UNIVERSITAT DE BARCELONA

# Isocyanide as a key functional group for the synthesis of biologically active compounds

Sònia Abás Prades

UNIVERSITAT DE BARCELONA

FACULTAT DE FARMÀCIA I CIÈNCIES DE L'ALIMENTACIÓ

DEPARTAMENT DE FARMACOLOGIA, TOXICOLOGIA I QUÍMICA TERAPÈUTICA

# ISOCYANIDE AS A KEY FUNCTIONAL GROUP FOR THE SYNTHESIS OF BIOLOGICALLY ACTIVE COMPOUNDS

**Sònia Abás Prades**

2018

UNIVERSITAT DE BARCELONA

FACULTAT DE FARMÀCIA I CIÈNCIES DE L'ALIMENTACIÓ

PROGRAMA DE DOCTORAT:
QUÍMICA ORGÀNICA

# ISOCYANIDE AS A KEY FUNCTIONAL GROUP FOR THE SYNTHESIS OF BIOLOGICALLY ACTIVE COMPOUNDS

Memòria presentada per Sònia Abás Prades per optar al títol de doctor per la Universitat de Barcelona

Directora i tutora:
Dra. Carmen Escolano Mirón

Doctoranda:
Sònia Abás Prades

**Sònia Abás Prades**
2018

Als meus pares,

Pedro i Maria Isabel.

El treball experimental recollit en aquesta memòria s'ha realitzat a la Secció de Química Terapèutica del Departament de Farmacologia, Toxicologia i Química Terapèutica de la Facultat de Farmàcia i Ciències de l'Alimentació de la Universitat de Barcelona, sota la direcció de la Dra. Carmen Escolano Mirón.

Aquest treball ha estat finançat pel Ministerio de Ciencia e Innovación (projectes CTQ2012-35250, BFU2015-66030-R i SAF2016-77703-C2-1-R). Per a la realització de la present Tesi Doctoral he gaudit d'una Beca Predoctoral d'ajuts destinats a universitats, centres de recerca i fundacions hospitalàries per a la contractació de personal investigador novell (FI-DGR 2014) de l'Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR) i un ajut per a estades predoctorals a l'estranger de la Fundació Pedro i Pons (abril-juliol 2016).

# Agraïments

En primer lloc, agrair a la meva directora i tutora d'aquesta present tesis, la Dra. Carmen Escolano, per donar-me l'oportunitat de realitzar aquesta tesi doctoral, per tota la confiança dipositada en mi, per la seva dedicació, per la seva ajuda, pel seu esforç i per orientar-me, no només al llarg de la tesi, sinó durant tots aquests anys que he tingut l'honor de treballar amb ella.

Al Dr. Carlos Arròniz, per haver sigut un GRAN mentor al laboratori, per ensenyar-me a ser perseverant, meticulós amb la feina i sobretot, per haver-me prestat la seva ajuda i els seu consells sempre que ho he necessitat.

Als Professors Dr. Joan Bosch i Dra. Merche Amat, per deixar-me forma part del seu grup. També, molt especialment, a les professores del grup: Dra. Núria LLor, Dra. Rosa Griera, Dra. Maria Pérez i la Dra. Anna Diez.

Al Professor Dr. Pelayo Camps, al Dr. Santiago Vázquez i al Dr. Diego Muñoz-Torrero per acollir-me en el seu equip aquest últim any.

Als estudiants que han participat directament en l'elaboració d'aquesta tesi: Juan, Judith, Marta, Àlex, Javier, Daniela, Gabi, Andrea, Anna i Ulrike. Molt especialment al Sergio. Sergio, gràcies per haver-te convertit en un magnífic company de tesi.

Als meus companys de laboratori, els de la unitat de química orgànica, on s'ha desenvolupat gran part d'aquesta tesi: gràcies Joan Andrés, Celeste, Guillaume, Álex, Fede, Roberto, Francesco, Elena, Carlos R, Carlos L, Magda, Agustín, Claudio, Sergi, Davinia, Aina, Míriam, Nihan ... i a l'Slavo, per ensenyar-me a fer els primers passets en un laboratori de Química Orgànica i a la Gisela per ser una gran amiga tan fora com dins del lab. Així mateix, tampoc oblidar els companys de la unitat de química farmacèutica que m'han acompanyat durant aquest darrer any. Gràcies Eugènica, Andreea, Sandra, Kàtia, Carla, Òscar... per acollir-me tan ràpid com un més. I aquells amb qui no he compartit lab., però si bons moments; gràcies Marta, Ane, Helena, Matías, Irene, Javi, Rosana. I molt especialment als Butiflers, perquè els matins sense el nostre cafè ja no són el mateix. Gràcies David per escoltar-me, gràcies Carles pels teus consells i sobretot, per la teva ajuda.

To all the people of Amsterdam. I would like to thank Prof Dr Romano Orru and Dr Eelco Ruijter for giving me the opportunity to work in their group. Thanks also to Vero, Barry, Elwin, Jordy, John, Răzvan, Gydo, Art, Jurriën and Giulia for their help and support and for providing a pleasurable work atmosphere. A l' Esther i Víctor per tota la infinita ajuda i consells. A Josep, gràcies per haver-me fet tan fàcil la convivència i a la Laia, gràcies per tot el teu temps compartit en mi, sempre és un magnífic plaer parlar amb tu de ciència.

Agrair a la Dra. Anna Linares per la seva ajuda en la realització dels experiments de Ressonància Magnètica Nuclear. A la Marina Fuentes per la realització dels espectres d'Infra Roig i la seva ajuda. I al Josep Galdón per preocupar-se i recordar-se sempre del meu buit.

Als col·laboradors, sense el treball dels quals gran part d'aquesta feina no hagués estat possible. Agrair al Dr. F. Javier Luque, la Dra. Carolina Estarellas i al Dr. Josep M. Campanera pels càlculs. Als Dr. Luis F. Callado, Dr. Jesús A. García-Sevilla, Dra. Amaia M. Erdozain, Dr. Benjamin Keller, Dra. Mercè Pallàs, Dr. Christian Griñan i Fotini Vasilopoulou pels estudis de farmacologia. Finalment, al Dr. Elies Molins per tots els rajos X i les seves classes de cristal·lografia.

Als meus amics, per estar al meu costat i per intentar entendre l'incomprensible, especialment quan he hagut de cancel·lar o fer tard a una quedada perquè la experimental s'ha complicat. Gràcies Mireia, Jessica, Enric, Joana, Rafel, Gemma, Nereida, Teresa i Marta.

I com no, moltes gràcies a la meva família. Al meu padrí per realitzar-me la portada d'aquesta present tesi i els seu consells. Als meus pares, a qui m'agradaria dedicar aquesta tesi, per sempre haver cregut en mi, per estar al meu costat i perquè sense la seva ajuda no hagués estat possible arribar fins aquí. I la meva germana per ser un gran suport.

Gràcies mami, gràcies papi, gràcies manger.

**ÍNDICE**

**PRÓLOGO**

Esta Tesis Doctoral se presenta como Compendio de Publicaciones. De acuerdo con la normativa vigente, la memoria incluye una introducción general en la que se presentan los trabajos y se detallan los objetivos, un resumen global de los resultados obtenidos, la discusión de los mismos y las conclusiones finales, junto con las copias completas de las publicaciones y una patente.

La presente tesis se ha dividido en 6 capítulos:

El capítulo 1 consiste en una introducción general a las características y reactividad del grupo isonitrilo y la importancia de los ésteres fosfónicos. En este capítulo inicial además se discute la organización de la memoria y se introducen los objetivos de cada uno de los trabajos realizados, los cuales quedan recogidos en los siguientes cinco capítulos. La agrupación de los resultados en base a unos objetivos concretos ha permitido la difusión de los mismos en revistas internacionales de reconocido prestigio.

El capítulo 2 comprende la publicación "*Access to the enantiopure pyrrolobenzodiazepine (PBD) dilactam nucleus via self-disproportionation of enantiomers*" (Sònia Abás, Carlos Arróniz, Elies Molins, Carmen Escolano, *Tetrahedron* **2018**, *74*, 867-871).

En el capítulo 3 se incluye la publicación "*First diastereoselective [3+2] cycloaddition reaction of diethyl isocyanomethylphosphonate and maleimides*" (Carlos Arróniz, Juan Molina, Sònia Abás, Elies Molins, Josep M. Campanera, F. Javier Luque, Carmen Escolano, *Org. Biomol. Chem*. **2013**, *11*, 1640-1649).

En el capítulo 4 se incorpora la publicación "*Easy access to (2-imidazolin-4-yl)phosphonates by a microwave assisted multicomponent reaction*" (Sònia Abás, Carolina Estarellas, F. Javier Luque, Carmen Escolano, *Tetrahedron* **2015**, *71*, 2872-2881).

En el capítulo 5 se adjunta el artículo "*Neuroprotective effects of a structurally new family of high affinity imidazoline $I_2$ receptor ligands*" (Sònia Abás, Amaia M. Erdozain,

Benjamin Keller, Sergio Rodríguez-Arévalo, Luis F. Callado, Jesús A. García-Sevilla, Carmen Escolano, *ACS Chem. Neurosci.* **2017**, *8*, 737-742).

El capítulo 6 contiene los resultados derivados de la preparación y caracterización farmacológica de una nueva familia de compuestos que han mostrado resultados excelentes como ligandos de receptores imidazólicos $I_2$ y neuroprotectores. Este capítulo está sometido a procesos de protección por parte de la Fundación Bosch i Gimpera y, tal y como determina la normativa, se encriptan los elementos que son indispensables para asegurar la protección o transferencia de los resultados (*Synthetic $I_2$ imidazoline receptor ligands for prevention or treatment of human brain disorders.* Escolano, C.; Pallàs, M.; Griñan, C. G.; Abás, S.; Callado, L. F.; García-Sevilla, J. A.. Eur. Pat. Appl. EP17382879.9, 2017).

Se recoge en el anexo un artículo publicado dentro del periodo de realización de la presente Tesis Doctoral que comparte como objetivo el estudio de las posibilidades sintéticas de isocianoacetatos pero no se enmarca en la obtención de compuestos complejos con posible interés terapéutico (*Facile microwave-assisted synthesis of thioformamides from isocyanides and carbon disulphide,* Sònia Abás, Ulrike Moens, Carmen Escolano *Tetrahedron Lett.* **2017**, *58*, 2768-2770).

## DIFUSIÓN DE LOS RESULTADOS

### Artículos por orden cronológico

**1. First diastereoselective [3+2] cycloaddition reaction of diethyl isocyanomethylphosphonate and maleimides**, Carlos Arróniz, Juan Molina, Sònia Abás, Elies Molins, Josep M. Campanera, F. Javier Luque, Carmen Escolano, *Org. Biomol. Chem*. **2013**, *11*, 1640-1649.

**2. Easy Access to (2-imidazolin-4-yl)phosphonates by a microwave assisted multicomponent reaction**, Sònia Abás, Carolina Estarellas, F. Javier Luque, Carmen Escolano, *Tetrahedron*, **2015**, *71*, 2872-2881.

**3. Neuroprotective effects of a structurally new family of high affinity imidazoline $I_2$ receptor ligands,** Sònia Abás, Amaia M. Erdozain, Benjamin Keller, Sergio Rodríguez-Arévalo, Luis F. Callado, Jesús A. García-Sevilla, Carmen Escolano, *ACS Chem. Neurosci.* **2017**, *8*, 737-742.

**4. Facile microwave-assisted synthesis of thioformamides from isocyanides and carbon disulfide** Sònia Abás, Ulrike Moens, Carmen Escolano, *Tetrahedron Lett.* **2017**, *58*, 2768-2770.

**5**. **Access to the enantiopure pyrrolobenzodiazepine (PBD) dilactam nucleus via self-disproportionation of enantiomers** Sònia Abás, Carlos Arróniz, Elies Molins, Carmen Escolano, *Tetrahedron* **2018**, *74*, 867-871.

*Special Issue Papers: Biogenetic Considerations in Complex Synthesis.*

**6. Synthetic $I_2$ imidazoline receptor ligands for prevention or treatment of human brain disorders.** Carmen Escolano, Mercè Pallàs, Christian G. Griñan, Luis F. Callado, Jesús A. García-Sevilla, *Eur. Pat. Appl.* EP17382879.9, 2017.

**CONGRESOS**

**1.** Christian Griñán-Ferré, Sònia Abás, Fotini Vasilopoulou, Amaia M. Erdozain, Benjamin Keller, Sergio Rodríguez-Arévalo, Luis F. Callado, Jesús A. García-Sevilla, Carmen Escolano, Mercè Pallàs.

*In vitro and in vivo neuroprotective evidences for new imidazoline $I_2$ Receptor ligands.*

17th National Congress of the Spanish Society of Neuroscience, Alicante (Spain), 2017.

Póster.

**2.** Mª Magdalena Alcover, Diana Berenguer, Berta Raventós, Sònia Abás, Sergio Rodríguez-Arévalo, Carmen Escolano, Roser Fisa, Cristina Riera.

*Efforts to design novel chemical entities to defeat leishmaniosis.*

XX Congreso de la Sociedad Española de Parasitología, San Cristóbal de la Laguna (Spain), 2017.

Póster.

**3.** Christian Griñán-Ferré, Sònia Abás, Fotini Vasilopoulou, Amaia M. Erdozain, Benjamin Keller, Sergio Rodríguez-Arévalo, Luis F. Callado, Jesús A. García-Sevilla, Carmen Escolano, Mercè Pallàs.

*Neuroprotective effect of novel imidazoline $I_2$ receptor ligands in the senescence accelerated mouse prone 8 (SAMP8).*

37 Congreso Sociedad Española de Farmacología, Barcelona (Spain), 2017.

Póster.

**4.** Sònia Abás, Sergio Rodríguez-Arévalo, Christian Griñán-Ferré, Amaia M. Erdozain, Benjamin Keller, Luis F. Callado, Jesús A. García-Sevilla, Mercè Pallàs, Carmen Escolano.

*Pharmacological characterization of a new family of (2-imidazolin-4-yl)phosphonates.*

IV Symposium of Young Researchers of the SEQT, Barcelona (Spain), 2017.

Comunicación oral.

**5.** Sònia Abás, Sergio Rodríguez-Arévalo, Christian Griñán-Ferré, Lara León-García, Francesc X. Sureda, Roser Fisa, Cristina Riera, Mercè Pallàs, Carmen Escolano.

*(2-Imidazolin-4-yl)methyl ester/phosphonates as leishmanicidal agents.*

IV Symposium of Young Researchers of the SEQT, Barcelona (Spain), 2017.

Póster.

**6.** Sònia Abás, Andrea Bagan, Amaia M. Erdozain, Benjamin Keller, Luis F. Callado, Jesús A. García-Sevilla, Carmen Escolano.

*A Structurally New Family of IBS Ligands.*

European Lead Factory- Early Career Researcher Event, Lisbon (Portugal), 2016.

Póster y comunicación oral.

**7.** Sònia Abás, Sergio Rodríguez-Arévalo, Amaia Erdozain, Benjamin Keller, Eduard Torrents, Luís F. Callado, Jesús A. García-Sevilla, Carmen Escolano.

*A Microwave assisted multicomponent reaction led to (2-imidazolin-4-yl)phosphonates.*

XXVII European Colloquium on Heterocyclic Chemistry, Amsterdam (Netherlands), 2016.

Póster.

**8.** Sònia Abás, Sergio Rodríguez-Arévalo, Amaia Erdozain, Benjamin Keller, Luís F. Callado, Jesús A. García-Sevilla, Carmen Escolano.

*(2-Imidazolin-4-yl)phosphonates as new imidazoline binding site ligands.*

III Symposium of Young Researchers of the SEQT, Barcelona (Spain), 2016.

Comunicación oral.

**9.** Juan Moreno-Felici, Sònia Abás, Eugènia Pujol, F. Javier Luque, Carmen Escolano, José Carlos Perales.

*The role of dibenzylxanthines as PEPCK-M inhibitors in diabetes and cancer.*

III Symposium of Young Researchers of the SEQT (Spain), Barcelona (Spain), 2016.

Póster.

**10.** Juan Moreno-Felici, Petra Hyroššová, Sònia Abás, A. Vidal-Alabró, F. Javier Luque, Carmen Escolano, Jose C. Perales.

*A drug discovery strategy to target PEPCK-M: implications in cancer and diabetes treatments.*

2nd MetNet annual Meeting: Adipose tissue metabolism in obesity, Barcelona (Spain), 2016.

Póster.

**11.** Sònia Abás, Carolina Estarellas, Sergio Rodríguez-Arévalo, F. Javier Luque, Carmen Escolano.

*A sustainable multicomponent microwave assisted reaction to prepare (2-imidazolin-4-yl)phosphonates.*

XII Simposio de Investigadores Jóvenes Químicos RSEQ-Sigma Aldrich, Barcelona (Spain), 2015.

Comunicación oral.

**12.** Sònia Abás, Carolina Estarellas, F. Javier Luque, Carmen Escolano.

*A green access to (2-imidazolin-4-yl)phosphonates.*

XXXV Bienal RSEQ 2015, A Coruña (Spain), 2015.

Póster y comunicación oral.

**13.** Sònia Abás, Juan Moreno, John M. Kelly, Lieve Naesens, F. Javier Luque, C. Escolano, Jose C. Perales.

*3-Alkyl-1,8-dibenzylxanthines as PEPCK-M inhibitors and other biological properties.*

II Symposium of Young Researchers of the SEQT, Madrid (Spain), 2015.

Póster.

**14.** Sònia Abás, Daniela Duver, Marie-France Herent, Carles Ciudad, Verónica Noé, Carmen Escolano.

*α-Iminophosphonates: synthesis, coordination studies and biological evaluation*.

II Symposium of Young Researchers of the SEQT, Madrid (Spain), 2015.

Póster.

**15.** Sònia Abás, Juan Molina, Carlos Arróniz, Marta Genís, Elies Molins, Josep M. Campanera, F. Javier Luque, Carmen Escolano.

*Synthetic and theoretical studies on the first diastereoselective [3+2] cycloaddition reaction of diethyl isocyanomethylphosphonate and maleimides.*

15$^{th}$ Tetrahedron Symposium, London (England), 2014.

Póster.

**16.** Sònia Abás, Judith Palà, Marie-France Herent and Carmen Escolano. *Studies on the reactivity and metal coordination properties of bicyclic α-aminophosphonates.*

15th Tetrahedron Symposium, London (England), 2014.

Póster.

**17.** Sonia Abás, Juan Molina, Carlos Arróniz, Judith Palà and Carmen Escolano. *Cyclic α-iminophosphonates by the first diastereoselective [3+2] cycloaddition reaction of diethyl isocyanomethylphosphonate and maleimides.*

VI Mediterranean Organic Chemistry Meeting, Granada (Spain), 2013.

Comunicación oral.

**18.** Josep M. Campanera, F. Javier Luque, Sònia Abás, Carmen Escolano.

*Theorical studies and transition states to compare the stereoselectivity in the formation of cyclic iminoesters vs. iminophosphonates.*

VI Mediterranean Organic Chemistry Meeting, Granada (Spain), 2013.

Póster.

**19.** Carlos Arróniz, Sònia Abás, Juan Molina, Mercedes Amat, Joan Bosch, Carmen Escolano.

*Isocyanoderivatives in 1,3-cycloaddition reactions.*

Organic and Medicinal Chemistry Workshop, Lisbon (Portugal), 2012.

Comunicación oral.

**ESTANCIA EN EL EXTRANJERO**

Del 1 de mayo de 2016 al 31 de julio de 2016 realicé una estancia predoctoral en la Vrije Universiteit en Amsterdam, bajo la supervisión del profesor Romano V. A. Orru trabajando en el tema "Use of diethyl isocyanomethylphosphonate in multicomponent reactions".

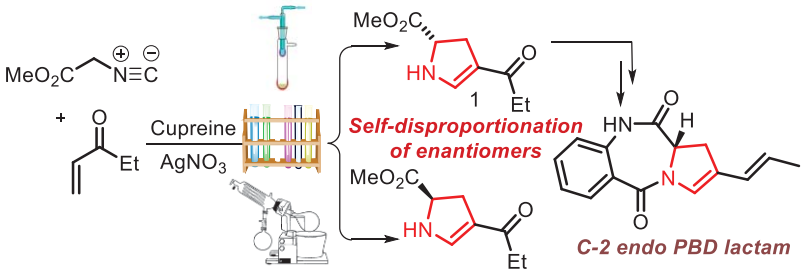
# Summary

The distinctive reactivity of isocyanides has been recognized as an advantageous characteristic for the formation of heterocyclic compounds. In the present manuscript new synthetic possibilities are explored involving the isocyanide group as the key point for the development of new reactions that allow the access to complex compounds. In particular, some of them show very promising pharmacological properties.

According to the different final products accessed, the pharmacological studies and the resulting publications, this dissertation has been classified into six sections.

**Access to the enantiopure pyrrolobenzodiazepine (PBD) dilactam nucleus via self-disproportionation of enantiomers** published in *Tetrahedron* **2018**, *74*, 867-871.

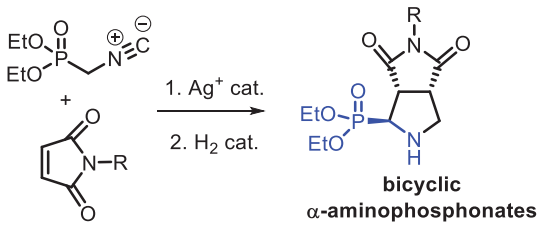
A short synthesis of an enantiopure pyrrolobenzodiazepine dilactam featuring early installation of the C2-C3 unsaturation is reported. An enantioselective cooperative catalytic cascade followed by self-disproportionation of enantiomers via sublimation afforded the enantiopure 2,3-dihydro-1*H*-pyrrole key intermediate, **1**. *N*-Acylation followed by reduction and lactam formation furnished the PBD dilactam.




**First diastereoselective [3+2] cycloaddition reaction of diethyl isocyanomethylphosphonate and maleimides** published in *Org. Biomol. Chem*. **2013**, *11*, 1640-1649.

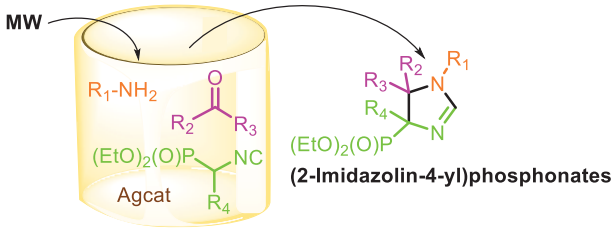
Bicyclic α-iminophosphonates were prepared *via* the first diastereoselective silver catalyzed [3+2] cycloaddition reaction of diethyl isocyanomethylphosphonate and diversely *N*-substituted maleimides. The reduction of the resulting imine by catalytic hydrogenation led to cyclic α-aminophosphonates, which are α-aminoester surrogates. The relative stereochemistry of the adducts was confirmed by X-ray crystallographic

analysis of one of the compounds. The diastereoselectivity of the cycloaddition reaction was rationalized by theoretical studies.




**Easy Access to (2-imidazolin-4-yl)phosphonates by a microwave assisted multicomponent reaction**, published in *Tetrahedron*, **2015**, *71*, 2872-2881.

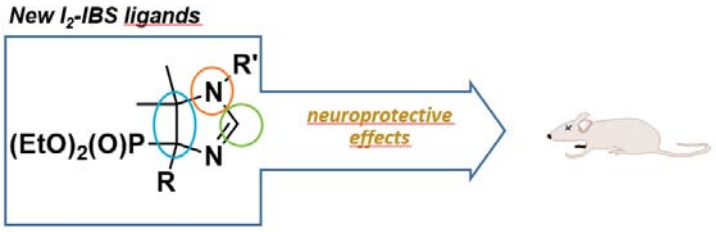
An efficient and user-friendly synthetic process involving the combination of multicomponent reaction methodology and microwave heating generates unprecedented (2-imidazolin-4-yl)phosphonates. This strategy presents a silver-catalyzed, operationally simple and environmentally friendly transformation without the need of anhydrous atmosphere or additional solvents.




**Neuroprotective effects of a structurally new family of high affinity imidazoline $I_2$ receptor ligands** published in *ACS Chem. Neurosci.* **2017**, *8*, 737-742.

The imidazoline $I_2$ receptors ($I_2$-IRs) are widely distributed in the brain, and $I_2$-IR ligands may have therapeutic potential as neuroprotective agents. Since structural data for $I_2$-IR remains unknown, the discovery of selective $I_2$-IR ligands devoid of $\alpha_2$-adrenoceptor ($\alpha_2$-AR) affinity is likely to provide valuable tools in defining the pharmacological characterization of these receptors. We report the pharmacological characterization of a new family of (2-imidazolin-4-yl)phosphonates. Radioligand binding studies showed that they displayed a higher affinity for $I_2$-IRs than idazoxan, and high $I_2/\alpha_2$ selectivity. In

vivo studies in mice showed that acute treatments with two of the compounds significantly increased p-FADD/FADD ratio (an index of cell survival) in the hippocampus when compared with vehicle-treated controls. Additionally, acute and repeated treatments with one compound markedly reduced hippocampal p35 cleavage into neurotoxic p25. The present results indicate a neuroprotective potential of (2-imidazolin-4-yl)phosphonates acting at $I_2$-IRs.




**Synthetic $I_2$ imidazoline receptor ligands for prevention or treatment of human brain disorders** Eur. Pat. Appl. EP17382879.9, 2017

# Capítulo 1

## Introducción

# 1.1
# INTRODUCCIÓN

La peculiar reactividad de los isocianuros representa una ventaja para su utilización en la síntesis de compuestos heterocíclicos. El átomo de carbono del grupo isocianuro es formalmente divalente, lo que posibilita reacciones tanto con electrófilos como con nucleófilos. En el caso de los derivados que contienen un grupo funcional isocianoacetato se reconoce una posición reactiva adicional, ya que el éster convierte en ácido al protón de la posición α-carbonílica (Figura 1).[1]


**Figura 1.** Reactividad de los isocianuros e isocianoacetatos.

Como objetivo global en esta Tesis, nos hemos planteado expandir el arsenal sintético de las reacciones que implican isocianuros y su aplicación en la preparación de heterociclos.

En este marco es interesante destacar que los heterociclos de cinco miembros que contienen nitrógeno son subestructuras importantes y ubicuas en una amplia gama de productos naturales y compuestos con interés biológico.[2] Dentro de este grupo de

[1] Para trabajos de revisión, ver: (a) Dömling, A. *Chem. Rev.* **2006**, *106*, 17-89; (b) Lygin, A.V.; de Meijere, A. *Angew. Chem. Int. Ed.* **2010**, *49*, 9094-9124; (c) Gulevich, A.V.; Zhdanko, A.G.; Orru, R.V.A.; Nenajdenko, V.G. *Chem. Rev.* **2010**, *110*, 5235-5331; (d) Tobisu, M.; Chatani, N. *Chem. Lett.* **2011**, *40*, 330-340; (e) Sadjadi, S.; Heravi, M.M. *Tetrahedron* **2011**, *67*, 2707-2752. (f) Bode, M.L.; Gravestock, D.; Rousseau, A.L. *Org. Prep. Proced. Int.* **2016**, *48*, 89–221; (g) Giustiniano, M.; Basso, A.; Mercalli, V.; Massarotti, A.; Novellino, E.; Tron, G.T.; Zhu, J. *Chem. Soc. Rev.* **2017**, *46*, 1295-1357.

[2] (a) Narayan, R.; Potowski, M.; Jia, Z.J.; Antonchick, A.P.; Waldmann, H. *Acc. Chem. Res.* **2014**, *47*, 1296–1310; (b) Arai, S.; Nakajima, M.; Nishida, A. *Angew. Chem. Int. Ed.* **2014**, *53*, 5569 –5572; (c) Vitaku, E.; Smith, D.T.; Njardarson, J.T. *J. Med. Chem.* **2014**, *57*, 10257–10274.

heterociclos, las pirrolinas (dihidropirroles) no solo son intermedios versátiles que brindan un acceso rápido a las pirrolidinas y pirroles, sino que también constituyen *building blocks* para la síntesis de compuestos con interés terapéutico e intermedios claves en la química actual.[3] En la literatura se han descrito diferentes enfoques sintéticos para obtener pirrolinas, pero la mayoría de las aproximaciones dan acceso a 3-pirrolinas.[4] La metodología sintética que permite la preparación de 2-pirrolinas está poco explorada a pesar de que estos heterociclos insaturados, que contienen una enamina, son importantes por su versatilidad en síntesis orgánica y en la preparación de compuestos con interés biológico.[5] Por tanto, el desarrollo de nuevas aproximaciones, versátiles y eficientes a 2-pirrolinas es un objetivo de elevado interés.

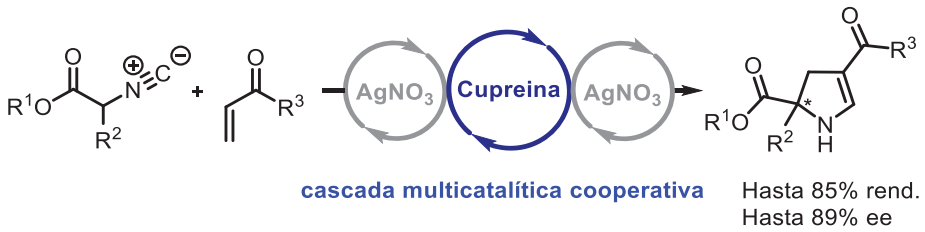
En el contexto de la Tesis Doctoral del Dr. Carlos Arróniz, se describió la primera reacción de cicloadición asimétrica formal [3+2] de isocianoacetatos y cetonas α,β-insaturadas que implicaba una secuencia de cascada multicatalítica cooperativa (Esquema 1).[6] Para desencadenar la reacción de cicloadición fue clave la combinación del organocatalizador, un derivado de los alcaloides cinchona que actuaba como base de Brønsted, con nitrato de plata que era un catalizador metálico y ácido de Lewis. Así pues, el proceso implicaba una cascada multicatalítica que permitió la formación de dos enlaces C-C y la generación de un centro estereogénico en un solo paso. Se exploraron diferentes patrones de sustitución en ambos materiales de partida para obtener un abanico de 2-pirrolinas enantioenriquecidas diferentemente sustituidas con rendimientos que llegaron hasta 85% y un exceso enantiomérico (ee) de hasta el 89%.

[3] (a) Humphrey, J.M.H.; Liao, Y.; Ali, A.; Rein, T.; Wong, Y.L.; Chen, H.J.; Courtney, A.K.; Martin, S.F. *J. Am. Chem. Soc.* **2002**, *124*, 8584-8592; (b) Herzon, S.B.; Myers, A.G. *J. Am. Chem. Soc.* **2005**, *127*, 5342-5344; (c) Bellina, F.; Rossi, R. *Tetrahedron* **2006**, *62*, 7213-7256. (d) Domagala, A.; Jarosz, T.; Lapkowski, M. *Eur. J. Med. Chem.* **2015**, *100*, 176-187.

[4] (a) Green, M.P.; Prodger, J.C.; Sherlock, A.E.; Hayes, C.J. *Org. Lett.* **2001**, *3*, 3377-3379; (b) Morita, N.; Krause, N. *Org. Lett.* **2004**, *6*, 4121-4123; (c) Tran, G.; Meier, R.; Harris, L.; Browne, D.L.; Ley, S.V. *J. Org. Chem.* **2012**, *77*, 11071-11078.

[5] (a) Grigg, R.; Lansdell, M.I.; Thornton-Pett, M. *Tetrahedron* **1999**, *55*, 2025-2044; (b) Kuwano, R.; Kashiwabara, M.; Ohsumi, M.; Kusano, H. *J. Am. Chem. Soc.* **2008**, *130*, 808-809; (c) Song, J.; Guo, C.; Chen, P.H.; Yu, J.; Luo, S.W.; Gong, L.Z. *Chem. Eur. J.* **2011**, *17*, 7786-7790.

[6] Arróniz, C.; Gil-González, A.; Semak, V.; Escolano, C.; Bosch, J.; Amat, M. *Eur. J. Org. Chem.* **2011**, 3755-3760.




**Esquema 1.** Primera reacción de cicloadición asimétrica formal [3+2] de isocianoacetatos y cetonas α,β-insaturadas que implica una secuencia de cascada multicatalítica cooperativa.

Con un nuevo método para la construcción rápida de 2,3-dihidropirrolinas enantioenriquecidas se propuso aplicar la metodología a la preparación enantioselectiva de moléculas complejas de interés biológico. Para ello, se escogieron como objetivo las moléculas denominadas pirrolobenzodiazepinas (PBDs) por su interés actual en la terapia del cáncer.

Descubiertas en la década de 1960, las PBDs son una clase importante de compuestos que interaccionan con el surco menor del ADN mediante unión covalente a bases de guanina.[7] Las PBDs tienen propiedades antibacterianas y citotoxicidad selectiva hacia las células tumorales y también exhiben una amplia gama de actividades biológicas (como antileishmania y herbicida).[8] Las PBDs naturales se han aislado de diversas especies de *Streptomyces* (por ejemplo, antramicina) y, más recientemente, de un *Micrococcus* (por ejemplo, las limazepinas).[9] Abarcan un rango de estructuras que se distinguen de la antramicina principalmente por la posición y el tipo de sustituyentes en los anillos A y C, y el grado y posición de los puntos de insaturación en el anillo C. Hasta la fecha, se han caracterizado trece PBDs naturales con diferentes patrones de sustitución en el anillo A y con el anillo C totalmente saturado (neotramicina A y B), C2/C3 *endo*-insaturado (antramicina) o C2-*exo*-insaturado (tomamicina) (Figura 2).

[7] (a) Antonow, D.; Thurston, D.E. *Chem. Rev.* **2011**, *111*, 2815-2864; (b) Varvounis, G. *Molecules* **2016**, *21*, 154-209.
[8] Hartley, J.A. *Expert Opin. Invest. Drugs*. **2011**, *20*, 733-744.
[9] Fotso, S.; Zabriskie, T.M.; Proteau, P.J.; Flatt, P.M.; Santosa, D.A.; Sulastri; Mahmud, T. *J. Nat. Prod.* **2009**, *72*, 690-695.

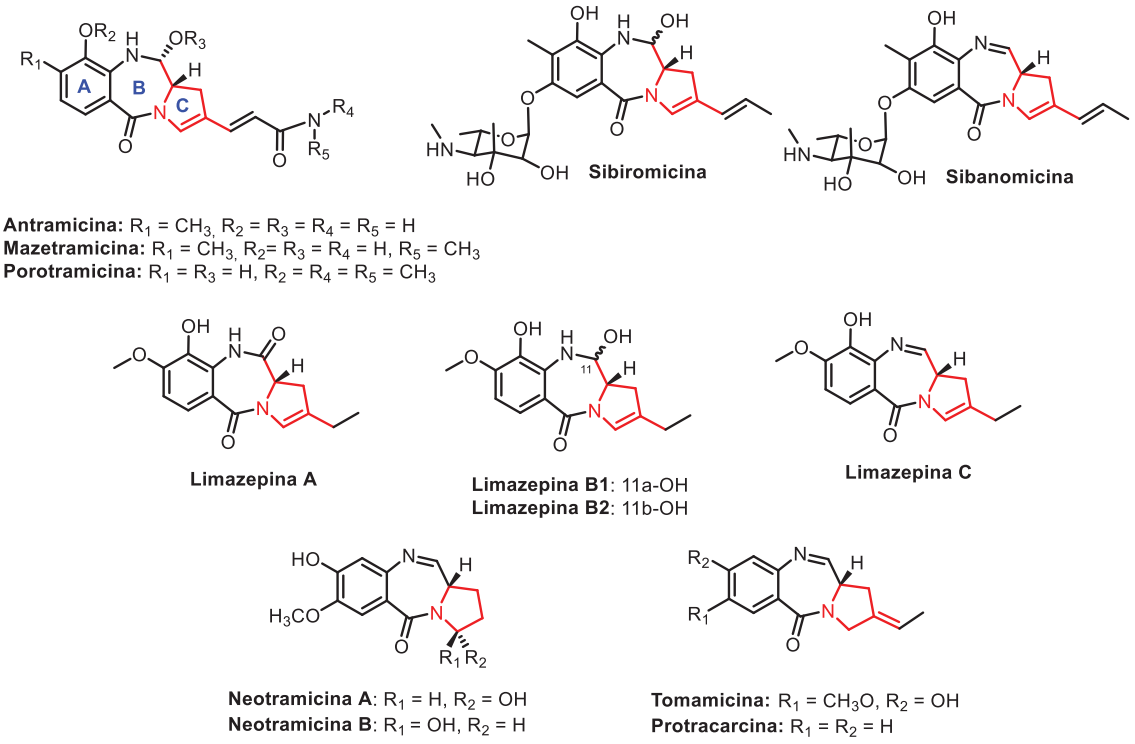



**Figura 2.** Pirrolobenzodiazepinas aisladas de fuentes naturales.

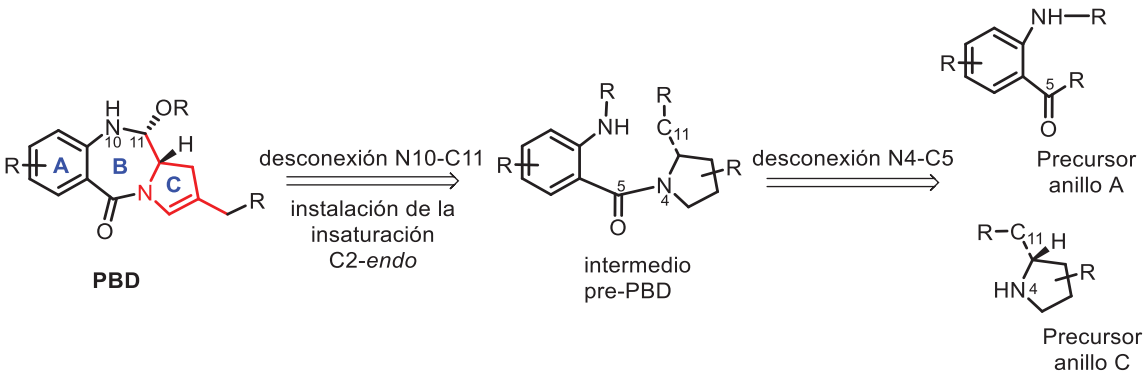
Desde el descubrimiento de la antramicina, la síntesis de las PBDs ha atraído la atención de muchos químicos debido a la compleja combinación de grupos funcionales, centros quirales, patrones de sustitución y su uso potencial como moléculas principales para el descubrimiento de fármacos anticancerígenos y antibacterianos.

Durante los últimos cuarenta años se han preparado una amplia gama de análogos sintéticos de PBDs, mediante síntesis que implican la formación tardía del enlace N10-C11 a partir de un intermedio pre-PBD resultante de la reacción de un precursor aromático adecuado del anillo A con un precursor del anillo C (Esquema 2).[10] La construcción del anillo C ha implicado generalmente la 2*S*-prolina y la 2*S*,4*R*-4-hidroxi-2-prolina derivada del éster metílico.

En las síntesis de PBDs C2-*endo* insaturadas publicadas hasta ahora, el doble enlace C2-*endo* se instala cuando el sintón pre-PBD ya está preparado o cuando el núcleo tricíclico

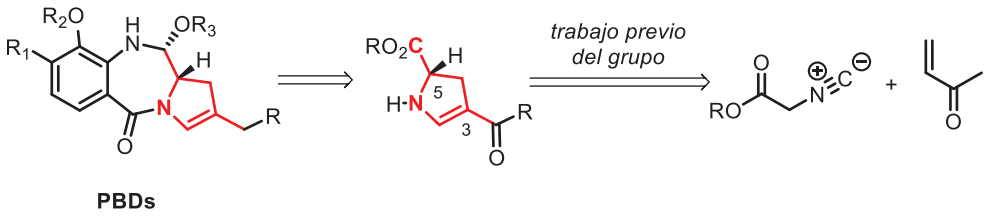
[10] (a) Biel, M.; Deck, P.; Giannis, A.; Waldmann, H. *Chem. Eur. J.* **2006**, *12*, 4121-4143; (b) Kamal, A.; Reddy K.L.; Devaiah, V.; Shankaraiah, N.; Reddy, G.S.K.; Raghavan, S. *Comb. Chem.* **2007**, *9*, 29-42.

ya está construido.[11] Ambos enfoques implican varios pasos sintéticos que, desafortunadamente, en la mayoría de los ejemplos constituyen un inconveniente, ya que se necesitan varias etapas sintéticas para incluir la insaturación sobre estructuras complejas.[12]




**Esquema 2.** Aproximación sintética general a las PBDs.

Para resolver el problema de la instalación del doble enlace en el anillo C en etapas de síntesis tardías propusimos una nueva estrategia sintética que se basa en utilizar las 3-acil-5-alcoxicarbonil-2-pirrolinas enantiopuras, recientemente sintetizadas por nuestro grupo como materiales de partida (Esquema 3). Por suerte, la alquilcetona en la posición 3 y el sustituyente éster en la posición 5 son los más adecuados para dar acceso a las PBDs a través de transformaciones orgánicas conocidas.




**Esquema 3**. Aproximación sintética propuesta en esta Tesis.

Se preparó el 4-propionil-2,3-dihidro-1*H*-pirrol-2-carboxilato de metilo siguiendo la metodología descrita en el grupo con un 74% de ee que tras la purificación por

[11] (a) Antonow, D.; Jenkins, T.C.; Howarda, P.W.; Thurstona, D.E. *Bioorg. Med. Chem.* **2007**, *15*, 3041-3053; (b) Kitamura, T.; Sato, Y.; Mori, M. *Tetrahedron* **2004**, *60*, 9649-9657.

[12] Para un trabajo de revisión de la biosíntesis, síntesis y actividades biológicas de las PBDs, ver: Gerratana, B. *Med. Res. Rev.* **2012**, *32*, 254-293.

sublimación llegó a un 99.9% de ee, sugiriendo que un fenómeno de *self-disproportonation of enantiomers* (SDE) había ocurrido.

El término SDE fue descrito por primera vez por Soloshonok en 2006.[13] La SDE constituye la propiedad no lineal de los compuestos quirales, que conduce a la separación espontánea o racionalmente diseñada de una muestra no racémica en fracciones más enantioenriquecidas y otras menos, en condiciones totalmente aquirales. Según los autores, este fenómeno está basado en la formación de fuerzas intramoleculares distintas que favorecen la formación preferencial de especies heteroquirales o homoquirales. Por ejemplo, partiendo de una muestra no racémica y en la que hay preferencia por las interacciones heteroquirales cabría esperar la formación de dímeros racémicos (*R*/*S*) y el exceso enantiomérico quedaría no emparejado en forma de monómero (Figura 3). Como el dímero y el monómero son identidades químicas distintas, estos podrían ser separados a través de condiciones aquirales. Lo mismo sucedería cuando haya preferencia para las interacciones homoquirales. Los enantiomeros *R* formarían una asociación y los *S* otra. Como hay cantidades distintas de cada enantiómero porque tenemos una muestra no racémica, esto formaría identidades distintas que podrían ser separadas por condiciones aquirales (Figura 3).[14]

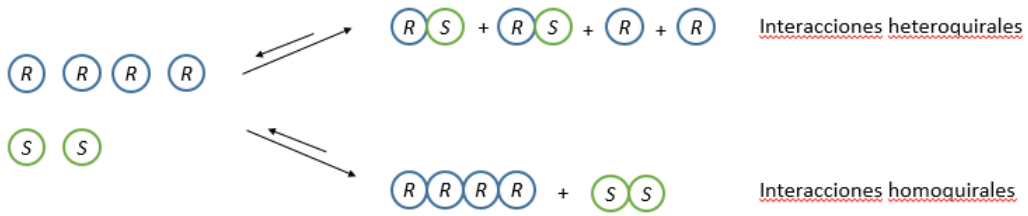
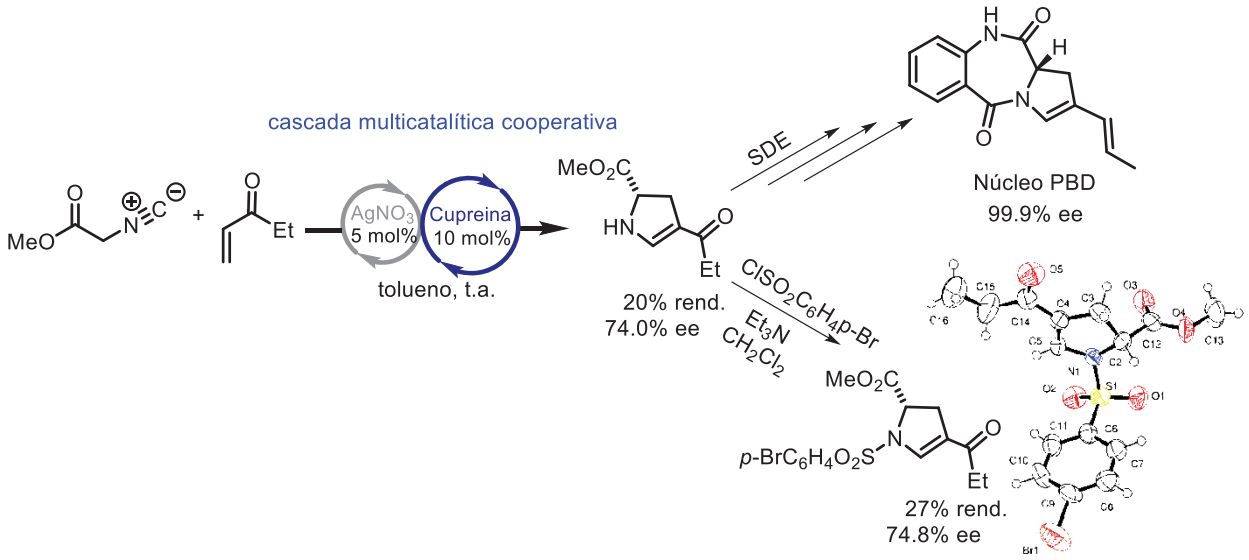



**Figura 3.** Representación de las interacciones heteroquirales y homoquirales.

[13] Soloshonok, V.A. *Angew. Chem. Int. Ed.* **2006**, *45*, 766-769.
[14] Para trabajos de revisión sobre SDE: (a) Soloshonok, V.A.; Roussel, C.; Kitagawa, O.; Sorochinsky, A.E. *Chem. Soc. Rev.* **2012**, *41*, 4180-4188; (b) Sorochinsky, A.E.; Aceña, J.L.; Soloshonok, V.A. *Synthesis* **2013**, *45*, 141-152; (c) Wzorek, A.; Sato, A.; Drabowicz, J.; Soloshonok, V.A. *Isr. J. Chem.* **2016**, *56*, 977-989; (d) Sorochinsky, A.E.; Soloshonok, V.A. *Top. Curr. Chem.* **2013**, *341*, 301-339.

La asignación del centro estereogénico presente en el 4-propionil-2,3-dihidro-1*H*-pirrol-2-carboxilato de metilo enantiopuro se realizó de manera indirecta preparando un derivado por acilación que contuviese un elemento pesado como el bromo (Esquema 4). La preparación de un monocristal de derivado bromado permitió la asignación absoluta e inequívoca del nuevo centro estereogénico generado en la reacción multicascada catalítica.

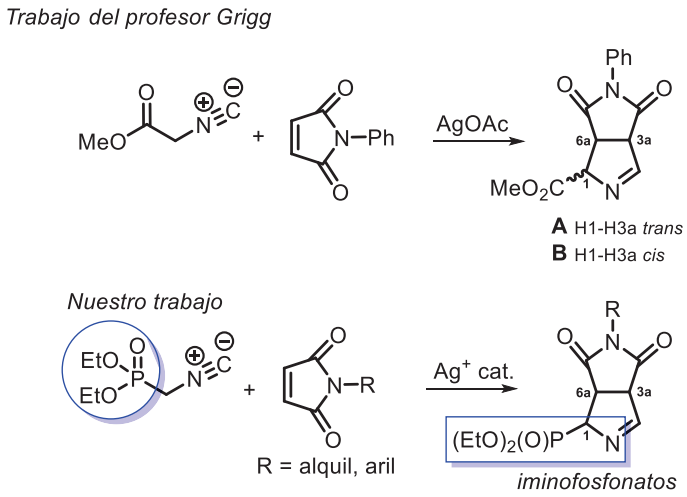



**Esquema 4.** Esquema sintético para acceder al núcleo de PBDs y estructura de Rayos X del (*S*)-1-[(4-bromofenil)sulfonil]-4-propionil-2,3-dihidro-1*H*-pirrol-2-carboxylato.

La configuración absoluta del centro estereogénico que se obtuvo con el uso de la cupreina como organocatalizador es la *S*, configuración requerida en el carbono 11a de todas las PBDs. Finalmente, una secuencia sintética sencilla condujo al núcleo tricíclico PBD constituyendo la primera aproximación a esta serie de compuestos complejos que implica la instalación del doble enlace del anillo C en etapas iniciales de la secuencia sintética.

A continuación, orientamos nuestros esfuerzos a expandir el potencial de los derivados de isocianoacetato inspirados en un trabajo previo del profesor Grigg y colaboradores en el que describían la reacción de cicloadición [3+2] entre isocianoacetato y aceptores de Michael para la síntesis de pirrolinas. En uno de los ejemplos se describe la reacción entre el isocianoacetato de metilo y la *N*-fenilmaleimida catalizada por acetato de plata

(Esquema 5).[15] En ella se formaban las 1-pirrolinas bicíclas correspondientes con una mezcla aproximada 2:1 de los dos posibles diastereómeros (**A** y **B**). Para el compuesto **A** la relación entre los hidrógenos de la posición 1 y 3a era *trans* y para el **B** la relación entre los hidrógenos de la posición 1 y 3a era *cis*.[16] Inspirados por este trabajo y con el objetivo de buscar nuevas estrategias para acceder a nuevos heterociclos, nos planteamos la posibilidad de llevar a cabo la reacción de cicloadición [3+2] sustituyendo el isocianoacetato de metilo por su análogo fosforado, el isocianometilfosfonato de dietilo (PhosMic) (Esquema 5).




**Esquema 5.** Cicloadición [3+2] entre derivados de isocianoacetato y maleimidas.

Los productos que resultarían de esta reacción serían iminofosfonatos cíclicos que son compuestos de elevado interés, ya que a parte de su valor intrínseco se pueden transformar por reducción en aminofosfonatos cíclicos ofreciendo un elevado rango de posibilidades sintéticas y de aplicación. Los α-aminofosfonatos son análogos estructurales de los α-aminoésteres, compuestos muy apreciados en la preparación de peptidomiméticos.[17] Además de su interés por su transformación sintética en otros

[15] Grigg, R.; Lansdell, M.I.; Thornton-Pett, M. *Tetrahedron* **1999**, *55*, 2025-2044.

[16] Es interesante destacar que recientemente el profesor Carretero ha descrito una versión enantioselectiva de esta reacción haciendo reaccionar un derivado de isocianoacetato de metilo y *N*-fenilmaleimida en presencia del complejo AuI/DTBM-segphos: Padilla, S.; Adrio, J.; Carretero, J.C. *J. Org. Chem.* **2012**, *77*, 4161-4166. Al mismo tiempo el profesor Shi y colaboradores han descrito otra versión enantioselectiva de la reacción con derivados de isocianoacetato y *N*-arilmaleimidas catalizada por plata y alcaloides de cinchona con un anillo de escuaramida en su estructura. Zhao, M.X.; Wei, D.K.; Ji, F.H.; Zhao, X.L.; Shi, M. *Chem. Asian J.* **2012**, *7*, 2777-2781.

[17] (a) Baylis, E.K.; Campbell, C.D.; Dingwall, J.G. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2845-2853. Para un artículo de revisión de fosfonatos azaheterocíclicos y su actividad biológica, ver: (b) Moone, K.; Laureyn,

derivados, en sí mismos tienen aplicaciones que van desde el área de la medicina hasta la agricultura. Así se han encontrado antibióticos, inhibidores enzimáticos, agentes anticancerosos, y herbicidas que tienen en común la existencia en su estructura de un grupo α-aminofosfónico.[18] El hecho de que compuestos que comparten dicho grupo funcional tengan propiedades biológicas interesantes se puede asociar en parte a la estructura tetrahédrica del grupo fosfonato que actúa como un análogo del estado de transición, por ejemplo en el caso de la hidrólisis peptídica enzimática. Por esta razón, estos compuestos se pueden considerar como inhibidores del estado de transición de peptidasas o esterasas específicas. De hecho, es conocido que los derivados de ácido α-aminofosfónico son inhibidores de numerosos enzimas implicados en el metabolismo endógeno peptídico. Todo este interés biológico comentado ha hecho que se hayan desarrollado diversos métodos enantio y diastereoselectivos para acceder a α-aminofosfonatos acíclicos ópticamente activos. Curiosamente, existen pocos métodos que permitan la síntesis diastereoselectiva de α-aminofosfonatos cíclicos ópticamente activos a pesar de que estos derivados son extremadamente atractivos desde el punto de vista farmacológico ya que son análogos del aminoácido prolina, conocidos como derivados fosfonoprolínicos (Figura 4).[19]

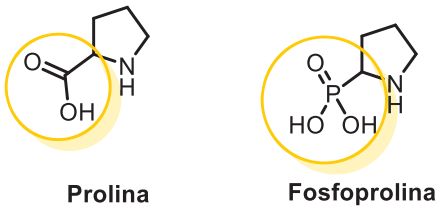



**Figura 4.** Representación de la prolina y fosfoprolina.

I.; Stevens, C.V. *Chem. Rev.* **2004**, *104*, 6177-6215. Para aplicaciones farmacológicas de ácidos α-aminofosfónicos y sus derivados, ver: (c) *Aminophosponic and Aminophosphinic Acids: Chemistry and Biological Activity*; Kukhar, V.P., Hudson, H.R., Eds; John Wiley & Sons; Chichester, UK, **2000**; (d) Orsini, F.; Sello, G.; Sisti, M. *Curr. Med. Chem.* **2010**, *17*, 264-289.

[18] (a) Atherton, F.R.; Hassall, C.H.; Lambert, R.W. *J. Med. Chem.* **1986**, *29*, 29-40; (b) De Lombaert, S.; Blanchard, L.; Tan, T.; Sakane, Y.; Berry, C.; Ghai, R.D. *Bioorg. Med. Chem. Lett.* **1995**, *5*,145-150; (c) Kafarski, P.; Lejczak, B. *Curr. Med. Chem.*: *Anti-Cancer Agents* **2001**, *1*, 301-312.

[19] Para la síntesis de fosfoprolinas, ver: (a) Groth, U.; Richter, L.; Schöllkopf, U. *Tetrahedron* **1992**, *48*, 117-122; (b) Davis, F.A.; Lee, S.H.; Xu, H. *J. Org. Chem.* **2004**, *69*, 3774-3781; (c) Hanessian, S.; Gauchet, C.; Charron, G.; Marin, J.; Nakache, P. *J. Org. Chem.* **2006**, *71*, 2760-2778; (d) Wuggening, F.; Schweifer, A.; Mereiter, K.; Hammerschmidt, F. *Eur. J. Org. Chem.* **2011**, 1870-1879; (e) Hirata, S.; Kuriyama, M.; Nomura, O. *Tetrahedron* **2011**, *67*, 9411-9416.

La reacción propuesta se llevó a cabo con maleimidas diferentemente sustituidas por grupos alquilo o arilo. Es de destacar que todas las reacciones fueron completamente diastereoselectivas ya que solo se observó uno de los dos posibles diastereoisómeros (H1-H3a *cis* o H1-H3a *trans*). La configuración relativa fue confirmada inequívocamente por análisis de cristalografía de rayos X de uno de los compuestos obtenido, que indica una relación *trans* entre los átomos de hidrógeno en las posiciones cabeza de puente y el átomo de hidrógeno en el carbono α del éster fosfónico (Figura 5).

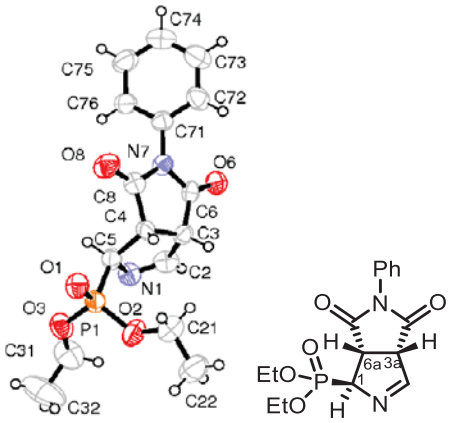



**Figura 5**. Estructura de rayos X de un iminofosfonato bicíclico.

Destacar que esta reacción de cicloadición [3+2] nos inspiró para la formación de una nueva librería de compuestos que resultaron tener una elevada afinidad para los receptores imidazólicos de tipo 2 ($I_2$-IR) (explicados con posterioridad en esta breve introducción), resultando tener un gran interés farmacológico como neuroprotectores. El trabajo se encuentra sometido a procesos de protección por parte de la Fundación Bosch i Gimpera de la Universidad de Barcelona.

Con el objetivo de buscar nuevas aplicaciones sintéticas para los derivados de isocianoacetatos y con la experiencia previa con el PhosMic y las maleimidas, decidimos estudiar el PhosMic en reacciones multicomponente (MCR).

Las MCR son aquellas en que participan tres o más reactivos para dar lugar en una sola operación a un único compuesto que contiene esencialmente todos los átomos presentes de los precursores.[20] Estos procesos se caracterizan por presentar una

[20] Para trabajos de revisión sobre reacciones multicomponente, ver: (a) Bienaymé, H.; Hulme, C.; Oddon, G.; Schmitt, P. *Chem.-Eur. J.* **2000**, *6*, 3321-3329; (b) Ruijter, E.; Scheffelaar, R.; Orru, R.V.A. *Angew. Chem.*

elevada economía de átomo, ya que todos los átomos de los reactivos de partida están en el producto final, son eficientes, porque se forma el producto en un solo paso en lugar de pasos múltiples, y exhiben un índice de formación de enlaces muy alto, debido a que se forman varios enlaces en una sola transformación sintética.

Los isocianuros son especialmente populares en este tipo de reacciones gracias a la capacidad que tienen de reaccionar tanto con electrófilos como nucleófilos, lo que les proporciona una gran flexibilidad reactiva. De hecho, el interés por explorar sus posibilidades sintéticas ha experimentado un incremento debido a su papel crucial en este tipo de reacciones, que en este caso particular se denominan reacciones multicomponente basadas en isocianuros (del inglés, isocyanide-base multicomponent reactions IMCRs).[21]

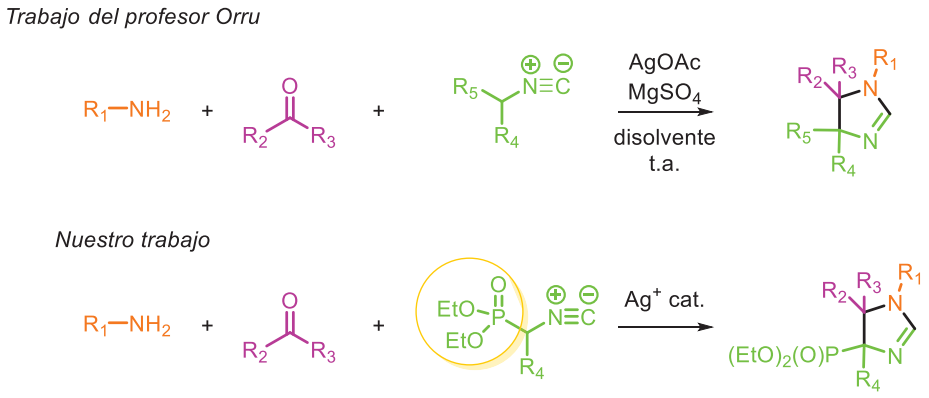
En un trabajo descrito por el profesor Orru y colaboradores se describe una reacción multicomponente eficiente y flexible para preparar 2-imidazolinas diversamente sustituidas, empleando isocianuros con un protón α-ácido, una amina y un compuesto carbonílico (Esquema 6).[22] Esta reacción se extrapoló al uso de PhosMic y derivados de PhosMic α-sustituidos (Esquema 6). Cabe mencionar, que aunque los derivados de isocianoacetatos se pueden encontrar ampliamente en la literatura como reactivos en MCR, el uso de PhosMic en IMCRs ha recibido menos atención.[23]

*Int. Ed.* **2011**, *50*, 6234-6246; (c) Dömling, A.; Wang, W.; Wang, K. *Chem. Rev.* **2012**, *112*, 3083-3135; (d) Van der Heijden, G.; Ruijter, E.; Orru, R.V.A. *Synlett* **2013**, *24*, 666-685.

[21] (a) Dömling, A. *Chem. Rev.* **2006**, *106*, 17-89; (b) Elders, N.; Ruijter, E.; Nenajdenko, V.G.; Orru, R.V. α-Acidic isocyanides in multicomponent chemistry. In *Isocyanide Chemistry Applications in Synthesis and Material Science*; Nenajdenko, V.G., Eds.; Wiley-VCH Verlag GmbH & Co. KGaA; Weinheim, Germany, **2012**, 75-108.

[22] (a) Bon, R.S.; van Vliet, B.; Sprenkels, N.E.; Schmitz, R.F.; de Kanter, F.J.J.; Stevens, C.V.; Swart, M.; Bickelhaupt, F.M.; Groen, M.B.; Orru, R.V.A. *J. Org. Chem.* **2005**, *70*, 3542-3553; (b) Elders, N.; Schmitz, R.F.; de Kanter, F.J.J.; Ruijter, E.; Groen, M.B.; Orru, R.V.A. *J. Org. Chem.* **2007**, *72*, 6135-6142; (c) Elders, N.; Ruijter, E.; de Kanter, F.J.J.; Groen, M.B.; Orru, R.V.A. *Chem.-Eur. J.* **2008**, *14*, 4961-4973.

[23] (a) Rueping, M.; Vila, C.; Bootwicha, T. *ACS Catal.* **2013**, *3*, 1676-1680; (b) Sinha, M.K.; Khoury, K.; Herdtweck, E.; Dömling, A. *Chem.-Eur. J.* **2013**, *19*, 8048-8052.




**Esquema 6.** IMCR propuesta con PhosMic.

Como las MCR se caracterizan por tener propiedades que se ajustan a la definición de síntesis ideal, nos planteamos buscar unas condiciones de reacciones que respetasen en la medida de lo posible ese concepto.[24] Según Wender y Miller la síntesis ideal consiste en la producción de una molécula concreta en una sola operación sintética a partir de materiales de partida fácilmente asequibles, con un rendimiento del 100% y sin formación de productos secundarios.[25] Así que para ello nos propusimos eliminar el disolvente de la reacción y realizar la reacción en un horno microondas, reduciendo notablemente el tiempo de reacción y la energía empleada para hacer que el proceso fuese respetuoso con el medio ambiente.[26]

Cabe destacar que la reacción propuesta daría acceso directo a las (2-imidazolin-4-il)fosfonatos, compuestos que contienen un anillo de 2-imidazolina en su estructura. Las 2-imidazolinas constituyen una clase importante de heterociclos que pueden encontrarse en la química de productos naturales, síntesis orgánica, química de coordinación y catálisis homogénea.[27] Además, se identifican como compuestos que

[24] (a) Gaich, T.; Baran, P.S. *J. Org. Chem.* **2010**, *75*, 4657-4673; (b) Wender, P.A. *Tetrahedron* **2013**, *69*, 7529-7550; (c) Cioc, R.C.; Ruijter, E.; Orru, R.V.A *Green Chem.* **2014**, *16*, 2958-2975.
[25] Wender, P.A.; Miller, B.L. *Nature* **2009**, *460*, 197-201.
[26] Para trabajos de revisión sobre microondas como un método dentro de la química verde, ver: (a) Moseley, J.D.; Kappe, C.O. *Green Chem.* **2011**, *13*, 794-806; (b) Majumder, A.; Gupta, R.; Jain, A. *Green Chem. Lett. Rev.* **2013**, *6*, 151-182. Para ver los principios de la química verde, ver: (c) Anastas, P.T.; Kirchhoff, M.M. *Acc. Chem. Res.* **2002**, *35*, 686-694.
[27] (a) Cherrn, J.W.; Liaw, Y.C.; Chen, C.S.; Rong, J.G.; Huang, C.L.; Chan, C.H.; Wang, A.H. *J. Heterocycles* **1993**, *36*, 1091-1103; (b) Weiss, M.E.; Fisher, D.F.; Xin, Z.Q.; Jautza, S.; Schweizer, W.B.; Peters, R. *Angew. Chem. Int. Ed.* **2006**, *45*, 5694-5699; (c) Ma, K.; You, J. *Chem.-Eur. J.* **2007**, *13*, 1863-1871; (d) Bon, R.S.; de Kanter, F.J.J.; Lutz, M.; Spek, A.L.; Jahnke, J.C.; Hahn, F.E.; Orru, R.V.A. *Organometallics* **2007**, *26*, 3639-3650; (e) Strassverger, Z.; Mooijman, M.; Ruitjer, E.; Alberts, A.H.; de Graaff, C.; Orru, R.V.A.; Rothenberg,

modulan los receptores $\alpha_2$-adrenérgicos, y a menudo muestran una alta afinidad por los receptores imidazólicos (IR).[28]

Los IR son lugares de unión que presentan una elevada afinidad para los compuestos que contienen una estructura de imidazolina. Como su estructura aún no se ha descrito han sido caracterizados gracias a su afinidad para una serie de radioligandos como [$^3$H] clonidina, [$^3$H] *p*-aminoclonidina e [$^3$H] idazoxan (figura 6). Así, podemos encontrar los receptores imidazólicos de tipo 1 ($I_1$-IR), que presentan una elevada afinidad para [$^3$H] clonidina, y los receptores imidazólicos de tipo 2 ($I_2$-IR), con una elevada afinidad para [$^3$H] idazoxan.[29] Finalmente, ha sido identificado un tercer subtipo de IR implicado en la secreción de insulina en las células β pancreáticas y designado como receptor imidazólico de tipo 3 ($I_3$-IR).[30]


**Figura 6.** Radioligandos usados para caracterizar los IR.

Tanto los $I_1$-IR como los $I_2$-IR están ampliamente distribuidos en el cerebro. Los $I_1$-IR, los receptores que han recibido más atención en el campo de los IR hasta la fecha, son responsables de los efectos simpático-inhibidores de fármacos antihipertensivos de acción central como la moxonidina o la rilmenidina.[31] Por otro lado, los $I_2$-IR, localizados especialmente en la glia del sistema nervioso central, se han estudiado por su posible implicación en trastornos cerebrales humanos, como la depresión,[32] demencia del tipo

G. *Appl. Organomet. Chem.* **2009**, *24*, 142-146; (f) Murai, K.; Fukushima, S.; Nakamura, A.; Shimura, M.; Fujioka, H. *Tetrahedron* **2011**, *67*, 4862-4868.

[28] Bousquet, P.; Feldman, J.; Schwartz, J.J. *Pharmacol. Exp. Ther.* **1984**, *230*, 232-236.

[29] (a) Dardonville, C.; Rozas, I. *Med. Res. Rev.* **2004**, *24*, 639-661; (b) Li, J.X.; Zhang, Y. *Eur. J. Pharmacol.* **2011**, *658*, 49-56; (c) Krasavin, M. *Eur. J. Med. Chem.* **2015**, *97*, 525-537; (d) Li, J.X. *Pharmacol. Ther.* **2017**, *178*, 48-56.

[30] Chan, S.L.; Brown, C.A.; Scarpello, K.E.; Morgan, N.G. *Br. J. Pharmacol.* **1994**, *112*, 1065-1070.

[31] Ernsberger, P.; Graves, M.E.; Graff, L.M.; Zakieh, N.; Nguyen, P.; Collins, L.A.; Westbrooks, K.L.; Johnson, G.G. *Ann. N. Y. Acad. Sci.* **1995**, *763*, 22-42.

[32] (a) Meana, J.J.; Barturen, F.; Martín, I.; García-Sevilla, J.A. *Biol. Psychiatry* **1993**, *34*, 498-501; (b) García-Sevilla, J.A.; Escribá, P.V.; Sastre, M.; Walzer, C.; Busquets, X.; Jaquet, G.; Reis, D.J.; Guimón, J. *Arch. Gen. Psychiatry* **1996**, *53*, 803-810.

Alzheimer,[33] enfermedad del Parkinson[34] y tumores gliales.[35] Cabe destacar que los $I_2$-IR podrían representar un grupo de proteínas heterogéneas que los ligandos del receptor $I_2$-IR como idazoxan reconocen. Por lo tanto, cuando se habla de los receptores $I_2$-IR, no se refiere a una identidad molecular específica, sino que de varias moléculas de proteínas diferentes y biológicamente diversas, por lo que sus ligando pueden dar lugar a distintos efectos terapéuticos.

Los compuestos con alta afinidad para los $I_2$-IR se han asociado a efectos farmacológicos como el analgésico,[36] reducción de la temperatura corporal (especialmente para procesos de isquemia cerebral)[37] y neuroprotección.[38] Pero la mayoría de estos compuestos presentan un problema y es que también son afines al receptor adrenérgico $\alpha_2$ ($\alpha_2$-AR), por lo que el desarrollo de nuevos compuestos con mejor afinidad y selectividad para los $I_2$-IR se vuelve crucial. Encontrar ligandos con mejor afinidad y selectividad permitiría caracterizar el receptor famacológicamente. Los ligandos $I_2$-IR pueden clasificarse en cuatro familias químicas: las imidazolinas, las guanidinas, las 2-aminoimidazolinas y las carbolinas (Figura 7).[39] De todos ellos, las imidazolinas son las más selectivas y dentro de ellos el idazoxan es el que se utiliza como referencia en muchos estudios.

[33] (a) Ruíz, J.; Martín, I.; Callado, L.F.; Meana, J.J.; Barturen, F.; García-Sevilla, J.A. *Neurosci. Lett.* **1993**, *160*, 109-112; (b) García-Sevilla, J.A.; Escribá, P.V.; Walzer, C.; Bouras, C.; Guimón, J. *Neurosci. Lett.* **1998**, *247*, 95-98.

[34] Gargalidis-Moudanos, C.; Pizzinat, N.; Javoy-Agid, F.; Remaury, A.; Parini, A. *Neurochem. Int.* **1997**, *30*, 31-36.

[35] (a) Martín-Gómez, J.I.; Ruíz, J.; Callado, L.F.; Garibi, J.M.; Aguinaco, L.; Barturen, F.; Meana, J.J. *NeuroReport* **1996**, *7*, 1393-1396; (b) Callado, L.F.; Martín-Gomez, J.I.; Ruiz, J.; Garibi, J.; Meana, J.J. *J. Neurol. Neurosurg. Psychiatry* **2004**, *75*, 785-787.

[36] (a) Li, J.X.; Zhang, Y. *Eur. J. Pharmacol.* **2011**, *658*, 49-56; (b) Li, J.X; Thorn, D.A.; Qiu, Y.; Peng, B.W.; Zhang, Y. *Br. J. Pharmacol.* **2014**, *171*, 1580-1590 ; (c) Thorn, D.A.; Zhang, Y.; Li, J.X. *Psychopharmacology* **2017**, *234*, 1871-1880.

[37] (a) Craven, J.A.; Conway, E.L. *Clin. Exp. Pharmacol. Physiol.* **1997**, *24*, 204-207; (b) Marion, D.W.; Penrod, L.E.; Kelsey, S.F.; Obrist, W.D.; Kochanek, P.M.; Palmer, A.M.; Wisniewski, S.R.; DeKosky, S.T. *N. Engl. J. Med.* **1997**, *336*, 540-546; (c) Thorn, D.A.; An, X.F.; Zhang, Y.; Pigini, M.; Li, J.X. *Br. J. Pharmacol.* **2012**, *166*, 1936-1945.

[38] Garau, C.; Miralles, A.; García-Sevilla, J.A. *J. Psychopharmacol.* **2013**, *27*, 123-134.

[39] Dardonville, C.; Rozas, I. *Med. Res. Rev.* **2004**, *24*, 639-661.

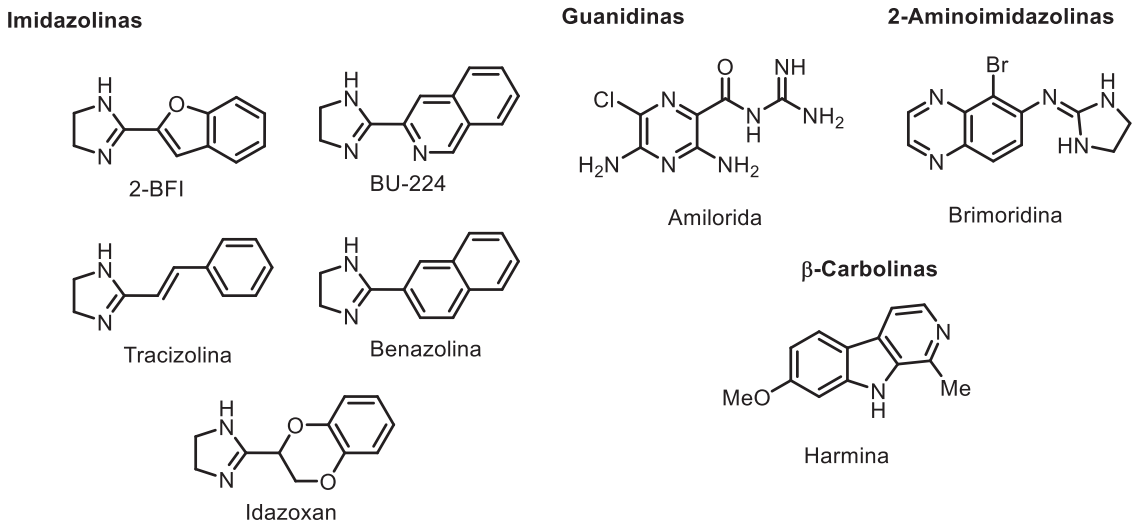
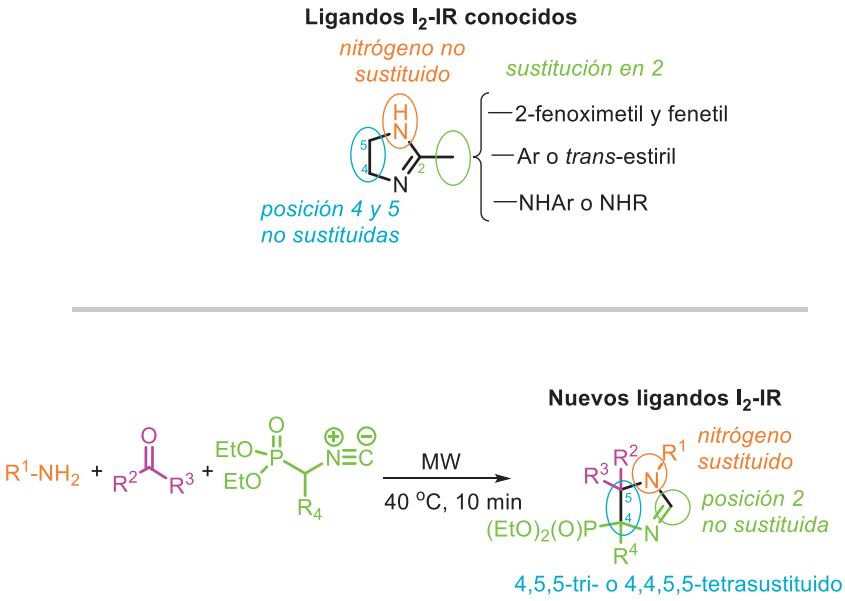



**Figura 7.** Ligandos $I_2$-IBS.

Tal y como se puede observar en la Figura 7, todos los ligandos del grupo de las imidazolinas presentan un núcleo de imidazolina sin sustituyentes en las posiciones 1, 4 y 5 y sustituidos en la posición 2 por heterociclos.

Las reacciones de IMCR propuestas conducen a compuestos imidazólicos con un patrón de sustitución en el núcleo imidazólico diferente a los ligandos $I_2$ descritos hasta el momento. En concreto, los nuevos compuestos tienen las posiciones 1, 4 y 5 sustituidas y sin sustituyentes en la posición 2 además de incluir un sustituyente fosfonato (Esquema 7).




**Esquema 7.** Estructura de los ligandos $I_2$-IR conocidos, nuevos ligandos y la reacción ICMR propuesta.

Posteriormente, se procedió a evaluar el perfil farmacológico y la selectividad de los nuevos (2-imidazolin-4-il)fosfonatos a través de estudios competitivos de unión con el radioligando selectivo de $I_2$-IR [$^3$H]-2-[(2-benzofuranil)-2-imidazolina] (2-BFI) y el radioligando selectivo del receptor $\alpha_2$-adrenérgico [$^3$H]-RX821002 (2-metoxiidazoxan). Finalmente, como las moléculas resultaron ser altamente selectivas para $I_2$-IR nos planteamos realizar estudios tanto *in vivo* como *in vitro* para determinar su perfil farmacológico. Así, se realizaron estudios de analgesia, de hipotermia y de neuroprotección (fosforilación de FADD[40] y la escisión del fragmento p35).[41]

[40] Ramos-Miguel, A.; García-Fuster, M.J.; Callado, L.F.; La Harpe, R.; Meana, J.J.; García-Sevilla, J.A. *Neuroscience* **2009**, *161*, 23-38.
[41] Yousuf, M.A.; Tan, C.; Torres-Altoro, M.I.; Lu, F.M.; Plautz, E.; Zhang, S.; Takahashi, M.; Hernández, A.; Kernie, S.G.; Plattner, F.; Bibb, J.A. *J. Neurochem.* **2016**, *138*, 317-327.

## 1.2
## ESTRUCTURA DE LA MEMORIA Y OBJETIVOS

Esta Tesis Doctoral se presenta como un Compendio de Publicaciones. La memoria está dividida en un resumen global de los resultados obtenidos, de la discusión de estos y de las conclusiones finales; una introducción general, donde se presentan los trabajos, se justifica la temática y se especifican los objetivos; cinco capítulos donde se presentan las copias completas de las publicaciones con el material suplementario correspondiente y las conclusiones.

Para una mayor comprensión de la memoria no se ha seguido el orden cronológico para la exposición de los trabajos realizados, sino que se han ordenado según el tipo de reacción que ha primado en el estudio. En todos los casos las estructuras sintéticas planteadas son novedosas y tienen interés farmacológico tal y como queda reflejado en la memoria.

El objetivo principal de esta Tesis es ampliar el arsenal sintético referido a reacciones que involucran isocianuros y, al mismo tiempo, la búsqueda de compuestos activos como pirrolinas e imidazolinas.

A un primer nivel los esfuerzos se han centrado en dos grupos principales de reacciones, las cicloadiciones y las reacciones multicomponente. En el primer caso y aprovechando la experiencia de una metodología desarrollada previamente en nuestro grupo para acceder a 2-pirrolidinas enantiopuras, nos planteamos el acceso enantioselectivo al núcleo de pirrolobenzodiazepina que se encuentra presente en compuestos de elevado interés para el tratamiento del cáncer (Capítulo 2).

Además, se ha descrito la primera reacción de cicloadición [3+2] diastereoselectiva entre isocianometilfosfonato de dietilo y maleimidas diferentemente sustituidas (Capítulo 3). En el segundo grupo de reacciones objetivo se encuentran las reacciones multicomponente en las que el grupo isocianuro ha mostrado una elevada versatilidad. En el estudio particular de esta Tesis se ha considerado la preparación de (2-imidazolin-4-il)fosfonatos mediante una reacción multicomponente promovida por microondas y satisfaciendo los principios de la química verde (Capítulo 4). Los compuestos preparados han resultado tener una excelente actividad como ligandos de los receptores

imidazólicos $I_2$, constituyendo un primer ejemplo de compuestos que tienen un esqueleto inédito y que no corresponde a los estereotipos estructurales descritos con anterioridad para este tipo de ligandos (Capítulo 5). La explotación de la diversidad estructural que puede englobar la cicloadición [3+2] ha llevado a la preparación de compuestos que han mostrado un elevado interés farmacológico como neuroprotectores (Capítulo 6). En la Figura 8 se esquematiza la presente memoria.

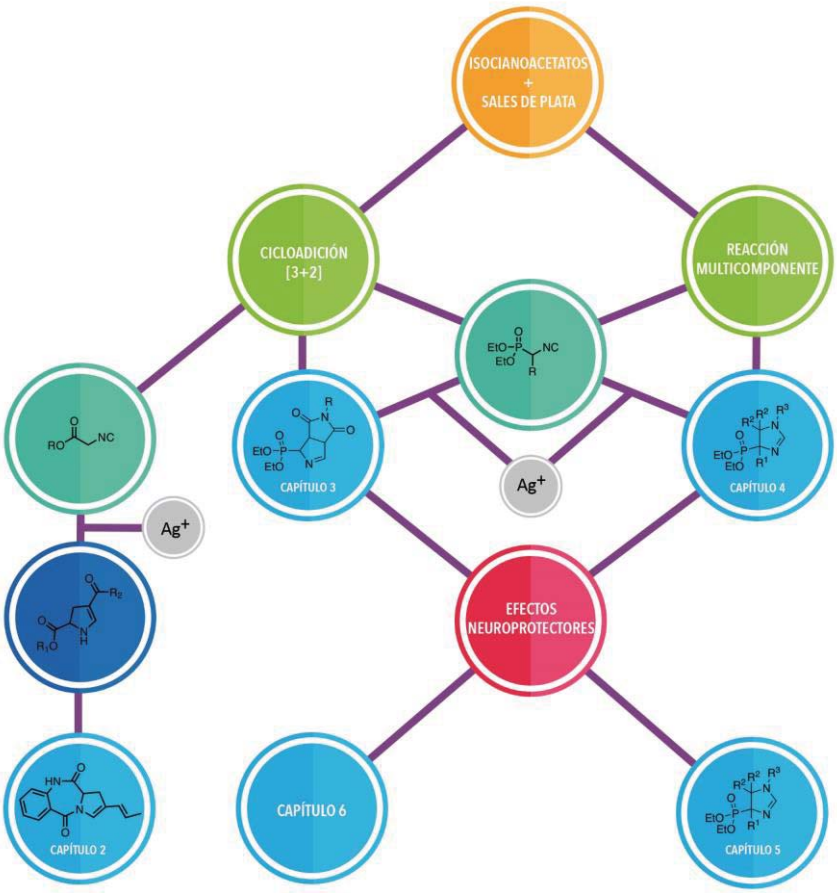


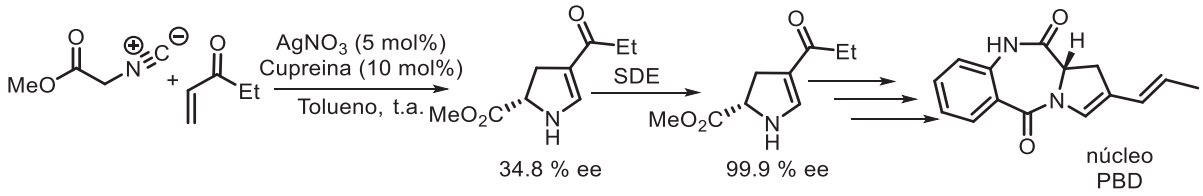

**Figura 8.** Estructura de la memoria.

A continuación, se indican los objetivos de cada uno de los diferentes estudios realizados en la presente Tesis Doctoral.

**Capítulo 2. Access to the enantiopure pyrrolobenzodiazepine (PBD) dilactam nucleus via self-disproportionation of enantiomers.**

Como extensión a los estudios previos realizados en el grupo, el objetivo principal de este capítulo fue el acceso enantioselectivo al núcleo de pirrolobenzodiazepina, compuestos con una excelente citotoxicidad para tumores celulares.

Para abordar esta síntesis enantioselectiva se partió de las dihidropirrolinas sintetizadas a través de la cascada multicatalítica cooperativa de un alcaloide cinchona y plata. En la reacción se obtuvieron las dihidropirrolinas correspondientes con un 89% de exceso enantiomérico. Para poder mejorar el exceso se planteó la utilización de métodos físicos como es la *self-disproportionation of enantiomers* (SDE) enriqueciendo el derivado de dihidropirrolina hasta un 99.9% ee. Finalmente, con el producto enantiopuro se preparó el núcleo de PBD de manera enantiopura mediante transformaciones sintéticas sencillas (Esquema 8).

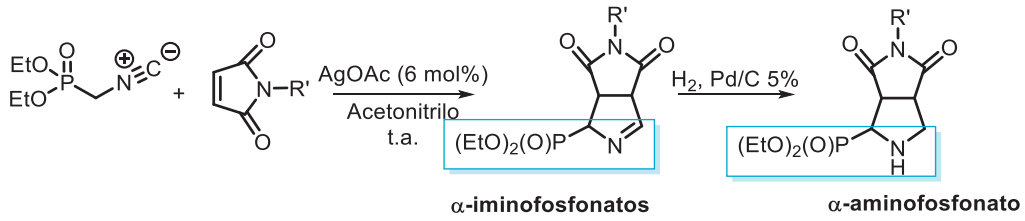



**Esquema 8.** Síntesis del núcleo de PBD.

**Capítulo 3. First diastereoselective [3+2] cycloaddition reaction of diethyl isocyanomethylphosphonate and maleimides.**

Los α-aminofosfonatos, análogos fosfónicos de los α-aminoésteres, han mostrado tener interés en diferentes ámbitos como son en la medicina y en agricultura. Esta actividad está asociada a la estructura tetraédrica del grupo fosfonato, que puede actuar como un "análogo del estado de transición" en la hidrólisis del péptido.

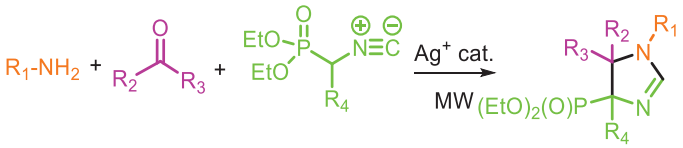
Así pues, como un primer objetivo nos propusimos la síntesis de α-aminofosfonatos bicíclicos a través de la primera cicloadición [3+2] entre PhosMic y maleimidas diversamente sustituidas. La reacción es altamente diastereoselectiva conduciendo a un único diastereoisómero (Esquema 9).




**Esquema 9.** Preparación de los α-aminofosfonatos a partir de PhosMic y maleimidas diversamente sustituidas.

**Capítulo 4. Easy Access to (2-imidazolin-4-yl)phosphonates by a microwave assisted multicomponent reaction.**

El objetivo de este capítulo es desarrollar una nueva reacción multicomponente en la que participe el PhosMic como uno de los componentes de la reacción, ya que su uso en las MCR está poco explorado. Para ello exploramos la reacción multicomponente entre PhosMic y derivados de PhosMic sustituidos en α, cetonas y aminas para dar lugar a las (2-imidazolin-4-il)fosfonato (Esquema 10). Además, nos planteamos que la reacción fuera respetuosa con el medio ambiente siguiendo consideraciones de química verde. Con este fin, se generó el calor de la reacción con un horno de microondas, que es una herramienta excepcional para la química orgánica sostenible, maximizando la eficiencia sintética, la diversidad y la complejidad.




**Esquema 10**. Síntesis de los (2-imidazolin-4-il)fosfonatos.

**Capítulo 5. Neuroprotective effects of a structurally new family of high affinity imidazoline $I_2$ receptor ligands**.

Los compuestos que contienen 2-imidazolinas constituyen una valiosa clase de agentes que son capaces de mostrar una elevada afinidad para los receptores imidazólicos (IBS). Por ello, en este Capítulo 5 nos planteamos la caracterización de las (2-imidazolin-4-il)fosfonatos como una nueva familia de ligandos de receptores imidazólicos de tipo 2 ($I_2$-IR). A su vez, también se realizaron estudios *in vivo* en ratón de analgesia, hipotermia

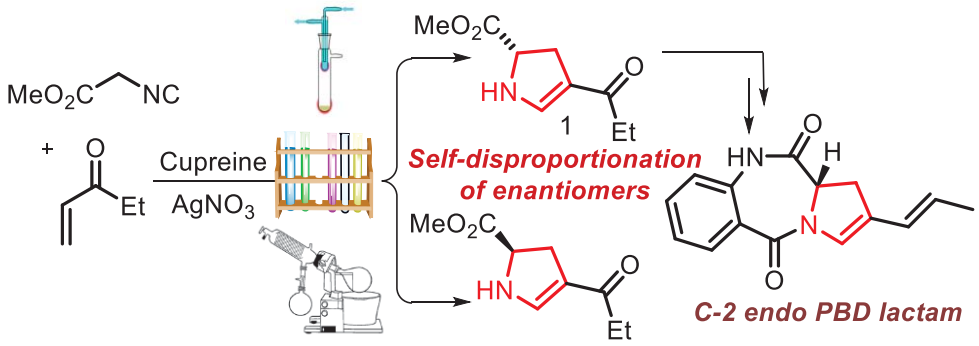
y neuroprotección (ratio p-FADD/FADD y transformación p35 en el compuesto neurotóxico p25).

**Capítulo 6. Synthetic $I_2$ imidazoline receptor ligands for prevention or treatment of human brain disorders.**

Después de los buenos resultados obtenidos con las (2-imidazolin-4-il)fosfonatos, nos propusimos describir el perfil farmacológico de una nueva familia. En un primer lugar intentamos ampliar la librería de compuestos para poder estudiar la estructura-actividad, basándonos con los resultados obtenidos en el capítulo 5. Posteriormente se realizaron los estudios *in vitro* y *in vivo* en un modelo de ratón para poder saber el posible rol que puedan tener estos nuevos ligandos frente a enfermedades neurodegenerativas como el Alzheimer.

# Capítulo 2

## Access to the enantiopure pyrrolobenzodiazepine (PBD) dilactam nucleus via self-disproportionation of enantiomers



Tetrahedron 74 (2018) 867–871

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

# Access to the enantiopure pyrrolobenzodiazepine (PBD) dilactam nucleus via self-disproportionation of enantiomers

Sònia Abás [a], Carlos Arróniz [a], Elies Molins [b], Carmen Escolano [a, *]

[a] Laboratory of Organic Chemistry and Laboratory of Medicinal Chemistry (Associated Unit to CSIC), Department of Pharmacology, Toxicology and Medicinal Chemistry, Faculty of Pharmacy and Food Sciences, and Institute of Biomedicine (IBUB), University of Barcelona, Av. Joan XXIII 27-31, E-08028, Barcelona, Spain

[b] Institut de Ciència de Materials de Barcelona (ICMAB-CSIC), Campus UAB, 08193, Cerdanyola, Spain

ARTICLE INFO

*Article history:*
Received 10 December 2017
Received in revised form
2 January 2018
Accepted 4 January 2018
Available online 8 January 2018

*Keywords:*
Self-disproportionation of enantiomers
Pyrrolobenzodiazepines
Sublimation
Cooperative catalysis
2,3-Dihydro-1*H*-pyrrole

ABSTRACT

A short synthesis of an enantiopure pyrrolobenzodiazepine (PBD) dilactam featuring early installation of the C2-C3 unsaturation is reported. An enantioselective cooperative catalytic cascade followed by self-disproportionation of enantiomers via sublimation afforded the enantiopure 2,3-dihydro-1*H*-pyrrole key intermediate, **1**. *N*-Acylation followed by reduction and lactam formation furnished the PBD dilactam.



## 1. Introduction

Pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are an important family of GC-rich-sequence-selective DNA minor-groove binding agents that react covalently with the C-2 amino group of guanine bases.[1] Through this mechanism of action PBDs exhibit both antibacterial properties and selective cytotoxicity toward tumor cells in the nanomolar range, and are considered key tools to understand DNA molecular basis for designing new biologically active compounds.[2] Structurally, PBDs are fused-tricyclic systems consisting in a diversely substituted benzene A-ring, a diazepinone B-ring and a 5-membered C-ring. In the naturally occurring PBDs so far reported, the C-ring can bear different substituents and can be either fully saturated (e.g., neothramycin A and B, chicamycin or abbeymycin), C2-*exo*-unsaturated (e.g., tomaymycin or prothracarcin), or unsaturated at C2/C3 (e.g., anthramycin, mazethramycin, porothramycin, sibiromycin, sibanomycin or limazepines) (Fig. 1). PBDs with either C2-*exo* or C2-*endo*-unsaturation are, on average, biologically more potent than their C-ring saturated analogs due to the relevance of the double bond for the optimal DNA binding.[3] All naturally occurring PBDs, isolated from various species of *Streptomyces* (e.g., anthramycin) and *Micrococcus* (e.g., limazepines A-F),[4] possess the (*S*)-configuration at C-11a, resulting in a right handed twist three dimensional conformation that allows them to fit perfectly within the minor groove of DNA. In addition to the naturally occurring PBDs, over the last 50 years a wide range of analogs have been synthesized, including SJG-136, a PBDs dimer that reached Phase II clinical trials for treating ovarian carcinoma, and PBD-based antibody drug conjugates, such as vadastuximab talirine (SGN-CD33A) and rovalpituzumab tesirine, currently in phase III clinical trials.[5]

Most of the reported synthesis of C2-*endo* unsaturated PBDs followed a common retrosynthetic approach involving the late functionalization of the N10-C11 bond in a dilactam, in turn generated by the intramolecular reaction of an amide containing a suitably substituted benzene A-ring precursor, e. g. an anthranilic acid, and a five membered C-ring precursor, typically a pre-functionalised 2*S*-proline derivative. The transformation of the dilactam —itself of biological interest,[6] into the final product, either imine or hemiaminal, also follows well-established protocols (Scheme 1).[7]

In sharp contrast with known synthetic approaches, which use multi-step sequences for a late introduction of the C2-*endo* double

* Corresponding author.
*E-mail address:* cescolano@ub.edu (C. Escolano).

https://doi.org/10.1016/j.tet.2018.01.006
0040-4020/

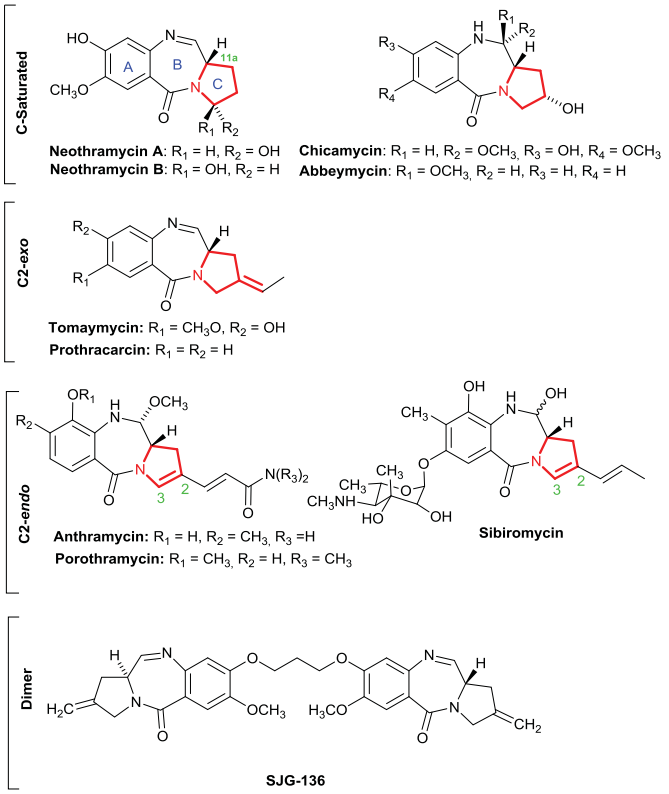
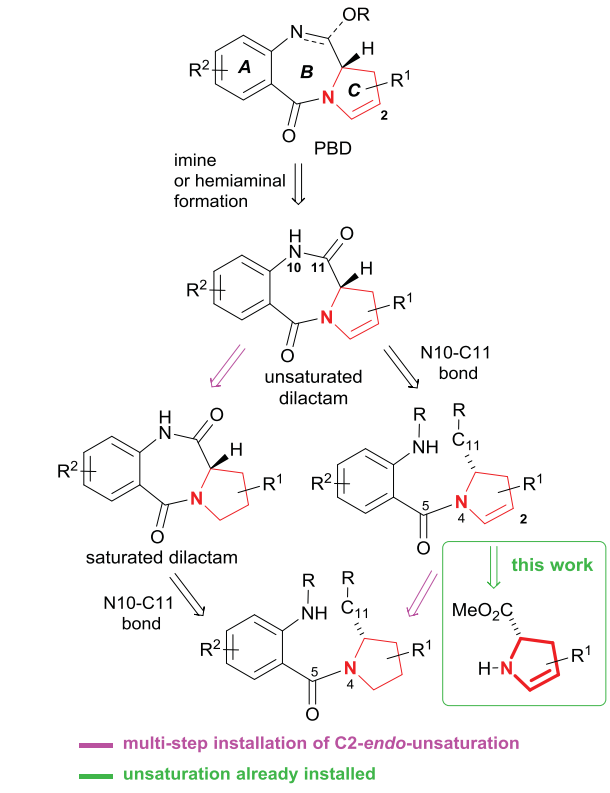



**Fig. 1.** Representative PBDs.




**Scheme 1.** General retrosynthesis and our approach to PBDs.

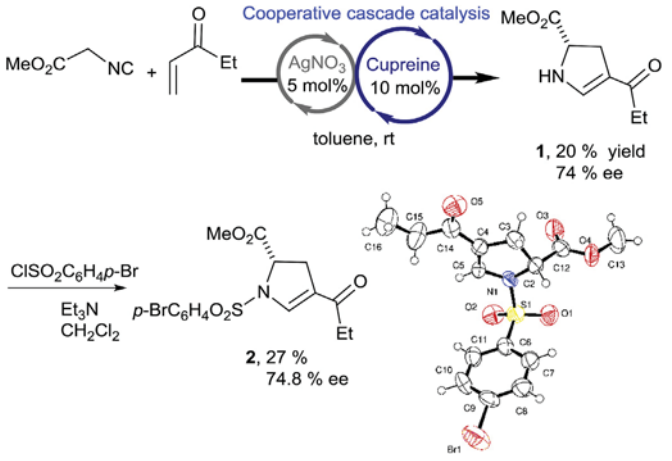
bond, the proposed biosynthetic pathway to PBDs involves a 4-vinyl-2,3-dihydro-1*H*-pyrrole-2-carboxylic acid intermediate that already incorporates the double bond featured in the final products.[8] Similarly, Stevens and co-workers published the synthesis of a dilactam PBD derivative *in the racemic series* using dimethyl 2,3-dihydro-1*H*-pyrrole-2,4-dicarboxylate as a key intermediate.[9]

Inspired by nature and this synthetic precedent, and bearing in mind the cumbersome installation of the double bond in the late stages of the synthesis, herein we describe a new synthetic strategy that relies on the beneficial use of suitably substituted enantiopure 2,3-dihydro-1*H*-pyrrole derivatives as key starting materials. To tackle this strategy we took advantage of our previous experience in the preparation of enantioenriched 3-acyl-5-alkoxycarbonyl-2,3-dihydro-1*H*-pyrroles that contain the required double bond, an acyl appendage at the 3-position and an ester substituent at the 5 position suitable to give access to the PBDs dilactam through known synthetic transformations (Scheme 3).[10]

## 2. Results and discussion

We began the synthesis of the key enantioenriched methyl 4-propionyl-2,3-dihydro-1*H*-pyrrole-2-carboxylate, **1**, by a cooperative multicatalytic cascade sequence involving methyl isocyanoacetate and ethyl vinyl ketone with the assistance of $AgNO_3$ and cupreine.[10] Mechanistically, the reaction is an asymmetric formal [3+2]cycloaddition that generates a new stereocenter in the 2,3-dihydro-1*H*-pyrrole-α-ester position. As all PBDs present the *S* configuration in the C-11a, before advancing the synthesis, efforts were devoted to the determination of the absolute stereochemistry of the newly created stereocenter. To this end, *N*-sulfonylation of an enantioenriched sample of **1** (74.8% enantiomeric excess, ee) with *p*-bromobenzenesulfonyl chloride[11] furnished sulfonamide **2** in 27% yield and without racemization (chiral HPLC analysis). X-ray diffraction analysis of a monocrystal of **2** obtained by crystallization from 2-propanol/pentane unequivocally established that its C-5 possessed the required absolute *S* configuration (Scheme 2 and supplementary data for the HPLC chromatograms and X-ray crystal data).

With the right absolute configuration determined, we attempted the enantiomeric total enrichment of **1** to continue with the proposed synthesis. To our surprise, during the preparation of compound **1** we observed that the distribution of enantiomers was altered after concentration in a rotary evaporator. To illustrate this fact, initial HPLC analysis of a solution of **1** in ethyl acetate revealed 76.3% ee. However, after concentrating the solution under reduced pressure to dryness we observed a gradient in the distribution of




**Scheme 2.** Preparation of compound **2** and Ortep view of its X-ray crystal structure.[12]

enantiomers from 67.7% ee when the solid sample was taken from the bottom of the flask to 81.9% ee when taken from the upper wall of the flask. Thus, a routine procedure as solvent evaporation and vacuum drying resulted in 14% ee difference in the *S* enantiomer enrichment of **1**. This observation suggested that self-disproportionation of enantiomers (SDE) could be occurring. SDE was coined by Soloshonok in 2006[13] as a phenomenon that occurs spontaneously without the assistance of any external chiral source for the optical purification of nonracemic (scalemic) chiral compounds. SDE leads to sample fractions containing enantiomeric enrichment or depletion in variable proportions. The basis of the enantiomer separation usually relies on the formation of homo- and heterochiral species that differ on the intermolecular forces stablished and, thus, exhibit different vapour pressures. Therefore, SDE can be classified as a green and practical optical purification method that involves low cost techniques such as fractional crystallization, sublimation, distillation and achiral chromatography.[14]

We then examined whether SDE occurs for **1** under chromatographic conditions in an automatic column Teledyne Isco® using mixtures of hexanes/ethyl acetate as eluent and collecting fractions of 6 mL. The loading substrate was enantioenriched **1** (81.4% ee) and 14 fractions were collected and analyzed by HPLC to give ee percentage values from 93.6% in the first fraction to 62.5% the last fraction. Thus, the difference in the ee amounted to 31%, thereby confirming that a SDE had occurred in the chromatographic process (see supporting information for further details). Although SDE via HPLC, MPLC, flash and gravity-driven achiral chromatography has been reported, to the best of our knowledge, the experiment that we have performed constitutes the first example of a SDE via an automatic driven achiral chromatography (Fig. 2 and Table S1 in supplementary data).[15]

At this point we were attracted by the possibility to access enantiopure **1** by sublimation, a well-known solvent-free purification technique, often associated with SDE processes. Soloshonok reported that due to the difference in the crystallographic structure of racemic and enantiopure crystals, the sublimation rates of racemic and enantiomerically pure crystals would be different.[16] Thus, we performed an experiment subjecting crude enantioenriched **1** (34.8% ee) to sublimation at 100 °C under vacuum (0.20 psi). From the sublimation apparatus two samples were analyzed, the white sublimate collected on the cold finger and the remaining gummy solid at the bottom of the apparatus. In both samples the ee was not altered, indicating that just a purification process was occurred. However, when the sublimate was submitted again to sublimation (100 °C, 0.20 psi) a SDE effect was observed. Whereas the HPLC analysis of the newly collected sublimate showed a decrease in ee (27.1%), the remaining solid at the bottom of the apparatus proved to be enantiomerically pure **1** (>99.9% ee, see supplementary data for HPLC chromatograms and further details).

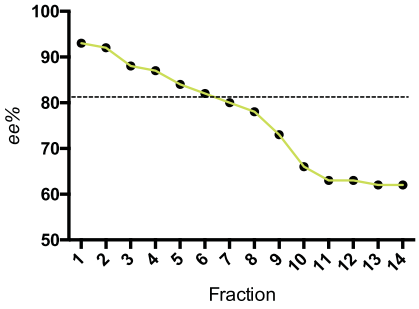



**Fig. 2.** Graphical representation of the progress of chromatography for the % *ee* of each fraction *vs* fraction.

Having in hand the key enantiopure methyl (2*S*)-4-propionyl-2,3-dihydro-1*H*-pyrrole-2-carboxylate **1**, targeted PBD dilactam was accessed by a short sequence of synthetic transformations.[17] Thus, reaction of **1** with 2-nitrobenzoyl chloride furnished **3** in 79% yield. Next, reduction of the ketone to alcohol followed by dehydration gave enamide **4** in a 40% yield over the two steps. Finally, one-pot reduction and cyclisation furnished in a 30% overall yield, the desired PBD dilactam **5** (Scheme 3).[18]

To confirm the stereochemical identity of the final product **5**, the synthesis was also carried out in the racemic series. Comparison of the HPLC chromatograms of the racemic and enantiopure dilactam **5** indicates that the stereocenter remains stable during the whole synthetic sequence (for HPLC chromatograms see supplementary data).

Further studies will be conducted to study the scope of the presented strategy and its application to synthesis of fashionable PBDs.

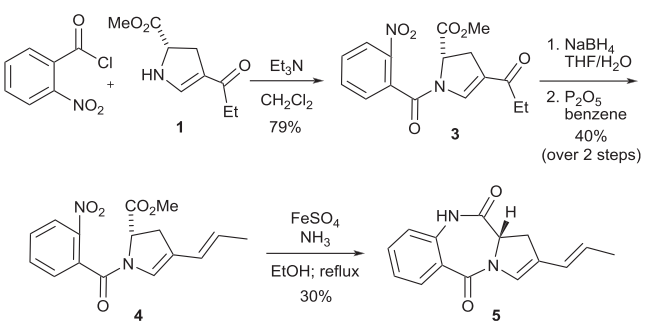
## 3. Conclusion

To sum up, we have described a synthetic approach to enantiopure PBD lactams with the advantage of using as a starting material compound **1** that in one synthetic step allows the introduction of the C2-C3 unsaturation featured in the final product and the stereocenter with the required configuration. Interestingly, enantiopure 2,3-dihydro-1*H*-pyrrole derivative **1** was accessed by a sublimation process involving a self-disproportionation phenomenon.

