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Regio-selective synthesis of polyazacyclophanes incorporating a pendant group as potential cleaving agents of mRNA 5′-*cap* structure

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Abstract—A terpyridine or an imidazole unit has been tethered to an *N*-protected polyazacyclophane to give the appropriate *N*-monofunctionalized polyazacyclophane. After mild deprotection, four polyazacyclophanes incorporating a pendant group were obtained in satisfactory yields. Their preliminary cleavage ability of mRNA 5′-*cap* model was studied at pH 7.2.
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1. Introduction

Recently we have been very interested in finding efficient catalysts for the cleavage of 5′-*cap* structure and evaluating their potential as constituents of artificial nucleases targeted towards the 5′-*cap* moiety of mRNA. The 5′-*cap* structure contains a unique dinucleoside 5′,5′-triphosphate moiety at its 5′-terminus, where one of the nucleosides is generally *N*7-methylguanosine (Fig. 1) and which plays an important role in the process of mRNA metabolism.[1–3] Chemical modifications of the *cap* structure retard the biosynthesis of the respective proteins[4–6] by lowering the affinity of the mRNA to ribosomes. Therefore, the 5′-*cap* structure is a potential target of artificial nucleases, chemical catalysts that sequence-selectively cleave intracellular RNA molecules.[7–9] Our previous study has shown that some polyazamacrocycles can cleave the triphosphate bridge and open the imidazole rings of the 5′-*cap* structure at physiological pH,[10,11] although at a lower rate compared with the hydrolysis of ATP by *O*-bisdien.[12] We also found that at 25 °C monosubstituted polyazacyclophanes enhance the cleavage of the mRNA 5′-*cap* model with the heteroaromatic pendant group playing an important role.[13]

Terpyridine and its derivatives are well-established chelates for transition metals, particularly the d^8 metals that form square planar complexes,[14] which can be used as cleavage agents and diagnostic agents in radiopharmacy. Bipyridine and terpyridine complexes of metal ions have also played an important role in the development of RNA hydrolysis agents.[15,16] Particularly, the Cu(II) complex of terpyridine was found to cleave RNA hydrolytically and is also efficient in the hydrolysis of the ApppA.[17] Terpyridine ethers, thioethers and related compounds have been found to play a prominent role in the production of sequence-specific RNA cleavage reagents.[18,19] Bashkin et al. have achieved sequence-specific hydrolytic cleavage of target RNA,[20] by linking bipyridine and terpyridine moieties to molecular recognition elements. The imidazole and its derivatives are also well-established nucleophiles, which can be used as cleavage agents at physiological conditions and have also played an important role in the development of RNA hydrolysis agents. Resulting from our efforts to increase the effectiveness of our cleavage agents, we sought to introduce the useful terpyridine and imidazole units into our macrocyclic systems to get more efficient cleavage ability. In this study, we designed and synthesized the terpyridine-containing macrocyclic polyamines **12a** and **12b** and two imidazole-containing macrocyclic polyamines **14a** and **14b**. The appealing feature of these monofunctionalized polyazamacrocycles is the tethered terpyridine or imidazole moiety,

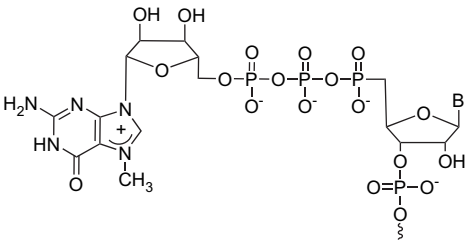

Figure 1. Cap structure.

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which is expected to introduce more efficient hydrolytic activity through the stronger aqueous metal complexes or extra nucleophiles, and the polyazamacrocyclophane, which is used as a vehicle for carrying the pendant residues close to the target sites with its hydrogen bonding ability to the triphosphate bridge of 5′-*cap* structure at physiological pH.

Although there are some successful examples[21–28] of the synthesis of monofunctionalized polyazamacrocycles, methods to synthesize monofunctionalized medium- to large-size polyazamacrocycles are still limited and the number of side arms introduced to the macrocyclic polyamines is also quite limited. In this report, a general synthetic method for *N*-monofunctionalized large polyazamacrocycles is established.

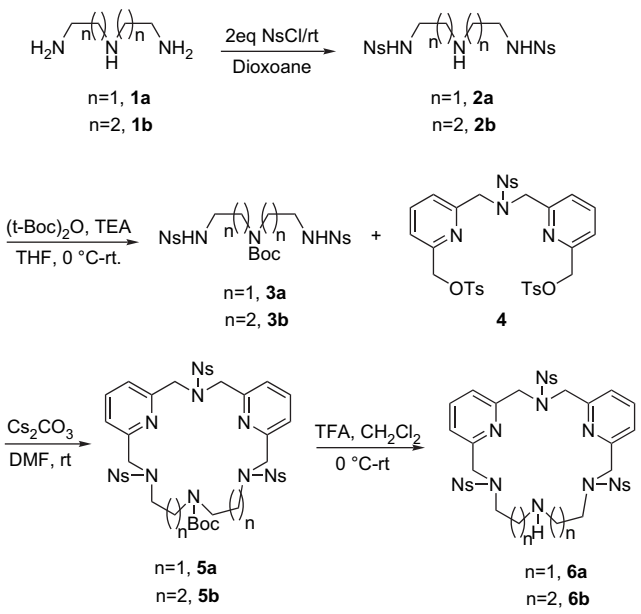
2. Results and discussion

The ligands **12a** and **12b** were obtained by regio-selective coupling of the terpyridine moiety to the polyazamacrocyclophane through conventional peptide coupling.[29] On the other hand, ligands **14a** and **14b** were obtained by regio-selective coupling of the imidazole to the polyazamacrocyclophane through conventional aldehyde reductive reaction.[30–33] For the macrocyclic polyamine synthesis we used the recently reported Ns strategy[34] and the cyclization method reported in our previous work.[13] The 2-nitrobenzenesulfonyl (Ns) groups acidify the amide proton similarly to tosyl protection, but can be removed easily with a soft nucleophile, thiophenol, in the presence of K_2CO_3 via a Meisenheimer complex at room temperature. Since the Ns protecting group is compatible with many other commonly used protecting groups, such as Boc, Bn, PMBz and Cbz,[35,36] it should also be compatible with the amide bond in our compounds.

