ChemPubSoc Europe

DOI: 10.1002/chem.201303867

CHEMISTRY
A European Journal
Communication

DNA Recognition

A Pyrimidopyrimidine Janus-AT Nucleoside with Improved Base-Pairing Properties to both A and T within a DNA Duplex: The Stabilizing Effect of a Second Endocyclic Ring Nitrogen

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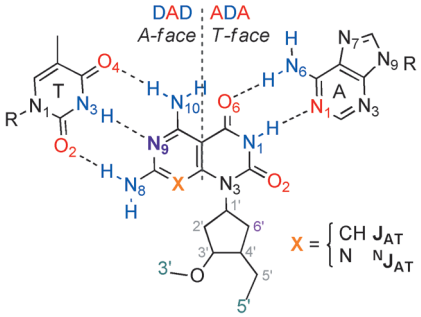
Abstract: Janus bases are heterocyclic nucleic acid base analogs that present two different faces able to simultaneously hydrogen bond to nucleosides that form Watson–Crick base pairs. The synthesis of a Janus-AT nucleotide analogue, $^{N}J_{AT}$, that has an additional endocyclic ring nitrogen and is thus more capable of efficiently discriminating T/A over G/C bases when base-pairing in a standard duplex-DNA context is described. Conversion to a phosphoramidite ultimately afforded incorporation into an oligonucleotide. In contrast to the first generation of carbocyclic Janus heterocycles, it remains in its unprotonated state at physiological pH and, therefore, forms very stable Watson–Crick base pairs with either A or T bases. Biophysical and computational methods indicate that $^{N}J_{AT}$ is an improved candidate for sequence-specific genome targeting.

DNA represents a therapeutic target, because it programs biological processes at the earliest step of gene expression. Hélène and co-workers[1] and Moser and Dervan[2] were the first to report the potential of an antigene strategy. Based on their seminal work, sequence-specific recognition of DNA has been extensively studied over the years, whereby DNA is recognized in a sequence-specific manner by oligonucleotides, peptides, and minor groove-binding ligands.[3] Oligonucleotides are attractive, because they have an inherent base-pairing capability that enables them to target potentially any sequence, usually by triplex formation by Hoogsteen or reverse Hoogsteen base pairing.[4] However, the number of hydrogen bonds contained within a Hoogsteen base pair is limited to two. Moreover, target sequences are limited largely to polypurine tracts. Alternatively, D-loop formation involves the invasion of a complementary strand with concomitant strand displacement; such might thus be preferred, because D-loop formation involves the formation of comparatively more stable Watson–Crick base pairs (particularly, G–C), the formation of which counterbalance the energetic cost of disrupting the double helix. Nevertheless, the displaced strand remains unpaired and is therefore poised to eject the inserted strand to recapture its position within the DNA duplex. This may explain the general thermodynamic instability of D-loops. To potentially overcome this thermodynamic ambiguity, Lehn and co-workers proposed the concept of Janus bases that would simultaneously hydrogen bond to bases on both strands by forming pseudo-Watson–Crick base pairs simultaneously with each base.[5] This concept was explored by several groups with oligonucleotides containing either singly-embedded Janus bases or tracts of Janus bases.[5] These examples provide important insight into the potential for such recognition. Yet, until recently, Janus bases that recognize a Watson–Crick base pair and not mismatched bases, have not been thoroughly investigated. Reasons for this are unclear, but may reflect synthetic challenges in creating and characterizing heterocycles with an inherent propensity for self-association and aggregation. Towards these ends, we recently synthesized and characterized a Janus nucleotide analogue, J_{AT}, capable of associating with A and T nucleotides, preferentially to G and C.[6] This first report detailed the synthesis of a pyrimidopyridine that base pairs with both A and T (Scheme 1). However, an oligonucleotide containing a single J_{AT} gave unusually wide UV melting transitions in presence of complementary DNA, particularly when juxtaposed across from A. This complicated the evaluation of the modified base and raised questions as to the extent of fully competent base pairing. Although both CD melting and extensive in silico



Scheme 1. J_{AT} and $^{N}J_{AT}$ structure, partial numbering, and putative hydrogen bonding with A and T (dashed lines). Janus-AT bases display two faces adapted to different hydrogen-bonding patterns (DAD and ADA; D = proton donor, A = proton acceptor). Purple stays for the potential protonation site (N9). The canonical ribose has been substituted with a carbocycle (C6′ instead of O4′). Shown is the antiparallel base pairing with bases in *anti*-conformation.

