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Tetrahedron Letters
Tetrahedron Letters 48 (2007) 7493–7497

Selective synthesis and ester cleavage property of $3^A,2^B$-anhydro-3^B-deoxy-3^B-thio-β-cyclodextrin

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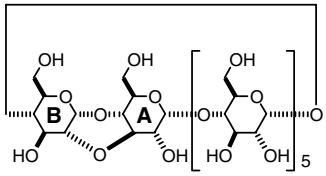
Received 26 July 2007; revised 10 August 2007; accepted 16 August 2007
Available online 22 August 2007

Abstract—The title compound was synthesized by the conversion of $2^A,3^A$-alloepoxy-β-cyclodextrin to the $2^A,3^A$-mannoepithio derivative with thiourea and subsequent ring-opening by intramolecular nucleophilic substitution. Its thiol group and the distorted cavity demonstrated good synergetic effect in promoting the cleavage of *m*-nitrophenyl acetate but did not cooperate with each other toward the *p*-isomer.

Appropriate distortion of the cyclodextrin (CD) cavity provides an interesting approach to get more defined accommodation of guest molecules in the cavities.[1] Among the various CD derivatives with distorted cavities,[2] $3^A,2^B$-anhydro-β-CD (**1**)[3] has been the only one host that demonstrated stronger binding ability toward methyl orange than β-CD itself.[2] Because the $3^A,2^B$-etheric bridge can be easily constructed by the intramolecular reactions of 2,3-mannoepoxy-CDs under strong alkaline conditions,[3,4] development of artificial enzymes based on **1** must be interesting if efficient methodology can be established to introduce additional functional groups onto the basic skeleton of **1**. Years ago we reported that the 3^A-deoxy-3^A-thio-β-CD (**2**) promoted the cleavage of *p*-nitrophenyl acetate 62 times more efficiently than β-CD.[5] In this Letter, we describe the regiospecific introduction of a thiol group to the $C3^B$ of the $3^A,2^B$-anhydro disaccharide residue (Scheme 1) and the enhanced catalytic ability of $3^A,2^B$-anhydro-3^B-deoxy-3^B-thio-β-CD (**5**).

The synthesis of **5** includes the conversion of $2^A,3^A$-alloepoxy-β-CD (**3**) to $2^A,3^A$-mannoepithio-β-CD (**4**)[6] and subsequent intramolecular nucleophilic opening reaction of the epithio-ring. Simply heating the alkaline solution of **4** selectively afforded $3^A,2^B$-anhydro-3^B-deoxy-3^B-thio-β-CD (**5**) in good yield.



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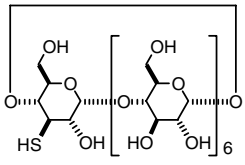

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In a typical synthesis, a solution of compound **4** (100 mg, 0.0883 mmol) in 1 M aqueous NaOH (10 mL) was stirred at 70 °C for 1 h, neutralized with 1 M HCl, and filtered. The filtrate was chromatographed on a Lobar column (Rp-18, size C) with a gradient elution from 3% aq EtOH to 7% aq EtOH (1 L for each) and then an elution of 30% aq EtOH (500 mL) to give **5** (68.5 mg, 68.5%). The TOF-MS spectrum of **5** showed a pseudo parent peak [M+Na^+] at *m*/*z* 1155, indicative of the isomeric relationship between **4** and **5**.[7] Because the thiol is unstable and susceptible to oxidation, **5** was converted to the corresponding disulfide dimer **6**[7] by the conventional treatment with aq DMSO.[8]

Structure **6** was characterized by NMR spectroscopy. *Only one* carbon (δ 48.8 ppm) signal was observed in the normal region where the S-bearing carbons should

