SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS IN THE CEFPIROME SERIES

II. ANALOGUES OF CEFPIROME WITH DIFFERENT 7-HETEROARYLACETAMIDO AND 3'-AMMONIUM SUBSTITUENTS†

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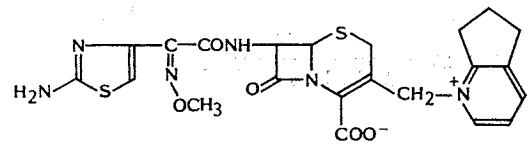
The synthesis and antibacterial activity *in vitro* of 7-(2-heteroarylacetamido)-3-[(2,3-cyclopentenopyridinium)methyl]cephalosporins and of some related compounds with different ammonium functions in 3'-position are described. The 7-[5-amino-1,2,4-thiadiazol-3-yl] and the 7-[4-aminopyrimidin-2-yl] analogues of cefpirome and compounds with 3-aliphatic ammoniummethyl functions have excellent antibacterial activity. Cephalosporins with different *N*-heterocycles other than pyridine in 3'-position are less active than their 3-pyridiniummethyl analogues. Attachment of a pyridinium group to a cephem at C-3 *via* a thiomethyl or an aminomethyl bridge causes reduction of antibacterial activity.

In the preceding paper[1] we described the synthesis and biological evaluation of pyridinium cephalosporins related to cefpirome (HR 810) (**II-1**, Fig. 1). The high antibacterial activity of many of these derivatives prompted us to extend our synthetic program to various other cephalosporins in which a) the thiazole ring in the 7-side chain is replaced by other heterocycles[2,3], b) the oxyimino moiety is replaced by neutral, acidic or basic functions, c) new heterocyclic[4] and aliphatic[5] nitrogen bases are introduced, and d) the pyridinium function is linked *via* nitrogen or sulfur bridges to the 3-CH_2 group. In this paper we report on the synthesis and biological activity of a selected number of such cephalosporins.

Fig. 1. Structure of cefpirome (HR 810, **II-1**).



Chemistry

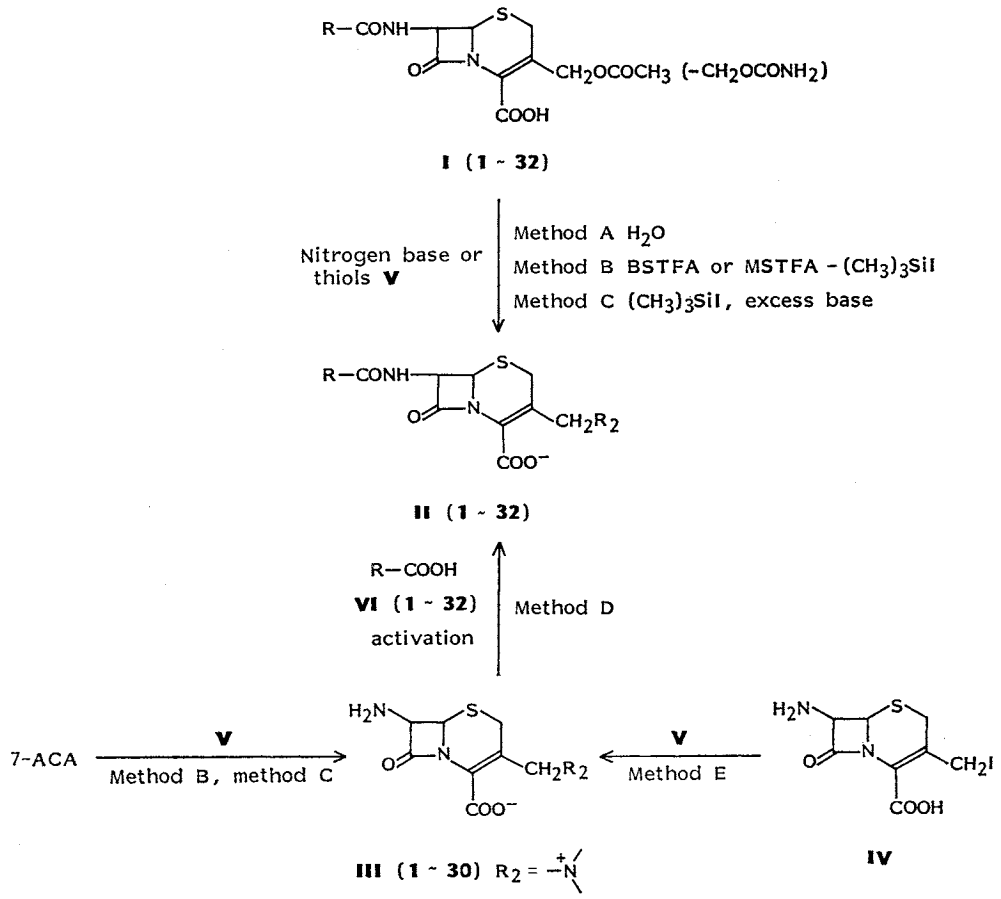
Most of the new compounds were prepared by two routes described in the preceding paper[1] (Scheme 1). 3'-Acetoxy- or 3'-carbamoyloxy-cephalosporins **I** reacted with nitrogen bases or thiols **V** either in water (Method A) or under non-aqueous conditions in the presence of iodotrimethylsilane. Silylation procedure B proceeds presumably *via* a 3-iodomethyl intermediate. Method C requires an excess of base. An alternative approach involves acylation of 7-amino-3-(ammoniomethyl)-

† Dedicated to Professor EDWARD C. TAYLOR on the occasion of his 65th birthday.

ceph-3-em-4-carboxylates **III** with activated side chain acids **VI**, *e.g.* acid chlorides or 1-hydroxybenzotriazole esters (Method D). Intermediates **III** were prepared from 7-aminocephalosporanic acid (7-ACA) and compounds **V** according to Methods B and C or from 7-amino-3-(iodomethyl)-ceph-3-em-4-carboxylic acid (**IV**) and **V** (Method E).

Easily accessible cephalosporins **I** were conveniently converted to **II** according to method A, *e.g.* preparation of **II-3**~**II-6**, **II-8**, **II-16**, and **II-17**. Compounds **II-18**~**II-22** were synthesized from cefotaxime (**I-1**) and the corresponding heterocycles, **II-32** from cefotaxime and 1-methyl-4(1*H*)-pyridine-

Scheme 1. Synthetic routes to cephalosporins **II**.



