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Syntheses of De(hydroxymethyl)desulfo Analogues of Bulgecins A, B and C

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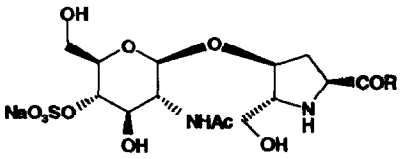
Abstract: The syntheses of de(hydroxymethyl)desulfo analogues of bulgecin A, B and C are described. Stereospecific β-glycosylation of aglycones was achieved using Schmidt's trichloroacetimidate methodology.

The bulgecins A (**1**), B (**2**) and C (**3**) are a group of *O*-sulfonated glycopeptides produced during the fermentation of *Pseudomonas acidophila* and *P. mesoacidophila.*[1] Although bulgecins themselves are devoid of antibacterial activity, in concert with β-lactam antibiotics, such as carbenicillin and sulfazecin, they induce a bulge formation in the cell wall of Gram-negative bacteria. As a result of this phenomenon the activity of these antibiotics is effectively potentiated.



1 R=$NHCH_2CH_2SO_3H$

2 R=$NHCH_2CH_2CO_2H$

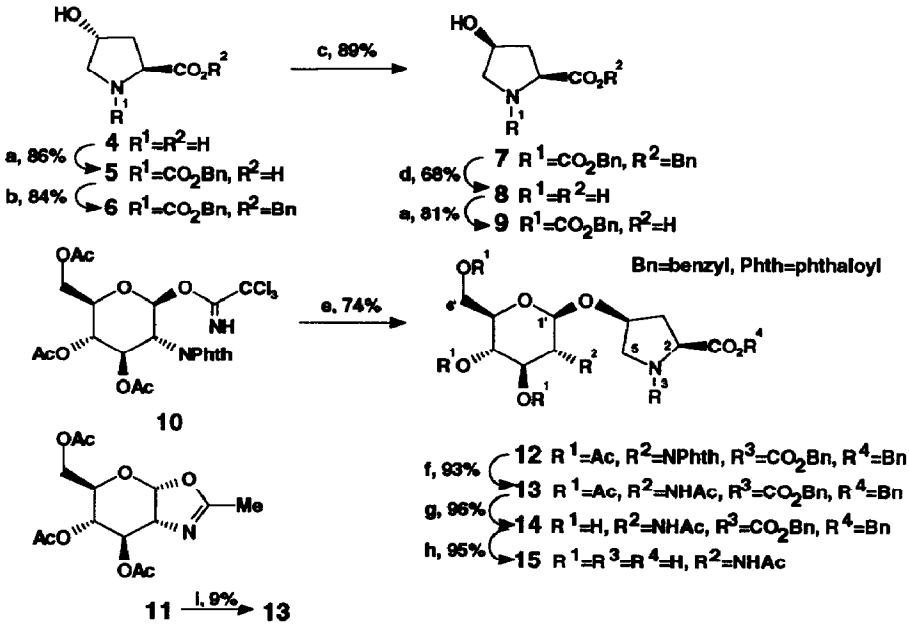
3 R=OH

Recently it has been shown in *E. coli* that the bulgecins specifically inhibit soluble lytic transglycosylase (SLT) in a noncompetitive manner.[2] This activity is believed to be responsible for the morphological effects produced by bulgecins in combination with a β-lactam agent because a similar bulge formation is observed when *slt* gene deletion mutants are treated with β-lactams. Although the specific function of SLT is undetermined, it is known to be an autolysin which cleaves the β-1,4-glycosidic bond between *N*-acetylmuramic acid and *N*-acetylglucosamine in peptidoglycan with formation of 1,6-anhydromuramic acid. It has been proposed that SLT has two distinct binding sites and that the sugar backbone of peptidoglycan initially binds to a secondary site on SLT before the enzyme can properly accomodate the bond to be cleaved in its active site.[2] Bulgecin glycopeptides, which show some structural analogy to the disaccharide subunit of peptidoglycan, might thereby bind to such a secondary site in SLT.

Preceding this discovery, a number of reports appeared relating to structure/activity of *O*-sulfonated bulgecin derivatives, both naturally isolated[3] and synthetically prepared.[4,5] In view of the current knowledge of the specific target of bulgecins and their structural similarity with the disaccharide subunit of peptidoglycan,

we now describe the syntheses of de(hydroxymethyl)desulfo analogues of bulgecins A, B and C as potential therapeutic agents.

