Extending the scope of the *C*-functionalization of cyclam via Copper(I)-catalyzed Alkyne-Azide Cycloaddition to bifunctional chelators of interest

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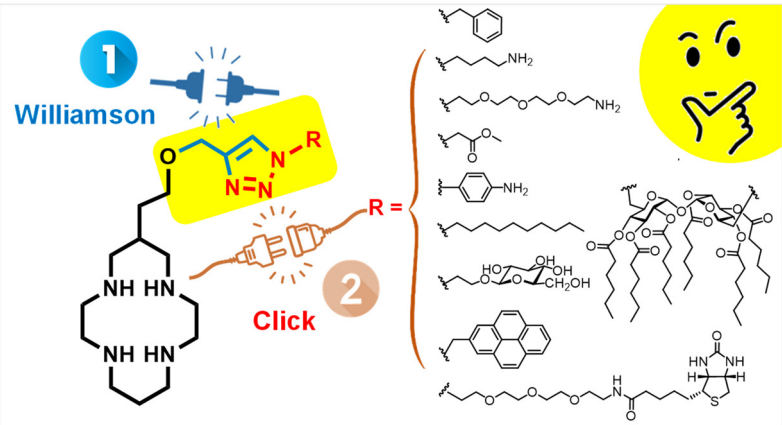
TOC Graphic



A robust two-step synthesis results in a versatile *C*-functionalization method of cyclams with broad functionalities for several applications.

Abstract

Cyclam, known for its potent chelation properties, is explored for diverse applications through selective *N*-functionalization, offering versatile ligands for catalysis, medical research, and materials science. The challenges arising from *N*-alkylation, that could decrease the coordination properties, are addressed by introducing a robust *C*-functionalization method. The facile two-step synthesis proposed here involves the click chemistry-based *C*-functionalization of a hydroxyethyl cyclam derivative using Cu(I)-catalyzed Alkyne-Azide Cycloaddition (CuAAC). Boc-protecting groups prevent undesired copper coordination, resulting in compounds with a wide range of functionalities. The optimized synthesis conditions enable *C*-functional cyclams to be obtained easily and advantageously, with high application potential in the previously cited fields. The methodology has been extended to Trehalose-based Siamese twin amphiphiles, enabling efficient gene delivery applications.

Introduction

Cyclam is a tetraazacycloalkane renowned for its remarkable chelation properties with a number of metallic cations including transition metals such as copper,[1] nickel,[2] cobalt[3] or even palladium.[4] Thanks to its secondary amines, cyclam can be selectively or fully *N*-functionalized to complete the coordination sphere of a metal, leading to other thermodynamic and kinetic properties.[5] The *N*-introduction of pendant functions or groups leads to so-called bifunctional chelating agents that provide a wide range of applications (Chart 1) such as modification of

thermal properties (compound **A**),[6] catalytic reduction of CO_2 (compound **B**),[2] but also for medical purposes, including cyclams bearing a bioconjugation function (compound **C**)[7] or a targeting group such as a biotin (compound **D**).[8] These are only selected examples of a larger list. More sophisticated structures with different functions on the nitrogen atoms have also been described, such as compound **E,** a nebramine-cyclam conjugate that enhances the efficacy of β-lactam antibiotics[9] or **F** which was developed for targeted positron emission tomography (PET) tumor imaging after radiolabeling with copper-64.[10]

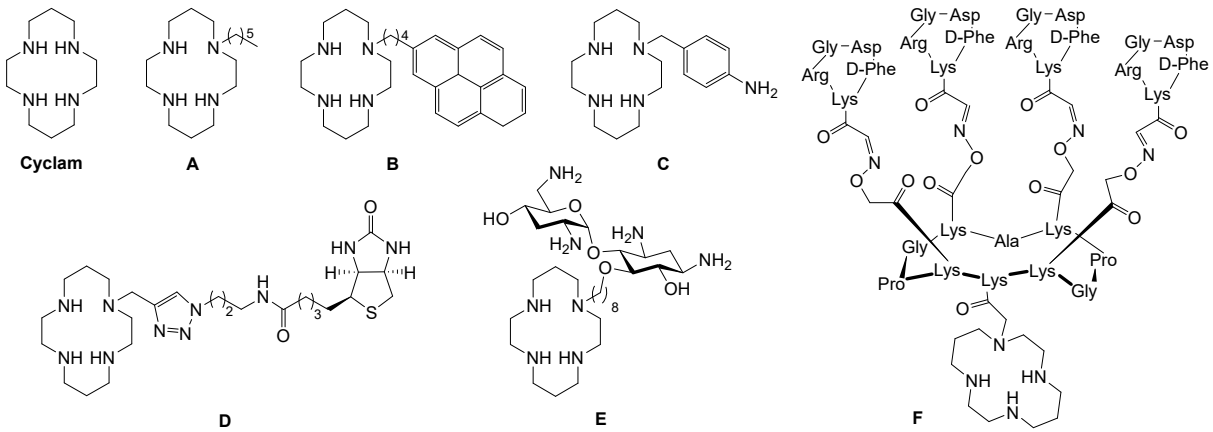


Chart 1. Examples of *N*-functionalized cyclams of interest described in the literature.

However, it is well established that *N*-alkylation of cyclam with non-coordinating groups can lead to a decrease in its coordination capacity which has an impact on thermodynamic stability and kinetic inertness of the corresponding complexes,[11] especially because of steric effects. Moreover, the resulting drop of the coordinating properties can be detrimental to the desired application, as in the case of compound **B**. Indeed, the authors explained that the loss of one secondary amine led to a decrease of selectivity towards CO_2 compared to non-alkylated cyclam. A judicious alternative is to introduce the function of interest on one carbon atom of the macrocycle, i.e. *C*-functionalization. But while *N*-alkylations are now well controlled by selective protection methods,[12] *C*-functionalization is much more challenging and requires reconsidering the synthesis from the beginning by cyclization between a preorganized linear tetraamine and a cyclizing agent that already contains the desired function or its precursor.

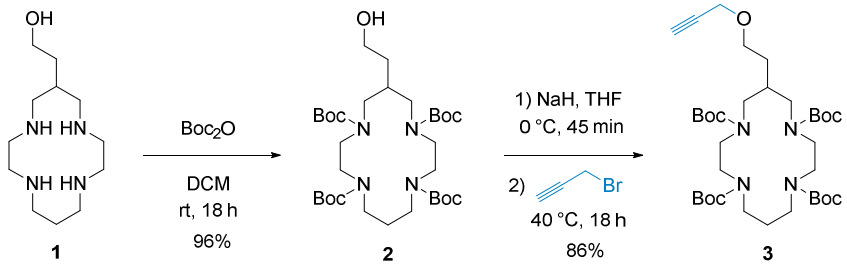
A decade ago, we described a versatile *C*-functionalization method using a bisaminal template for the synthesis of a cyclam bearing a hydroxyethyl chain, called EtOH-cyclam.[13] This method leads to the *C*-functional cyclam as easily and rapidly as the "naked" cyclam[14] and requires no chromatographic purification. This "[two-step/one week] synthesis", one of the most profitable to our knowledge, has not yet been exploited and deserves to be capitalized on. In order to address the problem raised above, we thought it might be very interesting to develop a simple and rapid method for modifying the hydroxyethyl function to extend the *C*-pendant arm to functions/functionalities of interest onto cyclam scaffold.

The synthesis of *C*-functionalized cyclams is essential for opening new avenues in applied research, enabling the design of innovative and targeted compounds, promising significant advancements in various fields. In this work, we then describe an easy and fast method for incorporating a wide variety of functions on the hydroxyethyl chain of EtOH-cyclam to broaden the range of possible applications of such compounds. To this end, we turned our attention to the click chemistry, in particular the Cu(I)-catalyzed Alkyne-Azide Cycloaddition (CuAAC) thanks to its numerous advantages.[15-17] However, in our case, the use of Cu(I) as a catalyst may be a major issue due to its unavoidable complexation with the macrocycle. An efficient solution, already used by other authors, is to convert the amines of the macrocycle to carbamate

functions, such as Boc groups, to prevent the coordination of copper ions.[18] In our case, the use of Boc-protected EtOH-cyclam is of particular interest because it has a dual role: *i*) preventing the coordination of copper ions during the CuAAC step, as indicated above, *ii*) inhibiting the reactivity of the amine functions, during the Williamson reaction, for the introduction of a terminal alkyne by *O*-alkylation of EtOH-cyclam. In a second time, the protected-cyclam derivative bearing the terminal alkyne can be coupled with a series of azides carrying functions of interest such as coupling functions, aromatic groups such as a pyrene, or targeting groups as glucose or biotin moieties. In the same context, and in order to propose a route towards more sophisticated compounds with greater added value and recognized biological potential, by means of a simple synthesis, we focused on the development of polycationic amphiphilic trehalose derivatives with Siamese[19] and Janus-macrocyclic structure (cyclotrehalans, CTs).[20] These terms refer specifically to α,α'-trehalose-based amphiphiles, which are symmetrical[19,21] or cyclic structures[22,23] formed from α,α'-trehalose (a non-reducing sugar composed of two linked glucose molecules). These structures have been studied for their ability to undergo co-assembly with plasmid DNA (pDNA) to form transfection complexes. By addition of different arrangements of polyamines such objects can enable strong pDNA complexation and potential transfection.[24-26] Thus, the introduction of naked cyclams able to preserve their structure through *C*-functionalization is of great interest, provided these compounds are available synthetically.

Results and discussion

The starting compound, EtOH-cyclam **1**, was easily synthesized as previously described.[13] The specific alkylation of the alcohol function of **1** required the prior protection of the secondary amino functions. This reaction was carried out with Boc_2O in dichloromethane (DCM) mixture at room temperature for 18 hours. The tetra-Boc EtOH-cyclam **2** was isolated as a yellow oil in 96% yield after column chromatography. Then, the *O*-alkylation was performed in the conditions of a Williamson ether synthesis in the presence of sodium hydride, in dry tetrahydrofuran (THF), at low temperature, followed by the direct addition of propargyl bromide leading, after heating at 40 °C overnight, to alkyne derivative **3** (Scheme 1). The different parameters of the Williamson reaction were optimized as summarized in Table 1.



Scheme 1. Synthesis of compound **3** from EtOH-cyclam **1**.

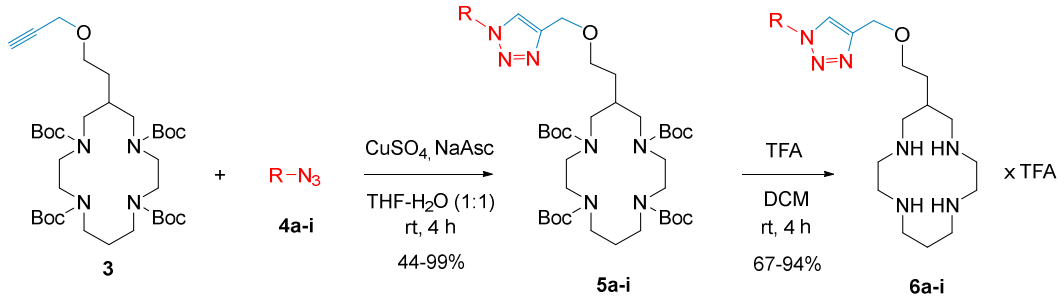
The conversions given in Table 1 were determined from NMR spectra. For this reaction, entries 1 and 2 show that temperature slightly increases the conversion. Higher amounts of sodium hydride and propargyl bromide also led to a better conversion, as shown in entries 3 and 4. Importantly, the number of sodium hydride equivalents had a greater impact on the conversion than those of propargyl bromide (RBr). Indeed, compared to entry 4 with 60% conversion (2 equiv NaH and 2 equiv RBr), conversion increased to 70% for entry 5 (2 equiv NaH and 8 equiv RBr) and 90% for entry 7 (4 equiv NaH and 2 equiv RBr). The reaction time of the deprotonation step with sodium hydride should also be considered in order to maximize the formation of the intermediate alkoxide anion. Indeed, entry 7 (deprotonation step in 45 min) led to better conversion than entry 6 (deprotonation step in 90 min). Taking all these parameters

into account, we managed to achieve a 100% conversion thanks to the conditions of entry 8, leading to compound **3** with 86% yield after purification. High-resolution mass spectra, ^{1}H, ^{13}C NMR spectra and other analysis are in experimental section and SI (Figures S1-S6).

Table 1. Optimization of the *O*-alkylation step of compound **2**.

Entry	NaH (equiv)	Time of deprotonation step (min)	Propargyl bromide (equiv)	T (°C)	NMR conversion (%)
1	1.2	45	1.2	rt	33
2	1.2	45	1.2	40	40
3	1.2	45	2	40	45
4	2	45	2	40	60
5	2	45	8	40	70
6	4	90	2	40	80
7	4	45	2	40	90
8	5	45	2	40	100

CuAAC click reaction was then performed on alkyne derivative **3** with the series of azides **4a-i** in a THF-H_2O solution (1:1) (Scheme 2). This reaction was optimized using azido benzene **4a** as shown on Table 2.



