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THE SYNTHESIS OF 3,6-DIAMINO-2,3,4,6-TETRADEOXY-DL-THREO-HEXOPYRANOSE DERIVATIVES, SUBSTRATES FOR THE SYNTHESIS OF NEGAMYCIN†

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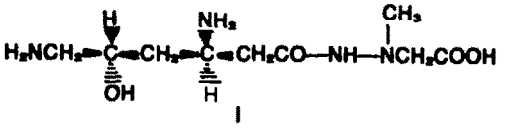
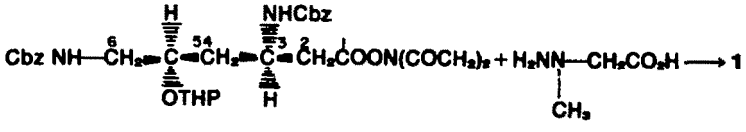
Abstract—Three synthetic routes to derivatives of 3,6 - diamino - 2,3,4,6 - tetradeoxy - DL - *threo* - hexopyranose were investigated. Addition of sodium azide in acetic acid to 6 - phthalimido - 5,6 - dihydro - 2 - pyrone gave 4-azido compound (7) of the *erythro* configuration. From methyl 2,4 - dideoxy - β - DL - *erythro* - hexopyranoside *threo* 4 - phthalimido - 6 - phthalimidomethyl - tetrahydro - 2 - pyrone (17) was obtained in three steps in low overall yield. Addition of sodium azide in acetic acid to butyl 6 - oxo - 2 - hydroxy - hex - 4 - enoate followed by methylation, amonolysis of the ester group, and reduction gave methyl 3,6 - diacetamido - 2,3,4,6 - tetradeoxy - α - DL - threo - hexopyranoside (26).

Negamycin (1), an antibiotic isolated from *Streptomyces purpeofuscus*,[1] is highly active against a broad spectrum of Gram-negative bacteria.[2] The structure of 1 was determined as N^1 - methyl - N^2 - (3R,6 - diamino - 5R - hydroxyhexanoyl) - hydrazinoacetic acid.[1]



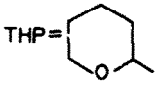
1

Negamycin was synthesized by condensation of N - hydroxysuccinimide ester of 3R,6 - di - N - benzyloxycarbonylamino - 5R - tetrahydropyranyloxyhexanoic acid (2) with N^1 - methyl - hydrazino - acetic acid (3)[3]:

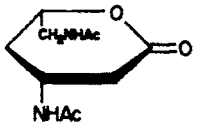


2 3

Cbz = C_6H_5OCO—



Compound 2 was, in turn, obtained from 3R,6 - diacetamido - 5*R* - hydroxy - hexanolactone (4).[3]



4

The other enantiomeric form of 1 was prepared in a similar way employing the antipode of 4;[3] the final product was *ca* 30 times less active than the original antibiotic. An aza analogue of 1, in which the C-3 methine group was replaced by a N atom was also synthesized;[4] the product was devoid of any antibacterial activity against several Gram-positive and Gram-negative bacteria even at a concentration of 200 μg/ml.

In view of the high activity and relatively simple structure of 1 we became interested in elaboration of a synthetic access to 4 (or its precursor 26) which is the key substrate for the preparation of 1.

Shibahara *et al.*[3] obtained lactone 4 from D-galacturonic acid in *ca* a 13-stage synthesis. The enantiomer of 4 was prepared from 3 - amino - 3 - deoxy - D - glucose in 11 steps.[3]

Our plan of synthesis was based on 6 - hydroxymethyl - 2 - methoxy - 5,6 - dihydro - 2H - pyran (5)[5] which was readily converted into 5,6 - dihydro - 6 - phthalimidomethyl - 2 - pyrone (6).[6] The simplest synthesis which could be devised is shown in Chart 1.

