MOLECULAR RECOGNITION OF NUCLEIC ACID COMPONENTS

by

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Dedicated to my wife and son

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Submitted to the Department of Chemistry
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requirements for the Degree of Doctor of Philosophy

ABSTRACT

Synthetic receptors for guanine, thymine, thymine photodimer and organic diacids are described. Self replicating systems are developed and experimentally demonstrated to show the autocatalytic behavior. These systems are prepared with either Kemp's triacid (**6**) or xanthene diacid **7**. The acyl amidine structure was useful for the recognition of guanine derivatives and selectivity of these systems toward different type of nucleotide bases was assessed with solubility titration. Receptors for thymine and thymine photodimer were prepared from xanthene diacid. Nuclear magnetic resonance (NMR) spectroscopy was used for the evaluation of the binding constants. Synthetic replicators are generated by covalent attachment of two complementary subunits to a self-complementary molecule. The new replicators involve hydrogen bonding of thymine derivatives to diaminotriazines as the recognition vehicle, and autocatalytic behavior is experimentally demonstrated. Diacid binders are prepared and found to bind simple organic diacids in non-polar organic solvents. Evidence for the complexation is presented. Also included are cleftlike molecules with urea and bipyridine functionality inside the cavity. These molecule may be used for hydrolysis of ester derivatives.

Thesis Supervisor: Julius Rebek, Jr.
Title: Professor of Chemistry

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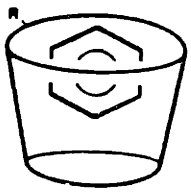
Introduction

Recognition at the molecular level is a fundamental and ubiquitous process in biochemical systems. It is the basis of enzyme-substrate specificity as well as the source of reagent selectivity in organic synthesis.[1]

An important goal in modern bioorganic chemistry concerns the design of synthetic molecules that mimic various aspects of enzyme chemistry. Detailed study of such models can lead not only to insights into the nature of enzyme action but might also lead to new chemical species with the specificity and speed normally associated only with enzymes. Central to the success of this endeavor is the development of molecular architecture in which catalytically useful functional groups are positioned in a well-defined array to provide a specific chemical microenvironment.[1]

For the last two decades, model bulding in bioorganic chemistry has generally been limited to macrocyclic molecules. This situation may result from their commecial availability (cyclodextrins **1**[2]), or their synthetic accessibility (cyclophanes **2**[4] and crown ethers **3**[5]).[3]



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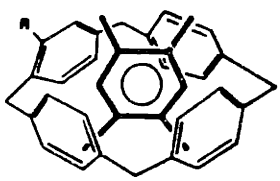


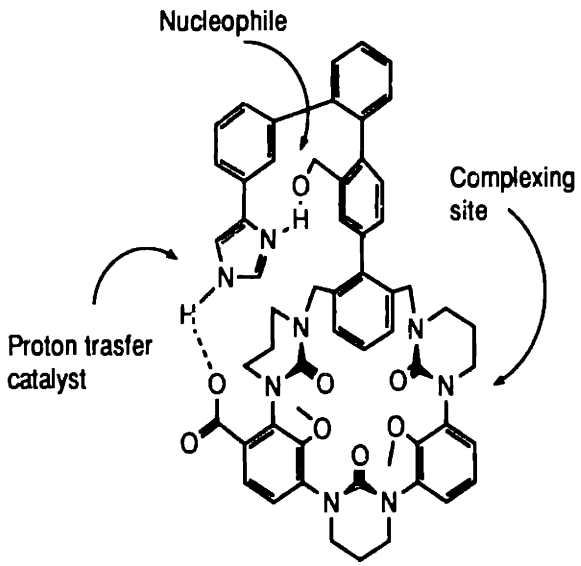
2

3

Macrocycles, however, have certain chemical disadvantages.[3] For molecular recognition to occur, host and guest must have complementarity, either in functionality, size, or in shape. In macrocyclic receptors, however, the major binding pattern is limited to size recognition, resulting in limited

selectivity, although crown ethers show exceptional affinity toward the protonated primary amine functionality. In contrast, natural systems often bind through functionality and/or shape recognition. A good example is the D-glucose binding protein[6] in which ten different amino acid side chains combine to create a cavity and bind D-glucose through a 13-hydrogen bond array.

Another problem with macrocyclic structures is that they are difficult to functionalize with reactive groups that *converge* on the guest molecule. The topology of such systems favors reagent approach to the outer surface and leads to auxiliary functionality which diverges; the functional groups introduced are directed away from the guest molecules. Focusing functional groups on the interior of the molecule requires a tremendous synthetic investment. A good example of this is macrocycle **4** designed by D. J. Cram *et al.*[7]



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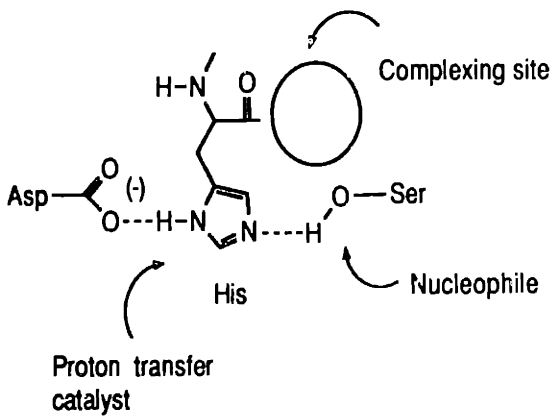
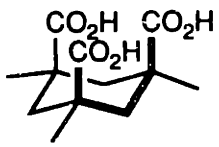


5

Molecule **4** is an active site model of chymotrypsin. The nucleophilic hydroxyl, an imidazole, and a carboxylic group in chymotrypsin is

preorganized largely by hydrogen bonds as depicted in **5**. Note that the divergent functionality in **4** is refocused back into the binding site by nearly U shaped geometry using both ortho and meta sustituted phenyl spacers. This molecule, in fact, is "much too complicated to synthesize without getting encouragement from simpler model compounds".[7]

In the late 1980s, this research group introduced Kemp's triacid (**6**)[8], originally synthesized by professor Daniel S. Kemp[9] at MIT, as a building block for convergent molecular clefts and other related compounds.



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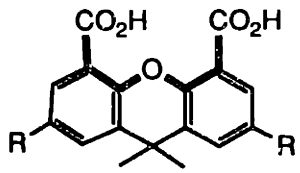
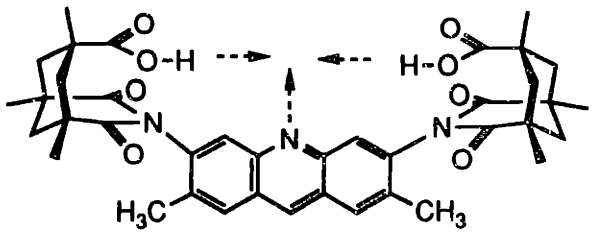


7

7a ; R = t- amyl
7b ; R = t-butyl

Kemp's triacid (**6**) has three carboxylate groups that show U-turn shape between any two functions. Another system introduced more recently is xanthene diacid **7**[10] which also has U-turn shape, but in a two dimensional array. From these molecules, depending on the mode of attachment and on the shape of spacers, varieties of different moleclules could be derived. These systems have been found to be useful in molecular recognition and other organic transfomations.[11] One of the most successful applictions of these compounds is the acridine diacid **8**[12], which has three convergent functional groups in a well defined microenvironment.

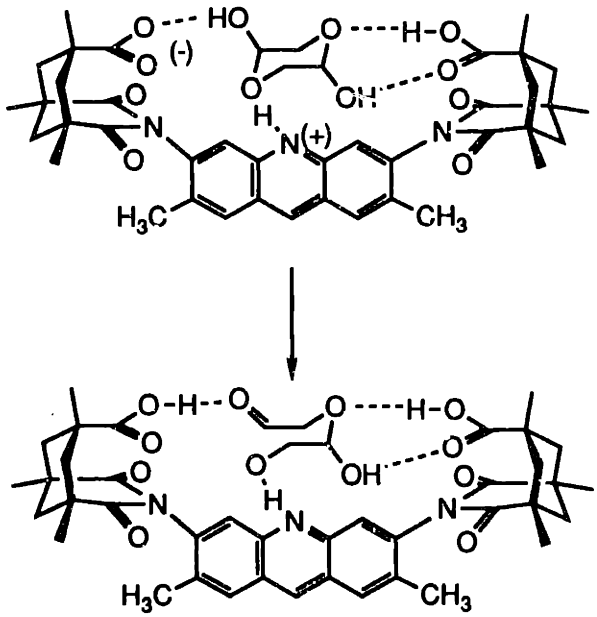


8

The importance of convergent functionality has been demonstrated by hemiacetal cleavage (**9** to **10**) catalysed by diacid **8**.[13]

9 **10**

The diacid cleft turned out to be by far the superior catalyst to the other systems prepared by combining similar functional groups with similar acidity and basicity.[13,14] The proposed mode of reaction is shown below, although the alternative mechanism involving catalysis by the single acid function cannot be ruled out.



The convergent functional group approach has been useful for the recognition of nucleotide bases such as adenine[34] and cytosine.[36] In the present study, we extend this approach to the recognition of guanine, thymine and the thymine photodimer. Complexation of other organic diacids in non-polar organic solvents, using Kemp's triacid (**6**) and xanthene diacid **7** derivatives is also described here.

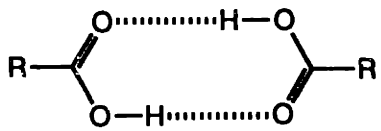
Chapter 1

Diacid binding with convergent diamine cleft.

1.1 Introduction

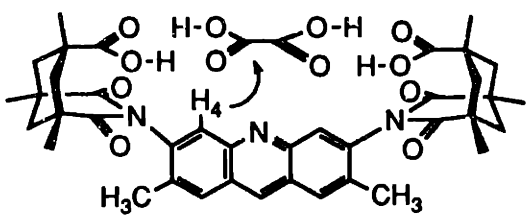
Organic diacids have been frequent targets in molecular recognition.[15-18] Carboxylic acids are known to form intermolecular hydrogen bonds through 8-membered rings (Fig 1). Since small diacids (C2-C6 and aromatic diacids) are usually insoluble in nonpolar organic solvents such as chloroform or methylene chloride, titration experiments cannot be performed. Instead, solid-liquid, liquid-liquid extraction experiments have generally been used in complexation studies.

Fig 1

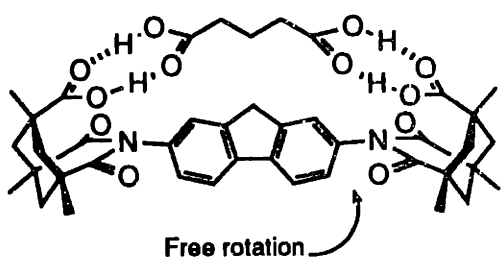


In 1987, Rebek, Nemeth, Ballester and Lin reported the binding of oxalic, malonic and glutaric acids with the diacid clefts **8** and **9** (Fig 2).[15]

Fig 2



8 + oxalic acid complex

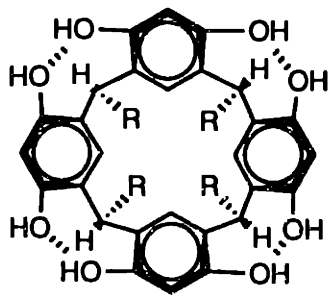


9 + glutaric acid complex

The acridine diacid **8** extracts one equivalent of oxalic acid, while the wider fluorene diacid cleft **9** binds glutaric acid. Unambiguous evidence of oxalic acid binding comes from intermolecular NOE experiment in which irradiation of the H4 proton of 1 : 1 complex results in 18 % enhancement of the ^{13}C-oxalic acid signal. NMR signals of fluorene diacid **9** are quite broad due to C_{aryl}-N_{imide} bond rotations and are temperature dependent, but upon complexation with glutaric acid the NMR signals sharpen up and are no longer temperature dependent.[15] It is interesting to note that the guest diacids bind the cleft diacid through hydrogen bonding while giving up two pairs of hydrogen bonds between themselves.

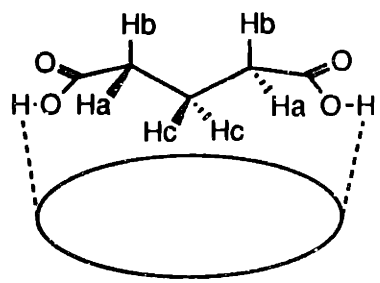
Binding of diacids through carboxylic acid-phenol interaction was also reported. Aoyama and his coworkers have prepared resorcinol-aldehyde cyclotetramer **10** (Fig 3).[16]

Fig 3



10a : R = $(CH_2)_{10}CH_3$
10b : R = $(CH_2)_3CH_3$

10



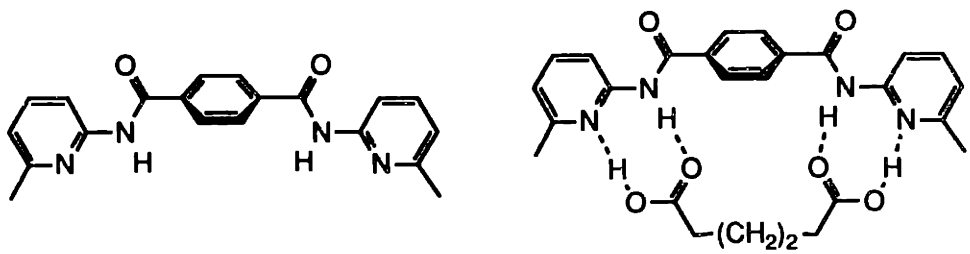
11

In $CDCl_3$, slightly soluble glutaric acid is readily solubilized in the presence of host **10**. The ^1H-NMR spectrum of the complex showed highly upfield-shifted CH proton resonances for bound glutaric acid at 0.70 (Ha) 0.10 (Hb) and -1.33 ppm (Hc). The stoichiometry of the complex **11** was established

by ^{1}H-NMR integration. The appearance of distinct signals for guest$_{(bound)}$ and guest$_{(free)}$ suggests that the exchange between these is slow at room temperature compared with NMR time scale. The host **10** also exhibited exceptional chain length selectivity.[16]

Hamilton *et al.* reported a different type of diacid receptor in which acylamino pyridine serves as two point contact binding site (Fig 4).[17] Diamide **12** was synthesized in one step from readily available starting materials.

Fig 4



12 **13**

The host **12** binds adipic acid quite tightly (Ka >10^5) and shows reasonable selectivity towards guest size. Notable is that the interaction between the acids and pyridines is not simple protonation, but hydrogen bonding. The structure of the complex is shown in **13** and this was proved unambiguously by X-ray crystallographic analysis.[17]

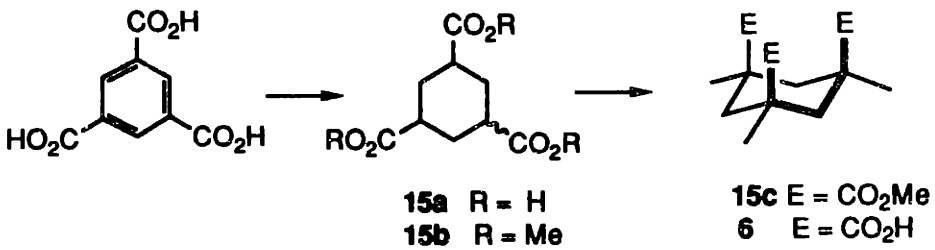
We have recently started to synthesize compounds containing convergent amino-groups held apart by aromatic spacers attached to Kemp's triacid derivatives. The architecture of these molecules is such that two secondary amines line a cavity which has as its base an aromatic surface. Modes of complexation of these substrates with aromatic and alkyl diacids were studied to ascertain the relationship between the length of the aromatic

spacer unit in the host and the distance between the two acid groups of the guest molecule.

1.2. Synthesis of Kemp's Triacid

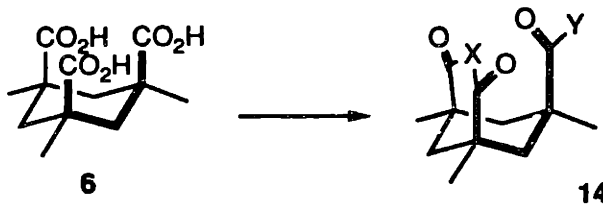
Synthesis of Kemp's triacid starts from trimesic acid (Scheme 1).[8] Trimesic acid was hydrogenated with Pd/C in a bomb hydrogenator at 160 °C under hydrogen pressure (1200 psi), and resulting crude triacid esterified with methanol to give the ester **15.** Trimethylation of **15b** with LDA then Me_2SO_4 in toluene followed by hydrolysis gave Kemp's triacid (**6**).

Scheme 1



Imide acid **14a** can be prepared by heating of the triacid **6** with urea in triglyme, while anhydride acid **14b** can be obtained by sublimation or refluxing in xylene (Scheme 2).

Scheme 2



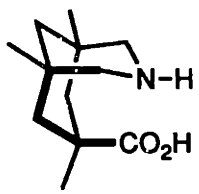
6

14a ; X = NH, Y = OH
14b ; X = O, Y = OH
14c ; X = NH, Y = Cl
14d ; X = O, Y = Cl

1.3 Synthesis

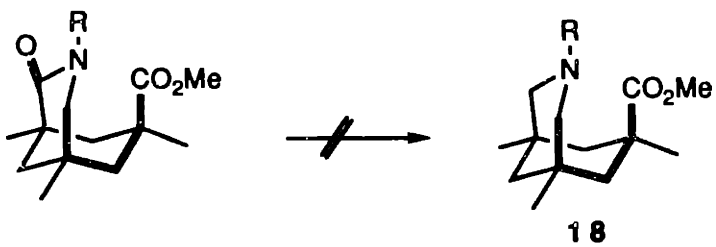
1.3.1 Preliminary study

To synthesize diamine clefts from Kemp's triacid, a bicyclic amine of Kemp's triacid derivative such as **16** is required.



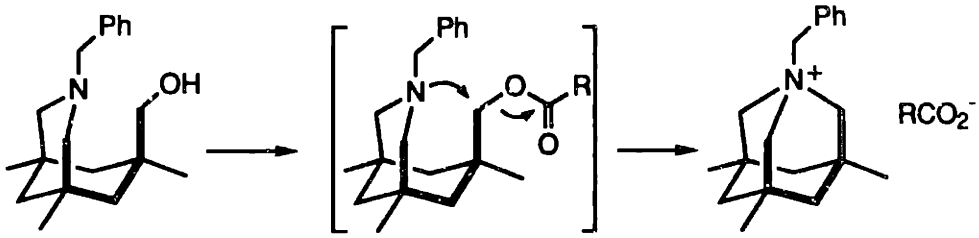
16

Unfortunately, all attempts to synthesize derivatives of the amine **16** were unfruitful. For instance, BH_3 reduction[19] of lactam ester **17**[20] or its imide ester gave none of the desired amine ester **18**.



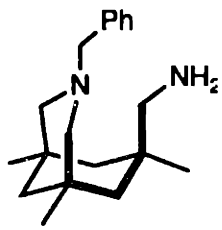
18

Another building block chosen was amine alcohol **19**, which was prepared by LAH reduction of the imide acid derivatives, but this compound cyclized upon acylation to azaadamantane **20**.[21]



19 **20**

In order to circumvent these difficulties, the amine **21** was synthesized.



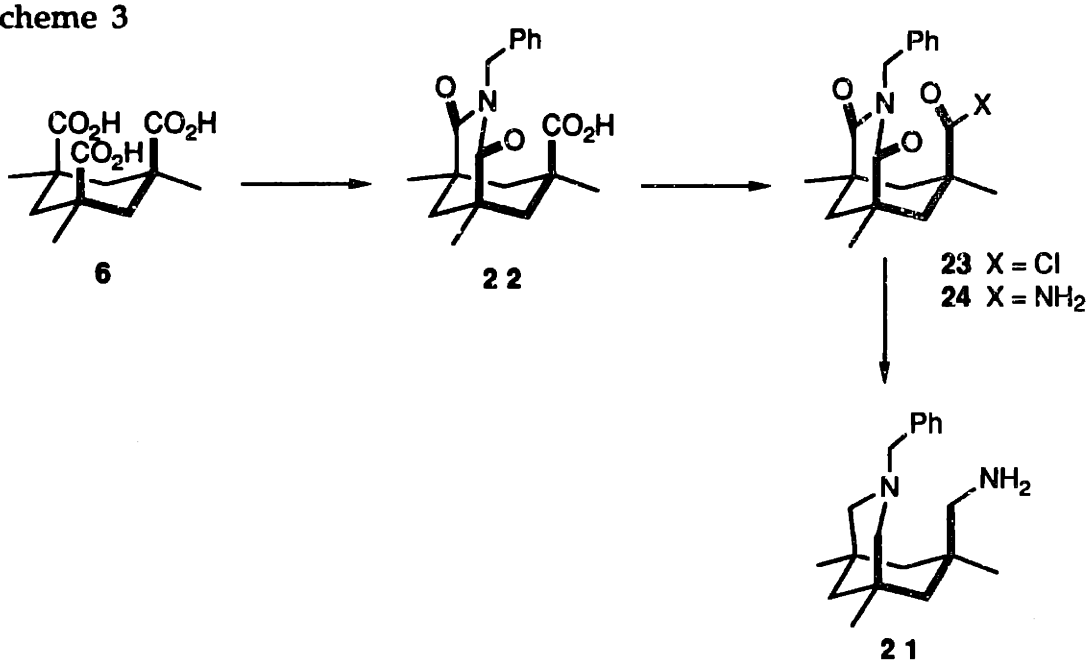
21

This chapter describes the synthesis of diamine clefts based on compound **21** and their binding behavior in organic solvents.

1.3.2 Synthesis of Diamine Clefts

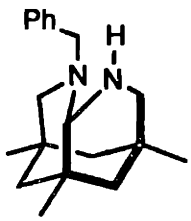
The molecules of interest were synthesized from Kemp's triacid in six steps. Heating of Kemp's triacid (**6**) (or the anhydride acid **14b**) with benzyl amine in pyridine gave the benzyl imide-acid **22** in quantitative yield (Scheme 3).

Scheme 3



6 22 21

The acid **22** was converted to amide **24** by heating with thionyl chloride followed by treatment with ammonia in THF. Heating of the amide **24** with a large excess of AlH_3 in THF[22] gave the diamine **21** in 75% yield. Reduction of imide amide **24** with LAH also leads to the desired diamine **21** but the yield is poor (ca. 30%) and the product is contaminated with numerous side products such as tricyclic diamine **25** and rearranged diamine **26.**



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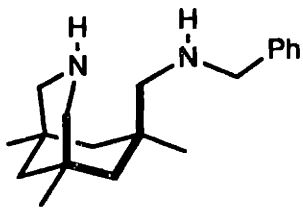
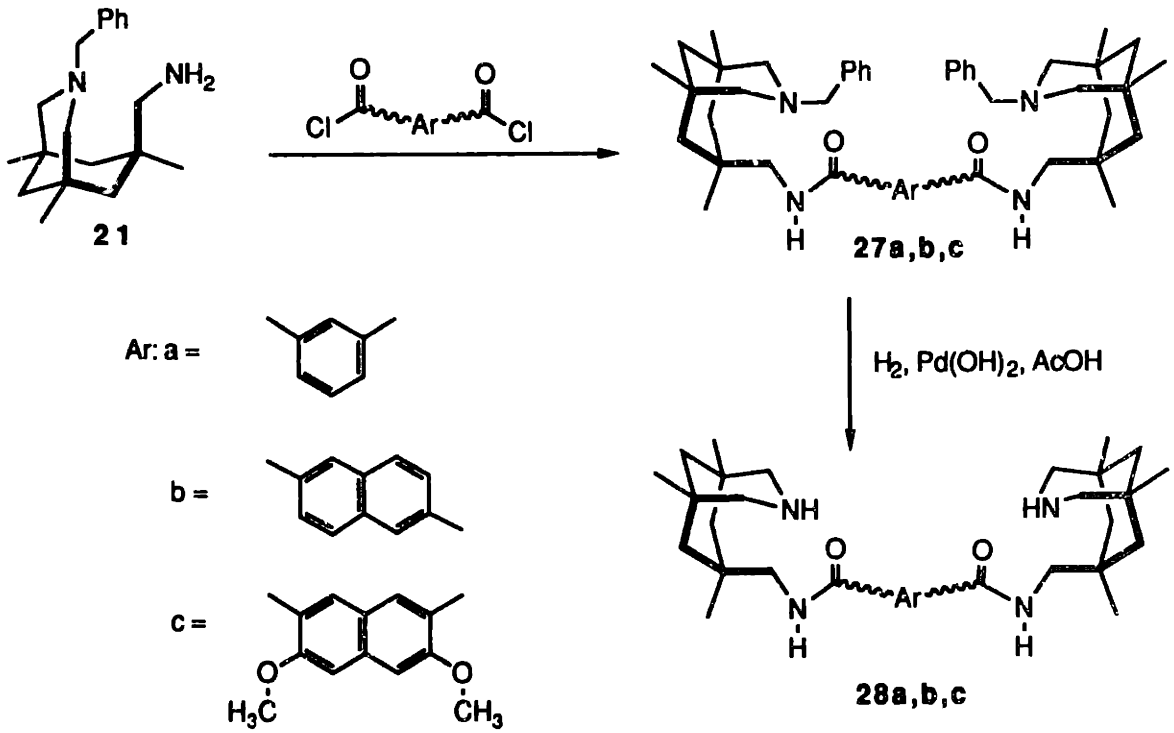


26

Condensation of the diamine **21** with aromatic diacid chlorides derived from diacids such as isophthalic, 2,6-naphthalene or 3,6-dimethoxy-2,7-naphthalene dicarboxylic acids[23] in CH_2Cl_2 in the presence of triethylamine

or diisopropylethylamine afforded the diamide **27a,b,c** in good yield (Scheme 4).

Scheme 4

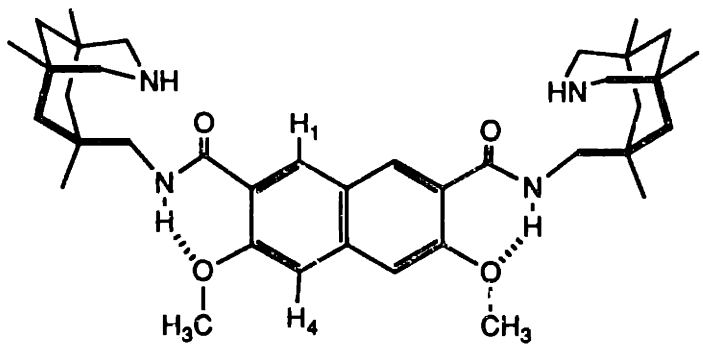


Treatment of the dibenzylamine-diamide **27** with Pearlman's catalyst[24] ($Pd(OH)_2$-C) in glacial acetic acid under a hydrogen atmosphere (40-60 psi) for 3-4 days then yielded the diamine-diamide **28** in good yield.

1.4 Complexation Studies

Even though three different diamine clefts were synthesized, most of binding studies were performed with cleft **28c**. This is partly because the reduced flexibility in the cleft **28c** (two possibilities for intramolecular hydrogen bonding) is thought to be better in binding and partly because the

clefts derived from isophthalic and 2,6-naphthalene diacids formed insoluble complexes rendering the studies difficult in $CDCl_3$ or CD_2Cl_2.



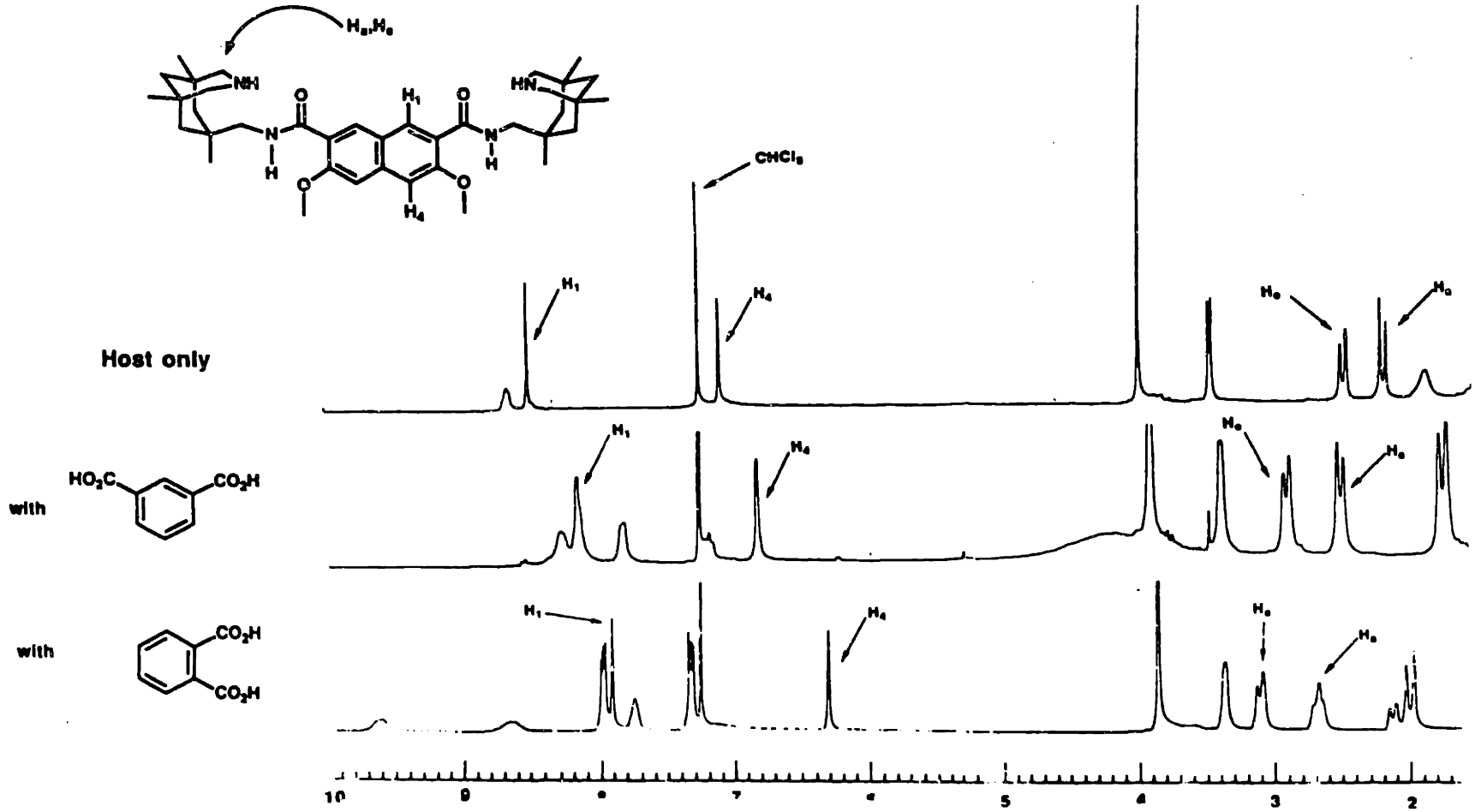
28c

As mentioned previously, solid-liquid extraction methods was used to study the complexation. A number of diacid-diamine pairs were investigated with this protocol. In solvents such as $CDCl_3$ and CD_2Cl_2 complexation predominates in which hydrogen bond donation and acceptance is important as is aryl stacking. In protic solvents such as CD_3OD, which support proton transfer, the molecules may be solvent separated. Evidence for this type of behavior is available from the ^{1}H-NMR. In $CDCl_3$ the 1:1 complex formed between **28c** and isophthalic acid gives an NMR spectrum in which the resonances of protons adjacent to the amine involved in hydrogen bonding are shifted downfield about 0.5 ppm while the protons lining the inside of the cleft are shifted upfield. Protons 1 and 4 resonate on the order of 0.3 ppm upfield of their normal position due to the deshielding region of the nearby isophthalic acid moiety in the complex. The aromatic surfaces may interact in either a face-to-face or edge-to-face mode.[25] In either fashion, the result would be an upfield shift of the protons on the aromatic spacer. The same diamine with phthalic acid gives a 2:1 complex which shows a larger upfield shift of the resonances due to protons 1, 0.6 ppm and 4, 0.9 ppm (see Fig 5).

Fig 5

Solid-Liquid Extraction Experiment

With 8 mM Host In $CDCl_3$

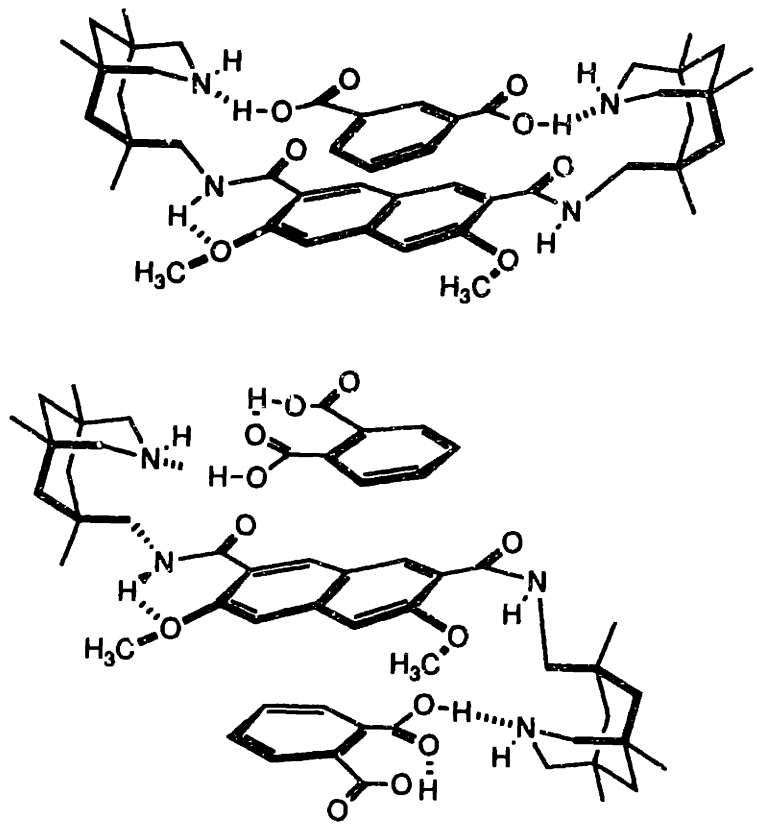


Upon fourfold dilution these spectra remain relatively unchanged, demonstrating the concentration independence of the interactions. This is an indication of the stability of the complex formed. In CD_3OD, however, only the resonances due to protons adjacent to the nitrogen are significantly shifted. The rationale is that in polar solvents proton transfer takes place to give the solvent separated carboxylate and ammonium ion pairs. In the case of solvents such as $CDCl_3$ which will not support ion pairs, hydrogen bonding and aromatic surface interactions are responsible for holding the complex together.

The stoichiometry of complexation was conveniently determined by extraction techniques. Typically, the diamine and the diamine-diacid complexes were soluble while the diacids themselves were insoluble in $CDCl_3$. Thus, an excess of the diacid was shaken with the diamine for 2-3 hr, the solutions were filtered, and the NMR spectra were recorded with peak integrations. Diacids such as maleic and phthalic which are constrained to intramolecular hydrogen bond in the monoanion exhibited 2:1 stoichiometry of diacid:diamine. In the NMR spectrum of these complexes two different signals are observed which were attributed to the protons involved in hydrogen bonding to the amine and the other being involved in an intramolecular hydrogen bond between the carboxylates. As a result of this well established intramolecular hydrogen bond, maleic and phthalic acid exhibit a larger ΔpK_a than other diacids. Linear diacids such as fumaric and succinic which either cannot intramolecularly hydrogen bond or must overcome entropic barriers to do so are extracted in 1:1 stiochiometry. The aromatic diacids isophthalic and terephthalic also exhibit 1:1 stoichiometry (Fig 6).

In the case of terephthalic acid the complex formed was insoluble in $CDCl_3$. In this instance the determination of stoichiometry was made by adding a large excess of the diamine receptor molecule to a slurry of the diacid. After shaking, the solution was filtered and the NMR spectrum of the precipitate was recorded in CD_3OD, a solvent in which both species are soluble. This revealed that terephthalic acid had caused 1 equivalent of otherwise soluble diamine to precipitate in the form of the complex. In the instances of these 1:1 extractions, the diacids may stretch out across the cavity such that each acid terminus interacts separately with the opposed amines. A schematic representation of the 2:1 and 1:1 complexes is given below.

Fig 6



Competition experiments were also carried out in which a slurry of two diacids was treated with diamine **28c**, the suspensions were filtered, and the

filtrates were analyzed. Within the group of aromatic acids, the binding selectivity was phthalic > isophthalic > terephthalic. Among the aliphatic acids the ordering was maleic > fumaric > succinic. The selectivity is a combination of acidity of the acid as well as its size relative to that of the cavity (Table 1).

Table 1; Selectivity (decreasing order) and stoichiometry of extraction.

Acid	Stoichiometry	pK_1[a]	ΔpK
maleic	1:2	1.910	4.422
phthalic	1:2	2.76	2.16
fumaric	1:1	3.095	1.507
succinic	1:1	4.206	1.184
isophthalic	1:1	3.70	0.9
terephthalic	1:1	3.5	0.84

a) pKa values are from "Ionization Constants of Organic Acids in Aqueous Solution" (IUPAC Chemical Data Series No 23) E. P. Sergeant and Boyd Dempesy, Pergamon Press, 1979.

Thus, in the experiment involving phthalic and maleic acid with **28c**, only the more acidic maleic acid was found in the $CDCl_3$ solution after shaking for 2 hr. However, in the case of isophthalic and succinic acid the less acidic succinic acid was selectively extracted. This is due to the difference in size of the two acids. The overall trend of extractability is maleic > phthalic > fumaric > succinic > isophthalic > terephthalic.

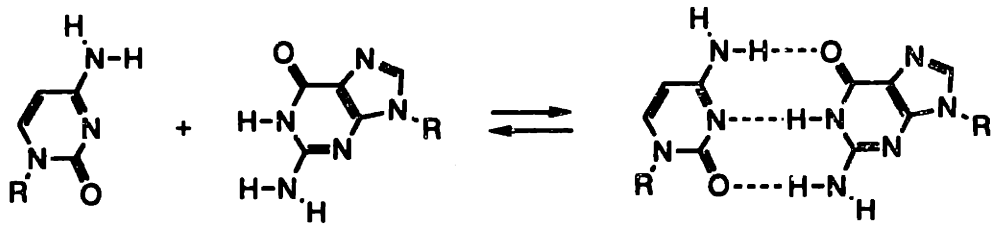
In summary, the synthetic receptor **28c** binds organic diacids with modest size selectivity. The upfield shifts of aromatic protons of the complex gives an evidence for complexation in nonpolar organic solvents.

Chapter 2

2 Receptors for Guanine Derivatives[26]

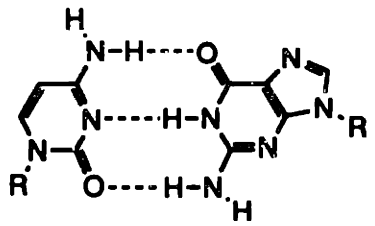
2.1 Introduction

Guanine (G) is a purine base found in nucleic acids. It basepairs with cytosine (C), a pyrimidine base.[27]



C G 29

It is known that DNA samples with high G-C content melt at higher temperature than samples with lower G-C content. This is ascribed to the higher number of hydrogen bonds in G-C pairs compared to adenine-thymine (A-T) system.[28] In contrast to the A-T system, the absolute hydrogen bonding ability in G-C pair and G-C-like systems is poorly understood. The binding constants of G-C pair **29** is known to be only 3.7 ± 0.6 M^{-1} in DMSO[29a] at 32 °C (^{13}C-NMR) and is estimated to be 10^4-10^5 M^{-1} in $CDCl_3$ (IR).[29b,c] Another complex **30** with similar bonding pattern, was recently shown to have a Ka of 1.7 x 10^4 M^{-1} in $CDCl_3$.[30]



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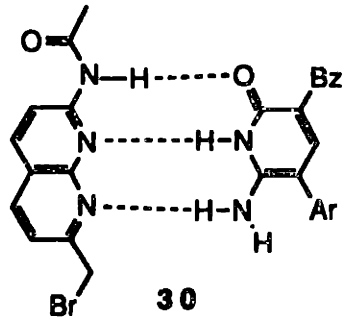

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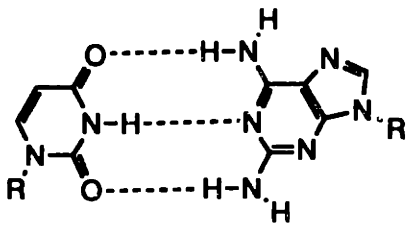
The benefit of a third hydrogen bond is apparent in view of the Ka's of 40-130 M^{-1} that are observed for complexes between nucleic bases with two hydrogen bonds. However, the situation is not so simple when other triply hydrogen bonded systems are considered. Thus, the Ka's for **31** and **32** in $CDCl_3$ are only 170 and 90 M^{-1}.[31]



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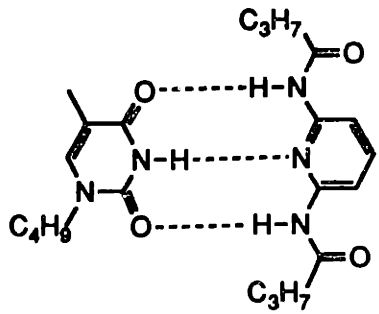
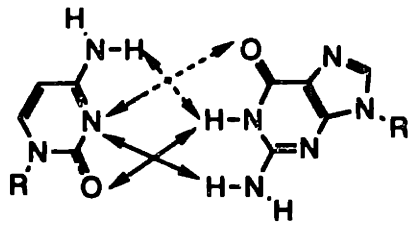


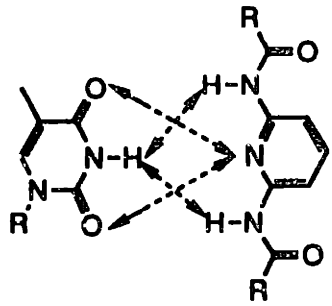
32

In both systems, there are the same three basic types of hydrogen bonds, NH_2···O, NH···N, and NH_2···O. Recently, Jorgensen and coworkers[32] have postulated the origin of the reduced binding of the latter systems (**31** and **32**) relative to **29** and **30**. They conclude that reduced binding in **31** and **32** is due to four unfavorable secondary interactions, while system **29** and **30** has only two, which are canceled out by two favorable ones.

Secondary interactions in hydrogen bonding



2 repulsive (◄-----►)
2 attractive (◄——►)

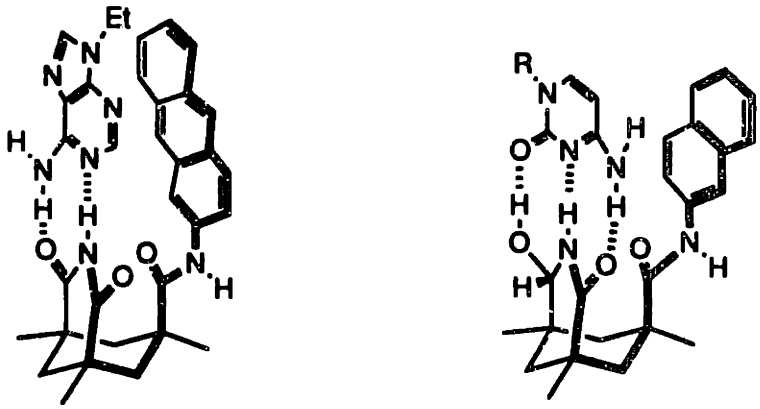


4 repulsive (◄-----►)

In contrast to A-T type receptor systems, only a limited number of guanine receptors are reported.[33] This seems due to the insolubility of guanine derivatives,[27b] difficulty in synthesizing the complementary binding function (acylated amidines), and to the complex binding pattern of G-C like systems as noted by Shaw, et al.[40]

Derivatives of Kemp's triacid have been useful for selective recognition of nucleotide derivatives. In particular, imides provide base-pairing toward adenine, and the U-shaped relationship with the other axial substituents presents possibilities for the other intermolecular contacts. Both hydrogen bonding and aromatic stacking interactions can be brought to bear on adenine derivatives (Fig. 7).[34,35] Selective receptors for cytosine derivatives[36] have been prepared by simple reduction of the imide to a hydroxy lactam function.

