JOC Article

Size-Tunable Trehalose-Based Nanocavities: Synthesis, Structure, and Inclusion Properties of Large-Ring Cyclotrehalans

David Rodríguez-Lucena,[†] Carmen Ortiz Mellet,[†] Carlos Jaime,[‡] Kepa K. Burusco,[‡] José M. García Fernández,[§] and Juan M. Benito*[,§]

Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, Apartado 553, E-41071 Sevilla, Spain, Departamento de Química, Universidad Autónoma de Barcelona, E-08193 Bellaterra, Spain, and Instituto de Investigaciones Químicas, CSIC - Universidad de Sevilla, Américo Vespucio 49, Isla de la Cartuja, E-41092 Sevilla, Spain

juanmab@iiq.csic.es

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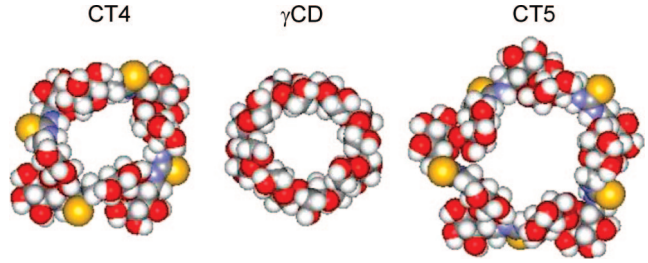

An efficient strategy toward the synthesis of large-ring cyclodextrin (CD) analogs alternating α,α′-trehalose disaccharide subunits and pseudoamide segments (cyclotrehalans, CTs), involving a bimolecular macrocyclization reaction as the key step, is reported. NMR and molecular modeling confirmed that the eight and ten α-D-glucopyranoside subunits in tetrameric and pentameric CT homologues (CT4 and CT5, respectively) are magnetically equivalent, as in the γ and εCD counterparts. Yet, the orientation of the monosaccharide constituents is reversed in CTs as compared with CDs, the β-face being directed to the inside of the nanometric cavity while the α-face remains in contact with the bulk solvent. Molecular mechanics and dynamics experiments revealed that the cyclooligosaccharide architecture in CT4 and CT5 is relatively flexible, which is in contrast to that previously observed for the first members of the CT series (CT2 and CT3 oligomers). Thus, although in their fully expanded conformation their cavity size is close to that of γCD, the higher mobility of the pseudoamide bridges as compared with classical glycosidic linkages endows these hosts with induced fitting capabilities toward smaller guests.

Introduction

Selective recognition of small molecules by their specific receptors is on the origin of fundamental biological processes. Efforts to unravel the molecular basis governing such supramolecular phenomena have prompted chemists to design a plethora of artificial systems meant to imitate associative/dissociative behaviors in a controlled fashion.[1] Naturally occurring macrocyclic structures have strongly inspired many of these designs.[2] Among them, the cyclomaltooligosaccharides (cyclodextrins, CDs), cyclic oligomers composed of α-(1→4)-linked D-glucopyranosyl units, hold a prominent position due to their innate ability to host hydrophobic molecules within their nanometric cavity while dissolved in polar solvents.[3] These favorable characteristics have been translated into applications in fields

* To whom correspondence should be addressed. Fax: +34 954460565. Tel: +34 954489560.
[†] Departamento de Química Orgánica, Universidad de Sevilla.
[‡] Universidad Autónoma de Barcelona.
[§] Instituto de Investigaciones Químicas, CSIC - Universidad de Sevilla.

(1) (a) Lehn, J.-M. *Supramolecular Chemistry, Concepts and Perspectives*; VCH: Weinheim, 1995. (b) Cooke, G. *Angew. Chem.* **2003**, *115*, 5008; *Angew. Chem., Int. Ed.* **2003**, *42*, 4860.

(2) Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Vögtle, F., Eds. *Comprehensive Supramolecular Chemistry*; Elsevier: Oxford, U.K., 1996; Vols. *1*−*11*.

(3) For a comprehensive review on cyclodextrins, see: Szejtli, J. *Chem. Rev.* **1998**, *98*, 1743.

(4) For review papers, see: (a) Svoboda, J.; König, B. *Chem. Rev.* **2006**, *106*, 5413. (b) Takahashi, K. *Chem. Rev.* **1998**, *98*, 2013.

(5) For recent publications, see: (a) Yang, C.; Mori, T.; Inoue, Y. *J. Org. Chem.* **2008**, *73*, 5786. (b) Lu, R.; Yang, C.; Cao, Y.; Wang, Z.; Wada, T.; Jiao, W.; Mori, T.; Inoue, Y. *Chem. Commun.* **2008**, 374. (c) Senra, J. D.; Malta, L. F. B.; de Souza, A. L. F.; Medeiros, M. E.; Aguiar, L. C. S.; Antunes, O. A. C. *Tetrahedron Lett.* **2007**, *48*, 8153.

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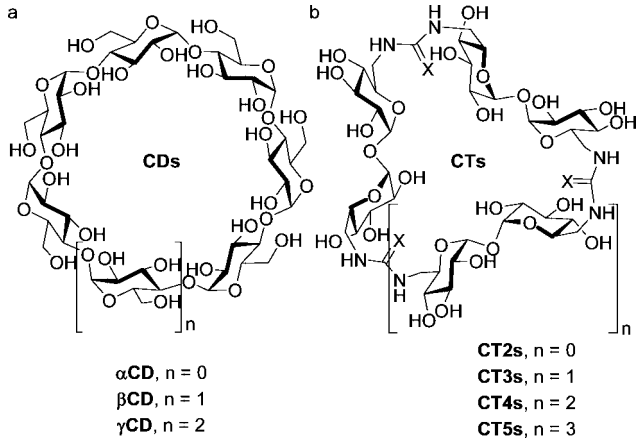


FIGURE 1. Schematic representation of cyclodextrin (CDs, a) and cyclotrehalan (CTs, b; X may represent S or O) family members.

such as molecular reactors,[4,5] drug delivery systems,[6,7] artificial enzymes,[8,9] catalysis,[10] molecular machines,[11,12] or supramolecular sensing,[13] to cite just a few. Commercial availability of the most representative CD family members, namely α, β, and γCD (Figure 1a, $n = 0$, 1, and 2, respectively), at reasonable prices has further fueled the burst of CD-based applications.[14,15]

In spite of the virtues of CDs as molecular hosts, the range of architectures available in terms of size and topology is rather restricted, which represents a serious limitation. Chemists have profusely developed CD derivatives displaying different functional groups at specific locations in the structure but have only limitedly succeeded to engineer the CD cavity interior.[16] The construction of tailor-made artificial glyconanocavities by de novo synthesis, an attractive alternative to overcome this confined scenario, remains a complicated challenge. The chemical methodologies at hand for the assembly of cyclooligosaccharide constructs generally require costly and time-consuming schemes.[17] Stimulated by their interesting supramolecular properties and potential applications, a range of novel glycooligomer host molecules with differently shaped internal cavities have been obtained by replacement of the natural glycosidic bonds by alternative linkages, including thioether,[18] acetylene,[19] amide,[20] amine,[21] 1,2,3-triazole,[22] or phosphate functionalities.[23]

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.[24] Like CDs, CTs feature a troncoconic structure with a hydrophobic cavity flanked by magnetically equivalent α-D-glucopyranoside units (Figure 1b). Yet, they were conceived as "reverse CDs", that is, to expose the β-face of the α-(1→1)-linked glucopyranosyl subunits to the inner cavity, instead of the α-face that coats the interior of the CD torus. The concept was originally demonstrated for the particular case of dimeric and trimeric macrocycles alternating α,α′-trehalose and thiourea moieties (Figure 1b, where $n = 0$ or 1 and X = S)[25] and further extended to the isosteric urea and guanidine analogues by exploiting the chemistry of carbodiimides.[26]

Whereas in the first members of the series, the cyclodimeric CTs (CT2s), the cavity is collapsed by the presence of strong intramolecular hydrogen bonds, the corresponding trimers (CT3s) exhibit a permanent cavity of hydrophobic nature, whose dimensions are intermediate between those of α and βCD. 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.[27] Here we report the preparation of higher

(6) For a recent review, see: Vyas, A.; Saraf, S.; Saraf, S. *J. Incl. Phenom. Macro.* **2008**, *62*, 23.

(7) (a) Hattori, K.; Kenmoku, A.; Mizuguchi, T.; Ikeda, D.; Mizuno, M.; Inazu, T. *J. Incl. Phenom. Macro.* **2006**, *56*, 9. (b) Benito, J. M.; Gómez-García, M.; Ortiz Mellet, C.; Baussanne, I.; Defaye, J.; García Fernández, J. M. *J. Am. Chem. Soc.* **2004**, *126*, 10355. (c) Mazzaglia, N.; Forde, D.; Garozzo, D.; Malvagna, P.; Ravoo, B. J.; Darcy, R. *Org. Biomol. Chem.* **2004**, *2*, 957. (d) Ortiz Mellet, C.; Defaye, J.; García Fernández, J. M. *Chem.—Eur. J.* **2002**, *8*, 1982.

(8) For a review, see: Villalonga, R.; Cao, R.; Fragoso, A. *Chem. Rev.* **2007**, *107*, 3088.

(9) (a) Yuan, D.-Q.; Kitagawa, Y.; Aoyama, Y.; Douke, T.; Fukudome, M.; Fujita, K. *Angew. Chem., Int. Ed.* **2007**, *46*, 5024. (b) Bjerre, J.; Nielsen, E. H.; Bols, M. *Eur. J. Org. Chem.* **2007**, 745. (c) Bjerre, J.; Fenger, T. H.; Marinescu, L. G.; Bols, M. *Eur. J. Org. Chem.* **2007**, 704. (d) Marinescu, L. G.; Bols, M. *Angew. Chem., Int. Ed.* **2006**, *45*, 4590. (e) Ortega-Caballero, F.; Rousseau, C.; Christensen, B.; Petersen, T. E.; Bols, M. *J. Am. Chem. Soc.* **2005**, *127*, 3238.

(10) (a) Zhou, Y.-H.; Zhao, M.; Mao, Z.-W.; Ji, L.-N. *Chem.—Eur. J.* **2008**, *14*, 7193. (b) Dong, Z.-Y.; Mao, S.-Z.; Liang, K.; Liu, J.-Q.; Luo, G.-M.; Shen, J.-C. *Chem.—Eur. J.* **2006**, *12*, 357. (d) Hapiot, F.; Tilloy, S.; Monflier, E. *Chem. Rev.* **2006**, *106*, 767. (c) Jeunesse, C.; Armspach, D.; Matt, D. *Chem. Commun.* **2005**, 5603.

(11) For a review, see: Wenz, G.; Han, B. H.; Muller, A. *Chem. Rev.* **2006**, *106*, 782.

(12) (a) Zhao, Y. L.; Dichtel, W. R.; Trabolsi, A.; Saha, S.; Aprahamian, I.; Stoddart, J. F. *J. Am. Chem. Soc.* **2008**, *130*, 11294. (b) Wang, Y.; Ma, N.; Wang, Z.; Zhang, X. *Angew. Chem., Int. Ed.* **2006**, *45*, 546. (c) Coulston, R. J.; Onagi, H.; Lincoln, S. F.; Easton, C. J. *J. Am. Chem. Soc.* **2006**, *128*, 14750. (d) Tian, H.; Wang, Q. C. *Chem. Soc. Rev.* **2006**, *35*, 364. (e) Smiljanic, N.; Moreau, V.; Yockot, D.; Benito, J. M.; García Fernández, J. M.; Djedaïni-Pilard, F. *Angew. Chem., Int. Ed.* **2006**, *45*, 546.

(13) (a) Zhang, L.; Wu, Y.; Brunsveld, L. *Angew. Chem., Int. Ed.* **2007**, *46*, 1798. (b) Medintz, I. L.; Clapp, A. R.; Mattoussi, H.; Goldman, E. R.; Fisher, B.; Mauro, J. M. *Nat. Mater.* **2003**, *2*, 630. (c) Tong, A. J.; Yamauchi, A.; Hayashita, T.; Zhang, Z.-Y.; Smith, B. D.; Teramae, N. *Anal. Chem.* **2001**, *73*, 1530.

(14) For technological applications, see: (a) Skriba, G. K. E. *J. Sep. Sci.* **2008**, *31*, 1991. (b) Araki, J.; Ito, K. *Soft Matter* **2008**, *3*, 1456. (c) Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Vötgel, F., Eds. *Comprehensive Supramolecular Chemistry*; Elsevier: Oxford, U.K., 1996; Vol. 3.

(15) For pharmaceutical and biomedical applications, see: (a) Davis, M. E.; Brewster, M. E. *Nat. Rev. Drug Discovery* **2004**, *3*, 1023. (b) Li, J.; Loh, X. J. *Adv. Drug. Delivery Rev.* **2008**, *60*, 1000. (c) Cal, K.; Centkowska, K. *Eur. J. Pharm. Biopharm.* **2008**, *68*, 467. (d) Carrier, R. L.; Miller, L. A.; Ahmed, I. *J. Control. Release* **2007**, *123*, 78.

(16) (a) Fukudome, M.; Shiratani, T.; Nogami, Y.; Yuan, D.-Q.; Fujita, K. *Org. Lett.* **2006**, *8*, 5733. (b) Fukudome, M.; Shiratani, T.; Immel, S.; Nogami, Y.; Yuan, D.-Q.; Fujita, K. *Angew. Chem., Int. Ed.* **2005**, *44*, 4201. (c) Nogami, Y.; Nasu, K.; Koga, T.; Ohta, K.; Fujita, K.; Immel, S.; Lindner, H. J.; Schmitt, G. E.; Lichtenthaler, F. W. *Angew. Chem., Int. Ed.* **1997**, *36*, 1899. (d) Gadelle, A.; Defaye, J. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 78.

(17) Gatuso, G.; Nepogodiev, S. A.; Stoddart, J. F. *Chem. Rev.* **1998**, *98*, 1919.

(18) Fan, L.; Hinsgaul, O. *Org. Lett.* **2002**, *4*, 4503.

(19) Bürli, R.; Vasella, A. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1852.