Scheme 2. Synthesis of a series of *C*-alkylated cyclam derivatives **6** from compound **3.**

Table 2. Optimization of the CuAAC between compound **3** and azido benzene **4a**.

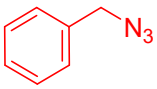
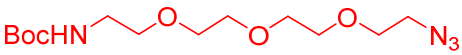
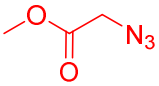
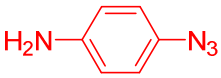
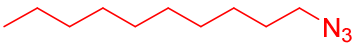
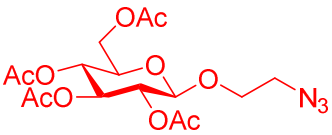
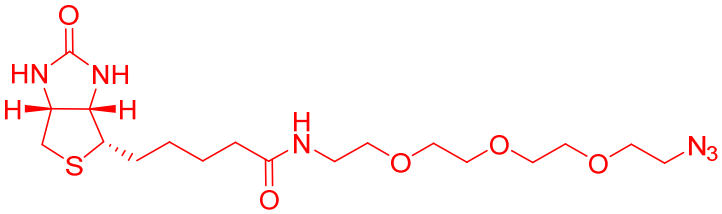
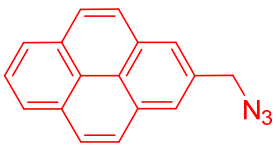
Entry	$CuSO_4$ (equiv)	Na ascorbate (equiv)	Ratio Na ascorbate/$CuSO_4$	Time (h)[a]	Yield (%)[b]
1	0.05	0.2	4	4	- [c]
2	0.05	0.2	4	18	- [c]
3	0.2	0.5	2.5	4	25
4	0.4	1	2.5	4	39
5	1.1	2	1.8	4	53
6	1.1	8	7.3	4	64

[a] Reactions performed at room temperature. [b] Yield after column chromatography. [c] No reaction.

Table 2 shows that classical conditions for CuAAC,[15] i.e. 0.05 equivalent of copper sulfate, 0.2 equivalent of sodium ascorbate, with stirring at room temperature (entries 1 and 2) do not work

for this coupling. When the number of copper sulfate equivalents increases, product **4a** starts to form. Entries 3 and 4 led to a mixture of product **4a** and starting alkyne **3**. The incomplete conversion could be explained by the trapping of part of the copper ions by the cyclam core, although Boc groups limit this phenomenon. Full conversion was reached for 1.1 equivalent of copper sulfate (entries 5 and 6). In parallel, increasing the stoichiometry of sodium ascorbate, and so the ratio sodium ascorbate/copper sulfate, maximized the proportion of Cu(I) in solution, preventing its oxidation to Cu(II) (entry 6). In these conditions, product **4a** was isolated with a 64% yield after chromatographic purification. These optimized conditions were then applied to the coupling between compound **3** and the series of azides **4a-i** (Table 3).

Table 3. Results of the CuAAC and deprotection step involving compound **3** and azides **4a-i**.

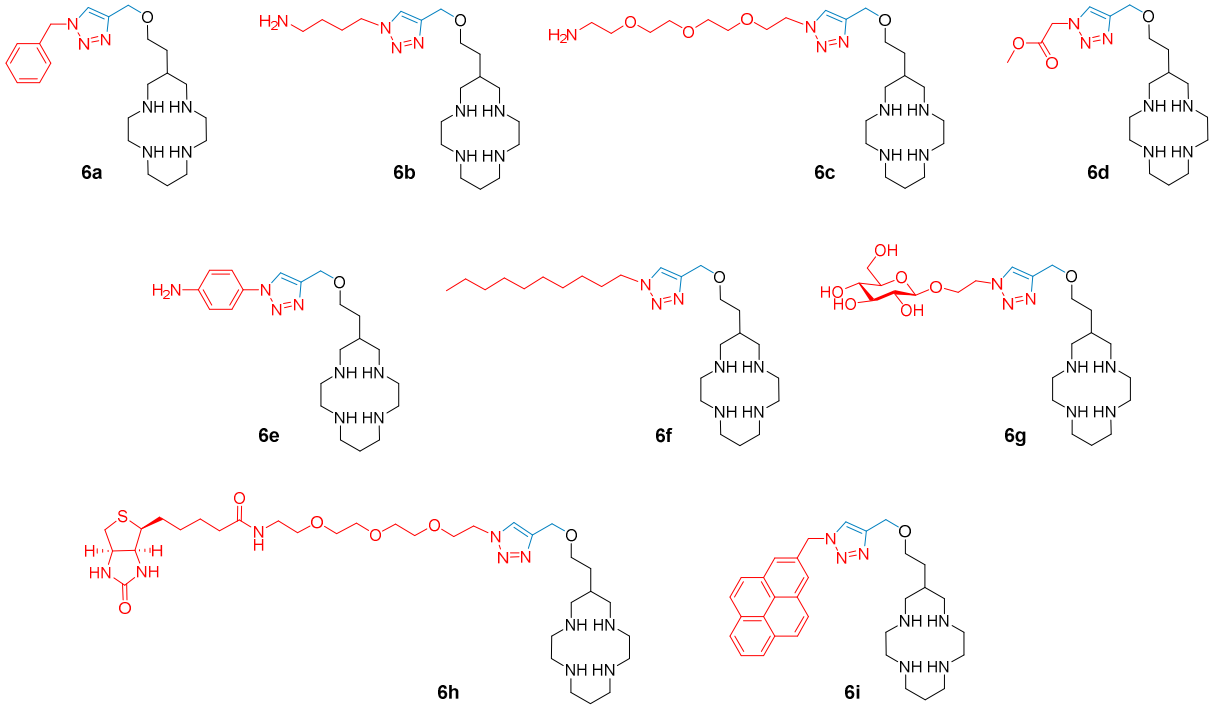
Entry	Azido derivatives 4a-i	Compounds 5a-i yields[a]	Compounds 6a-i yields
1	**4a** 	**5a** 64%	**6a** 72%
2	**4b** 	**5b** 44%	**6b** 94%
3	**4c** 	**5c** 57%	**6c** 88%
4	**4d** 	**5d** 74%	**6d** 68%
5	**4e** 	**5e** 61%	**6e** 90%
6	**4f** 	**5f** 58%	**6f** 67%
7	**4g** 	**5g**[b] 66%	**6g**[c] 53%
8	**4h** 	**5h** 99%	**6h** 79%
9	**4i** 	**5i**[b] 88%	**6i** 89%

[a] Yield after column chromatography. [b] Reaction performed at 40 °C for 18 h. [c] After further treatment with sodium methylate.

In a general trend, the optimized conditions for the CuAAC reaction (entry 6, Table 2) were suitable for most azides, including 1-(azidomethyl) pyrene **4i** (giving compound **5i** with 88% yield) and azido-PEG_3-biotin conjugate **4h** (leading to compound **5h** in 99% yield). The coupling reaction with azides **4b-f** led to products **5b-f** with acceptable to satisfying yields ranging from 44% to 74%. The limitations of these conditions relate to azides **4g** and **4i**, which were not fully converted into triazoles **5g** and **5i**. Nevertheless, we managed to overcome this lack of reactivity by carrying out the coupling at 40 °C for 18 hours, leading to compounds **5g** and **5i** in 66% and 88% yield respectively.

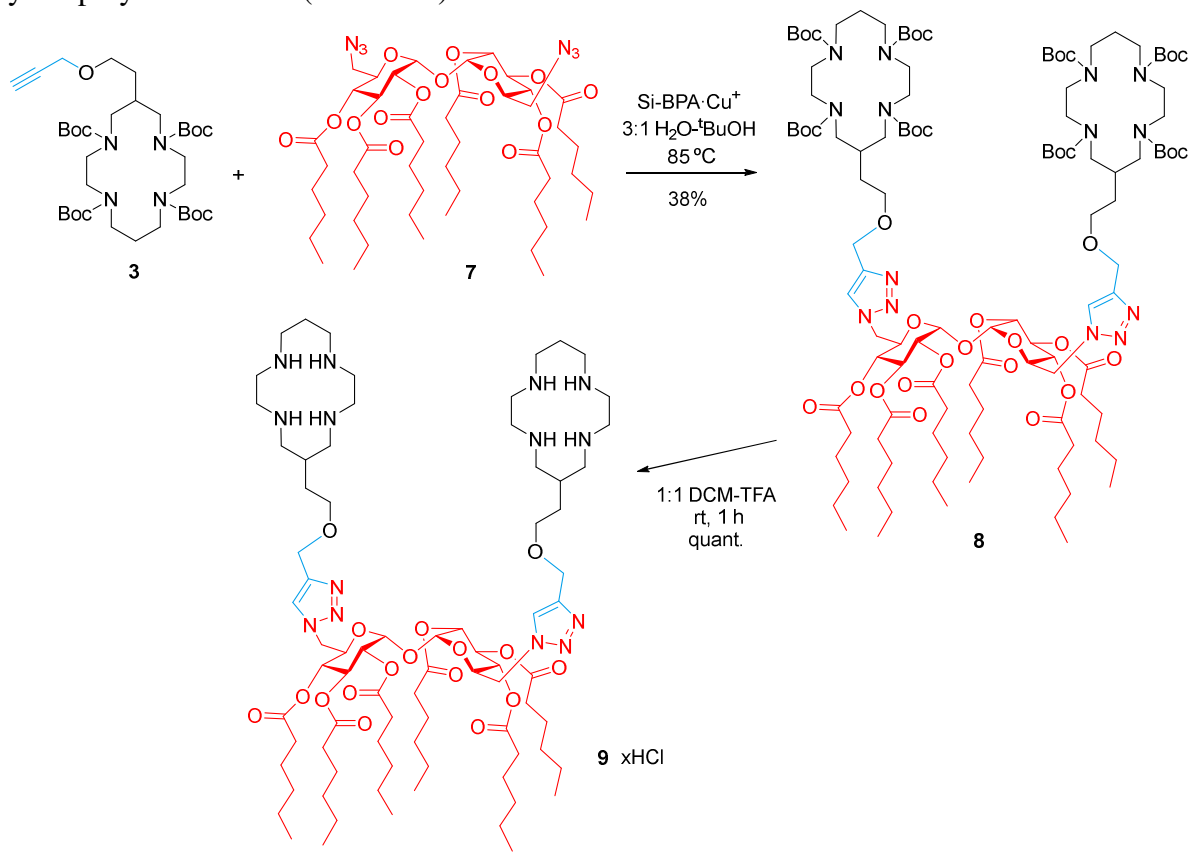
It should be noted that when the CuAAC reaction was carried out with the unprotected analogues of reagents **4b**-**4c**, bearing a "free" primary amine, a mixture of non-separable products was obtained. Indeed, ^{1}H NMR spectra showed a multiplet for the triazole proton instead of the expected characteristic singlet. Urankar *et al.* have previously reported that 3-azidopropylamine can assist the CuAAC reaction via a proximity effect, as the amine can participate as a ligand of Cu(I), reducing the reaction time.[27] In our case, the opposite effect might have occurred, meaning that Cu(I) and amine functions led to a complex that prevents the catalytic role of copper. This hypothesis was supported by the coupling with azide **4e**, that contains a non-protected aniline function, that went without a hitch as the lone pair of the nitrogen atom is less available due to mesomeric effect.

The final step of this synthesis was the removal of Boc groups with trifluoroacetic acid (TFA) in dry dichloromethane (DCM) at room temperature for 4 hours. The deprotected compounds **6a-i** were easily obtained after final evaporation and lyophilization in satisfactory yields ranging from 67% for compound **6f** to 90% for compound **6e**. This treatment efficiently removed Boc groups from compound **5g**, but a small fraction of acetate groups of the glucose moiety was also eliminated. We therefore chose to completely deprotect the glucose using sodium methylate in methanol for 18 hours at room temperature, leading to the fully deprotected compound **6g** in 73% yield (in 2 steps). The series of deprotected triazoles derivatives **6a-i** synthesized are shown in scheme 3. High-resolution mass spectra, ^{1}H, ^{13}C NMR spectra and other analysis of these last two steps are in experimental section and SI (Figures S7-S64).



