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COMMUNICATION

New imidazopyridopyrimidine:naphthyridine base-pairing motif, ImN^{N}:NaO^{O}, consisting of a DAAD:ADDA hydrogen bonding pattern, markedly stabilize DNA duplexes†

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The new imidazopyridopyrimidine:naphthyridine base-pairing motifs, ImO^{O}:NaN^{N} and ImN^{N}:NaO^{O}, were designed. Among the base pairs examined, DNA duplexes containing ImN^{N}:NaO^{O} pair(s) consisting of a DAAD:ADDA hydrogen bonding pattern (D = donor, A = acceptor) were markedly stabilized thermally and thermodynamically.

Hydrogen bonding (H-bonding) is one of the most important non-bonded molecular interactions. Therefore, it is important to understand the molecular recognition process in biology and in the design of functional organic supramolecules through H-bonding. In the field of nucleic acid chemistry, for example, specific H-bonding between adenine and thymine (A:T) and guanine and cytosine (G:C), the Watson–Crick base pairs, plays a critical role not only in conserving and transmitting genetic information but also in duplex stability. Furthermore, the development of an artificial base-pairing motif beyond the Watson–Crick base pairs is an area of active research with the aim of expanding biological, bioengineering, and therapeutic applications.[1–3]

We have been working on a project to develop new base-pairing motifs consisting of four hydrogen bonds (H-bonds) designed to stabilize and regulate DNA structures.[4–7] We have already prepared two sets of base-pairing motifs consisting of the imidazopyridopyrimidine:naphthyridine (Im:Na) pairs, that is, the ImO^{N}:NaN^{O} and ImN^{O}:NaO^{N} pairs shown in Fig. 1A and B.[4,5] Since the resulting base-pairing motifs were specific and markedly stabilized DNA duplexes (*ca.* +8 °C per pair relative to an A:T pair) independent of the sequence context, we attempted to develop thermally stabilized decoy molecules.[8] Furthermore, we have recently demonstrated the selective recognition of ImO^{N}:NaN^{O} and ImN^{O}:NaO^{N} pairs by DNA polymerases.[9,10] These successful results prompted us to develop new base-pairing motifs consisting of a series of Im:Na pairs. As can be seen in Fig. 1A and B, the previous ImO^{N}:NaN^{O} and ImN^{O}:NaO^{N} pairs have alternate H-bonding patterns of ADAD:DADA and DADA:ADAD, respectively (A = acceptor, D = donor). Since it has been suggested that the stability of hydrogen bonded complexes is affected by the arrangement of H-bonds arising from a secondary interaction,[11] we designed new base-pairing motifs, the ImO^{O}:NaN^{N} and ImN^{N}:NaO^{O} pairs (Fig. 1C and D), that possess ADDA:DAAD and DAAD:ADDA H-bonding patterns, which are expected to be more stable than the alternate H-bonding patterns.

In this communication, we report the synthesis of the new naphthyridine derivatives, NaN^{N} and NaO^{O}, and their base-pairing property with ImO^{O} and ImN^{N},[4] respectively. The base-pairing property of ImO^{O}:NaN^{N} was almost equal to those of the ImO^{N}:NaN^{O} and ImN^{O}:NaO^{N} pairs. In contrast, the ImN^{N}:NaO^{O} pair thermally and thermodynamically stabilized its duplex more than the ImO^{N}:NaN^{O} and ImN^{O}:NaO^{N} pairs. These results are also discussed.

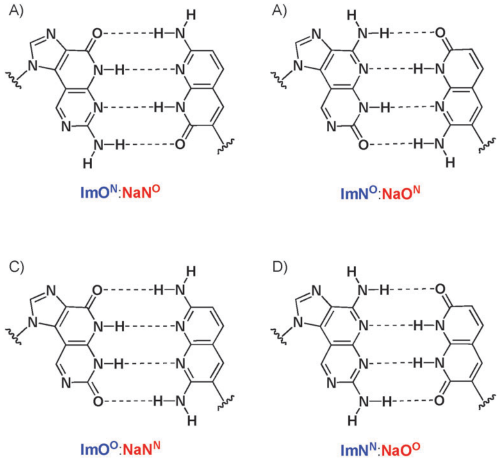

Fig. 1 Imidazopyridopyrimidine:naphthyridine (Im:Na) base-pairing motifs consisting of four H-bonds. (A) ImO^{N}:NaN^{O}, (B) ImN^{O}:NaO^{N}, (C) ImO^{O}:NaN^{N}, and (D) ImN^{N}:NaO^{O}.

