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Synthesis and antimicrobial properties of cephalosporin derivatives substituted on the C(7) nitrogen with arylmethyloxyimino or arylmethyloxyamino alkanoyl groups

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Abstract

Some 7-aminocephalosporanic acid (7-ACA) derivatives substituted on the C(7) nitrogen with 2-(arylmethyloxyimino)propionyl (**3a–f**), 2-(arylmethyloxyamino)propionyl (**4a–d**) and (arylmethyloxyamino)acetyl (**2a–d**) moieties were synthesized by reaction of the appropriate acylating agents with 7-ACA protected as a *t*-butyl ester, followed by removal of the *t*-butyl protecting group. The new compounds, tested in vitro for their antimicrobial activity against Gram-positive and Gram-negative bacteria, proved to possess a modest activity directed only against Gram-positive microorganisms.

Keywords: β-lactams; Cephalosporins; Antimicrobial activity

1. Introduction

By now, it is widely accepted that the antimicrobial properties of cephalosporinic β-lactam antibiotics and their stability to acids and resistance to enzyme inactivation, depend on various factors, of which, one of the most important is the chemical nature of the amidic substituent linked to the C(7) carbon of the β-lactam nucleus [1–5].

In the majority of cephalosporins of therapeutic interest, this amidic side-chain is substituted by an aryl or aromatic heterocycle, together with another more or less complex moiety, which, in many cases, contains an oximethereal group or a protonatable aminic moiety [6].

In a series of studies [7–12] in the field of cephalosporinic β-lactam antibiotics aiming at investigating the effects on the antimicrobial properties induced by certain structural modifications on the amidic side-chain linked to the C(7) carbon of the cephalosporanic nucleus, we previously described compounds **1a–g** [11], in which this side-chain contains an aryl-substituted [(methyloxy)imino]methyl moiety ($CH_2ON{=}C$, MOIMM). This oximethereal group was chosen on the basis of the fact that, in the field of β-adrenergic blocking drugs [13,14], it acted as a valid bioisoster of aryls, and therefore might also be able to effectively replace the aromatic moiety usually present on the amidic side-chains of the most active cephalosporins.

As compounds **1a–g** show a modest activity directed only against Gram-positive microorganisms, we thought it of interest to verify whether an increase in the polarity of the amidic side-chain might improve the antimicrobial properties of these types of compounds. Consequently, compounds **2a–d** were synthesized, in which the oximethereal moiety of compounds **1a–g** is replaced by the more polar hydroxylaminoethereal portion. This moiety, in addition to those present in some β-lactam antibiotics of clinical interest, possesses the basic characteristics needed to widen the activity spectrum and gives to the new compounds a stability to acids. Furthermore, in order to examine whether the antimicrobial activity of 2-arylmethyloxyimino (**1a–g**) and 2-arylmethyloxyamino (**2a–d**) compounds could be

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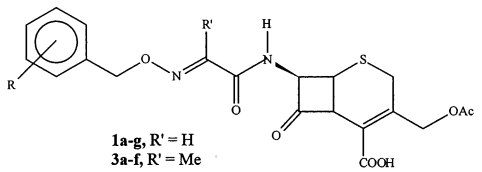
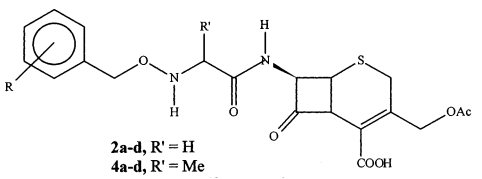
influenced by an increase in the steric bulk of the amidic side-chain, compounds **3a–f** and **4a–d** were also prepared, in which a methyl substituent is present on the oxime carbon of **1** or on the corresponding carbon of the saturated compounds **2**.



1a-g, R' = H
3a-f, R' = Me



2a-d, R' = H
4a-d, R' = Me

a, R = H; **b**, R = *o*-Cl; **c**, R = *m*-Cl; **d**, R = *p*-Cl; **e**, R = *o*-OMe; **f**, R = *m*-OMe; **g**, R = *p*-OMe

2. Chemistry

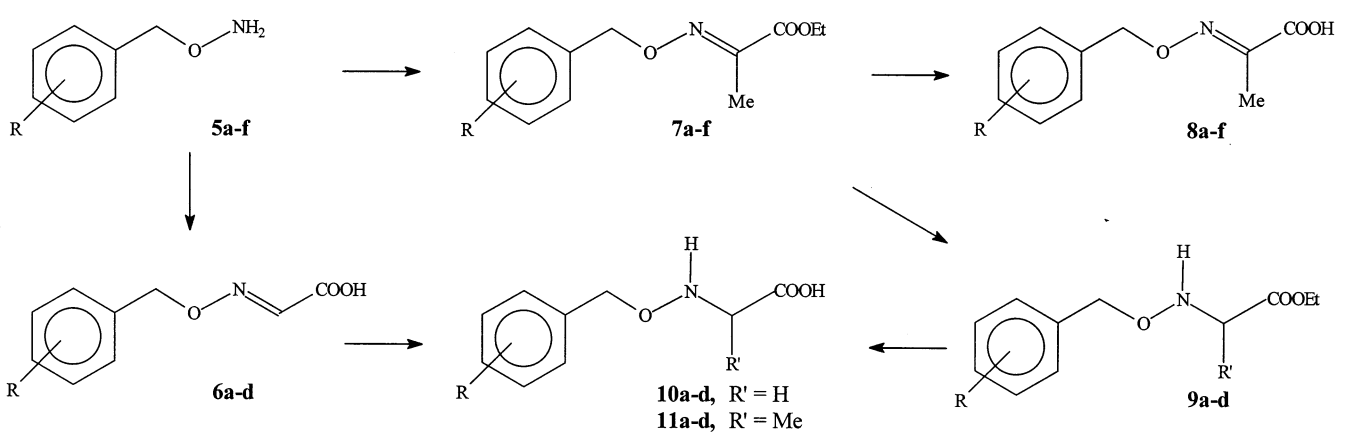
The *N*-(arylmethyloxy)iminoacids **8a–f** and the *N*-(arylmethyloxy)aminoacids **10a–d** and **11a–d** to be used as 7-aminocephalosporanic acid (7-ACA) acylating agents in the synthesis of the β-lactam compounds **3a–f**, **2a–d** and **4a–d**, were prepared as outlined in Scheme 1. For acids **10a–d** the synthetic route followed the one previously described [15], consisting of the reaction of **5a–d** with glyoxylic acid and subsequent reduction of the resulting oximic acids **6a–d**.