Scheme 3. Structures of the newly *C*-functional cyclams **6a-i.**

Given the structure of **6g**, we challenged ourselves to extrapolate the previous synthesis to provide more sophisticated macromolecular objects for supramolecular bioapplications, such as the development of trehalose-polyamine derivatives potentially able to form DNA nanocomplexes.[19,28,29] Advances in gene therapies rely on the effective delivery of nucleic acids into cells, with a main challenge being the development of carrier/targeting systems (often called vectors) capable of efficiently and specifically transporting therapeutic genetic material to the targeted sites.[30] Although viral vectors are effective, concerns about immunogenicity and integration into the host genome persist.[31] Non-viral vectors, such as those used in approved therapies like Patisiran and mRNA-based vaccines, are alternatives with fewer drawbacks.[32] However, many non-viral vectors lack conformational definition, hindering systematic studies for targeting process optimization. Efforts to obtain well-defined molecular targeting objects have led to the investigation of carbohydrate-based scaffolds, in particular α,α'-trehalose which has shown its potential in the emergence of molecular vectors with accurate chemical changes.[19,20,24-26] In order to understand how the molecular architecture of non-viral vectors can affect their complexation with DNA, and consequently influence cell selectivity and tissue tropism, it seems important to develop new molecular objects with significant impact on the selectivity of cell and organ transfection. In continuation of the research of some of us,[19] we have sought to take advantage of the previous general procedure that exploits click chemistry reactions on a *C*-functional cyclam to develop Siamese-trehalose-based vectors comprising cyclic polyamine heads (Scheme 4).



Scheme 4. Synthesis of triazole-linked cyclam-trehalose amphiphile **9**

The tetraBoc alkyne-cyclam **3** was then used in a click-coupling with the 6,6'-diazido-6,6'-dideoxy-α,α'-trehalose derivative **7**,[19] previously prepared, by adapting the reaction conditions to the nature/solubility of the reactant as previously described. Cyclam derivative **3** and trehalose **7** typically react in H_2O/*tert*-BuOH solution in presence of Cu-supported catalyst Si-BPA·Cu^+ [33] under reflux. After treatment and purification by column chromatography, the

coupled-protected compound **8** was obtained with 38% yield. The deprotection procedure was carried out classically in DCM/TFA (1:1) at room temperature. After solvent evaporation and treatment of the residue by H_2O/HCl 0.1 M (10:1), compound **9** was obtained as its hydrochloride salt with quantitative yield. This easy-to-run two-step reaction, starting from compounds **3** and **7** that can both be prepared as stocked reactants, highlights the versatility of the methodology that can be applied to more sophisticated macromolecular objects. All spectra are in SI (Figures S65-S70).

Preliminary complexation assays of the trehalose-biscyclam **9** with a plasmid encoding luciferase (pCMV-Luc VR1216, 6934 bp) at nitrogen (N) to phosphorous (P) stoichiometric ratio of 10 (N/P 10) in BHG buffer (HEPES 10 mM, pH 7.4, glucose 5% w/v) determined by dynamic light scattering (DLS) and ζ-potential measurements demonstrated the efficient DNA complexation into positive nanometric entities (D_h 157 ± 12 nm, ζ- potential +10 ± 5 mV) with potential protection abilities. The tendency of amphiphilic trehalose-biscyclam **9** to form mixed nanoparticles upon formulation with nucleic acids (nanoplexes) is a prerequisite to achieve efficient intracellular delivery and gene expression.[34] This paves the way for efficient gene protection and transfection.

Conclusion

Polyazacycloalkanes are remarkable coordinating ligands with numerous applications from catalysis to medicine. Among them, tacn, cyclen, and pyclen are widely used as chelating platforms, whether *N*-functionalized or not, for various metals, including transition metals, heavy metals and lanthanides. In some cases, not so rare, these macrocycles are also employed to interact with anions or macromolecules such as DNA. Cyclam is better suited for transition metals, and although it has been less produced/used on an industrial scale than its analogue cyclen, it has played an essential role in several processes mentioned in the introduction. The relatively recent emergence of the *C*-functionalization method of cyclam may explain why such compounds have been so rarely studied and developed to date. However, when the purpose of the *C*-functionalization is not simply to coordinate the metal centre, it can preserve (and sometimes improve) the macrocyclic structure and properties of the ligand. This has paved the way for several improvements in applications where the cyclam platform is coupled to an external moiety via *N*-functionalization. In this study, we have developed a highly efficient synthesis using the easily obtained *C*-functionalized hydroxyethyl cyclam, allowing the extension of the macrocycle with an alkyne function for CuAAC coupling reactions. The steps are straightforward and give ready-to-use bifunctional cyclams with good to excellent yields. This approach has opened the way to several potential improvements in targeting systems, interactions with nanomaterials for CO_2 reduction, or other conjugations.

As an extension of this work, we have applied the cyclam-coupling procedure to the synthesis of a trehalose derivative comprising two cyclam cores which could interact with plasmid DNA and form transfection complexes. First results shown the trehalose-biscyclam **9** vector forms nanometric formulations with pDNA that efficiently compact and protect gene material for selective cell transfection. This could open new avenues for targeted gene delivery without the requirement for additional ligands or components.

Current efforts are aimed at exploiting the derivatives obtained for some of the applications mentioned above with compounds **6** and investigations focused on evaluating gene protection and transfection in a set of polycationic amphiphilic trehalose derivatives are already accessible.[35] Another emerging challenge is to adapt this methodology to other cyclams of interest, N-functionalized with coordinating pendant arms. Once again, we are actively addressing this challenge.

Experimental section

Materials and methods

All commercial reagents were used as received from the suppliers unless otherwise indicated. The solvents were freshly distilled prior to use and according to the standard methods.

NMR Spectroscopic studies. Analytical ^{1}H and ^{13}C NMR spectra were recorded at the "Services communs" of the University of Brest on Bruker Avance 500 (500 MHz) or Bruker Avance 400 (400 MHz) spectrometers. The experiments were recorded at 25 °C, except those involving Boc-protected compounds which were recorded at 70 °C to improve resolution, and some of the deprotected final compounds were recorded at 80 °C. The signals are indicated as follows: chemical shift (ppm), multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; q, quadruplet; quint, quintuplet), coupling constants J in hertz (Hz). The abbreviations Ar and Cq stand for aromatic and quaternary carbon, respectively.

Chromatographic methods. All reactions were monitored by thin-layer chromatography, which was performed on aluminum sheets coated with silica gel 60 F254. TLC plates were revealed by UV and with 10% ethanolic H_2SO_4 or with ninhydrin in the absence of chromophore groups.

IR spectroscopy. IR spectra were recorded on a JASCO FTIR-410 instrument and were processed using the Jasco Spectra Manager ™ software. It has been recorded both in solid and in solution using an ATR MIRacleTM, presenting data indicating the numbers corresponding to the maximum absorption wavelength.

Mass spectrometry. Low-resolution mass spectra were performed on a Bruker Daltonics Esquire6000 ™ Using ESI as ionization source in the Instituto de Investigaciones Químicas (IIQ), CSIC. High-resolution mass spectra (HRMS) were performed on a Bruker maXis mass spectrometer by the SALSA platform from ICOA laboratory, using ESI for ionization.

Elemental analyses were performed at the Servicio de Microanálisis del Instituto de Investigaciones Químicas de Sevilla, Spain, with a Leco CHNS-932 elemental analyzer.

DNA condensation/ protection assays. 50 µL of paTrehalose/CTplexes were prepared in water at N/P ratio 5 and 10 to a final concentration of 50 µg/mL. Then, samples were electrophoresed for 30 min under 150 mV in 0.8% agarose gel. For protection assays, DNAse I (1U/µg pDNA) was added to each sample and stirred for 30 min at 37 ºC. 20 µL of EDTA 0.25 M was added to inactivate DNAse and the sample was vortexed and incubated for 5 min. 20 µL of SDS 25% was added and further incubated for 5 min. Samples were electrophoresed as described above. Plasmid integrity was compared with free pDNA treated and untreated.

Particle size and zeta-potential measurements. The size of the paTrehalose/CTplexes was measured by dynamic light scattering (DLS), and the overall charge by "Mixed Mode Measurement" phase analysis light scattering (M3-PALS) measurements using a Zeta Nano Series (Malvern Instruments, Spain). All measurements were performed in HEPES 10 mm, 5% glucose, pH 7.4, in triplicate. Size results are given as volume distribution of the major population by the mean diameter with its standard deviation.

Experimental protocols and Spectroscopic data of compounds

2-(1,4,8,11-tetra(*tert*-butoxycarbonyl)-1,4,8,11-tetraazacyclotetradecan-6-yl)ethanol (2). To a solution of 2-(1,4,8,11-tetraazacyclotetradecan-6-yl)ethanol or EtOH-cyclam **1** (203 mg, 0.83 mmol, 1 equiv) in DCM (60 mL) di-*tert*-butyl carbonate Boc_2O (796 mg, 3.65 mmol, 4.3 equiv) was added. The mixture was stirred at room temperature for 18 h. The solvent was removed by rotary evaporation, the oily residue dissolved in a minimum amount of diethyl ether and rapid stripping of all solvent under vacuum yielded an amorphous solid. The residue was purified by column chromatography on SiO_2 (eluent: EtOAc-petroleum ether, 1:1→2:1→4:1) to give **2** as a yellow oil (516 mg, 96%). Rf = 0.27 (EtOAc/petroleum ether 2:1). ^{1}H NMR (500 MHz, CD_3CN, 70 °C) δ (ppm): 3.68 (m, 2H, C*H*$_2$ α-N_{Boc}), 3.57 (m, 2H, CH_2-C*H*$_2$ α-O), [3.45 (m, 2H), 3.38–3.20 (m, 10H), 3.06 (m, 2H)] (C*H*$_2$ α-N_{Boc}), 2.59 (m, 1H, C*H* β-N_{Boc}), 1.87–1.67 (m, 2H, C*H*$_2$ β-N_{Boc}), 1.49–1.45 (m, 38H, C*H*$_2$-CH_2 α-O + C*H*$_3$ Boc).^{13}C NMR (125 MHz, CD_3CN, 70 °C) δ (ppm): [157.4 (x2), 156.9 (x2)] (*C*O Boc), [80.7 (×2), 80.5 (×2)] (*C*$_q$ Boc), 60.7 (CH_2-*C*H_2 α-O), [52.4 (×2), 49.4 (×2), 49.0 (×2), 48.0 (×2)] (*C*H_2 α-N_{Boc}), 36.0 (*C*H β-N_{Boc}), 34.3 (*C*H_2-CH_2 α-O), 29.7 (*C*H_2 β-N_{Boc}), 29.2 (×12) (*C*H_3 Boc). HRMS (ESI): *m/z* calcd for $C_{32}H_{61}N_4O_9^+$ $[M+H]^+$ 645.4433, found: 645.4432 $[M+H]^+$, 667.4250 $[M+Na]^+$, 545.3906 $[M+H – Boc]^+$.

1,4,8,11-tetra(*tert*-butoxycarbonyl)-6-[2-(prop-2-yn-1-yloxy)ethyl]-1,4,8,11-tetraazacyclotetradecane (3). To a solution of sodium hydride (NaH) (111 mg, 60% adsorbed on oil, 2.775 mmol, 5 equiv) in dry THF (1 mL) at 0 °C under an argon atmosphere was added dropwise a solution of compound **2** (358 mg, 0.555 mmol, 1 equiv) in dry THF (2 mL). The mixture was stirred for 45 min at 0 °C. Propargyl bromide was then added and the mixture was stirred for 18 h at 40 °C. After filtration on Celite® pad and washing with DCM, the filtrate was evaporated under reduced pressure to remove THF. The residue was taken up in water (15 mL) and extracted with $CHCl_3$ (3 × 20 mL). The organic layers were combined, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by chromatography on SiO_2 (eluent: EtOAc/hex from 0:1 to 1:0) to give the alkyne derivative **3** as a yellow oil (325 mg, 86%). Rf = 0.63 (EtOAc/hex 7:3). ^{1}H NMR (500 MHz, CD_3CN, 70 °C) δ (ppm): 4.08 (d, ^{4}J = 2.5 Hz, 2H, C_{sp}-C*H*$_2$ α-O), 3.64 (m, 2H, C*H*$_2$ α-N_{Boc}), 3.53 (d, ^{3}J = 6.4 Hz, 2H, CH_2-C*H*$_2$ α-O), [3.42 (m, 2H), 3.34–3.18 (m, 10H), 3.04 (m, 2H)] (C*H*$_2$ α-N_{Boc}), 2.62 (t, ^{4}J = 2.5 Hz, 2H, C_{sp}-C*H*), 2.03 (m, 1H, C*H* β-N_{Boc}), 1.84–1.66 (m, 2H, C*H*$_2$ β-N_{Boc}), 1.52 (m, 2H, C*H*$_2$-CH_2 α-O), 1.44 (s, 38H, C*H*$_3$ Boc). ^{13}C NMR (125 MHz, CD_3CN, 70 °C) δ (ppm): [157.3 (×2), 156.9 (×2)] (*C*O Boc), 81.7 (*C*$_{sp}$), [80.7 (×2), 80.6 (×2)] (*C*$_q$ Boc), 75.9 (C_{sp}-*C*H), 69.0 (CH_2-*C*H_2 α-O), 59.1 (*C*$_{sp}$-CH_2 α-O), [52.5 (×2), 49.5 (×2), 49.2 (×2), 48.2 (×2)] (*C*H_2 α-N_{Boc}), 36.2 (*C*H β-N_{Boc}), 31.5 (*C*H_2-CH_2 α-O), 29.9 (*C*H_2 β-N_{Boc}), 29.4 (×12) (*C*H_3 Boc). HRMS (ESI): *m/z* calcd for $C_{35}H_{63}N_4O_9^+$ $[M+H]^+$ 683.4590, found: 683.4583 $[M+H]^+$, 705.4406 $[M+Na]^+$, 583.4063 $[M+H – Boc]^+$.