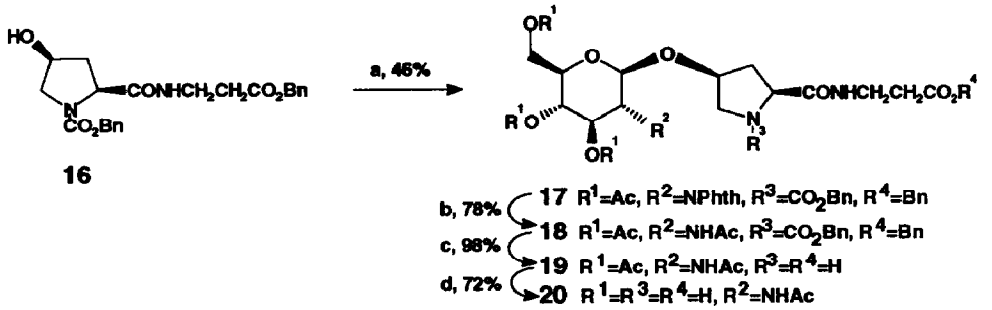
The common, commercially available starting material in these syntheses was *trans*-4-hydroxy-L-proline **4** (Scheme 1). This amino acid was protected as the carbamate **5** [6] by treatment with benzyloxycarbonyl chloride and aqueous sodium hydrogen carbonate. The carboxyl function in carbamate **5** was esterified to give the benzyl ester **6**. Inversion of the hydroxyl group in compound **6** was then carried out using the Mitsunobu procedure [7] to afford the *cis*-4-hydroxy-L-proline derivative **7** ($[\alpha]_D^{20}$ -19° {*c* 1,$CHCl_3$}) in 64% overall yield from compound **4**. Successful inversion of the hydroxyl group by this procedure was established by hydrogenolysis of compound **7** to give authentic *cis*-4-hydroxy-L-proline **8**, mp 255-6°C; $[\alpha]_D^{20}$ -58.0°(*c* 1, H_2O) (lit.[8] mp 248°C ; $[\alpha]_D^{20}$ -58.0°{*c* 2, H_2O}). We note here our observation that the ^1H NMR spectra of nearly all intermediates containing the NCO_2CH_2Ph moiety described herein are complicated by the doubling of some signals due to the existence of rotamers in such structures.



Scheme 1 **a) $PhCH_2OCOCl$, aq. $NaHCO_3$, 0°C —> rt, 3h; b) $PhCH_2Br$, K_2CO_3, DMF, 0°C, 2h; c) (i) $EtO_2CN{=}NCO_2Et$, Ph_3P, HCO_2H, THF, 0°C —> rt, 18h (ii) aq. NaOH, 1,4-dioxan, 0°C, 0.25h; d) H_2 (1 atm.), Pd/C, EtOH, rt, 2h; e) 7, $BF_3.Et_2O$, (0.2 equiv.), CH_2Cl_2, -20°C, 2h; f) (i) $N_2H_4.H_2O$ (excess), EtOH, reflux, 3h (ii) Ac_2O: pyridine (2:3), rt, 3h; g) NaOMe (0.02 equiv.), MeOH, rt, 5h; h) H_2 (1 atm), Pd/C, EtOH, rt, 4h; i) 7, pTsOH (anhydr., 0.2 equiv.), $ClCH_2CH_2Cl$, reflux, 2h.**

