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Note

Synthesis of biologically active pseudo-trehalosamine: [(1*S*)-(1,2,4/3,5)-2,3,4-trihydroxy-5-hydroxymethyl-1-cyclohexyl] 2-amino-2-deoxy-α-D-glucopyranoside*

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The title pseudo-disaccharide (**2**), one of the pseudo-sugar analogues of the antibiotic trehalosamine[2] (**1**), has been synthesised and its antimicrobial activity determined.

Condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-glucopyranosyl bromide[3] (**10**), under modified Königs–Knorr reaction conditions, with 2,3:4,7-di-*O*-isopropylidene-pseudo-α-DL-glucopyranose[4] (**3**) resulted in the formation mainly of the 3-glycosides[5] as a result of 2,3→1,2 acetal migration to give **4**. Therefore, an alternative aglycon, namely, 2,3,4,7-tetra-*O*-benzyl-pseudo-α-DL-glucopyranose (**8**) was used. Compound **8** was prepared from **3** by the following sequence: *O*-allylation (→**5**), *O*-deisopropylidenation then acetylation (→**6**), *O*-deacetylation then benzylation (→**7**), and *O*-deallylation (→**8**). The structure **6** was evident from the ^{1}H-n.m.r. spectrum, which contained coupled signals at δ 5.50 (t, *J* 9.8 Hz), 4.99 (dd, *J* 9.8, 10.8 Hz), and 4.83 (dd, *J* 3, 10.8 Hz), attributable to H-4, H-3, and H-2, respectively. Compound **8** was further characterised as the acetate **9**.

Coupling of **8** with 3 mol. equiv. of **10** in dichloromethane in the presence of silver carbonate, silver perchlorate, 2,4,6-trimethylpyridine, and calcium sulfate for 4 h at room temperature gave, after column chromatography, 63% of a mixture of **11A**, **11B**, and **14A**, and 29% of **14B**. Further fractionation of the mixture afforded **11B** (8%). Treatment of the mixture with Amberlite IRA-400 (HO^-) resin in methanol gave, after acetylation and then separation, the *N*-acetyl derivatives **12A** (8.8%), **12B** (3.8%), and **15A** (9.3%). Compounds **11B** and **14B** were also converted into the respective *N*-acetyl derivatives **12B** (7.1% total yield) and **15B** (9.2%). Hydrogenolysis (Pd/C) of **12A**, **12B**, **15A**, and **15B** in ethanol followed by acetylation gave the respective pseudo-disaccharide peracetates **13A**, $[\alpha]_D$ +113°

*Synthesis of Pseudo-trehalosamine and Related Pseudo-disaccharides, Part III. For Part II, see ref. 1.
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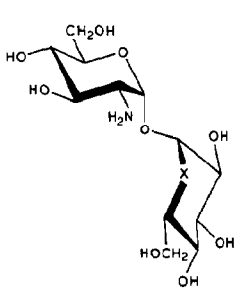
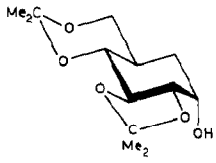


1 X = O
2A X = CH_2



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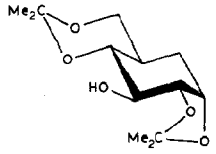

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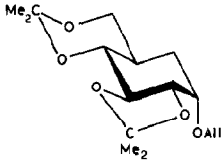

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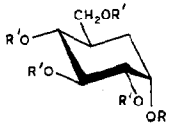
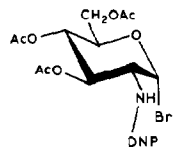
All = allyl
DNP = 2,4-dinitrophenyl



6 R = All, R′ = Ac
7 R = All, R′ = Bn
8 R = H, R′ = Bn
9 R = Ac, R′ = Bn



10

(chloroform); **13B**, $[\alpha]_D$ +35° (chloroform); **16A**, $[\alpha]_D$ +21° (chloroform); and **16B**, $[\alpha]_D$ −64° (chloroform); the structures of which were determined on the basis of the ^{1}H-n.m.r. data (Table I). The absolute configurations were assigned tentatively as shown, on the basis that the pseudo-α-D-glucopyranose moiety would provide a dextrorotatory contribution to the molecular rotation; pseudo-α-D-glucopyranose[6] had $[\alpha]_D$ +67° (water) and its penta-acetate had $[\alpha]_D$ +57° (chloroform).

