SELF-ASSEMBLY OF PURINES BY QUADRUPLE HYDROGEN BONDING: DESIGN, SYNTHESIS, AND STRUCTURE-PROPERTY RELATIONSHIPS

BY

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To my brother Tony Martin Jr., riding waves of peace (10-21-06); and to my nephew Curren
Nesta Martin
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<td>DAP</td>
<td>Diaminopurine</td>
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<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
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<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
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<tr>
<td>DP</td>
<td>Degree of polymerization</td>
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<tr>
<td>ESI-FT-ICR</td>
<td>Electrospray ionization-Fourier transform-ion cyclotron resonance</td>
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<tr>
<td>HRMS</td>
<td>High resolution mass spectrometry</td>
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<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
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<tr>
<td>NOESY</td>
<td>Nuclear Overhauser Spectroscopy</td>
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<td>PNA</td>
<td>Peptide nucleic acid</td>
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<tr>
<td>QHB</td>
<td>Quadruple hydrogen bond</td>
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<td>SMP</td>
<td>Supramolecular polymer</td>
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<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
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<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
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<tr>
<td>UDAP</td>
<td>Ureidodiaminopurine</td>
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<tr>
<td>VT</td>
<td>Variable temperature</td>
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The development of synthetically accessible, quadruple hydrogen bonded (QHB) systems that are built on nucleobase platforms has revolutionized the field of supramolecular chemistry in the last decade. Herein are introduced the first QHB systems built on a ureidodiaminopurine (UDAP) platform. This unit offers conformational stability with modest dimerization constants, $K_{\text{dim}} \sim 530–1100 \text{ M}^{-1}$, in chloroform. The structure-property relationships of five UDAPs were examined through synthetic modifications to the urea and N9 substituents. The urea substituents were varied from phenyl to hexyl to probe electronic effects. Likewise, the N9 substituent was varied from aryl to alkyl to investigate steric consequences. The synthesis of aryl UDAP is regioselective for N$_C$$_2$ substitution, interestingly, without isolation of an N$_C$$_6$ substituted product. However, substitution occurs exclusively at N$_C$$_6$ with hexyl isocyanate. From this reactivity difference, a study into the nucleophilicity of the amino groups of DAP with common acylating agents was undertaken. Characterization by $^1H$, $^{13}C$, $^1H$–$^{13}C$ gHMBC, $^1H$–$^{15}N$ gHMBC unambiguously assigned the site of acylation as N$_C$$_2$ or N$_C$$_6$. The nucleophilic reactivity of DAP was explored, thermodynamic equilibrium was eliminated, and the reactivity determined to favor N$_C$$_2$ kinetically with acetic anhydride and methoxy acetyl chloride.
CHAPTER 1
INTRODUCTION

Inspiration

Synthetic chemists often draw inspiration from the enormous complexity and specificity that has evolved in nature.\textsuperscript{1,2} From only a few atoms, nature forms a variety of functional groups, which together make up a small number of biological molecules (compared to the synthetic opportunities). These biological molecules (monomers) are combined to form macromolecules with exact molecular weight, with specific branching points, and with predisposed but dynamic conformations. Natural macromolecules are formed using a variety of functional groups that control polarity, electrostatic interactions, and hydrophobicity. Further, biology uses covalent and noncovalent interactions for assembly with precise control. These biological ensembles then elicit some unique function.

Common biomacromolecules or biopolymers that display function, which arises from complex structure, conformation, and assembly include DNA/RNA and polypeptides.\textsuperscript{3-6} The nucleobases of a single DNA strand are held together through covalent phosphate linkages, likewise polypeptides are held together by covalent amidic bonds. The primary sequence of the biopolymer represents the chemical information from which secondary structure emerges, that in turn leads to defined macromolecular architecture, functional group placement, and function.\textsuperscript{1} Under physiological conditions the amino acid side chains can link covalently (by disulfide bonds), or noncovalently, primarily by hydrogen bonds, to form local regions of ordered structure. In DNA the nucleobase sequence stores information, and specificity. Formation of tertiary structure is achieved through complementary pairing by hydrogen bonding between purine and pyrimidine bases. In a double helix the two strands are additionally held together by hydrophobic and electrostatic interactions. The noncovalent forces by which recognition or
association occurs in natural systems consists primarily of \( \pi-\pi \) stacking (aromatic interactions), van der Waals interactions, ionic interactions, and hydrogen bonding, which work in concert to stabilize the overall assembly.

**Supramolecular Chemistry**

It is through mimicking the interactions of biological systems that organic chemists can create synthetic materials with enhanced functions through the combination of covalent and noncovalent chemistry. There are many concepts in supramolecular chemistry which have been founded on the basis of modeling the interactions of nature, including a) molecular recognition, b) molecular self-assembly, and c) dynamic covalent chemistry.

Molecular recognition, involves two or more entities recognizing one another, and is, by definition an intermolecular process. Molecular recognition is ubiquitous in biological systems and is at the basis of the various physiological responses elicited by highly specific host-guest interactions at the cellular level (enzyme-substrate complexes, antibody/antigen, and membrane receptors). Study of molecular recognition extends to increasing the diversity of synthetic systems that are intended to mimic the host-guest interactions of biological systems. Molecular receptors are developed to be capable of selectively binding specific substrates by noncovalent interactions which rely on information storage, and processing information stored in the interacting species.

Examples of synthetic molecular recognition units include crown ethers, calixarenes, and molecular sensors. Association of metals with crown ethers is based on size, shape, and charge. Cavity size is proportional to ring size, thus selectivity of complexation is based on the size of the metal ion. For example, potassium ion, 2.66 Å, is nicely accommodated by 18-crown-6 with a cavity size of 2.6–3.2 Å, whereas, sodium, 1.94 Å, is accommodated by 15-crown-5 with a cavity size of 1.7–2.2 Å (figure 1-1). Sodium/potassium balance is critical to
cellular function, and disruption equates to cell death. This is important in terms of antimicrobial compounds. Hydrophilic ions cannot pass through hydrophobic cell membranes. Transport of ions across cell membranes, by crown ethers, results in bacterial death.

![Figure 1-1. Association of potassium and sodium by crown ethers](image)

Calixarenes are basket or cup-like structures that serve as hosts to guest from metals to neutral organic molecules by size complementarity and intermolecular interactions.\textsuperscript{12,13} As illustrated in figure 1-2, structures can vary from simple to complex by modification of the top or bottom rims, and are proposed as enzyme mimics and molecular capsules. These versatile binding pockets have helped to unravel many of the mysteries of molecular recognition and are useful for rational drug design.

Molecular sensors speak more to the analytical side of chemistry rather than the biological.\textsuperscript{14} Sensors are specifically designed molecules which change properties in response to the presence of a molecular complement. The specific reversible interaction of a guest with a host produces a change which can be readily monitored such as color, photoluminescence, or redox potential. The chosen method is photoluminescence; for example, crown ethers are tethered to luminescent moieties, and the specificity of crown ethers for metals is used to elicit a response upon binding (figure 1-3, left).\textsuperscript{15-17} Anions are typically bound using sets of hydrogen bond donors and acceptors as illustrated by the central structure of figure 1-3,\textsuperscript{18-20} where small molecules such as CO or O\textsubscript{2}, and organic compounds with donor atoms, can be coordinated to metal atoms.\textsuperscript{21}
Figure 1-2. Calixarene structures can vary from simple to complex and are proposed as mimics to enzyme binding pockets.

R = H, ether, alkyl, amides, acids, esters
R₁ = t-Bu, CH₃, H

Figure 1-3. Molecular sensors couple binding to an observable response (photoluminescence in these cases).

Molecular self-assembly falls under the umbrella of molecular recognition and is defined by components designed (programmed) or predisposed to reversibly organize themselves into desired patterns and functions (exemplified by protein folding or DNA/RNA duplex formation). Self-assembly can be inter- or intramolecular. The scope of self-assembly is broad and applies to biological chemistry, polymer chemistry, and materials science and engineering. Molecular self-assembly is within the general scope of this thesis and will be exemplified herein.

Dynamic covalent chemistry describes systems in which covalent bonds are formed reversibly, under thermodynamic control. The product composition is thus governed by the relative stability of the species. Figure 1-4 shows examples of such dynamic systems including mechanically interlocked molecular assemblies such as rotaxanes (dumbbell-shaped...
component with a trapped ring, left), and catenanes (two interlocked rings, right). These systems have been developed for molecular machines and electronics, and display readily controlled internal movements of one component with respect to the other.

Figure 1-4. Rotaxane (left) and catenane (right) are mechanically interlocked rings that can act as molecular machines or electronics.²²

Molecular electronics and molecular motors derived from catenanes and rotaxanes are formed by template directed synthesis, and since the noncovalent template component remains within the system they can be activated so that they respond to a stimulus. The recognition element can be chemical, electrical, or optically controlled to switch off and on. Molecular motors are conceptually similar; however, the response to stimuli results in the physical, reversible movement of the system components.

**Hydrogen Bonding in Supramolecular Chemistry**

Of the dynamic interactions that define supramolecular chemistry, hydrogen bonding interactions have a prominent role due to their intermediate strength, between van der Waals interactions (> 5 kJ mol⁻¹) and covalent bonds (< 250–800 kJ mol⁻¹).³⁰ Hydrogen bonding also offers directionality and specificity. Directionality is a consequence of an energetic preference for a hydrogen atom of a donor to arrange linearly, or close to linearly, with the lone pair of an acceptor atom (however, hydrogen bonding is dominated by electrostatics which allows for some
flexibility of the bond angle), and specificity is the result of the presence of a hydrogen bond donor and hydrogen bond acceptor (atoms which are more electronegative that H, generally N, O, or F, but in some cases C, P, S, Cl, Se, Br, and I). Hydrogen bond preferences can generally be discerned intuitively based on proton acidities and the basicity of the corresponding acceptor. Likewise, the strengths of individual hydrogen bonds can generally be related to donor strength (i.e., can be defined by the distance of X–H; X = donor) that follows a general ranking of O–H > N–H > S–H > C–H. As a general rule the donor strength is increased by neighboring electron-withdrawing groups and reduced by electron-donating groups. In consequence, the ranking of O–H donor strengths follows pKₐ, where H₃O⁺ > OCOH > PhOH > C(sp³)–OH > H₂O > OH.

When multiple hydrogen bonding entities are present the strength of association is considered additive, however, the additivity of the individual hydrogen bonds is complicated when multiple sites are linearly arranged. In the formation of complexes (even the dimer formed between thymine and adenine) there is a sizeable repulsive contribution by secondary interactions between adjacent sites of the complex. First stated by Jorgensen for simple homodimers, the interaction between identical sites at the H-bonding interface is repulsive (shown with black arrows, figure 1-5), while the interaction between different sites at the H-bonding interface is attractive (gray arrows, figure 1-5). Schneider later drew linear free-energy relationships that help to predict the strength of linear multiple hydrogen-bonded complexes in CDCl₃ (− 8 kJ/mol for each primary interaction and ± 2.9 kJ/mol for each attractive and repulsive secondary interaction). These values are truly estimates when conformational effects are considered, preorganization increases stability of the hydrogen-bonding motif by
reducing entropic penalties upon assembly and is an additional factor that can appear to increase the association strength between units of multiple hydrogen bonding arrays.39

Figure 1-5. Secondary interactions that result from adjacent sites in a hydrogen-bonded complex, and can be attractive (gray arrows) or repulsive (black arrows)

Supramolecular Materials and Self-Assembly

Supramolecular materials thus far have been described in terms of molecular recognition and dynamic covalent systems. Self-assembly of biological systems by H-bonding has inspired the development of synthetic systems which mimic these interactions to gain perspective on the interactions which control assembly, and also the pursuit of functional materials.

Functional materials that are self-assembled to obtain some degree of polymerization (DP), as one-dimensional units, in thermodynamic equilibrium are termed supramolecular polymers (SMPs).40-42 Supramolecular polymers/materials offer several advantages over their covalent counterparts that often include a simplified synthesis (reduced cost), self-assembly into the most thermodynamically stable form (“error checking” is inherent), and responsiveness (to external stimuli). These factors make supramolecular strategies attractive for materials applications in areas as diverse as electro-optics, thermoplastic materials, information storage, and biomedicine.43 Materials with tightly binding supramolecular units give elastic properties similar to covalent polymers, and with the temperature responsiveness of SMPs makes processability a great advantage.44 Self-assembling monomers are also of interest to biomedical areas where there is a need for biocompatible and biodegradable materials. Drug delivery,
polymer diagnostics, and implantable biomaterials for tissue or bone replacement engineering are appealing.

The strength of dimerization largely controls material properties. The degree of polymerization is directly related to the thermodynamic stability of the monomeric system or endgroup, and the relationship is such that a $K_a \sim 10^3$–$10^4$ in a 1 M solution gives a DP $\approx 100$, while a $K_a \sim 10^7$ offers a DP $\approx 100000$. Association strength, $K_a$, is also related to the association lifetime, and is thus relevant to viscoelastic properties in the bulk material. Strongly associating monomeric units are not the only way to obtain functional materials; the effects of weak hydrogen bonding interactions can be amplified by phase segregation (typically by aromatic interactions) to obtain higher degrees of polymerization by excluded volume effects. In general, the properties of SMPs are similar to those of traditional polymers with the exception of their reversibility which makes the materials thermally and chemically responsive.

Systems of two hydrogen bonds or more fall into two categories, complementary and self-complementary. Complementary systems require a bonding partner, and stoichiometry is a concern for maximizing interactions. Thus, self-complementary systems (necessarily involving two or four hydrogen bonds) offer an advantage in some materials applications in that a partner is not necessary for a recognition event to occur. In general, a higher number of hydrogen bonds increases the strength of association, but also importantly increases the specificity of recognition through preferential pairing.

Strength and Specificity of Linear Systems by Multiple Hydrogen Bonding

Systems of Two and Three Linear Hydrogen Bonds

Hydrogen bonding systems have evolved from systems with two linear hydrogen bonding sites to those with four. Early examples of the latter include those by Boucher and coworkers that
feature two pyridones connected through rigid acetylenic spacers (figure 1-6).\textsuperscript{50-52} Pyridones linked at the 3 and 6’ positions are self-complementary and strongly dimerize ($K_{\text{dim}} \sim 10^4 \text{ M}^{-1}$) into cyclic dimers as shown in figure 1-6, \textbf{A}. Conversely, linear polymeric aggregates are formed by pyridones which are not completely self-complementary, and are linked at the 6 and 6’ positions (\textbf{B}, figure 1-6). Expanded cyclic structures have also been prepared through further strategic reorganization of the pyridones about the spacer (not shown). In terms of dimerization motifs the dimers held together by four hydrogen bonds are $\sim$ two orders of magnitude more stable (\textbf{B}, $K_{\text{dim}} \sim 10^2 \text{ M}^{-1}$ based on the dimerization of single pyridone units).

![Figure 1-6](image.png)

Figure 1-6. Rigidly linked pyridone units are predisposed to assemble by the arrangement of hydrogen bond donors and acceptors. An acetylene spacer links two pyridones from the 3 and 6’ positions to arrange four hydrogen bonds for strong dimerization (\textbf{A}, $K_{\text{dim}} \sim 10^4 \text{ M}^{-1}$); incorrect “programming” gives mostly reversible polymers (\textbf{B}).\textsuperscript{50-52}

Functional systems with three hydrogen bonding sites included, early on, work with melamine and imide pairs, which were easily accessible, and dimerized on the order of $\sim 10^2 \text{ M}^{-1}$\textsuperscript{1,38,53,54} Related complexation of acylated diaminopyridines with thymine derivatives was explored by Lehn and coworkers and laid the groundwork for the quadruple hydrogen-bonded systems that are widely used today.\textsuperscript{46-49} They importantly discovered that the diacylpyridine/ N-propylthymine complex \textbf{B} ($K_a = 800 \text{ M}^{-1}$), figure 1-7, is an order of magnitude more stable than the complex formed from diaminopyridine and N-propylthymine, \textbf{A} ($K_a = 84 \text{ M}^{-1}$). Beijer and coworkers have similarly investigated diaminotriazines.\textsuperscript{55,56} Surprisingly with acyl substitution of
the diaminotriazine, the strength of association with propylthymine decreased from 890 to 6 M⁻¹, the apparent result of electrostatic repulsion between the heterocyclic nitrogens and amide oxygens (C, figure 2-1).

Figure 1-7. For the diacylpyridine/N-propylthymine complex B the $K_a$ of 800 M⁻¹ in CDCl₃ is an order of magnitude greater than the association of diaminopyridine with N-propylthymine ($K_a = 84$ M⁻¹, in CDCl₃) complex A. Acylation of diaminotriazine (C) resulted in a lowered $K_a \sim 6$ M⁻¹ due to electrostatic repulsion.

Repulsive effects in C (figure 1-2) led to unanticipated self-association via the mode illustrated in figure 1-8. When the amides are in the cis conformation, an ADADA array of five hydrogen bonding sites is formed. Four of the five sites can dimerize by a DADA QHB array ($K_{dim} = 37$ M⁻¹). This low value speaks to the unfavorable interactions even in this conformation, between the acetamide methyl groups and triazine nitrogen atoms. Nonetheless, the design was presented to explore systems using four hydrogen bonds.

Figure 1-8. Electrostatic repulsion of amide carbonyl groups yields a linear array of five hydrogen bond donors and acceptors and dimerization via four hydrogen bonds ($K_{dim} = 37$ M⁻¹, in CDCl₃).
Linear Arrays of Four Hydrogen Bonds

Subsequent work on QHB systems by Meijer and coworkers, particularly on self-complementary systems, has been profound. The dimerization strength of DADA QHB arrays is predicted to be $\sim 3.1 \times 10^2 \text{ M}^{-1}$ by Jorgensen's rules for secondary interactions; the $K_{\text{dim}}$ of $37 \text{ M}^{-1}$ for C in figure 1-7 was much lower. Dimer stability was increased by acylating only one amino group (F, figure 1-9) to reduce electrostatic repulsions to give a $K_{\text{dim}} = 530 \text{ M}^{-1}$. The strength of dimerization was further increased to $2 \times 10^4 \text{ M}^{-1}$ by preorganizing the QHB face via an intramolecular hydrogen bond of a urea hydrogen to a triazine nitrogen as shown in figure 1-9 F. Several amido and ureidopyrimidine units have been synthesized since then that have dimerization strengths between 170 and $2 \times 10^5 \text{ M}^{-1}$. The strength of dimerization predicted by Jorgensen’s rules have generally been far exceeded by these DADA self-complementary QHB systems; the examination of the remaining self-complementary QHB array, DDAA, naturally followed.

![Figure 1-9](image)

Figure 1-9. Strength of triazine dimerization was increased by structural modifications and preorganization. Dimerization strength was increased to $530 \text{ M}^{-1}$ and $2 \times 10^4 \text{ M}^{-1}$ in CDCl$_3$, for F and G, respectively.

The pursuit for a strong QHB hydrogen bonded DDAA array lead quickly to the discovery of a isocytosine-derived ureidopyrimidinone unit (UPy). This unit exists in three tautomeric forms, shown in figure 1-10 as H, I, and J; where the equilibrium lies depends on solvent,
concentration, and substitution at the C6 position. Two keto forms are accessible; **H** is preferred when R’ is electron donating, and is arranged for DDAA QHB dimerization \((K_{\text{dim}} \sim 10^7 \text{ M}^{-1})\).

![Diagram](image)

Figure 1-10. Tautomeric structures of UPy are preorganized for different modes of dimerization and association. Tautomer **H** forms a strong self-complementary DDAA motif \((K_{\text{dim}} \sim 10^7 \text{ M}^{-1})\). Tautomer **J** forms a strong DADA hydrogen-bonding motif \((K_{\text{dim}} \sim 10^5 \text{ M}^{-1})\). Tautomer **I** is organized for triple hydrogen bonding with an appropriate partner.

The keto tautomer **I** is preorganized for association by a triple hydrogen bonding array. The third tautomer, **J**, is an enol tautomer which is preferred when R’ is electron withdrawing, and is preorganized for DADA QHB association \((K_{\text{dim}} \sim 10^5 \text{ M}^{-1})\). The large dimerization stability of the UPy unit made it appealing for functionalization and for investigation as a building block in reversible polymeric materials.

Ureidopyrimidone (UPy) has been extensively explored in the area of functional materials, and is being commercially marketed by SupraPolix as *SupraB®*. A variety of low molecular weight (2000–3500 MW) hydroxyl terminated telechelic polymers including polyethers, polyesters, and polycarbonates have been functionalized with UPy end groups (figure 1-11). The resulting materials have the mechanical properties of covalent polymers, but the melt viscosity of small molecules. For example, polyethylene butylene (H1) is a viscous liquid at room temperature and when functionalized with UPy is a rubber-like solid.
Figure 1-11. Hydroxy telechelic polymers functionalized with UPy form materials with properties similar to covalent polymers but melt viscosity of small organic molecules.

The UPy unit has also been investigated in materials useful for tissue engineering. Bioactive materials were formed by incorporating cell adhesion promoting polypeptides functionalized with UPy that were then combined with UPy functionalized low molecular weight oligocaprolactone. Scaffolds were fabricated and implanted subcutaneously. Although the UPy–UPy dimerization is weak in an aqueous environment, hydrophobic shielding of the H-bonding face within the polymer film renders the dynamic binding strong. Cell adhesion was shown in vitro, and signaling of cells and angiogenesis were seen in vivo.

Synthetically modified nucleobases are interesting platforms for supramolecular materials. Natural nucleobases hardly self-associate, and weakly heteroassociate through two or three hydrogen bonds (adenine-thymine; $K_a = 50 \text{ M}^{-1}$, and guanine-cytidine; $K_a = 10^3 \text{ M}^{-1}$). Through simple modifications, multiple hydrogen bonding units can be formed from these heterocycles. This has been explained by Meijer and coworkers with the functional unit derived from isocytosine, UPy. Also Zimmerman and coworkers have introduced a complementary ureidoguanosine (UG) unit; the first purine derivative, shown in figure 1-12. With an appropriate partner, UG associates strongly; a stability of $5 \times 10^7 \text{ M}^{-1}$ is obtained with DAN (2,7-diamido-1,8-naphthyridine). Association of UG is decreased by competition from
Hoogsteen dimerization (involving N7), and a stronger dimer \((K_a \geq 10 \) times larger) is obtained by conversion of N7 to CH (DeUG)\(^{61}\).

Other nucleobases have been functionalized for use in multiple hydrogen bonding systems. Ditopic recognition units, termed Janus bases, have been introduced to recognize mismatches in DNA\(^{65}\). Further, Sessler and coworkers have introduced nucleobase-functionalized porphyrins as recognition units\(^{66-68}\).

![Diagram](image)

**Figure 1-12.** Ureidoguanosine (UG, top) dimerizes with DAN \(K_a \sim 5 \times 10^7 \) M\(^{-1}\) (CHCl\(_3\)), dimerization at the Hoogsteen face (bottom left) is eliminated with the synthesis of a deazaguanine (DeUG, \(K_a \sim 10^8\), in CDCl\(_3\), bottom right).
The allure of nucleobases is multifaceted. DNA or DNA-like materials offer opportunities for preparing controlled self-assembled structures.\(^6^9\) The heterocycles are inexpensive and readily available. Beyond this, purine bases introduce attractive advantages over pyrimidine bases, although these have been largely untapped. Purines possess polarizable aromatic surfaces which are available for $\pi-\pi$ and hydrophobic interactions (figure 1-13). These interactions can lend additional stability for functional materials. Further, purines have multiple sites for functionalization. The C2, C6, and C8 positions as well as N7 and N9 are all viable sites for modification. Further, purine nucleobases offer two H-bonding faces (Hoogsteen and Watson-Crick), which can provide homodimers. These additional H-bonding sites can play important roles in self-assembly.

![Diagram of purine structure with Hoogsteen and Watson-Crick faces indicated.](image)

Figure 1-13. Purines offer many opportunities for preparing controlled self-assembling structures.

