

DOI: 10.1002/cmdc.201000419

# Symmetry Complementarity-Guided Design of Anthrax Toxin Inhibitors Based on $\beta$ -Cyclodextrin: Synthesis and Relative Activities of Face-Selective Functionalized Polycationic Clusters

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Three new series of potential anthrax toxin inhibitors based on the  $\beta$ -cyclodextrin ( $\beta$ CD) scaffold were developed by exploiting face-selective Cu<sup>I</sup>-catalyzed azide-alkyne 1,3-cycloadditions, amine-isothiocyanate coupling, and allyl group hydroboration-oxidation/hydroxy  $\rightarrow$  amine replacement reactions. The molecular design follows the "symmetry-complementarity" concept between homogeneously functionalized polycationic  $\beta$ CD derivatives and protective antigen (PA), a component of

anthrax toxin known to form C<sub>7</sub>-symmetric pores on the cell membrane used by lethal and edema factors to gain access to the cytosol. The synthesis and antitoxin activity of a collection of  $\beta$ CD derivatives differing in the number, arrangement, and face location of the cationic elements are reported herein. These results set the basis for a structure-activity relationship development program of new candidates to combat the anthrax threat.

## Introduction

Anthrax is a zoonotic disease caused by *Bacillus anthracis*, long considered to be a potentially dangerous biological weapon. The resistance of spores to extreme conditions, their infectiveness, and the dreadful efficiency by which the anthrax toxin defeats host defenses are the main reasons of concern. Conventional antibiotic treatment alone is insufficient against anthrax, as the disease is rarely diagnosed at the early stages of infection, and when identified, the accumulation of toxins secreted by the bacteria are usually highly elevated. Therefore, improved treatments that combine antibiotic and antitoxin activity are still required to combat this threat.<sup>[1]</sup> Our understanding of the mechanisms exploited by anthrax toxin has grown tremendously over the last two decades.<sup>[2]</sup> More importantly, this knowledge has contributed to the identification of a number of potential therapeutic targets.<sup>[3]</sup> Anthrax toxin consists of three components: two toxic cytosolic proteases—edema factor (EF) and lethal factor (LF)—and protective antigen (PA), which mediates cell entry and access of the former two components into the cytosol. PA binds to certain host membrane receptors and self-assembles into heptameric pores that actively translocate EF and LF into the cell.<sup>[4]</sup> The strategies under investigation to tackle toxin action include blockage of PA binding to cell membrane receptors,<sup>[5]</sup> disruption of the pore assembly,<sup>[6]</sup> inhibition of EF<sup>[7]</sup> and LF activity,<sup>[8]</sup> and blockage of traffic through the pore. A strategy based on the installation of multiple copies of moderately binding PA ligands to polymeric platforms, initially envisioned by Collier and colleagues<sup>[9]</sup> and later implemented by others,<sup>[10]</sup> has rendered highly potent inhibitors of PA function. In this line, Karginov and collaborators recently demonstrated that the inhibition of

anthrax toxin in vitro and in vivo can be achieved by using small-molecule blockers of the PA pore, such as certain  $\beta$ -cyclodextrin (cyclomaltoheptaose,  $\beta$ CD) derivatives.<sup>[11]</sup>

$\beta$ CD is a natural cyclic oligosaccharide composed of seven  $\alpha(1\rightarrow4)$ -linked glucose units featuring a relatively rigid tronconic structure ( $\sim 15$  Å outer diameter) that matches the sevenfold symmetry and size ( $\sim 20$  Å inner diameter) of the anthrax PA pore (Figure 1). The installation of a homogeneous display of cationic elements at the primary hydroxy group rim was

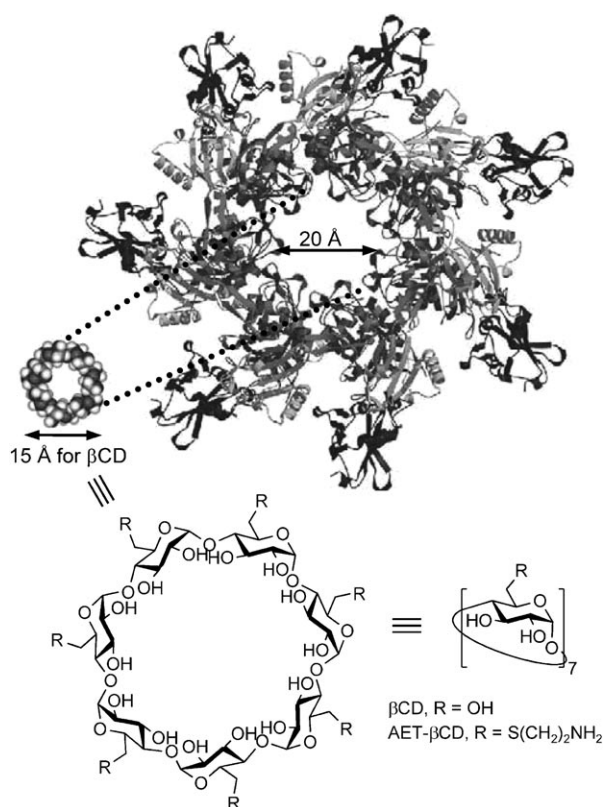
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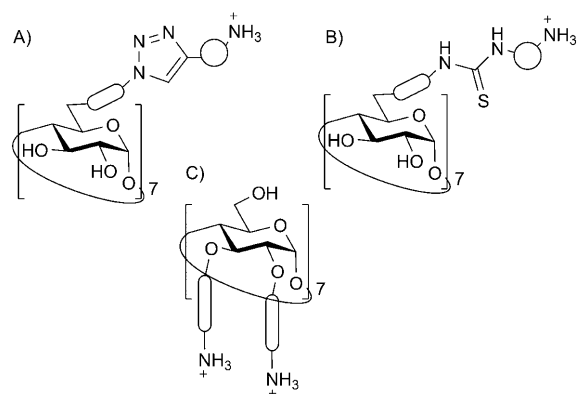
Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/cmdc.201000419>.



**Figure 1.** Schematic illustration of the structures of the anthrax PA pore (top),  $\beta$ CD and the heptacationic derivative heptakis(6-aminoethylthio) $\beta$ CD (AET- $\beta$ CD) (bottom).

shown to afford compounds with antitoxin activity, which was ascribed to the complementary electrostatic interactions with the negatively charged amino acids at the inner surface of the PA pore.<sup>[11a,c-f]</sup>

Many of the current examples of  $\beta$ CD-based anthrax toxin inhibitors are heptakis(6-aminoalkylthio) derivatives, such as heptakis(6-aminoethylthio-6-deoxy) $\beta$ CD (AET- $\beta$ CD; Figure 1),<sup>[11d-f,12]</sup> which were obtained by nucleophilic substitution reactions of conveniently protected  $\alpha,\omega$ -aminothiols and per-(C6)-halogenated  $\beta$ CD derivatives. Although this strategy provided a convincing proof of concept,<sup>[11a-e]</sup> the lack of flexible routes toward the preparation of a wide variety of  $\alpha,\omega$ -aminothiol building blocks and the difficulty in achieving full functionalization of the  $\beta$ CD scaffold was soon realized. To expand the range of potential antitoxin candidate compounds and to perform structure–activity relationship studies, the implementation of versatile  $\beta$ CD conjugation methodologies compatible with molecular-diversity-oriented strategies at relatively low synthetic cost is highly desirable. Herein we report the synthesis of three new families of multivalent cationic  $\beta$ CD derivatives that comply with those requirements, namely per-(C6)-“click” clusters, per-(C6)-aminothiourea clusters, and per-(O2,O3)-aminoalkyl clusters (Figure 2). The first two series retain the heptafunctionalization “jelly-fish”-like pattern<sup>[13]</sup> by exploiting Cu<sup>I</sup>-catalyzed azide–alkyne 1,3-dipolar cycloaddition reactions (“click chemistry”)<sup>[14,15]</sup> and the amine–isothiocyanate coupling reaction,<sup>[16,17]</sup> respectively: two of the most popular and efficient



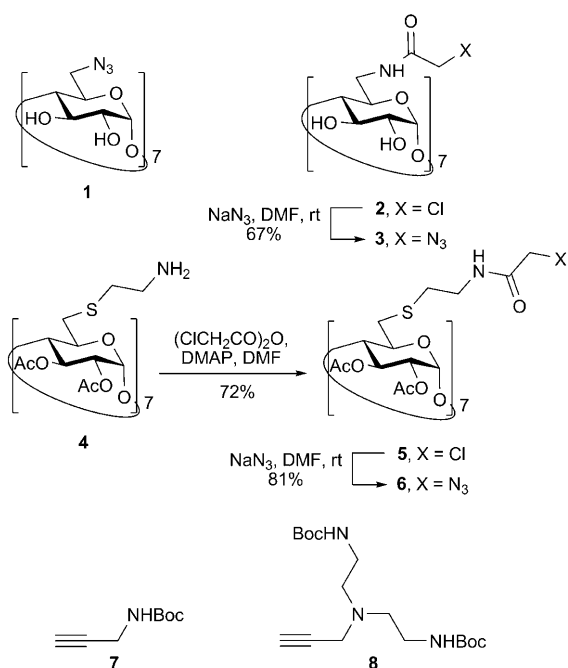
**Figure 2.** Schematic representation of the  $\beta$ CD scaffolded polycationic clusters prepared and evaluated as anthrax antitoxins in this study: A) per-(C6)-click clusters, B) per-(C6)-aminothiourea clusters, and C) per-(O2,O3)-aminoalkyl clusters.

methods for the preparation of multivalent neo-glycoconjugates. In the third prototype, the cationic centers are located at the terminal position of alkyl ether substituents homogeneously distributed at the 14 secondary hydroxy positions (“skirt”-type arrangement).<sup>[13]</sup> While in all cases the  $C_7$  symmetry of the  $\beta$ CD scaffold is preserved, a comparative assessment of the anthrax antitoxin activity indicates a strong influence of the structure of the bridging groups and the arrangement of cationic centers in their aptitude to fit into the anthrax PA pore.

## Results and Discussion

### Synthesis of per-(C6)-“click” clusters

For elaboration of a structurally diverse collection of polyamino  $\beta$ CDs by Cu<sup>I</sup>-catalyzed azide–alkyne cycloaddition, we selected the known per-(C6)-azido  $\beta$ CD **1** and the azidoacetamido  $\beta$ CD derivatives **3** and **6**, differing in the distance of the terminal azide groups to the  $\beta$ CD core, as the central building blocks. All of these can be obtained in a limited number of steps and in good yield from the commercially available  $\beta$ CD by primary face-selective functionalization methodologies.<sup>[18]</sup> Thus, nucleophilic displacement of halogen by azide anion in heptakis(6-chloroacetamido-6-deoxy) $\beta$ CD (**2**) provides the corresponding per-(C6)-azidoacetamido derivative **3**. Compound **6**, which incorporates a cysteamine segment, was obtained from the per-(O2,O3)-acetylated precursor **4**<sup>[19]</sup> by exhaustive N-chloroacetylation ( $\rightarrow$  **5**) and subsequent replacement of the chloro group by azide (Scheme 1). Temporary protection of the secondary hydroxy groups as the corresponding acetates was found to be advantageous in this case to prevent solubility problems. Commercially available *N*-(*tert*-butoxycarbonyl)propargylamine (**7**) and 3-bis[2-(*tert*-butoxycarbonylamino)ethyl]propargylamine (**8**)<sup>[15b]</sup> were chosen as the alkyne counterparts. The first would afford heptavalent cationic clusters, whereas the second would give rise to compounds having 14 primary amino groups.

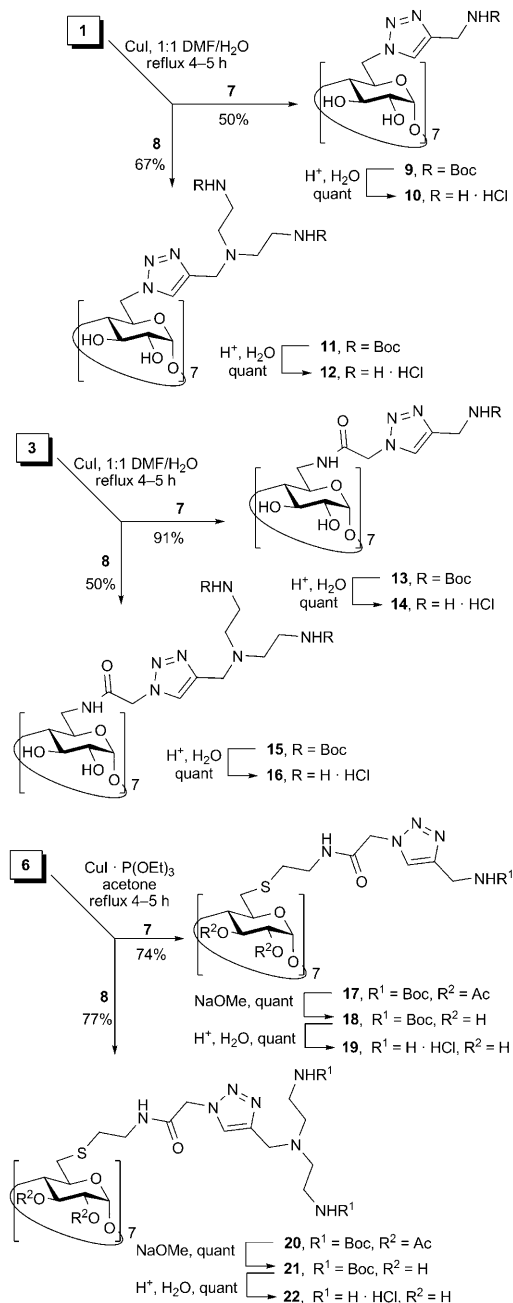


**Scheme 1.** Azide (compounds **1**, **3**, and **6**) and alkyne building blocks (compounds **7** and **8**) for the preparation of per-(C6)- $\beta$ CD click clusters.

The Cu<sup>I</sup>-catalyzed click coupling reactions proceeded smoothly in either 1:1 *N,N*-dimethylformamide/water or acetone under reflux to give the 4-substituted triazolyl hepta-adducts in 50–91% yield. The final removal of the protecting groups produced the target polyamino click clusters in quantitative yield, which were isolated as the corresponding hepta- or tetradecahydrochloride salts after freeze-drying from a dilute solution of HCl (Scheme 2). The NMR, ESIMS and combustion analysis data (Supporting Information) are consistent with the expected C<sub>7</sub>-symmetric arrangement for homogeneously substituted  $\beta$ CD derivatives, attesting to the efficiency of the synthetic scheme.

### Synthesis of per-(C6)-aminothiourea clusters

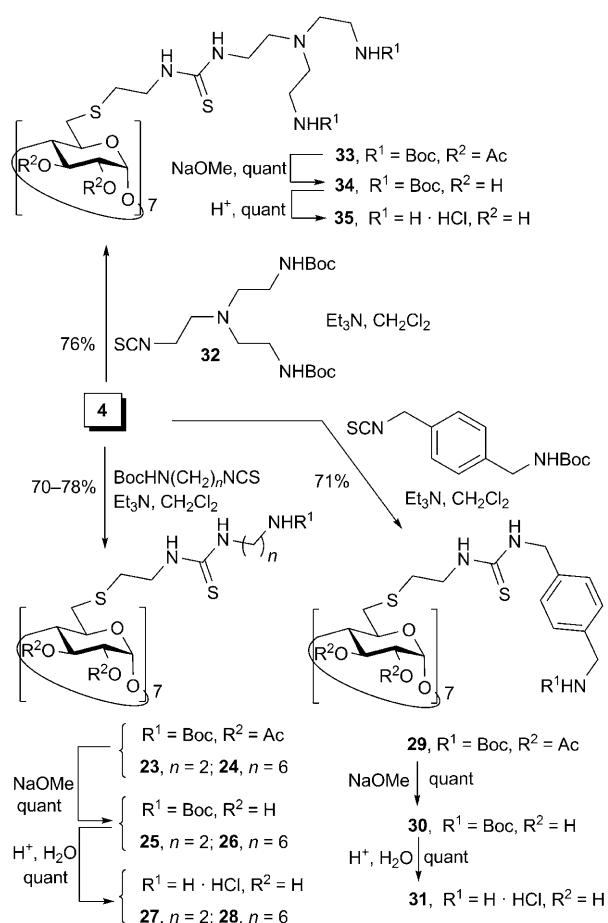
The heptacysteaminy  $\beta$ CD derivative **4** was efficiently coupled in dichloromethane to a set of readily available *N*-Boc-protected aminoisothiocyanates differing in chain length and rigidity to furnish the protected thiourea adducts **23**, **24**, and **29** in 70–78% yield. The branched 2-[3-bis[2-(*tert*-butoxycarbonylamino)ethyl]amino]ethyl isothiocyanate **32**<sup>[19]</sup> likewise furnished the dendritic derivative **33** (76%). Conventional deacetylation with sodium methoxide ( $\rightarrow$  **25**, **26**, **30**, **34**) and final acid hydrolysis of the carbamate groups afforded the corresponding hepta- (**27**, **28**, **31**) and tetradecaminothiourea (**35**)  $\beta$ CD clusters (Scheme 3). Notably, in contrast to what was observed in the triazole-linked series, prolonged acidic treatment of compounds **25**, **26**, **30**, and **34** may lead to partial hydrolysis of the glycosidic linkages of the  $\beta$ CD core. This was more evident in the case of compound **34**. Minimizing the reaction time for the Boc cleavage reaction is critical, in this case, to obtain a homogeneous product.



