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Synthesis of novel chiral spiro- β -lactams from nitrile oxides and 6-(*Z*)-(benzoylmethylene) penicillanate: batch, microwave-induced and continuous flow methodologies†

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The first examples of the diastereoselective 1,3-dipolar cycloaddition reaction of nitrile oxides and 6-alkylidene penicillanates leading to chiral spiroisoxazoline-penicillanates are reported. The synthesis of this new type of penicillanate involved the selective generation of two consecutive stereogenic centers, including a quaternary chiral center. Furthermore, the present work also describes the outcomes of these 1,3-dipolar cycloaddition reactions under three distinct reaction conditions (conventional heating, microwave irradiation and continuous flow). The successful use of the continuous flow technique as well as the proper selection of the reaction media allowed the development of a sustainable route to chiral spiroisoxazoline-penicillanates.

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Introduction

Since the discovery of penicillin, the β -lactam ring has been considered a very important moiety in synthetic and medicinal chemistry due to its highly synthetic versatility and biological properties.¹ Spiro- β -lactams are a subclass of β -lactams characterized by having an additional ring with the fusion of the two rings in one shared sp^3 carbon, the quaternary spiro carbon. Due to the tetrahedral nature of the spiro carbon, these molecules are endowed with a well-defined three-dimensional disposition, which allows an efficient interaction with a given molecular target. Therefore, these molecules have a wide spectrum of biological activities.²

The above-mentioned properties of β -lactams and spiro- β -lactams have attracted the interest of organic and medicinal chemists. Thus, many researchers have turned their focus to the development of alternative synthetic approaches aiming at the synthesis of novel spiro- β -lactam derivatives.³ The synthesis of chiral spirocyclic β -lactams derived from 6-aminopenicillanic acid (6-APA) has been explored by our research group, leading to a library of chiral spiro- β -lactams with significant structural diversity. Among the synthesized spiro- β -lactams, several were identified with potent activity against both HIV and *Plasmodium*, the causative agents of AIDS and malaria, respectively (Fig. 1).^{4,5} Infections by HIV and *Plasmodium* remain serious public health problems, preventing economic and social

progress in developing countries. The identification of spiro- β -lactams with potent activity against both infectious agents is particularly relevant due to considerable geographic overlap between HIV and *Plasmodium*, mainly in sub-Saharan Africa where co-infection is common, and the concerns regarding possible drug-drug interactions in patients receiving malaria and HIV treatment concomitantly.

The spiro- β -lactams synthesized by our research group were obtained by exploring 1,3-dipolar cycloaddition and [3 + 2] annulation reactions of 6-diazopenicillanates and 6-alkylidene penicillanates allowing the synthesis of novel chiral spiropenicillanates containing carbo- or heterocyclic rings, spirofused to the penicillin core.^{4,5} Using 6-alkylidenepenicillanates as 2 π -component, the phosphane-catalyzed [3 + 2] annulation with allenates or 2-butyneates, led to chiral spirocyclopentene- β -lactams,^{4b,5b,c} whereas 1,3-dipolar cycloaddition reactions with diazo compounds and nitrones afforded spiropyrazoline-penicillanates and spiroisoxazolidine-penicillanates, respectively (Scheme 1).^{5d,e}

The [3 + 2] nitrile oxide-alkene cycloaddition is an important and versatile synthetic route for the construction of isoxazoline rings.⁶ An important subclass of isoxazolines are the spiroisoxazolines, whose structural motif is present in natural occurring⁷ and synthetic compounds with biological activity, such as anticancer,^{7a,c,8} antibacterial,^{7b} antimalarial,⁹ or antiviral activity.¹⁰

Despite the known reports on the construction of the spiroisoxazoline core, to the best of our knowledge, few of them were dedicated to alkylidene- β -lactams. In late 80 s, Corbett and co-workers reported the synthesis of spirocarbapenems, from the 1,3-dipolar cycloaddition reaction of the corresponding

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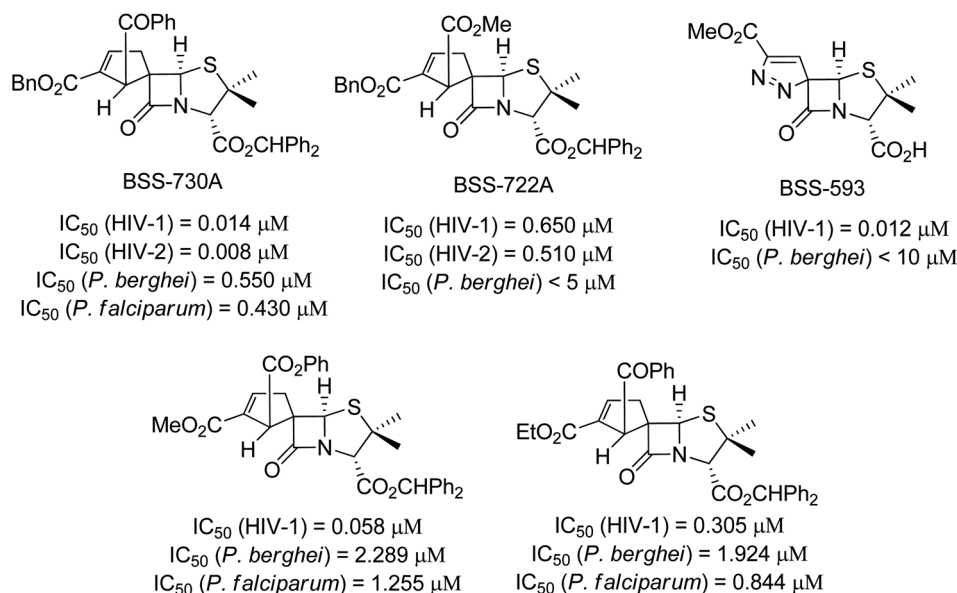
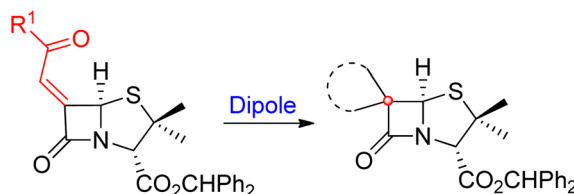
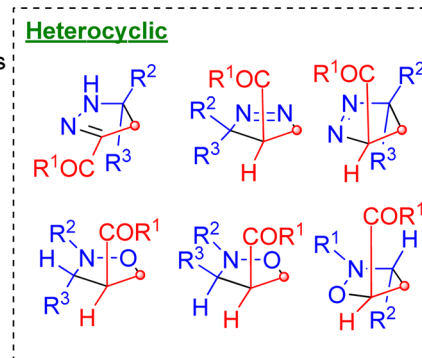
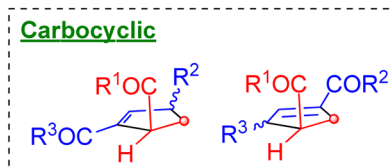


Fig. 1 Spiro- β -lactams with potent antimicrobial activity.

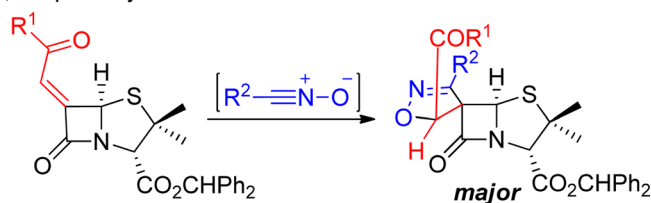
Previous work:



- Phosphane-catalyzed [3+2] cycloaddition of allenates or 2-butyneates (Pinho e Melo, 2013 & Pinho e Melo, 2021)
- 1,3-Dipolar cycloaddition with diazo compounds (Sheehan, 1978 & Pinho e Melo, 2014)
- 1,3-Dipolar cycloaddition with nitrones (Pinho e Melo, 2020)



This work: 1,3-Dipolar cycloaddition with nitrile oxides

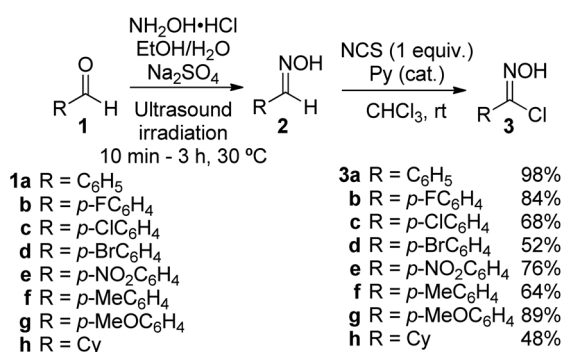


Scheme 1 Synthesis of different classes of chiral spiro- β -lactams from 6-alkylidene penicillanates.



alkylidene-carbapenem with a nitrile oxide.¹¹ Later, Otto and Liebscher's teams have explored the synthesis of spiro- β -lactams *via* 1,3-dipolar cycloaddition of nitrile oxides to α -alkylidene- β -monolactams.¹² Nevertheless, none of these studies were focused on the reactivity of alkylidenepenicillanates.

We envisaged that extending the library of spiropenicillanates by adding an isoxazoline ring, spiro-fused to the penicillin core's lactam ring, could lead to interesting new scaffolds with potential biological activity. In this context, the present work focused on the 1,3-dipolar cycloaddition reaction of a 6-alkylidenepenicillanate with a diversity of nitrile oxides as an approach to the first reported examples of chiral spiroisoxazoline-penicillanates, using batch, microwave irradiation and continuous flow techniques.



Scheme 2 Synthesis of hydroximoyl chlorides from aldehydes.

Results and discussion

1,3-Dipolar cycloaddition reactions *via* batch and microwave-induced methodologies

To explore the 1,3-dipolar cycloaddition of 6-(*Z*)-(benzoylmethylene)penicillanate to nitrile oxides, a library of nitrile oxide precursors, hydroximoyl chlorides **3**, was synthesized (Scheme 2). Initially, the reaction of hydroxylamine hydrochloride with the corresponding aromatic aldehydes **1a–g** was carried out in a mixture of ethanol/water with Na₂SO₄ as dehydrating agent under ultrasound irradiation. The condensation reaction led to aldoxime derivatives **2a–g** isolated with a simple workup procedure and used without further purification. Next, treatment of aldoximes **2** with *N*-chlorosuccinimide (NCS) in the presence of a catalytic amount of pyridine at room temperature afforded aromatic hydroximoyl chlorides **3a–g** in good to excellent yields, ranging from 52% to 98%. This synthetic approach also allowed the synthesis of aliphatic hydroximoyl chloride **3h** which was obtained in moderate yield (48%).