## 4. Experimental

### 4.1. General methods

All reactions were performed under an argon or nitrogen atmosphere with dry, freshly distilled solvents using standard procedures. Drying of organic extracts during the work-up of reactions was performed over anhydrous $Na_2SO_4$ or $MgSO_4$. Analytical grade solvents and commercially available reagents were used without further purification. Evaporation of solvents was accomplished with a rotatory evaporator. NMR spectra were recorded in $CDCl_3$ at 400 MHz ($^1H$) and 100.6 MHz ($^{13}C$), and chemical shifts are reported in $\delta$ values downfield from TMS or relative to residual chloroform ($\delta = 7.26$ ppm, 77.0 ppm) as an internal standard. Data are reported in the following manner: chemical shift, multiplicity, coupling constant, integrated intensity. Multiplicities are reported using the following abbreviations: s, singlet; dd, doublet of doublets; m, multiplet; br. s, broad signal. Evaporation of solvents was accomplished with a rotary evaporator. Thinlayer chromatography was done on $SiO_2$ (silica gel 60 F254), and the spots were located by UV and 1% aqueous $KMnO_4$. Chromatography refers to flash column




**Scheme 3.** Synthesis of enantiopure PBD dilactam **5**.

chromatography and was carried out on $SiO_2$ (silica gel 60, SDS, 230–400 mesh). Automatized chromatography was carried out with a Combiflash RF 150 psi from Teledyne Isco. Mass spectra were recorded with a LTQ spectrometer using electrospray ionization (ESI+) techniques. Elemental analyses were carried out at the IIQAB (CSIC) of Barcelona, Spain, in Thermofinnigan model Flash 1112 series elemental microanalyzers (A5) for C, H, and N determinations and in a Methrom model 808 titroprocessor for halogen determination. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. $[\alpha]_D$ values are given in $10^{-1}$ deg $cm^2$ $g^{-1}$. The enantiomeric excess (*ee*) of the products was determined by chiral stationary-phase HPLC (Daicel Chiralpak IA column).

### 4.2. Experimental procedures

#### 4.2.1. Procedure for the synthesis of racemic (rac-1) and optically active methyl (S)-4-propionyl-2,3-dihydro-1H-pyrrole-2-carboxylate (**1**)

For experimental data and spectral data for the racemic and enantioenriched **1**, see reference 10. $R_f$ (100% EtOAc) 0.18; M.p. (racemic) 92–93 °C; M.p. (enantiopure after sublimation) 120–122 °C; $[\alpha]_{22}^{D}$ +250.52 (c = 1, $CHCl_3$). The *ee* was determined by HPLC using a Chiralpak IA column [MtBE/EtOH (40:10)]; flow rate 0.5 mL/min; λ = 300 nm; $t_R$(major) = 11.6 min, $t_R$(minor) = 12.4 min.

#### 4.2.2. Procedure for the synthesis of racemic methyl 1-[(4-bromophenyl)sulfonyl]-4-propionyl-2,3-dihydro-1H-pyrrole-2-carboxylate (rac-2)

A solution of **1** (0.56 mmol, 102 mg) and $Et_3N$ (2.24 mmol, 310 μL) in $CH_2Cl_2$ (2.0 mL) was stirred for 5 h. Then, a solution of 4-bromobenzenesulfonyl chloride (3.35 mmol, 9 mg) in $CH_2Cl_2$ (4 mL) was added and the resulting solution was stirred at room temperature overnight. To quench the reaction $H_2O$ (6 mL) was added and the reaction mixture was extracted with $CH_2Cl_2$. The organic layers were combined, concentrated, and the residue was purified by column chromatography (hexane/EtOAc 1:2) to afford **2** (49 mg, 22%) as a white solid.

#### 4.2.2.1. Procedure for the synthesis of optically active methyl (S)-1-[(4-bromophenyl)sulfonyl]-4-propionyl-2,3-dihydro-1H-pyrrole-2-carboxylate (**2**).

A solution of **1** (0.27 mmol, 49 mg, 74% *ee*) and $Et_3N$ (1.08 mmol, 150 μL) in $CH_2Cl_2$ (1.0 mL) was stirred for 5 h. Then, a solution of 4-bromobenzenesulfonyl chloride (1.62 mmol, 4.4 mg) in $CH_2Cl_2$ (2 mL) was added and the resulting solution was stirred at room temperature overnight. To quench the reaction $H_2O$ (3 mL) was added and the reaction mixture was extracted with $CH_2Cl_2$. The organic layers were combined, concentrated, and the residue was purified by column chromatography (hexane/EtOAc 1:2) to afford **2** (29 mg, 27%, 74.8%*ee*) as a white solid. The solid was crystallized in pentane/2-propanol. $R_f$ (50% hexane/EtOAc) 0.38; M.p. 163–164 °C. IR (NaCl) 3090, 2946, 1746, 1651, 1613, 1573, 1435, 1367, 1049 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HSQC): δ = 1.10 (t, *J* = 7.2 Hz, $CH_2CH_3$), 2.60 (q, *J* = 7.2 Hz, 2H, $CH_2CH_3$), 2.88 (ddd, *J* = 16.4, 6.8, 1.6 Hz, 1H, H-3), 3.17 (ddd, *J* = 16.4, 12.0, 1.6 Hz, 1H, H-3), 3.73 (s, 3H, $OCH_3$), 4.53 (dd, *J* = 12.0, 6.8 Hz, 1H, H-2), 7.27 (t, *J* = 1.6 Hz, 1H, H-5), 7.73 (s, 4H, ArH); $^{13}C$ NMR (100.6 MHz): δ = 8.4 ($CH_2CH_3$), 31.9 ($CH_2CH_3$), 34.0 (C-3), 52.9 ($OCH_3$), 60.9 (C-2), 122.5 (C-4), 128.9 (2CHAr), 129.3 (C-*ipso*), 132.8 (2CHAr), 136.2 (C-*ipso*), 138.5 (C-5), 170.1 (COO), 196.1 (CO). Elemental analysis calculated for $C_{15}H_{16}BrNO_5S$: C, 44.79%; H, 4.01%; N, 3.48%; O, 19.89%; S, 7.97%. Found: C, 44.89%; H, 4.00%; N, 3.45%; S, 8.01%. The *ee* was determined by HPLC using a Chiralpak IA column [MtBE/EtOH (40:10)]; flow rate 0.5 mL/min; λ = 300 nm; $t_R$(major) = 7.7 min, $t_R$(minor) = 8.8 min (before crystallization 74.8% *ee*; crystals 99.0% *ee*; mother liquor 23.6% *ee*).

#### 4.2.3. Procedure for the synthesis of racemic methyl 1-(2-nitrobenzoyl)-4-propionyl-2,3-dihydro-1H-pyrrole-2-carboxylate (rac-3)

To a solution of **1** (3.17 mmol, 580 mg) and $Et_3N$ (13 mL) in $CH_2Cl_2$ (13 mL) was added a solution of 2-nitrobenzoyl chloride (3.8 mmol, 0.5 mL) in $CH_2Cl_2$ (50 mL). The reaction mixture was stirred at room temperature overnight. Then, $H_2O$ (25 mL) was added and the mixture was extracted with $CH_2Cl_2$. The organic layers were combined and concentrated to afford **3** (807 mg, 76%) as a yellow oil after column chromatography (hexane/0.5% $Et_3N$ in $CH_2Cl_2$ mixtures).

#### 4.2.3.1. Procedure for the synthesis of optically active methyl (S)-1-(2-nitrobenzoyl)-4-propionyl-2,3-dihydro-1H-pyrrole-2-carboxylate (**3**).

To a solution of **1** (0.27 mmol, 50 mg, >99,9% *ee*) and $Et_3N$ (1.2 mL) in $CH_2Cl_2$ (1 mL) was added a solution of 2-nitrobenzoyl chloride (0.32 mmol, 43 μL) in $CH_2Cl_2$ (5 mL). The reaction mixture was stirred at room temperature overnight. Then, $H_2O$ (3 mL) was added and the mixture was extracted with $CH_2Cl_2$. The organic layers were combined and concentrated to afford **3** (71 mg, 79%) as a yellow oil after column chromatography (hexane/0.5% $Et_3N$ in $CH_2Cl_2$ mixtures). $R_f$ ($CH_2Cl_2$/0.5% $Et_3N$/1%MeOH) 0.42; IR (NaCl) 2955, 1747, 1614, 1537, 1434, 1360, 1227 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$, HETCOR): δ = 0.98 (t, *J* = 7.6 Hz, 3H, $CH_2CH_3$), 2.39 (q, *J* = 7.6 Hz, 2H, $CH_2CH_3$), 2.92 (ddd, *J* = 16.8, 5.2, 1.6 Hz, 1H, H-3), 3.30 (ddd, *J* = 16.8, 12.0, 1.6 Hz, 1H, H-3), 3.80 (s, 3H, $OCH_3$), 5.13 (dd, *J* = 12.0, 5.2 Hz, 1H, H-2), 6.80 (br s, 1H, H-5), 7.58 (dd, *J* = 7.6, 1.2 Hz, 1H, ArH), 7.68 (m, 1H, ArH), 7.79 (td, *J* = 7.6, 0.8 Hz, 1H, ArH) and 8.22 (dd, *J* = 8.4, 0.8 Hz, 1H, ArH); $^{13}C$ NMR (100.6 MHz): δ = 8.1 ($CH_2CH_3$), 31.9 ($CH_2CH_3$), 32.3 (C-3), 52.9 ($OCH_3$), 58.8 (C-2), 124.2 (C-4), 125.0 (CHAr), 129.1 (CHAr), 130.4 (C-*ipso*), 131.4 (CHAr), 134.9 (CHAr), 136.8 (C-5), 145.5 (C-*ipso*), 164.5 (CONH), 170.3 (COO), 196.4 (CO); HRMS $C_{16}H_{17}N_2O_6$ $[M + H]^+$ 333.1081; found, 333.1081.

#### 4.2.4. Procedure for the synthesis of racemic methyl (E)-1-(2-nitrobenzoyl)-4-(prop-1-en-1-yl)-2,3-dihydro-1H-pyrrole-2-carboxylate (rac-4)

To a solution of **3** (0.74 mmol, 245 mg) in THF (10 mL) and $H_2O$ (160 μL), $NaBH_4$ (0.81 mmol, 31 mg) was added in small portions and the mixture was stirred at room temperature for 2 h. To quench the reaction, $H_2O$ (3 mL) was added and the organic solvent was removed under vacuum. The aqueous suspension was extracted with EtOAc. The organic layers were combined and concentrated to give an oily residue that was dissolved in benzene (7 mL). Then, $P_2O_5$ (0.26 mmol, 73 mg) was added and the mixture was heated at reflux for 2 h. After cooling, the benzene suspension was washed with an aqueous solution of $NaHCO_3$ and concentrated to afford **4** (75 mg, 32%) as a brown oil after column chromatography (hexane/EtOAc mixtures).

#### 4.2.4.1. Procedure for the synthesis of optically active methyl (S,E)-1-(2-nitrobenzoyl)-4-(prop-1-en-1-yl)-2,3-dihydro-1H-pyrrole-2-carboxylate (**4**).

To a solution of **3** (0.21 mmol, 70 mg, >99.9% *ee*) in THF (3 mL) and $H_2O$ (50 μL), $NaBH_4$ (0.23 mmol, 9 mg) was added in small portions and the mixture was stirred at room temperature for 2 h. To quench the reaction, $H_2O$ (1 mL) was added and the organic solvent was removed under vacuum. The aqueous suspension was extracted with EtOAc. The organic layers were combined and concentrated to give an oily residue that was dissolved in benzene (2 mL). Then, $P_2O_5$ (0.075 mmol, 21 mg) was added and the mixture was heated at reflux for 2 h. After cooling, the benzene suspension was washed with an aqueous solution of $NaHCO_3$ and concentrated

to afford **4** (26 mg, 40%) as a brown oil after column chromatography (hexane/EtOAc mixtures). $R_f$ (50% EtOAc/hexane) 0.30; IR (NaCl) 2955, 1746, 1644, 1531, 1434, 1348, 1213 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$, HETCOR): $\delta$ = 1.75 (dd, $J$ = 6.8, 1.2 Hz, 3H, $CH_3$), 2.80 (dd, $J$ = 16.0, 4.8 Hz, 1H, H-3), 3.23 (ddd, $J$ = 16.0, 11.6, 1.6 Hz, 1H, H-3), 3.84 (s, 3H, $OCH_3$), 5.11 (dd, $J$ = 11.6, 4.8 Hz, 1H, H-2), 5.52 (dq, $J$ = 15.6, 6.8 Hz, 1H, CH=*CH*$CH_3$), 5.90 (s, 1H, H-5), 5.97 (d, $J$ = 15.6 Hz, 1H, *CH*=$CHCH_3$), 7.57 (dd, $J$ = 7.6, 1.6 Hz, 1H, ArH), 7.64 (ddd, $J$ = 8.4, 7.6, 1.6 Hz, 1H, ArH), 7.76 (td, $J$ = 7.6, 1.2 Hz, 1H, ArH) and 8.21 (dd, $J$ = 8.4, 1.2 Hz, 1H, ArH); $^{13}C$ NMR (100.6 MHz): $\delta$ = 18.4 ($CH_3$), 33.3 (C-3), 52.7 ($OCH_3$), 58.1 (C-2), 123.6 (*CH*=$CHCH_3$), 124.0 (C-5), 124.1 (C-4), 124.7 (CHAr), 127.1 (CH=*CH*$CH_3$), 129.2 (CHAr), 130.5 (CHAr), 131.5 (C-*ipso*), 134.4 (CHAr), 145.4 (C-*ipso*), 162.8 (CONH) and 170.9 (COO). HRMS $C_{16}H_{17}N_2O_5$ $[M+H]^+$ 317.1127; found 317.1132.

*4.2.5. Procedure for the synthesis of racemic (E)-2-(prop-1-en-1-yl)-1,11a-dihydro-5H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-5,11(10H)-dione (rac-5)*

To a solution of **4** (0.24 mmol, 75 mg) in EtOH (2.5 mL) was added a solution of $FeSO_4 \cdot 7H_2O$ (3.61 mmol, 1 g) in $H_2O$ (2.4 mL) and 32% $NH_4OH$ aqueous solution (120 μL). The mixture was heated at reflux for 3 h. During this time period 32% $NH_4OH$ aqueous solution (0.86 mL) was added dropwise. The warm mixture was filtered and the EtOH was removed under vacuum. The suspension was extracted with EtOAc and the organic layers were combined and concentrated. The resulting solid residue was crystallized in EtOH to give **5** (16 mg, 27%) as white solid. M.p. 192–195 °C.

*4.2.5.1. Procedure for the synthesis of optically active (S,E)-2-(prop-1-en-1-yl)-1,11a-dihydro-5H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-5,11(10H)-dione (**5**).* To a solution of **4** (0.08 mmols, 25 mg, >99.9% *ee*) in EtOH (0.8 mL) was added a solution of $FeSO_4 \cdot 7H_2O$ (1.2 mmol, 338 mg) in $H_2O$ (0.8 mL) and 32% $NH_4OH$ aqueous solution (40 μL). The mixture was heated at reflux for 3 h. During this time period 32% $NH_4OH$ aqueous solution (0.3 mL) was added dropwise. The warm mixture was filtered and the EtOH was removed under vacuum. The suspension was extracted with EtOAc and the organic layers were combined and concentrated. The resulting solid residue was crystallized in EtOH to give **5** (6 mg, 30%) as white solid. $R_f$ (20% hexane/EtOAc) 0.37; IR (NaCl) 2925, 1693, 1633, 1480, 1443, 1415, 1263 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$, HETCOR): $\delta$ = 1.83 (d, $J$ = 6.4 Hz, 3H, $CH_3$), 2.92 (dd, $J$ = 16.0, 10.8 Hz, 1H, H-1), 3.70 (dd, $J$ = 16.0, 2.8 Hz, 1H, H-1), 4.51 (dd, $J$ = 10.8, 3.6 Hz, 1H, H-11a), 5.69 (dq, $J$ = 15.6, 6.4 Hz, 1H, CH=*CH*$CH_3$), 6.26 (d, $J$ = 15.6 Hz, 1H, *CH*=$CHCH_3$), 6.93 (s, 1H, H-3), 7.05 (d, $J$ = 8.0 Hz, 1H, ArH), 7.29 (t, $J$ = 7.6 Hz, 1H, ArH), 7.50 (t, $J$ = 7.6 Hz, 1H, ArH), 8.01 (d, $J$ = 7.6 Hz, 1H, ArH) and 8.59 (br s, 1H, NH); $^{13}C$ NMR (100.6 MHz): $\delta$ = 18.5 ($CH_3$), 29.8, (C-1), 56.4 (C-11a), 121.3 (CHAr), 123.2 (C-3), 124.2 (*CH*=$CHCH_3$), 125.4 (CHAr), 125.8 (C-2), 126.3 (C-*ipso*), 127.8 (CH=$CHCH_3$), 131.4 (CHAr), 132.7 (CHAr), 134.7 (C-*ipso*), 161.7 (CONH) and 169.3 (CONH). HRMS $C_{15}H_{15}N_2O_2$ $[M+H]^+$ 255.1127; found 255.1128. The *ee* was determined by HPLC using a Chiralpak IA column [MtBE/EtOH (40:10)]; flow rate 0.5 mL/min; $\lambda$ = 300 nm; $t_R$(minor) = 11.8 min, $t_R$(major) = 15.6 min (>99.9% *ee*). $[\alpha]_{22}^{D}$ +382.96 ($c$ = 0.49, $CHCl_3$).

## Acknowledgment

Financial support from the Spanish Ministry of Economy and Competitiveness MINECO (Projects BQU2015-66030-R, ENE2015-63969-C3-3-R, SEV2015-0615 and SAF2016-77703-C2-1-R) is grateful acknowledged.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.tet.2018.01.006.

## References

1. For reviews of synthesis of PBDs, see: a) Antonow D, Thurston DE. *Chem Rev*. 2011;111:2815;
b) Varvounis G. *Molecules*. 2016;21:154. and references cited therein.
2. Hartley JA. *Expet Opin Invest Drugs*. 2011;20:733.
3. Antonow D, Kaliszczak M, Kang G-D, et al. *J Med Chem*. 2010;53:2927.
4. Fotso S, Zabriskie TM, Proteau PJ, et al. *J Nat Prod*. 2009;72:690.
5. For a review of PBD-based molecules that are in clinical trials evaluation, see: Mantaj J, Jackson PJM, Rahman KM, Thurston DE *Angew Chem Int Ed*. 2017;56:462.
6. a) Biel M, Deck P, Giannis A, Waldmann H. *Chem Eur J*. 2006;12:4121;
b) Antonow D, Jenkins TC, Howarda PW, Thurstona DE. *Bioorg Med Chem*. 2007;15:3041;
c) Kamal A, Reddy KL, Devaiah V, Shankaraiah N, Reddy GSK, Raghavan S. *Comb Chem*. 2007;9:29;
d) Jiao RH, Xu H, Cui JT, Ge HM, Tan RX. *J Appl Microbiol*. 2013;114:1046.
7. For a synthesis of anthramycin with a key unsaturated five-membered ring intermediate that requires isomerization of the double bond from the C-1/C-2 position, see: Kitamura T, Sato Y, Mori M *Tetrahedron*. 2004;60:9649.
8. For a review on the biosynthesis, synthesis and biological activities of PBDs, see: a) Gerratana B. *Med Res Rev*. 2012;32:254;
b) For a recent paper on the alkylproline moiety and biosynthesis of PBDs, see: Kamenik Z, Kadlcik S, Gazak R, et al *ACS Chem Biol*. 2017;12:1993.
9. Stevens RV, Cory RM, Rossen S. *J Chem Soc Chem Commun*. 1975:742.
10. Arróniz C, Gil-González A, Semak V, Escolano C, Bosch J, Amat M. *Eur J Org Chem*. 2011:3755.
11. Murakami M, Hasegawa N, Hayaehi M, Ito Y. *J Org Chem*. 1991;56:7356.
12. Crystallographic data for 2 have been deposited with the CCDC 1576708.
13. a) Soloshonok VA. *Angew Chem Int Ed*. 2006;45:766;
b) For comments on the terminology related to SDE, see: Soloshonok VA, Klika KD *Helv Chim Acta*. 2014;97:1583.
14. a) Sorochinsky AE, Soloshonok VA. *Top Curr Chem*. 2013;341:301. and references cited therein;
b) Martens J, Bhushan R. *Helv Chim Acta*. 2014;97:161.
15. For reviews on the SDE via achiral chromatography, see: a) Soloshonok VA, Roussel C, Kitagawa O, Sorochinsky AE. *Chem Soc Rev*. 2012;41:4180;
b) Suzuki Y, Han J, Kitagawa O, Aceña JL, Klika KD, Soloshonok VA. *RSC Adv*. 2015;5:2988;
c) For discussion on SDE test, see: Soloshonok VA, Wzorek A, Klika KD *Tetrahedron Asymmetry*. 2017. https://doi.org/10.1016/j.tetasy.2017.08.020;
d) For representative examples, see: Sorochinsky AE, Katagiri R, Ono T, Wzorek W, Aceña J-L, Soloshonok VA *Chirality*. 2013;25:365;
e) Song W, Zhou Y, Fu Y, Xu W. *Tetrahedron Asymmetry*. 2014;24:909;
f) Wzorek A, Klika KD, Drabowicz J, Sato A, Aceña JL, Soloshonok VA. *Org Biomol Chem*. 2014;12:4738;
g) Wzorek A, Sato A, Drabowicz J, Soloshonok VA, Klika KD. *Helv Chim Acta*. 2015;98:1147;
h) Maeno M, Tokunaga E, Yamamoto T, et al. *Chem Sci*. 2015;6:1043;
i) Wzorek A, Sato A, Drabowicz J, Soloshonok VA. *Isr J Chem*. 2016;56:977;
j) Maeno M, Kondo H, Tokunaga E, Shibata N. *RSC Adv*. 2016;6:85058;
k) Wzorek A, Sato A, Drabowicz J, Soloshonok VA, Klika KD. *Amino Acids*. 2016;48:605;
l) Drabowicz J, Jasiak A, Wzorek A, Sato A, Solonoshok W. *Arkivoc*. 2017:557.
16. For a review on the SDE via sublimation, see: a) Han J, Nelson DJ, Sorochisnsky AE, Soloshonok VA. *Curr Org Synth*. 2011;8:310;
b) For representative examples, see: Soloshonok VA, Ueki H, Yasumoto M, Mekala S, Hirschi JS, Singleton DA *J Am Chem Soc*. 2007;129:12112;
c) Yasumoto M, Ueki H, Soloshonok VA. *J Fluorine Chem*. 2010;131:266;
d) Albrecht M, Soloshonok VA, Schrader L, Yasumoto M, Suhm MA. *J Fluorine Chem*. 2010;131:495;
e) Yasumoto M, Ueki H, Ono T, Katagiri T, Soloshonok VA. *J Fluorine Chem*. 2010;131:535;
f) Yasumoto M, Ueki H, Soloshonok VA. *J Fluorine Chem*. 2010;131:540;
g) Bellec A, Guillemin J-C. *J Fluorine Chem*. 2010;131:545;
h) Perry RH, Wu C, Nefliu M, Cooks RG. *Chem Commun*. 2007:1071;
i) Tarasevych AV, Sorochinsky AE, Kukhar VP, Guillemin J-C. *Org Life Evol Biosph*. 2013;43:129.
17. Massa S, De Martino G, Corelli F. *J Het Chem*. 1982;19:1497. Reference 7.
18. The configuration of the double bond in the propenyl C-2 chain is *trans* according to the 15.6 Hz coupling constant observed between the -*CH*=*CH*-$CH_3$ hydrogens and comparing with 1H RMN spectra reported for PBDs bearing an C-2 *trans* propenyl chain (ie. Ref 7).

# SUPPLEMENTARY DATA

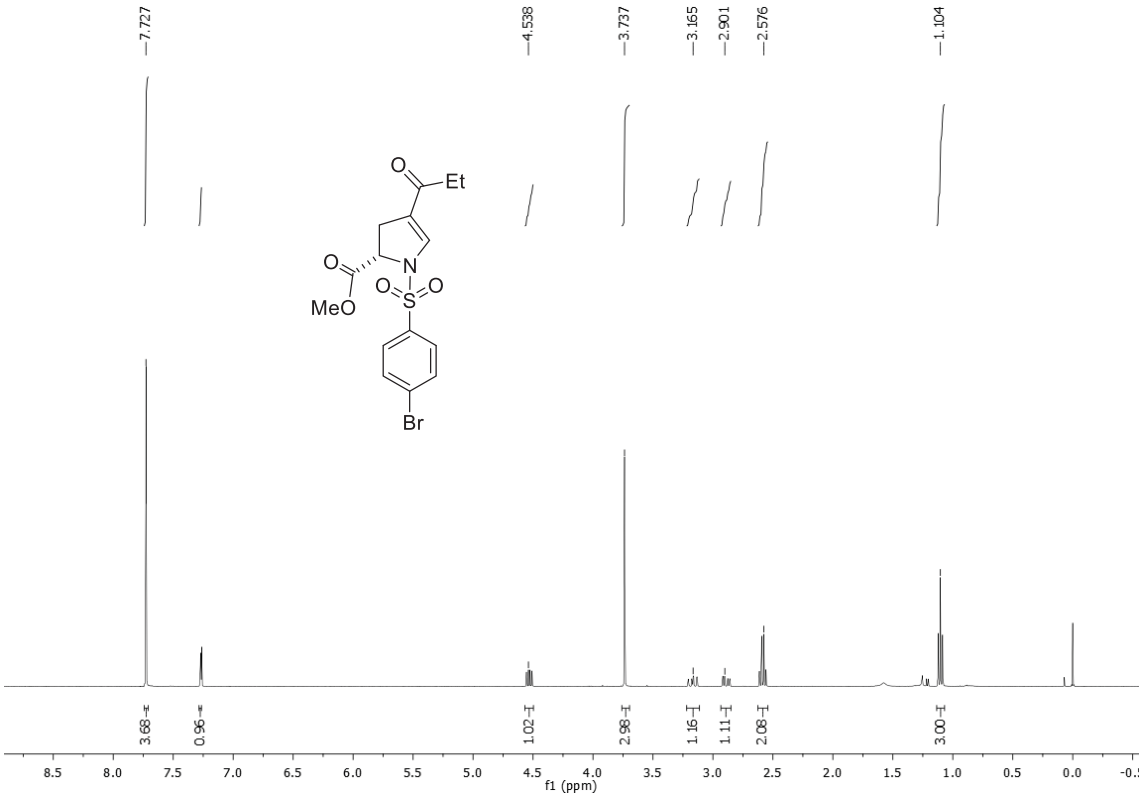
# Access to enantiopure pyrrolobenzodiazepine dilactam nucleus via self-disproportionation of enantiomers

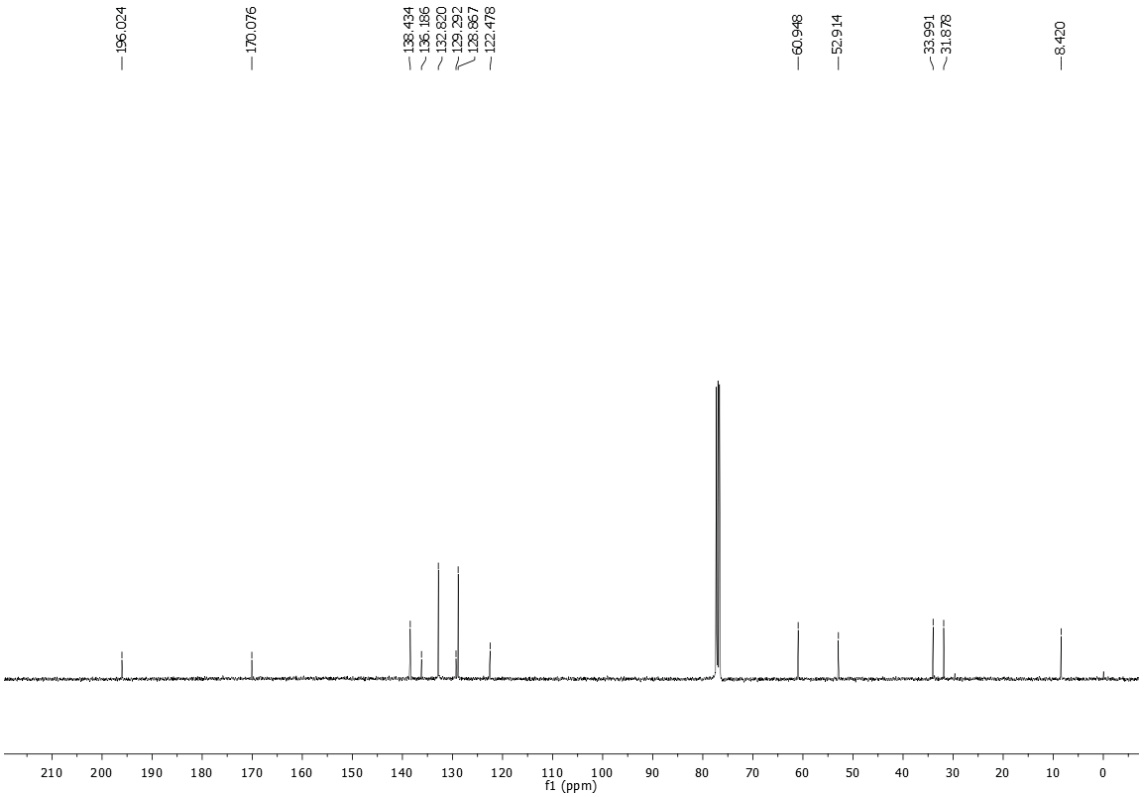
Sònia Abás,[a] Carlos Arróniz,[a] Elies Molins,[b] Carmen Escolano[a*]

[a]Laboratory of Medicinal Chemistry (Associated unit to CSIC), Department of Pharmacology, Toxicology and Medicinal Chemistry, Faculty of Pharmacy and Food Sciences, and Institute of Biomedicine (IBUB), University of Barcelona, Av. Joan XXIII 27-31, 08028, Barcelona, Spain. cescolano@ub.edu.

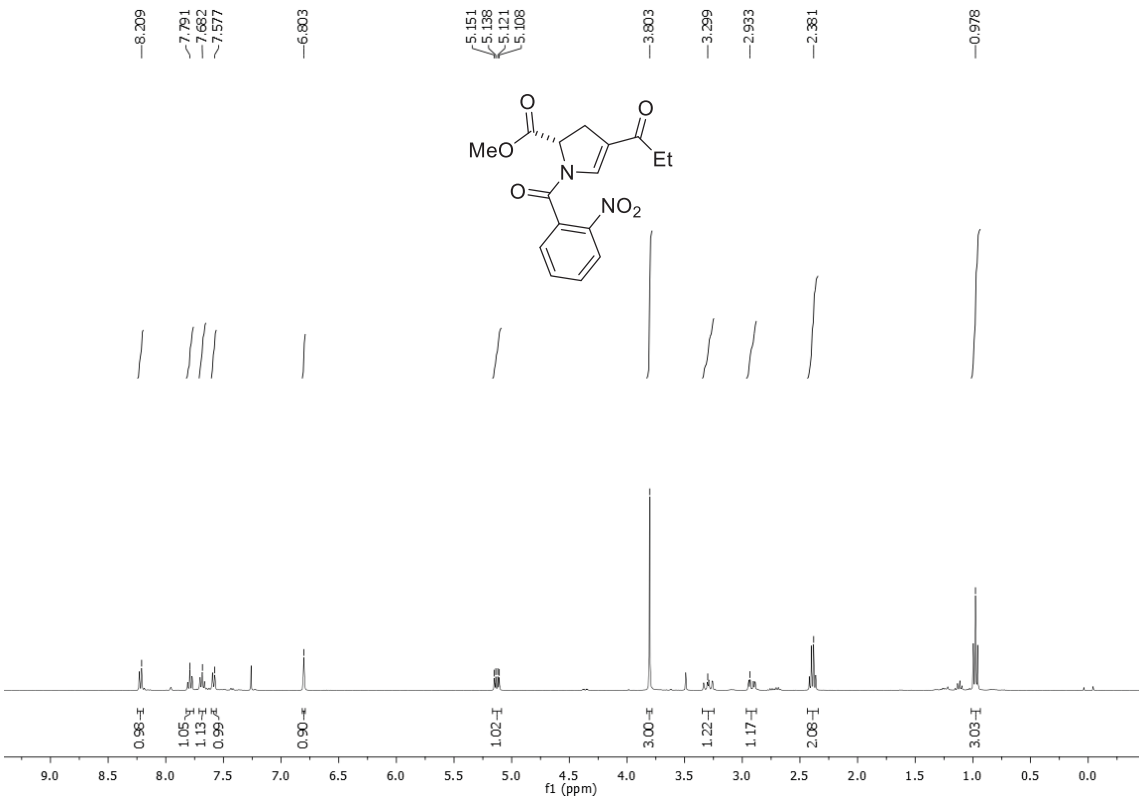
[b]Institut de Ciència de Materials de Barcelona (CSIC), Campus UAB, 08193-Cerdanyola, Spain.

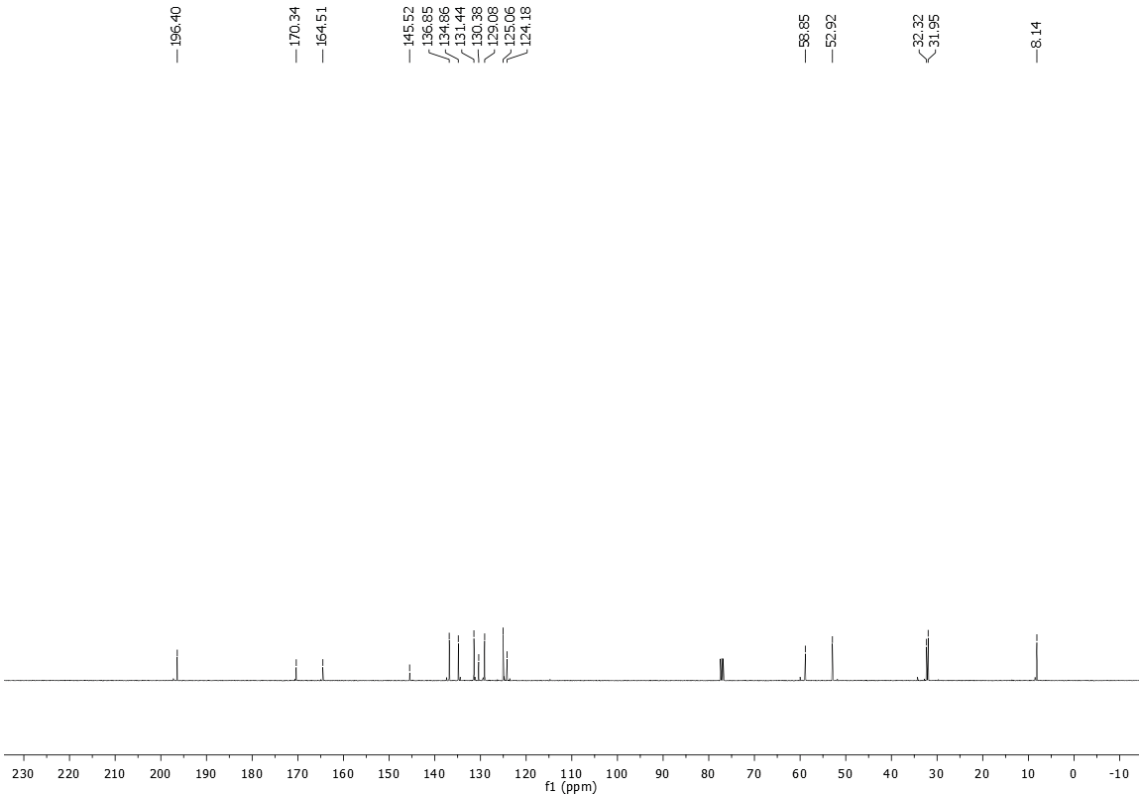
**CONTENTS**



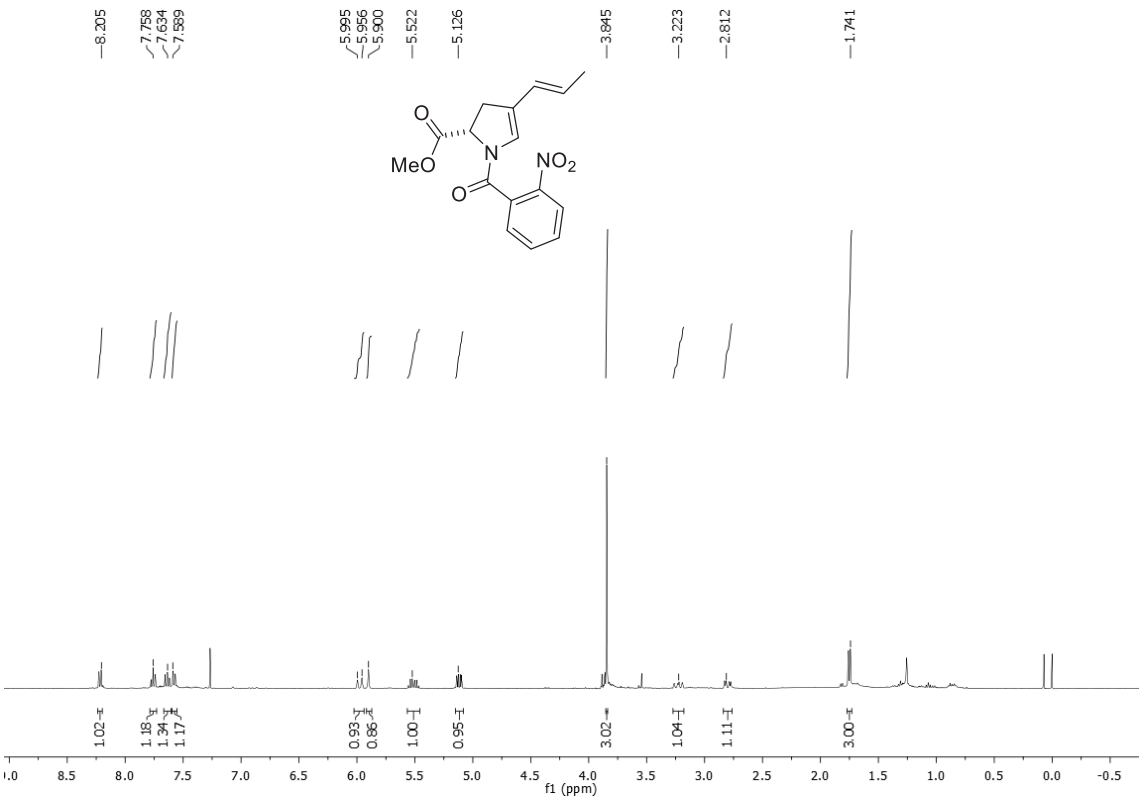



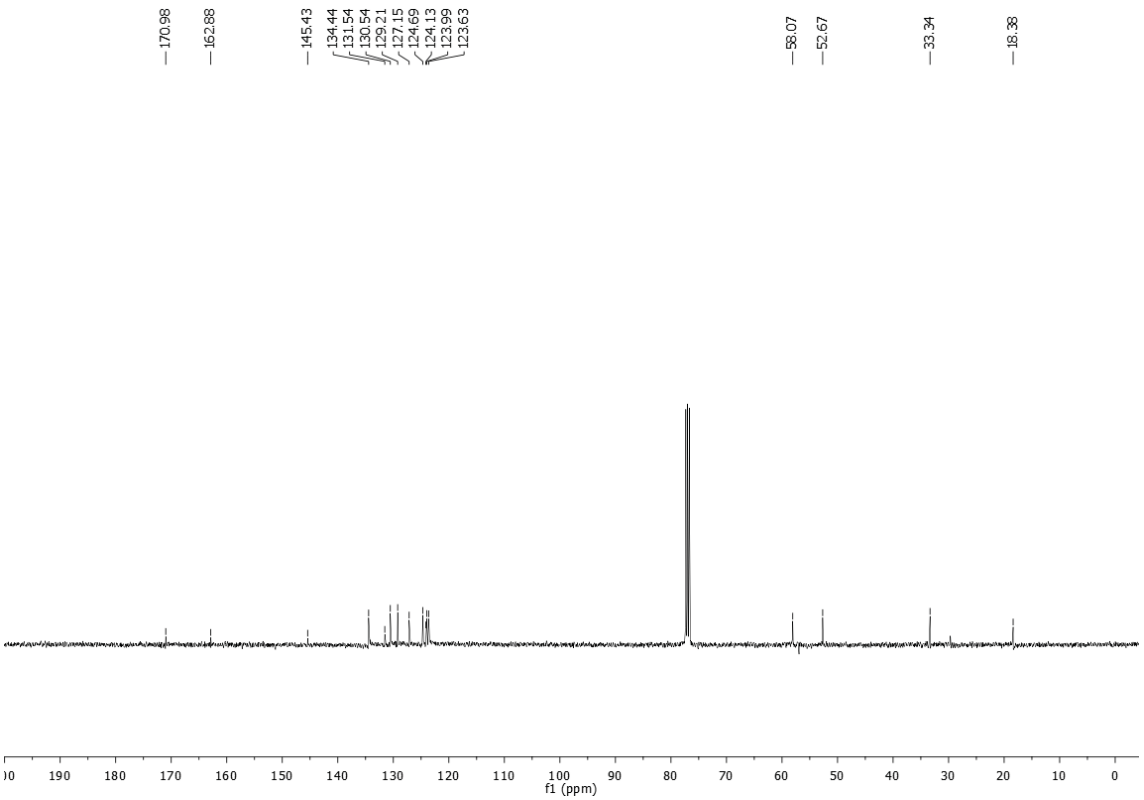

**Methyl (*S*)-1-[(4-bromophenyl)sulfonyl]-4-propionyl-2,3-dihydro-1*H*-pyrrole-2-carboxylate (2)**



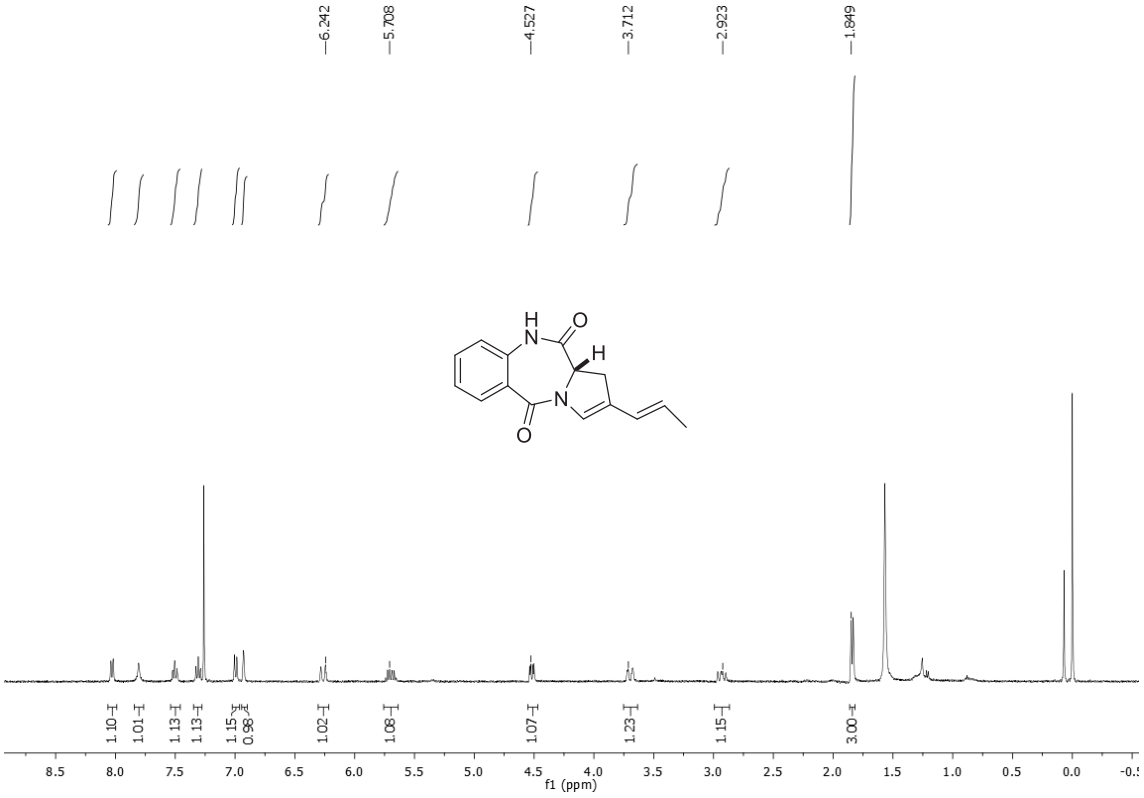



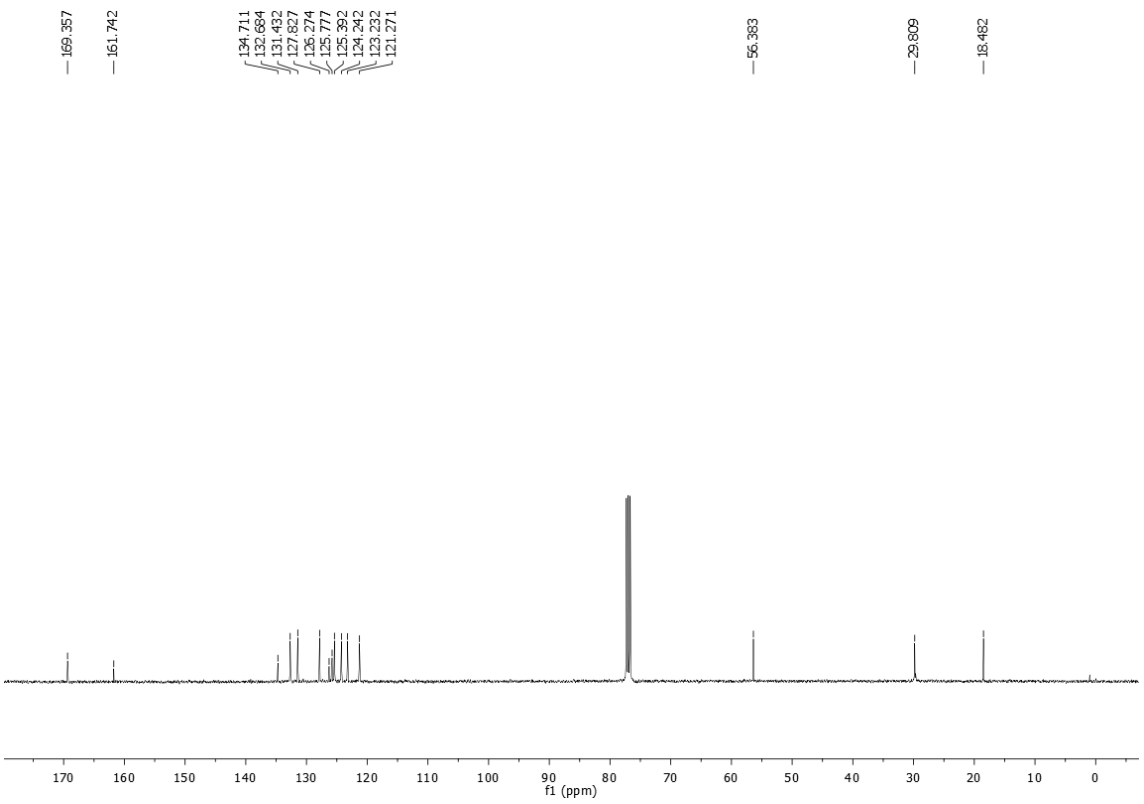

**Methyl (*S*)-1-(2-nitrobenzoyl)-4-propionyl-2,3-dihydro-1*H*-pyrrole-2-carboxylate (3)**



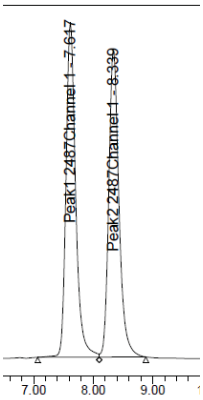

**Methyl (*S*,*E*)-1-(2-nitrobenzoyl)-4-(prop-1-en-1-yl)-2,3-dihydro-1*H*-pyrrole-2-carboxylate (4)**



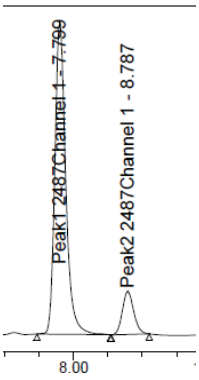




**(*S*,*E*)-2-(Prop-1-en-1-yl)-1,11*a*-dihydro-5*H*- benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepine-5,11(10*H*)-dione (5)**

## HPLC Chromatograms



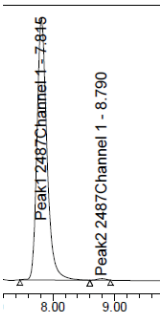

| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | Peak1 2487Channel 1 | 7.617 | 7778029 | 50.74 | 698356 |
| 2 | Peak2 2487Channel 1 | 8.339 | 7550685 | 49.26 | 643260 |




| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | Peak1 2487Channel 1 | 7.799 | 1439456 | 87.39 | 131903 |
| 2 | Peak2 2487Channel 1 | 8.787 | 207747 | 12.61 | 18147 |

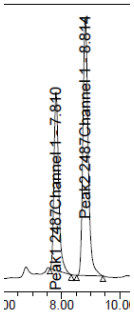
*rac*-**2**

**2** before crystallization (74.8% *ee*)



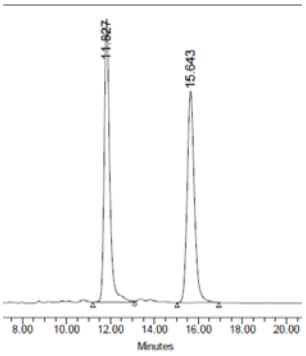

| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | Peak1 2487Channel 1 | 7.815 | 15885333 | 99.51 | 1419603 |
| 2 | Peak2 2487Channel 1 | 8.790 | 77492 | 0.49 | 8108 |

crystals of **2** (99.0%*ee*)



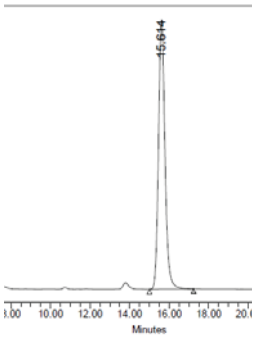

| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | Peak1 2487Channel 1 | 7.810 | 577119 | 38.20 | 51565 |
| 2 | Peak2 2487Channel 1 | 8.814 | 933498 | 61.80 | 74532 |

mother liquor of **2** (–23.6% *ee*)




| | RT | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 11.827 | 687818 | 50.78 | 39489 |
| 2 | 15.643 | 666557 | 49.22 | 29466 |

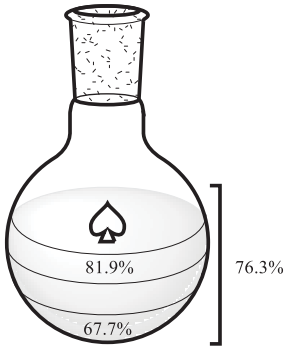
*rac*-**5**




| | RT | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 15.614 | 2585084 | 100.00 | 113666 |

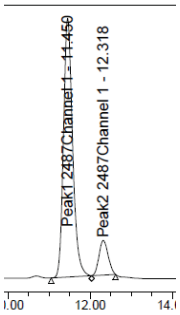
enantiopure **5** (>99.9%)

**SED by vacuum evaporation.**




A sample of **1** (20 mg) with 76.3% *ee* was placed into a round bottom flask of 250 mL, the solid was diluted in EtOAc and concentrated under vacuum (0.37 psi) at 40 ºC. Then, different samples were collected at different levels of the walls of the flask and their enantiomeric purity was analysed by HPLC chromatography.

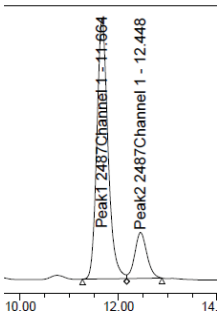
Representation of the different ee observed in the round bottom flask.




| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | Peak1 2487Channel 1 | 11.450 | 16797326 | 88.15 | 1093308 |
| 2 | Peak2 2487Channel 1 | 12.318 | 2258247 | 11.85 | 146831 |

**1** (76.3% *ee*)

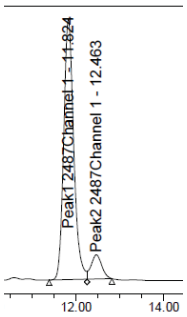
**a)**




| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | Peak1 2487Channel 1 | 11.664 | 694393 | 83.86 | 44126 |
| 2 | Peak2 2487Channel 1 | 12.448 | 133619 | 16.14 | 7779 |

bottom (67.7% *ee*)

**b)**



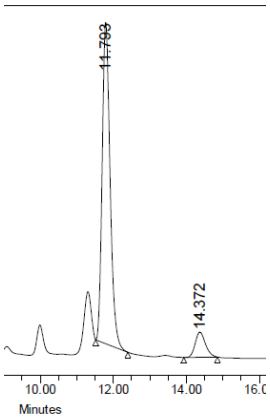

| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | Peak1 2487Channel 1 | 11.824 | 679707 | 90.95 | 43076 |
| 2 | Peak2 2487Channel 1 | 12.463 | 67654 | 9.05 | 3986 |

wall (81.9% *ee*)

**SED in automatic driven achiral column.**

**Table S1.** Chromatography of **1** with 81.4% *ee* (290 mg of compound **1**, 12 g of silica gel, GraceResolv™ Silica Flash Cartridges). Eluent: hexane/EtOAc 1:1 to 100% EtOAc.

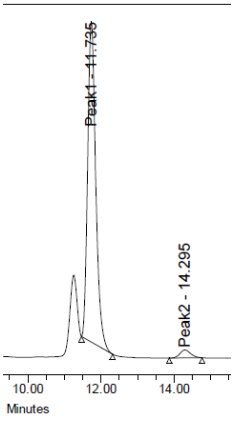
| Fraction | Volume (mL) | *ee*% |
|---|---|---|
| 1 | 6 | 93.6 |
| 2 | 12 | 92.5 |
| 3 | 18 | 92.3 |
| 4 | 24 | 87.4 |
| 5 | 30 | 84.2 |
| 6 | 36 | 81.9 |
| 7 | 42 | 80.2 |
| 8 | 48 | 78.5 |
| 9 | 54 | 73.0 |
| 10 | 60 | 66.8 |
| 11 | 66 | 63.6 |
| 12 | 72 | 63.0 |
| 13 | 78 | 62.7 |
| 14 | 84 | 62.5 |




| | RT | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 11.793 | 665242 | 90.72 | 44542 |
| 2 | 14.372 | 68014 | 9.28 | 3495 |

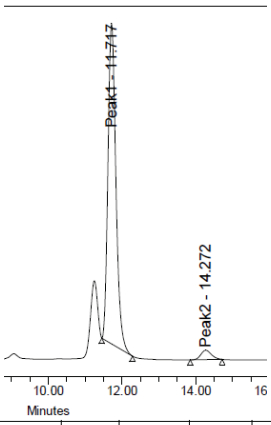
**1** (81.4% *ee*)

**1)**



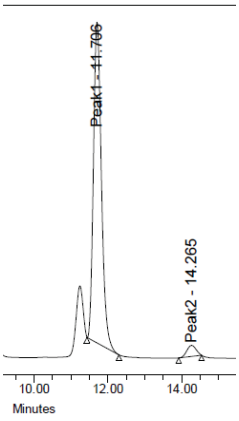

| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | Peak1 | 11.735 | 1217116 | 96.82 | 79813 |
| 2 | Peak2 | 14.295 | 40028 | 3.18 | 1979 |

**2)**



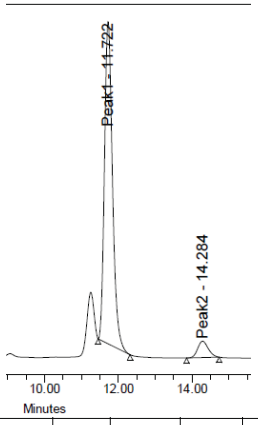

| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | Peak1 | 11.717 | 2042188 | 96.25 | 137795 |
| 2 | Peak2 | 14.272 | 79666 | 3.75 | 4028 |

**3)**



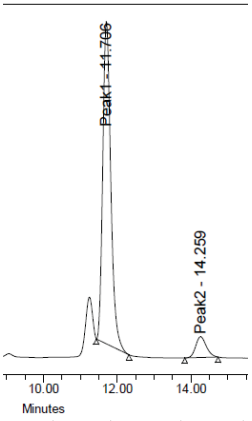

| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | Peak1 | 11.706 | 3631755 | 96.18 | 244250 |
| 2 | Peak2 | 14.265 | 144269 | 3.82 | 8391 |

**4)**



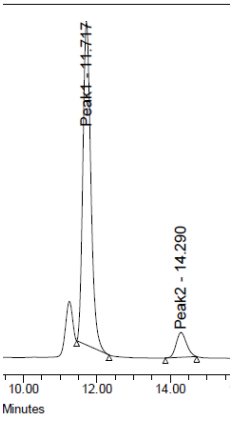

| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | Peak1 | 11.722 | 2980851 | 93.70 | 199283 |
| 2 | Peak2 | 14.284 | 200508 | 6.30 | 10120 |

**5)**



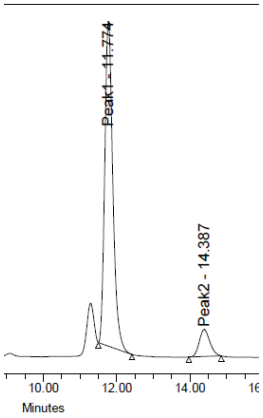

| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | Peak1 | 11.706 | 2154447 | 92.11 | 143480 |
| 2 | Peak2 | 14.259 | 184439 | 7.89 | 9230 |

**6)**



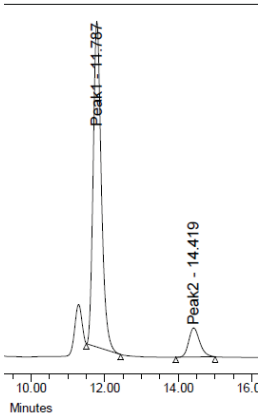

| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | Peak1 | 11.717 | 2417981 | 90.93 | 160885 |
| 2 | Peak2 | 14.290 | 241169 | 9.07 | 12333 |

**7)**



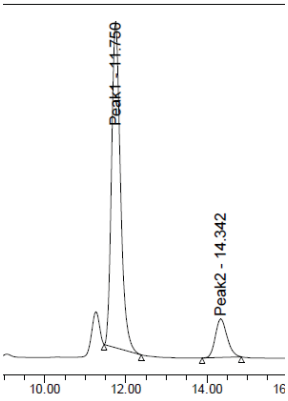

| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | Peak1 | 11.774 | 1778836 | 90.12 | 117210 |
| 2 | Peak2 | 14.387 | 195058 | 9.88 | 9720 |

**8)**



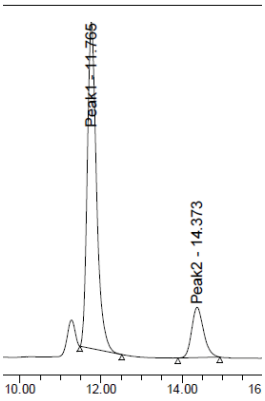

| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | Peak1 | 11.787 | 1725072 | 89.23 | 112726 |
| 2 | Peak2 | 14.419 | 208322 | 10.77 | 10081 |

**9)**



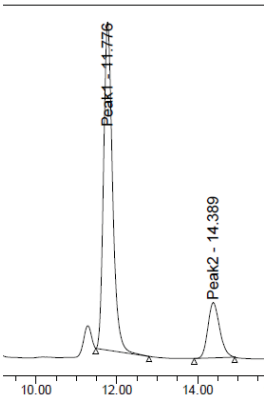

| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | Peak1 | 11.750 | 1207448 | 86.52 | 79349 |
| 2 | Peak2 | 14.342 | 188183 | 13.48 | 9346 |

**10)**



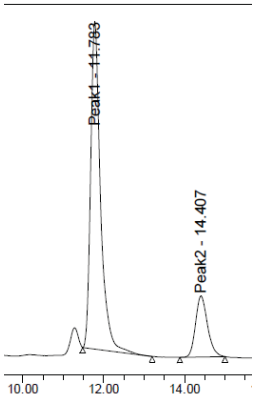

| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | Peak1 | 11.765 | 528697 | 83.40 | 33520 |
| 2 | Peak2 | 14.373 | 105215 | 16.60 | 5167 |

**11)**



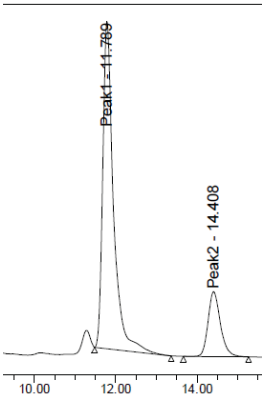

| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | Peak1 | 11.776 | 707487 | 81.79 | 46187 |
| 2 | Peak2 | 14.389 | 157497 | 18.21 | 7759 |

**12)**



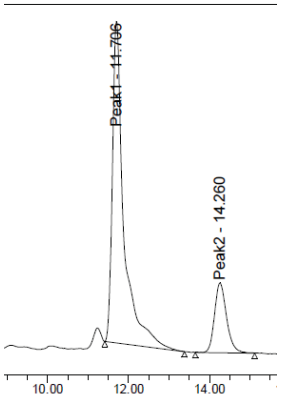

| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | Peak1 | 11.783 | 259006 | 81.50 | 15341 |
| 2 | Peak2 | 14.407 | 58811 | 18.50 | 2862 |

**13)**




| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | Peak1 | 11.789 | 255720 | 81.36 | 14077 |
| 2 | Peak2 | 14.408 | 58594 | 18.64 | 2793 |

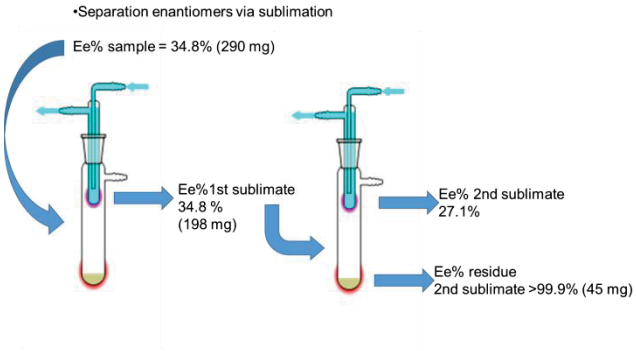
**14)**



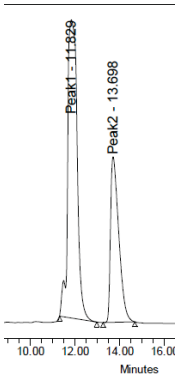

| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | Peak1 | 11.706 | 117056 | 81.26 | 6016 |
| 2 | Peak2 | 14.260 | 26991 | 18.74 | 1308 |

HPLC chromatograms of fractions from the column chromatography of **1** (81.4% *ee*); 1) HPLC of fraction 1 (93.6% *ee*); 2) HPLC of fraction 2 (92.5% *ee*); 3) HPLC of fraction 3 (92.3% *ee*); 4) HPLC of fraction 4 (87.4% *ee*); 5) HPLC of fraction 5 (84.2% *ee*); 6) HPLC of fraction 1 (81.9% *ee*); 7) HPLC of fraction 7 (80.2% *ee*); 8) HPLC of fraction 8 (78.5% *ee*); 9) HPLC of fraction 9 (73.0% *ee*); 10) HPLC of fraction 10 (66.8% *ee*); 11) HPLC of fraction 11 (63.6% *ee*); 12) HPLC of fraction 12 (63.0% *ee*); 13) HPLC of fraction 13 (62.7% *ee*); 14) HPLC of fraction 14 (62.5% *ee*).

**SDE by sublimation**

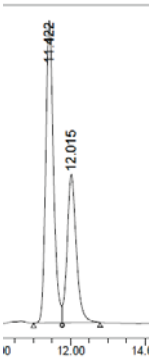



Compound **1** (290 mg, 34.8% *ee*) was placed in a sublimation apparatus, and heated at 100 °C under vacuum (0.20 psi) for 1 h. Then, the pure compound sublimated **1** (198 mg, 34.8 % *ee*) was treated again under the same conditions. The sublimate resulted in a 27.1 % *ee* and the remaining material (45 mg) in a >99.9% *ee*. The total yield of the two sublimate processes was 16 %.



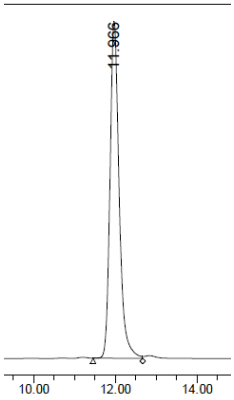

| | Peak Name | RT | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | Peak1 | 11.829 | 93237061 | 67.39 | 3171168 |
| 2 | Peak2 | 13.698 | 45110657 | 32.61 | 1759810 |

**1** (34.8% *ee*)




| | RT | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 11.422 | 8117739 | 63.55 | 559923 |
| 2 | 12.015 | 4656183 | 36.45 | 274294 |

sublimate (27.1% *ee*)




| | RT | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 11.966 | 9123059 | 100.00 | 587817 |

remaining material (> 99.9% *ee*)

**Single Crystal X-ray Diffraction**.

Crystallographic data for 2 were collected on an Enraf Nonius CAD4 diffractometer, using Mo-Kα radiation (λ = 0.71073 Å) and a graphite crystal monochromator. The cell parameters were obtained from 25 randomly obtained at high angle reflections. The data collection was done using ω-2ϑ scans. The structures were solved by direct methods, using the program SIR2014 (ref). The positional parameters and the anisotropic thermal parameters of the non-H atoms were refined using SHELXL-97 (ref). All hydrogen atoms were considered as ideal and geometrically placed. Selected crystal and data collection parameters are reported in the corresponding Tables (see Annex). The crystallographic plots were made with ORTEP3 and Mercury (refs). The calculations were made using WinGX and PLATON (refs).

**Table S2**. Crystal data and structure refinement for Jb126.

| | | |
|---|---|---|
| Identification code | Jb126 | |
| Empirical formula | C15 H16 Br N O5 S | |
| Formula weight | 402.26 | |
| Temperature | 294(2) K | |
| Wavelength | 0.71073 Å | |
| Crystal system | Orthorhombic | |
| Space group | P 21 21 21 | |
| Unit cell dimensions | a = 10.531(9) Å | α= 90°. |
| | b = 10.916(2) Å | β= 90°. |
| | c = 15.153(5) Å | γ = 90°. |
| Volume | 1741.9(16) $Å^3$ | |
| Z | 4 | |
| Density (calculated) | 1.534 $Mg/m^3$ | |
| Absorption coefficient | 2.502 $mm^{-1}$ | |
| F(000) | 816 | |
| Crystal size | 0.420 x 0.330 x 0.150 $mm^3$ | |
| Theta range for data collection | 2.299 to 25.014°. | |
| Index ranges | -12<=h<=0, -9<=k<=13, 0<=l<=18 | |
| Reflections collected | 2473 | |
| Independent reflections | 2228 [R(int) = 0.0434] | |
| Completeness to theta = 25.014° | 99.3 % | |
| Refinement method | Full-matrix least-squares on $F^2$ | |
| Data / restraints / parameters | 2228 / 0 / 210 | |
| Goodness-of-fit on $F^2$ | 0.985 | |
| Final R indices [I>2sigma(I)] | R1 = 0.0570, wR2 = 0.1206 | |
| R indices (all data) | R1 = 0.1560, wR2 = 0.1508 | |
| Absolute structure parameter | 0.01(3) | |
| Largest diff. peak and hole | 0.404 and -0.385 e.$Å^{-3}$ | |

**Table S3.** Atomic coordinates (x $10^4$) and equivalent isotropic displacement parameters ($Å^2$ x $10^3$) for Jb126. U(eq) is defined as one third of the trace of the orthogonalized $U^{ij}$ tensor.

| | x | y | z | U(eq) |
|---|---|---|---|---|
| Br(1) | 11404(1) | 2334(2) | 6797(1) | 101(1) |
| S(1) | 6789(3) | 4025(3) | 9245(2) | 55(1) |
| O(1) | 6280(8) | 5128(7) | 8898(5) | 71(2) |
| O(2) | 7197(7) | 3924(7) | 10146(5) | 68(2) |
| O(3) | 3181(8) | 4017(8) | 9217(5) | 76(3) |
| O(4) | 3528(8) | 4679(8) | 7851(5) | 72(3) |
| O(5) | 4159(9) | -863(8) | 8606(6) | 74(3) |
| N(1) | 5680(8) | 2995(8) | 9100(6) | 48(2) |
| C(2) | 4793(10) | 3052(10) | 8330(7) | 42(3) |
| C(3) | 4296(11) | 1733(10) | 8254(8) | 50(3) |
| C(4) | 5075(11) | 1036(11) | 8910(7) | 41(3) |
| C(5) | 5832(11) | 1770(11) | 9346(8) | 44(3) |
| C(6) | 8075(11) | 3556(10) | 8587(8) | 47(3) |
| C(7) | 8153(11) | 4005(11) | 7734(9) | 57(3) |
| C(8) | 9157(12) | 3642(13) | 7187(8) | 66(4) |
| C(9) | 10017(11) | 2835(12) | 7523(9) | 59(3) |
| C(10) | 9937(11) | 2365(12) | 8360(8) | 65(3) |
| C(11) | 8935(11) | 2748(12) | 8898(8) | 62(3) |
| C(12) | 3737(11) | 3965(11) | 8534(9) | 55(3) |
| C(13) | 2522(14) | 5604(14) | 7948(10) | 111(6) |
| C(14) | 4920(12) | -284(12) | 9031(8) | 55(3) |
| C(15) | 5707(15) | -905(12) | 9733(10) | 92(5) |
| C(16) | 5699(14) | -2247(12) | 9691(9) | 97(5) |

**Table S4.** Bond lengths [Å] and angles [°] for Jb126.

| | |
|---|---|
| Br(1)-C(9) | 1.909(12) |
| S(1)-O(1) | 1.419(8) |
| S(1)-O(2) | 1.436(8) |
| S(1)-N(1) | 1.636(10) |
| S(1)-C(6) | 1.758(12) |
| O(3)-C(12) | 1.190(13) |
| O(4)-C(12) | 1.314(13) |
| O(4)-C(13) | 1.471(14) |
| O(5)-C(14) | 1.206(14) |
| N(1)-C(5) | 1.398(12) |
| N(1)-C(2) | 1.497(13) |
| C(2)-C(12) | 1.525(16) |
| C(2)-C(3) | 1.537(14) |
| C(3)-C(4) | 1.497(14) |
| C(4)-C(5) | 1.309(14) |
| C(4)-C(14) | 1.462(16) |
| C(6)-C(11) | 1.349(15) |
| C(6)-C(7) | 1.385(14) |
| C(7)-C(8) | 1.400(15) |
| C(8)-C(9) | 1.363(17) |
| C(9)-C(10) | 1.371(16) |
| C(10)-C(11) | 1.398(15) |
| C(14)-C(15) | 1.509(16) |
| C(15)-C(16) | 1.466(18) |
| | |
| O(1)-S(1)-O(2) | 122.1(5) |
| O(1)-S(1)-N(1) | 105.3(5) |
| O(2)-S(1)-N(1) | 106.7(5) |
| O(1)-S(1)-C(6) | 109.2(6) |
| O(2)-S(1)-C(6) | 106.6(5) |
| N(1)-S(1)-C(6) | 105.9(5) |
| C(12)-O(4)-C(13) | 116.7(10) |
| C(5)-N(1)-C(2) | 108.6(9) |
| C(5)-N(1)-S(1) | 122.7(8) |
| C(2)-N(1)-S(1) | 121.4(7) |
| N(1)-C(2)-C(12) | 108.9(9) |

| | |
|---|---|
| N(1)-C(2)-C(3) | 103.4(8) |
| C(12)-C(2)-C(3) | 112.3(9) |
| C(4)-C(3)-C(2) | 103.9(9) |
| C(5)-C(4)-C(14) | 127.5(11) |
| C(5)-C(4)-C(3) | 110.9(10) |
| C(14)-C(4)-C(3) | 121.5(12) |
| C(4)-C(5)-N(1) | 112.5(10) |
| C(11)-C(6)-C(7) | 121.2(11) |
| C(11)-C(6)-S(1) | 120.6(10) |
| C(7)-C(6)-S(1) | 118.1(10) |
| C(6)-C(7)-C(8) | 119.8(11) |
| C(9)-C(8)-C(7) | 117.7(11) |
| C(8)-C(9)-C(10) | 123.1(11) |
| C(8)-C(9)-Br(1) | 118.6(10) |
| C(10)-C(9)-Br(1) | 118.3(10) |
| C(9)-C(10)-C(11) | 118.3(11) |
| C(6)-C(11)-C(10) | 119.9(11) |
| O(3)-C(12)-O(4) | 125.0(12) |
| O(3)-C(12)-C(2) | 124.5(12) |
| O(4)-C(12)-C(2) | 110.5(11) |
| O(5)-C(14)-C(4) | 121.6(12) |
| O(5)-C(14)-C(15) | 120.4(12) |
| C(4)-C(14)-C(15) | 118.0(12) |
| C(16)-C(15)-C(14) | 114.5(12) |

**Table S5**. Anisotropic displacement parameters ($Å^2$ x $10^3$) for Jb126. The anisotropic displacement factor exponent takes the form: $-2\pi^2[ h^2a^{*2}U^{11} + ... + 2 h k a^* b^* U^{12} ]$

| | $U^{11}$ | $U^{22}$ | $U^{33}$ | $U^{23}$ | $U^{13}$ | $U^{12}$ |
|---|---|---|---|---|---|---|
| Br(1) | 61(1) | 156(2) | 86(1) | -6(1) | 5(1) | 27(1) |
| S(1) | 53(2) | 52(2) | 60(2) | -7(2) | -2(2) | -5(2) |
| O(1) | 85(7) | 38(5) | 89(6) | 9(5) | 12(6) | 14(5) |
| O(2) | 68(5) | 81(6) | 56(6) | -6(5) | -1(5) | -11(5) |
| O(3) | 67(6) | 102(7) | 58(5) | 23(6) | 30(5) | 20(5) |
| O(4) | 66(6) | 89(6) | 59(6) | 36(5) | 4(5) | 21(6) |
| O(5) | 82(6) | 72(6) | 67(6) | -17(5) | -1(5) | -22(6) |
| N(1) | 49(6) | 46(6) | 50(6) | -1(5) | -6(5) | 0(5) |
| C(2) | 37(6) | 62(8) | 28(7) | 7(6) | -7(6) | 14(6) |
| C(3) | 40(6) | 55(9) | 56(8) | -11(7) | -12(7) | 4(6) |
| C(4) | 44(7) | 35(8) | 46(7) | -4(7) | 4(6) | 10(7) |
| C(5) | 37(7) | 46(9) | 47(8) | 9(6) | 4(6) | 15(6) |
| C(6) | 52(8) | 41(7) | 48(8) | -3(6) | -15(7) | -8(7) |
| C(7) | 40(7) | 59(8) | 71(9) | 20(8) | -12(7) | 5(7) |
| C(8) | 50(8) | 94(11) | 56(8) | 18(8) | -2(7) | 3(8) |
| C(9) | 33(6) | 78(9) | 67(9) | -5(8) | -3(7) | 9(8) |
| C(10) | 51(7) | 77(10) | 66(9) | 6(9) | -17(7) | 22(8) |
| C(11) | 50(8) | 69(9) | 67(8) | 4(8) | -15(7) | 6(8) |
| C(12) | 47(8) | 48(8) | 70(9) | 19(8) | -4(8) | -9(7) |
| C(13) | 100(12) | 107(13) | 125(14) | 48(10) | 19(10) | 72(11) |
| C(14) | 54(9) | 52(10) | 59(9) | -11(8) | 10(8) | 8(8) |
| C(15) | 120(12) | 51(10) | 104(11) | 25(9) | -30(10) | 9(9) |
| C(16) | 134(13) | 50(11) | 105(12) | -1(9) | 3(10) | 18(10) |

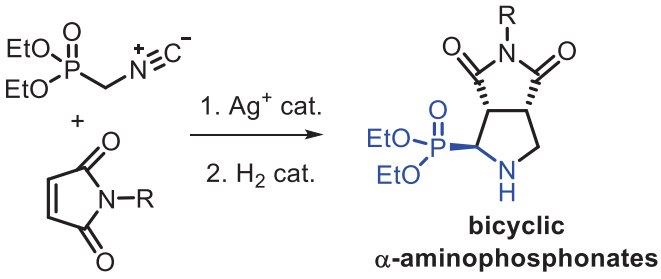
**Table S6.** Hydrogen bonds for Jb126 [Å and °].

| D-H...A | d(D-H) | d(H...A) | d(D...A) | <(DHA) |
|---|---|---|---|---|
| C(2)-H(2)...O(5)#1 | 0.98 | 2.40 | 3.351(14) | 164.8 |
| C(3)-H(3A)...O(2)#2 | 0.97 | 2.63 | 3.358(15) | 132.2 |
| C(5)-H(5)...O(3)#3 | 0.93 | 2.48 | 3.406(14) | 171.4 |
| C(7)-H(7)...O(5)#1 | 0.93 | 2.52 | 3.174(14) | 127.7 |
| C(11)-H(11)...O(3)#3 | 0.93 | 2.65 | 3.536(15) | 159.2 |

Symmetry transformations used to generate equivalent atoms:
#1 -x+1,y+1/2,-z+3/2 #2 x-1/2,-y+1/2,-z+2 #3 x+1/2,-y+1/2,-z+2

# Capítulo 3

## First diastereoselective [3+2] cycloaddition reaction of diethyl isocyanomethylphosphonate and maleimides




**bicyclic α-aminophosphonates**

Organic & Biomolecular Chemistry

RSC Publishing

PAPER

# First diastereoselective [3 + 2] cycloaddition reaction of diethyl isocyanomethylphosphonate and maleimides†

Cite this: *Org. Biomol. Chem.*, 2013, **11**, 1640

Carlos Arróniz,[a] Juan Molina,[a] Sonia Abás,[a] Elies Molins,[b] Josep M. Campanera,[c] F. Javier Luque[c] and Carmen Escolano*[a]

Received 7th September 2012,
Accepted 14th January 2013

DOI: 10.1039/c3ob26766k

**www.rsc.org/obc**

Bicyclic α-iminophosphonates were prepared *via* the first diastereoselective silver catalyzed [3 + 2] cycloaddition reaction of diethyl isocyanomethylphosphonate and diversely *N*-substituted maleimides. The reduction of the resulting imine by catalytic hydrogenation led to cyclic α-aminophosphonates, which are α-aminoester surrogates. The relative stereochemistry of the adducts was confirmed by X-ray crystallographic analysis of **4**. The diastereoselectivity of the cycloaddition reaction was rationalised by theoretical studies.

## Introduction

The distinctive reactivity of isocyanides has been recognised as an advantageous characteristic for the formation of heterocyclic compounds.[1] The carbon atom of the isocyanide group is formally divalent, which makes smooth reactions possible with both electrophiles and nucleophiles. Isocyanoacetate derivatives, in turn, present an additional reactive site since the ester functionality provides an acidic proton in the α-carbonyl position.

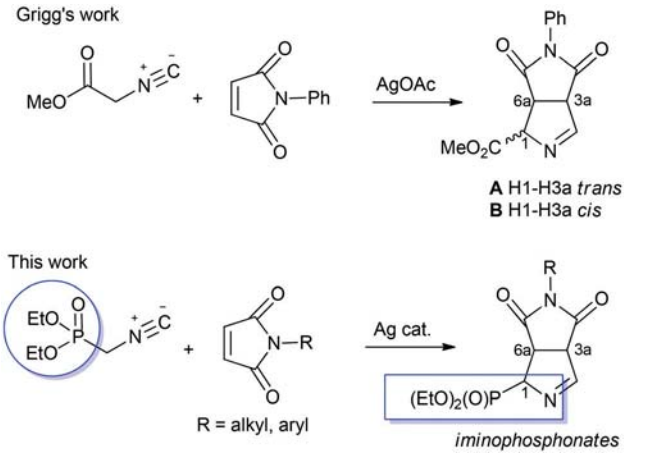
Amongst the synthetic possibilities that isocyanoacetates offer, [3 + 2] cycloaddition reactions with electron-deficient alkenes have been conceived as one of the most convergent approaches to the preparation of pyrrolines.[2] Of particular interest is the synthesis of 1-pyrrolines,[3] not only because this heterocycle is the backbone structure of important pharmacologically active compounds,[4] such as alkaloids, steroids, hemes and chlorophylls, but also because 1-pyrrolines are versatile templates for developing new drugs.[5]

In a pioneering work, Grigg and co-workers described an efficient formal [3 + 2] cycloaddition reaction of isocyanoacetates and Michael acceptors to yield pyrrolines.[6] In particular, the reaction of methyl isocyanoacetate and *N*-phenylmaleimide under the catalysis of silver acetate in acetonitrile gave an approximately 2 : 1 mixture of the two possible 1-pyrroline diastereoisomers **A** (H1–H3a *trans*) and **B** (H1 : H3a *cis*) (Scheme 1).

Recently, Carretero, Adrio and co-workers have reported an Au-catalyzed enantioselective version of this reaction.[7]

Seeking new applications of isocyano derivatives and new strategies for accessing 1-pyrroline compounds, we envisaged the possibility of performing a [3 + 2] cycloaddition reaction between diethyl isocyanomethylphosphonate (PhosMic) and maleimides (Scheme 1). This original reaction may give direct access to cyclic iminophosphonates, thus offering a wide range of synthetic possibilities. For example, reduction of the imine double bond can be easily performed to prepare cyclic aminophosphonates.

α-Aminophosphonates, the phosphonic analogues of α-aminoesters, are interesting as chiral building blocks for




**Scheme 1** Reaction of isocyano derivatives and maleimides.

[a]*Laboratory of Organic Chemistry, Faculty of Pharmacy, and Institute of Biomedicine (IBUB), University of Barcelona, 08028 Barcelona, Spain. E-mail: cescolano@ub.edu, amat@ub.edu; Fax: +34-93-402-4539*

[b]*Institut de Ciència de Materials de Barcelona (CSIC), Campus UAB, 08193 Cerdanyola, Spain*

[c]*Departament de Fisicoquímica and Institut de Biomedicina (IBUB), Facultat de Farmàcia, Universitat de Barcelona, Campus de l'Alimentació Torribera, Avgda. Prat de la Riba 171, 08921 Santa Coloma de Gramenet, Spain*

†Electronic supplementary information (ESI) available: Additional experimental information. Copies of $^{1}H$ and $^{13}C$ NMR spectra of new products. Cartesian coordinates and total energies for compounds **1a**, **1b**, **15a** and **15b**. CCDC 900421. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3ob26766k

constructing peptidomimetic structures.[8] Moreover, α-aminophosphonates have found applications ranging from medicine to agriculture, *e.g.* as antibiotics, enzyme inhibitors, anticancer agents, and herbicides.[9] These biological properties are mostly associated with the tetrahedral structure of the phosphonyl group acting as a "transition-state analogue", for example, in enzymatic peptide hydrolysis. For this reason, these compounds can be designed to be transition state inhibitors of specific peptidases or esterases. Indeed, α-aminophosphonic acid derivatives are known to be inhibitors of numerous enzymes involved in the metabolism of endogenous peptides. Due to their biological interest, several useful methods have been developed for the diastereo- and enantioselective synthesis of optically active acyclic α-aminophosphonates. However, there are fewer methods dealing with the diastereoselective synthesis of optically active cyclic α-aminophosphonates, which have found promising applications as surrogates of proline (also called phosphaproline).[10]

In this paper we disclose a general protocol for the diastereoselective synthesis of bicyclic conformationally constrained α-aminophosphonates.

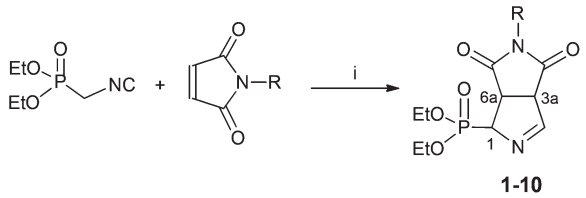
## Results and discussion

Silver has recently gained substantial attention because of its ability to activate various π-systems under mild conditions and at low-catalyst loadings.[11] To our delight, although active hydrogens in the α-isocyanophosphonate Schiff bases are less acidic than in α-isocyanoesters, the planned reaction occurred under silver catalytic conditions. Thus, the cycloaddition reaction of PhosMic and diversely *N*-substituted maleimides proceeded smoothly at room temperature in acetonitrile in the presence of catalytic amounts of silver acetate.[12]

The reaction was examined with maleimides bearing alkyl (R = Me, Et, Cy) or aryl substituents (Table 1). In the latter case, the effect of electron-donating and electron-withdrawing groups was studied.[13] The yields of the isolated final bicyclic iminophosphonates **1–10** ranged from medium to excellent (55–81%) (Scheme 2). The reaction also took place when silver fluoride was used as the catalyst, in dichloromethane or acetonitrile as the solvent, with comparable yields to those obtained under the standard silver acetate conditions.

**Table 1** Yields of the *N*-substituted bicyclic α-iminophosphonates **1–10**[14]

| Entry | R | Product | Yield (%) |
|---|---|---|---|
| 1 | Me | **1** | 72[15] |
| 2 | Et | **2** | 67 |
| 3 | Cyclohexyl | **3** | 69 |
| 4 | Ph | **4** | 69 |
| 5 | 4-$CF_3$Ph | **5** | 64 |
| 6 | (4-Phenyl)phenyl | **6** | 88 |
| 7 | 4-MeOPh | **7** | 66 |
| 8 | 4-FPh | **8** | 63 |
| 9 | 3-Cl, 4-FPh | **9** | 79 |
| 10 | 3-$NO_2$Ph | **10** | 51 |




**Scheme 2** Reagents and conditions: (i) *N*-substituted maleimide derivative (1.5 mmol), PhosMic (1 mmol), AgOAc (0.06 mmol), acetonitrile, rt, overnight; **1–10** (yield, see Table 1).

It is worthy of note that for all the maleimides studied, the process was completely diastereoselective as only one of the two possible diastereoisomers (H1–H3a *cis* or H1–H3a *trans*) was observed.

The final bicyclic iminophosphonates were found to be liquids except compound **4**, which could be recrystallized as a monocrystal from a mixture of solvents (EtOH–hexane). The relative configuration of **4** was unambiguously confirmed by X-ray crystallographic analysis, indicating a *trans* relationship between the hydrogen atoms on the bridged positions and the hydrogen atom at the α carbon of the phosphonic ester (Fig. 1).

With a straightforward method to access bicyclic iminophosphonates in hand, we considered their conversion to α-aminophosphonates. To this end, treatment of the bicyclic imines **1**, **3** and **4** with hydrogen in the presence of a catalytic amount of Pd/C furnished the corresponding aminophosphonates **12**, **13** and **14** in good yields (Scheme 3).

### Spectroscopic considerations

As expected, the $^1$H and $^{13}$C NMR spectra of the phosphonate derivatives **1–10** proved to be more complex than those of the ester derivative **A** due to additional coupling constants between $^1$H–P and $^{13}$C–P.

Table 2 shows the $^1$H and $^{13}$C chemical shifts for compounds **A** and **4** including both the multiplicity and the coupling constants.

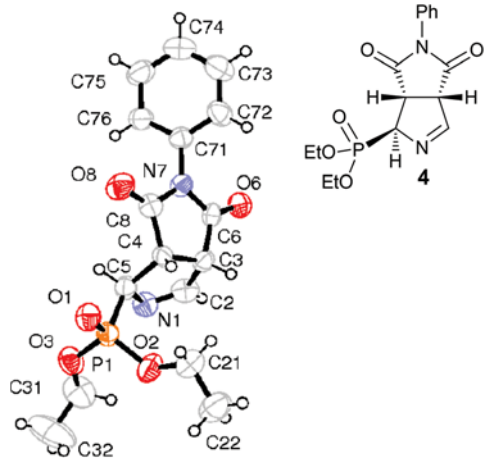
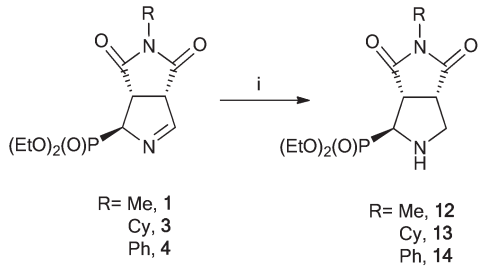



**Fig. 1** X-ray structure of **4**.




**Scheme 3** Reagents and conditions: (i) $H_2$, Pd/C 5%, ethanol, rt, overnight, 84%, **12**; 90%, **13**, and 87%, **14**.

**Table 2** Significant $^1$H and $^{13}$C NMR data for compounds **A** and **4**

| | H1 | H3 | H3a | H6a |
|---|---|---|---|---|
| **A** | 5.20 | 7.70 | 4.27 | 3.85 |
| | q | dd | ddd | dd |
| | 2.5 | 2.4, 1.2 | 8.4, 2.8, 1.2 | 8, 2.8 |
| **4** | 4.87 | 7.79 | 4.42 | 3.89 |
| | dq | ddd | dddd | dddd |
| | 19.5, 2.5 | 5, 2.5, 1 | 8.5, 4.5, 2.5, 1.0 | 17.5, 8.5, 2.5 |

| | C1 | C3 | C3a | C6a | C4 | C6 |
|---|---|---|---|---|---|---|
| **A** | 77.9 | 162.8 | 59.0 | 45.2 | 172.1 | 176.2 |
| **4** | 73.7 | 162.8 | 58.8 | 44.2 | 171.3 | 175.4 |
| | d | d | | | d | d |
| | 157 | 12 | | | 6 | 15 |

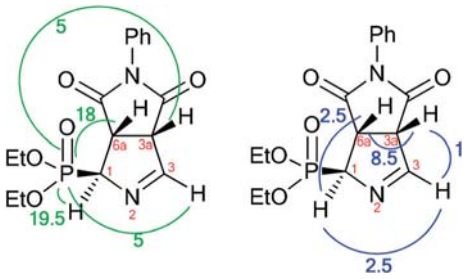


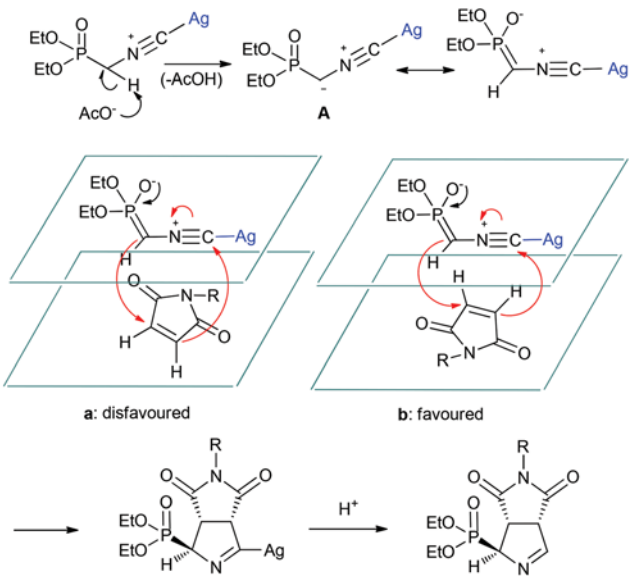

**Fig. 2** Coupling constants $^1$H–P (green) and $^1$H–$^1$H (blue).

In Fig. 2 the representative coupling constants, $^1$H–P (in green) and $^1$H–$^1$H (in blue), for compound **4** are depicted.

The above spectral data are in agreement with a *trans* relationship between H-3a and H-1, as confirmed by the X-ray crystallographic analysis of **4**.

### Proposed mechanism

Based on the stereochemistry of the final products a putative mechanism is proposed for this new [3 + 2] cycloaddition reaction. The silver complexation to the terminal carbon of PhosMic would increase the acidity of the α-proton, allowing deprotonation by the acetate ion to occur. After the α-deprotonation the 1,3-cycloaddition reaction with the *N*-substituted maleimide would take place. Two possibilities are considered for the approaching of the 1,3-dipole and the maleimide (Scheme 4, **a** and **b**). In case **a**, the phosphonate moiety is situated in an *endo* manner to the imide involving a certain degree



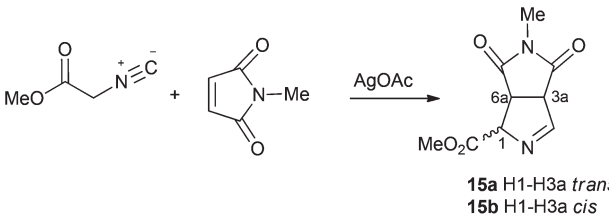

**Scheme 4** Putative mechanism for the [3 + 2] cycloaddition reaction.

of hindrance. In case **b**, the phosphonate moiety is situated in an *exo* fashion to the imide group, which is more favoured from the steric point of view. Finally, acetic acid protodemetallation would lead to the final isolated product, thereby regenerating the silver acetate catalyst.

## Theoretical studies

An interesting feature of the cycloadditions herein reported is their high diastereoselectivity in sharp contrast with the low diastereoselectivity previously observed by Grigg in his pioneering work with isocyanoacetates.[6a] In order to gain insight into these differences, theoretical calculations were carried out. To reduce the number of atoms involved in the theoretical studies the reaction of *N*-methyl maleimide and methyl isocyanoacetate was performed following the general experimental conditions (see the Experimental part and Scheme 5). As expected the reaction gave a mixture of diastereoisomers, the major isomer being **15a** with a H1–H3a *trans* relative configuration (Scheme 5).

Full geometry optimization of **15a** and its C-1 epimer (**15b**) was performed at the B3LYP/6-31+G(d) level. As indicated in




**Scheme 5** Reagents and conditions: (i) *N*-methylmaleimide (1.8 mmol), methyl isocyanoacetate (1.2 mmol), AgOAc (0.02 mmol), acetonitrile; **15a** : **15b** (3 : 1 GC/MS), 67%.

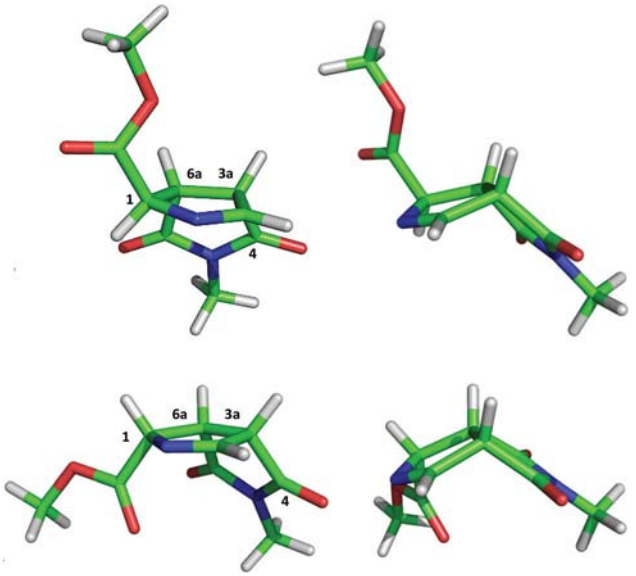



**Fig. 3** Two views of the most stable conformation of compounds **15a** (top) and **15b** (bottom).

Fig. 3, compound **15a** showed a completely planar disposition for both five-membered ring heterocycles with a dihedral angle C1–C6a–C3a–C4 of 120°. The ester substituent in C1 is eclipsed by the C6a–H6 bond. The same dihedral angle is found in the bicyclic system of compound **15b**, although in this case the hydrogens at positions 1, 6a and 3 are all eclipsed (Fig. 3). The methyl ester substituent occupies an outside position in the convex face of the bicyclic system and it is eclipsed by the C6a–C6 bond of the maleimide moiety. Both compounds proved to be energetically comparable, with only 0.4 kcal $mol^{-1}$ difference in favour of the **15a** isomer. In the H1–H3a *trans* isomer **15a** there is no steric congestion, whereas in isomer **15b** a slightly destabilizing interaction due to the proximity of the carbonyl group to the imide ring can be considered. This interaction might be responsible for the energetic destabilization observed.

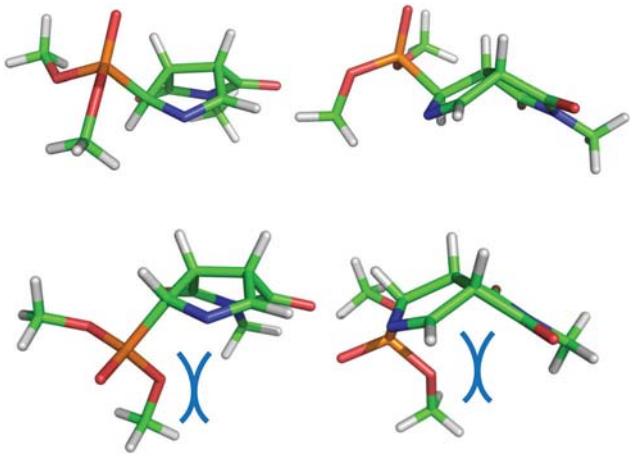


**Fig. 4** Two views of the most stable conformation of hypothetical compounds **1a** (top) and **1b** (bottom). Destabilizing interactions are indicated in blue.

In the phosphonate series, in order to reduce the calculation time, the methyl phosphonate derivatives rather than the ethyl phosphonate derivatives were minimized.[16] The relative conformation of the phosphonate derivatives **1a** and putative **1b** (Fig. 4) was similar to that of the ester derivatives related to the planar situation of the two cycles and to the dihedral angles between C1–C6a–C3a–C4 of 120°. The H1–H3a *trans* isomer **1a** was expected to be more stable than the H1–H3a *cis* derivative **1b** due to the steric hindrance caused by the orientation of one of the methoxy groups of the phosphonate

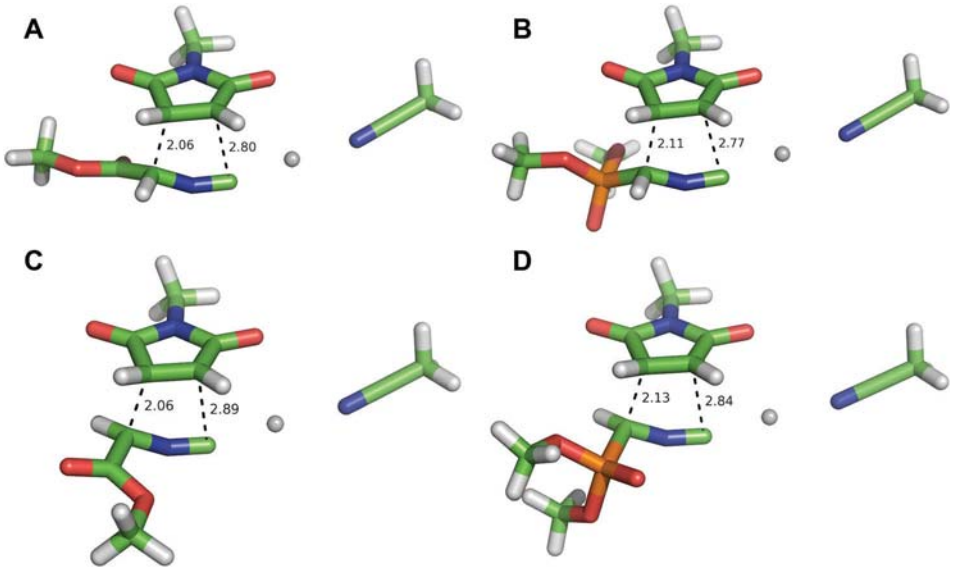



**Fig. 5** Representation of the transition states for the [3 + 2] reactions of methyl isocyanoacetate and *N*-methyl maleimide to give **15a** (A) and **15b** (C) and dimethyl isocyanomethylphosphonate and *N*-methyl maleimide to give **1a** (B) and **1b** (D) compounds for both *endo* (top) and *exo* (bottom) cycloadditions. The silver cation, which is bound to a molecule of acetonitrile, is shown as a sphere. Relevant distances of the forming bonds are shown in angstroms.

**Table 3** Relative energies (including zero-potential energy corrections; kcal $mol^{-1}$) between transition states of the [3 + 2] cycloaddition reactions of compounds **15b** and **1b** relative to **15a** and **1a**, respectively, determined at B3LYP, M06L and PB + ZORA levels

| | B3LYP | M06L | PB + ZORA |
|---|---|---|---|
| **15b** | 0.0 | 1.1 | 0.7 |
| **1b** | −2.8 | −2.1 | −4.5 |

at the concave face of the bicyclic system in the hypothetical derivative **1b**. Indeed, B3LYP/6-31+G(d) calculations indicate that the **1a** structure was more stable by 4 kcal $mol^{-1}$. This energetic difference might be enough to justify the excellent diastereoselectivity of the reaction described in this paper.

To further confirm the preceding trends, the transition states for the [3 + 2] cycloaddition reactions to yield compounds **15a**, **15b**, **1a** and **1b** were identified at the B3LYP/6-31+G(d) level. The effect of the catalyst was taken into account by adding a silver cation coordinated to acetonitrile. The transition state nature was confirmed by the single imaginary frequency of the stationary points in all cases. The geometries of the transition states reveal an asynchronous concerted process, where the length of the bond that involves the carbon atom 6a is around 0.7 Å shorter than the bond with carbon atom 3a (Fig. 5).

In order to determine the relative stability between the transition states for *endo* and *exo* cycloadditions, single-point calculations were performed using both M06L and PB + ZORA density functional (see Experimental). The results consistently show a distinctive trend for the addition of compounds bearing ester (**15**) and phosphonate (**1**) moieties: whereas the transition state leading to **15a** is found to be favoured by around 1 kcal $mol^{-1}$, the reverse trend is observed for the cycloaddition of compound **1**. Thus, the transition state that yields **1b** is destabilized by more than 2 kcal $mol^{-1}$, which can be attributed to the repulsive interaction between one of the phosphonate oxygens and the carbonyl unit of the maleimide reactant (Table 3).

Overall, the theoretical calculations are in agreement with the experimental data related to the different diastereoselectivity observed when the cycloaddition reaction was carried out with ester or phosphonate derivatives.

## Conclusions

In summary, we have reported the first [3 + 2] cycloaddition reaction of diethyl isocyanomethylphosphonate and maleimides *N*-substituted with groups of varying electronic character. The special feature of this experimentally simple reaction is the excellent selectivities observed in the products. The final bicyclic structures accessed were completely characterized from the stereochemical point of view based on the X-ray crystallographic analysis of compound **4**. Theoretical calculations were performed to justify the excellent diastereoselectivity observed when using diethyl isocyanomethylphosphonate compared to the reaction described with methyl isocyanoacetate and maleimides. Finally, conversion of three cyclic α-iminophosphonates **1**, **3** and **4** to the corresponding α-aminophosphonates was successfully carried out.

## Experimental

NMR spectra were recorded in $CDCl_3$ at 300 or 400 MHz ($^1H$) and 75.4 or 100.6 MHz ($^{13}C$), and chemical shifts are reported in $\delta$ values downfield from TMS or relative to residual chloroform (7.26 ppm, 77.0 ppm) as an internal standard. Data are reported in the following manner: chemical shift, multiplicity, coupling constant (*J*) in hertz (Hz), integrated intensity. Multiplicities are reported using the following abbreviations: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet; br s, broad signal, app, apparent. Evaporation of solvents was accomplished with a rotary evaporator. Melting points were determined in a capillary tube and are uncorrected. Thin-layer chromatography was done on $SiO_2$ (silica gel 60 $F_{254}$), and the spots were located by UV, 1% aqueous $KMnO_4$ or iodoplatinate (for tertiary amines). Chromatography refers to flash column chromatography and was carried out on $SiO_2$ (silica gel 60, SDS, 230–400 mesh) or $Al_2O_3$ (aluminium oxide 90 active basic, Merck). Mass spectra were recorded on an LTQ spectrometer using electrospray ($ES^+$) ionization techniques. *N*-Substituted maleimides, Scheme 1 R = 4-FPh, 4-MeOPh, 3-Cl, 4-FPh, were prepared according to literature procedures.[17]

### General procedure for the [3 + 2] cycloaddition reaction

To a solution of silver acetate (0.06 mmol) and maleimide (1.5 mmol) in acetonitrile was added PhosMic (1 mmol). The reaction mixture was stirred at room temperature for 8 h. Then, the mixture was filtered through a Celite® pad and concentrated and the residue was purified by column chromatography to afford pure product **1–10**.