In our previous study,[13] the trifluoroacetyl group has been used as a temporary primary amine protecting group versus secondary amines.[37] The alkylation of the secondary amine was then achieved with *tert*-butyl acetate, to afford the corresponding aminoalkanoates in high yields (90%). Then the Tf-deprotected intermediates were further reacted with NsCl to generate the corresponding dinosylated compounds **3a** and **3b**. The selectively protected asymmetric polyamines **3a** and **3b** were prepared in about 50% yield from the starting polyamine substrates.

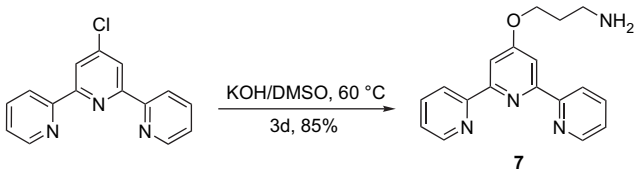
However, this route is rather tedious considering the selective protection and deprotection. According to the literature,[38,39] differentiation of primary and secondary amines of polyamines can be achieved by controlling the quantity of 2-nitrophenylsulfonyl chloride in $NaHCO_3$ basic conditions. This method was tried, but the yield of the selectively *N*-diprotected amine **2** is not as satisfactory as reported and the reaction mixture is rather complicated. Improvement of this synthetic route has been achieved by direct reaction of the polyamine with 2 equiv of nosyl chloride in dioxane without addition of any base. The idea is that the generated HCl selectively blocks the secondary amine or the unreacted polyamines to provide the selectivity between primary and secondary amines. This reaction took place at room temperature and the nosylated compounds precipitated out during the reaction. After workup and purification through silica gel chromatography, the selectively protected polyamine **2** was obtained in about 80% yield, with 10% totally protected polyamine. This method is rather direct, although the purification is not easy because of the low solubility of the mixture in common solvents, such as dichloromethane, acetone and methanol. In practice, the nosyl-protected mixture can be used for the next reaction directly without purification. After the mixture was reacted with $(t\text{-Boc})_2O$, the purification of compounds **3a** and **3b** is easy and they can be obtained in high yields.

Cyclization of **4**, which was easily prepared according to our previous reported method[13] with triamines **3a** and **3b** (Scheme 1), was accomplished at room temperature overnight in the presence of anhydrous Cs_2CO_3 as a base, with the Cs^+ cation serving as a cyclization template. The corresponding fully protected macrocycles, **5a** and **5b**, were obtained in 80% yields. Subsequently, the *t*-Boc protecting group was selectively removed by trifluoroacetic acid (TFA) at room temperature[40] and the intermediates, **6a** and **6b**, were obtained in almost quantitative yields.



Scheme 1.

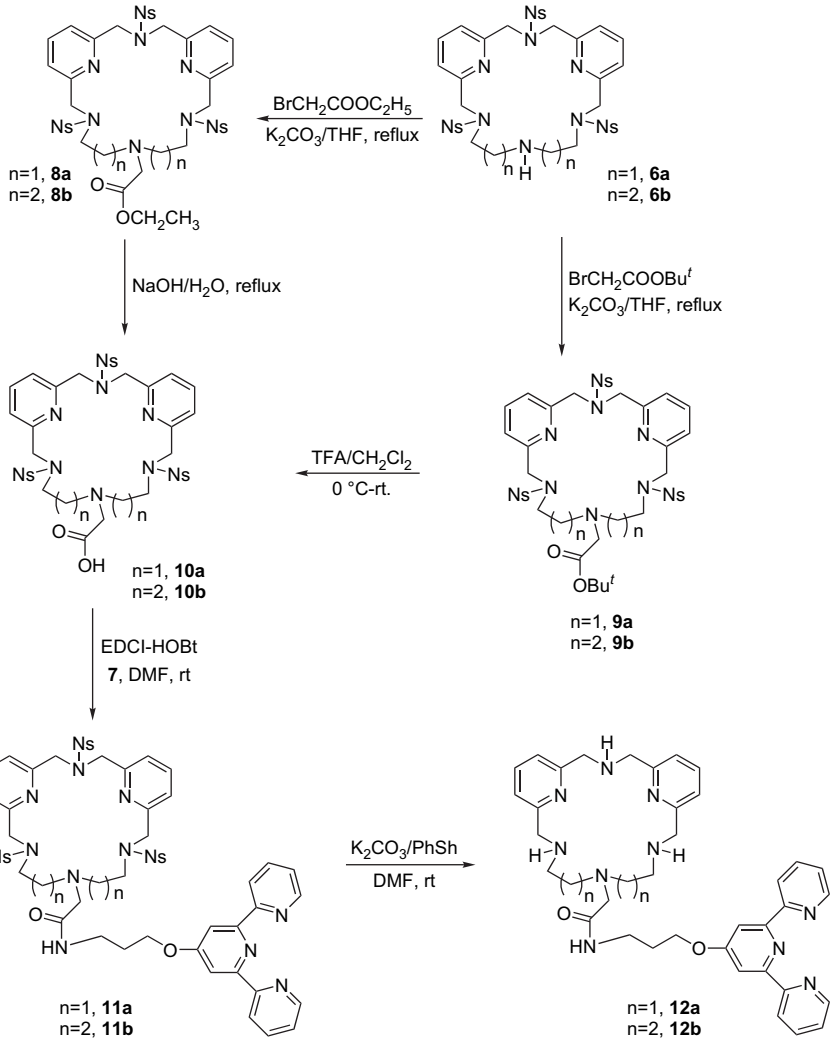
The amino-functionalized terpyridine **7** was obtained by the addition of 3-amino-1-propanol to a KOH/DMSO suspension, and the subsequent addition of 4′-chloro-2,2′:6,2″-terpyridine according to the literature method.[41] After purification, compound **7** was obtained in 90% yield (Scheme 2).



