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Preliminary communication

Synthesis of "dihydroacarbose", a potent α-D-glucosidase inhibitor

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Acarbose (**1**), having a pseudotetrasaccharide structure, is one of the microbial secondary metabolites that exhibit strong inhibitory activity against such α-D-glucosidases as sucrase, found in the wall of the small intestine[1]. The Bayer AG group also isolated[2], in only 2% yield, "dihydroacarbose"† (**2**) from the hydrogenation mixture of **1**, and found that it exhibited potent enzyme inhibitory activities[3] like those of **1**. Despite many efforts directed toward total synthesis of this type of enzyme inhibitor, the successful results so far reported[4], were confined to homologous pseudotrisaccharides, which are less potent inhibitors than **1** (ref. 1). We now describe the synthesis of **2**; this can be regarded as the first example of chemical construction of a pseudotetrasaccharide skeleton. A characteristic feature of this total synthesis of **2** was employment of a polysaccharide, namely pullulan (**3**)¶, as the actual starting material for preparation of the trisaccharide synthon, 4″-amino-1,6-anhydro-2,3,2′,3′,6′,2″,3″-hepta-*O*-benzyl-4″,6″-dideoxy-β-maltotriose (**13**).

Crystalline pentachlorophenyl 2,3,6,2′,3′,6′,2″,3″,4″,6″-deca-*O*-acetyl-β-maltotrioside (**4**), m.p. 109–112°, $[\alpha]_D^{23}$ +114° (*c* 0.63, $CHCl_3$); δ§ 5.26 (d, 1 H, $J_{1,2}$ 9.9 Hz, H-1) was prepared in 53% overall yield from **3** by a series of successive reactions with (*i*) 1% (w/w) equivalent of pullulanase (Hayashibara, Inc., 2000 units/g), (*ii*) Ac_2O–AcONa, (*iii*) HBr–AcOH, and (*iv*) sodium pentachlorophenolate–acetone. Similarly to our previous experiments with disaccharides[5], **4** was treated with aq. KOH, and the resulting 1,6-anhydromaltotriose (**5**) was per-*O*-acetylated for isolation of **6**, m.p. 159–161° (lit.[6] m.p. 156.5–157°). Basic treatment of **6** regenerated **5** as an amorphous powder which underwent benzylidenation at O-4″ and -6″ with $PhCH(OMe)_2$–TsOH–DMF, and per-*O*-benzylation of the product with BnBr–NaH–DMF gave **7** (67% from **5**), $[\alpha]_D^{23}$ +18° (*c* 0.28, $CHCl_3$).

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†This term is proposed as a trivial name for compound **2**.
¶Pullulan is extremely low-priced in comparison with its repeating unit, maltotriose.
§All ^1H-n.m.r. spectra were recorded at 400 MHz for solutions in $CDCl_3$, with Me_4Si as an internal standard unless otherwise specified.

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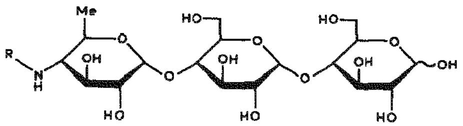
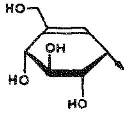


1 R =



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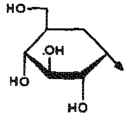
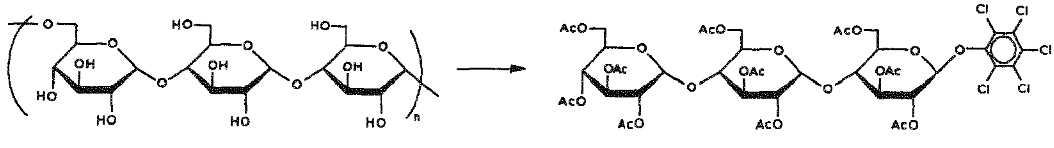
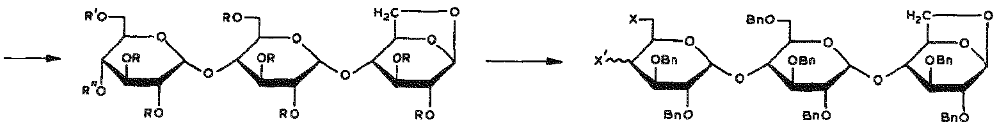


