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Degree in Chemistry

TRABAJO DE FIN DE GRADO

Development of protocols towards the synthesis of metabolites derived from Spidoxamat insecticide

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Abbreviations, acronyms and symbols

Ac	Acetyl	**Et**	Ethyl
ACC	Acetyl-coenzyme A carboxylase	**Et_3N**	Triethylamine
ADP	Adenosine diphosphate	**EtOAc**	Ethyl acetate
AIBN	Azobisisobutyronitrile	**equiv**	Equivalent
ATP	Adenosine triphosphate	**FID**	Flame ionization detector
aq.	Aqueous	**FRAC**	Fungicide Resistance Action Committee
BC	Biotin carboxylase	**GC**	Gas chromatography
BCCP	Biotin carboxyl carrier protein	**HPLC**	High Performance Liquid Chromatography
Bn	Benzyl	**HRAC**	Herbicide Resistance Action Committee
br s	Broad signal	**^{i}Pr**	Isopropyl
cat	Catalytic	**IRAC**	Insecticide Resistance Action Committee
CT	Carboxyltransferase	**L**	Ligand
Cy	Cyclohexyl	**LC**	Liquid Chromatography
d	Doublet	**m**	Multiplet
DAD	Diode Array Detector	**M^+**	Molecular ion (MS)
dba	Dibenzylideneacetone	**Me**	Methyl
DBU	1,8-Diazabicyclo [5.4.0]undec-7-ene	**MoA**	Mode of action
DMAc	*N,N*-Dimethylacetamide	**MS**	Mass Spectrometry
DMF	*N,N*-Dimethylformamide	**MSD**	Mass selective detector
DMSO	Dimethylsulfoxide	**MTBE**	Methyl *tert*-butyl ether
ed.	Edition	**m/z**	Mass to charge ratio
Ed(s).	Editor(s)	**NBS**	*N*-Bromosuccinimide
ESI	ElectroSpray Ionization	**NMP**	*N*-Methylpyrrolidone

NMR	Nuclear Magnetic Resonance
***n*-Pr**	Propyl
P_i	Inorganic phosphate
pp	Pages
R	Radical (group)
RT	Room temperature
s	Singlet
t	Triplet
T	Temperature
TBDPS	*tert*-Butyldiphenylsilyl
TBS	*tert*-Butyldimethylsilyl
^{t}Bu	*tert*-Butyl
^{t}BuO	*tert*-Butoxide
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
UV	Ultraviolet

ABSTRACT

The main goal of this work is to synthesize considerably big amounts of three metabolites derived from spidoxamat insecticide for their subsequent use in ecotoxicology studies and environmental risk evaluation. These studies are necessary in the development process of this new active ingredient. For that purpose, synthetic protocols have been designed for the fast and efficient preparation of the desired molecules.

The first metabolite synthesized in this work, spidoxamat-decyclohexylketone, has been prepared through the condensation of an amide with diethyl oxalate which leads to the formation of the maleimide ring present in the molecule. On the other hand, the required amount of the second metabolite, spidoxamat-dechlorohydroxy, has also been obtained thanks to the optimization of the necessary conditions for the palladium-mediated hydroxylation reaction of spidoxamat insecticide to form the product. Finally, the synthesis of spidoxamat-cyclohydroxy-benzylalcohol *(cis)* metabolite has been tackled. A longer pathway has been designed in this case due to the structural complexity of the metabolite, which includes a Dieckmann cyclization to form the tetramic-acid core of the molecule followed by its demethylation to give the product. This procedure has not been selective enough to prepare the desired amount of the metabolite. Nevertheless, alternative pathways have been proposed that could be helpful in future attempts to synthesize the molecule.

RESUMEN

El objetivo de este trabajo es sintetizar cantidades considerablemente grandes de tres metabolitos derivados del insecticida spidoxamato para su posterior uso en estudios ecotoxicológicos y de evaluación de riesgos medioambientales. Dichos estudios son necesarios en el proceso de desarrollo de este nuevo ingrediente activo. Para ello, se ha trabajado en el diseño de protocolos sintéticos para la preparación de estas moléculas de una manera rápida y efectiva.

El primer metabolito sintetizado en este trabajo, spidoxamato-desciclohexilcetona, ha sido preparado mediante la condensación de una amida con oxalato de dietilo para la formación del anillo de maleimida presente en la molécula. Por otro lado, la cantidad requerida del segundo metabolito, spidoxamato-desclorohidroxi, también ha sido obtenida gracias a la optimización de las condiciones necesarias para la hidroxilación del insecticida spidoxamato catalizada por paladio. Finalmente, se ha abordado la síntesis del tercer metabolito, spidoxamato-ciclohidroxi-bencilalcohol *(cis)*, para el cual se ha diseñado un procedimiento algo más largo debido a su complejidad estructural. Este incluye una condensación de Dieckmann para formar el anillo de ácido tetrámico que constituye la molécula y su posterior desmetilación para dar el producto. Este procedimiento ha resultado no ser lo suficientemente selectivo para sintetizar la cantidad deseada del metabolito. Sin embargo, se han propuesto rutas alternativas que podrían ser útiles en futuros intentos de sintetizar esta molécula.

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1. INTRODUCTION

1.1 The Agrochemical Industry

The Agrochemical Industry is a small but increasingly relevant part of the Chemical Industry, focused on the production and distribution of chemical formulations that allow the control of plant diseases, weeds, and pest insects, as well as on the supply of plant nutrients, with the main objective of increasing crop yields in the available farmland.[1] Agrochemical products include, mainly, fertilizers and pesticides such as herbicides, insecticides, and fungicides. Therefore, this industry plays an essential role in modern society as the global population is expected to increase to 9.0 billion inhabitants by 2050, which means that food demand will be doubled, highlighting the importance of the field.[1]

Many factors have been reported to be responsible for the continuous need of research and development in the area of the agrochemical industry; such as changes in the type of crops grown through the years and the introduction of genetically modified crops, what requires the use of new agrochemicals. The resistance of pests to already existing active ingredients is also a key concern in the development of new products. However, the upgrowing worry towards human and environmental safety is one of the most relevant factors in the current agrochemical research.[2]

Aiming for high quality and safe products, with a more favorable toxicological profile, involves a considerable time investment by companies. The work performed by our group is, certainly, a contribution to this part of the research for new Bayer Crop Science active ingredients.

1.2 Heterocyclic crop-protecting active ingredients

Crop-protecting active ingredients or pesticides are chemicals designed to fight target pests during the agricultural production process. As mentioned before, there are different types of pesticides (herbicides, insecticides, and fungicides) that are focused on specific pests. Insecticides kill or inhibit the growth of insects that cause losses in the production. The same is done by herbicides in the case of weeds, and fungicides are dedicated to fight pathogens that can affect plants.[1]

[1] a) Singh, K. N.; Merchant, K. The Agrochemical Industry. In *Handbook of Industrial Chemistry and Biotechnology*, 12th ed.; Kent, J.A., Ed.; Springer Science+Business Media: New York, 2012; Vol. 1, pp 643–698. b) Godfray, H. C. J.; Beddington, J. R.; Crute, I. R.; Haddad, L.; Lawrence, D.; Muir, J. F.; Pretty, J.; Robinson, S.; Thomas, S. M.; Toulmin, C. *Science* **2010**, *327*, 812–818.

[2] Sparks, T. C.; Lorsbach, B. A. *Pest Manag. Sci.* **2017**, *73*, 672–677.

Those active ingredients include a broad spectrum of molecules with very different chemical structures and modes of action (MoA). Herbicides[3], fungicides[4], and insecticides[5] are commonly classified in different groups depending on similarities in their MoA, that is, the way they act on the target pest. This classification is updated annually due to the release of new products to the market. In the case of insecticides, four big families are distinguished as they can act on the nervous, digestive or respiratory system of the insects as well as in their growth or development. Once the exact target site is identified, a more concrete classification can be done. They are currently divided in thirty-four groups (**Figure 1**), with obvious structural similarities between the compounds included in each of them.

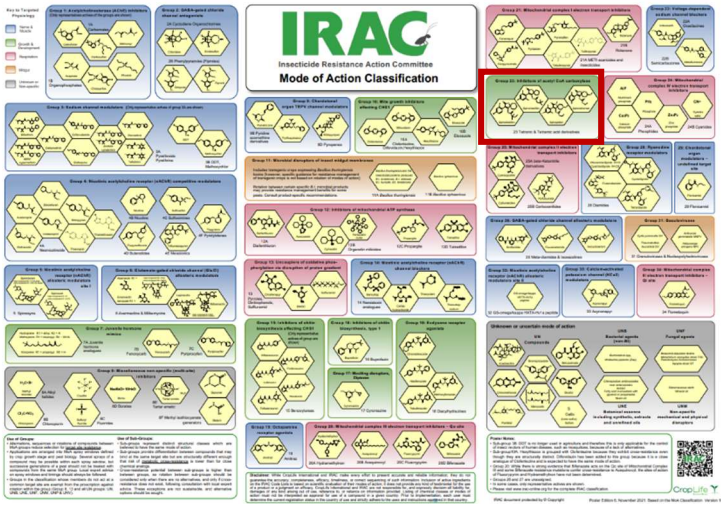


Figure 1. Insecticide Resistance Action Committee (IRAC) Mode of Action classification of insecticides.[5] The insecticides that affect the growth and development of the insects are classified in green. The ones that act on the respiratory system are marked in red, while the ones that affect the nervous system appear in blue. The two groups classified in orange act on the digestive system of the insect and, finally, the insecticides with and unknown or non-specific target system are marked in grey. Group 23, acetyl-CoA-carboxylase inhibitors, is indicated in a red box.

Despite the chemical heterogeneity present in the field, it has been reported that two-thirds of the active ingredients introduced in the past 20 years contained at least one heterocycle in their structure. The benefits provided by this kind of compounds include the synthetic accessibility of multi-substituted heterocycles and their improved physicochemical properties compared to carbacyclic analogs.[6] Important examples of these heterocyclic molecules are the tetramic and tetronic acid derivatives (**Figure 2**), group 23 in the classification of insecticides by their MoA.[5]

[3] HRAC Mode of Action Classification 2021 Map https://hracglobal.com/tools/hrac-mode-of-action-classification-2021-map (accessed Feb 18, 2022).
[4] FRAC | MoA Expert Panel https://www.frac.info/frac-teams/moa-expert-panel (accessed Feb 18, 2022).
[5] Mode of Action Classification | Insecticide Resistance Action Committee (IRAC) https://irac-online.org/mode-of-action/ (accessed Feb 18, 2022).
[6] Lamberth, C. *Pest Manag. Sci.* **2013**, *69*, 1106–1114.

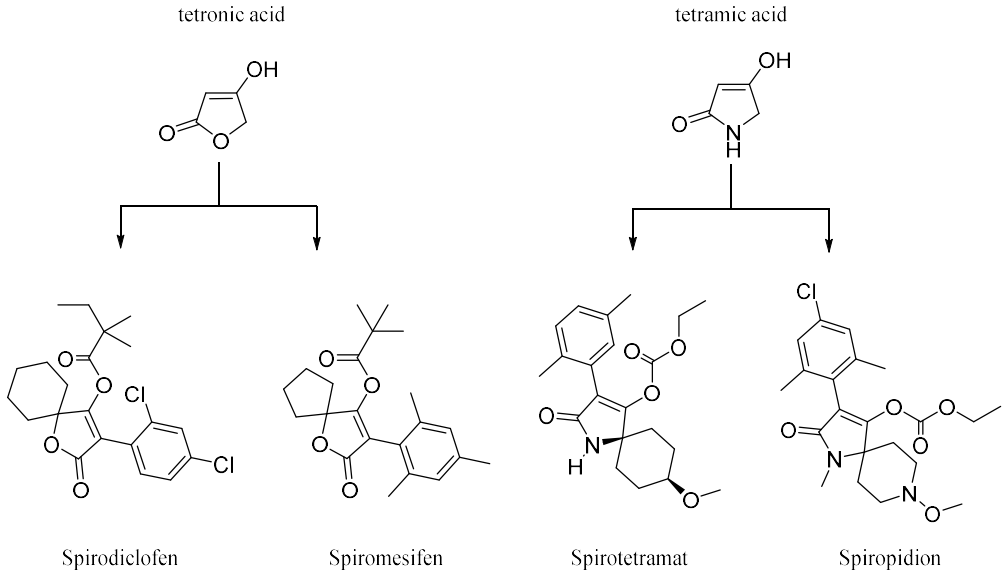


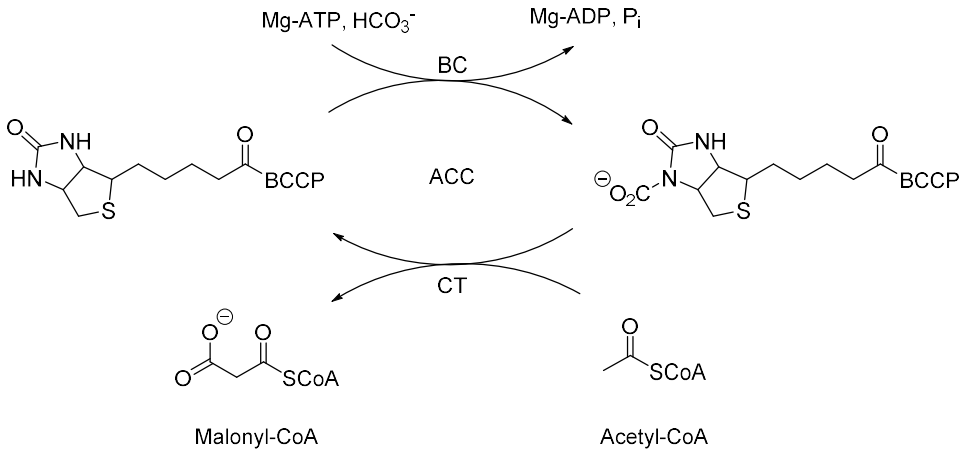
Figure 2. IRAC group 23. Inhibitors of acetyl CoA carboxylase. While spirodiclofen and spiromesifen are tetronic acid derivatives, spirotetramat and spiropidion come from tetramic acid.

These insecticides play a fundamental role in crop-protection industry acting as inhibitors of the acetyl-CoA-carboxylase (ACC) enzyme, which is crucial for the biosynthesis of fatty acids. ACC catalyzes the carboxylation of acetyl-CoA, which leads to malonyl-CoA in a two-step process.[7] First, biotin is carboxylated in the biotin carboxylase (BC) domain, followed by the transference of the carboxyl group to acetyl-CoA, forming malonyl-CoA in the carboxyltransferase (CT) domain (**Scheme 1**). Deep research carried out in the case of spirotetramat[8] (**Figure 2**) showed that the enolic derivative of spirotetramat acts as a mimic of acetyl-CoA, binding to an overlapping site in the CT domain and interfering, that way, in the transference of the carboxyl group to acetyl-CoA. This results in the inhibition of the growth and development of the insect.

This novel mode of action makes this family of insecticides essential in the fight against some pests that have developed resistance against most of the already existing insecticides. Spirodiclofen, the first member of the group, launched in 2002 by Bayer CropScience, showed efficacy against spider mites. Similar activity was detected for spiromesifen, introduced for the first time in 2003, which was also reported to be effective against whiteflies. Later, spirotetramat was discovered, which showed efficacy against a wider spectrum of pests, including aphids and whiteflies.[9,10,11] Spiropidion is the latest registered compound, and it was produced by Syngenta Group. This new active ingredient,

[7] Tong, L. *Cell. Mol. Life Sci.* **2005**, *62*, 1784–1803.
[8] Lümmen, P.; Khajehali, J.; Luther, K.; Van Leeuwen, T. *Insect Biochem. Mol. Biol.* **2014**, *55*, 1–8.
[9] Bretschneider, T; Fischer, R.; Nouen, R. Inhibitors of lipid synthesis (acetyl-CoA-carboxylase inhibitors). In *Modern Crop Protection Compounds*; Krämer, W.; Schirmer, U.; Eds.; WILEY-VCH GmbH & Co. KGaA: Weinheim, Germany, 2007; Vol. 3, pp 909-926.
[10] Marcic, D.; Peric, P.; Petronijevic, S.; Prijovic, M.; Drobnjakovic, T. *Pestic. fitomed.* **2011**, *26*, 185–195.
[11] Bretschneider, T.; Fischer, R.; Nauen, R.; Tetronic Acid Insecticides and Acaricides Inhibiting Acetyl-CoA Carboxylase. In *Bioactive Heterocyclic Compound Classes: Agrochemicals*, 1st ed; Lamberth, C.; Dinges, J., Eds., Wiley-VCH Verlag GmbH & Co. KGaA, 2012; pp 265-278.

containing a spiro *N*-methoxy-piperidine, is also effective against a broad spectrum of pests such as whiteflies, aphids, and mites.[12]



Scheme 1. Carboxylation of Acetyl-CoA catalyzed by ACC. Biotin carboxylation in the biotin carboxylase (BC) domain, followed by the carboxyl transference to acetyl-CoA forming malonyl-CoA in the carboxyl transferase (CT) domain. The second step is competitively inhibited by spirotetramat, which acts as a mimic of acetyl-CoA.

Nowadays, a new candidate is being studied by Bayer Crop Science for IRAC group 23, which is composed by cyclic keto-enols. This new active ingredient is called spidoxamat and it is another tetramic-acid derivative expected to bring huge benefits to the market (**Figure 3**). The work that will be described throughout the following sections is, essentially, a contribution to the development of the product with the goal of achieving a high-quality and safe compound.

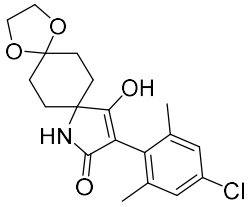


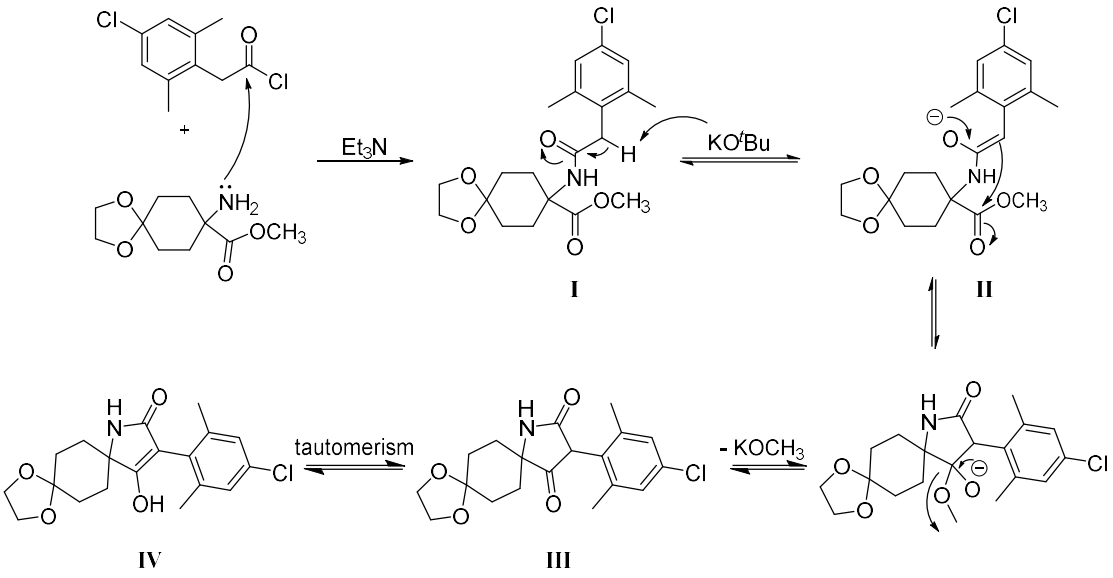
Figure 3. Chemical structure of the tetramic-acid-derived spidoxamat.

A common key step in the synthesis of all these tetramic-acid-derived cyclic keto-enols consists of an amide coupling followed by a Dieckmann cyclization that enables the formation of the tetramic ring.[9] This crucial process is exemplified in **Scheme 2** using spidoxamat.[13] The coupling between the amine and the acid chloride assisted by Et_3N generates amide **I**. Afterwards, a proton is abstracted by

[12] Muehlebach, M., et al. Spiropidion discovery: Broad spectrum control of sucking insects and mites for multicrop utility. In *Recent Highlights in the Discovery and Optimization of Crop Protection Products*. Maienfisch, P.; Mangelinckx, S.; Eds.; Academic Press; 2021; pp 241-260.

[13] Himmler, T.; Bruechner, P.; Lindner, W.; Hahn, J. J.; Moradi, W. A.; Fischer, R.; Dockner, M. Process for Preparing Substituted Cyclohexane Amino Acid Esters and Spiroketal-Substituted Cyclic Keto-Enols. US 2021/0032262 Al, February 4, 2021.

the base (KO^{t}Bu) from the α position to the carbonyl group and intermediate **II** is formed. A nucleophilic attack on the carbonyl group of the ester followed by the loss of methanol results in the cyclized product **III**. The keto-enol tautomeric equilibrium of the final product is highly displaced towards the enol compound **IV** due to conjugation with the aromatic ring and the carbonyl group from the amide.

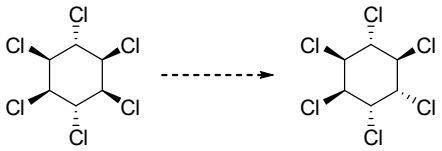


Scheme 2. Mechanism of the Dieckmann cyclization with KO^{t}Bu, key step in the synthesis of spidoxamat.

1.3 Environmental degradation of the active ingredients. Formation of metabolites

Once applied to the environment, pesticides undergo a series of biotic and abiotic transformations, resulting in significant amounts of metabolites and degradation products respectively. In the case of transformation products that represent more than 10% of the amount of the active ingredient, environmental studies are required.[14] The increasing concern towards human and environmental safety in the development of new products has led companies to carry out a more in-depth risk assessment of these degradation products. The toxicity of the metabolites is in most cases lower than that of the parent active ingredient, but this is not a general rule, and in some cases, they can be more toxic. An example of this is α-HCH, a transformation product of the organochlorine insecticide lindane (γ-HCH) (**Scheme 3**).[15] The use of this insecticide is currently very restricted.

[14] Sinclair, C. J.; Boxall, A. B. A. *Environ. Sci. Technol.* **2003**, *37*, 4617–4625.
[15] Andreu, V.; Picó, Y. *Trends Analyt. Chem*. **2004**, *23*, 772–789.



Lindane α-HCH

Scheme 3. Structure of lindane and its tranformation product α-HCH.

Degradation processes of active ingredients include a broad spectrum of chemical abiotic reactions and biotic transformations that can vary significantly from one pesticide to another depending mainly on their chemical structure and their likeliness to undergo certain kinds of reactions. A general scheme of the most frequently studied processes is presented in **Figure 4.**

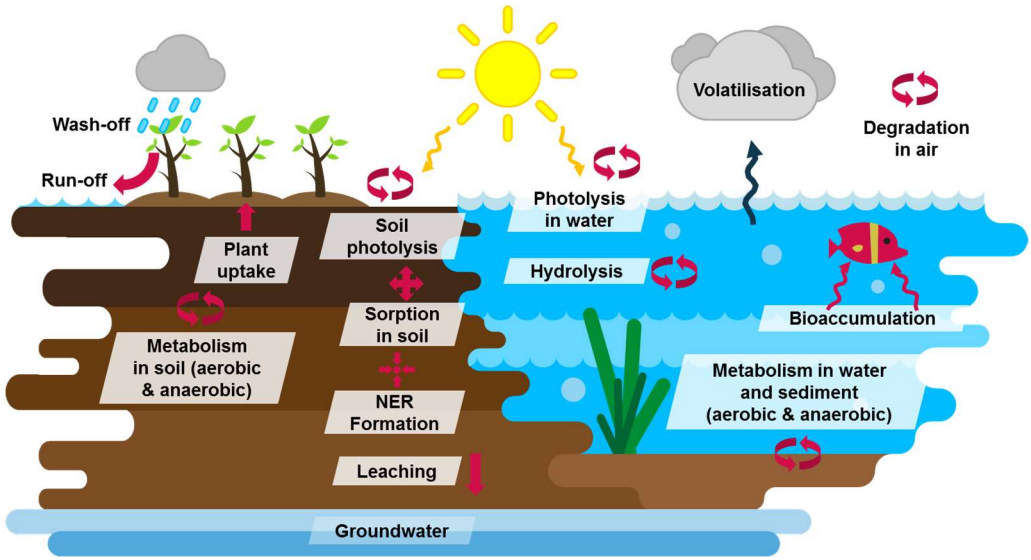


Figure 4. Environmental degradation processes.

