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Supramolecular Architecture through Self-Organization of Janus-Faced Homoazanucleosides

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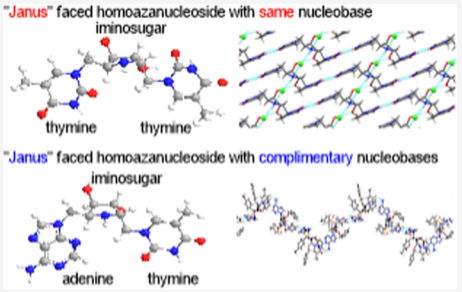
ABSTRACT: Design of Janus-faced or double-headed homoazanucleosides with the possibility to undergo self-organization through base pairing has been conceptualized and accomplished. The synthetic strategy demonstrates the unique ability to introduce two similar or complementary nucleobases on opposite arms of a chiral polyhydroxypyrrolidine while also ensuring that their faces are anti to each other to allow only intermolecular interactions between the nucleobases, an essential requisite for self-assembly. Single-crystal X-ray structures were determined for all three types of homoazanucleosides, one possessing two adenine molecules, the other with two thymine moieties, and the third containing both adenine and thymine. The crystal structures of all three display noncovalent interactions, including Watson−Crick base pairing, Hoogsteen H-bonding, and $\pi-\pi$ stacking, resulting in unusual supramolecular patterns. The most striking supramolecular motif among them, which emerged from the crystal structure of the homoazanucleoside containing both adenine and thymine, is a left-handed helix formed through Watson−Crick pairing between nucleobases. The present study thus forms a prelude to the design of Janus-faced building blocks to establish helical pillars as well as lateral branches that together define a three-dimensional (3D) lattice. The ready accessibility of these molecules is expected to spur the next generation of discoveries in the design of functional nanomaterials.



INTRODUCTION

Chemically modified oligonucleotides have emerged as nanostructures[1,2] useful for the design and assembly of both diagnostic[3] kits and therapeutic drugs.[4,5] Oligonucleotides are information-rich molecules where the size, shape, and topology[6,7] can be programmed due to inherent Watson−Crick (W−C)[8] base pairing. The precision and predictability of base-pairing rules (A-T/U and G-C) have provided tools to construct functional structures with a myriad of applications.[9] The self-assembly process relies on noncovalent interactions between molecules utilizing H-bonding, hydrophobic, and electrostatic interactions. The advantage of these weaker interactions is that they facilitate reversible assembly and disassembly of structures. Gratifyingly, the solid-phase automated synthesis of oligonucleotide has become a routine, delivering a large quantity of any modified sequence in a short period of time for research and commercial needs.[9−11] Tailored oligonucleotides that self-assemble have become a cornerstone for innovations in nanomaterials.

The base-pairing capabilities of the natural nucleobases have been further explored by the design and synthesis of Janus-faced modified nucleosides[12] to expand the repertoire of nucleic acid materials. Briefly, Janus-faced or double-headed nucleosides are molecules where two nucleobases are installed on a single sugar moiety such as 2-deoxyribose present in DNA. A growing number of these analogues have been synthesized and studied for their structural properties.[13] This concept of creating an information-rich functional molecule by anchoring two nucleobases has been a central theme in the research by Nielsen and associates[14] for over a decade. Thus, incorporating these double-headed nucleotides into short strands of DNA resulted in interesting structural features that are not accessible to native DNA. Bis-headed nucleotides featuring cytosine, guanine, thymine, adenine, hypoxanthine, and diaminopurine with a standard C1′-linked base combined with a second base attached to C2′ via a methylene linker (β-D-arabinoside) were studied inside DNA duplexes in terms of their pairing properties, and the structures of modified duplexes were analyzed using molecular dynamics (MD) simulations.[15] Bis-headed nucleotides adopt a C2′-*endo* sugar pucker and behave like dinucleotides, i.e., U_A (U at the C1′-position and A at the C2′-position) forms Watson−Crick pairs with A and U from the opposite strand. When bis-headed monomers were placed in opposite strands inside an 11mer duplex in a so-called (+1)-zipper arrangement, bases attached to C2′ can pair with each other and thus generate a 12th base pair.[16] Alternatively, bis-headed nucleotides can function as a dimer and pair with two standard nucleotides from the

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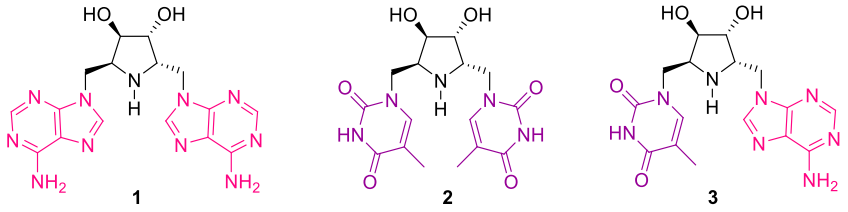
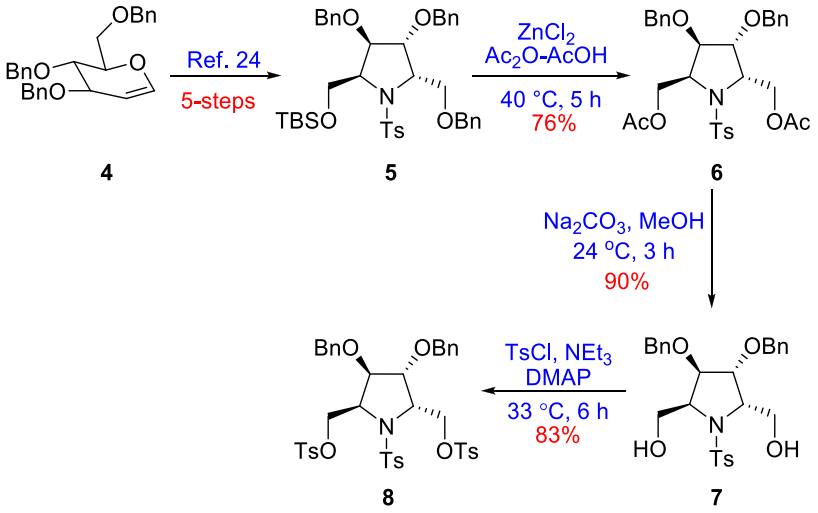


Figure 1. Structures of Janus-faced homoazanucleosides possessing the same nucleobases (**1** and **2**) and complementary nucleobases (**3**) on an iminosugar framework reported in this manuscript.

Scheme 1. Synthesis of Ditosylate 8 from Tri-*O*-benzyl-D-glucal 4



opposite strand, or they can interact with each other under the formation of two other base pairs when placed on opposite strands. Bis-headed nucleotides with an alternative configuration of the two bases, namely, a combination of the canonical C1′-linkage with a second 4′-Cα-methylene-linked base moiety, were investigated in regard to their effects on DNA duplex stability.[17] For example, combinations of bis-headed nucleotides, such as T_A/T_T, T_T/T_T, and T_A/T_A (the base at the 4′-Cα-position is represented with subscript font), were placed on opposite strands such that they were separated by a single A:T pair. In some cases, this resulted in an enhanced stability of the duplex and molecular modeling studies are suggestive of an interaction between the 4′-Cα-linked bases in the DNA minor groove.

In our research directed at the discovery of potential glycosidase inhibitors, we focused our attention on the synthesis of iminosugars.[18] In this vein, we reported the synthesis of several homoazanucleoside analogues starting from chiral iminosugars.[19] Our original work directed at the assembly of homoazanucleosides and concepts from the Nielsen group motivated us to design the Janus-faced functional molecules that could self-assemble. We chose the iminosugar framework because it offers multiple attractive attributes. (i) Iminosugars are ubiquitous in natural products[20] and key components of many pharmaceuticals.[21] (ii) Their ability to mimic carbohydrate structures[20,22] and their chemical and enzymatic stability make them ideal for drug discovery. (iii) Their structural resemblance to the oxocarbenium[23] ion transition state is responsible for PNP inhibition, leading to the potential treatment for cancer. (iv) Our existing approach to synthesize chiral homoazanucleosides on a gram scale, starting from an inexpensive glucal. (v) The two primary hydroxyl groups on either arm of the polyhydroxylated pyrrolidine iminosugar previously reported by us offer convenient points of attachment of either two identical or two complementary nucleobases to deliver structurally and biologically interesting azanucleosides. Therefore, we envisioned that these bioinspired building blocks may self-assemble into defined structures, with possible applications in medicine and biomaterials. Conspicuously, for these molecules to self-assemble through intermolecular H-bonding between the nucleobases, an inherent trans-stereochemistry at the C2 and C5 positions of the pyrrolidine is an essential requisite. The iminosugar reported from our lab has this intrinsic structural feature to address this issue as well.

Herein, we describe the synthesis of Janus-faced homoazanucleosides through the installation of the same nucleobases (**1** and **2**) or two complementary nucleobases (**3**) on a polyhydroxylated pyrrolidine framework (Figure 1) via an orthogonal protecting group strategy. The single-crystal X-ray structures of these Janus molecules exhibit their ability to self-assemble. These results are expected to open new avenues for the design of noncovalent architectures that facilitate the assembly of functional structures.

