REPLICATIONS AND RECOGNITIONS
WITH SYNTHETIC SYSTEMS

by

Qing Feng
B. S., Department of Chemistry
Nankai University, P. R. China

Submitted to the Department of Chemistry in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

at the
Massachusetts Institute of Technology
February 1994

© Qing Feng and Massachusetts Institute of Technology 1994
All rights reserved

Signature of Author...

Department of Chemistry
January 14, 1994

Certified by..................

Professor Julius Rebek, Jr.
Thesis Supervisor

Accepted by..................

Professor Glenn A. Berchtiold
Chairman, Departmental Committee on Graduate Students
This doctoral thesis has been examined by a Committee of the Department of Chemistry as follows:

Professor Stephen L. Buchwald.

........................
Chairman

Professor Julius Rebek, Jr. ...........

Thesis Supervisor

Professor Glenn A. Berchtold......
REPLICATIONS AND RECOGNITIONS WITH SYNTHETIC SYSTEMS

by

Qing Feng

Submitted to the Department of Chemistry on January 14, 1994 in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Chemistry

ABSTRACT

Synthetic self-replicating systems are described. One system is prepared from Kemp's triacid derivatives and adenosine amine to give a complementary hydrogen bond product which catalyzed its own formation. A kinetic model for the replication process is introduced, and equilibrium and rate constants are determined. Three kinetic pathways are elucidated. Predictions for the shapes of the product growth curves are made and compared with the experiments.

A photo-labile replicating system was developed. It showed the characteristic of competition, mutation and cooperation at the molecular level.

The second self-replicating system using hydrogen bonding of thymine derivatives to diaminotriazine as the recognition vehicle is discussed. Crossover experiments between the two synthetic replicators gave rise to new structure types. The ability (or inability) of the new recombinants to catalyze their own formation is shown to be a consequence of their molecular shapes.

Neutral synthetic receptors for anions and new larger receptors based on triptycene skeleton are also described. The binding affinities of these new receptors are studied by NMR titrations.

Thesis Supervisor: Dr. Julius Rebek, Jr.
Title: Camille Dreyfus Professor of Chemistry
ACKNOWLEDGMENTS

I would like to express my sincere gratitude to Professor Julius Rebek, Jr. for the invaluable advice and guidance he has provided me throughout the course of my research at MIT. His help and proofreading during the preparation of this thesis is also appreciated.

My thanks are also extended to the members of Rebek research group, especially to Dr. T. K. Park, Dr. A. Costa, Dr. J. I. Hong, and Ed Wintner for their cooperation. I am also grateful to Roland Pieters and Ken Shimizu who helped me proofread the thesis and gave me valuable advice.

Finally, I would like to thank my parents for their love and support, without whom this work would not have been possible.
# Table of Contents

Abstract ......................................................................................................................... 3
Acknowledgments ........................................................................................................... 4
Table of Contents ........................................................................................................... 5
List of Figures ................................................................................................................ 7
List of Schemes .............................................................................................................. 9
List of Tables .................................................................................................................. 10

Chapter One. Kinetic studies and modeling of a self-replicating system .......... 11
   1.1 Introduction ......................................................................................................... 11
   1.2 The development of a self-replicating system ................................................. 17
   1.3 Results ............................................................................................................... 21
       Association constants of the components ..................................................... 21
       Kinetics studies ............................................................................................. 22
       Control experiments ...................................................................................... 30
   1.4 Discussion ......................................................................................................... 34
       The background bimolecular reaction ......................................................... 34
       The base-pairing or preassociative mechanism ......................................... 35
       The termolecular template process ............................................................. 37
       Kinetic modeling of the replication process .............................................. 38
   1.5 Experimental section ..................................................................................... 47
References .................................................................................................................. 57

Chapter Two. New developments in replicating systems ......................... 60
   2.1 Competition, reciprocity and mutation at the molecular level: .......... 60
       Synthesis of photo labile amines: ............................................................... 61
       Improvement of the original self-replicating system ............................. 64
       Kinetic results: ......................................................................................... 69
   2.2 New synthetic replicators with thymine derivatives ............................ 76
   2.3 Crossover experiments ............................................................................... 81
   2.4 Experimental section ............................................................................... 87
References .................................................................................................................. 103
Chapter Three. Progress in triptycene receptors.................................................105

3.1 Triptycene cleft...............................................................................................105
   Introduction.........................................................................................................105
   Synthesis of the triptycene tetraacid cleft.......................................................106
   Titration of tetraacid with diamines.................................................................108
   Synthesis of a soluble version of triptycene tetraacid.................................110

3.2 Anion receptors...............................................................................................112
   Introduction.........................................................................................................112
   Synthesis of neutral bisurea anion receptor .................................................114
   Binding studies of the bisurea with some organic salts...............................116
   Synthesis of a dipyridyl bisurea derivatives.................................................120

3.3 Experimental section ......................................................................................125

References ...........................................................................................................142
# LIST OF FIGURES

## Chapter One

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>13</td>
</tr>
<tr>
<td>Figure 2</td>
<td>14</td>
</tr>
<tr>
<td>Figure 3</td>
<td>15</td>
</tr>
<tr>
<td>Figure 4</td>
<td>17</td>
</tr>
<tr>
<td>Figure 5</td>
<td>18</td>
</tr>
<tr>
<td>Figure 6</td>
<td>19</td>
</tr>
<tr>
<td>Figure 7</td>
<td>20</td>
</tr>
<tr>
<td>Figure 8</td>
<td>24</td>
</tr>
<tr>
<td>Figure 9</td>
<td>26</td>
</tr>
<tr>
<td>Figure 10</td>
<td>27</td>
</tr>
<tr>
<td>Figure 11</td>
<td>28</td>
</tr>
<tr>
<td>Figure 12</td>
<td>28</td>
</tr>
<tr>
<td>Figure 13</td>
<td>29</td>
</tr>
<tr>
<td>Figure 14</td>
<td>30</td>
</tr>
<tr>
<td>Figure 15</td>
<td>31</td>
</tr>
<tr>
<td>Figure 16</td>
<td>32</td>
</tr>
<tr>
<td>Figure 17</td>
<td>34</td>
</tr>
<tr>
<td>Figure 18</td>
<td>35</td>
</tr>
<tr>
<td>Figure 19</td>
<td>37</td>
</tr>
<tr>
<td>Figure 20</td>
<td>38</td>
</tr>
<tr>
<td>Figure 21</td>
<td>40</td>
</tr>
<tr>
<td>Figure 22</td>
<td>44</td>
</tr>
<tr>
<td>Figure 23</td>
<td>46</td>
</tr>
</tbody>
</table>

## Chapter Two

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>61</td>
</tr>
<tr>
<td>Figure 2</td>
<td>64</td>
</tr>
<tr>
<td>Figure 3</td>
<td>65</td>
</tr>
<tr>
<td>Figure 4</td>
<td>68</td>
</tr>
<tr>
<td>Figure 5</td>
<td>69</td>
</tr>
</tbody>
</table>
LIST OF SCHEMES

Chapter One
Scheme I .............................................................................................................. 21
Scheme II ................................................................. 22

Chapter Two
Scheme I .............................................................................................................. 63
Scheme II ................................................................. 66
Scheme III ................................................................. 67
Scheme IV ................................................................. 77

Chapter Three
Scheme I .............................................................................................................. 107
Scheme II ................................................................. 111
Scheme III ................................................................. 115
LIST OF TABLES

Chapter One
Table I .................................................................................................................. 29
Table II ................................................................................................................. 43

Chapter Two
Table I .................................................................................................................. 71
Table II ................................................................................................................. 74

Chapter Three
Table I .................................................................................................................. 109
Table II ................................................................................................................. 118
Table III ................................................................................................................. 123
Chapter One. Kinetic Studies and Modeling of a Self-Replicating System

1.1 Introduction

Replicating molecules stand on the boundary of chemistry and biology. The word 'replication' itself bears no structural content, yet the process invariably involves molecules that are complementary. Complementarity of size, shape and chemical surface are involved in molecular recognition and self-complementarity is one of the key features of biological macromolecules. This feature is apparent in the structure of double-stranded nucleic acids, viral coat proteins and allosteric enzymes. The assembly leads to an enormous variety of molecular shapes. We have used the principle of self-complementarity to study molecular recognition and catalysis.¹

Nonenzymatic molecular replication has seen considerable development in the past few years. The double-helical structure of DNA and mechanism of nucleic acid replication were established long before the growth of interest in molecular recognition as an area of organic chemistry. Consequently, early experiments on molecular replication used substrates closely related to polynucleotides².

There are in general two different types of replicating systems. The first type does not involve direct replication of a polymeric molecule, but may help to create such an environment favorable for replication. There have been some examples of using reverse micelles and vesicles to create the microenvironment for replication. The following replicating reverse micelles have been reported by Luisi.³ In this case the reverse micelles helped catalyze the hydrolysis of ester $\text{RCO}_2\text{R'}(\text{R} = \text{R'} = (\text{CH}_2)_6\text{CH}_3)$. The hydrolyzed products, octanoic acid salt and 1-octanol both became
incorporated into the micelles, and therefore an increase in the size of the micelles occurs, and one micelle eventually collapsed into two new micelles.

Reverse micelles replication

These examples are relevant to the origin of life, as they may provide mechanisms for keeping together soluble molecules that have been involved in each other's synthesis.

The second type of system involves the replication of polymers made up from two or more different monomers. Figure 1 shows a two-dimensional cartoon example of self-complementarity, and illustrates geometrically the minimal requirements of a self-replicating system. The system has two complementary components (A, E). They covalently react to form a self-complementary product T. The two starting components A and E both have functional groups complementary to each other. The product T also has complementary functional groups on both ends. It can act as a template for the assembly of another T from A and E. The complementary functional groups used are usually hydrogen bonding sites. Here, this concept was applied in the context of chemical structures.
The first successful demonstration of molecular replication was strictly modeled on nucleic acid replication which was developed by von Kiedrowski.\textsuperscript{2a-c} The two trinucleotide analogues I and II and the hexanucleotide analogue III were prepared by standard synthetic methods. The product III was formed by linking I and II by a phosphodiester bond. The sequences were chosen because they form the ternary complex T in which the 3'-phosphate of 1 is brought close to the 5'-hydroxyl of 2 (Figure 2). The addition of carbodiimide to the solution joins together I and II to form III, and converts the ternary complex T to the binary complex B. Thus a molecule of III catalyzes the production of another molecule of III from I and II. Another similar example based on three starting materials was also reported recently.\textsuperscript{4}
Figure 2.
In the last few years, we began experimental work on replication in a more general context as an aspect of self-organization. We started to focus on the possible role of simple organic molecules as self-replicating systems.

Not long ago, the first synthetic system capable of self-replication was introduced in this group.\textsuperscript{5} Unlike nucleic acids, replication occurs without the aid of enzymes or ribozymes,\textsuperscript{6} and unlike natural systems and other synthetic self-replicating molecules,\textsuperscript{2} this system involves the formation of an amide bond, rather than a phosphate bond, in the assembly step. Base pairing again provides the recognition and organization of the reacting components.

Subsequently, another system that involves relatively simple organic molecules was also developed by von Kiedrowski.\textsuperscript{7} Derivatives of 2-formylphenoxyacetic acid (A) and 3-aminobenzamidine (B), condense autocatalytically in DMSO to form anils C (Figure 3). Again, self-complementary functional groups were used to design the molecules from simple organic compounds.

![Figure 3.](image-url)
One goal of molecular recognition is to combine binding with catalytic steps; to make these events converge in space and in time. There are some successful systems developed in this groups in the area of binding various organic molecules.\textsuperscript{1} Synthetic receptors such as derivatives of the Kemp's triacid are capable of complexing adenine derivatives.\textsuperscript{8} The goal was to explore the possibility of using the binding step to catalyze a subsequent reaction. The specific objective was to convert an otherwise bimolecular reaction into an intramolecular or intracomplex one.

The binding in the adenine receptors involves both hydrogen bonding and aryl stacking interactions in organic solvent. The triaxial arrangement of the carboxyl groups of Kemp's triacid \textsuperscript{19} provides a U-turn shape and permits the construction of molecules which can "fold back" upon themselves. The imide \textsuperscript{2a} was prepared by condensation of Kemp's triacid with ammonia. It has a hydrogen bonding edge similar to that of thymine.\textsuperscript{8}

Aromatic esters or amides from the third carboxyl can provide aromatic stacking to guests involved in base pairing to the edge. Hydrogen bonding and aromatic stacking forces combine to provide a microenvironment complementary to adenine derivatives (Figure 4).

Base-pairing (hydrogen bonding) alone is not enough to bind two components together in water. The binding studies and kinetics were carried out mostly in CHCl\textsubscript{3} to magnify hydrogen bonding forces.
1.2 The Development of a Self-Replicating System

At first, two components were synthesized that can form a covalent bond and also bear complementary hydrogen bonding surfaces. The resulting molecule should be self-complementary and catalyze its own formation.

Kemp’s triacid imide was used as the starting material. It coupled with a phenyl spacer bearing a suitable leaving group to give 5. The other component used was 9-(3-aminopropyl)adenine. Its coupling with the p-nitrophenyl ester gave the product 8.

Competitive inhibition experiments indicated that coupling proceeded by way of the base-paired complex (6) (Figure 5). The structure of the product 8 was studied by NMR and indicated that 8 formed intramolecular base-pairing in CDCl₃. The molecule remained folded shut.
This early generation of self-replicating molecule 8 gave us some valuable lessons: The product formed has to be prevented from being folded shut. This means a long and rigid spacer is needed to be incorporated into the molecule (Kemp's imide 2a) so that the two ends cannot reach each other within the molecule, and it can remain extended in space with exposed and available binding surfaces (Figure 6). A more soluble version of the adenine derivative is also required, since the product formed from the above system is not very soluble. Accordingly, the possibility of building a naphthyl spacer into Kemp's imide 2a and using a ribosyl derivative as the adenine component was explored.
The 5'-amino adenosine derivative 9 was prepared via Mitsunobu reaction from 5'-hydroxy adenosine,\textsuperscript{11} and its coupling reactions to imides bearing 2,6-naphthalene spacer 10 were examined (Figure 7). The reaction with the acid chloride was too fast to follow conveniently, and that of the nitrophenylation ester was too slow. In either case the amide adduct 11a was formed. The product was self-complementary and formed a dimer in chloroform. Dilution studies gave a value for K_{diss} of 630 M\textsuperscript{-1} for the product.

Preliminary experiments with additives such as 9-ethyladenine slowed the coupling rate, but adding the reaction product speeded it up. This system seemed workable for replication. The active ester selected for the detailed studies was the pentafluorophenyl derivative. It gave a reasonable reaction rate to follow by HPLC.
The syntheses of the imide derivatives 10a - d and the N-methyl analogs 10e - h are shown in Scheme I. Kemp's triacid 1 was converted to the imide acid chloride 2b as previously described. Coupling of 2b to the protected spacer naphthol 12c generated ester 10a. Deprotection of the MOM ester under acidic conditions (H₃O⁺, acetone) gave the corresponding acid. It was converted to the acid chloride (SOCl₂), and reaction with pentafluorophenol and triethylamine gave the pentafluorophenyl ester 10d.

The N-methyl derivatives 10e - h were prepared in a parallel reaction sequence.
The protected spacer naphthol 12c was synthesized from bromonaphthol 12 to give 6-hydroxy-2-naphthoic acid 12a via lithiation and carboxylation. Protection of the hydroxy group in 12b by acetic anhydride, and further protection of the acid by MOM chloride produced ester 12b. The acetate was then deprotected under mildly basic conditions to give hydroxy naphthyl carboxylate 12c.

![Chemical structures and reactions]

**Scheme I.**

### 1.3 Results

**Association Constants of the Components.**

The association constants of the various components toward one another were determined by NMR titrations. The experimental data for associations were shown in the Scheme II. From these data, a value of $K_a \approx$
60 M\(^{-1}\) for the association of imides 10d or 11a with adenosyl derivatives seems suitable for the discussion that follows.

For template 11a, if the two ends showed identical affinity, the \(K_{\text{dim}}\) expected would be about \((K_a)^2\). It gave only a value of \(K_{\text{dim}} = 630\) M\(^{-1}\) from the dilution experiment. This decrease in binding affinity was probably due to the fact that two ribosyl groups in the middle of the dimer interact sterically, decreasing the stability of the dimer.

![Chemical Structures]

\[ K_a = 50 \text{ M}^{-1} \]
\[ K_a = 65 \text{ M}^{-1} \]
\[ K_a = 103 \text{ M}^{-1} \]
\[ K_a = 96 \text{ M}^{-1} \]

Scheme II.

**Kinetics Studies.**

The rates of the reactions were first monitored by proton NMR. But the problems like concentration-dependent shifts, broad NH resonances, and
overlapping multiplets prevented us from getting accurate integration necessary to determine the relative concentration of each component.

Instead, HPLC was used as the analytical method. In typical kinetic experiments, a solution of the reactants and template in CHCl₃ was analyzed periodically. Triethylamine was added to help prevent phenol from protonating the aminoadenosine during the course of the reaction. All the coupling reactions were performed with an excess of Et₃N present (generally 4 equiv). Identical rates were observed in the presence of 4 and 8 equiv of triethylamine.

Calibration curves were made to obtain the correlation between the HPLC area response and the template concentration. A linear relation between template concentration and template area response was observed.

Direct calibration curves (template concentration [T] vs. template peak area response) were used for the reaction, which was different from the previous used indirect calibration ([T/A] vs. template peak area response). For long time reactions and reactions at concentrated conditions, it was inappropriate to use the indirect calibration method. Different calibration curves were obtained for different reactant concentrations.

The concentration of the template at certain times during the reactions can be obtained through calibration curve from the area response of the template peak. A typical calibration curve is shown in Figure 8.
The difficulties of the kinetic reactions can be due to the fact that the reaction rate is more sensitive to changes in concentrations of reactants than of the added template. Also evaporation can occur over long reaction time. Experimental procedure was improved by preparing the stock solutions of reactants and templates, and by using Wheaton reaction vials sealed to decrease solvent evaporation. Each reaction was repeated at least three times.

Reactions of amine 9 and pentafluorophenyl ester 10d were studied at 1.65, 8.2 and 16.5 mM concentrations. In experiments in which template 11a was added, 0.2 or 0.5 equiv were used. The template peak is the convenient peak to follow. Competing processes, such as hydrolysis of the pentafluorophenyl ester by a trace of water (especially under dilute conditions or in longer runs), limited the accuracy of the kinetic reactions. Nevertheless, the kinetic behavior and the coupling rates were reproducible.
Preliminary studies were performed at 8.2 mM concentration of reactants 9 and 10d (Figure 9). The effect of added template and inhibitor upon the rates of reaction were examined. The initial rates of reaction were calculated by linear least squares analysis of data collected during the first 60 minutes of the reaction. In normal cases, the slopes of the fitted line are the initial rates. An initial rate of $1.00 \times 10^{-5}$ Mmin$^{-1}$ was observed for the reaction of 9 and 10d (Figure 9a).

In the presence of added template 11a, a significant rate acceleration occurs. When 0.20 equiv of 11a is added, the initial rate increases by 43% ($1.43 \times 10^{-5}$ Mmin$^{-1}$, Figure 9b); when 0.50 equiv is added, the rate increases by 73% ($1.73 \times 10^{-5}$ Mmin$^{-1}$, Figure 9c).

2,6-Di(acetylamino)pyridine inhibits the reaction. It competes the binding sites with amine 9. Addition of 1 equiv reduces the rate to $0.49 \times 10^{-5}$ Mmin$^{-1}$ (Figure 9e). The rate of reaction of the N-methyl imide 10h with aminoadenosine 9 is substantially slower than that of the parent (N-H) imide 10d ($0.15 \times 10^{-5}$ Mmin$^{-1}$, Figure 9d).
Figure 9.
Generation of template 11a or 11b as a function of time. All reactions were performed with initial concentrations: \([9] = [10d or h] = 0.0082 \text{ M in CHCl}_3\). Lines are linear least-squares fit of data, and correspond to the initial rates of reaction. Error bars represent standard deviations of multiple independent runs. (a) Reaction of 9 and 10d. (b) Reaction of 9 and 10d with 0.20 equiv of 11a added as autocatalyst. (c) Reaction of 9 and 10d with 0.50 equiv of 11a added as autocatalyst. (d) Reaction of 9 and the N-methylated 10h (single run). (e) Reaction of 9 and 10d with 1 equiv of 2,6-di(acetylamino)pyridine added as inhibitor.

The generation of product is essentially linear during the first 60 minutes of reaction at 8.2 mM, the rate slows as the reaction progresses. Figure 10 illustrates the formation of product over the course of the reaction of 9 and 10d at 8.2 mM. The failure of the reaction to approach completion (8.2 mM), may arise from hydrolysis of the pentafluoroester group of 10d.
The reaction of 9 and 10d is considerably more rapid at 16.5 mM. The initial rates of reaction were measured during the first 65 minutes of the reaction (Figure 11). Rates of $5.6 \times 10^{-5}$, $7.4 \times 10^{-5}$, and $8.8 \times 10^{-5}$ Mmin$^{-1}$ were observed for reaction in the presence of 0, 0.20, and 0.55 equiv of added template 11a, respectively. Figure 12 illustrates the prolonged course of the reaction of 9 and 10d.
Figure 11.
Generation of template 11a as a function of time. All reactions were performed with initial concentrations: $[9] = [10d] = 0.0165$ M in CHCl$_3$. Lines are linear least-squares fit of data, and correspond to the initial rates of reaction. Error bars represent standard deviations of multiple independent runs. (a) Reaction of 9 and 10d. (b) Reaction of 9 and 10d with 0.20 equiv of 11a added as autocatalyst. (c) Reaction of 9 and 10d with 0.55 equiv of 11a added as autocatalyst.

Figure 12.
Generation of template 11a as a function of time. The initial concentrations are: $[9] = [10d] = 0.0165$ M in CHCl$_3$. Error bars represent standard deviations of multiple independent runs.
At 1.65 mM, the reaction of 9 and 10d is far slower. Initial rates of 0.050 x 10^-5 and 0.065 x 10^-5 Mmin^-1 occurred in the presence of 0 and 0.20 equiv of added template 11a (Figure 13). Table I summarizes the rate data described in this section.