**Scope and Organization of Thesis**

It was the interest of the author for this thesis to contribute to the development of the biologically-inspired QHB systems for the better understanding of these types of systems. Herein is introduced the first adenine derived self-complementary QHB system through a DADA array. The rational design of this system, which is the topic of Chapter 2, includes structural, conformational, and synthetic considerations. These factors are discussed in detail, and followed with the synthesis of N9 aryl protected phenyl ureidodiaminopurine. The methods for solution phase characterization are presented, and followed by characterization of subsequent ureidodiaminopurines.
Reactivity issues complicated the synthesis of alkyl UDAP. These complications were overcome by alternative syntheses in Chapter 3, and the systems were characterized. The structure-property relationships were examined with alterations made to the urea and N9 substituents, to consider possible electronic and steric effects. The reactivity preferences of DAP that were encountered in Chapter 3 are the basis of Chapter 4. Reaction of DAP with common acylating agents was investigated to shed light on the differential reactivity of the N\textsubscript{C2} and N\textsubscript{C6} amino groups. Future directions are discussed in Chapter 5 in terms of applications of UDAP, and other interesting QHB platforms.
CHAPTER 2
DESIGN AND SYNTHESIS OF COMPLEMENTARY UREIDODIAMINOPURINES BY QUADRUPLE HYDROGEN BONDING

Nucleobases are readily available heterocycles as platforms for the construction of self-complementary quadruple hydrogen bonding (QHB) complexes. Of the nucleobases, the pyrimidines have been extensively explored as QHB systems.\textsuperscript{57,70-72} For example, in the last decade the UPy unit of Meijer et al. (derived from isocytosine) has found applications that span materials science due to its exceedingly high dimerization constant in organic solution ($K_{\text{dim}} \approx 10^7 \text{ M}^{-1}$ in CDCl\textsubscript{3}) via an accessible DDAA hydrogen bonding arrangement. Despite the extended $\pi$ surfaces, which can be used to control ring electronics, and additional sites for functionalization, purines have only recently been explored in this arrangement by Zimmerman et al. with a QHB ureideoguanine.\textsuperscript{61-64} In this discourse is presented the first discrete, self-complementary, QHB unit based on a DAP platform.

**Design: Structural, Conformational, and Synthetic Considerations**

**Structural and Synthetic Design**

The general design of a self-complementary QHB dimer derived from DAP is shown in figure 2-1; it entails formation of a DADA array along the Watson-Crick edge of the purine through regioselective urea formation at NC\textsubscript{2}. Intramolecular hydrogen bond formation between the H\textsuperscript{c} proton of the urea to N3 of the purine core serves to preorganize the monomer for dimer formation, similar to the strategy introduced for related systems in Chapter 1. Also apparent from the figure is that selection of the purine N9 (R) and urea (R\textsubscript{1}) substituents is important given that they can modulate solubility, conformational equilibria, and dimer stability.

Two requirements surface for the purine N9 substituent designated R; it must a) be sterically compatible with the urea substituent (R\textsubscript{1}) so as not to discourage formation of the desired folded conformation (conversely, an interaction which is complementary between R and
the urea substituent, such as an aromatic interaction, could confer additional stability to the desired folded conformation) and b) impart suitable solubility to the monomer and dimer to allow convenient spectroscopic study in solution. Regarding the latter, the purines are relatively polar heterocyclic structures and thus show problematic solubility in most organic solvents. While the nucleoside ribose hydroxyl groups of natural nucleobase precursors can be outfitted with organic protecting groups (e.g., TMS or TBDMS) for these studies, sugar substituents at N9 present challenges with respect to stability and size. These modifications are a topic of further discussion in Chapter 5. Benzyl substituents (CH₂–Ar), on the other hand, are a) known to increase the organic solubility of purines, b) chemically stable, and c) conveniently installed by Sₙ2 chemistry at N9.⁷³ Thus, for the initial design, benzylic N9 substituents were selected.

Figure 2-1. Dimerization equilibrium for UDAP, 1₁ᴺ³

The urea substituent, R₁, influences both intra- and intermolecular hydrogen bonding in the design. For example, an electron withdrawing group might promote preorganization of the monomer by rendering H⁺ more acidic (e.g., the pKₐ of an aryl urea (biphenyl urea) is ~20 versus ~27 for urea).⁷⁴ The same substituent could, however, diminish the basicity of the urea carbonyl group that is important for intermolecular H-bonding. Despite the risk that aromatic urea substituents might impair solubility via aromatic aggregation, phenyl was the initial choice for R₁ primarily for reasons of synthetic convenience. Ongoing studies in the lab revealed that aryl
isocyanates enjoy a considerable reactivity advantage over alkyl isocyanates in reaction with DAP (vide infra).

Further guiding the initial choice of R and R\textsubscript{1} groups were results from computation and X-ray crystallography. Four conformations can theoretically be accessed by the UDAP urea in the monomeric state, two designated \textit{syn} and two designated \textit{anti} (figure 2-2). Of these possible conformers two are expected to be preferred due to the formation of an enthalpically favored intramolecular hydrogen bond involving H\textsubscript{c} and either N1 (\textit{1\textsuperscript{N1}; anti I}) or N3 (\textit{1\textsuperscript{N3}; syn I}). These two conformers are interconverted through a single C–N bond rotation (figure 2-3) and their arrangement is prerequisite for the formation of a QHB dimer.

![Figure 2-2. Four possible low energy conformers of 9-methyl-2-N-phenylureidodiaminopurine (UDAP) considered computationally.](image)

A computational approach was taken to investigate the equilibrium among the four conformers (table 2-1). When a substituent in the N9 position is too small to interact appreciably with a urea phenyl group (R\textsubscript{1} = Ph), such as methyl (R = CH\textsubscript{3}), computation shows that the \textit{anti 2} and \textit{syn 2} conformers are \textasciitilde{} 5 kcal mol\textsuperscript{-1} higher in energy than the \textit{1\textsuperscript{N1} (anti I)} and \textit{1\textsuperscript{N3} (syn I)} conformers (Monte Carlo conformational searching using MacroModel v. 9.0 and the MCMM method; solvent (GB/SA) = CHCl\textsubscript{3}, force field = Amber*). Higher-level calculations were performed on the two lowest energy conformers, \textit{1\textsuperscript{N1} (anti I)} and \textit{1\textsuperscript{N3} (syn I)}, and show that they are essentially isoenergetic (\textasciitilde{} 0.55 kcal mol\textsuperscript{-1} in favor of the desired \textit{1\textsuperscript{N3} conformer}) in the gas
phase (Gaussian 03 (revision D.01), MP2/6–31G*'//HF/6–31G*). The results suggest that the
design shown in figure 2-1 is thermodynamically feasible, where the slight energetic preference
for \(1^{N3}\) will leave the monomer conformational equilibrium, and also likely \(K_{dim}\), easily
perturbed.

![Diagram of \(1^{N1}\) to \(1^{N3}\).]

Figure 2-3. Single C–N bond rotation interconverts \(1^{N1}\) to \(1^{N3}\).

Table 2-1. Relative energies (kcal mol\(^{-1}\)) of four possible UDAP conformers by computation

<table>
<thead>
<tr>
<th>Method</th>
<th>(1^{N3}) syn 1</th>
<th>(1^{N1}) anti 1</th>
<th>anti 2</th>
<th>syn 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amber*</td>
<td>0</td>
<td>–0.19</td>
<td>5.0</td>
<td>4.5</td>
</tr>
<tr>
<td>MM3*</td>
<td>0</td>
<td>0.06</td>
<td>5.7</td>
<td>5.5</td>
</tr>
<tr>
<td>HF/3-21G*</td>
<td>0</td>
<td>0.75</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HF/6-31G*</td>
<td>0</td>
<td>0.50</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MP2/6-31G*//</td>
<td>0</td>
<td>0.55</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HF/6-31G*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Further evidence for the accessibility of the \(1^{N3}\) conformer, when the N9 substituent is a
bulky aryl group, comes in the form of a crystal structure of model compound 2 (figure 2-4). The
dimethylamino substituent at C6 obviously prevents QHB formation, but the desired mode of
intramolecular hydrogen bonding nonetheless is obtained through a planar arrangement (where
N12···N3 = 2.74 Å). Interestingly, in the solid state there does not appear to be a steric
consequence for the bulky N9 and urea substituents, but what appears as a potentially favorable
interaction between the urea phenyl and the protecting group in the form of a near edge-to-face
Figure 2-4. X-ray crystal structure of model compound 2 (ellipsoids drawn at 50% probability level). The crystal structure shows an intramolecular hydrogen bond between N3 and N12 and a near edge-to-face interaction between the N9 and urea phenyl groups. A space-filling model illustrates the close contact between the aromatic N9 and urea substituents on the right.

Aromatic interaction (angle between the least-squares planes of the aromatic rings = 86.3°).

Further, the space filling model to the right of the ORTEP plot shows the extent of contact between the two aromatic groups. The rings are slightly offset (center-to-center distance = 5.42 Å) with respect to one another, however, still in close contact (the closest carbon–carbon distance equals 3.67 Å). Although assessing a priori whether this interaction would be important to the solution-phase conformation of the molecule is difficult, the solid-state structure does suggest that the desired conformation is sterically accessible. Indeed, consistent with the solid-state data, the chemical shift of the N12–H proton in CDCl₃ (~ 5 mM) is significantly deshielded to 11.4 ppm (relative to TMS); N10–H appears at ~ 7.2 ppm.

**Synthesis**

The three step synthesis of UDAP (figure 2-5) began from routinely prepared or commercially available 2-amino-6-chloropurine 3. Standard alkylation of N9 yielded compound 4a and 4b in the presence of K₂CO₃, overnight. The yields of the desired N9 product were
diminished by competitive N7 alkylation with the ratio of the two products sensitive to the size of the benzylic bromide; 4a (N9) was obtained in 40 % yield with its N7 regioisomer (4a') in

Figure 2-5. Regioselective synthesis of UDAP

12 %, while 4b (N9) was obtained in 85 %. For this step, benzyl bromides derived from mesitylene (a)\textsuperscript{77} and 3,5-heptyloxybenzene (b)\textsuperscript{78,79} were selected. The methyl substituents of the mesitylene-derived protecting group were intended to sterically disrupt aromatic interactions between dimers thereby increasing solubility, while the heptyloxy derivative featured long solubilizing arms. Subsequent addition of ammonia and elimination of the 6-chloro group afforded DAP, 5. Regioselective formation of 1 ensued by reaction of 5 with phenyl isocyanate in the presence of pyridine. This interesting regiochemical preference is the topic of Chapter 4.

**Solution Phase Characterization of 1a Supports the Mode of Dimerization**

Solution phase data was acquired by gHMBC, NOESY, and variable temperature (VT) NMR techniques. This data elucidates that the desired 1\textsuperscript{N3} conformer is populated in solution and participates in QHB.
The proton chemical shifts of 1a were unequivocally assigned by $^1$H–$^{13}$C gHMBC (figure 2-6, only pertinent information shown). The chemical shifts that are relevant to the discussion which follows are those of the urea $H^b$ ($\delta$ 8.92) and $H^c$ ($\delta$ 11.91), the methylene protons ($\delta$ 5.29; N9 CH$_2$, $\delta$ 2.32; CH$_3$) of the protecting group, and the ortho proton of the urea phenyl ($\delta$ 7.62). From these chemical shifts the most direct conformational evidence was observed by NOESY NMR (~ 2 mM, 25 °C, in CDCl$_3$). Key NOEs were found between the methylene protons of the N9 benzyl to $H^c$ and to the ortho phenyl proton. Significant NOEs were also seen from the methyl substituents of the protecting group to the ortho proton of the urea phenyl as well as to the urea proton $H^c$, which indicates the close spatial arrangement of the aromatic protecting group and urea phenyl in solution. Further, the chemical shift for the assignments of $H^b$ and $H^c$ ($\delta$ 11.91 ppm) is in agreement with the chemical shift of the model compound 2 ($H^c$, $\delta$ 11.4, ~ 5 mM, CDCl$_3$).

In considering the presence of the 1$^{N1}$ conformer in solution, one would expect to see exchange of the $H^c$ proton, as is seen for $H^b$. Exchange can occur with any other protic species in solution including solvent or H$_2$O, but such exchange is not readily apparent. Although, this is not conclusive evidence for the absence of the 1$^{N1}$ conformer it does speak to the stability of the 1$^{N3}$ conformer in solution at the concentration and temperature studied.

Conformational aspects were further clarified, and in addition, the mode of dimerization was supported by $^1$H VT-NMR (figure 2-7). Upon heating of a solution 1a in CDCl$_3$ from $-55$ °C to 55 °C, $H^c$ makes a moderate upfield shift from $\delta$ ~ 12.4 to $\delta$ ~ 11.8. On the contrary, $H^b$ makes a substantial upfield shift from $\delta$ ~ 10.2 to $\delta$ ~ 8.1, consistent with intermolecular hydrogen bonding. Also consistent with intermolecular hydrogen bonding is the broadening of the $H^b$ proton signal as the temperature increases. As the temperature is raised from $-55$ °C the
Figure 2-6. A NOESY spectrum (~ 2 mM, 25 °C, 500 MHz, in CDCl₃ relative to TMS) shows the desired $I_{N3}^{1}$ conformer (proton chemical shifts assigned by gHMBC, not shown). The signal broadens as the dimerization equilibrium becomes faster and then reaches the NMR time scale (between −5 and 15 °C). The rate of exchange gradually increases and the signal is sharpened at 55 °C. The intermolecularly hydrogen bonded amino proton signal for $H^a$, visible at $\delta \sim 9.3$ (−55 °C), shifts to $\delta \sim 8.4$ (5 °C) where the signal disappears by peak broadening through an intermediate exchange regime with $H'^a$; the latter remains at $\delta \sim 5.9$ until 5 °C. Fast exchange
of the amino protons relative to the NMR time scale is reflected by an averaged peak at $\delta \sim 6.2$ (55 °C). Interestingly, VT NMR of 1a’ did not indicate dimerization was occurring via QHB. This is likely a steric consequence of the mesitylene protecting group at N7 interacting with the amino protons.

A subtle but important point is that based on the trends of the chemical shifts for the amino protons, intermolecular hydrogen bonding via the Hoogsteen face is not a contributing mode of dimerization at the concentration and temperature range studied (figure 2-8, A). Zimmerman and coworkers encountered Hoogsteen binding as a competing motif in the association of ureidoguanine as described in the introductory chapter. Likewise, two-point hydrogen bonding between the urea groups in the 1$^{N1}$ conformation (B) is not consistent with the NOESY data and is expected to be much weaker than QHB in solution.

Figure 2-7. VT NMR of compound 1a (~ 2 mM, 500 MHz, in CDCl$_3$ relative to TMS)
Figure 2-8. The possible dimers that can be formed through two intermolecular hydrogen bonds via $^{N_1}$. Dimer $A$ is formed through the urea carbonyl and $H^b$ of one purine ($b$) to the Hoogsteen face of the partner ($a$). Complementary dimer $B$ is formed through hydrogen bonds between the urea carbonyl and $H^b$ proton.

**Intermolecular Hydrogen Bonding Strength**

The theory\textsuperscript{80-82} derived for a system consisting of a monomer and dimer in equilibrium begins with equation (1).

$$2M \rightleftharpoons D$$

(1)

and the dimerization constant described by (2).

$$K_{\text{dim}} = \frac{[D]}{[M]^2}$$

(2)

The concentration of monomer and dimer at a particular time sum to the initial concentration of monomer ($[M]_0$, Eq. 3).

$$[M]_0 = [M] + 2[D]$$

(3)

The chemical shift that is observed for $H^b$ is the chemical shift of the monomer less the change in chemical shift from dimer to monomer multiplied by the mole fraction of the initial monomer concentration that is dimer (Eq. 4a). The mole fraction of dimer can also be expressed in terms of the monomer concentration $[M]$ (Eq. 4b). Solving equations 4a and 4b for $[D]$ and $[M]$ and inserting them into the equilibrium expression and rearranging for $K_{\text{dim}}$ gives expression (5).
Taking the log of this expression and rearranging gives equation (6). A plot of the right side of this equation vs. \( \log [M]_0 \) will give a straight line with a y-intercept equal to \( \log K_{\text{dim}} + \log 2 - \log (\delta_D - \delta_M) \).

\[
\delta_{\text{obs}} = \delta_M + (\delta_D - \delta_M) \cdot 2 \frac{[D]}{[M]_0}, \quad \delta_{\text{obs}} = \delta_M + (\delta_D - \delta_M) \cdot (1 - \frac{[M]}{[M]_0}) \tag{4a and 4b}
\]

\[
K_{\text{dim}} = \frac{(\delta_D - \delta_M)(\delta_{\text{obs}} - \delta_M)}{2(\delta_D - \delta_{\text{obs}})^2[M]_0} \tag{5}
\]

\[
\log\left(\frac{2}{(\delta_D - \delta_M)}\right) + \log K_{\text{dim}} + \log [M]_0 = \log (\delta_{\text{obs}} - \delta_M) - 2 \log (\delta_D - \delta_{\text{obs}}) \tag{6}
\]

In order to determine the numerical value for \( K_{\text{dim}} \), it is apparent that one must know the endpoints, \( \delta_D \) and \( \delta_M \) with reasonable certainty. It is possible to extrapolate a value for \( \delta_D \) from a plot of chemical shift vs. concentration. It is also possible to obtain \( \delta_D \) at low temperature or high concentration. On the contrary, \( \delta_M \) can be determined by low concentration and/or high temperature. However, extrapolation to \( \delta_M \) is typically limited by NMR instrument sensitivity. It has been illustrated that the experimental chemical shift data obtained in CDCl\(_3\) and (CDCl\(_2\))\(_2\) are essentially equivalent.\(^6\) Tetrachloroethane is an appealing alternative for determining the limiting chemical shift values, as the higher boiling point (~ 146 °C) in comparison to chloroform (~ 62 °C) is helpful in achieving complete dissociation of the dimer into monomer units for measurement of \( \delta_M \).

The concentration range selected for \( K_{\text{dim}} \) determination should fall within the range of 20–80 % of saturation (ie. fraction of solution which is dimer), and is directly related to the accuracy of the experimental values obtained for \( \delta_M \) and \( \delta_D \).\(^8^3\) Outside of this saturation range the error associated increases exponentially.\(^8^3\) The relationship between \( K_{\text{dim}} \) and fraction of monomer can
be calculated by solving equation (3) for $[D]$ and inserting that value into the equilibrium expression to yield equation (7). It then follows that the fraction of monomer is $\frac{[M]}{[M]_0}$. The fraction of dimer can therefore be calculated as $1 - \frac{[M]}{[M]_0}$.

$$[M] = (1 + 8K_{\text{dim}}[M]_0)^{\frac{1}{2}} - \frac{1}{(4K_{\text{dim}})}$$  (7)

An observed chemical shift value which is central to $\delta_M$ and $\delta_D$, if accurate, will correspond to a solution in which the fraction of dimer is 0.5.

Dilution studies were performed at 25 °C, and the chemical shift of H$^b$ monitored by $^1$H NMR to obtain the intermolecular dimerization constant, $K_{\text{dim}}$ for 1a and 1b. The $K_{\text{dim}}$ values were determined as the average of four runs which were calculated based on the theory of Nogales$^{82}$ and then compared to values calculated using standard software.$^{84,85}$ Dilution studies were performed at starting concentrations between 3.5–4.7 mM for each of the four sets, and the chemical shift of H$^b$ monitored. A stock solution was diluted sequentially to a total volume of 1 mL in increments of 0.05 mL with 8–10 iterations (figure 2-9). The dimerization constants were calculated for each dilution set using the experimentally determined $\delta_D$ and $\delta_M$ and obtaining graphical representations of the data by equation 6 (figure 2-10), and the values averaged. The dimerization constants were calculated to be $820 \pm 160$ M$^{-1}$ (20–75 % dimer) and $980 \pm 290$ M$^{-1}$ (20–75 % dimer) for compounds 1a and 1b, respectively.

Dimerization constants were also calculated by non-linear least squares analysis using a program developed by Sanderson et al.$^{85}$ This program uses the same theoretical principles as described previously, with the exception that the $\delta_M$ and $\delta_D$ values are calculated by an iterative approach. The $K_{\text{dim}}$ values obtained from this method were in close agreement with the values
Figure 2-9. Representative dilution of **1a** (25 °C, 500 MHz, in CDCl₃).

Figure 2-10. Dilution data in figure 2-7 for **1a** and calculation of $K_{\text{dim}}$ calculated using experimentally determined endpoints. The average calculated values were 1100 ± 360 M⁻¹ (20–75 % dimer) for **1a** and 1600 ± 380 M⁻¹ (20–75 % dimer) for **1b**. The deviation in $K_{\text{dim}}$ is due to the calculated endpoints. However, the calculated monomer chemical shift ($\delta$ 7.2
± 0.1) was nearly equivalent to the experimental value (δ 7.2 ± 0.01; 120 °C, (CDCl₂)₂, ~ 1.5 mM)). The chemical shift of the fully dimerized species was calculated to be δ 10.1 ± 0.1 and experimentally determined to be δ 10.2 ± 0.01 (− 55 °C, (CDCl₂)₂, ~ 3 mM). Even a discrepancy of 0.1 ppm in δ₂₃ is enough to introduce significant uncertainty in the value of Kdim. Clearly the level of error by approximation is less favorable when using the previously developed program, however, the values for the equilibrium constant are in close agreement when the endpoint values are taken into consideration.

The values of Kdim for 1a and 1b are in close agreement given the experimental error. Chapter 3 discusses the structure-property relationships for this system in terms of R and R₁. It will become apparent that much larger groups can be accommodated than originally thought.

**Dimerization in the Solid State**

X-ray crystallography is a powerful tool for confirming hydrogen bonding modes. There are clear preferences for specific hydrogen bonding patterns in crystal structures. These preferences are combined hierarchally to form “empirical rules” which can be used for predicting hydrogen bond patterns from a limited number of functional groups.³² Further, in the absence of other forces, these rules can be used as “indicators” for hydrogen bond preferences in the solid state or in solution.³² In the solid state, compound 1a features both the 1N₁ and 1N₃ conformers and the expected hydrogen bonding and aromatic interactions. As per the empirical rules mapped out by Margaret Etter,³² all intramolecular hydrogen bonding possibilities are utilized, and all the hydrogen bond donor and acceptor sites available are satisfied in the crystal lattice.

The DADA QHB mode of dimerization is confirmed in the solid state (figure 2-11). The 1N₃ conformer is organized through an intramolecular hydrogen bond (2.678 Å, table 2-2), and intermolecular hydrogen bonds are present as expected. Intermolecular hydrogen bonds are seen
from N–H\(^{19}\)···O\(^1\) and N–H\(^{10}\)···N1 of each UDAP with averaged distances of 2.786 and 3.291, respectively. Other interesting conformational information is available. The plane of the urea phenyl is twisted with respect to the plane of the purine core by 20.6°. More interestingly, the N9 2,4,6-trimethylbenzyl substituent is in an \textit{anti} conformation 86.5° out of plane with the purine core, in contrast to the N9 benzyl substituent of model compound 2.

Figure 2-11. \(1^{N3}\) Conformer QHB motif in the solid state (hydrogens removed for clarity).

Table 2-2. Hydrogen bond distances (Å) for QHB \(1^{a}\) in the solid state.

<table>
<thead>
<tr>
<th>D–H···A</th>
<th>Distance (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N12–H···N3</td>
<td>2.678</td>
</tr>
<tr>
<td>N19–H···O1</td>
<td>2.786</td>
</tr>
<tr>
<td>N10–H···N1</td>
<td>3.291</td>
</tr>
</tbody>
</table>

The extended crystal structure of \(1^{a}\) also shows the presence of a preorganized (N12–H···N3) \(1^{N1}\) conformer. Hydrogen bonding from the urea of a preorganized \(1^{N1}\) conformer (a; N10–H···N7) to the Hoogsteen edge of a \(1^{N3}\) conformer (b; N19–H···O1) is seen (figure 2-12, for bond distances see table 2-3). Similar to the \(1^{N3}\) conformer, the N9 substituent is nearly perpendicular to the purine core (83.81°). It also follows that for the \(1^{N1}\) conformer the urea
phenyl is twisted out of the plane of the purine core by 25.86 degrees, similar to the intramolecular arrangement for $1^{N_3}$.

Figure 2-12. Hydrogen bonding to the Hoogsteen face of $1a$ in the $1^{N_3}$ conformation to the urea of a UDAP in the $1^{N_1}$ conformation

Table 2-3. Hydrogen bond distance of $1a$ participating in Hoogsteen dimerization in the solid state.

<table>
<thead>
<tr>
<th>D–H···A</th>
<th>Distance (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N12–H···N1</td>
<td>2.664</td>
</tr>
<tr>
<td>N10–H···N7</td>
<td>3.061</td>
</tr>
<tr>
<td>N19–H···O1</td>
<td>2.748</td>
</tr>
</tbody>
</table>

Summary

The general design and synthesis of a self-complementary QHB dimer derived from DAP has been outlined. Regioselective urea formation occurs at N$_{C2}$, and a DADA QHB motif is presented via preorganization of the urea into a $1^{N_3}$ syn 1 conformation. This conformation was shown to exist in solution by NOESY NMR in CDCl$_3$ (~ 2 mM). Further, by VT NMR, the conformation is again verified, and QHB is shown to be the predominant mode of assembly at
the temperature and concentration ranges studied. Dimerization constants were derived from sequential dilution and monitoring of $^1$H chemical shifts by NMR. Calculated $K_{\text{dim}}$ values were $820 \pm 160 \text{ M}^{-1}$ (20–75 % dimer) and $980 \pm 290 \text{ M}^{-1}$ (20–75 % dimer) for compounds 1a and 1b, respectively. X-ray crystallography confirmed QHB via the desired DADA motif, but also confirmed the possible Hoogsteen dimer.