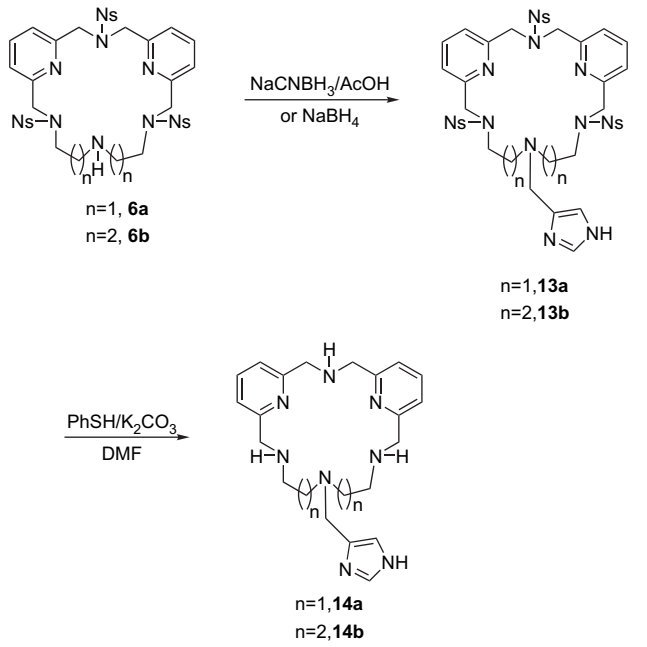
Scheme 2.

To conjugate this terpyridine building block **7** to the selectively deprotected cyclophane by an amide bond, the secondary amino group of **6a** and **6b** was derivatized with a carboxyethyl group to afford the acids **10a** and **10b** (Scheme 3). First, we attempted to prepare the two acids **10a** and **10b** through the basic hydrolysis of the corresponding ethyl esters **8a** and **8b**, which are easily prepared from the corresponding ethyl bromoacetate. Unfortunately, hydrolysis of the ethyl esters under basic condition is a very slow and complicated reaction and the yields of **8a** and **8b** are only around 20%. The Ns protecting group decomposed under longer exposure to basic conditions. Then, the *tert*-butyl esters were prepared, because they were expected to be hydrolyzed more easily and in high yield using trifluroacetic acid.[42] In the similar reaction conditions as for the preparation of **8a** and **8b**, the corresponding *tert*-butyl esters were obtained in high yields. Finally, 50% trifluoroacetic acid in CH_2Cl_2 was found to be an optimal condition to cleave the *tert*-butyl ester at 0 °C to room temperature, and under these conditions the expected acids **10a** and **10b** were obtained in almost quantitative yield. Lower concentrations of TFA would delay the reaction and cause incomplete cleavage. In the following process, acids **10a** and **10b** were reacted with the amine-functionalized terpyridine **7** to form the amide bond. Under amide bond coupling DCC/HOBt condition,[43] amides **11a** and **11b** were obtained in good yields. Then after the monofunctionalization of the polyazamacrocycles, K_2CO_3 and PhSH were used to remove the Ns protecting groups in DMF at room temperature overnight. After the workup and purification, the desired products **12a** and **12b** were obtained in good yields. According to our best knowledge this is the first time the terpyridine moiety has been introduced to polyazamacrocycles through a *N*-monofunctionalized side arm. This established synthetic method is practical and reproducible.

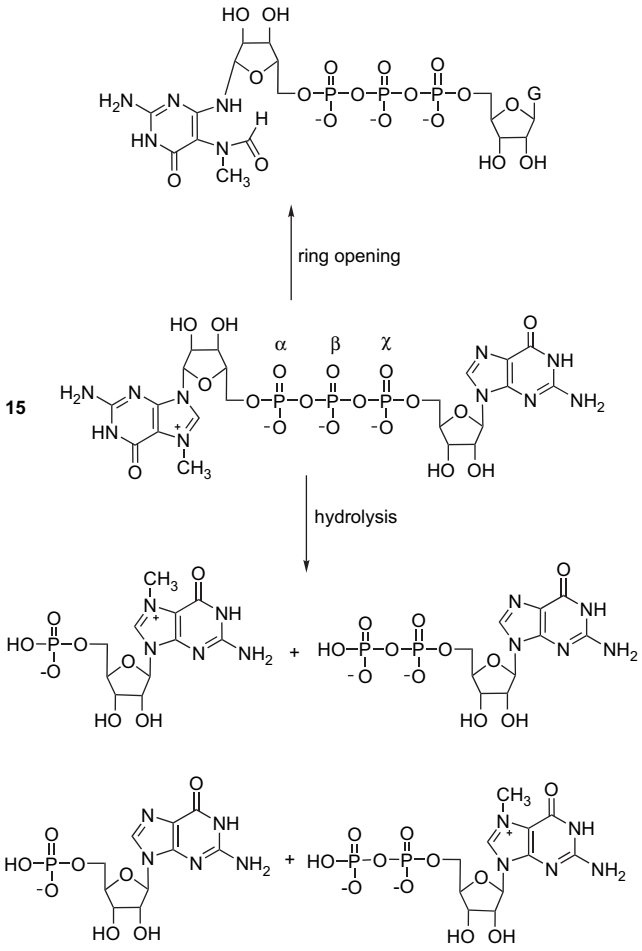
In order to introduce the imidazole functional group to our macrocycles, different conditions of reductive amination of 4-imidazolecarbaldehyde with macrocycles **6a** and **6b** were tried.[30–33] Finally, $NaCNBH_3$[31] with 2 equiv of AcOH and methanol as a solvent[32] was found to be an effective reductive method, and products **13a** and **13b** were obtained in 85% yield after purification (Scheme 4). On using $NaBH_4$ in situ for the reduction,[33] compounds **13a** and **13b** were obtained in about 90% yield. After monofunctionalization of the polyazamacrocycles, K_2CO_3 and PhSH were used to remove the Ns groups in DMF at room temperature overnight. Then the solvent was evaporated under high vacuum and the residue was purified directly on silica gel, using first dichloromethane, then methanol and finally



Scheme 3.