**Scheme 2.** Synthesis of per-(C6)- $\beta$ CD click clusters.

### Synthesis of per-(O2,O3)-aminoalkyl clusters

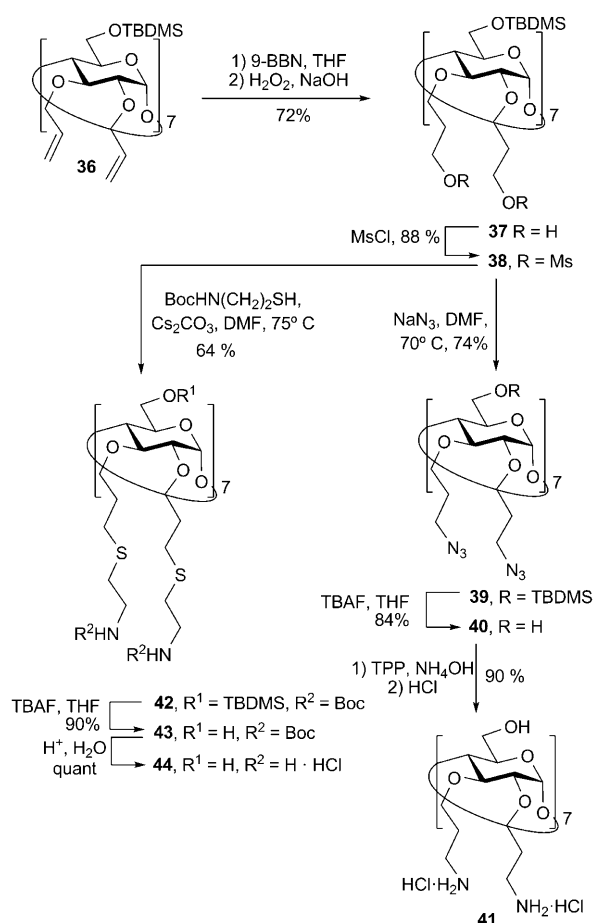
Selective secondary face functionalization of  $\beta$ CD represents a much more difficult challenge than functionalization at the primary positions. The steric constraints at this rim frequently lead to mixtures that contain undersubstituted conjugates. We developed an efficient strategy for the preparation of fully homogeneous per-(O2,O3)-substituted  $\beta$ CD derivatives in which the key step is the homologation of the secondary hydroxy groups through an allylation–hydroboration–oxidation reaction sequence, thereby enlarging the cavity and formally transforming the secondary hydroxy groups into 14 primary hydroxy groups. Prior protection of the OH-6 groups in the native

Scheme 3. Synthesis of per-(C6)-aminothiourea  $\beta$ CD clusters.

cyclo-oligosaccharide is necessary and can be achieved very conveniently by per-(O6)-*tert*-butyldimethylsilylation.<sup>[20]</sup> Further allylation of the secondary hydroxy groups OH-2 and OH-3 provides the face-differentiated derivative **36**,<sup>[20]</sup> which was used as starting material for the preparation of the “reverse”  $\beta$ CD-scaffolded polycationic clusters. The hydroboration–oxidation of the double bonds furnished the tetradecanol **37**,<sup>[21]</sup> which possesses 14 primary hydroxy groups, in 72% yield (Scheme 4). Exhaustive methylsulfonylation ( $\rightarrow$  **38**) followed by nucleophilic substitution by sodium azide ( $\rightarrow$  **39**) was found to be very convenient for the introduction of a nitrogen functionality at every position. Fluorolysis of the silyl ether groups and reduction of the azide groups with triphenylphosphine (TPP) afforded the per-(O2,O3)-(3-aminopropyl)  $\beta$ CD cluster **41** in 50% overall yield. Alternatively, the use of *N*-Boc-protected cysteamine as a nucleophile in reaction with the tetradecamesylate derivative **38** provided the corresponding polythioether **42**. Subsequent desilylation ( $\rightarrow$  **43**) and carbamate removal furnished the homologue tetradecaamine **44** in 58% overall yield (Scheme 4).

### In vitro antitoxin activity evaluation

To evaluate members of the aforementioned  $\beta$ CD library for their ability to inhibit anthrax toxin, a previously described in

Scheme 4. Synthesis of reversed cationic  $\beta$ CD derivatives **41** and **44**.

vitro cytotoxicity assay was used.<sup>[11b,d-f]</sup> Murine leukemic monocyte macrophage RAW 264.7 cells were chosen for this assay in order to be able to compare our results with published data. This cell line exhibits rapid growth and high sensitivity to anthrax lethal toxin (LeTx). The cells were exposed to increasing concentrations of each  $\beta$ CD derivative either in the absence or presence of LeTx (see Figure 3 as an example).

For comparative purposes, the heptacysteamyl derivative AET- $\beta$ CD (Figure 1), previously identified as a strong LeTx in-

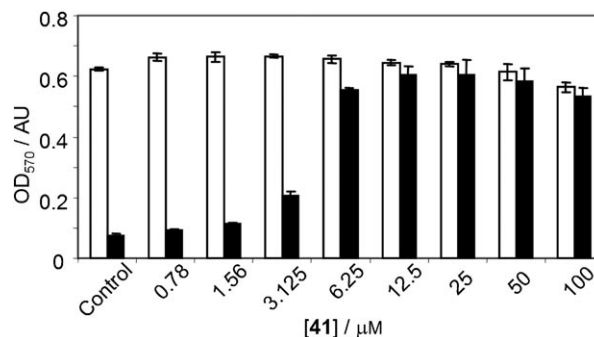


Figure 3. Protection of RAW 264.7 cells from LeTx-induced cell death by  $\beta$ CD derivative **41**. Cells were incubated with various concentrations of **41** in the absence (white bars) or presence (black bars) of LeTx in triplicate. Error bars represent standard deviations.

hibitor ( $IC_{50} = 6.6 \pm 2.4 \mu\text{M}$ ),<sup>[11d-f]</sup> was included in these assays. The results of the toxin inhibition experiments are summarized in Table 1.

The per-(C6)-click cationic clusters **10**, **12**, **14**, **16**, **19**, and **22** did not exhibit any relevant antitoxin activity in the range of concentrations tested (up to  $100 \mu\text{M}$ ). Independently of the density of the cationic display (either 7 or 14 ammonium groups) or connector length, antitoxin activity is just residual. Furthermore, a certain degree of toxicity associated with these cationic  $\beta\text{CD}$  derivatives was observed at concentrations  $> 50 \mu\text{M}$  (see Table 1 and Supporting Information).

The per-(C6)-aminothiourea clusters produced a totally different scenario. Compound **27**, a thiourea-containing analogue of **14**, exhibited an  $IC_{50}$  value of  $26 \pm 21 \mu\text{M}$  (Table 1). Increasing the length and flexibility of the spacer arm between the thiourea group and the terminal cationic centers was favorable in terms of antitoxin activity. Thus, compound **28**, which incorporates aminohexylthioureido in place of aminoethylthioureido segments, shows a much lower  $IC_{50}$  value ( $3.2 \pm 1.9 \mu\text{M}$ ) than

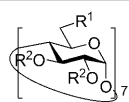
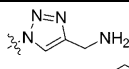
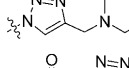
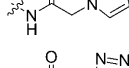
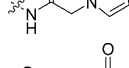
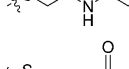
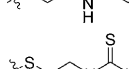
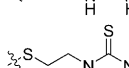
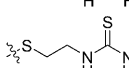
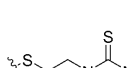
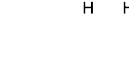
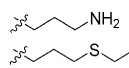
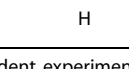
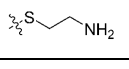
AET- $\beta\text{CD}$  (Table 1). Compound **31**, bearing an aromatic spacer of similar length, exhibited weaker antitoxin activity ( $IC_{50} = 20 \pm 14 \mu\text{M}$ ) pointing to the benefits of more flexible tethers (thiourea vs. triazole) to achieve a better induced fit in the pore. The tetradecacationic adduct **35** did not improve the efficiency observed for the heptavalent candidates, and furthermore exhibited significant cytotoxicity in control cells at relatively low concentrations ( $> 15 \mu\text{M}$ ; see Table 1 and Supporting Information).

The much higher cytotoxicity of compounds **12**, **16**, **32**, and **35**, which incorporate the *N,N*-bis(2-aminoethyl)amino structural moiety, is striking in comparison with compounds with linear arrangements of amino groups. Polycationic macromolecules are expected to interact with negatively charged proteoglycans at the cell surface through electrostatic interactions, which can trigger cell internalization.<sup>[22]</sup> The presence of tertiary amino groups in polycationic polymers such as branched polyethyleneimine (bPEI) has been hypothesized to facilitate subsequent endosome escape by buffering endosome acidification

prior to its fusion with a lysosome, the so-called proton-sponge effect.<sup>[23]</sup> A similar situation may occur in the case of the dendritic polycationic clusters of this work. Once in the cytosol, electrostatic interactions with negatively charged biomolecules may result in cell toxicity.

The secondary rim-functionalized per-(O2,O3)-aminoalkyl  $\beta\text{CD}$  clusters **41** and **44**, bearing a number of primary cationic centers identical to the dendritic cluster **35** but with the opposite orientation in the troncoconic structure and no tertiary amino groups, turned out to be highly efficient as antitoxin agents, with  $IC_{50}$  values of  $4.1 \pm 0.4$  and  $2.1 \pm 0.2 \mu\text{M}$ , respectively (Table 1). This skirt-type architectural design had not been explored before in the context of PA blockers. Alkylation or acylation of the secondary face in cationic clusters such as AET- $\beta\text{CD}$  (e.g. compound **4**) had been found to completely abolish the antitoxin activity (data not shown), which was ascribed to the distortion of the cyclo-oligosaccharide structure after breaking the intramolecular hydrogen bonds of  $\beta\text{CD}$ . As a result, the primary face-located arms are no longer able to fit in the pore cavity. The present results indicate that the

**Table 1.** Inhibition of LeTx cytotoxicity ( $IC_{50}$ ) and cell viability determined for cationic  $\beta\text{CD}$  derivatives in this study.

Compd			$IC_{50}$ [ $\mu\text{M}$ ] <sup>[a]</sup>	Viability [%] <sup>[b]</sup>
	R <sup>1</sup>	R <sup>2</sup>		
<b>10</b>		H	$> 100$	80 <sup>[c]</sup>
<b>12</b>		H	$> 100$	50 <sup>[c]</sup>
<b>14</b>		H	$> 100$	$> 95$ <sup>[c]</sup>
<b>16</b>		H	$> 100$	25 <sup>[c]</sup>
<b>19</b>		H	$> 100$	$> 95$ <sup>[c]</sup>
<b>22</b>		H	$> 100$	30 <sup>[c]</sup>
<b>27</b>		H	$26 \pm 21$	$> 95$
<b>28</b>		H	$3.2 \pm 1.9$	$> 95$
<b>31</b>		H	$20 \pm 14$	90
<b>35</b>		H	$> 100$	25 <sup>[d]</sup>
<b>41</b>	H		$4.1 \pm 0.4$	$> 95$
<b>44</b>	H		$2.1 \pm 0.2$	$> 95$
AET- $\beta\text{CD}$		H	$6.6 \pm 3.9$	$> 95$

[a]  $IC_{50}$  values are the means of at least three independent experiments. [b] Cell viability at  $IC_{50}$  unless another concentration is indicated: [c] at  $100 \mu\text{M}$ ; [d] at  $25 \mu\text{M}$ .

situation at the opposite secondary rim becomes just the reverse: amino groups located at the terminal positions of branches on this side of the distorted  $\beta$ CD platform are well suited to interact with the surface of the PA pore. Moreover, contrary to the dendritic counterpart **35**, compounds **41** and **44** were devoid of toxicity in the 0–100  $\mu$ m range in spite of their highly dense cationic display (see Table 1 and Supporting Information).

## Conclusions

The current body of results indicates that the development of new anthrax pore-blocking agents cannot rely exclusively on symmetric and electrostatic interactions. The comparison of the antitoxin results in the three series of compounds presented shows the tremendous impact of molecular architecture on biological activity. The symmetry complementarity concept alone does not warrant a good fit between the protein pore and the  $\beta$ CD cluster, as illustrated by the failure of per-(C6)-click clusters to inhibit LeTx-induced cell death. Some recent computational calculations on click glycoclusters point to the existence of conformational constraints associated with the presence of rigid triazole segments that might seriously jeopardize the binding of external ligands to specific protein receptors,<sup>[24]</sup> which may well be the situation in the present case. Flexibility seems to be essential to achieve a perfect pore- $\beta$ CD cluster fit, as observed from the results in the per-(C6)-aminothiourea clusters. Even in the case of the secondary face clusters, the antitoxin activity increased after increasing the spacer length by inserting a flexible segment. This skirt-type  $\beta$ CD design, featuring alternative functionalization patterns, may be considered a new antitoxin prototype. The modular synthetic strategies optimized here for the preparation of monodisperse compounds give high yield and full homogeneity and are very well suited for the diversity-oriented approaches in the search for more active derivatives. Research in that direction is currently being carried out in our research groups.

## Experimental Section

All reagents and materials were purchased from commercial sources, including *N*-Boc-2-aminoethyl isothiocyanate, *N*-Boc-6-aminoethyl isothiocyanate, and *N*-(*tert*-butoxycarbonyl)propargylamine (**7**). Heptakis[6-chloroacetamido-6-deoxy]cyclomaltoheptaose (**2**),<sup>[25]</sup> 3-bis[2-(*tert*-butoxycarbonylamino)ethyl]propargylamine (**8**),<sup>[15b]</sup> heptakis[6-(2-aminoethylthio)]cyclomaltoheptaose (**15**),<sup>[12]</sup> heptakis[6-(2-aminoethylthio)-2,3-di-*O*-acetyl]cyclomaltoheptaose (**4**),<sup>[19]</sup> heptakis[2,3-di-*O*-acetyl-6-(2-(*N'*-(2-*tert*-butoxycarbonylaminoethyl)-thioureido)ethylthio)]cyclomaltoheptaose (**23**),<sup>[19]</sup> heptakis[6-(2-(*N'*-(2-*tert*-butoxycarbonylaminoethyl)thioureido)ethylthio)]cyclomaltoheptaose (**25**),<sup>[19]</sup> heptakis[6-(2-(*N'*-(2-aminoethyl)thioureido)ethylthio)]cyclomaltoheptaose heptahydrochloride (**27**),<sup>[19]</sup> 4-(*tert*-butoxycarbonylaminoethyl)benzyl isothiocyanate,<sup>[26]</sup> 2-[3-bis[2-(*tert*-butoxycarbonylamino)ethyl]-amino]ethyl isothiocyanate (**32**),<sup>[19]</sup> and heptakis[2,3-*O*-diallyl-6-*O*-*tert*-butyldimethylsilyl]cyclomaltoheptaose (**36**)<sup>[20]</sup> were prepared as previously reported. Optical rotations were measured at room temperature in 1-dm tubes on a PerkinElmer 141 MC polarimeter. <sup>1</sup>H (and <sup>13</sup>C) NMR spectra were recorded at 300 (75.5 for <sup>13</sup>C), 400 (100.6 for <sup>13</sup>C), and 500

(125.7 for <sup>13</sup>C) MHz with Bruker 300, 400, and 500 DRX instruments; chemical shifts ( $\delta$ ) are reported in ppm. 1D <sup>1</sup>H TOCSY, 2D COSY, <sup>1</sup>H-<sup>13</sup>C HMQC, and HSQC experiments were used to assist NMR assignments. Thin-layer chromatography (TLC) was carried out on aluminum sheets coated with Kieselgel 60 F<sub>254</sub> (E. Merck), with visualization by UV light and by charring with 10% H<sub>2</sub>SO<sub>4</sub> or 0.2% ninhydrin. Column chromatography was carried out with Silica Gel 60 (E. Merck, 230–400 mesh). Electrospray ionization mass spectrometry (ESIMS) data were obtained with a Bruker Esquire 6000 instrument. The samples were dissolved in the appropriate solvent (H<sub>2</sub>O, CH<sub>3</sub>CN, MeOH, or mixtures thereof) at low micromolar concentrations. Elemental analyses were performed at the Instituto de Investigaciones Químicas (Sevilla, Spain). Triphenylphosphine, trifluoroacetic acid, 9-borabicyclo[3.3.1]nonane, 4-dimethylaminopyridine, tetra-*n*-butylammonium fluoride, *N,N*-diisopropylethylamine, and *N,N*-dimethylformamide are indicated by the acronyms TPP, TFA, 9-BBN, DMAP, TBAF, DIPEA, and DMF, respectively.

