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Total synthesis of 4-(D-alanylamino)-2-amino-2,3,4-trideoxy-DL-*threo*-pentose (3-deoxy-DL-prumycin)*

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ABSTRACT

3-Deoxy-DL-prumycin (**1**) was synthesized from 2-furanmethanol (2-furfuryl alcohol, **2**) in eleven steps in 15% total yield. Michael addition of azide anion to 2,3-dideoxy-DL-pent-2-enopyranos-4-ulose (**3**) and reduction *in situ* of the adduct afforded the key intermediates **5** and **6**. Introduction of a second azido group with inversion of configuration at C-4 afforded intermediates **14** and **17**, both of which had a *threo* configuration in regard to C-2 and C-4. Coupling with D-alanine and total deprotection yielded the title compound **1**.

INTRODUCTION

Prumycin, 4-(D-alanylamino)-2-amino-2,4-dideoxy-L-arabinose, is an amino sugar antibiotic isolated in 1971 by Hata and coworkers[1,2]. Structure–activity relationship studies showed that the spatial arrangement of the functional groups at positions C-2 and C-4 is closely related with the antimicrobial activity of prumycin[3]. On the other hand, 3-deoxyprumycin[4], 4-(D-alanylamino)-2-amino-2,3,4-trideoxy-L-*threo*-pentose (**1**), exhibits an inhibitory effect against a wider spectrum of phytopathogenic strains, including *Sclerotinia sclerotiorum* and *Botrytis cinerea*, as well as an antitumor activity against P-388 leukemia similar to that of prumycin.

In a continuation of our studies on the synthesis and pharmacology of derivatives of 2,3-dideoxy-DL-pent-2-enopyranos-4-uloses[5,6], we present herein a facile synthesis of 3-deoxy-DL-prumycin, utilizing a methodology for the introduction of two amino-substituents at positions C-2 and C-4 with a *threo* configuration on the "naked sugar" **3**.

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RESULTS AND DISCUSSION

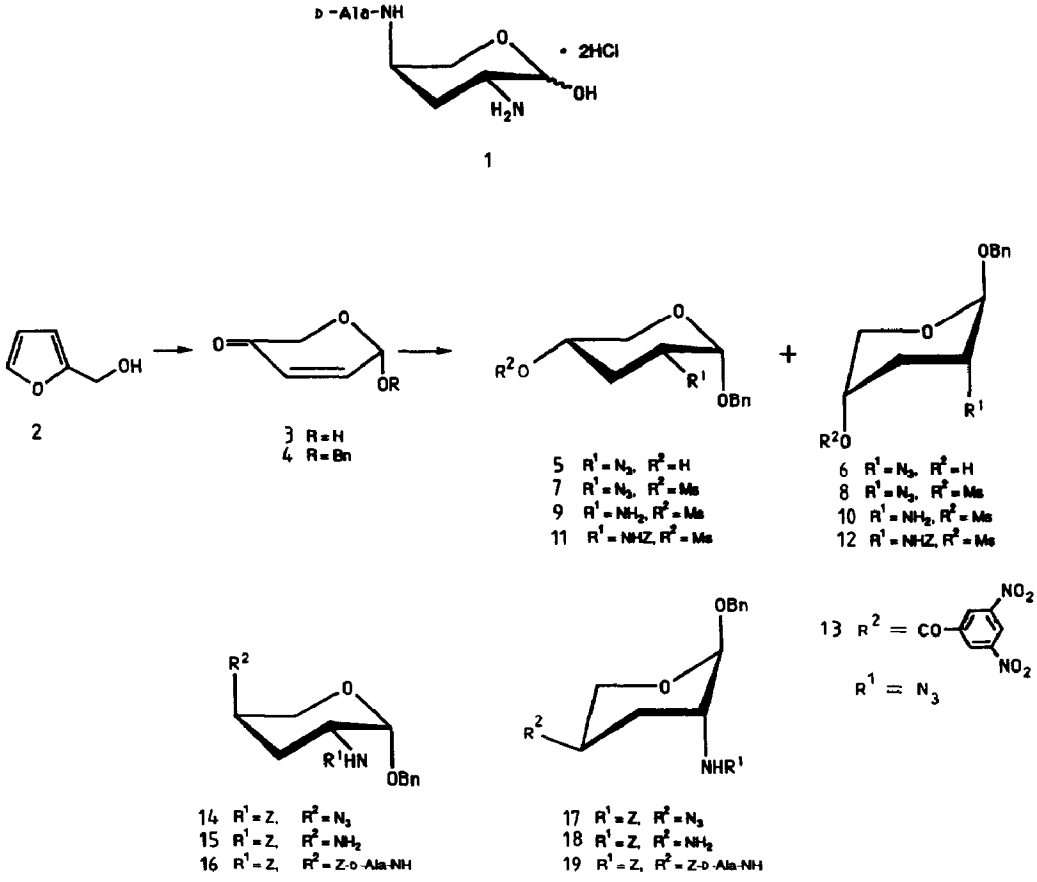
Oxidative rearrangement of 2-furanmethanol (2-furfuryl alcohol, **2**) with *m*-chloroperoxybenzoic acid (*m*-CPBA) afforded pyranulose **3** (ref. 7) in high yield. The anomeric hydroxy group was subsequently protected by conversion to the benzyl glycoside with $PhCH_2Br$–Ag_2O. Similar results were obtained using $PhCH_2OH$ in the presence of 70% $HClO_4$. Contrary to the literature[8], the direct benzylation is easily achieved in high yield. The small values for the allylic coupling constants obtained for compounds **3** and **4** indicates the axial orientation of the anomeric substituent[9]. Compound **3** (as well as its derivatives) is a racemic mixture, and, for the sake of simplicity, only one enantiomer is depicted throughout the paper.

