Inclusion and solubilization properties of 6-*S*-glycosyl-6-thio derivatives of β-cyclodextrin

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The synthesis and physico-chemical properties of branched β-cyclodextrins substituted by one or seven thioglycoside units at the primary hydroxy side are described. The solubilities in water of these compounds are strongly increased compared with the parent β-cyclodextrin although large differences are found between α- and β-anomers, the former exhibiting the larger solubility. The inclusion capacity of these derivatives has been investigated using NMR spectroscopy as the major analytical technique for various host–guest pairs. The apparent discrepancies between the intrinsic solubilities of these host molecules and their ability to solubilize hydrophobic hosts can be explained from geometrical considerations derived from detailed NMR studies. The respective roles of the side of inclusion, of steric effects and of stabilizing interactions are evidenced and allow an *a priori* selection of the optimal host derivative for a given guest molecule.

The continuing challenge of using cyclomaltooligosaccharides (cyclodextrins or CDs) for the solubilization, stabilization and targetting of drugs has led to the preparation of a wide variety of chemically modified derivatives dedicated to improve the properties of these host molecules and to override their adverse physiological effects, *e.g.* the hemolytic character.[1] When considering the most common and cheap cyclodextrin, cyclomaltoheptaose (β-cyclodextrin) the main feature to be improved is the solubility. This cyclic oligosaccharide is indeed much less soluble in water at room temperature than cyclomaltohexaose or cyclomaltooctaose (α- or γ-cyclodextrins, respectively).[2] The purpose is therefore to obtain derivatives of β-cyclodextrin exhibiting solubilities in the order of 100 g dm^{-3} or even more. The resulting increase in solubility is expected to be reflected in a much higher solubility of inclusion complexes.

It has been observed that 6-*O*-glycosyl cyclomaltooligosaccharides (branched cyclodextrins), obtained as by-products along with regular cyclodextrins in the transformation of amylose,[2] exhibit superior properties in terms of solubility in water with respective to the parent unbranched cyclodextrins.[3] These derivatives are, however, produced as complex mixtures with fairly low yields and their purification can be achieved only using tedious chromatographic techniques.[4] Their chemical synthesis requires a number of steps [5,6] and can therefore not be scaled-up to quantities required by potential pharmaceutical applications. Conversely, the synthesis of their 6-*S*-glycosyl thioanalogues can be achieved more readily and can afford mono- as well as per-substituted derivatives with full control of the anomeric configuration.

Previous results from one of our laboratories [7] have shown that 6^1-*S*-α- (**1**) as well as 6^1-*S*-β- (**2**) D-glucopyranosyl-6-thiocyclomaltoheptaose displayed an enhanced solubility in water compared with β-cyclodextrin. We now report on the synthesis of additional representatives in this series, namely the 6^1-*S*-α- (**3**) and the 6^1-*S*-β- (**4**) 6-thiomaltosyl homologues of **1** and **2** and the corresponding per-6-substituted analogues **5** and **6**.

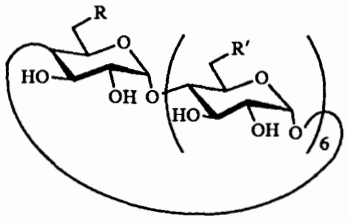
A detailed NMR spectroscopic investigation of the inclusion of model guests is performed in order to derive clues allowing the selection of the most appropriate derivative to solubilize a given guest. The respective roles of steric effects and the selection of mono- or per-substituted derivatives will be made possible by the determination of the stability and the structure of inclusion complexes by NMR spectroscopy.

Results and discussion

Synthesis of branched 6-*S*-glycosyl-6-thiocyclomaltoheptaose derivatives (Scheme 1)

The synthesis of branched derivatives **3–6** was achieved following the previously described procedure [7] involving nucleophilic reaction of a glycose 1-thiolate with a conveniently C-6 activated derivative of cyclomaltoheptaose in a polar aprotic solvent. In view of their ready availability, 6-*O*-*p*-tolylsulfonylcyclomaltoheptaose **7** [7,8] and heptakis(6-deoxy-6-iodo)cyclomaltoheptaose **8**,[9] both prepared in one step from cyclomaltoheptaose (β-cyclodextrin), were the respective starting materials for the mono- (**3**, **4**) and per- (**5**, **6**) 6-*S*-glycosyl-6-thiocyclomaltoheptaose derivatives (Scheme 2). Sulfur nucleophiles 1-thio-β-D-glucopyranose [10] and 1-thio-β-maltose [11] sodium salts were obtained following published procedures. The sodium salt of 1-thio-α-D-glucopyranose was conventionally prepared by sodium methoxide treatment of 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-α-D-glucopyranose.[12] The procedure used for the preparation of **14** [12] was extended to the preparation of the maltose homologue **12** (Scheme 3).

The key compound hepta-*O*-acetyl-2-hydroxymaltal **11** was obtained in 78% yield by dehydrobromination of the acetylated α-maltosyl bromide **10** with 1,5-diazabicyclo[5.4.0]undec-5-ene in *N*,*N*-dimethylformamide at ambient temperature. Addition of thioacetic acid to the acetylated hydroxyglycal **11**, in the presence of cumene hydroperoxide, proceeded smoothly to yield the expected hepta-*O*-acetyl-1-*S*-acetyl-1-thio-α-maltose derivative **12** which was converted into sodium α-maltose thiolate **13** by the conventional sodium methoxide procedure. Nucleophilic displacement at C-6 in either 6-*O*-*p*-tolylsulfonylcyclomaltoheptaose **7** or heptakis(6-deoxy-6-iodo)cyclomaltoheptaose **8** with the sodium salt of the corresponding anomer of 1-thioglycose was achieved in 1,3-dimethyl-2-oxohexahydropyrimidine (DMPU) at 50–60 °C for 3–7 h for the monothioglycosylated derivatives **3** and **4** while 17 h reaction time were needed for the per(thioglucosylated) derivatives **5–6** in order to get an optimal yield (Scheme 2). The higher yield (80–87%) of the perglycosylated derivatives **5–6** compared with the corresponding monofunctionalization **1–4** may be ascribed to the fact that the relatively bulky molecules **5–6** allowed the use of a tangential ultrafiltration process for the demineralization step of the reaction products, when ion exchange techniques and liquid chromatography had to be used for compounds **1–4**.[7]



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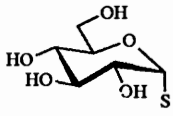
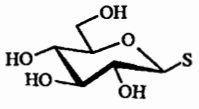
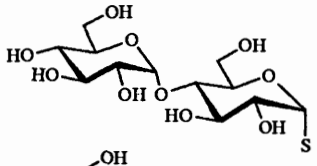
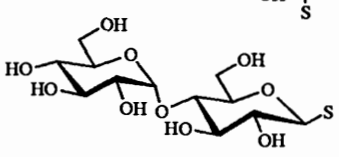
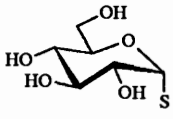
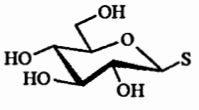
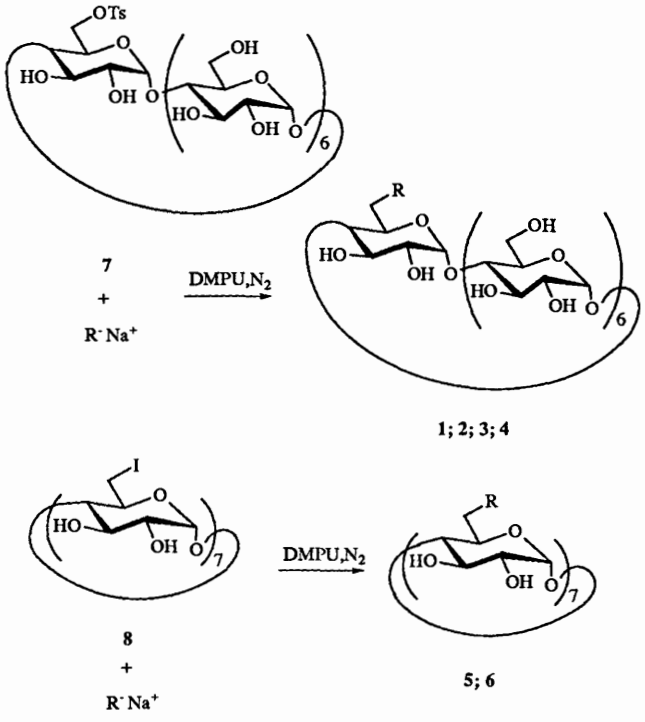
1		OH
2		OH
3		OH
4		OH
5		R
6		R

Scheme 1



Scheme 2

The chemical structures of compounds **3–6** was confirmed by elemental analysis and NMR spectra and mass spectrometry (see Experimental section).