Once pesticides are applied, they may undergo several mobility processes through the environment, being transported through the air (volatilization), water (wash-off, run-off) and soil (leaching). The likeliness of active ingredients to undergo one or many of these processes strongly depends on the properties of each compound and the transporting media. Besides, it must be taken into account that pesticides can be uptaken by plants and animals, what can lead to metabolic degradation.[16]

Simultaneously to their movement through the environment, pesticides undergo breakdown and decomposition processes, being these ones closely related to their location. Among the degradation events commonly observed, two important examples are photolysis and hydrolysis. The first one can occur when certain compounds are exposed to sunlight either on water or soil surfaces. Regarding the latter, it happens when pesticides are in water solution. Molecules that contain sensitive functional

[16] Fenner, K.; Canonica, S.; Wackett, L. P.; Elsner, M. *Science* **2013**, *341*, 752–758.

groups, such as esters, are more prone to suffer this kind of transformation. Active ingredients that are within the soil or located in sediments of the aquatic system tend to suffer biotic transformations to form metabolites. Microorganisms are crucial in biotic degradation, as they metabolize the active ingredients as food supply, sometimes through enzymatic reactions.[16]

Monitoring all these processes and evaluating the risk of the degradation products requires considerable work from companies. In Bayer Crop Science this research is divided in two main sections: Human Safety and Environmental Fate. In the Environmental Fate section all of the above mentioned (**Figure 4**) environmental abiotic transformations are investigated for a certain active ingredient, whereas Human Safety is focused on plant and animal metabolism in an attempt to keep track of the metabolites that could be harmful in case of human exposure. In both cases, the working procedure involves identification and structure elucidation, quantification and degradation-rate determination, investigation of the hazardousness for specific species and, finally, risk evaluation.

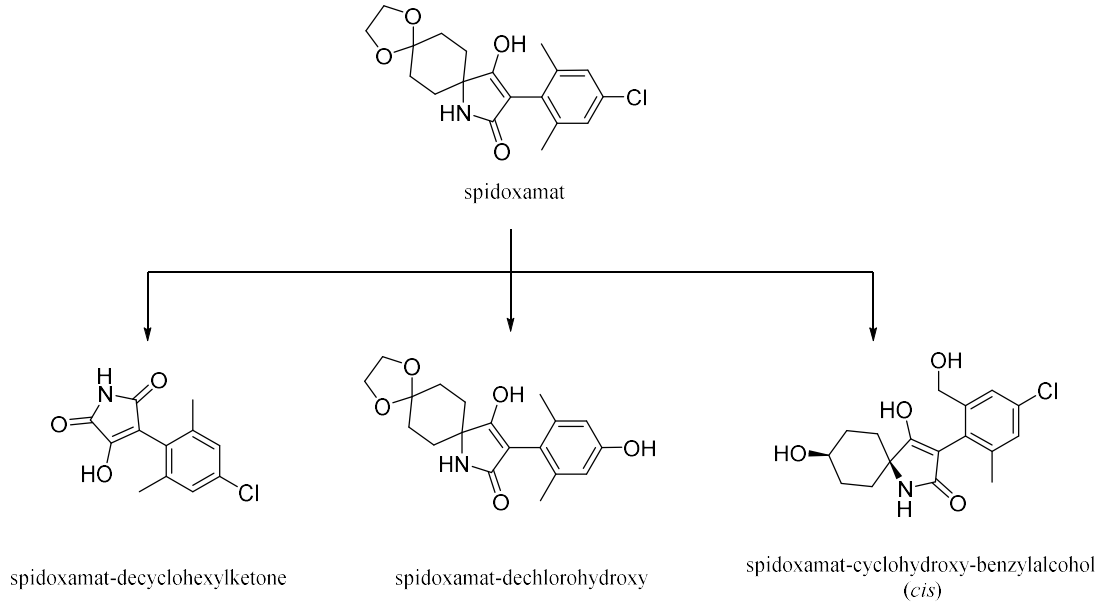
Radiolabels are used for the determination of the whole degradation pathway of a certain active ingredient in a complex environment. ^{14}C labels are placed in different parts of the parent molecule: in three parts in case of studying plant and animal metabolism and in four for environmental transformation studies. This way, the degradation pathways can be determined by following the radio signal. The general scheme followed in both of the investigations mentioned above is usually common: the labeled active ingredient is applied and after incubation and extraction of the formed transformation products, HPLC analysis of the extracted material is carried out for determination of the remaining active ingredient and the ratio of the formed products. Additionally, their structure is then elucidated using NMR and HPLC-MS/MS.

Once the major metabolites are found and their structure is determined, they are subjected to a series of ecotoxicology studies for risk evaluation. These studies require to use considerably big amounts of the metabolites being analyzed and, although they might have been classified as major metabolites (they represent more than 10% of the amount of the active ingredient), it is not possible to extract the required quantity of those molecules from the environment for this purpose. Therefore, they have to be prepared in the laboratory and that is why synthetic organic chemistry plays such a fundamental role in the field.

1.4 Transformation products of the Spidoxamat insecticide

In the case of the previously mentioned spidoxamat insecticide (**Figure 3**), several transformation products have been identified and are under evaluation. In **Scheme 4** these important metabolites derived from the mentioned insecticide can be observed. Spidoxamat-decyclohexylketone metabolite is formed from the active ingredient by the loss of the cyclohexane ring and formation of a carbonyl group in that position. The second metabolite, spidoxamat-dechlorohydroxy, is probably a direct aqueous photolysis transformation product of the active ingredient, where the chlorine substituent of

the phenyl group is replaced with a hydroxy group. Regarding spidoxamat-cyclohydroxy-benzylalcohol *(cis)* metabolite, detected in plant metabolism studies, a several-step transformation from the active ingredient occurs, including the loss of the acetal protecting group and the hydroxylation of one of the methyl groups of the phenyl ring.

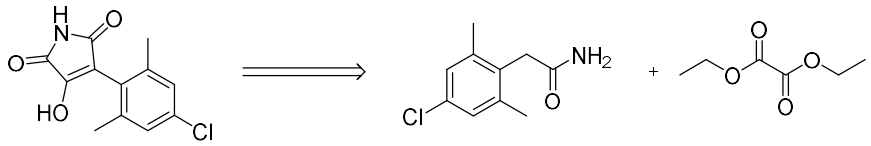


Scheme 4. Three metabolites identified for the spidoxamat insecticide.

2. OBJECTIVES

The main goal of this work was to design and develop synthetic protocols for the preparation of the three metabolites derived from spidoxamat insecticide that have been already mentioned in the previous section (**Scheme 4**). These molecules had to be prepared in considerably big amounts for their subsequent use in ecotoxicology studies and as analytical standards in Environmental Fate studies.

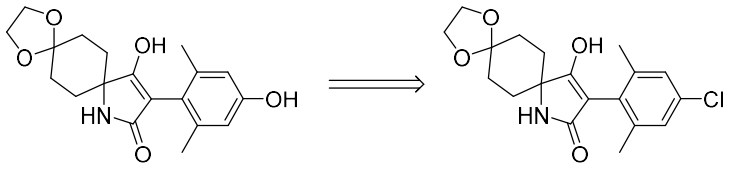
Firstly, it was proposed that spidoxamat-decyclohexylketone metabolite could be prepared starting from amide **V** and diethyl oxalate, whose condensation would lead to the formation of the maleimide ring (**Scheme 5**).



spidoxamat-decyclohexylketone **V**

Scheme 5. Retrosynthetic proposal for the preparation of spidoxamat-decyclohexylketone metabolite.

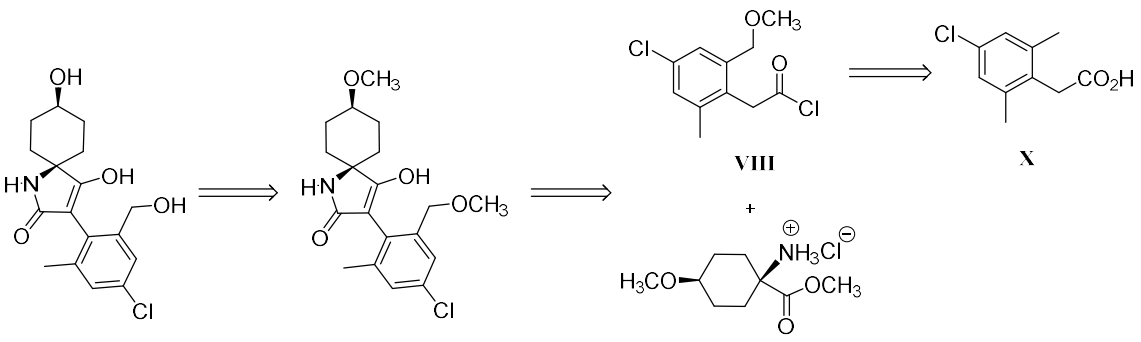
For spidoxamat-dechlorohydroxy metabolite, the viability of accomplishing a single-step synthesis *via* a palladium-mediated hydroxylation reaction was studied using the corresponding aryl chloride **VI** (spidoxamat) as the substrate (**Scheme 6**).



spidoxamat-dechlorohydroxy **VI**

Scheme 6. Retrosynthetic proposal for the preparation of spidoxamat-dechlorohydroxy metabolite.

Last but not least, it was proposed that spidoxamat-cyclohydroxy-benzylalcohol *(cis)* metabolite could be prepared *via* demethylation of compound **VII**, whose tetramic-acid core can be formed by means of the condensation between acid chloride **VIII** and amine **IX**. The former was hypothesized to be obtained from acid **X** (**Scheme 7**).

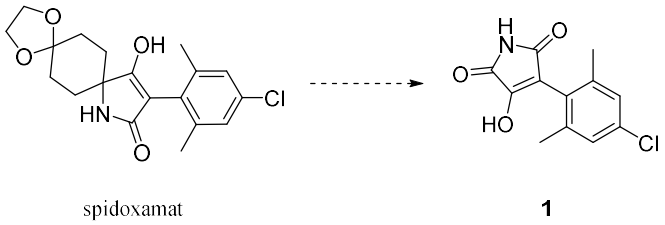


Scheme 7. Retrosynthetic proposal for the preparation of spidoxamat-cyclohydroxy-benzylalcohol *(cis)* metabolite.

3. RESULTS AND DISCUSSION

3.1 Design of a synthetic pathway for the preparation of Spidoxamat-decyclohexylketone metabolite 1

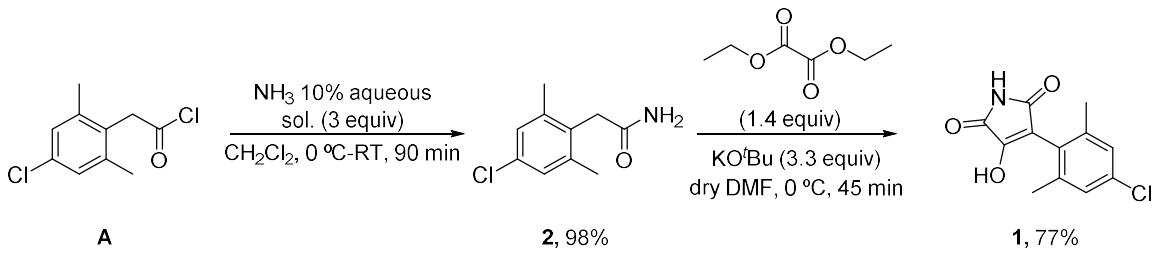
As it has been stated in the previous section, the first objective of the present work was to design an efficient methodology for the preparation of spidoxamat-decyclohexylketone metabolite **1** (**Scheme 8**).



spidoxamat **1**

Scheme 8. Spidoxamat-decyclohexylketone metabolite **1** derived from spidoxamat insecticide.

For that purpose, a two-step synthesis was proposed as indicated in **Scheme 9**, which involved the formation of an amide followed by its reaction with diethyl oxalate, that leads to the construction of the maleimide ring. Acid chloride **A** was reacted with ammonia to obtain amide **2** in very high yields. This procedure was successfully applied in a large scale to obtain more than 100 grams of the amide, for its subsequent use in the synthesis of big amounts of metabolite **1**.



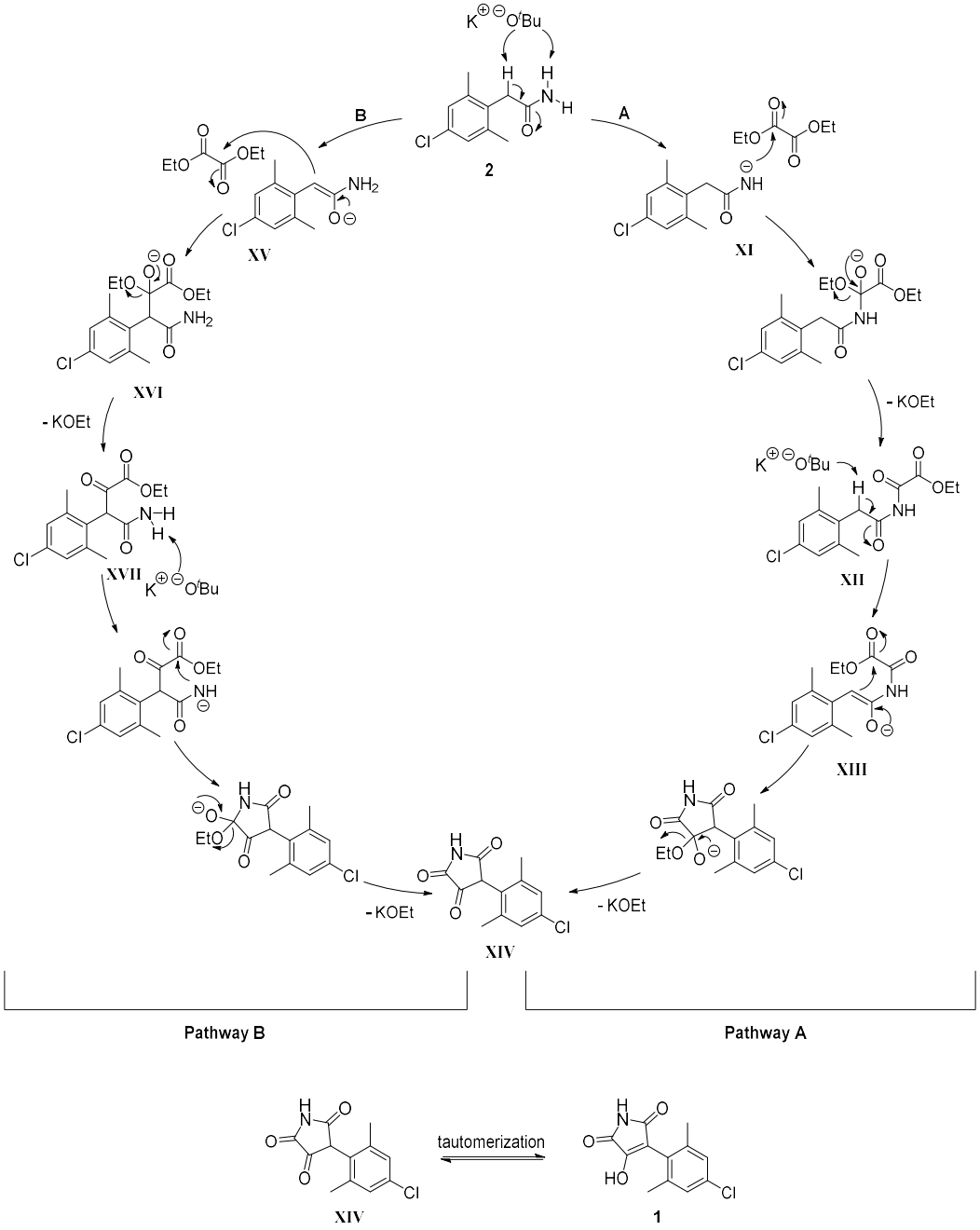
A **2**, 98% **1**, 77%

Scheme 9. Synthetic procedure for the preparation of compund **1**.

For the synthesis of maleimide **1** from amide **2**, conditions already reported in the literature for similar compounds were applied,[17] consisting of the KO^{t}Bu-promoted condensation of **2** with diethyl oxalate (**Scheme 9**). This reaction is proposed to follow the mechanism depicted in **Scheme 10**. First, N-H deprotonation of **2** takes place to form anionic species **XI**, which would undergo a nucleophilic attack towards diethyl oxalate, forming **XII** after release of ethoxide. Deprotonation of the other acidic proton would form enolate **XIII**, inducing cyclization *via* intramolecular nucleophilic

[17] Arnould, J.; Harris, C. S.; Boyle, F. T.; Gibson, K. H. 3,4-Disubstituted Maleimides for Use as Vascular Damaging Agents. WO/2005/102997 A1, November 3, 2005.

attack. This would lead to **XIV** after the loss of ethoxide. Tautomerization of this compound would generate the product (**Scheme 10, pathway A**).



Scheme 10. Mechanism proposal for the maleimide ring formation.

Nevertheless, it must be taken into account that both acid protons present in amide **2** (the one attached to the nitrogen and the one bound to the α-carbon) have a similar pKa (pKa~25.5 for the first one and pKa~26.6 in the case of the second one).[18] Due to this fact, a mechanism involving α-C-H deprotonation in the first step cannot be ruled out.

[18] Ripin, D. H.; Evans, D. A. pKa Table. http://evans.rc.fas. harvard.edu/pdf/evans_pKa_table.pdf (accessed March 22, 2022).

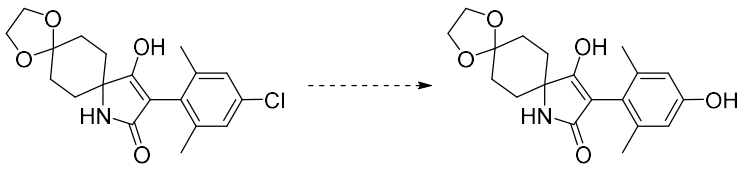
In this mechanistic pathway, enolate **XV** would carry out a nucleophilic attack to diethyl oxalate, forming **XVI**, which after extrusion of ethoxide, would lead to the formation of **XVII**. The subsequent deprotonation of the amide would promote the cyclization process leading to **XIV** after release of ethoxide (**Scheme 10, pathway B**).

Dry conditions were determined to be essential to ensure a good performance of the base and, thus, a complete cyclization. Otherwise, the reaction did not proceed satisfactorily, detecting signals that could be attributed to intermediates **XII** and **XVII** (**Scheme 10**) *via* mass spectrometry.

The optimized reaction conditions were then applied in a larger scale to succesfully obtain the desired amount of the metabolite with high purity, and its structure was confirmed by ^{13}C and ^{1}H-NMR spectroscopy. Due to the exorthermic character of the reaction, it was repeated several times in a 35-gram scale instead of carrying out a single 100-gram-scale reaction.

3.2 Design of a synthetic pathway for the preparation of Spidoxamat-dechlorohydroxy metabolite 3

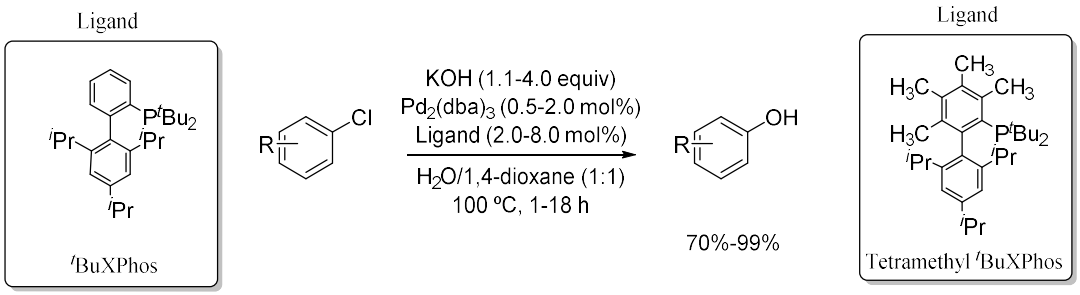
As it has been previously stated, the second objective of this work was to synthesize spidoxamat-dechlorohydroxy metabolite **3** (**Scheme 11**). It was hypothesized that this compound could be prepared in a single step through a Pd-catalyzed hydroxylation reaction of spidoxamat. This cross-coupling transformation, also known as the Buchwald-Hartwig reaction, has been extensively used in synthesis for C-N and C-O bond formation, allowing arylation of amines and alcohols employing aryl halides as coupling partners under palladium catalysis.[19]



Scheme 11. Spidoxamat-dechlorohydroxy metabolite **3** derived from spidoxamat insecticide.

In 2006, Buchwald and co-workers reported a methodology for the palladium-catalyzed hydroxylation of several aromatic halides using KOH as hydroxide source. This reaction proved to be efficient for the functionalization of aryl chlorides (**Scheme 12**).[20]

[19] Miyaura, N.; Buchwald, S. L.; Al, E. *Cross-Coupling Reactions: A Practical Guide*; Springer: Berlin, 2002; pp 131–209.
[20] Anderson, K. W.; Ikawa, T.; Tundel, R. E.; Buchwald, S. L. *J. Am. Chem. Soc.* **2006**, *128*, 10694-10695.



Scheme 12. General conditions reported in the literature.

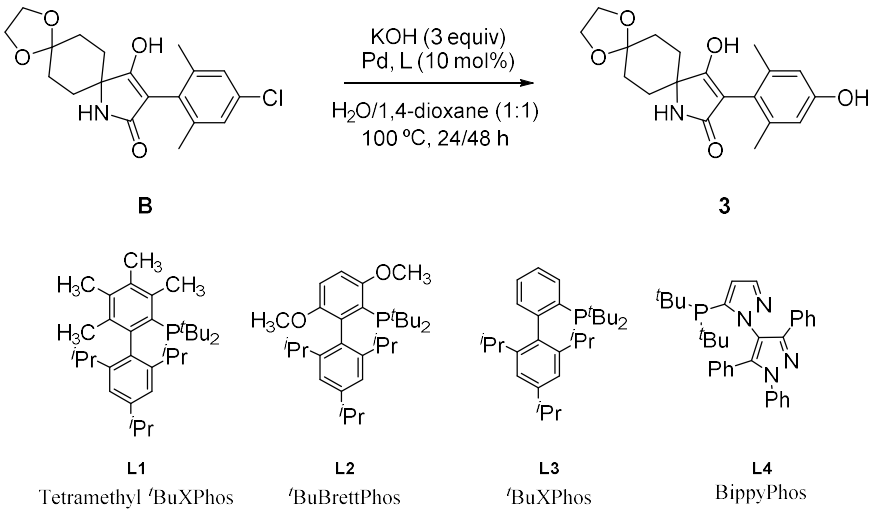
Bearing in mind that spidoxamat is essentially an aryl chloride it was decided to take the conditions reported as starting point for the synthesis of **3** using spidoxamat **B** as substrate (**Table 1**). Different palladium sources, commonly applied in this kind of cross-coupling reactions, were studied, in combination with four different phosphine ligands (**Table 1**).

As it can be observed in **Table 1**, while $Pd_2(dba)_3$ (Table 1, Entries 1-4) and $[Pd(cinnamyl)Cl]_2$ (Table 1, Entries 9-12) showed formation of the desired product, $Pd(OAc)_2$ (Table 1, entries 5-8) and $PdCl_2(CH_3CN)_2$ (Table 1, entries 13-16) failed to promote the reaction regardless of the ligand used.

In the case of $Pd_2(dba)_3$, poor results were observed employing **L3** and **L4** (Table 1, Entries 3 and 4, respectively). However, when **L1** and **L2** were used (Table 1, Entries 1 and 2, respectively), the outcome of the reaction improved dramaticaly, obtaining 77 area% of the product with **L2** after 48 h, which was the best result (Table 1, Entry 2).

For dimer $[Pd(cinnamyl)Cl]_2$, moderate yields were obtained in all the cases, observing the best result, 49 area% of the product, after 24 h when **L1** was employed (Table 1, Entry 9).

Table 1. Optimization of the reaction conditions for the synthesis of **3** starting from **B**.[a]

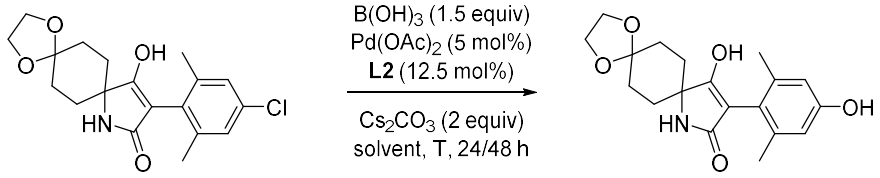


Entry	Catalyst	Ligand	Product % (HPLC)[b,c]
1	$Pd_2(dba)_3$	L1	60 (70)
2	$Pd_2(dba)_3$	L2	69 (77)
3	$Pd_2(dba)_3$	L3	17 (20)
4	$Pd_2(dba)_3$	L4	22 (23)
5	$Pd(OAc)_2$	L1	-
6	$Pd(OAc)_2$	L2	-
7	$Pd(OAc)_2$	L3	-
8	$Pd(OAc)_2$	L4	2 (2)
9	$[Pd(cinnamyl)Cl]_2$	L1	49 (33)
10	$[Pd(cinnamyl)Cl]_2$	L2	20 (31)
11	$[Pd(cinnamyl)Cl]_2$	L3	27 (28)
12	$[Pd(cinnamyl)Cl]_2$	L4	35 (39)
13	$PdCl_2(CH_3CN)_2$	L1	-
14	$PdCl_2(CH_3CN)_2$	L2	-
15	$PdCl_2(CH_3CN)_2$	L3	-
16	$PdCl_2(CH_3CN)_2$	L4	1 (1)

[a] Reaction conditions: **B** (0.3 mmol, 1 equiv), $Pd_2(dba)_3$ and $[Pd(cinnamyl)Cl]_2$ (2.5 mol%), $Pd(OAc)_2$ and $PdCl_2(CH_3CN)_2$ (5 mol%), ligand (10 mol%), KOH (3 equiv), H_2O/1,4-dioxane 1:1 (2 mL), 100 ºC, Ar atmosphere. [b] Determined by area% of the desired product, identified by mass spectrometry, in the HPLC-MS chromatogram of the crude reaction after 24 h. Area% of the desired product = (area of product × 100)/(area of unreacted **B** + area of product + area of other product related peaks) [c] In parentheses the percentage after 48 h of reaction.