The conversion of **4** to 2-azido-2,3-dideoxy-DL-*erythro*-pentopyranoside **5** and **6** was based on previous studies on similar hexopyranosiduloses. According to these studies[10,11], the introduction of an azide anion at position C-2 under mildly acidic conditions and prolonged reaction times occurs predominately from the thermodynamically favored side of the molecule, while a reduction *in situ* with $NaBH_4$ (ref. 11) stereoselectively affords the DL-*erythro* isomers in high yield. In our case, treatment of **4** with NaN_3 in an acetic acid–tetrahydrofuran–water mixture for 6 h at room temperature and subsequent reduction *in situ* with $NaBH_4$ produced only the DL-*erythro* compounds **5** and **6** in a 6:4 ratio.

The spin–spin splitting pattern of protons H-1, H-2 and H-4 (see Tables I and II) confirmed the α-DL-*erythro* configuration of compound **5**. The recorded values for the coupling constants $J_{2,3a}$, $J_{4,3a}$, and $J_{4,5a}$ (11.1, 9.3, and 9.3 Hz, respectively) were in agreement with the expected *trans*-diaxial orientation of protons H-2 and H-4. The assignments of chemical shifts of all the carbon atoms (Table III) were determined by the heteronuclear ^1H–^{13}C 2D COSY (360 MHz, $CDCl_3$) shift-correlation technique, while the assignments of chemical shifts of the protons were determined by decoupling experiments.

The assignment of the configuration of isomer **6** from the ^1H-n.m.r. spectrum is not as simple as that for **5** because of the interconverting chair conformers of this compound. The assignments can be inferred, however, from the differences obtained from the coupling constants in different solvents and temperatures (Tables II and IV). Comparison of the coupling constants $J_{2,3a}$, $J_{4,3a}$, and $J_{4,5a}$ (6.4, 5.0, and 4.8 Hz, respectively) of **6** in $CDCl_3$ at room temperature with the corresponding coupling constants of **5** suggested the equatorial orientation of protons H-2 and H-4. The ^{13}C-n.m.r. spectra showed that the C-5 signal of compound **5** is shifted upfield (Table III) relative to the corresponding signal of compound **6**, indicating β-DL-configuration of the latter. This is a result of the steric compression of a γ-gauche interaction. The homonuclear ^1H–^1H 2D COSY (500 MHz, CD_2Cl_2) spectrum of **13** indicated a long-range coupling constant $J_{H4,H1}$ which is in agreement with the usually observed zig-zag arrangement[12] for the *trans*-oriented 1,4 protons. Accordingly, the proposed configuration for **6** is β-DL-*erythro*.

Since column chromatographic separation of isomers **5** and **6** was tedious, we



Bn = benzyl
Ms = methanesulfonyl
Z = benzyloxycarbonyl

Scheme 1

carried out the reaction sequence from **4** to **14** (and **17**) working with both isomers, performing one final separation of the diastereomeric mixture at that stage.

Mesylation of the mixture of **5** and **6**, followed by catalytic hydrogenation in methanol using 10% Pd/C as a catalyst, afforded amines **9** and **10** in satisfactory yield. Treatment of **9** and **10** with benzyloxycarbonyl chloride yielded the key intermediates **11** and **12** in an overall yield of 84% from **5** and **6**.

