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An Expeditious Synthesis of 3'-α-Carboxymethyl-2'-O-Methyl Ribonucleosides

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Abstract: *3'-α-Carboxymethyl-2'-O-methyl ribonucleosides, which are important building blocks for the construction of high affinity amide modified antisense oligonucleotides, have been synthesized from the corresponding 2'-O-methyl ribonucleosides* via *catalytic hydrogenation of their 3'-deoxy 3'-methoxycarbonyl-methylidene derivatives. Reduction of the olefinic double bond proceeds in good to excellent yields and with α/β-product ratios > 9/1.*

The design and synthesis of modified nucleoside building blocks represents a fundamental prerequisite for the construction of nuclease resistant, biologically active antisense oligonucleotides.[1] One of the approaches that has been extensively pursued in this context is the alternating replacement of internucleoside phosphodiester groups by various types of uncharged 4-atom linkers (C(3')-X-Y-Z-CH_2-C(4')).[2] Efforts in our own laboratories have focused on amide-based phosphodiester replacements of type **I** (*Fig. 1*, X = CH_2, Y = C=O, Z = NH), which can significantly enhance the binding affinity of the corresponding oligonucleotide analogs for complementary RNA.[3] RNA-binding affinity strongly depends on the substitution pattern on the 2'-positions of the amide-modified dimer units and for those sequences investigated so far is highest, if both of these positions are substituted with 2'-O-methyl groups (*Fig. 1*, $R_1 = R_2 = OCH_3$).[3b]

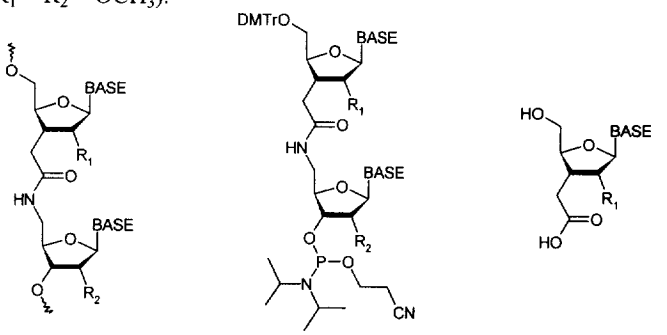

Figure 1 **I** **II** **III**

In the past, the incorporation of amide linkages of type **I** into oligonucleotide analogs has been based on the coupling of activated amide-modified dinucleotide analogs **II** as part of a standard oligonucleotide synthesis protocol.[3] More recently, we have also developed a truly *stepwise* solid-phase strategy for the synthesis of **I**-containing oligonucleotides that involves formation of the amide linkage on the solid support in the course of oligomer synthesis.[4] This latter approach reduces the number of building blocks required to access oligomers of any given base sequence from 16 (for the dimer approach) to eight (four acids and four amines) and thus represents a significant advance in the synthesis of amide-modified oligonucleotide analogs.[5]

An important prerequisite for the implementation of either one of the above strategies is the ready accessibility of appropriately protected and activated nucleoside-derived hydroxy acids of type **III**, either as precursors for dimer synthesis or directly for chain-elongation on the solid support. Regarding doubly 2'-O-methyl substituted modifi-