**Diethyl (1*RS*,3a*RS*,6a*RS*)-5-methyl-4,6-dioxo-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrrole-1-phosphonate (1).** Following the general procedure, AgOAc (2.4 mg, 0.02 mmol), *N*-methylmaleimide (66.6 mg, 0.6 mmol), acetonitrile (3 mL) and PhosMic (64 μL, 0.4 mmol) gave **1** (69 mg, 72%) as a brownish oil after column chromatography (EtOAc). IR (NaCl) 3470, 2984, 1701, 1435 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.38 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 1.39 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 2.96 (s, 3H, $NCH_3$), 3.71 (ddd, $J$ = 17.5, 8.5, 2.5 Hz, 1H, H-6a), 4.17–4.29 (m, 5H, $CH_2CH_3$, H-3a), 4.84 (dq, $J$ = 20.0, 2.5 Hz, 1H, H-1), 7.69 (m, 1H, H-3); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.3 ($CH_2CH_3$), 16.4 ($CH_2CH_3$), 25.2 ($NCH_3$), 44.0 (C-6a), 58.9 (C-3a), 63.1 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 63.7 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 73.0 (d, $J$ = 159.0 Hz, C-1), 162.9 (d, $J$ = 12.5 Hz, C-3), 172.4 (d, $J$ = 6.0 Hz, C-4), 176.3 (d, $J$ = 14.0 Hz, C-6); MS-EI *m*/*z* 288 $M^+$ (15), 260 (22), 230 (30), 203 (74), 175 (52), 147 (88); HRMS $C_{11}H_{18}N_2O_5P$ $[M + H]^+$ 289.0948; found, 289.0945. Anal. Cald for $C_{11}H_{17}N_2O_5P$ 3/4$H_2O$: C 43.79; H, 6.18; N, 9.28. Found: C, 43.64; H, 6.06; N, 9.24%.

**Diethyl (1*RS*,3a*RS*,6a*RS*)-5-ethyl-4,6-dioxo-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrrole-1-phosphonate (2).** Following the general procedure, AgOAc (27 mg, 0.16 mmol), *N*-ethylmaleimide (200 mg, 1.59 mmol), acetonitrile (8 mL) and PhosMic (169.9 μL, 1.06 mmol) gave **2** (215 mg, 67%) as a yellowish oil, after column chromatography (EtOAc–MeOH 99 : 1). IR (NaCl) 3250, 2924, 1705, 1402 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.13 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 1.38 (t, $J$ = 7.0, 0.5 Hz, 3H, $CH_2CH_3$), 1.39 (td, $J$ = 7.0, 0.5 Hz, 3H, $CH_2CH_3$), 3.53 (q, $J$ = 7.0 Hz, 3H, $NCH_2CH_3$), 3.69 (ddd, $J$ = 17.5, 8.5, 2.5 Hz, 1H, H-6a), 4.17–4.29 (m, 5H, $2CH_2CH_3$, H-3a), 4.84 (dq, $J$ = 20.0, 2.5 Hz, 1H, H-1), 7.69 (ddd, $J$ = 5.0, 2.5, 1.0 Hz, 1H, H-3); $^{13}C$ NMR (100.6 MHz) $\delta$ 12.8 ($NCH_2CH_3$), 16.3 ($CH_2CH_3$), 16.4 ($CH_2CH_3$), 34.3 ($NCH_2CH_3$), 44.0 (C-6a), 58.9 (C-3a), 63.1 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 63.7 (d, $J$ = 6 Hz, $CH_2CH_3$), 73.0 (d, $J$ = 159.0 Hz, C-1), 162.9 (d, $J$ = 12.0 Hz, C-3), 172.2 (d, $J$ = 6.0 Hz, C-4), 176.1 (d, $J$ = 14.0 Hz, C-6); MS-EI $m/z$ 302 $M^+$ (13), 287 (16), 273 (18), 230 (24), 203 (86), 175 (49), 147 (69); HRMS $C_{12}H_{20}N_2O_5P$ $[M + H]^+$ 303.1104; found, 303.1109.

**Diethyl (1*RS*,3a*RS*,6a*RS*)-5-cyclohexyl-4,6-dioxo-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrrole-1-phosphonate (3).** Following the general procedure, AgOAc (4 mg, 0.02 mmol), *N*-cyclohexylmaleimide (108 mg, 0.6 mmol), acetonitrile (3 mL) and PhosMic (64 μL, 0.4 mmol) gave **3** (98 mg, 69%) as a yellowish oil, after column chromatography (EtOAc–MeOH 99 : 1). IR (NaCl) 3464, 2933, 1702, 1371 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.12–1.30 (m, 2H, $CH_2$), 1.37 (t, $J$ = 7.0 Hz, $CH_2CH_3$), 1.39 (t, $J$ = 7.0 Hz, $CH_2CH_3$), 1.52 (m, 1H, $CH_2$), 1.64 (m, 1H, $CH_2$), 1.80 (m, 1H, $CH_3$), 2.06 (m, 1H, $CH_2$), 3.63 (ddd, $J$ = 17.5, 8.5, 2.5 Hz, 1H, H-6a), 3.89 (tt, $J$ = 12.5, 4.0 Hz, 1H, H-3a), 4.15–4.29 (m, 5H, NCH, $2CH_2CH_3$), 4.82 (dq, $J$ = 19.5, 2.5 Hz, 1H, H-1), 7.69 (ddd, $J$ = 5.0, 2.5, 1.0 Hz, 1H, H-3); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.3 [NCH-$(CH_2CH_2)_2CH_2$], 16.4 [$NCH(CH_2CH_2)_2CH_2$], 24.8 [$NCH(CH_2CH_2)_2CH_2$], 25.6 [$NCH(CH_2CH_2)_2CH_2$], 28.5 [NCH-$(CH_2CH_2)_2CH_2$], 28.7 [$NCH(CH_2CH_2)_2CH_2$], 43.6 (C-6a), 52.2 (C-3a), 58.5 (NCH), 63.1 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 63.6 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 73.6 (d, $J$ = 157.0 Hz, C-1), 163.3 (d, $J$ = 12.0 Hz, 1H, C-3), 172.0 (d, $J$ = 6.0 Hz, C-4), 176.4 (d, $J$ = 14.0 Hz, C-6); MS-EI $m/z$ 356 $M^+$ (7), 341 (2), 327 (2), 276 (12), 275 (100), 247 (19), 219 (25), 203 (14), 148 (10); HRMS $C_{16}H_{26}N_2O_5P$ $[M + H]^+$ 357.1574; found, 357.1574.

**Diethyl (1*RS*,3a*RS*,6a*RS*)-4,6-dioxo-5-phenyl-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrrole-1-phosphonate (4).** Following the general procedure, AgOAc (10 mg, 0.06 mmol), *N*-phenylmaleimide (208 mg, 1.2 mmol), acetonitrile (6 mL) and PhosMic (128.3 μL, 0.8 mmol) gave **4** (171 mg, 69%) as a white solid, after column chromatography (EtOAc). M.p. 104–106 °C (EtOH–hexane); IR (NaCl) 2982, 2928, 1718, 1380 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.39 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 1.40 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 3.89 (ddd, $J$ = 17.5, 8.5, 2.5 Hz, 1H, H-6a), 4.20–4.32 (m, 4H, $2CH_2CH_3$), 4.42 (dddd, $J$ = 8.5, 4.5, 2.5, 1.0 Hz, 1H, H-3a), 4.97 (dq, $J$ = 19.5, 2.5 Hz, 1H, H-1), 7.20–7.25 (m, 2H, HAr), 7.38–7.50 (m, 3H, ArH), 7.79 (ddd, $J$ = 5.0, 2.5, 1.0 Hz, 1H, H-3); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.4 ($CH_2CH_3$), 16.5 ($CH_2CH_3$), 44.2 (C-6a), 58.8 (C-3a), 63.2 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 63.8 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 73.7 (d, $J$ = 158.0 Hz, C-1), 126.2 (2CHAr), 129.0 (CHAr), 129.3 (2CHAr), 131.1 (C*i*), 162.8 (d, $J$ = 12.0 Hz, 1H, C-3), 171.3 (d, $J$ = 6.0 Hz, C-4), 175.4 (d, $J$ = 15.0 Hz, C-6); MS-EI $m/z$ 350 $M^+$ (21), 230 (19), 213 (19), 203 (44), 175 (25), 147 (29), 119 (25); HRMS $C_{16}H_{20}N_2O_5P$ $[M + H]$ 351.1104; found, 351.1102. Anal. Cald for $C_{16}H_{19}N_2O_5P$: C, 54.86; H, 5.47; N, 8.00. Found: C, 55.00; H, 5.58; N, 7.85%.

**Diethyl (1*RS*,3a*RS*,6a*RS*)-4,6-dioxo-5-(*p*-trifluoromethylphenyl)-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrrole-1-phosphonate (5).** Following the general procedure, AgOAc (4 mg, 0.02 mmol), *N*-(*p*-trifluoromethylphenyl)maleimide (145 mg, 0.6 mmol), acetonitrile (3 mL) and PhosMic (64 μL, 0.4 mmol) gave **5** (107 mg, 64%) as an orange oil, after column chromatography (AcOEt–MeOH 99 : 1). IR (NaCl) 3487, 2986, 1717, 1444 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.39 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 1.40 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 3.92 (ddd, $J$ = 17.5, 8.5, 2.5 Hz, 1H, H-6a), 4.22–4.29 (m, 4H, $2CH_2CH_3$), 4.44 (m, 1H, H-3a), 5.00 (dm, $J$ = 20.0 Hz, 1H, H-1), 7.42 (d, $J$ = 8 Hz, 2H, ArH), 7.73 (d, $J$ = 8 Hz, 2H, ArH), 7.80 (m, 1H, H-3); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.3 ($CH_2CH_3$), 16.4 ($CH_2CH_3$), 44.2 (C-6a), 58.8 (C-3a), 63.3 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 64.0 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 73.3 (d, $J$ = 158.0 Hz, C-1), 123.5 (q, $J$ = 123.5, $CF_3$), 126.3 (dd, $J$ = 7.5, 3.5 Hz, C-*m*), 126.5 (C-*o*), 130.7 (q, J = 33.0 Hz, C-*p*), 134.2 (C-*i*), 162.2 (d, $J$ = 12.0 Hz, C-3), 170.5 (d, $J$ = 6.0 Hz, C-4), 174.7 (d, $J$ = 6.0 Hz, C-6); MS-EI $m/z$ 418 $M^+$ (17), 230 (45), 203 (69), 187 (21), 175 (33), 147 (42), 109 (74), 94 (100); HRMS $C_{17}H_{19}F_3O_5P$ $[M + H]^+$ 419.0978; found, 419.0977.

**Diethyl (1*RS*,3a*RS*,6a*RS*)-4,6-dioxo-5-(*p*-phenylphenyl)-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrrole-1-phosphonate (6).** Following the general procedure, AgOAc (4 mg, 0.02 mmol), *N*-(*p*-phenylphenyl)maleimide (150 mg, 0.6 mmol), acetonitrile (3 mL) and PhosMic (64 μL, 0.4 mmol) gave **6** (150 mg, 88%) as a brownish oil, after column chromatography (EtOAc–MeOH 99 : 1). IR (NaCl) 3480, 2924, 1717, 1488 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.39 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 1.40 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 3.91 (ddd, $J$ = 17.5, 8.5, 2.5 Hz, 1H, H-6a), 4.20–4.30 (m, 4H, $CH_2CH_3$), 4.44 (dddd, $J$ = 8, 4, 2.5, 1.0 Hz, 1H, H-3a), 5.00 (dq, $J$ = 20.0, 2.5 Hz, 1H, H-1), 7.29–7.70 (m, 9H, HAr), 7.81 (ddd, $J$ = 5.0, 2.5, 1.0 Hz, 1H, H-3); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.4 ($CH_2CH_3$), 16.5 ($CH_2CH_3$), 44.2 (C-6a), 58.8 (C-3a), 63.3 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 63.8 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 73.6 (d, $J$ = 157.9 Hz, C-1), 126.5 (2CHAr), 127.2 (2CHAr), 127.8 (CHAr), 128.0 (2CHAr), 128.9 (2CH), 130.1 (C*i*), 140.0 (C*i*), 142.1 (C*i*), 162.9 (d, $J$ = 12.5 Hz, C-3), 171.4 (d, $J$ = 6.0 Hz, C-4), 175.4 (d, $J$ = 15.0 Hz, C-6); MS-EI $m/z$ 426 $M^+$ (35), 288 (15), 195 (35), 169 (9), 166 (9), 94 (100); HRMS $C_{22}H_{24}N_2O_5P$ $[M + H]^+$ 427.1417; found, 427.1421.

**Diethyl (1*RS*,3a*RS*,6a*RS*)-5-(*p*-methoxyphenyl)-4,6-dioxo-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrrole-1-phosphonate (7).** Following the general procedure, AgOAc (4 mg, 0.02 mmol), *N*-methoxyphenylmaleimide (122 mg, 0.6 mmol), acetonitrile (3 mL) and PhosMic (64 μL, 0.4 mmol) gave **7** (100 mg, 66%) as a colourless oil, after column chromatography

(EtOAc–MeOH 99 : 1). IR (NaCl) 3477, 2982, 1716, 1513 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.39 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 1.40 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 3.82 (s, 3H, $OCH_3$), 3.87 (ddd, $J$ = 17.5, 8.5, 2.5 Hz, 1H, H-6a), 4.20–4.32 (m, 4H, $2CH_2CH_3$), 4.39 (dddd, $J$ = 8.5, 4.5, 2.5, 1.0 Hz, 1H, H-3a), 4.96 (dq, $J$ = 19.5, 2.5 Hz, 1H, H-1), 6.96 (d, $J$ = 9.0 Hz, 2H, *o*-HAr), 7.13 (d, 2H, $J$ = 9.0 Hz, *m*-HAr), 7.79 (ddd, $J$ = 5, 2.5, 1 Hz, 1H, H-3); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.4 ($CH_2CH_3$), 16.5 ($CH_2CH_3$), 44.2 (C-6a), 55.5 ($OCH_3$), 58.8 (C-3a), 63.2 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 63.8 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 73.6 (d, $J$ = 158.0 Hz, C-1), 114.6 (2-*o*-CHAr), 123.6 (C*i*-O), 127.5 (2-*m*-CHAr), 159.7 (C*i*-O), 162.9 (d, $J$ = 12.0 Hz, 1H, C-3), 171.6 (d, $J$ = 5.5 Hz, C-4), 175.6 (d, $J$ = 14.0 Hz, C-6); MS-EI $m/z$ 380 $M^+$ (34), 242 (19), 149 (37), 134 (20); HRMS $C_{17}H_{22}N_2O_6P$ $[M + H]^+$ 381.1217; found, 381.1217.

**Diethyl (1*RS*,3a*RS*,6a*RS*)-5-(*p*-fluorophenyl)-4,6-dioxo-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrrole-1-phosphonate (8).** Following the general procedure, AgOAc (4 mg, 0.02 mmol), *N*-(*p*-fluorophenyl)maleimide (77 mg, 0.6 mmol), acetonitrile (3 mL) and PhosMic (64 μL, 0.4 mmol) gave **8** (90 mg, 63%) as an orange oil, after column chromatography (EtOAc–MeOH 99 : 1). IR (NaCl) 3481, 2985, 1718, 1511 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.39 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 1.40 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 3.89 (ddd, $J$ = 17.5, 8.5, 2.5 Hz, 1H, H-6a), 4.20–4.30 (m, 4H, $2CH_2CH_3$), 4.41 (dddd, $J$ = 8.0, 4.0, 2.5, 1 Hz, 1H, H-3a), 4.95 (dq, $J$ = 20.0, 2.5 Hz, 1H, H-1), 7.15 (t, $J$ = 8.5 Hz, 2H, HAr), 7.23 (dd, $J$ = 9.0, 5.0 Hz, 2H, HAr), 7.79 (m, 1H, H-3); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.4 ($CH_2CH_3$), 16.5 ($CH_2CH_3$), 44.2 (C-6a), 58.8 (C-3a), 63.3 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 63.8 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 73.6 (d, $J$ = 158.0 Hz, C-1), 116.3 (d, $J$ = 22.5 Hz, 2-*m*-CHAr), 126.9 (C*i*-O), 128.1 (2-*o*-CHAr), 162.3 (d, $J$ = 250.0 Hz, C*i*-F), 162.7 (d, $J$ = 12.0 Hz, 1H, C-3), 171.2 (d, $J$ = 6.0 Hz, C-4), 175.3 (d, $J$ = 15.0 Hz, C-6); MS-EI $m/z$ 368 $M^+$ (13), 231 (23), 203 (31), 186 (10), 175 (17), 147 (24), 137 (24), 109 (52); HRMS $C_{16}H_{19}FN_2O_5P$ $[M + H]^+$ 369.1011; found, 369.1084. Anal. Cald for $C_{16}H_{18}FN_2O_5P{\cdot}1/4H_2O$: C 51.55; H, 5.00; N, 7.51. Found: C, 51.89; H, 4.80; N, 7.31%.

**Diethyl (1*RS*,3a*RS*,6a*RS*)-5-(3-chloro-4-fluorophenyl)-4,6-dioxo-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrrole-1-phosphonate (9).** Following the general procedure, AgOAc (4 mg, 0.02 mmol), *N*-(3-chloro-4-fluorophenyl)maleimide (135 mg, 0.6 mmol), acetonitrile (3 mL) and PhosMic (64 μL, 0.4 mmol) gave **9** (128 mg, 79%) as an orange oil, after column chromatography (EtOAc–MeOH 99 : 1). IR (NaCl) 3487, 2985, 1717, 1501 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.38 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 1.40 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 3.89 (ddd, $J$ = 17.5, 8.5, 2.5 Hz, 1H, H-6a), 4.20–4.30 (m, 4H, $2CH_2CH_3$), 4.41 (dddd, $J$ = 8.0, 4.0, 2.5, 1.0 Hz, 1H, H-3a), 4.94 (dq, $J$ = 20.0, 2.5 Hz, 1H, H-1), 7.15 (ddd, $J$ = 8.5, 4.5, 3.0 Hz, 1H, HAr), 7.18 (d, $J$ = 8.0 Hz, 1H, HAr), 7.36 (dd, $J$ = 6.5, 2.5 Hz, 1H, HAr), 7.75 (ddd, $J$ = 5.0, 2.5, 1.0 Hz, 1H, H-3); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.4 ($CH_2CH_3$), 16.5 ($CH_2CH_3$), 44.2 (C-6a), 58.8 (C-3a), 63.3 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 63.8 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 73.6 (d, $J$ = 159.0 Hz, C-1), 117.0 (d, $J$ = 22.0 Hz, CHAr), 121.8 (d, $J$ = 19.0 Hz, C*i*), 126.2 (d, $J$ = 7.0 Hz, CH), 127.4 (d, $J$ = 4.0 Hz, C*i*), 128.7 (CHAr), 157.9 (d, $J$ = 250.0 Hz, C*i*-F), 162.4 (d, $J$ = 13.0 Hz, C-3), 170.9 (d, $J$ = 6.0 Hz, C-4), 175.0 (d, $J$ = 15.0 Hz, C-6); MS-EI $m/z$ 402 $M^+$ (28), 287 (10), 373 (10), 293 (11), 265 (16), 230 (40), 203 (68), 175 (20); HRMS $C_{16}H_{18}ClFN_2O_5P$ $[M + H]^+$ 403.0620; found, 403.0615.

**Diethyl (1*RS*,3a*RS*,6a*RS*)-5-(*m*-nitrophenyl)-4,6-dioxo-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrrole-1-phosphonate (10).** Following the general procedure, AgOAc (4 mg, 0.02 mmol), *N*-(*m*-nitrophenyl)maleimide (131 mg, 0.6 mmol), acetonitrile (3 mL) and PhosMic (64 μL, 0.4 mmol) gave **10** (80 mg, 51%) as an orange oil, after column chromatography (EtOAc–MeOH 99 : 1). IR (NaCl) 2984, 1722, 1484 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.39 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 1.42 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 3.94 (ddd, $J$ = 17.5, 8.5, 2.5 Hz, 1H, H-6a), 4.19–4.33 (m, 4H, $2CH_2CH_3$), 4.48 (ddd, $J$ = 20.0, 5.0, 2.5 Hz, 1H, H-3a), 5.00 (dq, $J$ = 19.5, 2.5 Hz, 1H, H-1), 7.65–7.70 (m, 2H, H-5, H-6), 7.80 (ddd, $J$ = 19.5, 5.0, 2.5 Hz, 1H, H-1), 8.23 (m, 1H, H-2), 8.28 (m, 1H, H-4); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.3 ($CH_2CH_3$), 16.4 ($CH_2CH_3$), 44.3 (C-6a), 58.8 (C-3a), 63.3 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 64.0 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 73.3 (d, $J$ = 157.9 Hz, C-1), 121.5 (C-4′), 123.6 (C-2′), 130.1 (C-5′), 132.0 (C-6′), 132.1 (C-1′), 148.0 (C-*i*), 162.2 (d, $J$ = 12.0 Hz, C-3), 170.5 (d, $J$ = 6.0 Hz, C-4), 174.7 (d, $J$ = 6.0 Hz, C-6); MS-EI $m/z$ 395 $M^+$ (8), 269 (11), 230 (42), 203 (52), 175 (27), 160 (11), 147 (32), 109 (66); HRMS $C_{16}H_{19}N_3O_7P$ $[M + H]^+$ 396.0955; found, 306.0958.

**Methyl (1*RS*,3a*SR*,6a*RS*)-5-methyl-4,6-dioxo-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate, (15a) and methyl (1*SR*,3a*SR*,6a*RS*)-5-methyl-4,6-dioxo-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrrole-1-carboxylate (15b).** Following the general procedure, AgOAc (4 mg, 0.02 mmol), *N*-methylmaleimide (200 mg, 1.8 mmol), acetonitrile (8 mL) and methyl isocyanoacetate (109 μL, 1.2 mmol) gave a 3 : 1 mixture (GC/MS) of **15a**:**15b** (32 mg, 67%), after column chromatography (hexane–EtOAc 50 : 50). **15a** (major, H1–H3a *trans*). IR (NaCl) 3465, 2954, 1699 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 3.00 (s, 3H, $NCH_3$), 3.83 (s, 3H, $OCH_3$), 3.85 (dd, $J$ = 8.0, 3.0 Hz, H-6a), 4.28 (ddd, $J$ = 9.0, 3.0, 1.5 Hz, H-3a), 5.20 (q, $J$ = 3.0 Hz, H-1), 7.70 (m, 1H, H-3); $^{13}C$ NMR (100.6 MHz) $\delta$ 25.2 ($CH_2CH_3$), 45.2 (C-6a), 53.2 ($OCH_3$), 59.1 (C-3a), 77.9 (C-1), 162.8 (C-3), 169.7 (COO), 172.1 (C-4), 176.2 (C-6); MS-EI $m/z$ 210 $M^+$ (22), 166 (19), 153 (77), 125 (51); **15b** (minor, H1–H3a *cis*) $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 2.96 (s, 3H, $NCH_3$), 3.72 (t, $J$ = 9.0 Hz, H-6a), 3.79 (s, 3H, $OCH_3$), 4.21 (dt, $J$ = 9.0, 1.5 Hz, H-3a), 5.15 (ddd, $J$ = 9.0, 3.0, 1.5 Hz, H-1), 7.78 (m, 1H, H-3); $^{13}C$ NMR (100.6 MHz) $\delta$ 25.8 ($CH_2CH_3$), 44.5 (C-6a), 52.8 ($OCH_3$), 59.5 (C-3a), 77.0 (C-1), 162.8 (C-3), 169.1 (COO), 171.8 (C-4), 174.9 (C-6); MS-EI $m/z$ 290 $M^+$ (22), 166 (19), 153 (77), 125 (51); HRMS $C_{11}H_{20}N_2O_5P$ $[M + H]^+$ 291.1104; found, 291.1103.

**Tetraethyl 1-phosphonatemethyl-1*H*-imidazole-4-phosphonate.** A mixture of PhosMic (50 μL, 0.31 mmol) and AgOAc (2.5 mg, 0.015 mmol) in acetonitrile (2 mL) was stirred at room temperature overnight. The reaction mixture was filtered through a Celite pad and the solvent was evaporated to give the title product as an oil (71% yield). IR (NaCl) 3465, 2983,

1524, 1239 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.30 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 1.33 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 4.08–4.20 (m, 4H, $CH_2CH_3$), 4.36 (d, $J$ = 13.0 Hz, $CH_2P$), 7.64 (s, 1H, H-5), 7.74 (m, 1H, H-2); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.1 (d, $J$ = 6.0 Hz, $CH_2CH_3$), 16.5 (d, $J$ = 6.0 Hz, $CH_2CH_3$), 43.7 (d, $J$ = 158.0 Hz, $CH_2P$), 62.3 (d, $J$ = 7.0 Hz, $OCH_2$), 63.2 (d, $J$ = 7.0 Hz, $OCH_2$), 129.3 (d, $J$ = 36.5 Hz, C-5), 132.0 (C-4), 140.2 (dd, $J$ = 24.0, 2.0 Hz, C-2); MS-EI $m/z$ 354 (6), 310 (4), 281 (41), 245 (100), 218 (21), 207 (15), 162 (17), 144 (13); HRMS $C_{12}H_{25}N_2O_6P_2$ $[M + H]^+$ 355.1182; found, 355.1188.

### General procedure for the hydrogenation reaction

5% Pd/C (wet 55%) was added to a solution of a bicyclic iminophosphonate derivative in EtOH and the mixture was stirred under a hydrogen atmosphere at room temperature for 24 h. Then, the crude mixture was filtered through Celite® and the solvent was evaporated to afford a residue, which was purified by column chromatography.

**Diethyl (1*RS*,3a*RS*,6a*RS*)-5-methyl-4,6-dioxo-1,2,3,3a,4,5,6,6a-octahydropyrrolo[3,4-*c*]pyrrole-1-phosphonate.** Following the general procedure, 5% Pd/C (wet 55%) (40 mg), **1** (200 mg, 0.69 mmol) and ethanol (15 mL) gave **12** (168 mg, 84%), after column chromatography (EtOAc–MeOH 95 : 5). IR (NaCl) 3461, 2987, 1701, 1437 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.37 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 3.00 (s, $NCH_3$), 3.34–3.40 (m, 2H, H-3a and H-2), 3.49–3.55 (m, 2H, H-2 and H-6a), 3.77 (dd, $J$ = 7.0, 2.0 Hz, 1H, H-3a), 4.09–4.28 (m, 4H, $CH_2CH_3$); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.4 ($CH_2CH_3$), 16.5 ($CH_2CH_3$), 25.3 ($NCH_3$), 45.9 (C-3a), 47.9 (d, $J$ = 2.0 Hz, C-6a), 49.7 (C-3), 57.9 (d, $J$ = 155.5 Hz, C-1), 62.3 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 63.0 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 177.7 (d, $J$ = 15.5 Hz, C-6), 178.7 (C-3); MS-EI $m/z$ 290 $M^+$ (3), 153 (100), 152 (5), 138 (4), 111 (12), 83 (7), 68 (27), 67 (8); HRMS $C_{11}H_{20}N_2O_5P$ $[M + H]^+$ 291.1104; found, 291.1103.

**Diethyl (1*RS*,3a*RS*,6a*RS*)-5-cyclohexyl-4,6-dioxo-1,2,3,3a,4,5,6,6a-octahydropyrrolo[3,4-*c*]pyrrole-1-phosphonate.** Following the general procedure, 5% Pd/C (wet 55%) (40 mg), **2** (200 mg, 0.56 mmol) and ethanol (15 mL) gave **13** (180 mg, 90%), after column chromatography (EtOAc–MeOH 95 : 5). IR (NaCl) 3454, 2933, 1700, 1452 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.13–1.26 (m, 3H, cyclohexyl), 1.31 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 1.32 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 1.50–1.61 (m, 2H, cyclohexyl), 1.75 (m, 2H, cyclohexyl), 2.01–2.12 (m, 3H, cyclohexyl), 3.23 (tm, $J$ = 8.0 Hz, 1H, H-3a), 3.30 (dm, $J$ = 11.0 Hz, 1H, H-3), 3.38 (dddd, $J$ = 15.0, 8.0, 1.5 Hz, 1H, H-6a), 3.46 (tm, $J$ = 10.0 Hz, 1H, H-3), 3.71 (dm, $J$ = 4.0 Hz, 1H, H-1), 3.88 (m, 1H, NCH), 4.08–4.20 (m, 4H, $CH_2CH_3$); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.4 ($CH_2CH_3$), 16.5 ($CH_2CH_3$), 24.9 ($CH_2$, cyclohexyl), 25.7 ($2CH_2$, cyclohexyl), 28.7 ($CH_2$, cyclohexyl), 45.6 (C-3a), 46.7 (C-6a), 50.0 (C-3), 52.1 (NCH), 58.0 (d, $J$ = 156.5 Hz, C-1), 62.2 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 63.1 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 177.7 (d, $J$ = 15.5 Hz, C-6), 178.7 (C-3); MS-EI $m/z$ 358 (1), 222 (14), 221 (3), 139 (55), 111 (10), 83 (10), 68 (39), 67 (11); HRMS $C_{16}H_{28}N_2O_5P$ $[M + H]^+$ 359.1730; found, 359.1732.

**Diethyl (1*RS*,3a*RS*,6a*RS*)-4,6-dioxo-5-phenyl-1,2,3,3a,4,5,6,6a-octahydropyrrolo[3,4-*c*]pyrrole-1-phosphonate.** Following the general procedure, 5% Pd/C (wet 55%) (40 mg), **4** (200 mg, 0.57 mmol) and ethanol (15 mL) gave **14** (175 mg, 87%), after column chromatography (EtOAc–MeOH 95 : 5). IR (NaCl) 3470, 2982, 1710, 1455 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.37 (t, $J$ = 7.0 Hz, 3H, $CH_2CH_3$), 3.47–3.52 (m, 2H, H-3a and H-2), 3.58–3.69 (m, 2H, H-2 and H-6a), 3.87 (d, $J$ = 7.0 Hz, 1H, H-3a), 4.14–4.26 (m, 4H, $CH_2CH_3$), 7.30 (d, $J$ = 8.0 Hz, 2H, HAr), 7.40 (t, $J$ = 7.0 Hz, 1H, Har), 7.50 (t, $J$ = 7.0 Hz, 2 H, HAr); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.4 ($CH_2CH_3$), 16.5 ($CH_2CH_3$), 46.0 (C-3a), 47.1 (d, $J$ = 2.5 Hz, C-6a), 50.2 (C-3), 58.3 (d, $J$ = 156.5 Hz, C-1), 62.4 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 63.1 (d, $J$ = 7.0 Hz, $CH_2CH_3$), 126.2 (2CHAr), 128.7 (CHAr), 129.1 (2CHAr), 131.8 (C-*i*), 176.7 (d, $J$ = 15.5 Hz, C-6), 177.7 (C-3); MS-EI $m/z$ 352 (3), 216 (14), 215 (100), 214 (49), 119 (33), 111 (20), 95 (16), 83 (18); HRMS $C_{16}H_{22}N_2O_5P$ $[M + H]^+$ 353.1261; found, 353.1264.

### Theoretical calculations

Initial geometries were obtained using the PCMODEL program.[18] Further geometry optimizations were carried out using the Gaussian 03 suite of programs on a Compaq HPC320 computer,[19] at Becke's three-parameter hybrid functional with the Lee, Yang and Parr correlation functional (B3LYP) level,[20] using the 6-31+G(d) basis set.[21] Transition states were determined for models that include a silver cation bound to acetonitrile. The LANL2DZ basis,[22] in conjunction with the effective core potential for inner electrons, was used for the metal cation. The nature of the stationary points was confirmed by inspection of the vibrational frequencies. Intrinsic reaction coordinate calculations[23] were carried out to check the connection between the transition states and the minimum energy structures. To further check the relative stabilities of transition states and correct the potential uncertainties of B3LYP calculations,[24] single-point calculations were performed using two strategies. First, M06L[25] calculations as implemented in Gaussian, where the silver cation was treated using the triple-zeta quality basis set and effective core potential described in ref. 26. Second, calculations were carried out with the ADF2009[27] program combining the local density approximation with Becke and Perdew (BP)[28] nonlocal corrections. Triple-$\zeta$ and polarization (TZP) basis sets were used for valence electrons of all atoms, and the inner core shells of C, N, O, P and Ag were treated by the frozen core approximation. The ZORA formalism[29] with corrected core potentials was used to account for relativistic corrections.

## Acknowledgements

Financial support from the Ministerio de Ciencia e Innovación (MICINN), Spain (Projects CTQ2009-12520-C03-03 and CTQ2009-07021/BQU), and the Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR), Generalitat de Catalunya (Grants 2009SGR-203 and 2009-SGR-1111) is gratefully acknowledged. We are deeply grateful to Dr Santiago Vázquez

for his invaluable advice and help in carrying out molecular calculations.

## Notes and references


1 (*a*) A. Dömling, *Chem. Rev.*, 2006, **106**, 17; (*b*) A. V. Lygin and A. de Meijere, *Angew. Chem., Int. Ed.*, 2010, **49**, 9094; (*c*) A. V. Gulevich, A. G. Zhdanko, R. V. A. Orru and V. G. Nenajdenko, *Chem. Rev.*, 2010, **110**, 5235; (*d*) M. Tobisu and N. Chatani, *Chem. Lett.*, 2011, **40**, 330.

2 For an example of the first asymmetric formal [3 + 2] cycloaddition reaction of isocyanoacetates and α,β-unsaturated ketones by means of a cooperative catalysis to access 2-pyrrolines, see: C. Arróniz, A. Gil-González, V. Semak, C. Escolano, J. Bosch and M. Amat, *Eur. J. Org. Chem.*, 2011, 3755.

3 For a review on the synthesis of 1-pyrrolines, see: M.-G. A. Shvekhgeimer, *Chem. Heterocycl. Compd.*, 2003, **39**, 405.

4 For some representative examples, see: (*a*) B. D. Morris and M. R. Prinsep, *J. Nat. Prod.*, 1999, **62**, 688; (*b*) B. Hao, W. Gong, T. K. Ferguson, C. M. Janes, J. A. Krzyckl and M. K. Chan, *Science*, 2002, **296**, 1462; (*c*) T. H. Jones, V. E. Zottig, H. G. Robertson and R. R. Snelling, *J. Chem. Ecol.*, 2003, **29**, 2721; (*d*) V. C. Clark, C. J. Raxworthy, V. Rakotomalala, P. Sierwald and B. L. Fisher, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 11617; (*e*) F. Bellina and R. Rossi, *Tetrahedron*, 2006, **62**, 7213.

5 (*a*) G. Dannhardt and W. Kiefer, *Arch. Pharm.*, 2001, **334**, 183.

6 (*a*) R. Grigg, M. I. Lansdell and M. Thornton-Pett, *Tetrahedron*, 1999, **55**, 2025. For some examples of cycloaddition reactions of azomethine ylides and maleimides, see: (*b*) R. Grigg, G. Donegan, H. Q. N. Gunaratne, D. A. Kennedy, J. F. Malone, V. Sridharan and S. Thianpatanagul, *Tetrahedron*, 1989, **45**, 1723–1746; (*c*) R. Grigg, M. Thornton-Pett, J. Xu and L.-H. Xu, *Tetrahedron*, 1999, **55**, 13841–13866. For examples of cycloadditions of azomethine ylides, generated from iminophosphonates and maleimides, see: (*d*) H. A. Dondas, Y. Durust, R. Grigg, M. J. Slater and M. A. B. Sarker, *Tetrahedron*, 2005, **61**, 10667; (*e*) D. Georgiou, V. Toutountzoglou, K. W. Muir, D. Hadjipavlou-Litina and Y. Elemes, *Bioorg. Med. Chem.*, 2012, **20**, 5103.

7 S. Padilla, J. Adrio and J. C. Carretero, *J. Org. Chem.*, 2012, **77**, 4161.

8 (*a*) E. K. Baylis, C. D. Campbell and J. G. Dingwall, *J. Chem. Soc., Perkin Trans. 1*, 1984, 2845. For a review on azaheterocyclic phosphonates and their biological activity see: (*b*) K. Moone, I. Laureyn and C. V. Stevens, *Chem. Rev.*, 2004, **104**, 6177–6215. For pharmacological applications of α-aminophosphonic acids and their derivatives, see: (*c*) *Aminophosponic and Aminophosphinic Acids: Chemistry and Biological Activity*, ed. V. P. Kukhar and H. R. Hudson, John Wiley & Sons, Chichester, UK, 2000; (*d*) F. Orsini, G. Sello and M. Sisti, *Curr. Med. Chem.*, 2010, **17**, 264–289.

9 (*a*) F. R. Atherton, C. H. Hassall and R. W. Lambert, *J. Med. Chem.*, 1986, **29**, 29; (*b*) S. De Lombaert, L. Blanchard, T. Tan, Y. Sakane, C. Berry and R. D. Ghai, *Bioorg. Med. Chem. Lett.*, 1995, **5**, 145; (*c*) P. Kafarski and B. Lejczak, *Curr. Med. Chem.: Anti-Cancer Agents*, 2001, **1**, 301.

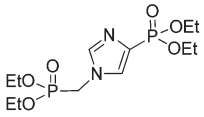
10 For the synthesis of phosphoproline, see: (*a*) U. Groth, L. Richter and U. Schöllkopf, *Tetrahedron*, 1992, **48**, 117; (*b*) F. A. Davis, S. H. Lee and H. Xu, *J. Org. Chem.*, 2004, **69**, 3774; (*c*) S. Hanessian, C. Gauchet, G. Charron, J. Marin and P. Nakache, *J. Org. Chem.*, 2006, **71**, 2760; (*d*) F. Wuggening, A. Schweifer, K. Mereiter and F. Hammerschmidt, *Eur. J. Org. Chem.*, 2011, 1870; (*e*) S. Hirata, M. Kuriyama and O. Nomura, *Tetrahedron*, 2011, **67**, 9411.

11 For a review on the silver-mediated synthesis of heterocycles, see: M. A. Corral, M. M. Dorado and I. R. Garcia, *Chem. Rev.*, 2008, **108**, 3174.

12 Other experimental conditions studied resulted in a decrease in the yield of the final product.

13 M. Sortino, F. Garibotto, V. C. Filho, M. Gupta, R. Enriz and S. Zaccino, *Bioorg. Med. Chem.*, 2011, **19**, 2823.

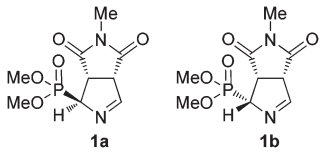
14 The cyclodimerization product of the PhosMic reaction was not observed in any of the reactions described. However, this compound was prepared as indicated in the Experimental section in order to confirm its non-formation.



.

15 Lowering the catalyst loading resulted in a considerable decrease in the yield of **1**.

16 For the sake of simplicity the numbers **1a** and **1b** have been assigned to the calculated structures.



**1a** **1b**

17 A. Sortino, F. Garibotto, V. C. Filho, M. Gupta, R. Enriz and S. Zacchino, *Bioorg. Med. Chem.*, 2011, **19**, 2823.

18 *PCMODEL, Version 8.00.1 for Windows*, Serena Software.

19 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman Jr., J. A. Montgomery, T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth,

P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez and J. A. Pople, *Theoretical calculations were carried out using Gaussian 03, Revision B.01*, Gaussian, Inc., Pittsburgh, PA, 2003.

20 (*a*) A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648; (*b*) C. Lee, W. Yang and R. G. Parr, *Phys. Rev. B: Condens. Matter*, 1988, **37**, 785.

21 (*a*) P. A. Hariharan and J. A. Pople, *Theor. Chim. Acta*, 1973, **28**, 213; (*b*) T. Clark, J. Chandrasekhar, G. W. Spitznagel and P. v. R. Schleyer, *J. Comput. Chem.*, 1983, **4**, 294; (*c*) M. J. Frisch, J. A. Pople and J. S. Binkley, *J. Chem. Phys.*, 1984, **80**, 3265.

22 (*a*) W. R. Wadt and P. J. Hay, *J. Chem. Phys.*, 1985, **82**, 284; (*b*) P. J. Hay and W. R. Wadt, *J. Chem. Phys.*, 1985, **82**, 299.

23 (*a*) C. Gonzalez and H. B. Schlegel, *J. Chem. Phys.*, 1989, **90**, 2154; (*b*) C. Gonzalez and H. B. Schlegel, *J. Phys. Chem.*, 1990, **94**, 5523.

24 (*a*) S. N. Pieniazek, F. R. Clemente and K. N. Houk, *Angew. Chem., Int. Ed.*, 2008, **47**, 7746; (*b*) R. S. Paton, S. Kim, A. G. Ross, S. J. Danishefsky and K. N. Houk, *Angew. Chem., Int. Ed.*, 2011, **50**, 10366.

25 Y. Zhao and D. G. Truhlar, *J. Chem. Phys.*, 2006, **125**, 194101.

26 N. Schultz, Y. Zhao and D. G. Truhlar, *J. Phys. Chem. A*, 2005, **109**, 4388; see also http://comp.chem.umn.edu/basissets/TZQ.basis

27 G. te Velde, F. M. Bickelhaupt, E. J. Baerends, C. Fonseca-Guerra, S. J. A. van Gisbergen, J. G. Snijders and T. Ziegler, *J. Comput. Chem*, 2001, **22**, 931; see also http://www.scm.com

28 (*a*) A. D. Becke, *Phys. Rev. A*, 1988, **38**, 3098; (*b*) J. P. Perdew, *Phys. Rev. B: Condens. Matter*, 1986, **33**, 8822.

29 E. van Lenthe, J. G. Snijders and E. J. Baerends, *J. Chem. Phys.*, 1996, **105**, 6505.

Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry


# Supporting Information

## First diastereoselective [3+2] cycloaddition reaction of diethyl isocyanomethylphosphonate and maleimides

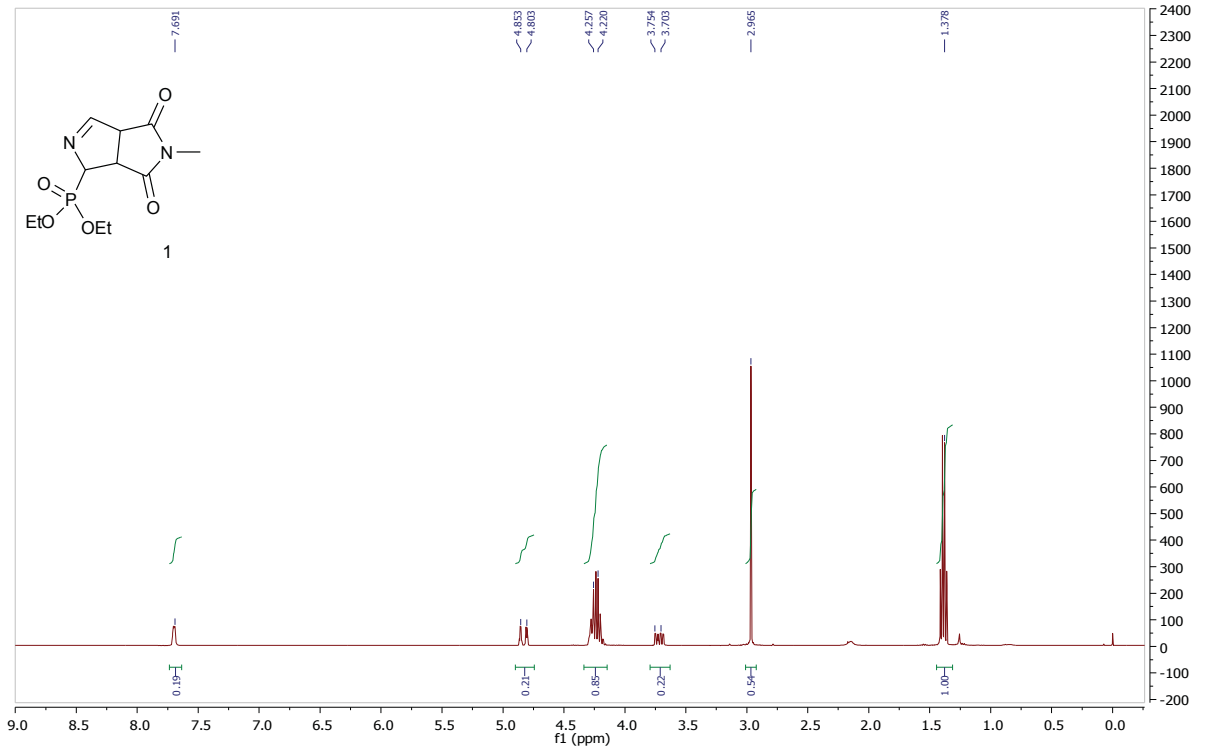
**Carlos Arróniz,[a] Juan Molina,[a] Sonia Abás,[a] Elies Molins,[b] Josep M. Campanera,[c] F. Javier Luque[c] and Carmen Escolano*[a]**

[a]*Laboratory of Organic Chemistry, Faculty of Pharmacy, and Institute of Biomedicine (IBUB), University of Barcelona, 08028 Barcelona, Spain. Fax: +34-93-402-4539; E-mail: cescolano@ub.edu*

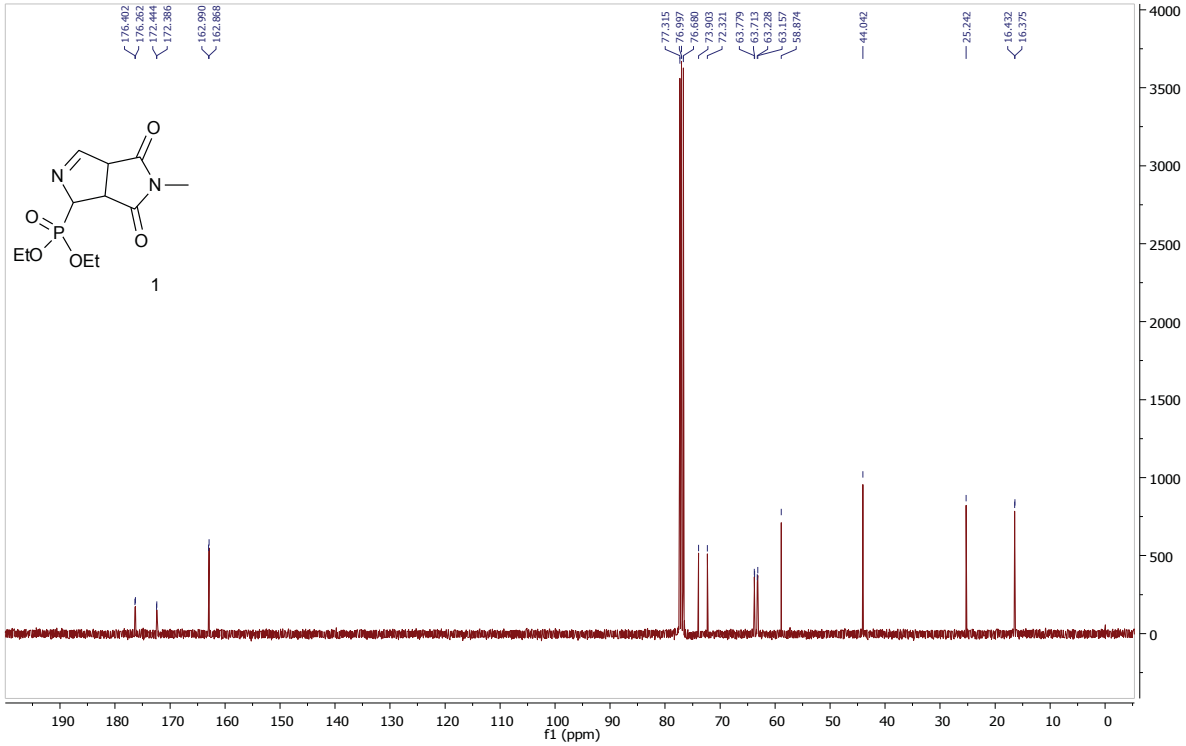
[b]*Institut de Ciència de Materials de Barcelona (CSIC), Campus UAB, 08193 Cerdanyola, Spain. E-mail: elias@icmab.es.*

[c]*Department de Fisicoquimica and Institut de Biomedicina (IBUB), Facultat de Farmàcia, Universitat de Barcelona, Campus de l'Alimentació de Torribera, Avgda. Prat de la Riba 171, 08921, Santa Coloma de Gramenet, Spain. E-mail: fjluque@ub.edu*

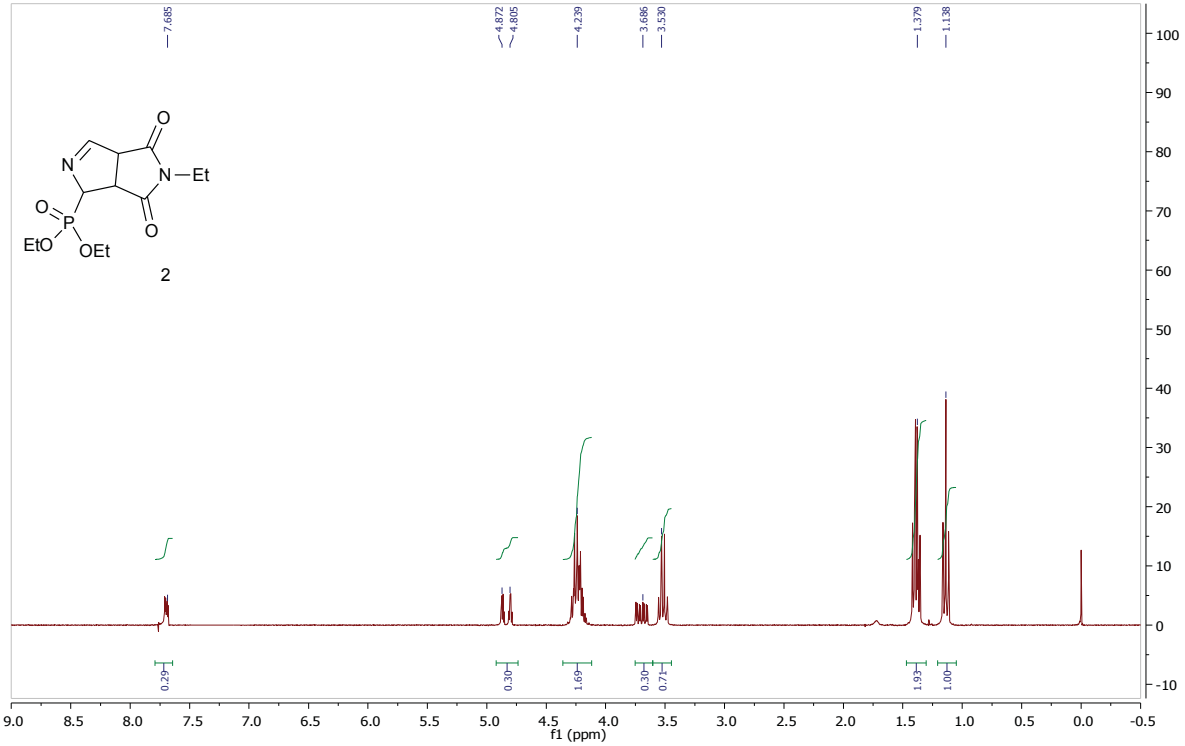
### Contents

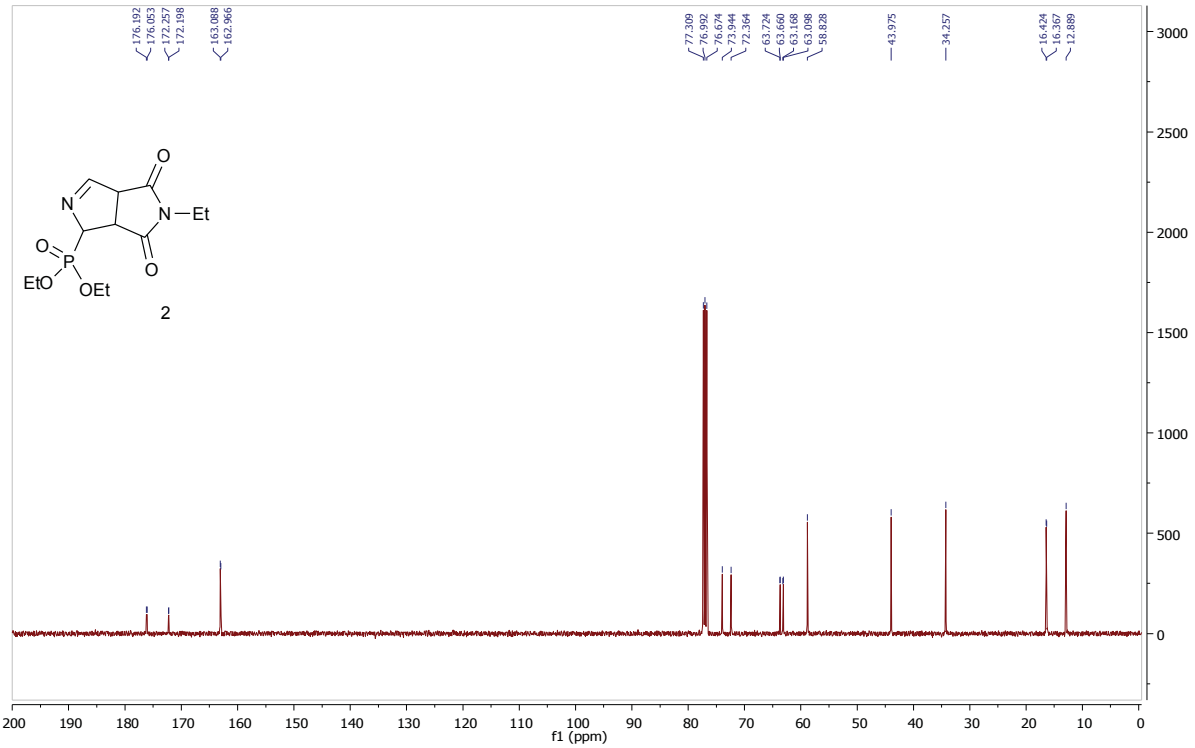


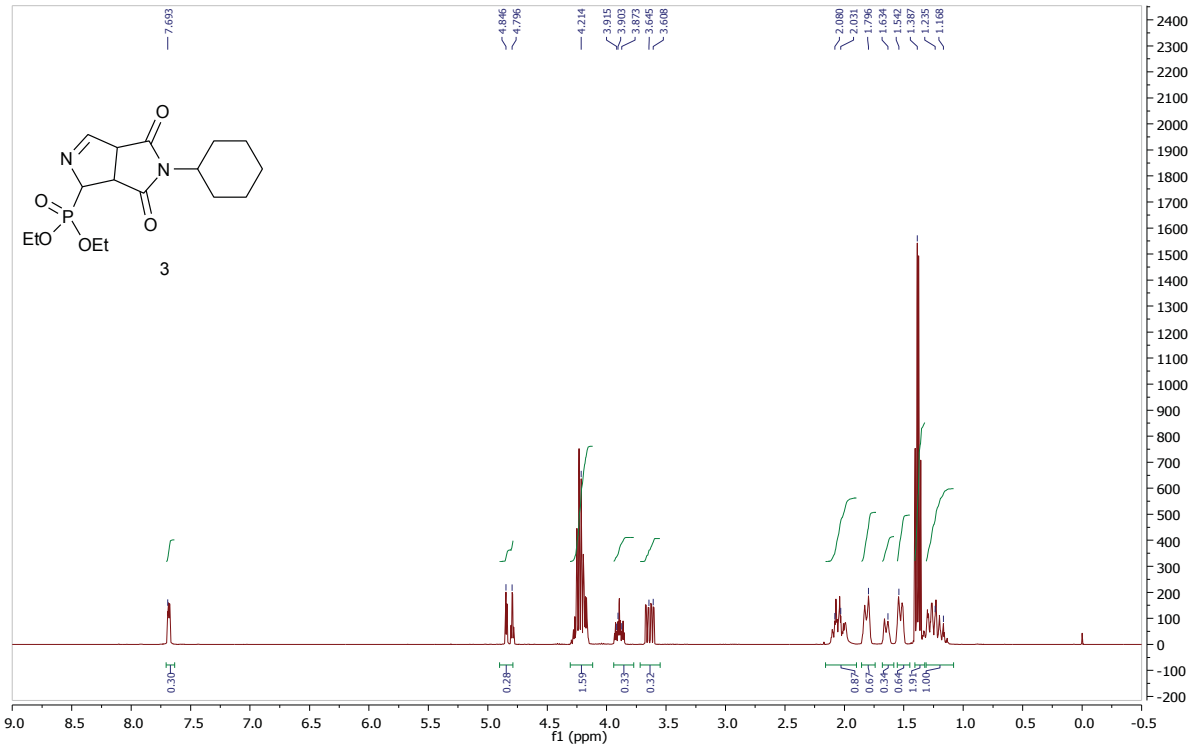

1

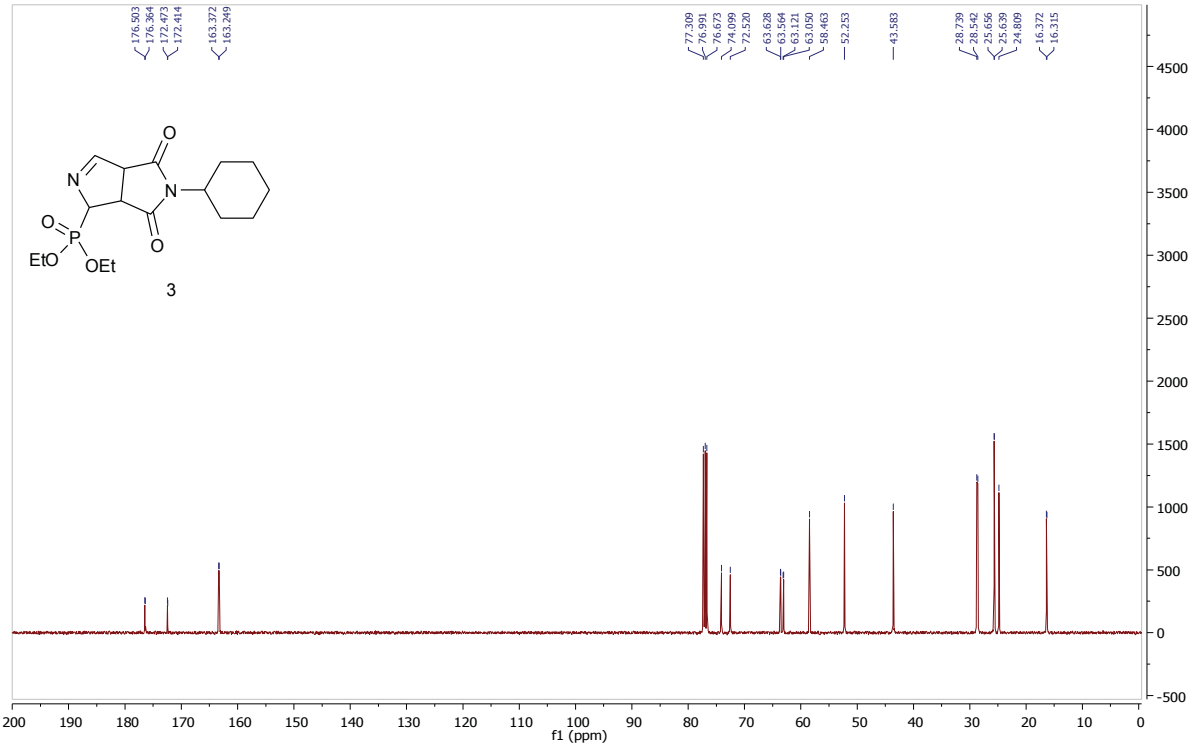


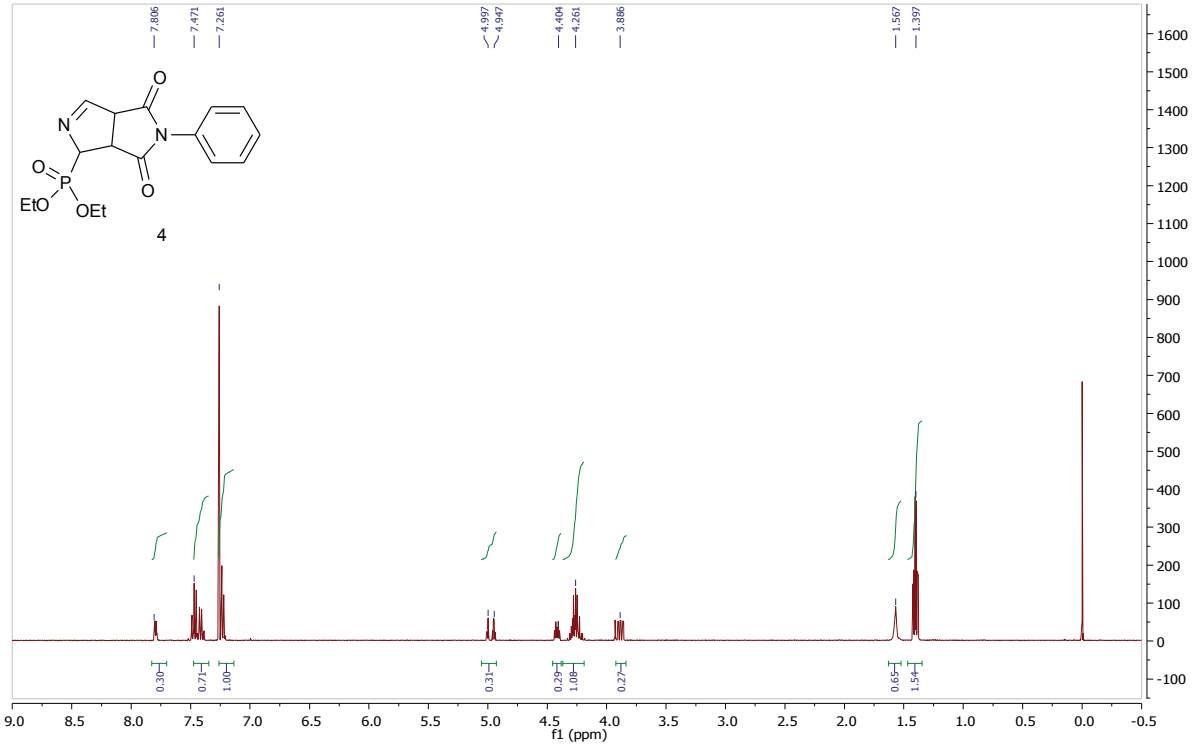

1

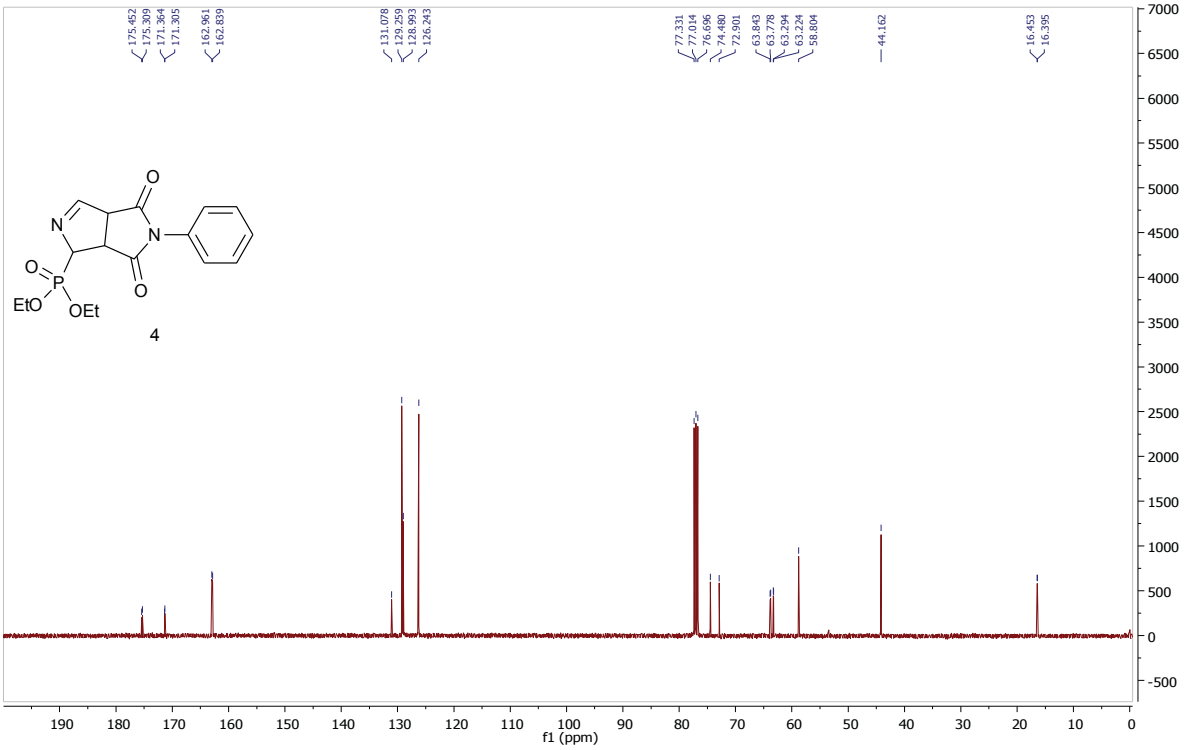


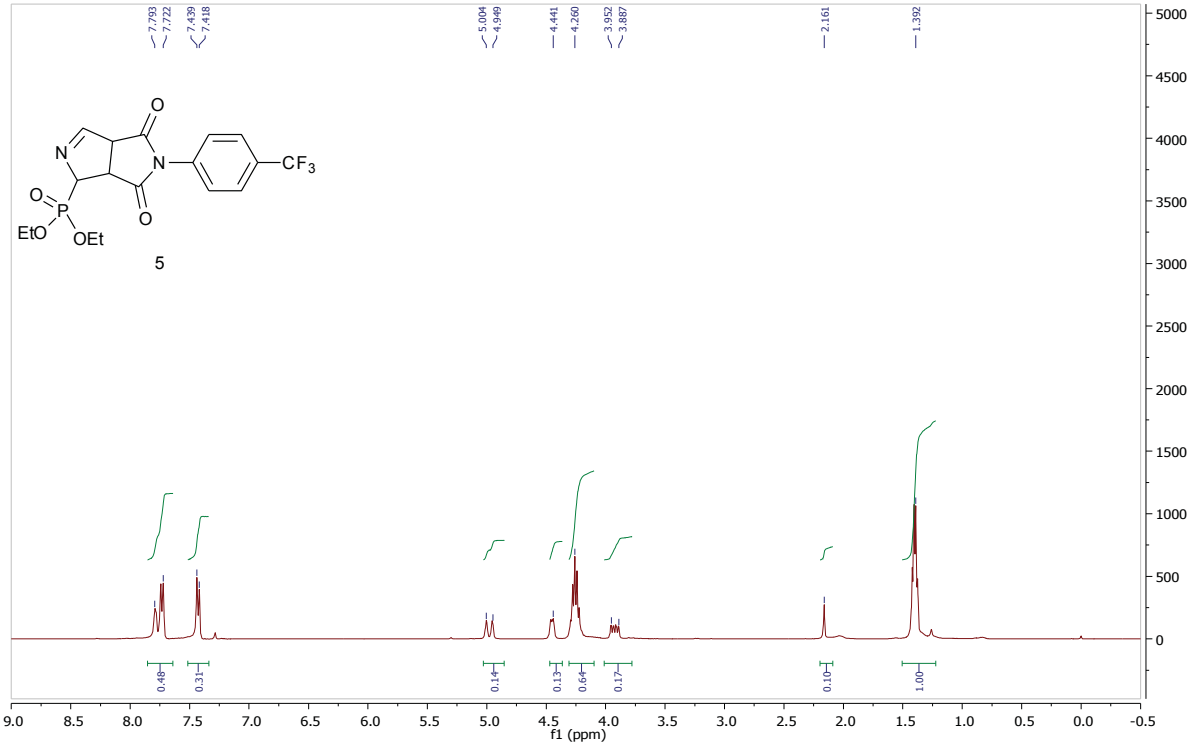



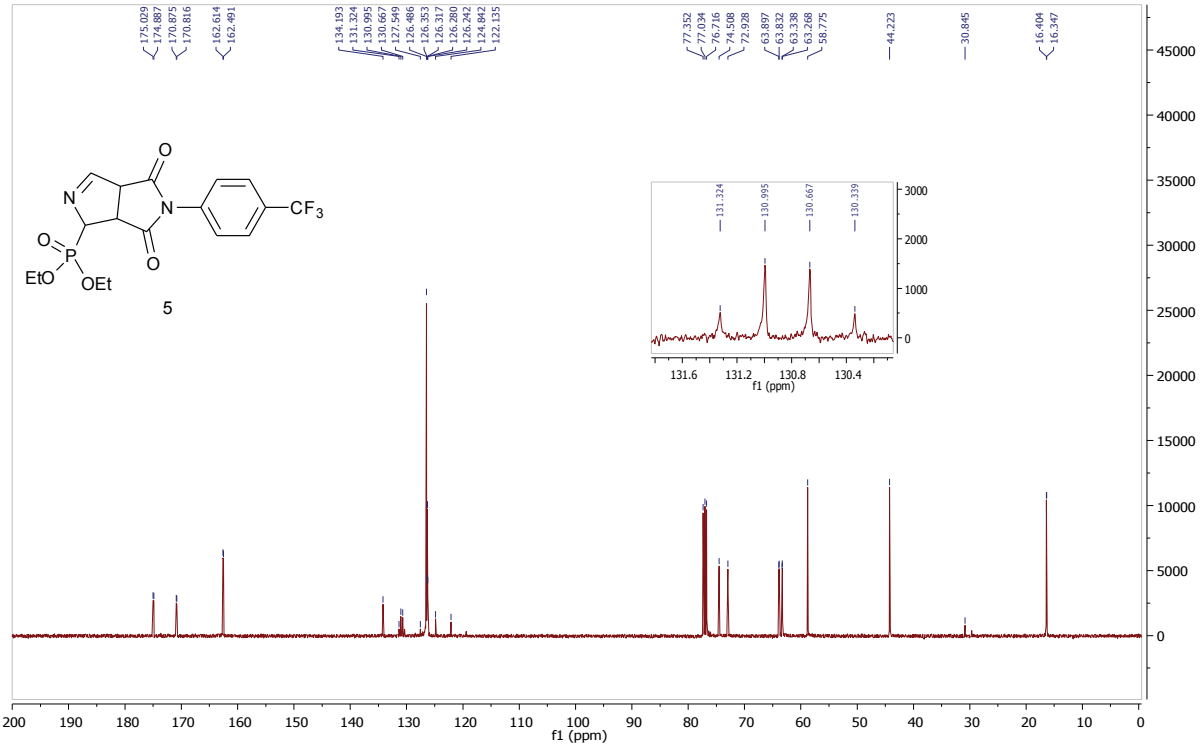


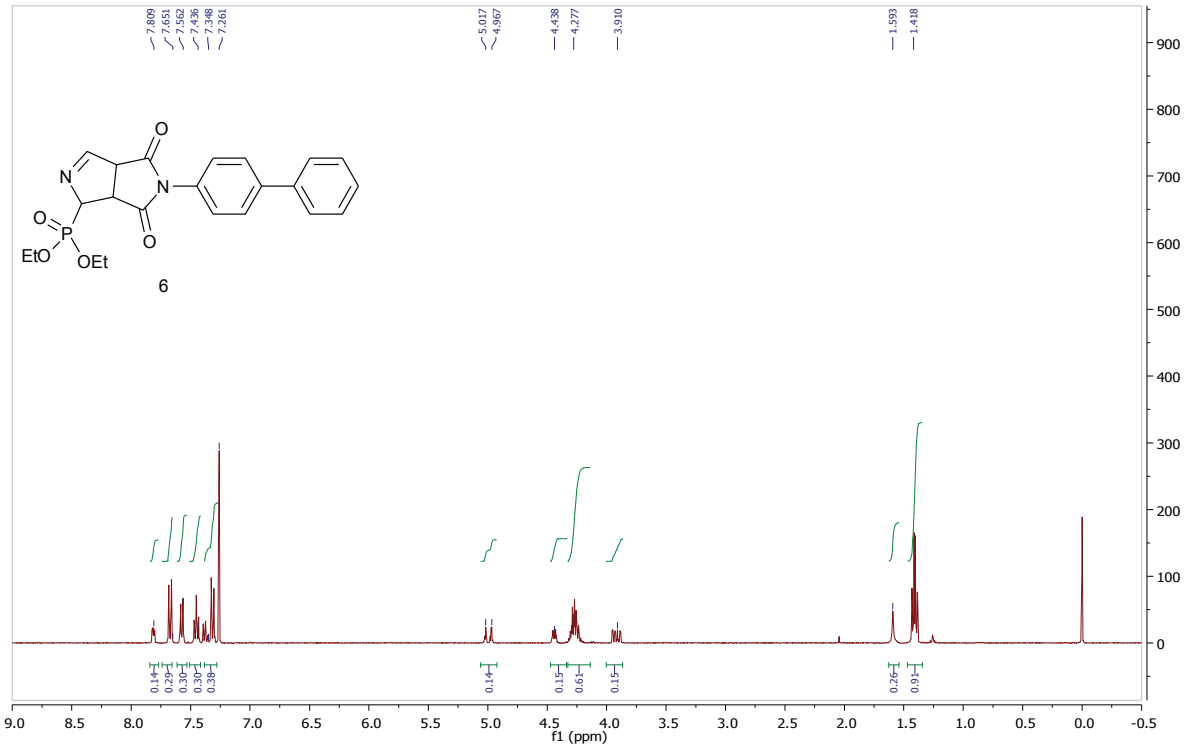



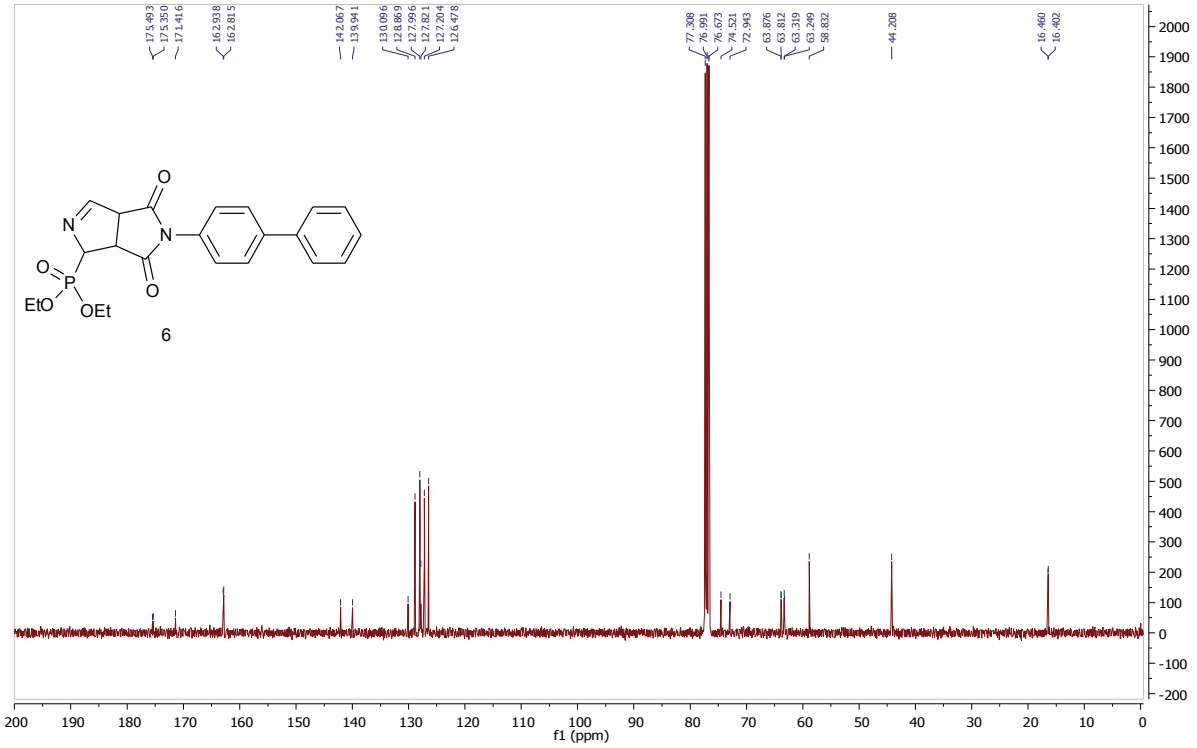


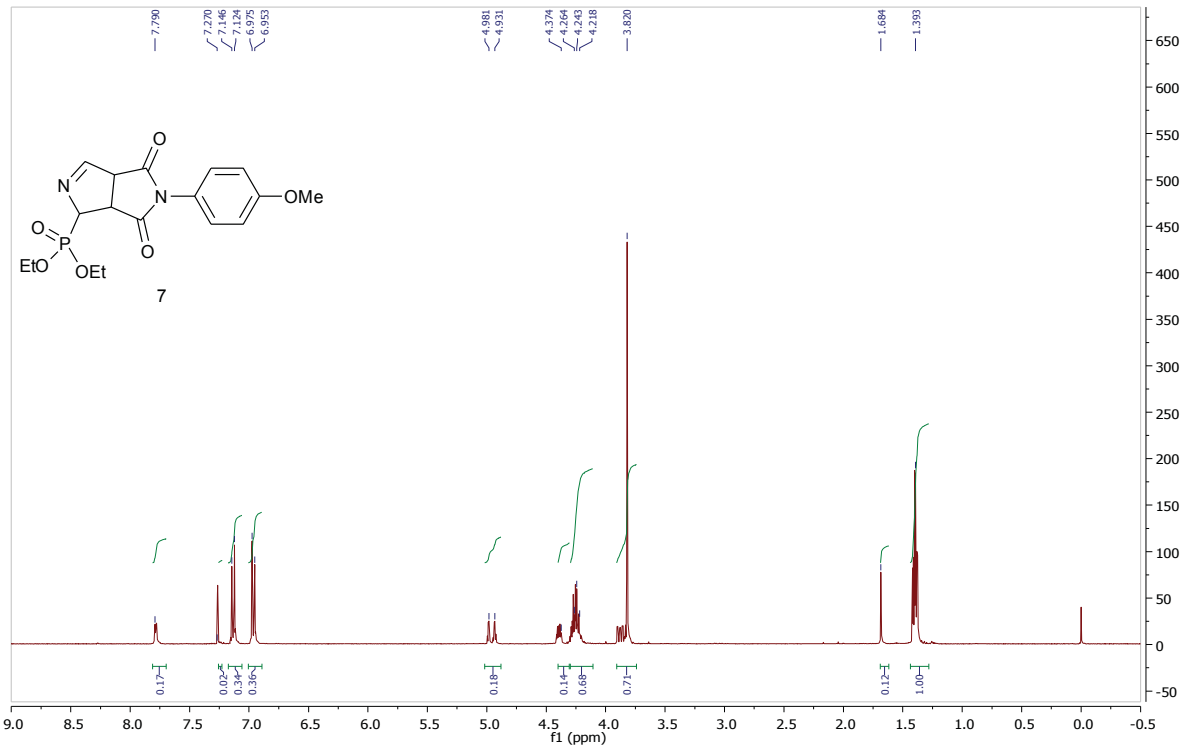



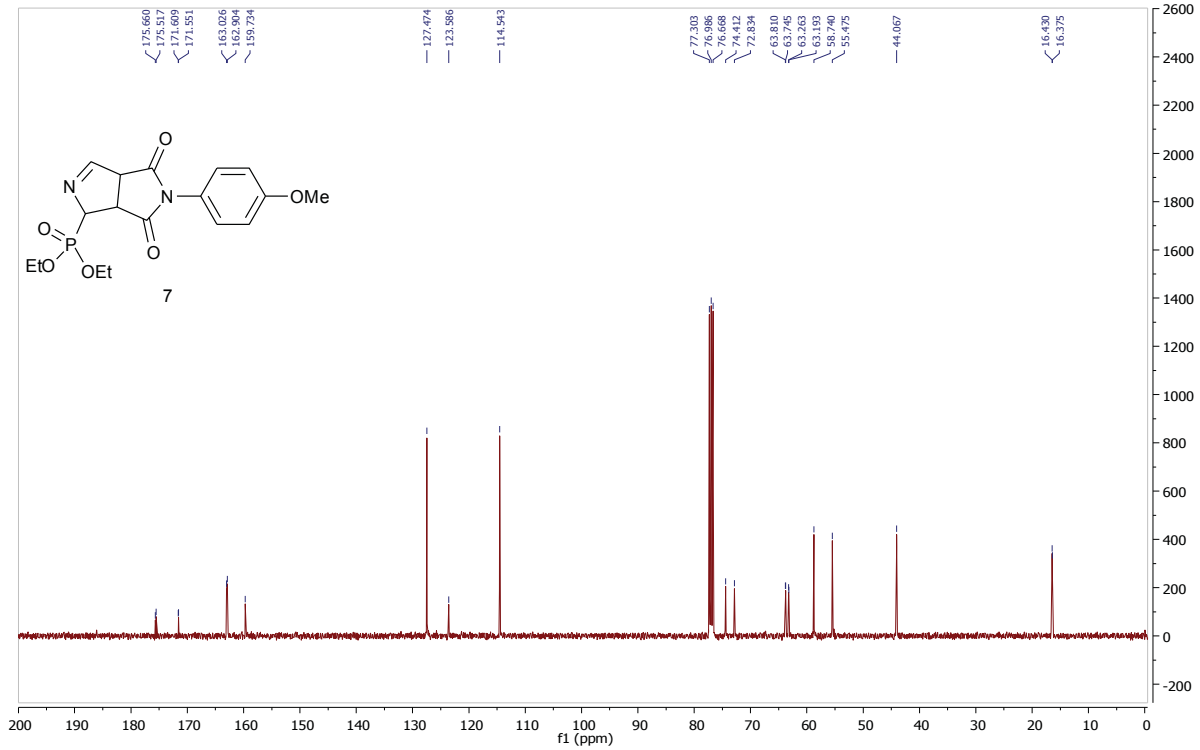

7.793
7.722
7.439
7.418
5.004
4.949
4.441
4.260
3.952
3.887
2.161
1.392
O
N
N
O
O
O
P
EtO
OEt
CF3
5
0.48
0.31
0.14
0.13
0.64
0.17
0.10
1.00
f1 (ppm)

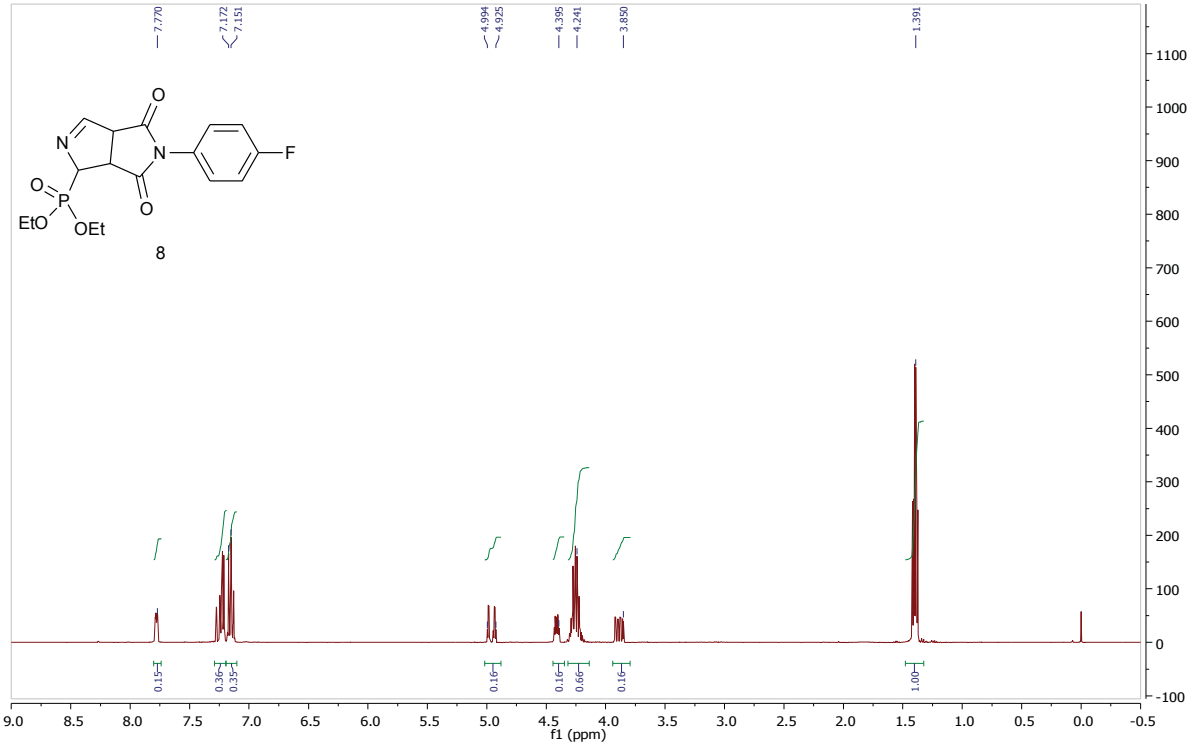

175.029
174.887
170.875
170.816
162.614
162.491
134.193
131.324
130.995
130.667
127.549
126.486
126.353
126.317
126.280
126.242
124.842
122.135
77.352
77.034
76.716
74.508
72.928
63.897
63.832
63.338
63.268
58.775
44.223
30.845
16.404
16.347
O
N
N
O
O
O
P
EtO
OEt
CF3
5
131.324
130.995
130.667
130.339
f1 (ppm)
f1 (ppm)

