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Synthesis, Structure, and Inclusion Capabilities of Trehalose-Based Cyclodextrin Analogues (Cyclotrehalans)

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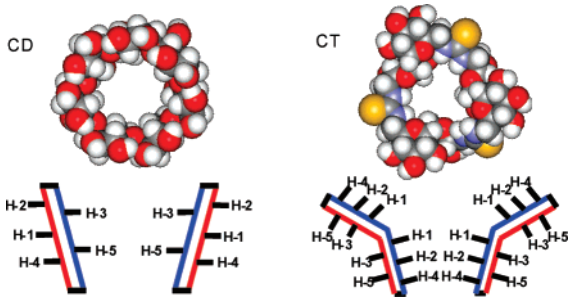

Concise and efficient strategies toward the synthesis of D_{2h}- and D_{3h}-symmetric cyclodextrin analogues alternating α,α′-trehalose disaccharide subunits and pseudoamide segments (cyclotrehalans, CTs) are reported. The conformational properties of these cyclooligosaccharides are governed by the rigidity of the α,α′-trehalose disaccharide repeating unit and the partial double-bond character of the N−(C═X) linkages. In contrast to the typical concave-shaped cavity of cyclodextrins (CDs), CTs feature a convex-shaped hydrophobic cavity in which the β-face of the monosaccharide subunits is oriented toward the inner side, as supported by NMR and modeling (molecular mechanics and dynamics) studies. In the case of cyclodimeric CTs (CT2s), the existence of intramolecular hydrogen bonds results in collapsed cavities, too small to allow the formation of inclusion complexes with organic molecules. Cyclotrimeric CTs (CT3s) display cavity sizes that are intermediate between those of αCD and βCD, ideally suited for the complexation of complementary guests with ternary symmetry such as adamantane 1-carboxylate (AC). The higher flexibility of the pseudoamide bridges as compared with classical glycosidic linkages endow these glyconanocavities with some conformational adaptability properties, making them better suited than CDs for complexation of angular guests, as seen from comparative inclusion capability experiments against the fluorescent probes 6-*p*-toluidinonaphthalene-2-sulfonate (TNS; linear) and 8-anilinonaphthalene-1-sulfonate (ANS; angular).

Introduction

Naturally occurring macrocyclic structures with diverse backbones have strongly motivated the design and synthesis of artificial hosts for selective recognition and catalysis.[1] Among them, cyclomaltooligosaccharides (cyclodextrins, CDs), cyclic

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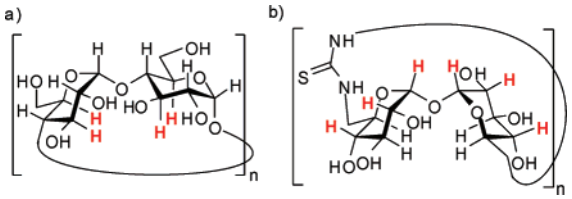

FIGURE 1. Schematic representation of cyclodextrins (CDs, a) and cyclotrehalans (CTs, b) with indication of the pyranoid protons directed to the inside of the cavity (red).

oligomers composed of α-(1→4)-linked D-glucopyranosyl units, hold a prominent position in supramolecular chemistry due to their ability to include hydrophobic molecules within their cavity while dissolved in polar solvents.[2] These favorable characteristics have been translated into applications in fields such as molecular reactors,[3] drug delivery systems,[4] artificial enzymes,[5] catalysis,[6] molecular machines,[7] or supramolecular sensing,[8] to cite just a few.

Organic chemistry offers relatively easy access to the construction of CD derivatives displaying the desired functional groups at specific locations in the structure.[9] Modifications of the CD cavity size and properties have been achieved by hydroxyl epimerization,[10] functional group modification,[11] or glucose $^4C_1 \rightarrow {}^1C_4$ chair inversion.[12] The topology and recognition features of the cavity are generally retained in such derivatives, the α-face of the monosaccharide constituents (i.e., the interglycosidic oxygens and the H-3 and H-5 protons in glucopyranosyl residues) being inside directed (Figure 1a). The tailor-made de novo synthesis of artificial glyconanocavities remains a far more complicated challenge. The chemical methodologies at hand generally require costly and time-consuming synthetic schemes.[13] Stimulated by their interesting supramolecular properties and potential applications, a range of novel host molecules with differently shaped internal cavities have been obtained by replacement of the natural glycosidic bonds by alternative linkages, including thioether,[14] acetylene,[15] amide,[16] amine,[17] 1,2,3-triazole,[18] or phosphate functionalities.[19] However, none of those cyclooligosaccharide hosts are suitable for the analysis of specific interactions involving the *β*-face of the monosaccharides, which represents an intrinsic limitation of the CD canon.

Cyclotrehalans (CTs) represent a unique family of cyclodextrin mimics in which the basic α-(1→4)-linked maltose disaccharide motif has been replaced by α,α′-trehalose building blocks.[20] Like CDs, CTs feature a troncoconic structure with a hydrophobic cavity (Figure 1b). Yet, they are exceptional in behaving as "reverse CDs"; that is, they expose the *β*-face of the α-(1→1)-linked glucopyranosyl subunits to the included guest, providing a complementary information on the ability of sugars to interact with other chemical species. The concept was originally demonstrated for the particular case of macrocycles alternating α,α′-trehalose and thiourea moieties (Figure 1b).[21] The development of efficient syntheses for the preparation of molecular diverse CTs remained, however, an important challenge.

We have recently reported the preparation of acyclic glycooligomers incorporating thiourea and carbodiimide intersaccharide bridges and their transformation into the corresponding urea- and guanidinium-tethered pseudooligosaccharide via carbodiimide intermediates.[22] Interestingly, such functional groups promoted defined folding patterns in the pseudooligosaccharide chain and imparted specific complexing properties.[23] Incorporating those features into the chiral cavity of cyclooligosaccharide hosts seemed particularly appealing. We have now implemented this general approach to the synthesis of a series of dimeric and trimeric cyclotrehalans (CT2s and CT3s) exhibiting D_{2h} and D_{3h} symmetry, respectively. Convergent as well as divergent strategies for accessing the pivotal *O*-protected macrocyclic

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thioureas and carbodiimides have been examined. The conformational properties of the final cyclooligosaccharides have been studied by dynamic NMR and computational methods. The preparation of the α,α′-trehalose building blocks, the scope and limitations of the synthetic schemes, and the complexing properties of the unprotected CTs as compared with CDs are discussed.

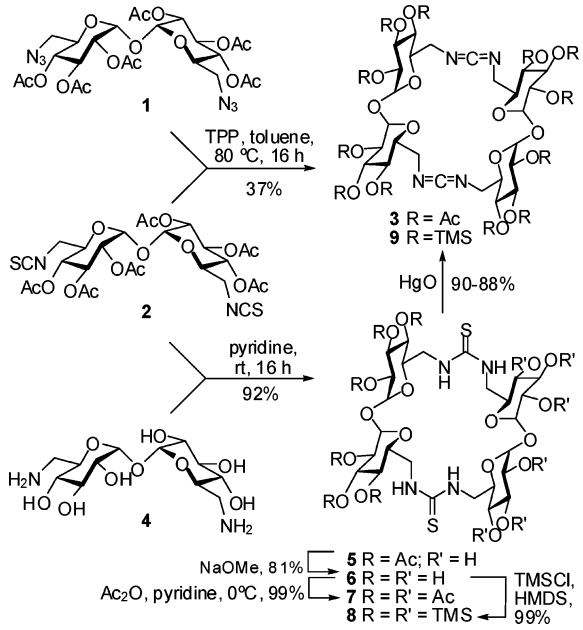
Results and Discussion

Two alternative synthetic pathways can be conceived, in principle, for the construction of the CT macrocyclic skeleton: (i) a convergent approach involving the coupling reaction of two C_2-symmetric precursors bearing complementary functionalities at their primary positions and (ii) a divergent route implying the construction of a linear pseudooligosaccharidic intermediate which must be desymmetrized in the last step in order to zip up the macrocyclic ring. On the other hand, generation of carbodiimide intersaccharide bridges in CTs can be undertaken either directly, by tandem Staüdinger−aza-Wittig type condensation of isothiocyanate- and azide-functionalized trehalose reagents or via a two-step procedure implying the nucleophilic addition of an amine to an isothiocyanate followed by desulfurization of the resulting thiourea adduct. In order to optimize the synthetic methodology, the advantages and limitations of all these possibilities were first explored in the smallest CT representatives, namely the cyclodimeric CT2s.

Synthesis of CT2s. The Staüdinger−aza-Wittig-type [2 + 2] cyclodimerization reaction of the per-*O*-acetylated 6,6′-diazido- and 6,6′-diisothiocyanato-α,α′-trehalose derivatives **1**[24] and **2**[25] was first attempted. The reaction proceeded to give the target bis(carbodiimide) **3** with high conversion yields, as seen from the MS and NMR spectra of the crude reaction mixtures. No formation of linear oligomers or higher cyclic homologues was detected. Separation of **3** from triphenylphosphine thioxide was found to be problematic, however, which seriously hampered isolation of the pure product (37% isolated yield; Scheme 1).

In view of the difficulties associated to the above procedure, the HgO-promoted desulfurization of preformed thiourea-linked CTs was considered. The convergent synthesis of the requested macrocyclic precursors from selectively *O*-protected diisothiocyanate and diamine building blocks required relatively long reaction sequences and suffered from complications associated to O → N protecting group migration, with the corresponding penalty in the global yield.[21a] Since previous results have shown a high chemoselectivity in the conjugation of amines and sugar isothiocyanates,[26] the use of unprotected building blocks was envisaged. Interestingly, double coupling reaction of **2** and the fully unprotected diamine **4**[25] proceeded smoothly in pyridine to give the corresponding hemiacetylated cyclic adduct **5** in 92% yield, offering a very convenient entry to this family of hosts.

SCHEME 1. Convergent Route to CT2s



Conventional deacetylation provided the unprotected CT2 **6** in 81% yield (seven steps from commercial α,α′-trehalose, 34% overall yield), which was further transformed into the per-*O*-acetate **7**[27] and the per-*O*-trimethylsilyl ether **8** in quantitative yields (Scheme 1).

Although very efficient, the above convergent strategy based on the use of symmetric α,α′-trehalose building blocks is limited exclusively to the preparation of CT2 derivatives. No higher linear or cyclic homologues were detected even using high concentrations and a big excess of either the diamine or the diisothiocyanate reagents. A priori, an alternative divergent approach entailing a linear trehalo-oligomer would be more general. It requires, however, the preparation of dissymmetric trehalose bulding blocks suitable for iterative synthetic schemes to grow a chain with the desired number of repeating disaccharide units before the final intramolecular macrocyclization reaction.

Our efforts to prepare α,α′-trehalose derivatives bearing orthogonal nitrogen functionalities at the two primary positions by statistic monoprotection of the amino groups of different 6,6′-diamino-6,6′-dideoxy-α,α′-trehalose derivatives afforded, invariably, low yields of the desired product after laborious purification.[28] Alternatively, statistic reduction of 6,6′-diazido-6,6′-dideoxy-α,α′-trehalose **10**[25] (two steps from α,α′-trehalose) was attempted. Treatment with Pd/C favored complete reduction even using low catalyst amounts and short reaction times. Analogous results were achieved when using triphenylphosphine (TPP),[29] dithiothreitol,[30] or 1,3-propanedithiol−triethylamine

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(27) Acetylation of thiourea adducts must be conducted at temperatures lower than 0 °C to prevent formation of *N*- or *S*-acetyl derivatives. See: García-Moreno, M. I.; Benito, J. M.; Ortiz Mellet, C.; García Fernández, J. M. *Tetrahedron: Asymmetry* **2000**, *11*, 161.

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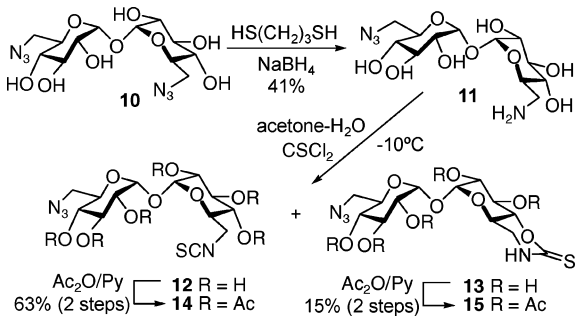
SCHEME 2. Synthesis of Dissymmetric α,α′-Trehalose Derivatives by Statistic Reduction of the Diazide Precursor 10



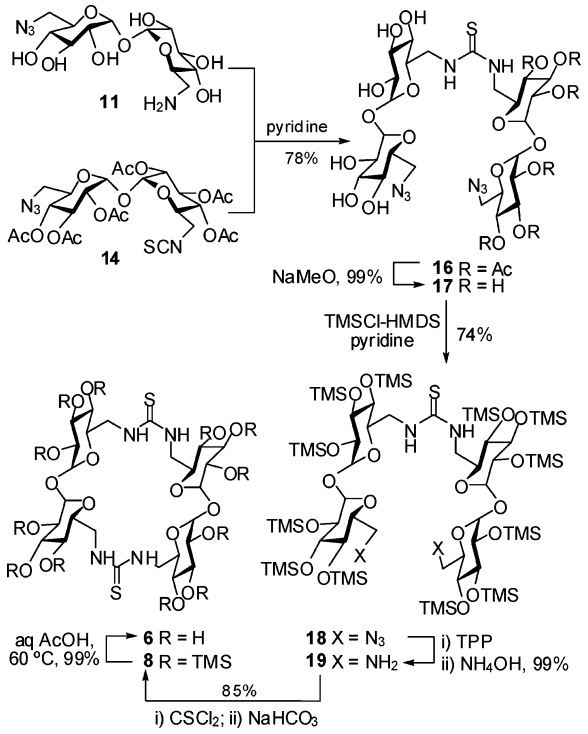
systems.[31] To our delight, when diazide **10** was treated with $NaBH_4$ and a catalytic amount of 1,3-propanedithiol,[32] reduction progressed slowly allowing, within 5 h, isolation of the dissymmetric derivative **11** in 41% yield (30% overall yield starting from α,α′-trehalose, Scheme 2). The reaction is reproducible in gram scale and the unreacted starting material can be easily recovered and recycled, with conversion yields that reach 85% referred to the transformed diazide.