1 ~ **9** R = Table 1

10 ~ **17** R = $R_1—C(=N—OCH_3)$— Table 2

18 ~ **26** R = 2-aminothiazol-4-yl-C(=N—OCH3)— Table 3

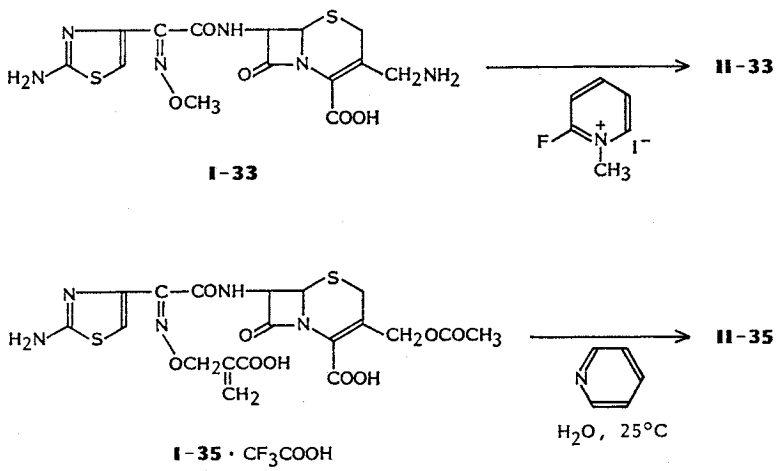
27 ~ **35** R = 2-aminothiazol-4-yl-C(=N—OR3)— Table 4

thione (**V-32**). Method B was applied for the preparation of the imidazopyridinium derivatives **II-23**~**II-26** and of the thio derivative **II-31**. Method C was used for the preparation of **II-10**. For the condensation of 7-amino-3-[(2,3-cyclopentenopyridinio)methyl]ceph-3-em-4-carboxylate (**III-1**)[1] with acids **VI** (Method D), activation *via* hydroxybenzotriazole (HOBT) esters was conveniently used (preparation of **II-2**, **II-9**, **II-11**~**II-15**). Yields varied between 15 and 40%. **II-34** was prepared by acylation of 7-ACA with the acid chloride of tritylated **VI-34**†. Intermediates **III** with aliphatic ammonium functions were preferably obtained from the 3-iodomethyl compound **IV** and aliphatic amines (Method E). The displacement reaction was carried out either in *N*,*N*-dimethylformamide or in dichloromethane after silylation, *e.g.* with bis(trimethylsilyl)acetamide. The reaction products **III** directly reacted with the HOBT ester of 2-(2-aminothiazol-4-yl)-2-(*Z*)-methoxyiminoacetic acid (**VI-1**) to give compounds **II-27**~**II-30**. After reaction of **IV** with *O*-benzyl-*N*,*N*-dimethylhydroxylamine, one product was isolated which showed two methyl groups but no benzyl in the NMR spectrum. Thus structure **II-30** is proposed.

Different procedures were applied to prepare compounds **II-7**, **II-33** and **II-35**. **II-7** was obtained by sulfonation of cefpirome (**II-1**) using the sulfur trioxide dioxane complex. Reaction of the 3-aminomethylcephalosporin **I-33** with 2-fluoro-1-methylpyridinium iodide gave **II-33**[7]. The cephalosporanic acid derivative **I-35** reacted with pyridine at room temperature preferably at the double bond of the methylene group to give **II-35** in high yield. No displacement of the 3′-acetate was observed (Scheme 2).

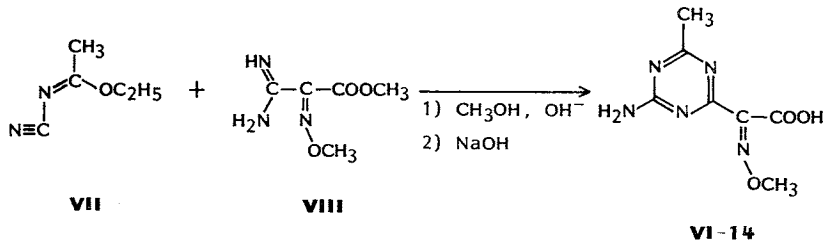
Most compounds of type **V** used in the 3′-exchange reaction were known. The bases **V-25** and **V-26** were obtained by catalytic reduction of the corresponding imidazopyridines with H_2 - PtO_2 in TFA. 1-(2-Mercaptoethyl)-2,3-cyclopentenopyridinium chloride (**V-31**) (solid, mp >100°C, dec) was synthesized from ethylene sulfide and 2,3-cyclopentenopyridine analogously to a published procedure[8]. The side chain acids **VI** were prepared according to procedures known from the literature. The hitherto unknown acid **VI-14** was obtained by condensation of ethyl *N*-cyanoacetimidate (**VII**)[9] with methyl (2-amidino-2-(*Z*)-methoxyimino)acetate (**VIII**) (Scheme 3). **VIII** was prepared from dimethyl methoxyiminomalonate analogously to a procedure described for the ethoxy compound[10].

Scheme 2. Synthesis of compounds **II-33** and **II-35**.



† **II-34** was prepared by a different way[6].

Scheme 3. Synthesis of the triazineacetic acid **VI-14**.



VII **VIII** **VI-14**

The oxazoleacetic acid derivative **VI-11** was synthesized from ethyl 4-bromo-2-(*Z*)-methoxyiminoacetoacetate and urea by a modification of a procedure from the literature[11].

Antibacterial Activity of Cephalosporins II

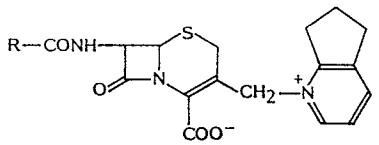
The examples of Table 1 illustrate the importance of the aminothiazole methoxyiminoacetic acid side chain of ammonio cephalosporins for outstanding antimicrobial activity. Compounds **2** and **3** lacking the methoxyimino group and compounds **4** and **5** without or with a blocked amino function respectively exhibit increased MIC values against Gram-negative bacteria, but retain good Gram-positive activity except against *Streptococcus faecium* D. Compound **6** without amino and methoxyimino groups is almost inactive against Gram-negative bacteria. Replacement of the 2-amino group by the acidic sulfonamino function in **7** and replacement of the *α-syn*-methoxyimino group by an acidic sulfo group in **8** reduces the antibacterial potency considerably. The *α*-amino substituted derivative **9** is nearly devoid of antibacterial activity.