The 1,3-dipolar cycloaddition reaction of nitrile oxide **4a**, generated *in situ* from benzaldehyde hydroximoyl chloride **3a** by the action of potassium carbonate, with 6-(*Z*)-(benzoylmethylene)penicillanate **5** was studied for the optimization of the batch and microwave-induced reaction conditions (Table 1). The use of an inorganic base ensures a slow dehydrohalogenation of hydroximoyl chlorides **3**, allowing a controlled formation of the corresponding nitrile oxide **4** which readily reacts with the desired dipolarophile. Thus, the reaction of the transient nitrile oxide **4a** with 6-(*Z*)-(benzoylmethylidene)

Table 1 Optimization of the 1,3-dipolar cycloaddition approach to spiroisoxazoline-penicillanates under batch and under microwave irradiation conditions

Reaction scheme showing the 1,3-dipolar cycloaddition of nitrile oxide **4a** (generated from **3a** and base) with spiroisoxazoline-penicillanate **5** to form products **6a**, **7a**, and **8a**.

Reagents: **3a** (2 equiv.), Solvent, Base (5 equiv.), **4a**.

Products: **6a**, **7a**, and **8a**.

	Entry	Base	Solvent	Reaction conditions	Isolated yields (ratio) ^a		Overall yield
Batch	1	K ₂ CO ₃	AcOEt	r.t., 7.5 h	6a , 60%	7a/8a , 32% (66 : 34)	92%
	2	K ₂ CO ₃	AcOEt	r.t., 24 h	6a , 49%	—	49%
	3	K ₂ CO ₃	AcOEt	Reflux, 5 h	6a , 59%	7a/8a , 38% (84 : 16)	97%
	4	K ₂ CO ₃	Toluene	80 °C, 4 h	6a , 65%	7a , 32%	97%
	5	NEt ₃	AcOEt	r.t., 24 h	6a , 47%	7a/8a , 51% (63 : 37)	98%
MW	6	NEt ₃	Toluene	80 °C, 4 h	6a , 11%	7a/8a , 37% (38 : 62)	48%
	7	K ₂ CO ₃	AcOEt	80 °C, 30 min	6a , 32%	7a , 28%	60%
	8	K ₂ CO ₃	AcOEt	80 °C, 1 h	6a , 35%	7a , 34%	69%
	9	K ₂ CO ₃	Toluene	80 °C, 30 min	6a , 42%	7a , 32%	74%
	10	K ₂ CO ₃	Toluene	120 °C, 10 min	—	—	— ^b
	11	NEt ₃	Toluene	80 °C, 30 min	Complex mixture		—

^a Ratio determined by ¹H NMR. ^b Trace amount of **6a**.



penicillanate **5** was carried out in ethyl acetate at room temperature for 7.5 h affording compound **6a** as major product in 60% yield. From this reaction an inseparable mixture of compounds **7a** and **8a** was also isolated in 32% yield (66:34 ratio) (entry 1). The increase of the reaction time to 24 h was counterproductive regarding the overall yield but led to compound **6a** as single product isolated in 49% yield (entry 2). However, better results were obtained by increasing the temperature. Carrying out the reaction of **4a** with **5** under refluxing ethyl acetate the desired products were obtained in excellent overall yield (97%) with a good selectivity for compound **6a** which was isolated in 59% yield (entry 3). Curiously, the same overall yield was obtained by performing the reaction in toluene at 80 °C (entry 4) with a slight increase in the yield of **6a** (entry 3: 59% vs. entry 4: 65%) and without the formation of **8a**. Additionally, an alternative base for the dehydrohalogenation of hydroximoyl chloride **3a** was also explored. Performing the reaction in the presence of triethylamine, the target spiro- β -lactams could also be obtained by carrying out the reaction either in ethyl acetate (in excellent overall yield, entry 5) or in toluene (in moderate overall yield, entry 6).

The reaction was also carried out under microwave irradiation (entries 7–11). It was observed that carrying out the reaction in ethyl acetate under microwave irradiation for 30 minutes at 80 °C afforded compounds **6a** and **7a** in 32% and 28% yield, respectively (entry 7). The yields were slightly improved using the same conditions for 1 hour, giving products **6a** and **7a** in 35% and 34% yield, respectively (entry 8). Changing the reaction solvent to toluene allowed the synthesis of **6a** and **7a** in 74% overall yield after 30 minutes of microwave irradiation at 80 °C, with compound **6a** being obtained as major product in 42% yield (entry 9). On the other hand, by increasing the temperature to 120 °C for 10 min, the consumption of the reagents was observed, but only a trace amount of **6a** was detected (entry 10). Finally, carrying out the microwave-induced reaction in toluene at 80 °C for 30 min using triethylamine as the dehydrohalogenating agent, a complex mixture was obtained. It should be noted that the cycloaddition reaction between alkylidenepenicillanate **5** and nitrile oxide **4a** under microwave irradiation proved to be more selective, without the formation of spiro- β -lactam **8a**.

The structural assignment of compounds **6a** and **7a** was supported by one and two-dimensional NMR spectra (^1H NMR, ^{13}C NMR and HSQC; see ESI†). As expected, the ^1H NMR spectrum of derivatives **6a** and **7a** show signals corresponding to two methyl groups from the penicillanate core, protons

corresponding to aromatic protons and to the benzhydryl proton (CHPh_2 , **6a**: 6.98 ppm; **7a**: 6.93 ppm). The ^1H NMR and HSQC spectra allowed us to assign the signals corresponding to protons H-3, H-5 of the penicillanate core and the proton of the isoxazoline ring of both compounds (Fig. 2). For compound **6a** the following chemical shifts were observed: H-3 at 4.65 ppm, H-5 at 5.86 ppm and isoxazoline proton at 6.17 ppm. Major difference was observed for the proton of the isoxazoline ring in the case of compound **7a** with a chemical shift of 5.67 ppm. This difference can be explained by the presence of an oxygen atom in a vicinal position to the proton in compound **6a** which promotes a downfield shift. Additionally, from the HSQC spectrum it was possible to confirm the assignment of the spirocyclic carbon of both spiro- β -lactams (**6a**: 76.6 ppm; **7a**: 102.0 ppm). The difference in the chemical shift of the spirocyclic carbons of these two regioisomers can be explained considering that only in the case of compound **7a** is the oxygen atom attached to the spirocyclic carbon. This NMR data is in agreement with the proposed structures of spiro- β -lactams **6a** and **7a** resulting from opposite regioselectivity, with **6a** and **7a** being a spiro[isoxazoline-4',6'-penicillanate] (major) and a spiro[isoxazoline-5',6'-penicillanate], respectively. It is known that due to the bicyclic β -lactam-thiazolidine ring system of the penicillin core which exists in a butterfly-like structure, the approach of a given reactant usually occurs by the convex face (α -side) of the penicillin derivative.^{3–5} This is in agreement with the observed stereoselectivity, with both compounds **6a** and **7a** resulting from the addition of the dipole to the less sterically hindered α -side of the penicillanates. The stereoselectivity observed in the formation of compound **8a** can be rationalized by an approach of the dipole through the β -side of the penicillanate, with the regioselective formation of the cycloadduct where the dipole's oxygen adds to the forthcoming spirocyclic carbon. The ^{13}C chemical shift of the spirocyclic carbons are similar for compounds **7a** and **8a** (**7a**: 102.2 ppm; **8a**: 100.1 ppm), as expected for diastereoisomers with the same regiochemistry.

The same stereo- and regioselectivity was previously described by Corbett, while exploring the 1,3-dipolar cycloaddition reaction of nitrile oxides with an alkylidene-carbapenem, aiming at the synthesis of spiroisoxazoline-carbapenem.¹¹ In this work, it was observed that three products were obtained from this cycloaddition reaction. Two of them were the expected cycloadducts from the addition of the dipole to the α -side of the carbapenem: one results from the attack of the dipole's oxygen to the terminal carbon of the exocyclic double bond of the alkylidene (major product) and the other from the addition of

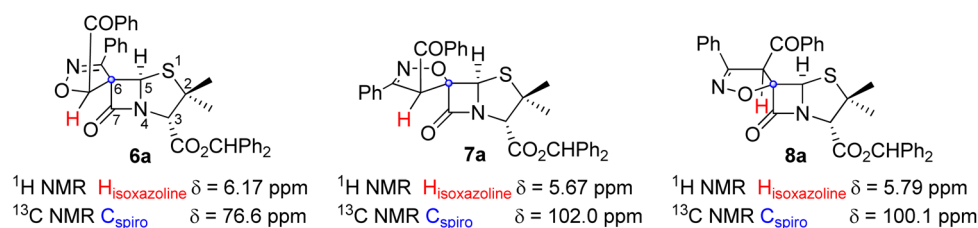


Fig. 2 Structures of selected spiro- β -lactams highlighting the most relevant ^1H and ^{13}C chemical shifts for the stereochemistry assignment.



the dipole's oxygen to the forthcoming spirocyclic carbon. The formation of a third cycloadduct was also observed, a diastereoisomer formed by the addition of the nitrile oxide to the β -side. It should be noted that the carbapenem bicyclic system is similar to the penicillanic core in terms of preferred conformation, leading to a more favorable addition of incoming reactants by the α -face.

Although a small difference was observed in the isolated yields of products of the 1,3-dipolar cycloaddition of 6-(*Z*)-(benzoylmethylene)penicillanate and phenyl-nitrile carrying out the reactions in ethyl acetate or in toluene, ethyl acetate was selected for further studies since it is a "greener" solvent than toluene.¹³ On the other hand, potassium carbonate was selected over triethylamine also aiming at developing a more sustainable synthetic methodology.

