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Hetero-bifunctional γ-cyclodextrins having dansylcysteine and tosyl groups at two adjacent sugar units: synthesis and determination of regio-chemistry

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Abstract—Ditosylation of two adjacent 6-positions of γ-cyclodextrin, subsequent mono-substitution with L-cysteine and final condensation with dansyl chloride afford the title compounds whose clockwise and counterclockwise isomers are separated from each other and their regio-chemistry is unambiguously determined by a new strategy based on the combination of enzyme degradation of the hetero-bifunctional γ-cyclodextrin to the corresponding disubstituted maltotriose and fragmentation analysis of the latter by PSD-MS technique.

Cyclodextrins (CDs) have been attracting worldwide interests in various fields relating host–guest recognition.[1] However, their fine functionalization is difficult and presents the bottleneck for molecular design based on them. The hetero-bifunctional CDs not only effect a three point recognition of asymmetric species, but also exercise strong asymmetric induction in catalyzing the chemical transformations such as transamination reactions.[2] However, these hetero-bifunctional CDs are very difficult to access. Methods for the preparation of hetero-bifunctional CDs are extremely scarce although some methodologies have become available for mono- or mono-facial functionalization.[3] For β-CD, several methods have been reported for the hetero-bifunctionalization, including random stepwise substitution with different reactants[4] and selective introduction of two different functional groups to two specific positions.[5] For γ-CD, which has a wider cavity and possesses quite different binding properties from β-CD, the only attempt to the introduction of two different groups was made by Hamada who substituted one tosylate group of 6^A,6^X-ditosyl-γ-CDs with a chromophore.[6] Except for the C_2 symmetrical 6^A,6^E-ditosyl-γ-CD, each of the other three ditosyl-γ-CDs generates a pair of clockwise and counterclockwise isomers which are hard to separate from each other, and the clockwise–counterclockwise isomeric mixtures were employed in molecular sensing. To the best of our knowledge, structurally well-characterized pure unsymmetrical hetero-bifunctional γ-CDs have not yet been reported. Herein, we describe the syntheses of pure hetero-bifunctional γ-CDs bearing a tosyl and a *N*-dansyl-L-cysteine residues at the two adjacent 6-positions and the application of MALDI-TOFMS in the determination of regio-chemistry. These bifunctional γ-CDs may serve as important intermediates for further functionalization.

The synthetic approach is depicted in Scheme 1. 6^A,6^B-Ditosylate **1** of γ-CD was synthesized by reacting γ-CD with tosyl chloride in pyridine[7] and separated from the other regio-isomers by chromatography on a preparative ODS-column. Treatment of ditosylate **1** with L-cysteine in DMF/H_2O alkaline solution gave 6^B-cysteine-6^A-tosyl-γ-CDs (**2**) and its counterclockwise regio-isomer **3**

