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Ligand and Cosubstrate Effects on the Hydrolysis of Phosphate Esters and DNA with Lanthanoids☆

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Attempts are described to enhance the catalytic activity of lanthanide ions by providing cosubstrates with an increasing number of nucleophilic groups or by attaching corresponding polyols covalently to stronger metal-binding centers like diazacrown ethers. Although the kinetic effects on the hydrolysis of bis(4-nitrophenyl) phosphate (**BNPP**) and of supercoiled plasmid DNA are only moderate, their dependence on cosubstrate structure and polyol configuration unambiguously demonstrates that these are involved in the slow step of the reactions. Thus, addition of gluconic acid to a $LaCl_3$ solution leads to a marked increase of DNA cleavage from 20 to 71%. Naphthyl units covalently bound to the diazacrown show little effect on **BNPP** hydrolysis, but enhance DNA hydrolysis again from e.g. 38% (RF II) to 60%. Preliminary affinity measurements with calf thymus DNA, using a fluorimetric assay with ethidium bromide, show for polyamines a dependence on the number of ligand charges as observed earlier. The naphthyl compound is characterized by a high affinity; preliminary NMR data indicate that the naphthyl units intercalate into DNA.

Lanthanoid ions belong to the strongest catalyst for the hydrolysis of phosphate diesters with experimentally realized rate accelerations by factors of up to 10^6 in the presence of around 1 mM cation concentration[1–6]. Natural nucleases, phosphatases etc. still exhibit a much higher activity, based on an array of functionalities in the active site together with e.g. one or more zinc atoms as activating metal ion[7]. We decribe here attempts to increase the activity of lanthanoids by providing suitable cosubstrates, in particular by attaching cosubstrates covalently to metal-binding centers or by DNA intercalating functionalities[5a,b].

The effect of additional nucleophilic centers such as hydroxyalkyl groups covalently bound to metal complexes on the hydrolysis of carboxylic esters was investigated by Kimura et al.[8] and on the hydrolysis of phosphate esters recently by Morrow et al.[4e]. However, reactions with such systems usually cannot, or only slowly, show turnover since after transesterification the liberation of the covalently bound residue is then the rate-determining step. In this paper we study the use of polyol-containing cosubstrates or corresponding functions bound to the metal complex. As outlined recently[5b] vicinal hydroxy groups may give rise to the formation of a cyclic phosphate ester in the first step, which can be produced very rapidly due to the proximity of the second nucleophilic group or high local concentrations. Furthermore, these cyclic esters are then cleaved much faster than the starting ester. The presence of a second vicinal hydroxy group is the reason for the much higher rate of RNA cleavage compared to DNA hydrolysis[7]. Instead of the second nucleophile in the *substrate* one should be able to provide it together with or within the *metal complex*. It should be advantageous to deliver not only two vicinal hydroxy groups: if there are more of these, the phosphate formed from diesters can "zip" along the polyol chain until the inorganic phosphate is liberated. As another activating possibility we briefly investigated also the introduction of additional groups of higher nucleophilicity into cosubstrates or metal complexes.

Results and Discussion

In order to obtain a variety of polyol chains covalently bound to metal-binding centers one could try to use *N*-glycosides. These can be prepared by standard procedures[9] from amine precursors like ethylenediamine. In view of the known hydrolytic instability of the glycosides[10] the lifetime of ethylenediamine glycoside[9] as model compound was tested by treating its 5 mM solution in D_2O with 2.5 mM of $EuCl_3$. After adjustment of the pH to 5.0 by addition of DCl the hydrolysis was complete within 5 min as revealed by NMR spectroscopy; even at pH 7.5 the stability was insufficient.

Derivatives containing several hydroxy groups which are hydrolytically stable in the presence of lanthanoids were then obtained by reductive aminations according to Hase et al.[11]; for these we found $NaBH_4$ to be superior to $NaBH_3CN$. The yields usually ranged between 50 and 70%;

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with increasing number of amino groups the yields decreased as a consequence of increasing Amadori rearrangements.

1,10-Diaza-18-crown-6 Derivatives

These derivatives were were obtained by nucleophilic substitution at the nitrogen atoms of the parent diazacrown[12,13] (also commercially available), e.g. with *p*-(bromomethyl)benzonitrile, and subsequent reduction to the amine, or similarly with other α-bromo-ω-cyano derivatives. The coupling with polyol chains was performed as described above. The intercalating naphthyl derivative **34e** was prepared by condensation of crown compound **38** bearing terminal amino groups with naphtaldehyde under reductive conditions.

The hydrolysis kinetics of bis(4-nitrophenyl) phosphate (**BNPP**) (Tables 1–3) and of double-stranded plasmid DNA was determined by spectrometry (**BNPP**) or by electrophoresis (DNA, Figure 1) as described earlier[14]. The kinetics of both compounds indicates excellent first-order[5a,b]. After having secured earlier[5a,b] first-order behavior also for the DNA cleavage, we describe in the present paper the catalytic effects with the nucleic acid only by percentage conversion under standard conditions with $5 \cdot 10^{-4}$ M Eu^{3+} (Tables 4, 5, Figure 1). While **BNPP** undoubtedly undergoes hydrolytic cleavage due to its excellent leaving group which is liberated as *p*-nitrophenolate, radical cleavage is known to be extremely fast in the formation of the open circular form RF II from the supercoiled form RF I of plasmid DNA[15] and could perhaps contribute also to the reactions catalyzed by lanthanoids. However, the hydrolytic pathway, which is the desired one for potential biotechnological and medical uses, is supported by several findings: [i] we never observed products except RF II, whereas radical cleavage leads to the formation of RF III and others[16]; [ii] the presence of hydrogen peroxide in concentrations up to 10^{-3} M does not affect the observed conversion to RF II in control runs with Eu^{3+}. In contrast, even 10^{-4} H_2O_2 with Cu^{2+} salts leads to a very large increase of conversion[16]; [iii] Komiyama et al.[3] report only on the formation of hydrolysis products from dinucleotides, even with Ce^{4+} ions which are known to be more redox-active than the lanthanoids used in the present investigation; [iv] similarly, Sessler et al. observed no products of oxidative RNS cleavages with Eu complexes of porphyrin analogs[17].

Cosubstrate Effects

A great number of cosubstrates (Scheme 1) were used, mostly at rather high concentrations in order to enhance the binding to the lanthanoid ion. It is known that polyols with structures such as **1** to **6**, or **9** bind lanthanoid ions very weakly in water at neutral pH[18], e.g. with $K = 4$ for sorbital (**6**) and Eu^{3+}; this leads to only about 16% complex formation under the conditions used. Nevertheless, the simple polyols exhibit interesting variations in rate with both **BNPP** and with DNA, unambiguously indicating *cosubstrate participation in the rate-limiting step of hydrolysis.* Addition of proton-abstracting bases like **12** or **13**[19] to the most effective sorbitol (**6**) leads to no further rate increases with **BNPP** (Table 1). With DNA a substantial increase in conversion (Table 4) is found with naturally occurring glycerol (**2**) (it should be kept in mind that the percentage appears to be only moderate, in view of the highest possible effect of 100% achievable with a very efficient catalyst like an enzyme). Generally, the cosubstrate effect decreases with increas efficiency of the chosen metal ion (Table 4).

