The first hetero-bifunctionalization of the secondary face of β-cyclodextrin: selective and efficient conversion of the A-ring of a $2^A,2^B$-disulfonate to $2^A,3^A$-epoxymannoside

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Received (in Cambridge, UK) 22nd February 2005, Accepted 22nd April 2005
First published as an Advance Article on the web 18th May 2005
DOI: 10.1039/b502573g

The A-ring of $2^A,2^B$-*O,O*-di(mesitylenesulfonyl)-β-cyclodextrin was converted to $2^A,3^A$-epoxymannoside without affecting the other sulfonylated residue, which affords the first approach to hetero-bifunctionalization at the secondary hydroxyl side of cyclodextrins.

Cyclodextrin (CD) derivatives bearing two different sorts of functionalities turn out to be interesting in developing artificial receptors and enzyme-like catalysts.[1] The hetero-bifuntional CDs not only effect a three point recognition of asymmetric species, but also exercise strong asymmetric induction in catalyzing chemical transformations such as transamination reactions.[2] However these hetero-bifunctional CDs are very difficult to access. The routine method includes stepwise substitution of $6^A,6^B$-dideoxy-$6^A,6^B$-diodo-β-CD with different nucleophiles.[2] An alternative method includes the sulfonylation of mono-substituted CDs.[3] In either case, the reaction is rather a random one and subject to competition from over-substitution. In this situation, we developed $6^A,6^B$-*O,O*-mesitylenedisulfonyl-capped β-CD which was subjected to a highly regioselective S_N2 reaction with imidazole on C-6^B (42% yield) compared to C-6^A (4%).[4] However, there has been no report about hetero-bifunctionalization at the secondary hydroxyl side.

We report here the first hetero-bifunctionalization at the secondary hydroxyl side of β-CD which includes selective conversion of the 2^A-*O*-sulfonylglucoside residue of $2^A,2^B$-*O,O*-di(mesitylenesulfonyl)-β-CD (**1**)[5] to the $2^A,3^A$-epoxymannoside residue while leaving the other sulfonylated residue unaffected, simply by stirring the disulfonate **1** in a buffer solution.

Compound **1** (516 mg), which was prepared by the reaction of β-cyclodextrin with mesitylenesulfonyl chloride in the presence of NaH in DMF, was added to 30 mL of a phosphate buffer solution (pH 12.0, 0.1 M) and kept at rt. The reaction progress was monitored by TLC.† After 1 h, the starting material **1** disappeared completely and monoepoxides **2** were predominate in the reaction products, while diepoxide **5** was only formed in a trace amount. The solution was neutralized with HCl and evaporated to dryness *in vacuo*. The residue was dissolved in 15% aq ethanol (400 mL),

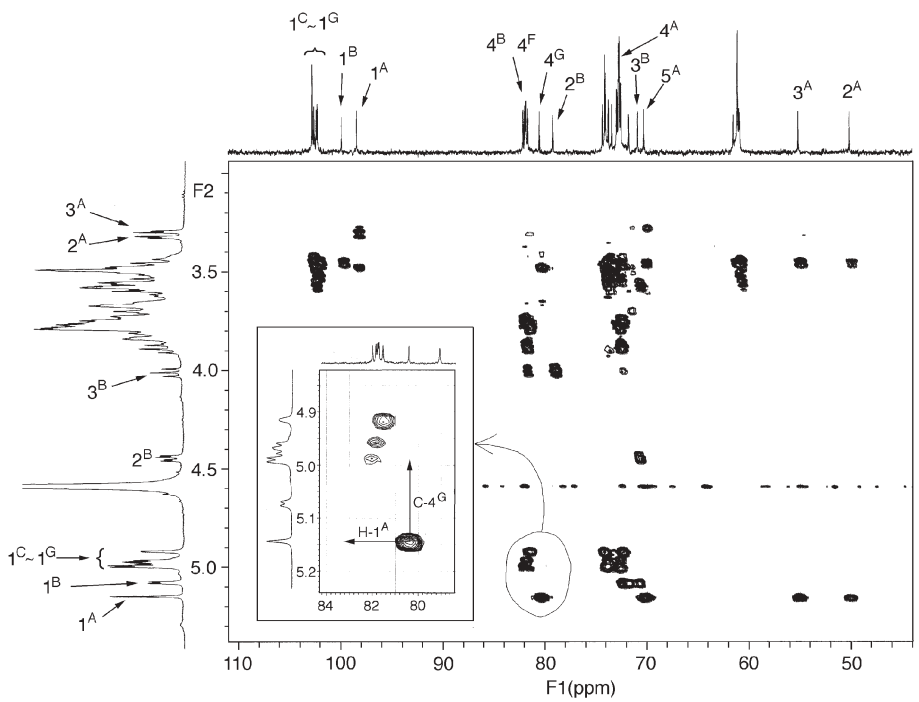


Fig. 1 HMBC NMR spectrum of the sugar part of compound **2a** in D_2O solution. The inset shows the (H-1^{X+1})–(C-4^X) correlations. Signal assignments were made based on ^1H–^1H and ^1H–^{13}C COSY spectra.