General procedure for the click reaction CuAAC leading to 5a-i (unless contrary indication)

Typical protocol for the synthesis of compound (5a). To a solution of alkyne derivative **3** (30 mg, 0.044 mmol, 1 equiv) in THF (0.6 mL) were added successively benzyl azide **4a** (6 µL, 0.048 mmol, 1.1 equiv), a solution of sodium ascorbate (70 mg, 0.352 mmol, 8 equiv) in ultrapure water (0.1 mL) and a solution of $CuSO_4.5H_2O$ (12 mg, 0.048 mmol, 1.1 equiv) in ultrapure water (0.1 mL). The mixture was stirred at room temperature for 4 h. After filtration on Celite® pad and washing with DCM, the filtrate was evaporated under reduced pressure to remove THF. The residue was dissolved in EtOAc and washed with a 10% NH_4OH solution. The organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure. The

residue was purified by chromatography on SiO_2 (eluent: EtOAc/hex from 1:1 to 1:0) to give the triazole derivative **5a** as a yellow oil (23 mg, 64%).

1,4,8,11-tetra(*tert*-butoxycarbonyl)-6-{2-[(1-(benzyl)-1*H*-1,2,3-triazol-4-yl)methoxy] ethyl}-1,4,8,11-tetraazacyclotetradecane (5a). This compound was obtained as a yellow oil (23 mg, 64%) after purification by chromatography on SiO_2 (eluent: EtOAc/hex from 1:1 to 1:0). Rf = 0.28 (EtOAc/hex 7:3). ^{1}H NMR (500 MHz, CD_3CN, 70 °C) δ (ppm): 7.68 (s, 1H, C*H*$_{triazole}$), 7.40–7.30 (m, 5H, C*H*$_{Ar}$), 5.53 (s, 2H, C*H*$_2$ α-N$_{triazole}$), 4.53 (s, 2H, C*H*$_2$ α-C$_{triazole}$), 3.63 (m, 2H, C*H*$_2$ α-N$_{Boc}$), 3.55 (t, ^3J = 6.3 Hz, 2H, CH$_2$-C*H*$_2$ α-O), [3.42 (m, 2H), 3.33–3.18 (m, 10H), 3.02 (m, 2H)] (C*H*$_2$ α-N$_{Boc}$), 2.05 (m, 1H, C*H* β-N$_{Boc}$), 1.82–1.63 (m, 2H, C*H*$_2$ β-N$_{Boc}$), 1.53 (m, 2H, C*H*$_2$-CH$_2$ α-O), 1.46 (s, 36H, C*H*$_3$ Boc). ^{13}C NMR (125 MHz, CD_3CN, 70 °C) δ (ppm): [157.3 (×2), 156.8 (×2)] (*C*O Boc), 146.7 (*C*$_{triazole}$), 137.3 (*C*$_{ipso}$ Ph), [130.2, 129.7 (×2), 129.3 (×2)] (*C*H$_{Ar}$), 124.4 (*C*H$_{triazole}$), [80.6 (×2), 80.5 (×2)] (*C*$_q$ Boc), 69.2 (CH$_2$-*C*H$_2$ α-O), 65.1 (*C*H$_2$ α-C$_{triazole}$), 54.9 (CH$_2$ α-N$_{triazole}$), [52.4 (×2), 49.3 (×2), 49.0 (×2), 48.0 (×2)] (*C*H$_2$ α-N$_{Boc}$), 36.1 (*C*H β-N$_{Boc}$), 31.4 (*C*H$_2$-CH$_2$ α-O), 29.6 (*C*H$_2$ β-N$_{Boc}$), 29.2 (×12) (*C*H$_3$ Boc). HRMS (ESI): *m/z* calcd for $C_{42}H_{70}N_7O_9^+$ $[M+H]^+$ 816.5230, found: 816.5222 $[M+H]^+$, 838.5044 $[M+Na]^+$.

1,4,8,11-tetra(*tert*-butyloxycarbonyl)-6-{2-[(1-(4-*tert*-butoxycarbonylaminobutyl)-1*H*-1,2,3-triazol-4-yl)methoxy]ethyl}-1,4,8,11-tetraazacyclotetradecane (5b). This compound was obtained as an orange oil (17 mg, 44%) after purification by chromatography on SiO_2 (eluent: EtOAc/hex from 1:4 to 1:0). Rf = 0.10 (EtOAc/hex 1:1). ^{1}H NMR (500 MHz, CD_3CN, 70 °C) δ (ppm): 7.68 (s, 1H, C*H*$_{triazole}$), 5.16 (broad s, 1H, N*H*Boc), 4.53 (s, 2H, C*H*$_2$ α-C$_{triazole}$), 4.35 (t, ^3J = 7.1 Hz, 2H, C*H*$_2$ α-N$_{triazole}$), 3.65 (m, 2H, C*H*$_2$ α-N$_{Boc}$), 3.56 (t, ^3J = 6.4l Hz, 2H, CH$_2$-C*H*$_2$ α-O), [3.42 (m, 2H), 3.35–3.19 (m, 10H)] (C*H*$_2$ α-N$_{Boc}$), 3.11–2.99 (m, 4H, C*H*$_2$ α-NHBoc and C*H*$_2$ α-N$_{Boc}$), 2.05 (m, 1H, C*H* β-N$_{Boc}$), 1.89 (m, 2H, C*H*$_2$ β-N$_{triazole}$), 1.84–1.65 (m, 2H, C*H*$_2$ β-N$_{Boc}$), 1.55 (m, 2H, C*H*$_2$-CH$_2$ α-O), 1.45 (m, 38H, C*H*$_2$ β-NHBoc and C*H*$_3$ Boc), 1.42 (s, 9H, C*H*$_3$ NHBoc). ^{13}C NMR (125 MHz, CD_3CN, 70 °C): δ (ppm): 157.5 (*C*O NHBoc), [157.3 (×2), 156.9 (×2)] (*C*O Boc), 146.3 (*C*$_{triazole}$), 124.2 (*C*H$_{triazole}$), [80.6 (×2), 80.5 (×2)] (*C*$_q$ Boc), 79.6 (*C*$_q$ NHBoc), 69.2 (CH$_2$-*C*H$_2$ α-O), 65.2 (*C*H$_2$ α-C$_{triazole}$), 50.9 (CH$_2$ α-N$_{triazole}$), [52.5 (×2), 49.3 (×2), 49.0 (×2), 48.0 (×2)] (*C*H$_2$ α-N$_{Boc}$), 41.0 (*C*H$_2$ α-NHBoc), 36.2 (*C*H β-N$_{Boc}$), 31.5 (*C*H$_2$-CH$_2$ α-O), 29.7 (*C*H$_2$ β-N$_{Boc}$), 29.2 (×12) (*C*H$_3$ Boc), 29.1 (×3) (*C*H$_3$ NHBoc), 28.6 (*C*H$_2$ β-N$_{triazole}$), 28.3 (*C*H$_2$ β-NHBoc). HRMS (ESI): *m/z* calcd for $C_{44}H_{81}N_8O_{11}^+$ $[M+H]^+$ 897.6019, found: 897.6020 $[M+H]^+$, 797.5499 $[M+H - Boc]^+$, 326.2666 $[M+H - BuNHBoc - 4\ Boc]^+$, 199.1734 $[M+2H - 5\ Boc]^{2+}$.

1,4,8,11-tetra(*tert*-butyloxycarbonyl)-6-{2-[(1-(11-*tert*-butoxycarbonylamino-3,6,9-trioxa undecyl)-1*H*-1,2,3-triazol-4-yl)methoxy]ethyl}-1,4,8,11-tetraazacyclotetradecane (5c). This compound was obtained as a colourless oil (25 mg, 57%) after purification by chromatography on SiO_2 (eluent: EtOAc/MeOH from 1:0 to 4:1). Rf = 0.55 (EtOAc/MeOH 9:1) ^{1}H NMR (500 MHz, CD_3CN, 70 °C) δ (ppm): 7.77 (s, 1H, C*H*$_{triazole}$), 5.21 (borad s, 1H, N*H*Boc), 4.54 (s, 2H, C*H*$_2$ α-C$_{triazole}$), 4.50 (t, ^3J = 5.3 Hz, 2H, C*H*$_2$ α-N$_{triazole}$), 3.87 (t, ^3J = 5.3 Hz, 2H, C*H*$_2$ β-N$_{triazole}$), 3.66 (m, 2H, C*H*$_2$ α-N$_{Boc}$), 3.60–3.51 (m, 10H, C*H*$_2$ α-O$_{PEG}$), 3.47 (t, ^3J = 5.7 Hz, 2H, CH$_2$-C*H*$_2$ α-O), 3.42 (m, 2H, C*H*$_2$ α-N$_{Boc}$), 3.33–3.17 (m, 12H, C*H*$_2$ α-NHBoc and C*H*$_2$ α-N$_{Boc}$), 3.02 (m, 2H, C*H*$_2$ α-N$_{Boc}$), 2.06 (m, 1H, C*H* β-N$_{Boc}$), 1.83–1.64 (m, 2H, C*H*$_2$ β-N$_{Boc}$), 1.55 (m, 2H, C*H*$_2$-CH$_2$ α-O), 1.46 (s, 36H, C*H*$_3$ Boc), 1.42 (s, 9H, C*H*$_3$ NHBoc). ^{13}C NMR (125 MHz, CD_3CN, 70 °C) δ (ppm): [157.3 (×2), 156.8 (×2)] (*C*O Boc), 156.9 (*C*O NHBoc), 146.1 (*C*$_{triazole}$), 125.0 (*C*H$_{triazole}$), [80.6 (×2), 80.5 (×2)] (*C*$_q$ Boc), 79.7 (*C*$_q$ NHBoc),

[71.6 (×2), 71.5, 71.4, 71.1] (CH_2 α-O_{PEG}), 70.5 (CH_2 β-$N_{triazole}$), 69.2 (CH_2-CH_2 α-O), 65.1 (CH_2 α-$C_{triazole}$), 52.4 (×2) (CH_2 α-N_{Boc}), 51.3 (CH_2 α-$N_{triazole}$), [49.3 (×2), 49.0 (×2), 48.0 (×2)] (CH_2 α-N_{Boc}), 41.8 (CH_2 α-NHBoc), 36.2 (CH β-N_{Boc}), 31.5 (CH_2-CH_2 α-O), 29.7 (CH_2 β-N_{Boc}), 29.2 (×12) (CH_3 Boc), 29.1 (CH_3 NHBoc). HRMS (ESI): *m/z* calcd for $C_{48}H_{89}N_8O_{14}^+$ $[M+H]^+$ 1001.6493, found: 1001.6490 $[M+H]^+$, 1023.6297 $[M+Na]^+$, 251.1977 $[M+2H - 5\ Boc]^{2+}$.

1,4,8,11-tetra(*tert*-butoxycarbonyl)-6-{2-[(1-(methylacetyl)-1*H*-1,2,3-triazol-4-yl) methoxy]ethyl}-1,4,8,11-tetraazacyclotetradecane (5d). This compound was obtained as a colourless oil (26 mg, 74%) after purification by chromatography on SiO_2 (eluent: EtOAc/hex from 1:1 to 1:0). Rf = 0.14 (EtOAc/hex 7:3). ^{1}H NMR (500 MHz, CD_3CN, 70 °C) δ (ppm): 7.77 (s, 1H, $CH_{triazole}$), 5.19 (s, 2H, CH_2 α-$N_{triazole}$), 4.57 (s, 2H, CH_2 α-$C_{triazole}$), 3.77 (s, 3H, CH_3 α-CO_{ester}), 3.70–3.62 (m, 2H, CH_2 α-N_{Boc}), 3.58 (t, ^3J = 6.3 Hz, 2H, CH_2-CH_2 α-O), [3.48–3.39 (m, 2H), 3.35–3.18 (m, 10H), 3.07–2.99 (m, 2H)] (CH_2 α-N_{Boc}), 2.10–2.03 (m, 1H, CH β-N_{Boc}), 1.85–1.65 (m, 2H, CH_2 β-N_{Boc}), 1.60–1.53 (m, 2H, CH_2-CH_2 α-O), 1.46 (s, 36H, CH_3 Boc). ^{13}C NMR (125 MHz, CD_3CN, 70 °C) δ (ppm): 168.5 (CO_{ester}), [157.4 (×2), 156.9 (×2)] (CO Boc), 146.6 ($C_{triazole}$), 125.8 ($CH_{triazole}$), [80.7 (×2), 80.5 (×2)] (C_q Boc), 69.3 (CH_2-CH_2 α-O), 65.1 (CH_2 α-$C_{triazole}$), 53.7 (CH_3 α-CO_{ester}), 52.5 (2×) (CH_2 α-N_{Boc}), 52.0 (CH_2 α-$N_{triazole}$), [49.4 (×2), 49.1 (×2), 48.1 (×2)] (CH_2 α-N_{Boc}), 36.2 (CH β-N_{Boc}), 31.5 (CH_2-CH_2 α-O), 29.7 (CH_2 β-N_{Boc}), [29.2 (×12)] (CH_3 Boc). HRMS (ESI): *m/z* calcd for $C_{38}H_{68}N_7O_{11}^+$ $[M+H]^+$ 798.4971, found: 798.4967 $[M+H]^+$, 820.4786 $[M+Na]^+$.