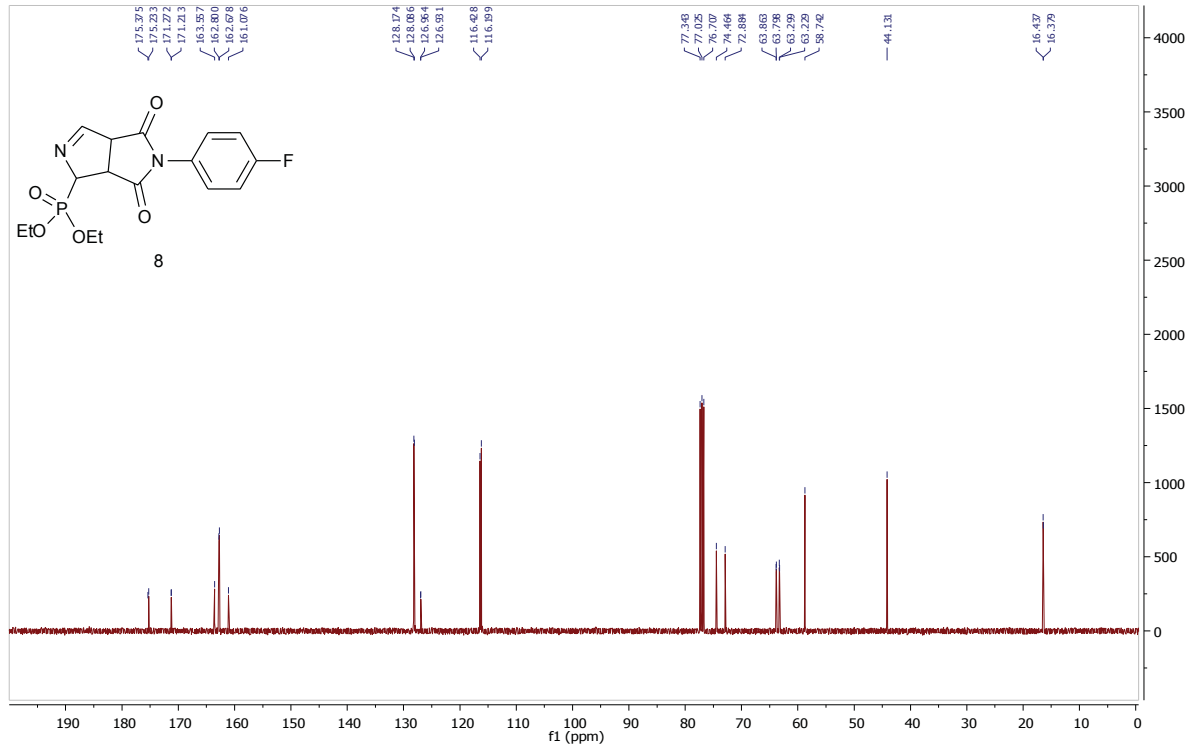


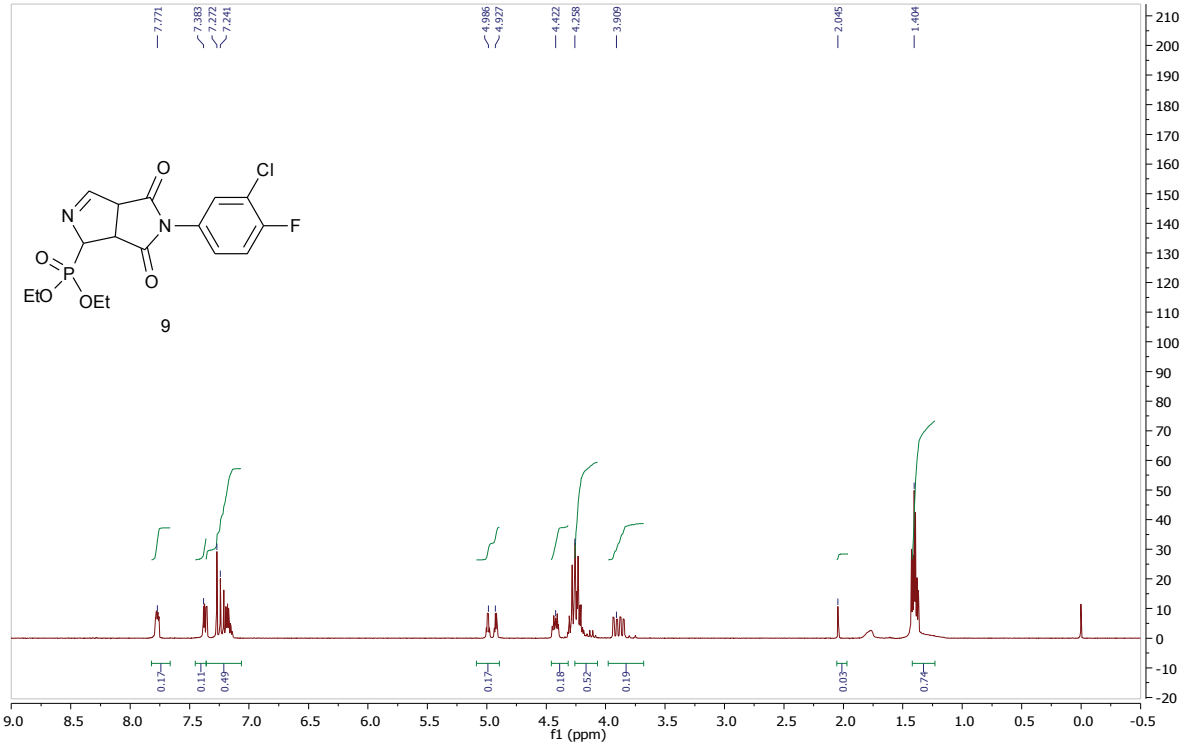



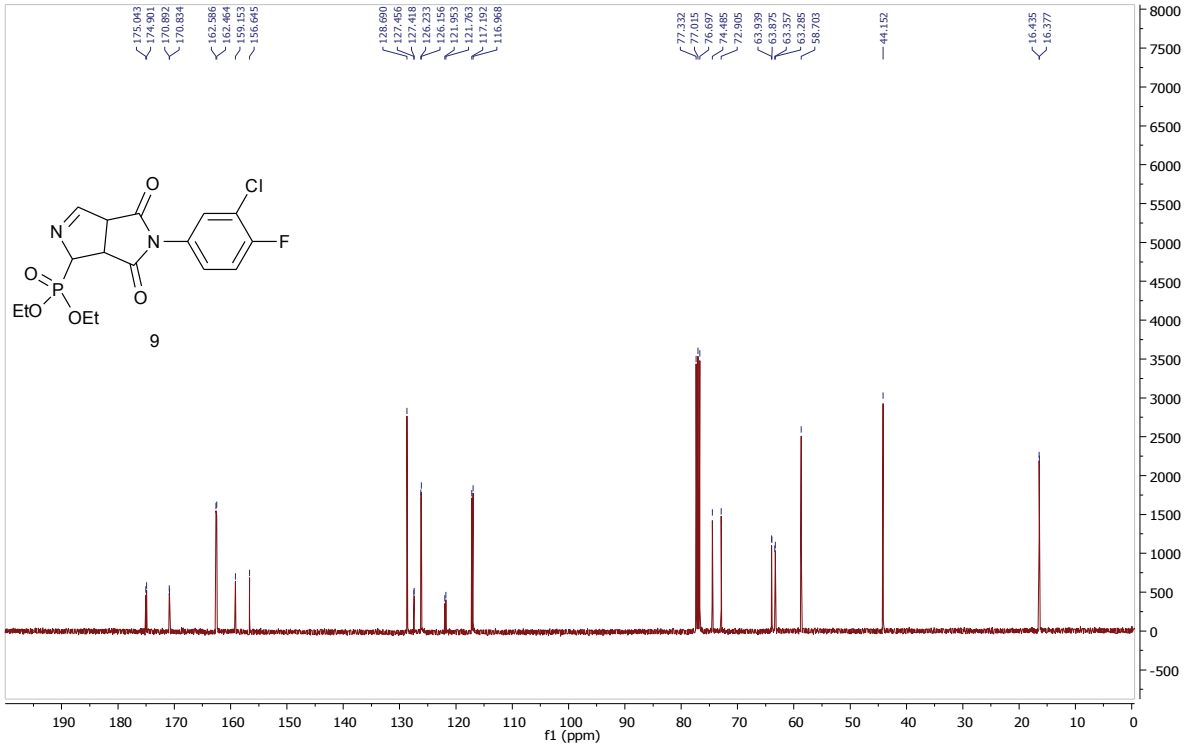


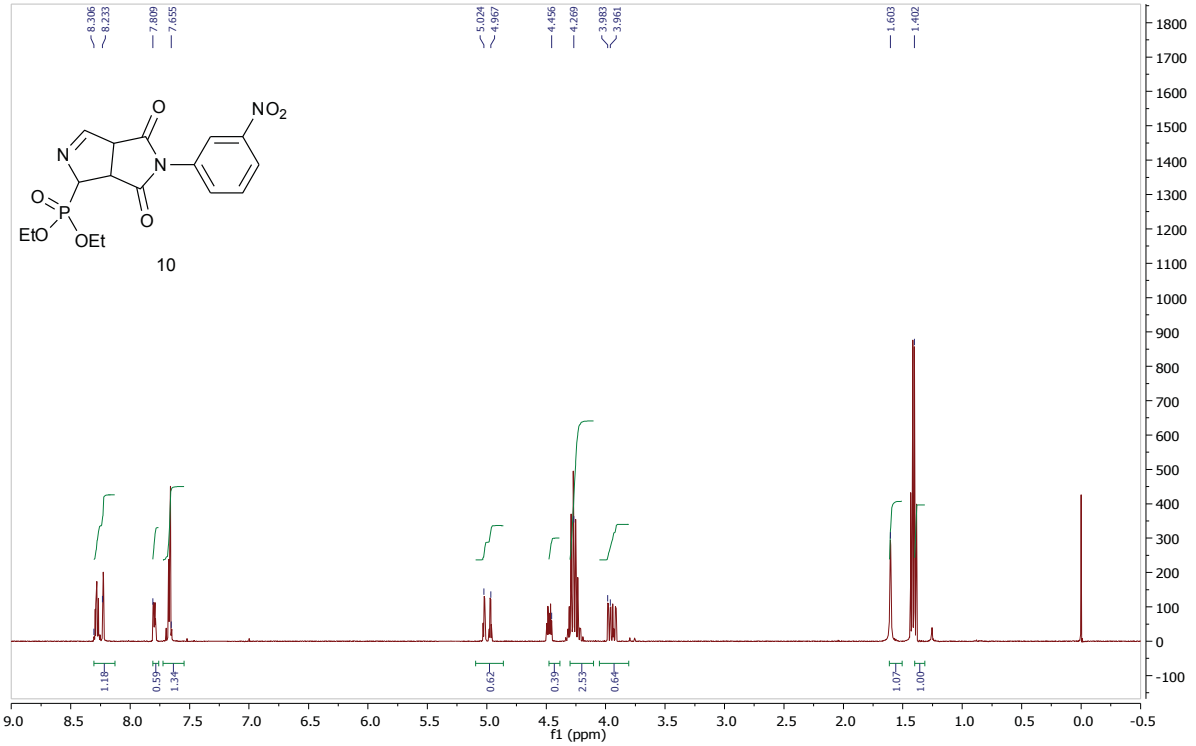



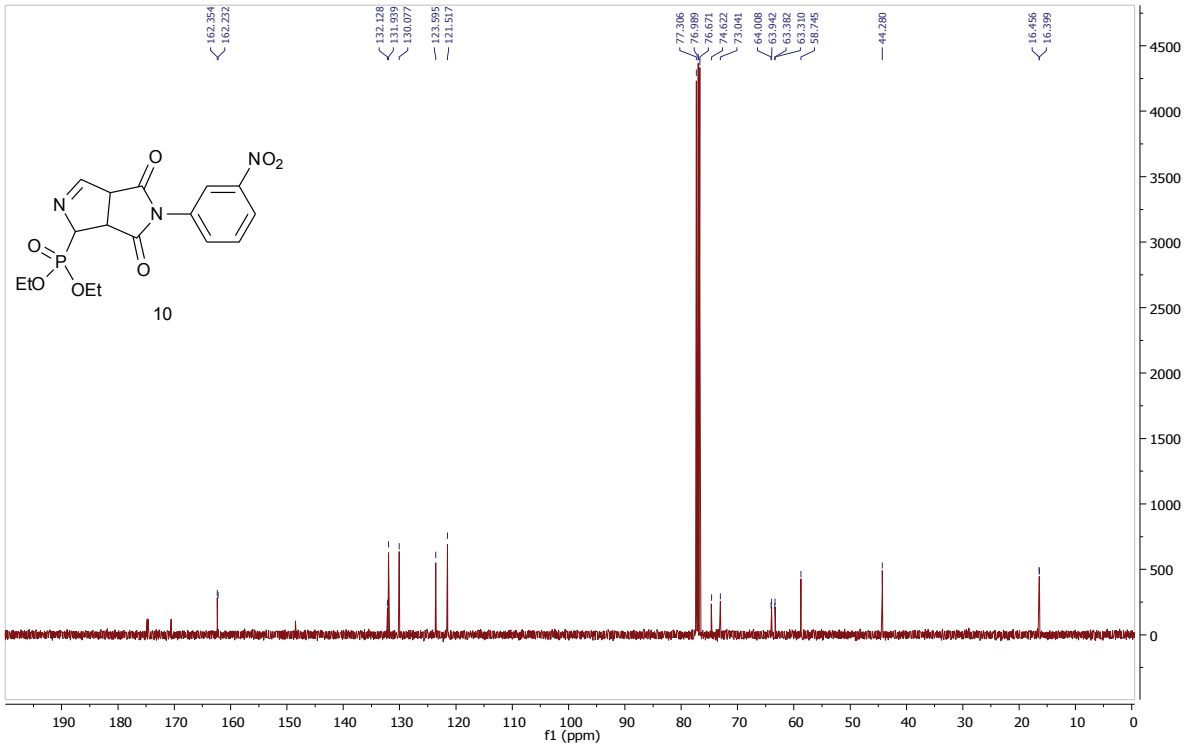


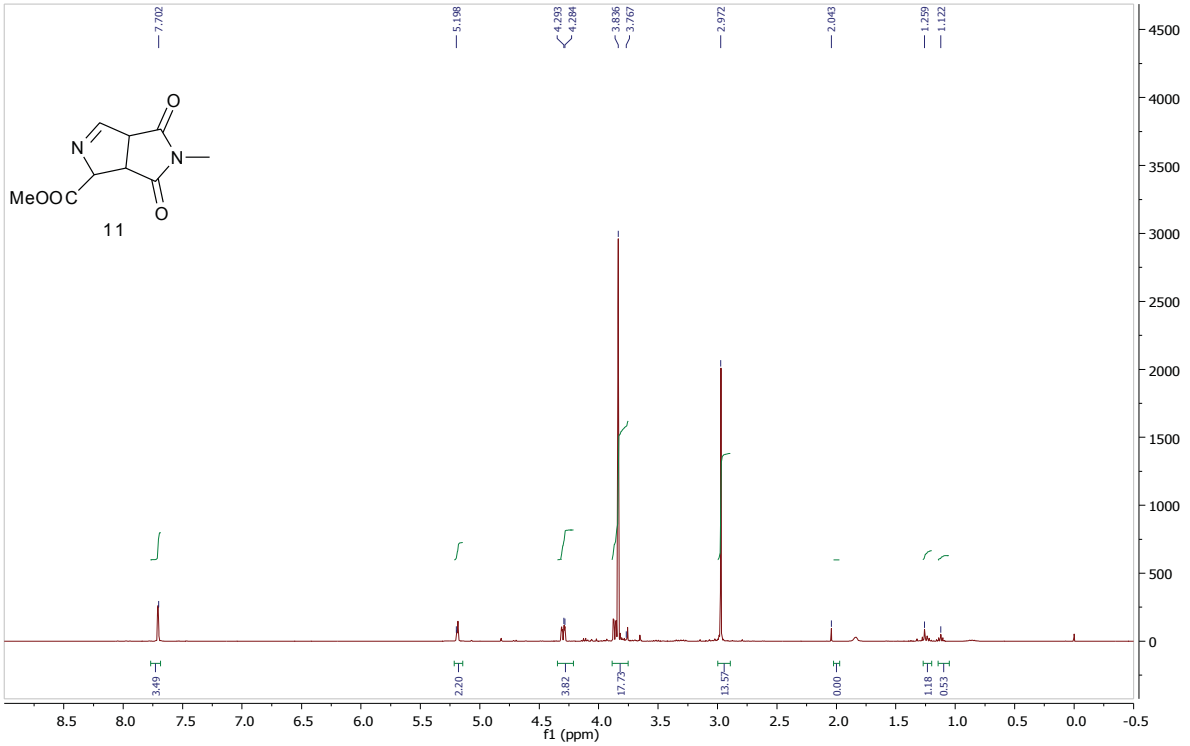



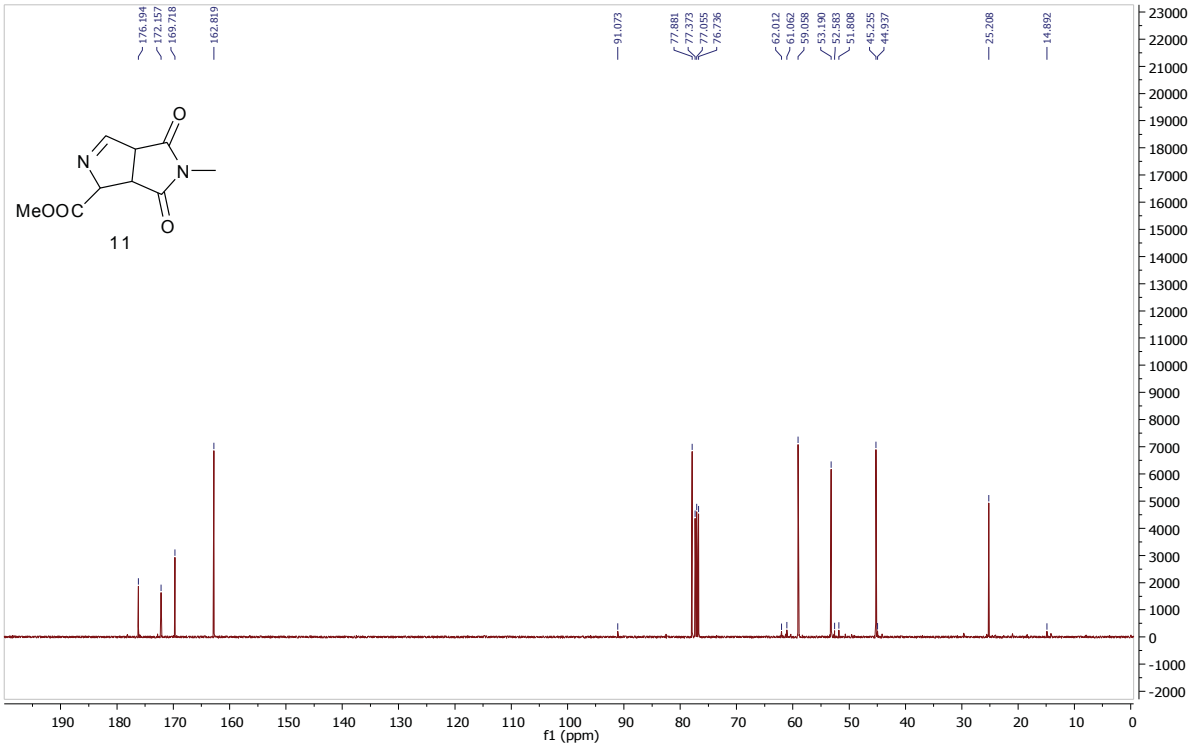


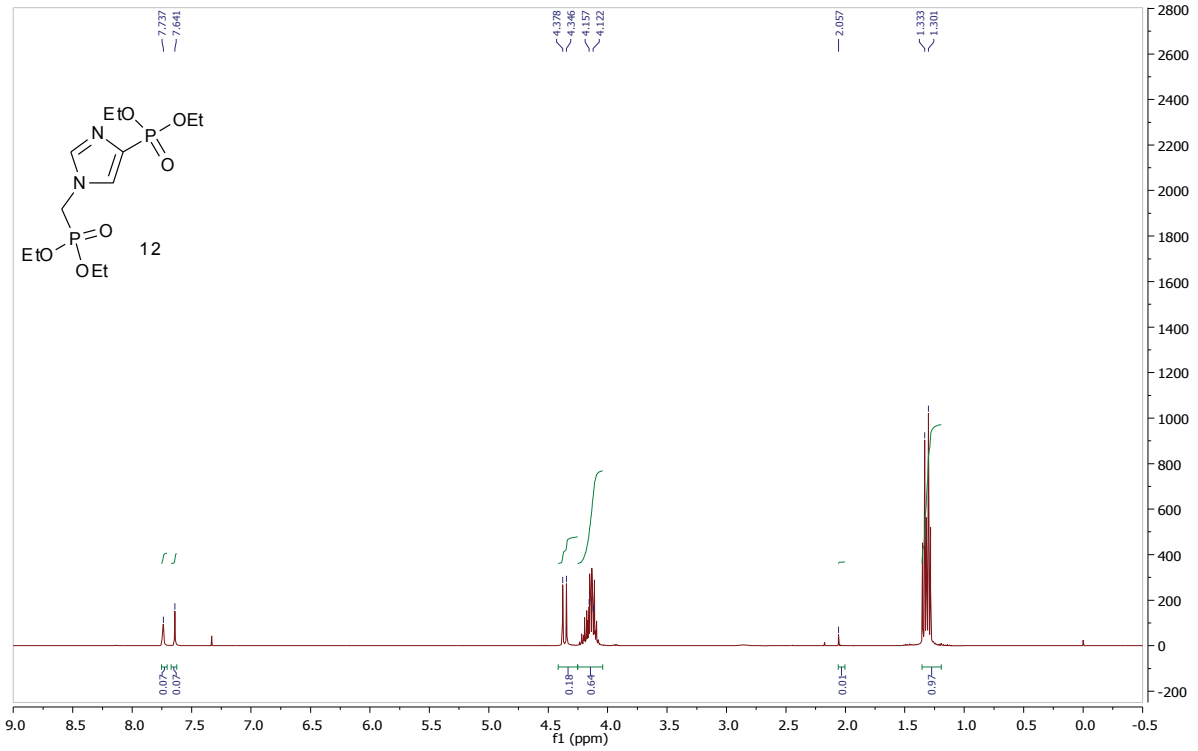



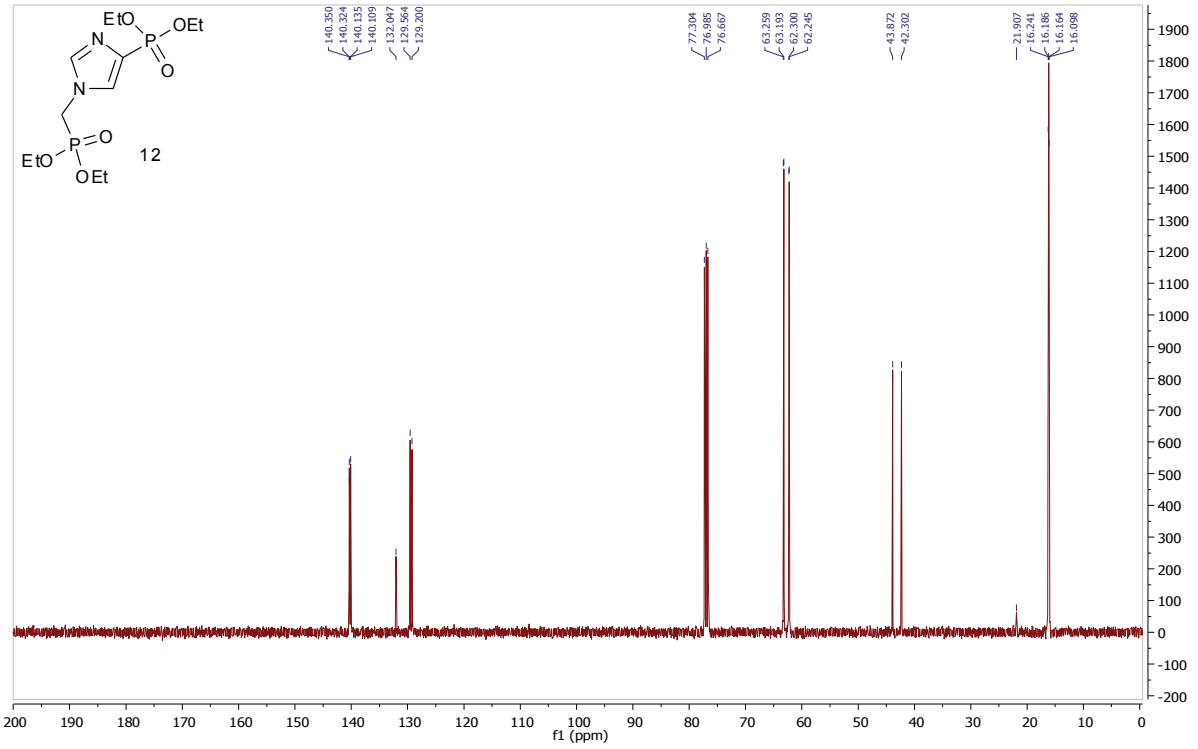


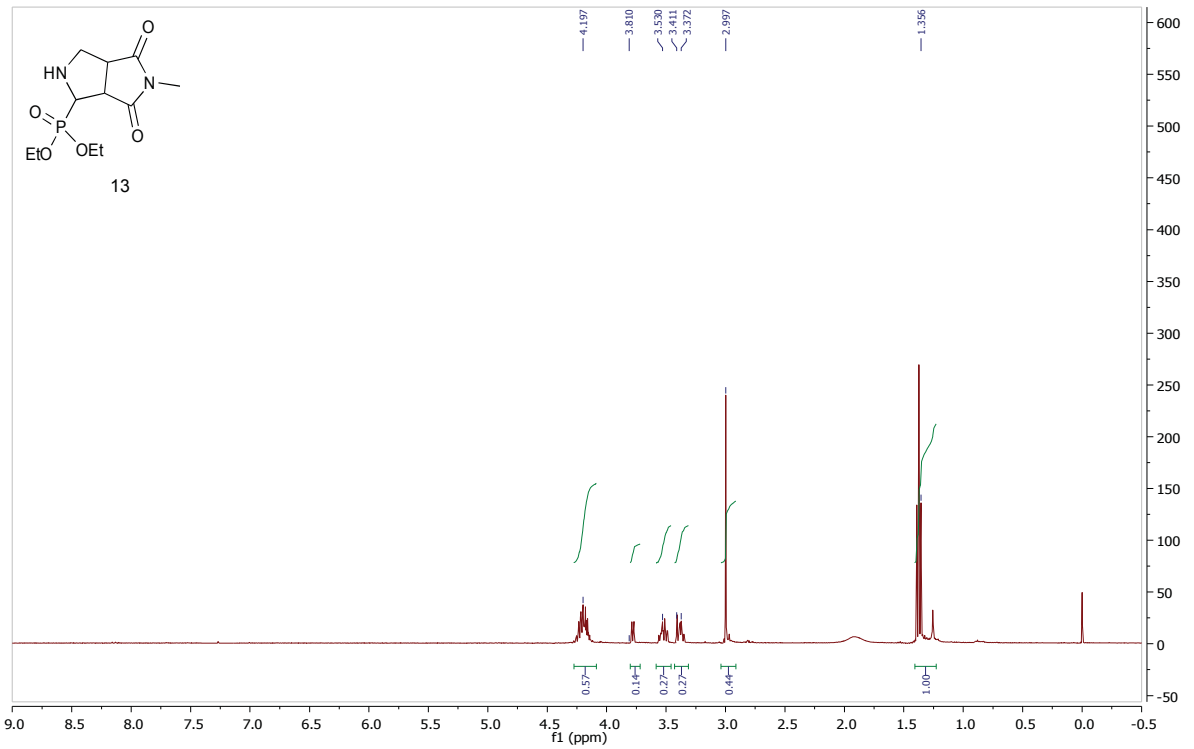



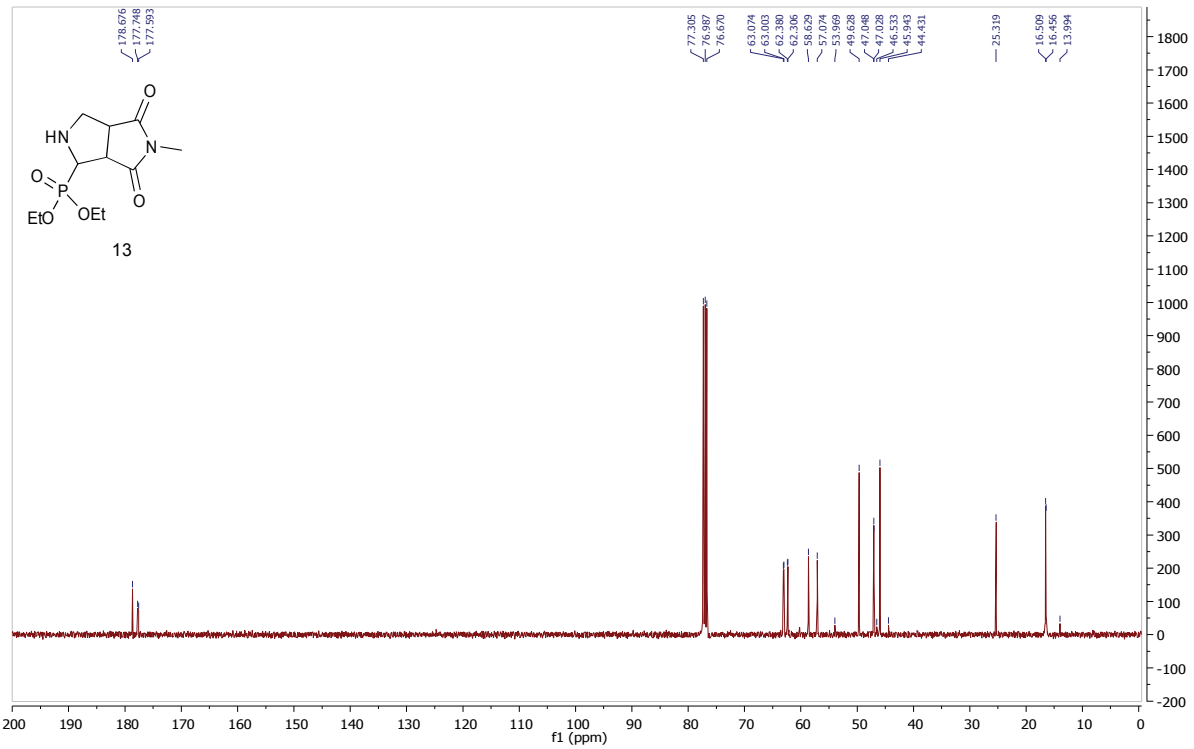


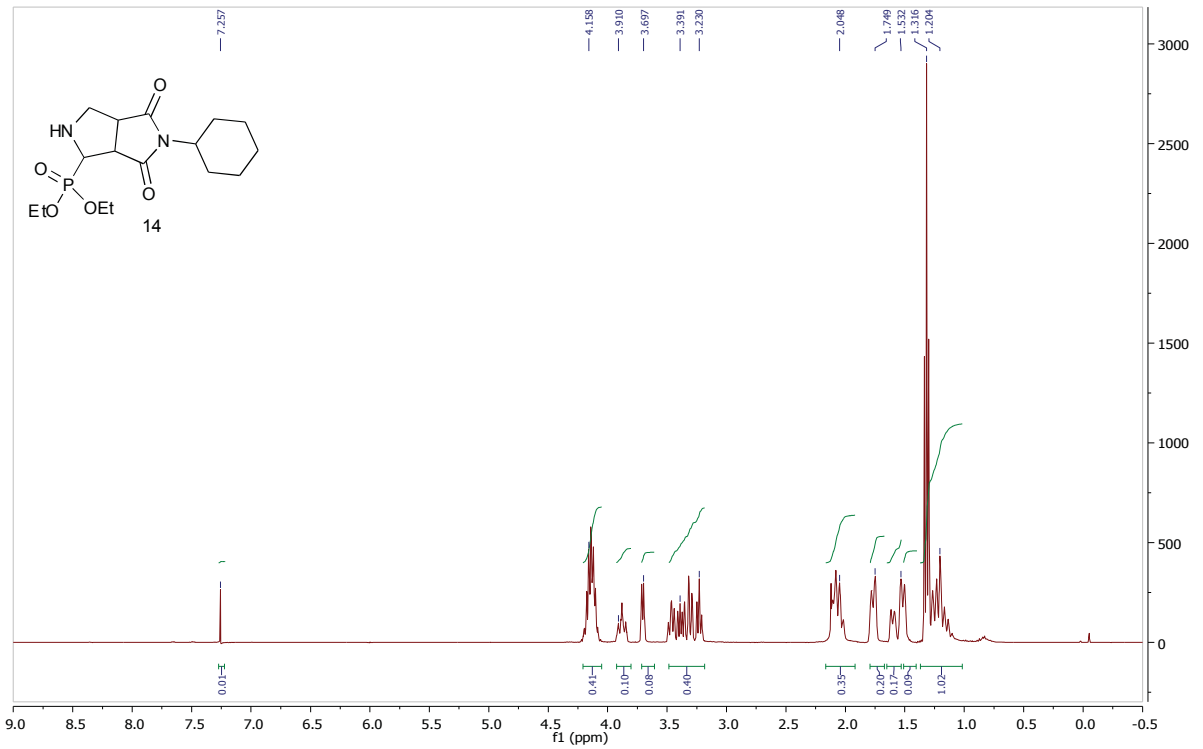



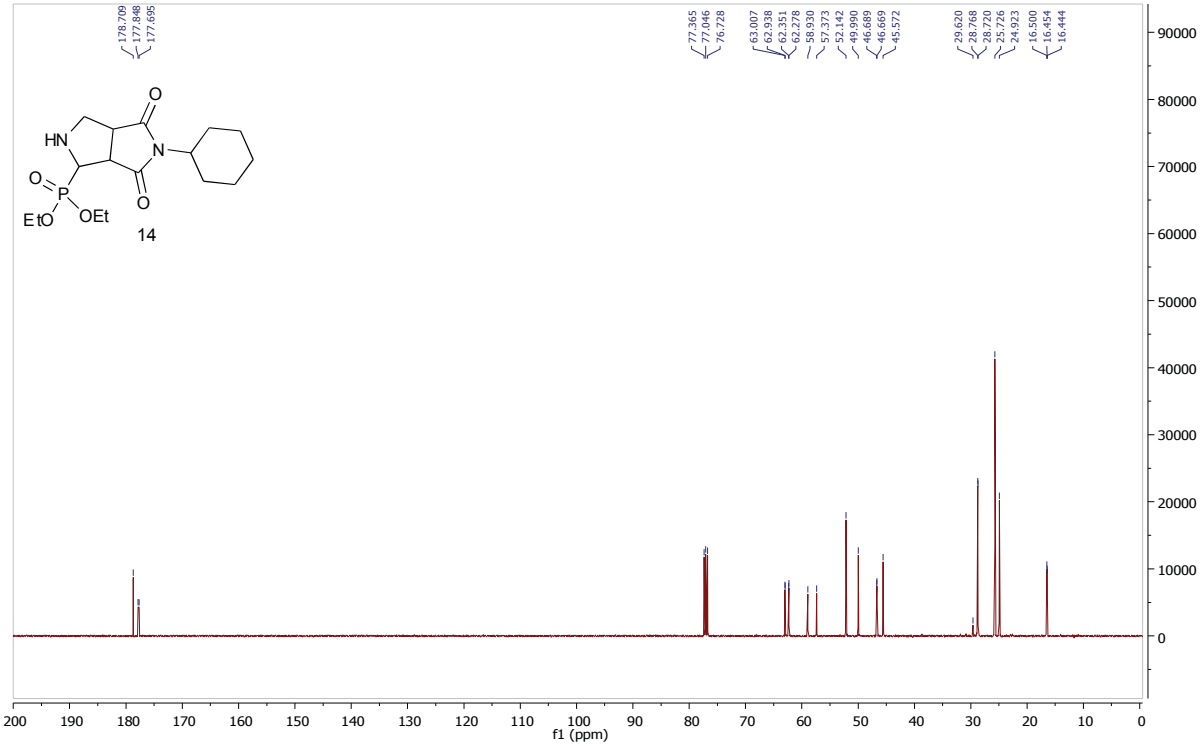


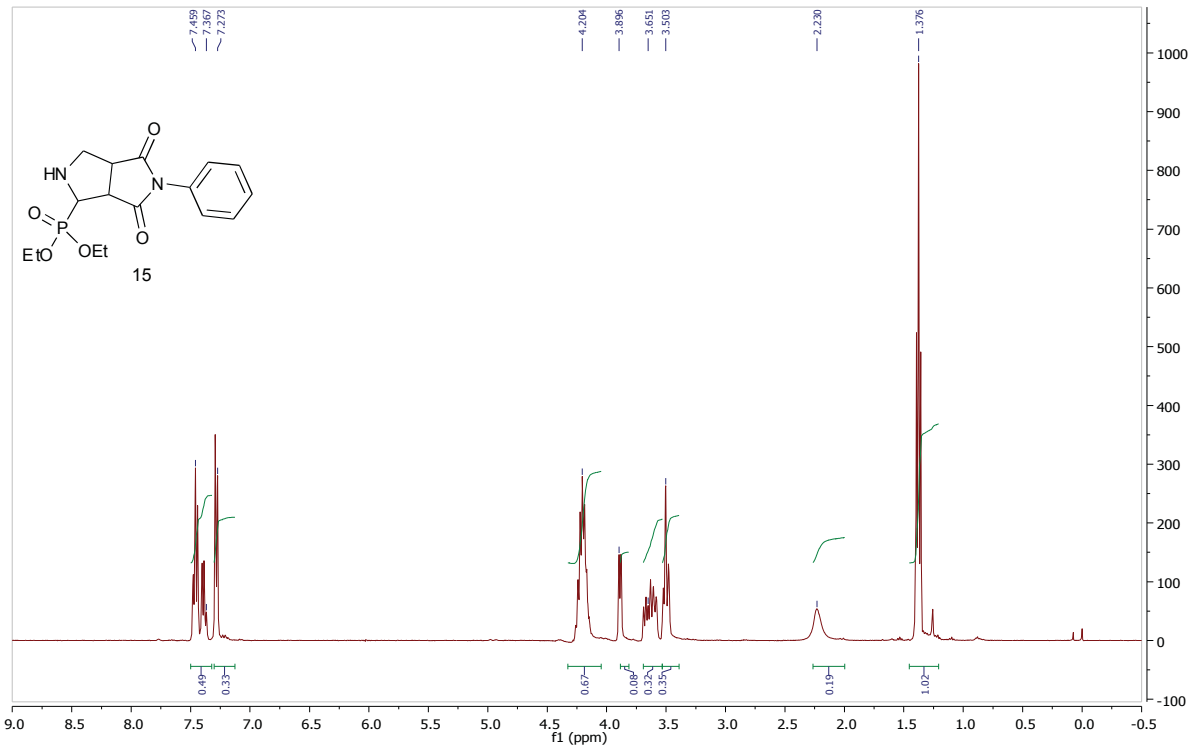



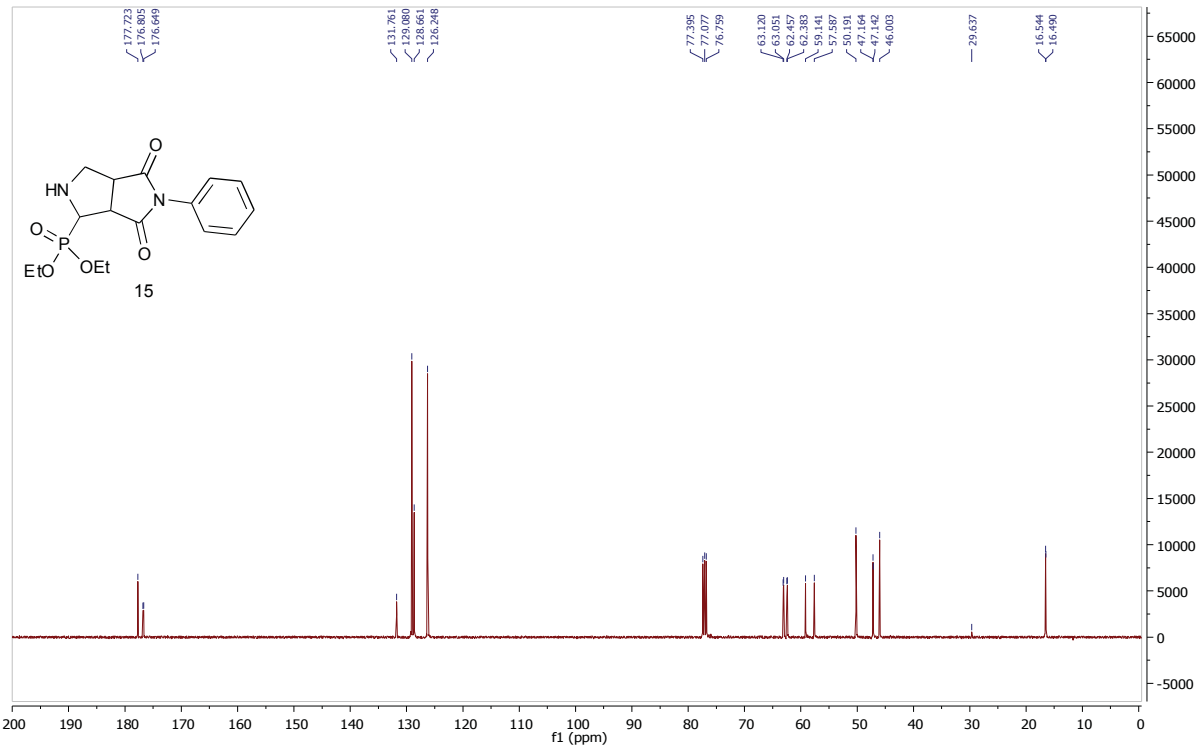






## 2. X-Ray crystallography

**Diethyl 4,6-dioxo-5-phenyl-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrrole-1-phosphonate, (4).**

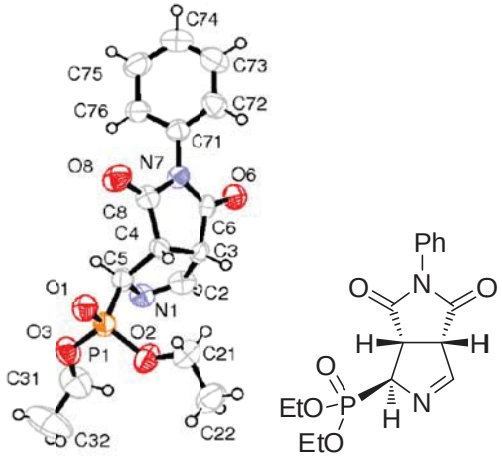



Table 1. Crystal data and structure refinement for **4**.

| | | |
|---|---|---|
| Identification code | Jb93 | |
| Empirical formula | C16 H19 N2 O5 P | |
| Formula weight | 350.30 | |
| Temperature | 294(2) K | |
| Wavelength | 0.71073 Å | |
| Crystal system | Triclinic | |
| Space group | P -1 | |
| Unit cell dimensions | a = 6.116(6) Å | α= 111.01(7)°. |
| | b = 11.264(7) Å | β= 92.49(7)°. |
| | c = 13.284(9) Å | γ = 101.66(8)°. |
| Volume | 829.9(10) Å$^3$ | |
| Z | 2 | |
| Density (calculated) | 1.402 Mg/m$^3$ | |
| Absorption coefficient | 0.195 mm$^{-1}$ | |
| F(000) | 368 | |
| Crystal size | 0.42 x 0.21 x 0.03 mm$^3$ | |
| Theta range for data collection | 1.66 to 24.98°. | |
| Index ranges | -7<=h<=7, -13<=k<=12, 0<=l<=15 | |
| Reflections collected | 3115 | |
| Independent reflections | 2918 [R(int) = 0.0595] | |
| Completeness to theta = 24.98° | 99.9 % | |
| Max. and min. transmission | 0.9942 and 0.9227 | |
| Refinement method | Full-matrix least-squares on F$^2$ | |
| Data / restraints / parameters | 2918 / 0 / 219 | |
| Goodness-of-fit on F$^2$ | 0.898 | |
| Final R indices [I>2sigma(I)] | R1 = 0.0659, wR2 = 0.1378 | |
| R indices (all data) | R1 = 0.1876, wR2 = 0.1696 | |
| Largest diff. peak and hole | 0.205 and -0.230 e.Å$^{-3}$ | |

Table 2. Atomic coordinates ( x $10^4$) and equivalent isotropic displacement parameters ($Å^2$x $10^3$) for jb93. U(eq) is defined as one third of the trace of the orthogonalized $U^{ij}$ tensor.

| | x | y | z | U(eq) |
|---|---|---|---|---|
| P(1) | -1021(3) | 3883(2) | 6326(1) | 57(1) |
| O(1) | -2033(6) | 4536(4) | 5726(3) | 62(1) |
| O(2) | 1266(6) | 4657(4) | 7066(3) | 75(1) |
| O(3) | -2592(6) | 3546(4) | 7124(3) | 71(1) |
| O(6) | 4413(6) | 193(4) | 3577(3) | 68(1) |
| O(8) | -1588(7) | 1739(4) | 2874(3) | 73(1) |
| N(1) | 526(8) | 1700(4) | 6003(4) | 60(1) |
| N(7) | 1329(7) | 770(4) | 2975(4) | 53(1) |
| C(2) | 2396(10) | 1541(5) | 5661(5) | 63(2) |
| C(3) | 3039(8) | 2014(5) | 4774(4) | 53(1) |
| C(4) | 1008(8) | 2524(5) | 4550(4) | 50(1) |
| C(5) | -547(9) | 2353(5) | 5413(4) | 55(2) |
| C(6) | 3076(8) | 875(5) | 3736(4) | 51(1) |
| C(8) | 6(9) | 1652(5) | 3378(5) | 57(2) |
| C(21) | 3107(11) | 5402(7) | 6771(5) | 88(2) |
| C(22) | 4609(11) | 6342(6) | 7701(5) | 91(2) |
| C(31) | -1996(12) | 3256(7) | 8054(5) | 93(2) |
| C(32) | -2996(16) | 3982(9) | 8979(6) | 149(4) |
| C(71) | 917(9) | -174(6) | 1877(4) | 55(1) |
| C(72) | 2620(10) | -227(6) | 1237(5) | 68(2) |
| C(73) | 2222(12) | -1097(7) | 178(5) | 82(2) |
| C(74) | 122(13) | -1899(7) | -242(5) | 82(2) |
| C(75) | -1612(11) | -1855(6) | 397(5) | 78(2) |
| C(76) | -1212(10) | -973(6) | 1482(5) | 65(2) |

Table 3. Bond lengths [Å] and angles [°] for **4**.

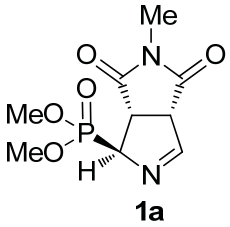
| | |
|---|---|
| P(1)-O(1) | 1.455(4) |
| P(1)-O(3) | 1.556(4) |
| P(1)-O(2) | 1.567(4) |
| P(1)-C(5) | 1.805(6) |
| O(2)-C(21) | 1.421(7) |
| O(3)-C(31) | 1.437(7) |
| O(6)-C(6) | 1.206(6) |
| O(8)-C(8) | 1.198(6) |
| N(1)-C(2) | 1.271(7) |
| N(1)-C(5) | 1.465(7) |
| N(7)-C(8) | 1.382(6) |
| N(7)-C(6) | 1.395(6) |
| N(7)-C(71) | 1.438(7) |
| C(2)-C(3) | 1.494(7) |
| C(3)-C(6) | 1.514(7) |
| C(3)-C(4) | 1.529(7) |
| C(4)-C(8) | 1.531(8) |
| C(4)-C(5) | 1.561(7) |
| C(21)-C(22) | 1.436(8) |
| C(31)-C(32) | 1.448(8) |
| C(71)-C(72) | 1.370(7) |
| C(71)-C(76) | 1.381(7) |
| C(72)-C(73) | 1.373(8) |
| C(73)-C(74) | 1.372(8) |
| C(74)-C(75) | 1.385(8) |
| C(75)-C(76) | 1.403(8) |
| | |
| O(1)-P(1)-O(3) | 111.4(2) |
| O(1)-P(1)-O(2) | 117.2(2) |
| O(3)-P(1)-O(2) | 103.7(2) |
| O(1)-P(1)-C(5) | 110.9(3) |
| O(3)-P(1)-C(5) | 106.5(3) |
| O(2)-P(1)-C(5) | 106.5(3) |
| C(21)-O(2)-P(1) | 125.4(4) |
| C(31)-O(3)-P(1) | 128.0(4) |
| C(2)-N(1)-C(5) | 109.6(5) |
| C(8)-N(7)-C(6) | 113.6(5) |
| C(8)-N(7)-C(71) | 122.8(4) |
| C(6)-N(7)-C(71) | 123.5(4) |
| N(1)-C(2)-C(3) | 116.9(5) |
| C(2)-C(3)-C(6) | 110.6(5) |
| C(2)-C(3)-C(4) | 102.5(4) |
| C(6)-C(3)-C(4) | 105.2(4) |
| C(3)-C(4)-C(8) | 105.1(4) |
| C(3)-C(4)-C(5) | 104.2(4) |
| C(8)-C(4)-C(5) | 113.8(4) |
| N(1)-C(5)-C(4) | 106.7(4) |
| N(1)-C(5)-P(1) | 111.5(4) |
| C(4)-C(5)-P(1) | 112.8(4) |
| O(6)-C(6)-N(7) | 125.4(5) |
| O(6)-C(6)-C(3) | 126.4(5) |
| N(7)-C(6)-C(3) | 108.2(4) |
| O(8)-C(8)-N(7) | 125.4(5) |
| O(8)-C(8)-C(4) | 126.7(5) |
| N(7)-C(8)-C(4) | 107.8(5) |
| O(2)-C(21)-C(22) | 112.3(6) |

| | |
|---|---|
| O(3)-C(31)-C(32) | 110.3(6) |
| C(72)-C(71)-C(76) | 121.6(6) |
| C(72)-C(71)-N(7) | 119.3(5) |
| C(76)-C(71)-N(7) | 119.1(5) |
| C(71)-C(72)-C(73) | 119.7(6) |
| C(74)-C(73)-C(72) | 120.2(6) |
| C(73)-C(74)-C(75) | 120.7(6) |
| C(74)-C(75)-C(76) | 119.4(6) |
| C(71)-C(76)-C(75) | 118.5(6) |

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for **4**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2 a^{*2} U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

| | $U^{11}$ | $U^{22}$ | $U^{33}$ | $U^{23}$ | $U^{13}$ | $U^{12}$ |
|---|---|---|---|---|---|---|
| P(1) | 48(1) | 65(1) | 59(1) | 25(1) | 7(1) | 14(1) |
| O(1) | 56(2) | 71(3) | 71(3) | 37(2) | 5(2) | 22(2) |
| O(2) | 55(2) | 88(3) | 77(3) | 35(3) | -6(2) | 0(2) |
| O(3) | 63(2) | 91(3) | 73(3) | 44(3) | 20(2) | 20(2) |
| O(6) | 57(2) | 77(3) | 70(3) | 25(2) | 7(2) | 25(2) |
| O(8) | 73(3) | 81(3) | 70(3) | 28(2) | -7(2) | 32(2) |
| N(1) | 63(3) | 66(3) | 62(3) | 33(3) | 9(2) | 17(3) |
| N(7) | 49(3) | 53(3) | 56(3) | 20(2) | 1(2) | 14(2) |
| C(2) | 78(4) | 59(4) | 59(4) | 30(3) | 4(3) | 19(3) |
| C(3) | 44(3) | 49(3) | 58(4) | 14(3) | -3(3) | 9(3) |
| C(4) | 47(3) | 49(3) | 51(3) | 16(3) | 9(3) | 13(3) |
| C(5) | 48(3) | 59(4) | 59(4) | 24(3) | 6(3) | 11(3) |
| C(6) | 39(3) | 52(3) | 62(4) | 25(3) | 4(3) | 4(3) |
| C(8) | 58(4) | 60(4) | 60(4) | 31(3) | 8(3) | 17(3) |
| C(21) | 72(5) | 104(6) | 76(5) | 25(4) | -4(4) | 13(4) |
| C(22) | 83(5) | 77(5) | 92(5) | 15(4) | -14(4) | 12(4) |
| C(31) | 109(6) | 114(6) | 90(5) | 68(5) | 28(4) | 43(5) |
| C(32) | 235(11) | 184(10) | 89(6) | 78(7) | 73(7) | 121(9) |
| C(71) | 58(4) | 56(4) | 52(4) | 23(3) | 3(3) | 12(3) |
| C(72) | 67(4) | 70(4) | 69(4) | 28(4) | 18(3) | 11(3) |
| C(73) | 94(5) | 81(5) | 70(5) | 26(4) | 25(4) | 18(4) |
| C(74) | 106(6) | 80(5) | 55(4) | 19(4) | 5(4) | 24(5) |
| C(75) | 73(4) | 75(5) | 68(5) | 12(4) | -8(4) | 10(4) |
| C(76) | 63(4) | 70(4) | 55(4) | 21(4) | 6(3) | 6(3) |

## 3. Computational details




**1a**

**COMPOUND 1a**

B3LYP/6-31+G(d): -1177.85946511 hartrees

Imaginary Frequencies: none found

Zero-point correction= 0.231518 (Hartree/Particle)

Thermal correction to Energy= 0.249234

Thermal correction to Enthalpy= 0.250179

Thermal correction to Gibbs Free Energy= 0.183860

Sum of electronic and zero-point Energies= -1177.627947

Sum of electronic and thermal Energies= -1177.610231

Sum of electronic and thermal Enthalpies= -1177.609286

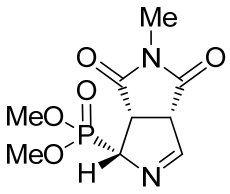
Sum of electronic and thermal Free Energies= -1177.675605

Standard orientation:

| Center Number | Atomic Number | Atomic Type | Coordinates (Angstroms) X | Y | Z |
|---|---|---|---|---|---|
| 1 | 7 | 0 | 2.938917 | 0.526998 | -0.210941 |
| 2 | 6 | 0 | 1.688650 | 1.145295 | -0.195778 |
| 3 | 6 | 0 | 0.707224 | 0.227875 | 0.523133 |
| 4 | 6 | 0 | -0.451575 | -0.275555 | -0.399142 |
| 5 | 7 | 0 | -0.179368 | -1.689232 | -0.693364 |
| 6 | 6 | 0 | 0.836486 | -2.082821 | -0.031274 |
| 7 | 6 | 0 | 1.515670 | -1.041578 | 0.842540 |
| 8 | 6 | 0 | 2.944121 | -0.732820 | 0.386840 |

| | | | | | |
|---|---|---|---|---|---|
| 9 | 6 | 0 | 4.132686 | 1.122545 | -0.798726 |
| 10 | 8 | 0 | 3.923725 | -1.442094 | 0.493578 |
| 11 | 8 | 0 | 1.443481 | 2.233287 | -0.682580 |
| 12 | 15 | 0 | -2.114917 | -0.078845 | 0.427655 |
| 13 | 8 | 0 | -2.018343 | -0.134488 | 1.909323 |
| 14 | 8 | 0 | -2.703924 | 1.296523 | -0.197881 |
| 15 | 6 | 0 | -2.400128 | 2.581510 | 0.388353 |
| 16 | 8 | 0 | -3.095948 | -1.173309 | -0.202013 |
| 17 | 6 | 0 | -3.377436 | -1.282546 | -1.612988 |
| 18 | 1 | 0 | 0.331870 | 0.733379 | 1.416125 |
| 19 | 1 | 0 | -0.494170 | 0.280263 | -1.342818 |
| 20 | 1 | 0 | 1.197360 | -3.106975 | -0.108201 |
| 21 | 1 | 0 | 1.527101 | -1.344864 | 1.894800 |
| 22 | 1 | 0 | 3.854673 | 2.098960 | -1.197660 |
| 23 | 1 | 0 | 4.907963 | 1.232736 | -0.035546 |
| 24 | 1 | 0 | 4.514604 | 0.482163 | -1.598606 |
| 25 | 1 | 0 | -3.113792 | 3.284528 | -0.044532 |
| 26 | 1 | 0 | -2.524189 | 2.542689 | 1.473899 |
| 27 | 1 | 0 | -1.380543 | 2.885107 | 0.128729 |
| 28 | 1 | 0 | -3.734702 | -0.326443 | -2.005779 |
| 29 | 1 | 0 | -2.483683 | -1.617885 | -2.146882 |
| 30 | 1 | 0 | -4.161699 | -2.036119 | -1.699385 |

---




**1b**

**COMPOUND 1b**

B3LYP/6-31+G(d): -1177.85224786 hartrees

Imaginary Frequencies: none found

Zero-point correction= 0.230956 (Hartree/Particle)

Thermal correction to Energy= 0.248877

Thermal correction to Enthalpy= 0.249821

Thermal correction to Gibbs Free Energy= 0.182828

Sum of electronic and zero-point Energies= -1177.621292

Sum of electronic and thermal Energies= -1177.603371

Sum of electronic and thermal Enthalpies= -1177.602427

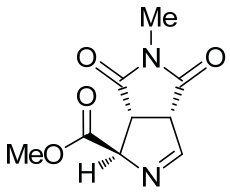
Sum of electronic and thermal Free Energies= -1177.669420

Standard orientation:

| Center Number | Atomic Number | Atomic Type | Coordinates (Angstroms) X | Y | Z |
|---|---|---|---|---|---|
| 1 | 7 | 0 | 2.191036 | -0.943152 | -0.167145 |
| 2 | 6 | 0 | 1.039223 | -0.867085 | -0.956185 |
| 3 | 6 | 0 | 0.746855 | 0.611688 | -1.243372 |
| 4 | 6 | 0 | -0.488360 | 1.317368 | -0.553654 |
| 5 | 7 | 0 | 0.045071 | 2.305594 | 0.393645 |
| 6 | 6 | 0 | 1.316207 | 2.316402 | 0.341303 |
| 7 | 6 | 0 | 1.957204 | 1.354219 | -0.638076 |
| 8 | 6 | 0 | 2.804598 | 0.281970 | 0.051556 |
| 9 | 6 | 0 | 2.728620 | -2.211624 | 0.304849 |

| | | | | | |
|---|---|---|---|---|---|
| 10 | 8 | 0 | 3.833624 | 0.459798 | 0.673926 |
| 11 | 8 | 0 | 0.450398 | -1.837208 | -1.382044 |
| 12 | 15 | 0 | -1.799215 | 0.330754 | 0.303176 |
| 13 | 8 | 0 | -2.881647 | 1.130532 | 0.933385 |
| 14 | 8 | 0 | -2.331743 | -0.721718 | -0.805282 |
| 15 | 6 | 0 | -3.616510 | -0.552848 | -1.437775 |
| 16 | 8 | 0 | -0.886494 | -0.579103 | 1.271553 |
| 17 | 6 | 0 | -1.506478 | -1.472804 | 2.220342 |
| 18 | 1 | 0 | 0.664056 | 0.714959 | -2.328040 |
| 19 | 1 | 0 | -1.056727 | 1.870154 | -1.313393 |
| 20 | 1 | 0 | 1.901202 | 2.982727 | 0.972703 |
| 21 | 1 | 0 | 2.591184 | 1.882325 | -1.360365 |
| 22 | 1 | 0 | 2.011441 | -2.692807 | 0.975764 |
| 23 | 1 | 0 | 2.915847 | -2.874857 | -0.544107 |
| 24 | 1 | 0 | 3.658536 | -2.002951 | 0.835608 |
| 25 | 1 | 0 | -3.533753 | 0.151594 | -2.272742 |
| 26 | 1 | 0 | -3.896513 | -1.536929 | -1.818088 |
| 27 | 1 | 0 | -4.357529 | -0.191371 | -0.720949 |
| 28 | 1 | 0 | -2.264948 | -0.942015 | 2.802687 |
| 29 | 1 | 0 | -0.707015 | -1.818526 | 2.877399 |
| 30 | 1 | 0 | -1.951553 | -2.322831 | 1.694146 |

---




15a

**COMPOUND 15a**

B3LYP/6-31+G(d): -759.427467391 hartrees

Imaginary Frequencies: none found

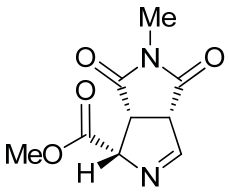
| | |
|---|---|
| Zero-point correction= | 0.192456 (Hartree/Particle) |
| Thermal correction to Energy= | 0.206345 |
| Thermal correction to Enthalpy= | 0.207289 |
| Thermal correction to Gibbs Free Energy= | 0.149964 |
| Sum of electronic and zero-point Energies= | -759.235011 |
| Sum of electronic and thermal Energies= | -759.221123 |
| Sum of electronic and thermal Enthalpies= | -759.220179 |
| Sum of electronic and thermal Free Energies= | -759.277504 |

Standard orientation:

| Center Number | Atomic Number | Atomic Type | Coordinates (Angstroms) X | Y | Z |
|---|---|---|---|---|---|
| 1 | 7 | 0 | -2.427503 | 0.360093 | -0.206070 |
| 2 | 6 | 0 | -1.290978 | 1.164531 | -0.290609 |
| 3 | 6 | 0 | -0.069300 | 0.261397 | -0.454523 |
| 4 | 6 | 0 | 0.948873 | 0.388793 | 0.724613 |
| 5 | 7 | 0 | 0.786986 | -0.823630 | 1.551423 |
| 6 | 6 | 0 | -0.026300 | -1.625295 | 0.987501 |
| 7 | 6 | 0 | -0.629725 | -1.165606 | -0.330326 |
| 8 | 6 | 0 | -2.149308 | -1.005085 | -0.248530 |

| | | | | | |
|---|---|---|---|---|---|
| 9 | 6 | 0 | -3.770817 | 0.909077 | -0.066656 |
| 10 | 8 | 0 | -2.976427 | -1.893332 | -0.205615 |
| 11 | 8 | 0 | -1.306610 | 2.378073 | -0.242696 |
| 12 | 6 | 0 | 2.407911 | 0.496512 | 0.273294 |
| 13 | 8 | 0 | 3.183111 | 1.343801 | 0.654999 |
| 14 | 8 | 0 | 2.722637 | -0.478740 | -0.603863 |
| 15 | 6 | 0 | 4.090347 | -0.498871 | -1.063507 |
| 16 | 1 | 0 | 0.391972 | 0.474626 | -1.421830 |
| 17 | 1 | 0 | 0.762058 | 1.272307 | 1.339857 |
| 18 | 1 | 0 | -0.276840 | -2.584239 | 1.438616 |
| 19 | 1 | 0 | -0.382234 | -1.854065 | -1.145343 |
| 20 | 1 | 0 | -3.975823 | 1.597860 | -0.890339 |
| 21 | 1 | 0 | -4.474782 | 0.076060 | -0.084943 |
| 22 | 1 | 0 | -3.856809 | 1.454128 | 0.877779 |
| 23 | 1 | 0 | 4.152304 | -1.345444 | -1.747338 |
| 24 | 1 | 0 | 4.330091 | 0.435431 | -1.577468 |
| 25 | 1 | 0 | 4.767773 | -0.633042 | -0.216601 |

---




**15b**

**COMPOUND 15b**

B3LYP/6-31+G(d): -759.426881110 hartrees

Imaginary Frequencies: none found

| | |
|---|---|
| Zero-point correction= | 0.192610 (Hartree/Particle) |
| Thermal correction to Energy= | 0.206398 |
| Thermal correction to Enthalpy= | 0.207343 |
| Thermal correction to Gibbs Free Energy= | 0.150339 |
| Sum of electronic and zero-point Energies= | -759.234271 |
| Sum of electronic and thermal Energies= | -759.220483 |
| Sum of electronic and thermal Enthalpies= | -759.219538 |
| Sum of electronic and thermal Free Energies= | -759.276542 |

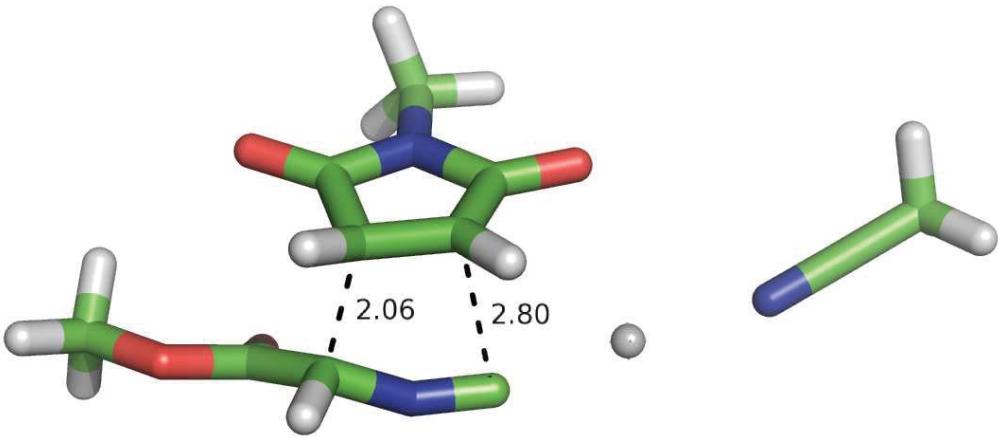
Standard orientation:

| Center Number | Atomic Number | Atomic Type | Coordinates (Angstroms) X | Y | Z |
|---|---|---|---|---|---|
| 1 | 7 | 0 | -1.709479 | -0.790007 | -0.597602 |
| 2 | 6 | 0 | -0.927684 | -0.853647 | 0.553640 |
| 3 | 6 | 0 | -0.633719 | 0.571394 | 1.020460 |
| 4 | 6 | 0 | 0.858971 | 1.058547 | 0.877737 |
| 5 | 7 | 0 | 0.860325 | 2.149332 | -0.109907 |
| 6 | 6 | 0 | -0.318613 | 2.341807 | -0.549151 |
| 7 | 6 | 0 | -1.409098 | 1.465503 | 0.036960 |
| 8 | 6 | 0 | -2.037243 | 0.506411 | -0.980800 |
| 9 | 6 | 0 | -2.105370 | -1.978188 | -1.342703 |

| | | | | | |
|---|---|---|---|---|---|
| 10 | 8 | 0 | -2.709682 | 0.810126 | -1.945610 |
| 11 | 8 | 0 | -0.577321 | -1.885253 | 1.091483 |
| 12 | 6 | 0 | 1.817637 | -0.031183 | 0.390865 |
| 13 | 8 | 0 | 1.722758 | -0.573690 | -0.689222 |
| 14 | 8 | 0 | 2.762112 | -0.303178 | 1.303074 |
| 15 | 6 | 0 | 3.696734 | -1.346440 | 0.951412 |
| 16 | 1 | 0 | -0.958384 | 0.662253 | 2.059816 |
| 17 | 1 | 0 | 1.233224 | 1.447901 | 1.827446 |
| 18 | 1 | 0 | -0.522654 | 3.102178 | -1.300977 |
| 19 | 1 | 0 | -2.209426 | 2.069026 | 0.481052 |
| 20 | 1 | 0 | -1.222571 | -2.450524 | -1.783460 |
| 21 | 1 | 0 | -2.591009 | -2.688780 | -0.669296 |
| 22 | 1 | 0 | -2.794301 | -1.665566 | -2.128504 |
| 23 | 1 | 0 | 4.366278 | -1.430659 | 1.807157 |
| 24 | 1 | 0 | 3.161501 | -2.283418 | 0.780301 |
| 25 | 1 | 0 | 4.248798 | -1.066945 | 0.050707 |

---

Cis
ITS




B3LYP/6•31+G(d): -1037.29810817

Imaginary Frequencies: 1 (-311.6939)

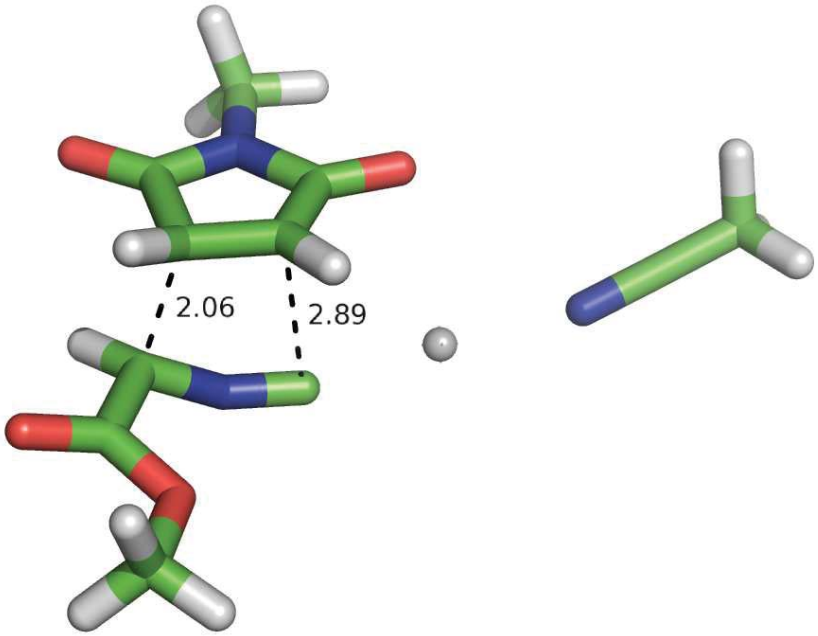
| | |
|---|---|
| Zero-point correction= | 0.221847 |
| Thermal correction to Energy= | 0.244158 |
| Thermal correction to Enthalpy= | 0.245103 |
| Thermal correction to Gibbs Free Energy= | 0.164754 |
| Sum of electronic and zero-point Energies= | -1037.076261 |
| Sum of electronic and thermal Energies= | -1037.053950 |
| Sum of electronic and thermal Enthalpies= | -1037.053006 |
| Sum of electronic and thermal Free Energies= | -1037.133354 |

Standard orientation:

| Center Number | Atomic Number | Atomic Type | Coordinates (Angstroms) X | Y | Z |
|---|---|---|---|---|---|
| 1 | 7 | 0 | -0.941970 | 1.940283 | -0.084414 |
| 2 | 6 | 0 | -2.164653 | 1.556128 | 0.435454 |
| 3 | 6 | 0 | -1.864744 | 0.563011 | 1.545620 |
| 4 | 6 | 0 | -2.347695 | -1.228657 | 0.660942 |
| 5 | 7 | 0 | -1.114334 | -1.633733 | 0.205742 |
| 6 | 6 | 0 | 0.053808 | -1.556385 | 0.096642 |
| 7 | 6 | 0 | -0.472848 | 0.636421 | 1.752628 |
| 8 | 6 | 0 | 0.121682 | 1.398841 | 0.694133 |
| 9 | 6 | 0 | -0.780697 | 2.813913 | -1.228698 |
| 10 | 8 | 0 | 1.318457 | 1.600103 | 0.402812 |
| 11 | 8 | 0 | -3.254330 | 1.953673 | 0.048377 |
| 12 | 6 | 0 | -3.376700 | -1.031767 | -0.387253 |
| 13 | 8 | 0 | -3.165465 | -0.880337 | -1.577178 |
| 14 | 8 | 0 | -4.604005 | -0.984879 | 0.181328 |
| 15 | 6 | 0 | -5.674285 | -0.564119 | -0.683089 |
| 16 | 1 | 0 | -2.602468 | 0.474397 | 2.336450 |
| 17 | 1 | 0 | -2.685020 | -1.799756 | 1.523429 |
| 18 | 1 | 0 | 0.100855 | 0.207068 | 2.562973 |
| 19 | 1 | 0 | -1.160242 | 3.818748 | -1.009874 |
| 20 | 1 | 0 | 0.286600 | 2.869390 | -1.453152 |
| 21 | 1 | 0 | -1.325792 | 2.414615 | -2.090112 |
| 22 | 1 | 0 | -6.570396 | -0.584009 | -0.061416 |
| 23 | 1 | 0 | -5.478012 | 0.447435 | -1.046612 |
| 24 | 1 | 0 | -5.775716 | -1.250722 | -1.528191 |
| 25 | 47 | 0 | 2.055473 | -0.925884 | -0.067293 |
| 26 | 7 | 0 | 4.168699 | -0.402662 | -0.241513 |
| 27 | 6 | 0 | 5.194977 | 0.127539 | -0.292700 |

| | | | | | |
|---|---|---|---|---|---|
| 28 | 6 | 0 | 6.482683 | 0.807628 | -0.354262 |
| 29 | 1 | 0 | 6.906380 | 0.716105 | -1.359355 |
| 30 | 1 | 0 | 6.348160 | 1.868012 | -0.117443 |
| 31 | 1 | 0 | 7.175227 | 0.364250 | 0.368144 |

Trans ITS




B3LYP/6•31+G(d): -1037.29817948

Imaginary Frequencies: 1 (-317.4587)

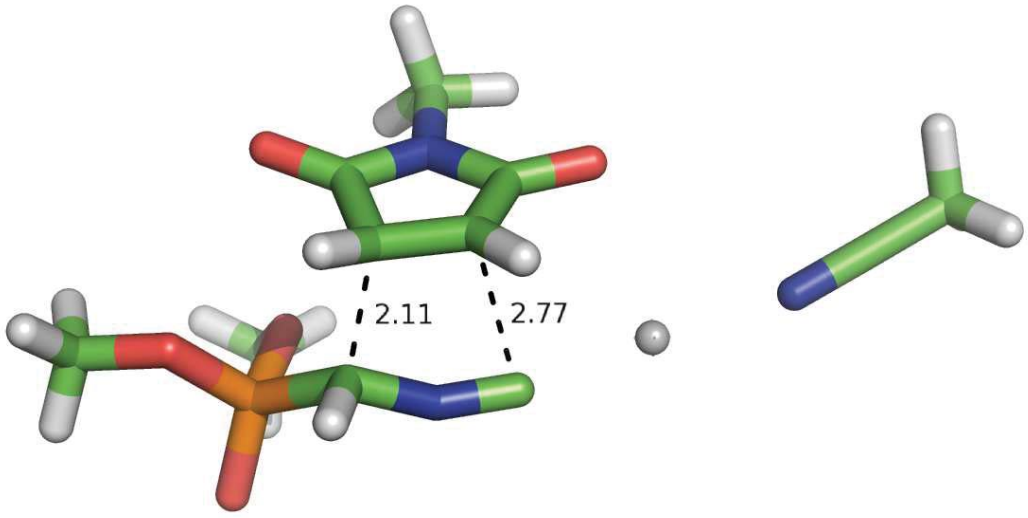
| | |
|---|---|
| Zero-point correction= | 0.221736 |
| Thermal correction to Energy= | 0.244151 |
| Thermal correction to Enthalpy= | 0.245095 |
| Thermal correction to Gibbs Free Energy= | 0.163847 |
| Sum of electronic and zero-point Energies= | -1037.076443 |
| Sum of electronic and thermal Energies= | -1037.054028 |
| Sum of electronic and thermal Enthalpies= | -1037.053084 |
| Sum of electronic and thermal Free Energies= | -1037.134333 |

Standard orientation:

| Center Number | Atomic Number | Atomic Type | Coordinates (Angstroms) X | Y | Z |
|---|---|---|---|---|---|
| 1 | 7 | 0 | -0.517561 | 2.545031 | 0.101175 |
| 2 | 6 | 0 | -1.885530 | 2.355041 | 0.187245 |
| 3 | 6 | 0 | -2.087711 | 1.044737 | 0.932977 |
| 4 | 6 | 0 | -2.673621 | -0.113950 | -0.667154 |
| 5 | 7 | 0 | -1.456909 | -0.595891 | -1.086603 |
| 6 | 6 | 0 | -0.305091 | -0.818570 | -1.017200 |
| 7 | 6 | 0 | -0.816619 | 0.668839 | 1.402397 |
| 8 | 6 | 0 | 0.174623 | 1.528179 | 0.825293 |
| 9 | 6 | 0 | 0.111690 | 3.661525 | -0.573629 |
| 10 | 8 | 0 | 1.421177 | 1.485338 | 0.860343 |
| 11 | 8 | 0 | -2.732009 | 3.101489 | -0.282805 |
| 12 | 6 | 0 | -3.634641 | -1.091057 | -0.115448 |
| 13 | 8 | 0 | -4.808674 | -0.831665 | 0.090235 |
| 14 | 8 | 0 | -3.059417 | -2.272129 | 0.225298 |
| 15 | 6 | 0 | -3.934975 | -3.232780 | 0.838186 |
| 16 | 1 | 0 | -3.018246 | 0.924241 | 1.478986 |
| 17 | 1 | 0 | -3.130321 | 0.616005 | -1.328731 |
| 18 | 1 | 0 | -0.573516 | -0.134763 | 2.084432 |
| 19 | 1 | 0 | -0.136073 | 4.607603 | -0.078518 |
| 20 | 1 | 0 | 1.191607 | 3.503152 | -0.536231 |
| 21 | 1 | 0 | -0.222864 | 3.719470 | -1.614872 |
| 22 | 1 | 0 | -3.313243 | -4.108344 | 1.029876 |

| | | | | | |
|---|---|---|---|---|---|
| 23 | 1 | 0 | -4.344342 | -2.837925 | 1.772314 |
| 24 | 1 | 0 | -4.758548 | -3.484934 | 0.164270 |
| 25 | 47 | 0 | 1.739161 | -0.793879 | -0.521820 |
| 26 | 7 | 0 | 3.863154 | -0.902864 | -0.032782 |
| 27 | 6 | 0 | 4.931965 | -0.729432 | 0.372871 |
| 28 | 6 | 0 | 6.274539 | -0.499609 | 0.891527 |
| 29 | 1 | 0 | 6.606822 | -1.368565 | 1.468233 |
| 30 | 1 | 0 | 6.971516 | -0.330611 | 0.064632 |
| 31 | 1 | 0 | 6.269438 | 0.381261 | 1.541658 |

Cis PTS




B3LYP/6•31+G(d): -1455.71652601

Imaginary Frequencies: 1 (-268.8965)

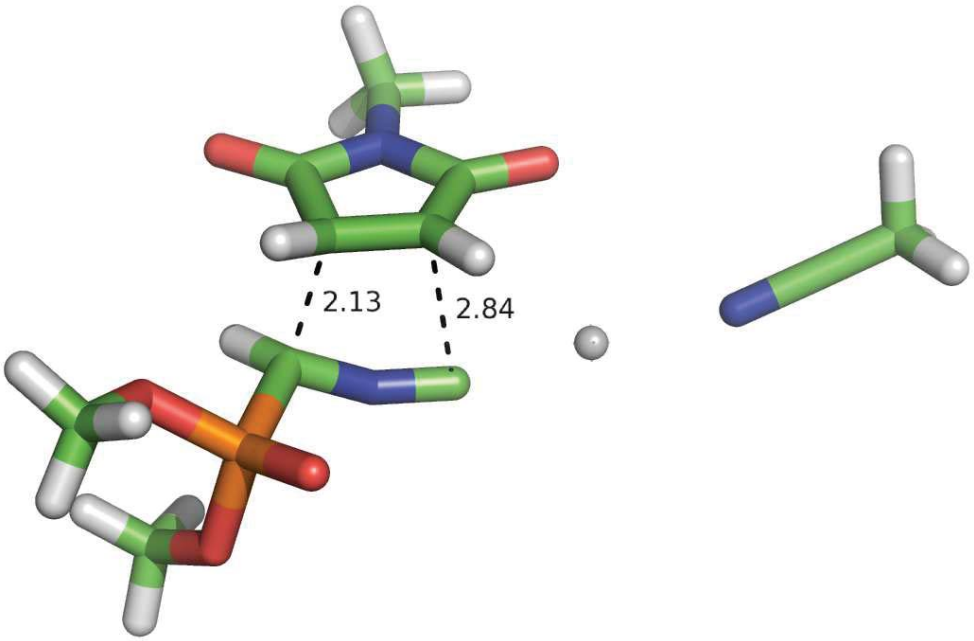
| | |
|---|---|
| Zero-point correction= | 0.260233 |
| Thermal correction to Energy= | 0.286809 |
| Thermal correction to Enthalpy= | 0.287753 |
| Thermal correction to Gibbs Free Energy= | 0.195809 |
| Sum of electronic and zero-point Energies= | -1455.456293 |
| Sum of electronic and thermal Energies= | -1455.429717 |
| Sum of electronic and thermal Enthalpies= | -1455.428773 |
| Sum of electronic and thermal Free Energies= | -1455.520717 |

Standard orientation:

| Center Number | Atomic Number | Atomic Type | Coordinates (Angstroms) X | Y | Z |
|---|---|---|---|---|---|
| 1 | 7 | 0 | 0.296192 | 2.140927 | 0.250240 |
| 2 | 6 | 0 | 1.503017 | 1.992358 | -0.424721 |
| 3 | 6 | 0 | 1.203261 | 1.129368 | -1.638192 |
| 4 | 6 | 0 | -0.197315 | 1.101792 | -1.740942 |
| 5 | 6 | 0 | -0.772388 | 1.640284 | -0.538209 |
| 6 | 6 | 0 | 0.140940 | 2.896689 | 1.475022 |
| 7 | 8 | 0 | -1.959461 | 1.694020 | -0.157519 |
| 8 | 8 | 0 | 2.555984 | 2.511325 | -0.095004 |
| 9 | 1 | 0 | 1.889560 | 1.199926 | -2.474158 |
| 10 | 1 | 0 | -0.795669 | 0.746682 | -2.569325 |
| 11 | 1 | 0 | 0.354481 | 3.960545 | 1.313711 |
| 12 | 1 | 0 | -0.892897 | 2.781350 | 1.807714 |
| 13 | 1 | 0 | 0.826283 | 2.518390 | 2.239860 |
| 14 | 6 | 0 | 1.850141 | -0.806147 | -1.106910 |
| 15 | 7 | 0 | 0.666065 | -1.340848 | -0.658317 |
| 16 | 6 | 0 | -0.497161 | -1.340882 | -0.474098 |
| 17 | 47 | 0 | -2.525370 | -0.916240 | -0.114985 |
| 18 | 7 | 0 | -4.660807 | -0.611123 | 0.221252 |
| 19 | 6 | 0 | -5.730402 | -0.204323 | 0.386822 |
| 20 | 6 | 0 | -7.074663 | 0.319880 | 0.593832 |
| 21 | 1 | 0 | -7.441985 | 0.027694 | 1.582675 |
| 22 | 1 | 0 | -7.056328 | 1.412474 | 0.527022 |
| 23 | 1 | 0 | -7.751785 | -0.074365 | -0.170480 |
| 24 | 15 | 0 | 3.251943 | -1.028791 | 0.011817 |
| 25 | 8 | 0 | 3.784321 | -2.397570 | 0.292162 |
| 26 | 8 | 0 | 4.291282 | -0.059728 | -0.755571 |

| | | | | | |
|---|---|---|---|---|---|
| 27 | 6 | 0 | 5.666159 | 0.020711 | -0.349541 |
| 28 | 8 | 0 | 2.745487 | -0.272355 | 1.354777 |
| 29 | 6 | 0 | 3.152341 | -0.710229 | 2.662295 |
| 30 | 1 | 0 | 2.107377 | -1.190008 | -2.096881 |
| 31 | 1 | 0 | 6.193510 | 0.530632 | -1.157935 |
| 32 | 1 | 0 | 5.751645 | 0.614241 | 0.567339 |
| 33 | 1 | 0 | 6.085167 | -0.979043 | -0.197723 |
| 34 | 1 | 0 | 3.342859 | -1.786765 | 2.670328 |
| 35 | 1 | 0 | 2.332665 | -0.463753 | 3.341488 |
| 36 | 1 | 0 | 4.053741 | -0.168092 | 2.968278 |

Trans PTS




B3LYP/6•31+G(d): -1455.72100294

Imaginary Frequencies: 1 (-280.6074)

| | |
|---|---|
| Zero-point correction= | 0.260289 |
| Thermal correction to Energy= | 0.286816 |
| Thermal correction to Enthalpy= | 0.287761 |
| Thermal correction to Gibbs Free Energy= | 0.196013 |
| Sum of electronic and zero-point Energies= | -1455.460714 |
| Sum of electronic and thermal Energies= | -1455.434187 |
| Sum of electronic and thermal Enthalpies= | -1455.433242 |
| Sum of electronic and thermal Free Energies= | -1455.524990 |

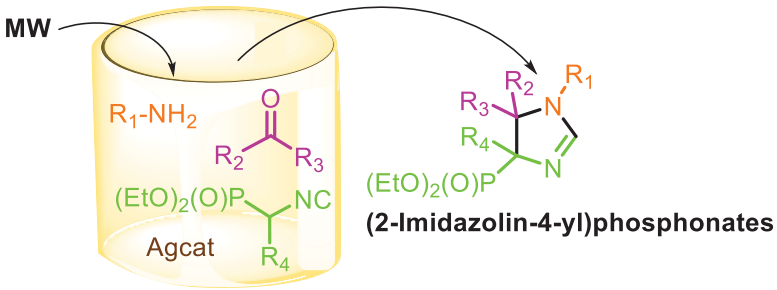
Standard orientation:

| Center Number | Atomic Number | Atomic Type | Coordinates (Angstroms) X | Y | Z |
|---|---|---|---|---|---|
| 1 | 7 | 0 | 0.274814 | 2.700370 | -0.236211 |
| 2 | 6 | 0 | -1.083593 | 2.592094 | 0.018872 |
| 3 | 6 | 0 | -1.243514 | 1.417911 | 0.963183 |
| 4 | 6 | 0 | 0.047760 | 1.039458 | 1.344498 |
| 5 | 6 | 0 | 1.005926 | 1.768422 | 0.556947 |
| 6 | 6 | 0 | 0.865940 | 3.680358 | -1.123788 |
| 7 | 8 | 0 | 2.245334 | 1.675503 | 0.485565 |
| 8 | 8 | 0 | -1.948103 | 3.310071 | -0.465258 |
| 9 | 1 | 0 | -2.121371 | 1.383286 | 1.596113 |
| 10 | 1 | 0 | 0.326993 | 0.333550 | 2.114414 |
| 11 | 1 | 0 | 0.705909 | 4.697199 | -0.746754 |
| 12 | 1 | 0 | 1.937498 | 3.475551 | -1.174319 |
| 13 | 1 | 0 | 0.424088 | 3.609755 | -2.123398 |
| 14 | 6 | 0 | -2.009148 | 0.058791 | -0.487586 |
| 15 | 7 | 0 | -0.840398 | -0.548969 | -0.854377 |
| 16 | 6 | 0 | 0.299739 | -0.833641 | -0.768846 |
| 17 | 47 | 0 | 2.358283 | -0.905751 | -0.369708 |
| 18 | 7 | 0 | 4.479851 | -1.114170 | 0.079975 |
| 19 | 6 | 0 | 5.578262 | -1.007795 | 0.424768 |
| 20 | 6 | 0 | 6.960189 | -0.863117 | 0.864940 |
| 21 | 1 | 0 | 7.201932 | -1.639397 | 1.597800 |

| | | | | | |
|---|---|---|---|---|---|
| 22 | 1 | 0 | 7.637178 | -0.954531 | 0.009711 |
| 23 | 1 | 0 | 7.096639 | 0.120136 | 1.326630 |
| 24 | 15 | 0 | -3.183874 | -1.035664 | 0.341078 |
| 25 | 8 | 0 | -2.687177 | -1.755583 | 1.541347 |
| 26 | 8 | 0 | -4.379060 | 0.037288 | 0.583616 |
| 27 | 6 | 0 | -5.409267 | -0.250928 | 1.548606 |
| 28 | 8 | 0 | -3.795774 | -2.103161 | -0.730403 |
| 29 | 6 | 0 | -4.372384 | -1.683779 | -1.975742 |
| 30 | 1 | 0 | -2.435999 | 0.713499 | -1.246032 |
| 31 | 1 | 0 | -6.053548 | -1.062499 | 1.192595 |
| 32 | 1 | 0 | -4.966367 | -0.527963 | 2.509370 |
| 33 | 1 | 0 | -5.991475 | 0.666667 | 1.650245 |
| 34 | 1 | 0 | -5.157317 | -0.936590 | -1.815581 |
| 35 | 1 | 0 | -3.604027 | -1.274071 | -2.641722 |
| 36 | 1 | 0 | -4.802428 | -2.579562 | -2.428726 |

# Capítulo 4

## Easy access to (2-imidazolin-4-yl)phosphonates by a microwave assisted multicomponent reaction



Tetrahedron 71 (2015) 2872–2881

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

# Easy access to (2-imidazolin-4-yl)phosphonates by a microwave assisted multicomponent reaction

Sònia Abás [a], Carolina Estarellas [b], F. Javier Luque [b], Carmen Escolano [a,*]

[a] Laboratory of Organic Chemistry, Faculty of Pharmacy, and Institute of Biomedicine (IBUB), University of Barcelona, Spain
[b] Departament de Fisicoquímica and Institut de Biomedicina (IBUB), Facultat de Farmàcia, Universitat de Barcelona, Campus de l'Alimentació Torribera, Av. Prat de la Riba 171, 08921 Santa Coloma de Gramenet, Spain

ARTICLE INFO

*Article history:*
Received 12 February 2015
Received in revised form 15 March 2015
Accepted 17 March 2015
Available online 23 March 2015

*Keywords:*
(2-Imidazolin-4-yl)phosphonate
Microwave
Multicomponent reaction
Theoretical calculations
Diethyl isocyanomethylphosphonate

ABSTRACT

An efficient and user-friendly synthetic process involving the combination of multicomponent reaction methodology and microwave heating generates unprecedented (2-imidazolin-4-yl)phosphonates **1**–**18**. This strategy presents a silver-catalysed, operationally simple and environmentally friendly transformation without the need of anhydrous atmosphere or additional solvents.



## 1. Introduction

2-Imidazoline-containing compounds constitute a valuable class of agents that modulate the α2-adrenergic receptors, and often show a high affinity for imidazoline binding sites (IBS) (Fig. 1).[1] The biological profile of these IBSs has been examined in pharmacological studies due to their involvement in the control of blood pressure, depression, insuline secretion, neurodegenerative disorders, and tolerance and dependence on opioids.[2] Moreover, 2-imidazolines are an important class of heterocyclic scaffolds that can be found in natural product chemistry, organic synthesis, coordination chemistry and homogeneous catalysis.[3]

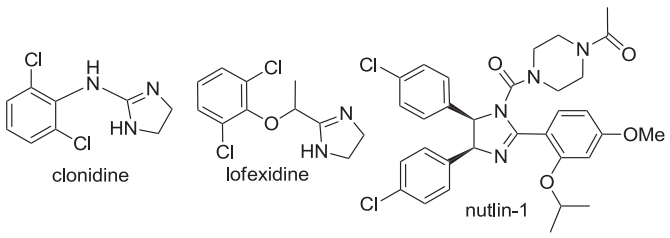



**Fig. 1.** Biologically active 2-imidazoline-containing compounds.

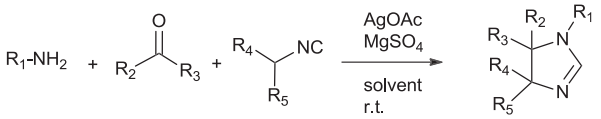
To meet the demand for 2-imidazoline-containing compounds, many efficient synthetic approximations have been developed, including the synthesis of 2-imidazolines from 1,2-diamines, β-hydroxyamides, *N*-acyldiamines, aziridines, alkenes, azalactones and imines, and isocyanides and imines.[4]

The distinctive reactivity of isocyanides has been recognized as an advantageous characteristic for the construction of nitrogen-containing heterocycles.[5] Their divalent nature makes smooth reactions possible with a myriad of reaction partners of either electrophilic or nucleophilic character. In addition, the facile coordination of isonitriles to metals increases the acidity of the adjacent hydrogen atoms. Deprotonation of metal-complexed isocyanides often proceeds with weak bases, leading to nucleophiles that can be used in, for example, cycloaddition reactions.[6] The rich chemistry of isonitriles has experienced a renaissance due to their crucial role in the fashionable and fascinating field of multicomponent reactions.[7] The isocyanide-based multicomponent reactions (IMCRs) have been widely investigated in organic synthesis owing to their inherent features such as atom- and step economy, convergence and diversity. Thus, IMCRs encode a set of privileged reactions in the toolbox of sustainable synthetic methodologies.[8]

In this framework, the pioneering paper by Orru and co-workers is a key contribution, as it describes an efficient and flexible multicomponent reaction of diversely substituted 2-imidazolines, employing isocyanides bearing an acidic α-proton (Scheme 1).[9]

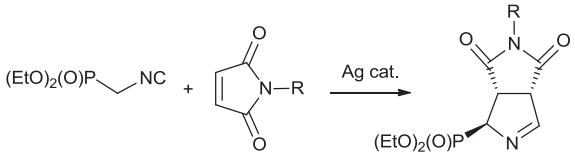
* Corresponding author. Tel.: +34 934024540; fax: +34 934024539; e-mail address: cescolano@ub.edu (C. Escolano).

http://dx.doi.org/10.1016/j.tet.2015.03.065
0040-4020/



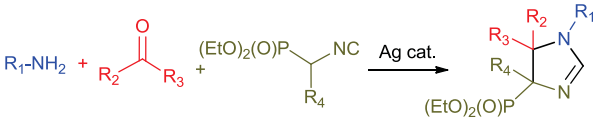

**Scheme 1.** Orru's synthesis of 2-imidazolines.

As part of our ongoing research in α-metallated isocyanides, we have recently published the first diastereoselective silver-catalysed [3+2]cycloaddition of diethyl isocyanomethylphosphonate (PhosMic) and diversely *N*-substituted maleimides, leading to bicyclic α-aminophosphonates (Scheme 2).[10]




**Scheme 2.** Reaction of PhosMic and maleimides.

Inspired by Orru's study and seeking new applications of isocyano derivatives, we envisaged the possibility of performing a multicomponent reaction between PhosMic or α-substituted PhosMic derivatives, ketones and amines (Scheme 3). Notably, although isocyano derivatives can be found extensively in the literature as reactants in multicomponent reactions, the use of PhosMic in IMCRs has received less attention.[11] It is worth noting that the proposed reaction would give direct access to (2-imidazolin-4-yl) phosphonate compounds. The potential biological interest of these new azaheterocyclic phosphonates is mostly associated with the tetrahedral structure of the phosphonyl group, which may act as a 'transition state analogue' in enzymatic peptide hydrolysis.[12]

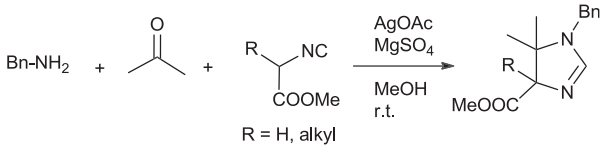



**Scheme 3.** Proposed reaction to access (2-imidazolin-4-yl)phosphonates.

This work addresses the synthesis of 2-(imidazolin-4-yl)phosphonates through an environmentally friendly multicomponent heterocyclic reaction following green chemistry considerations. To this end, we envisioned the use of microwave irradiation, which is an outstanding tool for sustainable organic chemistry, maximizing synthetic efficiency, diversity and complexity.[13] In particular, we disclose a general microwave protocol for a silver-catalysed three-component reaction towards the synthesis of (2-imidazolin-4-yl) phosphonates. The reactivity outcome is rationalized by theoretical calculations.

## 2. Results and discussion

As mentioned above, Orru and co-workers were able to obtain methyl 1-benzyl-5,5-dimethyl-4,5-dihydro-1*H*-imidazole-4-carboxylate (R=H, Scheme 4) in 89% yield by stirring a suspension of benzylamine, acetone and methyl isocyanoacetate in methanol for 5 h at room temperature in the presence of an equimolecular amount of magnesium sulfate. Using α-alkyl substituted isocyanate derivatives (R=alkyl, Scheme 4), the process required the presence of a catalytic amount of silver acetate and a considerable increase in the reaction time for the reaction to occur.

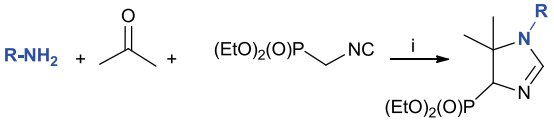



**Scheme 4.** Orru's examples with methyl isocyanoacetate derivatives.

In this work, an analogous multicomponent reaction using PhosMic was examined with the aim of finding the easiest to handle and eco-friendly reaction conditions different factors were considered. First of all, removal of methanol as the solvent and the use of acetone were studied. Thus, acetone would act both as reagent and solvent in a very concentrated reaction without exclusion of air and humidity. Secondly, the use of microwave irradiation was envisaged as an optimal technique to carry out the reaction in the shortest possible time.

Since active hydrogen atoms in the α-isocyanophosphonate Schiff bases are less acidic than their corresponding counterpart α-isocyanoesters, the proposed transformation needed to be performed under silver catalytic conditions. As previously reported, when PhosMic is stirred in the presence of silver acetate, a self-cyclodimerization product is observed.[14] In order to avoid this side product, a weaker base salt, $AgNO_3$, was employed as the catalyst. In this process, a series of experiments were conducted to study the impact of the temperature, the catalyst and the reaction time on the yield. Finally, the optimized reaction conditions consisted of irradiating a mixture of PhosMic (1 equiv), amine (1.5 equiv), $MgSO_4$ (1.8 equiv) and $AgNO_3$ (10%-mmol) and acetone in a microwave oven during 10 min at 40 °C.[15] The reaction was examined with a range of diversely substituted alkyl amines and the results are given in Table 1.

**Table 1**
Yields (%) of the phosphono-2-imidazolines **1**–**10**[a]




| Entry | $RNH_2$ | | With $MgSO_4$[b] | Without $MgSO_4$[b] |
|---|---|---|---|---|
| 1 | Propylamine | **1** | 30 | 53 |
| 2 | Cyclohexylamine | **2** | 57 | 62 |
| 3 | 2-Ethylhexylamine | **3** | 52 | 89 |
| 4 | 1-Adamantanemethylamine | **4** | 49 | 72 |
| 5 | Benzylamine | **5** | 62 | 66 |
| 6 | Phenethylamine | **6** | 91 | 89 |
| 7 | Tryptamine | **7** | 50 | 87 |
| 8 | 1-(1-Adamantyl)ethylamine | **8** | nd | 71[c] |
| 9 | 1-Methylbenzylamine | **9** | nd | 60[d] |
| 10 | 2-Methylphenethylamine | **10** | nd | 69 |

[a] Reagents and conditions: (i) PhosMic (1 equiv), amine (1.5 equiv), $MgSO_4$ (1.8 equiv) or without $MgSO_4$, $AgNO_3$ (10%-mmol) and acetone in a microwave oven 10 min at 40 °C; **1**–**10** (yields, see Table 1).
[b] Yields refer to isolated products.
[c] Reaction carried out at rt for 24 h.
[d] Reaction carried out at rt for 48 h. nd=not done.

Propylamine, cyclohexylamine and 2-ethylhexylamine gave expected final products **1**–**3** in 30, 57 and 52% yield, respectively (entries 1–3). Compound **3** was isolated as a single diastereoisomer. Notably, the reaction also occurred with the hindered adamantane moiety to furnish **4** in 49% yield (entry 4). When benzylamine, phenethylamine and tryptamine were employed, the reaction gave **5**–**7** in 62, 91 and 50% yield, respectively (entries 5, 6 and 7). After confirming that the reaction occurs with a considerable range of amines in the presence of acetone playing the dual role of reagent and solvent, removal of $MgSO_4$ was envisaged to achieve the

maximum reduction of the atoms used in the reaction (Table 1). Notably, in all cases removing the $MgSO_4$ reagent considerably increased the yield of the final product, thus satisfying the green criterion of atom economy and the E-factor.[16] In the case of the more sterically hindered α-methylsubstituted amines such as 1-(1-adamantyl)ethylamine (entry 8) and 1-methylbenzylamine (entry 9) the reaction did not occur under microwave irradiation and was conducted at rt overnight. After purification of the two crude reactions the two diastereoisomers of **8** and **9** were isolated in a 1:1 ratio, in 71 and 60% yield, respectively. When 2-methylphenethylamine was employed as the amine, **10** was obtained in 69% yield, carrying out the reaction under microwave irradiation. This indicates that the presence of a α-methyl substituent in the amine is not compatible with the use of microwaves to carry out the reaction, whereas a β-methyl substituent is compatible with the standard conditions described above (Fig. 2).

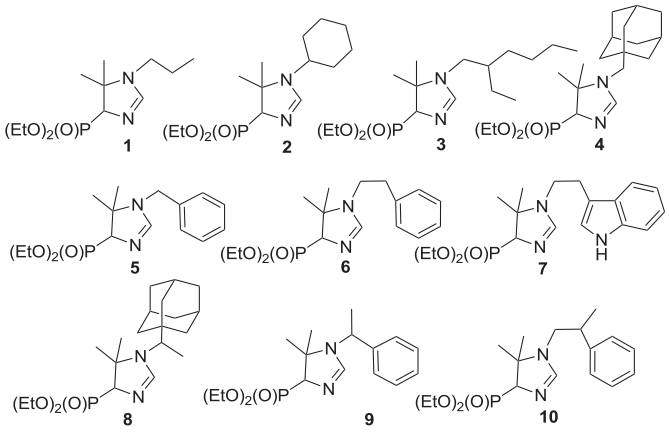



**Fig. 2.** (2-Imidazolin-4-yl)phosphonates **1**–**10**.

After evaluating the scope of the amine component, efforts were directed towards the carbonyl compound. To this end, the reaction with phenethylamine was undertaken using 2-butanone instead of acetone, taking place in 59% yield in a non-diastereoselective manner and the two possible diastereomers of compound **11** were isolated in 1:1 ratio. With cyclopentanone and cyclohexanone as one of the 3-MCR components, diazaspiro derivatives **12** and **13** were obtained in 48 and 61% yield, respectively (Fig. 3).[17]

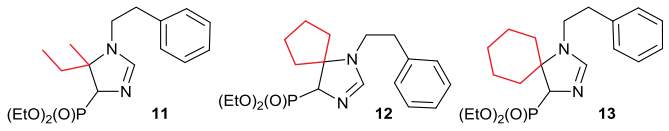



**Fig. 3.** (2-Imidazolin-4-yl)phosphonates **11**–**13**.

We then turned our attention to screening diethyl α-phenyl-isocyanomethylphosphonate.[18] The use of acetone with four amines (2-ethylhexylamine, benzylamine, phenethylamine and tryptamine) furnished compounds **14**–**17** in 83, 61, 78 and 67% yield, respectively. Also, the reaction with cyclohexanone and phenethylamine successfully provided compound **18** in 59% yield. Notably, these compounds possess two vicinal quaternary centres generated in a single step (Fig. 4). Employing diethyl α-methyl or α-benzylisocyanomethylphosphonate the reaction did not work (see theoretical calculations).

In order to compare the behaviour of PhosMic with diethyl isocyanoacetate, the optimized microwave irradiation conditions were applied to a mixture of benzylamine, methyl isocyanoacetate and acetone. With or without $MgSO_4$, the reaction finished in 10 min and the final product **19** was isolated in 92 and 94% yield,

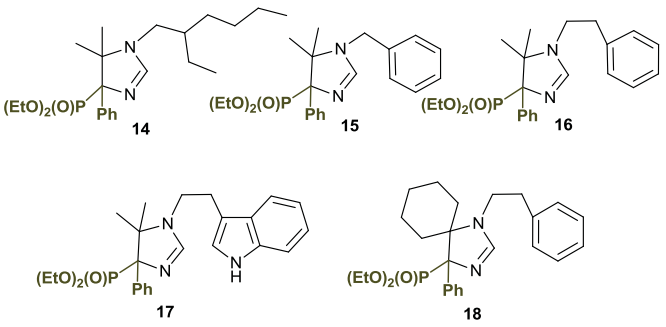



**Fig. 4.** (4-Phenyl-2-imidazolin-4-yl)phosphonates **14**–**18**.

respectively (Fig. 4). No silver catalyst was needed.[19] The reaction rates decreased as the steric bulk of the α-isocyanoacetate derivative increased. With methyl α-benzylisocyanoacetate, $AgNO_3$ as the catalyst and an increase of the reaction time to 20 min, **20** was obtained in 62% yield. On the other hand, 1-phenylethylamine led to **21**, as a mixture of diastereoisomers, under microwave irradiation and in the presence of $AgNO_3$ in 87% yield (Fig. 5).

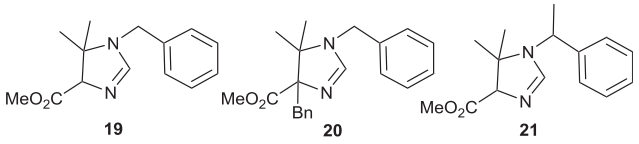



**Fig. 5.** (2-Imidazolin-4-yl)carboxylates from methyl isocyanoacetate derivatives.

Overall, the MCR studied is more favoured with methyl isocyanoacetate than with PhosMic. For example, compound **19** was formed with considerably better yield than compound **5** (Table 1, entry 5) without the necessity for a silver catalyst; compound **20** was also prepared, but the homologous reaction with diethyl α-benzylisocyanomethylphosphonate did not occur; and, finally, the 1-methylbenzylamine did not react with PhosMic under microwave irradiation, but with methyl isocyanoacetate the reaction did occur.

## 2.1. Spectroscopic considerations

As expected, the $^{1}H$ and $^{13}C$ spectra of the phosphonate derivatives **1**–**18** proved to be complex due to additional coupling constants between $^{1}H$–$^{31}P$ and $^{13}C$–$^{31}P$ (see Table in the Supplementary data section). In fact, the representative coupling constants in compounds **1**–**13** showed to be 15–16 Hz (H4 and P) and 2 Hz (H2 and P) in the $^{1}H$ NMR spectra. For compounds **1**–**18** the representative coupling constants are 145–170 Hz (C4–P), 13–18 Hz (C2–P) and 3–6 Hz (C5–P) in the $^{13}C$ NMR spectra (Fig. 6).

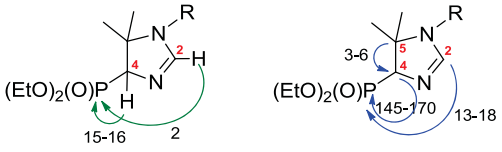
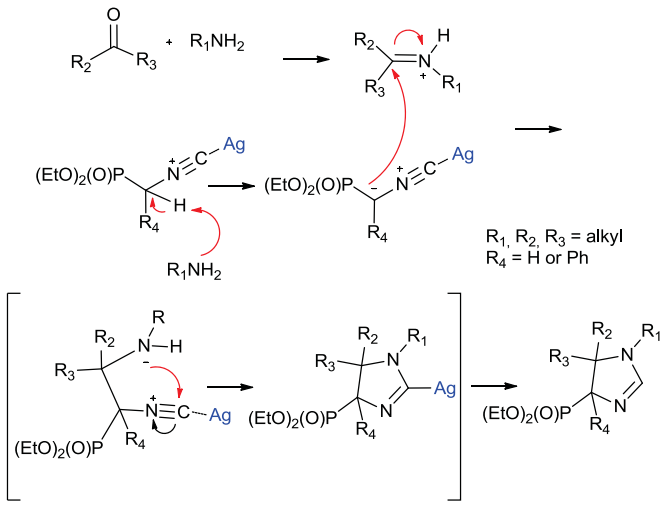



**Fig. 6.** Coupling constants (Hz) $^{1}H$–$^{31}P$ (green) and $^{13}C$–$^{31}P$ (blue).

## 2.2. Mechanistic and theoretical considerations

The proposed mechanism is depicted in Scheme 5. It is believed that the silver-activated PhosMic is deprotonated by the amine present in the reaction mixture.[9] The mechanism of this IMCR probably involves a Mannich-type like addition of the α-nucleophilic position of the PhosMic to a previous preformed iminium salt.

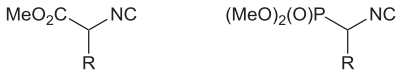



**Scheme 5.** Putative mechanism proposed for the IMCR.

Next, the ring closure can proceed by attack of the pair of electrons of the nitrogen to the silver-coordinated terminal carbon of the isonitrile. Finally, α-hydrogen PhosMic protodemetallation would lead to the final isolated product. Nevertheless, it must be stressed that the mechanistic details of MCR reactions and the effect played by the electronic features of reactants are still controversial. Hence, a concerted cycloaddition of the iminium salt and deprotonated and silver-coordinated PhosMic cannot be discarded.[9]

In order to compare the reactivity of methyl isocyanoacetate and PhosMic derivatives, we determined the free energy change for deprotonation of these compounds and their α-methyl and α-phenyl derivatives (Table 2; let us note that in computations the ethoxy groups of PhosMic and its derivatives were replaced by methoxy units). The deprotonation free energy was determined by using the thermodynamic cycle shown in Scheme 6, which combines the free energy of the deprotonation process in the gas phase and the solvation free energy in acetone, taking advantage of the cancellation of the contributions due to both neutral and protonated amine for comparison between isocyanide compounds. Moreover, since the reaction occurs under $AgNO_3$ catalysis, calculations were performed for the isolated compounds and for their complexes with silver cation, which in turn was modelled bound to acetone. To this end, calculations were performed at the M062X

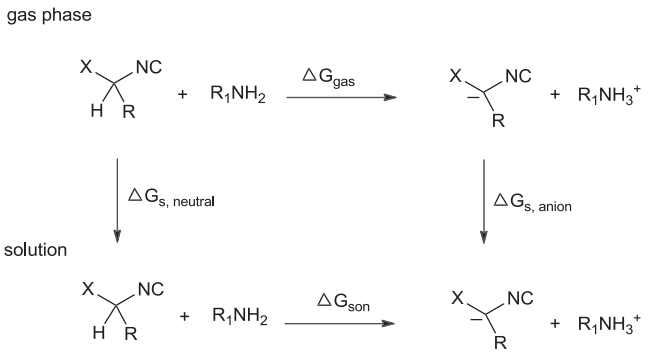
**Table 2**
Free energy changes (kcal/mol) for the deprotonation of methyl isocyanoacetate and dimethyl isocyanomethylphosphonate compounds. Values relative to the parent compound (**22a** and **23a**) are given in parenthesis[a]



**22a-c** **23a-c**

| Compound | $\Delta G_{gas}$ | $\Delta G_{son}$ |
|---|---|---|
| **22a** | 342.7 (0.0) | 301.2 (0.0) |
| **22b** | 343.8 (+1.1) | 302.7 (+1.5) |
| **22c** | 330.5 (−12.1) | 293.5 (−7.6) |
| **22a**+$Ag^+$ | 263.3 (0.0) | 294.3 (0.0) |
| **22b**+$Ag^+$ | 265.2 (+1.9) | 295.8 (+1.4) |
| **22c**+$Ag^+$ | 261.7 (−1.6) | 288.5 (−5.8) |
| **23a** | 344.4 (0.0) | 305.9 (0.0) |
| **23b** | 349.7 (+5.4) | 310.1 (+4.2) |
| **23c** | 332.6 (−11.8) | 297.5 (−8.4) |
| **23a**+$Ag^+$ | 270.0 (0.0) | 298.2 (0.0) |
| **23b**+$Ag^+$ | 270.0 (0.0) | 301.4 (+3.2) |
| **23c**+$Ag^+$ | 265.4 (−4.5) | 292.5 (−5.8) |

[a] a, R=H; b, R=methyl; c, R=phenyl.




**Scheme 6.** Thermodynamic cycle used for the calculation of relative free energies of deprotonation of methyl isocyanoacetate (X=$CO_2Me$) and dimethyl isocyanomethylphosphonate [X=$PO(OMe)_2$] derivatives.

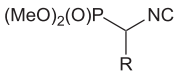
level[20] using the 6-31+G(d) basis[21] (LANLD2Z[22] for the silver cation), and solvent effects in acetone were taken into account by using the SMD[23] solvation model.

The results point out the relevant role of the silver cation in facilitating the deprotonation of both methyl isocyanacetate and dimethyl isocyanomethylphosphonate (Table 2), which reduces the free energy change in the gas phase by around 70 kcal/mol. This effect is slightly diminished in the reaction mixture, as expected from the destabilizing effect of solvation on the ionic pair formed in the metal complex compared to the same process in the absence of silver cation. Nevertheless, the overall stabilization arising from coordination of silver cation to the isocyanide moiety is close to 50 kcal/mol. Hence, the addition of $AgNO_3$ is important for promoting the loss of the proton from the neutral isocyanide reagent, and to enhance the α-position nucleophilicity required for the attack to the preformed iminium salt. Remarkably, the data in Table 2 shows that the isocyanoacetate compounds, **22**, are more acidic than the corresponding PhosMic derivatives, **23**, which agrees with the larger reactivity found experimentally by methyl isocyanoacetate compared with dimethyl isocyanomethylphosphonate (see above).

The results in Table 2 show that the phenyl derivative is found to be the more acidic compound in acetone within the series of isocyanoacetate and dimethyl isocyanomethylphosphonate compounds, and that this trend is not altered by coordination with silver cation. This finding reflects the stabilizing effect of the phenyl group due to the delocalization of the negative charge in the aromatic ring. It can then be hypothesized that this factor compensates the higher steric demand of the phenyl group in the addition reaction that leads to compounds **14**–**18** (Fig. 4). In contrast, the methyl derivative of dimethyl isocyanomethylphosphonate has the lowest acidity in acetone both in the presence and absence of the silver cation. This factor likely explains the failure of this compound to yield the expected product. Hence, activation of PhosMic by deprotonation via the amine present in the reaction mixture seems to be the key factor for the outcome of the studied IMCRs.

To confirm these trends, the transition state for the nucleophilic attack of the isocyanomethylphosphonate (R=H, Me, Ph) anion to the trimethylated iminium salt has been located. The geometrical features are similar in the three cases. Thus, the iminium salt stacks against the molecular plane of the isocyanomethylphosphonate, as noted in the angles (between 96 and 104°) defined by the C···C bond formed between the interacting reagents and the nitrogen atoms of the iminium and isocyanide moieties, which adopt an antiperiplanar arrangement (see Table 3 and Fig. 7). The formation of the C···C bond is slightly more advanced (by 0.37 Å) in the reaction with **b** (R=Ph), which is also noted in the larger pyramidalization of the carbon atoms in the interacting moieties. Compared to the unsubstituted isocyanomethylphosphonate, the barrier for the addition reaction to the phenyl derivative is increased by 3 kcal/

**Table 3**
Selected geometrical properties of the transition states obtained between the different isocyanomethylphosphonate (R=H, Me, Ph) anion and the iminium moiety




**23a-c**

| Property | 23a | 23b | 23c |
|---|---|---|---|
| $d$(C···C)[a] | 2.69 | 2.69 | 2.32 |
| $\alpha_1$(N–C···C)[b] | 103.6 | 100.8 | 104.0 |
| $\alpha_2$(C···C–N)[c] | 96.2 | 98.0 | 102.9 |
| $\gamma$(N–C···C–N)[d] | 179.2 | 178.5 | 172.7 |
| $\tau_1$($C_{isocyanide}$)[e] | 18.4 | 18.0 | 21.4 |
| $\tau_2$($C_{iminium}$)[f] | 7.3 | 8.3 | 17.2 |
| $\Delta G^{\#}$[g] | 6.8 | 5.6 | 9.9 |

a, R=H; b, R=methyl; c, R=phenyl.
[a] Distance (Å) between the carbon atoms involved in bond formation.
[b] Angle (degrees) formed between the carbon atoms of the forming bond and the nitrogen atom of the isocyanide moiety.
[c] Angle (degrees) formed between the carbon atoms of the forming bond and the nitrogen atom of the iminium moiety.
[d] Dihedral angle formed between N–C bonds in the isocyanide and imininum reagents.
[e] Pyramidalization (degrees) of the isocianide carbon atom of the forming bond.
[f] Pyramidalization (degrees) of the iminium carbon atom of the forming bond.
[g] Free energy barrier (kcal/mol) relative to separated reactants in solution.

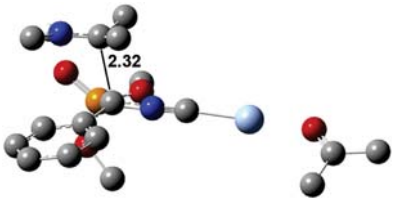



**Fig. 7.** Representation of the transition state for the addition reaction of the iminium salt (modelled with $R_1$=$R_2$=$R_3$=Me) to the phenyl derivative of PhosMic.

mol. Even though this finding reflects the larger steric hindrance arising from the bulkier phenyl group, the observed experimental outcome of these IMCRs confirms that the deprotonation of the corresponding isocyanomethylphosphonate derivative is the limiting factor of these reactions under the mild reaction conditions (MW, 10 min, 40 °C) used in this study.

## 3. Conclusions

In this work (2-imidazolin-4-yl)phosphonates are accessed by a MCR involving PhosMic. The combination of three reactants generates unprecedented (2-imidazolin-4-yl)phosphonate derivatives containing all the atoms of the starting materials. The better outcome obtained with methyl isocyanoacetate compared with PhosMic could be attributed to the increased acidity of the hydrogen atom at the α-position in the ester derivative, compared with the phosphonate derivative, which allowed the reaction to proceed smoothly. This factor also explains the larger reactivity of the phenyl derivative of PhosMic compared to the non-substituted and methyl-substituted counterparts. The atom economy, efficiency and mild conditions (MW, 10 min, 40 °C) in combination with no need of additional solvents would justify a place of this procedure in the toolbox of sustainable synthetic methodologies.

## 4. Experimental section

### 4.1. General experimental

NMR spectra were recorded in $CDCl_3$ at 400 MHz ($^1$H) and 100.6 MHz ($^{13}$C), and chemical shifts are reported in $\delta$ values downfield from TMS or relative to residual chloroform (7.26 ppm, 77.0 ppm) as an internal standard. Data are reported in the following manner: chemical shift, multiplicity, coupling constant ($J$) in hertz (Hz), integrated intensity. Multiplicities are reported using the following abbreviations: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet and br s, broad signal. Evaporation of solvents was accomplished with a rotary evaporator. Thin layer chromatography was done on $SiO_2$ (silica gel 60 F254), and the spots were located by UV, 1% aqueous $KMnO_4$. Chromatography refers to flash column chromatography and was carried out on $SiO_2$ (silica gel 60, SDS, 230–400 mesh). Mass spectra were recorded on an LTQ spectrometer using electrospray (ES+) ionization techniques. The CEM-Discover® Focused Monomode Microwave reactor (2450 MHz, 300 W) was used for microwave continuous flow irradiation. The temperature threshold was set at 40 °C.

### 4.2. General procedure

A mixture of silver nitrate (10%-mmol), ketone, amine (1.5 equiv) and PhosMic (1 equiv) was stirred under microwave irradiation at 40 °C for 10 min. The mixture was filtered and evaporated to yield a residue, which was purified by flash column chromatography ($CH_2Cl_2$/5% MeOH).

Following the general procedure but in the presence of $MgSO_4$ (1.8 equiv) compounds **1**–**10** were obtained with the yields depicted in Table 1.

*4.2.1. Diethyl (5,5-dimethyl-1-propyl-4,5-dihydro-1H-imidazol-4-yl) phosphonate (**1**).* Following the general procedure, $AgNO_3$ (6.8 mg, 0.04 mmol), acetone (3.0 mL), propylamine (50 μL, 0.6 mmol) and PhosMic (64 μL, 0.4 mmol) afforded **1** (58 mg, 53%) as a yellowish oil. IR (NaCl) 3423, 2973, 2934, 1629, 1376, 1245, 1024, 971 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 0.87 (t, $J$=7.0 Hz, 3H, $CH_2CH_3$), 1.25 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.27 (s, 3H, $CH_3$), 1.29 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.33 (s, 3H, $CH_3$), 1.49–1.58 (m, 2H, $NCH_2CH_2$), 2.85–2.98 (m, 2H, $NCH_2$), 3.87 (dd, $J$=16.0, 2.0 Hz, 1H, H-4), 4.09–4.19 (m, 4H, $OCH_2CH_3$), 6.89 (s, 1H, H-2); $^{13}$C NMR (100.6 MHz) $\delta$ 11.4 ($CH_2CH_2CH_3$), 16.4 (d, $J$=6.0 Hz, $OCH_2CH_3$), 16.5 (d, $J$=6.0 Hz, $OCH_2CH_3$), 21.1 (d, $J$=8.0 Hz, $CH_3$), 24.0 ($CH_2CH_2CH_3$), 27.7 (d, $J$=11.0 Hz, $CH_3$), 43.1 ($NCH_2$), 61.7 (d, $J$=7.0 Hz, $OCH_2CH_3$), 62.7 (d, $J$=7.0 Hz, $OCH_2CH_3$), 64.2 (d, $J$=3.0 Hz, C-5), 73.1 (d, $J$=164.0 Hz, C-4), 156.2 (d, $J$=13.0 Hz, C-2); MS-EI $m/z$ 276 (3), 261 (7), 140 (10), 139 (100), 97 (28); HRMS $C_{12}H_{26}N_2O_3P$ $[M+H]^+$ 277.1679; found, 277.1676.

*4.2.2. Diethyl (1-cyclohexyl-5,5-dimethyl-4,5-dihydro-1H-imidazol-4-yl)phosphonate (**2**).* Following the general procedure, $AgNO_3$ (6.8 mg, 0.04 mmol), acetone (3.0 mL), cyclohexylamine (69 μL, 0.6 mmol) and PhosMic (64 μL, 0.4 mmol) afforded **2** (79 mg, 62%) as a yellowish oil. IR (NaCl) 3439, 2932, 2856, 1623, 1586, 1244, 1028, 966 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.22 (m, 1H, H-3′/H-5′), 1.28 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.30 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.36 (s, 3H, $CH_3$), 1.38–1.42 (m, 1H, H-2′/H-6′), 1.44 (s, 3H, $CH_3$), 1.58–1.64 (m, 2H, H-4′), 1.75–1.83 (m, 2H, H-3′/H-5′ or H-2′/H-6′), 2.87 (dddd, 1H, $J$=12.0, 12.0, 3.0, 3.0 Hz, H-1′), 3.97 (dd, 1H, $J$=13.0, 2.0 Hz, H-4), 4.06–4.24 (m, 4H, $OCH_2CH_3$), 7.74 (br s, 1H, H-2); $^{13}$C NMR (100.6 MHz) $\delta$ 16.4 (d, $J$=6.0 Hz, $OCH_2CH_3$), 16.5 (d, $J$=6.0 Hz, $OCH_2CH_3$), 21.7 (d, $J$=7.0 Hz, $CH_3$), 24.8 (C-4′), 25.9 (d, $J$=3.0 Hz, C-3′ and C-5′), 28.2 (d, $J$=12.0 Hz, $CH_3$), 35.3 (d, $J$=24.5 Hz, C2′ and C-6′), 52.3 (C-1′), 62.4 (d, $J$=7.0 Hz, $OCH_2CH_3$), 63.4 (d, $J$=7.0 Hz, $OCH_2CH_3$), 66.3 (d, $J$=1.5 Hz, C-5), 68.2 (d, $J$=161.5 Hz, C-4), 154.3 (d, $J$=10.0 Hz, C-2); MS-EI $m/z$ 316 (10), 301 (26), 179 (100), 163 (10), 97 (78); HRMS $C_{15}H_{30}N_2O_3P$ $[M+H]^+$ 317.1994; found, 317.1989.

*4.2.3. Diethyl [1-(2-ethylhexyl)-5,5-dimethyl-4,5-dihydro-1H-imidazol-4-yl]phosphonate (**3**).* Following the general procedure, $AgNO_3$

(6.8 mg, 0.04 mmol), acetone (3.0 mL), 2-ethyl-1-hexylamine (98 μL, 0.6 mmol) and PhosMic (64 μL, 0.4 mmol) afforded **3** (123 mg, 89%) as a yellow oil. IR (NaCl) 3440, 2960, 2872, 1682, 1593, 1237, 1055, 965 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 0.87 (t, $J$=7.5 Hz, 3H, $CH_2CH_3$), 0.89 (t, $J$=7.5 Hz, 3H, $CH_2CH_3$), 1.25–1.39 (m, 9H), 1.34 (s, 6H, $CH_3$), 2.75–2.95 (m, 2H, $NCH_2$), 3.94 (dd, $J$=15.5, 2.0 Hz, 1H, H-4), 4.16–4.26 (m, 4H, $OCH_2CH_3$), 6.90 (d, $J$=1.0 Hz, 1H, H-2); $^{13}C$ NMR (100.6 MHz) $\delta$ 10.4 ($CH_3$), 14.0 ($CH_3$), 16.4 (d, $J$=5.5 Hz, $OCH_2CH_3$), 16.5 (d, $J$=5.5 Hz, $OCH_2CH_3$), 20.9 (dd, $J$=9.0, 7.0 Hz, $CH_3$), 22.9 (d, $J$=1.5 Hz, $CH_2$), 23.5 (d, $J$=7.5 Hz, $CH_2$), 27.4 (dd, $J$=11.5, 6.0 Hz, $CH_3$), 28.5 (d, $J$=1.5 Hz, $CH_2$), 30.3 (d, $J$=4.5 Hz, $CH_2$), 39.6 (CH), 45.2 (d, $J$=3.0 Hz, $NCH_2$), 61.8 (d, $J$=7.0 Hz, $OCH_2CH_3$), 62.5 (d, $J$=7.0 Hz, $OCH_2CH_3$), 64.5 (d, $J$=3.0 Hz, C-5), 73.0 (d, $J$=164.0 Hz, C-4), 157.0 (dd, $J$=14.0, 4.0 Hz, C-2); MS-EI $m/z$ 346 (3), 331 (15), 210 (15), 209 (100), 97 (39); HRMS $C_{17}H_{36}N_2O_3P$ $[M+H]^+$ 347.2457; found, 347.2458.

