Thionation of bicyclic β-lactam compounds by Lawesson's reagent

Andrés A. Poeylaut Palena and Ernesto G. Mata*

Instituto de Química Orgánica de Síntesis (CONICET - UNR), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, 2000 – Rosario, Argentina
E-mail: emata@fbioyf.unr.edu.ar

Dedicated to Prof. Rosa M. de Lederkremer on her 70th birthday

Abstract
We have developed a study on the thionation of cephalosporins and penicillins using Lawesson's reagent. Best results were obtained in the preparation of 7α-methoxy-8-thioxocephalosporins (70-98% isolated yield), and 6α-methoxy-7-thioxopenicillins (14% isolated yield).

Keywords: Lawesson's reagent, thionation, cephalosporins, penicillins

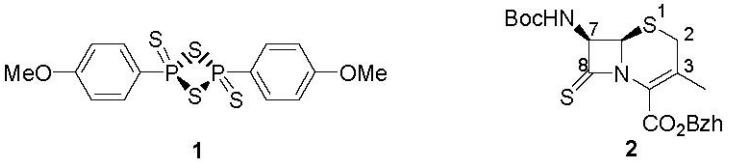
Introduction

It has been more than seven decades since Fleming observed the antibacterial action of penicillin[1] and about sixty years since penicillin was introduced into clinical practice. The subsequent development of other classes of β-lactam antibiotics (cephalosporins, cephamycins, carbapenems, monobactams) has made these compounds one of the most successful therapeutic agents to date and the most commonly prescribed antimicrobials. The remarkable biological effect of β-lactam antibiotics results from their capacity to disrupt the biosynthesis of the bacterial cell wall. Through the years, the effectiveness of β-lactam antibiotics has decreased due to the rapid evolution of bacterial resistance not only to single but also to multiple antibiotics, thanks to mutation and gene exchange.[2] Bacterial resistance to antibiotic drugs is also aggravated by the overuse of antibiotics in humans and animals and the noncompliance to the course of treatment by patients. The most common form of bacterial resistance to the β-lactam antibiotics is the production of β-lactamase enzymes in some bacteria.[3] These enzymes efficiently hydrolyze the amide bond of the β-lactam ring to give products that are devoid of antibacterial activity. Therefore, a current challenge in medicinal chemistry is the generation of new structures which will overcome the defense mechanisms of the bacteria.

To this end, β-thiolactams surge as an interesting class of non-β-lactamic analogues of penicillins and cephalosporins. Thionation, the conversion of a carbonyl group into a

thiocarbonyl group, can be performed by different methodologies. Phosphorus pentasulfide and boron sulfide were the most common thionating reagents until the development of 2,4-bis(4-methoxy-phenyl)-1,3-dithia-2,4-diphosphetane 2,4-disulfide, known as Lawesson's reagent (LR) (**1**) (Figure 1), an excellent reagent for converting carbonyl into thiocarbonyl compounds.[4]

Thionation of monocyclic β-lactams (monobactams) has been studied by different groups.[5] However, synthesis of bicyclic β-lactams such as penicillins and cephalosporin, has been scarcely reported. In 1975, Wojtkowski *et al.*[6] reported the thionation of some cephalosporins to form the corresponding 8-thioxocephalosporin using boron sulfide. The highest yield obtained was 20%. Similarly, thionation of penicillin derivatives was preformed with only 1% yield. Recently, the synthesis of the 8-thioxocephalosporin (**2**) was reported in good yield using Lawesson's reagent (Figure 1).[7]



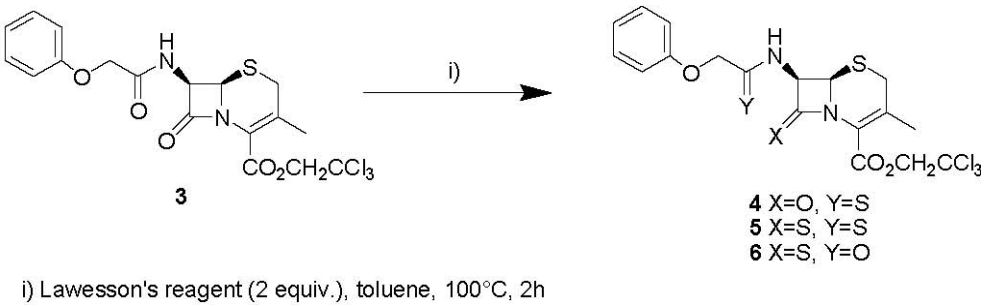
1 **2**

Figure 1

In order to obtain a general access to a variety of 8-thioxocephalosporins and 7-thioxopenicillins for biological screening and mechanistic studies, we became interested in the development of a reliable synthesis of these compounds. In this paper, we present a study about the scope and limitations of thionation of cephalosporins and penicillins using Lawesson's reagent.

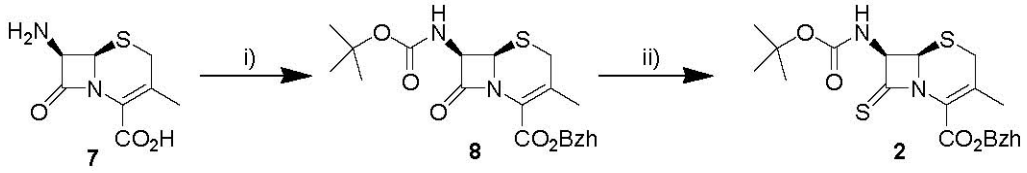
Results and Discussion

First, we selected cephalosporin **3** as the substrate for thionation studies (Scheme 1). Compound **3** was synthesized according to Chauvette *et al.*[8] Different conditions for thionation were attempted, starting material was recovered unless high temperature (100°C), two equivalents of Lawesson's reagent, and two hours of reaction were applied. Under these conditions, a compound was isolated in excellent yield (89%) after column chromatography. The isolated material was characterized as the thioamide **4** by comparison with the ^{1}H NMR data reported by Wojtkowski.[6] Due to their strained β-lactam ring, penicillins and cephalosporins have the normal amide resonance restricted. This causes an increase in the double bond character between the carbon and oxygen atoms, making their chemical behavior closer to that of a ketone group. Then, preparation of **4** was in agreement with Nishio *et al.*,[9] that established that the amide group tends to be more reactive to LR than ketones. The corresponding β-thiolactams **5** and **6** could not be obtained under any of the conditions employed.



