Synthesis of Artificial Receptors as Potential Candidates for Recognition and Binding of Pterin Analogs

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Open chain podands **2a-c** and macrocycles **2d-e** have been synthesized as potential candidates for 4-aminopterin recognition and binding, featuring H-bonding characteristics and "stacking interaction" **2e**. Preliminary binding studies between **6**, **7**, and **8** (simpler analogs of the above **2a-e**) as "hosts" and appropriate "guests", showed that carbamates **2a** and **2e** are the most promising receptors for 4-aminopterin binding.

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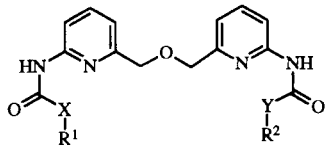
Introduction.

Since molecular recognition chemistry has become an area of current interest due to its possibility to mimic or approach nature [1], the idea to synthesize potential receptors for recognition and binding of pterin analogs appealed to us. Folic acid antagonists, such as the commonly used anti-cancer drug methotrexate, dichloroamethopterin, 10-deazamethotrexate, are 4-aminopterin analogs. Such toxic drugs could be selectively delivered to their site of action (tumor), if for example they had been attached as noncovalent complexes, through a receptor, to an antibody.

We have succesfully exploited the selective binding of those substrates showing complementary shape and H-bonding characteristics [2], as well as a stacking interaction [3], as a strategy for the design and synthesis of thymine [4a], guanine [4b], adenine [4c], barbiturates [5] and peptide receptors [4a]. Adopting this methodology, we have designed and synthesized three podands **2a-c** and two macrocycles **2d-e** as potential candidates for 4-aminopterin recognition (Scheme 1).

Scheme 1



2a-e

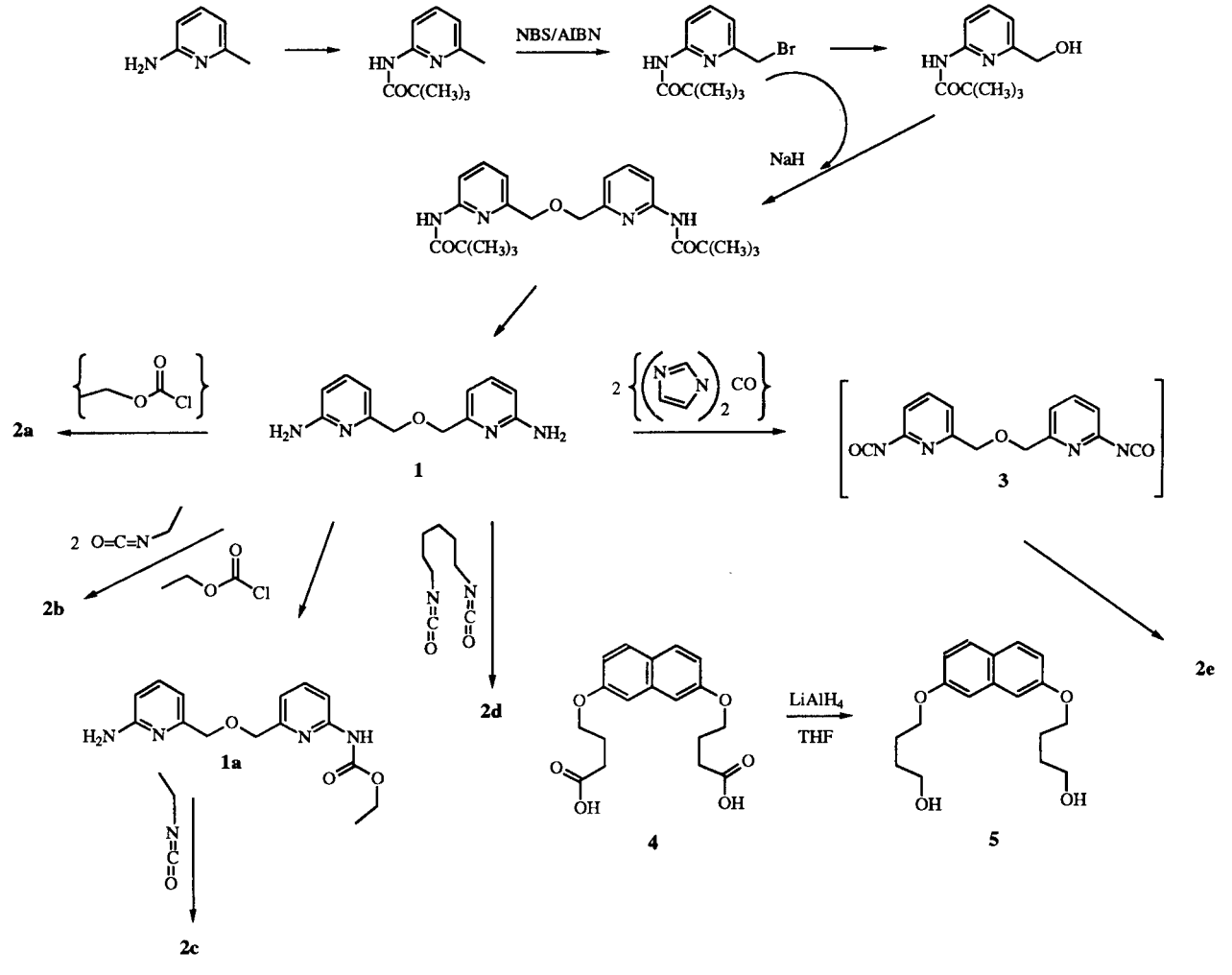
a: X=Y=O; R^1=R^2=Et
b: X=Y=NH; R^1=R^2=Et
c: X=O; Y=NH; R^1=R^2=Et
d: X=Y=NH; R^1–R^2=–$(CH_2)_6$–
e: X=Y=O; R^1–R^2=–$(CH_2)_4$–O–C$_6$H$_4$–O–$(CH_2)_4$–

Results and Discussion.