Solubilities in water of the branched cyclodextrins. The solubility in water at 25 °C has been determined for all six compounds depicted in Scheme 1. The results are displayed in Table 1 and have to be compared with solubility data of β-cyclodextrin under the same experimental conditions.

From the data presented, three major conclusions can be drawn. Branched derivatives with α-anomeric configuration exhibit a much greater solubility in water than the parent β-cyclodextrin. Branched derivatives with β-anomeric configuration exhibit a much lower solubility in water compared with corresponding α-anomers. In most cases, the gain achieved being within experimental error. Considering the gain in solubility only, persubstituted derivatives appear to be more favourable than the monosubstituted analogues, especially if α anomers are considered. The comparison of **1** and **5** is quite obvious in this respect.

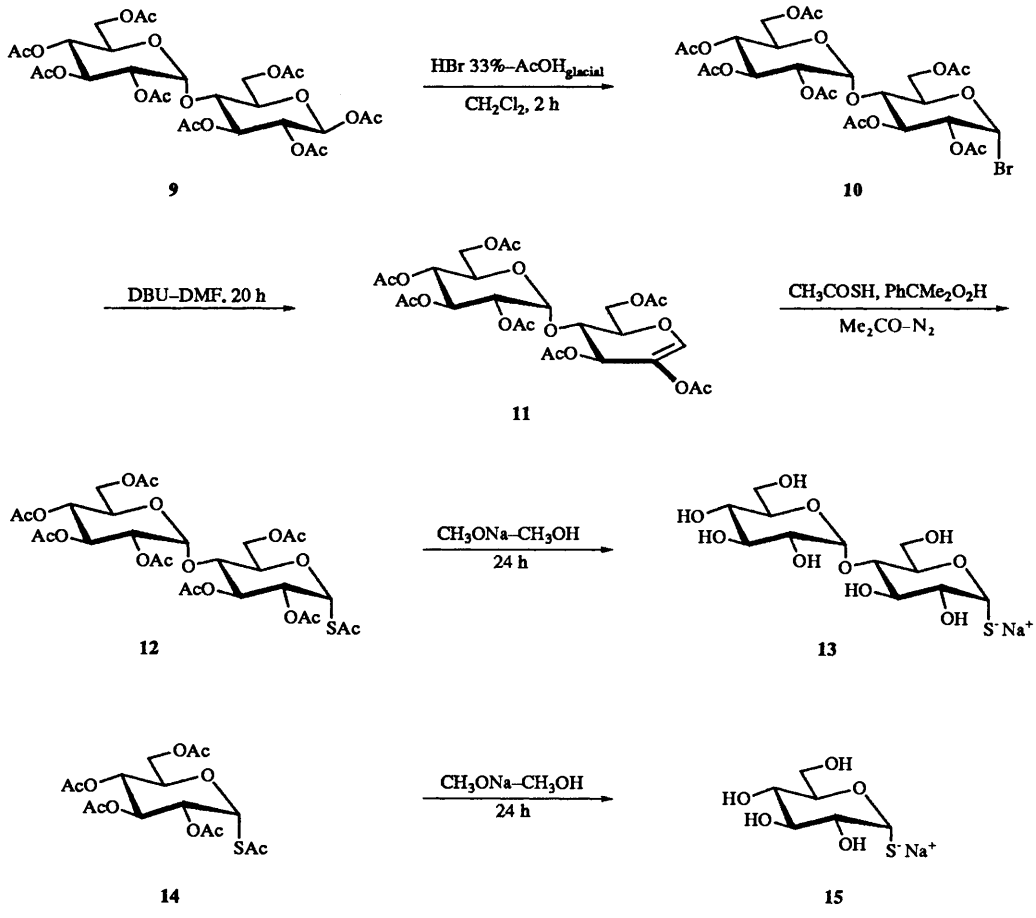
Solubilization power of branched 6-*S*-glycosyl-6-thiocyclomaltoheptaose derivatives for hydrophobic drugs. In order to estimate how the gain in solubility of the host can be reflected in the final inclusion complexes derived from hydrophobic drugs, a normalized solubility assay was performed using the hydrophobic anti-inflammatory steroid prednisolone. The solubilization of this drug is indeed well documented in this respect. It forms a 1:1 inclusion complex with β-and γ-CDs although the two derived complexes differ strongly in terms of geometry.[13]

The solubilization of prednisolone by the branched cyclodextrins was determined as described in the Experimental section and the results are reported in Table 1.

The following conclusions can be drawn from a detailed analysis of experimental data. The weaker intrinsic solubility of β-anomers is reflected in the low solubilization power of these derivatives towards prednisolone. This observation implies that β-anomers are not good candidates for the design of hosts of improved solubility and solubilization power. No clear explanation can be proposed at the present time to explain the considerable difference between the α- and β-anomers, however this observation is quite general. When comparing the effects of the number of units of the grafted moiety (with identical anomers), it appears that the maltosyl derivative **3** exhibits superior performances when compared with the glucosyl analogue **1**. The solubilization of a guest like prednisolone seems to follow the solubility of the host. This important point will be further discussed in another section of this report. Conversely, the solubility achieved with the persubstituted derivative **5** is far weaker than expected from the solubility of the host alone. The solubilization power of **5** for prednisolone is indeed weaker than the monosubstituted derivative **1** although their solubilities as free hosts follow a reverse order. This finding is of importance in the design of improved cyclodextrins and particularly in the selection of mono- *versus* per-substituted derivatives. The observed results will be tentatively explained in the following section in the light of a detailed NMR analysis.

NMR investigations of the complexation properties of persubstituted derivative 5. For the sake of clarity, definitions will be given here and retained throughout the next sections. In terms of cyclodextrin inclusion complexes, a very important parameter is the side from which the inclusion process takes place. Although it is generally admitted that the inclusion proceeds from the wider secondary alcohol groups side, a number of facts call for less definitive conclusions. The present work will give further evidence in this respect.

Fig. 1 represents the two possible general structures for inclusion of a guest molecule in a given cyclodextrin cavity. In type I complexes, inclusion of the guest molecule proceeds



9 10 11 12 13 14 15

Scheme 3

Table 1

Derivative	Solubility at 25 °C/ mmol dm^{-3}	Prednisolone solubility at 25 °C/ mmol dm^{-3}
β-Cyclodextrin	15	11
5	725 (×48)	181
6	35 (×2.3)	8.5
1	327 (×22)	164
2	23 (×1.5)	11.5
3	549 (×37)	275
4	23 (×1.5)	11.5

through the narrower primary hydroxy groups' side of the cyclodextrin, the most polar part being left outside in the vicinity of primary hydroxy groups. In type II complexes, a more 'classical' situation is encountered, the introduction of the guest taking place from the wider secondary hydroxy groups' side.