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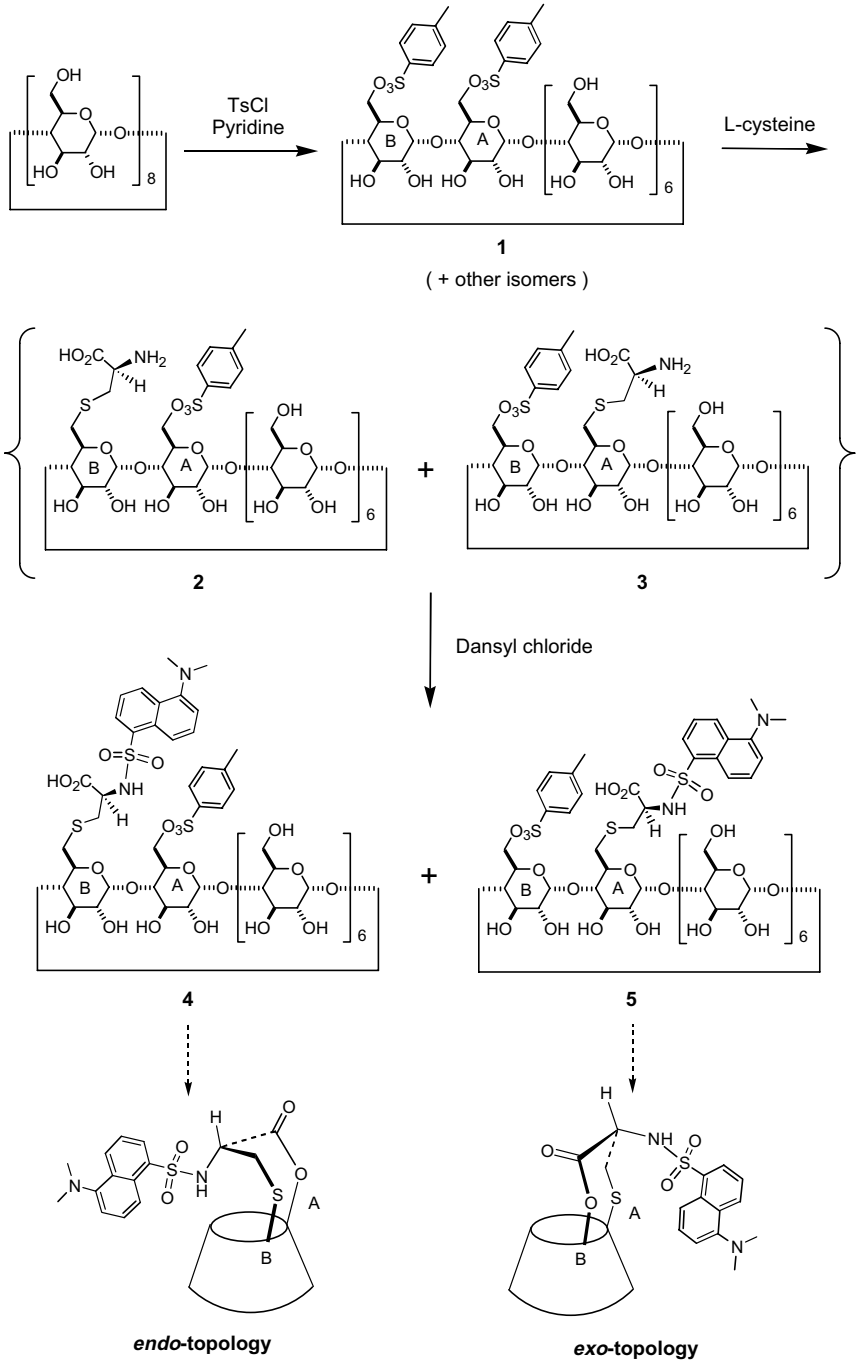


Scheme 1.

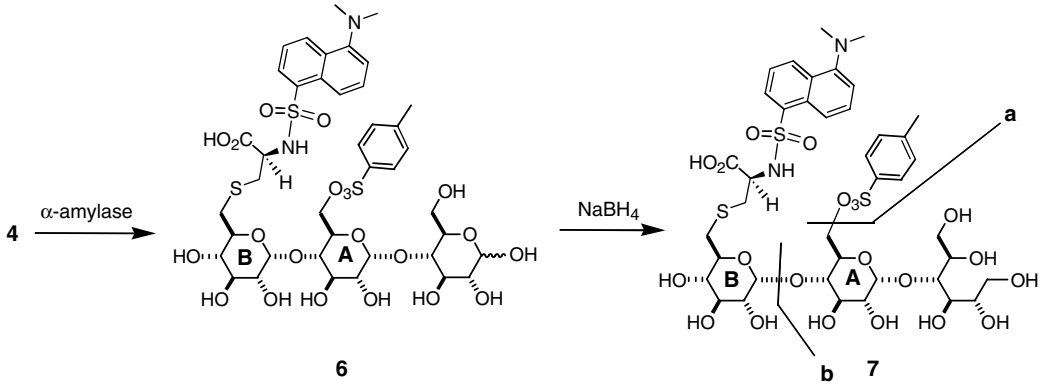
as an unseparable mixture. Sulfonylation of the amino group with dansyl chloride in alkali aqueous CH_3CN solution and subsequent chromatography on a reversed-phase column allowed the separation of the two regio-isomers **4** (with shorter retention time) and **5** (with longer retention time) in 36% and 25% yields, respectively.