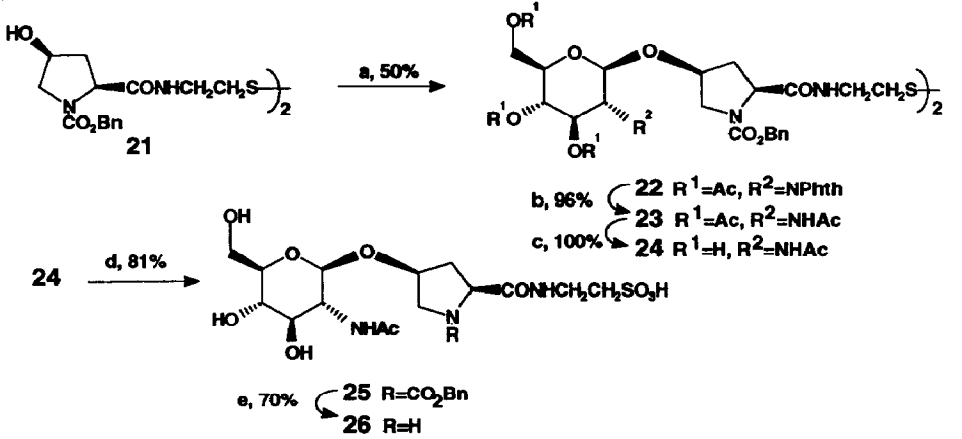
Glycosylation of the hydroxyl derivative **7** was best carried out using Schmidt's procedure with the trichloroacetimidate glycosyl donor **10** [9] under $BF_3.Et_2O$ catalysis to give, exclusively, the β-glycoside **12**. The required acetamidoglucose derivative **13** was prepared from the β-glycoside **12** by treatment with hydrazine hydrate followed by acetic anhydride in pyridine. The β-configuration of compound **13** was established from its ^1H NMR spectrum which featured a pair of doublets ($J_{1'2'}$ = 8.3 Hz for each rotamer) centred at δ 4.97 assigned to the anomeric proton in a *trans*-diaxial arrangement with the 2'-H proton. Although the acetamidoglucose derivative **13** could be prepared directly from the aglycone **7** using the oxazoline donor **11**,[10] the yield of this reaction was discouragingly low (9%). Deprotection of the β-glycoside **13** involved treatment with a catalytic amount of sodium methoxide in methanol to give *O*-deacetylated material **14** ([α

$]_D^{20}$ -25° {*c* 1, EtOH}), followed by hydrogenolysis of **14** to afford de(hydroxymethyl)desulfobulgecin C **15**[11] in 91% yield from compound **13**.



Scheme 2 **a) 10, $BF_3.Et_2O$ (0.2 equiv.), CH_2Cl_2, -20°C, 2h; b) (i) $N_2H_4.H_2O$ (excess), EtOH, reflux, 3.5h (ii) Ac_2O; pyridine (2:3), rt, 6h; c) H_2 (1 atm.), Pd/C, EtOH:H_2O (2:1), rt, 6h; d) aq.NH_3 880: MeOH (1:9$^v/_v$), rt, 21h.**

The corresponding bulgecin B derivative **20** (Scheme 2) was prepared from **9** *via* carbodi-imide coupling with β-alanine and subsequent glycosylation of the dipeptide **16** ($[\alpha]_D^{20}$ -16.4°{*c* 1,$CHCl_3$}) with the trichloroacetimidate **10**. The product **17** ($[\alpha]_D^{20}$ -10°{*c* 1,$CHCl_3$}) was smoothly converted to the acetamidoglucose compound **18** by the hydrazinolysis/acetylation procedure. However, *O*-deacetylation of compound **18** using a variety of methods (NaOMe/MeOH; NH_4OH/MeOH; KCN/MeOH[12]) successfully removed the *O*-acetyl groups but with unwanted, concomitant transesterification (R^4 = Bn—>Me). Therefore, intermediate **18** was first hydrogenolysed to give the amino acid **19** and was then *O*-deacetylated with conc. ammonium hydroxide in methanol to afford the glycopeptide **20**. [13]



Scheme 3 **a) 10 (2.1 equiv.), $BF_3.Et_2O$ (2.0 equiv.), CH_2Cl_2, -20°C →rt, 20h; b) (i) $N_2H_4.H_2O$ (excess), MeOH, reflux, 10h (ii) Ac_2O: pyridine (2:3), rt, 20h; c) aq. $NH_3$880:MeOH (1:9 $^v/_v$), rt, 16h; d) 30% H_2O_2, HCO_2H, rt, 16h; e) H_2 (1 atm.), Pd/C, H_2O, rt, 6h.**

De(hydroxymethyl)desulfobulgecin A **26** (Scheme 3) was synthesized *via* a disulphide oxidation strategy. The disulfide **21** ($[\alpha]_D^{20}$ -19.0° {*c* 1, $CHCl_3$}) was prepared in 71% yield from dicyclohexylcarbodi-imide/1-hydroxybenzotriazole-assisted coupling of the proline derivative **9** with cystamine. Treatment of the

disulfide **21** with trichloroacetimidate **10** gave the β-glycoside disulfide **22** ($[\alpha]_D^{20}$ -27° {*c* 1,$CHCl_3$}) which was converted to the corresponding acetamidoglucose compound **23** in the usual way. *O*-Deacetylation of compound **23** with ammonium hydroxide in methanol afforded an excellent yield of the polyhydroxylated disulfide **24**. Oxidation of the disulfide **24** with 30% hydrogen peroxide in formic acid [14] produced the sulfonic acid **25** which was hydrogenolysed to give the bulgecin A analogue **26**.[15]