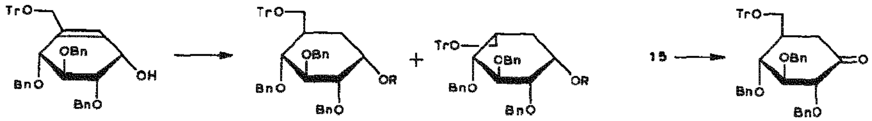


3 4



5 R = R' = R" = H
6 R = R' = R" = Ac
7 R = Bn, R', R" = PhCH
8 R = Bn, R' = R" = H

9 X = I, X' = OH (α)
10 X = H, X' = OH (α)
11 X = H, X' = I (β)
12 X = H, X' = N_3 (α)
13 X = H, X' = NH_2 (α)



14

15 R = H
16 R = Bz

17 R = H
18 R = Bz

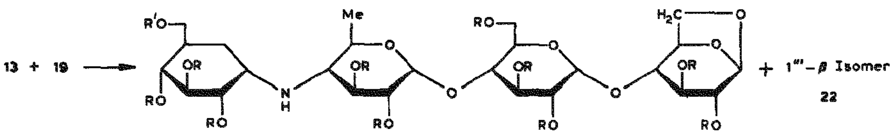
19

The benzylidene group was removed with aq. CF_3CO_2H, and the resulting diol **8**, $[\alpha]_D^{23}$ +25° (*c* 0.83, $CHCl_3$), was selectively iodinated with *N*-iodosuccinimide–Ph_3P–DMF, giving the 6"-iodide **9**, $[\alpha]_D^{18}$ +25° (*c* 0.83, $CHCl_3$); ν_{max}^{film} 3450 cm^{-1} (OH). Reduction of **9** with $LiAlH_4$ gave the 6"-deoxy compound **10** (72%), $[\alpha]_D^{24}$ +23° (*c* 1.1, $CHCl_3$); δ 1.15 (d, 3 H, $J_{6'',5''}$ 6.1 Hz, H-6"). Compound **10** underwent Garegg halogenation[7] with Ph_3P–triiodoimidazole–imidazole, to give **11** (82%), $[\alpha]_D^{20}$ +59° (*c* 1.2, $CHCl_3$); δ 4.36 (dd, 1 H, $J_{3'',4''}$ 3.7, $J_{4'',5''}$ 1.3 Hz, H-4") with configurational inversion. Further SN2 replacement of the iodide with azido anion was conducted, giving **12**, $[\alpha]_D^{21}$ +63° (*c* 0.50, $CHCl_3$); ν_{max}^{film} 2100 cm^{-1} (N_3); δ 3.03 (t, 1 H, $J_{3'',4''} = J_{4'',5''}$ = 9.8 Hz, H-4"). $LiAlH_4$ reduction of **12** resulted in **13** (60%), $[\alpha]_D^{23}$ +27° (*c* 0.60, $CHCl_3$); ν_{max}^{film} 3450 cm^{-1} (NH_2).

The other synthon of **2**, 2D-(2,4/3,5)-2,3,4-tri(benzyloxy)-5-(trityloxymethyl)-cyclohexanone (**19**) was prepared as follows. Catalytic hydrogenation of

1D-(1,2,4/3)-2,3,4-tri-*O*-benzyl-5-(trityloxymethyl)-5-cyclohexene-1,2,3,4-tetrol[8] (**14**) with PtO_2 afforded an inseparable mixture of 1D-(1,2,4/3,5) and 1D-(1,2,4,5/3) isomers (**15** and **17**) of pentasubstituted cyclohexane. Chromatographic separation of their 1-benzoates (**16** and **18**, 1:1 ratio) was achieved, and the isolated **16**, $[\alpha]_D^{22}$ +46° (*c* 1.70, $CHCl_3$); δ 1.96 (dt, 1 H, $J_{6a,1}$ 2.0, $J_{6a,6e}$ = $J_{6a,5}$ 11 Hz, H-6*a*), was treated with base, to regenerate **15**, $[\alpha]_D^{23}$ +17° (*c* 0.46, $CHCl_3$); ν_{max}^{film} 3500 cm^{-1} (OH). The hydroxyl group of **15** was oxidized in CH_2Cl_2 with Me_2SO–$(CF_3CO)_2O$–Et_3N, giving **19**, $[\alpha]_D^{23}$ +33° (*c* 1.1, $CHCl_3$); ν_{max}^{film} 1730 cm^{-1} (C=O).