### Table I.

**Initial Rates of Reaction of Amine 9 and Ester 10d or 10h**

<table>
<thead>
<tr>
<th>Reactants</th>
<th>Additive</th>
<th>Rate (Mmin^-1)^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.2 mM 9, 10d</td>
<td>none</td>
<td>1.00 x 10^-5 ± 0.06 x 10^-5</td>
</tr>
<tr>
<td>8.2 mM 9, 10d</td>
<td>0.20 equiv 11a</td>
<td>1.43 x 10^-5 ± 0.05 x 10^-5</td>
</tr>
<tr>
<td>8.2 mM 9, 10d</td>
<td>0.50 equiv 11a</td>
<td>1.73 x 10^-5 ± 0.12 x 10^-5</td>
</tr>
<tr>
<td>8.2 mM 9, 10d</td>
<td>1.00 equiv 2,6-di(acetylamino)pyridine</td>
<td>0.49 x 10^-5 ± 0.03 x 10^-5</td>
</tr>
<tr>
<td>8.2 mM 9, 10h</td>
<td>none</td>
<td>0.15 x 10^-5 b</td>
</tr>
<tr>
<td>16.5 mM 9, 10d</td>
<td>none</td>
<td>5.6 x 10^-5 ± 0.2 x 10^-5</td>
</tr>
<tr>
<td>16.5 mM 9, 10d</td>
<td>0.20 equiv 11a</td>
<td>7.4 x 10^-5 ± 0.4 x 10^-5</td>
</tr>
<tr>
<td>16.5 mM 9, 10d</td>
<td>0.55 equiv 11a</td>
<td>8.8 x 10^-5 ± 0.5 x 10^-5</td>
</tr>
<tr>
<td>1.65 mM 9, 10d</td>
<td>none</td>
<td>0.050 x 10^-5 ± 0.007 x 10^-5</td>
</tr>
<tr>
<td>1.65 mM 9, 10d</td>
<td>0.20 equiv 11a</td>
<td>0.065 x 10^-5 ± 0.007 x 10^-5</td>
</tr>
</tbody>
</table>

^a Uncertainties in rates represent standard deviations of multiple (2-4) independent runs.

^b Data from single run.

---

**Figure 13.**

Generation of template 11a as a function of time. All reactions were performed with initial concentrations: [9] = [10d] = 0.00165 M in CHCl₃. Lines are linear least-squares fit of data, and correspond to the initial rates of reaction. Error bars represent standard deviations of multiple independent runs. (a) Reaction of 9 and 10d. (b) Reaction of 9 and 10d with 0.20 equiv of 11a added as autocatalyst.
Control Experiments

The effect of triethylamine on the rate of the reaction was studied by performing the kinetic reactions at different amounts of triethylamine (Figure 14). It can been seen that the reaction rates are approximately the same for 4 equiv and 8 equiv added triethylamine within the experimental error (Figure 14a and 14b). This means that after certain concentration, the effect of triethylamine levels off. However, without added triethylamine the reaction was actually faster (Figure 14c). This is probably because that the released phenol has some beneficial effect on the reaction rate.

![Graph showing the effect of triethylamine](image)

**Figure 14.**
Effect of triethylamine on product formation. \([A_0] = [E_0] = 16.2 \text{ mM}\).
Reaction of 9 and 10d: a) 4 eq. of triethylamine added. b) 8 eq. triethylamine added. c) No triethylamine added.

In the previous section, it has been shown that the reaction of 9 and 10d was catalyzed by the addition of product 11a. This catalytic effect of the reactions has also been observed under different concentrations. But there
are still other possibilities that could be responsible for the catalysis. Does product 11a act as a template to direct the bond formation or it merely just act as a general catalyst? Is it possible that the reaction would still be catalyzed if the added compound had no binding function? To answer these questions, it is necessary to carry out some control experiments using the following related compounds (Figure 15)\textsuperscript{12}.

![Chemical structures](image)

**Figure 15.**

First, N-methyl imide template 11b was used as the additive. This molecule cannot form hydrogen bonds on the imide end; therefore, it cannot form termolecular intermediate (AET). There would be no catalysis with the addition 11b if the reaction is a template directed reaction. This was just what was observed. Addition of 0.2 equiv of N-methyl template 11b has almost no effect on reaction rate (Figure 16a), whereas addition of N-H substituted template 11a showed an increase the reaction rate (Figure 16c).
Figure 16.
Reaction of 9 and 10d: [A0] = [E0] = 16.0 mM. a) 0.2 eq. of N-Methyl template 11b added. b) no template added. c) template 11a added.

The possibility of trans-amide acting as a general catalyst to catalyze the amide bond formation was also excluded when the reaction was performed using naphthoyl adenosine imide 12 as an additive. This molecule is similar in structure to 11a, having the amide and adenosine portions but has no Kemp's imide portion. No rate increase was observed when it was added to the reaction (Figure 17c). This indicates that a trans-amide alone cannot catalyze the reaction.

The same is true for compound 14 with Kemp's imide and a naphthyl spacer, but without amide and adenosine function. This compound cannot form a termolecular intermediate either. No rate acceleration was observed when 14 was used as an additive (Figure 17d).

The 'template' 13 with a phenyl spacer was also used as an additive. This molecule has all the functional groups that template 11a has, but has a
shorter spacer. No rate acceleration was observed either (Figure 17e). This molecule probably does not have the appropriate size to be a template. It is possible that this molecule could also bind two starting components to form a termolecular intermediate. But it seems unlikely that the molecule could bring the two reacting functions close in space for rapid reaction, since it might not have the right length.

Ester template 15 was also synthesized from imide naphthyl acid 10b and adenosine alcohol by EDC coupling. It has a similar structure as template 11a, except that it has the ester group substituting for the amide group. This compound could act as a template, since it has the same binding functions and the same spacer.\textsuperscript{13} It turned out that this ester template is not a catalyst (Figure 17f). This observation can be explained in the following way: The amide group in the template 11a could probably stabilize the transition state in the bond formation step in the termolecular complex (AET); therefore it catalyzes the amide bond formation. But this catalytic effect would only be possible in the termolecular complex. The amide functional group alone is not the cause for catalysis, as in the cases mentioned above (11b, 12, 13).

In summary, the reaction between 9 and 11d is an autocatalytic reaction with the product 11a acting as a template. Figure 17 shows the results from the kinetic reactions.
Figure 17.
Reaction of 9 and 10d: [A₀] = [E₀] = 2.2 mM. Triethylamine (72 mM) was added as a general base. The additives are all 0.5 equiv. a) no additive; b) template 11a added; c) naphthoyl adenosine 12 added; d) methyl ester 14 added. e) phenyl spacer template 13 added; f) ester template 15 added.

1.4 Discussion

There are three major reactions contributing to the formation of the product: 1) the background bimolecular reaction; 2) the base-paired bimolecular reaction; 3) the termolecular template-catalyzed process. The third reaction is responsible for the replication process. The shape of the kinetic curves was determined by the combination effect of these three reactions.

The Background Bimolecular Reaction.

This is the simple bimolecular reaction between the amino adenosine 9 and the pentafluorophenyl ester 10d as shown in Figure 18. This reaction was determined by the reaction between the N-methyl imide derivative of the
pentafluorophenyl ester 10h and the amine 9. The rate constant for this process is 0.023 M⁻¹min⁻¹ (this is determined by the model discussed in the later section). An independent measure of the background bimolecular rate, using 2,6-di (acetylamo) pyridine as an inhibitor (Kₐ = 450 M⁻¹) in the reaction of 9 with the parent (NH) derivative 10d gave a similar value.

![Chemical structures](image)

**Figure 18.**

**The Base-Pairing or Preassociative Mechanism.**

The second pathway is the preassociative bimolecular pathway. In this pathway the two starting components amine 9 and ester 10d form a hydrogen bonded dimer AE prior to reaction. In this dimer, the amino and pentafluorophenyl groups are close in space, and readily react to generate the template in its cis-amide conformation cis-T. Isomerization to the more stable trans-amide occurs rapidly. This pathway plays a major role in the formation of template 11a (Figure 19).

This preassociative mechanism was supported by the following experiment. The NH derivative 10d reacts 6.5-fold more rapidly with the
aminoadenosine 9 than the N-methyl derivative 10h under the same conditions (Table I).

The inhibition of the reaction of 9 and 10d by 2,6-di(acetylamino)pyridine provides additional evidence. A decrease in rate of reaction was observed. The inhibitor 2,6-di(acetylamino)pyridine (I) binds to the imide ester 10d, competing for the binding sites with the amine 9. This corresponds to the decrease in the fraction of base-paired complex AE. Mere amide structures are not sufficient to catalyze the formation of the replicator.

From the base-pairing association constant between 9 and 10d (ca. 60 M$^{-1}$), it can be calculated that the initial mixture at 8.2 x 10$^{-3}$ M concentration involves 27 percent based-paired and 73 percent free components. The former contribute to the preassociative mechanism, whereas the latter contribute to the background reaction.

When 1 equiv of 2,6-di(acetylamino)pyridine (K$\alpha$ = 450 M$^{-1}$ with 10d) is added to this mixture, the fraction of free ester 10d decreases to 32%. While the ester that is complexed with 2,6-di(acetylamino)pyridine (54%) should still be reactive toward amine 9 via the background bimolecular pathway, it is unable to participate in the preassociative mechanism. The fraction of base paired complex is calculated to be 14% (51 percent of the value in the absence of the inhibitor), while the observed rate is 48% of that in the absence of inhibitor (Table I).
The Termolecular Template Process.

The template catalyzed, replicative process (Figure 20) was revealed by the enhanced rate of reaction of amine 9 and ester 10d upon addition of the template product 11a to coupling mixtures. In this mechanism, the amine, ester, and template first form a termolecular complex AET. The close proximity of the reactive amino and pentafluorophenyl ester groups then leads to amide formation. The resulting template dimer T₂ exists in
equilibrium with the free template. Control experiments with the N-methyl imide product 11b showed that its presence did not increase the rate of product formation.

![Chemical Structures](image)

**Figure 20.**

**Kinetic Modeling of the Replication Process.**

The self-replicating system contains a variety of species in rapid equilibrium. For the purposes of this discussion, some easily identified letter names have been assigned (shown in eq 1-9) to these species. At millimolar concentrations, there are substantial amounts of bimolecular species AE, AT, ET, and T₂ and some termolecular complex AET, in addition to the free amine A (9), ester E (10d), and template T (11a). Equations 1-9 represent all significant complexation processes. The values of the various equilibrium constants were determined by extrapolation from the related systems shown in Scheme II, from which K₁, K₂, and K₃ were each estimated to be 60 M⁻¹. K₄ and K₅ should have the similar values since the two binding ends of the
template are independent. The value of the self-association constant of the template, $K_{\text{dim}}$ is 630 M$^{-1}$

\[
\begin{align*}
K_1 & \quad A + E \rightleftharpoons AE \\
K_2 & \quad A + T \rightleftharpoons AT \\
K_3 & \quad E + T \rightleftharpoons ET \\
K_4 & \quad A + ET \rightleftharpoons AET \\
K_5 & \quad E + AT \rightleftharpoons AET \\
K_{\text{dim}} & \quad 2T \rightleftharpoons T_2
\end{align*}
\] (1) (2) (3) (4) (5) (6)

The three pathways by which template is generated were described above.

(1) In the background bimolecular pathway the amine $A$ and ester $E$ react without preassociation to give the template $T$ with a rate constant of $k_1$ (eq 7). Hydrogen bonded species (i.e., $AE$, $AT$, $ET$, and $AET$) can also react with each other by means of this bimolecular pathway.

(2) In the base-paired bimolecular pathway the hydrogen bonded complex $AE$ reacts to generate the template initially in an unstable cis-amide conformation with a rate constant of $k_2$ (eq 8). Isomerization to the more stable trans conformation is assumed to be a rapid, thermodynamically favorable process.

(3) In the autocatalytic, termolecular pathway the template forms via the complex $AET$ with a rate constant of $k_3$ (eq 9).

\[
\begin{align*}
k_1 & \quad A + E \rightarrow T \\
k_2 & \quad AE \rightarrow T
\end{align*}
\] (7) (8)
\[ k_3 \quad \text{AET} \rightarrow T_2 \quad (9) \]

Kinetic modeling of the overall process was performed using an iterative numerical method developed in this group.\textsuperscript{14} Fitting of the observed data from experiments performed at 8.2 x 10\textsuperscript{-3} M in amine 9 and ester 10d or 10h generates values of 0.023 M\textsuperscript{-1}min\textsuperscript{-1}, 0.0036 min\textsuperscript{-1}, and 0.070 min\textsuperscript{-1} for \(k_1\), \(k_2\), and \(k_3\), respectively. Rate constants were determined by minimizing the least squares difference between observed and calculated values, and also by matching of the calculated and experimental initial rates of reaction.

Figure 21 shows the calculated vs. experimental formation of template as a function of time. A satisfactory fit was obtained within the limits of precision of the data. An alternative model for the kinetics of self-replicating systems was introduced by von Kiedrowski which can be used for strongly dimerized molecules.\textsuperscript{15}

![Figure 21](image)

Experimental (points) and calculated (lines) generation of template 11a or 11b as a function of time. All reactions were performed with initial concentrations: \([9] = [10d \text{ or } h] = 0.0082 \text{ M in CHCl}_3\). (a) Reaction of 9 and 10d. (b) Reaction of 9 and 10d with 0.20 equiv of 11a added as autocatalyst. (c) Reaction of 9 and 10d with 0.50 equiv of 11a added as autocatalyst. (d) Reaction of 9 and the N-methylated 10h.
A number of systematic errors limit the accuracy of these rate constants, such as variations in temperature, loss of solvent by evaporation during prolonged runs, and slow hydrolysis of the ester by residual H₂O in CHCl₃.

The rate constants were found to fit other kinetic models only moderately well. The initially observed rate of reaction of 9 and 10d at 1.65 mM was calculated to be 18 times slower than that at 8.2 mM, whereas a 20-fold slower rate was observed (Table I).

The initially observed rate of reaction of 9 and 10d at 16.5 mM was calculated to be 3.3 times as fast as that at 8.2 mM, whereas a 5.6-fold greater rate was observed (Table I). Some of this inconsistency appears to arise from systematic errors, however. When the same stock solutions of 9 and 10d were used for both the 16 and the 8 mM runs, only a 4.2-fold greater rate was observed. In all cases, the kinetic model predicted the observed trends in reactivity quite well although it has some limitations.

The ratio of the rate constants of an intramolecular reaction and its intermolecular counterpart corresponds to the “effective molarity” of the reacting groups in the intramolecular reaction, and thereby give us some idea about the efficiency of the intramolecular process. In this system the preassociative bimolecule pathway and the replicating termolecular pathway (AE and AET in equations 8 and 9) are intramolecular processes which can be compared to the background bimolecular reaction (equation 7).

From the comparison it can be seen that the effective molarities of the amine and ester groups are actually higher in the termolecular complex AET (k₃/k₁ = 3.0 M) than in the bimolecular complex AE (k₂/k₁ = 0.16 M). The difference is about 19 fold. (It may reflect a higher activation energy for
formation of the \textit{cis} conformation of amide 11a from the bimolecular complex \textit{AE}, or a more favorable arrangement of functional groups in the termolecular complex \textit{AET}).

Although the effective molarity in the termolecular complex is greater than the preassociative bimolecular pathway, the product is formed mainly through the preassociative bimolecular pathway. Under typical reaction conditions (e.g., 10^{-2} M concentration of reactants), the concentration of the bimolecular complex far exceeds the concentration of the termolecular complex.

Table II illustrates the concentration of individual species (\textit{A}, \textit{E}, \textit{T}, \textit{AE}, \textit{AT}, \textit{ET}, \textit{AET}, \textit{T}_2) calculated from the kinetic model for certain reaction mixtures. \([\textit{A}]_{\text{tot}}, [\textit{E}]_{\text{tot}}, [\textit{T}]_{\text{tot}}\) represent the total concentration of the species containing \textit{A}, \textit{B}, \textit{T} respectively:

\[
[\textit{A}]_{\text{tot}} = [\textit{A}] + [\textit{AE}] + [\textit{AT}] + [\textit{AET}];
\]

\[
[\textit{E}]_{\text{tot}} = [\textit{E}] + [\textit{AE}] + [\textit{ET}] + [\textit{AET}];
\]

\[
[\textit{T}]_{\text{tot}} = [\textit{AT}] + [\textit{ET}] + [\textit{AET}] + 2[\textit{T}_2]).
\]

For example, in the presence of 0.3 equiv of added template (entry 3), 67% of the amine and ester are free, 27% are associated with each other, and only 1.5% are present as termolecular complex (\([\textit{A}]_{\text{tot}} = [\textit{E}]_{\text{tot}} = 0.010 \text{ M, } [\textit{T}]_{\text{tot}} = 0.003 \text{ M})). Under these conditions, the relative rates of the background bimolecular pathway (\(k_1\)), the preassociative pathway (\(k_2\)) and the template termolecular pathway (\(k_3\)) are 1.0 : 4.3 : 4.7, respectively. While the background bimolecular reaction contributes only slightly to the formation of product, both the preassociative (uncatalyzed) and the template (autocatalytic) mechanisms contribute substantially.
Table II.
Concentrations (M) of Species Present ([A]_{tot} = [E]_{tot} = 0.010 M)

<table>
<thead>
<tr>
<th>[T]_{tot}</th>
<th>[A] = [E]</th>
<th>[T]</th>
<th>[AE]</th>
<th>[AT] = [ET]</th>
<th>[AET]</th>
<th>[T_2]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00703</td>
<td>0</td>
<td>0.00297</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.001</td>
<td>0.00691</td>
<td>0.00040</td>
<td>0.00286</td>
<td>0.00017</td>
<td>0.00007</td>
<td>0.00010</td>
</tr>
<tr>
<td>0.003</td>
<td>0.00674</td>
<td>0.00095</td>
<td>0.00272</td>
<td>0.00038</td>
<td>0.00015</td>
<td>0.00057</td>
</tr>
<tr>
<td>0.010</td>
<td>0.00639</td>
<td>0.00216</td>
<td>0.00246</td>
<td>0.00083</td>
<td>0.00032</td>
<td>0.00293</td>
</tr>
</tbody>
</table>

Of particular interest is the shape of the product growth curve in the kinetic reactions. For an autocatalytic system which the product was formed only through autocatalytic mechanism, a sigmoidal curve is expected. But so far it has been difficult to observe sigmoidal curve in this system under different conditions. So far we have seen only one example with slight parabolic growth curve.\textsuperscript{2c}

In this system, as discussed above the autocatalytic pathway is only one of the three ways to form the product. Whether a sigmoidal curve can be observed depends on which pathway dominates the reaction. From the model we also know that there are several factors that will affect the shape of the curve.

In this system the initial concentration of reactants will probably affect the shape of the growth curve. At dilute concentrations of about 10^{-3} M, the concentration of the termolecular complex (AET) is very low compared with free amine (A) and ester (E) and bimolecular complex (AE). The contribution of the autocatalytic pathway is insignificant in this condition, and the product formation curve is similar to that of simple bimolecular kinetics (Figure 22a).

At initial concentrations in the order of 10^{-2} M, the percentage of the termolecular complex increases. A slight sigmoidal shape should be observed
(Figure 22b). Unfortunately, compared with the uncertainty in the data points the degree of sigmoidal curvature is too small to be observable.

At concentrations on the order of \(10^{-1} \text{ M}\), a more sigmoidal growth curve might be possible (Figure 22c). But the system is not soluble enough for us to run the reaction at this high concentration.

\begin{figure}[h]
\centering
\includegraphics[width=0.7\textwidth]{chart.png}
\caption{Figure 22. Calculated effect of reactant concentration upon the growth curve. All calculations were performed using the experimentally determined rate and equilibrium constants (see text). (a) top, initial concentration: \([9] = [10d] = 0.001 \text{ M}\). (b) middle, initial concentrations: \([9] = [10d] = 0.01 \text{ M}\). (c) bottom, initial concentrations: \([9] = [10d] = 0.1 \text{ M}\).}
\end{figure}
This model can also be used to simulate the reaction profiles at different rate constants (Figure 23). Curve \(a\) represents the reaction profile calculated for typical reaction conditions (0.010 M initial concentration of \(\text{9 and 10d}\)) using rate constants determined from the experimental data (\(k_1 = 0.023 \text{ M}^{-1}\text{min}^{-1}, k_2 = 0.0036 \text{ min}^{-1}, \text{and } k_3 = 0.070 \text{ min}^{-1}\)).

If the preassociative bimolecular pathway did not exist (i.e., \(k_2 = 0, k_1 \text{ and } k_3 \text{ are same as before}\)), then a highly sigmoidal growth curve \(b\) would be observed. If there is no autocatalytic pathway (i.e., \(k_3 = 0, k_1 \text{ and } k_2 \text{ are the same as before}\)) curve \(c\) would be observed. If the reaction proceeds only by a simple bimolecular mechanism (i.e., \(k_1 = 0.023 \text{ M}^{-1}\text{min}^{-1}, k_2 = 0, \text{and } k_3 = 0\)), curve \(d\) would be observed.

This information could give us some guidance for the modification of the present system and for future design of more effective self-replicating systems. The ideal replicating system would have no preassociative bimolecular pathway (\(k_2 = 0\)), and a background bimolecular reaction that is slow (\(k_1 \text{ is small}\)), and the termolecular reaction is fast (\(k_3 \text{ is large}\)).
Figure 23. Calculated effect of rate constants $k_1$, $k_2$, and $k_3$ upon the growth curve. All calculations were performed for initial concentrations: $[9] = [10d] = 0.01$ M using the experimentally determined equilibrium constants (see text). (a) $k_1 = 0.023 \text{ M}^{-1}\text{min}^{-1}$, $k_2 = 0.0036 \text{ min}^{-1}$, $k_3 = 0.070 \text{ min}^{-1}$. (b) $k_1 = 0.023 \text{ M}^{-1}\text{min}^{-1}$, $k_2 = 0$, $k_3 = 0.070 \text{ min}^{-1}$. (c) $k_1 = 0.023 \text{ M}^{-1}\text{min}^{-1}$, $k_2 = 0.0036 \text{ min}^{-1}$, $k_3 = 0$. (d) $k_1 = 0.023 \text{ M}^{-1}\text{min}^{-1}$, $k_2 = 0$, $k_3 = 0$.

In this chapter the design, synthesis, and kinetics of a molecular template 11a have been discussed.16 There are three reaction pathways leading to the formation of template. But only one, the template termolecular process, is responsible for replication. Kinetic modeling studies indicate that in this system both the preassociative mechanism and the template termolecular process contribute substantially to the formation of template. The preassociative mechanism is even more substantial than the termolecular process in product formation. In the later studies, this information will be used to modify the system in order to shut down or decrease the preassociative pathway. This will be discussed in the next chapter.
1.5 Experimental Section (Chapter One)

**General.** Mass spectra were obtained on a VG Instruments, a Se-20 mass spectrometer, or a Varian CH-5 instrument (high resolution). IR spectra were obtained on an IBM IR/32 FTIR. $^1$H NMR spectra were obtained on 250 Bruker and 300 MHz Varian instruments. THF was distilled from sodium benzophenone ketyl under argon. CH$_2$Cl$_2$ was distilled from CaH$_2$ under argon.

**N-Methyl Imide-Acid 2c.**

To 1.0 g of Kemp's triacid anhydride$^8$ (1.55 mmol) was added 15 mL of a 40% aq soln of methylamine, and the resulting solution was heated (60 °C) overnight. The solution was allowed to cool, and excess methylamine was evaporated under reduced pressure. The solution was acidified with cond HCl to pH = 1, and cooled to 0 °C. The resulting suspension was filtered, washed with water, and dried at 110 °C under vacuum for 3 h to afford 0.90 g of 2c as a white solid (80%):

mp 263 - 265 °C;

$^1$H NMR (300 MHz, CDCl$_3$) δ 11.0 (s, 1 H), 2.71 (s, 3 H), 2.55 (d, J = 13.6 Hz, 2 H), 1.99 (d, J = 13.3 Hz, 1 H), 1.37 (d, J = 13.3 Hz, 1 H), 1.27 (s, 6 H), 1.21 (s, 3 H), 1.21 (d, J = 13.5 Hz, 2 H).

