

A Trimer of Phenoxyethyl Penicillin Sulphone: Synthesis of a New β -Lactam Podand

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Nowadays, bacterial resistance (MRSA, VRE) towards antibiotics is no longer a local problem, but it is rather considered as a global question. Recent trends in the fight against resistant bacteria are the synthesis of the covalent di-, tri-, or oligomeric derivatives¹⁻⁷ of antibiotics, and the research of enzyme inhibitory compounds.

From the point of view of medicinal application, the leading group of the antibiotics is the family of β -lactams. Despite the huge number of the prepared semisynthetic penicillins and cephalosporins, only a few penicillanic acid sulphones, and clavam- and carbapenam-type β -lactamase inhibitory substances⁸) have been reported. At the same time, the number of the TEM-type inhibitor-resistant (IRT) β -lactamases is growing.

In continuation of our work in the field of β -lactam antibiotics, and in the light of those described above, the present paper deals with the synthesis of a penicillin-V podand with potential β -lactamase activity.

Our primary goal was the preparation of a trimer (**5**) of phenoxyethyl penicillin sulphone (Scheme 1), with the expectation that this podand would retain the favoured acid-stable properties of the parent antibiotic penicillin-V.

Accordingly, the benzyl ester of 6-aminopenicillanic acid (**2a**)⁹ was acylated with the crude acid chloride **1a** prepared from (3,5-bis-carboxymethoxy-phenoxy)-acetic acid (**1**)⁶ in dry dichloromethane in the presence of pyridine, and the produced trimeric product **3** was isolated in a moderate (42%) yield after chromatographic purification. Oxidation

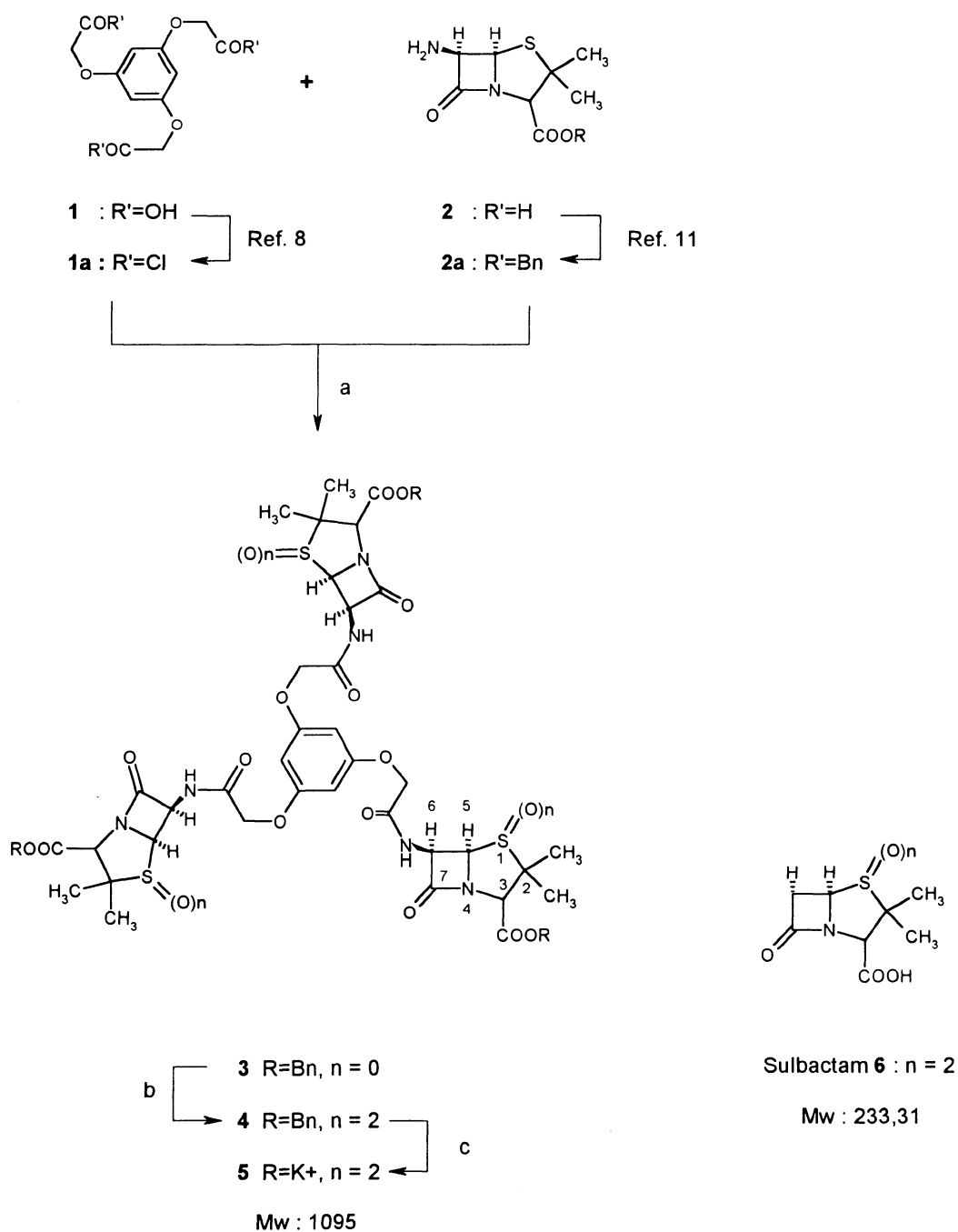
of **3** with potassium permanganate readily furnished the trisulfone **4**, whose benzyl protecting groups were removed by means of catalytic hydrogenation (over 10% Pd/C in ethyl acetate) to obtain the target trimeric antibiotic analogue in form of the potassium salt **5** with 76% yield.

