Synthesis and characterization of regioselectively monoderivatized maltooligosaccharides through a combination of tandem mass spectrometry and enzymatic hydrolysis studies

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Dedicated to Professor Richard R. Schmidt on the occasion of his 78th anniversary

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Abstract

Recently, we have designed chemical strategies leading to C-6 perfunctionalized linear maltoheptaose derivatives *via* the acetolysis of persubstituted β-cyclodextrin (β-CD) at the 6-positions. This approach has now been extended to the synthesis of monofunctionalized maltoheptaose derivatives. This report deals with the regioselectivity of the ring opening of monosubstituted β-CD and the use of electrospray ionization tandem mass spectrometry (ESI-MS/MS) to identify the substituted unit. In order to confirm our results, a controlled enzymatic degradation of the resulting derivatives, using the glucoamylase of *Aspergillus niger*, was performed and monitored by liquid chromatography / electrospray ionization mass spectrometry (LC/ESI-MS).

Keywords: Acetolytic fission, cyclodextrins, C-6 monofunctionalized maltoheptaose, ESI-MS/MS, LC/ESI-MS, Enzymatic degradation

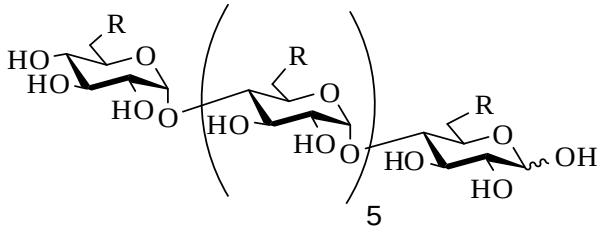
Introduction

Oligosaccharides and polysaccharides have become the focus of increasing interest in food and agriculture,[1] cosmetic[2,3] and pharmaceutical[3-5] applications. Consequently, there is an increasing need to access to modified oligosaccharides by more convenient and efficient routes. Up to now however, regioselective chemical modifications of linear oligosaccharide remain quite challenging and access to pure starting materials is extremely difficult.

The cost of native maltooligosaccharides, obtained by enzymatic degradation of amylose, with a degree of polymerization of 6 and 7 (dp 6 and 7) is still prohibitive on a gram-scale and the one of dp 8 is not yet commercially available. It is also noteworthy that their chemical synthesis is not adapted to operate at a preparative scale. Nevertheless, it's well know that their acetylated and benzoylated derivatives can be obtained through acetolysis of fully esterified cyclomaltooligosaccharides (α-, β-, and γ-cyclodextrins, α-, β- and γ-CDs).[6] We have recently extended the concept to the preparation of homologous series of C-6 persubstituted maltooligosaccharides, in high purity, starting from heptakis(2,3-di-*O*-acyl-6-bromo-6-deoxy)-β-CD or heptakis(2,3-di-*O*-acyl-6-azido-6-deoxy)-β-CD.[7] These compounds are highly suitable for further chemical modifications and are under investigation to open the way to a new class of polymeric materials such as anionic, cationic, zwitterionic or amphiphilic oligosaccharides.

In the pursuit of our work, and since the monosubstitution at the 6-position of cyclodextrin is well established,[8] we thought to explore routes to monosubstituted maltooligosaccharides by acetolysis of appropriate CDs. One important aspect in the design of synthetic routes to regioselectively modified linear maltooligosaccharides includes separation, purification and structural characterization of chemical reactions components using appropriate analytical tools. We report herein the synthesis of monosubstituted maltoheptaose derivatives **1** and **2** with azido and iodo groups (Figure 1) and their efficient purification by semi-preparative high performance liquid chromatography (semi-preparative HPLC) using a polymer-based NH_2 column. The location of the substituted unit during the chemical process was elucidated by:

- interpretation of MS/MS spectra obtained from collision-induced dissociation (CID) of their respective natriated precursor ions generated by electrospray ionization (ESI).[9]
- controlled enzymatic degradation in presence of a glucoamylase from *Aspergillus niger*[10] monitored by LC/ESI-MS.



1: R = 6 OH and 1 N_3; $C_{42}H_{71}N_3O_{35}$
(exact mass 1177.39)

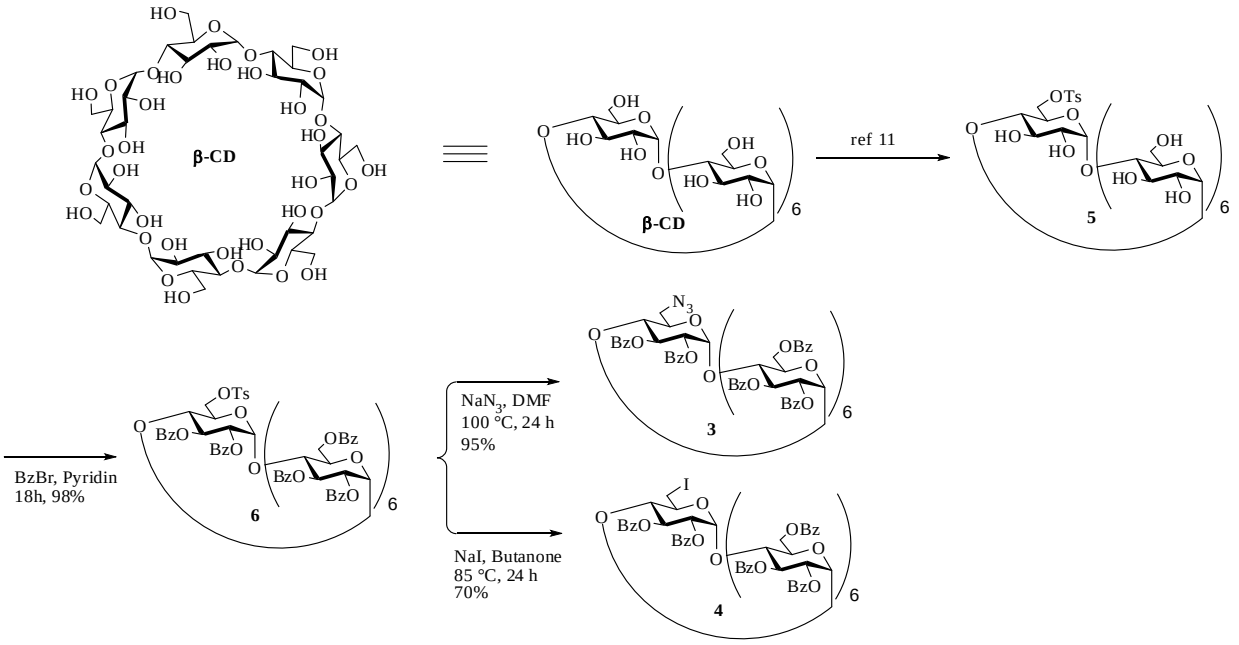
2: R = 6 OH and 1 I; $C_{42}H_{71}IO_{35}$
(exact mass 1262.28)