RESULTS AND DISCUSSION

Synthesis of Ditosylate 8. The starting material **5** required for the synthesis of Janus-faced homoazanucleosides was obtained in five steps from tri-*O*-benzyl-D-glucal **4** on a gram scale.[18b,24] It was thought that compound **5** would be an ideal substrate for the synthesis of a variety of double-headed homoazanucleosides. Interest in this area arose not only due to the novelty of the structure but also to study the base-pairing ability of such molecules. Along these lines, we initiated our

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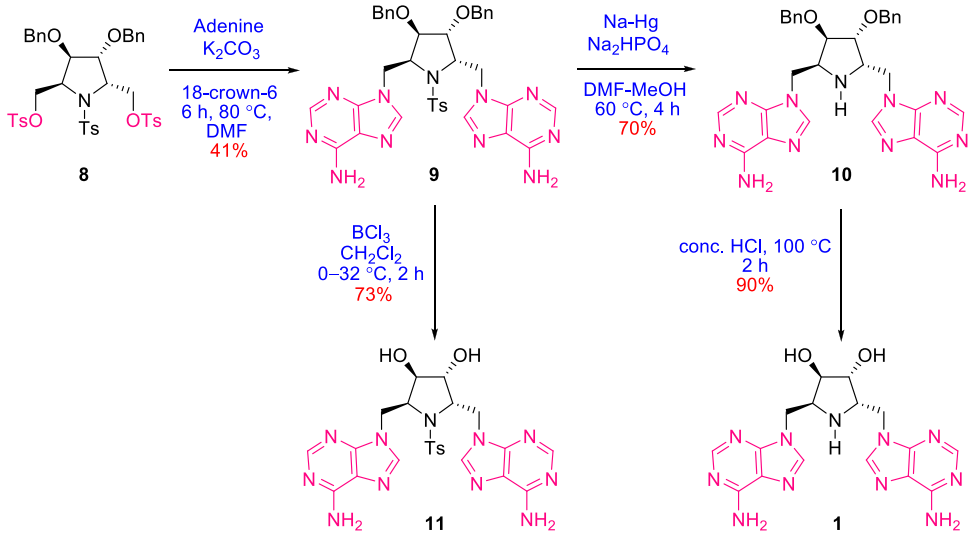
Scheme 2. Synthesis of Bis-adenine-Coupled Double-Headed Homoazanucleoside 1



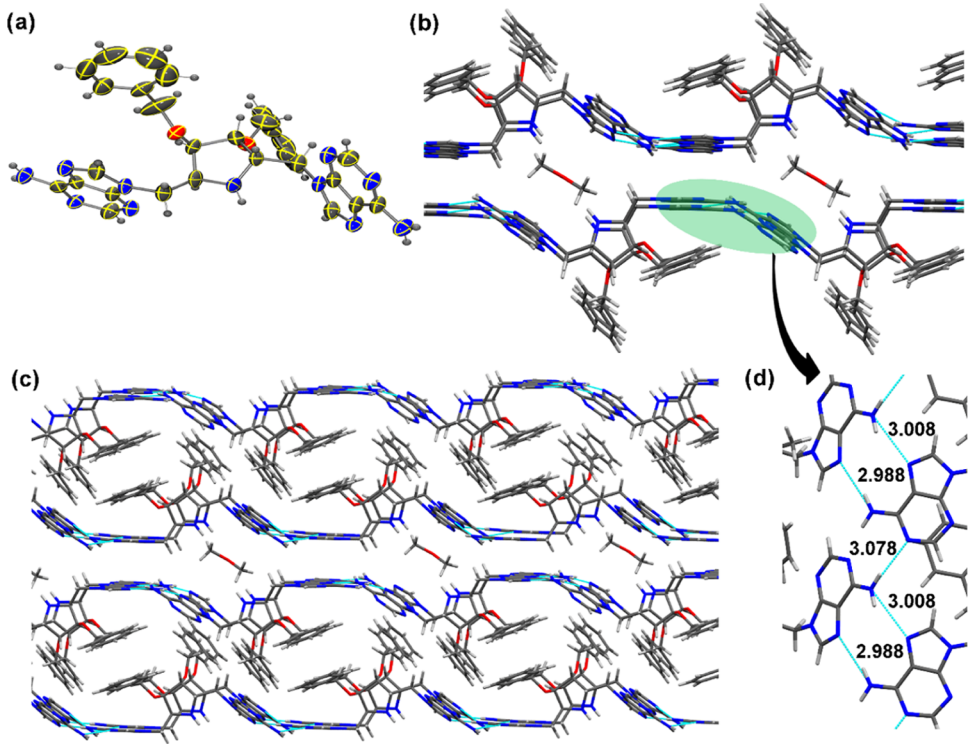


Figure 2. (a) Persistence of vision (POV)-ray representation of compound **10** with solvent molecules omitted for clarity; (b) X-ray superstructure illustrating H-bond-assisted formation of azanucleoside boxes; (c) view of the three-dimensional (3D) packing structure; (d) zoom view of intermolecular H-bonding interactions (cyan lines) involving both Watson−Crick and Hoogsteen edges of adenines. Distances are in angstrom.

research by performing selective debenzylative acetolysis of the primary benzyloxy group of **5** in the presence of the secondary ones. This was readily accomplished by treating compound **5** with $ZnCl_2$ and Ac_2O/AcOH (4:1) at room temperature.[18a,19a,25] In the process, the *tert*-butyldimethylsilyl (TBS) ether also underwent deprotective acetolysis to directly afford the diacetate **6** in 76% yield. Hydrolysis of the acetate group of compound **6** with Na_2CO_3 and MeOH at room temperature delivered the diol **7** in 90% yield. The diol was then reacted with *p*-toluenesulfonyl chloride in the presence of 4-dimethylaminopyridine (DMAP) and triethylamine at room temperature for 6 h to obtain the ditosylate **8** in 83% yield (Scheme 1).

Synthesis of Bis-adenine-Coupled Homoazanucleoside 1. As per our objective, the synthesis of bis-adenine-coupled homoazanucleoside **1** was initially attempted by treating the ditosylate **8** with 3.0 equiv of adenine in the presence of potassium carbonate and 18-crown-6-ether in dimethylformamide (DMF) at 80 °C.[19,26] The coupling reaction proceeded to give novel N^9-substituted bis-adenine derivative **9**. Compound **9** with orthogonally protected acid-sensitive benzyl and base-sensitive tosyl groups is ideally poised for selective deprotection. First, N-detosylation of

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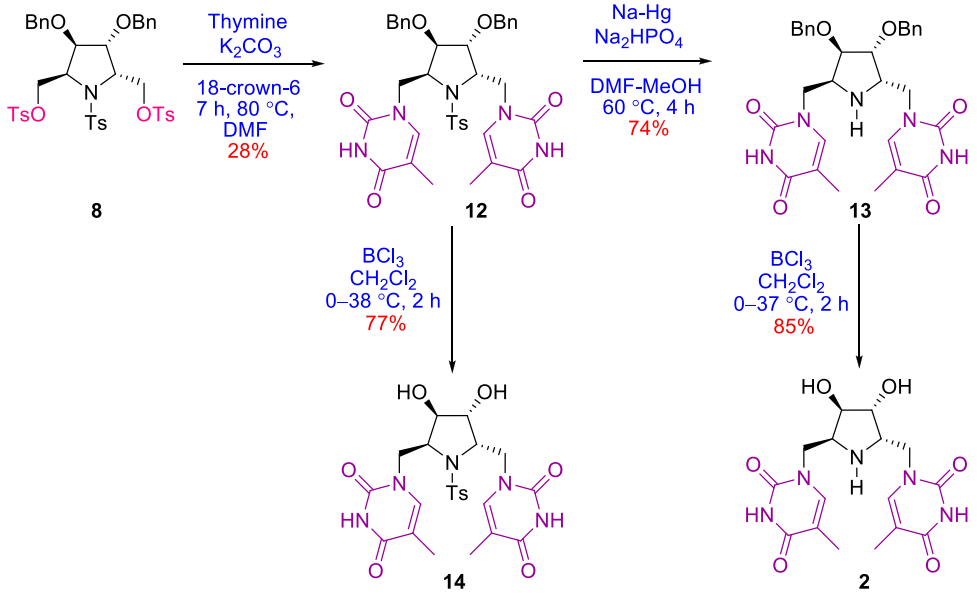
J. Org. Chem. 2021, 86, 367−378

Scheme 3. Synthesis of Bis-thymine-Coupled Double-Headed Homoazanucleoside 2



compound **9** was carried out with Na−Hg in Na_2HPO_4 in DMF and MeOH at 60 °C under an oil bath to obtain the free amine **10** in 70% yield. The two benzyl groups were then cleaved under acidic conditions by treating compound **10** with concentrated HCl at 100 °C to obtain the hitherto unreported adenine-coupled double-headed homoazanucleoside **1** in 90% yield. On the other hand, exposure of compound **9** to BCl_3 in dichloromethane resulted in a selective debenzylation reaction to provide the diol **11** in 73% yield (Scheme 2).

Self-Assembly of Homoazanucleoside 10 through Watson−Crick and Hoogsteen Base Pairing. The bis-adenine-coupled homoazanucleoside **10** was crystallized, and a single-crystal X-ray structure was obtained. The crystal structure revealed some interesting and intriguing information about the supramolecular assembly of **10**. The benzyl-protected bis-adenine homoazanucleoside crystallized in space group *P*1 with two molecules per unit cell along with two molecules of dimethyl sulfoxide (DMSO) and methanol. The iminosugars of the two independent nucleosides adopt different puckers, C2′-*endo* and C3′-*exo*, respectively, that represent neighboring 36° southern sectors in the pseudorotation phase cycle.[27] The two bis-adenines are related by a noncrystallographic dyad that runs approximately along the direction of the *a*-unit cell axis. The two nucleobases and benzyl protection groups of individual azanucleosides are arranged in a roughly tetrahedral manner (Figure 2a). Two adenine bases and iminosugars from independent azanucleoside box in methanol (two partially occupied molecules with overlapping positions of the oxygen), whereby N−H groups establish weak H-bonds to the CH_3OH oxygen from opposite sides. Both adenine moieties engage in H-bonding interactions with their Watson−Crick (W−C) and Hoogsteen edges (Figure 2d), such that the resulting H-bonds stitch bis-adenines together in roughly perpendicular directions. Half the adenines then form stacking interactions that extend in the third dimension (Figure 2b). Both adenines from individual nucleosides, including those not engaged in self-stacking, stack onto benzyl moieties. In this manner, undulating double sheets of adenines that are W−C H-bonded in one direction and Hoogsteen H-bonded in the other are held together by layers composed entirely of benzyls that are engaged in offset $\pi-\pi$ and edge-on stacking as well as hydrophobic interactions (Figure 2c). Adenine bilayers are disrupted by channels that harbor DMSO and CH_3OH molecules.

Adenine engaging in H-bonding interactions via both its Watson−Crick (N^1 and N^6H_2) and Hoogsteen edges (N^6H_2 and N^7) as seen in the structure of the bis-adenine azanucleoside is a commonly encountered pattern in crystal lattices of purines. Thus, in the crystal structure of anhydrous adenine, the two independent molecules in the asymmetric unit form layers in which individual adenines are stitched together by H-bonds involving not just their Watson−Crick and Hoogsteen edges but also the minor groove N^3 and N^9H functions.[28] The quintessential example of the involvement of both the Watson−Crick and Hoogsteen base edges for establishing intermolecular interactions are G-tetrads. A classic example is found in the quadruplex formed by the oligo-2′-deoxynucleotide 5′-AGAGAGATGGGTGCGTT-3′ with no fewer than 13 layers of base tetrads.[29] However, in an adjacent layer, an A-tetrad is held together by just four H-bonds that involve N^6H and N^3.