Fig 7

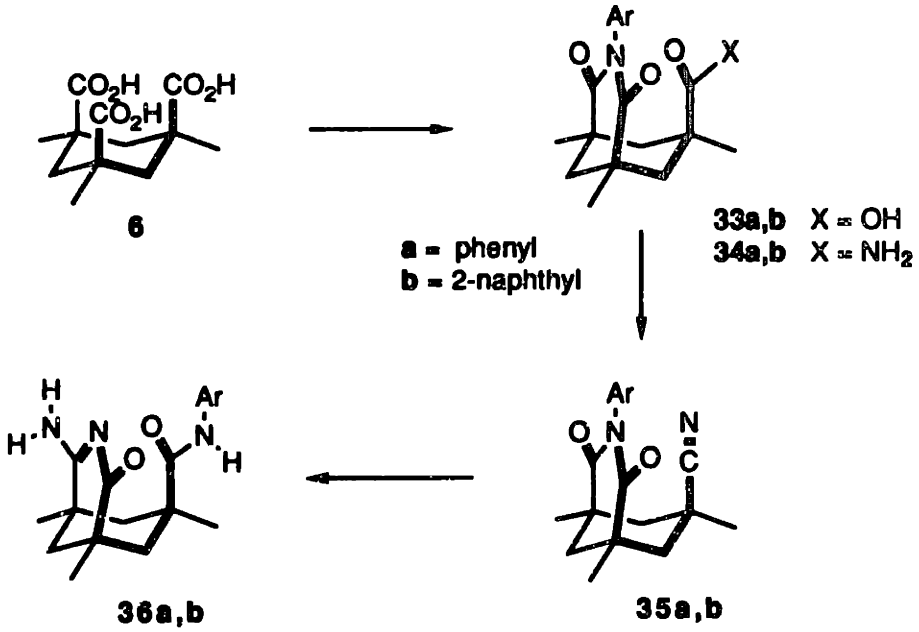


Here we introduce two systems capable of selective binding to guanosine derivatives, and apply solubility titration[37] to determine the association constants.

2.2 Synthesis

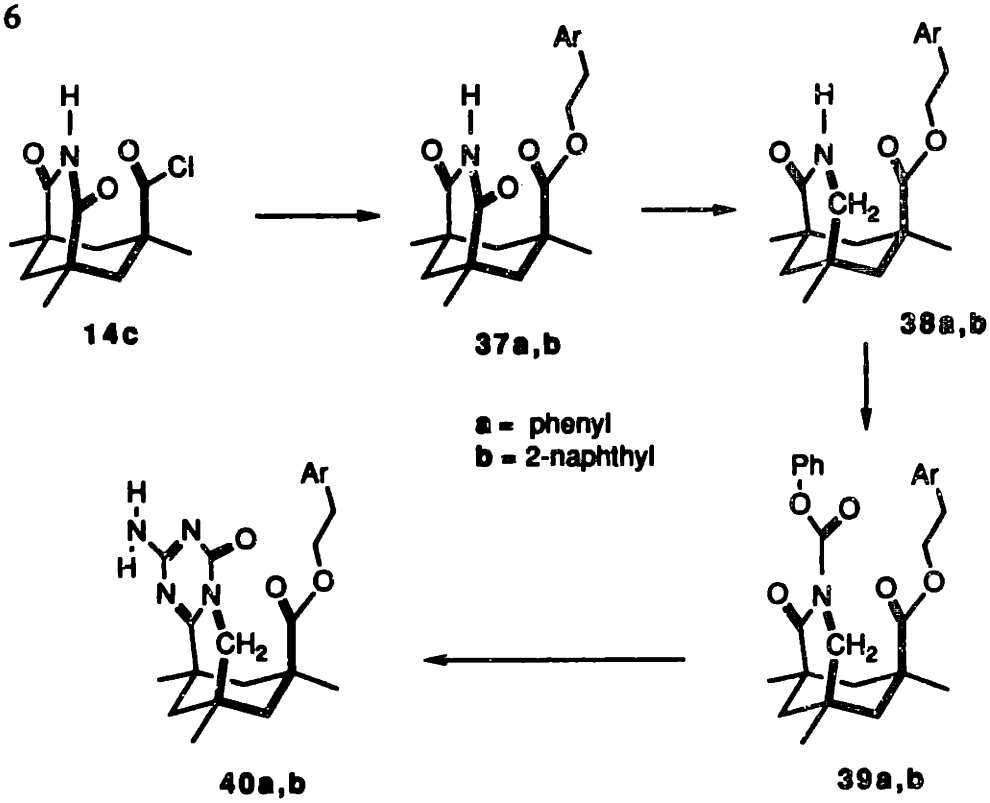
The synthetic receptors were prepared from Kemp's triacid **6** by conversion to the anhydride acid chloride **14b**, followed by condensation with aromatic amines to give the imide acids **33a** and **33b** (Scheme 5). Sequential treatment with $SOCl_2$ and NH_3, then dehydration with trifluoroacetic anhydride (TFAA), gave the nitriles **35a** and **35b**. Exposure to KNH_2 in THF/NH_3 rearranged these materials to the acylated amidines **36a** and **36b**.

Scheme 5



A second set of receptors was prepared from the imide acid chloride **14c** by esterification to the imide ester **37a** and **37b** (Scheme 7). The imide esters **37a** and **37b** were reduced to lactam esters **38a** and **38b** by sequential treatment with $NaBH_4$ (or $LiBH_4$) and triethylsilane in trifluoroacetic acid-CH_2Cl_2.[38] N-acylation with phenyl chloroformate-diisopropylethylamine (or triethylamine) in refluxing CH_2Cl_2 followed by condensation with guanidine hydrochloride then yielded the azacytosines **40a** and **40b**.[39]

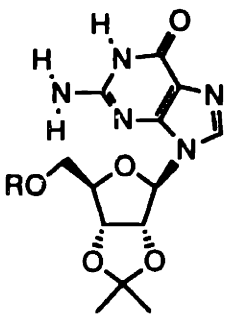
Scheme 6



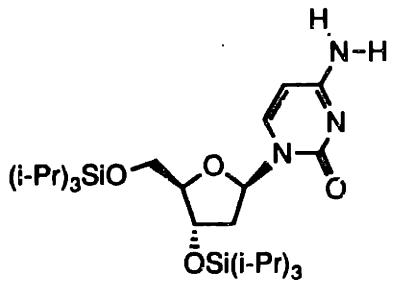
2.3 Complexation

Before describing the complexation experiments, we note that the self-association of guanosine derivatives, encountered by Shaw and others,[29a,40] cause solubility problems which render titration data difficult to interpret, particularly when diastereomeric complexes are formed. Accordingly, we have used the solubility titration method,[37] in which the synthetic receptor is used to solubilize the guanosine derivatives **41**. Solubility titrations were particularly well suited to study the association of our molecules with guanosine for several reasons. As previously mentioned, higher order aggregates of guanosines (G),[29a] are unlikely under these conditions, and the formation of G_2-C trimer[40] is minimal below 1 : 1 stochiometry. Also $(G\text{-}C)_2$ tetramers[40] are not present to any extent at low concentration (10^{-3} M).

Aggregation of guanosine can also be ignored, since limiting solubility (g_o) contains all the higher order guanosine species and is a constant throughout the titration.[37] To reach saturation in a typical NMR titration there must be a large exess of the guest (guanosine) component. However the analytical signal (i.e. the chemical shift of a relevant proton) is a composite of all the guanosine species in solution, which in the case of molecules which self-associate and aggregate is now the time average of more than the two relevant species, the free and host-bound guanosine. It is often impossible to deconvolute the signal and assign the partial contributions to the overall observations. In the solubility method the increase in the amount of guanosine present after the addition of a cytosine analog is due *only* to interactions with the host molecule making the analytical signal, in this case the HPLC peak, directly interpretable. In other words, we have excluded the guest-guest interactions which caused the main problem in this case from guest- host, guest-guest interactions.



41 : R = H
42 : R = Si(i-Pr)$_3$



43

Solubility titrations can be readily applied to a systems which are 1 : 1 stoichiometry.[45] In this case, association constant is defined as

$$Ka = [HG]/[H][G] \qquad (1)$$

where [H], [G] and [HG] are host, guest and host-guest complex concentration at equilibium, respectively. Host total (H_t) and guest total (G_t) concentrations are therefore:

$$H_t = [H] + [HG] \quad (2)$$

$$G_t = [G] + [HG] = g_o + [HG] \quad (3)$$

where g_o is the limiting solubility of the guest and is equal to free guest concentration. Elimination of [H] and [HG] in equation (1) using equations (2) and (3) gives:

$$G_t = g_o + \alpha H_t \quad (4)$$

where

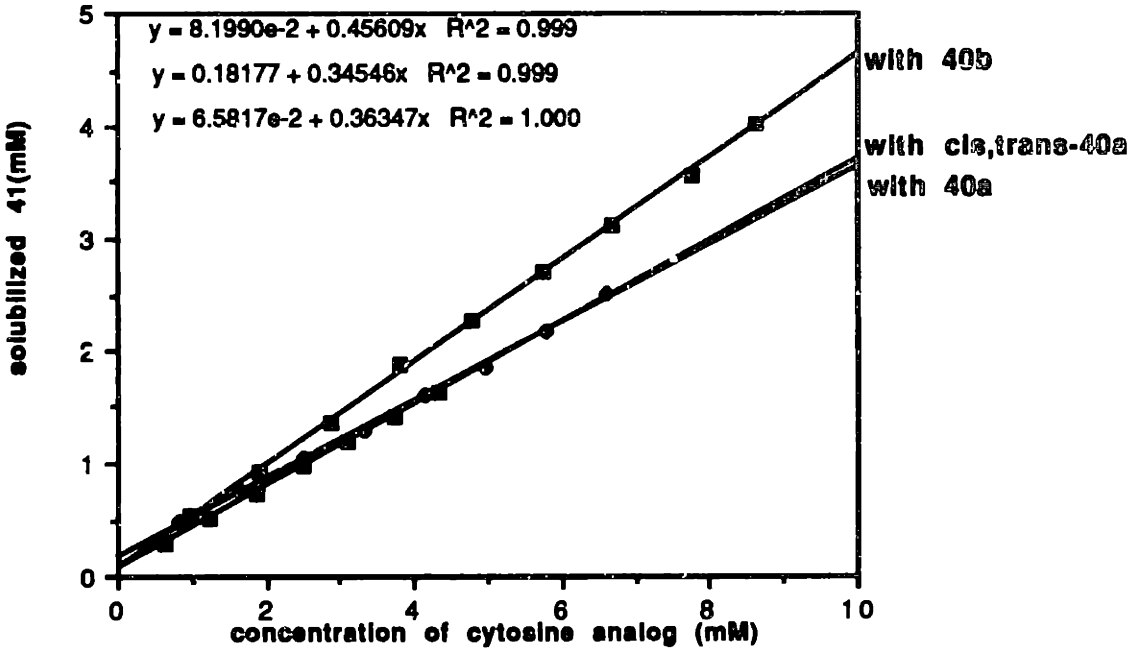
$$\alpha = g_o \cdot Ka/(1 + g_o \cdot Ka) \quad (5)$$

Thus a plot of total guest (in this case solubilized guanosine) concentration vs. total host (cytosine analog) concentration yields a straight line, and association constant (Ka) can be obtained from the slope (α) and y-intercept (g_o) as follows.

$$Ka = \alpha/(1- \alpha).g_o \quad (6)$$

The experiments are perfomed by preparing a stock soluton of the soluble component (cytosine analogs **36a, 37b, 40a, 40b**), and introducing increasing volumes of this solution into separate vials. These solutions are saturated with the insoluble guanosine **41**,[42] diluted to constant volume, and then shaken for at least 12 hr. After filtration, HPLC analysis of the solutions gives the total amount of each component. The synthetic receptors generally solubilize from 0.05 - 0.5 equivalents of the guanosine **41**, and at these concentrations, the formation of higher order aggregates is minimal and can be ignored in the analysis. The limiting solubility was also established for **41** by shaking in pure $CDCl_3$. Typical titration data is given Fig 8 using guanosine **41** as the solid component.

Fig 8 **Solubility titration Data(40a, 40b, cis,trans-40a)**



The association constants are given in Table 2, and reasonable agreement was obtained on g_o using the various receptors.

Table 2. Association Constants Measured by Solubility Titration in $CDCl_3$

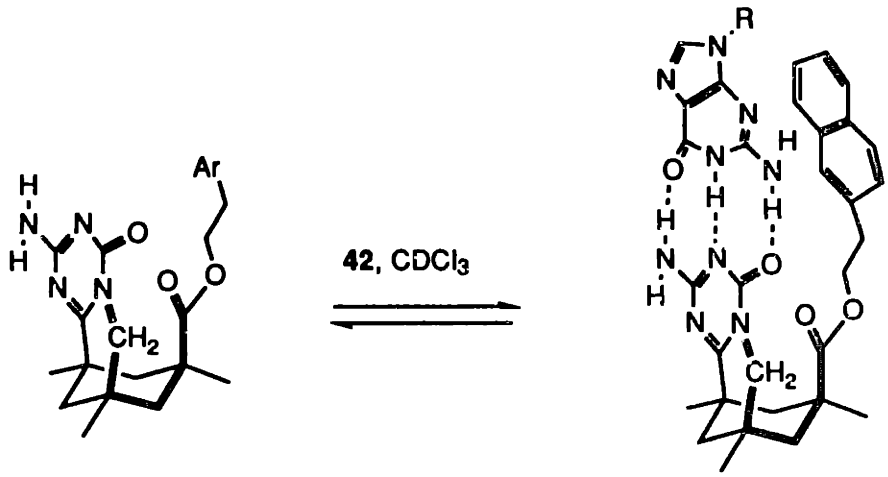
Host	Ar =	Ka (M^{-1})
36a	Phenyl	8000
36b	2-Naphthyl	5500
40a	phenyl	8000
40b	2-Naphthyl	13000
43		7900
cis,trans **40a**	phenyl	9000

2.4 Structure

Derivatized cytosine **43**,[43] which is unable to simultaneously hydrogen bond and aryl stack, exhibits a Ka of 7900 M^{-1}. A value of ΔH = -5.6 kcal/mol has been reported for a similarly derivatized G-C pair in $CHCl_3$.[46] Hydrogen bonding is the major contributor in low dielectric strength aprotic solvents like $CHCl_3$, while stacking is believed to dominate in water.[47]

Compound **36a** and **40a** in which the both interactions are possible associate to the same extent as cytosine **43**, suggesting that the phenyl ring is not suitably disposed to interact with the purine surface. Naphthylethyl ester **40b** shows the expected increase in binding upon favorable interactions with this aromatic surface. This may be due to the extra degrees of freedom allowed in the ethylene spacer (Fig 9).

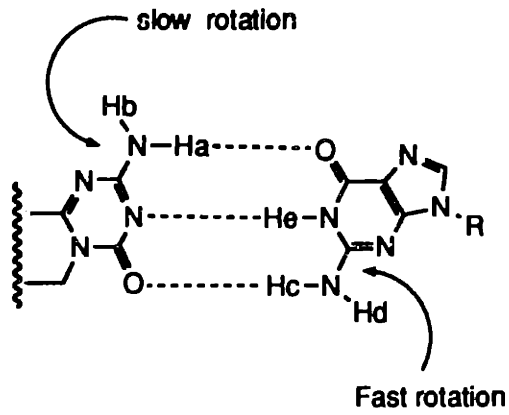
Fig 9



In the case of amide **36b** there are unfavorable steric interactions between the aromatic surface and the acetonide of the ribose on the guanosine which can be avioded in the more extended ester **40b**. Nonetheless, these models give association constants of a reasonable order of magnitude for a system containing three hydrogen bonds and enhancement of binding is observed in cases bearing a well placed aromatic surface.

While NMR did not allow quantification of the association constant, it did give some structural information regarding the complexes. Because synthetic receptors are racemic mixtures and the guanosines are optically active, they form diastereomeric complexes upon mixing. Interestingly, in the NMR spectrum of complex, the azacytosine **40a** and **40b** showed averaged signal when mixed with guanosine **42**[44], but acyl amidine **36a,b** resulted in a duplication of signals for protons which are in sufficiently different environments. In case of azacytosine **40a** and guanosine **42**, the NMR signals due to protons involved in hydrogen bonding can easily be followed (Fig 10).

Fig 10



The C-N bond in azacytosine rotates slowly and gives two separate signals (5.3 for Ha, 5.1 for Hb) (Fig 11). Upon addition of guest proton Ha moves downfield (5.3 to 9.1 ppm), while Hb remains relatively unchanged (5.1 to 5.7 ppm). In contrast, the C2-NH_2 bond in guanine rotates relatively freely on the NMR time scale,[41] and gives one broad singlet. The two protons (Hc and Hd) resonate ca 6.2 ppm when free and ca 7.3 ppm when fully complexed. The N1 proton of guanine (He) resonates about 12,1 ppm, and is quite concentration dependent (a good indication of self-aggregation), moves

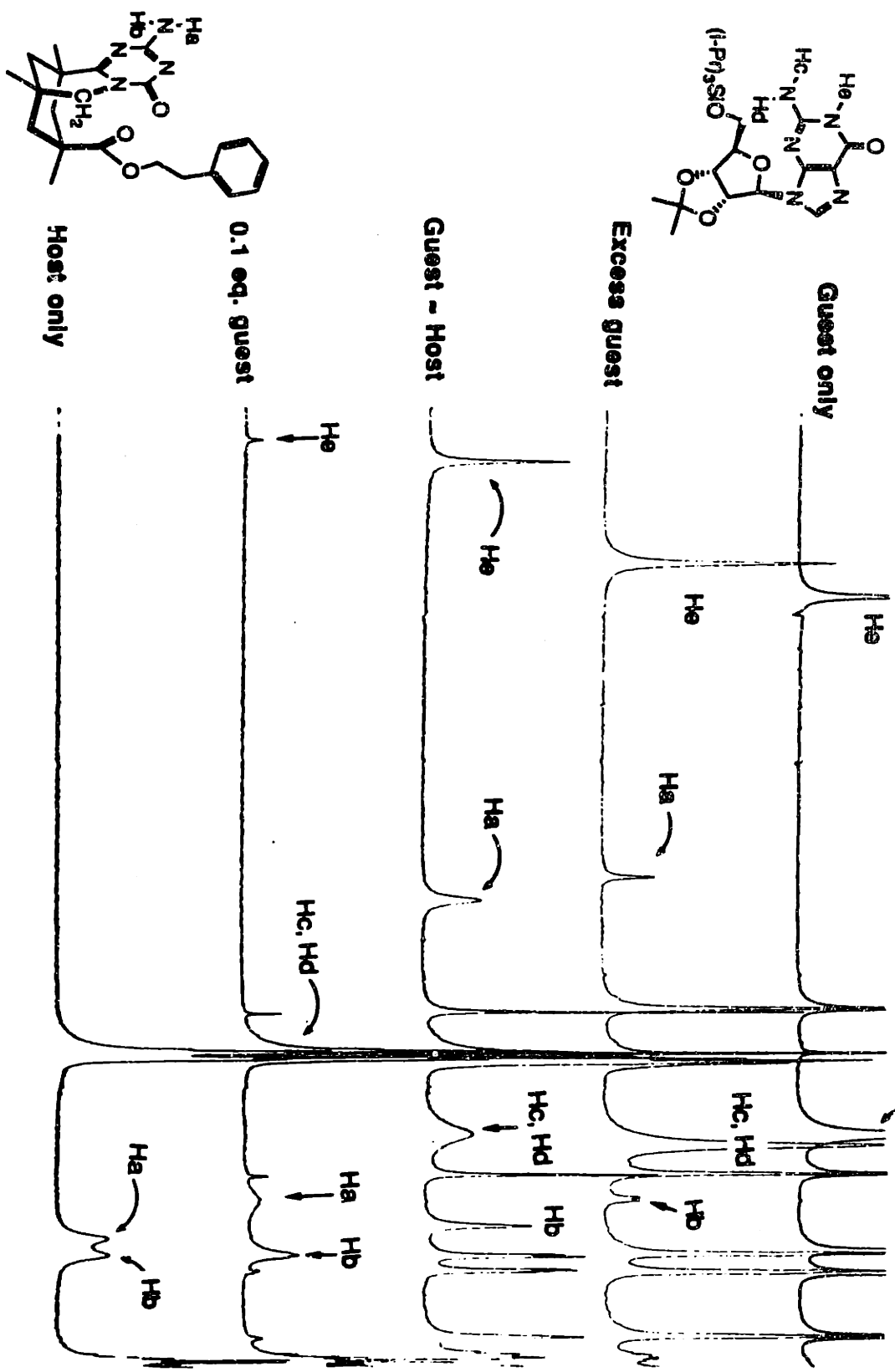
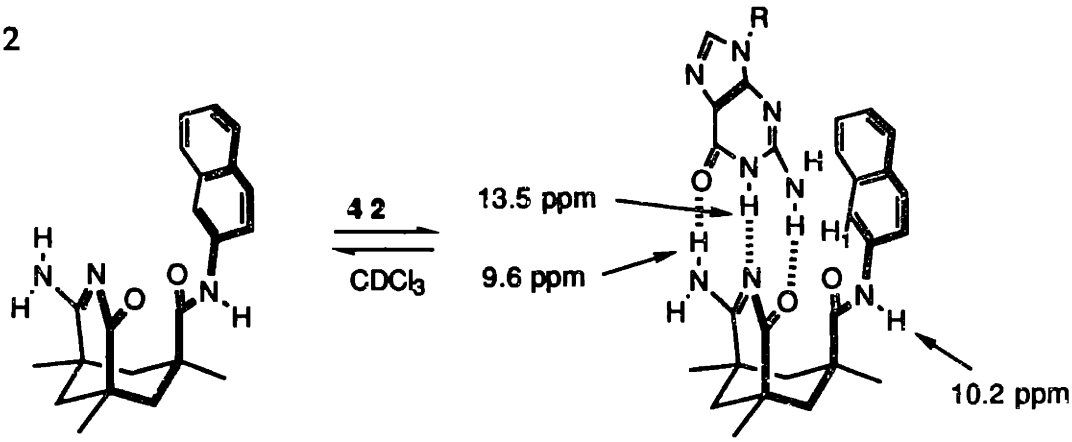

NMR Titration
Guest only
Excess guest
Guest + Host
0.1 eq. guest
Host only
Hc, Hd
Hb
Ha
He
(i-Pr)3SiO

Fig 11

downdfield to 13.7 ppm when bound. All of this behavior is in good agreement of previous reported data.[43]

The downfield region of the spectrum of a 1:1 mixture of **36b** and **42** taken at -25 °C contains five resonances, three of which are paired peaks from diastereomeric complexes, with the remaining two appearing as broadened signals.

Fig 12

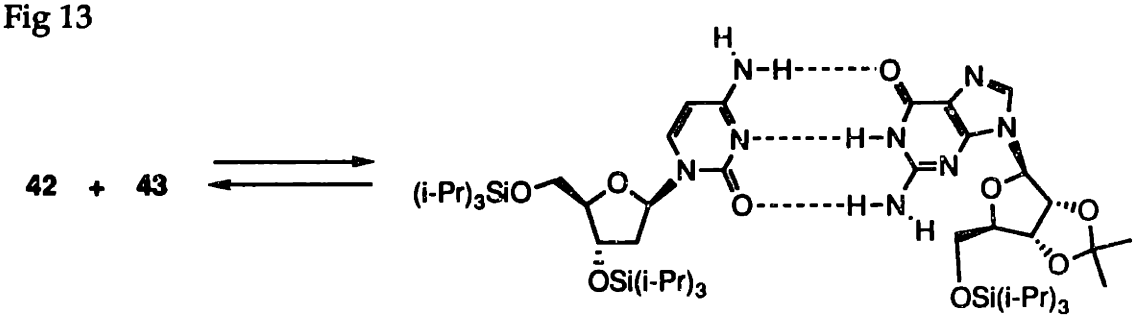


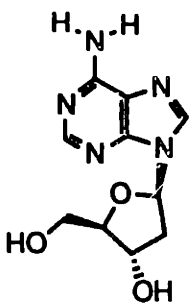
The resonances at 10.2 and 8.8 ppm were assigned as the amide NH and the H-1 proton of the naphthalene ring. The resonances at 13.5, 9.6 and 8.4 ppm were assigned as the imino NH of **42**, the amino NH of **36b**, and the a[illegible] NH of **42**, respectively. These assignments are also in agreement with values reported by Shaw for **42** complexed with **43**. A structure which is consistent with these findings is shown in Fig 12. A naphthalene configuration in which H-1 is adjacent to the amide carbonyl is sugested by its chemical shift.

It can be concluded that the simple acyl amidines express their affinity for guanosine derivatives by base pairing. The azacytosine, which features an extended ethylene spacer could permit favorable aromatic interactions and affinities twice that of the simple base pair derivatives.

The "natural" base pairing of guanosine plus cytosine (Fig 13) is reasonably well imitated by the new receptors, and their selectivity can also be assessed with the methodology here. Accordingly, a set of experiments was carried out to determine the extent to which **36a** would solubilize nucleoside bases to which it is not complementary. The insoluble components used were 2-deoxyadenosine (**44**) and the thymine (**45**). The technique gave limiting solubilities of 13 x 10^{-5} M for **44** and a Ka of 600 M^{-1} for **36a** while the corresponding numbers for **45** were 1.0 x 10^{-5} M and Ka of 850 M^{-1}. In either case, it appears that two hydrogen bonds are formed with some additional contact likely from aryl stacking. Thus the selectivity of the new receptors for guanosine is about one order of magnitude over the other bases under these conditions, probably reflecting the contribution of the third hydrogen bond.

Fig 13





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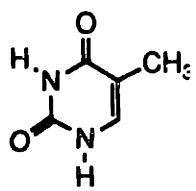


45

In summary, receptors for guanine derivatives were prepared. The binding constants between these receptors and guanine derivatives were measured using solubility titration. The receptors showed reasonable selectivity toward guanine over adenine and thymine.

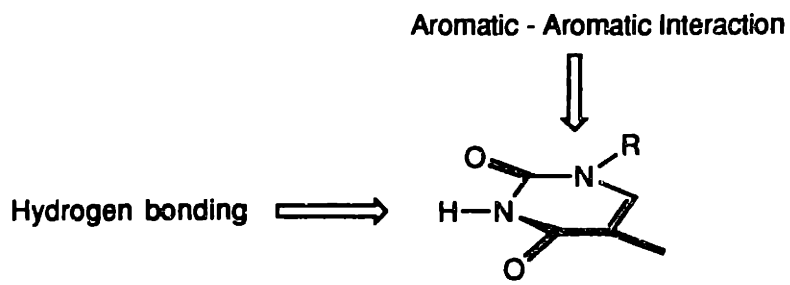
Chapter 3

3 Synthetic Receptors for Thymine and Thymine Photodimer[48]

3.1 Introduction

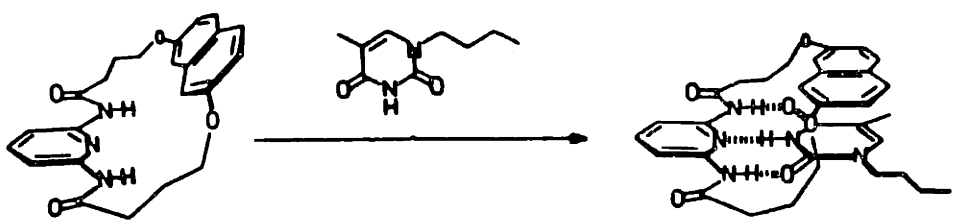
The combination of directed hydrogen bonding and π-stacking interactions offers a powerful approach for the recognition of planar heterocyclic substrates such as the nucleotide bases. The hydrogen bonding groups at the periphery of the heterocycle and the flat π surface at its top and bottom faces suggest a perpendicular convergence of binding interactions (Fig 14).

Fig 14



Recently, Hamilton et al reported a macrocyclic receptor for thymine using this strategy (Fig 15).[49]

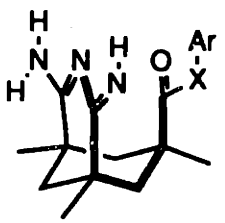
Fig 15



They have shown that the diacylaminopyridine provides a hydrogen bonding sites for thymine imide functionality. Depending on the electronic state of the naphthalene nucleus, the aromatic ring interacts either edge-to-face or face-to-face as evidenced by NMR and X-ray crystallographic analysis.[49]

From a strategic point of view, Kemp's triacid turned out to be a successful building block for such recognition. With this triacid, Rebek was able to build receptor molecules for adenine,[34] cytosine,[36] and guanine. Creation of receptors for thymine, however, turned out to be problematic. One possible binding surface complementary to thymine is that possessed by **50** (Fig 16).

Fig 16



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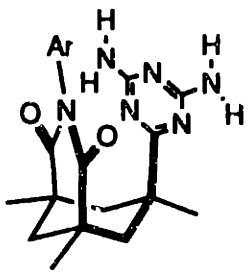

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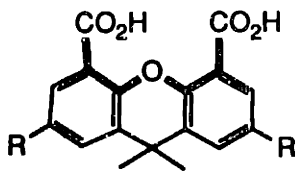

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Unfortunately, the functional group itself is quite difficult to make and susceptible to hydrolysis. Another possible alternative based on Kemp's triacid would be the diaminotriazine **51**. Attempt to form the triazine **51** through condensation with biguanide yielded only untractable mixtures. However, it is not suprising that the pivaloyl-type acid chloride in the imide would be quite unreactive. It seems obvious from this result that a less

hindered U-turn building block is required for the successful preparation of a triazine-based receptors.

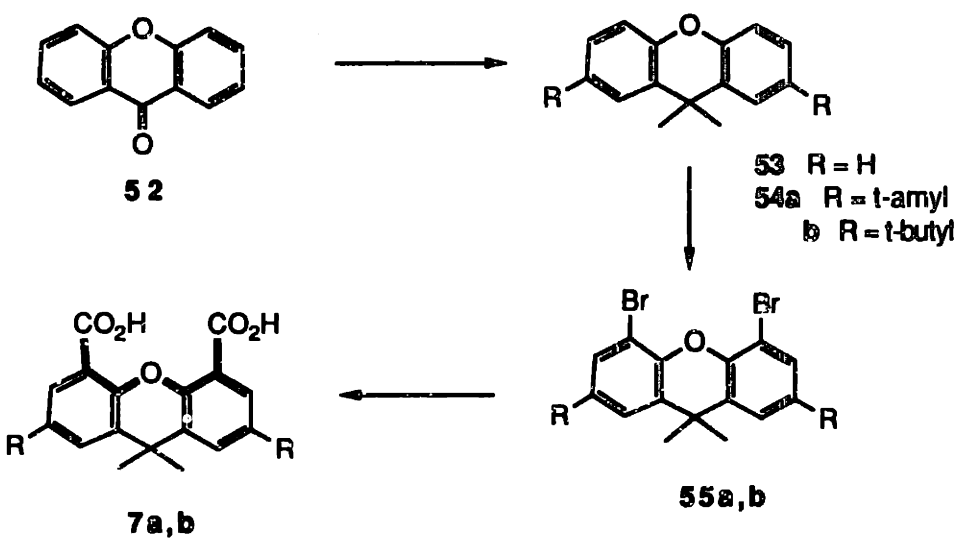
Recently, the synthesis of 2,7-dialkyl-9,9-dimethylxanthene-4,5-dicarboxylic acid (xanthene diacid) **7** was reported by Rebek et al.[10] This chapter describes the synthesis and binding studies of thymine receptor molecules based on the xanthene diacid **7**.

3.2 Synthesis and Binding Studies of Thymine

3.2.1 Synthesis of xanthene diacid[10]

Preliminary studies showed that anthracene-4,5-dicarboxylic acids provide U-shaped relationships between functions. Unfortunately, the low solubilities of these compounds and the clefts derived from them with diol or diamine spacers (e.g., biphenol) thwarted studies of their intermolecular complexes. Systems derived from xanthenes proved to be more soluble. By appending methyl groups to the 9-position, and *tert*-alkyl groups to the 2- and 7-positions of xanthene-4,5-dicarboxylic acid derivatives, highly soluble and readily accessible molecules were at hand. These *tert*-alkylated diacids are prepared from the commercially available xanthone **52** in four steps (Scheme 7).

Scheme 7

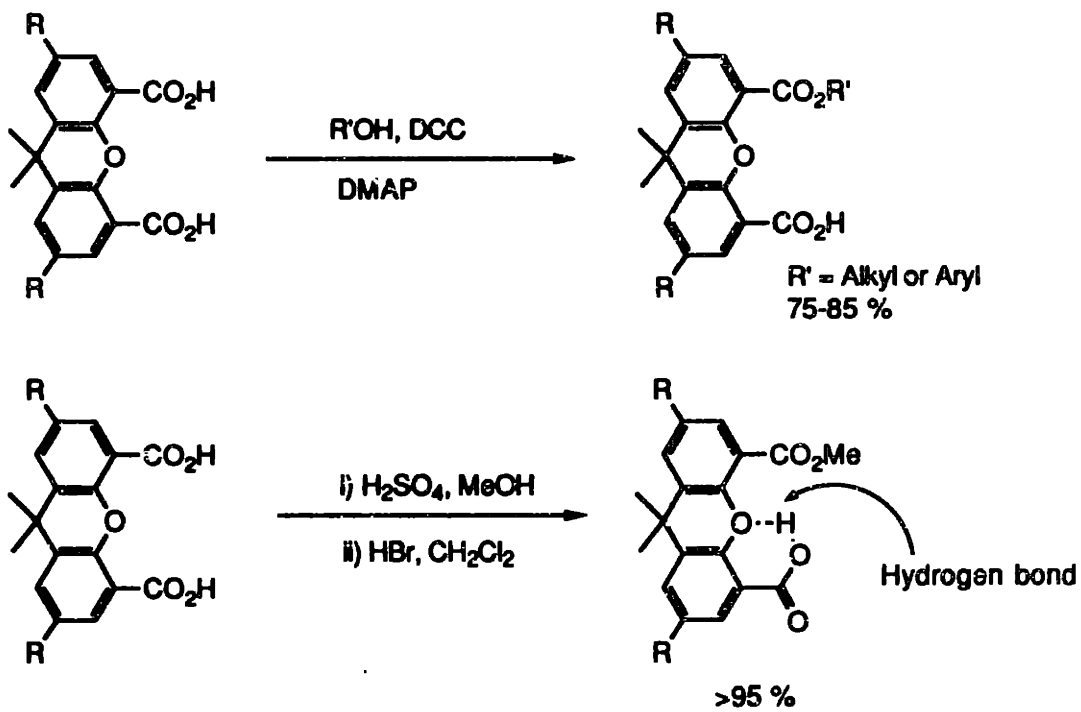


Treatment of xanthone (**52**) with Me_3Al in toluene, followed by Friedel-Crafts alkylation and bromination generates compounds **55a** and **55b**. These dibromo compounds were converted to diacids **7a,b** via their lithium derivatives. The di-*tert*-amylated compounds (**54a, 55a** and **7a**) were each found to contain ca. 20% of an impurity in which one of the *tert*-amyl groups was replaced by a *tert*-butyl group. These impurities appear to arise from fragmentation of the *tert*-amyl carbocation during the Friedel-Crafts alkylation step, and were not found to have a significant effect upon molecular recognition studies using derivatives of **7a**.

The solubility of the di-*tert*-butyl derivative **7b** in CH_2Cl_2 at 22 °C is 30 mg/mL, while the di-*tert*-amyl compound **7a** is even more soluble (52 mg/mL). The various host molecules derived from these diacid units were also found to possess excellent solubility in a variety of organic solvents.

The xanthene diacids **7a,b** were efficiently monoesterified with alcohols such as methanol, phenol, or benzyl alcohol using 1,3-dicyclohexylcarbodiimide (DCC) and catalytic 4-dimethylaminopyridine (DMAP) (Scheme 8).[50]

Scheme 8



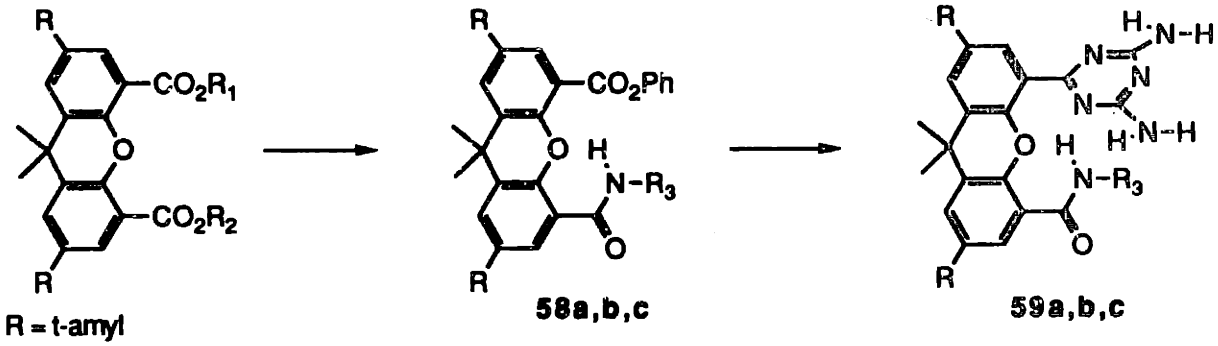
In larger scale preparations of monofunctionalized diacids, dimethyl ester formation (by refluxing in MeOH in the presence of sulfuric acid) followed by selective monodemethylation with gaseous HBr in CH_2Cl_2 proved suitable.[51] The selectivity in this demethylation is probably due to an intramolecular hydrogen bond in the monodeprotected acid, which is expected to be harder to protonate, thus slower in second step demethylation.

3.2.2 Preparation of Thymine Receptors

Creation of thymine binders began with di-t-amyl xanthene diacid **7a** (hereafter every xanthene diacid derived material is from di-t-amyl xanthene diacid unless noted). Xanthene diacid **7a** was monoesterified with phenol

using dicyclohexylcarbodiimide (DCC)[50] as coupling reagent in dry CH_2Cl_2 to afford the phenyl ester **56** in 75% yield (Scheme 9).

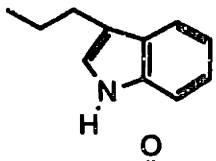
Scheme 9



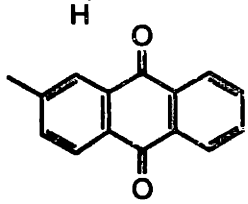
7a : $R_1 = R_2 = H$
56 : R_1=Ph, $R_2 = H$
57 : R_1= H, R_2 = 2-naphthylethyl

a =

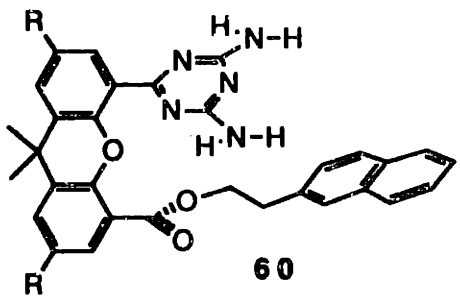
b =



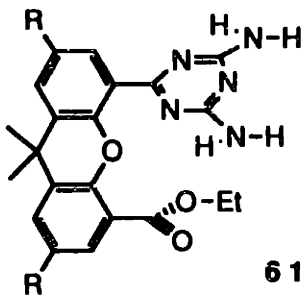
c =



57 —(i) $SOCl_2$; (ii) Biguanide→



7a —(i) $SOCl_2$; (ii) Biguanide→



The monoacid **56** was transformed into the corresponding acid chloride ($SOCl_2$, CH_2Cl_2, reflux), which was coupled with 2-β-naphthylethylamine

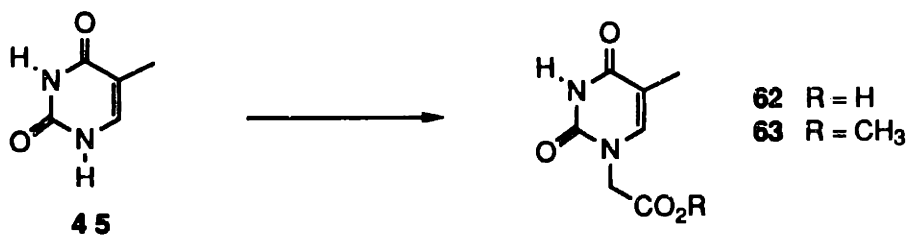
tryptamine, or 2-amino anthraquinone to give the amide phenyl esters **58a,b,c** in 73, 81, 72% yields, respectively. The 2-β-naphthylethylamine was obtained by reducing commercially available 2-naphthylacetonitrile with excess BH_3-THF at room temperature (76%). The triazine[53,54] amides **59a,b,c** were prepared by refluxing amide phenyl ester **58a,b,c** with 2 equivalents of free biguanide[52] in dry ethanol to give **59a** (67 %), **59b** (62 %) and **59c** (71 %). The acyl biguanide cyclized smoothly, and the only byproduct isolable was the corresponding ethyl ester.

The triazine β-naphthylethyl ester **60** was obtained from acid **57** by acid chloride formation followed by reaction with biguanide. The desired product was isolated in 39% yield from mixtures of ethyl-naphthylethyl ester and triazine ethyl ester.

The triazine ethylester **61** was isolated from attempted syntheses of the bis-triazine.

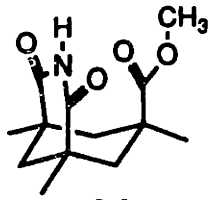
The thymine acetic acid methyl ester **63**[55]was prepared by reacting thymine with bromoacetic acid in refluxing aqueous KOH followed by esterification of the resulting acid **62** with diazomethane (Scheme 10).

Scheme 10



45

Imide methyl ester **64**[56] was obtained by esterification of the corresponding imide acid with diazomethane.



64

3.2.3 Binding Studies

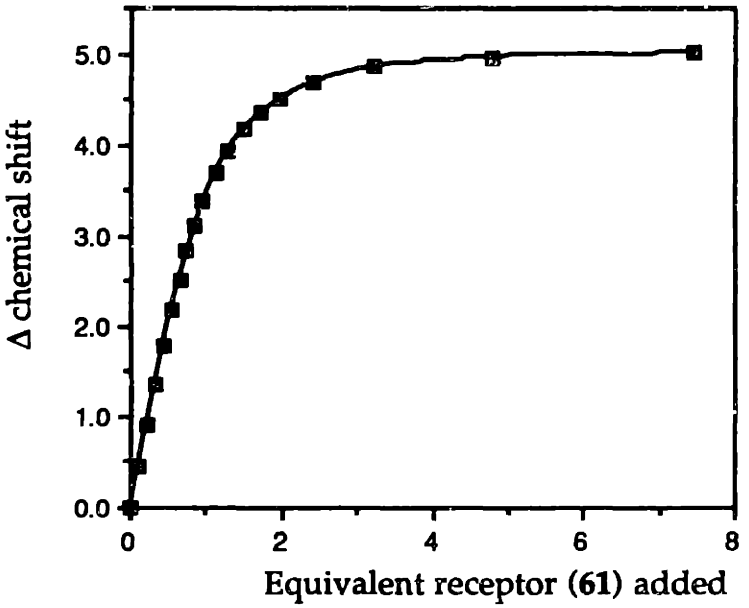
Since the receptor molecules prepared do not have suitable protons to follow by NMR, a reverse titration method was used in which imide served as host. Upon addition of triazine receptor to the thymine solution, the imide peak originally at ca 8.4 ppm moved downfield, a good indication of hydrogen bonding. After further addition of the receptor, the imide peak moved down to ca 13.2 ppm. The same trend was observed in Kemp's imide **64**, except the maximum chemical shift observed was 11.8 ppm. In most cases, the 95 % saturation was achieved. The association constants[57] were calculated from the titration data with Eadie-Hofstee treatment[58] (see Experimetal section for explanation), and are collected in Table 3.