Keywords: Cyclodextrin; Modification; Episulfide; Thiol; Catalysis.
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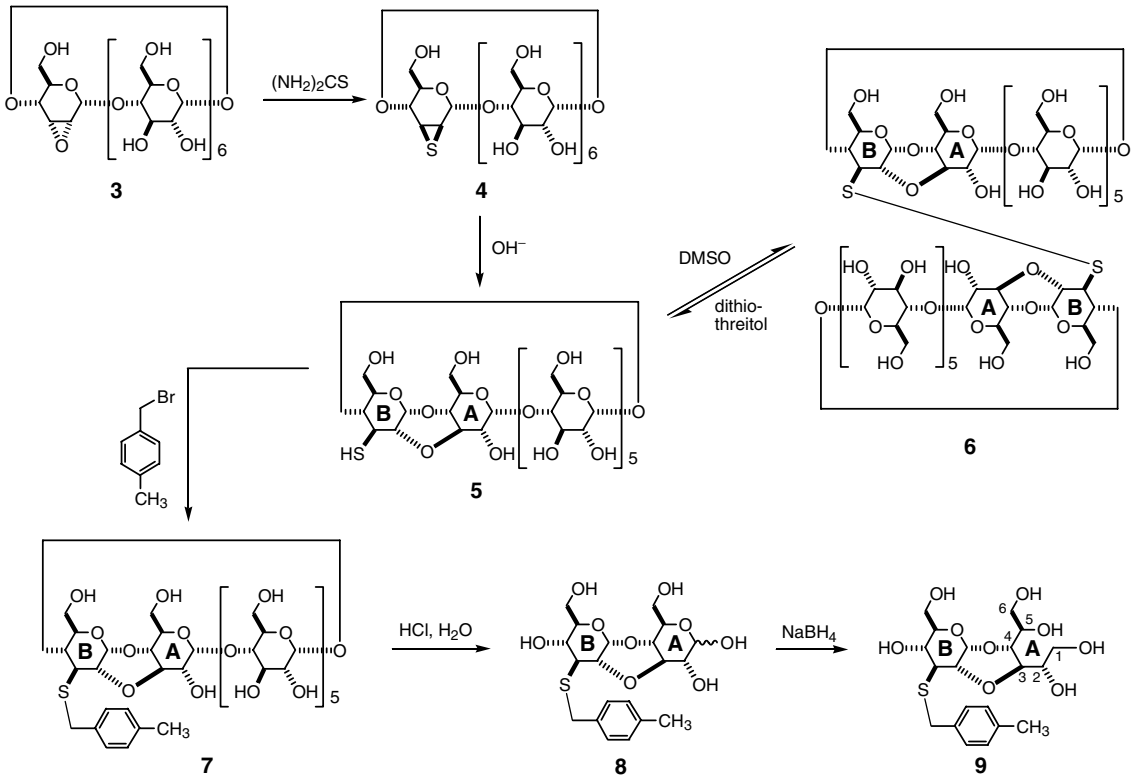
doi:10.1016/j.tetlet.2007.08.062



Scheme 1. Synthesis of $3^{A},2^{B}$-anhydro-3^{B}-deoxy-3^{B}-thio-β-CD **5** and chemical transformation of **5** to **9** for structural elucidation of the AB disaccharide residue.

appear, which is indicative of the opening of the epithio-ring by an O-nucleophile. TOF-MS spectrum of **6** showed the pseudo parent peaks [M+Na^+] at *m*/*z* 2285, suggesting that the nucleophile should be an intramolecular CD hydroxyl group rather than a water molecule. It is most likely that compound **4** has a similar reaction behavior to that of the analog $2^{A},3^{A}$-manno-epoxy-β-CD, which afforded compound **1** under similar reaction conditions. Therefore, it can be reasonably deduced that a $C2^{B}$–O–$C3^{A}$ bridge was formed and the S-atom is attached to $C3^{B}$. Reaction of **6** with dithiothreitol afforded monomer **5**, confirming the disulfide linkage between the two CD moieties.

The above structural assignment was further confirmed by the chemical transformation of **5** to **9** and subsequent detailed NMR spectral analysis. Maltitol **9** was obtained from **5** by alkylation of the thiol group (**7**), acid-hydrolysis to remove the unmodified glucoside units (**8**), and final reduction with $NaBH_4$.[9] The NMR spectra were recorded in DMSO-d_6 solution. The application of advanced NMR techniques including the ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, ^{1}H–^{13}C HMBC, TOCSY (1D and 2D), and ROESY (1D and 2D) spectrometry enabled the identification of all the protons (including OH) and carbon nuclei of compound **9** (Fig. 1). The very small chemical shift of $C3^{B}$ indicated that it is substituted by a sulfur atom. The ^{1}H–^{1}H COSY spectrum clearly demonstrated the correlation of $H4^{B}$ to an OH proton, strongly suggesting that residue B is a non-reducing terminal. Neither $H3^{A}$ nor $H2^{B}$ coupled with any OHs in the ^{1}H–^{1}H COSY spectrum. However, the two positions correlated each other by demonstrating a strong $C3^{A}$–$H2^{B}$ HMBC coupling, confirming the bridging of the two carbons by a single atom. Finally, the sequence of residues A and B was evidenced by the observation of $H4^{A}$–$C1^{B}$ HMBC signal. The TOF mass spectrum of **9** displayed the pseudo parent peak [M+Na^+] at *m*/*z* 469, which was in agreement with the structure determination.