Synthesis of Bis-thymine-Coupled Homoazanucleoside 2. Motivated by the success of the reaction of adenine with **8**, the protocol was extended for the installation of thymine as the nucleobase. The bis-thymine-coupled homoazanucleoside **2** and its *N*-tosyl derivative **14** were obtained following the same synthetic sequence as described for adenine (Scheme 3). The site for N^1 substitution was confirmed through the single-crystal X-ray structure.

Single-Crystal Structure of Bis-thymine-Coupled Homoazanucleoside 2. Single crystals of the bis-thymine-coupled homoazanucleoside **2** were grown by slow evaporation of 1 M HCl solution. Compound **2** crystallizes in the $P2_1$ space group, with one molecule per asymmetric unit together with a single water molecule. The iminosugar displays a southern C3′-*exo* conformation. Thymine bases adopt a virtually parallel orientation with a distance of ca. 4.4 Å along the normal to the two planes (Figure 2a). Thus, nucleobases are spaced along the vertical direction and also shifted laterally in their respective planes (by ca. 4.4 Å) in a way that precludes effective

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J. Org. Chem. 2021, 86, 367−378

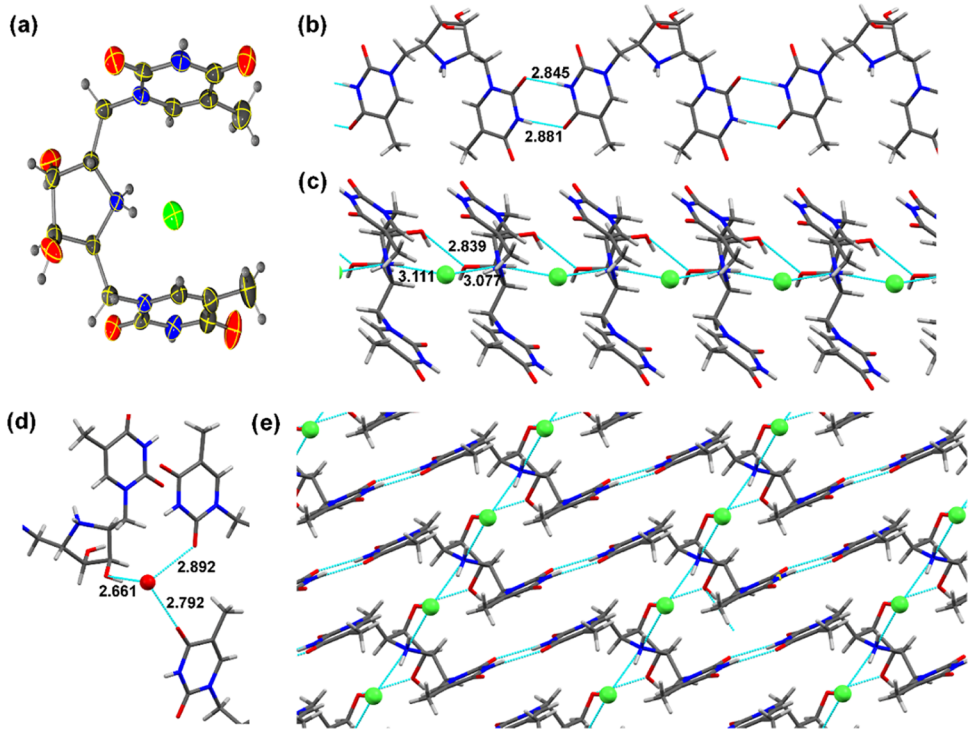

Figure 3. (a) POV-ray representation of compound **2**·HCl, solvent molecules have been omitted for clarity; (b) X-ray superstructure showing H-bond-assisted zigzag-shaped ribbons of T:T pairs linked by iminosugars; (c) view of H-bonding between the chloride anions and the protonated nitrogen of iminosugars from the adjacent nucleosides and additional interactions between sugar hydroxyl groups; (d) zoom view of H-bonded structure of the solvent−water molecule with two thymine bases and one sugar motif; (e) H-bond-assisted 3D zigzag packing, trapped water molecules have been omitted for clarity. Chloride anions and water molecules are shown in ball-and-stick representation and all H-bond distances are given in angstrom.

intramolecular stacking. In the crystal, the azanucleoside exhibits an approximate twofold rotational symmetry, whereby the noncrystallographic dyad runs through the sugar nitrogen and dissects the C2−C3 bond. This conformation results in W−C edges of thymine facing in opposite directions as envisioned by the Janus design principle. The space group symmetry generated a herringbone pattern of bis-thymine homoazanucleosides that are base-paired in a reverse pseudo W−C manner and joined by additional interactions between sugar hydroxyl groups, whereby H-bonds are oriented perpendicularly to the unique (*b*-) direction.

Zigzag-shaped ribbons of T:T pairs linked by iminosugars are then held together by stacking interactions featuring a thymine base from the first nucleoside present atop base-paired Ts from two additional nucleosides (Figure 3b). Chloride anions are H-bonded to the protonated nitrogen of iminosugars from the adjacent nucleosides such that each chloride is sandwiched between two thymines and is present halfway between the C^5 (C^5m)−C^6 edges of two further nucleobases (Figure 3c). Unlike in the case of the bis-adenine nucleoside crystal structure, packing interactions in the bis-thymine lattice involve an ionic component. Zigzag-shaped layers of homoazanucleosides stabilized by H-bonding and stacking interactions are stitched together along the crystallographic *b*-direction by tetrahedrally coordinated water molecules (Figure 3d). Each water establishes H-bonds to thymine O^4 and O^2 oxygens and a sugar hydroxyl group that is contributed by four different nucleosides.

The reverse W−C pairing type between thymines seen here is not encountered in antiparallel DNA duplexes. Thus, in a 15mer DNA hairpin stem−loop structure with a T·T mismatch pair flanked by various pairs on either side that was studied by solution NMR, the homothymine pairing type involved H-bonds between O^4 and N^3H; and N^3H and O^2.[30] Depending on the identity of base pairs flanking the T·T pair, either the T on the first strand used O^4 as the acceptor or the T on the second strand used O^2 or vice versa. However, the pairing type between Ts seen in the structure of the bis-thymine azanucleoside is also present in the NMR structures of parallel-stranded DNA duplexes with sequences d(CGATCG), d(TCGATCGA), and d(CGATCGATCG) at low pH that are held together by homo G·G, A·A, C·C, and T·T pairs.[31] Conversely, in the above quadruplex, adjacent thymines in the T-tetrad are only linked by single H-bonds between O^4 and N^3H.[29]

Synthesis of Thymine and Adenine-Coupled Homo-azanucleoside 3. Polyhydroxypyrrolidine **5**, being a distinctly unique orthogonally protected C_2 symmetric molecule, offers an unparalleled opportunity to introduce two different nucleobases in a single compound in a sequential manner. It was of particular interest to introduce two complementary nucleobases, such as adenine and thymine, on the two opposite arms (C2 and C5 carbons) of polyhydroxypyrrolidine **5** through a methylene bridge, thereby generating resemblance to an imino-*C*-nucleoside analogue. Conceptually, because the stereochemistry at C2 and C5 of the resulting double-headed homoazanucleoside will be trans, it should promote an intermolecular Watson−Crick type base pairing between the complementary bases, leading to a supramolecular assembly. Furthermore, the trans-stereochemistry also ensures the inability to form any intramolecular H-bonding between the complementary nucleobases. The synthesis of such a small

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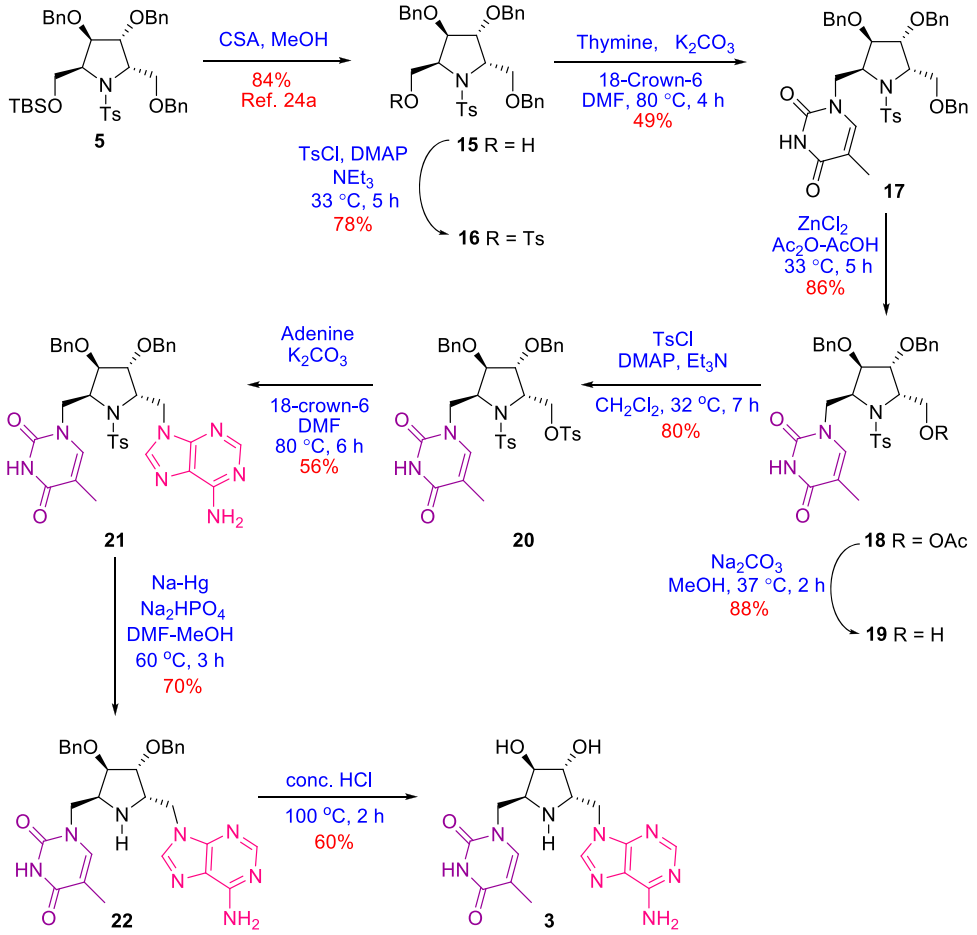
Scheme 4. Synthesis of Thymine- and Adenine-Coupled Double-Headed Homoazanucleoside 3



