Selective modification of β-cyclodextrin: an unexpected tandem reaction enables the cross-linking of $C2^A$ and $C2^B$ *via* a sulfur atom

Makoto Fukudome,[a] Kazuki Yoshikawa,[a] Kazutaka Koga,[b] De-Qi Yuan*[a] and Kahee Fujita*[a]

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2^A,3^A-Alloepithio-2^B-sulfonyl-β-cyclodextrin undergoes a tandem reaction to generate an unprecedented $C2^A$–S–$C2^B$-bridged glucosyl-3^A,6^A-anhydroglucoside segment.

Hetero-bifunctionalization of cyclodextrin (CD) has attracted much attention in artificial enzyme development.[1] However, even CDs hetero-bifunctionalized on the primary hydroxyl sides are very difficult to access,[2] except in only one case.[3] Moreover, no hetero-bifunctionalization of the secondary hydroxyl sides had been reported until we recently demonstrated the efficient synthesis of **2** from 2^A,2^B-*O*,*O*-di(mesitylenesulfonyl)-β-CD **1** (Scheme 1).[4] Herein we report the selective synthesis of alloepithio-sulfonate **3** and an unexpected tandem reaction of **3** to generate the first tetracyclic residue within the macrocycle belt.

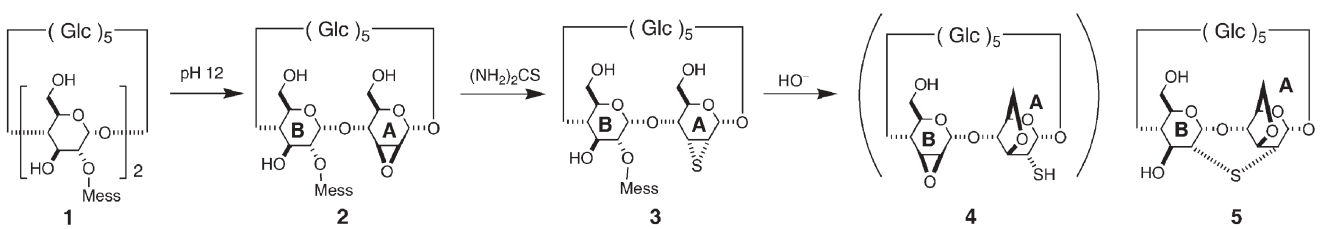
As part of a continuing project into the hetero-bifunctionalization of CDs, we attempted to synthesise the target structure **4**. Compound **3** can be obtained from **2** in 67.8% yield by stirring **2** and thiourea in 0.1 HCl solution at 70 °C, followed by treatment with $NaHCO_3$ (*cf.* Table 1 for the structural data).[5]† When treated by 1 M NaOH at 70 °C, **3** underwent a very clean reaction, affording a pure product in 73% yield.† However, the structural assignment revealed that this product takes the structure of **5** rather than the expected **4**!