Figure 1. Chemical structures of targeted linear maltooligosaccharide derivatives **1** and **2**

Results and Discussion

In order to obtain C-6 monofunctionalized maltoheptaoses, we focused on the synthesis of the mono-6-azido- (DP7-monoN_3) and the mono-6-deoxy-6-iodo-maltoheptaose (DP7-monoI) derivatives **1** and **2** starting from the $6^{\mathbf{I}}$-azido-$6^{\mathbf{I}}$-deoxy-cyclomaltoheptaose **3** and the $6^{\mathbf{I}}$-deoxy-$6^{\mathbf{I}}$-iodo-cyclomaltoheptaose **4** derivatives.

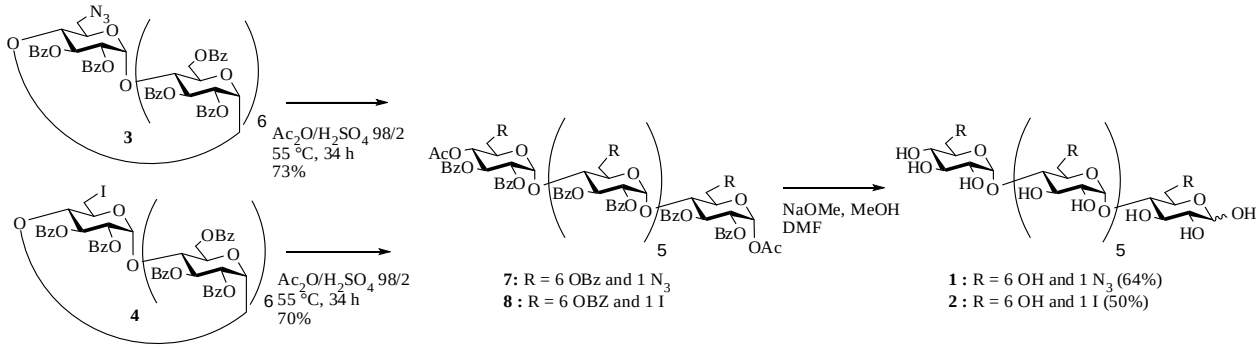
The most used method for monomodification at the 6-position of β-CD involves the known 6^{I}-tosyl-cyclomaltoheptaose **5**. Indeed, despite its 21 hydroxy groups, several methods[8] describe the introduction of a single *p*-toluenesulfonyl group at the upper rim of the β-CD with acceptable yields. Subsequent nucleophilic substitution reaction allowed the obtention of a wide range of monosubstituted β-CD. Thus in a first step 6^{I}-tosyl-cyclomaltoheptaose **5** was prepared as already described.[11] Then, the remaining secondary and primary hydroxyl groups were esterified in presence of benzoyl bromide to afford the desired benzoylated derivative **6** in 98% yield. At this stage, we chose to perform the nucleophilic substitution step of the sulfonate before the ring opening. The tosyl group was treated by sodium azide or sodium iodide, leading to the previously unknown 6^{I}-azido- and 6^{I}-iodo-benzoylated-β-CDs **3** and **4** respectively in 95% and 70% yield (Scheme1).



Scheme 1. Synthesis of 6^{I}-azido- and 6^{I}-iodo-benzoylated-β-CDs **3** and **4** from β-CD.

With **3** and **4** in hand, we undertook the investigation of their acetolysis. We found that the optimum reaction conditions were those described by Kuzuhara[6a,b] using acetic anhydride and sulfuric acid as catalyst. In contrast with the modest yields of ring opening obtained in the case of C-6 persubstituted β-CDs,[7] acetolysis of the monosubstituted derivatives **3** and **4** (Scheme 2),

occurred in acceptable yield (73% and 70% respectively) without loss of starting material. Indeed, unreacted monosubstituted β-CDs can be recovered in both cases, after their separation from the expected esterified and monosubstituted maltooligosaccharides **7** and **8**, by means of Kiesegel column chromatography. The purity control and the structure elucidation of both maltooligosaccharides were deduced from their ESI-MS analysis (Figure 2). The obtained spectra show abundant $[M+2Na]^{2+}$ ions at *m/z* 1695 and 1737. The $[M+Na]^{+}$ ions are also observed at *m/z* 3367 and 3452, respectively. Due to the fact that highly esterified oligosaccharides show more complicated MS/MS data than the deprotected derivatives, the structural investigations were not performed at this stage. Indeed, the main fragmentation process of natriated ions of benzoylated compounds involved principally the elimination of the ester groups. In that way, inter-glycosidic and cross-ring fragments ions, useful for the location of the substituents, are generated in lower abundance.[7b]



Scheme 2. Acetolysis of monsubstituted β-CD **3** and **4** and synthesis of linear derivatives **1** and **2.**

Finally, monosubstituted maltoheptaose derivatives **7** and **8** were submitted to basic methanolysis to give the desired linear maltoheptaose monofunctionalized DP7-monoN_3 **1** and DP7-monoI **2** in 64% and 50% yields respectively, after purification by semi-preparative HPLC (Scheme2). In order to obtain an efficient separation, the use of a polymeric-based NH_2 rather than a classical C_{18} column proved to be necessary. Indeed, its polar functionality is essential to counterbalance the hydrophobic interactions observed between the azido or iodo group and the alkylated bonded phase contained in the C_{18} one. The HPLC profiles and the ESI-MS spectra of the final batches are presented in Fig. 3, showing a high level of purity for these two compounds. The results of the elemental composition determinations of their $[M+Na]^{+}$ ions (*m/z* 1200 and *m/z* 1285) are in accordance with their structures (see experimental section).