The enzyme-inhibitory properties of the new β -lactam podand **5** towards the β -lactamase enzymes produced by *Bacillus cereus* 569/H, *Enterobacter cloacae* P99, *Proteus vulgaris* 1028/ β c, *Escherichia coli* R46, *Pseudomonas aeruginosa* cilote, and *Klebsiella oxytoca* 20 were investigated in 0.05 M phosphate buffer (pH=7.0) in the presence of the nitrocephine substrate by employing sulbactam (**6**) as the control material (Table 1). Considering the large difference between the molecular masses of compounds **5** and **6**, the 50% inhibitory concentration (I_{50}) values were calculated in a $\mu\text{mol/ml}$ unit instead of the usual $\mu\text{g/ml}$. Of the isolated enzymes, the trimeric podand **5** showed considerable inhibitory activity exclusively against the β -lactamase originating from *Enterobacter cloacae* P99: the I_{50} value of the podand **5** and sulbactam (**6**) for this strain were 0.018 $\mu\text{mol/ml}$ and 0.020 $\mu\text{mol/ml}$, respectively. At the same time, the I_{50} inhibitory effect of **5** against the β -lactamases produced by the other microorganisms was not significant. A low inhibition was observed in the case of the enzyme originating from *Proteus vulgaris*. It is remarkable that the β -lactamases of both *Enterobacter cloacae* and *Proteus vulgaris* are group 1 (class C) chromosomal cephalosporinases resistant to clavulanic acid.

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 F₂₅₄, 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 F₂₅₄ plates, layer thickness 2 mm. IR spectra (KBr discs) were recorded on a Perkin-Elmer 16 PC FT-IR spectrophotometer. Specific optical rotations were measured on a Perkin-Elmer 141 MC polarimeter at room

Scheme 1. Synthesis of a new penicillin-V podand sulphone.



temperature. ¹H NMR spectra: Bruker WP 200 SY (200 MHz) and Bruker AM 360 (360 MHz) instruments; tetramethylsilane as internal standard. ¹³C NMR spectra

were recorded on Bruker WP 200 SY (50 MHz). Electro spray (ESP) mass spectrometric measurements (positive and negative ion detections) were run on a

Table 1. Enzyme inhibitory activity of the trimeric podand **5** in 0.05 M phosphate buffer (pH=7) in the presence of nitrocephine substrate.