The introduction of the second group at C-4 was easily achieved by inversion of the configuration *via* heating **11** and **12** with sodium azide in hexamethylphosphoric triamide. The resulting mixture, after separation by column chromatography (85:15:0.5 hexane–ethyl acetate–triethylamine) yielded compounds **14** and **17** in an overall yield of 36% and 24% starting from **4**.

The ^1H–^{13}C 2D n.m.r. heteronuclear spectrum using the DEPT pulse sequence for

TABLE I

^{1}H-N-m.r. chemical shift data[a]

Compound	*H-1*	*H-2*	*H-3e*	*H-3a*	*H-4*	*H-5e*	*H-5a*	*PhCH$_2$*	*Other*
1	*b*	3.13	2.19	1.90	3.96	3.68	3.46		1.36(CH_3), 3.90(C*H*CH_3)
5	4.67	3.12	2.00	1.90	3.65	3.51	3.45	4.72, 4.50	2.64(OH), 7.25(Ph)
6	4.62	3.60	2.24	1.75	3.77	3.97	3.48	4.82, 4.56	2.78(OH), 7.37(Ph)
8	4.59	3.55	2.42	1.98	4.79	4.12	3.70	4.81, 4.60	3.05(Ms), 7.40(Ph)
13[c]	4.74	3.69	2.48	2.06	5.19	4.17	3.78	4.83, 4.62	9.21, 7.39(Ph)
14	4.86	4.17	2.02	1.92	3.75	3.86	3.62	4.72, 4.49	4.93(NH), 5.08(Z)[d], 7.32(Ph)
16	4.80	4.10	1.90	1.90	4.10	3.65	3.40	4.70, 4.50	1.40(CH_3)[e], 4.00(C*H*CH_3), 5.10(Z)[d]
17	4.62	3.98	2.08	2.08		— 3.65 —		4.79, 4.50	5.09(NH), 5.09(Z)[d], 7.32(Ph)
19	4.90	4.10	1.75	2.10	4.10	3.70	3.50	4.70, 4.50	1.40(CH_3)[e], 4.00(C*H*CH_3), 5.10(Z)[d]

[a] Spectra were determined at 360 MHz in $CDCl_3$. Shifts are reported in δ p.p.m. [b] Spectrum taken of the anomeric mixture. Chemical shifts for the anomeric proton: 5.12 p.p.m. for the α anomer and 4.71 p.p.m. for the β anomer. [c] Measured in CD_2Cl_2. [d] Resonance for PhCH_2O(C=O) . [e] A pair of doublets observed attributed to the mixture (1:1) of D-Ala-D-sugar and D-Ala-L-Sugar.

TABLE II

^{1}H-N.m.r. spin–spin coupling data[a]

Compound	$J_{1,2}$	$J_{2,3a}$	$J_{2,3e}$	$J_{3a,3e}$	$J_{4,3a}$	$J_{4,3e}$	$J_{4,5a}$	$J_{4,5e}$	$J_{5a,5e}$
1	*b*	—	—	—	—	—	—	—	
5	3.0	11.1	4.3	11.9	9.3	4.7	9.3	4.7	10.7
6	3.9	6.4	4.1	13.9	5.0	4.1	4.8	2.9	11.5
8	4.5	7.0	4.5	14.0	7.0	4.0	5.5	3.0	12.5
13	3.5	5.5	4.2	14.2	5.5	4.2	4.3	2.9	12.5
14	3.2	11.9	*c*	13.7	3.2	*c*	*c*	2.0	12.6
16	3.5	*d*	—	—	—	—	—	—	—
17	<1.0	—	—		(— — —)[e]		—	—	—
19	<1.0	—	—	—	—	—	—	—	—

[a] Spectra were determined at 360 MHz in $CDCl_3$. Coupling constants are given in Hz.
[b] Spectrum taken of the anomeric mixture. Calculated coupling constants for the anomeric proton: $J_{1,2}$ 2.9 Hz for the α anomer and $J_{1,2}$ 7.5 Hz for the β anomer.
[c] $J_{4,3a} + J_{4,3e} + J_{4,5a} + J_{4,5e} \simeq 8$ Hz; $J_{1,2} + J_{2,3a} + J_{2,3e} + J_{2,NH} \simeq 28$ Hz.
[d] Broad peak.
[e] $J_{4,3a} + J_{4,3e} + J_{4,5a} + J_{4,5e} \simeq 27$ Hz; $J_{1,2} + J_{2,3a} + J_{2,3e} + J_{2,NH} \simeq 16$ Hz.