Scheme 1

In order to avoid this competitive reaction, we decided to introduce the thiocarbonyl group before the introduction of the C-7 side chain in cephalosporin. Synthesis of the cephalosporin **8** from 7β-amino-3-deacetoxycephalosporanic acid (7-ADCA) (**7**) was then performed according to the literature (Scheme 2).[7]



Scheme 2

Table 1 Thionation of cephalosporin **8** with Lawesson's reagent (LR)

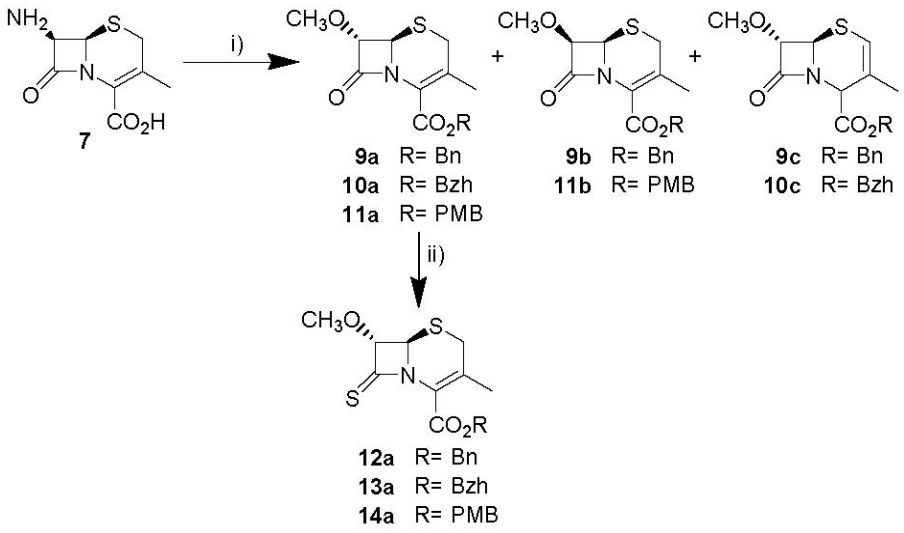
Entry	Conditions				Isolated products (%)
	Solvent	Equiv. of LR	temperature	time	
1	Toluene	1.0	100°C	20 h	starting material (**8**) (25%)[a]
2	Toluene	2.8	90°C	2 h	8-thioxocephalosporin (**2**) (20%) + **8** (20%)
3	Toluene	1.9	80°C	5 h	**2** (20%) + **8** (50%) + Δ^2 isomer of **8** (11%)
4	Toluene	1.9	80°C	3 h	**2** (30%)
5	THF	2.0	85°C	6 h	**8** (52%) + **2** (13%)
6	Benzene[b]	2.1	115°C	2.5 h	**8** (30%) + **2** (8.5%)

[a]Extended decomposition observed. [b]Dean-Stark trap was used.

Table 1 summarizes the conditions used for the thionation of **8** with LR. In our hands, the reported conditions (entry 1)[7] gave 25% of recovered starting material, together with decomposition products were obtained. The best approach for us was a shorter reaction time (3 h) and 1.9 equiv. of LR; thus, the 8-thioxocephalosporin **2** was obtained in 30% yield (entry 4), however this conditions were hard to reproduce. Moreover, compounds **8** and **2** have been shown to be unstable, decomposing in the order of days to weeks. Attempts to synthesize other ester derivatives of compound **8** and reacting them with LR, did not give rise to clear and reproducible results. These clearly demonstrate the difficulties of the preparation of 7β-aminoacyl-8-thioxocephalosporins as antibacterial analogues and we decided to abandon this approach.

7α-Alkoxycephalosporin derivatives have been reported as human leukocyte elastase (HLE) inhibitors.[10] Thus, different esters of 7α-methoxy-3-deacetoxycephalosporins (**9-11a**) were selected as substrates for thionation with LR. Esters **9-11a** were synthesized starting from 7-ADCA (**7**) (Scheme 3). Treatment of **7** with sodium nitrite and 70% $HClO_4$ in methanol,[11] followed by esterification gave a mixture of isomers that were separated by column chromatography. In the case of the benzyl ester derivative, three fractions were isolated: benzyl 7α-methoxy-3-deacetoxycephalosporinate (**9a**) (21% from 7-ADCA), benzyl 7β-methoxy-3-deacetoxycephalosporinate (**9b**) (5%) and the corresponding Δ^2 isomer (**9c**) (5%). For the benzhydryl (Bzh) ester derivative, benzhydryl 7α-methoxy-3-deacetoxycephalosporinate (**10a**) (12% from 7-ADCA) and the corresponding Δ^2 isomer (**10c**) (8%) were obtained. Finally, in the case of *p*-methoxybenzyl (PMB) esters, *p*-methoxybenzyl 7α-methoxy-3-deacetoxy-cephalosporinate (**11a**) (30% from 7-ADCA) and *p*-methoxybenzyl 7β-methoxy-3-deacetoxycephalosporinate (**11b**) (5%) were obtained.

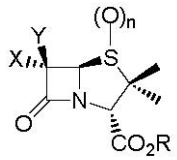
Esters **9-11a** were subjected to thionation with LR under different conditions. Best results were obtained when **9-11a** were treated with 1.8 equivalents of LR for 3 h at 90°C, hence, the corresponding 7α-methoxy-8-thioxocephalosporins (**12-14a**) were obtained in isolated yields ranging from 70 to 98%.



i) a.$NaNO_2$, 70% $HClO_4$ in MeOH, 16h; b. For R=Bn: BnCl, Et_3N, DMF, 45°C, 4h; for R=Bzh: benzhydryl bromide, Et_3N, Bu_4NI, Cl_3CH, 70°C, 6h; for R=PMB: PMBCl, Et_3N, DMF, 45°C, 4.5h. ii) Lawesson's reagent (1.8 equiv.), toluene, 90°C, 3h.

Scheme 3

Together with cephalosporins, penicillins are the most successful class of β-lactam antibiotics. Considering that the best yield reported for the synthesis of a 7-thioxopenicillin was 1%,[6] we decided to test the reaction of a series of penicillins with LR (Figure 2). Generally speaking, penicillins demonstrated to be less reactive than cephalosporins to LR. Benzyl 6,6-dihydropenicillanate 1,1-dioxide (**15**) and methyl 6,6-dibromopenicillanate (**16**), remained unreactive under conditions optimized for cephalosporins (toluene, 1.8 equiv. of LR, 90°C, 3 h) and, when conditions were more severe, an extended decomposition was observed. Furthermore, under the optimized conditions, thionation of benzyl 6α-chloropenicillanate (**17**) led to decomposition products.

