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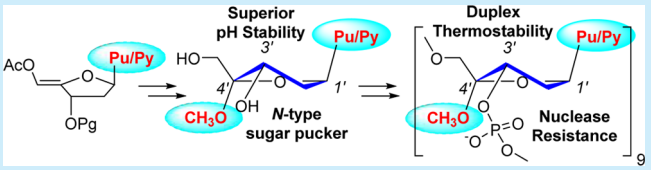
Straightforward Synthesis of Purine 4′-Alkoxy-2′-deoxynucleosides: First Report of Mixed Purine−Pyrimidine 4′-Alkoxyoligodeoxynucleotides as New RNA Mimics

Magdalena Petrová,* Ondřej Páv, Miloš Buděšínský, Eva Zborníková, Pavel Novák, Šárka Rosenbergová, Ondřej Pačes, Radek Liboska, Ivana Dvořáková, Ondřej Šimák, and Ivan Rosenberg*

Department of Bioorganic and Medicinal Chemistry, Institute of Organic Chemistry and Biochemistry Academy of Sciences of the Czech Republic v.v.i., Flemingovo nám. 2, 166 10 Praha 6, Czech Republic

Supporting Information

ABSTRACT: Purine and pyrimidine 4′-alkoxy-2′-deoxynucleosides were efficiently prepared from nucleoside 4′-5′-enol acetates in three steps by *N*-iodosuccinimide promoted alkoxylation, hydrolysis, and reduction followed by conversion to phosphoramidite monomers for the solid-phase synthesis of the oligonucleotides. Fully modified 4′-alkoxyoligodeoxynucleotides, which are characterized by a prevalent *N*-type (RNA-like) conformation, exhibited superior chemical and nuclease resistance as well as excellent hybridization properties with a strong tendency for RNA-selective hybridization, suggesting a potential application of 4′-alkoxy-oligodeoxynucleotides in antisense technologies.

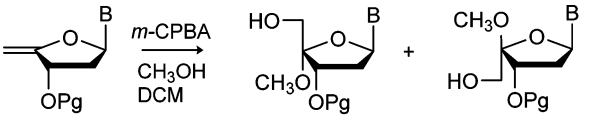


Chemically modified antisense oligonucleotides (AOs) are powerful tools for the regulation of gene expression.[1] AO modifications, which improve their affinity for cognate RNA by the conformational restriction of the sugar−phosphate backbone in the RNA-like C3′-*endo* conformation[2] (e.g., 2′-*O*-Me, 2′-*O*-MOE, and LNA[3]), are a powerful tool for antisense drug design, which target metabolic and cardiovascular diseases as well as cancer.[4] In addition, progress in AO chemistry is closely related to the development of RNAi technologies and the advance of siRNAs[5] and miRNAs[6] from basic research to therapeutic applications.[7]

A very simple modification of the sugar part of 2′-deoxynucleosides (dNs) was accomplished by 4′-alkoxy substitution, where *β*-D-*erythro* epimers preferentially adopt a C3′-*endo* conformation.[8] In 2011, we reported the synthesis of 4′-alkoxy oligothymidylates, whose hybridization properties with a complementary rA_{15} counterpart were superior to those of unmodified dT_{15}. Furthermore, the observed T_m enhancement was of similar magnitude to the regioisomeric 2′-*O*-Me oligoribothymidylate reference.[9] Recently, oligomers consisting of a 2′-*O*,4′-*C*-ethyleneoxy bridged 5-methyluridine derivative, which significantly stabilized duplex and triplex formation, were reported.[10]

The routine synthesis of epimeric 4′-methoxy dNs **2** is based on the transient epoxidation of 4′,5′-*exo*-methylene nucleoside precursors **1** with *m*-chloroperbenzoic acid followed by opening with methanol (MeOH) (Scheme 1).[8,9,11] Although the 4′-alkoxy thymidine derivatives were prepared in good yields, only trace amounts of the purine derivatives were produced.[11] The 4′-epimeric mixture of 2′-deoxy-4′-methoxyadenosine was alternatively obtained by the radical photolysis of a 4′-phenylselenide compound.[12] Therefore, for the preparation of mixed purine−pyrimidine 4′-methoxy oligodeoxynucleotides (MONs), a new, more robust synthetic approach that preferentially yields *β*-D-*erythro* epimers of purine derivatives would be highly useful.