(20) (a) Ménand, M.; Blais, J.-C.; Hamon, L.; Valéry, J.-M.; Xie, J. *J. Org. Chem.* **2005**, *70*, 4423. (b) Van Well, R. M.; Marinelli, L.; Erkelens, K.; van der Marel, G. A.; Lavecchia, A.; Overkleeft, H. S.; van Boom, J. H.; Kessler, H.; Overhand, M. *Eur. J. Org. Chem.* **2003**, 2303. (c) Locardi, E.; Stöckle, M.; Gruner, S.; Kessler, H. *J. Am. Chem. Soc.* **2001**, *123*, 8189.

(21) Ménand, M.; Blais, J.-C.; Valéry, J.-M.; Xie, J. *J. Org. Chem.* **2006**, *71*, 3295.

(22) (a) Bodine, K. D.; Gin, D. Y.; Gin, M. S. *J. Am. Chem. Soc.* **2004**, *126*, 1638. (b) Bodine, K. D.; Gin, D. Y.; Gin, M. S. *Org. Lett.* **2005**, *7*, 1371.

(23) (a) Di Fabio, G.; Randazzo, A.; D'Onofrio, J.; Ausín, C.; Pedroso, E.; Grandas, A.; De Napoli, L.; Montesarchio, D. *J. Org. Chem.* **2006**, *71*, 3395. (b) Coppola, C.; Aggiomo, V.; Di Fabio, G.; De Napoli, L.; Montesarchio, D. *J. Org. Chem.* **2007**, *72*, 9679. (c) D'Onofrio, J.; Coppola, C.; Di Fabio, G.; De Napoli, L.; Montesarchio, D. *Eur. J. Org. Chem.* **2007**, 3849.

(24) García Fernández, J. M.; Ortiz Mellet, C.; Defaye, J. *J. Inclusion Phenom. Macrocyclic Chem.* **2006**, *56*, 149.

(25) (a) García Fernández, J. M.; Jiménez Blanco, J. L.; Ortiz Mellet, C.; Fuentes Mota, J. *J. Chem. Soc., Chem. Commun.* **1995**, 57. (b) Jiménez Blanco, J. L.; Benito, J. M.; Ortiz Mellet, C.; García Fernández, J. M. *Org. Lett.* **1999**, *1*, 1217. (c) Benito, J. M.; Jiménez Blanco, J. L.; Ortiz Mellet, C.; García Fernández, J. M. *Angew. Chem., Int. Ed.* **2002**, *41*, 3674.

(26) Rodríguez-Lucena, D.; Benito, J. M.; Ortiz Mellet, C.; García Fernández, J. M. *J. Incl. Phenom. Macro.* **2007**, *57*, 147.

(27) Rodríguez-Lucena, D.; Benito, J. M.; Álvarez, E.; Jaime, C.; Pérez-Mirón, J.; Ortiz Mellet, C.; García Fernández, J. M. *J. Org. Chem.* **2008**, *73*, 2967.

CT homologues (CT4s and CT5s, Figure 1b, where $n = 2$ or 3) by taking advantage of highly efficient thiourea-forming bimolecular macrocyclization reactions involving trehalose-based building blocks. The conformational properties of these CTs, governed by the pseudoamide intersaccharidic linkages, have been studied by dynamic NMR and computational methods. Their molecular inclusion capabilities toward a variety of hydrophobic guests as compared with CDs are also discussed.

Results and Discussion

Some preliminary considerations are important when making the choice of the strategy for the preparation of large-ring CTs. In principle, de novo design of cyclooligosaccharidic receptors is circumscribed to one of two approaches, namely: (a) intramolecular cyclization of a suitable functionalized linear precursor that already contains all the elements of the final host (divergent approach) or (b) cyclooligomerization of building blocks that correspond to fragments of the target cyclooligosaccharide (convergent approach).[17] A priori there are not obvious advantages for any of these strategies. The choice is strongly dependent on the particular target structure and the properties and accessibility of the corresponding building blocks. In the case of cyclotrehalans, the α,α′-trehalose unit features a very rigid convex conformation in solution, due to the confluence of superimposed anomeric and exo-anomeric effects at the interglycosidic oxygen[28] that fits well in macrocyclic architectures. Moreover, the incorporation of pseudoamide intersaccharidic segments at the primary positions in linear trehalo-oligomers has been shown to promote folding patterns that favor the closure of macrocyclic rings by either divergent or convergent pathways.[25] Except for the case of the dimeric CT2s, the smallest CT representatives, which can be accessed from homobifunctional precursors, any of these routes imply dissymmetrization of the C_2-symmetric trehalose framework at a certain stage of the synthetic scheme. Lessons learned from previous work on the construction and intramolecular macrocyclization of linear trehalo-oligomers prompt us to discard this strategy for larger CTs.[27] Bimolecular macrocyclization of C_2-symmetric precursors bearing complementary functional groups at the terminal primary positions was the method of choice. Although the Staudinger−aza-Wittig type tandem reaction[29] of diazide and diisothiocyanate precursors has been shown to lead to the corresponding macrocyclic carbodiimide, the thiourea-bridging reaction between amines and isothiocyanates is found more convenient in terms of workup and final yield. Among the possible convergent schemes for assembling the cyclooctasaccharide and cyclodecasaccharide cores of CT4 and CT5 homologues, those involving the shorter linear precursors were preferred, that is, the [2 + 2] and the [3 + 2] approaches, respectively (Figure 2).

Synthesis of Thiourea-Linked Linear Trehalo-Oligomers. Our previous work on the comparative analysis of divergent and convergent strategies for the synthesis of small-ring CTs (CT2s and CT3s, Figure 1b) already paved the way for the preparation of linear dimeric and trimeric trehalo-oligomers incorporating thioureylene intersaccharide bridges.[27] The preparation of homobifunctional C_2-symmetric building blocks bearing amine and isothiocyanate groups at the primary C-6 positions

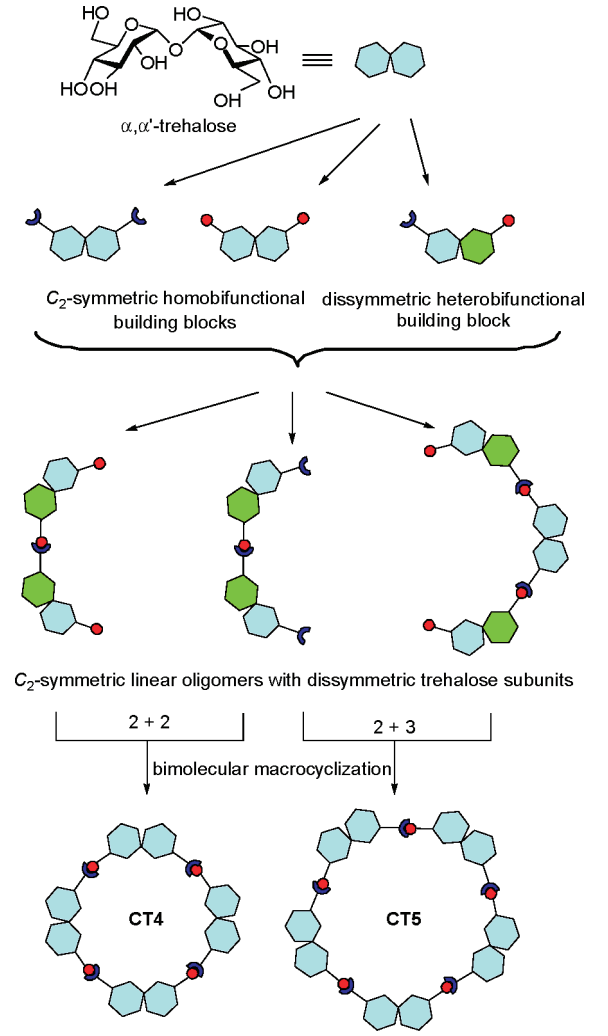


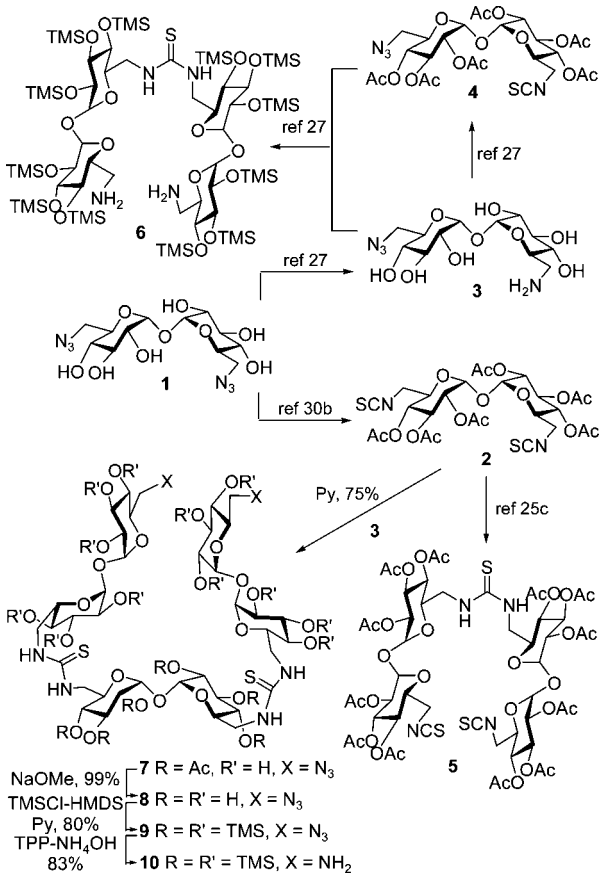
FIGURE 2. Schematic representation of the synthetic routes leading to large-ring CTs (CT4s and CT5s). Dissymmetric trehalose subunits are indicated by different colors.

is straight ahead from the pivotal 6,6′-diazido-6,6′-dideoxy-α,α′-trehalose precursor **1**.[30] Most importantly, the pyridine-promoted self-condensation reaction[31] of diisothiocyanate **2** can be stopped after formation of a single thiourea bridge, which provides a very convenient access to the linear dimer **5**,[25c,27] in which dissymmetrization of the trehalose moieties already took place.

Partial reduction of **1** with the 1,3-propanedithiol/sodium borohydride system[32] proved to be very convenient to access the heterobifunctional disaccharides **3** and **4**,[27] which are instrumental for the synthesis of the oligomeric linear diamines **6** and **10**. Coupling of **3** and **4** in pyridine, followed by protecting group manipulation and reduction of the terminal azido groups, provided the per-*O*-trimethylsilylated diamine linear dimer **6**.[27] For the preparation of the homologous diamine trimer, a bidirectional synthesis involving the coupling reaction of the symmetric diisothiocyanate **2** with the dissymmetric aminoazide **3** (→ **7**) was implemented. Removal of the acetyl

(28) Tvaroska, I.; Bleha, T. *Adv. Carbohydr. Chem. Biochem.* **1989**, *47*, 45.
(29) García-Moreno, M. I.; Díaz-Pérez, P.; Ortiz Mellet, C.; García Fernández, J. M. *J. Org. Chem.* **2003**, *68*, 8890.
(30) (a) Jiménez Blanco, J. L.; García Fernández, J. M.; Gadelle, A.; Defaye, J. *Carbohydr. Res.* **1997**, *303*, 367. (b) García Fernández, J. M.; Ortiz Mellet, C.; Jiménez Blanco, J. L.; Fuentes Mota, J.; Gadelle, A.; Coste-Sarguet, A.; Defaye, J. *Carbohydr. Res.* **1995**, *268*, 57.
(31) 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.
(32) Katajisto, J.; Karskela, T.; Heinonen, P.; Lönnberg, H. *J. Org. Chem.* **2002**, *67*, 7995.

SCHEME 1. Synthesis of Thiourea-Linked Linear Trehalo-Oligomeric Building Blocks

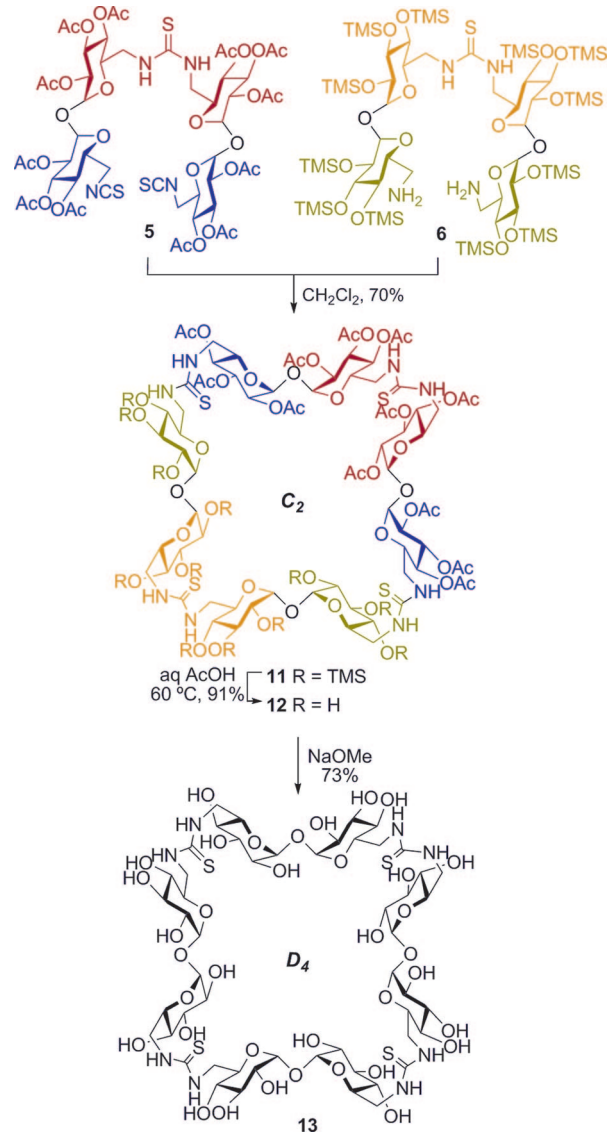


protecting groups in the adduct (→ **8**), followed by per-*O*-trimethylsilylation (→ **9**) and final reduction of the terminal azide functionalities by reaction with triphenylphosphine, afforded the requested pseudohexasaccharide diamine **10** (Scheme 1).

