$C_8H_8N_2$ (132.2): C, 72.71; H, 6.10; N, 21.21. Found: C, 72.7; H, 6.10; N, 21.3.

2-Methyl-1*H*-pyrrolo[3,2-*c*]pyridine (24a). Cyclization of crude **22a** according to the foregoing procedure and sublimation (1 mmHg) yielded 98% of **24a**: mp 210 °C;[13] ^{1}H NMR (90 MHz) (DMSO-d_6) δ 2.90 (s, 3 H, CH_3), 6.80 (s, 1 H, 3-H), 7.80 (d, 1 H, 7-H, J_{6-7} = 5 Hz), 8.60 (d, 1 H, 6-H), 9.20 (s, 1 H, 4-H), 11.90 (m, 1 H, NH); IR (KBr) 3420, 3200–2600, 1620, 1590, 1560, 1470, 1430 cm^{-1}. Anal. Calcd for $C_8H_8N_2$ (132.2): C, 72.71; H, 6.10; N, 21.21. Found: C, 72.6; H, 6.07; N, 21.2.

2-*tert*-Butyl-1*H*-pyrrolo[2,3-*b*]pyridine (12b). Cyclization of **20b** according to the foregoing procedure and crystallization from Et_2O yielded 90% of **12b**: mp 196 °C; ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9 H, *t*-Bu), 6.05 (s, 1 H, 3-H), 6.85 (dd, 1 H, 5-H, J_{4-5} = 8 Hz), 7.70 (dd, 1 H, 4-H, J_{4-6} = 2 Hz), 8.10 (dd, 1 H, 6-H), 12.10 (m, 1 H, NH); IR (KBr) 3140, 2970, 1570, 1510, 1380 cm^{-1}. Anal. Calcd for $C_{11}H_{14}N_2$ (174.2): C, 75.82; H, 8.10; N, 16.08. Found: C, 75.5; H, 8.14; N, 15.7.

2-*tert*-Butyl-1*H*-pyrrolo[2,3-*c*]pyridine (23b). Cyclization of **21b** according to the foregoing procedure and crystallization from Et_2O yielded 99% of **23b**: mp 204 °C; ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 9 H, *t*-Bu), 6.10 (s, 1 H, 3-H), 7.30 (d, 1 H, 4-H, J_{4-5} = 5 Hz), 7.95 (d, 1 H, 5-H), 8.55 (s, 1 H, 7-H), 11.20 (m, 1 H, NH); IR (KBr) 3440, 3240–2530, 1620, 1580, 1535, 1470, 1410 cm^{-1}. Anal. Calcd for $C_{11}H_{14}N_2$ (174.2): C, 75.82; H, 8.10; N, 16.08. Found: C, 75.8; H, 8.12; N, 16.0.

2-*tert*-Butyl-1*H*-pyrrolo[3,2-*c*]pyridine (24b). Cyclization of **22b** according to the foregoing procedure and crystallization from $CHCl_3$ yielded 98% of **24b**: mp >250 °C; ^{1}H NMR (90 MHz) (DMSO-d_6) δ 1.30 (s, 9 H, *t*-Bu), 6.15 (s, 1 H, 3-H), 7.20 (d, 1 H, 7-H, J_{6-7} = 5 Hz), 8.00 (m, 1 H, 6-H), 8.55 (m, 1 H, 4-H), 11.25 (m, 1 H, NH); IR (KBr) 3460, 3240–2400, 1620, 1585, 1550, 1470, 1430, 1400 cm^{-1}. Anal. Calcd for $C_{11}H_{14}N_2$ (174.2): C, 75.82; H, 8.10; N, 16.08. Found: C, 75.6; H, 8.08; N, 15.9.

2-*tert*-Butyl-1-methylpyrrolo[2,3-*b*]pyridine (13a). Cyclization of **9** according to the foregoing procedure and flash chromatography on silica gel (10% $Et_3N/CHCl_3$) yielded 70% of **13a** (oily product): ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9 H, *t*-Bu), 3.95 (s, 3 H, NCH_3), 6.20 (s, 1 H, 3-H), 6.85 (dd, 1 H, 5-H, J_{4-5} = 8 Hz, J_{5-6} = 5 Hz), 7.65 (dd, 1 H, 4-H, J_{4-6} = 2 Hz), 8.10 (dd, 1 H, 6-H); IR (film) 3050, 2960, 1590, 1390 cm^{-1}; mass calcd for $C_{12}H_{16}N_2$ 188.3, found 188.

1,2-Dimethylpyrrolo[2,3-*b*]pyridine (13b). Reaction between **5** and 2-propanone-derived enolate was achieved under UV illumination. Standard workup gave a crude product, which was cyclized as previously described. Flash chromatography on silica gel (10% $Et_3N/CHCl_3$) yielded 90% of **13b** (oily product): ^{1}H NMR ($CDCl_3$) δ 2.25 (s, 3 H, CH_3), 3.60 (s, 3 H, NCH_3), 6.00 (s, 1 H, 3-H), 6.80 (dd, 1 H, 5-H, J_{4-5} = 8 Hz, J_{5-6} = 5 Hz), 7.60 (dd, 1 H, 4-H, J_{4-6} = 2 Hz), 8.10 (dd, 1 H, 6-H); IR (film) 3060, 2960, 1560, 1510, 1420 cm^{-1}; mass calcd for $C_9H_{10}N_2$ 146.2, found 146.

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Registry No. 1, 372-48-5; 2, 113975-22-7; 3, 112197-15-6; 4, 104830-06-0; 5, 113975-23-8; 6, 113975-24-9; 7, 113975-25-0; 8, 113975-26-1; 9, 113975-27-2; 10, 113975-28-3; 11, 113975-29-4; 12a, 23612-48-8; 12b, 86847-74-7; 13a, 113975-30-7; 13b, 113975-38-5; 14, 86847-59-8; 15, 70298-88-3; 16, 70298-89-4; 17, 113975-31-8; 18, 113975-32-9; 19, 11375-33-0; 20a, 113975-34-1; 20b, 113975-39-6; 21a, 113975-35-2; 21b, 113975-40-9; 22a, 113975-36-3; 22b, 113975-41-0; 23a, 65645-56-9; 23b, 113975-42-1; 24a, 113975-37-4; 24b, 86847-76-9; pinacolone, 75-97-8; 2-hydroxyethanethiol, 60-24-2; 2-propanone, 67-64-1; acetaldehyde, 75-07-0.

Designed Water-Soluble Macrocyclic Esterases: From Nonproductive to Productive Binding

François Diederich,* Gregor Schürmann, and Ito Chao

Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90024-1569, and Max-Planck-Institut für medizinische Forschung, Abteilung Organische Chemie, Jahnstrasse 29, D-6900 Heidelberg, Federal Republic of Germany

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The synthesis and esterase properties of three water-soluble macrobicyclic hosts, designed as α-chymotrypsin mimics, are described. For supramolecular complexation in aqueous solution, host **1** possesses an apolar tetraoxa[6.1.6.1]paracyclophane binding cavity, while hosts **2** and **4** have a larger tetraoxa[8.1.8.1]paracyclophane binding site. A phenolic nucleophile is located atop the cavity of **1** and **2**, while an alcoholic hydroxyl group is attached to **4**. The multistep synthesis of hosts **1**, **2**, and **4** involves two macrocyclization reactions. A Williamson ether cyclization gives the tetraoxa[*n*.1.*n*.1]paracyclophanes, and an amide cyclization attaches the nucleophiles to the binding sites. ^{1}H NMR host–guest complexation analysis demonstrates that both **1** and **2** form complexes of high stability with naphthalene guests in aqueous solution. In aqueous phosphate buffer at pH 8, host **2**, with its partially ionized phenolic residue, is acylated much faster by complexed 4-nitro-1-naphthyl acetate (**26**) than host **4**, which possesses the nonionized alcoholic hydroxyl group. The acylation of **2** by the complexed ester **26** is much faster than the hydrolysis of the ester in the presence of **1**, a host with the same phenolic nucleophile but with a smaller binding site. The difference in esterase activity between **1** and **2** is explained in terms of productive versus nonproductive binding. The acylation of **2** by complexed ester **26** follows saturation kinetics whereas the hydrolysis of **26** in the presence of **1** obeys second-order kinetics. Host **2** shows a modest catalytic turnover in the hydrolysis of **26** in aqueous phosphate buffer at pH 8. The nature of catalysis provided by **2** is discussed.

Introduction. Ever since the fundamental studies of Cramer[1] on the catalytic properties of the cyclodextrins in the early fifties, chemists have been challenged by the prospect of developing catalysts that mimic enzymes by forming stoichiometric complexes with their substrates in a reversible equilibrium prior to the reaction steps.[2-11]

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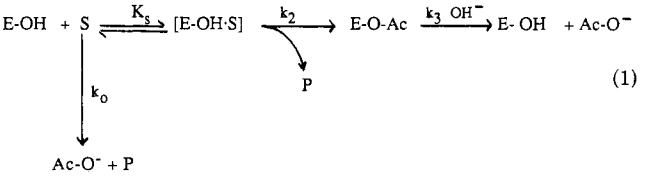
Hydrolase mimics have always been at the center of this bioorganic approach toward catalysis.[1-6,12-20] At the active sites of proteases, e.g., the serine proteases, the thiol proteases, or the aspartyl proteases, highly efficient catalytic mechanisms are generated through a specific array of functional groups. The isolated forms of these functionalities demonstrate little or no catalytic activity.[21,22] Only by the cooperative action of these groups in the specific active site do catalytic mechanisms such as transition-state binding and stabilization, acid–base catalysis, nucleophilic and electrophilic catalysis, and desolvation become effective. It is not surprising that the possibility of mimicking such catalytic mechanisms in designed supramolecular complexes has fascinated so many researchers.

For decades, natural and modified cyclodextrins were the only macrocycles capable of mimicking hydrolytic enzymes in aqueous solution.[1-3] Since Pedersen's discovery of the crown ethers,[23] a large number of designed macrocycles with hydrolase properties have been prepared. Esterase activity was demonstrated for neutral molecule binding cyclophane hosts in aqueous solution[6,7,13,18,19] as well as for functionalized ammonium ion binding crown ethers[4,5,15,17] and spherands[16] in organic solvents. Functionalized micelles are other systems that have been extensively studied.[24-29] The recent development of catalytic antibodies represents a promising new biological approach toward nonenzymatic hydrolases.[30,31]

Many macrocyclic hydrolase mimics model in some way α-chymotrypsin, the best studied member of the digestive serine proteases capable of hydrolyzing amides as well as esters.[1-3,6,14,16,18] We define *α-chymotrypsin mimics* as compounds that (a) complex their substrates (S) prior to reaction, (b) react according to the minimum mechanism for the α-chymotrypsin-catalyzed hydrolysis of esters and amides shown in eq 1, and (c) operate like the enzyme (E–OH) by the addition–elimination mechanism in both transacylation and deacylation steps of eq 1.[21] The deacylation step (k_3) is the rate-determining step in the ester hydrolysis catalyzed by α-chymotrypsin[32] or by its mimics. Large accelerations of the transacylation step (k_2), comparable to those observed for enzymes, have been achieved in designed supramolecular complexes.[14,16] Most designed systems, however, do not sufficiently accelerate the deacylation step (k_3) compared to the hydrolysis by pure buffer (k_0) to give catalytic turnover. While the work described in this paper was in full progress, a phenol-functionalized micelle[29] and an imidazole-functionalized spherand[16b] were described as α-chymotrypsin mimics showing modest catalytic turnover. More efficient turnover catalysis is, however, observed with functionalized micelles that operate in the deacylation step by catalyst-specific elimination mechanisms instead of by the addition–elimination deacylation mechanism accepted for α-chymotrypsin.[27,28] The development of efficient water-soluble α-chymotrypsin mimics therefore still represents a formidable challenge to bioorganic chemists.



(1)

In this paper, we describe the synthesis of the novel macrobicyclic hosts **1**, **2**, and **4**. The binding properties and esterase activity of these α-chymotrypsin models in aqueous solutions will be analyzed. It will be shown that the best of the three model systems gives a modest catalytic turnover in the hydrolysis of activated esters. A kinetic analysis will provide evidence that small changes in the size of preorganized macrobicyclic binding sites can lead from productive to entirely nonproductive substrate binding.

Design and Synthesis of the Macrobicyclic Esterase Models 1, 2, and 4. Over the past years, we have developed water-soluble tetraoxa[*n*.1.*n*.1]paracyclophane hosts derived from two diphenylmethane units with molecular binding sites of hydrophobic character.[34] In

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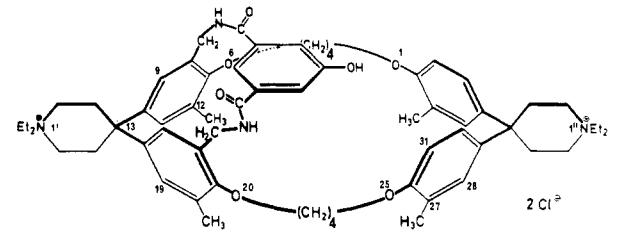
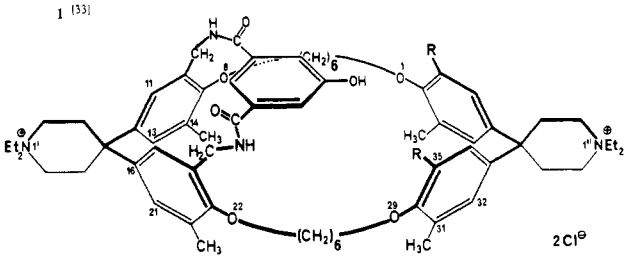
aqueous and also in organic solutions, these hosts form stable complexes with aromatic guests. The functionalized macrocycles **1**, **2**, and **4**, derived from these cyclophane

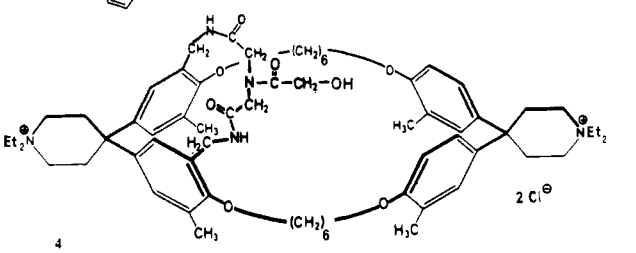


1 [33]



2 R = H [33]

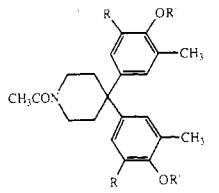
3 R = 



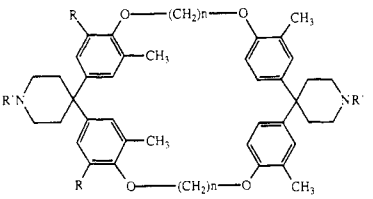
4

hosts, were designed to accelerate, via nucleophilic catalysis, the hydrolysis of complexed aromatic esters in aqueous solution near physiological pH. The macrobicyclic fixation of the hydroxylic nucleophiles was expected to enforce productive conformations of the nucleophiles atop the cavity binding sites. The macrocycles **1** and **2** differ from **4** in the nature of their reactive nucleophile, whereas compounds **1** and **2** are differentiated by the size of their cavity. After optimization of the nucleophile and binding cavity size of the synthetic esterases, it is planned to introduce imidazoles,[3,35] e.g., in **3**, to further promote both steps of the covalently catalyzed hydrolysis by general acid–base catalysis.

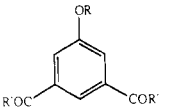
For the synthesis of the macrobicyclic systems **1**, **2**, and **4**, the diphenol **5**[34b] was brominated to give **6** (Br_2/HOAc, 99%). Dialkylation of **6** with 1,4-dichlorobutane or 1,6-dichlorohexane (KOH/*n*-BuOH) led to the dichlorides **7a** (86%) and **7b** (83%). The ether cyclizations of **7a/b** with **5** (Cs_2CO_3/DMF) yielded the macromonocycles **8a** (19%) and **8b** (13%). The reactions of **8a/b** with copper(I) cyanide in dimethylformamide afforded the dinitriles **9a** (83%) and **9b** (88%), which were subsequently reduced (BH_3/THF) to give the corresponding macrocyclic tetraamines **10a** (94%) and **10b** (93%).