**Heptakis[6-(4-*tert*-butoxycarbonylaminoethyl-1*H*-1,2,3-triazol-1-yl)-6-deoxy]cyclomaltoheptaose (**9**):** To a solution of heptakis(6-azido-6-deoxy)cyclomaltoheptaose (**1**, 0.2 g, 0.15 mmol) and *N*-(*tert*-butoxycarbonylamino)propargylamine (**7**, 0.18 g, 1.15 mmol, 1.1 equiv) in DMF (6 mL), CuI (70 mg, 0.37 mmol, 0.3 equiv) and H<sub>2</sub>O (6 mL) were added. The reaction mixture was held at reflux for 4 h. The solvent was eliminated under reduced pressure, and the residue was purified by column chromatography (CH<sub>3</sub>CN/H<sub>2</sub>O/NH<sub>4</sub>OH, 10:1:1  $\rightarrow$  10:2:1) to give **9** (0.18 g, 50%);  $R_f = 0.38$  (CH<sub>3</sub>CN/H<sub>2</sub>O/NH<sub>4</sub>OH, 10:2:1);  $[\alpha]_D = +17.0$  ( $c = 1.0$  in MeOH); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD, 323 K):  $\delta = 7.80$  (s, 7H, =CH), 5.18 (s, 7H, H-1), 4.61 (m, 7H, H-6a), 4.40 (bd, 7H,  $J_{6a,6b} = 8.6$  Hz, H-6b), 4.20 (m, 21H, CH<sub>2</sub>NHBoc, H-5), 3.89 (t, 7H,  $J_{2,3} = J_{3,4} = 9.3$  Hz, H-3), 3.48 (dd, 7H,  $J_{1,2} = 3.1$  Hz, H-2), 3.35 (t, 7H,  $J_{4,5} = 8.7$  Hz, H-4), 1.42 (s, 63H, CMe<sub>3</sub>); <sup>13</sup>C NMR (125.7 MHz, CD<sub>3</sub>OD, 323 K):  $\delta = 158.0$  (CO), 147.1 (C4 triazole), 126.0 (C5 triazole), 103.6 (C1), 84.5 (C4), 80.5 (C<sub>q</sub>), 74.3 (C3), 73.8 (C2), 71.7 (C5), 51.4 (C6), 37.0 (CH<sub>2</sub>NHBoc), 29.0 (CMe<sub>3</sub>); ESIMS:  $m/z$  1221 [ $M+2Na$ ]<sup>2+</sup>; Anal. calcd for C<sub>98</sub>H<sub>154</sub>N<sub>28</sub>O<sub>42</sub>: C 49.12, H 6.48, N 16.37, found: C 49.08, H 6.33, N 16.24.

**Heptakis[6-(4-(bis-(2-*tert*-butoxycarbonylaminoethyl)aminomethyl)-1*H*-1,2,3-triazol-1-yl)-6-deoxy]cyclomaltoheptaose (**11**):** A solution of heptakis[6-azido-6-deoxy]cyclomaltoheptaose (**1**, 107 mg, 82  $\mu$ mol), **8** (0.22 g, 0.63 mmol, 1.1 equiv) and CuI (33 mg, 0.17 mmol, 0.3 equiv) in DMF/H<sub>2</sub>O (1:1, 6 mL) was held at reflux for 5 h. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography (CH<sub>3</sub>CN/H<sub>2</sub>O/NH<sub>4</sub>OH, 20:1:1  $\rightarrow$  10:1:1). Yield: 204 mg (67%);  $R_f = 0.24$  (CH<sub>3</sub>CN/H<sub>2</sub>O/NH<sub>4</sub>OH, 10:2:1);  $[\alpha]_D = +12.7$  ( $c$  in 1.0 in MeOH); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD, 323 K):  $\delta = 7.89$  (s, 7H, =CH), 5.17 (d, 7H,  $J_{1,2} = 3.5$  Hz, H-1), 4.55 (m, 14H, H-6a, H-6b), 4.30 (m, 7H, H-5), 3.92 (t, 7H,  $J_{2,3} = J_{3,4} = 9.3$  Hz, H-3), 3.77, 3.68 (2d, 14H,  $^2J_{H,H} = 14.5$  Hz, CH<sub>2</sub>N), 3.44 (dd, 7H, H-2), 3.31 (t, 7H,  $J_{4,5} = 9.1$  Hz, H-4), 3.18–3.11 (m, 28H, CH<sub>2</sub>NHBoc), 2.64–2.56 (m, 28H, CH<sub>2</sub>CH<sub>2</sub>NHBoc), 1.46 (s, 126H, CMe<sub>3</sub>); <sup>13</sup>C NMR (125.7 MHz, CD<sub>3</sub>OD, 313 K):  $\delta = 158.2$  (CO), 145.5 (C4 triazole), 127.1 (C5 triazole), 103.8 (C1), 84.6 (C4), 80.0 (C<sub>q</sub>), 74.4 (C3), 74.0 (C2), 71.6 (C5), 54.5 (CH<sub>2</sub>CH<sub>2</sub>NHBoc), 51.5 (C6), 49.0 (CH<sub>2</sub>N), 39.5 (CH<sub>2</sub>NHBoc), 29.0 (CMe<sub>3</sub>); ESIMS:  $m/z$  1849.3 [ $M+Na+H$ ]<sup>2+</sup>, 1240 [ $M+2H+Na$ ]<sup>3+</sup>; Anal. calcd for C<sub>161</sub>H<sub>280</sub>N<sub>42</sub>O<sub>56</sub>: C 52.26, H 7.63, N 15.90. Found C 51.90, H 7.33, N 15.54.

**Heptakis[6-(4-aminomethyl-1*H*-1,2,3-triazol-1-yl)-6-deoxy]-cyclomaltoheptaose heptahydrochloride (**10**):** A solution of the carbamate **9** (80 mg, 33  $\mu$ mol) in TFA/H<sub>2</sub>O (1:1, 2 mL) was stirred at room temperature for 2 h, the solvents were evaporated and the residue was freeze-dried from a diluted HCl solution to quantitatively give **10**. Yield: 63 mg (99%);  $[\alpha]_D = +25.6$  ( $c = 1.0$  in H<sub>2</sub>O);

$^1\text{H}$  NMR (500 MHz,  $\text{D}_2\text{O}$ , 323 K):  $\delta$  = 8.08 (s, 7H, =CH), 5.08 (d, 7H,  $J_{1,2}$  = 2.5 Hz, H-1), 4.41 (dd, 7H,  $J_{6a,6b}$  = 14.2 Hz,  $J_{5,6a}$  = 3.0 Hz, H-6a), 4.30 (dd, 7H,  $J_{5,6b}$  = 6.5 Hz, H-6b), 4.20 (ddd, 7H,  $J_{4,5}$  = 9.5 Hz, H-5), 4.11, 4.09 (2d, 14H,  $^2J_{\text{H,H}}$  = 14.5 Hz,  $\text{CH}_2\text{NH}_2$ ), 3.97 (t, 7H,  $J_{2,3}$  =  $J_{3,4}$  = 9.5 Hz, H-3), 3.54 (dd, 7H, H-2), 3.30 (t, 7H, H-4);  $^{13}\text{C}$  NMR (125.7 MHz,  $\text{D}_2\text{O}$ , 323 K):  $\delta$  = 140.1 (C4 triazole), 127.5 (C5 triazole), 102.9 (C1), 82.5 (C4), 72.6 (C3), 72.1 (C2), 70.2 (C5), 50.7 (C6), 34.3 ( $\text{CH}_2\text{NH}_2$ ); ESIMS:  $m/z$  879.3 [ $M+2\text{Na}+\text{H}_2\text{O}$ ] $^{2+}$ ; Anal. calcd for  $\text{C}_{63}\text{H}_{105}\text{Cl}_7\text{N}_{28}\text{O}_{28}$ : C 38.79, H 5.43, N 20.10, found: C 38.47, H 5.34, N 20.03.

**Heptakis[6-(4-(bis-(2-aminoethyl)aminomethyl)-1H-1,2,3-triazol-1-yl)-6-deoxy]cyclomaltoheptaose tetradecahydrochloride (12).** A solution of the carbamate **11** (94 mg, 33  $\mu\text{mol}$ ) in TFA/ $\text{H}_2\text{O}$  (1:1, 2 mL) was stirred at room temperature for 2 h, the solvents were evaporated and the residue was freeze-dried from a diluted HCl solution to quantitatively give **12**. Yield: 102 mg (99%); [ $\alpha$ ] $_D$  = +17.0 ( $c$  = 0.9 in MeOH);  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ , 313 K):  $\delta$  = 8.02 (s, 7H, =CH), 5.16 (d, 7H,  $J_{1,2}$  = 3.5 Hz, H-1), 4.61 (dd, 7H,  $J_{6a,6b}$  = 15.3 Hz,  $J_{5,6a}$  = 3.6 Hz, H-6a), 4.55 (dd, 7H,  $J_{5,6b}$  = 4.9 Hz, H-6b), 4.28 (m, 7H, H-5), 3.97 (t, 7H,  $J_{2,3}$  =  $J_{3,4}$  = 9.2 Hz, H-3), 3.83, 3.76 (2d, 14H,  $^2J_{\text{H,H}}$  = 15.3 Hz,  $\text{CH}_2\text{N}$ ), 3.42 (dd, 7H, H-2), 3.26 (t, 7H,  $J_{4,5}$  = 9.3 Hz, H-4), 3.15 (t, 28H,  $^3J_{\text{H,H}}$  = 5.7 Hz,  $\text{CH}_2\text{NH}_2$ ), 2.79 (t, 28H,  $\text{CH}_2\text{CH}_2\text{NH}_2$ );  $^{13}\text{C}$  NMR (125.7 MHz,  $\text{CD}_3\text{OD}$ , 313 K):  $\delta$  = 143.3 (C4 triazole), 128.0 (C5 triazole), 103.5 (C1), 84.2 (C4), 74.0 (C3), 73.8 (C2), 71.1 (C5), 51.8 ( $\text{CH}_2\text{CH}_2\text{NH}_2$ ), 51.5 (C6), 47.3 ( $\text{CH}_2\text{N}$ ), 38.3 ( $\text{CH}_2\text{NH}_2$ ); ESIMS:  $m/z$  1149.7 [ $M+2\text{H}$ ] $^{2+}$ , 767.1 [ $M+3\text{H}$ ] $^{3+}$ ; Anal. calcd for  $\text{C}_{91}\text{H}_{182}\text{Cl}_{14}\text{N}_{42}\text{O}_{28}$ : C 38.91, H 6.53, N 20.94. Found C 38.52, H 6.39, N 20.61.

**Heptakis[6-(azidoacetamido)-6-deoxy]cyclomaltoheptaose (3).** To a solution of heptakis[6-(chloroacetamido)-6-deoxy]cyclomaltoheptaose (**2**, 1.68 g, 1.0 mmol) in dry DMF (11 mL), sodium azide (1.38 g, 21.2 mmol, 3 equiv) was added. The mixture was stirred at room temperature for 16 h. The solvent was evaporated and the resulting residue was washed with cold  $\text{H}_2\text{O}$  (7 mL). EtOAc was added (5 mL) and the residue was crushed to give **3** as a white solid. Yield: 1.13 g (67%);  $R_f$  = 0.31 ( $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{H}_2\text{O}$ , 30:10:1); [ $\alpha$ ] $_D$  = +103.6 ( $c$  = 1.0 in DMSO);  $^1\text{H}$  NMR (500 MHz, [ $\text{D}_6$ ]DMSO):  $\delta$  = 8.13 (m, 7H, NH), 5.89, 5.82 (m, 14H, OH-2, OH-3), 4.89 (d, 7H,  $J_{1,2}$  = 3.0 Hz, H-1), 3.92 (d, 7H,  $^3J_{\text{H,H}}$  = 15.6 Hz,  $\text{CH}_a$  amide), 3.89 (d, 7H,  $\text{CH}_b$  amide), 3.82 (m, 14H, H-6a, H-6b), 3.71 (bt, 7H,  $J_{4,5}$  =  $J_{5,6}$  = 7.1 Hz, H-5), 3.62 (t, 7H,  $J_{2,3}$  =  $J_{3,4}$  = 9.3 Hz, H-3), 3.38 (m, 7H, H-2), 3.29 (m, 7H, H-4);  $^{13}\text{C}$  NMR (125.7 MHz, [ $\text{D}_6$ ]DMSO):  $\delta$  = 168.6 (CO), 102.6 (C1), 83.8 (C4), 73.1 (C3), 72.6 (C2), 70.4 (C5), 51.2 ( $\text{CH}_2$  amide), 49.0 (C6); ESIMS:  $m/z$  874.3 [ $M+K+H$ ] $^{2+}$ , 1731.5 [ $M+Na$ ] $^{+}$ ; Anal. calcd for  $\text{C}_{56}\text{H}_{84}\text{N}_{28}\text{O}_{35}$ : C 39.35, H 4.95, N 22.94, found: C 39.12, H 4.82, N 22.65.