With the aim of getting higher yields, other conditions found in the literature were tried.[21] In this case, $B(OH)_3$ was used as hydroxide source (1.5 equiv) in combination with Cs_2CO_3 as the base. $Pd(OAc)_2$ was used as the catalyst together with ^{t}BuBrettPhos **L2**. Different solvents were screened (**Table 2**).

Table 2. Solvent screening for the Pd-catalyzed hydroxylation of **B** with $B(OH)_3$ as Hydroxyde source.[a]



B 3

Entry	Solvent	T (ºC)	Product % (HPLC)[b, c]
1	DMF	100	2 (3)
2	THF	80	-
3	NMP	100	1 (1)

[a] Reaction conditions: **B** (3 mmol, 1 equiv), $Pd(OAc)_2$ (5 mol%), ^{t}BuBrettPhos (12.5 mol%), $B(OH)_3$ (1.5 equiv), Cs_2CO_3 (2 equiv), solvent (40 mL), 100 ºC, Ar atmosphere. [b] Determined by area% of the desired product, identified by mass spectrometry, in the HPLC-MS chromatogram of the crude reaction after 24 h. Area% of the desired product = (area of product × 100)/(area of unreacted **B** + area of product + area of other product related peaks) [c] In parentheses the percentage after 48 h of reaction.

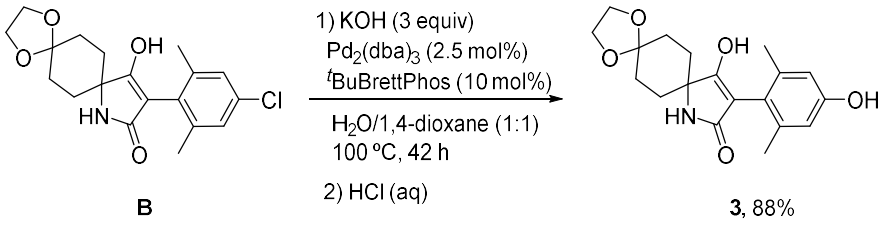
Due to the unsatisfactory results shown in **Table 2** the conditions described in Table 1, Entry 2 were selected as the optimal ones and applied to the large-scale synthesis of **3** starting form **B**.[22]

Once the reaction condictions had been selected, the next challenge was to find the best way to carry out the isolation of the product and the removal of the palladium impurities. Filtration with celite was first tried, however, big loss of product was detected due to its high insolubility at room temperature. Therefore, this method was dismissed, as well as column chromatography, because of the difficulties in dissolving the product in all the eluents tried.

After some studies, it was detected that the biphasic character of the reaction (a 1:1 mixture of dioxane and H_2O was used as solvent) could help in the isolation, as the product was mainly dissolved in the dioxane phase, whereas the rest of the impurities were contained in the aqueous phase. Separation of both phases before cooling down the reaction and precipitation of the product from the organic phase by addition of diluted aqueous HCl, resulted in the effective isolation of **3** with high

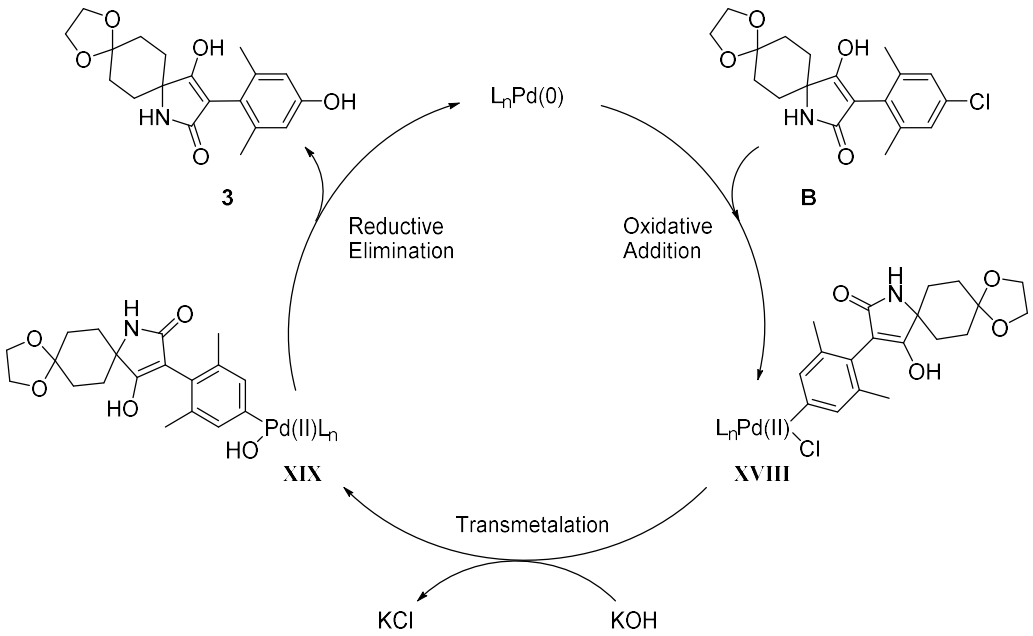
[21] Song, Z.-Q.; Wang, D.-H. *Org. Lett.* **2020**, *22*, 8470–8474.
[22] The response factors for the products and starting material in HPLC-MS are not considered when reporting the area% of the product. The yields reported as area% of product are not quantitative but still are good indicators for a fast qualitative evaluation of the conditions.

yield and without the need of further purification. After the optimization of the whole reaction procedure, it was applied to afford 25 g of the product in 88% yield (**Scheme 13**).



Scheme 13. Conditions for the synthesis of metabolite **3**.

A proposal of the mechanism that takes place in this reaction is shown in **Scheme 14**. First, the oxidative addition of the aryl halide **B** to the Pd(0) complex occurs, to give Pd(II) complex **XVIII**. The transmetalation between **XVIII** and KOH would provide species **XIX**, which undergoes a reductive elimination affording the phenolic metabolite **3** and regenerating the catalytically active species.



Scheme 14. Proposed mechanism for the Pd-catalyzed hydroxylation of spidoxamat **B** with KOH, leading to metabolite **3**.

The spirocyclic structure of product **3** gives an interesting pattern of four multiplets between 1 and 3 ppm that correspond to the protons attached to the cyclohexane ring. First of all, it is important to highlight that this molecule is completely symmetrical, so we just consider half of it to describe the coupling pattern. **Figure 5** shows how axial and equatorial protons attached to a same carbon in the cyclohexane ring are surrounded by different chemical environments. This explains the non-equivalence between those protons attached to a same carbon atom and the resulting multiplet pattern. Each of the hydrogen atoms (H_1, H_2, H_3 and H_4) is responsible for one of the signals when it couples to

the geminal proton and to each of the vicinal ones. This coupling results, in this case, in two doublets and two triplets of doublets.

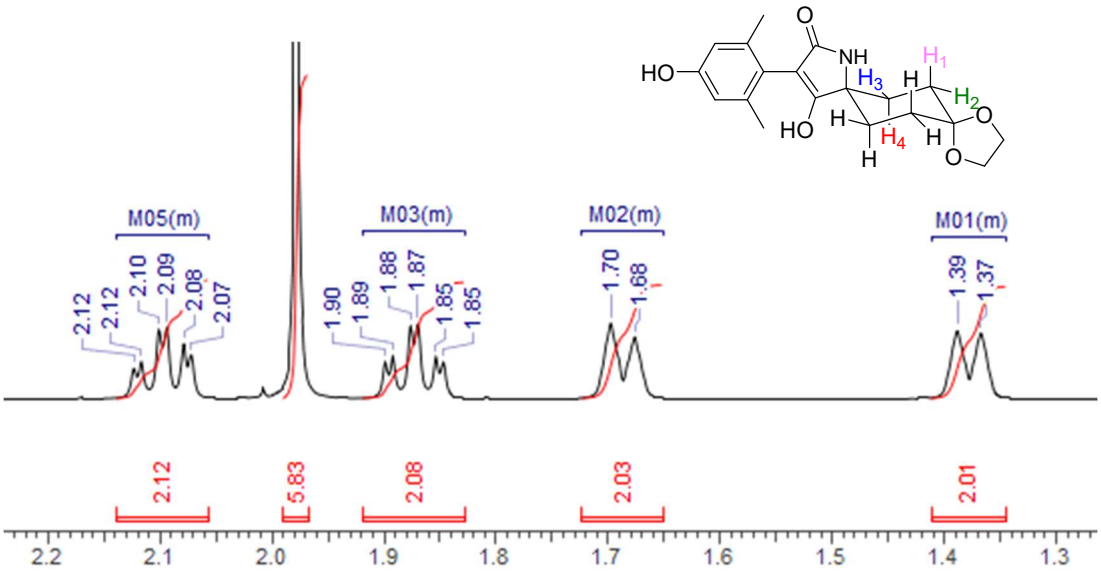
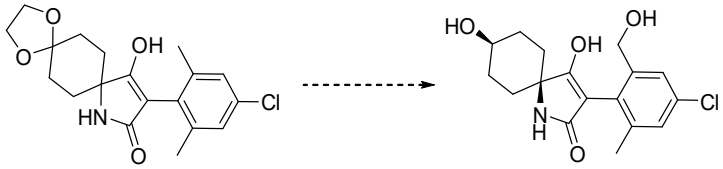


Figure 5. Multiplet pattern in ^{1}H-NMR of molecule **3**.

3.3 Design of a synthetic pathway for the preparation of Spidoxamat-cyclohydroxy-benzylalochol (*cis*) metabolite 4

Once the previous metabolites had been efficiently prepared, the synthesis of spidoxamat-cyclohydroxy-benzylalochol (*cis*) **4** was tackled (**Scheme 15**).

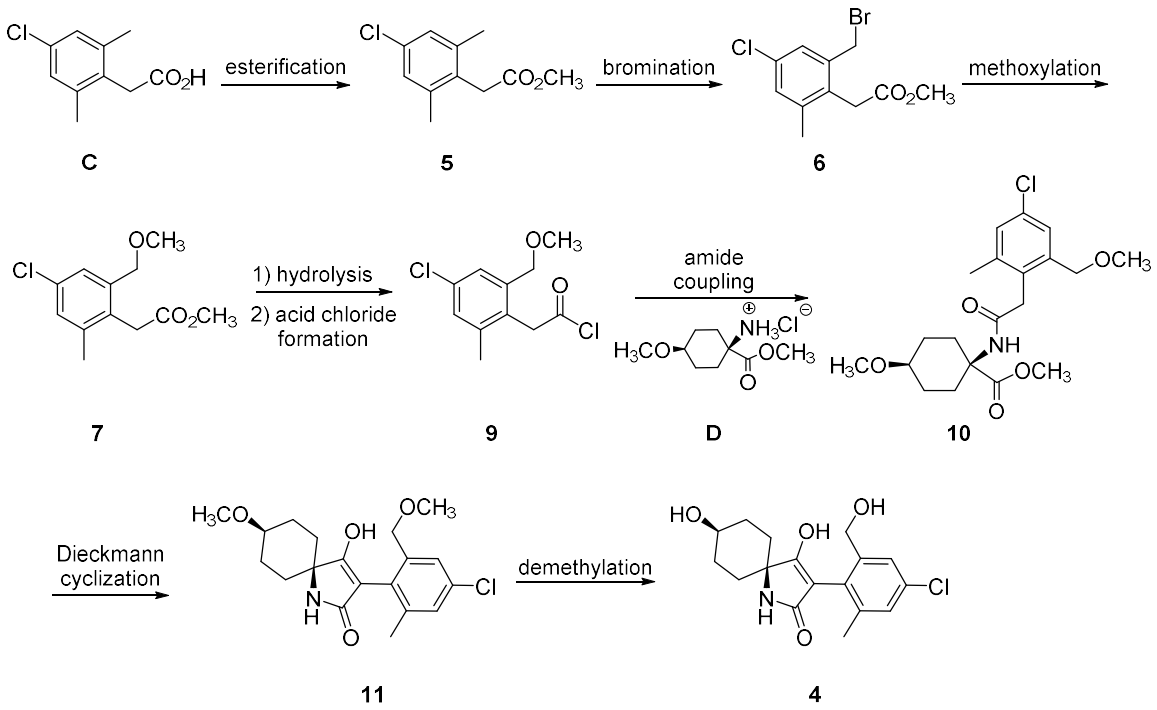


spidoxamat **4**

Scheme 15. Spidoxamat-cyclohydroxy-benzylalochol (*cis*) metabolite **4** derived from spidoxamat insecticide.

As it can be observed in **Scheme 15**, metabolite **4** is a product of the hydroxylation of one of the methyl substituents of the phenyl ring and it also contains a hydroxy substituent on the cyclohexane ring. These two alcohols need to be protected throughout the synthetic process owing to possible incompatibilities with the use of bases in the generation of the tetramic acid ring.

Due to the structural complexity of this compound, a synthetic pathway that was more complex than the ones mentioned before throughout this section had to be designed, starting from acid **C** (**Scheme 16**).

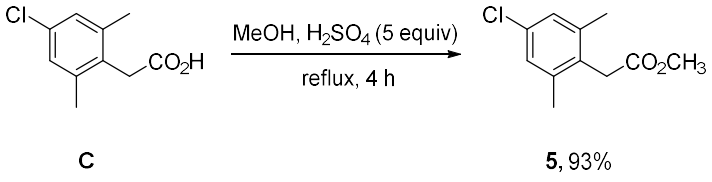


Scheme 16. Prorposed synthetic pathway for the synthesis of metabolite **4**.

With the aim of proving that the designed plan was acceptable for the synthesis of target molecule **4**, the conditions to prepare each of the proposed intermediates (**Scheme 16, Molecules 5-11**) were selected and optimized.

3.3.1 Esterification of phenylacetic acid C. Synthesis of ester 5

Carboxylic acid **C** was protected by esterification with methanol following the conditions indicated in **Scheme 17**. 77 g of ester **5** were obtained with 93% yield and high purity.



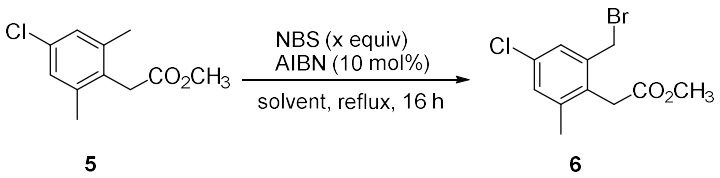
Scheme 17. Synthesis of the ester **5**.

3.3.2 Methoxylation of ester 5. Formation of methyl ether 7

For the introduction of the methoxy substituent in one of the methyl groups of molecule **5**, the desired position was first activated by bromination. NBS was selected as the brominating agent, combined with AIBN, which was observed to be a more effective radical initiator than benzoyl peroxide for this purpose, since the latter provided low conversion of the desired product. Ester **5** has three different benzylic positions that can be brominated in this reaction. The one α to the ester group is hindered and only has two hydrogen atoms, what makes bromination in this position less likely to

take place. The reaction conditions were optimized with the aim of obtaining the product **6** in high yields and avoiding the bromination of both methyl groups. The effect of the temperature, the amount of NBS and the solvent were studied for that purpose (**Table 3**).

Table 3. Screening of the conditions for the bromination reaction.[a]



5 **6**

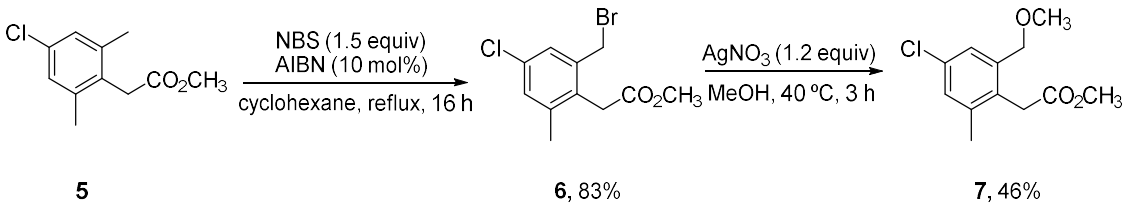
Entry	NBS Equiv	Solvent	Product (%)[b]
1	1	cyclohexane	62
2	1.5	cyclohexane	71
3	1	methylcyclohexane	45

[a] Reaction conditions: **5** (3 mmol, 1 equiv), NBS, AIBN (10 mol%), solvent (20 mL), reflux, 16 h. [b] Area% of the desired product in the HPLC chromatogram of the crude mixture.

First of all, the outcome of the reaction was studied using 1 equivalent of NBS in cyclohexane[23] (Table 3, Entry 1). Those reaction conditions resulted in a high amount of remaining starting material after 16 h of reaction. With the aim of increasing the conversion towards the desired product, an excess of NBS (1.5 equiv) was added (Table 3, Entry 2) what led to a satisfactory result, but also to an increased amount of the dibrominated product. The effect of the temperature was also investigated by using methylcyclohexane as solvent, which has a higher boiling point than cyclohexane (Table 3, Entry 3). Nevertheless, it did not seem to be an appropriate solvent for this reaction.

Therefore, the conditions in Table 3, Entry 2 were determined to be the most suitable ones for the reaction, obtaining a 7/2/1 ratio of the desired product, remaining starting material and dibrominated byproduct respectively. Once applied to a bigger scale, higher selectivities towards the desired product were seen, with a 8/1/1 ratio in this case (**Scheme 18**). The brominated product **6**, was used for the next step without further purification due to difficulties in the separation of the non-reacted starting material and the dibrominated byproduct from the desired ester **6**. 81.3 g of the mixture were obatined with 72% HPLC purity of **6**. The yield of the reaction was calculated based on the HPLC purity of the desired product.

[23] Page, P. C. B.; Buckley, B. R.; Farah, M. M.; Blacker, A. J. *Eur. J. Org. Chem.* **2009**, 3413–3426.

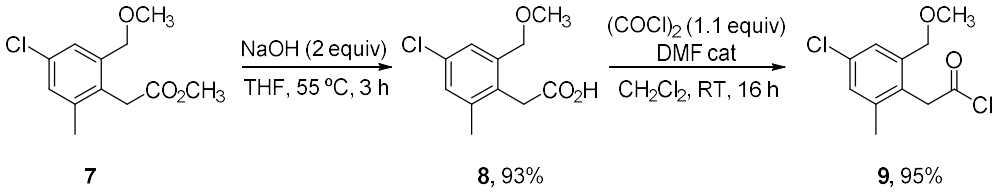


Scheme 18. Bromination of **5** and methoxylation of **6**. Synthesis of ester **7**.

Molecule **6** was then methoxylated by subjecting it to $AgNO_3$ in methanol. For the purification of this compound, distillation was selected as the optimal method. Methyl ether **7** was isolated with a 46% yield.

3.3.3 Formation of acid chloride 9 starting from phenylacetate 7

Once intermediate **7** had been synthesized, it had to be converted into an acid chloride to proceed with the formation of the tetramic acid ring. Hydrolisis of ester **7** with NaOH in THF was first carried out, obtaining carboxylic acid **8** with high yield (**Scheme 19**). This was then reacted with oxalyl chloride and a catalytic amount of DMF in CH_2Cl_2 to form acid chloride **9** in 95% yield with no need of purification (**Scheme 19**). It was stored under argon due to its inestability.

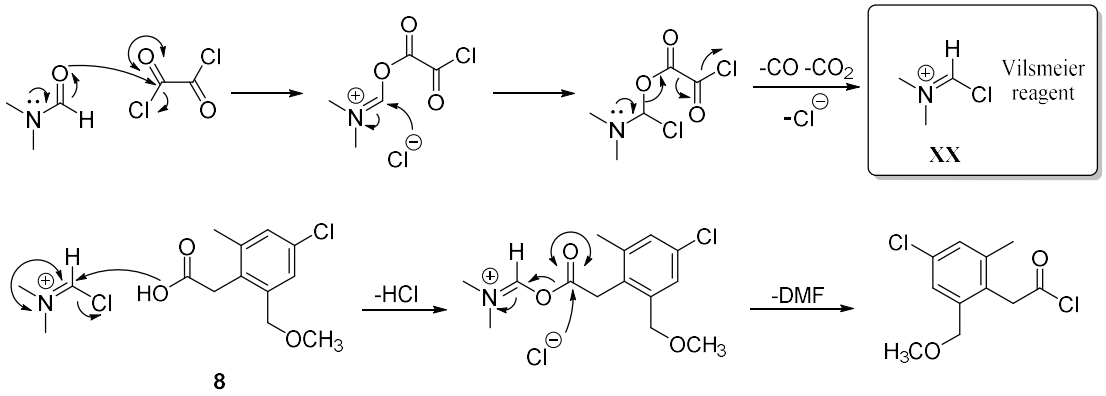


Scheme 19. Synthetic procedure for the formation of the acid chloride **9** from ester **7**.

Oxalyl chloride was selected as chlorinating agent due to its milder character compared to thionyl chloride. Besides, oxalyl chloride had afforded better results in previous synthesis of similar molecules in the group.

The mechanism of the herein shown chlorination process is represented in **Scheme 20**. Firstly, DMF reacts with oxalyl chloride to form the Vilsmeier reagent **XX**, an iminium salt with stronger chlorinating ability than $(COCl)_2$ itself. A nucleophilic attack of the carboxylic acid **8** on intermediate **XX**, results in the formation of acid chloride **9** and regeneration of DMF.[24]

[24] Mohammadkhani, L.; Heravi, M. M. *ChemistrySelect* **2019**, *4*, 6309–6337.

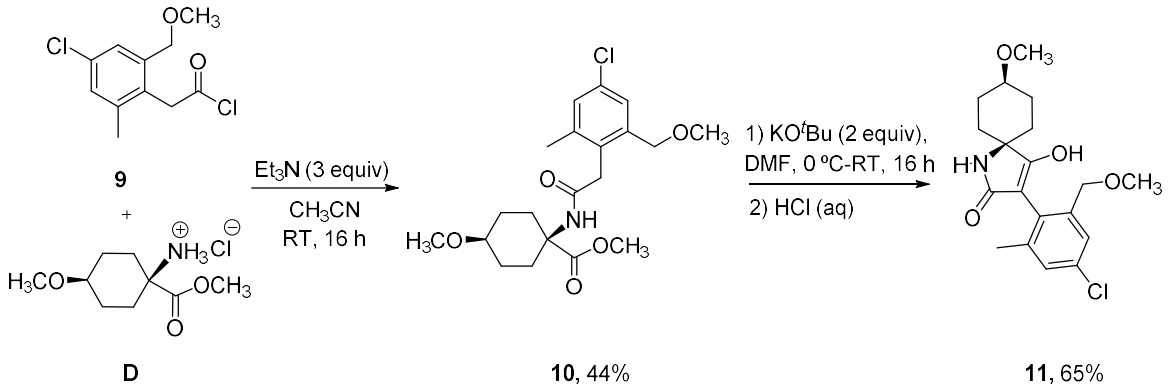


Scheme 20. Mechanism of the DMF-catalyzed chlorination reaction with $(COCl)_2$ for the formation of acid chloride **9.**

3.3.4 Formation of the tetramic acid ring. Synthesis of 11

As it has been previously mentioned in the Introductory section (page 12), the key step in the synthesis of all the tetramic-acid-based insecticides consists of an amidation reaction followed by a Dieckmann cyclization that leads to the formation of the heterocyclic core (**Scheme 2**).[9] This reaction was therefore employed to accomplish the synthesis of **11**, which was initially proposed to be an intermediate in the preparation of metabolite **4** (**Scheme 16**).

Once acid chloride **9** had been prepared, it was reacted with ammonium chloride **D**, which is also a key reagent for the synthesis of spirotetramat. The amidation reaction towards intermediate **10**, assisted by Et_3N in acetonitrile, occurred with 44% yield and the purity of the product (96.8%) was determined by HPLC. Product **10** was then treated with KO^{t}Bu and, after protonation of the enolic product with diluted HCl, the tetramic acid **11** was isolated in 65% yield (**Scheme 21**).

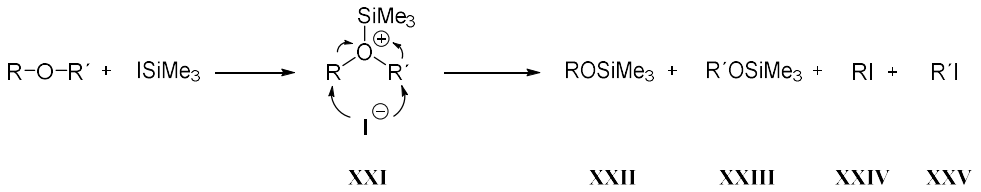


Scheme 21. Amide coupling and Dieckmann cyclization for the formation of the tetramic acid **11**.