	R	R'
5	H	H
6	Br	H
7a	Br	$(CH_2)_4$-Cl
7b	Br	$(CH_2)_6$-Cl



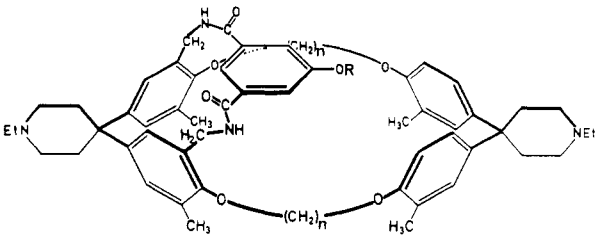
	R	R'	n
8a	Br	CH_3CO	4
8b	Br	CH_3CO	6
9a	CN	CH_3CO	4
9b	CN	CH_3CO	6
10a	CH_2NH_2	CH_3CH_2	4
10b	CH_2NH_2	CH_3CH_2	6
11	$CH_2NHCOCH_3$	CH_3CH_2	4



	R	R'
12	CH_3	O-Succinimide
13	$PhCH_2$	OH
14	$PhCH_2$	OC_6F_5
15	CH_3CO	OC_6F_5

We planned to incorporate the phenol caps in **1** and **2** via amide cyclizations of **10a/b** with the activated 5-hydroxyisophthalic acid derivatives **12**, **14**, or **15**. The bis(succinimido ester) **12** was prepared in 81% yield in the reaction of 5-methoxyisophthalic acid[36] with *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide (DCC).[34c,37] The benzyl-protected 5-hydroxyisophthalic acid **13** was obtained in 79% yield by hydrolysis (KOH/MeOH) of the corresponding dimethyl ester.[38] Reaction of **13** or of 5-acetoxyisophthalic acid with pentafluorophenol[39] in the presence of DCC afforded the activated derivatives **14** (85%) and **15** (78%).

The high-dilution amide cyclizations of **10a/b** with the activated esters **12** and **14** afforded the macrobicyclic systems **16** and **17** in yields of only ≈20%. The rather low yields[40] are due to severe conformational restrictions in the transition states of the closure of the 16-membered rings with 13 sp^2 centers.



	R	n
16	CH_3	4
17	$C_6H_5CH_2$	4
18a	CH_3CO	4
18b	CH_3CO	6
19a	H	4
19b	H	6

(33) The numbering of the atoms in the drawings is used in the description of the ^1H NMR spectra in the Experimental Section. This numbering differs from the numbering used in the systematic nomenclature of the macrobicycles.

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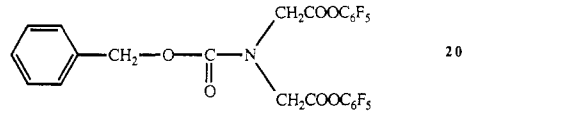
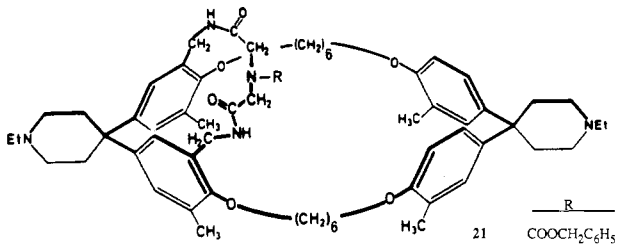
Selective removal of the phenolic protective group in **16** to give **19a** was not successful.[41] Demethylation with boron tribromide[42] or tetramethylsilyl iodide[43] or under nucleophilic conditions with EtS^-/DMF[44] or $PhCH_2Se^-$[45] in each case led to cleavage of the tetraoxa[*n*.1.*n*.1]paracyclophane skeleton. The catalytic removal of the benzylic protective group in **17** to give **19a** proved to be extremely tedious. At 1.3 atm of hydrogen gas in a Parr apparatus in the presence of palladium (10%) on charcoal in ethanol, the deprotection of **17** was only completed after 12 days (TLC). Higher hydrogen pressures of up to 80 atm did not significantly accelerate the reaction.

The cyclization of **15** with **10a** at first seemed to be very disappointing and gave only traces of the acetoxy macrocycle **18a**. Actually, the acetoxy derivative **18a** was not very stable. During the cyclization reaction in refluxing dioxane and the subsequent workup, partial deacylation of **18a** to the phenolic compound **19a** occurs. We finally were able to obtain the phenolic derivatives **19a** and **19b** in 20% yield from the cyclizations between **10a/b** and **15** without isolation of the acetoxy macrobicycles **18a/b**. Quaternization of **19a/b** with pure ethyl iodide followed by ion-exchange chromatography (Cl^-) afforded the target systems **1** and **2** as hygroscopic colorless solids.

For the preparation of **4**, iminodiacetic acid was protected with benzyl chloroformate (77%)[46] and then transformed into the bis(pentafluorophenyl ester) **20** (85%).[11] The amide cyclization of **20** with **10b** afforded the macrobicyclic system **21** in 41% yield. The 16-membered ring formed in this reaction only has 11 sp^2 centers. Catalytic deprotection (H_2, Pd/C, 10%) of **21** afforded the macrobicyclic triamine **22** in 60% yield. Reaction with α-bromoacetyl bromide yielded the bromomethyl derivative **23** in 82% yield, which was isolated and characterized as the dihydrobromide. The transformation of **23** to the hydroxymethyl compound **24** and its purification proved to be extremely difficult. Pure product **24** could only be isolated in 6% yield after medium-pressure chromatography from a reaction of **23** in acetone/water (7:3) in the presence of freshly precipitated silver oxide (24 h, 40 °C).[47] Quaternization of **24** with ethyl iodide and ion-exchange chromatography (Cl^-) gave the target compound **4** in 80% yield.



20



	R
21	$COOCH_2C_6H_5$
22	H
23	$COCH_2Br$
24	$COCH_2OH$

Orientation of the Nucleophiles at the Binding Sites of the Macrobicycles 1, 2, and 4. To correlate potential esterase activity with the geometries of the supramolecular complexes, it was of special interest to elucidate the orientation of the nucleophiles at the binding sites of **1**, **2**, and **4**. The four diphenylmethane benzene rings take the face-to-face conformation and shape the binding cavities. The macrobicyclic attachment prevents the phenol rings of **1** and **2** from entering the binding site.

CPK molecular model examinations indicate that a location of the phenol ring atop the binding cavity is sterically more favorable than a location of the ring in the opposite direction above the piperidinium ring or oriented perpendicular to the mean plane of the tetraoxa[*n*.1.*n*.1]paracyclophane. Only in a position atop the cavity, according to the models, can both amide linkages take a strain-free transoid conformation in planar conjugation[48] with the phenol ring.

The location of the phenolic ring atop the cavity in **1** and in the precursor molecules **16**, **17**, and **19a** is supported by ^1H NMR spectroscopy. In the spectra of **1** in Me_2SO-d_6 at various temperatures (T = 303–393 K) and at different spectrometer frequencies (80, 360, and 500 MHz), one highly resolved AMX spectrum is obtained for the two $ArCH_2NHCO$ linkages. At 393 K, the signals of the geminal methylene protons appear at δ 4.29 (A part, J = 14.2 and <1 Hz, 2 H), and 4.66 (M part, J = 14.2 and 10.2 Hz, 2 H), whereas the resonance of the NH protons is observed at δ 8.13 (X part, J = 10.2 and <1 Hz, 2 H). The exclusive and unusual appearance of a single, highly resolved AMX pattern even at 393 K suggests that the two amide linkages are symmetrically oriented and that the macrobicyclic phenol cap of **1** has a rigid orientation.

If the phenol ring was oriented preferentially in the direction opposite to the cavity above the piperidinium ring, a strong upfield shift of the protons 9-H and 3′-H would be expected. A comparison between the ^1H NMR spectra (360 MHz, Me_2SO-d_6, T = 350 K) of the diamide **11** and the phenol-bridged compound **16** demonstrates the presence of downfield rather than upfield shifts. The resonance of the aromatic proton 9-H appears in the spectrum of **11** at δ 7.01 and in the spectrum of **16** at δ 7.32, whereas the multiplet for 3′-H in both spectra appears at very similar positions around δ 2.3.

Strong experimental support for the preferred location of the phenol ring on the cavity side was obtained from ^1H NOE difference spectroscopy (360 MHz, CD_3CN, 303 K). Upon irradiation of the phenolic ring proton 4‴-H of **1**, a signal enhancement (≈3%) is observed exclusively for the resonance of the protons 28-H, 30-H, and 31-H of the diphenylmethane unit which is not bridged by the phenol ring.

Similar resonances of comparable protons in the ^1H NMR spectra of **1** and **2** suggest that the phenol ring in

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(42) (a) Benton, F. L.; Dillon, T. E. *J. Am. Chem. Soc.* **1942**, *64*, 1128–1129. (b) McOmie, J. F. W.; Watts, M. L.; West, D. E. *Tetrahedron* **1968**, *24*, 2289–2292.
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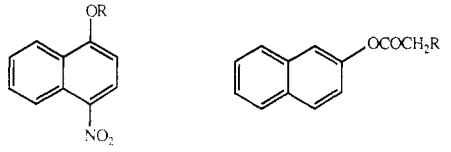
(44) Feutrill, G. I.; Mirrington, R. N. *Tetrahedron Lett.* **1970**, 1327–1328.
(45) Ahmad, R.; Saá, J. M.; Cava, M. P. *J. Org. Chem.* **1977**, *42*, 1228–1230.
(46) Gregory, H.; Morley, J. S.; Smith, J. M.; Smithers, M. J. *J. Chem. Soc. C* **1968**, 715–725.
(47) Geibel, K.; Hofman, H.; Kropf, H.; Thiem, J. In *Methoden der Organischen Chemie*, 4th ed.; Houben, J., Weyl, T., Müller, E., Eds.; Thieme: Stuttgart, 1979; Vol. VI/1a, Part 1, pp 174–179.
(48) Information on the energy required to bring the phenyl ring out of conjugation with the two amide linkages was not found in the literature. For acrylic acid, values for the rotational barrier for going from the s-trans to the s-cis conformer are reported between 3.8 kcal mol^{-1} (experimental) and 7.5 kcal mol^{-1} (theory); see: Loncharich, R. J.; Schwartz, T. R.; Houk, K. N. *J. Am. Chem. Soc.* **1987**, *109*, 14–23. Although acrylic acid is very different from the phenol cap in **1** and **2**, the data for acrylic acid suggest a high cost for taking the phenol ring out of coplanarity with the two carboxamide residues.

both macrobicycles takes a similar orientation. The cap in **2**, however, seems to possess higher flexibility. At 303 K in Me_2SO-d_6 (500 MHz), the AMX pattern for the two $ArCH_2NHCO$ linkages at δ 4.40, 4.60, and 8.39 is no longer resolved and broad signals are observed instead. The coalescence of the signals of the geminal methylene protons occurs already at 338 K. In the spectra of **1**, one highly resolved AMX pattern was observed up to temperatures as high as 393 K. No signal enhancement could be detected in ^1H NOE difference spectra upon irradiation of the phenol ring proton 4‴-H of **2**. The absence of efficient dipolar through-space coupling between the phenol ring protons of **2** and the protons 32-H, 34-H, and 35-H of the opposing diphenylmethane protons is not unexpected in view of the larger extension of the cavity of this macrobicycle.

With two additional methylene groups, the cap of macrobicycle **4** has a considerably higher conformational flexibility than the phenol cap in **1** or **2** and the hydroxyl nucleophile presumably can take various, energetically similar orientations at the binding site of **4**.

Host–Guest Interactions in Aqueous Solutions. The host–guest complexation analysis with **1** and **2** in aqueous solutions showed that the phenol ring does not interfere with the binding of aromatic guests in the macrocyclic cavity. Binding was studied qualitatively by monitoring ^1H NMR complexation shifts in deuteriated phosphate buffer (pD 8.4,[49] T = 303 K) below the ^1H NMR critical aggregation concentrations (cac)[34a] of the hosts (cac of **1** $\approx 1.5 \times 10^{-3}$ mol L^{-1}; cac of **2** $\approx 6.5 \times 10^{-4}$ mol L^{-1} under the given conditions). Large complexation shifts of both host and guest protons at very low concentration ranges, e.g., [host] = [guest] = 5×10^{-4} mol L^{-1}, indicated strong binding between **1** and benzene derivatives like *p*-nitrotoluene or *p*-tolunitrile or naphthalene derivatives like 2-methoxy-6-naphthonitrile, sodium 2-naphthalenesulfonate, and trimethyl(1-naphthyl)ammonium fluorosulfonate.[50] The larger hosts **2** and **4**[51] do not bind benzene guests efficiently but form complexes of high stability with naphthalene derivatives.

The comparison of the esterase activity of **1** and **2**, described below, involves naphthyl ester substrates since they can form stable complexes with both hosts. Since 4-nitro-1-naphthyl acetate (**26**) proved to be the most interesting substrate in these comparative studies, we determined quantitatively from ^1H NMR titrations the stability of the complexes formed between **1** and **2** and 4-nitro-1-naphthol (**25**), the apolar binding moiety of ester **26**. These ^1H NMR titrations ([host] = 1×10^{-4}–4×10^{-3} mol L^{-1}, [**25**] = 4×10^{-4} mol L^{-1} or 1×10^{-3} mol L^{-1}) were done at T = 298 K in D_2O/methanol-d_4 (70:30) (Figure 1).



25 R = H
26 R = $COCH_3$
27 R = H
28 R = Cl

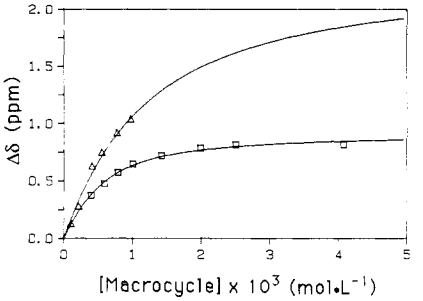


Figure 1. Determination of the association constants of the 1–4-nitro-1-naphthol complex (△) and of the **2**–4-nitro-1-naphthol complex (□) in D_2O/methanol-d_4 (70:30), T = 298 K, by computer-assisted nonlinear curve fitting of the experimental data of ^1H NMR titrations. The complexation-induced shifts $\Delta\delta$ observed for the guest proton 5-H are plotted against the host concentration.

A comparison of the binding strength in the pure aqueous buffers used in the kinetic runs was not possible. In the titrations with **1** in pure aqueous buffer, slow exchange broadened the signals of **25** to extent such that their positions could not be correctly assigned. From a nonlinear least-squares fit of the experimental titration data (Figure 1), the association constants were calculated for the **1**·**25** complex as $K_a = 1.5 \times 10^3$ L mol^{-1} ($-\Delta G$ = 4.35 kcal mol^{-1}) and for the **2**·**25** complex as $K_a = 3.5 \times 10^3$ L mol^{-1} ($-\Delta G$ = 4.80 kcal mol^{-1}). Hence, in aqueous methanol, the two complexes possess similar stability. This similarity can also be expected in aqueous phosphate buffer pH 8.0/Me_2SO (99:1 v/v), the solvent used in most of the kinetic runs.

Despite similar stability, the two complexes have different kinetic and geometric characteristics. In the spectra of the titration with the larger host **2**, the signals of both binding partners are highly resolved. In the spectra of the titration with the smaller host **1**, the signals of the guest are very broad due to slow decomplexation on the NMR time scale. Since the two complexes have similar stability, the complex of the smaller, more rigid host **1** forms at a slower rate than the complex of **2**. The complexation shifts of the individual protons in the spectra, taken from solutions of the two complexes, reflect different geometries of the two complexes. In the solutions of the **1**·**25** complex, the guest protons 5-H ($\Delta\delta_{satd,calcd}$ = +1.72 ppm) and 3-H show much larger complexation shifts than all the other guest protons (see experimental results). In the solutions of the **2**·**25** complex, only proton 3-H encounters a larger upfield shift whereas the residual protons including 5-H ($\Delta\delta_{satd,calcd}$ = +0.99 ppm) show smaller complexation shifts. The specific complexation shifts observed for the host protons support a time-averaged location of the guests in the plane passing through the two spiro centers of the host and perpendicular to the mean plane of the [*n*.1.*n*.1]-paracyclophane framework.[34] At the present time, however, it is not possible to deduce specific orientations of the guests in this plane from the observed shifts. The resonances of the protons of complexed **25** are influenced to an unknown extent by the anisotropic ring current of the phenol cap atop the binding sites of these novel host systems.

Esterase Activity of the Macrobicycles 1, 2, and 4. The activity of the novel macrobicyclic systems in the hydrolysis of the naphthyl esters **26**–**28** was studied in aqueous buffers under various reaction conditions (Table I). For the determination of the pseudo-first-order rate constants for hydrolysis in pure buffer, k_0 (eq 1), and for hydrolysis in the presence of the macrobicycles, k_{obsd}, we monitored the increase in optical density of the electronic

(49) pD = pH + 0.4. Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188–190.