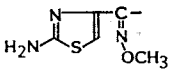
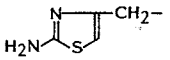
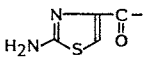
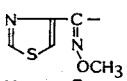
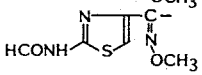
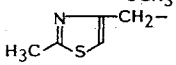
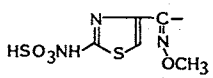
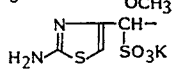
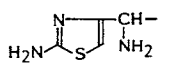
Replacement of the thiazole nucleus in cefpirome by other heterocyclic rings (Table 2) results in potent antibacterial compounds only in the case of the amino-1,2,4-thiadiazole derivative **10** and the aminopyrimidine compound **13**. Compound **10** is comparable to cefpirome with regard to its antibacterial properties *in vitro*. The isomeric amino-1,3,4-thiadiazole compound **12**, the aminooxazole analogue **11** or the 6-membered heterocyclic derivative **14** exhibit good activity against Gram-positive bacteria, but are much less active against Gram-negative strains. The deamino heterocyclic derivatives **15**, **16** and **17** display a similar disappointing activity pattern.

Replacement of the C-3′-pyridine nucleus of cefpirome by other heterocycles resulted in compounds **18**~**26** (Table 3) which have decreased activity mainly towards *Pseudomonas* and *Enterobacter cloacae* P 99. Thiazolium derivative **18** and imidazopyridinium derivative **24** are slightly inferior compared to the pyridinium compounds. All imidazopyridinium cephalosporins exhibit good Gram-positive and excellent activity against the K1 *β*-lactamase producing *Klebsiella aerogenes* 1082 E strain with the exception of **25**.

Table 4 summarizes the MICs of different ammonium substituted cephalosporins as well as analogues with pyridinium substituents linked *via* a nitrogen, a sulfur or a carbon atom. Aliphatic ammonium compounds, *e.g.* **27** (BMY-28142)[12] are excellent antibiotics. Similar to the pyridinium series beyond an optimal size, the activity is diminished with increasing size of the ammonium substituent (**28**→**29**). Introduction of an additional hydroxy group in compound **30** causes deterioration of activity against *Pseudomonas*. Cephalosporins **31**, **32** and **33**, in which the pyridinium nucleus is linked *via* a thio or nitrogen atom, are less active against *Pseudomonas*, but retain good activity in the Gram-positive area. The best overall activity is displayed by pyridiniumthio compound **32**.

Table 1. Antibacterial activity[a] of 2,3-cyclopentenopyridinium cephalosporins with different thiazole side chains.

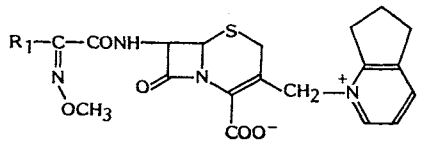


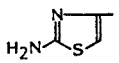
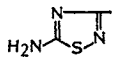
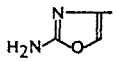
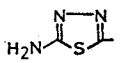
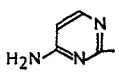
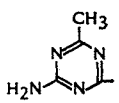
Compound	R	*S.a.* SG 511	*S.p.* 77 A	*S.f.* D	*P.a.* 1771	*P.a.* 1771 M	*E.c.* TEM	*K.a.* 1082 E	*K.a.* 1522 E	*E.cl.* P 99
1	 Cefpirome sulfate	0.19	<0.002	1.56	1.56	0.39	0.013	1.56	0.013	0.78
2		0.098	0.013	50	>100	3.13	12.5	>100	3.13	>100
3		0.78	0.025	>100	>100	50	25	>100	0.78	>100
4		0.19	0.004	>100	>100	0.19	0.19	12.5	0.19	12.5
5		0.19	0.007	25	12.5	0.39	0.39	12.5	0.098	25
6		0.098	0.049	>100	>100	>100	>100	>100	50	>100
7		3.13	0.39	>100	>100	25	12.5	>100	6.25	>100
8	 D, L	3.13	1.56	>100	25	3.13	25	>100	25	>100
9	 D, L	6.25	0.78	>100	>100	100	25	>100	25	>100

[a] MIC (μg/ml): Agar dilution test Mueller-Hinton agar (Difco); inoculum 5×10^4 cfu/spot.

S.a.: *Staphylococcus aureus*, *S.p.*: *Streptococcus pyogenes*, *S.f.*: *Streptococcus faecium*, *P.a.*: *Pseudomonas aeruginosa*, *E.c.*: *Escherichia coli*, *K.a.*: *Klebsiella aerogenes*, *E.cl.*: *Enterobacter cloacae*.

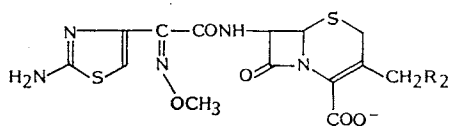
Table 2. Antibacterial activity[a] of 2,3-cyclopentenopyridinium cephalosporins, variation of the heterocycle in the 7-acyl side chain.

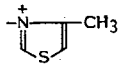
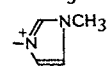
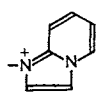
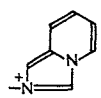
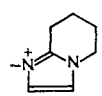
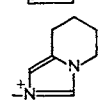


Compound	R_1	*S.a.* SG 511	*S.p.* 77 A	*S.f.* D	*P.a.* 1771	*P.a.* 1771 M	*E.c.* TEM	*K.a.* 1082 E	*K.a.* 1522 E	*E.cl.* P 99
1	 Cefpirome sulfate	0.19	0.002	1.56	1.56	0.39	0.013	1.56	0.013	0.78
10		0.39	0.004	1.56	3.13	0.78	0.098	3.13	0.098	3.13
11		0.78	0.004	100	>100	25	6.25	>100	0.39	100
12		0.19	0.049	>100	>100	25	6.25	>100	1.56	>100
13		0.39	0.002	>100	6.25	1.56	0.049	0.19	0.025	0.78
14		0.78	0.39	>100	>100	>100	50	>100	50	>100
15		1.56	0.049	>100	>100	>100	3.13	50	12.5	50
16		1.56	0.049	100	>100	100	12.5	>100	3.13	>100
17		0.098	0.002	>100	>100	100	3.13	>100	3.13	>100

[a] MIC (μg/ml): Agar dilution test, Mueller-Hinton agar (Difco); inoculum 5×10^4 cfu/spot.
Abbreviations: See footnote in Table 1.

