Tetrahedron Letters 54 (2013) 1080–1083

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

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

Synthesis of a nucleoside phosphorodithioate analogue responsive to microenvironmental changes through chiral induction

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ARTICLE INFO

Article history:
Received 19 October 2012
Revised 6 December 2012
Accepted 10 December 2012
Available online 25 December 2012

Keywords:
Phosphorodithioate
Chirality
Nucleotide
Phosphorous NMR
DNA

ABSTRACT

We have synthesized a 2′-aminomethyl branched-chain sugar nucleoside phosphorodithioate from 2,2′-anhydro uridine and subjected the material to a subsequent cyclization reaction under aqueous conditions using bi-functional linkers. The rate of the cyclization reaction was dependent on the leaving group on the bi-functional linkers. The generation of a chiral phosphorous peak from the achiral precursor, as indicated by ^{31}P NMR, was identified as a good indicator for potentially probing the local and global features of the DNA structure.

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Introduction

The chirality of a substance can play an essential role during its molecular recognition and structural formation processes.[1] Biomacromolecules in particular, such as nucleic acids, possess a variety of different substituents on their sugar–phosphate backbone that contribute to the formation of their typically helical structures (A-form and B-form duplexes), as well as some more unusual structures that are influenced by different binding molecules.[2] Nucleic acids themselves can also provide chiral environments to small molecules and work as molecular catalysts for a verity of different organic transformations, including the Diels–Alder, aldol, and nucleophilic substitution reactions. Stereo selective syntheses can be successfully accomplished using DNA based catalysis because the chiral environments created by the structural features of DNA effectively facilitate these transformations.[3] Furthermore, the chiral nucleic acid analogues, phosphorothioate DNA/RNA have been the subject of considerable attention as nucleic acid therapeutics because of their high levels of nuclease resistance.[4] The chirality of the phosphorous atom has a significant influence on the stability and sensitivity of the nucleic acid duplex to nucleolytic enzymes such as phosphodiesterase.[5] The chiral recognition of such enzymes is a particularly selective process and the stereoselective synthesis of phosphorothioate DNA has been developed to address which of the isomers is active and which one is not.[6] Phosphorodithioate DNA itself was developed more than two decades ago as a novel DNA analogue and showed significant levels of nuclease resistance and HIV reverse transcriptase (RT) inhibition.[7] From a structural perspective, and in similar to natural phosphodiesters, the phosphorodithioate possesses no chirality on their phosphorous atom. Alkylation of the phosphorodithioate on the sulfur atom, however, leads to the generation of chirality on the phosphorous atom, as shown in Figure 1. We were interested in developing an understanding of chiral generation reactions that are microenvironment dependent. At the fundamental level, our idea relies on the expectation that we can use these transformations to act as reporter reactions to probe the microenvironment

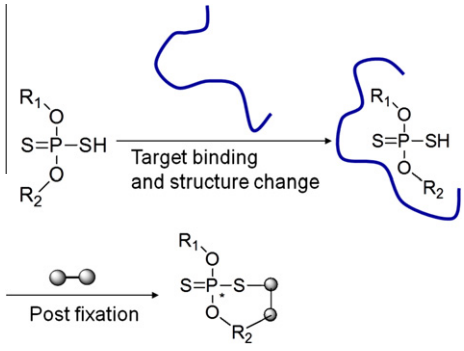

Figure 1. Alkylation of phosphorodithioate, with the resulting chirality acting as an indicator of the microenvironment.