(50) The complexation shifts observed in the deuteriated phosphate buffer are of similar magnitude to those reported in the preliminary communication (ref 18) for deuteriated borate buffer, pD 10.9.

(51) Macrobicyclic hosts such as **4** show strong complexation of naphthalene derivatives in aqueous solution similar to macromonocyclic tetraoxa[8.1.8.1]paracyclophanes and to host **2**. Lutter, H.-D.; Diederich, F., unpublished results.

Table I. Pseudo-First-Order Rate Constants for the Cleavage of Naphthyl Esters in the Presence or Absence of 1, 2, and 4 in Aqueous Buffer Solutions at Various pH Values[a]

run	pH	buffer	substrate	host	k_0 (10^{-4} s^{-1})	k_{obsd} (10^{-4} s^{-1})	k_{obsd}/k_0
1	8.0	phosphate	**26**	–	0.180		
2	8.0	phosphate	**26**	**1**		2.43	14
3	8.0	phosphate	**26**	**2**		32.1	178
4	8.0	phosphate	**26**	**2**		85.2[b]	472[b]
5	8.0	phosphate	**26**	**4**		1.67	9
6	6.88[c]	phosphate/Me_2SO (2:3)	**26**	–	0.450		
7	6.88[c]	phosphate/Me_2SO (2:3)	**26**	**2**		21.8	48
8	8.0	phosphate	**26**[d]	**2**[d]		0.205	1.1
9	8.0	phosphate	**26**[e]	**2**[e]		0.237	1.3
10	9.0	borate	**27**	–	0.151		
11	9.0	borate	**27**	**2**		0.195	1.3
12	9.0	borate	**28**	–	62.7		
13	9.0	borate	**28**	**2**		90.3	1.4
14	8.0	phosphate	**28**	–	6.35		
15	8.0	phosphate	**28**	**1**		6.48	1.0
16	8.0	phosphate	**28**	**2**		15.6	2.5
17	8.0	phosphate	**28**	**4**		11.3	1.8

[a] T = 293.0 K. If not otherwise stated, kinetic runs had initial concentrations of [host] = 5.0×10^{-4} mol L^{-1}; [substrate] = 2.0×10^{-5} mol L^{-1} and the solutions contained 1% (v/v) Me_2SO. [b] The pseudo-first-order rate constant, $k_{complex} = k_{obsd} - k_0$, calculated for acylation by fully bound substrate (see Figure 2) and the ratio $k_{complex}/k_0$ are shown. [c] pH of the aqueous buffer solution without Me_2SO; initial concentrations: [host] = 1.0×10^{-2} mol L^{-1}; [substrate] = 1.0×10^{-4} mol L^{-1}. [d] Run under turnover conditions; initial concentrations: [host] = 1.0×10^{-6} mol L^{-1}; [substrate] = 2.0×10^{-5} mol L^{-1}. [e] Run under turnover conditions; initial concentrations: [host] = 1.0×10^{-5} mol L^{-1}; [substrate] = 1.0×10^{-4} mol L^{-1}.

absorption bands of 2-naphthol and 4-nitro-1-naphtholate (product P in eq 1) formed during the reactions. In multiple runs, the reproducibility of the rate constants shown in Table I was ±8%.

With an estimated pK_a value of ≈15,[52] the primary hydroxyl group of **4** will not be dissociated to any significant extent in buffers at pH 8 or 9. The pK_a value of the phenolic nucleophile atop the cavity of **1** and **2** can be estimated as ≈8.4 from a linear free energy relationship plotting the known pK_a values of 3,5-disubstituted phenols[52] against the σ_m values of the substituents and taking for the amide substituents of the phenol in **1** and **2** the σ_m value of $CONH_2$ (0.28).[53] In pH 8 buffers, a considerable percentage of **1** and **2** will be dissociated and possess a reactive phenolate nucleophile.

Under the various reaction conditions shown in Table I, the following results were obtained:

1. Runs 1 and 3–5 at pH 8 in aqueous phosphate buffer/1% (v/v) Me_2SO under nonturnover conditions show that the cleavage of 4-nitro-1-naphthyl acetate (**26**) in the presence of **2** is 178 times faster than the hydrolysis in pure buffer, whereas the cleavage in the presence of **4** is only 8 times faster. Both macrocycles possess binding sites of similar complexation ability. The large difference in esterase activity is best explained by the difference in ionization state and reactivity of the two nucleophiles. At pH 8, a considerable fraction of **2** possesses a reactive phenolate nucleophile, whereas the alcoholic hydroxyl group of **4** is not ionized under these conditions. The rate dependency on nucleophile reactivity supports ester cleavage in the supramolecular complex that proceeds via nucleophilic attack and subsequent acyl transfer. For the acylation of **2** by complexed ester **26**, Michaelis–Menten type saturation kinetics is observed (Figure 2). From a nonlinear least-squares fit of the plot showing $k_{obsd} - k_0$ as a function of host concentration, the pseudo-first-order rate constant for acylation by fully bound substrate was calculated as $k_{complex} = 8.52 \times 10^{-3}$ s^{-1} and the Michaelis–Menten constant as $K_m = 7.6 \times 10^{-4}$ mol L^{-1}. Hence, at pH 8, the acylation by fully bound substrate is 472 times faster than the hydrolysis in pure buffer.

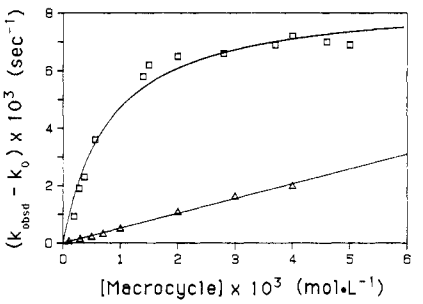


Figure 2. Plots of the pseudo-first-order rate constants ($k_{obsd} - k_0$) for the acyl transfer step in the hydrolysis of 4-nitro-1-naphthyl acetate (**26**) plotted as a function of host concentration. The reaction in the presence of host **2** (□) follows saturation kinetics (productive binding), and the reaction in the presence of host **1** (△) obeys second-order kinetics (nonproductive binding); T = 293 K, aqueous phosphate buffer, pH 8/1% (v/v) Me_2SO.

2. Runs 1–4 at pH 8 in aqueous phosphate buffer/1% (v/v) Me_2SO under nonturnover conditions show that the cleavage of **26** in the presence of the larger macrobicycle **2** is more accelerated against the hydrolysis in pure buffer than the cleavage in the presence of the smaller compound **1**. The reactions in the presence of the two very similar hosts differ entirely in their kinetics. Saturation kinetics is only observed in the presence of host **2**, whereas the cleavage of **26** in the presence of **1** strictly follows second-order kinetics as is shown in Figure 2. The efficiency of the cleavage in the presence of **1** and **2** is best compared by using second-order rate constants. The second-order rate constant for the bimolecular cleavage in the presence of **1**, $k_2 = 0.51$ s^{-1} L mol^{-1}, is obtained from the slope of the straight line obtained by plotting k_{obsd} as a function of the concentration of **1** (Figure 2). For the cleavage of **26** in the complex of **2**, a second-order rate constant $k_{complex}K_m^{-1} = 11.2$ s^{-1} L mol^{-1} is calculated. The comparison shows that the reaction in the supramolecular complex of **2** is 22 times faster than the transacylation in the presence of **1**.

The two macrobicycles possess the same phenolic nucleophile and, according to the binding studies described

(52) Grau, G. G. In *Landolt-Börnstein, Zahlenwerte und Funktionen aus Physik, Chemie, Astronomie, Geophysik und Technik*, 6th ed.; Hellwege, K.-H.; Hellwege, A. M.; Schäfer, K.; Lax, E., Eds.; Springer: Heidelberg, 1960; Vol II/7, pp 865–897.

(53) (a) Johnson, C. D. *The Hammett Equation*; Cambridge University: Cambridge, 1980. (b) Jaffé, H. H. *Chem. Rev.* **1953**, *53*, 191–261.

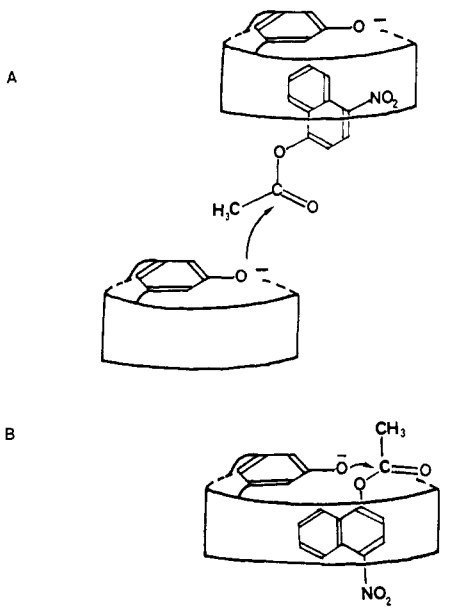


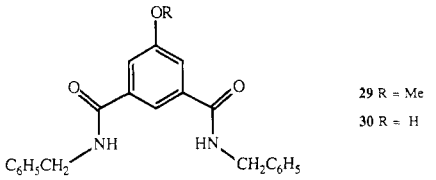
Figure 3. (A) Nonproductive binding of **26** by host **1** leads to intermolecular acyl transfer. (B) Productive binding of ester **26** by host **2** leads to supramolecular acyl transfer.

above (Figure 1), form complexes of comparable stability with the aromatic unit of substrate **26**. The results demonstrate that complexation alone is not sufficient to generate supramolecular catalysis. A proper orientation of nucleophile and ester residue is also required in the complex. Such a productive binding is only possible within the larger cavity of **2**. In the productive **2**·**26** complex, schematically shown in Figure 3, the ester residue of **26** can extend out of the cavity on the phenol side and have a favorable orientation for nucleophilic attack. In the supramolecular complex of the more rigid macrobicycle **1** with the smaller binding site, the phenol ring seems to completely block one side of the cavity, and only nonproductive complex conformations are possible. The ester residue of the complexed substrate **26** extends out of the cavity on the side opposite to the nucleophile and reacts in an intermolecular reaction with the phenoxide of another macrocycle (Figure 3).

For enzymatic reactions, the nonproductive binding at active sites in competition with the productive mode has been discussed as a mechanism to provide substrate selectivity.[54] In cyclodextrin chemistry, the faster hydrolysis of *m-tert*-butylphenyl acetate than of *p-tert*-butylphenyl acetate in the presence of α-cyclodextrin has been explained by a more productive binding of the meta-substituted substrate.[2a] In both enzymatic and cyclodextrin systems, however, saturation kinetics indicative of supramolecular reactions are observed. Our findings with hosts **1** and **2** provide an *example of nonproductive binding which is characterized by the complete absence of supramolecular reactivity.*

3. Runs 6 and 7 show that **2** also accelerates the cleavage of ester **26** in aqueous phosphate buffer pH 6.88/Me_2SO (2:3). Despite a considerably higher concentration of **2** ($c = 10^{-2}$ mol L^{-1}), the acceleration is smaller than in pure aqueous buffer (run 3), which we explain besides by the different pH mainly by weaker complexation in the more organic environment.[34d]

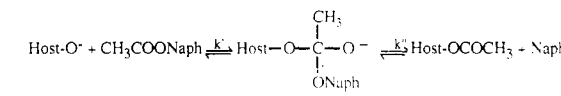
4. Runs 8 and 9 at pH 8 in aqueous phosphate buffer/1% (v/v) Me_2SO with ester substrate **26** in large excess show that **2** not only acts as transacylation reagent but that the deacylation step (k_3 in eq 1) is fast enough to allow for a weak but significant catalytic turnover. At [**2**] = 1.0×10^{-5} mol L^{-1} and [**26**] = 1.0×10^{-4} mol L^{-1} (run 9)[55] the hydrolysis of the ester is 1.3 times faster than the hydrolysis in pure buffer. The phenolate residue of **2** not only is a good nucleophile in the transacylation step but, with its estimated pK_a of ≈8.4, also represents a favorable leaving group in the deacylation step. Comparisons show that the catalytic turnover in the presence of **2** is not attributed exclusively to the presence of a phenolic group, which at pH 8 is both a good nucleophile and a good leaving group. Under conditions similar to those of run 9 ([ester] = 1.0×10^{-4} mol L^{-1}, [nucleophile] = 1.0×10^{-5} mol L^{-1}), the pseudo-first-order rate constant for the cleavage of **26** in the presence of the nonmacrocyclic comparison compound **30** is $k_{obsd} = 1.93 \times 10^{-5}$ s^{-1}, which only corresponds to a 1.08 times faster hydrolysis than in pure buffer. A significant extent of the higher catalytic turnover in the presence of **2** therefore must result from supramolecular catalysis.



29 R = Me
30 R = H

5. Runs 14–17 at pH 8 in aqueous phosphate buffer/1% (v/v) Me_2SO under nonturnover conditions with 2-naphthyl chloroacetate (**28**) provided an unexpected result. A comparison of the k_0 values for the pure buffer hydrolysis at pH 8 (runs 1 and 14) shows that the chloroacetate **28** ($k_0 = 6.35 \times 10^{-4}$ s^{-1}) is a far more activated ester than 4-nitro-1-naphthyl acetate (**26**; $k_0 = 1.8 \times 10^{-5}$ s^{-1}). However, the cleavage of **28** at pH 8 (run 16) in the presence of our most active esterase model **2** is only 2.5 times faster than the hydrolysis in the pure buffer. Runs 10–13 in aqueous borate buffer at pH 9 demonstrate that the cleavage rate of the activated chloroacetate **28** is not greater in the presence of **2** than the cleavage rate of 2-naphthyl acetate (**27**), a considerably less activated substrate. From the binding studies, it is clear that the absence of large supramolecular acceleration of the cleavage of **28** is not due to insufficient binding.

The large acceleration of the cleavage of **26** and the very weak acceleration of the cleavage of the activated ester **28** provide information on the nature of supramolecular catalysis in the complexes of **2**. The transacylation step proceeds via reversible addition and elimination steps (eq 2). Favorable proximity and orientation as well as microenvironment effects presumably lead to a lowering of the transition state for the nucleophilic addition step (k′ in eq 2) to the tetrahedral intermediate in the supramolecular complexes of all three esters **26**–**28**. Supramolecular catalysis, however, seems to be less efficient in lowering the transition state of the elimination step in the transacylation reaction (k″ in eq 2). A much faster cleavage is only observed for ester **26** with its good 4-nitro-1-



(2)

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(55) From the K_m value obtained in the evaluation of the transacylation runs, we calculated that 15% of host **2** is complexed under the conditions of run 9. The low solubility of the guest unfortunately did not allow studies at a higher degree of complexation.

naphtholate leaving group (pK_a of 4-nitro-1-naphthol = 6.60).[56,57] This is not surprising, since **2** does not provide electrophilic or general acid–base catalysis to assist the departure of a poorer leaving group. In the transacylation reaction of **26**, the very good leaving group lowers the transition state of the elimination step to an extent that the nucleophilic addition step (k′) under formation of the tetrahedral intermediate becomes rate determining. If this first transition state in eq 2 now becomes lowered in the supramolecular complex, this will be reflected in large rate accelerations. In the transacylation reaction of **27** and **28** with the poorer leaving groups, the elimination step must be rate determining. If the transition state of the elimination step is not lowered in the supramolecular complex, even a considerable stabilization of the addition step transition state will not lead to an observed acceleration.

In conclusion, we have prepared a series of novel macrobicyclic hosts with nucleophiles located atop apolar cavity binding sites. These α-chymotrypsin mimics form stable complexes with aromatic guests in aqueous solution. In aqueous phosphate buffer at pH 8, host **2** with its partially ionized phenolic residue is acylated much faster by complexed 4-nitro-1-naphthyl acetate (**26**) than host **4**, which possesses a nonionized alcoholic hydroxyl group. The comparison of the esterase activity of the two structurally related hosts **1** and **2**, both with phenolic nucleophiles atop the binding sites, has provided a convincing example of the importance of a productive binding geometry. A favorable orientation of the host–nucleophile and the carboxyl residue of ester **26** is only possible in the complex of the *larger* macrocycle **2**, and the observed saturation kinetics provides evidence for supramolecular transacylation. The complexation between the smaller host **1** and ester **26** is entirely nonproductive; the acyl transfer reaction is slower and obeys second-order kinetics. Host **2** shows a modest turnover in the supramolecular hydrolysis of 4-nitro-1-naphthyl acetate (**26**) in aqueous phosphate buffer at pH 8. Higher transacylation rates are observed in supramolecular complexes of esters with good leaving groups than of esters activated at the acyl side. Supramolecular catalysis by **2** seems to be more efficient in lowering the transition state of the nucleophilic addition step than in lowering the transition state of the elimination step of the transacylation reaction. With its optimized reactive site and a potent phenolate nucleophile which also acts as a good leaving group in the deacylation step, host **2** is a suitable system for further modifications, e.g., by introducing two imidazoles into **3**.