Scheme 1. Routine Synthesis of 4′-Methoxynucleosides



1 **2b** (β-D-*erythro*) **2a** (α-L-*threo*)

The concept of utilizing nucleoside 4′,5′-enol acetates as unsaturated derivatives to preserve the oxygen function at C5′ was introduced by Cook and Secrist in the late 1970s.[13] However, their attempts at direct alkoxylation failed. In this Letter, we disclose the true potential of 4′,5′-enol acetates for the introduction of alkoxy substituents, as demonstrated in the dNs series.

A modified[14] Moffat oxidation of *N*-acyl and 3′-*O*-*tert*-butyldiphenylsilyl (TBDPS) protected 2′-deoxyadenosine **3a** and 2′-deoxyguanosine **3b** afforded 5′-aldehydes **4a** (88%) and **4b** (81%), respectively, which were converted by reaction with acetic anhydride in the presence of K_2CO_3 in acetonitrile[13b] to 4′,5′-enol acetates **5a** (61%) and **5b** (43%), respectively. In consistency with the literature data,[13b] enol acetates **5** with *Z* configuration were exclusively formed. Surprisingly, the conversion of **5** using *N*-iodosuccinimide (NIS) and anhydrous

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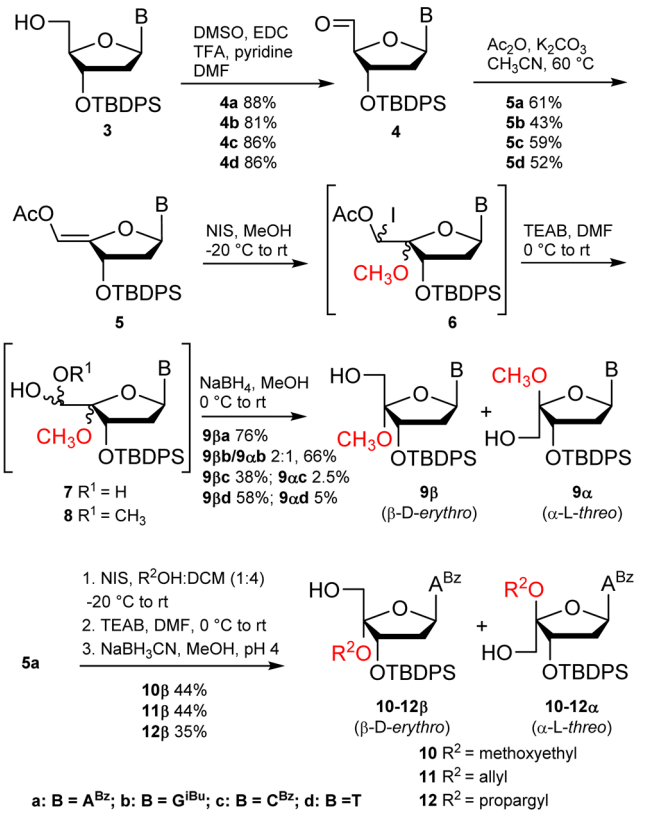
MeOH as the solvent readily afforded the corresponding 5′-acetoxy-5′-iodo-4′-methoxy intermediates **6**, which were directly subjected to hydrolysis (2 M aqueous triethylammonium bicarbonate−*N*,*N*-dimethylformamide mixture, TEAB−DMF, 1:10). The obtained complex mixture of acetals **7** and hemiacetals **8** was treated with sodium borohydride in a DMF−MeOH mixture to yield the desired 4′-methoxy products **9*β*a** (76%) and **9*β*b**/**9*α*b** (2/1, 66%) (Scheme 2). Similar results

Scheme 2. Synthesis of 4′,5′-Enol Acetates 5 and 4′-Methoxy dNs 9−12



were obtained for pyrimidine dNs: 86% yields for both 5′-aldehydes **4c** and **4d**; 59% and 52% for 4′,5′-enol acetates **5c** and **5d**, respectively; and 38%, 2.5%, 58%, and 5% for 4′-methoxy **9*β*c**, **9*α*c**, **9*β*d**, and **9*α*d**, respectively (Scheme 2).

The general usefulness of 4′,5′-enol acetates **5** as key intermediates in the synthesis of various 4′-alkoxy substituted dNs was demonstrated by the preparation of protected 4′-methoxyethoxy **10** (44%), 4′-allyloxy **11** (44%), and 4′-propargyloxy **12** (35%) derivatives of 2′-deoxyadenosine. The initial reaction with NIS also proceeds in a diluted mixture of alcohol and methylene chloride (1:4), and the final reduction is conveniently accomplished using sodium cyanoborohydride in MeOH at a pH of ∼4 (Scheme 2).