TABLE III

^{13}C-N.m.r. chemical shifts data[a]

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*CH_2(Bn)*	*NHCO*	*CH_2 (Z)[b]*
5	95.9	56.5	32.0	64.8	64.2	69.2		
6	98.2	57.8	31.5	64.2	66.2	69.7		
14	96.0	45.3	29.2	56.1	60.2	69.4	155.4	66.8
17	96.5	48.7	29.3	52.2	61.5	69.1	155.2	66.9

[a] Spectra were determined at 90 MHz in $CDCl_3$. Shifts are reported in δ p.p.m. [b] PhCH_2O(C=O)–.

TABLE IV

^{1}H-N.m.r. spin–spin coupling data (Hz) for **6** in CD_2Cl_2

Temp.°	$J_{1,2}$	$J_{2,3a}$	$J_{2,3e}$	$J_{4,3a}$	$J_{4,3e}$	$J_{4,5a}$	$J_{4,5e}$
10	4.8	8.8	2.8	6.8	4.1	5.6	3.1
−10	4.8	7.2	4.4	6.9	4.1	5.6	2.4
−30	6.1	—[a]	—	—	—	—	—

[a] Broad peak.

compound **14** indicated that the H-4 proton at δ_H 3.75 p.p.m. is coupled with the signal at δ_C 56.1 p.p.m. assigned to C-4. The H-5*e* and H-5*a* at δ_H 3.86 and 3.62 p.p.m., respectively, are coupled with the signal at δ_C 60.2 p.p.m., assigned to C-5. The methylene protons of the benzyl group at δ_H 4.72 and 4.49 p.p.m. are coupled with the signal at δ_C 69.4 p.p.m., whereas the methylene protons of the benzyloxycarbonyl group at 5.08 p.p.m. are coupled with the signal at δ_C 66.8 p.p.m. Subsequently, the homonu-

clear ^{1}H–^{1}H 2D COSY spectrum (360 MHz, $CDCl_3$) of **14** revealed that the signal at δ_H 4.93 p.p.m. (assigned to NH) is coupled with the signal at δ_H 4.17 p.p.m. assigned to H-2, which in turn is coupled with the signal at δ_H 4.86 p.p.m. (assigned to H-1). The long-range coupling of $J_{H4,H1}$ suggests the *trans* relationship between H-4 and H-1. The ^{1}H-n.m.r. and ^{13}C-n.m.r. spectra (Tables I, II and III) of compounds **14** and **17** are in agreement with the *β*- and α-DL-*threo* structures, respectively, which are depicted in Scheme 1. The signals for H-2 and H-4 for compounds **14** and **17** are not well resolved; however, the sum of their coupling constants with all neighbouring protons reveals their orientation. Thus H-4 is equatorial in compound **14** ($J_{4,3a} + J_{4,3e} + J_{4,5a} + J_{4,5e} = 8$ Hz) and axial in compound **17** ($J_{4,3a} + J_{4,3e} + J_{4,5a} + J_{4,5e} = 27$ Hz). On the contrary, H-2 is axial in compound **14** ($J_{1,2} + J_{2,3a} + J_{2,3e} + J_{2,NH} = 28$ Hz) and equatorial in compound **17** ($J_{1,2} + J_{2,3a} + J_{2,3e} + J_{2,NH} = 16$ Hz), (see Table II).

Since both diastereoisomers **14** and **17** have the DL-*threo* configuration in regard to C-2 and C-4, deprotection of the anomeric hydroxy group afforded identical compounds. In order to clarify any ambiguities on the assigned structures, compounds **14** and **17** were worked up separately. The reduction of the azide **14** under conditions of kinetic control[13], resulted in the desired monoprotected 2,4-diamino-2,3,4-trideoxy-*β*-DL-*threo*-pentopyranoside **15**. Accordingly, the isomeric diamine **18** was obtained from **17**.

The mixed carbonic anhydride method[14] was found to be the best method for coupling *N*-benzyloxycarbonyl-D-alanine with the amino sugar **15**. On the other hand, the method using *N*-*N'*-dicyclohexylcarbodiimide gave a considerable amount of the *N*-acylurea as by-product, while the combination of *N*,*N'*-dicyclohexylcarbodiimide–*N*-hydroxysuccinimide[15] did not remarkably increase the yield. Thus, optimum coupling conditions gave amide **16** in 75% yield. Amide **19** was prepared in the same yield from **18** using the carbonic anhydride method for coupling. Compounds **16** and **19** show by n.m.r. spectroscopy (Table I) a 1:1 diastereomeric mixture, although only one spot is observed by t.l.c. Finally, hydrogenolysis of **16** or **19** in 5:1 acetic acid–water in the presence of 10% Pd/C and treatment of the product with 0.1N HCl afforded the dihydrochloride of compound **1** as a mixture of anomers (4:3 α:*β*).