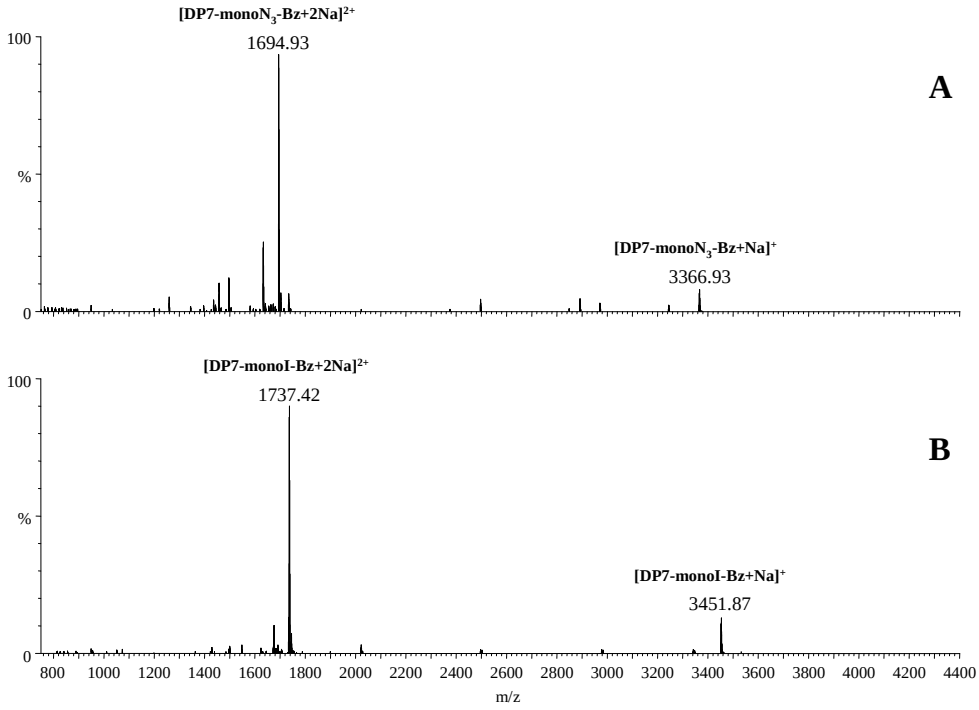


Figure 2. ESI-MS spectra of the benzoylated monofunctionalized maltooligosaccharides DP7-monoN$_3$-Bz **7** (A) and DP7-monoI-Bz **8** (B).

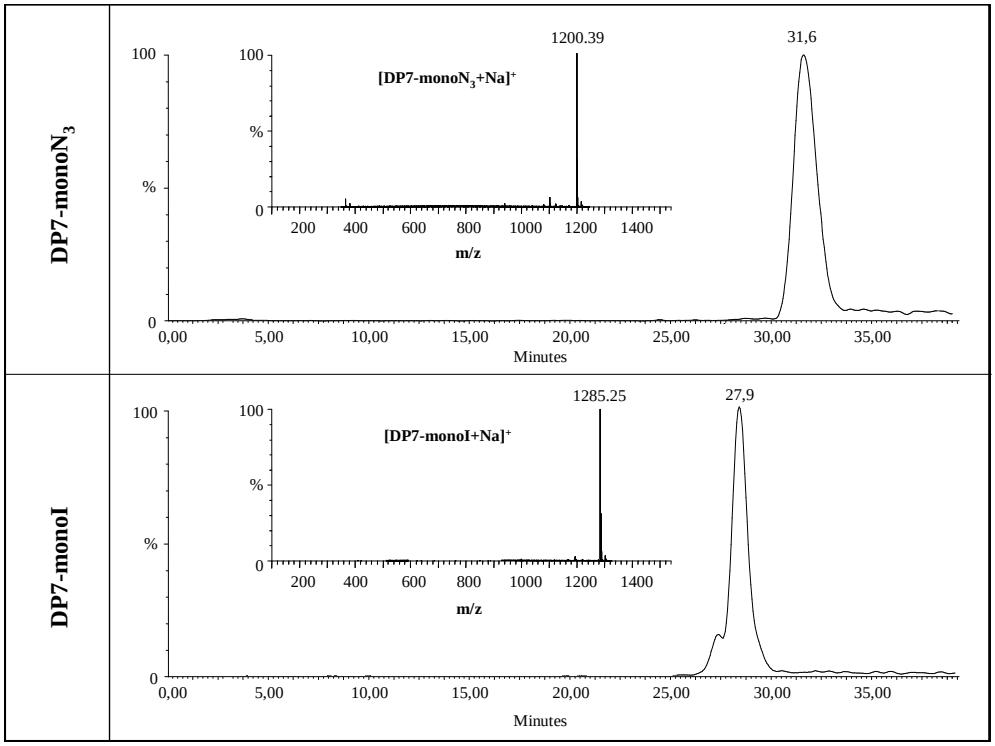


Figure 3. HPLC-ELSD chromatograms and ESI-MS spectra of pure DP7-monoN$_3$ (**1**, upper) and DP7-monoI (**2**, lower).

At this stage, the main challenge was to establish the regioselectivity of the acetolysis procedure in assigning precisely the localization of the azido and iodo groups on the respective linear oligosaccharides constituted of seven glucose monomer molecules $\alpha(1\rightarrow4)$ linked. Indeed, if the acidic cleavage is not influenced by the nature and location of the substituent at the 6-position, we should observe a mixture of isomers with the azido or iodo group distributed randomly on one or the other glucosidic unit. On the other hand, if the cleavage occurs in a specific way, we should obtain a single regioselectively C-6 monofunctionalized maltoheptaose derivative in each case. To this end, the sequence and branching points of both derivatives **1** and **2** were investigated by tandem mass spectrometry (MS/MS) experiments. Upon collision, their $[M+Na]^+$ ions yield B/Y, C/Z and A fragments ions, according to the nomenclature established by Domon and Costello.[12] The MS/MS spectra are dominated by inter-glycosidic cleavages (B/Y-ion and C/Z-ion series) that are observed for all glycosidic bonds and provide useful sequence information. The 162 Da mass increment between the subsequent B/Y and C/Z ions enabled the assignment of the seven glucose residues for both compounds. The third ion series (A-ion) corresponds to internal fragments arising from cross-ring cleavages and allows an identification of the linkage sites and also of the substituent position. In our case, we observed the $^{0,2}A_n$ and the $^{2,4}A_n$ series, which are known to be specific of $(1\rightarrow4)$ type glycosidic linkage.[13] The MS/MS spectrum obtained for the DP7-monoN_3 is presented Fig. 4.

