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Synthetic studies of bi-fluorescence-labeled maltooligosaccharides as substrates for α-amylase on the basis of fluorescence resonance energy transfer (FRET)

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ABSTRACT

A series of bi-fluorescence-labeled maltooligosaccharides that lead to fluorescence resonance energy transfer (FRET) was systematically synthesized. Effective FRETs were observed with all of the synthesized probes. Digestion of probes having tetra-, quintet-, hexa- or hepta-saccharidic chain lengths with human saliva α-amylase resulted in disappearance of FRET when an excitation wavelength of at 290 nm was used followed by detection at ca. 520 nm due to emission from the dansyl moiety. However, continuous FRET was observed when probes having di- or trisaccharidic chain lengths were used as substrates. In addition to the substrate characteristics based on saccharidic chain length, the reaction rates of digestion for the substrates by amylase were different and also depended on their saccharidic chain length.

1. Introduction

An analytical method based on fluorescence resonance energy transfer (FRET) is widely used not only in the biochemical field but also in the molecular biological field.[1] FRET is often applied to determine the distance between a donor fluorophore and an acceptor fluorophore on the basis of intramolecular FRET. The combination of donor–acceptor pair and the strategy for introduction of the probes into the substrate are important for selection of FRET probes. In carbohydrate chemistry, several FRET-based substrates having two independent fluorophores have been synthesized for analysis of the relation between the enzyme and carbohydrate. For example, chitopentaose having 5-(2-aminoethyl) amino-1-naphtalene-sulfonic acid (EDANS) and 4-dimethylaminoazobenzene (DAB) on each of the reducing and non-reducing end has been developed for the assay of chitinases.[2] A lactosyl derivative having dansyl fluorophore (DAN) and 2-naphthyl fluorophore (NAP) on both ends has also been developed for the assay of ceramide glycanase.[3] Furthermore, maltooligosaccharide-related fluorogenic substrates have been prepared for continuous assay of α-amylase.[4] Recently, the relationships between α-amylase isozymes and diseases such as myeloma[5] and diabetes[6] have been reported. Therefore, amylase substrates have been developed to elucidate amylase functions;[4] however, various amylase substrates are required for more detailed study of such diseases. In our previous study, we established a novel synthetic route for construction of bi-fluorescence-labeled maltohexaoside for α-amylase assay based on FRET.[7] In this paper, we describe the synthesis of a library of fluorogenic maltooligosaccharidic derivatives having different sugar chain lengths and comparison of the various derivatives as probes for α-amylase assay based on FRET.

2. Results and discussion

Since convenient and highly sensitive substrates for α-amylase were not available, fluorogenic substrates based on intramolecular FRET for α-amylase were systematically designed. Specific validation of FRET was required after adding amylase enzyme in order to function as an amylase probe. Thus, we designed a maltooligosaccharide derivative having two different fluorophores on both ends and having a non-modified saccharidic chain which can be hydrolyzed by amylase (Fig. 1).

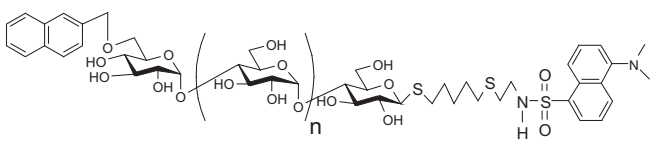

Figure 1. A series of bi-fluorescence-labeled maltooligosaccharides.

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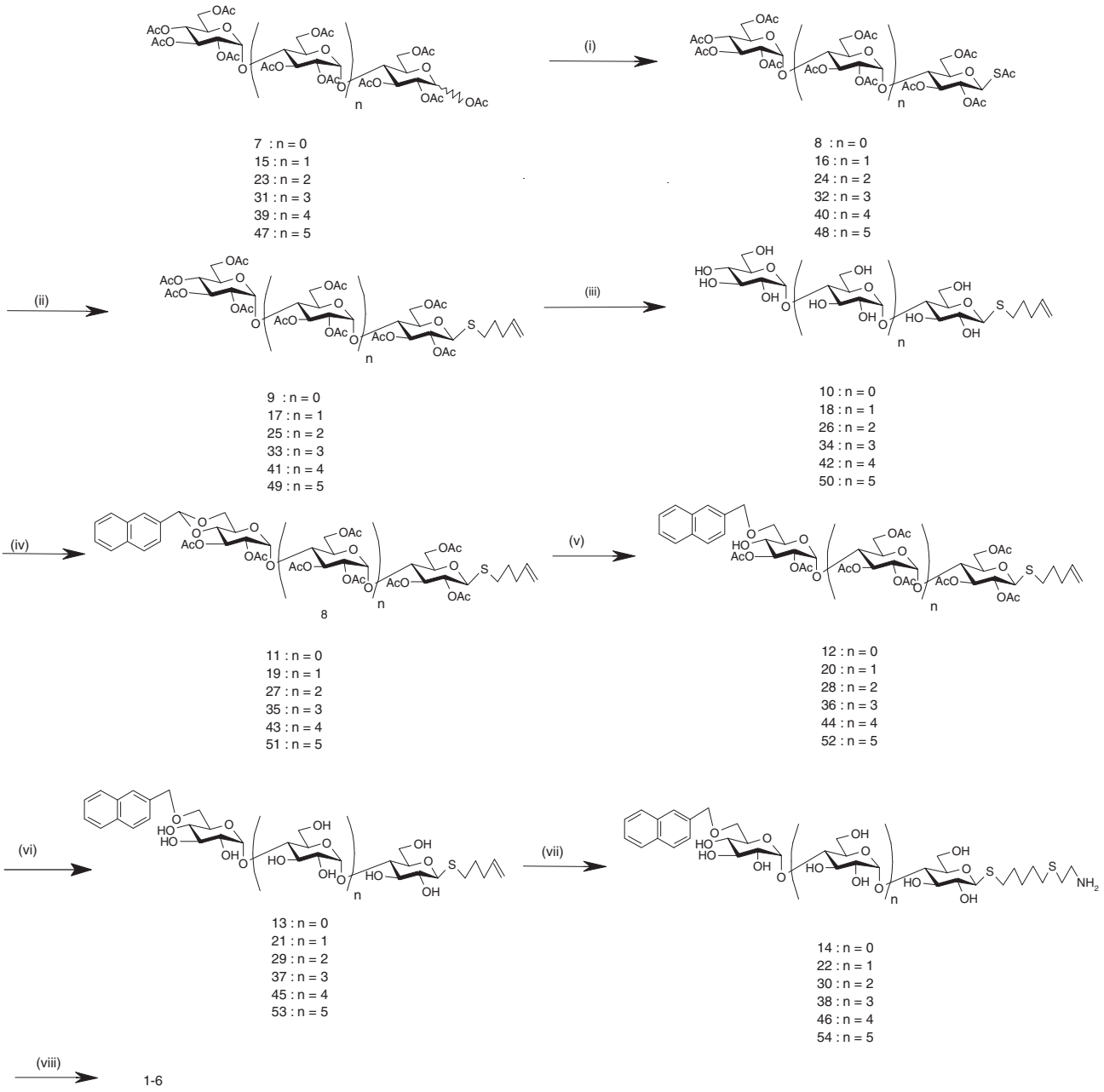
Acetates of maltotriaose **15**, maltotetraose **23** and maltopentaose **31** were readily isolated from a maltotetraose-rich maltodextrin (Tetrup-H; Hayashibara, Japan) and a maltopentaose-rich maltodextrin (Pentrup; Hayashibara, Japan) through freeze drying and subsequent acetylation. Acetates of maltohexaose **39** and maltoheptaose **47** were prepared from α- and β-cyclodextrin through ring opening acetolysis reaction, respectively.[8]

2.1. Synthesis of bi-fluorescence-labeled maltoside as a model for estimation of the synthetic route

Maltose was chosen as the simplest maltooligosaccharide having one α-1,4-linkage in order to establish the synthetic route for construction of the bi-fluorescence-labeled substrate. A naphthymethyl (NAP) residue and a dansyl (DAN) residue are selected as a fluorescent donor and an acceptor, respectively, because that pair shows suitable efficiency on FRET, stability in aqueous media and low cost for chemical synthesis.[9] In Scheme 1, we selected a readily available *S*-pentenyl derivative **10** as a key intermediate, where the C=C double bond at the terminal of the sugar aglycon is attractive for chemical modifications.

Anomeric β-thioacetate **8** was readily synthesized from maltose per acetate **7**[7] from α-bromomaltoside and potassium thioacetate by simple S_N2 reaction in 72% yield (two steps). A one-pot procedure involving anomeric de-S-acetylation with diethylamine and subsequent coupling reaction with *n*-pentenyl bromide afforded *n*-pentenyl thiomaltoside **9** in 91% yield. Ester exchange reaction of **9** using Zemplén's transesterification quantitatively gave the corresponding unprotected pentenyl thiomaltoside **10**. Because of the stability of the NAP residue under various reaction conditions,



Scheme 1. Reagents and conditions: (i) HBr/AcOH, Ac_2O, AcOH, rt, then AcSK, DMF; (ii) 5-bromopent-1-ene, DMF, diethylamine, −15 °C; (iii) NaOMe, MeOH, rt; (iv) 2-naphthaldehyde di-*i*-butyl acetal, CSA, reduced pressure, 50 °C, then Ac_2O-pyr, rt; (v) borane–trimethylamine complex, $AlCl_3$, MS4 Å, THF, rt; (vi) NaOMe, MeOH, rt; (vii) $HS(CH_2)_2NH_2HCl$, MeOH–H_2O, UV irradiation, rt; (viii) dansyl chloride, Et_3N, MeOH, rt.

we initially tried to introduce a naphthymethyl functional group at one of the ends. Thus, regioselective acetal exchange reaction with 2-naphtoaldehyde di-*i*-butylacetal (2-NADIBA)[3] and subsequent acetylation yielded cyclic acetal **11** as the sole product having 4^{II}, 6^{II}-*O*-naphthylymethyliden acetal at the non-reducing end in 45% yield. Reductive ring opening of the cyclic acetal in **11** in the presence of $BH_3{\cdot}NMe_3$–$AlCl_3$ in THF proceeded smoothly to yield 6^{II}-*O*-naphthylmethyl compound **12** in 74% yield. Quantitative transesterification of **12** using Zemplén's method gave the corresponding unprotected maltoside **13**. Radical addition of 2-mercaptoethylamine into the terminal C=C double bond of aglycon of **13** was carried out under UV irradiation to afford amine **14** with antiMarkovnikov's orientation in quantitative yield. In this radical-mediated reaction, a solvent of MeOH and water was required as a suitable solvent because of the different solubility of each starting material. In addition to the solubility, a highly concentrated solution was needed for enhancement of the yield on radical addition reaction. N-Dansylation of **14** was carried out by means of dansyl chloride in MeOH to yield bi-fluorescence-labeled maltoside **1** in quantitative yield.

2.2. Construction of a library of bi-fluorescence-labeled maltooligosaccharides having various saccharidic chain lengths

Since a synthetic route for construction of bi-fluorescence-labeled maltoside **1** was successfully established, we applied this method to the synthesis of maltooligosaccharide **2**–**5** having different saccharidic chain lengths against **1**. Anomeric thioacetates, **16**, **24**, **32**, **40** and **48**, were readily synthesized from corresponding acetates, **15**, **23**, **31**, **39** and **47**, respectively, via α-bromomaltooligosaccharides. The thioacetates were converted into corresponding *n*-pentenyl thioglycosides, **17**, **25**, **33**, **41** and **49**, by a method similar to Williamson ether synthesis. Quantitative de-O-acetylation of the thioglycosides was carried out by means of usual transesterification. For complete deprotection of maltooligosaccharides having a longer saccharidic chain than that of maltose, prolonged reaction time was required. Although 4,6-O-naphthylmethylidenation of nonreducing end glucose of the oligosaccharides with 2-NADIBA in the presence of an acid is a key step in this reaction route, the transacetalization could not proceed completely. Further trials using stronger reaction conditions gave a mixture including di- or tri-O-naphthylmethylidenated compounds. Thus, careful observation of the reaction mixture by TLC was needed, and isolation of the corresponding monocyclic acetal and starting material from the mixture was also carefully accomplished by means of chromatography on silica gel. Recovered starting materials could be recycled for this reaction after de-O-acetylation. Reductive ring opening of **19**, **27**, **35**, **43** and **51**, was performed using same protocol as that for **11** to give corresponding 6-*O*-naphthylmethyl compounds **20**, **28**, **36**, **44** and **52**, in good yields. De-O-acetylation of all of the acetates was carried out by transesterification in quantitative yields. Radical addition of 2-mercaptoethylamine and subsequent N-dansylation were successfully carried out to yield bi-fluorescence-labeled maltooligosaccharides **2–6**, respectively.

2.3. Enzymatic evaluation of the activities of bi-fluorescence-labeled maltooligosaccharides with human α-amylase

Since systematic synthesis of bi-fluorescence-labeled maltooligosaccharides was successfully accomplished, our attention was turned to evaluation of the fluorogenic compounds based on FRET. When a NAP fluorophore is excited at 290 nm, an emission of the NAP is observed around at 333 nm. Since the fluorescence emission field (333 nm) from NAP covers an excitation wavelength of a DAN fluorophore, the bi-fluorescence-labeled maltooligosaccharides give DAN emission at around 498–530 nm. When NAP and DAN

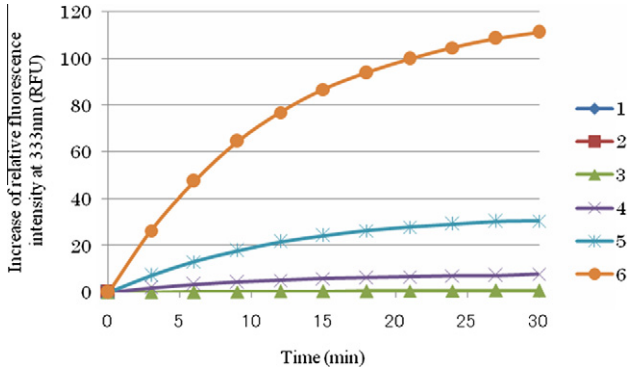


Figure 2. Time course of the hydrolysis of bi-fluorescence-labeled maltooligosaccharide by human saliva α-amylase.

fluorophores in the maltooligosaccharidic substrates are close to each other (less than 100 Å), FRET from NAP to DAN would be observed. Indeed, FRETs were observed from all synthesized probes when excitation at 290 nm was used. Therefore, it was estimated that both fluorophores are located less than 100 Å in aqueous media. Since FRET was certified using a series of fluorogenic substrates, continuous assay by means of FRET of the substrates was carried out using an α-amylase. Addition of human saliva α-amylase to the substrate solution decreased the FRETs when tetra-, penta-, hexa- and heptasaccharides were used as substrates. However, no change of fluorescence intensity was observed when di- and tri-saccharides were used as substrates. The results suggested that human saliva α-amylase hydrolyzed the oligosaccharide chain more than trisaccharidic chain length. Figure 2 shows the increase of relative fluorescence intensity at 333 nm which is corrected by initial dansyl intensity as 100 RFU. The results suggest that the rates of change in fluorescence intensity depended on the saccharidic chain length. Furthermore, a synthesized maltooligosaccharide having a large chain length was more rapidly hydrolyzed than that having a small chain length. This phenomenon is similar to that previously reported for a ^{14}C anomeric radiolabeled free maltoorigoside.[10]

3. Conclusion

We have systematically succeeded in the synthesis of a series of bi-fluorescence-labeled maltooligosaccharides having both DAN and NAP groups as FRET probes. Both derivatives of maltoside and maltotriaoside have resistance to human saliva α-amylase. Tetra-, penta-, hexa- and heptaosides undergo enzymatic digestion, and the reaction rate of heptaoside is the most rapid. We believe that these probes can be applied for study of various diseases related to amylases.

