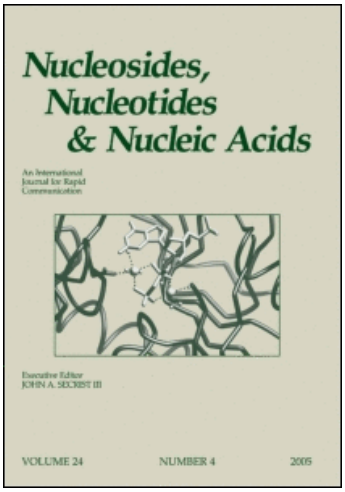


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

In Search of Acyclic Analogues as Universal Nucleosides in Degenerate Probes

A. Van Aerschot[a]; C. Hendrix[a]; G. Schepers[a]; N. Pillet[a]; P. Herdewijn[a]
[a] Laboratory of Medicinal Chemistry, Rega Institute for Medical Research, Leuven, Belgium

To cite this Article Van Aerschot, A. , Hendrix, C. , Schepers, G. , Pillet, N. and Herdewijn, P.(1995) 'In Search of Acyclic Analogues as Universal Nucleosides in Degenerate Probes', Nucleosides, Nucleotides and Nucleic Acids, 14: 3, 1053 — 1056
To link to this Article: DOI: 10.1080/15257779508012532
URL: http://dx.doi.org/10.1080/15257779508012532

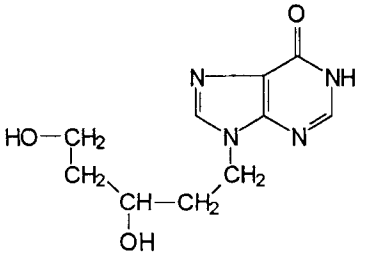
NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 1053-1056 (1995)

IN SEARCH OF ACYCLIC ANALOGUES AS UNIVERSAL NUCLEOSIDES IN DEGENERATE PROBES

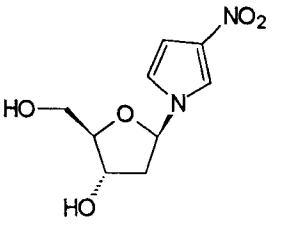
A. Van Aerschot, C. Hendrix, G. Schepers, N. Pillet, P. Herdewijn
Laboratory of Medicinal Chemistry, Rega Institute for Medical Research,
Minderbroedersstraat 10, B-3000 Leuven, Belgium.

Abstract: Five acyclic nucleoside analogues with unnatural base moieties have been synthesized of which three successfully were incorporated into oligonucleotides. The acyclic analogue containing the base 5-nitroindazole was the least discriminating and should be further pursued for use as a universal nucleoside analogue.

In view of the degeneracy of the genetic code, many research efforts have been devoted trying to devise a "lure" nucleoside analogue, capable of base-pairing equally well with two or more of the natural heterocyclic bases. Until now 2'-deoxyinosine - which was described first to fulfil this purpose - is still being used generally although results are not always satisfactory[1]. We recently described the synthesis of oligonucleotides containing acylic analogues with either a 3(*S*),5-dihydroxypentyl or a 4(*R*)-methoxy-3(*S*),5-dihydroxypentyl side chain. These acyclic nucleoside analogues discriminate less well than the natural 2'-deoxynucleosides due to their conformational flexibility[2]. 9-(3(*S*),5-Dihydroxypentyl)hypoxanthine (**12, ac-dI**) showed the least spreading in melting temperature on hybridization with the four natural 2'-deoxynucleosides, but suffers from destabilization of the duplex (about 5°C).