The $[M+Na]^+$ ion of DP7-monoN_3 **1** (*m/z* 1200) loss, as expected for a molecule carrying an azido group, a nitrogen molecule (28 Da) leading to the intermediate product ion (*m/z* 1172). Then, this ion generates the classical Y-ion (*m/z* 1010, 848, 686, 524, 362, 203) and B-ion (*m/z* 992, 833, 671, 509, 347, 185) series together with C-ion (*m/z* 1010, 851, 689, 527, 365, 203) and Z-ion (*m/z* 992, 830, 668, 506, 344, 185) series. The presence of a 159 Da difference between B_5 to B_6 and C_5 to C_6 ions as well as between Z_1 to Z_2 and Y_1 to Y_2 allows us to localize the azido group on the second hexose unit starting either from the reducing end or from the nonreducing end of the oligosaccharide. A careful examination of the $(1\rightarrow4)$ cross-ring fragments, the $^{0,2}A$ (*m/z* 1112, 950, 791, 629, 467, 305) and $^{2,4}A$ (*m/z* 1052, 893, 731, 569, 407, 245) series, confirm that the second hexose unit starting from the reducing end bears the azido group (see Figure 4).

As for DP7-monoN_3, the fragmentation pattern of the $[M+Na]^+$ ion (*m/z* 1285) of the DP7-monoI **2** was carefully analyzed. Abundant B (*m/z* 1105, 943, 671, 509, 347, 185), Z (m/z 1105, 943, 781, 619, 347, 185), C (*m/z* 1123, 961, 689, 527, 365, 203) and Y (*m/z* 1123, 961, 799, 637, 365, 203) fragments ions from inter-glycosidic bond cleavages are observed. The mass values of these different ions allow us to suggest that the iodo group is mainly localized on the third unit starting from the reducing end. However, this fragmentation is also possible (excepted for the Z_3 and Y_3 ions at *m/z* 619 and 637) for a substitution on the fourth hexose unit. Moreover, minor inter-glycosidic fragments ions at *m/z* 457 (Z_2), 475 (Y_2), 833 (B_5) and 851 (C_5) seem to indicate the probable presence of an oligosaccharide substituted on the second hexose unit. Finally, the complexity of the cross-ring fragmentation is in accordance with a mixture of monoiodo maltooligosaccharides.

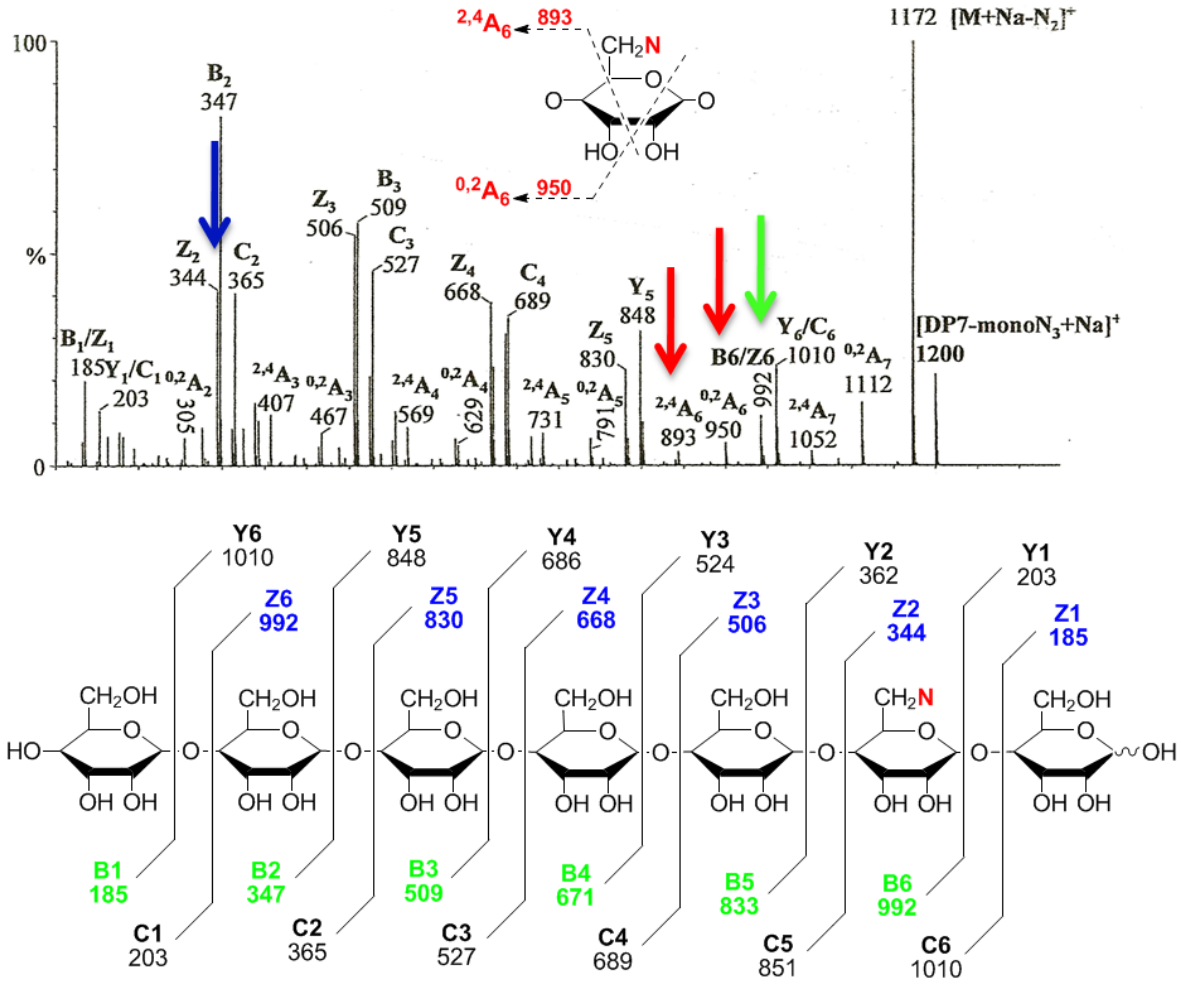


Figure 4. ESI-MS/MS spectrum of DP7-monoN$_3$ **1** showing the inter-glycosidic and cross-ring fragment ions.

The MS/MS fragmentation spectra being not entirely free from ambiguity, in particular for compound **2**, and the precise location of the substituted unit requiring confirmation, an enzymatic degradation of both compounds was performed. The α-glucoamylase G2 from *Aspergillus niger* is an exo-glycosidase which catalyzes the selective hydrolysis of α(1→4) glucosidic bonds starting from the nonreducing end of maltooligosaccharides. A chemical modification of the 6-position of one or the other unit should stop the hydrolysis process and should permit the identification of this residue. This has been validated by a kinetic study of the enzymatic degradation monitored by LC/ESI-MS of pure maltoheptaose and of the known 6$^{I\text{-}VII}$-heptaazido-6$^{I\text{-}VII}$-heptadeoxy-maltoheptaose.[7a] As expected, maltoheptaose was hydrolyzed to glucose in 40 min. while the azido derivative remains untouched for at least 27 hours (Figure 5).