Isothiocyanation of monoamine **11** with thiophosgene furnished azidoisothiocyanate **12**, with concomitant formation of the corresponding 4,6-cyclic thiocarbamate **13**. This side reaction was minimized by carrying the reaction at −10 °C. After conventional acetylation of the reaction mixture, the corresponding hexa-*O*-acetyl azidoisothiocyanate **14** could be isolated in 63% yield, together with a minor proportion of the pentaacetylated thiocarbamate **15** (15%).

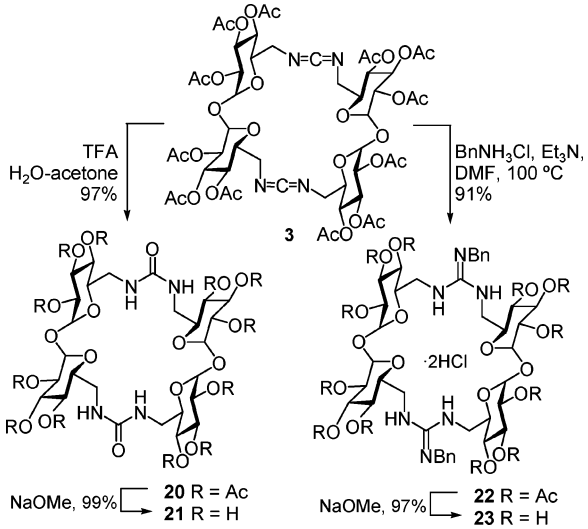
Coupling of aminoazide **11** and azidoisothiocyanate **14** in pyridine afforded the linear hemiacetylated adduct **16** in 78% yield, which was subsequently deacetylated to the C_2-symmetric thiourea-tethered pseudotetrasaccharide **17** (Scheme 3). Protection of the hydroxyl groups as the corresponding trimethylsilyl ethers (→ **18**) was considered convenient at this stage. First, purification of the highly polar polyhydroxylated derivatives is troublesome. Second, the TMS groups prevent formation of intramolecular thiocarbamates during isothiocyanation, a necessary transformation to promote the macrocyclization step, and are much less prone to O → N migration than acyl groups. Reduction of the azido groups in **18** with triphenylphosphine (TPP) provided the corresponding diamine **19**, a direct precursor of CT2 derivatives. Desymmetrization of **19** to the transient aminoisothiocyanate hydrochloride and subsequent intramolecular thiourea-forming reaction was effected in one pot by treatment with 0.5 equiv of thiophosgene followed by in situ adjustement at pH 8 with sodium bicarbonate. Compound **8** was thus obtained in 85% yield and further transformed into the fully unprotected CT2 dithiourea host **6** by acid hydrolysis of the TMS ether protecting groups (11 steps from commercial α,α′-trehalose, 18% overall yield).

Both, the dodeca-*O*-acetyl and the dodeca-*O*-trimethylsilyl derivatives **7** and **8** readily underwent desulfurization upon treatment with mercury(II) oxide (HgO), affording the corresponding macrocyclic bis(carbodiimide)s **3** and **9** in 90 and 88% yield, respectively (Scheme 1). Compound **9** exhibited an

SCHEME 3. Divergent Route to CT2s



SCHEME 4. Synthesis of Ureido and Guanidinium CT2s via Carbodiimides



astonishing inertness toward oxygen or amine nucleophiles. The presence of the bulky trimethylsilyl groups probably prevents nucleophile approaching. The dodeca-*O*-acetate **3** was also a stable compound both in the solid state and in solution, even in aqueous mixtures, at neutral pH. Nevertheless, it underwent addition of water at room temperature in acetone in the presence of catalytic amounts of trifluoroacetic acid to give the corresponding bis(urea) **20** in 97% yield. The preparation of the isosteric bis(*N*-benzylguanidine) derivative **22** by reaction of **3** with benzylamine hydrochloride required heating at 100 °C in *N*,*N*-dimethylformamide containing triethylamine. A final deacetylation step afforded the target fully unprotected CTs **21** and **23**, respectively, in virtually quantitative yields (Scheme 4).

Synthesis of CT3s. A first conclusion of the above-discussed survey of synthetic routes toward the target trehalose-based

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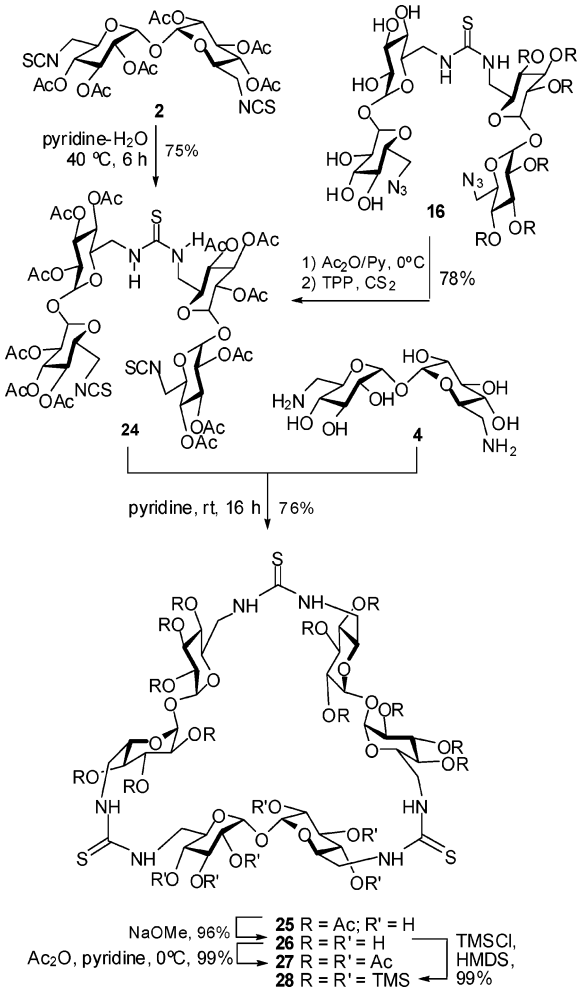
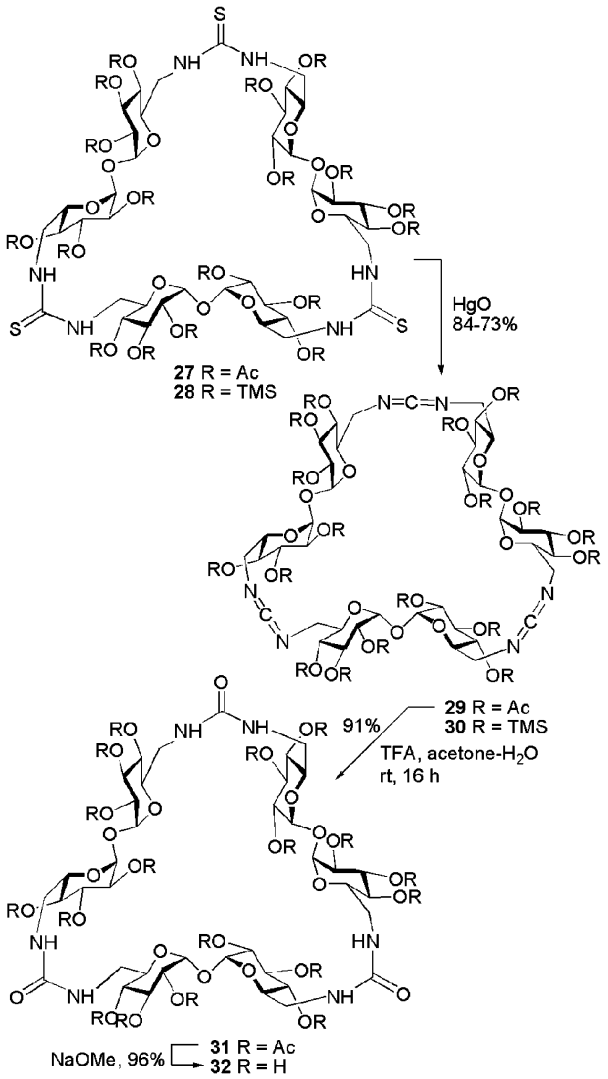
(32) For another successful application of the 1,3-propanedithiol−$NaBH_4$ system in statistic reduction of azides; see: Katajisto, J.; Karskela, T.; Heinonen, P.; Lönnberg, H. *J. Org. Chem.* **2002**, *67*, 7995.

SCHEME 5. Convergent Synthesis of CT3s



SCHEME 6. Synthesis of Ureido CT3s via Carbodiimides



macrocyclic hosts is that the two-step synthesis of carbodiimide-linked pseudooligosaccharides, via thiourea intermediates, is preferable in terms of efficiency to the direct formation of the heterocumulene bridges from azide and isothiocyanate precursors. Moreover, convergent strategies involving double intermolecular coupling are less costly than divergent approaches entailing intramolecular macrocyclization of a linear glycooligomer. From these considerations, the optimal retrosynthetic analysis in the case of the CT3 core would imply the unprotected diamine **4** in combination with pseudotetrasaccharide **24** as the diisothiocyanate partner (Scheme 5).

Although compound **24** exhibits C_2-symmetry, the constituent α,α′-trehalose moieties themselves are dissymmetric, meaning that the synthetic scheme must involve a desymmetrization step. Taking advantage of the selective reduction of the disaccharide diazide **10**, a synthesis of **24** via the linear dimeric diazide **16**, by acetylation followed by direct isothiocyanation with triphenylphosphine and carbon disulfide, was worked out. This approach involves, however, a relatively long synthetic route (eight steps from commercial α,α′-trehalose). Desymmetrization at the level of diisothiocyanate **2**, by exploiting the base-promoted self-condensation reaction of isothiocyanates to give symmetric thioureas,[33] significantly shortened the preparation of **24** (five steps from commercial α,α′-trehalose). Subsequent coupling of **4** and **24** in pyridine at room temperature took place with absolute chemoselectivity, furnishing the C_2-symmetric pseudocyclohexasaccharide adduct **25** in 76% yield. Zemplen deacetylation of **25** quantitatively yielded the fully unprotected D_{3h}-symmetric CT3 **26**, in which the six glucopyranosyl subunits become magnetically equivalents. The per-*O*-acetylated and per-*O*-silylated derivatives **27** and **28** were also prepared through standard procedures (Scheme 5).[27]

Treatment of the CT3-thioureas **27** and **28** with HgO afforded the corresponding macrocyclic tris(carbodiimides) **29** and **30** in 84% and 73% yield, respectively (Scheme 6). As previously observed for the corresponding silylated CT2 bis(carbodiimide) **9**, compound **30** exhibited a surprising inertness toward nucleophiles. The per-*O*-acetate **29** was also rather stable. Attempts to prepare the corresponding tris(*N*‴-benzylguanidine) by nucleophilic addition of benzylamine to the heterocumulene groups resulted in incomplete transformations at temperatures under 100 °C and in concomitant deacetylation above this temperature, leading to mixtures from which pure compounds could not be isolated. Addition of water, to provide the corresponding tris(urea) **31**, required the use of trifluoroacetic acid as catalyst and longer reaction times as compared to the CT2 series. Final deacetylation gave the unprotected CT3-urea **32** in 90% overall yield.

(33) Jiménez Blanco, J. L.; Saitz-Barría, C.; Benito, J. M.; Ortiz Mellet C.; Fuentes, J.; Santoyo-González, F.; García Fernández, J. M. *Synthesis* **1999**, 1907.

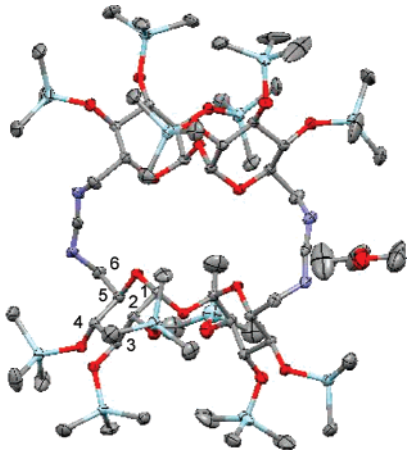


FIGURE 2. X-ray structure[37] of bis(carbodiimide) **9** (ORTEP; red: O; blue: N; light blue: Si; gray: C). A molecule of diethyl ether present in the cell unit is also shown.

Structure and Conformational Properties of CTs. Although the adaptability of the concave-shaped α,α′-trehalose molecule to macrocyclic architectures was predicted,[21c] the suitability of the rigid carbodiimide segments to be accommodated in such cyclooligosaccharide hosts was not anticipated. In principle, each of the heterocumulene groups can exist in either the *R* or *S* configuration. Consequently, up to three different diastereomers are possible for a macrocyclic D_{2h}-symmetric bis(carbodiimide), namely the (*R*,*R*), (*S*,*S*), and (*R*,*S*) isomers. The low interconversion barriers (about 7 kcal mol^{-1} for linear carbodiimides)[34] generally preclude the isolation of individual diastereomers. Achiral cyclic bis(carbodiimides) based on aromatic scaffolds have been previously synthesized and shown to exist in the (*R*,*S*)-*meso*-configuration in the solid state.[35] In contrast, the X-ray structure of compound **9** (see Figure 3) revealed that both carbodiimide groups adopt the *S* configuration, with N═C distances of 1.219−1.215 Å and valency angles for the carbodiimide carbon atoms of 170.7(2)° and 171.3(2)°. This geometry matches well the corresponding theoretical values for HN═C═NH (1.21−1.23 Å and 168−170°, respectively). Moreover, the torsional angles about the N═C bonds for **9** (130.7−148.1 Å) are closer to those reported for linear carbodiimides (112−142°) than to the reported values for conformationally constrained macrocyclic bis(carbodiimides) (134−156°),[33b] meaning that the macrocyclic architecture does not result in significant angular deformation. This is in agreement with the relatively high stability of **9** as compared with that reported for other cyclic carbodiimides suffering from angular tension.[33b] On the other hand, the separation between the carbodiimido groups imposed by the disaccharide scaffold prevents intramolecular [2 + 2] cycloaddition reactions, a common secondary reaction pathway in constrained bis(carbodiimides) (Figure 2).[36]

The α,α′-trehalose moieties in **9** keep the configuration dictated by the exo-anomeric effect at the glycosidic bonds (i.e., C-2−C-1−O-1−C-1′ and C-2′−C-1′−O-1′−C-1 dihedral angles close to 180°).[37] This geometry implies that the β-face of the monosaccharide subunits is directed to the inside of the cavity, in agreement with our original hypothesis. The C-5(5′)−C-6(6′) bonds adopt the gauche−gauche (gg) conformation (i.e., the carbodiimide substituent and H-5(5′) in anti relative disposition). Considering that the conformational behavior of D-glucopyranose derivatives about this bond generally implies an equilibrium between the gg and gt (gauche−trans) conformers, with rotameric populations close to 1:1,[38] that means that macrocyclization does not introduce torsional constrain at this level either. On the contrary, assuming a sequential coupling of trehalose building blocks, the gg (or gt) orientation in the (6→6)-linked linear trehalo-oligosaccharide intermediate would actually place in close proximity both chain ends, strongly favoring intramolecular versus intermolecular adducts, in agreement with the experimental results.

