CEPHALOSPORINS CONTAINING CARBOHYDRATES

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Among the recently known several ten thousands representatives of the semisynthetic β-lactam antibiotics only a very few carbohydrate-containing analogues have been reported[1~3].

To our knowledge no cephalosporin analogues substituted with carbohydrate units at any position have been hitherto synthesized. This paper deals with the preparation of novel cephalosporins bearing a thioglycosyl function either at position 7 or as the ester moiety. As 1-thio-D-glucose, occurring in several plants in form of glycosides[4], is atoxic it was assumed that the metabolism of the above-type cephalosporin antibiotics did not produce perilous materials.

For starting materials of the synthesis the known C-3 substituted 7-chloroacetamidocephalosporanic acids (7-ACA) (**1a~d**), 1-thio-2,3,4,6-tetra-*O*-acetyl-β-D-gluco- and -D-galactopyranose[5,6] (**2a, b**) and 1-thio-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranose[7] (**2c**) were applied.

The nucleophilic substitution of the chlorine atom of **1a~d** with **2a~c** (Method A) could be readily accomplished even at room temperature in aqueous acetone in the presence of potassium hydrogen carbonate and a novel type of cephalosporin compounds (**3a~l**) carrying thioglycoside unit in the 7-amino side chain were prepared**. Compounds **3a** and **3b** were obtained as crystals, whereas the additional products were isolated as chromatographically homogeneous amorphous materials. In the 200 MHz ^{1}H NMR spectra (in $CDCl_3$) of these derivatives the signal of the anomeric proton appeared at 4.60~4.80 ppm with coupling $J_{1,2}$ ~10 Hz unequivocally indicating the β-configuration of the linkage of the thioglycoside sidechain. Although no molecular ion could be detected in the mass spectra of the above compounds the observed fragments, characteristic of the major structural units, clearly supported the composition of the products.

The intense peak of the tetra-*O*-acetylglycosylium cation appears at *m/z* 331, whereas the carboxyl-free β-lactam fragment at *m/z* 169 is due to a moderate degradation of the ceph-3-em skeleton which is further decomposed into the *m/z* 115 aromatic methythiazolium cation. This latter is detected, without exception, in the mass spectrum of each cephalosporin molecule. The *m/z* 73 fragment in the spectrum of **3c** could be assigned to 1-methyltetrazole which degraded by the loss of HCN and CH_2N_3. Instead of the aforementioned fragment the peak of 2-methyl-1,3,4-thiadiazole was observed at *m/z* 97 in the case of compound **3d**.

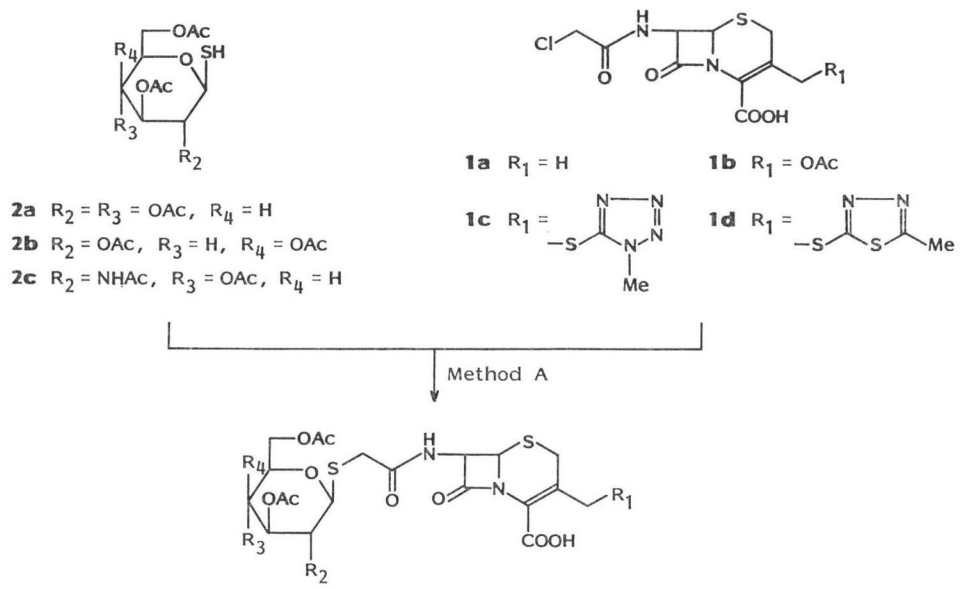
The above cephalosporins were also synthesized by an independent route (Method B). The reaction of **2a** with iodoacetic acid (in aqueous acetone in the presence of potassium hydrogen carbonate, 20°C) gave 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylthioacetic acid (**4**). Compound **4** was described earlier by Sokolov *et al.*[8] as a crystalline material but in our hands the product was syrupy and could be identified in form of its potassium salt. Esterification of **4** by treatment with pentachlorophenol and *N,N'*-dicyclohexylcarbodiimide in dichloromethane afforded the crystalline pentachlorophenyl ester **5**. Acylation of 7-ACA with this latter active ester resulted in **3b** which was identical, in every respect, with the product prepared from **1b** and **2a** by Method A.