The thiol of **5** shows no obvious absorption above 215 nm, while ionization of the thiol generates a new absorption band around 230 nm. Titration of the acidic solution of **5** with aq NaOH solution resulted in absorption changes at 230 nm, which enabled the determination of the pK_a of **5**. The pK_a value (7.4) of **5** was rather low compared with normal thiols (pK_a = 11–12), which might be the result of the inter-subunit hydrogen bonding, that is, $C3^{B}$–SH···HO–$C2^{C}$ and the electron-withdrawing effect of oxygen atoms around $C3^{B}$–SH. Very low pK_a values were also observed for 3^{A}-deoxy-3^{A}-thio-β-CD **2** (pK_a 7.7) and 3^{A}-deoxy-3^{A}-thio-α-CD (pK_a 7.7).[5] The decreased pK_a values allow the CD thiols to ionize almost completely to form the thiolate anions under pH 9.0 condition where neither the CD hydroxyl groups (pK_a = ca. 12 for 2-OHs and 3-OHs, pK_a = ca. 16 for 6-OHs) nor the normal thiols (pK_a = 11–12) undergo obvious ionization. Therefore, enhanced catalytic ability of the CD thiols can be expected even around neutral pH.

The cleavage of *p*- and *m*-nitrophenyl acetates by **5** was carried out at 25 °C in a carbonate buffer solution (pH