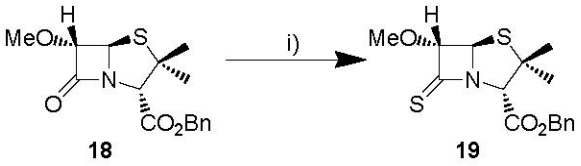


15 X=Y=H, n=2, R=Bn
16 X=Y=Br, n=0, R=Me
17 X=Cl, Y=H, n=0, R=Bn
18 X=MeO, Y=H, n=0, R=Bn

Figure 2

Only in the case of using benzyl 6α-methoxypenicillanate (**18**) as starting material, the expected β-thiolactam was isolated. Different conditions were attempted, mostly leading to decomposition; however, when compound **18** reacted with two equivalents of LR in toluene at

85°C for 1 h, the corresponding benzyl 6α-methoxy-7-thioxopenicillanate (**19**) was obtained in 14% isolated yield (Scheme 4).



18 19

i) Lawesson's reagent (2 equiv.), toluene, 90°C, 1h

Scheme 4

Conclusions

In summary, we report a study on the thionation of cephalosporins and penicillins using Lawesson's reagent. 7α-Methoxy-8-thioxocephalosporins were obtained in very high yield (70-98%), while 6α-methoxy-7-thioxopenicillins was synthesized in 14% yield. Other cephalosporin and penicillin substrates failed to give acceptable yields of the corresponding β-thiolactams.

From this study it can be concluded that, unlike monocyclic β-lactams, reaction of Lawesson's reagent with bicyclic β-lactams, such as cephalosporins and penicillins, is more difficult and very sensitive to even small changes in reaction conditions. Particularly, cephalosporins are more reactive than penicillins to LR. This observation can be explained by structural difference between cephalosporins and penicillins. The five-membered thiazolidine ring of penicillins is replaced by a six-membered dihydrothiazine ring in cephalosporins, as a consequence, the inhibition of the amide resonance is greater in penicillins enhancing the ketone character of the β-lactam's carbonyl group, which are, in turn, less reactive to LR than the amide group.

Experimental Section

General Procedures. Chemical reagents were purchased from commercial sources and were used without further purification unless noted otherwise. Solvents were analytical grade or were purified by standard procedures prior to use. Infrared spectra (IR) were recorded on a Shimadzu Prestige 21 spectrophotometer and only partial spectral data are listed. ^{1}H NMR spectra were recorded on a Bruker AC200 at 200 MHz in $CDCl_3$ unless otherwise stated, in the presence of TMS (0.00 ppm) as the internal standard. Conventional and gel-phase ^{13}C NMR spectra were recorded on the same apparatus at 50 MHz with $CDCl_3$ as solvent and reference (76.9 ppm), unless otherwise stated. ^{13}C NMR assignments were made on the basis of chemical shifts and proton multiplicities (from DEPT spectra). Analytical thin-layer chromatography (TLC) was

carried out with silica gel 60 F_{254} pre-coated aluminum sheets (Merck). Flash column chromatography was performed using Merck silica gel 60 (230-400 mesh).

(6*R*,7*R*)3-Methyl-8-oxo-7-(2-phenoxy-acetylamino)-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid 2,2,2-trichloro-ethyl ester (3).[8] $[\alpha]_D^{25}$= +231.2 (*c* 1.04, $CHCl_3$); IR (film) 3326 cm^{-1} (NH), 1782 cm^{-1} (C=O ester), 1737 cm^{-1} (C=O lactam), 1688 cm^{-1} (C=O amide), 1215 cm^{-1} (ester II Band); ^{1}H NMR δ 2.20 (s, 1H, CH_3 3'), 3.28 (d, *J*= 18.0 Hz, 1H, H2), 3.54 (d, *J*=18.0 Hz, 1H, H2'), 4.55 (s, 2H, C*H*$_2$CONH), 4.82 (d, *J*=12.0 Hz, 1H, C*H*CCl_3), 4.97 (d, *J*=12.0 Hz, 1H, C*H*CCl_3), 5.04 (d, *J*=4.7 Hz, 1H, H6), 5.88 (dd, J_1=4.7 Hz, J_2=9.1 Hz, 1H, H7), 6.9-7.5 (m, 6H, Ar and NH); ^{13}C NMR δ 20.2 (C3'), 30.4 (C2), 56.9 and 58.4 (C6 and C7), 67.2 and 74.8 (*C*H_2CCl_3 and PhO*C*H_2CO), 94.3 (CH_2*C*Cl_3), 114.7 (*o*-Ar), 121.7 (C4), 122.3 (*m*-Ar), 129.7 (*p*-Ar), 134.2 (C3), 156.9 (*α*-Ar), 160.4, 164.1 and 168.6 (ester, lactam and amide carbonyls).

(6*R*,7*R*) 3-Methyl-8-oxo-7-(2-phenoxy-thioacetylamino)-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid 2,2,2-trichloro-ethyl ester (4). cephalosporin **3** (95.6 mg, 0.20 mmol) was dissolved in anhydrous toluene (2.9 mL). Lawesson's reagent (**1**) (88.1 mg, 0.21 mmol, 2.1 equiv.) was previously dried in a vacuum desiccator over phosphorus pentoxide for 1 h, and then added to the solution of **3**. The mixture was stirred for 15 min. at room temperature, and then at 100ºC. After 40 min. of heating, monitored by TLC, the starting material has disappeared. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography eluting with hexane:ethyl acetate. One fraction of 88.0mg (89%) of pure **4** was obtained. A fraction of 42.0 mg was recrystallized from hexane-ethyl acetate (85:15%) to give 12.0 mg of a white amorphous solid. Mp=149-150ºC; $[\alpha]_D^{25}$= +199.1 (*c* 0.51, $CHCl_3$); IR (KBr) 3256 cm^{-1} (NH), 1773 cm^{-1} (C=O, ester), 1734 cm^{-1} (C=O, lactam); ^{1}H NMR δ 2.23 (s, 3H, CH_3 3'), 3.19 (d, *J*= 18.5 Hz, 1H, H2) , 3.51 (d, *J*= 18.5 Hz, 1H, H2'), 4.84 (d, *J*= 13.2 Hz, 1H, C*H*CCl_3), 4.96 (s, 2H, C*H*$_2$CONH), 4.99 (d, *J*= 13.2 Hz, 1H, C*H*CCl_3), 5.19 (d, *J*=4.6 Hz, 1H, H6), 6.20 (dd, J_1=4.6 Hz, J_2=7.8 Hz, 1H, H7), 6.9-7.5 (m, 6H, Ar and NH) ; ^{13}C NMR δ 20.3 (C3'), 30.6 (C2), 56.8 and 63.0 (C6 and C7), 73.8 and 74.8 (*C*H_2CCl_3 and PhO*C*H_2CO), 94.3 (CH_2*C*Cl_3), 114.9 (*o*-Ar), 121.7 (C4), 122.5 (*m*-Ar), 129.7 (*p*-Ar), 135.6 (C3), 156.6 (*α*-Ar), 160.2 and 162.6 (ester and lactam), 199.9 (thioamide).