Experimental Section

General. 1D ^1H NMR was carried out on Bruker WP80, HX360, and AM500 spectrometers. 2D COSY spectra were obtained at 360 and 500 MHz. All spectra of macromonocyclic and macrobicyclic structures were evaluated with the help of COSY spectra. Parameters included in the Bruker software version 850101 were applied to the 2D COSY and the 1D NOE difference spectroscopy.[58] All δ values (ppm) in the spectra to characterize new compounds refer to Me_4Si as internal standard. If not stated otherwise, the spectra were recorded at 303 K. EI mass spectra (70 eV) were carried out on a Du Pont CEC 21-492 instrument. FAB spectra (matrix: *m*-nitrobenzyl alcohol) were recorded on an AEI MS 902 and a VG FAB-SE spectrometer. Melting points (uncorrected) were measured on a Büchi (Dr. Tottoli) apparatus. IR spectra were recorded on a Perkin-Elmer PE 580 instrument. Elemental analysis was performed at Max-Planck-Institut für medizinische Forschung, Heidelberg. Analytical thin-layer chromatography (TLC) was conducted on Polygram SIL G/UV_{254} TLC cards, Macherey-Nagel. The following packing materials were used in column chromatography: E. Merck silica gel 60, 0.063–0.2 mm, for gravity chromatography; E. Merck silica gel 60, 0.04–0.063 mm, for flash chromatography at ≈1 bar. For medium-pressure liquid chromatography at 15–20 bar, thick glass columns (e.g., 25 × 600 mm) were packed with silica gel, 0.012–0.021 or 0.020–0.045 mm, from Labomatic. The columns were packed either by following a wet procedure[59] or by using a novel rapid dry packing procedure specifically developed for this work.[60]

Materials. Reagents were purchased and were used without further purification unless otherwise specified. Chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2), dimethylformamide (DMF), dioxane, and ether were purified for cyclization reactions by stirring over basic alumina, activity I, from E. Merck under N_2 followed by filtration. Nitromethane was dried over molecular sieves. Chloroform saturated with ammonia was prepared as a chromatographic eluant by extracting concentrated NH_4OH with chloroform and drying the resulting chloroform solution over sodium sulfate.

For ^1H NMR binding studies, *p*-tolunitrile, *p*-nitrotoluene, 2-methoxy-6-naphthonitrile, and sodium 2-naphthalenesulfonate, purchased from Aldrich Chemical Co., were recrystallized until a correct elemental analysis was obtained. Trimethyl(1-naphthyl)ammonium sulfonate[61] and 4-nitro-1-naphthol[62] were prepared according to literature procedures. Deuteriated buffers for ^1H NMR spectroscopy were prepared by freeze-drying 10 mL of the aqueous buffer solution followed by three cycles of adding D_2O (99.8 atom % D) and freeze-drying. After the last freeze-drying, 10 mL of D_2O (99.96 atom % D) was added to the residue.

For the kinetic runs, 2-naphthylacetate was purchased from Sigma. The other esters, 2-naphthyl α-chloroacetate[63] and 4-nitro-1-naphthyl acetate,[64] were prepared according to literature procedures. The pH 8 phosphate buffer (I = 0.19) was prepared by mixing 5.5 mL of 0.066 M KH_2PO_4 and 94.5 mL of 0.066 M Na_2HPO_4. The pH 9 borate buffer was purchased from Riedel-de-Haen. The pH 10.5 borate buffer (I = 0.21) was prepared by mixing 52.8 mL of aqueous borate solution containing 0.2 mol L^{-1} H_3BO_3 and 0.1 mol L^{-1} NaOH together with 47.2 mL of 0.1 M NaOH. The standard phosphate buffer, pH 6.88, of Riedel-de-Haen was used to prepare the phosphate buffer/Me_2SO (2:3) solutions (I = 0.04). The buffer solutions were always prepared with degassed, doubly distilled water. Solutions with pH ≥9 were saturated with argon and stored with the exclusion of carbon dioxide. Phosphate buffers were stored for a maximum of 7 days.

Synthesis. 1-Acetyl-4,4-bis(3-bromo-4-hydroxy-5-methylphenyl)piperidine (6). A total of 35.0 g (0.22 mol) of bromine in 200 mL of glacial acetic acid was added dropwise to a stirred suspension of 33.9 g (0.10 mol) of 1-acetyl-4,4-bis(4-hydroxy-5-methylphenyl)piperidine (**5**)[34b] in 500 mL of glacial acetic acid containing 500 mg of iodine. After the addition was completed, stirring was continued for 1 h. A total of 3 L of water was added, and the precipitated product was collected by filtration, washed to neutrality with water, and dried at 80 °C (10^{-3} Torr): 49.1 g (99%) of **6**, which was used without further purification in subsequent reactions. For elemental analysis, **6** was recrystallized from methanol: mp 228 °C; IR (KBr) ν (OH) 3380, (C=O), 1620 cm^{-1}; ^1H NMR (80 MHz, Me_2SO-d_6) δ 1.97 (s, 3 H, $NCOCH_3$), 2.19 (s, 6 H, aryl CH_3), 2.05–2.4 (m, 4 H, 3-H), 3.2–3.55 (m, 4 H, 2-H), 7.08 and 7.19 (AB, J = 2.5 Hz, 4 H, 2′-H, 6′-H), 8.83 (s, br, 2 H, OH); MS, m/z (relative intensity) 496 (100, M^+). Anal. Calcd for $C_{21}H_{23}Br_2NO_3$ (497.3): C, 50.72; H, 4.66; N, 2.82; Br, 32.14.

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Found: C, 50.68; H, 4.76; N, 2.84; Br, 32.04.

1-Acetyl-4,4-bis[3-bromo-4-(4-chlorobutoxy)-5-methylphenyl]piperidine (7a). A suspension of 49.8 g (0.10 mol) of **6** and 10.0 g (0.25 mol) of NaOH in 1 L of 1-butanol was heated to reflux with mechanical stirring. Upon addition of 70 mL of water, a clear solution was obtained. A total of 254.0 g (2.0 mol) of 1,4-dichlorobutane, dissolved in 350 mL of 1-butanol, and 60.0 g (0.43 mol) of K_2CO_3 was added, and the stirred mixture was heated to reflux for 2 days. The solvent and the excess of 1,4-dichlorobutane were removed under reduced pressure, and the residue was partitioned between CH_2Cl_2 and 2 N KOH. The organic layer was extracted two more times with 2 N KOH, washed three times with saturated NaCl, and dried over sodium sulfate. Evaporation of the CH_2Cl_2 gave crude **7a** as a yellow oil, which eventually started crystallizing. The addition of ligroin (40–80 °C)/ether (9:1) led to the completion of the crystallization, and the product was isolated by filtration and washed with ligroin (40–80 °C): 58.4 g (86%) of colorless **7a**; mp 145 °C; IR (KBr) ν (C=O) 1640 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.9–2.15 (m, 8 H, 3″-H, 4″-H), 2.06 (s, 3 H, $NCOCH_3$), 2.15–2.4 (m, 4 H, 3-H), 2.26 (s, 6 H, aryl CH_3), 3.25–3.75 (m, 8 H, 2-H, 5″-H), 3.90 (t, J = 5.5 Hz, 4 H, 2″-H), 6.88 and 7.19 (AB, J = 2.5 Hz, 4 H, 2′-H, 6′-H); MS, m/z (relative intensity) 677 (15, M^+), 91 (100). Anal. Calcd for $C_{29}H_{37}Br_2Cl_2NO_3$ (678.4): C, 51.35; H, 5.50; Br + Cl, 34.01; N, 2.06. Found: C, 51.58; H, 5.57; Br + Cl, 33.77; N, 2.10.

1-Acetyl-4,4-bis[3-bromo-4-(6-chlorohexoxy)-5-methylphenyl]piperidine (7b). When the procedure described above for the preparation of **7a** was followed, 49.7 g of **6** and 310 g (2 mol) of 1,6-dichlorohexane afforded 60.6 g (83%) of colorless **7b**: mp (MeOH) 108–110 °C; IR (KBr) ν (C=O) 1640 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.25–2.00 (m, 16 H, 3″-H, 4″-H, 5″-H, 6″-H), 2.06 (s, 3 H, $NCOCH_3$), 2.24 (s, 6 H, aryl CH_3), 2.25–2.4 (m, 4 H, 3-H), 3.25–3.75 (m, 4 H, 2-H), 3.52 (t, J = 6.8 Hz, 4 H, 7″-H), 3.87 (t, J = 6.0 Hz, 4 H, 2″-H), 6.87 and 7.20 (AB, J = 2.3 Hz, 4 H, 2′-H, 6′-H); MS, m/z (relative intensity) 730 (M^+). Anal. Calcd for $C_{33}H_{45}Br_2Cl_2NO_3$ (734.5): C, 53.97; H, 6.18; Br + Cl, 31.44; N, 1.96. Found: C, 54.19; H, 6.29; Br + Cl, 31.3; N, 2.21.

1′,1″-Diacetyl-8,16-dibromo-12,18,27,35-tetramethyldispiro[1,6,20,25-tetraoxa[6.1.6.1]paracyclophane-13,4′:32,4″-bispiperidine] (8a). A mixture of 18.0 g (53 mmol) of **5**, 35.9 g (53 mmol) of **7a**, and 26.0 g (80 mmol) of cesium carbonate in 3.3 L of dry DMF was stirred for 15 h at 90 °C under Ar. The cesium salts were removed by filtration, and the solvent was evaporated in vacuo. The residue was partitioned between $CHCl_3$ and water, and the organic phase was dried over sodium sulfate. The solvent was removed under reduced pressure, and the crude product was purified by two flash chromatographies on silica gel (l = 70 cm, d = 6 cm) from CH_2Cl_2/methanol (100:2). Upon addition of methanol to the product obtained as a viscous colorless oil after chromatography, analytically pure **8a** crystallized out: 9.5 g (19%); mp 279 °C dec; IR (KBr) ν (C=O) 1640 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 1.9–2.15 (m, 8 H, 3-H, 4-H), 2.06 and 2.08 (2 s, each 3 H, CH_3CON), 2.09 (s, 6 H, 27-CH_3), 2.15–2.35 (m, 8 H, 3′-H, 3″-H), 2.21 (s, 6 H, 12-CH_3), 3.4–3.7 (m, 8 H, 2′-H, 2″-H), 3.93 (t, J = 6.5 Hz, 4 H, 2-H), 4.02 (t, J = 5.7 Hz, 4 H, 5-H), 6.67 (d, J = 8.6 Hz, 2 H, 31-H), 6.87 (d, J = 1.9 Hz, 2 H, 11-H), 6.91 (d, J = 8.6 Hz, 2 H, 30-H), 6.92 (s, 2 H, 28-H), 7.13 (d, J = 1.9 Hz, 2 H, 9-H); MS, m/z (relative intensity) 942 (15, M^+), 144 (100). Anal. Calcd for $C_{50}H_{60}Br_2N_2O_6$ (944.9): C, 63.56; H, 6.40; Br, 16.92; N, 2.97. Found: C, 63.33; H, 6.49; Br, 16.86; N, 3.14.

1′,1″-Diacetyl-10,18-dibromo-14,20,31,39-tetramethyldispiro[1,8,22,29-tetraoxa[8.1.8.1]paracyclophane-15,4′:36,4″-bispiperidine] (8b). A mixture of 18.0 g (53.0 mmol) of **5** and 22.0 g (67.5 mmol) of cesium carbonate in 3 L of dry DMF was stirred for 2 h at 100 °C under Ar. After addition of a solution of 38.9 g (53.0 mmol) of **7b** in 300 mL of dry DMF, stirring was continued for 20 h at 100 °C. The cesium salts were removed by filtration, and the solvent was evaporated in vacuo. Chloroform was added to the residue, and polymeric materials were removed in a filtration through silica gel. The residue was partitioned between $CHCl_3$ and water, and the organic phase was dried over sodium sulfate. The solvent was removed under reduced pressure, and the crude product was chromatographed on silica gel [MPLC, 20 bars, l = 68 cm, d = 5.4 cm, SiO_2 (0.035–0.070 mm)] from $CHCl_3$/methanol (100:0.75). Recrystallization from methanol afforded 7.0 g (13%) of **8b**: mp 298 °C; IR (KBr) ν (C=O) 1640 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 1.4–1.6 (m, 8 H, 4-H, 5-H), 1.65–1.8 (m, 8 H, 3-H, 6-H), 1.95 (s, 6 H, CH_3CON), 2.06 (s, 6 H, 31-CH_3), 2.15–2.35 (m, 8 H, 3′-H, 3″-H), 2.20 (s, 6 H, 14-CH_3), 3.3–3.45 (m, 8 H, 2′-H, 2″-H), 3.80 (t, J = 6.6 Hz, 4 H, 2-H), 3.89 (t, J = 6.1 Hz, 4 H, 7-H), 6.75 (d, J = 8.6 Hz, 2 H, 35-H), 6.96 (dd, J = 8.6 and 2.0 Hz, 2 H, 34-H), 7.01 (d, J = 2.0 Hz, 2 H, 32-H), 7.14 (d, J = 2.1 Hz, 2 H, 13-H), 7.26 (d, J = 2.1 Hz, 2 H, 11-H); MS, m/z (relative intensity) 998 (4, M^+), 83 (100). Anal. Calcd for $C_{54}H_{68}Br_2N_2O_6$ (1000.9): C, 64.80; H, 6.85; Br, 15.97; N, 2.80. Found: C, 64.76; H, 7.02; Br, 15.89; N, 2.81.

1′,1″-Diacetyl-8,16-dicyano-12,18,27,35-tetramethyldispiro[1,6,20,25-tetraoxa[6.1.6.1]paracyclophane-13,4′:32,4″-bispiperidine] (9a). A stirred mixture of 5.83 g (6.0 mmol) of **8a** and 3.00 g (33.5 mmol) CuCN in 30 mL of dry DMF was heated under Ar to reflux for 12 h. After the mixture was cooled to 20 °C, 250 mL of concentrated NH_4OH was added. Water was added to give a total solution volume of 500 mL, and the precipitated crude product was collected by filtration. Chromatography on silica from $CHCl_3$/methanol (100:1) afforded 4.30 g (83%) of colorless **9a**, which was recrystallized from *n*-hexane: mp 296° dec; IR (KBr) ν (CN) 2233, (C=O) 1623 cm^{-1}; ^1H NMR (360 MHz, Me_2SO-d_6, T = 353 K) δ 1.8–2.05 (m, 8 H, 3-H, 4-H), 1.95 (s, 6 H, CH_3CON), 2.00 (s, 6 H, 27-CH_3), 2.05–2.45 (m, 8 H, 3′-H, 3″-H), 2.14 (s, 6 H, 12-CH_3), 3.3–3.45 (m, 8 H, 2′-H, 2″-H), 3.98 (t, J = 5.9 Hz, 4 H, 2-H), 4.09 (t, J = 6.5 Hz, 4 H, 5-H), 6.74 (d, J = 9.3 Hz, 2 H, 31-H), 6.98 (dd, J = 9.3 and 2.3 Hz, 2 H, 30-H), 6.99 (s, 2 H, 28-H), 7.44 (d, J = 2.8 Hz, 2 H, 11-H), 7.50 (d, J = 2.8 Hz, 2 H, 9-H); MS, m/z (relative intensity) 836 (100, M^+). Anal. Calcd for $C_{52}H_{60}N_4O_6$ (837.1): C, 74.61; H, 7.23; N, 6.69. Found: C, 74.43; H, 7.30; N, 6.61.

