A new synthesis of 3-amino-3-deoxy-α-D-mannopyranosyl 3-amino-3-deoxy-α-D-mannopyranoside, a potential antituberculous agent

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A practical, four-step synthesis of the title compound starting from 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-altropyranosyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-altropyranoside is described. Trifluoromethylsulfonylation followed by azide displacement furnished the corresponding α-D-*manno*,α-D-*manno* 3,3′-diazido-3,3′-dideoxy derivative, and subsequent hydrolytic deacetalation, followed by catalytic transfer hydrogenolysis to simultaneously reduce the azido groups and cleave the benzyl ether groups, gave the title disaccharide in 30% overall yield. As preceding work had provided the blocked starting disaccharide in a sequence of four facile operations departing from commercial α,α-trehalose (overall yield, 53%), the title compound can now be prepared conveniently on a multigram scale.

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Le 3-amino-3-désoxy-α-D-mannopyranosyl-3-amino-3-désoxy-α-D-mannopyranoside est obtenu commodément en quatre étapes partant du 2-*O*-benzyl-4,6-*O*-benzylidène-α-D-altropyranosyl-2-*O*-benzyl-4,6-*O*-benzylidène-α-D-altropyranoside. Une trifluorométhylsulfonylation suivie de déplacement par l'ion azide conduit au dérivé α-D-*manno*,α-D-*manno* 3,3′-diazido-3,3′-didésoxy qui, par hydrolyse des groupes protecteurs acétaliques, puis hydrogènolyse catalytique simultanée par transfert, des groupes azide et des groupes protecteurs benzyle, permet d'obtenir le disaccharide aminé attendu avec un rendement global de 30%. L'altroside de départ étant lui-même obtenu en quatre étapes commodes et un rendement global de 53% partant de l'α,α-tréhalose commercial, ce disaccharide aminé est donc désormais accessible à l'échelle préparative.

Introduction

Some years ago, we undertook chemical syntheses of several new, nitrogenous disaccharides structurally related to the important natural sugar α-D-glucopyranosyl α-D-glucopyranoside (α,α-trehalose, **1**). They included 3-amino-3-deoxy-α,α-trehalose (**1**) as well as 3,3′-diamino-3,3′-dideoxy-α,α-trehalose and its stereoisomers having the α-D-*gluco*,α-D-*manno* and α-D-*manno*,α-D-*manno* configurations (2). One point of interest attaching to these compounds relates to their use as substrate analogs for studies of the specificity and mechanism of action of the enzyme trehalase (3). Furthermore, the synthetic work was prompted by the fact that some naturally occurring amino disaccharides related to α,α-trehalose, which are produced as metabolites by certain *Streptomyces* species, had been found to show antibiotic activity (4). These were the 2-amino-2-deoxy (5) and 4-amino-4-deoxy (6) derivatives of **1**, and 2-amino-2-deoxy-α-D-glucopyranosyl α-D-mannopyranoside (7). It has subsequently been determined that one of the disaccharides obtained by us (2), namely 3-amino-3-deoxy-α-D-mannopyranosyl 3-amino-3-deoxy-α-D-mannopyranoside dihydrochloride (**11**), possesses a remarkable inhibitory activity (at 10 μg/mL in vitro) against *Mycobacterium tuberculosis* (human strain H37RV) and *Mycobacterium avium*, whereas the two stereoisomers mentioned showed no significant activity.[2] Unfortunately, the reported synthesis (2), based on the nitromethane cyclization methodology, had furnished chiefly the inactive isomers while **11** was formed as a minor product only, and was rather tedious to isolate. A more efficient procedure was therefore required in order to provide **11** conveniently in quantities which would enable detailed, pharmacological evaluation to be undertaken.

Several possible approaches were devised. One of them involved osmium tetraoxide-catalyzed *cis*-oxyamination, with chloramine-T, of a 2,2′-bis-enoside related to α,α-trehalose. This approach, expected to lead to diaminated disaccharides having the D-*manno*,D-*manno* configuration, did in fact afford **11** but, once again, in low yield, with the 2,3′- and 2,2′-diamino regioisomers constituting the major products (8). Although these latter disaccharides, which were previously unknown and have not thus far been evaluated biologically, command interest of their own, the problem of rendering **11** more readily available remained unsolved until an alternative synthesis, to be reported here, could be achieved.