(6*R*,7*R*) 7-*tert*-Butoxycarbonylamino-3-methyl-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid benzhydryl ester (8). 7β-amino-3-deacetoxycephalosporanic acid (7-ADCA) (**7**) (644.8 mg, 3.0 mmol) was dissolved in aqueous saturated solution of $NaHCO_3$ (20 mL) and a solution of di-tert-butyl dicarbonate (Boc_2O) (1.03 g, 4.8 mmol) in dioxane (20 mL) was added dropwise at 0°C. The reaction mixture was stirred at room temperature overnight. After adding water (20 mL), the mixture was washed with AcOEt (3 x 20 mL). The aqueous layer was acidified to pH=2 and extracted with AcOEt (4 x 20 mL). The organic layers were dried over Na_2SO_4 and evaporated under reduced pressure to give crude 7-(*tert*-butyloxycarbonyl)-3'-deacetoxy-cephalosporanic acid (730.0 mg) which was used without further purification. To a solution of the acid (730.0 mg) in anhydrous chloroform (16.8 mL) at 0ºC was added triethylamine (490 µl, 3.5 mmol, 1.17 equiv.). After this, a solution of tetrabutyl ammonium

iodide (460.0 mg, 1.25 mmol, 0.4 equiv.) and benzhydryl bromide (950.5 mg, 3.7 mmol, 1.2 equiv.) in chloroform (3.4 mL) were added. The mixture was stirred at reflux for 6 h. The reaction mixture was then diluted with ethyl acetate (200 mL) and washed with 1N HCl (2 x 5 mL), $NaHCO_3$ 5% w/w (2 x 5 mL) and brine. The organic layer was dried over Na_2SO_4, and solvent was evaporated under reduced pressure to give 1.025 g of brown oil as crude reaction product. A column chromatography was carefully performed eluting with hexane:ethyl acetate. Two fractions were obtained: 97.9 mg (7 % from 7-ADCA) of the Δ^2 isomerized product and 500.1 mg (35% from 7-ADCA) of the desired product **8**. $[\alpha]_D^{25}$= +23.9 (*c* 2.79, $CHCl_3$); IR (film) 3318 cm^{-1} (NH), 1774 cm^{-1} (C=O), 1719 cm^{-1} (C=O), 1159 cm^{-1} (ester II band); ^{1}H NMR δ 1.46 (s, 9H, *t*-Bu), 2.10 (s, 3H, H3'), 3.18 (d, *J*= 18.4 Hz, 1H, H2), 3.46 (d, *J*= 18.4 Hz, 1H, H2'), 4.93 (d, *J*= 4.8 Hz, 1H, H6), 5.37 (d, *J*= 9.5, 1H, NH), 5.59 (dd, J_1= 4.8 Hz, J_2= 9.5 Hz, 1H, H7), 6.91 (s, 1H, CH Bzh), 7.24-7.48 (m, 10H, Ar); ^{13}C NMR δ 20.1 (C3'), 28.1 (*t*-Bu), 30.2 (C2), 57.5 and 60.5 (C6 and C7), 78.9 (*t*-Bu), 80.9 (*C*HPh_2), 122.5 (C4), 126.9 (Ar), 127.3 (Ar), 127.8 (Ar), 128.3 (Ar), 128.4 (Ar), 133.2 (C3), 139.5 (Ar), 139.6 (Ar), 154.6, 161.2 and 165.2 (ester, lactam and carbamate).

(6*R*,7*R*) 7-*tert*-Butoxycarbonylamino-3-methyl-8-thioxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid benzhydryl ester (2). The starting material **8** (56.9 mg, 0.12 mmol) was placed in a round bottom flask, the system was purged with anhydrous nitrogen and the compound dissolved in anhydrous toluene (2.0 mL). Lawesson's reagent (**1**) (45.0 mg, 0.11 mmol, 1.9 equiv.) was previously dried in a vacuum desiccator over phosphorus pentoxide for 1 h, and then added to the solution of **8**. Once the reagent was added, the reaction mixture was stirred at room temperature for 15 min and then three hours at 90ºC. Solvent was removed under reduced pressure. The residue was then subjected to column chromatography (hexane:CH_2Cl_2, 17:83) to give pure product **2** (17.0 mg, 30%). $[\alpha]_D^{25}$= +103.2 (*c* 0.83, $CHCl_3$); IR (film) 3302 cm^{-1} (NH), 1715 cm^{-1} (C=O ester and carbamate), 1409 cm^{-1} (C=S thiolactam), 1248 cm^{-1} (ester II band); ^{1}H NMR δ 1.47 (s, 9H, *t*-Bu), 2.05 (s, 3H, H3'), 3.20 (d, *J*= 18.0 Hz, 1H, H2), 3.45 (d, *J*= 18.0 Hz, 1H, H2'), 5.15-5.30 (m, 2H, NH and H7), 5.40 (d, *J*= 3.9 Hz, 1H, H6), 6.99 (s, 1H, C*H*Ph_2), 7.26-7.41 (m, 10H, Ar); ^{13}C NMR δ 19.9 (C3'), 28.1 (*t*-Bu), 30.0 (C2), 60.3 and 64.6 (C6 and C7), 78.8 (*C*HPh_2), 80.9 (*t*-Bu), 124.9 (C4), 127.0 (Ar), 127.9 (Ar), 128.3 (Ar), 128.3 (Ar), 134.4 (C3), 139.0 (Ar), 139.5 (Ar), 154.8 and 160.9 (ester and carbamate carbonyls), 201.0 (thiolactam).