The pseudo-disaccharides **2A, 2B, 17A**, and **17B** were prepared by hydrazinolysis of the corresponding peracetates and assayed directly against three microorganisms. Compound **2A** had ~25% of the activity of 2-trehalosamine[2a] against *Klebsiella pneumoniae*, and the others were inactive. These results suggest that, in biologically active oligosaccharides, replacement of the pyranoid-ring oxygen of one sugar residue with a methylene group may not result in loss of activity.

EXPERIMENTAL

General methods. — Melting points were determined with a MEL-TEMP capillary melting-point apparatus and are uncorrected. Optical rotations were measured with a Jasco DIP-4 polarimeter. ^{1}H-N.m.r. spectra were recorded for solutions in $CDCl_3$ (internal Me_4Si) with Varian EM-390 (90 MHz) and Jeol FX-200 (200 MHz) instruments. T.l.c. was performed on Silica Gel 60 GF (Merck) with detection by charring with sulfuric acid. Column chromatography was conducted on Wakogel C-200 (200 mesh) or C-300 (300 mesh). Organic solutions were dried over anhydrous Na_2SO_4 and concentrated at <50° under diminished pressure.

TABLE I

^{1}H-N.M.R. DATA (200 MHz, $CDCl_3$) OF COMPOUNDS **13A, 13B, 16A**, AND **16B**

Proton	*Chemical shifts (δ)*					*Coupling constants (Hz)*			
	13A	**13B**	**16A**	**16B**		**13A**	**13B**	**16A**	**16B**
H-1	5.04(d)	5.03(d)	4.97(d)	5.05(d)	$J_{1,2}$	4	4	8	8
H-2	4.27(ddd)	4.36(ddd)	3.63(dt)	3.45(dt)	$J_{2,3}$	11.2	11.0	11.2	10.2
H-3	4.93(dd)	5.04(dd)	5.02(dd)	4.95(dd)	$J_{3,4}$	9.2	9.8	9.2	9.6
H-4	5.25(dd)	5.24(dd)	5.51(dd)	5.55(dd)	$J_{4,5}$	10.8	10.6	10.4	10.4
H-1′	—	—	—	4.36–4.28(m)	$J_{1',2'}$	3.2	3	2.4	3.8
H-2′	4.96(dd)	4.91(dd)	4.85(dd)	4.67(dd)	$J_{2',3'}$	10	10	10	10
H-3′	5.48(dd)	5.44(t)	5.39(t)	5.40(t)	$J_{3',4'}$	9.8	10	10	10
H-4′	5.12(t)	5.10(t)	5.03(t)	5.00(dd)	$J_{4',5'}$	9.8	10	10	10
NH	6.15(d)	6.01(d)	5.71(d)	5.76(d)	$J_{2,NH}$	8	9	8	8
$COCH_3$	2.10[a]	2.08	2.09	2.08					
	2.08	2.075	2.08	2.05					
	2.06	2.07	2.05	2.04					
	2.04	2.06	2.04	2.02					
	1.98	2.05	2.03[b]	2.01[b]					
		2.02	2.02	2.00					
		2.00[b]	2.00	1.92					

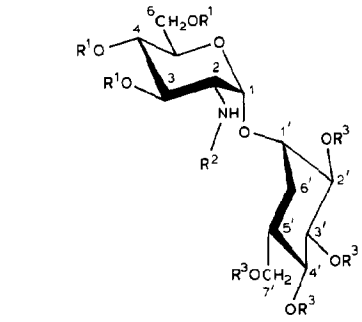
[a]Singlet for four methyl groups. [b]Singlet for two methyl groups.