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201303867.

modeling suggested that Watson–Crick-like base pairs to both A and T were achievable, we hypothesized that the anomalous behavior observed in standard T_m analysis may have been due to partial protonation of N9 (Scheme 1, purple) at physiological pH (pK_a was calculated to be ca. 7.5), leading to a dynamically equilibrating mixture of standard base-pairing interactions with the free base and pseudo-base-pairing interactions with a cationic (protonated) form (not shown).

Because further synthetic elaborations of heterocycles capable of acting as Janus-AT nucleosides are needed to probe unnatural base-pairing interactions, we expanded this work to include a second-generation Janus-AT nucleotide, in which the inclusion of an endocyclic nitrogen would render the heterocycle considerably less basic, thereby minimizing the potential for protonation to a cationic species capable of mispairing. Therefore, the optimized structure, referred to as $^{N}\mathbf{J}_{AT}$, differs by substitution of the C7–H group by a nitrogen atom to significantly lower the pK_a of the conjugate acid of N9, as suggested by empirical calculation (Scheme 1, orange).[7,8] This would more closely mimic the structure of a diaminopurine to provide an additional hydrogen bond. Finally, we substituted the canonical deoxyribose with a cyclopentane analogue to preclude potential depurination-like elimination, enhance association DNA, and increase nuclease stability.[9] With these aims in mind, synthetic design necessitated a new synthetic approach that has never been elaborated for this Janus-AT nucleoside analogue. Hereafter, we refer to the T-face and A-face by analogy with their base-pairing behavior (the T-face binds to A, and vice versa) as we report preliminary biophysical data, whereby we conclude that this new heterocycle has improved properties in terms of recognizing either a complementary A or T.

To gauge the capability of the $^{N}\mathbf{J}_{AT}$ for base pairing with A or T, we conducted BP86/TZ2P DFT geometry optimization on the *N*-methylated structures of A, T, and $^{N}\mathbf{J}_{AT}$ bases,[10] and their corresponding base pairs.[6,11] The $^{N}\mathbf{J}_{AT}$-A and $^{N}\mathbf{J}_{AT}$-T complex geometries feature quasi co-planar bases (10–15° between the planes defined by the interacting cycles, Figure 1), and electron densities before and after binding appear compatible with hydrogen bonding (Figures S1 and S2 in the Supporting Information). Interestingly, the intramolecular N_{10}–H···O_6 hydrogen bond observed for the isolated $^{N}\mathbf{J}_{AT}$ is disrupted upon binding with A and, to a lesser extent, T, consistent with the change in electron density as a consequence of intermolecular hydrogen bonding. Hydrogen bond donor/acceptor-atom distances (Table S1 in the Supporting Information), as well as the binding-induced deformations of the N–H and C=O bonds involved in hydrogen bonding only (Tables S2 and S3 in the Supporting Information), are consistent with extant hydrogen-bonding interactions. Notably, donor/acceptor distances of the A-face, but not of the T-face, are globally shorter than previously found for $\mathbf{J}_{AT}$, which is likely due to the inclusion of the ring nitrogen of this heterocycle. Finally, the complexes lie in the same energy range as an AT base pair, suggesting efficient hydrogen bonding (Table S4 in the Supporting Information). Notably, G or C bases cannot form co-planar complexes with either face of $^{N}\mathbf{J}_{AT}$, most likely because of important electronic repulsion due to electron-rich oxygen atoms and steric constraints due to primary amines. The potential of base pairing by $^{N}\mathbf{J}_{AT}$ with T or A is thus supported by 1) the compatibility of electrostatic potentials for hydrogen bonding; 2) the electron-density localization; 3) the deformation of the N–H and C=O bonds involved in hydrogen bonding; 4) the stabilization observed for the complexes; and 5) the quasi co-planarity of the bases. To test these in silico predictions, we tackled the synthesis of the $^{N}\mathbf{J}_{AT}$ nucleotide and its corresponding phosphoramidite that enabled its incorporation into a $^{N}\mathbf{J}_{AT}$-modified oligonucleotide.

