2′,3′-Cyclopropanated Nucleoside Dimers

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Abstract: Syntheses of three novel conformationally rigid dimers containing cyclopropyl -amide and -sulfonamide functionalities are described. Their incorporation into an oligonucleotide sequence resulted in considerable lowering of the T_m's in binding to their complementary RNA sequences.

Introduction. The synthesis of oligonucleotides with modified backbone linkages is currently an active area of research[1]. We[2] and others[3] have shown that dimeric nucleoside containing amide linkage **I**, when incorporated in oligonucleotides, hybridize to the complementary RNA with affinity and specificity similar to the unmodified DNA. We decided to improve the binding affinity[4] by exploring the effects of connecting the methylene group adjacent to the amide to the 2′-carbon of the sugar (dimers **1** and **2**) to increase the rigidity of the system, and of replacing the amide bond by a sulfonamide linkage (dimer **3**), as indicated by preliminary modeling studies[5].

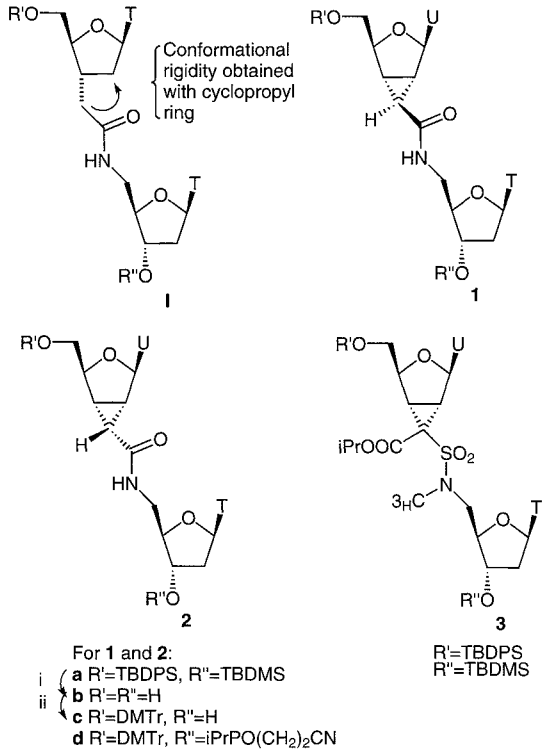


Figure 1

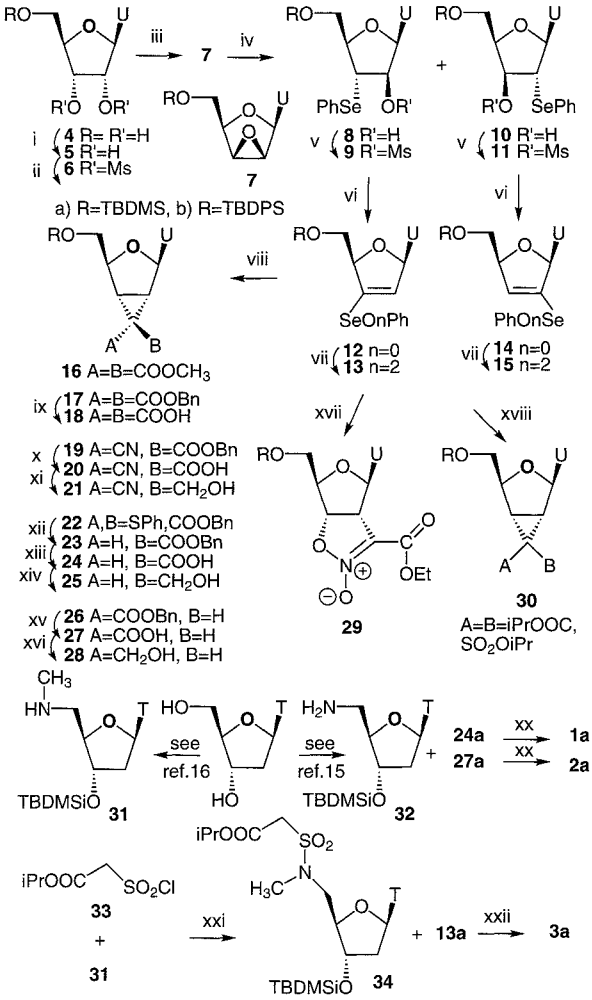
Herein we report the syntheses of novel conformationally rigid dimeric nucleoside building blocks of type **1**, **2** and **3** containing a cyclopropanated functionality and their incorporation into oligonucleotides. A similar synthesis has been reported by Haly *et al.*[6] The principle of conformational rigidity[7] in oligonucleotides has been extensively utilized by incorporation of modified nucleosidic residues[8].