DL-*(1,2,4/3,5)-5-Acetoxymethyl-2,3,4-tri-*O*-acetyl-1-*O*-allyl-1,2,3,4-cyclohexanetetrol* (**6**). — A mixture of (1*SR*,2*RS*,7*RS*,9*SR*,10*RS*)-4,4,12,12-tetramethyl-3,5,11,13-tetraoxatricyclo[8.3.0.0^{2,7}]tridecan-9-ol[4] (**3**; 0.30 g, 1.2 mmol) and allyl bromide (0.12 mL, 1.4 mmol) was stirred in *N,N*-dimethylformamide (12 mL) in the presence of 50% sodium hydride (85 mg, 1.8 mmol) for 1 h at room temperature. After treatment with methanol, the mixture was concentrated, and the residue was diluted with ethyl acetate, filtered, and concentrated to give the allyl ether **5** (0.39 g) as a yellow syrup. ^{1}H-N.m.r. data (90 MHz): δ 6.16–5.65 (m, 1 H, $CH_2CH{=}CH_2$), 5.39–4.94 (m, 2 H, $CH_2CH{=}CH_2$), 1.51 and 1.44 (2 s, 3 and 9 H, 2 CMe_2).

Compound **5** (0.39 g) was heated in aqueous 80% acetic acid (15 mL) for 1 h at 80°, and the mixture was concentrated. The residue was acetylated with acetic anhydride (4 mL) in pyridine (4 mL) to give a yellow syrupy product (0.53 g), which was eluted from a column of silica gel (C-200, 23 g) with acetone–hexane (1:5) to give **6** (0.41 g, 91% based on **3**), m.p. 79.5–81°. ^{1}H-N.m.r. data (90 MHz): δ 6.16–5.65 (m, 1 H, CH_2CHCH_2), 5.50 (t, 1 H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4), 5.38–4.94 (m, 2 H, $CH_2CH{=}CH_2$), 4.99 (dd, 1 H, $J_{2,3}$ 10.8 Hz, H-3), 4.83 (dd, 1 H, $J_{1,2}$ 3 Hz, H-2), 4.28–3.76 (m, 5 H, H-1, CH_2O and $CH_2CH{=}CH_2$), 2.04, 2.01, and 1.99 (3 s, 6, 3, and 3 H, 4 OAc).

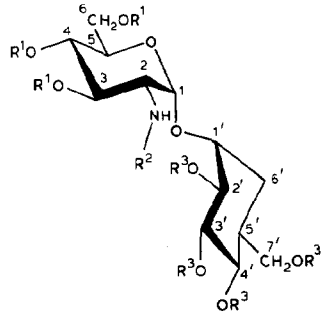
Anal. Calc. for $C_{18}H_{26}O_9$: C, 55.95; H, 6.78. Found: C, 56.10; H, 6.72.

DL-*(1,2,4/3,5)-2,3,4-Tri-*O*-benzyl-5-benzyloxymethyl-1,2,3,4-cyclohexanetetrol* (**8**). — Compound **6** (0.40 g, 1.0 mmol) was treated with methanolic M sodium