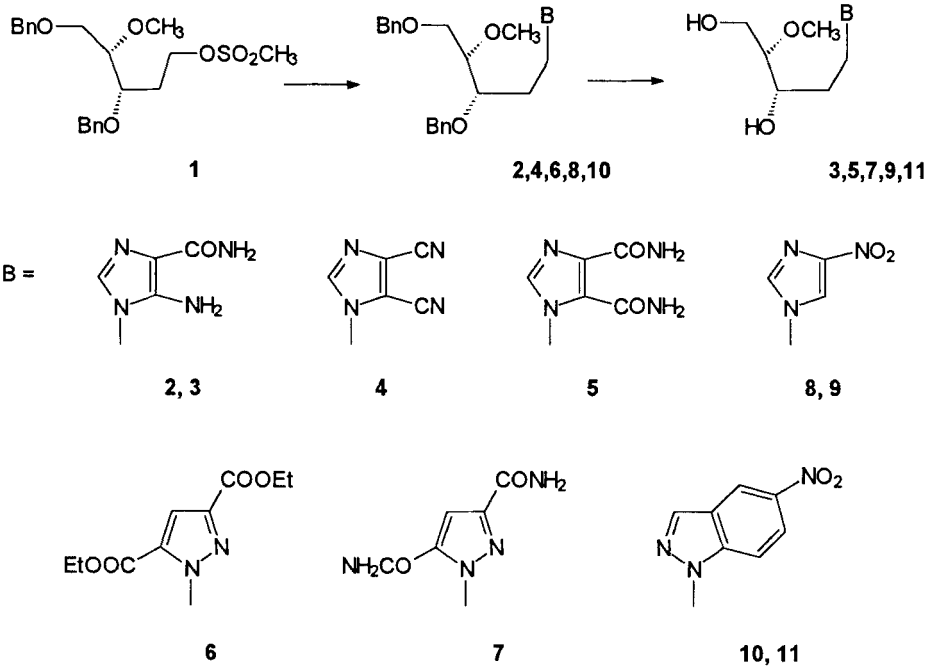
12



13

We now synthesized five new acyclic nucleoside analogues with unnatural base moieties to try to overcome this drawback. These include 4-amino-5-imidazole-carboxamide (**3**),

4,5-imidazoledicarboxamide (**5**), 3,5-pyrazoledicarboxamide (**7**), 4-nitroimidazole (**9**) and 5-nitroindazole (**11**). These compounds have a 4(*R*)-methoxy-3(*S*),5-dihydroxypentyl side chain instead of the 3(*S*),5-dihydroxypentyl group. When starting from 2-deoxy-D-ribose, the former nucleosides are easier to attain. The stereo-chemistry of both chiral centers is ascertained by the choice of the starting material. The compounds **7**, **9** and **11** were converted to their dimethoxytritylated phosphoramidites and were successfully incorporated into oligonucleotides after which the hybridizing properties of these oligonucleotides were evaluated.



1-*O*-Methanesulfonyl-3,5-di-*O*-benzyl-4-*O*-methyl-2-deoxy-D-ribitol (**1**)[3] was used as starting material for nucleophilic substitution with the different bases. Coupling of **1** with 4-amino-5-imidazolecarboxamide in the presence of NaH in DMF at 50°C was straightforward to yield 75% of the protected analogue **2**. Debenzylation by transfer hydrogenation with cyclohexene afforded 70% of **3** as an oil. Following 5'-*O*-dimethoxytritylation, phosphitylation took place primarily at the base moiety yielding mono- and diphosphitylated analogues. Protection of the base moiety thus seems necessary to incorporate this acyclic AICAR analogue in an oligonucleotide using regular phosphoramidite chemistry. Previously, AICAR was unexpectedly incorporated into oligonucleotides, using a protected derivative of 2-aza-2'-deoxyinosine[4]. All protecting

TABLE: Tm (°C) of the duplexes 5'-CACCGXCGGCGCC-3'
3'-GTGGCYGCCGCGG-5'

X \ Y	A	T	G	C	spreading of Tm (°C)
5	62.2	63.3	62.7	59.6	3.7
9	58.7	56.8	63.0	55.0	8.0
11	64.8	64.3	63.7	62.6	2.2
12 (ac-dI)	65.1	62.6	67.4	65.6	4.8
dI	70.2	64.6	65.1	68.5	5.6
complementary base	70.3 (T)	70.0 (A)	73.5 (C)	72.8 (G)	-

strategies that we tried (benzoyl, p-nitro-phenylethyloxycarbonyl, dimethylformamidine), failed, and incorporation of this analogue was not pursued any further.