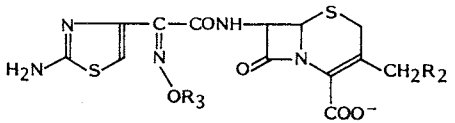
Table 3. Antibacterial activity[a] of cefpirome analogs, variation of the heterocycle in 3′-position.

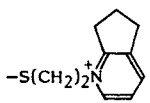
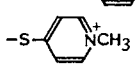
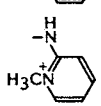
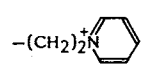
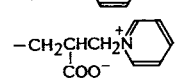


Compound	R_2	*S.a.* SG 511	*S.p.* 77 A	*S.f.* D	*P.a.* 1771	*P.a.* 1771 M	*E.c.* TEM	*K.a.* 1082 E	*K.a.* 1522 E	*E.cl.* P 99
18		0.78	<0.002	25	6.25	1.56	0.025	6.25	0.025	6.25
19		1.56	0.004	50	25	0.39	0.025	6.25	0.013	25
20		1.56	0.004	100	100	6.25	0.098	1.56	0.19	12.5
21		1.56	0.004	100	100	3.13	0.098	6.25	0.049	50
22		1.56	0.007	100	100	3.13	0.049	6.25	0.049	25
23		0.19	<0.002	50	25	1.56	0.007	0.78	0.007	25
24		0.39	<0.002	12.5	6.25	0.78	0.004	0.78	0.013	25
25		0.39	<0.002	50	100	3.12	0.098	6.25	0.098	25
26		0.78	0.049	100	100	12.5	0.025	0.78	0.025	25

[a] MIC (μg/ml): Agar dilution test, Mueller-Hinton agar (Difco); inoculum 5×10^4 cfu/spot.
Abbreviations: See footnote in Table 1.

Table 4. Antibacterial activity[a] of different ammonium substituted cephalosporins.



Compound	R_3	R_2	*S.a.* SG 511	*S.p.* 77 A	*S.f.* D	*P.a.* 1771	*P.a.* 1771 M	*E.c.* TEM	*K.a.* 1082 E	*K.a.* 1522 E	*E.cl.* P 99
27	CH_3		1.56	<0.002	>100	1.56	0.049	<0.002	1.56	<0.002	0.78
28	CH_3	$N^+(CH_3)_3$	1.56	<0.002	100	1.56	0.19	0.013	3.13	0.007	0.19
29	CH_3	$N^+(C_2H_5)_3$	1.56	0.007	>100	6.25	0.39	0.013	1.56	0.025	0.19
30	CH_3	$N^+(CH_3)_2OH$	0.78	<0.002	100	25	0.39	0.025	1.56	0.025	100
31	CH_3		0.19	<0.002	100	100	6.25	0.19	3.13	0.19	100
32	CH_3		0.19	0.002	12.5	12.5	3.13	0.002	0.19	0.002	12.5
33	CH_3		0.78	0.007	>100	100	12.5	0.39	1.56	0.19	100
34		$OCOCH_3$	6.25	0.007	>100	50	0.78	0.19	25	0.19	100
35		$OCOCH_3$	3.13	0.019	>100	100	0.62	0.31	7.8	0.039	100

[a] MIC (μg/ml): Agar dilution test, Mueller-Hinton agar (Difco); inoculum 5×10^4 cfu/spot.

Abbreviations: See footnote in Table 1.

Cephalosporins **34** and **35** bearing a pyridinium group in the alkoxyimino moiety are only moderately active both against Gram-positive bacteria and *Pseudomonas*.

In summary, in the series of aminothiazole methoxyimino cephalosporins optimum antibacterial activity is found when pyridinium substituents are directly attached to the 3-CH_2 group. Compounds with an amino-1,2,4-thiadiazole ring in the 7-side chain and compounds in which the pyridinium moiety is replaced by aliphatic ammonium groups show comparably high antibacterial activity.

Experimental

^{1}H NMR spectra were recorded on Bruker WP-60 and AM-270 spectrometers using TMS as internal standard. Medium pressure chromatography was conducted on Lobar silica gel columns obtained from Merck AG, Darmstadt, FRG. MP's are uncorrected. Acids **VI** or their esters respectively were either commercially available (**VI-2** and **VI-3**) or prepared by literature procedures (**VI-4**~**VI-10**, **VI-12**, **VI-13**, **VI-15** and **VI-16**).

7-[2-(2-Aminothiazol-4-yl)-2-(D,L)-sulfoacetamido]-3-[(2,3-cyclopenteno-1-pyridinio)methyl]ceph-3-em-4-carboxylate (**II-8**)

Method A: A mixture of 7-[2-(2-aminothiazol-4-yl)-2-(D,L)-sulfoacetamido]cephalosporanic acid (**I-8**)[13] (984 mg, 2 mmol), potassium thiocyanate (8 g, 82.5 mmol), 2,3-cyclopentenopyridine (1.2 g, 10 mmol) and water (4 ml) was heated at 70°C for 2 hours. The pH was adjusted to 6.5~6.8 with 85% aq H_3PO_4. The mixture was cooled, diluted with Me_2CO (30 ml) and chromatographed over a 4×50 cm column of silica gel. Salts and an excess of base were eluted with Me_2CO - H_2O (8:1) then **II-8** with Me_2CO - H_2O (2:1). After lyophilization, 200 mg (18%) of **II-8** as an amorphous colorless solid were obtained.

^{1}H NMR (60 MHz, CF_3COOD) δ 2.2~2.7 (2H, m, cyclopentene), 3.0~3.7 (6H, m, 4 cyclopentene-H, SCH_2), 5.15~6.0 (4H, m, 6-H, α-CH and CH_2N), 6.13 (1H, d, J=5 Hz, 7-H), 7.02 (1H, s, thiazole), 7.55~8.65 (3H, m, pyridine).

