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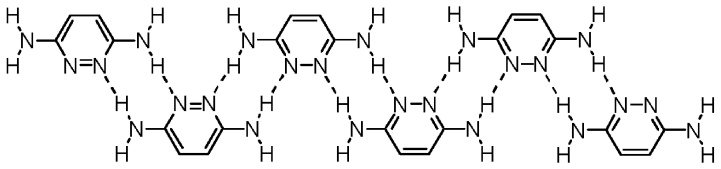
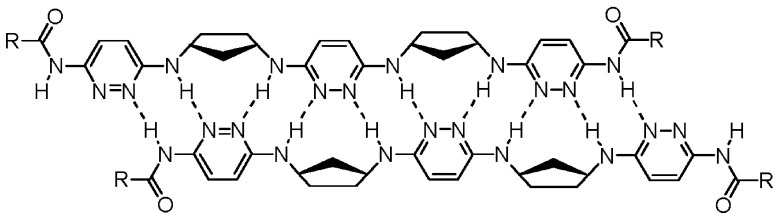
3,6-Diaminopyridazine Hydrogen Bonding Motif



High-Affinity Duplex Molecular Strands



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Duplex Molecular Strands Based on the 3,6-Diaminopyridazine Hydrogen Bonding Motif: Amplifying Small-Molecule Self-Assembly Preferences through Preorganization and Iterative Arrangement of Binding Residues

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Abstract: Structural parameters obtained through single-crystal X-ray diffraction analysis of the one-dimensional H-bonding motif expressed by 3,6-diaminopyridazine are applied to the design of related monomeric, dimeric, and trimeric duplex molecular strands. The mode of assembly and the interstrand affinity of the oligomers are established in solution by ^{1}H NMR dilution experiments, isothermal titration calorimetry (ITC), and vapor pressure osmometry. Single-crystal X-ray crystallographic analysis of the dimeric diaminopyridazine **2a** corroborates the intended duplex mode of assembly. Binding free energy per unimer ($-\Delta G°/n$) increases upon extension from monomer to dimer to trimer, signifying a positive cooperative effect. Micromolar binding affinity ($K_d = 1.25 \pm 0.1$ μM) was determined for the duplex trimer by ITC in 1,2-dichloroethane at 20 °C. These data provide further insight into the structural and interactional features of synthetic duplex oligomers required for high-affinity, high-specificity binding and define new recognition elements for use in nanoscale assembly.

Introduction

The design of functional synthetic oligomers and polymers requires reliable methods for the control of secondary and tertiary structure, as well as precise means of directing intermolecular aggregation. With the advent of foldamer chemistry, considerable progress toward the former objective has been made.[1] Progress in controlling noncovalent intermolecular aggregation of polymeric and oligomeric systems also has been made, as demonstrated by the design of polymers possessing noncovalent main chains[2,3] and the self-assembly of dendritic macromolecules,[4,5] block copolymers,[6,7] and polymers incorporating side-chain metal ion coordination sites,[8] side-chain H-bonding residues,[9] or even both![10] Recent cross-pollination between the fields of macromolecular and supramolecular chemistry has also stimulated investigations into the design of oligomers that assemble in a pairwise fashion through metal-ion coordination,[11] solvophobically driven π-stacking,[12] and interstrand hydrogen bond formation.[13,14]

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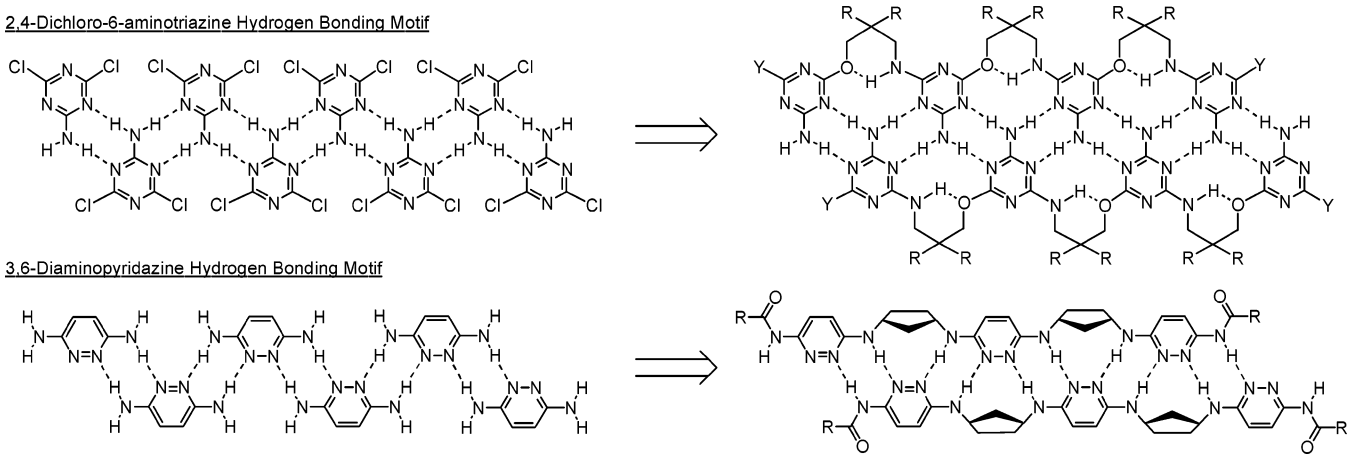


Figure 1. (Left) One-dimensional hydrogen-bonding motifs based on 2,4-dichloro-6-aminotriazine and 3,6-diaminopyridazine as established by single-crystal X-ray diffraction analysis. (Right) Corresponding duplex molecular strands.

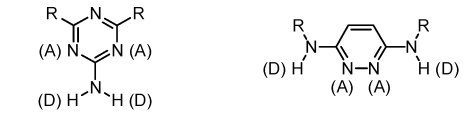
As part of a program in hydrogen-bond-mediated self-assembly, we recently introduced a strategy for the preparation of duplex molecular strands that assemble through the formation of interstrand hydrogen bonds.[15,16] Specifically, crystallographic data pertaining to monomers composing one-dimensional hydrogen-bonding motifs are used to define structural parameters for the design of corresponding oligomers (Figure 1). Given a commensurate relationship between the distances and geometries of the oligomer backbone and the noncovalent connectivities of the parent hydrogen-bonding motif, two-fold self-association of the oligomer should occur through formation of interstrand hydrogen bonds. Moreover, the inherent self-assembly preference of the monomeric subunits should be amplified through preorganization and cooperative binding, enabling formation of highly stable noncovalent assemblies.