**Experimental Methods**

**General protocols**

Reagents were purchased from Acros or Aldrich, and were used without further purification unless stated otherwise. Dry solvents were degassed and purified under an atmosphere of argon using the GlassContour solvent system (GlassContour, Inc.). Pyridine was distilled onto 3 Å activated molecular sieves. Column chromatography was carried out using Whatman 230–400 mesh silica gel. Thin layer chromatography (TLC) was performed on Duracil TLC aluminum sheets with visualization by UV light. Melting points (Mp) were determined on a MEL-TEMP melting apparatus and are uncorrected. $^1$H (300, 500 MHz) and $^{13}$C (75, 125 MHz) nuclear magnetic resonance (NMR) spectra were recorded on Varian Gemini 300, Mercury 300BB, and Inova 500 spectrometer at room temperature unless otherwise specified. Chemical shifts ($\delta$) are given in parts per million (ppm) relative to TMS and referenced to residual protonated solvent (CHCl$_3$: $\delta$ $^1$H 7.24 ppm, $\delta$ $^{13}$C 77.0 ppm; DMSO: $\delta$ $^1$H 2.49 ppm, $\delta$ $^{13}$C 39.5 ppm). Abbreviations used are singlet (s), doublet (d), triplet (t), multiplet (m), and broad (b). High resolution mass spectrometry (HRMS) spectra were recorded on a Finnigan LCQ-Ion Trap Spectrometer.
Synthetic details

2-Amino-6-chloro-N-9-(2,4,6-trimethylbenzyl)purine (4a). 6-Chloro-2-aminopurine\textsuperscript{76} 3 (2.08 g, 11.1 mmol), 2-bromomethyl-1,3,5-trimethyl-benzene\textsuperscript{77} (3.52 g, 16.5 mmol), and K\textsubscript{2}CO\textsubscript{3} (2.30 g, 16.6 mmol) were placed in an oven-dried round-bottomed flask and dried under argon. Dry DMF (180 mL) was added and the mixture was stirred overnight at room temperature. The solvent was removed under vacuum and the residue was purified by column chromatography on silica gel (5\% CH\textsubscript{3}OH/CH\textsubscript{2}Cl\textsubscript{2}) to yield a yellow/white powder (1.42 g, 40\%). Mp 205–208 °C; \textsuperscript{1}H NMR (CDCl\textsubscript{3}) \(\delta\) 2.25 (s, 6H), 2.31 (s, 4H), 5.17 (s, 4H), 6.94 (s, 2H), 7.25 (s, 1H); \textsuperscript{1}H NMR (DMSO-\textit{d}\textsubscript{6}) \(\delta\) 2.23 (s, 3H), 2.25 (s, 6H), 5.16 (s, 2H), 6.92 (s, 2H), 6.95 (s, 2H), 7.55 (s, 1H); \textsuperscript{13}C NMR (DMSO-\textit{d}\textsubscript{6}) \(\delta\) 19.4, 20.5, 123.1, 128.2, 128.3, 129.1, 129.2, 137.5, 141.7, 149.3, 153.9, 159.7; HRMS (ESI-FT-ICR) calculated for C\textsubscript{15}H\textsubscript{16}N\textsubscript{5}Cl (M + H)+ 302.1167, found 302.1166.

\[
\begin{align*}
\text{N} & \quad \text{N} \\
\text{Cl} & \quad \text{NH}_2
\end{align*}
\]

2-Amino-6-chloro-N-7-(2,4,6-trimethylbenzyl)purine (4a'). Using conditions identical to those designed for 4a, the N7 regioisomer was isolated as a yellow/white solid from 5.30 g (28.1 mmol) of starting material 3 to give 1.04 g (12\%) of 4a'. Mp 223–225 °C; \textsuperscript{1}H NMR (CDCl\textsubscript{3}) \(\delta\) 2.25 (s, 6H), 2.30 (s, 3H), 5.16 (s, 2H), 5.26 (bs, 2H), 6.94 (s, 2H), 7.25 (s, 1H); \textsuperscript{1}H NMR

\[
\begin{align*}
\text{N} & \quad \text{N} \\
\text{Cl} & \quad \text{NH}_2
\end{align*}
\]
(DMSO-$d_6$) $\delta$ 2.22 (s, 3H), 2.24 (s, 6H), 5.14 (s, 2H), 6.90 (s, 2H), 6.95 (bs, 2H), 7.54 (s, 1H);

$^{13}$C NMR (DMSO-$d_6$; 100 °C) $\delta$ 19.4, 20.5, 41.1, 123.2, 128.3, 129.2, 137.5, 137.6, 141.8, 149.4, 154.0, 159.8.

2,6-Diamino-N-9-(2,4,6-trimethylbenzyl)purine (5a). Vacuum dried starting material 4a (1.05 g, 3.30 mmol) was placed in a 330 mL pressure tube. Methanolic ammonia (95 mL, 7 N) was added to the solid followed by heating under pressure to 90 °C for 16 h. The solvent was removed under vacuum and the residue was purified by chromatography on silica gel (5% CH$_3$OH/CH$_2$Cl$_2$) to yield 5a (0.640 g, 50%) as a white powder. Mp 275–278 °C; $^1$H NMR (CDCl$_3$) $\delta$ 2.24 (s, 6H), 2.29 (s, 3H), 4.72 (bs, 2H), 5.11 (s, 2H), 5.30 (bs, 2H), 6.92 (s, 2H), 6.97 (s, 1H); $^1$H NMR (DMSO-$d_6$) $\delta$ 2.24 (s, 9H), 5.04 (s, 2H), 5.80 (bs, 2H), 6.65 (bs, 2H), 6.91 (s, 2H), 7.04 (s, 1H); $^{13}$C NMR (DMSO-$d_6$; 100 °C) $\delta$ 18.9, 20.1, 40.2, 112.9, 128.7, 128.7, 135.6, 136.9, 137.1, 151.7, 155.8, 159.9; HRMS (ESI-FT-ICR) calculated for C$_{15}$H$_{18}$N$_6$ (M + H)$^+$ 283.1666, found 283.1668.

2,6-Diamino-N-7-(2,4,6-trimethylbenzyl)purine (5a'). This compound was prepared by the same procedure as 5a from 1.00 g (3.11 mmol) of 4a to yield 5a' as a white solid (0.530 g,
42%). Mp 282–285 °C; $^1$H NMR (CDCl$_3$) $\delta$ 2.24 (s, 6H), 2.29 (s, 3H), 4.72 (bs, 2H), 5.11 (s, 2H), 5.29 (bs, 2H), 6.92 (s, 2H), 6.97 (s, 1H); $^1$H NMR (DMSO-d$_6$) $\delta$ 2.24 (s, 6H), 2.26 (s, 6H), 5.08 (s, 2H), 5.46 (bs, 2H), 6.27 (bs, 2H), 6.95 (s, 2H), 7.05 (s, 1H); $^{13}$C NMR (DMSO-d$_6$; 100 °C) $\delta$ 18.7, 19.9, 113.0, 128.5, 128.6, 135.5, 136.8, 136.9, 151.6, 155.7, 159.8.

6-Amino-N-9-(2,4,6-trimethylbenzyl)-2-N-(4-phenylamino)ureidopurine (1a).

Compound 5a (0.050 g, 0.21 mmol) was placed in an oven-dried two-necked round-bottomed flask and dried under vacuum. Under argon atmosphere CH$_2$Cl$_2$ (29 mL) was added to the solid and the mixture was heated to 50–55 °C to dissolve 5a. When the starting material was completely dissolved, the temperature was lowered to 40 °C. Pyridine (0.034 mL, 0.43 mmol) and phenyl isocyanate (0.051 mL, 0.47 mmol) were added. The mixture was stirred at 40 °C for 20.5 h followed by evaporation. Purification by column chromatography on silica gel (1% MeOH/CH$_2$Cl$_2$) afforded 1a (0.058 g, 70%). Mp 263–265 °C; $^1$H NMR (CDCl$_3$) $\delta$ 2.29 (s, 6H), 2.32 (s, 2H), 5.26 (s, 2H), 6.96 (s, 2H), 7.09 (t, $J = 7.1$ Hz, 3H), 7.16 (bs, 1H), 7.34 (t, $J = 7.3$ Hz, 2H), 7.59 (d, $J = 7.6$ Hz, 2H), 9.34 (bs, 1H), 12.04 (s, 1H); $^1$H NMR (DMSO-d$_6$) $\delta$ 2.24 (s, 3H), 2.26 (s, 6H), 5.20 (s, 2H), 6.92 (s, 2H), 7.02 (t, $J = 7.0$ Hz, 1H), 7.30 (t, $J = 7.3$ Hz, 2H), 7.37 (s, 1H), 7.61 (s, 2H), 7.73 (d, $J = 7.7$ Hz, 2H), 9.31 (s, 1H), 11.82 (s, 1H); $^{13}$C NMR (DMSO-d$_6$; 100 °C) $\delta$ 18.8, 19.9, 40.7, 114.6, 119.3, 122.2, 128.1, 128.7, 137.0, 137.1, 137.9, 138.6, 149.8, 151.4, 153.1, 155.2; HRMS (ESI-FT-ICR) calculated for C$_{19}$H$_{17}$N$_7$O (M + H)$^+$ 402.2037, found 402.2027; calculated for 2(C$_{19}$H$_{17}$N$_7$O) (2M + H)$^+$ 803.4001, found 803.4088.
6-Amino-N-7-(2,4,6-trimethylbenzyl)-2-N-(4-phenylamino)ureidopurine (1a'). Starting material 5a' (0.052 g, 0.22 mmol) was dried under vacuum in a 50 mL two-necked round-bottomed flask fitted with a reflux condenser. CH₂Cl₂ (45 mL) was added and the mixture was heated to reflux until 5a' was dissolved. The temperature was reduced to 40 °C. Without equilibration of the temperature, pyridine (0.035 mL, 0.44 mmol) was added followed by phenyl isocyanate (0.024 mL, 0.22 mmol). The mixture was stirred 24 h. The solvent was removed under vacuum and the residue was purified by column chromatography on silica gel (2% CH₃OH/CH₂Cl₂) to yield 1a' (0.064 g, 72%). Mp 243–246 °C; ¹H NMR (CDCl₃) δ 2.27 (s, 3H), 2.32 (s, 3H), 4.94 (s, 2H), 5.17 (s, 2H), 6.95 (s, 2H), 7.13 (t, J = 7.1 Hz, 1H), 7.35 (m, 2H), 7.36 (s, 1H), 7.59 (d, J = 7.5 Hz, 2H), 7.81 (s, 1H), 11.52 (s, 1H); ¹H NMR (DMSO-d₆) δ 2.23 (s, 3H), 2.26 (s, 6H), 5.13 (s, 2H), 6.83 (s, 2H), 6.92 (s, 1H), 7.05 (t, J = 7.0 Hz, 1H), 7.32 (t, J = 7.3 Hz, 2H), 7.38 (s, 1H), 7.75 (d, J = 7.7 Hz, 2H), 9.35 (s, 1H), 11.81 (s, 1H); ¹³C NMR (DMSO-d₆) δ 19.4, 20.6, 113.1, 119.9, 123.0, 128.6, 128.7, 129.1, 137.4, 137.5, 138.5, 138.9, 150.0, 151.2, 153.1, 158.6.
2-Amino-6-chloro-N-9-(3,5-bis-heptyloxybenzyl)purine (4b). 6-Chloro-2-aminopurine \(^{76}\) 3 (0.175 g, 0.931 mmol), 2-bromomethyl-3,5-bis-heptyloxybenzene \(^{78,79}\) (0.464 g, 1.16 mmol), and K\(_2\)CO\(_3\) (0.322 g, 2.33 mmol) were placed in an oven-dried round-bottomed flask and dried under argon. Dry DMF (50 mL) was added to the solid mixture and the mixture was stirred overnight at room temperature. The solvent was removed under vacuum and the residue was purified by column chromatography on silica gel (5% MeOH/CH\(_2\)Cl\(_2\)) to yield a yellow/white powder (0.386 g, 85%). Mp 110–111 °C; \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 0.86 (t, \(J = 6.6\) Hz, 6H), 1.35 (m, 16H), 1.72 (m, 4H), 3.87 (t, \(J = 3.9\) Hz, 4H), 5.14 (s, 2H), 5.20 (s, 2H), 6.34 (m, 3H), 7.74 (s, 1H); \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) 0.85 (t, \(J = 6.6\) Hz, 6H), 1.32 (m, 16H), 1.64 (m, 4H), 3.88 (t, \(J = 3.9\) Hz, 4H), 5.17 (s, 2H), 6.38 (s, 3H), 6.96 (s, 2H), 8.21 (s, 1H); \(^13\)C NMR (DMSO-\(d_6\)) \(\delta\) 13.9, 22.0, 25.4, 28.4, 28.6, 31.2, 38.7, 46.1, 67.4, 99.8, 105.7, 123.2, 138.7, 143.15, 149.43, 154.0, 159.9, 160.0; HRMS (ESI-FT-ICR) calculated for C\(_{26}\)H\(_{40}\)N\(_6\)O\(_2\) (M + H\(^+\)) 488.2787, found 488.2790.

![Chemical Structure](image)

2,6-Diamino-N-9-(3,5-bis-heptyloxybenzyl)purine (5b). Vacuum dried starting material 4b (0.323 g, 0.717 mmol) was placed in a 100 mL pressure tube. Methanolic ammonia (60 mL, 7 N) was added to the solid followed by heating under pressure to 90 °C for 19 h. The solvent was removed under vacuum and the residue was purified by column chromatography on silica gel (5% CH\(_3\)OH/CH\(_2\)Cl\(_2\)) to yield 5b (0.231 g, 69%) as a white powder. Mp 151–152 °C; \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 0.85 (t, \(J = 6.6\) Hz, 6H), 1.34 (m, 16H), 1.70 (m, 4H), 3.84 (t, \(J = 3.9\) Hz, 4H), 4.88...
(bs, 2H), 5.067 (s, 2H), 5.68 (bs, 2H), 6.33 (m, 3H), 7.44 (s, 1H); $^1$H NMR (DMSO-$d_6$) $\delta$ 0.85 (t, $J = 6.6$ Hz, 6H), 1.30 (m, 16H), 1.64 (m, 4H), 3.87 (t, $J = 3.9$ Hz, 4H), 5.06 (s, 2H), 6.34 (s, 2H), 6.66 (s, 2H), 7.75 (s, 1H); $^{13}$C NMR (CDCl$_3$) $\delta$ 14.3, 22.8, 26.2, 29.2, 29.4, 32.0, 46.9, 68.3, 100.8, 106.3, 114.4, 138.2, 152.4, 156.1, 160.2, 160.9; HRMS (ESI-FT-ICR) calculated for C$_{26}$H$_{40}$N$_6$O$_2$ (M + H)$^+$ 469.3286, found 469.3285.

![chemical structure](image)

**6-Amino-N-9-(3,5-bis-heptyloxybenzyl)-2-N-(4-phenylamino)ureidopurine (1b).**

Compound 5b (0.055 g, 0.12 mmol) was placed in an oven-dried round-bottomed flask and dried under vacuum. Methylene chloride (6 mL), pyridine (0.012 mL, 0.015 mmol), and phenyl isocyanate (0.017 mL, 0.015 mmol) were added sequentially. The mixture was stirred at room temperature for 19 h. Solvent was removed under vacuum. Purification of the residue was performed by column chromatography on silica gel (1% MeOH/CH$_2$Cl$_2$) to afford 1b (0.050 g, 73%). Mp 217–220 °C; $^1$H NMR (CDCl$_3$) 0.86 (t, $J = 6.6$ Hz, 6H), 1.26 (m, 16H), 1.69 (m, 4H), 3.80 (t, $J = 3.9$ Hz, 4H), 5.23 (s, 2H), 6.33 (m, 1H), 6.37 (m, 2H), 7.05 (t, $J = 7.0$ Hz, 1H), 7.27 (t, $J = 7.3$ Hz, 3H), 7.37 (d, $J = 7.4$ Hz 3H), 7.65 (s, 1H), 9.41 (s, 1H), 11.84 (s, 1H); $^1$H NMR (DMSO-$d_6$) $\delta$ 0.83 (t, $J = 6.6$ Hz, 6H), 1.22 (m, 16H), 1.59 (m, 4H), 3.82 (t, $J = 3.8$ Hz, 4H), 5.25 (s, 2H), 6.35 (s, 1H), 6.41 (s, 2H), 7.00 (t, $J = 7.0$ Hz, 1H), 7.26 (t, $J = 7.2$ Hz, 2H), 7.58 (d, $J = 7.6$ Hz, 2H), 7.61 (s, 1H), 8.11 (s, 1H), 9.30 (s, 1H), 11.75 (s, 1H); $^{13}$C NMR (DMSO-$d_6$; 100 °C) $\delta$ 13.3, 21.5, 25.0, 27.9, 28.2, 28.5, 30.7, 46.0, 67.4, 100.4, 105.7, 114.8, 119.2, 122.2, 128.2, 138.7, 139.7, 149.5, 151.4, 153.4, 155.5, 159.9; HRMS (ESI-FT-ICR) calculated for C$_{35}$H$_{45}$N$_7$O$_3$
(M + H)$^+$ 588.3657, found 588.3678; calculated for 2(C$_{33}$H$_{45}$N$_{7}$O$_{3}$) (2M + H)$^+$ 1175.7241, found 1175.7484.

![Chemical structure of N-9-Benzyl-6-dimethylamino-2-N-(4-phenylamino)ureidopurine (2).](image)

**N-9-Benzyl-6-dimethylamino-2-N-(4-phenylamino)ureidopurine (2).** Phenyl isocyanate (0.65 mL, 6.0 mmol) was added dropwise to 2-amino-6-dimethylamino-9-benzyl purine (0.10 g, 0.37 mmol) dissolved in dry pyridine (7.5 mL). After stirring at room temperature for 1 h the crude reaction mixture was concentrated under reduced pressure and the crude solid was recrystallized with ethanol to give a white solid (0.137 g, 94%). Mp 234–236 °C; $^1$H NMR (CDCl$_3$) $\delta$ 3.56 (bs, 6H), 5.31 (s, 2H), 7.16 (s, 1H), 7.33 (m, 10H), 7.61 (s, 1H) 11.39 (s, 1H); $^1$H NMR (DMSO-$d_6$) $\delta$ 3.62 (bs, 6H), 5.40 (s, 2H), 7.28 (m, 10H), 8.13 (s, 1H), 9.41 (1.37, 1H), 11.45 (s, 1H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 46.1, 60.0, 115.46, 119.17, 122.7, 126.8, 126.9, 127.6, 128.7, 128.8, 136.8, 138.7, 138.9, 151.8, 153.0, 154.1. HRMS (ESI-FT-ICR) calculated for C$_{21}$H$_{22}$N$_{7}$O (M + H)$^+$ 388.1886, found 388.1921.

**Computational details**

Monte Carlo conformational searching was done on a Dell PC (2.4 GHz) running the Fedora Core using MacroModel v. 9.0 (Schrodinger, LLC)$^{86}$ and the MCMM method (relevant parameters include: steps = 100, iterations = 2000, solvent (GB/SA) = CHCl$_3$, force field = Amber*). Only 1$^{N3}$ and 1$^{N1}$ were further refined using ab initio methods (using Gaussian 03 (revision D.01)$^{87}$) as implemented through the National Center for Supercomputing Applications, SGI Altix cluster “Cobalt” (http://www.ncsa.uiuc.edu/UserInfo/Resources/Hardware/SGIALtix/).
NMR Experimental parameters

General. NMR spectra were recorded at 25 °C on a Varian Inova spectrometer equipped with a 5 mm indirect detection probe, operating at 500 MHz for $^1$H and at 125 MHz for $^{13}$C. Chemical shifts are reported in ppm relative to TMS.

gHMBC. The gHMBC spectrum was recorded with the standard pulse sequence in vnmr, in 512 increments, each acquired in 16 transients. The number of points in the FIDs was 4k, and the same number was used for the spectrum in f2. The number of points for the spectrum in f1 was 2k. The preacquisition delay was 0.5 s. The 8 Hz spectrum was taken in CDCl$_3$ at an approximate concentration of 2 mM. All shifts are reported in ppm downfield of TMS, and $^{13}$C shifts are listed in bold.

NOESY. The NOESY spectrum was recorded at 25 °C with the standard pulse sequence in vnmr, in 2k increments, each acquired in 32 transients. The number of points in the FIDs was 4k, and the same number was used for the spectrum, in both f1 and f2. The preacquisition delay was 1 s and the mixing time 0.5 s. Spectrum was obtained in CDCl$_3$ at an approximate concentration of 2 mM.

VT-NMR. The variable temperature spectrum was recorded on a sample in CDCl$_3$ (~ 4 mM), on automation, arraying the temperature from −55 °C to 55 °C in steps of 10 °C. For each change in temperature, a delay of 300 s allowed for the temperature equilibration, followed by shimming z1–z2 on the lock level, then acquisition in 128 transients with an acquisition time of 5 s.
CHAPTER 3
STRUCTURE-PROPERTY RELATIONSHIPS IN UDAP DERIVATIVES

Introduction

Meijer and coworkers have developed self-complementary DADA quadruple hydrogen bonding (QHB) motifs from both triazines (A, figure 3-1) and pyrimidines (B, figure 3-1). The ureidotriazine unit (A) enjoys a $K_{\text{dim}}$ of $2 \times 10^4 \text{ M}^{-1}$ in CDCl$_3$; the pyrimidine unit B dimerizes even more efficiently, with a $K_a$ on the order of $10^5 \text{ M}^{-1}$ in CDCl$_3$. The increase in dimerization strength from A to B is apparently realized through a) the increased basicity of the pyrimidine nitrogen (diaminotriazine $pK_a \sim 2$, diaminopyrimidine $pK_a \sim 7$) and b) acylation of the 6-amino group that increases the acidity of the pyrimidine H-bond donor. Given the structural similarity of A and B to 1a and 1b, it is initially surprising that the UDAP systems dimerize at least an order of magnitude more weakly. To wit, the basicity of the nitrogen of interest for UDAP dimerization, N1, has a $pK_a$ (~ 5; diaminopurine) that falls between the values for triazine and pyrimidine. The goal of this chapter is to draw structure-property relationships within the UDAP systems that, once identified, will rationalize $K_{\text{dim}}$ and potentially suggest ways that it can be tuned.

Figure 3-1. Ureidotriazine (A; $K_{\text{dim}} = 2 \times 10^4 \text{ M}^{-1}$) and ureidopyrimidine (B; $K_{\text{dim}} = 2 \times 10^5 \text{ M}^{-1}$) of Meijer and coworkers form highly stable DADA QHB dimers.
Three new targets (7–9) were conceived to draw structure-property relationships in the UDAP platform (figure 3-2); each is specifically designed to probe how the structure of the urea and/or N9 substituent might alter conformational preferences, solubility, and ultimately $K_{\text{dim}}$. Exploring substituent effects more broadly should reveal a) the tolerance of the system to functionality in these positions that could potentially be used to covalently connect the UDAP group to a polymeric backbone or another UDAP unit and b) strategies to tune $K_{\text{dim}}$ by changes that are remote from the hydrogen bonding interface. The monocyclic ureidotriazines and pyrimidines, shown in figure 3-1, are not easily amenable to the latter studies.

![Figure 3-2. UDAP derivatives designed to probe structure-property relationships.](image)

An obvious difference between UDAPs 1a/1b and compounds A and B is the class of urea substituent. Alkyl ureas are typically used by Meijer, Zimmerman, and others for solubility reasons and synthetic feasibility. The poor reactivity of alkyl isocyanates with DAP precluded their use in the first UDAP molecules (Chapter 2). If suitable synthetic methods could be developed to prepare a compound like 7, that incorporates a hexyl urea substituent, two
important features could be explored. The contribution of a more basic urea carbonyl oxygen (and commensurately less acidic H^c proton) will highlight the role of electronic effects in the dimerization. A priori, it is difficult to determine which consequence, a weakened intramolecular hydrogen bond between H^c and N3 or a potentially strengthened intermolecular hydrogen bonding interface, is more important to the overall thermodynamics of the system. Secondly, an alkyl urea might expose the influence, if any, of the aromatic interaction (between the N9 substituent and the aryl urea) suggested by the NOESY NMR data for 1a (and crystal structure of 1a) by deleting this contact.