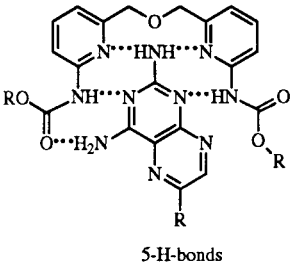
All of the above compounds feature a 2-amino-6-picolyl ether (**1**) as the basic moiety, symmetrically (**2a,b,d,e**) or unsymmetrically (**2c**) substituted on both amino-groups, to give carbamates, ureas or the combination of the two. Compound **1** was prepared in two different ways from which the most convenient is analytically described [6]. When 2-amino-6-picoline was acetylated with acetyl anhydride and then brominated with *N*-bromosuccinimide (NBS) in the presence of benzoyl peroxide or 2,2'-azobisisobutyronitrile (AIBN), the bromination occured at the 5-position of the pyridine ring rather than on the 6-methyl group [7]. However, when the bromination reaction was attempted on the pivaloylated 2-amino-6-picoline instead, the α-bromo- and the α,α-dibromo-derivatives were formed in 46% and 10% yields, respectively. Acidic hydrolysis of the monobromo-derivative led to formation of *N*-pivaloyl-2-amino-6-hydroxymethyl pyridine (50%) and 2-amino-6-hydroxymethyl pyridine (30%), while some unreacted starting material was also recovered.

The synthetic procedure for the preparation of the compounds **1** and **2a-e** is outlined in Scheme 2. The formation of ureas was almost quantative. It is worthwhile to be mentioned that macrocycle **2e** was synthesized in 20% yield in a one step reaction from the diisocyanate **3**, by coupling with 2,7-bis(4'-hydroxybutoxy)naphthalene. Compound **3** was formed *in situ*, when **1** was refluxed for 30 minutes in dichloromethane with 2 equivalents of 1,1'-carbonyldiimidazole. Among compounds **2a-e**, only compounds **2a**, **2c** and **2e** were soluble in chloroform and the binding constants (if any) could be determined by ^{1}H nmr titration. In the symmetrical systems **2a** and **2e**, there exists the possibility for 5 hydrogen bonds forming between "host" (H) and the amino-pterin "guest" (G), while in **2b**, **2d** and the unsymmetrical one, **2c**, there is

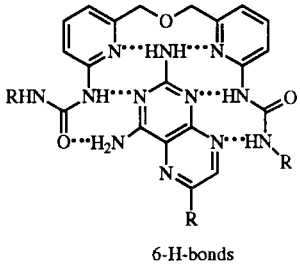
Scheme 2



Scheme 3

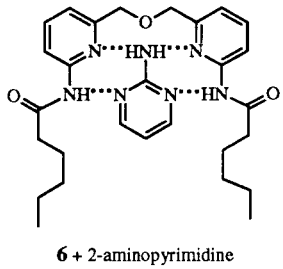


5-H-bonds

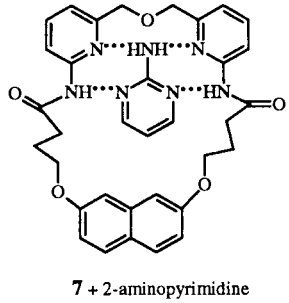


6-H-bonds

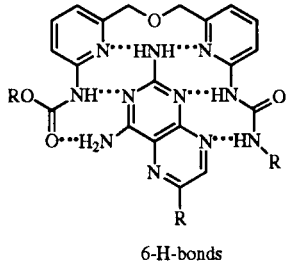
Scheme 4



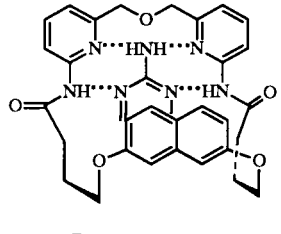
6 + 2-aminopyrimidine



7 + 2-aminopyrimidine

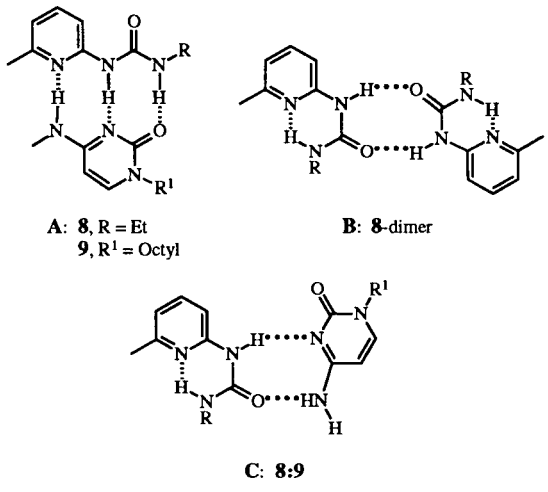


6-H-bonds



7 + 2-aminopyrimidine
(stacking interaction)