It was found that allylic C-3 acetoxy group of 7-(2,3,4,6-tetra-*O*-acetyl-β-D-glycopyranosyl)-thioacetamidocephalosporanic acid readily reacts with 1-methyl-5-mercapto-1*H*-tetrazole in aqueous acetone (reflux, 6 hours) at pH 6.0 and compound **3c**, identical with that obtained by Method A, was isolated.

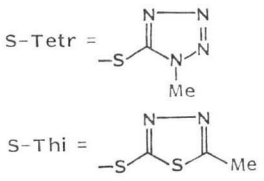
1-Thioglycoside derivatives were also utilized for the preparation of cephalosporin thioesters, potentially useful for purpose of oral administration. Consequently, 7-β-(thienylacetamido)-cephalosporanic acid (**7**), known as cephalothin in medicinal practice, was transformed with **2a**

** All the new compounds gave satisfactory elemental analyses.

Fig. 1.



	R_1	R_2	R_3	R_4
3a	H	OAc	OAc	H
3b	OAc	OAc	OAc	H
3c	S-Tetr	OAc	OAc	H
3d	S-Thi	OAc	OAc	H
3e	H	OAc	H	OAc
3f	OAc	OAc	H	OAc
3g	S-Tetr	OAc	H	OAc
3h	S-Thi	OAc	H	OAc
3i	H	NHAc	OAc	H
3j	OAc	NHAc	OAc	H
3k	S-Tetr	NHAc	OAc	H
3l	S-Thi	NHAc	OAc	H