**Heptakis[6-(2-(4-(tert-butoxycarbonylaminoethyl)-1H-1,2,3-triazol-1-yl)acetamido)-6-deoxy]cyclomaltoheptaose (13).** To a solution of **3** (0.1 g, 59  $\mu\text{mol}$ ) and (*N*-tert-butoxycarbonyl)propargylamine (**7**, 84 mg, 0.54 mmol, 1.3 equiv) in DMF/ $\text{H}_2\text{O}$  (1:1, 2 mL), CuI (8 mg, 41  $\mu\text{mol}$ , 0.1 equiv) was added and held at reflux for 4 h. Solvents were evaporated under reduced pressure and the residue was purified by column chromatography ( $\text{CH}_3\text{CN}/\text{H}_2\text{O}/\text{NH}_4\text{OH}$ , 10:1:1) to give **13** (0.10 g, 91%);  $R_f$  = 0.23 ( $\text{CH}_3\text{CN}/\text{H}_2\text{O}/\text{NH}_4\text{OH}$ , 10:2:1); [ $\alpha$ ] $_D$  = +72.5 ( $c$  = 1.0 in MeOH);  $^1\text{H}$  NMR (500 MHz, MeOD, 323 K):  $\delta$  = 7.81 (s, 7H, =CH), 5.10 (d, 7H,  $^3J_{\text{H,H}}$  = 16 Hz,  $\text{CH}_a$  amide), 5.04 (d, 7H,  $\text{CH}_b$  amide), 4.94 (d, 7H,  $J_{1,2}$  = 3.3 Hz, H-1), 4.29 (d, 7H,  $^3J_{\text{H,H}}$  = 15.4 Hz,  $\text{CH}_2\text{NHBoc}$ ), 4.24 (d, 7H,  $\text{CH}_2\text{NHBoc}$ ), 3.98 (m, 7H, H-5), 3.86 (t, 7H,  $J_{2,3}$  =  $J_{3,4}$  = 9.5 Hz, H-3), 3.53 (m, 7H, H-2), 3.47 (m, 14H, H-6a, H-6b), 3.32 (m, 7H, H-4), 1.45 (s, 63H,  $\text{CMe}_3$ );  $^{13}\text{C}$  NMR (125.7 MHz, MeOH, 313 K):  $\delta$  = 168.3 (CO amide), 158.2 (CO carbamate), 148.6 (C4 triazole), 126.8 (C5 triazole), 104.6 (C1), 85.5 (C4), 80.5 (C $_q$ ), 74.3 (C3, C2), 71.7 (C5), 53.4 ( $\text{CH}_2$  amide), 41.8 (C6), 37.0

( $\text{CH}_2\text{NHBoc}$ ), 29.1 ( $\text{CMe}_3$ ); ESIMS:  $m/z$  1409.7 [ $M+H+Na$ ] $^{2+}$ , 1417.6 [ $M+H+K$ ] $^{2+}$ , 1420.7 [ $M+2Na$ ] $^{2+}$ , 1428.1 [ $M+K+Na$ ] $^{2+}$ , 2817.2 [ $M+Na$ ] $^{+}$ ; Anal. calcd for  $\text{C}_{112}\text{H}_{175}\text{N}_{35}\text{O}_{49}$ : C 48.12, H 6.31, N 17.53, found: C 47.89, H 6.11, N 17.38.

**Heptakis[6-(2-(4-(bis-(2-tert-butoxycarbonylaminoethyl)aminoethyl)-1H-1,2,3-triazol-1-yl)acetamido)-6-deoxy]cyclomaltoheptaose (15).** To a solution of **3** (0.1 g, 59  $\mu\text{mol}$ ) and **8** (0.18 g, 0.53 mmol, 1.3 equiv) in DMF/ $\text{H}_2\text{O}$  (1:1, 4 mL), CuI (8 mg, 41  $\mu\text{mol}$ , 0.1 equiv) was added and held at reflux for 5 h. Solvents were evaporated under reduced pressure and the residue was purified by column chromatography ( $\text{CH}_3\text{CN}/\text{H}_2\text{O}/\text{NH}_4\text{OH}$ , 10:1:1) to give **15** (0.12 g, 50%);  $R_f$  = 0.53 ( $\text{CH}_3\text{CN}/\text{H}_2\text{O}/\text{NH}_4\text{OH}$ , 10:2:1); [ $\alpha$ ] $_D$  = +72.5 ( $c$  = 1.0 in MeOH);  $^1\text{H}$  NMR (500 MHz, MeOD, 313 K):  $\delta$  = 7.89 (s, 7H, =CH), 5.16 (d, 7H,  $^3J_{\text{H,H}}$  = 16.2 Hz,  $\text{CH}_a$  amide), 5.11 (d, 7H,  $\text{CH}_b$  amide), 4.96 (d, 7H,  $J_{1,2}$  = 3.4 Hz, H-1), 3.94 (m, 7H, H-5), 3.87 (t, 7H,  $J_{2,3}$  =  $J_{3,4}$  = 9.4 Hz, H-3), 3.78 (s, 14H,  $\text{CH}_2\text{N}$ ), 3.59 (m, 14H, H-6a, H-6b), 3.54 (dd, 7H, H-2), 3.35 (t, 7H,  $J_{4,5}$  = 9.3 Hz, H-4), 3.14 (t, 28H,  $^3J_{\text{H,H}}$  = 6.0 Hz,  $\text{CH}_2\text{NHBoc}$ ), 2.56 (t, 28H,  $\text{CH}_2\text{CH}_2\text{NHBoc}$ ), 1.45 (s, 126H,  $\text{CMe}_3$ );  $^{13}\text{C}$  NMR (100.6 MHz, MeOH):  $\delta$  = 168.3 (CO amide), 158.3 (CO carbamate), 145.2 (C4 triazole), 126.9 (C5 triazole), 103.6 (C1), 84.8 (C4), 80.0 (C $_q$ ), 74.3 (C3), 74.0 (C2), 71.7 (C5), 54.4 ( $\text{CH}_2\text{CH}_2\text{NHBoc}$ ), 53.2 ( $\text{CH}_2$  amide), 49.2 ( $\text{CH}_2\text{N}$ ), 41.5 (C6), 39.4 ( $\text{CH}_2\text{NHBoc}$ ), 29.2 ( $\text{CMe}_3$ ); ESIMS:  $m/z$  2050.7 [ $M+2\text{H}$ ] $^{2+}$ , 2061.7 [ $M+H+Na$ ] $^{2+}$ , 2069.7 [ $M+H+K$ ] $^{2+}$ , 2077.6 [ $M+2Na$ ] $^{2+}$ , 4100.1 [ $M+H$ ] $^{+}$ ; Anal. calcd for  $\text{C}_{175}\text{H}_{301}\text{N}_{49}\text{O}_{63}$ : C 51.27, H 7.40, N 16.74, found: C 50.96, H 7.31, N 16.42.

**Heptakis[6-(2-(4-aminomethyl-1H-1,2,3-triazol-1-yl)acetamido)-6-deoxy]cyclomaltoheptaose heptahydrochloride (14).** A solution of the carbamate **13** (94 mg, 34  $\mu\text{mol}$ ) in TFA/ $\text{H}_2\text{O}$  (1:1, 2 mL) was stirred at room temperature for 2 h, the solvents were evaporated and the residue was freeze-dried from a diluted HCl solution to give **14** (79 mg, 99%); [ $\alpha$ ] $_D$  = +68.9 ( $c$  = 1.0 in  $\text{H}_2\text{O}$ );  $^1\text{H}$  NMR (500 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 8.02 (s, 7H, =CH), 5.14 (s, 14H,  $\text{CH}_2$  amide), 4.96 (d, 7H,  $J_{1,2}$  = 3.6 Hz, H-1), 4.22 (s, 14H,  $\text{CH}_2\text{NH}$ ), 3.89 (m, 14H, H-5, H-3), 3.64 (m, 14H, H-6a, H-6b), 3.55 (dd, 7H,  $J_{2,3}$  = 9.7 Hz, H-2), 3.40 (t, 7H,  $J_{3,4}$  =  $J_{4,5}$  = 9.3 Hz, H-4);  $^{13}\text{C}$  NMR (125.7 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 167.6 (CO amide), 139.7 (C4 triazole), 126.7 (C5 triazole), 101.6 (C1), 82.3 (C4), 72.6 (C3), 71.9 (C2), 69.8 (C5), 52.0 ( $\text{CH}_2$  amide), 39.8 (C6), 33.9 ( $\text{CH}_2\text{NH}$ ); ESIMS:  $m/z$  1048.4 [ $M+2\text{H}$ ] $^{2+}$ , 1066.9 [ $M+K+H$ ] $^{2+}$ , 1079.9 [ $M+Na+K$ ] $^{2+}$ ; Anal. calcd for  $\text{C}_{77}\text{H}_{126}\text{Cl}_7\text{N}_{35}\text{O}_{35}$ : C 39.35, H 5.40, N 20.86, found: C 39.02, H 5.24, N 20.54.

**Heptakis[6-(2-(4-(bis-(2-aminoethyl)aminomethyl)-1H-1,2,3-triazol-1-yl)acetamido)-6-deoxy]cyclomaltoheptaose tetradecahydrochloride (16).** A solution of the carbamate **15** (118 mg, 29  $\mu\text{mol}$ ) in TFA/ $\text{H}_2\text{O}$  (1:1, 2 mL) was stirred at room temperature for 2 h, the solvents were evaporated and the residue was freeze-dried from a diluted HCl solution to give **16** (90 mg, 99%); [ $\alpha$ ] $_D$  = +37.7 ( $c$  = 1.0 in  $\text{H}_2\text{O}$ );  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 7.93 (s, 7H, =CH), 5.14 (m, 14H,  $\text{CH}_2$  amide), 4.90 (d, 7H,  $J_{1,2}$  = 3.8 Hz, H-1), 3.88 (m, 14H, H-5, H-3), 3.82 (s, 14H,  $\text{CH}_2\text{N}$ ), 3.73 (dd, 7H,  $J_{6a,6b}$  = 13.8 Hz,  $J_{5,6a}$  = 5.4 Hz, H-6a), 3.51 (m, 14H, H-6b, H-2), 3.34 (t, 7H,  $J_{3,4}$  =  $J_{4,5}$  = 9.6 Hz, H-4), 3.08 (t, 28H,  $^3J_{\text{H,H}}$  = 5.8 Hz,  $\text{CH}_2\text{NH}_2$ ), 2.72 (bt, 28H,  $\text{CH}_2\text{CH}_2\text{NH}_2$ );  $^{13}\text{C}$  NMR (100.6 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 167.5 (CO amide), 140.2 (C4 triazole), 127.2 (C5 triazole), 101.3 (C1), 81.9 (C4), 72.4 (C3), 71.9 (C2), 69.7 (C5), 51.9 ( $\text{CH}_2$  amide), 49.8 ( $\text{CH}_2\text{CH}_2\text{NH}_2$ ), 46.1 ( $\text{CH}_2\text{N}$ ), 39.4 (C6), 35.8 ( $\text{CH}_2\text{NH}_2$ ); ESIMS:  $m/z$  1350.1 [ $M+2\text{H}$ ] $^{2+}$ , 900.1 [ $M+3\text{H}$ ] $^{3+}$ ; Anal. calcd for  $\text{C}_{105}\text{H}_{203}\text{Cl}_{14}\text{N}_{49}\text{O}_{35}$ : C 46.74, H 7.06, N 25.44, found: C 50.96, H 7.31, N 16.42.

**Heptakis[6-deoxy-2,3-di-O-acetyl-6-(2-(chloroacetamido)ethylthio)]cyclomaltoheptaose (5).** To a solution of heptakis[2,3-di-O-acetyl-6-(2-aminoethylthio)]cyclomaltoheptaose heptahydrochlor-

ide (**4**, 0.15 g, 70  $\mu$ mol) in dry DMF (10 mL) under Ar atmosphere, DMAP was added (0.12 g, 1.0 mmol, 2 equiv) and stirred for 15 min. Chloroacetic anhydride (0.25 g, 1.5 mmol, 3 equiv) was added and the reaction mixture was stirred for 6 h. The solvent was removed under reduced pressure. The residue was dissolved in  $\text{CH}_2\text{Cl}_2$  (30 mL) and washed with  $\text{H}_2\text{O}$  ( $3 \times 10$  mL). The organic layer was dried, filtered and concentrated. The residue was purified by column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  30:1  $\rightarrow$  20:1) to give **5** (0.13 g, 72%):  $R_f = 0.56$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , 9:1);  $[\alpha]_D = +73.8$  ( $c = 0.8$  in  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 7.40$  (m, 7H, NH), 5.31 (t, 7H,  $J_{2,3} = J_{3,4} = 9.5$  Hz, H-3), 5.15 (d, 7H,  $J_{1,2} = 3.7$  Hz, H-1), 4.81 (dd, 14H, H-2), 4.20 (m, 7H, H-5), 4.11 (s, 14H,  $\text{CH}_2\text{Cl}$ ), 3.82 (t, 7H,  $J_{4,5} = 8.6$  Hz, H-4), 3.55 (m, 14H,  $\text{CH}_2\text{NH}_{\text{cyst}}$ ), 3.12 (bd, 7H,  $J_{6a,6b} = 11.9$  Hz, H-6a), 3.1 (bd, 7H, H-6b), 2.90–2.80 (m, 14H,  $\text{CH}_2\text{S}_{\text{cyst}}$ ), 2.10, 2.07 (2 s, 42H,  $\text{CH}_3$ );  $^{13}\text{C NMR}$  (100.6 MHz,  $\text{CDCl}_3$ ):  $\delta = 170.5$ , 170.3 (CO ester), 169.4 (CO amide), 96.8 (C1), 78.7 (C4), 71.2 (C5), 70.6 (C2), 70.5 (C3), 42.7 ( $\text{CH}_2\text{Cl}$ ), 39.1 ( $\text{CH}_2\text{N}_{\text{cyst}}$ ), 31.7 (C6), 29.5 ( $\text{CH}_2\text{S}_{\text{cyst}}$ ), 20.9, 20.8 ( $\text{CH}_3$ ); ESIMS:  $m/z$  1358.6  $[\text{M}+2\text{Na}]^{2+}$ , 2695.1  $[\text{M}+\text{Na}]^+$ ; Anal. calcd for  $\text{C}_{98}\text{H}_{140}\text{Cl}_7\text{N}_7\text{O}_{49}\text{S}_7$ : C 44.04, H 5.28, N 3.67, S 8.40, found: C 43.75, H 4.98, N 3.49, S 8.11.

**Heptakis[6-deoxy-2,3-di-O-acetyl-6-(2-(azidoacetamido)ethylthio)]cyclomaltoheptaose (6)**. To a solution of **5** (41.2 mg, 15  $\mu$ mol) in dry DMF (5 mL),  $\text{NaN}_3$  (21 mg, 0.3 mmol, 3 equiv) was added. The reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure. The residue was dissolved in  $\text{CH}_2\text{Cl}_2$  (20 mL) and washed with  $\text{H}_2\text{O}$  ( $3 \times 5$  mL). The organic layer was dried, filtered and concentrated. The residue was purified by column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , 9:1) to give **6** (33 mg, 81%):  $R_f = 0.57$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , 9:1);  $[\alpha]_D = +103$  ( $c = 1.0$  in  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H NMR}$  (400 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta = 5.40$  (t, 7H,  $J_{2,3} = J_{3,4} = 8.7$  Hz, H-3), 5.20 (d, 7H,  $J_{1,2} = 3.7$  Hz, H-1), 4.81 (dd, 7H, H-2), 4.24 (m, 7H, H-5), 4.00 (s, 14H,  $\text{CH}_2\text{N}_3$ ), 3.91 (t, 7H,  $J_{4,5} = 8.9$  Hz, H-4), 3.52 (m, 14H,  $\text{CH}_2\text{NH}_{\text{cyst}}$ ), 3.25 (dd, 7H,  $J_{6a,6b} = 11.9$  Hz,  $J_{6a,5} = 2.0$  Hz, H-6a), 3.11 (dd, 7H,  $J_{6b,5} = 6.3$  Hz H-6b), 2.90–2.70 (m, 14H,  $\text{CH}_2\text{S}_{\text{cyst}}$ ), 2.10 (bs, 42H,  $\text{CH}_3$ );  $^{13}\text{C NMR}$  (100.6 MHz,  $\text{CDCl}_3$ ):  $\delta = 170.8$ , 170.1 (CO ester), 168.5 (CO amide), 96.7 (C1), 78.8 (C4), 71.8 (C5), 70.9 (C2), 70.8 (C3), 51.9 ( $\text{CH}_2\text{N}_3$ ), 39.1 ( $\text{CH}_2\text{N}_{\text{cyst}}$ ), 33.5 (C6), 31.6 ( $\text{CH}_2\text{S}_{\text{cyst}}$ ), 19.8, 19.6 ( $\text{CH}_3$ ); ESIMS:  $m/z$  1381.8  $[\text{M}+2\text{Na}]^{2+}$ ; Anal. calcd for  $\text{C}_{98}\text{H}_{140}\text{N}_{28}\text{O}_{49}\text{S}_7$ : C 43.29, H 5.19, N 14.43, S 8.26, found: C 43.33, H 5.12, N 14.21, S 7.94.

