Urea Derivatives of 1,4,7,10-Tetraazacyclododecane − Synthesis and Binding Properties

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Dedicated to Prof. Henning Hopf on the occasion of his 60th birthday

Keywords: Azacrown ether / DNA binding / Macrocycles / Supramolecular chemistry / Urea

The reaction between 1,4,7,10-tetraazacyclododecane (cyclen) and partially protected derivatives and isocyanates gives macrocyclic ureas in good yields. Bridged biscyclens with aliphatic or aromatic spacers were obtained from the reaction between diisocyanates and partially protected cyclen. The substituted cyclen derivatives with one or two urea moieties coordinate zinc(II) and copper(II) ions to form stable mononuclear and dinuclear complexes. Potentiometric titration revealed that the urea substitution significantly changes the basicity of the remaining secondary amino groups of the macrocycle. In comparison with that of cyclen (**1**), which has two basic amino groups, the basicity of one amino group in 1,4,7,10-tetraazacyclododecane-1-carboxylic acid butylamide (**6b**) is reduced by 5 orders of magnitude. The binding affinity of cyclen (**1**), urea derivatives of cyclen, and metal complexes to double stranded DNA was measured under physiological conditions, using an ethidium bromide displacement assay. The binding affinities correlate with the positive charge of the protonated cyclic amines. Their metal complexes show even higher affinities, which presumably result from different binding motifs.

Introduction

The properties of azacrowns[1,2] such as 1,4,7,10-tetraazacyclododecane (cyclen) and 1,4,8,11-tetraazacyclotetradecane (cyclam) have been studied intensively over the last few years. The compounds are used as ligands for very stable metal complexes[3,4] in medicinal applications,[5,6,7] and their ammonium salts are receptors for binding anions,[8] including biological compounds such as ATP.[9] Some zinc complexes of azamacrocycles show dynamic anion recognition in water, mimicking enzyme properties.[10] This has been used to achieve self assembly under physiological conditions[11,12] and even to control gene expression in vivo.[13] To increase binding constants and binding selectivity, ditopic[14,15] and tritopic[16] complexes have been prepared. The major synthetic route for functionalization and covalent bridging of azamacrocycles[17] is *N*-alkylation or acylation[18−22] of their partially protected derivatives. Surprisingly, urea derivatives of cyclen, readily obtained by their treatment with isocyanates, have not yet, to the best of our knowledge, been reported in the literature. Although the introduction of carbamoyl groups at the nitrogen atoms reduces their basicity and binding ability irreversibly, the clean and high-yielding reaction is useful for receptor synthesis. In this paper we present the reactions between partially protected and unprotected cyclen and isocyanate and bisisocyanates, the determination of the structure and p*K*a values of the products, and a study of their binding properties towards double strand DNA under physiological conditions.

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Results and Discussion

Synthesis

Treatment of cyclen (**1**) with *n*-butylisocyanate in dichloromethane at room temperature yields macrocycle **2** in a smooth and clean conversion (Scheme 1). The compound was isolated as a white solid of high melting point (m.p. 204.5 °C). The simple ^{1}H and ^{13}C NMR spectra indicate unrestricted rotation of the urea moieties on the NMR timescale.[23] Compound **2** was crystallized from methanol and characterized by X-ray analysis (Figure 1), which revealed an inversion-symmetric conformation with all carbonyl groups pointing in one direction. The conformation is stabilized by four hydrogen-bonded methanol molecules bridging the carbonyl oxygen and the urea N−H moiety.

Under identical conditions the highly substituted cyclam **3**[24,25] was converted into **4**, although in lower yield. Cyclen derivatives **6b** and **8b**, with one or two urea moieties, are accessible from partially protected cyclens **5** and **7**.[26] The

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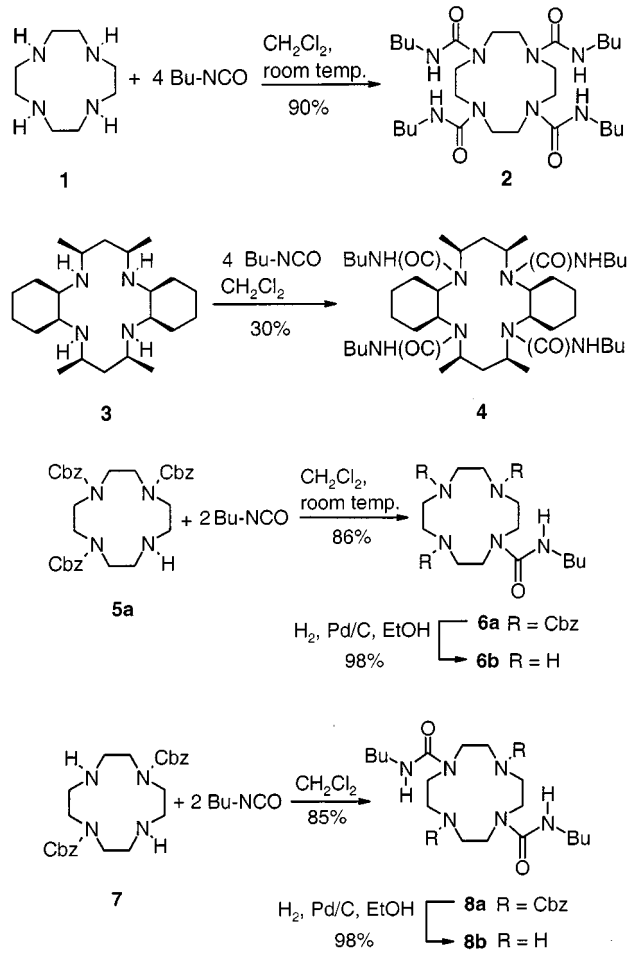