(6*S*,7*R*) 7-Methoxy-3-methyl-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid benzyl ester (9a). 7-ADCA (**7**) (860.0 mg, 4.0 mmol) was suspended in methanol (28.0 mL) and the reaction mixture was cooled to 0ºC. From an addition funnel, $HClO_4$ 70% (1.7 mL) was added dropwise into the reaction mixture for 20 min under vigorous stirring. After that, $NaNO_2$ (1.05 g, 15.2 mmol) was added in small portions. Temperature was maintained at 0ºC for 30 min, and then the mixture was stirred at room temperature for 4 h. Water was added and the crude was extracted with CH_2Cl_2 (10 x 10 mL), the combined organic layers were washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to give brown oil (752.2 mg) as crude 7-α-methoxy-3'-deacetoxy-cephalosporanic acid which was used without

further purification. A portion of the acid (421.2 mg, ~1.8 mmol) was dissolved in DMF (3.2 mL), and triethylamine (560 µl, 460 mg, 4.0 mmol, 2.2 equiv.) and benzyl chloride (450 µl, 495 mg, 3.92 mmol, 2.2 equiv.) were successively added dropwise at 0°C. The temperature was increased to 45ºC, and the reaction mixture was stirred for 4 h. The reaction was treated with water and extracted with AcOEt (5 x 25 mL). The combined organic layers were washed with 1N HCl (2 x 10 mL), 0.5N NaOH (2 x 10 mL), brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to give a brown oil (1.06 g) as crude reaction product. The residue was purified by flash column chromatography eluting with hexane and AcOEt. Three fractions were collected: 37.6 mg (5% from 7-ADCA) of the Δ^2-isomerized product **9c**, 39.8 mg (5% from 7-ADCA) of the β-epimer **9b**, and 150.0 mg (21% from 7-ADCA) of the expected product **9a**.

α-Epimer 9a. $[\alpha]_D^{25}$= +86.1 (*c* 1.6, $CHCl_3$); IR (film) 1773 cm^{-1} (C=O ester), 1724 cm^{-1} (C=O lactam) , 1223 cm^{-1} (ester II band); ^{1}H NMR δ 2.05 (s, 3H, $CH_3$3'), 3.13 (d, *J*= 17.8 Hz, 1H, H2), 3.45 (d, *J*= 17.8 Hz, 1H, H2'), 3.51 (s, 3H, CH_3O), 4.49 (d, *J*= 1.5 Hz, 1H, H7), 4.66 (d, *J*= 1.5 Hz, 1H, H6), 5.23 (d, *J*= 12.2 Hz, 1H, C*H*Ph), 5.33 (d, *J*= 12.2 Hz, 1H, C*H*'Ph), 7.26-7.45 (m, 5H, Ar); ^{13}C NMR δ 19.3 (C3'), 31.2 (C2), 55.8 (C6), 57.8 (CH_3O), 67.4 (*C*H_2Ph), 90.2 (C7), 123.6 (C4), 128.2 (Ar), 128.4 (Ar), 128.5 (Ar), 128.8 (Ar), 135.1 (C3), 161.1 and 161.9 (ester and lactam carbonyls).

β-Epimer 9b. $[\alpha]_D^{25}$= +28.3 (*c* 2.31, $CHCl_3$); IR (film) 1772 cm^{-1} (C=O ester), 1722 cm^{-1} (C=O lactam), 1223 cm^{-1} (ester II band); ^{1}H NMR δ 2.13 (s, 3H, $CH_3$3'), 3.20 (d, *J*= 18,5 Hz, 1H, H2), 3.44 (d, *J*= 18.5 Hz, 1H, H2'), 3.57 (s, 3H, CH_3O), 4.90 (s, 2H, H6 and H7), 5.22 (d, *J*= 12.2 Hz, 1H, C*H*Ph), 5.30 (d, *J*= 12.2 Hz, 1H, C*H*'Ph), 7.32-7.40 (m, 5H, Ar); ^{13}C NMR δ 20.0 (C3'), 30.3 (C2), 57.6 (CH_3O), 59.5 (C6), 67.3 (*C*H_2Ph), 84.8 (C7), 122.4 (C3), 128.2 (Ar), 128.4 (Ar), 128.5 (Ar), 133.1 (C4), 135.2 (Ar), 162.0 and 164.1 (ester and lactam carbonyls).

(6*S*,7*R*) 7-Methoxy-3-methyl-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid benzhydryl ester (10a). To a solution of the crude 7-α-methoxy-3'-deacetoxy-cephalosporanic acid (511.9 mg) in anhydrous chloroform (12 mL) at 0ºC was added triethylamine (350 µl, 2.5 mmol, 1 equiv.). After this, a solution of tetrabutyl ammonium iodide (330 mg, 0.9 mmol, 0.4 equiv.) and benzhydryl bromide (680 mg, 2.8 mmol, 1.1 equiv.) in chloroform (2.4 mL) were added. The mixture was stirred at reflux for 1.5 h. The reaction mixture was then diluted with ethyl acetate (200 mL) and washed with 1N HCl (2 x 5 mL), $NaHCO_3$ 5% w/w (2 x 5 mL) and brine. The organic layer was dried over Na_2SO_4, and solvent was evaporated under reduced pressure to give 1.025 g of brown oil as crude reaction product. A column chromatography was carefully performed eluting with hexane:ethyl acetate. Two fractions were obtained: 76.8 mg (8 % from 7-ADCA) of the Δ^2 isomerized product **10c** and 122.6 mg (12% from 7-ADCA) of the desired product **10a**.