Table 3 : Association Constants between Triazine Receptor and Imides.

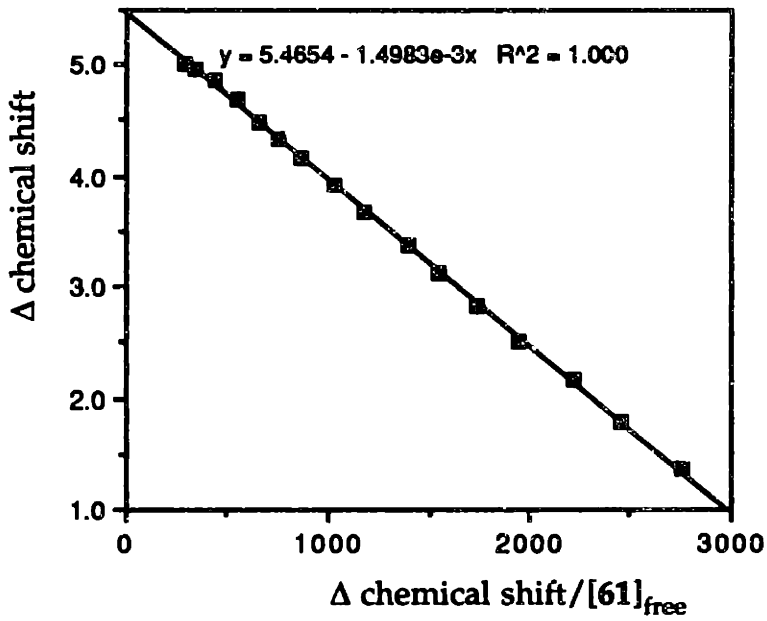
Imide	Receptor	Ka (M^{-1})
63	**61**	670
	59a	2000
	59b	2500
	59c	6700
	60	1100
64	**61**	330

The maximum chemical shifts of imides used in the calculation were either from extrapolation of observed data with non-linear regression analysis[59] or experimentally observed. Representative saturation curve and Eadie-Hofstee plot are shown in Fig 17.

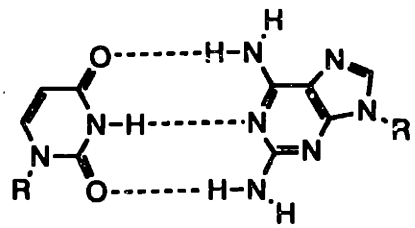
Fig 17 Saturation plot of titration data (**63** with **61**)



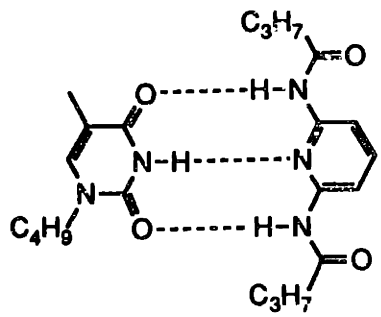
Eadie-Hofstee plot



The hydrogen bonding affinity of the diaminotriazine to thymine **63** is a little larger than that of diaminopurine (210 M^{-1}) and dibutyroyl pyridine (90 M^{-1}).[31]



31



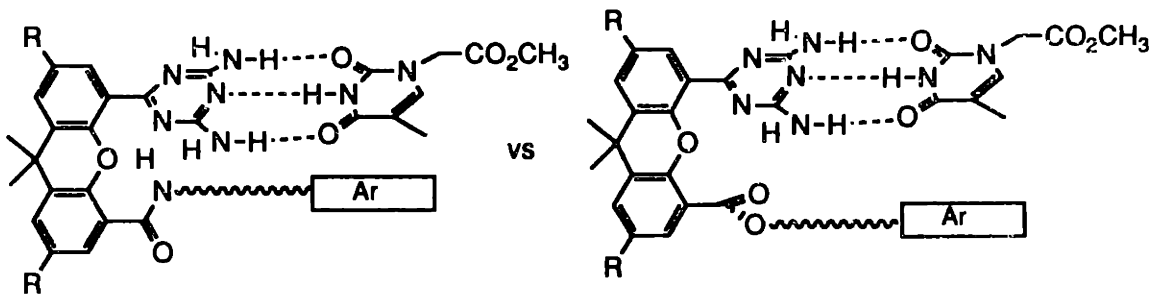
32

The lower affinity of triazine **61** to the saturated imide **64** is in accord with previous observation; reduced thymines (or uracils) bind less tightly to adenine than to itself,[31a] presumably because of reduced acidities.

Association constants increased 2-3 fold when the ethyl group in **61** was replaced with a flexible aromatic side chain (**59a,b** and **60**). The increase is probably from favorable aromatic stacking interactions. An additional 3-4 fold increase in Ka was observed when the two flexible methylene units were removed in the anthraquinone derivative **59c**.

Intramolecular hydrogen bonding between the amide NH and xanthene oxygen seems to be responsible for the higher affinity of the amide **59a** over ester **60** . Down field shifts of both amide proton (ca 10.5 ppm) and the proton ortho to the carbonyl group of the amide indicate the possibility of hydrogen bonding between amide N-H and xathene ring oxygen (Fig 18).

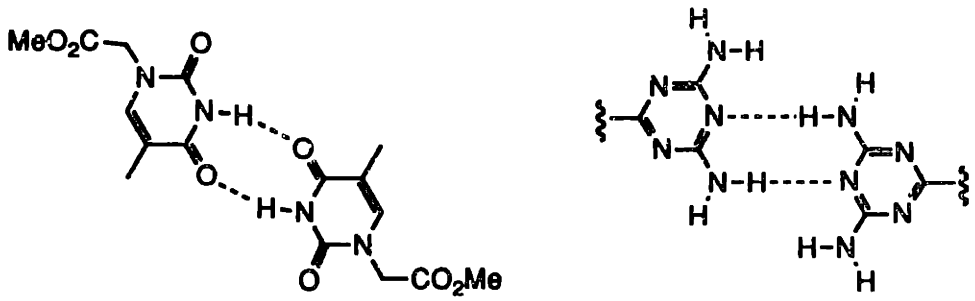
Fig 18



This bonding was recently established by crystallographic analysis.[60]

Self-dimerization of both thymine and triazine seems negligible under these conditions(Fig 19), a factor that simplifies the analysis of triazine data.

Fig 19; Self-dimerization of thymine and diaminotriazine



From the dilution study, dimerization constants were calculated using non-linear least square curve fitting program (systat 1:1) (Table 4).

Table 4; Dimerization constant of thymine and triazine (Dilution exp).

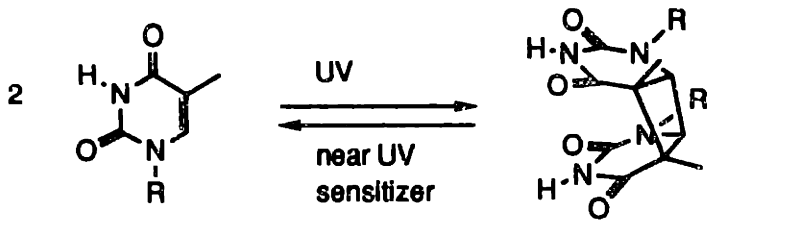
compd	Kd(M^{-1})	δ_{free}(cald)	δ_{bound}(cal'd)	con'n range studied
63	6.8±1.0	7.958 ppm	10.861 ppm	0.88-17.0 mM
59a	4.9±0.06	5.167 ppm	6.651 ppm	2.00-61.4 mM

3.3 Receptor for Thymine Photodimer

3.3.1 Introduction

Thymine dimers arise in DNA exposed to UV light as a consequence of a ($\pi^2_s+\pi^2_s$) photocycloaddition reaction between two stacked thymines in the double helix.[61]

Fig 20

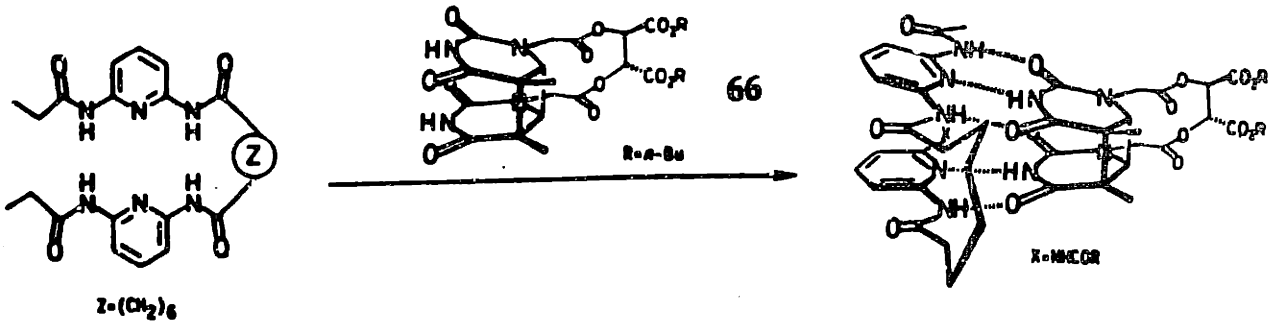


cis, syn - photodimer

In addition to thymine dimers, cytosine-thymine and cytosine-cytosine dimers of cis-syn type are also formed in UV irradiated DNA.[62] These dimers, which are cyclobutane derivatives, exert deleterious effects in living systems.[63] Repair on DNA photodamage in a wide variety of living systems is accomplished remarkably, by enzyme-catalysed splitting of the cyclobutane ring of the dimer to regenerate pyrimidines.[64] The enzymes responsible for dimer splitting are the DNA photolyases.[65] These exceptional enzymes utilize light in the visible and near-UV range of the spectrum to accomplish pyrimidine dimer cycloreversion.[66]

Despite intense interest[67] in these derivatives there has been little work on the binding properties of thymine dimers.[68] The structure of the cis,syn-dimer places two imide groups in close proximity at an angle of ~45° and a closest distance (4CO to 4CO) of 2.7 Å to each other. Hirst and Hamilton have prepared a thymine dimer receptor based on 2,6-diaminopyridine connected via flexible spacers (Fig 21).[68]

Fig 21



65

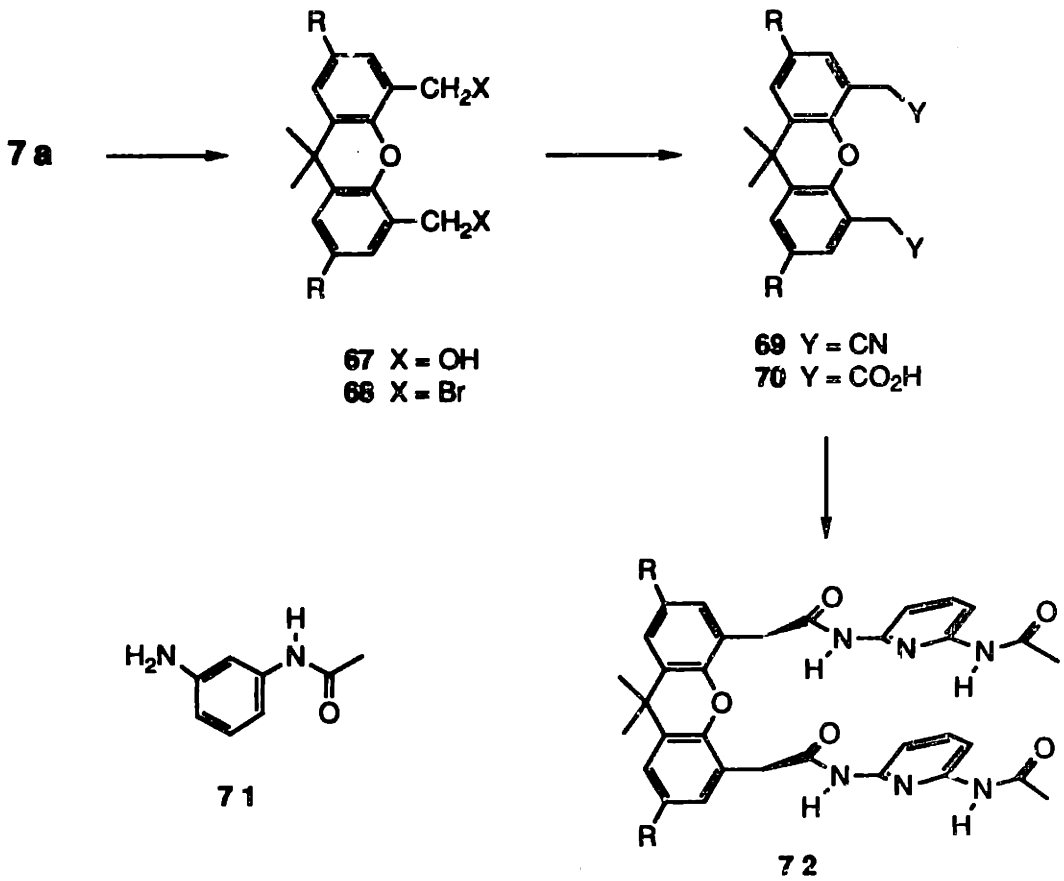
They found that the receptor **65** binds thymine dimer **66** with Ka of 2200 M^{-1}, the binding energy of which (-4.6 kcal mol^{-1}) is a little less than twice that of 2,6-dibutyramidopyridine and n-butylthymine (-2.7 kcal mol^{-1}).

3.3.1 Synthesis and Binding Studies of Thymine Photodimer Receptor.

Since the planes of the two imide functions in the thymine photodimer intersect ca. 45°, a ditopic receptor which could adjust the binding site angle to a complementary value seemed desirable. It is possible that two 2,6-diaminopyridine units can be linked so that the angle can be adjusted freely. Xanthene diacid **7a** was used as spacer. However, molecular model (CPK) examination revealed that if one of the amino functions in 2,6-diaminopyridine was attached directly to xanthene diacid **7a**, one of the imide carbonyls of the thymine photodimer would be placed directly on top of the electron-rich xanthene nucleus. Thus xanthene diacid was homologated as a one carbon unit.

Xanthene diacid **7a** was reduced with LAH in THF to the diol **67** (95 % yield) , which was then converted to the dibromide **68** by PBr_3 treatment in CCl_4 (quantitative) (Scheme 11).

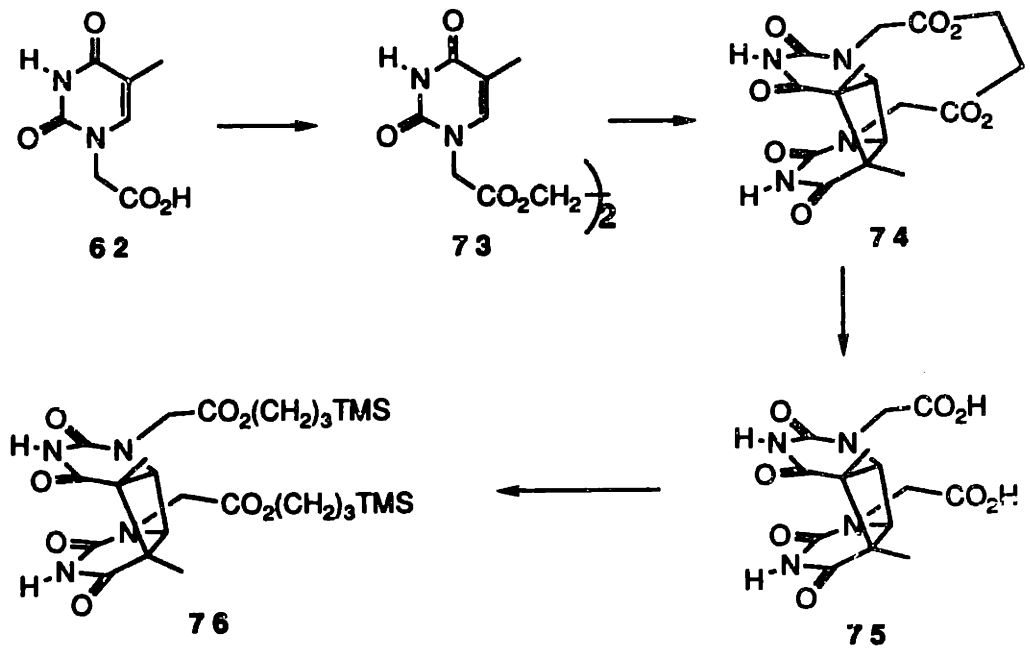
Scheme 11



Dibromide **68** was elongated at each position by the classical cyanide method. Treatment of the dibromide **68** with NaCN in aq. ethanol followed by hydrolysis gave the xanthene homodiacid **70**. Coupling of the diacid **70** with monoacetylated 2,6-diaminopyridine **71**[69] using DCC in THF gave the receptor **72** in 47 % yield.

Thymine photodimer was synthesized as follows.[70] Thymine-1-acetic acid **62** was transformed to bis thymine ester **73** with ethylene glycol mediated by carbonyl diimidazole (Scheme 12).

Scheme 12



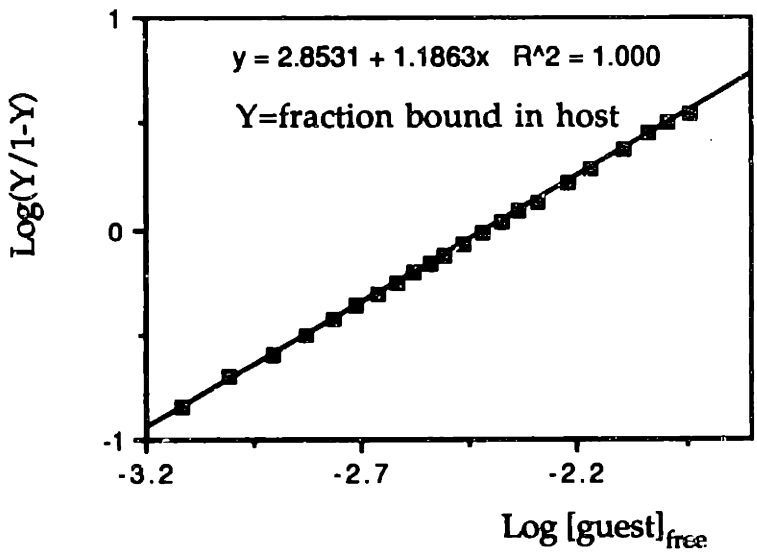
Irradiation of the bis ester **73** in aqueous acetone solution with a Hanovia immersion lamp filtered through a Pyrex sleeve (250 W, medium pressure) for 5 h gave the thymine photodimer **74** in quantitative yield. Hydrolysis (NaOH in aqueous methanol) followed by esterification with 3-trimethylsilylpropanol afforded the photodiomer **76**, which was highly soluble in both CH_2Cl_2 and $CHCl_3$.

3.3.3 Binding studies.

The receptor **72** was titrated first with the thymine **63** in $CDCl_3$. Both of the amide protons in the receptor **72** moved downfield upon addition of the thymine solution. Specifically, the acetamide NH shifted from 7.73 to 9.56 ppm, while the aryl acetamide NH moved from 8.71 to 9.98 ppm.

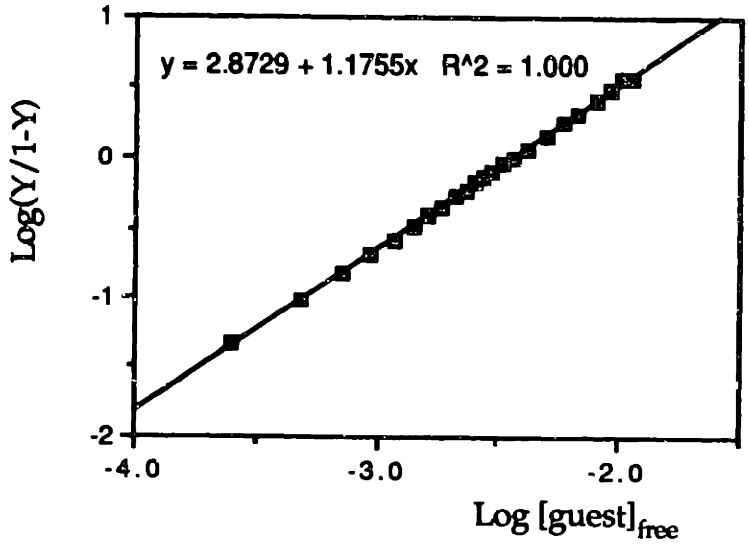
Assignment of the two amide proton comes from intramolecular NOE experiments in which irradiation of the benzylic proton gave a 14.8 % enhancement of the downfield NH signal, while irradiation of the acetyl group resulted in 7.1 % enhancement of the upfield NH signal. Thymine NH, which resonates ca 10.5 ppm when fully complexed move to 8.79 ppm upon addition of large exess thymine solution. The chemical shift data was analysed with Hill treatment[71] to yield K_1= 185, K_2=383 M^{-1} (statistically corrected) with a Hill coefficient of 1.18. These Ka's are the average from the two different amide protons. Results from both protons agree, as shown in Fig 22.

Fig 22 Hill plot of acetamide proton
K_1=174 M^{-1}, K_2=370 M^{-1} (statistically corrected)



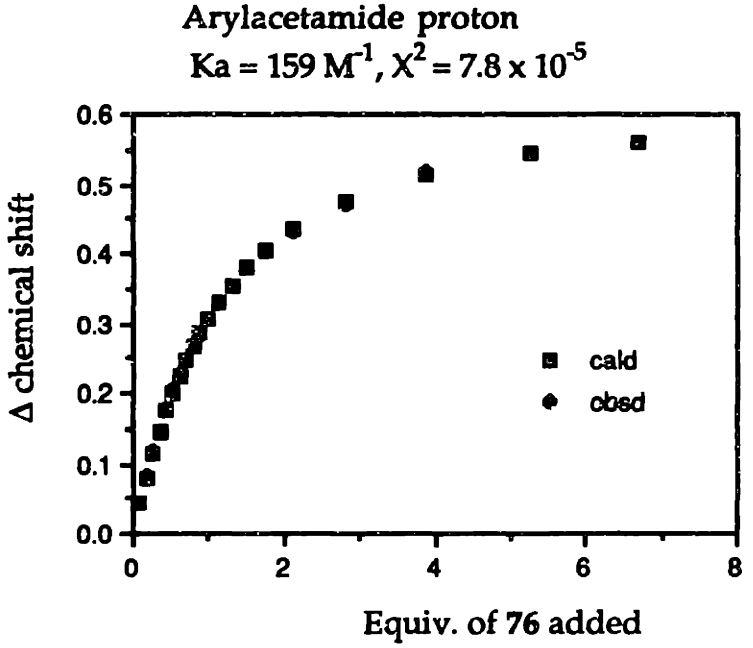
Hill plot of arylacetamide proton

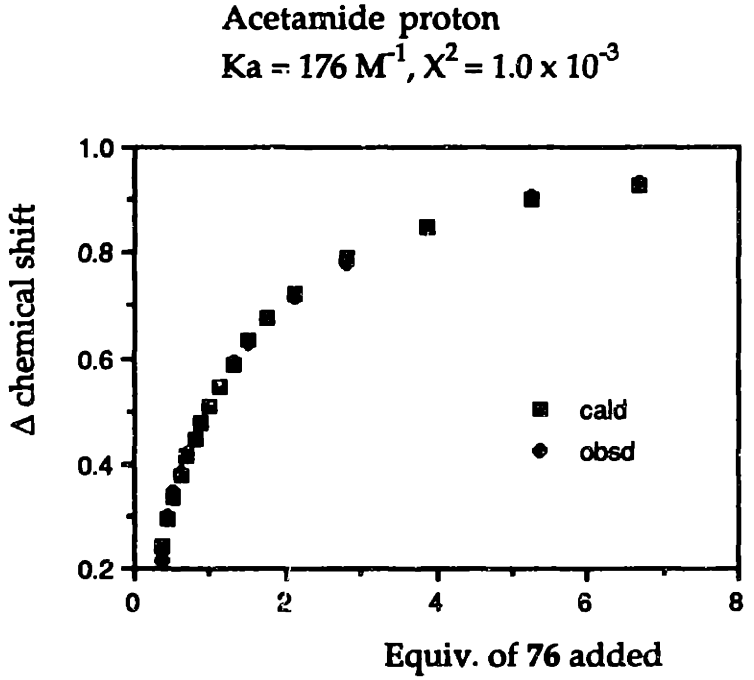
K_1=195 M^{-1}, K_2=396 M^{-1} (statistically corrected)



When the receptor **72** was titrated with the thymine photodimer **76**, the acetamide proton moved from 7.72 to 8.65 ppm, the arylacetamide proton moved from 8.71 to 9.28 ppm, while the imide protons in photodimer **76** originally at 9.00 ppm (fully complexed) shifted upfield to 7.90 ppm. However, binding constant was calculated to be only 171 M^{-1} *with a nice fit for 1:1 stoichiometry.* The binding constant is an average of nonlinear regression[59] and Eadie-Hofstee[58] analysis with both of the amide protons. Shown below are the saturation curve of the titration data, together with the calculated chemical shift using a 1:1 binding isotherm (Fig 23).

Fig 23: Saturation plot of Obs'd and Cal'd chemical shift with 1:1 model (72:76)

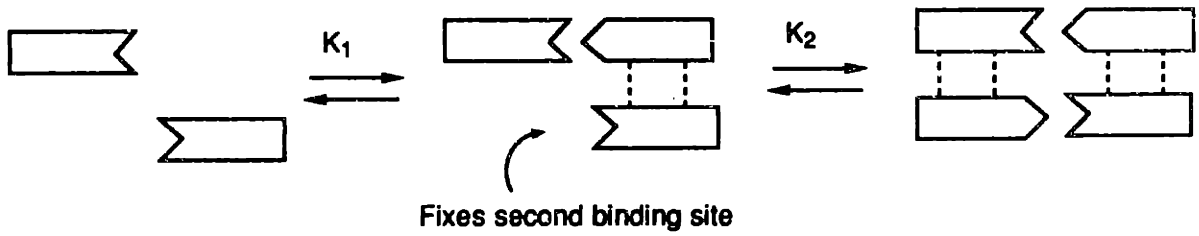




These results may be explained with the anti arrangement of the receptor **72**. Due to unfavorable dipole-dipole interaction, the majority of the receptor stays as anti conformation. In such a case, the two pyridine ring planes stay

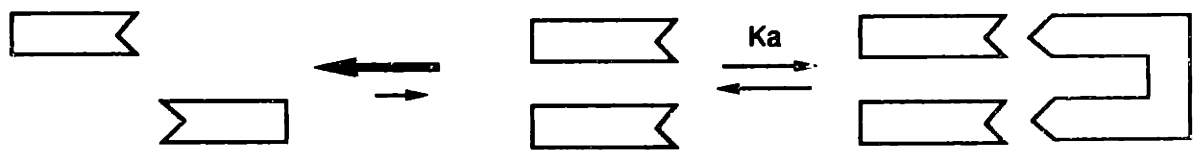
off center. Binding of one thymine will fix the conformation of the other pyridine binding site for the second thymine and thus result in positive cooperativity (Fig 24).

Fig 24



In order to bind thymine photodimer **76**, the receptor **72** has to overcome the bad geometry, which results in reduced binding (Fig 25). The anti form of receptor **72** does not seem to bind the dimer, which eventually would lead to a polymeric structure, because the methyl groups in the dimer **76** act as steric inhibitors.

Fig 25

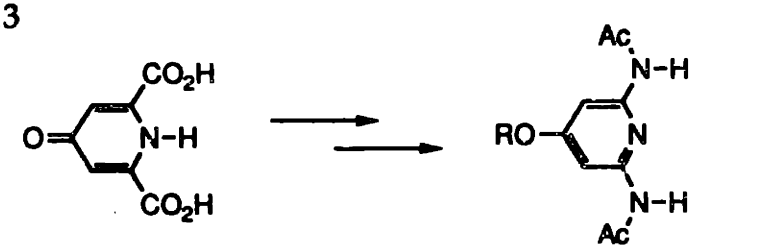


3.3.4 Synthesis of new photodimer receptor and binding studies.

Since the bipyridine receptor **72** did not exhibit high affinity toward the thymine dimer **76**, another type of receptor was prepared (Scheme 13). Chelidamic acid **77** was esterified to the corresponding diethyl ester, which

was protected with a benzyl group to give 4-be yloxypyridine-2,6-dicarboxylic acid diethyl ester (not shown).[72]

Scheme 13



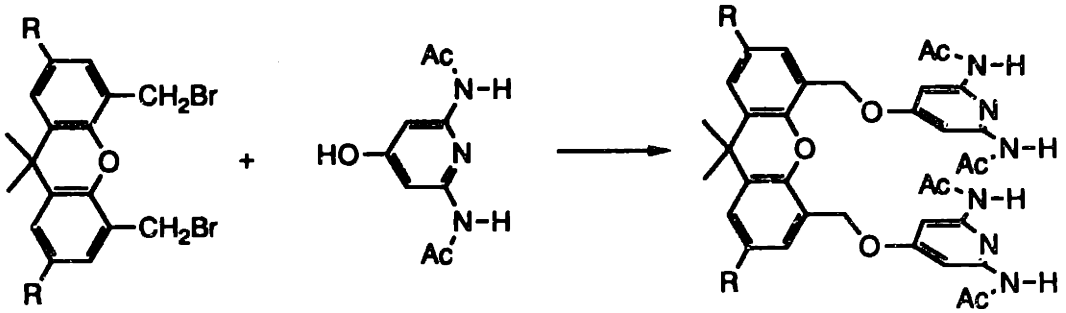
77

78 R = benzyl
79 R = H

The product was treated sequentially with hydrazine in refluxing ethanol, sodium nitrite in dilute hydrochloric acid and finally heated to reflux in ethanol to give 4-benzyloxy-2,6-diethoxycarbonylaminopyridine. Hydrolysis (KOH, ethanol, reflux) and acetylation (Ac_2O, pyridine) gave the diacetamidopyridine **78**, which was cleaved with hydrogen (Pd/C) to give 2,6-diacetamido-4-hydroxypyridine **79**.

Coupling of the hydroxy pyridine **79** with xanthene dibromide **68** in K_2CO_3-DMF[73] gave the desired receptor **80** in 51 % yield (Scheme 14).

Scheme 14



68 R = t-amyl
68a R = t-butyl

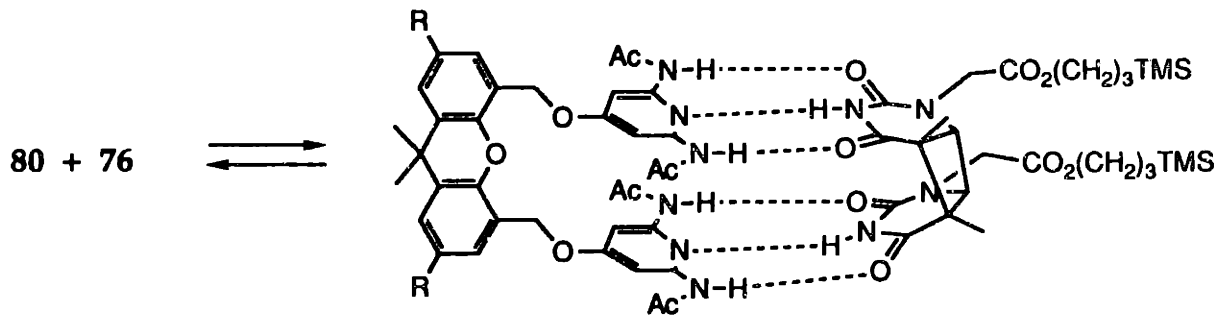
79

80 (51 %)
80a (49 %)

The binding of the receptor **80** toward the thymine **63** was studied. Since the receptor showed very broad NH and pyridine CH signals, which sharpen up with addition of the thymine **63**, the reverse titration method was employed. From the thymine imide chemical shift, the association constants were calculated using modified[74] Hill treatment. The binding constants were K_1=1250, K_2=400 M^{-1} (statistically corrected) with negative cooperativity (n_H=0.739). For comparison, thymine derivative **63** was titrated with the diacetamidopyridine **78** to give Ka of 960 M^{-1}. The slightly higher affinity of diacetamidopyridines (**78** and **80**) toward the thymine **63** probably results from electron donating properties of benzyloxy substituent in pyridine ring.[75]

The titration of the thymine dimer **76** with the receptor **80** showed down field shift of imide protons accompanied by guest peak sharpening. Treatment of the data with either Eadie-Hofstee or non-linear analysis gave Ka of ca 4800 M^{-1} (ΔG is 5 kcal mol^{-1}). Deviation of the Ka from the square of that with thymine monomer is probably due to the non-linear nature of the hydrogen bonds in a 1:1 complex, as shown in Fig 26.

Fig 26

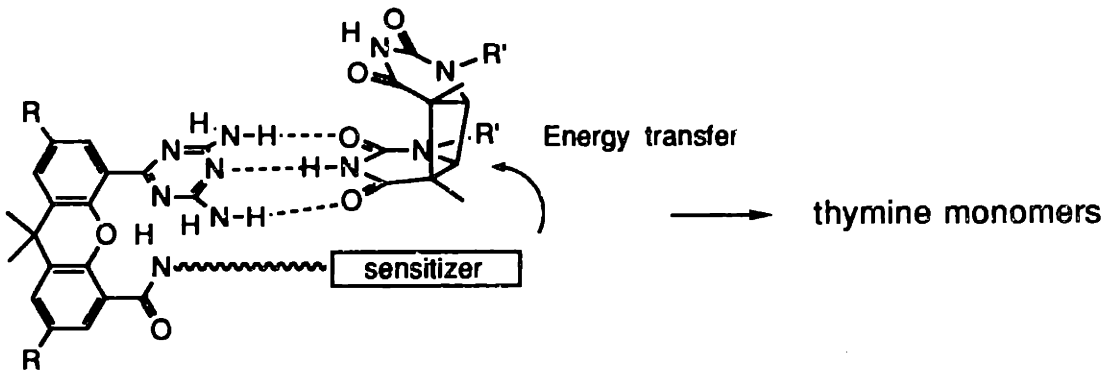


3.4 Attempted Photodimer Monomerization and Termolecular Reactions

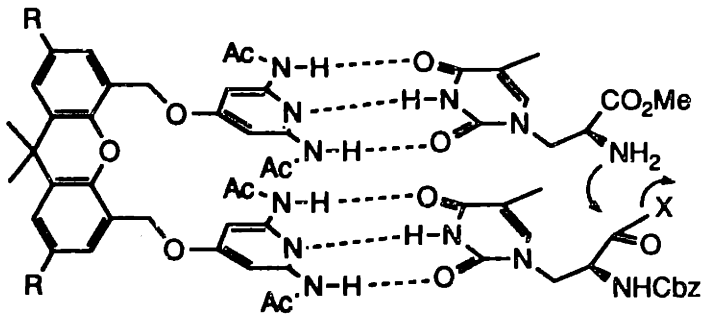
With systems that bind thymines and thymine dimers available, we started to study two reactions with these receptors. The first involved the thymine photodimer cycloreversion reactions that could be catalysed by the triazine receptors **59b,c** (Fig 27a). Our second choice was a reaction between the thymine side chains which could be catalysed by the receptor **80** in a termolecular complex[30] (Fig 27b), since thymine could be easily functionalized.

Fig 27

a



b

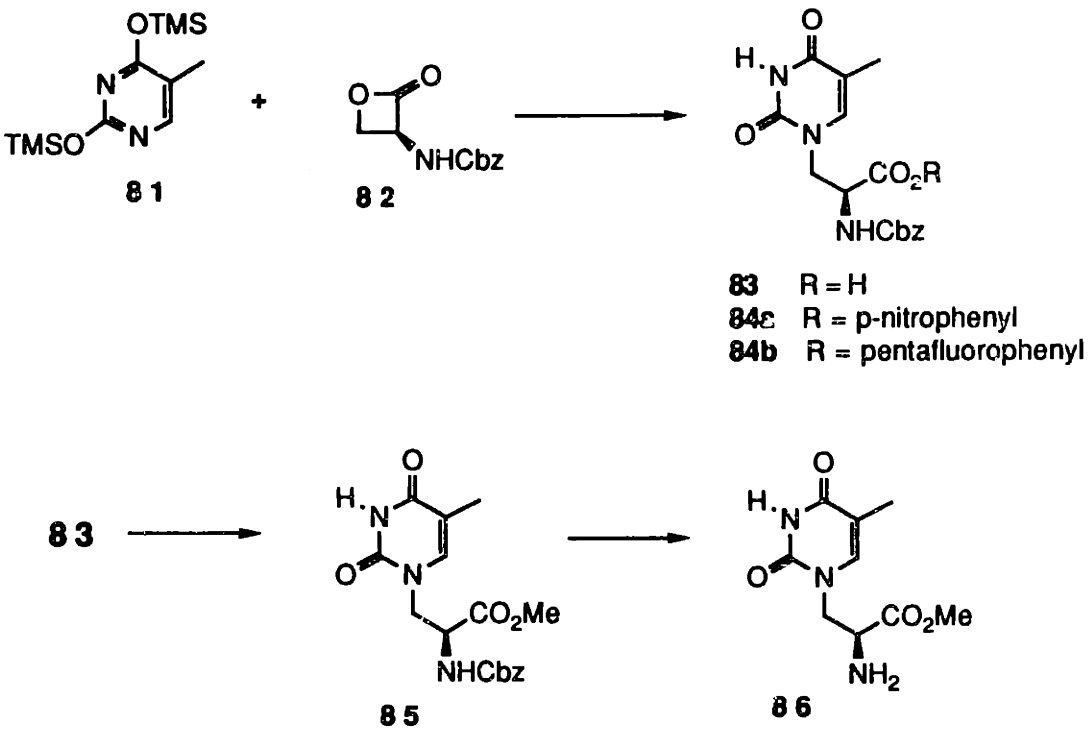


Although the exact mechanism is still unclear, thymine photodimers are known to be cleaved by photosensitized indoles,[76] quinones,[77] and flavins.[78] Flavins are, in fact, involved in DNA photolases. An intramolecular version of this photosensitized cycloreversion is also reported, in which a indole moiety is covalently attached to a pyrimidine photodimer and catalyses the monomerization process.[79] Since our triazine receptors have sensitizer

sidechains (indol in **59b** and anthraquinone in **59c**), it seemed possible that the cycloreversion might be catalysed by the receptors through an intramolecular photosensitization. Thus, the retro [2+2] reactions of thymine photodimer **76** were carried out in the presence of the receptors with light filtered though a $CoCl_2$ solution (DMF) which gives a bandpass of 300-450 nm. Unfortunately, all of the reaction performed in degassed $CDCl_3$, CD_3OD and CD_3CN with the dimer in the presence of the potential catalysts (**59b,c**) failed to give the monomer even under prolonged irradition (up to 6 h). However, a macrocyclic receptor having indole sidechains has recently been shown to catalyse this retro [2+2] reaction in CD_3CN with a similar strategy.[68b]

The template catalyzed amide bond formation required functionalized thymines and the synthesis is shown in Scheme 15.

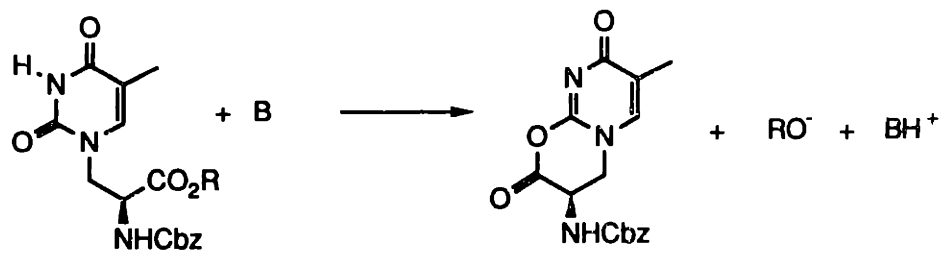
Scheme 15



Bis-O-trimethyl silyl thymine **81**[80] was alkylated with serine β-lactone **82**[81] to give the optically active thymine amino acid **83**.[82] Activation with phenol/DCC gave active ester **84a,b**,[83,84] while methyl ester formation followed by hydrogenolysis afforded the free amine **86**.

Equimolar mixtures of the two components were allowed to react, and the rate of *p*-nitrophenol (PNP) release was monitored by UV at 425 nm and found to be increased 3-4 fold when 0.5 equivalent of **80** was present. Unfortunately, the increased PNP release rate was catalysed by **80** but product formation was not. This was proved by performing the reaction without thymine amine **86**. The *p*-nitrophenol seems to be generated by base catalyzed cyclization of the active ester **84a** (Fig 28).