Scheme 1. Reaction between azamacrocycles and *n*-butyl isocyanate

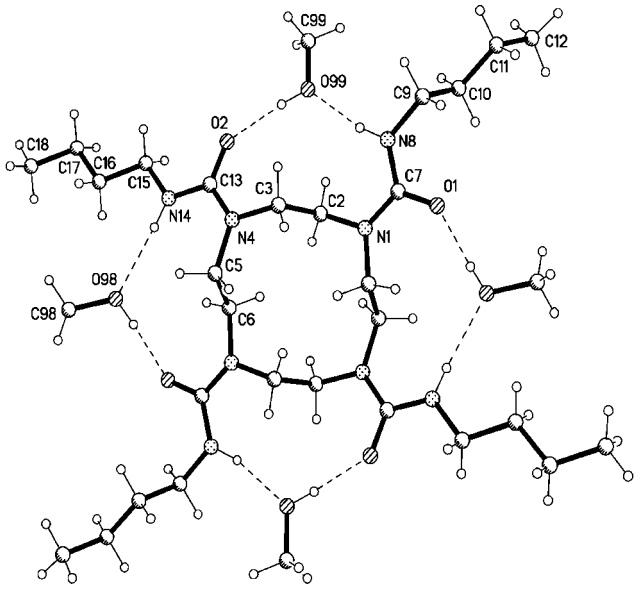
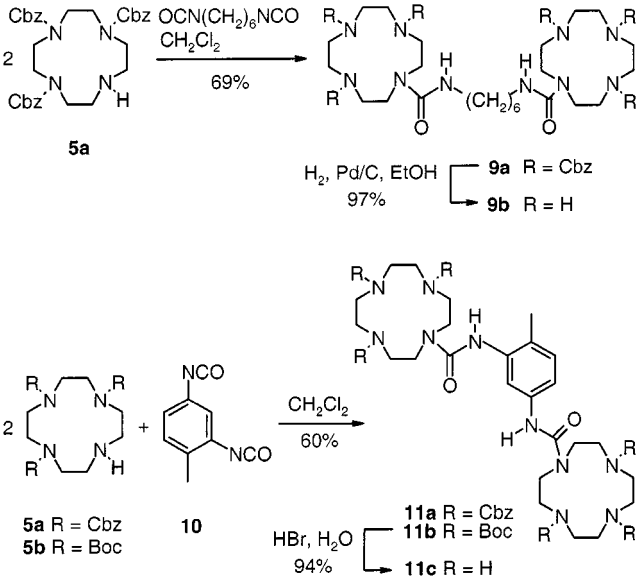


Figure 1. The structure of compound **2**·4CH_3OH in the crystal. Radii are arbitrary. H bonds are indicated by dashed lines. Dimensions of H bonds: N8−H08···O99 with N−H 0.87(3), H···O 2.05(3) Å, N−H···O 164(3)°; N14−H14···O98 with N−H 0.87(2), H···O 2.18(3) Å, N−H···O 172(3)°; O99−H99···O2 with O−H 0.84(4), H···O 1.84(4) Å, O−H···O 174(5)°; O98−H98···O1 ($-x+1$, $-y+1$, $-z$) with O−H 0.85(3), H···O 1.84(4) Å, O−H···O 177(3)°



Scheme 2. Synthesis of urea-bridged biscyclen macrocycles

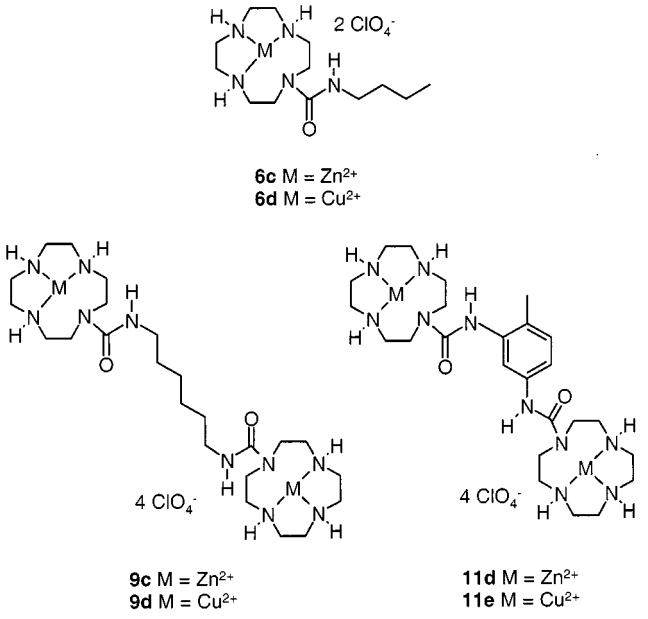
protecting groups were removed under standard conditions.[27]

Treatment of **5** with hexyl-1,6-diisocyanate and *m*-tolyl diisocyanate (**10**) at room temperature in dichloromethane accordingly gave biscyclens **9** and **11** (Scheme 2). While the Cbz protecting groups of **9a** were cleanly removed by catalytic hydrogenation to yield **9b**, the deprotection of **11a** to **11c** was difficult. Catalytic hydrogenation, even at elevated temperatures, did not remove the Cbz groups, and the molecule was destroyed under harsh hydrogenation conditions. However, starting from the BOC-protected compound **5b**, it was possible to obtain biscyclen **11c** by treatment with $HBr/H_2O/EtOH$.[28]

To examine their coordination ability, macrocycles **6b**, **9b**, and **11c** were refluxed with equimolar amounts of zinc(II) or copper(II) bisperchlorate in ethanol.[29] In the case of copper(II) bisperchlorate, an immediate color change of the solution indicated rapid complex formation. While all macrocyclic ligands are soluble in chloroform, the coordination compounds dissolve only in water and methanol. To investigate a possible participation of the urea moiety in metal ion coordination, IR spectra and ^{13}C carbonyl group resonance signals before and after complexation were carefully compared. However, no significant changes upon metal ion binding could be detected. The formation of the coordination compounds was confirmed by the observation of the corresponding molecular ions in FAB mass spectra and elemental analyses. Unfortunately it proved impossible to grow suitable crystals of the metal complexes to determine coordination geometries by X-ray analysis. (Scheme 3)

Determination of p*K*a Values by Potentiometric Titration

The effect of the urea moieties on the basicity of the nitrogen atoms of the cyclen macrocycle was investigated by



Scheme 3. Metal complexes of urea-functionalized cyclens

potentiometric titrations of compounds **6b** and **9b**. Figure 2 summarizes the results. In comparison to the parent cyclen system **1**, which shows two nitrogen atoms with high basicity (p*K*a 11.0 ±0.1 and 9.9 ±0.1) and two nitrogen atoms with low basicity (p*K*a < 2),[30] compound **6b** has one amino group with p*K*a of 11.3 ±0.1, one with reduced basicity (p*K*a 6.3 ±0.1), and one with low basicity (p*K*a < 2). Titration of biscyclen **9b** shows that protonation of both monosubstituted azamacrocycles is independent: The pH profile is nearly identical to **6b**, if equivalents of base (with respect to the number of cyclen moieties) are used (p*K*a 11.3 ±0.1 and p*K*a of 6.3 ±0.1). Introduction of a second urea moiety into the azamacrocycle, as in **8b**, produces a compound that is not stable under the conditions of the potentiometric titration.[31] Figure 3 shows the species distribution plot for compound **6b** at different pH values. At neutral pH in water, a mixture of monoprotonated and bisprotonated macrocycle is observed, while the formation of free **6b** requires a pH above 10 (Scheme 4).