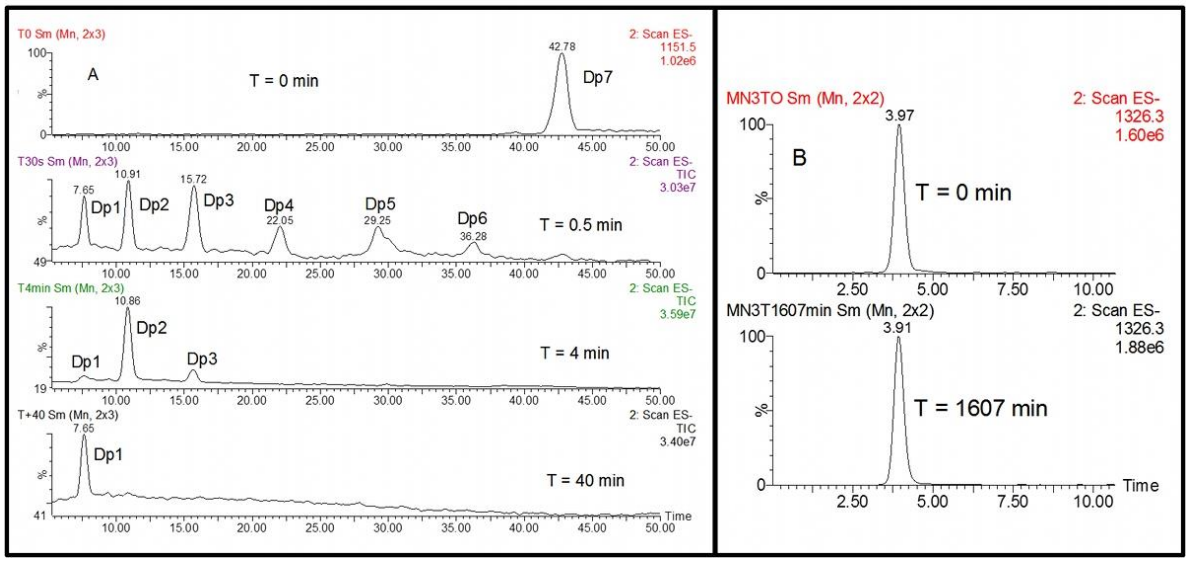


Figure 5. LC/ESI-MS monitoring of the enzymatic hydrolysis of maltoheptaose (A) and $6^{I\text{-}VII}$-heptaazido-$6^{I\text{-}VII}$-heptadeoxy-maltoheptaose (B) (horizontal axis : time in min.).

The method was then applied to monoiodo derivative **2**. The enzymatic hydrolysis of **2** in the presence of α-glucoamylase G2 leads in 40 min. to a mixture of glucose (dp1) and monoiodo substituted maltooligosaccharides of dp 2 to 4 (Figure 6).

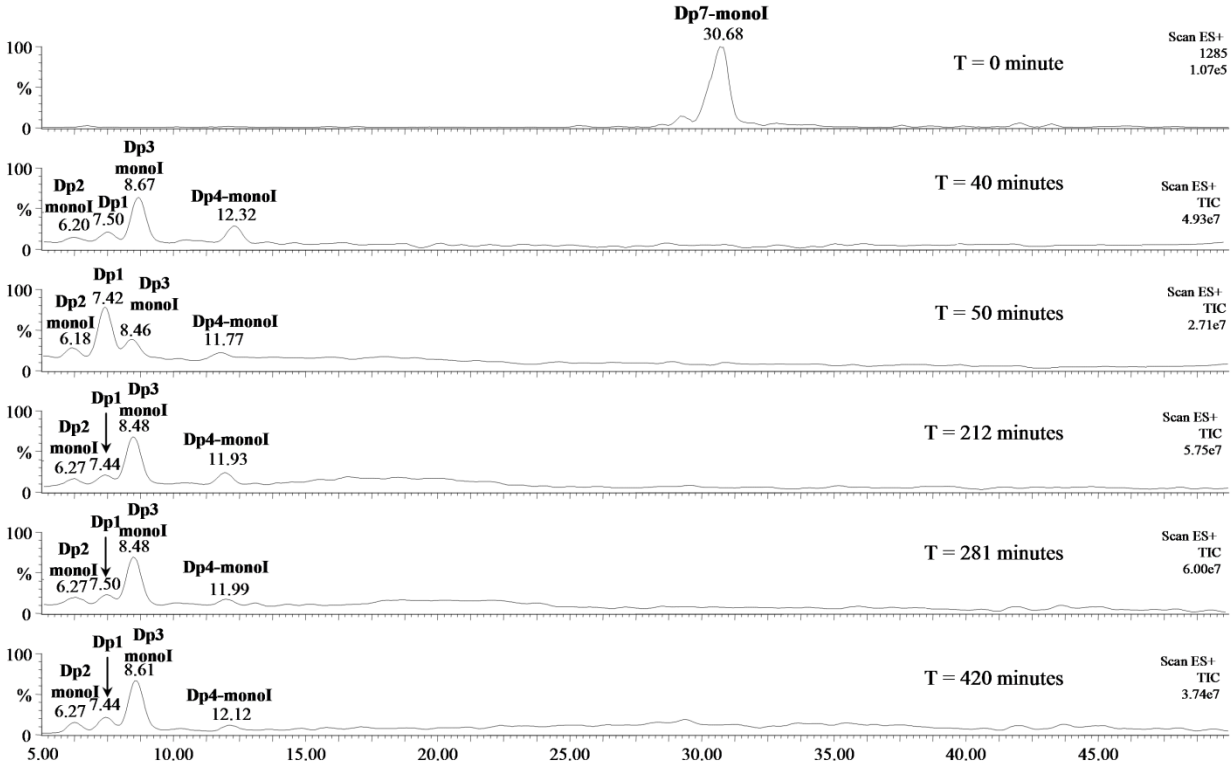
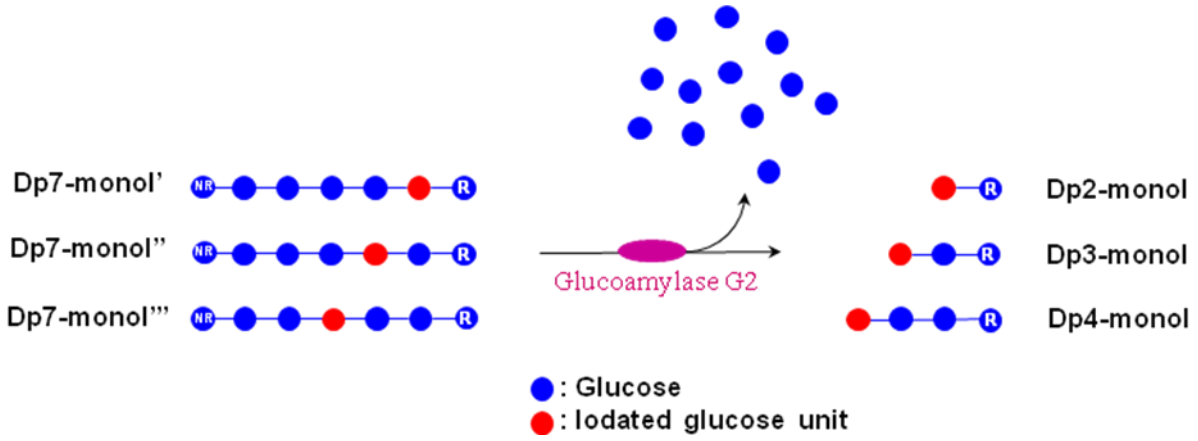