*4.2.4. Diethyl [1-(adamantan-1-yl)methyl-5,5-dimethyl-4,5-dihydro-1H-imidazol-4-yl]phosphonate (**4**).* Following the general procedure, $AgNO_3$ (6.8 mg, 0.04 mmol), acetone (3.0 mL), 1-adamantanemethylamine (106 μL, 0.6 mmol) and PhosMic (64 μL, 0.4 mmol) afforded **4** (110 mg, 72%) as a yellow oil. IR (NaCl) 3457, 2978, 2904, 2847, 1590, 1253, 1029, 964 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.30 (s, 6H, $2CH_3$), 1.35 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.36 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.48 (br s, 4H, $2CH_2$), 1.56–1.73 (m, 8H, $4CH_2$), 2.00 (br s, 3H, 3CH), 2.52 (d, $J$=14.5 Hz, 1H, $CH_2$), 2.65 (d, $J$=14.5 Hz, 1H, $CH_2$), 3.86 (dd, $J$=15.5, 2.0 Hz, 1H, H-4), 4.17–4.25 (m, 4H, $OCH_2CH_3$), 6.89 (t, $J$=2.0 Hz, 1H, H-2); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.4 (d, $J$=6.0 Hz, $OCH_2CH_3$), 16.5 (d, $J$=6.0 Hz, $OCH_2CH_3$), 20.7 (d, $J$=6.5 Hz, $CCH_3$), 26.7 (d, $J$=10.0 Hz, $CH_3$), 28.1 (3CH), 33.6 (C), 36.7 ($3CH_2$), 40.7 ($3CH_2$), 53.6 ($CH_2N$), 61.9 (d, $J$=6.8 Hz, $OCH_2CH_3$), 62.5 (d, $J$=6.8 Hz, $OCH_2CH_3$), 66.0 (d, $J$=3.5 Hz, C-5), 72.0 (d, $J$=164.0 Hz, C-4), 159.2 (d, $J$=18.0 Hz, C-2); MS-EI $m/z$ 382 (4), 367 (23), 245 (100), 149 (23), 135 (4); HRMS $C_{21}H_{37}N_2O_3P$ $[M+H]^+$ 383.2466; found, 383.2458.

*4.2.5. Diethyl (1-benzyl-5,5-dimethyl-4,5-dihydro-1H-imidazol-4-yl) phosphonate (**5**).* Following the general procedure, $AgNO_3$ (6.8 mg, 0.04 mmol), acetone (3.0 mL), benzylamine (65 μL, 0.6 mmol) and PhosMic (64 μL, 0.4 mmol) afforded **5** (85 mg, 66%) as a yellowish oil. IR (NaCl) 2980, 2932, 1719, 1677, 1594, 1389, 1231, 1052, 966, 711 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.23 (s, 3H, $CH_3$), 1.27 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.28 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.36 (s, 3H, $CH_3$), 3.94 (dd, $J$=16.0, 2.0 Hz, 1H, H-4), 4.08–4.20 (m, 6H, $OCH_2CH_3$, $CH_2Ar$), 6.78 (br s, 1H, H-2), 7.20–7.29 (m, 5H, ArH); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.4 (d, $J$=4.0 Hz, $OCH_2CH_3$), 16.5 (d, $J$=4.0 Hz, $OCH_2CH_3$), 21.2 (d, $J$=7.0 Hz, $CH_3$), 27.7 (d, $J$=7.0 Hz, $CH_3$), 45.6 ($CH_2Ph$), 61.8 (d, $J$=7.0 Hz, $OCH_2CH_3$), 62.7 (d, $J$=7.0 Hz, $OCH_2CH_3$), 64.2 (d, $J$=3.0 Hz, C-5), 73.6 (d, $J$=163.0 Hz, C-4), 127.5 (2CHAr), 127.6 (CHAr), 128.6 (2CHAr), 138.0 (C-*ipso*), 156.7 (d, $J$=13.5 Hz, C-2); MS-EI $m/z$ 324 (5), 309 (18), 188 (11), 187 (80), 92 (8), 91 (100), 65 (9); HRMS $C_{16}H_{26}N_2O_3P$ $[M+H]^+$ 325.1676; found, 325.1676.

*4.2.6. Diethyl [5,5-dimethyl-1-(1-phenethyl)-4,5-dihydro-1H-imidazol-4-yl]phosphonate (**6**).* Following the general procedure, $AgNO_3$ (6.8 mg, 0.04 mmol), acetone (3.0 mL), phenethylamine (75 μL, 0.6 mmol) and PhosMic (64 μL, 0.4 mmol) afforded **6** (120 mg, 89%) as a yellowish oil. IR (NaCl) 3444, 2978, 1718, 1592, 1391, 1244, 1054, 965 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, HETCOR) $\delta$ 1.29 (s, 3H, $CH_3$), 1.34 (t, $J$=6.8 Hz, 3H, $OCH_2CH_3$), 1.35 (t, $J$=6.8 Hz, 3H, $OCH_2CH_3$), 1.39 (s, 3H, $CH_3$), 2.85 (t, $J$=7.2 Hz, 2H, $CH_2Ar$), 3.18–3.34 (m, 2H, $NCH_2$), 3.90 (dd, $J$=16.5, 2.0 Hz, 1H, H-4), 4.15–4.26 (m, 4H, $OCH_2CH_3$), 6.82 (br d, 1H, H-2), 7.17–7.24 (m, 3H, ArH), 7.27–7.32 (m, 2H, ArH); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.4 (d, $J$=6.0 Hz, $OCH_2CH_3$), 16.5 (d, $J$=6.0 Hz, $OCH_2CH_3$), 21.2 (d, $J$=7.0 Hz, $CH_3$), 27.6 (d, $J$=11.0 Hz, $CH_3$), 37.7 ($CH_2Ar$), 43.1 ($CH_2N$), 61.8 (d, $J$=7.0 Hz, $OCH_2CH_3$), 62.9 (d, $J$=7.0 Hz, $OCH_2CH_3$), 64.3 (d, $J$=3.0 Hz, C-5), 73.0 (d, $J$=164.5 Hz, C-4), 126.7 (CHAr), 128.6 (2CHAr), 128.7 (2CHAr), 138.4 (C-*ipso*), 156.1 (d, $J$=14.0 Hz, C-2); MS-EI $m/z$ 339 (1), 338 (7), 323 (23), 247 (16), 202 (15), 201 (100), 109 (4), 105 (47), 97 (8), 91 (9); HRMS $C_{17}H_{27}N_2O_3P$ $[M+H]^+$ 339.1838; found, 339.1832.

*4.2.7. Diethyl [1-(2-(1H-indol-3-yl)ethyl-5,5-dimethyl-4,5-dihydro-1H-imidazol-4-yl]phosphonate (**7**).* Following the general procedure, $AgNO_3$ (6.8 mg, 0.04 mmol), acetone (3.0 mL), tryptamine (96 mg, 0.6 mmol) and PhosMic (64 μL, 0.4 mmol) afforded **7** (130 mg, 87%) as a yellowish oil. IR (NaCl) 3228, 2978, 1589, 1231, 1052, 967, 743 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.31 (s, 3H, $CH_3$), 1.32 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.33 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.42 (s, 3H, $CH_3$), 2.97 (t, $J$=7.0 Hz, 1H, $CH_2$), 3.28–3.38 (m, 2H, $CH_2N$), 3.95 (dd, $J$=16.0, 2.0 Hz, 1H, H-4), 4.14–4.25 (m, 4H, $OCH_2CH_3$), 6.81 (s, 1H, H-2), 7.02 (d, $J$=1.5 Hz, 1H, H-2′), 7.09 (t, $J$=7.0 Hz, 1H, H-5′), 7.16 (t, $J$=7.0 Hz, 1H, H-6′), 7.37 (d, $J$=8.0 Hz, 1H, H-7′), 7.52 (d, $J$=7.6 Hz, 1H, H-4′), 9.13 (br s, 1H, NH); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.4 (d, $J$=6.0 Hz, $OCH_2CH_3$), 16.5 (d, $J$=6.0 Hz, $OCH_2CH_3$), 21.4 (d, $J$=7.0 Hz, $CH_3$), 27.3 ($CH_2In$), 27.8 (d, $J$=12.0 Hz, $CH_3$), 42.0 ($CH_2N$), 62.0 (d, $J$=7.0 Hz, $OCH_2CH_3$), 63.0 (d, $J$=7.0 Hz, $OCH_2CH_3$), 64.5 (d, $J$=3.0 Hz, C-5), 72.3 (d, $J$=163.5 Hz, C-4), 111.6 (d, $J$=3.5 Hz, C-7′), 118.1 (C-4′), 119.2 (C-5′), 121.8 (C-6′), 122.9 (C-2′), 126.9 (CIn), 136.5 (CIn), 156.4 (d, $J$=13.0 Hz, C-2); MS-EI $m/z$ 377 (14), 249 (11), 248 (82), 247 (39), 235 (52), 218 (26), 206 (21), 144 (100), 143 (40), 138 (219), 130 (62), 111 (62), 97 (50); HRMS $C_{19}H_{29}N_3O_3P$ $[M+H]^+$ 378.1941; found, 378.1941.

*4.2.8. Diethyl [1-(1-(adamantan-1-yl)ethyl-5,5-dimethyl-4,5-dihydro-1H-imidazol-4-yl]phosphonate (**8**).* A mixture of $AgNO_3$ (8.00 mg, 0.05 mmol), acetone (3.6 mL), 1-(1-adamantyl)ethylamine (128 mg, 0.71 mmol) and PhosMic (75 μL, 0.47 mmol) was stirred at rt for 24 h. The reaction mixture was evaporated to afford a mixture 3:1 of the two diastereisomers of **8** calculated by NMR. The residue was purified by column chromatography (132 mg, 71%) and the diastereomers were separated for analytical purposes. *Diastereomer a*: IR (NaCl) 3445, 2905, 2849, 1589, 1236, 1055, 964 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.18 (d, $J$=7.0 Hz, 3H, $CHCH_3$), 1.20 (s, 3H, $CH_3$), 1.34 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.35 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.45–1.50 (m, 4H, $2CH_2$), 1.52 (s, 3H, $CH_3$), 1.55–1.72 (m, 8H, $4CH_2$), 2.01 (br s, 3H, 3CH), 2.60 (q, $J$=7.0 Hz, 1H, $CHCH_3$), 3.95 (dd, $J$=15.0, 2.0 Hz, 1H, H-4), 4.10–4.24 (m, 4H, $2OCH_2CH_3$), 7.07 (d, $J$=2.0 Hz, 1H, H-2); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.4 (d, $J$=6.5 Hz, $OCH_2CH_3$), 16.5 (d, $J$=6.5 Hz, $OCH_2CH_3$), 16.6 ($CHCH_3$), 22.6 (d, $J$=7.5 Hz, $CCH_3$), 28.0 (d, $J$=21.0 Hz, $CH_3$), 28.3 (3 CH), 29.6 (C), 36.6 ($2CH_2$), 36.8 ($2CH_2$), 39.1 ($2CH_2$), 56.4 ($CHCH_3$), 62.1 (d, $J$=7.0 Hz, $OCH_2CH_3$), 62.2 (d, $J$=7.0 Hz, $OCH_2CH_3$), 65.0 (d, $J$=4.0 Hz, C-5), 71.7 (d, $J$=152.0 Hz, C-4), 156.3 (d, $J$=7.0 Hz, C-2); MS-EI $m/z$ 396 (5), 381 (28), 262 (13), 261 (100), 260 (16), 259 (69), 245 (5), 163 (34), 124 (9), 123 (26), 107 (8), 93 (13); HRMS $C_{21}H_{37}N_2O_3P$ $[M+H]^+$ 397.2619; found, 397.2615. *Diastereomer b*: IR (NaCl) 2978, 2905, 2848, 1585, 1029, 962 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.18 (d, $J$=7.0 Hz, 3H, $CHCH_3$), 1.26 (s, 3H, $CH_3$), 1.36 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.37 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.40 (s, 3H, $CH_3$), 1.45–1.47 (m, 4H, $2CH_2$), 1.58–1.72 (m, 8H, $4CH_2$), 2.00 (br s, 3H, 3CH), 2.52 (q, $J$=7.0 Hz, 1H, $CHCH_3$), 3.81 (dd, $J$=16.0, 2.5 Hz, 1H, H-4), 4.23 (m, 4H, $2OCH_2CH_3$), 7.06 (s, 1H, H-2); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.3 ($CHCH_3$), 16.4 (d, $J$=6.0 Hz, $OCH_2CH_3$) 16.5 (d, $J$=6.0 Hz, $OCH_2CH_3$), 20.8 (d, $J$=6.0 Hz, $CH_3$), 27.4 (d, $J$=7.0 Hz, $CH_3$), 28.4 (3CH), 29.7 (C), 36.5 ($2CH_2$), 36.8 ($2CH_2$), 39.3 ($2CH_2$), 56.8 ($CHCH_3$), 61.8 (d, $J$=7.0 Hz, $OCH_2CH_3$), 62.8 (d, $J$=7.0 Hz, $OCH_2CH_3$), 67.1 (d, $J$=3.0 Hz, C-5), 71.3 (d, $J$=171.0 Hz, C-4), 156.3 (d, $J$=18.0 Hz,

C-2); MS-EI *m*/*z* 396 (9), 381 (830), 261 (100), 160 (19), 259 (91), 163 (43), 135 (11), 123 (29), 107 (10), 98 (33); HRMS $C_{21}H_{37}N_2O_3P$ $[M+H]^+$ 397.2620; found, 397.2615.

*4.2.9. Diethyl [5,5-dimethyl-1-(1-phenylethyl)-4,5-dihydro-1H-imidazol-4-yl]phosphonate (**9**).* A mixture of $AgNO_3$ (6.8 mg, 0.04 mmol), acetone (3.0 mL), 1-phenylethylamine (77 μL, 0.6 mmol) and PhosMic (64 μL, 0.4 mmol) was stirred at rt for 48 h. The reaction mixture was evaporated to afford a mixture 1:1 of the two diastereisomers of **9** calculated by GC/MS. The residue was purified by column chromatography (81 mg, 60%) and the diastereomers were separated for analytical purposes. *Diastereomer a*: IR (NaCl) 3457, 2978, 1716, 1589, 1251, 1028, 965, 702, 572 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.15 (s, 3H, $CH_3$), 1.36 (t, $J$=7.0 Hz, 6H, $OCH_2CH_3$), 1.48 (s, 3H, $CH_3$), 1.61 (d, $J$=7.0 Hz, 3H, $CH_3CH$), 3.95 (dd, $J$=16.5, 2.0 Hz, 1H, H-4), 4.15–4.23 (m, 2H, $OCH_2CH_3$), 4.22–4.29 (m, 2H, $OCH_2CH_3$), 4.34 (q, $J$=7.0 Hz, 1H, $CH_3CH$), 7.21–7.35 (m, 6H, ArH and H-2); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.4 (d, $J$=6.0 Hz, $OCH_2CH_3$), 16.5 (d, $J$=6.0 Hz, $OCH_2CH_3$), 21.5 (d, $J$=7.0 Hz, $CH_3$), 24.1 ($CH_3CH$), 28.5 (d, $J$=10.0 Hz, $CH_3$), 51.3 ($CH_3CH$), 61.8 (d, $J$=7.0 Hz, $OCH_2CH_3$), 62.8 (d, $J$=7.0 Hz, $OCH_2CH_3$), 64.8 (d, $J$=4.0 Hz, C-5), 73.3 (d, $J$=165.5 Hz, C-4), 125.8 (2CHAr), 127.3 (CHAr), 128.7 (2CHAr), 144.2 (C-*ipso*), 153.9 (d, $J$=15.0 Hz, C-2); MS-EI *m*/*z* 338 (8), 323 (15), 201 (40), 105 (100), 97 (619), 79 (129), 77 (10); HRMS $C_{17}H_{28}N_2O_3P$ $[M+H]^+$ 339.1837; found, 339.1832. *Diastereomer a*: 3444, 2979, 2932, 1681, 1589, 1455, 1241, 1052, 967 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.28 (s, 3H, $CH_3$), 1.29 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.32 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.39 (s, 3H, $CH_3$), 1.60 (d, $J$=7.0 Hz, 3H, $CH_3CH$), 4.02 (dd, $J$=16.0, 2.0 Hz, 1H, H-4), 4.12–4.20 (m, 4H, $2OCH_2CH_3$), 4.37 (q, $J$=7.0 Hz, 1H, $CH_3CH$), 7.20–7.35 (m, 6H, H-2, ArH); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.4 (d, $J$=5.5 Hz, $2OCH_2CH_3$), 22.4 (d, $J$=8.0 Hz, $CH_3$), 24.3 ($CH_3CH$), 28.3 (d, $J$=15.0 Hz, $CH_3$), 51.3 ($CH_3CH$), 62.0 (d, $J$=7.0 Hz, $OCH_2CH_3$), 62.6 (d, $J$=7.0 Hz, $OCH_2CH_3$), 64.6 (d, $J$=3.0 Hz, C-5), 72.6 (d, $J$=158.5 Hz, C-4), 125.9 (2CHAr), 127.4 (CHAr), 128.7 (2CHAr), 143.5 (C-*ipso*), 153.4 (d, $J$=11.0 Hz, C-2); MS-EI *m*/*z* 338 (3), 323 (8), 201 (30), 106 (9), 105 (100), 97 (68); HRMS $C_{17}H_{28}N_2O_3P$ $[M+H]^+$ 339.1832; found, 339.1836.

*4.2.10. Diethyl [5,5-dimethyl-1-(2-methyl-2-phenylethyl)-4,5-dihydro-1H-imidazol-4-yl]phosphonate (**10**).* Following the general procedure, $AgNO_3$ (6.8 mg, 0.04 mmol), acetone (3.0 mL), 2-methyl-2-phenylethylamine (87 μL, 0.6 mmol) and PhosMic (64 μL, 0.4 mmol) afforded **10** (97 mg, 69%) as a 1:1 mixture of diastereoisomers that could not be separated by column chromatography. $^1H$ NMR (400 MHz, $CDCl_3$, HETCOR) $\delta$ 1.16 (s, 3H, $CH_3$), 1.29–1.37 (m, 9H, $3CH_3$, $OCH_2CH_3$), 2.90 (m, 1H, $CHCH_3$), 3.02–3.24 (m, 2H, $NCH_2$), 3.84 (dd, $J$=13.0, 2.0 Hz, 1H, H-4 one diast), 3.89 (dd, $J$=13.0, 2.0 Hz, 1H, H-4 one diast), 4.12–4.22 (m, 4H, $OCH_2CH_3$), 6.60 (t, $J$=2.0 Hz, 1H, H-2 one diast), 6.74 (t, $J$=2.0 Hz, 1H, H-2 one diast), 7.17–7.34 (m, 5H, ArH); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.4 (d, $J$=6.0 Hz, $OCH_2CH_3$), 16.5 (d, $J$=6.0 Hz, $OCH_2CH_3$), 18.8 ($CHCH_3$ one diast), 19.0 ($CHCH_3$ one diast), 21.1 (d, $J$=7.5 Hz, $CH_3$ one diast), 21.1 (d, $J$=7.5 Hz, $CH_3$ one diast), 27.3 (d, $J$=11.0 Hz, $CH_3$ one diast), 27.5 (d, $J$=11.0 Hz, $CH_3$ one diast), 40.9 ($CHCH_3$ one diast), 41.0 ($CHCH_3$ one diast), 49.1 ($CH_2$Ar one diast), 49.2 ($CH_2$Ar one diast), 61.8 (d, $J$=7.0 Hz, $OCH_2CH_3$ one diast), 61.9 (d, $J$=7.0 Hz, $OCH_2CH_3$ one diast), 62.7 (d, $J$=7.0 Hz, $OCH_2CH_3$ one diast), 62.8 (d, $J$=7.0 Hz, $OCH_2CH_3$ one diast), 64.5 (d, $J$=6.0 Hz, C-5 one diast), 64.6 (d, $J$=6.0 Hz, C-5 one diast), 72.2 (d, $J$=164.5 Hz, C-4 one diast), 72.3 (d, $J$=163.0 Hz, C-4 one diast), 126.8 (CHAr one diast), 126.9 (CHAr one diast), 127.1 (2CHAr one diast), 127.2 (2CHAr one diast), 128.6 (2CHAr one diast), 128.7 (2CHAr one diast), 143.6 (C-*ipso*, one diast), 143.7 (C-*ipso*, one diast), 156.6 (d, $J$=6.0 Hz, C-2), 156.7 (d, $J$=6.0 Hz, C-2 one diast); MS-EI *m*/*z* 352 (11), 337 (23), 247 (100), 218 (25), 214 (76), 206 (26), 150 (11), 109 (11), 97 (44), 91 (61) one diast; 352 (10), 337 (25), 247 (100), 218 (26), 206 (25), 178 (10), 135 (11), 91 (64) one diastereoisomer.

*4.2.11. Diethyl [5-ethyl-5-methyl-1-(2-phenylethyl)-4,5-dihydro-1H-imidazol-4-yl]phosphonate (**11**).* Following the general procedure, $AgNO_3$ (14 mg, 0.08 mmol), 2-butanone (287 μL, 3.2 mmol), 2-phenylethylamine (151 μL, 1.2 mmol) and PhosMic (128 μL, 0.8 mmol) afforded a 1:1 mixture of the two diastereoisomers of **11** calculated by GC/MS. The residue was purified by column chromatography (168 mg, 59%) and the diastereoisomers were separated for analytical purposes. *Diastereomer a*: IR (NaCl) 2975, 2927, 1719, 1593, 1454, 1389, 1247, 1054, 965 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 0.93 (d, $J$=7.0 Hz, 3H, $CH_2CH_3$), 1.30–1.34 (m, 6H, $OCH_2CH_3$), 1.26 (s, 3H, $CH_3$), 1.72 (m, 1H, $CH_2CH_3$), 1.84 (m, 1H, $CH_2CH_3$), 2.80–2.87 (m, 2H $CH_2$Ar), 3.18 (m, 1H, $CH_2$N), 3.35 (m, 1H, $CH_2$N), 3.92 (dd, $J$=17.5, 1.5 Hz, 1H, H-4), 4.12–4.24 (m, 4H, $OCH_2CH_3$), 6.87 (s, 1H, H-2), 7.14–7.30 (m, 5H, ArH); $^{13}C$ NMR (100.6 MHz) $\delta$ 9.7 ($CH_2CH_3$), 16.4 (d, $J$=6.0 Hz, $OCH_2CH_3$), 16.5 (d, $J$=6.5 Hz, $OCH_2CH_3$), 25.8 (d, $J$=9.0 Hz, $CH_3$), 28.4 (d, $J$=7.0 Hz, $CH_2CH_3$), 37.9 ($CH_2$Ar), 43.8 ($CH_2$N), 61.7 (d, $J$=7.0 Hz, $OCH_2CH_3$), 62.8 (d, $J$=7.0 Hz, $OCH_2CH_3$), 68.4 (d, $J$=4.5 Hz, C-5), 73.0 (d, $J$=166.0 Hz, C-4), 126.7 (CHAr), 128.6 (2CHAr), 128.8 (2CHAr), 138.4 (C-*ipso*), 156.8 (d, $J$=15.0 Hz, C-2); MS-EI *m*/*z* 352 (5), 323 (28), 257 (20), 216 (16), 215 (100), 105 (51), 91 (8). *Diastereomer b*: IR (NaCl) 2975, 2928, 1596, 1454, 1247, 1055, 1029, 965 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 0.76 (d, $J$=7.5 Hz, 3H, $CH_2CH_3$), 1.25–1.30 (m, 6H, $OCH_2CH_3$), 1.44 (s, 3H, $CH_3$), 1.49–1.54 (m, 2H, $CH_2CH_3$), 2.78–2.83 (m, 2H $CH_2$Ar), 3.18 (t, $J$=7.0 Hz, 2H, $CH_2$N), 4.00 (dd, $J$=16.5, 1.5 Hz, 1H, H-4), 4.05–4.19 (m, 4H, $OCH_2CH_3$), 6.87 (s, 1H, H-2), 7.10–7.26 (m, 5H, ArH); $^{13}C$ NMR (100.6 MHz) $\delta$ 7.9 ($CH_2CH_3$), 16.4 (d, $J$=6.0 Hz, $OCH_2CH_3$), 16.5 (d, $J$=6.5 Hz, $OCH_2CH_3$), 20.8 (d, $J$=8.5 Hz, $CH_3$), 32.5 (d, $J$=12.5 Hz, $CH_2CH_3$), 37.5 ($CH_2$Ar), 43.2 ($CH_2$N), 61.8 (d, $J$=7.0 Hz, $OCH_2CH_3$), 63.0 (d, $J$=7.0 Hz, $OCH_2CH_3$), 67.0 (d, $J$=3.5 Hz, C-5), 69.1 (d, $J$=162.5 Hz, C-4), 126.7 (CHAr), 128.7 (2CHAr), 128.8 (2CHAr), 138.3 (C-*ipso*), 156.0 (d, $J$=11.5 Hz, C-2); MS-EI *m*/*z* 352 (7), 324 (8), 323 (100), 295 (13), 267 (37), 216 (16), 215 (100), 111 (10), 105 (61), 91 (13).

*4.2.12. Diethyl (1-phenethyl-1,3-diazaspiro[4.4]non-2-en-4-yl)phosphonate (**12**).* Following the general procedure, $AgNO_3$ (6.8 mg, 0.04 mmol), cyclopentanone (142 μL, 1.6 mmol), phenethylamine (75 μL, 0.6 mmol) and PhosMic (64 μL, 0.4 mmol) afforded **12** (70 mg, 48%) as a yellow oil after 20 min.

The general procedure in MeOH (0.8 mL) afforded **12** (70 mg, 48%) as a yellow oil. IR (NaCl) 3249, 2976, 1680, 1596, 1453, 1237, 1028, 965 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.33 (t, $J$=7.0 Hz, 3H, $CH_2CH_3$), 1.35 (t, $J$=7.0 Hz, 3H, $CH_2CH_3$), 1.60–1.85 (m, 7H, Hcyclop), 2.45 (m, 1H, Hcyclop), 2.86 (tm, $J$=8 Hz, 2H, $CH_2$Ph), 3.29 (tm, $J$=8.0 Hz, 2H, $CH_2$N), 4.04 (dd, $J$=15.0, 1.5 Hz, 1H, H-4), 4.14–4.25 (m, 4H, $OCH_2CH_3$), 6.85 (dd, $J$=3.0, 2.0 Hz, 1H, H-2), 7.18–7.32 (m, 5H, ArH); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.4 (d, $J$=6.0 Hz, $OCH_2CH_3$), 16.5 (d, $J$=6.0 Hz, $OCH_2CH_3$), 23.1 ($CH_2$cyclop), 24.0 ($CH_2$cyclop), 31.1 (d, $J$=7.5 Hz, $CH_2$cyclop), 37.8 ($CH_2$Ar), 39.0 (d, $J$=15.5 Hz, $CH_2$cyclop), 43.7 ($CH_2$N), 61.3 (d, $J$=7.0 Hz, $OCH_2CH_3$), 62.9 (d, $J$=7.0 Hz, $OCH_2CH_3$), 73.8 (d, $J$=157.0 Hz, C-4), 73.9 (d, $J$=2.0 Hz, C-5), 126.6 (CHAr), 128.6 (2CHAr), 128.7 (2CHAr), 138.4 (C-*ipso*), 156.3 (d, $J$=10.5 Hz, C-2); MS-EI *m*/*z* 364 (2), 355 (6), 341 (3), 335 (2), 282 (11), 281 (36), 267 (7), 228 (11), 227 (39), 208 (21), 207 (100), 191 (14), 135 (12), 133 (12), 124 (11), 105 (22), 96 (16); HRMS $C_{19}H_{30}N_2O_3P$ $[M+H]^+$ 365.1989; found, 365.1996.

*4.2.13. Diethyl (1-phenethyl-1,3-diazaspiro[4.5]dec-2-en-4-yl)phosphonate (**13**).* Following the general procedure, $AgNO_3$ (6.8 mg, 0.04 mmol), cyclohexanone (166 μL, 1.6 mmol), phenethylamine

(75 μL, 0.6 mmol) and PhosMic (64 μL, 0.4 mmol) afforded **13** (92 mg, 61%) as a yellow oil.

The general procedure in MeOH (1 mL) afforded **13** (119 mg, 79%) as a yellow oil. IR (NaCl) 3441, 2929, 2857, 1679, 1591, 1453, 1223, 1053, 964, 785 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.02 (m, 1H, Hcycloh), 1.27 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.31 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.21 (m, 1H, Hcycloh), 1.40 (m, 1H, Hcycloh), 1.54–1.71 (m, 5H, Hcycloh), 1.92 (m, 1H, cycloh), 2.28 (dm, $J$=13.5 Hz, 1H, cycloh) 2.73–2.84 (m, 2H, $CH_2Ar$), 3.13–3.31 (m, 2H, $CH_2N$), 4.05–4.20 (m, 5H, $OCH_2CH_3$ and H-4), 6.83 (d, $J$=2.5 Hz, H-2), 7.15–7.18 (m, 3H, ArH), 7.23–7.27 (m, 2H, ArH); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.4 (d, $J$=6.0 Hz, $OCH_2CH_3$), 16.5 (d, $J$=6.0 Hz, $OCH_2CH_3$), 22.0 ($CH_2$cycloh), 23.7 ($CH_2$cycloh), 25.3 ($CH_2$cycloh), 30.6 (d, $J$=8.0 Hz, $CH_2$cycloh), 33.0 (d, $J$=20.0 Hz, $CH_2$cycloh), 38.2 ($CH_2Ar$), 43.3 ($CH_2N$), 61.3 (d, $J$=7.0 Hz, $OCH_2CH_3$), 62.8 (d, $J$=7.0 Hz, $OCH_2CH_3$), 68.1 (d, $J$=2.5 Hz, C-5), 69.5 (d, $J$=150.0 Hz, C-5), 126.5 (CHAr), 128.5 (2CHAr), 128.7 (2CHAr), 138.4 (C-*ipso*), 156.9 (d, $J$=7.0 Hz, C-2); MS-EI $m/z$ 378 (3), 242 (18), 241 (100), 231 (4), 218 (6), 137 (5), 105 (23); HRMS $C_{20}H_{32}N_3O_3P$ $[M+H]^+$ 379.2142; found, 379.2145.

*4.2.14. Diethyl [1-(2-ethylhexyl)-5,5-dimethyl-4-phenyl-4,5-dihydro-1H-imidazol-4-yl]phosphonate (**14**).* Following the general procedure, $AgNO_3$ (6.8 mg, 0.04 mmol), acetone (1.5 mL), 2-ethylhexylamine (49 μL, 0.3 mmol) and α-PhPhosMic (51 mg, 0.2 mmol) afforded **14** (70 mg, 83%) as a yellowish oil. IR (NaCl) 3463, 2960, 1597, 1239, 1058, 961 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 0.51 (d, $J$=2.5 Hz, 3H, $CH_3$), 0.80–0.88 (m, 9H), 1.17–1.30 (m, 10H), 1.35–1.50 (m, 2H), 1.70 (s, 3H, $CH_3$), 2.64 (m, 1H, $CH_2N$), 2.85 (m, 1H, $CH_2N$), 3.30 (m, 1H, $OCH_2CH_3$), 3.60 (m, 1H, $OCH_2CH_3$), 3.95–4.11 (m, 2H, $2OCH_2CH_3$), 6.95 (dd, $J$=12.5, 4.0 Hz, 1H, H-2), 7.18–7.26 (m, 3H, ArH), 7.77–7.80 (m, 2H, ArH); $^{13}C$ NMR (100.6 MHz) $\delta$ 10.3 (d, $J$=13.0 Hz, $CH_3$), 14.0 ($CH_3$), 16.1 (d, $J$=5.0 Hz, $OCH_2CH_3$), 16.3 (d, $J$=5.0 Hz, $OCH_2CH_3$), 22.9 (dd, $J$=15.0, 3.0 Hz, $CH_3$), 23.0 ($CH_2$), 23.1 (d, $J$=38.0 Hz, $CH_2$), 24.1 (dd, $J$=28.0, 8.5 Hz, $CH_3$), 28.5 (d, $J$=7.0 Hz, $CH_2$), 30.2 (d, $J$=30.0 Hz, $CH_2$), 39.8 (d, $J$=10.0 Hz, CH), 45.7 (d, $J$=32.0 Hz, $CH_2N$), 62.4 (dd, $J$=9.0, 2.0 Hz, $OCH_2CH_3$), 63.3 (d, $J$=7.0 Hz, $OCH_2CH_3$), 68.7 (d, $J$=4.5 Hz, C-5), 82.0 (dd, $J$=145.0, 2.0 Hz, C-4), 127.2 (2CHAr), 127.3 (2CHAr), 128.5 (CHAr), 137.2 (d, $J$=4.0 Hz, C-*ipso*), 156.4 (dd, $J$=28.5, 6.0 Hz, C-2); MS-EI $m/z$ 422 (1), 287 (2), 286 (21), 285 (100), 173 (6), 146 (9); HRMS $C_{23}H_{40}N_2O_3P$ $[M+H]^+$ 423.2771; found, 423.2776.

*4.2.15. Diethyl (1-benzyl-5,5-dimethyl-4-phenyl-4,5-dihydro-1H-imidazol-4-yl)phosphonate (**15**).* Following the general procedure, $AgNO_3$ (4.2 mg, 0.024 mmol), acetone (1.8 mL), benzylamine (40 μL, 0.36 mmol) and α-PhPhosMic (62 mg, 0.24 mmol) afforded **15** (60 mg, 61%) as a yellowish oil after purification with column chromatography. IR (NaCl) 2977, 2928, 1599, 1493, 1390, 1241, 1057, 960 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 0.65 (s, 3H, $CH_3$), 0.95 (d, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.28 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.76 (s, 3H, $CH_3$), 3.67–3.47 (m, 1H, $OCH_2CH_3$), 3.68–3.78 (m, 1H, $OCH_2CH_3$), 4.00–4.18 (m, 2H, $OCH_2CH_3$), 4.17 (d, $J$=15.0 Hz, 1H, $CH_2Ar$), 4.26 (d, $J$=15.0 Hz, 1H, $CH_2Ar$), 7.00 (d, $J$=4.0 Hz, 1H, H-2), 7.25–7.39 (m, 8H, ArH), 7.78–7.93 (br d, $J$=27.5 Hz, 2H, ArH); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.1 (d, $J$=5.0 Hz, $OCH_2CH_3$), 16.4 (d, $J$=6.0 Hz, $OCH_2CH_3$), 23.0 (d, $J$=15.0 Hz, $CH_3$), 24.2 (d, $J$=8.5 Hz, $CH_3$), 46.1 ($CH_2Ar$), 62.8 (d, $J$=8.5 Hz, $OCH_2CH_3$), 63.3 (d, $J$=7.0 Hz, $OCH_2CH_3$), 68.6 (d, $J$=4.5 Hz, C-5), 82.0 (d, $J$=146.5 Hz, C-4), 127.3 (CHAr), 127.4 (d, $J$=2.5 Hz, CHAr), 127.5 (CHAr), 127.6 (CHAr), 128.2 (2CHAr), 128.6 (2CHAr), 137.2 (C-*ipso*), 138.1 (C-*ipso*), 156.3 (d, $J$=6.0 Hz, C-2); MS-EI $m/z$ 400 (0.1), 281 (1), 264 (20), 263 (100), 248 (2), 207 (2), 148 (2); HRMS $C_{22}H_{30}N_2O_3P$ $[M+H]^+$ 401.1989; found, 401.1990.

*4.2.16. Diethyl (5,5-dimethyl-1-phenethyl-4-phenyl-4,5-dihydro-1H-imidazol-4-yl)phosphonate (**16**).* Following the general procedure, $AgNO_3$ (3.4 mg, 0.02 mmol), acetone (1.5 mL), phenethylamine (38 μL, 0.3 mmol) and α-PhPhosMic (51 mg, 0.2 mmol) afforded **16** (64 mg, 78%) as a yellowish oil. IR (NaCl) 3060, 2978, 1698, 1595, 1494, 1391, 1239, 1030, 962 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, HETCOR) $\delta$ 0.61 (s, 3H, $CH_3$), 0.97 (t, $J$=7.5 Hz, 3H, $OCH_2CH_3$), 1.28 (t, $J$=7.5 Hz, 3H, $OCH_2CH_3$), 1.83 (s, 3H, $CH_3$), 2.86–2.96 (m, 2H, $CH_2Ar$), 3.19 (m, 1H, $CH_2N$), 3.28 (m, 1H, $CH_2N$), 3.45 (m, 1H, $OCH_2CH_3$), 3.70 (m, 1H, $OCH_2CH_3$), 4.03–4.07 (m, 2H, $OCH_2CH_3$), 7.07 (d, $J$=4.0 Hz, 1H, H-2), 7.20–7.34 (m, 8H, ArH), 7.82–7.84 (br s, 2H, ArH); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.1 (d, $J$=6.0 Hz, $OCH_2CH_3$), 16.5 (d, $J$=6.0 Hz, $OCH_2CH_3$), 23.3 (d, $J$=15.0 Hz, $CH_3$), 24.1 (d, $J$=9.0 Hz, $CH_3$), 37.9 ($CH_2Ar$), 43.8 ($CH_2N$), 62.8 (d, $J$=8.5 Hz, $OCH_2CH_3$), 63.2 (d, $J$=7.0 Hz, $OCH_2CH_3$), 68.5 (d, $J$=4.0 Hz, C-5), 81.5 (d, $J$=147.0 Hz, C-4), 126.7, 127.3, 128.6 (10CHAr), 137.1 (C-*ipso*), 138.5 (C-*ipso*), 155.6 (d, $J$=6.0 Hz, C-2); MS-EI $m/z$ 414 (0.3), 399 (1), 278 (25), 277 (100), 262 (2), 250 (1), 173 (2), 162 (3), 158 (2), 144 (2), 131 (3), 105 (14); HRMS $C_{23}H_{32}N_2O_3P$ $[M+H]^+$ 415.2145; found, 415.2146.

*4.2.17. Diethyl [1-(2-(1H-indol-3-yl)ethyl)-5,5-dimethyl-4-phenyl-4,5-dihydro-1H-imidazol-4-yl]phosphonate (**17**).* Following the general procedure, $AgNO_3$ (3.4 mg, 0.02 mmol), acetone (1.5 mL), tryptamine (48 mg, 0.3 mmol) and α-PhPhosMic (51 mg, 0.2 mmol) afforded **17** (61 mg, 67%) as a yellowish oil. IR (NaCl) 3226, 2978, 1593, 1457, 1230, 1056, 963, 743 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 0.62 (s, 3H, $CH_3$), 0.98 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.27 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.87 (s, 3H, $CH_3$), 2.98–3.11 (m, 2H, $CH_2Ar$), 3.26–3.43 (m, 2H, $CH_2N$), 3.52 (m, 1H, $OCH_2CH_3$), 3.78 (m, 1H, $OCH_2CH_3$), 4.03–4.20 (m, 2H, $OCH_2CH_3$) 7.09–7.10 (m, 2H, H-2, H-2′), 7.12 (ddd, $J$=8.0, 7.0, 1.0 Hz, 1H, H-5′), 7.19 (ddd, $J$=8.0, 7.0, 1.0 Hz, 1H, H-6′), 7.28–7.35 (m, 3H, HAr), 7.39 (dt, $J$=8.0, 1.0 Hz, 1H, H-7′), 7.56 (d, $J$=7.5 Hz, 1H, H-4′), 7.86 (br s, 2H, ArH), 8.86 (s, 1H, NH); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.2 (d, $J$=5.0 Hz, $OCH_2CH_3$), 16.6 (d, $J$=6.0 Hz, $OCH_2CH_3$), 23.5 (d, $J$=14.8 Hz, $CH_3$), 24.2 (d, $J$=8.5 Hz, $CH_3$), 27.5 ($CH_2Ar$), 42.7 ($CH_2N$), 63.0 (d, $J$=8.5 Hz, $OCH_2CH_3$), 63.2 (d, $J$=7.5 Hz, $OCH_2CH_3$), 68.6 (d, $J$=4.0 Hz, C-5), 81.5 (d, $J$=146.5 Hz, C-4), 111.5 (C-7′), 112.2 (CAr), 118.3 (C-4′), 119.3 (C-5′), 122.0 (C-6′), 122.5 (C-2′), 126.9 (CAr),127.4 (d, $J$=2.5 Hz, 3CHAr), 128.2 (CHAr), 128.7 (CHAr), 136.4 (CAr), 137.1 (CAr), 155.8 (d, $J$=6.0 Hz, C-2); MS-EI $m/z$ 453 (1), 317 (26), 316 (100), 173 (7), 144 (65), 130 (9), 115 (4); HRMS $C_{19}H_{29}N_3O_3P$ $[M+H]^+$ 454.2253; found, 454.2254.

*4.2.18. Diethyl (1-phenethyl-4-phenyl-1,3-diazaspiro[4.5]dec-2-en-4-yl)phosphonate (**18**).* Following the general procedure, $AgNO_3$ (3.4 mg, 0.02 mmol), cyclohexanone (83 μL, 0.8 mmol), phenethylamine (38 μL, 0.3 mmol) and α-PhPhosMic (51 mg, 0.2 mmol) in MeOH (0.4 mL) afforded **18** (54 mg, 59%) as a yellow oil. IR (NaCl) 2929, 1599, 1235, 1055, 1028, 961, 700 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 0.70 (m, 1H, Hcycloh), 0.92 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.00–1.15 (m, 3H, Hcycloh), 1.22 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.20–1.30 (m, 2H, Hcycloh), 1.65 (m, 1H, Hcycloh), 1.95 (m, 1H, Hcycloh), 2.10 (m, 1H, cycloh), 2.52 (m, 1H, Hcycloh), 2.78–2.95 (m, 2H, $CH_2Ph$), 3.44–3.55 (m, 3H, $CH_2N$, $OCH_2CH_3$), 3.80 (m, 1H, $OCH_2CH_3$), 3.99–4.12 (m, 2H, $OCH_2CH_3$), 7.08 (d, $J$=4.0 Hz, H-2), 7.10–7.30 (m, 8H, ArH), 7.74 (d, $J$=7.5 Hz, 1H, ArH), 8.08 (d, $J$=9.0 Hz, 1H, ArH); $^{13}C$ NMR (100.6 MHz) $\delta$ 16.1 (d, $J$=5.0 Hz, $OCH_2CH_3$), 16.5 (d, $J$=6.0 Hz, $OCH_2CH_3$), 21.5 ($CH_2$cycloh), 22.5 ($CH_2$cycloh), 24.7 ($CH_2$cycloh), 32.0 (d, $J$=8.5 Hz, $CH_2$cycloh), 33.2 (d, $J$=15.0 Hz, $CH_2$cycloh), 38.6 ($CH_2Ar$), 46.3 ($CH_2N$), 62.7 (d, $J$=8.5 Hz, $OCH_2CH_3$), 63.3 (d, $J$=7.0 Hz, $OCH_2CH_3$), 70.1 (d, $J$=3.0 Hz, C-5), 83.0 (d, $J$=144.0 Hz, C-4), 126.6 (CHAr), 126.9 (d, $J$=2.0 Hz, CHAr), 127.5 (d, $J$=2.0 Hz, CHAr), 128.1 (CHAr), 128.5 (2CHAr), 128.7 (2CHAr), 129.3 (d, $J$=3.0 Hz, CHAr), 129.5 (d, $J$=9.0 Hz, CHAr), 136.9 (C-*ipso*), 138.4 (C-*ipso*), 156.4 (d, $J$=6.0 Hz, C-2); MS-EI $m/z$ 454 (1), 339 (2), 319 (3), 318 (28), 317 (100), 202

(6), 105 (10); HRMS $C_{26}H_{36}N_2O_3P$ $[M+H]^+$ 455.2458; found, 455.2455.

*4.2.19. Methyl 1-benzyl-5,5-dimethyl-4,5-dihydro-1H-imidazole-4-carboxylate (**19**).* A mixture of $MgSO_4$ (80 mg, 0.66 mmol), acetone (3.0 mL), benzylamine (65 μL, 0.6 mmol) and methyl isocyanoacetate (36 μL, 0.4 mmol) was stirred under microwave irradiation at 40 °C for 10 min. The mixture was filtered and evaporated to give a residue, which was purified by column chromatography (EtOAc/5% MeOH/0.33% $Et_3N$) yield **19** (90 mg, 92%). Performance of the reaction in the absence of $MgSO_4$ furnished **19** (92 mg, 94%).[9b]

*4.2.20. Methyl 1,4-dibenzyl-5,5-dimethyl-4,5-dihydro-1H-imidazole-4-carboxylate (**20**).* A mixture of $AgNO_3$ (1.7 mg, 0.01 mmol), acetone (1.5 mL), benzylamine (33 μL, 0.3 mmol) and methyl 2-isocyano-3-phenylpropanoate (38 mg, 0.2 mmol) was stirred under microwave irradiation at 40 °C for 20 min. The mixture was filtered and evaporated to give a residue, which was purified by column chromatography (EtOAc/5% MeOH) yield **20** (41 mg, 62%).[9b]

*4.2.21. Methyl 5,5-dimethyl-1-(1-phenylethyl)-4,5-dihydro-1H-imidazole-4-carboxylate (**21**).* Following the general procedure, $AgNO_3$ (13.6 mg, 0.08 mmol), acetone (6 mL), 1-phenylethylamine (1.62 μL, 1.2 mmol), (38 μL, 0.3 mmol) and methyl isocyanomethylacetate afforded **21** (181 mg, 87%) as a 1:1 mixture of diastereoisomers. *Diastereoisomer a*: IR (NaCl) 2976, 1747, 1594, 1273, 1212 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 1.09 (s, 3H, $CH_3$), 1.12 (s, 3H, $CH_3$), 1.53 (d, $J$=7.0 Hz, 3H, $CHCH_3$), 3.70 (s, 3H, $OCH_3$), 4.23 (q, $J$=7.0 Hz, 1H, $CHCH_3$), 4.33 (d, $J$=2.0 Hz, 1H, H-4), 7.17–7.28 (m, 6H, ArH, H-2); $^{13}C$ NMR (100.6 MHz) $\delta$ 20.4 ($CH_3$), 24.0 ($CHCH_3$), 27.9 ($CH_3$), 51.6 and 51.8 ($CHCH_3$ and $OCH_3$), 65.2 (C-5), 78.6 (C-4), 125.8 (2CHAr), 127.3 (CHAr), 128.7 (2CHAr), 144.0 (C-*ipso*), 153.6 (C-2), 171.6 (CO); MS-EI $m/z$; 260 (5), 202 (4), 201 (33), 106 (9), 105 (100), 103 (8), 97 (42); HRMS $C_{15}H_{21}N_2O_2$ $[M+H]^+$ 261.1603; found, 261.1598. *Diastereoisomer b*: $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) $\delta$ 0.92 (s, 3H, $CH_3$), 1.48 (s, 3H, $CH_3$), 1.63 (d, $J$=7.0 Hz, 3H, $CHCH_3$), 3.70 (s, 3H, $CH_3$), 4.40 (q, $J$=7.0 Hz, 1H, $CHCH_3$), 4.46 (s, 1H, H-4), 7.23–7.36 (m, 5H, H-2), 7.64 (s, 1H, H-2); $^{13}C$ NMR (100.6 MHz) $\delta$ 21.5 ($CH_3$), 24.3 ($CHCH_3$), 27.4 ($CH_3$), 52.0 and 52.3 ($CHCH_3$ and $OCH_3$), 65.8 (C-5), 76.3 (C-4), 125.8 (2CHAr), 127.8 (CHAr), 128.9 (2CHAr), 142.8 (C-*ipso*), 154.8 (C-2), 170.4 (CO); MS-EI $m/z$; 260 (6), 202 (6), 201 (36), 106 (10), 105 (100), 103 (8), 97 (44).

### 4.3. Computational methods

Full geometry optimizations were performed with the M062X[20] density functional method by using the 6-31+G(d)[21] basis set. The LANLD2Z[22] basis, in conjunction with the effective core potential for inner electrons, was used for the silver cation. Solvent effects (acetone) were accounted for by means of the SMD version of the IEFPCM model.[23] The transition states corresponding to the addition of the iminium salt to the isocyanomethylphosphonate derivatives was determined by exploring different orientations of the interacting partners, as well as the attack through the two faces of the iminium compound. The nature of the stationary points was verified by inspection of the vibrational frequencies within the harmonic oscillator approximation. The suitability of this computational scheme is supported from the results determined for similar reactive processes.[10] Calculations were performed by using Gaussian 09.[24]

### Acknowledgements

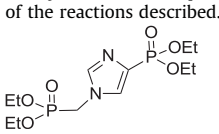
Financial support from the Spanish Ministry of Economy and Competitiveness (MINECO; Project CTQ2012-35250 and SAF2011-27642) and the AGAUR, Generalitat de Catalunya (Grant 2014-SGR-0155 and 2014-SGR-1189) is gratefully acknowledged. Thanks are also due to the MINECO for a fellowship to C. E. (FPDI-2013-15572). The Center for Scientific and Academic Services of Catalonia (CESCA) is acknowledged for the computational facilities.

### Supplementary data

Copies of $^1H$ and $^{13}C$ NMR spectra of new compounds **1**–**18** and **21**. Table with the $^1H$ and $^{13}C$ chemical shifts for compounds **1**–**18**. Optimized geometries of transition states. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2015.03.065.

### References and notes

1. Bousquet, P.; Feldman, J.; Schwartz, J. *J. Pharmacol. Exp. Ther.* **1984**, *230*, 232–236.
2. (a) Dardonville, C.; Rozas, I. *Med. Res. Rev.* **2004**, *24*, 639–661; (b) Vassilew, L. T.; Vu, B. T.; Graves, B.; Carvajal, D.; Padlaski, F.; Pilipovic, Z.; Kong, V.; Kammlott, U.; Lukacs, C.; Klein, C.; Fotouhi, N.; Liu, E. A. *Science* **2004**, *303*, 844–848; (c) Lowry, J. A.; Brown, J. T. *Clin. Toxicol.* **2014**, *52*, 454–469; (d) Krasavin, M. *Eur. J. Med. Chem.* **2014**, 1–13.
3. (a) Cherrn, J. W.; Liaw, Y. C.; Chen, C. S.; Rong, J. G.; Huang, C. L.; Chan, C. H.; Wang, A. H. J. *Heterocycles* **1993**, *36*, 1091–1103; (b) Weiss, M. E.; Fisher, D. F.; Xin, Z. Q.; Jautza, S.; Schweizer, W. B.; Peters, R. *Angew. Chem., Int. Ed.* **2006**, *45*, 5694–5699; (c) Ma, K.; You, J. *Chem.—Eur. J.* **2007**, *13*, 1863–1871; (d) Bon, R. S.; de Kanter, F. J. J.; Lutz, M.; Spek, A. L.; Jahnke, J. C.; Hahn, F. E.; Orru, R. V. A. *Organometallics* **2007**, *26*, 3639–3650; (e) Strassverger, Z.; Mooijman, M.; Ruitjer, E.; Alberts, A. H.; de Graaff, C.; Orru, R. V. A.; Rothenberg, G. *Appl. Organomet. Chem.* **2009**, *24*, 142–146; (f) Murai, K.; Fukushima, S.; Nakamura, A.; Shimura, M.; Fujioka, H. *Tetrahedron* **2011**, *67*, 4862–4868.
4. Liu, H.; Du, D.-M. *Adv. Synth. Catal.* **2009**, *351*, 489–519.
5. For a review of Isocyanides in the synthesis of nitrogen heterocycles, see: Lygin, A. V.; Meijere, A. *Angew. Chem., Int. Ed.* **2010**, *49*, 9094–9124.
6. For a review in catalytic isonitrile insertions and condensations initiated by RNC-X complexation, see: Chakrabarty, S.; Choudhary, S.; Doshi, A.; Liu, F.-Q.; Mohan, R.; Ravindra, M. P.; Shah, D.; Yang, X.; Fleming, F. F. *Adv. Synth. Catal.* **2014**, *356*, 2135–2196.
7. (a) Bienaymé, H.; Hulme, C.; Oddon, G.; Schmitt, P. *Chem.—Eur. J.* **2000**, *6*, 3321–3329; (b) Gaich, T.; Baran, P. S. *J. Org. Chem.* **2010**, *75*, 4657–4673; (c) Dömling, A.; Wang, W.; Wang, K. *Chem. Rev.* **2012**, *112*, 3083–3135; (d) Heijden, G.; Ruitjer, E.; Orru, R. V. A. *Synlett* **2013**, 666–685; (e) Wender, P. A. *Tetrahedron* **2013**, *69*, 7529–7550.
8. (a) Dömling, A. *Chem. Rev.* **2006**, *106*, 17–89; (b) Cioc, R. C.; Ruitjer, E.; Orru, R. V. A. *Green Chem.* **2014**, *16*, 2958–2975.
9. (a) Bon, R. S.; van Vliet, B.; Sprenkels, N. E.; Schmitz, R. F.; de Kanter, F. J. J.; Stevens, C. V.; Swart, M.; Bickelhaupt, F. M.; Groen, M. B.; Orru, R. V. A. *J. Org. Chem.* **2005**, *70*, 3542–3553; (b) Elders, N.; Schmitz, R. F.; de Kanter, F. J. J.; Ruijter, E.; Groen, M. B.; Orru, R. V. A. *J. Org. Chem.* **2007**, *72*, 6135–6142; (c) Elders, N.; Ruijter, E.; de Kanter, F. J. J.; Groen, M. B.; Orru, R. V. A. *Chem.—Eur. J.* **2008**, *14*, 4961–4973.
10. Arróniz, C.; Molina, J.; Abás, S.; Molins, E.; Campanera, J. M.; Luque, F. J.; Escolano, C. *Org. Biomol. Chem.* **2013**, *11*, 1640–1649.
11. (a) Rueping, M.; Vila, C.; Bootwicha, T. *ACS Catal.* **2013**, *3*, 1676–1680; (b) Sinha, M. K.; Khoury, K.; Herdtweck, E.; Dömling, A. *Chem.—Eur. J.* **2013**, *19*, 8048–8052.
12. Moonen, K.; Laureyn, I.; Stevens, C. V. *Chem. Rev.* **2004**, *104*, 6177–6215.
13. (a) Kruithof, A.; Ruitjer, E.; Orru, R. V. A. In *Microwaves in Organic Synthesis*; de la Hoz, A., Loupy, A., Eds.; Wiley: Weinheim, 2002; p 1099; (b) Kappe, C. O.; Dallinger, D. *Mol. Divers* **2009**, *13*, 71–76; (c) Kruithof, A.; Ruitjer, E.; Orru, R. V. A. *Curr. Org. Chem.* **2011**, *11*, 204–212; (d) Moseley, J. D.; Kappe, C. O. *Green Chem.* **2011**, *13*, 794–806; (e) Maumder; Gupta, R.; Jain, A. *Green Chem. Lett. Rev.* **2013**, *6*, 151–182.
14. The cyclodimerization product of the PhosMic reaction was not observed in any of the reactions described. However, this compound was prepared in Ref. 10.




15. Even though water has been reported to activate weakly acidic benzyl isocyanides, no significant difference was found in the yield of the reactions performed upon anhydrous conditions. See: Honey, M. A.; Yamashita, Y.; Kobayashi, S. *Chem. Commun.* **2014**, 3288–3291.
16. (a) Trost, B. M. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 259–281; (b) Trost, B. *Science* **1991**, *254*, 1471–1477; (c) Sheldon, R. A. *Green Chem.* **2007**, *9*, 1273–1283.

17. Performance of the reaction using equimolecular quantities of cyclopentanone and cyclohexanone/PhosMic in MeOH at 0.5 M concentration gave **12** and **13** in 48% and 79%, respectively.
18. (a) Bon, R. S.; Hong, C.; Bouma, M. J.; Schmitz, R. F.; de Kanter, F. J. J.; Lutz, M.; Spek, A. L.; Orru, R. V. A. *Org. Lett.* **2003**, *5*, 3759–3762; (b) Fayol, A.; Housseman, C.; Sun, X.; Janvier, P.; Bienaymé, H.; Zhu, J. *Synthesis* **2005**, 161–165.
19. Performance of the reaction by Orru's group in MeOH at rt 5 h gave 89% isolated yield of the final product (see Ref. 9b).
20. Zhao, Y.; Truhlar, D. G. *Theor. Chem. Acc.* **2008**, *120*, 215–241.
21. (a) Hariharan, P. C.; Pople, J. A. *Theor. Chim. Acta* **1973**, *28*, 213–218; (b) Clark, T.; Chandrasekhar, J.; Spitznagel, G. W.; Schleyer, P. V. R. *J. Comput. Chem.* **1983**, *4*, 294–301; (c) Frisch, M. J.; Pople, J. A.; Binkley, J. S. *J. Chem. Phys.* **1984**, *80*, 3265–3269.
22. (a) Wadt, W. R.; Hay, P. J. *J. Chem. Phys.* **1985**, *82*, 284–298; (b) Hay, P. J.; Wadt, W. R. *J. Chem. Phys.* **1985**, *82*, 299–310.
23. Marenich, A. V.; Cramer, C. J.; Truhlar, D. G. *J. Phys. Chem. B* **2009**, *113*, 6378–6396.
24. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Keith, T.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09, Revision C.01*; Gaussian: Wallingford CT, 2010.

# Easy access to (2-imidazolin-4-yl)phosphonates by a microwave assisted multicomponent reaction

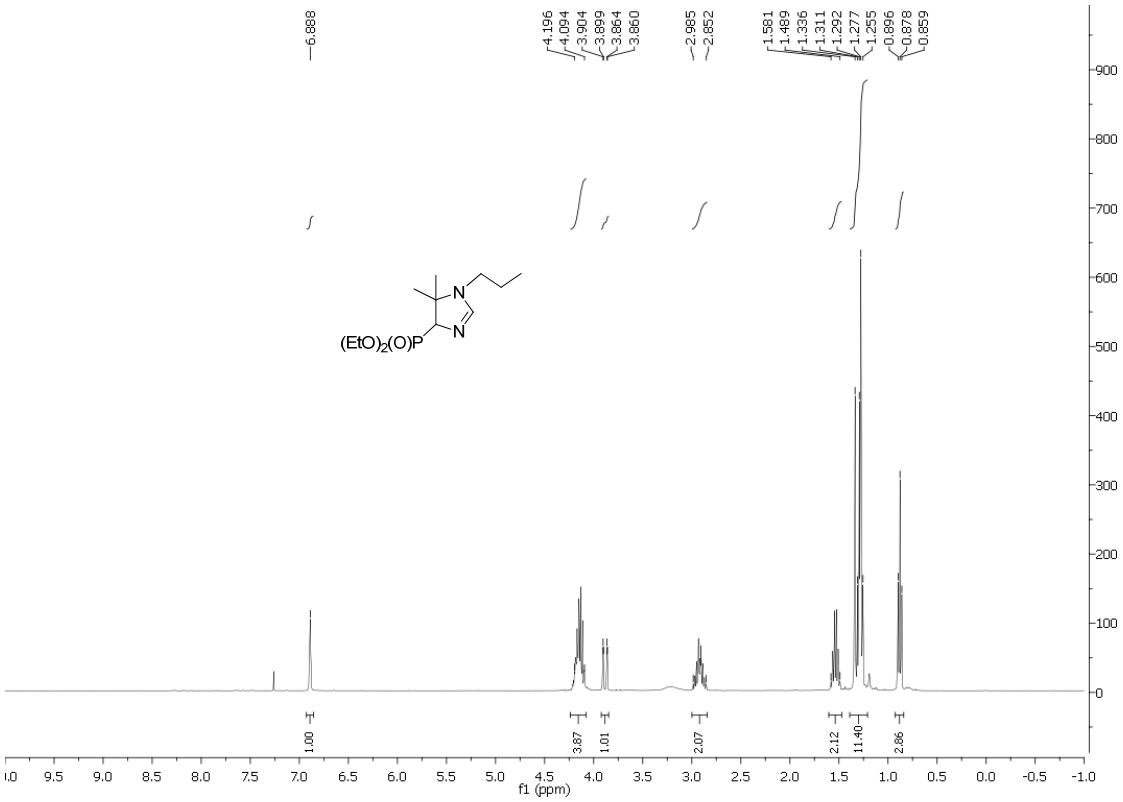
Sònia Abás,[a] Carolina Estarellas,[b] F. Javier Luque,[b] and Carmen Escolano*[,a]

[a] Laboratory of Organic Chemistry, Faculty of Pharmacy, and Institute of Biomedicine (IBUB), University of Barcelona, Spain. E-mail: cescolano@ub.edu.

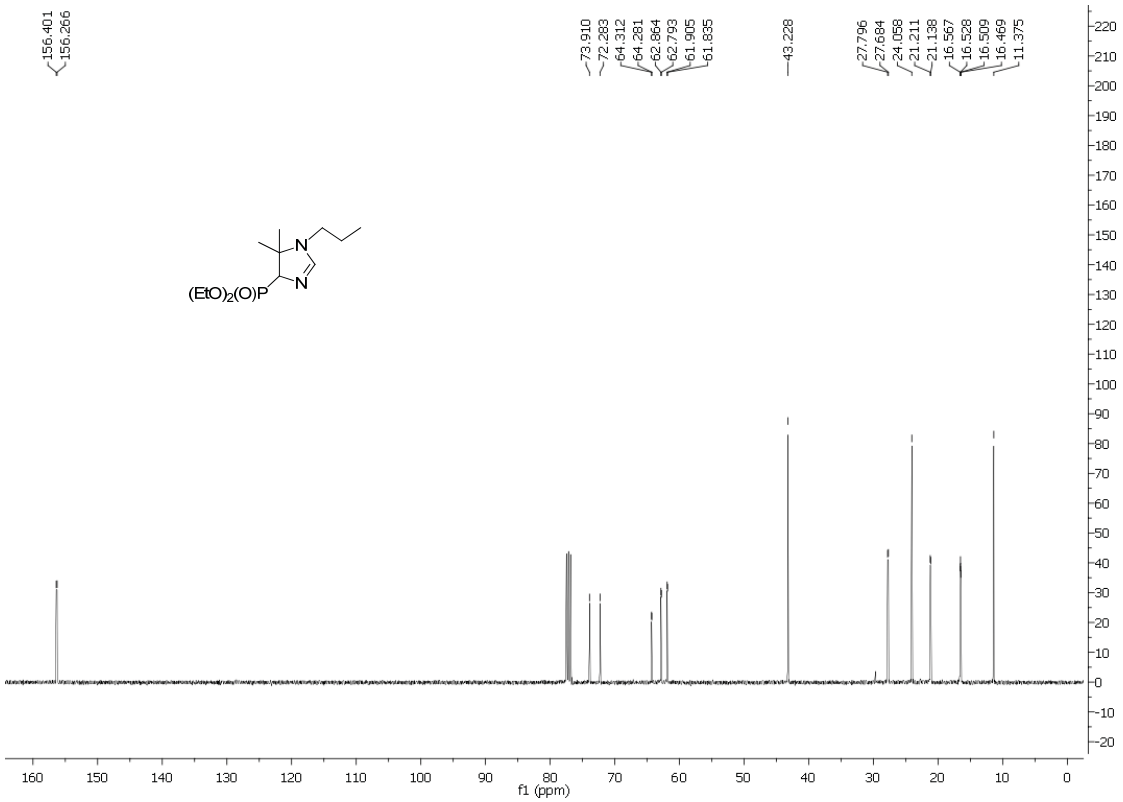
[b] Departament de Fisicoquímica and Institut de Biomedicina (IBUB), Facultat de Farmàcia, Universitat de Barcelona, Campus de l'Alimentació Torribera, Avdga. Prat de la Riba 171, 08921 Santa Coloma de Gramenet, Spain.

Preparation of diethyl α-phenylisocyanomethylphosphonate (ref. 16).

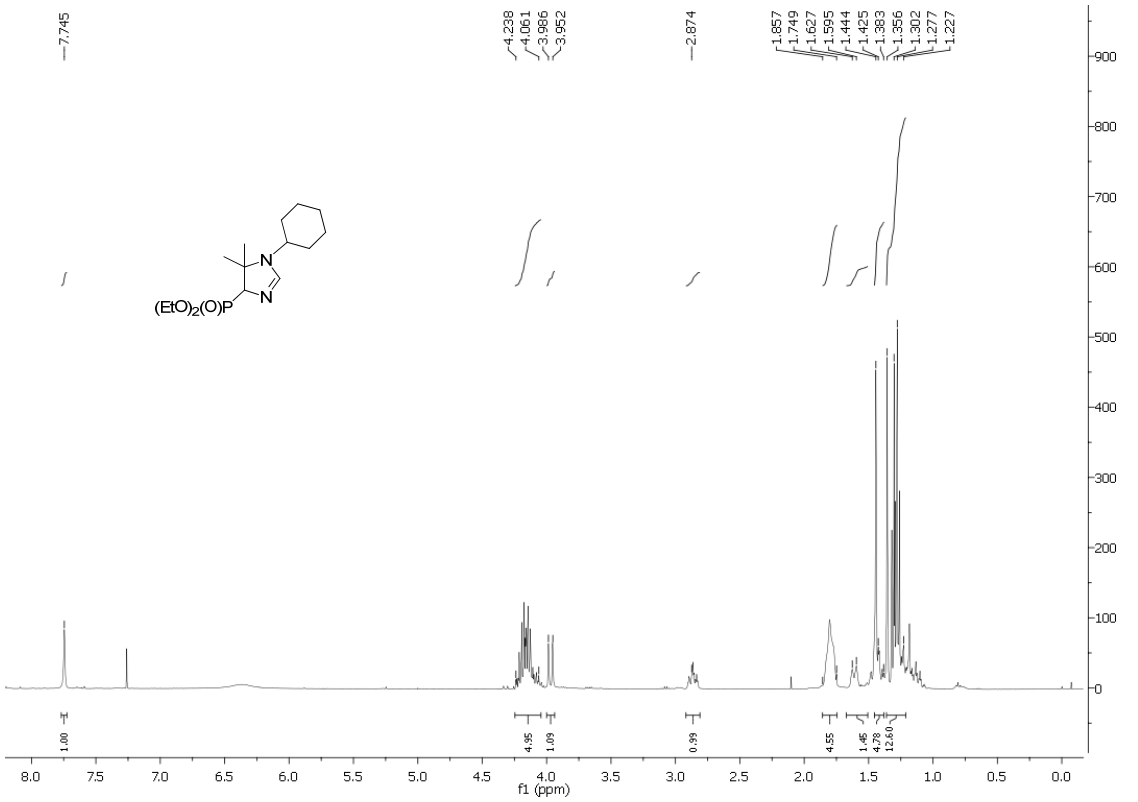
To a solution of diethyl(α-aminobenzyl)phosphonate (841 mg, 3.46 mmol) in HCOOH (6.5 mL), acetic anhydride (2.3 mL, 24.2 mmol) was added dropwise. The reaction was stirred at r.t. for 1 h and the mixture was concentrated *in vacuo* to give the corresponding formamide derivative. $^{1}$H NMR (400 MHz, $CDCl_3$, COSY, HETCOR) δ 1.07 (dd, *J* = 7.0, 0.5 Hz, 3H, $OCH_2CH_3$), 1.34 (dd, *J* = 7.0, 0.5 Hz, 3H, $OCH_2CH_3$), 3.60-3.72 (m, 1H, $OCH_2CH_3$), 3.84-3.94 (m, 1H, $OCH_2CH_3$), 4.11-4.22 (m, 2H, $OCH_2CH_3$), 5.63 (q, *J* = 10.0 Hz, CH), 7.29-7.36 (m, 3H, ArH), 7.47-7.51 (m, 2H, ArH), 8.23 (d, *J* = 1.0 Hz, 1H, H-2); $^{13}$C NMR (100.6 MHz) δ 16.0 ($CH_3$), 16.3 ($CH_3$), 49.2 (d, *J* = 155.0 Hz, $CHCH_3$), 63.2 (d, *J* = 7.0 Hz, $OCH_2$), 63.7 (d, *J* = 7.0 Hz, $OCH_2$), 128.1 (CHAr), 128.2 (CHAr), 128.3 (CHAr), 128.5 (CHAr), 128.6 (CHAr), 134.5 (C-*ipso*), 160.8 (d, *J* = 7.5 Hz, C-2). Then, $Et_3N$ (2.4 mL, 17.3 mmol) and $POCl_3$ (0.5 mL, 5.19 mmol) were added dropwise to a cooled solution (-78ºC) of the formamide in dry THF (9.3 mL), and the reaction mixture was allowed to warm to r.t.. After 2 h, ice cold water was added and the mixture was extracted with $Et_2O$. The combined organic layers were dried and concentrated *in vacuo* to give a residue which was purified by column chromatography (EtOAc-hexane (1.1 to 7:3) to afford α-PhPhosMic (739 mg, 84%) as a yellow oil. IR (NaCl) 2983, 2910, 2138, 1683, 1454, 1261, 1163, 976 $cm^{-1}$; $^{1}$H NMR (400 MHz, $CDCl_3$, COSY, HETCOR) δ 1.26 (td, *J* = 7.0, 1.0 Hz, 3H, $OCH_2CH_3$), 1.31 (td, *J* = 7.0, 1.0 Hz, 3H, $OCH_2CH_3$), 3.97-4.21 (m, 4H, $OCH_2CH_3$), 5.03 (d, *J* = 21.0 Hz, CH), 7.36-7.44 (m, 3H, ArH), 7.46-7.50 (m, 2H, ArH); $^{13}$C NMR (100.6 MHz) δ 16.1 (d, *J* = 5.5 Hz, $OCH_2CH_3$), 16.2 (d, *J* = 5.5 Hz, $OCH_2CH_3$), 54.8 (d, *J* = 152.0 Hz, CH), 64.2 (d, *J* = 7.0 Hz, $OCH_2CH_3$), 64.7 (d, *J* = 7.0 Hz, $OCH_2CH_3$), 127.3 (d, *J* = 5.0 Hz, 2 CHAr), 128.7 (d, *J* = 3.0 Hz, 2 CHAr), 128.9 (d, *J* = 3.0 Hz, CHAr), 129.8 (d, *J* = 6.0 Hz, C-*ipso*), 161.0 (d, *J* = 4.5 Hz, NC) MS-EI *m/z* 253.2 (22), 197. (48), 179 (49), 118 (10), 117 (100), 116 (49), 109 (75), 91 (34); HRMS $C_{12}H_{17}N_2O_3P$ $[M+H]^+$ 254.0941; found, 254.0949.



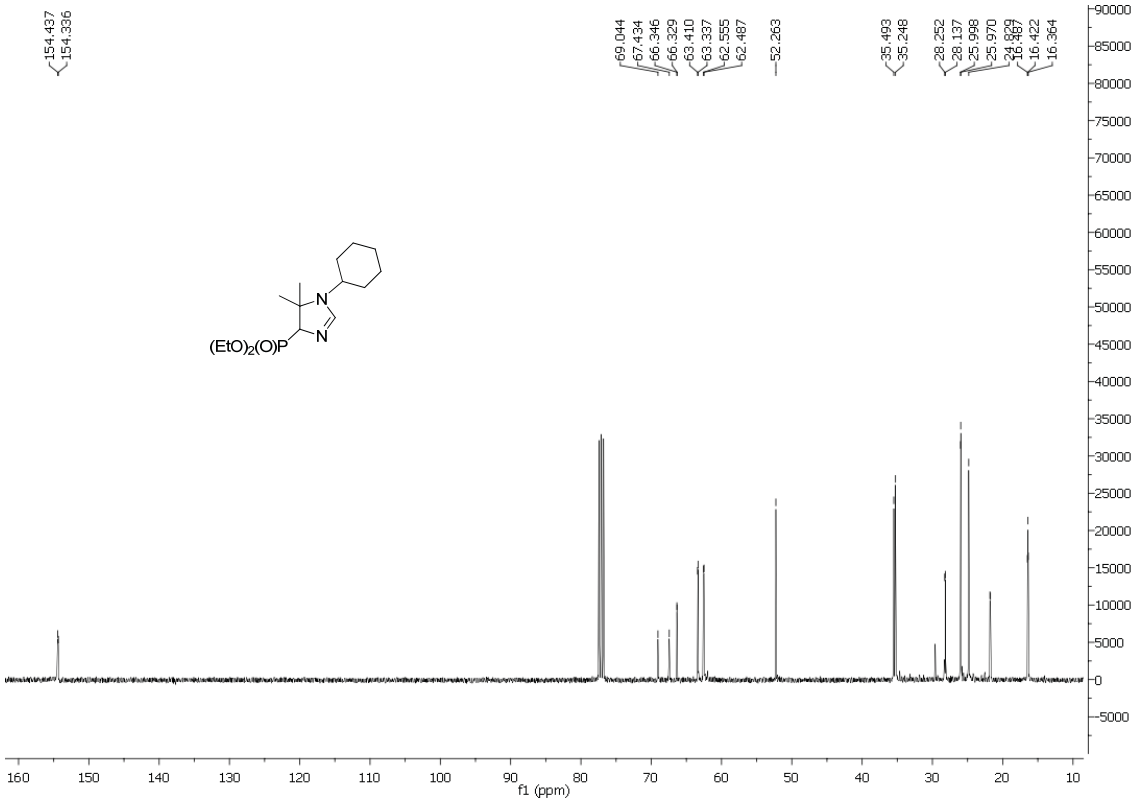

Diethyl (5,5-dimethyl-1-propyl-4,5-dihydro-1*H*-imidazol-4-yl)phosphonate (1)



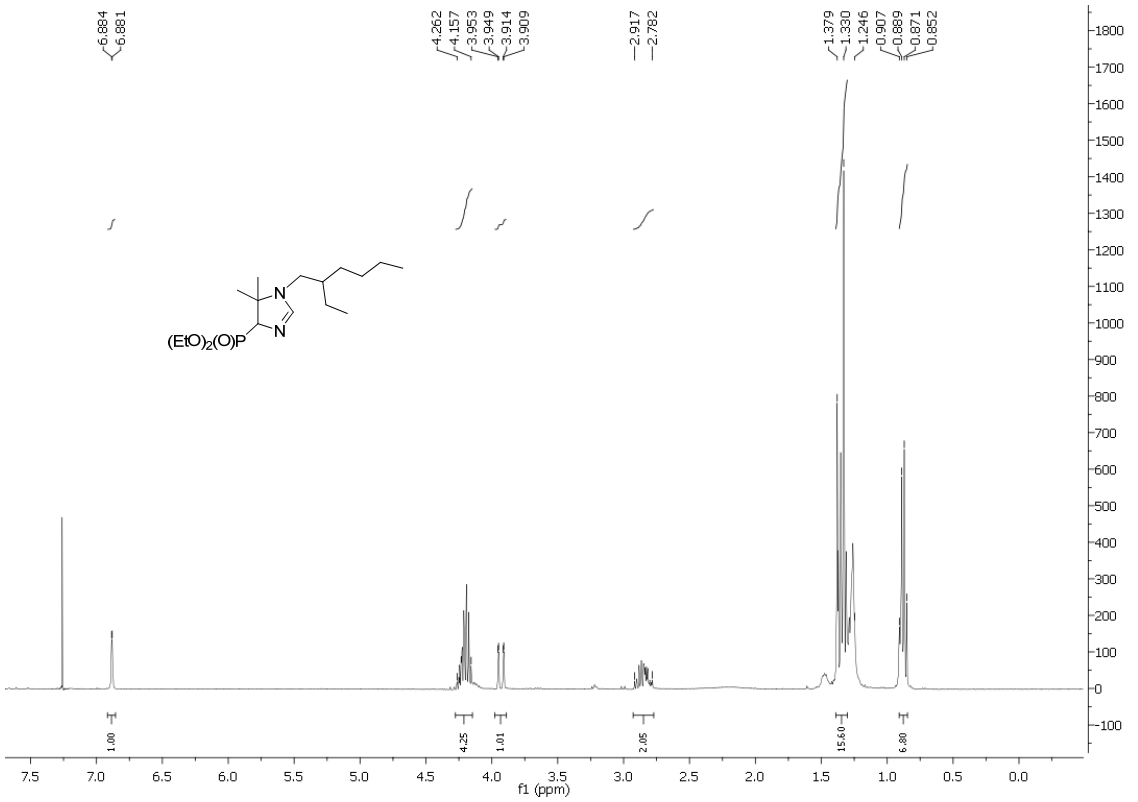

Diethyl (5,5-dimethyl-1-propyl-4,5-dihydro-1*H*-imidazol-4-yl)phosphonate (1)



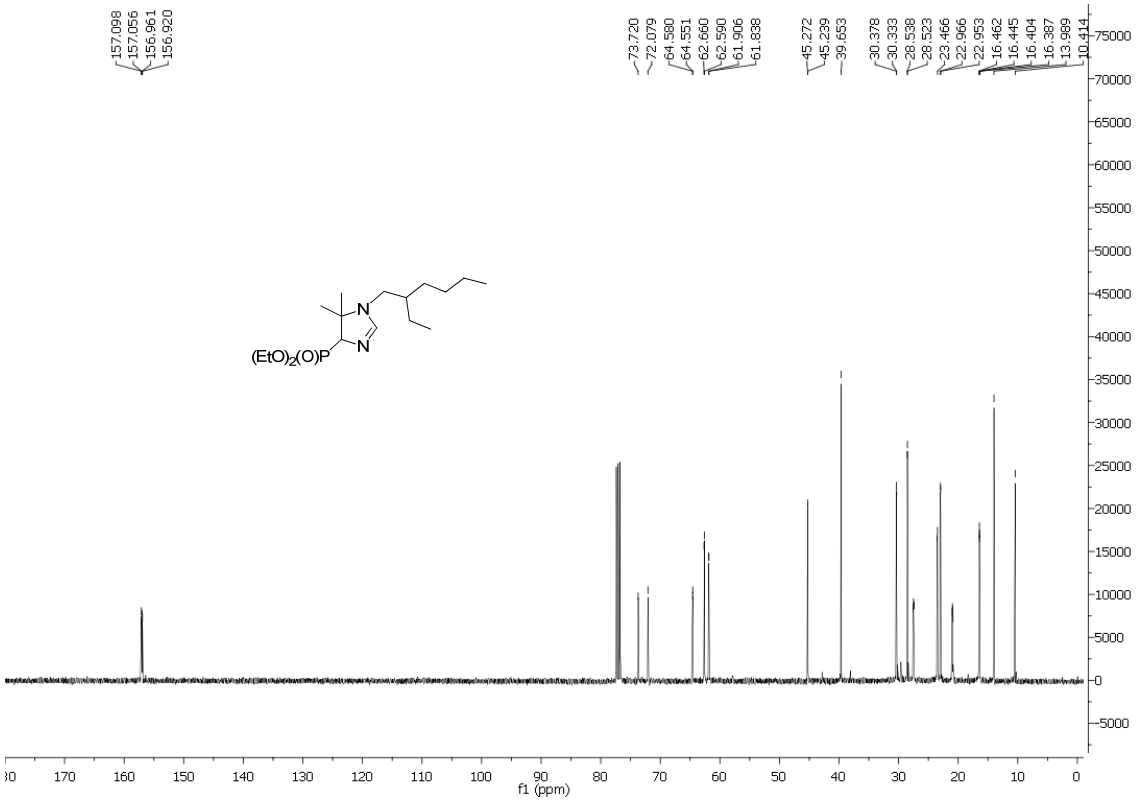

Diethyl (1-cyclohexyl-5,5-dimethyl-4,5-dihydro-1*H*-imidazol-4-yl)phosphonate (2)



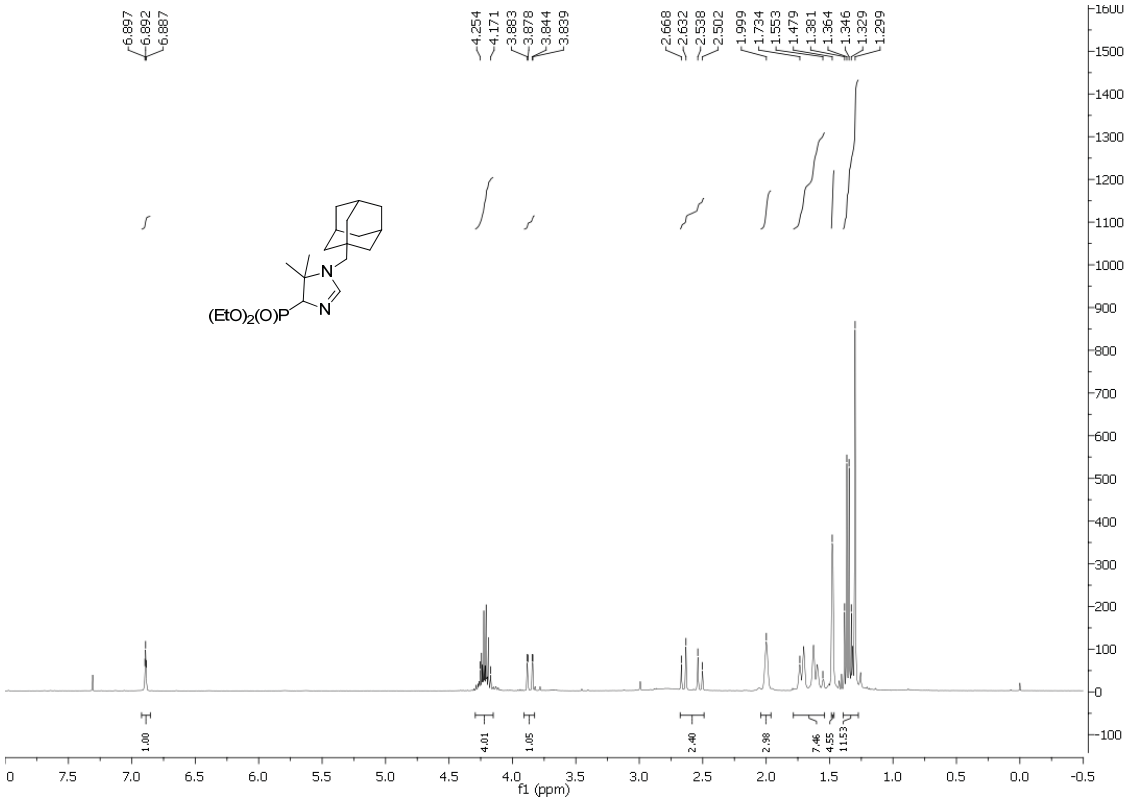

Diethyl (1-cyclohexyl-5,5-dimethyl-4,5-dihydro-1*H*-imidazol-4-yl)phosphonate (2)



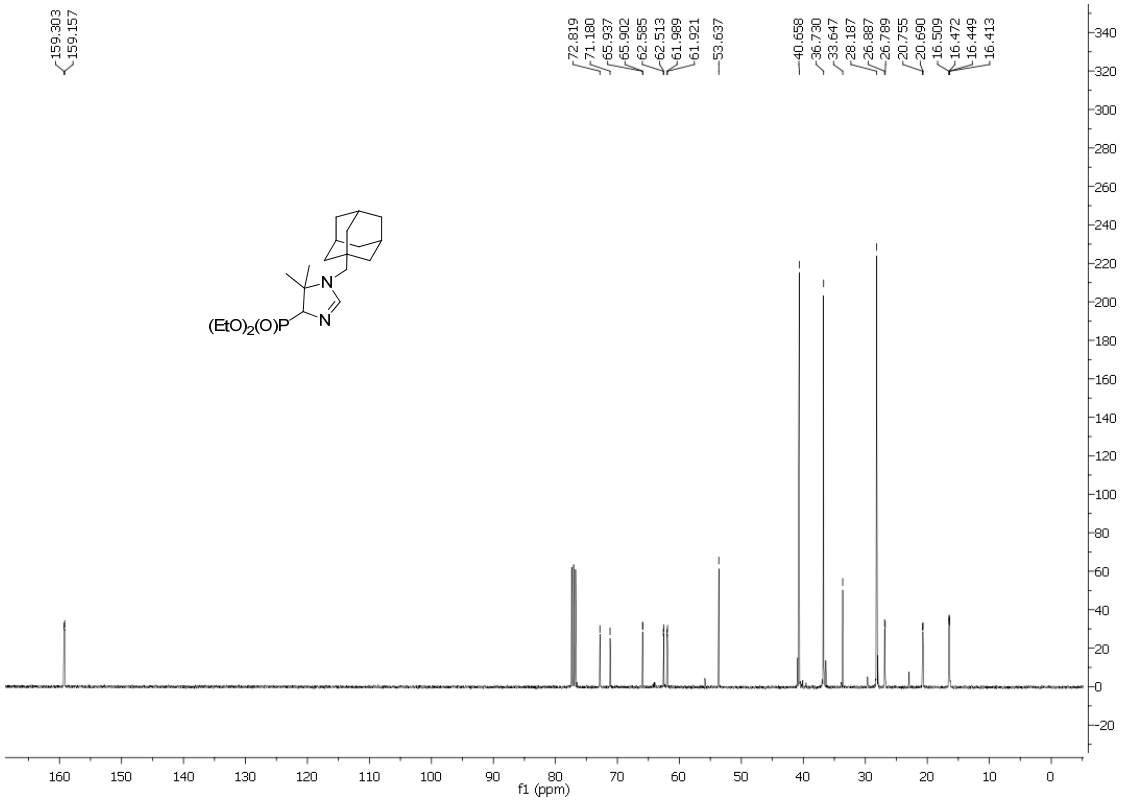

Diethyl [1-(2-ethylhexyl)-5,5-dimethyl-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (3)



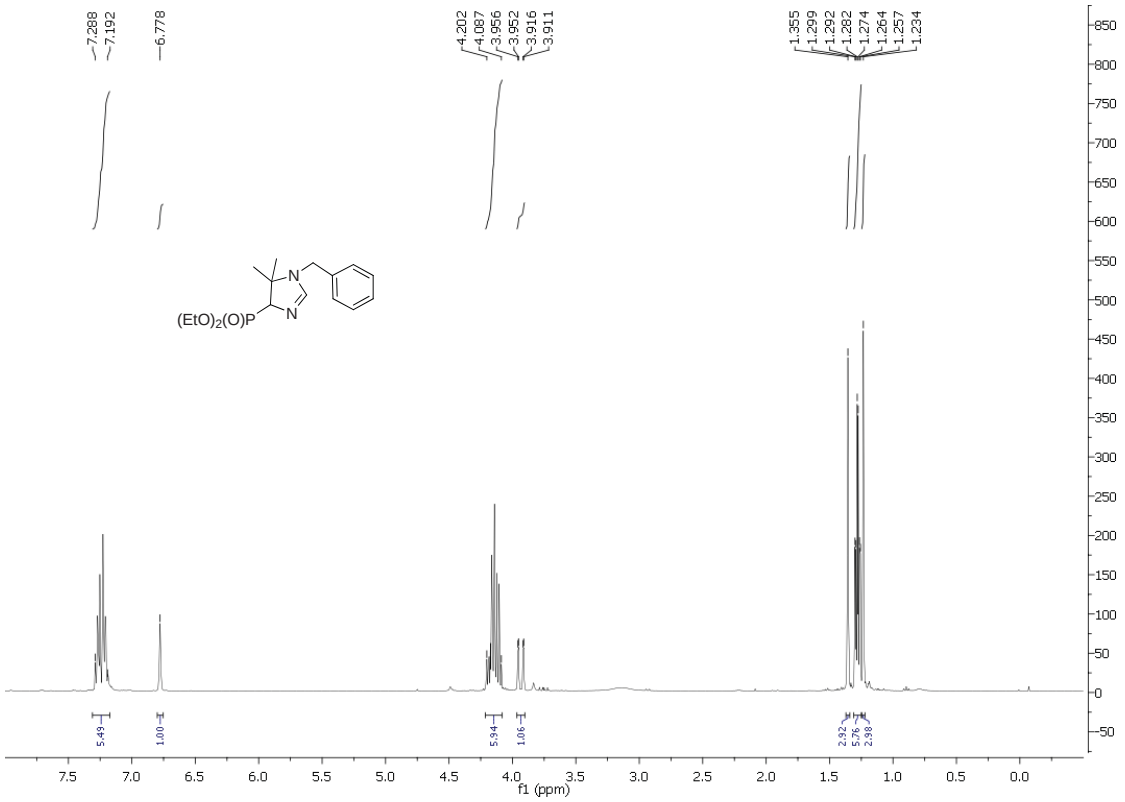

Diethyl [1-(2-ethylhexyl)-5,5-dimethyl-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (3)



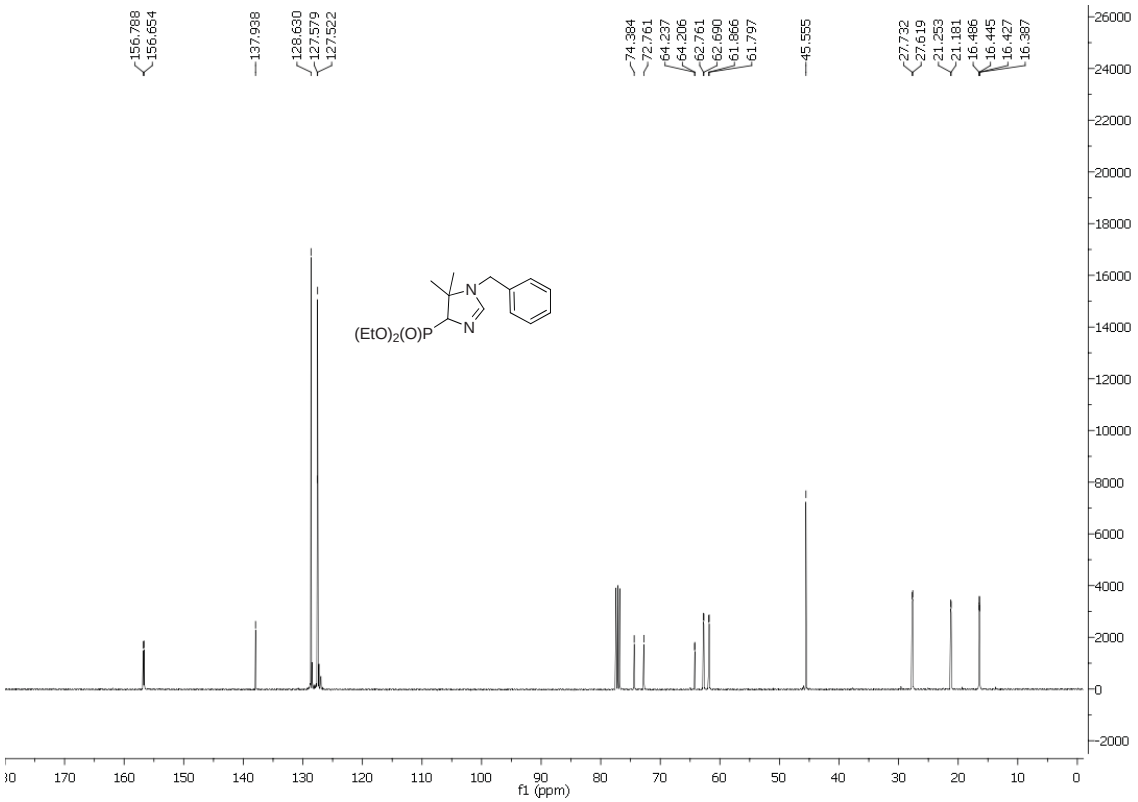

Diethyl [1-(adamantan-1-yl)methyl)-5,5-dimethyl-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (4)



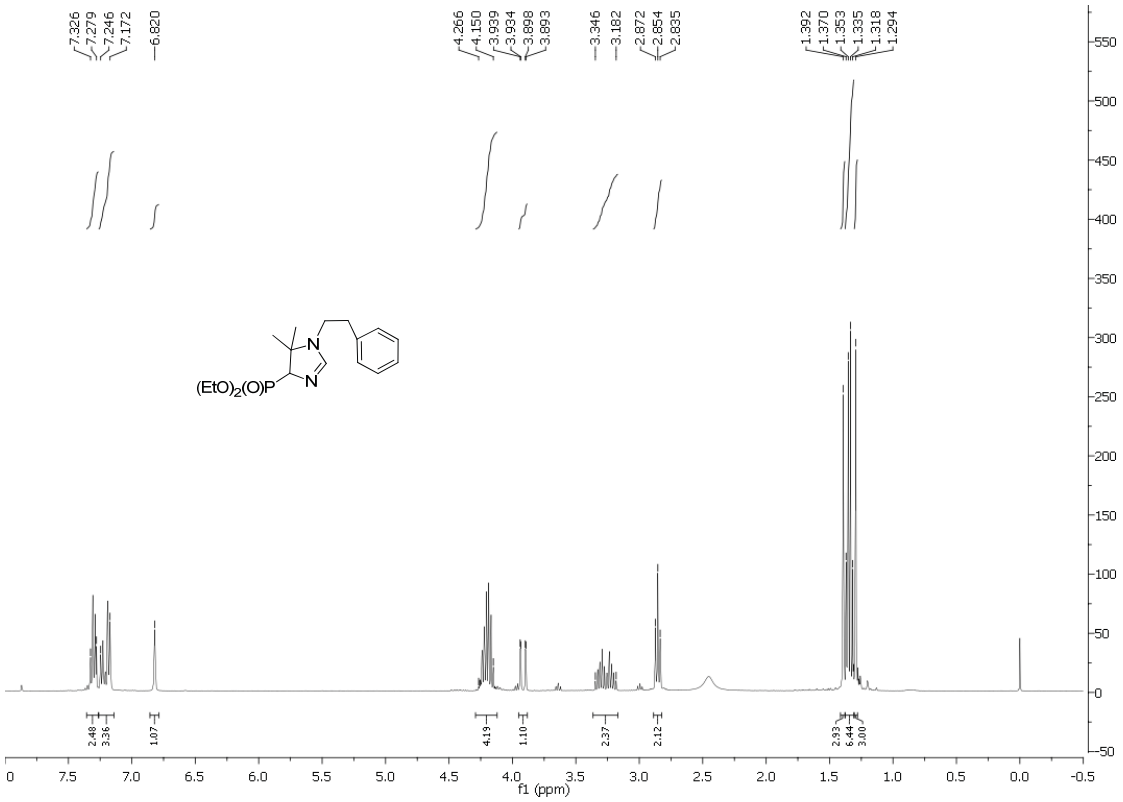

Diethyl [1-(adamantan-1-yl)methyl)-5,5-dimethyl-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (4)



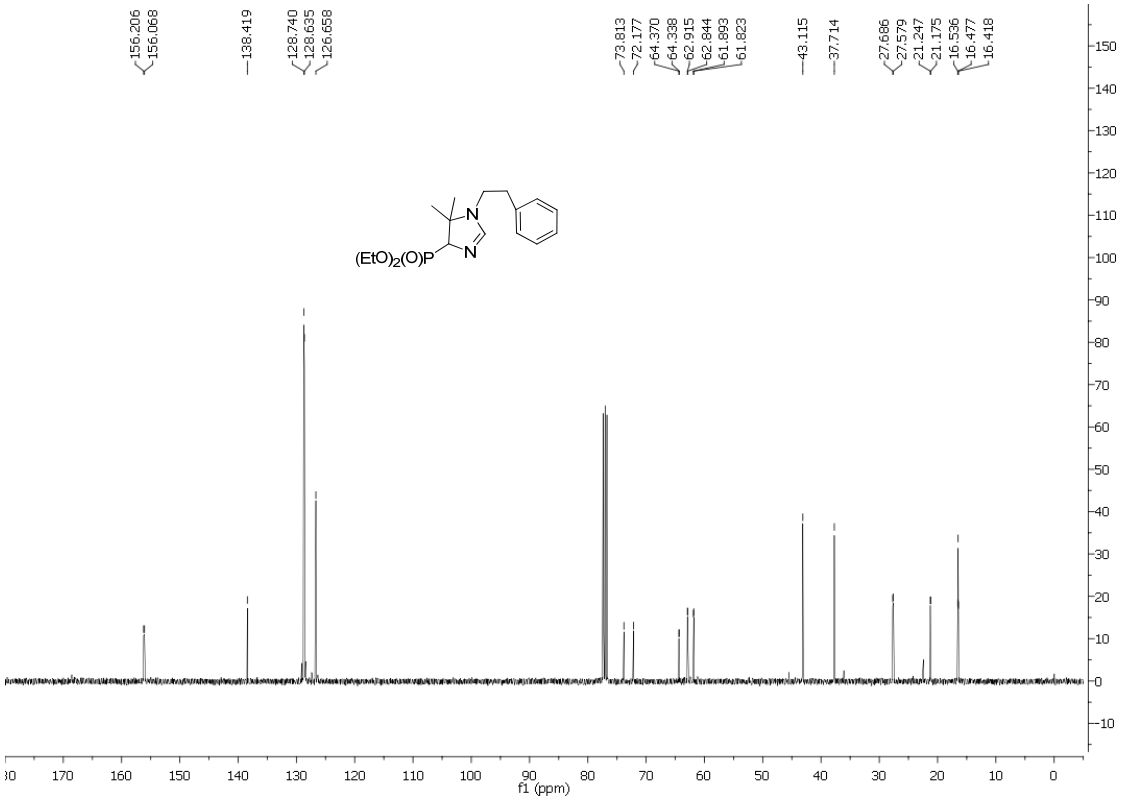

Diethyl (1-benzyl-5,5-dimethyl-4,5-dihydro-1*H*-imidazol-4-yl)phosphonate (5)



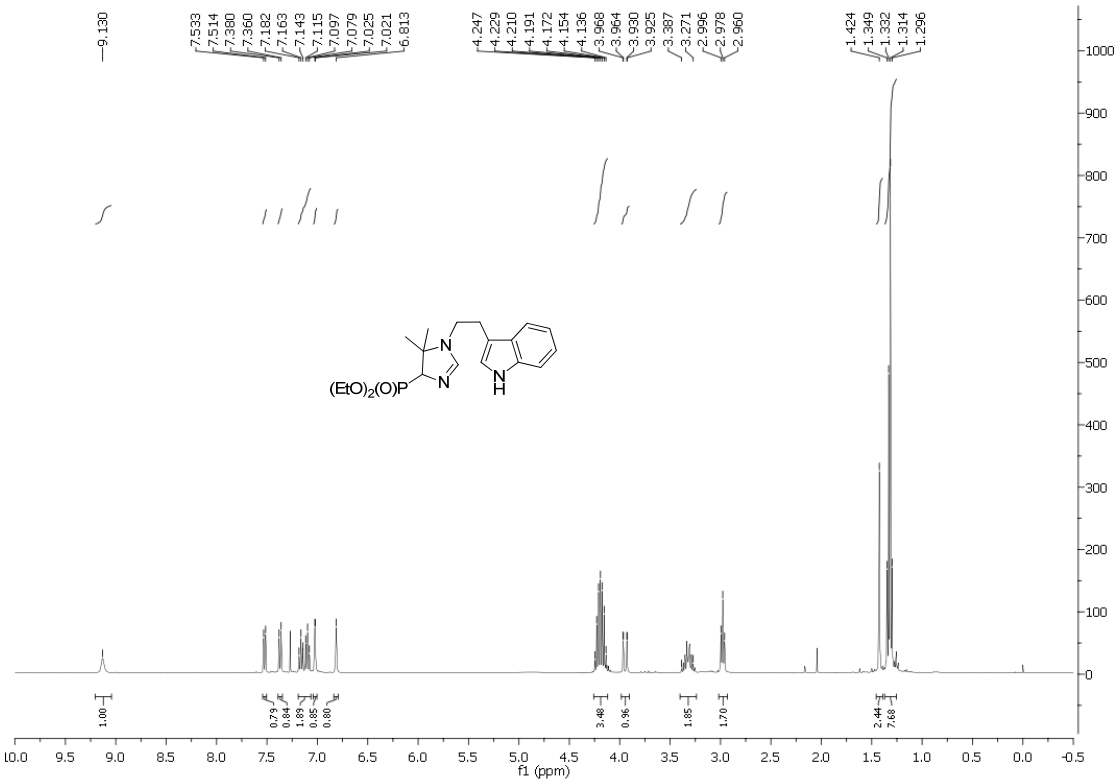

Diethyl (1-benzyl-5,5-dimethyl-4,5-dihydro-1*H*-imidazol-4-yl)phosphonate (5)



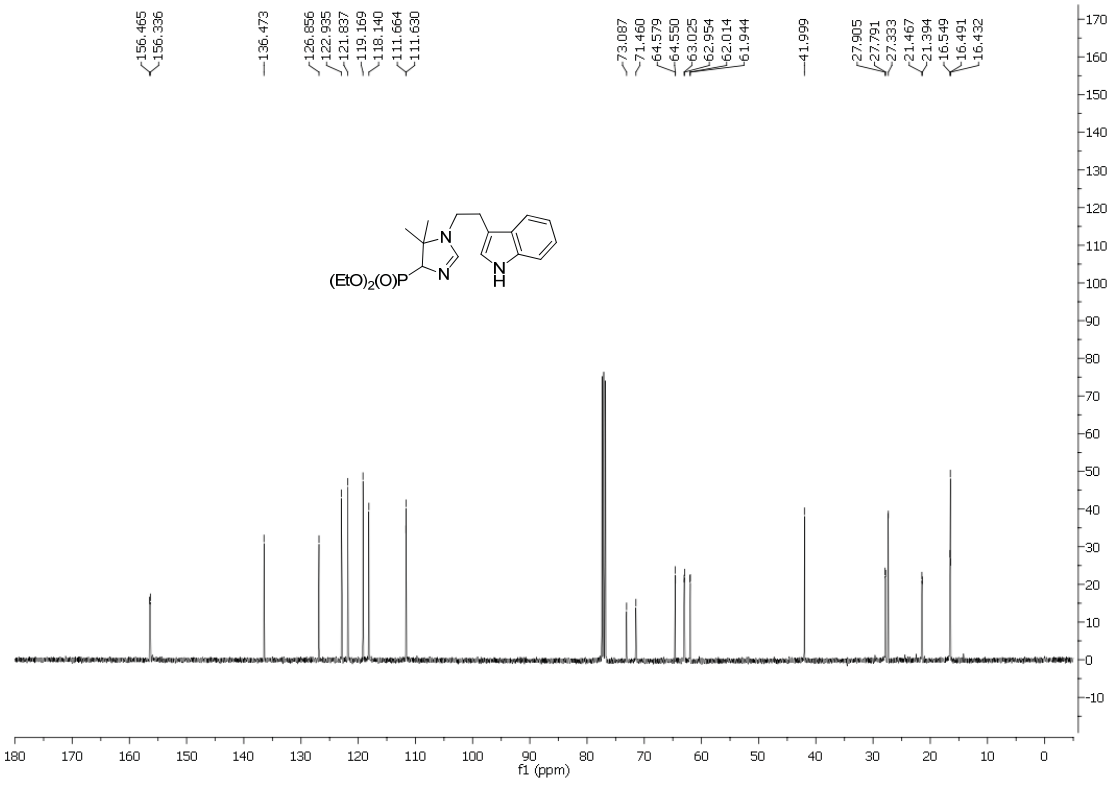

Diethyl [5,5-dimethyl-1-(1-phenethyl)-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (6)



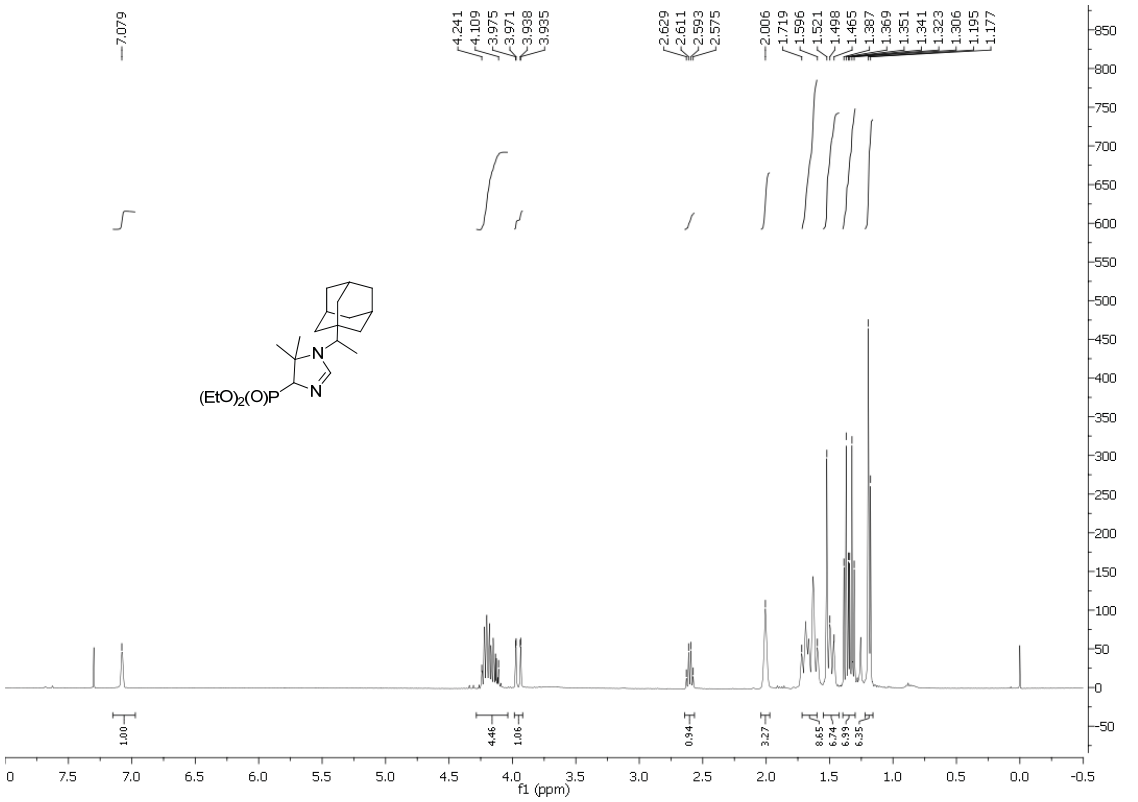

Diethyl [5,5-dimethyl-1-(1-phenethyl)-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (6)