Next, we extended the study of the reactivity of 6-(*Z*)-(benzoylmethylidene)penicillanate (**5**) to other nitrile oxides under batch and microwave irradiation optimized reaction conditions. However, reactions carried out under conventional heating were monitored by TLC and the reaction time determined by the total consumption of alkylidenepenicillanate **5**, ranging from 15 minutes to 8 hours.

The 1,3-dipolar cycloaddition reactions with nitrile oxides bearing electron-withdrawing and electron-donating substituents at *para*-position of the phenyl ring were explored (Table 2). To our delight, both types of dipole activation led to the

expected cycloadducts, with pure chiral spiroisoxazoline- β -lactams **6b–f** being obtained as major products, in yields ranging from 49–72% and 31–47%, under conventional heating and microwave irradiation, respectively (entries 1–12).

The reaction of alkylidenepenicillanate **5** with nitrile oxide **4b** ($R = p\text{-FC}_6\text{H}_4$) under conventional heating was highly selective, leading to the exclusive formation of a single product (**6b**) in 72% yield (entry 1). On the other hand, performing the same reaction under microwave irradiation (entry 2) led to the synthesis of the same spiro- β -lactam **6b** (39% yield) together with spiro- β -lactams **7b/8b** (32% yield). Carrying out the cycloaddition reaction with *p*-chloro or *p*-bromo substituted nitrile oxides (**4c** and **4d**, respectively) the corresponding spiroisoxazoline- β -lactams **6** (**6c** and **6d**) were obtained as major products under both methodologies in yields ranging from 41% to 49% (entries 3–6). From these reactions, mixtures of isomeric compounds **7c/8c** and **7d/8d** were also obtained leading to overall yields up to 92% (entries 3–6). The cycloaddition reaction of nitrile oxide **4e**, bearing a nitro group at the *para* position of the aromatic ring, with alkylidenepenicillanate **5** was also explored under the optimized conditions (entries 7 and 8). This reaction allowed the synthesis of **6e** as major product in 54% yield under conventional heating (entry 7) as well as under microwave conditions, with compound **6e** being obtained in 47% yield (entry 8). In both reactions, mixture of compounds **7e/8e** were also obtained in 32–35% yield (entries 7 and 8).

Table 2 1,3-Dipolar cycloaddition of 6-(*Z*)-(benzoylmethylidene)penicillanate **5** with nitrile oxides **4**

Reaction scheme showing the 1,3-dipolar cycloaddition of 6-(Z)-(benzoylmethylidene)penicillanate (**5**) with nitrile oxides (**3**) to form spiroisoxazoline- β -lactams (**6**, **7**, and **8**).

Reagents: **3** (2 equiv.), **4** ($R-C\equiv N-O^-$), EtOAc, K_2CO_3 .

Conditions: Batch: reflux; MW: 80 °C.

Entry	4	R	Methodology	Reaction time (h)	Isolated yields (ratio) ^a	Overall yield	
1	4b	<i>p</i> -FC ₆ H ₄	Batch	6	6b , 72%	7b , trace amounts	72%
2	4b	<i>p</i> -FC ₆ H ₄	MW	1	6b , 39%	7b/8b , 32% (84 : 16)	71%
3	4c	<i>p</i> -ClC ₆ H ₄	Batch	8	6c , 49%	7c/8c , 38% (71 : 29)	87%
4	4c	<i>p</i> -ClC ₆ H ₄	MW	1	6c , 41%	7c/8c , 29% (90 : 10)	70%
5	4d	<i>p</i> -BrC ₆ H ₄	Batch	2	6d , 49%	7d/8d , 43% (65 : 35)	92%
6	4d	<i>p</i> -BrC ₆ H ₄	MW	1	6d , 44%	7d/8d , 33% (58 : 42)	77%
7	4e	<i>p</i> -NO ₂ C ₆ H ₄	Batch	2	6e , 54%	7e/8e , 35% (34 : 66)	98%
8	4e	<i>p</i> -NO ₂ C ₆ H ₄	MW	1	6e , 47%	7e/8e , 32% (50 : 50)	79%
9	4f	<i>p</i> -MeC ₆ H ₄	Batch	5	6f , 50%	7f/8f , 38% (68 : 32)	88%
10	4f	<i>p</i> -MeC ₆ H ₄	MW	1	6f , 31%	7f/8f , 20% (85 : 15)	51%
11	4g	<i>p</i> -MeOC ₆ H ₄	Batch	0.5	6g/8g , 52% (69 : 31)		52%
12	4g	<i>p</i> -MeOC ₆ H ₄	MW	0.25	6g/8g , 36% (69 : 31)	7g , 21%	57%
13	4h	Cy	Batch	6	6h , 79%	7h/8h , 15% (17 : 83)	94%
14	4h	Cy	MW	1	6h , 60%	7h/8h , 10% (10 : 90)	70%

^a Ratio determined by ¹H NMR.



Notably, concerning the overall yields, the 1,3-dipolar cycloaddition reaction between **4e** and **5** was the most efficient under both conventional heating and microwave irradiation (overall yields: 98% and 79%, respectively).

The cycloaddition reaction of spiro- β -lactam **5** with *para*-methyl derivative **4f** also afforded the expected major spiroisoxazoline- β -lactam **6f** in 50% yield under conventional heating (entry 9), together with spiro- β -lactams **7f/8f** (38% yield). However, under microwave irradiation (entry 10) the overall yield of this reaction was modest, leading to compounds **6f** and **7f/8f** in 31% and 20% yield, respectively.

The 1,3-dipolar cycloaddition using as 1,3-dipole nitrile oxide **4g**, bearing a stronger electron-donating group ($R = p\text{-MeOC}_6\text{H}_4$), was extremely fast under conventional heating (15 minutes) leading to an inseparable mixture of **6g/8g** in 52% yield (entry 11). Under microwave irradiation for 30 minutes the same mixture (**6g/8g**) was isolated in 36% yield, together with pure spiro- β -lactam **7g** obtained in 20% yield (entry 12).

The synthesis of spiroisoxazoline- β -lactams derived from an alkyl-substituted nitrile oxide were also explored (entries 13 and 14). Under conventional heating, the 1,3-dipolar cycloaddition reaction of dipole **4h**, bearing a cyclohexyl moiety, with alkylidenepenicillanate **5** led to pure spiroisoxazoline- β -lactam **6h** in an exceptional yield of 79%. The formation of **7h/8h** was also observed in 15% yield. Conducting the same reaction under microwave irradiation allowed the synthesis of compound **6h** in 60% yield, together with the mixture of **7h/8h** isolated in low yield (10%). It should be noted that this cycloaddition was highly selective for the synthesis of spiro- β -lactam **6h** and the yields were comparable with the ones observed in the cycloaddition of spiro- β -lactam **5** with aryl-substituted nitrile oxides.

Briefly, both methodologies led to pure chiral spiroisoxazoline- β -lactams **6a–h** (with exception of **6g** which is obtained in a mixture with **8g**). To our surprise, the reaction under microwave irradiation took longer than expected, and in most cases, there were no substantial differences regarding reaction times using conventional heating or microwave irradiation. In addition, we should highlight the synthesis of spiro- β -lactams **6** in higher yields under conventional heating (conventional heating: 49–79% vs. microwave irradiation: 31–60%) as well as higher overall yields (conventional heating: 52–98% vs. microwave irradiation: 51–79%).

Continuous flow 1,3-dipolar cycloaddition reactions

Continuous flow chemistry is a term used to describe chemical reactions performed in a continuous manner.¹⁴ This approach has been widely used in the past years due to its advantages, such as faster reactions, improved safety, high reproducibility, scalability, facile automation, and a precise control of reaction conditions. Furthermore, the continuous flow technology also allows to carry out reactions in a safer, greener, and sustainable way, making flow systems extraordinary useful.¹⁵ Continuous flow apparatus enables the construction of different systems by coupling several and distinct reactors (*e.g.* coil reactor, microchips and packed-bed column) in any sequence. This characteristic extends the versatility of the methodology allowing to

carry out multistep transformations.¹⁶ As consequence, continuous flow chemistry has improved the synthesis of complex molecules such as pharmaceuticals¹⁷ and natural products.¹⁸

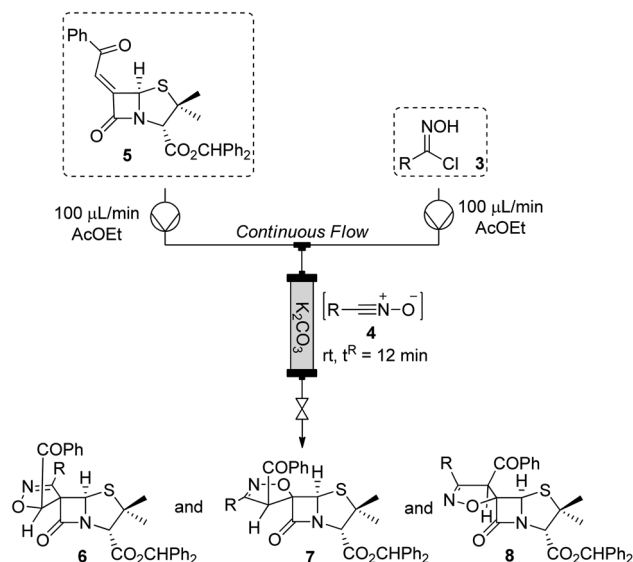
Thus, we have extended our efforts to the development of a continuous flow approach to chiral spiroisoxazoline-penicillanates *via* 1,3-dipolar cycloaddition reaction of alkylidenepenicillanate **5** to nitrile oxides **4** (Table 3). The approach for the dehydrohalogenation of hydroximoyl chlorides **3** leading to the *in situ* generation of the corresponding nitrile oxides **4**, relied on the use of a packed-bed reactor filled with fresh potassium carbonate.