Results

The synthesis departs from commercially available α,α,-trehalose (**1**), which is readily converted, in three high-yielding steps involving known procedures, into the bis-benzylidenated diepoxide **2**. As already reported (9), the latter reacted with sodium benzoxide in benzyl alcohol by predominantly diaxial opening of the oxirane ring, in accord with the Fürst–Plattner rule, affording the key intermediate **3**, 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-altropyranosyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-altropyranoside. The overall yield of **3** from **1** was 53% (9).

From **3**, the crystalline ditriflate **4** was obtained in 94% yield. The next step consisted of nucleophilic displacement with azide ion, to give the *manno,manno* diazide **5**. This crucial reaction was the only somewhat problematical point in the sequence of operations. The difficulty arose from a strong tendency for elimination to compete with substitution, resulting in the formation of the unwanted mono- and di-alkenes **6** and

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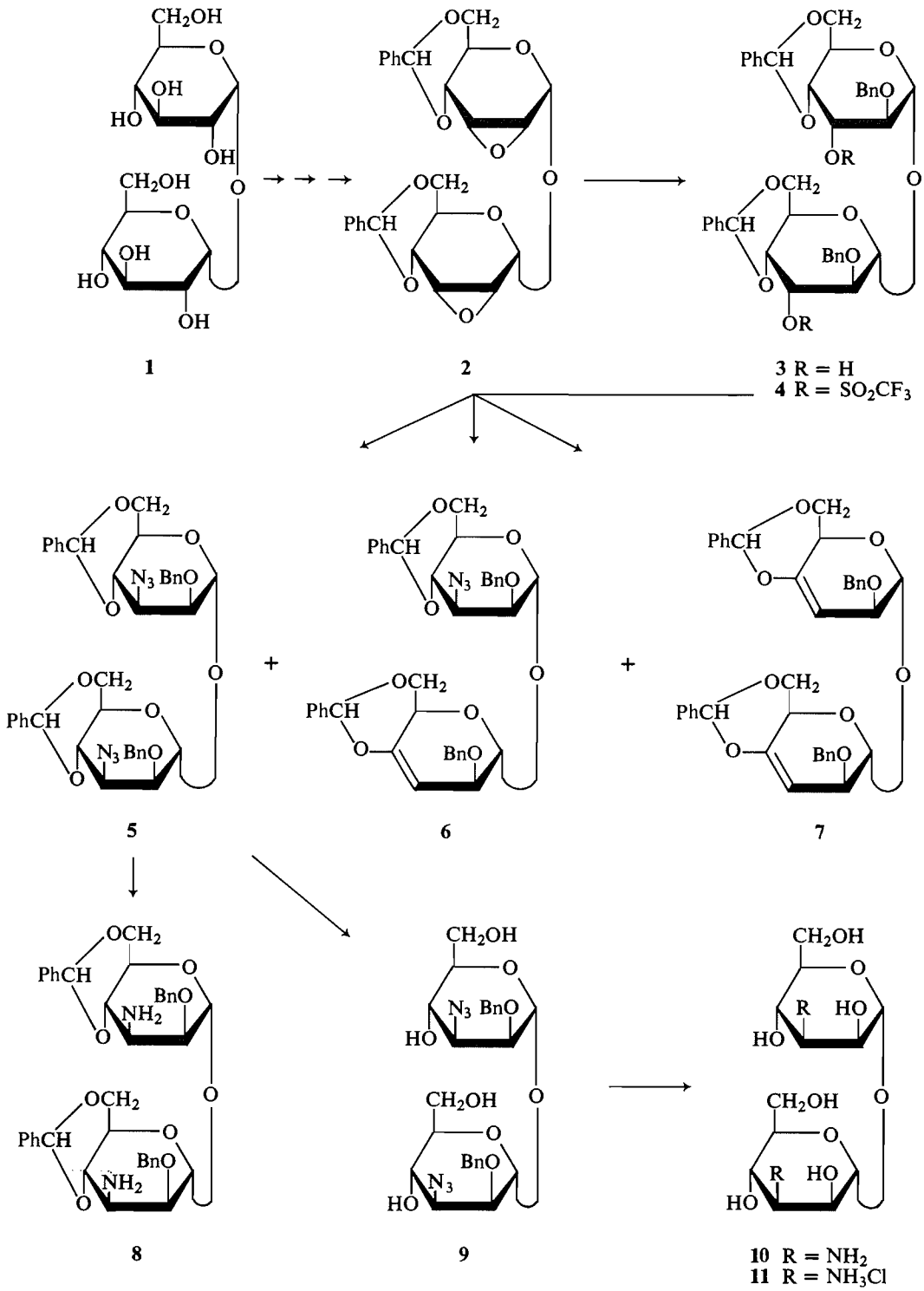
[2] The authors are indebted to Dr. H. L. David, Chef du Service de la Tuberculose à l'Institut Pasteur, Paris, for performing the assays. (Private communication, October 1979).