1,4,8,11-tetra(*tert*-butoxycarbonyl)-6-{2-[(1-(4-aminophenyl)-1*H*-1,2,3-triazol-4-yl) methoxy]ethyl}-1,4,8,11-tetraazacyclotetradecane (5e). To a solution of tetraBoc cyclam derivative **3** (30 mg, 0.044 mmol, 1 equiv) in THF (0.9 mL) were added successively 4-azidoaniline hydrochloride **4e** (8 mg, 0.048 mmol, 1.1 equiv), a solution of sodium ascorbate (70 mg, 0.352 mmol, 8 equiv) in ultrapure water (0.2 mL) and a solution of $CuSO_4.5H_2O$ (12 mg, 0.048 mmol, 1.1 equiv) in ultrapure water (0.1 mL). The mixture was stirred at room temperature for 18 h. Then diisopropylethylamine (8 µL, 0.048 mmol, 1.1 equiv) was added and the mixture was stirred at room temperature for 4 h. After filtration on Celite® pad and washing with DCM, the filtrate was evaporated under reduced pressure to remove THF. The residue was dissolved in EtOAc and washed with a 10% NH_4OH solution. The organic layer was dried ($MgSO_4$), evaporated under reduced pressure and chromatographed on SiO_2 (eluant: EtOAc/hex from 1:1 to 1:0) to give the triazole derivative **5e** as a yellow oil (22 mg, 61%). Rf = 0.31 (EtOAc/hex 4:1). ^{1}H NMR (500 MHz, CD_3CN, 70 °C) δ (ppm): 8.02 (s, 1H, $CH_{triazole}$), 7.45 (d, ^3J = 8.7 Hz, 2H, CH_{Ar}-C α-$N_{triazole}$), 6.78 (d, ^3J = 8.7 Hz, 2H, CH_{Ar}-C α-NH_2), 4.60 (s, 2H, CH_2 α-$C_{triazole}$), 3.64 (m, 2H, CH_2 α-N_{Boc}), 3.60 (t, ^3J = 6.4 Hz, 2H, CH_2-CH_2 α-O), [3.48–3.39 (m, 2H), 3.35–3.18 (m, 10H), 3.07–2.99 (m, 2H)] (CH_2 α-N_{Boc}), 2.10–2.03 (m, 1H, CH β-N_{Boc}), 1.85–1.65 (m, 2H, CH_2 β-N_{Boc}), 1.57 (m, 2H, CH_2-CH_2 α-O), 1.46 (s, 36H, CH_3 Boc). ^{13}C NMR (125 MHz, CD_3CN, 70 °C) δ (ppm): [157.3 (×2), 156.8 (×2)] (CO Boc), 150.1 (CH_{Ar}-C α-NH_2), 146.6 ($C_{triazole}$), 129.2 (CH_{Ar}-C α-$N_{triazole}$), 123.5 (CH_{Ar}-C α-$N_{triazole}$), 122.7 ($CH_{triazole}$), 116.0 (CH_{Ar}-C α-NH_2), [80.6 (×2), 80.5 (×2)] (C_q Boc), 69.2 (CH_2-CH_2 α-O), 65.1 (CH_2 α-$C_{triazole}$), [52.4 (2×), 49.3 (×2), 49.0 (×2), 48.0 (×2)] (CH_2 α-N_{Boc}), 36.1 (CH β-N_{Boc}), 31.5 (CH_2-CH_2 α-O), 29.7 (CH_2 β-N_{Boc}), [29.2 (×12)] (CH_3 Boc). HRMS (ESI): *m/z* calcd for $C_{41}H_{69}N_8O_9^+$ $[M+H]^+$ 817.5182, found: 817.5181 $[M+H]^+$, 839.5006 $[M+Na]^+$.

1,4,8,11-tetra(*tert*-butoxycarbonyl)-6-{2-[(1-(decyl)-1*H*-1,2,3-triazol-4-yl)methoxy]ethyl}-1,4,8,11-tetraazacyclotetradecane (5f). To a solution of tetraBoc cyclam derivative **3** (30 mg, 0.044 mmol, 1 equiv) in THF (0.6 mL) were added successively 1-azidodecane **4f** (in 0.5 M

solution in ^{t}BuOMe, 96 µL, 0 .048 mmol, 1.1 equiv), a solution of sodium ascorbate (70 mg, 0.352 mmol, 8 equiv) in ultrapure water (0.1 mL) and a solution of $CuSO_4.5H_2O$ (12 mg, 0.048 mmol, 1.1 equiv) in ultrapure water (0.1 mL). The mixture was stirred at room temperature for 18 h. After filtration on Celite® pad and washing with DCM, the filtrate was evaporated under reduced pressure to remove THF. The residue was dissolved in EtOAc and washed with a 10% NH_4OH solution. The organic layer was dried ($MgSO_4$), evaporated under reduced pressure and chromatographed on SiO_2 (eluant: EtOAc/hex from 1:1 to 1:0) to give the triazole derivative **5f** as a colourless oil (22 mg, 58%). Rf = 0.47 (EtOAc/hex 4:1). ^{1}H NMR (500 MHz, CD_3CN, 70 °C) δ (ppm): 7.66 (s, 1H, C*H*$_{triazole}$), 4.52 (s, 2H, C*H*$_2$ α-C$_{triazole}$), 4.32 (t, ^3J = 7.2 Hz, 2H, C*H*$_2$ α-N$_{triazole}$), 3.64 (m, 2H, C*H*$_2$ α-N$_{Boc}$), 3.55 (t, ^3J = 6.3 Hz, 2H, CH$_2$-C*H*$_2$ α-O), [3.43 (m, 2H), 3.33–3.18 (m, 10H), 3.01 (m, 2H)] (C*H*$_2$ α-N$_{Boc}$), 2.04 (m, 1H, C*H* β-N$_{Boc}$), 1.88 (m, 2H, C*H*$_2$-CH$_2$ α-N$_{triazole}$), 1.83–1.62 (m, 2H, C*H*$_2$ β-N$_{Boc}$), 1.54 (m, 2H, C*H*$_2$-CH$_2$ α-O), 1.45 (s, 36H, C*H*$_3$ Boc), 1.38–1.24 (m, 14H, CH$_2$ decane), 0,90 (t, ^3J = 6.7 Hz, 3H, C*H*$_3$).^{13}C NMR (125 MHz, CD_3CN, 70 °C) δ (ppm): [157.3 (×2), 156.8 (×2)] (*C*O Boc), 146.2 (*C*$_{ipso}$ triazole), 124.1 (*C*H triazole), [80.6 (×2), 80.5 (×2)] (*C*$_q$ Boc), 69.1 (CH$_2$-*C*H$_2$ α-O), 65.2 (*C*H$_2$ α-C$_{triazole}$), 52.4 (×2) (*C*H β-N$_{Boc}$), 51.2 (CH$_2$ α-N$_{triazole}$), [49.3 (×2), 49.0 (×2), 48.0 (×2)] (*C*H$_2$ α-N$_{Boc}$), 36.1 (*C*H β-N$_{Boc}$), 32.9 (*C*H$_2$-CH$_2$ α-N$_{triazole}$), 31.5 (*C*H$_2$-CH$_2$ α-O), [31.2, 30.5, 30.4, 30.2, 30.0] (*C*H$_2$ decane), 29.7 (*C*H$_2$ β-N$_{Boc}$), 29.2 (×12) (*C*H$_3$ Boc), [27.5, 23.6] (*C*H$_2$ decane), 14.6 (*C*H$_3$). HRMS (ESI): *m/z* calcd for $C_{45}H_{84}N_7O_9^+$ $[M+H]^+$ 866.6325, found: 866.6313 $[M+H]^+$, 888.6133 $[M+Na]^+$.

1,4,8,11-tetra(*tert*-butoxycarbonyl)-6-{2-[(1-(2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy]ethyl}-1,4,8,11-tetraazacyclotetradecane (5g). To a solution of tetraBoc cyclam derivative **3** (20 mg, 0.029 mmol, 1 equiv) in THF (0.6 mL) were added successively 2-azidoethyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside **4g** (13 mg, 0.032 mmol, 1.1 equiv), a solution of sodium ascorbate (57 mg, 0.290 mmol, 10 equiv) in ultrapure water (0.1 mL) and a solution of $CuSO_4.5H_2O$ (7 mg, 0.029 mmol, 1 equiv) in ultrapure water (0.1 mL). The mixture was stirred at 40 °C for 18 h. After filtration on Celite® pad and washing with DCM, the filtrate was evaporated under reduced pressure to remove THF. The residue was dissolved in EtOAc and washed with a 10% NH_4OH solution. The organic layer was dried ($MgSO_4$), evaporated under reduced pressure and chromatographed on SiO_2 (eluant: EtOAc/hex from 3:2 to 1:0) to give the triazole derivative **5g** as a yellow oil (21 mg, 66%). Rf = 0.25 (EtOAc/hex 7:3). ^{1}H NMR (500 MHz, CD_3CN, 70 °C) δ (ppm): 7.69 (s, 1H, C*H*$_{triazole}$), 5.20 (t, ^3J = 9.6 Hz, 1H, C$_3$*H*), 5.01 (t, ^3J = 9.7 Hz, 1H, C$_4$*H*), 4.87 (t, ^3J = 8.8 Hz, 1H, C$_2$*H*), 4.65 (d, ^3J = 8.0 Hz, 1H, C$_1$*H*), 4.55–4.49 (m, 4H, C*H*$_2$ α-C$_{triazole}$ + C*H*$_2$ α-N$_{triazole}$), 4.25–4.10 (m, 3H, C$_6$*H*$_2$ and N$_{triazole}$-CH$_2$-C*H*$_{2a}$ α-O), 4.01–3.95 (m, 1H, N$_{triazole}$-CH$_2$-C*H*$_{2b}$ α-O), 3.86–3.80 (m, 1H, C$_5$*H*), 3.71 3.63 (m, 2H, C*H*$_2$ α-N$_{Boc}$), 3.57 (t, ^3J = 6.3 Hz, 2H, CH$_2$-C*H*$_2$ α-O), [3.50–3.40 (m, 2H), 3.36–3.17 (m, 10H), 3.05–2.97 (m, 2H)] (C*H*$_2$ α-N$_{Boc}$), 2.05 (m, 1H, C*H* β-N$_{Boc}$), [2.05 (s, 3H), 2.03 (s, 3H), 1.99 (s, 3H), 1.95 (s, 3H)] (OAc), [1.84–1.76 (m, 1H), 1.71–1.63 (m, 1H)] (C*H*$_2$ β-N$_{Boc}$), 1.59–1.50 (m, 2H, C*H*$_2$-CH$_2$ α-O), 1.46 (s, 36H, C*H*$_3$ Boc). ^{13}C NMR (125 MHz, CD_3CN, 70 °C) δ (ppm): [171.5, 171.1, 170.8, 170.5] (*C*O Ac), [157.3 (×2), 156.9 (×2)] (*C*O Boc), 146.1 (*C*$_{ipso}$ triazole), 125.0 (*C*H$_{triazole}$), 101.8 (*C*$_1$), [80.6 (×2), 80.5 (×2)] (*C*$_q$ Boc), 73.9 (*C*$_3$), 73.2 (*C*$_5$), 72.5 (*C*$_2$), 70.1 (*C*$_4$), 69.3 (CH$_2$-*C*H$_2$ α-O), 69.2 (N$_{triazole}$-CH$_2$-*C*H$_2$ α-O), 65.1 (*C*H$_2$ α-C$_{triazole}$), 63.3 (*C*$_6$), 52.4 (×2) (*C*H$_2$ α-N$_{Boc}$), 51.1 (*C*H$_2$ α-N$_{triazole}$), [49.3 (×2), 49.0 (×2), 48.0 (×2)] (*C*H$_2$ α-N$_{Boc}$), 36.2 (*C*H β-N$_{Boc}$), 31.4 (*C*H$_2$-CH$_2$ α-O), 29.7 (*C*H$_2$ β-N$_{Boc}$), 29.2 (×12) (*C*H$_3$ Boc), [21.2, 21.1 (×3)] (*C*H$_3$ Ac). HRMS (ESI): *m/z* calcd for $C_{51}H_{86}N_7O_{19}^+$ $[M+H]^+$ 1100.5973, found: 1100.5963 $[M+H]^+$, 1122.5784 $[M+Na]^+$, 444.7139 $[M+2H - CO_2 - 3^tBu]^{2+}$, 416.6819 $[M+2H - CO_2 - 4^tBu]^{2+}$, 350.6977 $[M+2H - 4CO_2 - 4^tBu]^{2+}$.