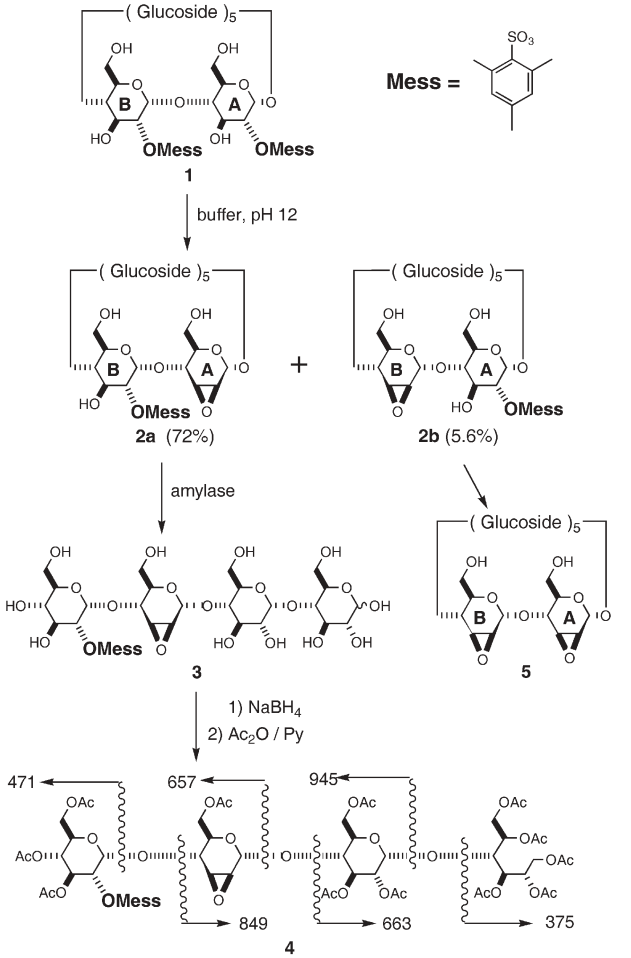
membrane-filtered (cellulose acetate, 0.2 μm), and chromatographed (Rp-18 Lobar column, size C) to give **2a** (323 mg, 72.2%) and **2b** (25 mg, 5.6%). **2a**: mp 189 °C (decomp); R_f = 0.51; $[\alpha]_D^{25}$ = +109 (c = 0.100 in H_2O); **2b**: mp 183 °C (decomp); R_f = 0.51;† $[\alpha]_D^{21}$ = +107 (c = 0.100 in H_2O).

Both compounds **2a** and **2b** gave the common pseudo parent peak [M + Na^+] at *m/z* 1321 in their FAB MS spectra, and therefore proved to be the mono-epoxy-mono-2-*O*-mesitylenesulfonyl-β-CDs, indicating that only one of the *O*-sulfonyl glucosides was selectively converted to the 2,3-epoxymannoside.

Fig. 1 shows the HMBC NMR spectrum of **2a** and the assignments were made based on ^1H,^1H- and ^1H,^{13}C-COSY NMR spectra. The epoxide residue of **2a** showed its C-2 at δ 50.2 and C-3 at δ 55.2 ppm. These very low chemical shifts together with the singlet pattern of H-1 (δ 5.15) indicated that the epoxide residue of **2a** is of a manno-type,[6,7] whose formation is expected from the stereochemistry of the intramolecular nucleophilic substitution reaction of **1**. The HMBC spectrum is powerful in determining the saccharide sequence by affording long range H–C hetero-nuclei correlations between the O-bridged positions of two adjacent sugar residues. The NMR signals of all the C-4 carbons and H-1 protons are far separated from those of other nuclei, and therefore the (H^{X+1}-1)-(C^X-4) cross peaks may provide reliable information about the (C^{X+1}-1)-O–(C^X-4) connections. As shown in Fig. 1, one singlet at δ 5.15 and one doublet at δ 5.08 were observed for the H-1 protons of the 2,3-epoxymannoside and 2^A-*O*-sulfonyl-glucoside residues, and they are clearly separated from other anomeric protons. Six peaks of C-4 and four (H^{X+1}-1)–(C^X-4) correlation islands were observed. Although not all of the C-4 signals could be definitely assigned based on the COSY spectra, the one resonating at δ 80.6 can be unambiguously assigned to an unmodified glucoside residue and this peak makes sense in determining the sequence of the two modified sugar units because it is clearly correlated to H-1 of the 2,3-epoxymannoside, suggesting the epoxymannoside-(1 → 4)-glucoside junction while ruling out the epoxymannoside-(1 → 4)-mesitylenesulfonylglucoside one. However, direct evidence for the sulfonylglucoside-(1 → 4)-epoxymannoside junction was not obtained because H-1 of the sulfonylglucoside residue did not correlate to any C-4 carbons. In order to gather additional evidence for the structural assignment, an enzymatic hydrolysis of **2a** was carried out.

