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NOTES

Cephalosporin Podand Derivatives

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In recent years the design and preparation of dendrimers and another multivalent molecules have emerged intensively studied subjects in organic synthesis[1]. Biologically important substances, such as carbohydrates[2~7], amino acids[8] often serve as the functional groups of these types of oligomers.

In many cases, related multivalent compounds possess remarkable biological effects. As an example, the so-called cluster effect in the case of the carbohydrate-containing substances is manifested in the enhanced affinity of the dendrimers towards another biomolecules due to weak, non-bonding interactions[9].

In the field of antibiotics, covalent oligomers or polymers have only been synthesized from vancomycin[10~12], and the complex-forming, and antibacterial properties of the derivatives obtained were found to be better than that of the parent, monomeric antibiotic[13].

Till now, no multivalent derivatives of the β-lactam antibiotics have been synthesized. In continuation of our work in the synthesis of podand molecules[6], the present paper describes the preparation of certain podand-type trimers of cephalosporin antibiotics (Scheme 1).

As the central block of the target trimers the tricarboxylic acid **3** was employed, which was obtained by the alkylation of phloroglucinol (**1**) with methyl bromoacetate and subsequent alkaline hydrolysis. The *tert*-butyl esters of 7-aminocephalosporanic acid (**5**)[14] and 7-aminodeacetoxycephalosporanic acid (**6**)[14] were acylated with the acid chloride **4** derived from **3** to obtain the trimers **9** and **11**, respectively. Removal of the *tert*-butyl protecting group of these products upon hydrolysis with trifluoroacetic acid followed by potassium salt formation furnished the target podands **10** and **12**.

It is well-known from the literature that certain cephalosporin sulfones possess β-lactamase enzyme inhibitory properties[15], and therefore, we decided to synthesize structurally related sulphone-trimers, as well. Thus, the *tert*-butyl ester of 7-aminocephalosporanic acid sulphone (**7**)[16] and the corresponding deacetoxy derivative **8** were acylated with the acid chloride **4** to give **13** and **15**. Subsequent removal of the ester protecting group by hydrolysis with trifluoroacetic acid then furnished the desired trimeric cephalosporin sulphones **14** and **16**.

The antibacterial activity of the cephalosporin trimers prepared was compared to that of a cefuroxime reference sample by using the agar-dilution method (Nutrient Broth). Against *Staphylococcus aureus* ATCC25923, cefuroxime showed a MIC of 1.5 μg/ml, but compounds **10** and **12** were active only in 12.5 and 50 μg/ml concentration, respectively. The podand **10** was considerably more active (MIC 3.12 μg/ml) against *Streptococcus faecalis* (OKI[†])80171 than cefuroxime (MIC 12.5 μg/ml), while the antibacterial effect of cefuroxime and of the trimer **12** was in the same range against this microorganism. The synthesized trimers were practically inactive against *Bacillus subtilis* ATCC6633, *Escherichia coli* (OKI)35034, *Proteus vulgaris* (OKI)60002 and *Pseudomonas aeruginosa* ATCC27853. In addition, the trimeric cephalosporin sulphone derivatives **13** and **15** did not show inhibitory effect (in 50 μmole concentration) on the β-lactamase enzymes produced by the strains *B. cereus*, *E. cloaceae*, *E. coli*, *P. vulgaris* and *P. aeruginosa*.

The expected cluster effect of our cephalosporin podands unfortunately could not be detected in their biological tests. Preparation of similar podands of other antibiotics is under study in our laboratory.

Dedicated to the memory of Sir EDWARD ABRAHAM.