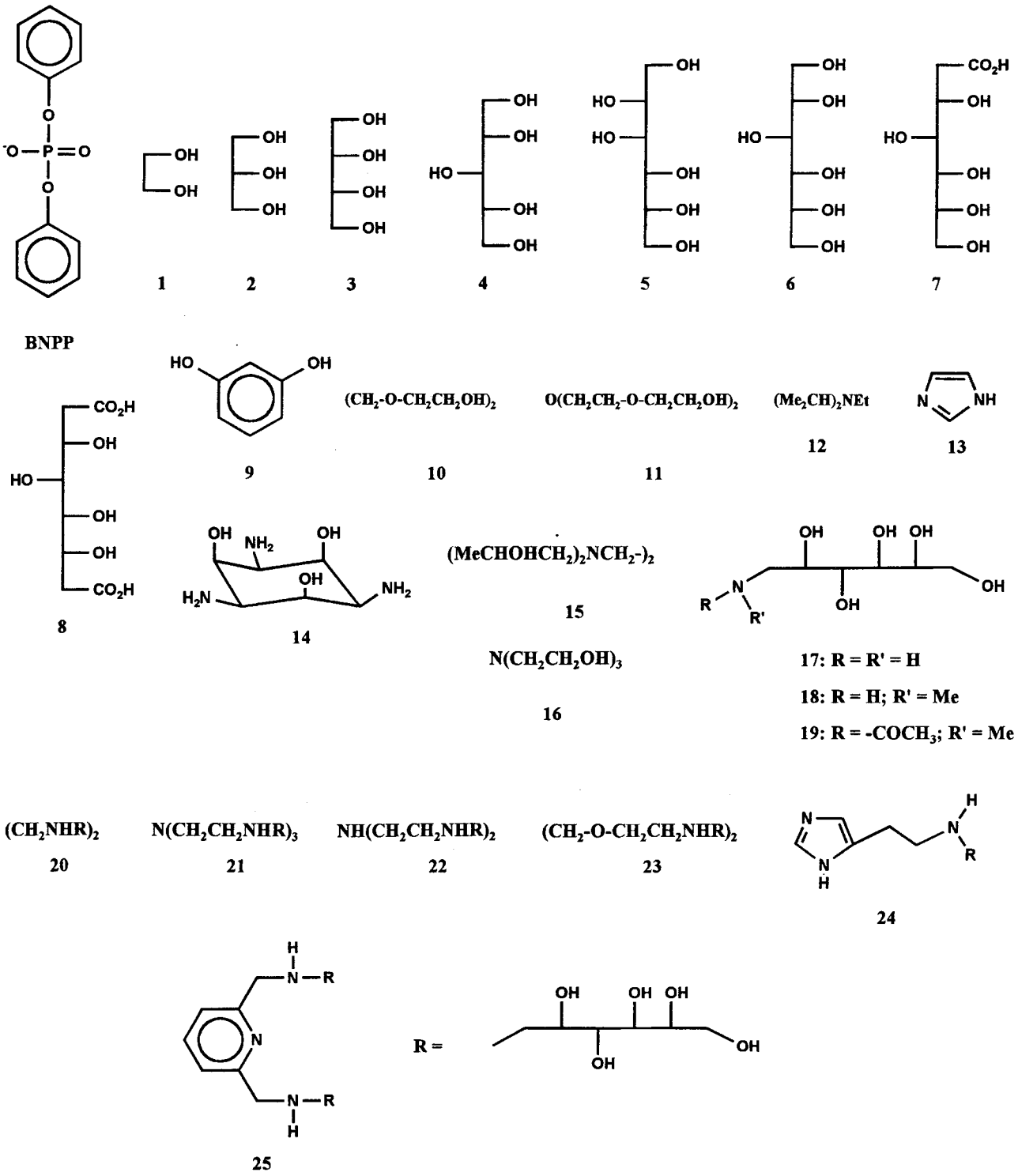
Negative charges in ligands like **7** and **8** enhance binding to the metal ions, but with **BNPP** usually lead to rate decreases due to electrostatic repulsion, or at least to a diminished positive charge in the vicinity of the metal ions which have to react with the negatively charged substrate. With DNA, however, an increase in the conversion (Table 4) from e.g. 20 to 71% RF II with La^{3+} was found in the presence of gluconic acid (**7**). Although this is reminiscent of the presence of acidic amino acids in the active centers of phosphatases[7], the inverted rate effects occurring with Eu^{3+} or Yb^{3+} show that there is no clear rationalization for these observations at the present time. The rate-decreasing effect of negative charges in cosubstrates was also observed with polyols like **14** ("taci"[20]), in which the hydroxy groups are partially deprotonated even in water at neutral pH. This may, besides conformational disadvantages, also be the reason why other covalent combinations of polyol with amine, amide[21], or thiol fragments in cosubstrates such as **15** to **33**, or **37** lead to rate decreases with **BNPP** *and* with DNA. The only exception weas found with **17** and **BNPP**. However, the efficiency does not really decrease as distinctly as it seems if one considers that *without* attached polyols many nitrogen-containing ligands like ethylenediamine alone show marked rate *de*creases[5a]. The often observed rate decrease caused by the addition of polyamines[5a,b] is attributed to the formation of ammonium ions in high concentrations upon protonation of the nitrogen functions, with subsequent competition between the substrate phosphate anions and the catalytically active lanthanoid ions. Only strong metal binders such as the cryptand **35** are not protonated under the conditions used, remain neutral and therefore retain the catalytic activity of the complex.

Polyols and Potential Intercalators Covalently Attached to Strong Metal Complexers

As a macrocycle which is known to have larger binding constants with lanthanoids[22] we chose the diaza crown ether **34**. The cryptand **35** would be more suitable because of its high thermodynamic and kinetic complex stability and yet surprisingly high catalytic activity toward **BNPP**[5a] (Table 3) and also DNA (Table 5). However, incorporation of polyol chains into the cryptand is synthetically more difficult and may well lead to rate decreases as a result of the completely occupied metal binding sites.

The presence of one hydroxy group in the side chain (compound **34b**) leads already to a rate increase with **BNPP** in accordance with a recent report of Morrow et al.[4e], but no increase in rate was found with DNA (Table 5). Incorporation of a polyol chain (**34d**) leads to negligible effects

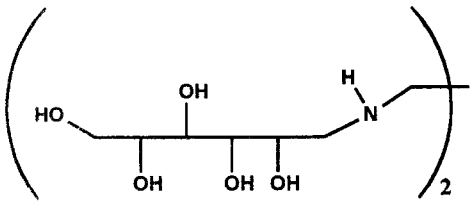
Scheme 1



with DNA, but to the highest ligand-induced rate increase by a factor of 4 with **BNPP** found so far.

Ligands **34c** and **34e** represent complexation agents which are potential intercalators into double-stranded DNA. In combinazion with **BNPP** they are only expected to show weak, if any effects by stacking between the aromatic parts of substrate and catalyst. This results from the small rate increase observed with the more lipophilic naphthalene-containing ligand **34e**. With DNA the largest increase in conversion from 38 to 60% was found with the same ligand **34e**, whereas the phenyl derivative **34c** shows only a weak effect. This is in line with the absence of intercalation with phenyl compounds, in contrast to the corresponding naphthalene systems (see below). In order to elucidate the origin of the catalytic efficiency of **34e**, rates were determined at different catalyst concentrations (Table

Scheme 1 (*continued*)



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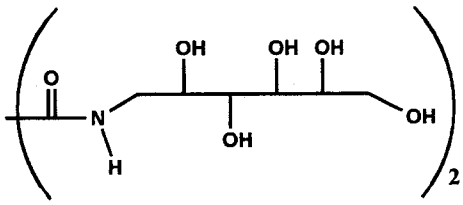

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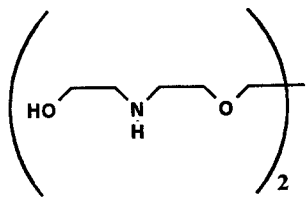

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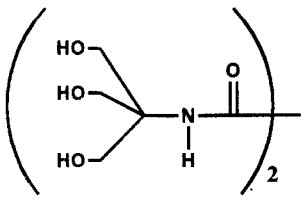

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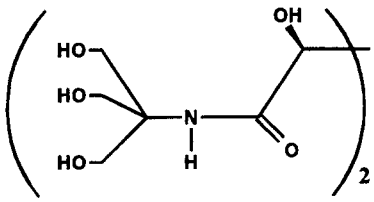

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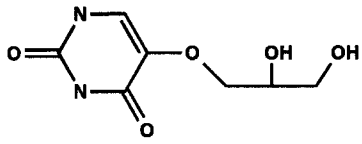

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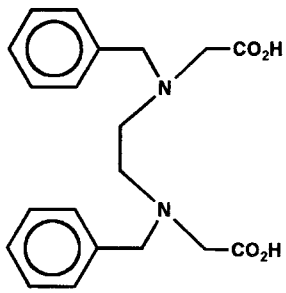

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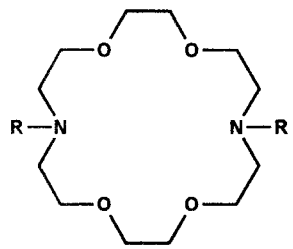
$(HO_2CNHCH_2)_2$

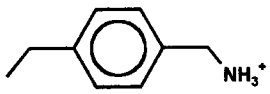
32



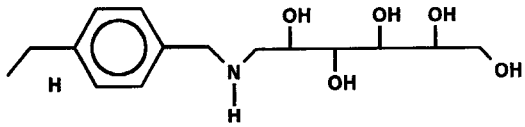
34a: R = H

34b: R = $-CH_2CH_2OH$

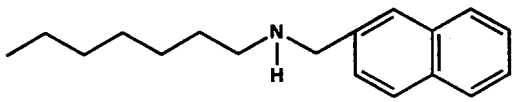
34c: R =



34d: R =

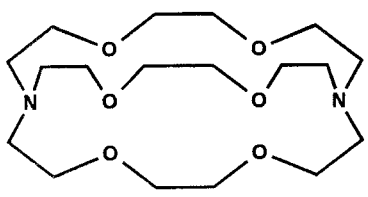


34e: R =



34f: R = $-CH_2CONH_2$

38: R = $-(CH_2)_6NH_2$



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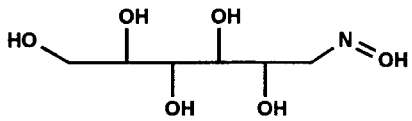

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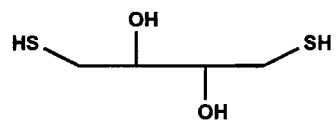

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6), yielding saturation kinetics of the Michaelis-Menten type[5b]. The resulting k_{cat} value indicated an almost 10-fold increase compared with the system containing only Eu^{3+} alone, and a K_M value smaller by a factor of about 2.