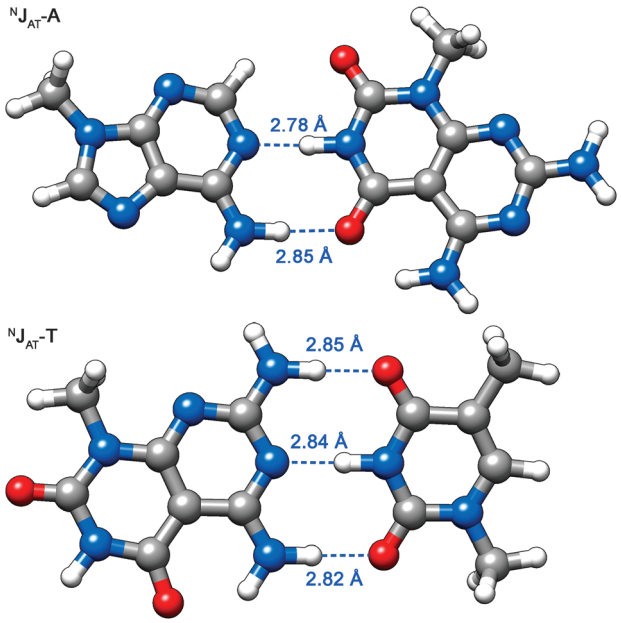
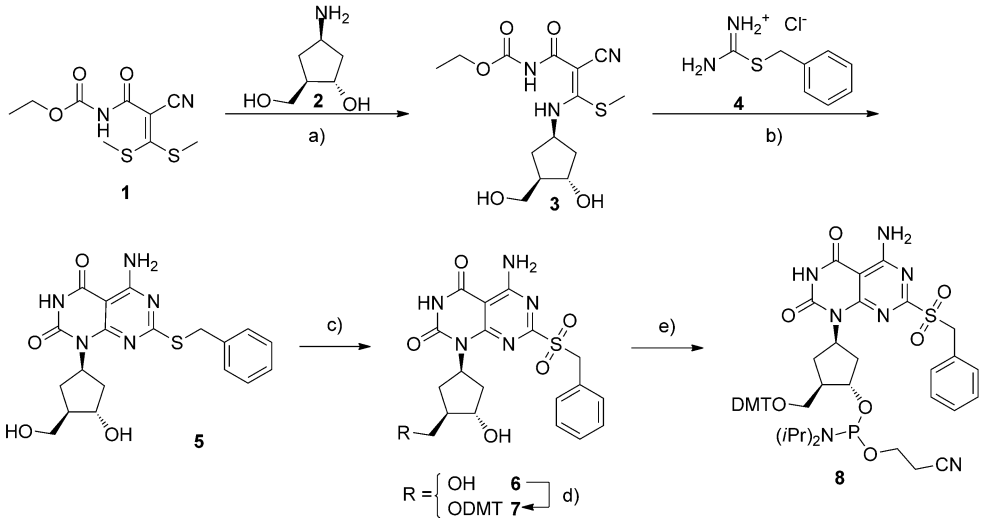


Figure 1. Geometries of the *N*-methylated $^{N}\mathbf{J}_{AT}$-A and $^{N}\mathbf{J}_{AT}$-T base pairs obtained by BP86/TZ2P DFT calculations. Carbon in grey, hydrogen in white, nitrogen in blue, oxygen in red, and computed hydrogen bonds as purple dashes. Bases are quasi co-planar, with acceptor-/donor-group distance consistent with Watson–Crick hydrogen bonding.

