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Synthesis of 1-(2′-*O*-methyl-β-D-ribofuranosyl)-5-nitroindole and its phosphoramidite derivative

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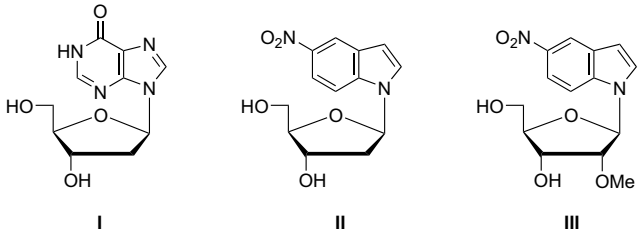
Abstract—The synthesis of 1-(2′-*O*-methyl-β-D-ribofuranosyl)-5-nitroindole, a new nucleoside containing the universal base 5-nitroindole, and its phosphoramidite derivative for incorporation into oligonucleotides is described.

The naturally occurring base hypoxanthine, as its ribonucleoside or deoxyribonucleoside **I** has been used for many years as a universal base analogue, that is an 'inert nucleotide' forming isoenergetic base pairs with each of the natural DNA bases (DNA nucleotide monomers).[1,2] This has led to applications in primers[3,4] and in probes for hybridization.[1,5,6] However, nucleotide **I** is not indiscriminate in its base pairing properties and a wide range of melting temperatures are found when it is paired opposite the natural bases in duplexes,[5,7] and multiple incorporations of **I** often induce a large decrease in the stabilities of the duplexes formed between these modified oligonucleotides and DNA complements.[7] These drawbacks have encouraged many efforts towards the discovery of new universal bases.[8] Among these, the 5-nitroindole 2′-deoxyribonucleoside **II** was found to be superior, giving more favourable duplex stabilities and behaving indiscriminately towards each of the four natural bases in duplex melting experiments (Fig. 1).[9]



I II III

Figure 1.

2′-*O*-Methyl-RNA[10,11] oligonucleotides are among the preferred modifications for antisense oligonucleotides. Their structure mimics the structure of RNA,[12] and we became interested in synthesizing the novel 5-nitroindole 2′-*O*-methylribonucleoside **III** to evaluate the properties of the 5-nitroindole base surrogate in an RNA-type oligonucleotide. For synthetic reasons, that is the convenience of not needing a protective group for the 2′-hydroxy group, we preferred the 2′-*O*-methyl-RNA derivative over the corresponding RNA derivative. To our knowledge, only one publication from a Russian group has dealt with the preparation of the ribo-analogue of **III**.[13] This synthetic sequence included glycosylation of 2,3-dihydro-5-nitroindole with a protected sugar, protection of the hydroxy groups, oxidation of the heterocyclic base, separation of the two anomers and finally complete deprotection. This process appeared rather long and tedious to us and furthermore furnished the RNA, and not the 2′-*O*-methyl-RNA, derivative. This letter reports the synthesis of the novel 2′-*O*-methyl-5-nitroindole ribofuranoside **III** and its phosphoramidite derivative suitable for incorporation into oligonucleotides.

Bromination of tetra-*O*-acetyl-β-D-ribofuranose **1** using trimethylsilyl bromide provided the bromo sugar derivative **2** as a mixture of the two anomers. The sodium salt of 5-nitroindole was formed by the action of sodium hydride on 5-nitroindole in acetonitrile, and was