To obtain free nucleosides **13**, the 3′-*O*-TBDPS protecting group from **9*β*** was removed with a 0.5 M solution of tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) followed by the removal of the *N*-acyl protecting groups from the nucleobases by heating in a 33% solution of methylamine in ethanol at 50 °C for 1−2 h (total yields for the two steps 66% **13a**, 86% **13b**, 57% **13c**; 92% **13d** only for the TBAF treatment). Similar results were obtained for the 4′-methoxyethoxy **14**, 4′-allyloxy **15**, and 4′-propargyloxy **16** derivatives of 2′-deoxyadenosine (51% each) (Figure 1a).

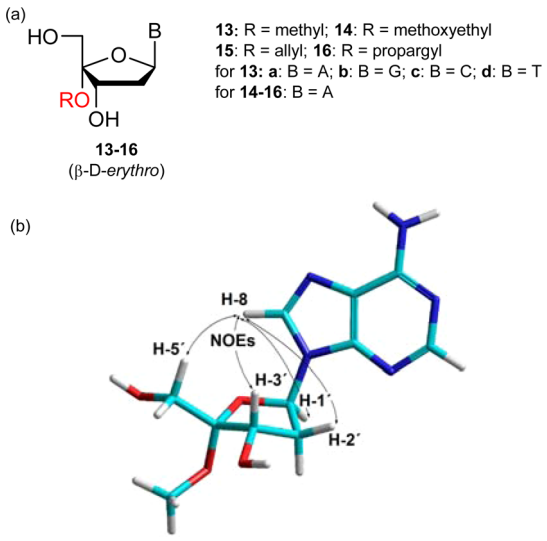


Figure 1. (a) 4′-Alkoxy substituted dNs **13**−**16** prepared in the present study. (b) Calculated conformation of 2′-deoxy-4′-methoxyadenosine **13a**.

The assumed preferential C3′-*endo* conformation of 2′-deoxy-4′-methoxyadenosine **13a** was confirmed by NMR spectroscopy (Figure 1b). The configuration at carbon C4′ and the preferred orientation of adenine were determined from the observed NOE contacts between the base and pentose protons. The absence of H-4′ does not allow for the use of a complete pseudorotational conformation calculation for the pentose ring. The application of the empirical relationship[16] for the population of *C*2′-*endo* and *C*3′-*endo* (*C*2′-*endo* = $[17.8 - (J(1',2'') + J(2'',3''))]/10.9$; *C*3′-*endo* = $[(J(1',2'') + J(2'',3')) - 6.9]/10.9$) and the observed vicinal coupling constants ($J(1',2'') = 7.6$ and $J(2'',3') = 9.2$ Hz) yields a population ratio for *C*2′-*endo*/*C*3′-*endo* of 9:91%. The use of $J(1',2'')$ and $J(2'',3')$ for guanine derivative **13b** (7.8 and 9.2 Hz) and cytosine derivative **13c** (7.9 and 9.1 Hz) gives the same ratio for *C*2′-*endo*/*C*3′-*endo* of 7:93%, which is in agreement with the data reported earlier for thymine derivative **13d**.[8]

Surprisingly, 4′-methoxy dNs exhibit exceptional stability in acidic media. Depurination of adenine and guanine derivatives **13a** and **13b** in 0.05 M HCl at 40 °C was monitored by the decreasing UV absorbance at wavelengths corresponding to the highest differences between the extinction coefficients of the appropriate nucleoside/nucleobase pairs. It was characterized by the initial depurination rate (IDR), expressed as the first derivative of time-dependent nucleobase cleavage curve at the initial time point zero, with obtained values 1.29×10^{-3} for 2′-deoxyadenosine compared to 1.29×10^{-4} for **13a** at $\lambda = 256$ nm, and 2.04×10^{-3} for 2′-deoxyguanosine compared to 2.32×10^{-4} for **13b** at $\lambda = 260$ nm (Figure 2). Concerning the mechanism of acidic hydrolysis of nucleosides, there are two mechanisms described in the literature.[17] The first mechanism assumes protonation of the furanose 4′-oxygen followed by breaking the C−O bond, attack of the water molecule on the newly formed Schiff base, and subsequent release of the nucleobase and (deoxy)ribose. A second, more recently claimed A-1 mechanism assumes protonation of the nucleobase, followed by slow breaking the C−N bond and formation of an oxocarbenium ion. The strong stabilizing effect of 2′-hydroxy and 2′-methoxy groups, as well as less stabilizing effect of 3′-hydroxy and 3′-methoxy groups are well established and explained in terms of