1′,1″-Diacetyl-10,18-dicyano-14,20,31,39-tetramethyldispiro[1,8,22,29-tetraoxa[8.1.8.1]paracyclophane-15,4′:36,4″-bispiperidine] (9b). A mixture of 12.8 g (12.8 mmol) of **8b** and 3.4 g (38.8 mmol) of CuCN in 60 mL of dry DMF was heated under Ar to reflux for 20 h. After cooling, the solvent was evaporated at 40 Torr, and the residue was dissolved in $CHCl_3$. The copper salts were removed by filtration and washed with $CHCl_3$. The combined $CHCl_3$ solutions were extracted three times with concentrated NH_4OH and dried over sodium sulfate. The solvent was removed in vacuo, and the residue was chromatographed on silica gel [MPLC, 20 bars, l = 68 cm, d = 5.4 cm, SiO_2 (0.035–0.070 mm)] from $CHCl_3$/methanol (100:0.75). Recrystallization from methanol afforded 10.0 g (88%) of colorless **9b**: mp 307 °C dec; IR (KBr) ν (CN) 2233, (C=O) 1640 cm^{-1}; ^1H NMR (360 MHz, Me_2SO-d_6, T = 353 K) δ 1.4–1.55 (m, 8 H, 4-H, 5-H), 1.65–1.8 (m, 8 H, 3-H, 6-H), 1.95 (s, 6 H, CH_3CON), 2.06 (s, 6 H, 31-CH_3), 2.1–2.45 (m, 8 H, 3′-H, 3″-H), 2.18 (s, 6 H, 14-CH_3), 3.3–3.5 (m, 8 H, 2′-H, 2″-H), 3.89 (t, J = 6.2 Hz, 4 H, 2-H), 4.00 (t, J = 6.5 Hz, 4 H, 7-H), 6.74 (d, J = 8.6 Hz, 2 H, 35-H), 6.95 (dd, J = 8.6 and 2.1 Hz, 2 H, 34-H), 7.02 (d, J = 2.1 Hz, 2 H, 32-H), 7.47 (s, 2 H, 13-H), 7.49 (d, J = 2.2 Hz, 2 H, 11-H); MS, m/z (relative intensity) 892 (29, M^+), 55 (100). Anal. Calcd for $C_{56}H_{68}N_4O_6$ (893.2): C, 75.24; H, 7.74; N, 6.27. Found: C, 75.31; H, 7.67; N, 6.27.

8,16-Bis(aminomethyl)-1′,1″-diethyl-12,18,27,35-tetramethyldispiro[1,6,20,25-tetraoxa[6.1.6.1]paracyclophane-13,4′:32,4″-bispiperidine] (10a). A total of 140 mL (140 mmol) of a 1 M solution of borane in THF was added under Ar to a solution of 5.9 g (7.0 mmol) of **9a** in 50 mL of THF. The mixture was stirred at room temperature for 4 h and then heated to reflux for 15 h. After cooling to 20 °C, 70 mL of THF/ethanol (1:1) was added carefully, and the solvents were removed in vacuo (40 Torr). The residue was dissolved in 100 mL of ethanol and 3 mL of concentrated sulfuric acid, and the solution was heated to reflux for 30 min. The solution was evaporated at 35 °C (40 Torr) to a residual volume of 20 mL, which was partitioned between 2 N NaOH and $CHCl_3$. The aqueous phase was extracted five times with $CHCl_3$, and the combined organic phases were dried over sodium sulfate. Evaporation of the solvent afforded 5.4 g (94%) of **10a** as a colorless glass, which was used for the subsequent amide cyclizations without further purification: IR (KBr) ν (NH) 3420, 3370 cm^{-1}; ^1H NMR (500 MHz, Me_2SO-d_6, T = 350 K) δ 0.92 and 0.93 (2 t, J = 7.1 Hz, each 3 H, CH_3CH_2N), 1.7–1.95 (m, 8 H, 3-H, 4-H), 2.02 (s, 6 H, 27-CH_3), 2.08 (s, 6 H, 12-CH_3), 2.18 and 2.19 (2 q, J = 7.1 Hz, each 2 H, CH_3CH_2N), 2.25–2.6 (m, 16 H, 2′-H, 2″-H, 3′-H, 3″-H), 3.62 (s, 4 H, CH_2NH_2), 3.73 (t, J =

6.5 Hz, 4 H, 5-H), 3.94 (t, J = 5.8 Hz, 4 H, 2-H), 6.70 (d, J = 8.4 Hz, 2 H, 31-H), 6.88 (d, J = 2.2 Hz, 2 H, 11-H), 6.92 (dd, J = 8.4 and 2.2 Hz, 2 H, 30-H), 6.98 (d, J = 2.2 Hz, 2 H, 28-H), 7.16 (d, J = 2.2 Hz, 2 H, 9-H); MS, m/z (relative intensity) 816 (7, M^+), 84 (100); HRMS m/z (M^+, $C_{52}H_{72}N_4O_4$) calcd 816.5553, obsd 816.5565.

10,18-Bis(aminomethyl)-1′,1″-diethyl-14,20,31,39-tetramethyldispiro[1,8,22,29-tetraoxa[8.1.8.1]paracyclophane-15,4′:36,4″-bispiperidine] (10b). Following the procedure described for **10a**, we reacted 5.7 g of **9b** with 127.6 mL (127 mmol) of a 1 M solution of borane in THF to give 5.2 g (93%) of **10b** as a colorless glass: IR (KBr) ν (NH), 3370, 3300 cm^{-1}; ^{1}H NMR (360 MHz, Me_2SO-d_6, T = 343 K) δ 0.93 and 0.94 (2 t, J = 7.1 Hz, each 3 H, CH_3CH_2N), 1.4–1.55 (m, 8 H, 4-H, 5-H), 1.6–1.75 (m, 8 H, 3-H, 6-H), 2.04 (s, 6 H, 31-CH_3), 2.11 (s, 6 H, 14-CH_3), 2.15–2.25 (m, 4 H, CH_3CH_2N), 2.25–2.45 (m, 16 H, 2′-H, 2″-H, 3′-H, 3″-H), 3.65 (s, 4 H, CH_2NH_2), 3.67 (t, J = 6.6 Hz, 4 H, 7-H), 3.87 (t, J = 6.2 Hz, 4 H, 2-H), 6.71 (d, J = 8.5 Hz, 2 H, 35-H), 6.88 (d, J = 2.3 Hz, 2 H, 13-H), 6.93 (dd, J = 8.5 and 2.3 Hz, 2 H, 34-H), 6.98 (d, J = 2.3 Hz, 2 H, 32-H), 7.15 (d, J = 2.3 Hz, 2 H, 11-H); MS (FAB; $C_{56}H_{80}N_4O_4$, m/z (relative intensity) 874 (45, M^+ + H), 873 (100, M^+), 872 (32, M^+ – H), 871 (29, M^+ – 2H).

8,16-Bis(acetamidomethyl)-1′,1″-diethyl-12,18,27,35-tetramethyldispiro[1,6,20,25-tetraoxa[6.1.6.1]paracyclophane-13,4′:32,4″-bispiperidine] (11). A suspension of 100 mg (0.12 mmol) of **10a** in 10 mL of acetic anhydride was stirred for 3 days at 20 °C, at which time a clear solution had formed. The excess of acetic anhydride was removed in vacuo. The residue was partitioned between $CHCl_3$ and 2 N Na_2CO_3, and the aqueous phase was extracted two times with $CHCl_3$. The combined organic phases were dried over sodium sulfate, and the solvent was removed by distillation. Medium-pressure liquid chromatography (MPLC) of the residue on silica gel from ethyl acetate as eluant gave 55 mg (51%) of **11**: mp 128–130 °C (ether); IR (KBr) ν (NH) 3280, (C=O) 1650 cm^{-1}; ^{1}H NMR (500 MHz, Me_2SO-d_6, T = 350 K) δ 0.95 (t, J = 7.1 Hz, 6 H, CH_3CH_2N), 1.75–2.0 (m, 8 H, 3-H, 4-H), 1.78 (s, 6 H, CH_3CO), 2.04 (s, 6 H, 27-CH_3), 2.10 (s, 6 H, 12-CH_3), 2.15–2.5 (m, 20 H, CH_3CH_2N, all piperidine H), 3.75 (t, J = 6.7 Hz, 4 H, 5-H), 3.94 (t, J = 6.1 Hz, 4 H, 2-H), 4.17 (d, J = 5.7 Hz, 4 H, CH_2NH), 6.71 (d, J = 8.6 Hz, 2 H, 31-H), 6.90 (d, J = 2.2 Hz, 2 H, 11-H), 6.92 (dd, J = 8.6 and 2.2 Hz, 2 H, 30-H), 6.99 (d, J = 2.2 Hz, 2 H, 28-H), 7.01 (d, J = 2.2 Hz, 2 H, 9-H), 7.78 (t, J = 5.7 Hz, 2 H, CH_2NH); MS (FAB), m/z (relative intensity) 900 (10, M^+), 899 (34, M^+ – H), 898 (48, M^+ – 2H), 84 (100). Anal. Calcd for $C_{56}H_{76}N_4O_6$ (901.2): C, 74.63; H, 8.50; N, 6.22. Found: C, 74.41; H, 8.68; N, 5.98.

1,3-Bis[(succinimidooxy)carbonyl]-5-methoxybenzene (12). A solution of 15.5 g (75 mmol) of dicyclohexylcarbodiimide (DCC) in 250 mL of dioxane was added at 20 °C to a stirred solution of 5.9 g (30 mmol) of 5-methoxyisophthalic acid[36] and 7.5 g (65 mmol) of N-hydroxysuccinimide in 250 mL of dioxane. After 2 h, the precipitated dicyclohexylurea was removed by filtration and washed twice with dioxane. After the filtrate and the dioxane washings were combined, the solvent was removed in vacuo. Recrystallization of the residue from 2-propanol afforded 9.5 g (81%) of **12**: mp 169–171 °C; IR (KBr) ν (C=O), 1780, 1740 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 2.92 (s, 8 H, 3′-H, 4′-H), 3.93 (s, 3 H, OCH_3), 7.91 (d, J = 1.4 Hz, 2 H, 4-H), 8.48 (t, J = 1.4 Hz, 1 H, 2-H); MS, m/z (relative intensity) 390 (2, M^+), 276 (100, $M^+ - C_4H_4NO_3$). Anal. Calcd for $C_{17}H_{14}N_2O_9$ (390.3): C, 52.31; H, 3.62; N, 7.18. Found: C, 52.60; H, 3.75; N 7.20.

5-(Benzyloxy)isophthalic Acid (13). A solution of 3.9 g (13 mmol) of dimethyl 5-(benzyloxy)isophthalate[38] and 30 g of potassium hydroxide in 300 mL of methanol was heated to reflux for 2 h. After cooling, the solution was acidified with 2 N HCl. The precipitated crude diacid **13** was collected by filtration and dried at 100 °C (40 Torr). Recrystallization from 2-propanol afforded 2.8 g (79%) of **13**: mp 252 °C dec; IR (KBr) ν (OH) 3500–2500, (C=O) 1690 cm^{-1}; ^{1}H NMR (80 MHz, Me_2SO-d_6) δ 5.42 (s, 2 H, CH_2), 7.35–7.65 (m, 5 H, $C_6H_5CH_2$), 7.74 (d, J = 1.6 Hz, 2 H, 4-H), 8.09 (t, J = 1.6 Hz, 1 H, 2-H), 13.25 (s, br, 2 H, COOH); MS, m/z (relative intensity) 272 (8, M^+), 91 (100, $C_7H_7^+$). Anal. Calcd for $C_{15}H_{12}O_5$ (272.3): C, 66.17; H, 4.44. Found: C, 66.08; H, 4.64.

Bis(pentafluorophenyl) 5-(Benzyloxy)isophthalate (14). A solution of 1.52 g (7.38 mmol) of DCC in 5 mL of dioxane was added dropwise at 20 °C to a stirred solution of 1.00 g (3.68 mmol) of **13** and 1.35 g (7.36 mmol) of pentafluorophenol in 30 mL of dioxane. After 1 h of stirring, the solution was cooled in an ice bath to 0 °C. The precipitated dicyclohexylurea was removed by filtration and washed once with dioxane. The filtrate and the washing liquor were combined, and the solvent was removed in vacuo. Recrystallization of the residue from n-heptane afforded 1.90 g (85%) of **14**: mp 110 °C; IR (KBr) ν (C=O) 1770 cm^{-1}; ^{1}H NMR (80 MHz, Me_2SO-D_6) δ 5.37 (s, 2 H, CH_2), 7.25–7.7 (m, 5 H, $C_6H_5CH_2$), 8.14 (d, J = 1.3 Hz, 2 H, 4-H), 8.43 (t, J = 1.3 Hz, 1 H, 2-H); MS, m/z (relative intensity) 604 (<1%, M^+), 421 (100, $M^+ - C_6F_5O$). Anal. Calcd for $C_{27}H_{10}F_{10}O_5$ (604.4): C, 53.66; H, 1.67. Found: C, 53.87; H, 1.45.

Bis(pentafluorophenyl) 5-Acetoxyisophthalate (15). A solution of 7.22 g (35 mmol) of DCC in 10 mL of dioxane was added dropwise at 20 °C to a solution of 3.81 g (17 mmol) of 5-acetoxyisophthalic acid[65] and 6.26 g (34 mmol) of pentafluorophenol in 120 mL of dioxane. After 1 h of stirring, the precipitated dicyclohexylurea was removed by filtration and washed once with dioxane. The filtrate and the washing liquor were combined, and the solvent was removed in vacuo. Recrystallization of the residue from n-heptane afforded 7.38 g (78%) of **15**: mp 103 °C; IR (KBr) ν (C=O) 1760 cm^{-1}; ^{1}H NMR (80 MHz, Me_2SO-d_6) δ 2.35 (s, 3 H, $COCH_3$), 8.41 (d, J = 1.6 Hz, 2 H, 4-H), 8.72 (t, J = 1.6 Hz, 1 H, 2-H); MS, m/z (relative intensity) 556 (1, M^+), 372 (100, $M^+ - C_6F_5O$ – H), 43 (50, CH_3CO^+). Anal. Calcd for $C_{22}H_6F_{10}O_6$ (556.3): C, 47.50; H, 1.09. Found: C, 47.55; H, 1.31.

11′,14′:16′,19′-Dietheno-1,1″-diethyl-35′,39′-metheno-37′-methoxy-3′,12′,18′,27′-tetramethyldispiro[piperidine-4,15′-[5,10,20,25]tetraoxa[33,41]diazatetracyclo[27.15.1.0^{4,43}.0^{26,31}]-pentatetraconta-1′,3′,11′,13′,16′,18′,26′,28′,30′,35′,37′,43′-dodecaene-45′,4″-piperidine]-34′,40′-dione (16). A solution of 1.20 g (1.47 mmol) of **10a** in 100 mL of degassed, dry dioxane and a solution of 0.57 g (1.47 mmol) of **12** in the same solvent were added under Ar dropwise and synchronously to 250 mL of dioxane heated to reflux. A high-dilution apparatus described by Vögtle[66] was used in this procedure. After the addition, stirring was continued for 2 h. The solvent was removed in vacuo, and the residue was partitioned between $CHCl_3$ and 2 N NaOH. The aqueous layer was extracted with four portions of $CHCl_3$, and the combined organic phases were dried over sodium sulfate. Evaporation of the solvent afforded a crude product, which was chromatographed (MPLC, l = 12 cm, d = 3 cm, 20 bars) on silica gel from ethyl acetate/methanol/triethylamine (100:10:5). Addition of ligroin (40–80 °C) to the combined pure product fractions ($R_f \approx 0.2$) afforded 0.30 g (21%) of **16** as a colorless solid: mp 178–180 °C; IR (KBr) ν (NH) 3340, (C=O) 1660 cm^{-1}; ^{1}H NMR (360 MHz, Me_2SO-d_6, T = 373 K) δ 0.90 and 0.91 (2 t, J = 7.1 Hz, each 3 H, CH_3CH_2N), 1.75–2.05 (m, 8 H, 3-H, 4-H), 1.99 (s, 6 H, 27-CH_3), 2.05–2.25 (m, 4 H, CH_3CH_2N), 2.17 (s, 6 H, 12-CH_3), 2.25–2.45 (m, 16 H, 2′-H, 2″-H, 3′-H, 3″-H), 3.65–3.75 and 3.8–3.9 (2 m, 4 H, 5-H), 3.87 (s, 3 H, OCH_3), 3.91 (t, J = 5.9 Hz, 4 H, 2-H), 4.42 and 4.47 (AB part of ABX, J = 15.0, 5.0, and 4.2 Hz, 4 H, CH_2NH), 6.58 (d, J = 8.5 Hz, 2 H, 31-H), 6.72 (dd, J = 8.5 and 2.3 Hz, 2 H, 30-H), 6.94 (d, J = 2.3 Hz, 2 H, 28-H), 7.11 (d, J = 2.0 Hz, 2 H, 11-H), 7.29 (d, J = 2.0 Hz, 2 H, 9-H), 7.34 (d, J = 1.2 Hz, 2 H, 4‴-H), 7.55 (s, 1 H, 2‴-H), 7.96 (X part of ABX, J = 5.0 and 4.2 Hz, 2 H, CH_2NH); MS (EI), m/z (relative intensity) 976 (7, M^+), 975 (8, M^+ – H), 84 (100). Anal. Calcd for $C_{61}H_{76}N_4O_7$ (977.3): C, 74.97; H, 7.84; N, 5.73. Found: C, 75.24; H, 7.76; N, 5.55.

