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Diastereoselective Synthesis of a Conformationally Restricted Dinucleotide with Predefined α and β Torsional Angles for the Construction of α,β-Constrained Nucleic Acids (α,β-CNA)

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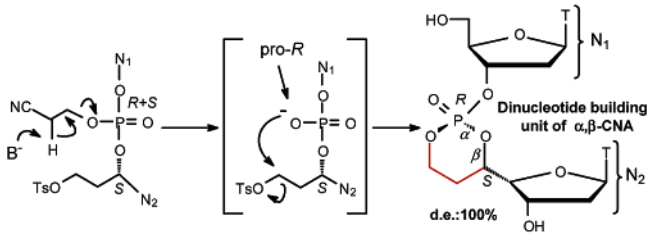
ABSTRACT



The synthesis of a diastereopure 1,3,2-dioxaphosphorinane linkage in which two, α and β, out of six torsion angles of the natural phosphodiester backbone are constrained with predefined values of ca. +60° (g$^+$) and 180° (t), respectively, is described. The stable and unstrained six-membered cyclic phosphotriester structure represents the smallest possible ring allowing the conformational locking of the α torsion angle at a significant positive value that is typical of many bulged regions of nucleic acids.

While the backbone organization of double-stranded DNA and RNA is normally quite regular, there are many other secondary and tertiary structures that DNA and RNA molecules can adapt in vivo.[1] It is now well established that the diverse biological functions of DNA and RNA are linked to their capacity to support a significant local conformational heterogeneity in the sugar−phosphate backbone. This conformational property allows RNA to adopt biologically important disparate structures such as bulges, hairpin loops, U-turns, adenosine platforms or branched junctions. An increasing number of studies indicate that these structural motifs are indeed characterized by a variety of backbone conformations that markedly differ from the regular conformational states of double-stranded DNA and RNA molecules.[2] The actual role played by the phosphate diester backbone in defining these structures is still not well understood. The flexibility of the phosphodiester linkage is expected to be an important component of this "second genetic code".

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(1) Belmont, P.; Constant, J-F.; Demeunynck, M. *Chem. Soc. Rev.* **2001**, *30*, 70 and references therein.

(2) See for example: (a) Ennifar, E.; Yusupov, M.; Walter, P.; Marquet, R.; Ehresmann, B.; Ehresmann, C.; Dumas, P. *Structure* **1999**, *7*, 1439. (b) Tereshko, V.; Wallace, S. T.; Usman, N.; Wincott, F. E.; Egli, M. *RNA* **2001**, *7*, 405. (c) Hung, L.-W.; Holbrook, E. L.; Holbrook, S. R. *Proc. Nat. Acad. Sci. U.S.A.* **2000**, *97*, 5107. (d) Scott, W. G.; Finch, J. T.; Klug, A. *Cell* **1995**, *81*, 991. (e) Ippolito, J. A.; Steitz, T. A. *Proc. Nat. Acad. Sci. U.S.A.* **1998**, *95*, 9819. (f) Cate, J. H.; Gooding, A. R.; Podell, E.; Zhou, K.; Golden, B. L.; Szewczak, A. A.; Kundrot, C. E.; Cech, T. R.; Doudna, J. A. *Science* **1996**, *273*, 1696.

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Considering the importance of the relationship between the local conformational and functional properties of nucleic acids, conformationally restricted oligonucleotides with modifications in the phosphodiester backbone unit, in the sugar unit and, to a limited extent, in the base units have been designed to efficiently sequester specific mRNA sequences with the background of antisense applications.[3] Since these modifications are introduced for the purpose of forming the strongest duplexes with the target complementary RNA, they do not lead to dramatic changes of the backbone torsion angles α−ζ (Figure 1) compared to the values

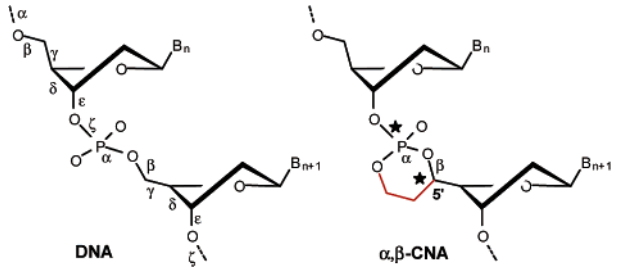


Figure 1. Six torsion angles α−ζ describing the sugar-backbone geometry of DNA. α,β-CNA (see ref 6) are nucleic acids in which the α and β torsion angles are conformationally constrained at predefined values.