Results and discussion. An obvious way to carry out the cyclopropanation of a nucleoside was to add a carbenoid species derived from ethyl diazoacetate to an appropriate olefin **12** (R = TBDMS, SeOnPh = H)[9]. All attempts to carry out this well documented reaction in an inter- or intramolecular mode, using rhodium acetate[10] or copper-based catalysts[11], failed. This approach was not further investigated since Samano and Robins reported unsuccessful results in their attempts to cyclopropanate a similar substrate under Simmons-Smith and related conditions[12].

Wu and Chattopadhyaya[13] had shown that vinyl selenones of type **13** (R = monomethoxytrityl, MMT) underwent efficient cyclopropanation when treated with the anion of dimethyl malonate or of nitromethane[14]. We chose the *tert*-butyldimethyl (TBDMS) and diphenyl (TBDPS) 5′-protecting groups instead of the MMT because of the acid instability of the latter. Silylated uridine dimesylates **6a** and **6b** were transformed to epoxides **7a** and **7b** with 3 equivalents of 1N NaOH in aqueous methanol[15]. Any excess of base resulted in substantial desilylation of the epoxides. Ring opening of epoxides **7a** and **7b** with phenylselenide anion yielded alcohols **8a, 10a** and **8b, 10b**, respectively in excellent yields. Their large scale chromatographic separation was difficult. Alcohols **8a, 10a, 8b** and **10b** were mesylated to their corresponding monomesylates **9a, 11a, 9b** and **11b** in quantitative yields. Alcohol **10b** required a catalytic amount of DMAP for successful mesylation. Treatment of monomesylate **9a** with potassium *tert*-butoxide in DMF for 4 hours at room temperature, as described by Wu and Chattopadhyaya gave a 1:1 mixture of silylated **12a** and desilylated olefin **12** (R = H), whereas in the TBDMS series, complete desilylation occurred. When TBDPS protected monomesylate **11b** was treated with potassium *tert*-butoxide in DMF at 0^0 C for 4 hours, only 30% of olefin **14b** was desilylated. To favor the bimolecular elimination over the desilylation process which we thought might proceed via an intramolecular process, we increased the concentration of reactants by dissolving monomesylates **9a** and **9b** in a minimal amount of DMF and added 3 eq. of potassium *tert*-butoxide at 0^0 C. The reactions were complete within 7 minutes. Any longer reaction times yielded a mixture of silylated and desilylated products. The unexpected base lability of silyl ethers **7a, 7b, 12a, 12b, 14a** and **14b** is possibly due to the fact that the imide anion formed during the base-catalysed reaction participates in the desilylation process. Similar results were recently reported by Le Hir de Fallois *et al.* They observed selective 5'-desilylation when 3',5'-di-*tert*-butyldimethylsilyl-2,2'-anhydrouridine was treated with ethanolic KOH[16]. *m*-Chloroperbenzoic acid treatment of olefins **12a, 12b** and **14a** yielded vinyl selenones **13a** (82%), **13b** (84%) and **15a** (90%) which underwent Michael addition reactions with dimethyl malonate anion to yield cyclopropano nucleoside **16a** (87%)or **16b** (85%).

Attempted base hydrolysis of nucleoside **16a** resulted in desilylation. The problem was circumvented by generating the dibenzyl malonate **17a**, which underwent smooth hydrogenolysis to provide diacid **18a** quantitatively. Coupling of **18a** with 5'-amino-5'-deoxythymidine[17] (1 eq) gave in excellent yield the bis-coupling product, indicating that the *exo* and *endo*-carboxylic acids had very similar reactivities in amide forming reactions. All attempts to decarboxylate **18a** failed to provide a monocarboxylic acid. Reaction of benzyl methyl malonate with **13b** gave an inseparable mixture of diastereomers.