Scheme 4.



Scheme 5.

methanolic ammonia as an eluent. After workup and purification, the desired products **14a** and **14b** were obtained in good yields.

With these four monofunctionalized polyazamacrocycles in hand, their preliminary cleavage of mRNA 5′-*cap* model (m^7GpppG, **15**) was studied in the presence of the synthesized free ligands and their Zn^{2+} and Cu^{2+} complexes at pH 7.2. At this pH, the macrocycles are partly protonated, and can efficiently bind with the triphosphate bridge through electrostatic interactions. The concentration of m^7GpppG was kept below 50 μM to maintain pseudo first-order conditions. Reactions were carried out in stoppered tubes in a water bath thermostated to 60.0±0.1 °C or 25±0.1 °C, and 10–15 aliquots were withdrawn to cover approximately two half-lives of total cleavage. Samples taken from reaction solutions were analyzed by capillary zone electrophoresis (CZE).[13] Two different reactions were observed to take place under the experimental conditions, the hydrolysis of the triphosphate bridge of m^7GpppG and the cleavage of the 7-methylguanosine base (Scheme 5). In the presence of the ligands only, the base cleavage predominates, whereas metal ion complexes enhance both the phosphate and base cleavage. Even though the rate-enhancements observed are modest, the results are promising in respect of the design of artificial nucleases targeted against the 5′-*cap* structure.

In conclusion, four polyazacyclophanes incorporating two pyridine units and a heteroaromatic pendant group have been synthesized using an *o*-nosyl group as an orthogonal protecting group and an efficient method for monofunctionalization has been established. Notable features of this method are the remarkable cyclization efficiency, mild reaction conditions, high yields and compatibility with the functionalities introduced. The synthetic ligands can be used as cleavage agents to destroy the mRNA 5′-*cap* structure through attacking either the phosphate or the positively charged base of mRNA 5′-*cap* structure. Further investigations of the cleavage ability are needed.

3. Experimental section

3.1. General

The reagents used in the synthetic work were products of Aldrich and Lab Scan, and they were used as supplied unless otherwise stated. DMF, CH_2Cl_2 and MeCN were distilled from CaH_2. Acetone was dried on 4 Å molecular sieves and distilled. THF was distilled from Na/benzophenone under nitrogen. All solvents were stored over 4 Å molecular sieves. TLC was carried out on Merck silica gel 60 F254 aluminium-backed plates. Air- and moisture-sensitive reactions were carried out under N_2 using oven-dried glassware and standard syringe/septa techniques. The ^{1}H NMR spectra were recorded on a JEOL JNM-GX 400 or Bruker 200 NMR magnetic resonance spectrometer in D_2O or $CDCl_3$. The chemical shifts are given as parts per million from Me_4Si, used as an external standard. The high-resolution mass spectroscopic data were obtained on a 7070E VG mass spectrometer.

3-(*tert*-Butoxycarbonyl)-N^1,N^5-bis(2-nitrobenzenesulfonyl)-3-azapentane-1,5-diamine (**3a**), 3-(*tert*-butoxycarbonyl)-N^1,N^7-bis(2-nitrobenzenesulfonyl)-4-azaheptane-1,7-diamine

(**3b**), *N*,*N*-bis{[6-(tosyloxymethyl)pyridin-2-yl]methyl}-2-nitrobenzenesulfonamide (**4**), 10-(*tert*-butoxycarbonyl)-3,7,13-tris(2-nitrobenzenesulfonyl)-1,5(2,6)-dipyridina-3,7,10,13-tetraazacyclotetradecaphane (**5a**), 11-(*tert*-butoxycarbonyl)-3,7,15-tris(2-nitrobenzenesulfonyl)-1,5(2,6)-dipyridina-3,7,11,15-tetraazacyclohexadecaphane (**5b**), 3,7,13-tris(2-nitrobenzenesulfonyl)-1,5(2,6)-dipyridina-3,7,10,13-tetraazacyclotetradecaphane (**6a**) and 3,7,15-tris(2-nitrobenzenesulfonyl)-1,5(2,6)-dipyridina-3,7,11,15-tetraazacyclohexadecaphane (**6b**) were synthesized as described previously.[13] m^7GpppG, required as a substrate in the kinetic studies, was synthesized according to a method described in the literature.[44]

3.2. 1,7-Bis(2-nitrophenylsulfonyl)-1,4,7-triazaheptane (2a)

A solution of 2-nitrophenylsulfonyl chloride (8.86 g, 40 mmol) was added dropwise to a solution of diethylenetriamine (2.06 g, 20 mmol) in dioxane (80 ml). The reaction mixture was stirred at room temperature overnight. The solvent was evaporated and H_2O (20 ml) was added. The mixture was extracted several times with large volume of CH_2Cl_2. The organic phase was combined and dried with anhydrous Na_2SO_4. After evaporation, the residue was purified by silica gel chromatography (CH_2Cl_2/CH_3OH=20:1) to afford 7.6 g of compound **1** as white solid. ^{1}H NMR (DMSO-d_6): 8.04–8.08 (m, 2H), 7.90–7.95 (m, 2H), 7.81–7.85 (m, 4H), 2.88 (t, 4H, *J*=6.4 Hz), 2.48 (t, 4H, *J*=6.4 Hz); ^{13}C NMR (DMSO-d_6): 43.10, 48.24, 124.75, 130.00, 133.02, 133.21, 134.39, 148.20. MS (FAB$^+$): 474 (M+1, 100%), 289 (M−Ns, 25%). In a similar way, compound **2b** can be obtained and used for the next reaction without purification. The spectral data obtained are consistent with those reported in the literature.[38,39]

3.3. 3-Aminopropanyl 4′-(2,2′:6′,2″-terpyridinyl)ether (7)