37′-(Benzyloxy)-11′,14′:16′,19′-dietheno-1,1″-diethyl-35′,39′-metheno-3′,12′,18′,27′-tetramethyldispiro[piperidine-4,15′-[5,10,20,25]tetraoxa[33,41]diazatetracyclo-[27.15.1.0^{4,43}.0^{26,31}]pentatetraconta-1′,3′,11′,13′,16′,18′,26′,-28′,30′,35′,37′,43′-dodecaene-45′,4″-piperidine]-34′,40′-dione (17). A total of 500 mg (0.61 mmol) of **10a** in 100 mL of CH_2Cl_2 and 369 mg (0.61 mmol) of **14** in 100 mL of CH_2Cl_2 were cyclized as described above for **16** by adding the two solutions dropwise to 250 mL of CH_2Cl_2 at reflux. Workup of the cyclization as de-

(65) Gumbley, S. J.; Stewart, R. *J. Chem. Soc., Perkin Trans. 2* **1984**, 529–531.

(66) (a) Vögtle, F. *Chem.-Ztg.* **1972**, *96*, 396–403. (b) Vögtle, F. *Chem. Ind. (London)* **1972**, 346.

scribed for **16** afforded, after chromatography, 134 mg (21%) of **17**: mp 175 °C (*n*-hexane); IR (KBr) ν (NH) 3330, (C=O) 1660 cm^{-1}; ^1H NMR (360 MHz, Me_2SO-d_6, T = 350 K) δ 0.88 and 0.91 (2 t, J = 7.1 Hz, each 3 H, CH_3CH_2N), 1.75–2.05 (m, 8 H, 3-H, 4-H), 1.98 (s, 6 H, 27-CH_3), 2.05–2.5 (m, 20 H, CH_3CH_2N, 2′-H, 2″-H, 3′-H, 3″-H), 2.17 (s, 6 H, 12-CH_3), 3.6–3.7 and 3.8–3.9 (2 m, 4 H, 5-H), 3.90 (t, J = 5.9 Hz, 4 H, 2-H), 4.40 and 4.48 (AB part of ABX, J = 15.0, 5.0, and 4.2 Hz, 4 H, CH_2NH), 5.25 (s, 2 H, $PhCH_2$), 6.58 (d, J = 8.5 Hz, 2 H, 31-H), 6.71 (d, J = 8.5 Hz, 2 H, 30-H), 6.93 (s, 2 H, 28-H), 7.13 (s, 2 H, 11-H), 7.31 (s, 2 H, 9-H), 7.3–7.5 (AA′BB′C, 5 H, $C_6H_5CH_2$), 7.44 (s, 2 H, 4‴-H), 7.54 (s, 1 H, 2‴-H), 8.06 (X part of ABX, J = 5.0 and 4.2 Hz, 2 H, CH_2NH); MS (EI), m/z (relative intensity) 1052 (15, M^+), 84 (100). Anal. Calcd for $C_{67}H_{80}N_4O_7$ (1053.4): C, 76.39; H, 7.66; N, 5.32. Found: C, 76.27; H, 7.70; N, 5.34.

11′,14′:16′,19′-Dietheno-1,1″-diethyl-37′-hydroxy-35′,39′-metheno-3′,12′,18′,27′-tetramethyldispiro[piperidine-4,15′-[5,10,20,25]tetraoxa[33,41]diazatetracyclo[27.15.1.0^{4,43}.0^{26,31}]-pentatetraconta-1′,3′,11′,13′,16′,18′,26′,28′,30′,35′,37′,43′-dodecaene-45′,4″-piperidine]-34′,40′-dione (19a). A total of 2.60 g (3.18 mmol) of **10a** in 500 mL of CH_2Cl_2 and 1.77 g (3.18 mmol) of **15** in 500 mL of CH_2Cl_2 were cyclized as described above for **16** by adding the two solutions at 20 °C under Ar to 100 mL of CH_2Cl_2. After the addition was completed, stirring of the reaction mixture was continued for 2 h. The solvent was evaporated in vacuo. The residue was taken up in 60 mL of ethanol, and after addition of 20 mL of concentrated NH_4OH, the mixture was heated to 70 °C for 30 min to remove the acetyl protective group. The volume of the solution was reduced to 5 mL by vacuum distillation. After addition of 50 mL of concentrated NH_4OH, the resulting suspension was extracted five times with $CHCl_3$. The combined organic extracts were dried over sodium sulfate, after which the solvent was evaporated in vacuo. Two successive chromatographies on silica gel [MPLC, 20 bars, l = 8 cm, d = 3.7 cm, SiO_2 (0.063–0.040 mm) followed by MPLC, 20 bars, l = 44 cm, d = 2.6 cm, SiO_2 (0.012–0.021 mm)] from chloroform, saturated with ammonia/EtOH (10:1), afforded 0.62 g (20%) of **19a** as a colorless solid: mp 210 °C dec; IR (KBr) ν (OH, NH) 3320, (C=O) 1655 cm^{-1}; ^1H NMR (360 MHz, Me_2SO-d_6, T = 350 K) δ 0.94 and 0.96 (2 t, J = 7.1 Hz, each 3 H, CH_3CH_2N), 1.75–2.05 (m, 8 H, 3-H, 4-H), 1.99 (s, 6 H, 27-CH_3), 2.1–2.5 (m, 20 H, CH_3CH_2N, 2′-H, 2″-H, 3′-H, 3″-H), 2.17 (s, 6 H, 12-CH_3), 3.6–3.7 and 3.75–3.85 (2 m, 4 H, 5-H), 3.85–3.95 (m, 4 H, 2-H), 4.37 and 4.49 (AB part of ABX, J = 15.0, 5.0, and 4.2 Hz, 4 H, CH_2NH), 6.60 (d, J = 8.5 Hz, 2 H, 31-H), 6.76 (d, J = 8.5 Hz, 2 H, 30-H), 6.94 (s, 2 H, 28-H), 7.13 (s, 2 H, 11-H), 7.24 (s, 2 H, 4‴-H), 7.32 (s, 2 H, 9-H), 7.38 (s, 1 H, 2‴-H), 8.03 (X part of ABX, J = 5.0 and 4.2 Hz, 2 H, CH_2NH); MS (FAB; $C_{60}H_{74}N_4O_7$), m/z (relative intensity) 965 (24, M^+ + 2H), 964 (65, M^+ + H), 963 (100, M^+), 962 (35, M^+ – H), 961 (30, M^+ – 2H).

13′,16′:18′,21′-Dietheno-1,1″-diethyl-41′-hydroxy-39′,43′-metheno-3′,14′,20′,31′-tetramethyldispiro[piperidine-4,17′-[5,12,22,29]tetraoxa[37,45]diazatetracyclo[31.15.1.0^{4,47}.0^{30,35}]-nonatetraconta-1′,3′,13′,15′,18′,20′,30′,32′,34′,39′,41′,47′-dodecaene-49′,4″-piperidine]-38′,44′-dione (19b). A total of 3.40 g (3.89 mmol) of tetraamine **10b** in 600 mL of CH_2Cl_2 and 2.17 g (3.89 mmol) of **15** in 600 mL of CH_2Cl_2 were cyclized as described above for **16** by adding the two solutions at 20 °C under Ar to 600 mL of CH_2Cl_2. The reaction workup following the procedure described for **19a** afforded 1.1 g (28%) of **19b**: mp 202 °C dec; IR (KBr) ν (OH, NH) 3335, (C=O) 1677 cm^{-1}; ^1H NMR (360 MHz, Me_2SO-d_6, T = 343 K) δ 0.91 and 0.93 (2 t, J = 7.1 Hz, each 3 H, CH_3CH_2N), 1.45–1.6 (m, 8 H, 4-H, 5-H), 1.6–1.8 (m, 8 H, 3-H, 6-H), 1.99 (s, 6 H, 31-CH_3), 2.1–2.25 (m, 4 H, CH_3CH_2N), 2.17 (s, 6 H, 14-CH_3), 2.25–2.4 (m, 16 H, 2′-H, 2″-H, 3′-H, 3″-H), 3.71 (t, J = 6.2 Hz, 4 H, 7-H), 3.83 (t, J = 6.2 Hz, 4 H, 2-H), 4.35–4.55 (m, 4 H, CH_2NH), 6.63 (d, J = 8.6 Hz, 2 H, 35-H), 6.81 (dd, J = 8.6 and 2.2 Hz, 2 H, 34-H), 6.90 (d, J = 2.2 Hz, 2 H, 32-H), 7.13 (s, 2 H, 13-H), 7.21 (s, 4 H, 4‴-H, 11-H), 7.48 (s, 1 H, 2‴-H), 7.95 (m, 2 H, CH_2NH) 9.83 (s, br, 1 H, OH); MS (FAB; $C_{64}H_{82}N_4O_7$), m/z (relative intensity) 1021 (26, M^+ + 2H), 1020 (70, M^+ + H), 1019 (100, M^+), 1018 (32, M^+ – H), 1017 (27, M^+ – 2H).

11′,14′:16′,19′-Dietheno-1,1,1″,1″-tetraethyl-37′-hydroxy-35′,39′-metheno-3′,12′,18′,27′-tetramethyldispiro[piperidinium-4,15′-[5,10,20,25]tetraoxa[33,41]diazatetracyclo-[27.15.1.0^{4,43}.0^{26,31}]pentatetraconta-1′,3′,11′,13′,16′,18′,26′,28′,30′,35′,37′,43′-dodecaene-45′,4″-piperidinium]-34′,40′-dione Dichloride (1). A total of 100 mg (0.10 mmol) of **19a** was dissolved under Ar in 5.82 g (37.32) of freshly distilled ethyl iodide, and the solution was stirred for 14 h at 20 °C. After removal of the excess of ethyl iodide in vacuo, the residue was dissolved in methanol. Upon addition of ether/acetone (4:1), the quaternized bis(ammonium iodide) precipitated. The purification by precipitation was repeated two more times, and after 3 days of drying at 100 °C (10^{-3} Torr), 116 mg (91%) of pure bis(ammonium iodide) was obtained as a hygroscopic colorless powder: mp 250 °C dec; IR (KBr) ν (OH, NH) 3270, (C=O) 1650 cm^{-1}; MS (FAB; $C_{64}H_{84}I_2N_4O_7$), m/z (relative intensity) 1148 (19, M^+ + H – I), 1147 (28, M^+ – I), 1061 (98, M^+ – I – $2C_2H_5$ – C_2H_4), 1033 (100, M^+ – I – $2C_2H_5$ – $2C_2H_4$). For ion exchange, 80 mg (0.063 mmol) of the bis(ammonium iodide) was chromatographed on Dowex ion-exchange resin (1 × 8, Cl^-) from H_2O–methanol (3:1). Recrystallization from methanol/ether and drying for 3 days at 100 °C (10^{-3} Torr) afforded 69 mg (90%) of **1**, mp 258 °C dec, as hygroscopic colorless microcrystals. For elemental analysis, the dried product was exposed to the atmosphere for 1 h, which led to the stoichiometric uptake of 2 equiv of water: IR (KBr) ν (OH, NH) 3270, (C=O) 1655 cm^{-1}; ^1H NMR (500 MHz, CD_3CN) δ 1.18 and 1.19 (2 t, J = 7.1 Hz, each 6 H, CH_3CH_2N), 1.55–1.6 (m, 2 H, 3-H), 1.85–2.3 (m, 6 H, 3-H, 4-H), 2.01 (s, 6 H, 27-CH_3), 2.21 (s, 6 H, 12-CH_3), 2.45–2.95 (m, 8 H, 3′-H, 3″-H), 3.05–3.45 (m, 16 H, CH_3CH_2N, 2′-H, 2″-H), 3.45–3.55 and 3.6–3.7 (2 m, each 2 H, 5-H), 3.7–3.9 and 4.0–4.15 (2 m, each 2 H, 2-H), 3.84 (A part of AMX, J = 14.2 and <1 Hz, 2 H, CH_2NH), 5.31 (M part of AMX, J = 14.2 and 10.2 Hz, 2 H, CH_2NH), 6.81 (d, J = 8.4 Hz, 2 H, 31-H), 6.91 (d, J = 8.4 Hz, 2 H, 30-H), 6.92 (s, 2 H, 28-H), 7.14 (s, 1 H, 2‴-H), 7.16 (d, J = 2.0 Hz, 2 H, 11-H), 7.62 (s, 2 H, 4‴-H), 8.40 (s, 2 H, 9-H), 9.10 (X part of AMX, J = 10.2, and <1 Hz, 2 H, CH_2NH), 10.76 (s, 1 H, OH); MS (FAB), m/z (relative intensity) 1089.7 (5 M^+ – H), 1062.7 (44, M^+ – C_2H_4), 1061.7 (75, M^+ – C_2H_5), 1055.7 (31, M^+ – Cl) 1020.7 (86, M^+ – 2Cl), 1019.7 (100, M^+ – H – 2Cl) 991.6 (58, M^+ – C_2H_5 – 2Cl). Anal. Calcd for $C_{64}H_{84}Cl_2N_4O_7 \cdot 2H_2O$ (1128.3): C, 68.13; H, 7.86; N, 4.97; Cl, 6.28. Found: C, 68.12; H, 7.80; N, 4.88; Cl, 6.11.

13′,16′:18′,21′-Dietheno-1,1,1″,1″-tetraethyl-41′-hydroxy-39′,43′-metheno-3′,14′,20′,31′-tetramethyldispiro[piperidinium-4,17′-[5,12,22,29]tetraoxa[37,45]diazatetracyclo-[31.15.1.0^{4,47}.0^{30,35}]nonatetraconta-1′,3′,13′,15′,18′,20′,30′,32′,-34′,39′,41′,47′-dodecaene-49′,4″-piperidinium]-38′,44′-dione Dichloride (2). A total of 600 mg (0.588 mmol) of **19b** was dissolved under Ar in 3 mL of freshly distilled ethyl iodide, and the solution was stirred for 14 h at 20 °C. After removal of the excess of ethyl iodide in vacuo, the residue was dissolved in 2 mL of methanol. Addition of 1 mL of acetone followed by 20 mL of ether led to the precipitation of the pure quaternized bis(ammonium iodide). After 3 days of drying at 100 °C (10^{-3} Torr), 720 mg (92%) of bis(ammonium iodide) was obtained as a hygroscopic colorless powder: mp 233 °C dec. For elemental analysis, the salt was exposed for 1 h to the atmosphere, which led to the stoichiometric uptake of 2 equiv of water: IR (KBr) ν (OH, NH) 3260, (C=O) 1650 cm^{-1}; MS (FAB), m/z (relative intensity) 1205 (11, M^+ + H – I), 1204 (23, M^+ – I), 1078 (19, M^+ + H – 2I), 1077 (53, M^+ – 2I), 1076 (79, M^+ – H – 2I), 1050 (22, M^+ – C_2H_4 – 2I), 1049 (61, M^+ – C_2H_5 – 2I), 1048 (100, M^+ – C_2H_5 – H – 2I), 1047 (18, M^+ – C_2H_5 – 2HI), 1046 (21, M^+ – C_2H_5 – H – 2HI). Anal. Calcd for $C_{68}H_{92}N_4O_7I_2 \cdot 2H_2O$ (1367.4): C, 59.73; H, 7.08; N, 4.10; I, 18.56. Found: C, 59.45; H, 6.79; N, 3.92; I, 18.57. For ion exchange, 300 mg (0.225 mmol) of the bis(ammonium iodide) was chromatographed on Dowex ion-exchange resin (1 × 8, Cl^-) from H_2O/methanol (4:1). Recrystallization from methanol/ether and drying for 3 days at 100 °C (10^{-3} Torr) afforded 222 mg (86%) of **2**, mp 248 °C dec, as hygroscopic colorless microcrystals. For elemental analysis, the dried product was exposed to the atmosphere for 1 h, which led to the stoichiometric uptake of 3.5 equiv of water: IR (KBr) ν (OH, NH) 3260, (C=O) 1650 cm^{-1}; ^1H NMR (500 MHz, Me_2SO-d_6, T = 303 K) δ 1.10 and 1.13 (2 t, J = 7.1 Hz, each 3 H, CH_3CH_2N), 1.4–1.6 (m, 8 H, 4-H, 5-H), 1.65–1.85 (m, 8 H, 3-H, 6-H), 2.02 (s, 6 H, 31-CH_3), 2.20 (s, 6 H, 14-CH_3), 2.4–2.9 (m, 8 H, 3′-H, 3″-H), 3.1–3.5 (m, 16 H, CH_3CH_2N, 2′-H, 2″-H), 3.6–3.8 (m, 4 H, 7-H), 3.8–3.95 (m, 4 H, 2-H), 4.3–4.5 and 4.5–4.7 (2 m, 4 H, CH_2NH), 6.70 (d, J = 8.3 Hz, 2 H, 35-H), 6.90 (d, J = 8.3 Hz, 2 H, 34-H), 7.02 (s,

2 H, 32-H), 7.24 (s, 4 H, 13-H, 4‴-H), 7.40 (s, 1 H, 2‴-H), 7.52 (s, 2 H, 11-H), 8.39 (m, 2 H, CH_2NH), 10.25 (s, 1 H, OH); MS (FAB), m/z (relative intensity) 1078 (6, $M^+ - 2Cl$), 1077 (49, $M^+ - H - 2Cl$), 1076 (61, $M^+ - 2HCl$), 1050 (85, $M^+ - C_2H_4 - 2Cl$), 1049 (63, $M^+ - C_2H_5 - 2Cl$), 1048 (100, $M^+ - C_2H_5 - H - 2Cl$). Anal. Calcd for $C_{68}H_{92}Cl_2N_4O_7 \cdot 3.5H_2O$ (1211.5): C, 67.42; H, 8.24; N, 4.62; Cl, 5.85. Found: C, 67.61; H, 8.27; N, 4.53; Cl, 5.57.