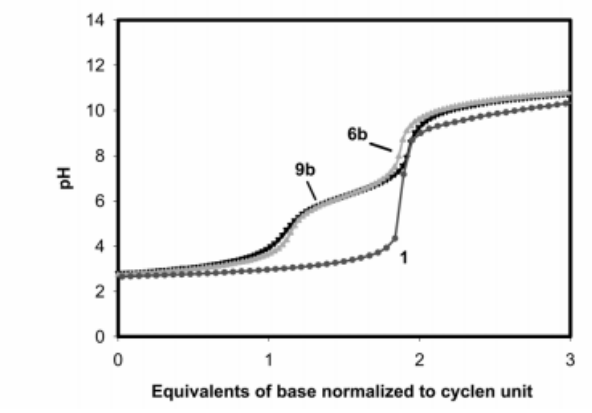


Figure 2. Potentiometric titration of compounds **1**, **6b**, and **9b**

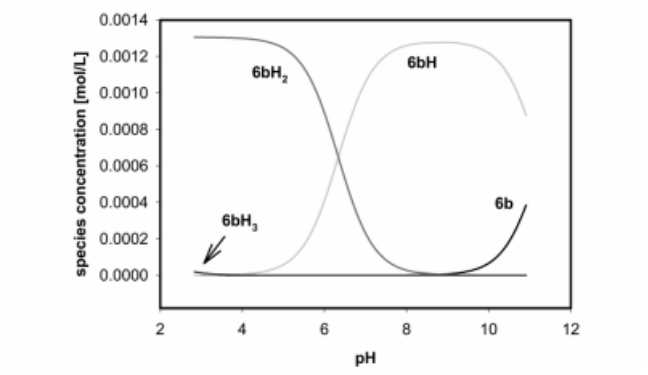
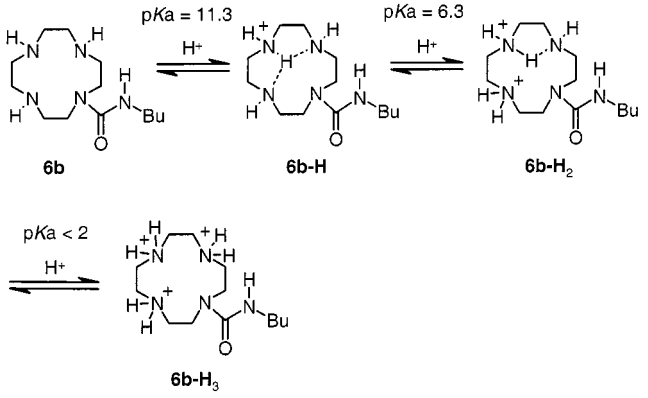


Figure 3. Species distribution plot for compound **6b** at different pH values



Scheme 4. Protonation equilibria of compounds **6b**

DNA Binding

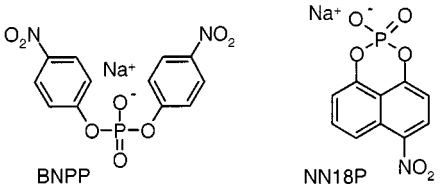
Protonated amines[32,33] and Lewis acid metal complexes[34] of azamacrocycles have been shown to bind to DNA. To compare relative binding affinities of the compounds presented in this paper, competitive ethidium bromide displacement titrations[35,36] were performed. The procedure provides a quick and reliable indicator of relative binding affinity.[37] Sonicated calf thymus DNA (CT-DNA) and plasmid DNA pUC 18[38] were used to determine apparent binding constants. The results are summarized in Table 1. To rule out any effect of metal complex-mediated phosphodiester hydrolysis of DNA in the assay, tests were made of the ability of compounds [Zn(cyclen)]$(ClO_4)_2$, [Cu(cyclen)]$(ClO_4)_2$, **6c**, and **6d** to hydrolyze the much more reactive phosphoric diesters **12**[39] and **13**[40] in buffered aqueous solution at pH 7.4 (Scheme 5). Within the error limits of the experiment, no hydrolysis of **12** and **13** could be detected, which clearly indicates that the much more resistant phosphodiester backbone of DNA is fully stable in the presence of the investigated coordination compounds.

Under the experimental conditions used, the determined apparent binding constants of the azamacrocycles **1**, **6b**, **9b**, and **11c** show a clear correlation with their charge. While, at pH 7.4, compounds **1** and **6b** bear up to two positive charges from protonation, which results in binding constants too small to be measured by this assay, compounds **9b** and **11c**, with two azamacrocyclic moieties, have up to

Table 1. C_{50} values and apparent binding constants K_{app} of azamacrocycles and metal complexes in binding to double stranded DNA in water at pH 7.4 and 50 mmol NaCl

compound	C_{50} value[a] CT-DNA [10^{-6} mol/L]	K_{app}[a] CT-DNA [10^3 L/mol]	C_{50} value pUC 18 [10^{-6} mol/L]	K_{app} pUC 18 [10^3 L/mol]
1	–	< 1	–	< 1
$[Zn(cyclen)]^{2+}$	3700	3.4	3725	3.4
$[Zn(cyclen)]^{2+}$[b]	–	–	–	3.5[b]
$[Cu(cyclen)]^{2+}$	189	68	223	57
6b	–	< 1	–	< 1
6c	3150	4.0	3200	3.9
6d	249	51	233	54
9b	547	23	550	22.5
9c	36	353	35	362
9d	19.5	650	20	635
11c	530	24	533	23.8
11d	36	350	36	350
11e	< 5	> 1000	< 5	> 1000