We next carried out the addition of the anion of benzyl cyanoacetate (4 eq, 1M in THF) to vinylselenone **13b**. The expected cyclopropanated species **19b** was obtained in 84% yield as a single diastereomer, with the protons at C2 and C3 appearing as AB quartets, centered at 3.04 and 2.79 ppm, J = 7 Hz. Its hydrogenolysis over Pd/C in MeOH gave the corresponding carboxylic acid **20b** quantitatively. NOe experiments on its diborane reduction product **21b** strongly suggested the *exo*-configuration for the hydroxymethyl group, thereby confirming the stereochemistry assigned to **19b** and **20b**. Having achieved the synthesis of a stereochemically well defined carboxylic acid, albeit with an α-cyano substituent, we next attempted to adapt the method to the addition of stabilised carbanions containing a removable substituent. A solution of ethyl nitroacetate containing 5 eq. of base was reacted with 1 eq. of **13b**. A 90% yield of a product not containing the characteristic cyclopropane protons and therefore isomeric with the expected one was obtained [$(M+H)^+$ = 580, FAB]. It was assigned structure **29b**, based in



series **a**, (i) TBDMSiCl/DMAP/Py., 0 °C, 2 h; (ii) MsCl/Py., 0 °C, 2 h; (iii) 1N NaOH, rt, 3 h; (iv) LAH/$(Ph)_2Se_2$/ THF, 0 °C, 30 min.; (v) MsCl/Py., 0 °C to rt, 24 h; (vi) t-BuOK/DMF, 0 °C, 7 min.; (vii) mCPBA/MeOH, 0 °C to rt, 12 h; (viii) A-CH_2-B/t-BuOK/ THF, 0 °C to rt, 3-6 h; (ix), (x), (xiii) and (xv) H_2/Pd/C, rt, 4 h; (xi), (xiv) and (xvi) $B_2H_6.Me_2S$, 0 °C to rt, 2 h; (xii) Bu_3SnH/AIBN/Benzene, reflux, 4h; (xvii) EtOOC-CH_2-NO_2, t-BuO K /THF, 0 °C to rt, 30 min.; (xviii) iPrOOC-CH_2-SO_2OiPr/ t-BuOK /THF, 0 °C, 4 h; (xx) BOP/Et_3N/DMF, rt, 2 h; (xxi) Py./CH_2Cl_2, 0 °C to rt, 1.5 h; (xxii) t-BuOK/THF, -78 °C to rt,2 h. Series **b**, same as **a** with R=TBDPS

Scheme 1

part by analogy to a similar compound obtained by Wu and Chattopadhyaya when adding ethyl acetoacetate to **13** (R = MMT).

The reaction of selenone **13b** for 8 hours with the anions of benzyl or methyl thiophenoxyacetate, generated at -78 °C by addition of butyl lithium in THF, gave a 75-85% yield of the desired cyclopropanes **22b** and the corresponding methyl ester (**22b**, Bn=Me) as single diastereoisomers, with the characteristic cyclopropane protons appearing as AB quartets at 3.03 and 2.95 and 3.09 and 3.00 ppm, respectively (J = 7.8, 8.0 Hz). Treatment of **22b** with Bu_3SnH and AIBN in refluxing benzene for 20 hours, followed by chromatography, gave *exo*-carboxylate **23b** and *endo*-carboxylate **26b** in 66% and 23% yields, respectively. The configurational assignments for **23b** and **26b** were carried out as described for **19b** and **20b** by converting them via their free acids **24b** and **27b** to the primary alcohols **25b** and **28b**, and by conducting appropriate nOe experiments. Reaction of the *exo*- and *endo*-acids **24** and **27** with 5'-amino-5'-deoxythymidine, using BOP as the condensing agent, gave dimers **1** and **2** in 90% yields[18].