Table 1. Hydrolysis of **BNPP** with polyhydroxy cosubstrates and Eu^{3+}[a]

Ligand	Concentration [M]	k_{obs} [1 10^4 s^{-1}]	k_{obs} / $k_{Eu^{3+}}$
-	-	1.67	1.0
1	5 10^{-2}	1.72	1.03
2	5 10^{-2}	1.93	1.15
3	5 10^{-2}	0.9	0.54
4	5 10^{-2}	3.3	2.0
5	5 10^{-2}	3.73	2.23
6	5 10^{-2}	4.22	2.52
	1 10^{-1}	4.1	2.45
6 + 12	5 10^{-2}/ 0.1	1.61	0.96
6 + 13	5 10^{-2}/ 0.01	3.42	2.05
7	5 10^{-2}	0.0092	0.0055
8	5 10^{-2}	< 0.002	0.0012
9	5 10^{-2}	1.48	0.89
10	5 10^{-3}	2.27	1.36
	5 10^{-2}	1.63	0.98
11	5 10^{-2}	1.47	0.88

[a] [**BNPP**] = 3.3 10^{-5} M, [Eu^{3+}] = 5 10^{-3} M, 50°C, pH 7.0; 0.01 M EPPS buffer, k_{obs} = ±1.5%, uncatalyzed rate (J. Chin, X. Zou, *J. Am. Chem. Soc.* **1988**, *110*, 223): k_o = 3 10^{-10} s^{-1}; precipitates 18-crown-6, with *m*-hydroxybenzoic acid and with 3,5-dihydroxybenzoic acid.

Table 2. Eu^3-catalysed hydrolysis of **BNPP** with different ligands; footnote see Table 1 (with [**29**] = [**33**] = 5 10^{-3} M: precipitates)

Ligand	Concentration [M]	k_{obs} (1 10^4 s^{-1})	k_{obs} / $k_{Eu^{3+}}$
--	--	1.67	1.0
14	5 10^{-3}	6.94 10^{-3}	4.1 10^{-3}
15	5 10^{-3}	1.58	0.95
	5 10^{-2}	1.03	0.62
16	5 10^{-3}	1.24	0.74
31	5 10^{-3}	0.845	0.51
17	5 10^{-2}	2.38	1.4
18	5 10^{-2}	1.23	0.74
19	5 10^{-2}	0.12	0.073
	5 10^{-3}	1.05	0.63
20	5 10^{-2}	1.17	0.7
21	5 10^{-3}	0.67	0.4
22	5 10^{-3}	0.0415	0.025
23	5 10^{-3}	1.28	0.77
24	5 10^{-2}	1.4	0.84
25	5 10^{-3}	0.015	0.0089
26	5 10^{-2}	0.73	0.44
27	5 10^{-2}	1.01	0.6
28	5 10^{-3}	1.45	0.87
30	5 10^{-2}	0.139	0.083
	5 10^{-3}	1.44	0.86
32	5 10^{-3}	0.14	0.082
36	5 10^{-2}	0.95	0.57
37	5 10^{-2}	1.46	0.87

Ligand Interactions with DNA

Ligands **20**–**27** and **34a**–**f** contain nitrogen atoms which by protonation are positively charged at neutral pH, and

Table 3. Hydrolysis of **BNPP** with Eu^{3+} and stronger complexing macrocycles; footnotes see Table 1

Ligand	Concentration [M]	k_{obs} (1·10^4 s^{-1})	k_{obs} / $k_{Eu^{3+}}$
--	--	1.67	1.0
34a	5 10^{-3}	1.10	0.66
34b	5 10^{-3}	2.62	1.6
34c	5 10^{-3}	0.67	0.4
34d	5 10^{-3}	6.7	4.0
34e	5 10^{-3}	2.45	1.5
34f	5 10^{-3}	0.321	0.19
35	5 10^{-3}	1.5	0.92

Table 4. Cleavage of plasmid DNA with different lanthanoids and ligands[a]

Lane	Ligand	La^{3+} RF II [%]	Eu^{3+} RF II [%]	Yb^{3+} RF II [%]
1, 13	--	0	0	0
2, 12	metal (alone)	20	38	62
3	**1**	21	39	65
4	**2**	30	54	68
5	**3**	24	37	61
6	**4**	23	34	31
7	**5**	21	33	36
8	**6**	21	28	25
9	**7**	71	32	11
10	**10**	24	38	63
11	**11**	22	36	63

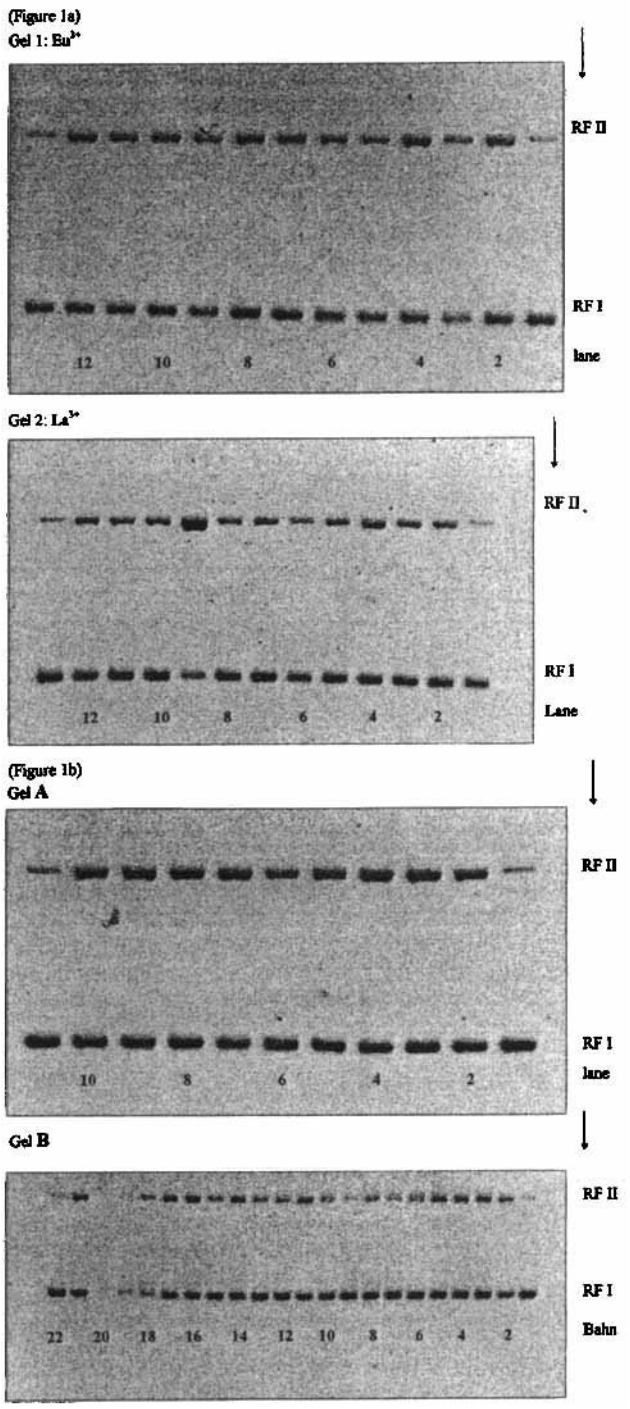
[a] % RF I: corrected for the 5% impurity of starting material RF II. Correction of RF I for decreased stainability by a factor of 1.22, see text. Double runs, error in % RF I or RF II: ±2.5%. Lane in electrophoresis, see Figure 1a. Incubation time 2 h, pH 7.0, 0.01 M EPPS buffer, 37°C, [DNA] = 1.9 10^{-5} M (base pair); [Eu^{3+}] = 5 10^{-4} M, [Ligand] = 5 10^{-3} M.

Table 5. Cleavage of plamid DNA with Eu^{3+} and different ligands; footnote see Table 4[a]

Gel	Lane	Ligand	RF II [%]	Gel	Lane	Ligand	RF II [%]
A	1, 11	--	0	**B**	1, 22	--	0
	2, 10	Eu^{3+}	38		2, 21	Eu^{3+}	38
	3	**23**	31		3	**17**	29
	4	**28**	35		4	**18**	33
	5	**24**	31		5	**19**	31
	6	**30**	22		6	**20**	22
	7	**31**	37		7	**26**	18
	8	**16**	33		8	**22**	17
	9	**9**	34		9	**25**	5
					10	**21**	26
					11	**15**	38
					12	**32**	26
					13	**27**	20
					14	**35**	39
					15	**34f**	22
					16	**34a**	40
					17	**34b**	39
					18	**34c**	45
					19	**34d**	34[a]
					20	**34e**	60[a]

[a] After treatment with ion exchanger; [Eu^{3+}] = 5 10^{-4} M, [Ligand] = 5 10^{-3} M, without: Gel **A** lane 3, 4, 7, 8 and Gel **B** lane 10, 12, 14–20: with [Ligand] = 5 10^{-4} M.