Hetero-bifunctional γ-CDs **4** and **5** share the molecular ions at *m*/*z* 1809 ($[M+H]^+$) and 1831 ($[M+Na]^+$) in their TOFMS spectra and displayed similar NMR spectra. The ^1H NMR spectrum of **4** demonstrated the anomeric protons of γ-CD, methyl protons of dansyl and methyl protons of tosyl moieties at ca. δ 4.87, 2.85 and 2.31 ppm, respectively, in integration ratios of 8/6/3. In the aromatic region, 6 doublets were recognized at δ 8.46 (d, $^3J = 8.52$ Hz, 1H), 8.22 (d, $^3J = 8.52$ Hz, 2H), 8.15 (d, $^3J = 7.14$ Hz, 1H), 7.76 (d, $^3J = 8.32$ Hz, 2H), 7.43 (d, $^3J = 8.32$ Hz, 1H), 7.22 (d, $^3J = 7.42$ Hz, 1H) and two triplets overlapped around δ 7.55 (2H). In addition to the basic chemical shift pattern of cyclodextrin,[8] the ^{13}C NMR spectrum of compound **4** demonstrated 14 peaks for the aromatic carbons (δ 110–160 ppm), and the carboxylic carbon, α- and β-carbons of cysteine moiety, and the methyl carbons of dansyl and tosyl groups were observed at δ 170.9, 57.5, 37.6, 45.0 and 21.0 ppm, respectively. A sugar carbon reso-

nates at very high field (δ 33.8), which can be assigned to the thio-substituted C6. These observations are consistent with the 1/1/1 ratio of γ-CD/tosyl/dansyl-L-cysteine. Similar results were also obtained with compound **5**. However, the NMR spectra are powerless towards the regio-chemistry of hetero-bifunctional γ-CDs **4** and **5**.

The only method available for the determination of the regio-chemistry of hetero-bifunctional CD derivatives includes enzymatic conversion of the macrocycle to linear oligosaccharide and fragmentation analysis of the latter by EI-MS.[9] However, the EI-MS measurement requires the acylation of all the hydroxyl groups of the oligosaccharides, which greatly increases the molecular weight of the analytes and makes fragmentations very complicated and difficult to define in some cases. Since TOFMS is powerful in sequencing bio-macromolecules,[10] we attempted to apply it in the structural determination of disubstituted CD derivatives. Combination of the amylase-promoted degradation of **4** and the post-source decay (PSD) TOFMS measurement[11] ensures a convenient and unambiguous assignment of the structure of **4** (Scheme 2). Compound **4** was decomposed to maltotriose **6** by the enzyme action of α-amylase and the latter, though good for PSD measurements at this stage, was reduced to **7** in order to remove the anomer equilibrium and facilitate the NMR analysis. The reduced triose structure of compound **7** was confirmed by TOFMS which displayed the pseudo-molecular ion at m/z 1019 (M+Na). The PSD-MS spectrum of **7** was very simple (Fig. 1). Apart from the mother peak at m/z 1019, only three fragment ions were observed at m/z 847, 520 and 350 as the sodium adducts. The former two fragment ions were generated by the one-site cleavages of type-a and type-b, respectively, while the latter one at m/z 350 in much lower abundance corresponds to the two-site cleavage of both type-a and type-b.[12] The type-b cleavage strongly evidences the sugar sequence of **7** and therefore its precursor **4**. Based on this assignment, the other isomer **5** should take the reversed arrangement for the two functional groups.

Because it is the first attempt to apply PSD-MS in the determination of the regio-chemistry of disubstituted CDs, the reliability of the above assignment is reconfirmed independently by NMR analysis of compound **7**. Thanks to the effect of the substituents, the resonances spread out widely both in the ^{1}H and ^{13}C NMR spectra of **7**, and all the signals relating the two substituted glucose residues can be assigned with the aid of 2D NMR measurements (Fig. 2). Both the C3 (δ 83.9 ppm) of the glucitol residue, which is linked to the middle glucose residue by an oxygen atom, and the C4 (δ 79.5 ppm) of the tosylglucoside appeared in the normal region (around δ 80 ppm) of the C4 carbons of CD derivatives. However, the C4 of the dansylcysteine-modified glucose residue demonstrated ca. 8 ppm



Scheme 2. Amylase-promoted degradation of the hetero-bifunctional γ-CD **4** and the fragmentation patterns of **7** observed in the PSD-MS spectrum.