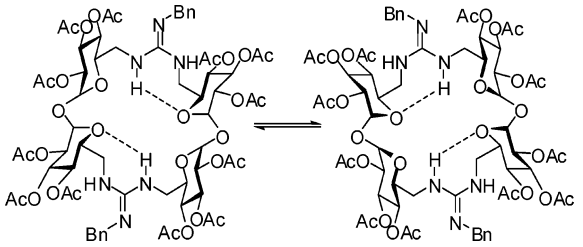


FIGURE 3. Schematic representation of the degenerated *Z*,*E*:*E*,*Z* ≡ *E*,*Z*:*Z*,*E* equilibrium in the CT2 derivative **22**.

The NMR spectra of the CT2-urea and CT2-benzylguanidine hexa-*O*-acetates **20** and **22** carried out at room-temperature exhibited significant line broadening, indicative of the existence of chemical exchange processes associated to slow rotations about the pseudoamide bonds. Line broadening was still evident at 40−60 °C in $CDCl_3$ or CD_3OD, though the spectra were consistent with a D_{2h}-symmetric macrocyclic architecture in which all glucopyranosyl subunits become magnetically equivalent. The bis(urea) derivative **20** aggregated at temperatures below 233 K, above the coalescence temperature, which prevented a complete characterization of the rotameric equilibrium. In the case of bis(guanidine) **22**, the NMR spectra carried out at low temperature were consistent with the presence of a major C_2-symmetric conformer having two distinct spin systems. The existence of characteristic NOE contacts between the magnetically nonequivalent glucopyranosyl units (H-1/H-1′; H-1/H-5′; H-5/H-1′) unambiguously pointed to the "crossed" *Z*,*E*:*E*,*Z* configuration at the pseudoamide N−C═NBn bonds, with two opposite NH protons directed to the inside of the cavity and the other two pointing to the outside, in analogy with the previously described bis(thiourea) derivative **7**.[21a] This structure is stabilized by two antiparallel seven-membered intramolecular hydrogen bonds,[39] resulting in relatively high rotational barriers for the degenerated *Z*,*E*:*E*,*Z* ≡ *E*,*Z*:*Z*,*E* equilibrium (Figure 3).

Line broadening was likewise evident in the NMR spectra of the cyclic (thio)urea-linked trehalose trimers **25**−**28**, **31**, and **32** at 298 K. At higher temperatures the spectral lines narrowed and were in agreement with the expected C_2 (**25**; three different

(34) Anet, F. A.; Jochims, J. C.; Bradley, C. H. *J. Am. Chem. Soc.* **1970**, *92*, 2557.

(35) (a) Molina, P.; Alajarín, M.; Sánchez-Andrada, P.; Elguero, J.; Jimeno, M. L. *J. Org. Chem.* **1994**, *59*, 7306. (b) Molina, P.; Alajarín, M.; Sánchez-Andrada, P.; Carrió, J. S.; Martínez-Ripoll, M.; Anderson, E. J.; Jimeno, M. L.; Elguero, J. *J. Org. Chem.* **1996**, *61*, 4289.

(36) Alajarin, M.; Molina, P.; Sanchez-Andrada, P.; Foces-Foces, C. *J. Org. Chem.* **1999**, *64*, 1121.

(37) CCDC-3765 contains the supplementary crystallographic data for **9**. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK; fax: (+44)1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk).

(38) (a) Kirschner, K. N.; Woods, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 10541. (b) Bock, K.; Duus, J.; Jens, O. *J. Carbohydr. Chem.* **1994**, *13*, 513.

spin systems) or D_{3h} symmetry (**26**−**28**, **31**, and **32**; a single spin system). The vicinal coupling constant (*J*) values about the pyranose rings confirmed the $^1C_4$ chair conformation, while the existence of NOE contacts between the two D-glucopyranosyl subunits in the disymmetric α,α′-trehalose moieties of **25** was in agreement with the exoanomeric orientation about the glycosidic bonds, supporting the reverse cyclodextrin-type nature of CT3 derivatives. On the basis of dynamic NMR experiments at low temperature for the *O*-protected derivatives, the existence of preferred rotameric patterns was discarded. Probably, the higher size of the cavity as compared with CT2 analogues is compatible with both the *Z*,*Z* and *Z*,*E* (or *E*,*Z*) configurations for each pseudoamide segment as well as all possible combinations between them.

In order to have a deeper insight on the cavity dimensions, shape, and properties of this family of carbohydrate-based hosts, molecular mechanics (MM) and molecular dynamics (MD) simulations were carried out on the fully unprotected thiourea-bridged derivatives **6** and **26**. For the generation of the initial structure of the α,α′-trehalose moieties, the X-ray coordinates obtained for compound **9** were used. The C-5−C-6 bonds of the α-D-glucopyranosyl subunits were set in the gauche−trans (gt) conformation and the thiourea groups in the *E*,*Z*-configuration for the CT2 derivative, according to the low-temperature NMR data for the *O*-protected derivative **7**[21a] and in the *Z*,*Z*-configuration, generally more stable in the absence of intramolecular hydrogen bonds,[40] for the CT3 homologue **26**. Preliminary simulations (500 ps) were carried out using the GBSA continuum solvent model for H_2O, monitoring the geometry every ps. The structures with the angles having the highest frequency were selected for the further MD simulations experiments (298 K, 1000 ps) both in the vacuum and in aqueous solution using an explicit solvent model.

For the dimeric cyclotrehalan **6**, the calculations confirmed that the constrain imposed by the macrocyclic structure forces the *Z*,*E* configuration at both thiourea segments. No significant differences were encountered between the MD results in vacuum and in water, pointing to a very rigid conformation stabilized by two intramolecular hydrogen bonds involving the NH protons of the *Z*-configured NH−(C═S) segments and the pyranoid oxygen O-5 of the corresponding glucose unit, in full agreement with the NMR data. In the case of the CT3 tris(thiourea) **26**, the MD simulation in vacuum showed an evolution from the open all-*Z*,*Z* conformation to a folded conformer stabilized by seven-membered intramolecular H-bonds analogous to those operating in CT2 derivatives. In the presence of explicit water, however, the open conformation remained stable all over the MD. The analysis of the time-averaged root-mean-square (rms) deviations of the moment of inertia from the last structure registered for each CT in water served to confirm their conformational stability (Figure 4).

The computational studies indicated that the cavity shape and dimensions remain virtually unchanged both in the dimer and in the trimer. In CT2s the cavity is actually collapsed by virtue

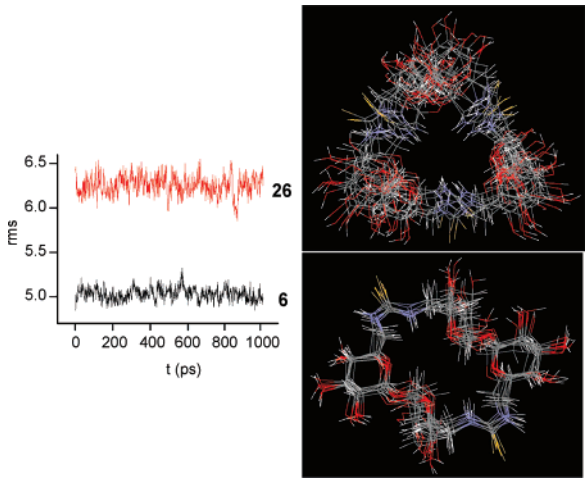


FIGURE 4. Superimposition of calculated conformations for **6** and **26** in explicit H_2O and root-mean-square (rms) deviations observed during calculations for **6** (black) and **26** (red) in H_2O.

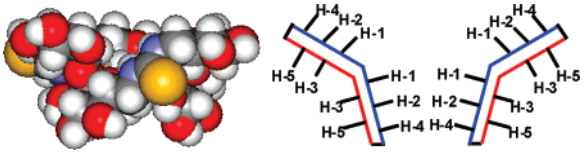


FIGURE 5. CPK side view of the minimized conformation of CT3 **26** (left) and schematic representation of the CH proton distribution (right).

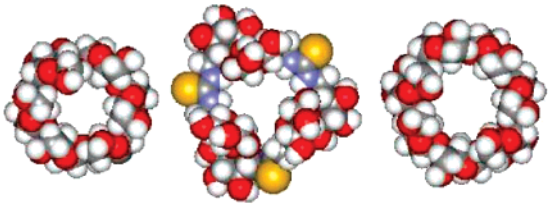


FIGURE 6. CPK top views of minimized conformations of αCD (left), CT3 **26** (center), and *β*CD (right).

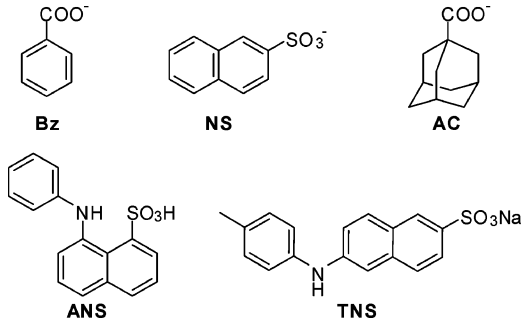
of the two intramolecular H-bonds above-mentioned.[41] In contrast, CT3 **26** features a funnel-like architecture that resembles the truncated cone conformation of CDs. Like in CDs, the hydroxyl groups are located at the two rims, pointing to the bulk solvent. The interior of the cavity is relatively hydrophobic, exposing the sugar H-1, H-2 and H-4 methine protons instead of the H-3 and H-5 typical of CDs (Figure 5). The dimensions of the cavity (7.1 Å internal medium diameter) are intermediate between those of α- and *β*CD (5.7 and 7.8 Å, respectively). A higher adaptability toward potential guest molecules of appropriate size could be expected on the basis of the capability to orientate the NH protons to the inside or outside of the cavity and the possibility to modify the wider and narrower rim diameters of the funnel, which experience fast interconvertion in solution as seen from NMR experiments, in a breathing-like molecular movement (Figures 5 and 6).

Inclusion Capabilities of CT3s. Preliminary NMR titration experiments of the CT3 tris(thiourea) host **26** against benzoate

(39) The formation of seven-membered NH···O intramolecular hydrogen bonds has already been found to be a dominant structural feature in sugar thioureas, being associated to the *E*,*Z* rotameric form. See ref 21b and: García Fernández, J. M.; Ortiz Mellet, C.; Jiménez Blanco, J. L.; Fuentes, J.; Diánez, M. J.; Estrada, M. D.; López-Castro, A.; Pérez Garrido, S. *Carbohydr. Res.* **1996**, *286*, 55.

(40) (a) Ortiz Mellet, C.; Moreno Marín, A.; Jiménez Blanco, J. L.; García Fernández, J. M.; Fuentes, J. *Tetrahedron: Asymmetry* **1994**, *5*, 2325. (b) Ortiz Mellet, C.; García Fernández, J. M. *Sulfur Rep.* **1996**, *19*, 61. (c) Ortiz Mellet, C.; García Fernández, J. M. *Adv. Carbohydr. Chem. Biochem.* **2000**, *55*, 35.

CHART 1. Structure of the Different Guests Used in the Comparative Inclusion Experiments



(Bz) and naphthalenesulfonate (NS) evidenced the formation of 1:1 complexes[42] with association constant (K_{as}) values (8 ± 2 and 235 ± 15 M^{-1}, respectively)[21c] that are closer to those reported for the corresponding complexes with αCD (10 ± 1 and 363 ± 8 M^{-1}) than to the respective *β*CD complexes (12 ± 2 and 2.3 × 10^5 M^{-1}).[43] However, in contrast to the typical displacement of the H-3 and H-5 resonances in CD inclusion complexes, in the case of **26** the signals for H-1, H-2, and H-4 protons were highfield shifted upon addition of the guest, supporting that the aromatic ring is bound in the cavity through hydrophobic interactions involving the *β*-face of the monosaccharide subunits. In the case of 1-adamantane carboxylate (AC), the corresponding **26**/AC complex (K_{as} 4.6 ± 0.4 × 10^4 M^{-1}) turned to be much more stable than the corresponding αCD/AC complex (K_{as} 141 ± 15 M^{-1}),[43] even more stable than the corresponding *β*CD/AC complex (K_{as} 3.9 × 10^4 M^{-1}),[43] thus illustrating the importance not only of the size-fit concept but also of the symmetry complementarity between host and guest in achieving efficient association (Chart 1).[44]

To gain further insight into the supramolecular properties of CT3s, the inclusion capabilities of the tris(thiourea) **26** and tris(urea) **32** toward the fluorescent probes 6-*p*-toluidino-2-naphthalenesulfonate and 8-anilino-1-naphthalenesulfonate (TNS and ANS, respectively; Chart 1) were investigated and compared to α- and *β*CD. TNS and ANS are excellent guests to probe molecular inclusion driven by nonpolar interactions in aqueous media. Their fluorescence intensity is intimately related to the milieu, increasing with the hydrophobicity of the environment.[45] Moreover, TNS and ANS being almost positional isomers, they feature very different steric volumes, corresponding to quasi-linear and angular molecular shapes, respectively, being very well suited to assess the conformational adaptability of different host partners. Thus, the intrinsic rigidity of CDs results in rather poor binding for the 1:1 CD/ANS complexes (K_{as} 115 M^{-1} both for α- and *β*CD).[43] In the case of TNS, a deeper inclusion in the larger *β*CD cavity is possible, resulting in a remarkable selectivity (K_{as} 83 and 2820 M^{-1} for αCD/TNS and *β*CD/TNS, respectively).[43] Inclusion is accompanied by a dramatic enhancement of the fluorescent intensity either for ANS or TNS, in agreement with the hydrophobic nature of the CD cavity.