The following compounds of Tables 1 and 2 were prepared in an analogous manner from the corresponding 3'-acetoxy cephalosporins and 2,3-cyclopentenopyridine. Compound and yield (%): **3** (25), **4** (15), **5** (30), **6** (28), **16** (9), **17** (40, cefuroxime as starting material). Starting from cefotaxime and the corresponding heterocycles, the following compounds were prepared: **18** (13), **19** (8), **20** (7), **21** (6) and **22** (11% yield).

7-[2-(2-Aminothiazol-4-yl)-2-(*Z*)-methoxyiminoacetamido]-3-[(1-methyl-4-pyridinio)thiomethyl]-ceph-3-em-4-carboxylate (**II-32**)

Method A: A mixture of cefotaxime (**I-1**) (685 mg, 1.5 mmol), 1-methyl-4(1*H*)-pyridinethione (400 mg, 3.2 mmol), potassium iodide (1 g, 6 mmol) and water (1.5 ml) was heated at 60°C for 7 hours. The mixture was chromatographed over a column of silica gel, eluting with Me_2CO - H_2O (2:1). The product fractions were lyophilized to give 125 mg (16%) of **II-32** as an amorphous solid.

^{1}H NMR (60 MHz, CF_3COOD) δ 3.81 (2H, br s, SCH_2), 4.30 (6H, br s, 2×CH_3), 4.70 and 5.02 (2H, AB, J=15 Hz, 3-CH_2S), 5.38 (1H, d, J=5 Hz, 6-H), 6.01 (1H, d, J=5 Hz, 7-H), 7.42 (1H, s, thiazole), 7.82 and 8.32 (4H, AA'XX', J=7 Hz, pyridine).

II-31 was similarly prepared from **I-1** and 1-(2-mercaptoethyl)-2,3-cyclopentenopyridinium chloride (**V-31**) in 15% yield.

^{1}H NMR (60 MHz, CF_3COOD) δ 2.2~2.8 (2H, m, cyclopentene-H), 3.0~3.7 (6H, m, 4 cyclopentene-H and SCH_2CH_2), 3.66 and 3.90 (2H, AB, J=18 Hz, SCH_2), 4.28 (3H, s, OCH_3), 4.2~4.6 (2H, AB, 3-CH_2S), 4.6~5.0 (2H, AB, CH_2N), 5.37 (1H, d, J=5 Hz, 6-H), 5.94 (1H, d, J=5 Hz, 7-H), 7.24 (1H, s, thiazole), 7.55~8.65 (3H, m, pyridine).

7-[2-(2-Aminothiazol-4-yl)-2-(*Z*)-methoxyiminoacetamido]-3-[(5,6,7,8-tetrahydroimidazo[1,5-a]-pyridinio-2-yl)methyl]ceph-3-em-4-carboxylate (**II-26**)

Method B: A mixture of cefotaxime **I-1** (0.91 g, 2 mmol), bis(trimethylsilyl)trifluoroacetamide

(BSTFA) (1.85 g, 7.2 mmol) and CH_2Cl_2 (5 ml) was heated under reflux for 1 hour while stirring. The solution was cooled to 20°C, iodotrimethylsilane (1.04 g, 5.2 mmol) was added and stirring was continued for 20 minutes at room temperature. The solvent was evaporated and the oily residue was dissolved in CH_3CN (4 ml). A solution of **V-26** (305 mg, 2.5 mmol) and BSTFA (1.23 g, 4.8 mmol) in CH_3CN (2 ml) was added. After 4 hours at room temperature, water (0.3 ml) was added which caused the precipitation of **II-26** monohydroiodide. The precipitate was filtered off, washed with CH_3CN, EtOH and Et_2O, and dried to give 1.0 g. The salt was dissolved in 10% $NaHCO_3$ and the dark colored solution chromatographed on a Lobar-B column of silica gel, eluting with Me_2CO - H_2O (3:1). The product fractions 22~29 (150 ml) were freeze dried to give **II-26** (175 mg, 17%) as a yellow amorphous solid.

^{1}H NMR (270 MHz, DMSO-d_6) δ 1.7~1.83 (2H, m, CH_2), 1.83~1.96 (2H, m, CH_2), 2.72~2.82 (2H, m, CH_2), 3.06 and 3.50 (2H, AB, J=18 Hz, SCH_2), 3.80 (3H, s, OCH_3), 4.12~4.22 (2H, m, CH_2), 4.66 and 5.14 (2H, AB, J=15 Hz, CH_2N), 5.00 (1H, d, J=5 Hz, 6-H), 5.59 (1H, dd, J=5 and 8 Hz, 7-H), 6.71 (1H, s, thiazole), 7.12 (2H, s, NH_2), 7.78 (1H, s, aromatic H), 9.14 (1H, s, aromatic H), 9.47 (1H, d, J=8 Hz, NH).

Compounds **II-23** and **II-24** (literature 14 examples 1 and 22 respectively) and **II-25** have been prepared analogously from **I-1** and the imidazopyridines **V-23**, **V-24** and **V-25** in 5, 7 and 23% yield.

II-25: ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.8~2.05 (4H, m, 2×CH_2), 2.95~3.05 (2H, m, CH_2), 3.13 and 3.38 (2H, AB, J=18 Hz, SCH_2), 3.81 (3H, s, OCH_3), 4.03~4.13 (2H, m, CH_2), 4.84 and 4.93 (2H, AB, J=15 Hz, CH_2N), 5.00 (1H, d, J=5 Hz, 6-H), 5.61 (1H, dd, J=5 and 8 Hz, 7-H), 6.70 (1H, s, thiazole), 7.18 (2H, s, NH_2), 7.54 (1H, d, J=2 Hz, aromatic H), 7.90 (1H, d, J=2 Hz, aromatic H), 9.50 (1H, d, J=8 Hz, NH).