Figure 1. Gel electrophoresis of DNA cleavage (plasmid DNA pBR 322) with different ligands; for identification of lane numbers see Tables 4–6; conditions: pH 7.0, 0.01 M EPPS, 37°C, incubation time 2 h, [DNA] = 1.9 10^{-5} M base pair; [Eu^{3+}] = 5 10^{-4} M, [Ligand] = 5 10^{-5} M, without: Gel **A** lane 3, 4, 7, 8, and Gel **B** lane 10, 12, 14–20 with [Ligand] = 5 10^{-4} M



therefore can contribute to the enhanced binding and in principle to the catalytic efficiency of cosubstrates or catalysts with DNA as substrate. Their interactions with double-stranded calf thymus (CT) DNA were briefly studied by the determination of melting point changes ΔT and by an affinity assay based on fluorescence measurements with ethidium bromide[23] (EB), where the molarity of the ligand necessary for lowering the EB fluorescence by 50% was measured as C_{50} value. For a great number of polyamines a fairly linear correlation between the number of ligand charges and C_{50} values was established e.g. with C_{50} around 10^{-2} M for 2 charges in the ligand and C_{50} around 10^{-6} for 4 charges such as in spermine[23,24a]. As shown earlier these values generally reflect polyamine binding to DNA with a free energy of 5+/−1 kJ per mol and per salt bridge[24]. The measurements (Table 7) were carried out as described before[14,24] and are in line with earlier results, indicating that largely the number of positive charges in the ligand determines the C_{50} values, with small and inconsisent effects on melting points[24,25]. Ligand **34e**, however, shows an unusually low C_{50} value, even in comparison with that of spermine (Table 7). The high affinity already suggests that the naphthyl units of this ligand are indeed intercalated as planned. We recently showed that NMR upfield shifts of intercalating ligand parts by CIS >0.1 ppm and an increase of the line width $W_{1/2}$ of the corresponding signals by >15 Hz unambiguously indicate intercalation[26]. Unfortunately, the application of this method was limited by the low solubility of the polyamine **34e**-CT-DNA complex. The solubility had to be increased by addition of NaCl (0.5 M concentration) which is known to lower the affinity. It was therefore necessary to perform measurements under conditions of incomplete complexation, which lowers the shift and $W_{1/2}$ changes correspondingly[24]. Nevertheless, with [**34e**] around 10^{-3} M and a similar DNA concentration per base pair an upfield shift of the signals of the naphthyl protons by up to 0.1 ppm with a line width around 15 Hz was observed. This confirms at least intercalation, however not bisintercalation in which both naphthyl rings are inserted into DNA.

Table 6. DNA cleavage with the Eu^{3+} complex of ligand **34e**: saturation kinetics; see footnote Table 4; lane in electrophoresis, see Figure 1

Lane	Ligand	Concentration of **34e** · Eu^{3+} [1·10^4 M]	RF II[a] [%]	k [1 10^2 min^{-1}]
1, 10	--	--	0	
2, 9	Eu^{3+}	--	38	
3	**34e**	25	--	
4		12.5	71	1.02
5		5	60	0.76
		2.5	42	0.45
		1.25	25	0.24
6		0.6	16	0.15

[a] After treatment with ion exchanger.

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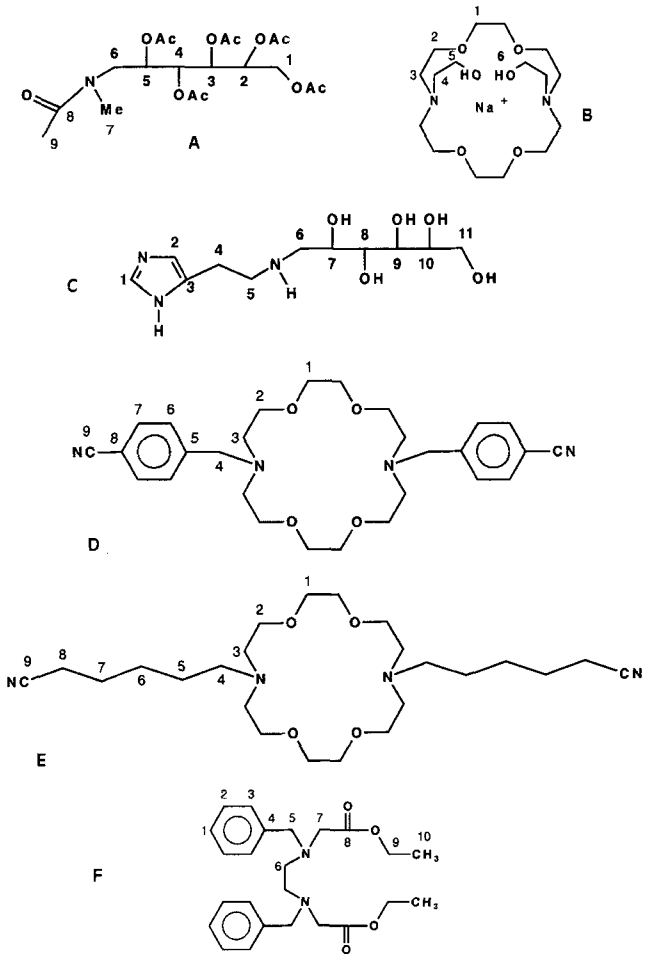
Experimental

Bis(4-nitrophenyl) phosphate (**BNPP**), reagent grade inorganic salt, buffers EPPS [*N*-(2-hydroxyethyl)piperazine-*N'*-propanesulfonic acid] and TRIS [tris(hydroxymethyl)aminomethane], glucose,

Table 7. Ligand interactions with CT-DNA[a]

Ligand	Number of charges in solution (pH 7.0)	C_{50} [M]	ΔT_M [°C]
20	2	$9.8\ 10^{-3}$	--
21	3	$1.3\ 10^{-5}$	--
22	2	$4.2\ 10^{-3}$	--
23	2	$5.6\ 10^{-4}$	1.5
24	2	$1.8\ 10^{-3}$	1.8
25	2	$2.5\ 10^{-4}$	--
26	2	$5.6\ 10^{-4}$	1.0
27	0	$7.6\ 10^{-2}$	--
34a	2	$1.3\ 10^{-3}$	--
34c	4	$1.9\ 10^{-6}$	3.5
34d	4	$1.2\ 10^{-5}$	
34e[b]	3	$2.0\ 10^{-7}$	--
34e	3	$4.0\ 10^{-7}$	2.0
34f	2	$3.3\ 10^{-3}$	--
$[H_2N(CH_2)_3NH\text{-}(CH_2)_2]_2$[c]	4	$1.2\ 10^{-6}$	5.2
$[Me_3N^+\text{-}(CH_2)_3\text{-}NMe_2^+\text{-}(CH_2)_2]_2$ [d]	4	$2.1\ 10^{-6}$	5.3

[a] C_{50} values in [L], concentrations [M] for 50% decrease of ethidium bromide fluorescence; ΔT_M change of DNA melting points upon addition of $1\ 10^{-6}$ M ligand. B-DNA (calf thymus) concentration corresponds in each case to A_{260} = 0.5 DNA, 0.01 M SHE buffer (9.4 mM NaCl, 10 mM EDTA, 2 mM HEPES), pH 7.0. – [b] Without EDTA. – [c] Spermine. – [d] Permethylated spermine [b) and c) see ref.[24a]].



ethylenediamine and tris(aminoethyl)amine were purchased from commercial sources and used without further purification. α-Bromo-*p*-tolunitrile[27], **34a** (Kryptofix2.2®)[13], **27**[28], **30**[28], **31**[29], **34f**[30], **35**[31], and **36**[32] were prepared according to literature methods. Compound **14** was a gift of Prof. Dr. *K. Hegetschweiler* (Saarbrücken); 2,6-pyridinedicarbaldehyde was a gift of *M. Hanisch* (Saarbrücken).

Reaction solutions were prepared by combining appropriate amounts of metal salt stock solutions, titrated with EDTA[33], ligand, and buffer and subsequent dilution with water to the correct volume. Because of the slow establishment of the complexation equilibrium, the solutions with the compounds **34a**–**f** and the metal salt were equilibrated for 8 d after which the pH was adjusted[22b,34,35] (Knick Digital pH Meter 646).

Kinetic Studies with **BNPP** and with DNA were carried out as described earlier[14]. The extinctions were measured continuously with a Kontron UVIKON 860 UV/Vis spectrometer thermostated with a Lauda RCS/RC 6.