Fig 28



84a

The exact mechanism was not studied further, but may also contribute to poor coupling of other thymines.[85]

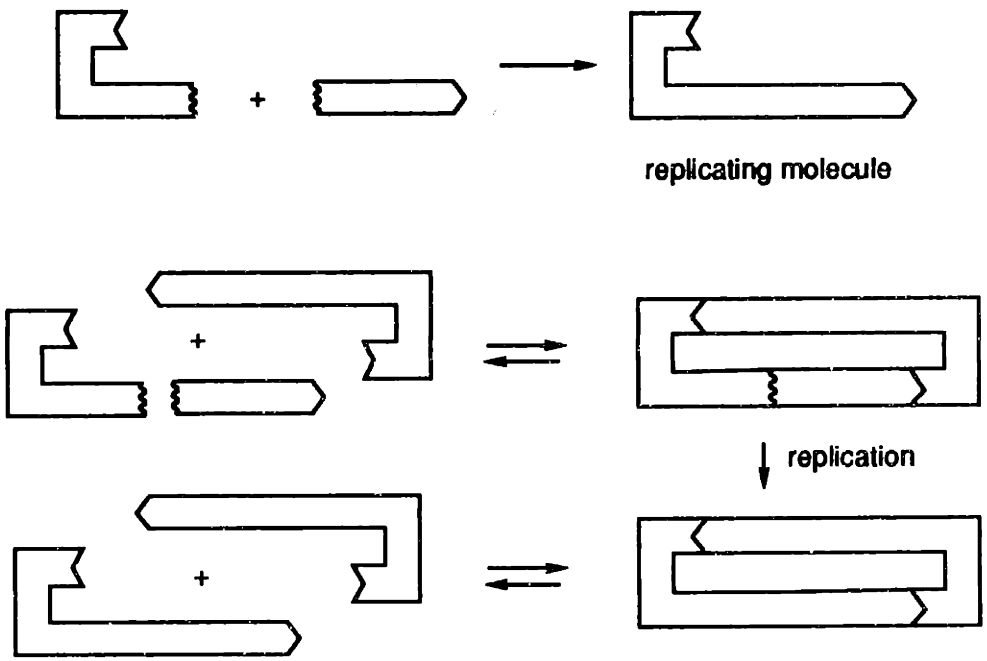
Chapter 4

4. Self Replication with Thymine derivatives.

4.1 New Synthetic Replicators with Thymine Derivatives.

Replicating molecules stand on the boundary of chemistry and biology.[87,88] Replication itself bears no structural content, yet the process invariably involves molecules that are complementary.[87,89] One goal of molecular recognition is to converge binding and catalytic steps in space and in time.[87] In principle, any molecule that can dimerize through complementary binding site can be divided into two pieces, which upon joining together can lead to replicating molecule (Fig 29).[87]

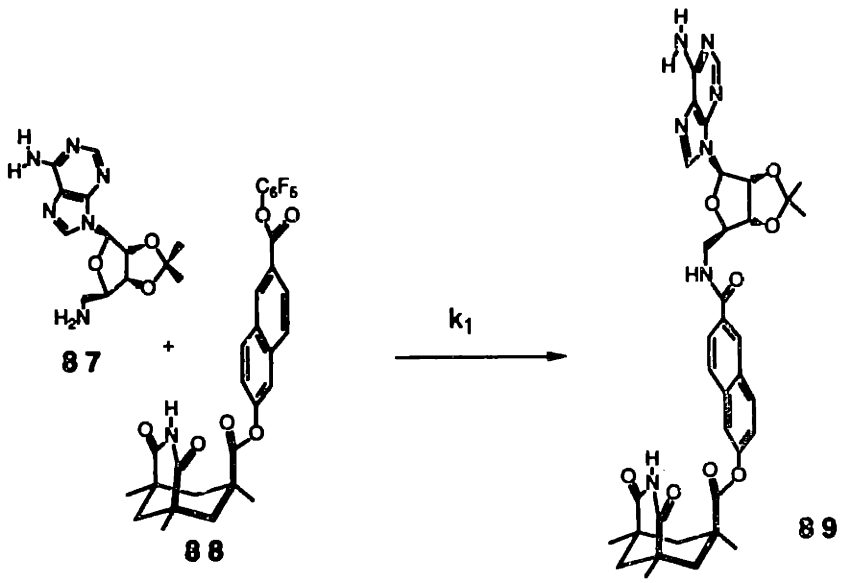
Fig 29

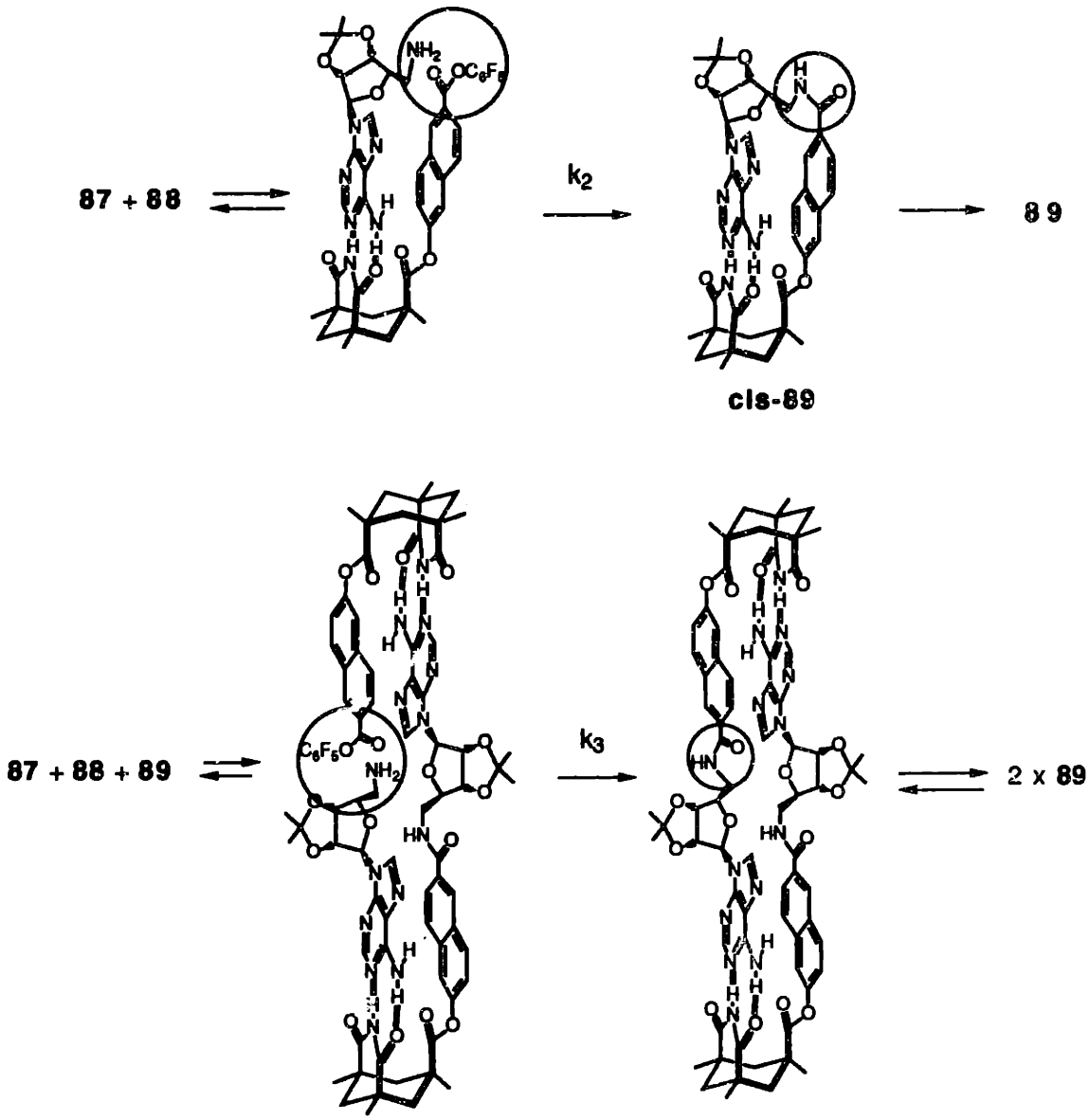


This group devised the first synthetic molecules that can self-replicate.[86,87] In this system, an imide provides a binding site for adenine, and naphthalene surface offers additional intramolecular contacts. The reaction involves an acyl transfer step to generate an amide. By perfoming the reaction in $CHCl_3$, they observed the initial rate increase of product formation after addition of the product (template) (Fig 30). This increased rate is attributed to the replicating behavior of the template.

Characteristic features of their system are as follows: i) The reaction exhibits autocatalysis. ii) The product is self-complementary. iii) The reactions of **88** proceed mainly through the formation of base-paired complexes. iv) Initial product of intramolecular acyl transfer reaction is *cis* amide.

Fig 30

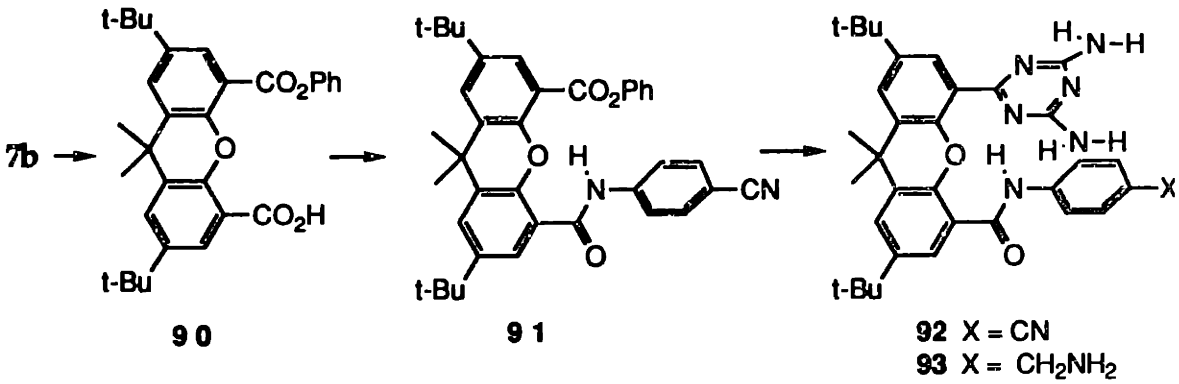




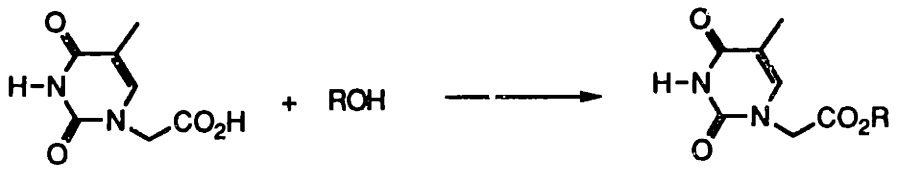
The product can be formed by three distinctive pathways; a bimolecular reaction (k_1), a preassociative bimolecular pathway (k_2), and the termolecular template-catalysed reaction (k_3), which is responsible for the autocatalytic behavior. When the template **89** is present in 20 % and 50 % in the reaction medium, the concentration of termolecular complexes are only ca 1-2 %, yet its presence increases the initial rate of product formation 40 % and 70 %, respectively.

In the present case, we designed a system where no preassocitive bimolecular pathway was likely. Xanthene diacid **7b** was monoesterified with phenol using DCC in the presence of catalytic DMAP (Scheme 16). The mono acid **90** was coupled with p-cyanoaniline via acid chloride to give **91** (95%). This was treated with biguanide in refluxing ethanol to give the triazine **92**.

Scheme 16

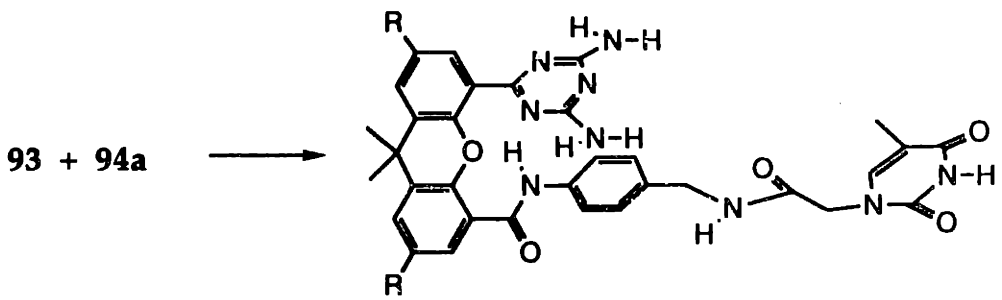


90 **91** **92** X = CN
93 X = CH_2NH_2



62

94a R = 2,4,5-trichlorophenyl
94b R = 2-chlorophenyl
94c R = phenyl
94d R = 4-t-butylphenyl
94e R = 4-methoxyphenyl

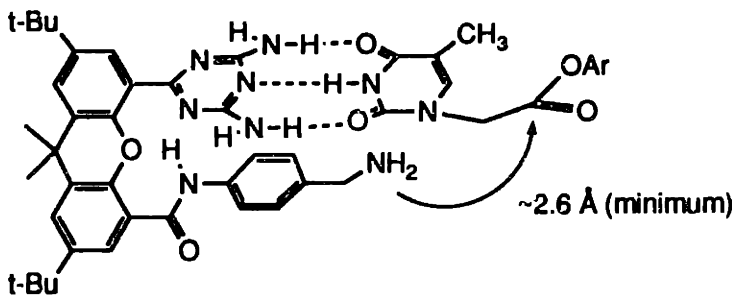


95

Nitrile group in **92** was reduced catalytically with hydrogen (Raney-nickel[90]) in the presence of large excess of ammonia to give **93** (quantitative). Activated thymine **93a-e** was prepared by reacting thymine acid **62** with phenol derivatives in the presence of a carbodiimide [N-ethyl N'-(3-dimethylaminopropyl) carbodiimide methiodide].[92]

Coupling of the triazine amine **93** with the active ester **93a**[91] gave template molecule **95**.

Even though molecular modeling of base paired complex (**93** and **94**) sugested that the distance between amino group and active ester carbonyl is longer than 2.6 Å, there seems to be a preassociative bimolecular reaction (background reaction rate between **93** and **94e** is relatively fast).



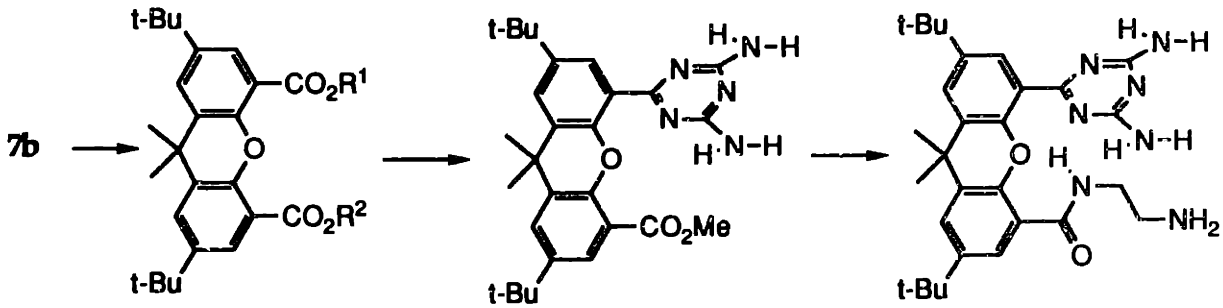
Slight deviation in hydrogen bonding angle from linearity could be the cause that shortens the distance between amino and ester carbonyl.

Since the two methylene protons (α to amide and benzylic) resonate in a position well separated from both of the reactants, the progress of reaction of this system was monitored with NMR by integration. However, the intrinsic inaccuracy of NMR integration and low solubility of the product (template) prevented the accurate measurement of the reaction. Rather, we created another system where both the reactant and the template are more soluble.

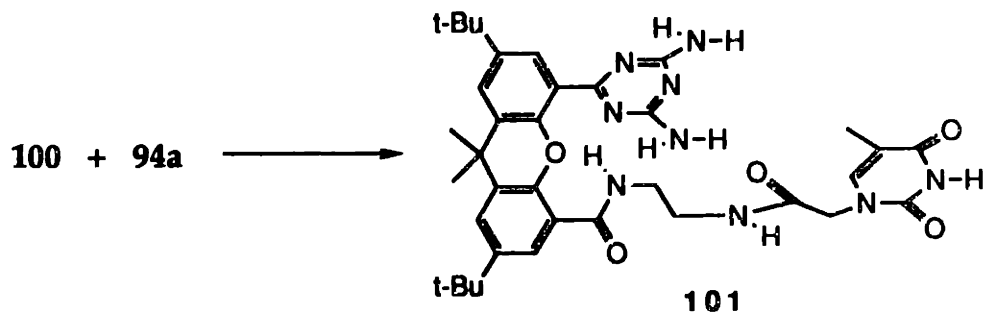
In addition we tried adjusting the spacer such that a preassociative bimolecular pathway was no longer possible.

Xanthene monomethyl ester **97**, prepared by selective deprotection of the dimethyl ester **96**, was converted to the monophenyl ester monomethyl ester **98**. As mentioned previously, this diester formation-selective deprotection route is preferred in large scale (> 5 gr) preparations. Ester **98** was reacted with biguanide to give the triazine methyl ester **99** (Scheme 17). Under the conditions of triazine formation, ester exchange reaction was generally observed, but only methyl ester **99** was obtained in this case, probably due to limited solubility of **99** in ethanol.

Scheme 17



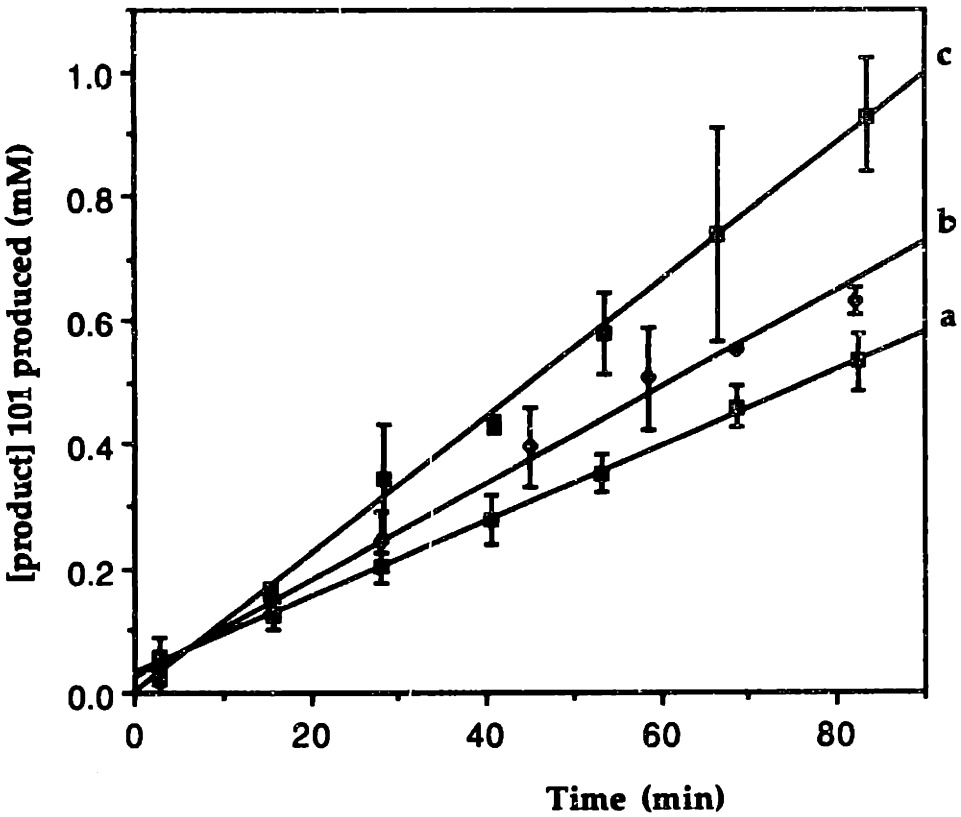
96 $R^1 = R^2 = Me$
97 $R^1 = H, R^2 = Me$
98 $R^1 = Ph, R^2 = Me$



Warming the triazine **99** in neat ethylenediamine afforded the triazine amine **100** in quantitative yield. Reaction of the amine **100** with thymine active ester **94a** gave the template **101**, which is quite soluble (>20 mM) in $CDCl_3$.

In initial studies using NMR, the new system repeatedly showed large deviations in rate, presumably due to errors associated with the NMR integration. However, this situation was neatly circumvented by HPLC. In the background reaction, a solution of equimolar concentration (each 8 mM) of the triazine amine **100** and the thymine phenyl ester **94c** in $CHCl_3$ with triethylamine (Et_3N) was allowed to react, and periodically aliquots were removed and analysed by HPLC. In order to find out the degree of autocatalysis, the template **101** (0.25 and 0.50 equivalant) was added to the background reaction mixture and analysed as the same manner as above. The reaction was monitored only initial part. A plot of the product **101** (concentration) *vs* time (min) is shown in Fig 31.

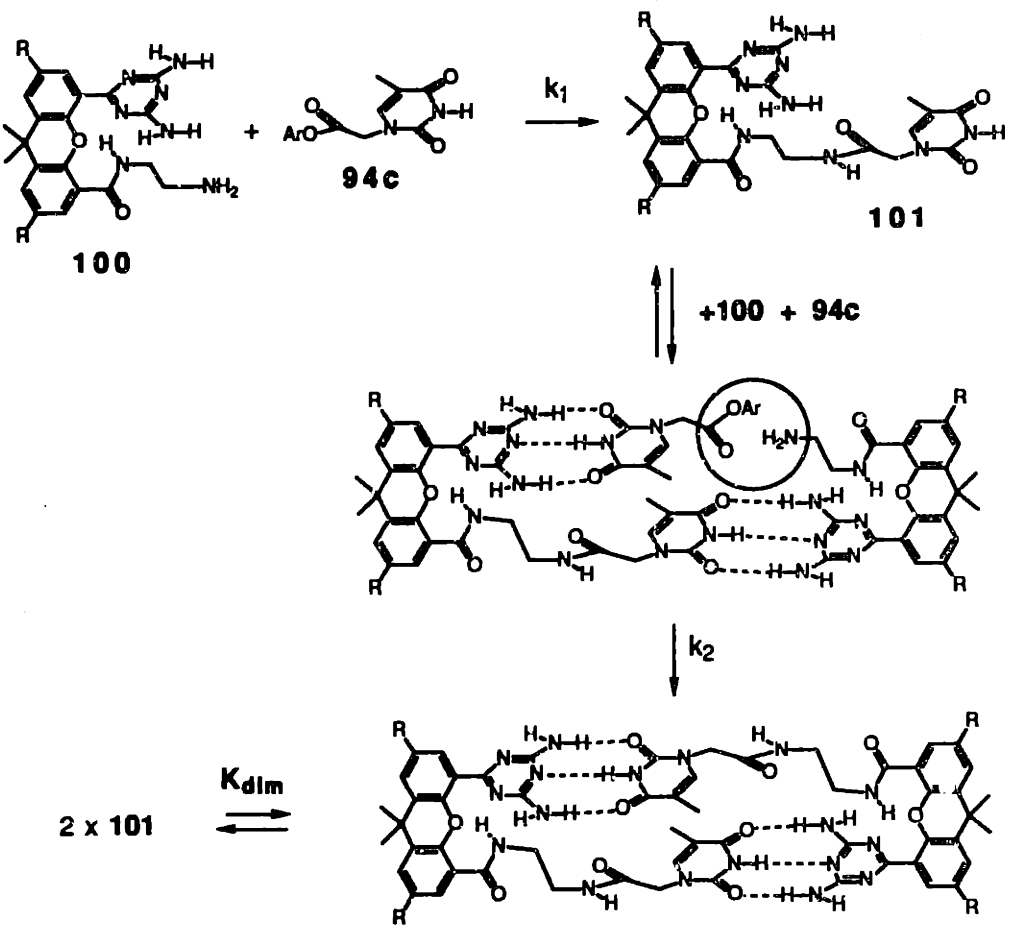
Fig 31



Appearence of **101** as a function of time. Initial concentrations are [**100**] = [**94c**] = 8.0 mM in $CHCl_3$. Et_3N (18 equiv) was present in all reaction solutions. Error bars represent standard deviations of multiple independent runs. a) no additive. b) 25 % **101** added. c) 50 % **101** added.

The result can be explained as follows. Two reactions contribute to the formation of product: the *background bimolecular* reaction and *termolecular template-catalysed* process (Fig 32). The latter is responsible for the replication process. The amino triazine **100** reacts with the phenyl ester **94c** by means of a bimolecular reaction generating the template **101**. This template binds with each of the reactants to form termolecular complex, which reacts and gives rise to two template molecule as dimerized form. The dimer dissociates into monomer template **101** which perfoms the catalysis.

Fig 32



Equilibrium constant between the triazine **101** and the thymine active ester **94c** was assumed to be 670 M^{-1} (see Chapter 4).

The dimerization constant (K_d) of the template was obtained from dilution study of the template. The imide peak in **101** is concentration dependent, moving from 12.92 ppm to 12.56 ppm when diluted from 12.7 mM to 1.8 mM with $CDCl_3$, indicating that the complementary binding sites dissociaciate. The K_d was estimated to be 30,000M^{-1} with computer aided calculation from the dilution data. According to the calculation, the chemical shift of unbound imide is 8.00 ppm, which is in good agreement with

previously obtained values for such structures (see Chapter 4 for free imide chemical shift in dilution experiment of **63**).

To substantiate the rate constants, multiple equilibrium constants are required. The individual binding constant between the triazine amine **100** (**A**), the thymine active ester **94c** (**E**) and template **101** (**T**) are defined as follows.

$$A + E \underset{}{\overset{K_1}{\rightleftharpoons}} AE$$

$$A + T \overset{K_2}{\rightleftharpoons} AT$$

$$E + T \overset{K_3}{\rightleftharpoons} ET$$

$$AT + E \overset{K_4}{\rightleftharpoons} AET$$

$$A + ET \overset{K_5}{\rightleftharpoons} AET$$

$$2\,T \overset{K_{dim}}{\rightleftharpoons} T_2$$

The rate constant of simple bimolecular reaction is k_1 and that of termolecular template catalysed process is k_2.

$$A + E \xrightarrow{k_1} T$$

$$AET \xrightarrow{k_2} T_2$$

In real calculation, it was assumed that the equilibrium constants K_2, K_3, K_4, K_5 were equal to $K_1 = 670\ M^{-1}$. From these values and dimerization constant of the template, initial concentration of termolecular complex was calculated to be only 5.1%, when both reactant concentrations are 8 mM and

template concentration is 2 mM (0.25 equivalent). The 5 % termolecular complex increases the initial reaction rate ca 30 % (Fig 31).[93]

4.2 Crossover Experiments

Synthetic replicators provide a means by which evolutionary behavior can be examined at the molecular level.[94] A recent example involves the coupling of adenine derivatives to suitably constructed imides derived from Kemp's triacid. Such systems can show sigmoidal growth,[95] reciprocity and even mutation[94]-features characteristic of evolution at the molecular level.

We introduce synthetic replicators capable of hybridization, and interpret their autocatalytic properties in terms of molecular shape and structure.

The minimalist requirement for replicators is a self-complementary structure[87] and we have described two such systems. The first involves adenine-imide hydrogen bonding[95] (Fig 33) and the second thymine-diaminotriazine hydrogen bonding[93] (Fig 34) as the molecular recognition components.

Fig 33

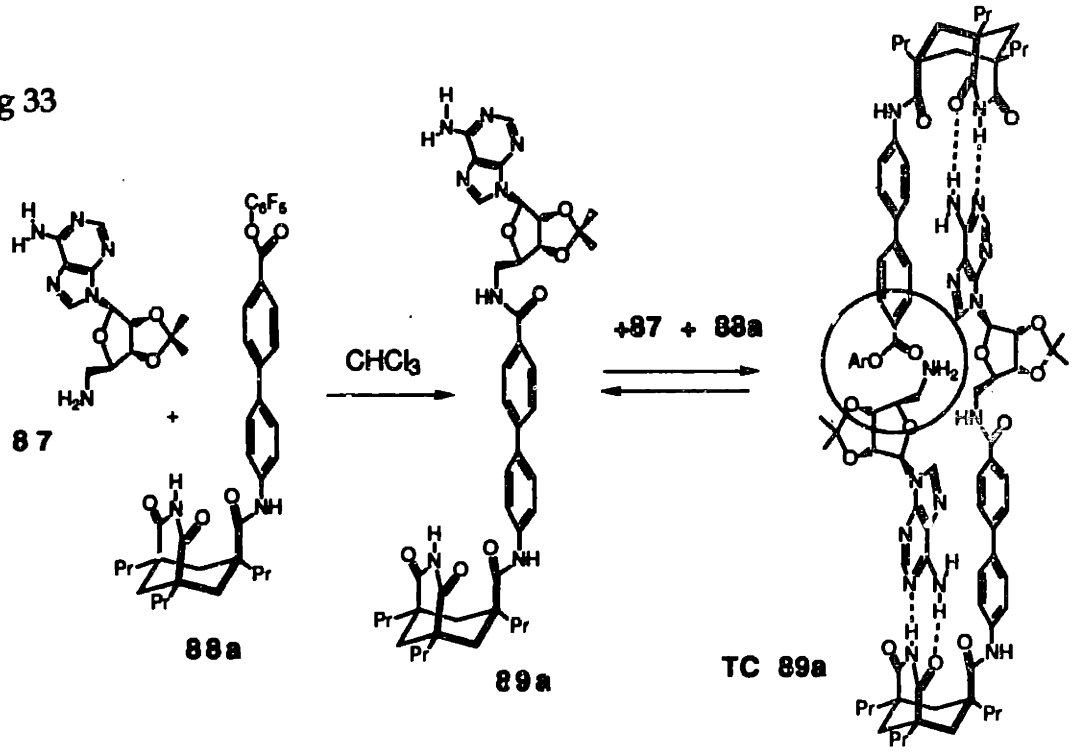
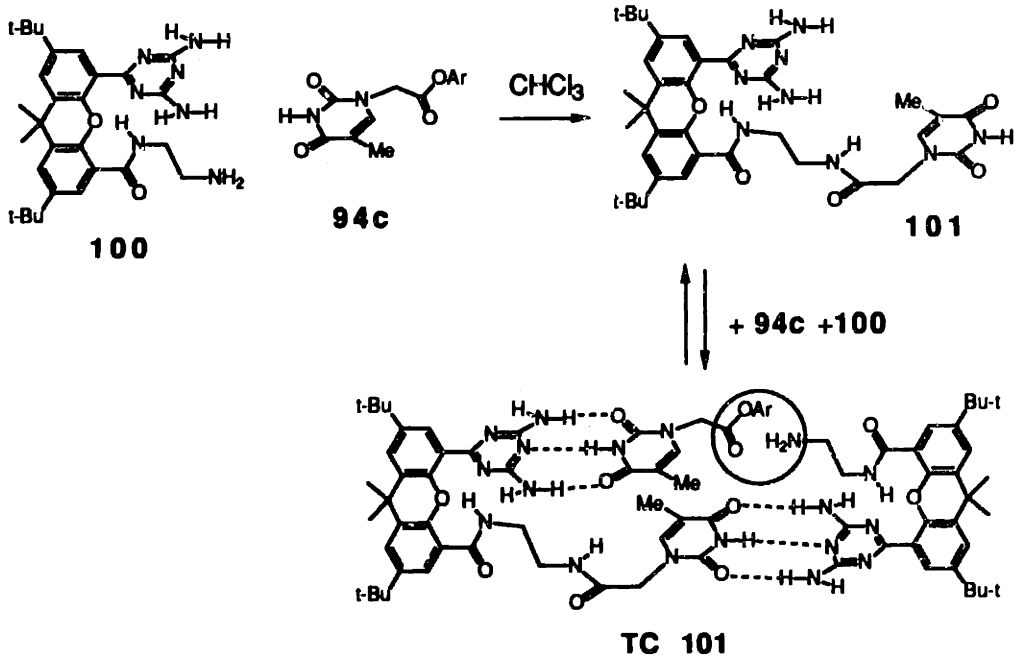


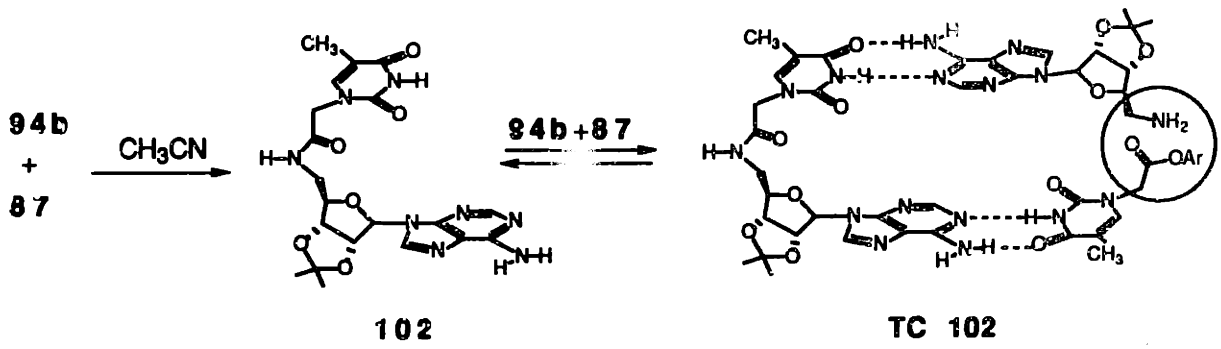
Fig 34



Both **89a** and **101** are replicators: they catalyse their own formation through the template effects suggested in termolecular complexes TC **89a** and TC **101.**

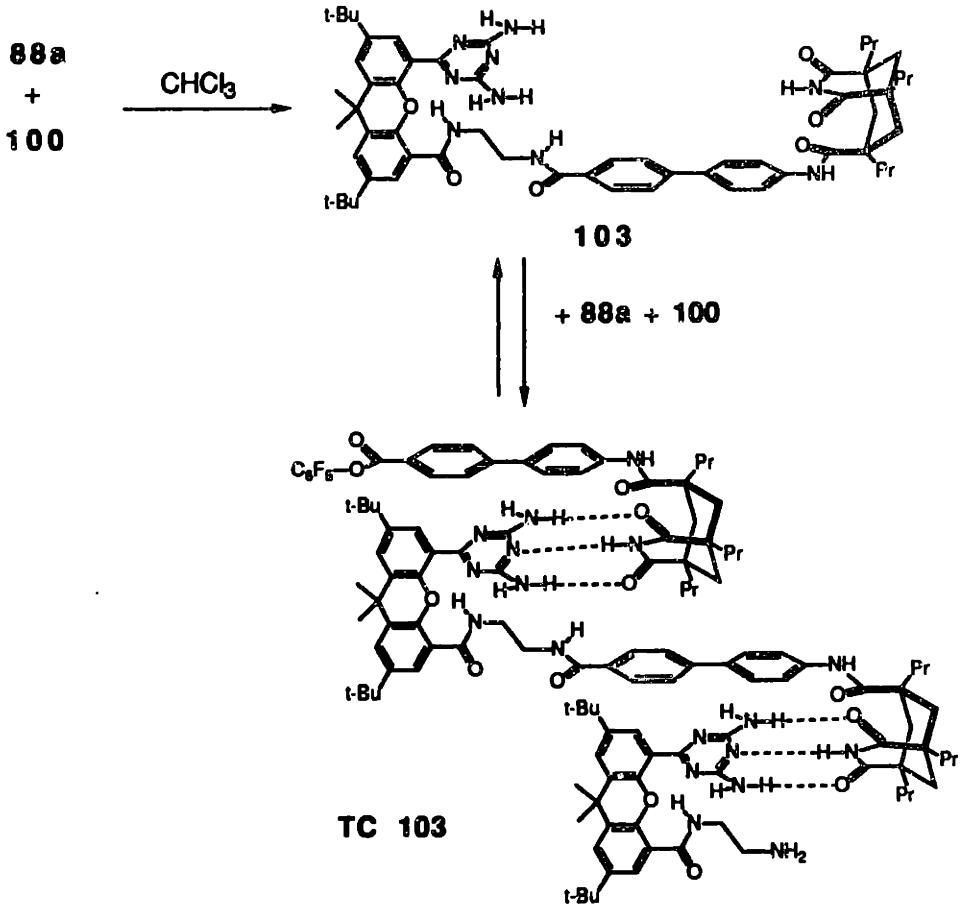
We staged a competition experiment in which hybrid (crossover) products were produced. Specifically, coupling of the adenine **87** with the thymine ester **94b** gave the dinucleotide analog **102** with a peptide/ribose backbone (Fig 35), whereas the corresponding reaction of **88a** with **100** gave **103** (Fig 36).

Fig 35



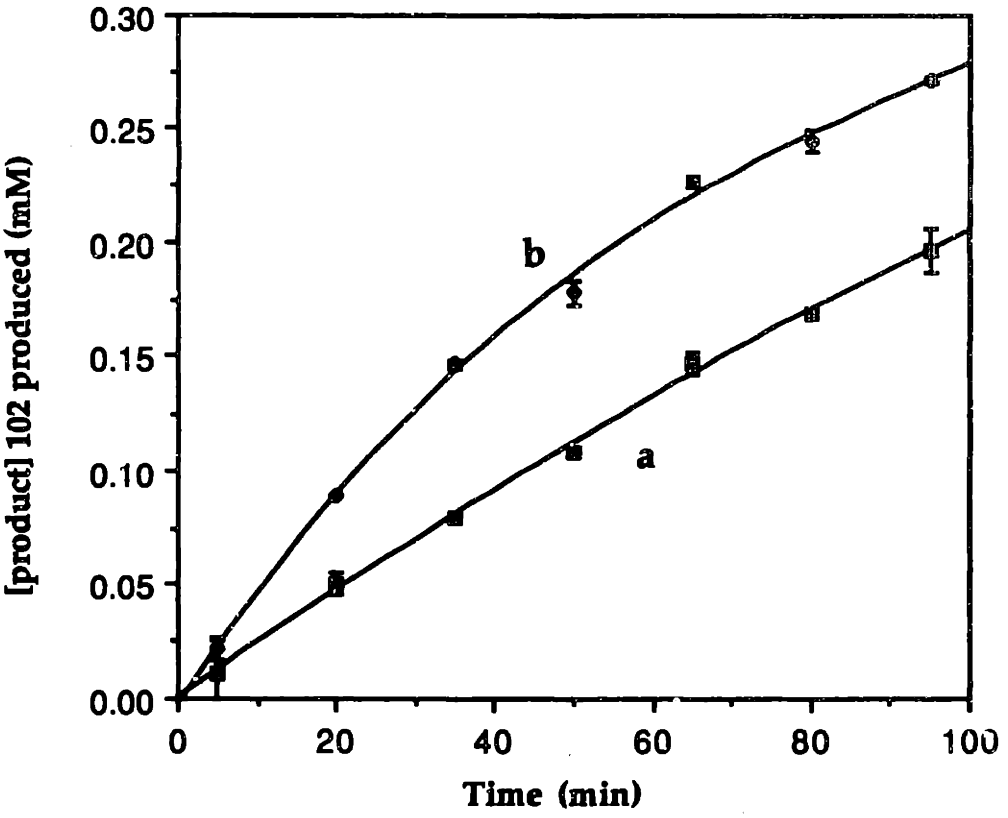
102 **TC 102**

Fig 36



At first glance, both hybrides might be expected to replicate. They bear complementary recognition surfaces which can gather their respective reaction components in termolecular complexes poised for intramolecular coupling. In fact, the adenine thymine hybrid **102** does so. Addition of **102** to mixtures of **87** and **94b** in CH_3CN led to the increased coupling rates (Fig 37) charicteristic of autocatalytic systems.[87] It is a fertile hybrid.

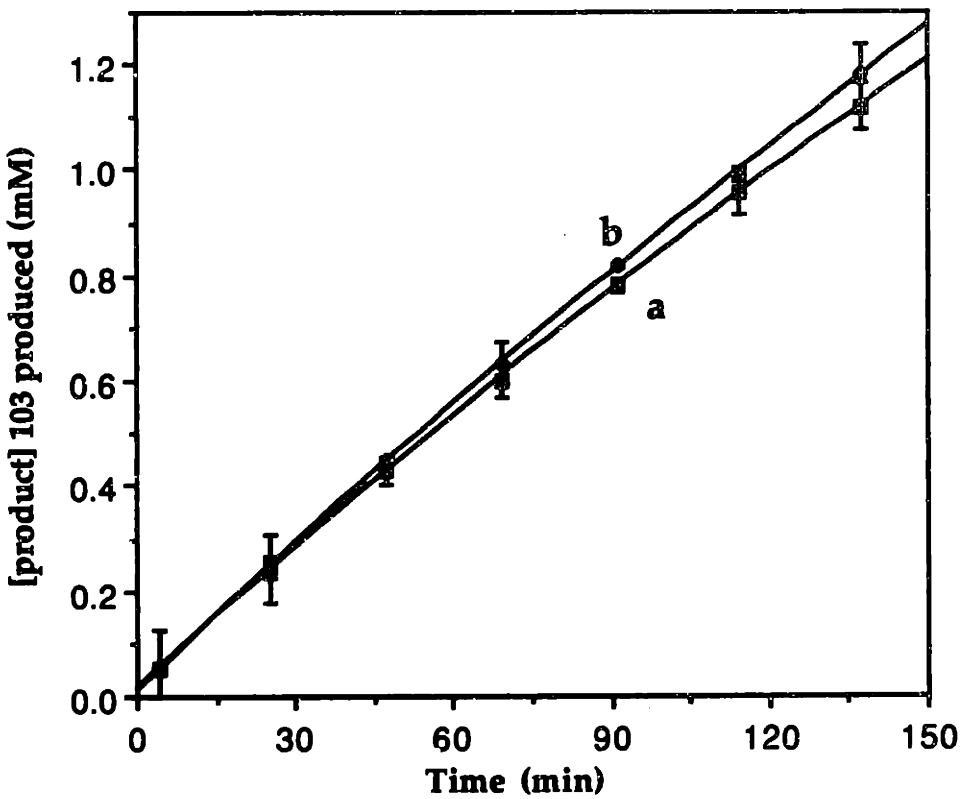
Fig 37



Appearence of **102** as a function of time. Initial concentrations are [**94b**] = [**87**] =3.0 mM in CH_3CN. Et_3N (18 equiv) was present in all reaction solutions. Error bars represent standard deviations of multiple independent runs. a) Reaction of **94b** and **87** without product **102** added. b) Reaction of **94b** and **87** with 0.07 equiv. **102** added.

This is not the case for the hybrid **103**. No increase in coupling rate for **88a** with **100** was observed on adding various amounts of the hybrid **103** (Fig 38). Accordingly, it can not catalyse its own formation; it is a sterile hybrid.

Fig 38



Appearence of **103** as a function of time. Initial concentrations are [**88a**] = [**100**] = 8.0 mM in $CHCl_3$. Et_3N (12 equiv) was present in all reaction solutions. Error bars represent standard deviations of multiple runs. a) with no added product **103** b) with 28 % **103** added.

The differences in behavior of **102** and **103** can be related to the orientations of their recognition surfaces. In **102** these can achieve a parallel arrangement that collects **94b** and **87** in a productive complex TC **102**. The initial product is the hydrogen bonded dimer of **102**.

The molecule **103** features two U-shaped modules - the Kemp's triacid and the xanthene diacid. Its overall configurations can be C- or S-shaped in which its recognition surface converge or diverge, respectively, in neither case can it simply dimerize. Rather self-complementarity is expressed in its ability to form chains (Fig 36).

In conclusion, orientation of recognition surfaces determines the supramolecular arrays available to self-complementary structures. When they permit a dimer to form, a replicating system can be devised. When they diverge, the molecular assemblies lead to mosaics and materials such as viral coat proteins. These notions are being persued in this lab with suitably oriented surfaces for controlled oligomerizations.

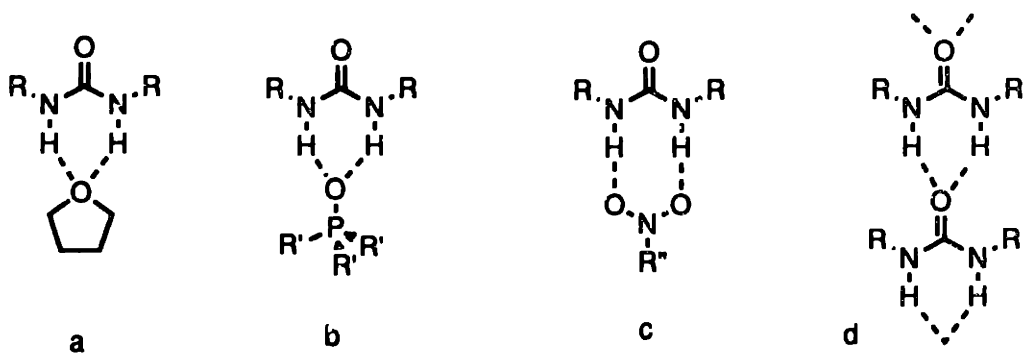
Chapter 5

5. Synthetic Studies Towards Cleft Molecules with Urea and Bipyridine Functionality Inside

5.1 Urea cleft

It is well known that many diaryl-, dialkyl-, and monoaryl-, or alkyl ureas readily form cocrystals.[96] Urea has hydrogen bond donors (NH hydrogens) and an acceptor (C=O). In N,N'-disubstituted ureas, the syn relationship of the two hydrogens seems to be ideally suited for simultaneous two-hydrogen bonding toward sp^2 or sp^3 hybridized heteroatom(s) such as oxygen. Molecules having functional groups such as ethers, trialkyl phosphines, sulfoxides, ketones and nitro, have been successfully cocrystalized with ureas (Fig 39). It is also reported that urea form self-hydrogen bonded polymeric structure such as in Fig.39d when substituents at nitrogens do not create severe steric hindrance.[96]

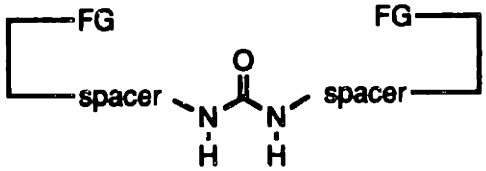
Fig 39



It seems also possible that enhanced nucleophilicity (basicity) of urea results from higher electron density at carbonyl oxygen compared to ketones,

amides, or esters. This may be exploited for the construction of cavities for metal ion binding sites. Thus we undertook to build a molecule with urea functionality set in the middle, as in Fig 40.