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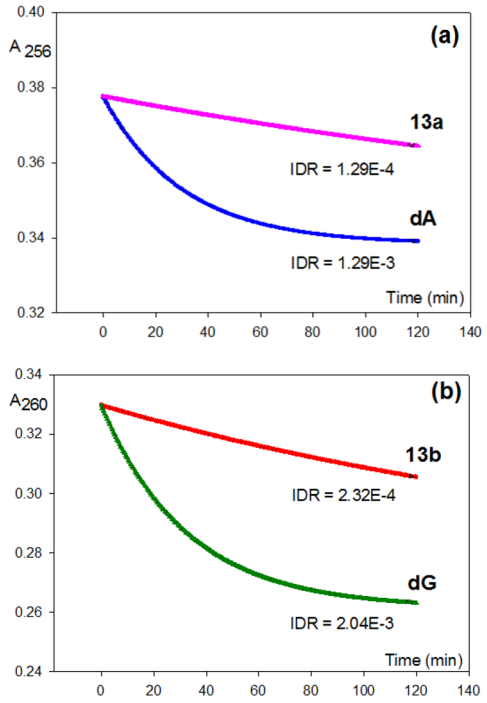


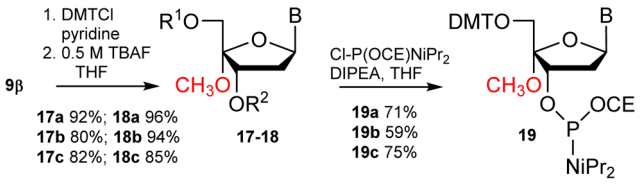
Figure 2. UV-monitored stability of 2′-deoxy-4′-methoxypurines in 0.05 M HCl at 40 °C. (a) 2′-Deoxy-4′-methoxyadenosine **13a** vs 2′-deoxyadenosine; λ = 256 nm. (b) 2′-Deoxy-4′-methoxyguanosine **13b** vs 2′-deoxyguanosine; λ = 260 nm.

the negative inductive effect of the substituents.[17a] A similar effect of 2′- and/or 3′-fluoro substituents is also attributed to the inductive effect leading to the destabilization of the oxocarbenium ion.[18] However, 4′-fluoro substituent led to a significant labilization of the glycosidic linkage.[19] Thus, there is a strong difference between the stability of nucleosides having 4′-alkoxy and 4′-fluoro substituents. This may conceivably be attributed to different electronegativity of these groups and their capability to increase or reduce, respectively, electron density of the furanose ring, i.e., to hamper or enhance, respectively, the formation of an oxocarbenium ion and the elimination of a nucleobase.

To prepare phosphoramidite monomers **19**, the dimethoxytrityl (DMT) group was introduced at the 5′-hydroxyl of **9*β***, followed by the removal of the 3′-*O*-TBDPS group from the 5′-*O*-DMT derivatives **17**. After purification of 3′-hydroxy derivatives **18** on reverse phase, reaction with 2-cyanoethoxy-*N*,*N*-diisopropylaminochlorophosphine in THF in the presence of diisopropylethylamine (DIPEA) gave 3′-phosphoramidites **19**. Because the complete removal of the chlorophosphine reagent residues using standard silica gel chromatography failed, the procedure based on RP chromatography using a CH_3CN gradient in TEAA buffer followed by rapid extraction with DCM upon cooling has been employed as the final purification step. Protected monomers were prepared in good yields (**19a** 71%, **19b** 59%, and **19c** 75%) (Scheme 3).

The ability of 4′-methoxyoligodeoxynucleotides (4′-MONs) to stabilize duplexes with complementary DNA and RNA was measured using fully modified 9-mers that were synthesized by a phosphoramidite method on solid phase.[9] Two types of sequences were evaluated: DNA-like nonamers featuring two 4′-methoxythymidine units (entries 3, 5, and 7), as well as RNA-like nonamers having two 2′-deoxy-4′-methoxyuridine units instead (entries 4 and 6). All of the studied oligomers enhanced

Scheme 3. Synthesis of Phosphoramidite Monomers 19



17: R^1 = DMT; R^2 = TBDPS; **18**: R^1 = DMT; R^2 = H
a: B = A^{Bz}; b: B = G^{iBu}; c: B = C^{Bz}

the duplex stability with varied potency. The results are summarized in Table 1.