Preliminary antifungal tests showed that free sugar **1**, derived either from **16** or **19** had the same activity against *Botrytis cinerea*. By the demonstrated procedure, which was free of tedious chromatographic separations, 3-deoxy-DL-prumycin was synthesized from 2-furanmethanol (**2**) in 15% total yield

EXPERIMENTAL

General Methods — Melting points were determined on a Büchi micro melting point apparatus and are uncorrected. Specific rotations were determined with a Perkin–Elmer 141 polarimeter using a 10-cm cell. N.m.r. spectra were recorded on a Varian EM 360 (60 MHz), a Bruker AM 360 (360 MHz), or Bruker AM 500 (500 MHz) spectrometer in deuteriochloroform containing tetramethylsilane as the internal reference, unless otherwise noted. I.r. spectra were recorded with a Perkin–Elmer 283B spectrophotom-

eter. Column chromatography was performed using silica gel. Freshly distilled 2-furanmethanol, methanesulfonyl chloride, isobutyl chloroformate, tetrahydrofuran, and acetone were used. *N*-Methylmorpholine was distilled from ninhydrin. Freshly prepared silver oxide was dried under vacuum for 24 h before use. Elemental analyses were carried out at the University of Thessaloniki.

*Preparation of 2,3-dideoxy-α,β-*DL*-pent-2-enopyranos-4-ulose (3).* — To a cold (0° bath), stirred solution of freshly distilled 2-furanmethanol (**2**) (60 g, 0.61 mol) in dichloromethane (1.2 L), was added portionwise *m*-chloroperoxybenzoic acid (120 g, 0.70 mol). The temperature was maintained between 10 and 15° during the addition. After stirring for 2 h at room temperature, the mixture was cooled to −10°, and the solid was filtered. The filtrate was concentrated under reduced pressure to one-third of its volume and again cooled to −10°. After removal of the solid that formed as before, hexane was added, and the precipitate was filtered, affording pure product **3** (61 g, 88%): m.p. 58° (lit.[1] 58–9°); υ_{max}^{KBr} 3400 (OH), 1700, 1685 (C=O), 1625 (C=C) cm^{-1}; ^{1}H-n.m.r. (60 MHz, $CDCl_3$): δ 5.50 (dd, 1 H, $J_{1,2}$ 3 Hz, $J_{1,3}$ 0.2 Hz, H-1), 6.80 (dd, 1 H, $J_{2,3}$ 10 Hz, H-2), 6.05 (d, 1 H, H-3), 4.55 (d, 1 H, $J_{5a,5e}$ 16 Hz, H-5*a*), 4.15 (d, 1 H, H-5*e*), 4.10 (OH).

*Preparation of benzyl 2,3-dideoxy-α,β-*DL*-pent-2-enopyranosid-4-ulose* (**4**). — *Method A.* To a cold solution of **3** (15 g, 0.13 mol) in anhydrous acetone (170 mL) were added Ag_2O (36 g, 0.15 mol) and $PhCH_2Cl$ (21.3 mL, 0.20 mol). The mixture was stirred at room temperature overnight, filtered through Celite, and evaporated to dryness. Compound **4** (11.8 g, 45%) was obtained as a colorless oil after distillation; b.p. 115°/0.3 torr (lit.[8] 115°/0.3 torr); υ_{max}^{KBr} 1700 (C=O), 1625 (C=C) cm^{-1}; ^{1}H-n.m.r. (60 MHz, $CDCl_3$), δ 5.10 (dd, 1 H, $J_{1,2}$ 3.5 Hz, $J_{1,3}$ 0.2 Hz, H-1), 6.65 (dd, 1 H, $J_{2,3}$ 10 Hz, H-2), 5.90 (d, 1 H, H-3), 4.45 (d, 1 H, $J_{5a,5e}$ 16 Hz, H-5*a*), 4.15 (d, 1 H, H-5*e*), 4.70, and 4.50 (ABq, 2 H, CH_2Ph).

Method B. To a solution of **3** (5.70 g, 0.049 mol) in acetone (25 mL) were added $PhCH_2OH$ (10 mL, 0.142 mol) and 70% $HClO_4$ (4.3 mL, 0.049 mol). The mixture was stirred for 5 min at room temperature and then poured into a saturated solution of $NaHCO_3$. The mixture was extracted with Et_2O, and the organic layer was washed to neutrality, dried ($MgSO_4$), and evaporated under reduced pressure. Pure product **4** (5.2 g, 51%) was obtained by distillation as described above. The product was identical by i.r. and n.m.r. spectroscopy with that obtained by Method A.

