Thioureido-β-cyclodextrins as molecular carriers

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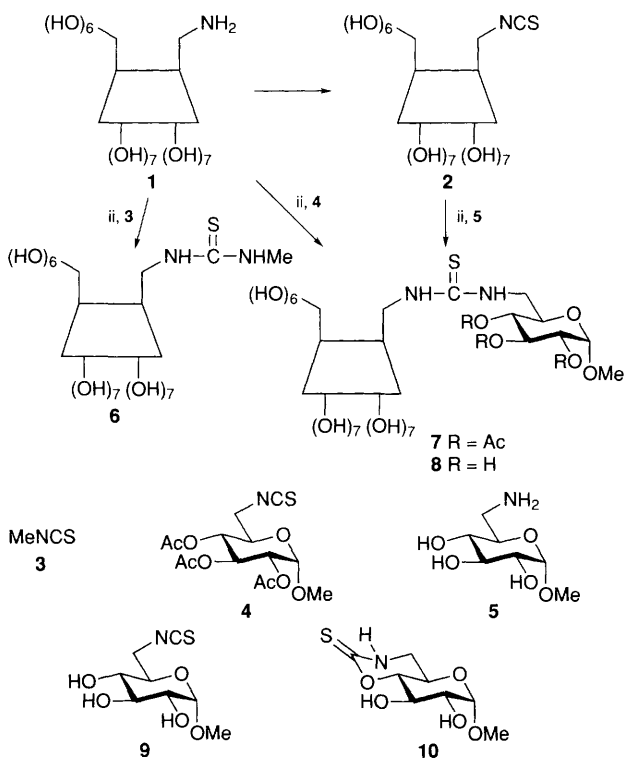
Alkyl, glycosyl and glycopeptidyl derivatives of 6^{I}-deoxy-6^{I}-thioureidocyclomaltoheptaose, prepared in high yield from either 6^{I}-amino-6^{I}-deoxycyclomaltoheptaose or the corresponding 6^{I}-isothiocyanate, exhibit considerably enhanced water solubilities as compared with the native β-cyclodextrin.

A variety of covalently modified cyclodextrins (CDs) has been synthesized over the last few years with the aim of improving their performance with regard of the bioavailability enhancement of drugs.[1] Some promising results towards the preparation of cyclomaltoheptaose (β-CD) derivatives with increased water solubility as well as the incorporation of biological markers for specific site delivery have been reported, involving the grafting of peptide[2] or saccharide antennae[3] onto the primary hydroxy rim of the CD core. Recent results have shown that the isothiocyanate functionality can be introduced at the primary position of non-reducing mono-[4] and oligo-saccharides[5] with no need for hydroxy protection. Using this approach, 6^{I}-deoxy-6^{I}-isothiocyanatocyclomaltoheptaose **2** has been prepared by isothiocyanation of the corresponding monoamine precursor[6] **1** with thiophosgene. Both **1** and **2** have been used in the preparation of a series of mono-thioureido-β-CDs bearing *N'*-alkyl, -glycosyl and -glycosyl amino acid substituents, bearing in mind that the thiourea functionality may behave, in the meantime, as semirigid bridging arm for controlled incorporation of a variety of haptens and as a hydrogen bond disruptor, preventing molecular aggregation in water.[7]

The resulting CD-thiourea adducts (Scheme 1) exhibited a water solubility several times higher than the native β-CD, independent of the hydrophilic (**6**, **7**) or hydrophobic character (**8**) of the *N'*-substituent.‡ The observed solubility values (Table 1) also compare favourably with data reported for singly branched glycosyl-β-CDs or their *S*-linked counterparts. They are in the range of the highly soluble per-6-branched β-CDs, with the *a priori* advantage of a higher accessibility of the cavity in monosubstituted derivatives.[3] Consequently, the water solubility of the anti-cancer drug Taxotère®[8] (0.004 g l^{-1}) was increased to 4.7 and 3.8 g l^{-1} in 64.2 and 57.3 mmol dm^{-3} solutions of the thiourea derivatives **6** and **7**, respectively.