Because of difficulties encountered in forming a sulfonamide when trying to prepare **30** following a pathway parallel to that just described for the carboxamides **1** and **2**, dimer **3** was prepared in the following manner. Chlorosulfonylacetyl chloride in CH_2Cl_2 was treated with isopropanol (1 eq), and gave, after evaporation and drying, sulfonyl chloride **33**. It (1.25 eq) was added to a CH_2Cl_2 solution of the 3'-TBDMS-derivative of 5'-deoxy-5'-methylaminothymidine[19] containing 25 eq of NEt_3 (-78 to 20 °C). Silica gel column chromatography gave sulfonamide **34**. Formation of its anion as described, and addition to vinylselenone **13a** gave dimer **3** in 67% yield[17]. Desilylation, dimethoxytritylation and phosphitylation reactions of **1**, **2** and **3** were carried out under standard conditions and provided dimers that were ready for incorporation into oligonucleotides using automated DNA synthesizer.

The modified oligomers **A-F** containing various cyclopropanated dimers (**1-3**) were prepared on a 1 µmol scale. The average coupling yield for most of the synthesis was found to be low (~85%). Introduction of an extended wait step (up to 2 minutes) during the coupling had little effect on the yield. After automated synthesis, the oligomers **A-F** were cleaved off the CPG support by NH_4OH treatment and purified by reverse phase HPLC. Subsequent detritylation and precipitation provided low amounts (~10 OD units) of **A-F** in reasonable purity (Table 1). The structural identity and purity of **A-F** was confirmed by ES-MS and CGE, respectively. The consistent mass range (0.5-1.3 units) between observed and the calculated values of the ES-MS provided unambiguous proof for the incorporations of the modified cyclopropanated dimers.

Table 1. Properties of the Modified Oligonucleotides

List of oligonucleotide sequences (5'♦3')

A. GCG TTTT U^1*T TTTT GCG

B. GCG U^1*T TT U^1*T TT U^1*T GCG

C. GCG TTTT U^2*T TTTT GCG

D. GCG U^2*T TT U^2*T TT U^2*T GCG

E. GCG TTTT U^3*T TTTT GCG

F. GCG U^3*T TT U^3*T TT U^3*T GCG

Oligo	HPLC RT[1]	CGE Purity[2]	ESMS: calc'd/exp. (Δ)[3]	ΔTm/mod.[4]
A	16.3	94	4820.3/4821.6 (1.3)	-6.2
B	16.5	84	4710.4/4711.2 (0.8)	-9.3
C	16.0	95	4820.3/4820.9 (0.6)	-6.0
D	16.4	35	4710.4.4710.9 (0.5)	nd
E	18.6	85	4956.5/4957.3 (0.8)	-3.2
F	24.3	88	5118.8/5119.9 (1.1)	-4.0

U^1*T = Dimer **1**; U^2*T = Dimer **2**; U^3*T = Dimer **3**;

[1] HPLC column: SupelcoLC18; 4.6 mm x 15 cm, 5µ, 5% to 25% CH_3CN in TEAA 0.05 M, pH 7.0, 1 ml/min, 260 nm;

[2] 12% Non crosslinked polyacrylamine (40 cm total/20 cm effective, 100 µm, I.D.) buffer 100 mM Bis-Trisborate, 7 M urea;

[3] Electrospray mass spectra were recorded according to reference 20;

[4] All modified oligonucleotides A-F were hybridized with complementary RNA of the same length and absorbance vs. temperature profiles were measured at 4 µM concentration of each strand in 100 mM Na^+, 10 mM phosphate, 0.1 mM EDTA at pH 7.0, see reference 21 for experimental details; nd = not determined due to low % purity.

The results of the Tm studies with oligomers **A-F** are summarized in Table 1. The study indicated that all oligomers containing cyclopropanated dimers (**1-3**) had a lower affinity for duplex formation with their complementary RNA sequences. The overall lower binding

affinities with all of the modifications studied herein discouraged us from pursuing the RNase H and nuclease stability studies of the modified oligonucleotides.

In summary, various synthetic routes for the preparation of cyclopropanated nucleosides has been accomplished. These modified nucleosides may not be useful for antisense constructs but may be of interest as potential candidates for nucleoside based therapeutics.

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