Compound 10a. $[\alpha]_D^{25}$= +57.9 (*c* 1.25, $CHCl_3$); IR (film) 1769 cm^{-1} (C=O, ester), 1714 cm^{-1} (C=O, lactam) , 1223 cm^{-1} (ester II band); ^{1}H NMR δ 2.05 (s, 3H, CH_3 3'), 3.15 (d, *J*= 17,1 Hz, 1H, H2), 3.39 (d, *J*= 17.1 Hz, 1H, H2'), 3.55 (s, 3H, CH_3O), 4.54 (d, *J*= 1.6 Hz, 1H, H7), 4.69 (d, *J*= 1.6 Hz, 1H, H6), 6.95 (s, 1H, C*H* Bzh), 7.25-7,46 (m, 10H, Ar Bzh); ^{13}C NMR δ 19.5 (C3'),

31.4 (C2), 56.3 (C6), 57.7 (CH_3O), 78.9 (*C*HPh_2), 90.5 (C7), 124.0 (C4), 127.0 (Ar), 127.4 (Ar), 127.8 (Ar), 128.4 (Ar), 128.4 (Ar), 131.8 (C3), 139.5 (Ar), 139.7 (Ar), 161.2 and 161.6 (ester and lactam carbonyls).

Δ^2-Isomer 10c. ^{1}H NMR δ 1.81 (s, 3H, CH_3 3'), 3.44 (s, 3H, CH3O), 4.61 (d, *J*= 0.9 Hz, 1H, H7), 4.84 (s, 1H, H4), 4.97 (d, *J*= 0.9 Hz, 1H, H6), 5.95 (s, 1H, H2), 6.89 (s, 1H, CH Bzh), 7.40-7,24 (m, 10H, Ar); ^{13}C NMR δ 21.9 (C3'), 51.9 (C4), 53.7 (C6), 57.3 (CH_3O), 78.7 (*C*HPh_2), 92.4 (C7), 114.9 (C2), 119.7 (C3), 126.6 (CH, Ar), 127.2 (CH, Ar), 128.1 (CH, Ar), 128.2 (CH, Ar), 128.2 (CH, Ar), 128.5 (CH, Ar), 139.0 (C, Ar), 139.0 (C, Ar), 162.2 and 166.4 (ester and lactam carbonyls).

(6*S*,7*R*) 7-Methoxy-3-methyl-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid 4-methoxy-benzyl ester (11a). To a solution of the crude 7-α-methoxy-3'-deacetoxy-cephalosporanic acid (663.3 mg) in anhydrous DMF (5.0 mL), triethylamine (870 µl, 631 mg, 6,2 mmol, 2,1 equiv.) and *p*-methoxybenzyl chloride (700 µl, 770 mg, 4,9 mmol, 1,7 equiv.) were successively added dropwise at 0°C. The temperature was increased to 45ºC, and the reaction mixture was stirred for 4.5 h. The reaction was treated with water and extracted with AcOEt (5 x 25 mL). The combined organic layers were washed with 1N HCl (2 x 10 mL), 0.5N NaOH (2 x 10 mL), brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to give a brown oil (1.12 g) as crude reaction product. The residue was purified by flash column chromatography eluting with hexane and AcOEt. Two fractions were obtained: the 7-β-epimerized product **11b** (75.1 mg, 5% from 7-ADCA) and the expected product **11a** (283.9 mg, 30% from 7-ADCA).

α-Epimer 11a. Mp= 112-113 ºC; $[\alpha]_D^{25}$= +60.1 (*c* 1.46, $CHCl_3$); IR (film) 1774 cm^{-1} (C=O ester), 1722 cm^{-1} (C=O lactam) , 1245 and 1224 cm^{-1} (C-O); ^{1}H NMR δ 2.05 (s, 3H, $CH_3$3'), 3.14 (d, *J*= 18.2 Hz, 1H, H2), 3.45 (d, *J*= 18.2 Hz, 1H, H2'), 3.53 (s, 3H, CH_3O), 3.80 (s, 3H, C*H*$_3$OPh), 4.49 (d, *J*= 1,2 Hz, 1H, H7), 4.65 (d, *J*= 1,2 Hz, 1H, H6), 5.17 (d, *J*= 11,9 Hz, 1H, C*H*Ph), 5.28 (d, *J*= 11.9 Hz, 1H, C*H'*Ph), 6,88 (d, *J*= 8.5 Hz, 2H, *m*-Ar), 7.36 (d, *J*= 8.5 Hz, 2H, *o*-Ar); ^{13}C NMR δ 19.4 (C3'), 31.3 (C2), 55.2 (CH_3O), 55.8 (C6), 57.9 (CH_3O), 67.3 (*C*H_2Ph), 90.3 (C7), 113.8 (*m*-Ar), 123.8, 127.3 and 128.4 (C3, C4 and *α*-Ar), 130.4 (*o*-Ar), 159.7 (*p*-Ar, CH_3O*C*), 161.1 and 162.0 (ester and lactam carbonyls).

β-Epimer 11b. $[\alpha]_D^{25}$= +22.5 (*c* 1.1, $CHCl_3$); IR (film) 1773 cm^{-1} (C=O ester), 1720 cm^{-1} (C=O lactam), 1246 and 1222 cm^{-1} (C-O); ^{1}H NMR δ 2.11 (s, 3H, $CH_3$3'), 3.18 (d, *J*= 18.0 Hz, 1H, H2), 3.43 (d, *J*= 18.0 Hz, 1H, H2'), 3.56 (s, 3H, CH_3O), 3.79 (s, 3H, CH_3OPh), 4.88 (s, 2H, H6 and H7), 5.15 (d, *J*= 11.8 Hz, 1H, C*H*Ph), 5.23 (d, *J*= 11.8 Hz, 1H, C*H'*Ph), 6.88 (d, *J*= 8.8 Hz, 2H, *m*-Ar), 7.34 (d, *J*= 8.8 Hz, 2H, *o*-Ar); ^{13}C NMR δ 20.0 (C3'), 30.2 (C2), 55.1 (CH_3O), 57.5 (C6), 59.5 (CH_3O), 67.1 (*C*H_2Ph), 84.7 (C7), 113.8 (*m*-Ar), 122.4, 127.3 and 132.6 (C3, C4 and *α*-Ar), 130.3 (*o*-Ar), 159.6 (*p*-Ar, CH_3O*C*), 162.1 and 164.0 (C, ester and lactam carbonyls).