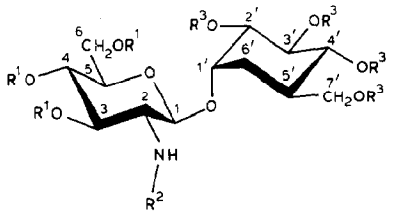


A

2, 11–13

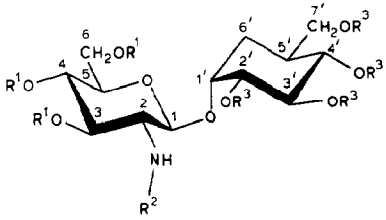


B



A

14–17



B

	R^1	R^2	R^3
11A,B; 14A,B	Ac	DNP	Bn
12A,B; 15A,B	Ac	Ac	Bn
13A,B; 16A,B	Ac	Ac	Ac
2A,B; 17A,B	H	H	H

DNP = 2,4-dinitrophenyl

methoxide (0.8 mL) in methanol (8 mL) for 1 h at room temperature. The mixture was neutralised with Amberlite IRA-120B (H^+) resin (0.8 mL) and then concentrated, and the syrupy residue (223 mg) was stirred with benzyl bromide (0.58 mL, 4.9 mmol) in *N,N*-dimethylformamide (9 mL) in the presence of 50% sodium hydride (0.29 g, 6.1 mmol) for 3 h at room temperature. The mixture was processed as described in the preparation of **5**. The product (0.70 g) was eluted from a column of silica gel (C-200, 35 g) with 2-butanone–toluene (1:20) to give the syrupy tetrabenzyl ether **7** (0.57 g, 95%). ^{1}H-N.m.r. data (90 MHz): δ 7.30 (s, 20 H, 4 Ph), 6.16–5.68 (m, 1 H, $CH_2CH{=}CH_2$), 5.46–5.02 (m, 2 H, $CH_2CH{=}CH_2$), 4.98–4.34 (m, 8 H, 4 CH_2Ph).

Compound **7** (0.57 g, 1.0 mmol) was treated with a boiling solution of selenium dioxide (165 mg, 1.5 mmol) in 1,4-dioxane (14 mL) in the presence of acetic acid (0.09 mL, 1.6 mmol) for 1.5 h. The mixture was then filtered and concentrated, and the syrupy residue (0.67 g) was eluted from a column of silica gel (C-300, 34 g) with 2-butanone–toluene (1:20) to give syrupy **8** (0.40 g, 75%). ^{1}H-N.m.r. data (90 MHz): δ 7.31 (s, 20 H, 4 Ph), 4.87 (s, 2 H), 4.87, 4.53 (2 d, each 1 H, *J* 12 Hz), 4.70 and 4.43 (2 s, each 2 H) (4 CH_2Ph), 4.22–4.00 (m, 1 H, H-1), 2.50–2.33 (m, 1 H, OH).

Compound **8** (20 mg, 0.04 mmol) was acetylated in the usual way to give the syrupy acetate **9** (23 mg, quant.). ^{1}H-N.m.r. data (90 MHz); δ 7.30 (s, 20 H, 4 Ph), 5.60–5.40 (m, 1 H, H-1), 5.08–4.36 (m, 8 H, 4 CH_2Ph), 2.09 (s, 3 H, OAc).

Anal. Calc. for $C_{37}H_{40}O_6$: C, 76.53; H, 6.94. Found: C, 77.02; H, 7.10.

[(1S)-(1,2,4/3,5)-2,3,4-Tribenzyloxy-5-benzyloxymethyl-1-cyclohexyl] 2-deoxy-2-(2,4-dinitrophenylamino)-α- (**11A**) *and -β-*D*-glucopyranoside triacetate* (**14A**), *and the respective (1*R*) diastereoisomers* (**11B** *and* **14B**). — A mixture of **8** (0.50 g, 0.9 mmol), powdered calcium sulfate (1 g), 2,4,6-trimethylpyridine (0.26 mL), and dichloromethane (14 mL) was stirred for 1 h at room temperature. Silver carbonate (0.27 g, 1.0 mmol), silver perchlorate (0.20 g, 1.0 mmol), and a solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenylamino)-α-D-glucopyranosyl bromide[3] (**10**; 1.60 g, 3.0 mmol) in dichloromethane (19 mL) were then added in turn. The mixture was stirred for 4 h, then neutralised with triethylamine, filtered, and concentrated. Elution of the mixture (2.5 g) of products from columns of silica gel [C-300, 60 g, with ethyl acetate–hexane (2:5); and C-300, 52 g, with 2-butanone–toluene (1:12)] gave, first, a mixture (0.61 g) of **11A, 11B**, and **14A** which was further fractionated by elution from a column of silica gel (C-300, 61 g) with ethyl acetate–chloroform (1:30) to give **11B** (76 mg, 8.3%), as a yellow amorphous powder, together with a mixture (0.51 g, 55%) of **11A, 11B**, and **14A**.

Compound **11B** had $[\alpha]_D^{23}$ +4.7° (*c* 3.4, chloroform). ^{1}H-N.m.r. data (90 MHz): δ 9.00–8.63 (m, 2 H, NH and H-3 of DNP), 8.10 (dd, 1 H, $J_{3,5}$ 3, $J_{5,6}$ 10 Hz, H-5 of DNP), 7.30 and 7.20–6.81 (s and m, 15 and 5 H, 4 Ph), 2.04, 2.00, and 1.78 (3 s, each 3 H, 3 OAc).