This strategy for oligomer assembly was successfully applied to the design of high-affinity duplex molecular strands based on the 2-amino-4,6-dichlorotriazine hydrogen-bonding motif.[15] As anticipated, interstrand affinity was found to be critically dependent on the structural features of the inter-triazine linkage. For example, neopentyl amino alcohol-linked dimers exhibit association constants 3 orders of magnitude greater than those of the corresponding neopentyl glycol-linked systems, due to preorganization of the oligomer backbone induced through formation of an intramolecular hydrogen bond. Trimeric strands possessing neopentyl amino alcohol linkages exhibit nanomolar binding affinities in dichloroethane, as determined by isothermal titration calorimetry (ITC).

Ultimately, through the "covalent casting" of alternative one-dimensional hydrogen-bonding motifs, a family of synthetic duplex molecular strands that embody orthogonal recognition motifs may be developed. With this broad goal in mind, covalent casting of the previously undescribed 3,6-diaminopyridazine hydrogen-bonding motif was explored. Here we disclose the design, synthesis, and characterization of homologous duplex oligomers based on 3,6-diaminopyridazine. These data provide further insight into the structural and interactional features of synthetic duplex oligomers required for high-affinity, high-specificity binding in solution and define new recognition elements for use in nanoscale assembly (Figure 1).

Design of 3,6-Diaminopyridazine-Based Oligomers

The energy of a single hydrogen bond is generally smaller than the energetic difference between aggregation states that may form competitively and reversibly under equilibrium conditions. To promote formation of a single construct, the cooperative formation of multiple hydrogen bonds is required. This may be achieved by amplifying the self-assembly preferences of small molecules through their preorganization and iterative presentation along an oligomer backbone, as in the case of DNA. As described above, this general approach was successfully applied to the construction of high-affinity duplex oligomers based on the aminotriazine hydrogen-bonding motif. For the aminotriazine subunit, the hydrogen bond donor (D)−acceptor (A) sites are arranged in an "ADDA" array. The juxtaposition of hydrogen bond donor−acceptor sites in 3,6-diaminopyridazine, "DAAD", is inverted with respect to that of aminotriazine. Hence, duplex oligomers based on 3,6-diaminopyridazine were sought, as such strands should possess orthogonal recognition characteristics with respect to the related aminotriazine-based systems.



3,6-Diaminopyridazine was prepared and crystallized from ethanol. The anticipated one-dimensional hydrogen-bonding motif was corroborated by single-crystal X-ray diffraction analysis (Figure 2, top). On the basis of this crystallographic data, the distance between amine nitrogens, $D_{N,N}$, for alternate adjacent pyridazines of the hydrogen-bonded tape was determined to be 5.05 Å (Figure 2). This distance is roughly commensurate with the inter-nitrogen distance, $D'_{N,N}$, of 4.88

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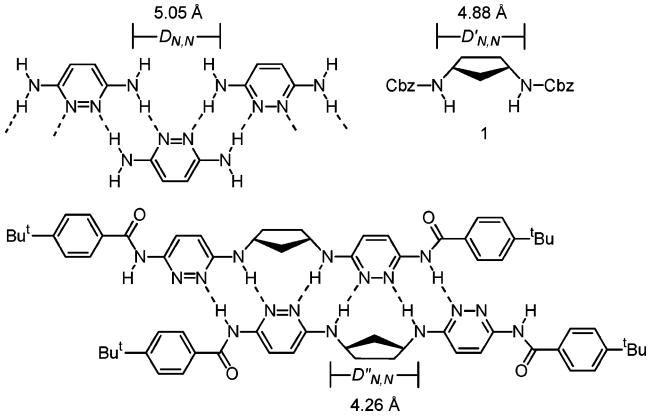

Figure 2. Defining a suitable inter-pyridazine linkage using interatomic distances (Å) taken from single-crystal X-ray diffraction data.

Å observed for the bis(Cbz-carbamate) **1** derived from *cis*-1,3-diaminocyclopentane as determined by single-crystal X-ray diffraction analysis (Figure 2). These distances are taken from molecules in the solid state and do not take into account distortions due to crystal packing forces. Nevertheless, these data suggest that the *cis*-1,3-cyclopentanyl moiety should serve as a suitable inter-pyridazine linkage. Additionally, the rigidity of the *cis*-1,3-cyclopentanyl linkage should discourage formation of intramolecular hydrogen bonds, thus promoting the desired duplex mode of assembly.

The veracity of this analysis is borne out by single-crystal X-ray diffraction analysis of the *cis*-1,3-diaminocyclopentane-linked bis(diaminopyridazine) **2a**, which reveals the intended duplex mode of assembly (Figure 2, bottom). Notably, the supramolecular connectivities evident in the parent 3,6-diaminopyridazine hydrogen-bonding motif persist upon introduction of a *cis*-1,3-cycopentanyl linkage. The "goodness-of-fit" of the covalent linkage may be approximated by comparing the geometries and interatomic distances found in the crystal structure of bis(diaminopyridazine) **2a** with those of the parent hydrogen-bonded tape. For **2a**, the distance, $D''_{N,N}$, between amine nitrogens of adjacent pyridazines is 4.26 Å. This distance is shorter than that found in the parent hydrogen-bonding motif ($D_{N,N}$ = 5.05 Å). The noncommensurate relationship between the covalent and noncovalent frameworks should cause strain to accumulate in higher oligomers, compromising positive cooperative effects. Again, this analysis is based on solid-state data and does not account for distortions due to crystal packing forces (Figure 3).

Synthesis of 3,6-Diaminopyridazine-Based Oligomers

For the preparation of homologous oligo(diaminopyridazines), a bidirectional synthetic strategy was envisioned. Concise access to bis(diaminopyridazines) **2a** and **2b** is achieved through the condensation of 6-chlorotetrazolo[1,5-*b*]pyridazine **3**[17] with *cis*-1,3-diaminocyclopentane[18] to provide bis(6-aminotetrazolo[1,5-*b*]pyridazine) **4**. Treatment of **4** with tributylphosphine,[19] followed by exposure of the isolable bis(iminophosphorane) to *p-tert*-butylbenzoic acid or 3,4,5-tributoxybenzoic acid, provides the bis(3,6-diaminopyridiazines) **2a** and **2b**, respectively (Scheme 1).[20]