Scheme 5



A: **8**, R = Et
9, R^1 = Octyl

B: **8**-dimer

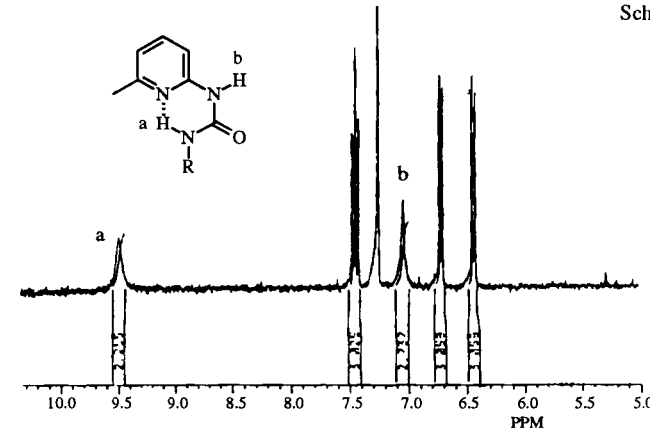
C: **8:9**

the possibility for 6 hydrogen bonds forming (Scheme 3). However, the advantage of the additional hydrogen bond in the unsymmetrical system **2c**, is probably balanced out by the statistical factor in the symmetrical ones. In other words, the probability for a favorable orientation of the guest in the unsymmetrical system decreases by half.

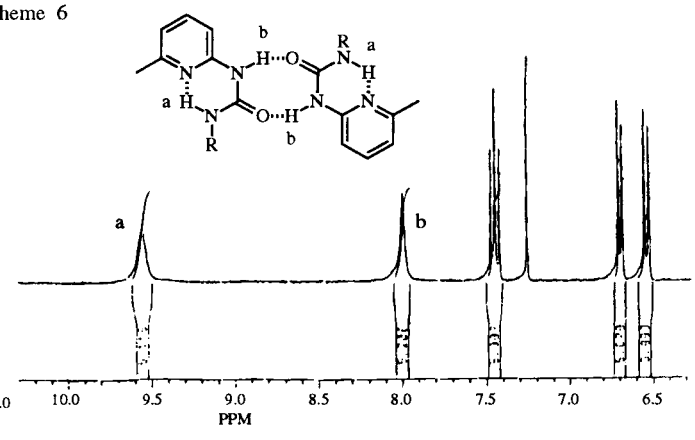
To test our binding hypothesis, we performed preliminary binding studies in the simpler systems **6** and **7** with 2-aminopyrimidine, which possess 4 sites for potential hydrogen bond formation (Scheme 4). Binding constants were calculated by ^{1}H nmr titration in deuteriochloroform, from the "host"-amidic hydrogen shifted peak [8]. Shifts of the amide protons display the largest variation among host resonances; this is understandable, since these are the protons most directly involved in binding. Shifting of the amidic hydrogen peak towards lower field values, confirms "host"-"guest" binding for both hosts. However, as one could expect [9], this shift is greater in the case of compound **7** ($\Delta\delta$ = 1.533 *versus* 0.9815 in **6**) presumably due to the coexisting "stacking" effect of the naphthalene ring, reflected by the upfield shift of the naphthalene- and amino- protons. The association constants (K_a) to 2-aminopyrimidine, of compounds **6** and **7** are 51 and 134 M^{-1}, respectively. The greater K_a value of **7** confirms also the present "stacking interaction".

If binding is observed when 4 sites of host's cavity are available for hydrogen bonding, one would expect an

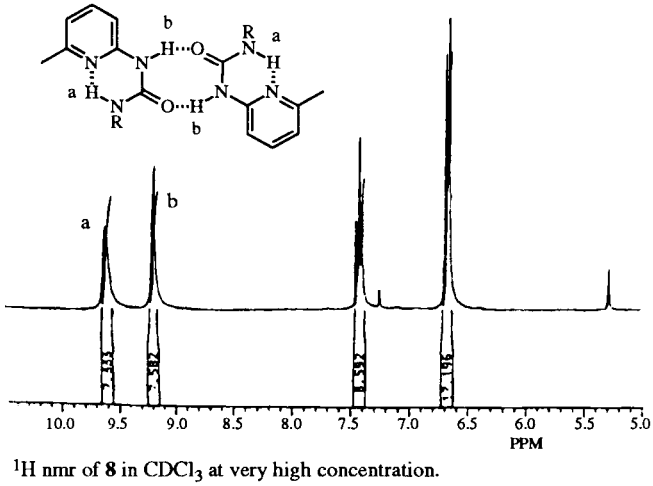
Scheme 6



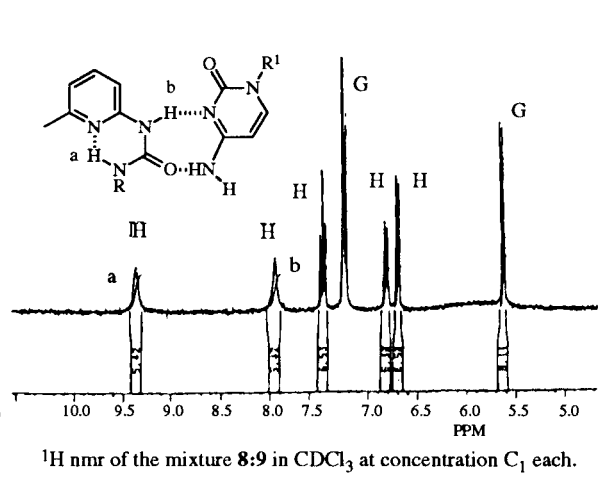
^{1}H nmr of **8** in $CDCl_3$ at concentration C_1 = 16.6 m*M*.