A mixture of **2a** (203 mg) and α-amylase (EC 3.2.1.1 from *Aspergillus oryzae*, Sigma) (203 mg) in 0.2 M acetate buffer (pH 5.5, 20 mL) containing 0.01 M $CaCl_2$ was allowed to stand for 2.5 days at 40 °C. After being heated for 10 min in a boiling water-bath, the mixture was membrane-filtered (cellulose acetate, 0.8 μm), diluted to 250 mL with water and chromatographed on a reversed-phase Lobar column (Rp-18, size C) to afford the major product **3** (104 mg, 80%). R_f = 0.65.†

Compound **3** gave the pseudo parent peak [M + Na^+] at *m/z* 853 in the MS spectrum, indicating that it was mono-epoxy-mono-*O*-mesitylenesulfony-maltotetraose. Compound **3** was reduced with $NaBH_4$ and subsequently acylated to give **4** which displayed the parent peak [M + Na^+] at *m/z* 1359 in the TOF-MS spectrum. The EI-MS spectrum of **4** was measured and analysis of the fragmentation patterns (Scheme 1) indicated that the sulfonylated sugar unit is located at the non-reducing end with the epoxy-sugar unit being next. This result confirms the structure figured out

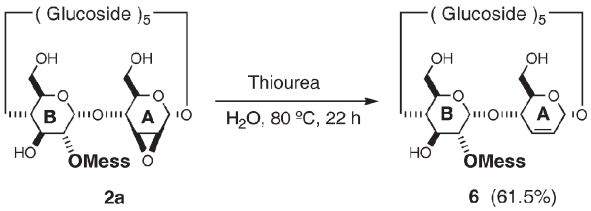


Scheme 1 Selective formation of epoxide **2a** from 2^A,2^B-disulfonate **1** and the fragmentation pattern (*m/z*) observed in the EI-MS spectrum of **4** which demonstrated the pseudo parent peak [M + Na^+] at *m/z* 1359 in its TOF-MS spectrum.

based on the above NMR spectral analysis. The formation of the tetraose structure during the enzymatic hydrolysis is consistent with the degradation pattern of α-amylase clarified in our previous study on the enzymatic hydrolysis of mono-mannoepoxy-β-CD and 2-*O*-sulfonylated β-CDs which gave 2″,3″-mannoepoxy-maltotetraose and 2″-*O*-sulfonylated maltotrioses, respectively.[8] The present result further builds up the database of this enzyme.

The positional alternative **2b** was structurally determined by transformation to diepoxide **5**, which gave the same spectral data as the authentic compound.[7]

A kinetics study indicated that, in comparison with 2-*O*-mesitylenesulfonyl-β-CD, the A-ring of **1** reacted 2.7 times



Scheme 2 Selective conversion of epoxide **2a** to olefin **6**.