Fluorescence intensity enhancement upon addition of the CT3s **26** or **32** to buffered aqueous solutions of either ANS or TNS was rather modest as compared to that observed for CDs. This qualitative observation points to a less hydrophobic environment in cyclotrehalan cavities, probably due to the hydrophilic character of the (thio)urea moieties. Nevertheless, the corresponding binding isotherms were indicative of association constants 8- to 5-fold higher for the 1:1 **26**/ANS and **32**/ANS complexes (K_{as} 839 ± 25 and 534 ± 17 M^{-1}, respectively),[46] while the corresponding **26**/TNS complex exhibited a K_{as} value (940 ± 23 M^{-1}) intermediate between those for α- and *β*CD. These results can be interpreted in terms of the higher conformational adaptability of trehalose-based macrocycles as compared with cyclodextrins. The breathing-like capability of the funnel-shaped CT cavity probably allows guest-induced fitting, therefore facilitating accommodation of sterically demanding angular guests such as ANS. On the other hand, such a mechanism implies an entropic penalty that is in agreement with the lower affinity of **26** toward the linear TNS guest as compared with *β*CD.

Conclusion

In summary, we have described a modular and efficient methodology for the synthesis of cyclooligosaccharide receptors consisting of α,α′-trehalose building blocks connected through pseudoamide bridges, namely cyclotrehalans (CTs). Molecular diversity can be introduced at the level of the intersaccharide connectors by exploiting the chemistry of macrocyclic carbodiimides, as demonstrated for dimeric (CT2s) and trimeric derivatives (CT3s). Like CDs, CTs show a high-symmetry that makes all the monosaccharide subunits magnetically equivalents, which strongly facilitates conformational and supramolecular studies. Whereas in CT2s the cavity is collapsed by the presence of intramolecular hydrogen bonds, CT3s exhibit a permanent convex cavity, relatively hydrophobic, whose dimensions are intermediate between those of α and *β*CD. Yet, they are unique in behaving as reverse CDs, exposing the *β*-face of the monosaccharide subunits to the inside. The inclusion capabilities toward hydrophobic guests depend on size match and, especially, on symmetry complementarity. The restricted conformational mobility of CT3s further endows these hosts with guest-induced fitting capabilities, which is translated into better affinities toward sterically demanding guests as compared with CDs. The extension of these studies to higher CT homologues is currently sought in our laboratories.

Experimental Section

For the computational, NMR titration, stoichiometry determination of complexes and fluorescent binding titration details, see the Supporting Information.

(41) The capabilities of the CT2 derivatives **6**, **21**, and **23** to complex mono (Na^+, K^+, Cs^+) and divalent metal cations (Ca^{2+}, Ba^{2+}, Zn^{2+}, Cu^{2+}) were examined by thin-layer ligand-exchange chromatography, following the protocol previously reported (cf. ref 21a). The formation of a strong complex was detected only for the bis(thiourea) compound **6** and the thiophile cation Cu^{2+} pair. The absence of complexation in the case of **21** and **23** supports the involvement of the thiocarbonyl sulfur atoms in complex formation. The existence of inclusion phenomena is unlike, anyway, in view of the discussed conformational properties.

(42) The 1:1 complex stoichiometry was confirmed by the continious variation method (Job plot). For a thorough description, see: Connors, K. A. *Binding Constants: The Measurement of Molecular Complex Stability*; Wiley: Chichester, U.K., 1987. (b) Job, P. *Ann. Chim.* **1928**, *9*, 113.

(43) For a complete survey on complexation thermodynamics of CDs, see: Rekharsky, M. V.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875.

(44) As a matter of fact, compound **26** behaved as a very efficient AC scavenger in competitive experiments with *β*CD derivatives, which has been exploited in the design of molecular switchers. See ref 8b.

(45) Turner, D. C.; Brand, L. *Biochemistry* **1968**, *7*, 3381.

(46) Binding constants were obtained at 32 °C in phosphate buffered media (10 mM, pH 7.3). Errors are estimated to be in the range of ±15%. Binding isotherms were fitted using the MicroMath Scientist Software.

Per-*O*-acetylated CT2 Bis(carbodiimide) (3). A solution of 2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-diazido-6,6′-dideoxy-α,α′-trehalose[24] (**1**, 0.29 g, 0.45 mmol) in toluene (15 mL) was stirred under nitrogen for 30 min. Then, a solution of 2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-dideoxy-6,6′-diisothiocyanato-α,α′-trehalose[25] (**2**, 0.3 g, 0.45 mmol) and TPP (0.27 g, 1.02 mmol, 1.1 equiv) in toluene (10 mL) was added dropwise. The reaction mixture was stirred at 80 °C for 24 h and concentrated under reduced pressure and the resulting residue was purified by column chromatography (1:1 → 3:1 EtOAc−petroleum ether) to furnish **3** (0.2 g, 37%) as an amorphous solid.

Alternatively, carbodiimide **3** was obtained following this procedure: Compound **5** (56 mg, 73 μmol) was acetylated by reaction with Ac_2O−pyridine (1:1, 1 mL) at 0 °C for 16 h to furnish **7**.[21c] Then, to a solution of compound **7** in a mixture of H_2O−CH_2Cl_2 (1:1, 6 mL) and HgO (95 mg, 0.44 mmol, 3 equiv) was added. The heterogeneous mixture was vigorously stirred at rt for 6 h, the reaction was diluted with CH_2Cl_2, and the organic phase separated, dried over $MgSO_4$, filtrated over Celite, and concentrated to furnish **3** in 90% yield (79 mg): R_f = 0.44 (3:1 EtOAc−petroleum ether); $[\alpha]_D$ = +173.6 (*c* 1.0, CH_2Cl_2); IR (KBr) ν_{max} 2146, 1755 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.50 (t, 4 H, $J_{2,3} = J_{3,4}$ = 10.3 Hz, H-3), 5.46 (d, 4 H, $J_{1,2}$ = 3.9 Hz, H-1), 5.16 (dd, 4 H, H-2), 5.00 (t, 4 H, $J_{4,5}$ = 10.2 Hz, H-4), 3.94 (ddd, 4 H, $J_{5,6a}$ = 7.4 Hz, $J_{5,6b}$ = 1.7 Hz, H-5), 3.49 (dd, 4 H, $J_{6a,6b}$ = 13.7 Hz, H-6a), 3.07 (dd, 4 H, H-6b), 2.13, 2.05, 2.04 (3 s, 36 H, 12 MeCO); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.0, 169.6, 169.5 (CO), 136.2 (NCN), 91.7 (C-1), 70.2 (C-5), 70.0 (C-3), 69.9 (C-4), 69.3 (C-2), 46.0 (C-6), 21.0, 20.7, 20.6 (MeCO); FAB-MS *m*/*z* 1223 ([M + Na]$^+$). Anal. Calcd for $C_{50}H_{64}N_4O_{30}$: C, 50.00; H, 5.37; N 4.66. Found: C, 49.80; H, 5.13; N, 4.56.

Hemiacetylated CT2 Bis(thiourea) (5). To a solution of 6,6′-diamino-6,6′-dideoxy-α,α′-trehalose[25] (**4**, 200 mg, 0.59 mmol) in pyridine (20 mL), 2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-dideoxy-6,6′-diisothiocyanato-α,α′-trehalose[25] (**2**, 0.4 g, 0.59 mmol) was added, and the reaction mixture was stirred at rt for 16 h. Then, the solvent was evaporated at reduced pressure, and the resulting residue was purified by column chromatography (MeCN → 4:1 MeCN−H_2O) to yield **5** (0.55 g, 92%) as an amorphous solid: R_f = 0.60 (6:1:1 MeCN−H_2O−NH_4OH); $[\alpha]_D$ = +159.0 (*c* 1.0, MeOH); ^1H NMR (500 MHz, DMSO-d_6, 343 K) δ 7.42 (m, 2 H, N^IH), 7.04 (m, 2 H, $N^{II}H$), 5.30 (t, 2 H, $J_{2,3} = J_{3,4}$ = 10.0 Hz, H-3^I), 5.13 (m, 2 H, H-1^I), 5.00 (dd, 2 H, $J_{1,2}$ = 3.5 Hz, H-2^I), 4.94 (t, 2 H, $J_{4,5}$ = 10.0 Hz, H-4^I), 4.89 (d, 2 H, $J_{1,2}$ = 4.0 Hz, H-1^{II}), 4.69 (m, 2 H, OH-4^I), 4.54 (m, 2 H, OH-2^I), 4.50 (m, 2 H, OH-3^I), 3.84 (ddd, 2 H, $J_{5,6b}$ = 6.5 Hz, $J_{5,6a}$ = 3.5 Hz, H-5^I), 3.83 (m, 2 H, H-5^{II}), 3.71 (m, 2 H, H-6^{I}a), 3.54 (t, 2 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3^{II}), 3.50 (bd, 2 H, $J_{6a,6b}$ = 16.5 Hz, H-6^{II}a), 3.42 (m, 2 H, H-6^{II}b), 3.35 (bd, 2 H, H-2^{II}), 3.29 (m, 2 H, H-6^{I}b), 3.06 (m, 2 H, H-4^{II}), 2.05−1.92 (3 s, 18 H, 6 MeCO); ^{13}C NMR (125.7 MHz, DMSO-d_6, 343 K): δ 184.6 (CS), 169.9−169.5 (CO), 94.3 (C-1^{II}), 90.4 (C-1^I), 72.9 (C-3^{II}), 71.8 (C-2^{II}, C-4^{II}), 71.3 (C-5^{II}), 70.6 (C-3^I), 69.6 (C-2^I), 69.5 (C-4^I), 69.3 (C-5^I), 46.0 (C-6^{II}), 44.7 (C-6^I), 21.0−20.7 (MeCO); FAB-MS *m*/*z* 1038 ([M + Na]$^+$). Anal. Calcd for $C_{38}H_{56}N_4O_{24}S_2$: C, 44.88; H, 5.55; N, 5.51; S, 6.31. Found: C, 44.91; H, 5.36; N ,5.51; S, 6.16.

CT2 Bis(thiourea) (6). To a solution of **5** (64 mg, 63 μmol) in dry MeOH (3 mL) and NaOMe (1 M) in MeOH (60 μL, until pH 9) was added. A white precipitate appeared immediately, and after 5 min, H_2O (2 mL) was added to redissolve it. The reaction mixture was further stirred for 30 min, and then neutralized with Amberlite IR-120 (H^+) ion-exchange resin and demineralized with Amberlite MB-9L (H^+, OH^-) mixed ion-exchange resin. The resin was filtered off and the clear solution was concentrated to furnish **6** (39 mg, 81%) as an amorphous solid: R_f = 0.31 (6:3:1 MeCN−H_2O−NH_4OH); $[\alpha]_D$ = +135.0 (*c* 0.6, H_2O); UV (MeOH) λ_{max} 238 nm (ϵ_{mM} 33.1); ^1H NMR (500 MHz, D_2O, 363 K) δ 5.61 (d, 4 H, $J_{1,2}$ = 3.9 Hz, H-1), 4.50 (ddd, 4 H, $J_{4,5}$ = 9.0 Hz, $J_{5,6b}$ = 5.4 Hz, $J_{5,6a}$= 4.4 Hz, H-5), 4.36 (t, 4 H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, H-3), 4.25 (m, 8 H, H-6a, H-6b), 4.17 (dd, 4 H, H-2), 3.92 (t, 4 H, H-4); ^{13}C NMR (75.5 MHz, D_2O, 358 K) δ 185.4 (CS), 96.3 (C-1), 75.7 (C-3), 74.2 (C-2), 73.9 (C-4), 73.8 (C-5), 47.9 (C-6). Anal. Calcd for $C_{26}H_{44}N_4O_{18}S_2$: C, 40.83; H, 5.80; N, 7.33. Found: C, 40.44; H, 5.51; N, 7.11.

Per-*O*-acetylated CT2 Bis(thiourea) (7). To a solution of **5** (0.8 g, 0.79 mmol) in pyridine (5 mL), at 0 °C, Ac_2O (5 mL) was added. The solution was stirred at 0 °C for 16 h. Conventional workup and purification by column chromatography using 100:1 → 30:1 CH_2Cl_2−MeOH afforded **7** (1.02 g, 99%) as an amorphous solid: R_f = 0.55 (20:1 CH_2Cl_2−MeOH). The analytical and spectroscopic data were in full agreement with published results.[21a]

Per-*O*-trimethylsilylated CT2 Bis(thiourea) (8). A solution of **6** (117 mg, 0.16 mmol) in pyridine (9 mL) was treated with a mixture of trimethylsilyl chloride and hexamethyldisilazane (1:2, 3.6 mL) at rt for 16 h. The solvents were removed under reduced pressure, and the residue was extracted with petroleum ether and concentrated to dryness. The resulting residue was purified by column chromatography using 1:9 EtOAc−petroleum ether to give **8** (251 mg, 99%) as an amorphous solid: R_f = 0.36 (1:9 EtOAc−petroleum ether).