None of the target bulgecin analogues **15, 20** or **26** showed any synergy with the β-lactam agent carbenicillin against a number of Gram-negative microorganisms. This demonstrates, in conjunction with other observations,[4] that both the sugar 4'-*O*-sulfo group and the aglycone 5-hydroxymethyl group are required for optimal synergistic activity. However, the inhibitory activity of these target compounds towards SLT has so far not been directly examined.

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11. Compound **15**: mp 194-5°C (EtOH/H_2O); $[\alpha]_D^{20}$-41° (*c* 1,H_2O); IR (KBr) 3350(br), 1660, 1605cm^{-1}; ^1H NMR (250 MHz, D_2O) δ2.03 (3H,s,Ac), 2.47-2.52 (2H,m,3-H), 3.36 (1H,dd,$J_{55'}$=12.8 Hz, J_{54}=4.2 Hz, 5-H), 3.52 (1H,d,*J*=12.8 Hz,5-H), 3.38-3.56 (3H,m,3'-H,4'-H,5'-H), 3.61 (1H,dd, $J_{2'1'}$=7.9 Hz,$J_{2'3'}$=10.0 Hz,2'-H), 3.69 (1H,dd,$J_{6'6'}$=12.4 Hz,$J_{6'5'}$=5.8 Hz,6'-H), 3.87 (1H,dd,*J*=12.4,$J_{6'5'}$=1.8 Hz,6'-H), 4.18 (1H,dd,J_{23}=5.6,8.8 Hz,2-H), 4.58 (1H,d,$J_{1'2'}$=7.9 Hz,1'-H), 4.58-4.63 (1H,m,4-H); MS (FAB) *m/z* 335 (MH^+,100%); Found: C,45.3; H, 6.7; N, 8.0. $C_{13}H_{22}N_2O_8.0.5H_2O$ requires C, 45.5; H, 6.8; N, 8.2%
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13. Compound **20**: mp 154-9°C (EtOH/H_2O); IR (KBr) 3285(br), 1654, 1560cm^{-1}; ^1H NMR (250 MHz,D_2O) δ2.02 (3H,s,Ac), 2.37 (3H,br t,*J*=6.5 Hz,3-H,β-ala CH_2), 2.59 (1H,ddd,J_{34}=5.2 Hz,J_{32}=9.9 Hz,J_{33}=14.8 Hz,3-H), 3.28-3.62 (8H,m), 3.68 (1H, dd,$J_{6'5'}$=5.4 Hz,$J_{6'6'}$=12.1 Hz,6'-H), 3.87 (1H,d,*J*=12.1,6'-H), 4.38 (1H,dd,J_{23}=4.2,9.8 Hz, 2-H), 4.54 (1H,d,$J_{1'2'}$=8.1 Hz,1'-H), 4.66 (1H,m,4-H); MS (FAB) *m/z* 406 (MH^+,28%).
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15. Compound **26**: mp 196-7°C (EtOH/H_2O); IR (KBr) 3372(br), 1670, 1560cm^{-1}; ^1H NMR (250 MHz, D_2O) δ2.04 (3H,s,Ac), 2.43 (1H,br d,J_{33}=14.8 Hz,3-H), 2.61 (1H,ddd,J_{34}=5.1 Hz,J_{32}=9.8 Hz,J_{33}=14.8 Hz,3-H), 3.05-3.11 (2H,m,taurine CH_2), 3.37-3.73 (9H,m), 3.88 (1H,d,$J_{6'6'}$=11.7 Hz,6'-H), 4.45 (1H,dd,J_{23}=4.0,9.8 Hz,2-H), 4.54 (1H,d,$J_{1'2'}$=8.1 Hz,1'-H), 4.69 (1H,m,4-H); MS (FAB) *m/z* 442 (MH^+,50%); Found: C, 39.3; H, 6.5; N, 8.8; S, 6.8. $C_{15}H_{27}N_3SO_{10}.H_2O$ requires C, 39.2; H, 6.4; N, 9.1; S, 7.0%

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