A key step in the preparation of the homologous tris(3,6-diaminopyridazine) **5** involves double substitution of a 3,6-dihalopyridazine using a monoprotected *cis*-1,3-diaminocyclopentane derivative. Under classical S_NAr conditions, the reaction of 3,6-dichloropyridazine with an excess of the mono-*tert*-butoxycarbamate (Boc) of *cis*-1,3-diaminocyclopentane provides the product of monosubstitution. Presumably, the initially introduced alkylamino residue deactivates the pyridazine nucleus toward further substitution, and even under forcing conditions (heating at >200 °C) only trace quantities of the desired disubstituted product are observed. Recently reported conditions for the palladium-catalyzed amination of resin-bound 3-aminoalkyl-6-chloropyridazines with aniline were examined next[21] but proved to be ineffective when applied to the double substitution of 3,6-dichloropyridazine using the mono-Boc-carbamate of *cis*-1,3-diaminocyclopentane. A promising result was obtained using Buchwald's method for copper-catalyzed *N*-arylation of primary amines with aryl halides, which employs ethylene glycol as ligand.[22] Here, using 3,6-diiodopyridazine,[23] a 9% yield of the desired bis(adduct) **6** is obtained, along with substantial quantities of *O*-arylation products derived from ethylene glycol addition. However, direct coupling of unprotected *cis*-1,3-diaminocyclopentane, followed by treatment of the crude reaction product with Boc-anhydride, affords a 74% isolated yield of the bis(adduct) **6** over the two-step sequence. Elaboration of **6** to the trimer **5** is achieved by acid-promoted cleavage of the Boc protecting groups, followed by condensation of the free diamine with 6-chlorotetrazolo[1,5-*b*]pyridazine **3**, to provide the bis(tetrazolopyridazine) **7**. Exposure of **7** to tributylphosphine, followed by condensation of the isolable bis(iminophosphorane) with 3,4,5-tributoxybenzoic acid, provides the tris(3,6-diaminopyridiazine) **5**. Although compounds **5**−**7** undoubtedly exist as equimolar mixtures of stereoisomers, that is, a chiral racemic isomer of C_2-symmetry and a corresponding meso-isomer, the ^{1}H and ^{13}C spectra corresponding to these isomers appear identical (Scheme 2). Finally, application of this synthetic strategy toward the design of higher oligomers is underway; however, due to issues of solubility, 1,4-diamino-6,7-dialkoxyphthalazine, which presents an identical array of hydrogen bond donor−acceptor sites, serves as the monomeric subunit.

Finally, the monomeric compounds 3,6-diaminopyridazine and **8** were prepared for the purpose of studying their modes of aggregation in the solid state and in solution, respectively. Although 3,6-diaminopyridazine is a known compound,[24] we found the condensation of 6-chlorotetrazolo[1,5-b]pyridazine **3** with ammonia, followed by Staudinger reduction, to be more convenient than the literature preparation. Monomer **8** was prepared in an analogous fashion (Scheme 3).

Self-Assembly in Solution

Isothermal Titration Calorimetry (ITC). The association of monomeric diaminopyridazine **8**, bis(diaminopyridazine) **2b**,

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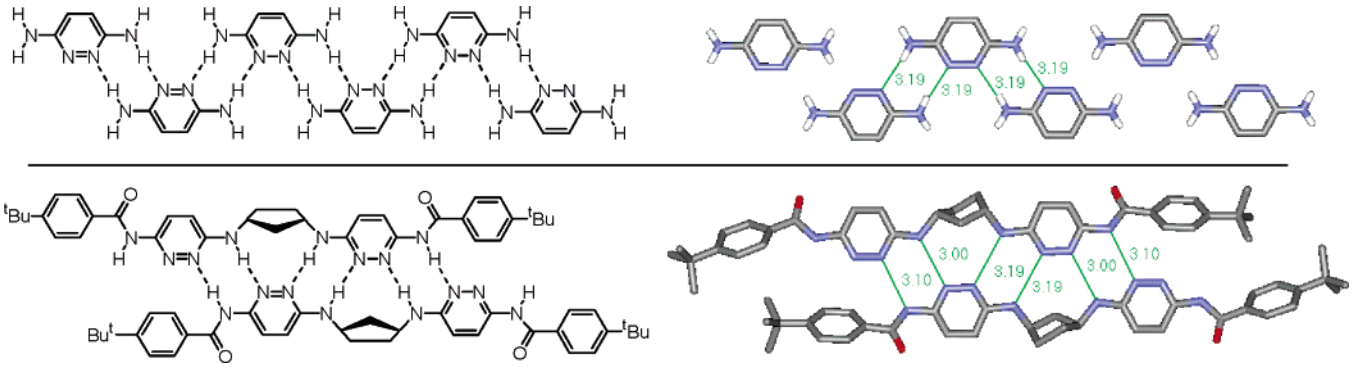
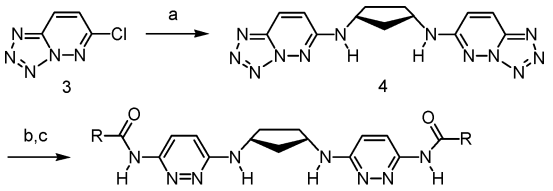


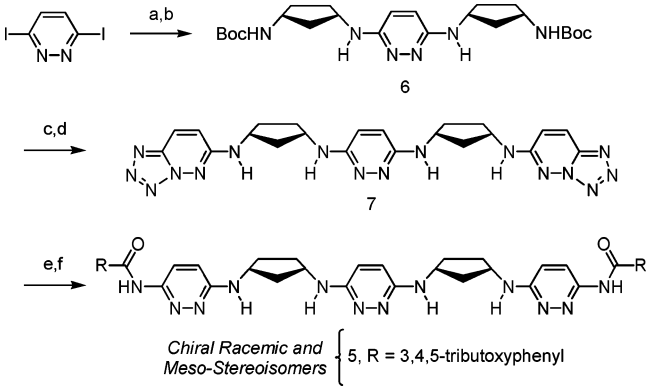
Figure 3. (Top) Anticipated one-dimensional hydrogen-bonding motif based on 3,6-diaminopyridazine as corroborated by single-crystal X-ray diffraction analysis. (Bottom) Anticipated duplex assembly of bis(3,6-diaminopyridazine) **2a** as corroborated by single-crystal X-ray diffraction analysis. The noncovalent connectivities persist upon introduction of the *cis*-1,3-cyclopentanyl linkage.

Scheme 1. Synthesis of Bis(3,6-diaminopyridazines) **2a** and **2b**[a]



[a] Reagents and conditions: (a) *cis*-1,3-diaminocyclopentane hydrochloride, K_2CO_3, CH_3CN, 180 °C, sealed tube, 85%. (b) *n*-Bu_3P, CH_3CN, 130 °C, sealed tube, 80%. (c) RCO_2H, $PhCH_3$, 150 °C, sealed tube, R = *p-tert*-butylphenyl, 30%, R = 3,4,5-tri-*n*-butoxyphenyl, 27%.