Compound 7 was obtained in high yield (*ca.* 80%) as a single stereoisomer of the undesired *erythro* configuration. Essentially the same stereochemical outcome was noticed on azide ion addition to 6 - acetoxymethyl - 5,6 - dihydro - 2 - pyrone (8) and to 6 - butoxycarbonyl - 5,6 - dihydro - 2 - pyrone (9): unstable 4-azido compounds (10 and 11, respectively) of the *erythro* configuration were the preponderant products of these additions. These results

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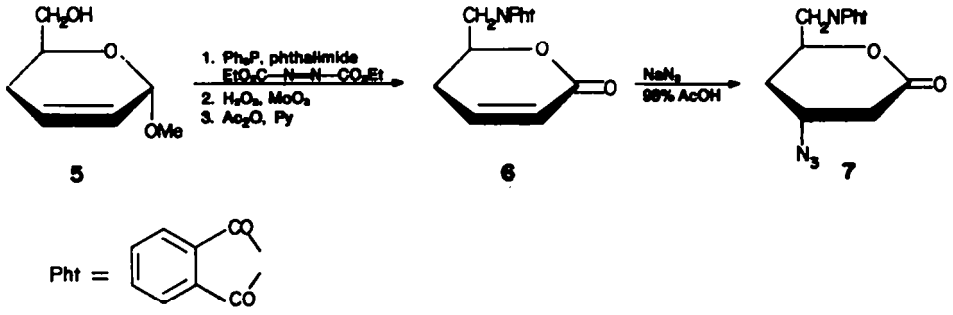
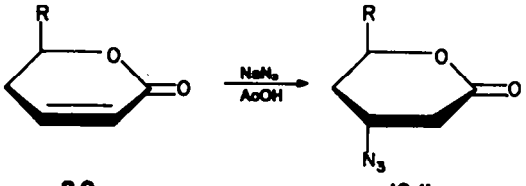


Chart 1.[a]

are in agreement with the conclusion that Michael type addition to 6-membered cyclic compounds proceed with axial attack of the entering anion.[7]



8,9 10,11

8, 10: R = CH_2OAc
9, 11: R = CO_2Bu

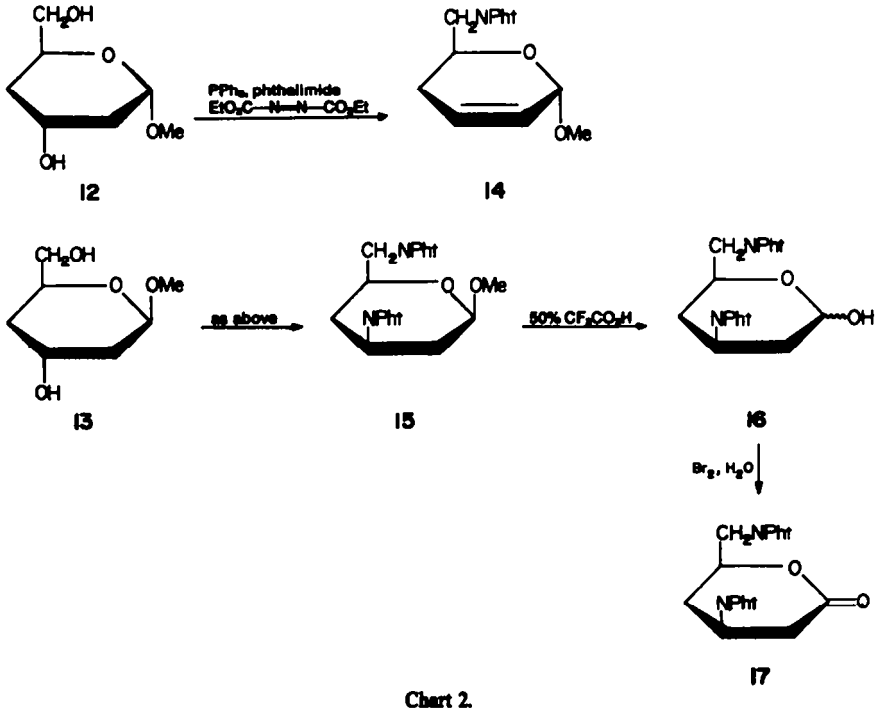
The next synthesis, leading to 17 (an analogue of lactone 4) started from methyl 2,4 - dideoxy - α - and β - DL - *erythro* - hexo - pyranosides (12 and 13).[8] Direct exchange of the OH groups at C-3 and C-6 against phthalimido residues according to Mitsunobu[9] succeeded for 13 only; 3,6 - dideoxy - 3,6 - diphthalimido derivative 15 of the *threo* configuration was obtained in 69% yield. In the case of α anomer 12 substitution at C-6 was accompanied by an elimination reaction leading to 2 - methoxy - 6 - phthalimidomethyl - 5,6 - dihydro - 2H - pyran (14).