As regards acids **8a–f** and **11a–d**, the reaction of the appropriate *O*-(arylmethyl)hydroxylamines (**5a–f**) with ethylpyruvate afforded the corresponding ethyl esters of the (*E*)-*N*-(arylmethyloxy)-2-iminopropionic acids (**7a–f**) as the only configurational isomer. Hydrolysis of **7a–f** with ethanolic NaOH yielded the corresponding iminoacids **8a–f**. Reduction of **7a–d** with borane-trimethylamine complex in anhydrous 8 N ethanolic hydrochloric acid afforded the aminoesters **9a–d** which were hydrolyzed with THF–H_2O NaOH to form the corresponding aminoacids **11a–d**.

The β-lactam derivatives **3a–f**, **2a–d** and **4a–d** were prepared as indicated in Scheme 2. Treatment of iminoacids **8a–f** and aminoacids **10a–d** and **11a–d** with 7-ACA, protected as a *t*-butyl ester, in the presence of [*N*-(3-dimethylaminopropyl)*N*-ethyl carbodiimide hydrochloride] (EDCI) as the coupling agent, afforded the corresponding β-lactam esters **12a–f**, **13a–d** and **14a–d**, which were purified by column chromatography and then hydrolyzed to the free acids **3a–f**, **2a–d** and **4a–d**, using trifluoroacetic acid and anisole.

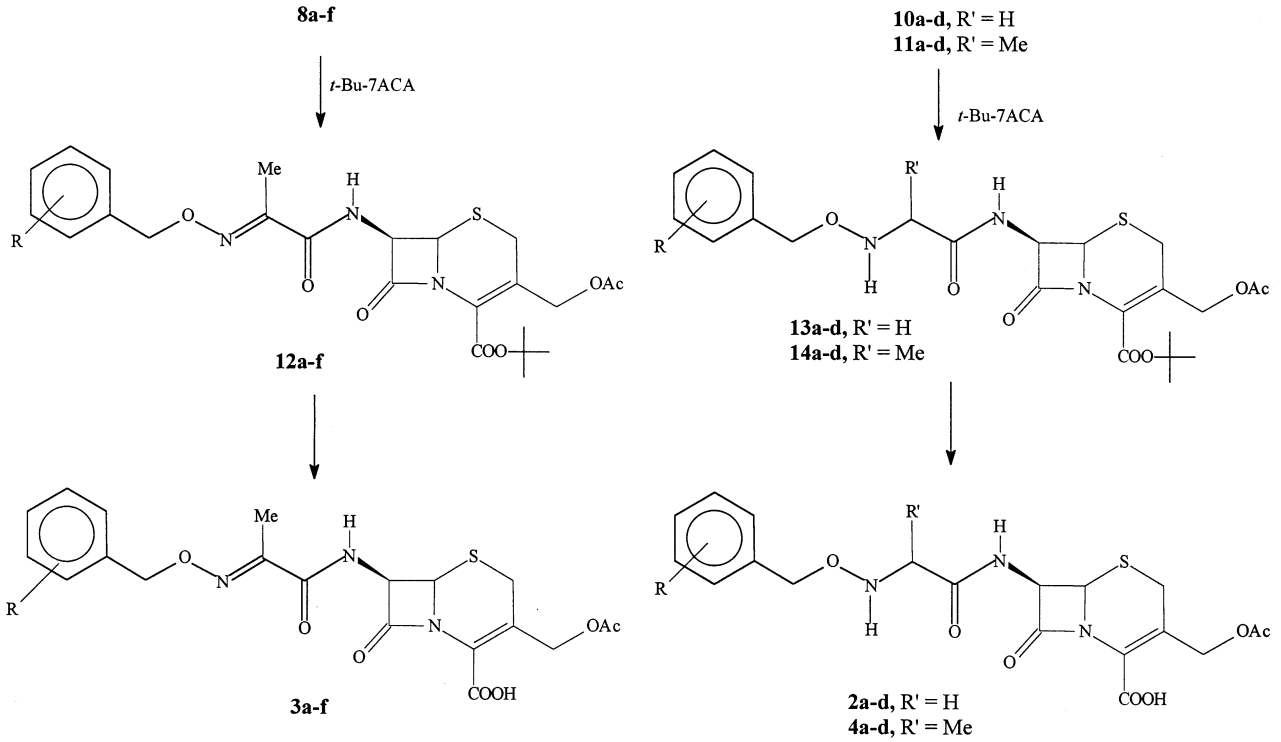
In view of the presence of the new chiral center at the level of the amidic side-chain, both the β-lactam esters **14a–d** and the corresponding acids **4a–d**, should be mixtures of two diastereoisomers, these proved to be inseparable by means of the usual separation methods.

The configuration around the oximic double bond of esters **7** was assigned by ^{1}H NMR study of the shielding induced by an anisotropic solvent (C_6D_6) on one of these esters (**7a**) in the unprotonated and protonated forms. For this compound, in agreement with findings for methylketone oxime ethers which present a *syn* relationship between the oximic oxygen and the methyl group [16], the protonation of the oximic nitrogen determines a shift of the signal of the methyl protons to a higher field, ranging from 0.14 to 0.26 ppm, depending on the quantity of acid added to the oximic compound **7a** (see Section 4). The very similar chemical shift value of the methyl signal for **7a** and for its analogs **7b–f** in the same experimental conditions, made it possible to also assign the same type of configuration (*E*) to **7b–f**. The *E* configuration around the iminic double bond of acids **8a–f**, of β-lactam esters **12a–f** and of the final compounds **3a–f** was assigned



a, R = H; **b**, R = *o*-Cl; **c**, R = *m*-Cl; **d**, R = *p*-Cl; **e**, R = *o*- OMe; **f**, R = *m*-OMe

Scheme 1.



a, R = H; **b**, R = *o*-Cl; **c,** R = *m*-Cl; **d,** R = *p*-Cl; **e,** R = *o*- OMe; **f**, R = *m*-OMe

Scheme 2.

on the basis of the observation that the value of the chemical shift of the signal of the methyl protons of these compounds is practically the same for the starting *E* oximes **7a**–**f**, acids **8a**–**f**, β-lactam esters **12a**–**f** and the corresponding acids **3a**–**f**.