The set-up was composed of two inlets, one containing a solution of 6-(*Z*)-(benzoylmethylidene)penicillanate **5** and another one containing a solution of the appropriate hydroximoyl chloride **3**, with both solutions being mixed right before entering the packed-bed reactor. Carrying out the reaction of alkylidene **5** with 1,3-dipole **4a** at room temperature with a flow rate of 100 $\mu\text{L min}^{-1}$ of each solution led to the expected spirocyclic compounds, in 89% overall yield, with the major compound **6a** being obtained in 54% yield (Table 3, entry 1). The reaction conditions were then optimized. The influence of the residence time, t^R , was investigated by tuning the flow rates of reagents (entry 2). Decreasing the flow rate of each solution to 50 $\mu\text{L min}^{-1}$, an improvement on the overall yield was observed (95%), however, with a slight decrease in the yield of the major compound **6a** (50%). Unlike what was observed for reactions carried out in batch or under microwave irradiation, the increase of the temperature to 80 $^\circ\text{C}$ under flow conditions did not lead to better results even with the simultaneous increase of nitrile oxide equivalents (entries 3 and 4). Considering the above-presented results, we selected a flow rate of 100 $\mu\text{L min}^{-1}$, together with room temperature and 2 equiv. of the nitrile oxide as the best conditions to continue our studies.

Next, continuous flow 1,3-dipolar cycloaddition of 6-(*Z*)-(benzoylmethylidene)penicillanate **5** to nitrile oxides **4b–h** was explored (Table 3, entries 5–10). Using *p*-halophenyl-nitrile oxides ($R = \text{F, Cl, Br}$) the expected spiro- β -lactams were obtained in excellent overall yields, ranging from 74% to 92% (entries 5–7). Chiral spiro- β -lactam **6b**, **6c** and **6d** were obtained as major products in 49%, 45% and 57%, respectively. The continuous flow also proved to be a viable approach for the synthesis of spiroisoxazoline-penicillanates using nitrile oxides containing electron-donating groups at the phenyl *para*-position, giving the expected cycloadducts in yields as good as under conventional heating (entries 8 and 9). Unfortunately, among the set of aryl-substituted nitrile oxides used in the present work it was not possible to study the behavior of nitrile oxide **4e** ($R = p\text{-NO}_2\text{C}_6\text{H}_4$) under flow conditions due to its partial insolubility in a wide range of solvents.

Finally, the 1,3-dipolar cycloaddition of alkyl-substituted dipole **4h** was studied. In this case, the major spirocyclic adduct **6h** was obtained in moderate yield (40%) together with a mixture of **7h/8h** (39 : 61 ratio) in 3% yield. Thus, the synthesis of chiral spiroisoxazoline-penicillanates **6h/7h/8h** under continuous flow conditions proved to be less efficient than under conventional heating or microwave irradiation.



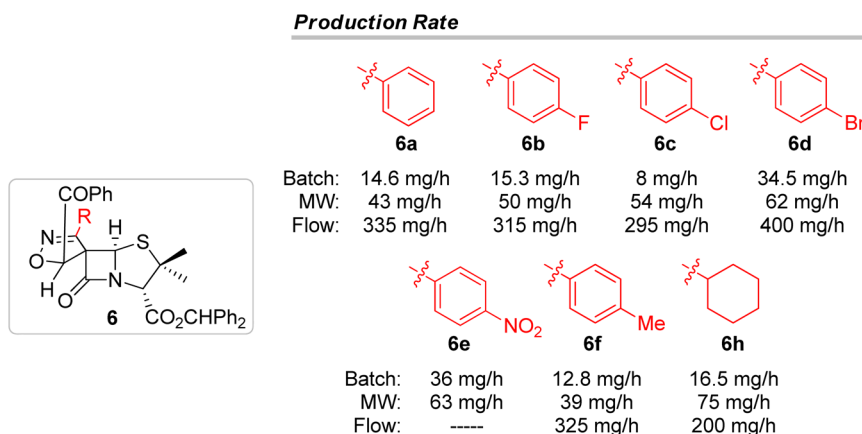
Table 3 Continuous flow 1,3-dipolar cycloaddition reaction of 6-(*Z*)-(benzoylmethylidene)penicillanate **5** with nitrile oxides **4**

Entry	4	R	Isolated yields (ratio) ^a	Overall yields
1	4a	C ₆ H ₅	6a , 54%	7a/8a , 35% (33 : 67)
2	4a ^b	C ₆ H ₅	6a , 50%	7a/8a , 45% (45 : 55)
3	4a ^c	C ₆ H ₅	6a , 10%	—
4	4a ^{c,d}	C ₆ H ₅	6a , 22%	—
5	4b	<i>p</i> -FC ₆ H ₄	6b , 49%	7b/8b , 38% (53 : 47)
6	4c	<i>p</i> -ClC ₆ H ₄	6c , 45%	7c/8c , 29% (65 : 35)
7	4d	<i>p</i> -BrC ₆ H ₄	6d , 57%	7d/8d , 35% (75 : 25)
8	4f	<i>p</i> -MeC ₆ H ₄	6f , 51%	7f/8f , 23% (39 : 61)
9	4g	<i>p</i> -MeOC ₆ H ₄	6g/8g , 54% (51 : 49)	—
10	4h	Cy	6h , 40%	7h/8h , 3% (39 : 61)

^a Ratio determined by ¹H NMR. ^b 50 μL min⁻¹ flow rate, *t*^R = 24 min. ^c Reaction carried out at 80 °C. ^d 4 equiv. of the nitrile oxide.

These results have shown that continuous flow is an interesting alternative approach to the other methodologies discussed in the present work, to carry out 1,3-dipolar cycloaddition reactions leading to spiro isoxazoline penicillanates. It is noteworthy that the continuous flow technique

allowed the synthesis of the target compounds with a very short reaction time (*t*^R = 12 minutes) having the great advantage of enabling easy scale-up processes. In fact, as outlined in Fig. 3, continuous flow proved to be the best methodology for the synthesis of the major spiro-β-lactams **6** with production rates

**Fig. 3** Production rates in mg h⁻¹ for the synthesis of the major products, spiro-β-lactams **6**.

ranging from 200 to 400 mg h⁻¹, values considerably higher than the ones observed for batch and microwave-induced methodologies.

Conclusion

Herein, a 1,3-dipolar cycloaddition-based approach to a new type of chiral spiropenicillanates has been disclosed. 6-(*Z*)-(Benzoylmethylidene)penicillanate reacted with *in situ* generated nitrile oxides to afford novel spiro isoxazoline penicillanates with the diastereoselective creation of two consecutive stereogenic centers, including a quaternary chiral center. Spiro[isoxazoline-4',6-penicillanates] were obtained as the major stereo- and regioisomers resulting from the addition of the dipole to the less sterically hindered α -side of the 6-alkylidene penicillanate and the attack of the dipole's oxygen to the terminal carbon of the exocyclic double bond of the alkylidene.

It was demonstrated that these spirocyclic adducts could be obtained using three different methodologies: conventional heating, microwave irradiation and continuous flow. Both conventional heating and continuous flow proved to be better approaches than the microwave-induced methodology leading to the major product in higher yields (conventional heating: 49–79%; continuous flow: 40–57%), and in high overall yields (conventional heating: 52–98%; continuous flow: 43–92%). Nevertheless, the continuous flow 1,3-dipolar cycloaddition reaction stands out for allowing very short reaction times, and by its inherent characteristics that ensure easy scale-up processes. In this study it was possible to observe that continuous flow conditions led to higher production rates than batch or microwave-induced methodologies, with the major products being obtained in up to 400 mg h⁻¹.

Experimental section

Thin-layer chromatography (TLC) analyses were performed using precoated silica gel plates. Flash column chromatography was performed with silica gel 60 as the stationary phase. ¹H Nuclear magnetic resonance (NMR) spectra (400 MHz) and ¹³C NMR spectra (100 MHz) were recorded using CDCl₃ as solvent. Chemical shifts are expressed in parts per million (ppm) relatively to internal tetramethylsilane (TMS) and coupling constants (*J*) are expressed in Hertz (Hz). Infrared spectra (IR) were recorded in a Fourier transform spectrometer coupled with a diamond Attenuated Total Reflectance (ATR) sampling accessory. High-resolution mass spectra (HRMS) were obtained on a Orbitrap q-Exactive Focus spectrometer coupled to a Vanquish HPLC with ESI. Melting points (mp) were determined in open glass capillaries. Optical rotations were measured on an Optical Activity AA-5 electrical polarimeter. 6-(*Z*)-(benzoylmethylidene)penicillanate **5** (ref. 5b) was prepared as described in the literature.

General procedure for the synthesis of hydroximoyl chlorides **3**

Hydroximoyl chlorides **3** were prepared based on previously developed procedures.¹⁹ The appropriate aldehyde (1 equiv.)

was dissolved in ethanol (15 mL). A solution of hydroxylamine hydrochloride (1.25 equiv.) in water (3 mL) and anhydrous sodium sulfate (1 equiv.) were added. The reaction mixture was irradiated in the ultrasonic bath at 30 °C for the time indicated in each case. The mixture was filtered, and the solvent was evaporated under reduced pressure. The residue was dissolved in CH₂Cl₂, washed with water, and extracted with CH₂Cl₂. The combined organic layers were dried over anhydrous NaSO₄, filtered, and the solvents were evaporated affording oximes **2** which were used without further purification. Oximes **2** were dissolved in chloroform (11 mL) and a drop of pyridine was added. After 5 min, *N*-chlorosuccinimide (1 equiv.) was added stepwise to the stirring reaction mixture. The reaction mixture was stirred for the time indicated in each case, monitored by TLC until completion. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography.

(Z)-N-Hydroxybenzimidoyl chloride (3a). Obtained from benzaldehyde (0.612 mL, 6 mmol) and hydroxylamine hydrochloride (521 mg, 7.5 mmol) as described in the general procedure after 10 minutes of ultrasonic irradiation. The crude mixture reacted overnight with *N*-chlorosuccinimide (799 mg, 6 mmol) as described in the general procedure. Purification by flash chromatography (hexane/ethyl acetate, 3 : 1) afforded compound **3a** as a yellow solid (917 mg, 5.89 mmol, 98%).