[a] See Experimental Section for details. – [b] This value was reported in the literature for binding to an oligonucleotide with 150 base pairs.[34]



Scheme 5. Activated sodium phosphodiester used in hydrolysis study

four positive charges and K_{app} of the order of $2 \cdot 10^4$ L/mol.[41] The apparent binding constants of the metal complexes are higher.[42] $[Zn(cyclen)](ClO_4)_2$ and **6c** show similar binding strengths, whereas the dinuclear zinc complexes **9c** and **11d** bind two orders of magnitude more strongly. The highest apparent binding constants for mononuclear and dinuclear complexes were determined for copper(II) salts $[Cu(cyclen)](ClO_4)_2$, **6d**, **9d**, and **11e**. Compound **11e**, with a K_{app} larger than 10^7 L/mol, shows especially strong affinity for DNA. The assay does not provide any information about the binding motif to DNA, which is probably very different for the different compounds. For zinc(II) cyclen complexes, binding to the imide moiety of thymine has been reported, while copper(II) complexes are expected to show preferential binding to the phosphate oxygen atoms of the DNA backbone.[43] A recent study on the influence of such complexes on DNA melting curves supports this view.[44]

Conclusion

We have shown that treatment of azamacrocycles and their partially protected derivatives with isocyanates yields macrocyclic ureas in clean reactions. Bridged biscyclens were obtained from treatment of diisocyanates with protected cyclens. In the case of **2**, the structure of the compound was confirmed by X-ray analysis. The urea substitution changes the basicity of the cyclen nitrogen atoms significantly, as determined by potentiometric titration. While in compound **1** two basic nitrogen atoms with p*K*a values around 11 are found, in compound **6b** the basicity of one of them is reduced to p*K*a 6.3. Substituted cyclens with one or two urea moieties still coordinate metal ions, such as zinc(II) or copper(II), and form stable and stoichiometric complexes, which were characterized by NMR, mass spectrometry, and elemental analysis. It was not possible to obtain single crystals to determine their coordination geometry by X-ray analysis. The affinities of cyclen, urea derivatives of **1**, and metal complexes for double stranded DNA under physiological conditions were determined by ethidium bromide displacement assay. The results show a clear correlation between binding affinity and the positive charge of the azamacrocycle under the experimental conditions. Metal complexes show higher affinities, which further increase in dinuclear complexes with rigid spacers. However, a direct comparison between the affinities of the tested compounds is difficult, because it is most likely that they interact with different functional groups in DNA and show individual binding motifs.

Experimental Section

General Remarks: Melting points were measured on a hotplate microscope apparatus and are not corrected. – NMR spectra were recorded at 400 MHz (^{1}H) and 100 MHz (^{13}C) in $CDCl_3$ solutions unless otherwise stated. The multiplicity of the ^{13}C signals was determined using the DEPT technique and quoted as: (+) for CH_3 or CH, (–) for CH_2, and (C_{quat}) for quaternary carbons. Compounds **3**,[25] **5a**,[26] and **7**[26] were prepared as previously described. Isocyanates were distilled before use. CC means column chromatography on SiO_2.

Potentiometric Titrations: Titrations were performed with a computer-controlled pH meter (WTW pH 3000) and dosimat (Metrohm Dosimat 655). Perchloric acid (0.1 M, Merck, 60%, p.a.) and 0.1 M tetraethylammonium hydroxide (TEAOH) (Merck, 20%) in water, with tetraethylammonium perchlorate to maintain an ionic strength of $I = 0.1$, were used for all titrations. TEAOH solutions were calibrated with monosodium phthalate. A titration of the perchloric acid with the TEAOH solution was used for calibration and to determine log K_w (–14.29) and A_I (–0.02).[45] All calculations were performed with the program HYPERQUAD.[46]

Ethidium Bromide Displacement Assay: All experiments were performed at room temperature in buffered aqueous solution (Tris-HCl, pH 7.4) containing 50 mmol/L NaCl. Buffered DNA stock solution (20 μL) with optical density 2.0 at 260 nm was added to ethidium bromide in buffer (2 mL). Compounds to be tested, dissolved in buffer, were added and the fluorescence intensity was recorded to determine the concentration necessary to reduce fluorescence intensity to 50% of the initial value (C_{50} value). The apparent binding constants were calculated from Equation (1). All data are average values from three titrations. The error limits of the method are +/– 10%.

$$K_{app} = K(EtBr) \cdot C(EtBr)/C_{50} \quad (1)$$

C(EtBr) = $1.26 \cdot 10^{-6}$ in mol/L (concentration ethidium bromide in buffer), K(EtBr) = $1 \cdot 10^{7}$ L/mol (binding constant of ethidium bromide to DNA under experimental conditions)[35]

Colored compounds may interfere with the assay. To determine whether the absorption of copper(II) complexes caused an error in determined binding constants, $[Cu(edta)]^{2-}$ was added to the DNA-ethidium bromide buffer. The negatively charged complex, which has an absorption spectrum similar to that of $[Cu(cyclen)]^{2+}$, cannot bind to the negatively charged DNA double strand. The addition of $[Cu(edta)]^{2-}$to the investigated compounds in comparable quantities did not produce any changes in emission intensity.

Phosphodiester Hydrolysis: Buffered aqueous solutions (Tris-HCl pH 7.4, 50 mM NaCl) of **12** or **13** with the metal complex to be tested were incubated at room temp. and the UV absorption at 400 nm was monitored. $[Co(cyclen)]^{3+}$, which is known to cleave activated phosphodiesters, was used as positive control (rate constant of hydrolysis under experimental conditions: $k = 8.8 \cdot 10^{-4}$ s^{-1}). None of the tested complexes $[Zn(cyclen)]^{2+}$, $[Cu(cyclen)]^{2+}$, **6c**, or **6d** displayed any detectable hydrolytic activity.