Janus-type nucleotides typically have very low solubility in organic solvents, which can impede the solid-phase synthesis of modified oligonucleotides.[12] Moreover, $\mathbf{J}_{AT}$ heterocycles proved refractory to standard acylation or phthaloylation under several conditions (data not shown) that otherwise would have afforded a protected heterocycle with increased solubility that would undergo normal deprotection with ammonia. In light of this, we opted for the synthesis of a sulfone-masked Janus-AT heterocycle that ultimately provided a phosphoramidite of much greater solubility, owing to a disrupted hydrogen-bonding pattern, hence avoiding self-aggregation (see below). The other advantage of such a target compound is that upon global deprotection following solid-phase oligonucleotide synthesis, the sulfone is converted to the desired exocylic amine by nucleophilic displacement.[6] Initially, we investigated the feasibility of a "west/east" disconnection analogous to the one we used for the $\mathbf{J}_{AT}$ nucleotide, that is, in which the T-face is elaborated first followed by closure of the A-face (Scheme S1 in the Supporting Information). The key intermediate **3** was prepared by condensation of carbamate **1** with the carbocyclic deoxyribose analogue **2**, by following a protocol that we and others reported previously.[9b,12] Un-



Scheme 2. a) Compound **2** (1 equiv), dry NEt_3 (2 equiv), absolute ethanol, reflux, 55%; b) compound **4** (1.2 equiv), NEt_3 (2 equiv), *i*PrOH, reflux, 61%; c) *m*-CPBA (3 equiv), MeOH, $CHCl_3$, 72%; d) dimethyltryptamine (DMT)-Cl (1.1 equiv), DIPEA (1.1 equiv), 1,4-dioxane, 51%; e) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (1.1 equiv), DIPEA (1.1 equiv), dry CH_2Cl_2, molecular sieves (4 Å), 60%.

fortunately, the T-face cyclization of **3** did not occur upon heating at reflux despite the use of a variety of conditions including acid, base, and nucleophilic catalysts, such as triethanolamine (TEA) and 1,4-diazabicyclo[2.2.2]octane (DABCO; data not shown). We hypothesized that this could be due to the steric hindrance of the thiomethyl group in the β position of the enone, as was described in literature.[13] Consequently, we attempted A-face cyclization by reacting **3** with **4**, the latter being easily obtained in high yield from thiourea and benzylbromide.[14] Interestingly, when **3** and **4** were combined at reflux in presence of triethylamine as a mild base, much to our delight, the bicyclic intermediate **5** was obtained directly. This critical step allowed us to construct the bicyclic compound in an original, convergent, and rapid manner. It was then straightforward to oxidize **5** with *meta*-chloroperoxybenzoic acid (*m*-CPBA) to obtain the sulfone-masked compound **6** (Scheme 2). However, attempted elaboration of the phosphoramidite in pyridine[9b] led to the rapid and untoward formation of a heretofore unknown Zincke salt **10** through facile displacement of the sulfone by pyridine (Scheme S2 in the Supporting Information).[15] Alternative solvent/base systems were screened, and fortunately, we identified 1,4-dioxane/*N*,*N*-diisopropylethylamine (DIPEA) to be highly suitable for accessing the protected nucleotide **7**, which was converted to phosphoramidite **8**.[9b] Overall, 15 steps were required, of which eleven are on the longest linear path with an overall yield of 4.1% (see the Supporting Information for detailed methods and $^1H/^{13}C$ NMR spectra). Compound **8** was used for solid-phase oligonucleotide synthesis to prepare the sequence 5′-GAGCGATG-$^{N}\mathbf{J}_{AT}$-GTAGCCAG-3′. Hereafter, all oligonucleotides have the sequence 5′-GAGCGATG-**Y**-GTAGCCAG-3′ ("strand I") or the complementary 5′-CTGGCTAC-**X**-CATCGCTC-3′ ("strand II"), in which **X** is either A, T, C, G, or the $^{N}\mathbf{J}_{AT}$ and **Y** is A, T, C or G, and are named "**X–Y**". A soluble, sulfone-masked, $^{N}\mathbf{J}_{AT}$ phosphoramidite can thus be prepared in a robust manner, ensuring its availability and compatibility with standard oligonucleotide solid-phase synthesis. MALDI-TOF analysis (see the Supporting Information) gave proof to its successful incorporation along with intended addition of the amine instead of the benzylsulfone.