Synthesis of CT4 and CT5 Derivatives. With all the necessary linear building blocks in hand, the corresponding bimolecular macrocyclization reactions were attempted. The use of silyl ether hydroxyl protecting groups in the diamine reagents was purposely chosen to avoid problems associated with O → N acyl migration. Acetylated diisothiocyanate **5** and silylated diamine **6** were efficiently coupled in CH_2Cl_2 to furnish the target thioureido CT4 **11** in an astonishing 70% yield, demonstrating the favorable orientation of the reacting groups (Scheme 2). No formation of linear oligomers or higher cyclic homologues was detected even when performing the reaction at relatively high concentration (7.5 mM). The corresponding ^{1}H NMR spectrum exhibited four different spin systems, in agreement with the C_2 symmetry of the cyclic octasaccharide structure as a result of the acetyl/silyl-protecting group pattern. Sequential acid-promoted hydrolysis of the silyl groups (→ **12**) and deacetylation afforded the fully unprotected thiourea-bridged CT4 **13** in 73% yield. The ^{1}H and ^{13}C NMR spectra confirmed the expected D_4 symmetry of **13**, where the eight glucopyranosyl units became magnetically equivalent.

The synthesis of thiourea-bridged CT5 derivatives followed a similar scheme, replacing the dimeric linear diamine **6** by the trimeric homologue **10** in the bimolecular macrocyclization step (→ **14**; 55% yield, Scheme 3). Both **14** and the hemiacetylated derivative **15** exhibited five different spin systems in their NMR

SCHEME 2. Convergent Synthesis of Thiourea-Bridged CT4 Derivatives[a]



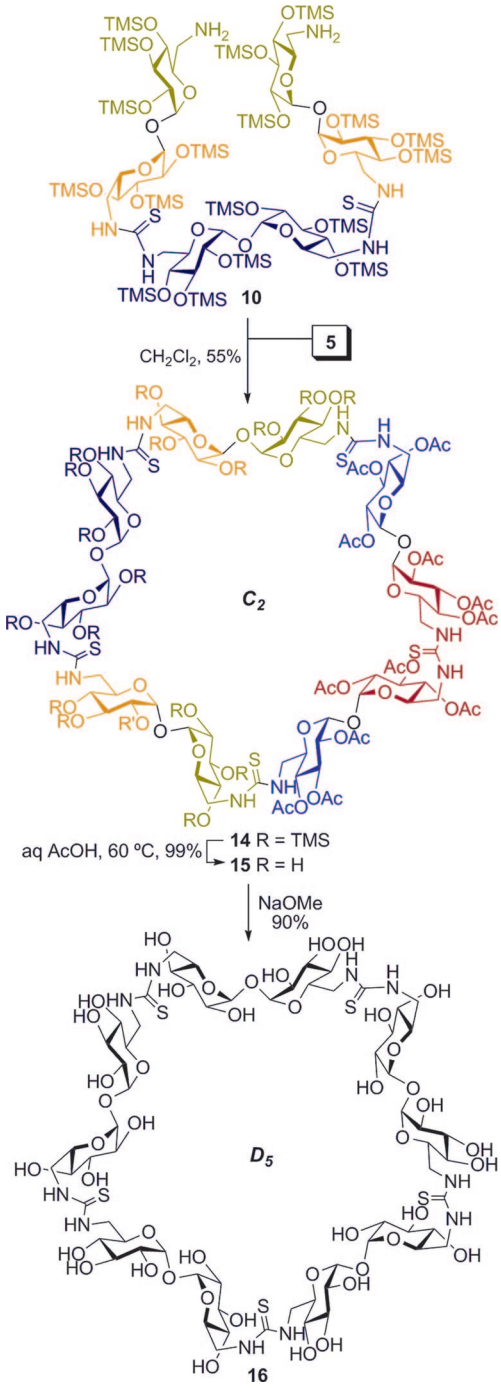
[a] Different colors indicate different spin systems.

spectra that collapsed to a single one after total deprotection (→ **16**), in agreement with the expected C_2 and D_5 symmetry, respectively. The efficiency of the synthetic methodology is remarkable considering the size of the molecules. Pentameric CTs, formally cyclodecasaccharides, are the higher homologues of the CT family reported so far and are among the biggest carbohydrate-based macrocycles obtained by chemical synthesis to date.

Since the semirigid pseudoamide intersaccharide groups could, in principle, modulate the macrocycle conformational flexibility and provide additional interactions with an included guest, the possibility to chemically modify the thiourea groups in the preformed thiourea-CTs was explored next. For such a purpose, the per-*O*-acetylated and per-*O*-silylated thiourea-bridged CT4 and CT5 derivatives **17**, **18** and **23**, **24** were first prepared from the unprotected compounds **13** and **16**, respec-

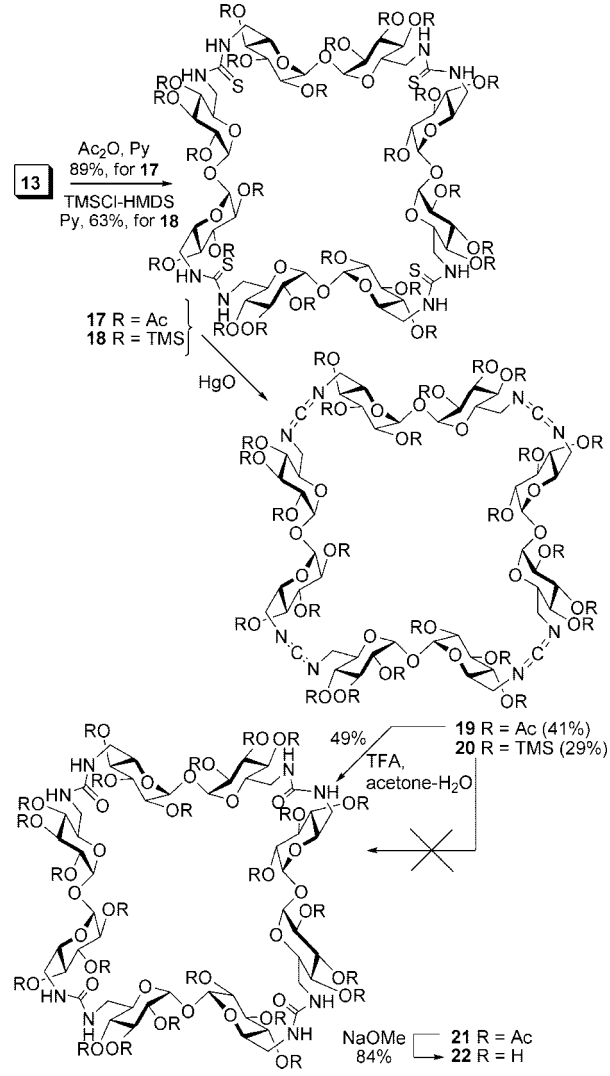
(33) Acetylation of thiourea adducts must be conducted at temperatures lower than 0 °C to prevent formation of *N*- or *S*-acetyl derivatives. See: García-Moreno, M. I.; Benito, J. M.; Ortiz Mellet, C.; García Fernández, J. M. *Tetrahedron: Asymmetry* **2000**, *11*, 161.

SCHEME 3. Convergent Synthesis of Thiourea-Bridged CT5 Derivatives[a]



[a] Different colors indicate different spin systems.

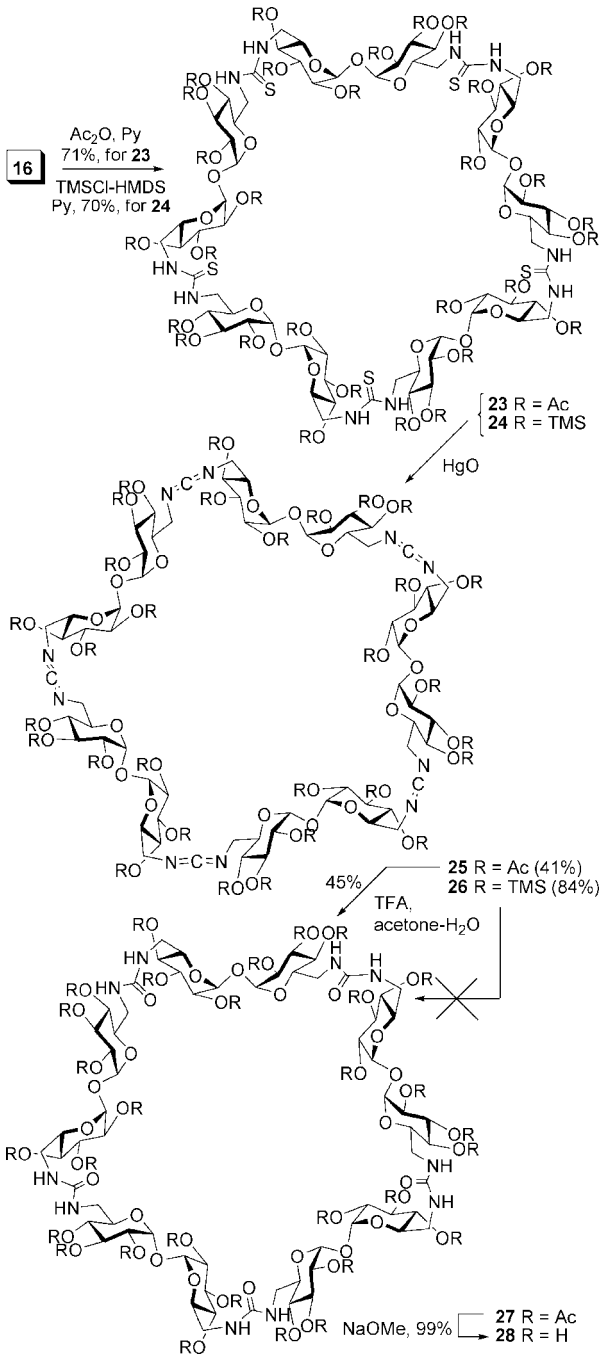
SCHEME 4. Synthesis of Urea-Bridged CT4s via Carbodiimide Derivatives



tively, through standard procedures (Schemes 4 and 5, respectively).[33] Treatment of the tetrakis- (**17**, **18**) or pentakis-thioureas (**23**, **24**) with HgO in a heterogeneous mixture of CH_2Cl_2 and H_2O afforded the corresponding macrocyclic carbodiimides **19**, **20** and **25**, **26**, respectively. Although conversion yields were high in all cases, isolated yields were variable, depending mainly on the particular chromatographical properties of the target compounds. The silyl ether derivatives **20** and **26** exhibited a surprising inertness toward oxygen or nitrogen nucleophiles, probably because the bulky trimethylsilyl groups prevent nucleophile approaching. The per-*O*-acetate **20** was also a stable compound both in the solid state and in solution, even in aqueous mixtures, at neutral pH. Nevertheless, it smoothly underwent addition of H_2O at room temperature in acetone in the presence of catalytic amounts of trifluoroacetic acid to give the corresponding tetrakis-urea **21** in 49% yield. Final deacetylation gave the unprotected ureido-CT4 **22** in 84% yield (Scheme 3). Analogously, acid-catalyzed H_2O addition to the per-*O*-acetyl carbodiimide-bridged CT5 **26** (→ **27**) and subsequent acetate cleavage furnished the pentakis-urea **28** (Scheme 5). Attempts to prepare the corresponding guanidines by nucleophilic addition of benzylamine hydrochloride to the heterocumulene groups in **20** and **26** resulted in incomplete transformations at rt and in concomitant deacetylation upon heating, leading to mixtures from which pure compounds could not be isolated (data not shown).

Structural and Conformational Properties of CT4s and CT5s. The NMR spectra at 298 K of the thiourea-bridged CT4s (**11**−**13** and **17**, **18**) and CT5s (**14**−**16** and **23**, **24**) exhibited significant line broadening, indicative of the existence of chemical exchange processes associated with slow rotations about the pseudoamide bonds. Line broadening was considerably alleviated at 313 K ($CDCl_3$) or 323 K (CD_3OD or D_2O). At such temperatures, all spectra were consistent with a C_2 symmetry featuring either four or five different spin systems

SCHEME 5. Synthesis of Urea-Bridged CT5s via Carbodiimide Derivatives



for CT4 (**11** and **12**) and CT5 (**14** and **15**) derivatives, respectively. Fully symmetric macrocyclic architectures (**13**, **17**, **18**, and **16**, **23**, **24**), in which all glucopyranosyl subunits become magnetically equivalent, showed a single spin system. The vicinal coupling constant (*J*) values about the pyranose rings confirmed the $^1C_4$ chair conformation. Variable temperature NMR experiments carried out on the *O*-protected derivatives discarded the existence of preferred rotameric patterns. Probably, the large macrocyclic architecture 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, with relatively low rotational barriers.

In order to have a deeper insight on the cavity dimensions, shape, and properties of this family of carbohydrate-based hosts,

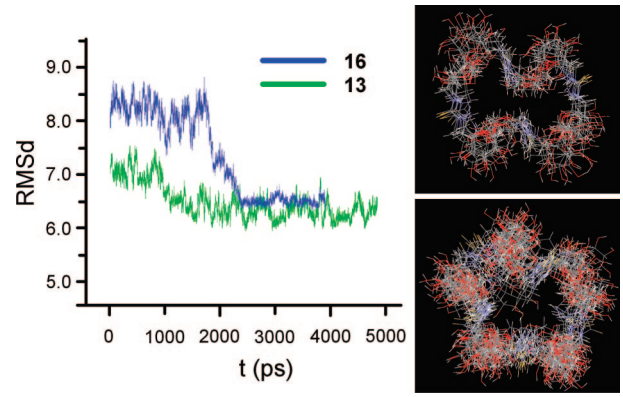


FIGURE 3. Root-mean-square (rms) deviations observed during MD calculations for **13** (green) and **16** (blue) in H_2O and superimposition of calculated conformations for **13** (0−1000 ps) and **16** (0−2000 ps) in explicit H_2O.

molecular mechanics (MM) and molecular dynamics (MD) simulations were carried out on the fully unprotected thiourea-bridged derivatives **13** and **16**. For the generation of the initial structure of the α,α′-trehalose moieties, the previously reported X-ray coordinates of bis-carbodiimido-CT2 were used.[27] The C-5–C-6 bonds of the α-D-glucopyranosyl subunits were set in the gauche−trans (gt) conformation and the thiourea groups were set in the *Z*,*Z*-configuration, generally more stable in the absence of intramolecular hydrogen bonds.[34] Preliminary simulations (500 ps) were carried out using the GBSA continuum solvent model for H_2O, monitoring the geometry every picosecond. The structures with the angles having the highest frequency were selected for the further MD simulation experiments (298 K, up to 5000 ps) both in the vacuum and in aqueous solution using an explicit solvent model.

