Nonnatural Nucleotides

Synthesis of 1,8-Naphthyridine C-Nucleosides and Their Base-Pairing Properties in Oligodeoxynucleotides: Thermally Stable Naphthyridine:Imidazopyridopyrimidine Base-Pairing Motifs**

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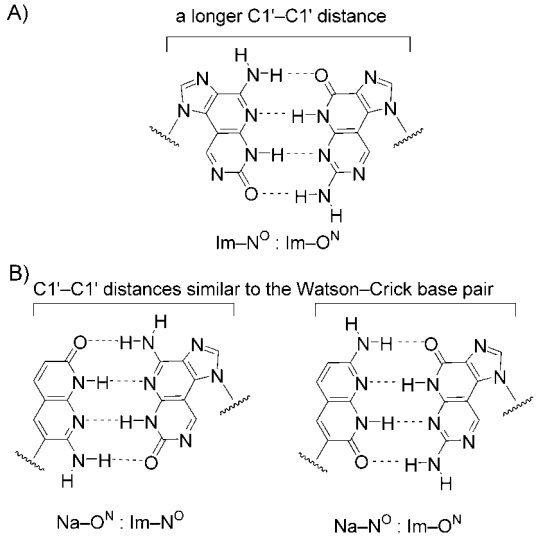
A number of nucleoside analogues that contain non-natural nucleobases have been synthesized and incorporated into oligodeoxynucleotides (ODNs) with the aim of biological, bioengineering, and therapeutic applications.[1,2] The development of new base-pairing motifs beyond the Watson–Crick hydrogen bonding (H bonding) model for thermal stability and specificity is therefore still an area of active research.[3,4] We recently reported the synthesis of imidazo[5′,4′:4,5]pyrido[2,3-*d*]pyrimidine nucleosides with the ability to form four H bonds and discussed their hybridization properties in ODNs (Scheme 1 A).[5,6] Accordingly, the Im-N^O:Im-O^N base pair markedly stabilized a duplex when three of the pairs were consecutively incorporated into ODNs. However, incorporation of one pair into ODNs resulted in destabilization of the duplex relative to those containing A:T and G:C base pairs. These results were explained by the conflicting effects of the Im-N^O:Im-O^N pair in ODNs, that is, the pair stabilizes the duplex with four H bonds, but it widens of the helix because the C1′–C1′ distance is longer than that in the Watson–Crick base pair—a destabilizing factor for the duplex that contains the pair. Since the goal of our continuing study is to develop base-pairing motifs that stabilize and regulate DNA structures, including a double-helix-independent mode of incorporation of the new base pair(s) (i.e., one pair, three nonconsecutive pairs, and three consecutive pairs in this study), the novel 1,8-naphthyridine C-nucleosides **7** (which bears an Na-N^O base) and **9** (which bears an Na-O^N base) were designed.[6] These C-nucleosides are expected to form two sets of naphthyridine:imidazopyridopyrimidine base-pairing motifs (Na-O^N:Im-N^O and Na-N^O:Im-O^N) with four hydrogen bonds when these are incorporated into ODNs



Scheme 1. A) Im-N^O:Im-O^N base-pairing motif. B) Newly designed naphthyridine:imidazopyridopyrimidine base-pairing motifs.

(Scheme 1 B). Furthermore, the new motifs can be regarded as an expanded pyrimidine:purine-type base pair (with C1′–C1′ distances similar to the Watson–Crick base pair), which, unlike the Im-N^O:Im-O^N pair, would not distort the helical structure.[5] Herein we describe the synthesis of the 1,8-naphthyridine C-nucleosides **7** and **9**, and the effects on the thermal stabilities of the ODNs containing the naphthyridine:imidazopyridopyrimidine base-pairing motifs.[7]

The synthetic route to the target compounds is illustrated in Scheme 2. The synthesis started from 2-amino-7-hydroxy-1,8-naphthyridine (**1**).[8] Iodination of **1** with *N*-iodosuccinimide (NIS) was followed by protection of the exocyclic amino group to give the 6-iodo-1,8-naphthyridine derivative **2**, a substrate for the synthesis of **7**. On the other hand, the synthesis of **9** requires the 3-iodo-1,8-naphthyridine derivative. Treatment of **1** with excess NIS, followed by protection of the exocyclic amino group gave the 3,6-diiodo derivative **3**, which was converted into the 3-iodo derivative **4** by treatment with a stoichiometric amount of tributyltin hydride in the presence of $[Pd(PPh_3)_4]$. This regioselective reduction of the 6-iodo group in **3** can be explained by the electron densities at C3 and C6, which were estimated from the ^{13}C NMR spectrum (C3: $\delta = 84.7$ ppm and C6: $\delta = 91.0$ ppm).[9] Heck coupling of the 6-iodo derivative **2** with the glycal **5**[10] was followed by deprotection and reduction[11] to afford the desired **6** in 78% overall yield (from **2**). In the same manner, the reaction of **4** with **5** afforded **8** in 76% yield. Treatment of **6** and **8** with methanolic ammonia gave the free nucleosides **7** and **9**, respectively. To incorporate both C-nucleosides **7** and **9** into ODNs, they were converted into the corresponding phosphoramidites **10** and **11**, respectively. For the conversion of **9**, the *N*-benzoyl group was the best choice as a protecting group for the exocyclic amino function,[12] and methyl *N*,*N*-diisopropylchlorophosphoramidite was used to give **11** because of purification problems that arose when 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite was used.