3a: mp 45.1–47.1 °C (lit.²⁰ 47–48 °C); ¹H NMR (CDCl₃, 400 MHz) δ = 7.40–7.46 (m, 3H), 7.85 (dd, *J* = 8.1 and 1.5 Hz, 2H), 8.25 (s, 1H). The ¹H NMR spectral data are in good agreement with the literature data.²¹

(Z)-4-Fluoro-N-hydroxybenzimidoyl chloride (3b). Obtained from 4-fluorobenzaldehyde (0.316 mL, 3 mmol) and hydroxylamine hydrochloride (260 mg, 3.75 mmol) as described in the general procedure after 30 minutes of ultrasonic irradiation. The crude mixture reacted overnight with *N*-chlorosuccinimide (400 mg, 3 mmol) as described in the general procedure. Purification by flash chromatography (hexane/ethyl acetate, 3 : 1) afforded compound **3b** as a white solid (438 mg, 2.52 mmol, 84%).

3b: mp 75.1–77.1 °C (lit.²² 74.5–75 °C); ¹H NMR (CDCl₃, 400 MHz) δ = 7.10 (t, *J* = 8.7 Hz, 2H), 7.84 (dd, *J* = 9.0 and 5.3 Hz, 2H), 7.91 (s, 1H). The ¹H NMR spectral data are in good agreement with the literature data.²³

(Z)-4-Chloro-N-hydroxybenzimidoyl chloride (3c). Obtained from 4-chlorobenzaldehyde (421 mg, 3 mmol) and hydroxylamine hydrochloride (260 mg, 3.75 mmol) as described in the general procedure after 30 minutes of ultrasonic irradiation. The crude mixture reacted overnight with *N*-chlorosuccinimide (400 mg, 6 mmol) as described in the general procedure. Purification by flash chromatography (hexane/ethyl acetate, 3 : 1) afforded compound **3c** as a white solid (389 mg, 2.05 mmol, 68%).

3c: mp 86.4–88.4 °C (lit.²⁰ 88.5–89.5 °C); ¹H NMR (CDCl₃, 400 MHz) δ = 7.39 (d, *J* = 8.8 Hz, 2H), 7.79 (d, *J* = 8.8 Hz, 2H), 27.96 (s, 1H). The ¹H NMR spectral data are in good agreement with the literature data.²⁴

(Z)-4-Bromo-N-hydroxybenzimidoyl chloride (3d). Obtained from 4-bromobenzaldehyde (555 mg, 3 mmol) and



hydroxylamine hydrochloride (260 mg, 3.75 mmol) as described in the general procedure after 30 minutes of ultrasonic irradiation. The crude mixture reacted overnight with *N*-chlorosuccinimide (400 mg, 3 mmol) as described in the general procedure. Purification by flash chromatography (hexane/ethyl acetate, 3 : 1) afforded compound **3d** as a white solid (363 mg, 1.55 mmol, 52%).

3d: mp 78.3–80.3 °C (lit.²⁵ 76–78 °C); ¹H NMR (CDCl₃, 400 MHz) δ = 7.55 (d, *J* = 8.7 Hz, 2H), 7.72 (d, *J* = 8.7 Hz, 2H), 7.84 (s, 1H). The ¹H NMR spectral data are in good agreement with the literature data.²³

(Z)-4-Nitro-*N*-hydroxybenzimidoyl chloride (3e). Obtained from 4-nitrobenzaldehyde (453 mg, 3 mmol) and hydroxylamine hydrochloride (260 mg, 3.75 mmol) as described in the general procedure after 15 minutes of ultrasonic irradiation. The crude mixture reacted overnight with *N*-chlorosuccinimide (400 mg, 3 mmol) as described in the general procedure. Purification by flash chromatography (hexane/ethyl acetate, 3 : 1) afforded compound **3e** as a yellow solid (460 mg, 2.29 mmol, 76%).

3e: mp 121.4–123.4 °C (lit.²⁰ 124–125 °C); ¹H NMR (CDCl₃, 400 MHz) δ = 8.05 (d, *J* = 9.0 Hz, 2H), 8.27 (d, *J* = 9.0 Hz, 2H), 8.32 (s, 1H). The ¹H NMR spectral data are in good agreement with the literature data.²³

(Z)-4-Methyl-*N*-hydroxybenzimidoyl chloride (3f). Obtained from 4-methylbenzaldehyde (0.353 mL, 3 mmol) and hydroxylamine hydrochloride (260 mg, 3.75 mmol) as described in the general procedure after 30 minutes of ultrasonic irradiation. The crude mixture reacted overnight with *N*-chlorosuccinimide (400 mg, 3 mmol) as described in the general procedure. Purification by flash chromatography (hexane/ethyl acetate, 3 : 1) afforded compound **3f** as a white solid (323 mg, 1.91 mmol, 64%).

3f: mp 67.4–69.4 °C (lit.²⁶ 71–72 °C); ¹H NMR (CDCl₃, 400 MHz) δ = 2.38 (s, 3H), 7.21 (d, *J* = 8.1 Hz, 2H), 7.65 (s, 1H), 7.73 (d, *J* = 8.3 Hz, 2H). The ¹H NMR spectral data are in good agreement with the literature data.²⁴

(Z)-4-Methoxy-*N*-hydroxybenzimidoyl chloride (3g). Obtained from 4-methoxybenzaldehyde (0.364 mL, 3 mmol) and hydroxylamine hydrochloride (0.260 mg, 3.75 mmol) as described in the general procedure after 3 hours of ultrasonic irradiation. The crude mixture reacted overnight with *N*-chlorosuccinimide (400 mg, 3 mmol) as described in the general procedure. Purification by flash chromatography (hexane/ethyl acetate, 3 : 1) afforded compound **3g** as a white solid (498 mg, 2.68 mmol, 89%).

3g: mp 85.7–87.7 °C (lit.²⁰ 87.5–89 °C); ¹H NMR (CDCl₃, 400 MHz) δ = 3.85 (s, 3H), 6.91 (d, *J* = 9.0 Hz, 2H), 7.65 (s, 1H), 7.79 (d, *J* = 9.0 Hz, 2H). The ¹H NMR spectral data are in good agreement with the literature data.²⁷

(Z)-*N*-Hydroxycyclohexanecarboximidoyl chloride (3h). Obtained from cyclohexanecarboxaldehyde (0.361 mL, 3 mmol) and hydroxylamine hydrochloride (260 mg, 3.75 mmol) as described in the general procedure after 30 minutes of ultrasonic irradiation. The crude mixture reacted overnight with *N*-chlorosuccinimide (400 mg, 3 mmol) as described in the general procedure. Purification by flash chromatography

(hexane/ethyl acetate, 3 : 1) afforded compound **3h** as a colorless oil (235 mg, 1.45 mmol, 48%).

3h: ¹H NMR (CDCl₃, 400 MHz) δ = 1.23–1.35 (m, 1H), 1.39–1.50 (m, 2H), 1.66–1.70 (m, 1H), 1.78–1.82 (m, 2H), 1.92–1.96 (m, 2H), 2.46 (tt, *J* = 11.5 and 3.4 Hz, 1H), 8.10 (s, 1H). The ¹H NMR spectral data are in good agreement with the literature data.²⁸

General procedure for the 1,3-dipolar cycloaddition of nitrile oxides with 6-(Z)-(benzoylmethylidene)penicillanate

Method A (Batch). A solution of the appropriate hydroximoyl chloride (2 equiv.) in ethyl acetate (2 mL) was added to a suspension of potassium carbonate (5 equiv.) and the 6-(Z)-(benzoylmethylidene)penicillanate **5** (1 equiv.) in ethyl acetate (3 mL). The reaction mixture was stirred under reflux for the time indicated in each case, monitored by TLC until completion and filtered through a pad of Celite, which was washed with ethyl acetate. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography.

Method B (MW irradiation). A suspension of hydroximoyl chloride (2 equiv.) with potassium carbonate (5 equiv.) and 6-(Z)-(benzoylmethylidene)penicillanate **5** (1 equiv.) in ethyl acetate (1 mL) was irradiated in a microwave reactor (CEM Focused Synthesis System, Discover S-Class) at 80 °C for the time indicated in each case. Upon completion, the crude mixture was filtered through a pad of Celite, which was washed with ethyl acetate. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography.

Method C (continuous flow chemistry). The flow system was set up according to the scheme in Table 2. A 0.2 M solution of 6-(Z)-(benzoylmethylidene)penicillanate **5** in ethyl acetate (1 mL) with a 100 μ L min^{−1} flow rate, was combined at a T-piece with a 0.4 M solution of the corresponding hydroximoyl chloride **3** in ethyl acetate (1 mL) with a 100 μ L min^{−1} flow rate. The combined solution was passed through a packed-bed column filled with fresh K₂CO₃ (\approx 2.1 g, 7 cm), kept at room temperature. The output solution was concentrated under reduced pressure and the crude product was purified by flash chromatography.

(4'R,5'S)-Benzhydryl spiro[[5-benzoyl-3-phenyl-isoxaxoline)-4',6-penicillanate] (**6a**) and *(4'S,5'S)*-benzhydryl spiro[[4-benzoyl-3-phenyl-isoxaxoline)-5',6-penicillanate] (**7a**) and *(4'S,5'R)*-benzhydryl spiro[[4-benzoyl-3-phenyl-isoxaxoline)-5',6-penicillanate] (**8a**). Obtained from hydroximoyl chloride **3a** (64 mg, 0.412 mmol) and 6-(Z)-(benzoylmethylidene)penicillanate **5** (100 mg, 0.206 mmol) as described in the general procedure. The crude product was purified by flash chromatography (hexane/ethyl acetate, 3 : 1). Method A, gave, in order of elution, **6a** as a white solid (73 mg, 0.121 mmol, 59%) and a mixture of **7a/8a** (84 : 16) as a yellow oil (47 mg, 0.078 mmol, 38%). Method B, gave, in order of elution, **6a** as a white solid (43 mg, 0.071 mmol, 35%) and **7a** as a yellow oil (42 mg, 0.070 mmol, 34%). Method C, gave, in order of elution, **6a** as a white solid (67 mg, 0.111 mmol, 54%) and a mixture of **7a/8a** (33 : 67) as a yellow oil (43 mg, 0.071 mmol, 35%).