Type I complexes with **5**.—*p*-Nitrophenol (pNp) is a well suited model for this purpose. It is well documented that it results in a Type I complex with β-cyclodextrin.[13] In order to check if this situation was retained in **5**, NMR experiments were performed. The result of the 'interaction' of pNp with **5** and with β-CD under the same conditions is shown in Fig. 2.

It is observed here that although pNp induces large shifts in resonance of the cavity protons (3-H and 5-H) of β-CD [Figs. 2(*a*) and (*b*)], almost no detectable shifts are experienced with **5** under the same conditions [Figs. 2(*c*) and (*d*)]. The shift variations are one order of magnitude weaker in the latter. This indicates that **5** cannot form a strong inclusion complex with this potential guest. Since it has been argued that pNp forms Type I complexes with β-CD, the absence of complexation by **5** is clearly related to the presence of a complete rim of bulky *S*-glucosyl substituents. The explanation for the lack of complexation of pNp with **5** is hence related only to steric hindrance effects.

Type II complexes with **5**.—As already indicated, **5** increases the solubility of prednisolone compared with β-CD although to a lesser extent than expected from the intrinsic solubility of this host molecule. The solubilization is the first clue for the formation of an inclusion complex. It is well documented that prednisolone forms a 1:1 inclusion complex (Type II) with β-CD, the apparent association constant being 2000 dm^3 mol^{-1} at 25 °C.[14] The same detailed NMR study has been performed using **5** as host. Comparison of the ^{1}H NMR spectra of **5** in the absence and in the presence of prednisolone [Figs. 3(*a*) and (*b*)] indicates the formation of an inlusion complex.

The seven-fold symmetry of **5** results in a readily assigned spectrum. Upon addition of prednisolone, shifts of protons 3-H and 5-H are observed confirming the formation of an inclusion complex. More detailed indications concerning the geometry of this inclusion complex can be derived from the evidence of spatial proximities between protons of the host and of the guest. This can be achieved by investigations of dipolar interactions using 2D ROESY experiments.[15] The corresponding contour plot is displayed in Fig. 4. Dipolar contacts (indicating spatial proximities) are observed between protons 1-H, 2-H, 4-H (ring

A) and 11-H (ring B) of prednisolone and protons 3-H, 5-H and 6-H of the host.

From this first set of results, it can be concluded that inclusion of the steroid molecule occurs from the wide secondary side (Type II complex) as in the parent cyclomaltoheptaose. However, since the geometrical similarities between prednisolone:β-CD and prednisolone:**5** do not explain the unexpected weak solubilization power of **5** for this drug, a more detailed analysis was performed using the continuous variation technique as described elsewhere.[16]

From the Job Plot analysis[17] (data not shown), the 1:1 stoichiometry was ascertained. A numerical simulation was used to derive the corresponding association constant and the same process was applied to the prednisolone:βCD complex.[18] Surprisingly, it was observed that the association constant K drops from 2000 dm^3 mol^{-1} for β-CD to 200 dm^3 mol^{-1} for **5**.

The key to the understanding of this important decrease can be found by considering that hydrogen bonds between the carbonyl group (position C-3) and primary hydroxy protons of β-CD play an important role in the stabilization of the complex. When β-CD is replaced by **5**, even if all geometrical parameters are retained, these interactions cannot be established anymore leading to a significant decrease in the association constant K. The presence of the stabilizing hydrogen bonds in the β-CD–prednisolone complex is obvious from the results of molecular modelling under NMR constraints.[18] Under these circumstances, the effective solubilization power of **5** for prednisolone is much weaker than expected since it is balanced between the positive effects of the solubility of the host and the weaker association constant.

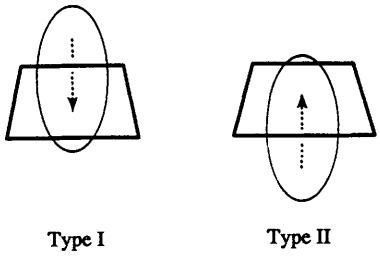


Fig. 1 General model for complexes of Type I and II in cyclodextrins. In this figure, the narrow side corresponds to the rim bearing primary hydroxy groups.

As a conclusion to this section, it appears that although they exhibit an extremely large solubility in water, per-(*S*-α-thioglycosylated) derivatives of cyclomaltoheptaose do not afford the expected performances for inclusion complexes of Type I and II. In view of the latter observation, a more appropriate behaviour is expected from the monosubstituted analogues.

Inclusion properties of mono-*S*-glycosylcyclomaltooligosaccharides 1 and 3. A similar strategy as presented for **5** was used for this purpose. Inclusion complexes of Type I and II were considered successively.

Type I complexes with **3**.—Type I complexes were evaluated using the anticoagulant molecule 2-phenylindane-1,3-dione as guest. This compound is indeed very sparingly soluble in water and its solubility can be improved by inclusion in cyclodextrins. It appears that β-cyclodextrin and compound **3** exhibit almost the same solubilization power towards 2-phenylindane-1,3-dione since the solubility of the latter is 1.5 and 1.8 mmol dm^{-3} in the presence of 10 mmol dm^{-3} solutions of β-cyclodextrin and **3**, respectively. NMR analysis was used to explain these results.

Fig. 5 shows a partial ^1H NMR spectrum of **3** in the absence and in the presence of 2-phenylindane-1,3-dione. As **3** lacks symmetry owing to the monosubstitution, the corresponding NMR spectrum appears quite complex and this does not allow us to measure the shifts of protons directly.[19] Dedicated 2D experiments were then required in order to investigate the structure of the inclusion complex. One important point is the good separation of most anomeric protons in the 5.10–5.55 ppm range. The complete assignment of this spectrum requires the use of dedicated 2D experiments which have been described in detail elsewhere.[20] The main purpose of the assignment task is to identify the positions of signals from protons 3-H and 5-H, these being the most prone to experience shifts upon the formation of an inclusion complex. Fig. 6 displays partial contour plots obtained by three steps relay experiments from anomeric protons allowing a stepwise identification along the coupled nuclei of each glucose unit. It is quite clearly observed that 3-H and 5-H experience large shifts upon inclusion of the 2-phenylindane-1,3-dione guest. Note that 5-H and 6-H show larger shifts than the proton 3-H. Moreover, a ROESY experiment performed (Fig. 7) on the inclusion complex, has clearly indicated strong interactions between protons of the

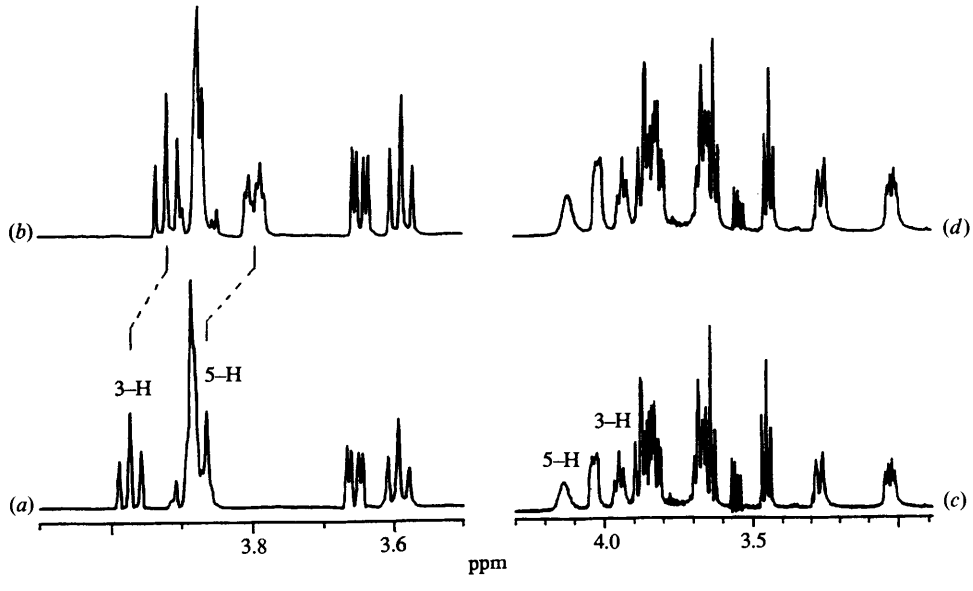


Fig. 2 Partial ^1H NMR spectra (600 MHz, 298 K) of β-cyclodextrin, 5 mmol dm^{-3} in D_2O in (*a*) the absence and in (*b*) the presence of 5 mmol dm^{-3} *p*-nitrophenol, and of compound **5**, 5 mmol dm^{-3} in D_2O in (*c*) the absence and in (*d*) the presence of 5 mmol dm^{-3} *p*-nitrophenol