*Benzyl 2-azido-2,3-dideoxy-α- and β-*DL*-*erythro*-pentopyranosides* (**5**) *and* (**6**). — To an ice-cooled and stirred solution of **4** (5 g, 0.024 mol) in tetrahydrofuran (100 mL) and acetic acid (40 mL) was added dropwise a solution of sodium azide (7 g, 0.108 mol) in water (20 mL). The mixture was stirred at room temperature for 6 h and then cooled to 0°. Sodium borohydride was added in portions until the reduction (monitored by t.l.c.) was complete. The mixture was extracted with CH_2Cl_2. The organic layer was washed with 10% $NaHCO_3$, water, dried ($MgSO_4$), and evaporated under reduced pressure, yielding an oil which was used without further purification in the next step. Column chromatography on silica gel (1:100) with 7:3 ether–hexane gave **5** (3.9 g, 64%) and **6** (2.2 g, 36%). Compound **5**: υ_{max}^{KBr} 3400 (OH), 2100 (N_3) cm^{-1}. See Tables I and II for

^{1}H-n.m.r. and Table III for ^{13}C-n.m.r. data. Compound **6**: υ_{max}^{KBr} 3400 (OH), 2100 (N_3) cm^{-1}. See Tables I, II and IV for ^{1}H-n.m.r. and Table III for ^{13}C-n.m.r. data.

*Benzyl 2-azido-2,3-dideoxy-4-*O*-methanesulfonyl-α- and -β-*DL-erythro-*pentopyranosides* (**7**) and (**8**). — To an ice-cold solution of **5** and **6** (5.5 g, 0.022 mol) in CH_2Cl_2 (40 mL) and Et_3N (3.0 mL, 0.04 mol) was added methanesulfonyl chloride (3 mL, 0.036 mol), and the mixture was stirred for 4 h at 0°. The mixture was washed successively with 10% $NaHCO_3$, water, 10% NH_4Cl, water, dried ($MgSO_4$), and evaporated to dryness under reduced pressure. Et_2O was added, and the solid was filtered to yield a mixture of compounds **7** and **8** (6.9 g, 95%): υ_{max}^{KBr} 2100 (N_3), 1360, 1170 (Ms) cm^{-1}. See Tables I and II for ^{1}H-n.m.r. data for compound **8**.

Anal. Calc. for $C_{13}H_{17}N_3O_5S$: C, 47.70; H, 5.23; N, 12.84. Found: C, 47.88; H, 5.29; N, 13.01.

*Benzyl 2-azido-2,3-dideoxy-4-*O*-(3,5-dinitrobenzoyl)-β-*DL-erythro-*pentopyranoside* (**13**). To an ice-cold solution of **6** (300 mg, 1.2 mmol) in pyridine (4 mL) was added 3,5-dinitrobenzoyl chloride (500 mg, 2 mmol), and the mixture was stirred for 4 h at 0°. The mixture was poured into ice, and the product was extracted with chloroform. Recrystallization from EtOAc–hexane afforded compound **13** (443 mg, 85%); m.p. 87°; υ_{max}^{KBr} 2120 (N_3), 1740 (C–O), 1555, 1350 (NO_2) cm^{-1}; See Tables I and II for ^{1}H-n.m.r. data.

Anal. Calc. for $C_{19}H_{17}N_5O_8$: C, 52.66; H, 3.95; N, 1616. Found: C, 52.83; H, 3.78; N, 15.98.

*Benzyl 2-(benzyloxycarbonyl)amino-2,3-dideoxy-4-*O*-methanesulfonyl-α- and -β-*DL-erythro-*pentopyranosides* (**11**) and (**12**). — A solution of **7** and **8** (6.0 g, 0.018 mol) in anhydrous MeOH (250 mL) was hydrogenated in the presence of 10% Pd/C catalyst (0.5 g). (Caution: pyrophoric mixture.) After 45 min the catalyst was removed by filtration, and the solvent was evaporated under reduced pressure, yielding a residue which was used directly for the next step: υ_{max}^{KBr} 3380 (NH_2), 1360, 1180 (Ms) cm^{-1}. To the above residue (4.11 g, 0.0136 mol), dissolved in 1,4-dioxane (40 mL), was added under cooling and stirring a solution of $NaHCO_3$ (2.22 g, 0.0264 mol) in water (60 mL), followed by benzyl chloroformate (3.7 ml, 0.0260 mol). The mixture was stirred for 1 h at room temperature and then poured onto ice (300 mL). The resulting solid was filtered and washed with water and Et_2O yielding compound **11** and **12** (5.8 g, 99%): υ_{max}^{KBr} 3360 (NH), 1715 (Z), 1360, 1180 (Ms) cm^{-1}.