7. In an analogous operation previously executed (1), a trehalose-derived 3-triflate had been very easily displaced at ambient to slightly elevated temperature, giving an 80% yield of the desired product and showing no evidence for elimination,[3] and that experience encouraged us in our present endeavor. However, the analogous substrate in question had possessed the D-*allo* configuration, with an equatorial benzoate ester function at C-2, and it was understandable that, in **4**, the axial benzyl ether group in that position would be apt to interfere seriously in the transition state of a parallel approach of nucleophile to C-3. We investigated the azide displacement in **4** under a wide variety of conditions, and invariably obtained mixtures of **5**, **6**, and **7**, generally with the desired diazide **5** present in an unsatisfactory proportion. However, we eventually succeeded in specifying conditions under which **5** was the main product. On a multigram scale, the reaction was best performed with sodium azide in a refluxing mixture of benzene

[3] The reaction was performed with lithium azide in a mixture of DMF and DMSO. The corresponding 3-mesylate reacted much more sluggishly (12 h at 105°C in DMSO–HMPA), and did suffer partial elimination.

and water, in the presence of tetrabutylammonium hydrogen sulfate as a phase transfer catalyst. The slow process was essentially complete after five days, and chromatographic separation of the products afforded crystalline **5** in 51.5% yield, and the unsymmetrical azidoalkene **6** and the dialkene **7**, both crystalline, in 40 and ~2% yield, respectively.

In order to convert **5** most efficiently into the target compound, several possibilities for sequential or simultaneous operations to reduce the azido functions and remove the protecting groups were considered, and tested in pilot experiments. Catalytic hydrogenation of **5**, or reduction with lithium aluminum hydride, gave the blocked diamino sugar **8**, but difficulties experienced in its complete deprotection caused us not to pursue this route any further. Instead, **5** was first debenzylidenated by hydrolysis with 80% acetic acid, affording the partially deprotected diazide **9**, crystalline in 91% yield. This product was then subjected to catalytic transfer hydrogenolysis (10) with cyclohexene in the presence of palladium on charcoal, which effected rapid reduction of the azido groups and simultaneously, although more slowly, cleavage of the benzyl ethers (11). The 3,3′diamino-3,3′-dideoxy disaccharide (**10**) was obtained as a crystalline monohydrate in 61% yield after purification. The crude **10** contained a small proportion of a by-product which was removed by recrystallization, and was judged to be a mono-*N*-ethyl derivative of **10**, on the basis of its ^{1}H nmr spectrum.[4] The free base **10** was converted into its dihydrochloride **11** that proved in every respect identical with previously synthesized (2, 8) material.

In summary, the present synthesis allows the convenient preparation of the title compound in four steps with ~30% overall yield from the intermediate (9) disaccharide **3**, or in eight steps from commercial trehalose with ~16% overall yield. The reactions can be performed on a multigram scale and require only common reagents and standard operations.

NOTE ADDED IN PROOF. The base **10** was submitted for testing to Dr. H. L. David, Institut Pasteur, Paris, and Dr. A. Laszlo, National Reference Centre of Tuberculosis, Ottawa. Neither laboratory was able to observe antimycobacterial activity as previously found[2] for the dihydrochloride **11** prepared by our first (2) method. The reason for this apparent discrepancy is being investigated.

Experimental

Instruments and general methods

Optical rotations were measured at ~25°C with a Perkin–Elmer 241 polarimeter. The ^{1}H nmr data refer to 300-MHz spectra recorded on a Varian XL-300 instrument, unless otherwise indicated. Melting points were determined with a Gallenkamp apparatus, and are uncorrected. Column chromatography was performed on Silica Gel 60, 230–400 mesh (E. Merck AG, West Germany), and the medium-pressure technique was usually employed for separations on a multigram scale. For tlc, precoated silica gel plates were used, and components were made visible by spraying the plates with 5% sulfuric acid in ethanol, and heating them briefly on a hot plate. Frequently used solvent combinations were ethyl acetate and hexane in the proportions (v/v) 1:9 (solvent A), 1:4 (solvent B), and 1:1 (solvent C).