6a: mp 74.0–76.0 °C; $[\alpha]_D^{25} = +360$ (c 0.5 in CH_2Cl_2); IR (ATR): $\nu = 881, 1083, 1156, 1449, 1496, 1586, 1678, 1740$ and 1780 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) $\delta = 1.15$ (s, 3H), 1.57 (s, 3H), 4.65 (s, 1H), 5.86 (s, 1H), 6.17 (s, 1H), 6.98 (s, 1H), 7.27–7.35 (m, 12H), 7.43 (t, $J = 7.5 \text{ Hz}$, 1H), 7.52–7.56 (m, 2H), 7.66 (t, $J = 7.4 \text{ Hz}$, 1H), 7.81–7.83 (m, 2H), 8.04–8.06 (m, 2H); ^{13}C NMR (CDCl_3 , 100 MHz) $\delta = 26.2, 32.1, 63.7, 68.0, 69.9, 76.6, 78.8, 81.8, 126.7, 127.3, 127.4, 127.5, 127.7, 128.5, 128.6, 128.6, 128.8, 128.9, 129.1, 129.2, 129.4, 131.0, 134.5, 135.0, 139.1, 139.2, 155.6, 166.5, 170.7, 191.6$; HRMS (ESI) m/z : calcd for $\text{C}_{36}\text{H}_{31}\text{N}_2\text{O}_5\text{S} [\text{M} + \text{H}]^+$ 603.1948; found 666.1941.

7a: mp low melting solid; $[\alpha]_D^{25} = +280$ (c 0.25 in CH_2Cl_2); IR (ATR): $\nu = 910, 1080, 1176, 1448, 1497, 1595, 1676, 1741$ and 1777 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): $\delta = 1.12$ (s, 3H), 1.46 (s, 3H), 4.60 (s, 1H), 5.67 (s, 1H), 5.68 (s, 1H), 6.93 (s, 1H), 7.22–7.24 (m, 2H), 7.30–7.35 (m, 11H), 7.48–7.55 (m, 4H), 7.63 (t, $J = 7.4 \text{ Hz}$, 1H), 7.95–7.97 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 25.5, 33.4, 57.9, 63.7, 68.9, 72.9, 77.5, 78.7, 102.2, 127.2, 127.3, 127.5, 127.6, 128.4, 128.6, 128.6, 128.7, 128.8, 128.8, 128.9, 129.0, 129.3, 131.0, 134.5, 137.2, 139.1, 139.1, 156.7, 166.2, 170.3, 194.6$; HRMS (ESI) m/z : calcd for $\text{C}_{36}\text{H}_{31}\text{N}_2\text{O}_5\text{S} [\text{M} + \text{H}]^+$ 603.1948; found 666.1945.

Recorded as a mixture of **7a/8a** (33 : 67): **8a:** ^1H NMR (CDCl_3 , 400 MHz) $\delta = 1.30$ (s, 3H), 1.33 (s, 3H), 4.46 (s, 1H), 5.56 (s, 1H), 5.79 (s, 1H), 6.93 (s, 1H), 7.22–7.24 (m, 2H), 7.30–7.35 (m, 11H), 7.48–7.55 (m, 4H), 7.63 (t, $J = 7.4 \text{ Hz}$, 1H), 8.11 (d, $J = 7.2 \text{ Hz}$, 2H).

(4*R*,5*S*)-Benzhydryl spiro[(5-benzoyl-3-(4-fluorophenyl)-isoxaxoline)-4',6-penicillanate] (**6b**) and (4*S*,5*S*)-benzhydryl spiro[(4-benzoyl-3-(4-fluorophenyl)-isoxaxoline)-5',6-penicillanate] (**7b**) and (4*S*,5*R*)-benzhydryl spiro[(4-benzoyl-3-(4-fluorophenyl)-isoxaxoline)-5',6-penicillanate] (**8b**). Obtained from hydroximoyl chloride **3b** (71 mg, 0.412 mmol) and 6-(*Z*)-(benzoylmethylidene)penicillanate **5** (100 mg, 0.206 mmol) as described in the general procedure. The crude product was purified by flash chromatography (hexane/ethyl acetate, 3 : 1). Method A gave **6b** as a white solid (92 mg, 0.148 mmol, 72%). Method B, gave, in order of elution, **6b** as a white solid (50 mg, 0.081 mmol, 39%) and **7b/8b** (84 : 16) as a yellow oil (41 mg, 0.066 mmol, 32%). Method C, gave, in order of elution, **6b** as a white solid (63 mg, 0.101 mmol, 49%) and a mixture of **7b/8b** (53 : 47) as a yellow oil (49 mg, 0.079 mmol, 38%).

6b: mp 167.1–169.1 °C; $[\alpha]_D^{25} = +380$ (c 0.5 in CH_2Cl_2); IR (ATR): $\nu = 838, 1081, 1156, 1449, 1509, 1598, 1676, 1736$ and 1778 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) $\delta = 1.17$ (s, 3H), 1.57 (s, 3H), 4.65 (s, 1H), 5.84 (s, 1H), 6.17 (s, 1H), 6.90 (t, $J = 8.7 \text{ Hz}$, 2H), 6.98 (s, 1H), 7.30–7.36 (m, 10H), 7.53–7.56 (m, 2H), 7.66 (t, $J = 7.4 \text{ Hz}$, 1H), 7.79–7.82 (m, 2H), 8.04–8.06 (m, 2H); ^{13}C NMR (CDCl_3 , 100 MHz) $\delta = 26.1, 32.3, 63.8, 68.0, 69.9, 76.6, 78.9, 81.8, 116.5$ (d, $J = 22 \text{ Hz}$), 123.4 (d, $J = 3 \text{ Hz}$), 127.2, 127.7, 128.5, 128.7, 128.8, 128.8, 129.0, 129.2, 129.4, 129.4, 129.5, 134.5, 134.9, 139.0 (d, $J = 2 \text{ Hz}$), 154.6, 164.4 (d, $J = 252 \text{ Hz}$), 166.5, 170.4, 191.6; ^{19}F NMR (CDCl_3 , 376 MHz) $\delta = -108.5$ (s, 1F); HRMS (ESI) m/z : calcd for $\text{C}_{36}\text{H}_{29}\text{N}_2\text{O}_5\text{FNaS} [\text{M} + \text{Na}]^+$ 643.1673; found 643.1671.

Recorded as a mixture of **7b/8b** (53 : 47): **7b:** ^1H NMR (CDCl_3 , 400 MHz) $\delta = 1.12$ (s, 3H), 1.46 (s, 3H), 4.60 (s, 1H), 5.63 (s, 1H),

5.66 (s, 1H), 6.91–6.96 (3H), 7.30–7.35 (m, 10H), 7.47–7.56 (m, 4H), 7.62–7.66 (m, 1H), 7.93–7.95 (m, 2H); **8b:** ^1H NMR (CDCl_3 , 400 MHz) $\delta = 1.30$ (s, 3H), 1.34 (s, 3H), 4.46 (s, 1H), 5.56 (s, 1H), 5.74 (s, 1H), 6.91–6.96 (3H), 7.30–7.35 (m, 10H), 7.47–7.56 (m, 4H), 7.62–7.66 (m, 1H), 8.08–8.10 (m, 2H).

(4*R*,5*S*)-Benzhydryl spiro[(5-benzoyl-3-(4-chlorophenyl)-isoxaxoline)-4',6-penicillanate] (**6c**) and (4*S*,5*S*)-benzhydryl spiro[(4-benzoyl-3-(4-chlorophenyl)-isoxaxoline)-5',6-penicillanate] (**7c**) and (4*S*,5*R*)-benzhydryl spiro[(4-benzoyl-3-(4-chlorophenyl)-isoxaxoline)-5',6-penicillanate] (**8c**). Obtained from hydroximoyl chloride **3c** (78 mg, 0.412 mmol) and 6-(*Z*)-(benzoylmethylidene)penicillanate **5** (100 mg, 0.206 mmol) as described in the general procedure. The crude product was purified by flash chromatography (hexane/ethyl acetate, 3 : 1). Method A, gave, in order of elution, **6c** as a white solid (64 mg, 0.100 mmol, 49%) and a mixture of **7c/8c** (71 : 29) as a yellow oil (50 mg, 0.078 mmol, 38%). Method B, gave, in order of elution, **6c** as a white solid (54 mg, 0.084 mmol, 41%) and **7c/8c** (90 : 10) as a yellow oil (38 mg, 0.21 mmol, 29%). Method C, gave, in order of elution, **6c** as a white solid (59 mg, 0.093 mmol, 45%) and a mixture of **7c/8c** (65 : 35) as a yellow oil (38 mg, 0.060 mmol, 29%).

6c: mp 198.1–199.4 °C; $[\alpha]_D^{25} = +450$ (c 0.5 in CH_2Cl_2); IR (ATR): $\nu = 975, 1086, 1156, 1203, 1450, 1492, 1595, 1667, 1736$ and 1779 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) $\delta = 1.17$ (s, 3H), 1.57 (s, 3H), 4.65 (s, 1H), 5.85 (s, 1H), 6.18 (s, 1H), 6.99 (s, 1H), 7.16 (d, $J = 8.7 \text{ Hz}$, 2H), 7.31–7.36 (m, 10H), 7.53–7.56 (m, 2H), 7.66 (t, $J = 7.4 \text{ Hz}$, 1H), 7.73–7.77 (m, 2H), 8.03–8.05 (m, 2H); ^{13}C NMR (CDCl_3 , 100 MHz) $\delta = 26.1, 32.8, 63.8, 68.0, 69.9, 76.5, 79.0, 81.9, 125.7, 127.2, 127.7, 128.6, 128.8, 129.0, 129.2, 129.4, 129.6, 134.6, 134.9, 137.2, 139.0, 154.6, 166.4, 170.4, 191.5$; HRMS (ESI) m/z : calcd for $\text{C}_{36}\text{H}_{29}\text{N}_2\text{O}_5\text{Cl}_2\text{S} [\text{M} + \text{Cl}]^+$ 671.1180; found 671.1183.