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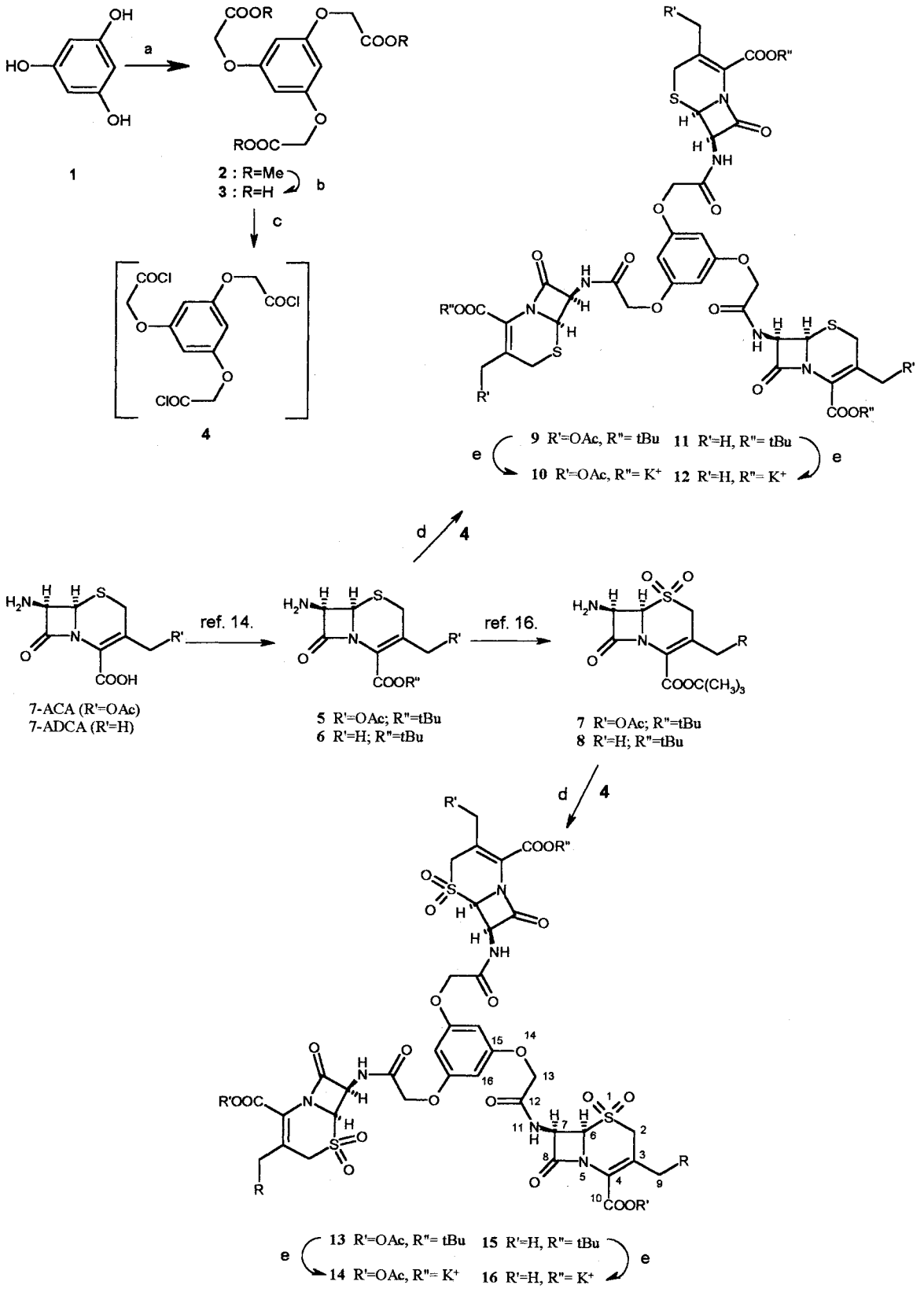
Scheme 1. Synthesis of cephalosporin podand derivatives.



a) $BrCH_2COOCH_3$, K_2CO_3, acetone; b) 10 % NaOH, 80 °C; c) $SOCl_2$; d) CH_2Cl_2, pyridine, 0 °C→ r.t.; e) i) 30 % TFAA/ CH_2Cl_2, ii) potassium α-ethylcaproate

Table 1. ^{1}H NMR data of new compounds.