For the tetrameric CT4 **13**, MD calculations confirmed the higher flexibility of the macrocyclic structure as compared with smaller CT analogs, which allows a wider range of conformational displays. Calculations in vacuo revealed intramolecular interactions that rapidly led to a collapse of the macrocycle cavity. The scenario changed when MD experiments were run in explicit H_2O. For the first 1000 ps of the simulation, the calculated time-averaged root-mean-square (rms) deviations of the moment of inertia remained stable, corresponding to a twisted conformation that is reminiscent of that encountered in large-ring cyclodextrins[35] (Figure 3, green). Longer simulation times resulted in a decrease on the rms deviation parameter as a result of molecular folding and cavity collapse. The pentameric homologue **16** showed a similar trend. A twisted conformation, featuring an open cavity, remained virtually unchanged for the initial 2000 ps during the simulation in explicit H_2O (Figure 3, blue). During the second half of the simulation, however, a significant drop in the rms deviation was observed, associated with the stabilization of a folded conformation in which the cavity was collapsed.

The above results are someway in contradiction with experimental observations. The folded conformation of compounds **13** and **16** would imply the adoption of the tg (trans-gauche) conformation about the C-5−C-6 bond in a significant propor-

(34) (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.

(35) Saenger, W.; Jacob, J.; Gessler, K.; Steiner, T.; Hoffmann, D.; Sanbe, H.; Koizumi, K.; Smith, S. M.; Takaha, T. *Chem. Rev.* **1998**, *98*, 1787.

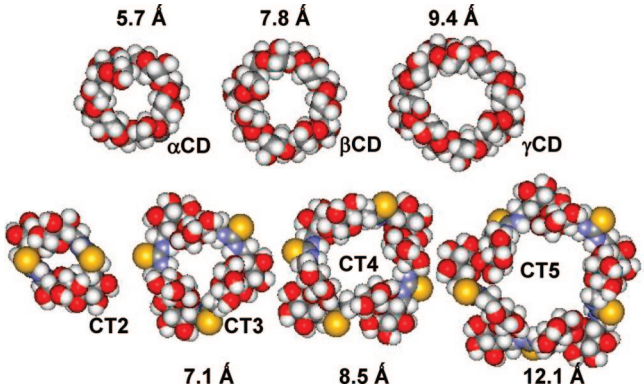


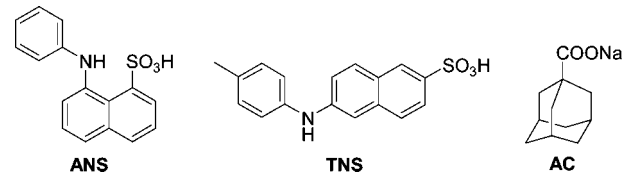
FIGURE 4. CPK top views of minimized conformations of CDs (top) and CTs (bottom) with indication of the average nanocavity diameter.

tion of the glucopyranosyl units, which is neither likely to occur in glucose derivatives[36] nor be visible in the NMR spectra. Furthermore, the absence of NOE contacts between the protons of magnetically unequivalent glucopyranosyl units in the C_2 symmetric CT4 derivative **12**, other that those that are diagnostic for the α-(1→1) glycosidic linkage of trehalose, discards proximity relationships in solution. Since the calculations were performed using Amber 7 and related force fields (see Supporting Information for details), which are optimized for proteins rather than for carbohydrates, it is probable that H-bond-driven intramolecular interactions are overestimated in our systems, leading to artifacts when using relatively long simulation times. Most probably, the ensemble of twisted conformations obtained after 1000 or 2000 ps, which keep an open cavity, are more representative for the conformational behavior of compounds **13** and **16** in H_2O solution.

In any case, the computational studies evidence that the cavity shape of the thiourea-bridged CT4 and CT5 is much less rigid than that of α-, *β*-, or *γ*CDs or that of the corresponding CT3 representative.[27] In spite of this flexible framework, the average cavity shape and size is conserved in the above discussed time frame limit. While mean dimensions of the CT3 cavity (7.1 Å internal medium diameter) were intermediate between those of α- and *β*CD (5.7 and 7.8 Å, respectively), CT4 **13** and CT5 **16** feature slightly smaller (8.5 Å) and larger (12.1 Å) nanocavities, respectively, as compared with *γ*CD (9.4 Å; Figure 4). Consequently, they are in principle presumed to form inclusion complexes in water with hydrophobic molecules of appropriate size.

Inclusion Capabilities of CT4 and CT5. The inclusion capabilities of cyclotrehalans **13** and **16** were evaluated against adamantane-1-carboxylate (AC), 6-*p*-toluidino-2-naphthalene-sulfonate (TNS), and 8-anilinonaphthalene-1-sulfonate (ANS, Chart 1). Thiourea-bridged CT3 and AC have been shown to form a strong inclusion complex with an association constant (K_{as}) value of 4.6×10^4 M^{-1}, even stronger than the *β*CD−AC complex (3.9×10^4 M^{-1}),[37] which was assigned to a perfect size match and ternary symmetry complementarity.[25c] For the pentameric CT **16**, the ^1H chemical shift variations induced by addition of increasing quantities of AC in D_2O were imperceptible and did not allow an accurate analysis. In the case of **13**, though complexation induced shifts (CIS) were still very small,

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



the NMR titration experiments in D_2O were highly reproducible and compatible with a 1:1 **13**−AC binding pattern. Least-squares fitting of the experimental binding isotherm provided a K_{as} value of 1.8×10^4 M^{-1} (see Supporting Information for details).[38] Although this value must be taken with care, it suggests that going from the CT3 to the CT4 derivative does not reduce significantly the binding affinity toward AC, supporting the existence of an open cavity in solution. The 2.5-fold decrease in the K_{as} is much smaller than that observed when going from the *β*CD−AC to the *γ*CD−AC complex (3.8×10^3 M^{-1}),[39] which might reflect the highest conformational adaptability of cyclotrehalans as compared with cyclodextrins, thereby compensating affinity losses due to size mismatching.

Because we were aware that the small CIS values (in the range of 0.01 ppm) seriously hamper the extraction of robust conclusions, the inclusion capabilities of the tetrakis-thiourea **13** and pentakis-thiourea **16** were further investigated toward TNS and ANS by fluorescence spectroscopy. 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.[40] Moreover, TNS and ANS are almost positional isomers; they feature very different steric volumes, corresponding to quasi-linear and angular molecular shapes, respectively, and are very well suited to assess the conformational adaptability of different host partners.

All four 1:1 complexes between CTs **13** and **16** and ANS and TNS were clearly identified in the corresponding negative-mode ESI-mass spectra for each host−guest pair.[41] The presence of complexes of higher stoichiometry was not detected for any of them. Job's plot[42] of each host−guest pair was compatible with this observation, discarding a significant contribution of 2:1 or 1:2 complexes to the overall binding (see Supporting Information).[43] Fluorescence intensity steadily increased upon addition of **13** or **16** to buffered aqueous solutions of either ANS or TNS, though variations were modest as compared with that observed for CDs. This fact points to a less hydrophobic environment in CT cavities, probably due to the hydrophilic

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

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

(38) The authors kindly thank Dr. C. Hunter for providing the titration isotherm curve fitting program. For a detailed description of the fitting methods and equations, see: (a) Bisson, A. P. Hunter, C. A. Morales, J. C. Young, K. *Chem.—Eur. J.* **1998**, *4*, 845. (b) Bisson, A. P. Carver, F. J. Eggleston, D. S. Haltiwanger, R. C. Hunter, C. A. Livingston, D. L. McCabe, J. F. Rotger, C. Rowan, A. E. *J. Am. Chem. Soc.* **2000**, *122*, 8856. Errors are estimated to be in the range of ±15%.

(39) Cromwell, W. C.; Bystrom, K.; Eftink, M. R. *J. Phys. Chem.* **1985**, *89*, 326.

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

(41) Recent studies of noncovalent binding in supramolecular complexes by ESI-MS/MS revealed that interactions existing in the gas phase are relatively reflective of their solution behavior. See: Nesatyy, V. J. *Int. J. Mass Spectrom.* **2002**, *221*, 147.

(42) The 1:1 complex stoichiometry was confirmed by the continuous variation method (Job plot). For a thorough description, see: (a) 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) These results parallel those reported for the CD series. See: Schneider, H.-J.; Blatter, T.; Simova, S. *J. Am. Chem. Soc.* **1991**, *113*, 1996.

TABLE 1. Association Constants (K_{as}, M^{-1}) for 1:1 Complexes Determined by Fluorescence Titration[43]

guest	αCD[a]	βCD[a]	γCD[a]	thiourea−CT3[b]	**13**	**16**
ANS	115	115	1260	839 ± 25	315 ± 20	75 ± 5
TNS	83	2820	79	940 ± 23	49 ± 2	7 ± 1

[a] K_{as} of CD complexes were extracted from ref 37, except γCD−ANS.[45] [b] See Figure 1b, $n = 1$, X = S.[27]

character of the intersaccharidic thiourea linkages. Nevertheless, the corresponding binding isotherms (see Supporting Information) were indicative of the formation of 1:1 complexes. The K_{as} values[44] (Table 1) are consistently lower than those previously obtained for the analogous thiourea-bridged CT3 with both guests. Moreover, the relative binding affinities are reversed, the complexes with ANS being now more stable than those with TNS. The stepwise decrease of binding efficiency on going from CT3 to CT4 (**13**) and CT5 (**16**) derivatives indicates that the larger the macrocyclic structure, the higher the energy cost of fitting the cavity to the guest, this effect being more pronounced for the linear guest TNS. When comparing with the rigid CD series,[37] the higher flexibility of CTs translates into much smaller differences in K_{as} values, which is also consistent with a lower dependence of the inclusion abilities on cavity size−guest size fitting.

Conclusions

In summary, we have implemented a modular methodology for the convergent synthesis of α,α′-trehalose-based cyclooligosaccharide receptors using pseudoamide intersaccharidic bridges, namely cyclotrehalans (CTs). The efficiency of the synthetic scheme is exemplified by the unprecedented yields obtained for bimolecular macrocyclization of pseudoocta(deca)saccharides CT4 and CT5 derivatives. Molecular diversity can be introduced at the level of the intersaccharide connectors by exploiting the chemistry of macrocyclic carbodiimides. Molecular modeling, when introducing a restriction in the simulation time, predicts the existence of a flexible, relatively hydrophobic cavity suited for molecular inclusion of nonpolar guests. NMR supports this fact and confirms the high symmetry of these hosts, where all glucopyranosyl units are magnetically equivalent as in cyclodextrins (CDs). The cavity sizes of the new CT4 and CT5 derivatives rank slightly below and above that of γCD, respectively. Determination of the inclusion capabilities toward a series of structurally diverse guests demonstrates that large-ring CTs are suited to form supramolecular complexes in water, the corresponding K_{as} trends are consistent with the existence of fluxional processes that are more pronounced for the larger members of the series.

Experimental Section

For the computational, NMR, and fluorescence spectroscopy binding titrations, stoichiometry determination of complexes, and MS experimental details see the Supporting Information.

Hemiacetylated Trimeric Linear Precursor 7. To a solution of 2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-dideoxy-6,6′-diisothiocyanato-α,α′-trehalose[30b] (**2**, 0.2 mg, 0.3 mmol) in pyridine (15 mL) was added 6-amino-6′-azido-6,6′-dideoxy-α,α′-trehalose[27] (**3**, 0.24 mg, 0.65 mmol, 1.1 equiv). The mixture was stirred at rt for 16 h, and then the solvent was evaporated under vacuum. The syrupy residue was purified by column chromatography (MeCN → 10:1 MeCN−H_2O) to afford **7** in 75% yield (0.32 g). R_f: 0.35 (6:1:1 MeCN−H_2O−NH_4OH). $[\alpha]_D$: +111.0 (*c* 1.0, MeOH). UV (MeOH): λ_{max} 242 nm ($\varepsilon_{mM} = 28.6$). IR (KBr): ν_{max} 2108, 1749 cm^{-1}. ^1H NMR (500 MHz, CD_3OD, 323 K): δ 5.46 (t, 2 H, $J_{2,3} = J_{3,4} = 10.0$ Hz, H-3), 5.33 (d, 2 H, $J_{1,2} = 4.0$ Hz, H-1), 5.09 (d, 2 H, $J_{1,2} = 4.0$ Hz, H-1^{II}), 5.08 (d, 2 H, $J_{1,2} = 4.0$ Hz, H-1^{III}), 5.06 (dd, 2 H, H-2), 4.93 (t, 2 H, $J_{4,5} = 10.0$ Hz, H-4), 4.00 (ddd, 2 H, $J_{4,5} = 9.5$ Hz, $J_{5,6b} = 6.5$ Hz, $J_{5,6a} = 2.5$ Hz, H-5^{III}), 3.99 (ddd, 2 H, $J_{5,6b} = 7.5$ Hz, $J_{5,6a} = 2.5$ Hz, H-5), 3.98 (m, 2 H, H-6a), 3.94 (ddd, 2 H, $J_{4,5} = 9.5$ Hz, $J_{5,6b} = 5.0$ Hz, $J_{5,6a} = 3.0$ Hz, H-5^{II}), 3.79 (m, 2 H, H-6^{II}a), 3.78 (t, 2 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3^{II}), 3.76 (t, 2 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3^{III}), 3.69 (da, 2 H, $J_{6a,6b} = 13.0$ Hz, H-6^{II}b), 3.54 (dd, 2 H, H-2^{II}), 3.53 (m, 2 H, H-6b), 3.52 (dd, 2 H, H-2^{III}), 3.51 (dd, 2 H, $J_{6a,6b} = 13.0$ Hz, H-6^{III}a), 3.41 (dd, 2 H, H-6^{III}b), 3.30 (t, 2 H, H-4^{III}), 3.25 (t, 2 H, H-4^{II}), 2.10−1.98 (3 s, 18 H, 6 MeCO). ^{13}C NMR (125.7 MHz, CD_3OD, 323 K): δ 184.0 (CS), 170.4−170.1 (CO ester), 94.5 (C-1^{III}), 94.3 (C-1^{II}), 90.9 (C-1), 73.1 (C-3^{III}), 72.9 (C-3^{II}), 71.8 (C-2^{III}), 71.7 (C-2^{II}), 71.6 (C-5^{III}), 71.4 (C-4^{II}), 71.3 (C-4^{III}), 71.2 (C-5^{II}), 70.3 (C-3), 70.0 (C-2), 69.8 (C-4), 69.4 (C-5), 51.5 (C-6^{III}), 45.0 (C-6^{II}), 44.6 (C-6), 19.5−19.2 (MeCO). MS (ESI): *m/z* 727.0 ($[M + 2Na]^{2+}$), 704.0 ($[M + 2H]^{2+}$). Anal. Calcd for $C_{50}H_{76}N_{10}O_{33}S_2$: C, 42.61; H, 5.44; N, 9.94. Found: C, 42.38; H, 5.33; N, 9.81.