Anal. Calc. for $C_{21}H_{25}NO_7S$: C, 57.92; H, 5.79; N, 3.22. Found: C, 58.11; H, 5.59; N, 3.30.

*Benzyl 4-azido-2-(benzyloxycarbonyl)amino-2,3,4-trideoxy-α- and -β-*DL-threo-*pentopyranosides* (**14**) *and* (**17**). — To a stirred solution of **11** and **12** (4.5 g, 0.01 mol) in hexamethylphosphoric triamide (10 mL) was added NaN_3 (1.4 g, 0.02 mol). The mixture was heated for 7–8 h at 80°, then cooled to room temperature and poured onto ice–water (200 mL). The resulting oil, after decantation, was dissolved in EtOAc, hexane was added, and the solid which formed was filtered off. The product was chromatographed on a silica gel column (1:100) with 15:85:0.5 EtOAc–hexane–Et_3N yielding **14** (1.2 g, 40%) and **17** (1.8 g, 60%). Compound **14**: m.p. 75°; υ_{max}^{KBr} 3320 (NH), 2100 (N_3), 1690 (Z) cm^{-1}. See Tables I and II for ^{1}H-n.m.r. and Table III for ^{13}C-n.m.r. data.

Anal. Calc. for $C_{20}H_{22}N_4O_4$: C, 62.82; H, 5.80; N, 14.64. Found: C, 62.96; H, 5.92; N, 14.47.

Compound **17**: m.p. 65°; υ_{max}^{KBr} 3320 (NH), 2100 (N_3), 1690 (Z) cm^{-1}. See Tables I and II for ^{1}H-n.m.r. and Table III for ^{13}C-n.m.r. data.

Anal. Calc. for $C_{20}H_{22}N_4O_4$: C, 62.82; H, 5.80; N. 14.64. Found: C, 63.02; H, 5.78; N, 14.46.

*Benzyl 4-(*N*-benzyloxycarbonyl-*D*-alanylamino)-2-(benzyloxycarbonyl)amino-2,3,4-trideoxy-β-*DL*-threo-pentopyranoside* (**16**). — To a mixture of 10% Pd/C (10 mg) in 1,4-dioxane (8 mL) and Et_3N (8 mL) was added compound **14** (100 mg, 0.26 mmol) at room temperature, and hydrogen was bubbled into the mixture for 15 min. The catalyst was filtered, and the filtrate was evaporated to dryness. The resulting free amine **15** was directly used for coupling as follows: *Method A*. To a stirred solution of *N*-benzyloxycarbonyl-D-alanine (80 mg, 0.36 mmol) in THF (10 mL) at −10°, was added *N*-methylmorpholine (0.04 mL, 0.37 mmol), followed by isobutyl chloroformate (0.05 mL, 0.37 mmol). After 5 min a precooled solution of **15** (103 mg, 0.29 mmol) in THF (4 mL) was slowly added, and the reaction mixture was stirred for 30 min at −10°, then overnight at room temperature. The solvent was removed under reduced pressure, and the resulting residue was dissolved in CH_2Cl_2. The organic phase was washed consecutively with water, N HCl, water, 5% aq. $KHCO_3$, water, dried ($MgSO_4$), evaporated, and chromatographed with 9.5:0.5 $CHCl_3$–MeOH to give **16** (122 mg, 75%): m.p. 44°; $[\alpha]_D^{20}$ −16.2° (*c* 1.95, $CHCl_3$); υ_{max}^{KBr} 3320 (NH), 1715 (Z), 1690 (NHCO) cm^{-1}. See Tables I and II for ^{1}H-n.m.r. data.

Anal. Calc. for $C_{31}H_{35}N_3O_7$: C, 62.82; H, 5.80; N. 14.64. Found: C, 62.96; H, 5.62; N, 14.47.

Method B. To a stirred solution of **15** (200 mg, 0.55 mmol), *N*-benzyloxycarbonyl-D-alanine (220 mg, 1 mmol), and *N*-hydroxysuccinimide (150 mg, 1.3 mmol) in tetrahydrofuran (3 mL) was added *N,N'*-dicyclohexylcarbodiimide (230 mg, 1.12 mmol) at −5°. The mixture was stirred overnight at room temperature. The solid which formed was filtered off, the filtrate was evaporated to dryness, and the resulting residue was dissolved in dichloromethane. Isolation and purification of the product was carried out as described in Method A to give **16** (174 mg, 55%).