[4] It may be assumed that ethanol, which was employed as a co-solvent, acted to some extent as a hydrogen donor (10) in the system, producing acetaldehyde that, with **10**, formed an imine whose hydrogenation gave the by-product. To optimize the transfer hydrogenation, it may be worthwhile to try modified conditions such as, for example, the use of 1,4-cyclohexadiene, which has been recommended (12) for the efficient debenzylation of protected peptides.

*2-O-Benzyl-4,6-O-benzylidene-3-O-trifluoromethylsulfonyl-α-D-altropyranosyl 2-O-benzyl-4,6-O-benzylidene-3-O-trifluoromethylsulfonyl-α-D-altropyranoside (**4**)*

Trifluoromethanesulfonic anhydride (7.3 mL, 43 mmol) in dry dichloromethane (200 mL) was added dropwise over a period of 30 min to a chilled (−17°C) solution of the diol (9) **3** (7.0 g, 10 mmol) in dry dichloromethane (70 mL) and pyridine (6 mL). After the end of the addition, spots of about equal strength were seen for starting **4** and what appeared to be its monotriflated derivative (tlc with chloroform). The triflation was then allowed to continue at room temperature and was complete after 2.5 h. The mixture was shaken with added water and, after phase separation, the organic layer was washed twice with sodium hydrogen carbonate solution and once with water. The combined aqueous phase and washings were extracted twice with dichloromethane. The combined organic phases were dried (Na_2SO_4), and evaporated (bath temperature, 35°C), and remnant pyridine was removed by two coevaporations with toluene. The residue was eventually obtained as a syrupy foam. Trituration of the material with ethanol (20 mL) gave a first crop (8.157 g) of slightly yellowish crystals, which were collected and washed with cold ethanol; mp 97–98.5°C, with decomposition starting at 91°C. The ethanolic mother liquor was diluted with several volumes of benzene, washed with aqueous $NaHCO_3$ solution, dried (Na_2SO_4), and evaporated, to give a thin syrup from which a second crop of **4** (0.644 g) was obtained by crystallization from ethanol. The combined crops were recrystallized by dissolution in benzene, evaporation of the solvent, and trituration of the residue with ethanol (20 mL), to give 8.61 g of pure **4**. The mother liquor of recrystallization was combined with the original mother liquor, and the solute was chromatographed on a short column of silica gel, with solvent A followed by solvent B as eluants, yielding another crop of crystalline **4** (0.246 g). The mother liquor therefrom was evaporated, and the residue was treated with triflic anhydride as described before (with appropriate proportions of reagents). Similar processing gave a final crop of **4** (0.239 g), for a total of 9.092 g (94.4%) of chromatographically homogeneous product.

An analytical sample was recrystallized twice more as described; mp 98–102°C, $[\alpha]_D$ +80.0° (*c* 0.5, chloroform); δ ($CDCl_3$): 7.44 (m, Ph), 5.64 (s, *Ph*-CH), 5.12 (narrow t, H-3), 4.66 (s, H-1), 4.82 and 4.57 (AB-q, J_{gem} = 12.0 Hz, *Ph*-CH_2), 4.13 (dd, $J_{5,6e}$ = 5.0, $J_{6a,6e}$ = 10.0 Hz, H-6e), 4.08 (dd, $J_{3,4}$ = 2.7, $J_{4,5}$ = 9.7 Hz, H-4), 3.91 (d, $J_{2,3}$ = 3.0 Hz, H-2), 3.72 (t, $J_{5,6a}$ = $J_{6a,6e}$ = 10.0 Hz, H-6a), 3.58 (dt, $J_{4,5}$ = 9.7, $J_{5,6a}$ = 10.0, $J_{5,6e}$ = 5.0 Hz, H-5). *Anal.* calcd. for $C_{42}H_{40}F_6O_{15}S_2$ (962.8): C 52.39, H 4.19, S 6.66; found: C 52.58, H 4.25, S 6.81.