Deprotected Trimeric Linear Precursor 8. To a solution of **7** (0.32 mg, 0.22 mmol) in MeOH (5 mL) was added 1 M methanolic NaOMe (0.13 mL). After 30 min at rt, a white precipitate appeared. The precipitate was dissolved by addition of H_2O (3 mL), and the clear solution was stirred for 1 h. Then, the reaction mixture was neutralized and demineralized with Amberlite IR-120 (H^+) and Amberlite MB-9L ion-exchange resins, respectively. Resins were filtered out and solvents were evaporated to furnish **8** in virtually a quantitative yield (0.26 g). R_f: 0.34 (6:3:1 MeCN−H_2O−NH_4OH). $[\alpha]_D$: +97.5 (*c* 0.8, H_2O). UV (H_2O): λ_{max} 238 nm ($\varepsilon_{mM} = 22.6$). ^1H NMR (500 MHz, D_2O, 323 K): δ 5.41 (d, 2 H, $J_{1,2} = 4.0$ Hz, H-1^{II}), 5.37 (d, 2 H, $J_{1,2} = 4.0$ Hz, H-1^{III}), 5.34 (d, 2 H, $J_{1,2} = 3.5$ Hz, H-1), 4.20 (ddd, 2 H, $J_{4,5} = 10.0$ Hz, $J_{5,6b} = 7.0$ Hz, $J_{5,6a} = 3.0$ Hz, H-5^{III}), 4.18 (ddd, 2 H, $J_{4,5} = 10.0$ Hz, $J_{5,6b} = 7.5$ Hz, $J_{5,6a} = 3.0$ Hz, H-5^{II}), 4.17 (ddd, 2 H, $J_{4,5} = 9.5$ Hz, $J_{5,6b} = 6.0$ Hz, $J_{5,6a} = 4.0$ Hz, H-5), 4.08 (m, 2 H, H-6^{II}a), 4.07 (t, 2 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3^{II}), 4.06 (t, 2 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 4.05 (t, 2 H, $J_{2,3} = J_{3,4} = 10.0$ Hz, H-3^{III}), 4.01 (m, 2 H, H-6a), 3.90 (m, 4 H, H-2^{II}, H-2^{III}), 3.88 (dd, 2 H, $J_{6a,6b} = 13.5$ Hz, H-6^{III}a), 3.87 (dd, 2 H, H-2), 3.86 (m, 4 H, H-6b, H-6^{II}b), 3.78 (dd, 2 H, H-6^{III}b), 3.68 (t, 2 H, H-4^{III}), 3.59 (t, 2 H, H-4^{II}), 3.58 (t, 2 H, H-4). ^{13}C NMR (125.7 MHz, D_2O, 323 K): δ 182.0 (CS), 94.2 (C-1^{II}), 94.1 (C-1^{III}), 94.0 (C-1), 72.9 (C-3^{III}), 72.8 (C-3, C-3^{II}), 71.6 (C-5, C-5^{II}, C-5^{III}), 71.5 (C-2, C-2^{II}, C-2^{III}), 71.4 (C-4, C-4^{II}), 71.1 (C-4^{III}), 51.5 (C-6^{III}), 45.4 (C-6, C-6^{II}). MS (ESI): *m/z* 1195.2 ($[M + K]^+$), 1179.3 ($[M + Na]^+$), 1157.3 ($[M + H]^+$). Anal. Calcd for $C_{38}H_{64}N_{10}O_{27}S_2$: C, 39.44; H, 5.58; N, 12.11. Found: C, 39.06; H, 5.26; N, 11.89.

Silylated Trimeric Linear Precursor 9. A solution of **8** (0.24 mg, 0.2 mmol) in pyridine (10 mL) was treated with a 1:2 mixture of trimethylsilyl chloride and hexamethyldisilazane (6 mL) at rt for 16 h. The reaction mixture was concentrated under vacuum, and the residue was extracted with petroleum ether. Then, the solvent was evaporated, and the residue was purified by column chromatography (1:9 Et_2O−petroleum ether) to yield **9** (0.4 g, 80%). R_f: 0.39 (1:9 Et_2O−petroleum ether). $[\alpha]_D$: +99.0 (*c* 1.0, CH_2Cl_2). UV (CH_2Cl_2): λ_{max} 250 nm ($\varepsilon_{mM} = 22.7$). IR (KBr): ν_{max} 3416, 2101 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, 313 K): δ 5.91 (m, 2 H, NH), 5.88 (m, 2 H, $N^{II}H$), 4.91 (d, 2 H, $J_{1,2} = 3.0$ Hz, H-1^{II}), 4.87 (d, 2 H, $J_{1,2} = 3.0$ Hz, H-1^{III}), 4.85 (d, 2 H, $J_{1,2} = 3.0$ Hz, H-1), 3.96 (ddd, 2 H, $J_{4,5} = 9.0$ Hz, $J_{5,6b} = 5.5$ Hz, $J_{5,6a} = 4.0$ Hz, H-5), 3.95 (m, 2 H, H-6^{II}a), 3.94 (m, 2 H, H-5^{II}), 3.93 (ddd, 2 H, $J_{4,5} = 9.0$ Hz, $J_{5,6b} = 5.0$ Hz, $J_{5,6a} = 3.0$ Hz, H-5^{III}), 3.91 (t, 2 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, H-3^{II}), 3.88 (t, 2 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, H-3), 3.86

(44) 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%.

(45) Franke, J.; Merz, F.; Lorenski, H. W.; Müller, W. M.; Werner, W.; Vögtle, F. *J. Inclusion Phenom.* **1985**, *3*, 471.

(t, 2 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, H-3^{III}), 3.70 (da, 2 H, $J_{6a,6b} = 12.5$ Hz, H-6a), 3.42 (dd, 2 H, H-2^{III}), 3.41 (dd, 2 H, H-2^{II}), 3.40 (dd, 2 H, H-2), 3.38 (t, 2 H, H-4^{III}), 3.37 (m, 2 H, H-6^{III}a), 3.35 (dd, 2 H, H-6b), 3.34 (m, 2 H, H-6^{II}b), 3.32 (dd, 2 H, $J_{6a,6b} = 13.0$ Hz, H-6^{III}b), 3.31 (t, 2 H, H-4), 3.30 (t, 2 H, H-4^{II}), 0.17−0.11 (6 s, 162 H, 18 Me_3Si). ^{13}C NMR (125.7 MHz, $CDCl_3$, 313 K): δ 183.7 (CS), 94.8 (C-1), 94.3 (C-1^{II}), 94.2 (C-1^{III}), 73.8 (C-4^{II}), 73.4 (C-4), 73.2 (C-3^{III}), 73.1 (C-3), 73.0 (C-3^{II}), 72.8 (C-4^{III}), 72.7 (C-2^{II}, C-2^{III}), 72.6 (C-5^{II}), 72.5 (C-2), 71.2 (C-5^{III}), 71.1 (C-5), 51.7 (C-6^{III}), 46.4 (C-6^{II}), 45.5 (C-6), 1.2−0.1 (Me_3Si). MS (ESI): *m*/*z* 2454.0 ([M + H]$^+$). Anal. Calcd for $C_{92}H_{208}N_{10}O_{27}S_2Si_{18}$: C, 44.98; H, 8.54; N, 5.70. Found: C, 44.75; H, 8.30; N, 5.65.

Diamine-Functionalized Trimeric Linear Precursor 10. To a solution of diazide **9** (0.94 g, 0.38 mmol) in a mixture of dioxane−MeOH (5:1, 12 mL) was added triphenylphosphine (0.3 g, 1.14 mmol, 1.5 equiv). The reaction was stirred at rt for 1 h. Then, 30% NH_4OH (2 mL) was added, and the reaction mixture was stirred for 16 h. Solvents were evaporated at reduced pressure, and the resulting residue was purified by column chromatography (50:1 → 20:1 CH_2Cl_2−MeOH) to obtain diamine **10** in 83% yield (0.76 g). R_f: 0.62 (9:1 CH_2Cl_2−MeOH). ^1H NMR (500 MHz, $CDCl_3$, 323 K): δ 6.05 (m, 4 H, NH, N^{II}H), 4.90 (d, 2 H, $J_{1,2} = 3.0$ Hz, H-1^{II}), 4.86 (d, 4 H, $J_{1,2} = 3.0$ Hz, H-1, H-1^{III}), 3.96 (m, 2 H, H-5), 3.94 (t, 2 H, $J_{2,3} = J_{3,4} = 9.1$ Hz, H-3^{II}), 3.93 (m, 2 H, H-5^{II}), 3.90 (t, 2 H, $J_{2,3} = J_{3,4} = 9.3$ Hz, H-3^{III}), 3.89 (t, 2 H, $J_{2,3} = J_{3,4} = 8.9$ Hz, H-3), 3.82 (m, 2 H, H-6^{II}a), 3.71 (ddd, 2 H, $J_{4,5} = 9.3$ Hz, $J_{5,6b} = 5.6$ Hz, $J_{5,6a} = 3.0$ Hz, H-5^{III}), 3.67 (m, 2 H, H-6a), 3.47 (m, 2 H, H-6b), 3.42 (dd, 2 H, H-2^{II}), 3.41 (dd, 2 H, H-2^{III}), 3.40 (dd, 2 H, H-2), 3.36 (t, 2 H, H-4^{III}), 3.32 (t, 2 H, $J_{4,5} = 8.8$ Hz, H-4), 3.31 (m, 2 H, H-6^{II}b), 3.30 (t, 2 H, $J_{4,5} = 9.0$ Hz, H-4^{II}), 2.91 (dd, 2 H, $J_{6a,6b} = 13.3$ Hz, H-6^{III}a), 2.72 (dd, 2 H, H-6^{III}b), 1.58 (m, 4 H, NH_2), 0.18−0.12 (5 s, 162 H, 18 $SiMe_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, 313 K): δ 184.1 (CS), 94.9 (C-1), 94.2 (C-1^{III}), 93.9 (C-1^{II}), 74.0 (C-5^{III}), 73.7 (C-4^{II}), 73.3 (C-3^{III}), 73.2 (C-3, C-4), 73.1 (C-3^{II}), 72.9 (C-2^{III}), 72.8 (C-2, C-2^{II}), 72.7 (C-4^{III}), 71.4 (C-5^{II}), 71.2 (C-5), 46.3 (C-6^{II}), 45.5 (C-6), 42.6 (C-6^{III}), 1.2−0.2 ($SiMe_3$). MS (ESI): *m*/*z* 2403.9 ([M + H]$^+$); 1202.9 ([M + 2H]$^{2+}$).

Hemiacetylated-Silylated Thioureido Cyclotrehalan CT4 (11). A solution of *N*,*N*′-bis-(2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-dideoxy-6′-isothiocyanato-α,α′-trehalos-6-yl)thiourea[25c] (**5**, 0.24 mg, 0.18 mmol) and *N*,*N*′-bis-(6′-amino-6,6′-dideoxy-2,3,4,2′,3′,4′-hexa-*O*-trimethylsilyl-α,α′-trehalos-6-yl)thiourea[27] (**6**, 0.29 mg, 0.18 mmol) in CH_2Cl_2 (24 mL) was stirred at rt for 14 h. The solvent was removed under reduced pressure, and the residue was purified by column chromatography (0.5% Et_3N containing 2:1 → 3:1 EtOAc−petroleum ether) to furnish **11** in 70% yield (364 mg). R_f: 0.57 (20:1 CH_2Cl_2−MeOH). $[\alpha]_D$: +125.9 (*c* 1.0, CH_2Cl_2). UV (CH_2Cl_2): λ_{max} 250 nm ($\varepsilon_{mM} = 60.0$). ^1H NMR (500 MHz, CD_3OD, 323 K): δ 5.46 (t, 2 H, $J_{2,3} = J_{3,4} = 10.0$ Hz, H-3), 5.45 (t, 2 H, $J_{2,3} = J_{3,4} = 10.0$ Hz, H-3^{II}), 5.31 (d, 2 H, $J_{1,2} = 4.0$ Hz, H-1^{II}), 5.29 (d, 2 H, $J_{1,2} = 3.5$ Hz, H-1), 5.02 (dd, 2 H, H-2^{II}), 4.98 (dd, 2 H, H-2), 4.96 (d, 2 H, $J_{1,2} = 3.0$ Hz, H-1^{IV}), 4.94 (d, 2 H, $J_{1,2} = 3.0$ Hz, H-1^{III}), 4.90 (dd, 2 H, $J_{4,5} = 9.5$ Hz, H-4^{II}), 4.87 (dd, 2 H, $J_{4,5} = 9.5$ Hz, H-4), 4.45 (da, 4 H, $J_{6a,6b} = 13.5$ Hz, H-6^{III}a, H-6^{IV}a), 4.21 (da, 4 H, $J_{6a,6b} = 14.0$ Hz, H-6a, H-6^{II}a), 3.99 (t, 2 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, H-3^{III}), 3.98 (t, 2 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, H-3^{IV}), 3.95 (ddd, 2 H, $J_{5,6b} = 8.0$ Hz, $J_{5,6a} = 3.5$ Hz, H-5), 3.92 (ddd, 2 H, $J_{5,6b} = 8.0$ Hz, $J_{5,6a} = 3.5$ Hz, H-5^{II}), 3.91 (ddd, 2 H, $J_{4,5} = 9.0$ Hz, $J_{5,6b} = 7.5$ Hz, $J_{5,6a} = 3.0$ Hz, H-5^{III}), 3.90 (ddd, 2 H, $J_{4,5} = 9.0$ Hz, $J_{5,6b} = 7.5$ Hz, $J_{5,6a} = 3.0$ Hz, H-5^{IV}), 3.49 (dd, 2 H, H-2^{IV}), 3.46 (dd, 2 H, H-2^{III}), 3.30 (t, 2 H, H-4^{IV}), 3.29 (t, 2 H, H-4^{III}), 3.27 (dd, 4 H, H-6b, H-6^{II}b), 3.11 (dd, 2 H, H-6^{III}b), 3.10 (dd, 2 H, H-6^{IV}b), 2.10−1.96 (6 s, 36 H, 12 MeCO), 0.19−0.14 (5 s, 108 H, 12 $SiMe_3$). ^{13}C NMR (125.7 MHz, CD_3OD, 323 K): δ 185.7, 185.4 (CS), 171.8−171.5 (CO), 95.1 (C-1^{III}, C-1^{IV}), 92.4 (C-1, C-1^{II}), 75.4 (C-4^{III}, C-4^{IV}), 74.5 (C-3^{III}, C-3^{IV}), 74.2 (C-2^{III}), 74.1 (C-2^{IV}), 73.7 (C-5^{IV}), 73.3 (C-5^{III}), 71.5 (C-3^{II}), 71.4 (C-3), 71.3 (C-2, C-2^{II}, C-4, C-4^{II}), 71.2 (C-5), 71.1 (C-5^{II}), 48.8, 48.2 (C-6^{III}, C-6^{IV}), 46.1, 45.9 (C-6, C-6^{II}), 21.2−20.6 (*Me*CO), 1.6−0.5 ($SiMe_3$). MS (ESI): *m*/*z* 2938.7 ([M + K]$^+$), 2921.7 ([M + Na]$^+$). Anal. Calcd for $C_{112}H_{208}N_8O_{48}S_4Si_{12}$: C, 46.38; H, 7.23; N, 3.86. Found: C, 46.41; H, 7.34; N, 3.88.