**N-Methyl Imide-Acid Chloride 2d.**

Reaction of N-methyl imide-acid 2c (5.0 g, 18.4 mmol) with SOCl$_2$ (100 mL) was performed as described for the preparation of 2b$^{11}$ to afford 5.0 g of 2d (97% ) as a pale yellow solid. An analytical sample was prepared by recrystallization from EtOAc as colorless crystals:

mp 120 - 122 °C;
IR (KBr) 3200, 3094, 2987, 1780, 1721, 1696, 1462, 1385, 1205, 924, 897, 833 cm⁻¹; 
¹H NMR (300 MHz, CDCl₃) δ 2.90 (s, 3 H), 2.78 (d, J = 14.6 Hz, 2 H), 2.04 (d, J = 13.4 Hz, 1 H), 1.43 (d, J = 14.5 Hz, 2 H), 1.37 (s, 3 H), 1.32 (s, 6 H), 1.27 (d, J = 13.3 Hz, 1 H).

**Imide-Ester 10a.**

A 200 mL round-bottomed flask was charged with imide-ester chloride 2b⁸ (0.38 g, 1.47 mmol), 12c (0.34 g, 1.47 mmol) and dry methylene chloride (ca. 50 mL). 4-(Dimethylamino)pyridine (ca. 10 mg) and 2 mL of triethylamine were added, and the resulting solution was stirred under nitrogen for 12 h. The solution was washed with 1 N HCl (ca. 20 mL), water (2 x 20 mL), and brine (20 mL), dried over MgSO₄, filtered, and evaporated to give an yellow solid. Column chromatography on silica gel (5% EtOAc in CHCl₃) afforded 0.54 g (81%) of 10a as a white solid:

mp 185 - 187 °C;

¹H NMR (300 MHz, CDCl₃) δ 8.64 (s, 1 H), 8.10 (dd, J = 1.4, 8.8 Hz, 1 H), 7.95 (d, J = 8.9 Hz, 1 H), 7.86 (d, J = 8.6 Hz, 1 H), 7.61 (s, 1 H), 7.59 (s, 1 H), 7.26 (m, 1 H), 5.55 (s, 2 H), 3.60 (s, 3 H), 2.88 (d, J = 14.2 Hz, 2 H), 2.08 (d, J = 13.2 Hz, 1 H), 1.49 (s, 3 H), 1.44 (d, J = 13.2 Hz, 1 H), 1.35 (d, J = 14.2 Hz, 2 H), 1.34 (s, 6 H);

HRMS m/z (M⁺) calcd 453.1787, obsd 453.1753.

**Imide-Acid 10b.**

A solution of imide-ester 10a (0.41 g, 0.90 mmol) in acetone (ca. 20 mL) and 5 drops of concentrated HCl was heated at 50 °C for 2 h. The solvent was evaporated, and a solid was obtained. The solid was dissolved in 50 mL EtOAc, and the solution was washed with brine, dried over MgSO₄, filtered, and concentrated to afford 0.33 g (89%) of 10b as a tan solid:

mp 295 - 300 °C;
$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 10.57 (s, 1 H), 7.88 (s, 1 H), 7.63 (m, 4 H), 7.19 (dd, J = 2.1, 8.7 Hz, 1 H), 2.90 (d, J = 13.8 Hz, 2 H), 2.14 (d, J = 13.5 Hz, 1 H), 1.50 (d, J = 13.5 Hz, 1 H), 1.47 (s, 3 H), 1.37 (s, 6 H), 1.29 (d, J = 13.2 Hz, 2 H);
HRMS m/z (M$^+$) calcd 409.1525, obsd 409.1533.

**Imide-Acid chloride 10c.**

A suspension of imide-acid 10b (0.33 g, 0.80 mmol), SOCl$_2$ (2 mL) and 1 drop of DMF in 25 mL of dry CH$_2$Cl$_2$ was heated at 40 °C for 0.5 h. The resulting clear solution was evaporated to give 0.34 g (100%) of 10c as a yellow solid:
mp 195 - 200 °C;
$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.73 (s, 1 H), 8.07 (dd, J = 1.5, 8.7 Hz, 1 H), 8.00 (d, J = 8.9 Hz, 1 H), 7.88 (d, J = 8.7 Hz, 1 H), 7.63 (s, 1 H), 7.62 (s, 1 H), 7.31 (dd, J = 2.1, 8.8 Hz, 1 H), 2.87 (d, J = 13.5 Hz, 2 H), 2.08 (d, J = 13.3 Hz, 1 H), 1.50 (s, 3 H), 1.44 (d, J = 13.3 Hz, 1 H), 1.36 (d, J = 13.5 Hz, 2 H), 1.33 (s, 6 H);
HRMS m/z (M$^+$) calcd 427.1186, obsd 427.1172.

**Imide-Ester 10d.**

A solution of acid chloride 10c (0.18 g, 0.42 mmol), pentafluorophenol (0.078 g, 0.42 mmol), triethylamine (0.5 mL) and dry methylene chloride was heated at reflux for 12 h. The solution was allowed to cool, and extracted with 10 mL of 1 N HCl and 10 mL of water, dried over MgSO$_4$, filtered, and concentrated to give a brownish residue. Column chromatography on silica gel (1:1 EtOAc-Hexanes) gave 0.23 g (95%) of 10d as a tan powder:
mp 150 - 152 °C;
$^1$H NMR (250 MHz, CDCl$_3$) $\delta$ 8.78 (s, 1 H), 8.16 (d, d, J = 1.8, 7.18 Hz, 1 H), 8.00 (d, J = 9.0 Hz, 1 H), 7.94 (d, J = 8.7 Hz, 1 H), 7.67 (s, 1 H), 7.64 (d, J = 2.2 Hz, 1 H), 7.30 (dd, J = 2.2, 8.8 Hz, 1H), 2.88 (d, J = 13.6 Hz, 2 H), 2.09 (d, J = 13.4 Hz, 1 H), 1.50 (s, 3 H), 1.44 (d, J = 13.3 Hz, 1 H), 1.36 (d, J = 13.5 Hz, 2 H), 1.33 (s, 6 H);
HRMS $m/z$ (M$^+$) calcd 575.1367, obsd 575.1365.

**N-Methyl Imide-Ester 10e.**

Reaction of imide-acid chloride 2d (0.65 g, 2.39 mmol) with naphthol 12c (0.56 g, 2.39 mmol) was performed as described for the preparation of 10a to give a yellowish oil. Column chromatography on silica gel (5% EtOAc in CHCl$_3$) afforded 0.96 g (86%) of 10e as a white solid:

$\text{mp} 158 - 160 \degree C$;

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.63 (s, 1 H), 8.10 (dd, $J = 1.5, 7.1$ Hz, 1 H), 7.95 (d, $J = 8.9$ Hz, 1 H), 7.86 (d, $J = 8.6$ Hz, 1 H), 7.62 (d, $J = 1.9$ Hz, 1 H), 7.27 (dd, $J = 2.2$, 8.7 Hz, 1 H), 5.55 (s, 2 H), 3.59 (s, 3 H), 2.85 (s, 3 H), 2.84 (d, $J = 7.7$ Hz, 2 H), 2.03 (d, $J = 7.9$ Hz, 1 H), 1.46 (d, $J = 7.9$ Hz, 1 H), 1.45 (s, 3 H), 1.35 (d, $J = 7.9$ Hz, 2 H), 1.34 (s, 6 H).

**N-Methyl Imide-Acid 10f.**

The ester 10e (0.87 g, 1.86 mmol) was converted to the acid 10f (0.65 g, 82%) as described for the preparation of 10b:

$\text{mp} 255 - 258 \degree C$;

$^1$H NMR 300 MHz, CDCl$_3$) $\delta$ 8.67 (s, 1 H), 8.10 (dd, $J = 1.9, 8.6$ Hz, 1 H), 7.96 (d, $J = 9.0$ Hz, 1 H), 7.88 (d, $J = 8.7$ Hz, 1 H), 7.63 (d, $J = 1.8$ Hz, 1 H), 7.30 (dd, $J = 2.0$, 8.9 Hz, 1 H), 2.86 (s, 3 H), 2.85 d, $J = 13.5$ Hz, 2 H), 2.04 (d, $J = 13.4$ Hz, 1 H), 1.48 (s, 3 H), 1.47 (d, $J = 13.7$ Hz, 2 H), 1.35 (s, 6 H), 1.28 (d, $J = 14.9$ Hz, 2 H);

HRMS $m/z$ (M$^+$) calcd 423.1682, obsd 423.1686.

**N-Methyl Imide-Acid Chloride 10g.**

The acid 10f (0.65 g, 1.53 mmol) was converted to acid chloride 10g (0.68 g, 100 %) as described for the preparation of 10c:

$\text{mp} 170 - 173 \degree C$;
$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.73 (s, 1 H), 8.07 (dd, $J$ = 1.9, 8.7 Hz, 1 H), 8.00 (d, $J$ = 8.9 Hz, 1 H), 7.88 (d, $J$ = 8.8 Hz, 1 H), 7.65 (d, $J$ = 2.1 Hz, 1 H), 7.34 (dd, $J$ = 2.2, 8.9 Hz, 1 H), 2.85 (d, $J$ = 13.0 Hz, 2 H), 2.84 (s, 3 H), 2.04 (d, $J$ = 13.2 Hz, 1 H), 1.48 (s, 3 H), 1.46 (d, $J$ = 13.1 Hz, 1 H), 1.35 (s, 6 H), 1.34 (d, $J$ = 14.3 Hz, 2 H);

HRMS $m/z$ (M$^+$) calcd 441.1343, obsd 441.1325.

**N-Methyl Imide-Ester 10h.** Acid chloride 10g (0.18 g, 0.421 mmol) was converted to pentafluorophenol ester 10h as described for the preparation of 10d. Column chromatography on silica gel (1:2:2 EtOAc-Hexanes-CHCl$_3$) gave 0.23 g (95%) 10h:

$^1$H NMR (250 MHz, CDCl$_3$) $\delta$ 8.79 (s, 1 H), 8.16 (dd, $J$ = 1.8, 7.2 Hz, 1 H), 8.00 (d, $J$ = 8.9 Hz, 1 H), 7.93 (d, $J$ = 8.7 Hz, 1 H), 7.68 (d, $J$ = 1.8 Hz, 1 H), 7.33 (dd, $J$ = 2.2, 8.8 Hz, 1 H), 2.86 (d, $J$ = 13.7 Hz, 2 H), 2.85 (s, 3 H), 2.05 (d, $J$ = 14.1 Hz, 1 H), 1.50 (s, 3 H), 1.44 (d, $J$ = 13.3 Hz, 1 H), 1.36 (d, $J$ = 13.5 Hz, 2 H), 1.33 (s, 6 H);

HRMS $m/z$ (M$^+$) calcd 589.1524, obsd 589.1524

**Imide-Amide 11a.**

To a solution of acid chloride 10c (0.21 g, 0.49 mmol) and amine 9$^{12}$ (0.15 g, 0.49 mmol) in 30 mL dry methylene chloride was added catalytic amount of 4-(dimethylamino)pyridine (ca. 20 mg) and 1.0 mL of triethylamine. The resulting solution was stirred at room temperature for 8 h, and then diluted with 30 mL methylene chloride, washed with water (3 x 50 mL) and brine (50 mL), dried over MgSO$_4$, filtered, and evaporated to give a tan product. Flash chromatography on silica gel (5% MeOH in CHCl$_3$) gave 0.28 g (82%) of 11a as a white powder:

mp 176 - 178 °C;

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 11.34 (s, 1 H), 8.14 (d, $J$ = 1.5 Hz, 1 H), 7.92 (d, $J$ = 6.3 Hz, 1 H), 7.84 (s, 1 H), 7.71 (dd, $J$ = 1.2, 8.4 Hz, 1 H), 7.67 (s, 1 H), 7.58 (d, $J$ = 9
Hz, 1 H), 7.54 (d, J = 1.8 Hz, 1 H), 7.45 (d, J = 8.7 Hz, 1 H), 7.16 (dd, J = 2.4, 7.2 Hz, 1 H), 6.33 (s, 2 H), 5.77 (d, J = 4.2 Hz, 1 H), 5.29 (dd, J = 4.2, 6.3 Hz, 1 H), 4.97 (dd, J = 3.0, 6.4 Hz, 1 H), 4.51 (dd, J = 3.0, 10.0 Hz, 1 H), 4.25 (m, 1 H), 3.58 (d, J = 14.7 Hz, 1 H), 2.89 (d, J = 13.8 Hz, 2 H), 2.11 (d, J = 13.2 Hz, 1 H), 1.62 (d, J = 14.0 Hz, 1 H), 1.59 (s, 3 H), 1.49 (d, J = 13.8 Hz, 1 H), 1.47 (s, 3 H), 1.37 (s, 2 H), 1.34 (s, 6 H), 1.33(s, 3 H);
HRMS m/z (M+) calcd 697.2860, obsd 697.2911.

6-N-Methyl Imide-Ester 11b.

Acid chloride 10g (0.68 g, 1.53 mmol) was converted to amide 11b as described for the preparation of 11a. Column chromatography on silica gel (5% MeOH in CHCl3) gave 0.80 g (73%) of 11b:
mp 175 - 180 °C;
1H NMR (300 MHz, CDCl3) δ 8.35 (s, 1 H), 8.23 (d, J = 6.3 Hz, 1 H), 7.94 (dd, J = 1.44, 8.6 Hz, 1 H), 7.86 (m, 4 H), 7.61 (d, J = 1.9 Hz, 1 H), 7.26 (dd, J = 2.2, 5.0 Hz, 1 H), 5.88 (d, J = 4.3 Hz, 1 H), 5.69 (s, 2 H), 5.39 (dd, J = 1.7, 4.4 Hz, 1 H), 5.03 (dd, J = 2.7, 3.6 Hz, 1 H), 4.59 (d, J = 2.7 Hz, 1 H), 4.35 (m, 1 H), 3.61 (dt, 14.4 Hz, J = 2.3, 1 H), 2.87 (s, 3 H), 2.05 (d, J = 12.5 Hz, 2 H), 2.85 (d, J = 12.0 Hz, 1 H), 1.64 (s, 3 H), 1.46 (s, 3 H), 1.45 (d, J = 12.5 Hz, 1 H), 1.36 (s, 3 H), 1.34 (s, 6 H), 1.31 (d, J = 14.0 Hz, 2 H);
HRMS m/z (M+) calcd 711.3016, calcd 711.3016.

6-Acetoxys-2-naphthoic acid 12a.

A mixture of 10 mL of acetic anhydride, 6-hydroxy-2-naphthoic acid (0.70 g, 3.72 mmol), and 3 mL of pyridine was stirred at room temperature for 2.5 h. The reaction mixture was quenched with water to afford a tan precipitate. The solid was isolated by filtration, and washed with 1 N HCl (15 mL) and
then with water (ca. 20 mL). The solid was dissolved in CH\textsubscript{2}Cl\textsubscript{2} (200 mL),
dried over MgSO\textsubscript{4}, and concentrated to afford 0.80 g (93\%) \textbf{12a} as a tan powder:
mp 208 - 210 °C;
\textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) \delta 8.70 (s, 1 H), 8.13 (dd, J = 1.5, 8.7 Hz, 1 H), 8.01 (d, J = 8.9 Hz, 1 H), 7.88 (d, J = 8.7 Hz, 1 H), 7.63 (d, J = 1.9 Hz, 1 H), 7.33 (\textsuperscript{3}d, J = 2.1, 8.9 Hz, 1 H), 2.38 (s, 3 H).

\textbf{Methoxymethyl 6-Acetoxy-2-naphthoate 12b.}
To a solution of acetoxy acid \textbf{12a} (0.70 g, 3.0 mmol) in dry THF (15 mL) and
triethylamine (1 mL), was slowly added methoxymethyl chloride (0.28 mL, 3.6 mmol). The resulting suspension was stirred at room temperature for 1 h.
The reaction mixture was quenched with ca. 5 mL of cold water, and the solvent was removed by rotary evaporation. The residue was dissolved in
EtOAc (50 mL), washed with water (3 x 50 mL) and brine (50 mL), dried over
MgSO\textsubscript{4}, filtered, and evaporated to afford 0.80 g (96\%) of \textbf{12b} as a brown oil
which solidified upon evaporation:
\textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) \delta 8.65 (s, 1 H), 8.11 (dd, J = 1.6, 8.7 Hz, 1 H), 7.99 (d, J = 8.9 Hz, 1 H), 7.86 (d, J = 8.6 Hz, 1 H), 7.62 (d, J = 1.6 Hz, 1 H), 7.32 (dd, J = 2.2, 8.9 Hz, 1 H), 5.56 (s, 2 H), 3.60 (s, 3 H), 2.38 (s, 3 H).

\textbf{Methoxymethyl 6-Hydroxy-2-naphthoate 12c.}
A solution of acetoxy ester \textbf{12b} (0.65 g, 2.37 mmol) in a mixture of 40 mL of
MeOH, 7 mL of saturated aq. NaHCO\textsubscript{3}, solution and 7 mL of H\textsubscript{2}O was stirred
at room temperature for 6 h. The reaction mixture was quenched with 1 N
HCl (ca. 10 mL) to afford a tan precipitate. MeOH was removed by rotary evaporation, and the solid was isolated by rapid filtration. The solid was
dissolved in EtOAc (50 mL), and the solution was washed with brine, dried
over MgSO₄, filtered, and concentrated to afford 0.57 g (97%) of 12c as a tan solid:
mp 130 - 132 °C;
¹H NMR (300 MHz, CDCl₃) δ 8.58 (s, 1 H), 8.05 (dd, J = 1.6, 8.6 Hz, 1 H), 7.80 (d, J = 8.6 Hz, 1H), 7.72 (d, J = 8.6 Hz, 1 H), 7.17 (m, 2 H), 5.55 (s, 2 H), 5.49 (s, 1 H), 3.60 (s, 3 H); HRMS m/z ([M-H]⁺) calcd 231.0657, obsd 231.0657.

**Aminoadenosine 9.** This can be synthesized according to the procedure by Kolb et al.¹¹

**Kinetics Studies.**
The reaction of imide esters 10d and 10h with amine 9 in the presence and absence of templates 11a and 11b was performed in CHCl₃ solution containing ca. 4 equiv Et₃N. A Waters 600 HPLC equipped with a UV detector (254 nm) was used for analysis of reaction mixtures. Analyses were performed using a mixture of water/methanol/Et₃N (30 : 70 : 0.1) as the mobile phase and a reverse phase column (Beckman C₁₈ column, Ultrasphere ODS dp, 5m, 4.6 mm, I.D x 25 cm, flow rate = 1.5 mL/min). The integrations and concentrations of all the peaks were calculated using an NEC computer and Waters 820 Baseline software. In case of prolonged reactions (> 100 min), a methanol flush was performed between injections to ensure complete elution of esters 10d or 10h. CHCl₃ was dried over molecular sieves or by passage through Al₂O₃ before use. All experiments were performed at ambient temperature (21.5 - 23.0 °C). Each run was performed in 2 - 4 times to obtain average values for data.

**Calibration of the HPLC.**
The HPLC was calibrated by injection of solutions of amine 9 and template 11a of varying concentration. A linear relationship between concentration and peak area was observed. Reaction mixtures were analyzed on the basis of either the area of the template peak or the ratio of the amine and template peaks.

Reaction Procedures.

During initial studies (data presented in Figures 9), reactions were performed in 100 mL solvent in 1-mL Wheaton serum vials equipped with aluminum caps and teflon coated silicone septa (Procedure A). During subsequent studies (data presented in Figures 10-13), 1-mL Wheaton screw-cap vials equipped with Mininert valves were used to minimize evaporative losses of solvent, and 500 mL of solvent was used to reduce changes in concentrations arising from solvent evaporation (Procedure B).

Typical Reaction Procedure A. Reaction of Imide-Ester 10d and Amine 9.

A 1-mL Wheaton serum vial equipped with a aluminum cap and a silicone rubber septum coated with teflon was charged with 20 μL CHCl₃, ca. 1 μL of Et₃N, 40 μL of amine 9 stock solution (2.05 x 10⁻² M) and 40 mL of pentafluoroester stock solution 10d (2.05 x 10⁻² M). The reaction mixture was shaken gently with a mechanical shaker. Aliquots (1.0 μL) were withdrawn periodically and analyzed by HPLC.

Typical Reaction Procedure B. Reaction of Imide-Ester 10d and Amine 9 in the Presence of Template 11a.

A 1-mL Wheaton reaction vial equipped with a Mininert valve and a stir vane was charged with 260 μL CHCl₃ and 4.6 μL of Et₃N. Stock solutions of amine 9 (100 μL, 8.2 x 10⁻² M), template 11a (40 μL, 4.1 x 10⁻² M), and
pentafluoroester 10d (100 μL, 8.2 x 10^{-2} M) were added by microliter syringe.

Aliquots (10 μL) were withdrawn from the reaction solution periodically, diluted with 90 μL of CHCl₃, and analyzed by HPLC (10 μL injected).
References (Chapter One)


12. The author thanks Mr. E. Wintner for helping the kinetic reactions in the control experiments.


14. The course of the reaction was treated as 100 time increments, $\Delta t$. Equilibrium concentrations of $A$, $E$, $T$, $AE$, $AT$, $ET$, $T_2$, and $AET$ were calculated at each time increment using successive approximations. The incremental formation of template was calculated by the equation: $\Delta [T]_{tot} = k_1[A]_{tot}[E]_{tot}\Delta t + k_2[AE]\Delta t + k_3[AET]\Delta t$, in which $[A]_{tot}$, $[E]_{tot}$, and $[T]_{tot}$ represent the total concentrations of amine, ester, and template present. Calculations were performed on a Macintosh computer using Microsoft Excel.

15. An alternative model for the kinetics of self-replicating systems was introduced by von Kiedrowski in 1986. (See references 5a-c.) In this model the rate is given as the sum of an autocatalytic term and a background term.

   The von Kiedrowski model provides a simple, elegant, and effective means of obtaining the kinetic parameters of autocatalytic systems without the need for equilibrium constants. The model used here accounts for the
mechanistic details of the reaction and accounts for the role of each of the
three mechanistic pathways (background bimolecular, preassociative, and
template termolecular). This model permits specific predictions about the
effects of changes in structure and binding affinities upon the rates of
reaction.