4. Experimental

4.1. Materials and methods

Unless otherwise stated, all commercially available solvents and reagents were used without further purification. Pyridine (Pyr) and *N*,*N*-dimethylformamide (DMF) were stored over molecular sieves (4 Å MS), and methanol (MeOH) was stored over 3 Å MS before use. Tetrahydrofuran (THF) was dried over sodium benzophenone ketyl under argon atmosphere and distilled prior to use. Optical rotations were determined with a JASCO DIP-1000 digital polarimeter. The IR spectra were obtained using a Shimadzu IR Prestage-21 spectrometer. The ^1H NMR spectra were recorded at 400 MHz for ^1H and 100 MHz for ^{13}C with a Bruker DPX-400 or a

Bruker DRX-400 or at 500 MHz for ^{1}H and 125 MHz for ^{13}C with a Bruker AVANCE 500 spectrometer in chloroform-*d* ($CDCl_3$). Chemical shifts are expressed as parts per million (ppm, δ) and are relative to an internal tetramethylsilane (TMS) in $CDCl_3$ (δ 0.0) or HDO in D_2O (δ 4.78) for ^{1}H, and $CDCl_3$ (δ 77.0), MeOD (δ 49.0) or acetone (δ 215.0) for ^{13}C. Ring-proton assignments in the ^{1}H NMR spectra were made by first-order analysis of the spectra and are supported by the results of homonuclear decoupling experiments and H–H or C–H COSY experiments. Elemental analyses were performed with a Fisons EA1108 on samples extensively dried at 50–60 °C over phosphorus pentoxide for 4–5 h. Fastatom bombardment mass (FABMS) spectra were recorded with a JEOL DX-303 spectrometer. Matrix-assisted laser desorption/ionization time-of-flight mass spectra (MALDI-TOF-MS) were obtained using a Bruker AutoflexIII spectrometer. Reactions were monitored by thin layer chromatography (TLC) on a precoated plate of Silica Gel 60 F_{254} (layer thickness, 0.25 mm; E. Merck, Darmstadt, Germany). For detection of the intermediates, TLC sheets were dipped in (a) a solution of 85:10:5 (v/v/v) MeOH–*p*-anisaldehyde–concd H_2SO_4 and heated for a few minutes (for carbohydrate) or (b) an aq solution of 5 wt % $KMnO_4$ and heated similarly (for detection of C=C double bonds). Column chromatography was performed on silica gel (Silica Gel 60; 63–200 μm, E. Merck). Flush column chromatography was performed on silica gel (Silica Gel 60, spherical neutral; 40–100 μm, E. Merck). All extractions were concentrated below 45 °C under diminished pressure.

4.1.1. 4-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-glucopyranose thioacetate (8)

Maltose per acetate **7** (30.0 g, 44 mmol) was dissolved in acetic acid (60.0 mL), and acetic anhydride (6.0 mL) was added to the solution. 30% HBr/AcOH was added to the solution and the reaction mixture was stirred at room temperature for 3 h. Then the solution was evaporated and co-evaporated with toluene to give maltosyl bromide, which was dissolved in DMF (300 mL) and cooled to 0 °C. Potassium thioacetate was added to the solution and the solution was stirred overnight at room temperature. The solution was extracted with EtOAc, and the organic layer was washed successively with cold H_2O, cold satd aq $NaHCO_3$ and brine, and then dried over anhyd Na_2SO_4, filtered, and evaporated in vacuo. Purification of the residue by silica gel column chromatography with 1:1 (v/v) hexane–EtOAc gave **8** (21.93 g, 71.9%).

R_f 0.43 [1:1 (v/v) ethyl acetate–toluene], 0.20 [1:1 (v/v) ethyl acetate–hexane]; $[\alpha]_D^{28}$ +76.2° (*c* 0.51, $CHCl_3$); IR (KBr) 1751 ($\nu_{C=O}$, ester), 1373 (ν_{C-H}), 1233 (ν_{C-O}, ester), 1045 (ν_{C-O}, ether) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.40 (d, 1H, $J_1^{II},_2^{II}$ = 4.0 Hz, H-1^{II}), 5.33 (m, 3H, H-1, H-3, H-3^{II}), 5.06 (t, 1H, $J_3^{II},_4^{II} = J_4^{II},_5^{II}$ = 9.9 Hz, H4^{II}), 4.98 (dd, 1H, *J* = 8.8 Hz, *J* = 10.4 Hz, H-2), 4.86 (dd, 1H, $J_1^{II},_2^{II}$ = 4.0 Hz, $J_2^{II},_3^{II}$ = 10.8 Hz, H-2^{II}), 4.45 (dd, 1H, $J_{5,6b}$ = 2.4 Hz, $J_{6a,6b}$ = 12.4 Hz, H-6b), 4.24 (dd, 1H, $J_{5,6}{}^{II}{}_b$ = 3.8 Hz, $J_6{}^{II}{}_{a,6}{}^{II}{}_b$ = 12.2 Hz, H-6^{II}b), 4.21 (dd, 1H, $J_{5,6a}$ = 4.0 Hz, $J_{6a,6b}$ = 12.4 Hz, H-6a), 4.02 (m, 2H, H-4, H-6^{II}a), 3.94 (m, 1H, H-5^{II}), 3.83 (m, 1H, H-5), 2.38 (s, 3H, S*Ac*), 2.13, 2.10, 2.06, 2.03, 2.01, 2.00 and 2.00 (each s, 21H, 7O*Ac*); MALDI-TOF-MS Calcd for $C_{76}H_{102}O_{50}S$ [M+Na]$^+$: 717.167, [M+K]$^+$: 733.141. Found: *m/z*: 717.158, *m/z*: 733.143.

Anal. Calcd for $C_{28}H_{38}O_{18}S$: C, 48.41; H, 5.51; N, 0.00. Found: C, 48.33; H, 5.50; N, 0.00.

4.1.2. *n*-Pentenyl 4-*O*-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-2,3,6-tri-O-acetyl-1-thio-β-D-glucopyranoside (9)

To a solution of maltose thioacetate **8** (20.0 g, 28.8 mmol) in DMF (200 mL) was added 5-bromopent-1-ene, and the mixture was cooled to −15 °C. To the solution was added diethyl amine (60.2 mL, 576 mmol) and then stirring was continued at room temperature for 2 h. The solution was diluted with EtOAc, and the organic layer was successively washed with cold H_2O, dried over anhyd Na_2SO_4, filtered, and evaporated in vacuo. Purification of the residue by silica gel column chromatography with 10:9 (v/v) hexane–EtOAc gave **9** (18.9 g, 90.9%).

R_f 0.38 [1:1 (v/v) ethyl acetate–hexane], 0.14 [1:2 (v/v) ethyl acetate–hexane]; $[\alpha]_D^{25}$ +44.1° (*c* 0.64, $CHCl_3$); IR (KBr): 2959 (ν_{C-H}), 1748 ($\nu_{C=O,}$ ester), 1373 (ν_{C-H}), 1238 (ν_{C-O}, ester), 1045 (ν_{C-O}, ether) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ ($CDCl_3$) 5.77 (m, 1H, –CH_2CH=CH_2), 5.41 (d, 1H, $J_1{}^{I},_2{}^{I}$ = 4.0 Hz, H-1^{I}), 5.36 (dd, 1H, *J* = 9.7 Hz, *J* = 10.3 Hz, H-3^{I}), 5.28 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, H-3), 5.03 (m, 3H, H4^{II}, –CH_2CH=CH_2), 4.86 (m, 2H, H-2, H-2^{II}), 4.53 (d, 1H, $J_{1,2}$ = 10.1 Hz, H-1), 4.46 (dd, 1H, $J_{5,6b}$ = 2.6 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6b), 4.25 (dd, 1H, $J_5{}^{II},_6{}^{II}{}_b$ = 4.0 Hz, $J_6{}^{II}{}_{a,6}{}^{II}{}_b$ = 12.2 Hz, H-6^{II}b), 4.21 (dd, 1H, $J_{5,6a}$ = 4.4 Hz, $J_{6a,6b}$ = 11.6 Hz, H-6a), 4.05 (dd, 1H, $J_5{}^{II},_6{}^{II}{}_a$ = 2.3 Hz, $J_6{}^{II}{}_{a,6}{}^{II}{}_b$ = 12.4 Hz, H-6^{II}a), 3.99 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.4 Hz, H-4), 3.95 (m, 1H, H-5^{II}), 3.69 (m, 1H, H-5), 2.66 (m, 2H, SCH_2CH_2–), 2.13, 2.10, 2.05, 2.03, 2.03, 2.01 and 2.00 (each s, 21H, 7O*Ac*), 2.17 (m, 2H, $SCH_2CH_2CH_2CH$=CH_2), 1.69 (m, 2H, SCH_2CH_2–); MALDI-TOF-MS Calcd for $C_{31}H_{44}O_{17}S$ [M+Na]$^+$: 743.219, [M+K]$^+$: 759.193. Found: *m/z*: 743.173, *m/z*: 759.153.

Anal. Calcd for $C_{31}H_{44}O_{17}S$: C, 51.66; H, 6.15; N, 0.00. Found: C, 51.71; H, 6.12; N, 0.00.

4.1.3. *n*-Pentenyl 4-*O*-(β-D-glucopyranosy)-1-thio-β-D-glucopyranoside (10)

To a solution of β-thioacetyl maltoside per acetate **9** (17.4 g, 24.0 mmol) in MeOH (348 mL) was added NaOMe (900 mg, 16.9 mmol), and the mixture was stirred overnight at room temperature. IR-120B (H^+) resin was added to the mixture to remove Na^+, and then the suspension was filtered and the filtrate was concentrated. Gel filtration of the residue using Sephadex G-25 with 5% aq AcOH gave **10** quantitatively.

R_f 0.53 [2:1 (v/v) chloroform–methanol], 0.03 [5:1 (v/v) chloroform–methanol]; $[\alpha]_D^{26}$ +49.6° (*c* 0.73, water); IR (KBr) 3360 (ν_{O-H}), 2928 (ν_{C-H}), 1639 ($\nu_{C=C}$), 1026 (ν_{C-O}) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ (D_2O) 5.80 (m, 1H, –CH_2CH=CH_2), 5.31 (d, 1H, $J_1{}^{II},_2{}^{II}$ = 3.4 Hz, H-1^{II}), 5.00 (d, 1H, J_{trans} = 17.3 Hz, one of –CH=CH_2), 4.93 (d, 1H, J_{cis} = 10.2 Hz, one of –CH=CH_2), 4.44 (d, 1H, $J_{1,2}$ = 9.9 Hz, H-1), 2.66 (m, 2H, SCH_2CH_2–), 2.08 (m, 2H, $SCH_2CH_2CH_2CH$=CH_2), 1.65 (m, 2H, SCH_2CH_2–); Fab-MS Calcd for $C_{17}H_{30}O_{10}S$ [M+Na]$^+$: 449.145. Found: *m/z*: 448.943.

Anal. Calcd for $C_{17}H_{30}O_{10}S{\cdot}0.8H_2O$: C, 46.31; H, 7.22; N, 0.00. Found: C, 46.06; H, 6.92; N, 0.00.

4.1.4. *n*-Pentenyl 4-*O*-(2,3-di-*O*-acetyl-4,6-*O*-naphthylmethylidene-β-D-glucopyranosyl)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (11)

To a solution of 2-naphthaldehyde diisobutyl acetal (2-NADIBA)[3c] (4.82 g, 16.8 mmol) and pentenyl thioglycoside **10** (2.39 g, 5.60 mmol) in DMF (24.0 mL) was added 10-(+)-camphorsulfonic acid (CSA) (650 mg, 2.80 mmol) and the solution was stirred at 50 °C under diminished pressure for 2 h. Then the solution was cooled to 0 °C, and triethylamine was added. After concentration of the solution, Ac_2O (24.0 mL) and pyridine (24.0 mL) were added and the mixture were stirred at room temperature for 4 days. After concentration of the solution followed by addition of cold water, the mixture was extracted with $CHCl_3$, and the organic layer was washed successively with cold H_2O, cold 1 M aq HCl, cold satd aq $NaHCO_3$ and brine, and then dried over anhyd Na_2SO_4, filtered, and evaporated in vacuo. Purification of the residue by silica gel column chromatography with 5:4 (v/v) hexane–EtOAc gave pure **11** (1.97 g, 45.4%).

R_f 0.42 [1:1 (v/v) ethyl acetate–hexane]; $[\alpha]_D^{26}$ +12.3° (*c* 0.51, $CHCl_3$); IR (KBr) 2941 (ν_{C-H}), 1744 ($\nu_{C=O}$, ester), 1373 (ν_{C-H}), 1230 (ν_{C-O}, ester), 1051 (ν_{C-O}, ether) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.90−7.46 (m, 7H, naphthyl), 5.77 (m, 1H, –CH_2CH=CH_2), 5.64 (s,

1H, naphthyl-*CH*OO), 5.49 (t, 1H, $J_{2^{II},3^{II}} = J_{3^{II},4^{II}}$ = 10.0 Hz, H-3^{II}), 5.37 (d, 1H, $J_{1^{II},2^{II}}$ = 4.2 Hz, H-1^{II}), 5.30 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, H-3), 5.02 (m, 2H, –CH=*CH*$_2$), 4.91 (dd, 1H, $J_{1^{II},2^{II}}$ = 4.1 Hz, $J_{2^{II},3^{II}}$ = 10.2 Hz, H-2^{II}), 4.87 (t, 1H, $J_{1,2} = J_{2,3}$ = 9.7 Hz, H-2), 4.54 (m, 1H, H-6), 4.53 (d, 1H, $J_{1,2}$ = 9.8 Hz, H-1), 4.30 (dd, 1H, $J_{5^{II},6^{II}}$ = 4.7 Hz, J_{gem} = 10.0 Hz, H-6^{II}), 4.28 (dd, 1H, $J_{5,6^{II}}$ = 4.2 Hz, J_{gem} = 12.1 Hz, H-6), 4.01 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.3 Hz, H-4), 3.91 (m, 1H, H-5^{II}), 3.79 (t, 1H, $J_{5^{II},6^{II}} = J_{gem}$ = 10.0 Hz, H-6^{II}), 3.71 (m, 1H, H-5), 3.69 (t, 1H, $J_{3^{II},4^{II}} = J_{4^{II},5^{II}}$ = 9.6 Hz, H-4^{II}), 2.66 (m, 2H, S*CH*$_2$CH$_2$–), 2.13 (m, 2H, SCH$_2$CH$_2$*CH*$_2$CH=CH$_2$), 2.12, 2.07, 2.05, 2.04 and 2.03 (5s, 15H, 5O*Ac*), 1.70 (m, 2H, SCH$_2$*CH*$_2$–); MALDI-TOF-MS Calcd for $C_{38}H_{46}O_{15}S$ [M+Na]$^+$: 797.245, [M+K]$^+$: 813.219. Found: *m/z*: 797.184, *m/z*: 813.154.