Scheme 2. Synthesis of Tris(3,6-diaminopyridazines) **6a** and **6b**[a]



[a] Reagents and conditions: (a) CuI (5 mol %), *cis*-1,3-diaminocyclopentane (600 mol %), $HOCH_2CH_2OH$ (200 mol %), K_3PO_4 (410 mol %), DMF (0.3 M), 95 °C. (b) Boc_2O (1200 mol %), 25 °C, 74% over two steps. (c) Trifluoroacetic acid, 0 °C. (d) chlorotetrazole **3** (210 mol %), K_2CO_3 (980 mol %), 95 °C, 80% over two steps. (e) PBu_3, CH_3CN, 180 °C, 60%. (f) Tri-*n*-butoxybenzoic acid (460 mol %), $PhCH_3$, 150 °C, sealed tube, 26%.

and tris(diaminopyridazine) **5** was investigated by isothermal titration calorimetry (ITC). ITC experiments were performed in 1,2-dichloroethane rather than chloroform to circumvent error incurred by evaporation. The dipole moment of $CDCl_3$ (μ = 1.1 D) is lower than that of 1,2-DCE (μ = 1.8 D). Consequently, hydrogen bond interactions are somewhat stronger in $CHCl_3$ than in 1,2-dichloroethane.[25] Concentrated analyte solutions were injected into a reservoir initially containing neat 1,2-dichloroethane, and enthalpy changes were monitored. A nonlinear least-

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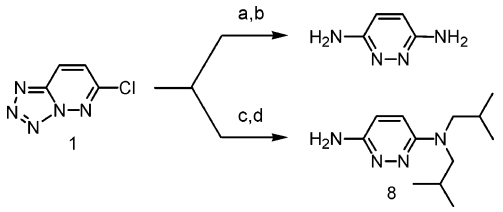
Scheme 3. Synthesis of 3,6-Diaminopyridazine and Monomer **7**[a]



[a] Reagents and conditions: (a) NH_4OH, 95 °C, 69%. (b) PBu_3, 180 °C, then HCl(aq), 100 °C, 40%. (c) $HN[CH_2CH(CH_3)_2]$, 130 °C, 90%. (d) PBu_3, CH_3CN, 180 °C, then HCl(aq), 100 °C, 80%.

squares minimization protocol was used to fit the experimental curve to a two-fold self-association model. Experiments were repeated a minimum of three times to ensure reproducibility. The average values obtained for K_d and $\Delta H°$ were used to calculate binding free energy ($\Delta G°$) and enthalpy ($T\Delta S°$).

The ITC analysis of monomeric diaminopyridazine **7** reveals an association constant of 5 M^{-1}. Upon strand extension from monomer **8** to dimer **2b**, an association constant of 870 M^{-1} is observed, representing a significant increase in binding affinity. Finally, for the homologous trimer **5**, ITC analysis indicates a binding constant of 8.0×10^5, representing an increase in binding affinity of approximately 3 orders of magnitude (Table 1).

^{1}H NMR Dilution Studies. To corroborate the association constants obtained by ITC analysis, the monomeric diaminopyridazine **8**, bis(diaminopyridazine) **2b**, and tris(diaminopyridazine) **5** were subjected to ^{1}H NMR dilution analysis in anhydrous ethanol-free $CDCl_3$ using a 300 MHz NMR spectrometer. Association constants were calculated by fitting the observed data to a two-fold self-association model using an iterative minimization protocol.[26]

^{1}H NMR dilution analysis of monomeric diaminopyridazine **8** reveals an association constant of 10 M^{-1} ($\log K = 1.0 \pm 0.1$), which is in good agreement with that determined by ITC. An association constant of 3.4×10^3 M^{-1} is calculated for the corresponding dimeric diaminopyridazine **2b** ($\log K = 3.5 \pm 0.8$). This value is somewhat higher than that determined by ITC, which is likely due to the aforementioned considerations of solvent polarity ($CDCl_3$, m = 1.1 D; 1,2-DCE, m = 1.8 D). Finally, tris(3,6-diaminopyridazine) **5** was subjected to ^{1}H NMR

(26) Dimerization constants were obtained using the computer program ChemEquili developed by Dr. Vitaly Solov'ev. For a detailed description, see: Solov'ev, V. P.; Baulin, V. E.; Strakhova, N. N.; Kazachenko, V. P.; Belsky, V. K.; Varnek, A. A.; Volkova, T. A.; Wipff, G. *J. Chem. Soc., Perkin Trans. 2* **1998**, 1489.

Table 1. Thermodynamic Parameters of Duplex Binding As Determined by ITC in Anhydrous 1,2-Dichloroethane at 20 °C[a]

compd	*n*	N_{HB}	K_d	K_a (M^{-1})	$\Delta G°$	$\Delta H°$	$T\Delta S°$	$\Delta G°/N_{HB}$
8	1	2	0.19 ± 0.01 M	5	−0.95	−7.5 ± 0.3	−6.5	−0.48
2b	2	6	1.14 ± 0.1 mM	870	−3.94	−8.8 ± 0.1	−4.9	−0.66
5	3	10	1.25 ± 0.12 μM	8.0×10^5	−7.92	−20.2 ± 0.7	−12.3	−0.79

[a] *n*, oligomer length; N_{HB}, number of H-bonds per duplex; K_d, dissociation constant; K_a, $1/K_d$; $\Delta G/n$, binding free energy per aminopyridazine; ΔG/HB, binding free energy per H-bond. Units of $\Delta G°$, $\Delta H°$, and $T\Delta S°$ are kcal mol^{-1}. Ethanol-free 1,2-dichloroethane was used.

Table 2. Vapor Pressure Osmometry (VPO) Results Based on Calibration with Sucrose Octaacetate (SO)[a]

compd	concn range (mM)	MW (duplex)	R^2	M_{obs}	M_{obs}/MW
2b	6.59−47.4	1854.36	0.928	1821.6	0.98
5	1.0−9.2	2206.8	0.952	2435.1	1.10

[a] VPO analysis was conducted at 40 °C in anhydrous, ethanol-free 1,2-dichloroethane.

dilution analysis. For **5**, no changes in chemical shift were observed upon dilution, suggesting persistence of the duplex at the limits of ^{1}H NMR detection.

Vapor Pressure Osmometry (VPO). To corroborate the intended mode of assembly, the binding stoichiometry of bis(diaminopyridazine) **2b** and tris(diaminopyridazine) **5** was investigated by vapor pressure osmometry (VPO) in anhydrous, ethanol-free 1,2-dichloroethane. Sucrose octaacetate (SO; MW = 678.6 g/mol) was used as the calibration standard. The ratio of observed molar mass (M_{obs}) to duplex molecular weight (MW) was calculated. For both bis(diaminopyridazine) **2b** and tris(diaminopyridazine) **5**, M_{obs}/MW values consistent with the intended duplex mode of aggregation were observed. That is, for both **2b** and **5**, M_{obs}/MW equaled approximately 1, suggesting formation of a 1:1 complex in solution. The correlation coefficient R^2 (from the linear regression used to analyze VPO data) is high for both **2b** and **5**, suggesting that the aggregation state does not vary over the concentration range studied (Table 2).