Fig 40

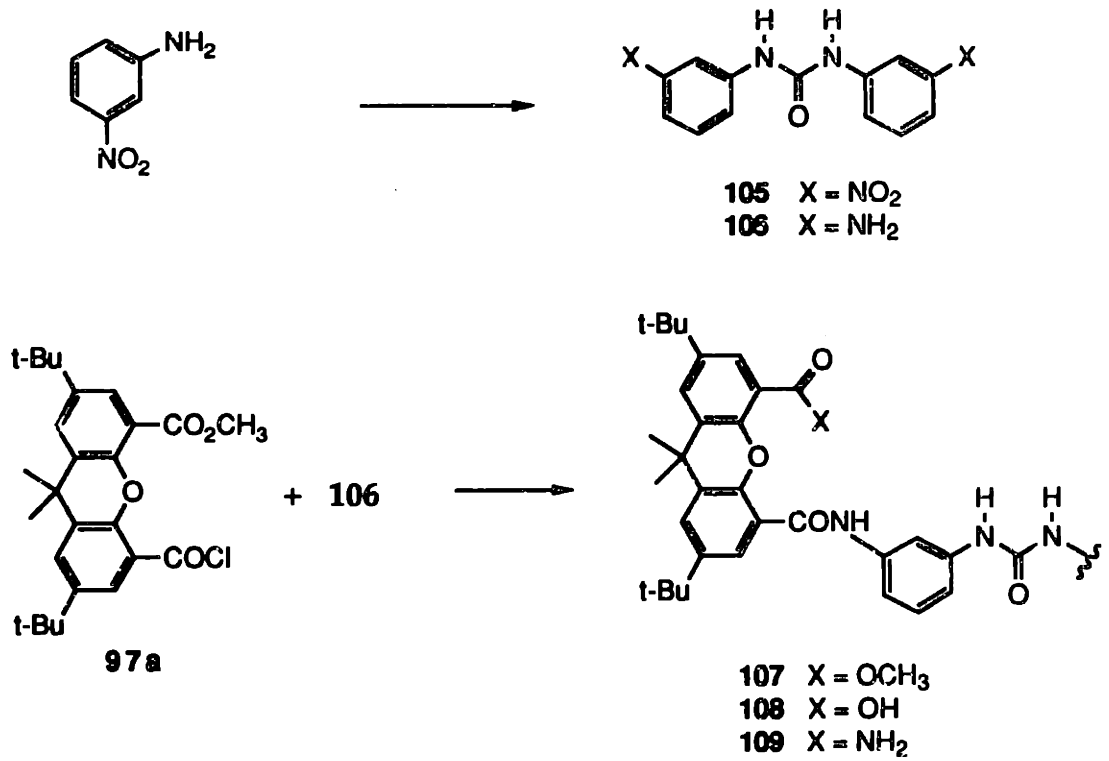


Since N,N' disustituted urea has ca 120° angle between two substituents, a meta substituted benzene ring was chosen as a spacer, and the xanthene diacid as a U-turn building block.

The synthesis of the target urea cleft is outlined in Scheme 18 and is quite straightfoward. Phenyl chloroformate was used as the urea carbonyl source.

Simple heating of phenyl chloroformate with two equivalent of m-nitroaniline followed by catalytic hydrogenation of the adduct **105** gave N,N'-bis (3-aminophenyl)urea **106** in 69 % yield. In both steps, the purification was performed by simple trituration in appropriate solvents.

Scheme 18



The diamine **106** was reacted with xanthene monoacid chloride **97a** to obtain urea cleft dimethyl ester **107** (77 %), which was hydrolysed (NaOH, MeOH-THF) to the urea cleft diacid **108** (97 %). The diacid **108** could be converted to the amide **109** by sequential treatment with carbonyldiimidazole and gaseous ammonia (92 %). The urea peak of **108** appeared at 7.96 ppm in $CDCl_3$ as broad singlet. With the urea cleft diacid **108**, a series of solid-liquid extraction experiments were performed using several aromatic and aliphatic diacids in $CDCl_3$. The results of extraction experiments is summarized in Table 5.

Table 5 : Summary of Solid-Liquid extraction with urea Cleft **108**

Guest	Eq. ext'd	Chemical shift change	Solubility

		Guest[a]	Host[b]	
glutaric	1.3	α(+0.08), β(+0.05)	+0.05	sol
β-ketoglutaric	0			insol
adipic	<0.17		~0	insol
pimelic	1.82		+0.02	sol
γ-ketopimelic	0.28	α(+0.07), β(~0)	-0.15	insol[c]
isophthalic	0			insol
terephthalic	0			insol

a) Chemical shift of α and β protons; + means upfield shift.

b) Host urea peak

c) Barely discernable from noise with 188 transients (AC 250 MHz).

The urea cleft **108** extracted glutaric, adipic, pimelic and γ-ketopimelic acid but not β-ketoglutaric, isophthalic and terephthalic acids. It seems that the cleft **108** does not exhibit any appreciable selectivity toward guest size; the host **108** can apparently self-adjust the cleft width (or length) toward guest diacids by rotation between benzene ring and urea nitrogens. Lack of preorganization is also confirmed from the low binding constant between the acid **108** and 4,4-bipyridine (Ka = 618 M^{-1}). Equivalents of guest extracted in this case seem a mere reflection of their solubility in $CDCl_3$.

Extractability (P) is function of association constant (Ka) and limiting solubility (g_o) of the guest, as discussed in Chapter 2.

$$P = [G]_t/[H]_t$$

$$\sim g_o \cdot Ka/(1+g_o \cdot Ka) = \alpha \text{ (slope of solubility titration) (when go } << 1)$$

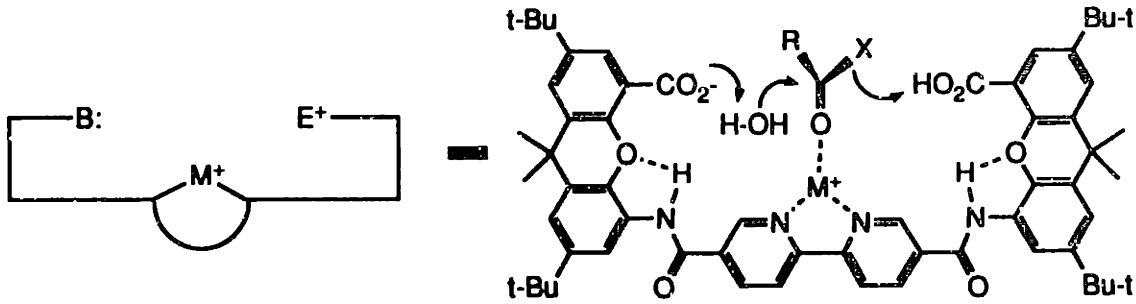
The above equation predicts that when $g_o \cdot Ka = 1$, host molecule extracts 0.5 equivalent of the guest in that medium. Note that with similar binding constants, the extractability is affected largely by limiting solubility of guest. When γ-ketopimelic acid was extracted, the urea peak shifted downfield 0.15 ppm, whereas upfield shift ocurred in all other cases. This result may be explained by hydrogen bonding of guest carbonyl to host urea NHs; otherwise it would be shifted upfield. The diamide cleft **109** seems to extract glutaric acid, but was not tried with the other guest diacids because the NMR of diamide cleft **109** was quite broad and complex, thus difficult to analyse.

5.2 Bipyridine Clefts

5.2.1 Bipyridine synthesis

2,2′-Bipyridine is one of the best metal chealating agents known.[97] Thus it was felt that an appropriately functionalized 2,2′-bipyridine could be used to build cleft-like molecules by incorporating U-turn building block such as Kemp's triacid or xanthene diacid. The advantage of cleft-like molecules having rigid spacers is that both acidic and basic component can coexist without collapsing upon one another. In an appropriate pH, the cleft will have three different convergent functional groups arranged in such a way that a protic acid, a base and Lewis acidic site create an active site for catalysis (Fig 41).

Fig 41

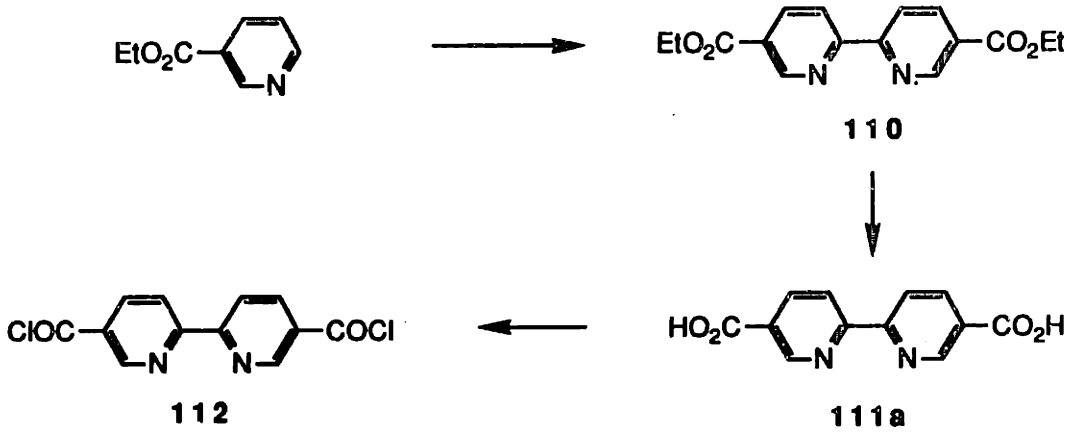


A metal chelated in the bipyridine site serves as Lewis acid, the carboxylate as general base, and the protic acid as a general acid. This type of arrangement might be exploited in hydrolysis of a carbonate or an ester.

2,2'-Bipyridine-4,4'-dicarboxylic acid is commercially available, but corresponding 5,5'-diacid is not. For building a binding site located inside cavity, 2,2'-bipyridine-5,5'-dicarboxylic acid seemed geometrically better than the 4,4'-diacid and thus needed to be synthesized.

The dimerization of substituted pyridine is well precedented,[98] the published method was followed for the required 5,5'-diacid. Diethyl 2,2'-bipyridine-5,5'-diacid **110** was obtained in 24 % yield by dimerization of ethyl nicotinate under heating (~130 ^{o}C) with Pd/C under vacuum (~14 mmHg) for 5 days (Scheme 19).

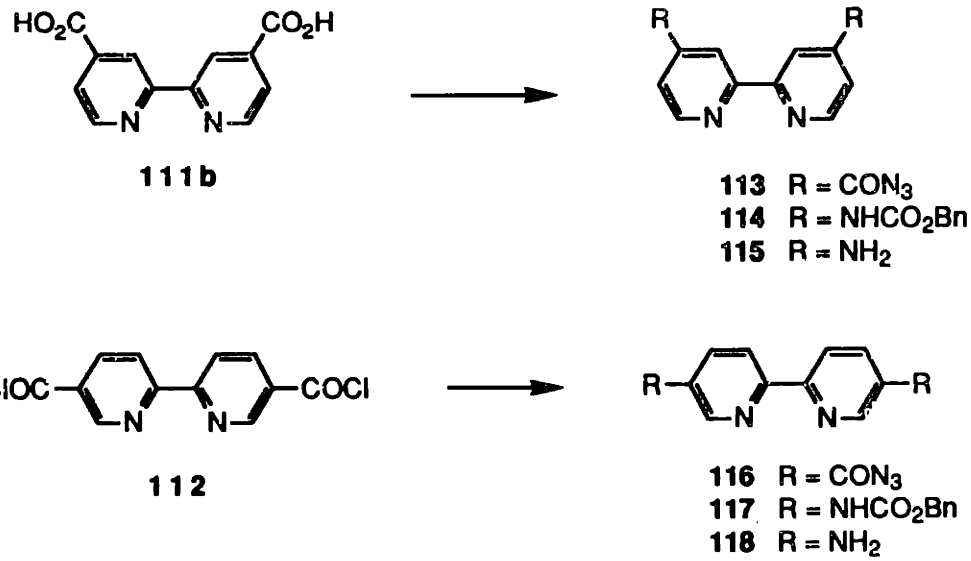
Scheme 19



Hydrolysis of **110** with KOH gave the bipyridine diacid **111a** in 81 % yield. Diacid **111a** is not soluble in most common organic solvents, but is soluble in aqueous acid and base. Transformation into diacid chloride **112** was achieved by heating in neat thionyl chloride for several hours.

In order to create a cleft shaped molecule using either a Kemp's triacid or a xanthene diacid, the degradation of the diacid **111a** was studied. As a model reaction, the commercial bipyridine diacid **111b** was subjected to Curtius degradation (Scheme 20).[99]

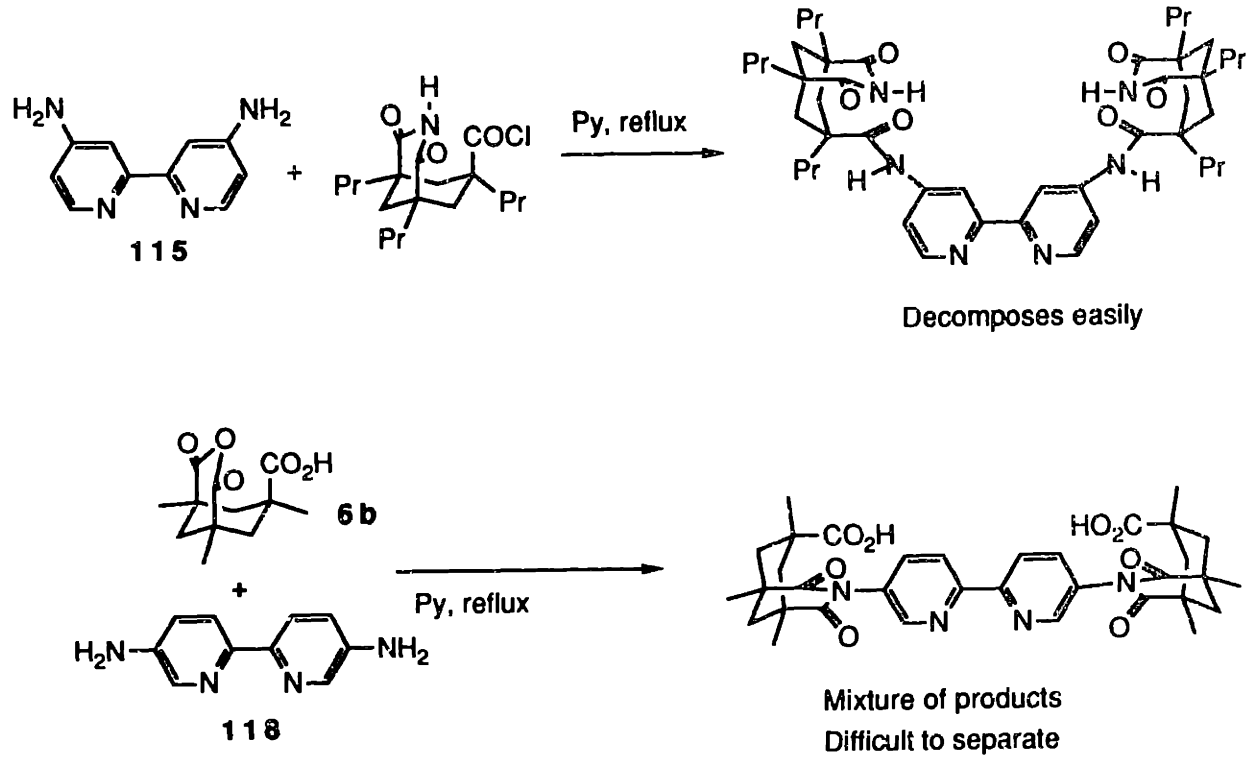
Scheme 20



The diacid chloride (obtained from **111b**) was suspended in acetone at 0 ^{o}C and treated with aqueous NaN_3. The acid azide **113** was filtered off and heated to reflux in toluene with slight exess of benzyl alcohol to give the dicarbamate **114**. Hydrogenolysis of the carbamate **114** in refluxing ethanol with Pd/C and 1,4-cyclohexadiene[100] then gave 4,4′-diamino-2,2′-bipyridine **115** in >70 % overall yield. The same sequence could be applied to the 5,5′-dicarboxylic acid chloride **112** with success; the overall yield of **118** was almost same as that of 4,4′-diamine **115** case. In both cases, rearrangement followed by transient protection-deprotection turned out to be excellent in both yield and purification. Direct rearrangement-hydrolysis often gives poor yield and is difficult because separation from side products such as ureas is problematic.

Unfortunately, when this diamine **115** was condensed with tripropylimide acid chloride (Scheme 21), there seems to be some desired product (nmr from crude), but it was found to be fairly unstable to aqueous workup and silica gel chromatography (from two separate attempts to do so).

Scheme 21

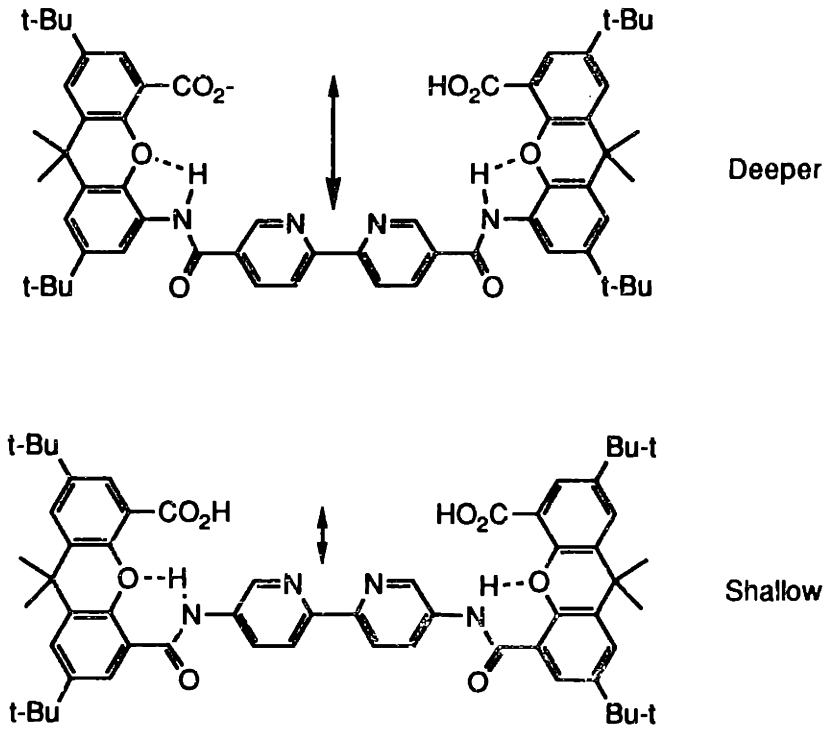


The diamine **118** was also condensed with the anhydride acid **6b** in refluxing pyridine but this resulted in mixture of products (by nmr of crude) which proved difficult to separate by crystallization or trituration.

5.2.2 Xanthene degradation

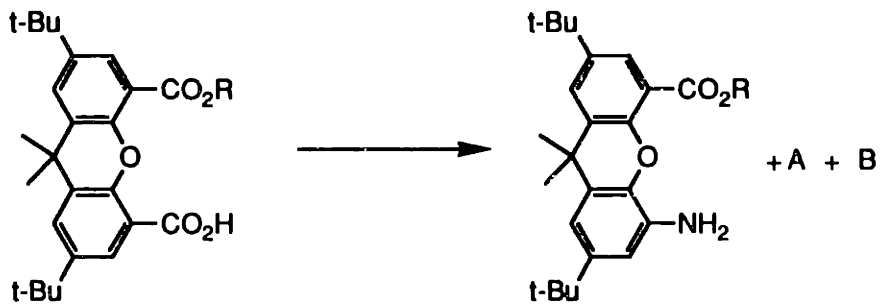
Attachment of xanthene diacid to the bipyridine **118** gives a cleftlike molecule. However, direct condensation of monofunctionalized xanthene with bipyridine diamine **118** results in a cleft which has shallow binding site, whereas the "reversed" amide gives a deeper cleft (Fig 42).

Fig 42



To attach the xanthene unit to the bipyridine diacid chloride **112**, one of the xanthene acid functional group must be converted to amino group. Thus xanthene monoacid monoester was subjected to classical Curtius degradation[99] by treatment with sodium azide followed by heating and hydrolysis (Scheme 22).

Scheme 22



97 R =Me
97b R = Bn

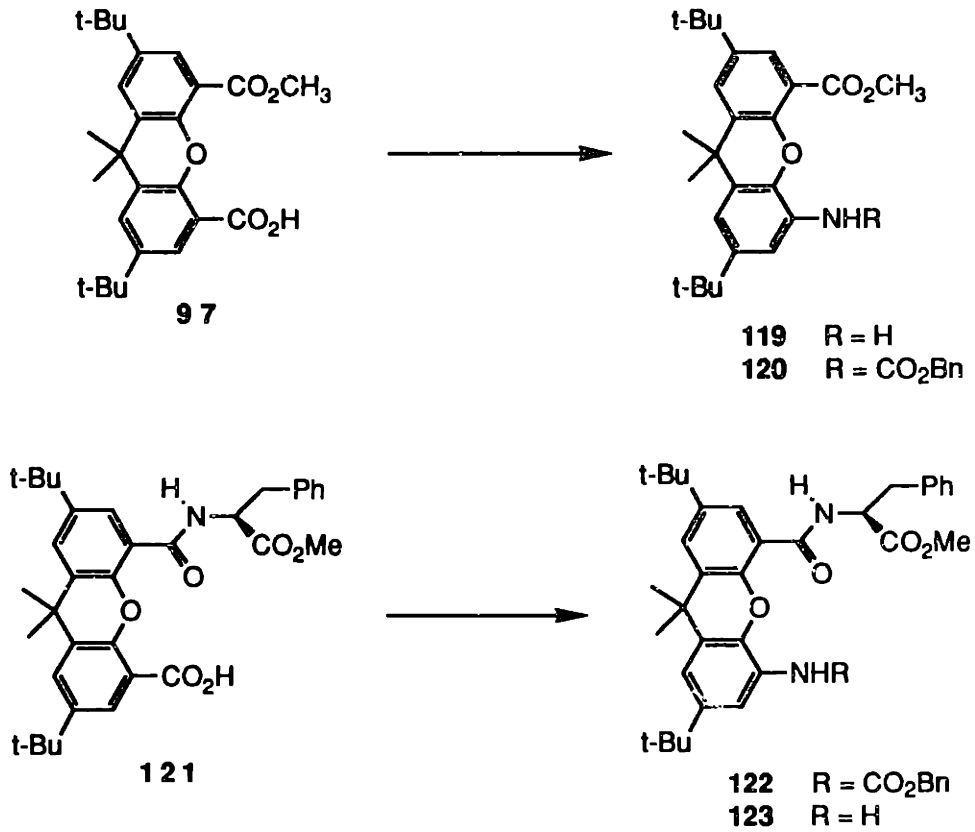
119 R = Me
119b R = Bn

This sequence resulted in three products; A(highest spot) : B(middle spot) : C(lowest spot) =49 : 157 : 199 (mass ratio). The material C turned out to be the desired amine; but the structures of byproducts A and B could not be assigned. According to ^{1}H and ^{13}C nmr spectra (see experimental section), both A and B are symmetric. One of them seems to be a symmetrical urea but could not be distinguished which is which.

Byproducts formation seems to be common in all three reactions tried (once with **97b** and the other two times with **97**) and seems to be due to the hydrophobic nature of xanthene in this case; during hydrolysis in water (or aqueous THF) the reactant stayed as oiiy droplets (mixture of amine + isocyanate), thus resulting in side reactions. However, this problem was nicely solved by employing diphenylphosphoryl azide (DPPA)[101] as reactant (Scheme 23).

Simple heating of monoacid **97** with DPPA and triethylamine (TEA) in toluene followed by benzylalcohol gave 56-70% of benzylcarbamate **120**. This could be easily hydrogenolized to the aminoxanthene **119** in quantitative yield. Heating the carboxylic acid with DPPA, TEA, benzyl alcohol altogether in toluene gave even higher yields. For example, the acid **121** could be degraded to the benzylcarbamate **122** in 97 % yield by this method.

Scheme 23

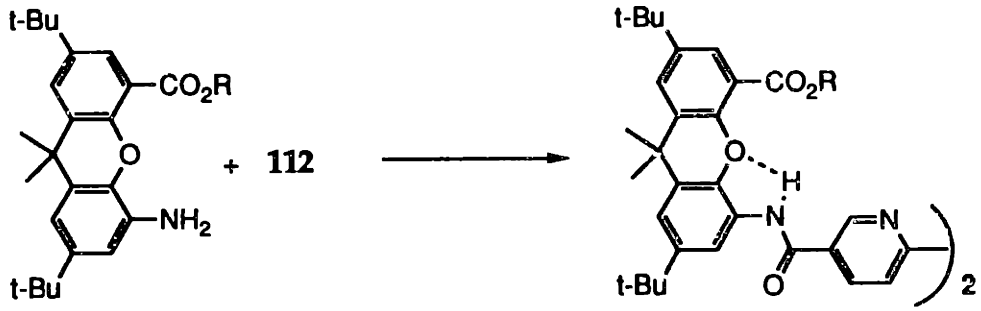


The benzyl carbamate **122** in this case was relatively unreactive toward hydrogenolysis. Aminoxanthene amide **123** could be obtained in only 76 % yield by prolonged heating of **122** in ethanol with quantities of paladium catalyst and 1,4-cyclohexadiene.

5.2.3 Synthesis of Bipyridine Clefts.

With both bipyridine acid chloride and aminoxanthene available, cleft synthesis could be started. Reaction of **119** and **119a** with bipyridine diacid chloride **112** gave the bipyridine cleft esters **124** and **125**, respectively (Scheme 24).

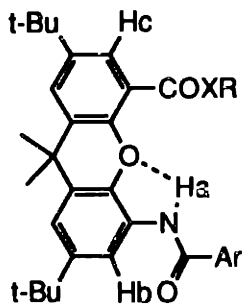
Scheme 24



119 R = Me
119a R = Bn

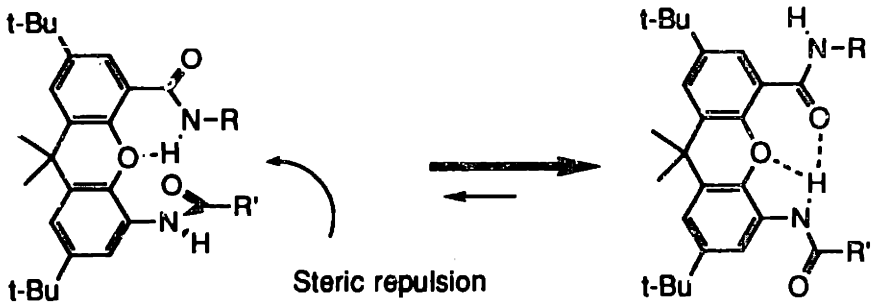
124 R = Me
125 R = Bn
126 R = H

Examination of amide NH chemical shift sugested that this amide proton is hydrogen bonded (via a five membered ring) to xanthene oxygen. In such cases, the xanthene proton next to amide is expected to be deshielded, and this was observed in all of the compounds prepared.



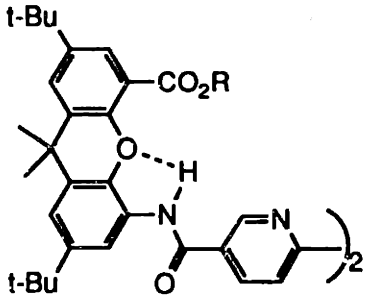
Amide proton Ha resonates 9.52-9.81 ppm and xanthene proton Hb 8.5-8.8 ppm, which is considerably deshielded than proton Hc. Even in cases where two amides are present, the five membered ring hydrogen bonding seems predominant in solution. This is perhaps due to the absence of steric repulsion in 5-membered ring hydrogen bonded structure (Fig 43).

Fig 43

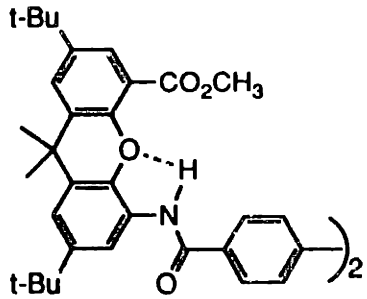


To verify this, these amide were crystalized, but it was difficult to obtain X-ray quality crystals. Several different molecules (Fig 44) were synthesized in attempt to obtain crystals but proved to be unfruitful. All of these compounds are microcrystalline.

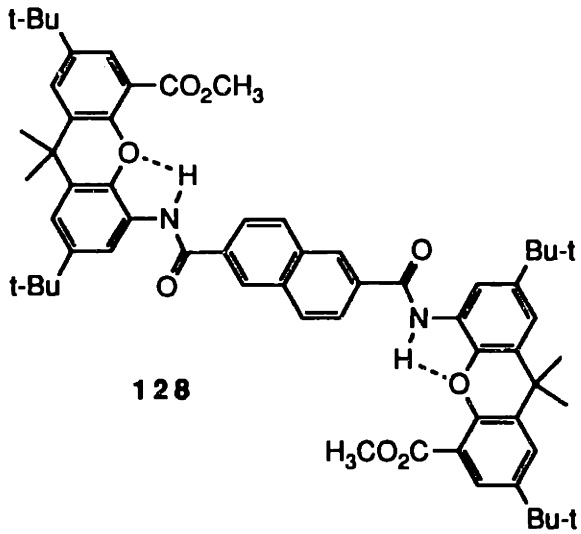
Fig 44



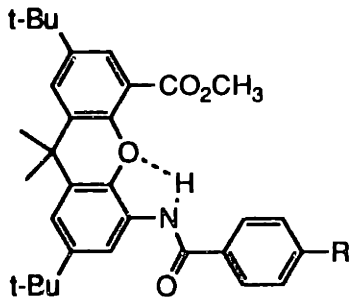
124 R = Me
125 R = Bn



127



128



129a R = H
129b R = Br

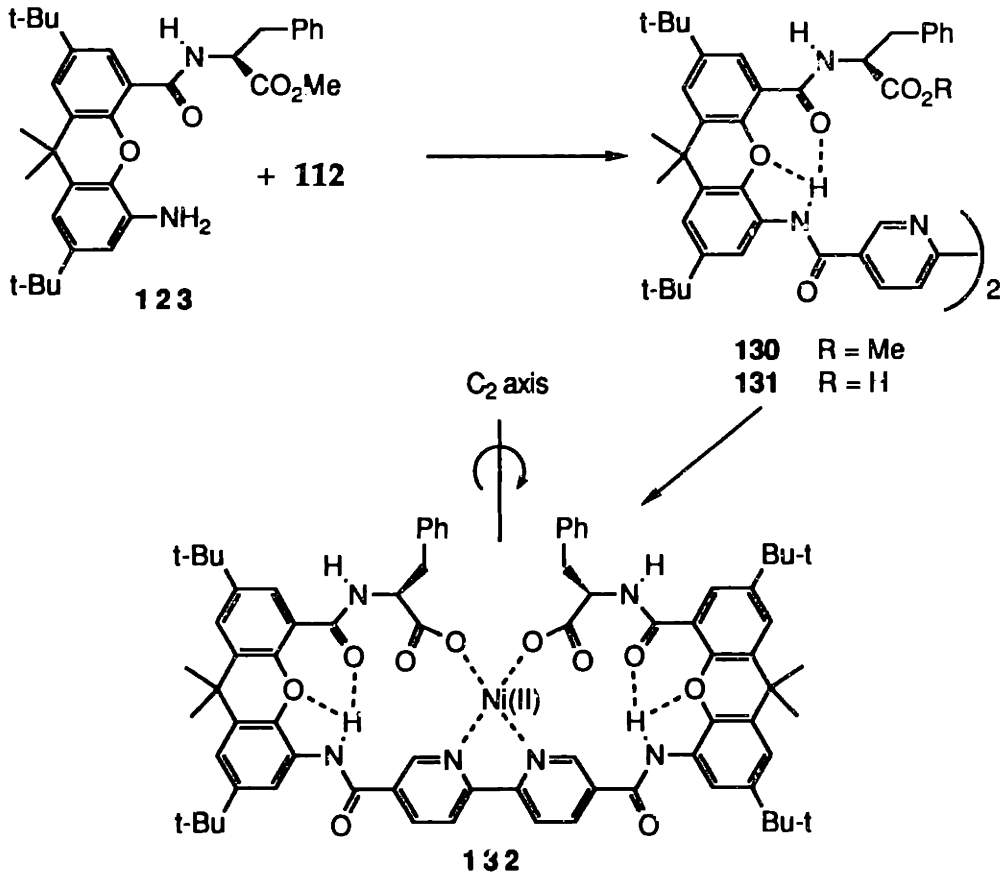
The next problem encountered was the hydrolysis of **124** to **126**. Attempts to hydrolyse with NaOH in MeOH (with or without THF), with HBr, with TMSI[102] (refluxing) in CH_2Cl_2, with NaOH - n-Bu_4NF in CH_2Cl_2-water all failed. To circumvent this difficulty, the corresponding benzyl ester series was prepared. Surprizingly, benzyl ester cleft **125** was resistant to hydrogenolysis. However, these problems (hydrolysis of cleft **124** and **125**) were solved with n-Bu_4NOH.

Even though there are two electron withdrawing substituents in the bipyridine unit, the ester **124** was found to bind metals. For example, a solution of **124** in $CDCl_3$ was treated with excess anhydrous $ZnCl_2$, stirred,

filtered, and the filtrate analysed by ^{1}H-NMR. All of the bipyridine protons were found to be deshielded (see experimental section). The acid cleft **126** also found to bind with $Zn(BF_4)_2$ in the bipyridine site, but not in the carboxylate position (mass spectroscopy).[103] The stoichiometry of the Zn complex is currently unknown, however.

A C2 symmetric tetradentate molecule can also be assembled from xanthene and bipyridine. Condensation of **123** with **112** gave chiral cleft **130**, which was hydrolysed with n-Bu_4NOH to give the acid cleft **131** (Scheme 25).

Scheme 25



By refluxing **131** with one equivalent of $Ni(OAc)_2 \cdot 4H_2O$ in ethanol greenish-yellow solution was obtained. This was concentrated, dissolved in CH_2Cl_2

(soluble, homogenious), filtered and concentrated to give **132**, whose structure is not yet established.

Since the cavity in **131** is chiral, extraction experiments were performed with phenylalanine, but the acid **131** did not extract phenylalanine at all (liq-liq, sol-liq). Metallic complexes like **132** may be useful for assymetric synthesis such as epoxidation or cyclopropanation. Studies along these lines are in progress within the group.

Experimentals

General

Melting points were determined by using either a Thomas-Hoover capillary melting point apparatus or Electrothermal series IA9100 digital melting point apparatus, and are uncorrected. Infrared spectra were obtained using a Mattson Cygnus 100 FT-IR spectrometer. ^{1}H-NMR spectra were measured with Bruker WM-250 (250 MHz) AC-250 (250 MHz), AM-300 (300 MHz), AM-500 (500 MHz)and GE-300 (300 MHz) spectrometer. Chemical shifts are reported in part per million (δ) relative to the residual nmr solvent peak: $CDCl_3$ (7.26 ppm), CD_2Cl_2 (5.29 ppm), CD_3OD (3.31 ppm), D_2O (4.80 ppm), DMSO-d_6 (2.49 ppm), acetone-d_6 (2.03 ppm), acetonitrile-d_3 (1.93 ppm), DMF-d_7 (2.91 ppm), pyridine-d_5 (8.71 ppm). Mass spectra were obtained on a VG instruments SE 20 (low resolution) and on a Finnegan MAT 8200 instrument (High resoluton). Elemental analyses were performed by Galbraith Laboratories Knoxville Tenn.

Materials

All commercial materials were used without further purification unless otherwise mentioned. The following solvents were dried and distilled using the following methods; ether and THF from sodium-benzophenone, CH_2Cl_2 and pyridine from CaH_2, and ethanol from Mg turnings. Anhydrous DMF was purchased from Aldrich as packed in Sure-Seal bottle. Reactions that require anhydrous and/or anaerobic condition were carried out under

nitrogen or argon atmosphere. Crude reaction products were purified by flash chromatography[104] with silica gel (Merck 9385-9, particle size 0.040-0.063 mm).

Experimentals for chapter 1

1,3,5-hexanetricarboxylic acid 15a

Trimesic acid (250 g, 1.19 mole) and palladium on carbon (10 g, 5 % Pd) were placed in a bomb hydrogenator. Water (250 mL) was added. The mixture were pressurerized to 800 psi and heated to 160 ^{o}C with efficient stirring. The hydrogen pressure was maintained to 1200 psi until no more hydrogen was taken up. The system was cooled and the pressure was relieved. The reaction was filtered through Celite, and the filterate concentrated and dried in vacuum to give 258 g (100 %) of white solid. This crude product was used directly in next step.

Trimethyl 1,3,5-hexanetricarboxylate 15b

In a 3 L 3-necked round bottomed flask equipped with air driven stirrer, $CaCl_2$ drying tube and 500 mL pressure equalizing addition funnel were placed hexahydro trimesic acid (**15a**) (270 g, 1.25 mol) and absolute grade methanol (1.5 L). The mixture was cooled with an ice bath and thionyl chloride (400 mL, 5.5 mol. 1.8 equiv) was slowly added (2 h). The reaction was allowed to warm to room temperature (2 h), heated to reflux overnight, cooled , and concentrated in vacuo. The residue was dissolved in 1.5 L of ether, washed with water, $NaHCO_3$ solution and brine, dried ($MgSO_4$) and concentrated.

Simple distilation under reduced pressure afforded 270 g (84 %) of triester **15b** as mixture of stereoisomers.

Kemp's triacid trimethyl ester 15c

In a 5L 3-necked round bottomed flask equipped with septum, pressure equalizing addition funnel (500 mL) and gas inlet tube were placed diisopropylamine (155 mL, distilled over CaH_2) and toluene (1600 ml, distilled over sodium/benzophenone) under argon atmosphere. The mixture was cooled with ice bath and n-BuLi (100 mL, 10 M) was added via cannula over a 1 h period. After 30 min, the triester **15b** (83 g, 0.32 mol, diluted to 500 mL with dry toluene) was added over a 70 min period and the mixture stirred additional 30 min. Dimethyl sulfate (118 mL, 3.87 mole equiv, in stoppered graduated cylinder under argon) was added via cannula over 20 min. The reaction was stirred for 2 h while allowing to warm up to room temperature, and quenched by addition of 1N HCl (1000 mL). Layers were separated and toluene layer was washed with 1N HCl (1000 mL), $NaHCO_3$ (500 mL) and sat'd NaCl (500 mL). Aqueous layer was extracted with ethyl acetate (1000 and 500 mL), washed with 1N HCl (500 mL), $NaHCO_3$ (500 mL), brine (500 mL). Combined organic layer was dried ($MgSO_4$) and concentrated. Vacuum distillation gave 75.23 g (78 %) of crude product as mixture of cis, cis and cis,trans isomer plus some dialkylated product (> 90 % cis, cis).

mp (cis,cis); 80-81 °C

^{1}H-NMR ($CDCl_3$, 300 MHz) of **15c**; δ 3.66 (s, 9 H), 2.74 (d, J = 14.3 Hz, 3 H), 1.21 (s, 9 H), 0.97 (d, J = 14.4 Hz, 3 H)

Kemp's triacid (6)

Distilled triester **15c** (78.3 g, 0.26 mol) was refluxed in NaOH (50 g, 1.25 mol) in 1:1 methanol-water (600 mL) for 13 h. Methanol was removed and the solution was acidified to ca pH=1 with conc HCl. The white precipitate was filtered, washed with 1N HCl (100 mL) and dried in vacuum to give 48 g (71 %) of pure cis,cis Kemp's triacid (**6**).

mp; 241-245 °C

IR (KBr); 3200-2200, 1690 and 1290 cm^{-1}.

^{1}H-NMR (pyridine-d_5, 300 MHz); δ 3.40 (d, J = 14.6 Hz, 3 H), 1.56 (d, J = 14.6 Hz, 3 H), 1.54 (s, 9H).

Imide acid 14a

Kemp's triacid (**6**) (10.00 g, 38.7 mmol) and urea (5.00 g) in triglyme (70 mL) was heated to ca 200 °C for 2 h. After cooling, 10% HCl (100 mL) was added, stirred and filtered. The solid was washed with 10 % HCl (50 mL) and with 1:1 ether-hexanes (100 mL), and dried in vacuum to give 8.174 g (88 %) of imide acid **14a**.

mp >300 °C

IR (KBr); 3300-2500, 1730 and 1717 cm^{-1}.

^{1}H-NMR ($CDCl_3$, 300 MHz); δ 10.48 (br s, 1 H), 2.71 (d, J = 13.2 Hz, 2 H), 1.97 (d, J = 13.3 Hz, 1 H), 1.36 (d, J = 13.3 Hz, 1 H), 1.26 (s, 3 H), 1.24 (s, 6 H), 1.15 (d, J = 14.2 Hz, 2 H).

Anhydride acid 14b

Kemp's triacid **6** was sublimed under vacuum to give **14b** (quantitative).

mp; 252-254 °C.

IR (KBr); 3300-3100 (br), 2968, 2874, 1794, 1770 and 1702 cm-1.

^{1}H-NMR (pyridine-d_5, 300 MHz); δ 2.95 (d, J = 14.1 Hz, 2 H), 2.05 (d, J = 13.2 Hz, 1 H), 1.41-1.21 (m, 12 H)

Imide acid chloride 14c

Imide acid (2.00 g, 8.34 mmol) in CH_2Cl_2 (50 mL) was treated with thionyl chloride (7 mL) and one drop of DMF and heated to reflux for 4 h. The cloudy solution was filtered while hot, and the filtrate was concentrated to about 5 mL. Fresh hexanes (50 mL) was added and concentrated to give 2.04 (95 %) of white solid.

mp; 182-184 °C (toluene).

IR (KBr); 1780, 1721 and 1696 cm^{-1}.

^{1}H-NMR ($CDCl_3$, 500 MHz); δ 7.65 (br s, 1 H), 2.75 (d, J = 13.9 Hz, 2 H), 2.02 (d, J = 13.4 Hz, 1 H), 1.39 (d, J = 13.4 Hz, 1 H), 1.35 (s, 3 H), 1.32 (d, J = 14.7 Hz, 2 H), 1.27 (s, 6 H).

Anhydride acid chloride 14d

Kemp's triacid in thionyl chloride was refluxed for several hours. Analytical sample was obtained by recrystalization from toluene.

^{1}H-NMR ($CDCl_3$, 300 MHz); δ 2.85 (d, J = 13.9 Hz, 2 H), 2.08 (d, J = 13.6 Hz, 1 H), 1.38-1.44 (m, 12 H).