**Heptakis[6-deoxy-2,3-di-O-acetyl-6-(2-(4-(tert-butoxycarbonylaminomethyl)-1H-1,2,3-triazol-1-yl)acetamido)ethylthio)]cyclomaltoheptaose (17)**. To a solution of **6** (70.5 mg, 26  $\mu$ mol) in acetone (7 mL), *N*-(tert-butoxycarbonyl)propargylamine (**7**, 37 mg, 0.24 mmol, 1.3 equiv),  $\text{CuI} \cdot \text{P}(\text{EtO})_3$  (6.5 mg, 18  $\mu$ mol, 0.1 equiv) and DIPEA (31  $\mu$ L, 0.18 mmol, 1 equiv) were added and the reaction mixture was held at reflux for 5 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , 30:1  $\rightarrow$  9:1) to give **17** (73 mg, 74%):  $R_f = 0.58$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , 9:1);  $[\alpha]_D = +70.4$  ( $c = 1.0$  in  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H NMR}$  (500 MHz,  $\text{CD}_3\text{OD}$ , 323 K):  $\delta = 7.87$  (s, 7H, =CH), 5.36 (bs, 7H, H-3), 5.19 (s, 7H, H-1), 5.14 (s, 14H,  $\text{CH}_2\text{CONH}$ ), 4.83 (d, 7H,  $J_{2,3} = 6.7$  Hz, H-2), 4.33 (s, 14H,  $\text{CH}_2\text{NHBoc}$ ), 4.25 (bs, 7H, H-5), 3.88 (s, 7H, H-4), 3.50 (bt, 14H,  $^3J_{\text{H,H}} = 5.9$ ,  $\text{CH}_2\text{NH}_{\text{cyst}}$ ), 3.29 (bs, 7H, H-6a), 3.09 (bs, 7H, H-6b), 2.86 (bt, 14H,  $\text{CH}_2\text{S}_{\text{cyst}}$ ), 2.09 (bs, 42H,  $\text{CH}_3$ ), 1.45 (s, 63H,  $\text{CMe}_3$ );  $^{13}\text{C NMR}$  (125.7 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta = 173.1$ , 172.6 (CO ester), 168.8 (CO amide), 159.0 (CO carbamate), 148.2 (C4 triazole), 126.5 (C5 triazole), 99.1 (C1), 81.4 (C4,  $\text{C}_q$ ), 73.1 (C5, C2, C3), 54.3 ( $\text{CH}_2\text{CONH}$ ), 41.6 ( $\text{CH}_2\text{N}_{\text{cyst}}$ ), 37.9 ( $\text{CH}_2\text{NHBoc}$ ), 35.9 (C6), 34.9 ( $\text{CH}_2\text{S}_{\text{cyst}}$ ), 29.8 ( $\text{CMe}_3$ ), 22.3 ( $\text{CH}_3$ ); ESIMS:  $m/z$  1291.3  $[\text{M}+3\text{Na}]^{3+}$ , 1224.6  $[\text{M}+2\text{Na}]^{2+}$ ; Anal. calcd for  $\text{C}_{154}\text{H}_{231}\text{N}_{35}\text{O}_{63}\text{S}_7$ : C 48.61, H 6.12, N 12.88, S 5.90, found: C 48.24, H 6.10, N 12.50, S 6.16.

**Heptakis[6-deoxy-2,3-di-O-acetyl-6-(2-(4-(bis-(2-tert-butoxycarbonylaminomethyl)-aminomethyl)-1H-1,2,3-triazol-1-yl)acetamido)ethylthio)]cyclomaltoheptaose (20)**. To a solution of **6** (68 mg, 25  $\mu$ mol) in acetone (7 mL), **8** (95 mg, 0.23 mmol, 1.3 equiv),  $\text{CuI} \cdot \text{P}(\text{EtO})_3$  (6 mg, 17  $\mu$ mol, 0.1 equiv) and DIPEA (30  $\mu$ L, 0.17 mmol, 1 equiv) were added and the reaction mixture was held at reflux for 4 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , 30:1  $\rightarrow$  9:1) to give **20** (98 mg, 77%):  $R_f = 0.62$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , 9:1);  $[\alpha]_D = +57.4$  ( $c = 1.0$  in  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H NMR}$  (400 MHz,  $\text{CD}_3\text{OD}$ , 323 K):  $\delta = 7.94$  (s, 7H, =CH), 5.33 (t, 7H,  $J_{2,3} = J_{3,4} = 10.7$  Hz, H-3), 5.19 (d, 7H,  $J_{1,2} = 4.0$  Hz, H-1), 5.18 (s, 14H,  $\text{CH}_2\text{CONH}$ ), 4.83 (dd, 7H, H-2), 4.25 (m, 7H, H-5), 4.84 (m, 21H,  $\text{CH}_2$ -triazole, H-4), 3.50 (m, 14H,  $\text{CH}_2\text{NH}_{\text{cyst}}$ ), 3.17 (t, 21H,  $^3J_{\text{H,H}} = 7.4$  Hz, H-6a,  $\text{CH}_2\text{NHBoc}$ ), 3.10 (dd, 7H,  $J_{6a,6b} = 17.0$  Hz,  $J_{6b,5} = 9.5$  Hz, H-6b), 2.86 (bs, 14H,  $\text{CH}_2\text{S}_{\text{cyst}}$ ), 2.61 (bs, 28H,  $\text{CH}_2\text{CH}_2\text{NHBoc}$ ), 2.10, 2.07 (2 s, 42H,  $\text{CH}_3$ ), 1.45 (s, 126H,  $\text{CMe}_3$ );  $^{13}\text{C NMR}$  (100.6 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta = 170.6$ , 169.8 (CO ester), 166.3 (CO amide), 156.8 (CO carbamate), 144.1 (C4 triazole), 125.2 (C5 triazole), 96.7 (C1), 78.6 (C4,  $\text{C}_q$ ), 70.8, 70.7, 70.5 (C2, C3, C5), 53.2 ( $\text{CH}_2\text{CONH}$ ), 51.8 ( $\text{CH}_2\text{CH}_2\text{NHBoc}$ ), 48.0 ( $\text{CH}_2$ -triazole), 39.2 ( $\text{CH}_2\text{N}_{\text{cyst}}$ ), 38.1 ( $\text{CH}_2\text{NHBoc}$ ), 33.0 (C6,  $\text{CH}_2\text{S}_{\text{cyst}}$ ), 27.4 ( $\text{CMe}_3$ ), 19.6 ( $\text{CH}_3$ ); ESIMS:  $m/z$  1725.8  $[\text{M}+3\text{Na}]^{3+}$ , 2577.0  $[\text{M}+2\text{Na}]^{2+}$ ; Anal. calcd for  $\text{C}_{217}\text{H}_{357}\text{N}_{49}\text{O}_{77}\text{S}_7$ : C 51.02, H 7.04, N 13.43, S 4.39, found: C 50.67, H 6.71, N 13.09, S 4.01.

**Heptakis[6-deoxy-6-(2-(2-(4-(2-aminomethyl)-1H-1,2,3-triazol-1-yl)acetamido)ethylthio)]cyclomaltoheptaose heptahydrochloride (19)**. To a solution of **17** (67 mg, 18  $\mu$ mol) in MeOH (2 mL), 1 M MeONa (25  $\mu$ L) was added and the reaction mixture was stirred at room temperature for 2 h. The solution was neutralized by addition of Amberlite IR120 ( $\text{H}^+$ ) ion-exchange resin. Then the resin was filtered off and the solvents were removed under reduced pressure. The residue was dissolved in 1:1 TFA/ $\text{H}_2\text{O}$  (1.6 mL) and stirred at room temperature for 3 h. The solvents were repeatedly co-evaporated with  $\text{H}_2\text{O}$ . The residue was suspended in diluted aqueous HCl solution and freeze-dried to give **19** as a white foam (49 mg, 98%):  $[\alpha]_D = +48.1$  ( $c = 0.6$  in  $\text{H}_2\text{O}$ );  $^1\text{H NMR}$  (400 MHz,  $\text{CD}_3\text{OD}$ , 323 K):  $\delta = 8.13$  (s, 7H, =CH), 5.22 (s, 14H,  $\text{CH}_2\text{CONH}$ ), 5.00 (d, 7H,  $J_{1,2} = 2.9$  Hz, H-1), 4.30 (s, 14H,  $\text{CH}_2\text{NH}_2$ ), 4.00 (m, 7H, H-5), 4.85 (t, 7H,  $J_{2,3} = J_{3,4} = 9.2$  Hz, H-3), 3.56–3.48 (m, 28H, H-4, H-2,  $\text{CH}_2\text{NH}_{\text{cyst}}$ ), 3.28 (bs, 7H, H-6a), 2.93 (dd, 7H,  $J_{6a,6b} = 14.3$  Hz,  $J_{6b,5} = 7.7$  Hz, H-6b), 2.87 (m, 14H,  $\text{CH}_2\text{S}_{\text{cyst}}$ );  $^{13}\text{C NMR}$  (100.6 MHz,  $\text{CD}_3\text{OD}$ , 323 K):  $\delta = 168.7$  (CO amide), 142.2 (C4 triazole), 128.2 (C5 triazole), 104.7 (C1), 87.3 (C4), 75.4–75.2 (C5, C2, C3), 53.3 ( $\text{CH}_2\text{CONH}$ ), 41.6 ( $\text{CH}_2\text{N}_{\text{cyst}}$ ), 36.6 ( $\text{CH}_2\text{NH}_2$ ), 35.7 (C6), 34.7 ( $\text{CH}_2\text{S}_{\text{cyst}}$ ); ESIMS:  $m/z$  1258.3  $[\text{M}+2\text{H}]^{2+}$ , 2516.7  $[\text{M}+\text{H}]^+$ ; Anal. calcd for  $\text{C}_{91}\text{H}_{154}\text{Cl}_7\text{N}_{35}\text{O}_{35}\text{S}_7$ : C 39.44, H 5.60, N 17.69, S 8.10, found: C 39.18, H 5.32, N 17.29, S 7.68.

**Heptakis[6-deoxy-6-(2-(2-(4-(bis-(2-aminoethyl)aminomethyl)-1H-1,2,3-triazol-1-yl)acetamido)ethylthio)]cyclomaltoheptaose tetradecahydrochloride (22)**. To a solution of **20** (84 mg, 16  $\mu$ mol) in MeOH (2 mL), 1 M MeONa (22  $\mu$ L) was added and the reaction mixture was stirred at room temperature for 2.5 h. The solution was neutralized by addition of Amberlite IR-120 ( $\text{H}^+$ ) ion-exchange resin. Then the resin was filtered and the solvent was removed under reduced pressure. The residue was dissolved in 1:1 TFA/ $\text{H}_2\text{O}$  (1.8 mL) and stirred at room temperature for 3 h. The solvents were repeatedly co-evaporated with  $\text{H}_2\text{O}$ . The residue was suspended in diluted aqueous HCl solution and freeze-dried to give **22** as a white foam (57 mg, 99%):  $[\alpha]_D = +31.6$  ( $c = 0.9$  in MeOH);  $^1\text{H NMR}$  (500 MHz,  $\text{CD}_3\text{OD}$ , 323 K):  $\delta = 8.05$  (s, 7H, =CH), 5.20 (s, 14H,  $\text{CH}_2\text{CONH}$ ), 5.00 (d, 7H,  $J_{1,2} = 2.9$  Hz, H-1), 4.05 (m, 7H, H-5), 3.90 (s, 14H,  $\text{CH}_2$ -triazole), 3.85 (t, 7H,  $J_{2,3} = J_{3,4} = 8.7$  Hz, H-3), 3.6–

3.48 (m, 28H, H-4, H-2,  $\text{CH}_2\text{NH}_{\text{cyst}}$ ), 3.20 (m, 7H, H-6a), 3.15 (t, 14H,  $^3J_{\text{H,H}} = 5.7$  Hz,  $\text{CH}_2\text{NH}_2$ ), 2.96 (m, 7H, H-6b), 2.86 (t, 42H,  $\text{CH}_2\text{CH}_2\text{NH}_2$ ,  $\text{CH}_2\text{S}_{\text{cyst}}$ );  $^{13}\text{C}$  NMR (125.7 MHz,  $\text{CD}_3\text{OD}$ , 323 K):  $\delta = 169.1$  (CO amide), 145.5 (C4 triazole), 128.5 (C5 triazole), 105.1 (C1), 87.3 (C4), 75.4–75.1 (C5, C2, C3), 54.5 ( $\text{CH}_2\text{CONH}$ ), 53.5 ( $\text{CH}_2\text{CH}_2\text{NH}_2$ ), 49.2 ( $\text{CH}_2$ -triazole) 42.0 ( $\text{CH}_2\text{N}_{\text{cyst}}$ ), 39.7 ( $\text{CH}_2\text{NH}_2$ ), 36.0 (C6,  $\text{CH}_2\text{S}_{\text{cyst}}$ ); ESIMS:  $m/z$  780.3  $[\text{M}+4\text{H}]^{4+}$ , 140.7  $[\text{M}+3\text{H}]^{3+}$ ; Anal. calcd for  $\text{C}_{119}\text{H}_{231}\text{Cl}_7\text{N}_{49}\text{O}_{35}\text{S}_7$ : C 39.38, H 6.42, N 18.91, S 6.18, found: C 39.01, H 6.11, N 18.54, S 5.81.

**Heptakis[2,3-di-O-acetyl-6-(2-(*N'*-(6-*tert*-butoxycarbonylamino-hexyl)thioureido)ethylthio)]cyclomaltoheptaose (24).** To a solution of heptakis[2,3-di-O-acetyl-6-(2-aminoethylthio)]cyclomaltoheptaose heptahydrochloride (4, 64 mg, 22  $\mu\text{mol}$ ) and  $\text{Et}_3\text{N}$  (54  $\mu\text{L}$ , 0.39 mmol, 2.5 equiv) in dry  $\text{CH}_2\text{Cl}_2$  (2.5 mL), a solution of 6-*tert*-butoxycarbonylamino-hexyl isothiocyanate (48 mg, 0.18 mmol, 1.2 equiv) in  $\text{CH}_2\text{Cl}_2$  (2.5 mL) was added. The mixture was stirred at room temperature for 24 h. Then the solvent was removed under vacuum and the residue was purified by column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , 20:1). Yield: 61 mg (70%);  $R_f = 0.48$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , 20:1);  $[\alpha]_{\text{D}} = +55.5$  ( $c = 1.0$  in  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ , 313 K):  $\delta = 6.84$  (bs, 14H, NHCS), 5.27 (t, 7H,  $J_{2,3} = J_{3,4} = 9.7$  Hz, H-3), 5.13 (bd, 7H,  $J_{1,2} = 3.7$  Hz, H-1), 4.80 (dd, 7H, H-2), 4.78 (bs, 14H, NHBoc), 4.20 (m, 7H, H-5), 3.79 (m, 21H, H-4,  $\text{CH}_2\text{CH}_2\text{S}_{\text{cyst}}$ ), 3.46 (m, 14H,  $\text{CH}_2\text{NHCS}$ ), 3.23 (bd, 7H,  $J_{6a,6b} = 12.5$  Hz, H-6a), 3.09 (t, 14H,  $^3J_{\text{H,H}} = 7.0$  Hz,  $\text{CH}_2\text{NHBoc}$ ), 3.05 (m, 7H, H-6b), 2.96 (m, 7H,  $\text{CH}_2\text{S}_{\text{cyst,a}}$ ), 2.85 (m, 7H,  $\text{CH}_2\text{S}_{\text{cyst,b}}$ ), 2.08, 2.04 (2 s, 42H,  $\text{COCH}_3$ ), 1.59 (m, 14H,  $\text{CH}_2\text{CH}_2\text{NHCS}$ ), 1.49 (m, 14H,  $\text{CH}_2\text{CH}_2\text{NHBoc}$ ), 1.44 (s, 63H,  $\text{CMe}_3$ ), 1.36 (m, 28H,  $\text{CH}_2\text{CH}_2\text{CH}_2\text{NHCS}$ ,  $\text{CH}_2\text{CH}_2\text{CH}_2\text{NHBoc}$ );  $^{13}\text{C}$  NMR (125.7 MHz,  $\text{CDCl}_3$ , 313 K):  $\delta = 182.1$  (CS), 170.9, 169.5 (2CO ester), 156.5 (CO carbamate), 97.0 (C1), 79.4 ( $\text{CMe}_3$ , C4), 71.8 (C5), 71.1 (C3), 70.8 (C2), 44.6 ( $\text{CH}_2\text{NHCS}$ ), 44.0 ( $\text{CH}_2\text{CH}_2\text{S}_{\text{cyst}}$ ), 40.7 ( $\text{CH}_2\text{NHBoc}$ ), 33.9 (C6), 33.2 ( $\text{CH}_2\text{S}_{\text{cyst}}$ ), 30.2 ( $\text{CH}_2\text{CH}_2\text{NHBoc}$ ), 29.2 ( $\text{CH}_2\text{CH}_2\text{NHCS}$ ), 28.7 ( $\text{CMe}_3$ ), 26.7, 26.6 ( $\text{CH}_2\text{CH}_2\text{CH}_2\text{NHCS}$ ,  $\text{CH}_2\text{CH}_2\text{CH}_2\text{NHBoc}$ ), 20.9 ( $\text{COCH}_3$ ); ESIMS:  $m/z$  1995.6  $[\text{M}+2\text{Na}]^{2+}$ ; Anal. calcd for  $\text{C}_{168}\text{H}_{287}\text{N}_{21}\text{O}_{56}\text{S}_{14}$ : C, 51.13; H, 7.33; N, 7.45, found: C, 50.79; H, 6.98; N, 7.20.