3. Results and discussion

Compounds **2a**–**d**, **3a**–**f** and **4a**–**d** were tested on 14 bacterial strains of Gram-positive and Gram-negative microorganisms; the results of these tests are expressed as MIC (minimum inhibitory concentration) values and are shown in Table 1, together with those obtained on the same strains for **1b** [11], one of the most active previously described compounds, cephaloram, a cephalosporin antibiotic active against Gram-positive bacteria [17], and ceftazidime, a wide-range cephalosporin antibiotic, particularly active against Gram-negative bacteria [18].

The 2-arylmethyloxyamino cephalosporins **2a**–**d**, like the previously reported 2-arylmethyloxyimino analogs **1a**–**g**, showed a low activity directed only towards two *Staphylococcus epidermidis* strains, and appeared to be completely inactive against the tested Gram-negative bacteria at concentrations lower than 128 μg/ml.

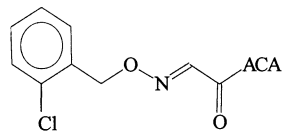
Compounds **3a**–**f** and **4a**–**d**, which differ from the 2-arylmethyloxyimino cephalosporins of type **1** and the 2-arylmethyloxyamino ones of type **2**, respectively, in the insertion of a methyl group on the MOIMM of **1** and on the corresponding carbon of the hydroxylaminoethereal portion of **2**, exhibited an antimicrobial profile similar to that of **1** and **2**, i.e. a modest activity towards some *Staphylococcus* strains, and a complete inactivity towards Gram-negative bacteria.

Compounds **2** were synthesized in order to verify whether the substitution of the oximethereal portion of **1** with the more polar hydroxylaminoethereal moiety which also possesses basic characteristics, might have a positive effect on the activity of these compounds, while compounds **3** and **4** were prepared with the aim of testing the effects on the antimicrobial activity of the insertion of a methyl group on the oximic or hydroxylaminic portions of **1** and **2**, respectively.

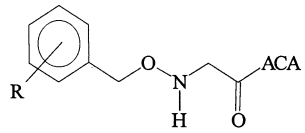
The results obtained showed that all three types of cephalosporinic derivatives synthesized (**2**–**4**) present similar antimicrobial characteristics to those of the type **1** compounds previously studied.

It may thus be concluded that the types of structural modifications which, starting from the structure of compound **1**, lead to compounds **2**, **3** and **4**, do not induce any improvement in the activity, and should not, therefore, be able to substantially modify any of the molecular parameters involved in the definition of the antimicrobial properties of those types of cephalosporinic derivatives.

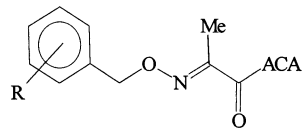
Table 1
Antimicrobial activity (MIC [a], μg/ml) of cephalosporins **1b**, **2a–d**, **3a–f** and **4a–d** against Gram-positive and Gram-negative bacteria



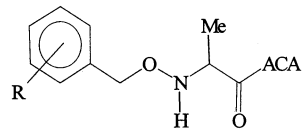
1b



2a-d



3a-f



4a-d

Microorganisms	CFL [b]	CFT [c]	**1b**	**2a**	**2b**	**2c**	**2d**	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**	**4a**	**4b**	**4c**	**4d**
Gram positive																	
Staphylococcus aureus [d]	0.036	128	8	>128	>128	64	>128	>128	>128	>128	>128	>128	>128	>128	64	>128	64
Staphylococcus aureus [e]	4	2	>128	>128	>128	>128	>128	32	8	32	8	32	8	2	2	1	2
Staphylococcus epidermidis [f]	0.036	8	4	2	4	1	4	16	8	16	16	32	32	2	2	1	1
Staphylococcus epidermidis [g]	8	32	4	32	64	32	64	>128	64	>128	64	>128	>128	32	16	16	16
Enterococco faecalis [h]	>128	128	64	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
Gram negative [i]																	
Geometric averages	>128	0.469	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128

[a] The in vitro antibacterial activities were evaluated by a two-fold serial dilution method with a multiinocular device (see Ref. [19]).
[b] Cephaloram.
[c] Ceftazidime.
[d] *S.a.* MPR 5.
[e] *S.a.* ATCC 6538.
[f] *S.e.* HCF Berset C.
[g] *S.e.* CPLH A2.
[h] *E.f.* LEP Br.
[i] Strain tested: *Escherichia coli* ATCC 8739, *Escherichia coli* ISF 432, *Enterobacter cloacae* OMNFI 153, *Proteus vulgaris* CUNR 6, *Providencia stuardi* CUNR 5, *Klebsiella pneumoniae* ATCC10031, *Shighella enteritidis*, *Pseudomonas aeruginosa* CNUR 4, *Pseudomonas aeruginosa* ATCC 9027.

4. Experimental

4.1. Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra, for comparison of compounds, were recorded on an FTIR Mattson 1000 Unicam spectrometer as Nujol mulls in the case of solid substances or as liquid film in the case of liquids. ^{1}H NMR spectra were recorded with a Varian CFT20 instrument operating at 80 MHz in ca. 3% $CDCl_3$ or DMSO-d_6 solutions. The proton magnetic resonance assignments were established on the basis of the expected chemical shifts and the multiplicity of the signals. Evaporations were made in vacuo (rotating evaporator). Analytical TLCs were carried out on 0.25 mm layer silica gel plates (Merck F254). Column chromatography was carried out on 70–230 mesh silica gel. $MgSO_4$ was always used as a drying agent. Elemental analyses were performed in our analytical laboratory and agreed with theoretical values to within $\pm 0.4\%$.

4.1.1. *N-(Arylmethyloxy)glycines (**10a–d**)*

These compounds were prepared following the synthetic route previously described [15]. Treatment of the appropriate *O*-(arylmethyl)hydroxylamine hydrochloride (**5a–d**) (0.041 mmol) with glyoxylic acid (0.049 mmol) in acetonitrile for 12 h at room temperature (r.t.) afforded the (*E*)-*N*-(arylmethyloxy)iminoacetic acids (**6a–d**), which by reduction with the borane–triethylamine complex in EtOH in the presence of 10% aqueous HCl, yielded, after the usual work-up, the aminoacids **10a–d**.