The TOF mass spectrum demonstrated a peak at *m*/*z* 1137 which is consistent with the [M + Na] ion of both **4** and **5**. The evidence for the structure of **5** was gathered from detailed NMR spectral analysis. As shown in Fig. 1, only two carbon signals can be recognized in the range δ 30–60 ppm where the 2,3-epoxy- or thio-bearing carbons should appear, one peak less than expected from the structure of **4**. ^1H–^1H and ^1H–^{13}C COSY experiments, which enabled the extraction of chemical shifts relating the nuclei of residues A and B (Table 1), indicated that the two peaks at δ 48.2 and 54.1 ppm correspond to the $C2^A$ of the residue A and the $C2^B$ of the residue B, respectively. The formation of a single S-bridge between the $C2^A$ and $C2^B$ was strongly suggested by the appearance of strong HMBC signals between $C2^A$ and $H2^B$ as well as between $H2^A$ and $C2^B$ (Fig. 1). The bridging of $C3^A$and $C6^A$ by an ether bond was suggested by the large downfield shifts of the two carbons and the strong HMBC signal between $C6^A$ and $H3^A$. The residue A closely resembles the modified residue of 3^A,6^A-anhydro-2^A-deoxy-2^A-thio-β-CD[6] both in chemical shifts and coupling patterns ($H1^A \sim 6^A$: δ 5.30, 3.48, 4.53, 3.99, 4.50, 3.92 and 4.22; $J_{1,2}$, $J_{2,3}$, $J_{3,4}$, $J_{4,5}$, $J_{5,6}$, $J_{5,6'}$, and $J_{6,6'}$ = *ca.* 4.8, 3.5, 2.2, 2.4, 0, and 11.2 Hz, respectively). Therefore, the residue A should be a 3^A,6^A-anhydro-2^A-deoxy-2^A-thioglucoside with the S-atom linked to $C2^B$ of the adjacent residue. Finally, the structure of the residue B was deduced on the basis that the chemical shifts of $C3^B$ (δ 70.5 ppm) and $C2^B$ (δ 54.1 ppm) of **5** are very close to the corresponding values δ 72.0 and 51.5 ppm of 2-benzylthio-2-deoxy-β-CD.[7] The small $^3J_{1,2}$ and large $^3J_{2,3}$ coupling constants (2.5 and 9.6 Hz) are consistent with equatorial–axial and *trans*-diaxial couplings, respectively, ruling out the formation of a mannoside structure *via* direct displacement of the mesitylenesulfonate group by the sulfur atom, which would result in a configuration inversion of C2.

The formation of **5** is somewhat astonishing because the C2 carbons of two adjacent saccharide residues in CD derivatives are normally separated far beyond the reachable range of a single atom! The bridging of $C2^A$ and $C2^B$ by a single sulfur atom should induce significant distortion of the CD cavity, which has proved interesting in defining the accommodation of guest molecules in the CD cavity.[8] The bridging of two adjacent methylene carbons of α-CD and cross linking of the $C3^x$ and $C2^{x+1}$ of β-CD by a single atom have been reported.[9] The present work represents the first example of bridging two neighboring C2 carbons by a single atom.

A plausible mechanism can be drawn for the formation of **5** by taking **6** as the reaction intermediate whose formation is followed

[a]*Department of Molecular Medicinal Sciences, Graduate School of Biomedical Sciences, Nagasaki University, Bunkyo-machi 1-14, Nagasaki 852-8521, Japan. E-mail: fujita@nagasaki-u.ac.jp; deqiyuan@nagasaki-u.ac.jp*
[b]*Nihon Pharmaceutical University, Kita-adachi, Saitama 362-0806, Japan*



Scheme 1 An unexpected tandem reaction enabled construction of a tetracyclic segment within the cyclodextrin belt (Mess = mesitylenesulfonyl).

Table 1 ^{1}H and ^{13}C NMR data relating the units A and B of **2**, **3**, **5** and **6** at 35 °C in D_2O[a]

	^{1}H, δ/ppm (coupling constant ^{3}J/Hz)				^{13}C, δ/ppm			
Nuclei	Compound **2**	Compound **3**	Compound **5**	Compound **6**	Compound **2**	Compound **3**	Compound **5**	Compound **6**
1^{A}	5.15 s	5.39 (5.3)	5.30 d (4.8)	5.49 d (5.2)	98.5	94.6	99.2	95.0
2^{A}	3.32 d (3.7)	*ca.* 3.55	3.41 dd (4.8, 3.5)	*ca.* 3.84	50.2	40.7	48.2	40.9
3^{A}	2.29 d (3.7)	3.21 dd (6.4, 4.3)	4.77 dd (3.5, 5.4)	*ca.* 3.72	55.2	38.7	77.7	38.7
4^{A}	*ca.* 3.46	4.00 dd (4.3, 9.2)	4.34 dd (5.4, 3.3)	4.42 dd (4.4, 9.3)	*ca.* 72.8	74.6	*ca.* 73.7	73.5
5^{A}	*ca.* 3.50	*ca.* 3.50	4.41 dd (3.3, 2.4)	*ca.* 3.66 m	70.3	68.3	76.8	68.1
6^{A}			3.92 dd (2.4, 11.2)				69.6	61.4
			4.18 d (11.2)					
1^{B}	5.08 d (3.7)	5.20 d (3.4)	5.32 d (2.5)	5.38 s	99.9	98.1	97.9	95.6
2^{B}	4.44 dd (3.7, 9.7)	4.50 dd (3.4, 9.8)	2.99 dd (2.5, 9.6)	3.35 d (3.6)	79.2	78.6	54.1	50.4
3^{B}	4.01 t (9.7)	4.04 t (9.8)	4.25 t (9.6)	*ca.* 3.52	70.9	71.3	70.5	54.2
4^{B}	*ca.* 3.58	*ca.* 3.60	*ca.* 3.57		79–82	82.2	79.0	
5^{B}			4.20 dbt (10.3, 3.4)	*ca.* 3.66 m			*ca.* 72.0	70.3