Anal. Calcd for $C_{38}H_{46}O_{15}S$: C, 58.90; H, 5.98; N, 0.00. Found: C, 58.89; H, 5.87; N, 0.00.

4.1.5. *n*-Pentenyl 4-*O*-(2,3-di-*O*-acetyl-6-*O*-naphthylmethyl-β-D-glucopyranosyl)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (12)

To a suspension of 4^{II},6^{II}-*O*-naphthylmethylidene maltoside **11** (1.97 g, 2.54 mmol) and 4 Å MS powder (3.90 g) in THF (30.0 mL) was added BH_3–NMe_3 complex (1.85 g, 25.4 mmol), and the mixture was stirred at room temperature for 1 h. After the suspension had been cooled to 0 °C, aluminum chloride (1.60 g, 12.3 mmol) was added, and then the mixture was stirred at room temperature for 2 h. The suspension was filtered, and the filtrate was diluted with $CHCl_3$ and washed successively with cold 1 M aq HCl, cold satd aq $NaHCO_3$ and brine, and then dried over anhyd Na_2SO_4, filtered, and evaporated in vacuo. Chromatographic purification of the residue by silica gel with 5:4 (v/v) hexane–EtOAc as the eluent gave **12** (1.48 g, 74.0%).

R_f 0.50 [2:1 (v/v) ethyl acetate–hexane]; $[\alpha]_D^{23}$ +39.9° (*c* 0.67, $CHCl_3$); IR (KBr) 2938 (ν_{C-H}), 1749 ($\nu_{C=O}$, ester), 1371 (ν_{C-H}), 1236 (ν_{C-O}, ester), 1049 (ν_{C-O}, ether) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.86–7.45 (m, 7H, naphthyl), 5.76 (m, 1H, –CH$_2$*CH*=CH$_2$), 5.37 (d, 1H, $J_{1^{II},2^{II}}$ = 4.0 Hz, H-1^{II}), 5.00 (m, 2H, –CH=*CH*$_2$), 4.15 (m, 2H, H-6), 3.98 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.2 Hz, H-4), 2.64 (m, 2H, S*CH*$_2$CH$_2$–), 2.13 (m, 2H, SCH$_2$CH$_2$*CH*$_2$CH=CH$_2$), 2.08, 2.05, 2.03, 2.00 and 1.99 (5s, 15H, 5O*Ac*), 1.68 (m, 2H, SCH$_2$*CH*$_2$–); MALDI-TOF-MS Calcd for $C_{38}H_{48}O_{15}S$ [M+Na]$^+$: 799.261, [M+K]$^+$: 815.235. Found: *m/z*: 799.164, *m/z*: 815.147.

Anal. Calcd for $C_{38}H_{48}O_{15}S$: C, 58.75; H, 6.23; N, 0.00. Found: C, 58.56; H, 6.15; N, 0.00.

4.1.6. *n*-Pentenyl 4-*O*-(6-*O*-naphthylmethyl-β-D-glucopyranosyl)-1-thio-β-D-glucopyranoside (13)

To a solution of 6^{II}-*O*-naphthylmethyl maltoside **12** (1.40 g, 1.80 mmol) in MeOH (328.0 mL) was added NaOMe (49 mg, 0.90 mmol), and the mixture was stirred overnight at room temperature. IR-120B (H$^+$) resin was added to the mixture to remove Na$^+$, and then the suspension was filtered and the filtrate was concentrated. Gel filtration of the residue using LH-20 with MeOH gave **13** quantitatively.

R_f 0.30 [5:1 (v/v) chloroform–methanol], 0.79 [2:1 (v/v) chloroform–methanol]; IR (KBr) 3385 (ν_{O-H}), 2924 (ν_{C-H}), 1638 ($\nu_{C=C}$), 1051 (ν_{C-O}) cm^{-1}; ^{1}H NMR (CD_3OD): δ 7.69–7.26 (m, 7H, naphthyl), 5.63 (m, 1H, –CH$_2$*CH*=CH$_2$), 5.01 (d, 1H, $J_{1^{II},2^{II}}$ = 3.7 Hz, H-1^{II}), 4.85 (d, 1H, J_{trans} = 17.1 Hz, one of –CH=*CH*$_2$), 4.77 (d, 1H, J_{cis} = 10.2 Hz, one of –CH=*CH*$_2$), 4.57 (s, 2H, naphthyl-*CH*$_2$O), 4.18 (d, 1H, $J_{1,2}$ = 9.7 Hz, H-1), 3.28 (dd, 1H, $J_{1^{II},2^{II}}$ = 4.0 Hz, $J_{2^{II},3^{II}}$ = 9.7 Hz, H-2^{II}) 3.07 (t, 1H, $J_{1,2} = J_{2,3}$ = 9.2 Hz, H-2), 2.54 (m, 2H, S*CH*$_2$CH$_2$–), 1.99 (m, 2H, SCH$_2$CH$_2$*CH*$_2$CH=CH$_2$), 1.54 (m, 2H, SCH$_2$*CH*$_2$–); MALDI-TOF-MS Calcd for $C_{28}H_{38}O_{10}S$ [M+Na]$^+$: 589.208, [M+K]$^+$: 605.182. Found: *m/z*: 589.108, *m/z*: 605.105.

4.1.7. Amino-ethyl thiopentyl 4-*O*-(6-*O*-naphthylmethyl-β-D-glucopyranosyl)-1-thio-β-D-glucopyranoside (14)

To a solution of free 6^{II}-*O*-naphtylmethyl maltoside **13** (200 mg, 0.353 mmol) in MeOH (0.5 mL) was added H_2O (0.5 mL) and 2-mercaptoethylamine hydrochloride (399 mg, 3.53 mmol). After UV irradiation for 4 h, the solution was directly applied to an LH-20 column with MeOH to give **14** quantitatively.

R_f 0.70 [5:5:1 (v/v) chloroform–methanol–water], 0.08 [2:1 (v/v) chloroform–methanol]; IR (KBr) 3408 (ν_{O-H}), 2926 (ν_{C-H}), 1622 (ν_{N-H}), 1051 (ν_{C-O}) cm^{-1}; ^{1}H NMR (D_2O): δ 7.88–7.45 (m, 7H, naphthyl), 5.27 (d, 1H, $J_{1^{II},2^{II}}$ = 3.8 Hz, H-1^{II}), 4.34 (d, 1H, $J_{1,2}$ = 9.9 Hz, H-1), 3.30 (m, 2H, H-3, H-2^{II}), 3.20 (t, 1H, $J_{1,2} = J_{2,3}$ = 9.4 Hz, H-2), 3.07 (t, 2H, *J* = 6.6 Hz, S*CH*$_2$CH$_2$N), 2.71 (t, 2H, *J* = 6.6 Hz, SCH$_2$*CH*$_2$N), 2.61 (m, 2H, G-S*CH*$_2$–), 2.47 (t, 2H, *J* = 7.1 Hz, –*CH*$_2$SCH$_2$CH$_2$N), 1.52 (m, 4H, G-SCH$_2$*CH*$_2$CH$_2$*CH*$_2$CH$_2$S), 1.37 (m, 2H, G-SCH$_2$CH$_2$*CH*$_2$–). MALDI-TOF-MS Calcd for $C_{30}H_{45}NO_{10}S_2$ [M+H]$^+$: 644.256. Found: *m/z*: 644.124.

4.1.8. Dansyl amido-ethyl thiopentyl 4-*O*-(6-*O*-naphthylmethyl-β-D-glucopyranosyl)-1-thio-β-D-glucopyranoside (1)

To a solution of amine **14** (68 mg, 0.10 mmol) derivatives in MeOH (3.4 mL) was added dansyl chloride (108 mg, 0.40 mmol) and triethyl amine (28 μL, 0.20 mmol), and then the mixture was stirred at room temperature for 2 h. Direct purification was performed by LH-20 with MeOH as an eluent to give **1** quantitatively.

R_f 0.44 [5:1 (v/v) chloroform–methanol], 0.79 [2:1 (v/v) chloroform–methanol]; IR (KBr) 3406 (ν_{O-H}), 2924 (ν_{C-H}), 1636 (ν_{N-H}), 1321 ($\nu_{S=O}$), 1144 ($\nu_{S=O}$), 1051 (ν_{C-O}) cm^{-1}; ^{1}H NMR (CD_3OD): δ 8.47–7.27 (m, 13H, naphthyl and dansyl), 5.01 (d, 1H, $J_{1^{II},2^{II}}$ = 3.7 Hz, H-1^{II}), 4.57 (s, 2H, naphthyl-*CH*$_2$O), 4.18 (d, 1H, $J_{1,2}$ = 9.7 Hz, H-1), 3.53 (dd, 1H, *J* = 6.4 Hz, *J* = 10.7 Hz), 3.01 (s, 6H, dansyl-N*Me*$_2$), 2.87 (t, 2H, *J* = 7.2 Hz, SCH$_2$*CH*$_2$N), 2.50 (m, 2H, G-S*CH*$_2$–), 2.25 (t, 2H, *J* = 7.2 Hz, S*CH*$_2$CH$_2$N), 2.12 (t, 2H, *J* = 6.5 Hz, –*CH*$_2$SCH$_2$CH$_2$N), 1.39 (m, 2H, G-SCH$_2$*CH*$_2$–), 1.22 (m, 4H, G-SCH$_2$CH$_2$*CH*$_2$*CH*$_2$CH$_2$S); MALDI-TOF-MS Calcd for $C_{42}H_{56}N_2O_{12}S_3$ [M+Na]$^+$: 899.289. Found: *m/z*: 899.241.

4.1.9. 2^{III},3^{III},4^{III},6^{III},2^{II},3^{II},6^{II},2,3,6,-Deca-*O*-acetyl-β-D-maltotriaose thioacetate (16)

Anomeric thioacetylation of **15** (7.00 g, 7.24 mmol) with potassium thioacetate (2.48 g, 21.0 mol) was carried out by the same method as that described for **8**. Purification was carried out by silica gel column chromatography with 3:5 (v/v) hexane–EtOAc to give **16** (5.21 g, 73.1%).

R_f 0.20 [1:1 (v/v) ethyl acetate–hexane], 0.26 [2:1 (v/v) ethyl acetate–hexane]; IR (KBr) 2960 (ν_{C-H}), 1748 ($\nu_{C=O}$, ester), 1236 (ν_{C-O}, ester), 1043 (ν_{C-O}, ether) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.41–5.25 (m), 5.07 (t, 1H, $J_{3^{III},4^{III}} = J_{4^{III},5^{III}}$ = 9.8 Hz, H4^{III}), 4.98 (t, 1H, $J_{1,2} = J_{2,3}$ = 9.6 Hz, H-2), 4.86 (dd, 1H, $J_{1^{III},2^{III}}$ = 3.8 Hz, $J_{2^{III},3^{III}}$ = 10.6 Hz, H-2^{III}), 4.74 (dd, 1H, *J* = 4.0 Hz, *J* = 10.4 Hz), 4.45 (m, 2H), 4.29 (dd, 1H, *J* = 4.0 Hz, *J* = 13.2 Hz), 4.25 (dd, 1H, *J* = 3.6 Hz, *J* = 13.2 Hz), 4.17 (dd, 1H, *J* = 2.8 Hz, *J* = 12.8 Hz) 4.05 (d, 1H, *J* = 10.8 Hz) 2.38–2.00 (11s, 33H, 10O*Ac*, S*Ac*); MALDI-TOF-MS. Calcd for $C_{40}H_{54}O_{26}S$ [M+Na]$^+$: 1005.252, [M+K]$^+$: 1021.226. Found: *m/z*: 1005.242, *m/z*: 1021.217.

4.1.10. *n*-Pentenyl 2^{III},3^{III},4^{III},6^{III},2^{II},3^{II},6^{II},2,3,6,-deca-*O*-acetyl-1-thio-β-D-maltotriaoside (17)

Anomeric extension of **16** (5.00 g, 5.09 mmol) with 5-bromopent-1-ene (0.88 mL, 10.2 mmol) and diethyl amine (10.7 mL, 102 mmol) was carried out by the same method as that described for **9**. Purification was carried out by silica gel column chromatography with 4:5 (v/v) hexane–EtOAc gave **17** quantitatively.

R_f 0.13 [1:1 (v/v) ethyl acetate–hexane], 0.39 [2:1 (v/v) ethyl acetate–hexane]; IR (KBr) 2957 (ν_{C-H}), 1751 ($\nu_{C=O}$, ester), 1236 (ν_{C-O}, ester), 1040 (ν_{C-O}, ether) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.78 (m,

1H, –CH_2CH=CH_2), 5.05 (m, 3H, –CH=CH_2), 4.74 (dd, 1H, J = 10.3 Hz, J = 4.1 Hz), 4.53 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-1), 4.46 (m, 2H), 4.30 (dd, 1H, J = 12.2 Hz, J = 4.3 Hz), 4.25 (dd, 1H, J = 12.5 Hz, J = 3.5 Hz), 4.18 (dd, 1H, J = 12.3 Hz, J = 3.4 Hz), 4.08 (near d, 1H, J = 12.5 Hz), 3.72 (m, 1H, H-5), 2.66 (m, 2H, SCH_2CH_2–), 2.16–1.99 (m, 32H, 10O*Ac*, $SCH_2CH_2CH_2CH$=CH_2), 1.70 (m, 2H, SCH_2CH_2–); ^{13}C NMR ($CDCl_3$): δ 171.17, 170.63, 170.58, 170.55, 170.47, 170.37, 170.04, 169.84, 169.69, 169.45, 137.40, 115.44, 95.73, 95.59, 83.13, 77.21, 76.21, 75.93, 73.71, 72.37, 71.65, 70.78, 70.38, 70.00, 69.28, 68.87, 68.43, 67.76, 63.13, 62.22, 61.28, 60.38, 32.55, 29.47, 28.81, 21.04, 20.86, 20.80, 20.67, 20.58, 14.16; MALDI-TOF-MS Calcd for $C_{43}H_{60}O_{25}S$ [M+Na]$^+$: 1031.304, [M+K]$^+$: 1047.278. Found: *m/z*: 1031.313, *m/z*: 1047.286.

4.1.11. *n*-Pentenyl 1-thio-β-D-maltotriaoside (18)

Deprotection of **17** (10.8 g, 10.7 mmol) was carried out by the same method as that described for **9** to give **18** (6.27 g, 99.5%).