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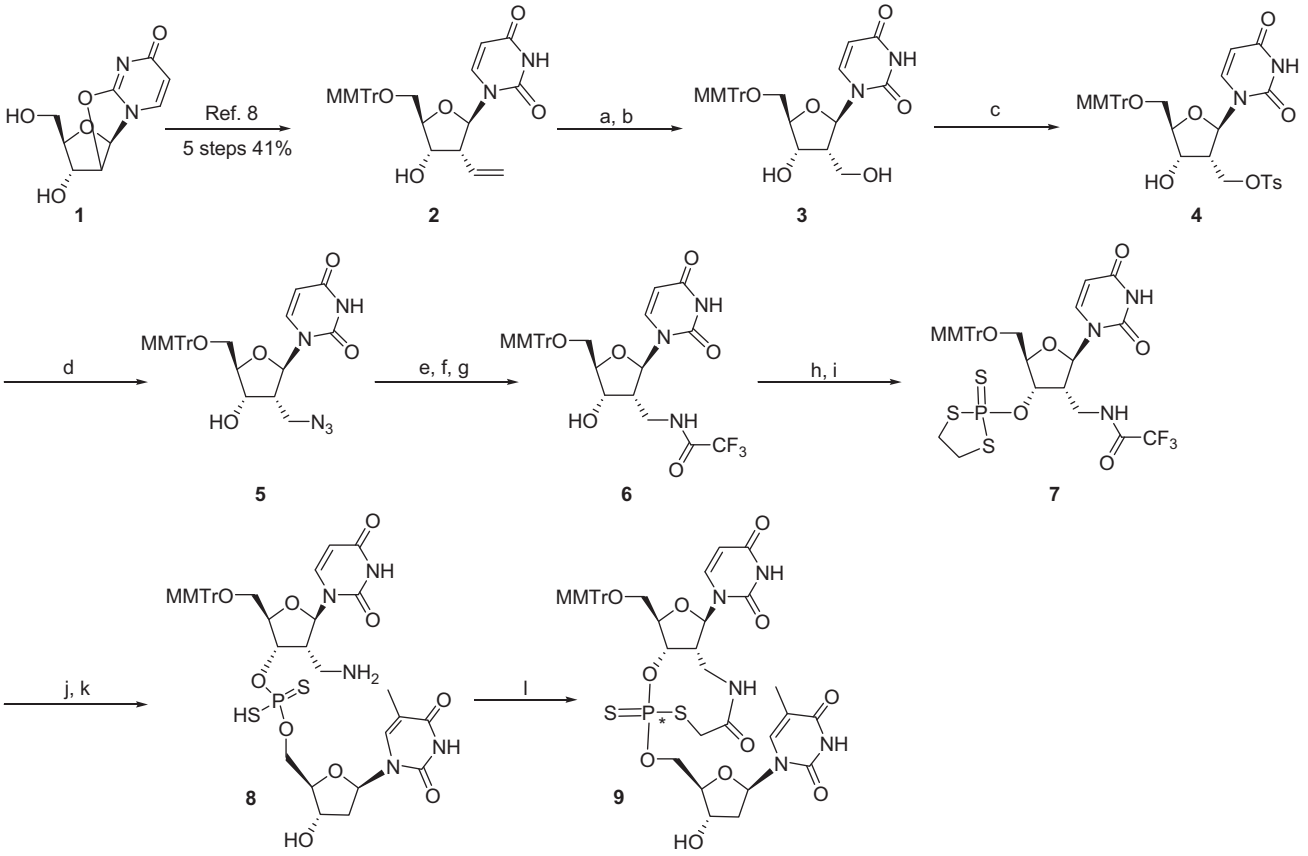
http://dx.doi.org/10.1016/j.tetlet.2012.12.028



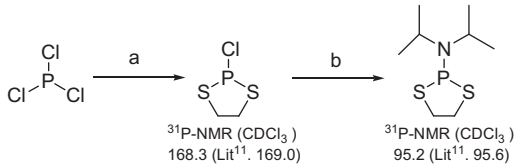
Scheme 1. Reagents and conditions: (a) OsO_4, NMO, 2,6-lutidine, dioxane-H_2O, rt, 3 h; (b) $NaBH_4$, MeOH, −78 °C to 0 °C, 2 h, 60% over 2 steps; (c) TsCl, pyridine, 0 °C, overnight, 72%; (d) NaN_3, DMF, 100 °C, 2 h, rt, overnight, 96%; (e) PPh_3, THF, 55 °C, 4 h; (f) NH_4OH, rt, 1 h; (g) $(CF_3CO)_2O$, pyridine, CH_2Cl_2, rt, 1 h, 86% over 3 steps; (h) (–SCH_2CH_2S–)$P(iPr)_2N$, 1*H*-tetrazole, CH_3CN, rt, 5 min; (i) elemental sulfur, sonication for 1 min and rt, 4 h, 92% over 2 steps; (j) 3′-*O*-Ac-thymidine, DBU, CH_3CN, rt, 5 min; (k) NH_4OH, rt, 9 h, 81% over 2 steps; (l) See Scheme 3.