1,4,8,11-tetra(*tert*-butoxycarbonyl)-6-{2-[(1-(11-((+)-biotinylamino)-3,6,9-trioxaundecyl)-1*H*-1,2,3-triazol-4-yl)methoxy]ethyl}-1,4,8,11-tetraazacyclotetradecane (5h).
This compound was obtained as a colourless oil (49 mg, 99%) after purification by chromatography on SiO_2 (eluent: EtOAc/MeOH + 1% NH_4OH from 10:0 to 7:3). Rf = 0.11 (EtOAc/MeOH 4:1). ^{1}H NMR (500 MHz, CD_3CN, 70 °C) δ (ppm): 7.78 (s, 1H, C$H_{triazole}$), 6.49 (s, 1H, NH amide), [5.33 (s, 1H), 5.13 (s, 1H)] (NH amide), 4.54 (s, 2H, CH_2 α-C$_{triazole}$), 4.51 (t, ^3J = 5.3 Hz, 2H, CH_2 α-N$_{triazole}$), 4.43 (m, 1H, CH_g), 4.26 (m, 1H, CH_f), 3.88 (t, ^3J = 5.3 Hz, 2H, CH_2 β-N$_{triazole}$), 3.65 (m, 2H, CH_2 α-N$_{Boc}$), 3.60–3.52 (m, 10H, 4 CH_2 α-O$_{PEG}$ + CH$_2$-CH_2 α-O), 3.49 (t, ^3J = 5.7 Hz, 2H, CH_2 α-O$_{PEG}$), 3.44 (m, 2H, CH_2 α-N$_{Boc}$), 3.34–3.15 (m, 13H, CH_2 α-N$_{Boc}$ + CH_2 α-N amide + CH_d), 3.01 (m, 2H, CH_2 α-N$_{Boc}$), [2.90 (dd, ^3J = 5.1 Hz, ^2J = 12.7 Hz, 1H), 2.67 (d, ^2J = 12.7 Hz, 1H)] (CH_{2e}), 2.15 (t, ^3J = 7.2 Hz, 2H, CH_2 α-CO amide), 2.04 (m, 1H, C*H* β-N$_{Boc}$), 1.82–1.74 (m, 1H, CH_2 β-N$_{Boc}$), 1.74–1.58 (m, 5H, CH_2 β-N$_{Boc}$ + CH_{2a} + CH_{2b}), 1.54 (m, 2H, CH_2-CH$_2$ α-O), 1.45 (s, 36H, CH_3 Boc), 1.31 (m, 2H, CH_{2c}). ^{13}C NMR (125 MHz, CD_3CN, 70 °C) δ (ppm): 174.1 (*C*O amide), 164.3 (*C*O urea), [157.2, 156.8] (*C*O Boc), 146.1 (*C*$_{triazole}$), 125.0 (*C*H$_{triazole}$), [80.6, 80.5] (*C*$_q$ Boc), [71.6 (×2), 71.5, 71.4, 70.8] (*C*H$_2$ α-O$_{PEG}$), 70.4 (CH_2 β-N$_{triazole}$), 69.2 (CH$_2$-CH_2 α-O), 65.1 (CH_2 α-C$_{triazole}$), 62.9 (*C*$_g$), 61.3 (*C*$_f$), 56.6 (*C*$_d$), 52.4 (×2) (*C*H$_2$ α-N$_{Boc}$), 51.3 (*C*H$_2$ α-N$_{triazole}$), 49.3 (×2) (*C*H$_2$ α-N$_{Boc}$), 49.0 (×2) (*C*H$_2$ α-N$_{Boc}$), 48.0 (×2) (*C*H$_2$ α-N$_{Boc}$), 41.4 (*C*H$_{2e}$), 40.3 (*C*H$_2$ α-N amide), 36.8 (*C*H$_2$ α-CO amide), 36.2 (*C*H β-N$_{Boc}$), 31.4 (*C*H$_2$-CH$_2$ α-O), [29.6, 29.5, 29.4] (*C*$_a$ + *C*$_c$ + *C*H$_2$ β-N$_{Boc}$), 26.7 (*C*$_b$). HRMS (ESI): *m/z* calcd for $C_{53}H_{95}N_{10}O_{14}S^+$ $[M+H]^+$ 1127.6744, found: 1127.6737 $[M+H]^+$, 1149.6554 $[M+Na]^+$, 514.3143 $[M+2H]^{2+}$.

1,4,8,11-tetra(*tert*-butoxycarbonyl)-6-{2-[(1-(pyrenylmethyl)-1*H*-1,2,3-triazol-4-yl) methoxy]ethyl}-1,4,8,11-tetraazacyclotetradecane (5i). To a solution of tetraBoc derivative cyclam **3** (30 mg, 0.044 mmol, 1 equiv) in THF (0.6 mL) were added successively 1-(azidomethyl) pyrene **4i** (12 mg, 0.048 mmol, 1.1 equiv), a solution of sodium ascorbate (70 mg, 0.352 mmol, 8 equiv) in ultrapure water (0.1 mL) and a solution of $CuSO_4.5H_2O$ (12 mg, 0.048 mmol, 1.1 equiv) in ultrapure water (0.1 mL). The mixture was stirred at 40 °C for 18 h. After filtration on Celite® pad and washing with DCM, the filtrate was evaporated under reduced pressure to remove THF. The residue was dissolved in EtOAc and washed with a 10% NH_4OH solution. The organic layer was dried ($MgSO_4$), evaporated under reduced pressure and chromatographed on SiO_2 (eluant: EtOAc/hex from 1:1 to 9:1) to give the triazole derivative **5i** as a yellow oil (36 mg, 88%). Rf = 0.56 (EtOAc/hex 4:1). ^{1}H NMR (500 MHz, CD_3CN, 70 °C) δ (ppm): [8.36 (d, ^3J = 9.3 Hz, 1H), 8.27–8.16 (m, 4H), 8.15–8.02 (m, 3H), 7.96 (d, ^3J = 7.9 Hz, 1H)] (CH_{Ar} pyrene), 7.62 (s, 1H, C$H_{triazole}$), 6.25 (s, 2H, CH_2 α-N$_{triazole}$), 4.46 (s, 2H, CH_2 α-C$_{triazole}$), 3.54 (m, 2H, CH_2 α-N$_{Boc}$), 3.46 (t, ^3J = 6.4 Hz, 2H, CH$_2$-CH_2 α-O), [3.42–3.33 (m, 2H), 3.25–3.08 (m, 10H), 2.94–2.86 (m, 2H)] (CH_2 α-N$_{Boc}$), 2.05 (m, 1H, C*H* β-N$_{Boc}$), 1.71–1.53 (m, 2H, CH_2 β-N$_{Boc}$), 1.48–1.43 (m, 2H, CH_2-CH$_2$ α-O), [1.42 (s, 18H), 1.38 (s, 18H)] (CH_3 Boc). ^{13}C NMR (125 MHz, CD_3CN, 70 °C) δ (ppm): [157.2 (×2), 156.8 (×2)] (*C*O Boc), 146.6 (*C*$_{ipso}$ triazole), [142.5, 133.2, 132.7, 132.0, 130.4, 126.1, 125.7] (*C*$_{sp2}$), [129.8, 129.3, 129.0, 128.6, 127.8, 127.1, 127.0, 126.4, 123.7] (*C*H$_{Ar}$), 124.4 (*C*H triazole), [80.5 (×2), 80.4 (×2)] (*C*$_q$ Boc), 69.1 (CH$_2$-*C*H$_2$ α-O), 65.1 (*C*H$_2$ α-C$_{triazole}$), 52.9 (CH$_2$ α-N$_{triazole}$), [52.3 (×2), 49.3 (×2), 48.9 (×2), 47.9 (×2)] (*C*H$_2$ α-N$_{Boc}$), 36.1 (*C*H β-N$_{Boc}$), 31.3 (*C*H$_2$-CH$_2$ α-O), 29.6 (*C*H$_2$ β-N$_{Boc}$), [29.2 (×6), 29.1 (×6)] (*C*H$_3$ Boc). HRMS (ESI): *m/z* calcd for $C_{52}H_{74}N_7O_9^+$ $[M+H]^+$ 940.5543, found: 940.5547 $[M+H]^+$, 962.5362 $[M+Na]^+$.

General procedure for the deprotection reaction leading to 6a-i (unless contrary indication)

Typical protocol for the synthesis of compound 6a. Trifluoroacetic acid (TFA) (0.5 mL) was added to a solution of compound **5a** (23 mg, 0.028 mmol, 1 equiv) in dry DCM (0.5 mL). The mixture was stirred at room temperature for 4 h under an argon atmosphere. The reaction mixture was evaporated under reduced pressure to afford compound **6a** as a yellow oil (17 mg, 72%) in its trifluoroacetate salt form.

6-{2-[(1-(benzyl)-1*H*-1,2,3-triazol-4-yl)methoxy]ethyl}-1,4,8,11-tetraazacyclotetradecane (6a). This compound was obtained as a yellow powder (17 mg, 72%). ^{1}H NMR (500 MHz, D_2O, 25 °C) δ (ppm): 8.08 (s, 1H, C*H*$_{triazole}$), 7.46–7.33 (m, 5H, C*H*$_{Ar}$), 5.63 (s, 2H, C*H*$_2$ α-N$_{triazole}$), 4.63 (s, 2H, C*H*$_2$ α-C$_{triazole}$), 3.60 (t, ^{3}J = 6.0 Hz, 2H, CH$_2$-C*H*$_2$ α-O), 3.12–2.70 (m, 12H) (*C*H$_2$ α-N), 2.90–2.74 (m, 4H) (*C*H$_2$ α-N), 2.04 (m, 1H, C*H* β-N), 1.88 (m, 2H, C*H*$_2$ β-N), 1.59 (m, 2H, C*H*$_2$-CH$_2$ α-O). ^{13}C NMR (125 MHz, D_2O, 25 °C) δ (ppm): 144.0 (*C*$_{triazole}$), 134.8 (*C*$_{ispo}$ Ar), [129.0 (×2), 128.7, 128.0 (×2)] (*C*H$_{Ar}$), 125.1 (*C*H$_{triazole}$), 66.8 (CH$_2$-*C*H$_2$ α-O), 62.4 (*C*H$_2$ α-C$_{triazole}$), 53.7 (CH$_2$ α-N$_{triazole}$), [53.3 (×2), 49.0 (×2), 46.0 (×2), 45.8 (×2)] (*C*H$_2$ α-N), 32.1 (*C*H β-N), 29.4 (*C*H$_2$-CH$_2$ α-O), 24.4 (*C*H$_2$ β-N). HRMS (ESI): *m/z* calcd for $C_{22}H_{38}N_7O^+$ $[M+H]^+$ 416.3132, found: 416.3127 $[M+H]^+$, 326.2659 $[M+H - Bn]^+$, 208.6603 $[M+2H]^{2+}$.

6-{2-[(1-(4-aminobutyl)-1*H*-1,2,3-triazol-4-yl)methoxy]ethyl}-1,4,8,11-tetraazacyclotetradecane (6b). This compound was obtained as a yellow powder (17 mg, 94%). ^{1}H NMR (500 MHz, D_2O, 25 °C) δ (ppm): 7.93 (s, 1H, C*H*$_{triazole}$), 4.55 (s, 2H, C*H*$_2$ α-C$_{triazole}$), 4.37 (t, ^{3}J = 7.0 Hz, 2H, C*H*$_2$ α-N$_{triazole}$), 3.54 (t, ^{3}J = 6.0 Hz, 2H, CH$_2$-C*H*$_2$ α-O), 3.22–3.04 (m, 14H, C*H*$_2$ α-N), 2.94–2.87 (m, 4H, C*H*$_2$ α-NH$_2$ and C*H*$_2$ α-N), 2.14 (m, 1H, C*H* β-N$_{Boc}$), 1.95 (m, 2H, C*H*$_2$ β-N), 1.87 (m, 2H, C*H*$_2$ β-N$_{triazole}$), 1.61 (q, ^{3}J = 6.0 Hz, 2H, C*H*$_2$-CH$_2$ α-O), 1.53 (m, 2H, C*H*$_2$ β-NH$_2$). ^{13}C NMR (125 MHz, D_2O, 80 °C) δ (ppm): 146.9 (*C*$_{triazole}$), 127.7 (*C*H$_{triazole}$), 70.3 (CH$_2$-*C*H$_2$ α-O), 65.5 (*C*H$_2$ α-C$_{triazole}$), 55.6 (×2) (*C*H$_2$ α-N), 52.5 (CH$_2$ α-N$_{triazole}$), [50.2 (×2), 48.2 (×2), 47.9 (×2)] (*C*H$_2$ α-N), 41.8 (*C*H$_2$ α-NH$_2$), 35.1 (*C*H β-N), 32.6 (*C*H$_2$-CH$_2$ α-O), 29.0 (*C*H$_2$ β-N$_{triazole}$), 26.6 (*C*H$_2$ β-NH$_2$), 26.2 (*C*H$_2$ β-N). HRMS (ESI): *m/z* calcd for $C_{19}H_{41}N_8O^+$ $[M+H]^+$ 397.3398, found: 397.3394 $[M+H]^+$, 326.2657 $[M+H - BuNH_2]^+$, 245.2332 $[M+H - BuNH_2 - triazole - methyl]^+$, 199.1735 $[M+2H]^{2+}$.