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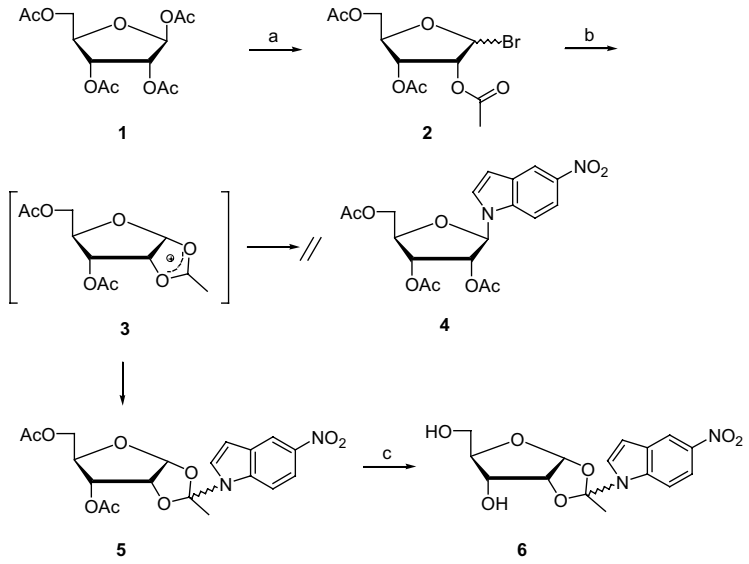
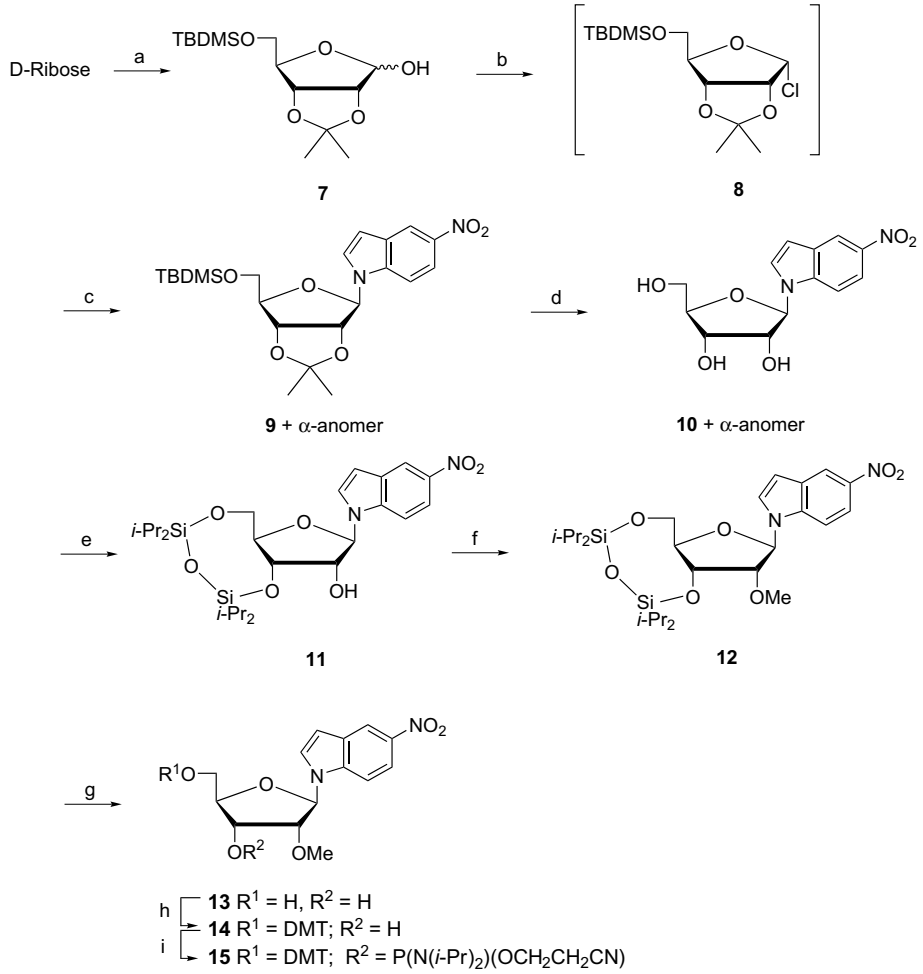


Scheme 1. Reagents and conditions: (a) TMSBr, $CHCl_3$, 0 °C; (b) sodium salt of 5-nitroindole, CH_3CN; (c) NH_3, MeOH, 78% from **1**, mixture of diastereoisomers.



Scheme 2. Reagents and conditions: (a) Ref. 17; (b) CCl_4, $P(NMe_2)_3$, THF, −80 °C; (c) sodium salt of 5-nitroindole, CH_3CN; (d) TFA/H_2O 9/1, 35% from **7**; (e) $TIPDSCl_2$, pyridine, 60%; (f) BDDDP, MeI, CH_3CN, 75%; (g) NEt_3/3HF, THF, 95%; (h) DMTCl, pyridine, 80%; (i) $ClP(N(i\text{-}Pr)_2)(OCH_2CH_2CN)$, DIPEA, CH_2Cl_2, 68%.