Chapter Two. New Developments in Replicating Systems

2.1 Competition, reciprocity and mutation at the molecular level: 
Irradiation of a synthetic replication generates a superior species

Self-replication is the ability of a system to catalyze its own formation. Replication, in contrast to simple autocatalytic processes, utilizes complementarity of shape, size, and intermolecular forces to bring together the reacting components to start reproductive process.¹ Synthetic self-replicating systems are closely related to biological systems, and may provide some insight into biotic and prebiotic chemical behavior.²-⁴

In the first chapter a self-replicating system that features phenomena of autocatalysis was described.¹ The fully-formed products could act as templates for the assembly of identical molecules. Our critics were quick to point out that this replication had severe limitations: the molecules would only make copies of themselves, and in prebiotic terms, this was a "dead end". Selection and consequent evolution in this system is impossible as there are no variants. Each template turned out identical product. As a result, the system has no way of evolving chemically.

One of the key features of living systems is the ability to mutate and evolve. For evolution to occur at the molecular level, the replicator must make mistakes⁵, allowing them to change structures that give rise to more effective replicators; those that were more fit in the environment of the experiments. The mutation and natural selection on a molecular level can be illustrated in Figure 1. One molecule is better than the other at replication in a given environment and takes over the resources of the system.
Synthesis of Photo labile amines:

In organic chemistry a mistake can be interpreted as a lack of selectivity, and with some modifications to our original system, we synthesized molecules that would catalyze not only their own formation, but of related structures. In this way a replicator makes mistakes.

In our system there are two starting components: an amine and a ester. In principle we can incorporate a difference into either one of them. Then an environmental change or stress applied to cause a heritable structural change (the mutation). Such an environmental stress can be a variety of different events including changes in temperature, pH, solvent, etc. In this case a photochemical change was chosen and a photo labile group was incorporated into the amine part. Specifically, molecules bearing an o-NO$_2$-benzyloxyxycarbonyl groups on them were used (Scheme I, 2b, 2c), and replicating molecules could have their activities enhanced by photo cleavage of the labile group.

The two substituted amines 2b and 2c were synthesized from the original unsubstituted amine 2a as shown in Scheme I$^6$. The adenosine amine 2a was first protected as a trifluoroamide 2d. Usually the Cbz group is introduced by using benzyl chloroformate under aqueous alkaline conditions. This condition is
not applicable to acylation of adenine amine. This is due to the low nucleophilic potential of the amino group. By using the substituted acylimidazolium salts (10a or 10b) adenosine 2d was easily acylated to give urethane-protected adenosine 2e and 2f. In this case the leaving group N-methyl imidazole is a good leaving group. Compound 2e and 2f were then deprotected under basic conditions to give the two substituted amines 2b and 2c.

The imidazolium chloride was synthesized from benzyl alcohol 8b to give benzyl chloroformate 9b which reacted with N-methyl imidazole to give the substituted imidazolium chloride 10b. Compound 10a can be obtained in a similar way.
The two substituted amines 2b and 2c are very similar in structure, but different in their photochemistry. Amine 2c has the o-NO2-benzyl carbamate moiety which can be photolytically removed, while amine 2b has only the unsubstituted benzyl carbamate and is not cleaved under UV irradiation. The photochemistry of 2c is shown in Figure 2. It involves the intramolecular hydrogen abstraction by the photo excited nitro group. The nitro group is then reduced to a nitroso group and an oxygen is inserted into the carbon-hydrogen bond located at the 2-position. This tetrahedral intermediate collapsed to the o-
nitroso benzaldehyde, liberating the amine. To prevent the phenyl aldehyde \textit{7} from reacting with the free amine it was quenched with excess p-tosyl hydrazide to form the corresponding hydrazone.

\textbf{Figure 2.} Photo cleavage reaction of the substituted amine \textit{2c} or \textit{3c}.

**Improvement of the original self-replicating system**

In the first chapter the synthesis and kinetic studies of the first generation of the self-replicating molecules were discussed. From the discussion we know that the system has three reaction pathways, but only the termolecular pathway contributes to the self-replication. To improve the replicating components, the bimolecular pathway has to be reduced or shut down. One way to achieve this is to build an even longer spacer in the molecule, so that in the base-pairing complex the two reacting centers cannot reach each other (Figure 3)
Figure 3. With a longer spacer the base-pairing (AE) bimolecular reaction pathway can be decreased.

The system described in Chapter One was modified by using a biphenyl spacer 11f and the propyl Kemp's acid derivative (imide acid chloride 12, to increase the solubility of the system).

The synthesis of biphenyl ester 1 was shown in Scheme II.\(^8\) It started from nitro biphenyl\(^9\) 11a; and after Friedel-Crafts acylation, 11b was obtained. This was converted to acid 11c with bromine in base. Acid 11c was then reacted with SOCl\(_2\) to give acid chloride 11d, which reacted with BnOH to give benzyl ester 11e. Reduction of the NO\(_2\) group by iron powder under acidic conditions gave 11f. Coupling of the imide acid chloride\(^10\) with the spacer 11f in the presence of DMAP yielded amide 13a. Hydrogenolysis of amide ester 13a went smoothly with Pearlman's catalyst (Pd(OH)\(_2\) on charcoal) to give the corresponding acid 13b. This was converted to active ester 1 by EDC coupling with pentafluorophenol (C\(_6\)F\(_5\)OH).
Scheme II. Synthesis of ester 1.

Coupling of the pentafluorocphenyl ester 1 (Scheme III) with different amines 2 in CHCl₃ yields the respective amides 3. The self-complementarity of these products leads to extensive self-association in the dimeric complex 4, and is the key to their replicative behavior. The unsubstituted template molecule can base-pair in both the Watson-Crick and Hoogsteen modes. The urethane-protected 2b and 2c are limited in this respect; the N-substituent hinders base-pairing in the Watson-Crick sense (Figure 4), and limits binding largely to the Hoogsteen modes.
Scheme III. Templates formation from three different amines.
This argument was further supported by NMR binding studies. From Figure 5, it can be seen that the dimerization constant for the unsubstituted template 3a is about 80 fold higher than that of the substituted template 3c. The binding constant between the ester 1 and the unsubstituted amine 2a is also 5 times higher than the binding between ester 13a and substituted amine (2b or 2c).
Figure 5. Binding constants of related compound.

Kinetic Results:

The reaction rates were again monitored by HPLC. Different solvent conditions and different flow rates were tried to separate these three structure closely related templates (3a-c). Finally the conditions were found that were able
to accomplish this task. For each template, a calibration curve was made which showed a linear relationship between template area response and concentration.

All three templates are replicators: they catalyze their own formation. For example, in Figure 6, the presence of 20% 3a (unsubstituted amine) increases the initial rate of coupling by 40%. In this biphenyl spacer system, compared with the naphthyl spacer system (Chapter One), the preassociative bimolecular pathway could be decreased. The dimerization constant for template 3a is 80,000 M\(^{-1}\). The binding constant for different other species (AE, AT, ET) is estimated to be 280 M\(^{-1}\).

![Graph](image)

**Figure 6.** Reaction of amine 2a + Ester 1 (0.050 M). a) no additive, b) 20% template 3a added.

From this information combined with the experimental data, the reaction constants were calculated from the kinetic model. 1) The bimolecular background reaction rate constant \(k_1\) is 0.036 M\(^{-1}\)min\(^{-1}\) (in naphthyl system \(k_1 =\) \(\ldots\)
0.023 M\(^{-1}\)min\(^{-1}\)). This slight increase in rate constant is presumably because that the biphenyl pentafluoro ester is more active than the corresponding naphthyl ester. 2) The preassociative bimolecular pathway still contributed to the product formation and \(k_2\) is 0.0005 M\(^{-1}\) (in naphthyl system \(k_2 = 0.0036\) min\(^{-1}\)). There is a significant decrease in rate constant. This longer spacer did work to some extent. 3) The rate constant for termolecular autocatalytic pathway is 0.06 min\(^{-1}\) which is similar to the naphthyl system (\(k_3 = 0.07\) min\(^{-1}\)). Table I summarizes the results.

<table>
<thead>
<tr>
<th>rate constant</th>
<th>naphthyl system</th>
<th>biphenyl system</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k_1) (background, M(^{-1})min(^{-1}))</td>
<td>0.023</td>
<td>0.036</td>
</tr>
<tr>
<td>(k_2) (base-pairing, min(^{-1}))</td>
<td>0.0036</td>
<td>0.0005</td>
</tr>
<tr>
<td>(k_3) (termolecular, min(^{-1}))</td>
<td>0.07</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Table I.

For the urethane-protected templates 3b and 3c, adding 20% of the product template to its respective reaction mixture enhances the initial coupling rate by ~30% (Figures 7 and 8).

In addition, the products make "mistakes" and catalyze the formation of each other. For example, the presence of 20% 3b during the coupling of 1 with 2c increases the rate of appearance of 3c by 18% (Figure 7), while the presence of 20% 3c during the coupling of 1 with 2b increases the rate of appearance of 3b by 10% (Figure 8). This reciprocal behavior is understandable due to the structural similarity of 3b and 3c. Consequently, the heterogeneous (mixed) termolecular species 5 (\(R_1 \neq R_2\)), will coexist with the homogeneous ones (\(R_1 = R_2\)). The unsubstituted compound (\(R = H\)) catalyzed the formation of both, as well as of
itself and is also a more efficient replication: addition of 20% of the template 3a increases the initial reaction rates by more than 2 fold (Figure 7 and 8).

**Figure 7.** Product 3b appearance as a function of time for the reaction of 2b + 1 at 0.050 M each in CHCl₃. a) no additive. b) 20% 3c added. c) 20% 3b added. d) 20% 3a added.
Figure 8. Product 3c appearance as a function of time for the reaction of 2c + 1 at 0.050 M each in CHCl₃, a) no additive, b) 20% 3b added, c) 20% 3c added, d) 20% 3a added.

Compounds 2c and 3c bearing photo labile blocking groups can be removed by irradiation at 350 nm (Figure 2). For example, a solution of coupling product 3c irradiated for 30 min (Rayonet reactor) is cleanly converted to the unsubstituted (and more efficient) replicator 3a; likewise amine 2c can be converted to 2a (Figure 2).

The resulting deblocked system is an excellent replicator: direct competition of the three amines 2a, 2b and 2c for a limited quantity of the ester 1 resulted in rapid formation of the efficient replicator 3a as the major product (Figure 9).
**Figure 9.** Products appearance as a function of time for the reaction of $2a + 2b + 2c + 1$ (0.042 M each) in CHCl₃. a) 3a. b) 3b. c) 3c.

**Table II** shows the summary of the kinetic results. It can be seen that when the same amount of template (20%) were used, the unsubstituted template 3a was in all cases the better replicator.¹¹

<table>
<thead>
<tr>
<th>Reaction type</th>
<th>added T</th>
<th>initial rate (x10⁻⁵)</th>
<th>rate increase</th>
<th>% reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitro-Z-Amine (1b) + Ester (4)</td>
<td>no T</td>
<td>3.8</td>
<td></td>
<td>11%</td>
</tr>
<tr>
<td></td>
<td>Z-T(20% 5c)</td>
<td>4.5</td>
<td>18%</td>
<td>13%</td>
</tr>
<tr>
<td></td>
<td>Nitro-Z-T(20% 5b)</td>
<td>4.8</td>
<td>26%</td>
<td>13%</td>
</tr>
<tr>
<td></td>
<td>NH₂-T(20% 5a)</td>
<td>8.5</td>
<td>124%</td>
<td>23%</td>
</tr>
<tr>
<td>Z-Amine (1c) + Ester (4)</td>
<td>no T</td>
<td>3.7</td>
<td></td>
<td>10%</td>
</tr>
<tr>
<td></td>
<td>Nitro-Z-T(20% 5b)</td>
<td>4.1</td>
<td>10.8%</td>
<td>11%</td>
</tr>
<tr>
<td></td>
<td>Z-T(20% 5c)</td>
<td>4.8</td>
<td>29.7%</td>
<td>13%</td>
</tr>
<tr>
<td></td>
<td>NH₂-T(20% 5a)</td>
<td>9.0</td>
<td>143%</td>
<td>19%</td>
</tr>
<tr>
<td>Amine (1a) + Ester (4)</td>
<td>no T</td>
<td>18.2</td>
<td></td>
<td>48%</td>
</tr>
<tr>
<td></td>
<td>NH₂-T(20% 5a)</td>
<td>26.0</td>
<td>43%</td>
<td>63%</td>
</tr>
</tbody>
</table>

**Table II.** Summary of the kinetic reactions.
This "mutable" system was used to develop a simple system capable of selection. Amines 2c and 2b (1 eq. each) were initially reacted with a limited quantity of ester 1 (1 eq). The amines and ester were allowed to react to completion, resulting in slightly more of the o-(NO2)-Cbz-protected template 3c and the CBz template 3b. Irradiation of mixture for 2 h gradually converted the amine 2c and the template 3c to their unsubstituted analogs. Additional ester 1 was added, and the reaction was allowed to proceed. The result is shown in Figure 10. Even though the "mutant" template 3a is initially at a lower concentration, its superior replicative ability allows it to dominate the system's resources, equaling, and then exceeding the concentration of template 3b. The "mutant" (3a, R = H) rapidly consumed the ester (Figure 10).12

Figure 10. Formation of templates 3a (T), 3b (z-T) and 3c (NO2-z-T): Reaction of ester 1 (20 mM) with amine 2b (50 mM) and amine 2c (50 mM), followed by irradiation, then addition of ester 1 (50 mM).

In summary, the replicators described here provide model systems for reciprocity and photochemically-induced mutation at the molecular level.
2.2 New Synthetic Replicators with Thymine Derivatives.

We have described earlier the first synthetic molecules that can self-replicate.\(^1\) (detailed discussion in Chapter 1). The product can be formed by three distinctive pathways; a bimolecular reaction \((k_1)\), a preassociative bimolecular pathway \((k_2)\), and the termolecular template-catalyzed reaction \((k_3)\), which is responsible for the autocatalytic behavior. The reactions proceed mainly through the formation of base-paired complexes.

In the present case, we synthesized another self-replicating system based on a different skeleton; namely, a xanthene diacid was used as the backbone structure and the hydrogen binding of thymines to diamino triazine was used as the recognition feature. At the same time we hoped to improve the autocatalysis by shutting down the preassociative bimolecular pathway (Figure 11).

![Figure 11.](image)

The preparation of the new system was as follows: xanthene diester \(20a\)\(^{13}\) was monofunctionalized with HBr gas to give \(20b\) which was esterified with phenol using DCC in the presence of catalytic DMAP to give \(20c\). Ester \(20c\) was reacted with biguanide to give the triazine methyl ester \(20d\) (Scheme IV).
Warming the triazine 20d in neat ethylenediamine afforded the triazine amine 21 in quantitative yield.
Activated thymine 23a-c was prepared by reacting thymine acid 22 with phenol derivatives in the presence of a carbodiimide [N-ethyl N’-(3-dimethylaminopropyl) carbodiimide methiodide]. Coupling of the triazine amine 21 with the active ester 23a gave template molecule 24.

Even though molecular modeling of the base-paired complex suggested that the distance between amino group and active ester carbonyl is longer than 2.6 Å (Figure 11), there seems to be a preassociative bimolecular reaction (Figure 12).

The reaction rates were again followed by HPLC. In the background reaction, a solution of equimolar concentration (each 8 mM) of the triazine amine 21 and the thymine phenyl ester 23c in CHCl₃ with triethylamine (Et₃N) was allowed to react, and periodically aliquots were removed and analysed by HPLC. In order to find out the degree of autocatalysis, the template 24 (0.25 and 0.50 equivalent) was added to the background reaction mixture and analyzed in the same manner as above. The reaction was also studied at 8 mM and was monitored only during the initial stage. A plot of product 24 (concentration) vs time (min) is shown in Figure 12.

The added template resulted in rate acceleration as in the previous replicating system. When 0.25 equiv. of template 24 was added, the initial rate increased by 28% (line b); when 0.5 equiv. was added, the rate increased by 80% (line c). The reaction between N-methyl thymine ester 23d and triazine amine 21 was much slower. Compared with the reaction between N-H thymine ester 23c and the same amine, the rate decreased by 50% (line d). A simple calculation shows that catalysis is proportional to the square root of the catalyst concentration.
Figure 12. Appearance of 24 as a function of time. (8.0 mM in CHCl₃) 
Et₃N(18 equiv.) was present in all reaction solutions. Error bars represent 
standard deviations of multiple independent runs. a) no additive. b) 25% 
24 added. c) 50% 24 added. d) Reaction of N-Methyl thymine ester with 21 were 
8.0 mM.

The results can be explained as follows. Three reactions contribute to the 
formation of product: the background bimolecular reaction, the base-pairing 
preassociative bimolecular reaction and termolecular template-catalyzed process 
(Figure 13). The latter is responsible for the replication process. The amino 
triazine 21 reacts with the phenyl ester 23c by means of a bimolecular reaction 
generating template 24. This template binds with each of the reactants to form 
termolecular complex 25, which reacts and gives rise to two template molecules 
as dimerized form. The dimer dissociates into monomer template 24 which acts 
as the catalyst. The decrease in rate for the N-methyl thymine ester suggested 
that in this system, the base-pairing preassociative bimolecular mechanism does 
exist.
Figure 13.

A control experiment was performed to find out whether the reaction is a template directed synthesis or whether triazine was merely acting as a base catalyst for the reaction, the reactions were carried out with a triazine bearing no active ester. Figure 14 shows the result. It is clear that triazine 26d has no effect on the product formation. This suggested that the reaction is not a general base (triazine amine) catalyzed reaction, but a template-directed reaction.16
The equilibrium constant between the triazine 24 and the thymine active ester 23c was measured to be 670 M\(^{-1}\). The dimerization constant (K_d) of the template was obtained from dilution study of the template. The imide peak in 24 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 dissociate. The K_d was estimated to be 30,000M\(^{-1}\) with the dilution data calculated from the Systat program.

### 2.3 Crossover Experiments

Synthetic replicators provide a means by which aspects of prebiotic behavior can be examined at the molecular level (see 2.1 of this chapter\(^5\). In the previous example we showed that the systems can have reciprocity and mutation features characteristic of evolution at the molecular level.
In this section the synthetic replicators capable of hybridization and recombination will be discussed, and their autocatalytic properties can be interpreted in terms of molecular shape and structure.

The minimal requirement for replicators is a self-complementary structure,\(^1\) and two such systems have been described in the previous chapters. The first involves adenine-imide hydrogen bonding\(^1,8,11\) (Figure 14) and the second thymine-diaminotriazine hydrogen bonding\(^16\) (Figure 15) as the molecular recognition components.

Figure 14. replicator with adenine-imide hydrogen bonding.
Figure 15. Replicator with thymine-diaminotriazine hydrogen bonding.

Both 28 and 24 are replicators: they catalyze their own formation through the template effects suggested in termolecular complexes 29 and 25.

With the two replicating systems in hand, we wondered: What would happen if we ran crossover experiments between the two systems? Specifically, coupling of the adenine 26 (the amine from the first system) with the thymine ester 23b (the ester from the second system) gave the crossover product dinucleotide analog 30 with a peptide/ribose backbone (Figure 16). The corresponding reaction of Kemp’s imide active ester 27 (the ester from the first system) with triazine amine 21 (the amine from the second system) gave another crossover product 31 (Figure 17).

Figure 16. Recombinant capable of replication.
At first glance, both hybrids might be expected to replicate. They both have complementary recognition surfaces which can gather their respective reaction components in termolecular complexes. In fact, the adenine thymine hybrid 30 does so. Addition of 30 to mixtures of 26 and 23b in CH$_3$CN led to the increased coupling rates (Figure 18) characteristic of autocatalytic systems.$^1$ It is a fertile hybrid.
Figure 18. Appearance of 30 as a function of time. Initial concentrations are \([26] = [23b] = 3.0 \text{ mM} \) in CH$_3$CN. Et$_3$N (18 equiv.) was present in all reaction solutions. Error bars represent standard deviations of multiple independent runs. a) no additive. b) 0.07 equiv of 30 added.

Figure 19  Appearance of 31 as a function of time. Initial concentrations are \([27] = [21] = 8.0 \text{ mM} \) in CHCl$_3$. Et$_3$N (12 equiv.) was present in all reaction solutions. Error bars represent standard deviations of multiple independent runs. a) Reaction of 27 and 21 without additive. b) Reaction of 27 and 21 with 0.28 equiv. of 31 added.
This is not the case for the hybrid 31. No increase in coupling rate for 27 with 21 was observed on adding various amounts of the hybrid 31 (Figure 19). Accordingly, it can not catalyze its own formation; it is a sterile hybrid.

The differences in behavior of hybrid 30 and 31 can be related to the orientations of their recognition surfaces. In 30 these can achieve a parallel arrangement that binds 23b and 26 in a productive termolecular complex 30a. The initial product is the hydrogen bonded dimer of 30.

The molecule 31 features two U-shaped parts - the Kemp's triacid and the xanthene diacid. Its overall configurations can be C- or S-shaped in which its recognition surfaces converge or diverge, respectively. In neither case can it simply dimerize. Rather, self-complementarity probably results in oligomerization to form chains (Figure 20)\(^{17}\).

![Diagram](image)

**Figure 20.** Schematic representation: (A) recombinant capable of replicating, the product 30 can dimerize. (B) and (C) are C- and S-shaped conformation of product 31. In either case, the product cannot form simple dimer.

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 achieved. When they diverge, the molecular assemblies lead to oligomers. These notions are being pursued in this lab with suitably oriented surfaces for controlled oligomerizations.\(^{18}\)
2.4 Experimental Section (Chapter Two)

**General.** Mass spectra were obtained on VG Instruments, a Se-20 mass spectrometer, or a Varian CH-5 instrument (high resolution). IR spectra were obtained on an IBM IR/32 FTIR. $^1$H NMR spectra were obtained on either AC 250 MHz Brucker or 300 MHz Varian instruments. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl. Melting point were determined on a Thomas Hoover melting point apparatus and were uncorrected.

5'-N-trifluoroacetyl adenosine amine (2d).

A solution of 5'-aminoadenosine 2a (535 mg, 1.75 mmol) in methanol (3.2 mL) was added triethylamine (0.25 ml, 1.79 mmol), and the resulting solution was cooled to 0 °C. Ethyl trifluoroacetate (0.26 mL, 2.18 mmol) was added dropwise to the reaction mixture. The resulting solution was stirred at room temperature for 36 hr. The solvent was removed, and the residue was chromatographed (2:98 = MeOH/CH$_2$Cl$_2$, then 5:95 = MeOH/CH$_2$Cl$_2$) to afford 2d as a colorless oil (quantitative yield):

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.44 (dd, J = 8.6, 1.7 Hz, 1 H), 8.29 (s, 1 H'), 7.80 (s, 1 H), 5.87 (br s, 2 H), 5.81 (d, J = 4.5 Hz, 1 H), 5.16 (dd, J = 6.2, 4.5 Hz, 1 H), 4.77 (d, J = 6.2, 2.4 Hz, 1 H), 4.54 (dd, J = 5.4, 2.4 Hz, 1 H), 4.23 (ddd, J = 14.7, 9.0, 3.1 Hz, 1 H), 3.43 (d, J = 14.7 Hz, 1 H), 1.61 (s, 3 H), 1.33 (s, 3 H);

IR (neat): 3327, 3184, 1720, 1650, 1600, 1479, 1378, 1217, 1098, 854, 758 cm$^{-1}$;

FAB HRMS: calcd (C$_{15}$H$_{17}$F$_3$N$_6$O$_4$) 402.1263 obsd 402.1262.