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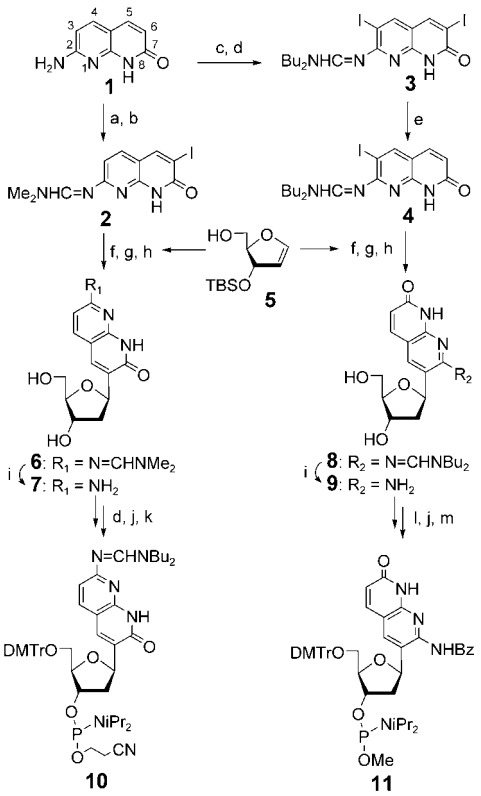

Scheme 2. Reagents and conditions: a) NIS (1.1 equiv), DMF; b) dimethylformamide dimethylacetal, DMF, 80 °C; c) NIS (2.9 equiv), DMF, 80 °C; d) dibutylformamide dimethylacetal, DMF; e) Bu_3SnH, $[Pd_2dba_3]\cdot CHCl_3$, PPh_3, DMF, 60 °C; f) **5**, $Pd(OAc)_2$, $AsPh_3$, Bu_3N, DMF, 60 °C; g) TBAF, THF; h) $NaBH(OAc)_3$, AcOH, CH_3CN; i) NH_3/MeOH, 80 °C; j) DMTrCl, pyridine; k) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoroamidite, iPr_2NEt, CH_2Cl_2; l) 1) TMSCl, pyridine then BzCl, 2) NH_4OH; m) methyl *N*,*N*-diisopropylchlorophosphoroamidite, iPr_2NEt, DMAP, CH_2Cl_2. NIS = *N*-iodosuccinimide; DMF = *N*,*N*-dimethylformamide; dba = dibenzylideneacetone; TBAF = tetrabutylammonium fluoride; DMTr = 4,4′-dimethoxytrityl; TMS = trimethylsilyl; DMAP = 4-(dimethylamino)pyridine.

To investigate the base-pairing properties of Na-N^O and Na-O^N, 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) that contain one X:Y pair in the center of the duplexes (containing Na-N^O, Na-O^N, Im-N^O, Im-O^N, or natural bases in their X or Y positions). The second class is made up of duplexes (a series of ODN III:ODN IV) that contain three nonconsecutive X:Y pairs, and the last class (a series of ODN V:ODN VI) is made up of three consecutive X:Y pairs. The thermal stability of all duplexes was measured by thermal denaturation in a buffer of 10 mM sodium cacodylate (pH 7.0) containing 1 mM NaCl.[13] The resulting melting temperatures T_ms and the ΔT_ms values calculated based on the T_m of the duplex (X:Y = A:T, common to ODN I:ODN II, ODN III:ODN IV, and ODN V:ODN VI) are listed in Table 1. As we expected, the Im-O^N:Na-N^O and Im-N^O:Na-O^N pairs stabilized the duplex by +9.4 °C and +8.6 °C, respectively, relative to that containing the A:T pair. In contrast, the Im-O^N:Im-N^O pair destabilized the duplex by −3.8 °C, which agreed with our previous

Table 1: Sequences of ODNs and hybridization data.