C,H,N Determinations: Elemental Analyzer Modell 1106, Carlo Elber. – Melting points: Apparatus from Gallenkamp. – NMR: Bruker AM 400. – Fluorescence measurements: F-2000 spectrometer from Hitachi.

Synthesis and Characterization of the Ligands

2,3,4,5,6-Penta-O-acetyl-1-deoxy-1-(N-methylacetamido)-D-glucitol: A solution of 0.5 g (2.6 mmol) of *N*-methylglucamine in 4.6 ml (57 mmol) of anhydrous pyridine is cooled in an ice bath, and 2.4 ml (25.4 mmol) of acetanhydride is added slowly. The mixture is stirred for 2 d at room temp. (TLC control with $CHCl_3$/MeOH, 3:1). The reaction mixture is poured into 50 ml of ice/water and extracted several times with 15 ml of $CHCl_3$. The combined organic layers are dried with Na_2SO_4, and the solvent is evaporated to yield 1.06 g (91%) of a colorless oil. – ^{1}H-NMR data of the *cis* isomer are identical with those reported by Patel and Poller[36], who, however, did not describe the synthesis. The reaction mixture contains the *cis* and *trans* isomers in a ratio of 3:1. The signals of 1a- and 1b-H are a well defined dd (reported in ref.[36] as multiplet only). – ^{1}H NMR ($CDCl_3$, TMS, compare formula **A**): *cis* Isomer: δ = 4.10 (dd, 1H, 1a-H), 4.28 (dd, 1H, 1b-H), 5.04 (m, 1H, 2-H), 5.48 (dd, 1H, 3-H), 5.29–5.34 (m, 1H, 4-H), 5.29–5.34 (m, 1H, 5-H), 3.55 (dd, 1H, 6a-H), 3.61 (dd, 1H, 6b-H), 3.0 (s, 3H, 7-H), 2.15 (s, 3H, 9-H), 2.04–2.12 (m, 18H, OAc). Coupling constants (Hz): $J_{6a,6b}$ = 14.4, $J_{6b,6a}$ = 14.3, $J_{1b,2}$ = 5.6, $J_{6b,5}$ = 7.5, $J_{1a,1b}$ = 12.4, $J_{3,2}$ = 6.8, $J_{6a,5}$ = 3.9, $J_{1a,2}$ = 3.1, $J_{3,4}$ = 4.0. – *trans* Isomer: δ = 4.13 (dd, 1H, 1b-H), 5.37 (dd, 1H, 3-H), 3.65 (dd, 1H, 6a-H), 3.42 (dd, 1H, 6b-H), 2.87 (s, 3H, 7-H), 2.12 (s, 3H, 9-H), 2.04–2.12 (m, 18H, OAc). Other signals were overlapping with the *cis* isomer. Coupling constants (Hz): $J_{6a,6b}$ = 15.6, $J_{6b,5}$ = 10.4, $J_{6a,5}$ = 2.6, $J_{1a,1b}$ = 12.6, $J_{1b,2}$ = 5.2, $J_{3,4}$ = 4.0, $J_{3,2}$ = 7.2.

1-Deoxy-1-(N-methylacetamido)-D-glucitol (**19**): 1.0 g (2.3 mmol) of the compound described above is dissolved in 10 ml of anhydrous methanol. A freshly prepared solution of sodium methoxide (13 mmol Na in 15 ml of methanol) is added under nitrogen at room temp. The solution is stirred for 2 d, and a small portion of a strong acidic ion exchanger (Amberlyst 15, Aldrich) is added. The mixture is allowed to stand at room temp. with occasional shaking for 3 h until the pH of the solution is neutral. Afterwards the ion exchanger is filtered off and the solvent evaporated. The obtained solid is recrystallized from ethanol to yield 552 mg (98%)

of colorless crystals, m.p. 119–120 °C (ref.[37] 119–121 °C). The *cis* and *trans* isomers are formed in a ratio of 1:1; no literature NMR-data are available for the trans-isomer. – ^{1}H NMR (D_2O, TMSP, the signals of the isomers overlap partially, numbering as above): δ = 4.13–4.20 (m, 2H, 5-H *cis* and *trans*), 3.85–3.97, 3.71–3.83, 3.57–3.64 (m, 14H, 1,3,4,5,6-H), 3.25 (s, 3H, 7-H *cis*), 3.07 (s, 3H, 7-H *trans*), 2.28 (s, 3H, 9-H *cis*), 2.25 (s, 3H, 9-H *trans*). – ^{13}C NMR: δ = 62.87 (C-1), 70.36 (C-2), 70.36 (C-3), 71.34 (C-4), 71.95 (C-5 *cis*), 71.74 (C-5 *trans*), 37.71 (C-7 *cis*), 34.19 (C-7 *trans*), 181.42 (C-8 *cis*), 174.81 (C-8 *trans*), 21.13 (C-9 *cis*), 20.76 (C-9 *trans*).

General Synthesis of the 1-Deoxy-D-glucitolyl Derivatives: The reactions of amines and glucose were carried out similarly to the method of Hase et al.[11]. To a solution of 1.0 g (5.5 mmol) of D-glucose in 10 ml of anhydrous methanol 2.5 mmol of diamine and 285 µl (5 mmol) of acetic acid are added. The mixture is stirred for 30 min at room temp., and 380 mg (10 mmol) of $NaBH_4$ is slowly added. The solution is stirred at room temp. for 2 d. The reaction is followed by TLC (MeOH/$CHCl_3$, 2:1, detection with 5% sulfuric acid in ethanol and warming). The solution is evaporated to dryness, the residue taken up in as a small amount of water as possible. Column chromatography is carried out on a strongly acidic ion exchanger (Amberlyst 15, H^+ form, column length: 30.0 cm, diameter: 1.0 cm). The column is eluted with water until the eluate is neutral. The compound is then eluted with 0.6 M ammonia, the product migrating with the ammonia front is collected when the pH of the eluate is basic. These fractions (about 100 ml) are evaporated to dryness. The residue is taken up in 25 ml of anhydrous methanol, and 2 ml of a 11 M methanolic HCl solution is added to the solution causing precipitation of the hydrochloride which is recrystallized from MeOH/H_2O. (The free amines are not stable enough for storage.)

N,N'-Bis(1-deoxy-D-glucitolyl)ethylenediamine Dihydrochloride (**20**): Yield 836 mg (72.5%) of a colorless powder of the hydrochloride, m.p. 191–192 °C. – NMR (D_2O, TMSP, assignment confirmed by a homo COSY): Free amine, ^{1}H NMR: δ = 3.61–3.65 (m, 4H, 1a, 1b-H), 3.74–3.78 (m, 2H, 2-H)*, 3.82 (dd, 2H, 3-H), 3.74–3.78 (m, 2H, 4-H)*, 3.89 (m, 2H, 5-H), 2.68 (dd, 2H, 6a-H), 2.75–2.80 (m, 2H, 6b-H), 2.75–2.80 (m, 4H, 7-H). – ^{13}C NMR: δ = 62.45 (C-1a,b), 70.67 (C-2), 70.67 (C-3), 70.67 (C-4), 70.5 (C-5), 50.4 (C-6a), 50.04 (C-6b), 47.08 (C-7). Coupling constants (Hz): $J_{6a,6b}$ = 12.4, $J_{6a,5}$ = 4.2, $J_{3,2}$ = 11.8, $J_{3,4}$ = 2.9. – Hydrochloride, ^{1}H MNR: δ = 3.65 (m, 4H, 1a,b-H), 3.76 (m, 2H, 2-H), 3.81 (dd, 2H, 3-H)*, 3.82–3.84 (m, 2H, 4-H)*, 4.13 (m, 2H, 5-H), 3.28 (dd, 2H, 6a-H), 3.36 (dd, 2H, 6b-H), 3.57 (s, 4H). – ^{13}C NMR: δ = 63.79 (C-1a,b), 71.56* (C-2), 71.82* (C-3), 72.08* (C-4), 69.26 (C-5), 51.37 (C-6a), 51.37 (C-6b), 44.39 (C-7). Coupling constants (Hz): $J_{6a,6b}$ = 12.7, $J_{6a,5}$ = 9.7, $J_{6b,5}$ = 3.2, $J_{3,4}$ = 2.8. – $C_{14}H_{34}Cl_2N_2O_{10}$ (461.3): calcd. C 36.45, H 7.43, N 6.07; found C 36.37, H 7.26, N 5.96.