5'-N-trifluoroacetyl cbz adenosine amine (2e)$^6$.

A mixture of 5'-N-trifluoroacetyl adenosine 2d (850 mg, 2.11 mmol) and 1-methyl-3-phenylmethoxycarbonylimidazolium chloride 10a (1.1 g, 4.35
mmol) in CH₂Cl₂ (24 mL) was stirred at room temperature for 24 hrs. The resulting solution was concentrated, and the residue was chromatographed (2:98 = MeOH/CH₂Cl₂ to 4:96 MeOH/CH₂Cl₂) to give 758 mg (67%) of 2e as a white foam:

mp 92-95 °C;

¹H NMR (300 MHz, CDCl₃) δ 8.83 (d, J = 7.5 Hz, 1 H), 8.72 (s, 1 H), 8.56 (s, 1 H), 7.91 (s, 1 H), 7.44 - 7.33 (m, 5 H), 5.79 (d, J = 4.2 Hz, 1 H), 5.31 (d, J = 12.0 Hz, 1 H), 5.26 (d, J = 12.0 Hz, 1 H), 5.16 (dd, J = 6.3, 4.5 Hz, 1 H), 4.80 (dd, J = 6.3, 2.6 Hz, 1 H), 4.52 (q, J = 2.8 Hz, 1 H), 4.18 (dd, J = 14.7, 8.6, 3.6 Hz, 1 H), 3.47 (d, J = 14.7 Hz, 1 H), 1.61 (s, 3 H), 1.33 (s, 3 H);

IR (neat): 3258, 2989, 1757, 1716, 1616, 1586, 1468, 1330, 1214, 1158, 1100, 754 cm⁻¹.

FAB HRMS: calcld (C₂₃H₂₃F₃N₆O₆) 536.1631; obsd 536.1630.

Cbz adenosine amine (2b).

A solution of adenosine 2e (620 mg, mmol) in MeOH (3.5 mL) was added 5% potassium carbonate in aq. methanol (v/v 2:5 water/methanol, 6 mL). The reaction mixture was stirred at room temperature for 45 min. The solvent was removed, and the residue was dissolved in a 1:1 mixture of methanol and chloroform solution (25 mL) and filtered through a short pad of Celite. The filtrate was concentrated, and the residue was chromatographed (10:90 = MeOH/CH₂Cl₂) to give 240 mg (47%) of 2b as a white foam:

mp 78-81 °C;

¹H NMR (300 MHz, CDCl₃) δ 8.74 (s, 1 H), 8.05 (s, 1 H), 7.44 - 7.32 (m, 5 H), 6.02 (d, J = 3.0 Hz, 1 H), 5.41 (dd, J = 6.3, 3.0 Hz, 1 H) 5.28 (s, 2 H), 4.99 (dd, J = 6.3, 3.5 Hz, 1 H), 4.26 (m, 1 H), 3.45 (s, 2 H), 3.01 (dd, J = 13.2, 4.5 Hz, 1 H), 2.92 (dd, J = 13.2, 5.9 Hz, 1 H), 1.60 (s, 3 H), 1.37 (s, 3 H);

IR (neat): 2991, 1751, 1616, 1586, 1406, 1383, 1231, 1088, 870, 766 cm⁻¹;
FAB HRMS [M+H]+: calcd 441.1886, obsd 441.1890.

5'-N-trifluoroacetyl (o-NO2)-Cbz adenosine (2f).
5'-N-trifluoroacetyladenosine 2d (747 mg, 1.85 mmol) was converted to Adenosine 2f in the same way as the preparation of 2e with carbonylimidazolium chloride 10b (1.1 g, 3.7 mmol). Column chromatography on silica gel (4:96 = MeOH/CH2Cl2) afforded 740 mg (69%) of 2f as a white foam;

1H NMR (300 MHz, CDCl3) δ 9.62 (br, 1 H), 8.76 (d, J = 5.4 Hz, 1 H), 8.67 (s, 1 H), 8.07 (s, 1 H), 8.04 (d, J = 8.1 Hz, 1 H), 7.68 (d, J = 7.8 Hz, 1 H), 7.59 (t, J = 7.5 Hz, 1 H), 7.46 (t, J = 7.5 Hz, 1 H), 5.92 (d, J = 4.2 Hz, 1 H), 5.64 (s, 2 H), 5.18 (dd, J = 6.3, 4.2 Hz, 1 H), 4.82 (dd, J = 6.3, 2.7 Hz, 1 H), 4.50 (br s, 1 H), 4.09 (m, 1 H), 3.50 (d, J = 14.7 Hz, 1 H), 1.59 (s, 3 H), 1.31 (s, 3 H);
IR (neat): 3247, 2992, 1761, 1719, 1616, 1528, 1470, 1344, 1214, 1158, 1093, 758 cm⁻¹;
FAB HRMS [M+H]+: calcd 582.15599, obsd 582.1555.

Cbz Adenosine amine (2c).
Adenosine amine 2f (610 mg, 1.05 mmol) was converted to adenosine 2c in the same way as the preparation of 2b. Column chromatography on silica gel (5:95 -10:90 MeOH/CH2Cl2) gave 254 mg (50%) of 2c as a white foam:

mp 79-82 °C;

1H NMR (250 MHz, CDCl3) δ 8.74 (s, 1 H), 8.13 (s, 1 H), 8.09 (dd, J = 8.2, 1.3 Hz, 1 H), 7.71 (dd, J = 7.7, 1.1 Hz, 1 H), 7.62 (ddd, J = 7.6, 7.6, 1.3 Hz, 1 H), 7.48 (ddd, J = 7.6, 7.6, 1.5 Hz, 1 H), 6.07 (d, J = 3.0 Hz, 1 H), 5.69 (s, 2 H), 5.42 (dd, J = 6.5, 3.0 Hz, 1 H), 4.99 (dd, J = 6.5, 3.4 Hz, 1 H), 4.25 (m, 1 H), 3.02 (dd, J = 13.4, 4.5 Hz, 1 H), 2.93 (dd, J = 13.4, 6.0 Hz, 1 H), 1.60 (s, 1 H), 1.36 (s, 1 H);
IR (neat): 2963, 1757, 1616, 1580, 1526, 1260, 1091, 1077, 1018, 797 cm⁻¹;
FAB HRMS [M+H]+: calcd 486.1737, obsd 486.1734.
2-Nitrobenzyloxy carbonyl chloride (9b).

A solution of 2-nitrobenzyl alcohol (3.3 g, 21.4 mmol) in 12 ml of 1,4-dioxane was added slowly to a solution of phosgene in toluene (1.93 M, 30 mL, 58 mmol). The resulting solution was stirred for 2.5 days at r. t. and concentrated under reduced pressure below 40 °C to give 9b as a brownish oil (quantitative yield);

\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 8.18 (dd, \(J = 8.4, 1.2\) Hz, 1 H), 7.72 (ddd, \(J = 8.4, 8.4, 1.2\) Hz, 1 H), 7.63 (d, \(J = 6.6\) Hz, 1 H), 7.56 (m, 1 H), 5.74 (s, 2 H);

IR (neat): 1778, 1579, 1530, 1448, 1345, 1140, 793, 729 cm\(^{-1}\);

\(^{1}\)AB HRMS: calcd (C\(_8\)H\(_6\)ClNO\(_4\)) 214.9985, obsd 214.9984.

1-Methyl-3-phenylmethoxycarbonylimidazolium chloride (10a)\(^6\).

A precooled (0 °C) solution of benzyl chloroformate (14 mL, 91 mmol) in CH\(_2\)Cl\(_2\) (200 mL, distilled from CaH\(_2\)) was added slowly to 1-methylimidazole (7.6 mL, 95 mmol) under argon. The resulting mixture was stirred at 0 °C for 15 min and another 30 min at room temperature. The solid was filtered, washed with dry CH\(_2\)Cl\(_2\), and dried under vacuo to give 17.3 g (75%) 10a as a white precipitate:

mp 83 - 85 °C;

\(^1\)H NMR (300 MHz, DMSO-d\(_6\)) \(\delta\) 9.91 (d, \(J = 1.8\) Hz, 1 H), 8.18 (t, \(J = 1.8\) Hz, 1 H), 7.87 (t, \(J = 1.8\) Hz, 1 H), 7.56 - 7.42 (m, 5 H), 5.55 (s, 2 H), 3.91 (s, 3 H);

IR (KBr): 3423, 3084, 1794, 1748, 1574, 1455, 1331, 1261, 1238, 1161, 1079, 743 cm\(^{-1}\).

1-Methyl-3-\(o\)-nitrophenylmethoxycarbonylimidazolium chloride (10b)\(^6\).

A precooled (0 °C) solution of 2-nitrobenzyloxy carbonyl chloride 9b (6.1 g, 28 mmol) in CH\(_2\)Cl\(_2\) (50 mL, distilled from CaH\(_2\)) was added 1-methylimidazole (2.4 mL, 30 mmol). The resulting mixture was stirred at 0 °C for 1 h and another 30 min at room temperature. The precipitate was collected by
suction, washed with dry CH₂Cl₂, and dried in vacuo to give 6.68 g (80%) \( \text{10b} \) as a white solid;
mp 91-93 °C;
\(^1\)H NMR (300 MHz, DMSO-d₆) \( \delta \) 10.05 (s, 1 H), 8.25 - 8.22 (m, 2 H), 7.98 - 7.69 (m, 4 H), 5.92 (s, 2 H), 3.95 (s, 3 H);
IR (KBr): 3432, 1739, 1530, 1342, 1330, 1240, 960, 756 cm⁻¹.

4-Nitrobiphenyl-1-benzylcarboxylic acid (11c).

This was synthesized according to a paper by Byron et al\(^9\) from 4-nitrobiphenyl 11a.

4-Nitrobiphenyl-1-benzylcarboxylate (11e).

A solution of 4-nitrobiphenyl-1-benzylcarboxylic acid 11a (1.501 g, 6.1 mmol) in THF (20 mL) and \( \text{SOCl}_2 \) was refluxed for 3 hrs. Solvent and excess \( \text{SOCl}_2 \) were removed. The resulting solid was dissolved in CH₂Cl₂ (20 mL), and the solution was cooled to 0 °C. Then benzyl alcohol (800 mg, 7.4 mmol) and triethylamine (1.721 g, 17 mmol) were added. The mixture was stirred for 20 min at 0 °C, 2 hr at room temperature, then poured into ether/EtOAc (50 mL/150 mL). The organic layer was washed with 1.2 N HCl (2 x 20 mL), saturated \( \text{NaHCO}_3 \) (1 x 20 mL), dried over \( \text{Na}_2\text{SO}_4 \) and concentrated to give 1.93 g (95%) of 11e as a pale brown solid:
mp 84-87 °C;
\(^1\)H NMR (300 MHz, CDCl₃) \( \delta \) 8.32 (d, J = 9.0 Hz, 2 H), 8.19 (d, J = 9.0 Hz, 2 H), 7.77 (d, J = 8.8 Hz, 2 H), 7.68 (d, J = 8.8 Hz, 2 H), 7.50 - 7.16 (m, 5 H), 5.40 (s, 2 H);
IR (neat): 1718, 1597, 1517, 1343, 1275, 1109, 845 cm⁻¹;
HRMS: calcd 333.1001, obsd 333.1002.

4-Aminobiphenyl-1-benzylcarboxylate (11f)
A suspension of iron (880 mg, 14.4 mmol) and nitro ester 11e (2.403 g, 7.2 mmol) in 90 mL THF was added concentrated HCl (2 mL) over 2 hr period. The resulting mixture was poured into water (30 mL), basified with solid NaOH and extracted with ether (2 x 100 mL). The organic layer was combined and concentrated to give 2.1 g (92%) 11f as a pale yellow solid:
mp 104-107 °C;

$^1$H NMR (300 MHz, CDCl$_3$) δ 8.07 (d, J = 8.7 Hz, 2 H), 7.58 (d, J = 8.7 Hz, 2 H), 7.44 (d, J = 8.6 Hz, 2 H), 7.44 - 7.32 (m, 5 H), 6.75 (d, J = 8.6 Hz, 2 H), 5.36 (s, 2 H);

IR (neat): 3375, 1708, 1599, 1498, 1275, 1189, 1102, 772 cm$^{-1}$;

FAB HRMS: calcd(C$_{20}$H$_{17}$NO$_2$) 303.1259, obsd 303.1255.

Imide acid chloride (12)

The synthesis of compound 12 can be found in our earlier paper$^{10}$.

Biphenyl benzyl ester (13a).

A solution of amine 11f (420 mg, 1.32 mmol), acid chloride 12 (528 mg, 1.55 mmol), triethylamine (0.5 mL) and DMAP (25 mg) was stirred at room temperature for 30 hr. The reaction mixture was poured into CH$_2$Cl$_2$ (150 mL), washed with 1 N HCl (20 mL), saturated NaHCO$_3$ (20 mL), dried over MgSO$_4$, filtered and concentrated to give a solid residue. It was chromatographed (5: 1, then 1:1 Hexane/EtOAc) to give 0.62 g (76%) of 13a as a white solid:
mp 140-142 °C;

$^1$H NMR (300 MHz, CDCl$_3$) δ 8.09 (d, J = 8.5 Hz, 2 H), 7.57 (d, J = 8.5 Hz, 2 H), 7.52 (s, 4 H), 7.46 - 7.32 (m, 5 H), 7.26 (br s, 1 H), 5.36 (s, 2 H), 2.58 (d, J = 13.8 Hz, 2 H), 2.20 (d, J = 13.2 Hz, 1 H), 2.01 - 1.90 (m, 2 H), 1.52 - 1.24 (m, 13 H), 0.92 (t, J = 7.0 Hz, 6 H), 0.84 (t, J = 7.2 Hz, 3H);

IR (neat): 3373, 2959, 1700, 1605, 1526, 1271, 1192, 1111, 772 cm$^{-1}$;
FAB HRMS: calcd (C\textsubscript{38}H\textsubscript{44}N\textsubscript{2}O\textsubscript{5}) 608.3250, obsd 608.3247.

**Biphenyl active ester (1):**

A suspension of 20% palladium hydroxide on carbon (Pearlman's catalyst, 400 mg) and benzyl ester 13a (620 mg, 1 mmol) in methanol (10 mL) was shaken under hydrogen (55 psi) for 16 hr. The suspension was filtered and the filtrate was concentrated to give 0.535 g (100%) of acid 13b as a fine white powder.

A suspension of acid 13b (0.536 g, 1 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (DEC, 370 mg, 1.23 mmol) and pentafluorophenol (230 mg, 1.25 mmol) in THF (10 mL) was stirred for 14 hr at room temperature. The suspension was filtered, and the filtrate was concentrated to give a yellow foam. It was chromatographed (3:1 = Hexane/EtOAc) to give 0.687 g (98%) pentafluoro ester 1 as a white solid: mp 148-151 °C;

\(^1\)H NMR (250 MHz, CDCl\textsubscript{3}) \(\delta\) 8.37 (s, 1 H), 8.24 (d, \(J = 8.4\) Hz, 2 H), 7.71 (d, \(J = 8.4\) Hz, 2 H), 7.59 (s, 4 H), 2.53 (d, \(J = 14.0\) Hz, 2 H), 2.24 (d, \(J = 13.5\) Hz, 1 H), 2.02 - 1.92 (m, 2 H), 1.51 - 1.17 (m, 13 H), 0.98 - 0.82 (m, 9H);

IR (neat): 2961, 1757, 1699, 1604, 1521, 1254, 1192, 1049, 1015, 763 cm\(^{-1}\).

**Biphenyl template (3a).**

A solution of active ester 1 (121 mg, 0.172 mmol), amine 2a (80 mg, 0.266 mmol) and triethylamine (100mg, 1 mmol) in CH\textsubscript{2}Cl\textsubscript{2} was stirred at room temperature for 16 hr. The resulting solution was concentrated and chromatographed (EtOAc, then 10:1:1 EtOAc/MeOH/Hexane) to give 123 mg (87%) of 3a as a white solid:

mp 160-162 °C;

\(^1\)H NMR (250 MHz, CDCl\textsubscript{3}) \(\delta\) 12.95 (br s, 1 H), 8.52 (br s, 1 H), 7.99 (s, 1 H), 7.95 (d, \(J = 8.2\) Hz, 2 H), \(\approx\) 6.5 (s, 1 H), 7.46 (d, \(J = 8.0\) Hz, 2 H), 7.40 (s, 1 H), 7.29 (d, \(J =\)
9.0 Hz, 2 H), 7.13 (d, J = 8.4 Hz, 2 H), 5.67 (d, J = 4.8 Hz, 1 H), 5.12 (br s, 1 H), 4.73 (d, J = 6.3 Hz, 1 H), 4.58 (s, 1 H), 4.56 - 4.43 (m, 1 H), 3.40 (d, J = 13.9 Hz, 1 H), 2.86 (d, J = 13.5 Hz, 1 H), 2.54 (d, J = 14.2 Hz, 2 H), 2.05 - 1.96 (m, 2 H), 1.46 - 1.16 (m, 13 H), 1.02 - 0.83 (m, 9H);
IR (neat) 2960, 1696, 1647, 1596, 1534, 1516, 1496, 1214, 1097, 756 cm⁻¹;
FAB HRMS [M+H]^+ calcd 807.4194, obsd 807.4197.

**Cbz-Template (3b).**

A mixture of template 3a (16 mg, 0.0198 mmol) and carbonylimidazolium chloride 10a (11 mg, 0.043 mmol) in 0.5 ml of CH₂Cl₂ was stirred at room temperature overnight. The solvent was removed, and the residue was chromatographed (1: 99 methanol / CH₂Cl₂, 2:98 methanol / CH₂Cl₂, 3 : 97 methanol / CH₂Cl₂ and 4 : 96 methanol / CH₂Cl₂) to give 15 mg (85%) of 3b as a white solid:
mp 158-160 °C;
¹H NMR (300 MHz, CDCl₃) δ 10.98 (br, 1 H), 9.23 (br s, 1 H), 8.40 (s, 1 H), 7.96 (s, 1 H), 7.89 (d, J = 8.3 Hz, 2 H), 7.52 (d, J = 8.3 Hz, 2 H), 7.52 - 7.50 (m, 2 H), 7.38 - 7.20 (m, 7 H), 5.70 (d, J = 4.8 Hz, 1 H), 5.34 (s, 2 H), 5.04 (m, 1 H), 4.80 (dd, J = 6.3, 2.1 Hz, 1 H), 4.49 (m, 1 H), 4.22 (m 1 H), 3.45 (d, J = 14.4 Hz, 1 H), 2.66 (d, J = 15.3 Hz, 1 H), 2.53 (d, J = 14.1 Hz, 1 H), 2.27 (d, J = 12.6 Hz, 1 H), 1.98 (m, 2 H), 1.60 - 1.20 (m, 13 H), 1.96 - 0.80 (m, 9 H);
IR (neat): ~960, 1702, 1698, 1612, 1532, 1516, 1212, 1069. 755 cm⁻¹;
FAB HRMS[M+H]^+ calcd 941.4562, obsd 941.4563.

**(o-NO₂)-Cbz Template (3c).**

To a solution of adenosine amine 2c (28 mg, 0.058 µmol) in CH₂Cl₂ (1.0 ml) was added pentafluorophenyl ester 1 (31 mg, 0.045 mmol) in one portion followed by triethylamine (63 µl, 0.45 mmol). The resulting mixture was
stirred at room temperature for 20 hr. The solvent was removed, and the residue was chromatographed (CH$_2$Cl$_2$, 1 : 99 MeOH / CH$_2$Cl$_2$, 2 : 98 MeOH / CH$_2$Cl$_2$, 3:97 MeOH / CH$_2$Cl$_2$ and 4:96 MeOH / CH$_2$Cl$_2$) to give 37 mg (83%) of 3c as a white solid:

mp 153-155 °C;

$^1$H NMR (300 MHz, CDCl$_3$) δ 12.38 (br, 1 H), 9.80 (br, 1 H), 8.33 (s, 1 H), 8.13 (dd, J = 8.1, 1.2 Hz, 1 H), 8.02 (s, 1 H), 7.96 (d, J = 7.8 Hz, 1 H), 7.86 (d, J = 8.2 Hz, 2 H), 7.66 (ddd, J = 7.7, 7.7, 1.3 Hz, 1 H), 7.46 (d, J = 8.2 Hz, 2 H), 7.30 (d, J = 3.7 Hz, 2 H), 7.11 (d, J = 8.7 Hz, 2 H), 5.78 (d, J = 14.7 Hz, 1 H), 5.71 (d, J = 14.7 Hz, 1 H), 5.63 (d, J = 4.8 Hz, 1 H), 4.92 (br, 1 H), 4.72 (dd, J = 6.5, 2.3 Hz, 1 H), 4.46 (d, J = 2.1 Hz, 1 H), 4.19 (m, 1 H), 3.42 (d, J = 14.7 Hz, 1 H), 2.77 (d, J = 13.5 Hz, 1 H), 2.56 (d, J = 14.7 Hz, 1 H), 2.28 (d, J = 12.9 Hz, 1 H), 1.96 (m, 2 H), 1.53 - 1.17 (m, 13 H), 0.91 - 0.81 (m, 9 H);

IR (neat): 2960, 1697, 1611, 1528, 1342, 1296, 1207, 1091, 1005 cm$^{-1}$;


**Kinetic Measurements (for 2.1 section)**

The coupling reactions were performed in CHCl$_3$ (dried over 3Å molecular sieves) at 22°C±1°C, in Wheaton reaction vials equipped with Minninert valves. The reaction mixtures were made up using stock solutions of the desired reagents. Aliquots were removed at the noted intervals and analyzed by HPLC.

**HPLC Analysis**

Separation was achieved using a 22:78:0.1 water/methanol/triethylamine solvent system for one product reaction and 35: 55: 10: 0.1= water / acetonitrile/ methanol/ triethylamine for more than one template system on a C18 reverse phase column (Beckman ODS, 4.6 mm x 25 cm) at a flow rate of
2-2.5 mL / min. The eluent was analyzed with a UV detector at 254 nm. The resulting chromatograms were integrated manually using the Waters Baseline 810 software package.

**Calibration of HPLC**

The response factors for the detector were calibrated by injecting known concentrations of the coupling products and measuring the resulting peak area. In all cases a linear relationship was observed. Internal precision within runs was maintained using 4-nitrobibiphenyl as an internal standard.

**Photolysis Experiment**

Irradiations were done in CHCl₃ at 25 °C in an RPR-100 apparatus (Rayonet the Southern Co., Middletown, Conn.) using 350 nm lamps and quartz equipment. (o-NO₂)-Cbz template 3c was irradiated at 10 mM for 1.5 hr to give completely deblocked 3a, identical with previously obtained material.

Amines 2c (17 mM each) were irradiated in the presence of p-toluenesulfonhydrazide (17 mM) as an aldehyde scavenger for 4.7 hr. More than 80% of 2c was converted to 2a.