The dependence of the dimerization properties on an interaction between the N9 substituent and the urea will be further tested by replacement of the N9 aryl group of 1a and 1b with a heptyl chain as in 8. Studies with this derivative could potentially also rationalize, in part, the lack of QHB observed for 1a' (N7 protected UDAP, Chapter 2). A final target, 9, that bears a tert-butyl acetate group at N9, will test the tolerance of UDAP dimerization to a functional group handle that has been remarkably successful in the peptide nucleic acid (PNA) field. Subsequent hydrolysis of the ester moiety and coupling to amino acids, for example, could provide a building block appropriate for chain extension (figure 3-3). The tert-butyl acetate group will also explore possible stabilization of the desired intramolecular hydrogen bonding conformation through introduction of a favorable electrostatic interaction between H^c and the ester carbonyl.

Discussed herein is how the three ureidodiaminopurine targets 7–9 have been synthesized by new methods, and studied in solution by the techniques outlined in Chapter 2. The results of the structure-property studies are then discussed with respect to K_{dim}, conformation, enthalpy and entropy effects, and secondary electrostatic interactions (e.g. Chapter 1) to leverage future optimization and application of these systems.
Figure 3-3. Ester hydrolysis of 9 and subsequent coupling with an amino acid residue for future application in a PNA.

Structure Property Relationships

Synthesis of 7, 8, and 9

The synthesis of UDAPs 1a and 1b was described in Chapter 2. The appropriate diaminopurine (DAP) was first prepared from N9 protected 2-amino-6-chloropurine, and the phenyl urea was subsequently formed regioselectively at N_{C2} by reaction with phenyl isocyanate to give 1a and 1b in fair yields. Preferential reactivity of acylating agents at N_{C2} of DAP has been demonstrated in the literature and is further discussed in depth in Chapter 4.^{95} It was observed, however, that alkyl isocyanates did not react readily at N_{C2} under the same, or even harsher conditions. The overall reactivity differences between aryl and alkyl isocyanates toward DAP can likely be rationalized on the basis of transition state stabilization and electronic effects.

Figure 3-4 shows the relative stabilities of the transition states. The reactivity difference originates from electronic differences between alkyl and aryl isocyanates and the respective intermediates. The phenyl isocyanate transition state is lower in energy due to resonance stabilization by the phenyl group in the reaction intermediate. Therefore the reaction is faster due to its lowered activation barrier. This interesting observation spurred an investigation into the nucleophilic reactivity difference of the amino group of 2,6-diaminopurine, and is the subject of
Chapter 4. It follows that formation of the relevant $\text{NC}_2$ hexylureidopurines 7, 8, and 9 was achieved by exploring other strategies to obtain hexyl ureidodiaminopurines.

Figure 3-4. The reaction of phenyl isocyanate with DAP is faster than reaction of hexyl isocyanate due to the lowered energy of the transition state.

To avoid the N7/N9 regioselectivity complications observed in the alkylation of 3 with benzylic bromides to form 4a and 4b (Chapter 2), a modified procedure was used for the synthesis of the heptyl (4c) and ester (4d) protected purines (figure 3-5). Conditions were used reported by Brik and coworkers for the synthesis of N9 alkylated purines under conditions mild enough for combinatorial reactions, where deprotonation and alkylation of N9 was afforded in minutes by TBAF. Similarly, 4c and 4d were both afforded in 75 % yield in about ten minutes (figure 3-5). Upon alkylation of 4c it was converted to DAP 5c by amination. Synthesis of 5d
was not amenable to the harsh conditions required for amination and was thus synthesized by direct alkylation of DAP.

![Synthesis diagram](image)

Figure 3-5. Synthesis of $4c$ and $4d$ using TBAF as base yields predominantly the N9 isomer in ten minutes. Amination of $4c$ yields $5c$, and $5d$ is prepared by alkylation of N9 directly.

The synthesis of UG by Zimmerman and coworkers was achieved by deprotonation of guanine with sodium hydride. Along this vein, deprotonation of DAP by a strong base was investigated. In accordance with the apparent increased nucleophilicity of N$_{C2}$, initial deprotonation occurred at N$_{C6}$ giving an N6-hexylureidopurine with $n$-BuLi or NaH (figure 3-6, experimental data presented in Chapter 4).

An alternative approach to access N$_{C2}$ alkyl urea derivatives involves reaction at N$_{C2}$ before introduction of the competitive amino group at C6, in other words, deprotonation of suitably N9 functionalized 2-amino-6-chloropurines $4a$–$d$. Formation of the 6-chloro-2-N-hexyl ureidopurines 7 and 8 was realized through deprotonation of 2-amino-6-chloropurine derivatives $6a$ and $6c$ by $n$-BuLi followed by reaction of the anion with hexyl isocyanate (figure 3-7).
Sodium hydride and \( n \)-BuLi reactions were performed concurrently, and \( n \)-BuLi was the preferred method for convenience. Subsequent formation of UDAP was achieved by displacement of the chloro group with ammonia (in methanol).

![Chemical structure](image)

\[
\text{R} = 5a; \text{2,4,6-trimethylbenzyl (} n \text{-BuLi; 89 \%, 10)}, \\
5c; \text{heptyl (} n \text{-BuLi; 97 \%, NaH; 96 \%, 11)}, \text{ or} \\
5d; \text{tert-butyl acetate, (} n \text{-BuLi; 46 \%, NaH; 83 \%, 12)}
\]

**Figure 3-6.** Deprotonation of DAP with \( n \)-BuLi or NaH and reaction of the anion with hexyl isocyanate gives 6-N-hexylureidopurine (for general procedures see Chapter 3 experimental).

![Chemical structure](image)

**Figure 3-7.** The synthesis of UDAP 7 and 8 by deprotonation, substitution with hexyl isocyanate, and displacement of the 6-chloro group

Formation of 7 and 8 were low-yielding reactions, 46 \% and 34 \%, respectively.

Interestingly, the reason for the low yields was the apparent displacement of the chloro substituent by methoxide formed in situ, the product of which was isolated in 34 \% for 13 and 56 \% for 14. The formation of the methoxy substituted products was initially overlooked, and was realized in attempts to synthesize heptyl protected N9 phenylureidopurines.
The presence of the N9 ester substituent prompted use of a different route for formation of 9. The pKₐ of the α-proton of an ester is ~ 25 while the pKₐ of the 2-amino group of 2-amino-6-chloropurine 4d is likely ~ 30 (value for Ph–NH₂). Not surprisingly, competitive deprotonation of the ester was observed when n-BuLi was used. Selective deprotonation of the more accessible amino group was afforded through the use of a bulkier base, LTMP, at low temperature. Treatment of this intermediate with hexyl isocyanate provided the isocyanate product 6d in respectable yield (69%, figure 3-8). Installation of the amino functionality at C6 was then achieved via a two-step procedure; displacement of the chloro group by sodium azide (to avoid disruption of the t-butyl ester) followed by catalytic transfer hydrogenation using ammonium formate as the hydrogen source.

Figure 3-8. The synthesis of 9 was achieved through deprotonation of 4d with LTMP followed by substitution with hexyl isocyanate to form 6d. The 6-chloro group was substituted by sodium azide and the azide subsequently reduced by ammonium formate.

**Properties of UDAP in Solution**

The solution-phase properties of 7, 8, and 9 were all investigated analogously to 1a and 1b (Chapter 2). Conformational considerations were addressed by NOESY NMR, while modes of dimerization were investigated by VT NMR. Likewise, solution-phase dimerization strength was quantified by monitoring the chemical shift of Hb upon sequential dilution in CDCl₃.

Ureidodiaminopurines 7–9 all populate the desired N3 hydrogen-bonded conformation in solution at mM concentrations in CDCl₃ at 25 °C based on NOESY NMR. The key NOEs
observed in each case are similar to \textbf{1a} and \textbf{1b}, between the urea H^c proton and the methylene protons at the N9 position. For \textbf{7}, this conformation was even found to be stable up to 75 °C in DMSO (a strong hydrogen-bond competitor). NOE contacts that would reflect the N1 conformation (e.g., between H^c and the 6-amino protons) have not been observed in any case. Even so, this conformation is presumably accessible in solution.

The trends identified for \textbf{7–9} by variable temperature NMR spectroscopy are similar to those found for \textbf{1a} and \textbf{1b}. Differences do arise in chemical shift as a consequence of conversion of the aryl urea to an alkyl urea. H^c, since less acidic, is comparatively upfield in \textbf{7–9} (e.g. δ at 25 °C and 2–4 mM: \textbf{1a}, 12.4; \textbf{7}, 11.8; \textbf{8}, 9.6; \textbf{9}, 9.4); this proton is also split into a triplet (\(^3J = 2.5\) Hz) from the neighboring methylene group making its assignment (versus H^b) trivial. A representative VT experiment is shown for \textbf{8} in Figure 3-9. At −55 °C, H^a appears at δ ~ 9.5, and H^a' δ ~ 5.9. The H^a resonance moves upfield with increasing temperature (as it responds to the dimer concentration) while H^a' remains stationary (again, good evidence for negligible Hoogsteen participation under these conditions). The amino protons H^a/a' then broaden into the baseline at ~ 0 °C and remain in an intermediate exchange regime through 55 °C; at this temperature a broad singlet is observed at ~ δ 7.1 (Figure 3-8, topmost spectrum). For \textbf{8}, H^a/a' appear as a broad peak at ~ δ 6.7 at 35 °C (similar to \textbf{1a}) and sharpens to a singlet at 55 °C (~ δ 6.3; not shown). Finally, in all cases, H^b moves upfield upon increasing the temperature (for \textbf{8} at 33 mM, from ~ δ 10 to 9.4).
Figure 3-9. \(^1\)H VT-NMR of compound 8 is similar to that of the other structures with the exception for coalescence of H\(^a\) and H\(^a'\) (bottom). Coalescence begins to appear at 55 °C (top). The top spectra shows a spectrum taken at 55 °C, it is unprocessed for visualization of coalescence of H\(^a,a'\) (CDCl\(_3\), ~ 33 mM, 300 MHz).

Solution-phase dimerization of the newly synthesized UDAP derivatives 7–9 were investigated by sequential dilution (a technique discussed in Chapter 2). Dimerization constants, \(K_{\text{dim}}\), were calculated based on experimentally determined \(\delta_D\) and \(\delta_M\) for H\(^b\) (table 3-1); the former was taken under conditions of complete dimerization (at high concentration (~ 3 mM) and low temperature (~ 55 °C) in (CD\(_2\)Cl\(_2\))\(_2\)) and the latter under conditions of dissociation (at lower concentration (~ 1.5 mM) and high temperature (120 °C) in (CD\(_2\)Cl\(_2\))\(_2\)). We and others\(^{82}\) have found (CD\(_2\)Cl\(_2\))\(_2\) to be a useful solvent analogue of CDCl\(_3\) in these limiting chemical shift determination experiments. The values determined for \(K_{\text{dim}}\) (and \(\Delta G^\circ\)) are similar within the estimated error in spite of the significant monomer structural modifications, with the exception of 9 that is noticeably weakened. At first glance, it appears that any introduced stabilizing and destabilizing interactions in the alkyl urea series (versus the aryl series) have been offset; signs of compensatory effects often observed in cooperative and reversible assembly. Rationalizing the
similar results and relative stabilities of 1a–b, 7, 8, and 9 is the emphasis of the remainder of this Chapter.

Table 3-1. Dimerization constants and free energy of formation data for 1a, 1b, and 7–9

<table>
<thead>
<tr>
<th>UDAP</th>
<th>1a</th>
<th>1b</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{\text{dim}}$ (M$^{-1}$)</td>
<td>820 ± 160</td>
<td>980 ± 290</td>
<td>1100 ± 250</td>
<td>820 ± 20</td>
<td>530 ± 40</td>
</tr>
<tr>
<td>$\Delta G^\circ$ (kcal/mol)</td>
<td>−4.0 ± 0.8</td>
<td>−4.1 ± 1.2</td>
<td>−4.1 ± 0.9</td>
<td>−4.0 ± 0.1</td>
<td>−3.7 ± 0.1</td>
</tr>
<tr>
<td>dilution range (% dimer)</td>
<td>20–69</td>
<td>23–74</td>
<td>22–75</td>
<td>63–83</td>
<td>27–65</td>
</tr>
<tr>
<td>$\delta_D$ (H$^b$)$_b$</td>
<td>10.23</td>
<td>10.23</td>
<td>9.89</td>
<td>9.97</td>
<td>9.91</td>
</tr>
<tr>
<td>$\delta_M$ (H$^b$)$_c$</td>
<td>7.22</td>
<td>7.22</td>
<td>7.04</td>
<td>7.23</td>
<td>6.98</td>
</tr>
<tr>
<td>$\delta$ (H$^c$)$_d$</td>
<td>12.04</td>
<td>11.82</td>
<td>9.58</td>
<td>9.69</td>
<td>9.38</td>
</tr>
</tbody>
</table>

$^a$ error calculated as an average deviation for four independently calculated $K_{\text{dim}}$ values $\Delta x_k = \frac{\sum |x_i - \bar{x}|}{N}$;  $^b$ Fully dimerized chemical shifts of H$^b$ protons, determined by cooling samples (~3 mM) to −55 °C in (CD$_2$Cl)$_2$/300 MHz.  $^c$ Chemical shifts of fully dissociated H$^b$ determined by heating diluted samples (~1.5 mM) to 120 °C in (CD$_2$Cl)$_2$/300 MHz.  $^d$ Chemical shift of H$^c$ determined at 25 °C in CDCl$_3$ at 500 MHz (~4 mM for 1a, 1b, 7, 9, and ~40 mM for 8).

Interactions between N9 and the Urea

Any enthalpically favorable interaction between the N9 substituent and the urea substituent might serve to stabilize the conformation required for dimerization, reduce the entropic penalty associated with assembly (preorganization), and result in a higher value of $K_{\text{dim}}$. During the design of the UDAP systems, interactions between the urea (e.g., phenyl) substituent and N9 (e.g., benzyl) groups were considered given that a potentially favorable aromatic interaction was identified for model compound 2 in the solid state. The solution-phase contribution of such an interaction in chloroform is of course much smaller. Hunter and coworkers have used chemical double mutant cycles and model systems to quantify these contacts between substituted phenyl groups.$^{98-100}$ Interaction energies ($\Delta G$) in chloroform can be as high as $−1.4 \pm 0.5$ kJ mol$^{-1}$ at 25 °C ($−0.33$ kcal mol$^{-1}$) for a benzoyl-diisopropyl aniline interacting with a tert-butyl aniline, but
typically range from destabilizing (0.29 kcal mol\(^{-1}\)) to stabilizing (−1.1 kcal mol\(^{-1}\)) depending on appropriate choice of aromatic ring substituents.

Comparison of \(1a\) and \(1b\) provides the starting point for the discussion where \(\Delta K_{\text{dim}} = 150\) M\(^{-1}\) (in favor of \(1b\)), a value that admittedly falls within the error bars. The ortho methyl (\(1a\)) substituents of the N9 protecting groups donate electron density inductively to the aromatic protecting group; similarly the alkoxy (\(1b\)) substituents donate by resonance.\(^98\) Both substituents are appropriate for edge-to-face aromatic interactions with the electron-deficient edge of the urea phenyl group. The conformations of \(1a\) and \(1b\) were explored by computational means using Monte Carlo methods (MacroModel, Amber* force field, CHCl\(_3\) solvation model) to see if edge-to-face interactions are geometrically favored, or even accessible. For these calculations the urea conformation was locked in the appropriate conformation for dimer formation. The lowest energy structures are represented in figure 3-10. The N9 substituent of compound \(1a\) appears to be restricted to an anti conformation. Edge-to-face interactions are likely prevented by the inability of the substituent to rotate without disrupting intramolecular H-bonding by ortho methyl substituents. The lack of ortho substituents and presence of meta substituents for \(1b\) (versus \(1a\)) does offer opportunity for some additional stabilization (long alkyl sitting under urea phenyl), but again this is difficult to quantify. However, comparison by \(^1\)H NMR indicates that \(1a\) populates the anti conformation more frequently than \(1b\). This is seen in the shielding of the C8 proton. The C8-H proton for \(1a\) appears at δ 7.58 ppm and appears at δ 7.65 ppm for \(1b\) in CDCl\(_3\) ~ 4 mM at 25 °C. This is not a significant difference, but does indicate the increased flexibility of a substituent with meta rather than ortho substitution.

The incorporation of a hexyl urea substituent in 7–9 deletes the aforementioned aryl interactions. That the \(K_{\text{dim}}\) of 7 is essentially the same as \(1a\) and \(1b\) begins to suggest that the
N9/urea interaction plays a secondary role (and that the large neighboring groups in 1a and 1b are not compromising the dimerization). This is further confirmed by the fact that the $K_{\text{dim}}$ for 9 ($530 \pm 40 \text{ M}^{-1}$), that bears two alkyl substituents, is equivalent to that of 1a ($K_{\text{dim}} = 820 \pm 160 \text{ M}^{-1}$).

Figure 3-10. Computational studies to probe possible edge-to-face aromatic interactions indicate that the N9 substituent for 1a occupies an anti conformation. The energy minimized structure of compound 1b shows that ortho substitution does offer some stabilization.

A comparison of 7 and 8 reveals shielding of the C8-H proton of 7, consistent with the preferred anti conformation of the mesitylene substituent (figure 3-11). Also identified is the similar chemical shift for $H^b$ at similar concentrations of 7 and 8; this of course suggests that the stability of the dimers of the two species is nearly similar, a fact borne out in their calculated $K_{\text{dim}}$ (Table 3-1). Finally, the values of $H^c$, which are also similar, show that the N9 substituent does not significantly perturb the intramolecular hydrogen bond.

Figure 3-11. Proton NMR of 7 (top) and 8 (bottom) show similar chemical shifts for $H^c$ ($\delta 9.59, \delta 9.60$) and $H^b$ ($\delta 9.07, \delta 9.29$) at concentrations of 4.53 and 4.55 mM, respectively. The imidazole proton, C8, is deshielded in 8 ($\delta 7.54$) in comparison to 7 ($\delta 7.11$), presumably due to the aromatic N9 substituent in the latter.
Electronics Effects

An alkyl urea substituent was introduced to explore electronic in addition to steric effects; consequences on the acidity of H^c (and intramolecular hydrogen bonding) and the basicity of the urea carbonyl oxygen (intermolecular hydrogen bonding). Indeed the acidity of H^c was decreased, as evidenced by an upfield chemical shift by ^1H NMR (figure 3-12; 1a; δ 12.01, 7; δ 9.60, table 3-1). From the values given in table 3-1, the desired effect on K_{dim} was not realized. This is consistent with the chemical shift of H^b remaining at similar chemical shifts at similar concentrations (~ 4 mM) for 1a and 7 (1a; δ 9.32, 7; δ 9.17; figure 3-13). In addition, the ^13C chemical shifts of the urea carbonyl groups are also similar for 1a and 7; δ 155.2, δ 155.3, respectively (experimental section). The consistency in the K_{dim} values is probably partially explained in terms of cooperative and offsetting electronic effects within the urea group and pyrimidine ring. Also, a potentially increased carbonyl basicity for 7 may be met with increased repulsive secondary interactions in the model of Jorgensen (Chapter 1).

![Figure 3-12. Proton NMR illustrates decreased acidity of H^c when the urea substituent is changed from aryl to alkyl, but the chemical shift of H^b remains approximately the same (CHCl3, 25 °C, 7 = 4.0 mM; 1a = 4.4 mM).](image)

Compound 9, which was synthesized with a tert-butyl acetate group at N9, saw the most noticeable change in dimerization constant (nearly a 50 % reduction in K_{dim}). In considering the ester substituent for N9, the possibility of a bifurcated hydrogen bond to H^c of the urea from the ester carbonyl and N3 appeared enthalpically appealing (illustrated in figure 3-13). Similar
motifs from the literature\textsuperscript{101} are shown that are, in general, stabilizing (indicated be the downfield shift of the N–H proton, up to ~ 0.5 ppm). If such a hydrogen bond was present in \textit{9}, by \textsuperscript{1}H NMR one may expect H\textsubscript{c} of \textit{9} to be more deshielded than H\textsubscript{c} of \textit{7} at similar concentration of dimer. This, however, is not the case as shown in figure 3-14 (H\textsubscript{c} = 9.37; \textit{8}, H\textsubscript{c} = 9.39; \textit{7}).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure.png}
\caption{Compound \textit{9} arranged for bifurcated hydrogen bonding of H\textsubscript{c} to N3 and the ester carbonyl and changes in chemical shift associated with the addition of a bifurcated hydrogen bond (CDCl\textsubscript{3}).\textsuperscript{101}}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{nmr.png}
\caption{Proton NMR of \textit{9} (bottom, 3.06 mM, 57 % dimer) and \textit{7} (top, 1.68 mM, 54 % dimer) shows no additional deshielding of H\textsubscript{c} (CDCl\textsubscript{3}, 25 °C, 500 MHz).}
\end{figure}

Molecular modeling again suggests reasons why this is the case. Simply put, unfavorable steric interactions between the bulky \textit{t}-butyl group and urea substituent preclude formation of the potentially stabilizing hydrogen bond. The reduced relative $K_{\text{dimer}}$ then additionally suggests that steric interactions involving the urea destabilize the conformation required for dimerization. Possible remedies include synthesis of a smaller methyl or ethyl ester, or perhaps even better, conversion of the group to an amide that would introduce a more basic carbonyl to the region.
Solubility

For the ureidopurines to be easily processed, they should be readily soluble. Ureidopurines 1a, 1b, and 7 were near saturation at 4.7–4.9 mM in deuterated chloroform at room temperature. That value equates to 0.5 g of 1a requiring almost 250 mL of chloroform for solvation. Compounds 8 and 9 were synthesized with a remedy in mind. It was envisioned that the ester at N9 on compound 9 would serve as a point for future functionalization and increased solubility. Solubility was expected to increase with a non-aromatic protecting group due to a decreased possibility of aggregation through π surfaces. Interestingly, the solubility was not significantly increased, with a saturated solution being obtained at ~ 4.5 mM in CDCl₃. It was apparently the alkyl chains for 8 which offered superior solubility relative to the other UDAP derivatives, affording a 38 mM solution in CDCl₃ (saturated solution at room temperature). Therefore, future synthetic designs might include an ester group with a longer methylene linker extending from N9.

Summary

Structure-property relationships were examined for five UDAP whose structural modifications were motivated by enhancing organic solubility, $K_{\text{dim}}$, and an understanding of the properties that control these phenomena. The urea and N9 substituents were varied to probe interactions between them to affect a change in $K_{\text{dim}}$ and $\Delta G^\circ$. Dimer stabilities were similar (within the error limits) with the exception of 7c, and appeared to be governed by cooperative interactions. Weakening of the dimer stability of 7c is the apparent result of steric interactions of the urea and bulky ester $t$-butyl group. The UDAP design is capable of accommodating bulky aromatic substituents on the urea and N9 positions without compromising the $K_{\text{dim}}$, an important observation when considering connection of the subunits to covalent scaffolds.
When comparing the UDAP systems to those of Meijer and coworkers, it appears that the differences in $K_{\text{dim}}$ largely derive from the differing electronic properties of the heterocyclic scaffolds. These subtleties will be explored in future UDAP designs.

**Experimental Methods**

![Chemical Structure](image)

6-Chloro-N-9-(2,4,6-trimethylbenzyl)-2-N-(4-hexylamino)ureidopurine (6a).

Compound 4a 6-chloro-9-(2,4,6-trimethylbenzyl)-2-aminopurine (0.225 g, 0.746 mmol) was placed in an oven dried two-necked round bottom flask and dried under vacuum. Tetrahydrofuran (20 mL) was added and the mixture cooled to $-78 \, ^\circ\text{C}$ under argon. $n$-BuLi (0.358 mL, 2.5 M in hexanes, 0.895 mmol) was added dropwise and the mixture stirred for 10 min. Hexyl isocyanate (0.095 mL, 0.895 mmol) was added dropwise and the mixture stirred at $-78 \, ^\circ\text{C}$ for 1.75 h. The reaction mixture was stirred an additional 10 min without cooling followed by quenching with saturated aqueous ammonium chloride. The product mixture was extracted with ethyl acetate and the solvent evaporated. Purification by column chromatography (5% CH$_3$OH/CH$_2$Cl$_2$) afforded 6a (0.230 g, 72 %). $^1$H NMR (CDCl$_3$) $\delta$ 0.86 (m, 3H), 1.30 (m, 4H), 1.63 (m, 2H), 2.24 (s, 6H), 2.30 (s, 3H), 3.40 (q, $J = 3.2$ Hz, 2H), 5.21 (s, 2H), 6.95 (s, 2H), 7.42 (s, 1H), 7.46 (s, 1H), 8.74 (t, $J = 2.9$ Hz, 1H). $^1$H NMR (DMSO-$d_6$) (50 °C) $\delta$ 0.083 (m, 3H), 1.28 (m, 8H), 2.24 (s, 9H), 2.49 (m, 2H), 3.24 (q, $J = 3.1$ Hz, 2H), 5.33 (s, 2H), 6.93 (s, 2H), 7.90 (s, 1H), 8.41 (t, $J = 2.8$ Hz, 1H), 9.68 (s, 1H). $^{13}$C NMR (DMSO-$d_6$) (50 °C) $\delta$ 13.5, 19.2, 20.3, 21.8, 25.9, 29.3, 30.8, 41.8, 125.8, 127.6, 129.1, 137.3, 137.5, 144.4, 149.3, 152.0, 153.1. HRMS (ESI-FT-ICR) calculated for C$_{22}$H$_{29}$ClN$_6$NaO (M + Na)$^+$ 451.1984 found 451.2014;
calculated for C_{44}H_{60}Cl_{2}N_{12}O_{2} (2M + H)^{+} 857.4256 found 857.4220; calculated for C_{44}H_{58}Cl_{2}N_{12}NaO_{2} (2M + Na)^{+} 879.4075 found 879.4230.