and global shape of DNA in the presence of target molecules. In the context of synthetic organic chemistry, the target molecule is expected to behave as a chiral ligand for the reporter reaction. Herein, we report the synthesis of a nucleoside phosphorodithioate dimer as a useful building block for the microenvironment dependent synthesis of conformationally restricted nucleotides.

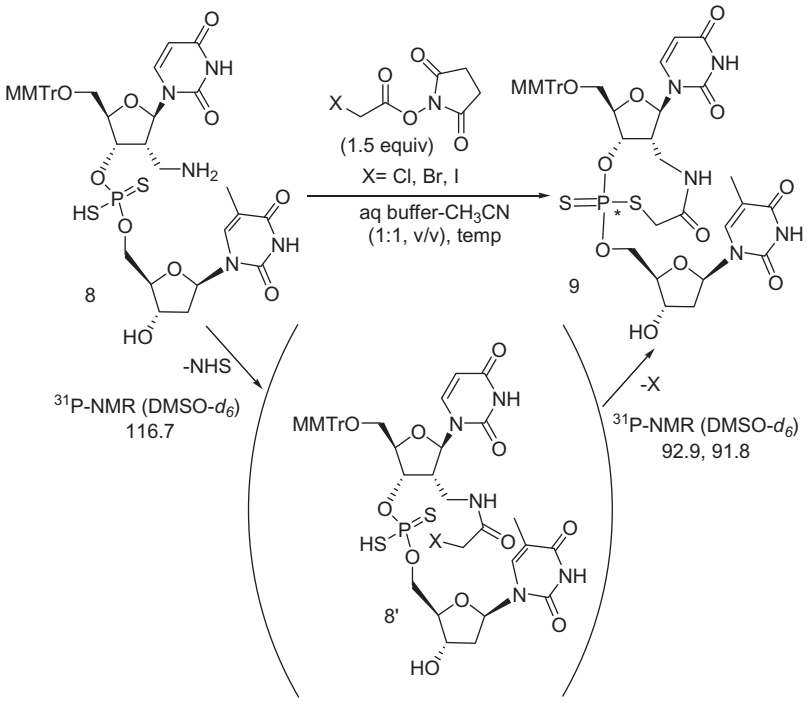
In Scheme 1, the synthetic work started from the commercially available 2,2′-cyclouridine **1**, which was converted into 2′-vinyl uridine **2** in 41% yield over 5 steps according to a procedure previously published by Sukeda.[8] The 2′-hydorxymethyl uridine derivative **3** was prepared according to a two stage procedure. Compound **2** was initially converted into the corresponding 2′-formyl intermediate by reaction with a combination of OsO_4, sodium periodate, and 2,6-lutidine according to a one pot procedure.[9] The 2′-formyl intermediate was found to be unstable and was therefore immediately reduced with $NaBH_4$ in MeOH to give the 2′-hydorxymethyl uridine derivative **3**. To introduce an azide group on branched side chain, the primary hydroxyl group was tosylated to give compound **4**, which was subsequently substituted with sodium azide in *N*,*N*-dimethylformamide to give compound **5**. The azide group was then reduced with PPh_3, and the resulting aza–ylide complex decomposed with NH_4OH to give the 2′-amino methyl modified nucleoside, which was acylated with trifluoroacetic anhydride to give compound **6**.