Compound 15 was hydrolyzed at C-1 to 16 which in turn, was oxidized with bromine in water to lactone 17 (Chart 2).

Although the synthesis of an analogue of lactone 4 was thus realized, the overall yield of 17 was rather low. We tried therefore a third route to 4. As substrate, butyl 2 - hydroxy - 6 - oxo - hex - 4 - enoate (19), is readily obtainable from Diels–Alder adduct 18 by mild acidic hydrolysis.[10] The sequence of reactions performed is shown in Chart 3.

Addition of hydrazoic acid to the α,β-unsaturated aldehyde 19 gave a mixture of stereoisomeric butyl 3 - azido - 2,3,4 - trideoxy - DL - hexopyranuronates (20). 20 was treated with methyl orthoformate and methanolic hydrogen chloride whereupon all four stereoisomeric methyl (methyl 3 - azido - 2,3,4 - trideoxy - DL - hexopyranosid) uronates (21–24) were obtained. This mixture was separated by column chromatography on silica gel into two fractions containing compounds 21 and 22 (fraction A) and 23 and 24 (fraction B). It was now

[a]For the sake of simplicity all formulae in this paper refer to monosaccharide D series although they represent, in fact, racemic compounds.



12 14 13 15 16 17

Chart 2.

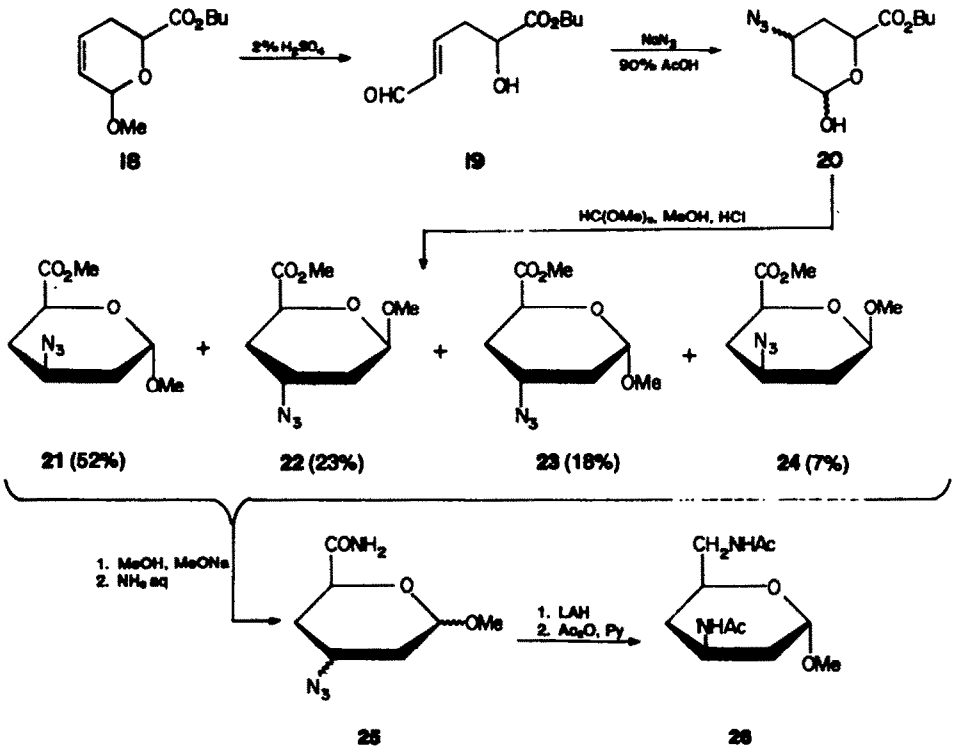
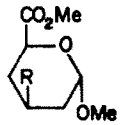
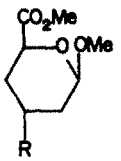
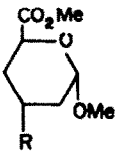
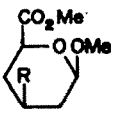