Aiming at a better evaluation of the scope of this methodology for the preparation of site specific drug delivery systems based on the β-CD inclusion ability, we have developed a convergent strategy for the coupling of glycopeptide entities to **2**. In the first approach, and taking into consideration their definitive importance in molecular recognition phenomena at the cell membrane level, we have focused in *N*-glycopeptides as suitable biological markers.[9] The preparation of the *N*-glycyl-β-D-glucopyranosylamine–β-CD conjugate **17** illustrates this possibility (Scheme 2). The bifunctional D-glucopyranosylamine derivative **13** plays a key role in the synthesis and is obtained in four steps from the readily available 1,2-*O*-isopropylidene-α-D-glucofuranose **11** *via* its 6-*O*-(toluene-*p*-sulfonyl) derivative, nucleophilic displacement of the tosyl group by azide anion, acid-catalysed deacetalation and reaction of the resulting 6-azido-6-deoxy-D-glucose **12** with ammonium hydrogen carbonate[10] (Scheme 2). Direct coupling of **13** with Boc-protected glycine, following the method of Anisfeld and Lansbury,[11] afforded the corresponding glucosylamino acid derivative **14**. Further Staudinger reduction of the azido group and nucleophilic addition of the resulting amine **15** to the CD-isothiocyanate **2** yielded the β-CD conjugate **16**. Final deprotection of the amino acid *N*-Boc protecting group§ with aqueous TFA gave the target compound **17**. The high efficiency of this convergent methodology may be applicable to the incorporation of more complex *N*-glycopeptide structures suitable for biological recognition as well as other bioactive molecules possessing an amino group.

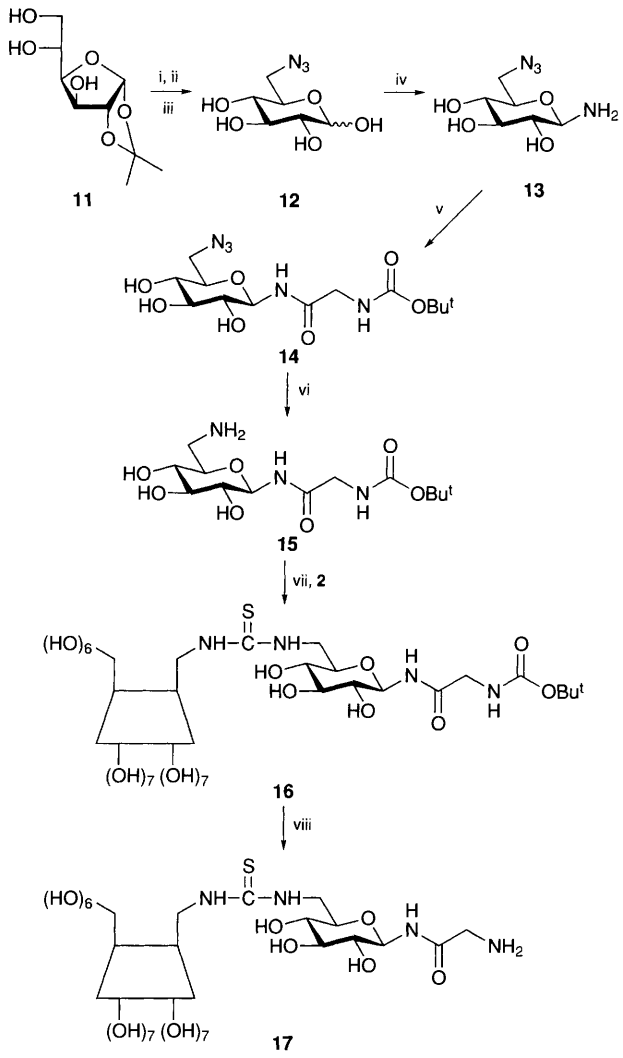
In conclusion, the condensation of **1** and **2** with complementary reagents to give thioureas **6–8** and **16** was efficiently achieved (>90% purity, HPLC) in pyridine at room temperature. Analytical samples were obtained after HPLC purification (RP Nucleosil C-18 column, 12:88 MeOH–H_2O). Although



Scheme 1 *Reagents and conditions*: i, $CSCl_2$, $CaCO_3$, 3:2 H_2O–acetone, room temp., 16 h, 65%; ii, pyridine, room temp., 48 h, >99% (HPLC)