Compound (solvent)	δ_H								
	2	**6**	**7**	**9**	**R"**	**R'**	**11**	**13**	**16**
2 ($CDCl_3$)						3.83 (s, 3H, OMe)		4.59 (s, 2H)	6.14 (s, 1H)
3 (D_2O)								4.40 (s, 2H)	6.09 (s, 1H)
9 ($CDCl_3$)	3.42 ; 3.48 (ABq, 2H)	5.02 (d, 1H)	5.93 (dd, 1H)	4.81 ; 5.1 (ABq, 2H)	1.55 (s, 9H)	2.10 (s, 3H, OAc)	7.22 (d, 1H)	4.55 (s, 2H)	6.18 (s, 1H)
11 ($CDCl_3$)	3.22 ; 3.51 (ABq, 2H)	5.01 (d, 1H)	5.87 (dd, 1H)	2.11 (s, 3H)	1.54 (s, 9H)		7.32 (d, 1H)	4.55 (s, 2H)	6.17 (s, 1H)
13 ($CDCl_3$)	3.82 ; 3.94 (ABq, 2H)	4.86 (d, 1H)	6.13 (dd, 1H)	4.74 ; 5.14 (ABq, 2H)	1.54 (s, 9H)	2.09 (s, 3H, OAc)	7.88 (d, 1H)	4.53 ; 4.60 (ABq, 2H)	6.17 (s, 1H)
15 ($CDCl_3$)	3.67 ; 3.82 (ABq, 2H)	4.80 (d, 1H)	6.06 (dd, 1H)	2.07 (s, 3H)	1.53 (s, 9H)		7.90 (d, 1H)	4.54 ; 4.66 (ABq, 2H)	6.19 (s, 1H)
10 (D_2O)	3.31 ; 3.58 (ABq, 2H)	5.06 (d, 1H)	5.66 (dd, 1H)	4.65 ; 4.84 (ABq, 2H)		2.05 (s, 3H, OAc)		4.7 (s, 2H)	6.28 (s, 1H)
12 (D_2O)	3.14 ; 3.50 (ABq, 2H)	5.02 (d, 1H)	5.57 (dd, 1H)	1.85 (s, 3H)				4.68 (s, 2H)	6.25 (s, 1H)
8 ($CDCl_3$)	3.43 ; 3.87 (ABq, 2H)	4.71 (d, 1H)	4.87 (d, 1H)	2.07 (s, 3H)	1.55 (s, 9H)		2.15 (m, 2H)		
14 (D_2O)	3.94 ; 4.24 (ABq, 2H)	5.31 (d, 1H)	6.00 (d, 1H)	4.70 ; 4.92 (ABq, 2H)		2.10 (s, 3H, OAc)		4.71 (s, 2H)	6.22 (s,1H)
16 (D_2O)	3.77 ; 4.12 (ABq, 2H)	5.21 (d, 1H)	5.91 (d, 1H)	1.87 (s, 3H)				4.64 (s, 2H)	6.14 (s, 1H)

Experimental

Solvents were distilled before use. Organic extracts were dried over magnesium sulphate. Solutions were concentrated at 35~40°C (bath) at *ca.* 17 mmHg. Melting points were determined in capillary tubes and are uncorrected. For thin layer chromatography precoated aluminum-backed plates (Silica gel 60 F_{254}, Merck, layer thickness: 0.2 mm) were used. The spots were visualized by spraying with 7% ammonium molybdate in 5% sulfuric acid and heating. Preparative TLC was carried out on Merck silica gel 60 F_{254} plates, layer thickness 0.25 and 0.5 mm. IR spectra (KBr discs) were recorded on a Perkin-Elmer 16 PC FT-IR spectrophotometer. Specific rotations were measured on a Perkin-Elmer 141 MC polarimeter at room temperature. ^{1}H NMR spectra: Bruker WP 200 SY (200 MHz) and Bruker AM 360 (360 MHz) instruments; tetramethylsilane as internal standard. ^{13}C NMR spectra were recorded on Bruker WP 200 SY (50 MHz) and on Bruker AM 360 (90 MHz) instruments (Table 1). Electrospray (ESP) mass spectrometric measurements (positive and negative ion detections) were run on a FINNIGAN TSQ 7000 triple quadrupole mass spectrometer equipped with API source; samples were introduced into 50% methanol solutions containing 0.1% acetic acid or 0.1% ammonium acetate. All compounds gave satisfactory elemental analysis data.