To complete the synthesis of phosphorodithioate, cyclic dithiophosphoramidite was employed as a phosphorylation reagent.[10] The cyclic dithiophosphoramidite reagent was prepared according to a literature procedure.[11] Briefly, phosphorus trichloride was treated with ethanedithiol and Et_3N to give chlorodithiophosphite, which was subsequently reacted with diisopropylamine in toluene (instead of benzene, as described in the previous paper) to give the target phosphoramidite derivative as shown in Scheme 2. The 3′-hydroxyl group of the nucleoside derivative **6** was phosphorylated in the presence of 1*H*-tetrazole and sulfidated with elemental sulfur to give the trithio cyclic phosphate derivative **7**. The subsequent ring opening coupling reaction with the appropriately protected thymidine derivative was performed with DBU to give coupling product **8**. ^{31}P NMR showed a single peak indicating the presence of a single isomer.

Next, we employed a tandem amidation–alkylation reaction sequence to allow for subsequent modification of the sugar–phosphate backbone, as in Scheme 3. Haloacetic acid *N*-hydroxysuccinimide ester was selected as the bi-functional reagent for the process. To test the reactivity of the material, three different types of linkers were used for the modification, as shown in Scheme 3. Using ESI-mass spectroscopic analysis,[12] we established that the amino group had reacted with the cross linker first followed by an intramolecular alkylation reaction. The alkylation reaction was



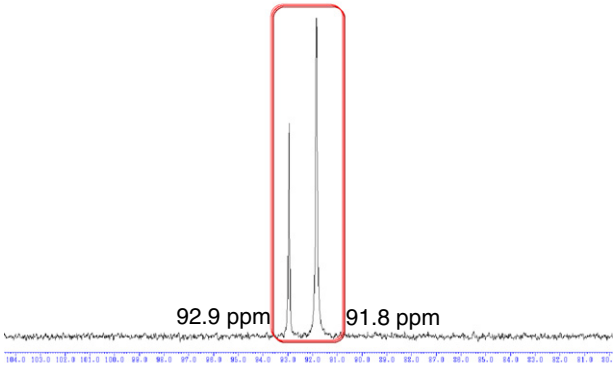
Scheme 2. Reagents and conditions: 1,2-ethanedithiol, Et_3N, Et_2O, rt, 20 h 73%; (b) diisopropylamine, toluene, rt, 2 h, 81%.



Scheme 3. Post synthesis cyclization under aqueous conditions using a bi functional linker.

found to be the rate determining step, with the rate being dependent on the nature of the leaving group as shown in Figure 2. The difference in the reactivity of the linker revealed that chlorine performed inefficiently as a leaving group, providing only 7% of the cyclized product even after 24 h. In contrast, the bromo and iodo leaving groups provided enhanced levels of reactivity to give the cyclized species after only 6 h. [13] The diastereomeric ratio of the products was found to be almost identical for each of the different leaving groups. This selectivity was attributed to the chirality of the nucleotide itself. The cyclization reaction was performed at different temperatures and pHs to clarify the reactivity and the diastereomeric ratio of the products. The results revealed that increasing the temperature to 37 °C enhanced the rate of the reaction, with the reaction reaching completion within 1 h. In contrast, the reaction proceeded at a slower rate when it was conducted at the lower temperature of 4 °C, and required 24 h to reach completion. Taken together, these results clearly indicated that the rate of the alkylation was controlled by the temperature as well as the nature of the leaving group. Interestingly, the temperature has no discernible impact on the diastereomeric ratio. Variations in the pH of the reaction mixture had a pronounced impact on the observed level of reactivity. Under acidic conditions, the reaction did not proceed at all because the protonation of the amino group on the nucleotide effectively subdued any reactivity. Under the basic conditions, the reaction also failed to provide any of the desired product probably because of the instability of the cyclized product **9** under the basic conditions. It is envisaged that future work conducted in the presence of target molecules, such as complementary DNA/RNA or other binding molecules, will reveal changes in the selectivity that will be dependent on the local conformation. The most intriguing property of these compounds was the characteristic NMR shift of phosphorodithioate triester that allowed for the effective discrimination of the product peaks from other phosphorous peaks such as those occurring in other natural compounds containing phosphodiesters, phosphomonoesters, phosphotriesters, pyrophosphates, and other phosphorothioates.