Table 1. Duplex Thermal Stability of Modified Oligonucleotides Compared to Their DNA and RNA Complements[a]

		T_m (ΔT_m per residue) °C	
entry	sequence (5′–3′)	vs DNA[b]	vs RNA[c]
1	d(GCA TAT CAC)	36.9	34.9
2	r(GCA UAU CAC)	34.5	46.1
3	d(GCA TAT CAC)	43.6 (+0.7)	52.2 (+1.9)
4	d(GCA UAU CAC)	39.9 (+0.3)	50.4 (+1.7)
5	d(GCA TAT CAC)	61.8 (+2.8)[d]	
6	d(GCA UAU CAC)	54.2 (+1.9)[e]	
7	PS-d(GCA TAT CAC)[f]	38.0 (+0.1)	47.5 (+1.4)

[a]Tm values were measured in 50 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl and 1 mM EDTA at a total strand concentration 4 μM. [b]Complementary DNA: 3′-d(CGT ATA GTG). [c]Complementary RNA: 3′-r(CGU AUA GUG). [d]Complementary strand 3′-d(CGT ATA GTG). [e]Complementary strand 3′-d(CGU AUA GUG). [f]Fully phosphorothioate.

Relative to natural oligomers, which form more stable DNA/DNA or RNA/RNA duplexes than hybrid DNA/RNA (entries 1 and 2), the modified oligonucleotides hybridized better with complementary RNA strands (entries 3, 4, and 7). These results correlate with the preferential RNA-type conformation of the sugar moieties in 4′-methoxy dNs. The thymine-containing oligomers (entries 3 and 5) exhibited a higher thermal stability than the uracil congeners (entries 4 and 6). The highest thermal stability was observed when both complementary strands were modified and contained thymine nucleobases (entry 5). The combination of fully 4′-methoxy and phosphorothioate modifications (entry 7) led to a significant decrease in duplex stability compared to DNA, with a T_m value comparable to the natural 9-mer (entry 7 vs entry 1). The duplex stabilization compared to RNA decreased only slightly and remained similar to that for the 4′-MONs with a phosphodiester backbone (entry 7 vs entries 3 and 4).

Fully phosphodiester 9-mer 5′-d(GTG ATA TGC) exhibited exceptional stability against cleavage by nucleases. The experiments were carried out under conditions in which the half-time cleavage of the natural DNA counterpart was less than 1 min. Complete stability was observed in the presence of a 10,000 g rat liver homogenate, phosphodiesterase II (EC 3.1.16.1), nuclease P1 (EC 3.1.3.16), and micrococcal nuclease (EC 3.1.31.1) during a 2 h incubation. Considerable stability (half-time cleavage of approximately 40 min) against cleavage was observed for phosphodiesterase I (EC 3.1.4.1).

In summary, we have developed a simple, *β*-D-*erythro*-preferential synthetic procedure for the previously unavailable

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purine 4′-methoxy dNs, and this method is fully applicable to the pyrimidine dNs series. The preferred C3′-*endo* conformation of the *β*-D-*erythro* epimers was confirmed by NMR (>90% for **13a**, **13b**, and **13c**). Fully modified oligonucleotides were synthesized on solid phase and exhibited superior chemical and nuclease stabilities. Moreover, their excellent hybridization properties with a remarkable tendency toward RNA-selective hybridization suggest the potential application of the 4′-MONs for the construction of AOs. In addition, we have demonstrated the general applicability of this synthetic procedure for 4′-alkoxy dNs. The use of a variety of 4′-alkoxy substituents in AOs is currently under investigation, and the results will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

Full experimental details, ^{1}H, ^{13}C, and ^{31}P NMR spectra of all new compounds, HPLC charts and MALDI-TOF-MS spectra of new oligonucleotides, and representative UV melting and nuclease stability data. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01430.

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: petrova@uochb.cas.cz.
*E-mail: rosenberg@uochb.cas.cz.

Notes

The authors declare no competing financial interest.

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(15) *Representative procedure (synthesis of compound* **9*β*a**): To a stirred solution of **5a** (1.60 g, 2.5 mmol) in absolute MeOH (25 mL) at −20 °C under argon, light-protected NIS was added (731 mg, 3.25 mml, 1.25 equiv.), and the mixture was allowed to stand as the temperature gradually increased to rt. After 2 h, the mixture was concentrated, and the residue was dissolved in DMF (25 mL). Upon cooling in an ice bath, 2 M TEAB was added (2.5 mL, 5 mmol, 2 equiv.). The mixture was stirred at rt overnight, concentrated and dissolved in DMF (25 mL). Absolute MeOH was added (1 mL), the mixture was cooled in an ice bath, and $NaBH_4$ was added in portions (315 mg, 7.25 mmol, 3.3 equiv. in total). The mixture was maintained at a low temperature overnight before being diluted with $CHCl_3$ and washed with brine. The dried organic layer was concentrated, and the residue was purified by flash chromatography ($CHCl_3$-10% EtOH) to afford *β*-D-*erythro* **9*β*a** (1.18 g, 1.90 mmol, 76%) as a white foam.

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