observed in a natural nucleotide unit in an A-form duplex.[4]

To our knowledge, much less attention has been paid to the design of conformationally restricted nucleosides with the aim of mimicking nucleic acid secondary structures containing non-Watson−Crick pairs or unpaired nucleotides.[5] We are interested in the development of conformationally constrained dinucleotide building units in which the backbone torsional angles α−ζ can have predefined values that are significantly different from the typical values observed in DNA and RNA duplexes. Herein, we report the diastereoselective synthesis of a conformationally fixed dinucleotide building unit {(S_C,R_P)-α,β-CNA,[6] Scheme 2} in which the α and β torsion angles are locked in a (g^+, t) conformation that frequently occurs in bulged regions of nucleic acids.[2a−c]

Our general strategy for the conformational locking of the phosphate backbone torsional angles is based on the introduction of the 1,3,2-dioxaphosphorinane ring structure at key positions along the phosphate backbone. To lock torsional angles α and β simultaneously, we selected the cyclic structure in which the P−O5′ and O5′−C5′ bonds are part of the dioxaphosphorinane system (Figure 1). In such a structure, both the phosphorus atom and the 5′-carbon atom are asymmetric centers. Thus, in the absence of a diastereoselective synthetic method, the α,β-constrained dinucleotide product (α,β-CNA) should consist of a mixture of four diastereoisomers.

It is well documented that six-membered phosphorus compounds containing endocyclic oxygen atoms adjacent to phosphorus have chair conformations that are strongly influenced by the ground-state anomeric (stereoelectronic) effect: exocyclic electronegative phosphorus substituents prefer an axial position (equatorial P═O), whereas electropositive and carbon substituents prefer an equatorial position (axial P═O).[8] Accordingly, it is expected that among the four possible diastereoisomers of α,β-CNA, those with the alkoxy group ON_1 axial (equatorial P═O) and the carbon group N_2 equatorial should have an energetically more favorable chair conformation due to *both* the sterically *and* anomerically favorable trans relationship between ON_1 and N_2 (Figure 2).[9]

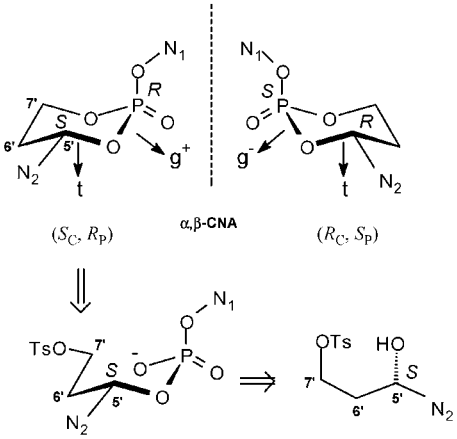


Figure 2. Retrosynthetic pathway for the diastereoselective synthesis of the (S_C,R_P)-stereoisomer of the α,β-CNA dinucleotide building unit with the (g^+, t) backbone conformation.

As shown in Figure 2, the trans (S_C,R_P)-stereoisomer has torsional angles α and β constrained to values ca. g^+ (+60°) and t (180°), whereas in the (R_C,S_P)-component, α and β are constrained to the (g^-, t) conformation, which is typical

(3) For a recent review, see: Leumann, C. J. *Bioorg. Med. Chem.* **2002**, *10*, 841.

(4) (a) Wengel, J. A. *Acc. Chem. Res.* **1999**, *32*, 301. (b) Petersen, M.; Bondensgaard, K.; Wengel, J.; Jacobsen, J. P. *J. Am. Chem. Soc.* **2002**, *124*, 5974. (c) Sørensen, A. M.; Nielsen, P. *Org. Lett.* **2000**, *2*, 4217. (d) Tarköy, M.; Leumann, C. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1432. (e) Marquez, V. E.; Siddiqui, M. A.; Ezzitouni, A.; Russ, P.; Wang, J.; Wagner, R. W.; Matteucci, M. D. *J. Med. Chem.* **1996**, *39*, 3739. (f) Singh, S. K.; Nielsen, P.; Koshkin, A. A.; Wengel, J. *Chem. Commun.* **1998**, 455. (g) Steffens, R.; Leumann, C. J. *J. Am. Chem. Soc.* **1999**, *121*, 3249. (h) Rajwanshi, V. K.; Håkansson, A. E.; Sørensen, M. D.; Pitsch, S.; Singh, S. K.; Kumar, R.; Nielsen, P.; Wengel, J. *Angew. Chem., Int. Ed.* **2000**, *39*, 1656.