(Benzyloxycarbonyl)bis[[(pentafluorophenoxy)carbonyl]methyl]amine (20).[11] Following the procedure described above for the preparation of **14**, we reacted 3 g (11.2 mmol) of *N*-(benzyloxycarbonyl)-*N*-(carboxymethyl)glycine[46] and 4.58 g (24.6 mmol) of pentafluorophenol in dioxane with 5.07 g (24.6 mmol) of DCC to give, after recrystallization from *n*-heptane, 5.72 g (85%) of **20**: mp 82 °C; IR (KBr) ν (C=O) 1775, 1695 cm^{-1}; ^1H NMR (80 MHz, Me_2SO-d_6) δ 4.71 (s, 2 H, NCH_2), 4.77 (s, 2 H, NCH_2), 5.15 (s, 2 H, Ar CH_2), 7.32 (m, 5 H, $C_6H_5CH_2$); MS, m/z (relative intensity) 599 (1, M^+), 416 (15, $M^+ - C_6F_5O$), 91 (100). Anal. Calcd for $C_{24}H_{11}F_{10}NO_6$ (599.4): C, 48.10; H, 1.85; N, 2.34. Found: C, 47.94; H, 1.86; N, 2.33.

40′-(Benzyloxycarbonyl)-13′,16′:18′,21′-dietheno-1,1″-diethyl-3′,14′,20′,31′-tetramethyldispiro[piperidine-4,17′-[5,12,22,29]tetraoxa[37,40,43]triazatetracyclo-[31.13.1.0^{4,45}.0^{30,35}]heptatetraconta-1′,3′,13′,15′,18′,20′,30′,-32′,34′,45′-decaene-47′,4″-piperidine]-38′,42′-dione (21). A total of 1.00 g (1.15 mmol) of tetraamine **10b** in 250 mL of CH_2Cl_2 and 0.69 g (1.15 mmol) of **20** in 250 mL of CH_2Cl_2 was cyclized as described above for **16** by adding the two solutions at 20 °C under Ar to 500 mL of CH_2Cl_2. The reaction workup following the procedure described for **16** afforded a crude product, which was purified by chromatography on silica gel [MPLC, l = 48 cm, d = 3.7 cm, SiO_2 (0.020–0.045 mm)] first from ethyl acetate/methanol (4:1) to remove pentafluorophenol and then from ethyl acetate/methanol/triethylamine (4:1:0.25). Recrystallization from ether/*n*-heptane afforded 0.52 g (41%) of colorless **21**: mp 144 °C dec; IR (KBr) ν (NH) 3250, (C=O) 1670 cm^{-1}; ^1H NMR (500 MHz, Me_2SO-d_6, T = 333 K) δ 0.93 (t, J = 7.1 Hz, 6 H, CH_3CH_2N), 1.35–1.55 (m, 8 H, 4-H, 5-H), 1.6–1.8 (m, 8 H, 3-H, 6-H), 2.02 and 2.04 (2 s, 6 H, 31-CH_3), 2.13 (s, 6 H, 14-CH_3), 2.15–2.5 (m, 20 H, CH_3CH_2N, 2′-H, 2″-H, 3′-H, 3″-H), 3.66 (t, J = 6.6 Hz, 4 H, 7-H), 3.8–3.95 (m, 4 H, 2-H), 4.11 and 4.13 (2 s, 4 H, NCH_2CO), 4.2–4.3 (m, 4 H, aryl CH_2NH), 5.04 (s, 2 H, aryl CH_2O), 6.66 and 6.70 (2 d, J = 8.4 Hz, 2 H, 35-H), 6.8–6.95 (m, 4 H, 32-H, 34-H), 6.94 (d, J = 1.6 Hz, 2 H, 13-H), 6.98 (s, 2 H, 11-H), 7.2–7.25 and 7.25–7.35 (2 m, 5 H, $C_6H_5CH_2O$), 8.9–9.0 (m, 2 H, CH_2NH); MS (FAB), m/z (relative intensity) 1107 (15, $M^+ + 3H$), 1106 (62, $M^+ + 2H$), 1105 (100, $M^+ + H$), 1104 (32, M^+), 1103 (27, $M^+ - H$). Anal. Calcd for $C_{68}H_{89}N_5O_8$ (1104.5): C, 73.95; H, 8.12; N, 6.34. Found: C, 74.02; H, 8.07; N, 6.22.

13′,16′:18′,21′-Dietheno-1,1″-diethyl-3′,14′,20′,31′-tetramethyldispiro[piperidine-4,17′-[5,12,22,29]tetraoxa-[37,40,43]triazatetracyclo[31.13.1.0^{4,45}.0^{30,35}]heptatetraconta-1′,3′,13′,15′,18′,20′,30′,32′,34′,45′-decaene-47′,4″-piperidine]-38′,42′-dione (22). A solution of 400 mg (0.362 mmol) of **21** in 50 mL of ethanol was stirred for 14 h at 20 °C in a hydrogen atmosphere (4 bars) in the presence of 400 mg of palladium (10%) on charcoal. The catalyst was removed by filtration and washed twice with ethanol. The residue, obtained by evaporation of the solvent in vacuo, was partitioned between CH_2Cl_2 and 2 N NaOH. The aqueous phase was extracted three more times with CH_2Cl_2. The combined organic phases were dried over sodium sulfate, and the solvent was distilled off. Chromatography of the crude product on silica gel [MPLC, l = 44 cm, d = 2.6 cm, SiO_2 (0.012–0.021 mm)] from ethyl acetate/methanol/triethylamine (100:20:5) followed by recrystallization from *n*-hexane afforded 210 mg (60%) of colorless **22**: mp 115 °C dec; IR (KBr) ν (NH) 3320, (C=O) 1675 cm^{-1}; ^1H NMR (500 MHz, Me_2SO-d_6, T = 333 K) δ 0.94 (t, J = 7.1 Hz, 6 H, CH_3CH_2N), 1.35–1.55 (m, 8 H, 4-H, 5-H), 1.6–1.8 (m, 8 H, 3-H, 6-H), 2.05 (s, 6 H, 31-CH_3), 2.13 (s, 6 H, 14-CH_3), 2.1–2.5 (m, 20 H, CH_3CH_2N, 2′-H, 2″-H, 3′-H, 3″-H), 3.14 (s, 4 H, NCH_2CO), 3.67 (t, J = 6.3 Hz, 4 H, 7-H), 3.86 (t, J = 6.2 Hz, 4 H, 2-H), 4.29 (d, J = 5.5 Hz, 4 H, aryl CH_2NH), 6.72 (d, J = 8.2 Hz, 2 H, 35-H), 6.94 (d, J = 8.2 Hz, 2 H, 34-H), 6.97 (s, 2 H, 32-H), 7.02 (d, J = 1.8 Hz, 2 H, 13-H), 7.05 (s, 2 H, 11-H), 8.28 (t, J = 5.5 Hz, 2 H, CH_2NH); MS, (EI), m/z (relative intensity) 970 (8, M^+), 886 (9), 84 (100). Anal. Calcd for $C_{60}H_{83}N_5O_6$ (979.4): C, 74.27; H, 8.62; N, 7.22. Found: C, 74.28; H, 8.79; N, 7.29.

40′-(Bromoacetyl)-13′,16′:18′,21′-dietheno-1,1″-diethyl-3′,14′,20′,31′-tetramethyldispiro[piperidine-4,17′-[5,12,22,29]tetraoxa[37,40,43]triazatetracyclo-[31.13.1.0^{4,45}.0^{30,35}]heptatetraconta-1′,3′,13′,15′,18′,20′,30′,-32′,34′,45′-decaene-47′,4″-piperidine]-38′,42′-dione (23). A solution of 500 mg (0.515 mmol) of the triamine **22** and 208 mg (1.03 mmol) of bromoacetyl bromide in dry CH_2Cl_2 was stirred for 1.5 h at 20 °C under Ar. The excess of bromoacetyl bromide was removed in vacuo, and upon addition of ether to the oily residue, the product crystallized out. After addition of 10 mL of *n*-hexane, the crystals were collected by filtration, washed twice with *n*-hexane, and dried at 20 °C (10^{-3} Torr): 530 mg (82%) of colorless **23** as dihydrobromide salt, which was used in the next step without further purification. For elemental analysis, **23** was further purified by chromatography on silica gel [MPLC, l = 44 cm, d = 2.6 cm, SiO_2 (0.012–0.021 mm)] from chloroform, saturated with ammonia/methanol (20:1). During the chromatographic purification, half of the total amount of **23** was lost due to decay on the column. The purified **23** was added to ether, saturated with dry HBr gas, and the dihydrobromide salt of **23** was isolated by filtration as a hygroscopic colorless solid. After being dried at 20 °C (10^{-3} Torr), the salt was exposed for 1 h to the atmosphere, which led to a stoichiometric uptake of 3.5 equiv of water. ^1H NMR, IR, and mass spectra were obtained from the free, thermolabile bis(tertiary amine) **23** after the MPLC purification: mp of dihydrobromide salt 186–188 °C dec; IR (KBr) ν (NH) 3260, (C=O) 1665 cm^{-1}; ^1H NMR (500 MHz, Me_2SO-d_6) δ 0.9–1.0 (m, 6 H, CH_3CH_2N), 1.4–1.55 (m, 8 H, 4-H, 5-H), 1.65–1.8 (m, 8 H, 3-H, 6-H), 2.05 (s, 6 H, 31-CH_3), 2.13 (s, 6 H, 14-CH_3), 2.1–2.5 (m, 20 H, CH_3CH_2N, 2′-H, 2″-H, 3′-H, 3″-H), 3.65 (t, J = 6.1 Hz, 4 H, 7-H), 3.87 (t, J = 6.1 Hz, 4 H, 2-H), 4.21 and 4.29 (2 d, J = 5.2 Hz, 4 H, aryl CH_2NH), 4.35 (s, 2 H, CH_2Br), 6.72 and 6.73 (2 d, J = 8.3 Hz, 2 H, 35-H), 6.83 (d, J = 8.3 Hz, 2 H, 34-H), 6.9–7.0 (m, 4 H, 13-H, 32-H), 7.02 (s, 2 H, 11-H), 8.69 and 9.15 (2 t, J = 5.2 Hz, 2 H, aryl CH_2NH); MS (FAB), m/z (relative intensity) 1092 (100, M^+ for ^{81}Br), 1090 (94, M^+ for ^{79}Br). Anal. Calcd for $C_{62}H_{84}N_5O_7Br \cdot 2HBr \cdot 3.5H_2O$ (1316.2): C, 56.54; H, 6.94; N, 5.20; Br, 18.49. Found: C, 56.58; H, 7.12; N, 5.32; Br, 18.21.

40′-[(Hydroxymethyl)carbonyl]-13′,16′:18′,21′-dietheno-1,1″-diethyl-3′,14′,20′,31′-tetramethyldispiro[piperidine-4,17′-[5,12,22,29]tetraoxa[37,40,43]triazatetracyclo-[31.13.1.0^{4,45}.0^{30,35}]heptatetraconta-1′,3′,13′,15′,18′,20′,30′,-32′,34′,45′-decaene-47′,4″-piperidine]-38′,42′-dione (24). A mixture of 300 mg (0.239 mmol) of **23** and 300 mg (1.295 mmol) of freshly prepared silver oxide[47] in 10 mL of acetone/water (7:3) was stirred at 40 °C for 24 h. After filtration of the silver salts and evaporation of the solvents in vacuo, the residue was partitioned between $CHCl_3$ and 2 N NaOH. The aqueous phase was extracted three times with $CHCl_3$, the combined organic extracts were dried over sodium sulfate, and the solvent was distilled off. Chromatography on silica gel [MPLC, l = 44 cm, d = 2.6 cm, SiO_2 (0.012–0.021 mm)] from ethyl acetate/methanol/triethylamine (20:4:1) afforded an oil, which, upon addition of *n*-hexane, gave 15 mg (6%) of **24** as a colorless solid: mp 152 °C dec; IR (KBr) ν (OH, NH), 3260, (C=O) 1660 cm^{-1}; ^1H NMR (500 MHz, Me_2SO-d_6) δ 0.96 and 0.97 (2 t, J = 7.1 Hz, 6 H, CH_3CH_2N), 1.4–1.55 (m, 8 H, 4-H, 5-H), 1.65–1.8 (m, 8 H, 3-H, 6-H), 2.05 (s, 6 H, 31-CH_3), 2.12 and 2.13 (2 s, 6 H, 14-CH_3), 2.15–2.5 (m, 20 H, CH_3CH_2N, 2′-H, 2″-H, 3′-H, 3″-H), 3.65 (t, J = 6.0 Hz, 4 H, 7-H), 3.86 and 3.87 (2 t, J = 6.0 Hz, 4 H, 2-H), 4.03 (s, br, 2 H, CH_2OH), 4.1–4.2 (m, 2 H, NCH_2CO), 4.2–4.25 (m, 4 H, NCH_2CO, aryl CH_2NH), 4.25–4.35 (m, 2 H, aryl CH_2NH), 4.79 (t, J = 5.3 Hz, 1 H, OH), 6.73 (2 d, J = 8.9 Hz, 2 H, 35-H), 6.84 (s, 2 H, 13-H), 6.93 (s, 2 H, 11-H), 6.95 (d, J = 8.9 Hz, 2 H, 34-H), 7.03 (s, 2 H, 32-H), 8.78 and 9.17 (2 t, J = 6.0 Hz, 2 H, aryl CH_2NH); MS (FAB; $C_{62}H_{85}N_5O_8$), m/z (relative intensity) 1030.6 (22, $M^+ + 2H$), 1029.6 (59, $M^+ + H$), 1028.6 (100, M^+), 1027.6 (23, $M^+ - H$), 1026.6 (17, $M^+ - 2H$).[67]

40′-[(Hydroxymethyl)carbonyl]-13′,16′:18′,21′-dietheno-1,1,1″,1″-tetraethyl-3′,14′,20′,31′-tetramethyldispiro[piperidinium-4,17′-[5,12,22,29]tetraoxa[37,40,43]triazatetracyclo-[31.13.1.0^{4,45}.0^{30,35}]heptatetraconta-1′,3′,13′,15′,18′,20′,30′,-32′,34′,45′-decaene-47′,4″-piperidinium]-38′,42′-dione Di-

(67) The purity of these compounds is ≥98% (500-MHz NMR). The elemental analysis was not done for reasons of small product quantities.