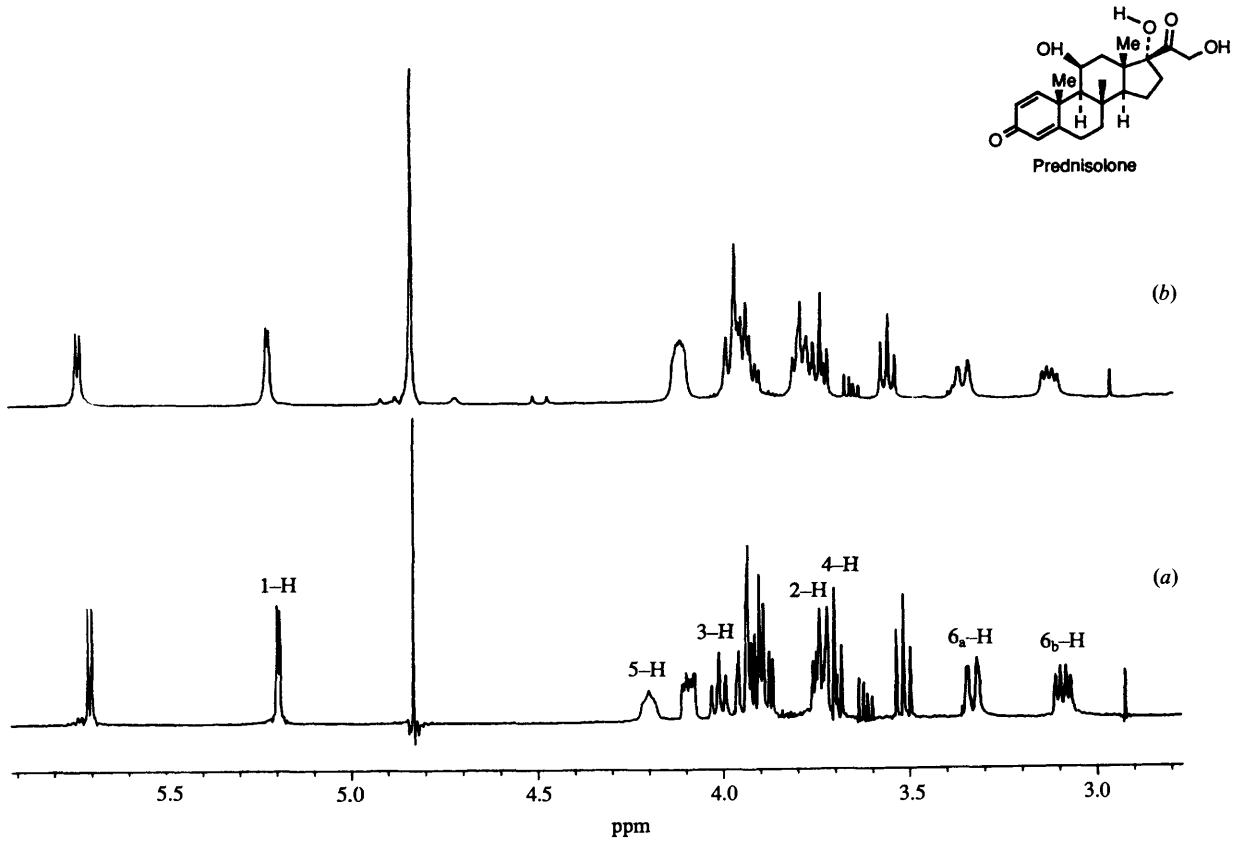


Fig. 3 Partial ^{1}H NMR spectra (500 MHz, 298 K) of compound **5**, 10 mmol dm^{-3} in D_2O in (*a*) the absence and in (*b*) the presence of 5 mmol dm^{-3} prednisolone

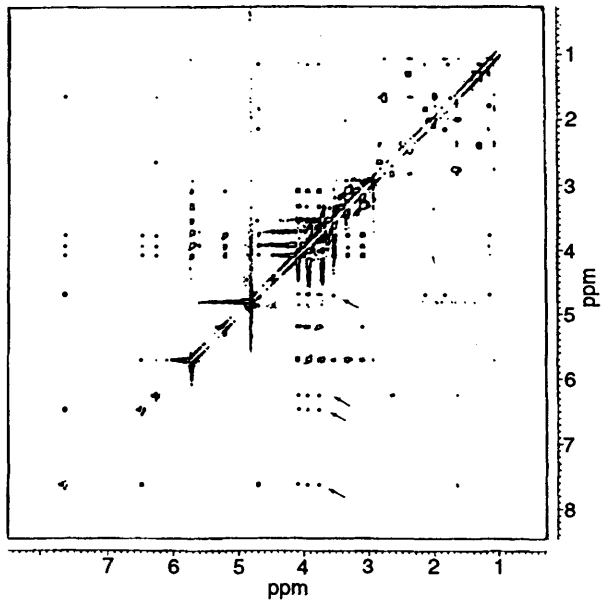


Fig. 4 ROESY experiment (500 MHz, 298 K, D_2O, 500 ms spin-lock time at 24 dB attenuation) performed on a mixture of **5** (10 mmol dm^{-3}) and prednisolone (5 mol dm^{-3}). Dipolar interactions between protons 3-H, 5-H and 6-H of **5** and protons 1-H, 2-H, 4-H and 11-H of prednisolone are indicated by arrows

aromatic part of the guest and protons 3-H, 5-H and 6-H of the β-cyclodextrin derivative.

All these results have confirmed that inclusion of 2-phenylindane-1,3-dione occurred from the primary side (Type I complex) as in the β-cyclodextrin. It was observed that the association constant drops from 1500 dm^3 mol^{-1} for β-cyclodextrin to 700 dm^3 mol^{-1} for **3**. The lower value of *K* for the complex 2-phenylindane-1,3-dione : **3** can be explained by steric hindrance effects. However the capacity of compound **3** to form a Type I complex is retained even if the association constant is weaker than that obtained with β-CD. This drawback is largely balanced by the higher solubility of derivative **3**.

Type II complexes with **1** *and* **3**.—A complete NMR study has been performed on the Type II complex obtained with compound **1** and dothiepine; this was described elsewhere.[21] It was found that no significant variation in the association constant (K = 1500 dm^3 mol^{-1}) was induced by the grafting of the sugar unit on the primary side. Moreover, a precise geometric model was derived from NMR data unambiguously showing the reality of the Type II complex.