3.3.5 Demethylation of tetramic acid 11

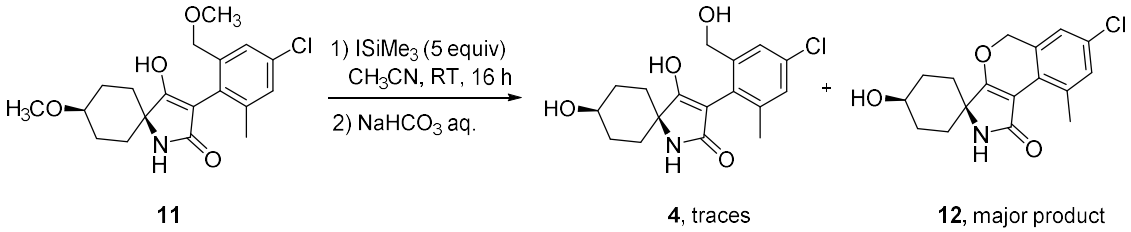
For the deprotection of molecule **11**, trimethylsilyl iodide was initially tried. The deprotection mechanism of this reagent is depicted in **Scheme 22**. Due to the interaction of the electron deficient silicon with the lone electron pair of the oxygen, the trimethylsilyl oxonium intermediate **XXI** is formed. A nucleophilic attack of the iodide to one of the carbons leads to the formation of the

corresponding alkyl iodide and a trimethylsilyl ether that is then hydrolyzed to the corresponding alcohol. In the case of unsymmetrical dialkyl ethers, mixtures of products **XXII**, **XXIII**, **XXIV** and **XXV** can be obtained and, although in the case of methyl ethers there is a greater tendency for the formation of methyl iodide, if an excess of $ISiMe_3$ is used, the alkyl iodides of both substituents are sometimes formed.[25]



Scheme 22. Ether deprotection mechanism with trimethylsilyl iodide.

From previous experience in the deprotection of cyclohexyl methyl ethers in the group, it was known that the reaction is quite selective towards the formation of cyclohexanol and it was selected as the most appropriate method for the synthesis of compounds containing such moiety. For that reason, it was applied in the deprotection of **11** and the reactivity of the benzylic methyl ether was also studied.



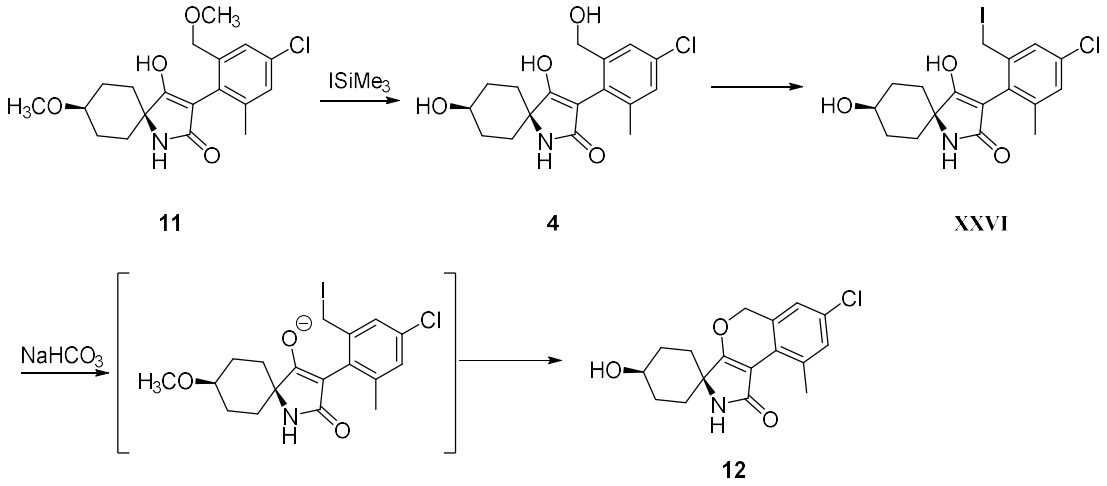
Scheme 23. Proposed conditions for the formation of product **4** starting from **11**.

The tetramic acid **11** was stirred in acetonitrile at room temperature in the presence of an excess of $ISiMe_3$ (5 equiv). After 16 h of reaction, a saturated aqueoues solution of sodium hydrogen carbonate was added to quench the reaction and the crude mixture was analyzed by mass spectrometry and NMR spectroscopy. Molecule **12** was determined to be the major product **(Scheme 23)**. On the other hand, it could be shown that the desired product **4** was also formed in the reaction by comparing it with the authentic metabolite sample, but this procedure was determined not to be efficient for the preparation of multigram quantities of the metabolite as only traces of **4** were detected.

To explain the formation of **12**, the reaction was followed and the formed intermediates were identified using mass spectrometry (**Scheme 24**). Although at the beginning of the reaction the desired product **4** could be detected, it was rapidly transformed into iodinated intermediate **XXVI** in the

[25] a) Olah, G. A.; Narang, S. C. *Tetrahedron* **1982**, *38*, 2225–2277. b) Jung, M. E.; Lyster, M. A. *Org. Synth.* **1979**, *59*, 35. c) Jung, M. E.; Lyster, M. A. *J. Org. Chem.* **1977**, *42*, 3761–3764.

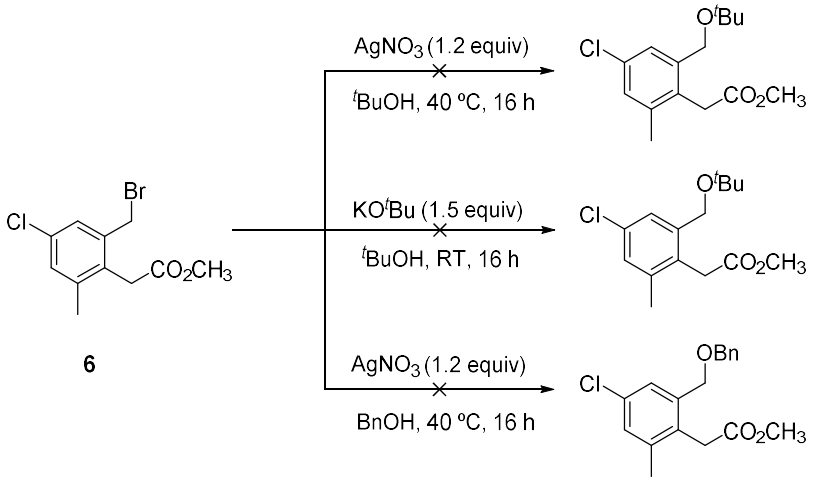
reaction media and, after treatment with aqueous $NaHCO_3$, final product **12** was formed. This occurs because the acidic enolic proton is substracted by the base and the cycle is formed by an intramolecular nucleophilic attack (Williamson ether formation).



Scheme 24. Explanation for the formation of molecule **12**.

Other Lewis acids such as BCl_3 and $SbCl_5$ were tried to accomplish the demethylation of the diether **11**. Although the latter has been reported to be very selective in the deprotection of benzyl methyl ethers to form the corresponding benzyl alcohols,[26] when it was reacted with **11**, the benzylic position was chlorinated. This showed the high tendecy of that position to undergo halogenation and the impossibility of synthesizing the metabolite through this procedure.

Due to the apparent reactivity of the benzylic methyl ether of **11**, the formation of different benzylic ethers starting from bromide **6** was tried. For that purpose, alcohols such as ^{t}BuOH and BnOH were used. However, none of the reactions tried afforded the desired ethers (**Scheme 25**).



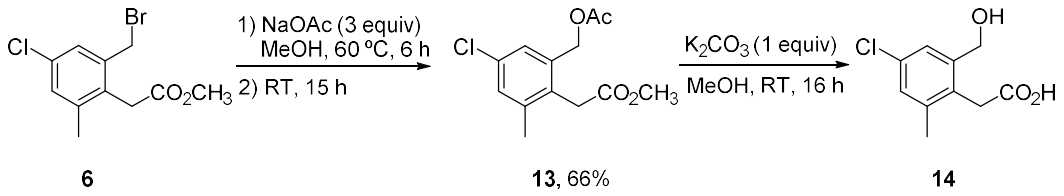
Scheme 25. Reactions with ^{t}BuOH and BnOH for the formation of the corresponding benzylic ethers.

[26] Saadati, F.; Meftah-Booshehri, H. *Synlett* **2013**, *24*, 1702–1706.

Due to the results obtained, we then focused on the introduction of a hydroxy group in the benzylic position. It was hypothesized that the benzyl alcohol obtained could be protected with different groups, such as silyl ethers, allowing the formation of the tetramic acid ring and the deprotection of the cyclohexyl methyl ether with $ISiMe_3$, without giving undersired side reaction. Finally, the introduced silyl ether would be cleaved with a fluoride source.

3.3.6 Hydroxylation of bromide 6. Synthesis of benzyl alcohol 14

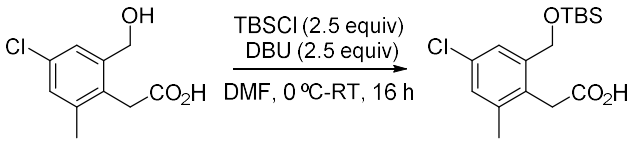
The best conditions found to carry out the hydroxylation of bromide **6** (**Scheme 26**) consisted of stirring the substrate in methanol in the presence of an excess of sodium acetate (3 equiv) to generate benzyl acetate **13** in 66% yield. In an attempt to selectively cleave the acyl group, intermediate **13** was treated with K_2CO_3 in methanol at room temperature, however, the methyl ester was also deprotected in the reaction to generate carboxylic acid **14**. A drawback of the obtainment of acid **14** was the risk of lactonization in the work-up due to the need of acidic conditions. As complete conversion was obtained in the reaction, the solvent was evaporated and the crude product was used in the next step without further purification.



6 **13**, 66% **14**

Scheme 26. Conditions for the formation of intermediate **14**.

3.3.7 TBS protection on benzyl alcohol 14. Preparation of 15



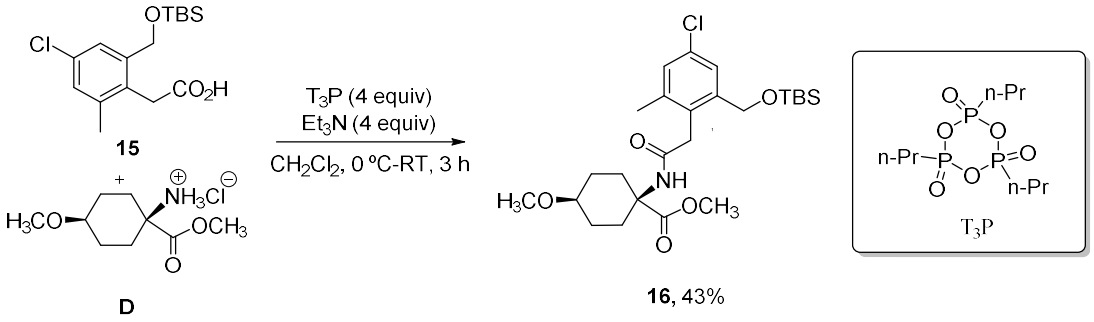
14 **15**, 97%

Scheme 27. Reaction conditions for the protection of intermediate **15**.

The final contribution of this work was to test the suitability of TBS as protecting group. To do so, alcohol **14** was protected using TBSCl (**Scheme 27**). After testing DBU and imidazole, DBU was determined to be a better base in this case, so 2.5 equivalents of it were used in the reaction. Product **15** was obtained in high yield.

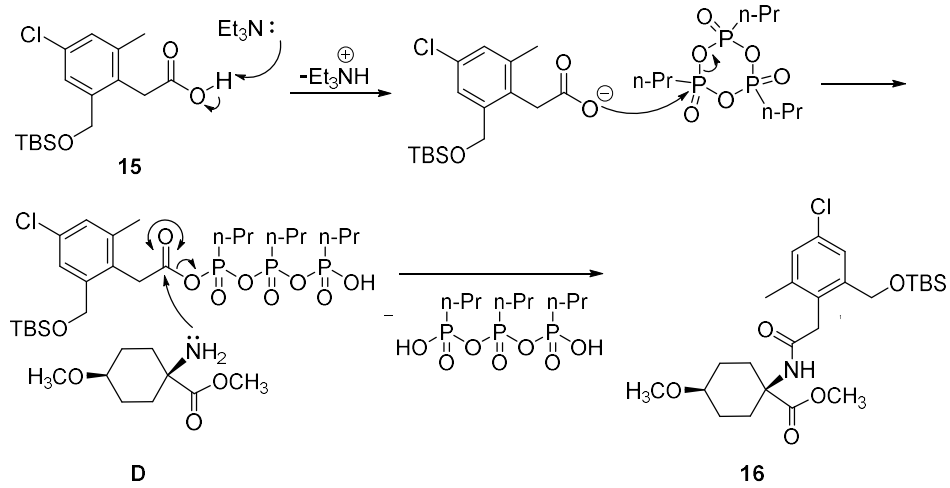
3.3.8 Formation of the tetramic acid ring. Synthesis of 17

In order to avoid any acidic conditions that could cleave the protecting group[27] and lead to the lactonization of the molecule, carboxylic acid **15** was directly coupled with ammonium chloride **D** by a T_3P-mediated amidation reaction[28] (**Scheme 28**). This reaction took place in 43% yield.



Scheme 28. T_3P-mediated amidation reaction for the generation of amide **16**.

The mechanism of the coupling reaction is shown is **Scheme 29**. After deprotonation with Et_3N, the carboxylic acid is activated by its coupling with T_3P. The attack of the amine to the carbonyl group leads to the generation of the amide upon extrusion of the leaving group.

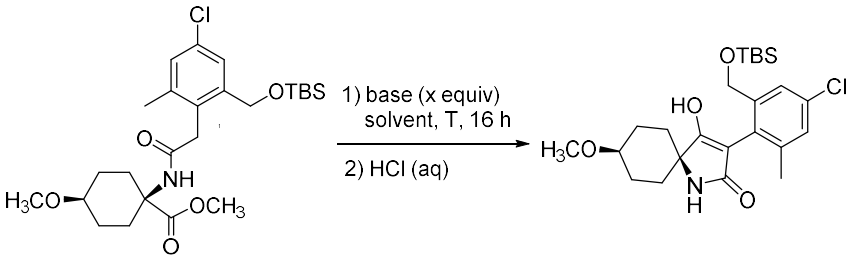


Scheme 29. Mechanism of the T_3P-mediated amidation reaction.

To proceed with the Dieckmann cyclization, amide **16** was treated with KO^{t}Bu in DMF, but, surprisingly, the general conditions used for the synthesis of most of the tetramic-acid-based active ingredients[9] that had been previously employed in this work (**Scheme 21**) were not effective in this case. There was no conversion to the desired product, so other combinations of bases and solvents were tried (**Table 4**).

[27] Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis.*, 3rd ed.; Wiley: New York, 1999.
[28] Wang, Z.; Barrows, R. D.; Emge, T. J.; Knapp, S. *Org. Process Res. Dev.* **2017**, *21*, 399–407.

Table 4. Screening of the conditions for the Dieckmann cyclization reaction of molecule **17**.[a]



16 **17**

Entry	Base	Equiv	Solvent	T (ºC)	Yield (%)[b]
1	KO^{t}Bu	2	DMF	RT	-
2	NaOMe	1.5	DMAc	RT	-
3	NaOH	1.5	DMAc	50	9

[a] Reaction conditions: **16** (0.8 mmol, 1 equiv), base, solvent (10 mL), RT, 16 h. [b] Isolated yields.

While the reactions with KO^{t}Bu and NaOMe did not show any conversion to the desired product (Table 4, Entries 1 and 2), the use of NaOH in DMAc allowed isolation of the product in 9% yield (Table 4, Entry 3). Unfortunatelly, it was determined that the protecting group tends to cleave under these conditions. The regulation of the temperature during the reaction could help reducing the deprotection but, due to the difficulties in finding the proper conditions for the reaction, the TBS protecting group was dismissed.

4. CONCLUSIONS

In the first place, the viability of the retrosynthetic proposal for the spidoxamat-decyclohexylketone metabolite **1** was probed in this work, consisting of a KO^{t}Bu-catalyzed annulative coupling between amide **2** and diethyl oxalate, leading to the formation of its maleimide core. Once the optimal conditions for the synthesis were determined, these were satisfactorily applied for the scale-up of the reaction to obtain more than 100 g of the product that could be used in ecotoxicology studies.

On the other hand, the optimal conditions for the synthesis of spidoxamat-dechlorohydroxy metabolite **3** *via* a palladium-mediated hydroxylation of spidoxamat **B** were determined. The use of $Pd_2(dba)_3$ together with ^{t}BuBrettPhos was the best combination found to catalyze the transformation. The discovery of such conditions allowed to perform in a single step the preparation of a product that would have required multiple steps on non-organometallic reactions for its synthesis. These conditions were applied successfully in a larger scale to obtain 25 g of the product with high yield and purity.

Lastly, the desired amount of spidoxamat-cyclohydroxy-benzylalochol (*cis*) metabolite **4** could not be synthesized through the initially proposed procedure since a selective demethylation of the benzylic methoxy group on intermediate **11** was not possible, leading to the formation of the byproduct **12** and very small amounts of **4**. Nevertheless, thanks to the preparation of other key intermediates, such as bromide **6** and acid **14**, it was possible to propose alternative procedures to continue with the synthesis of the metabolite. Starting from intermediate **14**, it was concluded that the TBS protecting group was not suitable for the proposed synthetic pathway, but, in future attempts, this could be replaced by other more stable groups such as TBDPS.

5. CONCLUSIONES

En primer lugar, en este trabajo se comprobó la viabilidad de la retrosíntesis propuesta para el metabolito spidoxamato-desciclohexilcetona **1**, que consiste en un acoplamiento entre la amida **2** y oxalato de dietilo catalizado por KO^{t}Bu, para la formación del anillo de maleimida presente en la molécula. Tras la determinación de las condiciones óptimas para la síntesis, se procedió a su escalado y se obtuvieron más de 100 g de producto que pudieron ser utilizados en estudios ecotoxocológicos.

Por otro lado, se determinaron las condiciones óptimas para sintetizar el metabolito spidoxamato-desclorohidroxi **3** mediante la hidroxilación del spidoxamato **B** catalizada por paladio. El uso de $Pd_2(dba)_3$ con ^{t}BuBrettPhos como ligando fue la mejor combinación encontrada para catalizar la transformación. El hallazgo de dichas condiciones permitió realizar en un solo paso la preparación de un producto que hubiera precisado de varias etapas con reacciones no organometálicas para su síntesis. Estas condiciones fueron aplicadas exitosamente a mayor escala para la obtención de 25 g de producto con gran rendimento y pureza.

Por último, la cantidad deseada del metabolito spidoxamato-ciclohidroxi-bencilalcohol (*cis*) no pudo ser sintetizada mediante el procedimiento planteado inicialmente, ya que no fue posible una desmetilación selectiva del grupo metoxi bencílico presente en el intermedio **11**, dando pie a la formación del subproducto **12** y cantidades muy pequeñas del producto **4**. A pesar de ello, gracias a la preparación de otros intermedios clave, como el bromuro **6** o el ácido **14**, se pudieron proponer procedimientos alternativos para continuar con la síntesis de este metabolito. Partiendo del intermedio **14**, se pudo concluir que el grupo protector TBS no era adecuado para el procedimiento sintético propuesto, pero este podría ser sustituido por otro más estable como TBDPS en futuras pruebas.

6. EXPERIMENTAL SECTION

6.1 General methods

Synthetic-grade solvents were employed and used as supplied from the manufacturer. Acetonitrile, ethyl acetate, dichloromethane and cyclohexane were obtained from Merck. All other solvents were purchased from Acros Organics. Some of the building blocks had previously been synthesized in the company. Commercial reagents were mainly obtained from Sigma-Aldrich, Acros Organics and Fluka and used without additional purification.

Elimination of the solvents was performed with *Büchi R-300* rotary evaporators, equipped with *Büchi V-300* vacuum pumps. Anhydrous magnesium sulfate was used to dry organic phases. All air- and moisture-sensitive reactions were performed under argon atmosphere. Low temperature reactions were performed using ice-baths (0 ºC). Heating plates and oil baths (under temperature control) were employed for reactions that required heating. The reaction screenings were performed in 10-mL Headspace Vials using electronic stirring plates (Variomag) or in 25-mL round-bottom flasks equipped with septums.

The reactions were monitored by HPLC in a *Shimadzu LC-20AD* chromatograph with a Zorbax Eclipse Plus C18 column and Shimadzu SPD-M20A Diode Array Detector (λ=210 nm). Gradient-grade acetonitrile and a 0.1% solution of H_3PO_4 in Millipore water were used for the mobile phase with a 3.5 mL/min flow. Alternatively, HPLC-MS was also used in an Agilent 1290 LC System together with Agilent MSD System and HTS PAL autosampler. This was equipped with a Zorbax Eclipse Plus C18 column (50 mm x 2.1 mm, 1.8 µm), a UV Diode Array Detector (DAD) and MSD detector with an electrospray ionization (ESI) source in positive or negative mode. A 0.1% solution of formic acid in acetonitrile and a 0.09% solution of formic acid in milipore water were used as eluents in a 1.0 mL/min flow. The samples were generally prepared in gradient grade acetonitrile and filtered with 0.20 µm pore-size teflon filters before injection.

Unless otherwise noted, the purification of the products was performed by flash column chromatography using a *CombiFlash Rf+ Lumen* system and RediSep Normal-phase Silica Flash or RediSep Rf Gold Normal-phase Silica columns. An adequate combination of solvents was chosen for each purification by thin layer chromatography (TLC) using Silica gel 60 F_{254} glass plates (Merck). These were visualized with a UV light (λ = 254 nm and 360 nm).

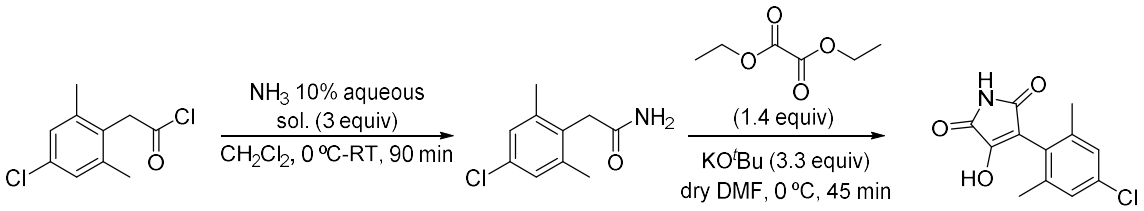
The purity of the products was determined using an Agilent 1260 HPLC system with Zorbax Eclipse Plus C18 4.6 x 50 mm 1.8 µm column, DAD detector (λ=210 nm) and using a 0.1% solution of H_3PO_4 in Millipore water/acetonitrile as the eluent system with a 2 mL/min flow. GC-MS was also used for this purpose employing a Thermo Trace 1310 system with a flame ionization detector (FID) and a mass spectrometer with a scan range between 50 m/z and 650 m/z.

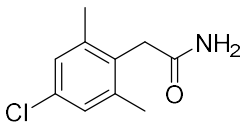
^{1}H-NMR was measured at a frequency of 600 MHz and ^{13}C-NMR at 151 MHz using *Bruker Avance* NEO 600 MHz and *Bruker Avance* III HD 600 MHz spectrometers. The samples were dissolved in DMSO-d_6. TMS was used as a reference for the ^{1}H-NMR measurements and the corresponding solvent signal in the case of ^{13}C-NMR, (δC 39.7 ppm for DMSO). Chemical shifts, δ, are indicated in parts per million (ppm) and coupling constants (*J*) in Hertz (Hz). The multiplicity of each peak is given as singlet (s), doublet (d), triplet (t), multiplet (m) or a combination of these. Broad signals are indicated as br s. All the spectra were processed with the program *ACD/spectrus processor.*

6.2 Synthetic procedures

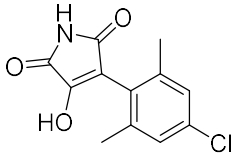
This section contains the description of the already optimized reaction conditions. In most cases, just the large-scale synthesis is described.