Anal. Calc. for $C_{53}H_{57}N_3O_{16}$: C, 64.17; H, 5.79; N, 4.24. Found: C, 64.01; H, 5.81; N, 4.08.

Eluted second was **14B** (0.27 g), isolated as a crude yellow syrup.

[(1S)-(1,2,4/3,5)-2,3,4-Tribenzyloxy-5-benzyloxymethyl-1-cyclohexyl] 2-acetamido-2-deoxy-α- (**12A**) *and -β-*D*-glucopyranoside triacetate* (**15A**), *and the respective (1*R*) diastereoisomers* (**12B** *and* **15B**). — The mixture (0.51 g) of **11A, 11B**, and **14A**, methanol (35 mL), acetone (21 mL), water (14 mL), and Amberlite IRA-400 (HO^-) resin (5 mL) was stirred for 3 days at room temperature, then filtered, and concentrated. The residue was acetylated and the syrupy product (225 mg) was eluted from a column of silica gel (C-300, 23 g) with ethyl acetate–chloroform (1:7) to give syrupy **12B** (31 mg, 3.8% based on **8**), then **12A** (72 mg) as a crude syrup, and, finally, amorphous **15A** (74 mg, 9.3% based on **8**).

Similarly, **11B** (76 mg, 0.08 mmol) and a crude syrup (0.27 g) of **14B** were converted, respectively, into syrupy **12B** (27 mg; total, 58 mg, 7.1% based on **8**) and amorphous **15B** (74 mg, 9.2% based on **8**).

Compound **12A** was obtained as a crude syrup. ^{1}H-N.m.r. data (90 MHz): δ 7.34 and 7.32 (2 s, each 10 H, 4 Ph), 5.82 (d, 1 H, $J_{2,NH}$ 9.3 Hz, NH), 2.01 and 1.81 (2 s, 9 and 3 H, NAc and 3 OAc).

Compound **12B** had $[\alpha]_D^{23}$ +6.7° (*c* 1.3, chloroform). ^{1}H-N.m.r. data (90 MHz): δ 7.34 and 7.27 (2 s, 5 and 15 H, 4 Ph), 6.17 (d, 1 H, $J_{2,NH}$ 10.2 Hz, NH), 2.07, 2.05, and 1.55 (3 s, 3, 6, and 3 H, NAc and 3 OAc).

Anal. Calc. for $C_{49}H_{57}NO_{13}\cdot 0.5\ H_2O$: C, 67.11; H, 6.67; N, 1.60. Found: C, 67.22; H, 6.72; N, 1.56.

Compound **15A** had $[\alpha]_D^{21}$ +31° (*c* 2.4, chloroform). ^{1}H-N.m.r. data (90 MHz): δ 7.44, 7.39, 7.35, and 7.33 (4 s, each 5 H, 4 Ph), 5.61 (d, 1 H, $J_{2,NH}$ 9 Hz, NH), 2.05, 2.00, 1.96, and 1.64 (4 s, each 3 H, NAc and 3 OAc).

Anal. Calc. for $C_{49}H_{57}NO_{13}$: C, 67.80; H, 6.62; N, 1.61. Found: C, 67.58; H, 6.61; N, 1.47.

Compound **15B** had $[\alpha]_D^{21}$ −35° (*c* 3.7, chloroform). ^{1}H-N.m.r. data (90 MHz): δ 7.30 (s, 20 H, 4 Ph), 5.71 (d, 1 H, $J_{2,NH}$ 9 Hz, NH), 1.98 and 1.77 (2 s, 9 and 3 H, NAc and 3 OAc).

Anal. Found: C, 67.08; H, 6.60; N, 1.42.