Alternatively, compound **8** was also obtained using the following divergent route: To a mixture of diamine **19** (100 mg, 63 μmol) and $CaCO_3$ (6.3 mg, 63 μmol, 1 equiv) in a heterogeneous mixture of H_2O−acetone (1:1, mL), Cl_2CS (2.5 μL, 31.5 μmol, 0.5 equiv) was added. The reaction was stirred vigorously for 30 min. Diluted $NaHCO_3$ was then added until pH 8−9, and the reaction mixture was further stirred for 16 h. The organic layer was decanted, the solvent was evaporated, and the resulting residue was purified by column chromatography (1:9 EtOAc−petroleum ether) to afford **8** (87 mg, 85%). The analytical and spectroscopic data were in full agreement with published results.[21a]

Per-*O*-trimethylsilylated CT2 Bis(carbodiimide) (9). A solution of **6** (122 mg, 0.16 mmol) in pyridine (20 mL) was treated with a mixture of trimethylsilyl chloride and hexamethyldisilazane (1:2, 6 mL) at rt for 16 h. The solvents were removed under reduced pressure, and the residue was extracted with petroleum ether and concentrated to dryness. To a solution of crude **8** thus obtained in a mixture of H_2O−CH_2Cl_2 (1:1, 10 mL) was added HgO (0.2 g, 0.96 mmol, 3 equiv). The heterogeneous mixture was vigorously stirred at rt for 6 h and then diluted with CH_2Cl_2 and the organic phase separated. The organic extract was dried over $MgSO_4$, filtrated over Celite, and concentrated to dryness to yield **9** (0.22 g, 88%) as an amorphous solid. White crystals suitable for X-ray diffractions were obtained from neat EtOAc: R_f = 0.83 (1:9 EtOAc−petroleum ether); $[\alpha]_D$ = +105.6 (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 2134 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.94 (d, 4 H, $J_{1,2}$ = 3.0 Hz, H-1), 3.87 (t, 4 H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, H-3), 3.81 (dt, 4 H, $J_{4,5}$ = 9.0 Hz, $J_{5,6}$ = 3.5 Hz, H-5), 3.43 (t, 4 H, H-4), 3.38 (dd, 4 H, H-2), 3.32 (d, 8 H, H-6), 0.16−0.11 (3 s, 108 H, 12 Me_3Si); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 141.5 (NCN), 94.0 (C-1), 73.4 (C-3), 72.9 (C-2), 72.3 (C-4), 71.2 (C-5), 47.1 (C-6), 1.1−0.3 (Me_3Si); ESI-MS *m*/*z* 1601.3 ([M + K]$^+$), 1584.4 ([M + Na]$^+$). Anal. Calcd for $C_{62}H_{136}N_4O_{18}Si_{12}$: C, 47.65; H, 8.77; N, 3.59. Found: C, 47.80; H, 8.54; N, 3.41.

6-Amino-6′-azido-6,6′-dideoxy-α,α′-trehalose (11). To a mixture of 6,6′-diazido-6,6′-dideoxy-α,α′-trehalose[25] (**10**, 1.62 g, 4.12 mmol), Et_3N (1.15 mL, 2 equiv), and 1,3-propanedithiol (40 μL, 0.1 equiv) in 2-propanol−DMF (2:1, 39 mL) was added $NaBH_4$ (171 mg, 1.1 equiv). The reaction mixture was stirred at rt for 5 h, and then H_2O (5 mL) was added and the solvents were evaporated at reduced pressure. The residue was purified by column chromatography (6:1:1 → 6:3:1 MeCN−H_2O−NH_4OH) to yield **11** (0.61 g, 41%) as an amorphous solid: R_f = 0.41 (6:3:1 MeCN−H_2O−NH_4OH); $[\alpha]_D$ = +93.8 (*c* 0.8, H_2O); IR (KBr) ν_{max} 2142 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 5.16 (d, 1 H, $J_{1,2}$ = 3.4 Hz, H-1^I), 5.13 (d, 1 H, $J_{1,2}$ = 3.5 Hz, H-1^{II}), 3.94 (ddd, 1 H, $J_{4,5}$ = 9.7 Hz, $J_{5,6b}$ = 8.8 Hz, $J_{5,6a}$ = 3.5 Hz, H-5^I), 3.91 (ddd, 1 H, $J_{4,5}$ = 9.5 Hz, $J_{5,6b}$ = 5.8 Hz, $J_{5,6a}$ = 2.6 Hz, H-5^{II}), 3.78 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.7

Hz, H-3^{I}), 3.76 (t, 1 H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3^{II}), 3.61 (dd, 2 H, H-2^{I}, H-2^{II}), 3.60 (dd, 1 H, $J_{6a,6b} = 14.5$ Hz, H-6^{II}a), 3.48 (dd, 1 H, H-6^{II}b), 3.38 (t, 1 H, H-4^{II}), 3.37 (dd, 1 H, $J_{6a,6b} = 13.1$ Hz, H-6^{I}a), 3.31 (t, 1 H, H-4^{I}), 3.09 (dd, 1 H, H-6^{I}b); ^{13}C NMR (125.7 MHz, D_2O) δ 93.9 (C-1^{I}, C-1^{II}), 72.4 (C-3^{II}), 72.1 (C-3^{I}), 71.5 (C-4^{I}), 71.1 (C-5^{II}), 71.0 (C-2^{II}), 70.8 (C-2^{I}), 70.5 (C-4^{II}), 68.3 (C-5^{I}), 51.0 (C-6^{II}), 40.6 (C-6^{I}); FAB-MS *m/z* 387 ([M + Na]$^+$). Anal. Calcd for $C_{12}H_{22}N_4O_9$: C, 39.34; H, 6.05; N, 15.29. Found: C, 39.26; H, 5.91; N, 14.97.

6-Azido-6,6′-dideoxy-6′-isothiocyanato-α,α′-trehalose (12) and 6-Amino-6′-azido-6,6′-dideoxy-α,α′-trehalose 6,4-(Cyclic thiocarbamate) (13). To a stirred suspension of **11** (0.25 g, 0.68 mmol) and $CaCO_3$ (205 mg, 2.04 mmol, 3.0 equiv) in a mixture of acetone−H_2O 1:1 (5 mL) at −10 °C was added $CSCl_2$ (0.08 mL, 1.02 mmol, 1.5 equiv). The mixture was stirred for 1 h at −10 °C, the solvents were evaporated under reduced pressure and the resulting residue was purified by column chromatography (45:5:3 EtOAc−EtOH−H_2O) to furnish, sequentially, the target isothiocyanate **12** (142 mg, 51%) and the cyclic thiocarbamate side product **13** (28 mg, 10%).

Data for **12**: $R_f = 0.48$ (45:5:3 EtOAc−EtOH−H_2O); $[\alpha]_D = +104.0$ (*c* 1.0, MeOH); IR (KBr) ν_{max} 2108 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 5.13 (d, 1 H, $J_{1,2} = 4.0$ Hz, H-1^{I}), 5.12 (d, 1 H, $J_{1,2} = 4.0$ Hz, H-1^{II}), 4.02 (ddd, 1 H, $J_{4,5} = 9.5$ Hz, $J_{5,6b} = 4.5$ Hz, $J_{5,6a} = 3.0$ Hz, H-5^{II}), 4.01 (ddd, 1 H, $J_{4,5} = 9.5$ Hz, $J_{5,6b} = 5.5$ Hz, $J_{5,6a} = 2.0$ Hz, H-5^{I}), 3.78 (m, 2 H, H-6^{II}), 3.77 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3^{II}), 3.74 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3^{I}), 3.51 (dd, 1 H, H-2^{II}), 3.50 (dd, 1 H, $J_{6a,6b} = 13.0$ Hz, H-6^{I}a), 3.49 (dd, 1 H, H-2^{I}), 3.40 (dd, 1 H, H-6^{I}b), 3.30 (t, 1 H, H-4^{I}), 3.29 (t, 1 H, H-4^{II}); ^{13}C NMR (125.7 MHz, CD_3OD): δ 134.0 (NCS), 95.8 (C-1^{II}), 95.6 (C-1^{I}), 74.4 (C-3^{I}), 74.3 (C-3^{II}), 73.1 (C-2^{I}, C-2^{II}), 72.6 (C-4^{I}, C-4^{II}), 71.9 (C-5^{I}, C-5^{II}), 52.6 (C-6^{I}), 47.1 (C-6^{II}); ESI-MS *m/z* 430.9 ([M + Na]$^+$), 409.0 ([M + H]$^+$). Anal. Calcd for $C_{13}H_{20}N_4O_9S$: C, 38.23; H, 4.94; N, 13.72. Found: C, 38.35; H, 4.94; N, 13.61.

Data for **13**: $R_f = 0.41$ (45:5:3 EtOAc−EtOH−H_2O); $[\alpha]_D = +95.0$ (*c* 1.0, H_2O); ^{1}H NMR (300 MHz, D_2O) δ 5.12 (d, 1 H, $J_{1,2} = 3.9$ Hz, H-1^{I}), 5.06 (d, 1 H, $J_{1,2} = 3.7$ Hz, H-1^{II}), 4.15 (m, 1 H, H-5^{II}), 4.00 (t, 1 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, H-3^{I}), 3.96 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz,H-4^{II}), 3.87 (m, 1 H, H-5^{I}), 3.68 (t, 1 H, $J_{2,3} = 9.5$ Hz, H-3^{II}), 3.67 (m, 1 H, H-2^{I}), 3.54 (dd, 1 H, $J_{6a,6b} = 13.5$ Hz, $J_{5,6a} = 3.4$ Hz, H-6^{I}a), 3.53 (dd, 1 H, H-2^{II}), 3.46 (dd, 1 H, $J_{6a,6b} = 12.0$ Hz, $J_{5,6a} = 6.0$ Hz, H-6^{II}a), 3.40 (dd, 1 H, $J_{5,6b} = 5.8$ Hz, H-6^{I}b), 3.32 (t, 1 H, $J_{3,4} = 9.5$ Hz, H-4^{I}), 3.18 (dd, 1 H, $J_{5,6b} = 10.0$ Hz, H-6^{II}b); ^{13}C NMR (75.5 MHz, D_2O): δ 186.9 (CS), 95.9 (C-1^{I}), 95.4 (C-1^{II}), 81.0 (C-4^{II}), 73.6 (C-3^{II}), 72.5 (C-5^{I}), 72.2 (C-2^{II}), 72.0 (C-2^{I}), 71.6 (C-4^{I}), 70.2 (C-3^{I}), 61.5 (C-5^{II}), 52.1 (C-6^{I}), 45.4 (C-6^{II}); ESI-MS *m/z* 430.9 ([M + Na]$^+$), 409.0 ([M + H]$^+$). Anal. Calcd for $C_{13}H_{20}N_4O_9S$: C, 38.23; H, 4.94; N, 13.72. Found: C, 38.15; H, 4.69; N, 13.48.

2,3,4,2′,3′,4′-Hexa-*O*-acetyl-6-azido-6,6′-dideoxy-6′-isothiocyanato-α,α′-trehalose (14) and 2,3,2′,3′,4′-Penta-*O*-acetyl-6-amino-6′-azido-6,6′-dideoxy-α,α′-trehalose 6,4-(Cyclic thiocarbamate) (15). Thiocarbonylation of **11** (0.25 g, 0.68 mmol) with $CSCl_2$ following the procedure as described above for the preparation of **12**, subsequent acetylation of the mixture of **12** and **13** thus obtained at 0 °C, and purification of the resulting per-*O*-acetates by column chromatography (1:2 → 2:1 EtOAc−petroleum ether) afforded, sequentially, isothiocyanate **14** (0.28 g, 63%) and the intramolecular thiocarbamate **15** (63 mg, 15%) as amorphous solids.

Data for **14**: $R_f = 0.30$ (1:1 EtOAc−petroleum ether); $[\alpha]_D = +144.2$ (*c* 1.0, CH_2Cl_2); IR (KBr) ν_{max} 2103 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.47 (t, 1 H, $J_{2,3} = J_{3,4} = 9.9$ Hz, H-3^{II}), 5.42 (t, 1 H, $J_{2,3} = J_{3,4} = 9.9$ Hz, H-3^{I}), 5.33 (d, 2 H, $J_{1,2} = 3.9$ Hz, H-1, H-1^{II}), 5.11 (dd, 1 H, H-2^{I}), 5.07 (dd, 1 H, H-2^{II}), 4.98 (t, 1 H, $J_{4,5} = 9.9$ Hz, H-4^{I}), 4.95 (t, 1 H, $J_{4,5} = 9.9$ Hz, H-4^{II}), 4.12 (ddd, 1 H, $J_{5,6a} = 6.7$ Hz, $J_{5,6b} = 3.3$ Hz, H-5^{II}), 4.07 (ddd, 1 H, $J_{5,6a} = 7.4$ Hz, $J_{5,6b} = 2.3$ Hz, H-5^{I}), 3.64 (dd, 1 H, $J_{6a,6b} = 14.8$ Hz, H-6^{II}a), 3.50 (dd, 1 H, H-6^{II}b), 3.35 (dd, 1 H, $J_{6a,6b} = 13.3$ Hz, H-6^{I}a), 3.14 (dd, 1 H, H-6^{I}b), 2.10−2.00 (6 s, 18 H, 6 MeCO); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.3−169.7 (CO), 136.6 (NCS), 93.6 (C-1^{II}), 93.4 (C-1^{I}), 70.4 (C-3^{I}, C-4^{II}), 70.3 (C-5^{I}), 70.0 (C-2^{II}), 69.9 (C-3^{II}, C-4^{I}), 69.8 (C-2^{I}), 69.0 (C-5^{II}), 51.3 (C-6^{I}), 46.4 (C-6^{II}), 21.0 (MeCO); FAB-MS *m/z* 683 ([M + Na]$^+$). Anal. Calcd for $C_{25}H_{32}N_4O_{15}S$: C, 45.45; H, 4.88; N, 8.48. Found: C, 45.21; H, 4.51; N, 8.44.

Data for **15**: $R_f = 0.18$ (1:1 EtOAc−petroleum ether); $[\alpha]_D = +103.4$ (*c* 1.0, CH_2Cl_2); IR (KBr) ν_{max} 2105, 1753 cm^{-1}; UV (CH_2Cl_2) λ_{max} 247 nm (ϵ_{mM} 7.3); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (d, 1 H, $J_{NH,6a} = 3.5$ Hz, NH), 5.57 (t, 1 H, $J_{2,3} = J_{3,4} = 10.0$ Hz, H-3^{II}), 5.39 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3^{I}), 5.31 (d, 1 H, $J_{1,2} = 4.0$ Hz, H-1^{I}), 5.26 (d, 1 H, $J_{1,2} = 4.0$ Hz, H-1^{II}), 5.03 (dd, 1 H, H-2^{II}), 4.96 (dd, 1 H, H-2^{I}), 4.94 (t, 1 H, $J_{4,5} = 9.5$ Hz, H-4^{I}), 4.15 (ddd, 1 H, $J_{4,5} = 10.0$ Hz, $J_{5,6b} = 9.5$ Hz, $J_{5,6a} = 4.5$ Hz, H-5^{II}), 4.12 (t, 1 H, H-4^{II}), 4.03 (ddd, 1 H, $J_{5,6a} = 7.5$ Hz, $J_{5,6b} = 2.0$ Hz, H-5^{I}), 3.43 (ddd, 1 H, $J_{6a,6b} = 12.0$ Hz, H-6^{II}a), 3.32 (dd, 1 H, $J_{6a,6b} = 13.0$ Hz, H-6^{I}a), 3.28 (dd, 1 H, H-6^{II}b), 3.15 (dd, 1 H, H-6^{I}b), 2.09−1.98 (5 s, 15 H, 5 MeCO); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 186.0 (CS), 169.9−169.5 (CO), 93.8 (C-1^{II}), 93.0 (C-1^{I}), 77.1 (C-4^{II}), 70.1 (C-2^{I}), 70.0 (C-5^{I}), 69.9 (C-2^{II}), 69.7 (C-3^{I}), 69.4 (C-4^{I}), 68.1 (C-3^{II}), 60.7 (C-5^{II}), 50.9 (C-6^{I}), 44.8 (C-6^{II}), 21.0−20.6 (*Me*CO); FAB-MS *m/z* 641 ([M + Na]$^+$). Anal. Calcd for $C_{23}H_{30}N_4O_{14}S$: C, 44.66; H, 4.89; N, 9.06. Found: C, 44.62; H, 4.81; N, 8.95.