General Procedure (*GP1*) for Treatment of Azacrowns with Isocyanates: The required stoichiometric amount of isocyanate, dissolved in as small a quantity as possible of dichloromethane, was slowly added at room temperature under nitrogen to a solution of 1 mmol of the azacrown derivative in 2 mL of dichloromethane. The reaction mixture was stirred for 12 h, the solvent was evaporated in vacuo, and the crude reaction products were purified by CC.

***N*,*N*′,*N*″,*N*‴-Tetrabutyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetracarboxamide (2):** Compound **1** (250 mg, 1.45 mmol) was allowed to react with *n*-butyl isocyanate (720 mg, 7.25 mmol) according to *GP1* and gave, after CC (CH_2Cl_2/MeOH 95:5; R_f = 0.4), 740 mg (90%) of **2** as a white solid, m.p. 190 °C. − ^{1}H NMR: δ = 0.92 (t, ^3J = 7.0 Hz, 12 H), 1.39 (m, 16 H), 3.17 (q, ^3J = 6.0 Hz, 8 H), 3.47 (s, 16 H), 5.02 (m, 4 H). − ^{13}C NMR: δ = 13.7 (+), 20.0 (−), 32.3 (−), 40.6 (−), 50.2 (−), 159.4 (C_{quat}). − IR (KBr): $\tilde{\nu}$ = 3355 cm^{-1}, 2864, 1630. − UV/Vis (CH_3CN): λ_{max} (lg ε) = 218 nm (3.668), 224 (3.204), 230 (2.720). − MS (EI, 70 eV): *m*/*z* (%) = 568 (4) [M^+], 468 (10) [$M^+ - C_5H_9NO$], 185 (100). − $C_{28}H_{56}N_8O_4$ (568.8): calcd. C 59.13; H 9.92; N 19.70; found C 59.17; H 9.94; N 19.57. Molecular mass 568 (MS).

***cis*,*cis*-2:3;9:10-Bis(tetramethylene)-*N*,*N*′,*N*″,*N*‴-tetrabutyl-1,4,8,11-tetraazacyclotetradecane-2,6,13,17-tetracarboxamide (4):** Compound **3** (165 mg, 0.45 mmol) was allowed to react with *n*-butyl isocyanate (225 mg, 2.25 mmol) according to *GP1* and gave, after CC (CH_2Cl_2/MeOH 95:5; R_f = 0.4), 103 mg (30%) of **4** as a white solid, m.p. 175 °C. − ^{1}H NMR: δ = 0.92 (m, 12 H), 1.3 (m, 48 H), 3.2 (m, 16 H). − IR (KBr): $\tilde{\nu}$ = 3365 cm^{-1}, 2871, 1691. − UV/Vis (CH_2Cl_2): λ_{max} (lg ε) = 240 nm (3.233), 276 (2.157), 230 (2.720). − MS (FAB): *m*/*z* (%) = 761 (4) [M^+ + 1], 662 (35) [$M^+ - C_5H_9NO$], 124 (100). − $C_{42}H_{80}N_8O_4$ (760.6): calcd. C 66.28; H 10.59; N 14.72; found C 66.04; H 10.81; N 14.46. Molecular mass 760 (MS).

Tribenzyl 10-Butylcarbamoyl-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate (6a): Compound **5a** (200 mg, 0.35 mmol) was allowed to react with *n*-butyl isocyanate (45 mg, 0.45 mmol) following *GP1* and yielded, after CC (EE; R_f = 0.4), 203 mg (86%) of **5a** as a white solid, m.p. 160 °C. − ^{1}H NMR: δ = 0.87 (t, ^3J = 7.0 Hz, 3 H), 1.30 (m, 4 H), 3.00 (m, 2 H), 3.30 (m, 16 H), 4.56 (br. s, 1 H), 5.04 (s, 2 H), 5.07 (s, 4 H), 7.30 (m, 15 H). − ^{13}C NMR δ = 13.8 (+), 20.0 (−), 32.2 (−), 40.6 (−), 50.4 (−), 50.6 (−), 67.2 (−), 128.1 (+), 128.28 (+), 128.29 (+), 128.4 (+), 136.1 (C_{quat}), 136.2 (C_{quat}), 156.5 (C_{quat}), 157.0 (C_{quat}), 159.1 (C_{quat}). − IR (KBr): $\tilde{\nu}$ = 3366 cm^{-1}, 2871, 1586, 1215. − UV/Vis (CH_3CN): λ_{max} (lg ε) = 204 nm (4.508), 258 (2.866). − MS (EI, 70 eV): *m*/*z* (%) = 673 (18) [M^+], 574 (100) [$M^+ - C_5H_9NO$]. − $C_{37}H_{47}N_5O_7 \cdot H_2O$ (691.8): calcd. C 64.24; H 7.14; N 10.12; found C 64.23; H 7.07; N 10.10. Molecular mass 673 (MS).

***N*-Butyl-1,4,7,10-tetraazacyclododecane-1-carboxamide (6b):** Compound **6a** (203 mg, 0.30 mmol) was hydrogenated (0.4 MPa pressure H_2) at room temp. for 24 h in 30 mL of ethanol over 80 mg Pd/C (5%) to yield **6b** (80 mg, 98%) as an oil. − ^{1}H NMR: δ = 0.92 (t, ^3J = 7.0 Hz, 3 H), 1.41 (m, 4 H), 2.68 (m, 4 H), 2.81 (m, 8 H), 3.19 (m, 2 H), 3.48 (m, 4 H), 5.69 (t, ^3J = 5.2 Hz, 1 H). − ^{13}C NMR δ = 13.7 (+), 20.0 (−), 32.1 (−), 40.6 (−), 45.4 (−), 47.9 (−), 48.4 (−), 48.7 (−), 160.0 (C_{quat}). − IR (KBr): $\tilde{\nu}$ = 3316 cm^{-1}, 2870, 1628. − UV/Vis (CH_3CN): λ_{max} (lg ε) = 216 nm (3.451), 220 (3.253). − MS (EI, 70 eV): *m*/*z* (%) = 271 (10) [M^+], 215 (80) [$M^+ - C_4H_8$], 128 (100).