chiral molecule with two complementary nucleobases has not been reported so far. Moreover, investigating its supramolecular assembly would be of great importance as it might shed light on the three-dimensional structural consequence arising from this base-pairing type. With this idea, we proceeded to synthesize a Janus-faced homoazanucleoside that contains the complementary nucleobases adenine and thymine in a single molecule. First, desilylation of **5** was carried out with a catalytic amount of camphorsulfonic acid in MeOH to obtain alcohol **15** in 84% yield. Tosylation of the hydroxyl group of **15** with *p*-toluenesulfonyl chloride in the presence of DMAP and triethylamine in dichloromethane at room temperature then delivered the corresponding tosylate **16** in 78% yield. Compound **16** was then subjected to coupling with thymine in the presence of K_2CO_3 and 18-crown-6-ether in DMF at 80 °C to give the thymine-coupled product **17** in 49% yield. With the monoheaded homoazanucleoside **17** in hand, the next step was to utilize the opportunity to selectively perform the debenzylative acetolysis of the primary benzyloxy group of **17** suitable for subsequent coupling with adenine. This was achieved by exposing compound **17** to $ZnCl_2$ in Ac_2O/AcOH (4:1) at 33 °C. The debenzylative acetolysis proceeded smoothly to deliver acetate **18** in 86% yield. It is noteworthy that the thymine base remains undisturbed during this transformation. Subjecting the acetate **18** to basic hydrolysis at 37 °C resulted in alcohol **19** in 88% yield. Compound **19** was then converted to its tosylate **20** in 80% yield by reacting it with *p*-toluenesulfonyl chloride in the presence of DMAP and triethylamine in dichloromethane at room temperature. Coupling of compound **20** with adenine in the presence of K_2CO_3 and 18-crown-6-ether in DMF at 80 °C afforded the novel double-headed homoazanucleoside **21** (56% yield) containing the complementary A and T nucleobases in a single molecule. N-Detosylation of compound **21** with Na−Hg in the presence of Na_2HPO_4 in DMF/MeOH (8:1) at 60 °C gave compound **22** in 70% yield. Finally, debenzylation of compound **22** under acidic conditions using concentrated HCl at 100 °C delivered, in 60% yield, the polyhydroxypyrrolidine **3** with two different nucleobases—a purine and a pyrimidine (Scheme 4). It is noteworthy that homoazanucleoside **3** is perfectly stable under acidic conditions at elevated temperatures. The extraordinary stability of these molecules may be a useful trait in the design of nanomaterials that will survive harsh reaction conditions.

Self-Assembly of Protected Homoazanucleoside 21 into a Left-Handed Helix through Watson−Crick Base Pairing. Single crystals of protected mixed A/T homoazanucleoside **21** were grown by slow evaporation of the compound from a solution of chloroform and methanol at room temperature. Compound **21** crystallizes in the $P4_3$ space group with a single molecule per asymmetric unit along with four solvent molecules (3 × chloroform and methanol). The iminosugar adopts the southern C3′-*exo* conformation. Adenine and thymine assume an orthogonal relative orientation with W−C edges facing away from each other (Figure 4a). One of the benzyl moieties stacks onto thymine,

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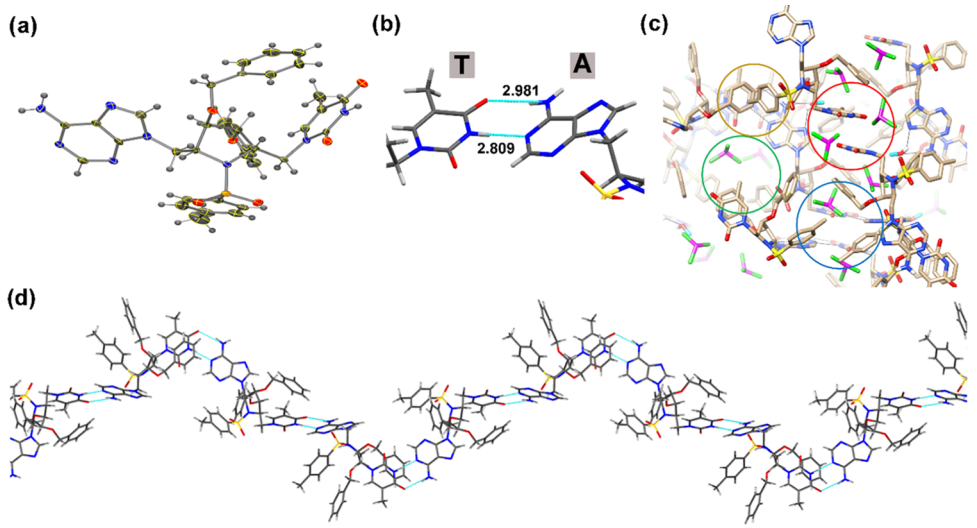

Figure 4. (a) POV-ray representation of compound **21**, solvent molecules have been omitted for clarity; (b) W−C pairing between adenine and thymine with H-bond distances in angstrom; (c) representation of various nonbonding interactions; and (d) A:T pair-assisted left-handed helix.

whereby the two rings exhibit a significant slant. The other benzyl forms an edge-on stacking interaction with the aromatic moiety of the tosyl group. The methanol donates an H-bond to N^7 of adenine. Closer inspection of the lattice interactions reveals several pertinent features. (i) Two thymines from adjacent nucleosides form an antiparallel stack (Figure 4c, red circle). (ii) A and T form W−C base pairs and two A:T pairs engage in a stacking interaction (blue circle). (iii) The methyl group of the tosyl moiety from one nucleoside sits above one of the benzyl rings from a neighbor (tan circle). (iv) Benzyl and tosyl rings from the adjacent nucleosides together with chloroform molecules generate a hydrophobic patch (green circle). The most striking supramolecular motif emerging from the crystal structure of the mixed A/T azanucleoside is a left-handed helix that consists of nucleoside units joined together by A:T base pairs (Figure 4d).

The left-handed helix formed by four mixed A/T homoazanucleoside has a pitch of 44.7 Å (length of the unit cell *c*-axis). This almost matches the 44.4 Å pitch of left-handed Z-DNA with twelve base pairs per turn.[32,33] Of course, there are many differences between the supramolecular structure formed by azanucleosides around the fourfold screw axis and the left-handed Z-DNA duplex. Thus, A:T pairs are formed roughly along the direction of the helical axis and the arrangement is single-stranded. In double-stranded Z-DNA, C:G pairs are perpendicular to the direction of the helical axis. An additional difference concerns the noncovalent nature of the interaction between Janus-faced units versus both phosphodiester linkages and base stacking interactions that stabilize Z-DNA. But from the perspective of DNA nanotechnology[34] and crystal engineering with DNA,[35] a key difference between self-assembling oligonucleotides that are typical of the right-handed B-form and the Janus-faced mixed A/T homoazanucleoside **21** helix is that the edges of base pairs are exposed in the latter. Conversely, base pairs are buried in the interior of B-form DNA and H-bonding functions along their edges tucked away in the grooves and thus not readily available for interactions with neighboring duplexes in a scaffold. One can envision the usage of Janus-faced building blocks to establish helical pillars as well as lateral branches that together define a 3D lattice.

CONCLUSIONS

We report here three novel Janus-faced homoazanucleosides that can be synthesized in an efficient manner, starting from a single chiral precursor. This is the first synthesis established for Janus-faced or double-headed homoazanucleosides, wherein either two identical or two complementary nucleobases were coupled onto a polyhydroxypyrrolidine moiety. We succeeded in determining single-crystal X-ray structures of the three double-headed homoazanucleosides **10**, **2**·HCl, and **21**, allowing us to study supramolecular interactions in their crystal lattices. Although oligonucleotide self-assembly, i.e., the formation of duplexes, triplexes, and quadruplexes, is well known, the ability of a single Janus-faced nucleoside analogue to self-assemble has no precedence. The process relies on noncovalent interactions between natural nucleic acid bases utilizing H-bonding, a hydrophobic protecting group, $\pi-\pi$ stacking, and protonation of the imino group. We anticipate that the facile accessibility of these molecules will inspire the next generation of discoveries in the design of functional nanomaterials.

EXPERIMENTAL SECTION

General Experimental Methods. All experiments were carried out in oven-dried apparatus under a nitrogen atmosphere in dry solvents unless otherwise indicated. Commercial-grade solvents were dried by known methods, and dry solvents were stored over molecular sieves (4 Å). IR spectra were recorded with KBr pellets, and the data are expressed in cm^{-1}. High-resolution mass spectra (HRMS) were recorded with a Q-TOF instrument, using electrospray ionization (ESI) as the ionization method. ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded using $CDCl_3$, DMSO-d_6, or D_2O as a solvent. Chemical shifts are reported in ppm downfield from tetramethylsilane, and coupling constants are expressed in hertz (Hz). Optical rotations were measured at the indicated temperatures in the indicated solvents. Commercially sourced thin-layer chromatography (TLC) plates were used, and the spots were visualized by exposure to iodine or dipping in $KMnO_4$ solution. Column chromatography was carried out on silica gel (230−400 mesh) using a mixture of hexane and EtOAc as an eluent unless otherwise indicated. Melting points are uncorrected.

(2S,3R,4R,5S)-2,5-Bis(acetoxymethyl)-3,4-bis(benzyloxy)-N-(p-toluenesulfonyl)pyrrolidine ***(6)***. Compound **5**[24] (2.00 g, 2.85 mmol) was dissolved in 30 mL of a mixture of acetic anhydride and acetic

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J. Org. Chem. 2021, 86, 367−378

acid (4:1). Anhydrous zinc chloride (1.94 g, 14.24 mmol) was added to it and the reaction mixture was stirred at 40 °C for 5 h, then quenched with water (50 mL), and extracted with EtOAc (3 × 30 mL). The combined organic layer was washed with the saturated sodium bicarbonate solution (3 × 100 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum. The resulting residue was purified by column chromatography over silica gel using a mixture of EtOAc and hexane (1:15) to obtain compound **6** (1.26 g, 76%) as a white solid (recrystallized from EtOAc and hexane). mp 82−84 °C; R_f: 0.53 (EtOAc−hexane, 1:2); $[\alpha]_D^{28}$ −18.0 (*c* 0.19, $CHCl_3$); IR (KBr): $\overline{\nu}$ 3030, 2936, 2881, 1737, 1454, 1375, 1330, 1238, 1140, 1037, 931, 749, 701, 662 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.65 (d, *J* = 7.8 Hz, 2H), 7.33−7.26 (m, 12H), 4.62 (m, 4H), 4.39 (dd, *J* = 11.7, 3.6 Hz, 2H), 4.25−4.21 (m, 4H), 4.01 (m, 2H), 2.41 (s, 3H), 1.70 (s, 6H); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ 169.9 (*C*), 143.5 (*C*), 137.5 (*C*), 129.7 (*CH*), 128.5 (*CH*), 128.0 (*CH*), 127.8 (*CH*), 127.0 (*CH*), 79.7 (*CH*), 73.3 (CH_2), 61.4 (CH_2), 56.7 (*CH*), 21.5 (CH_3), 20.7 (CH_3); HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{31}H_{35}NNaO_8S$ 604.1976; found 604.1995.