(6*S*,7*R*)7-Methoxy-3-methyl-8-thioxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid benzyl ester (12a). As a typical thionation procedure in this paper, the starting material **9a** (39.2 mg, 0.12 mmol) was placed in a round bottom flask, the system was purged with anhydrous nitrogen and the compound dissolved in anhydrous toluene (2.0 mL). Lawesson's

reagent (**1**) (44.1 mg, 0.10 mmol, 1.8 equiv.) was previously dried in a vacuum desiccator over phosphorus pentoxide for 1 h, and then added to the solution of **9a**. Once the reagent was added, the reaction mixture was stirred at room temperature for 15 min and then for 3 h at 90ºC. Solvent was removed under reduced pressure. This crude was purified by flash column chromatography (hexane-CH_2Cl_2, 40:60) to provide 39.2 mg (98%) of pure product **12a**. $[\alpha]_D^{25}$= +135.3 (*c* 1.21, $CHCl_3$); IR (film) 1728 cm^{-1} (C=O ester), 1417 cm^{-1} (C=S thiolactam) , 1246 cm^{-1} (ester II band); ^{1}H NMR δ 2.06 (s, 3H, $CH_3$3'), 3.17 (d, *J*= 17.6 Hz, 1H, H2), 3.42 (d, *J*= 17.6 Hz, 1H, H2'), 3.60 (s, 3H, CH_3O), 4.23 (d, *J*= 1.5 Hz, 1H, H7), 5.07 (d, *J*= 1.5 Hz, 1H, H6), 5.19 (d, *J*= 13.0 Hz, 1H, C*H*Ph), 5.36 (d, *J*= 13.0 Hz, 1H, C*H*'Ph), 7.32-7.44 (m, 5H, Ar); ^{13}C NMR δ 19.3 (C3'), 30.6 (C2), 57.3 (CH_3O), 62.7 (C6), 67.7 (*C*H_2Ph), 88.2 (C7), 125.1 (C3), 128.3 (CH, Ar), 128.4 (CH, Ar), 128.7 (CH, Ar), 130.9 (C3), 134.9 (C, Ar), 161.5 (ester carbonyl), 195.9 (C, thiolactam).

(6*S*,7*R*)7-Methoxy-3-methyl-8-thioxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid benzhydryl ester (13a). Following a similar procedure as that used for the preparation of the 8-thioxocephalosporin **12a**, compound **13a** was obtained in 82% yield. $[\alpha]_D^{25}$= +51.3 (*c* 1.4, $CHCl_3$); IR (film) 1726 cm^{-1} (C=O ester), 1416 cm^{-1} (C=S thiolactam) , 1225 cm^{-1} (ester II band); ^{1}H NMR δ 2.02 (s, 3H, CH_3 3'), 3.15 (d, *J*= 17.9 Hz, 1H, H2), 3.31 (d, *J*= 17.9 Hz, 1H, H2'), 3.61 (s, 3H, CH_3O), 4.28 (d, *J*= 1.1 Hz, 1H, H6), 5.13 (d, *J*= 1.1 Hz, 1H, H7), 6.97 (s, 1H, C*H*Ph_2), 7.46-7.25 (m, 10H, Ar); ^{13}C NMR δ 19.3 (CH_3 3'), 30.7 (C2), 57.0 (CH_3O), 63.8 (C6), 78.9 (*C*HPh_2), 88.6 (C7), 127.1 (CH, Ar), 127.7 (CH, Ar), 127.8 (CH, Ar), 127.9 (CH, Ar), 128.3 (CH, Ar), 128.3 (CH, Ar), 134.8, 139.2 and 139.6 (C, C3, C4 and Ar), 160.8 (C, ester), 196.9 (C, thiolactam).

(6*S*,7*R*)7-Methoxy-3-methyl-8-thioxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid 4-methoxy-benzyl ester (14a). Following a similar procedure as that used for the preparation of the 8-thioxocephalosporin **12a**, compound **14a** was obtained in 70% yield. Mp= 129-130 ºC; $[\alpha]_D^{25}$= +124.0 (*c* 0.39, $CHCl_3$); IR (film) 1728 cm^{-1} (C=O ester), 1416 cm^{-1} (C=S thiolactam) , 1246 cm^{-1} (ester II band); ^{1}H NMR δ 2.04 (s, 3H, $CH_3$3'), 3.16 (d, *J*= 17.6 Hz, 1H, H2), 3.42 (d, *J*= 17.6 Hz, 1H, H2'), 3.60 (s, 3H, CH_3O), 3.80 (s, 3H, C*H*$_3$OPh), 4.22 (d, *J*= 1.2 Hz, 1H, H7), 5.05 (d, *J*= 1.2 Hz, 1H, H6), 5.13 (d, *J*= 11.9 Hz, 1H, C*H*Ph), 5.29 (d, *J*= 11.9 Hz, 1H, C*H*'Ph), 6.88 (d, *J*= 8.7 Hz, 2H, *m*-Ar), 7.35 (d, *J*= 8.7 Hz, 2H, *o*-Ar); ^{13}C NMR δ 19.3 (C3'), 30.6 (C2), 55.2 (CH_3O), 57.4 (C6), 62.6 (CH_3O), 67.6 (C*H*$_2$Ph), 88.2 (C7), 113.8 (*m*-Ar), 125.2, 127.1 and 130.4 (C3, C4 and *α*-Ar), 130.6 (*o*-Ar), 159.8 (*p*-Ar, CH_3OC), 161.6 (C, ester), 195.8 (C, thiolactam).

(1*R*,4*R*,7*S*)7-Methoxy-3,3-dimethyl-6-oxo-2-thia-bicyclo[3.2.0]heptane-4-carboxylic acid benzyl ester (18). 6β-aminopenicillanic acid (6-APA) (1.0 g, 4.6 mmol) was suspended in anhydrous DMF (8.0 mL) at room temperature and triethylamine (1.3 mL) was added dropwise. The reaction mixture was heated at 45ºC and once the suspension becomes a solution (~20 min), benzyl chloride was added dropwise. The mixture was stirred at the same temperature for 4.5 h and then allowed to reach room temperature. Ethyl acetate (10 mL) was added and the triethylammonium chloride was filtered. The organic layer was washed with water (3 x10 mL),