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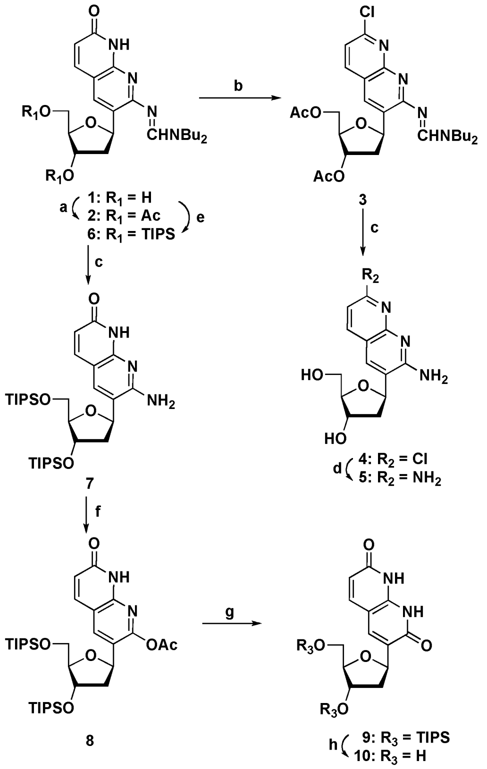


Scheme 1 Reagents and conditions: (a) Ac_2O, DMAP, Et_3N, DMF; (b) $POCl_3$; (c) NH_3/MeOH, 80 °C; (d) liq. NH_3, 120 °C; (e) TIPSCl, imidazole, DMF, 55 °C; (f) $NaNO_2$, AcOH; (g) NH_3/MeOH, 60 °C; (h) TBAF, THF.

The desired NaN^N and NaO^O (**5** and **10**) were prepared from the NaO^N derivative **1**[5] (Scheme 1). Thus, the 3′ and 5′-hydroxyl groups of **1** were protected by acetyl groups to give **2**. In order to convert the 7-oxo group of **2** into an amino group, **2** was treated with $POCl_3$ at room temperature to give the 7-chloro derivative **3** in 85% yield. The resulting **3** was treated with NH_3 in MeOH at 80 °C to afford the free nucleoside **4**. Since the 7-chloro group was inactive under the above conditions, **4** was treated with liq. NH_3 at 120 °C to give the desired NaN^N (**5**) in 88% yield.

For the synthesis of NaO^O (**10**), **1** was first converted into the di-*O*-TIPS derivative **6**. The protecting group of the exocyclic amino group was then removed by treatment with NH_3 in MeOH at 80 °C. If the resulting amino group could have been converted easily into an oxo group *via* diazotization, the desired NaO^O derivative **9** would have been available. However, the reaction of **7** with sodium nitrite in aqueous solution afforded a complex mixture, not the desired **9**. Consequently, **7** was treated with sodium nitrite in acetic acid to give the 2-acetate **8**, which was then deprotected by NH_3 in MeOH to give the NaO^O derivative **9** in 88% yield for the two steps. Deprotection of the TIPS groups of **9** was effected by treatment with TBAF in THF to give the desired NaO^O (**10**). The resulting nucleosides **5** and **10** were finally converted into the corresponding phosphoramidite units after appropriate protection of the nucleobase moieties for introduction into the oligodeoxynucleotides (ODNs) (see the ESI†).