The capability of $^{N}\mathbf{J}_{AT}$ to associate with A and T preferentially over G and C in a double-stranded (ds) DNA context was assessed by UV melting experiments. The melting curves displayed very clear single transitions for all four complementary sequences (Figure 2), supporting our initial hypothesis that lowering the pK_a of a hypothetical conjugate acid at N9 would lead to more consistent and well-behaved base-pairing interaction. As was expected, the $^{N}\mathbf{J}_{AT}$ base clearly prefers binding to T and A (T_m = 58.4 and 57.9 °C, respectively, close to the melting temperature of the canonical A-T: 58.3 °C) compared to G and C (54.9 and 53.1 °C). The large differences in stability between recognition of T versus C (5.3 °C) or between A and G (3.0 °C), as was usually observed with non-modified mismatches,[16] validates our design and suggests that $^{N}\mathbf{J}_{AT}$ oligonucleotides might be able to bind very preferentially to either A- and T-tracts. Circular dichroism (CD) analysis was then used to verify that the modified oligonucleotide forms a B-DNA duplex with cDNA. All spectra were characterized by two extremes consistent with a standard B-DNA helix (maximum/minimum 280/250 nm; Figure S3 in the Supporting Information),[17] which suggests that both strands undergo disassociation following a cooperative two-state model

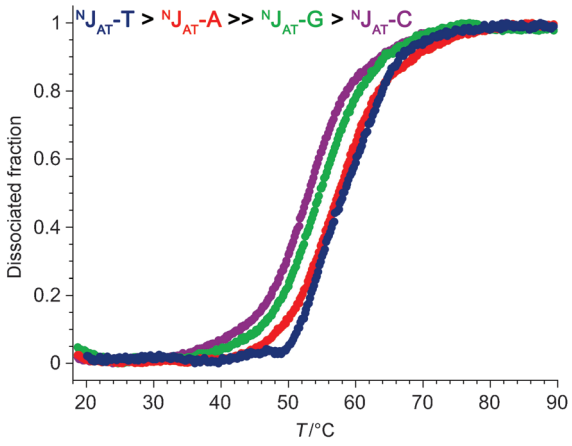


Figure 2. Dissociated fraction of $^{N}\mathbf{J}_{AT}$-**Y** (**Y** = A, T, G, C) oligonucleotides (1 μM strand concentration) as a function of temperature in sodium cacodylate (20 mM), NaCl (100 mM), and EDTA (0.5 mM) adjusted at pH 7.0.

when melted as a decrease of band intensities together with an isosbestic point around 260 nm where observed. Taken as a whole, results of biophysical experiments suggest that $^{N}\mathbf{J}_{AT}$ is capable of associating with T and A with no noticeable secondary-structure perturbation. This observation differs slightly from the previously reported pyrimidopyridine $\mathbf{J}_{AT}$ heterocycle, for which melting analysis suggested poorer base pairing to A. Equally important is that $^{N}\mathbf{J}_{AT}$-G/C base pairs lead to a significant destabilization of the dsDNA due to their mismatched nature, and can thus be easily discriminated against.

In an effort to acquire more structural insights, notably around the modified base-pair position in the duplex, we performed molecular-dynamics calculation on the $^{N}\mathbf{J}_{AT}$-A and $^{N}\mathbf{J}_{AT}$-T duplexes and compared the results with their canonical counterparts. Stochastic simulations were thus conducted in an AMBER force field with implicit water solvation at constant temperature (300 K) on a 2 ns timescale (Figures S4 and S5, Table S5 in the Supporting Information).[18] When comparing modified duplexes to their canonical counterpart, dihedral angles along the phosphate backbone and the pseudo *N*-glycosidic bond are mostly unchanged (Figure 3 and Table S6 in the Supporting Information), the only significant variation being observed for δ ($\Delta\delta = 18$ and 29° for $\mathbf{J}_{AT}$-A vs. T-A and $\mathbf{J}_{AT}$-T vs. A-T, respectively). This is consistent with what has been monitored for the first-generation $\mathbf{J}_{AT}$ and is believed to arise from the use of a carbocyclic deoxyribose analogue instead of the standard deoxyribose. Indeed, the carbocyclic deoxyribose analogue displays a nonstandard puckering ($P = 75°$ for $^{N}\mathbf{J}_{AT}$-A and 57° for $^{N}\mathbf{J}_{AT}$-T, respectively), which correspond respectively to C4′-*exo*/O4′-*endo* ($^{0}T_{4}$) and C4′-exo ($_{4}E$) conformations (Figure S6 in the Supporting Information). The *N*-glycosidic torsion angle χ (−126° and −132° for $^{N}\mathbf{J}_{AT}$-A and for $^{N}\mathbf{J}_{AT}$-T, respectively) is very similar to the non-modified sequence containing all deoxyribose linkages, that is, in a classical *anti* base conformation (Figure S7 in the Supporting Information). Notably, the *anti*-conformation implies that when $^{N}\mathbf{J}_{AT}$ associates with A, the T-face is largely positioned in the groove, which resulted in a slightly more solvent-accessible surface area (SASA) than when pairing with T (208.0 vs. 205.6 Å).

