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Author(s)	Kuramoto, Kazuyuki; Tarashima, Noriko; Hirama, Yasuyuki; Kikuchi, Yusaku; Minakawa, Noriaki; Matsuda, Akira
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COMMUNICATION

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

Kazuyuki Kuramoto,^a Noriko Tarashima,^b Yasuyuki Hirama,^a Yusaku Kikuchi,^b Noriaki Minakawa,^{*b} and Akira Matsuda^{*a}

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The new imidazopyridopyrimidine:naphthyridine base-pairing motifs, $\text{ImO}^{\text{O}}:\text{NaN}^{\text{N}}$ and $\text{ImN}^{\text{N}}:\text{NaO}^{\text{O}}$, were designed.

Among the base pairs examined, DNA duplexes containing $\text{ImN}^{\text{N}}:\text{NaO}^{\text{O}}$ 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}

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

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 structure.^{4–7} We have already prepared two sets of base-pairing motifs consisting of the imidazopyridopyrimidine:naphthyridine (Im:Na) pairs, that is, the $\text{ImO}^{\text{N}}:\text{NaN}^{\text{O}}$ and $\text{ImN}^{\text{O}}:\text{NaO}^{\text{N}}$ pairs shown in Figure 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 $\text{ImO}^{\text{N}}:\text{NaN}^{\text{O}}$ and $\text{ImN}^{\text{O}}:\text{NaO}^{\text{N}}$ 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 Figure 1A and B, the previous $\text{ImO}^{\text{N}}:\text{NaN}^{\text{O}}$ and $\text{ImN}^{\text{O}}:\text{NaO}^{\text{N}}$ 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 $\text{ImO}^{\text{O}}:\text{NaN}^{\text{N}}$ and $\text{ImN}^{\text{N}}:\text{NaO}^{\text{O}}$ pairs (Figure 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 $\text{ImO}^{\text{O}}:\text{NaN}^{\text{N}}$ was almost equal to those of the $\text{ImO}^{\text{N}}:\text{NaN}^{\text{O}}$ and $\text{ImN}^{\text{O}}:\text{NaO}^{\text{N}}$ pairs. In contrast, the $\text{ImN}^{\text{N}}:\text{NaO}^{\text{O}}$ pair thermally and thermodynamically stabilized its duplex more than the $\text{ImO}^{\text{N}}:\text{NaN}^{\text{O}}$ and $\text{ImN}^{\text{O}}:\text{NaO}^{\text{N}}$ pairs. These results are also discussed.

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 Supplementary Information).

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) TIPS-Cl, imidazole, DMF, 55 °C; f) NaNO_2 , AcOH; g) NH_3/MeOH , 60 °C; h) TBAF, THF

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_m s and the ΔT_m s 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 $\text{ImO}^{\text{N}}:\text{NaN}^{\text{O}}$ and $\text{ImN}^{\text{O}}:\text{NaO}^{\text{N}}$ 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 $\text{ImO}^{\text{O}}:\text{NaN}^{\text{N}}$ 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 $\text{ImO}^{\text{N}}:\text{NaN}^{\text{O}}$ and $\text{ImN}^{\text{O}}:\text{NaO}^{\text{N}}$ pairs. On the other hand, the $\text{ImN}^{\text{N}}:\text{NaO}^{\text{O}}$ pair stabilized the duplex by +11.4 °C, which was the highest among the four pairs. The preferable base-pairing motif by the $\text{ImN}^{\text{N}}:\text{NaO}^{\text{O}}$ pair was emphasized in the series of ODN III:ODN IV and ODN V:ODN VI. Thus, both duplexes containing three $\text{ImN}^{\text{N}}:\text{NaO}^{\text{O}}$ pairs were stabilized by ca. +40 °C, which is a dramatic improvement. In Table 1, the results of the base pairs illustrated in Figure 1 (i.e., 4 matched pairs) are listed. However, 16 combinations of the Im:Na pairs, for example $\text{ImN}^{\text{O}}:\text{NaN}^{\text{O}}$ and $\text{ImN}^{\text{N}}:\text{NaN}^{\text{N}}$ (i.e., an additional 12 mismatched pairs), are possible. To determine the specificity of these base pairs, we measured the T_m s for all possible combinations (see the Supplementary Information). As a result, $\text{ImO}^{\text{N}}:\text{NaN}^{\text{O}}$, $\text{ImN}^{\text{O}}:\text{NaO}^{\text{N}}$, $\text{ImO}^{\text{O}}:\text{NaN}^{\text{N}}$, and $\text{ImN}^{\text{N}}:\text{NaO}^{\text{O}}$ illustrated in Figure 1 were found to be well matched pairs, respectively, and the $\text{ImN}^{\text{N}}:\text{NaO}^{\text{O}}$ pair was the most thermally stable base-pairing motif among all possible combinations.

Table 1. Sequences of ODNs and hybridization data.

duplex	X	Y	T_m (°C) ^a	ΔT_m (°C) ^b
ODN I:ODN II	ImO^{N}	NaN^{O}	56.4	+7.8
	ImN^{O}	NaO^{N}	56.1	+7.5
	ImO^{O}	NaN^{N}	56.5	+7.9
	ImN^{N}	NaO^{O}	60.0	+11.4
	G	C	49.9	+1.3
ODN III:ODN IV	A	T	48.6	—
	ImO^{N}	NaN^{O}	81.4	+32.8
	ImN^{O}	NaO^{N}	79.6	+31.0
	ImO^{O}	NaN^{N}	80.5	+31.9
	ImN^{N}	NaO^{O}	88.0	+39.4
ODN V:ODN VI	G	C	56.7	+8.1
	ImO^{N}	NaN^{O}	79.0	+30.4
	ImN^{O}	NaO^{N}	80.1	+31.5
	ImO^{O}	NaN^{N}	81.3	+32.7
	ImN^{N}	NaO^{O}	88.9	+40.4
	G	C	55.2	+6.6

[a] Experimental conditions are described in the Supplementary Information. 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 the each duplex.

All the Im:Na base-pairing motifs illustrated in Figure 1 are expected to form base pairs with four H-bonds. Therefore, 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 $\text{ImO}^{\text{N}}:\text{NaN}^{\text{O}}$ pair has a DADA:ADAD H-bonding pattern (Figure 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 ADAD:DADA H-bonding pattern is expected to have the same overall strength of interaction (Figure 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 (Figure 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 Figure 1C, the overall strength of interaction of this pair can be estimated as four H-bonds and –2 of secondary interactions, which should be 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 (Figure 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 Supplementary Information).

Figure 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.

Conclusions

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 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.

Notes and references

- ^a Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan.
Fax: +81 (0)11-706-3228; Tel: +81 (0)11-706-4980
- ^b Graduate School of Pharmaceutical Sciences, The University of Tokushima, Shomachi 1–78–1, Tokushima 770–8505, Japan.
Fax: +81 (0)88-633-7288; Tel: +81 (0)88-633-7288; E-mail: minakawa@ph.tokushima-u.ac.jp
- † Electronic Supplementary Information (ESI) available: Experimental procedures and characterization data of all new compounds, and properties of oligodeoxynucleotides containing a series of Im:Na base pairs. See DOI: 10.1039/b000000x/
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