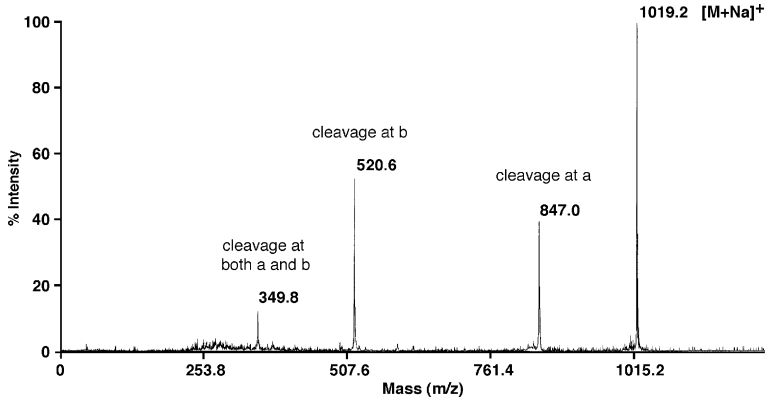


Figure 1. Post-source decay mass (PSD-MS) spectrum of compound **7**.

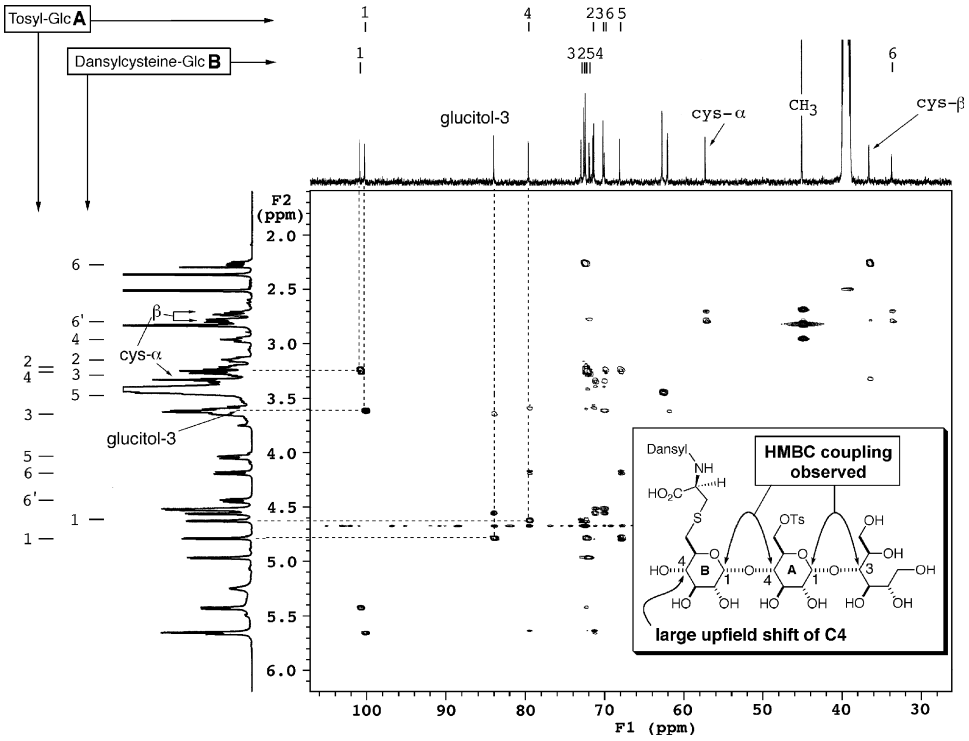


Figure 2. The ^{1}H, ^{13}C, and HMBC NMR spectra of triose **7** in DMSO-d_6 (only the sugar part is shown). The inset highlights the important information employed in the sequence determination.

upfield shift, and resonated at δ 71.8 ppm, a chemical shift within the typical range of the hydroxyl-substituted secondary carbons of saccharides. These observations imply that compound **7** should have the dansylcysteine-modified glucose as the terminal residue located at the non-reducing end, which is in consistence with the result of PSD-MS measurement. Heteronuclear multiple bond correlation (HMBC[13]) experiments were further carried out to confirm the saccharide sequence as it can provide long range H–C hetero-nuclei correlations between the O-bridged positions of two adjacent sugar residues. As shown in Figure 2, the C4 of the tosylglucose (δ 79.5 ppm) strongly correlated to the anomeric H1 proton of dansylcysteine-glucose (δ 4.63 ppm) while its own H1 (δ 4.79 ppm) coupled with the C3 of the glucitol residue that is generated by reduction of the glucose unit at the reducing end of **6**. Since these signals are all clearly separated from each other and do not overlap with any other ones, the observed multiple bond C–H correlations clarify unambiguously the sugar sequence of **7** and strongly support the results of PSD-MS.