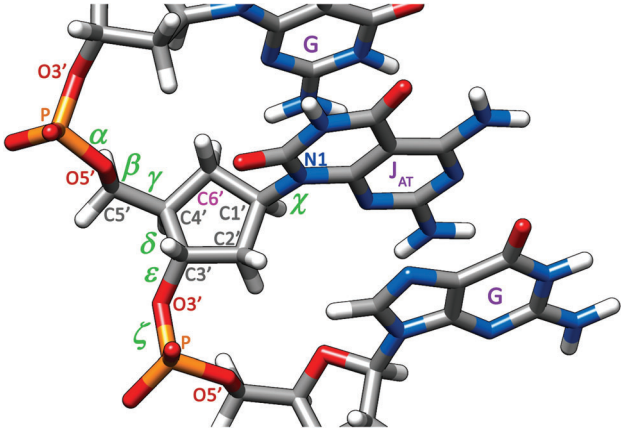


Figure 3. Backbone dihedral angles α, β, γ, δ, ε, and ξ, relative orientation of the bases versus the sugar (analogue) χ, and atom numbering of the $^{N}\mathbf{J}_{AT}$ nucleotide (average structure from the 2 ns molecular dynamics simulation, when pairing with T; strand II is not shown). $^{N}\mathbf{J}_{AT}$ stacks with the neighboring guanines in a canonical fashion (see also Figure S7 in the Supporting Information for more details).

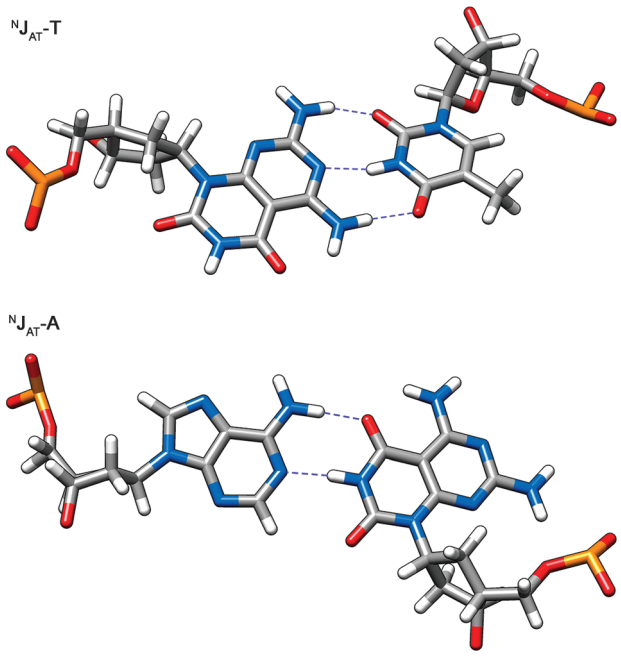


Figure 4. $^{N}\mathbf{J}_{AT}$-T and $^{N}\mathbf{J}_{AT}$-A base pairing (hydrogen bonds depicted as purple dashes) in average structures from molecular-dynamics results. Other atoms are not shown for clarity.

