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ORIGINAL ARTICLE

Trehalose-based cyclodextrin analogs: cyclotrehalans (CTs)

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Abstract A new concept for the de novo synthesis of artificial glyconanocavities is presented. The use of alternating α,α′-trehalose building blocks and (thio)urea segments allows the efficient synthesis of a new family of cyclooligosaccharides, namely *cyclotrehalans* (CTs), featuring a convex-shaped cavity with an apolar environment. CTs are designed to exhibit molecular inclusion abilities similar to that of cyclodextrins (CDs). Contrary to CDs, CTs expose the monosaccharide *β*-face to the inner cavity, while the (thio)urea tethers provides some conformational adaptability. High-yielding syntheses of a series of CTs and a preliminary evaluation of their inclusion properties are reported.

Keywords Cyclotrehalans · Glyconanocavities · Artificial receptor · Molecular inclusion · Glyconanotechnology · α,α′-Trehalose

Introduction

Interactions between carbohydrates and other biomolecules play a prominent role in many biological recognition processes [1]. The complexity of such phenomena has stimulated the use of model systems to unravel the factors influencing the binding specificity and the stability of carbohydrate-containing supramolecular entities [2]. Among sugar-derived model hosts, the commercially available cyclodextrins (CDs) have been by far the most extensively investigated for this purpose [3, 4]. CDs feature a rigid, hydrophobic cavity of nanometric dimensions that can accommodate a guest molecule of appropriate size. This straitjacket scenario is, however, an intrinsic limitation regarding guest complementarity. Yet, the drawbacks associated to de novo multistep oligosaccharide synthesis have consistently hampered the development of tailor-made cyclooligosaccharide analogs of CDs [5]. To circumvent these limitations, we have developed a conceptually different strategy for the synthesis of artificial glyconanocavities relying on alternating α,α′-trehalose building blocks and semi-rigid thiourea segments (*cyclotrehalans*, CTs) [6]. α,α′-Trehalose is a C_2-symmetric disaccharide having a rigid structure anchored by both anomeric and exo-anomeric effects, featuring a concave shape well suited for macrocycle construction. Furthermore, the use of achiral thiourea intersaccharidic linkages permits a convergent high-yielding synthetic scheme and might provide additional chelating points for molecular recognition and catalysis [7].

Experimental

All reagents and solvents were employed as supplied from commercial sources. Compounds **1–10** were prepared as previously described (see references cited below). ^{1}H and ^{13}C NMR spectra were recorded using Bruker DRX500 instrument. Electrospray mass spectra were recorded on a Bruker Esquire6000 instrument.

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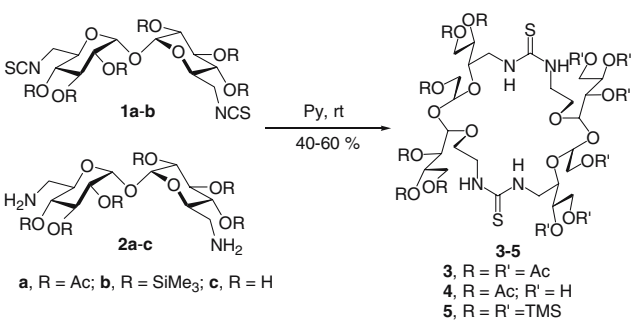
Cyclotris-[*N*,*N*′-bis-(6,6′-dideoxy-α,α′-trehalos-6,6′-diyl)]urea (**13**): To a solution of **7** (129 mg, 68 μmol) in 1:1 water-CH_2Cl_2 (6 mL) was added yellow HgO (129 mg, 0.612 mmol, 9 equiv). After 4 h of vigorous stirring at rt, the organic layer was separated, dried ($MgSO_4$), filtered through Celite, and concentrated to furnish the intermediate carbodiimide **11** (103 mg) in 84% yield. Then, a solution of **11** (50.5 mg, 28 μmol) and TFA (50 μl) in 1:1 acetone-water (3 mL) was stirred at rt for 16 h, then the solvents were evaporated and the residue was purified by column chromatography (45:5:3 EtOAc-EtOH-H_2O) to yield the per-*O*-acetylated triurea **12** (47.3 mg, 91%). Further deacetylation of **12** with MeONa in 1:1 MeOH-water (2 mL) yielded the target cyclotrehalan **13** (23 mg) as a white foam in 99 %. R_f = 0.48 (5:3:5 MeCN:H_2O:N-H_4OH); $[\alpha]_D$ = +136.4° (c 1.0, H_2O); ^1H RMN (500 MHz, D_2O): δ 5.04 (d, 6 H, $J_{1,2}$ = 3.5 Hz, H-1), 3.72 (t, 6 H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3), 3.70 (ddd, 6 H, $J_{4,5}$ = 9.5 Hz, $J_{5,6b}$ = 6.0 Hz, $J_{5,6a}$ = 2.5 Hz, H-5), 3.53 (dd, 6 H, H-2), 3.45 (dd, 6 H, $J_{6a,6b}$ = 14.5 Hz, H-6a), 3.23 (*t*, 6 H, H-4), 3.22 (dd, 6 H, H-6b); ^{13}C RMN (125.7 MHz, D_2O): δ 160.8 (CO), 93.4 (C-1), 72.4 (C-3), 71.4 (C-5), 71.2 (C-2), 70.8 (C-4), 40.5 (C-6); ES-MS: *m/z* 1137.1 ($[M + K]^+$); Anal. Calcd. for $C_{39}H_{66}N_6O_{30}$: C, 42.62; H, 6.05; N, 7.65. Found: C, 42.44; H, 5.84; N, 7.52.