To a suspension of powdered KOH in dry DMSO, 3-aminopropan-1-ol was added. The suspension was stirred at 60 °C for 1 h, and then 4′-terpyridine was added. The mixture was stirred at 60 °C for another three days. After cooling to room temperature, the mixture was evaporated. Water was added to the residue, and CH_2Cl_2 was used to extract the mixture several times. The organic phase was combined and dried with anhydrous Na_2SO_4, then concentrated under vacuum. The residue was column chromatographed eluting with 15% MeOH in CH_2Cl_2 to give **7** in 90% yield as a slight yellow solid. ^{1}H NMR ($CDCl_3$): 9.09 (d, 2H, *J*=4.4 Hz), 8.99 (d, 2H, *J*=8 Hz), 8.41 (s, 2H), 8.25–8.21 (m, 2H), 7.74–7.71 (m, 2H), 4.71 (t, 2H, *J*=5.6 Hz), 4.45–4.22 (m, 2H), 3.45–3.42 (m, 2H); ^{13}C NMR ($CDCl_3$): 166.7, 156.67, 155.6, 148.7, 136.5, 123.6, 120.9, 107.0, 65.7, 38.3, 31.2. MS: 306 (M, 15%), 276 (M−CH_2NH_2, 40%), 249 ((M+H)−$(CH_2)_3NH_2$, 90%). HRMS (FAB$^+$): required for $C_{18}H_{18}N_4O$ 306.1482, found 306.1481.

3.4. 10-(2-Ethyl-2-oxoethyl)-(3,7,13-tris(2-nitrobenzenesulfonyl)-1,5(2,6)-dipyridina-3,7,10,13-tetraza-cyclotetradecaphane) (8a)

To a solution of **6a** (700 mg, 0.795 mmol) in dry THF (15 ml), TEA (483 mg, 4.77 mmol) and ethyl bromoacetate (465.5 mg, 2.79 mmol) were added. The mixture was stirred and refluxed for about 5 h and then cooled to room temperature, water (2 ml) was added and the solution was extracted several times with CH_2Cl_2. The combined organic phases were dried using anhydrous Na_2SO_4. After concentration in vacuo, the crude product was purified by silica gel chromatography (CH_2Cl_2/MeOH=100:1). The compound was obtained as white foam in 95% yield. ^{1}H NMR ($CDCl_3$): 7.82–7.93 (m, 3H), 7.50–7.63 (m, 9H), 7.40–7.44 (m, 2H), 7.02–7.05 (m, 4H), 4.51 (s, 4H), 4.46 (s, 4H), 3.97 (q, 2H, *J*=6.8 Hz), 3.32 (t, 4H, *J*=7.2 Hz), 3.20 (s, 2H), 2.68 (t, 4H, *J*=7.2 Hz), 1.21 (t, 3H, *J*=6.8 Hz); ^{13}C NMR ($CDCl_3$): 170.8, 155.3, 147.9, 137.6, 134.1, 133.8, 132.9, 132.2, 131.9, 130.3, 124.2, 121.4, 60.4, 55.4, 53.8, 53.2, 52.8, 46.5, 14.2. MS (FAB$^+$): 968 (M+H, 50%). HRMS (FAB$^+$): required for $C_{40}H_{41}N_9O_{14}S_3$+H 968.2010, found 968.2013.

3.5. 10-(2-Ethyl-2-oxoethyl)-(3,7,13-tris(2-nitrobenzenesulfonyl)-1,5(2,6)-dipyridina-3,7,11,15-tetraza-cyclohexadecaphane) (8b)

Compound **8b** was obtained as white foam in 93% yield as described for compound **8a**. ^{1}H NMR ($CDCl_3$): 7.94–7.96 (m, 2H), 7.84 (dd, 1H, *J*=6.8 Hz, *J*=1.2 Hz), 7.50–7.71 (m, 11H), 7.23 (d, 2H, *J*=7.6 Hz), 7.15 (d, 2H, *J*=7.6 Hz), 4.68 (s, 4H), 4.46 (s, 4H), 3.37 (t, 4H, *J*=7.2 Hz), 3.05 (s, 2H), 2.40 (t, 4H, *J*=6.4 Hz), 1.50–1.57 (m, 4H), 1.21 (t, 3H, *J*=7.2 Hz); ^{13}C NMR ($CDCl_3$): 170.9, 155.9, 155.3, 147.9, 147.8, 137.7, 133.5, 131.7, 130.7, 124.1, 121.7, 121.0, 60.3, 53.8, 52.6, 50.9, 46.7, 30.9, 26.0, 14.3. MS (FAB$^+$): 996 (M+H, 100%). HRMS (FAB$^+$): required for $C_{42}H_{45}N_9O_{14}S_3$+H 996.2364, found 996.2326.

3.6. 10-(2-*tert*-Butoxy-2-oxoethyl)-(3,7,13-tris(2-nitrobenzenesulfonyl)-1,5(2,6)-dipyridina-3,7,10,13-tetraza-cyclotetradecaphane) (9a)

To a solution of **6a** (700 mg, 0.795 mmol) in dry THF (15 ml), TEA (483 mg, 4.77 mmol) and *tert*-butyl bromoacetate (465.5 mg, 2.39 mmol) were added. The mixture was stirred and refluxed for about 5 h and then cooled to room temperature. Water (2 ml) was added and the solution was extracted several times with CH_2Cl_2. The combined organic phases were dried using anhydrous Na_2SO_4. After concentration in vacuo, the crude product was purified by silica gel chromatography (CH_2Cl_2/MeOH=100:1). The compound was obtained as white foam in 93% yield. ^{1}H NMR ($CDCl_3$): 7.84–7.90 (m, 3H), 7.55–7.69 (m, 9H), 7.45–7.49 (m, 2H), 7.08–7.15 (m, 4H), 4.56 (s, 4H), 4.50 (s, 4H), 3.32 (t, 4H, *J*=7.2 Hz), 3.10 (s, 2H), 2.68 (t, 4H, *J*=7.2 Hz), 1.49 (s, 9H); ^{13}C NMR ($CDCl_3$): 170.3, 155.4, 147.9, 137.7, 133.9, 133.2, 133.0, 132.2, 131.8, 130.5, 124.2, 121.5, 81.2, 56.3, 53.6, 53.1, 52.8, 46.6, 28.1. HRMS (FAB$^+$): required for $C_{42}H_{45}N_9O_{14}S_3$+H 996.2298, found 996.2326.