Hydrogen-bond lengths and angles are analogous to the non-modified sequences, and very little if any base pairing disruption was observed over the course of the 2 ns simulation (Figure 4 and Table S7 in the Supporting Information). $^{N}\mathbf{J}_{AT}$ base-pairing isostery with natural bases was verified by using a set of virtual parameters, defined by Olson and co-workers (Table S6 in the Supporting Information).[19] The only significant discrepancy with the canonical base pair is the small desymmetrization observed for the interphosphate distances, likely due to the modification in phosphate torsion angles, most notably around the pseudo-sugar C3′–C4′ (see above). Overall, the $^{N}\mathbf{J}_{AT}$ nucleotide induces little to no distortion of the base pair nor of the whole duplex, compared to its canonical counterparts, consistent with DFT, CD, and UV melting (Figure S8 in the Supporting Information).

In conclusion, this new $^{N}\mathbf{J}_{AT}$ has been fittingly designed to base pair preferentially with T and A, and mismatch with G and C, as was theoretically verified by DFT by using *N*-methylated bases as model. Notably, our previous data suggested a new hypothesis based on the potential basicity of the pyrimidopyridine $\mathbf{J}_{AT}$ heterocycle. Hence, we used synthesis to test the issue of potential protonation of the A-face by introducing a second ring nitrogen to create the pyrimidopyrimidine $^{N}\mathbf{J}_{AT}$ herein. To mitigate the extremely low solubility of the phosphoramidite-$^{N}\mathbf{J}_{AT}$ that proved refractory to incorporation into oligonucleotides, we developed a synthetic strategy that involved the creation of a sulfone-masked precursor that we used successfully for the carbocyclic $\mathbf{J}_{AT}$ nucleoside. Nevertheless, the introduc-

tion of the sulphone required considerable synthetic iteration including the provision of a nucleophilic benzylthiourea that would displace the thiomethyl ether in a double ring closure in situ to give the desired pyrimidopyrimidine nucleus in a single step. Further elaboration to the phosphoramidite required skirting the formation of a Zincke salt, because the sulfone in this case proved to be quite delicate. Subsequently, successful incorporation into a DNA oligonucleotide by solid-phase synthesis was achieved and confirmed by MALDI-TOF analysis. UV melting experiments showed that $^{N}J_{AT}$ can indeed discriminate T and A over G and C, whereas CD and MD experiments confirmed that there is little to no secondary-structure modification for the duplexes formed with complementary DNA. Moreover, the sharp melting transitions observed for $^{N}J_{AT}$, unlike the broad ones of J_{AT}, suggest that the partial protonation of N9 was a likely issue, which we successfully addressed. Taken as a whole, biophysical and computational results suggest that $^{N}J_{AT}$ can efficiently base pair with T and A with comparable stabilities and structures, whereas the robust synthetic pathway ensures the availability of a soluble $^{N}J_{AT}$ phosphoramidite for DNA incorporation. Therefore, $^{N}J_{AT}$ is a very promising candidate for sequence-specific gene targeting, and evaluation of DNA and PNA $^{N}J_{AT}$-tracts are currently under study.

Acknowledgements

The authors acknowledge Suzanne C. Perry (Proteomics Core facility, UBC) for the MALDI-TOF analysis, Jane Cua for her kind technical assistance, NSERC for funding this project, and the Banting Postdoctoral Fellowship program for funding E.L.

