Pergamon

Tetrahedron Letters 42 (2001) 8777–8780

TETRAHEDRON LETTERS

2′-*O*-Carbamate-containing oligonucleotides: synthesis and properties

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Received 23 August 2001; revised 9 October 2001; accepted 10 October 2001

Abstract—In order to evaluate the effect of a new 2′-carbohydrate modification on the hybridization properties of oligonucleotides, uridine 2′-*O*-carbamates were synthesized and incorporated into DNA strands. The key intermediate in the synthesis, a mixed succinimide carbonate **2**, was treated with various amines to give 2′-*O*-carbamates **3**. Thermal melting studies of modified oligonucleotides revealed that the presence of the 2′-*O*-carbamate modification significantly destabilized DNA/RNA duplexes. A molecular-modeling study indicated that unfavorable steric interactions between the hydrogen of the NH group from the carbamate substituent and the anomeric hydrogen of the sugar residue on the same strand of the duplex may be the contributing factor causing destabilization.

Introducing different functional groups at the 2′-position of antisense oligonucleotides gives rise to a class of compounds collectively known as 'RNA mimetics'.[1,2] These modifications usually result in a C3′-*endo* sugar conformation leading to favorable binding affinity for the target RNA, improved nuclease resistance, altered pharmacokinetics, and increased chemical stability during oligonucleotide synthesis.[1–3] In addition, these modified oligonucleotides have improved oral absorption properties relative to phosphorothioate DNA as there is little possibility of depurination in the acidic pH of the gut. Some RNA mimics with these desirable properties are 2′-deoxy-2′-fluoro (2′-F),[4] 2′-*O*-(2-methoxyethyl) (2′-*O*-MOE),[5,6] 2′-*O*-(3-aminopropyl) (2′-*O*-AP)[7,8] and 2′-*O*-[2-(*N*,*N*-dimethylaminooxy)ethyl] (2′-*O*-DMAOE)[9] modified oligonucleotides (Fig. 1).

However, not all 2′-modifications stabilize hybrid duplex formation. Numerous destabilizing 2′-modifications have also been reported in the literature.[10] In such cases, enhanced nuclease resistance and lipophilicity, for example, may make these modifications useful. Herdewijn and his group have shown that the incorporation of 2′-acetamido-2′-deoxynucleosides (Fig. 1) in RNA and DNA strands destabilized RNA–RNA, DNA–DNA and mixed duplexes.[11] Polushin introduced various 2′-methoxyoxalamido functionalities (Fig. 1) on the 2′-amino substituent; however, no binding affinity data is available for this class of compounds.[12]

The 2′-*O*-carbamate modification (Fig. 2) is a simple, chemically feasible modification at the 2′-position of the sugar, which can be achieved via a 2′-*O*-carbonate intermediate. Various 2′-*O*-carbamates can be synthesized from a single precursor by using different amines. In addition, if a substituent with a free amino terminal group is used, further 2′-conjugation can be carried out. Although carbamate-containing backbone linkages[13] and solid supports are known,[14] sugar modifications are not.[15]

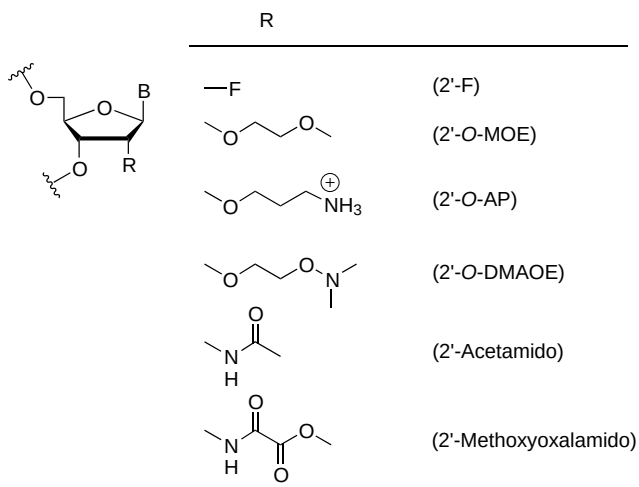

Figure 1.