Diethyl [1-(2-(1*H*-indol-3-yl)ethyl]-5,5-dimethyl-4,5-dihydro-*1H*-imidazol-4-yl)phosphonate (7)

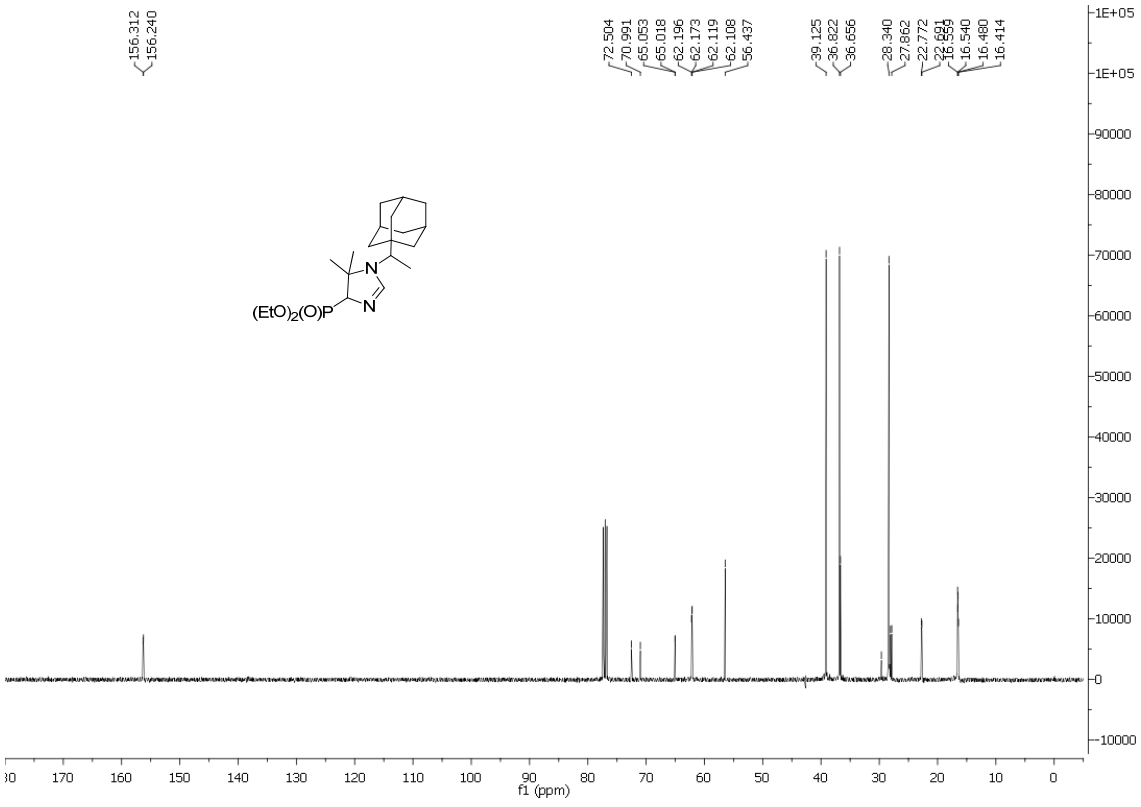



Diethyl [1-(2-(1*H*-indol-3-yl)ethyl]-5,5-dimethyl-4,5-dihydro-1*H*-imidazol-4-yl)phosphonate (7)



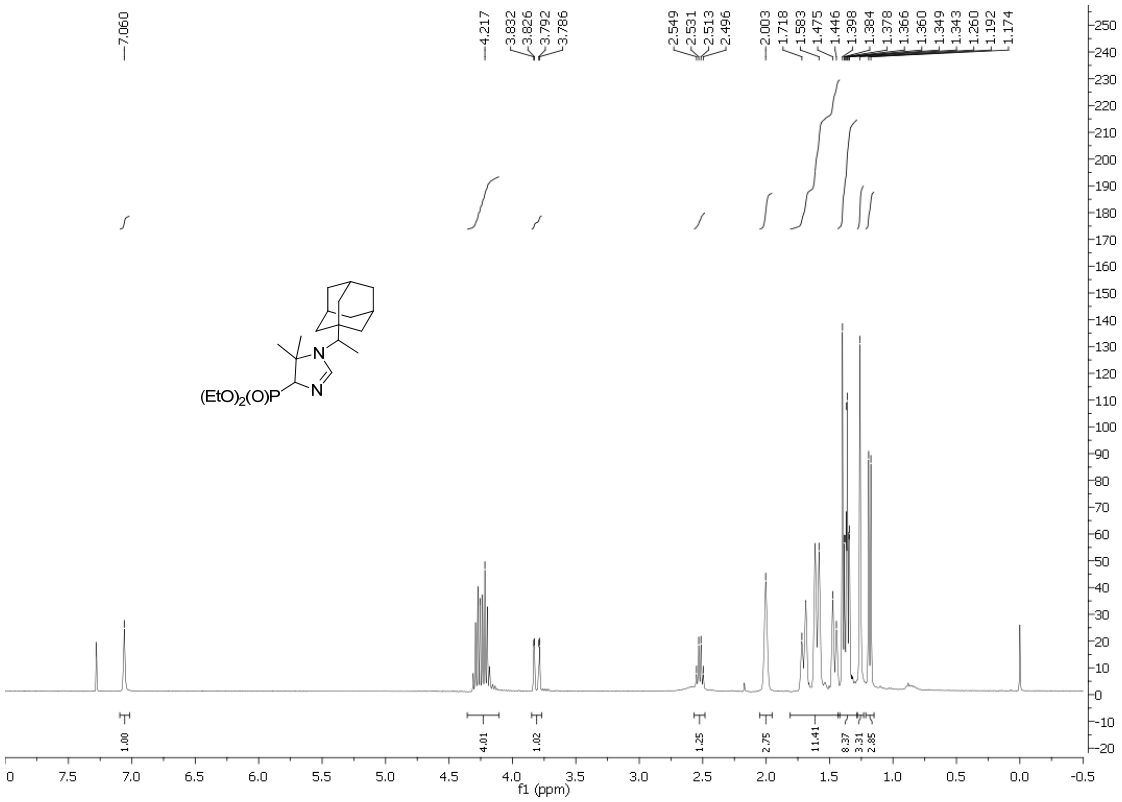

Diethyl [1-(1-(adamantan-1-yl)ethyl)-5,5-dimethyl-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (8)

*Diastereomer a*



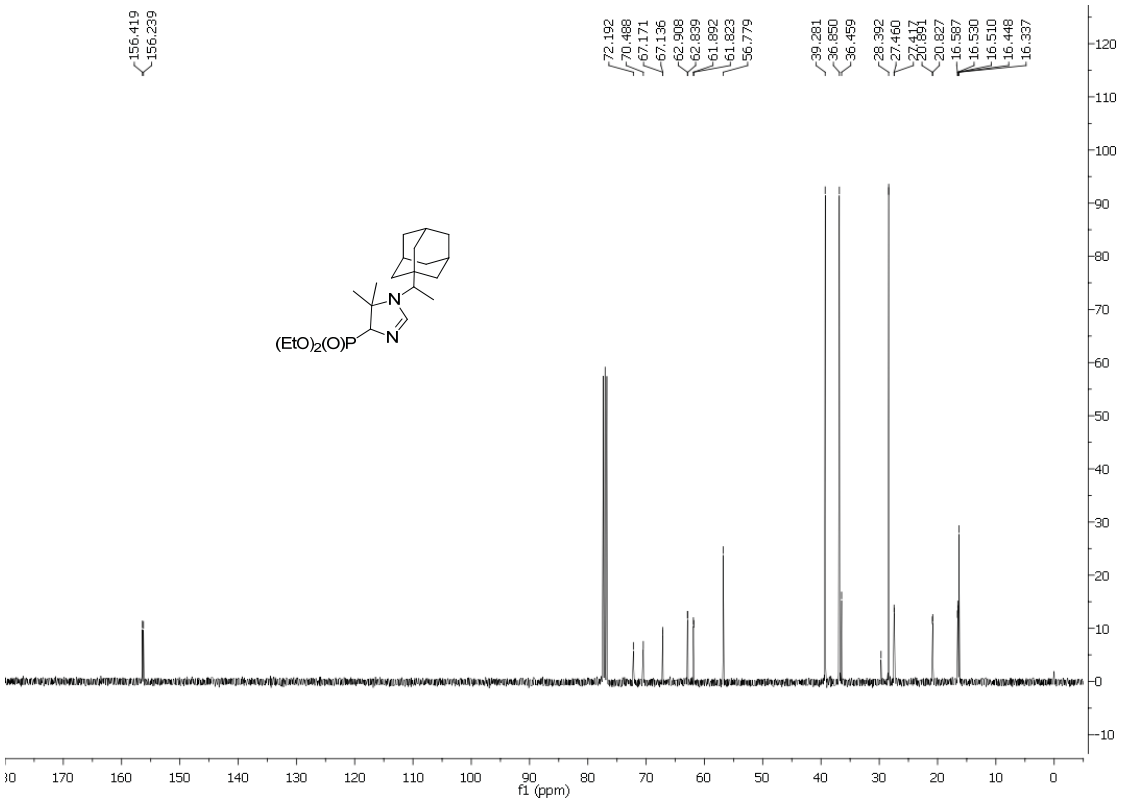

Diethyl [1-(1-(adamantan-1-yl)ethyl)-5,5-dimethyl-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (8)

*Diastereomer a*



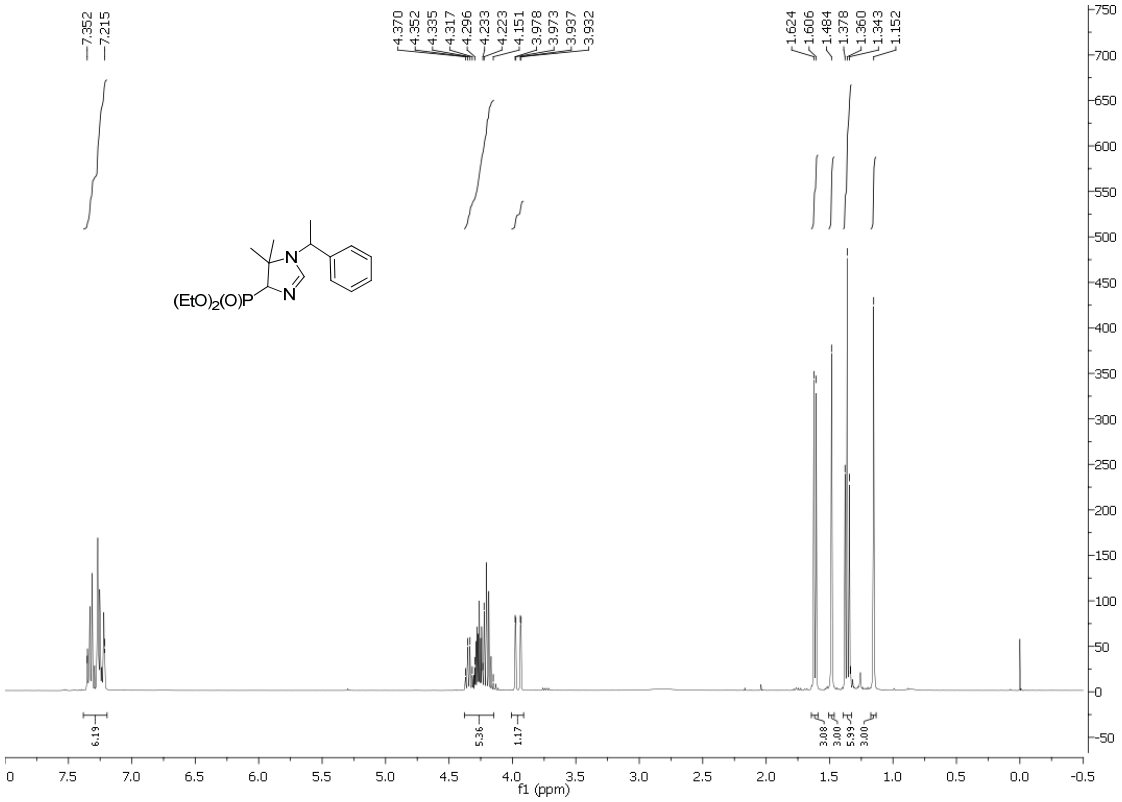

Diethyl [1-(1-(adamantan-1-yl)ethyl)-5,5-dimethyl-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (8)

*Diastereomer b*



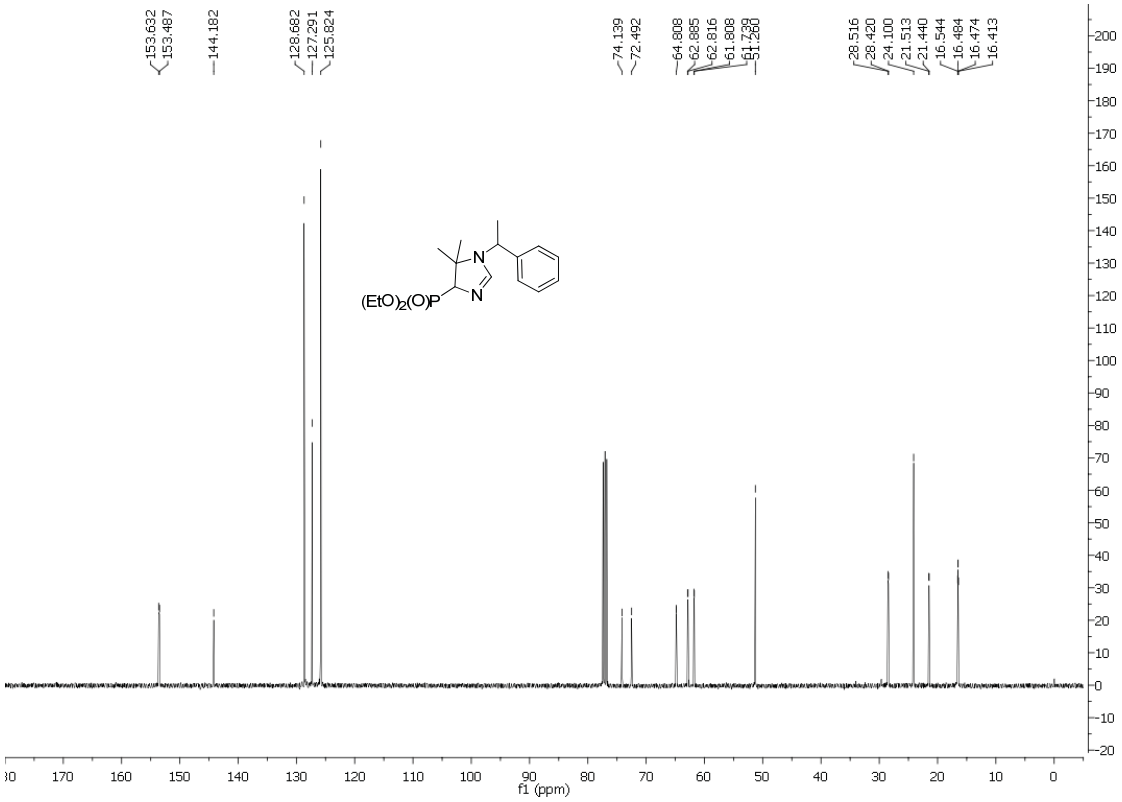

Diethyl [1-(1-(adamantan-1-yl)ethyl)-5,5-dimethyl-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (8)

*Diastereomer b*



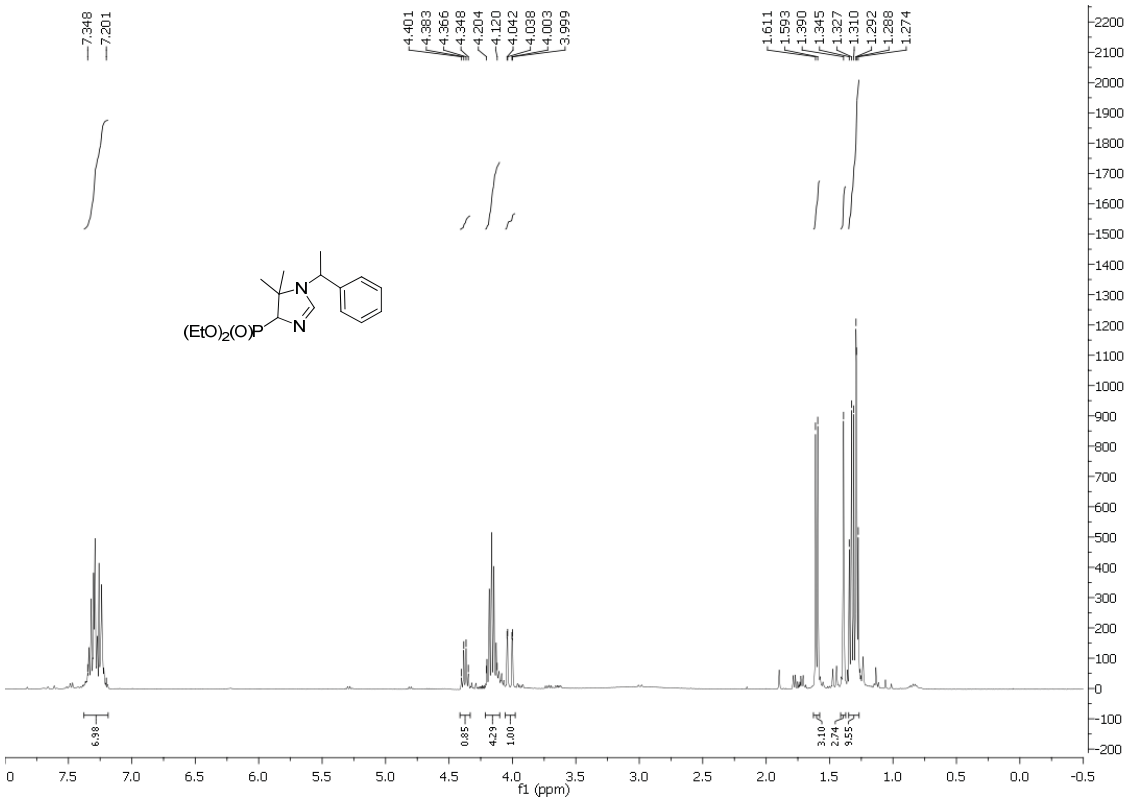

Diethyl [5,5-dimethyl-1-(1-phenylethyl)-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (9)

*Diastereomer a*



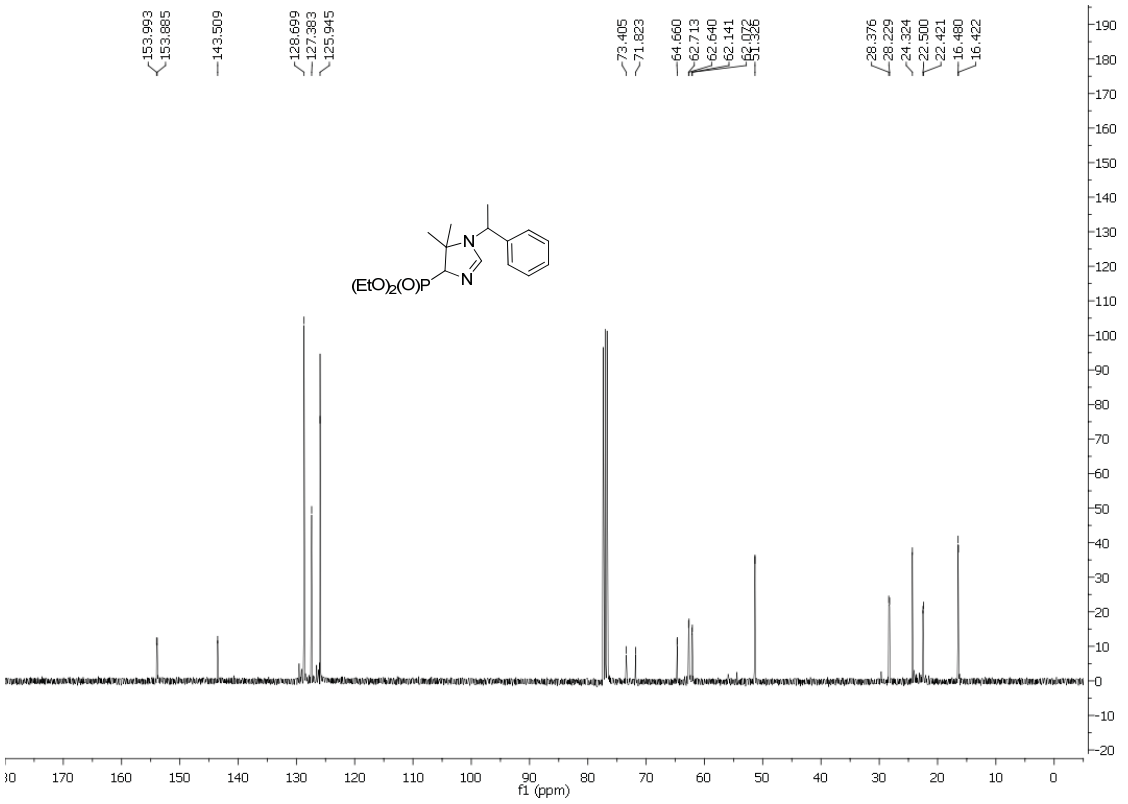

Diethyl [5,5-dimethyl-1-(1-phenylethyl)-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (9)

*Diastereomer a*



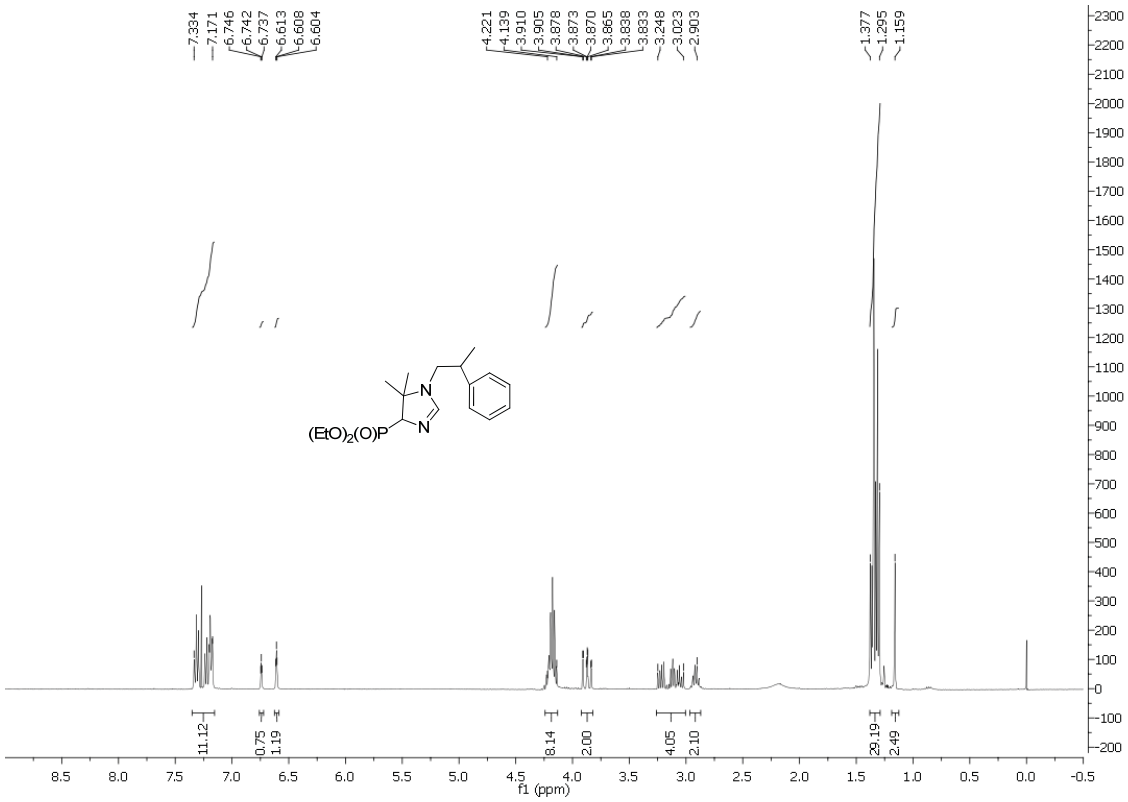

Diethyl [5,5-dimethyl-1-(1-phenylethyl)-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (9)

*Diastereomer b*

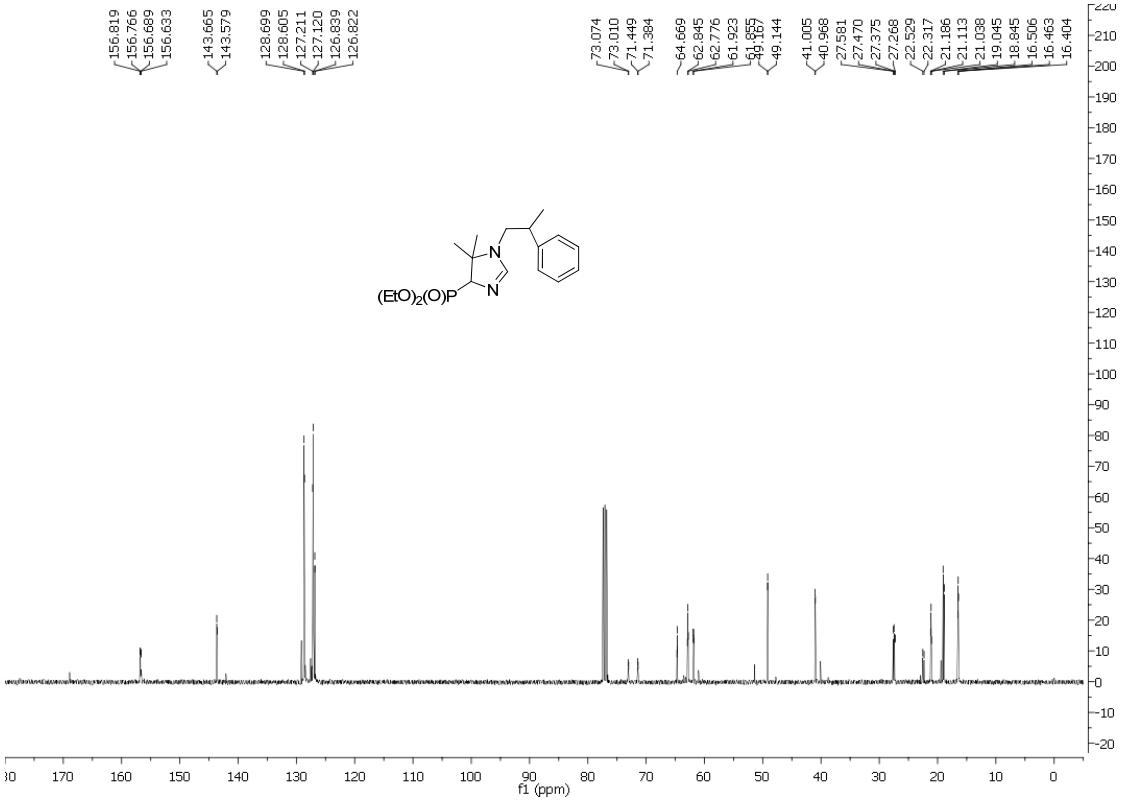



Diethyl [5,5-dimethyl-1-(1-phenylethyl)-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (9)

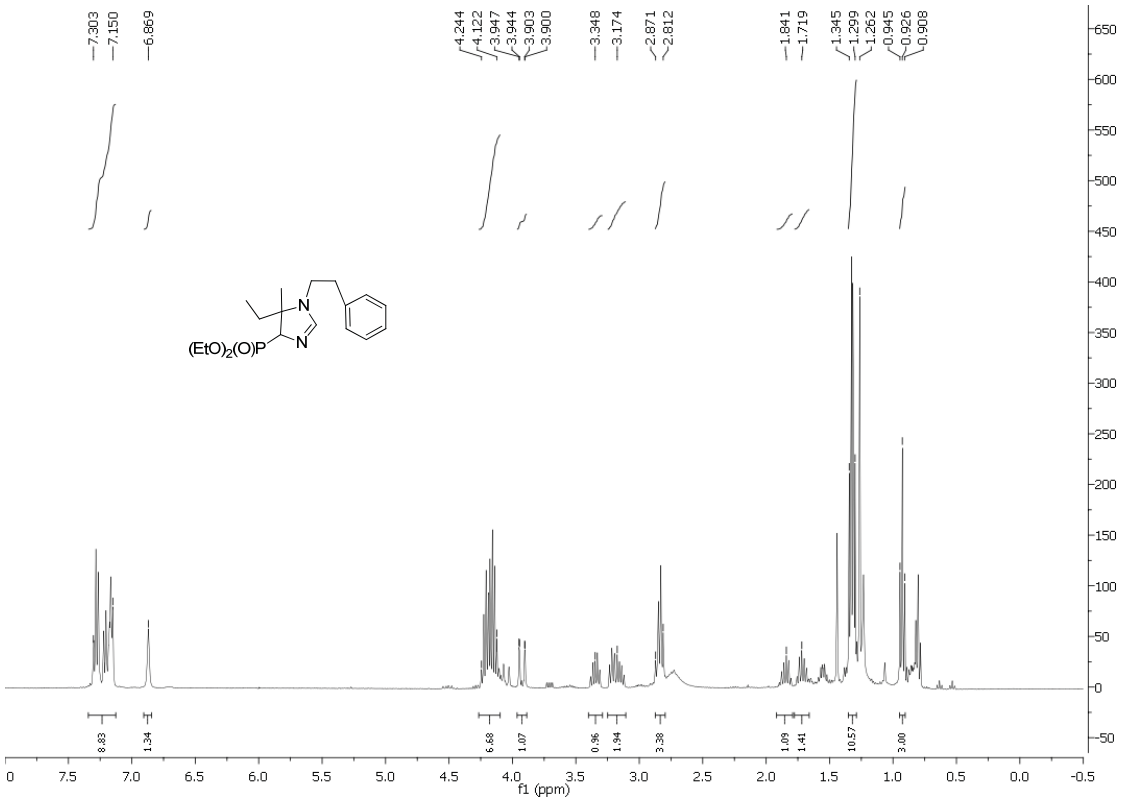
*Diastereomer b*




Diethyl [5,5-dimethyl-1-(2-methyl-2-phenylethyl)-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (10)

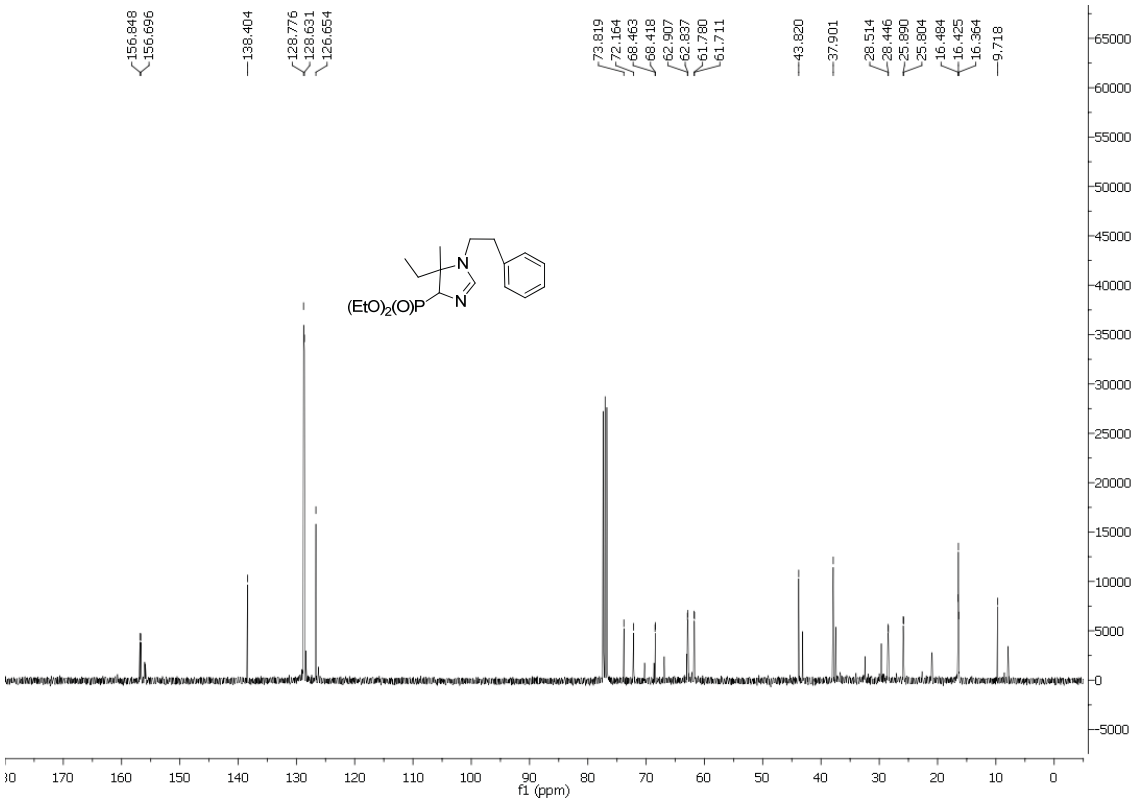



Diethyl [5,5-dimethyl-1-(2-methyl-2-phenylethyl)-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (10)



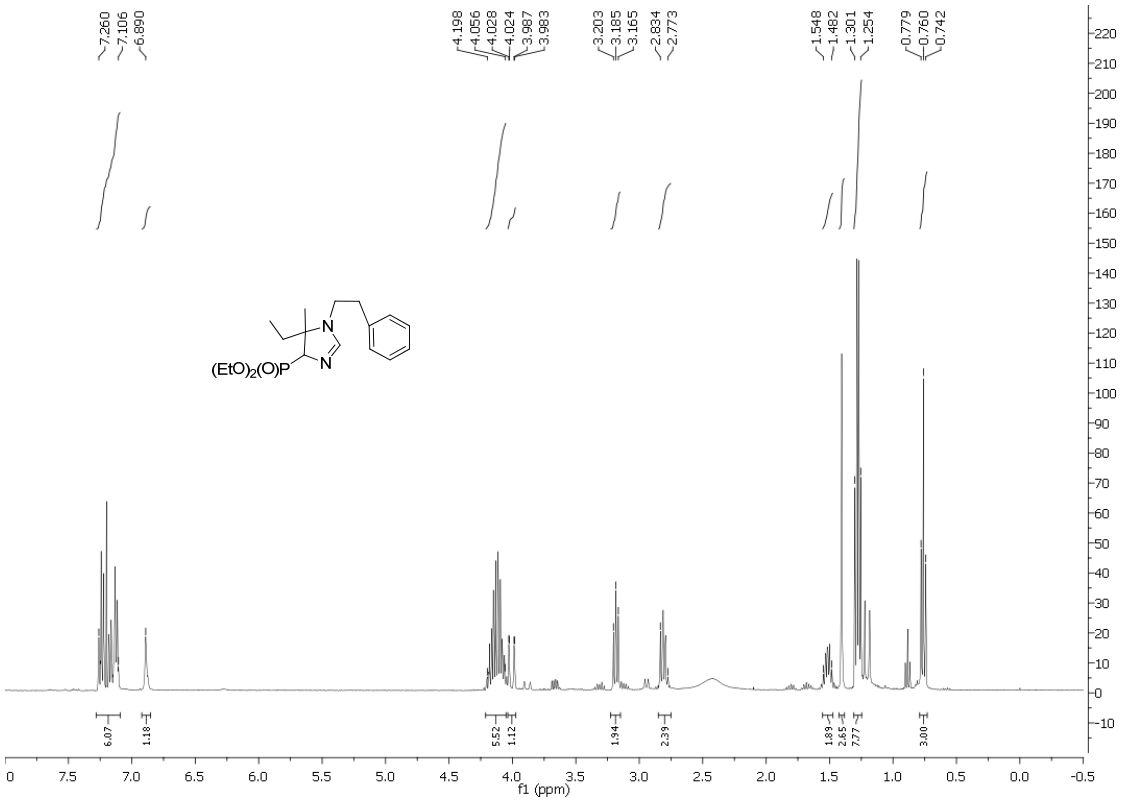

Diethyl [5-ethyl-5-methyl-1-(2-phenylethyl)-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (11)

*Diastereomer a*



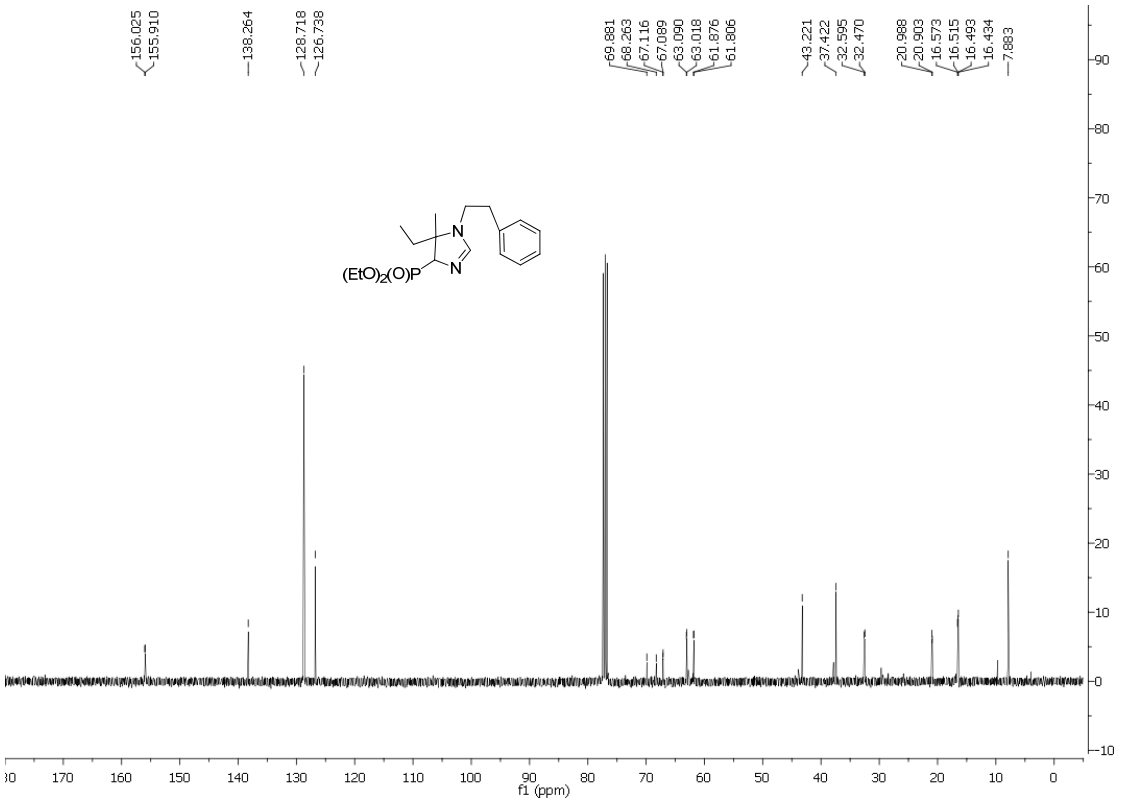

Diethyl [5-ethyl-5-methyl-1-(2-phenylethyl)-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (11)

*Diastereomer a*



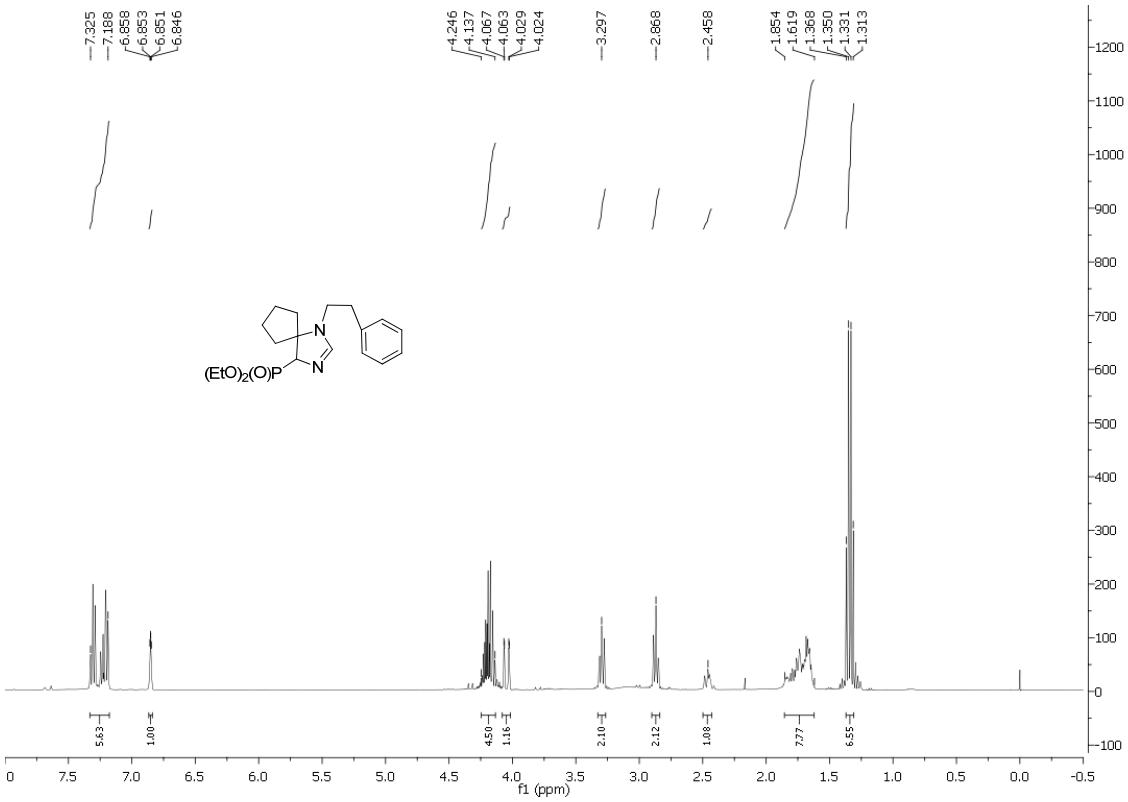

Diethyl [5-ethyl-5-methyl-1-(2-phenylethyl)-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (11)

*Diastereomer b*

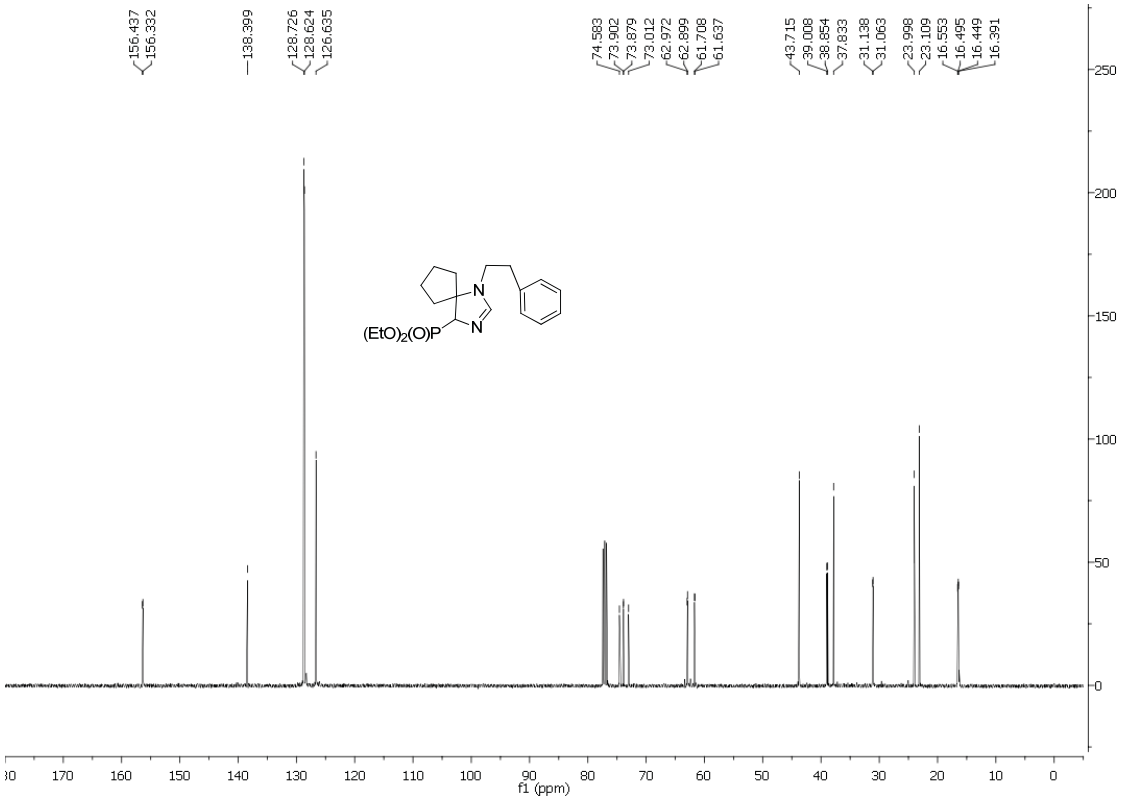



Diethyl [5-ethyl-5-methyl-1-(2-phenylethyl)-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (11)

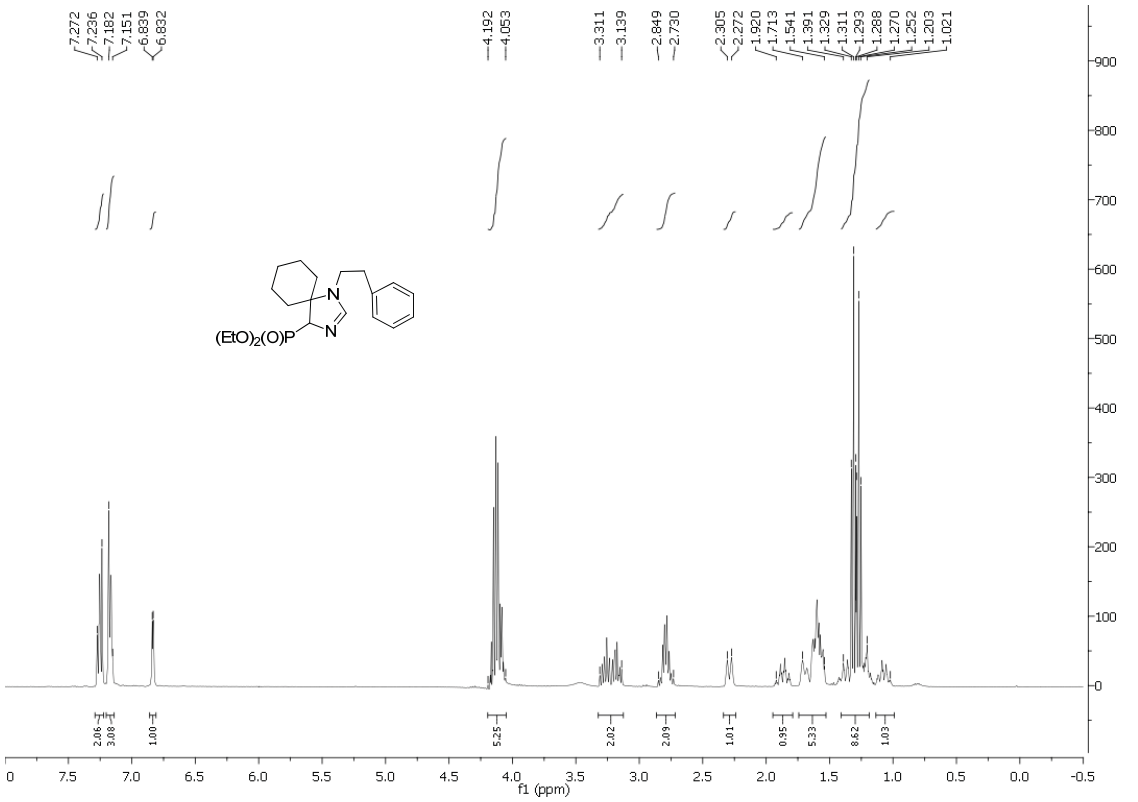
*Diastereomer b*



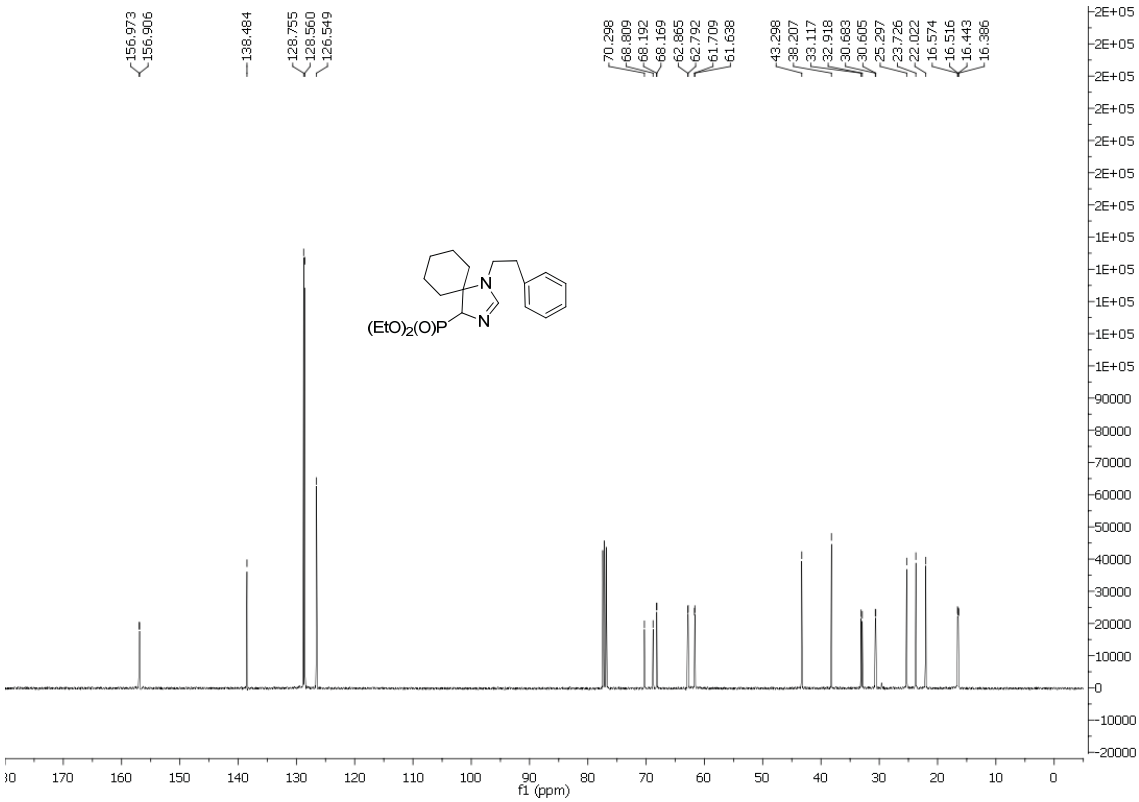

Diethyl (1-phenethyl-1,3-diazaspiro[4.4]non-2-en-4-yl)phosphonate (12)



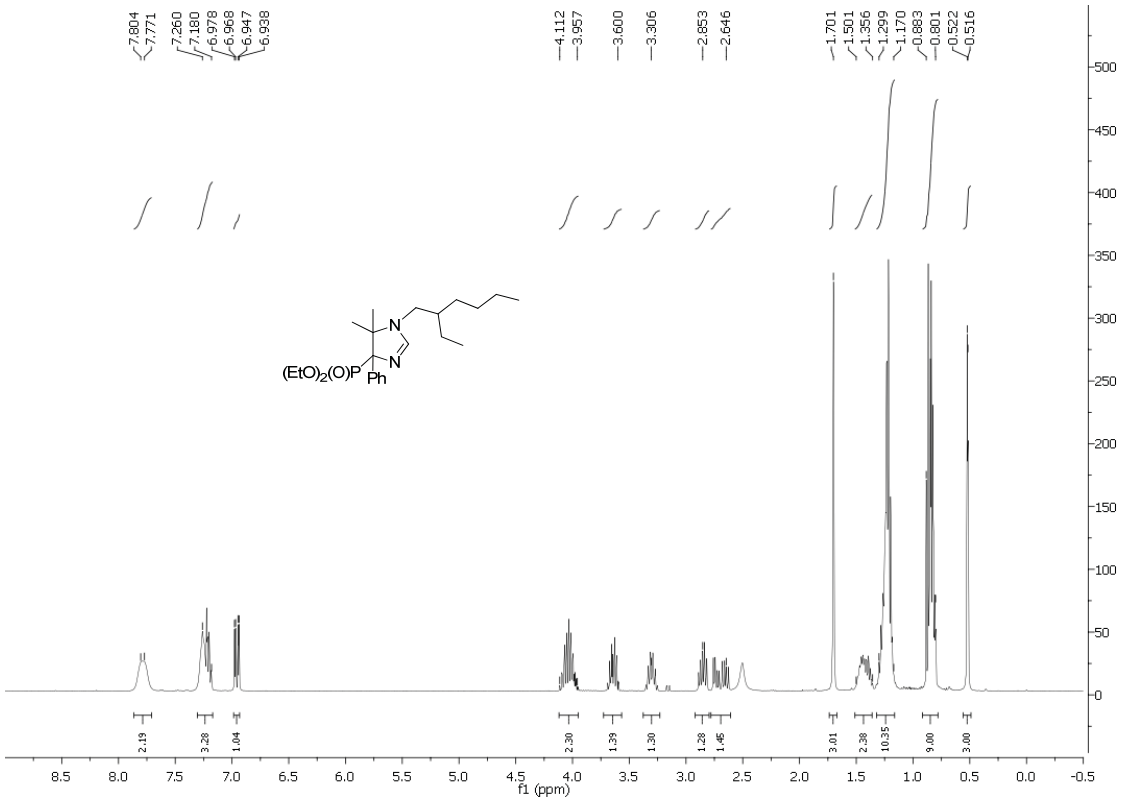

Diethyl (1-phenethyl-1,3-diazaspiro[4.4]non-2-en-4-yl)phosphonate (12)



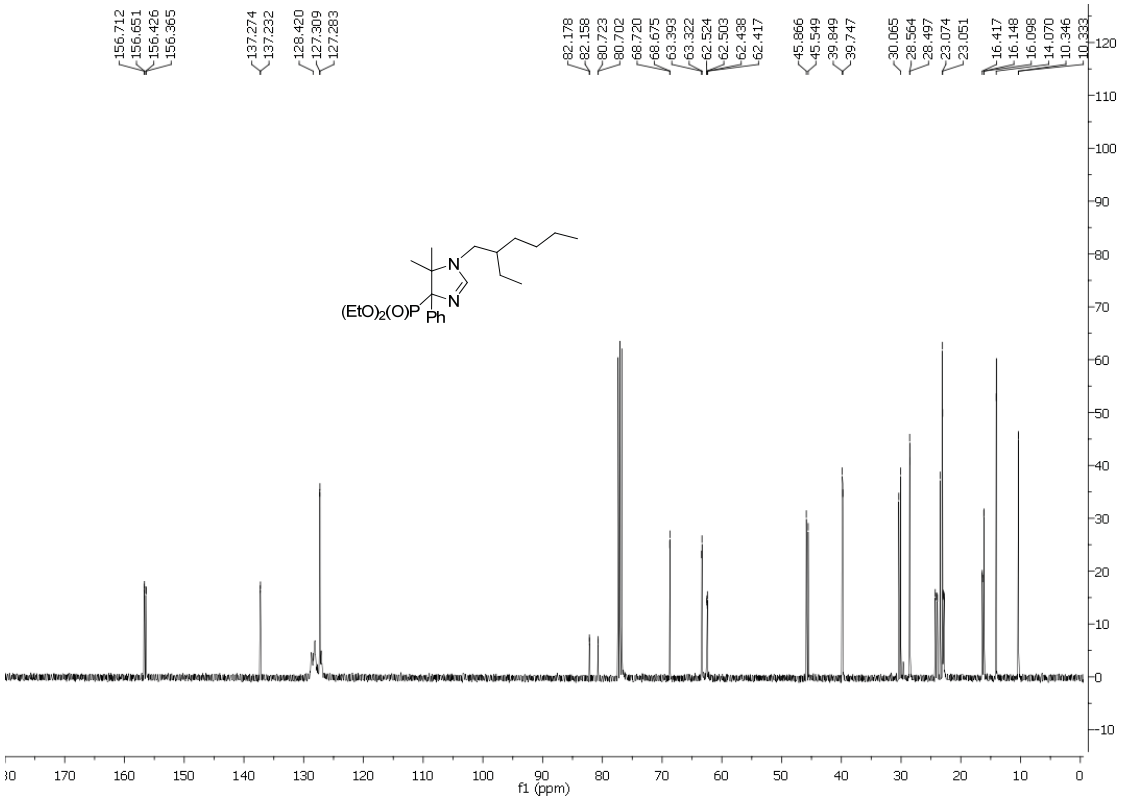

Diethyl (1-phenethyl-1,3-diazaspiro[4.5]dec-2-en-4-yl)phosphonate (13)



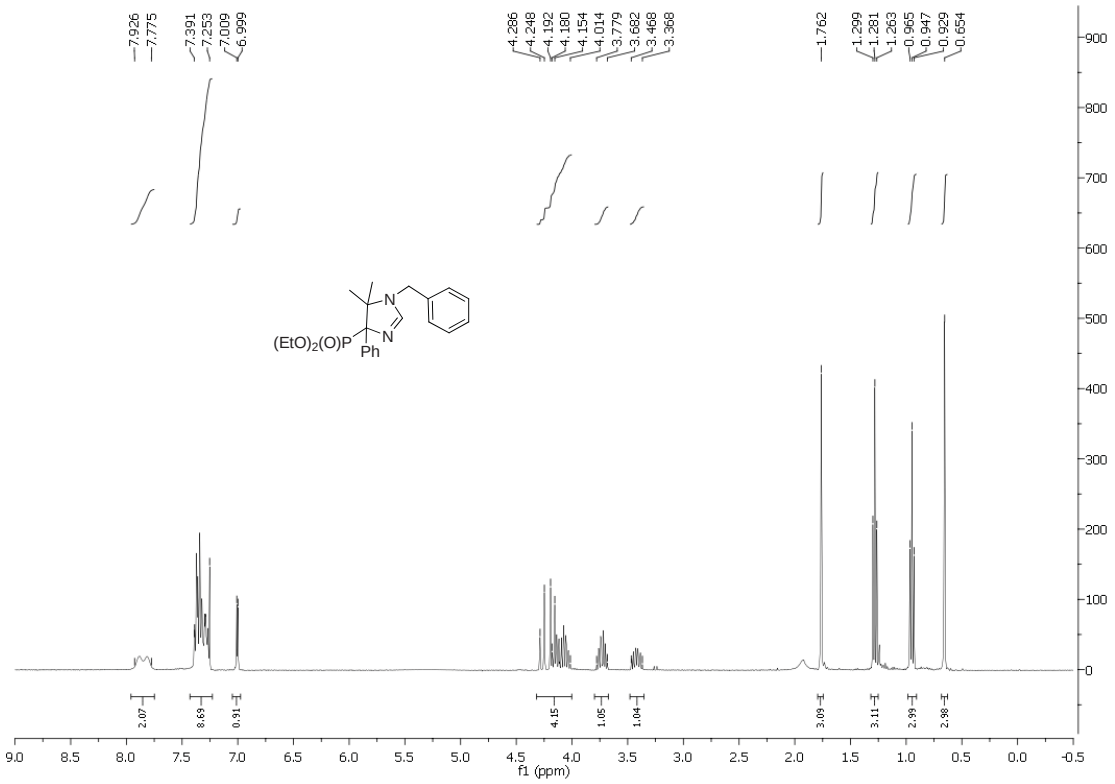

Diethyl (1-phenethyl-1,3-diazaspiro[4.5]dec-2-en-4-yl)phosphonate (13)



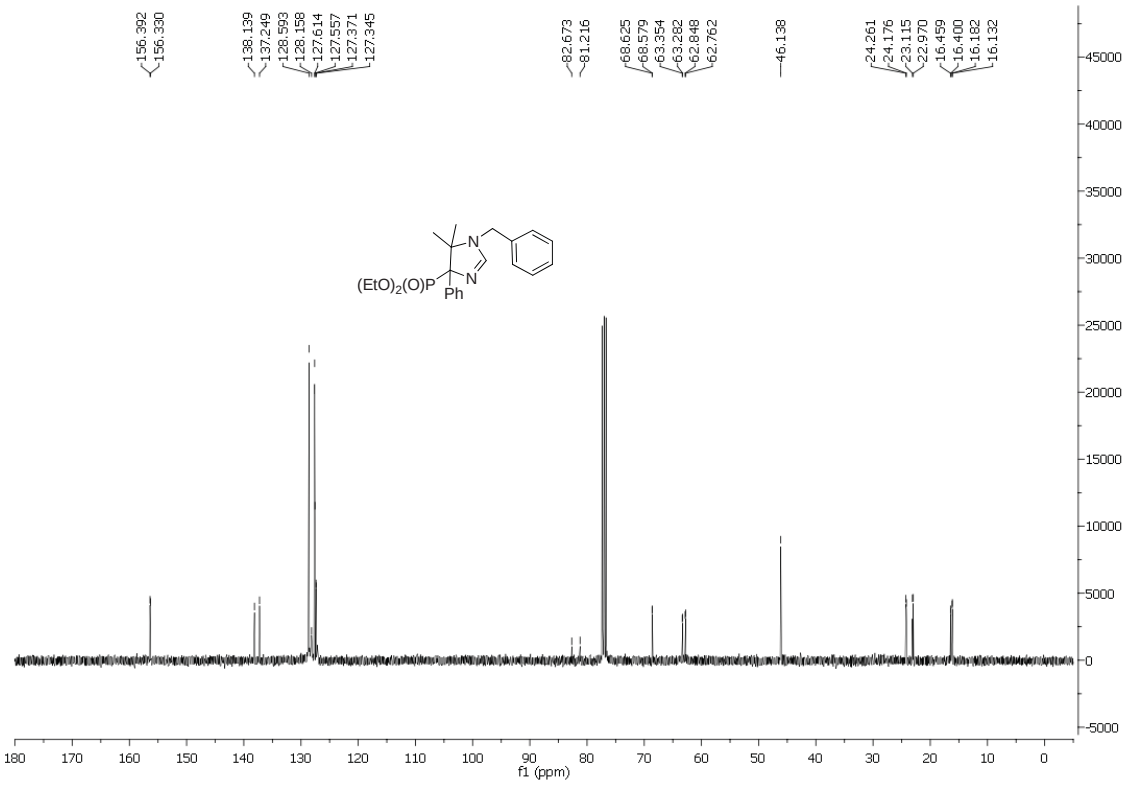

Diethyl [1-(2-ethylhexyl)-5,5-dimethyl-4-phenyl-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (14)



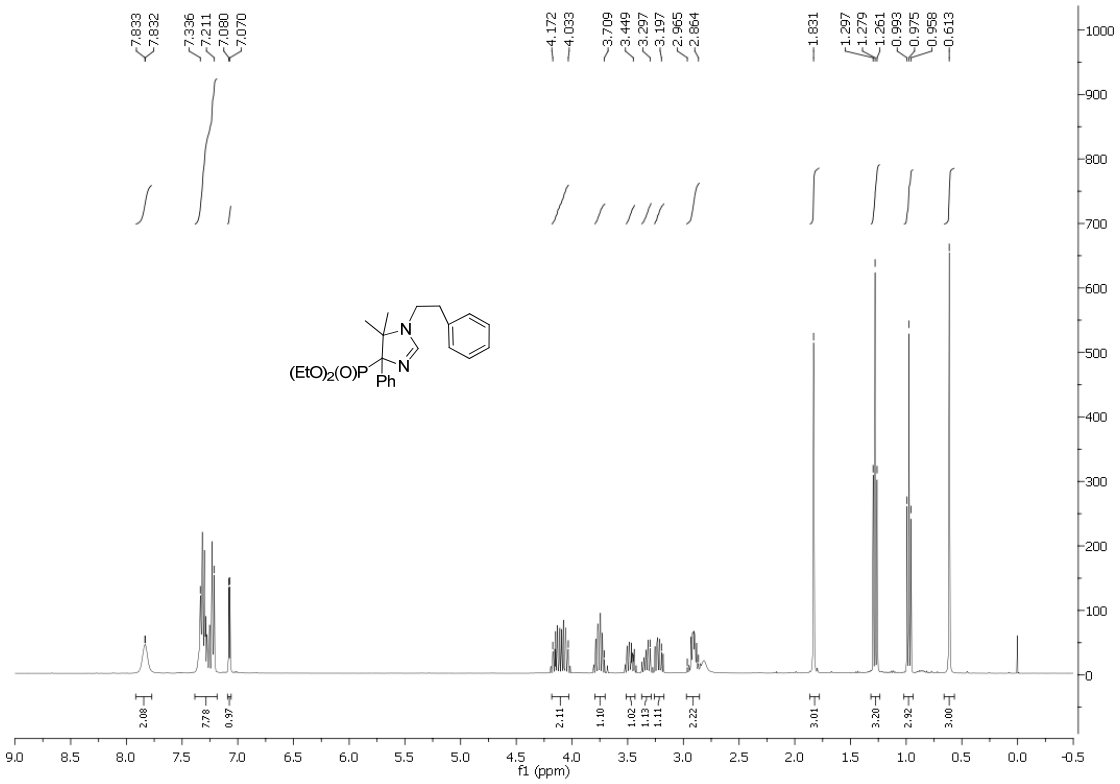

Diethyl [1-(2-ethylhexyl)-5,5-dimethyl-4-phenyl-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (14)



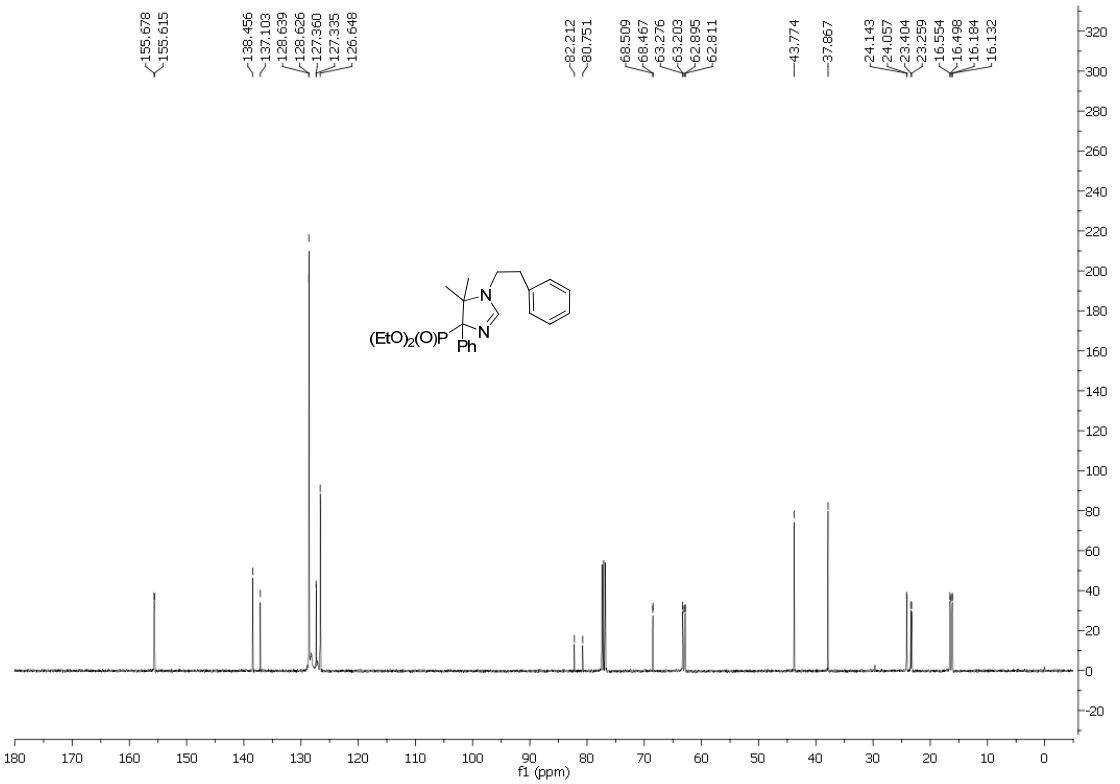

Diethyl (1-benzyl-5,5-dimethyl-4-phenyl-4,5-dihydro-1*H*-imidazol-4-yl)phosphonate (15)



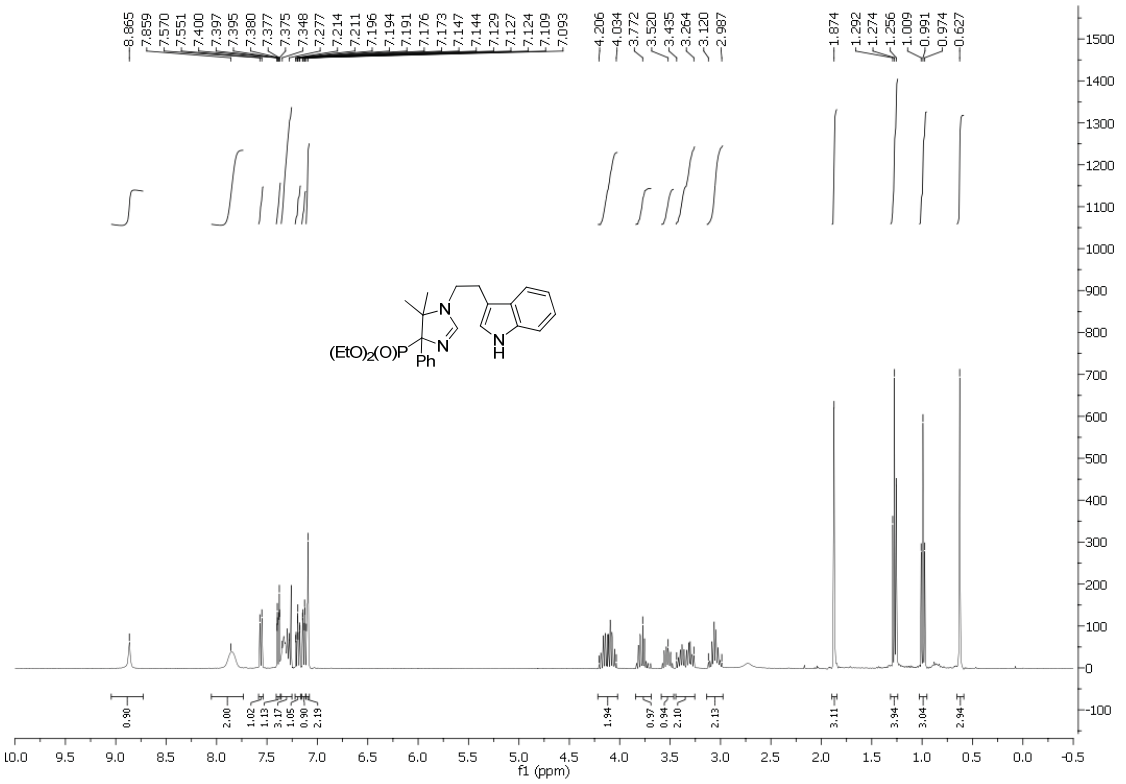

Diethyl (1-benzyl-5,5-dimethyl-4-phenyl-4,5-dihydro-1*H*-imidazol-4-yl)phosphonate (15)



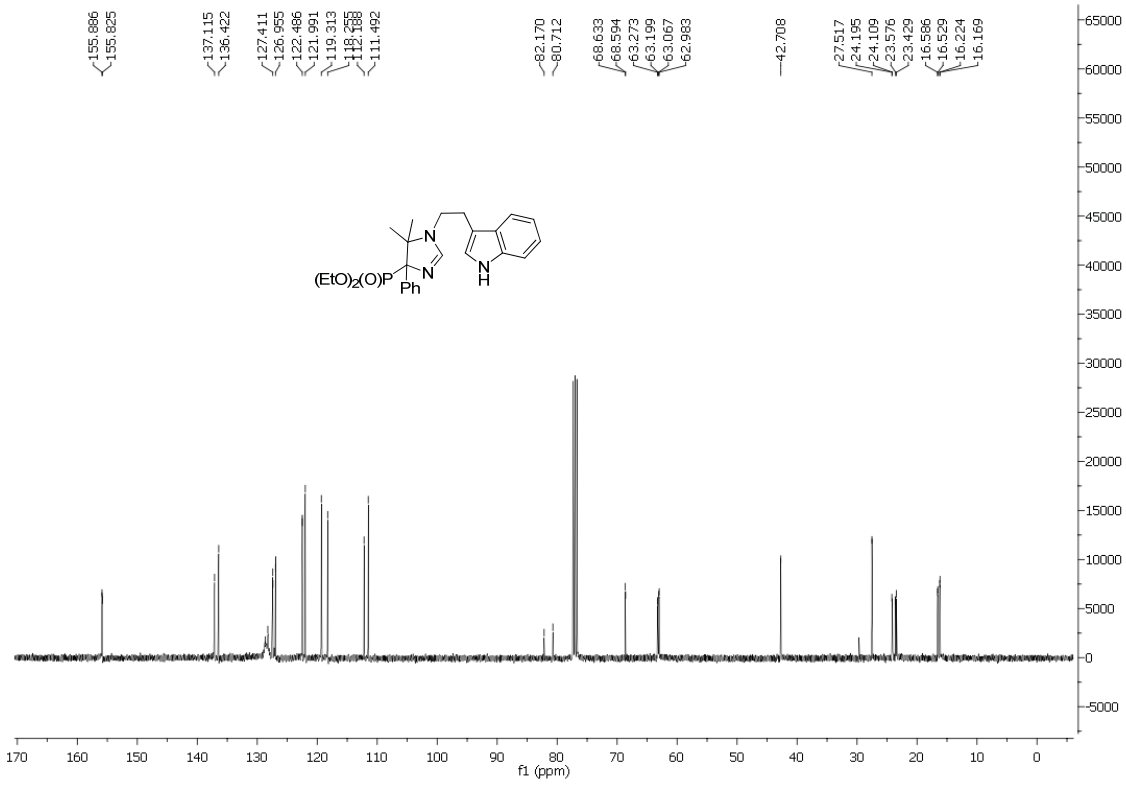

Diethyl (5,5-dimethyl-1-phenethyl-4-phenyl-4,5-dihydro-1*H*-imidazol-4-yl)phosphonate (16)



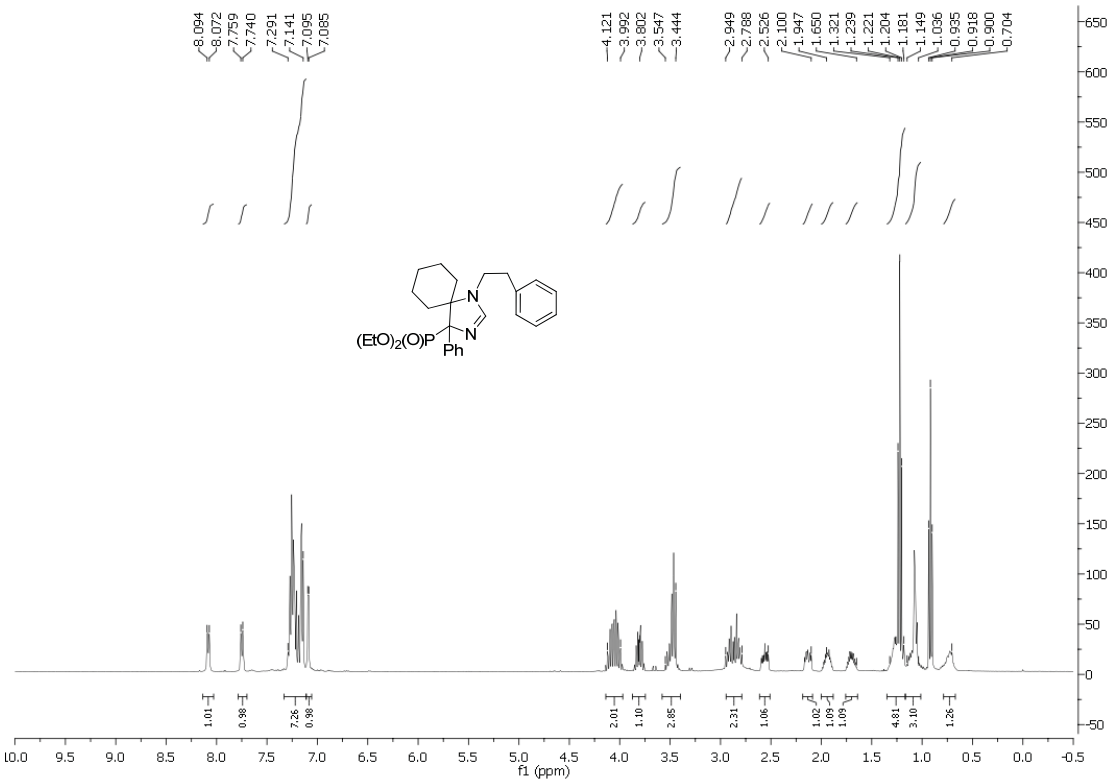

Diethyl (5,5-dimethyl-1-phenethyl-4-phenyl-4,5-dihydro-1*H*-imidazol-4-yl)phosphonate (16)



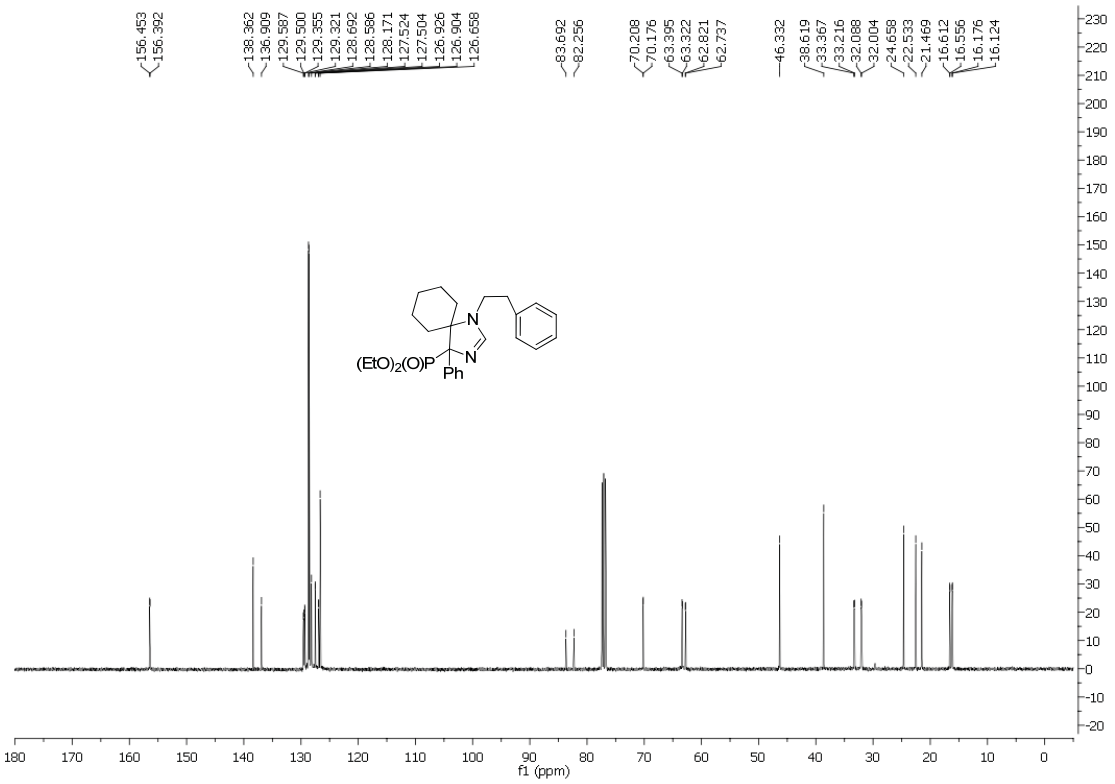

Diethyl [1-(2-(1*H*-indol-3-yl)ethyl)-5,5-dimethyl-4-phenyl-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (17)



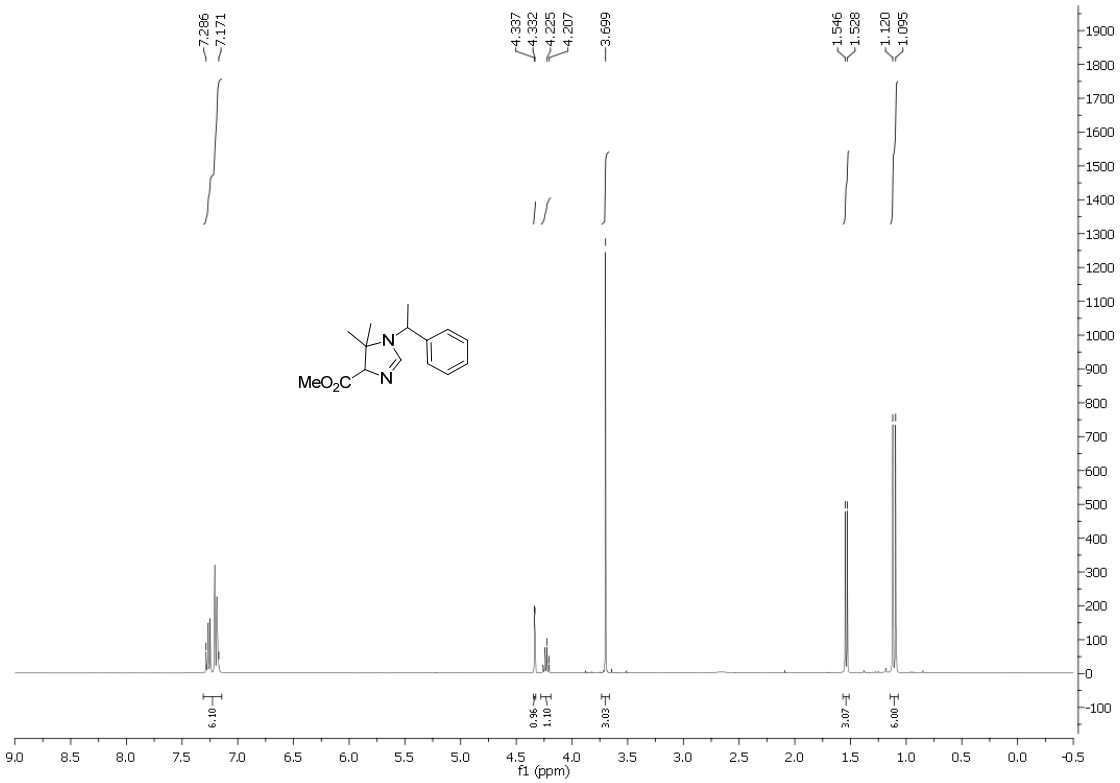

Diethyl [1-(2-(1*H*-indol-3-yl)ethyl)-5,5-dimethyl-4-phenyl-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate (17)



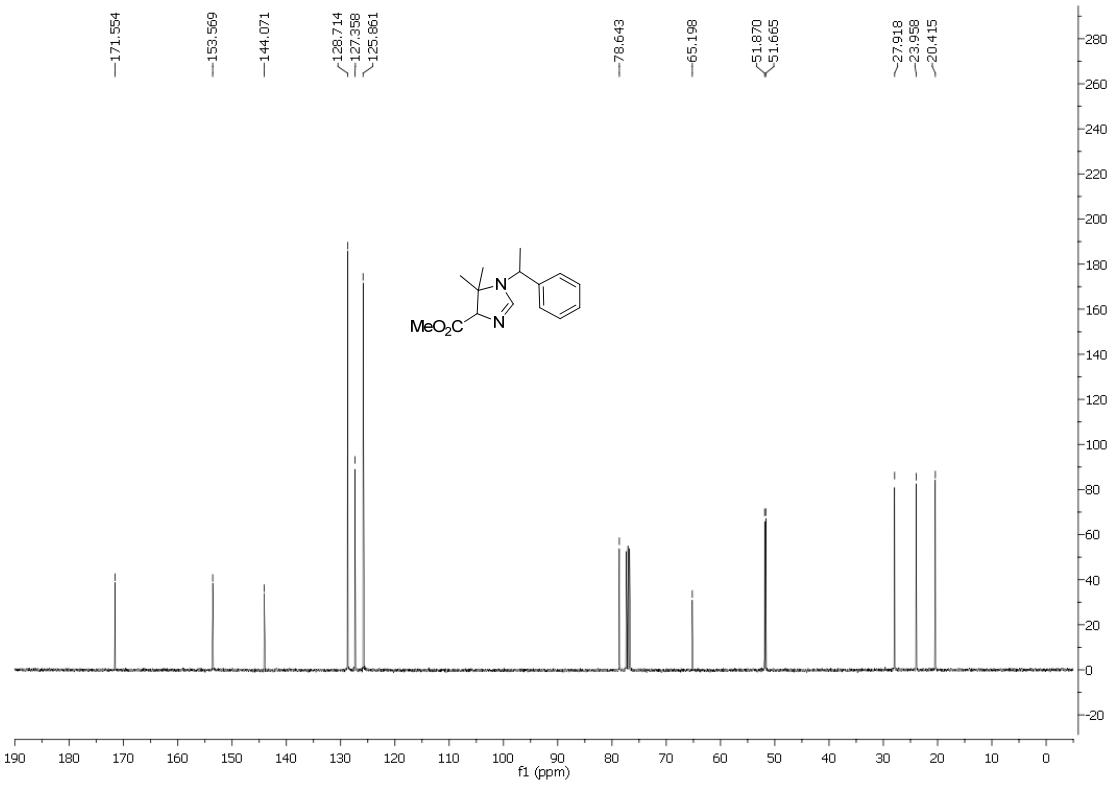

Diethyl (1-phenethyl-4-phenyl-1,3-diazaspiro[4.5]dec-2-en-4-yl)phosphonate (18)



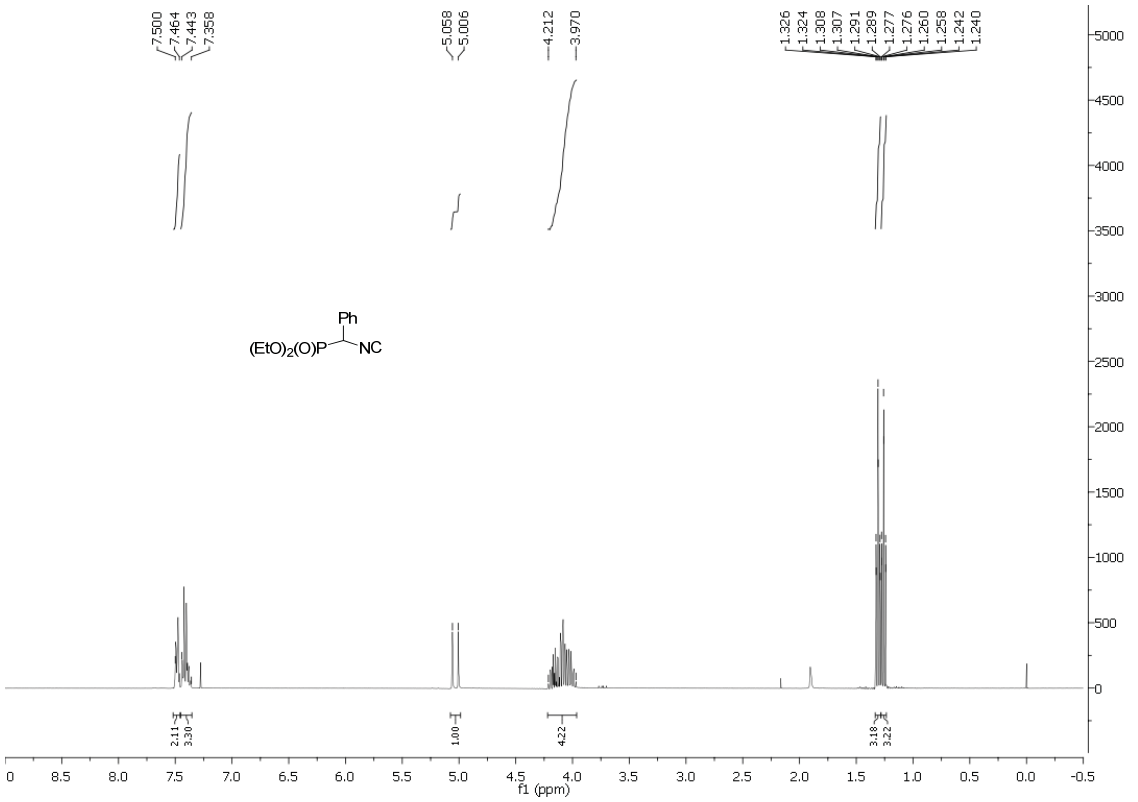

Diethyl (1-phenethyl-4-phenyl-1,3-diazaspiro[4.5]dec-2-en-4-yl)phosphonate (18)




Methyl 1-benzyl-5,5-dimethyl-4,5-dihydro-1*H*-imidazole-4-carboxylate (21)




Methyl 1-benzyl-5,5-dimethyl-4,5-dihydro-1*H*-imidazole-4-carboxylate (21)


7.500
7.464
7.443
7.358
5.058
5.006
4.212
3.970
1.326
1.324
1.308
1.307
1.291
1.289
1.277
1.276
1.260
1.258
1.242
1.240
Ph
(EtO)2(O)P
NC
2.11
3.30
1.00
4.22
3.18
3.22
f1 (ppm)

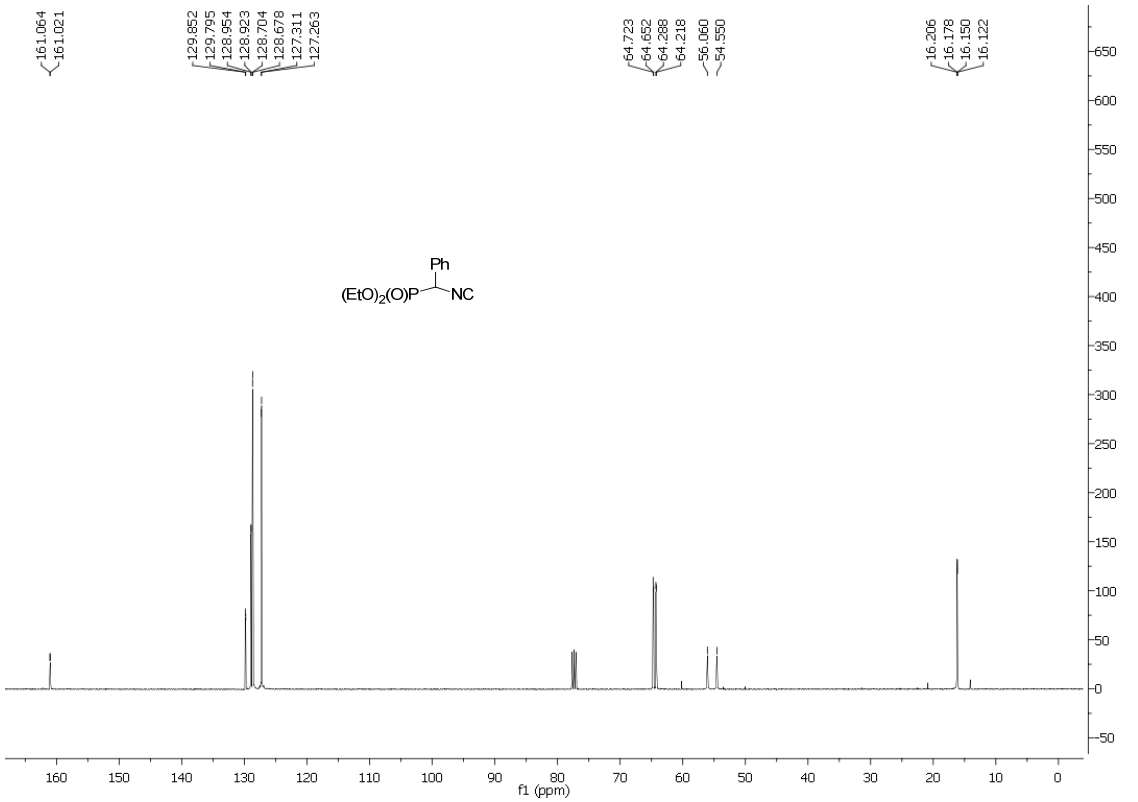

161.064
161.021
129.852
129.795
128.954
128.923
128.704
128.678
127.311
127.263
64.723
64.652
64.288
64.218
56.060
54.550
16.206
16.178
16.150
16.122
Ph
(EtO)2(O)P
NC
f1 (ppm)

Table. $^{1}H$ and $^{13}C$ chemical shifts for compounds **1**-**18** including both the multiplicity and the coupling constants.

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
|---|---|---|---|---|---|---|---|
| **H2** | 6.89, s | 7.74, br s | 6.90, d, 1.0 | 6.89, t, 2.0 | 6.78, br s | 6.82, br s | 7.02, d, 1.5 |
| **H4** | 3.87, dd, 16.0, 2.0 | 3.97, dd, 13.0, 2.0 | 3.94, dd, 15.5, 2.0 | 3.86, dd, 15.5, 2.0 | 3.94, dd, 16.0, 2.0 | 3.90, dd, 16.5, 2.0 | 3.95,dd, 16.0, 2.0 |
| **C2** | 156.2, d, 13.0 | 154.3, d, 10.0 | 157.0, dd, 14.0, 4.0 | 159.2, d, 18.0 | 156.7, d, 13.5 | 156.1, d, 14.0 | 156.4, d, 13.0 |
| **C4** | 73.1, d, 164.0 | 68.2, d, 161.5 | 73.0, d, 164.0 | 72.0, d, 164.0 | 73.6, d, 163.3 | 73.0, d, 164.5 | 72.3, d, 163.5 |
| **C5** | 64.2, d, 3.0 | 66.3, d, 1.5 | 64.5, d, 3.0 | 66.0, d, 3.5 | 64.2, d, 3.0 | 64.3, d, 3.0 | 64.5, d, 3.0 |

| | **8 (diast)** | **8(diast)** | **9(diast)** | **9(diast)** | **10(diast)*** | **10(diast)*** |
|---|---|---|---|---|---|---|
| **H2** | 7.07, d, 2.0 | 7.06, s | 7.21 | 7.20 | 6.60, t, 2.0 | 6.74, t, 2.0 |
| **H4** | 3.95, dd, 15.0, 2.0 | 3.81, dd, 16.0, 2.5 | 3.95, dd, 16.5, 2.0 | 4.02, dd, 16.0, 2.0 | 3.84, dd, 13.0, 2.0 | 3.89, dd, 13.0, 2.0 |
| **C2** | 156.3, d, 7.0 | 156.3, d, 18.0 | 153.9, d, 15.0 | 153.4, d, 11.0 | 156.6, d, 6.0 | 156.7, d, 6.0 |
| **C4** | 71.7, d, 152.0 | 71.3, d, 171.0 | 73.3, d, 165.5 | 72.6, d, 158.5 | 72.2, d, 164.5 | 72.3, d, 163.0 |
| **C5** | 65.0, d, 4.0 | 67.1, d, 3.0 | 64.8, d, 4.0 | 64.6, d, 3.0 | 64.5, d, 6.0 | 64.6, d, 6.0 |

| | **11(diast)** | **11(diast)** | **12** | **13** | **14** | **15** | **16** | **17** | **18** |
|---|---|---|---|---|---|---|---|---|---|
| **H2** | 6.87, s | 6.87, s | 6.85, dd, 3.0, 2.0 | 6.83, d, 2.5 | 6.95, dd, 12.5, 4.0 | 7.00, d, 4.0 | 7.07, d,4.0 | 7.90, m | 7.08, d, 4.0 |
| **H4** | 3.92, dd, 17.5, 1.5 | 4.00, dd, 16.5, 1.5 | 4.04, dd, 15.0, 1.5 | 4.05 | | | | | |
| **C2** | 156.8, d, 15.0 | 156.0, d, 11.5 | 156.3, d, 10.5 | 156.9, d, 7.0 | 156.4, dd, 28.5, 6.0 | 156.3, d, 6.0 | 155.6, d, 6.0 | 155.8, d, 6.0 | 156.4, d, 6 |
| **C4** | 73.0, d, 166.0 | 69.1, d, 162.5 | 73.8, d, 157.0 | 69.5, d, 150.0 | 82.0, d, 145.0 | 82.0, d, 146.5 | 81.5, d, 147.0 | 81.5, d, 146.5 | 83.0, d, 144.0 |
| **C5** | 68.4, d, 4.5 | 67.0, d, 3.5 | 73.9, d, 2.0 | 68.1, d, 2.5 | 68.7, d, 4.5 | 68.6, d, 4.5 | 68.5, d, 4.0 | 68.6, d,4.0 | 70.1, d,3.0 |

## Transition States

**b, R=H + $Ag^+$**

| Center Number | Atomic Number | Atomic Type | Coordinates (Angstroms) X | Y | Z |
|---|---|---|---|---|---|
| 1 | 6 | 0 | -1.989526 | -0.022867 | -0.927706 |
| 2 | 8 | 0 | -4.283575 | 0.773999 | 0.356719 |
| 3 | 8 | 0 | -1.913817 | 1.528092 | 1.161651 |
| 4 | 6 | 0 | -2.386353 | 2.448431 | 2.157703 |
| 5 | 1 | 0 | -3.312198 | 2.078414 | 2.606022 |
| 6 | 1 | 0 | -1.602168 | 2.517074 | 2.910881 |
| 7 | 1 | 0 | -2.564393 | 3.432242 | 1.713066 |
| 8 | 15 | 0 | -2.912586 | 1.189752 | -0.059861 |
| 9 | 8 | 0 | -3.035893 | 2.573469 | -0.874381 |
| 10 | 6 | 0 | -1.855465 | 3.193930 | -1.383201 |
| 11 | 1 | 0 | -2.180676 | 4.089380 | -1.912032 |
| 12 | 1 | 0 | -1.180764 | 3.469092 | -0.565404 |
| 13 | 1 | 0 | -1.336265 | 2.524036 | -2.077393 |
| 14 | 6 | 0 | 0.480779 | -0.249250 | -0.469411 |
| 15 | 7 | 0 | -0.653194 | -0.119283 | -0.715789 |
| 16 | 47 | 0 | 2.565419 | -0.338522 | -0.032153 |
| 17 | 8 | 0 | 4.692873 | -0.436826 | 0.562200 |
| 18 | 6 | 0 | 5.734106 | -0.003105 | 0.072357 |
| 19 | 6 | 0 | 7.042773 | -0.258756 | 0.755725 |
| 20 | 1 | 0 | 7.640142 | 0.657201 | 0.798654 |
| 21 | 1 | 0 | 6.881810 | -0.660947 | 1.755766 |
| 22 | 1 | 0 | 7.612341 | -0.983258 | 0.160610 |
| 23 | 6 | 0 | 5.741564 | 0.786819 | -1.203109 |
| 24 | 1 | 0 | 5.957236 | 1.835764 | -0.966089 |
| 25 | 1 | 0 | 6.538463 | 0.440248 | -1.868128 |
| 26 | 1 | 0 | 4.777068 | 0.727036 | -1.713848 |
| 27 | 6 | 0 | -2.994154 | -2.131703 | 0.396874 |
| 28 | 6 | 0 | -2.476310 | -1.638932 | 1.708512 |
| 29 | 1 | 0 | -2.432145 | -2.492799 | 2.397519 |
| 30 | 1 | 0 | -1.463641 | -1.242397 | 1.594983 |
| 31 | 1 | 0 | -3.121529 | -0.869115 | 2.136562 |
| 32 | 6 | 0 | -2.166616 | -3.106202 | -0.374247 |
| 33 | 1 | 0 | -1.110476 | -2.843027 | -0.276254 |
| 34 | 1 | 0 | -2.304249 | -4.107929 | 0.054909 |
| 35 | 1 | 0 | -2.432590 | -3.140244 | -1.431518 |
| 36 | 7 | 0 | -4.237289 | -1.911428 | 0.089405 |
| 37 | 1 | 0 | -4.668749 | -1.106715 | 0.559243 |
| 38 | 6 | 0 | -4.886688 | -2.340823 | -1.143126 |
| 39 | 1 | 0 | -5.941235 | -2.076564 | -1.081509 |
| 40 | 1 | 0 | -4.439428 | -1.823927 | -1.999729 |
| 41 | 1 | 0 | -4.792424 | -3.420606 | -1.268647 |
| 42 | 1 | 0 | -2.318022 | -0.298857 | -1.925605 |

**E(RM062X) = -1329.86781034**

**b, R=Met + $Ag^+$**

| Center Number | Atomic Number | Atomic Type | Coordinates (Angstroms) X | Y | Z |
|---|---|---|---|---|---|
| 1 | 6 | 0 | 1.903530 | -0.024737 | 0.845862 |
| 2 | 8 | 0 | 4.188581 | 0.705108 | -0.516478 |
| 3 | 8 | 0 | 1.848066 | 1.644671 | -1.184444 |
| 4 | 6 | 0 | 2.375006 | 2.515015 | -2.198719 |
| 5 | 1 | 0 | 3.227765 | 2.044793 | -2.695281 |
| 6 | 1 | 0 | 1.567075 | 2.683242 | -2.909831 |
| 7 | 1 | 0 | 2.689607 | 3.466252 | -1.758594 |
| 8 | 15 | 0 | 2.859473 | 1.171513 | -0.022380 |
| 9 | 8 | 0 | 3.098141 | 2.480248 | 0.887229 |
| 10 | 6 | 0 | 1.965957 | 3.166473 | 1.422593 |
| 11 | 1 | 0 | 2.354799 | 3.909789 | 2.117856 |
| 12 | 1 | 0 | 1.404851 | 3.661215 | 0.623611 |
| 13 | 1 | 0 | 1.304149 | 2.474626 | 1.956669 |
| 14 | 6 | 0 | -0.568573 | -0.161999 | 0.355272 |
| 15 | 7 | 0 | 0.571229 | -0.062705 | 0.599052 |
| 16 | 47 | 0 | -2.655296 | -0.152056 | -0.073843 |
| 17 | 8 | 0 | -4.788906 | -0.147013 | -0.667129 |
| 18 | 6 | 0 | -5.838033 | -0.077861 | -0.029479 |
| 19 | 6 | 0 | -7.151545 | -0.106974 | -0.749953 |
| 20 | 1 | 0 | -7.837288 | 0.639428 | -0.338239 |
| 21 | 1 | 0 | -7.006175 | 0.049632 | -1.818791 |
| 22 | 1 | 0 | -7.613208 | -1.089288 | -0.589658 |
| 23 | 6 | 0 | -5.851511 | 0.034785 | 1.466494 |
| 24 | 1 | 0 | -6.163456 | 1.050090 | 1.740299 |
| 25 | 1 | 0 | -6.585847 | -0.651803 | 1.898727 |
| 26 | 1 | 0 | -4.863070 | -0.161185 | 1.890104 |
| 27 | 6 | 0 | 2.750580 | -2.107537 | -0.633225 |
| 28 | 6 | 0 | 2.207000 | -1.494421 | -1.883119 |
| 29 | 1 | 0 | 2.118818 | -2.293537 | -2.631635 |
| 30 | 1 | 0 | 1.210382 | -1.078970 | -1.714689 |
| 31 | 1 | 0 | 2.867807 | -0.718899 | -2.274733 |
| 32 | 6 | 0 | 1.901413 | -3.093922 | 0.095430 |
| 33 | 1 | 0 | 0.873496 | -2.722139 | 0.139530 |
| 34 | 1 | 0 | 1.890249 | -4.038792 | -0.464320 |
| 35 | 1 | 0 | 2.258806 | -3.296431 | 1.106178 |
| 36 | 7 | 0 | 4.023884 | -1.999820 | -0.384701 |
| 37 | 1 | 0 | 4.481587 | -1.180394 | -0.802452 |
| 38 | 6 | 0 | 4.740574 | -2.659234 | 0.703399 |
| 39 | 1 | 0 | 5.799616 | -2.429296 | 0.594815 |
| 40 | 1 | 0 | 4.394718 | -2.294324 | 1.673755 |
| 41 | 1 | 0 | 4.602732 | -3.740587 | 0.643564 |
| 42 | 6 | 0 | 2.335206 | -0.377795 | 2.253972 |
| 43 | 1 | 0 | 1.898757 | 0.285286 | 3.014558 |
| 44 | 1 | 0 | 2.057034 | -1.405406 | 2.520465 |
| 45 | 1 | 0 | 3.423152 | -0.286558 | 2.339369 |

**E(RM062X) = -1369.16228669**

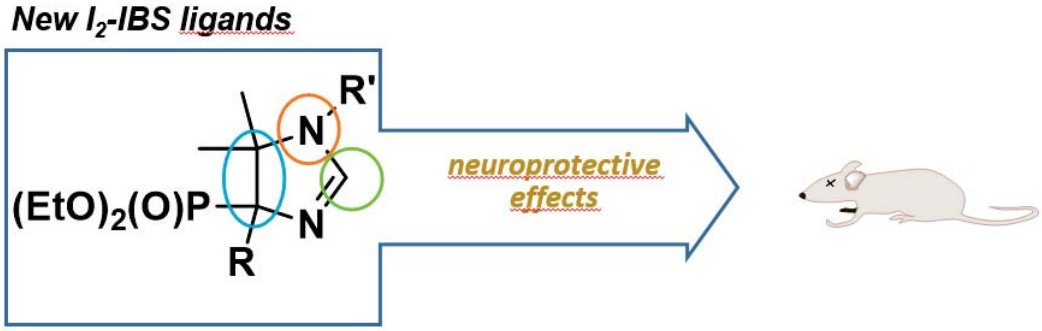
**b, R=Phe + $Ag^+$**

| Center Number | Atomic Number | Atomic Type | Coordinates (Angstroms) X | Y | Z |
|---|---|---|---|---|---|
| 1 | 6 | 0 | 1.567116 | -0.071601 | -0.062957 |
| 2 | 8 | 0 | 3.616077 | -1.925268 | 0.050497 |
| 3 | 8 | 0 | 1.135421 | -2.655898 | -0.344645 |
| 4 | 6 | 0 | 1.442867 | -4.052583 | -0.511340 |
| 5 | 1 | 0 | 2.291772 | -4.328517 | 0.118677 |
| 6 | 1 | 0 | 0.551046 | -4.599650 | -0.209073 |
| 7 | 1 | 0 | 1.675055 | -4.263502 | -1.559024 |
| 8 | 15 | 0 | 2.323544 | -1.606757 | -0.606008 |
| 9 | 8 | 0 | 2.518864 | -1.567441 | -2.197224 |
| 10 | 6 | 0 | 1.431844 | -1.167913 | -3.037466 |
| 11 | 1 | 0 | 1.746852 | -1.365993 | -4.061406 |
| 12 | 1 | 0 | 0.531566 | -1.747430 | -2.806657 |
| 13 | 1 | 0 | 1.233751 | -0.097836 | -2.914115 |
| 14 | 6 | 0 | -0.958081 | -0.120037 | 0.151792 |
| 15 | 7 | 0 | 0.198949 | -0.099012 | 0.018985 |
| 16 | 47 | 0 | -3.096180 | -0.163290 | 0.256764 |
| 17 | 8 | 0 | -5.304159 | -0.219231 | 0.333225 |
| 18 | 6 | 0 | -6.189096 | 0.500718 | -0.126760 |
| 19 | 6 | 0 | -7.627576 | 0.164806 | 0.122237 |
| 20 | 1 | 0 | -8.211021 | 0.263158 | -0.798342 |
| 21 | 1 | 0 | -7.722239 | -0.840027 | 0.533591 |
| 22 | 1 | 0 | -8.035514 | 0.888708 | 0.838410 |
| 23 | 6 | 0 | -5.874873 | 1.719528 | -0.942717 |
| 24 | 1 | 0 | -6.127123 | 1.514853 | -1.990439 |
| 25 | 1 | 0 | -6.490366 | 2.567137 | -0.626646 |
| 26 | 1 | 0 | -4.815822 | 1.981814 | -0.875693 |
| 27 | 6 | 0 | 2.260637 | -0.038455 | 2.147387 |
| 28 | 6 | 0 | 1.782573 | -1.411901 | 2.533788 |
| 29 | 1 | 0 | 1.754281 | -1.447268 | 3.631093 |
| 30 | 1 | 0 | 0.772664 | -1.601584 | 2.162195 |
| 31 | 1 | 0 | 2.457212 | -2.192745 | 2.178856 |
| 32 | 6 | 0 | 1.411566 | 1.112922 | 2.593729 |
| 33 | 1 | 0 | 0.352926 | 0.860256 | 2.500530 |
| 34 | 1 | 0 | 1.618633 | 1.309102 | 3.654079 |
| 35 | 1 | 0 | 1.615932 | 2.027508 | 2.031947 |
| 36 | 7 | 0 | 3.568446 | 0.150985 | 2.044216 |
| 37 | 1 | 0 | 4.108115 | -0.655906 | 1.732437 |
| 38 | 6 | 0 | 4.249275 | 1.440364 | 2.132340 |
| 39 | 1 | 0 | 5.261634 | 1.317568 | 1.747591 |
| 40 | 1 | 0 | 3.748834 | 2.204456 | 1.533243 |
| 41 | 1 | 0 | 4.300770 | 1.766938 | 3.176240 |
| 42 | 6 | 0 | 2.165859 | 1.182945 | -0.562696 |
| 43 | 6 | 0 | 3.509422 | 1.208755 | -0.981764 |
| 44 | 6 | 0 | 1.443968 | 2.388665 | -0.567915 |
| 45 | 6 | 0 | 4.106345 | 2.405426 | -1.367422 |
| 46 | 1 | 0 | 4.099281 | 0.296173 | -0.999029 |
| 47 | 6 | 0 | 2.051434 | 3.582623 | -0.947120 |
| 48 | 1 | 0 | 0.399695 | 2.394597 | -0.264931 |
| 49 | 6 | 0 | 3.388439 | 3.600531 | -1.343174 |
| 50 | 1 | 0 | 5.143720 | 2.400596 | -1.688989 |
| 51 | 1 | 0 | 1.474233 | 4.502512 | -0.936677 |
| 52 | 1 | 0 | 3.859732 | 4.531204 | -1.642509 |

E(RM062X) = -1560.84342814

# Capítulo 5

## Neuroprotective effects of a structurally new family of high affinity imidazoline $I_2$ receptor ligands



ACS Chemical Neuroscience

Letter

pubs.acs.org/chemneuro

# Neuroprotective Effects of a Structurally New Family of High Affinity Imidazoline $I_2$ Receptor Ligands

Sònia Abás,[†] Amaia M. Erdozain,[‡,§] Benjamin Keller,[∥] Sergio Rodríguez-Arévalo,[†] Luis F. Callado,[‡,§] Jesús A. García-Sevilla,[∥] and Carmen Escolano*[,†]

[†]Laboratory of Medicinal Chemistry (Associated Unit to CSIC), Department of Pharmacology, Toxicology and Medicinal Chemistry, Faculty of Pharmacy and Food Sciences, and Institute of Biomedicine (IBUB), University of Barcelona, Av. Joan XXIII 27-31, E-08028 Barcelona, Spain

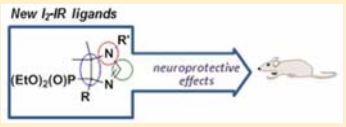
[‡]Department of Pharmacology, University of the Basque Country, UPV/EHU, E-48940 Leioa, Bizkaia, Spain

[§]Centro de Investigación Biomédica en Red de Salud Mental, CIBERSAM

[∥]Laboratory of Neuropharmacology, IUNICS/IdISPa, University of the Balearic Islands (UIB), Cra. Valldemossa km 7.5, E-07122 Palma de Mallorca, Spain

Supporting Information

**ABSTRACT:** The imidazoline $I_2$ receptors ($I_2$-IRs) are widely distributed in the brain, and $I_2$-IR ligands may have therapeutic potential as neuroprotective agents. Since structural data for $I_2$-IR remains unknown, the discovery of selective $I_2$-IR ligands devoid of $\alpha_2$-adrenoceptor ($\alpha_2$-AR) affinity is likely to provide valuable tools in defining the pharmacological characterization of these receptors. We report the pharmacological characterization of a new family of (2-imidazolin-4-yl)phosphonates. Radioligand binding studies showed that they displayed a higher affinity for $I_2$-IRs than idazoxan, and high $I_2/\alpha_2$ selectivity. In vivo studies in mice showed that acute treatments with **1b** and **2c** significantly increased p-FADD/FADD ratio (an index of cell survival) in the hippocampus when compared with vehicle-treated controls. Additionally, acute and repeated treatments with **2c**, but not with **1b**, markedly reduced hippocampal p35 cleavage into neurotoxic p25. The present results indicate a neuroprotective potential of (2-imidazolin-4-yl)phosphonates acting at $I_2$-IRs.