N,N',N''-Tris[2-(1-deoxy-D-glucitolylamino)ethyl]amine Tetrahydrochloride (**21**): Synthesis as described above for **20** with 1.0 g (5.5 mmol) of glucose, 0.244 g (1.67 mmol) of tris(2-aminoethyl)amine, and 286 µl (5 mmol) of acetic acid. Yield: 560 mg (53%) of a colorless powder (not recrystallized, precipitated by addition of diethyl ether and stirring in diethyl ether); m.p. 175 °C. – ^{1}H NMR (D_2O, TMSP): δ = 3.66 (m, 6H, 1a,b-H), 3.76 (m, 3H, 2-H), 3.82 (dd, 3H, 3-H)*, 3.84 (dd, 3H, 4-H)*, 4.14 (m, 3H, 5-H), 3.24 (dd, 3H, 6a-H), 3.26–3.34 (m, 3H, 6b-H), 3.26–3.34 (m, 6H, 7-H), 2.98 (t, 6H, J = 6.20, 8-H). – ^{13}C NMR (D_2O, TMSP): δ = 62.86 (C-1a,b), 71.22* (C-2), 70.99* (C-3), 70.68* (C-4), 68.17 (C-5), 50.04 (C-6a), 50.04 (C-6b), 48.85 (C-7), 44.63 (C-8). Coupling constants (Hz): $J_{6a,6b}$ = 12.9, $J_{6a,5}$ = 9.5, $J_{6b,5}$ = 3.4, $J_{4,5}$ = 2.17, $J_{3,4}$ = 2.93, $J_{3,2}$ = 12.6. – $C_{24}H_{58}Cl_4N_4O_{15}$ (784.6): calcd. C 36.74, H 7.45, N 7.14; found C 36.93, H 7.28, N 7.19.

N,N''-Bis(1-deoxy-D-glucitolyl)diethylenetriamine Trihydrochloride (**22**): Synthesis as described for **20** with 1.0 g (5.5 mmol) of glucose, 271.5 µl (2.5 mmol) of diethylenetriamine, and 286 µl (5 mmol) of acetic acid affording 600 mg (56%) of colorless powder; m.p. 209 °C (decomposition). – NMR (D_2O, TMSP): ^{1}H: δ = 3.66 (m, 4H, 1a,b-H), 3.76 (m, 2H, 2-H), 3.81 (dd, 2H, 3-H)*, 3.83 (dd, 2H, 4-H)*, 4.13 (m, 2H, 5-H), 3.28 (dd, 2H, 6a-H), 3.36 (dd, 2H, 6b-H), 3.50–3.55 (m, 4H, 7-H), 3.50–3.55 (m, 4H, 8-H). – ^{13}C: 65.2 (C-1a,b), 73.51* (C-2), 73.26* (C-3), 72.98* (C-4), 70.65 (C-5), 52.78 (C-6a), 52.78 (C-6b), 46.04# (C-7), 45.87# (C-8). Coupling constants (Hz): $J_{6a,6b}$ = 12.8, $J_{6a,5}$ = 9.6, $J_{6b,5}$ 3.0, $J_{4,5}$ = 2.3 $J_{3,4}$ = 2.7, $J_{3,2}$ = 11.8.

N,N'-Bis(1-deoxy-D-glucitolyl)-2,2'-ethylenebis(oxy)bis(ethylamine) Dihydrochloride (**23**): Synthesis as described for **20** with 1.0 g (5.5 mmol) of D-glucose, 402.7 µl (2.5 mmol) of ethylenebis(oxy)bis(ethylamine), and 286 µl (5 mmol) of acetic acid. After the solid residue has been taken up in methanol and reprecipitated with a methanolic HCl solution, it is recrystallized from ethanol/methanol to yield 1.2 g (80%) of a colorless powder, m.p. 112–113 °C. – NMR (D_2O, TMSP): ^{1}H: δ = 3.65 (m, 4H, 1a,b-H), 3.73–3.78 (m, 2H, 2-H), 3.79–3.85 (dd, 2H, 3-H)*, 3.82–3.85 (m, 2H, 4-H)*, 4.13 (m, 2H, 5-H), 3.23 (dd, 2H, 6a-H), 3.31 (dd, 2H, 6b-H), 3.36 (t, 4H, 7-H), 3.82–3.85 (m, 4H, 8-H), 3.75 (s, 4H, 9-H). – ^{13}C: δ = 63.3 (C-1a,b), 71.61* (C-2), 71.37* (C-3), 71.19* (C-4), 68.67 (C-5), 50.16 (C-6a), 50.16 (C-6b), 47.62 (C-7), 65.86 (C-8), 70.24 (C-9). Coupling constants (Hz): $J_{6a,6b}$ = 13.1, $J_{6a,5}$ = 9.7, $J_{6b,5}$ = 3.4, $J_{3,4}$ = 2.91, $J_{7,8}$ = 4.45. – $C_{18}H_{42}Cl_2N_2O_{10}$ (549.4): calcd. C 39.35, H 7.70, N 5.10; found C 39.15, H 7.55, N 5.03.

N-(1-Deoxy-D-glucitolyl)histamine (**24**): 1.0 g, (5.5 mmol) of glucose, 1.015 g (5.5 mmol) of histamine dihydrochloride, and 0.453 g (5.5 mmol) of sodium acetate are dissolved in 10 ml of methanol and the solution stirred for 30 min; then 0.4 g (10 mmol) of $NaBH_4$ is slowly added. After the column chromatography described above the solid is stirred several times with 5 ml of ethanol, filtered off and dried to yield 770 mg (51%) of a colorless powder; m.p. 140 °C (decomposition). – NMR (D_2O, TMSP): ^{1}H: δ = 7.68 (s, 1H, 1a,b-H), 6.93 (s, 1H, 2-H), 2.74–2.81 (m, 2H, 4-H)*, 2.87 (m, 2H, 5-H)*, 2.67 (dd, 1H, 6a-H), 2.74–2.81 (m, 1H, 6a-H), 3.89 (m, 1H, 7-H), 3.72–3.79 (m, 1H, 8-H)#, 3.86 (dd, 1H, 9-H)#, 3.72–3.79 (m, 1H, 10-H), 3.64 (m, 2H, 11a,b-H). – ^{13}C: δ = 138.2 (C-1a,b), 119.18 (C-2), 137.58 (C-3), 50.24 (C-4), 28.28 (C-5), 52.58 (C-6a), 52.58 (C-6a), 73.19* (C-7), 73.41* (C-8), 73.41* (C-9), 73.41* (C-10), 65.22 (C-11a,b). Coupling constants (Hz): $J_{6a,6b}$ = 12.6, $J_{6a,7}$ = 8.3, $J_{9,8}$ = 2.89, $J_{9,10}$ = 8.2, $J_{6b,7}$ = 3.63. – $C_{11}H_{21}N_3O_5$ (275.3): calcd. C 47.99, H 7.69, N 15.26; found C 47.88, H 7.62, N 14.99.

2,6-Bis[(1-deoxy-D-glucitolylamino)methyl]pyridine Trihydrochloride (**25**): To a solution of 0.27 g (2.0 mmol) of 2,6-pyridinedicarbaldehyde in 25 ml of methanol, 0.725 g (4.0 mmol) of glucamine is added. The solution is heated to 50 °C and stirred for 5 h. During this period a colorless precipitate forms. The solution is cooled to room temp., and 400 mg (10 mmol) of $NaBH_4$ is slowly added. The solution is stirred for further 18 h at room temp. and worked up as described above. After column chromatography and addition of the methanolic HCl, the solution is decanted and the solid dried, yielding 500 mg (54%) of a slightly yellow, very hygroscopic powder (CHN analysis not possible). – NMR (D_2O, TMSP): ^{1}H: δ = 3.66 (m, 4H, 1a,b-H), 3.76 (m, 2H, 2-H), 3.81 (dd, 2H, 3-H)*, 3.85 (dd, 2H, 4-H)*, 4.22 (m, 2H, 5-H), 3.30 (dd, 2H, 6a-H), 3.40 (dd, 2H, 6b-H), 4.57 (d, 2H, 6b-H), 4.57 (d, 2H,

7a-H), 4.51 (d, 2H, 7b-H), 7.49 (d, 2H, J = 7.85 Hz, 9-H), 7.96 (t, 1H, J = 7.8 Hz, 10-H). Coupling constants (Hz): $J_{6a,6b}$ = 12.9, $J_{4,5}$ = 4.8, $J_{7a,7b}$ = 15.1, $J_{6b,5}$ = 9.5, $J_{6a,5}$ = 3.28, $J_{7b,7a}$ = 15.0, $J_{4,3}$ = 2.3, $J_{3,4}$ = 2.9, $J_{3,2}$ = 11.6. – ^{13}C: δ = 63.48 (C-1a,b), 71.82* (C-2), 71.63* (C-3), 71.42* (C-4), 69.02 (C-5), 51.24 (C-6a), 51.24 (C-6b), 50.45 (C-7a), 50.45 (C-7b), 151.3 (C-8), 123.94 (C-9), 140.25 (C-10).