Table 1

Derivative	Water solubility at 25 °C/mmol dm^{-3}
β-Cyclodextrin	15
6	640 (× 42.6)
7	373 (× 24.8)
8	518 (× 34.5)
17	>567 (× 38.0)



Scheme 2 *Reagents and conditions*: i, toluene-*p*-sulfonyl chloride, pyridine, −15 °C, 16 h, 91%; ii, NaN_3, DMF, 120 °C, 20 h, 92%; iii, 1 : 1 TFA–H_2O, 40 °C, 30 min, 82%; iv, aq. NH_4OH (16 mol dm^{-3}), NH_4HCO_3, 40 °C, 30 h, 93%; v, *N*-*tert*-butoxycarbonylglycine, DMF, HBTU,HOBt, 16 h, room temp., 63%; vi, PPh_3, DMF, 5 h, then NH_4OH, 16 h, 90%; vii, pyridine, room temp., 72 h, 92%; viii, 9 : 1 TFA–H_2O, room temp., 1 h, 80% (after HPLC purification), (HBTU = *O*-benzotriazolyl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOBt = 1-hydroxybenzotriazole)

using **1** as the nucleophile is preferable in the case of simple, commercially available isothiocyanates (*e.g.* MeNCS **3**), the use of the β-CD-NCS reagent **2** was advantageous for the coupling reaction with fully unprotected sugar derived amines (*e.g.* **5**,[4b] **15**). In fact, the reverse procedure (**1** + **9**)[4b] results in the formation of an intramolecular cyclic thiocarbamate (**10**, Scheme 1) in the absence of C-4 hydroxy protecting group (*e.g.* **4**).[4b]¶

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Footnotes

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‡ ^{13}C NMR spectra (D_2O) of **6**–**8**, recorded at room temperature, showed broad signals characteristic of relatively slow chemical exchange processes, due to the hindered rotation about the thiourea NH–C(=S) bonds [see ref. 5(*a*) and 12]. Satisfactorily narrow signals were obtained at 50 °C.

§ The use of the benzyloxycarbonyl (Cbz, Z) or fluorenylmethyloxycarbonyl (Fmoc) *N*-protecting groups proved in convenient, leading to low yields in the coupling with **2** and in the final deprotection step. Inclusion phenomena involving aromatic substituents may account for this low reactivity.

¶ All new compounds gave microanalytical, mass spectral (FAB$^+$, glycerol–thioglycerol, NaI) and ^{13}C NMR data (50.3 MHz, D_2O) in agreement with the proposed structures.

Selected data for **2**: $[\alpha]_D$ +112.1 (*c* 0.7, pyridine); ^{13}C NMR δ 131.6 (NCS), 82.2 (C-4^I), 70.1 (C-5^I), 46.2 (C-6^I). For **6**: $[\alpha]_D$ +108.7 (*c* 1, H_2O); ^{13}C NMR δ 83.4 (C-4^I), 70.1 (C-5^I), 45.7 (C-6^I), 25.8 (Me). For **7**: $[\alpha]_D$ +116.1 (*c* 0.6, H_2O); ^{13}C NMR δ 95.0 (C-1′), 83.5 (C-4^I), 55.4 (OMe), 45.7 (C-6^I, C-6′), 19.0 (Ac). For **8**: $[\alpha]_D$ +96.9 (*c* 0.7, H_2O); ^{13}C NMR δ 100.0 (C-1′), 83.7 (C-4^I), 70.7 (C-5′), 70.3 (C-5^I), 55.8 (OMe), 45.9 (C-6^I, C-6′). For **16**: $[\alpha]_D$ +103.4 (*c* 0.9, H_2O); ^{13}C NMR δ 172.8 (CO amide), 157.0 (CO carbamate), 83.4 (C-4^I), 79.0 (C-1′), 76.0 (C-3′, 5′), 69.3 (C-5^I), 45.2 (C-6^I, C-6′), 42.8 (NCH_2), 28.1 (Me). For **17**: $[\alpha]_D$ +82.2 (*c* 1.8, H_2O); ^{13}C NMR δ 168.4 (CO amide), 83.3 (C-4^I), 79.3 (C-1′), 76.3, 75.7 (C-3′, 5′), 69.7 (C-5^I), 45.7, 44.8 (C-6^I, C-6′), 40.7 (NCH_2).

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