The properties of **3** were also investigated for Type II complexes. In order to allow a direct comparison with the persubstituted derivatives, prednisolone was used as a model guest. As indicated in Table 1, compound **3** has the highest solubilization power for this steroid and the comparison of **1** and **3** suggests that the differences are directly related to the intrinsic solubility of the host molecule. Fig. 8(*a*) shows NMR spectra obtained at various host–guest ratios. Shifts of several protons are clearly visible but, owing to the complexity of the NMR spectrum, a clear observation of the shifts experienced by 3-H and 5-H protons (which are the most prone to be affected by the inclusion process) is impossible owing to spectral overcrowding in the 3.5–4.1 ppm region. These important features can, however, be observed using dedicated experiments based upon stepwise magnetization transfers from anomeric protons.[22] Fig. 8(*b*) displays the results from one-step relay transfer from anomeric protons for the same samples as depicted in Fig. 8(*a*). In this example, the region of anomeric protons shown in Fig. 8(*a*) by a solid line was excited and magnetization was further transferred to 2-H and then to 3-H

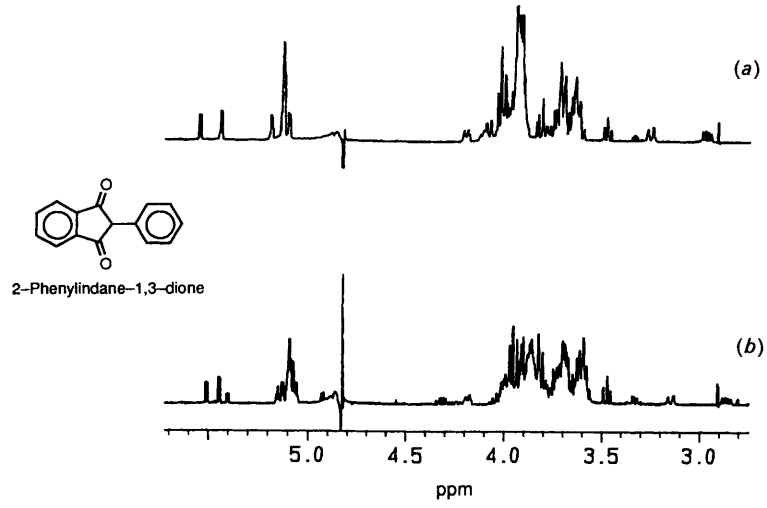


Fig. 5 Partial ^{1}H NMR spectra (500 MHz, 298 K, D_2O) of (*a*) compound **3** (5 mmol dm^{-3}) and in (*b*) a mixture of **3** (2.5 mmol dm^{-3}) and 2-phenylindane-1,3-dione (2.5 mmol dm^{-3})

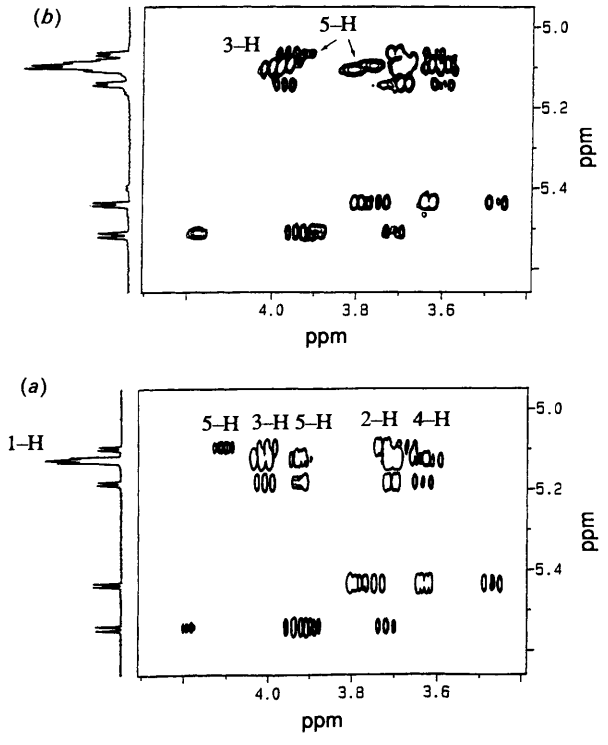


Fig. 6 Three steps RELAY experiment (500 MHz, 298 K, D_2O) of (*a*) compound **3** (5 mmol dm^{-3}) and in (*b*) a mixture of **3** (3 mmol dm^{-3}) and 2-phenylindane-1,3-dione (2 mmol dm^{-3})

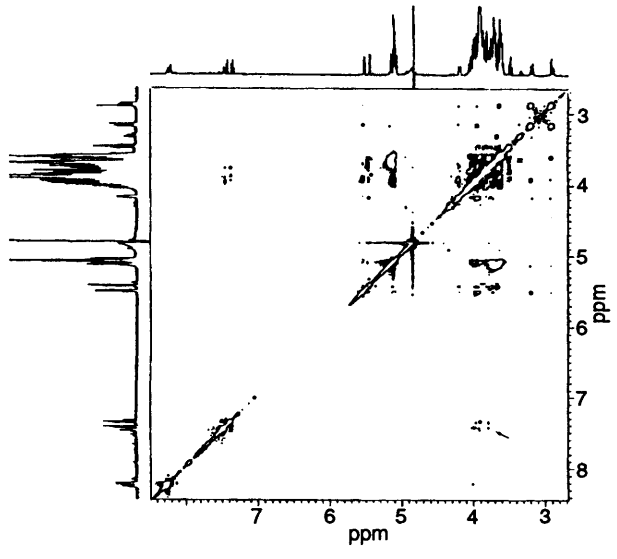


Fig. 7 ROESY experiment (500 MHz, 298 K, D_2O, 300 ms spin-lock time at 22 dB attenuation) performed on a sample containing 3 mmol dm^{-3} of **3** and 2 mmol dm^{-3} of 2-phenylindane-1,3-dione

protons. In the present case, owing to optimization of transfer parameters, no residual signals from 2-H protons are observed and only 3-H signals are observed. It is clear from the present data that 3-H protons experience large shifts upon addition of the steroid as a result of the formation of an inclusion complex.

The association constant of prednisolone with **3** was derived from the variation of the chemical shifts of selected signals from the host upon addition of variable concentrations of the host.[16] Signals exhibiting the largest variations were selected [they are indicated by arrows on Fig. 8(*a*)]. The average value for K was found to be 2200 dm^3 mol^{-1} in the present case. This value is virtually identical (within experimental uncertainties) to that found for β-CD under identical conditions (2000 dm^3 mol^{-1}). This fully supports that, in the case of Type II complexes, monosubstitution leaves the binding properties of the CD derivative unaffected relative to the original cyclodextrin.

The comparison of data derived from the formation of Type I and II complexes with monosubstituted cyclodextrin leads to the following conclusions. In the case of Type I complexes, steric hindrance effects generally result in a decrease of the association constant although complexes are obviously present. These reductions in affinity are, however, compensated by the larger solubility of the host resulting in an increase in the solubilization power for sparingly soluble guests. In the case of Type II complexes, no difference in the affinity is observed upon grafting one thioglycose moiety on the primary side of the cyclodextrin. In this case, the effect expected from the solubility properties alone is fully effective and the final solubility of complexes (expressed as solubilization power) is proportional to the intrinsic solubility of the pure host. Owing to the gain in solubility achieved for derivatives such as **3** relative to the parent CD, the final solubilization power obtained with such derivatives can be extremely high (up to a 25-fold increase).