N,N'-Bis(1-deoxy-D-galactitolyl)ethylenediamine Dihydrochloride (**26**): Synthesis as described for **20**; yield 500 mg (44%) of a colorless powder; m.p. 236°C. – NMR (D_2O, TMSP): ^{1}H: δ = 3.66–3.69 (m, 4H, 1-H), 3.96 (m, 2H, 2-H), 3.66–3.69 (m, 2H, 3-H), 3.61–3.67 (m, 2H, 3-H), 4.27–4.29 (m, 2H, 5-H), 3.3 (dd, 2H, 6a-H), 3.37 (dd, 2H, 6b-H), 3.45 (m, 2H, 7a-H), 3.49 (m, 2H, 7b-H). – ^{13}C: 63.24 (C-1), 70.47* (C-2), 70.01* (C-3), 69.44* (C-4), 65.88 (C-5), 51.40 (C-6a), 51.40 (C-6b), 43.46 (C-7a), 43.46 (C-7a). Coupling constants (Hz): $J_{6a,6b}$ = 13.1, $J_{6a,5}$ = 3.6, $J_{6b,5}$ = 9.8. – $C_{14}H_{34}N_2O_{10}$ (461.3): calcd. C 36.45, H 7.43, N 6.07; found C 34.97, H 7.54, N 6.41.

N,N'-Bis(1-deoxy-D-glucitolyl)oxamide (**27**): 7.48 g (41.27 mmol) of glucamine and 2.803 ml (20.6 mmol) of diethyl oxalate are heated in a 50-ml round-bottom flask at 140°C for 1 h. The solid is recrystallized from water/MeOH and placed in a refrigerator for about 12 h. The precipitate is filtered off and dried to furnish 6.5 g (76%) of a colorless powder, m.p. 221°C (dec.). Furhter purification is achieved by heating in anhydrous methanol, filtration and drying. – NMR (D_2O, TMSP): ^{1}H: δ = 3.69 (dd, 2H, 1a-H), 3.73 (dd, 2H, 1b-H, 2H), 3.78–3.85 (m, 2H, 2-H), 3.78–3.85 (m, 2H, 3-H), 3.78–3.85 (m, 2H, 4-H), 4.00 (m, 2H, 5a-H), 3.46 (dd, 2H, 6a-H), 3.58 (dd, 2H, 6b-H). – ^{13}C: δ = 63.74 (C-1a), 63.74 (C-1b), 71.27* (C-2), 71.8* (C-3), 72.1* (C-4), 72.37* (C-5a), 43.17 (C-6a), 43.17 (C-6b), 162.1 (C-7). Coupling constants (Hz): $J_{6a,6b}$ = 15.1, $J_{6a,5}$ = 7.9, $J_{6b,5}$ = 4.2, $J_{1a,1b}$ = 11.5, $J_{1a,2}$ = 5.9, $J_{1b,2}$ = 2.32. – $C_{14}H_{28}N_2O_{12}$ (416.4): calcd. C 40.38, H 6.78, N 6.73; found C 40.31, H 6.79, N 6.70.

1,10-Bis(2-hydroxyethyl)-4,7,13,16-tetraoxa-1,10-diazacyclooctadecane (**34b**) *as NaI Salt:* Prepared according to the procedure B) of Izatt et al.[38]; ^{1}H-NMR data and melting point are identical with those reported in the literature[38]; ^{13}C-NMR data have not been reported until now. – NMR ($CDCl_3$, TMS, compare formula **B**): ^{1}H: δ = 3.53 (s, 8H, 1-H), 3.46 (t, 8H, J = 4.4, 2-H), 2.51 (broad, 3-H), 2.55 (t, 4H, J = 5.0, 4-H), 3.59 (m, 4H, 5-H), 4.11 (t, 2H, J = 5.10, 6-H). – ^{13}C: δ = 68.66 (C-1), 68.66 (C-2), 53.28 (C-3), 57.81 (C-4), 67.41 (C-5).

1,10-Bis(2-hydroxyethyl)-4,7,13,16-tetraoxa-1,10-diazacyclooctadecane (**34b**): A solution of 1.0 g (2 mmol) of the NaI complex of 1,10-bis(2-hydroxyethyl)-4,7,13,16-tetraoxa-1,10-diazacyclooctadecane (see above) in a small amount of water is subjected to column chromatography as described above for **20**. The eluate is concentrated to yield 690 mg (98%) of a slightly brown oil. – NMR ($CDCl_3$, TMS): ^{1}H: δ = 3.57 (s, 8H, 1-H), 3.50 (t, 8H, J = 5.4, 2-H), 2.67 (t, 8H, J = 5.4), 2.56 (t, 4H, J = 5.0), 3.48 (t, 4H, J = 5.0). – ^{13}C: δ = 70.7 (C-1), 69.98 (C-2), 55.18 (C-3), 57.44 (C-4), 59.43 (C-5).

1,10-Bis(4-cyanobenzyl)-4,7,13,16-tetraoxa-1,10-diazacyclooctadecane: A solution of 750 mg (2.86 mmol) of 1,10-diaza-18-crown-6, 1.122 g (5.72 mmol) of α-bromo-*p*-tolunitrile, and 750 mg (7.07 mmol) of Na_2CO_3 in 40 ml of anhydrous acetonitrile is refluxed for 48 h. The solid is filtered off and the eluate evaporated to dryness. The residue is taken up in 30 ml of dichloromethane; the organic layer is extracted three times with 15 ml of water, dried with Na_2SO_4, and the solvent is evaporated to dryness. The solid is recrystallized from ethanol to yield 1.2 g (85%, ref.[39]: 91%) of a colorless solid; m.p. 102°C (ref.[39]: 103–104°C). – NMR ($CDCl_3$, TMS, compare formula **D**): δ = 3.59 (s, 8H, 1-H), 3.61 (t, 8H, 5.67, 2-H), 2.81 (t, 8H, J = 5.67, 3-H), 3.76 (s, 4H, 4-H), 7.50 (d, 4H, J = 8.20, 6-H), 7.59 (d, 4H, J = 8.20, 7-H). – ^{13}C: δ = (C-1), 70.01 (C-2), 54.21 (C-3), 59.64 (C-4), 146.0 (C-5), 129.21 (C-6), 132.04 (C-7), 110.69 (C-8), 119.01 (C-9).

1,10-Bis[4-(aminomethyl)benzyl]-4,7,13,16-tetraoxa-1,10-diazacyclooctadecane Tetrahydrochloride (**34c**): To 1.2 g (2.436 mmol) of the nitrile described above, 20 ml of a 1 M borane–THF solution is added under dry nitrogen in a 50-ml three-necked flask. The mixture is refluxed for 24 h, cooled, down and 5 ml of a 11 M methanolic HCl solution is added. Afterwards the formed boric acid trimethyl ester (b.p. 55–56°C) and THF are evaporated. The residue is taken up in 5 ml of anhydrous methanol and the solution evaporated to dryness. This step is repeated several times. After drying 1.45 g (92%) of a colorless powder is obtained; m.p. 202°C (dec.). – NMR (D_2O, TMSP): ^{1}H: δ = 3.90 (s, 8H, 1-H), 3.71 (s, 8H, 2-H), 3.50 (s, 8H, 3-H), 4.25 (s, 4H, 4-H), 7.57 (d, 4H, J = 8.3, 6-H), 7.60 (d, 4H, J = 8.5, 7-H), 4.52 (s, 4H, 9-H). – ^{13}C: δ = 72.48 (C-1), 66.45 (C-2), 55.47 (C-3), 45.27 (C-4), 131.88 (C-5), 132.31 (C-6), 134.86 (C-7), 132.53 (C-8), 60.12 (C-9). – $C_{28}H_{48}Cl_4N_4O_4$ (646.5): calcd. C 52.02, H 7.48, N 8.67; found C 51.49, H 7.41, N 8.10.

1,10-Bis[4-(4,5,6,7,8-pentahydroxy-2-azaoctyl)benzyl]-4,7,13,16-tetraoxa-1,10-diazacyclooctadecane (**34d**): Synthesis as described above for **20** with 0.5 g (0.773 mmol) of the above diamine, 280 mg (1.55 mmol) of D-glucose, and 5 ml of methanol. 112 mg of $NaBH_4$ is added slowly, and the mixture is stirred for 24 h at room temp. After the column chromatographic workup, as described in the general synthesis for the 1-deoxy-D-glucitolyl derivatives, the eluate is concentrated. 20 ml of anhydrous ethanol is added to the oily residue and the mixture stirred for 2 h. The ethanolic phase is decanted, and this step is repeated twice. 100 ml of anhydrous ethyl acetate is added to the combined ethanolic layers. The mixture is stored at –20°C for about 12 h. The precipitate is filtered off (hygroscopic) to yield 130 mg (20%) of a white hygroscopic powder; (CHN analysis not possible); m.p. 113°C (dec.). – NMR (D_2O, TMSP): Only ^{1}H-NMR data of the signals of 3,6,7,10a,11-H can be given as other overlap. δ = 2.68 (dd, 2H, 10a-H), 2.75–2.82 (m, 2H, 10b-H); 2.82 (s, 8H, 3-H); 3.57–3.85 (m, 34H, 1,2,4,9,12,13,14,15-H), 3.91 (m, 2H, 11-H), 7.63 (s, 8H, 6,7-H). – ^{13}C NMR: δ = 69.62 (C-1), 68.23 (C-2), 52.54 (C-3), 49.85 (C-4), 138.06 (C-5), 130.29 (C-6), 128.64 (C-7), 136.13 (C-8), 52.04 (C-9), 58.22 (C-10a), 58.22 (C-10b), 70.89 (C-11), 71.10 (C-12), 71.10 (C-13), 71.10 (C-14), 62.92 (C-15). Coupling constants (Hz): $J_{6a,6b}$ = 13.8, $J_{6b,5}$ = 8.3.