then reacted with **2** following the same procedure as reported by Loakes and Brown[9] for the synthesis of the corresponding 2′-deoxyribo-analogue. Interestingly, this procedure did not yield the expected ribofuranose **4**, but instead compound **5** was obtained as a mixture of two diastereoisomers. This result can be explained by intramolecular attack of the adjacent 2-*O*-acetyl group on the anomeric position to give intermediate **3**, followed by nucleophilic attack by the 5-nitroindolyl anion on the carbon atom of the O2-carbonyl moiety and not the C1-carbon as would be expected. Similar products have been reported in the literature by reacting other heterocyclic bases with a protected O2-benzoylated protected sugar.[14,15] The fully deacetylated compound **6** was obtained by treatment of **5** with saturated methanolic ammonia (78% from **1**, mixture of diastereoisomers) (Scheme 1). The structure of **6** was confirmed by high resolution mass spectroscopy, ^{1}H NMR, ^{13}C NMR and elementary analysis.[16]

In order to avoid the side reactions induced by the presence of the participating 2-*O*-acetyl group of the sugar moiety, the protected derivative **7**[17] was selected as starting material. After chlorination of ribofuranose **7** using CCl_4 and $P(NMe_2)_3$ at low temperature,[18] derivative **8** was reacted with the sodium salt of 5-nitroindole under the same conditions as applied above furnishing the protected 5-nitroindole ribofuranoside **9** and the corresponding α-anomer (inseparable by silica gel column chromatography) in a yield of 35% (from **7**; ~2:3 mixture of anomers according to ^{1}H NMR). This mixture was treated with aqueous TFA to cleave the protecting groups giving, in quantitative yield, a mixture of fully deprotected 1-(β-D-ribofuranosyl)-5-nitroindole **10** and the corresponding α-anomer.[19] In order to prepare for O2′-methylation, the 3′- and 5′-hydroxy groups of nucleoside **10** (and its α-anomer) were protected by reaction with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TIPDSCl_2$) in pyridine to give the desired β-anomer **11** in 60% yield after column chromatographic separation from a mixture of the two anomers. Attempts to O2′-methylate nucleoside **11** using NaH/MeI, Ag_2O/MeI or DBU/MeI failed. However, the use of BDDDP/MeI[20] provided the desired nucleoside in 75% yield. ^{1}H NOE experiments confirmed the β-D-ribo configuration of nucleoside **12**.[21] Cleavage of the silyl protective group afforded 1-(2′-*O*-methyl-β-D-ribofuranosyl)-5-nitroindole **13** in 95% yield.[22] In order to obtain the desired building block for automated oligonucleotide synthesis, nucleoside **13** was selectively protected at the primary hydroxy group using 4,4′-dimethoxytrityl chloride (DMTCl) in pyridine to give nucleoside **14** (80% yield), which was phosphitylated using the standard method to give the desired phosphoramidite derivative **15** in 68% yield (Scheme 2).[23]

In conclusion, we have developed a viable route to the novel 2′-*O*-methyl-5-nitroindole ribofuranoside and its phosphoramidite derivative suitable for automated oligonucleotide synthesis. Incorporation of this RNA-type 5-nitroindole nucleoside into oligonucleotides and evaluation of its potential as a universal base will be reported in due course.