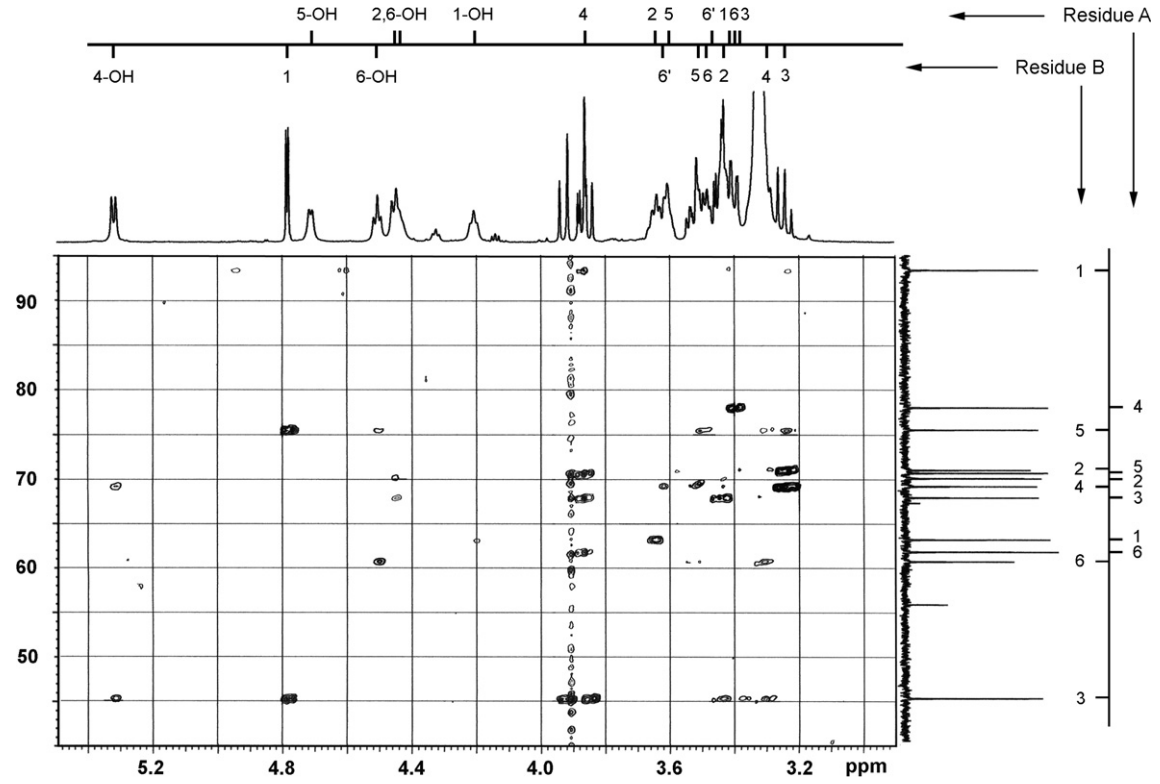


Figure 1. The sugar part of the ^{1}H–^{13}C HMBC spectrum of **9** in DMSO-d_6.

9.0, 0.1 M) and the release of the nitrophenolates was monitored at 400 nm. The kinetic parameters are summarized in Table 1 together with those of the reference compounds, β-CD, $3^{A},2^{B}$-anhydro-β-CD (**1**) and 3^{A}-deoxy-3^{A}-thio-β-CD (**2**). $3^{A},2^{B}$-Anhydro-β-CD (**1**) cleaves *p*- and *m*-nitrophenyl acetates 5 and 73 times faster than the buffer solution, slightly inferior to β-CD although it shows slightly increased binding affinities toward the ground states ($1/K_M$), which is in agreement with the results of the binding study. This result is reasonable because the formation of the 3^{A}-O-2^{B} bridge might distort the cavity and confine the orientation of the bound substrate so that some of the 2- or 3-OH can no longer actually contribute in promoting the reaction of the substrates. Compared with β-CD, compound **2**, which has a pseudo C7 symmetrical cavity and does not confine the substrate orientation, is 66 times more effective toward *p*-nitrophenyl acetate and 3 times more effective toward the *m*-isomer, strongly suggesting that the 3-SH group is catalytically important and actively engaged in the reaction. Compound **5** combines the structural feature of both **1** (distorted cavity) and **2** (catalytic thiol functionality). Its k_{cat} ($0.0054\ s^{-1}$) for *p*-nitrophenyl acetate, although a couple of times larger than that of **1**, is only one thirtieth that of **2**, implying that the introduction of the ether-bridge to **2** causes a dramatic loss of the catalytic ability of the thiol group. Comparison of the binding of **5** and **2** clearly indicated that the introduction of the ether-bridge to **2** resulted in a slight increase in the ground state binding ($1/K_M$) but a large decrease in the transition state binding ($1/K_{TS}$).[10] Obviously, the distorted cavity confined the ester group of *p*-nitrophenyl acetate to an orientation that was not good for the attack of the thiolate anion

Table 1. Kinetic parameters of the cleavage of *p*- and *m*-nitrophenyl acetates[a]

Catalyst	pK_a[b]	*p*-Nitrophenyl acetate				*m*-Nitrophenyl acetate				Substrate selectivity
		$k_{cat}/10^{-2}\ s^{-1}$	$K_M/10^{-3}$ M	k_{cat}/k_{un}	K_{TS}[c]$/10^{-5}$ M	$k_{cat}/10^{-2}\ s^{-1}$	$K_M/10^{-3}$ M	k_{cat}/k_{un}	K_{TS}[c]$/10^{-5}$ M	$(k_{cat}/K_M)_{para}/(k_{cat}/K_M)_{meta}$
β-CD		0.226	5.51	7.53	73.1	1.30	15.0	83.9	17.9	0.47
1		0.149	3.48	4.97	70.1	1.13	6.89	72.9	9.45	0.26
2	7.7	15.0	7.60	500	1.52	4.50	3.20	290	1.10	1.40
5	7.4	0.541	2.90	18.0	16.1	21.8	18.3	1410	1.30	0.16

[a] Reactions were carried out in 0.1 M carbonate buffer solutions (pH 9.0) at 25 °C; the release of nitrophenolates was monitored at 400 nm. Lineweaver–Burk treatment was applied to derive the k_{cat} and K_M values.

[b] Determined by UV titration at 230 nm of the acidic solution of a CD thiol with aq NaOH.