Results and discussion

Coupling of the per-*O*-protected diisothiocyanates **1a–b** with diamines **2a–c** [8] in pyridine furnished the target dimeric cyclotrehalans (CT2s) **3–5** in 40–60% yield (Scheme 1) [9]. The unusually high yields for bimolecular cyclization reactions are probably due to the favourable geometric characteristics of the α,α′-trehalose molecule and the favourable orientation of the reactive groups after formation of the first thiourea tether.



Scheme 1 Synthesis of dimeric cyclotrehalans (CT2)

The conformational properties of macrocycles **3–5** are governed by the presence of four slow-rotating NH—C(=S) bonds. Thus, only two C_2-symmetric conformational patterns are possible (Fig. 1), namely *Z*,*E*:*E*,*Z* (cross conformer) and *Z*,*E*:*Z*,*E* (parallel conformer). In both cases the three-dimensional structure is stabilised by the presence of two 7-membered intramolecular hydrogen-bonds that collapse the cavity. Consistently, CT2 **3** was shown to form complexes with benzoate anions in apolar solvents using exclusively the outside-directed NH protons, in stark contrast with per-*O*-acetylated bis(methyl α-D-glucopyranosid-6-yl)thiourea, an acyclic analogue, known to cooperatively bind carboxylates using four-centre bidentated H-bonds between thiourea and carboxylate groups [10].

It became evident from the above results that a route to higher CT homologues should be envisioned to probe the artificial glyconanocavity concept. A strategy for the synthesis of trimeric cyclotrehalans (CT3) requires, however, prior desymmetrization of the trehalose building blocks. Our solution to this problem involved the water-promoted self-condensation of isothiocyanates to furnish symmetric thioureas. This is a very efficient reaction that allows generation of an intersaccharide thiourea bridge without participation of any even transient amino nucleophile [11]. When applied to diisothiocyanate **1a**, these conditions directly afforded the linear pseudotetrasaccharide **6** in 45% yield, the key building block for the construction of higher CT homologues, in which the α,α′-trehalose subunits are already desymmetrized and appropriately functionalized [12]. Compound **6** can be in situ cyclized to give the cyclic dimer or, alternatively, coupled to diamine **2a** to furnish the fully symmetric CT3 **7**, although in modest yield (25%). In contrast, when silyl-protected diamine **2b** was coupled to **6** (→**8**) efficiency was raised up to 70% (Scheme 2).

The macrocycle **7** possesses a C_3 axis of symmetry perpendicular to the main plane of the nanocavity