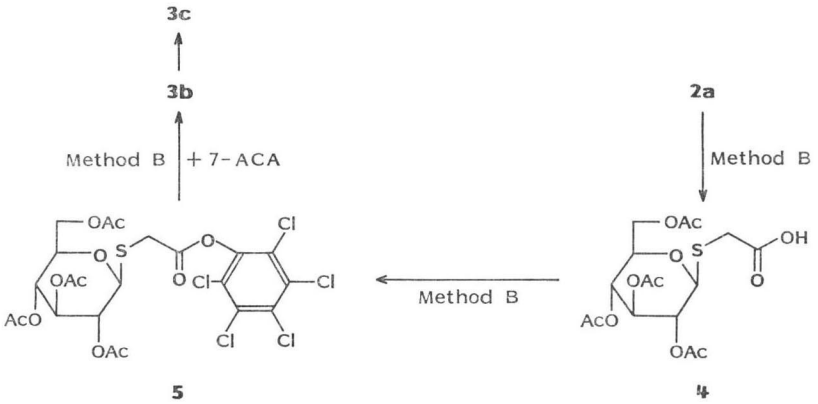
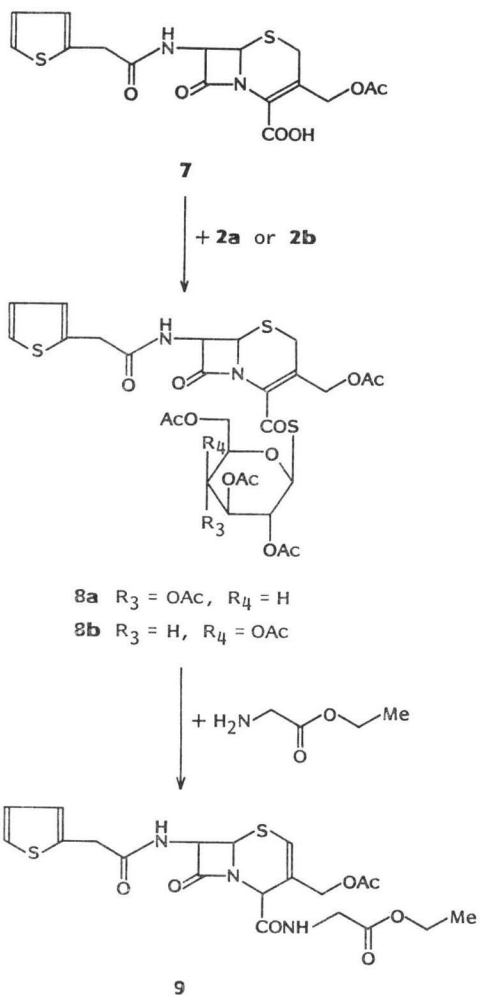


Fig. 2.



and **2b** and *N,N'*-dicyclohexylcarbodiimide in dichloromethane into the corresponding thioglycoxyl esters (**8a, b**).

TOMIĆ and KEGLEVIĆ[8] have reported that the 1-thio-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl esters of *N*-protected amino acids can be utilized as chemically active esters. Therefore, the acylation ability of **8a** and **8b** was considered worth of investigation.

The reaction of **8b** with ethyl glycinate (20°C, 24 hours) in dichloromethane readily afforded the known amide derivative (**9**) in good yield.

This finding clearly proved the active ester character of the cephalothin thioglycosyl esters. On the other hand, the ^{1}H NMR investigation of **9** indicated a non-desired $\Delta^3 \rightarrow \Delta^2$ isomerization of the double bond proceeding parallel with the acylation reaction.

The signals of the C-2 methylene protons, characteristic of Δ^3-cephems, were absent in the spectrum of **9**. Instead, peaks at δ=4.65 and 6.65 ppm correspondent to the C-4 and C-2 protons of the Δ^2-cephem skeleton were assigned. Similar interconversion has been reported by CHAUVETTE and FLYNN[9] by investigating of the reaction of cephalotonyloxyphthalimide and ethyl glycinate.

The *in vitro* activities of cephalosporin analogues **3a**~**l** were determined on eight test microorganisms. The compounds possessed activity only against Gram-positive microorganisms.

The best results were obtained with compounds **3b**~**d** on testing against *Streptococcus haemolyticus* (Lancefield A, 0.3~0.6 μg/ml). Additionally, the prepared compounds were practically inactive against β-lactamase producing *Staphylococcus* strains.

There is a linear connection between the *in vitro* and *in vivo* (in mice) activities of the examined cephalosporins. The comparison of the antibiotic activities of the synthesized thioglycosyl esters with those of Maripen* and cephalothin suggests that **8a** and **8b** are inactivated under the applied test-conditions and this is, most likely, due to a $\Delta^3 \rightarrow \Delta^2$ isomerization of the double bond (similarly to the case of the acylation of ethyl glycinate demonstrated before).

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* Acetoxymethyl ester of benzylpenicillin manufactured by Biogal Pharmaceutical Works, Debrecen, Hungary.

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