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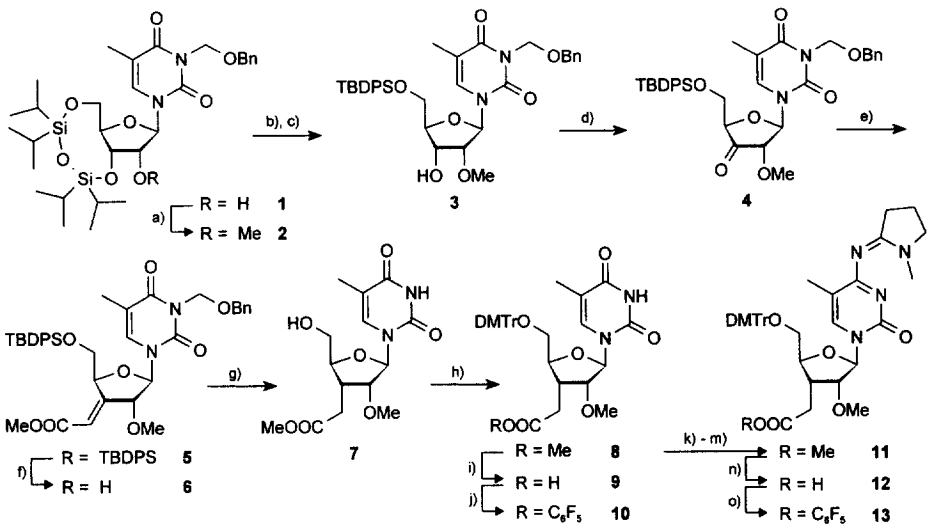
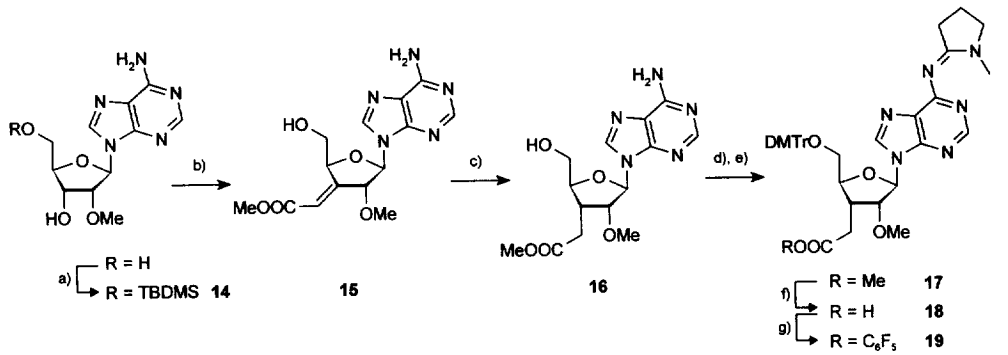


Scheme 1. a) 10 eq MeI, 1.2 eq NaH, DMF, 0°, 2 h. b) 2.5 eq Bu_4NF, THF, 0°, 3 h, 71% (2 steps). c) 1.1 eq TBDPS-Cl, 0.01 eq DMAP, pyridine, 50°, 24 h, 79%. d) 4 eq *Dess-Martin* periodinane, CH_2Cl_2, rt, 30 h, 99%. e) 1.2 eq Ph_3P=CHCOOMe, THF, 65°, 14 h, 70%. f) conc. HCl (aq)/MeOH (1/25), 50°, 36 h, 69%. g) 0.1 eq Pd-C (10%), H_2 (1 atm), MeOH, rt, 24 h, 98%. h) 1.3 eq DMTr-Cl, pyridine, rt, 15 h, 95%. i) 10 eq LiOH, MeOH/H_2O (8/1), rt, 6 h, quant. j) 1.2 eq $CF_3COOC_6F_5$, 1.5 eq pyridine, DMF, rt, 1 h, 79%. k) 2.5 eq $POCl_3$, 22 eq NEt_3, 20 eq 1,2,4-triazole, acetonitrile, 0° → rt, 1 h, 92%. l) 25% NH_4OH (aq)/dioxane (4/5), rt, 3 h, quant. m) 6 eq *N*-methyl pyrrolidine *O,O*-dimethyl acetal, 6 eq pyridine, MeOH, rt, 3 h, 88%. n) 15 eq LiOH, MeOH/H_2O (8/1), rt, 4 h, 71%. o) 1.2 eq $CF_3COOC_6F_5$, 1.5 eq pyridine, DMF, rt, 1 h, 85%.

cations of type **I** ($R_1 = R_2 = OCH_3$), only the 16-step synthesis of the corresponding "T-acid" has been described in the literature so far, which is based on the stereoselective radical allylation of 2,5-anhydro ribothymidine as the key step.[3b] Direct radical allylation of 2'-O-methyl ribothymidine on C-3', however, is hampered by the unsatisfactory selectivity observed in the radical addition step.[6] We have, therefore, sought alternative approaches to the synthesis of 2'-O-methyl substituted building blocks of type **III** that would (i) provide for improved stereoselectivity in the C(3')-CH_2 bond-forming step and (ii) would also involve direct extension at C(3') by the desired 2-carbon unit, rather than attachment of a 3-carbon unit and subsequent oxidative degradation. In this paper we now report on an efficient new strategy for the synthesis of all four base derivatives of modified nucleosides **III** ($R = OCH_3$), which is based on the stereoselective reduction of an α,β-unsaturated ester on C(3') as the key transformation[7] and meets both of the above criteria.