^{1}H nmr of **8** $CDCl_3$ at concentration $2C_1$.



^{1}H nmr of **8** in $CDCl_3$ at very high concentration.



^{1}H nmr of the mixture **8:9** in $CDCl_3$ at concentration C_1 each.

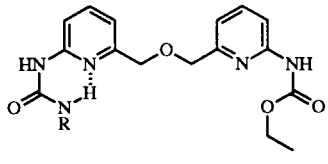
even greater binding in systems where 5 or 6 hydrogen bonds are formed, such as in the synthesized compounds **2a-e**.

For the comparative evaluation between ureas and carbamates of the compounds **2a-e**, we examined the binding in another simple model system. We prepared and used *N*-(6-methyl-2-pyridyl)-*N'*-ethylurea, **8**, as "host" and 1-octylcytosine, **9**, as the appropriate "guest". Compound **8** provides the necessary organization for 3 hydrogen bond formation with the cytosine derivative **9**, as is depicted in Scheme 5(A). However, ^{1}H nmr studies in deuteriochloroform showed that compound **8** prefers to form one intramolecular and one intermolecular hydrogen bond either with another molecule of **8** [formation of dimers, (B)] or with one molecule of **9** (C). Therefore, only the peak b, due to the amidic hydrogen adjacent to the pyridine ring, is significantly affected by the concentration of **9** (H) or **8** (G), since this hydrogen is available for intermolecular binding (Scheme 6). On the other hand the amidic hydrogen peak a is permanently shifted around 9.5 δ, indicating an intramolecular binding. In Scheme 6, the ^{1}H nmr pattern of a 1:1 mixture of **8** to **9** is almost identical, as concerns the amidic peaks (a,b) of **8**, with the ^{1}H nmr pattern of **8** itself in two fold concentration. The binding constant value, found for compound **8** is 30 M^{-1}.

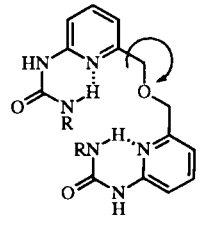
It is apparent that this possibility of intramolecular hydrogen bond formation exists also in compounds **2b**, **2c** and **2d** (Scheme 7). Indeed, in all three compounds one amidic hydrogen peak is permanently shifted towards lower field values (9.5-10 δ), which is only explicable in terms of intramolecular hydrogen bonding.

In conclusion, from the above discussion it is apparent that, besides insolubility problems, these ureas are not favorable as receptors, due to intramolecular hydrogen bonding. In contrast, however, carbamates and mainly the symmetrical macrocycle **2e** could be viewed as a promising candidate for recognition and binding to 4-aminopterins.

Scheme 7



2c



2b, 2d

EXPERIMENTAL

Melting points were determined on an open capillary Electrothermal Melting Point apparatus and are uncorrected. The ^{1}H nmr spectra were recorded mainly on a Bruker WM-300 (300 MHz) spectrometer (binding measurements), or on GEN-500 (500 MHz) or on a Gemini-300 (300 MHz) and reported in ppm (δ) relative to the solvent. Mass spectra were determined on a Varian CH-5 or VG7070 double focusing mass spectrometer at 70 eV or on a VG70-250SE model. Commercial reagents were utilized without further purification. Purity of synthesized compounds was checked by tlc and ^{1}H nmr analysis and was found to be 95% or greater. Compounds were repurified for elemental analysis by preparative tlc.

α,α'-Oxybis(6-amino-2-picoline) or Di(2-amino-6-picolyl) Ether or 2-Amino-6 picolyl Ether (**1**).

2-Amino-6-picoline was pivaloylated and then brominated [10] with NBS (2 equivalents) and AIBN (0.1 equivalent) in refluxing carbon tetrachloride to give *N*-pivaloyl-2-amino-6-bromomethylpyridine (46%), and some unreacted *N*-pivaloyl-2-amino-6-picoline. Products were separated/purified on preparative tlc plate (silica gel, 1000 μm, 15% ethyl acetate-85% petroleum ether). The monobromo-derivative was refluxed in 10% sulfuric acid for 1 hour to give some unreacted starting material, *N*-pivaloyl-2-amino-6-hydroxymethylpyridine (50%) and 2-amino-6-hydroxymethylpyridine (30%), [10]. All products were separated/purified on a preparative tlc plate (alumina, 1000 μm, ethyl acetate) as first, second and third band, respectively.

N-Pivaloyl-2-amino-6-hydroxymethylpyridine.

This compound had ^{1}H nmr (deuteriochloroform): δ 8.17 (d, J = 7.3 Hz, 1H, 3-aromatic H), 8.05 [s (br), 1H, -CONH-], 7.72 (t, J = 8 Hz, 1H, 4-aromatic H), 6.99 (d, J = 7.3 Hz, 1H, 5-aromatic H), 4.69 (s, 2H, -CH_2OH), 3.45 [s (br), 1H, -OH], 1.36 (s, 9H, -C(CH_3)$_3$); hrms Calcd. for $C_{11}H_{16}N_2O_2$: m/z 208.1212. Found: 208.1211.