Discussion and Summary

The previously unknown one-dimensional hydrogen-bonding motif based on 3,6-diaminopyridazine has been utilized as a template for the construction of related dimeric and trimeric duplex molecular strands **2a**,**b** and **5**, respectively. In solution, ITC, ^{1}H NMR, and VPO data strongly support the intended duplex mode of assembly for monomer **8**, dimer **2b**, and trimer **5**. Corroborative evidence for the duplex mode of assembly stems from single-crystal X-ray crystallographic analysis of the bis(diaminopyridiazine) **2a**, which clearly reveals the intended duplex mode of assembly. Higher oligomers were not investigated due to solubility issues.

It should be noted that isomeric "head-to-head" and "head-to-tail" binding modes are possible for bis(diaminopyridazine) **2b** and the meso form of tris(diaminopyridazine) **5**. Additionally, the chiral racemic form of trimer **5** may form diastereomeric duplex aggregates. The observance of a single apparent association constant for **2b** and **5** by ITC, and in the former case by ^{1}H NMR as well, suggests that these isomeric binding modes are roughly equienergetic.

As anticipated for hydrogen bond formation in organic media, duplex binding is enthalpically driven. The binding free energy per unimer ($-\Delta G°/n$) increases significantly upon extension from monomer to dimer to trimer, indicating a strong positive cooperative effect. Thus, the *cis*-1,3-cyclopentanyl linking group of the oligomer backbone preorganizes 3,6-diaminopyridazine residues, amplifying the inherent self-assembly preference of the monomers. While the *cis*-1,3-cyclopentanyl linkage is sufficiently rigid to mitigate or preclude intramolecularly folded states, it is of sufficient flexibility that compensation for any noncommensurate relationships between the covalent and noncovalent connectivities is possible.

In conclusion, dimeric and trimeric duplex molecular strands based on the hitherto unknown hydrogen-bonding motif of 3,6-diaminopyridazine have been developed, and their duplex mode of assembly has been corroborated in solution and in the solid state. Future work will focus on the "covalent casting" of alternative one-dimensional hydrogen-bonding motifs, as well as studies that establish the orthogonality of the diaminopyridazine molecular strands in relation to the previously developed aminotriazine-based oligomers.

Experimental Section

General. Reagents were used as received from Aldrich unless otherwise indicated. All reactions were carried out under an atmosphere of argon unless otherwise indicated. Anhydrous tetrahydrofuran (THF) and diethyl ether were distilled from sodium/benzophenone ketyl prior to use. Dry toluene was distilled over CaH_2 before use. Acetonitrile was used as HPLC grade from Fischer Scientific Co. Anhydrous dimethylformamide (DMF) was used from passage through two columns of activated molecular sieves and contained less than 50 ppm H_2O by Karl Fischer coulomeric moisture analysis. Copper(I) iodide was purchased from Strem Chemical. Anhydrous potassium phosphate was purchased from Fluka. Tri-*n*-butylphosphine (97%) was purchased from Aldrich. All other solvents were technical unless noted. Column chromatography was performed using Merck silica gel 60 as the solid support. All NMR spectra were recorded on Varian 300 MHz or 400 MHz or Bruker ACF-250 instruments at standard temperature and pressure. ^{1}H NMR and ^{13}C chemical shifts are reported in δ units, parts per million (ppm) relative to the chemical shift of residual solvent. Reference peaks for chloroform in ^{1}H NMR and ^{13}C NMR spectra were set at 7.27 and 77.0 ppm, respectively. Reference peaks for DMSO in ^{1}H NMR and ^{13}C NMR spectra were set at 2.49 and 39.5 ppm, respectively. Deuterated solvents were used as purchased from Cambridge Isotope Laboratories, Inc., and used as received. IR spectra were recorded on a Nicolet Avatar 380 FT-IR spectrometer. Low-resolution chemical ionization (CI) mass spectra were obtained on a Finnigan MAT TSQ-70 instrument, high-resolution mass spectra (HRMS) were obtained with a VG Analytical ZAB2-E instrument. Melting points were obtained on a Thomas-Hoover UniMelt apparatus and are uncorrected.

Vapor Pressure Osmometry (VPO). VPO analyses were performed on a Jupiter 833 vapor pressure osmometer thermostated at 40 °C. Sucrose octaacetate used for VPO calibration was purchased from Jupiter Instrument Co., Inc. 1,2-Dichloroethane was freshly distilled from P_2O_5 prior to use. VPO data were analyzed by linear regression using EXCEL software. Reported results represent single experimental runs.

Isothermal Titration Calorimetry (ITC). Analyses were performed on a MicroCal VP-ITC instrument thermostated to 20 °C. Reported results represent the average of three or more runs. 1,2-Dichloroethane

was prepared by distillation from P_2O_5, storage over molecular sieves, and filtration through a 0.45 μm nylon membrane. The solvent was degassed by sonication in vacuo prior to each run. Following is a summary of the mathematical model used for data analysis:

$$f_{\text{monomer}} + f_{\text{dimer}} = 1$$

$$f_{\text{dimer}} = 1 - K_d[(1 + 8A_0/K_d)^{1/2} - 1]/4A_0 \quad (1)$$

where f_{dimer} is the mole fraction in the associated state, K_d is the dissociation constant (mol/L), A_0 is the analyte concentration.

$$A_0(i-1) = A_0(i) - (\text{syr} \times V_{\text{inj}})/(V_{\text{inj}} + V_o) \quad (2)$$

$$q_{\text{obs}}(i) = {}^1/_2\Delta H^\circ[n(i)\, f_{\text{dimer}}(i) - n(i-1)\, f_{\text{dimer}}(i-1) - n(\text{inj})\, f_{\text{dimer}}(\text{inj})] \quad (3)$$

where ΔH° is the molar enthalpy of binding, $q_{\text{obs}}(i)$ is the integrated heat observed at injection i, $n(i)$ is the number of moles of analyte after injection i, $n(i-1)$ is the number of moles of analyte prior to injection i, $n(\text{inj})$ is the number of moles of analyte in V_{inj}, syr is the concentration of analyte in syringe, V_{inj} is the injection volume, and V_o is the sample cell volume.

Substitution of (1) and (2) into (3) yields an expression from which K_d and ΔH_o were computed from raw ITC data by nonlinear least-squares curve fitting in the Origin software package.

NMR Dilution Experiments. Dilution studies were carried out by incrementally diluting concentrated analyte samples while obtaining 300 MHz ^{1}H NMR spectra. For each experiment, if significant concentration-dependent shifting of spectral signals was observed, the data were analyzed in terms of a two-fold self-association model to obtain a value for log(K). For **8**, data from the amine hydrogens were processed. For **2b**, the internal pyridazine NH signals were processed.