**Xanthene diacid dimethyl ester 20a.**

Xanthene diacid ¹² (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 dimethyl ester 20a (14.56 g, 97%):

mp 220 °C;

¹H-NMR (250 MHz, CDCl₃) 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).

IR (KBr) 2964, 2905, 2870, 1730, 1707, 1445, 1316, 1276, 1101, 1009, 894 and 784 cm⁻¹;
Xanthene diacid monomethyl ester 20b.
Gaseous HBr was bubbled into CH₂Cl₂ at 0 °C for 25 min and dimethyl ester 20a (12.39 g, 28.25 mmol) was added at 0 °C. The strongly yellow colored solution was stirred for 2 h 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 CH₂Cl₂ (200 mL). The combined organic layers were dried (MgSO₄) and concentrated to give 11.89 g (99 %) of pure mono acid 20b: mp 178 °C;
IR (KBr) 3317 (COOH, sharp), 2963, 2908, 2872, 1717, 1444, 1324, 1270, 1245, 1118, 998 and 788 cm⁻¹;
¹H-NMR (250 MHz, CDCl₃) 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 20c.
Xanthene diacid monomethyl ester 20b (1.00 g, 2.36 mmol), phenol (666 mg, 3.0 eq), and DMAP (10 mg) in 20 mL dry CH₂Cl₂ was treated with DCC (535 mg, 1.1 equiv). After 6 h, the reaction was concentrated and chromatographed to give 1.11 g of impure product, which was contaminated with ~10 % of DCC derived impurities. This impure material was used directly for the next step:
¹H-NMR (250 MHz, CDCl₃); δ 7.82 (a, 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)

Diaminotriazine methyl ester 20d.
The impure xanthene phenyl ester methyl ester 20c (826 mg), biguanide (230 mg) 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 and washed
with 5 mL ethanol to give 547 mg of pure triazine methyl ester 20d. From the filtrate an additional 47 mg was obtained by flash chromatography (5 % MeOH in CH2Cl2). Total yield was 594 mg (69 % from xanthene diacid monomethyl ester 20c):

mp 335-336 °C;
IR (KBr) 3497, 3484, 3282, 3102, 2964, 2870, 1717, 1643, 1620, 1542, 1515,1437, 1394, 1273, 1260, 828 cm⁻¹;
¹H-NMR (250 MHz, CDCl₃) 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).

Diaminotriazine amine 21.

Diaminotriazine methyl ester 20d (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 21 as a white solid (quantitative):

mp 275 °C (dec.);
IR (KBr) 3350, 3195, 2962, 2869, 1653, 1617, 1539, 1437, 1394, 1266, 827 cm⁻¹;
¹H-NMR (250 MHz, CDCl₃) 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), 3.50 (q, J = 6.1 Hz, 2 H), 2.82 (t, J = 6.1 Hz, 2 H), 1.67 (s, 6 H), 1.58 (br, NH₂ + H₂O), 1.34 (s, 18 H);
HRMS calcd for C₂₉H₃₉N₇O₂: 517.3165, obsd 517.3162.

Thymine-1-acetic acid active ester 23.

Thymine-1-acetic acid 22 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 23a. 23% yield:**
mp 177-178 °C;
IR (KBr) 1775, 1696, 1457, 1350, 1234 cm⁻¹;
¹H-NMR (250 MHz, CDCl₃) 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).

**Phenyl ester 23c. 41% yield:**
mp 193-194 °C;
IR (KBr); 1756, 1696, 1652, 1457, 1203 cm⁻¹;
¹H-NMR (250 MHz, CDCl₃) 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);

**Diaminotriazine-thymine template 24**
Diaminotriazine amine 21 (61 mg, 0.118 mmol) and the trichlorophenyl ester 23a (43 mg, 1.0 eq) were allowed to react for 20 min in 6 mL of dry CH₂Cl₂ and Et₃N (100 µL) under argon. Flash chromatography of the concentrated residue with 5-10 % MeOH in CH₂Cl₂ gave 81 mg (100 %) of 24 as a white powder:
mp 200-205 °C;
IR (KBr) 3340, 3217, 2960, 2850, 1684, 1652, 1538, 1535, 1432 cm⁻¹;
¹H-NMR (250 MHz, CDCl₃) 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), 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), 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_{9}O_{5} 683.3544, obsd 683.3543.

**Kinetic Studies (for 2.2 and 2.3 sections)**

The reaction of thymine ester 23c with diaminotriazine amine 21 in the presence or absence of template 24 was performed in CHCl₃ solution containing 18 equivalents of triethylamine. A Waters 600 HPLC (Multisolvent Delivery System) equipped with a UV detector (Waters, Lambda-Max, Model 481 LC Spectrophotometer) set at 254 nm (auf = 1.0) was used for analysis of reaction mixtures. Analyses were performed using a reverse phase column (Beckman C18 column, Ultrasphere ODS dp, 5 μm, 4.6 mm, I.D x 25 cm, flow rate = 1.0 mL/min) and a mixture of water/methanol/TEA (16:84:0.6) as a mobile phase. The integration of peaks and calculation of concentrations were performed using an NEC computer and a Waters 820 Baseline software. Chloroform was dried over molecular sieves. All experiments were performed at ambient temperature (21.5-23.0 °C). Each run was performed 2-3 times to obtain average values for data.

**Typical Reaction Procedures.**

A Wheaton reaction vial (0.3 mL) equipped with a Microflex Miniert valve and Microflex stir vane was charged with 40 mL of diaminotriazine amine 21 stock solution (2.0 x 10⁻² M), 20 μL of CHCl₃, 2 μL of Et₃N, and finally with 40 μL of thymine ester 23c stock solution (2.0 x 10⁻² M). Aliquots (2.0 μL) were withdrawn periodically and analyzed by HPLC. The retention times of product (template) 24, diaminotriazine amine 21, and thymine active ester 23c were 7.4 min, >12 min, and 2.4 min respectively.

**Adenine-Thymine hybrid 30**
Amino adenosine 26 (57 mg, 0.186 mmol) and thymine active ester 23a (68 mg, 1.0 eq) under argon were diluted with dry CH₂Cl₂ (5 mL) and Et₃N (100 μL), and stirred for 20 min at room temperature. The suspension was concentrated and resuspended in CHCl₃, filtered and washed successively with EtOAc (2 mL), CHCl₃ (2 mL), then dried under vacuum to give 69 mg (78%) of 30 as a white powder.

mp 188-193 °C;
IR (KBr) 3486 (br), 2970, 1700 and 903 cm⁻¹;
¹H-NMR (250 MHz, DMSO-d₆) 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), 6.13 (d, J = 2.8 Hz, 1 H), 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, 2H), 1.73 (s, 3 H), 1.53 (s, 3 H), 1.30 (s, 3 H);

Triazine-Kemp's imide hybrid 31

A mixture of xanthene amine 21 (30 mg, 5.8 x 10⁻⁵ mol) and imide pentafluorophenyl ester 27 (ca 40 mg, 1.0 eq) under argon was diluted with were diluted with dry CH₂Cl₂ (5 mL) and Et₃N (50 μL). After stirring for 40 hr at room temperature, a white solid was formed and filtered. It was washed with 5 mL of hexane : CH₂Cl₂ (1:1) and with hexane (5 mL), and dried under vacuum to give 48 mg (81%) of 31 as a white powder.

mp 267-268 °C;
IR (KBr) 3461 (br), 2961, 1700, and 1539 cm⁻¹;
¹H-NMR (250 MHz, CDCl₃); (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), 6.3 (br, 2 H), 5.4 (br, 2 H), 3.2 -3.5 (m, 4 H), 2.67 (d, 2 H), 2.2 (d, 1 H), 0.8-2.0 (m, 48 H).

HPLC conditions for the reaction between triazine 21 and imide ester 27
A 0.5 mL Wheaton screw-cap reaction vial equipped with a Microflex Miniert valve and Microflex stir vane was charged with 40 μL of diaminotriazine amine 21 stock solution (2.0 x 10^{-2} M), 20 μL of CHCl3, 2 μL of Et3N, and finally with 40 μL of imide ester 27 stock solution (2.0 x 10^{-2} M). ([21] = [27] = 8.0 mM, 18 eq of Et3N). Aliquots (2.0 μL) were withdrawn periodically and analyzed by HPLC. Elution solvent was a mixture of MeOH-water (87:13) with 0.35% of Et3N. Flow rate was 1.5 mL/min with auf = 0.8 (254 nm). The retention times of the product (template) 31, diaminotriazine amine 21, and imide ester 27 were 12.6 min, 9.5 min, and > 17 min (broad) respectively.

**HPLC conditions for the reaction between aminoadenosine 26 and thymine 23b**

A 0.5 mL Wheaton screw-cap reaction vial equipped with a Microflex Miniert valve and Microflex stir vane was charged with 25 μL of adenosine amine 26 stock solution (1.2 x 10^{-2} M), 50 μL of CH3CN, 0.5 μL of Et3N, and finally with 25 μL of thymine 23b stock solution (1.2 x 10^{-2} M). ([26] = [23b] = 3.0 mM, 18 eq of Et3N). Aliquots (3.0 μL) were withdrawn periodically and analyzed by HPLC. Elution solvent was a mixture of MeOH-water (50:50) with 0.3% of AcOH. Flow rate was 1.0 mL/min with auf = 0.6 (254 nm) The retention times of the product (template) 30, adenosine amine 21, and thymine active ester 23b were 5.2 min, 9.0 min (broad), and 8.2 min respectively.
References (Chapter two):


7. a) For review, see Pillai, V. N. R. Organic Photochemistry, vol. 9, 225-323, Ed. by Padwa, E.


12. The author thanks Dr. J. I. Hong for helping this kinetic experiment.


Chapter Three. Progress in Triptycene Receptors

3.1 Triptycene cleft

Introduction

Molecules with convergent functional groups have been useful in the study of molecular recognition phenomena. In our laboratory, we have successfully used structures derived from Kemp's triacid.\(^1\),\(^2\) The U-shaped relationship that exists between any two carboxyl group combined with proper spacer groups permits the construction of molecules which can fold back upon themselves. These cleft shaped molecules have shown high affinities in binding a variety of smaller guest molecules.\(^1\)

Another series of related structures is based on xanthene-4,5-dicarboxylic acid derivatives. This building block also features dicarboxylic acids in a U-shaped relationship. Molecular clefts derived from them were able to complex considerably larger structures.\(^3\)

![Chemical structures](image)

Kemp's triacid  
Xanthene diacid  
Triptycene derivative

In order to develop a molecular framework with the three dimensional structure of Kemp's triacid and the size advantage of the xanthene diacid, we explored the possibility of using triptycene as a unit to build large three dimensional clefts.
Synthesis of the Triptycene Tetraacid Cleft

The synthesis of a triptycene skeleton started from 1, 8-dichloroanthrone 1 (Scheme I). Substitution of the two chlorine groups with nitriles using CuCN gave the dicyano substituted anthrone 1a, which upon hydrolysis in concentrated H₂SO₄ gave anthrone diacid 1b. Reduction of 1b with Zn under basic conditions gave anthracene diacid 2.⁴

Esterification with EtBr, followed by a Diels-Alder reaction with dibenzoquinone (Scheme I) gave the adduct 4. After aromatization under acidic conditions dihydroxy triptycene diester 5a was formed. Benzylolation of the phenols went smoothly to give dibenzyl triptycene diester 5b in good yield. Selective deprotection of the front benzyl group (front side is the side with two ester groups) was achieved by using HBr gas.
Scheme I. Synthesis of triptycene tetraacid 7b.
The selectivity of the reaction is due to the fact that the front benzyl group is close to two ester groups. After protonation of the ether, the front two ester groups can form hydrogen bonds with the proton, making this benzyl group more labile (Figure 1).

![Chemical structure](image)

Figure 1.

The biphenyl spacer 8c was synthesized from biphenyl dicarboxylic acid 8a. Reduction of the carboxylic acid 8a by borane in THF gave primary alcohol 8b cleanly. Substitution of the hydroxy group in 8b by PBr3 gave bromide 8c (Scheme 1).

An improved method for making 6 was found with the use of CF₃CO₂H at room temperature. A Williamson ether synthesis was used to join two triptycene skeletons (6) with biphenyl spacer 8c. This gave a tetra ester 7a which upon hydrolysis gave tetraacid 7b. This compound can assume a cleft-like conformation.

Unfortunately, the cleft was highly insoluble in most organic solvents, However, it was soluble in DMSO and some binding studies could be performed in that solvent.

**Titration of tetra acid 7b with diamine**

Complexation of sizable molecules bearing divergent amino groups was investigated with this receptor. As mentioned above, its low solubility in most organic solvents required titrations of the tetraacid in DMSO (Table 1).
The titrations were monitored by proton NMR using DMSO-d$_6$ as solvent. In standard proton NMR titrations, the host molecule protons are followed. In this case it is inconvenient to follow tetraacid proton shift. Instead, reverse titrations were carried out so that the amine proton could be followed.

For example, in titration of meta-xylene diamine A$_1$ with tetraacid 7b, the two benzyl protons in diamine A$_1$ shifted downfield (about 0.3 ppm) upon addition of the tetraacid. The proton shift was saturated upon addition of 0.5 equiv of tetraacid, suggesting that a 2 : 1 complex was formed. The titration data were fitted to 1 : 1 and 2:1 program, respectively. It fitted 1 : 1 binding curve poorly. A 2 : 1 binding program was fitted, and a reasonably good fit was obtained to give the binding constants (K$_1$ and K$_2$). A Hill plot was made giving a Hill coefficient$^5$ of about 1.5.

<table>
<thead>
<tr>
<th>Host</th>
<th>Guest</th>
<th>Ka</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A$_1$)</td>
<td>(7b)</td>
<td>K1=7755, K2=21316</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hill coeff=1.5366</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Host</th>
<th>Guest</th>
<th>Ka</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A$_2$)</td>
<td>(7b)</td>
<td>K1=2940, K2=8014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hill coeff=1.5351</td>
</tr>
</tbody>
</table>

**Table I.** Titration data (in DMSO-d).

In the titrations of tetraacid 7b with para and meta-xylene diamine, both cases gave a Hill coefficient$^5$ of about 1.5. This suggested that positive cooperativity was in operation. The receptor acid can have both C-shaped and S-shaped conformations. When there is no guest molecule, the receptor is probably in the S-shaped conformation to minimize unfavorable dipole-dipole interactions. In addition, other internal rotations can lead to conformations with divergent complexes on either end. Binding of one diamine molecule will fix the conformation to the C-shape, and thus makes
the binding of the second diamine entropically more favorable (Figure 2).
Further experiments are needed in other solvents to confirm this hypothesis.

![Diagram showing cooperative binding](image)

**Figure 2.** Cooperative binding between the tetra acid and a diamine.

**Synthesis of a soluble version of triptycene tetra acid 7a**

A modified synthesis was designed to make a soluble version of the tetraacid cleft (Scheme II). Selective reduction of anthraquinone 1 gave monoketone 9. A Grignard reaction with dodecyl magnesium bromide then gave anthracene 10. A similar procedure to the formation of triptycene diester 5a (in Scheme I) was used to form the corresponding diester 14 from anthracene 10 in six steps (Scheme II). Diester diphenol 14 was then reacted with the di-t-butyl benzyl bromide 16c to further increase the solubility of the system. The tetra acid cleft 18b was made by the usual coupling procedure. It is soluble in most organic solvents including chloroform.

This molecule showed a broad NMR spectrum in chloroform which may be due to the formation of aggregates. This makes proton NMR titration studies difficult in chloroform. The spectrum did sharpen upon addition of Et₃N and some other organic bases.

Benzyl bromide 16c was prepared from 3, 5-ditert-butyl benzoic acid 16a. Reduction of the carboxylic acid 16a by borane in THF gave clean primary alcohol 16b. Substitution of the hydroxy group in 16b by PBr₃ gave monobromide 16c (Scheme II).
Scheme II. Synthesis of soluble version of triptycene tetraacid.
3.2 Anion Receptors

Introduction:

Design and synthesis of receptor molecules for the selective complexation of anions has attracted a lot of attention during the past two decades. Phosphates, carboxylate and other oxy anions are a group of compounds that are very important in biological systems. Therefore, the preparation of artificial receptors for these molecules has been of interest to several groups of chemists and biologists.6
The following bis(acylguanidinium) type anion receptors have been prepared by other chemists. Göbel\textsuperscript{7} reported using compound I to bind apophosphodiester in DMF. Compound II has been synthesized by Hamilton\textsuperscript{8} to bind phosphates. A similar receptor for phosphate was also reported by Anslyn\textsuperscript{9}. They found compound III and IV can bind phosphate in a DMSO-water solvent system. The catalytic effects of these receptors has also been studied.

These systems used positively charged guanidinium ions to bind the anions. In most cases it was observed that one receptor will bind to more than one anion. This often makes analysis of binding affinities difficult to evaluate by titration.

![Chemical structures](image)

In biological systems, ion binding proteins act as ion carriers and channels across the cell membrane. They regulate the selective ion flow from and to the cell. It has been shown that the phosphate and sulfate anions are buried inside a cleft which is formed by two similarly folded globular protein
domains.\textsuperscript{10} Anion binding proteins do not use charged sites or metal centers to achieve complexation, but mainly make use of hydrogen bonding sites.

Recently, there are some published examples using mono urea derivatives to bind a carboxylate.\textsuperscript{11} Reinhoudt\textsuperscript{12} reported synthesizing the following neutral receptors \textbf{Va-c} for small anion binding. Hamilton\textsuperscript{13} also reported using bisurea molecule \textbf{VI} to bind carboxylates. In our group, the bisurea \textbf{VII} was also developed to explore the asymmetric binding of chiral carboxylates.\textsuperscript{14}

\begin{center}
\begin{minipage}{0.3\textwidth}
\centering
\includegraphics[width=\textwidth]{Va.png}
\textbf{Va}
\end{minipage}
\begin{minipage}{0.3\textwidth}
\centering
\includegraphics[width=\textwidth]{Vb.png}
\textbf{Vb}
\end{minipage}
\begin{minipage}{0.3\textwidth}
\centering
\includegraphics[width=\textwidth]{Vc.png}
\textbf{Vc}
\end{minipage}
\end{center}

\begin{center}
\begin{minipage}{0.4\textwidth}
\centering
\includegraphics[width=\textwidth]{VI.png}
\textbf{VI}
\end{minipage}
\begin{minipage}{0.4\textwidth}
\centering
\includegraphics[width=\textwidth]{VII.png}
\textbf{VII}
\end{minipage}
\end{center}

**Synthesis of neutral bisurea anion receptor**

In order to facilitate the recognition of an anion by the formation of multiple hydrogen bonds, a new scaffold for anion binding was developed. Again triptycene was used as a building block for the skeleton of the receptor. The synthesis of receptor \textbf{22} begins with the triptycene diester \textbf{14}. Benzylolation with different benzyl bromides (\textbf{BnBr}) using cesium carbonate in DMF.
yielded dibenzyl diester 19a-d. Shiorio reaction\textsuperscript{15} using
diphenylphosphorylazide (DPPA) and trimethylsilyl alcohol gave the
rearranged product dicarbamate 20a-d. Deprotection by Bu$_4$NF gave diamines
21a-d. Reaction of diamines 21a-d with phenyl isocyanate gave diureas 22a-d.
Several versions of diurea 22 were made (22a-d). For solubility reasons, 3, 5-
di-t-butylphenyl bisurea 22a was used for binding studies.

\begin{align*}
\text{14: } R & = \text{dodecyl} \\
\text{15a-d: } R_1 & = \text{Me} \\
\text{19a-d: } R_1 & = \text{H} \\
\text{20a-d} & \\
\text{21a-d} & \\
\text{22a: } R & = \text{dodecyl, } \text{Bn} = \begin{array}{c}
\text{Ph} \\
\text{t-Bu}
\end{array} \\
\text{22b: } R & = \text{dodecyl, } \text{Bn} = \begin{array}{c}
\text{Ph} \\
\text{CH}_2
\end{array} \\
\text{22c: } R & = \text{dodecyl, } \text{Bn} = \begin{array}{c}
\text{t-Bu} \\
\text{CH}_2
\end{array} \\
\text{22d: } R & = \text{dodecyl, } \text{Bn} = \begin{array}{c}
\text{N} \\
\text{CH}_2
\end{array}
\end{align*}

\textbf{Scheme III. Synthesis of bisurea receptors.}
Binding studies of bisurea with some organic salts

Titrations of triptycene bisurea 22a with different organic salts were carried out using $^1$H NMR in CDCl$_3$. In all cases, additions of the anions resulted in the downfield shift of more than 3 ppm of the urea N-H. The urea protons were very broad at the beginning and sharpened up as saturation was achieved. This suggested that the N-H bond of urea were actively involved in the binding of the anion. The urea protons were hard to follow at the beginning of the titration due to broadness of the NMR peaks. A convenient proton to follow is the CH$_2$ proton of the front benzyl group which gives a broader and shorter signal than that of the back benzyl group. It shifts 0.3-0.5 ppm downfield. A typical titration curve is shown below.

![Figure 3. Titration curve of bisurea 22a with phosphate.](image)
The titration results are shown in Table 1. The bisurea 22a did form complexes with most organic salts like phosphate, sulfate, carboxylate and different halides, etc. In almost all cases the counter ion used was tetrabutylammonium ion, and the corresponding salts are soluble in organic solvents such as chloroform. All the titration data were fitted to a 1 : 1 binding isotherm using Systat 5.2, a nonlinear regression analysis program, to give the binding constants. For binding constants greater than $10^5$, the error of the measurement increases. The stoichiometries for the bindings were supported by a Job's plot\textsuperscript{16} which gives a maximum in the curve at a mole ratio of 0.5 (corresponding to a 1 : 1 complex) for phosphate binding (Figure 4).

From the table it can be seen that bisurea 22a binds phosphate 29 better than p-tolylsulfonate 28. In addition, chloride, bromide, carboxylate and hydrogen sulfate bind strongly. In order to understand the origins of such strong binding, another series of binding studies was carried out with mono urea 23 under the same conditions (Table II).
<table>
<thead>
<tr>
<th>Host</th>
<th>Guest (Bu$_4$N$^+$)</th>
<th>Ka M$^{-1}$</th>
<th>$-\Delta G$ (298K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22a</td>
<td>Cl$^-$</td>
<td>$7.4 \times 10^6$</td>
<td>9.3</td>
</tr>
<tr>
<td></td>
<td>Br$^-$</td>
<td>$2.9 \times 10^5$</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>I$^-$</td>
<td>650</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>F$^-$</td>
<td>$1.0 \times 10^4$</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>H$_3$C-$\text{SO}_3^-$</td>
<td>$3.4 \times 10^3$</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>Ph$\text{-O-P(O)}$</td>
<td>$6.8 \times 10^3$</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>HSO$_4^-$</td>
<td>$2.8 \times 10^5$</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>$-\text{OAc}$</td>
<td>$2.1 \times 10^6$</td>
<td>8.6</td>
</tr>
<tr>
<td>23</td>
<td>Cl$^-$</td>
<td>$4.3 \times 10^3$</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>Br$^-$</td>
<td>1860</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>I$^-$</td>
<td>470</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>F$^-$</td>
<td>$2.8 \times 10^3$</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>H$_3$C-$\text{SO}_3^-$</td>
<td>$3.4 \times 10^3$</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>HSO$_4^-$</td>
<td>$1.5 \times 10^3$</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Table II. Titration result of bisurea 21a and monourea 23 with anions.
There are several reasons that can account for the difference in association constants. For bisurea 22a, if two urea functions have identical affinities, the binding constants for bisurea 22a and mono urea 23 have the following relation: \((K_{\text{bis}}) = (K_{\text{mono}})^2\). This is the case for Cl\(^-\), which suggested that both urea functions are involved in binding. For I\(^-\) and F\(^-\), \(K_{\text{bis}}\) are less than \((K_{\text{mono}})^2\). This can be explained by the fact that I\(^-\) is too bulky; the steric effect makes the binding affinity decrease. On the other hand, F\(^-\) is too small, binding is not so effective. However we cannot rule out the possibility that maybe two F\(^-\) are involved in binding one bisurea molecule.