As reductive amination of **19** with **13** competed with simple carbonyl reduction of **19**, a mixture of **13** and 2 mol. equiv. of **19** was treated in MeOH–CH_2Cl_2–AcOH at pH 6.2 with $NaBH_3CN$ in the presence of molecular sieves 3Å, following the model reaction of Köhn and Schmidt[9]. After chromatographic separation, the desired pseudotetrasaccharide (**20**); $[\alpha]_D^{22}$ +39° (*c* 1.6, $CHCl_3$); ν_{max}^{KBr} 3350 cm^{-1} (NH); δ 1.36 (dt, 1 H, $J_{6'''a,5'''}$ = $J_{6'''a,6'''e}$ = 13, $J_{1''',6'''a}$ <1 Hz, H-6‴*a*), was obtained in 30% yield, together with a 4% yield of 1‴-β isomer (**22**). The simple reduction products, **15** and its 1-β isomer, obtained in 50% yield, were oxidized, to regenerate **19** (*vide supra*). Compound **13** remaining in excess was also recovered, and could



20 R = Bn, R' = Tr
21 R = R' = Ac

(**20**:**22** = 8:1)

be recycled. Removal of all protecting groups from **20** by treatment with Na–liquid NH_3, and acetylation of the product with Ac_2O–pyridine at room temperature gave **21**; $[\alpha]_D^{24}$ +81° (*c* 0.60, $CHCl_3$); ν_{max}^{KBr} 3400 cm^{-1} (NH); δ 1.44 (dt, 1 H, $J_{6'''a,5'''}$ = $J_{6'''a,6'''e}$ = 14, $J_{1''',6'''a}$ = ~2 Hz, H-6‴*a*), and 4.89 (dd, 1 H, $J_{2''',3'''}$ 10.3, $J_{1''',2'''}$ 4.4 Hz, H-2‴). The imino group was never acetylated under these reaction conditions[4a,10]. Acetolysis of **21** was conducted at room temperature with 40:40:1 (v/v) Ac_2O–AcOH–conc. H_2SO_4, giving an anomeric mixture of per-*O*-acetylated **2** (84%, α:β = 9:1); δ 5.75 (d, 0.1 H, $J_{1,2}$ 8 Hz, β-H-1) and 6.25 (d, 0.9 H, $J_{1,2}$ 3.7 Hz, α-H-1). After deacetylation of the mixture by the Zemplén procedure, the product was desalted with CM-Sephadex C-25 (NH_4^+), giving **2** (87%); $[\alpha]_D^{25}$ +121° (*c* 0.17, H_2O, equilibrium) (lit.[2] $[\alpha]_D^{25}$ +141.3° (*c* 0.3, H_2O); δ[♀] (D_2O) 3.36 (dd, 1 H, $J_{1''',2'''}$ 4.0, $J_{2''',3'''}$ 10.0 Hz, H-2‴), 4.49 (d, 0.5 H, $J_{1,2}$ 8.05 Hz, H-1β), and 5.07 (d, 0.5 H, $J_{1,2}$ 4.15 Hz, H-1α); s.i.m.s. *m/z* 648 (M^+ + H). The ¹H-n.m.r. spectrum of synthetic **2** was in good conformity with that[2] of the hydrogenated products of natural **1**, except for their anomeric ratio.

[♀]Chemical shifts were referenced to the HOD peak as 4.67 p.p.m. at 20°.

This preparation exhibited strong inhibition (IC_{50} 1.2×10^{-6}M) of sucrase activity in the mucosa of small intestines obtained from rats.

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