Keywords: base pairing · circular dichroism · DNA · heterocycles · Janus bases

[1] T. Le Doan, L. Perrouault, D. Praseuth, N. Habhoub, J.-l. Decout, N. T. Thuong, J. Lhomme, C. Hélène, *Nucleic Acids Res.* **1987**, *15*, 7749 – 7760.
[2] H. Moser, P. Dervan, *Science* **1987**, *238*, 645 – 650.
[3] a) M. P. Knauert, P. M. Glazer, *Hum. Mol. Genet.* **2001**, *10*, 2243 – 2251; b) P. B. Dervan, A. T. Poulin-kerstien, E. J. Fechter, B. S. Edelson, *Top. Curr. Chem.* **2005**, *253*, 1 – 31.
[4] N. T. Thuong, C. Hélène, *Angew. Chem.* **1993**, *105*, 697 – 723; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 666 – 690.
[5] a) N. Branda, G. Kurz, J. M. Lehn, *Chem. Commun.* **1996**, 2443 – 2444; b) D. Chen, M. Meena, S. K. Sharma, L. W. McLaughlin, *J. Am. Chem. Soc.* **2004**, *126*, 70 – 71; c) H. Chen, Meena, L. W. McLaughlin, *J. Am. Chem. Soc.* **2008**, *130*, 13190 – 13191; d) D. Shin, Y. Tor, *J. Am. Chem. Soc.* **2011**, *133*, 6926 – 6929.
[6] E. Largy, W. Liu, A. Hassan, D. M. Perrin, *ChemBioChem* **2013**, *14*, 2199 – 2208.
[7] Two different empirical methods (see Ref. [8]) suggest that the pK_a of N9 drops from 6.21–6.78 to 3.81–3.99 when C7–H is substituted by N7.
[8] a) J. C. Shelley, A. Cholleti, L. L. Frye, J. R. Greenwood, M. R. Timlin, M. Uchimaya, *J. Comput.-Aided Mol. Des.* **2007**, *21*, 681 – 691; b) J. R. Greenwood, D. Calkins, A. P. Sullivan, J. C. Shelley, *J. Comput.-Aided Mol. Des.* **2010**, *24*, 591 – 604; c) Calculator Plugins for structure property prediction, Marvin 6.0.3, ChemAxon, Budapest (Hungary), **2013**.
[9] a) J. Sági, A. Szemzõ, J. Szécsi, L. Otvös, *Nucleic Acids Res.* **1990**, *18*, 2133 – 2140; b) M. Ober, H. Müller, C. Pieck, J. Gierlich, T. Carell, *J. Am. Chem. Soc.* **2005**, *127*, 18143 – 18149.
[10] Y. Yoshioka, Y. Kitagawa, Y. Takano, K. Yamaguchi, T. Nakamura, I. Saito, *J. Am. Chem. Soc.* **1999**, *121*, 8712 – 8719.
[11] C. Fonseca Guerra, F. M. Bickelhaupt, J. G. Snijders, E. J. Baerends, *Chem. Eur. J.* **1999**, *5*, 3581 – 3594.
[12] A. Asadi, B. O. Patrick, D. M. Perrin, *J. Org. Chem.* **2007**, *72*, 466 – 475.
[13] a) Y. Kanda, H. Saito, T. Fukuyama, *Tetrahedron Lett.* **1992**, *33*, 5701 – 5704; b) B.-R. Zhuang, G.-J. Hsu, K. Sung, *Bioorg. Med. Chem.* **2006**, *14*, 3399 – 3404; c) K. Sung, B.-R. Zhuang, P.-M. Huang, S.-W. Jhong, *J. Org. Chem.* **2008**, *73*, 4027 – 4033.
[14] H. R. Bolliger, *Helv. Chim. Acta* **1951**, *34*, 916 – 917.
[15] a) T. Zincke, G. Heuser, W. Möller, *Justus Liebigs Ann. Chem.* **1904**, *333*, 296 – 345; b) T. Zincke, G. Weißpfenning, *Justus Liebigs Ann. Chem.* **1913**, *396*, 103 – 131.
[16] A. Granzhan, E. Largy, N. Saettel, M. P. Teulade-Fichou, *Chem. Eur. J.* **2010**, *16*, 878 – 889.
[17] J. Kypr, I. Kejnovská, D. Renčiuk, M. Vorlícková, *Nucleic Acids Res.* **2009**, *37*, 1713 – 1725.
[18] a) W. D. Cornell, P. Cieplak, C. I. Bayly, I. R. Gould, K. M. Merz, D. M. Ferguson, D. C. Spellmeyer, T. Fox, J. W. Caldwell, P. A. Kollman, *J. Am. Chem. Soc.* **1995**, *117*, 5179 – 5197; b) T. Gaillard, D. A. Case, *J. Chem. Theory Comput.* **2011**, *7*, 3181 – 3198.
[19] Y. Xin, W. K. Olson, *Nucleic Acids Res.* **2009**, *37*, D83 – 88.

Received: October 3, 2013
Published online on January 17, 2014