6-Amino-N-9-(2,4,6-trimethylbenzyl)-2-N-(4-hexylamino)ureidopurine (7). Compound 6a (0.129 g, 0.301 mmol) was placed in a 75 mL pressure tube. Methanolic ammonia (40 mL) was added and the mixture heated to 75–85 °C. The mixture was stirred for 2 h. Solvent was evaporated and the product purified by column chromatography (3% CH_{3}OH/CH_{2}Cl_{2}) to yield 7a (0.054 g, 46 %). Mp 239–241 °C; \(^{1}\)H NMR (CDCl_{3}) \(\delta\) 0.83 (m, 5H), 1.26 (m, 4H), 1.59 (m, 2H), 2.54 (s, 6H), 2.60 (s, 3H), 3.39 (m, 2H), 4.07 (s, 2H) 5.17 (s, 2H), 6.94 (s, 2H), 7.00 (s, 1H), 9.16 (s, 1H), 9.60 (t, \(J = 2.4\) Hz, 1H). \(^{1}\)H NMR (DMSO-d_{6}) \(\delta\) 0.85 (m, 3H), 1.26 (m, 6H), 1.55 (q, \(J = 3.4\) Hz, 2H) 2.25 (s, 9H), 3.23 (q, \(J = 3.5\) Hz, 2H), 5.19 (s, 2H), 6.92 (s, 2H), 6.96 (s, 2H), 7.33 (s, 1H), 7.95 (s, 1H), 9.07 (t, \(J = 2.2\) Hz, 1H). \(^{13}\)C NMR (DMSO-d_{6}) (100 °C) \(\delta\) 13.0, 18.7, 19.1, 19.9, 25.6, 28.3, 30.4, 31.3, 40.5, 115.5, 128.0, 128.6, 136.8, 136.9, 151.0, 153.5, 155.3, 168.2. HRMS (ESI-FT-ICR) calculated for C_{22}H_{32}N_{7}O (M + H)^{+} 410.2663 found 410.2703.

N-9-(2,4,6-Trimethylbenzyl)- 6-methoxy -2-N-(4-hexylamino) ureidopurine (13).

Product was isolated from the same reaction mixture as compound 7. The product was isolated as a light yellow solid (0.044 g, 34%). Mp 239–241 °C \(^{1}\)H NMR (CDCl_{3}) \(\delta\) 0.83 (m, 3H), 1.28 (m,
6H), 1.61 (m, 2H), 3.39 (q, J = 3.4 Hz, 2H), 4.09 (s, 3H) 5.18 (s, 2H), 6.93 (s, 2H), 7.34 (s, 1H), 7.42 (s, 1H), 8.92 (t, J = 2.1 Hz, 1H). $^{13}$C NMR (CDCl$_3$) $\delta$ 13.9, 19.6, 20.9, 22.5, 26.7, 29.6, 29.8, 31.4, 42.1, 54.2, 126.9, 129.5, 129.6, 129.7, 137.5, 139.8, 152.2, 153.3, 154.3, 161.1.

HRMS (ESI-FT-ICR) calculated for C$_{23}$H$_{33}$N$_6$O$_2$ (M + H)$^+$ 425.2660 found 425.2647; calculated for C$_{46}$H$_{64}$N$_{12}$NaO$_4$ (2M + Na)$^+$ 871.5066 found 871.5111.

6-Chloro-N-9-heptyl-2-aminopurine (4c). Compound 3, 6-chloro-2-aminopurine (0.501 g, 2.67 mmol) was placed in a 250 mL oven dried round bottomed flask and suspended in dimethylformamide (100 mL). Tetrabutyl ammonium fluoride (5.60 mL, 5.60 mmol) and iodoheptane (0.813 mL, 3.59 mmol) were added dropwise. The mixture was stirred at room temperature for 20 minutes. The product was extracted with ethyl acetate and the solvent was evaporated. The residue was recrystallized from a 8:2 ratio of ethyl acetate:hexanes to yield the product (2.30 g, 75%). $^1$H NMR (CDCl$_3$) $\delta$ 0.35 (m, 3H), 0.74 (m, 8H), 1.32 (m, 2H), 3.55 (t, J = 3.5 Hz, 2H), 5.24 (s, 2H), 7.68 (s, 1H). $^1$H NMR (DMSO-$d_6$) $\delta$ 0.84 (s, 3H), 1.24 (m, 8H), 1.78 (m, 2H), 4.05 (t, J = 3.4 Hz, 2H), 6.89 (s, 2H), 8.14 (s, 1H). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 13.9, 22.0, 26.0, 28.2, 28.9, 31.1, 43.0, 123.4, 143.2, 149.3, 154.1, 159.8. HRMS (ESI-FT-ICR) calculated for C$_{12}$H$_{18}$ClN$_5$ (M + H)$^+$ 268.1323 found 268.1348.

2,6-Diamino-N-9-heptylpurine (5c). Vacuum dried 4c (0.207 g, 0.773 mmol) was placed in a pressure tube. Methanolic ammonia was added and the mixture heated to 65–70°C for 20 h.
Solvent was removed under vacuum and the product purified by column chromatography (5% CH₃OH/CH₂Cl₂) to yield 5c as a white solid (0.102 g, 50 %). Mp 227–230 °C; ¹H NMR
(DMSO-₆) δ 0.83 (m, J = 3.5 Hz, 3H), 1.22 (m, 8H), 1.71 (q, J = 3.4 Hz, 2H), 3.91 (t, J = 3.4 Hz, 2H), 5.74 (bs, 2H), 6.60 (bs, 2H), 7.68 (s, 1H). ¹³C NMR (DMSO-₆) δ 13.9, 22.0, 26.0, 28.2, 29.3, 31.1, 42.3, 113.2, 137.5, 151.7, 156.0, 160.1. HRMS (ESI-FT-ICR) calculated for C₁₂H₂₀N₆ (M + H)⁺ 249.1822 found 249.1828.

**6-Chloro-N-9-heptyl-2-N-(4-hexylamino)ureidopurine (6c).** 6-Chloro-9-heptyl-2aminopurine (0.519 g, 1.81 mmol) was placed in an oven dried two-necked round bottom flask and dried under vacuum. Tetrahydrofuran (15 mL) was added and the mixture cooled to −78 °C under argon. n-BuLi (1.25 mL, 1.6 M in hexanes, 1.99 mmol) was added dropwise and the mixture stirred for 30 minutes. Hexyl isocyanate (0.211 mL, 1.99 mmol) was added dropwise and the mixture stirred at −78 °C for 20 minutes. The reaction mixture was stirred an additional 10 minutes without cooling followed by quenching with saturated aqueous ammonium chloride. The product mixture was extracted with ethyl acetate and the solvent evaporated. Purification by column chromatography (5% CH₃OH/CH₂Cl₂) afforded 6c (0.610 g, 85 %). Mp 146–147 °C; ¹H NMR (CDCl₃) δ 0.86 (m, 5H) 1.30 (m, 4H), 1.63 (m, 2H), 2.24 (s, 6H), 2.30 (s, 3H), 3.40 (q, J = 3.38 Hz , 2H), 5.21 (s, 2H), 6.95 (s, 2H), 7.42 (s, 1H), 7.46 (s, 1H), 8.74 (t, J = 2.5 Hz, 1H). ¹³C NMR (DMSO-₆) δ 13.9, 14.0, 21.9, 22.1, 25.7, 26.0, 26.1, 28.2, 29.1, 29.5, 31.0, 31.2, 41.7, 114.6, 140.1, 149.8, 153.1, 155.1, 158.5. HRMS (ESI-FT-ICR) calculated for C₁₀H₁₄ClN₆O (M + H)⁺ 395.2321 found 395.2307.
6-Amino-N-9-heptyl-2-N-(4-hexylamino)ureidopurine (8). Compound 6c (0.604 g, 1.53 mmol) was placed in a 75 mL pressure tube. Methanolic ammonia (40 mL) was added and the mixture heated to 70 °C. The mixture was stirred for 3 h. Solvent was evaporated and the product purified by column chromatography (3% CH$_3$OH/CH$_2$Cl$_2$) to yield 7c (0.194 g, 34 %). Mp 229–231 °C; $^1$H NMR (CDCl$_3$) $\delta$ 0.87 (m, 6H), 1.27 (m, 14H), 1.42 (q, $J = 3.6$ Hz, 2H), 1.85 (q, $J = 3.4$ Hz, 2H), 3.38 (q, $J = 3.6$ Hz 2H), 4.03 (t, $J = 3.6$ Hz, 2H), 7.57 (s, 1H), 9.68 (s, 1H), 9.69 (t, $J = 2.4$,1H). $^1$H NMR (DMSO-$d_6$) $\delta$ 0.83 (m, 6H), 1.27 (m, 14H), 1.52 (q, $J = 3.5$ Hz, 2H), 1.75 (m, 2H), 3.71 (q, $J = 3.6$ Hz, 2H) 4.12 (t, $J = 3.5$ Hz, 2H), 6.87 (s, 2H), 7.94 (s, 1H), 8.64 (s, 1H), 9.36 (t, $J = 2.5$ Hz, 1H). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 13.9, 21.9, 22.1, 25.9, 26.0, 26.1, 28.1, 29.1, 29.5, 31.0, 31.1, 42.5, 113.1, 139.9, 150.2, 153.7, 158.0, 158.7. HRMS (ESI-FT-ICR) calculated for C$_{19}$H$_{33}$N$_7$O (M + H)$^+$ 376.2819 found 376.2829 C$_{19}$H$_{33}$N$_7$ONa (M + Na)$^+$ 398.2639 found 398.2648.

6-Chloro-N-9-heptyl-2-N-(4-phenylamino)ureidopurine (15). Tetrahydrofuran (10 mL) was added to an oven dried 50 mL two-necked, round bottomed flask followed by addition of 2,2,6,6-tetramethyl piperidine (0.095 mL, 0.559 mmol). The mixture was cooled to 0° C, and n-BuLi (0.467 mL, 0.560 mmol) was added dropwise. The cooled mixture was stirred for 30 minutes followed by cooling to –78 °C. Vacuum dried 2-amino-6-chloro-7-heptylpurine (0.125 g, 0.467 mmol) was dissolved in 20 mL THF and added dropwise. The reaction mixture was
stirred for 1 h. Phenylisocyanate (0.102 mL, 0.938 mmol) was added and stirred for 2 hours at –78 °C followed by stirring without cooling for 10 min. The reaction was quenched with aqueous saturated ammonium chloride. The mixture was extracted with chloroform and the organic layer washed with brine. Solvent was removed under reduced pressure. The product was purified by column chromatography (2% CH₃OH/ CH₂Cl₂) to yield the final product as a white solid (0.152 g, 84%). Mp 174–177 °C; ¹H NMR (CDCl₃) δ 0.84 (m, 3H), 1.28 (m, 8H), 1.89 (m, 2H), 4.18 (t, J = 3.5 Hz, 2H), 7.09 (t, J = 3.8 Hz, 1H), 7.33 (t, J = 4.9 Hz, 2H) 7.58 (d, J = 3.7 Hz, 2H), 7.78 (s, 1H), 7.94 (s, 1H), 11.05 (s, 1H). ¹³C NMR (CDCl₃) δ 13.9, 22.4, 26.6, 28.6, 29.6, 31.5, 44.5, 119.7, 123.8, 127.4, 129.0, 138.0, 144.4, 150.9, 151.0, 152.5, 152.6. HRMS (ESI-FT-ICR) calculated for C₁₉H₂₄ClN₆O (M + H)⁺ 387.1700 found 387.1691.

N-9-Heptyl-6-methoxy-2-N-(4-phenylamino)ureidopurine (16). Compound (15) (0.152 g, 0.414 mmol) was placed in an oven dried pressure tube to which methanolic ammonia (50 mL) was added. The mixture heated to 95 °C. The reaction was stirred for 3 h. Solvent was removed under reduced pressure. The product was purified by column chromatography (2% CH₃OH/ CH₂Cl₂) to yield the final product as a white solid (0.071 g, 49%). ¹H NMR (CDCl₃) δ 0.86 (m, 3H), 1.31 (m, 8H), 1.87 (q, J = 3.3 Hz 2H), 4.19 (t, J = 3.0 Hz, 5H), 7.12 (t, J = 3.7 Hz, 1H), 7.36 (t, J = 3.9 Hz, 2H) 7.42 (s, 1H), 7.58 (d, J = 3.8 Hz, 1H), 7.78 (s, 1H), 11.25 (s, 1H). ¹³C NMR (CDCl₃) δ 13.9, 21.9, 22.1, 25.9, 26.0, 26.1, 28.1, 29.1, 29.9, 30.0, 31.0, 31.1, 42.5, 113.1, 139.9, 150.2, 152.7, 153.7, 158.0, 158.7. HRMS (ESI-FT-ICR) calculated for C₂₀H₂₇N₆O₂ (M + H)⁺ 383.2195 found 383.2229.
6-Chloro-N-7-heptyl-2-N-(4-phenylamino)ureidopurine (15c'). Tetrahydrofuran (10 mL) was added to an oven dried 50 mL two-necked, round bottomed flask followed by addition of 2,2,6,6-tetramethyl piperidine (0.057 mL, 0.336 mmol). The mixture was cooled to 0° C, and n-BuLi (0.210 mL, 0.336 mmol) was added dropwise. The cooled mixture was stirred for 30 minutes followed by cooling to – 78 °C. Vacuum dried 2-amino-6-chloro-7-heptylpurine (0.075 g, 0.280 mmol) was dissolved in 12 mL THF and added dropwise. The reaction mixture was stirred for 1 h. Phenylisocyanate (0.061 mL, 0.560 mmol) was added and stirred for 2 h at – 78 °C followed by stirring without cooling for 10 min. The reaction was quenched with aqueous saturated ammonium chloride. The mixture was extracted with chloroform and the organic layer washed with brine. Solvent was removed under reduced pressure. The product was purified by column chromatography (1% CH3OH/ CH2Cl2) to yield the final product as a white solid (0.068 g, 63 %). Mp 151–155 °C; 1H NMR (CDCl3) δ 0.85 (m, 3H), 1.20 (m, 8H), 1.76 (m, 2H), 4.18 (t, J = 3.4 Hz, 2H), 6.99 (t, J = 3.4 Hz, 1H), 7.31 (t, J = 3.7 Hz, 2H), 7.48 (s, 1H), 7.58 (d, J = 3.4 Hz, 2H), 7.80 (s, 1H) 11.59 (s, 1H). 13C NMR (CDCl3) δ 13.9, 22.5, 26.3, 28.6, 31.0, 31.5, 47.8, 54.4, 119.8, 120.3, 123.5, 128.8, 129.0, 138.3, 152.0, 153.0, 157.8. HRMS (ESI-FT-ICR) calculated for C20H27N6O2 (M + H)+ 383.2195 found 383.2229; C20H26N6O2Na (M + Na)+ 405.2015 found 405.2030.
7-Heptyl-6-methoxy-2-N-(4-phenylamino)ureidopurine (16c'). Starting material, compound 15c' (0.068 g, 0.176 mmol), was placed in an oven-dried pressure tube. Methanolic ammonia (90 mL) was added and the vessel sealed. The mixture was heated to 90 °C for 18 h. Solvent was removed under vacuum and the product purified by column chromatography (1% CH3OH/CH2Cl2). The product was isolated as a white solid (0.027 g, 42%). Mp 179–181 °C; 1H NMR (CDCl3) δ 0.86 (m, 3H), 1.26 (m, 8H), 1.86 (m, 2H), 4.11 (s, 3H), 4.23 (t, J = 3.4 Hz, 2H), 7.06 (t, J = 3.4 Hz, 1H), 7.312 (t, J = 3.4 Hz, 2H), 7.46 (s, 1H), 7.66 (d, J = 3.7 Hz, 2H), 7.86 (s, 1H) 11.52 (s, 1H). 13C NMR (CDCl3) δ 14.0, 22.5, 26.7, 28.7, 29.7, 29.9, 31.6, 44.4, 54.6, 119.7, 123.7, 129.1, 137.1, 138.2, 151.5, 152.8, 156.4. HRMS (ESI-FT-ICR) calculated for C20H27N6O2 (M + H)+ 383.2195 found 383.2229.

2-Amino-6-chloro-9-(tert-butyl acetate)purine (4d). Dry 6-chloro-2-aminopurine (0.501 g, 2.67 mmol) was placed in a 250 mL oven dried round bottomed flask and suspended in dimethylformamide (100 mL). Tetrabutyl ammonium fluoride (5.60 mL, 5.60 mmol) and iodoheptane (0.813 mL, 3.59 mmol) were added dropwise. The mixture was stirred at room temperature for 20 minutes. The product was extracted with ethyl acetate and the solvent was evaporated. The residue was recrystallized from an 8:2 ratio of ethyl acetate:hexanes to yield the product (2.30 g, 75%). 1H NMR (CDCl3) δ 1.46 (s, 9H), 4.67 (s, 2H), 5.33 (s, 2H), 7.56 (s, 1H). 13C NMR (DMSO-d6) δ 27.6, 44.6, 82.3, 122.9, 143.5, 149.4, 154.3, 160.0, 166.7. HRMS (ESI-FT-ICR) calculated for C12H18ClN5 (M + H)+ 268.1323 found 268.1348.
**2,6-Diamino-9-(tert-butyl acetate)purine (5d).** 2,6-Diaminopurine (0.503 g, 3.35 mmol) was placed in a 100 mL oven dried round bottomed flask. The starting material was suspended in degassed dimethylformamide (20 mL). Sodium hydride (0.142 g, 60 % dispersion in mineral oil, 3.69 mmol) was added and the mixture stirred for 2.5 h. Bromo tert-butyl acetate (0.089 mL, 0.46 mmol) was added dropwise followed with stirring for 2.5 h. The product mixture was filtered through celite and the celite rinsed with hot ethyl acetate to give a pale orange solution. Solvent was removed under vacuum methylene chloride was added to the solid and the solid filtered to yield 5d as an off-white solid (0.447 g, 50 %). $^1$H NMR (DMSO-$d_6$) $\delta$ 1.42 (s, 9H), 4.74 (s, 2H), 5.82 (s, 2H), 6.71 (s, 2H), 7.67 (s, 1H). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 27.7, 44.0, 81.9, 112.7, 138.0, 152.0, 156.2, 160.4, 167.3. HRMS (ESI-FT-ICR) calculated for C$_{12}$H$_{20}$N$_6$ (M + H)$^+$ 249.1822 found 249.1828.

![Diagram of 2,6-Diamino-9-(tert-butyl acetate)purine (5d)](image)

**9-(tert-Butyl acetate)-6-chloro-2-N-(4-hexylamino)ureidopurine (6d).** Vacuum dried 6-chloro-9-heptyl-2aminopurine (4d) (0.224 g, 0.789 mmol) was placed in an oven dried round bottom flask and dried under vacuum. Tetrahydrofuran (10 mL) was added to a two necked round bottomed flask. $n$-BuLi (0.741 mL, 1.19 mmol, 1.6 M in hexanes) was added and the
mixture cooled to $-78 \, ^\circ C$ under argon. Tetramethylpiperidine (0.201 mL, 1.19 mmol) was added and the mixture stirred for 30 minutes. Hexyl isocyanate (0.100 mL, 0.947 mmol) was added dropwise and the mixture stirred at $-78 \, ^\circ C$ for 20 minutes. The reaction mixture was stirred an additional 10 min without cooling followed by quenching with saturated aqueous ammonium chloride. The product mixture was extracted with ethyl acetate and the solvent evaporated. Purification by column chromatography (1% CH$_3$OH/CH$_2$Cl$_2$) afforded 6d (0.610 g, 85 %). Mp 122–125 °C; $^1$H NMR (CDCl$_3$) $\delta$ 0.87 (m, 3H) 1.31 (m, 8H), 1.45 (s, 9H) 1.61 (m, 2H), 3.37 (q, $J = 2.4$ Hz, 2H), 4.77 (s, 2H), 7.55 (s, 1H), 7.97 (s, 1H), 8.68 (t, $J = 3.4$ Hz, 1H).

$^{13}$C NMR (CDCl$_3$) $\delta$ 14.0, 22.6, 26.7, 27.9, 29.6, 31.5, 40.1, 45.2, 77.0, 84.2, 126.6, 144.5, 150.8, 152.9, 153.3, 153.5, 165.3. HRMS (ESI-FT-ICR) calculated for C$_{18}$H$_{27}$ClN$_6$O$_3$ (M + H)$^+$ 411.1906 found 411.1896.

6-Azido-9-(tert-butyl acetate)-2-N-(4-hexylamino)ureidopurine (17). Compound 6d (0.107 g, 0.260 mmol) was placed in an oven dried round bottomed flask fitted with a reflux condenser. Dimethylformamide (9 mL) was added followed by sodium azide (0.034 g, 0.520 mmol). The mixture was heated 105 °C for 4 h. Product was purified by column chromatography (1% CH$_3$OH/CH$_2$Cl$_2$) isolated at a pink oil (0.052 g, 58%). $^1$H NMR (CDCl$_3$) $\delta$ 0.87 (m, 3H) 1.31 (m, 6H), 1.45 (s, 9H), 1.59 (m, 2H), 3.36 (q, $J = 2.4$ Hz, 2H), 4.74 (s, 2H), 7.50 (s, 1H), 7.85(s, 1H), 8.64 (t, $J =3.4$ Hz, 1H). $^{13}$C NMR (CDCl$_3$) $\delta$ 14.0, 22.5, 26.7, 27.9, 29.7, 31.5, 40.2,
45.2, 84.0, 119.4, 143.1, 153.1, 153.3, 153.5, 153.8, 165.4. HRMS (ESI-FT-ICR) calculated for C\textsubscript{11}H\textsubscript{16}N\textsubscript{6}O\textsubscript{2} (M + H\textsuperscript{+}) 418.2310 found 418.2355.

**6-Amino-9-(tert-butyl acetate)-2-N-(4-hexylamino)ureidopurine (9).** Starting material (9) (0.041 g, 0.097 mmol) was dried overnight in a 25 mL oven-dried two-necked round bottomed flask fitted with a reflux condenser. A 0.4 M solution of ammonium formate in methanol (2 mL) was added followed by 10 % palladium on carbon (9.0 mg). The mixture was heated to reflux for 1.5 h. The palladium was filtered and rinsed with excess methanol until black. Solvent was removed and the residue dissolved in CH\textsubscript{2}Cl\textsubscript{2} followed by washing with water 3 times. Purification by column chromatography (3% CH\textsubscript{3}OH/CH\textsubscript{2}Cl\textsubscript{2}) yielded compound 7d (0.028 g, 74 %). Mp 239–241 °C. \textsuperscript{1}H NMR (CDCl\textsubscript{3}) \(\delta\) 0.86 (m, 3H) 1.24 (m, 4H), 1.33 (m, 4H), 1.44 (s, 9H), 3.36 (q, \(J = 2.4\) Hz, 2H), 4.67 (s, 2H), 7.65 (s, 1H), 9.33 (s, 1H), 9.46 (t, \(J = 3.4\) Hz, 1H). \textsuperscript{1}H NMR (DMSO-\textit{d}\textsubscript{6}) (100 °C) \(\delta\) 0.86 (m, 3H), 1.29 (m, 6H), 1.41, (s, 9H), 1.49 (m, 2H), 3.20 (q, \(J = 2.5\) Hz, 2H), 4.83 (s, 2H), 7.10 (s, 2H), 7.88 (s, 1H), 8.18 (s, 1H), 9.14 (t, \(J = 3.3\) Hz, 1H). \textsuperscript{13}C NMR (DMSO-\textit{d}\textsubscript{6}) (100 °C) \(\delta\) 13.3, 21.6, 25.9, 27.5, 29.3, 30.7, 44.5, 81.9, 114.3, 139.8, 150.1, 153.9, 155.6, 166.3. HRMS (ESI-FT-ICR) calculated for C\textsubscript{18}H\textsubscript{29}N\textsubscript{7}O\textsubscript{3} (M + H\textsuperscript{+}) 392.2405 found 392.2407.
**General procedure for deprotonation of DAP by NaH.** Starting material was dried overnight under vacuum in an oven dried round bottomed flask and dissolved in THF. The solution was cooled to 0 °C and sodium hydride (1.2 eq.) added in portions. The mixture was then heated to 65–70 °C and stirred under Ar for 2.5 h followed with cooling again to 0 °C. Hexyl isocyanate (1.2 eq.) was added and the mixture stirred 2 h. Acid (0.2 M HCl) was added dropwise until the evolution of gas ceased. Solvent was evaporated and the residue purified by column chromatography. Characterization for N$_{C6}$ ureidopurines is given in Chapter 4, experimental methods.