***N*-(6′-Azido-6,6′-dideoxy-α,α′-trehalos-6-yl)-*N*′-(2,3,4,2′,3′,4′-tetra-*O*-acetyl-6′-azido-6,6′-dideoxy-α,α′-trehalos-6-yl)thiourea (16).** To a solution of azidoamine **11** (107 mg, 0.29 mmol) in pyridine (10 mL) was added azidoisothiocyanate **14** (0.19 g, 0.29 mmol), and the reaction mixture was stirred at rt for 16 h. The solvent was evaporated under reduced pressure, and the resulting syrup was purified by column chromatography (MeCN → 10:1 MeCN−H_2O) to afford **16** (0.23 g, 78%) as an amorphous solid: $R_f = 0.34$ (10:1:1 MeCN−H_2O−NH_4OH); $[\alpha]_D = +134.3$ (*c* 1.0, MeOH); IR (KBr) ν_{max} 3443, 2106, 1750 cm^{-1}; UV (MeOH) λ_{max} 270 nm ($\epsilon_{mM} = 9.1$); ^{1}H NMR (500 MHz, CD_3OD) δ 5.47 (t, 1 H, $J_{2,3} = J_{3,4} = 10.0$ Hz, H-3^{II}), 5.43 (t, 1 H, $J_{2,3} = J_{3,4} = 10.0$ Hz, H-3), 5.38 (d, 1 H, $J_{1,2} = 3.5$ Hz, H-1), 5.35 (d, 1 H, $J_{1,2} = 3.5$ Hz, H-1^{II}), 5.10 (m, 1 H, H-1^{IV}), 5.09 (d, 1 H, $J_{1,2} = 3.0$ Hz, H-1^{III}), 5.07 (dd, 2 H, H-2, H-2^{II}), 5.02 (t, 1 H, $J_{4,5} = 10.0$ Hz, H-4), 4.94 (t, 1 H, $J_{4,5} = 10.0$ Hz, H-4^{II}), 4.08 (m, 1 H, H-6^{III}a), 4.05 (ddd, 1 H, $J_{5,6a} = 7.0$ Hz, $J_{5,6b} = 2.5$ Hz, H-5), 3.99 (ddd, 1 H, $J_{4,5} = 9.5$ Hz, $J_{5,6b} = 6.0$ Hz, $J_{5,6a} = 2.5$ Hz, H-5^{IV}), 3.98 (ddd, 1 H, $J_{5,6a} = 5.0$ Hz, $J_{5,6b} = 2.0$ Hz, H-5^{II}), 3.91 (dt, 1 H, $J_{4,5} = 9.5$ Hz, $J_{5,6a} = J_{5,6b} = 4.0$ Hz, H-5^{III}), 3.79 (m, 1 H, H-6^{III}b), 3.77 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3^{III}), 3.75 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3^{IV}), 3.53 (dd, 1 H, H-2^{III}), 3.51 (dd, 1 H, H-2^{IV}), 3.49 (dd, 1 H, $J_{6a,6b} = 13.5$ Hz, H-6^{IV}a), 3.45 (dd, 1 H, $J_{6a,6b} = 13.5$ Hz, H-6a), 3.44 (m, 1 H, H-6^{II}a), 3.40 (dd, 1 H, H-6^{IV}b), 3.31 (m, 1 H, H-6^{II}b), 3.30 (dd, 1 H, H-6b), 3.29 (t, 1 H, H-4^{IV}), 3.25 (t, 1 H, H-4^{III}), 2.09−1.99 (5 s, 18 H, 6 MeCO); ^{13}C NMR (125.7 MHz, CD_3OD): δ 185.1 (CS), 171.8−171.3 (CO), 95.5 (C-1^{IV}), 95.4 (C-1^{III}), 93.2 (C-1^{II}), 93.1 (C-1), 74.3 (C-3^{III}, C-3^{IV}), 73.0 (C-2^{III}, C-2^{IV}, C-5^{IV}), 72.6 (C-4^{III}, C-4^{IV}), 71.4 (C-3), 71.3 (C-2, C-2^{II}, C-3^{II}, C-5), 71.1 (C-5^{II}, C-5^{III}), 70.9 (C-4^{II}), 70.8 (C-4), 52.6 (C-6^{IV}), 51.9 (C-6), 45.9, 46.5 (C-6^{II}, C-6^{III}), 20.9−20.6 (*Me*CO); ESI-MS *m/z* 1049.3 ([M + Na]$^+$). Anal. Calcd for $C_{37}H_{54}N_8O_{24}S$: C, 43.27; H, 5.30; N, 10.91. Found: C, 43.04; H, 5.29; N, 10.82.

***N*,*N*′-Bis-(6′-azido-6,6′-dideoxy-α,α′-trehalos-6-yl)thiourea (17).** To a solution of **16** (0.17 g, 0.16 mmol) in MeOH (3 mL) was added NaOMe (1 M) in MeOH (0.1 mL). After 5 min at rt, a white precipitate appeared and H_2O (1 mL) was added to redissolve it. The reaction mixture was stirred for 1 h, then neutralized using Amberlite IR-120 (H^+) ion-exchange resin, and demineralized using Amberlite MB-9L (H^+, OH^-) mixed ion-exchange resin. The resin was filtered off, and the solution was concentrated to dryness to give the fully unprotected derivative **17** (127 mg, 99%) as an amorphous solid: $R_f = 0.63$ (6:3:1 MeCN−H_2O−NH_4OH); $[\alpha]_D = +220.2$ (*c* 0.8, H_2O); UV (H_2O) λ_{max} 238 nm ($\epsilon_{mM} = 12.1$); ^{1}H NMR (500 MHz, D_2O, 313 K) δ 5.28 (d, 2 H, $J_{1,2} = 3.5$ Hz, H-1),

5.23 (m, 2 H, H-1′), 4.07 (ddd, 2 H, $J_{4,5}$ = 9.5 Hz, $J_{5,6b}$ = 6.5 Hz, $J_{5,6a}$ = 2.5 Hz, H-5′), 4.04 (m, 2 H, H-5), 3.99 (m, 2 H, H-6a), 3.94 (t, 2 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 3.93 (t, 2 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3′), 3.78 (dd, 2 H, H-2), 3.77 (dd, 2 H, $J_{1,2}$ = 3.0 Hz, H-2′), 3.76 (m, 2 H, H-6′a), 3.75 (m, 2 H, H-6b), 3.66 (dd, 2 H, $J_{6a,6b}$ = 13.5 Hz, H-6′b), 3.55 (t, 2 H, H-4′), 3.45 (t, 2 H, $J_{4,5}$ = 9.5 Hz, H-4); ^{13}C NMR (125.7 MHz, D_2O, 313 K): δ 182.7 (CS), 93.9 (C-1), 93.8 (C-1′), 72.7 (C-3, C-3′), 71.4 (C-5′), 71.3 (C-4, C-5), 71.2 (C-2, C-2′), 70.9 (C-4′), 51.3 (C-6′), 45.2 (C-6); ESI-MS *m*/*z* 797 ([M + Na]$^+$), 775 ([M + H]$^+$). Anal. Calcd for $C_{25}H_{42}N_8O_{18}S$: C, 38.76; H, 5.46; N, 14.46. Found: C, 38.75; H, 5.15; N, 14.14.

***N,N′*-Bis(6′-azido-6,6′-dideoxy-2,3,4,2′,3′,4′-hexa-*O*-trimethylsilyl-α,α′-trehalos-6-yl)thiourea (18).** A solution of **17** (0.26 g, 0.33 mmol) in pyridine (10.5 mL) was treated with a mixture of trimethylsilyl chloride and hexamethyldisilazane (1:2, 6 mL) at rt for 16 h. The solvents were evaporated, and the residue was extracted with petroleum ether and concentrated to dryness. The resulting residue was purified by column chromatography (1:15 → 1:9 Et_2O−petroleum ether) to furnish **18** (0.4 g, 74%) as an amorphous solid: R_f = 0.41 (1:9 Et_2O−petroleum ether); $[\alpha]_D$ = +105.7 (*c* 0.9, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 250 nm (ϵ_{mM} = 13.8); IR (KBr) ν_{max} 3417, 2101 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.01 (m, 2 H, NH), 4.89 (d, 2 H, $J_{1,2}$ = 3.0 Hz, H-1), 4.85 (d, 2 H, $J_{1,2}$ = 3.0 Hz, H-1′), 3.93 (ddd, 2 H, $J_{4,5}$ = 9.0 Hz, $J_{5,6b}$ = 5.0 Hz, $J_{5,6a}$ = 2.5 Hz, H-5′), 3.91 (m, 2 H, H-5), 3.88 (t, 2 H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, H-3), 3.86 (m, 2 H, H-6a), 3.83 (t, 2 H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, H-3′), 3.41 (dd, 2 H, H-2), 3.40 (dd, 2 H, H-2′), 3.38 (t, 2 H, H-4′), 3.37 (dd, 2 H, $J_{6a,6b}$ = 13.0 Hz, H-6′a), 3.33 (dd, 2 H, H-6′b), 3.29 (t, 2 H, $J_{4,5}$ = 9.0 Hz, H-4), 3.28 (m, 2 H, H-6b), 0.15−0.12 (6 s, 108 H, 12 $SiMe_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 183.6 (CS), 94.4 (C-1′), 94.3 (C-1), 73.4 (C-4), 73.2 (C-3′), 73.0 (C-3), 72.7 (C-2′), 72.6 (C-4′), 72.5 (C-2), 72.4 (C-5′), 71.3 (C-5), 51.6 (C-6′), 46.0 (C-6), 1.3−0.1 ($SiMe_3$); ESI-MS *m*/*z* 1678.4 ([M + K]$^+$), 1663.4 ([M + Na]$^+$). Anal. Calcd for $C_{61}H_{138}N_8O_{18}SSi_{12}$: C, 44.65; H, 8.48; N, 6.83. Found: C, 44.57; H, 8.22; N, 6.74.

***N,N′*-Bis(6′-amino-6,6′-dideoxy-2,3,4,2′,3′,4′-hexa-*O*-trimethylsilyl-α,α′-trehalos-6-yl)thiourea (19).** To a solution of diazide **18** (0.39 g, 0.24 mmol) in a mixture of dioxane−MeOH (5:1, 6 mL) was added TPP (0.38 g, 1.43 mmol, 3 equiv). After 1 h at rt, NH_4OH (30%, 0.5 mL) was added, and the reaction mixture was stirred at rt for further 16 h. Then solvents were removed under reduced pressure, and the solid residue was purified by column chromatography (50:1 → 20:1 CH_2Cl_2−MeOH) to yield **19** (0.37 g, 99%) as an amorphous solid: R_f = 0.40 (9:1 CH_2Cl_2−MeOH); $[\alpha]_D$ = +91.1 (*c* 1.0, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 250 nm (ϵ_{mM} = 15.6); ^{1}H NMR (500 MHz, $CDCl_3$, 313 K) δ 6.10 (m, 2 H, NH), 4.88 (d, 2 H, $J_{1,2}$ = 3.0 Hz, H-1), 4.83 (d, 2 H, $J_{1,2}$ = 2.5 Hz, H-1′), 3.91 (m, 2 H, H-5), 3.90 (t, 2 H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, H-3), 3.88 (t, 2 H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, H-3′), 3.82 (m, 2 H, H-6a), 3.70 (ddd, 2 H, $J_{4,5}$ = 9.0 Hz, $J_{5',6'b}$ = 5.0 Hz, $J_{5',6a}$ = 3.0 Hz, H-5′), 3.40 (dd, 2 H, H-2), 3.39 (dd, 2 H, H-2′), 3.37 (t, 2 H, H-4′), 3.30 (m, 2 H, H-6b), 3.29 (t, 2 H, $J_{4,5}$ = 9.0 Hz, H-4), 2.90 (dd, 2 H, $J_{6a,6b}$ = 13.0 Hz, H-6′a), 2.74 (dd, 2 H, H-6′b), 1.26 (m, 4 H, NH_2), 0.16−0.10 (6 s, 108 H, 12 $SiMe_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$, 313 K) δ 184.0 (CS), 94.2 (C-1′), 93.9 (C-1), 73.9 (C-5′), 73.5 (C-4), 73.3 (C-3′), 73.2 (C-3), 72.9 (C-2′), 72.8 (C-2), 72.5 (C-4′), 71.3 (C-5), 46.1 (C-6), 42.5 (C-6′), 1.1−0.2 ($SiMe_3$); ESI-MS *m*/*z* 1611.6 ([M + Na]$^+$), 1589.6 ([M + H]$^+$). Anal. Calcd for $C_{61}H_{142}N_4O_{18}SSi_{12}$: C, 46.11; H, 9.01; N, 3.53. Found: C, 46.10; H, 8.63; N, 3.54.