In summary, we have described for the first time the syntheses of isomerically pure hetero-bifunctional γ-CDs. A new method is established to determine the regio-chemistry of hetero-bifunctional CDs by the combination of enzyme-promoted degradation reaction and PSD-MS measurement, which is thought to be powerful and generally applicable in the structural determination of CD derivatives bearing two or more substituents. Hetero-bifunctional CDs **4** and **5** may serve as important intermediates for further functionalization. As an on-going project, the intramolecular displacement reaction of **4** and **5** was undertaken to provide an efficient approach to strictly control the topology of the functional CDs.[14]

Experimental

^{1}H and ^{13}C NMR spectra were determined with a Varian 500 MHz NMR spectrometer by setting the frequency at 500 MHz for ^{1}H spectra and at 125 MHz for ^{13}C spectra. MALDI-TOFMS spectra (positive) were recorded on Applied Biosystems Voyager System 6336 spectrometer using 2,5-dihydroxybenzoic acid as a matrix for reflector mode and α-cyano-2-hydroxycinnamic acid as a matrix for PSD mode.

Preparation of hetero-bifunctional γ-CDs 4 and 5

L-Cysteine hydrogen chloride (27 mg) and 6^{A},6^{B}-ditosyl-γ-CD[7] (620 mg) were dissolved in DMF (3 mL), and a 0.58 M aqueous Na_2CO_3 solution (10 mL) was added. After being stirred at rt for 2 h, the reaction mixture was neutralized, diluted with water, membrane filtered and chromatographed on a reversed-phase Lobar column (Rp-18, size C). Elution of the column with a gradient from water to 25% aqueous ethanol solution (a total 2 L) afforded **2** and **3** as an unseparable mixture (135 mg, 22.4 %) together with the recovery of the unreacted ditosylate (400 mg, 64.5%). TOFMS: *m*/*z* 1554 (M+H), 1576 (M+Na). The mixture of **2** and **3** (207 mg) was dissolved in 0.08 M aqueous $NaHCO_3$ solution (10 mL), and an acetonitrile solution (10 mL) containing dansyl chloride (36.2 mg) was added. The

mixture was stirred at rt for 30 min, and then it was neutralized, concentrated, precipitated with acetone and filtered. The precipitates were collected, dissolved in water and chromatographed (Lobar column, Rp-18, size C) after membrane filtration. Elution of the column with water (1 L) and a gradient from water to 30% aqueous ethanol solution (a total 2 L) enabled the separation of **4** and **5**.

Compound **4**: eluted faster, 86.3 mg, 36%. TOFMS: *m*/*z* 1809 ($[M+Na]^+$). ^{13}C NMR (DMSO-d_6, TMS int.): δ 170.9, 151.1, 144.7, 136.3, 132.3, 130.0, 129.1, 128.9, 127.7, 127.5, 127.4, 123.1 119.1, 114.8, 101.6, 101.5, 101.3, 84.1, 81.4, 80.9, 80.8, 80.6, 80.3, 72.8, 72.6, 72.4, 72.2, 72.0, 71.3 69.5, 68.8, 59.8, 57.5, 45.0, 37.6, 33.8, 21.0 ppm. Compound **5**: eluted slower, 60 mg, 25%. TOFMS: *m*/*z* 1809 ($[M+Na]^+$). ^{13}C NMR (DMSO-d_6, TMS int.): δ 169.3, 151.2, 145.4, 137.7, 129.5, 129.0, 128.9, 128.3, 128.0, 127.8, 125.5, 123.4, 119.3, 114.9, 102.4, 101.7, 101.2, 86.2, 82.1, 81.1, 81.0, 80.7, 80.4, 73.2, 72.9, 72.8, 72.7, 72.6, 72.5, 72.3, 72.2, 72.0, 71.9, 70.1, 69.9, 60.0, 59.8, 59.4, 57.9, 44.9, 33.8, 33.1, 20.7 ppm.