Chart 3.

possible to deduce the stereochemistry of 21–24 by means of ^{1}H NMR spectra (see Experimental, Table 1). Integration of appropriate signals in the ^{1}H NMR spectrum of the original mixture enabled the determination of the ratio of all four components. The important conclusion was that the mixture contained about 52% of the desired *α-threo* stereoisomer 21. Equilibration of the mixture with sodium methoxide in methanol raised the percentage of 21 to about 60%.[b] The mixture was converted with conc. ammonia into amide 25 which was subsequently reduced with LAH to the diamino compound. After acetylation pure methyl 3,6

[b] *α-Threo* stereoisomer 21 is certainly thermodynamically more stable than its partners in the mixture. Therefore equilibration under basic conditions increased the proportion of 21 in the mixture due to the conversion 22→21 (epimerization at C-5, *cf* Experimental).

Table 1. ^{1}HNMR data ($CDCl_3$) of stereoisomeric compounds 21–24 and 27

	R	δH-1	δH-5	δOMe	ΣJ_{H-1} Hz	ΣJ_{H-5} Hz
	N_3	4.98	4.38	3.38	6.5 $\left(\frac{W}{2}\right)$	14.7
	OMe	4.97	4.30	3.35	6.6 $\left(\frac{W}{2}\right)$	14.5
	N_3	4.70	. . .	3.48	9.5	. . .
	OMe	4.66	4.37	3.43	10.7	13.4
	N_3	4.86	4.63	3.42	6.2	13.3
	OMe	4.82	4.65	3.39	6.8	13.2
	N_3	4.36	4.02	3.51	11.5	14.2
	OMe	4.33	3.96	3.50	11.7	14.3

diacetamido - 2,3,4,6 - tetradeoxy - α - DL - threo - hexopyranoside (26) crystallized from the mixture in 21% yield. The remaining two steps leading to lactone 4: hydrolysis of the glycoside bond, and oxidation to lactone have been already described.[3]

Although also here the overall yield of the final product is not high, we regard the third route as practical because a simple preparative procedure is employed and laborious separations of stereoisomeric products at intermediate steps are avoided. Thus, preparations described here open an easy synthetic access to racemic negamycin.

EXPERIMENTAL

^{1}H NMR spectra were recorded for solns in $CDCl_3$ with Jeol JNM-4H-100 spectrometer (δ scale, TMS = 0 ppm). IR spectra were recorded on a Unicam SP-200 spectrophotometer. Tlc was performed with silica gel G Merck, and column chromatography with silica gel Merck (70–230 mesh).

Compounds 6, 8 and 9 were prepared according to Ref. 6. Compounds 12 and 13 were obtained from 6 - acetoxymethyl - 2 - methoxy - 5,6 - dihydro - 2H - pyran.[6] Compound 18 and its hydrolysis product 19 were obtained according to Ref. 10.

