A Trimer of Phenoxymethyl Penicillin Sulphone: Synthesis of a New \(\beta\)-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 derivatives\(^1\-^7\) 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 \(\beta\)-lactams. Despite the huge number of the prepared semisynthetic penicillins and cephalosporins, only a few penicillanic acid sulphones, and clavam- and carbapenam-type \(\beta\)-lactamase inhibitory substances\(^8\) have been reported. At the same time, the number of the TEM-type inhibitor-resistant (IRT) \(\beta\)-lactamases is growing.

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

Our primary goal was the preparation of a trimer (5) of phenoxymethyl 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-phenoxo)-acetic acid (1)\(^9\) 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 \(\beta\)-lactam podand 5 towards the \(\beta\)-lactamase enzymes produced by Bacillus cereus 569/H, Enterobacter cloacae P99, Proteus vulgaris 1028/\(\beta\)c, Escherichia coli R46, Pseudomonas aeruginosa citole, and Klebsiella oxytoca 20 were investigated in 0.05M 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 \(\beta\)-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 \(\beta\)-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 \(\beta\)-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 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 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.

Scheme 1:

- 1 : R'=OH
- 1a : R'=Cl
- 2 : R'=H
- 2a : R'=Bn

Reactions:

a.) CH₂Cl₂, pyridine, 0°C→r.t.;

b.) acetone-AcOH-H₂O, KMnO₄, 0°C;

c.) H₂/Pd, r.t., potassium α-ethylcaproate

Mw : 233.31

Mw : 1095

- 3 R=Bn, n = 0
- 4 R=Bn, n = 2
- 5 R=K+, n = 2

a) CH₂Cl₂, pyridine, 0°C→r.t.; b) acetone-AcOH-H₂O, KMnO₄, 0°C; c) H₂/Pd, r.t., potassium α-ethylcaproate

were recorded on Bruker WP 200 SY (200 MHz) and Bruker AM 360 (360 MHz) instruments; tetramethylsilane as internal standard. ¹³C NMR spectra 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.

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

The suspension of the acid 16) (250mg, 0.83mmol) in SOCl₂ (20ml) was refluxed for 2 hours under argon. The resulting clear mixture was evaporated and co-evaporated with abs. benzene (3~10ml). The crude acyl chloride (1a) was dissolved in dry. dichloromethane (2ml) and added dropwise to a cold solution (0°C) of the 6-aminopenicillanic acid benzyl ester 29) (850mg, 2.7mmol) in dichloromethane (10ml) and dry. pyridine (0.24ml, 3mmol). The stirred mixture was allowed to warm up to r.t. (2 hours), diluted with dichloromethane, extracted with 10% NaHSO₄, saturated. NaHCO₃ solutions and brine, dried and purified by column chromatography and preparative TLC (hexane: acetone 6:4) to give 3 (380mg, 42%) as a white powder. Mp. 96-97°C; FT-IR (KBr) νmax 1786, 1742, 1694, 1604, 1518cm⁻¹; [α]D²+136.9 (c 1.57, CHCl₃); ESP-MS: m/z 1182 (M+NH₄)+. 1H-NMR ¹H (ppm): 1.4 and 1.6 (2s, 6H, -Me), 4.5 (m, 3H, H-3, -CH₂-Ar), 5.2 (s, 2H, H-10), 5.6 (d, 1H, H-5), 5.75 (dd, 1H, H-6), 7.4 (s, 5H, phenyl).

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

The podand 3 (270mg, 0.23mmol) was dissolved in acetonitrile (15ml), acetic acid (1ml) and 290mg (1.84mmol) of potassium permanganate in water (10ml) 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 NaHCO₃ solution and brine and dried. Preparative TLC in hexane: acetone 1:1 gave 160mg (55%) of 4 as a white powder. Mp 115~116°C; FT-IR (KBr) νmax 1806, 1748, 1698, 1604, 1518 cm⁻¹; [α]D²+103.3 (c 1.0, CHCl₃); ESP-MS m/z: 1278 (M+NH₄)+. 1H-NMR ¹H (ppm): 1.3 and 1.6 (2s, 6H, -Me), 4.55 (m, 3H, H-3, -CH₂-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-[(3R,5R,6R)-3-benzyloxycarbonyl-penam-6-ylaminocarbonylmethoxy]-phenoxyacetamide-(3R,5R,6R)-penam-6-ylcarboxylate 1,1-Dioxide (5)

The podand 4 (105mg, 0.08mmol) was stirred in ethyl acetate (10ml and methanol (10ml) with 10% Pd/C (200mg) 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 (300mg) in dry. ether (50ml) and stirred for 16 hours. The ethereal phase was decanted, the remaining solid was triturated with dry ether (3×20ml), dissolved in distilled water (20ml), and filtered through a celite pad. Freeze-drying resulted in a hygroscopic flaky solid: 70mg (76%) of 5. FT-IR (KBr) νmax 1652, 1558, 1540, 1528, 1472, 1168, 1124 cm⁻¹; ESP-MS: m/z 989 (M-3K+2H)+, 494 (M-3K+H)+. ¹H-NMR ¹H (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).
phenyl). $^{13}$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