Recorded as a mixture of **7c/8c** (90 : 10): **7c:** ^1H NMR (CDCl_3 , 400 MHz) $\delta = 1.12$ (s, 3H), 1.46 (s, 3H), 4.60 (s, 1H), 5.64 (s, 1H), 5.67 (s, 1H), 6.93 (s, 1H), 7.21–7.23 (m, 2H), 7.30–7.36 (m, 10H), 7.46–7.53 (m, 4H), 7.62–7.66 (m, 1H), 7.93–7.95 (m, 2H); **8c:** ^1H NMR (CDCl_3 , 400 MHz) $\delta = 1.30$ (s, 3H), 1.34 (s, 3H), 4.46 (s, 1H), 5.56 (s, 1H), 5.74 (s, 1H), 6.93 (s, 1H), 7.21–7.23 (m, 2H), 7.30–7.36 (m, 10H), 7.46–7.53 (m, 4H), 7.62–7.66 (m, 1H), 8.07–8.09 (m, 2H).

(4*R*,5*S*)-Benzhydryl spiro[(5-benzoyl-3-(4-bromophenyl)-isoxaxoline)-4',6-penicillanate] (**6d**) and (4*S*,5*S*)-benzhydryl spiro[(4-benzoyl-3-(4-bromophenyl)-isoxaxoline)-5',6-penicillanate] (**7d**) and (4*S*,5*R*)-benzhydryl spiro[(4-benzoyl-3-(4-bromophenyl)-isoxaxoline)-5',6-penicillanate] (**8d**). Obtained from hydroximoyl chloride **3d** (97 mg, 0.412 mmol) and 6-(*Z*)-(benzoylmethylidene)penicillanate **5** (100 mg, 0.206 mmol) as described in the general procedure. The crude product was purified by flash chromatography (hexane/ethyl acetate, 3 : 1). Method A, gave, in order of elution, **6d** as a white solid (69 mg, 0.101 mmol, 49%) and a mixture of **7d/8d** (65 : 35) as a yellow oil (60 mg, 0.088 mmol, 43%). Method B, gave, in order of elution, **6d** as a white solid (62 mg, 0.091 mmol, 44%) and **7d/8d** (58 : 42) as a yellow oil (47 mg, 0.069 mmol, 33%). Method C, gave, in order of elution, **6d** as a white solid (80 mg, 0.117 mmol, 57%) and



a mixture of **7d/8d** (75 : 25) as a yellow oil (49 mg, 0.072 mmol, 35%).

6d: mp 196.7–198.4 °C; $[\alpha]_D^{25} = +410$ (c 0.5 in CH_2Cl_2); IR (ATR): $\nu = 866, 1086, 1155, 1203, 1450, 1492, 1594, 1667, 1736$ and 1779 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) $\delta = 1.18$ (s, 3H), 1.57 (s, 3H), 4.65 (s, 1H), 5.86 (s, 1H), 6.18 (s, 1H), 6.90 (t, $J = 8.7 \text{ Hz}$, 2H), 6.96 (s, 1H), 7.30–7.37 (m, 10H), 7.53–7.56 (m, 2H), 7.65–7.70 (m, 3H), 8.03–8.06 (m, 2H); ^{13}C NMR (CDCl_3 , 100 MHz) $\delta = 26.1, 32.4, 63.8, 68.0, 69.9, 76.5, 79.0, 81.9, 125.7, 126.2, 127.2, 127.7, 128.6, 128.7, 128.8, 129.0, 129.2, 129.4, 132.5, 134.6, 134.9, 139.0, 154.6, 166.4, 170.4, 191.5$; HRMS (ESI) m/z : calcd for $\text{C}_{36}\text{H}_{30}\text{N}_2\text{O}_5\text{BrS} [\text{M} + \text{H}]^+$ 681.1053; found 681.1049.

Recorded as a mixture of **7d/8d** (58 : 42): **7d**: ^1H NMR (CDCl_3 , 400 MHz) $\delta = 1.12$ (s, 3H), 1.46 (s, 3H), 4.60 (s, 1H), 5.64 (s, 1H), 5.66 (s, 1H), 6.93 (s, 1H), 7.31–7.35 (m, 10H), 7.38–7.40 (m, 3H), 7.47–7.53 (m, 3H), 7.63–7.66 (m, 1H), 7.93–7.95 (m, 2H); **8d**: ^1H NMR (CDCl_3 , 400 MHz) $\delta = 1.30$ (s, 3H), 1.34 (s, 3H), 4.46 (s, 1H), 5.56 (s, 1H), 5.73 (s, 1H), 6.94 (s, 1H), 7.31–7.35 (m, 10H), 7.38–7.40 (m, 3H), 7.47–7.53 (m, 3H), 7.63–7.66 (m, 1H), 8.07–8.09 (m, 2H).

(4*R*,5*S*)-Benzhydryl spiro[(5-benzoyl-3-(4-nitrophenyl)-isoxaxoline)-4',6-penicillanate] (**6e**) and (4*S*,5*S*)-benzhydryl spiro[(4-benzoyl-3-(4-nitrophenyl)-isoxaxoline)-5',6-penicillanate] (**7e**) and (4*S*,5*R*)-benzhydryl spiro[(4-benzoyl-3-(4-nitrophenyl)-isoxaxoline)-5',6-penicillanate] (**8e**). Obtained from hydroximoyl chloride **3e** (83 mg, 0.412 mmol) and 6-(*Z*)-(benzoylmethylidene)penicillanate **5** (100 mg, 0.206 mmol) as described in the general procedure. The crude product was purified by flash chromatography (hexane/ethyl acetate, 3 : 1). Method A, gave, in order of elution, **6e** as a white solid (72 mg, 0.111 mmol, 54%) and a mixture of **7e/8e** (34 : 66) as a yellow oil (47 mg, 0.072 mmol, 35%). Method B, gave, in order of elution, **6e** as a white solid (63 mg, 0.097 mmol, 47%) and **7e/8e** as a yellow oil (50 : 50) (43 mg, 0.066 mmol, 32%).

6e: mp 171.7–173.7 °C; $[\alpha]_D^{25} = +370$ (c 0.5 in CH_2Cl_2); IR (ATR): $\nu = 884, 1082, 1155, 1344, 1449, 1497, 1518, 1597, 1669, 1744$ and 1777 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) $\delta = 1.20$ (s, 3H), 1.58 (s, 3H), 4.68 (s, 1H), 5.88 (s, 1H), 6.25 (s, 1H), 7.00 (s, 1H), 7.33–7.38 (m, 10H), 7.54–7.58 (m, 2H), 7.69 (t, $J = 7.4 \text{ Hz}$, 1H), 7.98–8.06 (m, 6H); ^{13}C NMR (CDCl_3 , 100 MHz) $\delta = 26.1, 32.5, 64.0, 67.9, 69.9, 76.4, 79.2, 82.3, 124.4, 127.1, 127.8, 128.2, 128.6, 128.8, 128.8, 128.9, 128.9, 129.0, 129.3, 129.4, 133.4, 134.8, 138.8, 149.1, 153.8, 166.3, 169.9, 191.4$; HRMS (ESI) m/z : calcd for $\text{C}_{36}\text{H}_{30}\text{N}_3\text{O}_7\text{S} [\text{M} + \text{H}]^+$ 648.1799; found 648.1792.

Recorded as a mixture of **7e/8e** (50 : 50): **7e**: ^1H NMR (CDCl_3 , 400 MHz) $\delta = 1.13$ (s, 3H), 1.46 (s, 3H), 4.61 (s, 1H), 5.69 (s, 1H), 5.72 (s, 1H), 6.94 (s, 1H), 7.31–7.36 (m, 10H), 7.48–7.56 (m, 2H), 7.76–7.78 (m, 2H), 8.08–8.12 (m, 2H), 8.18–8.20 (m, 2H), 8.33–8.36 (m, 1H); **8e**: ^1H NMR (CDCl_3 , 400 MHz) $\delta = 1.31$ (s, 3H), 1.33 (s, 3H), 4.47 (s, 1H), 5.58 (s, 1H), 5.82 (s, 1H), 6.94 (s, 1H), 7.31–7.36 (m, 10H), 7.48–7.56 (m, 2H), 7.69–7.72 (m, 2H), 7.94–7.96 (m, 2H), 8.08–8.12 (m, 2H), 8.33–8.36 (m, 1H).

(4*R*,5*S*)-Benzhydryl spiro[(5-benzoyl-3-(4-methylphenyl)-isoxaxoline)-4',6-penicillanate] (**6f**) and (4*S*,5*S*)-benzhydryl spiro[(4-benzoyl-3-(4-methylphenyl)-isoxaxoline)-5',6-penicillanate] (**7f**) and (4*S*,5*R*)-benzhydryl spiro[(4-benzoyl-3-(4-methylphenyl)-isoxaxoline)-5',6-penicillanate] (**8f**). Obtained from hydroximoyl

chloride **3f** (70 mg, 0.412 mmol) and 6-(*Z*)-(benzoylmethylidene)penicillanate **5** (100 mg, 0.206 mmol) as described in the general procedure. The crude product was purified by flash chromatography (hexane/ethyl acetate, 3 : 1). Method A, gave, in order of elution, **6f** as a white solid (64 mg, 0.104 mmol, 50%) and a mixture of **7f/8f** (68 : 32) as a yellow oil (48 mg, 0.078 mmol, 38%). Method B, gave, in order of elution, **6f** as a white solid (39 mg, 0.063 mmol, 31%) and **7f/8f** (85 : 15) as a yellow oil (26 mg, 0.042 mmol, 20%). Method C, gave, in order of elution, **6f** as a white solid (65 mg, 0.105 mmol, 51%) and a mixture of **7f/8f** (39 : 61) as a yellow oil (29 mg, 0.047 mmol, 23%).

6f: mp 88.4–90.4 °C; $[\alpha]_D^{25} = +420$ (c 0.5 in CH_2Cl_2); IR (ATR): $\nu = 876, 1156, 1449, 1497, 1596, 1676, 1742$ and 1774 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) $\delta = 1.16$ (s, 3H), 1.56 (s, 3H), 2.34 (s, 3H), 4.65 (s, 1H), 5.88 (s, 1H), 6.16 (s, 1H), 6.98 (s, 1H), 7.04 (d, $J = 8.0 \text{ Hz}$, 2H), 7.26–7.36 (m, 10H), 7.52–7.55 (m, 2H), 7.65 (t, $J = 7.4 \text{ Hz}$, 1H), 7.72 (d, $J = 8.2 \text{ Hz}$, 2H), 8.04–8.06 (m, 2H); ^{13}C NMR (CDCl_3 , 100 MHz) $\delta = 21.6, 26.2, 32.1, 63.6, 68.0, 69.8, 76.6, 78.8, 81.6, 124.4, 127.3, 127.3, 127.6, 128.5, 128.5, 128.8, 128.9, 129.1, 129.4, 130.0, 134.4, 135.0, 139.1, 139.2, 141.3, 155.5, 166.6, 170.7, 191.6$; HRMS (ESI) m/z : calcd for $\text{C}_{37}\text{H}_{33}\text{N}_2\text{O}_5\text{S} [\text{M} + \text{H}]^+$ 617.2105; found 617.2098.