This is also the case for sulfonate. For p-toluene sulfonate, the binding constant is almost the same for both mono urea and bisurea. This suggests that the two ureas are not involved in binding at the same time. This also maybe due to the bulky size of p-toluenuyl sulfonate. For hydrogen sulfate 29,
the binding constant does increase 184 times for the bisurea compared with
the monoureia. In this case HSO$_4^-$ is smaller and both urea functions can be
involved in binding.

**Synthesis of dipyridyl bisurea**

In the previous section the binding of anions by a rigid bisurea receptor
was discussed. In triptycene bisurea **22a**, only two functional groups were
used for binding. The possibility of using a third functional group in the
triptycene molecule to build a three dimensional binding cavity will be
discussed in this section.

The first possibility was to change the front benzyl group in **22a** to
hydroxy group. The OH group would form an additional hydrogen bond
with the anion, thus increase the binding. The selective deprotection of the
front benzyl was not successful.

The second possibility was to include a basic functional group (like a
pyridyl group) in the molecule. In an apolar solvent such as chloroform,
when an acid and a base are in equilibrium with a contact ion pair, if a base
(in this case pyridyl group) has functional groups (for instance, urea groups)
capable of hydrogen bonding to a counteranion, the equilibrium can shift
towards the salt which is stabilized by additional hydrogen bonding.

Koga$^{17}$ has reported using the following receptor **VIII** to bind
phosphonic acid monoester **X** to form an ion pair in chloroform by three
hydrogen-bonding interactions. They found a large enhancement of salt
formation for **VIII** over **IX** in chloroform. This is due to the fact that the two
hydroxyl groups in **VIII** form a preorganized hydrogen-bond stabilized salt.
Another similar example was also reported by Koga recently.$^{18}$
With some simple modifications of our original system, a new receptor for the monoacid could also be built. In compound 22d (Scheme IV), the two benzyl groups in the previously described systems were replaced by two pyridyl groups. The synthesis of 22d from 14 is also included in Scheme IV.

The salt formation experiments were performed by $^1$H NMR spectroscopy. In a DMSO-d$_6$ solution of compound 22d, addition of one equivalent of diphenyl phosphate resulted in the 0.025 ppm downfield shift of both the front and the back CH$_2$ proton. The identical shift values suggest that proton affinity (basicity) of the two pyridyl groups are nearly the same in DMSO.

In chloroform, however, the selectivity between the front and the back pyridyl group was observed. In a CDCl$_3$ solution of compound 22d, addition
of one equivalent of diphenyl phosphate resulted in 0.25 ppm downfield shift for the front CH2, whereas the back CH2 showed no shift. This observation suggests that the ion-pair structure is stabilized by the front two urea groups which can form hydrogen bonds with diphenyl phosphate (Figure 5).

![Figure 5. Ion pair structure was stabilized by the two urea groups forming hydrogen bonds with the phosphate in 22d. Only the front pyridyl group shown in the figure.](image)

The binding affinities of receptor 22a and 22d were also compared with the same guest molecule. **Table III** list the results.
<table>
<thead>
<tr>
<th>Host</th>
<th>Guest (1 eq)</th>
<th>Shift (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22d</td>
<td>Ph–O–P(OH)</td>
<td>0.25 (CDCl₃)</td>
</tr>
<tr>
<td>22a</td>
<td>Ph–O–P(O)–29</td>
<td>0.023 (CDCl₃)</td>
</tr>
<tr>
<td>22d</td>
<td>Ph–O–P(O)–29</td>
<td>0.0015 (CDCl₃) upfield</td>
</tr>
<tr>
<td>22a</td>
<td>Ph–O–P(O)–29</td>
<td>0.35 (CDCl₃)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CH₂-front: 0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CH₂-back: no shift (CDCl₃)</td>
</tr>
<tr>
<td>22d</td>
<td>Ph–O–P(O)–OH</td>
<td>CH₂-front: 0.025</td>
</tr>
<tr>
<td></td>
<td>Ph–O–P(O)–29</td>
<td>CH₂-back: 0.025 (DMSO-d6)</td>
</tr>
</tbody>
</table>

Table III.

(1) A single equivalent of the guest molecule diphenyl phosphate 32 was added to 1 equivalent of the host 22a and 22d, dipyridyl urea 22d gave a 0.25 ppm downfield shift for the front CH₂ proton, whereas dibenzyl urea 22a gave only 0.023 ppm downfield shift for the front CH₂ proton. This suggested that diphenyl phosphate 32 can form an ion-pair salt with the front pyridyl group. This ion-pair was stabilized by the additional hydrogen-bonding of the
two urea functional groups. Host 22a has no basic sites to form an ion-pair with phosphate, the only binding forces come from the two urea groups.

(2) A single equivalent of the guest molecule diphenyl phosphate anion was added to 1 equivalent of the host 22a and 22d. Dibenzyl receptor 22a gave a 0.35 ppm downfield shift for the front CH2 proton, whereas dipyridyl urea 22d gave almost no shift for the front CH2 proton. This suggests that bisurea receptor 22a can bind the phosphate anion very well. The dipyridyl bisurea 22d has a lone pair on N which makes unfavorable interaction with the anion, and decreases the binding significantly (Figure 6).

![Diagram](image)

**Figure 6.** Schematic illustration: (a) compound 22d can form stabilized ion-pair structure with diphenyl phosphate 32. (b) compound 22d could not bind phosphate 29, due to lone pair-charge interaction.

In summary, a new skeleton (i.e. triptycene) was synthesized for molecular recognition, and the effort to build a three dimensional larger cleft was made. A neutral anion receptor based on triptycene was constructed which showed binding affinities for different kinds of anions. An ion-pair complex was stabilized by additional hydrogen bonds in an aprotic solvent.
3.3 Experimental section (Chapter Three)

Anthracene-1,8-dicarboxylic acid 2:
This compound was synthesized according to a procedure similar to Averill et al from 1 in three steps to give anthracene diacid 2.

Anthracene diester 3a:
A suspension of diacid 2 (10.64 g, 40 mmol), Cs₂CO₃ (16.3 g, 50 mmol), EtI (6.5 mL, 80 mmol) in 120 mL DMSO was stirred at r.t. for 12 h. The resulting mixture was diluted with 400 mL of H₂O, a white precipitate was formed. The precipitate was filtered and dissolved in 300 mL of CH₂Cl₂, washed with H₂O (150 mL x 3), brine (100 mL), dried over MgSO₄, filtered and concentrated to give 11 g (90%) of diester 3a.

¹H NMR (CDCl₃, 250 MHz): δ 10.71 (s, 1H); 8.56 (s, 1H); 8.33 (d, J = 8 Hz, 2 H), 8.24 (d, J = 8 Hz, 2 H), 8.21 (d, J = 8 Hz, 2 H), 7.57 (m, 2H) 4.60 (q, J = 8 Hz, 4 H), 1.58 (t, J = 8 Hz, 6 H);
mp: 58-60 °C.

Anthracene diester 3b:
To a suspension of diacid (10.64 g, 40 mmol) 2 in 200 mL MeOH was added 2 mL concentrated H₂SO₄, and the resulting mixture was refluxed under argon for 48 hr. The suspension changed to a clear solution. Water was added to the solution (ca. 5 mL) and the solution was concentrated under vacuo. The residue was extracted with CHCl₃ (ca. 300 mL), washed with H₂O, brine, dried over MgSO₄, filtered and concentrated to give 11 g (90%) of diester 3b as

¹H NMR (CDCl₃, 250MHz): δ 10.81 (s, 1H); 8.49 (s, 1H); 8.30 (d, J = 8 Hz, 2 H), 8.16 (d, J = 8 Hz, 2 H), 7.4-7.6 (m, 2 H), 4.09 (s, 6 H).
Diels-Alder Adduct 4:

A mixture of diester (5.3 g, 16.4 mmol) 3a and p-benzoquinone (5.30 g, 49.0 mmol) in 60 mL of benzene was refluxed under argon atmosphere for 8 h. The resulting mixture was allowed to cool to 10° C (ice bath) whereupon a precipitate was formed. The solid was filtered and washed with ethanol (2×10 mL) and dried under reduced pressure to give 5.6 g (77%) of the adduct 4 as small yellow needles.

mp (from CH₂Cl₂-MeOH): 195-196°C.

IR (KBr): 1737, 1709, 1668, 1278, 1267 cm⁻¹

¹H NMR (250 MHz, CDCl₃) δ 7.77 (dd, J = 1.2, 7.9 Hz, 1 H), 7.68 (dd, J = 1.4, 7.9 Hz, 1 H), 7.53 (dd, J = 1.7, 7.4 Hz, 1 H), 7.33 (dd, J = 1.4, 7.9 Hz, 1 H), 7.24 (dd, J = 7.4, 7.5 Hz, 1 H), 7.19 (d, J = 2.2 Hz, 1 H) 7.15 (dd, J = 7.4, 7.5 Hz 1 H) 6.37 (d, J = 9.8 Hz, 1 H), 6.29 (d, J = 10.2 Hz, 1 H), 4.93 (d, J = 2.4 Hz, 1 H), 4.46 (q, J = 6.9 Hz, 2 H), 4.44 (q, J = 6.9 Hz, 2 H), 3.18 (dd, J = 2.4, 7.8 Hz, 1 H), 3.16 (dd, J = 2.4, 8.0 Hz, 1 H), 1.49 (t, J = 7.2 Hz, 3 H), 1.47(t, J = 7.2 Hz, 3 H);

HRMS: cald 430.1416, obsd 430.1417.

Dihydroxytriptycene diester 5a:

A yellowish slurry of the adduct 5 (5.6 g) in 50 mL glacial acetic acid was added 30 drops of 20% HBr solution. The resulting slurry was stirred at room temperature for 15 hr. The precipitate was filtered and washed with H₂O. After drying it washed with CH₂Cl₂ to give 5.6 g (100%) of the aromatized product 5a.

¹H NMR (250 MHz, CDCl₃) δ 8.27 (s, 1H), 7.60 (d, J = 7.9 Hz, 2H), 7.56 (d, J = 7.3 Hz, 2 H), 7.07 (t, J=7.6Hz, 2 H), 7.07 (t, J = 7.6 Hz, 2 H), 6.51 (d, J = 8.5 Hz, 1 H), 6.36 (d, J = 8.6 Hz, 1 H), 5.9 (s, 1 H), 5.8(s, 1 H), 4.6 (s, 1 H), 4.46 (q, J = 7.0 Hz, 4 H), 1.47 (t, J = 7.1 Hz, 6 H);
mp: 269-270 °C
IR (KBr): 3595, 1710, 1690, 1265 cm⁻¹;

**Dibenzyldiptycene diester 5b**
A solution of diester 5a (5.0 g, 11.62 mmol) in 50 mL DMF was cooled to 0° C and t-BuOK (2.9 g, 25.2 mmol) was added to the solution. The solution turned deep red. After 15 min, BnBr (4 ml, 33.6 mmol) was added dropwise. The resulting mixture was stirred at room temperature for 6 h. The mixture was poured into ice-water, a tan solid was formed which was filtered and dissolved in CH₂Cl₂, washed with H₂O several times, washed with brine, dried over MgSO₄, and concentrated to give 6.1 g (86%) 5b as a white powder.
mp: 160-162 °C;
IR (KBr): 2980, 1717, 1496, 1384, 1261 cm⁻¹;
¹H NMR (250 MHz, CDCl₃) δ 8.40 (s, 1 H), 7.70-7.20 (m, 12 H), 7.04 (t, J = 7.5 Hz, 2 H), 6.55 (s, 2 H), 6.51 (d, J = 8.5 Hz, 1 H), 5.98 (s, 1 H), 5.9 (s, 1 H), 5.10 (s, 1 H), 5.04 (s, 1 H), 4.38 (q, J = 7.2 Hz, 4 H), 1.34 (t, J = 7.2 Hz, 6 H);
HRMS: cald 610.2355, obsd 610.2359.

**Monodeprotected triptycene diester 6**
A solution of dibenzyl diester 5b (1.0 g, 1.6 mmol) in 100 mL CH₂Cl₂ (distilled from CaH₂) was allowed to cool to 0° C. Hydrogen bromide gas was gently bubbled through the solution for 1.5 h. The solution was poured into H₂O, a tan precipitate formed. The solid was filtered, dissolved in CH₂Cl₂, washed with H₂O (4 x 200 mL), washed with brine, dried over MgSO₄ and concentrated to give a white precipitate which was purified by column chromatography (1% MeOH in CH₂Cl₂ on silica gel) to give 0.55 g (65%) deprotected product 6 as a white powder:
mp: 221-223 °C;
IR: 3452, 1697, 1476, 1240 cm\(^{-1}\);

\(^1\)H NMR (250 MHz, CDCl\(_3\)) \(\delta\) 8.28 (s, 1H), 7.60-7.40 (m, 12 H), 7.04 (t, J = 7.5 Hz, 2 H), 6.56 (q, J = 7.0, 1.9 Hz, 2 H), 5.96 (s, 1 H), 5.85 (s, 1 H), 5.03 (s, 1 H), 4.47 (q, J = 7.2 Hz, 4 H), 1.47 (t, J = 7.2 Hz, 6 H);

**Cleft tetraester 7a:**

A mixture of monodeprotected triptycene diester 7 (0.20 g, 0.384 mmol), spacer 8c (0.070 g, 0.206 mmol) and Cs\(_2\)CO\(_3\) (150 mg, 0.46 mmol) in 2 ml DMF was stirred for 12 h. The reaction mixture was poured into water, a white precipitate formed and was filtered. The solid was dissolved in CH\(_2\)Cl\(_2\) (50 mL) and was washed with water several times, dried over MgSO\(_4\), and concentrated to afford a white precipitate, which was purified by column chromatography (CH\(_2\)Cl\(_2\)) to give 0.21 g (89%) tetraester cleft 7a.

mp: 278-280 °C;
IR: 1244, 1121 cm\(^{-1}\);

\(^1\)H NMR (250 MHz, CDCl\(_3\)) \(\delta\) 8.45 (s, 2 H), 7.67-7.38 (m, 26 H), 7.05 (t, J = 7.5 Hz, 4 H), 6.57 (s, 4 H), 5.99 (s, 2 H), 5.16 (s, 4 H), 5.05 (s, 4 H), 4.44 (m, 8 H), 1.37 (t, J = 7.2 Hz, 12 H);
HRMS: cald 1218.4554, obsd 1218.4559.

**Cleft tetraacid 7b:**

A solution of cleft ester 7a (0.3 g, 0.24 mmol) and 2 M KOH Ethanol solution (3 mL) in 80 mL THF was refluxed for 1 h. A cloudy solution was formed, which was allowed to cool to r.t. and diluted with water. It was then acidified with 1 N HCl to pH = 1 and filtered. The precipitate was washed with H\(_2\)O (50 mL), MeOH (50 mL), CH\(_2\)Cl\(_2\) and dried under vacuo to give 0.25 g (95%) tetraacid 7b.

mp: > 350 °C;
$^1$H NMR (250 MHz, d-DMSO) δ 13.04 (bs, 4 H), 8.33 (s, 2 H), 7.70-7.35 (m, 26 H), 7.10 (t, J = 7.6 Hz, 4H), 6.73 (q, J = 1.5 Hz, 4 H), 6.09 (s, 2 H), 5.14 (s, 8 H);

**Synthesis of soluble version of tetraacid**

**4,5-dichloroanthrone 9:**

A solution of 1,8-Dichloroanthraquinone 1 (100 g, 0.36 mol) and metallic tin powder (100 g) suspended in glacial acetic acid (1100 mL) was heated to reflux while concentrated hydrochloric acid (150 mL) was added slowly. After refluxing for 3.5 hrs, the reaction was decanted and the decantate was cooled to room temp, and kept overnight. Crystals were formed and were filtered. The crystals were dissolved in boiling acetone (600 mL) and filtered while hot. The filtered residue was recrystalized from butanone to give 28 g (29%) product 9.

mp: 195-196 °C;

IR (KBr): 730, 1136, 1312, 1582, 1660, 3081 cm$^{-1}$;

$^1$H NMR (CDCl$_3$, 250MHz): δ 8.31 (dd, 2 H, J = 7.9, 1.2 Hz), 7.73 (dd, 2 H, J = 7.9, 1.2 Hz), 7.48 (t, 2 H, J = 7.9 Hz), 4.28 (s, 2 H);

**5-n-Dodecyl-1,8-dichloroanthracene 10:**

n-Dodecyl Grignard reagent was prepared from 1-bromododecane and magnesium turnings. A three-neck flask (500 mL) equipped with argon inlet and outlet, stir bar and Mg turnings (5.5 g, 228 mmol atom) was flame-dried under argon. Ether (distilled from Na, 20 mL) was added to the flask after it was cooled to room temp. The reaction was initiated by adding 1 mL dodecyl bromide. After initiation, more ether (100 mL) was added. Dodecyl bromide (55 mL, 228 mmol) was added dropwise to the flask over a one hour period. The solution was stirred at r.t. for another 1 hr. The reaction flask was cooled with a H$_2$O bath and anthrone 9 (20 g, 76 mmol) was added to the flask in one
portion. After the reaction subsided, the H₂O bath was removed; and the resulting solution was stirred overnight. The reaction solution was slowly poured into HCl solution (1 N, 300 mL) and extracted with CH₂Cl₂ (3 x 200 mL). The organic layers were combined and washed with brine, dried over MgSO₄, filtered and concentrated. Recrystallization from ethanol afforded 25 g (79%) of 10 as a yellow needle:

mp: 70-71 °C;
IR (KBr): 730, 789, 854, 1074, 1320, 1418, 2850, 2922, 2950 cm⁻¹;

¹H NMR (CDCl₃, 300MHz): δ 9.26 (s, 1 H), 8.20 (dd, 2 H, J = 8.6, 1.2 Hz), 7.63 (dd, 2 H, J = 6.8, 1.2 Hz), 7.44 (dd, 2 H, J = 9.3, 6.9 Hz), 3.58 (t, 2 H, J = 7.8 Hz), 1.8 (m, 2 H), 1.6 (m, 2 H), 1.3 (s, 16 H), 0.88 (t, 3 H, J = 6.4);

5-n-dodecyl-1,8-dicyanoanthrace 11

A suspension of dichloroanthracene 10 (28.4 g, 68.3 mmol), CuCN (15.3 g, 171 mmol, 2.5 eq.), in 500 mL N-methyl pyrrolidinone was refluxed under nitrogen for 40 hr. The resulting black slurry was cooled to r.t. and was poured into HCl solution (1 N, 200 mL) under stirring. A black solid was formed and was filtered. The solid was stirred in 20% ethylenediamine solution (300 mL) for 2 hr. The suspension was filtered and washed with H₂O until the filtrate was colorless. The solid was then recrystallized from EtOH to give 24 g (87%) of pure dicyano compound 11:

mp: 124-125 °C;
IR (KBr): 746, 800, 874, 1457, 2223, 2853, 2922, 2923, 2952 cm⁻¹;

¹H NMR (CDCl₃, 300MHz): δ 9.06 (s, 1 H), 8.54 (d, 2 H, J = 8.9, Hz), 8.04 (d, 2 H, J = 6.7 Hz), 7.62 (t, 2 H, J = 7.3 Hz), 3.63 (t, 2 H, J = 7.8 Hz), 1.8-1.2 (m, 20 H), 0.88 (t, 3 H, J = 6.4 Hz);

5-n-dodecyl-1,8-anthracene diacid 12a:
A suspension of dicyanoanthracene 11 (10 g, 24.8 mmol) in 70 mL ethylene glycol and 70 g (85%) of a KOH solution was refluxed at 160 °C for 48 hr. A yellow precipitate was formed and was filtered and washed with H₂O. The solid was dissolved in CHCl₃ + MeOH solution (100 mL each) and filtered. The filtrate was concentrated to give 10 g (93%) diacid 12a:

mp: >280 °C;
IR (KBr): 718, 1251, 1682, 2300-3300 cm⁻¹;
¹H NMR (CD₃COCD₃, 250 MHz): δ 9.57 (s, 1 H), 8.56 (d, 2 H, J = 9.1, Hz), 8.17 (d, 2 H, J = 6.2 Hz), 7.60 (dd, 2 H, J = 6.9, 8.8 Hz), 3.70 (t, 2 H, J = 6.8 Hz), 1.8 -1.2 (m, 20 H), 0.89 (t, 3 H, J = 6.4 Hz).

5-n-dodecyl-1,8-anthracene diester 12b:
To a suspension of 9 g (20.7 mmol) diacid 12a in 200 mL MeOH was added 2 mL of concentrated H₂SO₄ and the resulting mixture was refluxed under argon for 48 hr. The suspension changed to clear solution. The solution was added H₂O (ca. 5 mL) and concentrated under vacuo. The residue was extracted with CHCl₃ (ca. 300 mL), washed with H₂O, brine, dried over MgSO₄, filtered and concentrated to give 9.3 g (97%) of diester 12b as a brownish solid:

mp: 61-62 °C;
IR (KBr, cm⁻¹): 2919, 2849, 1713, 1260, 1120;
¹H NMR (CDCl₃, 250MHz): δ 10.50 (s, 1 H), 8.48 (d, 2 H, J = 9.0, Hz), 8.21 (d, 2 H, J = 6.8 Hz), 7.54 (dd, 2 H, J = 7.0, 8.9 Hz), 4.09 (s, 6 H), 3.62 (t, 2 H, J = 8.0 Hz), 1.8 -1.2 (m, 20 H), 0.88 (t, 3 H, J = 6.4 Hz);

Diels-Alder Adduct 13:
A mixture of diester (4.0 g) 12b and p-benzoquinone (4.0 g, excess) in 30 mL of benzene was refluxed under argon atmosphere for 5 hr, the reaction mixture was allowed to cool to 10°C (ice bath) whereupon the adduct precipitated.
The solid was filtered and washed with ethanol (2x10 ml) and dried under reduced pressure to give 5.1 g (84%) of the adduct 13:

mp: 141-142 °C;

IR (KBr, cm⁻¹): 3424, 3142, 2919, 2849, 1725, 1707, 1666, 1425, 1278;

¹H NMR (250 MHz, CDCl₃) δ 7.74 (dd, J = 0.8, 7.8 Hz, 1 H), 7.64 (d, J = 7.8 Hz, 1 H), 7.51 (d, J = 7.6 Hz, 1 H), 7.38 (d, J = 7.2 Hz, 1 H), 7.24 (m, 2H), 6.86 (d, J = 1.8 Hz, 1 H), 6.25 (d, J = 10.2 Hz, 1 H), 6.07 (d, J = 10.2 Hz, 1 H), 4.0 (s, 3 H), 3.97 (s, 3 H), 3.18 (q, J = 8.4 Hz, 2 H), 2.7-1.2 (m, 20 H) 0.88 (t, J = 6.4 Hz, 3 H);

HRMS: cald 570.2981, obsd 570.2983.