(5) (a) Seio, K.; Wada, T.; Sakamoto, K.; Yokoyama, S.; Sekine, M. *J. Org. Chem.* **1998**, *63*, 1429. (b) Seio, K.; Wada, T.; S.; Sekine, M. *Helv. Chim. Acta* **2000**, *83*, 162. (c) Sekine, M.; Kurasawa, O.; Shohda, K.; Seio, K.; Wada, T. *J. Org. Chem.* **2000**, *65*, 3572. (d) Sekine, M.; Kurasawa, O.; Shohda, K.; Seio, K.; Wada, T. *J. Org. Chem.* **2000**, *65*, 6515. An alternative synthetic method for a structure very similar to that previously described in ref 5d has been recently proposed. See: Børsting, P.; Nielsen, P. *Chem. Commun.*, **2002**, 2140.

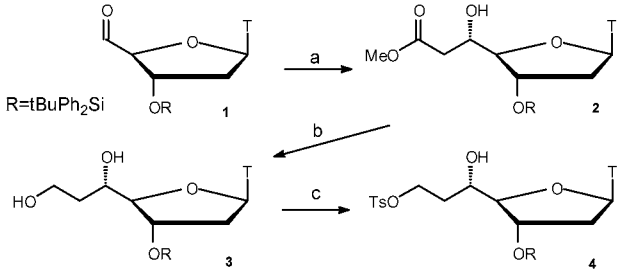
(6) We first wished to use the term α,β-LNA (α,β-locked nucleic acids) in the interest of semantic economy. The general term LNA (locked nucleic acid) was first introduced in reference to modifications in which the ribofuranose subunit of nucleic acids is locked (via covalent bridges between the 2′-oxygen and the 4′-carbon atoms) into the 3′-endo conformation with the aim of increasing the thermal stabilities of the corresponding duplexes formed with DNA and RNA. Therefore, the so-called LNA family is

of DNA and RNA duplexes. Therefore, the (S_C,R_P)-compound appears as the most interesting stereoisomer for the purpose of mimicking nucleic acid bulged structures.

We anticipated that the ground-state anomeric effect of the 1,3,2-dioxaphosphorinane system could be used as an essential driving force for the stereocontrolled chemical synthesis of this compound. According to our analysis, the pro-(*R*)-phosphate oxyanion of the (5′*S*)-isomer of the acyclic phosphate precursor shown in Figure 2 should attack much more readily the electrophilic 7′-carbon atom than the pro-(*S*)-oxyanion. If we are correct, then the cyclization reaction from the (5′*S*)-acyclic stereoisomer (referred to as compound **6** in Scheme 2) should occur stereospecifically to give the (S_C,R_P)-α,*β*-CNA as the dominant product. Finally, the key starting material that results from the retrosynthetic pathway shown in Figure 2 is a diastereopure (5′*S*)-C-tosyloxyethyl-substituted nucleoside.

The corresponding (5′*S*)-C-tosyloxyethylthymidine **4** was prepared from diastereopure diol **3** with tosyl chloride in the presence of pyridine (Scheme 1). Diastereomerically pure

Scheme 1. Diastereoselective Synthesis of the Key Starting Material 5′-C-Tosyloxyethylthymidine 4[a]