Recorded as a mixture of **7f/8f** (85 : 15): **7f**: ^1H NMR (CDCl_3 , 400 MHz) $\delta = 1.12$ (s, 3H), 1.46 (s, 3H), 2.27 (s, 3H), 4.59 (s, 1H), 5.66 (s, 1H), 5.66 (s, 1H), 6.93 (s, 1H), 7.04 (d, $J = 8.1 \text{ Hz}$, 2H), 7.30–7.35 (m, 10H), 7.43 (d, $J = 8.2 \text{ Hz}$, 2H), 7.46–7.52 (m, 2H), 7.62 (t, $J = 7.4 \text{ Hz}$, 1H), 7.94–7.96 (m, 2H); **8f**: ^1H NMR (CDCl_3 , 400 MHz) $\delta = 1.30$ (s, 3H), 1.34 (s, 3H), 2.32 (s, 3H), 4.46 (s, 1H), 5.56 (s, 1H), 5.75 (s, 1H), 6.93 (s, 1H), 7.13 (d, $J = 8.1 \text{ Hz}$, 2H), 7.30–7.35 (m, 10H), 7.42–7.44 (m, 2H), 7.46–7.52 (m, 2H), 7.56–7.58 (m, 1H), 8.09 (d, $J = 7.3 \text{ Hz}$, 2H).

(4*R*,5*S*)-Benzhydryl spiro[(5-benzoyl-3-(4-methoxyphenyl)-isoxaxoline)-4',6-penicillanate] (**6g**) and (4*S*,5*S*)-benzhydryl spiro[(4-benzoyl-3-(4-methoxyphenyl)-isoxaxoline)-5',6-penicillanate] (**7g**) and (4*S*,5*R*)-benzhydryl spiro[(4-benzoyl-3-(4-methoxyphenyl)-isoxaxoline)-5',6-penicillanate] (**8g**). Obtained from hydroximoyl chloride **3g** (76 mg, 0.412 mmol) and 6-(*Z*)-(benzoylmethylidene)penicillanate **5** (100 mg, 0.206 mmol) as described in the general procedure. The crude product was purified by flash chromatography (hexane/ethyl acetate, 3 : 1). The crude product was purified by flash chromatography (hexane/ethyl acetate, 3 : 1). Method A gave a mixture of **6g/8g** (69 : 31) as a yellow oil (68 mg, 0.107 mmol, 52%). Method B, gave, in order of elution, a mixture of **6g/8g** (69 : 31) as a yellow oil (47 mg, 0.074 mmol, 36%) and **7g** as a white solid (28 mg, 0.044 mmol, 21%). Method C gave a mixture of **6g/8g** (75 : 25) as a yellow oil (70 mg, 0.111 mmol, 54%).

Recorded as a mixture of **6g/8g** (69 : 31): **6g**: ^1H NMR (CDCl_3 , 400 MHz) $\delta = 1.16$ (s, 3H), 1.57 (s, 3H), 3.78 (s, 3H), 4.65 (s, 1H), 5.89 (s, 1H), 6.15 (s, 1H), 6.76 (d, $J = 8.9 \text{ Hz}$, 2H), 6.99 (s, 1H), 7.31–7.36 (m, 10H), 7.51–7.55 (m, 2H), 7.63–7.65 (m, 1H), 7.78–7.80 (m, 2H), 8.03–8.06 (m, 2H); **8g**: ^1H NMR (CDCl_3 , 400 MHz) $\delta = 1.16$ (s, 3H), 1.57 (s, 3H), 3.86 (s, 3H), 4.66 (s, 1H), 5.89 (s, 1H), 6.16 (s, 1H), 6.65 (d, $J = 8.9 \text{ Hz}$, 1H), 6.99 (s, 1H), 7.31–7.36 (m, 10H), 7.51–7.55 (m, 2H), 7.63–7.65 (m, 1H), 7.75 (dd, $J = 8.9$ and 2.2 Hz , 2H), 7.90 (d, $J = 2.2 \text{ Hz}$, 1H), 8.03–8.06 (m, 2H).



7g: mp 94.3–96.3 °C; $[\alpha]_D^{25} = +260$ (c 0.25 in CH₂Cl₂); IR (ATR): $\nu = 911, 1717, 1256, 1457, 1516, 1607, 1740$ and 1781 cm^{-1} ; ¹H NMR (CDCl₃, 400 MHz) $\delta = 1.12$ (s, 3H), 1.46 (s, 3H), 3.75 (s, 3H), 4.60 (s, 1H), 5.62 (s, 1H), 5.65 (s, 1H), 6.74 (d, $J = 8.9$ Hz, 2H), 6.92 (s, 1H), 7.30–7.35 (m, 10H), 7.47–7.51 (m, 4H), 7.62 (t, $J = 7.4$ Hz, 1H), 7.94–7.96 (m, 2H); ¹³C NMR (CDCl₃, 100 MHz) $\delta = 25.5, 33.4, 55.4, 58.5, 63.7, 68.9, 72.9, 78.7, 101.9, 114.4, 119.7, 127.3, 127.6, 128.4, 128.6, 128.7, 128.8, 128.8, 129.2, 129.3, 134.4, 137.2, 139.1, 139.1, 156.3, 161.7, 166.2, 170.5, 194.6$; HRMS (ESI) m/z : calcd for C₃₇H₃₃N₂O₆S [M + H]⁺ 633.2054; found 633.2048.

(4*R*,5'*S*)-Benzhydryl spiro[(5-benzoyl-3-cyclohexyl-isoxaxoline)-4',6'-penicillanate] (**6h**) and (4'*S*,5'*S*)-benzhydryl spiro[(4-benzoyl-3-cyclohexyl-isoxaxoline)-5',6'-penicillanate] (**7h**) and (4'*S*,5'*R*)-benzhydryl spiro[(4-benzoyl-3-cyclohexyl-isoxaxoline)-5',6'-penicillanate] (**8h**). Obtained from hydroximoyl chloride **3h** (67 mg, 0.412 mmol) and 6-(*Z*)-(benzoylmethylidene)penicillanate **5** (100 mg, 0.206 mmol) as described in the general procedure. The crude product was purified by flash chromatography (hexane/ethyl acetate, 3 : 1). The crude product was purified by flash chromatography (hexane/ethyl acetate, 3 : 1). Method A, gave, in order of elution, **6h** as a white solid (99 mg, 0.163 mmol, 79%) and a mixture of **7h/8h** as a colorless oil (13 : 87) (19 mg, 0.031 mmol, 15%). Method B, gave, in order of elution, **6h** as a white solid (75 mg, 0.123 mmol, 60%) and a mixture of **7h/8h** as a colorless oil (10 : 90) (13 mg, 0.021 mmol, 10%). Method C, gave, in order of elution, **6h** as a white solid (40 mg, 0.066 mmol, 35%) and a mixture of **7h/8h** (39 : 61) as a yellow oil (9 mg, 0.015 mmol, 8%).

6h: mp 182.7–185.0 °C; $[\alpha]_D^{25} = +340$ (c 0.25 in CH₂Cl₂); IR (ATR): $\nu = 882, 1154, 1176, 1450, 1495, 1597, 1668, 1746, 1773, 2856$ and 2390 cm^{-1} ; ¹H NMR (CDCl₃, 400 MHz) $\delta = 1.19$ (s, 3H), 1.22–1.26 (m, 3H), 1.53 (s, 3H), 1.61–1.79 (m, 5H), 1.97–2.00 (m, 2H), 2.47 (tt, $J = 11.4$ and 3.3 Hz, 1H), 4.59 (s, 1H), 5.84 (s, 1H), 5.94 (s, 1H), 6.97 (s, 1H), 7.32–7.38 (m, 10H), 7.49–7.53 (m, 2H), 7.63 (t, $J = 7.4$ Hz, 1H), 7.99–8.01 (m, 2H); ¹³C NMR (CDCl₃, 100 MHz) $\delta = 25.8, 26.0, 26.0, 26.2, 31.4, 31.9, 32.7, 36.1, 63.6, 68.0, 70.0, 78.2, 78.7, 80.0, 127.2, 127.6, 128.5, 128.7, 128.8, 128.9, 129.0, 129.4, 134.3, 135.1, 139.1, 139.3, 160.8, 166.5, 170.7, 192.0$; HRMS (ESI) m/z : calcd for C₃₆H₃₇N₂O₅S [M + H]⁺ 609.2418; found 609.2413.

Recorded as a mixture of **7h/8h** (10 : 90): **7h**: ¹H NMR (CDCl₃, 400 MHz) $\delta = 1.10$ (s, 3H), 1.38 (s, 3H), 1.40–1.88 (m, 10H), 2.03–2.10 (m, 1H), 4.40 (s, 1H), 5.40 (s, 1H), 5.46 (s, 1H), 6.91 (s, 1H), 7.29–7.36 (m, 10H), 7.53 (t, $J = 7.8$ Hz, 2H), 7.66 (t, $J = 7.4$ Hz, 1H), 7.95–7.97 (m, 2H); **8h**: ¹H NMR (CDCl₃, 400 MHz) $\delta = 1.10$ (s, 3H), 1.38 (s, 3H), 1.40–1.88 (m, 10H), 2.03–2.10 (m, 1H), 4.53 (s, 1H), 5.25 (s, 1H), 5.62 (s, 1H), 6.91 (s, 1H), 7.29–7.36 (m, 10H), 7.46–7.50 (m, 2H), 7.60 (t, $J = 7.4$ Hz, 1H), 8.02 (d, $J = 7.2$ Hz, 2H).

Conflicts of interest

There are no conflicts to declare.

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