4.1.2. *Ethyl esters of (E)-N-(arylmethyloxy)-2-iminopropionic acids (**7a–f**)*

A solution of the appropriate *O*-(arylmethyl)hydroxylamine hydrochloride (**5a–f**) (0.092 mol) and ethylpyruvate (0.092 mol) in anhydrous EtOH (74 ml) was treated dropwise, while stirring, with a solution of AcONa (0.138 mol) in anhydrous EtOH (74 ml). The resulting mixture was stirred at r.t. for 24 h and then evaporated at reduced pressure. The residue was added to H_2O and extracted with Et_2O. The organic phase was separated, washed (5% aqueous HCl, 10% aqueous $NaHCO_3$ and H_2O) and evaporated to dryness to yield a crude oily residue which was subjected to column chromatography on silica gel, eluting with a 4:1 hexane–AcOEt mixture to give **7** as only one of the two possible *E*/*Z* isomers (^{1}H NMR and GLC).

7a (85%): IR ν 1724 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.37 (t, 3H, $J = 7$ Hz), 2.07 (s, 3H), 4.30 (q, 2H, $J = 7$ Hz), 5.33 (s, 2H), 7.43 (s, 5H); **7b** (88%): IR ν 1726 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.30 (t, 3H, $J = 7$ Hz), 2.08 (s, 3H), 4.33 (q, 2H, $J = 7$ Hz), 5.43 (s, 2H), 7.16–7.66 (m, 4H); **7c** (75%): IR ν 1726 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.36 (t, 3H, $J = 7$ Hz), 2.11 (s, 3H), 4.36 (q, 2H, $J = 7$ Hz), 5.30 (s, 2H), 7.26–7.56 (m, 4H); **7d** (69%): IR ν 1726 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.35 (t, 3H, $J = 7$ Hz), 2.07 (s, 3H), 4.35 (q, 2H, $J = 7$ Hz), 5.3 (s, 2H), 7.37 (m, 4H); **7e** (81%): IR ν 1726 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.36 (t, 3H, $J = 7$ Hz), 2.13 (s, 3H), 3.86 (s, 3H), 4.36 (q, 2H, $J = 7$ Hz), 5.46 (s, 2H), 6.86–7.73 (m, 4H); **7f** (85%): IR ν 1726 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.35 (t, 3H, $J = 7$ Hz), 2.09 (s, 3H), 3.80 (s, 3H), 4.32 (q, 2H, $J = 7$ Hz), 5.28 (s, 2H), 6.81–7.30 (m, 4H).

^{1}H NMR study of the shielding effect induced by an anisotropic solvent on **7a** was carried out with a 5% solution (w/w) in C_6D_6 (0.5 ml) by adding increasing amounts (50, 100 and 1000 μl) of CF_3CO_2D. The spectrum was recorded for the free base and after each addition of acid. The chemical shifts of the methyl proton signals were 1.97 ppm for the free base and 1.83, 1.76 and 1.71 ppm for the protonated form, after the addition of 50, 100 and 1000 μl.

An analogous experiment carried out in the same conditions, using the solvent $CDCl_3$ instead of C_6D_6, did not reveal any appreciable effect on the chemical shift of the same methyl group.

4.1.3. *(E)-N-(Arylmethyloxy)-2-iminopropionic acid derivatives (**8a–f**)*

A 2 N NaOH ethanolic solution (73.2 ml) was added to a solution of the appropriate ethyl esters (**7a–f**) (22.6 mmol) in EtOH (4 ml). After stirring at 40°C for 30 min, the reaction mixture was evaporated at reduced pressure and the resulting residue was diluted with H_2O and washed with Et_2O. The aqueous phase was acidified with 10% aqueous HCl to pH $\cong$ 4 and extracted with AcOEt. Evaporation of the washed (H_2O) organic extracts gave the appropriate acids as solids which were purified by crystallization from hexane to yield exclusively the *E*-isomers **8a–f**.

8a (58%): m.p. 83–85°C; IR ν 1703 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.10 (s, 3H), 3.60–4.03 (br, 1H, D_2O exchangeable), 5.37 (s, 2H), 7.45 (s, 5H); **8b** (44%): m.p. 98–99°C; IR ν 1703 cm^{-1}; ^{1}H NMR ($CDCl_3$–DMSO-d_6) δ 2.04 (s, 3H), 5.26 (s, 2H), 7.34 (m, 4H); **8c** (68%): m.p. 104–105°C; IR ν 1749 cm^{-1}; ^{1}H NMR ($CDCl_3$–DMSO-d_6) δ 2.13 (s, 3H), 5.73 (s, 2H), 8.00 (m, 4H); **8d** (75%): m.p. 110–111°C; IR ν 1703 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.13 (s, 3H), 5.35 (s, 2H), 5.83–6.10 (br, 1H, D_2O exchangeable), 7.47 (m, 4H); **8e** (54%): m.p. 75–76°C; IR ν 1703 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.07 (s, 3H), 3.83 (s, 3H), 5.34 (s, 2H), 6.80–7.35 (m, 4H), 8.90 (br, 1H, D_2O exchangeable); **8f** (97%): m.p. 80–81°C; IR ν 1711 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.07 (s, 3H), 3.83 (s, 3H), 5.34 (s, 2H), 6.80–7.35 (m, 4H), 8.90 (br, 1H, D_2O exchangeable).

*4.1.4. Ethyl esters of N-(arylmethyloxy)alanines (**9a–d**)*

Ethanolic hydrochloric acid (8 N, 109 ml) was added dropwise to a stirred mixture (0°C) of borane–trimethylamine complex (21.9 mmol) and the appropriate oximether (**7a–d**) (14.6 mmol). Stirring was continued for 20 h, then the solvent was evaporated and the residue was dissolved in CH_2Cl_2 supplemented with solid $NaHCO_3$. After stirring for several hours, the suspension was filtered and the solvent was evaporated. The residue was filtered through a silica gel column eluting with a hexane/AcOEt (4:1) mixture. Evaporation of the final fraction yielded pure **9a–d** as oils (GLC).