6.2.1 Synthesis of Spidoxamat-decyclohexylketone metabolite 1





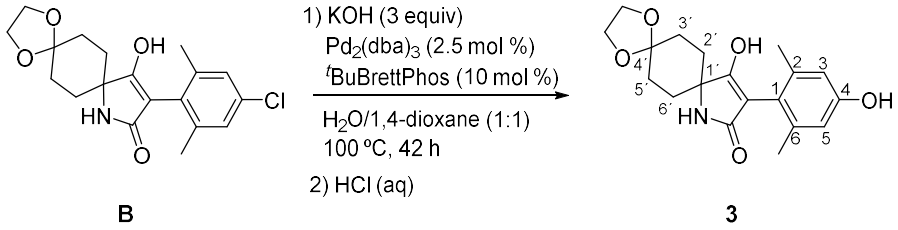
2-(4-Chloro-2,6-dimethylphenyl)acetamide (2). To a solution of 2-(4-chloro-2,6-dimethylphenyl)acetyl chloride **A** (185 g, 0.82 mol, 1 equiv) in CH_2Cl_2 (750 mL), during a period of 3 hours, a 10% aqueous solution of ammonia (438 mL, 2.5 mol, 3 equiv) was added dropwise while cooling to 0 °C. After the addition, the reaction was stirred for 90 minutes at room temperature. After complete conversion, the precipitate was filtered under vacuum and washed twice with water (100 mL) to give **2** (160 g, 98%, HPLC purity 99.2%) as a white solid. MS-ESI: 198.1 $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO-d_6) δ = 7.39 (br s, 1H, NH), 7.06 (s, 2H, H_3, H_5), 6.93 (br s, 1H, NH), 3.44 (s, 2H, CH_2), 2.23 (s, 6H, 2 x CH_3). ^{13}C NMR (151 MHz, DMSO-d_6) δ = 171.4 (CO), 139.5 (C_2, C_6), 133.1 (C_1), 130.3 (C_4), 127.1 (C_3, C_5), 35.5 (CH_2), 19.9 (2 x CH_3).



3-(4-Chloro-2,6-dimethylphenyl)-4-hydroxy-*1H*-pyrrole-2,5-dione (1). To a stirring solution of 2-(4-chloro-2,6-dimethylphenyl)acetamide **2** (35 g, 175.6 mmol, 1 equiv) in dry DMF (300 mL), diethyl oxalate (33.4 mL, 245.9 mmol, 1.4 equiv) was added dropwise under argon atmosphere and at 0 ºC. After stirring for 15 minutes, a 1.65 M THF solution of KO^{t}Bu (351.3 mL, 579.6 mmol, 3.3 equiv) was added. The mixture was stirred for 45 minutes at 0°C until complete conversion. The reaction was poured to a mixture of a diluted aqueous solution of HCl and ice water (200 mL). Afterwards, it was

extracted with ethyl acetate (2 x 300 mL). The combined organic layers were washed multiple times with water (300 mL). The organic phase was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The resulting solid was stirred in water, filtered under vacuum, and washed with water (3 x 150 mL) and cyclohexane (2 x 150 mL) to give **1** (34.4 g, 77%, HPLC purity 99.5%) as a light yellow solid. MS-ESI: 252.0 $[M+H]^+$. ^1H NMR (600 MHz, DMSO-d_6) δ = 10.65 (s, 1H, OH), 7.18 (s, 2H, H_3, H_5), 2.12 (s, 6H, 2 x CH_3). ^{13}C NMR (151 MHz, DMSO-d_6) δ = 171.5 (CO), 168.1 (CO), 154.1 (<u>C</u>OH), 140.2 (C_2, C_6), 132.7 (C_4), 127.0 (C_1), 126.8 (C_3, C_5), 108.0 (<u>C</u>=COH) , 19.9 (2 x CH_3).

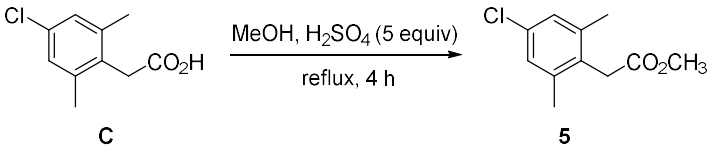
6.2.2 Synthesis of Spidoxamat-dechlorohydroxy metabolite 3



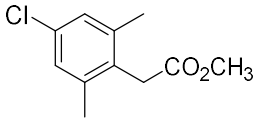
12-Hydroxy-11-(4-hydroxy-2,6-dimethylphenyl)-1,4-dioxa-9-azadispiro[4.2.4^8.2^5]tetradec-11-en-10-one (3). Over a solution of spidoxamat **B** (30 g, 82.3 mmol, 1 equiv) in a 1:1 mixture of dioxane and water (600 mL), a 47% aqueous solution of KOH (294.7 mL, 246.9 mmol, 3 equiv) was added. This mixture was flushed with argon for 30 minutes and it was then heated up to 100 ºC. Then, $Pd_2(dba)_3$ (1.94 g, 2.1 mmol, 2.5 mol%) and ^{t}BuBrettPhos (4.07 g, 8.2 mmol, 10 mol%) were added at that temperature. After stirring the reaction for 42 hours at 100 ºC and before cooling it down to room temperature, the reaction mixture was transferred to a separation funnel and the organic layer was separated from the aqueous one. The dioxane phase was poured to a mixture of a diluted aqueous solution of HCl and ice water (200 mL). The formed precipitate was filtered under vacuum and washed with water (3 x 150 mL) and cyclohexane (2 x 150 mL) to give **3** (25.5 g, 88%, HPLC purity 98.4%) as a white solid. MS-ESI: 346.2 $[M+H]^+$. ^1H NMR (600 MHz, DMSO-d_6) δ = 10.47 (s, 1H, OH), 9.03 (s, 1H, C_4OH), 8.05 (s, 1H, NH), 6.44 (s, 2H, H_3, H_5), 3.88 (s, 4H, 2 x CH_2), 2.10 (td, J = 13.5, 6.0 Hz, 2H, 2 x H_{Cy}), 1.98 (s, 6H, 2 x CH_3), 1.88 (td, J = 13.5, 6.0 Hz, 2H, 2 x H_{Cy}), 1.69 (d, J = 12.0 Hz, 2H, 2 x H_{Cy}), 1.38 (d, J = 12.0 Hz, 2H, 2 x H_{Cy}). ^{13}C NMR (151 MHz, DMSO-d_6) δ = 172.6 (<u>C</u>OH), 172.0 (CO), 156.2 (C_4), 138.9 (C_2, C_6), 120.8 (C_1), 113.6 (C_3, C_5), 107.3 ($C_{4'}$), 103.4 (<u>C</u>=COH), 63.6 (2 x CH_2), 58.8 ($C_{1'}$), 31.8 ($C_{2'}$, $C_{6'}$), 30.7 ($C_{3'}$, $C_{5'}$), 19.9 (2 x CH_3).

6.2.3 Synthesis of Spidoxamat-cyclohydroxy-benzylalcohol *(cis)* metabolite 4

6.2.3.1 Esterification of phenylacetic acid C. Synthesis of ester 5

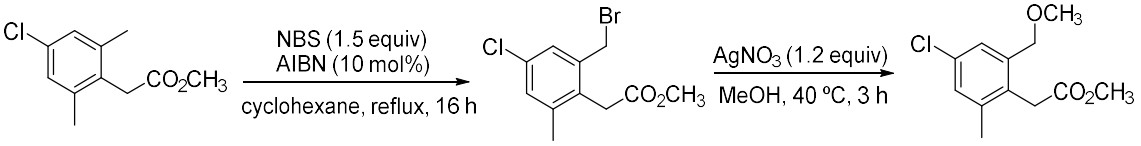


C 5

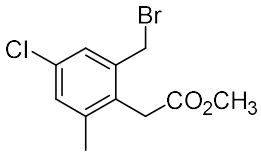


Methyl 2-(4-chloro-2,6-dimethylphenyl)acetate (5).[29] To a solution of 2-(4-chloro-2,6-dimethylphenyl)acetic acid **C** (81 g, 391.8 mmol, 1 equiv) in methanol (400 mL) sulfuric acid (110 mL, 2.0 mmol, 5 equiv) was added dropwise. The reaction mixture was stirred at reflux for 4 hours after the addition. Afterwards, it was cooled down to room temperature and poured into ice water (800 mL). The resulting mixture was extracted with MTBE (4 x 400 mL) and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure to give **5** (77.4 g, 93%) as a brown oil. MS-ESI: 213.1 $[M+H]^+$. ^1H NMR (600 MHz, DMSO-d_6) δ = 7.10 (s, 2H, H_3, H_5), 3.68 (s, 2H, CH_2CO), 3.61 (s, 3H, OCH_3), 2.23 (s, 6H, 2 x CH_3). ^{13}C NMR (151 MHz, DMSO-d_6) δ = 170.9 (CO), 139.4 (C_2, C_6), 131.1 (C_1), 130.9 (C_4), 127.2 (C_3,C_5), 51.8 (OCH_3), 34.1 ($\underline{C}H_2CO$), 19.5 (2 x CH_3).

6.2.3.2 Methoxylation of ester 5. Formation of methyl ether 7



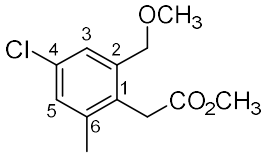
5 6 7



Methyl 2-[2-(bromomethyl)-4-chloro-6-methylphenyl]acetate (6). Over a solution methyl 2-(4-chloro-2,6-dimethyl-phenyl)acetate **5** (53 g, 242.2 mmol, 1 equiv) in cyclohexane (1.1 L), NBS (64.7 g, 363.3 mmol, 1.5 equiv) and AIBN (4.0 g, 24.2 mmol, 10 mol%) were added. The reaction mixture was refluxed for 16 hours. After complete conversion, it was cooled down to room temperature and extracted with EtOAc (4 x 300 mL) and water (3 x 200mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure, affording **6** (81.33 g, 83%, HPLC purity 72%) as an orange solid. This compound was used for the next step without further purification, due to

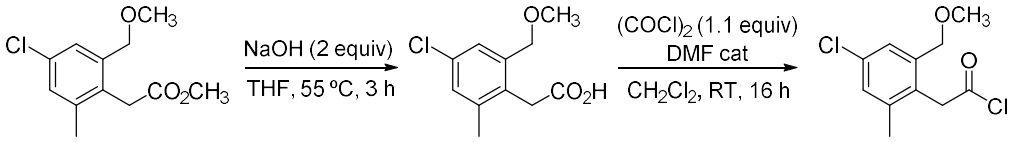
[29] Lieb, F.; Hagemann, H.; Widdig, A.; Ruther, M.; Fischer, R.; Bretschneider, T.; Erdelen, C.; Wachendorff-Neumann, U.; Dahmen, P.; Dollinger, M.; Santel, H.-J.; Alan, G.; Andersch, W. Preparation of 3-Phenylheterocycloalkyl-2,4-Dione Enols as Pesticides and Herbicides. DE 19603332 A1, January 2, 1997.

difficulties in the separation of the non-reacted starting material and the dibrominated byproduct from the product.

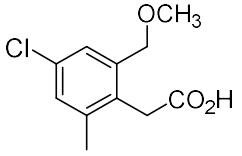


Methyl 2-[4-chloro-2-(methoxymethyl)-6-methylphenyl]acetate (7). Methyl 2-[2-(bromomethyl)-4-chloro-6-methylphenyl]acetate **6** (25 g, 66.6 mmol, 1 equiv) and silver (I) nitrate (13.6 g, 79.9 mmol, 1.2 equiv) were stirred in methanol (80 mL) for 3 hours at 40 ºC. After complete conversion, the reaction was allowed to cool down to room temperature, filtered and the solvent was evaporated. The residue was dissolved in ethyl acetate (300 mL), and this was washed with water (2 x 200 mL). The organic phase was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The crude product mixture was purified by distillation and **7** was obtained as a colourless oil (7.5 g, 46%). The yield was calculated considering the HPLC purity of **6**. MS-ESI: 243.0 $[M+H]^+$. ^1H NMR (600 MHz, DMSO-d_6) δ = 7.25 (d, *J* = 2.2 Hz, 1H, H_3), 7.23 (d, *J* = 2.2 Hz, 1H, H_5), 4.40 (s, 2H, CH_2O), 3.71 (s, 2H, CH_2CO), 3.59 (s, 3H, CO_2CH_3), 3.23 (s, 3H, OCH_3), 2.24 (s, 3H, C_6CH_3). ^{13}C NMR (151 MHz, DMSO-d_6) δ = 170.7 (CO), 140.1 (C_6), 139.2 (C_2), 131.1 (C_1), 131.0 (C_4), 129.0 (C_5), 125.9 (C_3), 71.6 (CH_2O), 57.5 (OCH_3), 51.7 ($CO_2\underline{C}H_3$), 33.4 ($\underline{C}H_2CO$), 19.2 ($C_6\underline{C}H_3$).

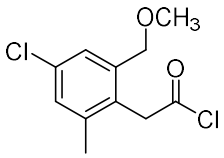
6.2.3.3 Formation of acid chloride 9 starting from phenylacetate 7



7 **8** **9**

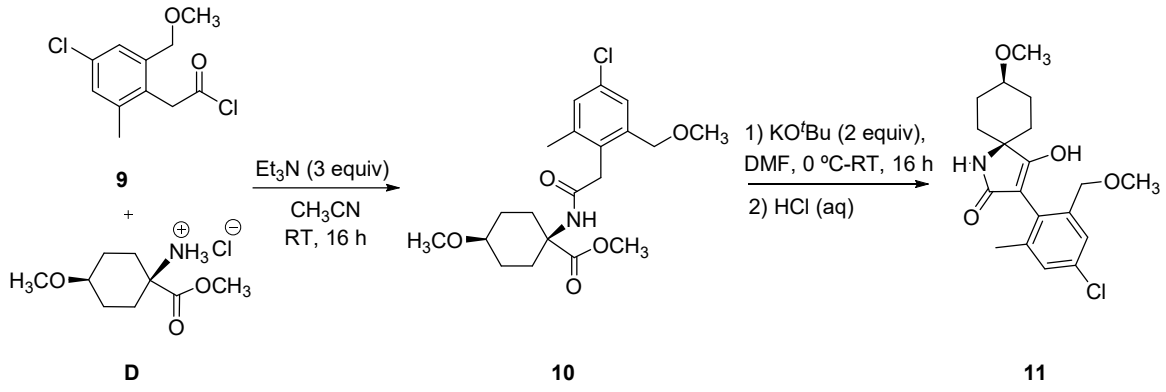


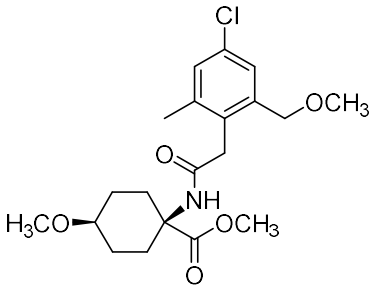
2-[4-Chloro-2-(methoxymethyl)-6-methylphenyl]acetic acid (8). Over a solution of methyl 2-[4-chloro-2-(methoxymethyl)-6-methylphenyl]acetate **7** (3.4 g, 13.1 mmol, 1 equiv) in THF (68 mL), a 1M aqueous solution of NaOH (26.2 mL, 26.2 mmol, 2 equiv) was added dropwise at room temperature. The reaction mixture was stirred at 55 ºC for 3 hours. After complete conversion, THF was evaporated and the aqueous phase was acidified to pH 1 with diluted aqueous HCl. This was extracted with CH_2Cl_2 (3 x 75 mL). The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure to give **8** (2.78 g, 93%) as a white solid. MS-ESI: 227.0 $[M-H]^-$. ^1H NMR (600 MHz, DMSO-d_6) δ = 12.35 (br s, 1H, OH), 7.23 (s, 1H, H_3), 7.22 (s, 1H, H_5), 4.40 (s, 2H, CH_2O), 3.61 (s, 2H, CH_2CO), 3.26 (s, 3H, OCH_3), 2.25 (s, 3H, C_6CH_3). ^{13}C NMR (151 MHz, DMSO-d_6) δ = 171.9 (CO), 140.2 (C_6), 139.3(C_2), 131.8 (C_1), 131.0 (C_4), 129.0 (C_5), 125.9 (C_3), 71.8 (CH_2O), 57.8 (OCH_3), 33.9 ($\underline{C}H_2CO$), 19.4 ($C_6\underline{C}H_3$).



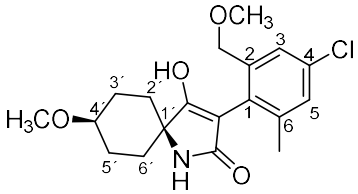
2-[4-Chloro-2-(methoxymethyl)-6-methylphenyl]acetyl chloride (9). Over a solution of 2-[4-chloro-2-(methoxymethyl)-6-methylphenyl]acetic acid **8** (2.78 g, 12.2 mmol, 1 equiv) in CH_2Cl_2 (23 mL) a solution of oxalyl chloride (1.2 mL, 13.4 mmol, 1.1 equiv) in CH_2Cl_2 (7 mL) was added dropwise. Three drops of catalytic DMF were also added. The reaction was stirred overnight at room temperature. After complete conversion, the solvent and the excess of oxalyl chloride were evaporated, the residue was dissolved in cyclohexane and this solution was concentrated under reduced pressure to give **9** (2.85 g, 95%) as an oil. Due to the inestability of this compound, to prove the formation of the product, a sample was dissolved in methanol and the mass of the formed methyl ester was determined by mass spectrometry, MS-ESI: 243.0 $[M+H]^+$. The NMR data of the formed methyl ester is consistent with that for compound **7**.

6.2.3.4 Formation of the tetramic acid ring. Synthesis of 11



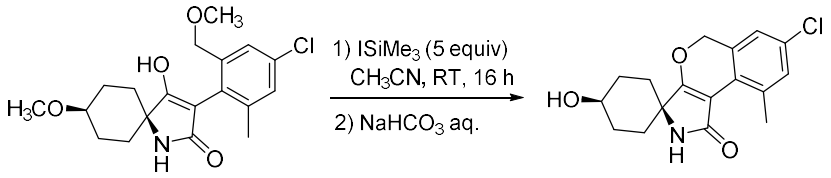


***(cis)*-Methyl 1-{2-[4-chloro-2-(methoxymethyl)-6-methylphenyl] acetamido}-4-methoxycyclohexane-1-carboxylate (10).** Over a solution of methyl *cis*-1-amino-4-methoxycyclohexane-1-carboxylate hydrochloride **D** (3.1 g, 13.8 mmol, 1.2 equiv) in acetonitrile (40 mL), Et_3N (4.8 mL, 34.6 mmol, 3 equiv) was added. Afterwards, a solution of 2-[4-chloro-2-(methoxymethyl)-6-methylphenyl]acetyl chloride **9** (2.85 g, 11.5 mmol, 1 equiv) in acetonitrile (40 mL) was added to the reaction flask dropwise. The reaction was stirred overnight at room temperature. Then, it was concentrated and the resulting solid was dissolved in ethyl acetate (200 mL) and washed with a diluted aqueous solution of HCl (100 mL). The organic phase was washed with a saturated aqueous solution of $NaHCO_3$ (75 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The product was purified by flash column chromatography (silica gel, cyclohexane/MTBE 1:1) to give **10** (2.03 g, 44%) as a white solid. MS-ESI: 398.2 $[M+H]^+$.

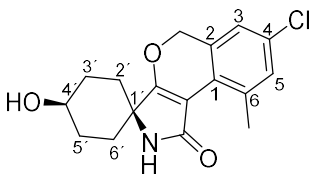


(*cis*)-3-[4-Chloro-2-(methoxymethyl)-6-methylphenyl]-4-hydroxy-8-methoxy-1-azaspiro[4.5]dec-3-en-2-one (11). A 1.65 M solution of KO^{t}Bu in THF (6.8 mL, 11.2 mmol, 2 equiv) was added dropwise at 0 ºC to a solution of *cis*-methyl 1-{2-[4-chloro-2-(methoxymethyl)-6-methylphenyl]aceta-mido}-4-methoxycyclohexane-1-carboxylate **10** (2.3 g, 5.6 mmol, 1 equiv) in dry DMF (50 mL) under argon atmosphere. The mixture was stirred at that temperature for 1h. Afterwards, it was allowed to warm up to room temperature and stirred at that temperature overnight. After complete conversion, the reaction was poured to a mixture of a diluted aqueous solution of HCl and ice water (50 mL) and extracted with ethyl acetate (2 x 75 mL). The combined organic layers were washed several times with water (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The resulting solid was stirred in water, filtered under vacuum and washed with water (50 mL) and cyclohexane (50 mL) to give **11** (1.32 g, 65%) as a white solid. MS-ESI: 366.2 $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO-d_6) δ = 10.86 (br s, 1H, OH), 8.16 (s, 1H, NH), 7.23 (s, 1H, H_3), 7.22 (s, 1H, H_5), 4.24 (d, *J* = 13.3 Hz, 1H, CH_2O), 4.18 (d, *J* = 13.3 Hz, 1H, CH_2O), 3.26 (s, 3H, $C_{4'}OCH_3$), 3.25 (s, 3H, CH_2OCH_3), 3.18 - 3.09 (m, 1H, $H_{4'}$), 2.09 (s, 3H, C_6CH_3), 2.01 - 1.95 (m, 2H, 2 x H_{Cy}), 1.93 - 1.85 (m, 2H, 2 x H_{Cy}), 1.56 - 1.49 (m, 2H, 2 x H_{Cy}), 1.49 - 1.41 (m, 2H, 2 x H_{Cy}). ^{13}C NMR (151 MHz, DMSO-d_6) δ = 173.5 (COH), 171.0 (CO), 141.0 (C_2), 140.6 (C_6), 131.8 (C_4), 127.9 (C_1), 127.6 (C_5), 123.4 (C_3), 101.2 (C=COH), 77.6 ($C_{4'}$), 71.0 (CH_2O), 59.6 ($C_{1'}$), 58.0 (CH_2OCH_3), 54.9 ($C_{4'}OCH_3$), 32.2 ($C_{2'}$), 32.1 ($C_{6'}$), 27.5 ($C_{3'}$), 27.5 ($C_{5'}$), 19.3 (C_6CH_3).

6.2.3.5 Demethylation of tetramic acid 11. Formation of 12



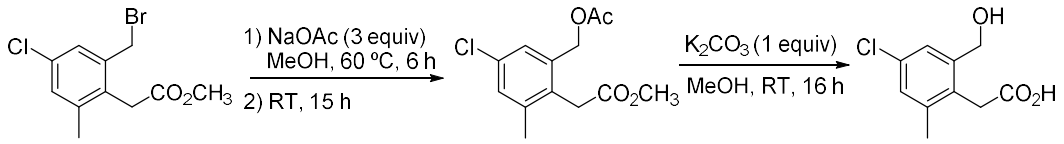
11 **12**



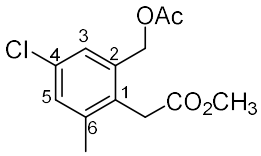
(*cis*)-7'-Chloro-4-hydroxy-9'-methyl-5'H-spiro[cyclohexane-1,3'-isochromeno(3,4-c)pyrrol]-1'(2'H)-one (12). Trimethylsilyl iodide (18.0 mmol, 5 equiv) was added dropwise at 10 ºC to a solution of (*cis*)-3-[4-chloro-2-(methoxymethyl)-6-methylphenyl]-4-hydroxy-8-methoxy-1-azaspiro[4.5]dec-3-en-2-one **11** (1.32 g, 3.6 mmol, 1 equiv) in acetonitrile (18 mL) under argon atmosphere. Afterwards, the reaction was allowed to warm up to room temperature and stirred at that temperature overnight. Then, it was treated with a saturated aqueous solution of $NaHCO_3$ (10 mL). The phases were separated and the aqueous one was acidified with diluted aqueous HCl and extracted with ethyl acetate (2 x 30 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. This product was not obtained pure. Spectroscopic data

corresponding to the major product **12**: MS-ESI: 320.1 $[M+H]^+$. ^1H NMR (600 MHz, DMSO-d_6) δ = 7.19 (d, *J* = 1.7 Hz, 1H, H_3), 7.15 (d, *J* = 1.7 Hz, 1H, H_5), 5.22 (s, 2H, OCH_2), 3.47 (br s, 1H, OH), 3.46 - 3.42 (m, 1H, $H_{4'}$), 1.91 (s, 3H, CH_3), 1.82 - 1.46 (m, 8H, 8 x H_{Cy}).

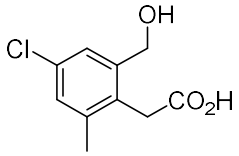
6.2.3.6 Hydroxylation of bromide 6. Synthesis of benzyl alcohol 14



6 **13** **14**

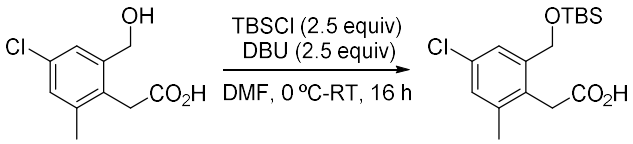


Methyl 2-[2-(acetoxymethyl)-4-chloro-6-methylphenyl]acetate (13). To a solution of methyl 2-[2-(bromomethyl)-4-chloro-6-methylphenyl]acetate **6** (30 g, 74.1 mmol, 1 equiv) in methanol (300 mL), sodium acetate (18.23 g, 222.2 mmol, 3 equiv) was added and the mixture was stirred at 60 ºC for 6 hours. Afterwards, it was allowed to cool down to room temperature and stirred at that temperature overnight. After complete conversion, the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (600 mL) and washed with water (2 x 300 mL). The organic phase was dried over $MgSO_4$, concentrated under reduced pressure and the product was purified by flash column chromatography (silica gel, cyclohexane/MTBE 9:1 to 1:1) to give **13** (13.2 g, 66%, GC purity 100%) as a white solid. ^1H NMR (600 MHz, DMSO-d_6) δ = 7.31 (d, *J* = 2.2 Hz, 1H, H_3), 7.29 (d, *J* = 2.2 Hz, 1H, H_5), 5.07 (s, 2H, CH_2O), 3.74 (s, 2H, CH_2CO), 3.61 (s, 3H, OCH_3), 2.26 (s, 3H, C_6CH_3), 2.00 (s, 3H, $COCH_3$). ^{13}C NMR (151 MHz, DMSO-d_6) δ = 170.9 ($CH_2\underline{C}O$), 170.1 ($\underline{C}OCH_3$), 140.6 (C_6), 137.3 (C_2), 131.6 (C_1), 131.3 (C_4), 129.8 (C_5), 127.1 (C_3), 63.9 (CH_2O), 52.0 (OCH_3) , 33.8 ($\underline{C}H_2CO$) , 20.7 ($CO\underline{C}H_3$) , 19.5 ($C_6\underline{C}H_3$).