Dibenzyl 4,10-Dibutylcarbamoyl-1,4,7,10-tetraazacyclododecane-1,7-dicarboxylate (8a): Compound **7** (350 mg, 0.795 mmol) was allowed to react with *n*-butyl isocyanate (196 mg, 2 mmol) as described for **5a** and gave, after CC (EE; R_f = 0.4), 430 mg (85%) of **8a** as a white solid, m.p. 80 °C. − ^{1}H NMR: δ = 0.89 (t, ^3J = 7.1 Hz, 6 H), 1.29 (m, 8 H), 3.06 (q, ^3J = 6.8 Hz, 4 H), 3.35 (m, 16 H), 4.69 (br. s, 2 H), 5.12 (s, 4 H), 7.33 (m, 10 H). − ^{13}C NMR: δ = 13.8 (+), 20.0 (−), 32.3 (−), 40.5 (−), 49.9 (−), 50.8 (−), 67.4 (−), 128.36 (+), 128.4 (+), 128.6 (+), 136.1 (C_{quat}), 157.1 (C_{quat}). − IR (KBr): $\tilde{\nu}$ = 3388 cm^{-1}, 2872, 1587, 1215. − UV/Vis (CH_3CN): λ_{max} (lg ε) = 198 nm (4.656), 218 (4.037). − MS (EI, 70 eV): *m*/*z* (%) = 638 (10) [M^+], 539 (24) [$M^+ - C_5H_9NO$], 91 (100).

***N*,*N*′-Dibutyl-1,4,7,10-tetraazacyclododecane-1,7-dicarboxyamide (8b):** Compound **8a** (430 mg, 0.68 mmol) was hydrogenated as described for **6a** to yield 245 mg (98%) of **8b** as an oil. − ^{1}H NMR: δ = 0.92 (t, ^3J = 7.3 Hz, 6 H), 1.33 (m, 4 H), 1.50 (m, 4 H), 2.90 (m, 8 H), 3.19 (m, 4 H), 3.49 (m, 8 H), 5.27 (m, 2 H). − ^{13}C NMR: δ = 13.7 (+), 20.0 (−), 32.09 (−), 32.1 (+), 40.5 (−), 40.6 (−), 48.7 (−), 49.3 (−), 158.39 (C_{quat}), 158.42 (C_{quat}). − IR (KBr): $\tilde{\nu}$ = 3426 cm^{-1}, 3388, 2865, 1684. − UV/Vis (CH_3CN): λ_{max} (lg ε) = 268 nm (3.169), 276 (3.168). − MS (EI, 70 eV): *m*/*z* (%) = 370 (8) [M^+], 315 (10) [$M^+ - C_4H_8$], 56 (100).

***N*,*N*′-(Hexane-1,6-diyl)-Bridged 1,6-Bis(4,7,10-tribenzylcarboxyl-1,4,7,10-tetraazacyclododecane-1-carboxamide) (9a):** A mixture of **5a** (314 mg, 0.55 mmol) and hexane-1,6-diisocyanate (46 mg, 0.27 mmol) was allowed to react according to *GP1* to yield, after CC (CH_2Cl_2/MeOH 95:5; R_f = 0.3), 250 mg (69%) of **9a** as a white solid, m.p. 170 °C. − ^{1}H NMR: δ = 1.26 (m, 8 H), 2.99 (m, 4 H), 3.28 (m, 16 H), 3.40 (m, 16 H), 4.89 (br. s, 2 H), 5.03 (s, 2 H), 5.04 (s, 2 H), 5.07 (s, 4 H), 5.08 (s, 4 H), 7.30 (m, 30 H). − ^{13}C NMR: δ = 26.4 (−), 30.1 (−), 30.3 (−), 40.5 (−), 49.9 (−), 50.5 (−), 50.7 (−), 67.25 (−), 67.27 (−), 128.2 (+), 128.3 (+), 128.5 (+), 128.56 (+), 136.2 (C_{quat}), 136.3 (C_{quat}), 156.6 (C_{quat}), 157.1 (C_{quat}), 171.1 (C_{quat}). − IR (KBr): $\tilde{\nu}$ = 3375 cm^{-1}, 2929, 1648, 1217. − UV/Vis (CH_3CN): λ_{max} (lg ε) = 240 nm (3.165), 258 (3.052). − MS (FAB): *m*/*z* (%) = 1318 (5) [M^+ + 1], 1341 (5) [M + Na^+], 136 (100). − $C_{72}H_{88}N_{10}O_{14}$ (H_2O + MeOH) (1381.6): calcd. C 64.11; H 6.93; N 10.24; found C 64.44; H 7.39; N 10.65.

***N*,*N*′-(Hexane-1,6-diyl)-Bridged 1,6-Bis(1,4,7,10-tetraazacyclododecane-1-carboxamide) (9b):** A mixture of **9a** (250 mg, 0.19 mmol) and 80 mg of Pd/C (5%) in 30 mL of ethanol was hydrogenated at room temp. for 24 h (0.4 MPa pressure H_2) to give 94 mg (97%) of **9b** as an oil. − ^{1}H NMR: δ = 1.28 (br. s, 4 H), 1.44 (br. s, 4 H),

2.63 (m, 8 H), 2.76 (m, 16 H), 3.12 (m, 4 H), 3.44 (br. s, 8 H), 5.58 (br. s, 2 H). − ^{13}C NMR: δ = 26.0 (−), 29.9 (−), 40.3 (−), 45.8 (−), 48.1 (−), 48.9 (−), 159.7 (C_{quat}). − IR (KBr): $\tilde{\nu}$ = 3332 cm^{-1}, 2883, 1625. − UV/Vis (CH_3CN): λ_{max} (lg ε) = 224 nm (3.357), 332 (3.061). − MS (FAB): *m*/*z* (%) = 513 (24) [M$^+$], 382 (88) [M$^+$ − $C_8H_{20}N_4$], 55 (100).