**General procedure for deprotonation of DAP by n-BuLi.** Vacuum dried DAP was placed in an oven dried round bottomed flask. Tetrahydrofuran was added and the solution cooled to −78 °C. The mixture was stirred under inert atmosphere as n-BuLi (1.1 eq) was added dropwise. Hexyl isocyanate (1.2 eq.) was added after 30 minutes and stirred for 30 minutes longer. The ice bath was removed and the mixture stirred without cooling for 5 minutes followed with quenching with saturated aqueous ammonium chloride. The product mixture was extracted with ethyl acetate and the solvent removed. The product residue was then purified by column chromatography. Characterization for N$_{C6}$ ureidopurines is given in Chapter 4, experimental methods.

**Computational Details**

Monte Carlo conformational searching was done on a Dell PC (2.4 GHz) running the Fedora Core using MacroModel v. 9.0 (Schrodinger, LLC)$^{86}$ and the MCMM method (relevant parameters include: steps = 100, iterations = 2000, solvent (GB/SA) = CHCl$_3$, force field = Amber*). Only 1$^{N3}$ and 1$^{N1}$ were further refined using ab initio methods (using Gaussian 03 (revision D.01)$^{87}$) as implemented through the National Center for Supercomputing Applications, SGI Altix cluster “Cobalt” (http://www.ncsa.uiuc.edu/UserInfo/Resources/Hardware/SGIAltix/).
CHAPTER 4
REACTIVITY OF 2,6-DIAMINOPURINE

Introduction

Diaminopurine is a useful synthon, and serves as a platform for applications which span materials to pharmaceuticals (figure 4-1). These applications use diaminopurine with various modified amino groups at N9, C2, and C6 of the purine core. Interestingly, few are obtained by nucleophilic substitution with DAP. Rather these molecules are obtained through electrophilic substitution of purines halogenated at C2 and C6, which incorporates additional steps into a synthetic route. Generating a greater understanding of the reactivity of the amino groups will assist in advancing purine chemistry and serves as the thrust for this chapter.

Figure 4-1. DAP derivatives investigated in pharmaceutical research as specific kinase inhibitors

A second thrust involves the systematic characterization of diaminopurine derivatives by NMR, particularly 2-D and $^{15}$N (e.g., $^{1}$H–$^{13}$C gHMBC, and $^{1}$H–$^{15}$N gHMBC), for the purposes of elucidating substitution patterns on the core. This level of advanced NMR characterization for diaminopurines is sparse in the literature, and to date $^{15}$N chemical shift data has only been reported by isotopic labeling of purine fragments. Described here is the full characterization data for differently functionalized diaminopurines, including $^{15}$N chemical shifts (collected at natural abundance), that may serve as a useful reference for future derivatives.
Syntheses of aryl and alkyl UDAPs have been described in Chapters two and three, respectively. Phenyl UDAP is formed through regioselective reaction of phenyl isocyanate at N\textsubscript{C2}, without the isolation of an N\textsubscript{C6} product. This result is consistent with literature precedent (one case) for preferential acylation at N\textsubscript{C2}.\textsuperscript{111} Porcher and coworker proposed that 2-aminoadenosine was acylated by methoxyacetyl chloride at N\textsubscript{C2} in 86 %; no mention was made of the N\textsubscript{C6} substituted product (figure 4-2, top). The N\textsubscript{C2} acylated product was then subjected to a second acylation step at N\textsubscript{C6} with isobutyryl chloride. This work is one of just a couple examples of the apparent differential reactivity of the amino groups of 2,6-diaminopurine (N\textsubscript{C2} versus N\textsubscript{C6}) reported in the literature.

![Chemical structures](image)

Figure 4-2. Examples of DAP acylation found in the literature\textsuperscript{111,112}

Synthesis of the alkyl UDAPs required a different approach due to an apparent reactivity difference between aryl and alkyl isocyanates. This ultimately involved deprotonation of N9-protected 2-amino-6-chloro purine (4), followed by reaction with hexyl isocyanate to yield compounds 6a, c, and d, and then subsequent amination. Recall that direct deprotonation of DAP yielded the N\textsubscript{C6}-substituted purine upon treatment with an alkyl isocyanate. Interestingly, treatment of DAP with hexyl isocyanate at elevated temperature (in a pressure tube), in pyridine,
was also found to afford the \( \text{NC}_6 \) regioisomer in 97% yield (based on \( ^1\text{H} \) and NOESY NMR analysis). This result, in connection with work from Nielsen and coworkers, which reported the exclusive \( \text{NC}_6 \) substitution of DAP to form a carbamate via reaction with \( \text{N}-\text{benzyloxy carbonyl-N-methylimidazolium triflate (rt, dioxane, 20 h, 88 \%)} \),\(^{112}\) Rappaport’s reagent, speaks to a more complicated story involving \( \text{NC}_2 \) versus \( \text{NC}_6 \) substitution. In other words, the two best literature examples of DAP electrophilic substitution chemistry provide disparate results, as do examples from our own experiments.

Issues related to \( \text{NC}_2 \) versus \( \text{NC}_6 \) substitution quickly expose the additional problem of assigning the (monosubstitution) regiochemistry with certainty by NMR. NOE (from 1-D or 2-D techniques) and routine chemical shift data (\( ^1\text{H} \) and \( ^{13}\text{C} \)) are largely inconclusive, the latter since attempts to establish chemical shift trends among the isomers fail due to unpredictable shift changes upon introduction of different electrophiles. In fact, the work of Nielsen and the work of Porcher offers unsatisfying evidence with respect to regiochemical assignments.

In addition to changes in solvent, electrophile, and temperature, DAP structure is also a mitigating factor. In particular, functionalization at the N9 position that could, in theory, affect reaction at \( \text{NC}_2 \). It has also been demonstrated that the solubility of the diaminopurines is affected by the N9 substituent (Chapter 3, \( 8 \) is \( \sim 10 \) times more soluble than \( 7 \)), and solubility could also affect apparent rates and selectivities. Finally, largely unexplored is the extent to which acylation reactions involving DAP might be reversible. Drawing general conclusions about DAP regioselectivity requires a more systematic study.

**Reactivity of 2,6-Diaminopurine**

**Acylation with Methoxy Acetyl Chloride**

Acylation of diaminopurine was studied using common acylating agents with three N9 protected DAPs (\( 5a, 5c, \) and \( 5d \)), each previously introduced in the synthesis of UDAP. The
three DAPs were intended to probe solvent, steric, or electronic effects related to the N9 substituents. Experiments were performed in two different solvent systems (3:1 solvent mixture of CH₂Cl₂ or CH₃CN to pyridine) with 2.5 eq. methoxy acetyl chloride (A), a common acylating agent (figure 4-3, table 4-1). The reactions were stirred until the starting material disappeared, and the monosubstituted (x or y) and disubstituted products (z) were isolated. By the ratio of the products obtained the reactivity difference between the two amino groups under these conditions might be revealed (assuming irreversible bond formation).

![Figure 4-3](image_url)

Figure 4-3. Reaction of DAP with 2.5 eq. methoxy acetyl chloride in a 3:1 ratio of CH₂Cl₂ or CH₃CN to pyridine. Products 18x, 18y, and 18z were isolated and characterized.

The time for the reactions to reach completion varied from 0.5 h to 1.5 h for CH₂Cl₂ or CH₃CN, respectively (table 4-1). From each of the reaction mixtures two components were isolated in similar ratios. The major product was the monosubstituted product (x or y in Figure 4-1) and the minor product was the disubstituted product, z (as determined by ¹H NMR).

Table 4-1. Reaction conditions and outcomes for nucleophilic substitution of DAP with methoxy acetyl chloride.

<table>
<thead>
<tr>
<th>Purine</th>
<th>R₁</th>
<th>time (h)</th>
<th>Solvent</th>
<th>t (°C)</th>
<th>Yield</th>
<th>x:y:z</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>CH₂OCH₃</td>
<td>0.5</td>
<td>CH₂Cl₂/C₅H₅N</td>
<td>Rt</td>
<td>95</td>
<td>9:1:0</td>
</tr>
<tr>
<td>5a</td>
<td>CH₂OCH₃</td>
<td>1.5</td>
<td>CH₃CN/C₅H₅N</td>
<td>Rt</td>
<td>95</td>
<td>9:1:0</td>
</tr>
<tr>
<td>5c</td>
<td>CH₂OCH₃</td>
<td>0.5</td>
<td>CH₂Cl₂/C₅H₅N</td>
<td>Rt</td>
<td>97</td>
<td>9:1:0</td>
</tr>
<tr>
<td>5c</td>
<td>CH₂OCH₃</td>
<td>1.5</td>
<td>CH₃CN/C₅H₅N</td>
<td>Rt</td>
<td>98</td>
<td>4:1:0</td>
</tr>
<tr>
<td>5d</td>
<td>CH₂OCH₃</td>
<td>0.5</td>
<td>CH₂Cl₂/C₅H₅N</td>
<td>Rt</td>
<td>98</td>
<td>9:1:0</td>
</tr>
<tr>
<td>5d</td>
<td>CH₂OCH₃</td>
<td>1.5</td>
<td>CH₃CN/C₅H₅N</td>
<td>Rt</td>
<td>97</td>
<td>4:1:0</td>
</tr>
</tbody>
</table>

Reactions were performed at a concentration of 4.0 mM with 2.5 eq. of electrophile. Yields are based on total product masses isolated.
**Determination of Substitution Site**

The protocol for determining the site of nucleophilic substitution as $N_C^2$ or $N_C^6$ of DAP (in all of the studies) involved the comprehensive characterization of the N9 protected 2-amino-6-chloropurines (4a, 4c, and 4d), their corresponding diaminopurines (5a, 5c, and 5d), and the monosubstituted reaction products by $^1H$, $^13C$, $^1H–^13C$ gHMBC, and $^1H–^15N$ gHMBC NMR (in addition to mass spec). The NMR data was obtained at 25 °C, in a common solvent (DMSO-$d_6$), and at natural abundance of $^{15}N$ (NMR spectra were recorded on a Varian Inova spectrometer equipped with a 5 mm indirect detection probe, operating at 500 MHz at 50 MHz for $^{15}N$). Concentrated samples (0.1 M) were prepared in DMSO-$d_6$, however, peak broadening was an issue. Although the issue of peak broadening was not completely remedied (occurrences were seen in relation to NH$_2$ and NH resonance), chemical shift correlations were still accessible.

Chemical shift data for 4c, 5c, and 12c are representative and discussed here (figure 4-4 and table 4-2); information for other compounds is provided in Appendix A. The chemical shift of the C8-H and $\alpha$-CH$_2$ protons (at N9) were easily assigned on the basis of chemical shift and integration. These protons were further characterized by their one- and multiple-bond heteronuclear couplings. For H8, H8-C8 coupling was observed by $^1H–^13C$ gHMQC, H8-C5 and H8-C4 coupling by $^1H–^13C$ gHMBC, and H8-N7 and H8-N9 coupling by $^1H–^15N$ gHMBC. These correlations are shown schematically in figure 4-2.

![Figure 4-4. NMR coupling assignments of the imidazole portion of 2-amino-6-chloropurine by $^1H–^13C$ and $^1H–^15N$ gHMBC](image)

4c, R = hexyl, R$_1$ = Cl, R$_2$ = NH$_2$
5c, R = hexyl, R$_1$ = R$_2$ = NH$_2$
18cx, R = hexyl, R$_1$ = NH$_2$, R$_2$ = COCH$_2$OCH$_3$
18cy, R = hexyl, R$_1$ = COCH$_2$OCH$_3$, R$_2$ = NH$_2$
Table 4-2. Chemical shifts of the imidazole portion of the bicyclic purine compounds 4c (2-amino-6-chloro-9-heptylpurine), 5c (2,6-diamino-9-heptylpurine), and 18c (monosubstituted product 18cx or 18cy)

<table>
<thead>
<tr>
<th>Purine</th>
<th>C4a</th>
<th>C5a</th>
<th>C8a</th>
<th>H8a</th>
<th>αH9a</th>
<th>N7b</th>
<th>N9b</th>
</tr>
</thead>
<tbody>
<tr>
<td>4c</td>
<td>154.1</td>
<td>123.4</td>
<td>143.2</td>
<td>8.14</td>
<td>4.05</td>
<td>237.8</td>
<td>159.0</td>
</tr>
<tr>
<td>5c</td>
<td>151.7</td>
<td>113.2</td>
<td>137.7</td>
<td>7.70</td>
<td>3.94</td>
<td>237.4</td>
<td>157.1</td>
</tr>
<tr>
<td>18c</td>
<td>150.3</td>
<td>115.0</td>
<td>140.3</td>
<td>8.03</td>
<td>4.07</td>
<td>237.2</td>
<td>159.8</td>
</tr>
</tbody>
</table>

Chemical shifts at natural abundance in 0.1 M DMSO-d_6 solutions at 25 °C. a H and C values are relative to TMS; b 15N values given on the ammonia scale relative to nitromethane (379.5 ppm).

The chemical shift assignments of the pyrimidine ring for the compounds of this study were made similarly to the imidazole assignments. The C2 amino groups (N_C2) of compounds 4c and 5c were assigned by long-range coupling of the amino protons to two nitrogens in the H–^{15}N gHMBC spectra, assigned as N1 and N3 (assigned by chemical shifts,^{110} experimental shifts given in table 4-3). Likewise, the N_C6 amino group of 5c was assigned based on the long-range coupling to only one nitrogen, N1. To further solidify the assignment of N_C6 and N_C2, coupling was seen for the amino protons N_C6H and N_C2H to C5 and C2 (respectively) of the purine core in the H–^{13}C gHMBC spectrum.

Assignment for the point of nucleophilic substitution was made by the simple line of reasoning demonstrated thus far. In all six of the reactions the point of substitution was the same, and the assignment was made for substitution at N_C2. The amidic proton of N_C2 was coupled to two nitrogen in the H–^{15}N gHMBC spectra (scheme shown, figure 4-5, and spectrum shown, figure 4-6), N1 and N3. Similarly the N_C6 amino protons showed long range coupling to both N1 and to C5 (H–^{13}C gHMBC, not shown, see Appendix A).

With the site of monosubstitution confirmed (table 4-1), the results of this study indicate that solvent, but more importantly N9 protecting groups, are not factors in the reactivity of DAP under fairly routine acylation conditions. The results further suggest that N_C2 reacts completely and much more quickly than N_C6 (the N_C6 monosubstituted product was not identified) in
agreement with the results of Porcher and coworkers;\(^{111}\) the N\(_{C6}\) reacts next to provide the disubstituted product under these conditions.

![Diagram](image)

**Figure 4-5.** Correlations proved the point of substitution to be N\(_{C2}\). Long-range coupling is seen from the amide proton to N1 and N3, and from the amino protons of N\(_{C6}\) to N1 in the \(^{1}H–^{15}N\) gHMBC spectrum. Long-range coupling is also seen from N\(_{C6}\)H to C5 in the \(^{1}H–^{13}C\) gHMBC spectrum (chemical shifts given tables 4-1 and 4-2).

<table>
<thead>
<tr>
<th>Purine</th>
<th>C2</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
<th>N(_{C2})H</th>
<th>N(_{C6})H</th>
<th>N1</th>
<th>N(_{C2})</th>
<th>N3</th>
<th>N(_{C6})</th>
</tr>
</thead>
<tbody>
<tr>
<td>4c</td>
<td>149.3</td>
<td>154.1</td>
<td>123.4</td>
<td>159.8</td>
<td>6.89</td>
<td>--</td>
<td>232.9</td>
<td>81.0</td>
<td>196.9</td>
<td>--</td>
</tr>
<tr>
<td>5c</td>
<td>156.2(^a)</td>
<td>151.7</td>
<td>113.2</td>
<td>160.4(^a)</td>
<td>6.69</td>
<td>5.81</td>
<td>197.7(^b)</td>
<td>75.6</td>
<td>178.4(^b)</td>
<td>75.8</td>
</tr>
<tr>
<td>18cx</td>
<td>156.1</td>
<td>150.3</td>
<td>116.0</td>
<td>152.3</td>
<td>7.22</td>
<td>9.60</td>
<td>209.8</td>
<td>139.5</td>
<td>197.2</td>
<td>79.8</td>
</tr>
</tbody>
</table>

\(^a\) Chemical shift value was not measured for compound 5c due to peak broadening. Value given is the chemical shift for the corresponding atom of 5d.\(^b\) Value given was not measured for 5c, but was obtained for 5a and 5d, which were averaged to give the corresponding value.

**Substitution at N\(_{C6}\) and Reversibility**

Introduced earlier, when DAP (\(~ 40 \text{ mM}\)) was reacted with hexyl isocyanate at 100 °C in a sealed reaction vessel in pyridine (figure 4-7, table 4-4) N\(_{C6}\) monosubstitution was predominantly observed. The increased concentration of substrate for this reaction was coincidentally similar to the concentration of DAP that provided the N\(_{C6}\) carbamate upon reaction with N-benzyloxycarbonyl-N-methylimidazolium triflate\(^{95}\) in dioxane at room temperature (20 h), reported by Nielsen.\(^{112}\) The literature reaction was reproduced in our
laboratory, and the products from both reactions were studied thoroughly by the NMR techniques discussed above. Although the conditions (e.g. electrophiles, solvents, etc.) were quite different, characterization proved the products to be N$_{c6}$ substituted (11cz, 11dz and 12dz). Furthermore, the products were obtained in high yield with complete regioselectivity (table 4-4).

Figure 4-6. Spectrum showing $^1$H–$^{15}$N gHMBC correlations for 18cx proves the site of substitution to be N$_{c2}$. Chemical shifts are at natural abundance in a 0.1 M DMSO-$d_6$ solution at 25 °C. $^{15}$N values are given on the ammonia scale relative to nitromethane (379.5 ppm).

Figure 4-7. Electrophiles A–C were reacted with DAP and the reaction products characterized. Reaction conditions and outcomes are given in table 4-4.
Table 4-4. Reaction outcomes for high concentration (40 mM) reactions of 5c and 5d with electrophiles A, B, and C.

<table>
<thead>
<tr>
<th>purine</th>
<th>E⁺</th>
<th>R₁</th>
<th>time (h)</th>
<th>Solvent</th>
<th>temp (°C)</th>
<th>yield</th>
<th>x:y:z</th>
</tr>
</thead>
<tbody>
<tr>
<td>5c</td>
<td>B</td>
<td>NHC₆H₁₃</td>
<td>3.5</td>
<td>C₅H₆N</td>
<td>100</td>
<td>97</td>
<td>0:0:10</td>
</tr>
<tr>
<td>5d</td>
<td>B</td>
<td>NHC₆H₁₃</td>
<td>3.5</td>
<td>C₅H₆N</td>
<td>100</td>
<td>97</td>
<td>0:0:10</td>
</tr>
<tr>
<td>5d</td>
<td>C</td>
<td>OBn</td>
<td>20</td>
<td>C₄H₈O₂</td>
<td>97</td>
<td>97ᵃ</td>
<td>0:0:10</td>
</tr>
<tr>
<td>5d</td>
<td>A</td>
<td>CH₂OCH₃</td>
<td>0.3</td>
<td>CH₂Cl₂/C₅H₅N</td>
<td>99</td>
<td>99</td>
<td>9:1:0</td>
</tr>
</tbody>
</table>

ᵃ Reaction yield based on recovered starting material (product yield was 81 %).

Before speculating on the origin of the regioselectivity differences, we took the opportunity to evaluate whether any of these acylations were reversible under the reaction conditions tested. Returning to the methoxy acetyl chloride reactions, a 4 mM solution of DAP 5d in a 3:1 mixture of CH₂Cl₂:pyridine was cooled to −35 °C and treated with the electrophile (2.5 equiv). The starting material completely disappeared within 3 h (determined to be NC₂ substituted product, 18dx). Within 4 h TLC showed a second product spot, the disubstituted product (18dy). The reaction was left to stir overnight and showed no change in product ratio or conversion by TLC. More importantly, the reaction at −30(−35) °C yielded the same product ratio (9:1:0, x:y:z) that was isolated from the reaction at room temperature. This result is most consistent with the NC₂ regioisomer being the kinetic product. In a second variable temperature experiment, product 18dx was dissolved in a 3:1 mixture of methylene chloride:pyridine and heated to 75 °C for 17 h in a sealed reaction vessel. Detectable displacement of the acyl group by pyridine (or a purine) would establish a case for an equilibrium. No evidence arose to support an acyl migration from NC₂ to NC₆ under these conditions, or reaction of the starting material with pyridine; the NC₂ product was recovered in 94% yield. A similar reaction was performed with compound 8 (6-amino-2N-hexyl-9-heptylureidopurine). There was also no visible evidence for exchange seen here (8 was recovered in 97% yield).
Reactivity of DAP with Acetic Anhydride

Reaction of DAP with acetic anhydride was next pursued to a) explore regioselectivity trends using a generic electrophile and b) shed further light on potential reversibility issues (thermodynamic effects) in a reaction rate regime that was particularly amenable to NMR analysis. Reaction of a 4 mM solution of 5d in a 3:1 ratio of CH2Cl2:pyridine with acetic anhydride (2.5 eq.) offered a much slower reaction rate (figure 4-8, a discussion follows which addresses the factors involved in the comparatively decreased reaction rate, including significantly different reactive intermediates). After 48 h both monosubstituted products were isolated in a 4.5:1 ratio (NC2(19x):NC6(19z)) in an overall yield of 91 % based on recovered starting material. With the formation of NC2 and NC6 products from the same reaction mixture the study of kinetic and thermodynamic effects was simplified.

Figure 4-8. Reaction of DAP with acetic anhydride yielded a mixture of NC2 and NC6 products. To determine if an equilibrium was present under the reaction conditions two experiments were performed. Compound 19x was heated to reflux with deuterated acetic anhydride (2.5 eq.) in a methylene chloride/pyridine mixture. No deuterium was incorporated into the NC2 position nor was any NC6 monosubstituted product isolated. The experiment showed that under these conditions, the acylated NC2 product was kinetically stable. Further support for NC2 as the kinetic product was obtained by modifying the concentration of starting material in the reaction mixture. A 40 mM solution of compound 5d was prepared in a 3:1 mixture of CH2Cl2:pyridine and 2.5
equiv of methoxy acetyl chloride was added. Consistent with a kinetic effect, the reaction rate was increased and the same 9:1 product mixture of N\textsubscript{C2}:disubstituted product was isolated (table 4-4). The increased concentration also ruled out possible associative effects in the formation of N\textsubscript{C6} substituted products, 11z and 12z.

A thermodynamic equilibrium was eliminated conclusively by monitoring the reaction progress by NMR. Diaminopurine, 5d, was treated with acetic anhydride in a 3:1 mixture of CDCl\textsubscript{3}:pyridine (6.6 mM). After ~ 48 h the reaction had only progressed to ~ 30 % consumption of starting material, however, the N\textsubscript{C2}:N\textsubscript{C6} product ratios remained 4:1 throughout the course of the reaction (figure 4-9). Based on this result, the reaction of DAP with a common acylating agent, acetic anhydride, is a kinetically governed reaction with N\textsubscript{C2} substitution being favored.

![Graph showing reaction progress](image)

**Figure 4-9.** Reaction of 5d with acetic anhydride in 3:1 CDCl\textsubscript{3}:pyridine to yield 15dx and 15dz. A relative product ratio of 4:1 ratio is maintained throughout the course of reaction indicating a kinetic distribution of products.

The results thus far point to a kinetically controlled acylation of N\textsubscript{C2} under standard conditions that involve pyridine and either an acid chloride or simple anhydride. Complicating
the overall analysis is what could be different acylation mechanisms related to the identity of the active acylating agent under the conditions employed. Acylations catalyzed by pyridine are well-established that proceed through an acyl pyridinium intermediate.\textsuperscript{113} It is also possible, for methoxy acetyl chloride, that pyridine will facilitate elimination of HCl to form a ketene; this has been illustrated in the literature (specifically for methoxy acetyl chloride) in the presence of tertiary amines.\textsuperscript{114,115} Thus, the reaction by which \textbf{18x} is formed could involve three different electrophilic species: a ketene, a pyridinium cation, or an acid chloride. Likewise, formation of \textbf{19x} and \textbf{19z} can occur through two likely pathways: displacement of a pyridinium cation, or displacement of acetate. Interestingly, reaction of \textbf{5d} with acetic anhydride in the absence of pyridine gave a different product composition. The \textit{N}_{6} product was isolated in the absence of the \textit{N}_{2} product after 56 h at room temperature in 23 % yield (53\% based on recovered starting material; \~{}5 \% of the composition was found to be disubstituted product by \textsuperscript{1}H NMR). The known catalysis by pyridine in acylation reactions is thus perhaps an important factor in the regioselectivity of DAP. Finally, also uncertain is the extent to which deprotonated DAP (generated at high temperature in the presence of pyridine) might lead to preferential reaction at \textit{N}_{6} in the simple acylation reactions.