In conclusion, we have synthesized a 2′-aminomethyl branched-chain sugar nucleoside phosphorodithioate and subjected this



Entry	X	Time (h)	Temp (°C)	pH[a]	Yield (%)[b] (diastereomer ratio: 91.8, 92.9 ppm)
1	Cl	24	rt	7.4	7 (75:25)
2	Br	6	rt	7.4	58 (69:31)
3	I	6	rt	7.4	63 (69:31)
4	I	1	37	7.4	75 (65:35)
5	I	24	4	7.4	51 (68:32)
6	I	6	rt	5.0	0
7	I	6	rt	9.0	0

(a) pH 7.4 PBS buffer; pH 5.0 or 9.0 50 mM phosphate buffer
(b) Isolated yield

Figure 2. ^{31}P NMR spectrum of the cyclic phosphorodithioate triester **9** and cyclization conditions.

material to a subsequent cyclization reaction using bi-functional linkers. The current reaction involves the simple chiral generation reaction of an achiral phosphorous atom. It is envisaged that the reaction will be microenvironment dependent when conducted in the presence of a target strand or other nucleic acid binding materials such as distamycin antibiotics and intercalators. Furthermore, this reaction could represent a new method for monitoring nucleic acid hybridization and nucleic acid–ligand interaction by ^{31}P NMR. Further studies of this reaction in the presence of different oligonucleotide strands are currently underway.

Acknowledgments

H.S., H.A., and Y.I. were financially supported by Ministry of Education, Culture, Sports, Science and Technology (MEXT) and New Energy and Industrial Technology Development Organization (NEDO). We are grateful for the support received from the Brain Science Institute (BSI) Research Resource Center for mass spectrum analysis.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.12.028.

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12. *LRMS data for intermediate* ***8'****:* Cl: calcd for ($C_{42}H_{44}ClN_5O_{12}PS_2^-$) $[M-H]^-$ 940.18, found 940.07; Br: calcd for ($C_{42}H_{46}BrN_5O_{12}PS_2^+$) $[M+H]^+$ 986.15, found 986.13; I: calcd for ($C_{42}H_{44}IN_5O_{12}PS_2^-$) $[M-H]^-$ 1032.12, found 1031.99.
13. *Post cyclization reaction for the synthesis of* ***9*** *under aqueous conditions:* Compound **8** (30 mg, 29 μmol) was dissolved in PBS–CH_3CN (15 mL, 1:1, v/v). The iodo acetic acid NHS ester (12 mg, 42 μmol) was then added and the resulting mixture stirred at, rt for 6 h. The reaction mixture was diluted with EtOAc and washed with H_2O. The organic solution was dried (Na_2SO_4), filtered and evaporated in vacuo. The residue was purified by column chromatography eluting with $CHCl_3$/MeOH (10:1) to give **9** (17 mg, 63%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.53–11.34 (3H, m), 7.62–7.26 (15H, m), 6.94–6.92 (2H, d, J = 8.69), 6.23–6.21 (1H, m), 5.93 (1H, t, J = 9.65), 5.66–5.32 (2H, m), 4.80–4.24 (4H, m), 4.11–3.72 (5H, m), 3.51–3.42 (2H, m), 3.17–2.98 (2H, m), 2.24–2.08 (2H, m), 1.79 (3H, s); ^{31}P NMR (DMSO-d_6) δ 92.9, 91.8; HRMS (ESI) calcd for ($C_{42}H_{44}N_5NaO_{12}PS_2^+$) $[M+Na]^+$ 928.2058, found 928.2066.