The syntheses of thymidine and 5-methyl cytidine derivatives of type **III** are outlined in *Scheme 1*. Benzyloxymethyl (BOM) protection of the thymine moiety in 1,1,3,3-tetra-isopropyldisiloxane (TIPDS)-protected 5-methyl uridine (ribothymidine)[8] initially led to **1**, which was converted to its 2'-O-methyl derivative **2** by treatment with methyl iodide and sodium hydride in N,N-dimethylformamide (DMF) at 0 °C. TIPDS-cleavage and selective O(5')-silylation with TBDPS-Cl afforded **3**, which was cleanly oxidized to the C(3')-keto derivative **4** by the use of 4 equivalents of 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one (*Dess-Martin* periodinane)[9] in near quantitative yield. Wittig reaction with methyl (triphenyl-phosphoranylidene)-acetate afforded **5** as a single double bond isomer with the olefin geometry as shown.[10]

Desilylation of the highly base sensitive **5** proved to be unexpectedly difficult and was only achieved under rather harsh acidic conditions (conc. HCl/MeOH, 50°). Remarkably, these forcing conditions did not induce double bond isomerization or elimination of the heterocyclic base (followed by aromatization to the corresponding furan), both of which were observed upon treatment of **5** with Bu_4NF or HF-pyridine. Hydrogenation of the double bond in **6** with concomitant removal of the BOM protecting group (Pd/C in MeOH) afforded **7** stereoselectively in 98% isolated yield.[11] It is noteworthy that **6** did not act as a substrate for directed homogeneous hydrogenation with either $[Ir(coc)py(PCy_3)]PF_6$ or $[Rh(nbd)(di\text{-}phos\text{-}4)]BF_4$.[12] Dimethoxytritylation of **7** led to **8**, which underwent

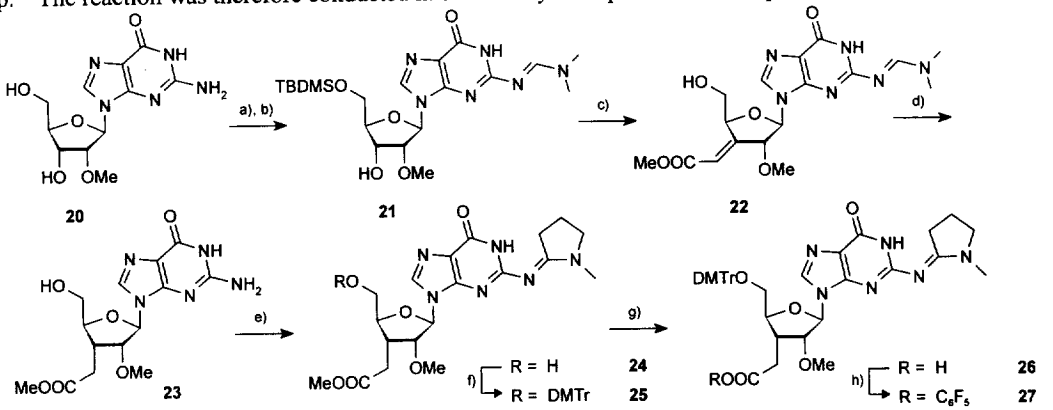


Scheme 2. a) 1.1 eq TBDMS-Cl, 1.1 eq NEt_3, 0.05 eq DMAP, DMF/CH_2Cl_2 (1/1), rt, 8 h, 89%. b) i) 3 eq CrO_3, 6 eq pyridine, 3 eq Ac_2O, CH_2Cl_2, rt, then toluene (excess); ii) 2 eq Ph_3P=CHCOOMe, toluene, 0° → rt; iii) 1 eq TBAF/2 eq AcOH, rt, 18h, 26 - 41%. (3 steps). c) Pd-C (10%), H_2 (3 atm), MeOH/THF (2/1), rt, 100 h, 75% (cryst.). d) 3 eq N-methyl pyrrolidine *O,O*-dimethyl acetal, MeOH, rt, 4h, quant. e) 1.1 eq DMTr-Cl, 1.3 eq NEt_3, cat. DMAP, pyridine, 0°, 4 h, 86%. f) 1N NaOH (2 eq), $MeOH/H_2O$ (8/1), rt, 7 h, 85%. g) 1.2 eq $CF_3COOC_6F_5$, 1.5 eq pyridine, DMF, rt, 1 h, 62%.