9a [20] (69%): IR ν 3269, 1742 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.03–1.50 (m, 6H), 3.40–3.96 (m, 1H), 4.26 (q, 2H, $J = 7$ Hz), 4.76 (s, 2H), 6.05 (br, 1H, $J = 9$ Hz, D_2O exchangeable), 7.40 (s, 5H); **9b** (73%): IR ν 3269, 1742 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.10–1.50 (m, 6H), 3.53–4.00 (m, 1H), 4.26 (q, 2H, $J = 7$ Hz), 4.90 (s, 2H), 5.93–6.38 (br, 1H, D_2O exchangeable), 7.16–7.60 (m, 4H); **9c** (83%): IR ν 3269, 1742 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.10–1.50 (m, 6H), 3.42–3.97 (m, 1H), 4.25 (q, 2H, $J = 7$ Hz), 4.70 (s, 2H), 6.03 (br, 1H, $J = 8$ Hz, D_2O exchangeable), 7.26–7.53 (m, 4H); **9d** (86%): IR ν 3269, 1742 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.10–1.50 (m, 6H), 3.4–4.03 (m, 1H), 4.25 (q, 2H, $J = 7$ Hz), 4.70 (s, 2H), 6.02 (br, 1H, $J = 9$ Hz, D_2O exchangeable), 7.37 (m, 4H).

*4.1.5. N-(Arylmethyloxy)alanines (**11a–d**)*

A stirred THF/H_2O (7:3) solution (10 ml) of the appropriate ester (**9a–d**) (1.37 mmol) was cooled at 0°C and then treated dropwise with 1 N aqueous NaOH (1.37 mmol). At the end of the reaction, monitored by TLC (AcOEt/hexane 3:2), the THF was evaporated and the aqueous solution was acidified to pH ≅ 3–4 with aqueous H_3PO_4 and extracted with AcOEt. The organic phase was evaporated to dryness to give the appropriate alanine derivatives (**11 a–d**) as a solid residue which was purified by crystallization from hexane.

11a (61%): m.p. 121–122°C (lit. [21] m.p. 123–124°C); IR ν 3236, 1719 cm^{-1}, ^1H NMR ($CDCl_3$) δ 1.27 (m, 3H, $J = 7$ Hz), 3.56–4.03 (m, 1H, $J = 7$ Hz), 4.82 (s, 2H), 7.43 (m, 5H), 8.68 (br, 2H, D_2O exchangeable); **11b** (77%): m.p. 130–131°C; IR ν 3236, 1719 cm^{-1}, ^1H NMR ($CDCl_3$) δ 1.26 (d, 3H, $J = 7$ Hz), 3.50–4.03 (m, 1H, $J = 7$ Hz), 4.90 (s, 2H), 7.23–8.05 (m, 6H); **11c** (60%): m.p. 119–120°C; IR ν 3236, 1719 cm^{-1}, ^1H NMR ($CDCl_3$) δ 1.24 (d, 3H, $J = 7$ Hz), 3.50–4.00 (m, 1H, $J = 7$ Hz), 4.77 (s, 2H), 7.20–7.56 (m, 4H), 7.63–8.10 (br, 2H, D_2O exchangeable); **11d** (50%): m.p. 129–130°C; IR ν 3236, 1719 cm^{-1}, ^1H NMR ($CDCl_3$) δ 1.24 (d, 3H, $J = 7$ Hz), 3.47–3.93 (m, 1H, $J = 7$ Hz), 4.73 (s, 2H), 7.38 (m, 4H), 8.08–8.58 (br, 2H, D_2O exchangeable).

*4.1.6. t-Butyl esters of the 7β-{(E)-[N-(arylmethyloxy)imino]-2-propionamido}-3-(acetoxymethyl)-3-cephem-4-carboxylic acid derivatives (**12a–f**)*

A stirred solution of the appropriate acid (**8a–f**) (0.763 mmol) and the *t*-butyl ester of 7-ACA (0.738 mmol) in anhydrous CH_2Cl_2 (10 ml) was cooled at 0°C and then treated portionwise with EDCI (0.738 mmol). After stirring for 24 h at r.t. the reaction mixture was washed (5% aqueous HCl, saturated aqueous $NaHCO_3$ and brine), filtered and evaporated to give an oil which was purified by silica gel column chromatography (hexane/2-pentanone 2:1). Evaporation of the middle fractions yielded **12a–f** as a vitreous product.

12a (67%): ^1H NMR ($CDCl_3$) δ 1.55 (s, 9H), 2.03 (s, 3H), 2.07 (s, 3H), 3.30 and 3.55 (2d, 2H, $J = 18.1$ Hz), 4.97 (d, 1H, $J = 4.8$ Hz), 5.82 (dd, 1H, $J = 4.2$ and 9.0 Hz); **12b** (55%): ^1H NMR ($CDCl_3$) δ 1.55 (s, 9H), 2.09 (s, 6H), 3.29 and 3.63 (2d, 2H, $J = 18.6$ Hz), 4.79 (d, 1H, $J = 12.6$ Hz), 5.01 (d, 1H, $J = 4.2$ Hz), 5.11 (d, 1H, $J = 12.6$ Hz), 5.33 (s, 2H), 5.86 (dd, 1H, $J = 4.2$ and 9.0 Hz), 7.31 (m, 5H); **12c** (61%): ^1H NMR ($CDCl_3$) δ 1.55 (s, 9H), 2.06 (s, 3H), 2.08 (s, 3H), 3.29 and 3.65 (2d, 2H, $J = 18.6$ Hz), 4.79 (d, 1H, $J = 12.6$ Hz), 5.0 (d, 1H, $J = 4.8$ Hz), 5.10 (d, 1H, $J = 12.6$ Hz), 5.17 (s, 2H), 5.86 (dd, 1H, $J = 4.8$ and 9.6 Hz), 7.27 (m, 5H); **12d** (70%): ^1H NMR ($CDCl_3$) δ 1.54 (s, 9H), 2.03 (s, 3H), 2.08 (s, 3H), 3.29 and 3.65 (2d, 2H, $J = 18.6$ Hz), 4.79 (d, 1H, $J = 12.6$ Hz), 5.0 (d, 1H, $J = 4.8$ Hz), 5.10 (d, 1H, $J = 12.6$ Hz), 5.17 (s, 2H), 5.86 (dd, 1H, $J = 4.8$ and 9.6 Hz), 7.27 (m, 5H); **12e** (55%): ^1H NMR ($CDCl_3$) δ 1.54 (s, 9H), 2.03 (s, 3H), 2.08 (s, 3H), 3.30 and 3.60 (2d, 2H, $J = 17.5$ Hz), 3.82 (s, 3H), 4.78 (d, 1H, $J = 12.6$ Hz), 4.96 (d, 1H, $J = 4.8$ Hz), 5.07 (d, 1H, $J = 12.6$ Hz), 5.24 (s, 2H), 5.82 (dd, 1H, $J = 4.8$ and 8.8 Hz), 6.80–7.41 (m, 5H); **12f** (65%): ^1H NMR ($CDCl_3$) δ 1.54 (s, 9H), 2.04 (s, 3H), 2.08 (s, 3H), 3.30 and 3.59 (2d, 2H, $J = 17.6$ Hz), 3.79 (s, 3H), 4.78 (d, 1H, $J = 12.8$ Hz), 4.97 (d, 1H, $J = 5.6$ Hz), 5.07 (d, 1H, $J = 12.8$ Hz), 5.15 (s, 2H), 5.82 (dd, 1H, $J = 5.6$ and 8.8 Hz), 6.86–7.35 (m, 5H).