(2S,3R,4R,5S)-3,4-Bis(benzyloxy)-2,5-bis(hydroxymethyl)-N-(p-toluenesulfonyl)pyrrolidine ***(7)***. To a solution of compound **6** (0.50 g, 0.86 mmol) in 10 mL of MeOH, sodium carbonate (0.27 g, 2.58 mmol) was added and stirred at 24 °C for 3 h, after which the solid was filtered through a Buchner funnel. The filtrate was dried over anhydrous sodium sulfate and concentrated under vacuum. The resulting residue was purified by column chromatography over silica gel using a mixture of EtOAc and hexane (1:15) to obtain the diol **7** as a colorless viscous liquid (0.38 g, 90%); R_f: 0.43 in (EtOAc−hexane, 1:2); $[\alpha]_D^{28}$ −19.0 (*c* 0.81, $CHCl_3$); IR (KBr): $\overline{\nu}$ 3520, 3033, 2931, 1600, 1455, 1326, 1151, 1096, 1028, 815, 740, 704, 663 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.78 (d, *J* = 8.1 Hz, 2H), 7.34−7.21 (m, 12H), 4.66 (d, *J* = 11.4 Hz, 2H), 4.58 (d, *J* = 11.7 Hz, 2H), 4.37 (m, 2H), 3.99−3.92 (m, 4H), 3.78 (d, *J* = 11.7 Hz, 2H), 2.58 (br s, 2H exchangeable with D_2O), 2.37 (s, 3H); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ 143.7 (*C*), 137.3 (*C*), 136.7 (*C*), 129.7 (*CH*), 128.5 (*CH*), 127.9 (*CH*), 127.6 (*CH*), 127.2 (*CH*), 81.6 (*CH*), 73.5 (CH_2), 60.2 (CH_2), 59.7 (*CH*), 21.5 (CH_3); HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{27}H_{31}NNaO_6S$ 520.1764; found 520.1748.

(2S,3R,4R,5S)-3,4-Bis(benzyloxy)-2,5-bis(p-toluenesulfonyloxymethyl)-N-(p-toluenesulfonyl)pyrrolidine ***(8)***. To a solution of **7** (6.70 g, 13.47 mmol) in dichloromethane (70 mL), triethylamine (7.50 mL, 53.87 mmol), DMAP (1.64 g, 13.47 mmol), and *p*-TsCl (7.70 g, 40.40 mmol) were added and the reaction mixture was stirred at 33 °C for 6 h. It was then diluted with water (150 mL) and extracted with EtOAc (3 × 100 mL). The combined organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum to give a residue, which was purified by column chromatography over silica gel using a mixture of EtOAc and hexane (1:15) as an eluent to obtain **8** (9.00 g, 83%) as a viscous liquid. R_f: 0.39 (EtOAc−hexane, 1:2); $[\alpha]_D^{28}$ +2.8 (*c* 0.98, $CHCl_3$); IR (KBr): $\overline{\nu}$ 3034, 2925, 1598, 1493, 1453, 1357, 1178, 1096, 980, 860, 815, 706, 662 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.64 (d, *J* = 7.8 Hz, 6H), 7.33−7.11 (m, 16H), 4.38−4.35 (m, 6H), 4.16−4.10 (m, 4H), 3.90 (br m, 2H), 2.40 (s, 3H), 2.34 (s, 6H); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ 144.7 (*C*), 144.0 (*C*), 137.3 (*C*), 136.0 (*C*), 132.6 (*C*), 129.9 (*CH*), 129.6 (*CH*), 128.4 (*CH*), 128.3 (*CH*), 128.1 (*CH*), 127.9 (*CH*), 127.8 (*CH*), 127.3 (*CH*), 79.8 (*CH*), 73.5 (CH_2), 66.3 (CH_2), 57.2 (*CH*), 21.6 (CH_3), 21.5 (CH_3); HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{41}H_{43}NNaO_{10}S_3$ 828.1941; found 828.1915.

(2S,3R,4R,5S)-2,5-Bis(9-adeninylmethyl)-3,4-bis(benzyloxy)-N-(p-toluenesulfonyl)pyrrolidine ***(9)***. Compound **8** was dissolved (8.00 g, 9.93 mmol) in dry dimethylformamide (80 mL). Potassium carbonate (4.11 g, 29.78 mmol), 18-crown-6-ether (5.24 g, 19.85 mmol), and adenine (4.02 g, 29.78 mmol) were added successively and the reaction mixture was heated at 80 °C (oil bath) for 6 h. The reaction mixture was diluted with water (150 mL) and extracted with EtOAc (3 × 150 mL). The combined organic layer was washed with brine (100 mL) and dried over anhydrous sodium sulfate. Evaporation of the solvent under vacuum gave a residue, which was purified by column chromatography over silica gel using a mixture of methanol and chloroform (1:16) as an eluent to obtain compound **9** (3.00 g, 41%) as a white solid (recrystallized from MeOH and $CHCl_3$). mp 93−95 °C; R_f: 0.37 (MeOH−$CHCl_3$, 1:4); IR (KBr): $\overline{\nu}$ 3324, 3139, 2922, 1651, 1600, 1479, 1417, 1330, 1304, 1248, 1159, 1089, 796, 752, 731, 700, 657 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.23 (s, 2H), 8.00 (d, *J* = 7.8 Hz, 2H), 7.73 (s, 2H), 7.33 (d, *J* = 8.1 Hz, 2H), 7.24 (br s, 4H exchangeable with D_2O), 7.21−7.14 (m, 6H), 6.92 (d, *J* = 6.9 Hz, 4H), 4.92 (dd, *J* = 12.9, 3.9, 2H), 4.71−4.68 (m, 2H), 4.54−4.47 (m, 2H), 4.14 (m, 4H), 3.43 (d, *J* = 3.3 Hz, 2H), 2.34 (s, 3H); $^{13}C\{^1H\}$ NMR (75 MHz, DMSO-d_6): δ 156.0 (*C*), 152.5 (*C*), 149.6 (*C*), 143.2 (*C*), 140.7 (*CH*), 137.3 (*C*), 136.2 (*C*), 129.5 (*CH*), 128.0 (*CH*), 127.5 (*CH*), 127.5 (*CH*), 127.3 (*CH*), 118.7 (*C*), 78.1 (*CH*), 71.2 (CH_2), 60.7 (*CH*), 41.1 (CH_2), 21.0 (CH_3); HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{37}H_{38}N_{11}O_4S$ 732.2823; found 732.2827.

(2S,3R,4R,5S)-2,5-Bis(9-adeninylmethyl)-3,4-bis(benzyloxy)-pyrrolidine ***(10)***. To a solution of **9** (1.80 g, 2.46 mmol) in 30 mL of a mixture of DMF and methanol (8:1), disodium hydrogen phosphate (1.74 g, 12.30 mmol) and 3% Na−Hg (37.71 g, 49.19 mmol) were added and the reaction mixture was stirred for 4 h at 60 °C (oil bath). On completion, the reaction mixture was diluted with water (100 mL) and extracted with EtOAc (3 × 100 mL). The combined organic layer was washed with brine (50 mL), dried over anhydrous sodium sulfate, and the solvent was concentrated under vacuum to give a residue, which was purified by column chromatography over silica gel using a mixture of methanol and chloroform (1:18) as an eluent to obtain **10** (1.00 g, 70%) as a white solid (recrystallized from MeOH and $CHCl_3$). mp 172−174 °C; R_f: 0.17 (MeOH−$CHCl_3$ 1:4); IR (KBr): $\overline{\nu}$ 3562, 3311, 3242, 3124, 2926, 1683, 1646, 1607, 1576, 1483, 1418, 1332, 1305, 1247, 1075, 740, 698, 647 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.09 (s, 2H), 7.95 (s, 2H), 7.37−7.30 (m, 10H), 7.16 (s, 4H exchangeable with D_2O), 4.62 (d, *J* = 11.7 Hz, 2H), 4.52 (d, *J* = 11.7 Hz, 2H), 4.29 (dd, *J* = 13.8, 4.8 Hz, 2H), 4.12−4.05 (m, 4H), 3.87 (m, 2H), 2.84 (s, 1H exchangeable with D_2O); $^{13}C\{^1H\}$ NMR (75 MHz, DMSO-d_6): δ 155.8 (*C*), 152.1 (*CH*), 149.5 (*C*), 141.0 (*CH*), 138.0 (*C*), 128.2 (*CH*), 127.6 (*CH*), 127.5 (*CH*), 118.6 (*C*), 82.1 (*CH*), 71.2 (CH_2), 58.1 (*CH*), 43.5 (CH_2); HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{30}H_{32}N_{11}O_2$ 578.2735; found 578.2709.

(2S,3R,4R,5S)-2,5-Bis(9-adeninylmethyl)-3,4-(dihydroxy)-pyrrolidine ***(1)***. Compound **10** (0.15 g, 0.26 mmol) was dissolved in concentrated HCl (2 mL) and heated (oil bath) at 100 °C for 2 h. After completion, hydrochloric acid was evaporated in a rotatory evaporator and the residue was quenched with ammonium hydroxide solution (4 mL). Evaporation of ammonium hydroxide solution under vacuum gave a residue, which was purified by column chromatography over silica gel using a mixture of ammonium hydroxide solution and acetonitrile (1:19) as an eluent to obtain **1** (0.093 g, 90%) as a white solid (recrystallized from MeOH and $CHCl_3$). mp 201−203 °C; R_f: 0.25 (NH_4OH−acetonitrile, 1:4); IR (KBr): $\overline{\nu}$ 3143, 1653, 1608, 1480, 1403, 1336, 1305, 1252, 1208, 1083, 647 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.20 (s, 2H), 8.15 (s, 2H), 7.43−7.25 (br s, 4H exchangeable with D_2O), 6.10 (s, 2H exchangeable with D_2O), 4.43 (br m, 4H), 4.20 (br m, 2H), 4.07 (br m, 2H); $^{13}C\{^1H\}$ NMR (75 MHz, DMSO-d_6): δ 156.1 (*C*), 152.8 (*CH*), 149.8 (*C*), 141.7 (*CH*), 118.9 (*C*), 75.0 (*CH*), 60.6 (*CH*), 41.8 (CH_2); HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{16}H_{20}N_{11}O_2$ 398.1796; found 398.1804.

