Tetrahedron Letters 49 (2008) 6068–6070

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

De novo approach to L-anhydrohexitol nucleosides as building blocks for the synthesis of L-hexitol nucleic acids (L-HNA)

Daniele D'Alonzo [a], Annalisa Guaragna [a,*], Arthur Van Aerschot [b], Piet Herdewijn [b], Giovanni Palumbo [a]

[a] Dipartimento di Chimica Organica e Biochimica, Università di Napoli Federico II, Via Cinthia, 4 I-80126 Napoli, Italy
[b] Katholieke Universiteit Leuven, Rega Institute for Medical Research, Minderbroederstraat 10, B-3000 Leuven, Belgium

ARTICLE INFO

Article history:
Received 9 July 2008
Revised 24 July 2008
Accepted 29 July 2008
Available online 31 July 2008

Keywords:
1,6-Anhydro-L-sugars
Domino reactions
Nucleosides analogues
HNA
Oligonucleotides

ABSTRACT

A stereoselective and scalable route to 1,5-anhydrohexitol nucleoside analogues belonging to L-series as building blocks for L-HNA oligonucleotide synthesis has been efficiently tuned. Key to the successful outcome of our approach is the development of a DDQ-mediated domino reaction, which leads to the formation of an unsaturated 1,6-anhydrosugar derivative. Sugar elaborations and base insertion then enable to synthesize six-membered nucleosides.

During the last decade considerable efforts have been devoted to the development of sugar-modified oligonucleotide analogues, such as those containing a six-membered sugar backbone, to be employed as a source of information for a deeper understanding of the DNA and RNA pairing behaviour.[1] Expansion of the sugar ring size of nucleic acids has increased the structural diversity beyond the classical A- and B-forms of DNA duplexes, introducing a great number of new structures capable of selective cross-communication in parallel or antiparallel orientation.[2] Due to an improved hybridization aptitude and a remarkable enzymatic stability, several examples of six-membered oligonucleotide analogues have been found to be good candidates for antisense purposes, as observed for Hexitol Nucleic Acids[3] (D-HNA, Fig. 1). In addition, they have been considered in the aptamer field,[4] in investigating life's origin,[1] as well as in biotechnology and diagnostics.[5]

In this context, the opportunity to modify the sugar backbone for creating oligonucleotide architectures belonging to L-series is a relatively unexplored area. Indeed, nucleic acids with L-configuration are unknown in nature, but exhibited extraordinary resistance to biological degradation.[6] For this reason, investigations on L-oligonucleotides (the so called Spiegelmers, from the German 'spiegel', for mirror) have been convincingly carried out in recent times for different purposes.[7] Some of them effectively displayed unprecedented strong antiparallel hybridisation with both DNA and RNA complements.[8] Likewise, research in the aptamer field has led to the construction of several oligonucleotide spiegelmers, which were successfully used as highly specific binders.[9]

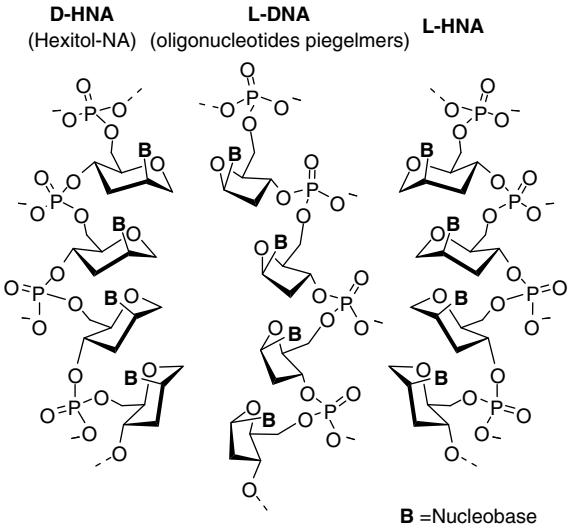
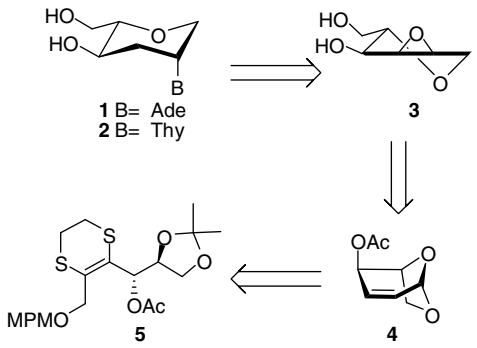


Figure 1. D-/L-HNA and L-DNA as sugar-modified nucleic acids.