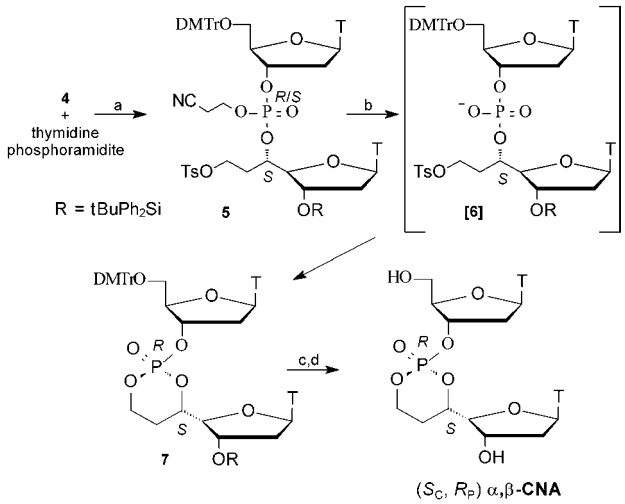


[a] Reaction conditions: (a) Methyl acetate silylketene, $BiCl_3/ZnI_2$ cat, CH_2Cl_2, 90%; (b) $NaBH_4$, EtOH, 85%; (c) TsCl, pyridine, 90%. T = thymin-1-yl.

diol **3** has been obtained starting from 5′-C-aldehyde thymidine **1**, which, via a Mukaïyama's reaction with the silylketene of methyl acetate catalyzed by $BiCl_3/ZnI_2$,[10] provided compound **2** with a (*S*)-configuration at the newly created asymmetric center 5′-C in 90% yield (Scheme 1).[11] Reduction of the ester function by $NaBH_4$ produced **3** in 85% yield.

(5′*S*)-C-Tosyloxyethylthymidine **4** was coupled with the commercially available thymidine phosphoramidite (Scheme 2) using standard phosphoramidite technology[12] to give two

Scheme 2. Diastereoselective Synthesis of the (S_C,R_P)-α,*β*-CNA Dinucleotide[a]



[a] Reaction conditions: (a) 1*H*-tetrazole, CH_3CN, 20 min, then collidine, I_2/H_2O−THF, 89%; (b) Et_3N, DMF, 95%; (c) $^nBu_4NF$, THF, 90%; (d) 2% TFA in CH_2Cl_2, 95%. T = thymin-1-yl.

diastereoisomeric dinucleotides **5** in an equimolar ratio (characterized by two ^{31}P NMR signals at δ_P −2.6 and −2.5). The expected ring closure reaction occurred by treatment of **5** with triethylamine in dry dimethylformamide at 90 °C for 2 h. Cyclic phosphotriester **7** was obtained in 95% yield as a single diastereoisomer as observed by ^{31}P (δ_P −9.0), ^1H, and ^{13}C NMR. The subsequent removal of the protective groups (using fluoride ion and trifluoroacetic acid) provided the final compound (S_C,R_P)-α,*β*-CNA. Finally, it should be noted that the cyclic structure described here is perfectly compatible with the chemistry of nucleotide structures.[13]

The (S_C,R_P)-α,*β*-CNA dinucleotide product was fully characterized by elemental analysis and by ^1H, ^{13}C, and ^{31}P NMR spectroscopy.[14] The chair conformation of this compound is evident from the ^1H NMR spectra,[15] with no detectable coupling constant between the 5′-H involved in

characterized by the *extra* conformational locking of only one out of six torsion angles of the sugar−phosphate backbone, the *δ* torsion angle, and could thus have been referred to as *δ*-LNA according to this nomenclature. However, to avoid any confusion with α-L-*ribo*-configured locked nucleic acids (termed α-L-LNA[7]) and the natural *β*-D-*ribo* configuration of LNA, we used the term "constrained" instead of "locked" to assign the meaning of torsional angle to α and *β* when they precede the acronym CNA (constrained nucleic acids).

(7) See: 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 and references therein.

(8) Gorenstein, D. G. *Chem. Rev.* **1987**, *87*, 1047 and references therein.

(9) The two remaining stereoisomers, (R_C,R_P) and (S_C,S_P), with a cis relationship between ON_1 and N_2 have *either* a sterically *or* anomerically favorable chair conformation.

(10) Le Roux, C.; Gaspard Iloughmane, H.; Dubac, J.; Jaud, J.; Vignaux, P. *J. Org. Chem.* **1993**, *58*, 1835.

(11) (a) Banuls, V.; Escudier, J.-M. *Tetrahedron* **1999**, *55*, 5831. (b) Escudier, J.-M.; Tworkowski, I.; Bouziani, I.; Gorrichon, L. *Tetrahedron Lett.* **1996**, *50*, 4689.

(12) Caruthers, M. H. *Acc. Chem. Res.* **1991**, *24*, 278.

(13) The uncharged (S_C,R_P)-α,*β*-CNA dimer has been successfully incorporated at preselected positions in otherwise unmodified oligonucleotides using the conventional automatic solid-phase technology based on phosphoramidite chemistry.[12] Unpublished results.