Diisobutyltetrazolo[1,5-*b*]pyridazin-6-ylamine. To a round-bottomed flask (25 mL) charged with 6-chlorotetrazolo[1,5-*b*]pyridazine **1** (0.2 g, 1.3 mmol) was added diisobutylamine (1 mL). The reaction mixture was stirred for 3.5 h at 130 °C. After the mixture was cooled to 25 °C, water (20 mL) was added. The aqueous phase was extracted with EtOAc (3 × 50 mL). The combined organic phases were dried ($MgSO_4$), filtered, and evaporated directly onto silica. The crude material was subjected to flash chromatography (SiO_2:20% EtOAc in hexanes) to give the title compound as a white solid (0.29 g, 1.2 mmol, 90%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.93 (d, J = 9.9 Hz, 1H), 7.23 (d, J = 9.9 Hz, 1H), 3.26 (d, J = 7.5 Hz, 4H), 1.90−2.00 (m, 2H), 0.75 (d, J = 6.6 Hz, 12H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.2, 139.3, 123.0, 117.2, 57.9, 26.7, 19.9. IR (KBr): 3056, 2960, 2869, 1620, 1562, 1507, 1466, 1428, 1367, 1237, 1089 cm^{-1}. HRMS (CI): m/z [M + 1]$^+$ found 249.1835, calcd 249.1828 for $C_{12}H_{21}N_6$. Mp = 108−110 °C.

[3-(Benzyloxycarbonylamino)cyclopentyl]carbamic Acid Benzyl Ester (1). To a suspension of *cis*-1,3-diaminocyclopentane hydrogen chloride salt (0.2 g, 1.16 mmol, 100 mol %) in CH_3CN (10 mL) was added potassium carbonate (0.8 g, 5.81 mmol, 500 mol %), followed by benzyl chloroformate (0.793 g, 4.65 mmol, 400 mol %). The reaction was stirred at 25 °0 for 22 h, at which point a precipitate was formed and was collected by filtration. The mother liquor was partitioned between water and EtOAc. The organic layer was separated, dried ($MgSO_4$), filtered, and evaporated to provide a solid residue, which was combined with the earlier precipitate. The combined solid residues were recrystallized from EtOAc to afford the title compound as a white solid (0.36 g, 1.0 mmol, 90%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.25−7.30 (m, 10H), 5.00 (s, 2H), 3.75−3.90 (m, 2H), 2.10−2.25 (m, 1H), 1.77−1.85 (m, 2H), 1.48−1.55 (m, 2H), 1.25−1.45 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 155.4, 137.2, 128.3, 133.3,127.8, 65.1, 50.2, 30.5. IR (KBr): 1645, 1262 cm^{-1}. HRMS (CI): m/z [M + 1]$^+$ found 369.1799, calcd 369.1814 for $C_{21}H_{25}N_2O_4$. Mp = 139−142 °C.

***N,N′*-Bis(tetrazolo[1,5-*b*]pyridazin-6-yl)cyclopentane-1,3-diamine (4).** To a sealed tube apparatus charged with *cis*-1,3-cyclopentyldiamine dihydrochloride salt (0.5 g, 2.89 mmol, 100 mol %) and potassium carbonate (1.6 g, 11.56 mmol, 400 mol %) was added 6-chlorotetrazolopyridazine **3** (0.95 g, 6.07 mmol, 210 mol %), followed by CH_3CN (2 mL). The reaction vessel was sealed, placed in a 130 °C oil bath, and allowed to stir for 6 h. The reaction mixture was removed from the heating bath and allowed to cool to 25 °C. To the reaction mixture were added water and EtOAc. The reaction mixture was filtered, and the solid was collected and dried in vacuo to afford **2** as a brown solid (0.83 g, 2.46 mmol, 85%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.21 (s, 2H), 8.21 (d, J = 9.6 Hz, 2H), 7.17 (d, J = 9.6, 2H), 4.0−4.4 (m, 2H), 2.64−2.73 (m, 1H), 2.00−2.30 (bm, 2H), 1.70−2.00 (bm, 2H), 1.57−1.66 (m, 1H). ^{13}C NMR (63 MHz, DMSO-d_6): δ 155.2, 139.9, 122.9, 121.0, 50.9, 38.2, 30.2. IR (KBr): 3088, 3056, 2924, 1627, 1581, 1507, 1360, 1289, 1237, 1100, 838 cm^{-1}. HRMS (CI): m/z [M + 1]$^+$ found 339.1541, calcd 339.1543 for $C_{13}H_{15}N_{12}$. Mp = 280 °C (dec).

Bis(iminophoshorane) en Route to 2a and 2b. To an oven-dried sealed tube apparatus charged with bis(tetrazolo[1,5-*b*]pyridazine) **4** (0.4 g, 1.18 mmol, 100 mol %) was added tri-*n*-butylphosphine (1.43 g, 7.08 mmol, 600 mol %), followed by CH_3CN (4 mL). The reaction vessel was sealed, placed in a 180 °C oil bath, and allowed to stir for 3 h. The reaction mixture was removed from the heating bath and allowed to cool to 25 °C. A precipitate was formed and was collected by filtration. The precipitate was then washed with EtOAc (5 × 20 mL). The residue was dissolved in dichloromethane (DCM), and the solid impurities were removed by filtration. Upon removal of the DCM in vacuo, the title compound was obtained as a light brown solid (0.65 g, 0.94 mmol, 80%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.75 (d, J = 9.6 Hz, 2H), 6.51 (dd, J = 2.1 Hz, J = 9.6 Hz, 2H), 4.23 (bs, 4H), 2.46−2.55 (m, 1H), 2.00−2.20 (m, 14H), 1.60−1.80 (m, 2H), 1.30−1.60 (m, 25H), 0.91 (t, J = 7.2 Hz, 18H). ^{31}P (121.5 MHz, $CDCl_3$): δ 33.00. ^{13}C (75 MHz, $CDCl_3$): δ 160.6, 152.5, 125.6, 125.3, 117.8, 52.3, 41.3, 31.9, 24.3, 24.2, 24.1, 24.0, 23.9, 23.5, 13.6. IR (KBr): 3115, 3055, 2957, 2933, 2872, 1574, 1448, 1377, 1293, 1002, 837 cm^{-1}. HRMS (CI): m/z [M + 1]$^+$ found 687.5113, calcd 687.5120 for $C_{37}H_{69}N_8P_2$. Mp = 218−222 °C.