[c] $K_{TS} = K_M k_{un}/k_{cat}$, cf. Ref. 10.

of **5** to develop the transition state, that is, the distorted cavity and the catalytic thiol group cannot efficiently cooperate in promoting the reaction of *p*-nitrophenyl acetate. Interestingly, compound **5** behaves quite differently toward *m*-nitrophenyl acetate. It accelerates the cleavage of *m*-nitrophenyl acetate by a factor of $k_{cat}/k_{un} = 1400$, ca. 20 times that of **1** and five times that of **2**, revealing the good *synergistic effect* of the orientation confining of substrate and the catalysis of the thiol functionality. Because the binding strength of the ground state was greatly decreased while that of the transition state was not significantly altered, the improved catalytic ability might be attributed primarily to the decrease in binding strength of the ground state rather than the improvement of transition state binding. These results may imply that the combination of the distorted cavity and the thiol group prevents against deep inclusion of the substrate and the $C3^B$ position, even not the most preferred, should be among the ones that the ester group of the bound substrate can easily access.

The transfer of the acyl group of the substrates to the S-atom of **5** was confirmed by isolation and structural determination of the reaction product of **5** in the cleavage of the substrates.[11] The HPLC of the catalytic reaction mixture of **5** showed only one peak for the acylated CD species. The TOF mass spectrum of the isolated CD product demonstrated the parent peaks at *m*/*z* 1197 and 1213, which were consistent with the $[M+Na^+]$ and the $[M+K^+]$ ions of the acylated **5**. Evidence for the thioacetate structure was obtained in the ^{13}C NMR spectrum. The chemical shifts of both the methyl carbon and the carbonyl carbon (δ 199.7 ppm) of the CD acetate were in the normal regions (δ ca. 31 and 194 ppm, respectively) of thioacetates, but much larger than the corresponding normal values of acetates (δ ca. 21 and 170 ppm, respectively).

Only when SH is put in the right position with the correct conformation required to access the transition state can it significantly accelerate the reaction. The $C3^B$ of $3^A,2^B$-anhydrodisaccharide residue seems to be among the proper candidates of such positions in the case of *m*-nitrophenyl acetate as the substrate but it is obviously not good for promoting the reaction of the *p*-isomer. As a result, compound **5** demonstrated improved *m*-selectivity.

In summary, we described a synthetic strategy for the functionalization of the $C3^B$ of $3^A,2^B$-anhydro-β-CD, which has a distorted cavity to confine the guest orientation, and demonstrated that enhanced catalytic ability can be obtained only when the introduced catalytic group can cooperate with the distorted cavity in developing the transition state of the catalytic reaction.

Acknowledgment

The authors are grateful to the Japan Maize Products Co. Ltd, for the generous gift of cyclodextrins.