chloride (4). Compound **24** (5 mg, 0.0048 mmol) was stirred in 1 mL of freshly distilled ethyl iodide under Ar for 14 h at 20 °C. The excess of ethyl iodide was removed in vacuo. Addition of 0.5 mL of methanol and 5 mL of ether to the residue led to the precipitation of the product, which was isolated by filtration and dried at 60 °C (10^{-3} Torr): 5.9 mg (95%) of colorless bis(quaternary ammonium iodide); mp 208 °C dec; IR (KBr) ν (OH, NH) 3260, (C=O) 1660 cm^{-1}; MS (FAB; $C_{66}H_{95}I_2N_5O_8$), m/z (relative intensity) 1215 (44, $M^+ + 2H - I$), 1214 (100, $M^+ + H - I$), 1213 (90, $M^+ - I$), 1212 (17, $M^+ - HI$). For ion exchange, 4.1 mg (0.0032 mmol) of the bis(ammonium iodide) was chromatographed on Dowex ion-exchange resin (1 × 8, Cl^-) from doubly distilled water. Recrystallization from methanol/ether and drying for 3 days at 80 °C (10^{-3} Torr) afforded 3.0 mg (84%) of **4**, mp 214 °C dec, as hygroscopic colorless microcrystals: IR (KBr) ν (OH, NH) 3270, (C=O) 1660 cm^{-1}; ^1H NMR (500 MHz, Me_2SO-d_6, T = 343 K) δ 1.15 and 1.16 (2 t, J = 7.1 Hz, each 6 H, CH_3CH_2N), 1.4–1.55 (m, 8 H, 4-H, 5-H), 1.65–1.8 (m, 8 H, 3-H, 6-H), 2.09 (s, 6 H, 31-CH_3), 2.16 (s, 6 H, 14-CH_3), 2.55–2.7 (m, 8 H, 3′-H, 3″-H), 3.2–3.3 (m, 8 H, 2′-H, 2″-H), 3.35 and 3.36 (2 q, J = 7.1 Hz, 8 H, CH_3CH_2N), 3.67 (t, J = 6.6 Hz, 4 H, 7-H), 3.89 (t, J = 6.3 Hz, 4 H, 2-H), 4.04 (s, 2 H, CH_2OH), 4.1–4.35 (m, 8 H, Ar CH_2NH, NCH_2CO), 6.77 (d, J = 8.7 Hz, 2 H, 35-H), 6.88 (s, 2 H, 13-H), 7.00 (d, J = 8.7 Hz, 2 H, 34-H), 7.01 (s, 2 H, 11-H), 7.11 (s, 2 H, 32-H), 8.65–8.75 and 9.05–9.15 (2 m, 2 H, Ar CH_2NH); MS (FAB; $C_{66}H_{95}Cl_2N_5O_8$), m/z (relative intensity) 1086.5 (23, $M^+ - 2Cl$), 1085.5 (58, $M^+ - H - 2Cl$), 1084.5 (79, $M^+ - 2HCl$), 1057.5 (63, $M^+ - C_2H_5 - 2Cl$), 1056 (100, $M^+ - C_2H_5 - H - 2Cl$).[67]

3,5-Bis(benzylcarbamoyl)anisole (29). A solution of 450 mg (4.2 mmol) of freshly distilled benzylamine in 50 mL of dry ether was dropped into a solution of 233 mg (1.0 mmol) of 3,5-bis(chloroformyl)anisole[36] in 50 mL of dry ether. The crystalline product formed during the reaction was isolated by filtration, washed with ether, and recrystallized from methanol: 266 mg (71%) of **29**, mp 169 °C; IR (KBr) ν (NH) 3235, (C=O) 1635 cm^{-1}; ^1H NMR (360 MHz, Me_2SO-d_6, T = 333 K) δ 3.85 (s, 3 H, OCH_3), 4.48 (d, J = 5.9 Hz, 4 H, $C_6H_5CH_2$), 7.2–7.4 (m, 10 H, $C_6H_5CH_2$), 7.56 (d, J = 1.5 Hz, 2 H, 4-H), 7.98 (t, J = 1.5 Hz, 1 H, 2-H), 8.96 (t, J = 5.9 Hz, 2 H, NH); MS (EI), m/z (relative intensity) 374 (100, M^+), 241 (80). Anal. Calcd for $C_{23}H_{22}N_2O_3$ (374.4): C, 73.78; H, 5.92; N, 7.48. Found: C, 73.85; H, 6.09; N, 7.27.

3,5-Bis(benzylcarbamoyl)phenol (30). A total of 3.25 mL (3.25 mmol) of a 1 M solution of boron tribromide in CH_2Cl_2 was added dropwise under Ar at 20 °C to a solution of 50 mg (0.13 mmol) of **29** in 10 mL of CH_2Cl_2. After being stirred for 3 h, the mixture was evaporated in vacuo to dryness. Upon careful addition of 2 N HCl to the residue, the crude product crystallized out. The crystals were collected by filtration and washed with water. Recrystallization from methanol/water (1:1) followed by drying at 60 °C (10^{-3} Torr) afforded 31 mg (67%) of colorless **30**: mp 189 °C; IR (KBr) ν (OH) 3400, (NH) 3230, (C=O) 1650 cm^{-1}; ^1H NMR (500 MHz, Me_2SO-d_6, T = 333 K) δ 4.46 (d, J = 5.9 Hz, 4 H, $C_6H_5CH_2$), 7.2–7.35 (m, 10 H, $C_6H_5CH_2$), 7.40 (d, J = 1.4 Hz, 2 H, 4-H), 7.80 (t, J = 1.5 Hz, 1 H, 2-H), 8.85 (t, J = 5.9 Hz, 2 H, NH), 9.75 (s, 1 H, OH); MS (EI), m/z (relative intensity) 360 (100, M^+), 106 (70). Anal. Calcd for $C_{22}H_{20}N_2O_3$ (360.4): C, 73.32; H, 5.59; N, 7.77. Found: C, 73.14; H, 5.71; N, 7.67.

^1H NMR Complexation Studies. ^1H NMR complexation shifts observed in some of the qualitative binding studies with host **1** and benzene or naphthalene derivatives are included in ref 18. The binding between hosts **1** and **2** and 4-nitro-1-naphthol (**25**) was determined quantitatively by a nonlinear least-squares fit of ^1H NMR titration data obtained in D_2O/methanol-d_4 at T = 298 K, [host] = 1×10^{-1}–4×10^{-3} mol L^{-1}, [**25**] = 4×10^{-4} or 1×10^{-3} mol L^{-1}, CH_3OD as internal reference (Figure 1). Titrations with host **1** in pure aqueous buffers could not be evaluated since the upfield moving resonances of the guest protons become very broad due to slow exchange on the ^1H NMR time scale. Methanol was added to obtain comparable binding data for the complexes of both hosts. Complexation shifts of the host protons observed during the titration to determine the stability of the **1·25** complex at [**1**] = [**25**] = 4.0×10^{-4} mol L^{-1}: $\Delta\delta$ = +0.26 (3-H), +0.23 (4-H), +0.07 (12-CH_3), +0.12 (27-CH_3), −0.08 (3″-H), +0.35 and +0.45 (5-H), +0.25 (2-H), +0.10 and −0.03 (CH_2NH), +0.05 (31-H), −0.08 (30-H), −0.06 (28-H), −0.09 (11-H), −0.16 (2‴-H), −0.12 (9-H), −0.07 (4‴-H). Complexation shifts of the guest protons at [**1**] = 2×10^{-4} mol L^{-1}, [**25**] = 4×10^{-4} mol L^{-1}: $\Delta\delta$ = +0.15 (2-H), +0.43 (3-H), +0.28 (5-H), +0.09 (6-H), +0.12 (7-H), +0.13 (8-H). Study of the **2·25** complex:complexation shifts of the host protons observed during the titration to determine the stability of the **2·25** complex at [**2**] = 8.0×10^{-4} mol L^{-1}, [**25**] = 1.0×10^{-3} mol L^{-1}: $\Delta\delta$ = ≈+0.23 (4,5-H), +0.14 (3,6-H), +0.07 (31-CH_3), +0.07 (14-CH_3), +0.33 (7-H), +0.13 (2-H), +0.24 (CH_2NH), −0.06 (32-H), −0.08 (34-H), −0.05 (13-H), −0.11 (11-H). Complexation shifts of the guest protons at [**2**] = 1.8×10^{-3} mol L^{-1}, [**25**] = 1.0×10^{-3} mol L^{-1}: $\Delta\delta$ = +0.83 (2-H), +1.36 (3-H), +0.79 (5-H), +0.91 (7-H), +0.94 (6-H), +0.73 (8-H).

Kinetic Measurements. Preparation of a Sample Solution Containing [host] = 5.0×10^{-4} mol L^{-1} and [ester] = 2.0×10^{-5} mol L^{-1}. A 2.5×10^{-4} M solution of the ester was freshly prepared for each kinetic run by dissolving the ester in 1.25 mL of Me_2SO and adding 8.75 mL of degassed doubly distilled water. A 5.435 × 10^{-4} M stock solution of host was prepared by dissolving the host in the desired aqueous buffer. The solution for the kinetic run ([host] = 5.0×10^{-4} mol L^{-1} and [ester] = 2.0×10^{-5} mol L^{-1} in aqueous buffer/1% (v/v) Me_2SO) was prepared by adding 0.16 mL of the ester solution to 1.84 mL of the host solution.

Kinetics. Reactions were followed in a Cary 17 UV spectrometer in 1-cm quartz cells at 20.0 (±0.1) °C. A total of 1.84 mL of pure buffer solution or of buffer solution containing the host was added to the cell, which was equilibrated and maintained at 20 °C in the thermostated cell holders (15 min). A total of 0.16 mL of ester solution was added. The cells, fitted with Teflon brand stoppers, were taken out of the cell holders and shaken thoroughly for a short time. The cell was placed back into the cell holder, and optical densities were measured continuously at a fixed wavelength depending on the ester to be hydrolyzed. To monitor the formation of 2-naphthol in the cleavage of **27** and **28**, we set the wavelength at λ = 329 nm. The two esters **27** and **28** do not show any absorption above $\lambda \geq 320$ nm.[68] The leaving group, predominately present as 2-naphthol at pH 8 (pK_a = 9.51 at 25 °C),[69] has an absorption maximum at λ = 329 nm with ϵ = 2000,[70] which allows the optical detection of its formation. At the given concentration ranges, 1-naphthyl acetate and 1-naphthyl chloroacetate could not be used as substrates. Above the end absorption of the two esters at λ = 320,[68] the extinction coefficient of the cleavage product 1-naphthol (pK_a at 25 °C ≈ 9.34)[69] predominately present at pH 8 is too small ($\epsilon \approx 60$ at 329 nm)[70] to allow optical detection of its formation. To monitor the formation of 4-nitro-1-naphtholate in the cleavage of **26**, we set the wavelength at λ = 480 nm for runs in pure aqueous buffer and at λ = 515 nm for runs in phosphate buffer/Me_2SO (2:3). The addition of the ester solution into the cell was chosen as time t = 0, and the first recordings were made after ≈25–30 s. Endpoints were noted at 5–8 half-lives and were stable within experimental error. Ten experimental data points were taken from the plot of the optical density as a function of time. The optical density at the endpoint was taken as 100% relative absorbance (A_∞), and the optical densities at the other data points at time t were changed into relative absorbances (A_t). For the calculation of the pseudo-first-order rate constants k_0 and $k_{obs}d$, the experimental data were fitted to the equation $y = a(1 - e^{-kt})$ by using a computer-assisted nonlinear least-squares fit ($y = A_t$; $a = A_\infty$). All rate constants given in Table I were obtained from triplicate runs, and their reproducibility was ±8%.

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Registry No. **1**·2H$^-$, 114274-42-9; **1**·2Cl$^-$, 108787-19-5; **1**·2Cl$^-$/**25**, 114274-56-5; **1**·2Cl$^-$/*p*-nitrotoluene, 114274-58-7; **1**·2Cl$^-$/*p*-tolunitrile, 108787-28-6; **1**·2Cl$^-$/2-methoxy-6-naphthonitrile,

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108824-13-1; 1·2Cl⁻/sodium 2-naphthylenesulfonate, 114274-59-8; 1·2Cl⁻/trimethyl(1-naphthyl)ammonium fluorosulfonate, 114274-60-1; 2·2I⁻, 114274-52-1; 2·2Cl⁻, 114274-35-0; 2·2Cl⁻/25, 114274-57-6; 4·2Cl⁻, 114274-36-1; 4·2I⁻, 114274-55-4; 5, 99407-79-1; 6, 108787-21-9; 7a, 108787-22-0; 7b, 114274-48-5; 8a, 108787-23-1; 8b, 114274-49-6; 9a, 108787-24-2; 9b, 114274-50-9; 10a, 108787-25-3; 10b, 114274-51-0; 11, 114274-37-2; 12, 114274-38-3; 13, 114274-39-4; 14, 114299-60-4; 15, 108787-26-4; 16, 114274-40-7; 17, 114274-41-8; 19a, 108787-27-5; 19b, 114274-53-2; 20, 105400-47-3; 21, 114274-43-0; 22, 114274-44-1; 23, 114274-54-3; 23·2HBr, 114299-61-5; 24, 114274-45-2; 25, 605-62-9; 26, 6549-14-0; 27, 1523-11-1; 28, 26177-06-0; 29, 114274-46-3; 30, 114274-47-4; α-chymotrypsin, 9004-07-3; 1,4-dichlorobutane, 110-56-5; 1,6-dichlorohexane, 2163-00-0; 5-methoxyisophthalic acid, 46331-50-4; N-hydroxysuccinimide, 6066-82-6; dimethyl 5-(benzyloxy)isophthalate, 53478-04-9; pentafluorophenol, 771-61-9; 5-acetoxyisophthalic acid, 90466-78-7; N-(benzoyoxycarbonyl)-N-(carboxymethyl)glycine, 17335-88-5; bromoacetyl bromide, 598-21-0; 3,5-bis(chloroformyl)anisole, 35227-77-1; 2-naphthol, 135-19-3; p-nitrotoluene, 99-99-0; p-tolunitrile, 104-85-8; 2-methoxy-6-naphthonitrile, 67886-70-8; sodium 2-naphthylenesulfonate, 532-02-5; trimethyl(1-naphthyl)ammonium fluorosulfonate, 93254-42-3.

Aspartame Decomposition and Epimerization in the Diketopiperazine and Dipeptide Products as a Function of pH and Temperature

Susan M. Gaines and Jeffrey L. Bada*

Amino Acid Dating Laboratory (A-012B), Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093

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Cyclization and hydrolysis of aspartame were studied over a range of pH and temperatures by using an HPLC method which allows simultaneous analysis of the diastereomeric dipeptide and diketopiperazine products. The pH dependence of aspartic acid and phenylalanine racemization rates in the dipeptide–diketopiperazine system resulting from aspartame decomposition was determined. On the basis of these studies a general scheme of relative epimerization rates of amino acids in diketopiperazines and in the various positions and ionic states of peptides is presented. This scheme is discussed in terms of the carbanion mechanism of amino acid racemization and found to be consistent with it. Racemization rates in the diketopiperazine were greater than those of all ionic forms of the free amino acids and dipeptides except for fully protonated free amino acids and protonated terminal amino acids of peptides. In the neutral pH range the relative racemization rates in the DKP and dipeptides were DKP > amino terminal > carboxy terminal. Apparently contradictory results reported in the literature from dipeptide heating experiments were reanalyzed in terms of dipeptide–diketopiperazine-inverted dipeptide conversions. Viewed in this light, the literature is self-consistent and supports the generality of our scheme of relative racemization rates and mechanistic conclusions.

Intramolecular aminolysis of dipeptides and their derivatives to form cyclic dipeptides (diketopiperazines or DKPs) occurs readily in aqueous solution.[1,2] The ubiquitous nature of this reaction has become apparent to workers in the fields of peptide chemistry and biogeochemistry. Rapid rates of internal aminolysis via DKP formation at the amino terminal of peptides has lead to the suggestion that this process may play a major role in the abiotic decomposition of proteins in fossils.[3,4] Kinetic and mechanistic studies of peptide hydrolysis[5] and amino acid racemization in proteins[2,6] have been complicated by DKP formation and peptide sequence inversion. In order to properly interpret results from studies wherein DKPs may form, a clear understanding of the dipeptide–DKP system is necessary.

The purpose of this study was to develop a general qualitative scheme of relative epimerization rates in the various positions and ionic forms of peptides. Such a scheme will assist in the interpretation of results from investigations of the geochemical decomposition of proteins, as well as in the design of experiments to model peptide decomposition and epimerization. The literature contains a number of contradictory conclusions about relative epimerization rates in DKPs and dipeptides, with widely differing mechanistic interpretations.[2,6,7] We have drawn together results from the literature and shown that they are consistant with our general scheme and with the accepted mechanistic explanation of amino acid epimerization.

In this study the decomposition and subsequent epimerization of aspartame (L-aspartyl-L-phenylalanine methyl ester) have been investigated. We have determined the rates of decomposition of this dipeptide derivative, the relative stability of the DKP and dipeptide products, and overall epimerization rates over the pH range 3–10 at temperatures ranging from 6 °C to 100 °C. Besides its low cost and ready availability, aspartame (AP-OMe) was chosen as the model dipeptide for a number of reasons. The presence of two chiral centers permits chromatographic separation of diastereomers without derivatization or hydrolysis. The Phe moiety provides a chromophore for UV detection at a convenient wavelength so that peptide hydrolysis can be followed during heating experiments. These properties enabled us to develop an analytical method for the direct, simultaneous analysis of the methyl ester and its various diastereomeric decomposition products.[8] This affords an insight into the relative rates of epimerization of the DKP and dipeptide products not accessible from analysis of D/L ratios for the amino acids in hydrolyzed samples.

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