*3-Azido-2-O-benzyl-4,6-O-benzylidene-3-deoxy-α-D-mannopyranosyl 3-azido-2-O-benzyl-4,6-O-benzylidene-3-deoxy-α-D-mannopyranoside (**5**), 3-azido-2-O-benzyl-4,6-O-benzylidene-3-deoxy-α-D-mannopyranosyl 2-O-benzyl-4,6-O-benzylidene-3-deoxy-α-D-*threo*-hex-3-enopyranoside (**6**), and 2-O-benzyl-4,6-O-benzylidene-3-deoxy-α-D-*threo*-hex-3-enopyranosyl 2-O-benzyl-4,6-O-benzylidene-3-deoxy-α-D-*threo*-hex-3-enopyranoside (**7**)*

A solution of **4** (8.158 g) in benzene (1.6 L) and a solution of sodium azide (10.0 g) and tetrabutylammonium hydrogen sulfate (3.0 g) in water (200 mL) were mixed with efficient stirring and heated under reflux for 2 days. (Smaller volumes of solvent tended to lead to decreased yields of **5**.) Additional sodium azide (10.0 g) and tetrabutylammonium salt (3.0 g), suspended in 25 mL of water, were introduced and refluxing continued for 3 more days. Progress of the reaction was monitored by two-dimensional tlc using solvent B in the first direction, and double irrigation with chloroform in the second. For processing, solvent was distilled from the reaction mixture until 1250 mL of distillate (~1075 mL of benzene and 175 mL of water) had been collected. The remaining mixture was cooled, the phases were separated, and the benzene phase was washed 4 times with water. The aqueous phase and washings were back-extracted with benzene. The combined benzene solutions were again washed repeatedly with water (as nmr spectroscopy had revealed the presence of remnant tetrabutylammonium salt), then dried with Na_2SO_4, and

evaporated to give a mixture of carbohydrate material (7.34 g). The mixture was applied to a column of silica gel (58 × 4.5 cm) with the aid of a small amount of benzene, and chromatographed with solvent A. The effluent fractions were examined by tlc with solvent B. Appropriate fractions were combined and gave 3.278 g (51.5%) of the diazide **5** (R_f 0.4) as an amorphous material, 2.575 g of a mixture containing chiefly the azidoalkene **6** (R_f 0.3), and 0.295 g of a mixture rich in the dialkene **7** (R_f 0.25).

For analysis, a sample of **5** was dissolved in a large proportion of methanol and recovered as a white, gummy precipitate by partial evaporation of the solvent. The gum was washed with a little methanol, and dried under high vacuum with occasional rubbing by spatula to give a brittle solid that melted to a glass at 78–87°C; $[\alpha]_D$ +42.4° (*c* 0.7, chloroform); ν_{max} neat: 2115 (azide) cm^{-1}; δ ($CDCl_3$): 7.43 (m, Ph), 5.67 (s, *Ph*-CH), 4.85 (d, $J_{1,2}$ = 1.4 Hz, H-1), 4.86 and 4.63 (AB-q, J_{gem} 12.0 Hz, *Ph*-CH_2), 4.21 (dd, $J_{4,5}$ = 9.5, $J_{3,4}$ = 10.5 Hz, H-4), 4.09 (dd, $J_{5,6e}$ = 5.0, $J_{6a,6e}$ = 10.5 Hz, H-6e), 3.81 (t, $J_{5,6a} \approx J_{6a,6e}$ = 10.5 Hz, H-6a), 3.76 (dd, $J_{2,3}$ = 3.4, $J_{3,4}$ = 10.5 Hz, H-3), 3.69 ($J_{1,2}$ = 1.4, $J_{2,3}$ = 3.3 Hz, H-2), 3.46 (~dt, *J* = 5.0, 9.5, and 10.5 Hz, H-5). *Anal.* calcd. for $C_{40}H_{40}N_6O_9$ (748.8): C 64.16, H 5.38, N 11.22; found: C 64.29, H 5.44, N 11.31.

The aforementioned column eluate (2.575 g) containing **6** (R_f 0.3) was crystallized from hot methanol (200 mL), yielding pure **6** (1.852 g, plus 0.53 g by concentration of the mother liquor; 39.7%); mp 110–112°C, unchanged after recrystallization; $[\alpha]_D$ +118° (*c* 0.5, chloroform); ν_{max}: 2111 (azide) and 1694 (vinyl ether) cm^{-1}; δ ($CDCl_3$) for the azido moiety: 5.71 (s, *Ph*-CH), 4.93 (s, H-1), 4.92 and 4.67 (AB-q, J_{gem} 12.0 Hz, *Ph*-CH_2), 4.25 (H-4, not separated from H-6e and H-6′e), and 3.81 (dd, $J_{2,3}$ = 3.4, $J_{3,4}$ = 11.0 Hz, H-3); δ for the alkenic moiety: 5.63 (s, *Ph*-CH), 5.46 (d, $J_{2,3}$ = 5.5 Hz, H-3), 5.17 (s, H-1), 4.64 (AB-q having the inner lines at 4.66 and 4.62; *Ph*-CH_2); δ for both moieties: 7.45 (m, Ph), 4.27 (m, H-6e and H-6′e), 3.90 and 3.77 (2 m, H-2,2′, 5,5′, 6a,6′a). (The assignments were made by comparison with the data for the symmetrical derivatives **5** and **7**). *Anal.* calcd. for $C_{40}H_{39}N_3O_9$ (705.7): C 68.07, H 5.57, N 5.95; found: C 67.94, H 5.43, N 5.89.