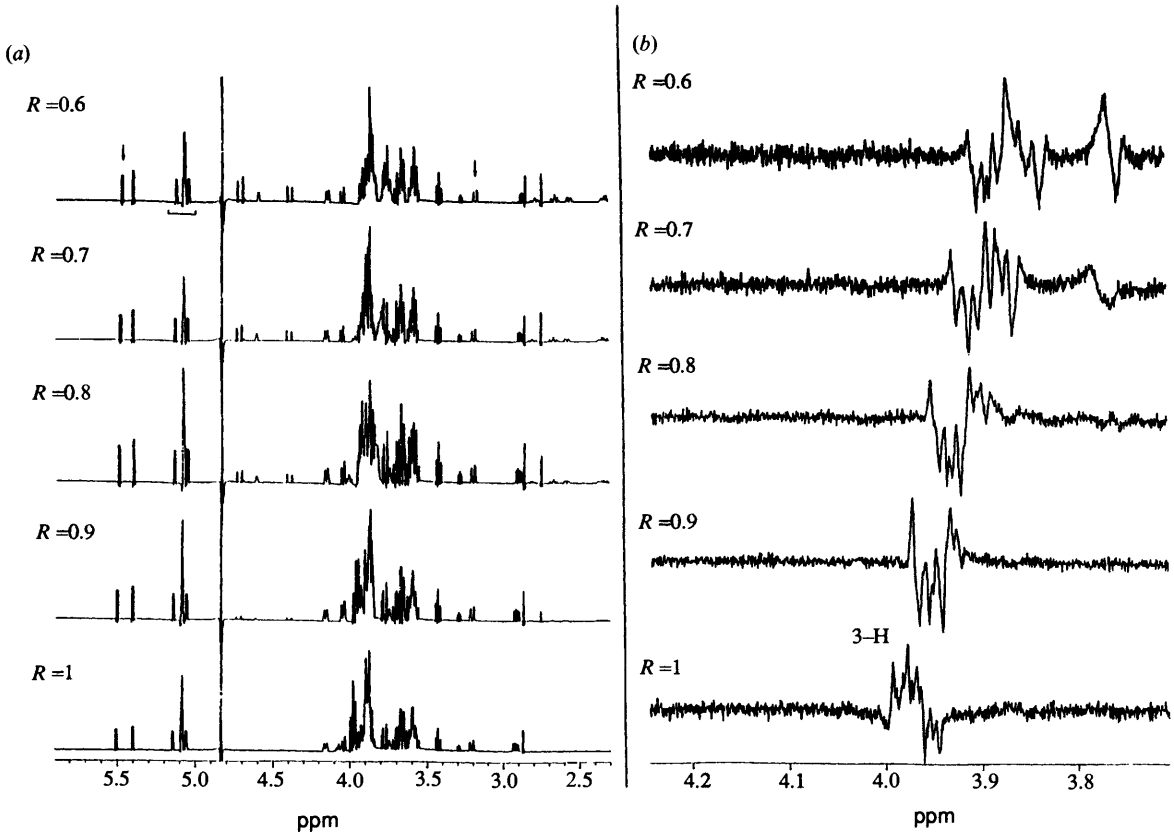


Fig. 8 Influence of the prednisolone:**3** molar ratio on (*a*) the partial 500 MHz proton spectra (298 K) of **3** or on (*b*) the one step relay transfer from anomeric protons (*b*); the total concentration of species is 10 mmol dm^{-3} and the molar ratios are indicated as [**3**]/[**3** + prednisolone]

Experimental

Materials and methods

TLC was performed on Silicagel 60 plates (E. Merck) followed by charring with 10% H_2SO_4. Separation experiments were carried out by flash or open column chromatography on Silicagel 60, 230–400 mesh (E. Merck) using the eluent selected by TLC. LC of the unacetylated derivatives was performed on a Perkin-Elmer 250 pump fitted to a Nucleosil C_{18} (250 × 6.2 mm, 5μ) column and an LC-30 refractive index detector with 88:12 water:methanol as eluent and a flow rate of 2.5 cm^3 min^{-1}. Preparative LC was carried out with a PREP LC/500 chromatograph (Waters Associates), equipped with a refractometric detector and a Prep Pak 500/C_{18}-bonded silica column, by elution with 88:12 water:methanol at 100 cm^3 min^{-1}. Tangential flow–filtration was carried out with a Minitan-S system (Millipore Co.) equipped with a cellulose acetate membrane PCAC 1 K. Melting points were determined with a Büchi 535 capillary device and are corrected. Optical rotations were measured using a Jobin–Yvon Micropolarimeter. FAB mass spectra (Cs gun, acceleration potential 8 kV) were measured in the positive mode with a VG ZAB-SEQ instrument using a 1:1 glycerol–thioglycerol matrix to which a drop of a 0.1 mol dm^{-3} solution of NaI or KI was added. Elemental analysis for cyclomaltoheptaose derivatives were obtained from samples previously dried at 140 °C/1.33 Pa for 48 h in the presence of P_2O_5. ^1H NMR experiments were performed using Bruker AM400, AMX500 and AMX600 spectrometers operating at 400, 500 and 600 MHz, respectively. ^{13}C NMR spectra were recorded with a Bruker AM400 spectrometer operating at 100.6 MHz. 1D NMR spectra were collected using 16 K data points. All 2D experiments were acquired using 2 K data points and 256 time increments. Scalar correlations (COSY, Relay and Double-Quantum correlation experiments) were processed in the absolute value mode after-zero filling resulting in a 1 K × 1 K (real points) data matrix. For dipolar correlations (ROESY experiments) the phase sensitive (TTPI) sequence was used and processing resulted in 1 K × 1 K (real–real) matrix. The probe temperature was carefully controlled to within 0.1 °C by means of a Haake exchange device. Chemical shifts are given in ppm downfield from external tetramethylsilane (TMS). D_2O and [$^2H_6$]DMSO were obtained from Euriso-Top (France). Details concerning experimental conditions are given in the figure captions.

Preparation of inclusion complex samples

The potential for cyclodextrins to improve the solubility in water of prednisolone was estimated using a preliminary assay. To a saturated aqueous solution of the pertinent β-cyclodextrin derivatives at 25 °C, a 10 mmol dm^{-3} solution of prednisolone in acetone was added dropwise. The mixture was stirred and acetone was allowed to evaporate slowly under a stream of nitrogen. Additions were repeated up to saturation characterized by the formation of a turbid solution.

2,2′,3,3′,4,6,6′-Hepta-*O*-acetyl-α-maltosyl bromide 10. To a solution of 1,2,2′,3,3′,4′,6,6′-octa-*O*-acetyl-β-maltose[23] **9** (15 g, 22.1 mmol) in CH_2Cl_2 (57 cm^3), 33% HBr in glacial AcOH (33 cm^3, 185.5 mmol) was added dropwise at 0 °C. The resulting mixture was left at room temperature for 2 h and poured into ice-cold water (200 cm^3). The aqueous layer was extracted with CH_2Cl_2. The organic layer was further washed with water and saturated $NaHCO_3$, dried (Na_2SO_4) and concentrated under reduced pressure. The crude bromide **10** was recrystallized from ether–light petroleum (14.73 g, 88%), mp 114.5–115.5 °C (lit.,[24] 112–113 °C); $[\alpha]_D^{20}$ +180 (*c* 1, $CHCl_3$), [lit.,[24] +180.1 ($CHCl_3$)].