[a] The marks s, d, t, dd, dbt, and m denote singlet, doublet, triplet, double doublet, double broad triplet, and multiplet, respectively.

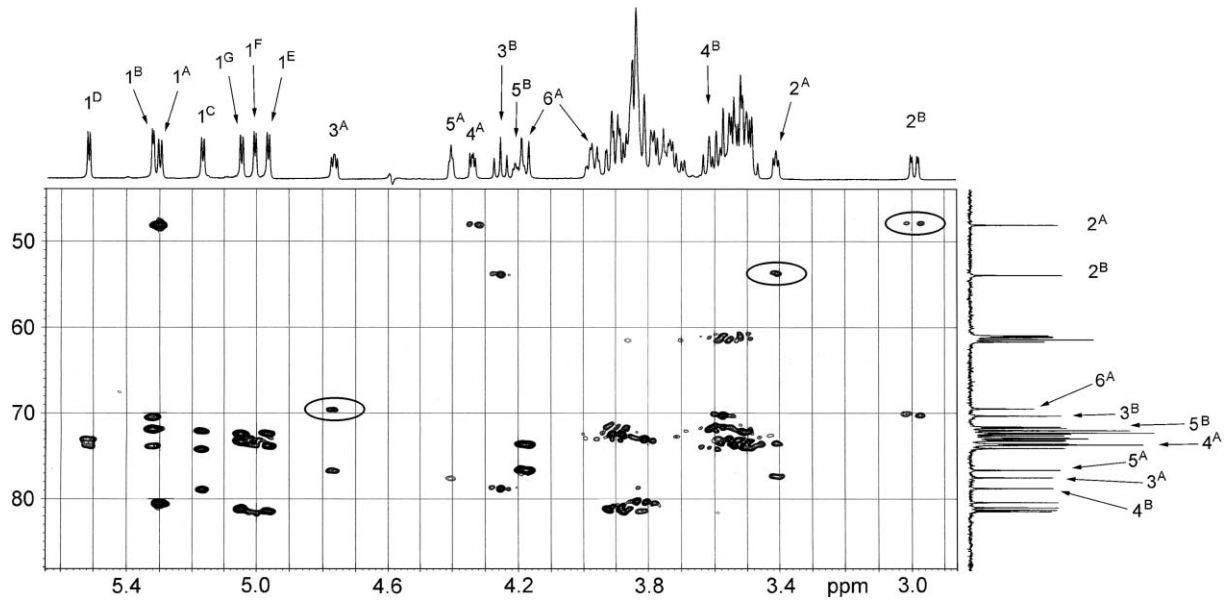
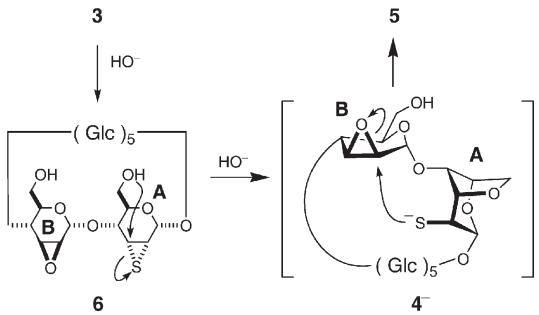