6-{2-[(1-(11-amino-3,6,9-trioxaundecyl)-1*H*-1,2,3-triazol-4-yl)methoxy]ethyl}-1,4,8,11-tetraazacyclotetradecane (6c). This compound was obtained as a white powder (24 mg, 88%). ^{1}H NMR (500 MHz, D_2O, 25 °C) δ (ppm): 8.05 (s, 1H, C*H*$_{triazole}$), 4.65 (s, 2H, C*H*$_2$ α-C$_{triazole}$), 4.61 (t, ^{3}J = 5.0 Hz, 2H, C*H*$_2$ α-N$_{triazole}$), 3.94 (t, ^{3}J = 5.0 Hz, 2H, C*H*$_2$ β-N$_{triazole}$), 3.70 (t, ^{3}J = 5.1 Hz, 2H, CH$_2$-C*H*$_2$ α-O), 3.66–3.57 (m, 10H, C*H*$_2$ α-O$_{PEG}$), 3.49–3.22 (m, 14H, C*H*$_2$ α-N), 3.19-3.11 (m, 4H, C*H*$_2$ α-N and C*H*$_2$ α-NH$_2$), 2.39 (m, 1H, C*H* β-N), 2.14 (m, 2H, C*H*$_2$ β-N), 1.79 (m, 2H, C*H*$_2$-CH$_2$ α-O). ^{13}C NMR (125 MHz, D_2O, 80 °C) δ (ppm): 146.5 (*C*$_{triazole}$), 128.1 (*C*H$_{triazole}$), [72.4, 72.3 (×2), 72.2, 71.3] (*C*H$_2$ α-O$_{PEG}$), 70.0 (*C*H$_2$ β-N$_{triazole}$), 69.0 (CH$_2$-*C*H$_2$ α-O), 65.5 (*C*H$_2$ α-C$_{triazole}$), 53.7 (×2) (*C*H$_2$ α-N), 53.0 (*C*H$_2$ α-N$_{triazole}$), [47.7 (×2), 46.4 (×2), 46.0 (×2)] (*C*H$_2$ α-N), 42.0 (*C*H$_2$ α-NH$_2$), 34.2 (*C*H β-N), 32.5 (*C*H$_2$-CH$_2$ α-O), 24.5 (*C*H$_2$ β-N). HRMS (ESI): *m/z* calcd for $C_{23}H_{49}N_8O_4^+$ $[M+H]^+$ 501.3871, found: 501.3868 $[M+H]^+$, 523.3690 $[M+Na]^+$, 251.1974 $[M+2H]^{2+}$, 167.8006 $[M+3H]^{3+}$.

6-{2-[(1-(methylacetyl)-1*H*-1,2,3-triazol-4-yl)methoxy]ethyl}-1,4,8,11-tetraazacyclotetradecane (6d). This compound was obtained as a white powder (19 mg, 68%).

^{1}H NMR (500 MHz, D_2O, 25 °C) δ (ppm): 8.08 (s, 1H, C$H_{triazole}$), 5.41 (s, 2H, CH_2 α-$N_{triazole}$), 4.68 (s, 2H, CH_2 α-$C_{triazole}$), 3.80 (s, 1H, CH_3 α-CO_{ester}), 3.65 (t, ^{3}J = 5.9 Hz, 2H, CH_2-CH_2 α-O), 3.28–3.07 (m, 14H, CH_2 α-N), 3.01–2.93 (m, 2H, CH_2 α-N), 2.19 (m, 1H, CH β-N), 2.03 (m, 2H, CH_2 β-N), 1.70 (m, 2H, CH_2-CH_2 α-O). ^{13}C NMR (125 MHz, D_2O, 25 °C) δ (ppm): 170.0 (CO_{ester}), 144.8 ($C_{triazole}$), 126.7 ($CH_{triazole}$), 67.6 (CH_2-CH_2 α-O), 63.1 (CH_2 α-$C_{triazole}$), 53.8 (CH_3 α-CO_{ester}), 52.7 (×2) (CH_2 α-N), 51.2 (CH_2 α-$N_{triazole}$), [47.2 (×2), 45.3 (×2), 45.0 (×2)] (CH_2 α-N), 32.4 (CH β-N), 30.2 (CH_2-CH_2 α-O), 23.4 (CH_2 β-N). HRMS (ESI): *m/z* calcd for $C_{18}H_{36}N_7O_3^+$ $[M+H]^+$ 398.2874, found: 398.2872 $[M+H]^+$, 245.2337 [EtOH-cyclam+H]$^+$.

6-{2-[(1-(4-aminophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy]ethyl}-1,4,8,11-tetraazacyclotetradecane (6e). This compound was obtained as a yellow powder (24 mg, 90%). ^{1}H NMR (500 MHz, D_2O, 25 °C) δ (ppm): 8.38 (s, 1H, C$H_{triazole}$), 7.59 (d, ^{3}J = 8.8 Hz, 2H, CH_{Ar}-C α-$N_{triazole}$), 7.07 (d, ^{3}J = 8.8 Hz, 2H, CH_{Ar}-C α-NH_2), 4.72 (s, 2H, CH_2 α-$C_{triazole}$), 3.69 (t, ^{3}J = 6.2 Hz, 2H, CH_2-CH_2 α-O), 3.15–2.78 (m, 16H, CH_2 α-N), 2.09 (m, 1H, CH β-N), 1.88 (m, 2H, CH_2 β-N), 1.65 (m, 2H, CH_2-CH_2 α-O). ^{13}C NMR (125 MHz, D_2O, 25 °C) δ (ppm): 148.5 (CH_{Ar}-C α-NH_2), 147.1 ($C_{triazole}$), 132.2 (CH_{Ar}-C α-$N_{triazole}$), 126.5 ($CH_{triazole}$), 125.5 (CH_{Ar}-C α-$N_{triazole}$), 120.5 (CH_{Ar}-C α-NH_2), 70.0 (CH_2-CH_2 α-O), 65.5 (CH_2 α-$C_{triazole}$), [56.4 (×2), 52.0 (×2), 49.0 (×2), 48.8 (×2)] (CH_2 α-N), 35.2 (CH β-N), 32.5 (CH_2-CH_2 α-O), 27.4 (CH_2 β-N). HRMS (ESI): *m/z* calcd for $C_{21}H_{37}N_8O^+$ $[M+H]^+$ 417.3090, found: 417.3086 $[M+H]^+$.

6-{2-[(1-(decyl)-1*H*-1,2,3-triazol-4-yl)methoxy]ethyl}-1,4,8,11-tetraazacyclotetradecane (6f). This compound was obtained as a white powder (16 mg, 67%). ^{1}H NMR (500 MHz, D_2O, 25 °C) δ (ppm): 7.97 (s, 1H, C$H_{triazole}$), 4.60 (s, 2H, CH_2 α-$C_{triazole}$), 4.38 (t, ^{3}J = 7.0 Hz, 2H, CH_2 α-$N_{triazole}$), 3.58 (t, ^{3}J = 6.1 Hz, 2H, CH_2-CH_2 α-O), 3.19–2.82 (m, 16H, CH_2 α-N), 2.11 (m, 1H, CH β-N), 1.94 (m, 2H, CH_2 β-N), 1.84 (m, 2H, CH_2-CH_2 α-$N_{triazole}$), 1.60 (m, 2H, CH_2-CH_2 α-O), 1.27-1.13 (m, 14H, CH_2 decane), 0.80 (t, ^{3}J = 6.8 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, D_2O, 25 °C) δ (ppm): 146.6 ($C_{triazole}$), 127.4 ($CH_{triazole}$), 70.0 (CH_2-CH_2 α-O), 65.6 (CH_2 α-$C_{triazole}$), 55.9 (×2) (CH_2 α-N), 53.2 (CH_2 α-$N_{triazole}$), [51.0 (×2), 48.5 (×2), 48.2 (×2)] (CH_2 α-N), 35.1 (CH β-N), 34.4 (CH_2-CH_2 α-$N_{triazole}$), 32.6 (CH_2-CH_2 α-O), [32.5, 31.9, 31.8, 31.7, 31.4, 28.7] (CH_2 decane), 26.7 (CH_2 β-N), 25.1 (CH_2 decane), 16.4 (CH_3). HRMS (ESI): *m/z* calcd for $C_{25}H_{52}N_7O^+$ $[M+H]^+$ 466.4228, found: 466.4230 $[M+H]^+$, 245.2342 [M+H –], 233.7151 $[M+2H]^{2+}$.

6-{2-[(1-(2-(β-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy]ethyl}-1,4,8,11-tetraazacyclotetradecane (6g). To a solution of compound **5g** (21 mg, 0.019 mmol, 1 equiv) in dry DCM (0.5 mL) was added TFA (0.5 mL). The reaction mixture was stirred at room temperature for 4 h under argon atmosphere. The reaction mixture was evaporated under reduced pressure. The residue was dissolved in MeOH (0.5 mL) and MeONa (4.1 mg, 0.076 mmol, 4 equiv) was added. The mixture was stirred at room temperature for 18 h and then evaporated under reduced pressure. The salts were removed using a Sep-Pak® C18 cartridge to afford compound **6g** as an off-white powder (5 mg, 53% on 2 steps). ^{1}H NMR (500 MHz, D_2O, 25 °C) δ (ppm): 8.12 (s, 1H, C$H_{triazole}$), 4.71 (t, ^{3}J = 5.1 Hz, 2H, CH_2 α-$N_{triazole}$), 4.67 (s, 2H, CH_2 α-$C_{triazole}$), 4.44 (d, ^{3}J = 7.9 Hz, 1H, C_1H), [4.32, 4.13] (m, 2H, $N_{triazole}$-CH_2-CH_2 α-O), 3.89 (dd, ^{3}J = 12.3 Hz , ^{3}J = 2.3 Hz, 1H, C_6H_{2a}), 3.73–3.68 (m, 1H, C_6H_{2b}), 3.66 (t, ^{3}J = 6.2 Hz, 2H, CH_2-CH_2 α-O), 3.49–3.41 (m, 2H, C_5H and C_3H), 3.35 (m, 1H, C_4H), 3.23 (dd, ^{3}J = 9.4 Hz , ^{3}J = 7.9 Hz, 1H, C_2H), 3.16-2.80 (m, 16H, CH_2 α-N), 2.09 (m, 1H, CH β-N), 1.89 (m, 2H, CH_2 β-

N), 1.63 (m, 2H, CH_2-CH$_2$ α-O). ^{13}C NMR (125 MHz, D$_2$O, 25 °C) δ (ppm): 143.7 ($C_{triazole}$), 125.8 ($CH_{triazole}$), 102.4 (C_1), 75.9 (C_3), 75.6 (C_5), 72.9 (C_2), 69.5 (C_4), 68.0 ($N_{triazole}$-CH$_2$-CH$_2$ α-O), 67.2 (CH$_2$-CH$_2$ α-O), 62.6 (CH$_2$ α-$C_{triazole}$), 60.6 (C_6), 53.6 (×2) (CH$_2$ α-N), 50.3 (CH$_2$ α-$N_{triazole}$), [49.1 (×2), 46.2 (×2), 46.0 (×2)] (CH$_2$ α-N), 32.3 (CH β-N), 29.7 (CH$_2$-CH$_2$ α-O), 24.5 (CH$_2$ β-N). HRMS (ESI): *m/z* calcd for $C_{23}H_{46}N_7O_7^+$ [M+H]$^+$ 532.3453, found: 532.3457 [M+H]$^+$, 370.2930 [M+H – glucose]$^+$, 185.6503 [M+2H – glucose]$^{2+}$.