N-Pivaloyl-2-amino-6-hydroxymethylpyridine (1 equivalent) was converted to its sodium salt by treating with sodium hydride (1 equivalent, 60% in mineral oil) in dry tetrahydrofuran and coupled with *N*-pivaloyl-2-amino-6-bromomethylpyridine (1 equivalent) by stirring at room temperature overnight. The pivaloylated form of compound **1** was separated from the reaction mixture by preparative tlc (alumina, 1000 μm, dichloromethane, second band), yield 48%; ^{1}H nmr (deuteriochloroform): δ 8.17 (d, J = 7.4 Hz, 2H, 3,3'-aromatic H), 7.98 [s (br), 2H, -CONH-], 7.70 (t, J = 8.0 Hz, 2H, 4,4'-aromatic H), 7.19 (d, J = 7.6 Hz, 2H, 5,5'-aromatic H), 4.62 (s, 4H, -OCH_2-), 1.31 [s, 18H, -C(CH_3)$_3$]; hrms Calcd. for $C_{22}H_{31}N_4O_3$ [M^++H]: m/z 399.2396. Found: 399.2370.

Compound **1** was readily obtained in 86% yield from the pivaloylated precursor by heating a solution of the latter in dioxane with 1*N* hydrochloric acid, as a white viscous mass; ^{1}H nmr (deuteriochloroform): δ 7.39 (t, J = 7.8 Hz, 2H, 4,4'-aromatic H), 6.78 (d, J = 7.3 Hz, 2H, 3,3'-aromatic H), 6.37 (d, J = 8.2 Hz, 2H, 5,5'-aromatic H), 4.52 [s (br), 8H, -OCH_2- plus -NH_2];

hrms Calcd. for $C_{12}H_{14}N_4O$: m/z 230.1167. Found: 230.1162.

α,α'-Oxybis[6-(ethoxycarbonyl)amino-2-picoline] or [*N,N'*-Dicarbethoxy][2-amino-6-picolyl Ether] (**2a**).

To a dichloromethane solution (3 ml) of di(2-amino-6-picolyl) ether **1** (91 mg, 0.396 mmole) and triethylamine (0.11 ml, 0.8 mmole), ethyl chloroformate (88 mg, 0.8 mmole) in dichloromethane was added dropwise at room temperature. The reaction mixture was stirred under argon atmosphere for 48 hours, after which 0.11 ml of triethylamine and 88 mg of ethyl chloroformate were added, and the reaction mixture was heated under reflux for 3 hours (no observation of the diamine on the tlc plate). The reaction mixture was then evaporated under reduced pressure, dissolved in methylene chloride, washed with saturated sodium bicarbonate and water, dried with sodium sulfate and after evaporation the residue was separated in preparative tlc (alumina, 1000 μm, dichloromethane- 4% methanol). The first band was compound **2a**, as a yellow oil (slowly crystallized), soluble in ethyl acetate, dichloromethane, chloroform or acetone, 18 mg (12%); ^{1}H nmr (deuteriochloroform): δ 7.87 (d, J = 8.3 Hz, 2H, 3,3'-aromatic H), 7.69 (t, J = 7.7 Hz, 2 H, 4,4'-aromatic H), 7.37 (s, 2H, -CONH-), 7.14 (d, J = 7.3 Hz, 2H, 5,5'-aromatic H), 4.6 (s, 4H, -OCH_2-), 4.23 (q, J = 7.1 Hz, 4H, -CH_2CH_3), 1.31 (t, J = 7.1 Hz, 6H, -CH_2CH_3); hrms Calcd. for $C_{16}H_{17}N_4O_4$: m/z 329.1250 [M^+-OEt]. Found: 329.1251.

Anal. Calcd. for $C_{18}H_{22}N_4O_5$: C, 57.75; H, 5.92; N, 14.96. Found: C, 57.51; H, 5.70; N, 14.94.

The second band was the monocarbamate **1a** from which we prepared the unsymmetrical compound **2c**. It was an oil, soluble in dichloromethane, chloroform or ethyl acetate, 5 mg (4%); ^{1}H nmr (deuteriochloroform): δ 7.84 [d, J = 8.3 Hz, 1H, 3'-aromatic H (-C_5H_3N-NHCOOEt)], 7.67 (t, J = 7.8 Hz, 1H, 4'-aromatic H), 7.50 (s, 1H, -CONH-), 7.43 [t, J = 7.8 Hz, 1H, 4-aromatic H (-C_5H_3N-NH_2)], 7.14 (d, J = 7.3 Hz, 1H, 5'-aromatic H), 6.80 (d, J = 7.4 Hz, 1H, 3-aromatic H), 6.39 (d, J = 8.1 Hz, 1H, 5-aromatic H), 4.6 (s, 2H, -CONH-C_5H_3N-CH_2O-), 4.55 (s, 2H, -OCH_2-C_5H_3N-NH_2), 4.49 (br, 2H, -NH_2), 4.22 (q, J = 7.1 Hz, 2H, -CH_2CH_3), 1.30 (t, J = 7.1 Hz, 3H, -CH_2CH_3); hrms Calcd. for $C_{15}H_{19}N_4O_3$: m/z 303.1441 [M^++H]. Found: 303.1443.

α,α'-Oxybis[6-(ethylaminocarbonyl)amino-2-picoline] or α,α'-Oxybis[6-(3'-ethylureido)-2-picoline] or [*N,N'*-Diethylcarbamoyl][2-amino-6-picolyl Ether] (**2b**).