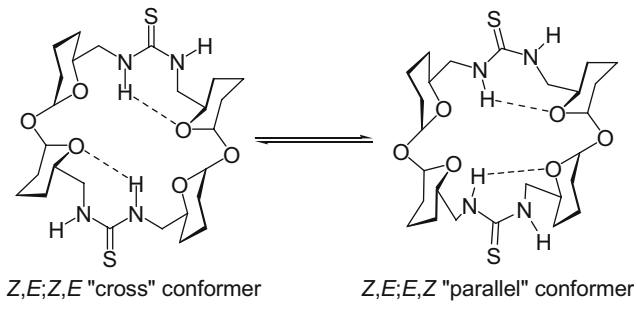
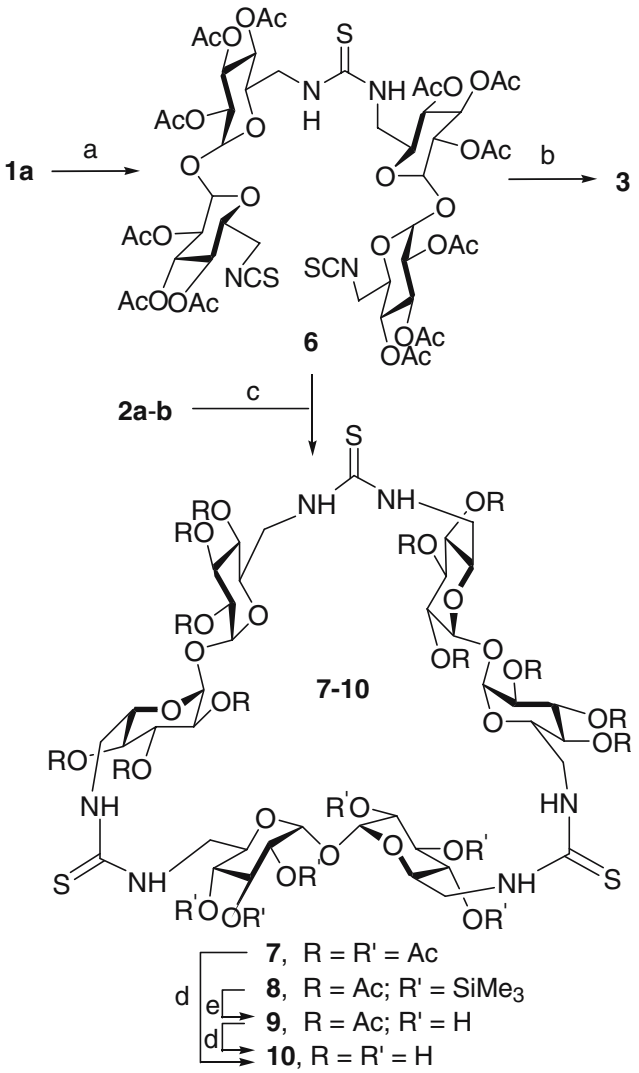


Fig. 1 Possible conformations in CT2 stabilised by 7-membered intramolecular H-bonds



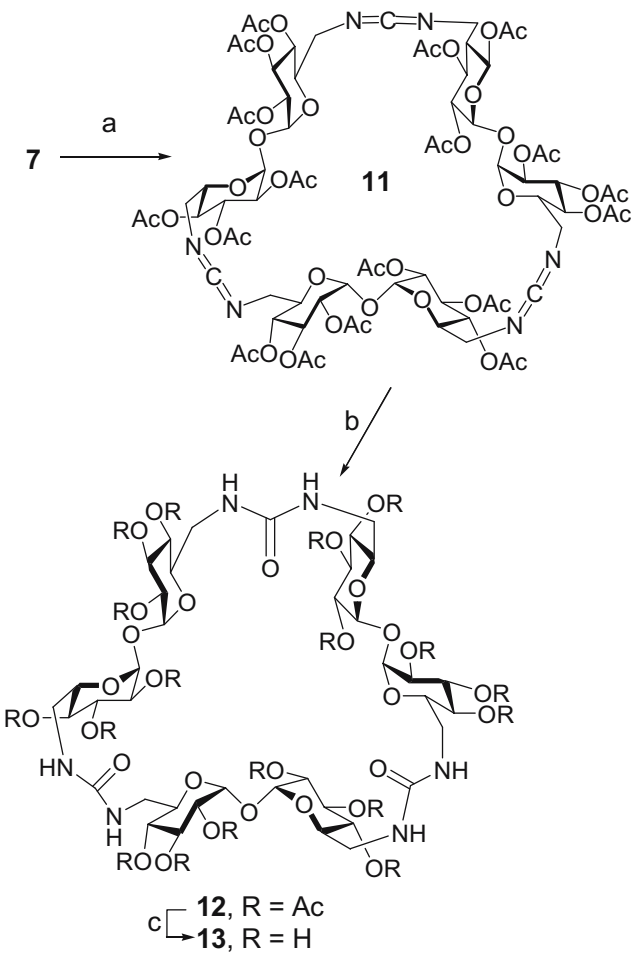
Scheme 2 Synthesis of trimeric cyclotrehalans (CT3). Reagents and conditions: (**a**) Pyridine-water 9:1, 40°C, 6 h, 45%; (**b**) Pyridine-water 9:1, 60°C, 48 h, 35%; (**c**) Pyridine or CH_2Cl_2, rt, 12 h, 70–25%; (**d**) 10% AcOH in 1:3:4 water-MeOH-CH_2Cl_2, 99%; (**e**) MeONa, MeOH-water, 98%