3.7. 10-(2-*tert*-Butoxy-2-oxoethyl)-(3,7,13-tris(2-nitrobenzenesulfonyl)-1,5(2,6)-dipyridina-3,7,11,15-tetraza-cyclohexadecaphane) (9b)

Compound **9b** was obtained as white foam in 92% yield as described for compound **9a**. ^{1}H NMR ($CDCl_3$): 7.91 (d, 2H, *J*=8.0 Hz), 7.81 (d, 1H, *J*=8.0 Hz), 7.57–7.68 (m,

8H), 7.49–7.54 (m, 3H), 7.18 (d, 2H, J=7.6 Hz), 7.11 (d, 2H, J=7.6 Hz), 4.65 (s, 4H), 4.44 (s, 4H), 3.34 (t, 4H, J=7.2 Hz), 2.92 (s, 2H), 2.37 (m, 4H), 1.40–1.51 (m, 4H), 1.35 (s, 9H); ^{13}C NMR ($CDCl_3$): 170.3, 155.9, 155.3, 147.9, 137.7, 133.7, 133.4, 131.8, 130.6, 124.1, 121.7, 121.0, 80.9, 54.7, 53.6, 52.6, 50.9, 46.8, 28.1, 26.0. HRMS (FAB$^+$): required for $C_{44}H_{49}N_9O_{14}S_3$+H 1024.2594, found 1024.2639.

3.8. (3,7,13-Tris(2-nitrobenzenesulfonyl)-1,5(2,6)-dipyridina-3,7,10,13-tetrazacyclotetradecaphane)-10-acetic acid (10a)

To a solution of **9a** (600 mg, 0.6 mmol) in CH_2Cl_2 (8 ml), TFA (8 ml) was added dropwise at 0 °C. The mixture was stirred at room temperature for another 6 h, after which the mixture was evaporated in vacuo. The residue was dissolved in methanol (10 ml) and saturated $NaHCO_3$ was added to neutralize the acid. The mixture was extracted several times with CH_2Cl_2. The organic phase was dried (anhydrous Na_2SO_4) and concentrated affording the crude solid. After purification by silica gel chromatography, the compound was obtained quantitatively as white solid. ^{1}H NMR ($CDCl_3$): 8.05 (d, 2H, J=6.4 Hz), 7.64–7.94 (m, 12H), 7.23 (d, 2H, J=6.0 Hz), 7.17 (d, 2H, J=6.0 Hz), 4.69 (s, 4H), 4.67 (s, 4H), 3.91 (br, 4H), 3.83 (s, 2H), 3.44 (br, 4H); ^{13}C NMR ($CDCl_3$): 205.8, 155.7, 148.2, 137.9, 134.3, 132.9, 132.3, 132.0, 130.3, 124.2, 121.7, 121.4, 54.2, 53.1, 52.7, 51.6, 47.0. MS (FAB$^+$): m/z 940 (M+H, 10%). HRMS (FAB$^+$): required for $C_{38}H_{36}N_9O_{14}S_3$+H 940.1690, found 940.1700.

3.9. (3,7,15-Tris(2-nitrobenzenesulfonyl)-1,5(2,6)-dipyridina-3,7,11,15-tetrazacyclohexadecaphane)-11-acetic acid (10b)

Compound **10b** was obtained quantitatively as white solid, as described for compound **10a**. ^{1}H NMR (CD_3COCD_3): 8.00–8.05 (m, 3H), 7.77–7.82 (m, 8H), 7.68–7.76 (m, 3H), 7.21–7.26 (m, 4H), 4.78 (s, 4H), 4.47 (s, 4H), 3.46 (br, 4H), 3.31 (br, 2H), 2.78 (br, 4H), 1.77 (br, 4H); ^{13}C NMR (CD_3COCD_3): 205.8, 156.1, 155.7, 148.1, 137.9, 134.2, 133.3, 132.2, 130.6, 124.2, 121.8, 121.3, 56.2, 54.1, 52.5, 51.0, 47.6, 24.9. MS (FAB$^+$): m/z 968 (M+H, 10%). HRMS (FAB$^+$): required for $C_{40}H_{41}N_9O_{14}S_3$+H 968.2010, found 968.2013.

3.10. 10-{3-Aza-2-oxo-6-[(2,2′:6′,2″-terpyridin-4′-yl)-oxy]hexyl}-3,7,13-tris(2-nitrobenzenesulfonyl)-1,5(2,6)-dipyridina-3,7,10,13-tetrazacyclotetradecaphane (11a)

To a solution of compound **10a** (259 mg, 0.276 mmol) in dry DMF (6 ml), dicyclohexylcarbodiimide (DCC, 56.8 mg, 0.275 mmol) and 1-hydroxybenzotriazole (1-HOBt, 37.2 mg, 0.275 mmol) were added. The reaction mixture was stirred at room temperature for 1 h and compound **7** (84.2 mg, 0.275 mmol) was added. The reaction mixture was stirred for 72 h, after which the precipitate was filtered off. The filtrate was concentrated in vacuo affording the crude product. After silica gel chromatography (CH_2Cl_2/MeOH=20:1), **11a** was obtained as yellow foam in 85% yield. ^{1}H NMR ($CDCl_3$): 8.66 (d, 2H, J=4.4 Hz), 8.59 (d, 2H, J=8.0 Hz), 7.98 (s, 2H), 7.83–7.88 (m, 6H), 7.50–7.65 (m, 12H), 7.30–7.35 (m, 3H), 7.09–7.27 (m, 4H), 4.66 (s, 4H), 4.42 (s, 4H), 4.28 (t, 2H, J=6.0 Hz), 3.49 (t, 2H, J=6.0 Hz), 3.42 (t, 4H, J=7.2 Hz), 3.02 (s, 2H), 2.59 (t, 4H, J=7.2 Hz), 2.03–2.09 (m, 2H); ^{13}C NMR ($CDCl_3$): 170.2, 167.0, 156.9, 155.6, 149.1, 147.9, 137.9, 136.9, 133.7, 132.7, 132.0, 131.8, 130.7, 124.2, 124.0, 122.0, 121.6, 121.3, 120.6, 107.4, 66.5, 58.6, 53.4, 53.2, 52.5, 45.9, 36.5, 28.8. MS (FAB$^+$): m/z 1228.3 (M+1, 10%). HRMS (FAB$^+$): required for $C_{56}H_{53}N_{13}O_{14}S_3$+H 1228.3044, found 1228.3075.