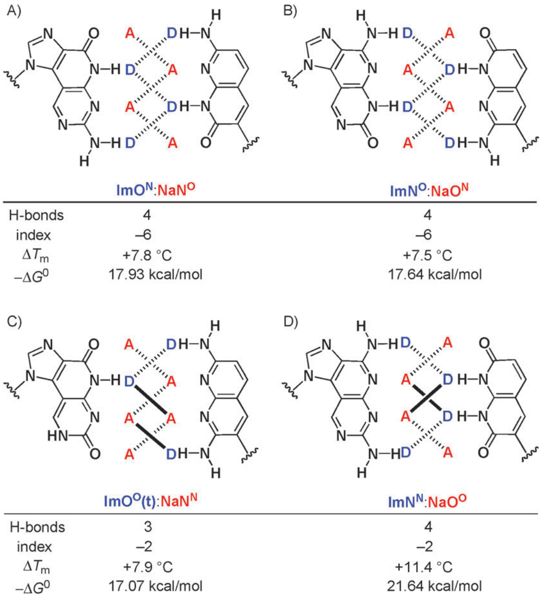
In order to investigate the base-pairing properties of NaN^N and NaO^O, three classes of complementary duplexes were synthesized. As shown in Table 1, the first class consists of duplexes (a series of ODN I:ODN II) containing one X:Y pair at the center of the duplexes (with the noncanonical and natural bases in their X or Y positions). The second class is made up of duplexes (a series of ODN III:ODN IV) having three non-consecutive X:Y pairs, and the third class (a series of ODN V:ODN VI) is made up of three consecutive X:Y pairs. These duplexes were used in a series of studies,[4,5,7] where the measurement of the thermal stability was carried out in a buffer of 10 mM sodium cacodylate (pH 7.0) containing 1 mM NaCl. The resulting melting temperature T_ms and the ΔT_ms values calculated based on the T_m of the duplex (X:Y = A:T, common to all duplexes) were listed in Table 1.[12] In the ODN I:ODN II case, the ImO^N:NaN^O and ImN^O:NaO^N pairs stabilized the duplexes by +7.8 °C and +7.5 °C, respectively, relative to the one containing the A:T pair. These results are consistent with our previous data.[5] When X:Y was substituted to the ImO^O:NaN^N pair, one of the newly designed base-pairing motifs, the ΔT_m value was +7.9 °C, which is almost equal to those of the ImO^N:NaN^O and ImN^O:NaO^N pairs. On the other hand, the ImN^N:NaO^O pair stabilized the duplex by +11.4 °C, which was the highest among the four pairs. The preferable base-pairing motif by the ImN^N:NaO^O pair was emphasized in the series of ODN III:ODN IV and ODN V:ODN VI. Thus, both duplexes containing three ImN^N:NaO^O pairs were stabilized by *ca.* +40 °C, which is a dramatic improvement. In Table 1, the results of the base pairs illustrated in Fig. 1 (*i.e.*, 4 matched pairs) are listed. However, 16 combinations of the Im:Na pairs, for example ImN^O:NaN^O and ImN^N:NaN^N (*i.e.*, an additional 12 mismatched pairs), are possible. To determine the specificity of these base pairs, we measured the T_ms for all possible combinations (see the ESI†). As a result, ImO^N:NaN^O, ImN^O:NaO^N, ImO^O:NaN^N, and ImN^N:NaO^O illustrated in Fig. 1 were found to be well matched pairs, respectively, and the ImN^N:NaO^O pair was the most thermally stable base-pairing motif among all possible combinations.

All the Im:Na base-pairing motifs illustrated in Fig. 1 are expected to form base pairs with four H-bonds. Therefore, the

Table 1 Sequences of ODNs and hybridization data

duplex	X	Y	T_m(ÞC)[a]	ΔT_m(ÞC)[b]
ODN I:ODN II	ImO^N	NaN^O	56.4	+7.8
	ImN^O	NaO^N	56.1	+7.5
5′-GCACCGAA**X**AAACCACG-3′	ImO^O	NaN^N	56.5	+7.9
3′-CGTGGCTT**Y**TTTGGTGC-5′	ImN^N	NaO^O	60.0	+11.4
	G	C	49.9	+1.3
	A	T	48.6	—
ODN III:ODN IV	ImO^N	NaN^O	81.4	+32.8
	ImN^O	NaO^N	79.6	+31.0
5′-GC**X**CCGAA**X**AAACC**X**CG-3′	ImO^O	NaN^N	80.5	+31.9
3′-CG**Y**GGCTT**Y**TTTGG**Y**GC-5′	ImN^N	NaO^O	88.0	+39.4
	G	C	56.7	+8.1
ODN V:ODN VI	ImO^N	NaN^O	79.0	+30.4
	ImN^O	NaO^N	80.1	+31.5
5′-GCACCGA**XXX**AACCACG-3′	ImO^O	NaN^N	81.3	+32.7
3′-CGTGGCT**YYY**TTGGTGC-5′	ImN^N	NaO^O	88.9	+40.4
	G	C	55.2	+6.6

[a] Experimental conditions are described in the ESI.† The data presented are average of triplicates. [b] The ΔT_m values were obtained by subtracting data for the T_m possessing X:Y = A:T from each duplex.