Keywords: carbonates; carbamates; nucleosides; antisense; oligonucleotides.

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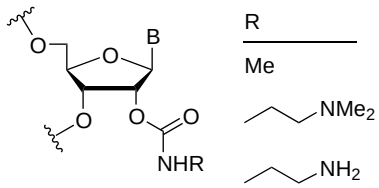
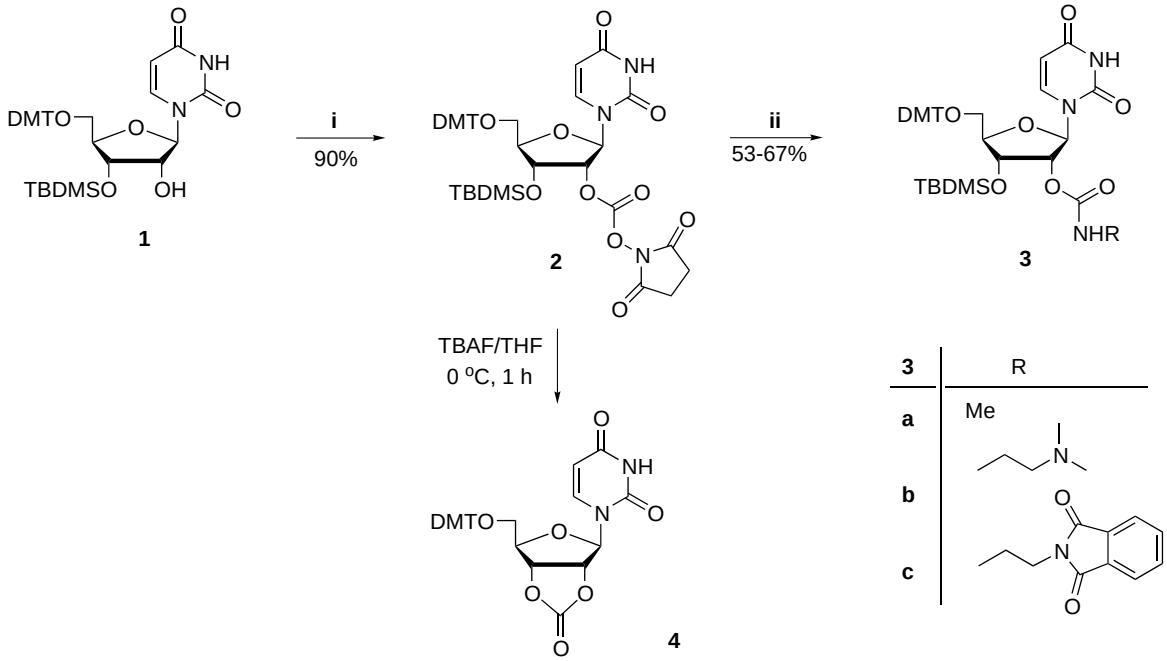


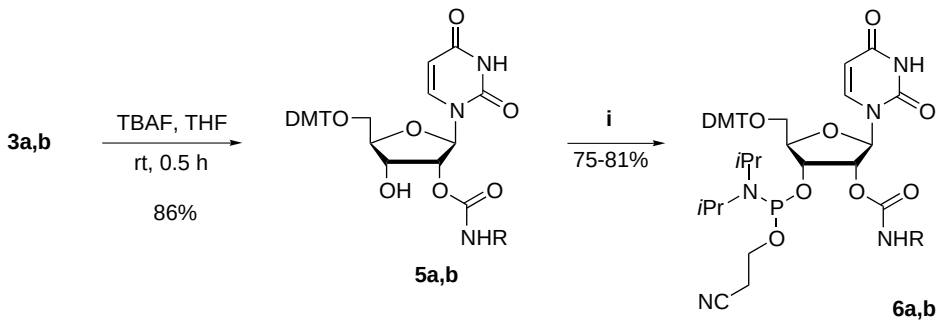
Figure 2.