Addition of hydrazoic to 6 - substituted 5,6 - dihydro - 2 - pyrones 6, 8, and 9

4 - *Azido* - 6 - *phthalimidomethyl* - *tetrahydro* - 2 - *pyrone* 7. A soln of 6 (2.6 g, 10 mmoles) in 10 ml of 98% aq AcOH was left at room temp. for 72 hr. The soln was diluted with 10 ml water and extracted with $CHCl_3$. Concentration under diminished pressure gave crude 7 (2.4 g, *ca.* 80%), m.p. 150–152° (after crystallization from a mixture of EtOAc and hexane). ν_{max}^{KBr} 2200, 1720, 1400, 1250, 1040, 1030, 960, 910, 720 cm^{-1} ^{1}H–NMR data: δ 7.82 (m, 4H, aromatic), 4.90 (m, 1H, H–6), 4.25 (quintet, 1H, ΣJ = 17.5 Hz, H–4), 4.05 and 3.86 (AB system, 2H, $J_{A,6}$ = 7.2 Hz, $J_{B,6}$ = 5.6 Hz, $J_{A,B}$ = 14.0 Hz, CH_AH_BNPht), 2.83 (pd, 1H, $J_{3,4}$ = 5.4 Hz, $J_{3,3'}$ = 17.4 Hz, H–3), 2.65 (pd, 1H, $J_{3',4}$ = 4.2 Hz, H–3'), 2.12 (pt, $J_{5,4}$ = 4.0 Hz, $J_{5,6}$ = 4.5 Hz, $J_{5,5'}$ = 14.5 Hz, H–5), 1.92 (pq 1H, $J_{3',4}$ = 4.0 Hz, $J_{3',6}$ = 10.0 Hz, H–5').

The *erythro* configuration was assigned to 7 on the basis of ΣJ of H–4: 17.5 Hz. It had been found[6] that for 3,6 - di - O - acetyl - 2,4 - dideoxy - DL - hexono - 1,5 - lactones ΣJ of H–3 amounted to 15.2 Hz for the *eyrthro* and 27 Hz for the *threo* stereoisomer.

Compound 7 was unstable; it slowly eliminated hydrazoic acid on standing at room temp. The starting lactone 6 was again formed. Analytical data of 7 showed constantly a too high (*ca.* 1%) C and too low N content.

Compounds 10 and 11 were prepared in the same way from lactones 8 and 9, respectively. Both compounds were non-distillable oils. Column chromatography converted 10 and 11 into the starting lactones, therefore no correct analytical data have been obtained.

10: ν_{max}^{film}: 2150, 1750, 1230, 1090, 1050 cm^{-1}. ^{1}H NMR data: δ 4.79 (m, 1H, H–6), 4.00–4.40 (m, 3H, H–4, CH_2OAc), 2.84 (pd, 1H, $J_{3,4}$ = 5.0 Hz, $J_{3,3'}$ = 17.0 Hz, H–3), 2.69 (pd, 1H, $J_{3',4}$ = 4.5 Hz, H–3'), 2.12 (s, 3H, OAc), 2.05 (m, 2H, H–5, H–5'). Weak signals at δ 6.03 and 6.95 indicated the presence of small amount of lactone 8 in the sample. The *erythro* configuration of 10 was assigned on the basis of H–3 and H–3' signals which resembled those of 7.

11: ν_{max}^{film} 2200, 1740, 1220, 1110 cm^{-1}. ^{1}H NMR data: δ 4.24 (c. of m., 4H, H–4, H–6, $-OCH_2-$), 2.95 (pd, > 1H, $J_{3,4}$ = 5.9 Hz, $J_{3,3'}$ = 17.5 Hz, H–3), 2.64 (m, > 2H, H–3, H–3'), 0.98–2.31 (m, 9H, H–5, H–5', C_3H_7–).

2 - *Methoxy* - 6 - (N - *phthalimido*) *methyl* - 5,6 - *dihydro* - 2H - *pyran* (14). Compound 12 (0.81 g), triphenylphosphine (2.62 g) and phthalimide (1.47 g) were dissolved in THF (50 ml), and diethyl azodicarboxylate (1.9 g) was added. A slight exothermic effect was noticed. The mixture was left for 12 hr at room temp. and subsequently was evaporated under reduced pressure to dryness. The remaining substance was separated on a silica gel column with a mixture of benzene and ether (9:1, v/v) as eluent. Compound 14 (1.02 g, 75%), m.p. 123–124° was obtained, identical TLC, ^{1}H NMR IR (with an original sample obtained earlier).[8]

Methyl 2,3,4,6 - *tetradeoxy* - 3,6 - *diphthalimido* - β - DL - *threo hexo* - *pyranoside* (15). By the procedure described for 14 compound 13 (0.81 g) was converted into 15 (1.45 g, 69%), m.p. 197–200° from EtOH. ν_{max}^{KBr} 1770, 1710, 1615 cm^{-1}. ^{1}H NMR data δ 7.8–8.2 (m, 8H, aromatic H), 3.7–4.7 (m, 5H, H–1, H–3, H–5, H–6, H–6'), 3.47 (S, 3H, OCH_3), 1.6–2.8 (m, 4H, H–2, H–2', H–4, H–4'). (Found: C, 65.8; H, 5.0; N, 6.5. Calc. for $C_{23}H_{20}N_2O_6$ C, 65.7; H, 4.8; N, 6.7).