(2S,3R,4R,5S)-2,5-Bis(9-adeninylmethyl)-3,4-(dihydroxy)-N-(p-toluenesulfonyl)pyrrolidine ***(11)***. To a suspension of compound **9** (0.40 g, 0.55 mmol) in dichloromethane (1 mL), BCl_3 (2.73 mL, 1 M solution in dichloromethane) was added dropwise at 0 °C under an argon atmosphere. The reaction mixture was stirred at 32 °C for 2 h. The reaction was stopped, and the solvent was evaporated in a rotatory evaporator. The resulting residue was quenched with 2 mL of ammonium hydroxide solution. After evaporation of ammonium hydroxide solution, the crude product was purified by column chromatography over silica gel using a mixture of ammonium hydroxide solution and acetonitrile (1:32) as an eluent to obtain **11** (0.220 g, 73%) as a white solid (recrystallized from MeOH and $CHCl_3$). mp 228−230 °C; R_f: 0.60 (NH_4OH−acetonitrile, 1:4); IR (KBr): $\overline{\nu}$ 3339, 3179, 1645, 1602, 1478, 1414, 1323, 1245, 1055, 801, 659 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): 8.22 (s, 2H), 8.07 (d, *J* =

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J. Org. Chem. 2021, 86, 367−378

8.1 Hz, 2H), 7.97 (s, 2H), 7.45 (d, J = 8.1 Hz, 2H), 7.27 (s, 4H exchangeable with D_2O), 5.56 (d, J = 2.4 Hz, 2H exchangeable with D_2O), 5.06 (dd, J = 12.9, 3.0 Hz, 2H), 4.43−4.29 (m, 4H), 3.34 (s, 2H signal merged with the water signal of DMSO) 2.41 (s, 3H); $^{13}C\{^1H\}$ NMR (75 MHz, DMSO-d_6): δ 156.0 (*C*), 153.0 (*CH*), 150.0 (*C*), 143.9 (*C*), 142.2 (*CH*), 137.2 (*C*), 130.2 (*CH*), 127.6 (*CH*), 118.8 (*C*), 72.8 (*CH*), 62.4 (*CH*), 41.5 (CH_2), 21.4 (CH_3); HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{23}H_{25}N_{11}NaO_4S$ 574.1704; found 574.1711.

(2S,3R,4R,5S)-3,4-Bis(benzyloxy)-2,5-bis(1-thyminylmethyl)-N-(p-toluenesulfonyl)pyrrolidine ***(12)***. Compound **8** was dissolved (3.40 g, 4.22 mmol) in dry DMF (40 mL). Potassium carbonate (1.74 g, 12.65 mmol), 18-crown-6-ether (2.22 g, 8.44 mmol), and thymine (1.59 g, 12.65 mmol) were added successively and the reaction mixture was heated (oil bath) at 80 °C for 7 h. The reaction mixture was diluted with water (100 mL) and extracted with EtOAc (3 × 150 mL). The combined organic layer was washed with brine (50 mL) and dried over anhydrous sodium sulfate. Evaporation of the solvent under vacuum gave a residue, which was purified by column chromatography over silica gel using a mixture of methanol and chloroform (1:24) as an eluent to obtain compound **12** (0.85 g, 28%) as a white solid. mp 195−198 °C; R_f: 0.59 (MeOH−$CHCl_3$, 1:4); $[\alpha]_D^{28}$ +73.3 (c 0.12, $CHCl_3$); IR (KBr): $\overline{\nu}$ 3462, 3211, 3060, 2921, 1679, 1464, 1344, 1247, 1158, 1093, 812, 760, 702, 668 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 9.43 (s, 2H exchangeable with D_2O), 7.75 (d, J = 8.1 Hz, 2H), 7.29−7.11 (m, 12H), 6.87 (s, 2H), 4.54−4.30 (m, 8H), 3.79−3.90 (m, 2H), 3.78 (d, J = 3.0 Hz, 2H), 2.36 (s, 3H), 1.71 (s, 6H); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ 164.7 (*C*), 151.4 (*C*), 143.7 (*C*), 141.8 (*CH*), 137.3 (*C*), 136.9 (*C*), 129.8 (*CH*), 128.6 (*CH*), 128.2 (*CH*), 127.6 (*CH*), 127.3 (*CH*), 110.1 (*C*), 79.6 (*CH*), 72.3 (CH_2), 60.8 (*CH*), 47.1 (CH_2), 21.6 (CH_3), 12.1 (CH_3); HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{37}H_{39}N_5NaO_8S$ 736.2411; found 736.2405.

(2S,3R,4R,5S)-3,4-Bis(benzyloxy)-2,5-bis(1-thyminylmethyl)-pyrrolidine ***(13)***. To a solution of **12** (1.20 g, 1.68 mmol) in 20 mL of a mixture of DMF and methanol (8:1), Na_2HPO_4 (1.19 g, 8.40 mmol) and 3% Na−Hg (25.77 g, 33.62 mmol) were added and the reaction mixture was stirred for 4 h at 60 °C (oil bath). On completion, the reaction mixture was diluted with water (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layer was washed with brine (50 mL), dried over anhydrous sodium sulfate filtered, and the solvent was concentrated under vacuum to give a residue, which was purified by column chromatography over silica gel using a mixture of methanol and chloroform (1:16) as an eluent to obtain **13** (0.700 g, 74%) as a white solid. mp 160−162 °C; R_f: 0.44 (MeOH−$CHCl_3$, 1:4); $[\alpha]_D^{28}$ −5.7 (c 0.21, $CHCl_3$); IR (KBr): $\overline{\nu}$ 3469, 3335, 3163, 3030, 2828, 1680, 1471, 1427, 1362, 1226, 1088, 903, 757, 737, 703 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 9.27 (br s, 1H exchangeable with D_2O), 7.37−7.26 (m, 10H), 7.01 (s, 2H), 4.61 (d, J = 11.4 Hz, 2H), 4.43 (d, J = 11.7 Hz, 2H), 4.01 (d, J = 3.3 Hz, 2H), 3.89 (dd, J = 12.0, 2.7, 2H), 3.80−3.68 (m, 4H), 1.98 (br s, 2H exchangeable with D_2O), 1.78 (s, 6H); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$) δ 164.5 (*C*), 151.5 (*C*), 141.9 (*CH*), 137.5 (*C*), 128.7 (*CH*), 128.3 (*CH*), 127.9 (*CH*), 110.0 (*C*), 82.3 (*CH*), 72.4 (CH_2), 58.1 (*CH*), 49.1 (CH_2), 12.2 (CH_3); HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{30}H_{34}N_5O_6$ 560.2504; found 560.2522.

(2S,3R,4R,5S)-3,4-Dihydroxy-2,5-bis(1-thyminylmethyl)-pyrrolidine ***(2)***. To a solution of compound **13** (0.50 g, 0.89 mmol) in dry dichloromethane (2 mL), BCl_3 (4.47 mL, 1 M solution in dichloromethane) was added dropwise at 0 °C under an argon atmosphere. The reaction mixture was stirred at 37 °C for 2 h. The reaction was stopped, and the solvent was evaporated in a rotatory evaporator. The resulting residue was quenched with 10 mL of ammonium hydroxide solution. After evaporation of ammonium hydroxide, the crude product was purified by column chromatography over silica gel using a mixture of ammonium hydroxide solution and acetonitrile (1:19) as an eluent to obtain **2** (0.290 g, 85%) as a white solid (recrystallized from MeOH and $CHCl_3$). mp 250−252 °C; R_f: 0.20 (NH_4OH−acetonitrile, 1:4); IR (KBr): $\overline{\nu}$ 3448, 3151, 3044, 2830, 1685, 1472, 1406, 1333, 1224, 1075, 877, 758, 711 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 11.35 (s, 2H exchangeable with D_2O), 9.74 (s, 1H exchangeable with D_2O), 7.69 (s, 2H), 6.11 (s, 2H exchangeable with D_2O), 4.07−3.97 (m, 8H), 1.73 (s, 6H); $^{13}C\{^1H\}$ NMR (75 MHz, DMSO-d_6): δ 164.7 (*C*), 151.5 (*C*), 142.0 (*CH*), 109.1 (*C*), 74.4 (*CH*), 60.6 (*CH*), 45.9 (CH_2), 12.2 (CH_3); HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{16}H_{22}N_5O_6$ 380.1565; found 380.1582.

(2S,3R,4R,5S)-3,4-Dihydroxy-2,5-bis(1-thyminylmethyl)-N-(p-toluenesulfonyl)pyrrolidine ***(14)***. To a solution of compound **12** (0.33 g, 0.46 mmol) in dichloromethane (1 mL), BCl_3 (2.31 mL, 1 M solution in dichloromethane) was added dropwise at 0 °C under an argon atmosphere. The reaction mixture was stirred at 38 °C for 2 h. The reaction was stopped, and the solvent was evaporated in a rotatory evaporator. The resulting residue was quenched with 5 mL of ammonium hydroxide solution. After evaporation of ammonium hydroxide, the crude product was purified by column chromatography over silica gel using a mixture of ammonium hydroxide solution and acetonitrile (1:32) as an eluent to obtain **14** (0.190 g, 77%) as a white solid (recrystallized from MeOH and $CHCl_3$). mp 232−235 °C; R_f: 0.35 (NH_4OH−acetonitrile, 1:4); IR (KBr): $\overline{\nu}$ 3290, 3084, 3027, 2925, 2824, 1672, 1477, 1426, 1380, 1325, 1221, 1161, 1090, 893, 814, 765, 679 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 11.31 (s, 2H exchangeable with D_2O), 7.85 (d, J = 8.1 Hz, 2H), 7.38 (d, J = 8.1 Hz, 2H), 7.33 (s, 2H), 5.40 (d, J = 2.1 Hz, 2H exchangeable with D_2O), 4.68 (dd, J = 12.9, 3.9 Hz, 2H), 4.13−4.10 (m, 2H), 3.68−3.60 (m, 4H), 2.40 (s, 3H), 1.72 (s, 6H); $^{13}C\{^1H\}$ NMR (75 MHz, DMSO-d_6): δ 164.9 (*C*), 151.5 (*C*), 143.5 (*C*), 142.9 (*CH*), 137.6 (*C*), 130.0 (*CH*), 127.4 (*CH*), 108.6 (*C*), 72.8 (*CH*), 61.4 (*CH*), 46.4 (CH_2), 21.4 (CH_3), 12.3 (CH_3); HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{23}H_{27}N_5NaO_8S$ 556.1472; found 556.1492.