The column eluate (0.295 g) containing **7** (R_f 0.25) was chromatographed again on silica gel, using 1% ethyl acetate in dichloromethane as the eluant, and fractions further enriched in **7** were eluted. The syrupy material obtained from these was crystallized in part, from ethanol, by inoculation with seed crystals of **7** that had formed in a previous, syrupy preparation after storage for several months. The mother liquor of crystallization was combined with the less-pure fractions from the column, and preparative tlc (3 irrigations with dichloromethane) furnished additional **7** that was recrystallized from ethanol, for a total yield of 116 mg (2%). The crystals still contained an impurity (R_f 0.2) even after several subsequent recrystallizations, and the entire material was therefore purified once more by ptlc (solvent B), to give 87 mg (1.5%) of pure **7**; mp 106–108°C; $[\alpha]_D$ +176.7° (*c* 0.5, chloroform); ν_{max}: 1692 (vinyl ether; azide band absent) cm^{-1}; δ ($CDCl_3$): 7.43 (m, Ph), 5.67 (s, *Ph*-CH), 5.49 (d, $J_{2,3}$ = 5.4 Hz, H-3), 5.28 (s, H-1), 4.72 and 4.65 (AB-q, J_{gem} = 12.0 Hz, *Ph*-CH_2), 4.40 (dd, $J_{5,6e}$ = 6.6, $J_{6a,6e}$ = 10.8 Hz, H-6e), 4.14 (m, H-5), 3.97 (dd, $J_{2,3}$ = 5.4, $J_{2,5}$ = 1.8 Hz, H-2), 3.89 (t, $J_{6a,6e} = J_{5,6a}$ = 10.8 Hz, H-6a). *Anal.* calcd. for $C_{40}H_{38}O_9$ (662.7): C 72.49, H 5.78; found: C 72.36, H 5.71.

By-product

The methanolic mother liquor from the crystallization of **6** showed a spot at R_f 0.52 in addition to one for residual **6** (tlc with CH_2Cl_2). After standing for several days at room temperature, partial decomposition was noticed (appearance of an immobile spot, and smell of benzaldehyde), but the component R_f 0.52 was still visible. The methanol was evaporated and the residue dissolved in dichloromethane, washed with aqueous $NaHCO_3$, dried, and subjected to ptlc, with CH_2Cl_2 as irrigant. About 20 mg of the fast-moving compound was isolated. Its ir spectrum was similar to that of the ditriflate **4** in the 1550–600 cm^{-1} region but also showed a strong azide band at 2112 cm^{-1}. The 300-MHz nmr spectrum revealed the structure of an unsymmetrically substituted disaccharide, showing the essential features of both **4** and **5**, with a narrow, one-proton triplet at δ 5.1 characteristic for the 3-triflate moiety. Evidently, the substance was the monoazidomonotriflate intermediate of the reaction **4** → **5**.

3-Amino-2-O-benzyl-4,6-O-benzylidene-3-deoxy-α-D-mannopyranosyl 3-amino-2-O-benzyl-4,6-O-benzylidene-3-deoxy-α-D-mannopyranoside (8)

Compound **5** (140 mg) in ethanol (25 mL) was hydrogenated with 10% palladium on charcoal (200 mg) for 17 h at room temperature, in a Parr flask under 400 kPa of hydrogen pressure. The solution thereafter showed one major product spot, R_f 0.24, in tlc using 95:5 ethyl acetate – ethanol. Isolation of the product by ptlc with the same solvents, but 98:2, gave ~100 mg of **8**. The ir spectrum showed no azido-group absorption. The ^1H nmr data (200 MHz, $CDCl_3$), δ: 7.41 (m, Ph), 5.56 (s, *Ph*-CH), 5.05 (s, H-1), 4.69 (s, *Ph*-CH_2), 4.12 (dd, $J_{6a,6e}$ = 10.0, $J_{5,6e}$ = 4.5 Hz, H-6e), 3.84–3.63 (m, H-2,4,6a), 3.55 (m, $J_{4,5} = J_{5,6a}$ = 10, $J_{5,6e}$ = 4.5 Hz, H-5), 3.18 (dd, $J_{2,3}$ = 3.7, $J_{3,4}$ = 10.0 Hz, H-3), and 1.70 (broad s, removable by D_2O exchange, NH_2).