Hydrolysis of methyl glycoside 15 (0.85 g) with 60% aq. trifluoroacetic acid (25 ml) at room temp. during 1 hr gave 16 (0.49 g, 60%, m.p. 185–190°) from acetonitrile. ν_{max}^{KBr} 3500, 1770, 1750, 1620, 1380, 1080, 1040, 910, 720 cm^{-1}. (Found: C, 65.1; H, 4.4; N, 6.9. Calc. for $C_{22}H_{18}N_2O_6$: C, 65.0; H, 4.5; N, 6.9%).

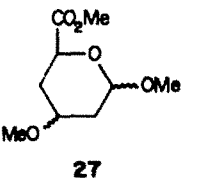
4 - *Phthalimido* - 6 - *phthalimidomethyl* - *tetrahydro* - 2 - *pyron* (17). Compound 16 (0.2 g) was oxidized with dimethylsulfoxide (2 ml) and Ac_2O (1 ml). After 24 hr at room temp. the mixture was diluted with water whereupon crystals of 17 separated (0.08 g 40%) m.p. 118–120° (from acetonitrile). ν_{max}^{Nujol} 1770, 1720, 1610, 1380, 1060, 710 cm^{-1}. (Found: C, 64.7; H, 4.0: N, 6.6. Calc. for $C_{22}H_{16}N_2O_6$: C, 65.3; H, 4.0; N, 6.9%).

Butyl 3 - *azido* - 2,3,4 - *trideoxy* - DL - *hexopyranuronates* (20). A soln of 19 (2.0 g, 10 mmoles) and sodium azide (3.2 g, 50 mmoles) in 90% aq AcOH (50 ml) was stirred at 5° for 12 hr. The mixture was diluted with an equal volume of water and extracted several times with $CHCl_3$. The $CHCl_3$ soln was dried ($MgSO_4$) and concentrated under lowered pressure affording crude 20 as a syrup in quantitative yield (2.3 g). A sample of 20 was purified by chromatography on silicon gel. ν_{max}^{film} 3500, 2150, 1740, 1250, 1140, 1060, 1040, 990, 900 cm^{-1}. ^{1}H NMR spectrum of 20 contained signals of anomeric protons at δ 5.51 (broad s.), 5.32 (t, ΣJ = 7.5 Hz), and 5.12 (pd $J_{1,2a}$ = 7.5 Hz, $J_{1,2e}$ = 3.0 Hz) which were assigned to α-*threo*-α-*erythro* and β-*erythro* stereoisomers. Integration of these signals pointed at the proportion 2.1:1:1.3, respectively. The remaining signals were complex multiplets. (Found: C, 49.4; H, 7.1; N, 16.0. Calc. for $C_{10}H_{17}N_3O_4$: C, 49.4; H, 7.0; N 17.3%).

Methyl (*methyl* 3 - *azido* - 2,3,4 - *trideoxy* - DL - *hexopyranosid*) *uronates* (21–24). A solon of 20 (8.0 g) and methyl orthoformate (10 ml) in MeOH (100 ml) containing 1.5% HCl was refluxed for 5 hr whereupon it was diluted with water (100 ml) and extracted with $CHCl_3$. The $CHCl_3$ soln was dried and the solvent was removed under diminished pressure. The residue was distilled at 85–94° (0.4 Torr) affording mixture of stereoisomers 21–24 as colorless liquid, 6.0 g (84.7%).