7-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(*Z*)-methoxyiminoacetamido]-3-[(2,3-cyclopenteno-1-pyridinio)methyl]ceph-3-em-4-carboxylate (**II-10**)

Method C: To a suspension of 7-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(*Z*)-methoxyiminoacetamido]cephalosporanic acid trifluoroacetate (**I-10**)[10] (57 mg, 0.1 mmol) in CH_2Cl_2 (1.5 ml) was added 2,3-cyclopentenopyridine (0.12 ml, 1 mmol) followed by iodotrimethylsilane (0.1 ml, 0.7 mmol) while stirring. The resulting solution was heated under reflux for 1.5 hours. Water (2 ml) was added while stirring and the mixture adjusted to pH 6.8 by the addition of $NaHCO_3$. The aqueous layer was separated and chromatographed over a Lobar-A column of silica gel, eluting with Me_2CO - H_2O (4:1). After freeze drying of the product fractions 31 mg (60%) of **II-10** was obtained as an amorphous colorless solid.

^{1}H NMR (60 MHz, CF_3COOD) δ 2.2~2.7 (2H, m, cyclopentene), 3.05~3.7 (6H, m, 4 cyclopentene-H and SCH_2), 4.30 (3H, s, OCH_3), 5.40 (1H, d, J=5 Hz, 6-H), 5.45 and 5.91 (2H, AB, J=15 Hz, CH_2N), 6.31 (1H, d, J=5 Hz, 7-H), 7.65~8.65 (3H, m, pyridine).

7-[2-(2-Amino-4-methyl-1,3,5-triazin-6-yl)-2-(*Z*)-methoxyiminoacetamido]-3-[(2,3-cyclopenteno-1-pyridinio)methyl]ceph-3-em-4-carboxylate (**II-14**)

Method D: A mixture of 2-(2-amino-4-methyl-1,3,5-triazin-6-yl)-2-(*Z*)-methoxyiminoacetic acid hydrochloride (**VI-14**) (124 mg, 0.5 mmol), pyridine (0.04 ml, 0.5 mmol), 1-hydroxybenzotriazole hydrate (84 mg, 0.55 mmol), dicyclohexylcarbodiimide (110 mg, 0.53 mmol) and DMF (3 ml) was stirred for 3 hours at room temperature. A solution of 7-amino-3-[(2,3-cyclopenteno-1-pyridinio)methyl]ceph-3-em-4-carboxate (**III-1**) hydroiodide[1] (230 mg, 0.5 mmol) in DMF (2.8 ml) and water (1.4 ml) was added, and the mixture was stirred overnight at room temperature. Dicyclohexylurea was filtered off, and the solvent was removed *in vacuo*. The residue was chromatographed over a Lobar-B column of silica gel, eluting with Me_2CO - H_2O (3:1). Freeze drying of the product fractions afforded 103 mg (38%) of **II-14** as an amorphous solid.

^{1}H NMR (60 MHz, CF_3COOD) δ 2.2~2.7 (2H, m, cyclopentene), 2.79 (3H, s, CH_3), 3.05~3.8 (6H, m, 4 cyclopentene-H and SCH_2), 4.33 (3H, s, OCH_3), 5.40 and 5.93 (2H, AB, J=15 Hz, CH_2N), 5.43 (1H, d, J=5 Hz, 6-H), 6.33 (1H, d, J=5 Hz, 7-H), 7.6~8.65 (3H, m, pyridine).

Compounds **II-2** (yield 24%), **11** (28), **12** (17), **13** (15) and **15** (20), were similarly prepared by

acylation of **III-1** with the corresponding HOBT active esters **VI**. *tert*-Butoxycarbonyl (BOC)-protected **VI-9** gave the protected **II-9** (yield 23%) that was deprotected with TFA to give the **II-9** bistrifluoroacetate.

3-Acetoxymethyl-7-[2-(2-aminothiazol-4-yl)-2-(*Z*)-(2-pyridinioethoxyimino)acetamido]ceph-3-em-4-carboxylate (**II-34**)

Method D: A solution of phosgene in toluene (2.1 ml, 2.1 mmol) was added to a stirred mixture of 2-(2-tritylaminothiazol-4-yl)-2-(*Z*)-(2-pyridinioethoxyimino)acetate (tritylated **VI-34**[6]) (536 mg, 1 mmol), water (11 mg), *N*,*N*-dimethylacetamide (0.23 ml, 2.5 mmol), and CH_2Cl_2 (5 ml). Stirring was continued for 2 hours at −5°C, then the solution was cooled to −15°C, and a solution of 7-ACA (326 mg, 1.2 mmol) and triethylamine (0.29 ml, 2.1 mmol) in CH_2Cl_2 (4 ml) was added. The mixture was stirred for 2 hours at 5°C, diluted with CH_2Cl_2 (20 ml) and washed with 5% aq $NaHCO_3$ (10 ml). The organic layer was evaporated to dryness. The residue (0.6 g) was dissolved in 80% aq HCOOH (10 ml) and stirred at room temperature for 2 hours. Triphenylcarbinol was filtered off, and the filtrate was evaporated to dryness. The residue was chromatographed over a Lobar-A column of silica gel, eluting with THF - H_2O (3 : 1). The product fractions were lyophilized to give 15 mg (9%) of **II-34** as a colorless solid.

^{1}H NMR (60 MHz, CF_3COOD) δ 2.28 (3H, s, $COCH_3$), 3.70 (2H, s, SCH_2), 4.65~5.3 (6H, m, 3-CH_2 and OCH_2CH_2), 5.30 (1H, d, J=5 Hz, 6-H), 5.99 (1H, d, J=5 Hz, 7-H), 7.38 (1H, s, thiazole), 7.6~8.8 (5H, m, pyridine).

7-[2-(2-Aminothiazol-4-yl)-2-(*Z*)-methoxyiminoacetamido]-3-(trimethylammoniomethyl)ceph-3-em-4-carboxylate (**II-28**)

Method E: A solution of **IV**[1] (678 mg, 2 mmol) and trimethylamine (355 mg, 6 mmol) in DMF (40 ml) was stirred for 3 hours at room temperature. 2-(2-Aminothiazol-4-yl)-2-(*Z*)-methoxyiminoacetic acid (**VI-1**) HOBT active ester[1] (700 mg, 2.2 mmol) was then added and stirring was continued for 3 hours. The solvent was removed *in vacuo* and the residue chromatographed over a Lobar-B column of silica gel. The product was eluted with Me_2CO - H_2O (3 : 1). After freeze drying 110 mg (12%) of **II-28** as an amorphous solid was obtained.