Analogous coupling of dicyanoimidazole with **1** required a temperature of 90°C to yield 45% of the protected analogue **4**. Conversion to the double amide was accomplished with hydrogen peroxide in a biphasic system in 31% yield, followed by transfer hydrogenation to yield 70% of **5**.

Pyrazole-3,5-dicarboxilic acid was converted to the diethyl ester with ethylchloroformiate in 90% yield. Coupling to **1** in the presence of potassium carbonate for 16 h at 60°C afforded 73% of the protected analogue **6**. Treatment with anhydrous ammonia in ethanol for 48 h at 100°C in a pressurized bottle, followed by debenzylation afforded 32% of **7**, which awaits further incorporation into oligonucleotides. 4-Nitroimidazole and 5-nitropyrazole were condensed with **1** in a moderate 37% and 32% yield respectively, with the aid of NaH. Debenzylation to **9** and **11** was accomplished with boron trichloride 1 M in methylene chloride at -78°C (75% yield) leaving the nitro substituent untouched.

The analogues **5**, **9** and **11** were 5'-*O*-dimethoxytritylated and phosphytilated following standard procedures and the obtained phosphoramidites were used for incorporation of these analogues into oligonucleotides. Hereto, a 0.13 M solution was used as compared to the usual 0.1 M solution of phosphoramidites. This gave good coupling efficiency as reflected by the HPLC ion exchange profile (not shown). The target sequence was a 13-mer with the modification positioned in the middle. Melting temperatures with the four complementary sequences were determined. Whereas the

acyclic inosine analogue **12** gave a spreading of 4.8°C, the 5-nitroindazole **11** and the 4,5-imidazoledicarboxamide **5** gave a spreading of only 2.2°C and 3.7°C, respectively. Excluding the C-oligonucleotide as complement, the spreading is even only 1.1°C for both analogues. This narrow range in Tm for oligonucleotides containing **11**, warrants this analogue to be pursued further as a universal nucleoside at ambiguous sites in DNA primers. Melting temperatures, however, are considerably lower when compared to incorporation of a natural Watson-Crick base-pair, but only marginally lower when compared to incorporation of the well-known 2'-deoxyinosine. It would be of interest to compare the recently reported analogue **13** containing a 3-nitropyrrole substituent[5] with our acyclic analogues **11** and **5**. This analogue **13** is reported to be a perfect universal nucleoside, and oligonucleotides containing **13** at several sites were used as primers for sequencing and the polymerase chain reaction. However, melting temperatures of these oligonucleotides in comparison with their control sequences were not given.

REFERENCES

1 E. Ohtsuka, S. Matsuki, M. Ikehara, Y. Takahashi and K. Matsubara, *J. Biol. Chem.* **1985**, *260*, 2605-2608.

2 F. Vandendriessche, K. Augustyns, A. Van Aerschot, R. Busson, J. Hoogmartens and P. Herdewijn, *Tetrahedron* **1993**, *49*, 7223-7238.

3 F. Vandendriessche, R. Snoeck, G. Janssen, J. Hoogmartens, A. Van Aerschot, E. De Clercq and P. Herdewijn, *J. Med. Chem.* **1992**, *35*, 1458-1465.

4 D. Fernandez-Forner, R. Eritja, F. Bardella, C. Ruiz-Perez, X. Solans, E. Giralt and E. Pedroso, *Tetrahedron* **1991**, *47*, 8917-8930.

5 R. Nichols, P.C. Andrews, P. Zhang and D.E. Bergstrom, *Nature* **1994**, *369*, 492-493.