Enzyme inhibitory activity (I_{50}) in $\mu\text{g/ml}$		
Strain (source of enzyme)	Compound 5	Subactam (6)
<i>Bacillus cereus</i> 569/H	> 500	2.40
<i>Enterobacter cloacae</i> P99	20 (0.018 $\mu\text{mol/ml}$)	5.00 (0.02 $\mu\text{mol/ml}$)
<i>Proteus vulgaris</i> 1028/ β c	196 (0.22 $\mu\text{mol/ml}$)	0.12 (0.0048 $\mu\text{mol/ml}$)
<i>Escherichia coli</i> R46	> 500	0.33
<i>Pseudomonas aeruginosa</i> cilote	> 500	0.24
<i>Klebsiella oxytoca</i> 20	> 500	2.50

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.

Benzyl 6-{3,5-Bis-[(3*R*,5*R*,6*R*)-3-benzyloxycarbonylpenam-6-ylaminocarbonylmethoxy]}-phenoxyacetamide-(3*R*,5*R*,6*R*)-penam-6-ylcarboxylate (**3**)

The suspension of the acid **1**⁶⁾ (250 mg, 0.83 mmol) in SOCl_2 (20 ml) was refluxed for 2 hours under argon. The resulting clear mixture was evaporated and co-evaporated with abs. benzene (3×10 ml). The crude acyl chloride (**1a**) was dissolved in dry. dichloromethane (2 ml) and added dropwise to a cold solution (0°C) of the 6-aminopenicillanic acid benzyl ester **2**⁹⁾ (850 mg, 2.7 mmol) in dichloromethane (10 ml) and dry. pyridine (0.24 ml, 3 mmol). The stirred mixture was allowed to warm up to r.t. (2 hours), diluted with dichloromethane, extracted with 10% NaHSO_4 , saturated. NaHCO_3 solutions and brine, dried and purified by column chromatography and preparative TLC (hexane:acetone 6:4) to give **3** (380 mg, 42%) as a white powder. Mp. $96\sim 97^\circ\text{C}$ (dec.); FT-IR (KBr) ν_{max} 1786, 1742, 1694, 1604, 1518 cm^{-1} ; $[\alpha]_{\text{D}}^{23} +136.9$ (c 1.57, CHCl_3); ESP-MS: m/z 1182 ($\text{M}+\text{NH}_4^+$). $^1\text{H-NMR}$ δ (ppm): 1.4 and 1.6 (2s, 6H, -Me), 4.5 (m, 3H, H-3, $-\text{CH}_2\text{-Ar}$), 5.2 (s, 2H, H-10), 5.6 (d, 1H, H-5), 5.75 (dd, 1H, H-6), 6.2 (s, 1H, H-12), 7.4 (s, 5H, phenyl).

Benzyl 6-{3,5-Bis-[(3*R*,5*R*,6*R*)-3-benzyloxycarbonylpenam-6-ylaminocarbonylmethoxy]}-phenoxyacetamide-(3*R*,5*R*,6*R*)-penam-6-ylcarboxylate 1,1-Dioxide (**4**)

The penicillin podand **3** (270 mg, 0.23 mmol) was

dissolved in acetone (15 ml), acetic acid (1 ml) and 290 mg (1.84 mmol) of potassium permanganate in water (10 ml) was added dropwise to the solution at 0°C . Three hours later 10% hydrogen peroxide solution was added to diminish the purple colour. The reaction mixture was evaporated, dissolved in dichloromethane, washed with saturated NaHCO_3 solution and brine and dried. Preparative TLC in hexane:acetone 1:1 gave 160 mg (55%) of **4** as a white powder. Mp $115\sim 116^\circ\text{C}$; FT-IR (KBr) ν_{max} 1806, 1748, 1698, 1604, 1518 cm^{-1} ; $[\alpha]_{\text{D}}^{23} +103.3$ (c 1.0, CHCl_3); ESP-MS m/z : 1278 ($\text{M}+\text{NH}_4^+$). $^1\text{H-NMR}$ δ (ppm): 1.3 and 1.6 (2s, 6H, -Me), 4.55 (m, 3H, H-3, $-\text{CH}_2\text{-Ar}$), 4.8 (d, 1H, H-5), 5.25 (ABq, 2H, H-10), 6.25 (s, 2H, H-6, H-12), 7.4 (s, 5H, phenyl), 8.05 (d, 1H, NH).