6-{2-[(1-(11-((+)-biotinylamino)-3,6,9-trioxaundecyl)-1*H*-1,2,3-triazol-4-yl)methoxy]ethyl}-1,4,8,11-tetraazacyclotetradecane (6h). This compound was obtained as a yellow powder (41 mg, 79%). ^{1}H NMR (500 MHz, D$_2$O, 25 °C) δ (ppm): 8.10 (s, 1H, C$H_{triazole}$), 4.65 (m, 4H, CH_2 α-$C_{triazole}$ + CH_2 α-$N_{triazole}$), 4.59 (m, 1H, CH_g), 4.42 (m, 1H, CH_f), 3.99 (t, ^3J = 5.0 Hz, 2H, CH_2 β-$N_{triazole}$), 3.70–3.58 (m, 14H, CH_2 α-N_{Boc} + 5 CH_2 α-O_{PEG} + CH$_2$-CH_2 α-O), 3.38 (t, ^3J = 5.3 Hz, 2H, CH_2 α-N amide), 3.32 (m, 1H, CH_d), 3.17–2.96 (m, 12H, CH_2 α-N_{Boc}), 2.94 (m, 1H, CH_{2e}), 2.91–2.82 (CH_2 α-N_{Boc}), 2.77 (m, 1H, CH_{2e}), 2.26 (t, ^3J = 7.3 Hz, 2H, CH_2 α-CO amide), 2.09 (m, 1H, CH β-N_{Boc}), 1.89 (m, 2H, CH_2 β-N_{Boc}), 1.77–1.52 (m, 6H, CH_2-CH$_2$ α-O + CH_{2a} + CH_{2b}), 1.41 (m, 2H, CH_{2c}). ^{13}C NMR (125 MHz, D$_2$O, 25 °C) δ (ppm): 176.8 (*C*O amide), 165.1 (*C*O urea), 142.8 ($C_{triazole}$), 125.6 ($CH_{triazole}$), [69.5, 69.4, 69.3 (×2), 68.6] (CH$_2$ α-O_{PEG}), 68.3 (CH_2 β-$N_{triazole}$), 66.9 (CH$_2$-CH_2 α-O), 62.5 (CH_2 α-$C_{triazole}$), 62.0 (C_g), 60.2 (C_f), 55.2 (C_d), 50.5 (CH$_2$ α-$N_{triazole}$), [46.6 (×2), 41.5 (×2), 39.8 (×2)] (CH$_2$ α-N_{Boc}), 39.5 (CH_{2e}), 39.1 (×2) (CH$_2$ α-N_{Boc}), 38.8 (CH$_2$ α-N amide), 35.2 (CH$_2$ α-CO amide), 29.5 (CH$_2$-CH$_2$ α-O), 28.7 (CH β-N_{Boc}), [27.7, 27.6] (C_a + C_c), 25.0 (C_b), 18.8 (CH$_2$ β-N_{Boc}). HRMS (ESI): *m/z* calcd for $C_{33}H_{63}N_{10}O_6S^+$ [M+H]$^+$ 727.4647, found: 727.4641 [M+H]$^+$, 364.2357 [M+2H]$^{2+}$, 243.1600 [M+3H]$^{3+}$.

6-{2-[(1-(pyrenylmethyl)-1*H*-1,2,3-triazol-4-yl)methoxy]ethyl}-1,4,8,11-tetraazacyclotetradecane (6i). This compound was obtained as a yellow powder (34 mg, 89%). ^{1}H NMR (500 MHz, D$_2$O, 25 °C) δ (ppm): 7.60 (s, 1H, C$H_{triazole}$), [7.36 (d, ^3J = 9.1 Hz, 1H), 7.31–7.07 (m, 6H), 6.93 (m, 2H)] (CH_{Ar} pyrene), 5.46 (s, 2H, CH_2 α-$N_{triazole}$), 4.24 (s, 2H, CH_2 α-$C_{triazole}$), 3.19 (t, ^3J = 5.6 Hz, 2H, CH$_2$-CH_2 α-O), [2.77–2.46 (m, 12H), 2.37 (m, 2H), 2.19 (t, ^3J = 11.3 Hz, 2H)] (CH_2 α -N_{Boc}), 1.70–1.54 (m, 3H, CH_2 β-N_{Boc} + CH β-N_{Boc}), 1.48–1.11 (m, 2H, CH_2-CH$_2$ α-O). ^{13}C NMR (125 MHz, D$_2$O, 25 °C) δ (ppm): 143.9 (C_{ipso} triazole), [130.3, 129.7, 129.2, 127.4, 126.6, 122.9, 122.6] (C_{sp2}), [127.5, 126.9, 126.8, 126.2, 125.5, 124.8 (×2), 124.2 (×2)] (CH_{Ar}), 121.0 (*C*H triazole), 66.9 (CH$_2$-CH$_2$ α-O), 62.4 (CH$_2$ α-$C_{triazole}$), 53.0 (×2) (CH$_2$ α-N_{Boc}), 50.9 (CH$_2$ α-$N_{triazole}$), [48.6 (×2), 45.5 (×2), 45.4 (×2)] (CH$_2$ α-N_{Boc}), 31.7 (CH β-N_{Boc}), 29.3 (CH$_2$-CH$_2$ α-O), 24.0 (CH$_2$ β-N_{Boc}). HRMS (ESI): *m/z* calcd for $C_{32}H_{42}N_7O^+$ [M+H]$^+$ 540.3445, found: 540.3454 [M+H]$^+$, 326.2663 [M+H – pyrene]$^+$.

6,6'-Bis[*O*-[2-(3,6,10,13-tetrakis(*tert*-butoxycarbonyl)-3,6,10,13-tetraaza-cyclotetradecanyl)ethyl]methyl-1*H*-1,2,3-triazol-1-yl]-6,6'-dideoxy-2,3,2',3'-tetra-*O*-hexanoyl]-α,α'-trehalose (8). To a solution of **7** (78 mg, 0.080 mmol) and tetraBoc cyclam derivative **3** (120 mg, 0.18 mmol) in a H$_2$O-^{t}BuOH (3:1) solution (8 mL) the Cu-supported catalyst Si-BPA·Cu$^+$ (5.4 mg) was added and the reaction mixture was refluxed at 110 ºC overnight. The reaction mixture was diluted with DCM (8 mL), the catalyst was filtered, the organic phase was separated, dried (MgSO$_4$), filtered and the concentrated. The residue was purified by column chromatography (1:2 → 4:1 EtOAc/Cyclohexane) to give **8** with 38% yield (72 mg) as a white powder. R_f = 0.57 (2:1 EtOAc-cyclohexane). $[\alpha]_D$ = +40.9 (*c* 1.0, DCM). IR: ν_{max} = 2957, 2932, 2864, 1746, 1687, 1156 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$, 298 K): δ =

7.57 (s, 2 H, =CH), 5.47 (t, 2 H, $J_{2,3} = J_{3,4} = 10.1$ Hz, H-3), 4.95 (dd, 2 H, $J_{1,2} = 3.8$ Hz, H-2), 4.86 (t, 2 H, $J_{4,5} = 10.1$ Hz, H-4), 4.85 (d, 2 H, H-1), 4.56 (s, 4 H, CH_2 triazole), 4.46 (bd, 2 H, $J_{6a,6b} = 12.6$ Hz, H-6a), 4.30 (dd, 2 H, $J_{5,6b} = 8.2$ Hz, H-6b), 4.22 (dd, 2 H, H-5), 3.76-2.88 (m, 32 H, cyclam), 3.53 (t, 4 H, $^3J_{H,H} = 6.3$ Hz, CH_2O), 2.39-2.16 (m, 12 H, H-2_{Hex}), 1.93 (m, 2 H, cyclam), 1.71 (m, 4 H, cyclam), 1.65-1.50 (m, 16 H, CH_2CH_2O, H-3_{Hex}), 1.44 (bs, 54 H, Cme_3), 1.32-1.23 (m, 24 H, H-4_{Hex}, H-5_{Hex}), 0.91-0.86 (m, 18 H, H-6_{Hex}). ^{13}C NMR (75.5 MHz, $CDCl_3$, 298 K): δ = 172.4, 172.2, 172.1 (CO ester), 155.9, 155.6 (CO carbamate), 145.3 (C-4 triazole), 123.8 (C-5 triazole), 91.5 (C-1), 79.8, 79.7 (*C*me_3), 69.3 (C-3, C-4), 68.9 (C-2), 68.7 (C-5), 68.2 (CH_2O), 64.0 (*C*H_2 triazole), 53.4, 52.1 (cyclam), 50.4 (C-6), 48.2, 46.7, 35.4 (cyclam), 33.9, 33.7 (C-2_{Hex}), 31.2 (C-4_{Hex}), 29.9 (*C*H_2CH_2O), 29.6, 29.3 (cyclam), 28.4 (Cme_3), 28.2 (cyclam), 24.4, 24.3 (C-3_{Hex}), 22.2 (C-5_{Hex}), 13.8 (C-6_{Hex}). ESI-MS: *m/z* = 2369.3 $[M + Na]^+$, 1196.3 $[M + 2Na]^{2+}$. Anal. Calcd for $C_{118}H_{204}N_{14}O_{33}$: C, 60.39; H, 8.76; N, 8.36; found: C, 60.46; H, 8.83; N, 8.09.

6,6'-Bis[*O*-[2-(3,6,10,13-tetrahydrochloride-3,6,10,13-tetraaza-cyclotetradecanyl)ethyl]methyl-1*H*-1,2,3-triazol-1-yl]-6,6'-dideoxy-2,3,2',3'-tetra-*O*-hexanoyl]-α,α'-trehalose (9). Compound **8** (70 mg, 0.030 mmol) was treated with a DCM-TFA (1:1) solution (4 mL) at rt for 30 min. Then the solvent was removed under reduced pressure and co-evaporated several times with water. The residue was dissolved in H_2O-HCl 0.1N (10:1) and freeze-dried to yield **9** as its hydrochloride salt with 100% yield (55 mg) as a white solid. $[\alpha]_D$ = +382.3 (*c* 1.0, DCM). IR: ν_{max} = 2962, 2932, 2859, 1751, 1673, 1126 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD, 298 K): δ = 7.96 (s, 2 H, =CH), 5.50 (t, 2 H, $J_{2,3} = J_{3,4} = 9.9$ Hz, H-3), 5.05 (dd, 2 H, $J_{1,2} = 3.7$ Hz, H-2), 4.99 (t, 2 H, $J_{4,5} = 9.9$ Hz, H-4), 4.92 (d, 2 H, H-1), 4.63 (dd, 2 H, $J_{6a,6b} = 14.9$ Hz, $J_{5,6a} = 2.8$ Hz, H-6a), 4.60 (s, 4 H, CH_2 triazole) 4.56 (dd, 2 H, $J_{5,6b}$ = 7.8 Hz, H-6b), 4.30 (ddd, 2 H, H-5), 3.61 (t, 4 H, $^3J_{H,H} = 5.9$ Hz, CH_2O), 3.19-2.87 (m, 32 H, cyclam), 2.46-2.24 (m, 12 H, H-2_{Hex}), 2.19 (m, 2 H, cyclam), 1.94 (m, 4 H, cyclam), 1.67-1.54 (m, 16 H, CH_2CH_2O, H-3_{Hex}), 1.42-1.29 (m, 24 H, H-4_{Hex}, H-5_{Hex}), 0.97-0.92 (m, 18 H, H-6_{Hex}). ^{13}C NMR (100.6 MHz, CD_3OD, 298 K): δ = 174.0, 173.7, 173.4 (CO ester), 145.9 (C-4 triazole), 126.4 (C-5 triazole), 92.3 (C-1), 71.4 (C-3), 70.6 (C-4), 70.2 (C-2, C-5), 68.6 (CH_2O), 64.5 (*C*H_2 triazole), 54.9 (cyclam), 51.4 (C-6), 50.1, 47.5, 47.3 (cyclam), 34.9, 34.8 (C-2_{Hex}), 34.4 (cyclam), 32.5, 32.4 (C-4_{Hex}), 31.7 (*C*H_2CH_2O), 26.0 (cyclam), 25.6, 25.5, 25.4 (C-3_{Hex}), 23.4 (C-5_{Hex}), 14.3, 14.2 (C-6_{Hex}). ESI-MS: *m/z* = 773.9 $[M - 6H - 8Cl]^{2+}$. Anal. Calcd for $C_{78}H_{148}Cl_8N_{14}O_{17}$: C, 50.98; H, 8.12; N, 10.67; found: C, 50.81; H, 7.93; N, 10.32.

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Acknowlegements

R.T. and N.L.B. acknowledge the Ministère de l'Enseignement Supérieur et de la Recherche, and the Centre National de la Recherche Scientifique. C.O. is grateful to the Région Bretagne, the Ministère de l'Enseignement Supérieur et de la Recherche, and the MAC-group (UBO). We acknowledge the Ministerio de Ciencia, Innovación y Universidades and the Agencia Estatal de Investigación (projects RTI2018-097609-B-C21 and RTI2018-097609-B-C22), AEI/10.13039/501100011033 and ''ERDF A way of making Europe'' (PID2021-124247OB-C21 and PID2022-141034OB-C21) and the COST action GLYCONanoPROBES (CM18132). J.M.G.F. acknowledges funding by the European Union's Horizon Europe research and innovation program under the Marie Skłodowska-Curie grant agreement 101130235-Bicyclos. This work was supported by Grant RYC2021-034263-I (A. M.-A.), founded by the Ministry of Science and Innovation/State Research Agency/10.13039/501100011033 and the European Union "NextGenerationEU/Recovery, Transformation and Resilience Plan" The CITIUS (University of Seville) is also acknowledged for technical support.

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