*4.1.7. 7β-{(E)-[N-(Arylmethyloxy)imino]-2-propionamido}-3-(acetoxymethyl)-3-cephem-4-carboxylic acid derivatives (**3a–f**)*

Trifluoroacetic acid (1.3 ml) was added dropwise to a stirred and cooled (0°C) solution of the appropriate ester (**12a–f**) (0.29 mmol) in anisole (0.15 ml) and anhydrous CH_2Cl_2 (1 ml). After stirring at 0°C for 5 h, the solution was concentrated at reduced pressure, diluted with AcOEt and extracted with 10% aqueous $NaHCO_3$. The aqueous phase was cooled at 0°C, acidified at pH ≅ 3 with 10% aqueous HCl and then extracted with AcOEt. Evaporation of the washed (H_2O) organic extracts gave the pure acids **3a–f** as oils.

3a (82%): ^{1}H NMR ($CDCl_3$) δ 2.03 (s, 3H), 2.07 (s, 3H), 3.34 and 3.60 (2d, 2H, $J = 18.4$ Hz), 5.18 (s, 2H), 5.84 (dd, 1H, $J = 4.2$ and 9.6 Hz), 7.20–7.70 (m, 5H); **3b** (82%): ^{1}H NMR ($CDCl_3$) δ 2.03 (s, 3H), 2.08 (s, 3H), 3.34 and 3.72 (2d, 2H, $J = 17.7$ Hz), 4.80 (d, 1H, $J = 12.7$ Hz), 5.02 (d, 1H, $J = 9.3$ Hz), 5.28 (d, 1H, $J = 12.7$ Hz), 5.31 (s, 2H), 5.84 (dd, 1H, $J = 4.9$ and 9.3), 7.20–7.60 (m, 5H); **3c** (87%): ^{1}H NMR ($CDCl_3$) δ 2.06 (s, 3H), 2.09 (s, 3H), 3.40 and 3.62 (2d, 2H, $J = 17.7$ Hz), 4.85–5.38 (m, 5H), 5.84 (dd, 1H, $J = 5.0$ and 9.6 Hz), 7.00–7.55 (m, 5H); **3d** (98%): ^{1}H NMR ($CDCl_3$) δ 2.03 (s, 3H), 2.09 (s, 3H), 3.35 and 3.60 (2d, 2H, $J = 18.4$ Hz), 5.15 (s, 2H), 5.45–6.10 (m, 6H), 7.20–7.80 (m, 5H); **3e** (60%): ^{1}H NMR ($CDCl_3$–DMSO-d_6) δ 2.04 (s, 3H), 2.09 (s, 3H), 3.79 (s, 3H), 5.01 (d, 1H, $J = 4.9$ Hz), 5.25 (s, 2H), 5.85 (dd, 1H, $J = 4.9$ and 9.4 Hz), 6.80–7.60 (m, 5H); **3f** (93%): ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 3H), 2.02 (s, 3H), 3.33 and 3.52 (2d, 2H, $J = 18.5$ Hz), 3.73 (s, 3H), 5.11 (s, 2H), 5.81 (dd, 1H, $J = 4.9$ and 9.6 Hz), 6.70–7.30 (m, 4H).

*4.1.8. t-Butyl esters of 7β-{[N-(arylmethyloxy)amino]-acetamido} (**13a–d**) and of 7β-{[N-(arylmethyloxy)-amino]propionamido}-3-(acetoxymethyl)-3-cephem-4-carboxylic acid derivatives (**14a–d**)*

A stirred and cooled (0°C) solution of the appropriate acid (**9a–d** or **10a–d**) (0.55 mmol) and the *t*-butyl ester of 7-ACA (0.55 mmol) in anhydrous CH_2Cl_2 (10 ml) was treated portionwise with EDCI (0.55 mmol). The mixture was stirred at 20°C for 12 h, diluted with CH_2Cl_2, washed with aqueous $NaHCO_3$ and brine, filtered and evaporated to give a semisolid which, after purification by column chromatography (hexane–AcOEt 1:1), yielded pure **13a–d** or **14a–d** as oils.