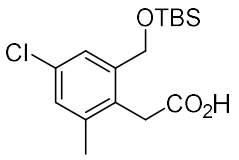


2-[4-Chloro-2-(hydroxymethyl)-6-methylphenyl]acetic acid (14). Over a solution of methyl 2-[2-(acetoxymethyl)-4-chloro-6-methylphenyl]acetate **13** (8.87 g, 32.7 mmol, 1 equiv) in methanol (85mL), K_2CO_3 (4.52 g, 32.7 mmol, 1 equiv) was added. The reaction mixture was stirred at room temperature overnight. After complete conversion, it was concentrated and the crude product was used in the next step due to risk of lactonization upon acidic work-up. The final solid **14** was contaminated with some solvent and the yield could not be calculated. MS-ESI: 213.2 $[M-H]^-$. ^1H NMR (600 MHz, DMSO-d_6) δ = 7.12 (s, 1H, H_3), 7.09 (s, 1H, H_5), 4.37 (s, 2H, CH_2O), 4.25 (br s, 1H, OH), 3.31 (s, 2H, CH_2CO), 2.35 (s, 3H, CH_3). ^{13}C NMR (151 MHz, DMSO-d_6) δ = 172.3 (CO), 143.0 (C_2), 139.6 (C_6), 136.8 (C_1), 129.0 (C_4), 128.3 (C_5), 126.6 (C_3), 63.1 (CH_2O), 40.6 ($\underline{C}H_2CO$), 19.8 (CH_3).

6.2.3.7 TBS protection on benzyl alcohol 14. Preparation of 15

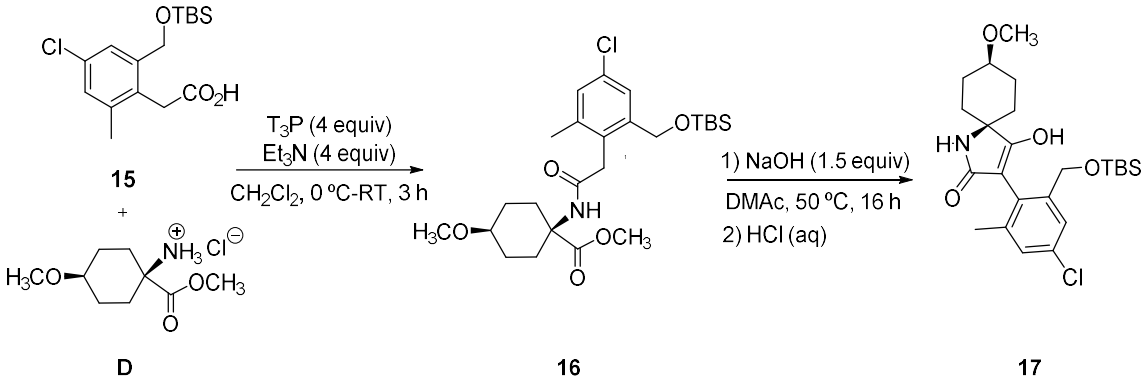


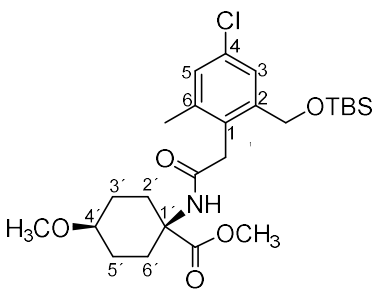
14 15



2-{2-[((*tert*-Butyldimethylsilyl)oxy)methyl]-4-chloro-6-methylphenyl}acetic acid (15). Over a solution of TBSCl (19.98 g, 132.5 mmol, 2.5 equiv) in dry DMF (75 mL), a solution of 2-[4-chloro-2-(hydroxymethyl)-6-methylphenyl]acetic acid **14** (11.4 g, 53.0 mmol, 1 equiv) in dry DMF (75 mL) was added under argon atmosphere. DBU (20.18 g, 132.5 mmol, 2.5 equiv) was added to the mixture at 0 ºC and it was stirred at room temperature overnight. After complete conversion, the reaction was poured to a mixture of a diluted aqueous solution of formic acid and ice water (200 mL). It was extracted with MTBE (2 x 150 mL) and washed with water (200 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure to give **15** (16.9 g, 97%) as a solid. ^{1}H NMR (600 MHz, DMSO-d$_6$) δ = 12.31 (br s, 1H, OH), 7.18 (d, *J* = 2.1 Hz, 1H, H_3), 7.12 (d, *J* = 2.1 Hz, 1H, H_5), 4.62 (s, 2H, CH_2O), 3.52 (s, 2H, CH_2CO), 2.16 (s, 3H, C_6CH_3), 0.82 (s, 9H, $C(CH_3)_3$), 0.00 (s, 6H, 2 x $SiCH_3$). ^{13}C NMR (151 MHz, DMSO-d$_6$) δ = 171.9 (CO), 142.1 (C_2), 139.9 (C_6), 131.1 (C_4), 130.5 (C_1), 128.3 (C_5), 124.0 (C_3), 62.5 (CH_2O), 33.6 ($\underline{C}H_2CO$), 26.0 ($C(\underline{C}H_3)_3$), 19.3 ($C_6\underline{C}H_3$), 18.1 ($\underline{C}(CH_3)_3$), -5.3 (2 x $Si\underline{C}H_3$)

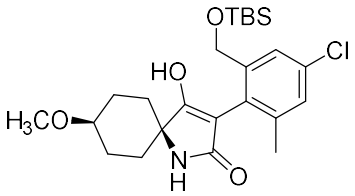
6.2.3.8 Formation of the tetramic acid ring. Synthesis of 17





Methyl (*cis*)-1-{2-[2-(((tert-butyldimethylsilyl) oxy)methyl)-4-chloro-6-methylphenyl]acetamido}-4-methoxycyclohexane-1-carboxylate (16). Over a suspension of methyl (*cis*)-1-amino-4-methoxycyclohexane-1-carboxylate **D** (2.62 g, 11.7 mmol, 1.1 equiv) in CH_2Cl_2 (100 mL), Et_3N (4.3 g, 42.6 mmol, 4 equiv) was added dropwise. A solution of 2-{2-[((tert-butyldimethylsilyl)

oxy)methyl]-4-chloro-6-methylphenyl} acetic acid **15** (3.5 g, 10.6 mmol, 1 equiv) in CH_2Cl_2 (60 mL) was added to the reaction flask and it was cooled down to 0 ºC. Once at this temperature, a 50 wt.% solution of T_3P in ethyl acetate (25.3 mL, 42.6 mmol, 4 equiv) was also added dropwise and, after the addition, the reaction was allowed to warm up to room temperature. The reaction mixture was stirred at this temperature for 3 hours. Afterwards, it was treated with a saturated aqueous solution of $NaHCO_3$ (130 mL) and the aqueous phase was extrated with CH_2Cl_2 (2 x 50 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The product was purified by flash column chromatography (silica gel, cyclohexane/MTBE 9:1 to 1:1) to give **16** (2.37 g, 43%, HPLC purity 95.6%) as a white solid. MS-ESI: 498.3 $[M+H]^+$. ^1H NMR (600 MHz, DMSO-d_6) δ = 8.17 (s, 1H, NH), 7.15 (s, 1H, H_3), 7.05 (d, *J* = 2.0 Hz, 1H, H_5), 4.61 (s, 2H, CH_2O), 3.45 (s, 2H, CH_2CO), 3.42 (s, 3H, CO_2CH_3), 3.14 (s, 3H, $C_{4'}OCH_3$), 3.09 - 3.03 (m, 1H, $H_{4'}$), 2.15 (s, 3H, C_6CH_3), 1.97 - 1.90 (m, 2H, 2 x H_{Cy}), 1.76 - 1.69 (m, 2H, 2 x H_{Cy}), 1.63 - 1.52 (m, 2H, 2 x H_{Cy}), 1.35 - 1.31 (m, 2H, 2 x H_{Cy}), 0.82 (s, 9H, $C(CH_3)_3$), 0.00 (s, 6H, 2 x $SiCH_3$). ^{13}C NMR (151 MHz, DMSO-d_6) δ = 174.2 ($\underline{C}O_2CH_3$), 169.2 (CONH), 142.5 (C_2), 139.8 (C_6), 131.0 (C_1), 130.9 (C_4), 127.9 (C_5), 123.3 (C_3), 77.0 ($C_{4'}$), 62.3 ($\underline{C}H_2O$), 57.7 ($C_{1'}$), 55.1 ($C_{4'}O\underline{C}H_3$), 51.9 ($CO_2\underline{C}H_3$), 34.0 ($\underline{C}H_2CO$), 29.8 ($C_{2'}$, $C_{6'}$), 26.7 ($C_{3'}$, $C_{5'}$), 26.0 ($C(\underline{C}H_3)_3$), 19.4 ($C_6\underline{C}H_3$) , 18.0 ($\underline{C}(CH_3)_3$), -5.3 (2 x $Si\underline{C}H_3$).



(*cis*)-3-{2-[((*tert*-Butyldimethylsilyl)oxy)methyl]-4-chloro-6-methylphenyl}-4-hydroxy-8-methoxy-1-azaspiro[4.5]dec-3-en-2-one (17). Over a solution of methyl (*cis*)-1-{2-[2-(((tert-butyldimethylsilyl)oxy)methyl)-4-chloro-6-methylphenyl]acetamido}-4-methoxycyclohexane-1-carboxylate **16** (0.4 g, 0.8 mmol, 1 equiv) in DMAc (10 mL) at 50 ºC, NaOH (46.8 mg, 1.2 mmol, 1.5 equiv) was added and the reaction was stirred overnight at this temperature. The reaction was poured to a mixture of a diluted aqueous solution of HCl and ice water (10 mL) and extracted with cyclohexane (4 x 15 mL). The combined organic phases were washed with water (2 x 15 mL), dried over $MgSO_4$ and concentrated under reduced pressure to give **17** (30 mg, 9%). MS-ESI: 464.3 $[M-H]^-$.

APPENDIX

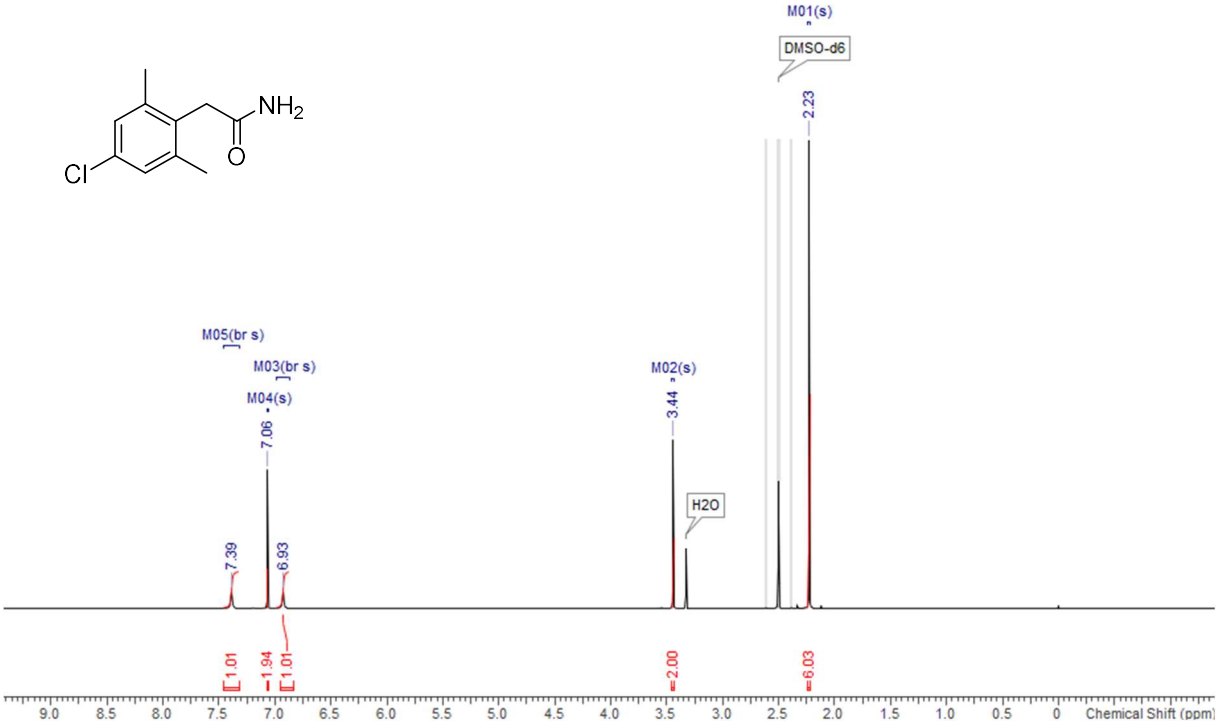


Figure 6. ^{1}H NMR (600 MHz, DMSO-d_6) for compound **2**.

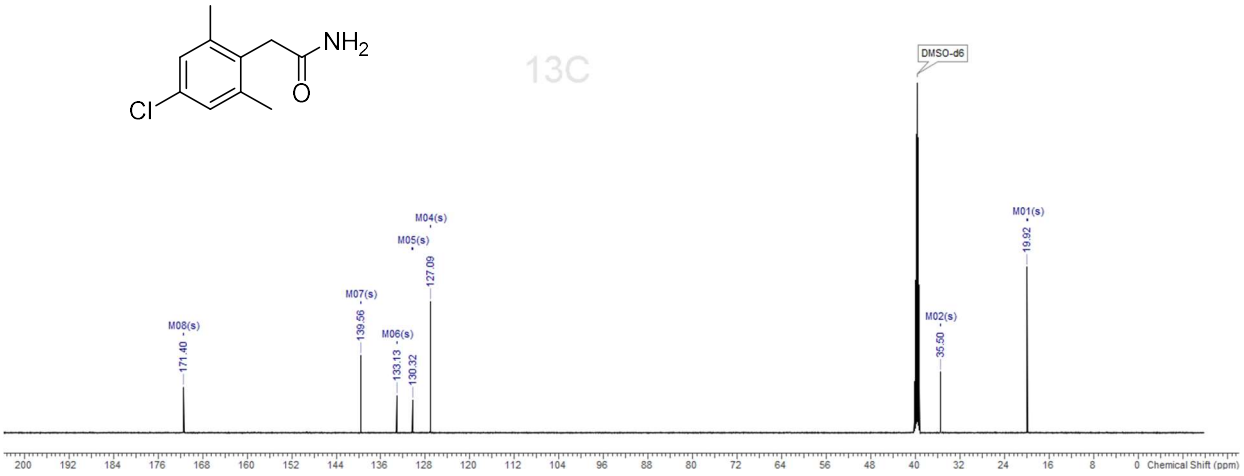


Figure 7. ^{13}C NMR (151 MHz, DMSO-d_6) for compound **2**.

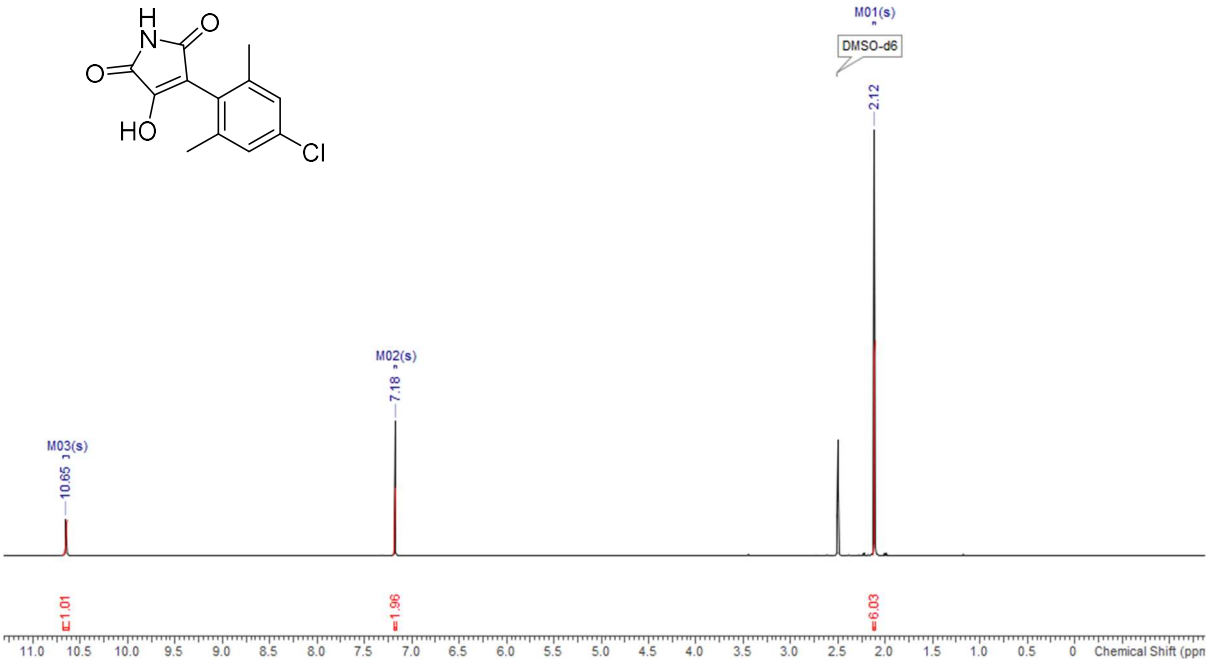


Figure 8. ^{1}H NMR (600 MHz, DMSO-d$_6$) for compound **1**.

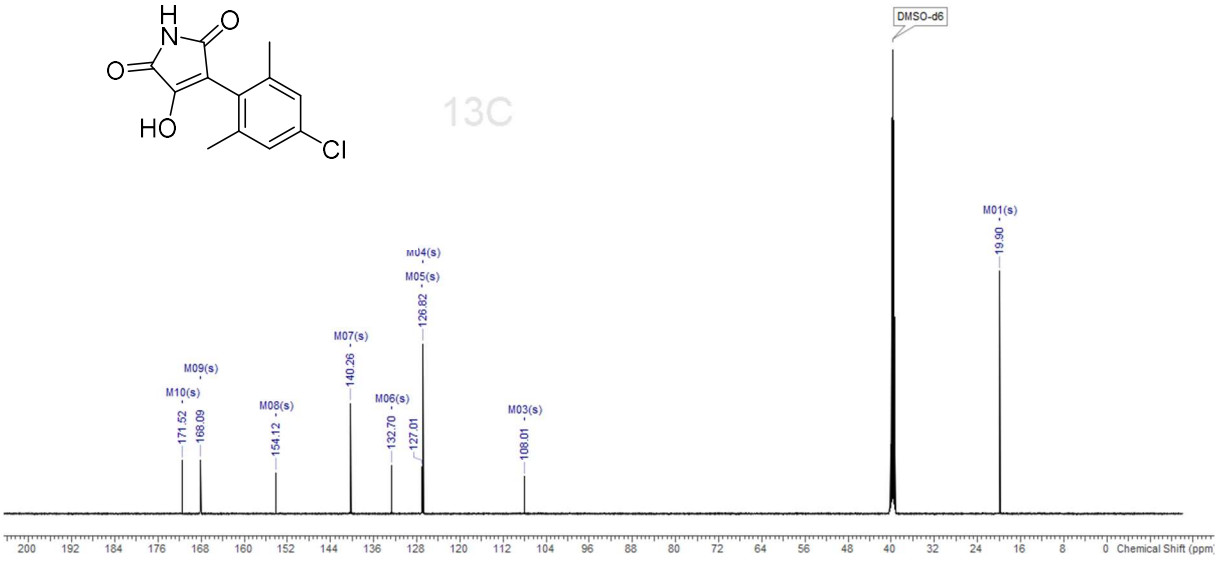


Figure 9. ^{13}C NMR (151 MHz, DMSO-d$_6$) for compound **1**.

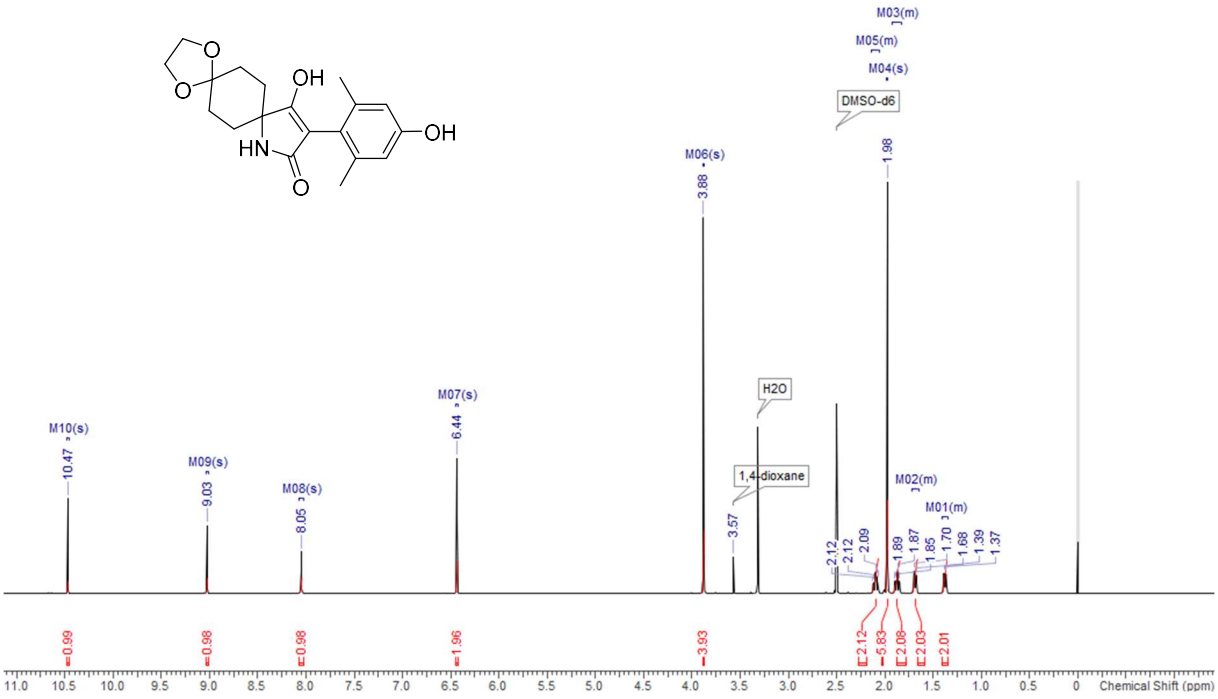


Figure 10. ^{1}H NMR (600 MHz, DMSO-d$_6$) for compound **3**.

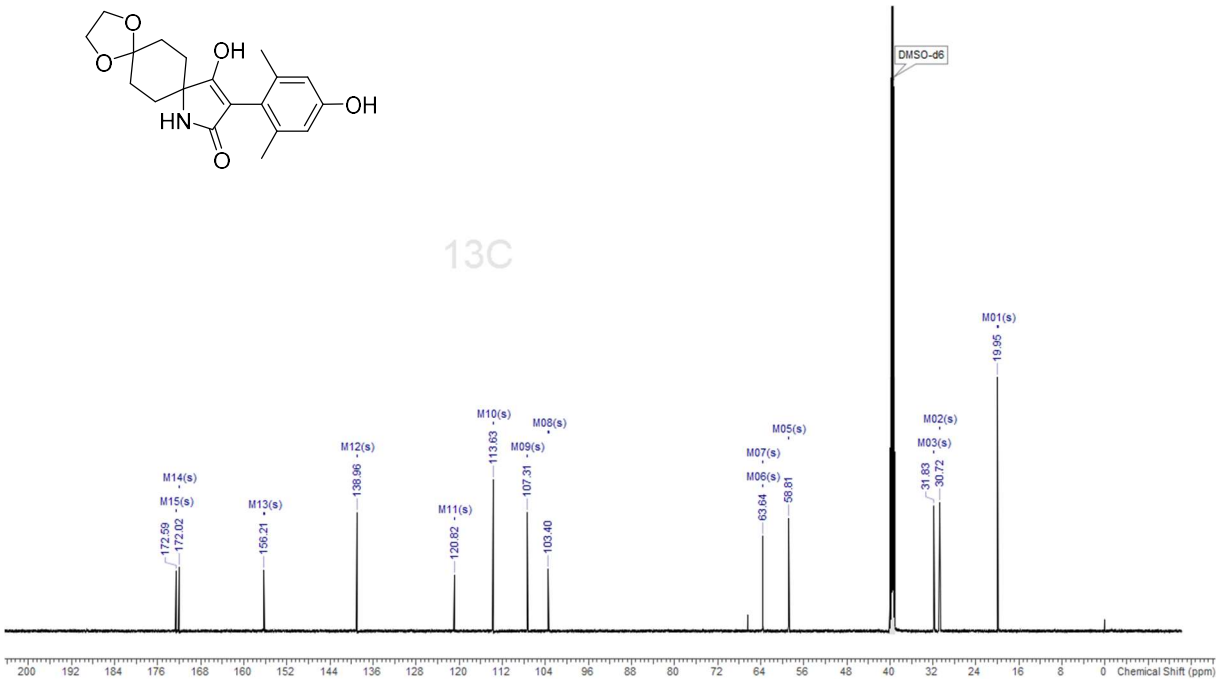


Figure 11. ^{13}C NMR (151 MHz, DMSO-d$_6$) for compound **3**.

