First Regiospecific Synthesis of $6^A,6^C,6^E$-Tri-*O*-methylcyclomaltohexaose

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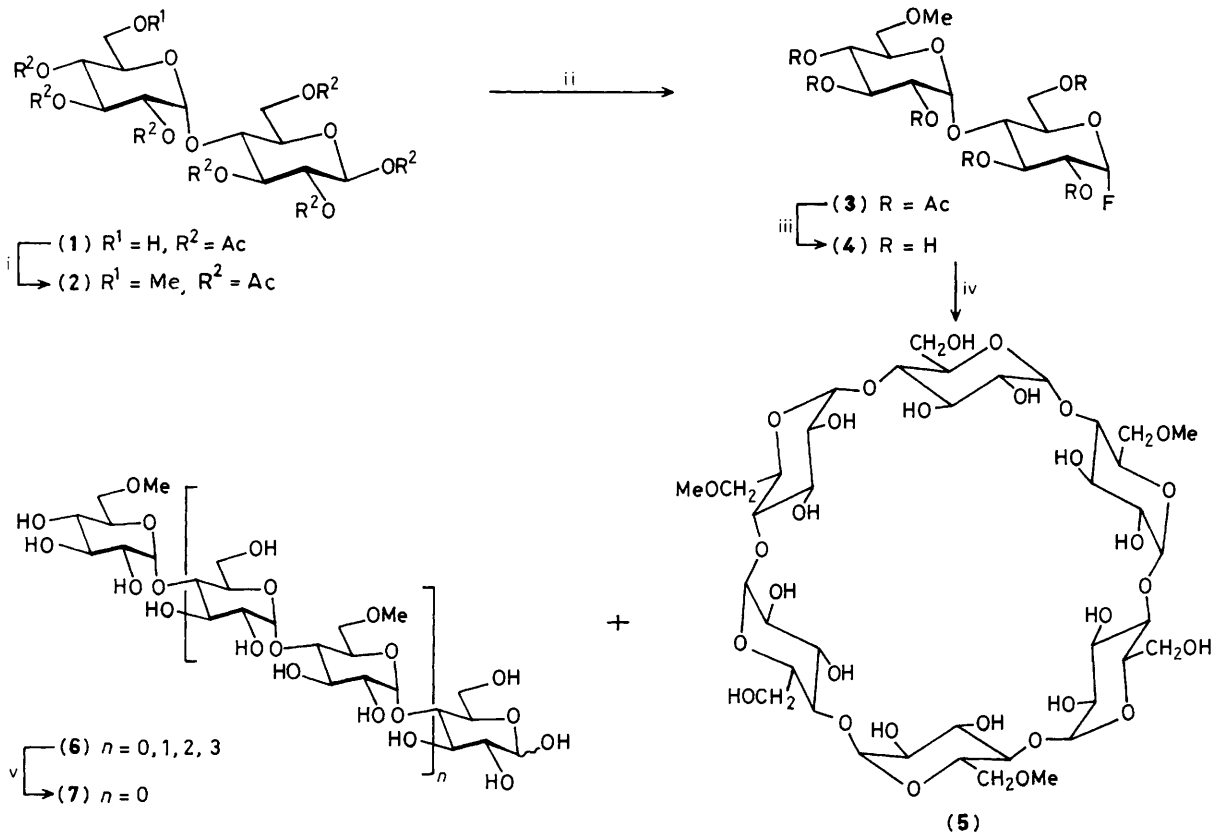
The title compound (**5**) was obtained in good yield by the effective condensation and cyclisation of 6′-*O*-methyl-α-maltosyl fluoride (**4**) with cyclodextrin glucosyltransferase.

In the last decade, specific preparations of primary di- or tri-substituted cyclodextrins have been studied in order to construct sophisticated models of enzymes.[1] However, difficulties in the purification and characterization of these compounds have hampered progress in this field. A total chemical synthesis could be theoretically possible since the first chemical synthesis of a natural cyclodextrin was published in 1985.[2] However, the poor overall yield is a limitation of this method. In the course of the studies of the specificity of the active site of cyclodextrin glycosyltransferase (CGTase), the regiospecific synthesis of $6^A,6^C,6^E$-tri-*O*-methylcyclomaltohexaose is reported.

To examine the structural requirements of both acceptor and donor parts of the active site of CGTase, modified



Scheme 1. *Reagents and conditions:* i, $MeOS(O_2)CF_3$, 2,6-di-t-butylpyridine, CH_2Cl_2, 2.5 h, 80 °C, 97%; ii, HF–pyridine (70:30 v/v), pyridine, 25 min, 0 °C, 85%; iii, (a) MeONa–MeOH (1 M, 1%), 2 h, room temp., (b) IRN77 H^+, 100%; iv, CGTase phosphate buffer (pH 6.5, 0.1 M) 20 h, 50 °C; v, Taka-amylase phosphate buffer (pH 6.5, 0.1 M) 1 h, 40 °C.

α-maltosyl fluorides were used. This strategy was suggested by the pioneering work of Hehre *et al.*[3] in which CGTase was found to be able to utilize α-maltosyl fluoride for *de novo* synthesis of natural cyclodextrins.

Methylation with methyl trifluoromethanesulphonate[4] of the known[5] 1,2,2′,3,3′,4′,6-hepta-*O*-acetyl-β-maltose (**1**) gave the crystalline 6′-*O*-methyl derivative (**2**).† Treatment of (**2**) with HF–pyridine afforded the key compound (**3**) in good yield from maltose. De-*O*-acetylation of the crystalline fluoride (**3**) gave the corresponding compound (**4**). In a typical enzyme experiment, to the fluoride (**4**) (0.82 mmol) in phosphate buffer (15 ml), CGTase (635 U/ml, 400 μl) was added and the decrease of the fluoride (**4**) was monitored by h.p.l.c. (NH_2-μ-Bondapack; acetonitrile–water, 70:30). The reaction mixture was then heated (100 °C, 1 min), centrifuged (12 000 rev./min; 15 min), and the supernatant was evaporated. Separation of compounds (**5**) and (**6**) was achieved by p.l.c. on modified silica gel (C-18) by fractional elution with aqueous methanol. Elution with 20% and 50% methanol afforded (**6**) (49%) and (**5**) (43%), respectively. The ^{13}C n.m.r. spectrum of (**5**) shows a simple pattern compatible with a regular disaccharide repeating unit, and also matches the C_3 molecular symmetry. The expected substitution effect of methyl groups at C-6 (δ 71.7 *vs.* 61.4) was assigned by using the DEPT (distortionless enhancements by polarisation techniques) sequence.

† All new compounds reported here gave spectral and analytical data consistent with assigned structures, except for (**4**), which was shown to be pure by h.p.l.c.

Further treatment of (**6**) with Taka-amylase (34 U/mg, 5 mg) in the above buffer (5 ml) led quantitatively to (**7**). This result is in good agreement with the action of Taka-amylase on mono-, di-, and tri-substituted cycloamyloses.[6—8]

Thus the present enzymatic synthesis affords the easily purified tri-*O*-methyl cyclomaltohexose (**5**) in an overall yield of *ca.* 12% from the commercially available maltose.

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