(3,5-Bis-methoxycarbonyl-methoxy-phenoxy)acetic Acid Methyl Ester (**2**)

Phloroglucinol (2.00 g, 15.9 mmol) was dissolved in the suspension of abs. acetone (100 ml) and potassium carbonate (10.0 g). Methyl bromoacetate (6.80 ml, 71.4 mmol) was added to the well-stirred slurry and the mixture was refluxed for 12 hours. The solid was filtered off, washed with dicloromethane and the organic solvents were evaporated. The residue was dissolved in dichloromethane (100 ml), extracted with 10% $NaHSO_4$

solution and brine, dried and evaporated. The product crystallized by adding abs. ether, 3.22 g (59%), mp: 131~132°C; FT-IR (KBr) 1770 cm^{-1}; ESP-MS (*m/z*): 343 $(M+H)^+$; ^{13}C NMR (50 MHz, $CDCl_3$) δ: 52.3, 65.2, 95.2, 159.6, 168.9.

(3,5-Bis-carboxymethoxy-phenoxy)acetic Acid (**3**)

Compound **2** (2.13 g, 6.23 mmol) was dissolved in 10% NaOH solution (15 ml) and warmed at 80°C for 12 hours. After cooling, the clear solution was extracted with ethyl acetate and the aqueous phase was acidified carefully with 10% HCl solution. The precipitate was collected by filtration, washed with cold water and dried in a desiccator, 1.77 g of white powder (95%), mp 304~305°C (dec.); FT-IR (KBr) 1740 cm^{-1}; ESP-MS (*m/z*): 301 $(M+H)^+$; ^{13}C NMR (50 MHz, $D_2O+K_2CO_3$) δ: 67.5, 95.1, 160.3, 167.6.

tert-Butyl 7-Amino-deacetoxycephalosporanoate 1,1-Dioxide (**8**, R=H)

The reaction was carried out starting from **6** as it is described for *tert*-butyl 7-amino-cephalosporanoate 1,1-dioxide (**7**). Yield 77%; FT-IR (KBr) ν_{max} 3414, 1754, 1712, 1652 cm^{-1}; $[\alpha]_D^{23}+14.7$ (*c* 1.11, $CHCl_3$); ESP-MS *m/z*: 303 $(M+H)^+$, 301 $(M-H)^-$.

General Procedure for the Acylation of β-Lactam Esters

The suspension of acid **3** (0.20 mmol) in $SOCl_2$ (2 ml) was refluxed for 2 hours under Ar. The resulting clear mixture was evaporated and co-evaporated with abs. benzene (3×5 ml). The crude acyl chloride **4** was dissolved in abs. dichloromethane (2 ml) and added dropwise to the cooled solution of a β-lactam ester (0.60 mmol) in dichloromethane (2 ml) and abs. pyridine (1.20 mmol). The stirred mixture was allowed to warm up to r.t. (2 hours), diluted with dichloromethane, extracted with 10% $NaHSO_4$, sat. $NaHCO_3$ solutions and brine, dried and purified by column chromatography or preparative TLC.

tert-Butyl 7-(3,5-Bis-(((6*R*,7*R*)3-acetoxymethyl-4-*tert*-butoxycarbonyl-cephem-7-ylcarbamoyl)-methoxy))-phenoxyacetamido Acetoxycephalosporanoate (**9**)

Starting from **5**, yield 50%, mp 166~167°C (dec.); FT-IR (KBr) ν_{max} 1786, 1728, 1602, 1522 cm^{-1}; $[\alpha]_D^{23}+26.1$ (*c* 1.89, $CHCl_3$); ESP-MS: *m/z* 1248 $(M+NH_4^+)$.

tert-Butyl 7-(3,5-Bis-(((6*R*,7*R*)4-*tert*-butoxycarbonyl-3-methyl-cephem-7-ylcarbamoyl)-methoxy))-phenoxyacetamido Deacetoxycephalosporanoate (**11**)

Starting from **6** (R=H)[1], yield 49%; mp 195~196°C (dec.); FT-IR (KBr) ν_{max} 1782, 1716, 1602, 1522 cm^{-1}; $[\alpha]_D^{23}+71.8$ (*c* 1.02, $CHCl_3$); ESP-MS *m/z*: 1074 $(M+NH_4)^+$, 1079 $(M+Na)^+$, 1095 $(M+K)^+$.

tert-Butyl 7-(3,5-Bis-(((6*R*,7*R*)3-acetoxymethyl-4-*tert*-butoxycarbonyl-1,1-dioxo-cephem-7-ylcarbamoyl)-methoxy))-phenoxyacetamido Acetoxycephalosporanoate 1,1-Dioxide (**13**)