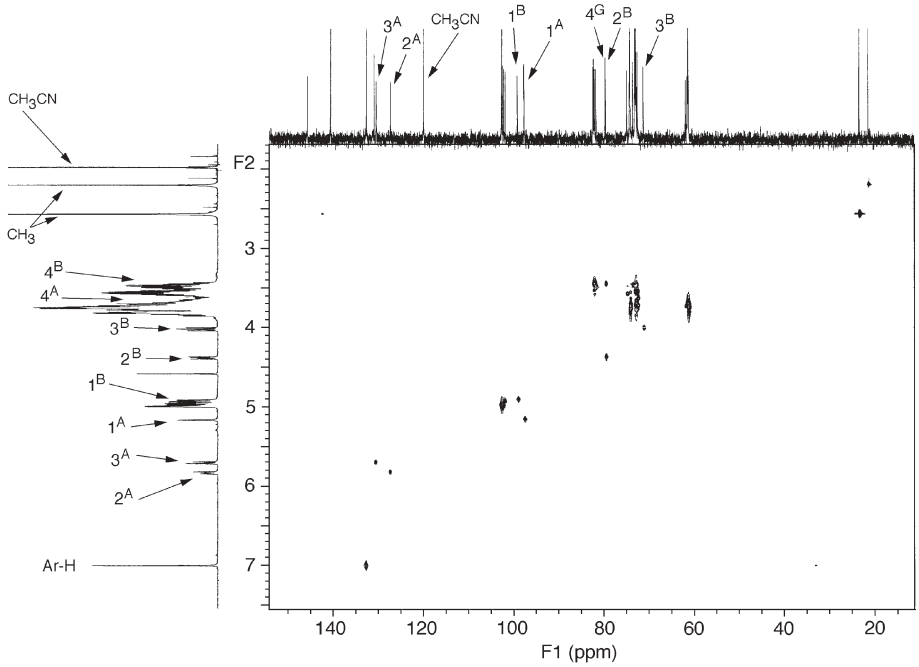


Fig. 2 $^{1}H,^{13}C$-COSY NMR spectrum of olefin **6** in D_2O solution. CH_3CN was used as internal standard.

faster while the B-ring reacted 5.0 times slower in a phosphate buffer solution (pH 12.0, 0.1 M) at 15 °C. The A,C- and A,D-regioisomers of **1** did not display meaningful ring-preference, and the corresponding intermediate epoxy-sulfonates did not accumulate as the major products.

Compound **2a** may serve as a versatile starting material for further functionalization since epoxide and sulfonate have basically different reactivities and are both susceptible to diverse chemical transformations. As one example, compound **2a** was converted to the corresponding olefin species **6** simply by heating an aqueous solution of **2a** in the presence of thiourea (Scheme 2). A solution of **2a** (200 mg) and thiourea (587 mg) in 10 mL of water was stirred at 80 °C for 22 h and then poured into 190 mL of acetone. The formed precipitate was membrane-filtered (cellulose acetate, 0.2 mm), dissolved in 400 mL of water and chromatographed on a reversed-phase Lobar column (Rp-18, size C) with an elution of 20% aq ethanol (1000 mL) and then a gradient elution from 20% to 50% aq ethanol (1000 mL each) to give **6** (121 mg, 61.5%). Compound **6** gave the pseudo parent peak $[M + Na^+]$ at *m*/*z* 1305 in the TOF MS spectrum. Fig. 2 shows the $^{1}H–^{13}C$ COSY NMR spectrum of **6** and assignments were made based on $^{1}H–^{1}H$, $^{1}H–^{13}C$ COSY and HMBC NMR spectra. The mesitylenesulfonyl group is clearly demonstrated in the spectrum and the sugar unit bearing this substituent has the same chemical shift patterns as those of unit B of the precursor **2a** (*cf.* also Fig. 1). However, the chemical shift patterns (both ^{1}H and ^{13}C) of unit A are remarkably different from those of the corresponding unit in **2a**, with the H-2^A, H-3^A, C-2^A and C-3^A nuclei being all shifted from the highest field to the lowest field in the sugar region, which is consistent with the formation of the olefin functionality.‡[9] The result indicates that the 2^B-sulfonate functionality is stable enough to sustain until the completion of the conversion of the epoxide residue to an olefin function. Thus, for the first time, we succeeded in hetero-bifuctionalization at the secondary hydroxyl side of β-CD.

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Notes and references

† TLC was performed on pre-coated silica gel with a mixed solvent of *n*-C_3H_7OH–AcOEt–H_2O: 7 : 7 : 5.

‡ The chemical shifts of the units A and B of compound **6**: δ 5.17 (d, J = 2.6 Hz; H-1^A), 5.83 (dt, J = 10.5, *ca.* 1.7 Hz; H-2^A), 5.70 (d, J = 10.5 Hz; H-3^A), 4.92 (d, J = 3.7 Hz; H-1^B), 4.38 (dd, J = 9.9, 3.5 Hz; H-2^A), 4.02 (t, J = 9.4 Hz; H-3^A); δ 97.3 (C-1^A), 127.4 (C-2^A), 131.1 (C-3^A), 98.8 (C-1^B), 79.3 (C-2^B), and 71.0 (C-3^B).

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