	ImO^N:NaN^O	ImN^O:NaO^N
H-bonds	4	4
index	−6	−6
ΔT_m	+7.8 °C	+7.5 °C
$-\Delta G^0$	17.93 kcal/mol	17.64 kcal/mol

	ImO^O(t):NaN^N	ImN^N:NaO^O
H-bonds	3	4
index	−2	−2
ΔT_m	+7.9 °C	+11.4 °C
$-\Delta G^0$	17.07 kcal/mol	21.64 kcal/mol

Fig. 2 Consideration of thermal and thermodynamic stability of a series of Im:Na base-pairing motifs. The dotted line represented repulsive secondary interaction and the bold line represented attractive secondary interaction. The index represented the sum of both secondary interactions. The listed ΔT_m and $-\Delta G^0$ values were obtained from a series of ODN I:ODN II.

complementarity of H-bonds is one of the important factors for duplex stability. However, the thermal stability of these Im:Na base pairs was different. As described in the introduction, Jorgensen and Pranata suggested the importance of secondary interaction for the stability of the hydrogen bonded complexes.[11] The validity of their consideration is well demonstrated and evaluated in many complexes possessing a variety of H-bonding patterns.[13–16] Our results can also be understood in view of their hypothesis. Thus, the ImO^N:NaN^O pair has a DADA:ADAD H-bonding pattern (Fig. 2A). In this pair, six repulsive secondary interactions (represented by dotted lines) arising from D–D and A–A repulsion have to be considered together with four primary H-bonds. Accordingly, the overall strength of interaction of the ImO^N:NaN^O pair can be estimated as four primary H-bonds and six repulsive secondary interactions (−6) represented as "index". The ImN^O:NaO^N pair possessing an ADAD:DADA H-bonding pattern is expected to have the same overall strength of interaction (Fig. 2B). This estimation agrees well with the calculated T_m values of the ImO^N:NaN^O and ImN^O:NaO^N pairs (+7.8 °C *vs.* +7.5 °C). Since the ImN^N:NaO^O pair has a DAAD:ADDA H-bonding pattern (Fig. 2D), this pair is expected to have four repulsive and two attractive secondary interactions (represented by bold lines) together with four primary H-bonds. Accordingly, the overall strength of interactions of the ImN^N:NaO^O pair can be estimated as four primary H-bonds and –2 of secondary interactions, which was, in fact, thermally more stable than the ImO^N:NaN^O and ImN^O:NaO^N pairs (+11.4 °C *vs.* +7.8 and +7.5 °C). For the ImO^O:NaN^N pair, if this pair has an ADDA:DAAD H-bonding pattern as illustrated in Fig. 1C, the overall strength of interaction of this pair can be estimated as four H-bonds and −2 of secondary interactions, which should be the same as those of the ImN^N:NaO^O pair. However the thermal stability of this pair (+7.9 °C) was rather low compared with the ImN^N:NaO^O pair and almost the same as those of the ImO^N:NaN^O and ImN^O:NaO^N pairs. In our previous study,[4] we suggested that the ImO^O exists as a tautomeric form, represented as ImO^O(t), possessing an ADAA H-bonding pattern. This being the case, then the base pair between ImO^O(t) and NaN^N should have four repulsive and two attractive secondary interactions together with three primary H-bonds (Fig. 2C). Accordingly, the overall strength of interaction of the ImO^O(t):NaN^N pair can be estimated as three H-bonds and −2 of secondary interactions, which was thermally similar to those of four H-bonds and −6 of secondary interactions. The attached $-\Delta G^0$ data also support these considerations. Thus, the ImN^N:NaO^O pair by giving rise to a favorable contribution to the enthalpy of formation was most thermodynamically stable, and the remaining pairs showed similar thermodynamic parameters (see the ESI†).

The synthesis of the new 1,8-naphthyridine nucleosides NaN^N (**5**) and NaO^O (**10**) and their incorporation into ODNs have been accomplished. Comparison of the base-pairing properties of a series of Im:Na pairs revealed that the ImN^N:NaO^O pair possessing a DAAD:ADDA H-bonding pattern was most thermally and thermodynamically stable. Our results showed the importance of the secondary interaction, depending on the arrangement of H-bonds, together with the number of H-bonds and the stacking effect of the expanded aromatic surfaces in the duplex stability.[17] Application of this markedly stable base-pairing motif toward developing alternative stable base pairs other than the Watson–Crick base pairs is underway.

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