R_f 0.11 [1:1 (v/v) chloroform–methanol], 0.72 [5:5:1 (v/v) chloroform–methanol–water]; $[\alpha]_D^{17}$ +96.7° (*c* 0.302, water); IR (KBr) 3381 (ν_{O-H}), 2930 (ν_{C-H}), 1638 ($\nu_{C=C}$), 1026 (ν_{C-O}) cm^{-1}; ^{1}H NMR (D_2O): δ 5.81 (m, 1H, –CH_2CH=CH_2), 5.31 (d, 2H, J = 3.9, H-1^{II}, H-1^{IIII}), 5.01 (d, 1H, J_{trans} = 17.3 Hz, J_{gem} = 1.7 Hz, one of –CH=CH_2), 4.94 (d, 1H, J_{cis} = 10.2 Hz, J_{gem} = 1.1 Hz, one of –CH=CH_2), 4.45 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-1), 3.34 (t, 1H, J = 9.4 Hz), 3.26 (t, 1H, J = 9.5 Hz, H-2), 2.68 (m, 2H, SCH_2CH_2–), 2.09 (q, 2H, J = 7.1 Hz, $SCH_2CH_2CH_2CH$=CH_2), 1.66 (m, 2H, SCH_2CH_2–); MALDI-TOF-MS Calcd for $C_{23}H_{40}O_{15}S$ [M+Na]$^+$: 611.195. Found: *m/z*: 611.191.

Anal. Calcd for $C_{23}H_{40}O_{15}S{\cdot}H_2O$: C, 46.22; H, 6.91; N, 0.00. Found: C, 46.19; H, 6.77; N, 0.00.

4.1.12. *n*-Pentenyl 4^{III},6^{III}-*O*-naphthylmethylidene-2^{III},3^{III},2^{II},3^{II},6^{II},2,3,6,-octa-*O*-acetyl-1-thio-β-D-maltotripyranoside (19)

Naphthylmethylidenation and subsequent acetylation of pentenyl thio-maltotriaoside (3.00 g, 5.10 mmol) with 2-NADIBA (4.40 g, 15.3 mmol) and CSA (592 mg, 2.55 mmol) was carried out by the same method as that described for **11**. Purification was carried out by silica gel column chromatography with 10:9 (v/v) EtOAc–hexane to give **19** (1.36 g, 25.1%).

R_f 0.63 [2:1 (v/v) ethyl acetate–hexane], 0.54 [1:1 (v/v) ethyl acetate–toluene]; IR (KBr) 2947 (ν_{C-H}), 1751 ($\nu_{C=O}$, ester), 1371 (ν_{C-H}), 1236 (ν_{C-O}, ester), 1051 (ν_{C-O}, ether) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.91–7.47 (m, 7H, naphthyl), 5.77 (m, 1H, –CH_2CH=CH_2), 5.63 (s, 1H, naphthyl-*CH*OO), 5.49 (t, 1H, $J_2^{III},_3^{III} = J_3^{III},_4^{III}$ = 10.0 Hz, H-3^{III}), 5.42 (dd, 1H, J = 10.2 Hz, J = 8.6 Hz), 5.37 (d, 1H, $J_1^{III},_2^{III}$ = 4.1 Hz, H-1^{III}), 5.01 (m, 2H, –CH=CH_2), 4.90 (dd, 1H, $J_1^{III},_2^{III}$ = 4.1 Hz, $J_2^{III},_3^{III}$ = 10.2 Hz, H-2^{III}), 4.85 (t, 1H, $J_{1,2} = J_{2,3}$ = 9.6 Hz, H-2), 4.75 (dd, 1H, J = 10.3 Hz, J = 4.1 Hz), 4.53 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-1), 4.25 (dd, 1H, J = 12.4 Hz, J = 3.3 Hz), 2.66 (m, 2H, SCH_2CH_2–), 2.17–1.99 (m, 26H, 8O*Ac*, $SCH_2CH_2CH_2CH$=CH_2), 1.70 (m, 2H, SCH_2CH_2–); MALDI-TOF-MS Calcd for $C_{50}H_{62}O_{23}S$ [M+Na]$^+$: 1085.329, [M+K]$^+$: 1101.303. Found: *m/z*: 1085.297, *m/z*: 1101.297.

4.1.13. *n*-Pentenyl 6^{III}-*O*-naphthylmetyl-2^{III},3^{III},2^{II},3^{II},6^{II},2,3,6,-octa-*O*-acetyl-1-thio-β-D-maltotripyranoside (20)

Reductive ring opening of **19** in THF was carried out with BH_3–NMe_3 complex (254 mg, 3.48 mmol) and 4 Å MS powder by the same method as that described for **12**. Purification was carried out by silica gel column chromatography with 3:2 (v/v) EtOAc–hexane to give **20** (333 mg, 90.0%).

R_f 0.53 [2:1 (v/v) ethyl acetate–hexane], 0.21 [3:2 (v/v) ethyl acetate–hexane]; IR data (KBr) 3472 (ν_{O-H}), 2938 (ν_{C-H}), 1749 ($\nu_{C=O}$, ester), 1371 (ν_{C-H}), 1238 (ν_{C-O}, ester), 1045 (ν_{C-O}, ether) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.85–7.45 (m, 7H, naphthyl), 5.77 (m, 1H, –CH_2CH=CH_2), 5.38 (dd, 1H, J = 10.3 Hz, J = 8.1 Hz), 5.35 (d, 1H, $J_1^{III},_2^{III}$ = 3.9 Hz, H-1^{III}), 5.28 (d, 1H, J = 3.9 Hz), 5.26 (d, 1H, $J_1^{II},_2^{II}$ = 3.9 Hz, H-1^{II}), 5.21 (m, 1H), 5.05 (m, 2H, –CH=CH_2), 4.85 (t, 1H, $J_{1,2} = J_{2,3}$ = 9.8 Hz, H-2), 4.82 (dd, 1H, J = 10.5 Hz, J = 4.0 Hz), 4.75 (d, 1H, J = 3.0 Hz), 4.71 (dd, 1H, J = 10.3 Hz, J = 3.9 Hz), 4.52 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-1), 4.46 (d, 2H, J = 12.1 Hz), 4.27 (dd, 1H, J = 12.2 Hz, J = 4.8 Hz), 2.66 (m, 2H, SCH_2CH_2–), 2.15 (m, 2H, $SCH_2CH_2CH_2CH$=CH_2), 2.14–1.99 (8s, 24H, 8O*Ac*), 1.70 (m, 2H, SCH_2CH_2–); MALDI-TOF-MS Calcd for $C_{50}H_{64}O_{23}S$ [M+Na]$^+$: 1087.345, [M+K]$^+$: 1103.319. Found: *m/z*: 1087.299, *m/z*: 1103.282.

4.1.14. *n*-Pentenyl 6^{III}-*O*-naphthylmetyl-1-thio-β-D-maltotriaoside (21)

Deprotection of **20** (739 mg, 0.69 mmol) was carried out by the same method as that described for **9** to give **21** quantitatively.

R_f 0.80 [5:5:1 (v/v) chloroform–methanol–water], 0.65 [2:1 (v/v) chloroform–methanol], $[\alpha]_D^{23}$ +50.0° (*c* 0.46, MeOH); IR (KBr) 3408 (ν_{O-H}), 2924 (ν_{C-H}), 1634 ($\nu_{C=C}$), 1049 (ν_{C-O}) cm^{-1}; ^{1}H NMR (CD_3OD): δ 7.73–7.27 (m, 7H, naphthyl), 5.64 (m, 1H, –CH_2CH=CH_2), 4.99, 4.98 (2d, 2H, J = 3.9 Hz, J = 3.8 Hz, H-1^{II}, H-1^{III}), 4.85 (dd, 1H, J_{trans} = 17.1 Hz, J_{gem} = 1.8 Hz, one of –CH=CH_2), 4.79 (dd, 1H, J_{cis} = 10.2 Hz, J_{gem} = 0.9 Hz, one of –CH=CH_2), 4.57 (s, 2H, naphthyl-CH_2O), 3.06 (t, 1H, $J_{1,2} = J_{2,3}$ = 9.2 Hz, H-2), 2.55 (m, 2H, SCH_2CH_2–), 1.99 (q, 2H, J = 7.1 Hz, $SCH_2CH_2CH_2CH$=CH_2), 1.54 (m, 2H, SCH_2CH_2–); MALDI-TOF-MS Calcd for $C_{34}H_{48}O_{15}S$ [M+Na]$^+$: 751.261. Found: *m/z*: 751.234.

Anal. Calcd for $C_{34}H_{48}O_{15}S$: C, 56.03; H, 6.64; N, 0.00. Found: C, 55.96; H, 6.76; N, 0.00.

4.1.15. Amino-ethyl thiopentyl 6^{III}-*O*-naphthylmethyl-1-thio-β-D-maltotriaoside (22)

Radical addition of 2-mercaptoethylamine (209 mg, 1.85 mmol) to pentenyl glycoside **21** (135 mg, 0.185 mmol) was carried out by the same method as that described for **14** to give **22** quantitatively.

R_f 0.32 [5:5:1 (v/v) chloroform–methanol–water], 0.03 [2:1 (v/v) chloroform–methanol].

IR (KBr) 3383 (ν_{O-H}), 2930 (ν_{C-H}), 1624 (ν_{N-H}), 1045 (ν_{C-O}) cm^{-1}; ^{1}H NMR (D_2O): δ 7.83–7.44 (m, 7H, naphthyl), 5.15 (m, 2H, H-1^{II}, H-1^{III}), 4.36 (d, 1H, $J_{1,2}$ = 9.7 Hz, H-1), 2.78 (t, 2H, J = 6.8 Hz, SCH_2CH_2N), 2.70 (m, 2H, G-SCH_2–), 2.59 (t, 2H, J = 7.2 Hz, –$CH_2SCH_2CH_2N$), 1.64 (m, 4H, G-$SCH_2CH_2CH_2CH_2CH_2S$), 1.54 (m, 2H, G-$SCH_2CH_2CH_2$–). MALDI-TOF-MS Calcd for $C_{36}H_{55}NO_{15}S_2$ [M+H]$^+$: 806.309, [M+Na]$^+$: 828.291, [M+K]$^+$: 844.264. Found: *m/z*: 806.246, *m/z*: 828.229, *m/z*: 844.218.

4.1.16. Dansylamido-ethyl thiopentyl 6^{III}-*O*-naphthylmetyl-1-thio-β-D-maltotriaoside (2)

Dansylation of amine **22** (100 mg, 0.119 mmol) with dansyl chloride (384 mg, 1.428 mmol) and triethylamine (100 μL, 0.714 mmol) was carried out by the same method as that described for **1** to give **2** quantitatively.

R_f 0.48 [5:1 (v/v) chloroform–methanol]; IR (KBr) 3310 (ν_{O-H}), 2938 (ν_{C-H}), 1645 (ν_{N-H}), 1144 ($\nu_{S=O}$), 1036 (ν_{C-O}) cm^{-1}; ^{1}H NMR (CD_3OD): δ 8.47–7.30 (m, 13H, naphthyl and dansyl), 5.02 (m, 2H, H-1^{II}, H-1^{III}), 4.60 (s, 2H, naphthyl-CH_2O), 4.22 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-1), 2.89 (m, 2H, SCH_2CH_2N), 2.28 (t, 2H, J = 6.0 Hz, SCH_2CH_2N), 2.14 (t, 2H, J = 6.4 Hz, –$CH_2SCH_2CH_2N$), 1.42 (m, 2H, G-SCH_2CH_2–), 1.24 (m, 4H, G-$SCH_2CH_2CH_2CH_2CH_2S$); ^{13}C NMR (CD_3OD): δ 137.64, 135.25, 134.94, 132.11, 131.30, 131.27, 131.19, 131.07, 129.56, 129.54, 129.44, 129.17, 128.22, 127.90, 127.76, 127.63, 127.41, 127.36, 126.28, 103.43, 87.56, 82.13, 81.59, 81.02, 79.83, 75.66, 75.37, 75.03, 74.70, 74.53, 74.27, 74.27, 73.87, 72.12, 71.56, 62.82, 47.85, 47.13, 44.40, 43.29, 38.91, 32.87, 32.79, 31.26, 31.01, 30.58, 29.33.

MALDI-TOF-MS Calcd for $C_{48}H_{66}N_2O_{17}S_3$ [M+Na]$^+$: 1061.321, [M+K]$^+$: 1077.316. Found: *m/z*: 1061.323, *m/z*: 1077.298.

4.1.17. $2^{IV},3^{IV},4^{IV},6^{IV}$, $2^{III},3^{III},6^{III},2^{II},3^{II},6^{II}$,2,3,6,-Trideca-*O*-acetyl-β-D-maltotetraose thioacetate (24)

Anomeric thioacetylation of **23** (45.2 g, 35.9 mmol) with potassium thioacetate (12.3 g, 0.108 mol) was carried out by the same method as that described for **8**. Purification was carried out by silica gel column chromatography with 1:1 (v/v) toluene–EtOAc gave **24** (32.0 g, 70.2%).

R_f 0.35 [2:1 (v/v) ethyl acetate–toluene]; IR (KBr) 2961 (ν_{C-H}), 1751 ($\nu_{C=O}$), 1371 (ν_{C-H}), 1236 (ν_{C-O}), 1040 (ν_{C-O}) cm^{-1}; ^1H NMR (CD_3OD): δ 5.10 (t, 1H, J = 10.0 Hz, H-4^{II}), 4.98 (t, 1H, J = 9.1 Hz, H-2), 4.86 (dd, 1H, J = 10.5 Hz, J = 4.0 Hz, H-2^{II}), 4.73 (dd, 1H, J = 10.3 Hz, J = 4.0 Hz), 2.38 (s, 3H, SAc), 2.18–1.93 (13s, 39H, 13OAc); MALDI-TOF-MS Calcd for $C_{52}H_{70}O_{34}S$ $[M+Na]^+$: 1293.336, $[M+K]^+$: 1309.310. Found: *m/z*: 1293.212, *m/z*: 1309.155.

4.1.18. *n*-Pentenyl $2^{IV},3^{IV},4^{IV},6^{IV},2^{III},3^{III},6^{III},2^{II},3^{II},6^{II}$,2,3,6,-trideca-*O*-acetyl-1-thio-β-D-maltotetrapyranoside (25)

To a solution of maltotetraosyl thioacetate **24** (31.7 g, 24.9 mmol) in DMF (317 mL) was added 5-bromopent-1-ene (4.30 mL, 49.9 mmol), and the mixture was cooled to −15 °C. Then to the solution was dropwise added diethyl amine (52.1 mL, 499 mmol), and stirring was continued at room temperature for 2 h. The solution was extracted with EtOAc, and the organic layer was washed with cold H_2O, dried over anhyd Na_2SO_4, filtered, and evaporated in vacuo. Purification of the residue by silica gel column chromatography with 3:2 (v/v) toluene–EtOAc gave **25** (28.2 g, 87.3%).