(14) Selected data for (S_C,R_P)-α,*β*-CNA. Elemental analysis: found (calcd) C, 46.35 (46.26); H, 5.08 (5.11); N, 9.65 (9.79). ^{31}P NMR δ_P (81 MHz, CD_3OD): −6.50. ^1H NMR δ_H (400 MHz, CD_3OD): $H_{5'b}$ 4.85; $J_{5'b/6'bax}$ = 11.9 Hz, $J_{5'b/6'beq}$ = 1.9 Hz, $J_{5'b/4'b}$ = 2.0 Hz, $J_{5'b/P} \approx 0$ Hz. For full characterization, see Supporting Information.

(15) Independent preparation of the (R_C,S_P)-stereoisomer of α,*β*-CNA (de 70%) was carried out directly from the cyclization reaction of the (5′*R*)-isomer of the acyclic phosphate precursor shown in Figure 2. The structure and geometry of (R_C,S_P)-α,*β*-CNA was confirmed by ^1H, ^{13}C, and ^{31}P NMR spectroscopy and ultimately by X-ray crystallography, establishing the chair conformation and the trans relationship between the ON_1 (axial) and N_2

the dioxaphosphorinane system and phosphorus, which is characteristic of an axial position of the 5′-H proton.[16]

The present results clearly demonstrate that it is possible to introduce the specific {α(g$^+$), β(t)} conformation into the phosphodiester linkage by simultaneously constraining torsional angles α and β into a dioxaphosphorinane structure. To our knowledge, we have reported the first example of a (100%) diastereoselective synthesis of a cyclic phosphotriester linkage. This very simple strategy that consists of using both steric and anomeric effects to stereocontrol the ring-closing reaction allowed us to independently prepare cyclic phosphotriester linkages with either an {α(g$^-$), β(t)} conformation,[15] which is typical of RNA and DNA duplexes, or an {α(g$^+$), β(t)} conformation, which frequently occurs in many bulged regions of nucleic acids (Figure 2).

Six-membered cyclic phosphotriesters are resistant compounds relative to both hydrolysis and phosphohydrolase enzymes.[17] Thus, synthetically accessible dinucleotides that are conformationally restricted by a dioxaphosphorinane system are expected to play an important role for the artificial stabilization of biologically important nucleic acid secondary structures.[18] In this respect, the dioxaphosphorinane-CNA family might provide new and promising tools for the structural analysis of complex RNA edifices and might ultimately contribute to the design of specific ligands to target particular RNA structures in the hope of treating a variety of chronic and degenerative diseases.

Further efforts in synthetic chemistry and detailed structural studies of potentially interesting dioxaphosphorinane-CNA dinucleotides are in progress in our laboratory.

Acknowledgment. We thank Dr. Stéphane Massou for his assistance in NMR spectroscopy.

Supporting Information Available: Experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(equatorial) groups. From the ^{31}P NMR spectra, the axial position of 5′-H is revealed by the absence of a P-coupled 5′-CH signal. Chemical shifts of δ_P −6.5 and −5.1 were conclusively assigned to the (S_C,R_P)- and (R_C,S_P)-diastereoisomers of α,β-CNA, respectively. Unpublished results.

(16) Typical upper and lower limits observed for the $^3J_{H/P}$ coupling constants for dioxaphosphorinane structures in chair conformation are $^3J_{Hax/P}$ < ca. 3 Hz and $^3J_{Heq/P}$ > ca. 20 Hz, respectively. See: Gorenstein, D. G.; Rowell, R.; Findlay, J. *J. Am. Chem. Soc.* **1980**, *102*, 5077.

(17) (a) Westheimer, F. H. *Acc. Chem. Res.* **1968**, *1*, 70. (b) Thatcher, G. R. J.; Kluger, R. *Adv. Phys. Org. Chem.* **1989**, *25*, 99. (c) Farquhar, D.; Chen, R.; Khan, S. *J. Med. Chem.* **1995**, *38*, 488.

(18) The simple fact that the α,β-CNA dinucleotide unit has no charge could in itself bring about a significant change in the conformation of the nucleic acid. The resulting positive or negative effect of that aspect in the possible induction of bulged regions will be discussed elsewhere (full paper in preparation).