Duplex	X	Y	T_m [°C][a]	ΔT_m [°C][b]
ODN I:ODN II	Im-O^N	Na-N^O	57.2	+9.4
	Im-N^O	Na-O^N	56.4	+8.6
5′-GCACCGAAXAAACCACG-3′	Im-O^N	Im-N^O	44.0	−3.8
3′-CGTGGCTTYTTTGGTGC-5′	Na-N^O	Na-O^N	50.1	+2.3
	G	C	49.1	+1.3
	A	T	47.8	
ODN III:ODN IV	Im-O^N	Na-N^O	82.2	+34.4
	Im-N^O	Na-O^N	80.9	+33.1
5′-GCXCCGAAXAAACCXCG-3′	Im-O^N	Im-N^O	53.3	+5.5
3′-CGYGGCTTYTTTGGYGC-5′	Na-N^O	Na-O^N	48.9	+1.1
	G	C	56.7	+8.9
ODN V:ODN VI	Im-O^N	Na-N^O	80.2	+32.4
	Im-N^O	Na-O^N	81.0	+33.2
5′-GCACCGAXXXAACCACG-3′	Im-O^N	Im-N^O	70.4	+22.6
3′-CGTGGCTYYYTTGGTGC-5′	Na-N^O	Na-O^N	68.1	+20.3
	G	C	55.2	+7.4

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

results.[5] Although the Na-N^O:Na-O^N pair stabilized the duplex by +2.3 °C, the value was much less than those of Im-O^N:Na-N^O and Im-N^O:Na-O^N, and similar to that of the G:C pair. The preferable base-pairing motifs by Im-O^N:Na-N^O and Im-N^O:Na-O^N were emphasized in a series of ODN III:ODN IV. Both pairs stabilized the duplexes by more than +30 °C, and the effects of Im-O^N:Im-N^O and Na-N^O:Na-O^N were insufficient, despite the expected base-pairing motifs with four H bonds. In the series ODN V:ODN VI, not only the Im-O^N:Na-N^O and Im-N^O:Na-O^N pairs but also the Im-O^N:Im-N^O and Na-N^O:Na-O^N pairs stabilized the duplexes much more than G:C and A:T pairs, although the first pairs are generally considered more effective for thermal stability. From these results, it can be concluded that the newly designed base pairing motifs Im-O^N:Na-N^O and Im-N^O:Na-O^N thermally stabilized the duplex by nearly 10 °C more per pair than the A:T pair and 8 °C more than the G:C pair independent of the mode of incorporation of the new base pair(s) into the ODNs. This effect is presumably caused by the noncanonical base pairs consisting of four H bonds and the stacking effect of the expanded aromatic surfaces.[14] Furthermore, the fact that the shape of the pairs resembles a pyrimidine:purine base pair (i.e., shape complementarity) would also be critical for their effect because of the sequence-dependent thermal stabilizing effect of the Im-O^N:Im-N^O and Na-N^O:Na-O^N pairs. As we expected, the Im-O^N:Na-N^O and Im-N^O:Na-O^N pairs did not cause the disruption of the helical structure, unlike the Im-O^N:Im-N^O pair. Although some shift in the base-pairing phase from the usual pyrimidine:purine base pairing could occur to complete the base pairing of Im-O^N:Na-N^O and Im-N^O:Na-O^N (see Scheme 1B), the effect of this shift should be negligible for the thermally stable duplex formation, since

both pairs stabilized the duplex, irrespective of the mode of incorporation.

To clarify the specificity of the naphthyridine:imidazopyridopyrimidine base pairs, the base-pairing properties of Na-N^O with natural bases, as an example, were examined in a series of ODN I:ODN II and ODN V:ODN VI. As can be seen in Table 2, the resulting T_ms were all lower than that of A:T

Table 2: Sequences of ODNs and hybridization data of Na-N^O with natural bases.

duplex	X	Y	T_m [°C][a]	ΔT_m [°C][b]
ODN I:ODN II	Na-N^O	A	61.0	−2.6
	Na-N^O	G	58.4	−5.2
5′-GCACCGAAXAAACCACG-3′	Na-N^O	C	54.3	−9.3
3′-CGTGGCTTYTTTGGTGC-5′	Na-N^O	T	59.0	−4.6
	G	C	64.8	+1.2
	A	T	63.6	
ODN V:ODN VI	Na-N^O	A	60.3	−3.3
	Na-N^O	G	55.6	−8.0
5′-GCACCGAXXXAACCACG-3′	Na-N^O	C	45.2	−18.4
3′-CGTGGCTYYYTTGGTGC-5′	Na-N^O	T	60.0	−3.6
	G	C	69.0	+5.4

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

pair. Although the adenine base is expected to form a base pair with Na-N^O like the A:T pair, Na-N^O:A pair also destabilized the duplex.

In conclusion, the novel 1,8-naphthyridine C-nucleosides **7** and **9** with the ability to form four H bonds were synthesized through Heck coupling. The ODNs containing **7** and **9** formed extremely stable duplexes by the base-pairing motifs Im-O^N:Na-N^O and Im-N^O:Na-O^N. Furthermore, these motifs are specific, so that these would be versatile in stabilizing and regulating a variety of DNA structures.

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[12] As the reaction of **8** with DMTrCl gave the desired product in poor yield, a protecting group for the exocyclic amino function was examined.
[13] In a buffer containing 100 mM NaCl (conditions used in the previous paper[5]), some duplexes showed T_ms higher than 95 °C. Therefore, 1 mM NaCl was used in this study.
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