\textbf{Summary and Conclusions}

Nucleophilic substitution reactions involving DAP have been studied using various electrophiles, conditions, and DAP protecting groups (at \textit{N}9). For monosubstitution, the site of substitution has for the first time been unambiguously assigned by NMR methods including \textsuperscript{1}H, \textsuperscript{13}C, \textsuperscript{1}H–\textsuperscript{13}C $g$HMQC, and \textsuperscript{1}H–\textsuperscript{15}N $g$HMBC at natural abundance. The characterization has provided a protocol and small database of \textsuperscript{15}N chemical shift data, through the study of fourteen purine derivatives, that could be used in the structural elucidation of future DAP substitution products.
Reaction of DAP with standard acylating reagents, acetic anhydride or methoxy acetyl chloride, in the presence of pyridine, gave exclusively $N_C^2$ monosubstitution. The selectivity was insensitive to temperature, concentration, solvent, or DAP N9 substituent. Thermodynamic effects were ruled out, and the reaction was shown to be kinetically governed. Unactivated acetic anhydride, alkyl isocyanates, and Rappoport’s reagent give primarily $N_C^6$ monosubstitution. Substitution at this position is also observed upon formal deprotonation of DAP with $n$-BuLi. Further studies are required to determine whether the selectivity changes mirror mechanism changes that could involve deprotonation of the purine (under certain conditions) or the nature of the electrophile. Also, subtle differences between the reactive centers may need to be analyzed more closely. For example, although nitrogen data does not suggest different chemical environments for $N_C^2$ and $N_C^6$, the $^{13}$C chemical shift of C6 is slightly more deshielded than C2 ($\delta$ 160.4 and 156.2 in DMSO-$d_6$, 25 °C for 5d).

**Experimental Methods**

\[
\begin{align*}
\text{N} & \text{N} \text{N} \text{N} \text{H} \text{N} \\
\text{C}_6\text{H}_{13} & \text{N} \text{H} \text{O} \\
\end{align*}
\]

**6-Amino-N-9-heptyl-6-N-(4-hexylamino)ureidopurine (11).** 2,6-Diamino-9-heptylpurine (0.0191 g, 0.0769 mmol) was placed in an oven-dried pressure tube and dissolved in pyridine (2 mL). Hexylisocyanate (0.123 mL, 1.15 mmol) was added and the mixture was heated to 100 °C. The reaction was stirred for 3.5 h. The solvent was removed under reduced pressure, and the product was purified by column chromatography (1% CH$_3$OH/CH$_2$Cl$_2$) to yield 11 as a beige solid (0.0281, 97%). Mp 148–149 °C; $^1$H NMR (CDCl$_3$) $\delta$ 0.85 (m, 6H), 1.37 (m, 14H), 1.42 (q, $J = 3.7$ Hz, 2H), 1.85 (q, $J = 3.6$ Hz, 2H) 3.38 (q, $J = 3.0$ Hz, 2H), 4.02 (t, $J = 3.8$ Hz, 2H), 4.84
(s, 2H), 7.68 (s, 1H), 7.88 (s, 1H), 9.16 (t, J = 2.7 Hz, 1H). \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) 0.83 (m, 6H), 1.27 (m, 14H), 1.54 (q, J = 3.4 Hz, 2H), 1.75 (q, J = 3.4 Hz, 2H), 3.23 (t, J = 3.4 Hz, 2H), 3.99 (t, J = 3.6 Hz, 2H), 4.43 (s, 2H), 6.60 (s, 2H), 7.95 (s, 1H), 8.76 (s, 1H), 9.58 (t, J = 2.8 Hz, 1H).

\(^{13}\)C NMR (DMSO-\(d_6\)) \(\delta\) 13.9, 21.9, 22.1, 25.9, 26.0, 26.1, 28.1, 29.1, 29.9, 30.0, 31.0, 31.1, 42.5, 113.1, 139.9, 150.2, 152.7, 153.7, 158.0, 158.7. HRMS (ESI-FT-ICR) calculated for C\(_{19}\)H\(_{4}\)N\(_7\)O (M + H\(^+\)) 376.2819, found 376.2829.

6-Amino-N-9-(tert-butyl acetate)-6-N-(4-hexylamino)ureidopurine (12). 2,6-Diamino-9-heptylpurine (0.086 g, 0.325 mmol) was placed in an oven dried pressure tube and dissolved in pyridine (9 mL). Hexylisocyanate (0.517 mL, 4.88mmol) was added and the mixture heated to 100 °C. The reaction was stirred for 3.5 h. Solvent was removed under reduced pressure. The product was purified by column chromatography (1% CH\(_3\)OH/ CH\(_2\)Cl\(_2\)) to yield the final product as a beige solid (0.124 g, 97 %). Mp 242–245 ºC; \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 0.88 (m, 3H), 1.30 (m, 8H), 1.45 (s, 9H), 1.60 (q, J = 3.5 Hz, 2H), 3.37 (t, J = 2.9 Hz, 2H), 4.68 (s, 2H), 4.84 (s, 2H), 7.69 (s, 1H), 7.88 (s, 1H), 9.16 (t, J = 2.7 Hz, 1H). \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) 0.86 (m, 3H), 1.30 (m, H), 1.42 (s, 9H), 1.55 (m, 2H), 3.24 (t, J = 3.0 Hz, 2H), 4.82 (s, 2H), 7.93 (s, 1H), 8.88 (s, 1H), 9.58 (t, J = 2.8 Hz, 1H). \(^{13}\)C NMR (DMSO-\(d_6\)) \(\delta\) 13.9, 21.9, 22.1, 25.9, 26.0, 26.1, 28.1, 29.1, 29.9, 30.0, 31.0, 31.1, 42.5, 113.1, 139.9, 150.2, 152.7, 153.7, 158.0, 158.7. HRMS (ESI-FT-
ICR) calculated for C\textsubscript{18}H\textsubscript{30}N\textsubscript{7}O\textsubscript{3} (M + H)\textsuperscript{+} 392.2404, found 392.2409 calculated for C\textsubscript{18}H\textsubscript{29}N\textsubscript{7}O\textsubscript{3} (M + Na)\textsuperscript{+} 414.2224, found 414.2245.

![Chemical Structure](image)

6-Amino-N-9-(2,4,6-trimethylbenzyl)-2-N-(methoxy-acetamide)purine (18ax).

Compound 5a (0.0510 g, 0.181 mmol) was placed in an oven-dried three necked-round-bottomed flask and dried overnight. The starting material was dissolved in a 3:1 mixture of acetonitrile (46 mL) and pyridine (15 mL). Methoxy acetyl chloride (0.0410 mL, 0.450 mmol) was added. The reaction mixture was stirred until the starting material was consumed (1.5 h). The solvent was evaporated under a flow of nitrogen and the product was purified by column chromatography (2%-3% CH\textsubscript{3}OH/CH\textsubscript{2}Cl\textsubscript{2}). Purine 18ax was isolated as a white solid (0.056 g, 88%).

\[ ^1H \text{NMR (CDCl}_3 \text{)} \delta 2.25 (s, 6H), 2.30 (s, 3H), 3.51 (s, 3H), 4.16 (s, 2H), 5.24 (s, 2H), 5.27 (s, 2H), 6.93 (s, 2H), 7.17 (s, 1H), 8.86 (s, 1H) \]

\[ ^1H \text{NMR (DMSO-}d_6 \text{)} (100 °C) \delta 2.24 (s, 3H), 2.28 (s, 6H), 3.40 (s, 3H), 4.20 (s, 2H), 5.22 (s, 2H), 6.81 (s, 2H), 6.91 (s, 2H), 7.42 (s, 1H), 9.05 (s, 1H). \]

\[ ^13C \text{NMR (DMSO-}d_6 \text{)} (100 °C) \delta 18.7, 19.9, 40.6, 58.0, 71.7, 115.6, 128.2, 128.6, 136.9, 137.0, 138.5, 150.1, 151.7, 155.7, 167.7. \]

HRMS (ESI-FT-ICR) calculated for C\textsubscript{18}H\textsubscript{22}N\textsubscript{6}O\textsubscript{2} (M + H)\textsuperscript{+} 355.1877, found (M + Na)\textsuperscript{+} 377.1696.
N-9-(2,4,6-Trimethylbenzyl)-2,6-N-(methoxy-acetamide)purine (18ay). The product was isolated as an off-white solid (0.009 g, 14 %) from the same reaction mixture as compound 18ax. Mp 265–269 °C (decomp); \(^1\)H NMR (CDCl\(_3\)) \(\delta \) 2.24 (s, 6H), 2.30 (s, 3H), 3.51 (s, 3H), 3.52 (s, 3H), 4.17 (s, 2H), 4.25 (s, 2H), 5.31 (s, 2H), 6.93 (s, 2H), 7.35 (s, 1H), 8.95 (s, 1H), 9.18 (s, 1H). \(^1\)H NMR (DMSO-\(d_6\)) (100 °C) \(\delta \) 2.24 (s, 3H), 2.28 (s, 6H), 3.40 (s, 3H), 3.41 (s, 3H), 4.28 (s, 2H), 4.35 (s, 2H), 5.31 (s, 2H), 6.92 (s, 2H), 7.76 (s, 1H), 9.76 (s, 1H), 9.94 (s, 1H). \(^1\)H NMR (DMSO-\(d_6\)) (100 °C) \(\delta \) 18.8, 19.9, 42.9, 58.0, 58.1, 71.8, 72.4, 108.6, 127.7, 128.7, 137.1, 137.1, 139.6, 144.3, 148.4, 149.1, 168.1, 168.1. \(^{13}\)C NMR (DMSO-\(d_6\)) (100 °C) \(\delta \) 13.0, 21.2, 25.4, 27.4, 28.6, 30.4, 42.4, 58.0, 71.9, 82.4.

HRMS (ESI-FT-ICR) calculated for C\(_{21}\)H\(_{26}\)N\(_6\)O\(_4\) (M + H\(^+\)) 427.2088 found (M + Na\(^+\)) 449.1908.

6-Amino-N-9-heptyl-2-N-(methoxy-acetamide)purine (18cx). Compound 5c (0.030 g, 0.121 mmol) was placed in an oven dried three necked-round-bottomed flask and dried overnight. The starting material was dissolved in a 3:1 mixture of acetonitrile (31 mL) and pyridine (10 mL). Methoxy acetyl chloride (0.028 mL, 0.76 mmol) was added. The reaction mixture was stirred until the starting material was consumed (1.5 h). The solvent was evaporated under a flow of nitrogen and the product was purified by column chromatography (2% CH\(_3\)OH/CH\(_2\)Cl\(_2\)). The product was isolated as a white solid (0.032 g, 83%). Mp 201–202 °C (decomp); \(^1\)H NMR (CDCl\(_3\)) \(\delta \) 0.85 (m, 3H), 1.26 (m, 8H), 1.86 (m, 2H), 3.50 (s, 3H), 4.09 (t, J = 3.4 Hz, 2H), 4.29 (s, 2H), 7.68 (s, 1H). \(^1\)H NMR (DMSO-\(d_6\)) (100 °C) \(\delta \) 0.85 (t, 3H), 1.26 (m, 8H), 1.83 (m, 2H), 3.39 (s, 3H), 4.07 (t, J = 3.3 Hz, 2H), 4.23 (s, 2H), 6.78 (s, 2H), 7.94 (s, 1H), 9.05 (s, 1H). \(^{13}\)C NMR (DMSO-\(d_6\)) (100 °C) \(\delta \) 13.0, 21.2, 25.4, 27.4, 28.6, 30.4, 42.4, 58.0, 71.9,
115.8, 139.8, 150.1, 151.7, 155.6, 167.8. HRMS (ESI-FT-ICR) calculated for C_{13}H_{24}N_{6}O_{2} (M + H)^{+} 321.2034 found (M + H)^{+} 321.2037.

![Image](https://via.placeholder.com/150)

**N-9-Heptyl-2,6-N-(methoxy-acetamide)purine (18cy).** The disubstituted product was isolated as an off-white solid (0.006 g, 16 %) from the same reaction mixture as compound 18cx. Mp 204–206 °C; $^{1}$H NMR (CDCl$_3$) $\delta$ 0.85 (m, 3H), 1.27 (m, 8H), 1.89 (m, 2H), 3.50 (s, 3H), 3.53 (s, 3H), 4.15 (m, 2H), 4.18 (s, 2H), 4.26 (s, 2H), 7.89 (s, 1H), 8.92 (s, 1H), 9.25 (s, 1H). $^{1}$H NMR (DMSO-$d_6$) $\delta$ 0.83 (m, 3H), 1.22 (m, 8H), 1.78 (m, 2H), 3.34 (s, 3H), 3.37 (s, 3H), 4.12 (t, $J = 3.5$ Hz, 2H), 4.32 (s, 2H), 4.39 (s, 2H), 8.34 (s, 1H), 10.21 (s, 1H), 10.40 (s, 1H). $^{13}$C NMR (DMSO-$d_6$) (100 °C) $\delta$ 13.0, 21.2, 25.4, 27.4, 28.4, 30.4, 35.9, 42.8, 58.1, 58.2, 71.8, 71.9, 75.0, 118.9, 143.1, 148.2, 151.0, 160.9, 168.1. HRMS (ESI-FT-ICR) calculated for C$_{18}$H$_{28}$N$_{6}$O$_{4}$ (M + H)^{+} 393.2245 found (M + Na)^{+} 415.2064.

![Image](https://via.placeholder.com/150)

**6-Amino-N-9-(tert-butyl acetate)-6-N-(methoxy-acetamide)purine (18cd).** Compound 5d (0.0415 g, 0.157 mmol) was placed in an oven-dried three-necked- round bottomed flask and dried overnight. The starting material was dissolved in a 3:1 mixture of acetonitrile (40 mL) and
pyridine (13 mL). Methoxy acetyl chloride (0.0358 mL, 0.393 mmol) was added. The reaction mixture was stirred until the starting material was consumed (1.5 h). Solvent was evaporated under a flow of nitrogen and product purified by column chromatography (2% CH3OH/ CH2Cl2). The product was isolated as a white solid (0.044 g, 83%). Mp 196–197 °C; 1H NMR (CDCl3) δ 1.47 (s, 9H), 3.49 (s, 3H), 4.12 (s, 2H), 4.79 (s, 2H), 5.94 (s, 2H), 7.78 (s, 1H), 8.81 (s, 1H). 1H NMR (DMSO-d6) (100 °C) δ 1.43 (s, 9H), 3.38 (2, 3H), 4.22 (s, 2H), 6.85 (s, 2H), 7.93 (s, 1H), 9.08 (s, 1H). 13C NMR (DMSO-d6) (100 °C) δ 27.2, 29.8, 44.2, 58.0, 115.2, 140.3, 150.3, 151.9, 155.6, 166.1, 167.9. HRMS (ESI-FT-ICR) calculated for C14H20N6O4 (M + H)+ 337.1619, found (M + Na)+ 359.1438.

9-(tert-buty acetate)-2,6-N-(methoxy-acetamide)purine (18dy). The disubstituted product was isolated as an off-white solid (0.007 g, 13 %) from the same reaction mixture as compound 18dx. Mp 196–198 °C; 1H NMR (CDCl3) δ 1.47 (s, 9H), 3.50 (s, 3H), 3.36 (s, 3H), 4.14 (s, 2H), 4.24 (s, 2H), 4.87 (s, 2H), 7.97 (s, 1H), 8.94 (s, 1H), 9.26 (s, 1H). 1H NMR (DMSO-d6) δ 1.43 (2, 9H), 3.39 (s, 3H), 3.43 (s, 3H), 4.28 (s, 2H), 4.36 (s, 2H), 4.95 (s, 2H), 8.23 (s, 1H), 9.67 (s, 1H), 9.85 (s, 1H). 13C NMR (DMSO-d6) (100 °C) δ 27.2, 44.4, 58.0, 58.1, 71.7, 71.9, 82.0, 108.6, 118.4, 143.5, 148.3, 151.3, 165.8, 168.2. HRMS (ESI-FT-ICR) calculated for C17H24N6O6 (M + H)+ 409.1830, found 409.1819.
2-Amino-6-N-(benzylcarboxy amino)-N-9-(tert-butyl acetate)purine (12z). 2,6-Diamo-9-(tert-butyl acetate)purine (5d) (0.065 g, 0.25 mmol) was placed in an oven-dried two-necked round-bottomed flask. Dry degassed 1,4-dioxane (2 mL) was added followed by N-benzylloxycarbonyl imidazolium triflate95 (0.124 g, 0.356 mmol). The mixture was stirred for 20 h at room temperature. The solvent was removed under vacuum and the residue was purified by column chromatography (3% CH3OH/CH2Cl2). The product was isolated as a white powder (0.079 g, 81%). Mp 269–271 °C; 1H NMR (CDCl3) δ 1.45 (s, 9H), 4.63 (s, 2H), 5.19 (s, 2H), 5.24 (s, 2H), 7.36 (m, 5H), 7.65 (s, 1H), 8.80 (s, 1H). 13C NMR (CDCl3) δ 28.1, 44.8, 67.7, 83.6, 128.6, 128.7, 128.8, 135.9, 140.8, 150.2, 151.4, 153.4, 160.1, 163.3, 166.4. HRMS (ESI-FT-ICR) calculated for C_{17}H_{24}N_{6}O_{6} (M + H)^{+} 399.1775, found 399.1757.

6-Amino-2-N-(acetyl)-N-9-(tert-butyl acetate)purine (19x). Compound 5d (0.052 g, 0.196 mmol) was placed in an oven-dried two-necked round-bottomed flask. Methylene chloride (50 mL) was added followed by pyridine (17 mL). Acetic anhydride (0.046 mL) was added and the mixture was stirred for 43 h at room temperature. The solvent was removed under vacuum
and the residue purified by column chromatography (3% CH$_3$OH/CH$_2$Cl$_2$) to yield the product as
a white solid (0.040 g, 67% (77% based on recovered starting material)). $^1$H NMR (DMSO-$d_6$) $\delta$
1.41 (s, 9H), 2.18 (s, 3H), 4.85 (s, 2H), 7.19 (s, 2H), 7.97 (s, 1H), 9.74 (s, 1H). $^{13}$C NMR
(DMSO-$d_6$) $\delta$ 27.7, 43.6, 44.4, 82.0, 115.3, 150.5, 153.0, 156.0, 167.0. HRMS (ESI-FT-ICR)
calculated for C$_{13}$H$_{19}$N$_6$O$_3$ (M + H)$^+$ 307.1513 found 307.1518 calculated for C$_{13}$H$_{18}$N$_6$NaO$_3$ (M + Na)$^+$ 329.1333, found 329.1346.

2-Amino-6-N-(acetyl)-9-(tert-butyl acetate)purine (19z). The product was isolated from
the above reaction mixture as a yellow solid (0.009 g, 15%). Mp 259–262 °C; $^1$H NMR (CDCl$_3$)
$\delta$ 1.46 (s, 9H), 2.54 (s, 3H), 4.70 (s, 2H), 4.97 (s, 2H), 7.67 (s, 1H), 8.53 (s, 1H). $^1$H NMR
(DMSO-$d_6$) $\delta$ 1.39 (s, 9H), 2.20 (s, 3H), 4.780 (s, 2H), 6.37 (s, 2H), 7.88 (s, 1H), 10.05 (s, 1H).
$^{13}$C NMR (DMSO-$d_6$) $\delta$ 24.4, 27.6, 44.1, 82.0, 116.3, 149.7, 159.9, 167.0, 169.1. HRMS (ESI-
FT-ICR) calculated for C$_{13}$H$_{19}$N$_6$O$_3$ (M + H)$^+$ 307.1522 found (M + H)$^+$ 307.1522 calculated for
C$_{26}$H$_{36}$N$_{12}$NaO$_6$ (2M + Na)$^+$ 635.2773 found (2M + Na)$^+$ 635.2713.

NMR Experimental Parameters

NMR spectra were recorded on a Varian Inova spectrometer equipped with a 5 mm
indirect detection probe, operating at 500 MHz for $^1$H, 125 MHz for $^{13}$C, and at 50 MHz for $^{15}$N.
The samples were prepared as 0.1 M solutions in dimethyl sulfoxide-$d_6$, containing an equivalent
amount of tetramethylsilane and nitromethane. The $^1$H and $^{13}$C chemical are referenced to
tetramethylsilane, 0 ppm. The $^{15}$N chemical shifts are given on the liquid ammonia scale, and are referenced to internal nitromethane, 379.5 ppm. The temperature was 25 ºC.

Four experiments were run for each sample:

$^{1}$H spectrum was run in 1 transient, with an acquisition time of 5 s, and transformed with no apodization.

$^{13}$C spectrum was run in 1024 transients, with an acquisition time of 1.3 s, and transformed with a line broadening of 0.5 Hz.

The $^{1}$H – $^{13}$C gHMBC had in $f_2$ a spectral window from 10 ppm to -0.5 ppm and 4096 points, yielding a digital resolution of 1.3 Hz. The relaxation delay was 1 s. In $f_1$, the spectral window was from 170 ppm to 5 ppm, and 512 increments were acquired in 1 transient. The total acquisition time was 14 minutes. The experiment was optimized for a $^{1}$H – $^{13}$C long range coupling of 8 Hz. The number of points in the $f_1$ dimension was set to 2048, to provide a digital resolution of 0.085 ppm/point. In both $f_2$ and $f_1$ shifted Gaussian functions were applied, $g_f = 0.261$, $g_{fs} = 0.095$, $g_{f1} = 0.011$, $g_{fs1} = 0.010$.

The $^{1}$H – $^{15}$N gHMBC had in $f_2$ a spectral window from 10 ppm to – 0.5 ppm and 4096 points, yielding a digital resolution of 1.3 Hz. The relaxation delay was 1 s. In $f_1$, the spectral window was from 400 ppm to 50 ppm, and 1024 increments were acquired in 1 transient. The total acquisition time was 27 minutes. The experiment was optimized for a $^{1}$H – $^{15}$N long range coupling of 4 Hz. The number of points in the $f_1$ dimension was set to 4096, to provide a digital resolution of 0.085 ppm/point. In both $f_2$ and $f_1$ shifted Gaussian functions were applied, $g_f = 0.131$, $g_{fs} = 0.069$, $g_{f1} = 0.034$, $g_{fs1} = 0.019$. 
CHAPTER 5
CONCLUSIONS AND FUTURE DIRECTIONS

Summary and Conclusions

The general design, synthesis, and structure-property relationships of self-complementary QHB ureidodiaminopurines have been described. Urea formation at N_C2 affords a DADA motif, which is preorganized into a N3 conformer. The existence of this conformer has been verified by NOESY and VT NMR. Variable temperature studies also revealed QHB via the N3 conformer to be the predominant mode of assembly at the concentration studied. The N3 conformer and its associated dimer were further identified in the solid state. The stability of association ($K_{\text{dim}}$) was measured by sequential dilution and monitoring the intermolecular hydrogen-bonded urea proton by $^1$H NMR. The dimerization constants were calculated based on the chemical shift of the urea proton using well-known theory, and found to be $820 \pm 160 \text{ M}^{-1}$ and $980 \pm 290 \text{ M}^{-1}$ for $1a$ and $1b$, respectively.

The dimerization constants of the UDAPs were one to two orders of magnitude lower than the comparable ureidodiaminotriazine ($10^4 \text{ M}^{-1}$) and ureidodiaminopyrimidine ($10^5 \text{ M}^{-1}$) systems developed by Meijer and coworkers. Three new UDAPs were synthesized to derive structure-property relationships and understand how modifications at and remote from the hydrogen-bonded interface could influence self-association. The urea and N9 substituents were varied to see potential differences between alkyl and aryl ureas, and probe interactions between the N9 and urea substituents with respect to $K_{\text{dim}}$.

Dimers of various structures proved to have similar solution stabilities ($K_{\text{dim}} = 820–1100 \text{ M}^{-1}$), within the margin of error, with the exception of $9$. The consistency speaks to the compensating effects that typify most self-assembling systems. Apparent steric and electrostatic interactions between the bulky ester N9 substituent and the urea substituent of $9$ decreased $K_{\text{dim}}$. 
by almost half (530 ± 40 M\(^{-1}\)). Despite the lowered dimerization constant the ester substituent is an attractive point for future functionalization. The heptyl N9 substituent of 8 afforded a nice increase in solubility, and it is foreseen that functionalization of UDAP with a tert-butyl-7-heptanoate (or conversion of the tert-butyl to the methyl ester) will relieve unfavorable steric interactions, and afford nice solubility with a point of functionalization. Additionally, the results indicate that N9 can accommodate substituents of larger size than initially anticipated, even with an aryl urea present.