R_f 0.21 [1:1 (v/v) ethyl acetate–toluene], 0.47 [2:1 (v/v) ethyl acetate–toluene]; IR (KBr) 2959 (ν_{C-H}), 1751 ($\nu_{C=O}$, ester), 1371 (ν_{C-H}), 1236 (ν_{C-O}, ester), 1136 (ν_{C-O}, ether); ^1H NMR ($CDCl_3$): δ 5.78 (m, 1H, –CH_2CH=CH_2), 5.42 (d, 1H, $J_1^{II},_2^{II}$ = 4.2 Hz, H-1^{II}), 4.86 (m, 2H), 4.75 (dd, 1H, J = 3.9 Hz, J = 3.0 Hz), 4.72 (dd, 1H, J = 3.9 Hz, J = 3.0 Hz), 4.35 (dd, 1H, J = 12.3 Hz, J = 4.2 Hz), 4.25 (dd, 1H, J = 12.3 Hz, J = 3.3 Hz), 4.17 (d, 1H, J = 10.3 Hz), 3.72 (m, 1H, H-5), 2.67 (m, 2H, SCH_2CH_2–), 2.19–1.99 (13s, 39H, 13O*Ac*), 2.14 (m, 2H, $SCH_2CH_2CH_2CH$=CH_2), 1.70 (m, 2H, SCH_2CH_2–); ^{13}C NMR ($CDCl_3$): δ 170.54, 170.45, 170.43, 170.36, 170.02, 169.77, 169.67, 169.62, 169.59, 169.46, 137.81, 137.40, 128.98, 128.17, 125.24, 115.42, 95.74, 95.65, 95.52, 83.10, 72.16, 71.68, 71.39, 70.77, 70.44, 70.32, 69.98, 69.26, 68.89, 68.86, 68.36, 67.76, 63.12, 62.38, 62.06, 61.23, 32.53, 29.42, 28.79, 21.41, 20.86, 20.84, 20.77, 20.65, 20.56, 20.53; MALDI-TOF-MS Calcd for $C_{55}H_{76}O_{33}S$ $[M+Na]^+$: 1319.388, $[M+K]^+$: 1335.362. Found: *m/z*: 1319.424, *m/z*: 1335.384.

4.1.19. *n*-Pentenyl 1-thio-β-D-maltotetraoside (26)

Deprotection of **25** (14.7 g, 11.3 mmol) was carried out by the same method as that described for **10** to give **26** (8.17 g, 96.3%) quantitatively.

R_f 0.51 [2:1 (v/v) chloroform–methanol], 0.57 [5:5:1 (v/v) chloroform–methanol–water]; $[\alpha]_D^{27}$ +113.1° (*c* 0.60, water); IR (KBr) 3404 (ν_{O-H}), 2932 (ν_{C-H}), 1634 ($\nu_{C=C}$), 1028 (ν_{C-O}) cm^{-1}; ^1H NMR (D_2O): δ 5.79 (m, 1H, –CH_2CH=CH_2), 4.99 (d, 1H, J_{trans} = 17.3 Hz, one of –CH=CH_2), 4.92 (d, 1H, J_{cis} = 10.2 Hz, J_{gem} = 0.9 Hz, one of –CH=CH_2), 4.44 (d, 1H, $J_{1,2}$ = 9.9 Hz, H-1), 3.32 (t, 1H, J = 9.3 Hz), 3.24 (t, 1H, J = 9.2 Hz), 2.66 (m, 2H, SCH_2CH_2–), 2.07 (m, 2H, $SCH_2CH_2CH_2CH$=CH_2), 1.64 (m, 2H, SCH_2CH_2–); MALDI-TOF-MS Calcd for $C_{29}H_{50}O_{20}S$ $[M+Na]^+$: 773.251. Found: *m/z*: 773.170.

Anal. Calcd for $C_{29}H_{50}O_{20}S{\cdot}3H_2O$: C, 43.28; H, 7.01; N, 0.00. Found: C, 43.24; H, 6.71; N, 0.00.

4.1.20. *n*-Pentenyl $4^{IV},6^{IV}$-*O*-naphthylmethylidene-2^{IV}, $3^{IV},2^{III},3^{III},6^{III},2^{II},3^{II},6^{II}$,2,3,6,-undeca-*O*-acetyl-1-thio-β-D-maltotetrapyranoside (27)

Naphthylmethylidenation and subsequent acetylation of pentenyl thio-maltotetraoside **26** (4.29 g, 5.71 mmol) with 2-NADIBA (4.91 g, 17.1 mmol) and CSA (662 mg, 2.85 mmol) was carried out by the same method as that described for **11**. Purification was carried out by silica gel column chromatography with 5:4 (v/v) toluene–EtOAc to give **27** (4.71 g, 61.0%). Acetate of starting material **26** (760 mg, 10.3%) was also recovered.

R_f 0.69 [2:1 (v/v) ethyl acetate–toluene]; IR (KBr) 2951 (ν_{C-H}), 1751 ($\nu_{C=O}$, ester), 1639 ($\nu_{C=C}$), 1371 (ν_{C-H}), 1236 (ν_{C-O}, ester), 1033 (ν_{C-O}, ether) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.91–7.47 (m, 7H, naphthyl), 5.77 (m, 1H, –CH_2CH=CH_2), 5.63 (s, 1H, naphthyl-*CH*OO), 5.49 (t, 1H, $J_2^{IV},_3^{IV}$ = $J_3^{IV},_4^{IV}$ = 10.0 Hz, H-3^{IV}), 5.02 (m, 2H, –CH=CH_2), 4.90 (dd, 1H, $J_1^{IV},_2^{IV}$ = 4.4 Hz, $J_2^{IV},_3^{IV}$ = 10.4 Hz, H-2^{IV}), 4.85 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 10.0 Hz, H-2), 4.53 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-1), 2.66 (m, 2H, SCH_2CH_2–), 2.20–1.99 (11s, 33H, 11O*Ac*), 2.17 (m, 2H, $SCH_2CH_2CH_2CH$=CH_2), 1.70 (m, 2H, SCH_2CH_2–); MALDI-TOF-MS Calcd for $C_{62}H_{78}O_{31}S$ $[M+Na]^+$: 1373.414, $[M+K]^+$: 1389.388. Found: *m/z*: 1373.309, *m/z*: 1389.289.

4.1.21. *n*-Pentenyl 6^{IV}-*O*-naphthylmethyl-2^{IV}, $3^{IV},2^{III},3^{III},6^{III},2^{II},3^{II},6^{II}$,2,3,6-undeca-*O*-acetyl-1-thio-β-D-maltotetrapyranoside (28)

Reductive ring opening of **27** (4.00 g, 2.96 mmol) in THF was carried out with BH_3–NMe_3 complex (2.16 g, 29.6 mmol) and 4 Å MS powder by the same method as that described for **12**. Purification was carried out by silica gel column chromatography with 1:1 (v/v) toluene–EtOAc to give **28** (2.37 g, 59.1%).

R_f 0.56 [2:1 (v/v) ethyl acetate–toluene]; IR (KBr) 3468 (ν_{O-H}), 2943 (ν_{C-H}), 1751 ($\nu_{C=O}$, ester), 1638 ($\nu_{C=C}$), 1371 (ν_{C-H}), 1238 (ν_{C-O}, ester), 1042 (ν_{C-O}, ether) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.85–7.45 (m, 7H, naphthyl), 5.78 (m, 1H, –CH_2CH=CH_2), 5.20 (d, 1H, J = 10.4 Hz, J = 8.8 Hz, H-3), 5.02 (m, 2H, –CH=CH_2), 4.53 (d, 1H, J = 10.0 Hz, H-1), 4.35 (dd, 1H, J = 12.2 Hz, J = 4.2 Hz), 4.22 (dd, 1H, J = 12.3 Hz, J = 3.6 Hz), 4.15 (dd, 1H, J = 12.3 Hz, J = 2.7 Hz), 2.64 (m, 2H, SCH_2CH_2–), 2.17–1.98 (11s, 33H, 11O*Ac*), 2.14 (m, 2H, $SCH_2CH_2CH_2CH$=CH_2), 1.70 (m, 2H, SCH_2CH_2–); MALDI-TOF-MS Calcd for $C_{62}H_{80}O_{31}S$ $[M+Na]^+$: 1375.430, $[M+K]^+$: 1391.404. Found: *m/z*: 1375.400, *m/z*: 1391.381.

4.1.22. *n*-Pentenyl 6^{IV}-*O*-naphthylmethyl-1-thio-β-D-maltotetraoside (29)

Deprotection of **28** (2.17 g, 1.61 mmol) was carried out by the same method as that described for **13** to give **29** (1.33 g, 93.0%).

R_f 0.82 [5:5:1 (v/v) chloroform–methanol–water]; $[\alpha]_D^{22}$ +76.6° (*c* 0.60, MeOH); IR (KBr) 3387 (ν_{O-H}), 2926 (ν_{C-H}), 1638 ($\nu_{C=C}$), 1369 (ν_{C-H}), 1028 (ν_{C-O}) cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.89–7.43 (m, 7H, naphthyl), 5.76 (m, 1H, –CH_2CH=CH_2), 4.64 (s, 1H, one of naphthyl-CH_2O), 4.63 (s, 1H, one of naphthyl-CH_2O), 4.25 (d, 1H, $J_{1,2}$ = 9.6 Hz, H-1), 3.01 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 9.1 Hz, H-2), 2.58 (m, 2H, SCH_2CH_2–), 2.07 (m, 2H, $SCH_2CH_2CH_2CH$=CH_2), 1.60 (m, 2H, SCH_2CH_2–); MALDI-TOF-MS Calcd for $C_{28}H_{38}O_{10}S$ $[M+Na]^+$: 913.313. Found: *m/z*: 913.304.

Anal. Calcd for $C_{40}H_{58}O_{20}S{\cdot}0.4H_2O$: C, 53.49; H, 6.60; N, 0.00. Found: C, 53.45; H, 6.49; N, 0.00.

4.1.23. Amino-ethyl thiopentyl 6^{IV}-*O*-naphthylmethyl-1-thio-β-D-maltotetraoside (30)

Radical addition of 2-mercaptoethylamine (254 mg, 2.24 mmol) to pentenyl glycoside **29** (200 mg, 0.224 mmol) was carried out by the same method as that described for **14** to give **30** (187 mg, 83.1%).

R_f 0.48 [5:5:1 (v/v) chloroform–methanol–water]; IR (KBr) 3387 (ν_{O-H}), 2926 (ν_{C-H}), 1636 (ν_{N-H}), 1028 (ν_{C-O}) cm^{-1}; ^1H NMR (D_2O): δ 7.88–7.46 (m, 7H, naphthyl), 5.26 (m, 3H, H-1^{II}, H-1^{III}, H-1^{IV}), 4.36 (d, 1H, $J_{1,2}$ = 9.9 Hz, H-1), 3.85 (t, 2H), 3.22 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 10.0 Hz, H-2), 3.11 (t, 2H, J = 6.7 Hz, SCH_2CH_2N), 2.75 (t, 2H, J = 6.6 Hz, SCH_2CH_2N), 2.65 (m, 2H, G-SCH_2–), 2.51 (t, 2H, J = 7.2 Hz, –$CH_2SCH_2CH_2$N), 1.54 (m, 4H, G-$SCH_2CH_2CH_2CH_2CH_2$S), 1.40 (m,

2H, G-$SCH_2CH_2CH_2$–); MALDI-TOF-MS Calcd for $C_{42}H_{65}NO_{20}S_2$ $[M+H]^+$: 968.361, $[M+Na]^+$: 990.343, $[M+K]^+$: 1006.317. Found: *m/z*: 968.337, *m/z*: 990.365, *m/z*: 1006.330.

4.1.24. Dansyl amide-ethyl thiopentyl 6^{IV}-*O*-naphthylmethyl-1-thio-β-D-maltotetraoside (3)

Dansylation of amine **30** (60 mg, 0.0597 mmol) with dansyl chloride (64 mg, 0.24 mmol) and with triethylamine (67 μL, 0.48 mmol) was carried out by the same method as that described for **1** to give **3** (18 mg, 25.1%).

R_f 0.77 [5:5:1 (v/v) chloroform–methanol–water]; $[\alpha]_D^{26}$ +142.3° (*c* 0.067, MeOH); IR (KBr) 3310 (ν_{O-H}), 2930 (ν_{C-H}), 1321 ($\nu_{S=O}$), 1146 ($\nu_{S=O}$), 1028 (ν_{C-O}) cm^{-1}; ^1H NMR (CD_3OD): δ 8.47–7.11 (m, 13H, naphthyl and dansyl), 5.00 (m, 3H, H-1^{II}, H-1^{III}, H-1^{IV}), 4.58 (s, 2H, naphthyl-CH_2O), 4.19 (d, 1H, $J_{1,2}$ = 9.7 Hz, H-1), 3.08 (t, 1H, *J* = 9.3 Hz, H-2), 2.84 (t, 2H, *J* = 7.3 Hz, SCH_2CH_2N), 2.72 (s, 6H, dansyl-NMe_2), 2.52 (m, 2H, G-SCH_2–), 2.23 (t, 2H, *J* = 7.3 Hz, SCH_2CH_2N), 2.10 (t, 2H, *J* = 6.9 Hz, –$CH_2SCH_2CH_2N$), 1.41 (m, 2H, G-SCH_2CH_2–), 1.21 (m, 4H, G-$SCH_2CH_2CH_2CH_2CH_2S$); MALDI-TOF-MS Calcd for $C_{54}H_{76}N_2O_{22}S_3$ $[M+Na]^+$: 1223.392, $[M+K]^+$: 1239.368. Found: *m/z*: 1223.397, *m/z*: 1239.371.

Anal. Calcd for $C_{54}H_{76}N_2O_{22}S{\cdot}H_2O$: C, 53.19; H, 6.45; N, 2.30. Found: C, 53.06; H, 6.42; N, 2.35.

4.1.25. 2^V,3^V,4^V,6^V,2^{IV},3^{IV},6^{IV},2^{III},3^{III},6^{III},2^{II},3^{II},6^{II},2,3,6,-Hexadeca-*O*-acetyl-β-D-maltopentaose thioacetate (32)

Anomeric thioacetylation of **31** (60.0 g, 38.8 mmol) with potassium thioacetate (13.3 g, 0.12 mol) was carried out by the same method as that described for **8**. Purification was carried out by silica gel column chromatography with 2:3 (v/v) toluene–EtOAc to give **32** (37.1 g, 61.2%).

R_f 0.41 [2:1 (v/v) ethyl acetate–toluene]; IR (KBr) 1751 ($\nu_{C=O}$, ester), 1371 (ν_{C-H}), 1236 (ν_{C-O}, ester), 1038 (ν_{C-O}, ether) cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.42–5.27 (m), 5.07 (t, 1H, $J_3{}^V{}_{,4}{}^V = J_4{}^V{}_{,5}{}^V$ = 10.0 Hz, H4^V), 4.97 (t, 1H, *J* = 9.0 Hz, *J* = 10.5 Hz, H-2), 4.86 (dd, 1H, $J_1{}^V{}_{,2}{}^V$ = 4.0 Hz, $J_2{}^V{}_{,3}{}^V$= 10.5 Hz, H-2^V), 4.34 (dd, 1H, *J* = 3.5 Hz, *J* = 10.5 Hz), 2.38 (s, 3H, S*Ac*), 2.20–1.99 (16s, 48H, 16O*Ac*); MALDI-TOF-MS Calcd for $C_{64}H_{86}O_{42}S$ $[M+Na]^+$: 1581.421, $[M+K]^+$: 1597.395. Found: *m/z*: 1581.330, *m/z*: 1597.318.