13a (20%): ^{1}H NMR ($CDCl_3$) 1.55 (s, 9H), 2.08 (s, 3H), 3.30 (d, 1H, $J = 17.6$ Hz), 3.58 (m, 2H), 3.60 (d, 1H, $J = 17.6$ Hz), 4.75 (s, 2H), 4.78 (d, 1H, $J = 12.8$ Hz), 4.98 (d, 1H, $J = 5.6$ Hz), 5.06 (d, 1H, $J = 12.8$ Hz), 5.87 (dd, 1H, $J = 5.6$ and 9.6 Hz), 7.0–7.80 (m, 6H); **13b** (15%): ^{1}H NMR ($CDCl_3$) 1.55 (s, 9H), 2.08 (s, 3H), 3.30 and 3.58 (2d, 2H, $J = 17.6$ Hz), 3.62 (m, 2H), 4.55–5.55 (m, 5H), 5.87 (dd, 1H, $J = 4.8$ and 9.6 Hz), 7.00–7.55 (m, 5H); **13c** (22%): ^{1}H NMR ($CDCl_3$) 1.48 (s, 9H), 2.08 (s, 3H), 3.32 and 3.59 (2d, 2H, $J = 17.6$ Hz), 3.60 (m, 2H), 4.71 (s, 2H), 4.79 (d, 1H, $J = 12.8$ Hz), 4.97 (d, 1H, $J = 4.8$ Hz), 5.09 (d, 1H, $J = 12.8$ Hz), 5.87 (dd, 1H, $J = 4.8$ and 8.8 Hz), 7.10–7.80 (m, 5H); **13d** (32%): ^{1}H NMR ($CDCl_3$) 1.54 (s, 9H), 2.07 (s, 3H), 3.30 and 3.58 (2d, 2H, $J = 17.6$ Hz), 3.57 (m, 2H), 4.70 (s, 2H), 4.90 (d, 1H, $J = 4.8$ Hz), 4.78 (d, 1H, $J = 13.6$ Hz), 4.96 (d, 1H, $J = 4.8$ Hz), 5.05 (d, 1H, $J = 13.6$ Hz), 5.85 (dd, 1H, $J = 5.6$ and 9.6 Hz), 7.15–7.45 (m, 4H); **14a** (42%): ^{1}H NMR ($CDCl_3$) 1.25 (m, 3H), 1.55 (s, 9H), 2.08 (s, 3H), 3.10–3.85 (m, 3H), 4.65–5.20 (m, 6H), 5.83 (dd, 1H, $J = 4.8$ Hz, 8.8 Hz), 7.10–7.70 (m, 6H); **14b** (42%): ^{1}H NMR ($CDCl_3$) 1.26 (m, 3H), 1.54 (s, 9H), 2.07 (s, 3H), 3.25 and 3.62 (2d, 2H, $J = 18.4$ Hz), 3.70 (m, H), 4.65–5.25 (m, 6H), 5.82 (m, H), 7.05–7.60 (m, 5H); **14c** (43%): ^{1}H NMR ($CDCl_3$) 1.25 (m, 1H), 1.55 (s, 9H), 2.08 (s, 3H), 3.10–3.90 (m, 3H), 4.50–5.75 (m, 6H), 5.80 (m, H), 7.00–7.70 (m, 5H); **14d** (31%): ^{1}H NMR ($CDCl_3$) 1.24 (m, 3H), 1.55 (s, 9H), 2.08 (s, 3H), 3.15–3.80 (m, 3H), 4.40–5.20 (m, 6H), 5.82 (m, H), 7.00–7.55 (m, 5H).

*4.1.9. 7β-{[N-(Arylmethyloxy)amino]acetamido} (**2a–d**) and 7β-{[N-(arylmethyloxy)amino]-2-propionamido}-3-(acetoxymethyl)-3-cephem-4-carboxylic acid derivatives (**4a-d**)*

Trifluoroacetic acid (1.15 ml) was added dropwise to a stirred and cooled (0°C) solution of the appropriate ester (**13a–d** or **14a–d**) (0.81 mmol) in a mixture of anisole (0.18 ml) and anhydrous CH_2Cl_2 (1 ml). After 12 h at the same temperature, the reaction mixture was evaporated and the oily residue was dissolved in AcOEt and extracted with 10% aqueous $NaHCO_3$. The aqueous phase was cooled to 0°C, acidified at pH $\cong$ 2.5 with 10% aqueous HCl and extracted with AcOEt. Evaporation of the washed (H_2O) organic extract gave an amorphous solid which was purified by trituration to yield **2a–d** ($CHCl_3$/hexane) and **4a–d** (Et_2O).

2a (65%): ^{1}H NMR ($CDCl_3$) 2.07 (s, 3H), 3.31 and 3.60 (2d, 2H, $J = 17.6$ Hz), 3.58 (m, 2H), 4.14 (br, 1H, D_2O exchangeable), 4.73 (s, 2H), 4.80–5.35 (m, 3H), 5.87 (m, H), 7.00–7.65 (m, 5H), 7.86 (d, 1H, $J = 9.6$ Hz, D_2O exchangeable); **2b** (88%): ^{1}H NMR ($CDCl_3$) 2.07 (s, 3H), 3.17–3.85 (m, 4H), 4.65–5.25 (m, 5H), 5.65 (m, 1H, D_2O exchangeable), 5.80 (m, H), 7.10–7.50 (m, 5H); **2c** (78%): ^{1}H NMR ($CDCl_3$) 2.07 (s, 3H), 3.29 (d, 1H, $J = 17.6$ Hz), 3.59 (m, 2H), 3.60 (d, 1H, $J = 17.6$ Hz), 4.69 (s, 2H), 4.80–5.42 (m, 3H), 5.85 (dd, 1H, $J = 4.8$ and 9.6 Hz), 7.10–7.45 (m, 4H), 7.84 (d, 1H, $J = 9.6$ Hz, D_2O exchangeable); **2d** (88%): ^{1}H NMR ($CDCl_3$) 2.07 (s, 3H), 3.31–3.80 (m, 4H), 3.55–3.95 (m, 2H), 4.70 (s, 2H), 4.91–5.30 (m, 5H), 5.52 (br, 1H, D_2O exchangeable), 5.86 (dd, 1H, $J = 4.8$ and 9.6 Hz), 7.20–7.45 (m, 5H), 7.67 (d, 1H, $J = 9.6$ Hz); **4a** (24%): ^{1}H NMR ($CDCl_3$) 1.24 (m, 3H), 2.06 (s, 3H), 3.20–3.65 (m, 3H), 4.70–5.15 (m, 5H), 5.65–6.00 (m, 2H), 7.15–7.55 (m, 6H); **4b** (22%): ^{1}H NMR ($CDCl_3$, DMSO-d_6) 1.26 (m, 3H), 2.08 (s, 3H), 3.25 and 3.70 (m, 3H), 4.50–6.20 (m, 6H), 6.80–7.55 (m, 5H); **4c** (86%): ^{1}H NMR ($CDCl_3$–DMSO-d_6) 1.29 (m, 3H), 2.08 (s, 3H), 3.15 and 3.80 (m, 3H), 3.65–3.90 (m, H), 4.50–5.20 (s, 5H), 5.80 (m, 2H), 7.15–7.40 (m, 5H); **4d** (41%): ^{1}H NMR ($CDCl_3$–DMSO-d_6) 1.23 (m, 3H), 2.07 (s, 3H), 3.30 (d, 1H, $J = 17.6$ Hz), 3.45–4.00 (m, 2H), 4.66 (s, 2H), 4.73–5.20 (m, 3H), 5.80 (dd, 1H, $J = 4.8$ and 9.6 Hz), 7.20–7.40 (m, 4H), 7.78 (d, 1H, $J = 9.6$ Hz).