***N*,*N*′-(6-Methyl-1,3-phenylene)-Bridged 1,3-Bis(*N*′,*N*′′,*N*′′′-tribenzylcarbamoyl-1′,4′,7′,10′-tetraazacyclododecane-*N*-carboxamide) (11a):** Compound **5a** (680 mg, 0.87 mmol) was allowed to react with 1,3-diisocyanato-4-methylbenzene (**10**) (77 mg, 0.44 mmol) following *GP1*. CC (EE R_f = 0.4) yielded 470 mg (60%) of **11a** as a white solid, m.p. 150 °C. − ^{1}H NMR: δ = 1.94 (s, 3 H), 3.24 (br. s, 16 H), 3.35 (br. s, 16 H), 4.91 (s, 2 H), 4.95 (s, 2 H), 4.97 (s, 4 H), 5.03 (s, 4 H), 6.19 (br. s, 2 H), 7.16 (m, 2 H), 7.19 (m, 30 H), 7.78 (1 H). − ^{13}C NMR: δ = 17.0 (+), 49.6 (−), 50.3 (−), 50.5 (−), 67.2 (−), 67.3 (−), 67.5 (−), 113.0 (+), 114.8 (+), 128.1 (+), 128.2 (+), 128.29 (+), 128.3 (+), 128.4 (+), 128.42 (+), 128.5 (+), 130.4 (+), 136.0 (C_{quat}), 136.1 (C_{quat}), 136.3 (C_{quat}), 137.0 (C_{quat}), 137.5 (C_{quat}), 157.2 (C_{quat}), 171.1 (C_{quat}), 171.1 (C_{quat}). − IR (KBr): $\tilde{\nu}$ = 3089 cm^{-1}, 1598, 1240. − UV/Vis (CH_3CN): λ_{max} (lg ε) = 215 nm (4.920), 262 (3.756). − MS (ESI): *m*/*z* (%) = 1323 (15) [M$^+$ + 1], 1345 (100) [M + Na]$^+$. − $C_{73}H_{90}N_{10}O_{15}$ (1341): calcd. C 65.36; H 6.31; N 10.44; found C 65.52; H 6.24; N 10.31. Molecular mass 1322 (MS).

***N*,*N*′-(6-Methyl-1,3-phenylene)-Bridged 1,3-Bis[*N*′,*N*′′,*N*′′′-tri(*tert*-butyl)carbamoyl-1′,4′,7′,10′-tetraazacyclododecane-*N*-carboxamide] (11b):** Compound **5b** (300 mg, 0.63 mmol) and 1,3-diisocyanato-4-methylbenzene (**10**) (55 mg, 0.32 mmol) were treated following *GP1* and gave, after CC (EE R_f = 0.4), 219 mg (62%) of **11b** as a white solid, m.p. 75 °C. − ^{1}H NMR: δ = 1.46 (br. s, 54 H), 2.22 (s, 3 H), 3.50 (br. s, 32 H), 6.47 (s, 2 H), 7.03 (d, ^3J = 8.3 Hz, 1 H), 7.10 (d, ^3J = 8.1 Hz, 1 H), 7.70 (s, 1 H). − ^{13}C NMR δ = 17.4 (+), 28.3 (+), 28.39 (+), 28.42 (+), 49.8 (−), 50.7 (−), 79.82 (C_{quat}), 79.89 (C_{quat}), 115.0 (+), 116.1 (+), 123.9 (C_{quat}), 130.2 (+), 136.8 (C_{quat}), 137.2 (C_{quat}), 156.0 (C_{quat}), 156.6 (C_{quat}). − IR (KBr): $\tilde{\nu}$ = 3427 cm^{-1}, 2871, 1597. − UV/Vis (CH_3CN): λ_{max} (lg ε) = 222 nm (4.547), 250 (4.457), 264 (4.260). − MS (FAB): *m*/*z* (%) = 1118 (6) [M$^+$], 1141 (14) [M + Na]$^+$, 57 (100).

***N*,*N*′-(6-Methyl-1,3-phenylene)-Bridged 1,3-Bis[1,4,7,10-tetraazacyclododecane-*N*-carboxamide] Hydrobromide (11c):** Compound **11b** (150 mg, 0.34 mmol) was allowed to react with HBr (48% in H_2O, 10 mL) in ethanol (20 mL) at room temp. for 3 h. The product was filtered off to yield **11c** (320 mg, 94%) as a white solid, m.p. 255 °C (dec.). − ^{1}H NMR (D_2O): δ = 2.22 (s, 3 H), 3.31 (m, 24 H), 3.76 (m, 8 H), 7.21 (m, 2 H), 7.45 (s, 1 H). − ^{13}C NMR: δ = 17.5 (+), 44.7 (−), 45.1 (−), 45.3 (−), 47.3 (−), 47.8 (−), 47.9 (−), 48.2 (−), 123.5 (+), 124.2 (+), 131.7 (+), 133.3 (C_{quat}), 136.5 (C_{quat}), 136.6 (C_{quat}), 159.7 (C_{quat}), 160.1 (C_{quat}). − IR (KBr): $\tilde{\nu}$ = 3419 cm^{-1}, 2928, 1637. − MS (FAB): *m*/*z* (%) = 962 (1) [M − HBr + K]$^+$, 541 (5) [M − 6 HBr + Na]$^+$, 519 (3) [M − 6 HBr + 1]$^+$, 153 (100).

General Procedure (*GP2*) for Complex Formation: Equimolar amounts of ligand and metal salt were heated to reflux in methanol solution for 2 h, the solvent was removed, and the coordination compound was recrystallized from ethanol and dried in high vacuum.

(*N*-Butyl-1,4,7,10-tetraazacyclododecane-1-carboxamide)zinc(II) Perchlorate (6c): Compound **6b** (100 mg, 0.37 mmol) and zinc(II) perchlorate hexahydrate (138 mg, 0.37 mmol) in methanol (25 mL) were allowed to react according to *GP2* to yield **6c** (185 mg, 93%) as an oil. − ^{1}H NMR: δ = 0.92 (t, ^3J = 7.3 Hz, 3 H), 1.34 (m, 2 H), 1.51 (m, 2 H), 2.92 (m, 2 H), 3.10 (m, 14 H), 3.50 (m, 2 H). − ^{13}C NMR: δ = 13.9 (+), 20.8 (−), 32.1 (−), 41.9 (−), 44.6 (−), 44.7 (−), 45.3 (−), 46.3 (−), 162.9 (C_{quat}). − IR (KBr): $\tilde{\nu}$ = 3533 cm^{-1}, 3465, 2873, 1623. − UV/Vis (CH_3CN): λ_{max} (lg ε) = 218 nm (2.062), 230 (1.635). − MS (FAB): *m*/*z* (%) = 436 (100) [M − ClO_4]$^+$. − $C_{13}H_{29}N_5Cl_2O_9Zn$ (536): calcd. C 29.15; H 5.46; N 13.07; found C 29.02; H 5.40; N 12.91. Molecular mass 536 (MS).