Inspired by such combined findings, we are engaging in a study based on the synthesis of L-pyranosyl oligonucleotide analogues and their hybridization with complementary nucleic acids. In this preliminary Letter, attention has been focused on the study of

* Corresponding author. Tel./fax: +39 081 674 119.
E-mail address: annalisa.guaragna@unina.it (A. Guaragna).

0040-4039/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2008.07.159



Scheme 1. Retrosynthetic path.

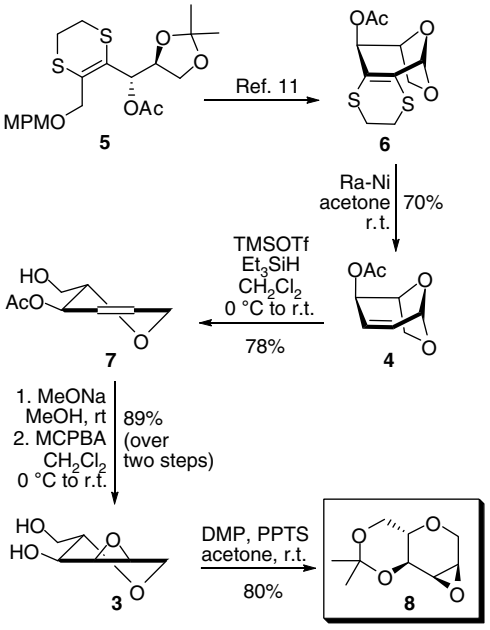
L-hexitol-based (L-HNA) oligonucleotide systems (Fig. 1). More specifically, synthesis of L-hexitol nucleoside monomers **1** and **2** (Fig. 2) as building blocks for the construction of L-HNA structures is proposed herein, by exploiting a recently reported procedure,[10,11] already devoted to the synthesis of rare sugars.

As depicted in Scheme 1, compound **4** represented the key intermediate of the whole synthetic strategy. Indeed, it presents a 1,6-anhydrosugar skeleton, which can be easily cleaved under reductive conditions to give the required 1-deoxy sugar moiety. Our strategy therefore includes the following steps: (a) preparation of the 1,6-anhydro-L-sugar derivative **4** by a domino reaction from intermediate **5**; (b) reductive 1,6-anhydrosugar ring-opening of **4**; (c) creation of a *C*-2 electrophilic site (such as in **3**) and base insertion to afford anhydrohexitol nucleosides **1** and **2**. To test the breadth of our methodology, the study of nucleosides containing thymine (T) and adenine (A) (as pyrimidine and purine base models, respectively) has been preliminarily undertaken.

The synthesis began with the preparation of the 1,6-anhydro derivative **7** by a domino reaction,[11] treating acetate **5**[12] with DDQ in a 18:1 CH_2Cl_2/H_2O emulsion (Scheme 2). Formally, by this procedure the following reactions took place: MPM group removal, oxidation of the resulting primary hydroxyl function to aldehyde, isopropylidene group removal and acetalation of the aldehyde function by an intramolecular double cyclization. Then, dithioethylene bridge removal on **6** by means of Ra-Ni in acetone at room temperature afforded the olefin **4** (70% yield). Driven by the need to obtain a 1,5-anhydrohexitol backbone, the 2,3-didehydro-2,3-dideoxy-β-L-1,6-anhydrohexose **4** was treated with triethylsilane in acidic medium,[13,14] to give the pseudo-glucal **7**.

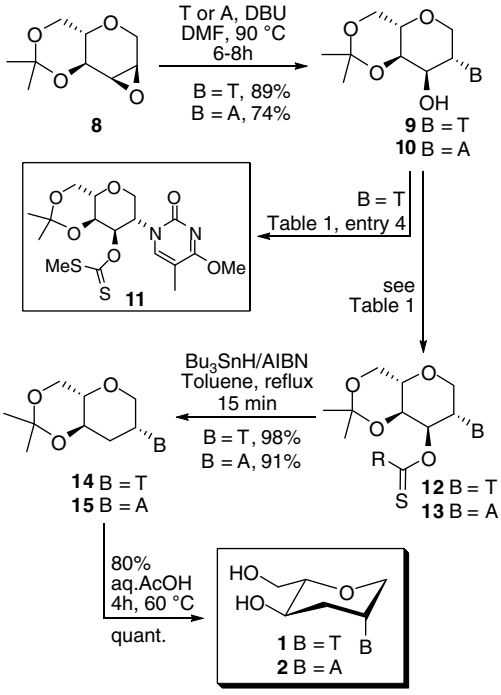
Once 1,6-anhydro ring cleavage was achieved, our attention was turned to the construction of an electrophilic site on *C*-2 position by creating an *allo*-epoxide function, suitable for the insertion of an axially-oriented nucleobase moiety. Thus, **7** was deacetylated under Zemplén conditions (Scheme 2). Treatment of the resulting allylic alcohol with *m*CPBA provided epoxide **3** in good yield (89% from olefin **7**). Finally, isopropylidene protection of **3** under mild conditions[15] afforded key intermediate **8** in 80% yield.