Dihydroxytriptycene diester 14:

A yellowish slurry of the adduct 13 (4.0 g) from the last step in 15 ml of glacial acetic acid was added 1 ml of 20% HBr aq. solution. The resulting suspension was stirred at room temperature for 15 hrs. The precipitate was filtered, washed with H₂O and redissolved in CHCl₃ (ca. 200 ml). The CHCl₃ layer was washed with H₂O, brine, dried over MgSO₄, filtered and concentrated to give 3.8 g (95%) of 14 as a white solid:

mp: 163-164 °C;

IR (KBr, cm⁻¹): 3436, 2919, 2849, 1702, 1496, 1437, 1326, 1273, 1114;

¹H NMR (250 MHz, CDCl₃) δ 8.18 (s, 1 H), 7.62 (m, 4 H), 7.12 (t, J = 7.8 Hz, 2 H), 6.49 (b, 1 H), 6.28 (b, 1 H), 6.23 (d, J = 8.5 Hz, 1 H), 4.42 (s, 1 H), 5.8 (s, 1 H), 4.00 (s, 6 H), 3.25 (b, 2 H), 2.00 (b, 2 H), 1.8-1.2 (m, 18 H), 0.88 (t, J = 6.4 Hz, 3 H);

HRMS: cald 570.2981, obsd 570.2980.

Dibenzyltriptycene diester 15a:

A solution of diester 14 (0.50 g, 0.88 mmol), 3,5-di-t-Butyl benzyl bromide (0.5 g, 1.8 mmol), Cs₂CO₃ (0.70 g, 2.1 mmol) in 3 mL DMF was stirred at r.t for 3 hrs. The mixture was quenched with ice-water (50 mL), a white solid was
formed. It was filtered, washed with H₂O and dissolved in CHCl₃ (ca. 150 mL). The chloroform layer washed with H₂O (3 x 100 mL), brine, dried over MgSO₄, filtered and concentrated. Column chromatography on silica gel (CHCl₃/Hexane = 7: 3) afforded 0.7 g (82%) 15a as a white powder.

mp: 198-199 °C;
IR (KBr, cm⁻¹): 3436, 3412, 2954, 2919, 2355, 1719, 1596, 1490, 1461, 1396, 1361, 1255, 1114;
¹H NMR (250 MHz, CDCl₃) δ 8.24 (s, 1 H), 7.54 (m, 4 H), 7.42 (s, 4 H), 7.25 (s, 2 H) 7.06 (t, J = 7.8 Hz, 1 H), 6.64 (s, 2 H), 4.98 (s, 2 H), 4.92 (s, 2 H), 3.70 (s, 6H), 3.3 (b, 2 H), 1.8 (b, 2 H), 1.6-1.1 (m, 54 H), 0.88 (t, J = 6.4 Hz, 3 H);
HRMS: cald 974.6424, obsd 974.6417.

Monodeprotected triptycene diester 17:
A solution of dibenzyl diester 15a obtained from last step (0.3 g, 0.31 mmol) in CH₂Cl₂ (ca. 20 mL) was added 0.5 mL CF₃CO₂H, the resulting solution was stirred at r.t. for 10 hrs. The solution was added more CH₂Cl₂ and was washed with H₂O (2 x 100 mL), brine, dried over MgSO₄, filtered and concentrated. Column chromatography on silica gel (CHCl₃) afforded 0.21 g (88%) 17 as a white powder:

mp: 82-83 °C;
IR (KBr, cm⁻¹): 3460, 3142, 2943, 2860, 1719, 1702, 1596, 1467, 1431, 1396, 1284, 1249, 1214, 1108, 1079;
¹H NMR (250 MHz, CDCl₃) δ 8.20 (s, 1 H), 7.56 (t, J = 7.9 Hz, 2 H), 7.40 (t, J = 0.6 Hz, 1 H), 7.11 (m, 6 H), 6.19 (s, 1 H), 6.36 (s, 1 H), 4.01 (s, 6H), 3.2 (b, 2 H), 1.8 (b, 2 H), 1.5-1.1 (m, 36 H), 0.88 (t, J = 6.4 Hz, 3 H);
HRMS: cald 772.4703, obsd: 772.4709.

Tetra ester cleft 18a
A suspension of monoprotected diester 17 (95 mg, 0.123 mmol), spacer (21 mg, 0.062 mmol) and Cs$_2$CO$_3$ (50 mg, 0.15 mmol) in 1 mL DMF was stirred at room temp. for 10 hrs. The mixture was quenched with H$_2$O (ca. 20 mL). A white precipitate was formed. It was taken in CHCl$_3$. The CHCl$_3$ solution was washed with H$_2$O (3 x 50 mL), brine, dried over MgSO$_4$, filtered and concentrated. Column chromatography on silica gel (CHCl$_3$/Hexane = 7: 3) afforded 54 mg (57%) 18a as a white powder:

$^1$H NMR (300 MHz, CDCl$_3$) δ 8.23 (s, 2 H), 7.67-7.38 (m, 30 H), 6.45 (s, 2 H), 4.95 (s, 4 H), 4.77 (s, 4 H), 3.90 (s, 12 H), 1.1-1.6 (m, 80 H), 1.37 (t, J = 7.2 Hz, 6 H);

Tetraacid 18b:

A solution of tetra ester cleft 18a (50 mg) in 10 mL THF was added 1 mL KOH/EtOH solution (2M). The solution was refluxed under argon for 1 hr and was cooled to r. t, concentrated under reduced pressure. The residue was added HCl solution (1N, 10 mL). A white solid was formed which was filtered and washed with H$_2$O. The solid was dissolved in CHCl$_3$ (ca. 100 mL) and the chloroform layer was washed with brine, dried over MgSO$_4$, filtered and concentrated to give 45 mg (94%) tetra acid 18b.

mp: > 300 °C

IR: (KBr, cm$^{-1}$): 3600-3200, 2960, 2924, 2854, 1689, 1600, 1465, 1421, 1394, 1296, 1248;

$^1$H NMR (250 MHz, DMSO-d$_6$) δ 13.02 (s, 4 H), 8.28 (s, 2 H), 8.0 -6.9 (m, 30 H), 6.74 (s, 2 H), 5.88 (s, 4 H), 4.88 (s, 4 H), 1.1-1.6 (m, 80 H), 0.85 (t, J = 7.2 Hz, 6 H);

The NMR is very broad in CDCl$_3$.

4,4'-dihydroxymethyl biphenyl 8b

A flame-dried three-neck flask was charged with 10 mL THF (distilled from Na), 1 g diacid 8a. BH$_3$ solution (5 mL, 1 M) was added slowly to the flask. The
resulting suspension was refluxed for 4 h. The resulting clear solution was allowed to cool down to room temperature. The solution was neutralized with 5 drops of 1 N HCl solution, and THF removed under reduced pressure. The resulting residue was washed with water and dried in vacuo to give 0.88 g (100%) dialcohol 8b.

$\text{H NMR (250 MHz, CDCl}_3 \delta 7.60 (d, J = 8.0 \text{ Hz, 4 H}), 7.45 (d, J = 7.8 \text{ Hz, 4 H}), 4.75 (s, 4 H).}$

4,4'-dibromomethyl biphenyl 8c

A suspension of diol 8b (0.5 g, 2.34 mmol) in 15 mL CCl$_4$ and 3 mL CH$_2$Cl$_2$ was added PBr$_3$ (0.7 mL, 7.0 mmol). The resulting solution was stirred at rt overnight. The suspension was filtered and the filtrate was washed with H$_2$O, saturated NaHCO$_3$, and with brine, dried over MgSO$_4$, concentrated to give 0.45 g (57%) pure 8c.

$\text{H NMR (250 MHz, CDCl}_3 \delta 7.56 (d, J = 8.3 \text{ Hz, 4 H}), 7.47 (d, J = 8.2 \text{ Hz, 4 H}), 4.55 (s, 4 H).}$

Synthesis of 3, 5-di-t-butyl Benzyl Bromide 16c:

3, 5-di-t-butyl benzyl alcohol 16b:

A flame-dried flask charged with acid 16a (2.0 g, 8.5 mmol), THF (10 mL, distilled from Na) was slowly added Borane THF solution (6 mL, 1 N THF solution). Bubbles were formed. After addition, the solution was stirred at r.t. for another 3 hrs. The reaction was then quenched with several drops of 1 N HCl solution (slowly until no bubbles was formed). The solution was concentrated under reduced pressure. The residue was taken in CHCl$_3$ and washed with 1 N HCl, brine, dried over MgSO$_4$, filtered and concentrated to give 1.8 g (96%) of alcohol 16b as a liquid.
\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.38 (t, \(J = 1.4\) Hz, 1 H), 7.22 (t, \(J = 1.4\) Hz, 2 H), 4.68 (s, 2 H), 1.65 (s, 1 H), 1.33 (s, 18 H).

3, 5-di-t-butyl Benzyl Bromide 16c:
A solution of alcohol 16b (1.8 g, 8.2 mmol) obtained from last step in 30 mL CCl\(_4\) was added PBr\(_3\) (0.8 mL, 8.4 mmol). The resulting solution was stirred at r.t. for 30 min. when check TLC the reaction was done. The solution was washed with H\(_2\)O (50 mL), NaHCO\(_3\) (20 mL, 1 N), brine, dried over MgSO\(_4\), filtered and concentrated to give 1.8 g (78%) of 16c:
\[^1\)H NMR (250 MHz, CDCl\(_3\)) \(\delta\) 7.36 (t, \(J = 1.8\) Hz, 1 H), 7.23 (d, \(J = 1.8\) Hz, 2 H), 4.52 (s, 2 H), 1.33 (s, 18 H).

Triptycene diacid 19a:
Hydrolysis of diester 15a (0.2 g, 0.2 mmol) was performed as described for the preparation of tetra acid 7b to give 0.19 g (100%) of 19a as a solid. It was used for the next step without further purification.

Triptycene dicarbamate 20a:
A flame-dried flask (25 mL) was charged with a stir bar, reflux condenser, argon inlet and outlet, diacid 19a (0.176 g, 0.186 mmol), toluene (20 mL, distilled from Na), triethylamine (60 \(\mu\)L, 0.430 mmol, 2.3 eq), Diphenylphosphoryl azide (DPPA, 90 \(\mu\)L, 0.419 mmol, 2.3 eq) and 2-(trimethylsilyl) ethanol (120 \(\mu\)L, 0.837 mmol, 4.5 eq). The resulting solution was stirred at 85 °C for 3 hrs. It was cooled to room temperature and concentrated. The residue was taken in CHCl\(_3\) (ca. 50 mL) and washed with 1 N HCl solution (ca. 30 mL), NaHCO\(_3\) solution (1 N, 30 mL), brine, dried over MgSO\(_4\), filtered and concentrated to give a yellowish residue. Column
chromatography (CHCl₃ / Hexane = 6: 4) afforded 0.14 g (63%) of 20a as a yellow solid:
mp: 159-160 ºC;
IR (KBr, cm⁻¹): 3330, 2954, 2919, 2860, 2155, 1731, 1707, 1602, 1519, 1484, 1425,
1360, 1249, 1220, 1061, 861, 838;
¹H NMR (250 MHz, CDCl₃) δ 7.5 (bs, 2 H), 7.4-6.9 (m, 12 H), 6.38 (q, J = 9.0 Hz, 2 H), 6.28 (s, 1 H), 4.91 (s, 2 H), 4.81 (s, 2 H), 4.24 (m, 4 H), 3.17 (b, 2 H), 1.8-0.7 (m,
63 H), 0.0 (s, 18 H);
HRMS: cald 1176.7746, obsd: 1176.7757.

Triptycene diamine 21a:
A solution of dicarbamate 20a (0.12 g, 0.10 mmol) obtained from last step in 20 mL THF and Bu₄NF in THF solution (1 N, 2 mL) was refluxed under argon for 3 hrs. The solution was cooled to room temperature and concentrated under vacuo. The residue was taken in CHCl₃ (ca. 50 mL), and was washed with H₂O (2 x 50 mL), brine, dried over MgSO₄, filtered and concentrated to give a yellowish residue. Column chromatography (1% MeOH in CHCl₃) afforded 0.09 g (quantitative) of 21a as a shining flaky crystal.
mp: 93-94 ºC;
IR (KBr, cm⁻¹): 3436, 3366, 3213, 3072, 2955, 2919, 2860, 1602, 1484, 1361, 1296,
1249, 1202, 1155, 1061, 873;
¹H NMR (250 MHz, CDCl₃) δ 7.4 (m, 2 H), 7.35 (d, J= 1.7 Hz, 2 H), 7.26 (d, J= 1.7 Hz, 2 H), 6.95 (d, J = 7.6 Hz, 2 H), 6.80 (t, J = 7.9 Hz, 2 H), 6.60 (t, J = 8.9 Hz, 2 H),
6.38 (d, J = 7.8 Hz, 2 H), 6.17 (s, 1 H), 5.00 (s, 2 H), 4.89 (s, 2 H), 3.9-2.8 (b, 6 H),
1.91 (bs, 2 H), 1.6-1.1 (m, 54 H), 0.88 (t, J = 6.3 Hz, 3 H);
HRMS: cald 888.6532, obsd: 888.6528.

Triptycene bisurea 22a:
A solution of diamine 21a (95 mg, 0.11 mmol) obtained from last step was added phenyl isocyanate (25 µl, 0.23 mmol). The resulting solution was stirred at room temperature overnight. More CHCl₃ was added and the reaction mixture was washed with 1 N HCl solution (ca. 20 mL), H₂O, brine, dried over MgSO₄, filtered and concentrated to give 70 mg (58%) of 22a:

mp: 175-176 °C;

IR (KBr, cm⁻¹): 3236, 2955, 2908, 1655, 1596, 1549, 1478, 1243;

¹H NMR (250 MHz, CDCl₃) δ 7.67 (bs, 2 H), 7.5-6.9 (m, 22 H), 6.58 (m, 3 H), 4.93 (s, 2 H), 4.76 (s, 2 H), 3.24 (bs, 2 H), 1.83 (bs, 2 H), 1.6-1.1 (m, 54 H), 0.88 (t, J = 6.3 Hz, 3 H);


4-Pyridyl methyl bromide hydrobromide:
This was prepared according to Niemann¹⁸ from 4-pyridylcarbinol (4.5 g, 41.2 mmol) to give 6.3 g 4-Pyridyl methyl bromide hydrobromide (60%).

Dipyridyltriptycene diester 15d:
A solution of diester 14 (0.80g, 1.40 mmol), 4-pyridyl methyl bromide hydrobromide (0.71 g, 2.80 mmol), Cs₂CO₃ (2 g, 6.2 mmol) in 3 mL DMF was stirred at r.t for 5 hrs. The mixture was quenched with ice-water (50 mL), a redish solid was formed. It was filtered, washed with H₂O and dissolved in CHCl₃ (ca. 150 mL). The chloroform layer washed with H₂O (3 x 100 mL), brine, dried over MgSO₄, filtered and concentrated. Column chromatography on silica gel (2.5% MeOH in CH₂Cl₂) afforded 0.6 g (57%) 15d as a redish solid: mp: 177-178 °C;

IR (KBr, cm⁻¹): 3422, 2922, 2853, 1720, 1490, 1461, 1431, 1289, 1260, 1111, 1067, 749, 711;
$^1$H NMR (250 MHz, CDCl$_3$) $\delta$: 9.0-8.5 (m, 4 H), 8.30 (s, 1 H), 8.08 (d, J = 7.8 Hz, 1 H), 7.2 -7.8 (m, 7 H), 7.09 (t, J = 7.7 Hz, 2 H), 6.59 (s, 2 H), 5.05 (s, 2 H), 4.96 (s, 2 H), 3.88 (s, 6 H), 3.3 (bs, 2 H), 2.2-1.1 (m, 20 H), 0.88 (t, J = 6.3 Hz, 3 H);

**Dipyridyltriptycene diacid 19d:**

It was synthesized in the same way as 19a to give quantitative diacid 19d. This compound was used without further purification.

$^1$H NMR (250 MHz, DMSO-d) $\delta$: 8.97 (s, 1 H), 8.87 (s, 1 H), 8.89 (d, J = 4.3 Hz, 1 H), 8.73 (d, J = 3.8 Hz, 1 H), 8.30 (s, 1 H), 8.25 (m, 2 H), 8.0 -7.3 (m, 6 H), 7.15 (t, J = 6.3 Hz, 2 H), 6.83 (s, 2 H), 5.22 (s, 2 H), 5.10 (s, 2 H), 3.8 (bs 2 H), 3.2 (bs, 2 H), 1.9-1.1 (m, 20 H), 0.85 (t, J = 6.3 Hz, 3 H).

**Dipyridyltriptycene dicarbamate 20d:**

The same procedure was used as synthesis of 20a starting from diacid 19d (0.118 g, 0.16 mmol). Column chromatography in 5% MeOH in CH$_2$Cl$_2$ gave 0.102 g (66%) 20d as an off-white solid.

mp: 175-176 °C;

IR (KBr, cm$^{-1}$): 3286, 2954, 2924, 2852, 1709, 1599, 1526, 1490, 1425, 1250, 1067, 1027, 938, 860, 837;

$^1$H NMR (250 MHz, CDCl$_3$) $\delta$: 8.69 (m 2 H), 8.63 (m, 1 H), 8.57(m, 1 H), 7.81 (m, 4 H), 7.4 (m, 4 H), 7.05 (m, 4 H), 6.48 (s, 2 H), 6.36 (s 1 H),4.93 (s, 2 H), 4.92 (s, 2 H), 4.3 (m, 4 H), 3.2 (bs, 2 H), 2.0 - 1.0 (m, 24 H), 0.91 (t, J = 6.3 Hz, 3 H), 0.07 (s, 18 H).

**Dipyridyltriptycene diamine 21d:**

The same procedure was used as synthesis of 21a starting from dicarbamate 20d (0.5 g, 0.52 mmol) to give 0.33 g (95%) diamine 21d as yellow solid:

mp: 77-78 °C;
IR (KBr, cm\(^{-1}\)): 3500, 3350, 3200, 2922, 2851, 1596, 1488, 1428, 1400, 1300, 1260;
\(^1\)H NMR (250 MHz, CDCl\(_3\)) 8: 8.79 (s, 1 H), 8.68 (d, J = 1.5 Hz, 1 H), 8.62 (m, 2 H),
7.82 (m, 2 H), 7.35 (m, 2 H), 6.96 (d, J = 7.5 Hz, 2 H), 6.83 (t, J = 6.5 Hz, 2 H), 6.5
(m, 4 H), 6.15 (m, 1 H), 5.06 (s, 2 H), 4.93 (s, 2 H), 3.2 (b, 2 H), 2.5 (b, 4 H), 2.0 - 1.0
(m, 20 H), 0.91 (t, J = 6.3 Hz, 3 H).

**Dipyridyltriptycene bisurea 22d:**

The same procedure was used as in the synthesis of 22a starting from diamine
21a (0.24 g, 0.36 mmol). Column chromatography in 3% MeOH in CH\(_2\)Cl\(_2\)
gave 0.181 g (57%) 22d as an off-white solid.

mp: > 260 °C;
IR (KBr, cm\(^{-1}\)): 3386, 3240, 3054, 2922, 2851, 1657, 1600, 1552, 1488, 1440, 1420,
1294, 1224, 1054;
\(^1\)H NMR (250 MHz, CDCl\(_3\)) 8: 8.69 (s, 1 H), 8.64 (m, 1 H), 8.26 (m, 2 H), 7.85 (d,
2 H), 7.6 - 6.6 (m, 22 H), 6.36 (m, 3 H), 4.86 (s, 2 H), 4.47 (s, 2 H), 3.2 (bs, 2 H), 2.0 -
1.0 (m, 20 H), 0.90 (t, J = 6.3 Hz, 3 H);

HRMS [M + H]: cald 905.4754, obsd 905.4761.

**Formation of salts for NMR titration with triptycene bisurea:**

**Tetrabutylammonium phosphate 29:**

A solution of diphenyl phosphate 32 (200 mg, 0.8 mmol) in 5 mL MeOH was
added 0.8 mL 1 N Bu\(_4\)NOH in MeOH solution. The solution was stirred for
another half hour and concentrated to dryness.

2. Bu\(_4\)NF, Bu\(_4\)NSO\(_4\), etc. were made in a similar way as above.

**Titration studies:**

(a) Stoichiometry.
The complex of bisurea 22a with phosphate 29 was determined by using Job's method.\textsuperscript{16} Solutions of 22a and 29 (5.0 x 10^{-3} M each) were prepared in separate vials. In eleven separate NMR tubes mixtures of each solution were added such that the stoichiometry of each component varied but the total volume was 600 \( \mu \)L. For example, the first tube contained only the receptor 22a; the second tube contained 50 \( \mu \)L of the solution of 29 and 550 \( \mu \)L of the solution 22a. Likewise, the last tube contained only the salt 29, and the tube before that contained 50 \( \mu \)L of the solution of 22a and 550 \( \mu \)L of the solution 29. The \(^1\text{H}\) NMR spectra were obtained for each tube, and the chemical shift of the benzyl CH\(_2\) (which on the same side as urea groups) was used to calculate the complex concentration. This value was plotted against the mole fraction of the bisurea 22a. The resulting Job plot showed a maximum at 0.5 mole fraction (Figure 4).

(b) Titrations:

The host (22a) concentration remained constant during the titration. For a specific example, the titration of bisurea 22a with phosphate 29 will be described here. A 1.3 x 10^{-3} M solution of 22a (4.55 mg in 3.109 mL of CDCl\(_3\)) was prepared in a vial. A 9.1 x 10^{-3} M solution of 29 (10.35 mg in 2.313 mL of the above 22a CDCl\(_3\) solution) was also prepared in another vial. An initial NMR spectrum of 22a was obtained, and the initial chemical shift of the benzyl CH\(_2\) (which on the same side as urea groups) was recorded. The guest 29 was initially added in 10 \( \mu \)L portion, and the chemical shift of the benzyl CH\(_2\) was recorded again. After 500 \( \mu \)L of guest 29 was added, the chemical shift of the benzyl CH\(_2\) was almost the same as the previous spectra, indicating the maximum chemical shift has been reached.
References (Chapter Three):