The synthesis of uridinyl-2′-carbamate is summarized in Scheme 1. 5′-*O*-Dimethoxytrityl-3′-*O*-*tert*-butyldimethylsilyluridine (**1**) was transformed into the 2′-*O*-carbonate **2** with di-(*N*-succinimidyl)carbonate (DSC) and Et_3N according to the procedure by Ghosh et al.[16,17] The carbonate **2** was further treated with an amine to give the 2′-*O*-carbamate **3** in good yield. If the carbonate **2** is treated with TBAF, the cyclic carbonate **4** is formed. A short treatment of **3** with TBAF in THF at room temperature afforded the 3′-*O*-deprotected nucleoside **5**, which was further phosphitylated into the phosphoramidite **6** using conventional procedures (Scheme 2). Compound **6** was used in oligonucleotide synthesis in an automated DNA synthesizer at 0.5 M concentration in MeCN under standard conditions. Oligonucleotides were cleaved from solid support and deprotected after an overnight treatment in 30% aqueous ammonia at 55°C. They were then purified by RP HPLC, followed by size-exclusion chromatography, and were analyzed by HPLC, CE and ESMS. The properties of the different oligomers synthesized, all of which had phosphodiester backbones, are summarized in Table 1.

Helix to coil transition melting temperatures were measured for duplexes formed with a complementary RNA oligonucleotide (Table 1). In all cases, a substantial decrease in T_m was observed in comparison to unmodified oligonucleotides indicating that carbamate modifications lead to a destabilization of the duplex. Possible reasons for the loss of affinity are lack of rotational freedom of the carbamate substituent due to C-sp^2 hybridization, van der Waals repulsion (Fig. 3) and steric bulk due to the carbamate group.



Scheme 1. (i) Disuccinimidyl carbonate/Et_3N/MeCN/rt/5 h; (ii) RNH_2/Et_3N/MeCN/rt/1 h.



Scheme 2. (i) 2-Cyanoethyl *N*,*N*-bisdiisopropylaminophosphorodiamidite/1*H*-tetrazole/iPr_2NH/CH_2Cl_2/rt/16 h.

Table 1. Properties of 2′-*O*-carbamate-containing oligonucleotides

Oligo	Sequence (5′→3′)	Modif. U[a]	Retention time (min)[b]	ESMS calcd	ESMS found	T_m (°C)	T_m (°C) (unmodif.)[c,d]	ΔT_m/mod. (°C)
I	CTC GTA CCU TTC CGG TCC	Ua	18.68	5437.3	5435.7	58.3	64.0	−5.76
II	UCC AGG UGU CCG CAU C	Ua	17.47	5069.9	5070.4	51.3	60.6	−2.34
III	CTC GTA CUU UUC CGG TCC	Ua	17.74	5629.3	5629.4	48.0	60.7	−3.17
IV	GCG UUU UUU UUU UGC G	Ua	18.67	5466.0	5465.9	NC[e]	50.2	
V	CTC GTA CCU TTC CGG TCC	Ub	18.66	5494.3	5495.3	58.3	64.0	−5.68

[a] Ua: 2′-*O*-(*N*-methylcarbamoyl)uridine, Ub: 2′-*O*-[*N*-(2-*N*,*N*-dimethylaminoethyl)carbamoyl]uridine.
[b] Column: C18 Waters Delta Pak (15 μm; 3.8×300 mm); buffer A: 50 mM triethylammonium acetate pH 7.0; buffer B: MeCN; a linear gradient from 5 to 60% B in 60 min.
[c] Melting temperature of the parent unmodified oligonucleotide (U = T).
[d] Ref. 10.
[e] Non-cooperative transition was observed.