The same product was obtained by treatment of **5** in ether with lithium aluminum hydride for 15 min.

3-Azido-2-O-benzyl-3-deoxy-α-D-mannopyranosyl 3-azido-2-O-benzyl-3-deoxy-α-D-mannopyranoside (9)

A suspension of **5** (3.73 g) in 80% acetic acid (50 mL) was agitated on a steam bath, whereby the initially formed oil dissolved within 10 min. The debenzylidenation was complete after 30 min, as judged by tlc (solvent C). The solution was cooled, and evaporated repeatedly with added portions of ethanol until the smell of acetic acid was no longer noticeable, and a dry foam was obtained (~2.94 g). The foam crystallized on treatment with dichloromethane (20 mL), giving 2.167 g of **9** (R_f 0.17), mp 140–142°C, contaminated by a trace of a product having R_f 0.34. The mother liquor showed components having R_f 0.17, 0.34, and 0.43 (solvent C). Upon removal of the solvent, this mixture was subjected to hydrolysis as previously described, using 10 mL of 80% acetic acid, which caused disappearance of the component having R_f 0.43. After processing as before, the material was heated on the steam bath for 15 min in a mixture (10 mL) of methanol–water–triethylamine (5:3.5:1.5). Evaporation, followed by crystallization from dichloromethane, gave an additional 308 mg of **9**, plus 153 mg from the mother liquor after its passage through a short column of silica gel (solvent C). The combined crops (2.628 g, 92.5% yield) were recrystallized as described, by treating with dichloromethane the dry foam obtained on evaporation of an ethanolic solution of the compound. Pure **9** so prepared (2.589 g, 91.1%) had mp 141–143°C; $[\alpha]_D$ +83.2° (*c* 0.5, ethanol); ν_{max}: 3350 (OH) and 2111 (N_3) cm^{-1}; δ (CD_3COCD_3): 7.46 (m, Ph), 5.18 (d, $J_{1,2}$ = 1.8 Hz, H-1), 4.78 (d, $J_{4,OH}$ = 6.4 Hz, OH-4), 4.76 (s, 4H, 2 *Ph*-CH_2), 4.17 (dt, $J_{4,OH}$ = 6.4, $J_{3,4} \approx J_{4,5}$ = 10.2 Hz, H-4), 4.03 (dd, $J_{1,2}$ = 1.8, $J_{2,3}$ = 3.2 Hz, H-2), 4.00–3.90 (m, 6H, H-6, H-6′, and OH-6), 3.85 (dd, $J_{2,3}$ = 3.2, $J_{3,4}$ = 10.4 Hz, H-3), and 3.78 (octet, H-5). When D_2O was added to the solution, the OH-4 signal disappeared, H-4 gave a clear triplet, the *Ph*-CH_2 singlet became an AB quartet (J_{gem} = 12.6 Hz), the δ 4.0–3.9 region was resolved and revealed H-6 at δ 4.00 (dd, $J_{5,6}$ = 2.6, $J_{6,6'}$ = 12 Hz) as well as H-6′ at δ 3.88 (dd, $J_{5,6'}$ = 5.6 Hz). All assignments were verified by spin decoupling experiments. *Anal.* calcd. for $C_{26}H_{30}N_6O_9$ (570.5): C 54.73, H 5.30, N 14.73; found: C 54.58, H 5.40, N 14.88.

3-Amino-3-deoxy-α-D-mannopyranosyl 3-amino-3-deoxy-α-D-mannopyranoside (10) and its dihydrochloride (11)