3.11. 11-{3-Aza-2-oxo-6-[(2,2′:6′,2″-terpyridin-4′-yl)-oxy]hexyl}-3,7,15-tris(2-nitrobenzenesulfonyl)-1,5(2,6)-dipyridina-3,7,11,15-tetrazacyclohexadecaphane (11b)

Compound **11b** was obtained as a yellow foam in 86% yield, as described for compound **11a**. ^{1}H NMR ($CDCl_3$): 8.63–8.55 (m, 3H), 7.77–7.89 (m, 6H), 7.43–7.65 (m, 12H), 7.15–7.33 (m, 7H), 4.68 (s, 4H), 4.35 (s, 4H), 4.19 (t, 2H, J=7.2 Hz), 3.24–3.42 (m, 6H), 2.99 (s, 2H), 2.40 (br, 4H), 2.20 (t, 2H, J=7.2 Hz), 1.62 (br, 4H); ^{13}C NMR ($CDCl_3$): 166.9, 156.9, 155.8, 155.3, 149.0, 147.8, 137.9, 137.0, 133.8, 133.6, 132.9, 131.8, 130.7, 128.3, 126.1, 125.1, 124.0, 121.9, 121.5, 121.2, 66.2, 58.0, 53.5, 52.8, 52.3, 46.6, 36.5, 28.9, 25.2. MS (FAB$^+$): m/z 1255 (M+1, 10%). HRMS: required for $C_{58}H_{56}N_{13}O_{14}S_3$+H 1256.3455, found 1256.3388.

3.12. 10-{3-Aza-2-oxo-6-[(2,2′:6′,2″-terpyridin-4′-yl)-oxy]hexyl}-1,5(2,6)-dipyridina-3,7,10,13-tetrazacyclotetradecaphane (12a)

Thiophenol (265 mg, 2.40 mmol) was added to a stirred mixture of compound **11a** (586 mg, 0.48 mmol) and anhydrous K_2CO_3 (1.110 g, 8.04 mmol) in 10 ml of anhydrous DMF. The resulting mixture was stirred overnight at room temperature. The solvent was evaporated and the residue was partitioned between water and CH_2Cl_2. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried (Na_2SO_4) and concentrated to a syrup, which was purified on silica gel applying stepwise elution: CH_2Cl_2, CH_2Cl_2/MeOH (20:1; v/v), MeOH, MeOH/concd aq NH_3 (30:1; v/v). Compound **12a** was obtained in 85% yield (273 mg) as pale yellow solid foam. ^{1}H NMR (DMSO-d_6): 8.74–8.75 (m, 2H), 8.62–8.65 (m, 2H), 8.01–8.05 (m, 2H), 7.88 (s, 2H), 7.73 (t, 2H, J=7.6 Hz), 7.47–7.53 (m, 2H), 7.18–7.24 (m, 4H), 4.11 (s, 4H), 4.09 (s, 4H), 3.51–3.72 (s, 8H), 3.11–3.34 (m, 4H), 2.60–2.78 (m, 6H), 2.35–2.43 (m, 2H), 1.66–1.69 (m, 2H); ^{13}C NMR (DMSO-d_6): 174.9, 166.9, 159.1, 157.2, 155.3, 149.8, 139.1, 137.9, 128.3, 125.1, 124.3, 121.3, 120.9, 107.1, 66.0, 57.7, 54.0, 53.6, 47.3, 40.6, 36.6, 28.4. MS (FAB$^+$): m/z 710 (M+K, 30%), 747 (M+2K, 100%). HRMS (FAB$^+$): required for $C_{38}H_{43}N_{10}O_2$+H 673.4016, found 673.4039.

3.13. 11-{3-Aza-2-oxo-6-[(2,2′:6′,2″-terpyridin-4′-yl)-oxy]hexyl}-1,5(2,6)-dipyridina-3,7,11,15-tetrazacyclohexadecaphane (12b)

Compound **12b** was obtained in the same way as for **12a** in 85% yield. ^{1}H NMR (DMSO-d_6): 8.68 (d, 2H, J=4.0 Hz), 8.59 (d, 2H, J=8.0 Hz), 7.92–8.00 (m, 4H), 7.62–7.66 (m, 2H), 7.45–7.49 (m, 2H), 7.22 (d, 2H, J=7.6 Hz), 7.15 (d, 2H, J=7.6 Hz), 4.17 (t, 2H, J=6.4 Hz), 3.80 (s, 8H),

3.20–3.34 (m, 2H), 2.93 (s, 2H), 2.59 (t, 2H, J=6.0 Hz), 2.43 (t, 2H, J=6.0 Hz), 1.83–1.86 (m, 2H), 1.55–1.58 (m, 4H); ^{13}C NMR (DMSO-d_6): 171.4, 167.1, 162.8, 159.2, 157.6, 157.1, 155.3, 149.7, 137.8, 137.3, 124.9, 121.3, 121.0, 107.2, 66.3, 58.2, 53.7, 53.3, 52.8, 49.0, 47.0, 36.2, 35.7, 31.2, 29.2, 25.7. MS (FAB$^+$): m/z 701 (M+H, 100%). HRMS (FAB$^+$): required for $C_{40}H_{47}N_{10}O_2$+H 701.4016, found 701.4039.