(*N*-Butyl-1,4,7,10-tetraazacyclododecane-1-carboxamide)copper(II) Perchlorate (6d): Compound **6b** (100 mg, 0.37 mmol) and copper(II) perchlorate hexahydrate (137 mg, 0.37 mmol) in methanol (25 mL) were allowed to react according to *GP2* to yield **6c** (192 mg, 95%) as a blue solid, m.p. 155 °C. − IR (KBr): $\tilde{\nu}$ = 3533 cm^{-1}, 3465, 2873, 1623. − UV/Vis (CH_3CN): λ_{max} (lg ε) = 192 nm (1.1820), 292 (0.7685), 610 (0.0626). − MS (FAB): *m*/*z* (%) = 434 (100) [M − ClO_4]$^+$.

***N*,*N*′-(Hexane-1,6-diyl)-Bridged 1,6-Bis(1,4,7,10-tetraazacyclododecane-1-carboxamide)dizinc(II) Perchlorate (9c):** A mixture of **9b** (51 mg, 0.1 mmol) and copper(II) bisperchlorate hexahydrate (74 mg, 0.2 mmol) in methanol (10 mL) was refluxed for 2 h to yield **9c** (80 mg, 77%) as an oil. − UV/Vis (CH_3CN): λ_{max} (lg ε) = 210 nm (3.375), 684 (1.945). − MS (FAB): *m*/*z* (%) = 939 (1) [M − ClO_4]$^+$, 136 (100).

***N*,*N*′-(Hexane-1,6-diyl)-Bridged 1,6-Bis(1,4,7,10-tetraazacyclododecane-1-carboxamide)dicopper(II) Perchlorate (9d):** A mixture of **9b** (51 mg, 0.1 mmol) and copper(II) bisperchlorate hexahydrate (74 mg, 0.2 mmol) in methanol (10 mL) was refluxed for 2 h to yield **9d** (80 mg, 77%) as an oil. − UV/Vis (CH_3CN): λ_{max} (lg ε) = 210 nm (3.375), 684 (1.945). − MS (FAB): *m*/*z* (%) = 938 (1) [M − ClO_4]$^+$, 136 (100). − $C_{24}H_{52}Cl_4Cu_2N_{10}O_{18}$ (1037.6): calcd. C 27.78; H 5.05; N 13.50; found C 28.12; H 5.20; N 13.81. Molecular mass 1038 (MS).

***N*,*N*′-(6-Methyl-1,3-phenylene)-Bridged 1,3-Bis(1,4,7,10-tetraazacyclododecane-1-carboxamide)dizinc(II) Tetraperchlorate (11d):** A solution of **11c** (225 mg, 0.43 mmol) and zinc(II) bisperchlorate hexahydrate (318 mg, 0.86 mmol) in methanol (25 mL) was treated according to GP2 to yield **11d** (440 mg, 95%) as a white solid. − ^{1}H NMR (D_2O): δ = 2.23 (s, 3 H), 3.32 (m, 24 H), 3.77 (m, 8 H), 7.23 (m, 2 H), 7.34 (s, 1 H). − ^{13}C NMR: δ = 17.4 (+), 44.4 (−), 45.3 (−), 45.4 (−), 47.0 (−), 47.2 (−), 47.8 (−), 48.2 (−), 123.2 (+), 123.9 (+), 131.7 (+), 133.2 (C_{quat}), 136.6 (C_{quat}), 136.8 (C_{quat}), 159.0 (C_{quat}), 160.1 (C_{quat}). − IR (KBr): $\tilde{\nu}$ = 3419 cm^{-1}, 2928, 1637. − MS (FAB): *m*/*z* (%) = 947 (1) [M − ClO_4^-]$^+$, 99 (100).

***N*,*N*′-(6-Methyl-1,3-phenylene)-Bridged 1,3-Bis(1,4,7,10-tetraazacyclododecane-1-carboxamide)dicopper(II) Tetraperchlorate (11e):** A solution of **11c** (20 mg, 0.04 mmol) and copper(II) bisperchlorate hexahydrate (29 mg, 0.08 mmol) in methanol (25 mL) was treated according to GP2 to yield **11e** (40 mg, 95%) as a blue solid. − MS (FAB): *m*/*z* (%) = 943 (8) [M − ClO_4^-]$^+$, 99 (100).

X-ray Crystallographic Study: Crystal structure determination of compound **2**·4CH_3OH. Crystal data: $C_{32}H_{72}N_8O_8$, triclinic, $P\bar{1}$, *a* = 6.518(2), *b* = 12.424(4), *c* = 13.498(4) Å, α = 67.58(2), β = 88.09(2), γ = 88.96(3)°, *U* = 1009.9 Å^3, *Z* = 1, *T* = −130 °C. Data collection: 3899 reflections were recorded to $2\theta_{max}$ 50° (Mo-K_α radiation) on a Stoe STADI-4 diffractometer equipped with a Siemens LT-2 low temperature device. Merging equivalents gave 3562 unique reflections, which were used for all calculations. Structure refinement: The structure was refined against F^2 (program SHELXL-93, G.M. Sheldrick, Univ. of Göttingen) to *wR*2 = 0.148, *R*1 = 0.0609 for 237 parameters and 201 restraints; *S* = 1.06, max Δρ = 0.20 e Å^{-3}. H atoms were refined according to type, variously

freely (NH and OH), as rigid methyl groups, or using a riding model.

Crystallographic data (excluding structure factors) for the structure(s) included in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-151717. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) + 44-(0)1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft (grant Ko 1537/2-1). M. P. thanks the State of Niedersachsen for a graduate fellowship. We thank Mrs. Helga Leffler-Schuster for experimental support and Prof. R. Moss, Rutgers University, U.S.A., for his help in phosphodiester hydrolysis measurements.

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Received November 6, 2000
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