^{1}H NMR (60 MHz, CF_3COOD) δ 3.38 (9H, s, 3×CH_3), 3.82 (2H, s, CH_2S), 4.28 (3H, s, OCH_3), 4.65 and 4.93 (2H, AB, J=14 Hz, CH_2N), 5.48 (1H, d, J=5 Hz, 6-H), 5.95 (1H, d, J=5 Hz, 7-H), 7.43 (1H, s, thiazole).

In an analogous way **IV** and *N*-methylpyrrolidine and triethylamine respectively gave **II-27** (23%) and **II-29** (14%).

7-[2-(2-Aminothiazol-4-yl)-2-(*Z*)-methoxyiminoacetamido]-3-(dimethylhydroxyammoniomethyl)-ceph-3-em-4-carboxylate (**II-30**)

Method E: A mixture of **IV** (678 mg, 2 mmol), *O*-benzyl-*N*,*N*-dimethylhydroxylamine (0.9 g, 6 mmol), bis(trimethylsilyl)acetamide (1 ml, 4.1 mmol) and CH_2Cl_2 (50 ml) were stirred for 3 hours at room temperature. CH_2Cl_2 was evaporated, the residue dissolved in DMF - water (3 : 1, 20 ml) and **VI-1** HOBT active ester (1 g, 3.15 mmol) was added. The mixture was stirred for 4 hours at room temperature, concentrated *in vacuo* and chromatographed over a Lobar-B column of silica gel, eluting with Me_2CO - H_2O (3 : 1). The product fractions were lyophilized to give 95 mg (10%) of **II-30** as a colorless solid.

^{1}H NMR (60 MHz, CF_3COOD) δ 3.10 (3H, s, CH_3), 3.18 (3H, s, CH_3), 3.70 and 3.95 (2H, AB, J=18 Hz, SCH_2), 4.10 and 4.48 (2H, AB, J=14 Hz, CH_2N), 4.30 (3H, s, OCH_3), 5.36 (1H, d, J=5 Hz, 6-H), 6.12 (1H, d, J=5 Hz, 7-H), 7.41 (1H, s, thiazole).

6-[(2,3-Cyclopenteno-1-pyridinio)methyl]-7-[2-(*Z*)-methoxyiminoacetamido-2-(2-sulfoaminothiazol-4-yl]ceph-3-em-4-carboxylate (**II-7**)

Cefpirome (**II-1**) (2.05 g, 4 mmol) was dissolved in a solution of SO_3 (1 g, 12.5 mmol) in DMF (10 ml). Triethylamine (1.7 ml, 12 mmol) in DMF (5 ml) was added dropwise during 15 minutes while cooling, maintaining the temperature at 25°C. After stirring for 3 hours at room temperature, the

red colored solution was diluted with diisopropyl ether (100 ml), whereupon a solid separated. The supernatant was discarded and the residue was dissolved in Me_2CO - H_2O (1 : 1). The resulting solution was chromatographed over a Lobar-C column of silica gel, eluting with Me_2CO - H_2O (4 : 1). Freeze drying of the product fractions gave **II-7** (980 mg, 41%) as an amorphous colorless solid.

^1H NMR (60 MHz, DMSO-d_6) δ 2.1~2.3 (2H, m, cyclopentene), 2.9~3.6 (6H, m, 4 cyclopentene-H and SCH_2), 3.83 (3H, s, OCH_3), 5.07 (1H, d, J=5 Hz, 6-H), 5.23 and 5.47 (2H, AB, J=15 Hz, CH_2N), 5.67 (1H, dd, J=5 and 8 Hz, 7-H), 6.90 (1H, s, thiazole), 7.88 (1H, dd, J=7 Hz, pyridine), 8.37 (1H, d, J=7 Hz, pyridine), 9.23 (1H, d, J=7 Hz, pyridine), 9.60 (1H, d, J=8 Hz, NH).

7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(1-methyl-2-pyridinio)aminomethyl]-ceph-3-em-4-carboxylate (**II-33**)

A solution of 2-fluoro-1-methylpyridinium iodide (286 mg, 1.2 mmol) in water (2 ml) was added dropwise during 30 minutes at room temperature to a stirred solution of **I-33** trifluoroacetate[7] in water (10 ml) and 1 N NaOH (1.6 ml). The pH was maintained at 8.5 by simultaneous addition of 1 N NaOH. The brownish solution was cooled for 1 hour at 5°C and freeze dried. The residue was chromatographed over a Lobar-C column of silica gel, using Me_2CO - H_2O (3 : 1). Freeze drying of the product fractions yielded 150 mg (30%) of **II-33** as an amorphous colorless solid.

^1H NMR (60 MHz, DMSO-d_6) δ 3.33 (2H, s, SCH_2), 3.80 (6H, br s, 2×CH_3), 4.15 and 4.60 (2H, AB, J=15 Hz, CH_2NH), 4.93 (1H, d, J=5 Hz, 6-H), 5.55 (1H, dd, J=5 and 8 Hz, 7-H), 6.71 (1H, s, thiazole), 6.88 (1H, d, J=7 Hz, NH), 7.17 (2H, s, NH_2), 7.66~8.33 (4H, m, pyridine), 9.52 (1H, d, J=8 Hz, NH).

7-[2-(2-Aminothiazol-4-yl)-2-(Z)-(2-carboxy-3-(1-pyridinio)prop-1-yl-oxyimino)acetamido]cephalosporanate (**II-35**)

a) 7-[2-(2-Aminothiazol-4-yl)-2-(Z)-(2-carboxy-2-propen-1-yl-oxyimino)acetamido]cephalosporanic Acid (**I-35**): 2-(Z)-(2-*tert*-Butoxycarbonyl-2-propen-1-yl-oxyimino)-2-(2-tritylaminothiazol-4-yl)acetic acid[1] (2.9 g, 5 mmol), HOBT (0.8 g, 5.2 mmol) and dicyclohexylcarbodiimide (1.1 g, 5.3 mmol) in THF (20 ml) were stirred at room temperature for 3 hours. 7-ACA-*tert*-butyl ester (1.64 g, 5 mmol) was added and stirring was continued for 6 hours. The mixture was filtered and the filtrate chromatographed over a column of silica gel eluting with toluene - EtOAc (2 : 1). The product fractions were evaporated to give 1.76 g (40%) of trityl protected **I-35** bis-*tert*-butyl ester as an amorphous solid. This solid was dissolved in 90% aq TFA (50 ml). After 1 hour at room temperature the solvent was evaporated, the residue triturated several times with Et_2O and dried (KOH) to give **I-35** trifluoroacetate (0.9 g, 70%).