A solution of **9** (2.567 g) in 95% ethanol (40 mL) together with 10% palladium on charcoal (2.5 g) was heated with magnetic stirring, and cyclohexene (20 mL) was added when refluxing started. Progress of the reaction was monitored by tlc using 5:4:1 methanol–chloroform – ammonium hydroxide (concentrated, aqueous) as the irrigant. After 6 min, the presence of 5 products was indicated: R_f 0.10 (**10**), 0.34 (diaminomonobenzyl derivative), 0.44 (weak; monoaminomonoazidomonobenzyl derivative), 0.73 (diaminodibenzyl derivative), and 0.86 (**9**). After 12 min, the product having R_f 0.44 was no longer

detectable. To promote the reaction, the following was added to the boiling mixture: cyclohexene (10 mL, after 45 min), water (2 mL, after 1 h), and Pd catalyst (0.5 g, after 2 h). About 140 min after the start of the reaction only a trace of **9** had remained, and after 3 h there were intense spots for **10** (R_f 0.1) and a by-product (R_f 0.2), accompanied only by weak spots of more-mobile intermediates. Refluxing was stopped, the mixture was filtered, and the catalyst was washed with hot 95% ethanol followed by hot 90% ethanol (100 mL each). Evaporation of the filtrate and washings gave ~1.6 g of a material, which was dissolved in a methanol–water mixture. The solution was evaporated to a thin syrup to which some fresh methanol was added, and scratching with a glass rod then initiated crystallization of **10** (1.045 g) contaminated by a trace of the compound having R_f 0.2. Twice recrystallized from methanol, the substance (988 mg, 61.3%) was chromatographically homogeneous (R_f 0.11), but faintly yellowish. The coloration was removed by vacuum filtration of the compound, in concentrated aqueous solution (3 mL of water), through a plug of activated carbon and Celite. The recovered, colorless compound (874 mg, plus 95 mg from the mother liquor; see below) had mp 177–180°C; $[\alpha]_D$ +105.7° (*c* 0.7, water); δ (D_2O, acetone lock signal): 4.95 (d, $J_{1,2}$ = 2.0 Hz, H-1), 3.74 (narrow m, H-2), 3.72 (dd, $J_{5,6}$ = 2.0, $J_{6,6'}$ = 12.0 Hz, H-6), 3.56 (dd, $J_{5,6'}$ = 7.0, $J_{6,6'}$ = 12 Hz, H-6′), 3.47 (m, *J* = 2.0, 7.0, and 10.0 Hz, H-5), 3.29 (t, $J_{3,4} = J_{4,5}$ = 10 Hz, H-4), 3.15 (dd, $J_{2,3}$ = 3.0, $J_{3,4}$ = 10.0 Hz, H-3). All assignments were confirmed by spin decoupling; ^{13}C nmr data, δ (H_2O, with acetone lock signal 29.8 ppm from TMS): 94.5 (C-1), 73.2, 69.5, and 67.3 (C-2, -4, and -5), 60.6 (C-6), and 51.5 (C-3). *Anal.* calcd. for $C_{12}H_{24}N_2O_9 \cdot H_2O$ (358.3): C 40.22, H 7.31, N 7.82; found: C 40.20, H 7.18, N 7.66.

A sample of **10** was converted into its dihydrochloride **11** by precipitation from methanolic solution with ether containing anhydrous hydrogen chloride (2); mp 199.5°C (dec.), $[\alpha]_D$ + 84.2°, in excellent agreement with the reported (2, 8) values. The ^{13}C chemical shift data, differing slightly from those of the free base, above, completely matched those reported (8) for **11**.

The N*-ethyl derivative as by-product*

The mother liquors from the isolation and purification of **10** were combined and evaporated. The residue was chromatographed on a column of powdered cellulose by means of 85:13:2 ethanol–water–ammonium hydroxide (concentrated, aqueous) as the eluant. The fractions containing the faster-moving (R_f ~0.25) by-product were inhomogeneous, whereas from subsequent fractions some additional **10** was obtained in crystalline form (95 mg). The inhomogeneous fractions were passed through the same column again, with a 90:8:2 mixture of the previous solvents, and 45 mg of the compound showing R_f 0.25 could thereby be isolated pure. The ^1H nmr spectrum (D_2O, acetone lock signal) indicated unequal substitution of the disaccharide due to the presence of one *N*-ethyl group; δ: 5.05 and 5.02 (two d, *J* = 2 Hz, H-1 and H-1′), 4.12 and 3.98 (two narrow dd, H-2 and H-2′), 3.8–3.5 (unresolved m, 8H, H-4,4′, 5,5′, 6a,6a′, 6b,6b′), 3.38 and 3.32 (two dd, $J_{2,3}$ and $J_{2',3'}$ = 3.5 Hz, $J_{3,4}$ and $J_{3',4'}$ = 10.5 Hz, H-3 and H-3′), 3.04 (complex symm. multiplet, 2H, CH_2 of Et), and 1.14 (t, 3H, CH_3 of Et). The assignments were verified by spin decoupling.

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