Acknowledgements

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References and notes

1. Othsuka, E.; Matsuki, S.; Ikehara, M.; Takahashi, Y.; Matsubara, K. *J. Biol. Chem.* **1985**, *260*, 2605–2608.
2. Takahashi, Y.; Kato, K.; Hayashizaki, Y.; Wakabayashi, T.; Ohtsuka, E.; Matsuki, S.; Ikehara, M.; Matsubara, K. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1931–1935.
3. Liu, H.; Nichols, R. *Biotechniques* **1994**, *16*, 24–26.
4. Kamaya, H.; Sakaguchi, T.; Murata, N.; Fujimuro, M.; Miura, H.; Ishikawa, K.; Shimizu, M.; Inoue, H.; Nishimura, S.; Matsukage, A.; Masutani, C.; Hanaoka, F.; Ohtsuka, E. *Chem. Pharm. Bull.* **1992**, *40*, 2792–2795.
5. Martin, F. H.; Castro, M. M.; Aboul-ela, F.; Tinoco, I. *Nucleic Acids Res.* **1985**, *13*, 8927–8938.
6. Van Aerschot, A.; Peeters, B.; Van Derhaegue, H. *Nucleosides Nucleotides* **1987**, *6*, 437–439.
7. Kawase, Y.; Iwai, S.; Inoue, H.; Miura, K.; Ohtsuka, E. *Nucleic Acids Res.* **1986**, *14*, 7727–7736.
8. Loakes, D. *Nucleic Acids Res.* **2001**, *29*, 2437–2447.
9. Loakes, D.; Brown, D. M. *Nucleic Acids Res.* **1994**, *22*, 4039–4043.
10. Inoue, H.; Hayase, Y.; Imura, A.; Iwai, S.; Miura, K.; Ohtsuka, E. *Nucleic Acids Res.* **1987**, *15*, 6131–6148.
11. Lesnik, E. A.; Guinosso, C. J.; Kawasaki, A. M.; Sasmor, H.; Zounes, M.; Cummins, L. L.; Ecker, D. J.; Cook, P. D.; Freier, S. M. *Biochemistry* **1993**, *32*, 7832–7838.
12. Blommers, M. J. J.; Pieles, U.; De Mesmaeker, A. *Nucleic Acids Res.* **1994**, *22*, 4187–4194.
13. Mukhanov, V. I.; Miniker, T. D.; Preobrazhenskaya, M. N. *Zh. Org. Khim.* **1977**, *13*, 214–218.
14. Ramasamy, K.; Robins, R. K.; Revankar, G. R. *J. Heterocycl. Chem.* **1987**, *24*, 863–868.
15. Cristalli, G.; Franchetti, P.; Grifantini, M.; Nocentini, G.; Vittori, S. *J. Med. Chem.* **1989**, *32*, 1463–1466.
16. Compound **6**. Elemental analysis calcd for $C_{15}H_{16}N_2O_7$: C, 53.57, H, 4.79, N, 8.33; found C, 53.58, H, 4.87, N, 8.08. HR-MS (MALDI) $[M+Na]^+$ calcd: 359.0849; found 359.0848.
17. Kane, P. D.; Mann, J. *J. Chem. Soc., Perkin Trans. 1* **1984**, 657–660.
18. Wilcox, C. S.; Otoski, R. M. *Tetrahedron Lett.* **1986**, *27*, 1011–1014.
19. The signals in the ^{1}H NMR spectrum originating from one of the anomers were identical to data reported earlier for compound **10**.[12]
20. Gotfredsen, C. H.; Jacobsen, J. P.; Wengel, J. *Bioorg. Med. Chem.* **1996**, *4*, 1217–1225.
21. Compound **12**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.59–6.69 (5H, m, base), 6.01 (1H, s, H-1′), 4.56–4.52 (1H, m, H-3′), 4.29–4.04 (3H, m, H-4′, H-5′, H-5″), 3.70 (1H, d, H-2′, $J = 4.6$ Hz), 3.66 (3H, s, OMe), 1.57–0.99 (28H, m, TIPDS). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 142.1, 137.5, 128.6, 127.5, 118.2, 117.5, 109.3, 104.7, 89.7, 84.3, 81.1, 70.2, 59.8, 17.5, 17.4, 17.3, 17.1, 17.0, 16.9, 13.4, 13.0, 12.6. HRMS (MALDI) $[M+Na]^+$ calcd: 573.2428; found: 573.2420. Key results of NOE experiments: irradiation of H-3′ gives 7.3% enhancement of the H-2′ signal and 3.1% of the H-6 signal. Irradiation of H-6 gives 2.9% enhancement of the H-3′ signal and 1.4% of the H-2′ signal.
22. Compound **13**: ^{1}H NMR (CD_3OD, 300 MHz) δ 8.66–6.88 (5H, m, base), 6.24 (1H, d, H-1′, $J = 6.3$ Hz), 4.56–4.53

(1H, m, H-3′), 4.25–4.21 (2H, m, H-2′, H-4′), 3.99–3.87 (2H, m, H-5′, H-5″), 3.48 (3H, s, OMe). ^{13}C NMR (CD_3OD, 75 MHz) δ 143.0, 140.2, 129.6, 129.3, 118.5, 118.1, 111.1, 106.1, 88.8, 86.9, 85.1, 70.5, 62.7, 58.7. HRMS (MALDI) $[M+Na]^+$: calcd: 331.0901; found: 331.0905.

23. Compound **15**: ^{31}P NMR (DMSO-d_6, 121.5 MHz) δ 151.4, 150.9.