**Heptakis[2,3-di-O-acetyl-6-(2-(*N'*-(4-(*tert*-butoxycarbonylamino-methyl)benzyl)thioureido)ethylthio)]cyclomaltoheptaose (29).** To a solution of heptakis[2,3-di-O-acetyl-6-(2-aminoethylthio)]cyclomaltoheptaose hydrochloride (4, 52 mg, 17.6  $\mu\text{mol}$ ) and  $\text{Et}_3\text{N}$  (34  $\mu\text{L}$ , 0.25 mmol, 2 equiv) in dry  $\text{CH}_2\text{Cl}_2$  (1.5 mL), a solution of 4-(*tert*-butoxycarbonylamino-methyl)benzyl isothiocyanate (39 mg, 0.14 mmol, 1.1 equiv) in dried  $\text{CH}_2\text{Cl}_2$  (1.5 mL) was added dropwise. The mixture was stirred at room temperature for 24 h, dried ( $\text{Na}_2\text{SO}_4$ ), concentrated and purified by column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , 20:1). Yield: 51 mg (71%);  $R_f = 0.52$  ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , 10:1);  $[\alpha]_{\text{D}} = +48.4$  ( $c = 0.98$  in  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , 313 K):  $\delta = 7.15$ , 7.14 (2 s, 28H, Ph), 6.96 (bs, 14H, NHCS), 5.28 (t, 7H,  $J_{2,3} = J_{3,4} = 8.8$  Hz, H-3), 5.25 (bs, 7H, NHBoc), 5.15 (bs, 7H, H-1), 4.80 (dd, 7H,  $J_{1,2} = 3.2$  Hz, H-2), 4.52 (bs, 14H,  $\text{PhCH}_2\text{NHCS}$ ), 4.19 (m, 21H, H-5,  $\text{CH}_2\text{NHBoc}$ ), 3.77 (t, 7H,  $J_{4,5} = 8.0$  Hz, H-4), 3.69 (bs, 14H,  $\text{CH}_2\text{CH}_2\text{S}_{\text{cyst}}$ ), 3.25 (bd, 7H,  $J_{6a,6b} = 12.8$  Hz, H-6a), 3.02 (m, 7H, H-6b), 2.90 (m, 7H,  $\text{CH}_2\text{S}_{\text{cyst,a}}$ ), 2.79 (m, 7H,  $\text{CH}_2\text{S}_{\text{cyst,b}}$ ), 2.10, 2.07 (2 s, 42H,  $\text{COCH}_3$ ), 1.44 (s, 63H,  $\text{CMe}_3$ );  $^{13}\text{C}$  NMR (125.7 MHz,  $\text{CDCl}_3$ , 313 K):  $\delta = 182.2$  (CS), 170.7, 169.3 (2CO ester), 156.2 (CO carbamate), 138.4, 136.6, 127.9, 127.5 (Ph), 96.8 (C1), 79.6 ( $\text{CMe}_3$ ), 79.4 (C4), 71.7 (C5), 70.8 (C3), 70.6 (C2), 48.0 ( $\text{PhCH}_2\text{NCS}$ ), 44.3 ( $\text{CH}_2\text{NHBoc}$ ), 43.9 ( $\text{CH}_2\text{CH}_2\text{S}_{\text{cyst}}$ ), 33.7 (C6), 33.0 ( $\text{CH}_2\text{S}_{\text{cyst}}$ ), 28.4 ( $\text{CMe}_3$ ), 20.7 ( $\text{COCH}_3$ ); ESIMS:  $m/z$  2071.1  $[\text{M}+2\text{Na}]^{2+}$ , 1388.1  $[\text{M}+3\text{Na}]^{3+}$ ; Anal. calcd for  $\text{C}_{182}\text{H}_{259}\text{N}_{21}\text{O}_{56}\text{S}_{14}$ : C, 53.50; H, 6.39; N, 7.20; S, 10.99, found: C, 53.28; H, 6.12; N, 6.97; S, 10.77.

**Heptakis[6-(2-(*N'*-(6-*tert*-butoxycarbonylamino-hexyl)thioureido)ethylthio)]cyclomaltoheptaose (26).** To a solution of 24 (50 mg, 12.7  $\mu\text{mol}$ ) in MeOH (2 mL), 1 M MeONa (18  $\mu\text{L}$ ) was added and the reaction mixture was stirred for 2.5 h at room temperature. The solution was neutralized by addition of Amberlite IR-120 ( $\text{H}^+$ ) ion-exchange resin. Then the resin was filtered and the solvent was removed under reduced pressure. Yield: 41.5 mg (98%);  $R_f = 0.55$  ( $\text{CH}_3\text{CN}/\text{H}_2\text{O}/\text{NH}_4\text{OH}$ , 10:2:1);  $[\alpha]_{\text{D}} = +48.1$  ( $c = 0.3$  in MeOH);  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ , 323 K):  $\delta = 4.99$  (d, 7H,  $J_{1,2} = 3.0$  Hz, H-1), 4.04 (m, 7H, H-5), 3.83 (t, 7H,  $J_{2,3} = J_{3,4} = 9.3$  Hz, H-3), 3.74 (bt, 14H,  $^3J_{\text{H,H}} = 6.3$  Hz,  $\text{SCH}_2\text{CH}_2\text{NHCS}$ ), 3.5 (m, 28H,  $\text{CH}_2\text{NHCS}$ , H-2, H-4), 3.25 (dd, 7H,  $J_{6a,6b} = 13.8$  Hz, H-6a), 3.06 (t, 14H,  $^3J_{\text{H,H}} = 6.7$  Hz,  $\text{CH}_2\text{NHBoc}$ ), 2.98 (m, 21H, H-6b,  $\text{CH}_2\text{S}_{\text{cyst}}$ ), 1.47 (m, 119H,  $\text{CMe}_3$ );  $^{13}\text{C}$  NMR (125.7 MHz, MeOD, 323 K):  $\delta = 183.3$  (CS), 158.4 (CO), 104.1 (C1), 86.3 (C4), 80.0 ( $\text{CMe}_3$ ), 74.6 (C3), 74.4 (C2), 73.5 (C5), 45.3, 45.0 ( $\text{CH}_2\text{NHCS}$ ), 41.6 ( $\text{CH}_2\text{NHBoc}$ ), 34.8 (C6), 34.1 ( $\text{CH}_2\text{S}_{\text{cyst}}$ ), 31.1 ( $\text{CH}_2\text{CH}_2\text{NHBoc}$ ), 30.3 ( $\text{CH}_2\text{CH}_2\text{NHCS}$ ), 29.1 ( $\text{CMe}_3$ ), 27.7 ( $\text{CH}_2\text{CH}_2\text{CH}_2\text{NHBoc}$ ,  $\text{CH}_2\text{CH}_2\text{CH}_2\text{NHCS}$ ); ESIMS:  $m/z$  1706.2  $[\text{M}+\text{Na}+\text{K}]^{2+}$ , 1155.9  $[\text{M}+3\text{K}]^{3+}$ ; Anal. calcd for  $\text{C}_{140}\text{H}_{259}\text{N}_{21}\text{O}_{42}\text{S}_{14}$ : C, 50.08; H, 7.78; N, 8.76, found: C, 49.94; H, 7.50; N, 8.61.

**Heptakis[6-(2-(*N'*-(4-(*tert*-butoxycarbonylamino-methyl)benzyl)thioureido)ethylthio)]cyclomaltoheptaose (30).** To a solution of 29 (55 mg, 13  $\mu\text{mol}$ ) in MeOH (2 mL), 1 M MeONa (19  $\mu\text{L}$ ) was added and the reaction mixture was stirred for 2.5 h at room temperature. The solution was neutralized by addition of Amberlite IR-120 ( $\text{H}^+$ ) ion-exchange resin. Then the resin was filtered and the solvent was removed under reduced pressure. Yield: 45.3 mg (96%);  $R_f = 0.58$  ( $\text{CH}_3\text{CN}/\text{H}_2\text{O}/\text{NH}_4\text{OH}$ , 10:2:1);  $[\alpha]_{\text{D}} = +37.7$  ( $c = 0.8$  in MeOH);  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ , 323 K):  $\delta = 7.20$  (m, 28H, Ph), 4.92 (d, 7H,  $J_{1,2} = 3.4$  Hz, H-1), 4.60 (m, 14H,  $\text{PhCH}_2\text{NHCS}$ ), 4.19 (m, 14H,  $\text{CH}_2\text{NHBoc}$ ), 4.01 (m, 7H, H-5), 3.77 (t, 7H,  $J_{2,3} = J_{3,4} = 9.3$  Hz, H-3), 3.64 (m, 28H,  $\text{CH}_2\text{NHCS}$ ), 3.48 (m, 14H, H-2, H-4), 3.14 (m, 7H, H-6a), 2.93 (dd, 7H,  $J_{6a,6b} = 14.4$  Hz,  $J_{5,6} = 7.8$  Hz, H-6b), 2.82 (m, 14H,  $\text{CH}_2\text{S}_{\text{cyst}}$ ), 1.42 (s, 63H,  $\text{CMe}_3$ );  $^{13}\text{C}$  NMR (125.7 MHz,  $\text{CD}_3\text{OD}$ , 323 K):  $\delta = 182.3$  (CS), 156.9 (CO), 138.7, 137.2, 127.4, 127.2, 127.1, 127.0 (Ph), 102.6 (C1), 84.8 (C4), 79.0 ( $\text{C}_q$ ), 73.2 (C3), 72.9 (C2), 72.1 (C5), 46.8 ( $\text{PhCH}_2\text{NCS}$ ), 43.7 ( $\text{CH}_2\text{NHBoc}$ ), 33.3 ( $\text{CH}_2\text{S}_{\text{cyst}}$ ), 32.6 (C6), 27.7 ( $\text{CMe}_3$ ); ESIMS:  $m/z$  1771.0  $[\text{M}+2\text{Na}]^{2+}$ , 1188.9  $[\text{M}+3\text{Na}]^{3+}$ ; Anal. calcd for  $\text{C}_{154}\text{H}_{231}\text{N}_{21}\text{O}_{42}\text{S}_{14}$ : C, 52.88; H, 6.66; N, 8.41, found: C, 52.53; H, 6.30; N, 8.24.

**Heptakis[6-(2-(*N'*-(6-amino-hexyl)thioureido)ethylthio)]cyclomaltoheptaose heptahydrochloride (28).** Compound 26 (40 mg, 11.9  $\mu\text{mol}$ ) was dissolved in 1:1 TFA/ $\text{H}_2\text{O}$  (2 mL) and stirred at room temperature for 1 h. The solvents were repeatedly co-evaporated with  $\text{H}_2\text{O}$ . The residue was suspended in diluted aqueous HCl solution and freeze-dried to give 28 as a white foam (34.7 mg, 99%);  $[\alpha]_{\text{D}} = +20.9$  ( $c = 0.5$  in  $\text{H}_2\text{O}$ );  $^1\text{H}$  NMR (500 MHz,  $\text{D}_2\text{O}$ , 333 K):  $\delta = 5.41$  (d, 7H,  $J_{1,2} = 3.5$  Hz, H-1), 4.33 (m, 7H, H-5), 4.17 (t, 7H,  $J_{2,3} = J_{3,4} = 8.6$  Hz, H-3), 4.02 (m, 21H,  $\text{SCH}_2\text{CH}_2\text{NHCS}$ , H-2), 3.87 (t, 7H, H-4), 3.73 (m, 14H,  $\text{CH}_2\text{NHCS}$ ), 3.60 (dd, 7H,  $J_{6a,6b} = 13.5$  Hz, H-6a), 3.33 (t, 14H,  $^3J_{\text{H,H}} = 7.6$  Hz,  $\text{CH}_2\text{NH}_2$ ), 3.27 (m, 7H, H-6b), 3.25 (t, 14H,  $^3J_{\text{H,H}} = 6.6$  Hz,  $\text{CH}_2\text{S}_{\text{cyst}}$ ), 2.00 (m, 14H,  $\text{CH}_2\text{CH}_2\text{NH}_2$ ), 1.93 (m, 14H,  $\text{CH}_2\text{CH}_2\text{NHCS}$ ), 1.72 (m, 28H,  $\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$ ,  $\text{CH}_2\text{CH}_2\text{CH}_2\text{NHCS}$ );  $^{13}\text{C}$  NMR (125.7 MHz,  $\text{D}_2\text{O}$ , 333 K):  $\delta = 180.2$  (CS), 102.3 (C1), 85.1 (C4), 73.5 (C3), 72.5 (C2), 72.2 (C5), 44.4, 44.1 ( $\text{CH}_2\text{NHCS}$ ), 40.1 ( $\text{CH}_2\text{NH}_2$ ), 34.0 ( $\text{CH}_2\text{S}_{\text{cyst}}$ ), 32.6 (C6), 28.7, 27.1, 26.1, 25.9 ( $\text{NHCSCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$ ); ESIMS:  $m/z$  1329.0  $[\text{M}+2\text{H}]^{2+}$ , 886.1  $[\text{M}+3\text{H}]^{3+}$ , 664.8  $[\text{M}+4\text{H}]^{4+}$ ; Anal. calcd for  $\text{C}_{105}\text{H}_{210}\text{Cl}_7\text{N}_{21}\text{O}_{28}\text{S}_{14}\cdot 7\text{H}_2\text{O}$ : C, 41.51; H, 7.43; N, 9.68; S, 14.78, found: C, 41.54; H, 7.39; N, 9.65; S, 14.65.

**Heptakis[6-(2-(*N'*-(4-(aminomethyl)benzyl)thioureido)ethylthio)]cyclomaltoheptaose heptahydrochloride (31).** Compound 30

(33 mg, 9.4  $\mu\text{mol}$ ) was dissolved in 1:1 TFA/H<sub>2</sub>O (2 mL) and stirred for 1 h at room temperature. The solvents were repeatedly co-evaporated with H<sub>2</sub>O. The residue was suspended in diluted aqueous HCl solution and freeze-dried to give **31** as a white foam (29 mg, 99%):  $[\alpha]_{\text{D}} = +11.2$  ( $c = 1.0$  in H<sub>2</sub>O); <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O, 323 K):  $\delta = 7.64, 7.56$  (bs, 28H, Ph), 5.24 (bs, 7H, H-1), 4.84 (bs, 14H, PhCH<sub>2</sub>NHCS), 4.39 (m, 14H, CH<sub>2</sub>NH<sub>2</sub>), 4.19 (bs, 7H, H-5), 3.90 (m, 28H, H-3, CH<sub>2</sub>NHCS, H-2), 3.69 (bs, 7H, H-4), 3.48 (bs, 7H, H-6a), 3.11 (m, 21H, H-6b, CH<sub>2</sub>S<sub>cyst</sub>); <sup>13</sup>C NMR (100.6 MHz, D<sub>2</sub>O, 323 K):  $\delta = 183.5$  (CS), 141.6, 134.4, 131.7, 131.6, 130.3, 130.2 (Ph), 104.5 (C1), 87.2 (C4), 78.0 (C3), 75.5 (C2), 74.6 (C5), 49.5 (PhCH<sub>2</sub>NCS), 45.8 (CH<sub>2</sub>NHCS), 45.5 (CH<sub>2</sub>NH<sub>2</sub>), 36.1 (C6), 34.6 (CH<sub>2</sub>S<sub>cyst</sub>); ESIMS:  $m/z$  1399.3  $[M+2H]^{2+}$ , 932.6  $[M+3H]^{3+}$ , 699.7  $[M+4H]^{4+}$ ; Anal. calcd for C<sub>119</sub>H<sub>182</sub>Cl<sub>7</sub>N<sub>21</sub>O<sub>28</sub>S<sub>14</sub>: C, 46.83; H, 6.01; N, 9.64; S, 14.71, found: C, 46.51; H, 6.12, N, 9.33; S, 14.36.