and three C_2 axes in the plane, making all the six glucose subunits equivalents. Consequently, the NMR spectra showed a single spin system. However, symmetry is reduced in CT3 **8** to a single C_2 axis resulting in the presence of three distinct spin systems in the NMR spectra. NOE experiments indicate close contacts between the H-1/H-1′, H-1/H-5′, and H-5/H-1′ protons of the magnetically non-equivalent D-glucopyranose moieties. This unequivocally points to a rigid conformation about the glycosidic linkages dictated by the exoanomeric effect, a feature that is conserved in the hemi- (**9**) and fully deprotected (**10**) macrocycles. In contrast with native CDs, the β-face of the α-D-glucopyranose subunits is now oriented to the inside of a convex-shaped hydrophobic cavity as a result of the particular conformational properties of α,α′-trehalose.

The overall shape of **10** exhibits remarkable similarities with that of α, β and γCD. The geometry also corresponds to a truncated cone structure. But the situation is like if we turn inside-out a CD, so that the outside becomes the inside. Now the H-3 and H-5 protons are outside directed while H-1, H-2 and H-4 point to the inside. The hydroxyl groups are limiting the two rims of the cavity while the interior is comparatively apolar. In addition, to the six equivalent glucopyranosyl subunits, the macrocycle incorporates three thiourea groups that can adopt *Z*,*Z* or *Z*,*E* rotameric arrangements, thus imparting a restricted flexibility to the structure. However, it should not modify substantially either the topology or the size of the internal cavity (medium internal diameter 7.1 Å), which is estimated to be intermediate between that of α- and βCD (5.7 and 7.8 Å, respectively). Binding of benzoate anion in water supports this hypothesis. A 1:1 stoichiometry (Job plot) [13] and an association constant (K_{as}) of 8 ± 2 M^{-1} were determined by NMR titration, in the range of data reported for α and βCD (10–11 M^{-1}). The ROESY spectra showed intermolecular crossed peaks between the aromatic protons and the H-1, H-2 and H-4 protons of the α,α′-trehalose subunits in agreement with the predicted complex structure and that fully confirmed the convex nature of the cavity.

Since the thiourea intersaccharide groups could, in principle, provide additional interactions with an included guest [14, 15], their chemical manipulation might arise as an interesting strategy to optimize host-guest affinity. Thus, transformation of the thiourea bridges of CT3 derivatives into isosteric functionalities has been considered. Mercury oxide-mediated desulfurization of CT3 **7** successfully furnished the cyclic tricarbodiimide **11** in 73% yield. Subsequent TFA-catalysed water addition (→**12**) and deacetylation afforded ureido CT3 **13** (90%, two steps, Scheme 3).

In a preliminary assessment, CT3 **13** preserved the average structural features of the thioureido-analogue **7**, that is, a convex-shaped cavity coated by the H-1, H-2, and H-4 protons of the glucose subunits. However, the lower rotational barrier around NH—C(=O) bonds should provide a greater degree of conformational adaptability. Furthermore, the lower acidity of the NH urea protons, as compared with thiourea, should result in decreased H-bond-donating capabilities, while the carbonyl group is a more efficient acceptor than the thiocarbonyl counterpart.



Scheme 3 Synthesis of isosteric ureido-CT3. Reagents and conditions: (**a**) HgO in 1:1 CH_2Cl_2-water, rt, 24 h, 73%; (**b**) TFA in 1:1 acetone-water, 91%; (**c**) MeONa, MeOH-water, 99%

Optimization of host-guest fitting can be further explored by modulating not only the nature of the intersaccharide connector, but also the macrocycle size. Current investigations in the synthesis of higher CT homologues, with a range of possible applications in fields like artificial enzymes and catalysis, are in progress in our laboratory.

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