dried over Na_2SO_4 and concentrated to a half of its original volume. After that, a solution of *p*TsOH (880 mg) in AcOEt (15 mL) was added at 0ºC to give a yellow solid which was filtered and washed successively with AcOEt and ethyl ether to afford, after drying under reduced pressure, 1.17g (51%) of the ammonium salt of the benzylated 6-APA as a white solid. A portion of this white solid (337.9 mg, 0.71 mmol) was placed in a round bottom flask and water (20 mL) and CH_2Cl_2 (20 mL) were added with vigorous stirring. Then, $NaNO_2$ (800 mg, 11.6 mmol) and *p*TsOH (160 mg , 0.93 mmol) were added dropwise at 0ºC. After 10 min at 0ºC, the layers were separated in a separatory funnel. The organic layer was washed with brine and dried over Na_2SO_4 to give 338.9 mg of the benzyl 6-diazopenicillanate as a deep yellow oil. This oil was dissolved in methanol and stirred overnight at room temperature. Solvent was evaporated under reduced pressure and the residue was purificated by chromatography column to give 74.0 mg (33% from the ammonium salt) of pure product **18**. $[\alpha]_D^{25}$= 143.0 (*c* 1.98, $CHCl_3$); IR (film) 2964 cm^{-1} (C-H), 1778 cm^{-1} (C=O) , 1745 cm^{-1} (C=O); ^{1}H NMR δ 1.39 (s, 3H, CH3 2'), 1.54 (s, 3H, CH_3 2''), 3.52 (s, 3H, CH_3O), 4.52 (s, 1H, H3), 4.57 (d, *J*=1.1 Hz, 1H, H5), 5.19 (s, 2H, benzylic), 5.31 (d, *J*=1.1 Hz, 1H, H6), 7.37 (s, 5H, Ar); ^{13}C NMR δ 25.5 and 33.6 (CH_3, 2'α and 2'β), 57.6 (CH_3O), 64.2 (CH), 67.3 (Ph*C*H_2), 68.3 (CH), 68.9 (CH), 92.4 (C2), 128.5 (CH, Ar), 128.6 (CH, Ar), 134.7 (C, Ar), 167.1 and 169.3 (ester and lactam carbonyls).

(1*R*,4*R*,7*S*)7-Methoxy-3,3-dimethyl-6-thioxo-2-thia-bicyclo[3.2.0]heptane-4-carboxylic acid benzyl ester (19). penicillin **18** (90.8 mg, 0.28 mmol) was placed in a round bottom flask, the system was purged with anhydrous nitrogen and the compound dissolved in anhydrous toluene (4.5 mL). Lawesson's reagent (**1**) (113 mg, 0.28 mmol, 2.0 equiv.) was previously dried in a vacuum desiccator over phosphorus pentoxide for 1 h, and then added to the solution of **18**. Once the reagent was added, the reaction mixture was stirred at room temperature for 15 min and then for 1 h at 85ºC. Solvent was removed under reduced pressure. The crude product was filtered over silica gel using CH_2Cl_2 as eluting solvent. The solvent was evaporated and then the residue was purified by chromatography column (hexane-AcOEt) to provide 12.2 mg (14%) of pure product **19**. $[\alpha]_D^{25}$= 144.4 (*c* 0.79, $CHCl_3$); IR (film) 2927 cm^{-1} (C-H), 1743 cm^{-1} (C=O) , 1402 cm^{-1} (C=S); ^{1}H NMR δ 1.41 (s, 3H, CH_3 2'), 1.61 (s, 3H, CH_3 2''), 3.60 (s, 3H, C*H*$_3$O), 4.28 (d, *J*= 1 Hz, 1H, H5), 4.81 (s, 1H, H3), 5.21 (s, 2H, benzylics), 5.67 (d, *J*= 1 Hz, 1H, H6), 7.37 (s, 5H, Ar); ^{13}C NMR δ 25.9 and 33.3 (CH_3, 2'α and 2'β), 57.1 (CH_3O), 64.7 (CH), 67.5 (CH), 70.3 (Ph*C*H2), 74.4 (CH), 90.7 (C2), 128.5 (CH, Ar), 128.6 (CH, Ar), 134.6 (C, Ar), 166.5 (C, ester carbonyl), 206.6 (C, thiolactam carbonyl).

Supplementary Information

The ^{1}H and ^{13}C NMR spectra of the 8-thioxocephalosporins, 7-thioxopenicillin, and their starting materials are included.

Acknowledgements

The authors would like to thank CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas - Argentina), Agencia Nacional de Promoción Científica y Tecnológica (Argentina); Universidad Nacional de Rosario (Argentina), The Royal Society of Chemistry (U.K.) and Fundación Antorchas (Argentina) for financial support.

References

1. Fleming, A. *Br. J. Exp. Pathol.* **1929**, *10*, 226.
2. Chu, D. T. W.; Plattner, J. J.; Katz, L. *J. Med. Chem.* **1996**, *39*, 3853.
3. Bush, K. *Curr. Pharm. Design* **1999**, 5, 839.
4. Jesberger, M.; Davis, T. P.; Barner, L. *Synthesis* **2003**, 1929.
5. (a) Commercon A; Ponsinet, G. *Tetrahedron Lett*. **1983**, *24*, 3725. (b) Verkoyen, C.; Rademacher, P. *Chem. Ber.* **1985**, *118*, 653. (c) Förster, W. R.; Isecke, R.; Spanka, C.; Schaumann, E. *Synthesis* **1997**, 942. (d) Méndez, L.; Delpiccolo, C. M. L.; Mata, E. G. *Synlett* **2005**, 1563.
6. Wojtkowski, P. W.; Dolfini, J. E.; Kocy, O.; Cimarusti, Ch. M. *J. Am. Chem. Soc.* **1975**, *97*, 5628.
7. Tsang, W. Y.; Dhanda, A.; Schofield, Ch. J.; Page, M. I. *J. Org. Chem.* **2004**, *69*, 339.
8. Chauvette, R. R.; Pennington, P. A.; Ryan, C. W.; Cooper, R. D. G.; Jose, F. L.; Wright, I. G.; Van Heyningen, E. M.; Huffman, G. W. *J. Org. Chem.* **1971**, 36, 1259.
9. Nishio *et al.* have established a reactivity order towards LR: alcohol > amide > ketone > ester; see: Nishio, T.; Ori, M. *Heterocycles* **2000**, *52*, 111.
10. Mascaretti, O. A.; Boschetti, C. E.; Danelon, G. O.; Mata, E. G.; Roveri, O. A. *Curr. Med. Chem.* **1995**, *1*, 441.
11. Alpegiani, M.; Bissolino, P.; D'Anello, M.; Rivola, G.; Borghi, D.; Perrone, E. *Tetrahedron Lett.* **1991**, *32*, 3883.