To a dichloromethane solution (5 ml) of the diamine **1** (90 mg, 0.391 mmole), ethyl isocyanate (0.11 ml, excess) was added dropwise at 0°. After one night stirring at room temperature a white solid had precipitated. It was filtered, washed with dichloromethane and identified as **2b**. It is insoluble in chloroform, acetone, methylene chloride and ether, 102 mg (70%), mp 220-224° dec; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 9.23 (s, 2H, -CH_2N*H*CO-), 8.14 (s, 2H, -CON*H*-C_5H_3N-), 7.68 (t, J = 7.8 Hz, 2H, 4,4'-aromatic H), 7.24 (d, J = 8.2 Hz, 2H, 3,3'-aromatic H), 7.00 (d, J = 7.3 Hz, 2H, 5,5'-aromatic H), 4.58 (s, 4 H, -OCH_2-), 3.16 (t, J = 5.8 Hz, 4H, -CH_2CH_3), 1.05 (t, J = 7.1 Hz, 6H, -CH_2CH_3); hrms Calcd. for $C_{18}H_{24}N_6O_3$: m/z 372.1886. Found: 372.1885.

Anal. Calcd. for $C_{18}H_{24}N_6O_3$: C, 58.05; H, 6.5; N, 22.57. Found: C, 57.80; H, 6.41; N, 22.56.

α-[6'-(Ethylaminocarbonyl)amino-2'-picolyloxy]-6-(ethoxycarbonyl)amino-2-picoline or Ethyl 6-[α-[6'-(*N'*-Ethylureido)-2'-picolyloxy]-2-picolyl]carbamate or [*N*-Carbethoxy-*N'*-ethylcarbamoyl][2-amino-6-picolyl Ether] (**2c**).

This was prepared in an analogous fashion to compound **2b**, using the monocarbamate **1a** as starting material (20 mg of **1a**, 0.01 ml of ethyl isocyanate, 5 ml of dichloromethane). The final solid was washed many times with ether, 23.5 mg (95%); ^{1}H nmr (deuteriochloroform): δ 9.36 (s, 1H, -CH_2N*H*CO-), 8.55 (s, 1H, -CON*H*-C_5H_3N-), 7.88 [d, J = 8.1 Hz, 1H, 3-aromatic H (-OCONH-C_5H_3N-)], 7.70 (t, J = 8.1 Hz, 1H, 4-aromatic H), 7.58 [t, J = 8.1 Hz, ^{1}H, 4'-aromatic H (-C_5H_3N-NHCONH-)], 7.48 (s, 1H, -OCONH-), 7.15 (d, J = 7.5 Hz, 1H, 5-aromatic H), 7.00 (d, J = 7.5 Hz, 1H, 3'-aromatic H), 6.73 (d, J = 8.1 Hz, 1H, 5'-aromatic H), 4.64 (s, 2H, -OCH_2-), 4.62 (s, 2H, -OCH_2-), 4.23 (q, J = 8.1 Hz, 2H, -OCH_2CH_3), 3.49-3.35 (quint, 2H, -$HNCH_2CH_3$), 1.31 (t, J = 7.2 Hz, 3H, -OCH_2CH_3), 1.21 (t, J = 7.2 Hz, 3H, -$HNCH_2CH_3$); hrms Calcd. for $C_{18}H_{23}N_5O_4$: m/z 373.1730. Found: 373.1730. (also [M^++H] = 374.1808, Found: 374.1809).

Anal. Calcd. for $C_{18}H_{23}N_5O_4$: C, 57.9; H, 6.21; N, 18.76. Found: C, 57.89; H, 6.02; N, 18.70.

6,6'-(α,ω-Hexanediureido)-2,2'-(2-oxaethylene)bispyridine or [*N,N'*-(α,ω-Hexamethylenedicarbamoyl)][2-amino-6-picolyl Ether] (**2d**).

In dry dichloromethane (100 ml) and under an argon atmosphere, a solution of the diamine **1** (100 mg, 0.435 mmole) in dichloromethane (100 ml) and a solution of 1,6-diisocyanatohexane (0.07 ml) in dichloromethane (100 ml) were added dropwise from two additional funnels over a 12 hour period, and the mixture was stirred for another 24 hours at room temperature. The solvent was evaporated under reduced pressure and the residue was triturated with ether. A white solid was precipitated and identified as compound **2d**. It is insoluble in ether or dichloromethane and slightly soluble in chloroform, 107 mg (62%), mp 208-210° dec; ^{1}H nmr (deuteriochloroform): δ 9.46 (s, 2H, -CONH-), 8.88 (s, 2H, -CONH-), 7.58 (t, J = 7.6 Hz, 2H, 4,4'-aromatic H), 7.01 (d, J = 7.4 Hz, 2H, 3,3'-aromatic H), 6.76 (d, J = 8.2 Hz, 2H, 5,5'-aromatic H), 4.64 (s, 4H, -OCH_2-), 3.39-3.24 (m, 4H, -$HNCH_2$-), 1.58-1.20 [m, 8H, -$(CH_2)_4$-]; hrms Calcd. for $C_{20}H_{26}N_6O_3$: m/z 398.2066. Found: 398.2065.

Anal. Calcd. for $C_{20}H_{26}N_6O_3$: C, 60.29; H, 6.58; N, 21.09. Found: C, 60.08; H, 6.85; N, 20.9.

6,6'-[2,7-Naphthalenedi(oxybutoxycarbonylamino)]-2,2'-(2-oxaethylene)bispyridine (**2e**).