(2S,3R,4R,5S)-3,4-Bis-benzyloxy-5-(benzyloxymethyl)-2-(p-toluenesulfonyloxymethyl)-N-(p-toluenesulfonyl)pyrrolidine ***(16)***. To a solution of **15**[24a] (5.10 g, 8.68 mmol) in dichloromethane (40 mL), triethylamine (2.40 mL, 17.35 mmol), DMAP (2.12 g, 17.35 mmol), and p-TsCl (3.30 g, 17.35 mmol) were added and the reaction mixture was stirred at 33 °C for 5 h. It was then diluted with water (100 mL) and extracted with EtOAc (3 × 100 mL). The combined organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum to give a residue, which was purified by column chromatography over silica gel using a mixture of EtOAc and hexane (1:9) as an eluent to obtain **16** (5.00 g, 78%) as a colorless solid. mp 53−55 °C; R_f: 0.83 (EtOAc−hexane, 1:2); $[\alpha]_D^{28}$ −10.3 (c 0.28, $CHCl_3$); IR (KBr): $\overline{\nu}$ 3033, 2872, 1598, 1454, 1357, 1145, 982, 864, 814, 730, 660 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.73 (d, J = 8.1 Hz, 2H), 7.66 (d, J = 8.1 Hz, 2H), 7.29−7.13 (m, 17H), 7.00−6.99 (m, 2H), 4.59 (d, J = 11.7 Hz, 1H), 4.51 (d, J = 11.7 Hz, 1H), 4.46−4.38 (m, 4H), 4.25−4.08 (m, 4H), 3.92−3.90 (m, 2H), 3.79−3.76 (dd, J = 9.9, 3.0 Hz, 1H), 3.54 (d, J = 9.9 Hz, 1H), 2.37 (s, 3H), 2.32 (s, 3H); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ 144.6 (*C*), 143.5 (*C*), 138.2 (*C*), 138.0 (*C*), 137.8 (*C*), 137.3 (*C*), 132.9 (*C*), 129.7 (*CH*), 129.6 (*CH*), 128.4 (*CH*), 128.4 (*CH*), 128.3 (*CH*), 128.1 (*CH*), 127.9 (*CH*), 127.8 (*CH*), 127.6 (*CH*), 127.3 (*CH*), 127.1 (*CH*), 80.5 (*CH*), 80.3 (*CH*), 73.6 (CH_2), 73.3 (CH_2), 72.8 (CH_2), 67.5 (CH_2), 66.1 (CH_2), 58.5 (*CH*), 57.3 (*CH*), 21.7 (CH_3), 21.5 (CH_3); HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{41}H_{43}NNaO_8S_2$ 764.2322; found 764.2340.

(2S,3R,4R,5S)-3,4-Bis(benzyloxy)-5-(benzyloxymethyl)-2-(1-thyminylmethyl)-N-(p-toluenesulfonyl)pyrrolidine ***(17)***. Compound **16** (5.50 g, 7.41 mmol) was dissolved in dry DMF (50 mL). Potassium carbonate (1.53 g, 11.12 mmol), 18-crown-6-ether (1.95 g, 7.41 mmol), and thymine (1.40 g, 11.12 mmol) were added successively and the reaction mixture was heated at 80 °C (oil bath) for 4 h. The reaction mixture was cooled to room temperature, diluted with water (150 mL), and extracted with EtOAc (3 × 100 mL). The combined organic layer was washed with brine (50 mL) and dried over anhydrous sodium sulfate. Evaporation of the solvent under vacuum gave a residue, which was purified by column chromatography over silica gel using a mixture of EtOAc and hexane (1:1.5) as an eluent to obtain compound **17** (2.50 g, 49%) as a colorless solid. mp 52−54 °C; R_f: 0.40 (EtOAc−hexane, 1:1); $[\alpha]_D^{29}$ +43.0 (c 0.20, $CHCl_3$); IR

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J. Org. Chem. 2021, 86, 367−378

(KBr): $\bar{\nu}$ 3156, 3038, 2925, 1677, 1461, 1337, 1153, 905, 815, 742, 700, 663 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 10.35 (s, 1H exchangeable with D_2O) 7.83 (d, J = 8.1 Hz, 2H), 7.26−7.11 (m, 15H), 6.88−6.87 (m, 3H), 4.65 (d, J = 11.7 Hz, 1H), 4.50−4.44 (m, 3H), 4.35−4.28 (m, 3H), 4.11−3.93 (m, 4H), 3.76−3.54 (m, 3H), 2.16 (s, 3H), 1.51 (s, 3H); $^{13}C\{^1H\}$ (75 MHz, $CDCl_3$): δ 164.8 (*C*), 151.3 (*C*), 143.3 (*CH*), 143.1 (*C*), 137.6 (*C*), 137.4 (*C*), 137.2 (*C*), 136.7 (*C*), 129.3 (*CH*), 128.2 (*CH*), 128.2 (*CH*), 128.1 (*CH*), 127.9 (*CH*), 127.7 (*CH*), 127.6 (*CH*), 127.5 (*CH*), 127.4 (*CH*), 127.1 (*CH*), 126.9 (*CH*), 109.0 (*C*), 81.0 (*CH*), 79.9 (*CH*), 73.1 (CH_2), 73.0 (CH_2), 72.5 (CH_2), 65.0 (CH_2), 59.1 (*CH*), 55.5 (*CH*), 50.6 (CH_2), 21.2 (CH_3), 11.7 (CH_3); HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{39}H_{41}N_3NaO_7S$ 718.2557; found 718.2552.

(2S,3R,4R,5S)-5-(Acetoxymethyl)-3,4-bis(benzyloxy)-2-(1-thyminylmethyl)-N-(p-toluenesulfonyl)pyrrolidine ***(18)***. Compound **17** (2.5 g, 3.59 mmol) was dissolved in 20 mL of a mixture of acetic anhydride and acetic acid (4:1). Anhydrous zinc chloride (2.45 g, 17.96 mmol) was added to it and the reaction mixture was stirred at 33 °C for 5 h. It was then quenched with water (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layer was washed with saturated sodium bicarbonate solution (3 × 100 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum. The resulting residue was purified by column chromatography over silica gel using a mixture of EtOAc and hexane (1:2) to obtain compound **18** (2.00 g, 86%) as a colorless solid. mp 72−74 °C; R_f: 0.29 (EtOAc−hexane, 1:1); $[\alpha]_D^{29}$ +39.6 (c 0.33, $CHCl_3$); IR (KBr): $\bar{\nu}$ 3176, 3035, 2928, 1738, 1682, 1460, 1342, 1237, 1153, 1091, 1037, 904, 813, 744, 701, 664 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.85 (s, 1H exchangeable with D_2O), 7.84−7.80 (m, 2H), 7.33−7.18 (m, 12H), 6.95 (s, 1H), 4.60−4.53 (m, 4H), 4.46 (dd, J = 11.4, 2.7 Hz, 1H), 4.41−4.36 (m, 1H), 4.31−4.27 (m, 1H), 4.18−4.09 (m, 4H), 3.77 (dd, J = 13.2, 8.1 Hz, 1H), 2.36 (s, 3H), 1.60 (s, 3H), 1.46 (d, J = 2.7 Hz, 3H); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ 169.6 (*C*), 164.5 (*C*), 151.4 (*C*), 143.7 (*C*), 143.2 (*CH*), 137.1 (*C*), 137.1 (*C*), 136.9 (*C*), 129.8 (*CH*), 128.7 (*CH*), 128.6 (*CH*), 128.3 (*CH*), 128.2 (*CH*), 127.9 (*CH*), 127.9 (*CH*), 127.5 (*CH*), 109.6 (*C*), 80.8 (*CH*), 79.1 (*CH*), 73.5 (2 × CH_2), 59.9 (CH_2), 57.7 (*CH*), 55.6 (*CH*), 50.8 (CH_2), 21.5 (CH_3), 20.3 (CH_3), 12.0 (CH_3); HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{34}H_{37}N_3NaO_8S$ 670.2193; found 670.2191.

(2S,3R,4R,5S)-3,4-Bis(benzyloxy)-5-(hydroxymethyl)-2-(1-thyminylmethyl)-N-(p-toluenesulfonyl)pyrrolidine ***(19)***. To a solution of compound **18** (1.15 g, 1.77 mmol) in MeOH (10 mL), sodium carbonate (0.37 g, 3.55 mmol) was added and stirred at 37 °C for 2 h, after which the solid was filtered through a Buchner funnel. The filtrate was dried over anhydrous sodium sulfate and concentrated under vacuum. The resulting residue was purified by column chromatography over silica gel using a mixture of EtOAc and hexane (1:2) to obtain **19** as a colorless solid (0.95 g, 88%). mp 54−57 °C; R_f: 0.25 in (EtOAc−hexane, 1:1); $[\alpha]_D^{26}$ +32.0 (c 0.41, $CHCl_3$); IR (KBr): $\bar{\nu}$ 3459, 3182, 3034, 2925, 1680, 1461, 1334, 1222, 1156, 1092, 1037, 906, 812, 741, 703, 668 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 9.15 (s, 1H exchangeable with D_2O), 7.81 (d, J = 8.1 Hz, 2H), 7.31−7.16 (m, 12H), 6.92 (s, 1H), 4.60−4.41 (m, 5H), 4.28 (dd, J = 14.1, 5.1 Hz, 1H), 4.18−4.08 (m, 2H), 4.05−4.03 (m, 1H), 3.93−3.86 (m, 3H), 2.36 (s, 3H), 1.67 (s, 3H); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ 164.4 (*C*), 151.3 (*C*), 143.8 (*C*), 142.3 (*CH*), 137.1 (2 × *C*), 137.1 (*C*), 129.8 (*CH*), 128.7 (2 × *CH*), 128.3 (*CH*), 128.2 (*CH*), 127.8 (*CH*), 127.7 (*CH*), 127.4 (*CH*), 109.9 (*C*), 81.1 (*CH*), 80.7 (*CH*), 73.3 (CH_2), 73.2 (CH_2), 62.3 (*CH*), 59.6 (CH_2), 57.3 (*CH*), 49.3 (CH_2), 21.6 (CH_3), 12.1 (CH_3); HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{32}H_{35}N_3NaO_7S$ 628.2088; found 628.2088.