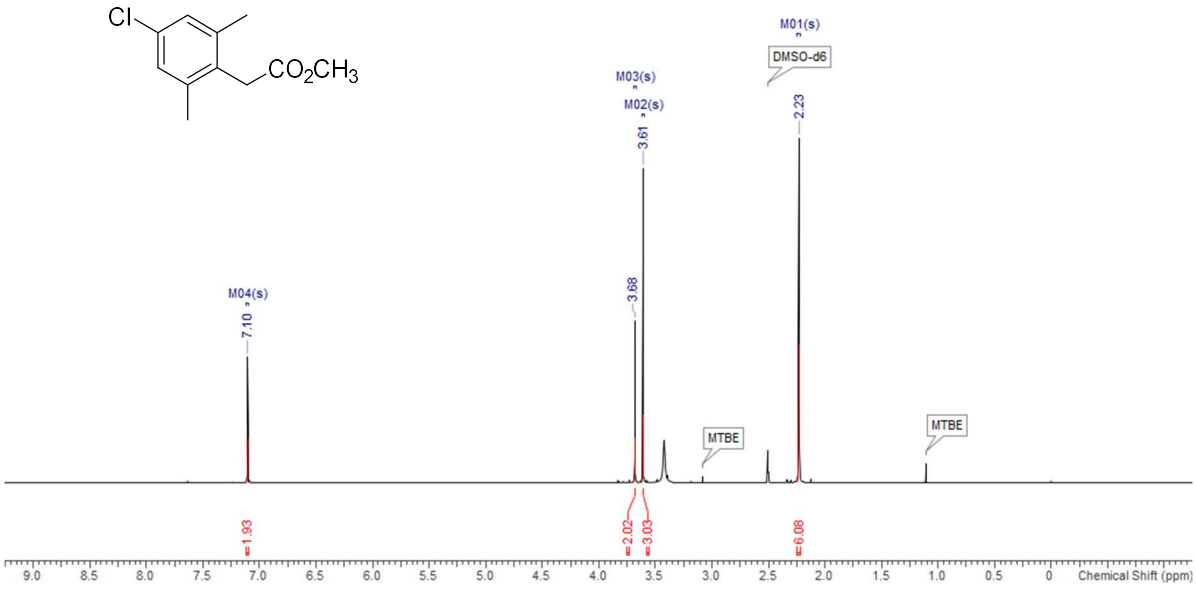


Figure 12. ^{1}H NMR (600 MHz, DMSO-d$_6$) for compound **5**.

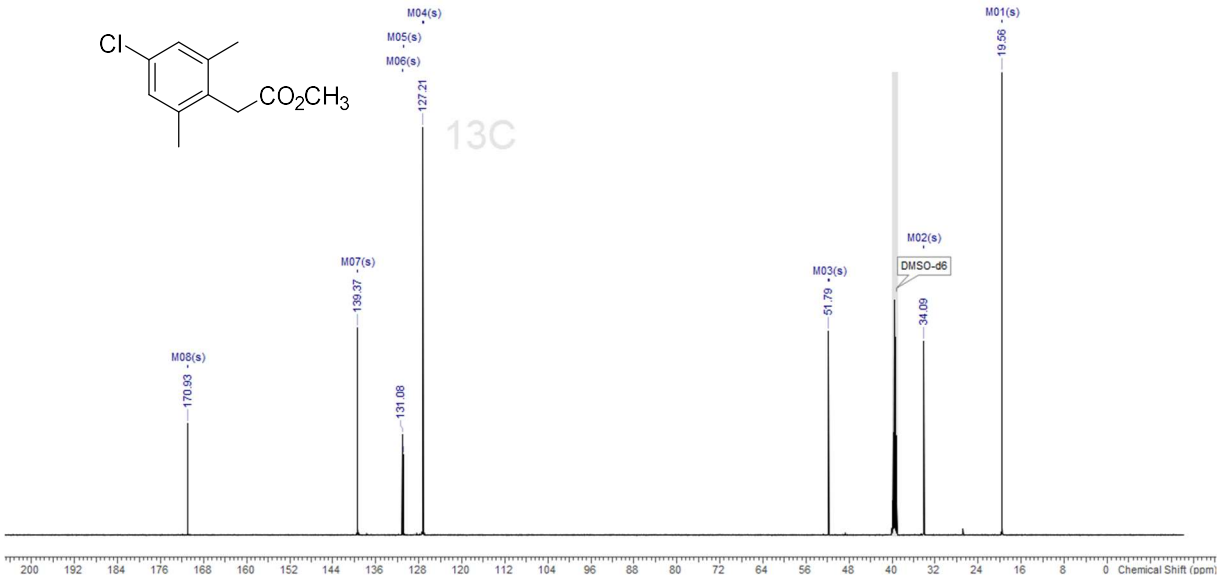


Figure 13. ^{13}C NMR (151 MHz, DMSO-d$_6$) for compound **5**.

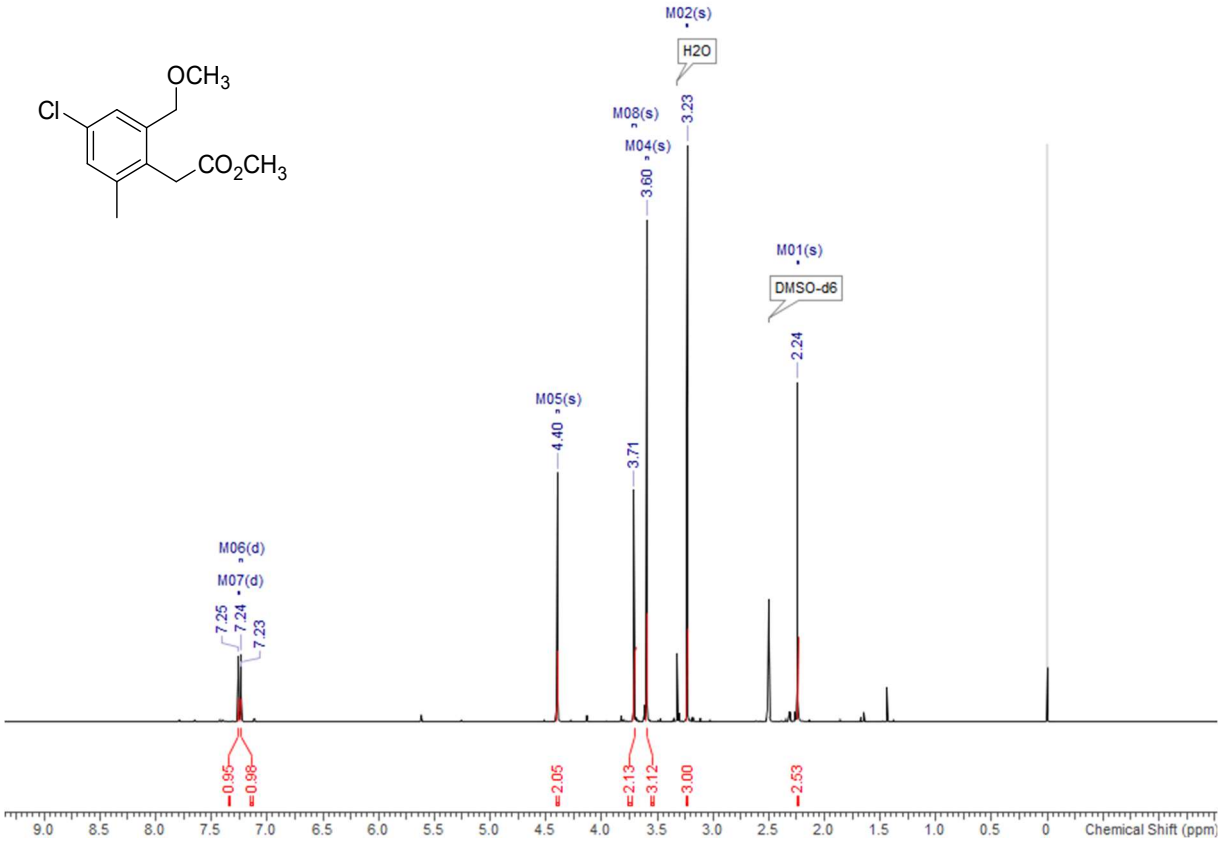


Figure 14. ^{1}H NMR (600 MHz, DMSO-d_6) for compound **7**.

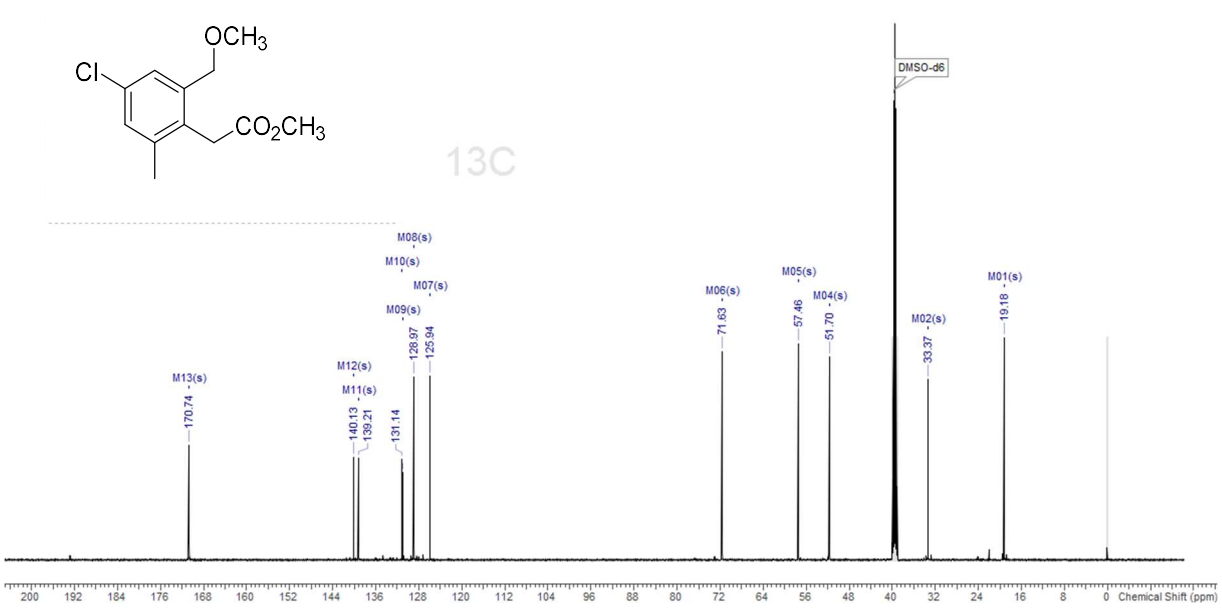


Figure 15. ^{13}C NMR (151 MHz, DMSO-d_6) for compound **7**.

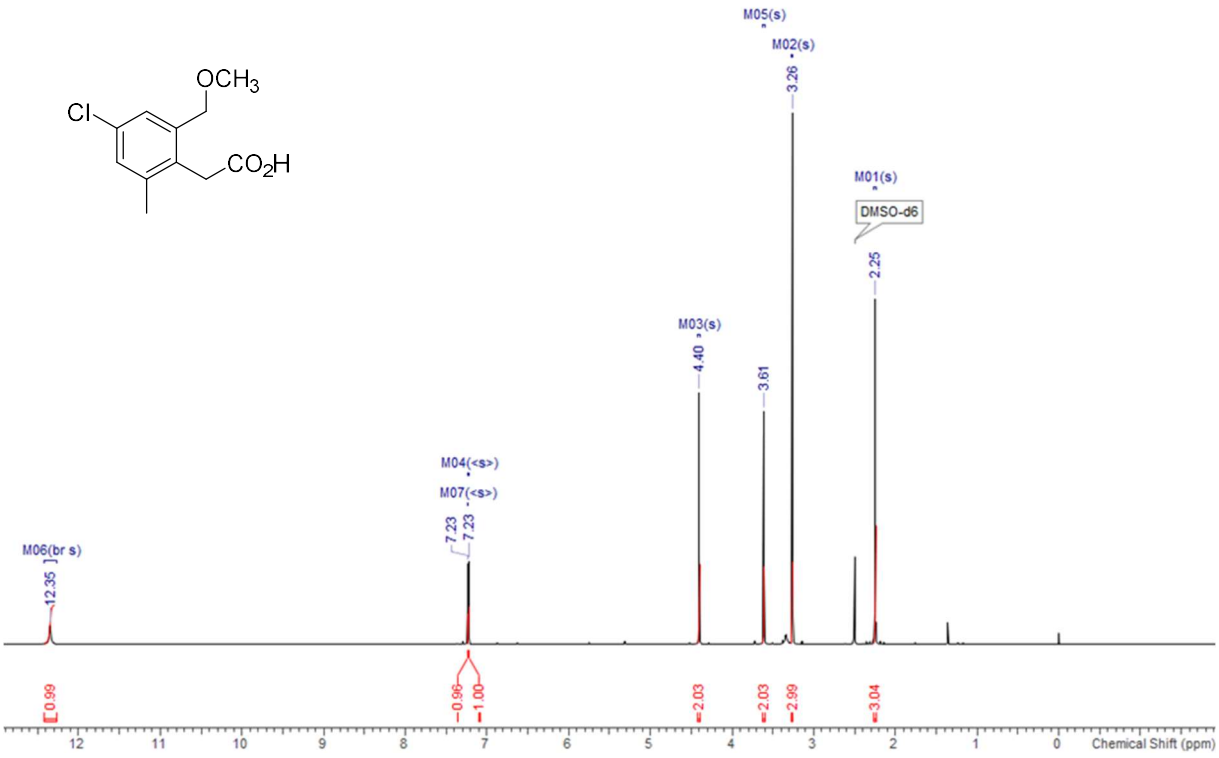


Figure 16. ^{1}H NMR (600 MHz, DMSO-d_6) for compound **8**.

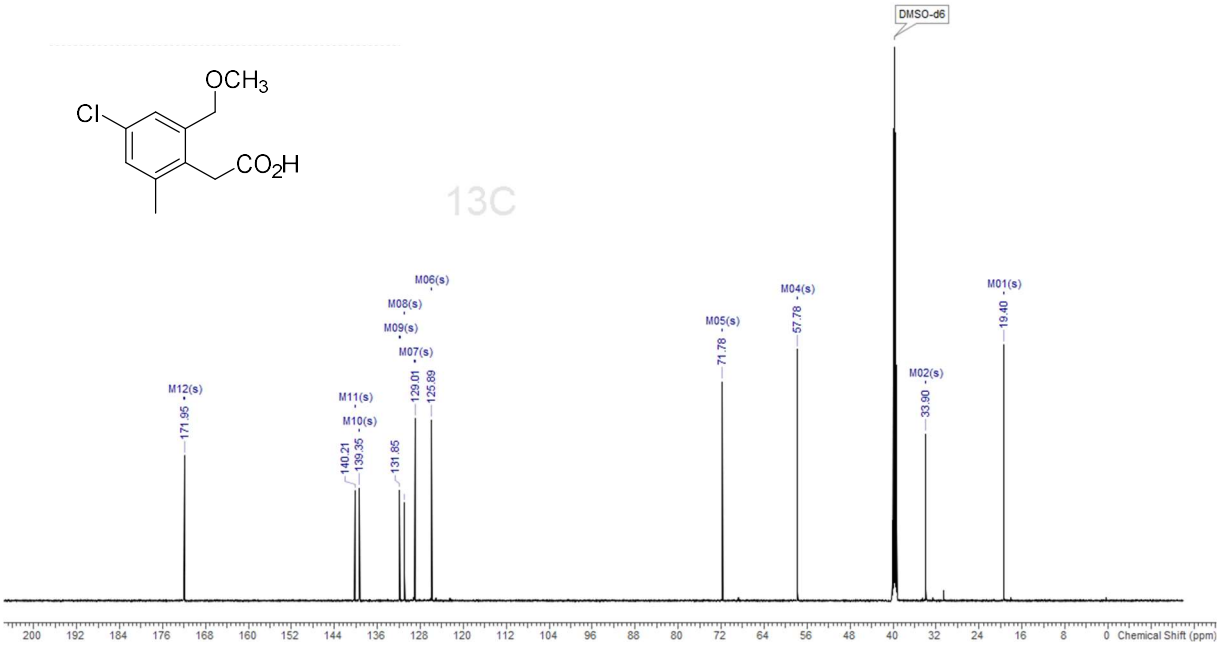


Figure 17. ^{13}C NMR (151 MHz, DMSO-d_6) for compound **8**.

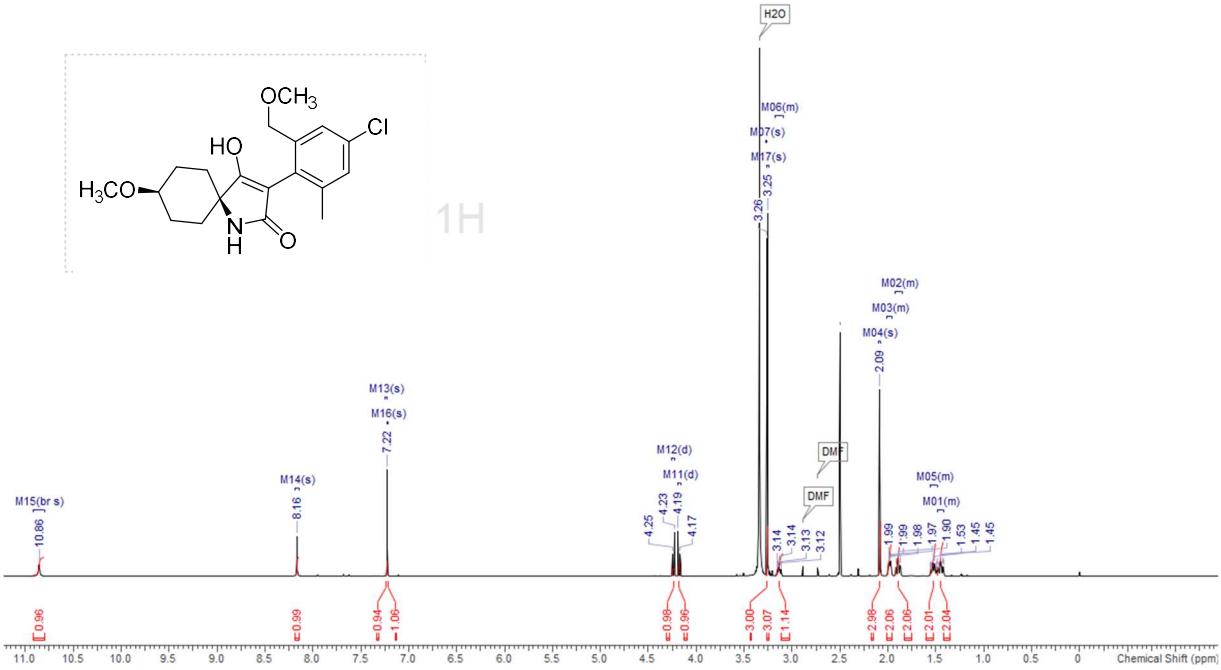


Figure 18. ^{1}H NMR (600 MHz, DMSO-d$_6$) for compound **11**.

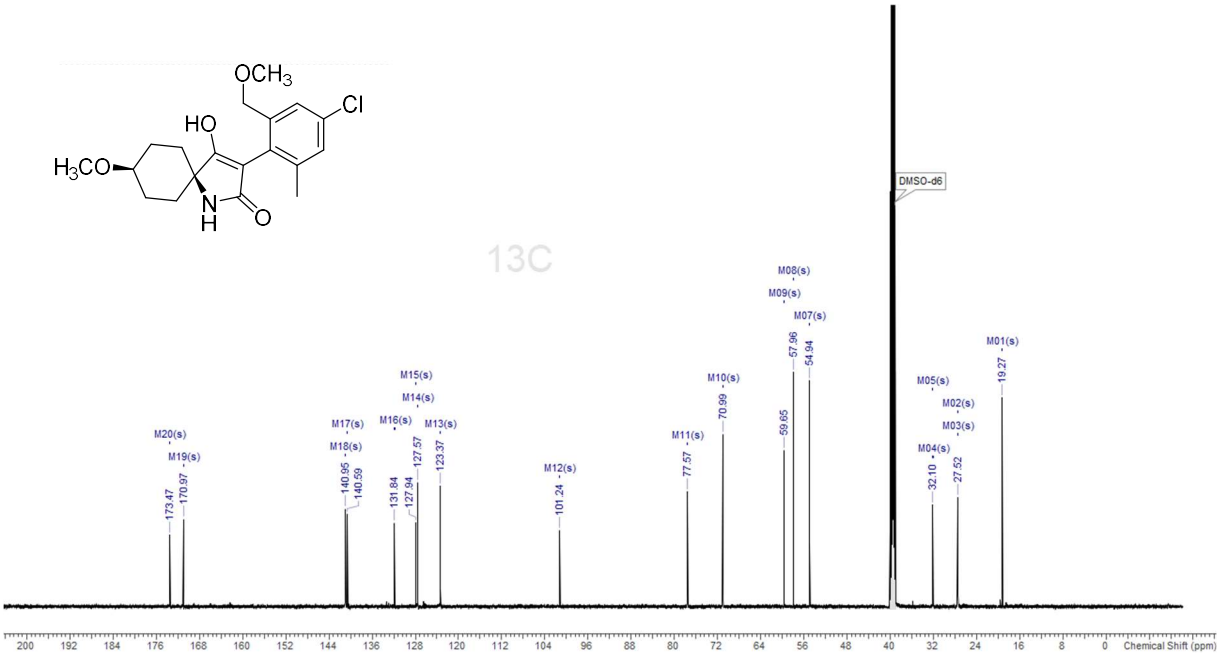


Figure 19. ^{13}C NMR (151 MHz, DMSO-d$_6$) for compound **11**.

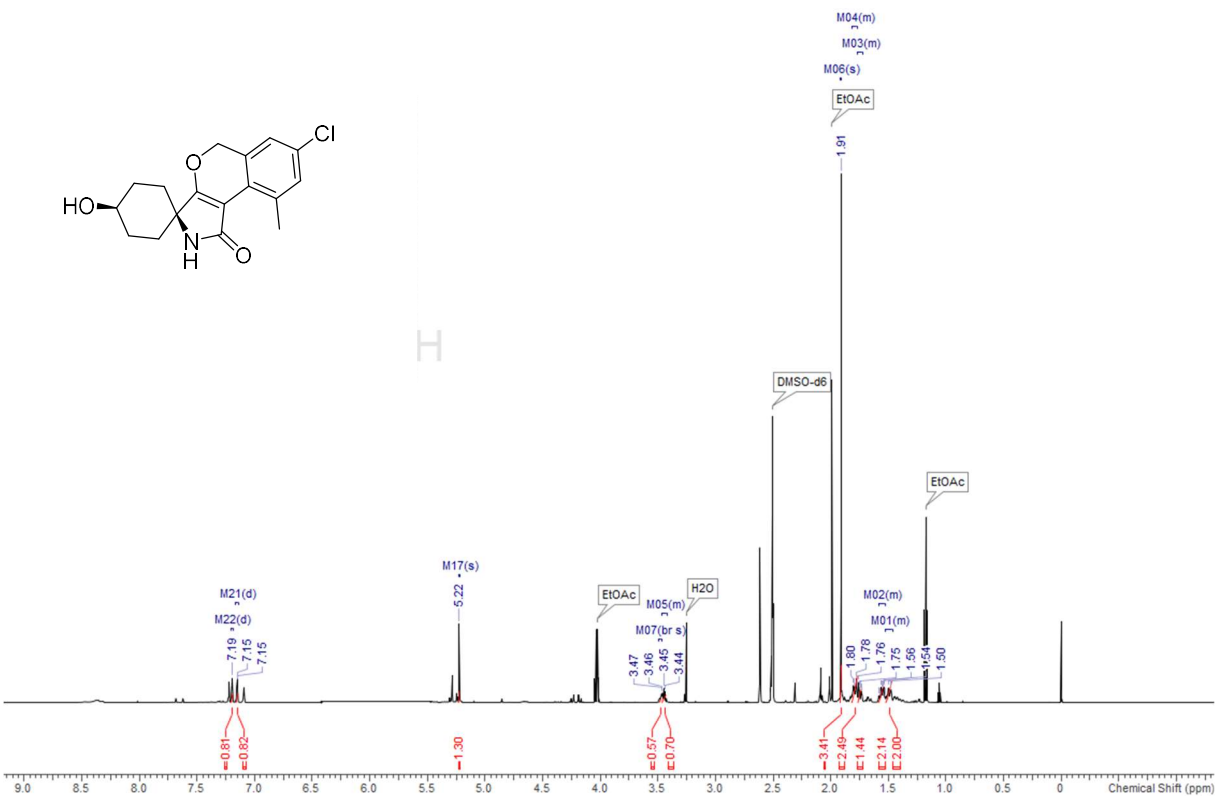


Figure 20. ^{1}H NMR (600 MHz, DMSO-d_6) for compound **12**.