Starting from **7**, yield 47%, mp 169~170°C (dec.); FT-IR (KBr) ν_{max} 1806, 1724, 1608, 1522 cm^{-1}; $[\alpha]_D^{23}-39.0$ (*c* 1.29, $CHCl_3$); ESP-MS: *m/z* 1344 $(M+NH_4^+)$.

tert-Butyl 7-(3,5-Bis-(((6*R*,7*R*)4-*tert*-butoxycarbonyl-1,1-dioxo-3-methyl-cephem-7-ylcarbamoyl)-methoxy))-phenoxyacetamido Deacetoxycephalosporanoate 1,1-Dioxide (**15**)

Starting from **8** (R=H), yield 57%, mp 191~192°C (dec.); FT-IR (KBr) ν_{max} 1796, 1716, 1608, 1522 cm^{-1}; $[\alpha]_D^{23}+26.1$ (*c* 0.57, $CHCl_3$); ESP-MS *m/z*: 1170 $(M+NH_4)^+$, 1175 $(M+Na)^+$, 1191 $(M+K)^+$.

General Procedure for the Synthesis of the Potassium Cephalosporanoate Podands

tert-Butyl cephalosporanoate podand (0.10 mmol) was dissolved in 30% trifluoroacetic acid in dichloromethane (9 ml) and stirred at r.t. for 1.5 hours. The solvents were evaporated and co-evaporated with abs. benzene (3×5 ml). The residue was suspended in a solution of potassium α-ethylcaproate (2 equiv.) in abs. ether (10 ml) and stirred for 16 hours. The etheral phase was decanted, the remaining solid was decanted with abs. ether (3×5 ml), dissolved in distilled water (10 ml), and filtered trough a celite pad. Lyophilization resulted in hygroscopic flaky solids.

7-(3,5-Bis-(((6*R*,7*R*)3-acetoxymethyl-4-carboxy-cephem-7-ylcarbamoyl)-methoxy))-phenoxyacetamido Acetoxycephalosporanoic Acid Tripotassium salt (**10**)

Starting from **9**, yield 72%; FT-IR (KBr) ν_{max} 1762, 1684, 1608, 1558, 1540 cm^{-1}; $[\alpha]_D^{23}+77.9$ (*c* 0.97, H_2O); ESP-MS: *m/z* 1061 $(M-H^-)$.

7-(3,5-Bis-(((6*R*,7*R*)4-carboxy-3-methyl-cephem-7-ylcarbamoyl)-methoxy))-phenoxyacetamido Deacetoxycephalosporanoic Acid Tripotassium Salt (**12**)

Starting from compound **11**, yield: 82%; FT-IR (KBr) ν_{max} 1750, 1684, 1602, 1558, 1540 cm^{-1}; $[\alpha]_D^{23}+130.2$ (*c* 0.94, H_2O); ESP-MS *m/z*: 906 $(M+NH_4)^+$, 911 $(M+Na)^+$, 887 $(M-H)^+$.

7-(3,5-Bis-(((6*R*,7*R*)3-acetoxymethyl-4-carboxy-1,1-dioxo-cephem-7-ylcarbamoyl)-methoxy))-phenoxyacetamido Acetoxycephalosporanoic Acid 1,1-Dioxide Tripotassium Salt (**14**)

Starting from **13**, yield 78%; FT-IR (KBr) ν_{max} 1784, 1684, 1616, 1540, 1522 cm^{-1}; $[\alpha]_D^{23}$+24.7 (*c* 0.85, H_2O); ESP-MS: *m/z* 1157 $(M-H)^-$.

7-(3,5-Bis-(((6*R*,7*R*)4-carboxy-1,1-dioxo-3-methyl-cephem-7-ylcarbamoyl)-methoxy))-phenoxyacetamido deacetoxycephalosporanoic Acid 1,1-Dioxide Tripotassium Salt (**16**)

Starting from **15**, yield 81%; FT-IR (KBr) ν_{max} 1772, 1684, 1608, 1558, 1540 cm^{-1}; $[\alpha]_D^{23}$+46.2 (*c* 1.23, H_2O); ESP-MS *m/z*: 983 $(M-H)^-$, 491 $(M-H)^{2-}$.

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