N-benzylimide acid 22

Anhydride acid **14b** (245 mg, 1.02 mmol) dissolved in dry pyridine (10 mL) was treated with benzylamine (150 μL, 1.35 equiv.) and heated to reflux overnight. Pyridine was removed in vacuo and the resulting residue was dissolved in dilute hydrochloric acid (1N, 15 mL) and extracted with EtOAc (3 x 20 mL). Combined extracts were washed with brine containing dilute HCl then dried with Na_2SO_4. Concentration gave **22** as a white solid (332 mg, 99 %). (Reaction of Kemp's triacid (**6**) with benzylamine under similar condition gives the same result.)

mp; 225-226 °C.
IR (KBr); 2972, 1732, 1702, 1462 cm^{-1}.
^{1}H-NMR ($CDCl_3$, 500 MHz); δ 1.19 (d, J = 14.3 Hz, 2 H), 1.24 (s, 3 H), 1.27 (s, 6 H), 1.36 (d, J = 13.2 Hz, 1 H), 1.93 (d, J = 13.3 Hz, 1 H), 2.67 (d, J = 13.4 Hz, 2 H), 4.71 (s, 2 H), 7.23-7.40 (m, 5 H).
HRMS calcd for $C_{19}H_{23}NO_4$: 329.1627, obsd:329.1627.

N-benzylimide amide 24

Imide-acid **22** (2.3 g, 6.8 mmol) was refluxed overnight in $SOCl_2$ (15 mL). Excess $SOCl_2$ was removed under vacuum and the residue was taken up in dry THF (25 mL). Ammonia was bubbled through the solution for 30 min

then discontinued while the reaction was allowed to stir 1 hr at room temperature. The reaction was concentrated to dryness and the residue extracted with hot EtOAc (3 x 50 mL). The combined organic extracts were concentrated to give the imide amide **24** in good yield which was used without further purification.

mp; 225-227 °C.

IR (KBr): 3465, 3364, 2968, 1725, 1669 and 1602 cm^{-1}.

^{1}H-NMR (500 MHz, $CDCl_3$); δ 7.33-7.35 (m, o-Ar), 7.20-7.29 (m, p-Ar), 5.38 (br s, NH), 4.95 (br s, NH), 4.75 (s, 2 H), 2.52 (d, J = 13.5 Hz, 2 H), 1.93 (d, J = 13.5 Hz, 1 H), 1.37 (d, J = 13.0 Hz, 2 H), 1.27 (s, 6 H), 1.25 (d, J = 17.0 Hz, 1 H), 1.22 (s, 3 H).

Anal. calcd for $C_{19}H_{24}N_2O_3$: C, 69.49; H, 7.37, found: C, 69.75; H, 7.48.

HRMS calcd for $C_{19}H_{24}N_2O_3$: 328.1787, obsd: 328.1786.

N-benzylamine primary amine 21

To a solution of N-benzylimide amide **24** (3.0 g, 9.15 mmol) in dry THF (100 mL) at room temperature was added slowly 0.5 M solution of AlH_3 in THF (175 mL). The reaction was heated to reflux for 45 hr, cooled, hydrolyzed with H_2O (10 mL), and then filtered. After concentration, the crude material was purified by flash chromatography with silica gel (30 % CH_3OH in CH_2Cl_2) to give 1.97 g (75 %) of colorless liquid.

IR (neat); 3300 (br), 3028, 2947, 2796, 2753, 1453, 1365, 748, 699 cm^{-1}.

^{1}H-NMR ($CDCl_3$, 250 MHz); δ 0.75-0.85 (d, 1 H), 0.80 (s, 6 H) , 0.88 (s, 3 H), 0.96-1.02 (d, J = 14.2 Hz, 2 H), 1.30-1.60 (m, 5 H), 2.33-2.38 (d, J = 11 Hz, 2 H), 2.75 (s, 2 H), 3.34 (s, 2 H), 7.20-7.28 (m, 5 H).

HRMS calcd for $C_{19}H_{30}N_2$: 286.2409, obsd: 286.2409.

Di-N-benzylamine diamide 27c

To a solution of 3,6-dimethoxy naphthalene-2,7-diacid chloride (473 mg, 1.51 mmol) and dry triethylamine (2 mL) in dry CH_2Cl_2 (10 mL) was added the diamine **21** (875 mg, 3.05 mmol) in dry CH_2Cl_2 (35 mL) with stirring. After 22 h, the reaction was diluted to 100 mL with CH_2Cl_2 and washed with sat'd $NaHCO_3$ (2 x 50 mL). The combined aqueous layers were extracted with CH_2Cl_2 (50 mL), which was further washed with satd $NaHCO_3$ (50 mL). The combined organic layers were dried ($MgSO_4$) and concentrated. Flash chromatography on silica gel (20 % EtOAc in CH_2Cl_2) gave **27c** as a white crystalline solid (870 mg, 71 %).

mp; 189-191 °C.

IR (KBr); 3408,3129, 2947, 2841, 1654, 1617., 1534, 1457,1411, 1219 cm^{-1}.

^{1}H-NMR ($CDCl_3$, 270 MHz); δ 0.86-0.97 (m, 14 H), 1.04 (s, 6 H), 1.10-1.35 (m, 6 H), 1.60 (d, J = 11 Hz, 4 H), 1.64 (d, J = 11.5 Hz, 4 H), 2.50 (d, J = 10.6 Hz, 4 H), 3.40 (s, 4 H), 3.90-4.00 (m, 10 H), 7.05-7.25 (m, 12 H), 7.85 (br, 2 H), 8.80 (s, 2 H).

Diamine diamide 28c

Dibenzylamine diamide **27c** (385 mg, 0.47 mmol) in acetic acid (20 mL) was treated with $Pd(OH)_2$-C (408 mg) and shaken for 4 days under hydrogen atmosphere (60 psi). After filtration and evaporation, the crude acetate salt was dissolved in CH_2Cl_2 (150 mL) and washed with sat'd $NaHCO_3$ (2 x 50 mL), dried (Na_2SO_4) and concentrated. Passing the residue through a short

silica column (10-30 % MeOH in CH_2Cl_2) gave 260 mg (87%) of **28c** as a white solid.

mp; 220-221 °C.

IR (KBr); 3393, 2946, 2920, 2863, 1649, 1543, 1463, 1221 cm^{-1}.

^{1}H-NMR ($CDCl_3$, 250 MHz); δ 0.86 (s, 12 H), 0.91-0.96 (d, J = 12 Hz, 2 H), 1.10 (s, 6 H), 1.21-1.26 (d, J = 13.5 Hz, 4 H), 1.46-1.51 (d, J = 11.9 Hz, 2 H), 1.54-1.60 (d, J = 15 Hz, 4 H), 2.24-2.28 (d, J = 10.5 Hz, 4 H), 2.53-2.57 (d, J = 10.5 Hz, 4 H), 3.51-3.53 (d, J = 5.0 Hz, 4 H), 4.04 (s, 6 H), 7.10 (s, 2 H), 8.52 (s, 2 H), 8.67 (br, 2 H).

Extraction experiment.

A solution of the diamine cleft **28c** in $CDCl_3$ (8 mM) was treated with excess diacid (maleic, succinic, phthalic and isophthalic) and vibramixed for 2-3 h at room temperature. The solution was filtered through Millex GV_4 filtertip and the filtrate analysed by NMR with peak integration. In cases when the complex is not soluble (**28c** with fumaric or terephthalic), one equivalent of the diacid was treated with excess **28c**, shaken and filtered. The filter residue was dissolved in CD_3OD and analysed with NMRwith integration of appropriate. Selective extraction was performed by treating **28c** with excess amount of two diacids (1:1 mole ratio), filtered and analysed. In all diacid combinations, only one component was found in the solution except succinic-isophthalic acid pair, where some isophthalic acid were found in solution.

Experimentals for Chapter 2

N-β naphthylimide-acid 33b

Anhydride acid chloride **14d** (0.9 g, 3.5 mmol), β-naphthylamine (500 mg, 3.5 mmol), and pyridine (290 µl, 3.6 mmol) were dissolved in CH_2Cl_2 and stirred at room temperature for 2 h. The mixture was diluted with pyridine (10 mL) and refluxed overnight. The pyridine was removed under vacuum and the residue was treated with 10 % HCl (5 mL). The precipitate was filtered yielding the imide acid **33b** (1.1 g) in 85 % yield.

mp; 250 °C(dec)

IR (2 % KBr); 3445, 3146, 1730, 1714, and 1678 cm^{-1}.

^{1}H-NMR ($CDCl_3$); δ 7.75-7.84 (m, 4 H-Ar), 7.39-7.43 (m, 3 H- Ar), 2.73 (d, J = 13.8 Hz, 2 H), 2.17 (d, J = 13.8 Hz, 1 H), 1.49 (d, J = 13.2 Hz, 1 H), 1.35 (s, 6 H), 1.20 (d, J = 12.6 Hz, 2 H), 1.18 (s, 3 H).

naphthylimide amide 34b

Imide acid **33b** (0.9 g, 2.5 mmol) was refluxed overnight in $SOCl_2$ (10 mL). Excess $SOCl_2$ was removed under vacuum and the residue was taken up in dry THF (10 mL). Ammonia was bubbled through the solution for 30 min then discontinued while the reaction was allowed to stir 1 hr at room temperature. The reaction was concentrated to dryness and the residue extracted with hot $CHCl_3$ (2 x 50). The combined organic extracts were concentrated to give the imide-amide **34b** (1 g) in quantitative yield, which was used without further purification.

IR (2 % KBr); 3480, 1728, 1680, and 1192 cm^{-1}.

^{1}H-NMR ($CDCl_3$); δ 7.83-7.86 (m, 4 H-Ar), 7.44-7.49 (m, 3 H- Ar), 5.28 (br s, NH_2), 2.56 (d, J = 14.1 Hz, 2 H), 2.18 (d, J = 13.2 Hz, 1 H), 1.49 (d, J = 13.2 Hz, 1 H), 1.35 (s, 6 H), 1.28 (d, J = 12.6 Hz, 2 H), 1.20 (s, 3 H).

Naphthylimide nitrile 35b

To a suspension of amide **34b** (0.9 g, 2.5 mmol) and pyridine (490 μL, 6.0 mmol) in THF (10 mL) was added dropwise TFAA (425 μL, 3.0 mmol) at room temperature. After 2 h the mixture became homogeneous, at which time it was diluted with 10 % HCl (60 mL) and EtOAc (100 mL). The organic and aqueous layers were separated and the aqueous layer extracted with EtOAc (3 x 40 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to give imide nitrile **35b** in quantitative yield.

mp; 232-233 °C.
IR (2 % KBr); 2231, 1732, and 1682 cm^{-1}.
^{1}H-NMR ($CDCl_3$); δ 7.80-7.90 (m, 4 H-Ar), 7.44-7.49 (m, 3 H- Ar), 2.50 (d, J = 13.5 Hz, 2 H), 2.30 (d, J = 13.2 Hz, 1 H), 1.54 (s, 3 H), 1.51 (d, J = 13.2 Hz, 1 H), 1.41 (d, J = 14.4 Hz, 2 H), 1.39 (s, 3 H).
Anal. calcd for $C_{22}H_{22}N_2O_2$: C, 76.28; H, 6.39. found: C, 75.95; H, 6.52.

Naphthylamide 36b

Potassium amide was prepared by dissolving potassium metal (225 mg, 5.8 mmol) in liquid ammonia (25 mL) and adding a small amount of $FeCl_3$. At this point the solution turned from blue to grey. The nitrile (250 mg, 0.72 mmol) is added as a slurry in THF (10 mL). The reaction was stirred at reflux

for 3 h before quenching with NH_4Cl. The ammonia was allowed to boil off and the residue was taken up in aqueous acid, then washed with CH_2Cl_2 (2 x 60 mL). The aqueous layer was then made basic (pH = 12) causing the product to precipitate. Filtration gave 217 mg of crude material which could be further purifed by recrystallization from $CHCl_3$.

mp; 266-268 °C.

IR (2 % KBr); 3333, 3192, 1682, 1603, and 1540 cm^{-1}.

^1H-NMR (300 MHz, CD_3OD); δ 8.06 (s, NH), 7.75 (d, 1-Ar, J = 8.4 Hz), 7.51 (dd, 2 Ar, J = 2.1 and 8.7 Hz), 7.40 (m, 4-Ar), 2.76 (d, J = 14.4 Hz, 2 H), 1.83 (d, J = 13.0 Hz, 1 H), 1.20-1.53 (m, 3 H), 1.30 (s, 3 H), 1.28 (s, 3 H), 1.19 (s, 3 H).

^1H-NMR (300 MHz, $CDCl_3$); δ 8.15 (s, NH), 7.72 (m, 3 Ar), 7.38-7.46 (m, 4 Ar), 2.71 (d, J = 14 Hz, 1 H), 2.53 (d, J = 14 Hz, 1 H), 1.36-1.58 (m, 3 H), 1.32 (s, CH_3), 1.27 (s, CH_3), 1.22 (s, CH_3).

HRMS calcd for $C_{22}H_{25}N_3O_2$: 363.147. found: 363.1946.

Phenylimide acid 33a

Kemp's triacid (**6**) (2.0 g, 7.75 mmol) and aniline (720 µL, 7,75 mmol) were refluxed overnight in pyridine (10 mL). The pyridine was removed under vacuum and the residue was partitioned between 10 % HCl (40 mL) and EtOAc (50 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 x 40 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to give imide acid **33a** which was used without further purification (2.07 g, 82 % yield).

mp; 251-252 °C.

IR (2 % KBr); 3196, 2980, 1730, 1709, and 1678 cm^{-1}.

^{1}H-NMR (300 MHz, $CDCl_3$); δ 7.29-7.39 (m, m and p-Ar), 7.05-7.08 (m, o-Ar), 2.82 (d, J = 13.5 Hz, 2 H), 2.14 (d, J = 13.5 Hz, 1 H), 1.49 (d, J = 13.5 Hz, 1 H), 1.34 (s, 6 H), 1.33 (s, 3 H), 1.28 (d, J = 14.4 Hz, 2 H).

Anal calcd for $C_{18}H_{21}NO_4$: C, 68.40; H, 6.90. found: C, 68.55; H, 6.71.

Phenylimide amide 34a

Imide acid **33a** (2.0 g, 6.3 mmol) was refluxed for 5 h in $SOCl_2$ (15 mL). Excess $SOCl_2$ was removed under vacuum and the residue was taken up in dry THF (30 mL). Ammonia was bubbled through the solution for 30 min then discontinued while the reaction was allowed to stir overnight at RT. The mixture was diluted with H_2O (50 mL) and EtOAc (60 mL) and the organic and aqueous layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 x 40 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to give the imide amide (1.4 g, 71 % yield) which was used without further purification.

mp; 273-275 °C

IR (2 % KBr); 3404, 3200, 1732, and 1684 cm^{-1}.

^{1}H-NMR (300 MHz, $CDCl_3$); δ 7.34-7.43 (m, m, p-Ar), 7.20 (br s, o-Ar), 5.55 (br s, NH), 5.23 (br s, NH), 2.68 (d, J = 13.9 Hz, 2 H), 2.17 (d, J = 13.2 Hz, 1 H), 1.50 (d, J = 13.2 Hz, 1 H), 1.36 (s, 6 H), 1.35 (d, J = 14.9 Hz, 2 H), 1.31 (s, 3 H).

Anal calcd for $C_{18}H_{22}N_2O_3$: C, 68.53; H, 7.01. found: C, 68.77; H, 7.05.

Phenylimide nitrile 35a

To a suspension of amide **34a** (1.1 g, 3.5 mmol) and pyridine (550 μL, 7.2 mmol) in THF (25 mL) was added dropwise TFAA (500 μL, 3.5 mmol) at room temperature. After 2 h the mixture became homogeneous at which time it was diluted with 10 % HCl (50 mL) and EtOAc (70 mL). The organic and aqueous layers were separated and the aqueous layer extracted with EtOAc (3 x 40 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to give imide nitrile **35a** (860 mg, 83 % yield).

mp; 206-208 °C.

IR (2 % KBr); 2971, 2227, 1731, and 1682 cm-1.

^{1}H-NMR (300 MHz, $CDCl_3$); δ 7.34-7.46 (m, o, m, p-Ar), 2.47 (d, J = 14.9 Hz, 2 H), 2.26 (d, J = 13.2 Hz, 1 H), 1.52 (s, 3 H), 1.49 (d, J = 13.2 Hz, 1 H), 1.41 (d, J = 14.9 Hz, 2H), 1.38 (s, 6 H).

Anal calcd for $C_{18}H_{20}N_2O_2$: C, 72.95; H, 6.80. found: C, 72.47; H, 6.68.

Phenylamide amidine 36a

Potassium amide was prepared by dissolving potassium metal (800 mg, 20.7 mmol) in liquid ammonia (40 mL) and adding a small amount of $FeCl_3$. At this point the solution turns from blue to grey. The nitrile **35a** (770 mg, 2.58 mmol) is added as a slurry in THF (12 mL). The reaction was stirred at reflux for 3 h before quenching with NH_4Cl. The ammonia was allowed to boil off and the residue was partitioned between Et_2O (50 mL) and H_2O (50 mL). The aqueous layer was made acidic with 10 % HCl and extracted with Et_2O (2 x 25 mL). The aqueous layer was then made basic (pH = 12) with K_2CO_3 causing the product to precipitate. Filtration gave 762 mg of crude material (95 %

yield, ~95 % purity) which could be further purifed by recrystallization from $CHCl_3$.

mp; 276-278 °C.

IR (1 % KBr); 3338, 3175, 2961, 1676, 1599, and 1536 cm^{-1}.

1H-NMR ($CDCl_3$, 300 MHz); δ 7.47 (d, o-Ar, J = 6.9 Hz), 7.27 (dd, m-Ar, J = 7.0 Hz), 7.0 (t, p-Ar, J = 7 Hz,), 2.74 (d, J = 15.0 Hz, 1 H), 2.52 (d, J = 13.0 Hz, 1 H), 1.96 (d, J = 13.0 Hz, 1 H), 1.23-135 (m, 3 H and 3 CH_3).

HRMS calcd for $C_{18}H_{23}N_3O_2$: 313.1789. found 313.1789.

3,5-bis(triisopropylsilyl)-2-deoxycytosine 43

Cytosine (100 mg, 0.39 mmol) and imidazole (235 mg, 3.46 mmol) were dissolved in dry DMF (1 mL). Triisopropyl silyl chloride (337 μL, 1.57 mmol) was added neat and dropwise with stirring at room temperature. After stirring overnight the reaction mixture was diluted with H_2O (15 mL) and extracted with $CHCl_3$ (5 x 30 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated. Chromatography on SiO_2 eluting with 5 % MeOH/$CHCl_3$ gave material which was further recrystallized from $CHCl_3$/Hex to give 75 mg (36 % yield) of pure material.

mp; 193-194 °C.

IR (1 % KBr); 3327, 3107, 2944, 2866, 1723, and 1652 cm^{-1}.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 9.48 (br s, NH), 8.52 (br s, NH), 7.93 (d, 1 H, J = 7.5 Hz), 6.35 (d, 1H, J = 7.5 Hz), 6.20 (dd, OCHN, J = 6.8 and 6.8 Hz), 4.57 (ddd, J = 22 Hz, HCOSi), 4.03 (d, J = 2.3 Hz, C<u>H</u>CH_2), 3.93 (dd, J = 11.5 and 2.7 Hz, C<u>H</u>HO),

3.87 (dd, J = 11.5 and 2.1 Hz, CHHO), 2.44 (ddd, J = 21.6 Hz, CHH), 1.98 (ddd, J = 6.2, 6.2, and 12.4 Hz; CHH), 1.01-1.12 (m, 2Si(CH(CH3)2)3.

Imide β-naphthylethyl ester 37b

Imide acid chloride **14c** (1.50 g, 5.8 mmol), β-naphthylethyl alcohol (1.50 g, 8.7 mmol) and 4-(N,N-dimethylamino)pyridine (DMAP) (1.42 g, 11.6 mmol) in dry CH_2Cl_2 (30 mL) under nitrogen atmosphere was heated to reflux for 44 h. Acetyl chloride (350 μL) was added in one portion and refluxed for 20 min. The reaction was poured into 1 N HCl (50 mL) and extracted with $CHCl_3$ (3 x 50 mL); washed with satd $NaHCO_3$ (50mL); dried; and concentrated. Flash chromatography with silica gel ($CHCl_3$:Et_2O = 4:1) gave 2.037 g (89%) of the desired ester **37b** as a white solid.

mp; 187-188 °C (recrystalized from toluene)
IR ($CHCl_3$, thin film); 1729, 1696 cm^{-1}.
^{1}H-NMR (300 MHz, CDCl3); δ 1.10 (s, CH_3), 1.14 (d, J = 14.3 Hz, 2 H), 1.26 (s, 2 CH_3), 1.36 (d, J = 13.4 Hz, 1 H), 1.98 (d, J = 13.4 Hz, 1 H), 2.70 (d, J = 13.0 Hz, 2 H), 3.08 (t, J = 7.1 Hz, 2 H), 4.30 (t, J = 7.2 Hz, 2 H), 7.32-7.82 (m, 8 H).
HRMS calcd for $C_{24}H_{27}NO_4$: 393.1940. found: 393.1939.

Lactam β-naphthylethyl Ester 38b

A solution of imide β-naphthylethyl ester **37b** (1.23 g, 3.13 mmol) in ethanol at 0 °C was treated with $NaBH_4$ (2.03 g). After 6 h, the reaction was neutralized with 6 N HCl, and extracted with CH_2Cl_2 (3 x 100 mL), which was washed with sat'd NaCl (100 mL). Combined extracts were washed with satd

$NaHCO_3$ and dried ($MgSO_4$). Concentration followed by drying on vacuum gave 1.20 g of corresponding hydroxy lactam.

mp; 182-183 °C

^{1}H-NMR (300 MHz, $CDCl_3$); δ 0.94 (d, J = 15.6 Hz, 1 H), 1.06 (s, CH_3), 1.10 (s, CH_3), 1.14 (d, J = 14.0 Hz, 1 H), 1.17 (s, CH_3), 1.29 (d, J = 13.3 Hz, 1 H), 1.77 (d, J = 13.3 Hz, 1 H), 2.63 (d, J = 13.3 Hz, 2 H), 3.13 (t, J = 7.1, 2 H), 4.26-4.46 (m, 2 H), 4.54 (d, J = 13.0 Hz, 1 H), 4.71 (d, J = 13.0 Hz, 1 H), 5.43 (br s, NH), 7.34-7.82 (m, 7 H).

LRMS (readily eliminates 2-vinylnaphthalene); 154 (M - 2-vinylnaphthalene).

Above hydroxylactam β-naphthylethyl ester (840 mg, 2.12 mmol) in dry CH_2Cl_2 (15 mL) was treated with trifluoroacetic acid (1.5 mL) and triethylsilane (1.5 mL). After 3 h at room temperature, the reaction was made basic with satd $NaHCO_3$ (50 mL), extracted with CH_2Cl_2 (3 x 50 mL), washed satd $NaHCO_3$ (50 mL) and dried ($MgSO_4$). Flash chromatography with CH_2Cl_2 then CH_2Cl_2:ether (1:1) yielded 800 mg (99%) of the lactam **38b**.

mp; 186-187 °C

IR ($CHCl_3$, thin film); 1725, 1665 cm^{-1}.

^{1}H-NMR (300 MHz, CDCl3); δ 0.90 (s, CH_3), 1.02 (d, J = 13.9 Hz, 2 H), 1.08 (s, CH_3), 1.16 (s, CH_3), 1.20 (d, J = 13.0 Hz, 1 H), 1.68 (d, J = 13.0 Hz, 1 H), 2.46 (d, J = 14.0 Hz, 1 H), 2.62 (d, J = 13.9 Hz, 1 H), 2.82-2.95 (m, 2 H,), 3.12 (t, J = 7.0 Hz, 2 H), 4.22-4.36 (m, 2 H), 5.08 (br s, NH), 7.35-7.82 (m, 7 H).

HRMS calcd for $C_{24}H_{29}NO_3$: 379.2147. found: 379.2148.

N-Phenyloxycarbonylactam β-Naphthylethyl Ester 39b

A solution of lactam **38b** (519 mg, 1.37 mmol), phenylchloroformate (800 μL), and diisopropylethyl amine (800 μL) in dry CH_2Cl_2 (25 mL) was heated to reflux for 15 h. The reaction was diluted with CH_2Cl_2 (100 mL), washed with 1N HCl (50 mL), dried ($MgSO_4$) and concentrated. Flash chromatography (Hex : CHCl3 : ether = 5 : 2 : 1) gave 608 mg (89 %) of the N-phenoxycarbonyl lactam **39b** as a white solid.

mp; 110.5-112 °C

^{1}H-NMR (300 MHz, $CDCl_3$); δ 1.04 (s, CH_3), 1.08 (s, CH_3), 1.16 (d, J = 12.5 Hz, 2H), 1.25 (s, CH_3), 1.35 (dd, J = 13.1 and 2.6 Hz, 1 H), 1.84 (d, J = 13.1 Hz, 1 H), 2.60 (d, J = 14.0 Hz, 1 H), 2.72 (d, J = 14.1 Hz, 1 H), 3.02-3.08 (m, 2 H), 3.40 (dd, J = 12.0 and 1.8 Hz, 1 H), 3.80 (dd, J = 12.4 and 2.6 Hz, 1 H), 4.26-4.33 (m, 2 H), 7.17-7.79 (m, 12 H).

5-Azacytosine β-naphthyl ester 40b

In a flame dried 2-necked 50 mL flask equipped with a condenser were placed guanidine hydrochloride (73 mg, 0.76 mmol) and 60 % NaH (30.5 mg). Dry THF (13 mL) was added (vigorous hydrogen evolution). After 5 min N-phenoxylactam **39b** (380 mg, 0.76 mmol) in dry THF (25 mL) was added via double tipped needle. After 5 min diisopropylethylamine (663 μL) was added. The flask was heated for 17 h at 90 °C. The reaction was cooled, filtered and concentrated. The resulting crude product was loaded on silica gel column and side products including lactam **4a** were removed by washing with ether. Elution with 5% ethanolic chloroform gave 256 mg (75%) of amorphous solid **40b**.

mp; 227-228 °C

IR ($CHCl_3$, thin film); 1721, 1622, 1581, 1539, 1514, 1462 cm^{-1}.

^{1}H-NMR (300 MHz, $CDCl_3$); δ 1.03-1.72 (m, 13 H), 2.45 (d, J = 14.2 Hz, 1 H), 2.61 (d, J = 13.7 Hz, 1 H), 3.05 (t, J = 6.9 Hz, 2 H), 3.20 (d, J = 15.9 Hz, 1 H), 3.99 (d, J = 12.8 Hz, 1 H), 4.11-4.21 (m, 2 H), 5.13 (br s, NH_2), 7.34-7.81 (m, 7 H).

HRMS calcd for $C_{26}H_{30}N_4O_3$: 446.2318. found: 446.2319.

Imide phenethyl ester 37a

A mixture of imide acid chloride **14c** (1.00 g, 3.90 mmol), DMAP (952 mg, 7.8 mmol), and phenethyl alcohol (1.5 mL) in dry CH_2Cl_2 (20 mL) under a nitrogen atmosphere was heated to reflux for 66 h. The reaction was poured into 1 N HCl (50 mL) and the layers separated. The aqueous layer was extracted with CH_2Cl_2 (2 x 60 mL). Combined extracts were washed with 1 N HCl (50 ml), dried ($MgSO_4$) and concentrated. Chromatography on silica gel with CH_2Cl_2 resulted in partial separation. Phenethyl alcohol was easily removed by recrystalization from *n*-hexane. This gave 1.07 g (80 %) of imide ester **37a**.

mp; 160-161 °C

IR ($CHCl_3$, thin film); 1734, 1701, and1718 cm^{-1}.

^{1}H-NMR (300 MHz, $CDCl_3$); δ 1.12 (s, CH_3), 1.15 (d, J = 16.8 Hz, 2 H), 1.26 (s, 2 CH_3), 1.36 (d, J = 13.3 Hz, 1 H), 1.98 (d, J = 13.3 Hz, 1 H), 2.68 (d, J = 13.3 Hz, 2 H), 2.91 (t, J = 7.2 Hz, 2 H), 4.21 (t, J = 7.3 Hz, 2 H), 7.19-7.32 (m, 5 H), 7.47 (br s, NH).

LRMS (m/z) =344 (MH^+) by chemical ionization with NH_3 matrix.

Lactam phenethyl ester 38a

Imide phenethyl ester **37a** (604 mg, 1.76 mmol) and $LiBH_4$ (310 mg) at 0 °C were dissolved in dry THF (15 mL). After 4 h at 0 °C additional portions of $LiBH_4$ (110 mg) and THF (15 mL) were added. The reaction was kept in refrigerator overnight. The reaction was worked up with CH_2Cl_2 and sat'd NaCl solution. Flash chromatography on silica gel column (CH_2Cl_2 : ether = 9 : 1 to 4:1) gave starting material **37a** (112 mg, slightly contaminated with phenethyl alcohol) and the corresponding hydroxylactam (418 mg, 68 %).

^{1}H-NMR (300 MHz, $CDCl_3$); δ 0.94 (d, J = 16.2 Hz, 1 H), 1.05 (s, CH_3), 1.12 (s, CH_3), 1.16 (s, CH_3), 1.29 (d, J = 13.5 Hz, 2 H), 1.76 (d, J = 13.4 Hz, 1 H), 2.62 (d, J = 13.7 Hz, 2 H), 2.96 (t, J = 7.0 Hz, 2 H), 4.15-4.35 (m, 2 H), 4.53-4.70 (d, J = 13.0 Hz, 1 H) 4.70 (d, J = 7.0 Hz, 1 H), 5.39 (br s, NH), 7.18-7.33 (m, 5 H).
HRMS calcd for $C_{20}H_{25}NO_3$ (M-H2O): 327.1834. found: 327.1832.

Above hydroxylactam phenethyl ester (405 mg, 1.17 mmol) in dry CH_2Cl_2 (5 mL) was treated with trifluoroacetic acid (500 μL) and triethyl silane (500 μL). After 3.5 h, the reaction was diluted with CH_2Cl_2 (30 mL) and poured into satd $NaHCO_3$ (50 ml). The aqueous and organic layers were separated, the aqueous layer was extracted with CH_2Cl_2 (3 x 50 mL). The combined organics were washed with sat'd $NaHCO_3$ (50 mL), dried ($MgSO_4$), and concentrated to give 379 mg (98 %) of **38a**.

mp; 145-146 °C
IR ($CHCl_3$, thin film); 1726 and 1665 cm^{-1}.

^{1}H-NMR (300 MHz, $CDCl_3$); δ 0.94 (s, CH_3), 1.03 (d, J = 13.9 Hz, 2 H), 1.09 (s, CH_3), 1.16 (s, CH_3), 1.22 (d, J = 12.8 Hz, 1 H), 1.70 (d, J = 12.9 Hz, 1 H), 2.46 (d, J = 14.0 Hz, 1 H), 2.61 (d, J = 13.9 Hz, 1 H), 2.93-2.98 (m, 4 H), 4.10-4.31 (m, 2 H), 5.1 (br s, NH), 7.18-7.31 (m, 5 H).
HRMS calcd for $C_{20}H_{27}NO_3$: 329.1991. found: 329.1990.

N-phenoxycarbonyllactam phenethyl ester 39a

A mixture of lactam **38a** (519 mg, 1.37 mmol), phenylchloroformate (800 μl) and diisopropylethyl amine (800 μL) in dry CH_2Cl_2 (25 mL) was heated to reflux for 15 h. The reaction was diluted to 100 mL with CH_2Cl_2, washed with 1N HCl (50 mL), dried ($MgSO_4$) and concentrated. Chromatographic separation (Hex : $CHCl_3$: ether = 5 : 2 : 1) gave 608 mg (89 %) of **39a** (solidifies upon standing when pure) .

IR ($CHCl_3$, thin film); 1786 and 1728 cm^{-1}.
^{1}H-NMR (300 MHz, $CDCl_3$); δ 1.05 (s, CH_3), 1.09 (s, CH_3), 1.16 (d, J = 12.0 Hz, 2 H), 1.24 (s, CH_3), 1.35 (dd, J = 13.0 and 2.4 Hz, 1 H), 1.84 (d, J = 13.0 Hz, 1 H), 2.59 (d, J = 14.2 Hz, 1 H), 2.71 (d, J=14.2 Hz, 1 H), 2.84-2.90 (m, 2 H), 3.40 (d, J=12.3 Hz, 1 H), 3.79 (dd, J = 12.4 and 2.4 Hz, 1 H), 4.21 (t, J = 7.1 Hz, 2 H), 7.08-7.37 (m, 10 H).
HRMS calcd for $C_{21}H_{26}NO_4$ (M-PhO): 356.1862. found; 356.1862.

Azacytosine phenethyl ester 40a

To a suspension of guanidine hydrochloride (143 mg, 1.46 mmol) and sodium hydride (60 % dispersion, 60 mg, 1.46 mmol) in dry THF (25 mL) under

nitrogen atmosphere were slowly added N-phenoxycarbonyl lactam phenethyl ester **39a** (655 mg, 1.46 mmol) and triethylamine (1.2 mL). The resulting yellowish solution was heated to reflux for 20 h. The reaction was concentrated and chromatographed directly. Elution with ether followed by 5% ethanol in chloroform gave 291 mg (50%) of azacytosine **40a** as a white solid.

mp; 184-185 °C

IR ($CHCl_3$, thin film); 3300 (br), 3200 (br), 1721, 1684, 1622, 1581, and 1458 cm^{-1}.

^{1}H-NMR ($CDCl_3$, 300 MHz); δ 1.05-1.36 (m, 12 H), 1.70 (d, J = 13.0 Hz, 1 H), 2.47 (d, J = 14.2 Hz, 1 H), 2.60 (d, J = 13.8 Hz, 1 H), 2.87 (t, J = 7.1 Hz, 2 H), 3.20 (dd, J = 14.6 and 1.4 Hz, 1 H), 3.94-4.12 (m, 3 H), 5.30 (br s, 1 H), 6.06 (br s, 1 H), 7.17-7.30 (m, 5 H).

HRMS calcd for $C_{22}H_{28}N_4O_3$: 396.2161. found: 396.2163.

cis, trans azacytosine phenethyl ester (cis,trans 40a)

Synthesized as similarly as in cis compound case. 81% yield from the corresponding N-phenoxycarbonyl lactam.

mp; 218-220 °C

^{1}H-NMR($CDCl_3$, 270 MHz): δ 0.91 (s, CH_3), 1.11 (s, CH_3), 1.31 (s, CH_3), 1.31-1.81 (m, 6H), 2.94 (t, J = 6.9 Hz, 2H), 3.48 (d, J = 14.5 Hz, 1 H), 3.79 (d, J = 15.1 Hz, 1H), 4.29 (t, J = 6.9 Hz, 2 H), 5.25 (br s, 1 H), 5.60 (br s, 1 H), 7.18-7.33(m, 5 H).

LRMS(m/z) = 396.

Guanosine-2',3'-acetonide 41

Yield 67%

^{1}H-NMR (DMSO-d6, 300 MHz); δ 1.30 (s, 3 H), 1.50 (s, 3 H), 3.50 (m, 2 H), 4.10 (m, 1 H), 4.95 (m, 1 H), 5.17 (m, 1 H), 5.91 (d, J = 2.7 Hz, 1 H), 6.50 (br, 2 H), 7.90 (s, 1 H), 10.66 (s, 1 H).

Guanosine-5'-triisopropylsilyl-2',3'-acetonide 42

Yield=77%

mp; 260-261 °C (CH_2Cl_2-hexane).

IR ($CHCl_3$, thin film); 3320, 3154, 2944, 2867, 1694, and 1374 cm^{-1}.

^{1}H-NMR ($CDCl_3$, 300 MHz); δ 1.04-1.15 (m, 21 H), 1.40 (s, 3 H), 1.62 (s, 3 H), 3.89 (s, 2 H), 4.33 (m, 1 H), 4.98 (m, 1 H), 5.15 (m, 1 H), 6.01 (d, J = 2.4 Hz, 1 H), 6.06 (br s, 2 H), 7.73 (s, 1 H), 12.07(s, 1 H).

HRMS calcd for $C_{22}H_{37}N_5O_5Si$: 479.2564. found: 479.2566.

Solubility Titration

A solution of azacytosine (several milimolar) in $CDCl_3$ was distributed in increasing volume in separate vials containing 2-3 mg of the guanosine 41. The total volume of the solution was adjusted to 700 µL by addition of $CDCl_3$. The vials were capped tightly and shaken for 12 - 24 h at room temperature and filtered through Millex-GV_4 filtertip (0.22 µm) just before HPLC analysis. HPLC solvent system was mixture of methanol and water (both HPLC grade) containing 0.1 % distilled triethyl amine. Beckmann ultrasphere 4.6 mm x 25 cm ODS column (part number 235329) was used for the analysis. In all cases, flow rate was 1 mL/min. and UV detector set at 254 nm (with auf 1.0) was

used throughout the titration. Total guanosine concentrations were calculated from integrated peak areas and calibration curve, and were the average of 4 - 5 injections per sample solution. A calibration curve was obtained with a standard solution containing each component in $CDCl_3$ - MeOH (80 : 20, V/V) at same HPLC condition. Limiting solubility g_o used for the calculation was estabilished from saturated solution in $CDCl_3$ and found to be 6.8×10^{-5} M.

Naphthylethyl ester azacytosine ; MeOH : H_2O = 90 : 10 (with 0.1 % Et_3N), retention time (guanosine **41** = 2.4, azacytosine **40b** = 3.8 min.)

cis,cis-Phenethyl ester azacytosine ; MeOH : H_2O = 80 : 20 (with 0.1 % Et_3N), retention time (guanosine **41** = 2.4, azacytosine **40a** = 4.4 min.)

cis, trans Phenethyl ester azacytosine ; MeOH : H_2O = 75 : 25 (with 0.1 % Et_3N), retention time (guanosine **41** = 2.5, azacytosine **cis, trans 40a** = 5.7 min.)

Experimentals for chapter 3

9,9-Dimethylxanthene 53.

A 1 L, three-necked, round-bottomed flask equipped with an argon inlet adapter, an addition funnel, a rubber septum, and a magnetic stirring bar was charged with a suspension of 9-xanthone (**52**) (50.0 g, 0.255 mol) in 300 mL of toluene. The apparatus was evacuated and filled with argon three times and then cooled in an ice bath while trimethylaluminum solution (2.0 M in toluene, 320 mL, 0.640 mol) was added over 50 min. The resulting solution was allowed to warm

to room temperature over ca. 3 h and stirred further for 14 h. The reaction mixture was transferred via cannula into a manually stirred mixture of 250 mL of conc. HCl and 4 L of ice. The organic phase was separated, dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to afford 51.5 g (96%) of dimethyl xanthene **53** as a yellow oil, which was used without further purification.

2,7-Bis(1,1-dimethylethyl)-9,9-dimethylxanthene (54b).

An ice-cooled, 1 L, three-necked, round-bottomed flask equipped with a drying tube, an addition funnel, a glass stopper, and a magnetic stirring bar was charged with 9,9-dimethylxanthene **53** (51.5 g, 0.245 mol), 500 mL of CH_2Cl_2, and $FeCl_3$ (2.0 g, 0.012 mol). *tert*-Butyl chloride (65 mL, 0.60 mol) was added over 50 min. The reaction mixture was allowed to warm to room temperature over ca. 3 h and stirred further for 15 h. The reaction mixture was extracted with 1 L of H_2O, dried over $MgSO_4$, and concentrated by rotary evaporation to afford a grey-green solid. The solid was suspended in 400 mL of absolute EtOH, and the mixture was boiled, allowed to cool, and filtered to afford 41.9 g (53%) of **54b** as a white solid. Concentration of the filtrate afforded an additional 3.9 g (5%) of **54b**.

mp; 191-192 °C

IR ($CHCl_3$); 3009, 2967, 2908, 2871, 1489, 1407, 1365, 1295, 1268, 1134, 1122, 1088, 849, 826, and 753 cm^{-1}

^{1}H-NMR (250 MHz, $CDCl_3$); δ 7.40 (d, J = 2.4 Hz, 2 H), 7.21 (dd, J = 8.6, 2.3 Hz, 2 H), 6.96 (d, J = 8.5 H, 2 H), 1.65 (s, 6 H), and 1.33 (s, 18 H).

^{13}C-NMR (75 MHz, $CDCl_3$); δ 148.3, 145.4, 129.2, 124.3, 122.7, 115.6, 34.4, 34.3, 32.5, and 31.6

HRMS *m/e* calcd for $C_{23}H_{30}O$: 322.2297, found: 322.2298.

2,7-Bis(1,1-dimethylpropyl)-9,9-dimethylxanthene (54a).

An ice-cooled, 2 L, three-necked, round-bottomed flask equipped with a gas inlet adapter connected to a mineral oil bubbler, a glass stopper, and a mechanical stirrer was charged with 9,9-dimethylxanthene **53** (52.2 g, 0.248 mol), 500 mL of CH_2Cl_2, and 2-chloro-2-methylbutane (92 mL, 0.75 mol). $FeCl_3$ (4.0 g, 0.025 mol) was added in several portions over 30 min, and then the reaction mixture was allowed to warm to room temperature over ca. 3 h and stirred further for 14 h. $FeCl_3$ (4.0 g, 0.025 mol) was then added in one portion, and the reaction mixture was stirred for an additional 6 h. The reaction mixture was extracted with 1 L of H_2O, dried over $MgSO_4$, and concentrated by rotary evaporation to afford 88.2 g (101%) of impure **54a** as a yellow-black semisolid. An analytical sample of **54a** was prepared by recrystallization two times from EtOH

mp; 130-131 °C
IR ($CHCl_3$); 3008, 2968, 2932, 2879, 1488, 1467, 1407, 1387, 1379, 1364, 1293, 1260, 1133, 1088, 843, 825, 767, and 759 cm^{-1}
^{1}H-NMR (250 MHz, $CDCl_3$); δ 7.34 (d, J = 2.3 Hz, 2 H), 7.14 (dd, J = 8.5, 2.4 Hz, 2 H), 6.95 (d, J = 8.5 Hz, 2 H), 1.64 (s, 6 H), 1.63 (q, J = 7.4 Hz, 4 H), 1.29 (s, 12 H), and 0.69 (t, J = 7.4 Hz, 6 H)
HRMS *m/e* calcd for $C_{25}H_{34}O$: 350.2610, found: 350.2607.

4,5-Dibromo-2,7-bis(1,1-dimethylethyl)-9,9-dimethylxanthene (55b).

An ice-cooled, 1 L, three-necked, round-bottomed flask equipped with a gas inlet adapter connected to a mineral oil bubbler, an addition funnel, and a mechanical

stirrer was charged with a solution of 2,7-bis(1,1-dimethylethyl)-9,9-dimethylxanthene **54b** (45.4 g, 0.141 mol) in 500 mL of CCl_4. Bromine (16 mL, 0.31 mol) was added dropwise over 25 min and the reaction mixture was allowed to warm to room temperature over ca. 3 h and stirred further for 23 h. Fe powder (0.39 g, 0.007 mol) was then added in one portion and the reaction mixture was stirred for an additional 16 h. Bromine (5 mL, 0.10 mol) was then added, and the reaction mixture was stirred for an additional 10 h. The reaction mixture was diluted with 1 L of CH_2Cl_2 and extracted with 500 mL of H_2O. The organic phase was separated, dried over $MgSO_4$, filtered, and concentrated to afford a yellow solid. Recrystallization from THF-EtOH afforded 56.7 g (84%) of **55b** as two crops of white crystals.

mp; 259-260 °C

IR ($CHCl_3$); 3011, 2969, 2909, 2872, 1588, 1479, 1448, 1404, 1366, 1301, 1276, 1186, 1099, 875, 866, and 752 cm^{-1}

^{1}H-NMR (300 MHz, $CDCl_3$); δ 7.48 (d, J = 2.0 Hz, 2 H), 7.34 (d, J = 2.3 Hz, 2 H), 1.63 (s, 6 H), and 1.32 (s, 18 H).