Potassium 6-{3,5-Bis-[(3*R*,5*R*,6*R*)-3-benzyloxycarbonylpenam-6-ylaminocarbonylmethoxy]}-phenoxyacetamide-(3*R*,5*R*,6*R*)-penam-6-ylcarboxylate 1,1-Dioxide (**5**)

The podand **4** (105 mg, 0.08 mmol) was stirred in ethyl acetate (10 ml) and methanol (10 ml) with 10% Pd/C (200 mg) for 15 hours under hydrogen. The catalyst was filtered off and the solvents were evaporated. The residue was suspended in a solution of potassium α -ethylcaproate (300 mg) in dry. ether (50 ml) and stirred for 16 hours. The ethereal phase was decanted, the remaining solid was triturated with dry ether (3×20 ml), dissolved in distilled water (20 ml), and filtered through a celite pad. Freeze-drying resulted in a hygroscopic flaky solid: 70 mg (76%) of **5**. FT-IR (KBr) ν_{max} 1652, 1558, 1540, 1472, 1168, 1124 cm^{-1} ; ESP-MS: m/z 989 ($\text{M}-3\text{K}+2\text{H}$)⁻, 494 ($\text{M}-3\text{K}+\text{H}$)²⁻. $^1\text{H-NMR}$ δ (ppm): 1.4 and 1.6 (2s, 6H, -Me), 4.35 (s, 1H, H-3), 4.65 (s, 2H, H-10), 5.2 (d, 1H, H-5), 6.05 (d, 1H, H-6), 6.3 (s, 1H, H-12), 7.4 (s, 5H,

phenyl). $^{13}\text{C-NMR}$ δ (ppm): 18.1, 20.2 (-Me); 55.1, 57.7, 66.0 (C-3, C-5, C-6); 66.4, 67.4 (C-2, C-10); 88.9 (C-12); 159.3 (C-11), 171.1, 173.2, 175.4 (3 C=O).

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References

- 1) RAD, J. & G. M. WHITESIDES: Tight binding of a dimeric derivative of vancomycin with dimeric L-Lys-D-Ala-D-Ala. *J. Am. Chem. Soc.* 119: 10286~10290, 1997
- 2) STAROSKE, TH. & D. H. WILLIAMS: Synthesis of covalent head-to-tail dimers of vancomycin. *Tetrahedron Lett.* 39: 4917~4920, 1998
- 3) SZTARICKAI, F.; GY. BATA, Z. DINYA, O. V. MIROSHNIKOVA, M. N. PREOBRAZHENSKAYA, F. HERNÁDY, Á. KONCZ & Z. BODA: Chemical modification of the eremomycin antibiotic. Formation a cyclic covalent dimer. *J. Antibiotics* 54: 314~319, 2001
- 4) NICOLAOU, K. C.; R. HUGHES, S. Y. CHO, N. WINSSINGER, H. LABISCHINSKI & R. ENDERMAN: Synthesis and biological evaluation of vancomycin dimers with potent activity against vancomycin-resistant bacteria: Target-accelerated combinatorial synthesis. *Chem. Eur. J.* 7: 3824~3843, 2001
- 5) SALEM, A.-B. & J. B. REGNOUF-DE-VAINS: Synthesis and characterisation of a new podand based on a calixarene and a β -lactam. *Tetrahedron Lett.* 42: 7033~7036, 2001
- 6) KOVÁCS-KULYASSA, Á.; P. HERCZEGH & F. SZTARICKAI: Cephalosporin podand derivatives. *J. Antibiotics* 53: 1207~1211, 2000
- 7) ARIMOTO, H.; K. NISHIMURA, T. KINUMI, I. HAYAKAWA & D. UEMURA: Multi-valent polymer of vancomycin: Enhanced antibacterial activity against VRE. *Chem. Comm.* 1361~1362, 1999
- 8) LIVERMORE, D. M.: β -Lactamase-mediated resistance and opportunities for its control. *J. Antimicrobial Chemoth.* 41 (Suppl. D): 25~41, 1998
- 9) BRAIN, E. G.; I. McMILLIAN, J. H. C. NAYLER, R. SOUTHGATE & P. TOLLIDAY: The chemistry of penicillanic acids. Part. III. A route to 1,2-secopenicilline. *J. Chem. Soc. Perkin I.* 562~567, 1975