Diamine **1** (130 mg, 0.565 mmole) and carbonyl diimidazole (201 mg, 1.243 mmole) were heated under reflux in dry dichloromethane (5 ml) for half an hour to give the corresponding diisocyanate **3**, which was used without isolation for the macrocyclization. The same procedure as in the case of **2d** was followed for this step: funnel A contained a methylene chloride solution of the above diisocyanate (100 ml) [we assumed that 55 mg of the diisocyanate existed in the solution, since according to the literature [11], the preparative yield of isocyanates with this method is about 35%]; funnel B contained a methylene chloride solution of 2,7-bis (4'-hydroxybutoxy)naphthalene (59 mg, 0.194 mmole, 100 ml). After the addition was completed, the stirring was continued overnight, the solvent was evaporated and preparative tlc was followed (alumina, 1000 μm, ethyl acetate). The second band was compound **2e** as a white solid, soluble in chloroform, methylene chloride, acetone or ethyl acetate, 23 mg (20%); ^{1}H nmr (deuteriochloroform): δ 7.82 [d, J = 5 Hz, 2H,

3,3'-aromatic H (pyridinic)], 7.68 [t, J = 4.6 Hz, 2H, 4,4'-aromatic H (pyridinic)], 7.62 [d, J = 5.3 Hz, 2H, 3,6-aromatic H (naphthalenic)], 7.33 (s, 2H, -CONH-), 7.12 [d, J = 4.4 Hz, 2H, 5,5'-aromatic H (pyridinic)], 7.09 [s, 2H, 1,8-aromatic H (naphthalenic)], 6.96 [d, J = 4.3 Hz, 2H, 4,5-aromatic H (naphthalenic)], 4.58 (s, 4H, -OCH_2-C_5H_3N-), 4.28 (t, J = 3.3 Hz, 4H, -CH_2OCO-), 4.18 (t, J = 3.8 Hz, 4H, -CH_2-$OC_{10}H_6O$-), 2.05-1.89 (m, 8H, -$OCH_2(CH_2)_2CH_2O$-); ms: fab in glycerol or in *m*-nitrobenzoic acid for $C_{32}H_{34}N_4O_7$: 587 = [M^++H].

Anal. Calcd. for $C_{32}H_{34}N_4O_7$: C, 65.52; H, 5.84; N, 9.55. Found: C, 65.32; H, 5.98; N, 9.22.

2,7-Bis(3'-carboxypropyloxy)naphthalene (**4**).

This was prepared from 2,7-dihydroxynaphthalene and ethyl 4-bromobutyrate according to the literature [3a].

2,7-Bis(4'-hydroxybutoxy)naphthalene (**5**).

In a two-necked flask equipped with a condenser and a dropping funnel, were placed a 1*M* lithium aluminum hydride solution [12] in tetrahydrofuran (2.5 ml, 2.5 mmoles) and 5 ml of dry tetrahydrofuran. From the dropping funnel was added in a period of 45 minutes a tetrahydrofuran solution (8 ml) of 2,7-bis(3'-carboxypropyloxy)naphathalene (**4**) [3a] (332 mg, 1 mmole). The mixture in the flask started to bubble and a white solid was formed. After 15 minutes water was added cautiously to decompose the hydride excess, and 10% sulfuric acid solution was added to solubilize the mixture. Extraction with chloroform followed by drying and evaporation, gave the desired diol 252 mg (83%) as a white solid, soluble in ethyl acetate, chloroform or dichloromethane, mp 92-93°; ^{1}H nmr (deuteriochloroform): δ 7.62 (d, J = 8.8 Hz, 2H, 3,6-aromatic H), 7.00 (s, 2H, 1,8-aromatic H), 6.97 (d, J = 8.8 Hz, 2H, 4,5-aromatic H), 4.07 (t, J = 6.1 Hz, 4H, -C$H_2OC_{10}H_6$-), 3.73 (s, 2H, -OH), 3.69 (t, J = 6.3 Hz, 4H, -CH_2OH), 1.94-1.74 (m, 8H, -$OCH_2(CH_2)_2CH_2$OH); hrms Calcd. for $C_{18}H_{24}O_4$: m/z 304.1675. Found: 304.1674.

Anal. Calcd. for $C_{18}H_{24}O_4$: C, 71.03; H, 7.95. Found: C, 71.01; H, 7.99.

α,α'-Oxybis(6-hexanoylamino-2-picoline) or (*N,N'*-Dihexanoyl)-2-amino-6-picolyl Ether (**6**).

Hexanoyl chloride (0.122 ml, 0.87 mmole), the diamine **1** (100 mg, 0.435 mmole) and triethylamine (0.12 ml) were stirred for 2 hours at room temperature, in a dichloromethane solution (5 ml). The compound **6** was formed, 176 mg (95%), as a white solid, soluble in dichloromethane, chloroform or acetone, mp 113-115°; ^{1}H nmr (deuteriochloroform): δ 8.13 (d, J = 8.9 Hz, 2H, 3,3'-aromatic H), 7.84 (s, 1H, -CONH-), 7.71 (t, J = 7.9 Hz, 2H, 4,4'-aromatic H), 7.17 (d, J = 7.5 Hz, 2H, 5,5'-aromatic H), 4.61 (s, 4H, -OCH_2-), 2.37 (t, J = 7.5 Hz, 4H, -$COCH_2$-), 1.75-1.31 (m, 12H, -$(CH_2)_3CH_3$), 0.90 (t, J = 6.5 Hz, 6H, -$(CH_2)_3CH_3$); hrms Calcd. for $C_{24}H_{34}N_4O_3$: m/z 426.2631. Found: 426.2632.

Anal. Calcd. for $C_{24}H_{34}N_4O_3$: C, 67.58; H, 8.03; N, 13.13. Found: C, 67.37; H, 8.11; N, 13.16.

6,6'-[2,7-Naphthalenedi(4"-oxypropamido)]-2,2'-(2-oxaethylene)bispyridine (**7**).