4.1.26. *n*-Pentenyl 2^V,3^V,4^V,6^V,2^{IV},3^{IV},6^{IV},2^{III},3^{III},6^{III},2^{II},3^{II},6^{II},2,3,6,-hexadeca-*O*-acetyl-1-thio-β-D-maltopentaoside (33)

Anomeric extension of **32** (37.6 g, 23.7 mmol) with 5-bromopent-1-ene (4.10 mL, 47.4 mmol) and diethyl amine (49.5 mL, 474 mmol) was carried out by the same method as that described for **9**. Purification was carried out by silica gel column chromatography with 1:1 (v/v) toluene–EtOAc to give **33** (35.5 g, 94.4%).

R_f 0.24 [1:1 (v/v) ethyl acetate–toluene], 0.42 [2:1 (v/v) ethyl acetate–toluene]; $[\alpha]_D^{28}$ +103.3° (*c*.0.22, $CHCl_3$); IR (KBr) 2959 (ν_{C-H}), 1751 ($\nu_{C=O}$, ester), 1371 (ν_{C-H}), 1236 (ν_{C-O}, ester), 1138 (ν_{C-O}, ether) cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.78 (m, 1H, –CH_2CH=CH_2), 5.04 (m, 3H, –CH_2CH=CH_2, –CH), 4.86 (m, 2H), 4.74 (m, 3H), 4.37 (dd, 1H, *J* = 12.3 Hz, *J* = 3.9 Hz), 4.30 (dd, 1H, *J* = 12.5 Hz, *J* = 3.4 Hz), 4.17 (d, 1H, *J* = 11.7 Hz), 3.71 (m, 1H, H-5), 2.66 (m, 2H, SCH_2CH_2–), 2.35–1.99 (m, 50H, 16O*Ac*, $SCH_2CH_2CH_2CH$=CH_2), 1.70 (m, 2H, SCH_2CH_2–); MALDI-TOF-MS Calcd for $C_{67}H_{92}O_{41}S$ $[M+Na]^+$: 1607.473, $[M+K]^+$: 1623.447. Found: *m/z*: 1607.519, *m/z*: 1623.487.

Anal. Calcd for $C_{67}H_{92}O_{41}S$: C, 50.76; H, 5.85; N, 0.00. Found: C, 50.81; H, 5.79; N, 0.00.

4.1.27. *n*-Pentenyl 1-thio-β-D-maltopentaoside (34)

Deprotection of **33** was carried out by the same method as that described for **10** to give **34** quantitatively.

R_f 0.07 [1:1 (v/v) chloroform–methanol], 0.43 [5:5:1 (v/v) chloroform–methanol–water]; IR (KBr) 3397 (ν_{O-H}), 2930 (ν_{C-H}), 1636 ($\nu_{C=C}$), 1026 (ν_{C-O}) cm^{-1}; ^1H NMR (D_2O): δ 5.79 (m, 1H, –CH_2CH=CH_2), 4.99 (dd, 1H, J_{trans} = 17.3 Hz, J_{gem} = 1.5 Hz, one of –CH=CH_2), 4.93 (d, 1H, J_{cis} = 10.2 Hz, one of –CH=CH_2), 4.44 (d, 1H, $J_{1,2}$ = 9.9 Hz, H-1), 3.32 (t, 1H, *J* = 8.8 Hz), 3.24 (t, 1H, $J_{1,2} = J_{2,3}$ = 9.2 Hz, H-2), 2.66 (m, 2H, SCH_2CH_2–), 2.07 (q, 2H, *J* = 7.0 Hz, $SCH_2CH_2CH_2CH$=CH_2), 1.65 (m, 2H, SCH_2CH_2–); MALDI-TOF-MS Calcd for $C_{35}H_{60}O_{25}S$ $[M+Na]^+$: 935.304, $[M+K]^+$: 951.278,. Found: *m/z*: 935.285, *m/z*: 951.264.

4.1.28. *n*-Pentenyl 4^V,6^V-*O*-Naphthylmethyliden-2^V,3^V,2^{IV},3^{IV},6^{IV},2^{III},3^{III},6^{III},2^{II},3^{II},6^{II},2,3,6,-tetradeca-*O*-acetyl-1-thio-β-D-maltopentaoside (35)

Naphthylmethylidenation and subsequent acetylation of pentenyl thio-maltopentaoside **34** (1.00 g, 1.10 mmol) with 2-NADIBA (945 mg, 3.30 mmol) and CSA (128 mg, 0.55 mmol) was carried out by the same method as that described for **11**. Purification was carried out by silica gel column chromatography with 1:1 (v/v) toluene–EtOAc to give **35** (750 mg, 41.7%). Acetate of starting material **33** (260 mg, 14.9%) was also recovered.

R_f 0.56 [2:1 (v/v) ethyl acetate–toluene]; IR (KBr) 2955 (ν_{C-H}), 1751 ($\nu_{C=O}$, ester), 1371 (ν_{C-H}), 1236 (ν_{C-O}, ester), 1033 (ν_{C-O}, ether) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.91–7.47 (m, 7H, naphthyl), 5.78 (m, 1H, –CH_2CH=CH_2), 5.63 (s, 1H, naphthyl-*CH*OO), 5.50 (t, 1H, $J_2{}^V{}_{,3}{}^V = J_3{}^V{}_{,4}{}^V$ = 10.1 Hz, H-3^V), 5.02 (m, 2H, –CH=CH_2), 4.89 (dd, 1H, $J_1{}^V{}_{,2}{}^V$ = 4.2 Hz, $J_2{}^V{}_{,3}{}^V$ = 10.1 Hz, H-2^V), 4.86 (t, 1H, $J_{1,2} = J_{2,3}$ = 9.2 Hz, H-2), 2.66 (m, 2H, SCH_2CH_2–), 2.22–1.98 (14s, 42H, 14O*Ac*), 2.14 (m, 2H, $SCH_2CH_2CH_2CH$=CH_2), 1.70 (m, 2H, SCH_2CH_2–); MALDI-TOF-MS Calcd for $C_{74}H_{94}O_{39}S$ $[M+Na]^+$: 1661.499, $[M+K]^+$: 1677.472. Found: *m/z*: 1661.471, *m/z*: 1677.449.

4.1.29. *n*-Pentenyl 6^V-*O*-naphthylmethyl-2^V,3^V,2^{IV},3^{IV},6^{IV},2^{III},3^{III},6^{III},2^{II},3^{II},6^{II},2,3,6,-tetradeca-*O*-acetyl-1-thio-β-D-maltopentaoside (36)

Reductive ring opening of **35** (700 mg, 0.427 mmol) in THF was carried out with BH_3–NMe_3 complex (311 mg, 4.27 mmol) and aluminum chloride (568 mg, 4.27 mmol) by the same method as that described for **12**. Purification was carried out by silica gel column chromatography with 4:5 (v/v) toluene–EtOAc to give **36** (459 mg, 65.5%).

R_f 0.31 [3:2 (v/v) ethyl acetate–toluene]; IR (KBr) 3480 (ν_{O-H}), 2945 (ν_{C-H}), 1751 ($\nu_{C=O}$, ester), 1639 ($\nu_{C=C}$), 1371 (ν_{C-H}), 1238 (ν_{C-O}, ester), 1036 (ν_{C-O}, ether) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.85–7.45 (m, 7H, naphthyl), 5.78 (m, 1H, –CH_2CH=CH_2), 5.20 (t, 1H, *J* = 9.6 Hz), 5.04 (dd, 1H, J_{trans} = 17.3 Hz, J_{gem} = 1.7 Hz, one of –CH=CH_2), 4.99 (d, 1H, J_{cis} = 10.2 Hz, one of –CH=CH_2), 2.64 (m, 2H, SCH_2CH_2–), 2.20–1.98 (m, 44H, 14O*Ac*, $SCH_2CH_2CH_2CH$=CH_2), 1.70 (m, 2H, SCH_2CH_2–); MALDI-TOF-MS Calcd for $C_{74}H_{96}O_{39}S$ $[M+Na]^+$: 1663.514, $[M+K]^+$: 1679.488. Found: *m/z*: 1663.428, *m/z*: 1679.418.

4.1.30. *n*-Pentenyl 6^V-*O*-naphthylmethyl-1-thio-β-D-maltopentaoside (37)

Deprotection of **36** (400 mg, 0.243 mmol) was carried out by the same method as that described for **13** to give **37** quantitatively.

R_f 0.77 [5:5:1 (v/v) chloroform–methanol–water]; $[\alpha]_D^{24}$ +86.8 (*c* 0.71, MeOH); IR (KBr) 3387 (ν_{O-H}), 2926 (ν_{C-H}), 1639 ($\nu_{C=C}$), 1369 (ν_{C-H}) cm^{-1}; ^1H NMR (CD_3OD): δ 7.64–7.24 (m, 7H, naphthyl), 5.60 (m, 1H, –CH_2CH=CH_2), 4.53 (s, 2H, naphthyl-CH_2O), 4.14 (d, 1H, $J_{1,2}$ = 9.7 Hz, H-1), 2.50 (m, 2H, SCH_2CH_2–), 1.95 (q, 2H, *J* = 6.8 Hz, $SCH_2CH_2CH_2CH$=CH_2), 1.50 (m, 2H, SCH_2CH_2–); MALDI-TOF-MS Calcd for $C_{46}H_{68}O_{25}S$ $[M+Na]^+$: 1075.366, $[M+K]^+$: 1091.340. Found: *m/z*: 1075.290, *m/z*: 1091.253.

Anal. Calcd for $C_{46}H_{68}O_{25}S{\cdot}H_2O$: C, 51.58; H, 6.58; N, 0.00. Found: C, 51.83; H, 6.49; N, 0.00.

4.1.31. Amino-ethyl thiopentyl 6^{V}-*O*-naphthylmethyl-1-thio-β-D-maltopentaoside (38)

Radical addition of 2-mercaptoethylamine (230 mg, 2.04 mmol) to pentenyl glycoside **37** (215 mg, 0.204 mmol) was carried out by the same method as that described for **14** to give **38** (181 mg, 76.1%).

R_f 0.07 [5:5:1 (v/v) chloroform–methanol–water]; IR (KBr) 3381 (ν_{O-H}), 2928 (ν_{C-H}), 1636 (ν_{N-H}), 1028 (ν_{C-O}) cm^{-1}; ^1H NMR (D_2O): δ 7.85–7.44 (m, 7H, naphthyl), 5.24, 5.20, 5.19, 5.11 (4 d, 4H, J = 3.7 Hz, H-1^{II}, H-1^{III}, H-1^{IV}, H-1^{V}), 4.24 (d, 1H, $J_{1,2}$ = 9.8 Hz, H-1), 3.36 (dd, 1H, J = 9.8 Hz, J = 3.8 Hz), 3.09 (m, 2H, S$CH_2$$CH_2$N), 2.73 (t, 2H, J = 5.8 Hz, SCH_2CH_2N), 2.62 (m, 2H, G-SCH_2–), 2.49 (t, 2H, J = 6.7 Hz, –$CH_2SCH_2CH_2$N), 1.52 (m, 4H, G-$SCH_2CH_2CH_2CH_2CH_2$S), 1.39 (m, 2H, G-$SCH_2CH_2CH_2$–); MALDI-TOF-MS Calcd for $C_{48}H_{75}NO_{25}S_2$ [M+H]$^+$: 1130.414, [M+Na]$^+$: 1152.396, [M+K]$^+$: 1168.370. Found: *m/z*: 1130.274, *m/z*: 1152.242, *m/z*: 1168.221.

4.1.32. Dansyl amide-ethyl thiopentyl 6^{V}-*O*-naphthylmethyl-1-thio-β-D-maltopentaoside (4)

Dansylation of amine **30** (80 mg, 0.069 mmol) with dansyl chloride (146 mg, 0.55 mmol) and with triethylamine (37 μL, 0.27 mmol) was carried out by the same method as that described for **1** to give **4** quantitatively.

R_f 0.32 [5:5:1 (v/v) chloroform–methanol–water]; IR (KBr) ν 3401 (ν_{O-H}), 2935 (ν_{C-H}), 1636 (ν_{N-H}), 1146 ($\nu_{S=O}$), 1034 (ν_{C-O}) cm^{-1}; ^1H NMR (CD_3OD): δ 8.36–7.27 (m, 13H, naphthyl and dansyl), 4.98 (m, 4H, H-1^{II}, H-1^{III}, H-1^{IV}, H-1^{V}), 4.56 (s, 2H, naphthyl-CH_2O), 4.17 (d, 1H, $J_{1,2}$ = 9.6 Hz, H-1), 2.88 (t, 2H, J = 7.5 Hz, SCH_2CH_2N), 2.49 (m, 2H, G-SCH_2–), 2.28 (t, 2H, J = 7.0 Hz, SCH_2CH_2N), 2.16 (t, 2H, J = 6.6 Hz, –$CH_2SCH_2CH_2$N), 1.39 (m, 2H, G-SCH_2CH_2–), 1.23 (m, 4H, G-$SCH_2CH_2CH_2CH_2CH_2$S); MALDI-TOF-MS Calcd for $C_{60}H_{86}N_2O_{27}S_3$ [M+Na]$^+$: 1385.447, [M+K]$^+$: 1401.421. Found: *m/z*: 1385.393, *m/z*: 1401.366.

4.1.33. 2^{VI},3^{VI},4^{VI},6^{VI},2^{V},3^{V},6^{V},2^{IV},3^{IV},6^{IV},2^{III},3^{III},6^{III},2^{II},3^{II},6^{II},2,3,6,-Nonadeca-*O*-acetyl-β-D-maltohexaose thioacetate (40)

Anomeric thioacetylation of **39** (604 mg, 0.33 mmol) with potassium thioacetate (75 mg, 0.66 mmol) was carried out by the same method as that described for **8**. Purification was carried out by silica gel column chromatography with 1:2 (v/v) toluene–EtOAc to give **40** (561 mg, 92.1%).

R_f 0.39 [3:1 (v/v) ethyl acetate–toluene]; IR (KBr) 1749 ($\nu_{C=O}$, ester), 1371 (ν_{C-H}), 1238 (ν_{C-O}, ester), 1038 (ν_{C-O}, ether) cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.42–5.27 (m), 5.07 (t, 1H, $J_3^{VI},_4^{VI} = J_4^{VI},_5^{VI}$ = 9.8 Hz, H-4^{VI}), 4.85 (t, 1H, $J_{1,2} = J_{2,3}$ = 9.6 Hz, H-2), 4.86 (dd, 1H, $J_1^{VI},_2^{VI}$ = 4.0 Hz, $J_2^{VI},_3^{VI}$ = 10.5 Hz, H-2^{VI}), 2.37–1.98 (m, S*Ac*, O*Ac*); MALDI-TOF-MS Calcd for $C_{76}H_{102}O_{50}S$ [M+Na]$^+$: 1869.505, [M+K]$^+$: 1885.479. Found: *m/z*: 1869.482, *m/z*: 1885.461.