Figure 6. LC/ESI-MS monitoring of the enzymatic hydrolysis of DP7-monoI **2** (horizontal axis : time in min.)

No significant change was observed during the following 6 hours. In line with the results of mass spectrometry locating the iodo group mainly on unit 3, but not excluding the presence of other iodo derivatives, this study confirmed the lack of regioselectivity of the acetolysis of 6^{I}-iodo-benzoylated-β-CD (Scheme 3). These observations are in agreement with the HPLC analysis (Figure 3), showing a shoulder on the peak at t_R = 27.9 min for the DP7-monoI **2**. In contrast, the enzymatic treatment of monoazido derivative **1**, conducted under the same conditions, led to a mixture of glucose and monoazido substituted maltooligosaccharide of dp2, consistent with the experiments results of LC/ESI-MS.



Scheme 3. Enzymatic degradation pathway of the three regioisomers of Dp7-monoI **2**.

Conclusions

This work deals with the acetolysis of C-6 monosubstituted β-CDs using Kuzuhara's conditions. The lack of regioselectivity observed in the case of the monoiodo β-CD was disappointing. Nevertheless, getting a single regioisomer when monoazido β-CD was submitted to the ring opening encourages us to other investigations of this reaction. We have also demonstrated that ESI-MS, in combination with the high resolution (HRMS) and tandem mass spectrometry (MS/MS) capabilities of a Q-TOF mass spectrometer, constitutes a key analytical tool and that monitoring of their enzymatic degradation can be an excellent means to confirm the structural determination of this kind of modified oligosaccharides.

Experimental Section

General. β-cyclodextrin and other chemical reagents were purchased from Wacker (Munich, Germany) and Sigma-Aldrich (St. Louis, MO, USA), respectively. HPLC grade acetonitrile and water were purchased from Fisher Scientific (Loughborough, UK). All reactions were monitored by TLC on Kieselgel 60 F254 (E. Merck). Detection was achieved by charring with vanillin. Silica gel (E. Merck, 240-400 mesh) was used for chromatography. Solutions were concentrated under reduced pressure. Optical rotation was measured with a JASCO DIP-370 digital polarimeter, using a sodium lamp (λ = 589 nm) at 20 °C. All NMR experiments were performed at 300.13 MHz using Bruker DMX300 spectrometer equipped with a Z-gradient unit for pulsed-field gradient spectroscopy. Chemical shifts are given relative to external TMS with calibration involving the residual solvent signals.

Mass spectrometry. Electrospray ionization mass spectra (ESI-MS) in the positive ion mode were obtained on a Q-TOF Ultima Global hybrid quadrupole/time-of-flight instrument (Waters-Micromass, Manchester, U.K.), equipped with a pneumatically assisted electrospray (Z-spray) ion source and an additional sprayer (Lock Spray) for the reference compound used for external and internal mass calibration. The maltooligosaccharides samples were dissolved (0.1 $mg.mL^{-1}$) in water and the solutions directly introduced (5 $\mu L.mn^{-1}$) through an integrated syringe pump into the electrospray source. The source and desolvation temperatures were kept at 80 and 150 °C, respectively. Nitrogen was used as the drying and nebulizing gas at flow rates of 350 and 50 $L.h^{-1}$, respectively. The capillary voltage was 3.5 kV, the cone voltage 100 V and the RF lens1 energy was optimised for each sample (50 to 150 V). For collision-induced dissociation (CID) experiments, argon was used as collision gas at an indicated analyser pressure of 5.10^{-5} Torr and the collision energy was optimised for each parent ion (60 to 80 V). Lock mass correction, using appropriate cluster ions of sodium iodide $(NaI)_nNa^+$, was applied for accurate mass measurements. The mass range was typically 50-4500 Da and spectra were recorded at 4 s/scan in the profile mode at a resolution of 10000 (FWMH). Data acquisition and processing were performed with MassLynx 4.0 software.

Chromatography. The crude reaction mixtures containing the mono functionalized linear maltoheptaoses were purified on a Waters LC4000 system (Milford, MA, USA). The sample solutions were prepared in HPLC grade water at a concentration of 10 $mg.mL^{-1}$. The Prevail Carbohydrate ES semi-preparative (250 mm x 10 mm i.d., 5 μm particle size) column used for the separation was purchased from Alltech (Deerfield, IL, USA). The column was kept at room temperature and the sample injection volume was 100 μL. A gradient elution was applied at a flow rate of 4.7 $mL.min^{-1}$: 70% acetonitrile - 30% water to 50% acetonitrile - 50% water in 50 min followed by a 10 min hold at 50% acetonitrile - 50% water. Oligosaccharide signals were monitored using an evaporative light scattering detector PL-ELS 1000 (Polymer Labs, Amherst, MA, USA) with a nitrogen gas flow of 1.5 $mL.min^{-1}$, a nebulization temperature of 80 °C and an evaporation temperature of 90 °C. Data acquisition was performed using Empower software. The collected fractions were directly freeze dried to afford DP7-monoN_3 **1** and DP7-monoI **2**.