Hemiacetylated Thioureido Cyclotrehalan CT4 (12). A solution of compound **11** (0.2 g, 69 μmol) in CH_2Cl_2−MeOH−H_2O (4:3:1, 8 mL) was treated with 10% aq AcOH (0.17 mL) at 60 °C for 4 h. The solvents were evaporated under reduced pressure, and AcOH was coevaporated with H_2O to furnish **12** (128 mg, 91%). R_f: 0.35 (6:1:1 MeCN−H_2O−NH_4OH). $[\alpha]_D$: +82.2 (*c* 0.6, MeOH). UV (MeOH): λ_{max} 241 nm ($\varepsilon_{mM} = 93.9$). ^1H NMR (500 MHz, CD_3OD, 323 K): δ 5.48 (t, 2 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 5.45 (t, 2 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3^{II}), 5.40 (da, 2 H, $J_{1,2} = 3.5$ Hz, H-1), 5.38 (m, 2 H, H-1^{II}), 5.12 (dd, 2 H, $J_{1,2} = 4.0$ Hz, H-2^{II}), 5.11 (d, 4 H, $J_{1,2} = 4.0$ Hz, H-1^{III}, H-1^{IV}), 5.02 (dd, 2 H, H-2), 4.93 (t, 2 H, $J_{4,5} = 9.5$ Hz, H-4^{II}), 4.92 (t, 2 H, $J_{4,5} = 9.5$ Hz, H-4), 4.20 (m, 4 H, H-6a, H-6^{II}a), 4.00 (m, 2 H, H-5), 3.95 (ddd, 2 H, $J_{4,5} = 9.5$ Hz, $J_{5,6b} = 5.0$ Hz, $J_{5,6a} = 4.0$ Hz, H-5^{IV}), 3.93 (m, 2 H, H-6^{IV}a), 3.88 (m, 4 H, H-5^{II}, H-5^{III}), 3.87 (m, 2 H, H-6^{III}a), 3.79 (t, 2 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3^{III}), 3.78 (t, 2 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3^{IV}), 3.75 (m, 2 H, H-6^{II}b), 3.70 (m, 2 H, H-6^{IV}b), 3.56 (dd, 2 H, H-2^{III}), 3.49 (dd, 2 H, H-2^{IV}), 3.29 (m, 4 H, H-6b, H-6^{II}b), 3.24 (t, 4 H, $J_{4,5} = 9.5$ Hz, H-4^{III}, H-4^{IV}), 2.07−1.98 (6 s, 36 H, 12 MeCO). ^{13}C NMR (125.7 MHz, CD_3OD, 323 K): δ 184.0 (CS), 170.7−170.6 (CO), 93.9 (C-1^{III}, C- 1^{IV}), 90.9 (C-1, C-1^{II}), 72.9 (C-3^{IV}), 72.6 (C-3^{III}), 71.9 (C-2^{IV}), 71.6 (C-2^{III}), 71.5 (C-5, C-5^{II}), 71.2 (C-4^{III}, C-4^{IV}), 70.2 (C-2, C-3^{II}, C-4, C-5^{III}, C-5^{IV}), 70.0 (C-3), 69.9 (C-2^{II}), 69.8 (C-4^{II}), 44.7 (C-6, C- 6^{II}, C-6^{III}, C-6^{IV}), 19.8−19.2 (*Me*CO). MS (ESI): *m*/*z* 2072.5 ([M + K]$^+$), 2055.5 ([M + Na]$^+$), 2033.5 ([M + H]$^+$). Anal. Calcd for $C_{76}H_{112}N_8O_{48}S_4$: C, 44.88; H, 5.55; N, 5.51. Found: C, 44.71; H, 5.40; N, 5.47.

Tetrakis-[*N*,*N*′-bis-(6,6′-dideoxy-α,α′-trehalos-6,6′-diyl)]thiourea (13). To a solution of **12** (0.1 g, 50 μmol) in MeOH (5 mL) was added 1 M methanolic NaOMe (60 μL). After 30 min at rt, a white precipitate appeared. The precipitate was dissolved by addition of H_2O (3 mL), and the clear solution was stirred for 1.5 h. Then, the reaction mixture was neutralized and demineralized with Amberlite IR-120 (H^+) and Amberlite MB-9L ion-exchange resins, respectively. Resins were filtered out and solvents were evaporated to afford **13** in 73% yield (57 mg). R_f: 0.43 (5:3:5 MeCN−H_2O−NH_4OH). $[\alpha]_D$: +123.4 (*c* 1.0, H_2O). UV (H_2O): λ_{max} 238 nm ($\varepsilon_{mM} = 47.6$). ^1H NMR (500 MHz, D_2O, 323 K): δ 5.35 (d, 8 H, $J_{1,2} = 3.5$ Hz, H-1), 4.15 (m, 8 H, H-5), 4.06 (t, 8 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 4.12−3.81 (m, 16 H, H-6a, H-6b), 3.88 (dd, 8 H, H-2), 3.58 (t, 8 H, $J_{4,5} = 9.5$ Hz, H-4). ^{13}C NMR (125.7 MHz, D_2O, 323 K): δ 182.1 (CS), 94.0 (C-1), 72.8 (C-3), 71.5 (C-2, C-4, C-5), 45.5 (C-6). MS (ESI): *m*/*z* 1551.0 ([M + Na]$^+$). Anal. Calcd for $C_{52}H_{88}N_8O_{36}S_4$: C, 40.83; H, 5.80; N, 7.33. Found: C, 40.73; H, 5.61; N, 7.12.

Hemiacetylated-Silylated Thioureido Cyclotrehalan CT5 (14). To a solution of **10** (95 mg, 39 μmol) in CH_2Cl_2 (5 mL) was added *N*,*N*′-bis-(2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-dideoxy-6′-isothiocyanato-α,α′-trehalos-6-yl)thiourea[25c] (**5**, 51 mg, 39 μmol), and the reaction was stirred at rt for 16 h. Then, the solvent was evaporated under reduced pressure, and the syrupy residue was purified by column chromatography (0.5% Et_3N containing 1:1 EtOAc−petroleum ether) to yield **14** (80 mg, 55%). R_f: 0.71 (2:1 EtOAc−petroleum ether). $[\alpha]_D$: +118.3 (*c* 1.0, CH_2Cl_2). ^1H NMR (500 MHz, CD_3OD, 323 K): δ 5.45 (t, 2 H, $J_{2,3} = J_{3,4} = 10.0$ Hz, H-3^{II}), 5.44 (t, 2 H, $J_{2,3} = J_{3,4} = 10.0$ Hz, H-3), 5.34 (d, 2 H, $J_{1,2} = 3.5$ Hz, H-1^{II}), 5.32 (d, 2 H, $J_{1,2} = 3.5$ Hz, H-1), 5.03 (dd, 2 H, H-2), 4.98 (dd, 2 H, H-2^{II}), 4.97 (d, 2 H, $J_{1,2} = 2.5$ Hz, H-1^{III}), 4.95, 4.94 (2 d, 2 H, $J_{1,2} = 3.5$ Hz, H-1^{IV}, H-1^{V}), 4.91 (t, 2 H, $J_{4,5} = 10.0$ Hz, H-4), 4.88 (t, 2 H, $J_{4,5} = 10.0$ Hz, H-4^{II}), 4.24 (da, 2 H, $J_{6a,6b} = 14.0$ Hz, H-6^{II}a), 4.10 (m, 2 H, H-6a), 4.00, 3.99 (2 t, 4 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, H-3^{IV}, H-3^{V}), 3.98 (t, 2 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, H-3^{III}), 3.94 (m, 4 H, H-6^{IV}a, H-6^{V}a), 3.93 (ddd, 2 H, $J_{5,6b} = 8.0$ Hz, $J_{5,6a} = 1.5$ Hz, H-5^{II}), 3.91 (ddd, 2 H, $J_{5,6b} = 8.5$ Hz, $J_{5,6a} = 3.0$ Hz, H-5), 3.90 (m, 4 H, H-5^{IV}, H-5^{V}), 3.87 (m, 2 H, H-6^{III}a), 3.86 (ddd, 2 H, $J_{4,5} = 9.0$ Hz, $J_{5,6b} = 8.5$ Hz, $J_{5,6a} = 3.0$ Hz, H-5^{III}), 3.49, 3.48 (2

dd, 4 H, H-2^{IV}, H-2^{V}), 3.52 (dd, 2 H, H-2^{III}), 3.39 (m, 6 H, H-6b, H-6^{IV}b, H-6^{V}b), 3.38 (t, 4 H, H-4^{IV}, H-4^{V}), 3.24 (dd, 2 H, H-6^{II}b), 3.33 (t, 2 H, H-4^{III}), 3.16 (dd, 2 H, $J_{6a,\ 6b}$ = 13.5 Hz, H-6^{III}b), 2.09−1.98 (6 s, 36 H, 12 MeCO), 0.19−0.15 (7 s, 162 H, 18 $SiMe_3$). ^{13}C NMR (125.7 MHz, CD_3OD, 323 K): δ 184.3−184.1 (CS), 170.5−170.2 (CO), 93.5 (C-1^{IV}, C-1^{V}), 93.4 (C-1^{III}), 91.3 (C-1), 91.2 (C-1^{II}), 73.9 (C-4^{III}), 73.6 (C-4^{IV}, C-4^{V}), 73.5 (C-3^{III}), 73.4, 73.2 (C-3^{IV}, C-3^{V}), 72.7 (C-2^{III}, C-2^{IV}, C-2^{V}), 72.2 (C-5^{III}), 71.6 (C-5^{IV}, C-5^{V}), 70.2 (C-5), 70.1 (C-5^{II},C-3), 70.0 (C-2^{II}, C-3^{II}), 69.9 (C-2), 69.8 (C-4^{II}), 69.7 (C-4), 46.0 (C-6^{III}, C-6^{IV}, C-6^{V}), 45.0 (C-6), 44.7 (C-6^{II}), 19.8−19.4 (*Me*CO), 0.3−0.0 ($SiMe_3$). MS (ESI): *m/z* 1888.3 ([M + Na + K]$^{2+}$), 1880.3 ([M + 2Na]$^{2+}$). Anal. Calcd for $C_{143}H_{278}N_{10}O_{57}S_5Si_{18}$: C, 46.22; H, 7.54; N, 3.77. Found: C, 46.11; H, 7.52; N, 3.62.

Hemiacetylated Thioureido Cyclotrehalan CT5 (15). To a solution of **14** (56 mg, 15 μmol) in CH_2Cl_2−MeOH−H_2O (4:3:1, 2 mL) was added 10% aq AcOH (40 μL). The reaction mixture was stirred at 60 °C for 16 h. The solvents were evaporated under reduced pressure, and AcOH was coevaporated with H_2O to furnish **15** (38 mg, 99%). R_f: 0.64 (6:3:1 MeCN−H_2O−NH_4OH). $[\alpha]_D$: +94.7 (*c* 1.0, pyridine). UV (MeOH): λ_{max} 242 nm (ε_{mM} = 55.8). ^1H NMR (500 MHz, CD_3OD, 323 K): δ 5.46 (t, 2 H, $J_{2,3} = J_{3,4}$ = 10.0 Hz, H-3^{II}), 5.45 (t, 2 H, $J_{2,3} = J_{3,4}$ = 10.0 Hz, H-3), 5.39 (d, 2 H, $J_{1,2}$ = 3.5 Hz, H-1^{II}), 5.38 (m, 2 H, H-1), 5.14 (dd, 2 H, $J_{1,2}$ = 3.5 Hz, H-2), 5.10 (d, 2 H, $J_{1,2}$ = 4.0 Hz, H-1^{V}), 5.09 (d, 2 H, $J_{1,2}$ = 4.0 Hz, H-1^{III}), 5.08 (d, 2 H, $J_{1,2}$ = 4.0 Hz, H-1^{IV}), 5.05 (dd, 2 H, H-2^{II}), 4.95 (t, 2 H, $J_{4,5}$ = 10.0 Hz, H-4^{II}), 4.93 (t, 2 H, $J_{4,5}$ = 10.0 Hz, H-4), 4.22 (da, 2 H, $J_{6a,6b}$ = 14.5 Hz, H-6a), 4.11 (m, 2 H, H-6^{II}a), 3.98 (bdd, 2 H, $J_{5,6b}$ = 7.5 Hz, H-5^{II}), 3.97 (m, 2 H, H-5^{IV}), 3.94 (m, 2 H, H-6^{III}a), 3.93 (m, 2 H, H-6^{IV}a), 3.91 (m, 2 H, H-5^{V}), 3.90 (m, 2 H, H-5^{III}), 3.89 (bdd, 2 H, $J_{5,6b}$ = 8.0 Hz, H-5), 3.81 (m, 2 H, H-6^{V}a), 3.80 (t, 4 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3^{III}, H-3^{V}), 3.78 (t, 2 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3^{IV}), 3.72 (m, 2 H, H-6^{III}b), 3.65 (m, 2 H, H-6^{V}b), 3.57 (m, 2 H, H-6^{IV}b), 3.58 (dd, 2 H, H-2^{III}), 3.56 (dd, 2 H, H-2^{V}), 3.54 (dd, 2 H, H-2^{IV}), 3.38 (m, 2 H, H-6^{II}b), 3.35 (m, 2 H, H-6b), 3.27 (t, 2 H, $J_{4,5}$ = 9.0 Hz, H-4^{III}), 3.24 (t, 2 H, $J_{4,5}$ = 10.0 Hz, H-4^{V}), 3.23 (t, 2 H, $J_{4,5}$ = 9.5 Hz, H-4^{IV}), 2.11−1.98 (4 s, 36 H, 12 MeCO). ^{13}C NMR (125.7 MHz, CD_3OD, 313 K): δ 185.2−184.8 (CS), 172.1−171.6 (CO), 95.6 (C-1^{III}, C-1^{V}), 95.2 (C-1^{IV}), 92.2 (C-1, C-1^{II}), 74.2, 73.9 (C-3^{III}, C-3^{IV}, C-3^{V}), 73.1 (C-2^{III}, C-2^{IV}, C-2^{V}), 73.0−71.5 (C-5, C-5^{II}, C-5^{III}, C-5^{IV}, C-5^{V}), 72.4 (C-4^{III}, C-4^{IV}, C-4^{V}), 71.6 (C-3, C-3^{II}), 71.4 (C-2^{II}), 71.2 (C-2), 70.8 (C-4, C-4^{II}), 46.0 (C-6, C-6^{II}, C-6^{III}, C-6^{IV}, C-6^{V}), 21.1−20.6 (*Me*CO). MS (ESI): *m/z* 2453.5 ([M + K]$^+$), 2437.6 ([M + Na]$^+$). Anal. Calcd for $C_{89}H_{134}N_{10}O_{57}S_5$: C, 44.24; H, 5.59; N, 5.80. Found: C, 44.20; H, 5.45; N, 5.65.