With the protected epoxide **8** in our hand, base insertion was next explored. The easiest way for gaining access to hexitol nucleosides was by oxirane ring-opening, under alkaline conditions, using unprotected nucleobases that arranged into the required *C*-2 axial position. As already studied for similar substrates,[16] the mildest conditions for nucleobase attack consisted in the use of 1,8-diazabicylo[5.4.0]undec-7-ene (DBU).[17] Indeed, treatment of **8** with thymine and DBU in DMF at 90 °C for 8 h afforded protected L-altritol nucleoside[18] **9** in 89% yield (Scheme 3). Analogously, reaction of **8** with adenine under the same conditions gave **10** in a few lower yields (74%).



Scheme 2. Synthesis of the protected *syn*-epoxide **8**.

Hence, 3-OH group removal was examined by testing several deoxygenation procedures (Table 1) involving hydroxyl group



Scheme 3. Synthesis of 1,5-anhydro-2-(thymin-1-yl)-2,3-dideoxy-L-*arabino*-hexitol (**1**) and 1,5-anhydro-2-(adenin-9-yl)-2,3-dideoxy-L-*arabino*-hexitol (**2**).

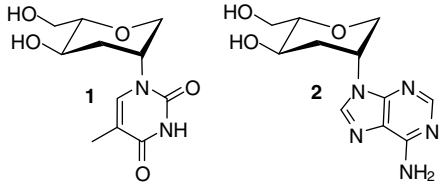


Figure 2. Anhydrohexitol nucleoside analogues **1** and **2** as starting material for L-HNA synthesis.

Table 1
3-OH activation of compounds **9**–**10**

Entry	Base	R	Conditions	Time (h)	Yield (%)
1	T	PhO	PhOC(S)Cl/Py	48	N.R.
2	T	PhO	PhOC(S)Cl/ DMAP/CH_3CN	48	N.R.
3	T	PhO	PhOC(S)Cl/ DMAP/Py	48	15
4	T	MeS	NaH/CS_2/MeI/DMF	0.5	>95[a]
5	T	$CN(CH_2)_2S$	NaOH(aq)/CS_2/ $BrCH_2CH_2CN$/DMSO	2	82
6	A	$CN(CH_2)_2S$	NaOH(aq)/CS_2/ $BrCH_2CH_2CN$/DMSO	0.5	<10
7	A	Im	$(Im)_2CS$/toluene	48	N.R.
8	A	PhO	PhOC(S)Cl/DMAP/Py	48	18
9	A	MeS	NaH/CS_2/MeI/DMF	0.5	21
10	A	CH_3CH_2S	NaOH(aq)/CS_2/ $BrCH_2CH_3$/DMF	0.5	93

[a] Compound **11** was isolated as the only product.

activation (as xanthate or thiocarbonate) and its removal under radical conditions (Bu_3SnH/AIBN). Alcohol **9**, which was treated with phenoxythiocarbonyl chloride (PhOC(S)Cl) under various conditions[19] did not show almost any reactivity (Table 1, entries 1–3). Conversely, classical xanthate formation by Barton–McCombie method[20] (entry 4) afforded, in almost quantitative yield, the undesired derivative **11** (Scheme 3), which careful structure analysis clarified that an extra-methylation on the nucleobase occurred. Looking for an alternative procedure, the combination $NaOH_{(aq)}$, CS_2, $BrCH_2CH_2CN$[21] in DMSO (entry 5) furnished the nucleoside **12** in a satisfying 82% yield. On the other hand, as far as it concerns the adenine nucleoside **10** (Scheme 3), the latter procedure did not give analogous results, the product being obtained in low yields[22] (entry 6). Likewise, previously reported procedures (entries 7–9) failed. Eventually, good results were obtained by replacing the alkylating agent with the less electrophilic bromoethane. In fact, treatment of **10** with the $NaOH_{(aq)}/CS_2/BrCH_2CH_3$/DMF mixture at 0 °C (entry 10) gave, already after 30 min, the desired product **13** in 93% yield.