**KEYWORDS:** *(2-Imidazolin-4-yl)phosphonates, imidazoline $I_2$ receptors, neuroprotection*

Two types of nonadrenergic receptors for imidazolines (imidazoline receptors, IRs),[1] namely, the $I_1$- and $I_2$-types, are widely distributed in the brain. The $I_1$-IRs are responsible for sympatho-inhibitory effects of second generation, centrally acting, antihypertensive drugs such as moxonidine or rilmenidine.[2] The $I_2$-IRs have been studied for their possible involvement in human brain disorders such as depression,[3,4] Alzheimer's type dementia,[5,6] Parkinson's disease,[7] and glial tumors.[8,9] Additionally, a third IR subtype involved in insulin secretion has been identified in pancreatic $\beta$-cells and designated as $I_3$-IR.[10]

Compounds with high affinity for the $I_2$-IR have been associated with diverse actions, including analgesic,[11] anti-inflammatory,[12] and neuroprotective effects, e.g., through regulation of apoptotic signaling[13] or inhibition of p35 cleavage into neurotoxic p25.[14,15] It has been also proposed the existence of a binding site with high affinity for $I_2$-IR ligands on monoamine oxidase B enzyme.[16] Therefore, the heterogeneity of the IRs and their implication in so many different physiological and pathological processes emphasize their pharmacological importance and, then, the development of new compounds with better affinity and selectivity for these receptors becomes crucial to identify the targets connected to each biological effect.

Since structural data for $I_2$-IR remains unknown, the discovery of selective $I_2$-IR ligands devoid of $\alpha_2$-adrenoceptor ($\alpha_2$-AR) affinity is likely to have enhanced efficacy and serve as valuable tools in defining the pharmacological characterization of these receptors. Diverse research teams took this challenge. From a structural point of view, the nature of known IR ligands seems relatively restricted.[17] In particular, the $I_2$-IR ligands arsenal includes a plethora of 2-imidazolines attached to heterocycles such as benzofuran, benzoxazine, and benzodioxane; 2-phenoxymethyl and phenethylimidazolines; *trans*-styrylimidazolines, 2-aryl and 2-heterocyclic imidazolines; and 2-aminoimidazolines, apart from "agmatine-like" aliphatic guanidines. Figure 1 collects some selective $I_2$-IR ligands including idazoxan, a compound with a well-established affinity for $I_2$-IR (Figure 1).

As outlined in Figure 1, all $I_2$-IR ligands reported to date feature a 2-substituted imidazoline nucleus without additional decoration in the 1-, 4-, and 5-positions.[17] As the effects of introducing substituents in these positions have remained so far unexplored, a medicinal chemistry program aimed to this goal may lead to $I_2$-ligands with enhanced pharmacological proper-

**Received:** December 6, 2016
**Accepted:** December 28, 2016
**Published:** December 28, 2016





DOI: 10.1021/acschemneuro.6b00426
*ACS Chem. Neurosci.* 2017, 8, 737−742

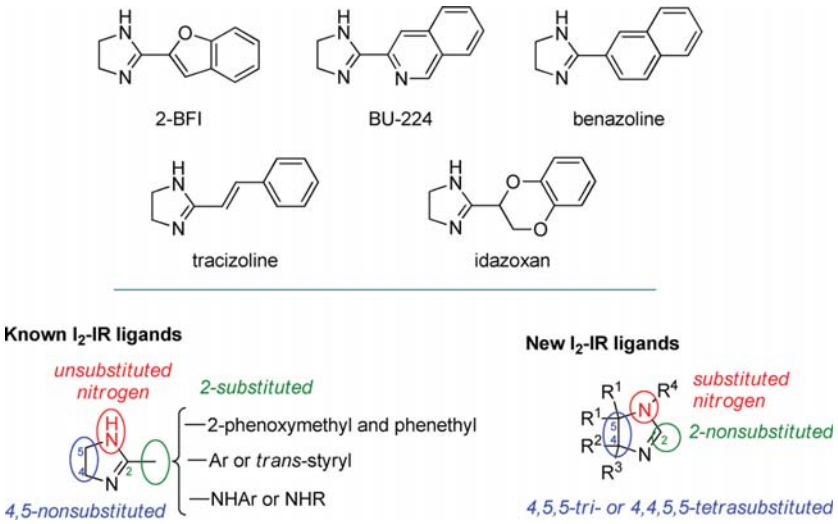
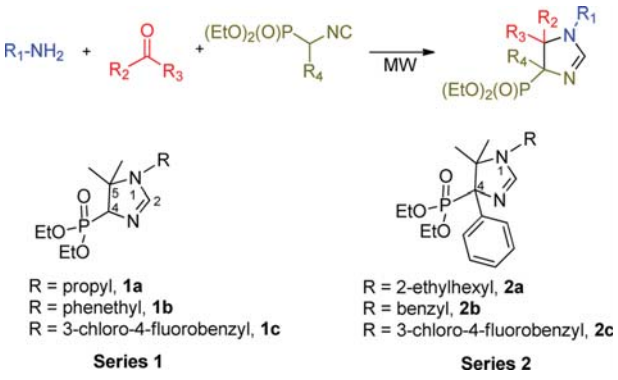



**Figure 1.** Representative $I_2$-IR ligands (up), general structure of previously described $I_2$-IR ligands (bottom, left), and proposed new $I_2$-IR ligands (bottom, right).

ties. Additionally, the introduction of substituents in these positions may be combined with the removal of the substituent from the 2-position.

Driven by the above-mentioned interest and to meet the demand for highly substituted 2-imidazoline-containing compounds, we recently published, using an isocyanide-based multicomponent reaction under microwave irradiation, the first synthesis of diversely substituted (2-imidazolin-4-yl)-phoshonates (Scheme 1).[18]

**Scheme 1. General Synthesis of the (2-Imidazolin-4-yl)phosphonates and Examples Herein Studied**




Hence, we assessed the pharmacological profile and selectivity of these substituted (2-imidazolin-4-yl)phoshonates through competition binding studies against the selective $I_2$-IR radioligand [$^3$H]-2-[(2-benzofuranyl)-2-imidazoline] (2-BFI) and the $\alpha_2$-adrenergic receptor selective radioligand [$^3$H]-RX821002 (2-methoxyidazoxan). Furthermore, we characterized the pharmacological effects of them both in vivo and in vitro looking at their analgesic and neuroprotective effects.

## RESULTS AND DISCUSSION

Initially, we evaluated two series of (2-imidazolin-4-yl)-phoshonates: (a) series 1 with variations at position 1 and monosubstituted in position 4 by a diethylphosphonate group; and (b) series 2 with variations at position 1 and bearing an extra phenyl substituent in the 4-position leading to a quaternary carbon (Scheme 1). Both series were dimethylsubstituted in the 5-position and, in sharp contrast with known $I_2$-IR ligands, did not present a C-2 substituent. Regarding N-1, alkyl groups (**1a** and **2a**), phenethyl (**1b**), and benzyl substituents, either nonhalogenated (**2b**) or halogenated (3-Cl-4-F-benzyl, **1c** and **2c**) were considered.

**Chemistry.** Compounds were prepared using our previously optimized conditions involving the irradiation of a solution of diethyl isocyanomethylphosphonate (**1a**, **1b**, **1c**) or diethyl $\alpha$-phenylisocyanomethylphosphonate (**2a**, **2b**, **2c**) (1 equiv), $AgNO_3$ (10% mmol), and a suitable primary amine (propylamine, **1a**; 2-ethylhexenylamine, **2a**; phenethylamine, **1b**; benzylamine, **2b**; 3-chloro-4-fluorobenzylamine, **1c** and **2c**) (1.5 equiv) in acetone in a microwave oven during 10 min at 40 °C. The synthesis of **3**, bearing a bulkier substituent in the 5-C, was performed following the general procedure replacing acetone by cyclohexanone. Compound **4**, endowing a methyl ester at the 4-C instead of a phosphonate ester, was accessed following the general reaction conditions using methyl $\alpha$-phenylisocyanoacetate. Noteworthy, compounds **1c**, **2c**, and **4** were first synthesized and fully characterized in this work (Figure 2). The yields and experimental procedures for the preparation of these derivatives are provided in the Supporting Information.

**Pharmacology and Structure−Activity Relationships.** The pharmacological activity of the prepared compounds was evaluated through competition binding studies against the selective $I_2$-IR radioligand [$^3$H]-2-BFI or the selective $\alpha_2$-AR radioligand [$^3$H]RX821002. The studies were performed in

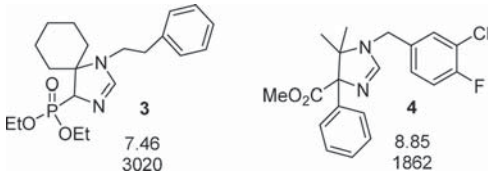



**Figure 2.** Modified proposed structures.



DOI: 10.1021/acschemneuro.6b00426
ACS Chem. Neurosci. 2017, 8, 737−742

membranes from postmortem human frontal cortex, a brain area that shows an important density of $I_2$-IRs and $\alpha_2$-ARs.[5,19] The inhibition constant ($K_i$) for each compound was obtained and are expressed as the corresponding p$K_i$ (Table 1).

**Table 1. $I_2$-IR and $\alpha_2$-AR Receptor Binding Affinities (p$K_i$) of the New Compounds**

| compd | p$K_i$ [$^3$H]-2-BFI, $I_2$ | p$K_i$ [$^3$H]-RX821002, $\alpha_2$ | selectivity $I_2/\alpha_2$[a] |
|---|---|---|---|
| idazoxan | 7.27 ± 0.07 | 7.51 ± 0.07 | |
| **1a** | 7.84 ± 0.26 | 3.95 ± 0.26 | 7762 |
| **1b** | 8.19 ± 0.27 | 4.18 ± 0.09 | 10 233 |
| **1c** | 8.31 ± 0.30 | 5.81 ± 0.38 | 316 |
| **2a** | 7.61 ± 0.29 | 4.63 ± 0.11 | 955 |
| **2b** | 8.89 ± 0.29 | 7.52 ± 0.45 | 23 |
| **2c** | 9.42 ± 0.16 | 6.76 ± 0.22 | 457 |
| **3** | 7.46 ± 0.24 | 3.98 ± 0.12 | 3020 |
| **4** | 8.85 ± 0.21 | 5.58 ± 0.14 | 1862 |

[a]Selectivity $I_2$-IR/$\alpha_2$-AR expressed as antilog(p$K_i$ $I_2$-IR − p$K_i$ $\alpha_2$-ARs).

Idazoxan, a compound with well-established affinity for $I_2$-IR (p$K_i$ = 7.27 ± 0.07) and $\alpha_2$-ARs (p$K_i$ = 7.51 ± 0.07), was used as reference. The competition binding assays for $\alpha_2$-ARs were carried out also for all compounds **1**−**4**. The selectivity between receptors was expressed by the $I_2/\alpha_2$ index. This index is calculated as the antilogarithm of the difference between p$K_i$ values for $I_2$-IR and p$K_i$ values for $\alpha_2$-ARs (see Table 1).

Of note, all the compounds tested showed higher affinity to $I_2$-IR than the standard idazoxan (p$K_i$ $I_2$ = 7.27). In particular, in series 1, compound **1a**, bearing a propyl group in N1, displays a p$K_i$ ($I_2$) affinity of 7.84 with an $I_2/\alpha_2$ selective ratio of 7762. Substitution of the *N*-alkyl group by a phenethyl group gave rise to **1b** with a markedly benefit in the $I_2$-IR affinity (p$K_i$ $I_2$ = 8.19), and the selectivity profile was greatly enhanced to an $I_2/\alpha_2$ ratio of 10233. Preparation of congener **1c** was driven by the interest in considering the effect than halogens in an aromatic N1 substituent can have in the $I_2$-IR binding activity, resulting in an increase in the p$K_i$ to 8.31 but with a considerable drop in the $I_2/\alpha_2$ ratio selectivity value to 316.

Next, the effect of having a phenyl substituent in position 4 of the 2-imidazolin nucleus was tested with compounds of series 2. The presence of alkyl group, 2-ethylhexyl, as N1 substituent in **2a** led to a slight decrease in the affinity (p$K_i$ $I_2$ = 7.61) compared with the homologues in series 1 **1a** (p$K_i$ $I_2$ = 7.84) but still with better activity binding than that recorded for the reference idazoxan (p$K_i$ $I_2$ = 7.27, $K_i$ = 53.70 nM). Regarding the $I_2/\alpha_2$, a 955 ratio for **2a** was encountered. As in the previous series, the introduction of a substituent in the N1 bearing an aromatic group, a benzyl in **2b**, improved the affinity value to p$K_i$ $I_2$ = 8.89 ($K_i$ = 1.29 nM), even more (1.28 times) than that previously seen in series 1 (0.35). An opposite trend was found with regard to the $I_2/\alpha_2$ selectivity, which increased dramatically in series 1 from **1a** to **1b**, and reduced from alkyl N1 substituted **2a** to aryl bearing N1 substituent in **2b**, where the $\alpha_2$ affinity was comparable with that for idazoxan. In agreement with the observation made in series 1, the presence of halogen atoms (3-chloro-4-fluoro) in the aromatic ring results in an increase in the $I_2$-IR affinity. Of note, in compound **2c**, the affinity raised to an outstanding level (p$K_i$ $I_2$ = 9.42, $K_i$ = 0.38 nM). The $I_2/\alpha_2$ selectivity ratio is comparable in both analogous compounds **1c** and **2c**.

Taking into account the aforementioned results two compounds emerged as the more promising ones: **1b**, endowed with an outstanding $I_2/\alpha_2$ selectivity, and **2c**, with an excellent affinity for $I_2$-IR (Scheme 1 and Table 1). Both compounds were briefly examined for further structural modifications. In order to explore the effect of bulkier substituents in 5-C we synthesized **3** (Figure 2). Although **3** exceeded both the affinity and selectivity of idazoxan, it did not improve the results of **1b**, thus suggesting that the presence of a bulkier substituent in 5-C is deleterious for both the affinity and selectivity.

Besides, replacement of the phosphonate ester of **2c** by a methyl ester gave rise to **4** (Figure 2). Although the affinity of **4** for both the $I_2$-IR (p$K_i$ = 8.85, $K_i$ = 1.41 nM) and $\alpha_2$-AR (p$K_i$ = 5.58) was lower than those of **2c**, a 4-fold increase in the $I_2/\alpha_2$ selectivity was observed as a consequence of a stronger decrease in $\alpha_2$-AR affinity.

The potential role of these new compounds as agonists or antagonists of $I_2$-IRs is not elucidated in the present study because the lack of appropriate functional assays. This may be due to the fact that the physiological effects mediated by the $I_2$-IRs are not fully understood.

**Analgesic Effects of 1b and 2c.** Based on the high affinity/selectivity for the $I_2$-IR of **1b** and **2c**, the analgesic effects of these compounds (5, 10, and 20 mg/kg) were studied in mice using the hot plate test. As shown in Figure 3A, both **1b** and **2c** increased the latency time to the first lick in mice, but this effect was only statistically significant for **2c** at 20 mg/kg. Consistently, the latency time to jump was also significantly increased by **2c** at the same dose (Figure 3B), suggesting that this compound presents some analgesic properties. Note the analgesic effect of morphine 5 mg/kg, used as control.

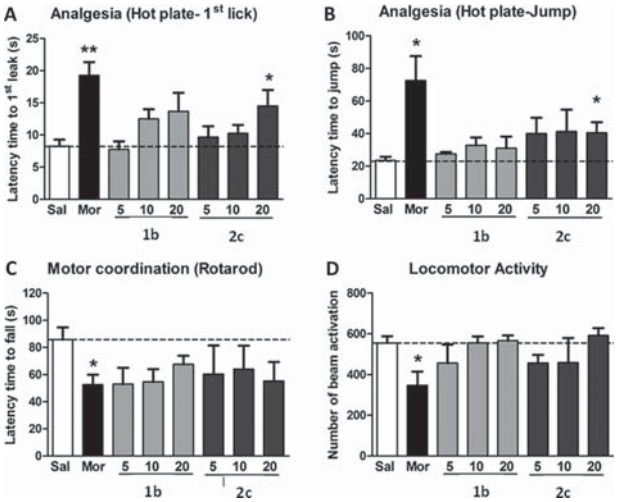



**Figure 3.** Measurement of the effects on analgesia, motor coordination and locomotor activity of **1b** (5, 10, and 20 mg/kg, i.p.) and **2c** (5, 10, and 20 mg/kg, i.p.) in mice. Morphine (5 mg/kg, i.p.) was used as a reference. (A) Latency time to the first lick induced by the acute administration of **1b**, **2c**, morphine, or vehicle control. (B) Latency time to jump induced by the acute administration of **1b**, **2c**, morphine, or vehicle control. (C) Effects on motor coordination induced by the acute administration of **1b**, **2c**, morphine, or vehicle control. (D) Effects on locomotor activity induced by the acute administration of **1b**, **2c**, morphine, or vehicle control. Columns represent mean ± SEM of 4−5 mice per group. *$p$ < 0.05, **$p$ < 0.005 when compared with the saline control group (ANOVA).



DOI: 10.1021/acschemneuro.6b00426
ACS Chem. Neurosci. 2017, 8, 737−742

Since the latency time to jump could be due not only to the analgesic effect, but also to a neuromuscular impairment produced by the drug, the effect of **1b** and **2c** on motor coordination and locomotor activity were also studied. As shown in Figure 3C, both compounds induced a little impairment in the motor coordination that did not reach statistical significance. Similarly, nor **1b** or **2c** did significantly affect the locomotor activity (Figure 3D).

$I_2$-IR ligands are effective analgesics in tonic inflammatory and neuropathic pain models. However, they show less effect in acute phasic pain models.[11] Altogether our present data suggest that **2c** produces an analgesic effect, as observed in the hot plate test, as previously demonstrated by other $I_2$-IR selective ligands.

**Hypothermic Effects of Compounds 1b and 2c in Mice.** In adult male CD1 mice and compared with vehicle-treated controls, the compounds **1b** and **2c** induced mild hypothermia as assessed by moderate reductions (−0.6 to −2.2 °C) of the rectal temperature at 1 h after injection with similar effects of the tested doses of 5 and 20 mg/kg (Figure 4A and B,

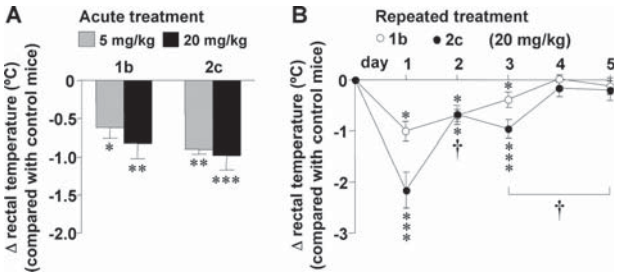



**Figure 4.** Acute and repeated measurement of the hypothermic effects of compounds **1b** and **2c** in mice. (A) Effects of acute treatments with **1b** and **2c** (5−20 mg/kg, i.p.) on the rectal body temperature in mice. Columns are means ± SEM of the difference (Δ, 1 h, basal value) in body temperature (°C) for **1b**/5-treated mice compared with vehicle-treated control (C) mice. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ when compared with the C group (Student's $t$ test). (B) Effects of repeated (5 days) treatments with **1b** (20 mg/kg, i.p., open circles) and **2c** (20 mg/kg, i.p., closed circles) on the rectal body temperature in mice. Circles are means ± SEM of the difference (Δ, 1 h, basal value) in body temperature (°C) for **1b**/5-treated mice compared with vehicle-treated controls. *$p < 0.05$, ***$p < 0.001$ when compared with the C group (ANOVA), $^{\dagger}p < 0.05$ when compared with the corresponding data of treatment day 1 (repeated measures ANOVA).

day 1). Repeated (5 days) administration (20 mg/kg) revealed significantly attenuated hypothermic effects of **1b** from day 3 and of **2c** from day 2 of treatment (repeated measures ANOVA; Figure 4B), indicating the induction of tolerance to the hypothermic effects of both drugs.

It has previously reported that other $I_2$-IR selective ligands as idazoxan or 2-(4,5-dihydroimidazol-2-yl)quinoline (BU224) induce also hypothermia in rats.[15,20,21] Hypothermia is well established as having a neuroprotective effect in cerebral ischemia and even mild temperature drops cause significant neuroprotection.[20] In the clinic, hypothermia has been used to improve the neurological outcome under various pathological conditions, including stroke and traumatic brain injury.[22,23] Thus, the hypothermic effects showed by our compounds might be a relevant feature that could mediate neuroprotection. In this sense, it has been proposed that some of the neuroprotective effect of $I_2$-IR selective ligands as idazoxan may be related to hypothermic effects of the drug.[20] The mechanism explaining the neuroprotective effect mediated by hypothermia may be related to the inhibition of glutamate release.[24]

**Effects of Acute and Repeated Treatments with 1b, 2c, and BU224 on the Ratio of p-Ser191 FADD to FADD in the Hippocampus of Mice.** The ratio of oligomeric fas-associated protein with death domain (FADD) phosphorylated at ser191 (p-FADD) to total dimeric FADD has been introduced as an index of cell survival and neuroplasticity.[25] Acute treatments of mice with **1b** (20 mg/kg), **2c** (5 mg/kg), and BU224 (20 mg/kg, used as reference $I_2$-IR compound) significantly increased (40−70%, $p < 0.05$) the p-FADD/FADD ratio in the hippocampus when compared with vehicle-treated controls (Figure 5A, left panel). After repeated (5 days) administration and again compared with control mice, **1b** and **2c** (5 and 20 mg/kg) as well as BU224 (20 mg/kg) did not alter the hippocampal p-FADD/FADD ratio (Figure 5A, right panel).

FADD is an adaptor of death receptors that can also induce antiapoptotic actions through its phosphorylated form (p-FADD). In the extrinsic (Fas receptor) apoptotic pathway, the balance between proapoptotic FADD adaptor and its non-apoptotic p-Ser191/194 FADD form has a critical role (p-FADD/FADD ratio) to determine cell death or survival actions in the brain.[26−28] The significantly increase of the p-FADD/FADD ratio in the hippocampus suggests that the tested compounds are able to induce novel nonapoptotic (e.g., neuroplastic) actions in the mice brain.

**Effects of Acute and Repeated Treatments with 1b, 2c, and BU224 on p35 Cleavage into Neurotoxic p25.** The contents of p35 and p25 (cdk5 activators), expressed as p25/p35 ratios (Figure 5B), were assessed in the hippocampus of mice treated with **1b**, **2c** and BU224 as a reference. Single (5 and 20 mg/kg) or repeated doses (20 mg/kg) of **1b** had no effect on the levels of p35 or those of its calpain-dependent cleavage product p25 (Figure 5B). In turn, **2c** did not affect the levels of p35, but markedly reduced (61−72%, $p < 0.01$) the contents of p25 and accordingly the p25/p35 ratios after a single dose (5 and 20 mg/kg; Figure 5B). This inhibitory action on p25 protein in the mouse hippocampus persisted after repeated (5 days) treatment with **2c** (20 mg/kg) without reaching statistical significance (due to the error size in the control group). Similarly, acute but not repeated BU224 (20 mg/kg) decreased (69%, $p < 0.01$) the p25/p35 ratio (Figure 5B).[15]

After neuronal injury or neurotoxic insult, there is a subsequent increase in intracellular calcium levels that induce p35 to convert to its N-terminal truncated product p25. Calpain mediated cleavage of Cdk5 activator p35 generates the p25 fragment, which is responsible for aberrant Cdk5 activity and neurotoxicity.[29] Thus, increased levels of p25 are found in the brains of patients with Alzheimer's disease[14] or ischemic stroke.[30] It has been also reported that neurons undergoing certain forms of nonexcitotoxic damage also require calpain-mediated production of p25.[31,32] A previous report of our group has demonstrated that the $I_2$-IR selective ligand BU224 display neuroprotective effects in an animal model of excitotoxicity.[15] This effect could be related to the reduced contents of p25 as a result of reduced calpain activity. The inhibitory effects of IR drugs on calpain activity (reduced fragmentation of p35) appear to result from decreased intracellular $Ca^{2+}$ concentrations and not from direct enzyme inhibition.[15] All these data suggest that the reduction in the


DOI: 10.1021/acschemneuro.6b00426
ACS Chem. Neurosci. 2017, 8, 737−742

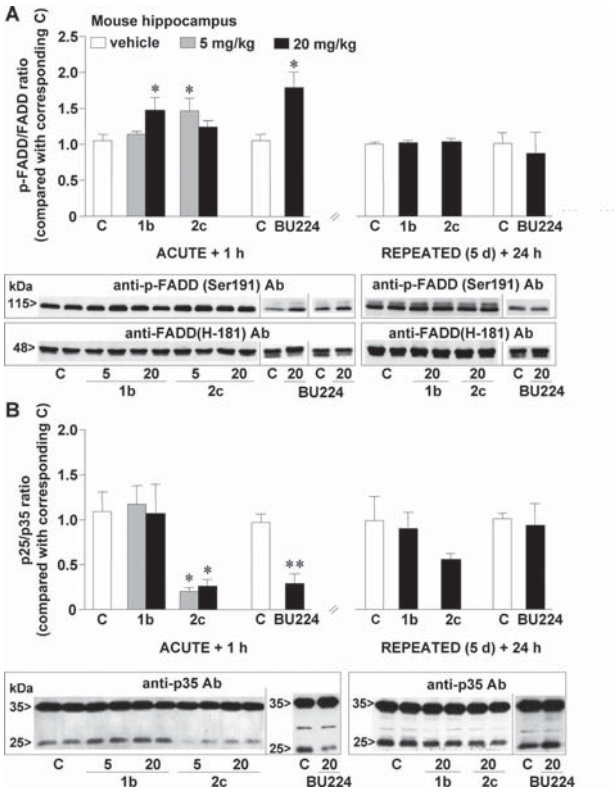



**Figure 5.** Effects of acute and repeated treatments with **1b**, **2c**, and BU224 on the ratio of p-Ser191 FADD to FADD (A) and on p35 cleavage into neurotoxic p25 (B) in the hippocampus of mice. (A) Effects of acute (5−20 mg/kg, i.p., $n$ = 3−6) and repeated (20 mg/kg, i.p., $n$ = 4−5) **1b**, **2c**, and BU224 on the contents of oligomeric FADD phosphorylated at Ser191 and total dimeric FADD (expressed as the ratio of p-FADD to FADD) in the hippocampus (HC) of mice. Columns are means ± SEM of p-FADD/FADD ratios in **1b**-, **2c**-, BU224-, and vehicle (control, C, $n$ = 5−6)-treated groups. *$p$ < 0.05, when compared with the corresponding C group (one- or two-tailed Student's $t$ test). Below: representative immunoblots for p-FADD and FADD in mouse HC after acute/repeated **1b** and **2c**. (B) Effects of acute (5−20 mg/kg, i.p., $n$ = 3−5) and repeated (20 mg/kg, i.p., $n$ = 5) **1b**, **2c**, and BU224 on cleavage of p35 into p25 (expressed as the ratio of p25 to p35) in the hippocampus (HC) of mice. Columns are means ± SEM of p25/p35 ratios in **1b**-, **2c**-, BU224- and vehicle (control, C, $n$ = 5−6)-treated groups. *$p$ < 0.05, when compared with the corresponding C group (ANOVA with Bonferroni's post hoc test), **$p$ < 0.01, when compared with the corresponding C group (two-tailed Student's $t$ test). Below: representative immunoblots for p35 and p25 in mouse HC after acute/repeated **1b**, **2c** and BU224. The apparent molecular masses (kDa) of target proteins were estimated from referenced standards.

contents of p25 induced by **2c** in the present study could support its putative therapeutic role as a neuroprotective drug.

## CONCLUSIONS

Owing the outstanding high $I_2$-IR affinity of **2c** and the high $I_2$-IR/$\alpha_2$ selectivity of **1b**, markedly better than any described $I_2$-IR ligand to date, (2-imidazolin-4-yl)phoshonates might be considered as suitable scaffolds for designing novel $I_2$-IR ligands. This new family of selective $I_2$ ligands is likely to enhance the therapeutic efficacy in pathological processes involving $I_2$-IR. In addition, (2-imidazolin-4-yl)phoshonate $I_2$ ligands are useful tools to define the functional role (e.g., neuroprotection) of imidazoline $I_2$ receptors.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.6b00426.

General methods for the chemical syntheses and pharmacology methods (PDF)

## AUTHOR INFORMATION

### Corresponding Author

*Phone: +34 934024542. E-mail: cescolano@ub.edu.

### ORCID

Carmen Escolano: 0000-0002-9117-8239

### Author Contributions

S.A. and S.R.-A. synthesized and characterized compounds chemically. A.M.E. and B.K. performed the biological experiments. L.F.C., J.A.G.-S., and C.E. designed the study, supervised the project, and wrote the manuscript. All authors have given approval to the final version of the manuscript.

### Funding

This study was supported by the *Ministerio de Economía y Competitividad* and *Fondo Europeo de Desarrollo Regional* (MINECO-FEDER) (Projects SAF2011-29918, SAF2013-48586-R, SAF2014-55903-R, SAF2016-77703-C2-1-R, BFU2015-66030-R), *Generalitat de Catalunya* (Grant 2014-SGR-0155), and the Basque Government (IT616/13). B.K. was supported by a predoctoral contract from Redes Temáticas de Investigación Cooperativa en Salud−Red de Trastornos Adictivos (RETICS-RTA, RD12/0028/0011), Instituto de Salud Carlos III (ISCIII, MINECO/FEDER), Spain. J.A.G.-S. is a member of the Institut d'Estudis Catalans (Barcelona, Catalonia, Spain). A.M.E. was recipient of a postdoctoral fellowship from the Basque Government.

### Notes

The authors declare no competing financial interest.

## ABBREVIATIONS

$I_2$-IRs, $I_2$ receptors; $\alpha_2$-AR, $\alpha_2$-adrenoceptor; [$^3$H]-2-BFI, [$^3$H]-2-[(2-benzofuranyl)-2-imidazoline]; [$^3$H]RX821002, 2-methoxyidazoxan; BU224, 2-(4,5-dihydroimidazol-2-yl)quinolone

## REFERENCES

(1) Bousquet, P., Feldman, J., and Schwartz, J. (1984) Central cardiovascular effects of alpha adrenergic drugs: differences between catecholamines and imidazolines. *J. Pharmacol. Exp. Ther. 230*, 232−236.

(2) Ernsberger, P., Graves, M. E., Graff, L. M., Zakieh, N., Nguyen, P., Collins, L. A., Westbrooks, K. L., and Johnson, G. G. (1995) I1-imidazoline receptors. Definition, characterization, distribution, and transmembrane signaling. *Ann. N. Y. Acad. Sci. 763*, 22−42.

(3) Meana, J. J., Barturen, F., Martín, I., and García-Sevilla, J. A. (1993) Evidence of increased non-adrenoreceptor [$^3$H]idazoxan binding sites in the frontal cortex of depressed suicide victims. *Biol. Psychiatry 34*, 498−501.

(4) García-Sevilla, J. A., Escribá, P. V., Sastre, M., Walzer, C., Busquets, X., Jaquet, G., Reis, D. J., and Guimón, J. (1996) Immunodetection and quantitation of imidazoline receptor proteins in platelets of patients with major depression and in brains of suicide victims. *Arch. Gen. Psychiatry 53*, 803−810.



DOI: 10.1021/acschemneuro.6b00426
*ACS Chem. Neurosci.* 2017, 8, 737−742

(5) Ruíz, J., Martín, I., Callado, L. F., Meana, J. J., Barturen, F., and García-Sevilla, J. A. (1993) Non-adrenoreceptor [$^3$H] idazoxan binding sites ($I_2$-imidazoline sites) are increased in postmortem brain from patients with Alzheimer's disease. *Neurosci. Lett. 160*, 109−112.
(6) García-Sevilla, J. A., Escribá, P. V., Walzer, C., Bouras, C., and Guimón, J. (1998) Imidazoline receptor proteins in brains of patients with Alzheimer's disease. *Neurosci. Lett. 247*, 95−98.
(7) Gargalidis-Moudanos, C., Pizzinat, N., Javoy-Agid, F., Remaury, A., and Parini, A. (1997) $I_2$-imidazoline binding sites and monoamine oxidase activity in human postmortem brain from patients with Parkinson's disease. *Neurochem. Int. 30*, 31−36.
(8) Martín-Gómez, J. I., Ruíz, J., Callado, L. F., Garibi, J. M., Aguinaco, L., Barturen, F., and Meana, J. J. (1996) Increased density of $I_2$-imidazoline receptors in human gliobastomas. *NeuroReport 7*, 1393−1396.
(9) Callado, L. F., Martín-Gomez, J. I., Ruiz, J., Garibi, J., and Meana, J. J. (2004) Imidazoline $I_2$ receptors density increases with the malignancy of human gliomas. *J. Neurol., Neurosurg. Psychiatry 75*, 785−787.
(10) Chan, S. L., Brown, C. A., Scarpello, K. E., and Morgan, N. G. (1994) The imidazoline site involved in control of insulin secretion: characteristics that distinguish it from $I_1$- and $I_2$-sites. *Br. J. Pharmacol. 112*, 1065−1070.
(11) Li, J. X., and Zhang, Y. (2011) Imidazoline $I_2$ receptors: target for new analgesics? *Eur. J. Pharmacol. 658*, 49−56.
(12) Regunathan, S., Feinstein, D. L., and Reis, D. J. (1999) Anti-proliferative and anti-inflammatory actions of imidazoline agents. Are imidazoline receptors involved? *Ann. N. Y. Acad. Sci. 881*, 410−419.
(13) Garau, C., Miralles, A., and García-Sevilla, J. A. (2013) Chronic treatment with selective $I_2$-imidazoline receptor ligands decreases the content of pro-apoptotic markers in rat brain. *J. Psychopharmacol. 27*, 123−134.
(14) Patrick, G. N., Zukerberg, L., Nikolic, M., de la Monte, S., Dikkes, P., and Tsai, L. H. (1999) Conversion of p35 to p25 deregulates Cdk5 activity and promotes neurodegeneration. *Nature 402*, 615−622.
(15) Keller, B., and García-Sevilla, J. A. (2016) Inhibitory effects of imidazoline receptor ligands on basal and kainic acid-induced neurotoxic signalling in mice. *J. Psychopharmacol. 30*, 875−886.
(16) McDonald, G. R., Olivieri, A., Ramsay, R. R., and Holt, A. (2010) On the formation and nature of the imidazoline $I_2$ binding site on human monoamine oxidase-B. *Pharmacol. Res. 62*, 475−488.
(17) Dardonville, C., and Rozas, I. (2004) Imidazoline binding sites and their ligands: an overview of the different chemical structures. *Med. Res. Rev. 24*, 639−641.
(18) Abás, S., Estarellas, C., Luque, F. J., and Escolano, C. (2015) Easy Access to (2-imidazolin-4-yl)phosphonates by a microwave assisted multicomponent reaction. *Tetrahedron 71*, 2872−2881.
(19) Grijalba, B., Callado, L. F., Meana, J. J., García-Sevilla, J. A., and Pazos, A. (1996) $\alpha_2$-Adrenoceptor subtypes in the human brain: a pharmacological delineation of [$^3$H]RX-821002 binding to membranes and tissue sections. *Eur. J. Pharmacol. 310*, 83−93.
(20) Craven, J. A., and Conway, E. L. (1997) Effects of alpha 2-adrenoceptor antagonists and imidazoline 2-receptor ligands on neuronal damage in global ischemia in the rat. *Clin. Exp. Pharmacol. Physiol. 24*, 204−207.
(21) Thorn, D. A., An, X. F., Zhang, Y., Pigini, M., and Li, J. X. (2012) Characterization of the hypothermic effects of imidazoline $I_2$ receptor agonists in rats. *Br. J. Pharmacol. 166*, 1936−1945.
(22) Marion, D. W., Penrod, L. E., Kelsey, S. F., Obrist, W. D., Kochanek, P. M., Palmer, A. M., Wisniewski, S. R., and DeKosky, S. T. (1997) Treatment of traumatic brain injury with moderate hypothermia. *N. Engl. J. Med. 336*, 540−546.
(23) Maier, C. M., Ahern, K. V., Cheng, M. L., Lee, J. E., Yenari, M. A., Steinberg, G. K., and Kirsch, J. R. (1998) Optimal depth and duration of mild hypothermia in a focal model of transient cerebral ischemia: effects on neurologic outcome, infarct size, apoptosis, ad inflammation. *Stroke 29*, 2171−2180.
(24) Illievich, U. M., Zornow, M. H., Choi, K. T., Scheller, M. S., and Strnat, M. A. (1994) Effects of hypothermic metabolic suppression on hippocampal glutamate concentrations after transient global cerebral ischemia. *Anesth. Analg. 78*, 905−911.
(25) Ramos-Miguel, A., Esteban, S., and García-Sevilla, J. A. (2010) The time course of unconditioned morphine-induced psychomotor sensitization mirrors the phosphorylation of FADD and MEK/ERK in rat striatum: role of PEA-15 as a FADD-ERK binding partner in striatal plasticity. *Eur. Neuropsychopharmacol. 20*, 49−64.
(26) García-Fuster, M. J., Ramos-Miguel, A., Rivero, G., La Harpe, R., Meana, J. J., and García-Sevilla, J. A. (2008) Regulation of the extrinsic and intrinsic apoptotic pathways in the prefrontal cortex of short- and long-term human opiate abusers. *Neuroscience 157*, 105−119.
(27) Ramos-Miguel, A., García-Fuster, M. J., Callado, L. F., La Harpe, R., Meana, J. J., and García-Sevilla, J. A. (2009) Phosphorylation of FADD at serine 194 is increased in the prefrontal cortex of opiate abusers: relation to mitogen activated protein kinase, phosphoprotein enriched in astrocytes of 15 kDa, and Akt signaling pathways involved in neuroplasticity. *Neuroscience 161*, 23−38.
(28) Álvaro-Bartolomé, M., La Harpe, R., Callado, L. F., Meana, J. J., and García-Sevilla, J. A. (2011) Molecular adaptations of apoptotic pathways and signaling partners in the cerebral cortex of human cocaine addicts and cocaine-treated rats. *Neuroscience 196*, 1−15.
(29) Yousuf, M. A., Tan, C., Torres-Altoro, M. I., Lu, F. M., Plautz, E., Zhang, S., Takahashi, M., Hernández, A., Kernie, S. G., Plattner, F., and Bibb, J. A. (2016) Involvement of aberrant Cdk5/p25 activity in experimental traumatic brain injury. *J. Neurochem. 138*, 317−327.
(30) Mitsios, N., Pennucci, R., Krupinski, J., Sanfeliu, C., Gaffney, J., Kumar, P., Kumar, S., Juan-Babot, O., and Slevin, M. (2007) Expression of cyclin-dependent kinase 5 mRNA and protein in the human brain following acute ischemic stroke. *Brain Pathol. 17*, 11−23.
(31) Kusakawa, G., Saito, T., Onuki, R., Ishiguro, K., Kishimoto, T., and Hisanaga, S. (2000) Calpain-dependent proteolytic cleavage of the p35 cyclin-dependent kinase 5 activator to p25. *J. Biol. Chem. 275*, 17166−17172.
(32) Nath, R., Davis, M., Probert, A. W., Kupina, N. C., Ren, X., Schielke, G. P., and Wang, K. K. (2000) Processing of cdk5 activator p35 to its truncated form (p25) by calpain in acutely injured neuronal cells. *Biochem. Biophys. Res. Commun. 274*, 16−21.


DOI: 10.1021/acschemneuro.6b00426
*ACS Chem. Neurosci.* 2017, 8, 737−742

# Neuroprotective effects of a structurally new family of high affinity imidazoline $I_2$ receptor ligands

*Sònia Abás,[†] Amaia M. Erdozain,[‡] Benjamin Keller,[ǂ] Sergio Rodríguez-Arévalo,[†] Luis F. Callado,[‡] Jesús A. García-Sevilla,[ǂ] Carmen Escolano[†,]**

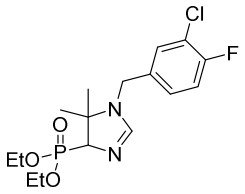
[†]Laboratory of Medicinal Chemistry (Associated Unit to CSIC), Department of Pharmacology, Toxicology and Medicinal Chemistry, Faculty of Pharmacy and Food Sciences, and Institute of Biomedicine (IBUB), University of Barcelona, Av. Joan XXIII 27-31, E-08028 Barcelona, Spain.

[‡]Department of Pharmacology, University of the Basque Country, UPV/EHU, E-48940 Leioa, Bizkaia, and Centro de Investigación Biomédica en Red de Salud Mental, CIBERSAM, Spain.

[ǂ]Laboratory of Neuropharmacology, IUNICS/IdISPa, University of the Balearic Islands (UIB), Cra. Valldemossa km 7.5, E-07122 Palma de Mallorca, Spain.

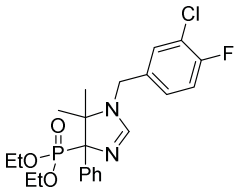
**Table of contents**

**Chemical synthesis.** NMR spectra were recorded in $CDCl_3$ at 400 MHz ($^1H$) and 100.6 MHz ($^{13}C$), and chemical shifts are reported in δ values downfield from TMS or relative to residual chloroform (7.26 ppm, 77.0 ppm) as an internal standard. Data are reported in the following manner: chemical shift, multiplicity, coupling constant (J) in hertz (Hz), integrated intensity. Multiplicities are reported using the following abbreviations: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet and br s, broad signal. Evaporation of solvents was accomplished with a rotary evaporator. Thin layer chromatography was done on $SiO_2$ (silica gel 60 F254), and the spots were located by UV, 1% aqueous $KMnO_4$. Chromatography refers to flash column chromatography and was carried out on $SiO_2$ (silica gel 60, SDS, 230–400 mesh). Mass spectra were recorded on an LTQ spectrometer using electrospray (ES+) ionization techniques. The CEM-Discover® Focused Monomode Microwave reactor (2450 MHz, 300 W) was used for microwave continuous flow irradiation. The temperature threshold was set at 40ºC.



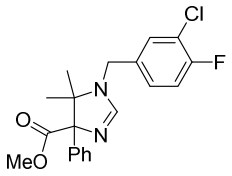

**Diethyl [1-(3-chloro-4-fluorobenzyl)-5,5-dimethyl-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate, 1c**. A mixture of $AgNO_3$ (10%-mmol, 6.8 mg, 0.04 mmol), acetone (3.0 mL), 3-chloro-4-fluorobenzylamine (63.8 mg, 0.4 mmol) and diethyl isocyanomethylphosphonate (64 μL, 0.4 mmol) was stirred under microwave irradiation at 60 ˚C for 20 min. The mixture was evaporated and the resulting residue was purified by column chromatography ($CH_2Cl_2$/5% MeOH) to give **1c** (77.7 mg, 51%) as a yellowish oil. IR (NaCl) 2982, 2906, 1678, 1597, 1391,

1249, 1029, 967, 773 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) δ 1.30 (s, 3H, $CH_3$), 1.36 (t, *J* = 7.0 Hz, 3H, $OCH_2CH_3$), 1.37 (t, *J* = 7.0 Hz, 3H, $OCH_2CH_3$), 1.42 (s, 3H, $CH_3$), 4.00 (dd, *J* = 16.4, 2.0 Hz, 1H, H-4), 4.13-4.28 (m, 6H, $OCH_2CH_3$, $CH_2Ar$), 6.89 (t, *J* = 2.0 Hz, 1H, H-2), 7.13 (t, *J* = 8.5 Hz, 1H, ArH), 7.19 (m, 1H, ArH), 7.34 (dd, *J* = 6.8, 2.0 Hz, 1H, ArH); $^{13}C$ NMR (100.6 MHz) δ 16.4 (d, *J* = 5.5 Hz, $OCH_2CH_3$), 16.5 (d, *J* = 5.5 Hz, $OCH_2CH_3$), 21.2 (d, *J* = 7.0 Hz, $CH_3$), 27.6 (d, *J* = 12.0 Hz, $CH_3$), 44.6 ($CH_2Ar$), 62.0 (d, *J* = 7.0 Hz, $OCH_2CH_3$), 62.9 (d, *J* = 7.0 Hz, $OCH_2CH_3$), 64.4 (d, *J* = 3.0 Hz, C-5), 73.5 (d, *J* = 163.0 Hz, C-4), 116.9 (d, *J* = 21.0 Hz, CHAr), 121.3 (d, *J* = 18.0 Hz, CAr), 127.0 (d, *J* = 7.0 Hz, CHAr), 129.5 (CHAr), 135.1 (d, *J* = 3.5 Hz, C-*ipso*), 156.7 (d, *J* = 13.0 Hz, C-2), 157.5 (d, *J* = 249.0 Hz, CAr); MS-EI *m*/*z* 376 $M^+$ (6), 361 (22), 305 (6), 239 (100), 143 (96), 107 (7), 81 (4); HRMS $C_{16}H_{24}ClFN_2O_3P$ $[M+H]^+$ 377.1203; found, 377.1192.



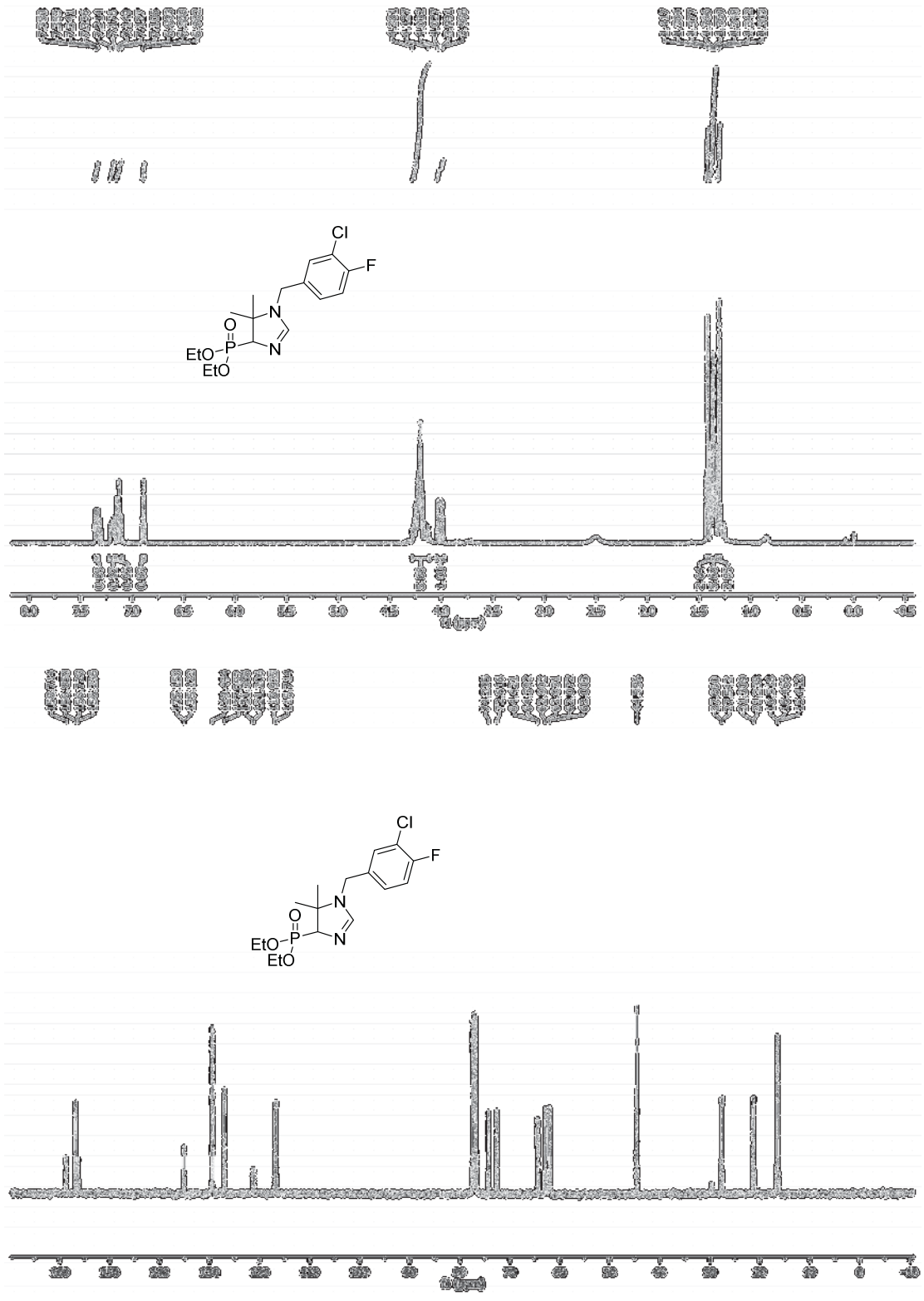

**Diethyl [1-(3-chloro-4-fluorobenzyl)-5,5-dimethyl-4-phenyl-4,5-dihydro-1*H*-imidazol-4-yl]phosphonate, 2c**. A mixture of $AgNO_3$ (10%-mmol, 6.8 mg, 0.04 mmol), acetone (3.0 mL), 3-chloro-4-fluorobenzylamine (95.7 mg, 0.6 mmol) and diethyl α-phenylisocyanomethylphosphonate (101.2 mg, 0.4 mmol) was stirred under microwave irradiation at 40 ˚C for 10 min. The mixture was evaporated and the resulting residue was purified by column chromatography ($CH_2Cl_2$/5% MeOH) to give **2c** (137.7 mg, 76%) as a colorless oil. IR (NaCl) 2924, 2906, 1603, 1500, 1391, 1247, 1057, 962, 754 $cm^{-1}$; $^1H$ NMR (400

MHz, $CDCl_3$, COSY, HETCOR) δ 0.66 (s, 3H, $CH_3$), 0.98 (d, *J* = 7.0 Hz, 3H, $OCH_2CH_3$), 1.32 (t, *J* = 7.0 Hz, 3H, $OCH_2CH_3$), 1.74 (s, 3H, $CH_3$), 3.44 (m, 1H, $OCH_2CH_3$), 3.74 (m, 1H, $OCH_2CH_3$), 4.08-4.23 (m, 2H, $OCH_2CH_3$), 4.12 (d, *J* = 16.0 Hz, 1H, $CH_2Ar$), 4.26 (d, *J* = 16.0 Hz, 1H, $CH_2Ar$), 7.01 (d, *J* = 4.0 Hz, 1H, H-2), 7.13 (t, *J* = 8.8 Hz, 1H, ArH), 7.27-7.39 (m, 4H, ArH), 7.47 (dd, *J* = 7.0, 2.5 Hz, 1H, ArH), 7.85 (br d, *J* = 35.2 Hz, 2H, ArH); $^{13}C$ NMR (100.6 MHz) δ 16.2 (d, *J* = 5.0 Hz, $OCH_2CH_3$), 16.5 (d, *J* = 5.5 Hz, $OCH_2CH_3$), 22.9 (d, *J* = 14.5 Hz, $CH_3$), 24.2 (d, *J* = 8.5 Hz, $CH_3$), 45.1 ($CH_2Ar$), 62.8 (d, *J* = 8.5 Hz, $OCH_2CH_3$), 63.4 (d, *J* = 7.0 Hz, $OCH_2CH_3$), 68.8 (d, *J* = 5.0 Hz, C-5), 81.9 (d, *J* = 146.0 Hz, C-4), 116.7 (d, *J* = 21.0 Hz, CHAr), 121.2 (d, *J* = 18.0 Hz, CAr), 126.9 (CHAr), 127.0 (CHAr), 127.4 (d, *J* = 2.5 Hz, CHAr), 128.1 (CHAr), 128.7 (2CHAr), 129.4 (CHAr), 135.5 (d, *J* = 3.5 Hz, C-*ipso*), 137.0 (C-*ipso*), 156.2 (d, *J* = 6.5 Hz, C-2); 157.5 (d, *J* = 248.5 Hz, CAr); MS-EI *m*/*z* 452 $M^+$ (0.2), 315 (100), 200 (2), 143 (46), 107 (2); HRMS $C_{22}H_{28}ClFN_2O_3P$ $[M+H]^+$ 453.1509; found, 453.1505.

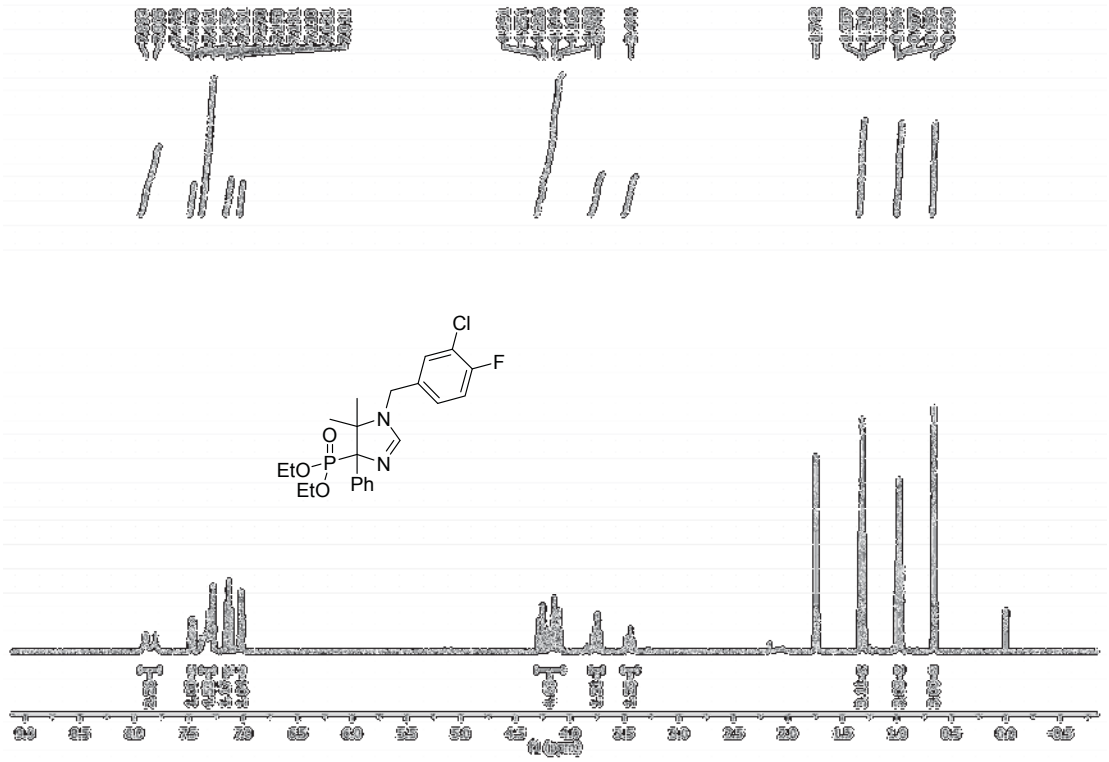


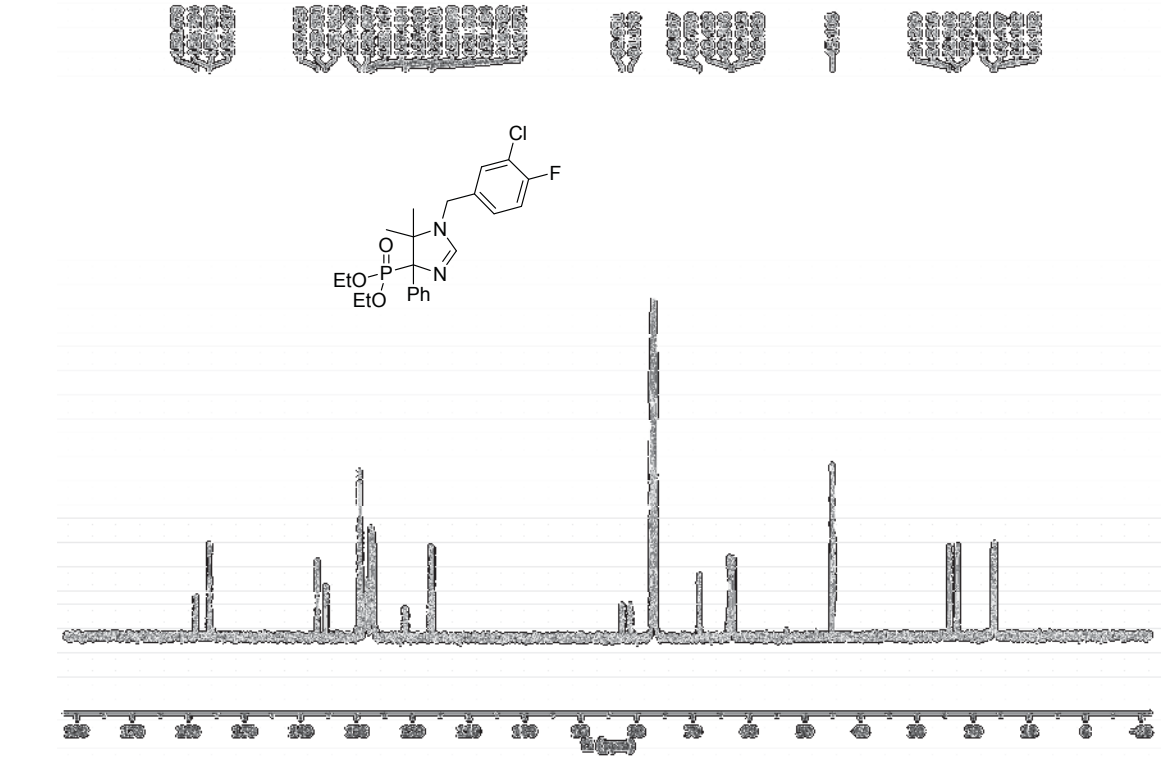

**Methyl [1-(3-chloro-4-fluorobenzyl)-5,5-dimethyl-4-phenyl-4,5-dihydro-1*H*-imidazole-4-yl]carboxylate, 4.** A mixture of $AgNO_3$ (10%-mmol, 6.8 mg, 0.04 mmol), acetone (3.0 mL), 3-chloro-4-fluorobenzylamine (95.7 mg, 0.6 mmol) and methyl α-phenylisocyanoacetate (70.1 mg, 0.4 mmol) was stirred under microwave irradiation at 40 ˚C for 10 min. The mixture was evaporated and the resulting residue was purified by column chromatography ($CH_2Cl_2$/5% MeOH) to give **4** (127.9 mg, 71%) as a yellowish oil. IR (NaCl) 2806, 1733, 1602, 1500, 1249,

1058, 750 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) δ 0.78 (s, 3H, $CH_3$), 1.36 (s, 3H, $CH_3$), 3.76 (s, 3H, $OCH_3$), 4.16 (s, 2H, $CH_2Ar$), 7.06-7.11 (m, 2H, ArH), 7.17 (s, 1H, H-2), 7.30-7.37 (m, 4H, ArH), 7.61 (br d, *J* = 7.2 Hz, 2H, ArH); $^{13}C$ NMR (100.6 MHz) δ 22.2 ($CH_3$), 22.8 ($CH_3$), 44.8 ($CH_2Ar$), 52.4 ($OCH_3$), 69.3 (C-5), 84.3 (C-4), 116.7 (d, *J* = 21.5 Hz, CHAr), 121.2 (d, *J* = 17.5 Hz, CAr), 126.7 (d, *J* = 7.0 Hz, CHAr), 127.5 (2CHAr), 127.7 (CHAr), 127.8 (2CHAr), 129.3 (CHAr), 135.4 (d, *J* = 4.0 Hz, C-*ipso)*, 136.6 (C-*ipso*), 156.3 (C-2); 157.5 (d, *J* = 249.0 Hz, CAr), 171.7 (CO); MS-EI *m*/*z* 374 $M^+$ (1), 315 (100), 231 (6), 200 (12), 164 (11), 143 (70), 104 (11); HRMS $C_{20}H_{21}ClFN_2O_2$ $[M+H]^+$ 375.1275; found, 375.1270.

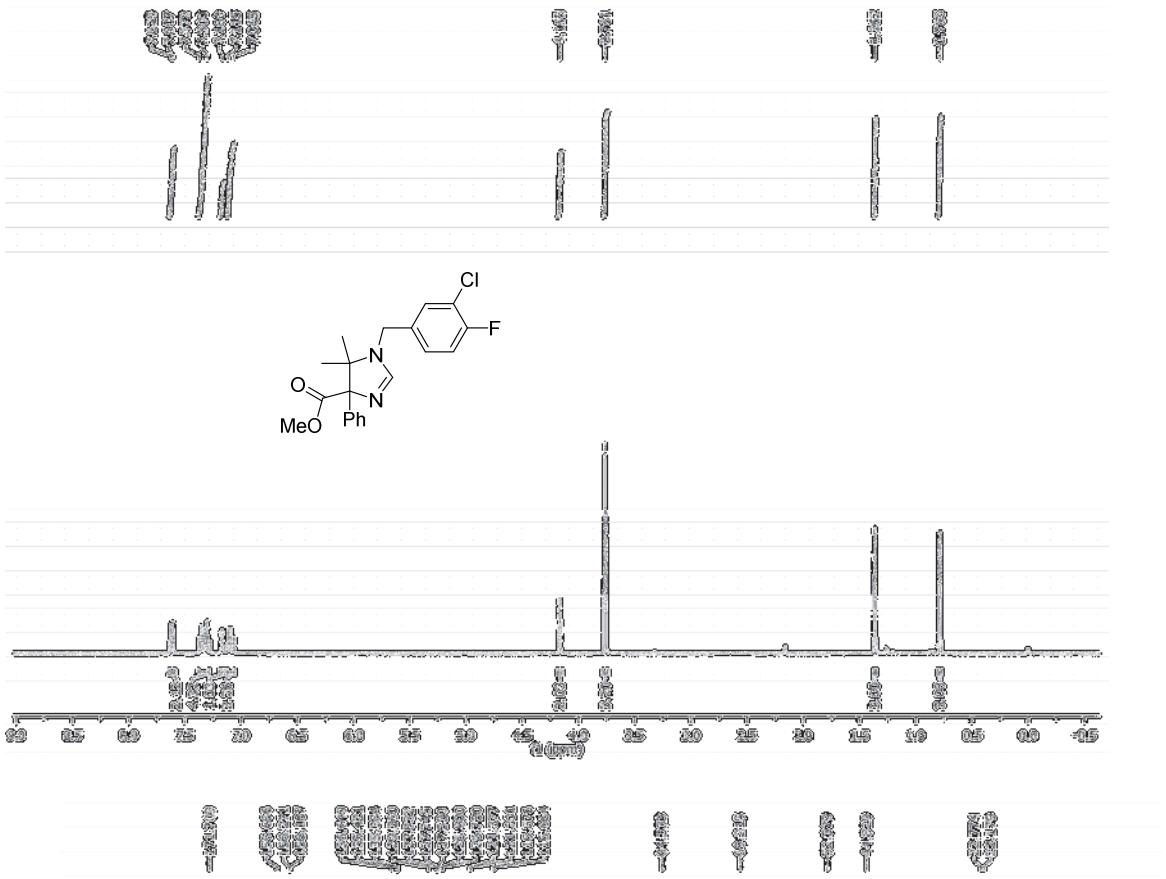


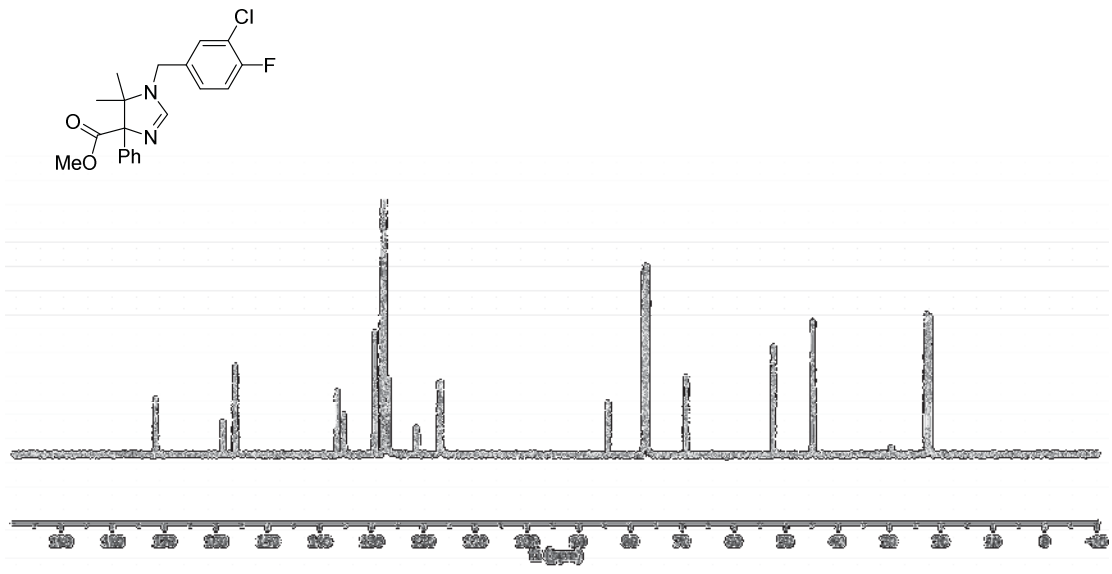

*Diethyl [1-(3-chloro-4-fluorobenzyl)-5,5-dimethyl-4,5-dihydro-1H-imidazol-4-yl]phosphonate*




*Diethyl [1-(3-chloro-4-fluorobenzyl)-5,5-dimethyl-4-phenyl-4,5-dihydro-1H-imidazol-4-yl]phosphonate*




*Methyl [1-(3-chloro-4-fluorobenzyl)-5,5-dimethyl-4-phenyl-4,5-dihydro-1H-imidazole-4-yl]carboxylate*

## Pharmacology

**Preparation of post-mortem human prefrontal cortex membranes.** Human brain samples from subjects who died by sudden and violent causes (motor-vehicle accidents) were obtained at autopsy in the Basque Institute of Legal Medicine, Bilbao, Spain. Samples from the prefrontal cortex (Brodmann's area 9) were dissected at the time of autopsy and immediately stored at -70ºC until assay. The study was developed in compliance with policies of research and ethical review boards for postmortem brain studies. Postmortem human frontal cortex samples of 3-4 subjects (~1 g) were homogenized using an ultraturrax in 30 volumes of homogenization buffer (0.25 M sucrose, 1mM $MgCl_2$, 5mM Tris–HCl, pH 7.4). The crude homogenate was centrifuged for 5 min at 1000 g (4 °C) and the supernatant was centrifuged again for 10 min at 40,000 g (4 °C). The resultant pellet was washed twice in 20 volumes of homogenization buffer and recentrifuged in similar conditions. Protein content was measured according to the method of Bradford using BSA as standard.

**[$^3$H]BFI and [$^3$H]RX821002 Binding Assays.** The pharmacological activity of the compounds was evaluated through competition binding studies against the selective $I_2$-IR radioligand [$^3$H]BFI [2-(2-benzofuranyl)-2- imidazoline] or the $\alpha_2$-AR selective radioligand [$^3$H]RX821002 (2-methoxy idazoxan). Specific binding was measured in 0.25 mL aliquots (50 mM Tris-HCl, pH 7.5) containing 100 μg of human cortical membranes, which were incubated in 96-well plates either with [$^3$H]BFI (2 nM) for 45 min at 25 °C or with [$^3$H]RX821002 (1 nM) for 30 min at 25°C, in the absence or presence of the competing compounds ($10^{-12}$–$10^{-3}$ M, 10 concentrations). Incubations were terminated by separating free ligand from bound ligand by rapid filtration under vacuum (1450 Filter Mate Harvester, PerkinElmer) through GF/C glass

fiber filters. The filters were then rinsed three times with 300 μL of binding buffer, air-dried (120 min), and counted for radioactivity by liquid scintillation spectrometry using a MicroBeta TriLux counter (PerkinElmer). Specific binding was determined and plotted as a function of the compound concentration. Nonspecific binding was determined in the presence of idazoxan ($10^{-5}$ M), a compound with well-established affinity for $I_2$-IRs and $\alpha_2$-AR. Analyses of competition experiments to obtain the inhibition constant ($K_i$) were performed by nonlinear regression using the GraphPad Prism program. All experiments were analyzed assuming a one-site model of radioligand binding. $K_i$ values were normalized to $pK_i$ values. $I_2/\alpha_2$ selectivity index was calculated as the antilogarithm of the difference between pKi values for $I_2$-IR and pKi values for $\alpha_2$-ARs.

**Animals.** Adult male CD-1 IGS mice (7 weeks old; 30-35 g; n=61) were used to assess the pharmacological effects of the compounds **1b** and **2c**. Mice were kept in methacrylate cages (Panlab S.L., Barcelona, Spain) with wood shavings for nesting material (Ultrasorb, Panlab S.L.) under controlled environmental conditions (20±2ºC; 50-60% humidity; 12 h light/dark cycle, light period from 8.00 to 20.00 h) and had free access to standard diet (Panlab A04) and tap water. The animals were handled for several days to reduce stress during drug testing. The animals were allowed to habituate to the laboratory environment for at least 2 h before any experiment was initiated. They were used only once, killed by cervical dislocation after testing, and brains were kept at -70ºC for future biochemical analysis. All animal experiments were conducted in accordance with the NIH Guidelines for Care and Use of Laboratory Animals (1985) and the European Communities Council Directive 1986 (86/609/EEC), and approved by the local Bioethical Committee of the UIB. All efforts were made to minimize the number of animals used and their suffering.

**Drug treatments.** Groups of randomly allocated mice received a single or repeated (5 days, one per day) intraperitoneal (i.p.) injection/s (2 ml/kg body weight) of the compounds **1b** and **2c** (acute: 5 and 20 mg/kg, n=3-5; repeated: 20 mg/kg, n=5). Dose range and regimen were deduced from those of other $I_2$-selective compounds (with similar affinities for $I_2$-IRs), associated with maximal effects in previous functional studies.[1-3] Control mice (acute: n=5, repeated: n=5) received equal volumes of the vehicle (DMSO/saline, 2 ml/kg, i.p.) in parallel to drug treatments. Assessment of hypothermic effects has been introduced as a functional assay for the study of $I_2$-IR activation in vivo.[4] Thus, in the present study the animals' rectal temperature was measured before (basal) and 1 h after vehicle/drug injection by use of a rectal probe (RET-3: Physitemp Instruments, Clifton, NJ) and a digital thermometer (EcoScan Temp JKT; Thermo Fisher Scientific, Waltham, MA). In additional experiments, further groups of mice (n=4-6) received a single or repeated (5 d) doses (20 mg/kg, i.p.) of the $I_2$-selective ligand BU224 2-(4,5-dihydroimidazol-2-yl)quinoline hydrochloride; Tocris, Bristol, UK), used as a reference $I_2$-compound, or the drug vehicle (saline, 2 ml/kg, i.p.). All mice were killed at 1 h after the acute treatments, or at 24 h after the last injection of repeated treatments by decapitation without anaesthesia (to avoid possible effects on target proteins) and the hippocampus was dissected on ice, frozen in liquid nitrogen and stored at -80°C until neurochemical analyses.

**Hot-plate test for analgesia.** Mice were placed in a hot plate (LE7406, Panlab S.L.; Spain) at 55 ± 0.1 °C as a nociceptive stimulus and the time of latency until the 1st lick and jumping were taken as the end points, with a cut-off time of 120 s. Animals were tested 30 min after the i.p. injection of separated doses of the compounds **1b** or **2c** (5, 10 and 20 mg/kg, n = 4-5), or the same volume of vehicle (10 ml/kg, n = 4). Morphine (5 mg/kg, i.p.) was used as a positive control. All drugs were disolved in 50% DMSO, 50% saline.

**Rotarod test for locomotor coordination.** Once the analgesia test was performed, mice were placed on a rotarod (LE8200, Panlab S.L.; Spain) with the maximum acceleration in 5 minutes, and latency to fall from the rotarod was recorded. Each animal was tested twice, separated by a 1□min interval, and the mean value was calculated. Animals had previously been habituated to the rod, the day before, rotating at a low speed of 12 rpm.

**Locomotor activity test.** After the rotarod test, mice were placed in a locomotor activity cage (LE886, Panlab S.L.; Spain), and movements were recorded for 5 min using infrared beam breaks in the horizontal plane.

**Preparation of mouse brain total homogenates.** Total homogenates of both hippocampi were prepared for Western blot analysis of target proteins as described elsewhere.[2-3] Tissue preparations contained a mixture of six protease inhibitors (AEBSF, aprotinin, leupetin, bestatin, pepstain A, E-64; Protease Inhibitor Cocktail, P-8340, Sigma, St Louis, MO) and various phosphatase inhibitors (Phosphatase Inhibitor Cocktail 3, P-0044, Sigma). BCA protein assay (Pierce Biotechnology, Rockford, IL) was used to assess the sample protein concentrations.

**Immunoblot assays and quantification of target proteins.** Brain sample proteins (40 μg) were separated by sodium dodecyl sulphate polyacrylamide electrophoresis (SDS-PAGE) on 10-12 % polyacrylamide minigels (Bio-Rad) and transferred onto nitrocellulose membranes by standard Western blot procedures as described.[2] The membranes were incubated overnight with the following specific primary antibodies (Ab, dilution range: 1/1000 to 1/10,000): anti-FADD(H-181) Ab, #sc-5559, lot: D0109 (Santa Cruz Biotechnology, Santa Cruz, CA); anti-phospho-FADD (Ser191) Ab, #2785, lot: 2 (Cell Signalling, Danvers, MA) anti-p35 (C-19) Ab, #sc-820, lot: K0409 (Santa Cruz Biotechnology); β-actin (clone AC-15) Ab, #A1978, lot: 118K4827

(Sigma). Appropriate horseradish peroxidase-conjugated secondary antibodies (anti-rabbit, anti-mouse) and ECL detection system (Amersham, Buckinghamshire, UK) were used to visualize the target proteins on autoradiographic films (Amersham ECL Hyperfilm). Upon densitometric scanning (GS-800 Imaging Densitometer, Bio-Rad) of immunoreactive bands (integrated optical density, IOD) the amount of target protein in brain samples of mice from different treatment groups was compared with that of vehicle-treated controls (100%) in the same gel. Quantification of β-actin contents served as a loading control (no differences between treatment groups, data not shown). Each brain sample (and target protein) was quantified in 2-4 gels and the mean value was used as a final estimate.

**Data processing and statistical analysis.** The program GraphPad Prism$^{TM}$, version 5.0 (GraphPad Software, San Diego, CA) was used for data analysis. Data were expressed as mean values±standard error of the mean (SEM). One-way analysis of variance (ANOVA, also repeated measures ANOVA) with Bonferroni's post hoc multiple comparison test and one-/two-tailed Student's *t*-test were applied for statistical evaluations. The level of significance was set to $p<0.05$ in all statistical evaluations.

**References.**


1. Garau, C., Miralles, A., García-Sevilla, J. A. (2013) Chronic treatment with selective $I_2$-imidazoline receptor ligands decreases the content of pro-apoptotic markers in rat brain. *J. Psychopharmacol. 2*, 123-134.
2. Keller, B., García-Sevilla, J. A. (2015) Immunodetection and subcellular distribution of imidazoline receptor proteins with three antibodies in mouse and human brains: Effects of treatments with $I_1$- and $I_2$-imidazoline drugs. *J. Psychopharmacol. 29*, 996-1012.

3. Keller B., García-Sevilla J. A. (2016) Inhibitory effects of imidazoline receptor ligands on basal and kainic acid-induced neurotoxic signalling in mice. *J. Psychopharmacol. 30*, 875-886.
4. Thorn, D. A., An, X. F., Zhang, Y., Pigini, M., Li, J. X. (2012) Characterization of the hypothermic effects of imidazoline $I_2$ receptor agonists in rats. *Br. J. Pharmacol. 166*, 1936-1945.

# Capítulo 6

## Synthetic $I_2$ imidazoline receptor ligands for prevention or treatment of human brain disorders

MINISTERIO
DE INDUSTRIA, ENERGÍA
Y TURISMO

Oficina Española
de Patentes y Marcas

# Acknowledgement of receipt

We hereby acknowledge receipt of your request for grant of a European patent as follows:

| | | |
|---|---|---|
| Submission number | 300258950 | |
| Application number | EP17382879.9 | |
| File No. to be used for priority declarations | EP17382879 | |
| Date of receipt | 21 December 2017 | |
| Your reference | UBTT0327 | |
| Applicant | UNIVERSITAT DE BARCELONA | |
| Country | ES | |
| Title | SYNTHETIC I2 IMIDAZOLINE RECEPTOR LIGANDS FOR PREVENTION OR TREATMENT OF HUMAN BRAIN DISORDERS | |
| Documents submitted | package-data.xml | ep-request.xml |
| | application-body.xml | ep-request.pdf (5 p.) |
| | OLF-ARCHIVE.zip\UBTT0327 Specification.zip | SPECTRANONEP.pdf\UBTT0327 Resumen.pdf (1 p.) |
| | SPECEPO-1.pdf\UBTT0327 Specification.pdf (21 p.) | OTHER-1.pdf\UBTT0327 Signature Applicant 2.pdf (1 p.) |
| | OTHER-2.pdf\UBTT0327 Signature Applicant 3.pdf (1 p.) | f1002-1.pdf (2 p.) |
| | feesheetint.pdf (1 p.) | |
| Submitted by | CN=Pascual-Marcos Segura Camara 45901 | |
| Method of submission | Online | |
| Date and time receipt generated | 21 December 2017, 12:59:10 (CET) | |

| | |
|---|---|
| Official Digest of Submission | 32:0A:96:AD:6D:97:A7:97:2C:41:72:8F:DA:87:3B:2D:8D:70:1C:14 |

/Madrid, Oficina Receptora/

Europäisches Patentamt
European Patent Office
Office européen des brevets

# Request for grant of a European patent

| *For official use only* | | |
|---|---|---|
| **1** Application number: | MKEY | |
| **2** Date of receipt (Rule 35(2) EPC): | DREC | |
| **3** Date of receipt at EPO (Rule 35(4) EPC): | RENA | |
| **4** Date of filing: | | |

**5** Grant of European patent, and examination of the application under Article 94, are hereby requested. ☒

Request for examination in an admissible non-EPO language: Se solicita el examen de la solicitud según el artículo 94.

**5.1** The applicant waives his right to be asked whether he wishes to proceed further with the application (Rule 70(2)) ☐

Procedural language: en

Description and/or claims filed in: en

**6** Applicant's or representative's reference: UBTT0327

Filing Office: ES

**Applicant 1**

**7-1** Name: UNIVERSITAT DE BARCELONA

**8-1** Address:
Centre de Patents de la UB
Baldiri Reixac 4 - Torre D
08028 Barcelona
Spain

**10-1** State of residence or of principal place of business: Spain

Telephone: +34 934034511

Fax: +34 934034517

E-mail: nuriasans@ub.edu

**Applicant 1**

**Applicant 2**

| | | |
|---|---|---|
| 7-2 | Name: | UNIVERSIDAD DEL PAÍS VASCO |
| 8-2 | Address: | C/ Barrio de Sarriena<br>48940 Leioa<br>Spain |
| 10-1 | State of residence or of principal place of business: | Spain |
| | Telephone: | +34 946012203 |

**Applicant 3**

| | | |
|---|---|---|
| 7-3 | Name: | UNIVERSITAT DE LES ILLES BALEARS |
| 8-3 | Address: | Carretera de Valldemossa, km. 7.5<br>07122 Palma<br>Spain |
| 10-1 | State of residence or of principal place of business: | Spain |
| | Telephone: | +34 971173000 |

14.1 The/Each applicant hereby declares that he is an entity or a natural person under Rule 6(4) EPC. ☒

**Representative(s)**

21- General authorisation has been registered under No. 636920.1

**Inventor(s)**

23 Inventor details filed separately ☒

**24 Title of invention**

Title of invention: SYNTHETIC I2 IMIDAZOLINE RECEPTOR LIGANDS FOR PREVENTION OR TREATMENT OF HUMAN BRAIN DISORDERS

**25 Declaration of priority (Rule 52)**

A declaration of priority is hereby made for the following applications

25.2 This application is a complete translation of the previous application ☐

25.3 It is not intended to file a (further) declaration of priority ☒

**26 Reference to a previously filed application**

**27 Divisional application** ☐

**28 Article 61(1)(b) application** ☐

**29 Claims**

Number of claims: 14

**29.1** ☒ as attached

**29.2** ☐ as in the previously filed application (see Section 26.2)

**29.3** ☐ The claims will be filed later

**30 Figures**

It is proposed that the abstract be published together with figure No.

**31 Designation of contracting states**

All the contracting states party to the EPC at the time of filing of the European patent application are deemed to be designated (see Article 79(1)).

**32 Different applicants for different contracting states**

**33 Extension/Validation**

This application is deemed to be a request to extend the effects of the European patent application and the European patent granted in respect of it to all non-contracting states to the EPC with which extension or validation agreements are in force on the date on which the application is filed. However, the request is deemed withdrawn if the extension fee or the validation fee, whichever is applicable, is not paid within the prescribed time limit.

**33.1** It is intended to pay the extension fee(s) for the following state(s):

**33.2** It is intended to pay the validation fee(s) for the following state(s):

**34 Biological material**

**38 Nucleotide and amino acid sequences**

The European patent application contains a sequence listing as part of the description ☐

The sequence listing is attached in computer-readable format in accordance with WIPO Standard ST.25 ☐

The sequence listing is attached in PDF format ☐

**Further indications**

**39** Additional copies of the documents cited in the European search report are requested

Number of additional sets of copies:

**40** Refund of the search fee under to Article 9 of the Rules relating to Fees is requested ☐

Application or publication number of earlier search report:

**42 Payment**

Method of payment: Not specified

| **44-A Forms** | | Details: | System file name: |
|---|---|---|---|
| **A-1** | Request | | as ep-request.pdf |
| **A-2** | 1. Designation of inventor | 1. Inventor | as f1002-1.pdf |

| **44-B Technical documents** | | Original file name: | System file name: |
|---|---|---|---|
| **B-1** | Specification | UBTT0327 Specification.pdf<br>Description; 14 claims; abstract | SPECEPO-1.pdf |
| **B-2** | Pre-conversion archive | UBTT0327 Specification.zip | OLF-ARCHIVE.zip |
| **B-3** | Translation of description, claims, abstract and drawings in Spanish | UBTT0327 Resumen.pdf | SPECTRANONEP.pdf |

| **44-C Other documents** | | Original file name: | System file name: |
|---|---|---|---|
| **C-1** | Signature Applicant 2 | UBTT0327 Signature Applicant 2.pdf | OTHER-1.pdf |
| **C-2** | Signature Applicant 3 | UBTT0327 Signature Applicant 3.pdf | OTHER-2.pdf |

**Annotations**

**Title (Author):**

**1Note (for EPO)**

(COMMON REPRESENTATIVE: UNIVERSITAT DE BARCELONA)

Universitat de Barcelona is the common representative regarding this patent application

**45** Employee under Article 133(3), having a general authorisation: SEGURA CAMARA, Mr. Pascual-Marcos

General authorisation: 636920.1

**46 Signature(s)**

| | |
|---|---|
| Place: | **Barcelona** |
| Date: | **21 December 2017** |
| Signed by: | **Pascual-Marcos Segura Camara 45901** |
| Employee name (Art. 133 EPC): | **Pascual-Marcos SEGURA CAMARA** |
| General authorisation number (Art. 133(3) EPC): | **636920.1** |
| Capacity: | **(Employee of UNIVERSITAT DE BARCELONA)** |

Title of invention: Synthetic $I_2$ imidazoline receptor ligands for prevention or treatment of human brain disorders
Applicant's ref: UBTT0327

## SIGNATURE OF APPLICANT 2

On behalf of Applicant 2

UNIVERSIDAD DEL PAÍS VASCO
c/ Barrio de Sarriena
48940 Leioa, Bizkaia
Spain

Signed by

Arturo Muga Villate
Vice-Chancellor for Scientific Development and Transfer
Universidad del País Vasco

Leioa, 14 December 2017

Title of invention: Synthetic $I_2$ imidazoline receptor ligands for prevention or treatment of human brain disorders
Applicant's ref: UBTT0327

SIGNATURE OF APPLICANT 3

On behalf of Applicant 3

UNIVERSITAT DE LES ILLES BALEARS
Carretera de Valldemossa, km. 7.5
07122 Palma
Spain

Signed by

Jordi Llabrés Bordoy
Vice-Rector for Innovation and Institutional Relations
Universitat de les Illes Balears

Palma, 14 December 2017

# Form 1002 - 1: Public inventor(s)

## Designation of inventor

User reference: UBTT0327
Application No:

Public

| | | |
|---|---|---|
| | **Inventor** | |
| | Name: | ESCOLANO MIRÓN María Carmen |
| | Company: | UNIVERSITAT DE BARCELONA |
| | Address: | Centre de Patents de la UB<br>Baldiri Reixac 4<br>08028 Barcelona<br>Spain |
| | The applicant has acquired the right to the European patent: | As employer |
| | **Inventor** | |
| | Name: | PALLÀS LLIBERIA Mercè |
| | Company: | UNIVERSITAT DE BARCELONA |
| | Address: | Centre de Patents de la UB<br>Baldiri Reixac 4<br>08028 Barcelona<br>Spain |
| | The applicant has acquired the right to the European patent: | As employer |
| | **Inventor** | |
| | Name: | GRIÑÁN FERRE Cristian Gaspar |
| | Company: | UNIVERSITAT DE BARCELONA |
| | Address: | Centre de Patents de la UB<br>Baldiri Reixac 4<br>08028 Barcelona<br>Spain |
| | The applicant has acquired the right to the European patent: | As employer |
| | **Inventor** | |
| | Name: | ABÁS PRADES Sònia |
| | Company: | UNIVERSITAT DE BARCELONA |
| | Address: | Centre de Patents de la UB<br>Baldiri Reixac 4<br>08028 Barcelona<br>Spain |
| | The applicant has acquired the right to the European patent: | Under agreement: 14 December 2017 |

| | | |
|---|---|---|
| | **Inventor** | |
| | Name: | CALLADO HERNANDO Luis-Felipe |
| | Company: | UNIVERSIDAD DEL PAÍS VASCO |
| | Address: | c/ Barrio Sarriena s/n<br>48940 Leioa<br>Spain |
| | The applicant has acquired the right to the European patent: | As employer |
| | **Inventor** | |
| | Name: | GARCÍA SEVILLA Jesús A. |
| | Company: | UNIVERSITAT DE LES ILLES BALEARS |
| | Address: | Carretera de Valldemossa, km. 7.5<br>07122 Palma<br>Spain |
| | The applicant has acquired the right to the European patent: | As employer |

**Signature(s)**

| | |
|---|---|
| Place: | **Barcelona** |
| Date: | **21 December 2017** |
| Signed by: | **Pascual-Marcos Segura Camara 45901** |
| Employee name (Art. 133 EPC): | **Pascual-Marcos SEGURA CAMARA** |
| General authorisation number (Art. 133(3) EPC): | **636920.1** |
| Capacity: | **(Employee of UNIVERSITAT DE BARCELONA)** |

# Synthetic $I_2$ imidazoline receptor ligands for prevention or treatment of human brain disorders

## TECHNICAL FIELD

The present invention relates to the field of compounds with high affinity for imidazoline receptors of the $I_2$-type (compounds referred to as $I_2$-IR ligands in the following), which have been associated with prevention or treatment of human brain disorders.

## BACKGROUND ART

In the research in the field of imidazoline receptors much attention was focused on $I_1$-type receptors and potential clinical implication such as hypertension. However, the understanding of $I_2$-IR and its potential functionality had always been elusive until recently. Although there is a lack of consensus regarding the nature of $I_2$-IR, it seems clear that $I_2$-IR may represent a group of heterogeneous proteins recognized by $I_2$-IR ligands such as idazoxan, the ligand mainly used to characterize $I_2$-IR. $I_2$-IR are involved in human brain disorders, such as depression and Alzheimer's disease (AD), pain modulation, and inflammation. $I_2$-IR ligands show antihyperalgesic properties in a murine model of inflammatory and neuropathic pain. In AD brain patiens the density (Bmax) of $I_2$ imidazoline sites is significantly higher than in control, and neuroprotectant properties have been demonstrated (cf. S. Abas et al., "Neuroprotective Effects of a Structurally New Family of High Affinity Imidazoline $I_2$ Receptor Ligands", *ACS Chemical Neuroscience* 2017, vol. 8, pp. 737-742; and references therein). It is believed that $I_2$-IR ligands are a new first-in-class of pharmacotherapeutics for the management of brain disorders such as neuropathic pain and inflammation. Selective $I_2$-IR ligands devoid of $\alpha_2$-adrenergic receptor ($\alpha_2$-AR) activity are considered particularly promising as active pharmaceutical ingredients (API), as illustrated in recent reviews (cf. e.g. J.-X. Li, "Imidazoline $I_2$ receptors: An update", *Pharmacology & Therapeutics* 2017. vol. 178, pp. 48-56; J.-X. Li et al., "Imidazoline $I_2$ receptors: Target for new analgesics?", *European Journal of Pharmacology* 2011, vol. 658, pp. 49-56; and references therein), in which it is also shown that most synthetic $I_2$-IR ligands have an imidazoline moiety. Thus, prior art suggests that it is desirable to provide new $I_2$-IR ligands useful as API.

## SUMMARY OF INVENTION

An aspect of the present invention relates to the provision of a compound selected from the group consisting of the enantiomer of formula I, its mirror-image enantiomer, and a

mixture of both enantiomers, wherein:

$R_1$ is ethyl or phenyl;

$R_2$ is methyl or phenyl; and

$R_3$ is a radical selected from the group consisting of: $(C_1\text{-}C_6)$-alkyl, $(C_1\text{-}C_6)$-cycloalkyl, $\text{-}[CH_2]_n$-phenyl, and $\text{-}[CH_2]_n$-[substituted phenyl]; wherein [substituted phenyl] is a phenyl radical with one, two or three substituents which are radicals independently selected from the group consisting of: F, Cl, Br, $(C_1\text{-}C_3)$-alkyl, $(C_1\text{-}C_3)$-alkyloxy, phenyl, phenoxy, $\text{-}CF_3$ , $\text{-}OCF_3$ , nitro, -CN, $\text{-CO-}(C_1\text{-}C_3)$-alkyl, and benzoyl; and n is an integer between 0 and 4.

As illustrated by the accompanying experimental results of Table 1, the compounds of the present invention are $I_2$-IR ligands, and consequently they are applicable in the prevention or treatment of brain disorders. The supplementary experimental results of Tables 2-9 further illustrate their promising potentialities in this respect. For instance, results obtained in cognitive evaluation test in mice indicate that I-06 improves cognitive abilities in a model of aging gated to cognitive decline and early AD and in a murine model of AD. Moreover, molecular and biochemical measures in treated mice hippocampus (an important area for learning and also one of early affected in AD) show a potent and efficient anti-inflammatory effect in both tested models.

Another aspect of the present invention refers to the compounds of the present invention for use as an API. Another aspect of the present invention refers to the compounds of the present invention for use in the prevention or treatment of a brain disorder in an animal, including a human. In a particular embodiment, the brain disorder is a neurodegenerative disorder; and in another particular embodiment, the neurodegenerative disorder is Alzheimer's disease (AD). All these aspects of the present invention are related to the use of the compounds of the present invention for the preparation of medicaments for the prevention or treatment of a brain disorder in animals, including humans, particularly neurodegenerative disorders, and more particularly AD. These aspects of the present invention are also related to a method of prevention or treatment of brain disorders, particularly neurodegenerative disorders, and more particularly AD, in animals including humans.

Preparation process of racemic mixtures corresponding to compounds I

Racemic mixtures of compounds I and their respective mirror-image enantioners are prepared by a process involving a diastereoselective [3+2] cycloaddition [redacted] a process which is summarized in the accompanying schemes, and further illustrated in accompanying preparative examples 1-12. The starting materials are either commercially available or prepared as it is here indicated. [redacted] Along the description and claims, specific racemic mixtures are referred to by I-#, wherein # is an arbitrary Arabic numeral.

Enantiomers of formula I and their mirror-image enantiomers can be prepared from their respective mixtures by methods known in the art, e.g. by preparative chiral HPLC.

Additional objects, advantages and features of the invention will become apparent to those skilled in the art upon examination of the description or may be learned by practice of the invention. The following examples are provided by way of illustration, and they are not intended to be limiting of the present invention. Furthermore, the present invention covers all possible combinations of particular and preferred embodiments described herein.

DESCRIPTION OF EMBODIMENTS

In a particular embodiment the compound of the present invention is a mixture of both enantiomers, and more particularly a racemic mixture of them. In another particular embodiment the substituted phenyl is a phenyl radical with one or two substituents. In another particular embodiment n is an integer between 0 and 2. In another particular embodiment $R_3$ is selected from the group consisting of: methyl, cyclohexyl, phenyl, 3-nitrophenyl, 3-chloro-4-fluorophenyl, 4-methoxyphenyl, (1,1'-biphenyl)-4-yl, benzyl, and phenethyl. In another particular embodiment $R_1$ is ethyl; and in another one, $R_2$ is phenyl.

Particular embodiments are the following racemic mixtures, prepared for the first time as illustrated in the accompanying examples, as well as their corresponding separated enantiomers:

General process for the [3 + 2] cycloaddition reaction to prepare the racemic mixtures corresponding to compounds I (specific racemic mixtures are referred to by I-#, wherein # is an arbitrary Arabic numeral)

Reagents, solvents and starting materials were acquired from commercial sources. The term "concentration" refers to the vacuum evaporation using a Büchi rotavapor. When indicated, the reaction products were purified by "flash" chromatography on silica gel (35-70 μm) with the indicated solvent system. The melting points were measured in a MFB 59510M Gallenkamp instruments. IR spectra were performed in a spectrophotometer Nicolet Avantar 320 FTR-IR, and only noteworthy IR absorptions ($cm^{-1}$) are listed.
The accurate mass analyses were carried out using a LC/MSD-TOF spectrophotometer. The elemental analyses were carried out in a Flash 1112 series Thermofinnigan elemental microanalyzator (A5) to determine C, H, and N.

To a solution of [redacted] (0.06 mmol) and [redacted] (1.0 or 1.5 mmol) in acetonitrile, 1.0 mmol of [redacted] was added. The reaction mixture was stirred at room temperature overnight, concentrated, and the resulting residue was purified by column chromatography to afford racemic mixtures I-#.

Example 1. (I-25)

Following the general procedure, (20 mg, 0.12 mmol), (222 mg, 2.0 mmol), (15 mL) and (384 mg, 2.0 mmol) gave I-25 (239 mg, 40%) as a yellowish oil, after column chromatography (EtOAc).

Example 2. (I-31)

Following the general procedure, (8 mg, 0.05 mmol), (208 mg, 1.2 mmol), (6 mL) and (153 mg, 0.8 mmol) gave I-31 (35 mg, 12%) as a coloured oil, after column chromatography (EtOAc/hexane 8:2 to 9:1).

Example 3. (I-30)

Following the general procedure, (13.3 mg, 0.08 mmol), (133 mg, 1.2 mmol), (6 mL) and (202 mg, 0.8 mmol) gave I-30 (184 mg, 64%) as a yellowish oil after column chromatography (EtOAc/hexane 95:5).

Example 4. (I-07)

Following the general procedure, (15 mg, 0.09 mmol), (403 mg, 2.25 mmol), (12 mL) and (380 mg, 1.5 mmol) gave I-07 (494 mg, 76%) as a white solid after column chromatography (EtOAc). M.p. 128-132 $^{o}$C (EtOAc).

Example 5. (I-29)

Following the general procedure, (4.0 mg, 0.02 mmol), (112 mg, 0.6 mmol), (3 mL) and (101 mg, 0.4 mmol) gave I-29 (139 mg, 79%) as a yellowish oil, after column chromatography (EtOAc/hexane 9:1).

Example 6. (I-28)

Following the general procedure, (4.0 mg, 0.02 mmol), (131 mg, 0.6 mmol), (3 mL) and (101.3 mg, 0.4 mmol) gave I-28 (101 mg, 54%) as a white solid, after column chromatography (EtOAc). M.p. 192-195 ˚C (EtOAc).

Example 7. (I-16)

Following the general procedure, (4 mg, 0.02 mmol), (104 mg, 0.6 mmol), (3.0 mL) and (101 mg, 0.4 mmol) gave I-16 (108 mg, 64%) as a white solid after column chromatography (EtOAc). M.p. 158-160 $^{o}$C (EtOAc).

Example 8. (I-06)

Following the general procedure, (8 mg, 0.05 mmol), (250 mg, 1.11 mmol), (6 mL) and (187 mg, 0.74 mmol) gave I-06 (189 mg, 54%) as white needles after column chromatography (EtOAc). M.p. 185-186 ˚C (EtOAc).

Example 9. (I-22)

Following the general procedure, (4 mg, 0.02 mmol), (122 mg, 0.6 mmol), (3 mL) and (102 mg, 0.4 mmol) gave I-22 (119 mg, 65%) as a white solid after column chromatography (EtOAc). M.p. 167 °C (EtOAc).

Example 10. (I-26)

Following the general procedure, (2 mg, 0.012 mmol), (52 mg, 0.3 mmol), (2 mL) and (70 mg, 0.2 mmol) gave I-26 (73 mg, 70%) as a yellow solid after column chromatography (hexane/EtOAc 4:1 to 7:3).

Example 11. (I-33)

Following the general procedure, (7 mg, 0.04 mmol), (200 mg, 1.00 mmol) (5 mL) and (170 mg, 0.67 mmol) gave I33 (110 mg, 36%) as an oil after column chromatography (EtOAc/hexane 1:1).

Example 12. (I-32)

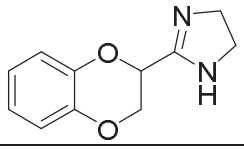
Following the general procedure, (5.0 mg, 0.03 mmol), (200 mg, 0.80 mmol) (4 mL) and (134 mg, 0.53 mmol) gave I32 (129 mg, 49%) as a

yellowish oil after column chromatography (EtOAc).