Preparation and reduction of maltotriose 6

A mixture of **2** (100 mg) and α-amylase (EC 3.2.1.1 from *Aspergillus oryzae*, Sigma) (100 mg) in 0.1 M acetate buffer (pH 5.5, 10 mL) containing 0.02 M $CaCl_2$ was allowed to stand for 3 days at 40 °C. After being neutralized with NaOH solution and 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, eluted with a gradient of 20–50% aqueous EtOH) to afford a major product **6** (29 mg, 52%). TOFMS: *m*/*z* 1017 ($[M+Na]^+$).

Compound **6** (30 mg) and $NaBH_4$ (30 mg) were dissolved in water (3 mL) and the resultant mixture was stirred at rt for 1 h. The reaction solution was then subjected to dilution with 25% ethanol, membrane filtration and chromatography on a reversed-phase Lobar column (Rp-18, size C). Elution of the column with water (1 L) and a gradient of 10–30% aqueous ethanol (a total 2 L) afforded compound **7** (23 mg, 76.5%). TOFMS: *m*/*z* 1019 ($[M+Na]^+$). ^1H NMR (DMSO-d_6, TMS int.): δ 8.45 (d, $^3J = 8.47$ Hz, 1H), 8.25 (d, $^3J = 8.70$ Hz, 1H), 8.20 (d, $^3J = 7.10$ Hz, 1H), 7.74 (d, $^3J = 8.30$ Hz, 2H), 7.61 (m, 2H), 7.40 (d, $^3J = 8.30$ Hz, 2H), and 7.25 (d, $^3J = 7.10$ Hz, 1H) (Ar–H); 5.65 (m, 2H), 5.43 (d, $^3J = 6.87$ Hz, 1H), ca. 5.4 (v br, 1H), 3.25 (br, 1H), 4.97 (d, $^3J = 4.81$ Hz, 1H), 4.56 (d, $^3J = 3.89$ Hz, 1H), and 4.52 (m, 2H) (OH); 4.79 (d, $^3J = 3.66$ Hz, 1H; 1^A), 4.63 (d, $^3J = 3.66$ Hz, 1H; 1^B), 4.44 (dd, $J = 10.86$, 3.10 Hz, 1H; $6^{A'}$), 4.18 (d, $J = 9.39$ Hz, 1H; 6^A), 4.04 (m, 1H; 5^A), 3.64–3.57 (m, 4H; 3^A, $3^{glucitol}$, 2H of glucitol), 3.45–3.32 (m, 7H; 5^B, cys-α, 5H of glucitol), 3.29–3.12 (m, 4H; 3^B, 4^A, 2^A, 2^B), 2.96 (t, $^3J = 9.38$ Hz, 1H; 4^B), 2.83 (s, 6H; $N(CH_3)_2$), 2.81–2.77 (m, 2H; cys-β′, $6^{B'}$), 2.72 (dd, $J = 12.60$, 4.35 Hz, 1H; cys-β), 2.36 (s, 6H; Ts-CH_3), 2.27 ppm (dd, $J = 14.20$, 7.10 Hz, 1H; 6^B). ^{13}C NMR (DMSO-d_6, TMS int.): δ 170.9 (COOH), 151.2, 144.6, 135.9, 132.2, 129.9, 129.2, 129.0, 127.8, 127.7, 127.6, 123.3, 119.1, and 115.0 (Ar); 100.9 (1^B), 100.2 (1^A), 83.9 ($3^{glucitol}$), 79.5 (4^A), 72.9 (3^B), 72.5 (2^B), 72.3 (5^B), 71.8 (4^B), 71.4 (2^A), 71.2, 70.1 (3^A), 69.9 (6^A), 68.0 (5^A), 62.6, and 61.9 (1 and $6^{glucitol}$), 57.2 (cys-α), 45.0 (NCH_3), 36.5 (cys-β), 33.6 (6^B), 21.0 (Ts-CH_3) ppm.

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