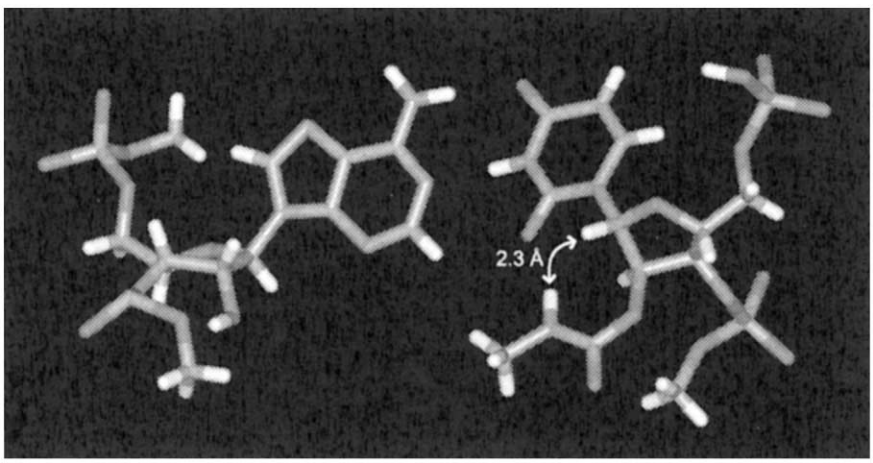


Figure 3. Energy minimized molecular-modeling diagram of 2′-*O*-(*N*-methylcarbamte) containing uridine (right) base paired with adenosine in the A form of an RNA/DNA duplex.

Molecular-modeling calculations were carried out to understand the molecular origins of the instability of an RNA:DNA duplex containing an RNA r(GCGAGCG) strand and DNA d(CGCUCGC) strand with and without the 2′-*O*-(*N*-methylcarbamate) (Ua) modification. The 7-mer RNA–DNA duplex was constructed in the A-form geometry and the energy was minimized (AMBER force filed, MSI software package) for comparison purposes with an RNA:DNA duplex containing the 2′-modified nucleotide. The modified nucleotide was incorporated at the center of the DNA strand and energy minimization was performed. Fig. 3 shows the base pair between the uridine with the carbamate modification and the adenine on the RNA strand. The model suggests that there is a steric clash between the anomeric hydrogen and the hydrogen of the NH group from the carbamate substituent (2.3 Å). Further calculations and structural studies will be required to dissect the roles of the various energetic contributions (hydration, dipole moment of the 2′-substituent, etc.) that give rise to the observed duplex instability.

It is interesting to note that while a single Ua modification in oligomer I leads to a destabilization of 5.8°C, multiple substitutions (as in the oligomers II and III) lead to less destabilization per modification. While we do not have any structural data to explain these differences, one possible explanation is that the introduction of ‘kinks’ at the junction point of the modification generates maximum steric perturbation with a single modification, and the helix accommodates such perturbations better with multiple modifications leading to less drastic changes. It is worth pointing out that most of the ‘destabilizing’ 2′-modifications (such as 2′-sulfur-

or 2′-α-alkyl-linked modifications) studied so far exhibit a similar trend.[10]

In summary, a simple chemical approach for the synthesis of 2′-*O*-carbamate-containing nucleosides and oligonucleotides is presented. The modified oligonucleotides destabilize the duplexes formed with the target RNA as compared to unmodified controls. Nevertheless, the modification is worth evaluating for the following reasons. The therapeutic properties of nucleosides with 2′-*O*-carbamates are not known yet. In addition, the 2′-*O*-carbamate chemistry may be used (with an intermediate such as compound **3c**) for further conjugation of ligands to oligonucleotides, either for enhancement of antisense properties or for use as diagnostic agents. Finally, placement of a 2′-*O*-carbamate moiety at the 5′- or 3′-terminus may not cause extensive destabilization due to the 'fraying' of the duplex, but might enhance nuclease resistance.

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