Per-*O*-acetylated CT2 Bis(urea) (20). To a solution of the bis(carbodiimide) **3** (100 mg, 0.08 mmol) in a mixture of acetone−H_2O 1:1 (5 mL) was added TFA (5 μL). The mixture was stirred at rt for 7 h, and then the solvents were removed under reduced pressure and the residue purified by column chromatography (EtOAc → 45:5:3 EtOAc−EtOH−H_2O) to yield **20** (101 mg, 97%) as an amorphous solid: R_f = 0.47 (45:5:3 EtOAc−EtOH−H_2O); $[\alpha]_D$ = +159.8 (*c* 1.0, MeOH); ^{1}H NMR (500 MHz, CD_3OD, 333 K) δ 5.48 (t, 4 H, $J_{2,3} = J_{3,4}$ = 9.7 Hz, H-3), 5.30 (m, 4 H, H-1), 5.17 (m, 4 H, H-2), 5.01 (m, 4 H, H-4), 3.84 (m, 4 H, H-5), 3.36 (bd, 4 H, $J_{6a,6b}$ = 14.0 Hz, H-6a), 3.06 (m, 4 H, H-6b), 2.11, 2.02, 2.00 (3 s, 36 H, 12 MeCO); ^{13}C NMR (125.7 MHz, CD_3OD, 333 K) δ 170.4, 170.3 (CO ester), 162.3 (CO urea), 90.5 (C-1), 70.1 (C-2, C-3, C-4, C-5), 40.4 (C-6), 19.4, 19.3, 19.2 (MeCO); FAB-MS *m*/*z* 1259 ([M + Na]$^+$). Anal. Calcd for $C_{50}H_{68}N_4O_{32}$: C, 48.54; H, 5.54; N, 4.53. Found: C, 48.43; H, 5.38; N 4.45.

CT2 Bis(urea) (21). To a solution of **20** (81 mg, 65 μmol) in MeOH (2 mL), NaOMe 1 m in MeOH was added until pH 9. After 30 min, a white precipitate appeared, and H_2O (0.1 mL) was added to redissolve it. The solution was stirred at rt for 5 min and then neutralized with Amberlite IR-120 (H^+) ion-exchange resin, demineralized with Amberlite MB-9L (H^+, OH^-) mixed ion-exchange resin, and concentrated to furnish **21** (43 mg, 91%) as an amorphous solid: R_f = 0.47 (5:3:5 MeCN−H_2O−NH_4OH); $[\alpha]_D$ = +135.0 (*c* 1.0, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 5.06 (d, 4 H, $J_{1,2}$ = 4.0 Hz, H-1), 3.84 (m, 4 H, H-5), 3.78 (t, 4 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 3.60 (dd, 4 H, H-2), 3.46 (dd, 4 H, $J_{6a,6b}$ = 14.7 Hz, $J_{5,6a}$= 2.0 Hz, H-6a), 3.30 (t, 4 H, $J_{4,5}$ = 9.5 Hz, H-4), 3.27 (dd, 4 H, $J_{5,6b}$ = 8.0 Hz, H-6b); ^{13}C NMR (125.7 MHz, D_2O): δ 161.7 (CO), 92.9 (C-1), 72.6 (C-3), 71.3 (C-2, C-4), 70.7 (C-5), 41.2 (C-6); FAB-MS *m*/*z* 755 ([M + Na]$^+$). Anal. Calcd for $C_{26}H_{44}N_4O_{20}$: C, 42.62; H, 6.05; N, 7.65. Found: C, 42.45; H, 6.06; N, 7.46.

Per-*O*-acetylated CT2 Bis(*N″*-benzylguanidine) Dihydrochloride (22). A mixture of carbodiimide **3** (100 mg, 83 μmol) and dry $BnNH_2$·HCl (179 mg, 1.24 mmol, 7.5 equiv) was dissolved in DMF (4 mL) and Et_3N (0.17 mL, 1.24 mmol) under argon. The mixture was vigorously stirred at 100 °C for 20 min. Then, the solvent was removed under reduced pressure, and the resulting residue was partitioned with H_2O−CH_2Cl_2 (15 mL). The organic layer was washed with H_2O, 0.1 N HCl was added until pH 5, and the solution was evaporated under reduced pressure. The residue was purified by column chromatography using 5:1 EtOAc−MeOH affording **22** (112 mg, 91%) as an amorphous solid: R_f = 0.42 (10:1:1 MeCN−H_2O−NH_4OH); $[\alpha]_D$ = +196.6 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (500 MHz, CD_3OD, 333 K) δ 7.40−7.30 (m, 10 H, 2 Ph), 5.60 (bs, 4 H, H-1), 5.54 (m, 4 H, H-3), 5.04 (m, 8 H, H-2, H-4), 4.53 (d, 2 H, $^2J_{H,H}$ = 15.7 Hz, CH_2a), 4.46 (d, 2 H, CH_2b), 3.89 (m, 4 H, H-5), 3.51 (m, 8 H, H-6a, H-6b), 2.15−1.98 (3 s, 36 H, 12 MeCO); ^{13}C NMR (125.7 MHz, CD_3OD, 333 K) δ 170.2 (CO), 157.2 (CN), 135.4−127.1 (Ph), 91.7 (C-1), 70.9−68.1 (C-2, C-3, C-4, C-5), 45.1 (*C*H_2Ph), 41.1 (C-6), 19.4−19.2 (MeCO); ^{1}H NMR (500 MHz, CD_3OD, 233 K) δ 7.42−7.31 (m, 10 H, 2 Ph), 5.90 (d, 2 H, $J_{1',2'}$ = 4.0 Hz, H-1′), 5.62 (d, 2 H, $J_{1,2}$ = 4.1 Hz, H-1), 5.56 (t, 2 H, $J_{2',3'} = J_{3',4'}$ = 10.0 Hz, H-3′), 5.38 (t, 2 H, $J_{2,3} = J_{3,4}$ = 9.7 Hz, H-3), 5.22 (dd, 2 H, H-2′), 5.16 (t, 2 H, $J_{4',5'}$ = 9.8 Hz, H-4′), 5.06 (dd, 2 H, H-2), 4.93 (t, 2 H, $J_{4,5}$ = 9.8 Hz, H-4), 4.49 (d, 2 H, $^2J_{H,H}$ = 16.0 Hz, CH_2a), 4.44 (d, 2 H, $^2J_{H,H}$ = 16.0 Hz, CH_2b), 3.92 (m, 2 H, H-5′), 3.82 (m, 2 H, H-5), 3.63 (dd, 2 H, $J_{6a,6b}$ = 13.6 Hz, $J_{5,6a}$ = 5.4 Hz, H-6a), 3.53 (d, 2 H, H-6b), 3.47 (bd, 2 H, $J_{6a,6b}$ = 13.6 Hz, H-6a′), 3.36 (bd, 2 H, H-6b′), 2.18−2.02 (3 s, 36 H, 12 MeCO); ^{13}C NMR (125.7 MHz, CD_3OD, 233 K) δ 170.4−170.0 (CO), 157.1 (CN), 135.8−127.1 (Ph), 91.8 (C-1), 91.6 (C-1′), 71.6 (C-2′), 69.7 (C-5), 69.6 (C-3), 69.1 (C-2), 68.3 (C-3′), 67.9 (C-5′), 67.6 (C-4), 66.9 (C-4′), 44.8 (*C*H_2Ph), 40.4 (C-6), 39.1 (C-6′), 19.6−19.3 (MeCO); FAB-MS *m*/*z* 1415 ([M + H − 2 HCl]$^+$). Anal. Calcd for $C_{64}H_{84}N_6O_{30}Cl_2$ · 2 H_2O: C, 50.43; H, 5.82; N, 5.51. Found: C, 50.49; H, 5.86; N, 5.47.

CT2 Bis(*N″*-benzylguanidine) Dihydrochloride (23). To a solution of **22** (16 mg, 10 μmol) in MeOH (1 mL) was added NaOMe (1 M) in MeOH until pH 9. After 1 h, the reaction mixture was neutralized with Amberlite IR-120 (H^+) ion-exchange resin, and the solvent was removed under reduced pressure to furnish **23** (9.5 mg, 97%) as an amorphous solid: R_f = 0.57 (7:7:4:5 EtOAc−2-propanol−H_2O−30% NH_4OH); $[\alpha]_D$ = +161.0 (*c* 1.0, H_2O); ^{1}H NMR (500 MHz, D_2O, 353 K) δ 8.00−7.89 (m, 10 H, Ph), 5.43 (bs, 4 H, H-1), 5.15, 5.07 (2 d, 2 H each, $^2J_{H,H}$ = Hz, CH_2Ph), 4.42 (bd, 4 H, $J_{4,5}$ = 9.6 Hz, H-5), 4.33 (t, 4 H, $J_{2,3} = J_{3,4}$ = 9.6

Hz, H-3), 4.15 (bs, 8 H, H-6), 3.97 (bd, 4 H, $J_{1,2}$ = 9.0 Hz, H-2), 3.85 (t, 1 H, H-4); ^{13}C NMR (125.7 MHz, D_2O, 333 K) δ 157.2 (CN), 136.9−126.9 (Ph), 93.3 (C-1), 73.0 (C-3), 71.6, 71.4 (C-2, C-4), 71.1 (C-5), 44.8 (*C*H_2Ph), 42.7 (C-6); FAB-MS *m/z* 911 ([M − 2HCl + H]$^+$). Anal. Calcd for $C_{40}H_{60}N_6O_{18}Cl_2\cdot 2H_2O$: C, 47.11; H, 6.33; N, 8.24. Found: C, 47.11; H, 6.50; N, 8.17.

***N,N′*-Bis(2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-dideoxy-6′-isothiocyanato-α,α′-trehalos-6-yl)thiourea (24).** A solution of 2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-dideoxy-6,6′-diisothiocyanato-α,α′-trehalose (**2**, 0.4 g, 0.6 mmol) in a mixture of pyridine−H_2O (10:1, 15 mL) was stirred at 40 °C for 6 h. Then, the solvents were evaporated under reduced pressure and the resulting residue was purified by column chromatography using 30:1 CH_2Cl_2−MeOH as eluent to afford **24** (162 mg, 43%): R_f = 0.14 (30:1 CH_2Cl_2−MeOH); $[\alpha]_D$ = +97.3 (*c* 0.7, CH_2Cl_2); IR (KBr) ν_{max} 2100 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.32 (bt, 2 H, $J_{NH,6}$ = 6.9 Hz, NH), 5.61 (d, 2 H, $J_{1,2}$ = 3.6 Hz, H-1^{II}), 5.49 (t, 2 H, $J_{2,3}$ = $J_{3,4}$ = 9.4 Hz, H-3^{I}), 5.42 (t, 2 H, $J_{2,3}$ = $J_{3,4}$ = 9.4 Hz, H-3^{II}), 5.35 (d, 2 H, $J_{1,2}$ = 4.0 Hz, H-1^{I}), 5.10 (dd, 2 H, H-2^{II}), 4.97 (t, 2 H, $J_{4,5}$ = 9.3 Hz, H-4^{I}), 4.93 (t, 2 H, $J_{4,5}$ = 9.3 Hz, H-4^{II}), 4.84 (dd, 2 H, H-2^{I}), 4.06 (m, 2 H, H-5^{I}), 4.02 (m, 2 H, H-6^{II}a), 3.85 (bt, 2 H, $J_{5,6b}$ = 9.3 Hz, H-5^{II}), 3.64 (dd, 2 H, $J_{6a,6b}$ = 14.8 Hz, $J_{5,6a}$ = 6.6 Hz, H-6^{I}a), 3.54 (dd, 2 H, $J_{5',6b'}$ = 3.4 Hz, H-6^{I}b), 3.31 (m, 2 H, H-6^{II}b), 2.10, 2.07, 2.03, 2.00 (4 s, 36 H, 12 MeCO); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 184.4 (CS), 169.9, 169.7, 169.6, 169.3 (CO), 136.2 (NCS), 92.8 (C-1^{II}), 92.2 (C-1^{I}), 70.8 (C-2^{I}), 70.0 (C-3^{II}), 69.9 (C-4^{I}), 69.6 (C-5^{II}), 69.5 (C-2^{II}, C-4^{II}), 69.2 (C-3^{I}), 68.5 (C-5^{I}), 46.0 (C-6^{I}), 45.1 (C-6^{II}), 20.7, 20.6, 20.5 (*Me*CO); ESI-MS *m/z* 1333 [M + Na]$^+$; HRFAB-MS *m/z* 1310.292101, calcd for $C_{51}H_{66}N_4O_{30}S_3$ 1310.292403.

Hemiacetylated CT3 Tris(thiourea) (25). To a solution of 6,6′-diamino-6,6′-dideoxy-α,α′-trehalose[25] (**4,** 105 mg, 0.3 mmol) in pyridine (10 mL) was added diisothiocyanate **24 (**203 mg, 0.15 mmol), and the reaction mixture was stirred at rt for 16 h. The solvent was evaporated under reduced pressure, and the resulting residue was purified by column chromatography (45:5:3 EtOAc−EtOH−H_2O) to yield **25** (188 mg, 76%) as an amorphous solid: R_f = 0.34 (45:5:3 EtOAc−EtOH−H_2O); $[\alpha]_D$ = +117.3 (*c* 1.0, MeOH); ^{1}H NMR (500 MHz, MeOD, 323 K) δ 5.47 (t, 2 H, $J_{2,3}$ = $J_{3,4}$ = 10.6 Hz Hz, H-3^{I}), 5.46 (t, 2 H, $J_{2,3}$ = $J_{3,4}$ = 10.1 Hz Hz, H-3^{II}), 5.39 (d, 2 H, $J_{1,2}$ = 3.6 Hz Hz, H-1^{II}), 5.38 (d, 2 H, $J_{1,2}$ = 3.7 Hz Hz, H-1^{II}), 5.11 (d, 2 H, $J_{1,2}$ = 3.7 Hz, H-1^{III}), 5.05 (dd, 2 H, H-2^{II}), 5.01 (dd, 2 H, H-2^{I}), 4.94 (t, 2 H, $J_{4,5}$ = 10.1 Hz Hz, H-4^{I}), 4.93 (t, 2 H, $J_{4,5}$ = 10.6 Hz Hz, H-4^{II}), 4.11 (bd, 2 H, $J_{6a,6b}$ = 15.1 Hz, H-6^{I}a), 3.95 (ddd, 2 H, $J_{5,6b}$ = 6.6 Hz, $J_{5,6a}$ = 2.5 Hz, H-5^{I}), 3.95 (ddd, 2 H, $J_{5,6b}$ = 6.5 Hz, $J_{5,6a}$ = 3.0 Hz, H-5^{III}), 3.93 (ddd, 2 H, $J_{5,6a}$ = 7.0 Hz, $J_{5,6b}$ = 3.8 Hz, H-5^{II}), 3.88 (bd, 2 H, $J_{6a,6b}$ = 12.6 Hz, H-6^{II}a), 3.81 (m, 2 H, H-6^{III}a), 3.76 (t, 2 H, $J_{2,3}$ = $J_{3,4}$ = 9.9 Hz, H-3^{III}), 3.69 (m, 2 H, H-6^{III}b), 3.61 (bd, 2 H, H-6^{II}b), 3.53 (dd, 2 H, H-2^{III}), 3.42 (dd, 2 H, H-6b^{I}), 3.26 (t, 2 H, $J_{4,5}$ = 9.9 Hz, H-4^{III}), 2.10, 2.09, 2.04, 2.02, 2.00, 1.98 (6 s, 36 H, 12 MeCO); ^{13}C NMR (125.7 MHz, MeOD, 323 K) δ 184.1, 184.0 (CS), 170.5, 169.7, 169.6, 169.5 (CO), 95.0 (C-1^{III}), 91.3 (C-1^{I}, C-1^{II}), 72.8 (C-3^{III}), 71.9 (C-2^{III}), 71.6 (C-4^{III}), 71.1 (C-5^{III}), 70.1 (C-2^{I}, C-2^{II}), 70.0 (C-3^{I}, C-3^{II}), 69.7(C-4^{I}), 69.6 (C-4^{II}), 69.4 (C-5^{I}, C-5^{II}), 45.6 (C-6^{III}), 44.6 (C-6^{I}), 44.1 (C-6^{II}), 20.7, 20.6, 20.5, 20.4 (MeCO). Anal. Calcd. for $C_{63}H_{90}N_6O_{39}S_3$: C, 45.81; H 5.49; N, 5.09. Found: C, 45.65; H, 5.42; N, 4.96.