Activity upon $I_2$ imidazoline receptors

The pharmacological activity of the prepared compounds was evaluated through competition binding studies against the selective $I_2$-IR radioligand [$^3$H]-2-BFI or the selective $\alpha_2$-AR radioligand [$^3$H]RX821002. The studies were performed in membranes from post-mortem human frontal cortex, a brain area that shows an important density of $I_2$-IRs and $\alpha_2$-AR. The inhibition constant ($K_i$) for each compound was obtained and is expressed as the corresponding $pK_i$ (Table 1). Idazoxan, a compound with well-established affinity for $I_2$-IR ($pK_i$ = 7.27 ± 0.07) and $\alpha_2$-AR ($pK_i$ = 7.51 ± 0.07) was used as a reference. The selectivity between receptors was expressed by the $I_2/\alpha_2$ index. This index is calculated as the antilogarithm of the difference between $pK_i$ values for $I_2$-IR and $pK_i$ values for $\alpha_2$-AR (see Table 1).

Table 1. $I_2$-IR and $\alpha_2$-AR receptor binding affinities ($pK_i$)

| Compound | | [$^3$H]-2-BFI<br>$I_2$ | [$^3$H]-RX821002<br>$\alpha_2$ | Selectivity<br>$I_2/\alpha_2$ |
|---|---|---|---|---|
| Idazoxan |  | 7.27±0.07 | 7.51±0.07 | - |
| I-06 | | 8.56±0.32 | 6.27±0.56 | 195 |
| I-07 | | 9.74±0.29 | 9.01±0.49 | 5 |
| I-16 | | 10.28±0.37 | 10.38±0.22 | 1 |
| I-22 | | 6.65±1.27 | 4.59±0.22 | 115 |
| I-25 | | 8.57±0.39 | 7.29±2.47 | 19 |

| | | | | |
|---|---|---|---|---|
| I-26 |  | 5.86±1.22 | 11.64±0.45 | - |
| I-28 |  | 6.81±0.27 | 10.18±0.41 | |
| I-29 |  | 5.26±0.22 | 8.11±0.28 | |

Parallel Artificial Membrane Permeation Assays- Blood-Brain Barrier (PAMPA-BBB)

To evaluate the brain penetration of the different compounds, a parallel artificial membrane permeation assay for blood-brain barrier was used, following the method described by Di et al. (cf. Table 2). The *in vitro* permeability ($P_e$) of fourteen commercial drugs through lipid extract of porcine brain membrane together with the test compounds were determined. Commercial drugs and assayed compounds were tested using a mixture of PBS:EtOH (70:30). Assay validation was made by comparing the experimental permeability with the reported values of the commercial drugs by bibliography and lineal correlation between experimental and reported permeability of the fourteen commercial drugs using the parallel artificial membrane permeation assay was evaluated ($y = 1.5481x-1.0128$; $r^2 = 0,9405$). From this equation and taking into account the limits established by Di et al. for BBB permeation, inventors have established the ranges of permeability as compounds of high BBB permeation (CNS+): *P*e ($10^{-6}$ cm $s^{-1}$) > 5,179; compounds of low BBB permeation (CNS-): *P*e ($10^{-6}$ cm $s^{-1}$) < 2,083 and compounds of uncertain BBB permeation (CNS+/-): 5,179 > *P*e ($10^{-6}$ cm $s^{-1}$) > 2,083. Table 2 shows permeability results from the different commercial and assayed compounds (three different experiments in triplicate) and predictive penetration in the CNS.

Table 2. Permeability (*Pe* 10-6 cm $s^{-1}$) in the PAMPA-BBB assay of 14 commercial drugs and tested compounds and predictive penetration in the CNS. Bibliography values are taken from L. Di et al., "High throughput artificial membrane permeability assay for blood-brain barrier", *Eur. J. Med.Chem.* 2003, vol. 38, pp. 223-232. * = poor dissolution. SD = Standard Deviation.

| Compound | Bibliography value | Experimental value ± SD (n=3) | CNS Prediction |
|---|---|---|---|
| verapamil | 16.0 | 26.4±0.5 | |
| testosterone | 17.0 | 24.9±0.4 | |
| costicosterone | 5.1 | 6.7±0.1 | |
| clonidine | 5.3 | 6.50±0.05 | |
| ofloxacin | 0.8 | 0.99±0.04 | |
| lomefloxacin | 0.0 | 0.70±0.04 | |
| progesterone | 9.3 | 16.8±0.3 | |
| promazine | 8.8 | 13.8±0.3 | |
| imipramine | 13.0 | 12.3±0.1 | |
| hidrocortisone | 1.9 | 1.40±0.05 | |
| piroxicam | 2.5 | 1.90±0.02 | |
| desipramine | 12.0 | 17.8±0.1 | |
| cimetidine | 0.0 | 0.70±0.03 | |
| norfloxacine | 0.1 | 0.90±0.02 | |
| I-06 | | 9.7±0.7 | CNS+ |
| I-07 | | 25.9±0.6 | CNS+ |
| I-16 | | 7.8±0.6 | CNS+ |
| I-22 | | 3.90±0.15 | CNS+/- |
| I-25 | | 4.6±0.7 | CNS+/- |
| I-28* | | 4.50±0.25 | CNS+/- |
| I-29* | | >30 | CNS+ |
| I-30 | | 0.75±0.13 | CNS- |
| I-31 | | 5.13±0.95 | CNS+/- |

Strains

SAMP8 is one of the non transgenic mouse model used in AD preclinical studies. It is an inbred strain with shortened lifespan and accelerated aging phenotype, with elevated levels of endogenous APP, but not plaques, and tau hyperphosphorylation. 5XFAD is an

early-onset mouse transgenic model which overexpress mutant human APP(695) with the Swedish (K670N, M671L), Florida (I716V, and London (V717I) Familial Alzheimer's Disease (FAD) mutations along with human PS1 harboring two FAD mutations, M146L and L286V. Both transgenes are regulated by the mouse Thy1 promoter to drive overexpression in the brain. 5XFAD mice recapitulate major features of AD amyloid pathology and may be a useful model of intraneuronal Abeta-42 induced neurodegeneration and amyloid plaque formation.

Behavioral test: In vivo model for assessing the efficacy of a test compound in learning and memory impairment (Novel Object Recognition Test; NORT) (cf. Tables 3-6)

Mice were placed in a 90°, two-arm, 25-cm-long, 20-cm-high, 5-cm-wide black maze. The walls could be lifted off for easy cleaning. Light intensity in the middle of the field was 30 lux. The objects to be discriminated were made of plastic and were chosen not to frighten the mice, and objects with parts that could be bitten were avoided. Before performing the test, the mice were individually habituated to the apparatus for 10 min during 3 days. On day 4, the animals were submitted to a 10-min acquisition trial (first trial), during which they were placed in the maze in the presence of two identical, novel objects (A+A or B+B) at the end of each arm. A 10-min retention trial (second trial) was carried out 2 h later. During this second trial, objects A and B were placed in the maze and the behavior of the mice was recorded with a camera. Time that the mice explored the new object (TN) and Time that the mice explored the Old object (TO) were measured. A Discrimination Index (DI) was defined as (TN−TO)/(TN+TO). In order to avoid object preference biases, objects A and B were counterbalanced so that one half of the animals in each experimental group were exposed first to object A and then to object B, whereas the remaining half saw object B first and then object A. The maze and the objects were cleaned with 70º ethanol after each test in order to eliminate olfactory cues. The learning & memory paradigm is based on spontaneous exploratory activity of rodents and does not involve rule learning or reinforcement. The object recognition paradigm has been shown to be sensitive to the effects of ageing and cholinergic dysfunction among others (cf. C. Scali et al., "Nerve growth factor increases extracellular acetylcholine levels in the parietal cortex and hippocampus of aged rats and re- stores object recognition", *Neurosci. Lett.* 1994, vol. 170, pp 117-120; L. Bartolini et al., "Aniracetam restores object recognition impaired by age, scopolamine and nucleus basalis lesions", *Pharmacol. Biochem. Behav*. 1996, vol. 53, pp. 277-283). This model has been adapted to mice and validated using pharmacological agents (cf. X. Bengoetxea et al., "Object recognition test for studying cognitive impairments in animal models of Alzheimer's disease", *Front. Biosci. (Schol. Ed.)* 2015, vol. 7, pp 10-29). Evaluation of I-06 neuroprotective properties in both mice

models by NORT, showed a reduced memory deficits in treated groups compared to the control groups and in case of 5xFAD treated group recovered DI levels of the Wild Type (Wt) control group. Therefore, I-06 is able to improve cognitive capabilities in two murine models of neurodegeneration and brain disorders.

Table 3. Values of DI of NORT 2h in female mice at 12 months control SAMR1 (SAMR1-Ct), control SAMP8 (SAMP8-Ct), and SAMP8 treated with I-06 (SAMP8-I-06). Data are observed mean ± Standard Error of the Mean (SEM) (*n* = 8 for each group). ****p<0.0001 compared to SAMR1-Ct group. $^{\#\#}$p<0.01 compared to SAMP8-Ct group.

| NORT 2h | | |
|---|---|---|
| Group | Number of mice | DI (-1,1) |
| SAMR1-Ct | 8 | 0.37±.0.0427 |
| SAMP8-Ct | 7 | -0.023±0.039**** |
| SAMP8-I-06 (5 mg/kg) | 7 | 0.33±0.071$^{\#\#}$ |

Table 4. Values of DI of NORT 24h in female mice at 12 months control SAMR1 (SAMR1-Ct), control SAMP8 (SAMP8-Ct), and SAMP8 treated with I-06 (SAMP8-I-06). (*n* = 8 for each group). **p<0.01 compared to SAMR1-Ct group. $^{\#\#}$p<0.01 compared to SAMP8-Ct group.

| NORT 24h | | |
|---|---|---|
| Group | Number of mice | DI (-1,1) |
| SAMR1-Ct | 8 | 0.295±0.057 |
| SAMP8-Ct | 7 | -0.052±0.091** |
| SAMP8 I-06 (5 mg/kg) | 7 | 0.273±0.042$^{\#\#}$ |

Table 5. Values of DI of NORT 2h in female mice at 6 months control Wt (Wt-Ct), control 5XFAD (5XFAD-Ct) and 5XFAD treated with I-06 (5XFAD-I-06). (*n* = 8 for each group). *p<0.05 compared to Wt-Ct group. #p<0.05 compared to 5xFAD-Ct group.

| NORT 2h | | |
|---|---|---|
| Group | Number of mice | DI (-1,1) |
| Wt-Ct | 14 | 0.12±0.33 |
| 5xFAD-Ct | 16 | -0.032±0.067* |
| 5xFAD I-06 (5 mg/kg) | 18 | 0.199±0.053$^{\#}$ |

Table 6. Values of DI of NORT 24h in female mice at 6 months control Wt (Wt-Ct), control 5XFAD (5XFAD-Ct) and 5XFAD treated with I-06 (5XFAD-I-06). (*n* = 8 for each group). *p<0.05 compared to Wt-Ct group. #p<0.05 compared to 5xFAD-Ct group.

| NORT 24h | | |
|---|---|---|
| Group | Number of mice | DI (-1,1) |
| Wt-Ct | 14 | 0.211±0.054 |
| 5xFAD-Ct | 16 | -0.060±0.076* |
| 5xFAD I-06 (5 mg/kg) | 18 | 0.229±0.051# |

Molecular analysis: RNA extraction and gene expression determination for inflammatory markers and beta amyloid processing

Total RNA isolation was carried out by means of Trizol reagent following the manufacturer's instructions. RNA content in the samples was measured at 260 nm, and sample purity was determined by the A260/280 ratio in a NanoDrop™ ND-1000 (Thermo Scientific). Samples were also tested in an Agilent 2100B Bioanalyzer (Agilent Technologies) to determine the RNA integrity number. Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was performed as follows: 2 µg of messenger RNA (mRNA) was reverse-transcribed using the High Capacity complementary DNA (cDNA) Reverse Transcription Kit (Applied Biosystems). Normalization of expression levels was performed with actin.

SYBER Green, real-time PCR was performed in the Step One Plus Detection System (Applied Biosystems) employing the SYBR Green PCR Master Mix (Applied Biosystems). Each reaction mixture contained 7.5 µL of cDNA, whose concentration was 2 µg, 0.75 µL of each primer (whose concentration was 100 nM), and 7.5 µL of SYBR Green PCR Master Mix (2X).

Data were analyzed utilizing the comparative Cycle threshold (Ct) method (Fold Change), where the actin transcript level was used to normalize differences in sample loading and preparation. Each sample (n = 4-5) was analyzed in triplicate, and results represented the n-fold difference of transcript levels among different samples.

Effect of I-06 on inflammatory markers (cf. Tables 7-8)

Molecular evaluation of inflammatory markers in brain by gene expression determination shows that I-06 reduced two markers of neuroinflammation such as *Il-6* and *Cxcl10* , both in SAMP8 and 5xFAD.

Table 7. Values of gene expression of Inflammatory Markers for *Il-6* and *Cxcl10* in female mice at 12 months control SAMR1 (SAMR1-Ct), control SAMP8 (SAMP8-Ct), and SAMP8 treated with I-06 (SAMP8-I-06). Gene expression levels were determined by real-time PCR. Mean ± SEM from five independent experiments performed in triplicate are represented (*n* = 4 for each group). *p<0.05 compared to SAMR1-Ct group. ****p<0.0001 compared to SAMR1-Ct group. $^{\#}$p<0.05 compared to SAMP8-Ct group.

| Group | Gene Name | Fold Change |
|---|---|---|
| SAMR1-Ct | *Il-6* | 0.486±0.0228 |
| | *Cxcl10* | 0.563±0.125 |
| SAMP8-Ct | *Il-6* | 1.006±0.078**** |
| | *Cxcl10* | 1.02±0.158* |
| SAMP8-I-06 (5 mg/kg) | *Il-6* | 0.701±0.070$^{\#}$ |
| | *Cxcl10* | 0.660±0.012 |

Table 8. Values of gene expression of Inflammatory Markers for *Il-6* and *Cxcl10* in female mice at 6 months control Wt (Wt-Ct), control 5XFAD (5XFAD-Ct), and 5XFAD treated with I-06 (5XFAD-I-06). Gene expression levels were determined by real-time PCR. Mean ± SEM from five independent experiments performed in triplicate are represented (*n* = 4 for each group). *p<0.05 compared to Wt-Ct group.

| Group | Gene Name | Fold Change |
|---|---|---|
| Wt-Ct | *Il-6* | 1.002±0.036 |
| | *Cxcl10* | 1.193±0.413 |
| 5xFAD-Ct | *Il-6* | 1.2474±0.101* |
| | *Cxcl10* | 3.541±1.153 |
| 5xFAD-I-06 (5 mg/kg) | *Il-6* | 1.35±0.136 |
| | *Cxcl10* | 2.07±0.647 |

qPCR: APP processing and degradation markers (cf. Table 9)

With the aim to describe the amyloid precursor protein (APP) processing and Abeta degradation, inventors evaluated the gene expression of Adam10, Neprilysin (*Nep*) and Insuline Degrading Enzyme (*Ide*). Significantly increases in gene expression of *Adam10* and *Ide* (two enzymes related with non-amiloidogenic processing of APP) in SAMP8 treated group compared to SAMP8-Ct group were obtained.

Table 9. Values of gene expression of APP processing and degradation Markers for *Adam10, Nep* and *Ide* in female mice at 12 months control SAMR1 (SAMR1-Ct), control SAMP8 (SAMP8-Ct), and SAMP8 treated with I-06 (SAMP8-I-06). Gene expression levels were determined by real-time PCR. Results indicated improved processing pathway for APP through a reduction in beta amyloid production. Mean ± SEM from five independent experiments performed in triplicate are represented (*n* = 4 for each group). *p<0.05 compared to SAMR1-Ct group. $^{\#}$p<0.05 compared to SAMP8-Ct group.

| Group | Gene Name | Fold Change |
|---|---|---|
| SAMR1-Ct | *Adam10* | 1.467±0.198 |
| | *Nep* | 1.614±0.057 |
| | *Ide* | 1.263±0.036 |
| SAMP8-Ct | *Adam10* | 1.044±0.209 |
| | *Nep* | 1.035±0.269* |
| | *Ide* | 1.021±0.114 |
| SAMP8-I-06 (5 mg/kg) | *Adam10* | 1.88±0.222$^{\#}$ |
| | *Nep* | 1.772±0.226 |
| | *Ide* | 1.3436±0.074$^{\#}$ |

CITATION LIST

Non Patent Literature

- S. Abas et al., "Easy access to (2-imidazolin-4-yl)phosphonates by a microwave assisted multicomponent reaction", *Tetrahedron* 2015, vol. 71, pp. 2872-2881.
- S. Abas et al., "Neuroprotective Effects of a Structurally New Family of High Affinity Imidazoline $I_2$ Receptor Ligands", *ACS Chemical Neuroscience* 2017, vol. 8, pp. 737-742.
- C. Arroniz et al., "First diastereoselective [3+2] cycloaddition reaction of diethyl isocyanomethylphosphonate and maleimides", *Org. Biomol. Chem.* 2013, vol. 11, pp. 1640-1649.
- L. Bartolini et al., "Aniracetam restores object recognition impaired by age, scopolamine and nucleus basalis lesions", *Pharmacol. Biochem. Behav*. 1996, vol. 53, pp. 277-283.
- X. Bengoetxea et al., "Object recognition test for studying cognitive impairments in animal models of Alzheimer's disease", *Front. Biosci. (Schol. Ed.)* 2015, vol. 7, pp 10-29.
- L. Di et al., "High throughput artificial membrane permeability assay for blood-brain barrier", *Eur. J. Med.Chem.* 2003, vol. 38, pp. 223-232.
- J.-X. Li, "Imidazoline $I_2$ receptors: An update", *Pharmacology & Therapeutics* 2017. vol. 178, pp. 48-56.
- J.-X. Li et al., "Imidazoline $I_2$ receptors: Target for new analgesics?", *European Journal of Pharmacology* 2011, vol. 658, pp. 49-56.
- J. Rachon et al., "Syntheses with $\alpha$-metalated isocyanides. XLVIII. Phosphorus analogs of amino acids and peptides. V. Synthesis of 1-aminoalkylphosphonic acids by alkylation of $\alpha$-metalated diethyl isocyanomethylphosphonate", *Liebigs Ann. Chem.* 1981, pp. 709-718.
- C. Scali et al., "Nerve growth factor increases extracellular acetylcholine levels in the parietal cortex and hippocampus of aged rats and re- stores object recognition", *Neurosci. Lett.* 1994, vol. 170, pp 117-120.
- M. Sieńczyk, "Synthesis of isocyanide derivatives of α-aminoalkylphosphonate diphenyl esters", *Tetrahedron Lett.* 2006, vol. 47, pp. 4209-4211.
- M. Sortino et al. "Antifungal, cytotoxic and SAR studies of a series of N-alkyl, N-aryl and N-alkylphenyl-1,4-pyrrolediones and related compounds", *Bioorg. Med. Chem.* 2011, vol. 19, pp. 2823-2834.

CLAIMS

1. A compound selected from the group consisting of the enantiomer of formula I, its mirror-image enantiomer, a mixture of both enantiomers, wherein:
$R_1$ is ethyl or phenyl;
$R_2$ is methyl or phenyl; and
$R_3$ is a radical selected from the group consisting of: $(C_1\text{-}C_6)$-alkyl, $(C_1\text{-}C_6)$-cycloalkyl, $\text{-}[CH_2]_n$-phenyl, and $\text{-}[CH_2]_n$-[substituted phenyl]; wherein [substituted phenyl] is a phenyl radical with one, two or three substituents which are radicals independently selected from the group consisting of: F, Cl, Br, $(C_1\text{-}C_3)$-alkyl, $(C_1\text{-}C_3)$-alkyloxy, phenyl, phenoxy, $\text{-}CF_3$ , $\text{-}OCF_3$ , nitro, -CN, $\text{-CO-}(C_1\text{-}C_3)$-alkyl, and benzoyl; and n is an integer between 0 and 4.

I

2. The compound according to claim 1, which is a mixture of both enantiomers.

3. The compound according to claim 2, wherein the mixture is a racemic mixture.

4. The compound according to any one of the claims 1-3, wherein the substituted phenyl is a phenyl radical with one or two substituents,

5. The compound according to any one of the claims 1-4, wherein n is an integer between 0 and 2.

6. The compound according to any one of the claims 1-5, wherein $R_3$ is selected from the group consisting of: methyl, cyclohexyl, phenyl, 3-nitrophenyl, 3-chloro-4-fluorophenyl, 4-methoxyphenyl, (1,1'-biphenyl)-4-yl, benzyl, and phenethyl.

7. The compound according to any one of the claims 1-6, wherein $R_1$ is ethyl.

8. The compound according to any one of the claims 1-7, wherein $R_2$ is phenyl.

9. The compound according to claim 3, which is a racemic mixture selected from:

- 
 (I-06);
- 
 (I-25);
- 
 (I-31);
- 
 (I-30);
- 
 (I-07);
- 
 (I-29);
- 
 (I-28);
- 
 (I-16);
- 
 (I-22);
- 
 (I-26);
- 
 (I-33); and
- 
 (I-32).

10. 
 (I-06).

11. A compound as defined in any one of the claims 1-10, for use as a pharmaceutical active ingredient.

12. A compound as defined in any one of the claims 1-10, for use in the prevention or treatment of a brain disorder in an animal, including a human.

13. The compound for use according to claim 12, wherein the brain disorder is a neurodegenerative disorder.

14. The compound for use according to claim 13, wherein the neurodegenerative disorder is Alzheimer's disease (AD).

ABSTRACT

**Synthetic $I_2$ imidazoline receptor ligands for prevention or treatment of human brain disorders**

Compounds of formula I, their respective mirror-image enantiomers, and mixtures -preferably racemic- of both enantiomers, wherein $R_1$ is ethyl or phenyl; $R_2$ is methyl or phenyl; $R_3$ is selected from the group consisting of: $(C_1\text{-}C_6)$-alkyl, $(C_1\text{-}C_6)$-cycloalkyl, $\text{-}[CH_2]_n$-phenyl, and $\text{-}[CH_2]_n$-[substituted phenyl]; wherein [substituted phenyl] is a phenyl radical with one, two or three substituents independently selected from: F, Cl, Br, $(C_1\text{-}C_3)$-alkyl, $(C_1\text{-}C_3)$-alkyloxy, phenyl, phenoxy, $\text{-}CF_3$ , $\text{-}OCF_3$ , nitro, -CN, $\text{-}CO\text{-}(C_1\text{-}C_3)$-alkyl and benzoyl; and n is an integer between 0 and 4; have a high affinity for imidazoline receptors of the $I_2$ type, i.e. they are $I_2$-IR ligands. Consequently they are applicable in the prevention or treatment of brain disorders in animals, including humans, particularly of neurodegenerative disorders, and more particularly of Alzheimer's disease (AD).

RESUMEN

**Ligandos sintéticos para el receptor $I_2$ de imidazolina, para la prevención o tratamiento de desórdenes cerebrales en humanos**

Los compuestos de fórmula I, sus respectivos enantiómeros imágenes especulares y las mezclas -preferiblemente racémicas- de ambos enantiómeros, donde $R_1$ es etilo o fenilo; $R_2$ es metilo o fenilo; y $R_3$ se selecciona del grupo que consiste en: ($C_1$-$C_6$)-alquilo, ($C_1$-$C_6$)-cicloalquilo, -$[CH_2]_n$-fenilo, y -$[CH_2]_n$-[fenilo sustituido]; donde [fenilo sustituido] es un radical fenilo con uno, dos o tres sustituyentes independientemente seleccionados entre: F, Cl, Br, ($C_1$-$C_3$)-alquilo, ($C_1$-$C_3$)-alquiloxi, fenilo, fenoxilo, -$CF_3$ , -$OCF_3$ , nitro, -CN, -CO-($C_1$-$C_3$)-alquilo y benzoilo; y n es un número entero entre 0 y 4; tienen una alta afinidad para los receptores de imidazolina del tipo $I_2$ , o sea, son ligandos $I_2$-IR. Consecuentemente, son aplicables en la prevención o el tratamiento de desórdenes cerebrales en animales, incluidos seres humanos, particularmente de desórdenes neurodegenerativos, y más particularmente de la enfermedad de Alzheimer (Alzheimer's disease, AD).

# Conclusiones

La metodología desarrollada a lo largo de la presente Tesis ha contribuido a extender la aplicabilidad de los derivados de isocianoacetatos en la síntesis de compuestos con interés farmacológico. Las conclusiones de los cinco proyectos de esta tesis son las siguientes:

1. El uso de la 3-acil-5-alcoxicarbonil-2-pirrolina, obtenida a través de una cascada multicatalítica cooperativa, proporciona en una sola etapa la estereoquímica y la insaturación requerida para la preparación de las pirrolobenzodiazepinas. Además, el hecho de que el producto pueda sufrir un proceso de *Self-Disproportonation of Enantiomers* permite sintetizar de manera enantiopura el núcleo de Pirrolobenzodiazepina y reducir notablemente los pasos de síntesis.

2. El uso de PhosMic ha permitido obtener de manera diastereoselectiva iminofosfonatos biciclos. La reacción tiene lugar a través de una cicloadición [3+2] entre el PhosMic y maleimidas *N*-sustituidas. Gracias al estudio de rayos X se ha podido saber la configuración relativa de los compuestos y a través de estudio teórico el posible mecanismo que da lugar a la diastereoselectividad observada. Finalmente, la reducción del doble enlace a través de hidrogenación permitió obtener los α-aminofosfonatos correspondientes.

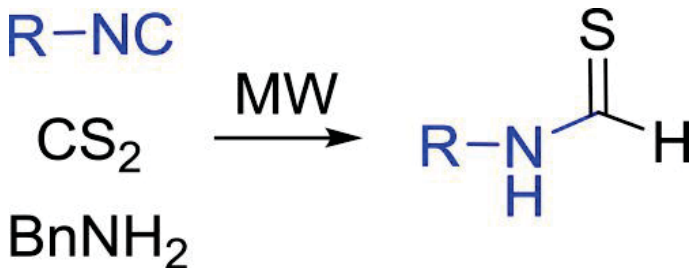
3. Se ha presentado una nueva IMCR con PhosMic que gracias a su combinación con una amina y una cetona nos ha permitido obtener los (2-imidazolin-4-il)fosfonatos. La reacción transcurre con una buena economía del átomo, no se utiliza disolvente, es eficiente y gracias al uso del microondas se permiten unas condiciones suaves de reacción (10 min, 40 $^{o}$C).

4. Los (2-imidazolin-4-il)fosfonatos obtenidos a través de la IMCR con PhosMic han revelado una elevada afinidad $I_2$-IR y buena selectividad $I_2$-IR/$\alpha_2$-AR. Por lo tanto, estas nuevas imidazolinas, con un patrón de sustitución distinto a los ligandos descritos con anterioridad, se convierten en buenos candidatos para caracterizar los $I_2$-IR. Asimismo, los efectos farmacológicos observados *in vitro* e *in vivo* demuestran la eficacia de ellos como posibles herramientas terapéuticas.

5. Se ha caracterizado una nueva familia sin estructura de imidazolina con elevada afinidad para los $I_2$-IR. Demostrando, además, que pueden ser buenos candidatos para la prevención o el tratamiento de desórdenes neurodegenerativos como la Enfermedad del Alzheimer.

# Anexo

*Facile microwave-assisted synthesis of thioformamides from isocyanides and carbon disulphide*



Tetrahedron Letters xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

# Facile microwave-assisted synthesis of thioformamides from isocyanides and carbon disulfide

Sònia Abás, Ulrike Moens, Carmen Escolano *

*Laboratory of Medicinal Chemistry (Associated Unit to CSIC), Department of Pharmacology, Toxicology and Medicinal Chemistry, Faculty of Pharmacy and Food Sciences, and Institute of Biomedicine (IBUB), University of Barcelona, Av. Joan XXIII 27-31, E-08028 Barcelona, Spain*

ARTICLE INFO

*Article history:*
Received 17 April 2017
Revised 29 May 2017
Accepted 1 June 2017
Available online xxxx

*Keywords:*
Thioamide
Microwave assisted organic synthesis
Carbon disulfide
Isocyanide
Environmentally friendly chemistry

ABSTRACT

A new, fast, solvent-free and efficient method is provided to prepare thioformamides by reacting isocyanides derivatives, carbon disulfide and benzylamine under microwave irradiation.



Microwave-assisted organic synthesis (MAOS) is one of the cornerstones in modern synthetic organic chemistry. In fact, microwave instruments are becoming part of the equipment in numerous laboratories providing researchers with a new tool to enhance the arsenal of reactions. The increasing relevance of MAOS in accessing new entities providing efficient and cleaner procedures has been compiled in several reviews.[1]

Recently, we have contributed to MAOS's popularity describing a solventless access to (2-imidazolin-4-yl)phosphonates through a multicomponent reaction between diethyl isocyanomethylphosphonate, ketones and amines involving microwave irradiation in only 10 min at 40 °C.[2] The resulting 2-imidazolin compounds probed to be a structurally new family of high affinity imidazoline $I_2$ receptor ligands showing neuroprotective properties.[3] One of the key points for the success of the reaction was the divalent nature that exhibit isocyanides making possible reactions with a myriad of reaction partners of either electrophilic or nucleophilic character.[4]

Our interest in exploring isocyanides as an extremely useful class of organic compounds for synthetic chemistry, combined with our knowledge in the development of microwave assisted methodologies, prompted us to explore the preparation of thioformamides from isocyanides.

Thioformamides constitute a particular functional group embodying a high reactivity profile. They can be found as key intermediates in the synthesis of *N*-thioformyl peptides,[5] in the preparation of structurally diverse thioheterocycles some displaying fluorescence properties,[6] in the preparation of relevant heterocycles,[7] in the reaction with organometallic reagents giving access to diverse organic entities[8] and playing a starring role in the discovery of their physicochemical properties.[9]

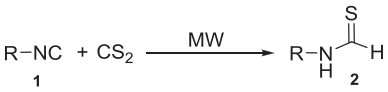
To the best of our knowledge few methods have been described for the synthesis of thioformamides being the most popular the reaction of isocyanides with thioacids.[10]

In this paper we add a new, efficient and safe methodology for the preparation of thioformamides using isocyanides and carbon disulfide, as the source of sulfur atoms, under microwave irradiation and in the absence of solvent (Scheme 1).

As a proof of concept of our approach, a model reaction using methyl isocyanoacetate as substrate, carbon disulfide and benzylamine, was optimized. Most gratifyingly, the desired product **2a** was obtained in a 71% yield when an equimolecular mixture of reagents was irradiated in the microwave oven at 40 °C during 10 min. Neither excess of benzylamine (entry 2) nor excess of both benzylamine and carbon disulfide (entry 3) increased the yield. In order to rule out a catalytic role for the base, a substoichiometric amount of amine, 0.5 equivalents, was examined. In agreement with the putative mechanism of the reaction (see below), under this condition the yield dropped to 48% (entry 4). Finally, neither shorter reaction time (entry 5) nor higher reaction temperature increased the yield (Table 1).

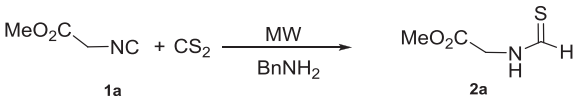
* Corresponding author.
*E-mail address:* cescolano@ub.edu (C. Escolano).

http://dx.doi.org/10.1016/j.tetlet.2017.06.001
0040-4039/




**Scheme 1.** Reaction studied.

**Table 1**
Optimization of the reaction conditions.




| Entry | $BnNH_2$ equivalents | $CS_2$ equivalents | MW conditions T/time | Yield[a] % |
|---|---|---|---|---|
| 1 | 1.0 | 1.0 | 40 °C/10 min | 71 |
| 2 | 1.5 | 1.0 | 40 °C/10 min | 70 |
| 3 | 1.5 | 1.5 | 40 °C/10 min | 70 |
| 4 | 0.5 | 1.0 | 40 °C/10 min | 48 |
| 5 | 1.0 | 1.0 | 40 °C/5 min | 54 |

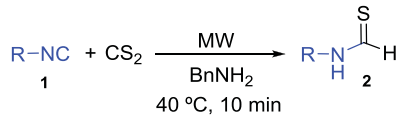
[a] Isolated yield of **2a**.

Therefore, the optimized conditions are microwave irradiation of a mixture of carbon disulfide, benzylamine and an isocyanide derivative in equimolecular quantities at 40 °C for 10 min in the absence of solvent. With the standard conditions in hand, we surveyed the functional group compatibility and scope of the present microwave-assisted synthesis of thioformamides directing the efforts towards the structural diversity of the isocyanide compound.

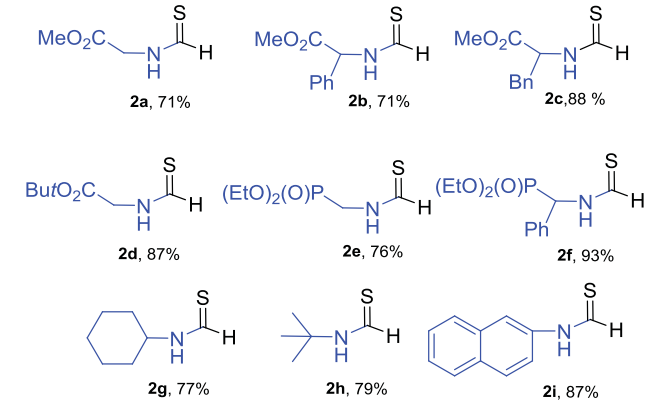
To this end, reactions with isocyanides substituted in the α position were undertaken. Thus, the use of methyl-αphenylisocyanoacetate and methyl-α-benzylisocyanoacetate furnished thioamides **2b** and **2c** with 71 and 88% yields, respectively. Also the putative influence of the alkylgroup in the ester was evaluated by performing the reaction with *t*-butyl isocyanoacetate to furnish the product **2d** in an excellent 87% yield. Worthy of note, the reaction took place with a 76% yield when diethyl isocyanomethylphosphonate was used as reagent to produce **2e**. Similarly to the methyl ester series, the formation of the thioformamide **2f** also occurs in excellent yields, 93%, when diethyl α-phenylisocyanometylphosphonate, an isocyanide substituted in the α-position, was employed.

The use of alkyl isocyanide derivatives was also examined and cyclohexylisocyanide gave **2g** in 77% yield. When *t*-butylisocyanide, an isocyanide tetrasubstituted in the αposition was used, **2h** was isolated in 79% yield. Finally, the inclusion of aromatic isocyanides was undertaken and the naphtylisocyanide successfully afforded **2i** in 87% yield.[11] (Scheme 2).

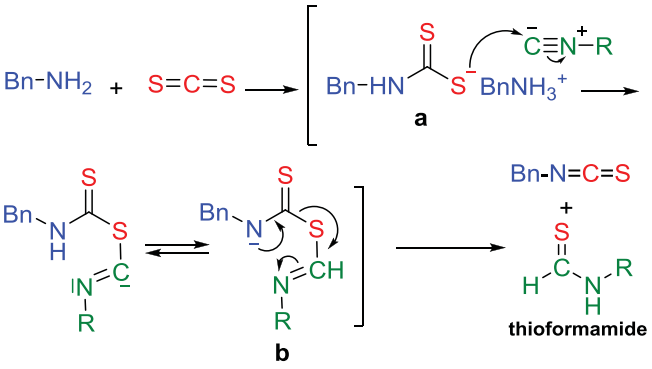
Of note, the reaction of carbon disulfide with amines has been applied in the last years for obtaining symmetrical thioureas.[12] However, in the presence of isocyanides the reaction evolved to give thioformamides. Taking into account that, in agreement with earlier reports,[13] one equivalent of benzylamine was needed for the formation of the dithiocarbamate salt **a**, which was observed within few minutes after the nucleophilic attack of benzylamine to carbon disulfide, a putative mechanism for this novel reaction is shown in Scheme 3. Very likely, under our usual reaction conditions, the intermediate **a** might attack the isocyanide to give the species **b** that would suffer fragmentation to deliver the final thioformamide and benzylisothiocyanate. Note that benzylisothiocyanate is produced in stoichiometric amount and therefore, the described procedure also covers an easy solvent free route to isothiocyanate derivatives. NMR spectra and HMRS probed the formation of intermediate **a** and benzylisothiocyanate. Considering the propose mechanism, with the sole exception of the sulfur atom, in the final thioamide all the atoms stem from the isocyanate reagent. Considering that a myriad of isocyanates are currently available, a very broad range of thioformamides can be accessed following this new methodology.




**1a**, R = $MeCO_2CH_2$-
**1b**, R = $MeCO_2CH(Ph)$-
**1c**, R = $MeCO_2CH(Bn)$-
**1d**, R = $tBuCO_2CH_2$-
**1e**, R = $(EtO)_2(O)PCH_2$-
**1f**, R = $(EtO)_2(O)PCH(Ph)$-
**1g**, R = cyclohexyl-
**1h**, R = *t*Bu-
**1i**, R = naphtyl-




**Scheme 2.** Synthesis of thioformamides **2a**–**2i**.




**Scheme 3.** Putative mechanism.

To summarize, the work presented in this manuscript provides a mild, rapid and efficient method for accessing thioformamides compatible with a range of diversely substituted isocyanides. This method, contributes to enlarge the repertoire of useful reactions to be performed under microwave irradiation.

## Acknowledgments

Financial support from the Spanish Ministry of Economy and Competitiveness MINECO: Project BQU2015-66030-R and SAF2016-77703-C2-1-R and Generalitat de Catalunya Grant 2014-SGR-01555 is grateful acknowledged.

## Supplementary data

Supplementary data (detailed experimental procedures and copies of NMR spectra for all the new compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2017.06.001.

## References

1. (a) Kruithof A, Ruitjer E, Orru RVA. In: de la Hoz A, Loupy A, eds. *Microwaves in Organic Synthesis*. Wiley; 2002:1099;
(b) Kappe CO, Dallinger D. *Mol Divers*. 2009;13:71–193;
(c) Caddick S, Fitzmaurice R. *Tetrahedron*. 2009;65:3325–3355;
(d) Kruithof A, Ruijter E, Orru RVA. *Curr Org Chem*. 2011;15:204–236;
(e) Moseley JD, Kappe CO. *Green Chem*. 2011;13:794–806;
(f) Majumder A, Gupta R, Jain A. *Green Chem Lett Rev*. 2013;6:151–182.
2. Abás S, Estarellas C, Luque FJ, Escolano C. *Tetrahedron*. 2015;71:2872–2881.
3. Abás S, Erdozain AM, Keller B, et al. *ACS Chem Neurosci*. 2017. http://dx.doi.org/10.1021/acschemneuro.6b00426.
4. (a) Gulevich AV, Zhdanko AG, Orru RVA, Nenajdenko VG. *Chem Rev*. 2010;110:5235–5331;
(b) Tobisu M, Chatani N. *Chem Lett*. 2011;40:330–340;
(c) . *Isocyanide Chemistry Applications in Synthesis and Materials Science*. Weinheim: Wiley-VCH; 2012;
(d) Bode ML, Gravestock D, Rousseau AL. *Org Prep Proced Int*. 2016;48:89–221;
(e) Wang Y, Kumar RK, Bi X. *Tetrahedron Lett*. 2016;57:5730–5741;
(f) Giustiniano M, Basso A, Valentina M, et al. *Chem Soc Rev*. 2017;46:1295–1357.
5. (a) Yuan Y, Zhu J, Li X, Wu X, Danishefsky SJ. *Tetrahedron Lett*. 2009;50:2329–2333;
(b) Fowler BS, Mikochik PJ, Miller SJ. *J Am Chem Soc*. 2010;132:2870–2871.
6. (a) Murai T, Hori F, Maruyama T. *Org Lett*. 2011;13:1718–1721;
(b) Yamaguchi K, Murai T, Hasegawa S, et al. *J Org Chem*. 2015;80:10742–10756.
7. Zou JP, Zeng RS, Lu ZE, Chen KQ. *Trends Heterocycl Chem*. 2001;7:107–116.
8. (a) Murai T, Asai F. *J Am Chem Soc*. 2007;129:780–781;
(b) Murai T, Kazuki U, Narengerile K. *J Org Chem*. 2009;74:5703–5706;
(c) Murai T, Hori R. *Bull Chem Soc Jpn*. 2010;83:52–57;
(d) Murai T, Matsushita K. *Phosphorus Sulfur Silicon Relat Elem*. 2011;186:1094–1103;
(e) Murai T, Mutoh Y. *Chem Lett*. 2012;41:2–8;
(f) . For a review, see:Wei J, Liu L, Zhan M, Xu L, Zhang WX, Xi Z. *Angew Chem Int Ed*. 2014;53:5634–5638;
(g) Murai T, Mutoh N. *J Org Chem*. 2016;81:8131–8134.
9. Hutton DA, Shang J, Wille U. *Chem Eur J*. 2016;22:3163–3169.
10. (a) Chupp JP, Leschinsky KL. *J Org Chem*. 1975;40:66–71;
(b) Ref. 5a.;
(c) Stockdill JL, Wu X, Danishefsky SJ. *Tetrahedron Lett*. 2009;50:5152–5155;
(d) Ramazani A, Joo SW, Nasrabadi FZ. *Turk J Chem*. 2013;37:405–412.
11. Compound **1i** is a solid, therefore, in the preparation of **2i** the use of ethanol was required.
12. Jangale AD, Kumavat PP, Wagh YB, Tayade YA, Mahulikar PP, Dalal DS. *Synth Commun*. 2015;45:236–244.
13. Munch H, Hansen JS, Pittelkow M, Christensen JB, Boas U. *Tetrahedron Lett*. 2008;49:3117–3119.

## Supplementary material

# Facile microwave-assisted synthesis of thioformamides from isocyanides and carbon disulfide

Sònia Abás, Ulrike Moens and Carmen Escolano

*Laboratory of Medicinal Chemistry (Associated unit to CSIC), Department of Pharmacology, Toxicology and Medicinal Chemistry, Faculty of Pharmacy and Food Sciences, and Institute of Biomedicine (IBUB), University of Barcelona, Av. Joan XXIII 27-31, E-08028, Barcelona, Spain*

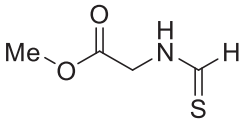
## General information

NMR spectra were recorded in $CDCl_3$ at 400 MHz ($^1H$) and 100.6 MHz ($^{13}C$), and chemical shifts are reported in δ values downfield from TMS or relative to residual chloroform (7.26 ppm, 77.0 ppm) as an internal standard. Data are reported in the following manner: chemical shift, multiplicity, coupling constant (J) in hertz (Hz), integrated intensity. Multiplicities are reported using the following abbreviations: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet; q, quadruplet and br s, broad signal. Evaporation of solvents was accomplished with a rotary evaporator. Thin layer chromatography was done on $SiO_2$ (silica gel 60 F254), and the spots were located by UV and 1% aqueous $KMnO_4$. Chromatography refers to flash column chromatography was carried out on $SiO_2$ (silica gel 60, SDS, 230–400 mesh). Automatized chromatography was carried out with a Biotage Isolera System Single Channel, standard arm, variable detector (ISO-1SV) using Biotage® SNAP Cartridge, 10 g. Mass spectra were recorded on an LTQ spectrometer using electrospray (ES+) ionization techniques. The CEM-Discover® Focused Monomode Microwave reactor (2450 MHz, 300 W) was used for microwave continuous flow irradiation. The temperature threshold was set at 40ºC.

### General Procedure

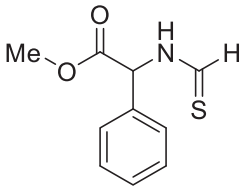
A mixture of carbon disulfide (1 eq.), benzylamine (1 eq.) and isocyanide derivative (1 eq) was stirred under microwave irradiation at 40 °C for 10 min. The solvent of the mixture was evaporated under reduced pressure to yield a residue which was purified by flash column chromatography.

**Methyl thioformylglycinate 2a**



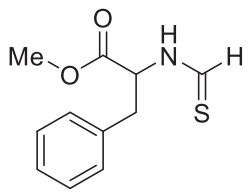
Following the general procedure, carbon disulfide (48 µL, 0.8 mmol), benzylamine (87 µL, 0.8 mmol) and methyl isocyanoacetate **1a** (72 µL, 0.8 mmol) gave **2a** as a red oil (75.0 mg, 71%) after column chromatography (Hexane/EtOAc mixtures). IR (NaCl) 3307, 2928, 2855, 1747, 1676, 1437, 1261, 1215, 802, 702 $cm^{-1}$; $^{1}H$ NMR (COSY, HETCOR) δ 3.84 (s, 3H, OC*H*$_3$), 4.44 (dd, *J* = 4.6, 0.8 Hz, 2H, C*H*$_2$), 7.94 (br s, 1H,N*H*), 9.51 (dm, *J* = 6.0 Hz, 1H, CS*H*)**;** $^{13}C$ NMR δ 43.8 (O*C*H$_3$), 51.8 (*C*H$_2$), 168.2 (*C*O), 188.5 (*C*S). HRMS $C_6H_8NO_2S$ $[M + H]^+$ 134,1768; found, 134,0268.

**Methyl thioformylglycinate 2b**



Following the general procedure, carbon disulfide (36 µL, 0.6 mmol), benzylamine (65 µL, 0.6 mmol) and methyl 1-phenyl isocyanoacetate[ii] **1b** (105 mg, 0.6 mmol) gave **2b** as a white crystals (89.5 mg, 71%) after column chromatography (Hexane/EtOAc mixtures). IR (NaCl) 3315, 2954, 2360, 1742, 1498, 1436, 1261, 1213, 897, 698 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) δ 3.74 (s, 3H, OC*H*$_3$), 6.16 (d, *J* = 6.4 Hz, 1H, C*H*), 7.34 – 7.40 (m, 5H, Ar*H*), 8.60 (br s, 1H, N*H*), 9.44 (dd, *J* = 6.4, 0.8 Hz, 1H, CS*H*); $^{13}C$ NMR (100.6 MHz) δ 53.2 (O*C*H$_3$), 59.2 (*C*H), 127.7 (2*C*Ar), 128.9 (*C*Ar), 129.0 (2*C*Ar), 134.5 (*C*$_{ipso}$), 170.3 (*C*O), 188.8 (*C*S); HRMS $C_{10}H_{12}NO_2S$ $[M + H]^+$ 210.2728; found, 210.0574. M. p.: 62 – 64°C.

**Methyl thioformylphenylalaninate 2c**

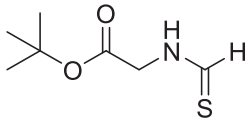


Following the general procedure, carbon disulfide (36 µL, 0.6 mmol), benzylamine (65 µL, 0.6 mmol) and methyl 2-isocyano-3-phenylpropanoate[1] **1c** (124 mg, 0.6 mmol) gave **2c** as a yellow oil (118.9 mg, 88%) after column chromatography (Hexane/EtOAc

[i] Bon RS, Hong S, Bouma MJ, Schmitz RF, Kanter FJJ, Lutz M, Spek AL, Orru RVA, *Org. Lett*. 2003; 5: 3759-3762. Fayol A, Housseman C, Sun X, Janvier P, Bienaymé H, Zhu J. *Synthesis*. 2005; 1: 161-165.

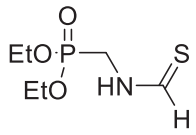
mixtures). IR (NaCl) 3314, 3029, 2952, 2360, 1742, 1497, 1438, 1273, 1217, 699 $cm^{-1}$; $^{1}H$ NMR (COSY, HETCOR) δ 3.23 (dd, *J* = 14.0, 4.4 Hz, 1H, C$H_2$), 3.45 (dd, *J* = 14.0, 6.0 Hz, 1H, C$H_2$), 3.78 (s, 3H, OC$H_3$), 5.51 (m, 1H, C*H*), 7.08 – 7.10 (m, 2H, Ar*H*), 7.25 – 7.30 (m, 3H, Ar*H*), 7.75 (br s, 1H, N*H*), 9.43 (dd, *J* = 6.4, 1.2 Hz, 1H, CS*H*); $^{13}C$ NMR δ 35.9 (O*C*$H_3$), 52.7 (*C*H), 56.0 (*C*$H_2$), 127.4 (*C*HAr), 128.7 (2*C*HAr), 129.3 (2*C*HAr), 135.1 ($C_{ipso}$), 170.9 (*C*O), 188,6 (*C*S); HRMS $C_{11}H_{12}NO_2S$ $[M - H]^-$ 222,2835; found, 222,0596.

**_Tert_-butyl thioformylglycinate 2d**



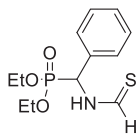
Following the general procedure, carbon disulfide (48 µL, 0.8 mmol), benzylamine (87 µL, 0.8 mmol) and *tert*-butyl isocyanoacetate **1b** (116 µL, 0.8 mmol) gave **2d** as a red oil (121.7 mg, 87%) after column chromatography (Hexane/EtOAc mixtures). IR (NaCl) 3314, 2983, 2364, 1745, 1677, 1370, 1233, 1156, 845, 772 $cm^{-1}$; $^{1}H$ NMR (COSY, HETCOR) δ 1.50 (s, 9H, C$H_3$), 4.32 (s, 2H, C$H_2$), 8.39 (br s, 1H, N*H*), 9.47 (s, 1H, CS*H*); $^{13}C$ NMR δ 28.0 (3*C*$H_3$), 45.7 (*C*$H_2$), 83.3 (*C*-*t*Bu), 167.8 (*C*O), 189.0 (*C*S); HRMS $C_7H_{12}NO_2S$ $[M - H]^-$ 174.2407; found, 174.0594.

**Diethyl (methanethioamidomethyl)phosphonate 2e**



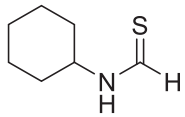
Following the general procedure, carbon disulfide (48 µL, 0.8 mmol), benzylamine (87 µL, 0.8 mmol) and diethyl isocyanomethylphosphonate **1e** (128 µL, 0.8 mmol) gave **2e** as a yellow oil (129.3 mg, 76%) after column chromatography (EtOAc/Hexane mixtures). IR (NaCl) 3215, 2984, 2743, 2363, 1683, 1541, 1448, 1236, 1023, 978, 779 $cm^{-1}$; $^{1}H$ NMR (COSY, HETCOR) δ 1.36 (t, *J* = 6.8 Hz, 6H, OC$H_2$C$H_3$), 4.13 (q, *J* = 7.0 Hz, 2H, OC$H_2$C$H_3$), 4.15 (q, *J* = 7.0 Hz, 2H, OC$H_2$C$H_3$), 4.24 (dd, *J* = 13.6, 5.6 Hz, 2H, C$H_2$), 9.45 (d, *J* = 5.6 Hz, 1H, CS*H*), 9.74 (br s, 1H, N*H*); $^{13}C$ NMR δ 16.3 (d, *J* = 6.0 Hz, OC$H_2$C$H_3$), 38.0 (d, *J* = 155.0 Hz, *C*H), 63.2 (d, *J* = 6.5 Hz, OC$H_2$C$H_3$), 189.7 (d, *J* = 9.0 Hz, *C*S); HRMS $C_{11}H_{13}NO_3PS$ $[M - H]^-$ 210.0361; found, 210.0359**.**

**Diethyl [methanethioamido(phenyl)methyl]phosphonate 2f**



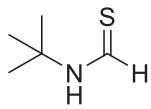
Following the general procedure, carbon disulfide (36 µL, 0.6 mmol), benzylamine (65 µL, 0.6 mmol) and diethyl isocyano(phenyl)methylphosphonate[1] **1f** (152 mg, 0.6 mmol) gave **2f** as a yellow solid (161 mg, 93%) after column chromatography, (EtOAc/Hexane mixtures). IR (NaCl) 3186, 2995, 2358, 1537, 1446, 1226, 1020, 975, 790 $cm^{-1}$; $^1H$ NMR (COSY, HETCOR) δ 1.07 (dt, *J* = 7.0, 0.8 Hz, 3H, $OCH_2CH_3$), 1.35 (dt, *J* = 7.0, 0.8 Hz, 3H, $OCH_2CH_3$), 3.70 (m, 1H, $OCH_2CH_3$), 3.90 (m, 1H, $OCH_2CH_3$), 4.12-4.25 (m, 2H, $OCH_2CH_3$), 6.42 (dd, *J* = 19.6, 9.2 Hz, 1H, *H*α), 7.33-7.39 (m, 3H, Ar*H*), 7.49-7.52 (m, 2H, Ar*H*), 9.37 (br s, 1H, N*H*), 9.37 (dd, *J* = 6.0, 0.8 Hz, 1H, CS*H*); $^{13}C$ NMR δ 16.1 (d, *J* = 6.0 Hz, $OCH_2CH_3$), 16.4 (d, *J* = 6.0 Hz, $OCH_2CH_3$), 52.8 (d, *J* = 154.0 Hz, *C*H), 63.6 (d, *J* = 7.0 Hz, $OCH_2CH_3$), 64.1 (d, *J* = 7.0 Hz, $OCH_2CH_3$), 128.5 (d, *J* = 2.5 Hz, *C*HAr), 128.6 (*C*HAr), 128.7 (*C*HAr), 128.8 (d, *J* = 3.5 Hz, *C*HAr), 128.9 (d, *J* = 2.5 Hz, *C*HAr), 133.4 (d, *J* = 2.0 Hz, $C_{ipso}$), 189.1 (d, *J* = 10.5 Hz, CS); HRMS $C_{12}H_{19}NO_3PS$ $[M + H]^+$ 288.0819; found, 288.018. M. p.: 114-115°C

**_N_-Cyclohexylmethanethioamide 2g**



Following the general procedure, carbon disulfide (48 µL, 0.8 mmol), benzylamine (87 µL, 0.8 mmol) and cyclohexylisocyanide **1g** (100 µL, 0.8 mmol) gave **2g** as a yellow oil (1:1 ratio of rotamer)(88.0 mg, 77%) after column chromatography (Hexane/EtOAc mixtures). IR (NaCl) 3197, 3020, 2932, 2855, 1538, 1451, 1318, 1286, 966, 873 $cm^{-1}$; $^1H$ NMR (COSY, HETCOR) δ 1.15 – 1.48 (m, 5H, $CH_2$), 1.66 (m, 1H, $CH_2$), 1.73 – 1.81 (m, 2H, $CH_2$), 1.97 (m, 1H, $CH_2$), 2.07 (m, 1H, $CH_2$), 3.46 (m, 1H, C*H* rotamer 1), 4.50 (m, 1H, C*H* rotamer 2), 7.44 (br s, 1H, S*H* rotamer 2), 8.13 (br s, 1H, N*H*, rotamer 1), 9.20 (d, *J* = 15.6 Hz, CS*H* rotamer 2), 9.34 (dd, *J* = 6.4, 0.8 Hz, CS*H* rotamer 1); $^{13}C$ NMR δ 24.4 ($CH_2$ rotamer 1), 24.5 ($CH_2$ rotamer 2), 24.8 ($2CH_2$ rotamer 1), 25.3 ($2CH_2$ rotamer 2), 31.4 ($2CH_2$ cyclo rotamer 1), 33.4 ($2CH_2$ rotamer 2), 51.6 (C*H* rotamer 1), 58.8 (C*H* rotamer 2), 187.4 (CS*H* rotamer 1), 189.0 (CS*H* rotamer 2); HRMS $C_7H_{12}NS$ $[M - H]^-$ 142.2419; found, 142.0693.

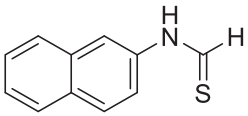
**_N-Tert_-butylmethanethioamide 2h**



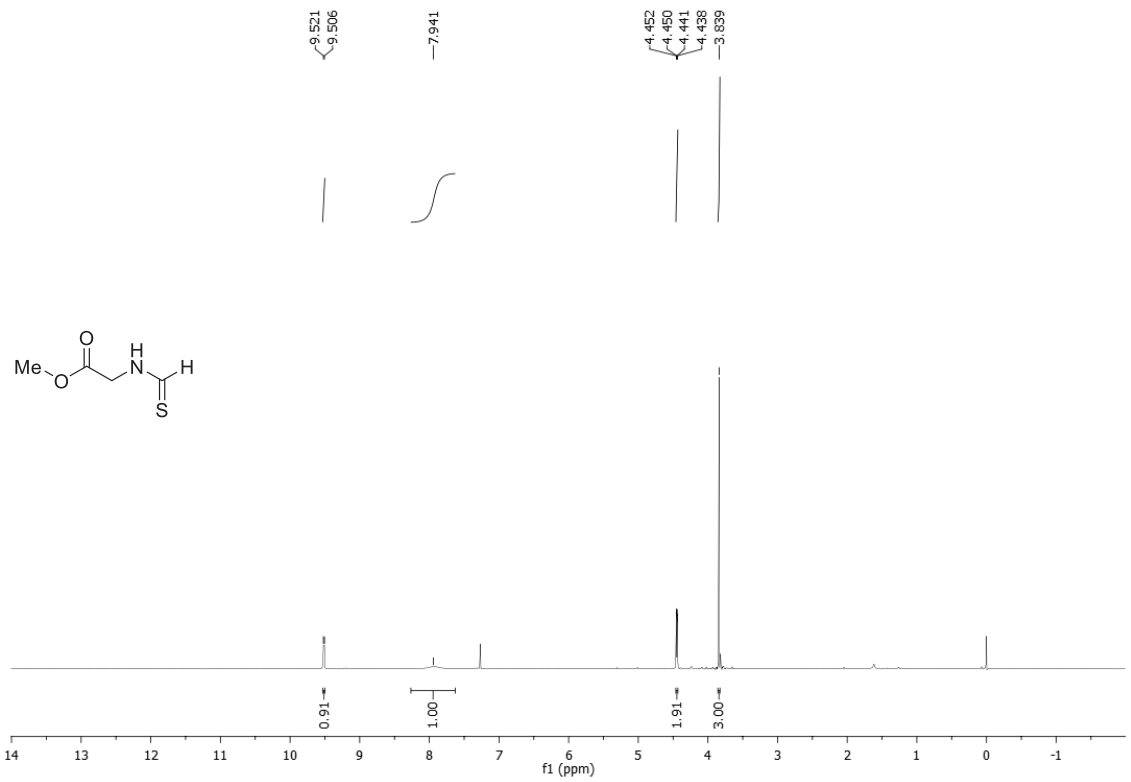
Following the general procedure, carbon disulfide (48 µL, 0.8 mmol), benzylamine (87 µL, 0.8 mmol) and *N-tert*-butylisocyanide **1h** (90 µL, 0.8 mmol) produced **2h** as a white solid (73.9 mg, 79%) after column

chromatography (Hexane/EtOAc mixtures). IR (NaCl) 2985, 2364, 1745, 1670, 1634, 1577, 1474, 1371, 1241, 948 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$, COSY, HETCOR) δ 1.38 (s, 9H, C*H*$_3$), 8.85 (br s, 1H, N*H*), 9.30 (d, *J* = 16.0 Hz, 1H, CS*H*); $^{13}C$ NMR δ 29.7 (3*C*$H_3$), 56.1 (*C*-*t*Bu), 187.1 (*C*S). HRMS $C_5H_{12}NS$ $[M + H]^+$ 118,2205; found, 118,0686. M. p.: 119-121°C.

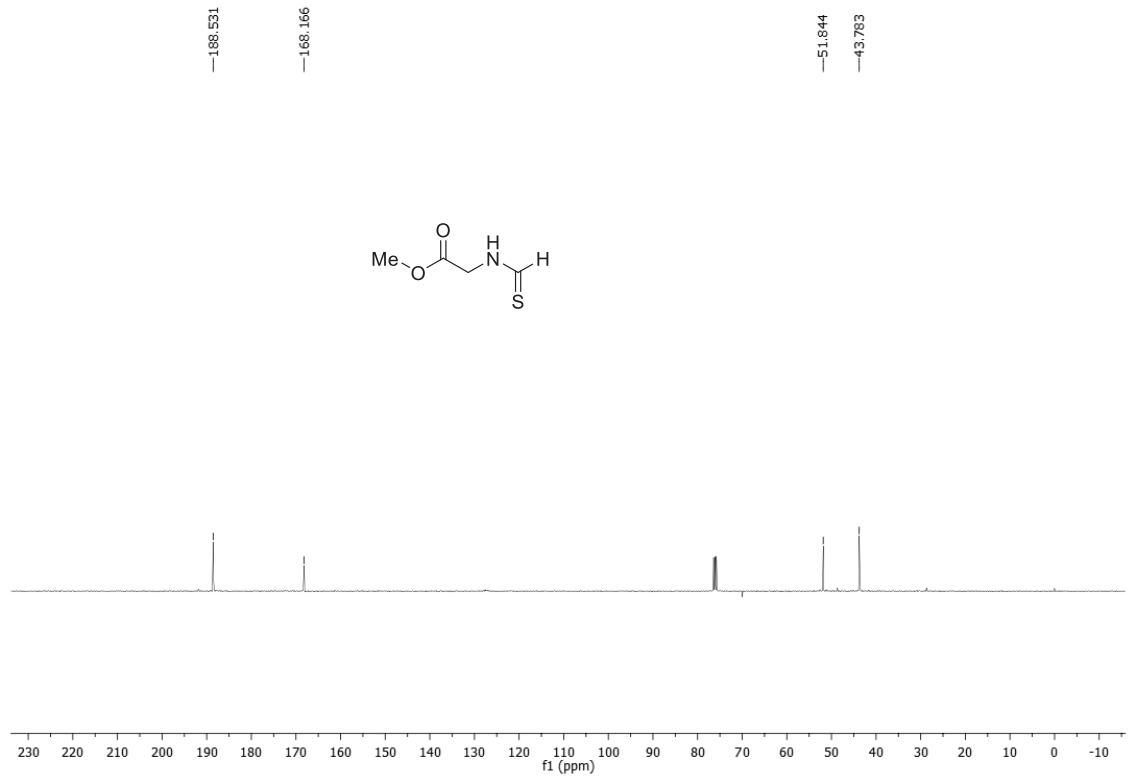
***N*-(Naphtalen-2-yl)methanethioamide 2i**

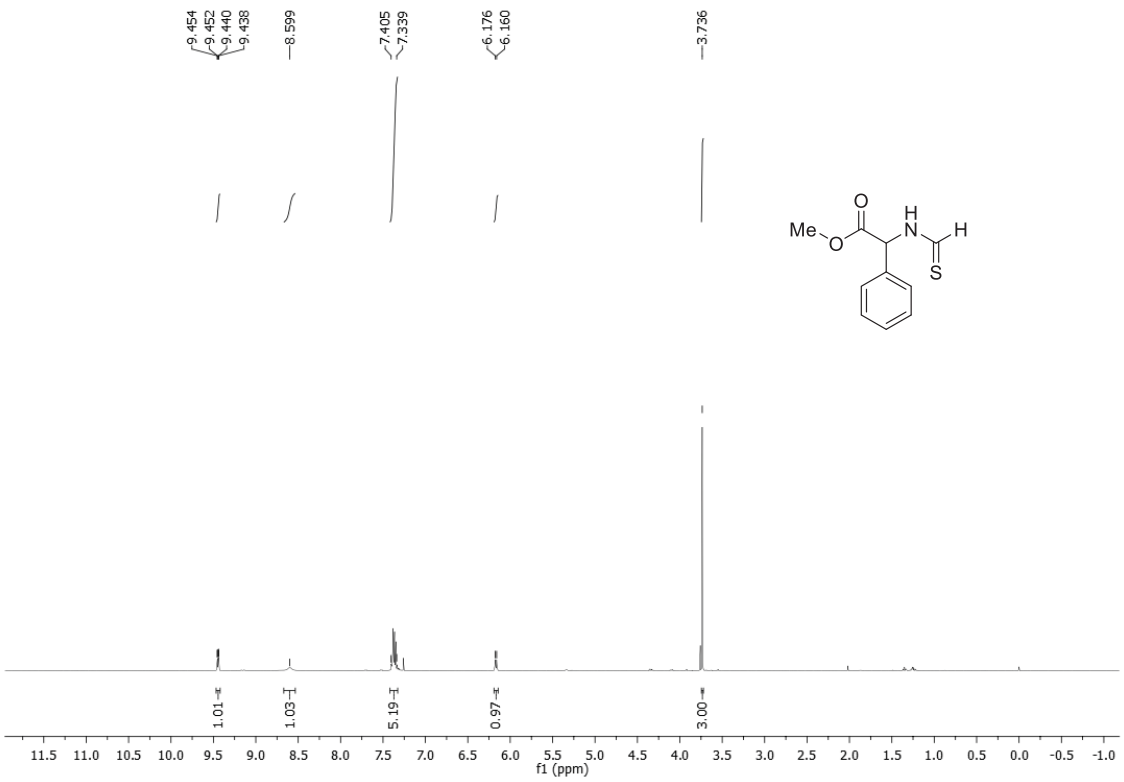



Following the general procedure, carbon disulfide (50 µL, 0.8 mmol), benzylamine (87 µL, 0.8 mmol) in ethanol (1.6 mL) and 2-naphtylisocyanide **1i** (122 mg, 0.8 mmol) gave **2i** as a white slightly brown solid (130.8 mg, 87%) after column chromatography (Hexane/EtOAc mixtures). IR (NaCl) 3443, 3046, 2799, 2365, 1573, 1378, 1327, 945, 801, 462 $cm^{-1}$; $^{1}H$ NMR (COSY, HETCOR) δ 7.29 (dd, *J* = 8.8, 2.4 Hz, 1H, Ar*H*), 7.46 – 7.56 (m, 3H, Ar*H*), 7.79 – 7.88 (m, 3H, Ar*H*), 9.55 (br s, 1H, N*H*), 9.94 (s, 1H, CS*H*); $^{13}C$ NMR δ 114.4 (*C*HAr), 116.9 (*C*HAr), 126.0 (*C*HAr), 127.4 (*C*HAr), 127.5 (*C*HAr), 127.9 (*C*HAr), 130.3 (*C*HAr), 131.5 (*C*Ar), 133.6 (*C*Ar), 135.9 (*C*Ar), 187.4 (*C*S). HRMS $C_{11}H_8NS$ [M - H]- 186,2529; found, 186,0381. M. p.: 141-145°C.

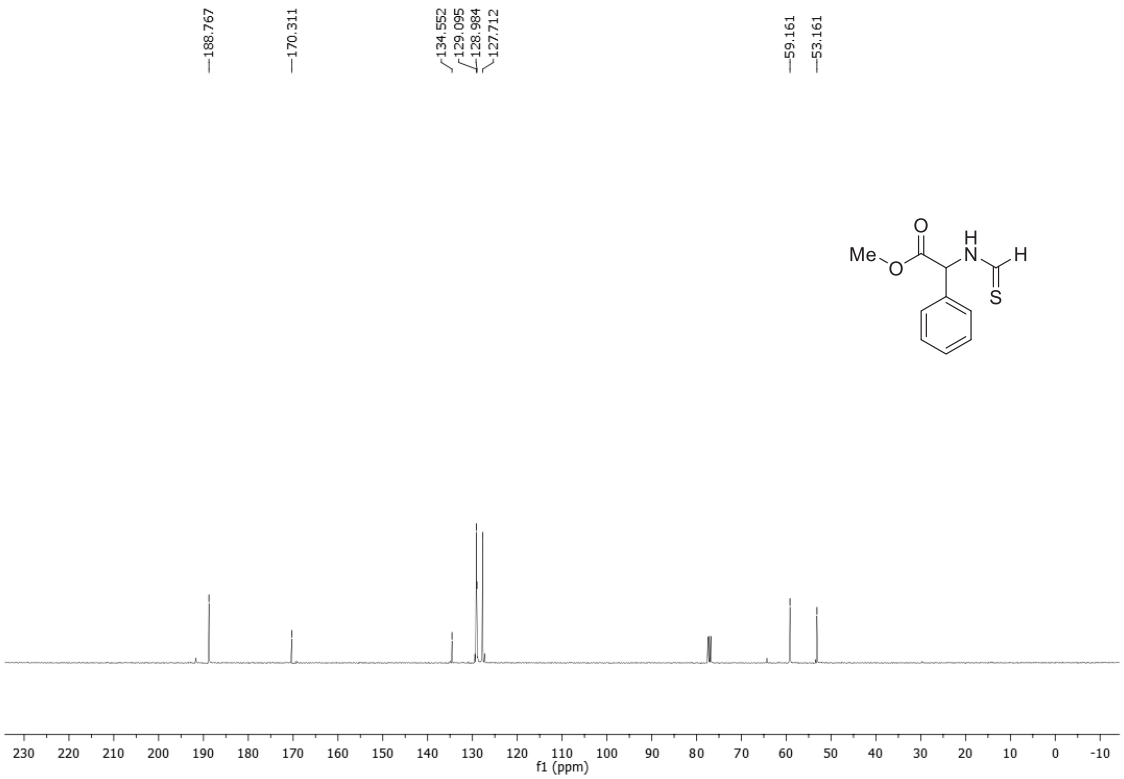


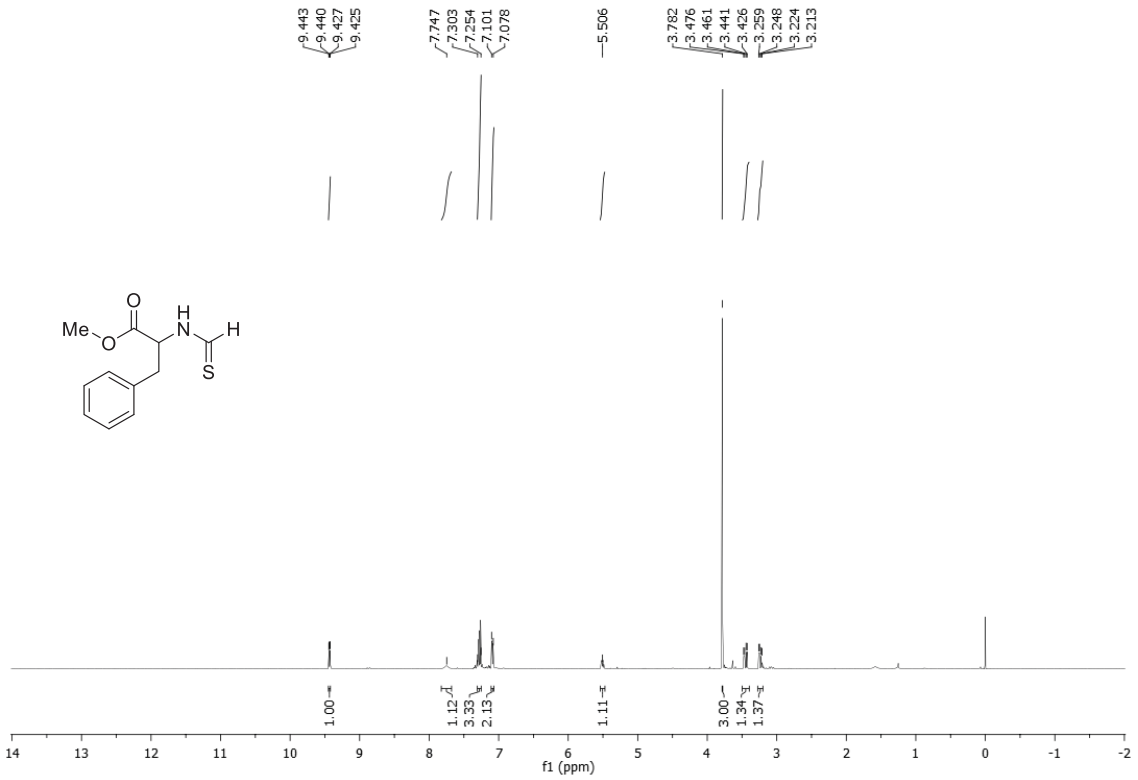

**Methyl thioformylglycinate 2a**



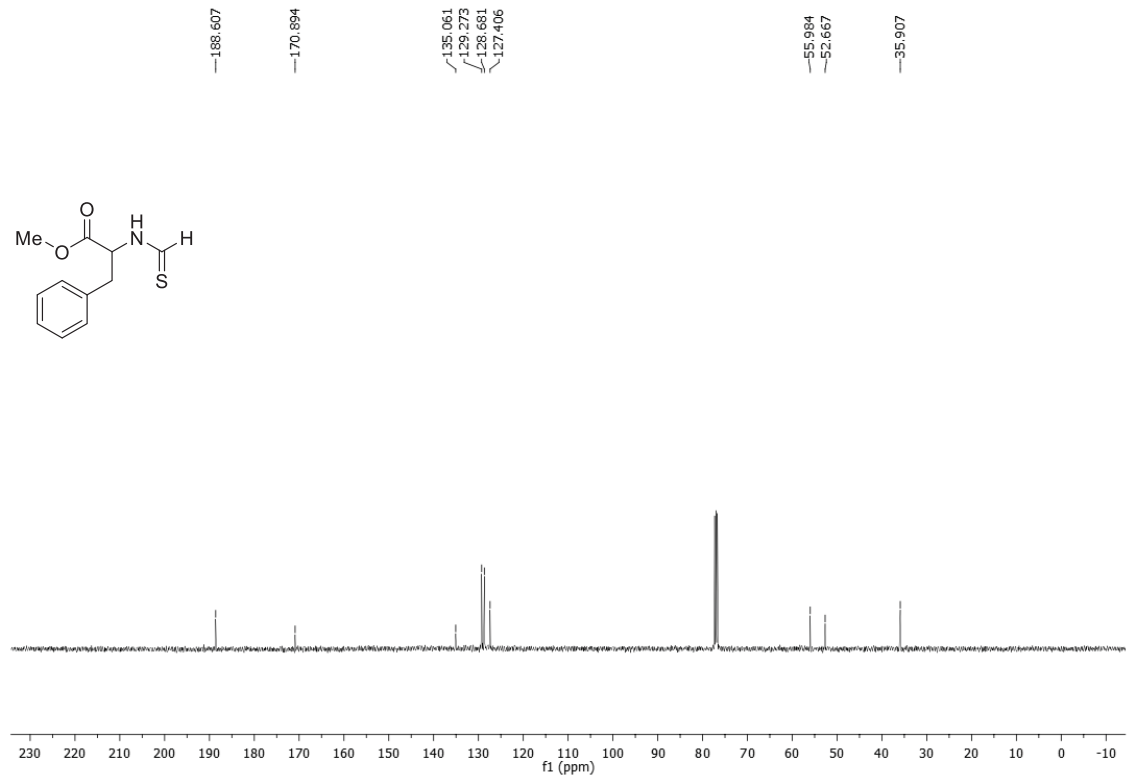


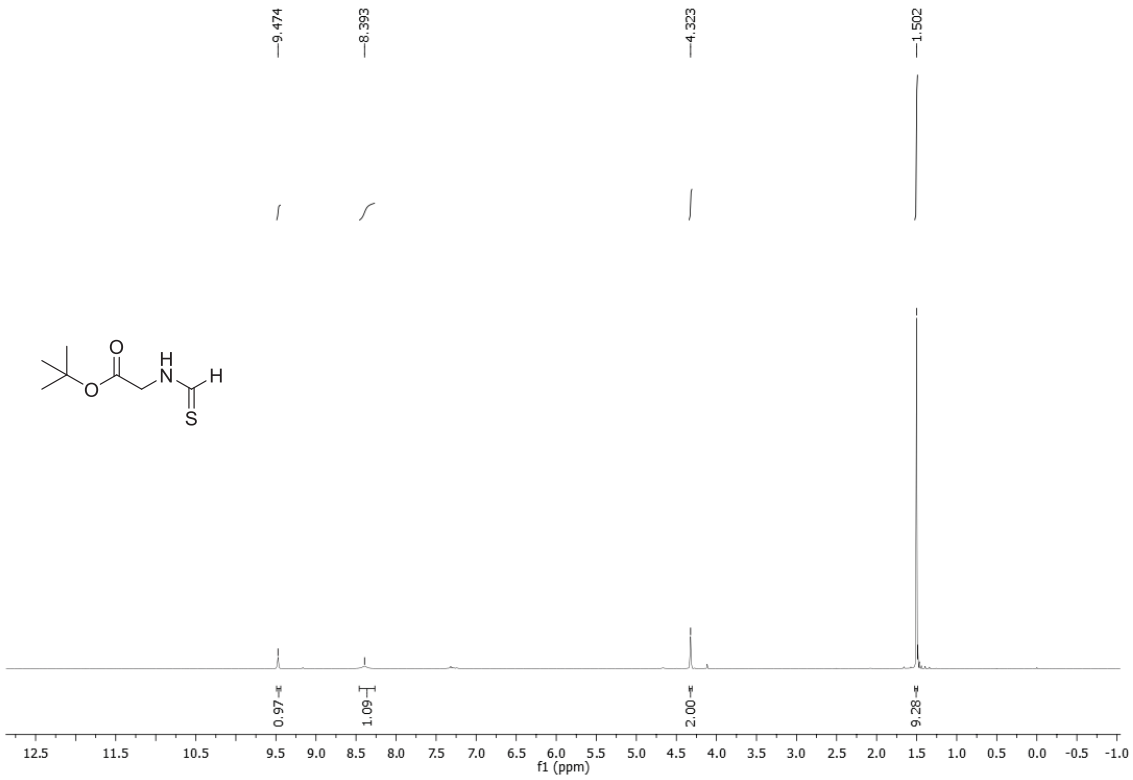

**Methyl thioformylglycinate 2b**



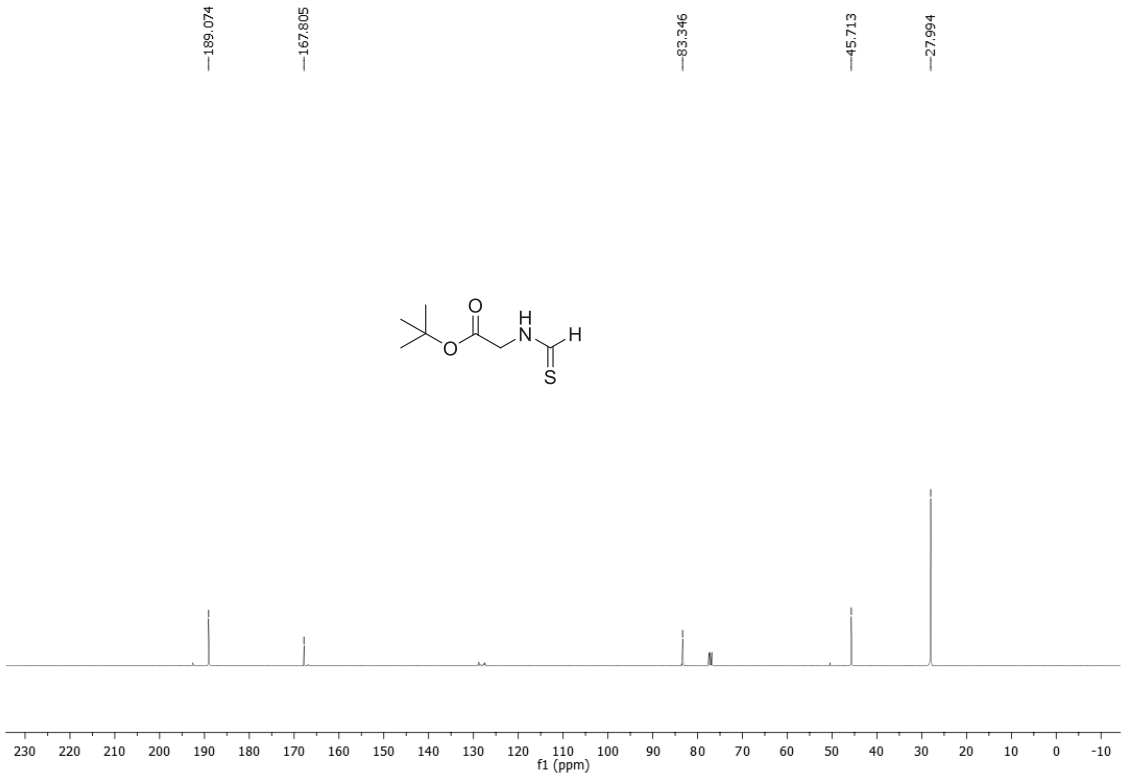


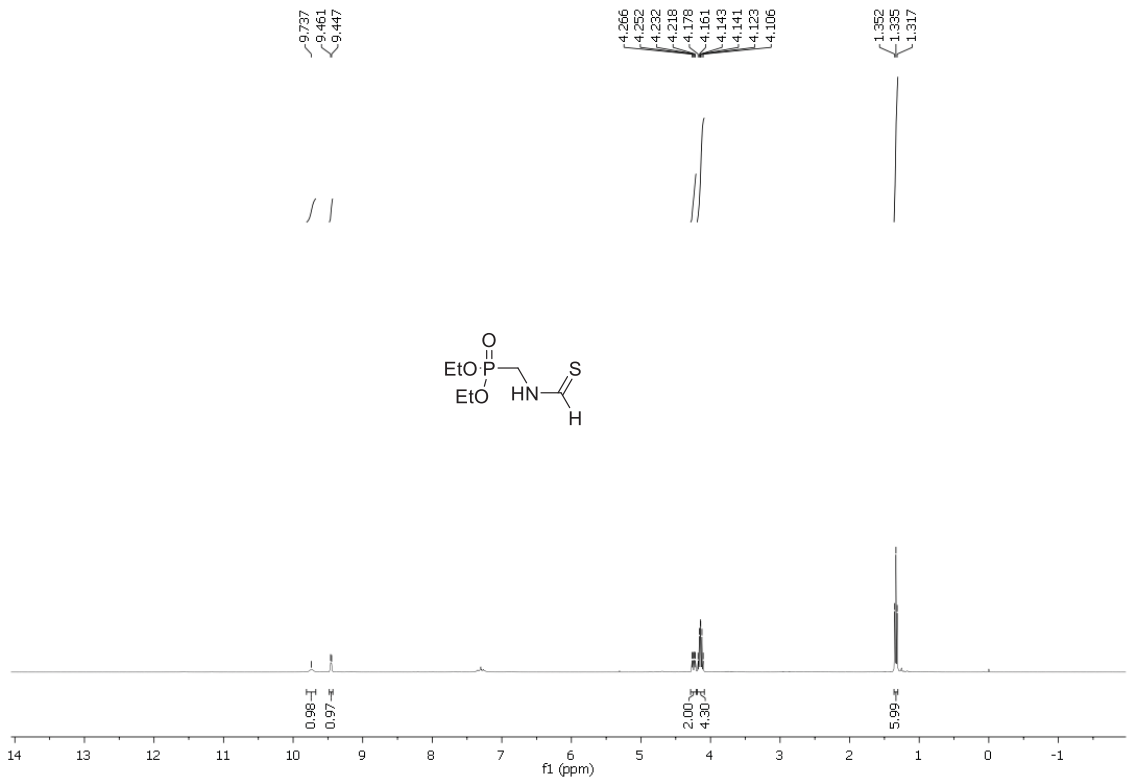

**Methyl thioformylphenylalaninate 2c**



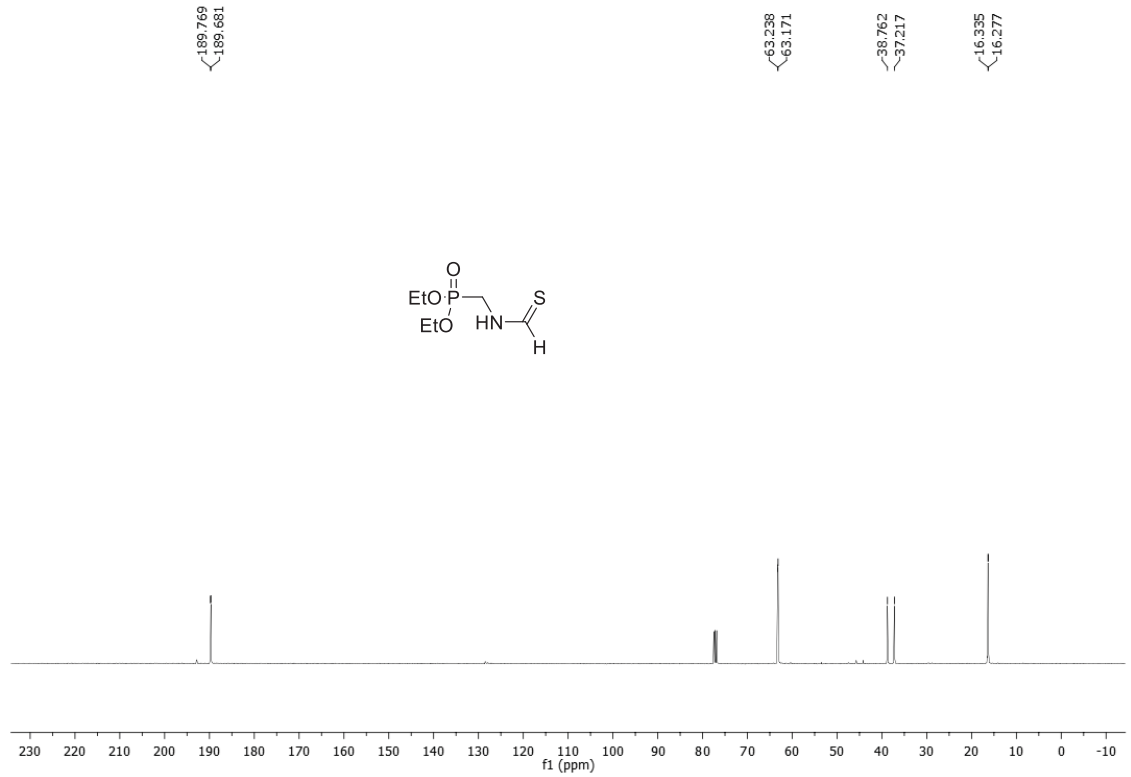


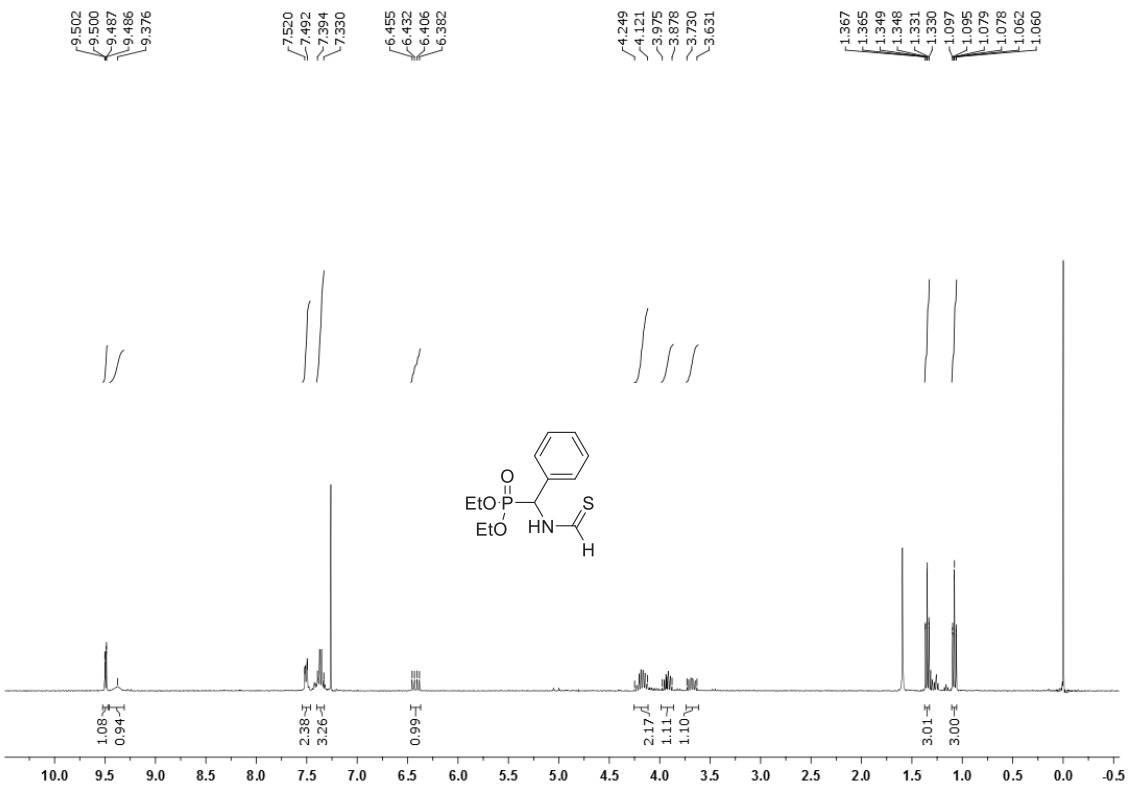

**_Tert_-butyl thioformylglycinate 2d**



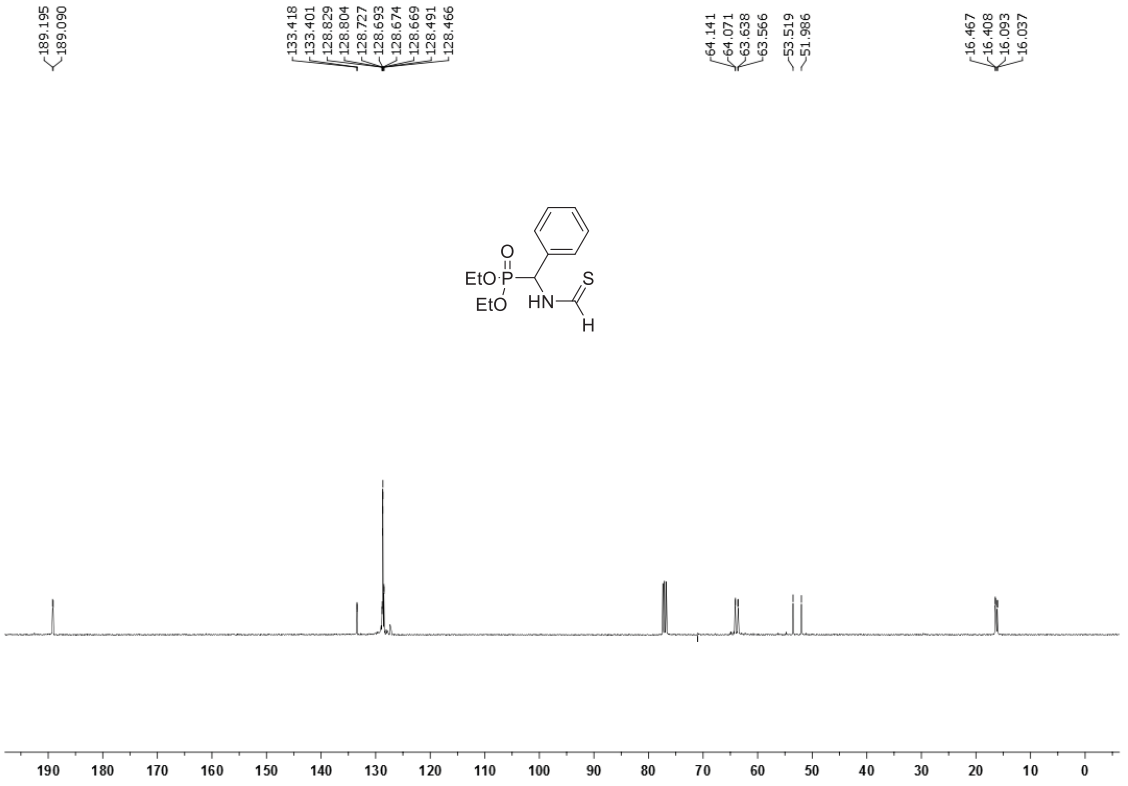


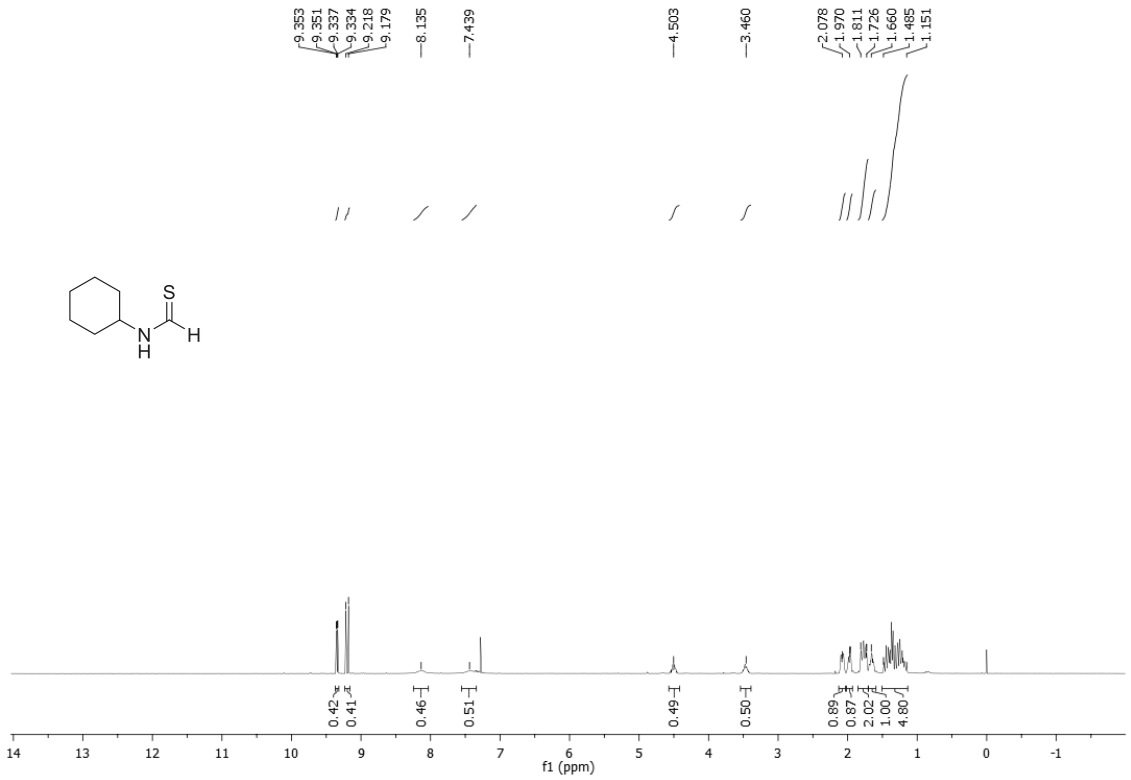

**Diethyl (methanethioamidomethyl)phosphonate 2e**



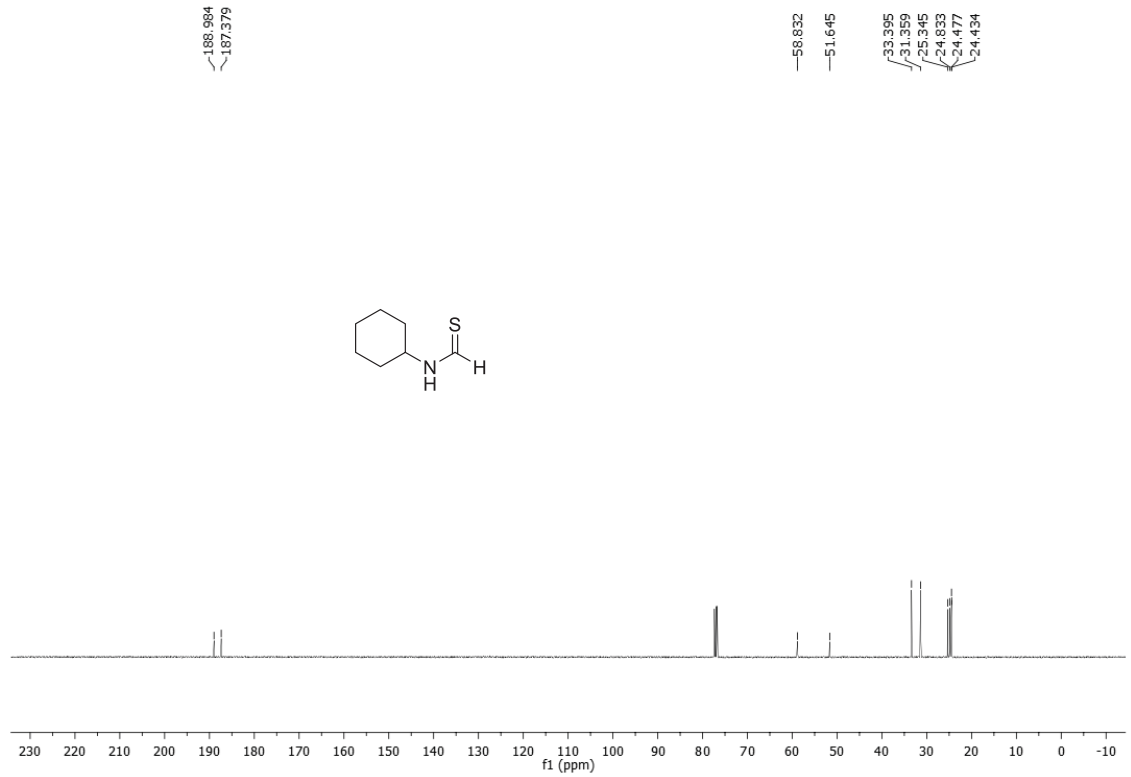


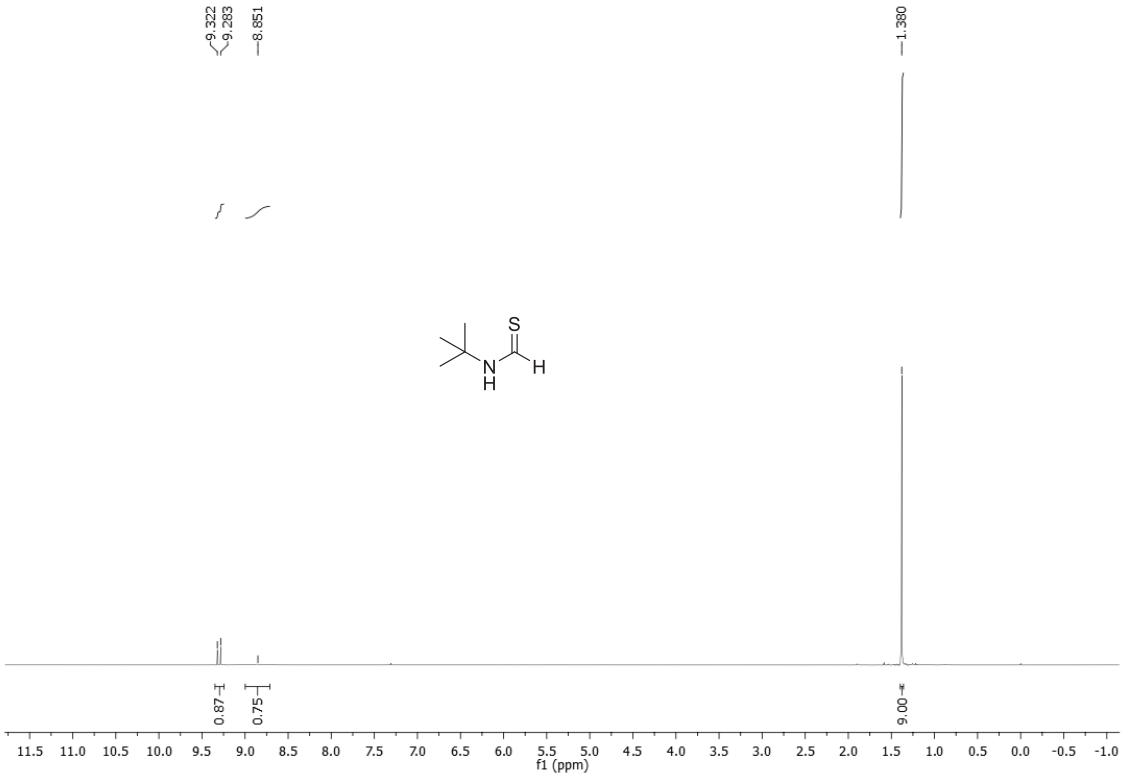

**Diethyl [methanethioamido(phenyl)methyl]phosphonate 2f**



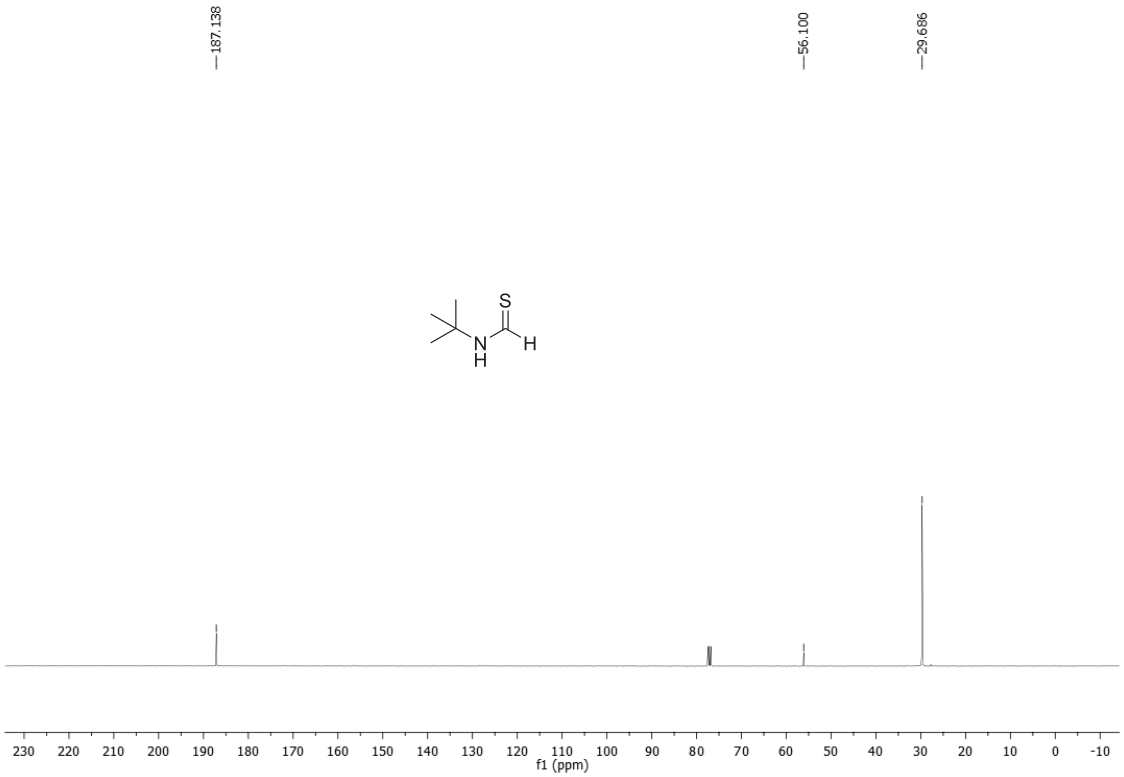


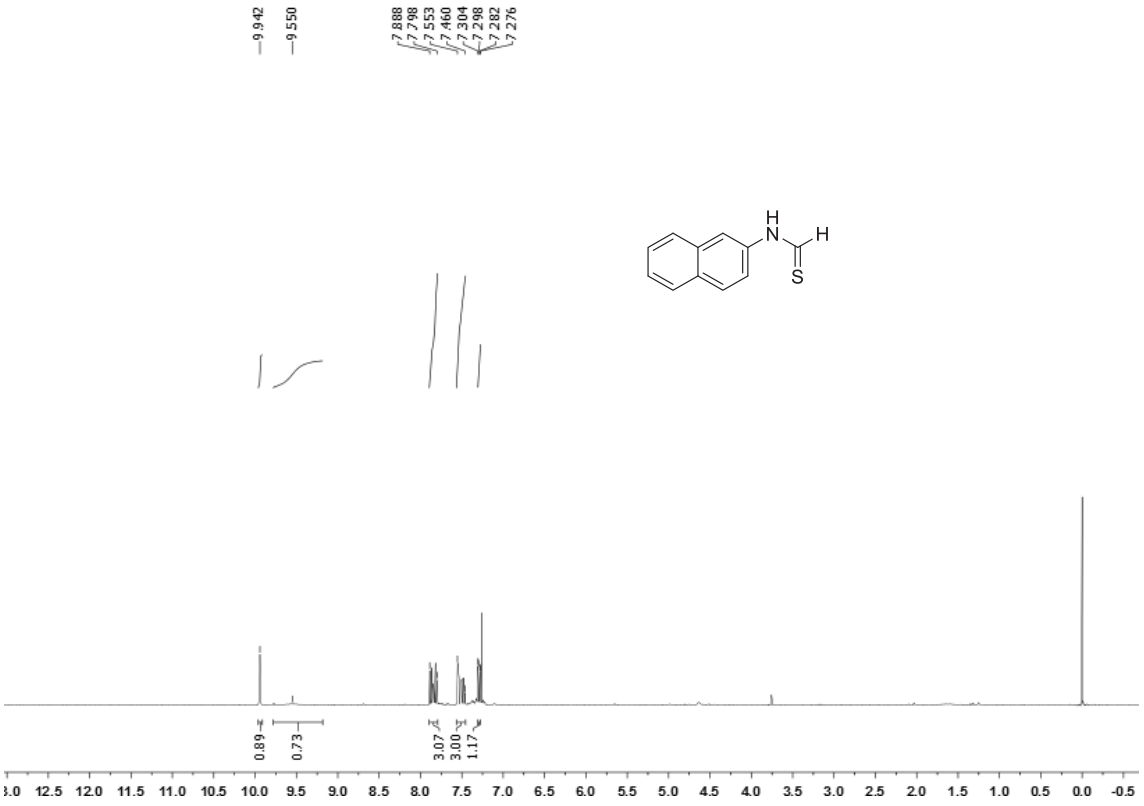

**_N_-Cyclohexylmethanethioamide 2g**






**_N-Tert_-butylmethanethioamide 2h**






***N*-(Naphtalen-2-yl)methanethioamide 2i**