**Heptakis[2,3-di-O-acetyl-6-(2-(N-(2-(N,N-bis(2-tert-butoxycarbonylamino)ethyl)amino)ethyl)thioureido)ethylthio]cyclomaltoheptaose (33)**. To a solution of heptakis[2,3-di-O-acetyl-6-(2-aminoethylthio)cyclomaltoheptaose heptahydrochloride (**4**, 80 mg, 28  $\mu\text{mol}$ ) and Et<sub>3</sub>N (68  $\mu\text{L}$ , 0.49 mmol, 2.5 equiv) in dry CH<sub>2</sub>Cl<sub>2</sub> (2.5 mL), a solution of 2-[bis[2-(tert-butoxycarbonylamino)ethyl]amino]ethyl isothiocyanate (**32**, 92 mg, 0.24 mmol, 1.2 equiv) in dried CH<sub>2</sub>Cl<sub>2</sub> (2.5 mL) was added dropwise. The mixture was stirred at room temperature for 24 h, concentrated and purified by column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 20:1). Yield: 105 mg (76%);  $R_f = 0.5$  (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 9:1);  $[\alpha]_{\text{D}} = +51.0$  ( $c = 0.98$  in CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, 313 K):  $\delta = 7.23, 7.14$  (2bs, 14H, NHCS), 5.26 (t, 7H,  $J_{2,3} = J_{3,4} = 9.0$  Hz, H-3), 5.21 (bs, 14H, NHBoc), 5.10 (d, 7H,  $J_{1,2} = 3.6$  Hz, H-1), 4.77 (dd, 7H, H-2), 4.17 (m, 7H, H-5), 3.82 (t, 7H,  $J_{4,5} = 8.7$  Hz, H-4), 3.75 (bs, 14H, CH<sub>2</sub>CH<sub>2</sub>S<sub>cyst</sub>), 3.52 (bs, 14H, NCH<sub>2</sub>CH<sub>2</sub>NHCS), 3.13 (m, 42H, H-6a, H-6b, CH<sub>2</sub>NHBoc), 2.87 (m, 14H, CH<sub>2</sub>S<sub>cyst</sub>), 2.65 (bt, 14H, NCH<sub>2</sub>CH<sub>2</sub>NHCS), 2.56 (bt, 28H, CH<sub>2</sub>CH<sub>2</sub>NHBoc), 2.06, 2.02 (2s, 42H, COCH<sub>3</sub>), 1.43 (s, 126H, CMe<sub>3</sub>); <sup>13</sup>C NMR (75.5 MHz, CDCl<sub>3</sub>, 313 K):  $\delta = 184.8$  (CS), 172.9, 171.6 (2CO ester), 158.8 (CO carbamate), 99.0 (C1), 81.6 (CMe<sub>3</sub>), 81.5 (C4), 73.0 (C5, C3, C2), 56.7 (CH<sub>2</sub>CH<sub>2</sub>NHBoc), 56.3 (NCH<sub>2</sub>CH<sub>2</sub>NHCS), 44.6 (CH<sub>2</sub>CH<sub>2</sub>S<sub>cyst</sub>), 41.0 (NCH<sub>2</sub>CH<sub>2</sub>NHCS, CH<sub>2</sub>NHBoc), 36.0 (C6), 35.3 (CH<sub>2</sub>S<sub>cyst</sub>), 30.6 (CMe<sub>3</sub>), 23.0, 22.9 (COCH<sub>3</sub>); ESIMS:  $m/z$  2463.4  $[M+2Cl]^{-}$ , 2458.8  $[M+2Na]^{2+}$ ; Anal. calcd for C<sub>203</sub>H<sub>357</sub>N<sub>35</sub>O<sub>70</sub>S<sub>14</sub>: C, 50.20; H, 7.41; N, 10.09; S, 9.24, found: C, 50.01; H, 7.22; N, 9.81; S, 8.94.

**Heptakis[6-(2-(N-(2-(N,N-bis(2-(tert-butoxycarbonylamino)ethyl)amino)ethyl)-thioureido)ethylthio]cyclomaltoheptaose (34)**. To a solution of **33** (76 mg, 15.7  $\mu\text{mol}$ ) in MeOH (2 mL), 1 M MeONa (22  $\mu\text{L}$ ) was added and the reaction mixture was stirred at room temperature for 2.5 h. The solution was neutralized by addition of Amberlite IR-120 (H<sup>+</sup>) ion-exchange resin. Then the resin was filtered and the solvent was removed under reduced pressure. Yield: 45.5 mg (99%);  $R_f = 0.50$  (CH<sub>3</sub>CN/H<sub>2</sub>O/NH<sub>4</sub>OH, 10:1:1);  $[\alpha]_{\text{D}} = +33.3$  ( $c = 0.8$  in MeOH); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD, 313 K):  $\delta = 4.96$  (d, 7H,  $J_{1,2} = 3.5$  Hz, H-1), 4.03 (m, 7H, H-5), 3.79 (t, 7H,  $J_{2,3} = J_{3,4} = 8.8$  Hz, H-3), 3.74 (bt, 14H, CH<sub>2</sub>CH<sub>2</sub>S<sub>cyst</sub>), 3.51 (m, 28H, H-2, H-4, NCH<sub>2</sub>CH<sub>2</sub>NHCS), 3.24 (m, 7H,  $J_{6a,6b} = 14.3$  Hz,  $J_{5,6a} = 1.8$  Hz, H-6a), 3.12 (bt, 14H,  $^3J_{\text{H,H}} = 6.0$  Hz, CH<sub>2</sub>NHBoc), 2.97 (m, 7H,  $J_{5,6b} = 5.9$  Hz, H-6b), 2.87 (m, 14H, CH<sub>2</sub>S<sub>cyst</sub>), 2.69 (bt, 14H,  $^3J_{\text{H,H}} = 5.6$  Hz, NCH<sub>2</sub>CH<sub>2</sub>NHCS), 2.59 (bt, 28H, CH<sub>2</sub>CH<sub>2</sub>NHBoc), 1.44 (bs, 126H, CMe<sub>3</sub>); <sup>13</sup>C NMR (125.7 MHz, CD<sub>3</sub>OD, 313 K):  $\delta = 183.6$  (CS), 158.5 (CO), 104.1 (C1), 86.2 (C4), 80.3 (C<sub>q</sub>), 74.5 (C3), 74.4 (C2), 73.6 (C5), 55.7 (CH<sub>2</sub>CH<sub>2</sub>NHBoc), 54.8 (NCH<sub>2</sub>CH<sub>2</sub>NHCS), 45.3 (CH<sub>2</sub>CH<sub>2</sub>S<sub>cyst</sub>), 43.6 (NCH<sub>2</sub>CH<sub>2</sub>NHCS), 40.1 (CH<sub>2</sub>NHBoc), 34.9 (C6), 34.2 (CH<sub>2</sub>S<sub>cyst</sub>), 29.1 (CMe<sub>3</sub>); ESIMS:  $m/z$  2135.1  $[M+2H]^{2+}$ , 1441.3  $[M+3Na]^{3+}$ ; Anal.

calcd for C<sub>175</sub>H<sub>329</sub>N<sub>35</sub>O<sub>56</sub>S<sub>14</sub>: C, 49.24; H, 7.77; N, 11.48, found: C, 49.25; H, 7.71; N, 11.37.

**Heptakis[6-(2-(N-(2-(N,N-bis(2-aminoethyl)amino)ethyl)thioureido)ethylthio]cyclo-maltoheptaose tetradecahydrochloride (35)**. Compound **34** (44 mg, 10.3  $\mu\text{mol}$ ) was dissolved in 1:1 TFA/H<sub>2</sub>O (2 mL) and stirred at room temperature for 1 h. The solvents were repeatedly co-evaporated with H<sub>2</sub>O. The residue was suspended in diluted aqueous HCl solution and freeze-dried to give **35** as a white foam (34.7 mg, 99%):  $[\alpha]_{\text{D}} = +20.3$  ( $c = 1.0$  in H<sub>2</sub>O); <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O, 333 K):  $\delta = 5.46$  (d, 7H,  $J_{1,2} = 3.3$  Hz, H-1), 4.30 (m, 7H, H-5), 4.26 (t, 7H,  $J_{2,3} = J_{3,4} = 9.5$  Hz, H-3), 4.09 (m, 28H, NCH<sub>2</sub>CH<sub>2</sub>NHCS, CH<sub>2</sub>CH<sub>2</sub>S<sub>cyst</sub>), 4.02 (dd, 7H, H-2), 3.98 (t, 7H, H-4), 3.65 (m, 28H, CH<sub>2</sub>NH<sub>2</sub>), 3.52 (m, 35H, CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>, H-6a), 3.40 (m, 21H, H-6b, CH<sub>2</sub>S<sub>cyst</sub>), 3.29 (m, 14H, NCH<sub>2</sub>CH<sub>2</sub>NHCS); <sup>13</sup>C NMR (75.5 MHz, D<sub>2</sub>O, 333 K):  $\delta = 181.9$  (CS), 102.2 (C1), 84.8 (C4), 73.3 (C3), 72.4 (C2, C5), 53.4 (NCH<sub>2</sub>CH<sub>2</sub>NHCS), 50.9 (NCH<sub>2</sub>CH<sub>2</sub>NHCS), 44.2 (CH<sub>2</sub>CH<sub>2</sub>S<sub>cyst</sub>), 39.8 (CH<sub>2</sub>NH<sub>2</sub>), 35.2 (CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>), 33.8 (C6), 32.4 (CH<sub>2</sub>S<sub>cyst</sub>); ESIMS:  $m/z$  1434.1  $[M+2H]^{2+}$ , 956.4  $[M+3H]^{3+}$ , 717.2  $[M+4H]^{4+}$ ; Anal. calcd for C<sub>105</sub>H<sub>231</sub>Cl<sub>14</sub>N<sub>35</sub>O<sub>28</sub>S<sub>14</sub>: C, 37.34; H, 6.89; N, 14.52, found: C, 37.01; H, 6.67; N, 14.22.

**Heptakis[6-O-tert-butylidimethylsilyl-2,3-di-O-(3-methanesulfonyloxypropyl)cyclomaltoheptaose (38)**. To a solution of **37** (141 mg, 60  $\mu\text{mol}$ ) in CH<sub>2</sub>Cl<sub>2</sub> (2.4 mL), Et<sub>3</sub>N (0.21 mL, 1.44 mmol) and MsCl (1.2 mL, 1.44 mmol) were added at 0 °C. The reaction mixture was stirred at room temperature for 5 h. The solvent was removed under vacuum and the resulting residue was purified by column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 30:1) to give **38** (0.2 g, 88%);  $R_f = 0.18$  (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 30:1);  $[\alpha]_{\text{D}} = +36.0$  ( $c = 1.0$  in CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, 313 K):  $\delta = 5.19$  (d, 7H,  $J_{1,2} = 3.2$  Hz, H-1), 4.37–4.30 (m, 4H, 2 × CH<sub>2</sub>OMs), 4.13 (bd, 7H,  $J_{6a,6b} = 10.6$  Hz, H-6a), 3.97 (m, 7H, OCH<sub>3</sub>), 3.79–3.76 (m, 7H, OCH<sub>3</sub>), 3.78 (t, 7H,  $J_{3,4} = J_{4,5} = 9.2$  Hz, H-4), 3.75–3.68 (m, 14H, OCH<sub>2</sub>), 3.62 (bd, 7H, H-6b), 3.61 (t, 7H,  $J_{2,3} = 9.8$  Hz, H-3), 3.53 (d, 7H, H-5), 3.11 (dd, 7H, H-2), 3.00, 2.99 (2s, 42H, CH<sub>3</sub>SO<sub>2</sub>), 2.07–2.02 (m, 28H, CH<sub>2</sub>), 0.87 (s, 63H, SiCMe<sub>3</sub>), 0.02, 0.01 (2s, 42H, SiMe<sub>2</sub>); <sup>13</sup>C NMR (125.7 MHz, CDCl<sub>3</sub>, 313 K):  $\delta = 97.6$  (C1), 80.7 (C2), 80.4 (C3), 77.3 (C4), 72.5 (C5), 69.5 (OCH<sub>2</sub>), 68.2, 67.4 (CH<sub>2</sub>OMs), 67.2 (OCH<sub>2</sub>), 62.2 (C6), 37.3, 37.2 (CH<sub>3</sub>SO<sub>2</sub>), 30.2, 30.1 (CH<sub>2</sub>), 25.9 (SiCMe<sub>3</sub>), 18.2 (SiCMe<sub>3</sub>), –4.8, –5.2 (SiMe<sub>2</sub>); ESIMS:  $m/z$  1940.7  $[M+2Na]^{2+}$ ; Anal. calcd for C<sub>140</sub>H<sub>280</sub>O<sub>77</sub>S<sub>14</sub>Si<sub>7</sub>: C 43.78, H 7.35, found: C 43.38, H 7.16.

**Heptakis[2,3-di-O-(3-azidopropyl)-6-O-tert-butylidimethylsilyl]cyclomaltoheptaose (39)**. A solution of **38** (137 mg, 36  $\mu\text{mol}$ ) and NaN<sub>3</sub> (66 mg, 1.01 mmol, 2 equiv) in dry DMF (2 mL) was stirred at 70 °C under Ar atmosphere. The solvent was removed under vacuum and the resulting residue was purified by column chromatography (EtOAc/petroleum ether, 1:8 → 1:7) to give **39** (82 mg, 74%);  $R_f = 0.35$  (EtOAc/petroleum ether, 1:6);  $[\alpha]_{\text{D}} = +58.6$  ( $c = 0.5$  in CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta = 5.19$  (d, 7H,  $J_{1,2} = 3.1$  Hz, H-1), 4.14 (d, 7H,  $J_{6a,6b} = 10.3$  Hz, H-6a), 3.96 (m, 7H, OCH<sub>3</sub>), 3.79 (t, 7H,  $J_{3,4} = J_{4,5} = 9.2$  Hz, H-4), 3.73 (m, 7H, OCH<sub>3</sub>), 3.69–3.63 (m, 14H, OCH<sub>2</sub>), 3.59 (d, 7H, H-6b), 3.59 (t, 7H,  $J_{2,3} = 9.2$  Hz, H-3), 3.50 (d, 7H,  $J_{5,6} = 9.5$  Hz, H-5), 3.43, 3.39 (2t, 14H,  $^3J_{\text{H,H}} = 6.7$  Hz, CH<sub>2</sub>N<sub>3</sub>), 3.09 (dd, 7H, H-2), 1.92–1.83 (m, 28H, CH<sub>2</sub>), 0.87 (s, 63H, SiCMe<sub>3</sub>), 0.01, 0.00 (2s, 42H, SiMe<sub>2</sub>); <sup>13</sup>C NMR (125.7 MHz, CDCl<sub>3</sub>, 313 K):  $\delta = 97.3$  (C1), 80.7 (C2), 80.5 (C3), 77.3 (C4), 72.4 (C5), 70.4, 68.2 (OCH<sub>2</sub>), 62.2 (C6), 48.5, 48.2 (CH<sub>2</sub>N<sub>3</sub>), 29.8, 29.6 (CH<sub>2</sub>), 25.9 (SiCMe<sub>3</sub>), 18.3 (SiCMe<sub>3</sub>), –4.8, –5.2 (SiMe<sub>2</sub>); ESIMS:  $m/z$  1571.4  $[M+2Na]^{2+}$ , 3118.2  $[M+Na]^{+}$ ; Anal. calcd for C<sub>126</sub>H<sub>238</sub>N<sub>42</sub>O<sub>35</sub>Si<sub>7</sub>: C 48.85, H 7.74, N 18.99, found: C 48.72, H 7.57, N 18.76.