*Benzyl 4-(*N*-benzyloxycarbonyl-*D*-alanylamino)-2-(benzyloxycarbonyl)amino-2,3,4-trideoxy-α-*DL*-threo-pentopyranoside* (**19**). — Compound **17** (55 mg, 0.14 mmol) was hydrogenated as described for compound **14**, and the resulting free amine **18** was coupled with *N*-benzyloxycarbonyl-D-alanine (50 mg, 0.23 mmol) as described for compound **15** in Method A, yielding **19** (60 mg, yield 74%): m.p. 42°; $[\alpha]_D^{20}$ −33.7° (*c* 0.5, $CHCl_3$); υ_{max}^{KBr} 3320 (NH), 1715 (Z), 1690 (NHCO) cm^{-1}. See Tables I and II for ^{1}H-n.m.r. data.

*Preparation of 4-(*D*-alanylamino)-2-amino-2,3,4-trideoxy-*DL*-threo-pentopyranose dihydrochloride* (**1**). — To a stirred solution of **16** (118 mg, 0.22 mmol) dissolved in HOAc (10 mL) and water (2 mL) was added 10% Pd/C (118 mg). Hydrogen was bubbled through the mixture for 20 h at room temperature. The catalyst was filtered off, the filtrate was evaporated, and 0.1N HCl (10 mL) was added. The solution was

evaporated below 30°, and the resulting hygroscopic solid was lyophilized (46 mg, 84%): m.p. 145–147° (dec); [lit.[4]: m.p. 145–152° (dec)]; $[\alpha]_D^{20}$ −3.6° (*c* 0.65, H_2O); [lit.[4]: $[\alpha]_D$ −14° (*c* 0.69, H_2O]; υ_{max}^{KBr} 3600–2700 (NH_3^+), 1680 (NHCO) cm^{-1}.

Compound **19** gave, upon treatment as in the foregoing, product **1** in 86% yield: m.p. 145–147° (dec); $[\alpha]_D^{20}$ −3.4° (*c* 0.65, H_2O); υ_{max}^{KBr} 3600–2700 (NH_3^+), 1680 (NHCO) cm^{-1}. See Table I and II for ^{1}H-n.m.r. data.

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REFERENCES

1 T. Hata, S. Ohmura, M. Katagiri, K. Atsumi, J. Awaya, S. Higashikawa, K. Yasui, H. Terada, and S. Kuyama, *J. Antibiot.*, 24 (1971) 900–901.
2 S. Ohmura, M. Katagiri, K. Atsumi, T. Hata, A. A. Jukubowski, E. B. Springs, and M. Tishler, *J. Chem. Soc., Perkin Trans. 1*, (1974) 1627–1631.
3 H. Hashimoto, K. Araki, and J. Yoshimura, *Nippon Kagaku Kaishi*, (1981) 805–812.
4 H. Hashimoto, K. Araki, K-I. Miyazawa, and J. Yoshimura, *Carbohydr. Res.*, 99 (1982) 59–69.
5 M. P. Georgiadis, *J. Med. Chem.*, 19 (1976) 346–349.
6 M. P. Georgiadis, in H. van der Goot, G. Domany, L. Pallos, and H. Timmerman (Eds), *Proc. Xth Int. Symp. Med. Chem.*, Elsevier, 1989, pp. 197–215.
7 R. Laliberte, G. Medawar, and Y. Lefebvre, *J. Med. Chem.*, 16 (1973) 1084–1089.
8 G. Grynkiewicz, B. Barszczak, and A. Zamojski, *Synthesis*, (1979) 364.
9 O. Achmatowicz, JR., P. Bukowski, B. Szechner, Z. Zwierzchowska, and A. Zamojski, *Tetrahedron*, 27 (1971) 1973–1996.
10 J. Cleophax, S. Gero, and J. Leboul, *J. Chem. Soc., Chem. Commun.*, (1973) 710–711.
11 M. P. Georgiadis, and E. A. Couladouros, *J. Heterocyclic. Chem.*, 28 (1991) 1325–1337.
12 Atta-Ur-Rahman, *Nuclear Magnetic Resonance*, Springer-Verlag, Berlin, 1986, pp. 85.
13 A. Hasegawa, N. Aritake, and M. Kiso, *Carbohydr. Res.*, 52 (1976) 137–149.
14 J. Meienhofer, in E. Gross and J. Meienhofer (Eds.), *The Peptides*, Vol. 1, Academic Press, New York, 1979, pp. 263–314.
15 E. Wunsch and F. Dress, *Chem. Ber.*, 99 (1966) 110–120.