A sample (0.9 g) of this mixture was separated on a silica gel column with light petroleum and ether (9:1 v/v) as eluent. Two fractions were obtained: fraction A (less polar, 0.44 g) consisted of 21 (70%) and 22 (30%), fraction B (more polar, 0.15 g) contained 23 (72%) and 24 (28%).

Configuration of steroisomers 21–24 was deduced from ^{1}H NMR spectra which showed close analogy to those of 2,4 -



27

dimethoxy - 6 - methoxycarbonyl - tetra - hydropyrans (27) investigated in detail earlier.[11] Selected data from ^{1}H NMR spectra of 21–24 and all stereoisomeric 27 are compared in Table 1.

Fraction A: b.p. 140°/0.4 Torr; ν_{max}^{film} 2200, 1750, 1260, 1165, 1120, 1040, 970, 880 cm^{-1}. (Found: C, 44.8; H, 6.3; N, 19.9. Calc. for $C_8H_{13}N_3O_4$: C, 44.6; H, 6.1; N, 19.5%).

Fraction B: b.p. 140°/0.4 Torr; ν_{max}^{film} 2200, 1750, 1690, 1220, 1130, 1060, 970 cm^{-1}. (Found: C, 45.0; H, 6.0; N, 19.4. Calc. for $C_8H_{13}N_3O_4$: C, 44.6; H, 6.1; N, 19.5%. Equilibration of the mixture 21–24 with 1% NaOMe in abs MeOH (24 hr, room temp.) increased the content of 21 to about 60% (^{1}H NMR spectrum).

Equilibration of a sample of fraction A under the same conditions gave a mixture containing *ca.* 85% of **21** and 15% of **22**.

Methyl 3,6 - *diacetamido* - 2,3,4,6 - *tetradeoxy* - *α* - DL - *theo* - *hexopyranoside* (**26**). Equilibrated mixture of stereoisomers **21–24** (2.0 g) was shaken with conc aqueous ammonia (20 ml) during 4 hr. Evaporation of the soln to dryness gave crude amide **25** (2.0 g) as a colorless syrup. In the ^{1}H NMR spectrum of **25** signals of three anomeric protons were visible: 4.91 (d, $J_{1,2}$ = 3.0 Hz, 4.81 (t, ΣJ = 5.5 Hz), and 4.37 ($\Sigma J \approx 11.5$ Hz). These signals were ascribed to *α-threo* (preponderant component of the mixture), *α*- and *β-erythro* - stereoisomers of 4 - azido - 2 - methoxy - tetrahydropyran - 6 - carboxamide.

Crude amide **25** (1.3 g) was dissolved in THF (20 ml) and slowly added to gently refluxing suspension of LAH (2.8 g) in 50 ml THF. After completion of addition the mixture was refluxed for 3 hr. The excess of LAH was decomposed with water and 5% NaOHaq. The soln was filtered, dried and evaporated to dryness. The residue was treated with Ac_2O and pyridine. The acetylated product was crystallized from a mixture of EtOAc and hexane giving 0.2 g of pure compound **26**, m.p. 182–183°. ν_{max}^{KBr} 3300, 1640, 1560, 1130, 1050, 970, 940 cm^{-1}. ^{1}H NMR data: ≡ 6.25 (m, 2H, 2NH), 4.80 (d, 1H, $J_{1,2a}$ = 3.0 Hz, H–1), 4.29 (m, 1H, ΣJ = 36 Hz, H–5), 3.86 (m, 1H, ΣJ = 26 Hz, H–3), 3.34 (s + m, 5H, OCH_3, H–6, H–6'), 2.04 and 1.96 (two s overlapped with m, 8H, two OAc, H–2e, H–4e), 1.48 (pt, 1H; $J_{1,2a}$ = 3.0 Hz, $J_{2a,3}$ = 7.5 Hz, H–2a), 1.23 (pt, 1H, $J_{4a,5} \approx J_{4a,3} \approx 11.7$ Hz, $J_{4a,4e}$ = 12.0 Hz, H–4a). (Found: C, 54.1; H, 8.2; N 11.4. Calc. for $C_{11}H_{20}N_2O_4$: C, 54.1; H, 8.3; N, 11.5%).

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