Bis(3,6-diaminopyridazine) (2a). To an oven-dried sealed tube apparatus charged with the bis(iminophosphorane) derived from **4** (1.0 g, 1.46 mmol, 100 mol %) and 4-*tert*-butylbenzoic acid (1.04 g, 5.84 mmol, 400 mol %) was added dry toluene (10 mL). The reaction vessel was sealed, placed in a 150 °C oil bath, and allowed to stir for 24 h. The reaction mixture was removed from the heating bath and allowed to cool to 25 °C. The resulting mixture was directly subjected to flash column chromatography (SiO_2:3% 2-propanol in DCM) to afford the title compound as a white solid (0.27 g, 0.44 mmol, 30%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.80 (s, 2H), 8.00 (d, J = 7.5 Hz, 4H), 7.90 (d, J = 9.6 Hz, 2H), 7.53 (d, J = 6.6 Hz, 4H), 6.93 (d, J = 7.5 Hz, 4H), 4.25 (s, 2H), 2.62 (bs, 1H), 2.08 (bs, 2H), 1.69 (bs, 2H), 1.46 (bs, 1H), 1.31 (s, 18H). ^{13}C (75 MHz, DMSO-d_6): δ 165.5, 157.1, 154.7, 148.0, 131.1, 127.8, 125.1, 122.9, 116.1, 50.7, 24.7, 30.9, 30.7. IR (KBr): 2960, 2867, 1666, 1608, 1497, 1365, 1314, 1270 cm^{-1}. HRMS (CI): m/z [M + 1]$^+$ found 607.3500, calcd 607.3509 for $C_{35}H_{43}N_8O_2$. Mp = 260 °C (dec).

Bis(3,6-diaminopyridazine) (2b). To an oven-dried sealed tube apparatus charged with the bis(iminophoshorane) derived from **4** (0.5 g, 0.73 mmol, 100 mol %) and 3,4,5-tri-*n*-butoxybenzoic acid (0.99 g, 2.92 mmol, 400 mol %) was added dry toluene (5 mL). The reaction vessel was sealed, placed in a 150 °C oil bath, and allowed to stir for 24 h. The reaction mixture was removed from the heating bath and allowed to cool to 25 °C. The resulting mixture was directly subjected to flash column chromatography (SiO_2:40% EtOAc in hexanes) to afford the title compound as a light yellow solid (0.18 g, 0.22 mmol, 27%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.99 (bs, 2H), 8.28 (d, J = 9.9 Hz, 2H), 8.16 (bs, 2H), 7.19 (s, 4H), 6.88 (d, J = 9.9 Hz, 2H), 3.80−4.10

(m, 14H), 2.70−2.90 (m, 1H), 1.80−2.10 (m, 5H), 1.60−1.80 (m, 12H), 1.25−1.60 (m, 12H), 0.80−1.00 (18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.4, 159.1, 153.1, 148.5, 141.7, 128.7, 114.0, 106.8, 73.3, 69.0, 52.6, 39.3, 32.5, 32.0, 31.5, 19.4, 19.3, 14.1, 14.0. IR (KBr): 2958, 2872, 1669, 1583, 1495, 1427, 1332, 1210, 1110 cm^{-1}. HRMS (CI): *m*/*z* [M + 1]$^+$ found 927.5707, calcd 927.5708 for $C_{51}H_{75}N_8O_8$. Mp = 98−100 °C.

Bis(3,6-diaminopyridazine) (6). To an oven-dried sealed tube apparatus charged with *cis*-1,3-cyclopentyldiamine (0.19 g, 1.8 mmol, 600 mol %), 3,6-diiodopyridazine (0.1 g, 0.3 mmol, 100 mol %), CuI (2.9 mg, 0.015 mmol, 5 mol %), and K_3PO_4 (0.27 g, 1.27 mmol, 410 mol %) was added anhydrous ethylene gylcol (0.037 g, 0.6 mmol, 200 mol %), followed by DMF (1 mL). The reaction vessel was sparged with argon, sealed, and placed in a 95 °C oil bath for 12 h. The reaction mixture was removed from the heating bath and allowed to cool to 25 °C, at which point Boc-anhydride (0.79 g, 3.6 mmol, 1200 mol %) was added to the reaction mixture, followed by EtOAc (5 mL). The reaction mixture was stirred for 1 h at 25 °C. The reaction was then partitioned between dilute aqueous NH_4OH solution (5 mL) and EtOAc (25 mL). The organic layer was collected, and the aqueous layer was washed with EtOAc (2 × 10 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated onto silica in vacuo. The resulting residue was subjected to flash chromatography (SiO_2:50% EtOAc in hexanes → neat EtOAc) to afford the title compound as a pale yellow solid (0.11 g, 0.22 mmol, 74%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.87 (d, J = 7.8 Hz, 2H), 6.59 (s, 2H), 5.88 (d, J = 6.6 Hz, 2H), 3.88−3.98 (m, 2H), 3.68−3.78 (m, 2H), 2.18−2.38 (m, 2H), 1.68−1.98 (m, 4H), 1.38−1.68 (m, 4H), 1.36 (s, 18 H), 1.18−1.38 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 154.9, 153.0, 118.3, 77.3, 50.6, 49.9, 30.6, 28.2. IR (KBr): 2972, 2868, 1687, 1528, 1476, 1390, 1365, 1291, 1250, 1170, 1012, 828 cm^{-1}. HRMS (CI): *m*/*z* [M + 1]$^+$ found 477.3174, calcd 477.3189 for $C_{24}H_{41}N_6O_4$. Mp = 180 °C (dec).

Bis(tetrazolopyridazine) (7). To a 50 mL dry round-bottom flask in an ice−water bath charged with bis(adduct) **6** (0.11 g, 0.23 mmol, 100 mol %) was added trifluoroacetic acid (1 mL). The reaction mixture was allowed to stir for 0.5 h at 0 °C, at which point the trifluoroacetic acid was removed in vacuo. The residue was dissolved in dry DMF (1 mL). Potassium carbonate (0.31 g, 2.25 mmol, 980 mol %) was added, followed by 6-chlorotetrazolo[1,5-*b*]pyridazine **3** (0.077 g, 0.49 mmol, 210 mol %). The reaction mixture was placed under an argon atmosphere and allowed to stir for 8 h at 95 °C. The reaction mixture was removed from the heating bath and allowed to cool to 25 °C. A precipitate was formed. Water and EtOAc were added to the mixture. The precipitate was collected by filtration and dried in vacuo to afford **7** as a brown solid (0.094 g, 0.184 mmol, 80%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.23 (d, J = 9.6 Hz, 2H), 8.23 (s, 2H), 7.16 (d, J = 9.6 Hz, 2H), 6.67 (s, 2H), 6.09 (d, J = 6 Hz, 2H), 2.56−2.70 (m, 2H), 2.00−2.20 (m, 4H), 1.60−1.80 (m, 4H), 1.40−1.60 (m, 2H). ^{13}C NMR (63 MHz, DMSO-d_6): δ 155.2, 153.0, 139.9, 122.7, 121.1, 118.8, 51.1, 51.0, 30.6, 30.3. IR (KBr): 3089, 2960, 1623, 1577, 1496, 1360, 1289, 1241, 1099, 826, 755 cm^{-1}. HRMS (CI): *m*/*z* [M + 1]$^+$ found 515.2598, calcd 515.2605 for $C_{22}H_{27}N_{16}$. Mp = 179−181 °C.