Radical deoxygenation and acetonide deprotection reactions were finally accomplished. Protected L-altritol xanthates **12** (R = SCH_2CH_2CN) and **13** (R = SCH_2CH_3) underwent radical deoxygenation by means of Bu_3SnH in refluxing toluene, using AIBN as radical initiator. In both cases, reduction promptly occurred, with products **14** and **15** being obtained in excellent yields (98% and 91%, respectively). Hence, exposure of **14** and **15** to 80% aqueous acetic acid at 60 °C for 4 h afforded, after common purification procedures, the pure 1,5-anhydro-2-(thymin-1-yl)-2,3-dideoxy-L-*arabino*-hexitol[23] (**1**) and 1,5-anhydro-2-(adenin-9-yl)-2,3-dideoxy-L-*arabino*-hexitol[24] (**2**) in quantitative yields (Scheme 3).

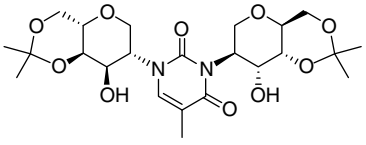
In summary, a stereoselective procedure for the synthesis of L-hexitol thymine and adenine nucleoside analogues **1** and **2** via 1,6-anhydro-β-L-hexopyranose **4** has been conveniently reported. Further experiments aimed to incorporate nucleosides **1** and **2** in oligonucleotide strands (i.e. L-HNA), for determining their hybridization aptitude with natural DNA sequences are currently ongoing and will be published in due course.

Acknowledgements

^1H and ^{13}C NMR spectra were performed at Centro Interdipartimentale di Metodologie Chimico-Fisiche (CIMCF), Università di Napoli Federico II. The Varian Inova 500 MHz instrument is the property of Consorzio Interuniversitario Nazionale La Chimica per l'Ambiente (INCA) and was used in the frame of a project by INCA and M.I.U.R. (L. 488/92, Cluster 11-A). D.D. is indebted to Professor Bogdan Doboszewski for his helpful suggestions and to Dr Natalia Dyubankova for having contributed to structure elucidation of compound **16** [Ref. 18] with 2D NMR experiments.

References and notes

1. Eschenmoser, A. *Chimia* **2005**, *59*, 836–850.
2. Lescrinier, E.; Froeyen, M.; Herdewijn, P. *Nucleic Acids Res.* **2003**, *31*, 2975–2989.
3. Hendrix, C.; Rosemeyer, H.; Verheggen, I.; Seela, F.; Van Aerschot, A.; Herdewijn, P. *Chem. Eur. J.* **1997**, *3*, 110C–120C; Lescrinier, E.; Esnouf, R.; Schraml, J.; Busson, R.; Heus, H. A.; Hilbers, C. W.; Herdewijn, P. *Chem. Biol.* **2000**, *7*, 719–731; Kang, H.; Fisher, M. H.; Xu, D.; Miyamoto, Y. J.; Marchand, A.; Van Aerschot, A.; Herdewijn, P.; Juliano, R. L. *Nucleic Acids Res.* **2004**, *32*, 4411–4419.
4. Kolb, G.; Reigadas, S.; Boiziau, C.; Van Aerschot, A.; Arzumanov, A.; Gait, M. J.; Herdewijn, P.; Toulmé, J. J. *Biochemistry* **2005**, *44*, 2926–2933.
5. Crey-Desbiolles, C.; Ahn, D.-R.; Leumann, C. J. *Nucleic Acids Res.* **2005**, *33*, e77.
6. Schumacher, T. N.; Mayr, L. M.; Minor, D. L.; Milhollen, M. A.; Burgess, M. W.; Kim, P. S. *Science* **1996**, *271*, 1854–1857.
7. (a) Sooter, L. J.; Ellington, A. D. *Chem. Biol.* **2002**, *9*, 857–858; (b) Kim, Y.; Yang, C. J.; Tan, W. *Nucleic Acids Res.* **2007**, *5*, 7279–7287.
8. Sørensen, M. D.; Kværnø, L.; Bryld, T.; Håkansson, A. E.; Verbeure, B.; Gaubert, G.; Herdewijn, P.; Wengel, J. *J. Am. Chem. Soc.* **2002**, *124*, 2164–2176.
9. Purschke, W. G.; Eulberg, D.; Buchner, K.; Vonhoff, S.; Klussmann, S. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 5173–5178.
10. Guaragna, A.; Napolitano, C.; D'Alonzo, D.; Pedatella, S.; Palumbo, G. *Org. Lett.* **2006**, *8*, 4863–4867.
11. D'Alonzo, D.; Guaragna, A.; Napolitano, C.; Palumbo, G. *J. Org. Chem.* **2008**, *73*, 5636–5639.
12. Compound **5** has been prepared starting from our heterocyclic homologating agent. See: Guaragna, A.; Pedatella, S.; Palumbo, G. In *e-Encyclopedia of Reagents for Organic Synthesis (e-EROS)*; Paquette, L. A., Ed.; John Wiley & Sons: New York, US, 2008. doi:10.1002/047084289X.rn00831.
13. Jung, M. E.; Kiankarimi, M. *J. Org. Chem.* **1998**, *63*, 8133–8144.
14. Both boron trifluoride diethyl ether complex ($BF_3{\cdot}OEt_2$) and TMSOTf were attempted, the last one successfully driving the reaction in 2 h at 0 °C (78% yield).
15. Takahata, H.; Banba, Y.; Ouchi, H.; Nemoto, H. *Org. Lett.* **2003**, *5*, 2527–2529.
16. Allart, B.; Busson, R.; Rozenski, J.; Van Aerschot, A.; Herdewijn, P. *Tetrahedron* **1999**, *55*, 6527–6546.
17. DBU was preferred to other commonly used bases, such as NaH or LiH, because not strictly anhydrous conditions were required.
18. After chromatographic purification a UV detectable byproduct was also isolated (10% yield): complete NMR characterization (COSY, HMBC, HSQC-TOCSY) unambiguously determined its structure as the bis-alkylated thymine, corresponding to the molecule reported below:



16

19. Chong, Y.; Chu, C. K. *Carbohydr. Res.* **2002**, *337*, 397–402.
20. Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1574–1585.
21. Chu, C. K.; Bhadti, V. S.; Doboszewski, B.; Gu, Z. P.; Kosugi, Y.; Pullaiah, K. C.; Van Roey, P. *J. Org. Chem.* **1989**, *54*, 2217–2225.
22. During the reaction, an inseparable mixture of two products was formed (TLC). Although an unambiguous identification of the structure was not possible, a second alkylation on the nucleobase moiety is assumed to have occurred, as already reported elsewhere [see Ref. 21].
23. Compound **1**: ^1H NMR (400 MHz, DMSO-d_6): δ 1.63–1.77 (m, 4H), 2.00–2.10 (m, 1H), 3.12–3.15 (m, 1H), 3.49–3.65 (m, 3H), 3.72 (dd, J = 12.9 Hz, J = 3.5 Hz, 1H), 3.97 (d, J = 12.9 Hz, 1H) 4.49 (s, 1H), 4.67 (br s, 1H), 4.97 (br s, 1H), 7.87 (s, 1H), 11.21 (br s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 12.4, 35.2, 50.0, 60.2, 60.6, 66.9, 82.4, 108.3, 139.0, 151.0, 163.8. Anal. Calcd for $C_{11}H_{16}N_2O_5$: C, 51.56; H, 6.29; N, 10.93. Found: C, 51.38; H, 6.31; N, 10.97.
24. Compound **2**: ^1H NMR (400 MHz, DMSO-d_6): δ 1.87 (dddd, J = 15.3 Hz, J = 13.7 Hz, J = 11.1 Hz, J = 4.3 Hz, 1H), 2.22–2.34 (m, 1H), 3.14–3.22 (m, 1H), 3.42–3.54 (m, 1H), 3.56–3.62 (m, 1H), 3.63–3.71 (m, 1H), 3.85 (dd, J = 12.6 Hz, J = 2.5 Hz, 1H), 4.21 (br d, J = 12.6 Hz, 1H), 4.66 (t, J = 5.9 Hz, 1H), 4.77 (s, 1H), 4.92 (d, J = 5.2 Hz, 1H), 7.22 (s, 2H), 8.17 (s, 1H), 8.30 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 36.4, 50.5, 60.9, 61.1, 68.5, 83.4, 118.7, 140.0, 149.8, 152.7, 156.5. Anal. Calcd for $C_{11}H_{15}N_5O_3$: C, 49.81; H, 5.70; N, 26.40. Found: C, 49.96; H, 5.72; N, 26.48.