^{13}C-NMR (75 MHz, $CDCl_3$); δ 147.5, 145.5, 131.3, 128.7, 121.7, 110.5, 35.6, 34.4, 31.7, and 31.2

Anal. calcd for $C_{23}H_{28}Br_2O$: C, 57.52; H, 5.88. found: C, 57.28; H, 5.75.

4,5-Dibromo-2,7-bis(1,1-dimethylpropyl)-9,9-dimethylxanthene (55a).

An ice-cooled, 2 L, three-necked, round-bottomed flask equipped with a gas inlet adapter connected to a mineral oil bubbler, an addition funnel, and a mechanical stirrer was charged with a solution of crude 2,7-bis(1,1 dimethylpropyl)-9,9-dimethylxanthene **54a** (88.2g, 0.252 mol) in 500 mL of CCl_4. Bromine (32 mL, 0.62

mol) was added dropwise over 30 min and the reaction mixture was allowed to warm to room temperature over ca. 4 h and stirred further for 8 h.[10] Fe powder (1.38 g, 0.025 mol) was then added in one portion and the reaction mixture was stirred for an additional 11 h and then poured into 1 L of H_2O. The aqueous phase was separated and extracted with a 100 and a 20 mL portion of CH_2Cl_2, and the combined organic phases were dried over $MgSO_4$, filtered, and concentrated to afford 152.7 g of dark grey-green semisolid. Recrystallization three times from abs EtOH afforded 34.8 g (28%) of **55a** as white crystals. Concentration of the second and third mother liquors, followed by recrystallization three times from abs EtOH, afforded a second crop of 5.4 g (4%) of **55a** as a tan solid

mp; 177-178 °C

IR ($CHCl_3$); 3011, 2969, 2938, 2931, 2880, 1587, 1451, 1405, 1388, 1380, 1365, 1309, 1274, 1188, 1099, 874, and 864 cm^{-1}

^{1}H-NMR (300 MHz, $CDCl_3$); δ 7.42 (d, J = 2.1 Hz, 2 H), 7.27 (d, J = 2.0 Hz, 2 H), 1.63 (q, J = 7.5 Hz, 4 H), 1.62 (s, 6 H), 1.28 (s, 12 H), and 0.69 (t, J = 7.4 Hz, 6 H)

HRMS *m/e* calcd for $C_{25}H_{32}{}^{79}Br_2O$: 506.0821, found: 506.0817.

2,7-Bis(1,1-dimethylethyl)-9,9-dimethylxanthene-4,5-dicarboxylic acid (7b).

A 2 L, three-necked round-bottomed flask equipped with an argon inlet adapter, an addition funnel, and a mechanical stirrer was charged with a solution of 4,5-dibromo-2,7-bis(1,1-dimethylethyl)-9,9-dimethylxanthene **55b** (56.3 g, 0.117 mol) in 1.4 L of THF. The flask was cooled with a dry ice-acetone bath (-78 °C), *n*-BuLi solution (2.5 M in hexanes, 140 mL, 0.350 mol) was added over 15 min, and the resulting suspension was stirred for an additional 30 min. The addition funnel was replaced by a gas inlet tube, and dry CO_2 (61 g, 1.39 mol) was bubbled into the

reaction mixture. The clear solution was allowed to warm to room temperature and then poured into a mixture of 300 mL of ice, 100 mL of concd aq HCl, and 500 mL of diethyl ether. The organic phase was separated and extracted with 200 mL of saturated NaCl solution, dried over $MgSO_4$, filtered, and concentrated to afford an oily white solid. The solid was washed with ca. 500 mL of hexanes and dried at 1 mmHg to yield 44.2 g (92%) of **7b** as a white solid.

mp; 307-309 °C

IR ($CHCl_3$); 2500-3600, 3320 (br), 3029, 2969, 2910, 2874, 1715, 1693, 1615, 1446, 1398, 1367, 1336, 1303, 1267, 1217, 1122, 898, 856, 766, 750, and 713 cm^{-1}

^{1}H-NMR (300 MHz, $CDCl_3$); δ 11.86 (br s, 2 H), 8.19 (d, J = 2.5 Hz, 2 H), 7.73 (d, J = 2.5 Hz, 2 H), 1.71 (s, 6 H), and 1.39 (s, 18 H).

^{13}C-NMR (75 MHz, $CDCl_3$); δ 169.1, 147.6, 147.0, 130.5, 129.1, 128.8, 116.5, 34.5, 34.3, 32.3, and 31.1

Anal. calcd for $C_{25}H_{30}O_5$: C, 73.15; H, 7.30. found: C, 73.13; H, 7.30.

2,7-Bis(1,1-dimethylpropyl)-9,9-dimethylxanthene-4,5-dicarboxylic acid (7a).

A 2 L, three-necked, round-bottomed flask equipped with an argon inlet adapter, a rubber septum, and a mechanical stirrer was charged with a solution of 4,5-dibromo-2,7-bis(1,1-dimethylpropyl)-9,9-dimethylxanthene **55a** (26.0 g, 0.0511 mol) in 500 mL of THF. The flask was cooled with a dry ice-acetone bath (-78 °C), *n*-BuLi solution (1.6 M in hexanes, 96 mL, 0.154 mol) was added by syringe over 6 min, and the resulting clear solution was stirred for an additional 30 min. The septum was replaced by a gas inlet tube, and dry CO_2 (44 g, 1.00 mol) was bubbled into the reaction mixture. The clear solution was allowed to warm to 5 °C, quenched with 200 mL of 1 M HCl solution, and partitioned between 300 mL of

diethyl ether and 1 L of H_2O. The organic phase was separated and extracted with 1 L of saturated NaCl solution, dried over $MgSO_4$, filtered, and concentrated to afford an oily, pale yellow solid. The solid was washed with 200 mL of hexanes and dried at 1 mmHg to yield 20.9 g (93%) of **7a** as a cream colored solid.

mp; 255-256 °C

IR ($CHCl_3$); 2400-3500, 3320 (br), 3029, 2969, 2938, 2880, 1714, 1694, 1615, 1446, 1390, 1366, 1339, 1331,1303, 1258, 1246, 1217, 1121, 757, and 753 cm^{-1}

^{1}H-NMR (300 MHz, $CDCl_3$); δ 12.10 (br s, 2 H), 8.13 (d, J = 2.4 Hz, 2 H), 7.66 (d, J = 2.4 Hz, 2 H), 1.70 (q, J = 7.4 Hz, 4 H), 1.70 (s, 6 H), 1.36 (s, 12 H), and 0.72 (t, J = 7.4 Hz, 6 H).

^{13}C-NMR (75 MHz, $CDCl_3$) d 168.7, 147.2, 145.1, 130.2, 129.5, 129.3, 116.3, 37.9, 36.7, 34.4, 32.4, 28.4, and 9.1

Anal. calcd for $C_{27}H_{34}O_5$: C, 73.95; H, 7.81. found: C, 73.65; H, 7.61.

All of the compounds based on the xanthene nucleus were contaminated ca. 10 % of mono tert-butylated material. NMR chemical shifts data are reported only for the major component.

NMR titrations.

All of the NMR titrations were performed in a 5 mm OD nmr tube in dry $CDCl_3$ using reverse titration; Imides were used as hosts and triazines as guests. Concentration of imides ranged from 11-17 mM; triazines 10-32 mM. Dilution studies were performed in 1-17 mM (thymine acetic acid methyl ester **63**) and in 2-61 mM (triazine **59a**) concentration range.

Phenyl ester monoacid 56

To a solution of xanthene diacid **7a** (1.00 g, 2.28 mmol), DMAP (28 mg) and phenol (644 mg, 3.0 equiv.) in dry CH_2Cl_2 (25 mL) under nitrogen atmosphere at 0 °C was added DCC (480 mg, 1.02 equiv) in one portion. After ca 2 min. ice bath was removed and stirred for 20 h at room temperature. The reaction was diluted with n-hexane and filtered. Filterate was concentrated and chromatographed with ethyl acetate in hexane (5-30%) to give **56** (878 mg, 75%) as a white crystalline solid.

mp; 70-71 °C.

IR (KBr); 1194, 1445, 1727, 2877, 2966, and 3351 (relatively sharp OH peak) cm^{-1}.

^{1}H-NMR ($CDCl_3$); δ 0.65-0.75 (m, 6 H), 1.32 (s, 6 H), 1.37 (s, 6 H), 1.63-1.76 (m, 10 H), 7.24-7.47 (m, 5 H), 7.61 (d, J = 2.4 Hz, 1 H), 7.71 (d, J = 2.4 Hz, 1 H), 8.14 (d, J = 2.4 Hz, 1 H), 8.18 (d, J = 2.4 Hz, 1 H), 11.77 (br, 1 H).

HRMS calcd for $C_{33}H_{37}O_4$ $[M\text{-}OH]^+$: 497.2692, found: 497.2692.

2-Naphthylethyl ester monoacid 57

Almost same condition as above (70% yield). Product was isolated after conversion of excess naphthylethyl alcohol to corresponding acetate with acetyl chloride.

mp; 57-58 °C

IR (KBr); 1118, 1236, 1445, 1615, 1713, 2876, 2965, and 3321 (3100-3400) cm^{-1}.

^{1}H-NMR ($CDCl_3$); δ 0.61-0.71 (m, 6 H), 1.24 (s, 6 H), 1.32 (s, 6 H), 1.60-1.68 (m, 10 H), 3.28 (t, J = 6.8 Hz, 2 H), 4.71 (t, J = 6.8 Hz, 2 H), 7.36-7.83 (m, 10 H), 8.18 (d, J = 2.4 Hz, 1 H), 11.97 (br, 1 H).

HRMS calcd for $C_{39}H_{44}O_5$: 592.3189, found: 592.3188.

Triazine 2-naphthylethyl ester 60

Mono acid **57** (396 mg, 0.668 mmol) was refluxed with $SOCl_2$ (1 mL) in CH_2Cl_2 (10 mL) for 3 h. Solvent and residual $SOCl_2$ were removed by rotary evaporation. The acid chloride obtained and free biguanide (150 mg, 2.22 equiv.) was mixed and charged with nitrogen. Dry ethanol (15 mL) was introduced at 0 °C. Ice bath was removed and stirred for 3 h at room temperature. The resulting white suspension was heated to 100-110 °C (bath temp) for 2 h. After concentration, the product was chromatographed with 30% ethyl acetate in CH_2Cl_2 to give 231 mg of triazine ester which contained 19% of ethyl ester by nmr. Another chromatography with same solvent system gave 170 mg (39%) of the desired triazine **60** as a white powder.

mp; 238-239 °C.

IR (KBr); 3491, 2965,1718, 1542, 1442, 747 cm^{-1}.

^{1}H-NMR ($CDCl_3$); δ 0.61-0.71 (m, 6 H), 1.23 (s, 6 H), 1.29 (s, 6 H), 1.59-1.64 (m, 10 H), 3.03 (t, J = 7.2 Hz, 2 H), 4.48 (t, J = 7.2 Hz, 2 H), 5.50 (br, 4 H), 7.36-7.76 (m, 11 H).

HRMS calcd for $C_{41}H_{47}N_5O_3$: 657.3679, found: 657.3677.

Amide phenyl esters

All were prepared through activation of acid functional group ($SOCl_2$, CH_2Cl_2, reflux) followed by reaction with amines in CH_2Cl_2 in the presence of pyridine at room temperature.

Tryptamine amide phenyl ester 58b

81% yield as a white solid.

mp; 198-199 °C.

IR (KBr); 1089, 1196, 1440, 1538, 1648, 1740, 2877, 2965, 3061, 3225 (broad), and 3380 (sharp) cm^{-1}.

^{1}H-NMR ($CDCl_3$); δ 0.66-0.78 (m, 6 H), 1.33 (s, 6 H), 1.37 (s, 6 H), 1.64-1.73 (m, 10 H), 2.99 (t, J = 7.7 Hz, 2 H), 3.70 (m, 2 H), 6.86-7.48 (m, 10 H), 7.56 (d, J = 2.4 Hz, 1 H), 7.70 (d, J = 2.4 Hz, 1 H), 7.86 (br, 1 H), 8.09 (d, J = 2.4 Hz, 1 H), 8.37 (d, J = 2.4 Hz, 1 H), 9.61 (br, 1 H)

HPMS calcd for $C_{43}H_{48}N_2O_4$: 656.3614, found: 656.3613.

2-Aminoanthraquinone amide phenyl ester 58c

72 % yield as a yellow crystalline solid

mp; 204-206 °C.

IR (KBr); 1296, 1417, 1520, 1594, 1675, 1733, 2877, 2965, and 3329 (NH) cm^{-1}.

^{1}H-NMR ($CDCl_3$); δ 0.69-0.79 (m, 6 H), 1.36 (s, 6 H), 1.38 (s, 6 H), 1.69-1.73 (m, 10 H), 6.97-7.17 (m, 5 H), 7.59-8.52 (m, 11 H), 10.82 (s, 1 H)

HRMS calcd for $C_{47}H_{45}NO_6$: 719.3247, found: 719.3243.

2-Naphthylethyl amide phenyl ester 58a

73% yield as a white crystalline solid

mp; 87-89 °C

IR (KBr); 1090, 1184, 1257, 1439, 1530, 1654, 1736, 2876, 2965, and 3386 (NH) cm^{-1}.

^{1}H-NMR ($CDCl_3$); δ 0.66-0.78 (m, 6 H), 1.34 (s, 6 H), 1.38 (s, 6 H), 1.62-1.77 (m, 10 H), 2.96 (t, J = 8.0 Hz, 2 H), 3.63 (m, 2 H), 7.07-7.81 (m, 13 H), 8.10 (d, J = 2.4 Hz, 1 H), 8.30 (d, J = 2.5 Hz, 1 H), 9.63 (t, J = 5.8 Hz, 1 H)

HRMS calcd for $C_{45}H_{49}NO_4$: 667.3362, found: 667.3357.

Triazine tryptamine amide 59b (a general procedure)

The tryptamine amide phenyl ester **58b** (232 mg, 0.360 mmol) and biguanide (79 mg, 2.1 equiv.) under nitrogen were added dry ethanol (10 mL) and heated to 110-120 °C (bath temp) for 4 h. After removal of ethanol, the crude product was directly loaded on silica gel column and eluted with 75% ethyl acetate in CH_2Cl_2 to obtain 152 mg (67%) of the triazine **59b** as a white solid.

mp; 284-285 °C

IR (KBr); 3483, 3387, 2963, 1636, 1602 and1537 cm^{-1}.

^{1}H-NMR ($CDCl_3$); δ 0.65-0.75 (s, 6 H), 1.31 (s, 6 H), 1.32 (s, 6 H), 1.67 (m, 10 H), 2.87 (t, J = 7.8 Hz, 2 H), 3.66 (m, 2 H), 5.14 (br, 4 H), 7.07-8.25 (m, 11 H).

HRMS calcd for $C_{39}H_{47}N_7O_2$: 645.3791, found: 645.3789.

Triazine 2-(9,10-dioxo) anthryl amide 59c

62% yield as a yellow crystalline solid

mp; >310 °C

IR (KBr); 714, 826, 1297, 1439, 1583, 1592, 1674, 2877, 2965, 3130, 3193, 3361, and 3495 cm^{-1}.

^{1}H-NMR ($CDCl_3$); δ 0.67-0.76 (m, 6 H), 1.34 (s, 12 H), 1.65-1.71 (m, 10 H), 4.84 (br, 4 H), 7.53-8.35 (m, 11 H), 9.94 (br, 1 H).

HRMS calcd for $C_{43}H_{44}N_6O_4$: 708.3424, found: 708.3420.

Triazine naphthylethyl amide 59a

71% yield as a white solid

mp; 168-169 °C

IR (KBr); 749, 826, 1250, 1436, 1608, 2876, 2965, 3196, 3329, and 3492 cm^{-1}.

^{1}H-NMR ($CDCl_3$); δ 0.64-0.74 (m, 6 H), 1.30 (s, 6 H), 1.31 (s, 6 H), 1.62-1.70 (m, 10 H), 2.85 (t, J = 7.8 Hz, 2 H), 3.64 (m, 2 H), 5.29 (br, 4 H), 7.36-7.80 (m, 10 H), 8.12 (d, J = 2.4 Hz, 1 H), 8.34 (br, 1 H).

HRMS calcd for $C_{41}H_{48}N_6O_2$: 656.3839, found: 656.3838.

Triazine ethyl ester 61

Separated (20 %) from attempted bistriazine synthesis starting from xanthene diacid chloride and biguanide sulfate in the presence of sodium ethoxide in ethanol at reflux.

mp: 283-285 °C

IR (KBr); 3500, 1716, 1618, 1542 and 1444 cm^{-1}.

^{1}H-NMR ($CDCl_3$); δ 0.64-0.72 (m, 6 H), 1.20-1.34 (m, 15 H), 1.61-1.69 (m, 10 H), 4.26 (q, J = 7.1 Hz, 2 H), 5.20 (br, 4 H), 7.4-7.5 (m, 4 H).

HRMS calcd for $C_{31}H_{41}N_5O_3$ [M-CH_3]: 516.2975, found: 516.2974.

Thymine acetic acid 62

Prepare a slurry of thymine (5 g, 40 mmol) and bromoacetic acid (11 g, 79 mmol) in 100 mL of H_2O. Addition of NaOH (6.3 g, 150 mmol) causes the mixture to become homogeneous. The reaction is stirred at reflux for 4 h then concentated by distillation at reduced pressure to a volume of 30 mL. Addition of conc HCl until pH = 1 followed by cooling causes the product to precipitate. Filtration gave 5 g (70 %) of alkylated thymine which was used without further purification.

mp; 264-265 °C (lit 270 °C)
IR (1 % KBr); 3180-2962 br, 1662, and 1485 cm^{-1}.
^{1}H-NMR (d^6-DMSO, 250 MHz); δ 13.2 (s, CO_2H), 11.4 (s, NH), 7.48 (d, J = 1 Hz, CH), 4.36 (s, CH_2), 1.74 (d, J = 1 Hz, CH_3).

Thymine acetic acid methyl ester 63

Prepared by treating thymine acetic acid **62** with diazomethane. Byproduct formed is N-methyl thymine ester which can be removed easily with chromatography (1:1 EtOAc-$CHCl_3$).

mp; 195-196 °C
IR (KBr); 3176, 1738, 1694, and 1651 cm^{-1}.
^{1}H-NMR ($CDCl_3$); δ 1.93 (s, 3 H), 3.80 (s, 3 H), 4.44 (s, 2 H), 6.93 (s, 1 H), 8.38 (br, 1 H).
HRMS calcd for $C_8H_{10}N_2O_4$: 198.0641, found: 198.0640.

Kemp's imide methyl ester 64

Imide acid **14a** (300 mg) in THF (30 mL) was treated with excess diazomethane. After removal of excess diazomethane with acetic acid and of THF, the residue was dissolved in CH_2Cl_2 (50 mL) and washed $NaHCO_3$ (2 x 50 mL). The concentrated product was purified further by chromatography (20 % EtOAc in CH_2Cl_2) to give 284 mg (89 %) of **64**.

General titration procedure

Titration of thymine **63** with receptor **61** is representative.
A solution of thymine **63** was prepared by dissolving 2.45 mg with $CDCl_3$ to 1 mL in volumetric flask (12.36 mM). Triazine ester **61** (28.02 mg) was dissolved and diluted to 2 mL with $CDCl_3$ in 2 mL volumetric flask (26.35 mM). Four hundred microliter of **63** solution was transfered into 5 mm o.d. NMR tube and the NMR recorded. Twenty microliters of **61** solution was added and the NMR spectrum recorded. Twenty microliter aliquot addition was continued until total receptor solution added exceeded 180 µL. After each addition NMR spectra was recorded. Aliquot size added increased to 30 40, finally to 500 µl until no significant chemical shift change had taken place.

Eadie-Hofstee Treatment of the titration data.

In case of 1:1 binding, the association constant (Ka) is defined as:

$$Ka = [HG]/[H][G] \qquad (1)$$

where [H], [G] and [HG] are concentrations of host, guest and host-guest complex at equilibrium, respectively.

The quantitative treatment starts with the statement that the observed chemical shift δ is the average of the chemical shifts of the proton in host and host-guest complex, weighted by the fractional occupancy of these states:

$$\delta_{obs} = \{[H]\cdot\delta_o + [HG]\cdot\delta_{1:1}\}/[H_t] \qquad (2)$$

where δ_{obs}, δ_o and $\delta_{1:1}$ are the observed, free host and 1:1 complex chemical shifts, respectively. Since $[H_t] = [H] + [HG]$, equation (2) can be written:

$$\delta_{obs} = (\delta_{1:1} - \delta_o)\cdot[HG]/[H_t] + \delta_o \qquad (3)$$

Defining chemical shift differences

$$\Delta = \delta_{obs} - \delta_o \qquad (4)$$

$$\Delta_{max} = \delta_{1:1} - \delta_o \qquad (5)$$

allows equation (3) to be expressed as:

$$\Delta = \Delta_{max}\cdot[HG]/[H_t] \qquad (6)$$

Combining equations (2) and (6) gives:

$$\Delta = \Delta_{max}\cdot Ka\cdot[G]/\{1 + Ka\cdot[G]\} \qquad (7)$$

Equation (7) is the NMR 1:1 binding isotherm. Rearranging equation (7) yields:

$$\Delta = \Delta_{max} - (1/Ka)\cdot\{\Delta/[G]\} \qquad (8)$$

Thus plotting Δ vs Δ/[G] gives straight line, and the association constant can be calculated from the slope (Ka = -1/slope). In order to get reasonable estimate of Δ_{max}, reasonable chemical shifts of δ_o's and $\delta_{1:1}$'s are required. In the present study, δ_o's used were directly from initial chemical shift of host and $\delta_{1:1}$'s were from observed maximum chemical shift where large exess of guests are present or extrapolation of the observed data with nonlinear regression analysis. Typical titration data are shown below.

Table 6 Titration data of **63** with **61** (Initial host volume 400 μL).

Entry #	Total **61** added (μL)	Chemical shift (ppm)
1	0	8.33023
2	20	8.79847
3	40	9.24411
4	60	9.69876
5	80	10.1115
6	100	10.5023
7	120	10.8502
8	140	11.1691
9	160	11.4547
10	180	11.7103
11	210	12.0108
12	240	12.2460
13	280	12.4894
14	320	12.6673
15	370	12.8274
16	450	13.0041
17	600	13.1181
18	900	13.2916
19	1400	13.3344

Xanthene diol 67

Xanthene diacid **7a** (2.00 g, 4.56 mmol) in dry THF (90 mL) was reduced with LAH (560 mg) by refluxing for 2.5 h. Workup as usual (15 % NaOH) gave the diol (1.670 g, 89 %) as practically pure solid (by nmr).

mp; 169-171 ^{o}C

IR (KBr); 3500-3100, 2965, 2875, 1472, 1457, 1285, 1243, 1212, 1031 and 878 cm^{-1}.

^{1}H-NMR ($CDCl_3$, 250 MHz); δ 0.69 (t, J = 7.4 Hz, 6 H), 1.29 (s, 12 H), 1.59-1.68 (m, 10 H), 3.08 (br, 2H, OH), 4.80 (d, J = 4.6 Hz, 4 H), 7.11 (d, J = 2.3 Hz, 2 H), 7.33 (d, J = 2.3 Hz, 2 H).

Xanthene dibromide 68

To a clear, homogeneous solution of the diol **67** (2.00 g, 4.54 mmol) in regular CCl_4 (100 mL) was added slowly PBr_3 (1.00 mL, 2.34 mol equiv) with stirring. After 18 h at room temperature, the reaction was washed with $NaHCO_3$ solution and dried ($MgSO_4$). Evaporation of solvent afforded **68** (quantitative).

mp; 130-132 ^{o}C

IR (KBr); 2965, 2928, 2873, 1464, 1282, 1207 and 758 cm^{-1}.

^{1}H-NMR ($CDCl_3$, 250 MHz); δ 0.70 (t, J = 7.4 Hz, 6 H), 1.29 (s, 12 H), 1.59-1.65 (m, 10 H), 4.82 (s, 4 H), 7.19 (d, J = 2.3 Hz, 2 H), 7.32 (d, J = 2.3 Hz, 2 H).

Xanthene dinitrile 69

A solution of dibromide **68** (1.551 g, 2.89 mmol) in ethanol (5 mL) was added NaCN (425 mg, 3 mol equiv) in water (2 ml). The mixture was refluxed for 4

h, cooled and extracted with CH_2Cl_2 - hexanes. Chromatography of the evaporation residue with 50-70% CH_2Cl_2 in hexanes gave 893 mg (72 %) of **69**.

1H-NMR ($CDCl_3$, 250 MHz); δ 0.70 (t, J = 7.3 Hz, 6 H), 1.30 (s, 12 H), 1.60-1.69 (m, 10 H), 3.90 (s, 4 H), 7.16 (d, J = 2.2 Hz, 2 H), 7.35 (d, J = 2.2 Hz, 2 H).

Xanthene homodiacid 70

Dinitrile **69** (630 mg, 1.47 mmol) in ethanol (5 mL) was treated with KOH (2.00 g) in water (2 mL) and refluxed for 24 h. Mixture was poured into water, acidified with dil HCl, and extracted with EtOAc + hexanes (2 x 70 mL), then washed with 2N HCl, dried ($MgSO_4$). Concentration gave 633 mg (93 %) of **70**.

mp; 290-292 °C
IR (KBr); 1223, 1286, 1415, 1467, 1709,2500-3400, and 2968 cm^{-1}.
1H-NMR ($CDCl_3$, 250 MHz); δ 0.68 (t, J = 7.3 Hz, 6 H), 1.26 (s, 12 H), 1.55-1.65 (m, 10 H), 3.78 (s, 4H), 6.99 (d, J = 2.1 Hz, 2 H), 7.60 (d, J = 2.1 Hz, 2H).

Xanthene homodiacid bis pyridine receptor 72

To a solution of the diacid **70** (100 mg, 0.215 mmol) and mono protected pyridine **71** (97 mg, 3.0 mol equiv) and DMAP (several mg) in dry CH_2Cl_2 (8 mL) at 0 °C was added DCC (106 mg, 2.4 mol equiv) in one portion. After 5 min, the cooling bath was romoved and stirred for 3.5 h (TLC). Additional DCC (100 mg) was added and stirred overnight (total reaction time 20 h). Concentrated and chromatographed directly with 30-80 % EtOAc in hexanes gave 75 mg (48 %) of **72**.

mp; 251-253 °C

IR (KBr); 802, 1154, 1244, 1300, 1452, 1512, 1588, 1689, 2877, 2966, 3281, and 3413 cm^{-1}.

^{1}H-NMR ($CDCl_3$, 250 MHz); δ 0.72 (t, J = 7.3 Hz, 6 H), 1.30 (s, 12 H), 1.59-1.68 (m, 10 H), 2.15 (s, 6 H), 3.81 (s, 4 H), 7.13 (d, J = 2.1 Hz, 2 H), 7.30 (d, J = 2.1 Hz, 2 H), 7.64 (t, J = 8.0 Hz, 2 H), 7.80 (br, 2 H), 7.84 (d, J = 8.0 Hz, 2 H), 7.95 (d, J = 8.0 Hz, 2 H), 8.73 (br s, 2 H).

Tethered thymine 73

Thymine acetic acid **62** (3.5 g, 19 mmol) was dissolved in dry DMF (100 mL). The solution is cooled to 0 °C and carbonyl diimidazole (CDI, 3.1 g, 19 mmol) is added in small batches. The solution was allowed to warm to room temperature with stirring for 45 min. Ethylene glycol (540 μL, 9.6 mmol) is then added dropwise via syringe. The reaction mixture is reluxed for 4 h, then concentrated to near dryness by distillation at reduced pressure. Trituation with H_2O and cooling causes the product to precipitate. Filtration gave 2.15 g (57 %) of **73** which was used without further purification. Additional product could be obtained by evaporation of the mother liquor and chromatography.

mp; 215-217 °C

IR (1 % KBr); 3286, 3009, 1675, and 1420 cm^{-1}.

^{1}H-NMR (d^6-DMSO, 250 MHz); δ 11.42 (s, NH), 7.49 (d, CH), 4.51 (s, CH_2), 4.33 (s, CH_2), 1.76 (d, J = 1 Hz, CH_3).

Thymine photodimer 74

The tethered thymine **73** (2.15 g, 5.5 mmol) is dissolved in 700 mL of 10 % aqueous acetone and degassed by bubbling argon through the solution for 30 min. The solution is photolyzed using a Hanovia immersion lamp filtered through a Pyrex sleeve (250 W, high pressure). After 5 h the solution is concentrated to give a quantitative yield of the cyclobutane derivative **74** .

mp; > 320 °C

IR (1 % KBr); 3378, 3190, 1751, 1691, and 1476 cm^{-1}.

^{1}H-NMR (d^6-DMSO, 250 MHz); δ 10.67 (s, NH), 4.85 (d, J = 9.6 Hz, CH_2), 4.45 (d, J = 18 Hz, CH_2), 4.03 (d, J = 9.7 Hz, CH_2), 3.96 (s, CH), 3.61 (d, J = 18 Hz, CH_2), 1.24 (s, CH_3).

Thymine photoimer diacetic acid 75

Tethered thymine dimer **74** (1.68 g, 4.26 mmol) and NaOH (680 mg, 17 mmol) is dissolved in 25 % aqueous MeOH (25 mL). The solution is refluxed for 4 h. The MeOH was removed by rotary evaporation and the remaining solution made acidic with conc HCl. Cooling caused precipitation of the product which was isolated by filtration yielding 0.9 g (58 %).

mp decomposed at 350 °C

IR (1 % KBr); 3436, 3132, 2992, 1702, 1638, and 1401 cm^{-1}.

^{1}H-NMR (d^6-DMSO, 250 MHz); δ 10.60 (s, NH), 4.03 (d, J = 17.9 Hz, CH_2), 3.98 (d, J = 17.5 Hz, CH2), 3.89 (s, CH),1.25 (s, CH_3).

Thymine Photodimer Ditrimethylsilylpropyl ester 76

Thymine dimer acetic acid **75** (0.9 g, 2.45 mmol) was dissolved in dry DMF (10 mL). The solution was cooled to 0 °C and carbonyl diimidazole (CDI, 0.79 g, 4.89 mmol) was added in small batches. The solution was allowed to warm to room temperature with stirring for 45 min. 3-Trimethylsilyl propanol (780 μL, 4.89 mmol) was then added dropwise via syringe. The reaction mixture was reluxed for 4 h then concentrated to near dryness by distillation at reduced pressure. Trituation with H_2O and cooling caused the product to precipitate. Filtration and washing with hot H_2O gave 0.93 g (64 %) of **76**.

mp; 222-223 °C

IR (1 % KBr); 3284, 2954, 1740, 1687, and 1472 cm^{-1}.

^{1}H-NMR ($CDCl_3$, 250 MHz); δ 7.62 (s, NH), 4.31(d, J = 17.5 Hz, NCH_2), 4.16 (dt, J = 10,5 and 7.2 Hz, OCH_2), 4.02 (d, J = 17.5 Hz, NCH_2), 3.96 (dt, J = 9.6 and 7.2 Hz, OCH_2), 3.87 (s, CH), 1.58-1.71 (m, $OCH_2\underline{CH_2}$), 1.51 (s, CH_3), 0.46-0.53 (m, $CH_2\underline{CH_2}Si$), 0.17 (s, $Si(CH_3)_3$).

Table 7: Titration data of the receptor **72** with the thymine **63**; ([host]=5.9352 mM, [guest]=15.754 mM, initial host volume=400 μL)

entry	total guest added (μL)	Chemical shift of H_L (ppm)	Chemical shift of H_R(ppm)	Chemical shift of **63** (ppm)
1	0	8.71178	7.73071	N/A
2	20	8.78258	7.83744	~10.5
3	40	8.85360	~7.94	10.4454
4	60	8.92150	8.02500	10.3957
5	80	8.98660	8.12100	10.3369
6	100	9.04770	8.20430	10.2864

7	120	9.10410	8.28940	10.2310
8	140	9.16050	8.36860	10.1697
9	160	9.21400	8.44320	10.1174
10	180	9.26240	8.51340	10.0635
11	200	9.30570	8.57650	10.0114
12	220	9.35065	8.63835	9.96245
13	240	9.39176	8.69457	9.90938
14	260	9.42853	8.74631	9.86200
15	290	9.47886	8.81731	9.79341
16	320	9.52225	8.87937	9.72940
17	360	9.57204	8.95082	9.64397
18	400	N/A	9.01912	9.57267
19	450	9.66739	9.08414	9.48872
20	550	9.74332	9.19227	9.35031
21	650	9.79939	9.27129	9.24121
22	850	9.87552	9.38630	9.07322
23	1100	9.93271	9.47016	8.93562
24	1400	9.9730	9.53053	8.84096
25	1900	9.98465	9.55697	8.79017

H_L; aryl acetamide proton.

H_R; acetamide proton.

Imide proton peak was followed in guest.

4-Benzyloxy-2,6-diacetylamidopyridine 78

Prepared according to published procedure (Markees *et al. J. Med. Chem.* **1968, *11*,** 126) in seven steps starting from chelidamic acid monohydrate (**77**).

mp; 136-137 ^{o}C

^{1}H-NMR (250 MHz, $CDCl_3$); δ 7.62 (br, 2 H), 7.32-7.52 (m, 7 H), 5.14 (s, 2 H), 2.17 (s, 6 H).

2,6-Diacetylamido-4-hydroxypyridine 79

4-benzyloxy-2,6-diacetylamido-pyridine **78** (439 mg, 1.47 mmol) and Pd/C (10 % Pd, 58 mg) in ethanol (11 mL) was stirred for 2h under hydrogen pressure (baloon). The resulting slury was dissolved by addition of methanol, filtered and evaporated to give 268 mg (90 %) of greyish white solid.

mp; >290 °C
^{1}H-NMR (250 MHz, DMSO-d_6); δ 10.51 (br, 1 H), 9.84 (br, 2 H), 7.23 (s, 2 H), 2.06 (s, 6 H).

Di-t-amyl receptor 80

Di-t-amylxanthene dibromide **68** (100 mg, 0.186 mmol), 2,6-diacetylamido-4-hydroxypyridine (**79**) (94 mg, 2.4 mol equiv.) and K_2CO_3 (62 mg, 2.4 mol equiv.) in dry DMF (5 mL) under argon was heated to 83-90 °C for 22 h. DMF was removed and residue taken up with EtOAc (100 mL), washed with water (100 mL) and dried (Na_2SO_4). Chromatography on silica gel with 5-10 % methanol in CH_2Cl_2 gave 76 mg (51 %) of the desired product as white solid.

mp; 163-164 °C
IR (KBr); 3447(br), 1695, 1433, 1232 cm-1.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 7.38 (d, J = 2.0 Hz, 2 H), 7.24 (d, J = 2.0 Hz, 2 H), 5.11 (s, 4 H), 2.16 (s, 6 H), 1.60-1.67 (m, 10 H), 1.29 (s, 12 H), 0.69 (t, J = 7.4 Hz,6 H).

Di-t-butyl receptor 80a was synthesized similarly.

mp; 175-176 °C

IR (KBr); 3430(br), 1695, 1433 cm-1.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 7.45 (d, J = 2.0 Hz, 2 H), 7.31 (d, J = 2.0 Hz, 2 H), 5.12 (s, 4 H), 2.17 (s, 12 H), 1.68 (s, 6 H), 1.34 (s, 18 H).

In both cases (t-amyl and t-butyl template) all four amide NHs and four pyridine CHs are very broad (~8.4 and ~7.5 ppm, respectively) and sharpen up when guest molecules, e.g. soluble thymine photodimer, are added.

(L)-3- (1-Thymine)-N-benzyloxycarbonyl alanine 83

A suspension of (L)-N-benzyloxycarbonyl serine β-lactone (**82**) (1.00 g, 4.52 mmol) and bis-O-trimethylsilyl thymine **81** (6.113 g, 5.0 equiv.) in 120 mL acetonitrile (from sure-seal bottle) under argon was stirred for 5 days at 45-50 °C. Water (30 mL) was added to the yellowish homogeneous solution. Solvent was removed and the residue taken up with dil. HCl saturated with NaCl, extracted with 60 % EtOAc in $CHCl_3$ (3 x 150 mL) and dried (Na_2SO_4). Trituration of the concentrated crude product in CH_2Cl_2-hexanes gave 957 mg (61 %) of the desired product **83** (ca 90 % purity by nmr).

^{1}H-NMR (250 MHz, CD_3CN); δ 7.2-7.4 (m, 5 H), 7.09 (d, 1 H), 4.99-5.08 (m, 3 H), 4.48-4.54 (dd, Ja = 4.44 Hz, Jb = 9.0 Hz, 1 H), 4.17-4.25 (dd, Ja = 4.4 Hz, Jb = 14.1 Hz, 1 H), 3.70-3.79 (dd, Ja = 9.2 Hz, Jb = 14.2 Hz, 1 H), 1.73 (d, J = 0.9 Hz, 3 H). Typical impurity (dihydro DEAD) peaks are at 4.1 (q) and 1.2 (t).

(L)-3-(1-Thymine)-N-benzyloxycarbonyl alanine methyl ester 85

Thymine amino acid **83** (525 mg, 1.52 mmol) in 25 mL of abs. MeOH was treated with thionyl chloride (2 mL) and stirred for 15 h. Chromatographic separation of the concentrated product followed by trituration in CH_2Cl_2-hexanes gave 524 mg (96 %) of the methyl ester **85** as white powder.

mp; 163-165 °C
^{1}H-NMR (250 MHz, $CDCl_3$); δ 8.12 (br, 1 H), 7.2-7.4 (m, 5 H), 6.89 (s, 1 H), 5.67 (d, 1 H), 5.04-5.17 (dd, Ja = 12.2 Hz, 2 H), 4.51-4.59 (dd, Ja = 6.0 Hz, Jb = 12.7 Hz, 1 H), 4.0-4.2 (m, 2 H), 3.79 (s, 3 H), 1.82 (d, J = 0.9 Hz, 3 H).
HRMS (FAB, 3-nitrobenzyl alcohol) cald for $C_{17}H_{20}N_3O_6$ (MH^+): 362.1352, obsd: 362.1352.

(L)-3-(1-Thymine)-alanine methyl ester 86

A solution of (L)-3-(1-thymine)-N-benzyloxycarbonyl alanine methyl ester **85** (61 mg, 0.169 mmol) in 11 mL of t-butanol-THF (10:1) was stirred with 28 mg of 10 % Pd/C under hydrogen baloon pressure. After 7 h, additional catalyst (22 mg) was added and hydrogen baloon was replaced. Stirring overnight (total reaction about 24 h) showed complete reaction by TLC. The reaction

was filtered and concentrated. Chromatographic separation with 5 % methanol in chloroform gave 28 mg (74%) of the product **86** as white powder.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 7.10 (d, J = 1.3 Hz, 1 H), 4.00-4.07 (dd, Ja = 4.1 Hz, Jb = 12.5 Hz, 1 H), 3.72-3.87 (m, 5 H), 1.90 (s, 3 H).
$[\alpha]_D^{20}$; +23.7 ($CHCl_3$).
HRMS (FAB, glycerol) cald for $C_9H_{14}N_3O_4$ (MH^+): 228.0984, obsd: 228.0984.

(L)-3-(1-Thymine)-N-benzyloxycarbonyl alanine p-nitrophenyl ester 84a

Thymine amino acid **83** (300 mg, 0.87 mmol) and p-nitrophenol (121 mg, 1.0 equiv.) in 5 mL dry THF under argon at room temperature was treated in one portion with DCC (179 mg, 1.0 equiv.). After 6 h, DCU was filtered off, and the DCU rinced with several mililiters of ether. Filterated was concentered and redissolved with 2-3 mL of EtOAc and ca 10 mL of ether and kept in refrigerator. Precipitate (mostly DCU) was filtered off. The filterate was concentrated and reprecipitated with EtOAc and cyclohexane (refrigerator) to give 136 mg of pure p-nitrophenyl ester **84a**. From the mother liquor, a second crop (44 mg) was obtained.(44 % total yield).

mp; 166-167 °C
^{1}H-NMR (250 MHz, $CDCl_3$); δ 8.37 (br, 1 H), 8.27 (d, J = 9.1 Hz, 2 H), 7.27-7.40 (m, 7 H), 6.93 (s, 1 H), 5.82 (d, J = 6.4 Hz, 1 H), 5.09-5.22 (dd, Ja = 12.1 Hz, Jb = 19.5 Hz, 2 H), 4.74-4.81 (dd, Ja = 5.84 Hz, Jb = 11.6 Hz, 1 H), 4.20-4.42 (m, 2 H), 1.82 (s, 3 H).

Pentafluorophenyl ester **84b** was prepared with similar procedure.

mp; 158-159 °C

^{1}H-NMR (250 MHz, $CDCl_3$); δ 8.14 (br, 1 H), 7.35 (br s, 5 H), 6.95 (s, 1 H), 5.89 (d, J = 7.67 Hz, 1 H), 5.14 (s, 2 H), 4.9-5.0 (m, 1 H), 4.33-4.41 (dd, Ja = 4.8 Hz, Jb = 14.1 Hz, 1 H), 4.15-4.25 (dd, Ja = 7.5 Hz, Jb = 14.1 Hz, 1 H), 1.85 (s, 3 H).

Experimentals for Chapter 4

All of xanthene diacid used from now on is di-t-butyl version unless otherwise noted.

Xanthene phenyl ester *p*-cyanophenyl amide 91

Xanthene phenyl ester mono acid **90** (500 mg, 1.03 mmol) in 15 mL CH_2Cl_2 was refluxed with thionyl chloride (4 mL) for 2 h, concentraeted and dried in vacuum. The acid chlorode and *p*-aminobenzonitrile (245 mg, 2.0 equiv) under argon was diluted with dry CH_2Cl_2 (20 mL) and Et_3N (1 mL). After 10 min, the reaction was concentrated and chromatographed with 15% EtOAc in hexanes to give 575 mg (95%) of pure **91** as white solid.

mp; 246-247 °C

IR (KBr); 3313, 2960, 2861, 2226, 1727, 1659, 1599, 1577, 1507, 1442, 1312, 1230, 1184, 1084 cm^{-1}.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 10.58 (s, 1 H), 8.34 (d, J = 2.3 Hz, 1 H), 8.12 (d, J = 2.4 Hz, 1 H), 7.77-7.81 (m, 3 H), 7.66 (d, J = 2.3 Hz, 1 H), 7.35-7.41 (m, 5 H), 7.09-7.12 (m, 2 H), 1.73 (s, 6 H), 1.43 (s, 9 H), 1.39 (s, 9 H).

HRMS calcd for $C_{38}H_{38}N_2O_4$: 586.2832, found 586.2829.

Xanthene triazine p-cyanophenylamide 92

Xanthene phenyl ester *p*-cyanophenyl amide **91** (500 mg, 0.852 mmol) and free biguanide (172 mg, 2.0 equiv) under argon was diluted with absolute ethanol (17 mL) and slowly heated to reflux. Additional ethanol (6 mL) was added for efficient stirring. After 2.5 h reflux, the white solid was filtered to give 331 mg of pure **92**. Flash chromatography of the filtrate residue ($CHCl_3$ then 40 % EtOAc in CH_2Cl_2) afforded a 42 mg of second crop (total yield=76 %).

mp; >340 °C

IR (KBr); 3393, 3302, 3106, 2964, 2871, 2227, 1672, 1631, 1608, 1592, 1544, 1440 cm^{-1}

^{1}H-NMR (250 MHz, $CDCl_3$); δ 9.76 (s, 1 H), 8.13 (d, J = 2.46 Hz, 1 H), 7.43-7.65 (m, 7 H), 4.96 (br, 4 H), 1.72 (s, 6 H), 1.39 (s, 9 H), 1.37 (s, 9 H).