Fig. 1 ^{1}H–^{13}C HMBC spectrum of the tetracyclic segment-incorporated cyclodextrin **5** in D_2O.

by a tandem reaction initiated by the attack of 6^{A}-OH on the $C3^{A}$ of the epithio residue. The ring opening of the latter generates an thiolate anion (**4**$^{-}$) which attacks the $C2^{B}$ of the neighboring 2^{B},3^{B}-mannoepoxy residue to form compound **5** (Scheme 2).

The intermediate **6** was isolated in 84.4% yield by performing the reaction in a pH 12 buffer solution at rt (Table 1).† Treatment of **6** with a NaOH solution at 70 °C afforded **5**, confirming that



Scheme 2 A plausible pathway for the formation of **5** from **3**.

the sulfur atom is introduced to the $C2^{B}$ *via* epoxide ring opening of the unit B.

The attack of 2^{A}-S^{-} on the 2^{B},3^{B}-mannoepoxide in intermediate **4**$^{-}$ was hardly supposed to occur because of the long distance between the $C2^{B}$ and the S^{-} group. However, trapping this intermediate was not successful, which may imply that **4**$^{-}$ is highly reactive and does not accumulate in the reaction. Molecular model speculation indicated that the 3^{A},6^{A}-anhydro-2^{A}-deoxy-2^{A}-thioglucoside residue takes the $^{1}C_4$ conformation while the normal glucoside residues prefer the $^{4}C_1$ conformation. The combination of the $^{1}C_4$ conformation of the residue A with the $^{4}C_1$ conformation of the residue B generates a bridge consisting of two axial C–O bonds between the two residues, which is different from the normal axial–equatorial linkage that bridges two neighboring glucoside residues in CD molecules. Such an axial–axial linkage makes the AB disaccharide segment sharply bent, allowing the 2^{A}-SH, which is axially disposed inside the CD cavity, to get close to the inner side of the residue B. This means that the 2^{A}-SH may not be as far from the residue B as it was supposed. This inference is evidenced by the ^{1}H-NMR spectrum of

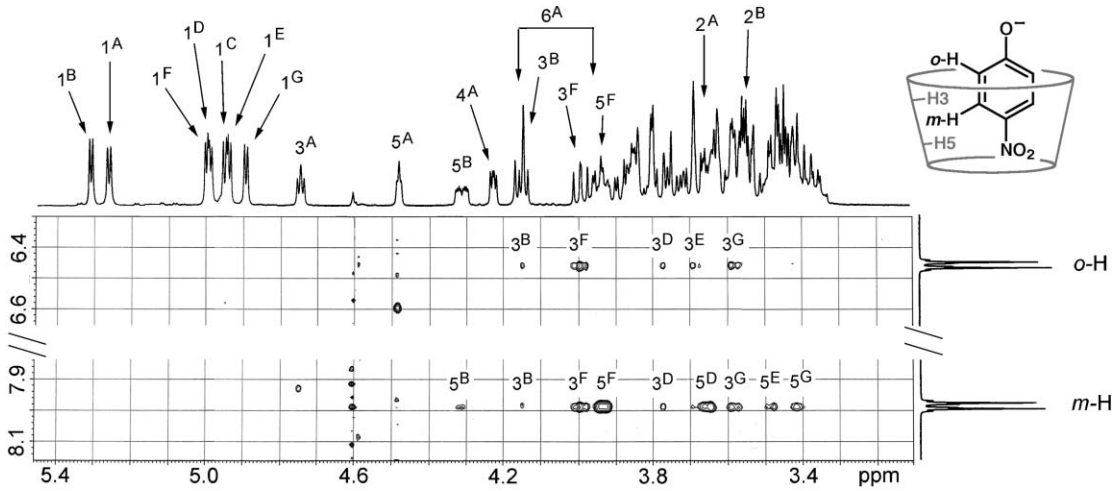


Fig. 2 ROESY NMR spectrum of **5** (23 mM) and sodium *p*-nitrophenolate (120 mM) in D_2O.