2,2′,3,3′,4′,6,6′-Hepta-*O*-acetyl-2-hydroxymaltal 11. To an ice-cold solution of **10** (9.00 g, 12.9 mmol) in dry DMF (33 cm^3),

1,5-diazabicyclo[5.4.0]undec-5-ene (1.95 cm^3) was added dropwise. The solution was set aside for 20 h at room temperature and slowly poured into ice–water (250 cm^3). The solid formed was filtered, dried under vacuum and recrystallized from EtOH–H_2O yielding **11** (6.04 g, 78%), mp 121.9–122.9 °C; $[\alpha]_D^{20}$ +68 (*c* 1.3, $CHCl_3$); FABMS: *m/z* 641.4 (48, $[M + Na]^+$), 559.3 (80, $[M - OAc]^+$) and 331.2 (41, $[M - C_{12}H_{15}O_8]^+$) (Found: C, 50.60; H, 5.28. Calc. for $C_{26}H_{34}O_{17}$: C, 50.49; H, 5.50%); δ_H(400 MHz; CD_3COCD_3) 6.72 (1-H, d, $J_{1,3}$ 0.5), 5.43 (1′-H, d, $J_{1',2'}$ 4.0), 5.41 (3′-H, ddd, $J_{3',4'}$ 9.5, $J_{3',5'}$ 0.5), 5.35 (3-H, ddd, $J_{3,4}$ 3.0, $J_{3,5}$ 1.5), 5.03 (4′-H, dd, $J_{4',5'}$ 10.0), 4.85 (2′-H, dd, $J_{2',3'}$ 10.5), 4.61 (5-H, t, $J_{5,6a}$ 3.5, $J_{5,6b}$ 7.5), 4.46 (6b-H, dd), 4.29 (6a-H, dd, $J_{6a,6b}$ 12.0), 4.25 (4-H, dd, $J_{4,5}$ 3.5) and 4.16 (5′-H, 6′a-H, 6′b-H); δ_C(100 MHz; CD_3COCD_3) 170 (C=O), 139.8 (C-1), 128.1 (C-2), 96.8 (C-1′), 75.6 (C-5), 74.1 (C-4), 71.1 (C-2′), 70.5 (C-3′), 69.5 (C-4′), 69.3 (C-5′), 67.1 (C-3), 62.8 (C-6′), 61.8 (C-6) and 20.5 (CH_3).

2,2′,3,3′,4′,6,6′-Hepta-*O*-acetyl-1-*S*-acetyl-1-thio-α-maltose 12. The glycal **11** (2.0 g, 3.2 mmol) and thioacetic acid (12 cm^3, 168.6 mmol) were dissolved in acetone (40 cm^3) under N_2 and 2-phenylprop-2-yl hydroperoxide (8.6 cm^3, 54 mmol) was added dropwise at 0 °C. The resulting solution was kept at room temperature for 40 h under N_2. After concentration of acetone at 20 °C, the crude residual material was purified on a silica gel column eluted at first with hexane. Further elution with EtOAc–hexane (1:1) gave **12** (1.28 g, 57%) after recrystallization from EtOH, mp 134.7–136.1 °C (lit.,[25] 134–136 °C); $[\alpha]_D^{20}$ +139 (*c* 0.50, $CHCl_3$) [lit.,[25] +143 (*c* 0.46, $CHCl_3$)]; FABMS: *m/z* 717.3 (19.5, $[M + Na]^+$), 695 (4, $[M + H]^+$), 619.3 (16, $[M - SAc]^+$) and 331.1 (51, $[M - C_{12}H_{15}O_8]^+$) (Found: C, 48.55; H, 5.44; S, 4.61. Calc. for $C_{28}H_{38}O_{18}S$: C, 48.41; H, 5.51; S, 4.61%); δ_H(400 MHz; CD_3COCD_3) 6.12 (1-H, d, $J_{1,2}$ 5.3), 5.38 (3′-H, dd, $J_{3',4'}$ 9.6), 5.37 (1′-H, d, $J_{1',2'}$ 4.0), 5.23 (3-H, dd, $J_{3,4}$ 7.5), 5.10 (2-H, dd, $J_{2,3}$ 9.6), 5.07 (4′-H, t, $J_{4',5'}$ 9.6), 4.89 (2′-H, dd, $J_{2',3'}$ 10.6), 4.47 (6b-H, dd), 4.35 (5′-H, ddd, $J_{5',6'a}$ 2.4, $J_{5',6'b}$ 4.0), 4.28 (6a-H, dd, $J_{6a,6b}$ 12.4), 4.23 (6′b-H, dd), 4.09 (4-H, dd, $J_{4,5}$ 9.2), 4.09 (6′a-H, dd, $J_{6'a,6'b}$ 11.9) and 4.03 (5-H, ddd, $J_{5,6a}$ 4.6, $J_{5,6b}$ 2.5); δ_C(100 MHz; CD_3COCD_3) 168.8–170.1 (*C*=O, 8s), 96.3 (C-1′), 79.8 (C-1), 73.7 (C-4), 72.3 (C-3), 72.3 (C-5), 70.1 (C-2′), 69.5 (C-2), 69.2 (C-3′), 68.7 (C-4′), 68.2 (C-5′), 62.8 (C-6), 61.6 (C-6′), 30.5 (*C*H_3–S) and 20.1–19.6 (*C*H_3–O).

1-Thio-α-maltose sodium salt 13. To a suspension of **12** (1.57 g, 2.26 mmol) in MeOH (22 cm^3) under N_2 was added methanolic 1 mol dm^{-3} MeONa (3.4 cm^3, 3.4 mmol). The solution was kept at room temperature for 24 h, then the solvent was removed under reduced pressure and the resulting amorphous powder was dried (vacuum dessicator, P_2O_5). It was used without further purification.

1-Thio-α-D-glucopyranose sodium salt 15. To a suspension of 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-α-D-glucopyranose[12] **14** (1.5 g, 3.7 mmol) in MeOH (31 cm^3) under N_2, was added methanolic 1 mol dm^{-3} MeONa (4.5 cm^3, 4.5 mmol). The solution was kept at room temperature for 12 h, then the solvent was removed under reduced pressure and the resulting amorphous powder was dried (vacuum dessicator, P_2O_5). It was used without further purification.

6[1]-*S*-α-Maltosyl-6-thiocyclomaltoheptaose 3 and 6[1]-*S*-β-maltosyl-6-thiocyclomaltoheptaose 4: general procedure. To a solution of 6-*O*-*p*-tolylsulfonylcyclomaltoheptaose **7**[7,8] (1 mmol) in 1,3-dimethyl-2-oxohexahydropyrimidine (DMPU) (5 cm^3) either anomer of the 1-thiomaltose sodium salt (1.5 mmol) was added and the reaction mixture was stirred for 18 h at 50 °C under N_2. After cooling to room temperature, addition of acetone resulted in the formation of a solid which was filtered out, washed with acetone and redissolved in water (15 cm^3). The aqueous solution was desalted by passing it through a column of amberlite MB-6113 (H^+, OH^-) ion exchange resin (10 cm^3), then freeze-dried to give a solid which contained almost pure **3** or **4** (LC). Final purification was achieved by preparative LC to afford either **3** or **4** as an amorphous powder after freeze-drying.

3.—1.43 g (65%) from 6-*O*-*p*-tolylsulfonylcyclomaltoheptaose **7**[7,8] (1.94 g, 1.5 mmol) and 1-thio-α-maltose sodium salt **13** (0.85 g, 2.26 mmol); mp 283 °C (decomp.); $[\alpha]_D^{20}$ +170 (*c*, 1, H_2O); FABMS: *m/z* 1497 (100, $[M + Na]^+$), 1475 (20, $[M + H]^+$), 1139 (27, $[M - C_{12}H_{21}O_{10}S + Na]^+$) and 1173 (25, $[M - C_{12}H_{21}O_{10} + 2H + Na]^+$) (Found: C, 40.44; H, 6.79; S, 1.95. Calc. for $C_{54}H_{90}O_{44}S \cdot 7H_2O$: C, 40.50; H, 6.55; S, 2.00%). δ_H(600 MHz; D_2O) 5.54 (1′-H, d, $J_{1',2'}$ 5.10), 5.44 (1″-H, d, $J_{1'',2''}$ 3.90), 5.18 (1-G-H), d, $J_{1,2}$ 3.80), 5.13 (1B,C,D,E,F-H, d, $J_{1,2}$ 3.80), 5.10 (1A-H, d, $J_{1,2}$ 3.80), 4.19 (5′-H, dt), 4.1 (5A-H, dt), 4.08 (6_bG-H, dd), 4.02 (3B,C,D,E,F-H, t), 4.01 (3G-H, t), 4.00 (3A-H, t), 3.93 (3′-H and 6″-H, m), 3.92 (5G-H, 6′-H and 5B,C,D,E,F-H, m), 3.95–3.90 ($6_{a,b}$ B,C,D,E,F-H, m), 3.88 (2′-H, dd), 3.80 (5″-H, dt), 3.75 (3″-H, t), 3.72 (2B,C,D,E,F-H, m), 3.73 (2A-H, dd), 3.72 (2G-H, dd), 3.71 (4′-H, t), 3.67 4A-H, t), 3.63 (4G-H and 2″-H, m), 3.64–3.61 (4B,C,D,E,F-H), 3.48 (4″-H, t), 3.25 (6_bA-H, dd) and 2.98 (6_aA-H, dd).