**Heptakis[2,3-di-O-(3-azidopropyl)cyclomaltoheptaose (40)**. To a solution of **39** (0.26 g, 84  $\mu\text{mol}$ ) in dry THF (5 mL) under Ar atmos-

phere was added a solution of TBAF (0.22 g, 71 mmol) in dry THF (3.4 mL). The reaction was stirred at room temperature for 1 day. The solvent was removed, and the resulting residue was dissolved in  $\text{CH}_2\text{Cl}_2$  and washed with brine. The organic phase was dried ( $\text{MgSO}_4$ ), filtered and purified by column chromatography (EtOAc/EtOH/ $\text{H}_2\text{O}$ , 45:5:3) to give **39** (0.14 g, 84%):  $R_f=0.35$  (EtOAc/EtOH/ $\text{H}_2\text{O}$ , 45:5:3);  $[\alpha]_D^{25} = +103.0$  ( $c=1.0$  in  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta=5.13$  (d, 7H,  $J_{1,2}=3.1$  Hz, H-1), 4.66 (bs, 7H, OH), 3.97 (bd, 7H,  $^2J_{\text{H,H}}=10.2$  Hz, H-6a), 3.93 (dt, 14H,  $^2J_{\text{H,H}}=9.2$  Hz,  $^3J_{\text{H,H}}=6.2$  Hz,  $\text{CH}_2\text{O}$ ), 3.80–3.75 (m, 21H, H-5, H-6b, OCH), 3.73–3.68 (m, 14H, OCH), 3.64–3.58 (m, 14H, H-3, H-4), 3.53–3.45 (m, 14H,  $\text{CH}_2\text{N}_3$ ), 3.42 (t, 14H,  $^3J_{\text{H,H}}=6.6$  Hz,  $\text{CH}_2\text{N}_3$ ), 3.33 (dd, 7H,  $J_{2,3}=8.7$  Hz, H-2), 1.93–1.84 (m, 28H,  $\text{CH}_2$ );  $^{13}\text{C NMR}$  (125.7 MHz,  $\text{CDCl}_3$ ):  $\delta=97.4$  (C1), 80.5 (C2), 79.8 (C3), 78.0 (C4), 72.9 (C5), 70.2, 68.2 (OCH<sub>2</sub>), 61.7 (C6), 48.4, 48.1 ( $\text{CH}_2\text{N}_3$ ), 29.7, 29.5 ( $\text{CH}_2$ ); ESIMS:  $m/z$  1169.1  $[\text{M}+\text{H}+\text{K}]^{2+}$ ; Anal. calcd for  $\text{C}_{84}\text{H}_{140}\text{N}_{14}\text{O}_{35}$ : C 43.90, H 6.14, N 25.60, found: C 43.73, H 5.81, N 25.43.

**Heptakis[2,3-di-O-(3-aminopropyl)]cyclomaltoheptaose tetradecahydrochloride (41).** To a solution of **40** (0.17 g, 75  $\mu\text{mol}$ ) in DMF (2 mL), TPP (0.62 g, 2.37 mmol) was added. After stirring at room temperature for 4 h,  $\text{NH}_4\text{OH}$  (25%, 0.7 mL) was added and the mixture was further stirred at room temperature for 1 day. The solvent was removed, and the resulting residue was partitioned between  $\text{Et}_2\text{O}$  and  $\text{H}_2\text{O}$ . The aqueous phase was acidified with diluted HCl and lyophilized to give **4** (0.16 g, 90%):  $[\alpha]_D^{25} = +66.8$  ( $c=0.5$  in  $\text{H}_2\text{O}$ );  $^1\text{H NMR}$  (500 MHz,  $\text{D}_2\text{O}$ ):  $\delta=5.28$  (d, 7H,  $J_{1,2}=3.3$  Hz, H-1), 3.99 (dt, 7H,  $^2J=9.5$  Hz,  $^3J=5.5$  Hz, OCH<sub>2</sub>), 3.90 (bd, 7H,  $J_{6a,6b}=11.0$  Hz, H-6a), 3.81–3.72 (m, 49H, H-3, H-4, H-5, H-6b, OCH<sub>2</sub>), 3.51 (dd, 7H,  $J_{2,3}=9.3$  Hz, H-2), 3.09 (m, 28H,  $^3J_{\text{H,H}}=7.5$  Hz,  $\text{CH}_2\text{NH}_2$ ), 1.99 (m, 28H,  $\text{CH}_2$ );  $^{13}\text{C NMR}$  (125.7 MHz,  $\text{CDCl}_3$ ):  $\delta=96.4$  (C1), 80.0 (C3), 78.6 (C2), 75.9 (C4), 71.7 (C5), 70.3, 68.2 (OCH<sub>2</sub>), 60.6 (C6), 37.9, 37.0 ( $\text{CH}_2\text{NH}_2$ ), 27.6, 27.5 ( $\text{CH}_2$ ); ESIMS:  $m/z$  1934.2  $[\text{M}+\text{H}]^+$ ; Anal. calcd for  $\text{C}_{84}\text{H}_{182}\text{Cl}_{14}\text{N}_{14}\text{O}_{35}$ : C 41.27, H 7.50, N 8.02, found: C 40.89, H 7.332, N 7.67.

**Heptakis(6-O-tert-butylidimethylsilyl-2,3-di-O-3-(2-N-tert-butoxycarbonylaminoethylthio)propyl)cyclomaltoheptaose (42).** A suspension of **38** (60 mg, 16  $\mu\text{mol}$ ),  $\text{Cs}_2\text{CO}_3$  (0.22 g, 0.67 mmol) and  $\text{BocNH}(\text{CH}_2)_2\text{SH}$  (0.11 mL, 0.67 mmol) in dry DMF (4 mL) was heated, under Ar atmosphere at  $75^\circ\text{C}$  for 20 h. The solvent was removed under vacuum and the residue was purified by column chromatography (EtOAc/petroleum ether, 1:2  $\rightarrow$  1:1) to give **42** (51 mg, 64%):  $R_f=0.38$  (EtOAc/petroleum ether, 1:2);  $[\alpha]_D^{25} = +47.8$  ( $c=1.0$  in  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta=5.46$ , 5.32 (2bs, 14H, NH), 5.20 (bs, 7H, H-1), 4.15 (d, 7H,  $J_{6a,6b}=10.8$  Hz, H-6a), 3.98 (m, 7H, OCH<sub>2</sub>), 3.79 (t, 7H,  $J_{3,4}=J_{4,5}=9.1$  Hz, H-4), 3.75–3.68 (m, 21H, OCH<sub>2</sub>), 3.61 (d, 7H, H-6b), 3.57 (t, 7H, H-3), 3.50 (d, 7H,  $J_{5,6b}=3.5$  Hz, H-5), 3.32 (d, 28H,  $^3J_{\text{H,H}}=7.9$  Hz,  $\text{CH}_2\text{NH}$ ), 3.12 (dd, 7H,  $J_{1,2}=3.0$  Hz,  $J_{2,3}=9.8$  Hz, H-2), 2.69–2.61 (m, 56H, SCH<sub>2</sub>), 1.95–1.87 (m, 28H,  $\text{CH}_2$ ), 1.45 (s, 126H,  $\text{CMe}_3$ ), 0.89 (s, 63H,  $\text{SiCMe}_3$ ), 0.04, 0.02 (2s, 42H,  $\text{SiMe}_2$ );  $^{13}\text{C NMR}$  (100.6 MHz,  $\text{CDCl}_3$ ):  $\delta=155.9$  (CO), 97.5 (C1), 80.6 (C3), 80.4 (C2), 79.2, 79.1 (C<sub>q</sub>, C4), 72.4, 72.3 (OCH<sub>2</sub>), 69.9 (C5), 62.2 (C6), 40.1, 39.9 ( $\text{CH}_2\text{NH}$ ), 32.1 ( $\text{CH}_2\text{S}$ ), 30.7, 30.1 ( $\text{CH}_2$ ), 28.8, 28.2 ( $\text{CH}_2\text{S}$ ), 28.5, 28.4 ( $\text{CMe}_3$ ), 26.0 ( $\text{SiCMe}_3$ ), 18.3 ( $\text{SiCMe}_3$ ), -4.5, -5.1 ( $\text{SiMe}_2$ ); ESIMS:  $m/z$  2516.2  $[\text{M}+\text{Na}+\text{K}]^{2+}$ ; Anal. calcd for  $\text{C}_{224}\text{H}_{434}\text{N}_{14}\text{O}_{63}\text{S}_{14}\text{Si}_7$ : C 54.05, H 8.79, N 3.94, found: C 53.74, H 8.88, N 3.88.

**Heptakis[2,3-di-O-3-(2-N-tert-butoxycarbonylaminoethylthio)propyl)cyclomaltoheptaose (43).** To a solution of **42** (0.49 g, 0.1 mmol) in dry THF (10 mL) under Ar atmosphere was added a solution of TBAF (0.26 g, 0.82 mmol) in dry THF (4 mL). The reaction was stirred at room temperature for 1 day. The solvent was removed, and the resulting residue was dissolved in  $\text{CH}_2\text{Cl}_2$  and

washed with brine. The organic phase was dried ( $\text{MgSO}_4$ ), filtered and purified by column chromatography (EtOAc/EtOH, 20:1  $\rightarrow$  EtOAc/EtOH/ $\text{H}_2\text{O}$ , 45:5:3) to give **43** (0.38 g, 90%):  $R_f=0.52$  (EtOAc/EtOH/ $\text{H}_2\text{O}$ , 45:5:3);  $[\alpha]_D^{25} = +57.2$  ( $c=1.0$  in  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ , 333 K):  $\delta=5.15$  (d, 7H,  $J_{1,2}=3.5$  Hz, H-1), 4.01–3.93 (m, 14H, H-6a, OCH<sub>2</sub>), 3.82–3.77 (m, 21H, H-5, H-6b, OCH<sub>2</sub>), 3.72 (t, 14H,  $^3J_{\text{H,H}}=6.2$  Hz, OCH<sub>2</sub>), 3.64–3.60 (m, 14H, H-3, H-4), 3.35–3.31 (m, 28H,  $\text{CH}_2\text{NH}(\text{Boc})$ ), 3.26 (dd, 7H,  $J_{2,3}=8.7$  Hz, H-2), 2.70–2.62 (m, 56H,  $\text{CH}_2\text{S}$ ), 1.93–1.90 (m, 28H,  $\text{CH}_2$ ), 1.48, 1.47 (2s, 126H,  $\text{CMe}_3$ );  $^{13}\text{C NMR}$  (100.6 MHz,  $\text{CDCl}_3$ , 313 K):  $\delta=155.9$  (CO), 97.5 (C1), 80.6 (C3), 80.2 (C2), 79.3, 79.2 (C<sub>q</sub>), 78.5 (C4), 72.8 (C5), 72.4, 70.0 (OCH<sub>2</sub>), 61.9 (C6), 40.1 ( $\text{CH}_2\text{NH}$ ), 32.2 ( $\text{CH}_2\text{S}$ ), 30.6, 30.0 ( $\text{CH}_2$ ), 28.7, 28.3 ( $\text{CH}_2\text{S}$ ), 28.5 ( $\text{CMe}_3$ ); ESIMS:  $m/z$  2115.8  $[\text{M}+\text{Na}+\text{K}]^{2+}$ ; Anal. calcd for  $\text{C}_{182}\text{H}_{336}\text{N}_{14}\text{O}_{63}\text{S}_{14}$ : C 52.33, H 8.11, N 4.69, found: C 52.35, H 7.84, N 4.52.

**Heptakis[2,3-di-O-3-(2-aminoethylthio)propyl)cyclomaltoheptaose tetradecahydrochloride (44).** Treatment of carbamate **43** (50 mg, 12  $\mu\text{mol}$ ) with 1:1 TFA- $\text{CH}_2\text{Cl}_2$  (2 mL) at room temperature for 1 h, followed by evaporation of the solvents and freeze-drying from diluted HCl solution, gave pure **44** (42 mg, 99%):  $R_f=0.07$  ( $\text{CH}_3\text{CN}/\text{H}_2\text{O}/\text{NH}_4\text{OH}$ , 6:3:1);  $[\alpha]_D^{25} = +57.7$  ( $c=0.5$  in  $\text{H}_2\text{O}$ );  $^1\text{H NMR}$  (500 MHz,  $\text{D}_2\text{O}$ ):  $\delta=5.24$  (s, 7H, H-1), 3.91–3.87 (m, 14H, H-6a, OCH<sub>2</sub>), 3.80–3.73 (m, 35H, H-6b, OCH<sub>2</sub>), 3.72–3.67 (m, 21H, H-3, H-4, H-5), 3.37 (bd, 7H, H-2), 3.18 (m, 28H,  $^3J_{\text{H,H}}=6.8$  Hz,  $\text{CH}_2\text{NH}_2$ ), 2.83, 2.65 (2dt, 56H,  $\text{CH}_2\text{S}$ ), 1.88 (m, 28H,  $\text{CH}_2$ );  $^{13}\text{C NMR}$  (100.6 MHz,  $\text{D}_2\text{O}$ ):  $\delta=96.7$  (C1), 80.3 (C3), 78.9 (C2), 76.2 (C4), 71.8 (OCH<sub>2</sub>), 69.6 (OCH<sub>2</sub>, C5), 60.4 (C6), 38.5 ( $\text{CH}_2\text{NH}$ ), 29.4, 29.3 ( $\text{CH}_2$ ), 28.2, 28.1, 27.6, 27.4 ( $\text{CH}_2\text{S}$ ); ESIMS:  $m/z$  926.1  $[\text{M}+3\text{H}]^{3+}$ ; Anal. calcd for  $\text{C}_{112}\text{H}_{238}\text{Cl}_{14}\text{N}_{14}\text{O}_{35}\text{S}_{14}$ : C 40.93, H 7.30, N 5.97, found: C 40.94, H 7.48, N 5.86.

### Cell-based assays

**Cells and cell culture.** Recombinant *B. anthracis* lethal factor (rLF) and protective antigen (rPA) were acquired from List Biological Laboratories (Campbell, CA, USA). The murine RAW 264.7 monocyte-macrophage cell line (ATCC TIB-71) was obtained from American Type Culture Collection (Manassas, VA, USA). The cells were cultured in phenol-red-free Dulbecco's modified Eagle's medium (DMEM, Mediatech, Herndon, VA, USA) supplemented with 10% heat-inactivated fetal bovine serum,  $100 \text{ U mL}^{-1}$ :  $100 \mu\text{g mL}^{-1}$  penicillin-streptomycin, 0.1 mM nonessential amino acids, and 0.5 mM of 2-mercaptoethanol at  $37^\circ\text{C}$  under a 5%  $\text{CO}_2$  atmosphere. The cells were harvested by gentle scraping with a cell scraper and were then washed once with media. RAW 264.7 cells were plated in 96-well flat-bottomed tissue culture plates from Becton-Dickinson (San Jose, CA, USA) at a concentration of  $10^5$  cells per well in DMEM and incubated overnight at  $37^\circ\text{C}$  under 5%  $\text{CO}_2$ .

**Cytotoxicity neutralization assay.** RAW 264.7 cells were pre-incubated with various concentrations of the tested compounds in DMEM for 1 h at  $37^\circ\text{C}$  under a 5%  $\text{CO}_2$  atmosphere. Then, DMEM or LeTx (LF =  $32 \text{ ng mL}^{-1}$ ; PA =  $500 \text{ ng mL}^{-1}$  in the media) was added, and the plate was incubated under the same conditions for 4 h. Cell viability was estimated by using the MTS kit from Promega (Madison, WI, USA). A micro-Quant spectrophotometer from Bio-Tek Instruments (Winooski, VT, USA) was used to obtain  $\text{OD}_{570}$  readings.

## Acknowledgements

This work was supported by grant number 2R44AI052894-02 from the National Institute of Allergy and Infectious Diseases (USA), grants CTQ2007-61180/PPQ and CTQ2010-15848/BQU from the Spanish Ministry of Science and Education, the European Union (FEDER), and the Junta de Andalucía. A.D.-M. and A.M.-A. are grateful to the CSIC and the Spanish Ministry of Science and Education, respectively, for pre-doctoral fellowships.

**Keywords:** anthrax · click chemistry · cyclodextrins · symmetry complementarity · toxin inhibitors

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Received: October 1, 2010

Revised: November 5, 2010

Published online on December 7, 2010