Enzymatic degradation studies. A solution of glucoamylase G2 (E. C. 3. 2. 1. 3) of *Aspergillus niger* (166.2 μL, 1 mg/mL in an acetate buffer at pH 4.2) was added to a solution of maltoheptaose, 6^{I-VII}-heptaazido-6^{I-VII}-heptadeoxy-maltoheptaose, compound **1** or **2** (2 mL, 1.76 g/L in an acetate buffer at pH 4.2). The reaction mixture was gently stirred in a closed test tube at 37 °C. An aliquot (20 μL) was taken at regular time interval and analyzed by LC/ESI-MS. The LC/ESI-MS experiments were performed on a ZQ 4000 simple quadrupole mass spectrometer (Waters-Micromass, Manchester, UK), coupled with a Waters 2695 Alliance HPLC system (Milford, MA, USA). Samples (20 μL) were loaded on a Prevail Carbohydrate ES analytical column (250 mm x 4.6 mm i.d., 5 μm particle size) purchased from Alltech (Deerfield, IL, USA) regulated to 25 °C. A gradient elution was applied at a flow rate of 1 $mL.min^{-1}$: 70% acetonitrile - 30% water to 50% acetonitrile - 50% water in 50 min followed by a 10 min hold at 50% acetonitrile - 50% water. The effluent was flow-split via a peek tee with 1/4 of the flow directed toward the electrospray source (Z-spray) of the mass spectrometer operated in the positive ion mode and the residual directed to the waste. Capillary voltage and cone voltage were set at 3 kV and 40 V, respectively. The nebulizing (100 L/h) and drying (450 L/h) gas was nitrogen. The source and desolvatation temperatures were kept at 120 °C and 250 °C, respectively. Data were recorded using a mass range of 150 to 1550 amu at 1 scan/s and were collected in the profile mode and analyzed with MassLynx 4.0 software.

2^{I-VII},3^{I-VII},6^{II-VII}-Eicosa-*O*-benzoyl-6^{I}-deoxy-6^{I}-*p*-toluenesulfonyl-cyclomaltoheptaose (6). To a solution of 6^{I}-deoxy-6^{I}-*p*-toluenesulfonyl-cyclomaltoheptaose **5** (5 g, 3.8 mmol) in pyridine (30 mL) was added benzoyl bromide (18 mL, 155 mmol) at 0 °C. The reaction mixture was stirred at rt under inert atmosphere for 18 h. The solution was concentrated to dryness and the subsequent residue was suspended in MeOH / Water (50 mL, 10 : 1 v/v) to give a precipitate which was filtered to afford compound **6** (12.9 g, 98%) : ^{13}C NMR δ (75 MHz, $CDCl_3$) 166.0, 164.5 ($C_6H_5\underline{C}O$), 145.3, 141.1, 133.2, 135.6, 132.3, 129.9, 129.6, 128.7, 128.3, 128.0, 127.7, 126.8 ($\underline{C}_6H_5CO$ and $CH_3\underline{C}_6H_4SO_2$), 97.2 (C-$1^{I-VII}$), 77.4 (C-$4^{I-VII}$), 71.3, 70.0 (C-$2^{I-VII}$, C-$3^{I-VII}$, C-$5^{I-VII}$), 70.5 (C-$6^{I}$), 63.3 (C-$6^{II-VII}$), 22.1 ($\underline{C}H_3C_6H_4SO_2$); HRMS ($ESI^+$) Calcd. for $C_{189}H_{156}O_{57}SNa$ (MNa^+) 3391.8927. Found 3391.8931.

6^{I}-Azido-2^{I-VII},3^{I-VII},6^{II-VII}-eicosa-*O*-benzoyl-6^{I}-deoxy-cyclomaltoheptaose (3). A solution of tosylate derivative **6** (12 g, 3.6 mmol) and sodium azide (1.157 g, 17.8 mmol) in dry DMF (20 mL) was stirred for 24 h. at 100 °C. The solution was concentrated to dryness and the subsequent residue was suspended in MeOH / Water (50 mL, 10 : 1 v/v) to give a precipitate which was filtered to afford compound **3** (11 g, 95%) : ^{13}C NMR δ (75 MHz, $CDCl_3$) 166.4, 164.9 ($C_6H_5\underline{C}O$), 133.8, 133.1, 132.8, 130.4, 130.2, 129.1, 128.2, 128.1 ($\underline{C}_6H_5CO$), 97.9 (C-1^{I-VII}), 78.1 (C-4^{I-VII}), 71.9, 71.8, 70.5 (C-2^{I-VII}, C-3^{I-VII}, C-5^{I-VII}), 63.9 (C-6^{II-VII}), 51.4 (C-6^{I}); HRMS (ESI^+) Calcd. for $C_{182}H_{149}N_3O_{54}Na$ (MNa^+) 3262.8903. Found 3262.8899.

2^{I-VII},3^{I-VII},6^{II-VII}-Eicosa-*O*-benzoyl-6^{I}-deoxy-6^{I}-iodo-cyclomaltoheptaose (4). A solution of tosylate derivative **6** (5 g, 1.5 mmol) and sodium azide (2.22 g, 15 mmol) in butanone (50 mL) was stirred for 24 h. at 85 °C. The solution was concentrated to dryness and the subsequent

residue was suspended in MeOH / Water (50 mL, 10 : 1 v/v) to give a precipitate which was filtered to afford compound **4** (3.45 g, 70%) : ^{13}C NMR δ (75 MHz, $CDCl_3$) 166.0, 165.9,164.4 ($C_6H_5\underline{C}O$), 133.4, 133.2, 132.5, 132.2, 130.0, 129.6, 129.4, 128.7, 128.6, 128.2, 127.7, 127.6 ($\underline{C}_6H_5CO$), 97.3 (C-1$^{I\text{-}VII}$), 78.1 (C-4$^{I\text{-}VII}$), 71.4, 70.1 (C-2$^{I\text{-}VII}$, C-3$^{I\text{-}VII}$, C-5$^{I\text{-}VII}$), 63.3 (C-6$^{II\text{-}VII}$), 9.8 (C-6^{I}); HRMS (ESI$^+$) Calcd. for $C_{182}H_{149}IO_{54}Na$ (MNa^+) 3347.7856. Found 3347.7876.