References

[1] K.E. Price, Structure activity relationships of semisynthetic penicillins (supplement), in: D. Perlman (Ed.), Structure Activity Relationships Among the Semisynthetic Antibiotics, vol. 12, Academic Press, New York, 1977, pp. 61–68.

[2] M.L. Sassiver, A. Lewis, Structure activity relationships of semisynthetic cephalosporins. I. The first generation of compounds, in: D. Perlman (Ed.), Structure Activity Relationships Among the Semisynthetic Antibiotics, vol. 12, Academic Press, New York, 1977, pp. 87–160.

[3] J.A. Webber, W.J. Wheeler, Antimicrobial and pharmacokinetic properties of newer penicillins and cephalosporins, in: R.B. Morin, M. Gorman (Eds.), Chemistry and Biology of β-Lactam Antibiotics, vol. I, Academic Press, New York, 1982, pp. 371–436.

[4] D.M. Boyd, Theoretical and physicochemical studies on β-lactam antibiotics, in: R.B. Morin, M. Gorman (Eds.), Chemistry and Biology of β-Lactam Antibiotics, vol. I, Academic Press, New York, 1982, pp. 437–545.

[5] C.M. Cimarusti, Dependence of β-lactamase stability on substructure within β-lactam antibiotics, J. Med. Chem. 27 (1984) 247.

[6] W. Durckheimer, F. Adam, C. Fisher, R. Kirrstetter, in: B. Testa (Ed.), Advances in Drug Research, vol. 17, Academic Press, New York, 1988, pp. 61–234.

[7] A. Balsamo, B. Macchia, F. Macchia, A. Rossello, R. Giani, G. Pifferi, M. Pinza, G. Broccali, Synthesis and antibacterial activities of new (α-hydrazinobenzyl)cephalosporins, J. Med. Chem. 26 (1983) 1648.

[8] A. Balsamo, G. Broccali, A. Lapucci, B. Macchia, F. Macchia, E. Orlandini, A. Rossello, Synthesis and antimicrobial properties of substituted β-aminoxypropionyl penicillins and cephalosporins, J. Med. Chem. 32 (1989) 1398.

[9] A. Balsamo, B. Macchia, A. Martinelli, E. Orlandini, A. Rossello, F. Macchia, G. Broccali, P. Domiano, Synthesis and antimicrobial properties of substituted 3-aminoxy-(*E*)-2-methoxyiminopropionyl penicillins and cephalosporins, Eur. J. Med. Chem. 25 (1990) 227.

[10] A. Balsamo, B. Macchia, E. Orlandini, A. Rossello, F. Macchia, G. Broccali, W. Fonio, Synthesis and antimicrobial properties of substituted 3-aminoxypropionyl and 3-aminoxy-(*E*)-2-methoxyiminopropionyl monobactams, Farmaco 45 (1990) 879.

[11] M. Macchia, E. Menchini, E. Orlandini, A. Rossello, G. Broccali, M. Visconti, Synthesis and antimicrobial activity of 7β-[*N*-(arylmethyloxyimino)acetamido]cephalosporanic acid derivatives, Farmaco 50 (1995) 713.

[12] M. Macchia, E. Menchini, E. Orlandini, A. Rossello, G. Broccali, M. Visconti, Synthesis and antimicrobial activity of 7β-(*S*)- and 7β-[(*R*)-3-(methylenaminoxy)-2-methylpropionamido]substituted cephalosporanic acid derivatives, Farmaco 51 (1996) 283.

[13] B. Macchia, A. Balsamo, M.C. Breschi, G. Chiellini, M. Macchia, A. Martinelli, C. Martini, S. Nencetti, A. Rossello, R. Scatizzi, The (methyloxymino)methyl moiety as bioisoster of aryl. A novel class of completely aliphatic β-adrenergic receptor antagonist, J. Med. Chem. 37 (1994) 1518.

[14] A. Balsamo, M.C. Breschi, G. Chiellini, L. Favero, M. Macchia, A. Martinelli, C. Martini, A. Rossello, R. Scatizzi, Synthesis and β-adrenergic properties of (*E*)-*N*-[3-(alkylamino)-2-hydroxypropylidene](methyloxy)amines substituted with an aromatic group on their [(methyloxy)imino]methyl moiety (MOIMM): an investigation into the biopharmacological effects of an aryl substitution in the class of MOIMM β-blocking drugs, Eur. J. Med. Chem. 30 (1995) 743.

[15] A. Balsamo, M.S. Belfiore, M. Macchia, C. Martini, S. Nencetti, E. Orlandini, A. Rossello, Synthesis and aldose reductase inhibitory activity of *N*-(arylsulfonyl)- and *N*-(aroyl)-*N*-(arylmethyloxy)glycines, Eur. J. Med. Chem. 29 (1994) 787.

[16] B.L. Fox, J.E. Reboulet, Assignment of ketoxime stereochemistry by a nuclear magnetic resonance method, J. Org. Chem. 35 (1970) 4234.

[17] M.L. Sassiver, A. Lewis, R.G. Shepperd, in: G.L. Hobby (Ed.), Antimicrobial Agent and Chemotherapy-1968, Williams and Wilkins, Baltimore MD, 1969, pp. 101–105.

[18] C.E. Newall, Recent Advances in the Chemistry of β-lactam Antibiotics, Third International Symposium, The Royal Society of Chemistry, 1984, p. 4.

[19] E. Steers, E.L. Foltz, B.S. Graves, Antimicrobial Chemother. 9 (1959) 307.

[20] M.W. Tijhuis, J.D.M. Herscheid, H.C.J. Ottenheijm, A practical synthesis of N-hydroxy- α-amino acid derivatives, Synthesis 11 (1980) 890.

[21] T. Kolasa, A. Chimiak, O-Protected derivatives of *N*-hydroxyamino acids, Tetrahedron 30 (1974) 3591–3595.