(2S,3R,4R,5S)-3,4-Bis(benzyloxy)-2-(1-thyminylmethyl)-2-(p-toluenesulfonyloxymethyl)-N-(p-toluenesulfonyl)pyrrolidine ***(20)***. To a solution of **19** (1.40 g, 2.31 mmol) in dichloromethane (15 mL), triethylamine (0.32 mL, 2.31 mmol), DMAP (0.28 g, 2.31 mmol), and *p*-TsCl (0.66 g, 3.47 mmol) were added and the reaction mixture was stirred at 32 °C for 7 h. It was then diluted with water (100 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum to give a residue, which was purified by column chromatography over silica gel using a mixture of EtOAc and hexane (1:2) as an eluent to obtain **20** (1.40 g, 80%) as a colorless solid. mp 59−61 °C; R_f: 0.33 (EtOAc−hexane, 1:1); $[\alpha]_D^{30}$ +49.5 (c 0.20, $CHCl_3$); IR (KBr): $\bar{\nu}$ 3424, 3177, 3037, 2926, 1682, 1600, 1458, 1353, 1171, 1095, 978, 850, 814, 743, 703, 667 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.87 (m, 1H exchangeable with D_2O), 7.74 (d, J = 8.0 Hz, 2H), 7.64 (d, J = 8.0 Hz, 2H), 7.32−7.15 (m, 10H), 7.15 (d, J = 6.8 Hz, 2H), 7.05 (d, J = 5.6 Hz, 2H), 6.88 (s, 1H), 451−4.28 (m, 8H), 4.14 (m, 1H), 4.03 (t, J = 6.0 Hz, 1H), 3.94 (t, J = 6.0 Hz, 1H), 3.77 (dd, J = 13.6, 8.4 Hz, 1H), 2.38 (s, 6H), 1.61 (s, 3H); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ 164.4 (*C*), 151.2 (*C*), 145.0 (*C*), 144.0 (*C*), 142.5 (*CH*), 137.0 (*C*), 137.0 (*C*), 136.2 (*C*), 132.5 (*C*), 129.9 (*CH*), 129.8 (*CH*), 128.7 (*CH*), 128.5 (*CH*), 128.2 (*CH*), 128.2 (*CH*), 128.1 (*CH*), 127.9 (*CH*), 127.7 (*CH*), 127.6 (*CH*), 109.8 (*C*), 80.5 (*CH*), 78.8 (*CH*), 73.4 (CH_2), 73.1 (CH_2), 65.5 (CH_2), 59.0 (*CH*), 57.4 (*CH*), 49.2 (CH_2), 21.7 (CH_3), 21.7 (CH_3), 12.0 (CH_3); HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{39}H_{41}N_3NaO_9S_2$ 782.2176; found 782.2175.

(2S,3R,4R,5S)-5-(9-Adeninylmethyl)-3,4-bis(benzyloxy)-2-(1-thyminylmethyl)-N-(p-toluenesulfonyl)pyrrolidine ***(21)***. Compound **20** (1.98 g, 2.61 mmol) was dissolved in dry DMF (20 mL). Potassium carbonate (0.54 g, 3.91 mmol), 18-crown-6-ether (0.69 g, 2.61 mmol), and adenine (0.53 g, 3.91 mmol) were added successively and the reaction mixture was heated at 80 °C (oil bath) for 6 h. The reaction mixture was diluted with water (100 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layer was washed with brine (50 mL) and dried over anhydrous sodium sulfate. Evaporation of the solvent under vacuum gave a residue, which was purified by column chromatography over silica gel using a mixture of methanol and chloroform (1:19) as an eluent to obtain compound **21** (1.05 g, 56%) as a colorless solid (recrystallized from $CHCl_3$). mp 131−132 °C; R_f: 0.51 (MeOH−$CHCl_3$, 1:9); $[\alpha]_D^{26}$ +84.3 (c 0.13, $CHCl_3$); IR (KBr): $\bar{\nu}$ 3332, 3189, 3058, 3031, 2925, 1687, 1599, 1457, 1338, 1246, 1159, 1092, 906, 806, 746, 702, 669 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 11.21 (s, 1H exchangeable with D_2O), 8.41 (s, 1H), 7.79 (m, 1H), 7.72 (d, J = 7.8 Hz, 2H), 7.27−7.20 (m, 7H), 7.16−7.14 (m, 3H), 7.05−7.04 (m, 2H), 6.82 (s, 1H), 6.29 (s, 2H exchangeable with D_2O), 4.98 (dd, J = 13.2, 5.1 Hz, 1H), 4.77−4.71 (m, 1H), 4.55−4.48 (m, 3H), 4.40−4.23 (m, 4H), 3.99−3.95 (m, 1H), 3.73 (br s, 1H), 3.67 (br s, 1H), 2.34 (s, 3H), 1.71 (s, 3H); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ 165.4 (*C*), 155.8 (*C*), 153.0 (*CH*), 151.8 (*C*), 150.1 (*C*), 143.7 (2 × *C*), 141.7 (*CH*), 141.4 (*CH*), 137.0 (*C*), 136.7 (*C*), 129.7 (*CH*), 128.6 (*CH*), 128.5 (*CH*), 128.3 (*CH*), 128.2 (*CH*), 128.0 (*CH*), 127.5 (*CH*), 127.4 (*CH*), 118.9 (*C*), 110.2 (*C*), 79.7 (*CH*), 79.6 (*CH*), 72.6 (CH_2), 72.3 (CH_2), 61.2 (*CH*), 60.7 (*CH*), 47.1 (CH_2), 41.9 (CH_2), 21.6 (CH_3), 12.1 (CH_3); HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{37}H_{39}N_8O_6S$ 723.2708; found 723.2719.

(2S,3R,4R,5S)-5-(9-Adeninylmethyl)-3,4-(dihydroxy)-2-(1-thyminylmethyl)-N-(p-toluenesulfonyl)pyrrolidine ***(22)***. To a solution of **21** (0.39 g, 0.54 mmol) in 10 mL of a mixture of DMF and methanol (8:1), Na_2HPO_4 (0.48 g, 2.69 mmol) and 3% Na−Hg (8.25 g, 10.76 mmol) were added and the reaction mixture was stirred for 3 h at 60 °C (oil bath). On completion, the reaction mixture was diluted with water (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layer was washed with brine (50 mL) dried over anhydrous sodium sulfate filtered and the solvent was concentrated under vacuum to give a residue, which was purified by column chromatography over silica gel using a mixture of methanol and chloroform (1:16) as an eluent to obtain **22** (0.215 g, 70%) as a colorless solid (recrystallized from MeOH and $CHCl_3$). mp 180−182 °C; R_f: 0.24 (MeOH−$CHCl_3$, 1:9); IR (KBr): $\bar{\nu}$ 3395, 3342, 3255, 3219, 2925, 2857, 2776, 1706, 1670, 1604, 1451, 1426, 1380, 1334, 1201, 1106, 1060, 1021, 937, 756, 700 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 11.20 (s, 1H exchangeable with D_2O), 8.10 (s, 1H), 7.99 (s, 1H), 7.38−7.29 (m, 11H), 7.18 (s, 2H, exchangeable with D_2O), 4.66−4.47 (m, 4H), 4.33 (dd, J = 12.9, 3.3 Hz, 1H), 4.15−4.05 (m, 3H), 3.85−3.81 (m, 2H), 3.64 (br m, 1H), 3.55−3.48 (m, 1H), 2.78 (br s, 1H exchangeable with D_2O), 1.63 (s, 3H); $^{13}C\{^1H\}$ NMR (75 MHz, DMSO-d_6): δ 164.3 (*C*), 155.9 (*C*), 152.2 (*CH*), 151.1 (*C*), 149.6 (*C*), 142.3 (*CH*), 141.1 (*CH*), 138.1 (2 × *C*), 128.3 (2 ×

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J. Org. Chem. 2021, 86, 367−378

CH), 127.7 (2 × CH), 127.6 (2 × CH), 118.6 (*C*), 107.5 (*C*), 82.2 (CH), 82.0 (CH), 71.1 (CH_2), 71.1 (CH_2), 58.3 (CH), 57.5 (CH), 47.9 (CH_2), 43.3 (CH_2), 11.8 (CH_3); HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{30}H_{33}N_8O_4$ 569.2619; found 569.2599.

(2S,3R,4R,5S)-5-(9-Adeninylmethyl)-3,4-(dihydroxy)-2-(1-thyminylmethyl)pyrrolidine ***(3)***. Compound **22** (0.150 g, 0.264 mmol) was dissolved in concentrated HCl (2 mL) and heated (oil bath) for 2 h at 100 °C. On completion, the reaction was stopped and cooled to room temperature. HCl was evaporated in a rotatory evaporator, and the resulting residue was quenched with ammonium hydroxide solution (4 mL). Evaporation of the ammonia solution under vacuum gave a residue, which was purified by column chromatography over silica gel using a mixture of ammonium hydroxide solution and acetonitrile (1:13) as an eluent to obtain **3** (0.061 g, 60%) as a colorless solid (recrystallized from MeOH and $CHCl_3$). mp 198−200 °C; R_f: 0.30 (ammonia solution−acetonitrile, 1:4); IR (KBr): $\bar{\nu}$ 3471, 3320, 3192, 3139, 1697, 1663, 1404, 1252, 1068, 969, 885, 668 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 11.32 (s, 1H exchangeable with D_2O), 8.15 (s, 1H), 8.09 (s, 1H), 7.52 (s, 2H, 1H exchangeable with D_2O), 7.22 (s, 2H exchangeable with D_2O), 6.01−5.56 (m, 2H exchangeable with D_2O), 4.36 (d, J = 5.7 Hz, 2H), 4.09−3.75 (m, 6H), 1.66 (s, 3H); $^{13}C\{^1H\}$ NMR (75 MHz, DMSO-d_6): δ 164.3 (*C*), 155.9 (*C*), 152.2 (CH), 151.2 (*C*), 149.5 (*C*), 142.0 (CH), 141.2 (CH), 118.7 (*C*), 108.0 (*C*), 75.6 (CH), 75.5 (CH), 59.7 (CH), 59.1 (CH), 47.0 (CH_2), 42.3 (CH_2), 11.9 (CH_3); HRMS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{16}H_{21}N_8O_4$ 389.1680; found 389.1680.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.0c02140.

Copies of ^1H and ^{13}C NMR spectra of all new compounds; ORTEP and associated X-ray crystallographic data for compounds **2**, **10**, and **21** (PDF)

Accession Codes

CCDC 2023656−2023658 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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