smooth lithium hydroxide-mediated methyl ester hydrolysis to **9**. Acid **9** could be converted to its pentafluorophenyl ester **10** in 85% yield by reaction with pentafluorophenyl trifluoroacetate.[13, 14] The synthesis of 5-methyl cytidine derivative **13** (*Scheme 1*) was performed in high yield according to standard procedures.[14, 15]

The preparation of 2'-O-methyl adenosine derived α,β-unsaturated ester **15** (*Scheme 2*) was achieved most efficiently, if the corresponding C(3') ketone precursor (accessed by oxidation of **14** with CrO_3/Pyridine/Ac_2O 1/2/1[16]), was not isolated, but was reacted *in situ* with methyl (triphenyl-phosphoranylidene)-acetate. The resulting olefin was isolated as a single double bond stereoisomer,[10] and removal of the O(5')-TBDMS protecting group with TBAF/AcOH[17] led to **15.** Hydrogenation of **15** over Pd/C furnished saturated ester **16** in 75% yield as a single diastereoisomer after recrystallization. Base protection and subsequent dimethoxytritylation gave **17** in 86% yield, which could be converted to the free carboxylic acid **18** by treatment with aqueous NaOH/MeOH without cleavage of the base protecting group. Reaction of **18** with pentafluorophenyl trifluoroacetate[13] provided pentafluorophenyl ester **19**[14] in 87 % yield.

In contrast to adenosine derivative **15**, complete hydrogenation of the C=C double bond in guanosine derivative **22** (*Scheme 3*) was uniformly accompanied by partial partial loss of the dimethyl-formamidine base protecting group.[18] The reaction was therefore conducted in such a way as to provide base-unprotected saturated ester **23**,



Scheme 3. a) 3 eq $(CH_3)_2NCH(OCH_3)_2$, MeOH, rt, 18 h, 98%. b) 1.1 eq TBDMS-Cl, 1.1 eq NEt_3, 0.05 eq DMAP, DMF/CH_2Cl_2 (1/1), rt, 18 h, 82% (cryst.). c) i) 3 eq CrO_3, 6 eq pyridine, 3 eq Ac_2O, CH_2Cl_2, then toluene (excess); ii) 3 eq Ph_3P=CHCOOMe, 10 °C, 3.5 h; iii) 1.1 eq TBAF/AcOH (2/1), THF, rt, 5 h, 24% (3 steps). d) Pd-C (10%), H_2 (2 atm), $MeOH/H_2O$ (10/1), 50 °C, 5 d, 74% (cryst.). e) 3 eq N-methyl pyrrolidine O,O-diethyl acetal, EtOH, rt, 4h, 79%. f) 2 eq DMTr-Cl, pyridine, rt, 18 h, 84%. g) 1N LiOH (3 eq), THF/H_2O (8/1), rt, 7 h, 96%. h) 1.2 eq $CF_3COOC_6F_5$, 1.5 eq pyridine, DMF, rt, 1 h, 87%.

which was obtained in 74% yield as a single diastereoisomer. Reprotection of the base by reaction with *N*-methyl pyrrolidine *O,O*-diethyl acetal[18] was followed by O(5')-dimethoxytritylation, LiOH-induced methyl ester cleavage and activation to the pentafluorophenyl ester **27**[14] in good overall yield.

Pentafluorophenyl esters **10, 13, 19** and **27** have been successfully employed for the stepwise solid-phase synthesis of backbone-modified oligonucleotide analogs of type **II** (*Fig. 1*). The synthesis of these oligonucleotides and their RNA binding properties will be the subject of future reports.[4]