This was prepared according to the literature [6] .

N-(6-Methyl-2-pyridyl)-*N'*-ethylurea (**8**).

In dry methylene chloride (5 ml), 2-amino-6-picoline (0.1 g, 0.907 mmole) and ethyl isocyanate (0.075 ml, 0.93 mmole) were stirred overnight under argon atmosphere at room temperature. From the reaction mixture a white crystalline solid was isolated, soluble in chloroform, dichloromethane or ethyl acetate, 164 mg (99%), mp 135-137°; ^{1}H nmr (deuteriochloroform): δ (in a 149 m*M* concentration) 9.61 (s, 1H, -CH_2N*H*CO-), 9.20 (s, 1H, -C_5H_3N-N*H*CO-), 7.43 (t, J = 7.8 Hz, 1H, 4-aromatic H), 6.67 (d, J = 7.4 Hz, 4H, 3,5-aromatic H), 3.43 (quint, J = 6.9 Hz, 2H, -NHCH_2CH_3), 2.42 (s, 3H, CH_3-C_5H_3N-), 1.25 (t, J = 7.2 Hz, 3H, -$NHCH_2CH_3$); hrms Calcd. for $C_9H_{13}N_3O$: m/z 179.1059. Found: 179.1058.

Anal. Calcd. for $C_9H_{13}N_3O$: C, 60.32; H, 7.31; N, 23.45 . Found: C, 59.98; H, 7.42; N, 22.97.

1-Octylcytosine (**9**).

This was prepared according to 1-butylcytosine [13], from *N*-acetylcytosine [14] (0.500 g), octyl bromide (0.564 ml) and potassium carbonate (0.495 g), and then methanolic ammonia (30 ml). It is a white solid, slightly soluble in chloroform, 335 mg (46%), mp 240-242°; ^{1}H nmr (deuteriochloroform): δ 7.24 [d, J = 7 Hz, 1H, 6-H (pyrimidinic)], 7.62 [d, J = 7 Hz, 1H, 5-H (pyrimidinic)], 5.38 (br, 2H, -NH_2), 3.75 (t, J = 7.2 Hz, 2H, >NCH_2-), 1.70-1.68 (m, 2H, >NCH_2CH_2-), 1.30-1.25 (m, 10H, -$(CH_2)_5CH_3$), 0.89 (t, J = 6.9 Hz, 3H, -$(CH_2)_5CH_3$); hrms Calcd. for $C_{12}H_{21}N_3O$: m/z 223.1698. Found: 223.1698.

Anal. Calcd. for $C_{12}H_{21}N_3O$: C, 64.54; H, 9.48; N, 18.82. Found: C, 64.32; H, 9,44; N, 18.85 .

Association Constant (K_a) Determination.

A standarized experimental protocol was used for the determination of binding constants of compounds **6** and **7** with 2-aminopyrimidine or of compound **8** with 1-octylcytosine, by ^{1}H nmr titration. Ten μmoles of purified host were dissolved in 1 ml of deuteriochloroform to give a 10 m*M* solution. A 1 *M* standard solution of 2-aminopyrimidine was prepared by adding 1 ml of deuteriochloroform to 95 mg (1 mmole). Similarly, a 1 *M* standard solution of 1-octylcytosine was prepared by dissolving 223 mg (1 mmole) in 1 ml of deuteriochloroform. A microliter syringe was used to withdraw guest solution through a plastic septum and measure it into the 5 mm nmr tube containing the host solution. Typically 10 to 15 data points between 0.0 and 10 equivalents of guest were taken. Changes in the chemical shift of the macrocyclic amidic protons were tabulated. The δ and Δδ data from these protons were used in subsequent calculations and the binding constants (K_a) were calculated from the Scatchard [8b] plot: $\Delta\delta/[G_0] = \Delta\delta max \cdot K_a - \Delta\delta \cdot K_a$ as the negative slope of the obtained line: $\Delta\delta/[G_0]$ versus Δδ. ($\Delta\delta = \delta - \delta_0$, where δ_0 is the chemical shift of noncomplexed host and δ is the chemical shift being observed in the complexed host. Δδ = Δδmax, when δ becomes maximum; $[G_0]$ is the initial (total) concentration of guest; K_a is the association or binding constant).

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[6] S. Goswami and A. D. Hamilton; Unpublished results. Briefly, compound **1** was also prepared as follows: 2-amino-6-picoline was acetylated with acetyl chloride and oxidized to the corresponding picolinic acid with potassium permanganate in water at 55°. The acetyl group was removed by sodium hydroxide and the cesium salt was formed by reacting with cesium carbonate. The benzyl ester of the 2-amino-6-picolinic acid was formed by reacting its cesium salt with benzyl bromide in dimethyl sulfoxide suspension; the latter was reduced to the corresponding alcohol with lithium aluminum hydride in ether. This intermediate was selectively acetylated on the amino group by reacting with acetic anhydride, followed by overnight stirring with potassium carbonate in methanol. Part of the amino-protected alcohol was converted to the corresponding bromide by phosphorus tribromide treatment. Then the 2-acetylamino-6-hydroxymethylpyridine was coupled with 2-acetylamino-6-bromomethylpyridine by sodium hydride/tetrahydrofuran treatment, to give the acetylated form of compound **1**. Hydrolysis of the latter with sodium hydroxide gave compound **1**.

[7] The ^{1}H nmr peak at 6.87 δ, representing the proton on the C-4 position of the pyridine ring had disappeared after bromination, while no methylene group was observed.

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