^1H NMR (60 MHz, DMSO-d_6) δ 2.02 (3H, s, $COCH_3$), 3.52 (2H, br s, SCH_2), 4.5~4.95 (4H, m, CH_2OAc and $NOCH_2$), 5.12 (1H, d, J=5 Hz, 6-H), 5.55~5.95 (2H, m, 7-H and $=CH_2$), 6.15 (1H, br s, $=CH_2$), 6.70 (1H, s, thiazole), 7.20 (2H, br s, NH_2), 9.62 (1H, d, J=8 Hz, NH).

b): A mixture of **I-35** trifluoroacetate (1.28 g, 2 mmol), KI (13.3 g, 80 mmol), pyridine (4 ml, 50 mmol) and 2 N HCl (10 ml) was stirred for 68 hours at room temperature. The solution was chromatographed on silica gel. KI was eluted with Me_2CO - H_2O (8 : 1) then **II-35** with Me_2CO - H_2O (4 : 1). Yield 700 mg (58%) of an amorphous solid.

^1H NMR (60 MHz, DMSO-d_6) δ 2.00 (3H, s, $COCH_3$), 3.30 (2H, br s, SCH_2), 4.1~5.1 (8H, m, 6-H, $=NCH_2CHCH_2$, 3-CH_2), 5.58 (1H, dd, J=5 and 8 Hz, 7-H), 6.68 (1H, s, thiazole), 7.30 (2H, br s, NH_2), 7.8~9.1 (5H, m, pyridine), 9.65 (1H, d, J=8 Hz, NH).

2-(2-Amino-4-methyl-1,3,5-triazin-6-yl)-2-(Z)-methoxyiminoacetic Acid (**VI-14**)

Ethyl-*N*-cyanoacetimidate (**VII**) (1.6 g, 14.2 mmol) was added to a stirred solution of methyl 2-amidino-2-(Z)-methoxyiminoacetate (**VIII**) (2 g, 12.5 mmol) in MeOH (10 ml) and concd NaOH (0.1 ml) at 5°C. Crystallization of the **VI-14** methyl ester begun after 30 minutes. The mixture was left overnight in the refrigerator. The crystals were filtered off, washed with cold MeOH, and dried to give 1.6 g (57%), mp 151~152°C.

^1H NMR (60 MHz, $CDCl_3$) δ 2.43 (3H, s, CH_3), 3.90 (3H, s, OCH_3), 4.12 (3H, s, $COOCH_3$), 5.95 (2H, br s, NH_2).

1.35 g (6 mmol) of the **VI-14** methyl ester was dissolved in MeOH (15 ml) and 1 N NaOH (15 ml). After 5 hours at room temperature the solution was acidified with 2 N HCl (15 ml) and evaporated to dryness. The residue was stirred with MeOH (40 ml). NaCl was filtered off and the filtrate concentrated to a volume of 2 ml. Et_2O (20 ml) was added to precipitate **VI-14**. The crystals were filtered off, washed with Et_2O, and dried to give 1.32 g (88%) of **VI-14** hydrochloride, mp 140°C (dec).

^{1}H NMR (60 MHz, DMSO-d_6) δ 2.30 (3H, s, CH_3), 3.90 (3H, s, OCH_3), 7.55 (2H, br s, NH_2).

2-(2-Aminooxazol-4-yl)-2-(*Z*)-methoxyiminoacetic Acid (**VI-11**)

A mixture of ethyl 4-bromo-2-(*Z*)-methoxyiminoacetoacetate (50.4 g, 0.2 mol), urea (150 g, 2.5 mol) and DMF (500 ml) was stirred for 3 hours at 100°C. The solution was cooled, diluted with water (2 liters), and extracted with EtOAc (3×500 ml). The organic phase was washed with water (3× 300 ml) and brine (2×200 ml), dried ($MgSO_4$) and concentrated to a volume of 70 ml. Diisopropyl ether (100 ml) was added while stirring. The crystalline solid was filtered off, washed with Et_2O, and dried to yield 3.9 g, mp 148~149°C. Concentration of the mother liquor gave another crop of 2.9 g of **VI-11** ethyl ester, mp 146~148°C (literature 11, mp 138~140°C) (yield 16%). Saponification with 2 N NaOH - dioxane at room temperature gave the free acid **VI-11** as an amorphous solid after acidification, filtration and drying.

^{1}H NMR (60 MHz, DMSO-d_6) δ 3.83 (3H, s, OCH_3), 6.87 (2H, br s, NH_2), 7.72 (1H, s, oxazole).

5,6,7,8-Tetrahydroimidazo[1,5-a]pyridine (**V-26**)

PtO_2 (1 g) was added to a solution of imidazo[1,5-a]pyridine (**V-24**) (6 g, 50.8 mmol) in TFA (100 ml) and the mixture was shaken at room temperature in an atmosphere of hydrogen at a slight positive pressure. The reaction was complete in 5 hours, whereupon 2,300 ml of gas were taken up. The catalyst was removed by filtration. TFA was evaporated and the residue was made strongly alkaline by the addition of concd aq NaOH. The resulting slurry was triturated several times with CH_2Cl_2. The organic solution was dried ($MgSO_4$) and evaporated to give 6.4 g of a colorless oil. Purity 93% according to GC.

^{1}H NMR (60 MHz, $CDCl_3$) δ 1.5~2.1 (4H, m, 2×CH_2), 2.5~3.0 (2H, m, CH_2), 3.75~4.1 (2H, m, CH_2), 6.73 (1H, s, aromatic H), 7.37 (1H, s, aromatic H).

5,6,7,8-Tetrahydroimidazo[1,2-a]pyridine (**V-25**) was similarly prepared from imidazo[1,2-a]-pyridine (**V-23**) and obtained as an colorless oil of 99% purity.

^{1}H NMR (60 MHz, $CDCl_3$) δ 1.7~2.2 (4H, m, 2×CH_2), 2.5~3.1 (2H, m, CH_2), 3.6~4.1 (2H, m, CH_2), 6.80 (1H, d, J=2 Hz, aromatic H), 6.98 (1H, d, J=2 Hz, aromatic H).

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