References and Notes

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5. Throughout this paper, the term "amide-modified oligonucleotide" refers to modified analogs with a *mixed* amide-phosphodiester backbone with no two contiguous modifications. Fully modified analogs in this context would thus be characterized by an alternating arrangement of amide and phosphodiester linkages (*cf.* ref. 3b).
6. A α/β product ratio of ≈ 4/1 is observed for the radical allylation of 2'-O-methyl-5'-O-TBDPSi-3'-O-(p-methyl phenoxythiocarbonyl) thymidine with $Bu_3SnCH_2CH{=}CH_2$/AIBN: von Matt, P.; De Mesmaeker, A., unpublished.
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10. This is contrary to a recent report by Lee & Wiemer, who have investigated the reaction of 3'-keto 2',5'-O-bis-TBDMS adenosine with methoxycarbonyl phosphorane and have observed selective formation of the opposite double bond isomer: Lee, K.; Wiemer, D. F. *J. Org. Chem.* **1993,** *58*, 7808 - 7812.
11. As hydrogenation of the olefinic double bond in **5** using Pd/C proved to be poorly reproducible, we focused our atttention on the reduction of the corresponding homoallylic free alcohol **6**.
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14. **10**: ^{1}H-NMR (400 MHz, $CDCl_3$): 8.45 (s, 1H, NH); 7.74 (s, 1H, H-6); 7.42 (m, 2H, DMTr); 7.31 (m, 7H, DMTr); 6.84 (m, 4H, DMTr); 5.95 (s, 1H, H-1'); 4.09 (ddd, 1H, H-4'); 4.00 (dd, 1H, H-2'); 3.79 (s, 6H, DMTr); 3.73 (dd, 1H, H-5'); 3.55 (s, 3H, 2'- OCH_3); 3.25 (dd, 1H, H-5'); 2.90 (dd, 1H, C(3')-CH_2 and m, 1H, H-3'); 2.38 (dd, 1H, 1H, C(3')-CH_2); 1.46 (s, 3H, C(5)-CH_3).5-Me-C(NMPA) **13**: ^{1}H-NMR (400 MHz, $CDCl_3$): 7.82 (s, 1H, H-6); 7.46 (d, 2H, DMTr); 7.40 - 7.21 (m, 7H, DMTr); 6.85 (m, 4H, DMTr); 6.08 (s, 1H, H-1'); 4.11 (ddd, 1H, H-4'); 4.00 (dd, 1H, H-2'); 3.80 (s, 6H, DMTr); 3.70 (dd, 1H, H-5'); 3.65 (s, 3H, 2'-OCH_3); 3.44 (ddd, 2H, NMPA); 3.25 (dd, 1H, H-5' and ddd, 1H, NMPA); 3.12 (ddd, 1H, NMPA); 3.04 (s, 3H, N-CH_3); 2.89 (dd, 1H, C(3')-CH_2); 2.81 (m, 1H, H-3'); 2.35 (dd, 1H, C(3')-CH_2); 2.05 (ddd, 2H, NMPA); 1.62 (s, 3H, C(5)-CH_3). **19**: ^{1}H-NMR (400 MHz, $CDCl_3$): 8.53 (s, 1H, H-2); 8.18 (s, 1H, H-8); 7.43 (d, 2H, DMTr); 7.30 (m, 7H, DMTr); 6.81 (d, 4H, DMTr); 6.25 (s, 1H, H-1'); 4.30 (d, 1H, H-2'); 4.17 (m, 1H, H-4'); 3.79 (s, 6H, DMTr); 3.58 (s, 4H, 2'-OCH_3 and m, H-5'); 3.49 (t, 2H, NMPA); 3.38 (dd, 1H, H-5'); 3.15 (s, 3H, NMPA); 3.05 - 2.09 (m, 4H, H-3' + NMPA + C(3')-CH_2); 2.53 (dd, 1H, C(3')-CH_2); 2.05 (m, 2H, NMPA). **27**: ^{1}H-NMR (400 MHz, $CDCl_3$): 8.86 (s, br, 1H, NH); 7.82 (s, 1H, H-8); 7.342 (d, 2H, DMTr); 7.30 (m, 7H, DMTr); 6.82 (d, 4H, DMTr); 6.05 (s, 1H, H-1'); 4.21 (d, 1H, H-2'); 4.09 (m, 1H, H-4'); 3.79 (s, 6H, DMTr); 3.52 (dd, 1H, H-5'); 3.47 (m, 2H, NMPA + s, 3H, 2'-OCH_3); 3.38 (dd, 1H, H-5'); 3.14 (m, 2H, NMPA); 3.04 (s, 3H, NMPA); 2.90(m, 2H, H-3' + C(3')-CH_2); 2.50 (dd, 1H, C(3')-CH_2); 2.05 (m, 2H, NMPA).
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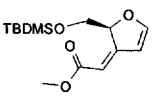
17. Attempted cleavage of the O(5')-TBDMS group with TBAF in the absence of AcOH led to exclusive formation of the elimination product **15a** and adenine:



15a

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