Pentakis-[*N*,*N*′-bis-(6,6′-didesoxi-α,α′-trehalos-6,6′-diyl)]thiourea (16). To a solution of **15** (40 mg, 13 μmol) in MeOH (1 mL) was added 1 M methanolic NaOMe (60 μL). After 5 min at rt, a white precipitate appeared. The precipitate was dissolved by addition of H_2O (1 mL), and the clear solution was stirred for 30 min. Then, the reaction mixture was neutralized and demineralized with Amberlite IR-120 (H^+) and Amberlite MB-9L ion-exchange resins, respectively. Resins were filtered out and solvents were evaporated to furnish **16** in 90% yield (22 mg). R_f: 0.53 (5:3:5 MeCN−H_2O−NH_4OH). $[\alpha]_D$: +101.0 (*c* 1.0, H_2O). UV (H_2O): λ_{max} 238 nm (ε_{mM} = 92.6). ^1H NMR (500 MHz, D_2O, 333 K): δ 5.43 (d, 10 H, $J_{1,2}$ = 4.0 Hz, H-1), 4.24 (ddd, 10 H, $J_{4,5}$ = 9.5 Hz, $J_{5,6b}$ = 6.0 Hz, $J_{5,6a}$ = 3.0 Hz, H-5), 4.15 (t, 10 H, $J_{3,2} = J_{3,4}$ = 9.5 Hz, H-3), 4.10 (m, 20 H, H-6a, H-6b), 3.96 (dd, 10 H, H-2), 3.67 (t, 10 H, H-4). ^{13}C NMR (125.7 MHz, D_2O, 333 K): δ 182.4 (CS), 94.1 (C-1), 73.0 (C-3), 71.7 (C-2, C-5), 71.5 (C-4), 45.6 (C-6). MS (ESI): *m/z* 1933.1 ([M + Na]$^+$), 975.1 ([M + K + H]$^{2+}$). Anal. Calcd for $C_{65}H_{110}N_{10}O_{45}S_5$: C, 40.83; H, 5.80; N, 7.33. Found: C, 40.70; H, 5.64; N, 7.21.

Tetrakis-[*N*,*N*′-bis-(2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-dideoxy-α,α′-trehalos-6,6′-diyl)]thiourea (17). Compound **17** was obtained by conventional acetylation of **13** (0.2 g, 0.14 mmol) at 0 °C, followed by column chromatography purification (50:1 → 20:1 CH_2Cl_2−MeOH). Yield: 0.31 g (89%). R_f: 0.67 (9:1 CH_2Cl_2−MeOH). $[\alpha]_D$: +156.5 (*c* 1.0, CH_2Cl_2). UV (CH_2Cl_2): λ_{max} 250 nm (ε_{mM} = 50.1). ^1H NMR (500 MHz, $CDCl_3$, 313 K): δ 6.46 (m, 8 H, NH), 5.46 (t, 8 H, $J_{2,3} = J_{3,4}$ = 9.7 Hz, H-3), 5.39 (m, 8 H, H-1), 5.00 (m, 8 H, H-2), 4.97 (t, 8 H, $J_{4,5}$ = 9.7 Hz, H-4), 3.90 (m, 8 H, H-6a), 3.89 (m, 8 H, H-5), 3.41 (m, 8 H, H-6b), 2.09−2.01 (3 s, 72 H, 24 MeCO). ^{13}C NMR (125.7 MHz, $CDCl_3$, 313 K): δ 184.8 (CS), 170.5−169.7 (CO), 92.1 (C-1), 70.3 (C-2), 69.7 (C-3), 69.4 (C-4, C-5), 44.7 (C-6), 20.8−20.6 (*Me*CO). MS (ESI): *m/z* 2576.1 ([M + K]$^+$), 1288.1 ([M + K + H]$^{2+}$). Anal. Calcd for $C_{100}H_{136}N_8O_{60}S_4$: C, 47.32; H, 5.40; N, 4.41. Found: C, 47.20; H, 5.41; N, 4.34.

Tetrakis-[*N*,*N*′-bis-(6,6′-dideoxy-2,3,4,2′,3′,4′-hexa-*O*-trimethylsilyl-α,α′-trehalos-6,6′-diyl)]thiourea (18). A solution of **13** (0.11 g, 74 μmol) in pyridine (15 mL) was treated with a 1:2 mixture of trimethylsilyl chloride and hexamethyldisilazane (5.2 mL) at rt for 16 h. The reaction mixture was concentrated under vacuum, and the residue was extracted with petroleum ether. Then, the solvent was evaporated, and the residue was purified by column chromatography (1:8 → 1:5 EtOAc−petroleum ether) to furnish **18** in 63% yield (0.15 g). R_f: 0.51 (1:5 EtOAc−petroleum ether). $[\alpha]_D$: +103.5 (*c* 1.0, CH_2Cl_2). UV (CH_2Cl_2): λ_{max} 250 nm (ε_{mM} = 116.4). ^1H NMR (500 MHz, $CDCl_3$, 313 K): δ 5.94 (m, 8 H, NH), 4.84 (d, 8 H, $J_{1,2}$ = 3.0 Hz, H-1), 3.96 (td, 8 H, $J_{4,5} = J_{5,6b}$ = 9.0 Hz, $J_{5,6a}$ = 5.0 Hz, H-5), 3.90 (t, 8 H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, H-3), 3.74 (m, 8 H, H-6a), 3.46 (m, 8 H, H-6b), 3.38 (dd, 8 H, H-2), 3.34 (t, 8 H, H-4), 0.18−0.13 (3 s, 216 H, 24 $SiMe_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, 313 K): δ 184.1 (CS), 94.7 (C-1) 73.4 (C-4), 73.2 (C-3), 72.8 (C-2), 71.4 (C-5), 45.8 (C-6), 1.2−0.3 ($SiMe_3$). MS (ESI): *m/z* 3282.7 ([M + Na]$^+$). Anal. Calcd for $C_{124}H_{280}N_8O_{36}S_4Si_{24}$: C, 45.66; H, 8.65; N, 3.44. Found: C, 45.56; H, 8.55; N, 3.40.

Tetrakis-[*N*,*N*′-bis-(2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-dideoxy-α,α′-trehalos-6,6′-diyl)]carbodiimide (19). To a solution of **17** (0.29 g, 0.11 mmol) in a mixture of H_2O−CH_2Cl_2 (1:1, 14 mL) was added HgO (0.29 g, 1.33 mmol, 3 equiv). The resulting heterogeneous mixture was vigorously stirred at rt for 16 h. Then, the mixture was diluted with CH_2Cl_2 (10 mL), and the organic layer was decanted, dried ($MgSO_4$), filtered over Celite, and concentrated. The resulting residue was purified by column chromatography (0.5% Et_3N containing 100:1 CH_2Cl_2−MeOH) to yield **19** in 48% yield (0.13 g). R_f: 0.34 (CH_2Cl_2−MeOH 20:1). $[\alpha]_D$: +131.1 (*c* 1.0, CH_2Cl_2). IR: ν_{max} 2142, 1755 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 5.46 (t, 8 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 5.29 (d, 8 H, $J_{1,2}$ = 3.9 Hz, H-1), 5.03 (dd, 8 H, H-2), 4.90 (t, 8 H, $J_{4,5}$ = 9.5 Hz, H-4), 3.83 (ddd, 8 H, $J_{5,6a}$ = 7.9 Hz, $J_{5,6b}$ = 3.2 Hz, H-5), 3.42 (dd, 8 H, $J_{6a,6b}$ = 13.4 Hz, H-6a), 3.15 (dd, 8 H, H-6b), 2.09−2.01 (3 s, 72 H, 24 MeCO). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 169.9−169.7 (CO), 138.7 (NCN), 91.5 (C-1) 70.6 (C-3), 69.9 (C-5), 69.8 (C-4), 69.4 (C-2), 47.1 (C-6), 20.8−20.7 (*Me*CO). MS (ESI): *m/z* 2440.6 ([M + K]$^+$), 2423.6 ([M + Na]$^+$), 1239.9 ([M + 2K]$^{2+}$), 1231.4 ([M + Na + K]$^{2+}$). Anal. Calcd for $C_{100}H_{128}N_8O_{60}$: C, 50.00; H, 5.37; N, 4.66. Found: C, 49.85; H, 5.23; N, 4.55.

Tetrakis-[*N*,*N*′-bis-(6,6′-dideoxy-2,3,4,2′,3′,4′-hexa-*O*-trimethylsilyl-α,α′-trehalos-6,6′-diyl)]carbodiimide (20). To a solution of **18** (0.14 g, 41 μmol) in a mixture of H_2O−CH_2Cl_2 (1:1, 5 mL) was added HgO (0.1 g, 0.49 mmol, 3 equiv). The resulting heterogeneous mixture was vigorously stirred at rt for 10 h. Then, the mixture was diluted with CH_2Cl_2 (10 mL), and the organic layer was decanted, dried ($MgSO_4$), filtered over Celite, and concentrated. The resulting residue was purified by column chromatography (1:15 → 1:9 EtOAc−petroleum ether) to afford **20** in 29% yield (37 mg). R_f: 0.73 (EtOAc−petroleum ether 1:9). $[\alpha]_D$: +138.9 (*c* 1.0, CH_2Cl_2). IR (KBr): ν_{max} 2139 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 4.90 (d, 8 H, $J_{1,2}$ = 3.5 Hz, H-1), 3.87 (t, 8 H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, H-3), 3.85 (m, 8 H, H-5), 3.51 (t, 8 H, $J_{4,5}$ = 9.0 Hz, H-4), 3.45 (d, 16 H, $J_{5,6}$ = 2.5 Hz, H-6a, H-6b), 3.41 (dd, 8 H, H-2), 0.17−0.11 (3 s, 216 H, 24 $SiMe_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 139.3 (NCN), 94.9 (C-1) 73.5 (C-3), 72.7 (C-2), 71.9 (C-4), 71.5 (C-5), 46.4 (C-6), 1.1−0.2 ($SiMe_3$). MS (ESI): *m/z* 3162.4 ([M +

K]$^+$). Anal. Calcd for $C_{124}H_{272}N_8O_{36}Si_{24}$: C, 47.65; H, 8.77; N, 3.59. Found: C, 47.70; H, 8.63; N, 3.45.

Tetrakis-[*N,N′*-bis-(2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-dideoxy-α,α′-trehalos-6,6′-diyl)]urea (21). To a solution of **19** (45 mg, 19 μmol) in acetone−H_2O (2:1, 6 mL) was added TFA (50 μL). The solution was stirred at rt for 16 h, and then, the solvent was evaporated under vacuum. The resulting residue was purified by column chromatography (EtOAc → 20:1 EtOAc−EtOH) to yield **21** (23 mg, 49%). R_f: 0.56 (45:5:3 EtOAc−EtOH−H_2O). $[\alpha]_D$: +144.4 (*c* 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.44 (t, 8 H, $J_{2,3} = J_{3,4} = 9.9$ Hz, H-3), 5.31 (d, 8 H, $J_{1,2} = 2.8$ Hz, H-1), 4.93 (m, 8 H, H-2), 4.92 (t, 8 H, $J_{4,5} = 9.9$ Hz, H-4), 3.78 (m, 8 H, H-5), 3.49 (da, 8 H, $J_{6a,6b} = 13.5$ Hz, H-6a), 3.06 (dd, 8 H, $J_{5,6b} = 6.6$ Hz, H-6b), 2.04−2.00 (2 s, 72 H, 24 MeCO). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 172.8−172.2 (CO ester), 160.3 (CO urea), 93.9 (C-1), 72.4 (C-2), 72.0 (C-3), 71.9 (C-5), 71.8 (C-4), 42.4 (C-6), 23.0 (MeCO). MS (ESI): *m/z* 2495.7 ([M + Na]$^+$), 1259.4 ([M + 2Na]$^{2+}$). Anal. Calcd for $C_{100}H_{136}N_8O_{64}$: C, 48.54; H, 5.54; N, 4.53. Found: C, 48.47; H, 5.71; N, 4.37.