Bis(iminophoshorane) en Route to 5. To an oven-dried sealed tube apparatus charged with bis(tetrazolo[1,5-*b*]pyridazine) **7** (0.25 g, 0.36 mmol, 100 mol %) and tri-*n*-butylphosphine (0.73 g, 3.62 mmol, 1000 mol %) was added CH_3CN (5 mL). The reaction vessel was sealed, placed in a 180 °C oil bath, and allowed to stir for 3 h. The reaction mixture was removed from the heating bath and allowed to cool to 25 °C. A precipitate was formed and was collected by filtration. The precipitate was washed with EtOAc (5 × 20 mL). The residue was dissolved in DCM, and the solid impurities were removed by filtration. The DCM was removed in vacuo to afford the title compound as a light brown solid (0.19 g, 0.22 mmol, 60%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.81 (d, J = 9.6 Hz, 2H), 6.60 (s, 2H), 6.55 (d, J = 9.6 Hz, 2H), 4.85 (bs, 2H), 4.60 (bs, 2H), 4.20 (m, 4H), 2.56−2.63 (m, 2H), 2.0−2.2 (bm, 18H), 1.6−1.9 (4H), 1.3−1.6 (m, 24H), 0.90 (t, J = 6.9 Hz, 18H). ^{31}P (121.5 MHz, $CDCl_3$): δ 32.90. ^{13}C (75 MHz, $CDCl_3$): δ 160.1, 154.1, 152.9, 125.7, 125.4, 117.8, 117.6, 52.3, 40.6, 31.9, 31.7, 28.0, 27.1, 24.2, 24.1, 23.9, 23.7, 23.4, 13.6. IR (KBr): 3117, 3025, 2962, 2938, 2864, 1594, 1451, 1370, 1331, 1135, 1059, 834 cm^{-1}. HRMS (CI): *m*/*z* [M + 1]$^+$ found 863.6179, calcd 863.6182 for $C_{46}H_{81}N_{12}P_2$. Mp 136−138 °C.

Tris(3,6-diaminopyridazine) (5). To an oven-dried sealed tube apparatus charged with the bis(iminophosphorane) derived from **7** (1.0 g, 1.15 mmol, 100 mol %) and 3,4,5-tri-*n*-butoxybenzoic acid (1.8 g, 5.3 mmol, 460 mol %) was added dry toluene (10 mL). The reaction vessel was sealed, placed in a 150 °C oil bath, and allowed to stir for 24 h. The reaction mixture was removed from the heating bath and allowed to cool to 25 °C. The reaction mixture was directly subjected to flash column chromatography (SiO_2:neat EtOAc → Et_3N:2-propanol:DCM = 2:10:88) to afford the title compound as a yellow solid (0.33 g, 0.30 mmol, 26%). Further purification prior to ITC studies was performed by preparative TLC, eluting with 30% 2-propanol in DCM. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.85 (s, 2H), 7.86 (d, J = 9.6 Hz, 2H), 7.36 (s, 4H), 6.94 (d, J = 6.4 Hz, 2H), 6.90 (d, J = 9.6 Hz, 2H), 6.68 (s, 2H), 6.10 (s, 2H), 4.14−4.25 (m, 2H), 4.05−4.15 (m, 2H), 4.00 (q, J = 5.6 Hz, 8H), 3.90 (t, J = 5.6 Hz, 4H), 2.50−2.62 (m, 2H), 1.92−2.30 (m, 4H), 1.53−1.80 (m, 16H), 1.30−1.53 (m, 14H), 0.80−1.00 (m, 18H). ^{13}C NMR (63 MHz, DMSO-d_6): δ 164.9, 157.1, 153.0, 152.3, 148.1, 140.2, 128.5, 122.8, 118.8, 116.0, 106.2, 72.1, 68.1, 51.0, 50.9, 31.8, 30.9, 18.8, 18.7, 13.7. IR (KBr): 2956, 2932, 2871, 1666, 1582, 1492, 1427, 1331, 1210, 1112, 1023, 831, 757 cm^{-1}. HRMS (CI): *m*/*z* [M + 1]$^+$ found 1103.6775, calcd 1103.6770 for $C_{60}H_{87}N_{12}O_8$. Mp 214−216 °C.

***N,N*-Diisobutylpyridazine-3,6-diamine (8).** To a sealed tube apparatus charged with 6-diisobutyltetrazolo[1,5-*b*]pyridazin-6-ylamine (0.5 g, 2.0 mmol, 100 mol %) and tri-*n*-butylphoshine (0.82 g, 4.0 mmol, 200 mol %) was added CH_3CN (2 mL). The reaction vessel was sealed, placed in a 180 °C oil bath, and allowed to stir for 3 h. The reaction mixture was removed from the heating bath and allowed to cool to 25 °C. An aqueous solution of HCl (37%, 10 mL) was added, and the reaction mixture was allowed to stir at 100 °C for 6 h. After cooling to 25 °C, the reaction mixture was transferred to a beaker and neutralized with solid K_2CO_3. The aqueous phase was extracted with EtOAc (2 × 50 mL), and the combined organic extracts were dried ($MgSO_4$), filtered, and evaporated directly onto silica. The residue was loaded onto a silica gel column and subjected to flash chromatography (SiO_2:10% 2-propanol in DCM) to afford the desired product **7** as a sticky oil (0.36 g, 1.60 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.76 (d, J = 6.9 Hz, 1H), 6.72 (d, J = 6.9 Hz, 1H), 4.32 (bs, 2H), 3.28 (d, J = 7.2 Hz, 4H), 2.00−2.14 (m, 2H), 0.88 (d, J = 6.6 Hz, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.6, 151.8, 117.9, 116.1, 57.5, 26.5, 20.0. IR (NaCl): 3254, 2963, 1570, 1500, 2863, 1460, 1006 cm^{-1}. HRMS (CI): *m*/*z* [M + 1]$^+$ found 223.1922, calcd 223.1923 for $C_{12}H_{23}N_4$.

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Supporting Information Available: Spectral data for all new compounds, including scanned images of ^{1}H and ^{13}C NMR spectra; crystallographic data for 3,6-diaminopyridazine, bis-(diaminopyridazine) **2a**, and bis(Cbz-carbamate) **1** (PDF, CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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