$3^A,6^A$-anhydro-2^A-deoxy-2^A-thio-β-CD[6] which demonstrated an unexpected, large downfield shift ($\Delta\delta$ *ca.* 0.3 ppm) and an abnormal double triplet coupling pattern (J = 10, 2.7 Hz) for the $H5^B$ of the unmodified residue B. Such influence should stem from the 2^A-SH of the residue A because it is similarly demonstrated by **5** ($H5^B$ at δ 4.20 ppm, double triplet, J = 10.3, 3.4 Hz) but not by $3^A,6^A$-anhydro-β-CD.[10] Therefore, it is clear that the 2^A-S^- of $\mathbf{4}^-$ should be located in close proximity to the residue B. As soon as it is generated it attacks the $C2^B$ of the $2^B,3^B$-mannoepoxy residue to complete the tandem reaction.

The 3,6-bridge of sugar residue A distorts the cavity shape by straightening the A–G disaccharide and bending the A–B disaccharide sharply while the $C2^A$–S–$C2^B$ bridge narrows significantly the cavity portal of the secondary side. Both units A and B turned their secondary side toward the cavity. Such distortion of the cavity slightly decreases the binding affinity of **5** toward *p*-nitrophenol (PNP, K_a = 160 M^{-1} in aqueous solution, *ca.* half that of β-CD). However, **5** exhibits more refined binding: both the orientation and binding depth of the guest in the cavity are exactly controlled. Upon binding PNP, **5** demonstrated significant changes in its ^1H NMR spectrum (Fig. 1 for free **5** and Fig. 2 for the PNP·**5** complex). Assignments of the signals allowed the mapping of the anisotropic effects of PNP on **5**: residues F and B in the deshielding region and residues G, E and D in the shielding region. The *meta*-H of the PNP exhibited strong NOE correlations with the H5 and H3 protons of **5**, while the *ortho*-H of the PNP correlated only to the H3 protons of **5**, both with the strongest correlation going to residue F. These observations clearly indicated that the PNP molecule was accommodated in the cavity of **5** by directing its NO_2 group to the primary side and the two edges of the benzene ring toward the residues F and B, respectively. The PNP molecule did not bind very deeply in the cavity but at such a depth that the proton pairs of *meta*-H···*ortho*-H and H5···H3 of **5** form an offset arrangement with the *meta*-H being located between the H5 and H3.

In conclusion, we have described an unexpected tandem reaction on a hetero-bifunctional β-CD, which allows the construction of a tetracyclic structural segment within the CD belt and a unique, distorted cavity. The reaction mechanism was elucidated on the basis of experimental evidence. The product of the tandem reaction was demonstrated to have the ability to finely control the orientation and location of the guest molecules bound in its cavity.

Notes and references

† *Synthesis of* ***3***: A modification of the previously reported procedure was employed to synthesize **3** from **2** (67.8%).

Synthesis of ***5***: A solution of **3** (100 mg, 0.076 mmol) in 1 M NaOH (10 ml) was stirred at 70 °C for 4 h. After cooling in a cold water bath, the reaction mixture was neutralized with HCl, membrane filtered, and chromatographed on a Lobar column (Rp-18, size B), eluting with water (500 ml) and then a gradient elution from water to 30% aq. EtOH (500 ml for each) to give **5** (62 mg, 73%).

Synthesis of ***6***: A solution of **3** (1.5 g, 1.1 mmol) in phosphate buffer (150 ml, pH 12.0, 0.1 M) was stirred at rt. After the complete disappearance of **3** (it took one day) was confirmed by TLC, the reaction was terminated by neutralization with HCl. Routine work-up of the reaction solution afforded **6** (1.07 g, 84.4%).

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