4.1.34. *n*-Pentenyl 2^{VI},3^{VI},4^{VI},6^{VI},2^{V},3^{V},6^{V},2^{IV},3^{IV},6^{IV},2^{III},3^{III},6^{III},2^{II},3^{II},6^{II},2,3,6,-nonadeca-*O*-acetyl-1-thio-β-D-maltohexaoside (41)

Anomeric extension of **40** (550 mg, 0.30 mmol) with 5-bromo-pent-1-ene (40 μL, 0.45 mmol) and diethyl amine (0.62 mL, 6.0 mmol) was carried out by the same method as that described for **9**. Purification was carried out by silica gel column chromatography with 1:2 (v/v) toluene–EtOAc to give **41** (458 mg, 81.5%).

R_f 0.33 [3:1 (v/v) ethyl acetate–toluene]; $[\alpha]_D^{30}$ +106.4° (*c* 0.58, $CHCl_3$); IR (KBr) 2957 (ν_{C-H}), 1751 ($\nu_{C=O}$, ester), 1636 ($\nu_{C=C}$), 1371 (ν_{C-H}), 1236 (ν_{C-O}, ester), 1038 (ν_{C-O}, ether) cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.78 (m, 1H, –CH_2CH=CH_2), 5.42–5.26 (m), 5.08 (t, 1H, J = 9.6 Hz, H-4^{VI}), 5.04 (dd, 1H, J_{trans} = 17.2 Hz, J_{gem} = 1.8 Hz, one of –CH_2CH=CH_2), 5.00 (d, 1H, J_{cis} = 10.2 Hz, one of –CH_2CH=CH_2), 4.85 (m, 2H), 4.73 (m, 3H), 4.37 (dd, 1H, J = 12.3 Hz, J = 3.9 Hz), 3.71 (m, 1H), 2.66 (m, 2H, SCH_2CH_2–), 2.21–1.98 (m, O*Ac*, $SCH_2CH_2CH_2CH$=CH_2), 1.72 (m, 2H, SCH_2CH_2–); MALDI-TOF-MS Calcd for $C_{79}H_{108}O_{49}S$ [M+Na]$^+$: 1895.557, [M+K]$^+$: 1911.531. Found: *m/z*: 1895.625, *m/z*: 1911.579.

Anal. Calcd for $C_{79}H_{108}O_{49}S$: C, 50.64; H, 5.81; N, 0.00. Found: C, 50.45; H, 5.75; N, 0.00.

4.1.35. *n*-Pentenyl 1-thio-β-D-maltohexaoside (42)

Deprotection of **41** (11.0 g, 5.87 mmol) was carried out by the same method as that described for **10** to give **42** quantitatively.

R_f 0.47 [5:5:1 (v/v) chloroform–methanol–water]; $[\alpha]_D^{18}$ +176.4° (*c* 0.05, water); IR (KBr) 3381 (ν_{O-H}), 2926 (ν_{C-H}), 1639 ($\nu_{C=C}$), 1026 (ν_{C-O}) cm^{-1}; ^1H NMR (D_2O): δ 5.78 (m, 1H, –CH_2CH=CH_2), 5.00 (d, 1H, J_{trans} = 17.3 Hz, one of –CH=CH_2), 4.93 (d, 1H, J_{cis} = 10.2 Hz, one of –CH=CH_2), 4.44 (d, 1H, $J_{1,2}$ = 9.9 Hz, H-1), 3.32 (t, 1H, J = 9.4 Hz), 3.25 (t, 1H, $J_{1,2} = J_{2,3}$ = 8.7 Hz, H-2), 2.66 (m, 2H, SCH_2CH_2–), 2.08 (q, 2H, J = 6.9 Hz, $SCH_2CH_2CH_2CH$=CH_2), 1.65 (m, 2H, SCH_2CH_2–); Fab-MS Calcd for $C_{41}H_{70}O_{30}S$ [M+Na]$^+$: 1097.36. Found: *m/z*: 1097.64.

Anal. Calcd for $C_{41}H_{70}O_{30}S{\cdot}2H_2O$: C, 44.32; H, 6.71; N, 0.00. Found: C, 44.40; H, 6.64; N, 0.00.

4.1.36. *n*-Pentenyl 4^{VI},6^{VI}-*O*-naphthylmethylidene-2^{VI},3^{VI},2^{V},3^{V},6^{V},2^{IV},3^{IV},6^{IV},2^{III},3^{III},6^{III},2^{II},3^{II},6^{II},2,3,6,-heptadeca-*O*-acetyl-1-thio-β-D-maltohexapyranoside (43)

Naphthylmethylidenation and subsequent acetylation of pentenyl thio-maltohexaoside **42** (2.50 g, 2.32 mmol) with 2-NADIBA (1.38 g, 4.64 mmol) and CSA (265 mg, 1.16 mmol) was carried out by the same method as that described for **11**. Purification was carried out by silica gel column chromatography with 1:1 (v/v) toluene–EtOAc to give **43** (984 mg, 22.0%). Acetate of starting material **41** (2.31 g, 53.1%) was also recovered.

R_f 0.63 [3:1 (v/v) ethyl acetate–toluene]; IR (KBr) 2957 (ν_{C-H}), 1751 ($\nu_{C=O}$, ester), 1639 ($\nu_{C=C}$), 1371 (ν_{C-H}), 1238 (ν_{C-O}, ester), 1036 (ν_{C-O}, ether) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.91–7.47 (m, 7H, naphthyl), 5.78 (m, 1H, –CH_2CH=CH_2), 5.63 (s, 1H, naphthyl-*CH*OO), 5.49 (t, 1H, J = 9.9 Hz), 5.03 (dd, 1H, J_{trans} = 18.8 Hz, J_{gem} = 1.4 Hz, one of –CH=CH_2), 4.99 (d, 1H, J_{cis} = 11.9 Hz, one of –CH=CH_2), 4.90 (dd, 1H, J = 10.5 Hz, J = 4.5 Hz), 4.85 (t, 1H, J = 9.8 Hz), 2.66 (m, 2H, SCH_2CH_2–), 2.30–1.97 (m, O*Ac*, $SCH_2CH_2CH_2CH$=CH_2), 1.69 (m, 2H, SCH_2CH_2–); MALDI-TOF-MS Calcd for $C_{86}H_{110}O_{47}S$ [M+Na]$^+$: 1949.583, [M+K]$^+$: 1965.557. Found: *m/z*: 1949.640, *m/z*: 1965.601.

4.1.37. *n*-Pentenyl 6^{VI}-*O*-naphthylmethyl-2^{VI},3^{VI},2^{V},3^{V},6^{V},2^{IV},3^{IV},6^{IV},2^{III},3^{III},6^{III},2^{II},3^{II},6^{II},2,3,6,-heptadeca-*O*-acetyl-1-thio-β-D-maltohexapyranoside (44)

Reductive ring opening of **43** (1.70 g, 0.88 mmol) in THF was carried out with BH_3–NMe_3 complex (897 mg, 12.3 mmol) and aluminum chloride (1.60 g, 12.3 mmol) by the same method as that described for **12**. Purification was carried out by silica gel column chromatography with 2:3 (v/v) toluene–EtOAc to give **44** (1.30 g, 76.5%).

R_f 0.38 [3:1 (v/v) ethyl acetate–toluene]; $[\alpha]_D^{30}$ +108.7° (*c* 0.79, $CHCl_3$); IR (KBr) 3478 (ν_{O-H}), 2955 (ν_{C-H}), 1748 ($\nu_{C=O}$, ester), 1639 ($\nu_{C=C}$), 1371 (ν_{C-H}), 1236 (ν_{C-O}, ester), 1036 (ν_{C-O}, ether) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.85–7.46 (m, 7H, naphthyl), 5.78 (m, 1H, –CH_2CH=CH_2), 5.03 (d, 1H, J_{trans} = 18.9 Hz, one of –CH=CH_2), 5.00 (d, 1H, J_{cis} = 11.3 Hz, one of –CH=CH_2), 2.66 (m, 2H, SCH_2CH_2–), 2.20–1.86 (m, O*Ac*, $SCH_2CH_2CH_2CH$=CH_2), 1.70 (m, 2H, SCH_2CH_2–); MALDI-TOF-MS Calcd for $C_{86}H_{112}O_{47}S$ [M+Na]$^+$: 1951.599, [M+K]$^+$: 1967.573. Found: *m/z*: 1951.637, *m/z*: 1967.627.

Anal. Calcd for $C_{86}H_{112}O_{47}S{\cdot}2H_2O$: C, 52.54; H, 5.95; N, 0.00. Found: C, 52.43; H, 5.65; N, 0.00.

4.1.38. *n*-Pentenyl 6^{VI}-*O*-naphthyl-1-thio-β-D-maltohexaoside (45)

Deprotection of **44** (1.18 g, 0.61 mmol) was carried out by the same method as that described for **13** to give **45** quantitatively.

R_f 0.77 [5:5:1 (v/v) chloroform–methanol–water]; $[\alpha]_D^{27}$ +95.3° (*c* 0.79, MeOH); IR (KBr) 3385 (ν_{O-H}), 2926 (ν_{C-H}), 1636 ($\nu_{C=C}$), 1028 (ν_{C-O}) cm^{-1}; ^1H NMR (CD_3OD): δ 7.71–7.30 (m, 7H, naphthyl), 5.64 (m, 1H, $-CH_2CH{=}CH_2$), 4.86 (dd, 1H, J_{trans} = 17.1 Hz, J_{gem} = 1.6 Hz, one of $-CH{=}CH_2$), 4.80 (d, 1H, J_{cis} = 10.2 Hz, one of $-CH{=}CH_2$), 4.59 (s, 2H, naphthyl-CH_2O), 4.21 (d, 1H, $J_{1,2}$ = 9.7 Hz, H-1), 3.09 (t, 1H, $J_{1,2} = J_{2,3}$ = 9.2 Hz, H-2), 2.56 (m, 2H, SCH_2CH_2-), 2.00 (q, 2H, $SCH_2CH_2CH_2CH{=}CH_2$), 1.56 (m, 2H, SCH_2CH_2-); MALDI-TOF-MS Calcd for $C_{52}H_{78}O_{30}S$ $[M+Na]^+$: 1237.419, $[M+K]^+$: 1253.393. Found: *m/z*: 1237.486, *m/z*: 1253.476.

Anal. Calcd for $C_{52}H_{78}O_{30}S{\cdot}3H_2O$: C, 49.21; H, 6.67; N, 0.00. Found: C, 49.22; H, 6.58; N, 0.00.

4.1.39. Amino-ethyl thiopentyl 6^{VI}-*O*-naphthylmethyl-1-thio-β-D-maltohexaoside (46)

Radical addition of 2-mercaptoethylamine (283 mg, 2.47 mmol) to pentenyl glycoside **45** (300 mg, 0.247 mmol) was carried out by the same method as that described for **14** to give **46** quantitatively.

R_f 0.12 [5:5:1 (v/v) chloroform–methanol–water], 0.68 [1:1:1 (v/v) ethyl acetate–acetic acid–water]; $[\alpha]_D^{28}$ +97.8° (*c* 0.56, water); IR (KBr) 3385 (ν_{O-H}), 2928 (ν_{C-H}), 1638 (ν_{N-H}), 1028 (ν_{C-O}) cm^{-1}; ^1H NMR (D_2O): δ 7.76–7.38 (m, 7H, naphthyl), 4.61 (s, 2H, naphthyl-CH_2O), 4.21 (d, 1H, $J_{1,2}$ = 9.8 Hz, H-1), 3.19 (t, 1H, $J_{1,2} = J_{2,3}$ = 8.9 Hz, H-2), 3.09 (t, 2H, *J* = 6.4 Hz, SCH_2CH_2N), 2.72 (t, 2H, *J* = 6.4 Hz, SCH_2CH_2N), 2.59 (m, 2H, G-SCH_2-), 2.45 (t, 2H, *J* = 7.1 Hz, $-CH_2SCH_2CH_2N$), 1.48 (m, 4H, G-$SCH_2CH_2CH_2CH_2CH_2S$), 1.34 (m, 2H, G-$SCH_2CH_2CH_2-$); MALDI-TOF-MS Calcd for $C_{54}H_{85}NO_{30}S_2$ $[M+Na]^+$: 1314.449, $[M+K]^+$: 1330.423. Found: *m/z*: 1314.492, *m/z*: 1330.473.

Anal. Calcd for $C_{54}H_{86}NO_{30}S_2Cl{\cdot}1.5H_2O$: C, 47.83; H, 6.62; N, 0.00. Found: C, 47.73; H, 6.56; N, 0.00.

4.1.40. Dansyl amide-ethyl thiopentyl 6^{VI}-*O*-naphthylmethyl-1-thio-β-D-maltohexaoside (5)

Dansylation of amine **46** (100 mg, 0.077 mmol) with dansyl chloride (83 mg, 0.308 mmol) and with triethylamine (21 μL, 0.154 mmol) was carried out by the same method as that described for **1** to give **5** quantitatively.

R_f 0.70 [5:5:1 (v/v) chloroform–methanol–water]; $[\alpha]_D^{29}$ +76.9° (*c* 0.53, MeOH); IR (KBr) 3375 (ν_{O-H}), 2928 (ν_{C-H}), 1638 (ν_{N-H}), 1028 (ν_{C-O}) cm^{-1}; ^1H NMR (CD_3OD): δ 8.39–7.19 (m, 13H, naphthyl and dansyl), 4.56 (s, 2H, naphthyl-CH_2O), 4.18 (d, 1H, $J_{1,2}$ = 9.7 Hz, H-1), 2.82 (t, 2H, *J* = 7.3 Hz, SCH_2CH_2N), 2.77 (s, 6H, naphthyl-NMe_2), 2.49 (m, 2H, G-SCH_2-), 2.22 (t, 2H, *J* = 7.3 Hz, SCH_2CH_2N), 2.07 (t, 2H, *J* = 6.6 Hz, $-CH_2SCH_2CH_2N$), 1.38 (t, 2H, *J* = 6.8 Hz, G-SCH_2CH_2-), 1.19 (m, 4H, G-$SCH_2CH_2CH_2CH_2CH_2S$); MALDI-TOF-MS Calcd for $C_{66}H_{96}N_2O_{32}S_3$ $[M+Na]^+$: 1547.500, $[M+K]^+$: 1563.474. Found: *m/z*: 1547.557, *m/z*: 1563.534.

Anal. Calcd for $C_{66}H_{96}N_2O_{32}S_3{\cdot}1.5H_2O$: C, 51.05; H, 6.43; N, 1.80. Found: C, 51.00; H, 6.35; N, 1.50.

4.1.41. 2^{VII},3^{VII},4^{VII},6^{VII},2^{VI},3^{VI},6^{VI},2^{V},3^{V},6^{V},2^{IV},3^{IV},6^{IV}, 2^{III},3^{III},6^{III},2^{II},3^{II},6^{II},2,3,6,-Docosa-*O*-acetyl-β-D-maltoheptaose thioacetate (48)

Anomeric thioacetylation of **47** (22.7 g, 10.7 mmol) with potassium thioacetate (3.66 g, 32.1 mmol) was carried out by the same method as that described for **8**. Purification was carried out by silica gel column chromatography with 1:2 (v/v) toluene–EtOAc to give **48** (15.8 g, 69.1%).