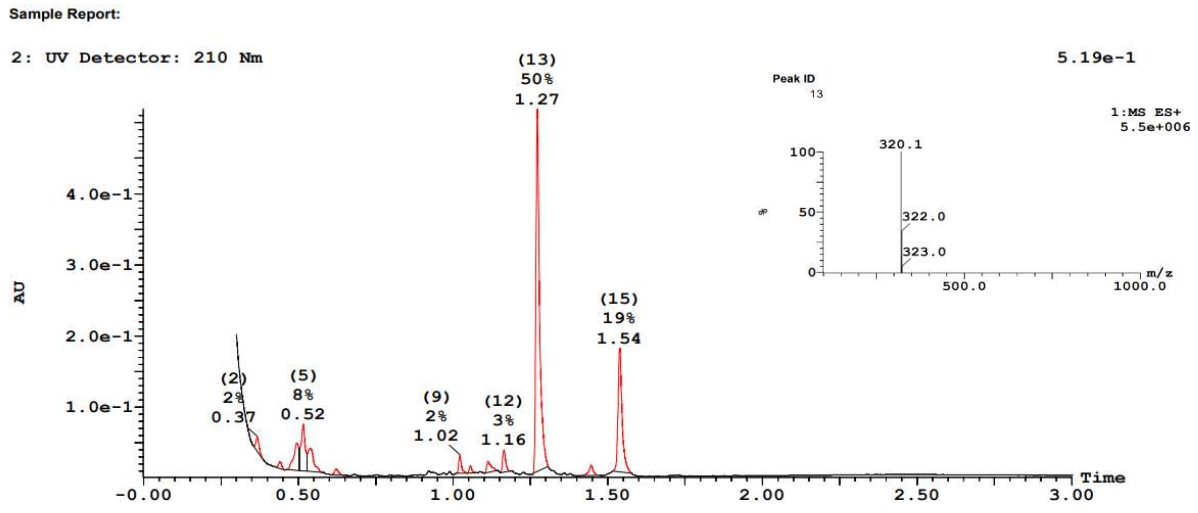


Figure 21. HPLC chromatogram together with MS-ESI in positive mode report for the major peak corresponding to compound **12**.

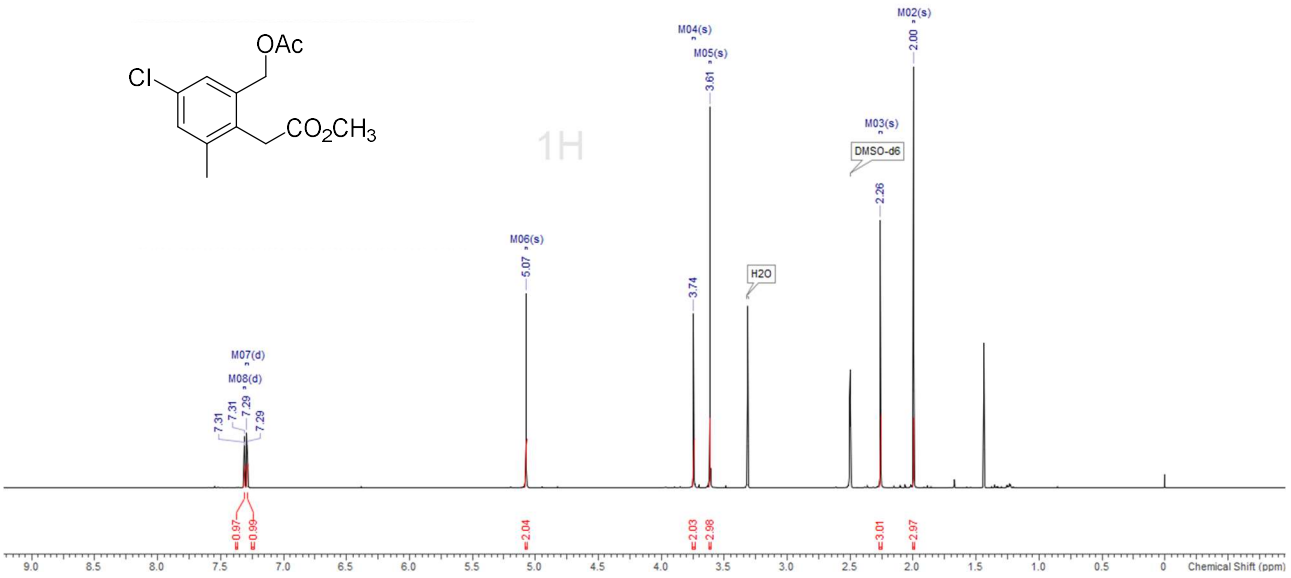


Figure 22. ^{1}H NMR (600 MHz, DMSO-d_6) for compound **13**.

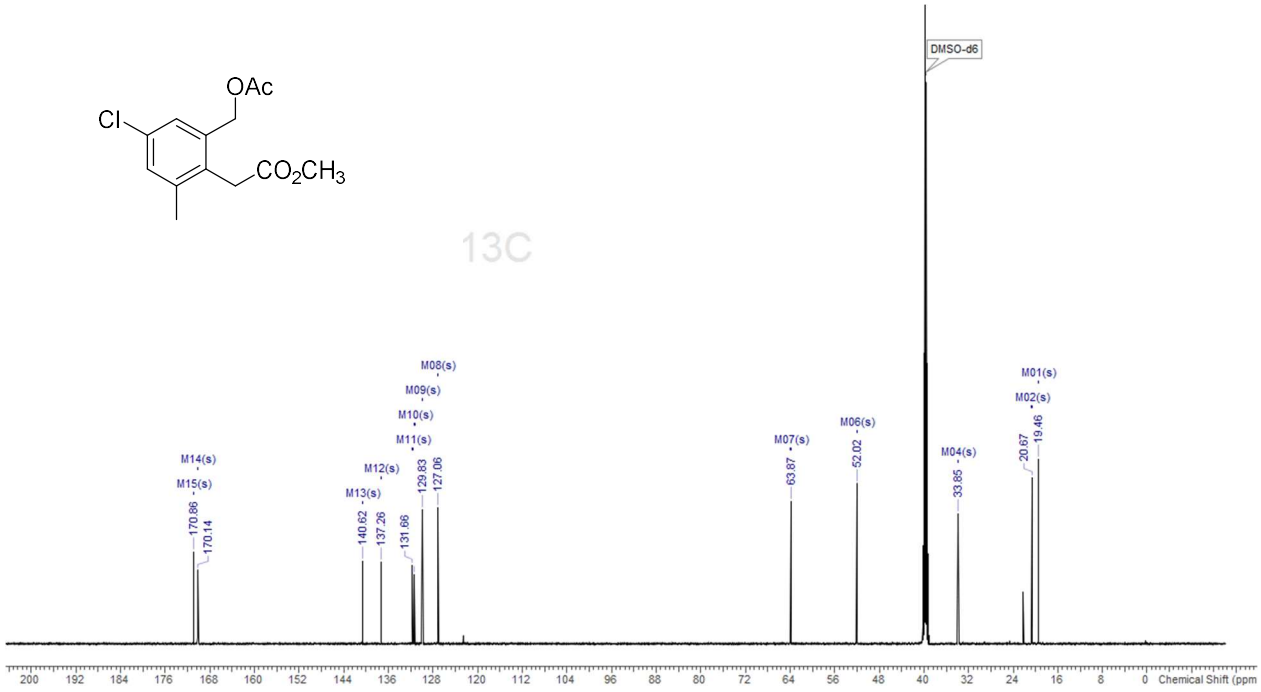


Figure 23. ^{13}C NMR (151 MHz, DMSO-d_6) for compound **13**.

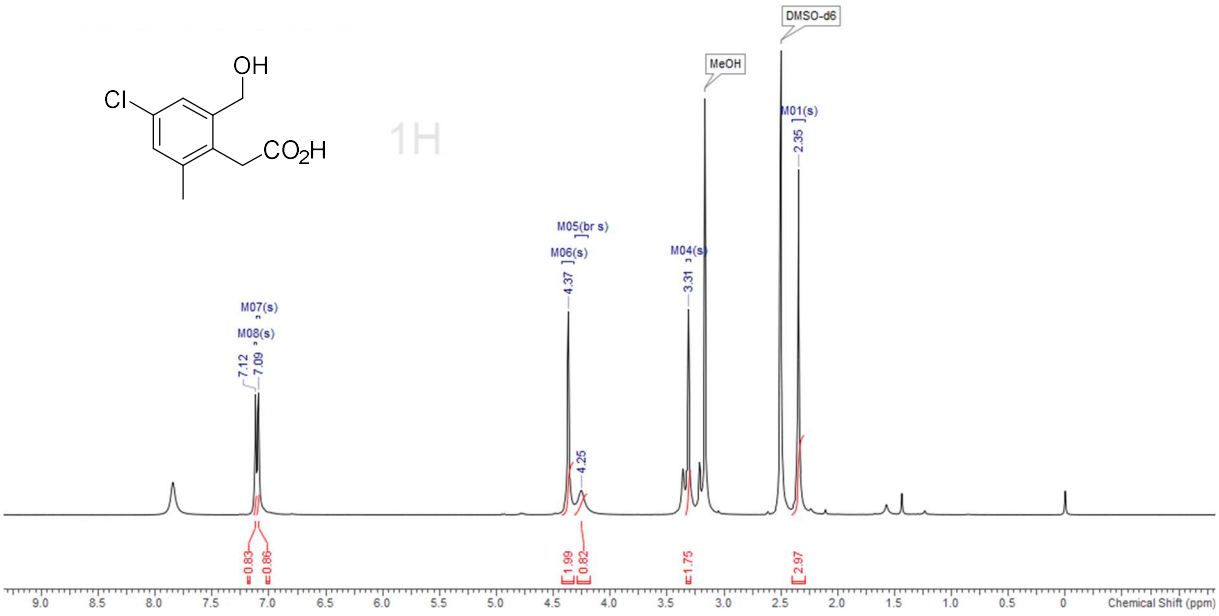


Figure 24. ^{1}H NMR (600 MHz, DMSO-d_6) for compound **14**.

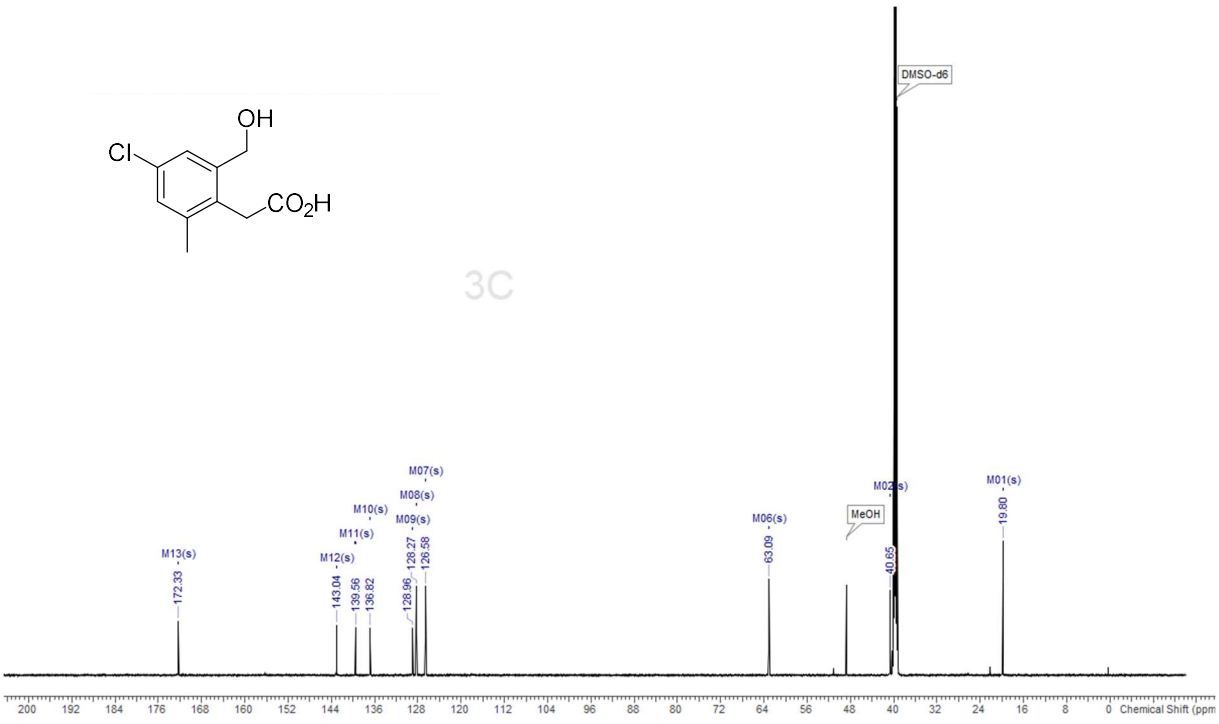


Figure 25. ^{13}C NMR (151 MHz, DMSO-d_6) for compound **14**.

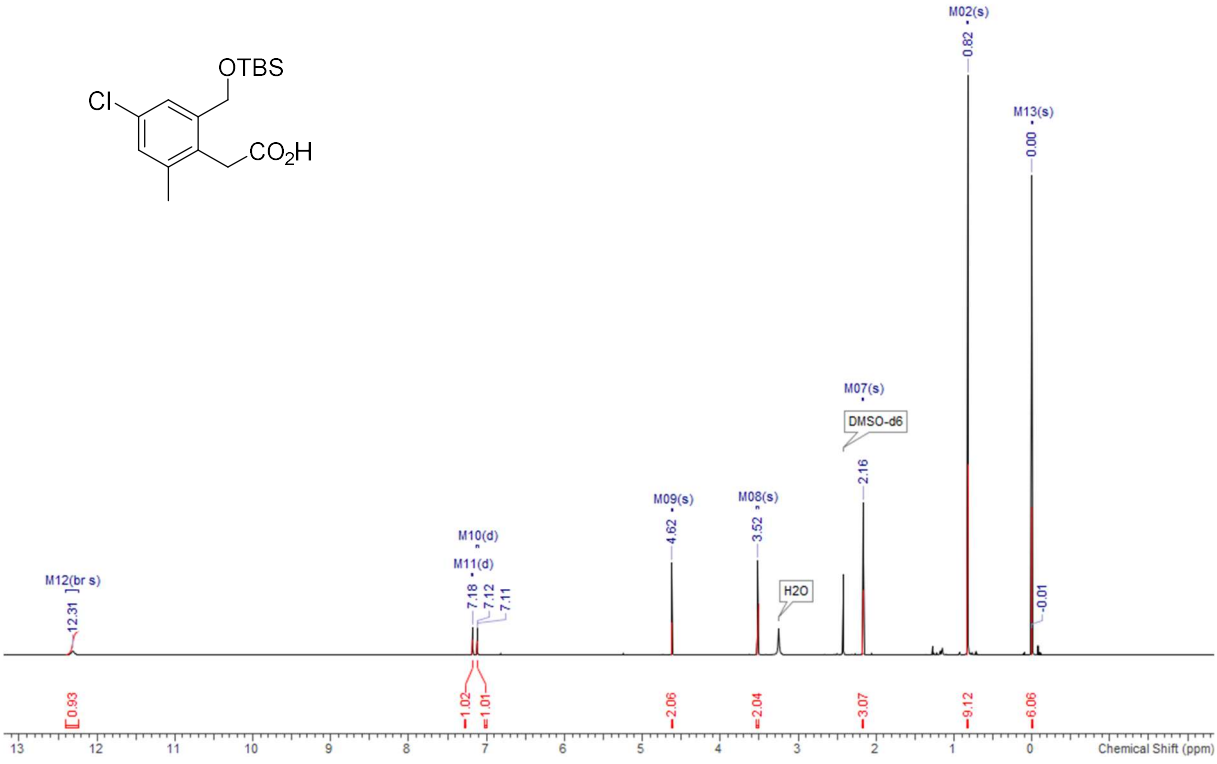


Figure 26. ^{1}H NMR (600 MHz, DMSO-d$_6$) for compound **15**.

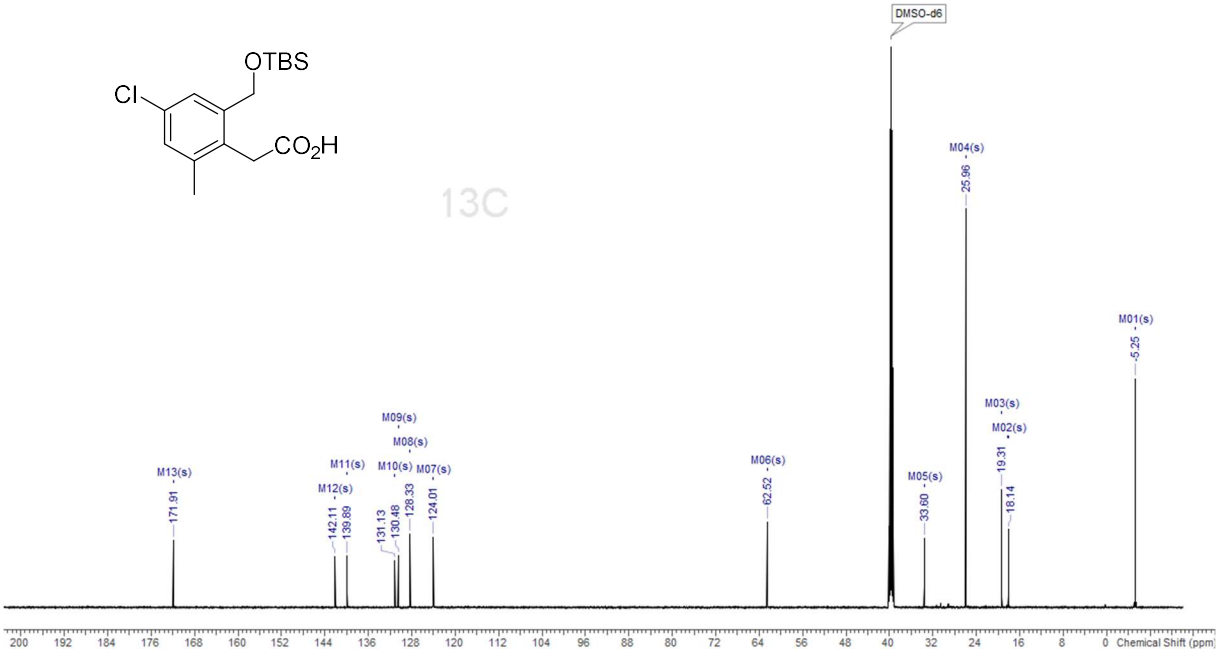


Figure 27. ^{13}C NMR (151 MHz, DMSO-d$_6$) for compound **15**.

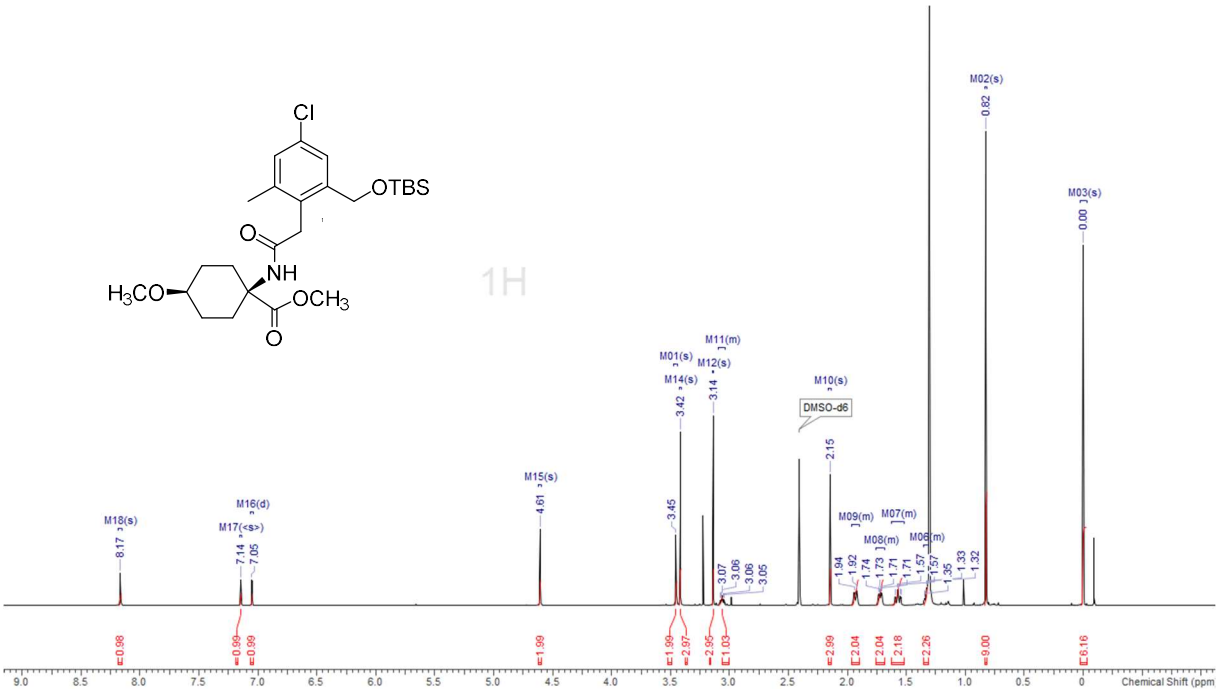


Figure 28. ^{1}H NMR (600 MHz, DMSO-d$_6$) for compound **16**.

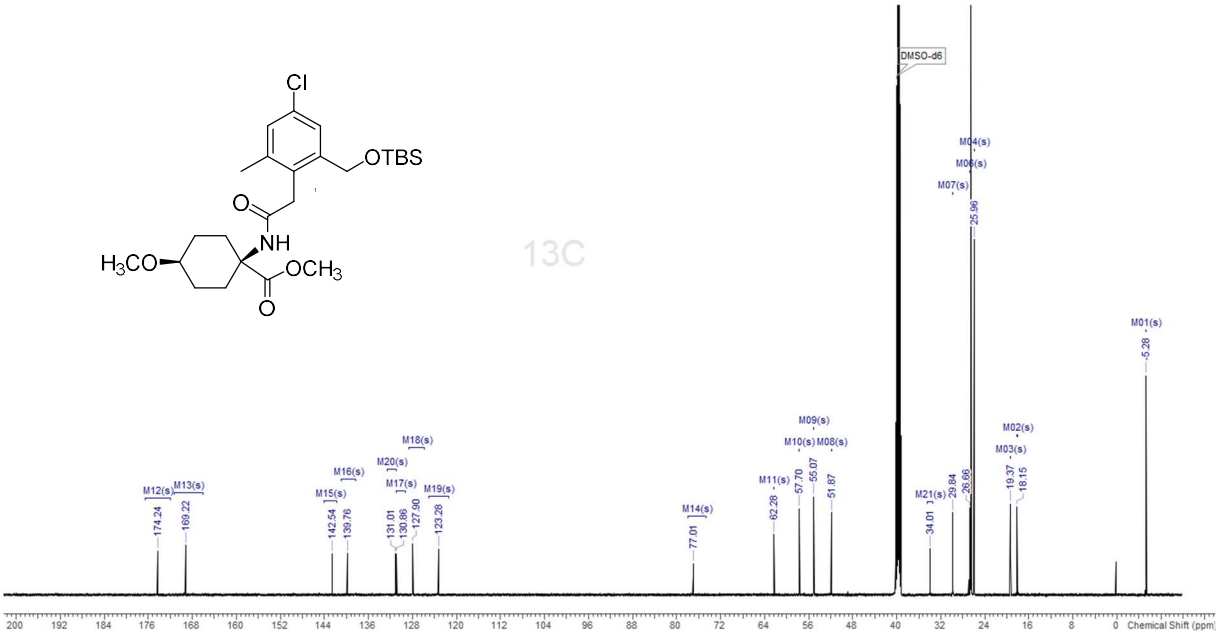


Figure 29. ^{13}C NMR (151 MHz, DMSO-d$_6$) for compound **16**.