Tetrakis-[*N,N′*-bis-(6,6′-dideoxy-α,α′-trehalos-6,6′-diyl)]urea (22). To a solution of **21** (23 mg, 9.2 μmol) in MeOH (2 mL) was added 1 M methanolic NaOMe (60 μL). After 30 min at rt, a white precipitate appeared. The precipitate was dissolved by addition of H_2O (3 mL), and the clear solution was stirred for 1.5 h. Then, the reaction mixture was neutralized and demineralized with Amberlite IR-120 (H$^+$) and Amberlite MB-9L ion-exchange resins, respectively. Resins were filtered out, and solvents were evaporated to furnish **22** in 84% yield (11 mg). R_f: 0.41 (2:1:1 ^{i}PrOH−AcOH−H_2O). $[\alpha]_D$: +108.0 (*c* 1.0, H_2O). ^{1}H NMR (500 MHz, D_2O, 313 K): δ 5.18 (d, 8 H, $J_{1,2} = 3.9$ Hz, H-1), 3.88 (t, 8 H, $J_{2,3} = J_{3,4} = 9.6$ Hz, H-3), 3.87 (m, 8 H, H-5), 3.68 (dd, 8 H, H-2), 3.58 (dd, 8 H, $J_{6a,6b} = 14.6$ Hz, $J_{5,6a} = 2.5$ Hz, H-6a), 3.40 (dd, 8 H, $J_{5,6b} = 6.5$ Hz, H-6b), 3.39 (t, 8 H, $J_{4,5} = 9.6$ Hz, H-4). ^{13}C NMR (125.7 MHz, D_2O, 313 K): δ 161.1 (CO), 93.5 (C-1) 72.7 (C-3), 71.4 (C-2, C-5), 71.3 (C-4), 40.9 (C-6). MS (ESI): *m/z* 752.2 ([M + K + H]$^{2+}$). Anal. Calcd for $C_{52}H_{88}N_8O_{40}$: C, 42.62; H, 6.05; N, 7.65. Found: C, 42.34; H, 6.03; N, 7.53.

Pentakis-[*N,N′*-bis-(2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-dideoxy-α,α′-trehalos-6,6′-diyl)]thiourea (23). Compound **23** was obtained by conventional acetylation of **16** (0.22 g, 92 μmol) at 0 °C, followed by column chromatography purification (20:1 CH_2Cl_2−MeOH). Yield: 0.21 g (71%). R_f: 0.31 (20:1 CH_2Cl_2−MeOH). $[\alpha]_D$: +151.6 (*c* 1.0, CH_2Cl_2). UV (CH_2Cl_2): λ_{max} 250 nm ($\varepsilon_{mM} = 70.0$). ^{1}H NMR (500 MHz, $CDCl_3$, 323 K): δ 6.59 (m, 10 H, NH), 5.46 (t, 10 H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 5.36 (m, 10 H, H-1), 5.01 (m, 10 H, H-2), 4.97 (m, 10 H, H-4), 3.96 (m, 10 H, H-6a), 3.87 (m, 10 H, H-5), 3.45 (m, 10 H, H-6b), 2.10−2.00 (3 s, 90 H, 30 MeCO). ^{13}C NMR (125.7 MHz, $CDCl_3$, 323 K): δ 187.6 (CS), 170.7−169.6 (CO), 91.9 (C-1), 70.3 (C-2), 69.9 (C-3, C-4), 69.5 (C-5), 44.6 (C-6), 20.7−20.5 (*Me*CO). MS (ESI): *m/z* 1616.4 ([M + Na + K]$^{2+}$), 1597.9 ([M + Na + H]$^{2+}$). Anal. Calcd for $C_{125}H_{170}N_{10}O_{75}S_5$: C, 47.32; H, 5.40; N, 4.41. Found: C, 47.24; H, 5.30; N, 4.23.

Pentakis-[*N,N′*-bis-(6,6′-dideoxy-2,3,4,2′,3′,4′-hexa-*O*-trimethylsilyl-α,α′-trehalos-6,6′-diyl)]thiourea (24). A solution of **16** (0.25 g, 0.13 mmol) in pyridine (30 mL) was treated with a 1:2 mixture of trimethylsilyl chloride and hexamethyldisilazane (9 mL) at rt for 16 h. The reaction mixture was concentrated under vacuum, and the residue was extracted with petroleum ether. Then, the solvent was evaporated, and the residue was purified by column chromatography (0.5% Et_3N containing 1:9 EtOAc−petroleum ether) to obtain **24** in 70% yield (0.37 g). R_f: 0.43 (1:9 EtOAc−petroleum ether). $[\alpha]_D$: +52.0 (*c* 1.0, CH_2Cl_2). UV (CH_2Cl_2): λ_{max} 250 nm ($\varepsilon_{mM} = 72.7$). ^{1}H NMR (500 MHz, $CDCl_3$, 313 K): δ 5.85 (m, 10 H, NH), 4.86 (d, 10 H, $J_{1,2} = 3.0$ Hz, H-1), 3.94 (td, 10 H, $J_{4,5} = J_{5,6b} = 9.0$ Hz, $J_{5,6a} = 5.0$ Hz, H-5), 3.90 (t, 10 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, H-3), 3.82 (m, 10 H, H-6a), 3.41 (dd, 10 H, H-2), 3.38 (m, 10 H, H-6b), 3.31 (t, 10 H, H-4), 0.17−0.13 (3 s, 270 H, 30 $SiMe_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$, 313 K): δ 183.9 (CS), 94.5 (C-1) 73.8 (C-4), 73.1 (C-3), 72.8 (C-2), 71.2 (C-5), 46.0 (C-6), 1.2−0.3 ($SiMe_3$). MS (ESI): *m/z* 2060.7 ([M + 2Na]$^{2+}$). Anal. Calcd for $C_{155}H_{350}N_{10}O_{45}S_5Si_{30}$: C, 45.66; H, 8.65; N, 3.44. Found: C, 45.58; H, 8.52; N, 3.34.

Pentakis-[*N,N′*-bis-(2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-dideoxy-α,α′-trehalos-6,6′-diyl)]carbodiimide (25). To a solution of **23** (0.1 g, 32 μmol) in H_2O−CH_2Cl_2 (1:1, 5 mL) was added HgO (0.1 g, 0.48 mmol, 3 equiv). The resulting heterogeneous mixture was vigorously stirred at rt for 16 h. Then, the mixture was diluted with CH_2Cl_2 (5 mL), and the organic layer was decanted, dried ($MgSO_4$), filtered over Celite, and concentrated. The resulting residue was purified by column chromatography (0.5% Et_3N containing 100:1 CH_2Cl_2−MeOH) to yield **25** (39 mg, 41%). R_f: 0.32 (20:1 CH_2Cl_2−MeOH). $[\alpha]_D$: +170.0 (*c* 1.0, CH_2Cl_2). IR (KBr): ν_{max} 2142, 1754 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.45 (t, 10 H, $J_{2,3} = J_{3,4} = 9.9$ Hz, H-3), 5.32 (d, 10 H, $J_{1,2} = 3.8$ Hz, H-1), 5.07 (dd, 10 H, H-2), 5.01 (t, 10 H, $J_{4,5} = 9.8$ Hz, H-4), 3.96 (ddd, 10 H, $J_{5,6a} = 6.2$ Hz, $J_{5,6b} = 2.8$ Hz, H-5), 3.38 (dd, 10 H, $J_{6a,6b} = 13.5$ Hz, H-6a), 3.28 (dd, 10 H, H-6b), 2.09−2.02 (3 s, 90 H, 30 MeCO). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 170.1−169.7 (CO), 139.8 (NCN), 93.0 (C-1) 70.2 (C-3), 69.8 (C-5), 69.6 (C-4), 69.3(C-2), 46.5 (C-6), 20.8 (*Me*CO). MS (ESI): *m/z* 3039.0 ([M + K]$^+$), 1520.8 ([M + K + H]$^{2+}$). Anal. Calcd for $C_{125}H_{160}N_{10}O_{75}$: C, 50.00; H, 5.37; N, 4.66. Found: C, 50.11; H, 5.37; N, 4.53.

Pentakis-[*N,N′*-bis-(6,6′-didesoxi-2,3,4,2′,3′,4′-hexa-*O*-trimetilsilil-α,α′-trehalos-6,6′-diyl)]carbodiimide (26). To a solution of **24** (0.37 g, 91 μmol) in H_2O−CH_2Cl_2 (1:1, 30 mL) was added HgO (0.3 g, 1.36 mmol, 3 equiv). The resulting heterogeneous mixture was vigorously stirred at rt for 16 h. Then, the mixture was diluted with CH_2Cl_2 (20 mL), and the organic layer was decanted, dried ($MgSO_4$), filtered over Celite, and concentrated. The resulting residue was purified by column chromatography (0.5% Et_3N containing 1:9 EtOAc−petroleum ether) to furnish **26** in 84% yield (0.3 g). R_f: 0.39 (1:9 Et_2O−petroleum ether). $[\alpha]_D$: +135.2 (*c* 1.0, CH_2Cl_2). IR (KBr): ν_{max} 2143 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.88 (d, 10 H, $J_{1,2} = 3.0$ Hz, H-1), 3.84 (m, 10 H, H-5), 3.83 (t, 10 H, $J_{2,3} = J_{3,4} = 9.1$ Hz, H-3), 3.51 (t, 10 H, $J_{4,5} = 9.1$ Hz, H-4), 3.47 (m, 10 H, H-6a), 3.44 (m, 10 H, H-6b), 3.40 (dd, 10 H, H-2), 0.15−0.09 (3 s, 270 H, 30 $SiMe_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 139.5 (NCN), 95.2 (C-1) 73.5 (C-3), 72.7 (C-2), 71.9 (C-4), 71.5 (C-5), 46.4 (C-6), 1.1−0.2 ($SiMe_3$). MS (ESI): *m/z* 1965.2 ([M + Na + K]$^{2+}$). Anal. Calcd for $C_{155}H_{340}N_{10}O_{45}Si_{30}$: C, 47.65; H, 8.77; N, 3.59. Found: C, 47.39; H, 8.68; N, 3.53.

Pentakis-[*N,N′*-bis-(2,3,4,2′,3′,4′-hexa-*O*-acetyl-6,6′-dideoxy-α,α′-trehalos-6,6′-diyl)]urea (27). To a solution of **25** (26 mg, 8.7 μmol) in acetone−H_2O (2:1, 1.5 mL) was added TFA (50 μL). The reaction mixture was stirred at rt for 16 h, and then, the solvents were removed under reduced pressure, and the residue was purified by column chromatography (EtOAc → 20:1 EtOAc−EtOH). Yield: 12 mg (45%). R_f: 0.53 (45:5:3 EtOAc−EtOH−H_2O). $[\alpha]_D$: +112.6 (*c* 1.0, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 5.45 (t, 10 H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 5.33 (d, 10 H, $J_{1,2} = 3.6$ Hz, H-1), 5.07 (dd, 10 H, H-2), 4.95 (t, 10 H, $J_{4,5} = 9.7$ Hz, H-4), 3.48 (m, 10 H, H-5), 3.52 (da, 10 H, $J_{6a,6b} = 13.2$ Hz, H-6a), 3.01 (dd, 10 H, $J_{5,6b} = 7.5$ Hz, H-6b), 2.07−2.01 (3 s, 90 H, 30 MeCO). ^{13}C NMR (100.6 MHz, CD_3OD): δ 171.8−171.5 (CO ester), 159.9 (CO urea), 92.4 (C-1), 71.5 (C-2), 71.2 (C-3), 71.1 (C-5), 71.0 (C-4), 41.3 (C-6), 20.7 (MeCO). MS (ESI): *m/z* 1576.2 ([M + K + Na]$^{2+}$), 1566.4 ([M + 2Na]$^{2+}$), 1557.1 ([M + Na + H]$^{2+}$), 1545.0 ([M + 2H]$^{2+}$). Anal. Calcd for $C_{125}H_{170}N_{10}O_{80}$: C, 48.54; H, 5.54; N, 4.53. Found: C, 48.55; H, 5.42; N, 4.43.

Pentakis-[*N,N′*-bis-(6,6′-dideoxy-α,α′-trehalos-6,6′-diyl)]urea (28). To a solution of **27** (8.5 mg, 2.8 μmol) in MeOH (1 mL) was added 1 M methanolic NaOMe (50 μL). After 5 min at rt, a white precipitate appeared. The precipitate was dissolved by addition of H_2O (2 mL), and the clear solution was stirred for 1 h. Then, the reaction mixture was neutralized and demineralized with Amberlite IR-120 (H$^+$) and Amberlite MB-9L ion-exchange resins, respectively. Resins were filtered out, and solvents were evaporated to furnish **28** in virtually aquantitative yield (5 mg). R_f: 0.38 (2:1:1

^{i}PrOH−AcOH−H_2O). $[\alpha]_D$: +102.4 (*c* 1.0, H_2O). ^{1}H NMR (500 MHz, D_2O): δ 5.15 (d, 10 H, $J_{1,2}$ = 3.8 Hz, H-1), 4.87 (t, 10 H, $J_{2,3} = J_{3,4}$ = 9.4 Hz, H-3), 3.84 (m, 10 H, H-5), 3.66 (dd, 10 H, H-2), 3.54 (dd, 10 H, $J_{6a,6b}$ = 14.5 Hz, $J_{5,6a}$ = 2.0 Hz, H-6a), 3.40 (dd, 10 H, $J_{5,6b}$ = 6.6 Hz, H-6b), 3.36 (t, 10 H, $J_{4,5}$ = 9.3 Hz, H-4). ^{13}C NMR (125.7 MHz, D_2O): δ 160.9 (CO), 93.2 (C-1), 72.4 (C-3), 71.3 (C-5), 71.2 (C-2), 71.0 (C-4), 40.5 (C-6). MS (ESI): *m*/*z* 971.2 ($[M + Cl + Br]^{2-}$), 963.3 ($[M + SO_4]^{2-}$), 932.3 ($[M - H + Cl]^{2-}$). Anal. Calcd for $C_{65}H_{110}N_{10}O_{50}$: C, 42.62; H, 6.05; N, 7.65. Found: C, 42.66; H, 5.93; N, 7.58.

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Supporting Information Available: General experimental methods, including computational, NMR titration, and fluorescent binding titration details, as well as copies of the NMR spectra for compounds **7**−**28** and binding isotherms for the 1:1 **13**−AC, **13**−TNS, **13**−ANS, **16**−TNS, and **16**−ANS complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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