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7. Structural data of compound **5**: $R_f = 0.48$ (*n*-PrOH/EtOAc/H_2O = 7:3:6 by volume). TOF-MS: *m*/*z* 1155 ($M+Na^+$), 1171 ($M+K^+$). ^1H NMR (D_2O, CH_3CN int.): δ 5.08 (d, $J = 2.8$ Hz, 1H, $H1^B$), 5.10 (d, $J = 4.0$ Hz, 1H), 5.06 (d, $J = 3.5$ Hz, 1H), 5.04 (d, $J = 4.0$ Hz, 1H), 5.02 (d, $J = 3.5$ Hz, 1H), 4.99 (d, $J = 4.0$ Hz, 1H), and 4.97 (d, $J = 4.0$ Hz, 1H, H1); 4.3–3.4 (m, 42H) ppm. ^{13}C NMR (D_2O, CH_3CN int.): δ 103.0, 102.5, 102.5, 102.3, 101.9, and 100.7 (C1); 94.6 ($C1^B$); 82.0, 81.9, 81.8, 81.6, and 80.7 (C4); 80.0 ($C4^B$); 76.4 ($C2^B$); 75.4, 74.2, 74.1, 74.0, 74.0, 73.7, 73.6, 73.5, 73.0, 72.9, 72.8, 72.7, 72.7, 72.6, 72.5, 72.3, 71.0, 70.7, and 70.0 (C5, C3, C2); 61.3, 61.3, 61.2, 61.2, 61.0, 60.9, and 60.3 (C6); 40.2 ($C3^B$) ppm.
Structural data of compound **6**: $R_f = 0.36$ (*n*-PrOH/EtOAc/H_2O = 7:3:6 by volume). TOF-MS: *m*/*z* 2285 ($M+Na^+$), 2301 ($M+K^+$). ^1H NMR (D_2O, CH_3CN int.): δ 5.18 (d, $J = 3.7$ Hz, 1H, $H1^B$), 5.11 (d, $J = 2.7$ Hz, 1H, $H1^A$), 5.09 (d, $J = 3.5$ Hz, 1H), 5.07 (d, $J = 3.8$ Hz, 1H), 5.04 (d, $J = 3.9$ Hz, 1H), 4.98 (d, $J = 3.8$ Hz, 1H), and 4.97 (d, $J = 3.7$ Hz, 1H, H1); 4.22 (dd, $J = 11.0$, 3.6 Hz, 1H, $H2^B$); 4.01 (dd, $J = 12.6$, 3.7 Hz, 1H, $H6^B$); 3.96–3.64 (m, 28H, $H2^A$, $H2^B$, $H4^B$, H6, H5, H3), 3.64–3.47 (m, 12H, H2, H4, $H3^B$) ppm. ^{13}C NMR (D_2O, CH_3CN int.): δ 103.1 ($C1^A$), 102.5, 102.5, 102.4, 101.5, and 100.0 (C1); 95.1 ($C1^B$); 82.0, 81.9, 81.8, 81.7, and 79.4 (C4); 75.7 ($C4^B$); 74.6 ($C5^B$); 74.2, 74.2, 74.1, 74.0, 74.0, 73.7, 73.6, 73.0, 72.9, 72.8, 72.7, 72.6, 72.5, 72.3, and 71.0 (C5, C3, $C4^A$, C2); 70.7 ($C2^A$); 70.5 ($C2^B$); 70.1 ($C3^A$); 61.4, 61.2, 61.1, 61.0, and 60.4 (C6); 48.8 ($C3^B$) ppm.
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9. Compound **5** (70 mg), *p*-methylbenzyl bromide 140 mg, and Cs_2CO_3 were added to DMF (3.5 mL) and the resultant mixture was stirred at 70 °C for 30 min. After being neutralized with 1 M HCl, the reaction mixture was concentrated in vacuo, and the residue was dissolved in 5% aq EtOH and chromatographed on Lobar column (Rp 18, size C). Elution of the column with 5% aq EtOH (500 mL) and then a gradient from 5% to 30% aq EtOH (500 mL, each) gave compound **7** (60.4 mg) in 79.1% yield. The TOF-MS and ^1H NMR spectra of **7** confirmed the S-benzylation. Compound **7** (100 mg) was hydrolyzed with 2 M HCl (10 mL) at 80 °C for 4 h. The reaction solution was neutralized with 1 M NaOH, and then chromato-

graphed on a reversed-phase Lobar column. Elution of the column with water (500 mL) and a subsequent gradient from water to 50% aq EtOH (500 mL each) gave a maltose derivative **8** (13.4 mg) in 45.7% yield together with tri, tetra, and pentasaccharide derivatives that were respectively shown to possess the basic structure of **8** by their TOF-MS spectra. The reducing anomeric carbon of **8** was reduced by reacting **8** (8.4 mg) with 1% aq $NaBH_4$ (840 μL) for 30 min and purified compound **9** was obtained by the chromatograph of the reaction mixture on a reversed-phase Lobar column (eluted with a gradient from water to 50% aq EtOH).

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11. Structural data of the isolated thioester of **5**: TOF-mass: *m/z* 1197 ($M+Na^+$), 1213 ($M+K^+$). ^{13}C NMR (D_2O, CH_3CN int.): δ 199.7, 103.1, 102.5, 102.3, 100.8, 94.8, 81.9, 81.6 81.5, 81.3, 76.9, 76.3, 74.3, 74.2, 74.0, 73.8 73.4, 73.1, 72.9, 72.8, 72.7, 72.6, 72.5, 72.4, 72.3, 70.9, 70.4, 61.3, 61.2, 60.9, 60.3, 44.3, 31.3 ppm.