CT3 Tris(thiourea) (26). To a solution of **25** (188 mg, 0.11 mmol) in MeOH (4 mL) was added NaOMe (1 M) in MeOH (0.13 mL). After 5 min at rt, a white precipitate appeared, and H_2O (3 mL) was added to redissolve it. The reaction mixture was stirred for additional 30 min. Then reaction was neutralized using Amberlite IR-120 (H^+) ion-exchange resin and demineralized using Amberlite MB-9L (H^+, OH^-) mixed ion-exchange resin. The resin was filtered off, and the solution was concentrated to furnish the fully deprotected derivative **26** (126 mg, 96%): R_f = 0.39 (2:1:1 BuOH−AcOH−H_2O); $[\alpha]_D$ = +60.0 (*c* 1.0, H_2O); ^{1}H NMR (500 MHz, D_2O, 313 K) δ 5.47 (d, 6 H, $J_{1,2}$ = 3.8 Hz Hz, H-1), 4.27 (ddd, 6 H, $J_{4,5}$ = 9.8 Hz, $J_{5,6b}$ = 7.0 Hz, $J_{5,6a}$ = 2.9 Hz, H-5), 4.24 (m, 6 H, H-6a), 4.18 (t, 6 H, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, H-3), 4.03 (m, 6 H, H-6b), 3.99 (dd, 6 H, H-2), 3.71 (t, 6 H, H-4); ^{13}C NMR (125.7 MHz, D_2O, 323 K): δ 183.7 (CS), 95.5 (C-1), 74.2 (C-2), 72.9 (C-3, C-4, C-5), 46.8 (C-6). Anal. Calcd for $C_{39}H_{66}N_6O_{27}S_3$: C, 40.83; H, 5.80; N, 7.32. Found: C, 40.51; H, 5.88; N, 7.25.

Per-*O*-acetylated CT3 Tris(thiourea) (27). To a solution of **26** (0.2 g, 0.17 mmol) in pyridine (5 mL) at 0 °C was added Ac_2O (5 mL). The solution was stirred at 0 °C for 16 h. Conventional workup and purification by column chromatography using 100:1 → 30:1 CH_2Cl_2−MeOH afforded **27** (0.33 g, 99%) as an amorphous solid: R_f = 0.23 (20:1 CH_2Cl_2−MeOH); $[\alpha]_D$ = +131.0 (*c* 0.3, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, 313 K) δ 6.11 (bs, 6 H, NH), 5.48 (t, 6 H, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz Hz, H-3), 5.43 (d, 6 H, $J_{1,2}$= 3.6 Hz, H-1), 4.95 (t, 6 H, $J_{4,5}$ = 9.6 Hz, H-4), 4.92 (dd, 6 H, H-2), 3.97 (m, 6 H, H-6a), 3.92 (m, 6 H, H-5), 3.56 (m, 6 H, H-6b), 2.10, 2.09, 2.08, 2.03 (4 s, 54 H, 18 MeCO); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 184.1 (CS), 171.1, 169.9 (CO), 92.2 (C-1), 70.4 (C-2), 69.5 (C-3), 69.3 (C-4), 69.2 (C-5), 44.6 (C-6), 20.8, 20.7 (MeCO); FAB-MS *m/z* 1926 [M + Na]$^+$. Anal. Calcd for $C_{75}H_{102}N_6O_{45}S_3$: C, 47.31; H, 5.40; N, 4.41; S, 5.05. Found: C, 47.35: H, 5.27; N, 4.30; S, 4.97.

Per-*O*-trimethylsilylated CT3 Tris(thiourea) (28). A solution of **26** (126 mg, 0.11 mmol) in pyridine (9 mL) was treated with a mixture of trimethylsilyl chloride and hexamethyldisilazane (1:2, 3 mL) at rt for 16 h. Then, the solvents were evaporated, and the residue was extracted with petroleum ether. The solvent was removed under reduced pressure and the resulting residue was purified by column chromatography (1:9 EtOAc−petroleum ether) to furnish **28** (0.27 g, 99%) as an amorphous solid: R_f = 0.35 (1:9 EtOAc−petroleum ether); $[\alpha]_D$ = +82.9 (*c* 1.0, CH_2Cl_2). UV (CH_2Cl_2) λ_{max} 249 nm (ϵ_{mM} = 61.9); ^{1}H NMR (500 MHz, $CDCl_3$, 313 K): δ 5.72 (m, 6 H, NH), 4.80 (d, 6 H, $J_{1,2}$ = 2.5 Hz, H-1), 4.43 (m, 6 H, H-6a), 3.94 (t, 6 H, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, H-3), 3.89 (bt, 6 H, $J_{4,5}$ = $J_{5,6b}$ = 9.0 Hz, $J_{5,6a}$ = 3.5 Hz, H-5), 3.40 (dd, 6 H, H-2), 3.26 (t, 6 H, H-4), 3.05 (m, 6 H, H-6b), 0.20−0.13 (3 s, 162 H, 18 $SiMe_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 183.2 (CS), 93.7 (C-1), 74.0 (C-4), 72.9 (C-3), 72.7 (C-2), 71.5 (C-5), 46.6 (C-6), 1.1−0.0 ($SiMe_3$); ESI-MS *m/z* 2415.0 ([M − TMS + K]$^+$), 2342.0 ([M − 2 TMS + K]$^+$). Anal. Calcd for $C_{93}H_{210}N_6O_{27}S_3Si_{18}$: C, 45.66; H, 8.65; N, 3.44. Found: C, 45.69; H, 8.48; N, 3.41.

Per-*O*-acetylated CT3 Tris(carbodiimide) (29). To a solution of **27** (166 mg, 68 μmol) in H_2O−CH_2Cl_2 (1:1, 6 mL) was added HgO (129 mg, 0.612 mmol, 3 equiv). The resulting mixture was vigorously stirred at rt for 4 h and then diluted with CH_2Cl_2, the organic layer was decanted, dried over anhydrous $MgSO_4$, and filtered over Celite, and the solvent was evaporated to dryness to furnish **29** (103 mg, 84%) as an amorphous solid: R_f = 0.78 (9:1 CH_2Cl_2−MeOH); $[\alpha]_D$ = +105.3 (*c* 1.0, CH_2Cl_2); IR (KBr) ν_{max} 2143, 1753 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.44 (t, 6 H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3), 5.35 (d, 6 H, $J_{1,2}$ = 4.0 Hz, H-1), 5.17 (dd, 6 H, H-2), 5.07 (t, 6 H, $J_{4,5}$ = 9.5 Hz, H-4), 4.08 (m, 6 H, H-5), 3.38 (dd, 6 H, $J_{6a,6a}$ = 14.5 Hz, $J_{5,6a}$ = 7.5 Hz, H-6a), 3.06 (d, 6 H, H-6b), 2.08−2.01 (3 s, 54 H, 18 MeCO); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 170.2−169.5 (CO), 138.7 (NCN), 93.7 (C-1), 70.2 (C-3), 69.9 (C-5), 69.6 (C-4), 69.4 (C-2), 45.8 (C-6), 20.8−20.6 (MeCO); ESI-MS *m/z* 1839.5 ([M + K]$^+$), 1823.6 ([M + Na]$^+$), 1801.6 ([M + H]$^+$). Anal. Calcd. for $C_{75}H_{96}N_6O_{45}$: C, 50.00; H, 5.37; N, 4.66. Found: C, 49.95; H, 5.47; N, 4.57.

Per-*O*-trimethylsilylated CT3 Tris(carbodiimide) (30). To a solution of **28** (115 mg, 47 μmol) in H_2O−CH_2Cl_2 (1:1, 4 mL) was added HgO (89 mg, 0.42 mmol, 3 equiv) was added. The resulting mixture was vigorously stirred at rt for 4 h and then diluted with CH_2Cl_2, the organic layer decanted, dried over $MgSO_4$, and filtered over Celite, and the solvent was evaporated to dryness to furnish **30** (80 mg, 76%): R_f = 0.61 (1:9 EtOAc−petroleum ether); $[\alpha]_D$ = +127.0 (*c* 1.0, CH_2Cl_2); IR (KBr) ν_{max} 2139 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.91 (d, 6 H, $J_{1,2}$ = 3.0 Hz, H-1), 3.88 (t, 6 H, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, H-3), 3.83 (m, 6 H, H-5), 3.51 (t, 6 H, $J_{4,5}$ = 9.0 Hz, H-4), 3.42 (m, 12 H, H-6a, H-6b), 3.41 (dd, 6 H, H-2),

0.17−0.10 (3 s, 162 H, 18 Me_3Si); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 139.6 (NCN), 94.2 (C-1), 73.5 (C-3), 72.7 (C-2), 71.9 (C-4), 71.5 (C-5), 46.3 (C-6), 1.1−0.2 (Me_3Si); ESI-MS *m*/*z* 2345.0 ([M + H]$^+$). Anal. Calcd for $C_{93}H_{204}N_6O_{27}Si_{18}$: C, 47.65; H, 8.77; N, 3.59. Found: C, 47.62; H, 8.82; N, 3.44.

Per-*O*-acetylated CT3 Tris(urea) (31). To a solution of **29** (50.5 mg, 28 μmol) in 1:1 acetone−H_2O (3 mL) was added TFA (50 μL). The reaction mixture was stirred at rt for 16 h, and then the solvents were evaporated under reduced pressure and the residue was purified by column chromatography (EtOAc → 45:5:3 EtOAc−EtOH−H_2O) to give tris(urea) **31** (47.3 mg, 91%) as an amorphous solid: R_f = 0.50 (45:5:3 EtOAc−EtOH−H_2O); $[\alpha]_D$ +136.6 (*c* 1.0, MeOH); ^1H NMR (500 MHz, CD_3OD) δ 5.51 (t, 6 H, $J_{2,3} = J_{3,4}$ = 10.0 Hz, H-3), 5.46 (d, 6 H, $J_{1,2}$ = 3.5 Hz, H-1), 5.04 (dd, 6 H, H-2), 4.97 (t, 6 H, $J_{4,5}$ = 10.0 Hz, H-4), 3.90 (ddd, 6 H, $J_{5,6b}$ = 6.5 Hz, $J_{5,6a}$ = 3.0 Hz, H-5), 3.34 (dd, 6 H, $J_{6a,6b}$ = 14.0 Hz, H-6a), 3.28 (dd, 6 H, H-6b), 2.14−2.01 (3 s, 54 H, 18 MeCO); ^{13}C NMR (125.7 MHz, CD_3OD): δ 171.9−171.2 (CO ester), 160.0 (CO urea), 92.6 (C-1), 71.5 (C-2), 71.4 (C-3), 70.9 (C-5), 70.7 (C-4), 40.6 (C-6), 20.8−20.6 (MeCO); ESI-MS *m*/*z* 1877.6 ([M + Na]$^+$). Anal. Calcd for $C_{75}H_{102}N_6O_{48}$: C, 48.54; H, 5.54; N, 4.53. Found: C, 48.41; H, 5.63; N, 4.53.

CT3 Tris(urea) (32). To a solution of **31** (39 mg, 21 μmol) in MeOH (1 mL) was added methanolic 1 M NaOMe until pH 9. After 5 min at rt, a white precipitate appeared. H_2O (1 mL) was then added to redissolve it, and the reaction mixture was further stirred for 30 min. Then the reaction was neutralized using Amberlite IR-120 (H^+) ion-exchange resin and demineralized using Amberlite MB-9L (H^+, OH^-) mixed ion-exchange resin. The resin was filtered off, and the solution was concentrated to furnish the fully deprotected derivative **32** (23 mg, 99%): R_f = 0.48 (5:3:5 MeCN−H_2O−NH_4OH); $[\alpha]_D$ = +136.4 (*c* 1.0, H_2O); ^1H NMR (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 NMR (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); ESI-MS *m*/*z* 1137.1 ([M + K]$^+$), 1121.2 ([M + Na]$^+$), 569.1 ([M + K + H]$^{2+}$). 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.

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Supporting Information Available: General experimental methods, including computational, NMR titration, and stoichiometry determination of complexes and fluorescent binding titration details, ORTEP plot for **9** with full numbering system, Tables S1−S6 with relevant crystallographic data, as well as the complete X-ray crystallographic data in CIF format, copies of the NMR spectra for compounds **3**, **5**−**9**, and **11**−**32**, and binding isotherms for the 1:1 **26**/TNS and **26**/ANS complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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