4.—0.068 g (68%) from 6-*O*-*p*-tolylsulfonylcyclomaltoheptaose **7**[7,8] (0.090 g, 0.07 mmol) and 1-thio-β-maltose sodium salt[11] (0.040 g, 0.11 mmol); mp 285 °C (decomp.); $[\alpha]_D^{20}$ +84 (*c* 1, H_2O); FABMS: *m/z* 1497 (80, $[M + Na]^+$), 1475 (100, $[M + H]^+$) (Found: C, 38.78; H, 6.46; S, 1.71. Calc. for $C_{54}H_{90}O_{44}S \cdot 11H_2O$: C, 38.75; H, 6.74; S, 1.91%); δ_H(500 MHz; D_2O) 5.45 (1″-H, d, $J_{1'',2''}$ 3.90), 5.18 (1G-H, d, J_{1-2} 3.90), 5.12 (1B,C,D,E,F-H, d, $J_{1,2}$ 3.80), 5.09 (1A-H, d, $J_{1,2}$ 3.60), 4.68 (1′-H, d, $J_{1',2'}$ 9.90), 4.12 (5A-H, m), 4.01 (3A,B,C,D,E,F,G-H, t), 4.03–3.88 (5B,C,D,E,F,G-H, 6B,C,D,E,F,G-H, 6′$_b$-H, 6″$_b$-H, m), 3.81 (3′-H, 6′$_a$-H, 6″$_a$-H, m), 3.75 (5″-H, m), 3.72 (3″-H, t), 3.71 (4′-H, 2A-H, m), 3.70 (2B,C,D,E,F,G-H, 4A-H, m), 3.68–3.60 (4B,C,D,E,F,G-H, 2″-H, m), 3.61 (5′-H, m), 3.46 (4″-H, t, $J_{3'',4''} = J_{4'',5''}$ 9.5), 3.42 (6_bA-H, m), 3.40 (2′-H, t, $J_{2',3'}$ 9.70) and 3.16 (6_aA-H, dd, $J_{6a,6b}$ 13.40, $J_{5,6a}$ 6.80).

Heptakis(6-*S*-α-D-glucopyranosyl-6-thio)cyclomaltoheptaose 5 and heptakis(6-*S*-β-D-glucopyranosyl-6-thio)cyclomaltoheptaose 6: general procedure. To a solution of heptakis(6-deoxy-6-iodo)cyclomaltoheptaose **8**[9] (1 mmol) in DMPU (15 cm^3), either anomer of the 1-thio-D-glucose sodium salt (1.5 mol equiv.) was added and the reaction mixture was stirred for 5 h (α-anomer) or 7 h (β-anomer) at 70 °C under N_2. Addition of acetone to the cooled solution resulted in a precipitate which was recovered by filtration, washed with acetone and dried. It was then dissolved in water (3 dm^3) and processed by tangential flow-filtration up to a volume of 25 cm^3. Freeze-drying led to analytically pure **5** or **6** as an amorphous powder.

5.—2.11 g (87%) from heptakis(6-deoxy-6-iodo)cyclomaltoheptaose **8**[9] (1.92 g, 1 mmol) and 1-thio-α-D-glucopyranose sodium salt **15** (2.31 g, 10.62 mmol), mp 266 °C (decomp.); $[\alpha]_D^{20}$ +205 (*c* 0.68, H_2O); FABMS: *m/z* 2403.2 (100, $[M + Na]^+$) (Found: C, 41.04; H, 5.99; S, 9.13. Calc. for $C_{84}H_{140}O_{63}S_7 \cdot 4H_2O$: C, 41.11; H, 6.04; S, 9.14%); δ_H(400 MHz, D_2O) 5.70 (1′-H, d, $J_{1',2'}$ 5.5), 5.20 (1-H, d, $J_{1,2}$ 3.5), 4.14 (5-H, ddd, $J_{5,6b}$ 6.0), 4.11 (5′-H, ddd, $J_{5',6'b}$ 5.0), 4.06 (3-H, dd, $J_{3,4}$ 9.0), 3.94 (2′-H, dd, $J_{2',3'}$ 9.5), 3.94 (6′$_a$-H, dd, $J_{5',6'a}$ 2.5), 3.90 (6′$_b$-H, dd, $J_{6'a,6'b}$ −13.0), 3.79 (4-H, t, $J_{4,5}$ 9.0), 3.73 (2 H, dd, $J_{2,3}$ 9.5), 3.70 (3′-H, t, $J_{3',4'}$ 9.5), 3.53 (4′-H, t, $J_{4',5'}$ 9.5), 3.38 (6$_a$-H, dd, $J_{5,6a}$ 2.5) and 3.13 (6$_b$-H, dd, $J_{6a,6b}$ −13.5); δ_C(62.9 MHz; D_2O) 103.3 (C-1), 87.8 (C-1′), 85.6 (C-4), 75.3 (C-3′), 74.3 (C-3), 74.1 (C-5′), 73.6 (C-2), 72.8 (C-5, C-2′), 71.4 (C-4′), 62.5 (C-6′) and 33.1 (C-6).

6.—1.92 g (80%) from heptakis(6-deoxy-6-iodo)cyclomaltoheptaose[9] (1.92 g, 1 mmol) and 1-thio-β-D-glucopyranose sodium salt **13**[26] (2.31 g, 10.62 mmol), mp 248 °C (decomp.); $[\alpha]_D^{20}$ +23 (*c* 0.70, H_2O); FABMS: *m/z* 2418.8 (88, $[M + K]^+$) (Found: C, 40.05; H, 6.21; S, 8.80. Calc. for

$C_{84}H_{140}O_{63}S_7 \cdot 8H_2O$: C, 39.94; H, 6.18; S, 8.87%); δ_H(400 MHz, D_2O) 5.24 (1-H, d, $J_{1,2}$ 3.5), 4.75 (1'-H, d, $J_{1',2'}$ 9.5), 4.18 (5-H, dd, $J_{5,6b}$ 5.5), 4.02 (3-H, t, $J_{3,4}$ 9.5), 4.00 ($6'_a$-H, dd, $J_{6'a,6'b}$ −12.5), 3.84 (4-H, t, $J_{4,5}$ 9.5), 3.82 ($6'_b$-H, dd, $J_{5',6b}$ 4.0), 3.77 (2-H, dd, $J_{2,3}$ 9.5), 3.61 (3'-H, t, $J_{3',4'}$ 9.0), 3.60 (5'-H, dd), 3.52 (4'-H, t, $J_{4',5'}$ 9.0), 3.48 (6_a-H, d), 3.46 (2'-H, dd, $J_{2',3'}$ 9.0) and 3.29 (6_b-H, dd, $J_{6a,6b}$ −14.0); δ_C(62.9 MHz; D_2O) 103.1 (C-1), 87.5 (C-1'), 84.6 (C-4), 81.2 (C-5'), 78.6 (C-3'), 74.1 (C-3, C-2'), 73.3 (C-2), 72.1 (C-5), 71.0 (C-4'), 62.5 (C-6') and 33.2 (C-6).

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