1^{I},4^{VII}-Di-*O*-acetyl-6^{II}-azido-2$^{I\text{-}VII}$,3$^{I\text{-}VII}$,6$^{I,III\text{-}VII}$-eicosa-*O*-benzoyl-6^{II}-deoxy-α-maltoheptaose (7). Benzoylated β-CD-monoN_3 **3** (2 g, 0.61 mmol) was dried at 60 °C for 24 h under diminished pressure and dissolved in 98:2 Ac_2O-H_2SO_4 (14 mL). The mixture was stirred at 55 °C for 24 h, cooled, and the reaction was quenched by the addition of pyridine (10 mL). The solution was evaporated under diminished pressure. The residue was diluted in EtOAc (40 mL) and washed with water. The aqueous layer was extracted with EtOAc. The organic layers were concentrated and the residue was subjected to silica gel column chromatography (20:2 toluene-EtOAc then 15:1 toluene-EtOAc and 10:1 toluene-EtOAc) to afford unchanged **3** (0.2 g, 10%) and **7** (1.5 g, 73%) : $[\alpha]_D$ + 84 (*c* 2.2, EtOAc); ^{13}C NMR δ (75 MHz, $CDCl_3$) 169.2, 168.8 ($CH_3\underline{C}O$), 166.1, 165.6, 165.3, 164.7, 164.5 ($C_6H_5\underline{C}O$), 133.2, 133.0, 129.9, 129.6, 129.4, 128.9, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8 ($\underline{C}_6H_5CO$), 96.9, 96.7 (C-1$^{II\text{-}VII}$), 88.9 (C-1^{I}), 74.4, 74.1, 73.5, 73.2, 71.9, 71.7, 71.4, 70.7, 70.4, 70.0, 68.8, 68.1, 68.0 (C-2$^{I\text{-}VII}$, C-3$^{I\text{-}VII}$, C-4$^{I\text{-}VII}$,C-5$^{I\text{-}VII}$), 63.0, 62.6, 62.3, 62.1, 61.6 (C-6$^{I,III\text{-}VII}$), 50.3 (C-6^{II}), 20.4 ($\underline{C}H_3CO$); HRMS (ESI$^+$) Calcd. for $C_{186}H_{155}N_3O_{57}Na$ (MNa^+) 3364.9407. Found 3364.9368.

1^{I},4^{VII}-Di-*O*-acetyl-2$^{I\text{-}VII}$,3$^{I\text{-}VII}$,6$^{I,II\text{-}IV\text{-}VII}$-eicosa-*O*-benzoyl-6^{III}-deoxy-6^{III}-iodo-α-maltoheptaose (8). Benzoylated β-CD-monoI **4** (2 g, 0.60 mmol) was dried at 60 °C for 24 h under diminished pressure and dissolved in 98:2 Ac_2O-H_2SO_4 (14 mL). The mixture was stirred at 55 °C for 24 h, cooled, and the reaction was quenched by the addition of pyridine (10 mL). The solution was evaporated under diminished pressure. The residue was diluted in EtOAc (40 mL) and washed with water. The aqueous layer was extracted with EtOAc. The organic layers were concentrated and the residue was subjected to silica gel column chromatography (20:2 toluene-EtOAc then 15:1 toluene-EtOAc and 10:1 toluene-EtOAc) to afford unchanged **4** (0.18 g, 9%) and **8** (1.44 g, 70%) : ^{13}C NMR δ (75 MHz, $CDCl_3$) 169.2, 168.9 ($CH_3\underline{C}O$), 165.7, 165.4, 165.3, 164.5 ($C_6H_5\underline{C}O$), 133.3, 133.0, 129.9, 129.7, 129.4, 128.9, 128.5, 128.4, 128.3, 128.2, 128.0, 127.8 ($\underline{C}_6H_5CO$), 97.0, 96.7, 96.4 (C-1$^{II\text{-}VII}$), 89.0 (C-1^{I}), 74.0, 73.6, 73.3, 73.1, 72.0, 71.7, 71.0, 70.8, 70.5, 70.0, 69.8, 68.8, 68.0 (C-2$^{I\text{-}VII}$, C-3$^{I\text{-}VII}$, C-4$^{I\text{-}VII}$,C-5$^{I\text{-}VII}$), 65.2, 62.6, 62.1, 61.7 (C-6$^{I,II,IV\text{-}VII}$), 20.9, 20.4 ($\underline{C}H_3CO$), 8.75 (C-6^{III}); HRMS (ESI$^+$) Calcd. for $C_{186}H_{155}IO_{57}Na$ (MNa^+) 3449.8174. Found 3449.81799.

6^{II}-Azido-6^{II}-deoxy-maltoheptaose (1). A solution of sodium methoxide (7.2 mL, 1 M in MeOH) was added dropwise at 0 °C to a solution of **7** (1 g, 0.3 mmol) in MeOH (30 mL) and DMF (20 mL). The reaction mixture was stirred at room temperature for 22 h. An acidic resin (Amberlite IR 120 H^+) was added to neutralise MeONa. The resin was filtered and the solvent removed. The residue was subjected to High Performance Liquid Chromatography to afford DP7-monoN_3 **1** (220 mg, 64%) : ^{13}C NMR δ (75 MHz, D_2O) 99.9 (C-1$^{II\text{-}VII}$), 96.2 (C-1^{I}β), 92.0

(C-$1^{I}\alpha$), 77.1, 73.7, 73.2, 71.8, 71.5, 69.7 (C-2^{I-VII}, C-3^{I-VII}, C-4^{I-VII},C-5^{I-VII}), 60.8 (C-$6^{I,\ III-VII}$), 50.4 (C-6^{II}); HRMS (ESI^+) Calcd. for $C_{42}H_{71}N_3O_{35}Na$ (MNa^+) 1200.3766. Found 1200.3785.

6^{III}-Deoxy-6^{III}-iodo-maltoheptaose (2). A solution of sodium methoxide (7.2 mL, 1 M in MeOH) was added dropwise at 0 °C to a solution of **8** (1 g, 0.29 mmol) in MeOH (30 mL) and DMF (20 mL). The reaction mixture was stirred at room temperature for 22 h. An acidic resin (Amberlite IR 120 H^+) was added to neutralise MeONa. The resin was filtered and the solvent removed. The residue was subjected to High Performance Liquid Chromatography to afford DP7-monoI **2** (184 mg, 50%) : ^{13}C NMR δ (75 MHz, D_2O) 99.9, 99.7 (C-1^{II-VII}), 96.0 (C-$1^{I}\beta$), 92.2 (C-$1^{I}\alpha$), 81.3, 81.0, 77.1, 77.0, 76.3, 74.8, 74.3, 73.6, 73.1, 73.0, 72.0, 71.8, 71.7, 71.6, 71.4, 70.2, 69.6, 69.4 (C-2^{I-VII}, C-3^{I-VII}, C-4^{I-VII},C-5^{I-VII}), 60.9, 60.7 (C-$6^{I,II-IV-VII}$), 8.02 (C-6^{III}); HRMS (ESI^+) Calcd. for $C_{42}H_{71}IO_{35}Na$ (MNa^+) 1285.2718. Found 1285.2679.

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