[(1S)-(1,2,4/3,5)-2,3,4-Trihydroxy-5-hydroxymethyl-1-cyclohexyl] 2-acetamido-2-deoxy-α- (**13A**) *and -β-*D*-glucopyranoside hepta-acetate* (**16A**)*, and the respective (1*R*) diastereoisomers* (**13A** *and* **16B**). — A solution of crude **12A** (72 mg) in ethanol (10 mL) was hydrogenated in the presence of 10% Pd/C (50 mg) in a Parr shaker apparatus (initial hydrogen pressure of 3.4 kg/cm^2) for 2.5 h at room temperature, then filtered, and concentrated. The residue was acetylated and the product was eluted from a column of silica gel (C-300, 6.2 g) with 2-butanone–toluene (1:2) to give amorphous **13A** (26 mg, 4.2% based on **8**), $[\alpha]_D^{21}$ +113° (*c* 1.3, chloroform).

Likewise, **12B** (58 mg, 0.07 mmol), **15A** (75 mg, 0.09 mmol), and **15B** (74 mg, 0.09 mmol) were converted, respectively, into **13B** (41 mg, 92%), m.p. 170–171° (from EtOH), $[\alpha]_D^{21}$ +35° (*c* 1, chloroform); **16A** (48 mg, 82%), m.p. 174–175° (from EtOH), $[\alpha]_D^{21}$ +21° (*c* 1, chloroform); and **16B** (50 mg, 87%), m.p. 194–194.5° (from EtOH), $[\alpha]_D^{22}$ −64° (*c* 1, chloroform). For the ^{1}H-n.m.r. data (200 MHz) of these compounds, see Table I.

Anal. Calc. for $C_{29}H_{41}NO_{17}$: C, 51.55; H, 6.12; N, 2.07. Found: **13A**, C, 51.42; H, 6.09; N, 1.96; **13B**, C, 51.30; H, 6.06; N, 2.18. Calc. for $C_{29}H_{41}NO_{17}\cdot 0.5\ H_2O$: C, 50.88; H, 6.18; N, 2.05. Found: **16A**, C, 50.99; H, 6.04; N, 2.01; **16B**, C, 50.77; H, 5.94; N, 1.89.

[(1S)-(1,2,4/3,5)-2,3,4-Trihydroxy-5-hydroxymethyl-1-cyclohexyl] 2-amino-2-deoxy-α- (**2A**) *and -β-*D*-glucopyranoside* (**17A**)*, and the respective (1*R*) diastereoisomers* (**2B** *and* **17B**). — Compound **13A** (22 mg, 0.03 mmol) was heated in 80% hydrazine hydrate (0.75 mL) for 0.5 h at 70°. After cooling, the mixture was concentrated. The products (29 mg) were eluted from a column of Dowex 50W-X2 (H^+) resin (2.9 mL) with methanol to give amorphous **2A** (12 mg, quant.), $[\alpha]_D^{23}$ +156° (*c* 0.6, methanol).

Likewise, **13B** (28 mg, 0.04 mmol), **16A** (40 mg, 0.06 mmol), and **16A** (42 mg, 0.06 mmol) were converted, respectively, into **2B** (15 mg, quant.), $[\alpha]_D^{23}$ +50° (*c* 0.8, methanol); **17A** (20 mg, quant.), $[\alpha]_D^{23}$ +21° (*c* 1, methanol); and **17B** (20 mg, 96%), $[\alpha]_D^{23}$ −73° (*c* 1, methanol); isolated as amorphous powders.

These amines, without further purification, were assayed[7] against *Klebsiella pneumoniae* No. 126, *Staphylococcus aureus* 209P, and *Bacillus subtilis* PCI-219.

AGAR DIFFUSION ASSAY OF **2A** AND 2-TREHALOSAMINE

Organism	*Medium*	*Inhibition zone (mm) at 2 mg/mL*	
		2A	*2-Trehalosamine*
Klebsiella pneumoniae No. 126	No. 1003[a]	13[W]	24[W]
Staphylococcus aureus 209P	NA[b]	—	27[W]
Bacillus subtilis PCI-219	NA	—	25

[a]Peptone, 10 g; meat extract, 5 g; and agar, 12 g/litre. [b]NA, nutrient agar; W, hazy inhibition zone; —, no inhibition zone.

ACKNOWLEDGMENTS

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