HRMS calcd for $C_{34}H_{37}N_7O_2$: 575.3009, found 575.3006.

Xanthene triazine p-aminomethylphenyl amide 93

Xanthene triazine *p*-cyanophenyl amide **92** (117 mg, 0.203 mmol) in 20 mL ethanol containing 733 mg of ammonia (210-fold exess) was stirred under hydrogen atmosphere (balloon) with ca. 100 mg of Raney nickel (W2, washed twice with absolute ethanol) for 21 h at room temperature. Filtration followed by concentration gave 115 mg (97 %) of the product as a white solid.

mp; 269-271 °C

IR (KBr); 3324, 3182, 3118, 2962, 2869, 1662, 1609, 1540, 1437, 1266, 826 cm^{-1}.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 9.50 (s, 1 H), 8.14 (d, J = 2.4 Hz, 1 H), 7.56-7.59 (m, 3 H), 7.19-7.30 (ABq, J = 8.6 Hz, 4 H), 5.07 (br, 4 H), 3.86 (s, 2 H), 1.78 (br, 2 H), 1.70 (s, 6 H), 1.35 (s, 18 H).

HRMS calcd for $C_{34}H_{41}N_7O_2$: 579.3322, found 579.3320.

Thymine-1-acetic acid active ester

Thymine-1-acetic acid **62** and phenol derivative (2-3 equiv) in dry THF under argon at 0 °C was treated with N-ethyl-N'-3-dimethylaminopropyl carbodiimide methodide (1.0 equiv) and stirred overnight while allowing to warm to room temperature. The supernatent solution was concentrated and flash chromatographed with appropriate solvent. The product obtained was triturated in hexanes.

2,4,5-Trichlorophenyl ester 94a

23 % yield

mp; 177-178 °C

IR (KBr); 1775, 1696, 1457, 1350, 1234 cm^{-1}.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 8.30 (br, 1 H), 7.58 (s, 1 H), 7.37 (s, 1 H), 7.01 (m, 1 H), 4.73 (s, 2 H), 1.96 (d, J = 1.3 Hz, 3 H).

***o*-Chlorophenyl ester 94b**

13 % yield

mp; 217-218 °C

IR (KBr); 1764, 1700, 1652, 1477, 1234, 1216, 1184 cm^{-1}.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 8.20 (br, 1 H), 7.18-7.48 (m, 4 H), 7.03 (m, 1 H), 4.76 (s, 2 H), 1.96 (d, J = 0.9 Hz, 3 H).

Phenyl ester 94c

41 % yield

mp; 193-194 °C

IR (KBr); 1756, 1696, 1652, 1457, 1203 cm^{-1}.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 8.35 (br, 1 H), 7.40 (t, J = 7.3 Hz, 2 H), 7.26 (t, J = 7.4 Hz, 1 H), 7.13 (d, J = 7.4 Hz, 2 H), 7.02 (m, 1 H), 4.69 (s, 2 H), 1.95 (d, J = 1.0 Hz, 3 H).

HRMS calcd for $C_{13}H_{12}N_2O_4$: 260.0797, found: 260.0795.

***p*-*t*-Butylphenyl ester 94d**

47 % yield

mp; 97-100 °C

IR (KBr); 1772, 1685, 1509, 1467, 1181 cm^{-1}.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 8.32 (br, 1 H), 7.37-7.41 (m, 2 H), 7.01-7.06 (m, 3 H). 4.68 (s, 2 H), 1.95 (d, J = 1.3 Hz, 3 H), 1.31 (s, 9 H).

***p*-Methoxyphenyl ester 94e**

43 % yield

mp; 188-189 °C

IR (KBr); 1758, 1719, 1683, 1506, 1370, 1205 cm^{-1}.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 8.51(br, 1 H), 6.87-7.06 (m, 5 H), 4.67 (s, 2 H), 3.80 (s, 3 H), 1.95 (d, J = 1.3 Hz, 3 H).

Xanthene triazine - thymine template 95

Xanthene triazine *p*-aminomethylphenyl amide **93** (30 mg, 0.052 mmol) and the trichlorophenyl ester **94a** (19 mg, 1.0 equiv) were allowed to react in CH_2Cl_2 (5.17 mL) and Et_3N (14.4 μL). The reaction was completed within 2 h, generating white suspension. The crude product was chromatographed with 10 % MeOH in $CHCl_3$ to give 35 mg (93 %) of a white solid.

mp; >340 °C

IR (KBr); 3345,3211, 2963, 2869, 1696, 1685, 1539, 1437, 1266, 827 cm^{-1}.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 13.41 (s, 1 H), 9.46 (s, 1 H), 8.08 (br, 1 H), 7.0 - 7.7 (m, 11 H), 5.16 (br, 2 H, triazine NH_2 not involved in hydrogen bonding), 4.31 (d, J = 5.0 Hz, 2 H), 4.26 (s, 2 H), 1.94 (s, 3 H), 1.69 (s, 6 H), 1.36 (s, 9 H), 1.35 (s, 9 H).

HRMS calcd for $C_{41}H_{47}N_9O_5$: 745.3700, found 745.3693.

di-t-butylxanthene diacid dimethyl ester 96

Xanthene diacid **7b** (13.28 g, 32.3 mmol) was refluxed in methanol (500 mL) and sulfuric acid (20 mL) for 4 h. After cooling, the precipitate was filtered off, washed with cold methanol and dried to yield the dimethyl ester **96** (14.56 g, 97 %).

mp; 220 °C

IR (KBr); 2964, 2905, 2870, 1730, 1707, 1445, 1316, 1276, 1101, 1009, 894 and 784 cm^{-1}.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 7.63 (d, J = 2.4 Hz, 2 H), 7.54 (d, J = 2.4 Hz, 2 H), 3.97 (s, 6 H), 1.64 (s, 6 H), 1.33 (s, 18 H).

Di-t-butylxanthene diacid monomethyl ester 97

Gaseous HBr was bubbled into CH_2Cl_2 at 0 °C for 25 min and dimethyl ester **96** (12.39 g, 28.25 mmol) was added at 0 °C. The strongly yellow colored solution was stirred for 2h at 0 °C (TLC). The reaction mixture was poured into ice water (500 mL). The aqueous layer was saturated with NaCl and extracted twice with CH_2Cl_2 (200 mL), the combined organic layers wrere dried (MgSO4), and concentrated to give 11.89 g (99 %) of pure mono acid **97**.

mp; 178 °C

IR (KBr); 3317(COOH, sharp), 2963, 2908, 2872, 1717, 1444, 1324, 1270, 1245, 1118, 998 and 788 cm^{-1}.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 11.92 (s, 1 H), 8.25 (d, J = 2.5 Hz, 1 H), 7.98 (d, J = 2.4 Hz, 1 H), 7.70 (d, J = 2.4 Hz, 1 H), 7.67 (d, J = 2.6 Hz, 1 H), 4.01 (s, 3 H), 1.68 (s, 6 H), 1.37 (s, 9 H), 1.36 (s, 9 H).

Xanthene phenyl ester methyl ester 98

Xanthene monomethyl ester mono acid **97** (1.00 g., 2.36 mmol), 666 mg of phenol (3.0 eq), and DMAP (10 mg) in 20 mL dry CH_2Cl_2 was treated with DCC (535 mg, 1.1 equiv). After 6 h, the reaction was concentrated and

chromatographed to give 1.112 g of impure product, which was contaminated with ~ 10 % DCU. This impure material was used directly for the next step.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 7.82 (d, J = 2.4 Hz, 1 H), 7.63 (d, J = 2.4 Hz, 1 H), 7.58 (d, J = 2.4 Hz, 1 H), 7.55 (d, J = 2.4 Hz, 1 H), 7.23 - 7.47 (m, 4 H), 3.63 (m, 3 H), 1.67 (s, 6 H), 1.37 (s, 9 H), 1.33 (s, 9 H)

Xanthene triazine methyl ester 99

Xanthene phenyl ester methyl ester **98** (826 mg, 1.65 mmol), biguanide (230 mg, 1.38 eq) and triethylamine (500 μL) in 15 ml absolute EtOH under argon were heated to reflux for 90 min., the white solid was filtered while hot, washed ~5 mL ethanol to give 547 mg of pure triazine methyl ester **99**. From the filtrate an additional 47 mg was obtained by flash chromatography (5 % MeOH in CH_2Cl_2).
Total yield was 594 mg (69 % from xanthene monomethyl ester **97**).

mp; 335-336 °C
IR (KBr); 3497, 3484, 3282, 3102, 2964, 2870, 1717, 1643, 1620, 1542, 1515,1437, 1394, 1273, 1260, 828 cm^{-1}.
^{1}H-NMR (250 MHz, $CDCl_3$); δ 7.48 - 7.55 (m, 4 H), 5.21 (br, 4 H), 3.76 (s, 3 H), 1.65 (s, 6 H), 1.34 (s, 9 H), 1.32 (s, 9 H).

Xanthene triaxine ethylene diamine hemiamide 100

Xanthene triazine methylester **99** (275 mg, 0.562 mmol) was suspended in anhydrous ethylene diamine (20 mL) under argon and heated to 46° C for 13

h. The reaction was heated additional 2 h at 67 °C. Removal of ethylene diamine under reduced pressure gave **100** as a white solid (quantitative).

mp; 275 °C (dec.)
IR (KBr); 3350, 3195, 2962, 2869, 1653, 1617, 1539, 1437, 1394, 1266, 827 cm^{-1}.
^{1}H-NMR (250 MHz, $CDCl_3$); δ 8.45 (t, J = 6.1 Hz, 1 H), 8.16 (d, J = 2.4 Hz, 1 H), 7.53-7.56 (m, 3 H), 5.88 (br, 4 H), 3.5 0 (q, J = 6.1 Hz, 2 H), 2.82 (t, J = 6.1 Hz, 2 H), 1.67 (s, 6 H), 1.58 (br, NH_2 + H_2O), 1.34 (s, 18 H).
HRMS calcd for $C_{29}H_{39}N_7O_2$: 517.3165, found: 517.3162.

Xanthene tiazine thymine template with ethylene spacer 101

Xanthene triazine ethylene diamine hemiamide **100** (61 mg, 0.118 mmol) and the trichlorophenyl ester **94a** (43 mg, 1.0 eq) were allowed to react for 20 min in 6 mL of dry CH_2Cl_2 and Et_3N (100 μL) under argon. Flash chromatography of the concentrated residue with 5-10 % MeOH in CH_2Cl_2 gave 81 mg (100 %) of product as a white powder.

mp; 200-205 °C
IR (KBr); 3340, 3217, 2960, 2850, 1684, 1652, 1538, 1535, 1432 cm^{-1}.
^{1}H-NMR (250 MHz, $CDCl_3$); δ 12.9 (br, 1 H), 8.34 (t, 1 H), 8.08 (d, J = 2.2 Hz, 1 H), 7.75 (br, 2 H: hydrogen bonded triazine NH_2), 7.57 (d, J = 2.3 Hz, 1 H), 7.52 (d, J = 2.1 Hz, 1 H), 7.47 (d, J = 2.1 Hz, 1 H), 7.31 (br, 1 Hr), 7.07 (s, 1 H), 5.43 (br, 2 H, non-hydrogen bonded triazine NH_2), 4.31 (s, 2 H), 3.3-3.5 (m, 4 H), 1.90 (s, 3 H), 1.67 (s, 6 H), 1.34 (s, 18 H).
HRMS calcd for $C_{36}H_{45}N_9O_5$: 683.3544, found 683.3543.

Kinetic Studies.

The reaction of thymine ester **94c** with triazine amine **100** in the presence and absence of template **101** was performed in $CHCl_3$ solution containing ca. 18 equivalent triethylamine (TEA). A Waters 600 HPLC equipped with a UV dectector (254 nm, auf = 1.0) was used for analysis of reaction mixtures. Analyses were performed using mixture of water/methanol/TEA (16 : 84 : 0.6) as a mobile phase and a reverse phase column (Beckman C18 column, Ultrasphere ODS dp, 5 μ, 4.6 mm, I.D x 25 cm, flow rate = 1.0 mL/min). The integrations and concentrations of all the peaks were calculated using an NEC computer and Waters 820 Baseline software. Chloroform was dried over molecular sieves. All experiments were performed at ambient temperature. Each run was performed 2-3 times to obtain average values for data. Wheaton screw-cap vials (0.5-mL) equipped with Mininert valves were used to minimize evaporative losses of solvent.

Typical Reaction Procedures.

A 0.5-mL Wheaton screw-cap vial equipped with a Mininert valve was charged with 20 μL of $CHCl_3$, 2 μL of TEA, 40 μL of triazine **100** stock solution (2.0×10^{-2} M) and 40 μL of thymine ester **94c** stock solution (2.0×10^{-2} M). The reaction mixture was stirred gently with a magnetic stirrer. Aliquots (2.0 μL) were withdrawn periodically and analysed by HPLC. The retention times of product (template) **101**, triazine amine **100**, and thymine ester **94c** were 7.4 min, >12 min, and 2.4 min respectively.

Adenine-Thymine hybrid 102

Amino adenosine **87** (57 mg, 0.186 mmol) and thymine active ester **94a** (68 mg, 1.0 equiv) under argon were diluted with dry CH_2Cl_2 (5 mL) and Et_3N (100 μL), and stirred for 20 min at room temperature. The suspension was concentrated and resuspended in $CHCl_3$, filtered and washed succesively with EtOAc (2 mL), $CHCl_3$ (2 mL) then dried under vacuum to give 69 mg (78 %) of **102** as white powder.

mp; 188-193 °C.

IR (KBr); 3486 (br), 2970, 1700 and 903 cm^{-1}.

^{1}H-NMR (DMSO-d_6, 250 MHz); δ 11.27 (s, 1 H, imide), 8.49 (t, 1 H, amide), 8.34 (s, 1 H), 8.19 (s, 1 H), 7.40 (m, 3 H, NH_2+thymine methine), 6.13 (d, J = 2.8 Hz), 1H), 4.88 (ABq, J = 6.3 and 2.9 Hz, 1 H) 4.40 (ABq, J = 6.2 and 2.9 Hz, 1 H), 4.30 (s, 2 H), 4.18 (m, 1 H), 3.39 (t, J = 5.3 Hz, 2 H), 1.73 (s, 3 H), 1.53 (s, 3 H), 1.30 (s, 3 H)

Triazine-Kemp's imide hybrid 103

A mixture of xanthene amine **100** (30 mg, 5.8 x 10^{-5} mol), and imide pentafluorophenyl ester **88a** (ca 40 mg, 1.0 equiv) under argon was diluted with dry CH_2Cl_2 (5 mL) and Et_3N (50 μL). After stirring for 40 h at room temperature, white solid formed was filtered off, washed with 5 mL of CH_2Cl_2-hexanes (1:1) and with hexanes (5 mL) then dried under vacuum to give 48 mg (81 %) of **103** as white powder.

mp; 267-268 °C.

IR (KBr); 3461 (br), 2961, 2870, 1700 and 1539 cm^{-1}.

^{1}H-NMR ($CDCl_3$, 250 MHz); (all of the exchangeable protons were integrated less than they should be) δ 9.9 (br, amide), 8.5 (br, amide), 8.13 (s, 1 H), 7.2-7.8 (m, 11 H), ca 6.3 (br, two triazine NH's involved in H-bond), ca 5,4 (br, two triazine NH's not involved in H-bond), 3.2-3.5 (m, 4 H), 2.67 (d, 2 H, equatorial), 2.20 (d, 1 H, axial), 0.8-2.0 (m, 48 H).

HPLC conditions for reaction between triazine 100 and imide 88a.

A 0.5-mL Wheaton screw-cap vial equipped with a Mininert valve was charged with 20 μL of $CHCl_3$, 2 μL of TEA, 40 μL of triazine **100** stock solution (2.0×10^{-2} M) and 40 μL of imide **88a** stock solution (2.0×10^{-2} M) ([**88a**] = 8.0 mM, [**100**] = 8.0 mM, 18 equiv of Et_3N). The reaction mixture was stirred gently with a magnetic stirrer. Aliquots (2.0 μL) were withdrawn periodically and analysed by HPLC. Elution solvent was mixtures of methanol-water (87:13) with 3.5 μL of Et_3N. Flow rate was 1.5 mL/min with auf = 0.8 (254 nm). The retention times of product **103**, triazine amine **100**, and imide ester **88a** were 12.6 min, 9.5 min, and >17 min (broad), respectively.

HPLC conditions for reaction between amino adenosine 87 and thymine 94b.

A 0.5-mL Wheaton screw-cap vial equipped with a Mininert valve was charged with 50 μL of CH_3CN, 0.5 μL of TEA, 25 μL of adenosine **87** stock solution (1.2×10^{-2} M) and 25 μL of thymine **94b** stock solution (1.2×10^{-2} M) ([**87**] = [**94b**] = 3.0 mM). The reaction mixture was stirred gently with a magnetic stirrer. Aliquots (3.0 μL) were withdrawn periodically and analysed by HPLC. Elution solvent was mixtures of methanol-water (50:50) containing 0.3 % AcOH. Flow rate was 1.0 mL/min with auf = 0.6 (254 nm). The

retention times of product (template) **102**, adenosine **87**, and thymine ester **94b** were 5.2 min, >9.0 min (broad), and 8.2 min, respectively.

Experimentals for chapter 5

N,N'-bis (3-nitrophenyl) urea 105

To a yellow homogeneous solution of 3-nitroaniline (2.00 g, 14.5 mmol) and triethylamine (2 mL) in dry THF (20 mL) under argon at room temperature was added slowly phenylchloroformate (908 µL). The resultant suspension was refluxed overnight. Solvent was removed and the residue taken up with EtOAc and dil HCl, washed twice with 1N-HCl, twice with 1N-NaOH. After concentration, the crude product was purified by triturating in 1:1 CH_2Cl_2-hexanes to give 1.651 g (75%) of yellow powder.

mp; 247-248 °C
IR (KBr); 3331(br), 1661 cm-1.
^{1}H-NMR (250 MHz, DMSO-d_6); δ 9.39 (s, 2 H, urea NH), 8.55 (t, J = 2.2 Hz, 2 H), 7.74-7.87 (m, 4 H), 7.58 (t, J = 8.19 Hz, 2 H).

N,N'-bis (3-aminophenyl) urea 106

A suspension of N,N'-bis (3-nitrophenyl) urea **106** (800 mg, 2.65 mmol) in 30 mL absolute ethanol was hydrogenated with Pd/C (100 mg, 5% Pd) under atmospheric hydrogen pressure (balloon) for 24 h. The grey slurry was diluted with methanol (product soluble in methanol), filtered and the filtrate

concentrated. Trituration in CH_2Cl_2 gave 590 mg (92%) of greyish cottonlike solid.

mp; 185 °C(dec)
IR (KBr); 3394, 3274(br), 1634 cm-1.
^{1}H-NMR (250 MHz, DMSO-d_6); δ 8.24 (s, 2 H, urea NH), 6.86 (t, J = 7.9 Hz, 2 H), 6.75 (t, J = 2.0 Hz, 2 H), 6.53 (m, 2 H), 6.16 (m, 2 H), 5.12 (br, 4 H, amino NH).

Urea cleft dimethyl ester 107

Diamino urea **106** (143 mg, 0.59 mmol) was reacted with xanthene mono acid chloride **97a** (derived from corresponding mono acid (500 mg, 1.18 mmol)) in THF (20 mL) in the presence of triethyl amine (100 μL) for 2 h. The concentrated crude product was chromatographed (10-15 % EtOAc in CH_2Cl_2) to give 480 mg (77 %) of white solid.

mp; 187-198 °C.
IR (KBr); 3331 (br), 1719, 1437 and 1237 cm^{-1}.
^{1}H-NMR (250 MHz, $CDCl_3$); δ 10.63 (s, 2 H), 8.33 (d, J = 2.5 Hz, 2 H), 7.81 (d, J = 2.5 Hz, 2 H), 7.74 (s, 2 H), 7.65 (d, J = 2.4 Hz, 2 H), 7.59 (d, J = 2.5 Hz, 2 H), 7.18-7.50 (m, 8 H), 3.75 (s, 6 H), 1.67 (s, 12 H), 1.34 (s, 36 H).

Diacid cleft 108

Dimethyl ester **107** (156 mg, 0.148 mmol) was treated with NaOH (230 mg) in 6 mL of MeOH-THF (5:1) and heated to reflux for 1 h. After removal of solvent, the residue was taken up with $CHCl_3$-1N HCl, extracted twice with $CHCl_3$,

washed with 1N HCl, dried over Na_2SO_4. Concentration gave 150 mg (99 %) of **108** as white powder.

mp; 218-222 °C

IR (KBr); 3500-2700 (br), 1717, 1529, 1434 and 1264 cm^{-1}

^{1}H-NMR (250 MHz, $CDCl_3$); δ 10.80 (br, 2 H), 8.31 (d, J = 2.4 Hz, 2 H), 7.97 (br, 2 H), 7.87 (s, 2 H), 7.58-7.65 (br, 8 H), 7.22 (m, 2 H), 6.91 (br, 2 H), 1.68 (s, 12 H), 1.34 (s, 18 H), 1.30 (s, 18 H).

Cleft diamide 109

A mixture of diacid **108** (125 mg, 0.121 mmol) and carbonyl diimidazole (98 mg, in exess) in dry CH_2Cl_2 (5 mL) under argon was heated to reflux for 1 h, and cooled to room temperature. Gaseous ammonia was bubbled in for short period, stirred for 30 mim. The reaction was diluted with EtOAc (50 mL), washed with 1N HCl (2 x 50 mL) and dried (Na_2SO_4). The crude product was purified by flash chromatography with 25-50 % EtOAc in CH_2Cl_2 to give 115 mg (92%) of the diamide cleft **109** as white powder.

mp; 242-245 °C

IR (KBr); 3600-3000 (br), 1650, 1436 and 1262 cm^{-1}

^{1}H-NMR (250 MHz, DMSO-d_6); δ 10.49 (s, 2 H), 8.79 (s, 2 H), 8.02 (s, 2 H), 7.92 (s, 2 H), 7.80 (d, J = 2.5 Hz, 2 H), 7.72 (m, 4 H), 7.61 (s, 2 H), 7.54 (d, J = 2.2 Hz, 2 H), 7.21-7.36 (m, 6 H), 1.68 (s, 12 H), 1.35 (s, 18 H), 1.31 (s, 18 H).

Extraction Experiment.

Extraction experiments (between urea cleft **108, 109** and organic diacids) were perfomed as described in Chapter 1.

Diethyl 2,2′-bipyridine-5,5′-dicarboxylate 110

Ethyl nicotinate (30 g, 0.20 mol) and 10 % Pd/C (10 g) in 100 mL round bottomed flask equipped with reflux condenser was heated to 125-130 ^{o}C under ca 14 mmHg for 5 days. The hot reaction mixture was filtered and washed with methylene chloride. Filtrate was concentrated and the residue was crystalized by addition of hexanes. Yellowish crystals were redissolved in EtOAc and treated with activated charcoal, filtered and concentrated. Trituration in hexanes containing water (ca 10 mL) gave 5.818 g of product as white needles. Second crop (1.400 g) was obtained from mother liquid (total 24 % yield).

^{1}H-NMR (250 MHz, $CDCl_3$); δ 9.25 (d, J = 1.6 Hz, 2 H), 8.57 (d, J = 8.3 Hz, 2 H), 8.41 (dd, Ja = 8.3 Hz, Jb = 2.0 Hz, 2 H), 4.41 (q, J = 7.0 Hz, 4 H), 1.41 (t, J = 7.0 Hz, 6 H).

2,2′-bipyridine-5,5′-dicarboxylic acid 111a

Diethyl 2,2′-bipyridine-5,5′-dicarboxylate **110** (4.828 g, 16 mmol) suspended in 150 mL of ethanol was treated with KOH (2.71 g) in 10 mL water and refluxed for 2 h. Solvent was removed in vacuo, the residue dissolved in warm water (ca 60 mL) and the pH was adjusted to ca 6 by addition of conc. HCl. After cooling, the white white precipitate was collected by filteration, washed with water (80 mL) and ethanol (30 mL), then dried in vacuum desicator for 3 days

to give 3.191 g (81 %) of white powder. This diacid was analysed after conversion to corresponding diacid chloride.

2,2′-bipyridine-5,5′-diacid chloride 112 (as x hydrochloride)

Bipyridine diacid **111a** (200 mg, 0.819 mmol) in 6 mL of $SOCl_2$ was refluxed for 4 h. The resulting yellow homogeneous solution was concentrated in vacuo, then dried under high vacuum for 1 h to give yellowish crystalline solid.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 9.38 (d, J = 2.0 Hz, 2 H), 8.72 (d, J = 8.5 Hz, 2 H), 8.54 (dd, Ja = 8.4 Hz, Jb = 2.2 Hz, 2 H).

5,5′-Dibenzyloxycarbonylamino-2,2′-bipyridine 117

2,2′-Bipyridine-5,5′-dicarboxylic acid **111a** (650 mg, 2.66 mmol) in 10 mL $SOCl_2$ was refluxed for 3 h. Thionyl chloride was removed and dried in vacuum. The acid chloride suspended in reagent grade acetone (27 mL) at 0 °C was treated slowly with 1.03 g of NaN_3 in 3 mL of water. After 30 min. the reaction was diluted to 50 mL with water, filtered, washed with water (20 mL) and dried in vacuum (856 mg as crude). The crude azide **116** suspended in toluene (32 mL) and benzyl alcohol (1.10 mL, 4.0 mol equiv.) under argon was refluxed overnight. Solvent removal followed by trituration in chloroform (30 mL) gave 962 mg (80 %) of the dicarbamate **117** as yellowish powder.

mp; 255 °C (dec).

IR (KBr); 3467 (br), 3313, 1701 1523, 1240, 1217 cm-1.

^{1}H-NMR (250 MHz, DMSO-d_6); δ 10.27 (s, 2 H), 8.72 (d, J = 2.4 Hz, 2 H), 8.25 (d, J = 8.7 Hz, 2 H), 8.03 (dd, Ja = 8.8 Hz, Jb = 2.4 Hz, 2 H), 7.30-7.46 (m, 10 H), 5.19 (s, 4 H).

5,5′-Diamino-2,2′-bipyridine 118

Stirring the dicarbamate **117** (930 mg, 2.04 mmol) and 10 % Pd/C (150 mg) in 100 mL of ethanol-THF (1:1) under hydrogen baloon for 28 h showed almost no hydrogenolysis had taken place (by TLC). 1,4-Cyclohexadiene (3 mL) and additional catalyst (50 mg) was added and refluxed. After 5 h, additional 1,4-cyclohexadiene (2 mL) was added and refluxed for an additional 15 h. The reaction was filtered and concentrated. Trituration of the residue in $CHCl_3$-hexanes (10 mL, 1:1) gave 351 mg (92 %) of the diamino bipyridine **118** as yellow powder.

^{1}H-NMR (250 MHz, DMSO-d_6); δ 7.84-7.91 (m, 4 H), 6.97 (d, J = 8.5 Hz, 2 H), 5.46 (br, 4 H).

The Curtius degradation of commercially available 2,2′-bipyridine-4,4′-dicarboxylic acid using identical procedure gave similar results.

4,4′-Dibenzyloxycarbonylamino-2,2′-bipyridine 114

72 % yield

mp; 213-215 °C

^{1}H-NMR (250M Hz, DMSO-d_6); δ 10.36 (s, 2 H), 8.51 (d, J = 2.0 Hz, 2 H), 8.47 (d, J = 5.6 Hz, 2 H), 7.50 (dd, Ja = 5.6 Hz, Jb = 2.2 Hz, 2 H), 7.30-7.45 (m, 10 H), 5.20 (s, 4 H).

4,4'-Diamino-2,2'-bipyridine 115

100 % yield

mp; 253 °C (dec).

^{1}H-NMR (250 MHz, DMSO-d_6); δ 8.00 (d, J = 5.4 Hz, 2 H), 7.51 (d, J = 2.0 Hz, 2 H), 6.42 (dd, Ja = 5.5 Hz, Jb = 2.3 Hz, 2 H), 6.02 (br, 4 H).

Amino xanthene methyl ester 119

Xanthene monomethyl ester **97** (1.050 g, 2.47 mmol) in dry toluene (8 mL) under argon was refluxed for 2 h with diphenyl phosphoryl azide (DPPA) (586 μL, 1.1 equiv.) and triethyl amine (379 μL, 1.1 equiv). Benzyl alcohol (512 μL, 2.0 equiv.) was added and refluxed overnight. Toluene was removed, the residue was diluted with CH_2Cl_2 (100 mL), washed with 1N HCl (100 mL), $NaHCO_3$ (100 mL) and dried (Na_2CO_3). Chromatographic separation (40 % CH_2Cl_2 in hexanes) gave 705 mg (56 %) of the benzylcarbamate **120**.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 8.25 (s, 1 H), 8.15 (br, 1 H), 7.89 (d, J = 2.4 Hz, 1 H), 7.62 (d, J = 2.4 Hz, 1 H), 7.32-7.48 (m, 5 H), 7.07(d, J = 2.4 Hz, 1 H), 5.29 (s, 2 H), 3.92 (s, 3 H), 1.62 (s, 6 H), 1.34 (s, 18 H).

The benzylcarbamate **120** (705 mg) in MeOH-THF (20 mL, 1:1) was refluxed for 2 h with 10 % Pd/C (73 mg) and 1,4-cyclohexadiene (1 mL). Filtration

followed by concentration gave 526 mg (quantitative) of the amino xanthene methyl ester **119**.

mp; 144-146 °C

IR (KBr); 3447, 3356, 2965, 2951, 2903, 2867, 1717, 1695,1457, 1436, 1280 and 1009 cm^{-1}.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 7.83 (d, J = 2.4 Hz, 1 H), 7.63 (d, J = 2.4 Hz, 1 H), 6.81 (d, J = 2.4 Hz, 1 H), 6.74 (d, J = 2.4 Hz, 1 H), 4.15 (br, 2 H), 3.96 (s, 3 H), 1.64(s, 6 H), 1.32 (s, 9 H).

Bipyridine cleft dimethyl ester 124

Amino xanthene methyl ester **119** (526 mg, 1.33 mmol), 2,2'-bipyridine-5,5'-diacid chloride **112** (211 mg, 0.5 equiv. assumed as dihydrochloride) and triethyl amine (370 μL) in dry CH_2Cl_2 (30 mL) was stirred for 1 h. Concentration followed by trituration in ethanol gave 660 mg (99 %) of bipyridine cleft dimethyl ester **124** as (yellowish) powder.

mp; >320 °C

^{1}H-NMR (250 MHz, $CDCl_3$); δ 9.81 (s, 2 H), 9.56 (d, J = 2.3 Hz, 2 H), 8.82 (dd, Ja = 10.6 Hz, Jb = 2.3 Hz, 2 H), 8.68-8.71 (m, 4 H), 7.89 (d, J = 2.3 Hz, 2 H), 7.67 (d, J = 2.3 Hz, 2 H), 7.20 (d, J = 2.3 Hz, 2 H), 4.00 (s, 6 H), 1.67 (s, 12 H), 1.41 (s, 18 H), 1.37 (s, 18 H).

Corresponding $ZnCl_2$ complex was prepared by stirring with exess $ZnCl_2$ in $CDCl_3$. Exess $ZnCl_2$ was removed by filtration, the filtrate concentrated, redissolved in $CDCl_3$, and analysed by nmr.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 10.18 (s, 2 H), 9.87 (s, 2 H), 9.18 (d, J = 8.3 Hz, 2 H), 8.64 (d, J = 8.4 Hz, 2 H), 8.54 (s, 2 H), 7.90 (d, J = 2.3 Hz, 2 H), 7.65 (d, J = 2.3 Hz, 2 H), 7.24 (d, J = 2.3 Hz, 2 H), 4.01 (s, 6 H), 1.65 (s, 12 H), 1.36 (s, 36 H).

Aminoxanthene benzyl ester 119a

mp; 138-140 °C

IR (KBr); 3476, 3377, 2963, 2905, 2867, 1717, 1700, 1627, 1457, 1280, 1246, 1194 and 1103 cm^{-1}.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 7.90 (d, J = 2.3 Hz, 1 H), 7.62 (d, J = 2.3 Hz, 1 H), 7.37-7.50 (m, 5 H), 6.77 (d, J = 1.8 Hz, 1 H), 6.64 (d, J = 2.0 Hz, 1 H), 5.42 (s, 2 H), 3.65 (br, 2 H), 1.63 (s, 6 H), 1.36 (s, 9 H), 1.29 (s, 9 H).

Bipyridine cleft dibenzyl ester 125

Prepared similarly as in corresponding dimethyl ester case (85 % yield).

^{1}H-NMR (250 MHz, $CDCl_3$); δ 9.78 (s, 2 H), 9.61 (s, 2 H), 8.68-8.72 (m, 6 H), 7.92 (d, J = 2.2 Hz, 2 H), 7.67 (d, J = 2.4 Hz, 2 H), 7.25-7.50 (m, 10 H), 7.19 (d, J = 1.9 Hz, 2 H), 5.35 (s, 4 H), 1.66 (s, 12 H), 1.40 (s, 18 H), 1.36 (s, 18 H)

Naphthalene cleft dimethyl ester 128

mp; >310 °C

^{1}H-NMR (250 MHz, $CDCl_3$); δ 9.86 (s, 2 H), 8.99 (s, 2 H), 8.78 (d, J = 1.9 Hz, 2 H), 8.38 (d, 8.6 Hz, 2 H), 8.25 (d, J = 8.6 Hz, 2 H), 7.89 (d, J = 2.3 Hz, 2 H), 7.68 (d, J =

2.3 Hz, 2 H), 7.20 (d, J = 2.0 Hz, 2 H), 3.91 (s, 6 H), 1.68 (s, 12 H), 1.42 (s, 18 H), 1.37 (s, 18 H).

Aminoxanthene methylester Benzamide 129a

mp; 177-179 °C

^{1}H-NMR (250 MHz, $CDCl_3$): δ 9.60 (s, 1 H), 8.71 (d, J = 2.2 Hz, 1 H), 8.26-8.31 (m, 2 H), 7.85 (d, J = 2.4 Hz, 1 H), 7.66 (d, J = 2.4 Hz, 1 H), 7.5-7.6 (m, 3 H), 7.17 (d, J = 2.2 Hz, 1 H), 3.90 (s, 3 H), 1.66 (s, 6 H), 1.39 (s, 9 H), 1.36 (s, 9 H).

Biphenyl cleft dimethylester 127

mp; >310 °C

^{1}H-NMR (250 MHz, $CDCl_3$); δ 9.69 (s, 2 H), 8.74 (d, J = 1.93 Hz, 2 H), 8.43 (d, J = 8.3 Hz, 4 H), 7.87 (d, J = 2.3 Hz, 2 H), 7.82 (d, J = 8.2 Hz, 4 H), 7.67 (d, J = 2.3 Hz, 2 H), 7.18 (d, J = 1.9 Hz, 2 H), 3.96 (s, 6 H), 1.67 (s, 12 H), 1.41 (s, 18 H), 1.37 (s, 18 H).

Bipyridine cleft diacid 126

Bipyridine cleft dimethyl ester **124** (105 mg, 0.105 mmol) suspended in THF (15 mL) was treated with tetrabutylammonium hydroxide (227 mg, 1.67 equiv., 40 % solution in water). Immediately, the reaction turned to a yellow homogeneous solution. After 4 h, the reaction was acidified with dil HCl (30 mL), concentrated and redissolved in dil HCl (30 mL) + EtOAc (30 mL). The solid formed during shaking was filtered out to give 46 mg of yellow powder, which appears to be the hydrochloride salt.

mp; >320 °C

^{1}H-NMR (250 MHz, $CDCl_3$-CD_3OD = 9:1); δ 9.72 (d, J = 1.8 Hz, 2 H), 9.24 (dd, Ja = 8.3 Hz, Jb = 1.7 Hz, 2 H), 9.11 (d, J = 8.5 Hz, 2 H), 8.50 (d, J = 1.8 Hz, 2 H), 7.92 (d, J = 2.4 Hz, 2 H), 7.60 (d, J = 2.4 Hz, 2 H), 7.20 (d, J = 2.2 Hz, 2 H), 1.61 (s, 12 H), 1.33 (s, 18 H), 1.30 (s, 18 H).

From the remaining organic layer, 32 mg of yellow powder (after trituration in CH_2Cl_2-hexanes) was obtained (**126**).

^{1}H-NMR (250 MHz, $CDCl_3$-CD_3OD = 9:1); δ 9.50 (d, J = 2.2 Hz, 2 H), 8.74 (dd, Ja = 8.4 Hz, Jb = 2.1 Hz, 2 H), 8.56 (d, J = 2.0 Hz, 2 H), 8.48 (d, J = 8.3 Hz, 2 H), 7.90 (d, J = 2.4 Hz, 2 H), 7.61 (d, J = 2.4 Hz, 2 H), 7.17 (d, J = 2.1 Hz, 2 H), 1.62 (s, 12 H), 1.35 (s, 18 H), 1.32 (s, 18 H).

Xanthene diacid O-methylphenylalanine monoamide 121

To a homogeneous solution of xanthene diacid **7b** (800 mg, 1.95 mmol), phenylalanine methyl ester hydrochloride (441 mg, 1.05 mol equiv), triethylamine (150 µL, 1.05 mol equiv.) and several crystals of DMAP in dry CH_2Cl_2 (30 mL), under argon at 0 °C was treated with DCC (442 mg, 1.1 mol equiv.), and ice bath removed , stirred for 40 min at room temperature. The reaction was diluted with n-hexanes (100 mL), filtered. The filtrate was concentrated and the residue chromatographed with EtOAc in CH_2Cl_2. Traces of DCU contaminant were removed by precipitating with CH_2Cl_2-hexanes. The desired monoamide **121** (551mg, 49 %) was obtained as white powder.

mp; 118-125 °C

^{1}H-NMR (250 MHz, $CDCl_3$); δ 8.00 (d, J = 2.4 Hz, 1 H), 7.96 (br, 1 H), 7.74 (d, J = 2.4 Hz, 1 H), 7.64 (d, J = 2.5 Hz, 1 H), 7.56 (d, J = 2.4 Hz, 1 H), 7.18-7.30 (m, 5 H), 5.20 (q, J = 7.7Hz, 1 H), 3.77 (s, 3 H), 3.33 (m, 2 H), 1.70 (s, 3 H), 1.61 (s, 3 H), 1.35 (s, 9 H), 1.32 (s, 9 H).

Amino xanthene O-methylphenylalanine amide 123

Xanthene diacid O-methylphenylalanine monoamide **121** (444 mg, 0.777 mmol), DPPA (218 μL, 1.3 equiv.), triethylamine (141 μL, 1.3 equiv) and benzyl alcohol (105 μL, 1.3 equiv.) in dry toluene (9 mL) under argon was refluxed for 1.5 h. Concentration followed by chromatographic separation (CH_2Cl_2-5 % EtOAc in CH_2Cl_2) gave 493 mg (94 %) of the benzylcarbamate **122** as colorless viscous liquid (solidified very very slowly).

^{1}H-NMR (250 MHz, $CDCl_3$); δ 7.99 (br, 1 H), 7.90 (br, 1 H), 7.69 (d, J = 2.3 Hz, 1 H), 7.57 (d, J = 2.4 Hz, 1 H), 7.12-7.46 (m, 12 H), 5.26 (s, 2 H), 5.00 (m, 1 H), 3.66 (s, 3 H), 3.05-3.25 (m, 2 H), 1.73 (s, 3 H), 1.63 (s, 3 H), 1.39 (s, 9 H), 1.36 (s, 9 H).

Hydrogenolysis of benzylcarbamate **122** (607 mg, 0.897 mmol) required prolonged (~2.5 days) heating in ethanol (or MeOH-THF) with Pd/C and 1,4-cyclohexadiene (with periodic addition of more catalyst and more 1,4-cyclohexadiene). Chromatographic purification (5 % EtOAc in CH_2Cl_2) gave 369 mg (76 %) of unblocked amine **123** as oil, which solidified upon standing.

mp; 125-127 °C.

IR (KBr); 2954, 2870, 1743, 1653, 1436, 1363 and 898 cm^{-1}.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 7.96 (d, J = 7.5 Hz, 1 H), 7.90 (d, J = 2.5 Hz, 1 H), 7.54 (d, J = 2.4 Hz, 1 H), 7.18-7.30 (m, 5 H), 6.82 (d, J = 1.9 Hz, 1 H), 6.65 (d, J = 2.0 Hz, 1 H), 5.14 (m, 1 H), 3.81 (br, 2 H), 3.75 (s, 3 H), 3.17-3.38 (m, 2 H), 1.62 (s, 6 H), 1.33 (s, 9 H), 1.29 (s, 9 H).

Bipyridine cleft O-methylphenylalanine diamide 130

Obtained from reaction of amino xanthene O-methylphenylalanine amide **123** (225 mg, 0.415 mmol) and 2'2-bipyridine-5,5-diacidchloride (dihydrochloride) (66 mg, 0.5 mol equiv.) in CH_2Cl_2 (10 mL) in the presence of triethylamine (173 μL). Product was purified by filtering through short silica column after concentration (10 % EtOAc in CH_2Cl_2) to give 266 mg (99 %) of the product.

mp; 156-158 °C.

IR (KBr); 2963, 2906, 2869, 1751, 1734, 1675, 1653, 1539 and 1436 cm^{-1}.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 9.57 (d, J = 1.6 Hz, 2 H), 9.54 (s, 2 H), 8.78 (d, J = 8.3 Hz, 2 H), 8.70 (dd, Ja = 8.3 Hz, Jb = 2.1 Hz, 2 H), 8.55 (d, J = 1.9 Hz, 2 H), 7.56 (d, J = 2.3 Hz, 2 H), 7.09-7.24 (m, 14 H), 6.49 (d, J = 7.8 Hz, 2 H), 5.29 (m, 2H), 3.66 (s, 6H), 3.17-3.37 (m, 4 H), 1.70 (s, 6 H), 1.60 (s, 6 H), 1.40 (s, 18 H), 1.33 (s, 18 H).

HRMS calcd for $C_{80}H_{88}N_6O_{10}$: 1292.6562, found 1292.6555.

Bipyridine cleft phenylalanine diamide 131

Bipyridine cleft O-methylphenyl-alanine diamide **130** (97 mg, 0.0736 mmol) in THF (2 mL) was treated with n-$(Bu)_4NOH$ (40 % water solution, 116 μL, 2.4

mol equiv.) and stirred for 1 h. The reaction was acidified by addition of conc HCl, concentrated. Dissolved in EtOAc, washed several times with dil HCl and water. Concentration of organic layer gave 81 mg (87 %) of **131** as yellow powder.

^{1}H-NMR (250 MHz, $CDCl_3$); δ 9.67 (s, 2 H), 9.52 (d, J = 1.5 Hz, 2 H), 8.72 (dd, Ja = 8.2 Hz, Jb = 1.9 Hz, 2 H), 8.56 (d, J = 2.1 Hz, 2 H), 8.49 (d, J = 8.3 Hz, 2 H), 7.53 (d, J = 2.1 Hz, 2 H), 7.20-7.30 (m, 10 H), 7.19 (d, J = 1.9 Hz, 2 H), 7.00 (d, J = 2.0 Hz, 2 H), 6.31 (d, J = 6.6 Hz, 2 H), 4.83 (m, 2 H), 3.40 (dd, Ja = 14.1 Hz, Jb = 5.1 Hz, 2 H), 3.12 (dd, Ja = 14.0 Hz, Jb = 8.8 Hz, 2 H), 1.73 (s, 6 H), 1.56 (s, 6 H), 1.39 (s, 18 H), 1.26 (s, 18 H).

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