R_f 0.33 [3:1 (v/v) ethyl acetate–toluene]; IR (KBr) 2961 (ν_{C-H}), 1748 ($\nu_{C=O}$, ester), 1638 ($\nu_{C=O}$, SAc), 1371 (ν_{C-H}), 1236 (ν_{C-O}, ester), 1038 (ν_{C-O}, ether) cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.07 (t, 1H, $J_3^{VII}{}_{,4}^{VII}$ = $J_4^{VII}{}_{,5}^{VII}$ = 9.9 Hz, H-4^{VII}), 4.85 (t, 1H, $J_{1,2} = J_{2,3}$ = 9.7 Hz, H-2), 4.86 (dd, 1H, $J_1^{VII}{}_{,2}^{VII}$ = 4.0 Hz, $J_2^{VII}{}_{,3}^{VII}$ = 10.5 Hz, H-2^{VII}), 2.37–1.98 (m, *SAc*, *OAc*); MALDI-TOF-MS Calcd for $C_{88}H_{118}O_{58}S$ $[M+Na]^+$: 2157.590, $[M+K]^+$: 2173.564. Found: *m/z*: 2157.494, *m/z*: 2173.463.

4.1.42. *n*-Pentenyl 2^{VII},3^{VII},4^{VII},6^{VII},2^{VI},3^{VI},6^{VI},2^{V},3^{V},6^{V},2^{IV},3^{IV}, 6^{IV},2^{III},3^{III},6^{III},2^{II},3^{II},6^{II},2,3,6-docosa-*O*-acetyl-1-thio-β-D-maltoheptaoside (49)

Anomeric extension of **48** (2.13 g, 1.00 mmol) with 5-bromopent-1-ene (173 μL, 2.00 mmol) and diethylamine (2.1 mL, 20 mmol) was carried out by the same method as that described for **9**. Purification was carried out by silica gel column chromatography with 2:3 (v/v) toluene–EtOAc to give **49** (1.34 g, 62.2%).

R_f 0.34 [3:1 (v/v) ethyl acetate–toluene]; $[\alpha]_D^{23}$ +122.3° (*c* 0.43, $CHCl_3$); IR (KBr) 2957 (ν_{C-H}), 1751 ($\nu_{C=O}$, ester), 1645 ($\nu_{C=C}$), 1371 (ν_{C-H}), 1240 (ν_{C-O}, ester), 1036 (ν_{C-O}, ether) cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.78 (m, 1H, $-CH_2CH{=}CH_2$), 5.05 (m, 2H), 5.00 (d, 1H, *J* = 10.3 Hz), 4.85 (m, 2H), 4.37 (dd, 1H, *J* = 12.2 Hz, *J* = 3.8 Hz), 2.66 (m, 2H, SCH_2CH_2-), 2.21–1.98 (m, *OAc*, $SCH_2CH_2CH_2CHCH_2$), 1.70 (m, 2H, SCH_2CH_2-); MALDI-TOF-MS Calcd for $C_{91}H_{124}O_{57}S$ $[M+Na]^+$: 2183.642, $[M+K]^+$: 2199.616. Found: *m/z*: 2183.608, *m/z*: 2199.577.

4.1.43. *n*-Pentenyl 1-thio-β-D-maltoheptaoside (50)

Deprotection of **49** (8.00 g, 3.70 mmol) was carried out by the same method as that described for **10** to give **50** quantitatively.

R_f 0.18 [5:5:1 (v/v) chloroform–methanol–water]; $[\alpha]_D^{27}$ +138.7° (*c* 0.52, water); IR (KBr) 3385 (ν_{O-H}), 2928 (ν_{C-H}), 1649 ($\nu_{C=C}$), 1026 (ν_{C-O}) cm^{-1}; ^1H NMR (D_2O): δ 5.80 (m, 1H, $-CH_2CH{=}CH_2$), 5.31 (m, 6H, H^{II}, H^{III}, H^{IV}, H^{V}, H^{VI}, H^{VII}), 5.00 (d, 1H, J_{trans} = 17.3 Hz, one of $-CH{=}CH_2$), 4.94 (dd, 1H, J_{cis} = 10.2 Hz, J_{gem} = 1.0 Hz, one of $-CH{=}CH_2$), 4.45 (d, 1H, $J_{1,2}$ = 9.9 Hz, H-1), 2.67 (m, 2H, SCH_2CH_2-), 2.08 (q, 2H, *J* = 6.8 Hz $SCH_2CH_2CH_2CH{=}CH_2$), 1.66 (m, 2H, SCH_2CH_2-); MALDI-TOF-MS Calcd for $C_{47}H_{80}O_{35}S$ $[M+Na]^+$: 1259.409, $[M+K]^+$: 1275.461. Found: *m/z*: 1259.491, *m/z*: 1275.383.

Anal. Calcd for $C_{47}H_{80}O_{35}S{\cdot}3H_2O$: C, 43.72; H, 6.71; N, 0.00. Found: C, 43.43; H, 6.67; N, 0.00.

4.1.44. *n*-Pentenyl 4^{VII},6^{VII}-*O*-naphthylmethylidene-2^{VII},3^{VII},2^{VI},3^{VI},6^{VI},2^{V},3^{V},6^{V},2^{IV},3^{IV},6^{IV},2^{III},3^{III},6^{III},2^{II},3^{II},6^{II},2,3,6-eicosa-*O*-acetyl-1-thio-β-D-maltoheptaoside (51)

Naphthylmethylidenation and subsequent acetylation of pentenyl thio-maltoheptaoside **50** (1.77 g, 1.4 mmol) with 2-NADIBA (850 mg, 2.8 mmol) and CSA (162 mg, 0.70 mmol) was carried out by the same method as that described for **11**. Purification was carried out by silica gel column chromatography with 1:1 (v/v) toluene–EtOAc to give **51** (518 mg, 16.3%). Acetate of starting material **49** (1.84 g, 59.3%) was also recovered.

R_f 0.55 [3:1 (v/v) ethyl acetate–toluene]; IR (KBr) 1751 ($\nu_{C=O}$, ester), 1371 (ν_{C-H}), 1238 (ν_{C-O}, ester), 1036 (ν_{C-O}, ether) cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.91–7.47 (m, 7H, naphthyl), 5.78 (m, 1H, $-CH_2CH{=}CH_2$), 5.63 (s, 1H, naphthyl-*CH*OO), 5.49 (t, 1H, *J* = 10.0 Hz), 5.04 (dd, 1H, J_{trans} = 17.2 Hz, J_{gem} = 2.0 Hz, one of $-CH{=}CH_2$), 5.00 (d, 1H, J_{cis} = 10.0 Hz, one of $-CH{=}CH_2$), 4.90 (dd, 1H, *J* = 10.2 Hz, *J* = 4.2 Hz), 4.85 (dd, 1H, *J* = 10.2 Hz), 2.66 (m, 2H, SCH_2CH_2-), 2.36–1.98 (m, *OAc*, $SCH_2CH_2CH_2CHCH_2$), 1.70 (m, 2H, SCH_2CH_2-); MALDI-TOF-MS Calcd for $C_{98}H_{126}O_{55}S$ $[M+Na]^+$: 2237.668, $[M+K]^+$: 2253.641. Found: *m/z*: 2237.633, *m/z*: 2253.616.

4.1.45. *n*-Pentenyl 6^{VII}-*O*-naphthylmethyl-2^{VII},3^{VII},2^{VI},3^{VI},6^{VI},2^{V},3^{V},6^{V},2^{IV},3^{IV},6^{IV},2^{III},3^{III},6^{III},2^{II},3^{II},6^{II},2,3,6-eicosa-*O*-acetyl-1-thio-β-D-maltoheptaoside (52)

Reductive ring opening of **51** (518 mg, 0.233 mmol) in THF was carried out with BH_3–NMe_3 complex (239 mg, 3.27 mmol) and aluminum chloride (435 mg, 3.27 mmol) by the same method as that described for **12**. Purification was carried out by silica gel column chromatography with 4:5 (v/v) toluene–EtOAc to give **52** (230 mg, 44.6%).

R_f 0.36 [3:1 (v/v) ethyl acetate–toluene]; IR (KBr) 3468 (ν_{O-H}), 2945 (ν_{C-H}), 1751 ($\nu_{C=O}$, ester), 1371 (ν_{C-H}), 1238 (ν_{C-O}, ester), 1036 (ν_{C-O}, ether) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.85–7.45 (2m, 7H,

naphthyl), 5.78 (m, 1H, $-CH_2CH{=}CH_2$), 5.02 (m, 2H, $-CH{=}CH_2$), 4.85 (t, 1H, J = 9.5 Hz), 4.81 (dd, 1H, J = 10.5 Hz, J = 3.9 Hz), 4.36 (dd, 1H, J = 12.2 Hz, J = 3.9 Hz), 2.66 (m, 2H, SCH_2CH_2-), 2.20–1.98 (m, OA*c*, $SCH_2CH_2CH_2CHCH_2$), 1.70 (m, 2H, SCH_2CH_2-); MALDI-TOF-MS Calcd for $C_{98}H_{128}O_{55}S$ $[M+Na]^+$: 2239.683, $[M+K]^+$: 2255.657. Found: *m/z*: 2239.674, *m/z*: 2255.642.

4.1.46. *n*-Pentenyl 6^{VII}-*O*-naphthylmethyl 1-thio-β-D-maltoheptaoside (53)

Deprotection of **52** (110 mg, 0.05 mmol) was carried out by the same method as that described for **13** to give **53** quantitatively.

R_f 0.62 [5:5:1 (v/v) chloroform–methanol–water]; $[\alpha]_D^{24}$ +101.7° (*c* 0.56, methanol); IR (KBr) 3399 (ν_{O-H}), 2924 (ν_{C-H}), 1638 ($\nu_{C=C}$), 1026 (ν_{C-O}) cm^{-1}; ^1H NMR (CD_3OD): δ 7.70−7.27 (2 m, 7H, naphthyl), 5.63 (m, 1H, $-CH_2CH{=}CH_2$), 4.85 (dd, 1H, J_{trans} = 17.2 Hz, J_{gem} = 1.9 Hz, one of $-CH{=}CH_2$), 4.78 (d, 1H, J_{cis} = 10.2 Hz, one of $-CH{=}CH_2$), 4.58 (s, 2H, naphthyl-CH_2O), 4.20 (d, 1H, $J_{1,2}$ = 9.7 Hz, H-1), 3.07 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 9.2 Hz, H-2), 2.55 (m, 2H, SCH_2CH_2-), 1.99 (q, 2H, $SCH_2CH_2CH_2CHCH_2$), 1.54 (m, 2H, SCH_2CH_2-); MALDI-TOF-MS Calcd for $C_{58}H_{88}O_{35}S$ $[M+Na]^+$: 1399.472, $[M+K]^+$: 1415.446. Found: *m/z*: 1399.492, *m/z*: 1415.469.

Anal. Calcd for $C_{58}H_{88}O_{35}S{\cdot}5H_2O$: C, 47.47; H, 6.73; N, 0.00. Found: C, 47.25; H, 6.58; N, 0.00.

4.1.47. Amino-ethyl thiopentyl 6^{VII}-*O*-naphthylmethyl-1-thio-β-D-maltoheptaoside (54)

Radical addition 2-mercaptoethylamine (107 mg, 0.946 mmol) to pentenyl glycoside **53** (136 mg, 0.0946 mmol) was carried out by the same method as that described for **14** to give **54** (114 mg, 80.9%).

R_f 0.05 [5:5:1 (v/v) chloroform–methanol–water], 0.87 [1:1:1 (v/v) ethyl acetate–acetic acid–water]; $[\alpha]_D^{23}$ +109.3° (*c* 0.66, Water); IR (KBr) 3396 (ν_{O-H}), 2933 (ν_{C-H}), 1633 (ν_{N-H}), 1028 (ν_{C-O}) cm^{-1}; ^1H NMR (D_2O–CD_3OD): δ 7.88−7.46 (m, 7H, naphthyl), 4.34 (d, 1H, $J_{1,2}$ = 9.6 Hz, H-1), 3.11 (t, 2H, J = 6.4 Hz, SCH_2CH_2N), 2.74 (t, 2H, J = 6.6 Hz, SCH_2CH_2N), 2.64 (m, 2H, G-SCH_2-), 2.49 (t, 2H, J = 7.2 Hz, $-CH_2SCH_2CH_2N$), 1.54 (m, 4H, G-$SCH_2CH_2CH_2CH_2CH_2S$), 1.40 (m, 2H, G-$SCH_2CH_2CH_2-$); MALDI-TOF-MS Calcd for $C_{60}H_{95}O_{35}S_2$ $[M+H]^+$: 1454.520, $[M+Na]^+$: 1476.502. Found: *m/z*: 1454.488, *m/z*: 1476.511.

Anal. Calcd for $C_{60}H_{96}NO_{35}S_2Cl{\cdot}5H_2O$: C, 46.64; H, 6.65; N, 0.91. Found: C, 46.63; H, 6.48; N, 1.17.

4.1.48. Dansyl amido-ethyl thiopentyl 6^{VII}-*O*-naphthylmethyl-1-thio-β-D-maltoheptaoside (6)

Dansylation of amine **54** (50 mg, 0.033 mmol) with dansyl chloride (36 mg, 0.134 mmol) and with triethylamine (9.3 μL, 0.067 mmol) was carried out by the same method as that described for **1** to give **6** (47 mg, 83.2%).

R_f 0.86 [5:5:1 (v/v) chloroform–methanol–water]; IR (KBr) 3391 (ν_{O-H}), 2934 (ν_{C-H}), 1634 (ν_{N-H}), 1030 (ν_{C-O}) cm^{-1}; ^1H NMR (CD_3OD): δ 8.43−7.12 (m, 13H, naphthyl and dansyl), 4.59 (s, 2H, naphthyl-CH_2O), 4.21 (d, 1H, $J_{1,2}$ = 9.7 Hz, H-1), 2.84 (t, 2H, J = 7.2 Hz, SCH_2CH_2N), 2.73 (s, 6H, naphthyl-NMe_2), 2.52 (m, 2H, G-SCH_2-), 2.24 (t, 2H, J = 7.2 Hz, SCH_2CH_2N), 2.09 (br, 2H, $-CH_2SCH_2CH_2N$), 1.40 (br, 2H, G-SCH_2CH_2-), 1.16 (br, 4H, G-$SCH_2CH_2CH_2CH_2CH_2S$); MALDI-TOF-MS Calcd for $C_{72}H_{106}N_2O_{37}S_3$ $[M+Na]^+$: 1709.553, $[M+K]^+$: 1725.527. Found: *m/z*: 1709.712, *m/z*: 1725.703.

4.2. Enzymatic evaluation

Alpha-amylase from human saliva was purchased from Cosmo Bio Japan. 10 mM HEPES-NaOH (pH 7.2), 50 mM NaCl, 10 mM $CaCl_2$, 0.01% NaN_3, and 4.2 μM synthesized bi-fluorescence-labeled maltooligosaccharide with/without α-amylase (0.03 U (caraway method)/vial) were incubated at 37 °C (total 3 mL).[7] Fluorescent intensities were continuously monitored every 3 min for 30 min.

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