The synthesis of the alkyl UDAPs required new methodology as the reactivity of DAP with alkyl isocyanates was found to be sluggish (versus aryl isocyanates), a result rationalized in terms of transition state stabilization. This work ultimately produced the desired alkyl ureas, but found that the site of substitution was N\(_{c6}\) under various conditions. This unexpected regiochemistry spurred a study into the apparent differential nucleophilicity of the DAP amino groups. For this purpose an NMR protocol was developed to rigorously confirm the substitution position (as N\(_{c6}\) or N\(_{c2}\)). \(^1\)H, \(^{13}\)C, \(^1\)H–\(^{13}\)C, and \(^1\)H–\(^{15}\)N 1-D and 2-D NMR techniques nicely elucidated that alkyl isocyanates react regioselectively at N\(_{c6}\) under more pressing conditions (100 °C in a pressure tube).

Diaminopurine is a useful synthon for pharmaceutical and materials applications; thus, understanding the reactivity of DAP has practical implications for the development of purine chemistry. The reactivity of DAP was studied under common acylation conditions. The reaction with methoxy acetyl chloride occurred most rapidly at N\(_{c2}\) in agreement with the results of Porcher and coworkers;\(^{111}\) the ratio of the monosubstitution to disubstitution products (9:1) was invariant with solvent (from CH\(_3\)CN to CH\(_2\)Cl\(_2\)) and N9 substituent. These results, along with the seemingly disparate results of Neilsen and coworkers (N\(_{c6}\) substitution of DAP by a
benzyloxy carbonyl triflate), prompted a thermodynamic/kinetic study. Acylation of DAP with acetic anhydride and monitoring the reaction progress by NMR generally showed that acylation at the NC2 position was kinetically controlled under standard pyridine-catalyzed conditions. The conditions that were found to afford NC6 monosubstitution will require further study to elucidate the potential role of deprotonated purine intermediates, N7 transition state stabilization, and other more subtle effects.

**Future Directions**

**Reversible Materials**

Purines are functional aromatic heterocycles with the capacity for core functionalization at multiple sites. Functionalization can be achieved through palladium chemistry at C8, by alkylation at N9, and through nucleophilic/electrophilic substitution at C2 and C6. Not discussed much in this dissertation, but the subject of considerable laboratory investigation by the author, is covalent functionalization at C8. This chemistry is much less general than the previously mentioned N9 functionalization of DAP through routine alkylation. This position is the most promising, particularly given the apparent insensitivity of the dimerization to its substituents, for connection of UDAP to other scaffolds.

Extension at N9 via the ester moiety for UDAP application in PNA chemistry (figure 3-3) could be an early first goal. PNAs are nucleobase analogs that are composed of neutral peptide or peudopeptide backbones with regular or modified nucleobases. The pseudopeptide backbone typically consists of N-(2-aminoethyl) glycine and replaces the sugar-phosphate backbone of DNA/RNA, which is negatively charged under physiological conditions. The neutral backbone promotes increased binding affinity for the minor groove of DNA or RNA by reducing electrostatic repulsion. The PNA backbone features six atoms that separate the...
nucleobases to mimic DNA. PNAs are useful drug targets for gene therapy when the molecular basis of a disease is well known.

Our interest would be to see whether connection of the UDAPs to the PNA scaffold would provide them a framework within which to form self-complementary oligomers, much in the fashion of DNA and RNA. The binding properties of these oligomers with natural nucleobase oligomers could also be probed. Synthesis of the PNA oligomers would be done in two parts. The synthetic protocol for UDAP has been described herein. Saponification of the ester moiety will deliver a unit which is suitable for peptide coupling to ethyl-N-(2-t-butyloxy carbonylaminoethyl)glycinate (DCC and DhbtOH) to afford the PNA monomer after basic hydrolysis of the ester (figure 3-3). PNA oligomers would be formed through the Merrifield method on solid support.

Functionalization at N9 is also appealing for forming linear reversible polymeric systems. Although the degree of polymerization (DP) for UDAP is expected to be low based on $K_{\text{dim}}$, excluded volume interactions (phase segregation) can render these interactions stronger thereby increasing the DP. Recently Sivakova and coworkers introduced weakly associating nucleobase derivatives (1.5 ± 1 M$^{-1}$ for 6-N-(4-methoxy-benzoyl)-9-(dodecyl)adenine, and 5 ± 2 M$^{-1}$ for 1-(methoxycarbonylmethyl)-4-N-(4-tert-butylbenzoyl)cytosine, in CDCl$_3$) as self-assembling units to study phase segregation aided supramolecular polymerization. Low molecular weight amine terminated THF (a soft waxy material, MP = 20 °C) was functionalized with the nucleobase derivatives to yield thermoresponsive films (increased MP to 126–135 °C). Attaching the more strongly dimerizing UDAP units to a low molecular weight THF would be a nice extension of this work.
To do this, the N9 ester substituent would serve as a point of functionalization. Attachment of the purine moiety would involve saponification of the ester and then coupling to the amino functionalized poly(THF). Coupling may be realized by traditional peptide coupling as in the PNA, or through the method presented by Sivakova and coworkers (coupling of the purine to amino terminated poly(THF) via anhydride formation of the carboxylic acid and trimethylacetyl chloride and N-methyl morpholine; figure 5-1).  

![Figure 5-1. Synthesis of UDAP functionalized low molecular weight (< 2000 g mol\(^{-1}\)) poly(THF) for functional materials](image)

In addition to the ester functionality, a ribose group at the N9 position can serve as a point for functionalization. Zimmerman and coworkers have explored this option with a ureidoguanosine unit, and shown that the ribose nicely accommodates functionalization from the 5′ hydroxyl group without disrupting preorganization of the QHB motif. A ureido-2-aminoadenosine (UA, 23) counterpart is an interesting concept. The results of 1a suggest that a phenyl urea is sterically compatible with bulky N9 substituents. The synthesis of a phenyl ureido-2-aminoadenosine has been initially explored.

The synthesis started from commercially available 2,6-diaminoadenosine, 20. The ribose hydroxyl groups were protected following a procedure published by Beigelman and coworkers (figure 5-2). The 3′ and 5′ hydroxyl groups were protected by 1,3-
dichlorotetraisopropyldisiloxane (TIPDSiCl₂) to yield 21, and the 2’ hydroxyl group was methylated (22) under standard conditions. Urea formation was afforded through regioselective reaction at N₉ with phenyl isocyanate (23). Initial results were promising for this unit and further exploration is warranted.

Figure 5-2. Original synthesis of compound 23 (UA)

The limiting step in the synthesis of 23 was the methylation of the 2’ hydroxyl group that employed NaH as the base. As discovered in Chapter 3, the amino groups may also be deprotonated by sodium hydride. Hence the yield suffered as a consequence (31%, 22). The proposed remedy is to deprotonate the hydroxyl groups with pyridine, and protect the 2’, 3’, and 5’ hydroxyl groups in one step with trimethylsilyl chloride to yield 24 (figure 5-3).

Ureidodiaminopurines are new scaffolds for supramolecular applications. The quadruple hydrogen bonding face resembles those of systems created by Meijer and coworkers. The UDAPs, however, offer unique structural features that cannot be mimicked by the monocyclic ureidotriazines and ureidopyrimidines. Although the dimerization is weaker by an order of
magnitude, the extended aromatic surfaces offer additional sites for functionalization toward the preparation of functional materials.

![Diagram of chemical structures](image)

Figure 5-3. Proposed synthesis of UA

**Alternative QHB Platforms**

A new class of nucleobase-like complementary and self-complementary quadruple hydrogen bonding systems composed of [4,5]-fused pyrimidine rings were initially explored as a new generation of QHB units (figure 5-4). Urea functionalization of these systems derived from 2,4,6,8-tetrachloro[4,5-d]pyrimido pyrimidine (26) show potential as interesting supramolecular structures that mimic the ureidopyrimidine units of guanine (28) and diaminopurine (27) (figure 5-4). The urea variants would be the most rapidly accessed monomers for supramolecular polymeric systems yet reported, side-stepping the typical requirement for linking QHB recognition units together by a covalent tether (as shown in figure 5-1).

It has been confirmed through synthesis beginning from 26 that the 4 and 8 chloro groups are readily displaced by nucleophiles in a matter of minutes at low temperatures. Conversely, the 2 and 6 positions require elevated temperatures and extended reaction times for substitution. Based on the reactivity differences between the 2,6, and 4,8 positions of the core, it is expected that there will be a cooperative effect between the two faces of the monomer (essentially a chain effect, or communication from one pyrimidine unit to the next) upon formation of polymeric structures. The reactivity differences around the pyrimidopyrimidine would also be the basis for
possibly differentiating the two hydrogen bonding faces of the molecule; a hybrid structure of 27 and 28 is conceivable.

Figure 5-4. Pyrimidine rings fused at the [4,5] carbons show potential for a new class of ditopic self-assembling molecules.

The synthesis of the self-complementary ditopic ureidodiaminopurine mimic, 27, was proposed to be a simple two step procedure (figure 5-5). The starting material, 2,4,6,8-tetrachloro[4,5-d]pyrimido pyrimidine, was to be heated in methanolic ammonia in a sealed reaction vessel with a copper catalyst to yield 29. The tetraamino product would then be reacted with phenyl isocyanate to yield the final ditopic DADA QHB motif (27).

Preliminary results obtained suggest that harsher conditions are required for the substitution of the C6 amino group. At 185 °C in a sealed reaction vessel three of the chloro groups were substituted by methanolic ammonia even upon long reaction times (figure 5-6). A suggested remedy is to use higher temperatures with a stainless steel reaction vessel rather than a thick-walled glass vessel in the interest of safety.

Despite triamino substitution, 30 was reacted with phenyl isocyanate to yield 31 (although the position of phenyl urea has not been rigorously confirmed, only predicted based on the reactivity of 2,6-diaminopurines with phenyl isocyanate). Limited product solubility necessitated solution studies in DMSO-$d_6$ by $^1$H NMR for compound 31, where information on hydrogen
bonding could not be obtained. Solid phase analysis (IR or solid-state NMR) might be useful to characterize this polar system.

![Chemical Structures](image)

**Figure 5-5.** Synthesis of 4,8-diamino[4,5-d]pyrimido-2,6-bisphenylureidopyrimidine by a simple two-step process.

![Chemical Structures](image)

**Figure 5-6.** Products obtained in preliminary synthesis aimed at forming 4,8-diamino-6-chloro[4,5-d]pyrimido-2-N-phenylureidopyrimidine.

The proposed synthesis of the complementary QHB fused guanine mimic also began with 2,4,6,8-tetrachloro[4,5-d]pyrimido pyrimidine 26 (figure 5-7). In this case the differential reactivity of the 2,6 and 4,8 positions was used advantageously to place the benzyloxy groups at the 4 and 8 positions to yield 32 quantitatively. The amino groups were placed in the 2 and 6 positions by copper sulfate catalyzed substitution with ammonia in methanol to yield 33. Compound 34 was formed (74 %) by heating compound 24 with hexyl isocyanate and pyridine to 100 °C in a sealed reaction vessel. Reduction of the benzyloxy groups at C4 and C8 to give 28 was attempted by hydrogenation with Pd/C and H2 as used in the debenzylation of protected guanine. The desired product was not visibly present in the inseparable product mixture, and decomposition was evident.
Figure 5-7. Synthesis of 4,8-dibenzyloxy\[4,5-d\]pyrimido-2,6-N-hexylureidopyrimidine (34) and unsuccessful reduction by hydrogenation.

A possible remedy is to use a different hydrogen donor in the reduction (figure 5-8). Ammonium formate reduction has been shown to be compatible with the urea in the azide reduction to form compound 9. It should therefore be feasible for the formation of compound 28.

A mono-faced counterpart to the ditopic systems derived from 2,4-dichloroquinazoline (35) was also explored (figure 5-9). Compound 36 was visualized as a chain stopper for the self-complementary ditopic diaminopurine mimic (figure 5-9), but a mono-faced guanine mimic (37) will provide an equally interesting platform. Also, a substituent in the position indicated “R” could be used to explore interactions with the neighboring urea (e.g. an OCH₃ group).

Functionalization elsewhere on the quinazoline ring of either 36 or 37 will transition these monomers into functional materials. It was envisioned that the synthesis of these units from
dichloroquinazoline 35 would afford similar reactivity patterns at the C2 and C4 positions that were observed in the synthesis of DAP derivatives.

Figure 5-8. The suggested synthesis for 28 by catalytic hydrogen transfer from ammonium formate in refluxing methanol

Figure 5-9. The mono-faced counterparts to the ditopic systems 36 and 37 derived from 2,4-chloroquinazolines.

The 2,4-dichloroquinazoline starting material (35) was obtained from commercially available 2-aminobenzonitrile (39, figure 5-11). Compound 39 in the presence of diphosgene forms a phenyl isocyanate which undergoes ring formation by acetonitrile incorporation and subsequent chlorination to form compound 35.127 As expected, the reaction of compound 35 with ammonia in methanol did appear to show the reactivity preference encountered in the synthesis of compounds 29/30. Sites C2 and C4 react at different rates, and compound 40 was obtained. It is projected that harsher conditions will afford the desired 2,4-diaminoquinazoline (38), which can be urea-functionalized to yield a DADA QHB motif 36. Additionally, the synthesis of the
quinazoline-derived guanine analog is proposed to follow the same reaction sequence as the
ditopic system (28).

![Figure 5-10](image)

Figure 5-10. The proposed synthesis of 4-amino-2-N-phenylureidoquinazoline is similar to the
ditopic counterpart.

![Figure 5-11](image)

Figure 5-11. Synthesis of 2,6-dichloroquinazoline 29 from 2-aminobenzonitrile 27

Preliminary Experimental Results

2,6-Diamino-9-[3', 5'-O-tetraisopropyldisiloxane-1, 3-diyl]-β-D-ribofuranosyl]purine (21)

Vacuum-dried 2,6-diamino-9- (β-D-ribofuranosyl]purine (0.499 g, 0.177 mmol) was placed
in an oven-dried round bottomed flask. Under argon, DMF (5 mL) and pyridine (8 mL) were
added, and the suspension was cooled to 0 °C. The temperature was maintained at 0 °C with the
dropwise addition (over 30 min) of TIPDSiCl₂ (0.700 g, 2.12 mmol). The mixture was allowed
to warm to room temperature and the reaction was monitored to completion by TLC. The
reaction was quenched with ethanol (1 mL). The solvent was evaporated and the residue was
dissolved in EtOAc and rinsed with saturated aq NaHCO₃. Upon evaporation of the solvent, the product was purified by column chromatography (EtOAC/hexanes/EtOH 2:1:1) to afford 21 as a white solid (0.929 g, quantitative). ¹H NMR (DMSO- d₆) δ 1.02 (d, J = 3.7 Hz, 12H), 1.06 (m, 16H), 3.33 (d, J = 0.5 Hz, 2H), 4.00 (m, 3H), 4.29 (t, J = 4.8 Hz, 2H), 4.44 (q, J = Hz, 1H), 5.58 (d, J = 4.8 Hz, 2H), 5.71 (d, J = 1.4 Hz, 1H), 5.77 (s, 1H), 6.77 (s, 2H), 7.77 (s, 1H). LRMS (ESI-FT-ICR) calculated for C₂₂H₄₁N₆O₅Si₂ (M + H)⁺ 525, found 525.

![2,6-Diamino-9-[3', 5'-O-tetraisopropylsiloxane-1, 3-diy]-2'-O-methyl-β-D-ribofuranosyl]purine (22). Under a blanket of argon to a solution of compound 21 (0.375 g, 0.710 mmol) in DMF (7.3 mL) was added CH₃I (0.133 g, 2.10 mmol). The reaction was cooled to 0 °C and NaH (0.043 g, 1.8 mmol) was added. The reaction was stirred for 40 min at 0 °C, and then quenched with ethanol (1mL). Cold CH₂Cl₂ was added to the reaction mixture and the solution washed with saturated aq NH₄Cl and brine. The solvent was removed and the yellow oil recrystallized with ethanol/water (1:1) to yield a white solid (0.121 g, 31%). ¹H NMR (CDCl₃) δ 1.07 (m, 29H), 1.25 (m, 2H), 3.67 (s, 2H), 3.72 (d, J = 3.4 Hz, 1H), 3.98 (d, J = 3.2 Hz, 1H), 4.02 (d, J = 4.8 Hz, 1H), 4.10 (d, J = 4.3 Hz, 1H), 4.20 (d, J = 6.0 Hz, 1H), 4.60 (q, J = 4.5 Hz, 1H), 4.73 (s, 2H), 5.40 (s, 2H), 5.86 (s, 1H), 7.81 (s, 1H). LRMS (ESI-FT-ICR) calculated for C₂₃H₄₃N₆O₅Si₂ (M + H)⁺ 535 found 535.
6-Amino-2-N-(phenylamino)carbonyl-9-[3′, 5′-O-tetraisopropyldisiloxane-1, 3-diyl]-2′-O-methyl-β-D-ribofuranosyl]purine (23). Previously vacuum-dried 22 (0.05 g, 0.09 mmol) was placed in a 25 mL oven-dried two-necked round bottomed flask and stirred under vacuum for approximately one hour followed by 15 min of stirring under Ar. By syringe, CH$_2$Cl$_2$ (4.0 mL) was added followed by pyridine (0.01 mL) and phenylisocyanate (0.01 mL, 0.12 mmol). The mixture was allowed to stir at room temperature for 1.75 h until there was no observable change by TLC. The solvent was removed under vacuum and the residue was purified by column chromatography (5% MeOH, CH$_2$Cl$_2$) to yield 23 as a white solid (0.070 g, 89 %). $^1$H NMR (CDCl$_3$) δ 1.02 (m, 34H), 1.24 (s, 2H), 3.56 (s, 3H), 3.85 (d, $J = 4.68$ Hz, 1H), 4.06 (m, 1H), 4.28 (m, 2H), 4.55 (m, 1H), 6.05 (s 1H), 7.08 (t, $J = 8.06$ Hz, 2H), 7.35 (t, $J = 7.68$ Hz, 3H), 7.68 (t, $J = 7.78$ Hz, 2H), 8.04 (s, 1H), 11.8 (s, 1H). $^{13}$C NMR (CDCl$_3$) δ 13.0, 13.4, 13.9, 30.2, 59.9, 60.4, 70.1, 81.6, 84.0, 88.2, 115.5, 137.5, 151.6, 155.3, 159.1. HRMS (ESI-FT-ICR) calculated for C$_{30}$H$_{47}$O$_6$N$_7$Si$_2$Na (M + Na)$^+$ 680.3019, found 680.3017.
**4,8-Dibenzyloxy-2,6-dichloro-pyrimidino[5,4-d]pyrimidine (32).** Tetrahydrofuran (25 mL) was transferred by syringe to an oven dried two necked-round-bottomed flask under argon. Benzyl alcohol (0.314 mL, 3.02 mmol) and sodium hydride (0.123 g, 3.02 mmol, 60% dispersion in mineral oil) were added and the mixture was stirred for 30 min at room temperature. 2,4,6,8-Tetrachloropyrimido[5,4-d]pyrimidine (0.160 g, 0.610 mmol) was dissolved in THF (10 mL) and added dropwise to the reaction mixture. The mixture was stirred for 50 min after which the reaction was quenched by the addition of water. The product was extracted with chloroform and washed with brine. The organic layers were combined and the solvent was evaporated. The product was used without further purification (0.269 g, quant.). $^1$H NMR (CDCl$_3$) $\delta$ 5.68 (s, 4H), 7.35 (m, 6H), 7.53 (m, 4H). $^{13}$C NMR (CDCl$_3$) $\delta$ 71.1, 128.7, 128.9, 129.3, 134.2, 136.5, 156.6, 165.8. HRMS (ESI-FT-ICR) calculated for C$_{20}$H$_{15}$Cl$_2$N$_4$O$_2$ (M + H)$^+$ 413.2568, found 413.2664; calculated for C$_{20}$H$_{14}$Cl$_2$N$_4$O$_2$Na (M + Na)$^+$ 435.0391, found 435.0385.

![Image of 4,8-Dibenzyloxy-2,6-dichloro-pyrimidino[5,4-d]pyrimidine](image)

**2,6-Diamino-4,8-dibenzyloxy-pyrimidino[5,4-d]pyrimidine (33).** Compound 32 (0.054 g, 0.13 mmol) was placed in a pressure tube. Methanolic ammonia (15 mL) was added along with copper(II)sulfate (0.002 g, 0.01 mmol). The mixture was heated to 140–150 °C and stirred 19 h. The solvent was evaporated and the product was purified by column chromatography (5% CH$_3$OH/CH$_2$Cl$_2$) to yield a yellow/white powder (0.058 g, quant.). $^1$H NMR (DMSO-$d_6$) $\delta$ 5.38 (s, 4H), 7.36 (m, 10H), 7.44 (m, 4H). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 67.5, 127.6, 127.9, 128.3, 137.5,
159.6, 162.4. HRMS (ESI-FT-ICR) calculated for C_{20}H_{18}N_{6}O_{2}Na (M + Na)^+ 397.1383, found 397.1397; calculated for C_{20}H_{18}N_{6}O_{2}Na_{2} (2M + Na)^+ 771.2880, found 771.2894.

![Chemical structure](image)

2,6-N-dihexylamino-4,8-dibenzyloxyureidopyrimidino[5,4-d]pyrimidine (34).

Compound 33 (0.020 g, 0.053 mmol) was placed in a 75 mL pressure tube. Pyridine (2 mL) and hexylisocyanate (0.057 mL, 0.037 mmol) were added. The reaction mixture was heated to 100 °C and stirred 20 h. Solvent was removed under reduced pressure and the product purified by column chromatography (1% CH₃OH/CH₂Cl₂) to yield the product as a white solid (0.0303 g, 89%). \(^1\)H NMR (CDCl₃) \(\delta\) 0.86 (m, 6H), 1.29 (m, 12H), 1.59 (m, 4H), 3.38 (q, \(J = 3.2\) Hz 4H), 5.42 (s, 4H), 7.42 (m, 10H), 8.47 (s, 2H), 8.99 (t, \(J = 2.7\) Hz, 2H). \(^{13}\)C NMR (CDCl₃) \(\delta\) 14.2, 22.8, 26.9, 29.7, 31.7, 40.0, 128.2, 128.6, 128.9, 130.2, 135.9, 152.8, 157.2, 159.0. HRMS (ESI-FT-ICR) calculated for C_{34}H_{44}N_{8}O_{4}Na (M + Na)^+ 651.3377, found 651.3416; calculated for C_{34}H_{44}N_{8}O_{4}Na_{2} (2M + Na)^+ 1279.6863, found 1279.6957.
H1_DMSO_50_C

8-methoxy-[2,4,8-trimethylbenzy]-2N-phenyletidopurine_CDCB esp
6-chloro-9-heptyl-2-N-phenylureidoopine_{CDCl3}.jpg

9-heptyl-6-methoxy-2-N-phenylureidoopine_{CDCl3}.jpg
**APPENDIX B**  
**SOLID STATE DATA**

Table 1. Crystal data and structure refinement for 1a.

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BIOGRAPHICAL SKETCH

Alisha Michelle Martin was born on February 12, 1975, in Gainesville, Florida. She was the middle child of two brothers, and grew up in Jacksonville, Florida. She was always an independent soul who wanted to do things her way without question. She always held an appreciation for nature, and the way things work. Whether it be the physical or the mechanical, Alisha was always curious. She would sit for hours and ponder such realities. All she needed was encouragement to pursue the formal education necessary to make the most of her favorite past times; thinking, questioning, and dreaming.

Alisha had her own way of thinking. To view the world through her own eyes, but obtain the knowledge of the wiser world that made her pursuit challenging and therefore fulfilling. After high school Alisha attended Florida Community College at Jacksonville for a gain in momentum. From there she went on to earn her bachelors degree in Chemistry from the University of North Florida. Upon graduation from UNF, in spring of 2001, she obtained a position as an analytical chemist at an environmental testing company in Jacksonville, Florida, Environmental Conservation Laboratories (ENCO). It was quickly apparent that this would not satisfy her need to search for the answers to the bigger questions, and decided that continuing education was the best option. She began studies at the University of Florida in fall 2002, as a post-baccalaureate with Dr. Castellano. Alisha remained under the advisement of Ron Castellano to obtain a Ph. D. in organic chemistry in the Fall of 2007. It is through the diversity of her experiences and education that she has become a well rounded individual.