3.14. General procedure for reductive amination of imidazole-4-carbaldehyde with azacyclophanes

A mixture of imidazole-4-carbaldehyde and 1.3–1.6 equiv of $NaBH_4$ in MeOH was stirred at room temperature overnight. Then macrocyclic amine was added and the mixture was stirred at room temperature for another 2 h. When the reaction was completed, 1 M HCl was added to terminate the reaction. Then saturated $NaHCO_3$ was added to adjust the pH value of the solution to pH 8. The solution was extracted several times with $CHCl_3$. The combined organic phases were dried (Na_2SO_4), filtered and evaporated to give the crude products. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/CH_3OH=50:1) to give the product in 80–85% yield.

3.14.1. 10-[(Imidazol-4-yl)methyl]-3,7,13-tris(2-nitrobenzenesulfonyl)-1,5(2,6)-dipyridina-3,7,10,13-tetraazacyclotetradecaphane (13a). ^{1}H NMR ($CDCl_3$): 7.94 (d, 1H, J=7.7 Hz), 7.85 (d, 2H, J=6.8 Hz), 7.50–7.78 (m, 13H), 7.14 (t, 4H, J=7.2 Hz), 6.75 (s, 1H), 4.56 (s, 4H), 4.46 (s, 4H), 3.49 (s, 2H), 3.36 (t, 4H, J=6.4 Hz), 2.47 (t, 4H, J=6.4 Hz); ^{13}C NMR ($CDCl_3$): 155.3, 147.9, 137.9, 135.3, 134.0, 133.8, 133.0, 132.2, 132.0, 130.6, 124.4, 124.2, 121.7, 121.4, 53.5, 52.7, 52.3, 49.6, 45.7. MS (FAB$^+$): m/z 962 (M+H, 100%), 882 (22%). HRMS (FAB$^+$): required for $C_{40}H_{39}N_{11}S_30_{12}$+H 962.1975, found 962.2020.

3.14.2. 11-[(Imidazol-4-yl)methyl]-3,7,15-tris(2-nitrobenzenesulfonyl)-1,5(2,6)-dipyridina-3,7,11,15-tetraazacyclohexadecaphane (13b). ^{1}H NMR ($CDCl_3$): 7.89–7.91 (m, 1H), 7.82–7.84 (m, 2H), 7.48–7.68 (m, 13H), 7.13–7.19 (m, 3H), 7.07 (d, 1H, J=7.6 Hz), 4.70 (s, 2H), 4.60 (s, 2H), 4.39 (s, 2H), 4.36 (s, 2H), 3.32–3.38 (m, 6H), 2.47 (t, 2H, J=6.4 Hz), 2.01 (t, 2H, J=6.4 Hz), 1.58–1.61 (m, 2H), 1.40–1.50 (m, 2H); ^{13}C NMR ($CDCl_3$): 156.1, 155.2, 148.1, 147.8, 137.8, 135.3, 133.8, 133.2, 133.0, 131.8, 130.7, 124.1, 121.8, 121.1, 53.4, 52.6, 50.9, 46.9, 46.1, 27.5, 26.0. MS (FAB$^+$): m/z 990 (M+H, 50%), 910 (65%). HRMS (FAB$^+$): required for $C_{42}H_{43}N_{11}0_{12}S_3$+H 990.2366, found 990.2333.

3.15. 10-[(Imidazol-4-yl)methyl]-1,5(2,6)-dipyridina-3,7,10,13-tetraazacyclotetradecaphane (14a)

Thiophenol (265 mg, 2.40 mmol) was added to a stirred mixture of compound **13a** (548 mg, 0.57 mmol) and anhydrous K_2CO_3 (1.11 g, 8.04 mmol) in anhydrous DMF (10 ml). The resulting mixture was stirred overnight at room temperature. The solvent was evaporated and the residue was partitioned between water and CH_2Cl_2. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried (Na_2SO_4) and concentrated to a syrup, which was purified on silica gel applying stepwise elution: CH_2Cl_2, CH_2Cl_2/MeOH (20:1; v/v), MeOH, MeOH/concd aq NH_3 (30:1; v/v). Compound **14a** was obtained in 80% yield (185.6 mg) as a yellow solid. ^{1}H NMR (D_2O): 7.75 (t, 2H, J=7.6 Hz), 7.31 (d, 2H, J=7.6 Hz), 7.22–7.25 (m, 3H), 6.97 (s, 1H), 4.09 (s, 4H), 4.07 (s, 4H), 3.55 (s, 2H), 3.10 (t, 4H, J=7.2 Hz), 2.82 (t, 4H, J=7.2 Hz); ^{13}C NMR (D_2O): 170.2, 154.9, 152.3, 138.7, 136.2, 122.6, 121.8, 117.1, 51.5, 50.6, 49.0, 47.7, 44.9. MS (FAB$^+$): m/z 407 (M+H, 100%), 429 (M+Na$^+$, 35%), 445 (M+K$^+$, 55%). HRMS (FAB$^+$): required for $C_{22}H_{30}N_8$+H 407.2659, found 407.2672.

3.16. 11-[(Imidazol-4-yl)methyl]-1,5(2,6)-dipyridina-3,7,11,15-tetraazacyclotetradecaphane (14b)

Compound **14b** was obtained in the same way as for **14a** in 80% yield. ^{1}H NMR (D_2O): 7.66 (t, 2H, J=7.5 Hz), 7.46 (s, 1H), 7.23 (d, 2H, J=7.5 Hz), 7.18 (d, 2H, J=8.0 Hz), 6.74 (s, 1H), 3.79 (s, 4H), 3.71 (s, 4H), 3.43 (s, 2H), 2.50 (t, 4H, J=7.2 Hz), 2.35 (t, 4H, J=7.2 Hz), 1.56–1.60 (m, 4H); ^{13}C NMR (D_2O): 161.6, 160.6, 159.6, 137.2, 135.1, 120.8, 120.7, 120.1, 54.5, 53.9, 51.5, 47.3, 30.5, 26.9. MS (FAB$^+$): m/z 435 (M+H, 100%), 473 (M+K$^+$, 10%). HRMS (FAB$^+$): required for $C_{24}H_{34}N_8$+H 435.2979, found 435.2985.

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