1,10-Bis(5-cyanopentyl)-4,7,13,16-tetraoxa-1,10-diazacyclooctadecane: A solution of 0.671 g (3.82 mmol) of 6-bromocapronitrile, 0.5 g (1.9 mmol) of 1,10-diaza-18-crown-6, and 0.848 g (8 mmol) of Na_2CO_3 in 40 ml of anhydrous acetonitrile is refluxed for 2 d. The precipitate is filtered off, and the filtrate is evaporated to dryness. Recrystallization of the residue from a small amount of ethyl acetate yields 500 mg (58%) of a yellow solid, m.p. 64–65°C. – NMR ($CDCl_3$, TMS, compare formula **E**): ^{1}H: δ = 3.61 (s, 8H, 1-H), 3.69 (t, 8H, J = 4.9 Hz, 2-H), 3.05 (s, 8H, 3-H), 2.38 (t, 4H, J = 7.0 Hz, 4-H), 1.65 (m, 4H, 5-H), 1.47 (m, 4H, 6-H), 1.70 (m, 4H, 7-H), 2.78 (m, 4H, 8-H). – ^{13}C: 70.47 (C-1), 70.47 (C-2), 53.86 (C-3), 54.00 (C-4), 25.03 (C-5), 17.06 (C-6), 26.2 (C-7), 67.95 (C-8), 119.5 (C-9).

1,10-Bis(6-aminohexyl)-4,7,13,16-tetraoxa-1,10-diazacyclooctadecane Tetrahydrochloride: Preparation similar to that of **34c** from 560 mg (1.23 mmol) of the nitrile as described above and 13 ml of

a 1 M borane–THF solution. Yield 750 mg (92%) of a colorless powder; hygroscopic (CHN analysis not possible); m.p. 235°C (decomposition). – NMR (D_2O, TMSP): ^1H: δ = 3.77 (s, 8H, 1-H), 3.90 (m, 8H, 2-H), 3.50 (m, 8H, 3-H), 3.01 (t, J = 7.60 Hz, 4H, 4-H), 1.76 (m, 4H, 5-H)*, 1.43–1.45 (m, 4H, 6-H), 1.43–1.45 (m, 4H, 7-H), 1.70 (m, 4H, 8-H)*, 3.26 (t, J = 8.1, 4H, 9-H). – ^{13}C: 67.9 (C-1), 61.8 (C-2), 50.9 (C-3), 51.4* (C-4), 51.5* (C-5), 23.2# (C-6), 23.3# (C-7), 37.4 (C-8), 24.5 (C-9).

1,10-Bis[8-(2-naphthyl)-7-azaoctyl]-4,7,13,16-tetraoxa-1,10-diazacyclooctadecane (**34e**): 750 mg (1.23 mmol) of the previous compound and 524 mg (4.95 mmol) of Na_2CO_3 are stirred for 30 min in 30 ml of anhydrous methanol. 386 mg (2.47 mmol) of β-Naphthaldehyde is added, the solution is refluxed for 1 h, cooled to room temp. and stirred for further 16 h. 100 mg of $NaBH_4$ is added, and the solution is stirred for 24 h at room temp. The precipitate is filtered off and the filtrate evaporated to dryness. The residue is taken up in 10 ml of 2 M HCl and the solution several times extracted with 5 ml of dichloromethane. The organic layer containing 2-(hydroxymethyl)naphthalene is discarded, the aqueous layer is made alkaline and 4 times extracted with 10 ml of dichloromethane. The combined extracts are dried with Na_2SO_4, and the solvent is evaporated to afford 378 mg (41%) of a colorless oil (CHN analysis not possible). – NMR ($CDCl_3$, TMS): ^1H: δ = 3.59 (s, 8H, 1-H), 3.58 (t, J = 6.2 Hz, 8H, 2-H), 2.74 (t, J = 6.0 Hz, 8H, 3-H), 2.65 (t, J = 7.25 Hz, 4H, 4-H), 1.51 (m, 4H, 5-H), 1.28 (m, 4H, 6-H), 1.26 (m, 4H, 7-H), 1.43 (m, 4H, 8-H), 2.46 (t, J = 7.5 Hz, 4H, 9-H), 3.94 (s, 4H, 10-H), 7.74 (s, 2H, 11-H), 7.41–7.48 and 7.79–7.81 (m, 12H, 15,16-H). – ^{13}C: 70.7* (C-1), 70.03* (C-2), 54.00 (C-3), 54.09 (C-4), 30.08 (C-5), 27.26 (C-6), 27.26 (C-7), 27.34 (C-8), 55.90# (C-9), 49.39# (C-10), 125.38** (C-11), 138.09 (C-12), 133.44## (C-19), 132.61## (C-20), 125.85** (C-13), 126.28** (C-14), 126.52** (C-15), 127.56** (C-16), 127.61** (C-17), 127.92** (C-18).

Diethyl N,N'-Dibenzyl-N,N'-ethylenediaminediacetate: Synthesized analogously to the method of Rana[40]: 6.0 g (25 mmol) of *N,N'*-Dibenzylethylenediamine is dissolved in 50 ml of anhydrous acetonitrile. 6 ml (51 mmol) of ethyl 2-bromoacetate and 8.6 ml (51 mmol) of ethyldiisopropylamine are added to the solution. The mixture is refluxed for 22 h, cooled, and the solvent is evaporated. The residue is taken up in 100 ml of diethyl ether, the solution stirred for about 12 h and filtered. The filtrate is diluted with 6 ml of toluene and extracted several times with a phosphate buffer solution (pH 2). The organic layer is dried with molecular sieve and evaporated to furnish 8.0 g (75%) of a slightly brown oil (CHN analysis not possible). – NMR ([D4]DMSO, TMS, compare formulae **F**): ^1H: δ = 7.22–7.31 (m, 10H, 1,2,3-H), 2.70 (s, 4H, 5-H), 3.32 (s, 4H, 6-H), 3.69 (s, 4H, 7-H), 4.05 (q, J = 7.1 Hz, 4H, 9-H), 1.17 (t, J = 7.1 Hz, 6H, 10-H). – ^{13}C: δ = 126.77 (C-1), 128.49 (C-2), 127.96 (C-3), 138.92 (C-4), 53.93 (C-5), 50.98 (C-6), 57.74 (C-7), 170.68 (C-8), 59.5 (C-9), 13.98 (C-10).

N,N'-Dibenzyl-N,N'-ethylenediaminediacetic Acid (**33**): To a solution of 1.548 g (3.6 mmol) of the compound described above in 40 ml of ethanol a solution of 0.37 g (9.25 mmol) of NaOH in 40 ml water is added. The mixture is stirred for 3 d at room temp. The major part of the ethanol is evaporated, the solution is acidified (pH 2), and the precipitate is filtered off to yield 920 mg (60%) of a colorless solid; m.p. 240°C (dec.). – NMR ([D4]DMSO, TMS): ^1H: δ = 7.27 (m, 10H, 1,2,3-H), 2.76 (s, 4H, 5-H), 3.22 (s, 4H, 6-H), 3.74 (s, 4H, 7-H). – ^{13}C: δ = 127.10 (C-1), 128.19 (C-2), 128.88 (C-3), 138.43 (C-4), 54.07 (C-5), 51.12 (C-6), 57.78 (C-7), 171.94 (C-8). – $C_{20}H_{24}N_2O_4$ (356.4): calcd. C 67.40, H 6.79, N 7.86; found C 66.63, H 6.67, N 7.67.

6,9-Dioxa-3,12-diaza-1,14-tetradecanediol (**28**): Synthesized according to the method of Krespan et al.[41]. – ^1H-NMR data and melting point are identical with those reported in the literature; ^{13}C-NMR data are not known. – NMR ($CDCl_3$, TMS): ^1H: δ = 3.55 (s, 4H, 1-H), 3.54 (t, J = 5.0 Hz, 4H, 2-H), 2.68 (t, J = 5.0 Hz, 4H, 3-H), 2.74 (t, J = 5.0 Hz, 4H, 4-H), 3.60 (t, J = 5.0 Hz, 4H, 5-H). – ^{13}C: δ = (C-1), 70.18 (C-2), 48.78 (C-3), 51.33 (C-4), 60.65 (C-5).

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