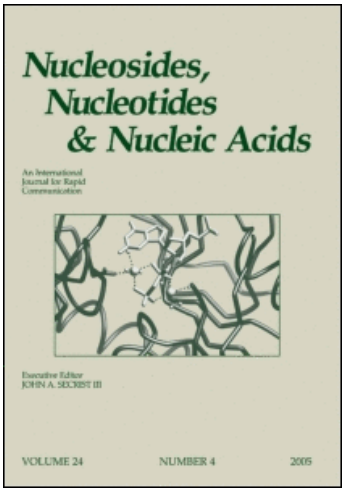

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SYNTHESIS OF A 2′-*O*-(CARBOMOYLMETHYL)RIBONUCLEOSIDE H-PHOSPHONATE BUILDING BLOCK AND A MODEL DINUCLEOTIDE

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□ *In order to obtain higher quality 2′-O-carbamoylmethyl oligoribonucleotides we are conducting studies of this modification. Here we present synthesis of 2′-O-carbamoylmethyl containing H-phosphonate building blocks as well as synthesis of model dinucleotides needed for these studies.*

Keywords Carbamoylmethyl; modified RNA; 2′-*O*-alkyl; H-phosphonate; dinucleotide

Several 2′-modifications of DNA oligonucleotides have been shown to increase the DNA:RNA duplex stability.[1,2] The 2′-*O*-carbamoylmethyl modification provide a small electronegative hydrophilic group that increases the duplex stability significantly.[3] In the first published study 2′-*O*-carbamoylmethyl oligonucleotides were synthesized from 2′-*O*-cyanomethyl oligonucleotides by hydrolysis. In order to explore the potential of this modification further we decided to develop methods for synthesis of 2′-*O*-carbamoyl building blocks, and to make dimer models for studies on the chemical stability and potential side-reactions of this modification.

The first attempt to get the desired 2′-*O*-carbamoylmethyl-adenosine was through alkylation of unprotected adenosine (**1**) with sodium hydride and iodoacetamide in DMF (Scheme 1). This yielded the product (**2**) as a 2′, 3′ isomeric mixture (in about 6/4 ratio). The yield and selectivity was, however, quite sensitive to moisture, amount of base, amount of alkyl halide and varied substantially. The product mixture of the 2′ and the 3′ isomers was more polar than adenosine and not readily separated. Isomer separation was therefore done at a later stage. Base protection (*N*-6-butyryl) of the isomeric mixture gave compound (**3**) and monomethoxytrityl protection of the 5′-OH gave compound (**4**). Finally phosphonylation with PCl_3/imidazole gave

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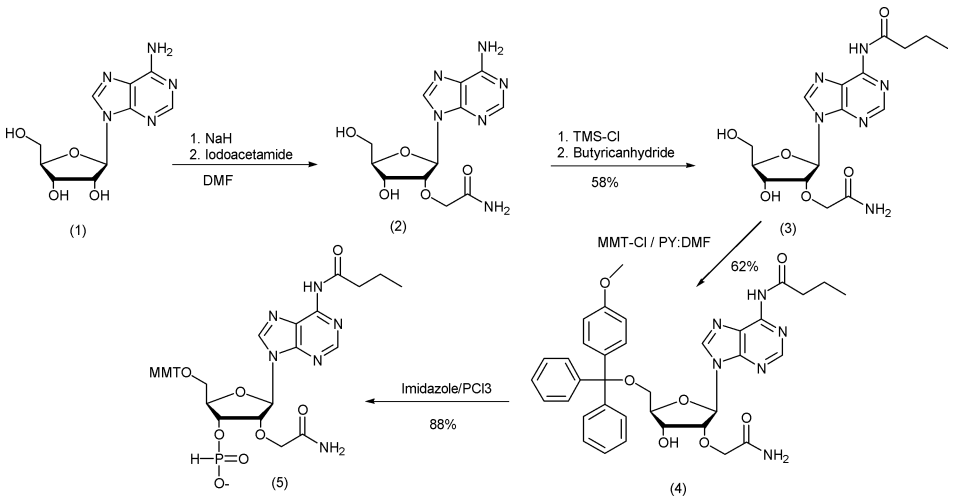
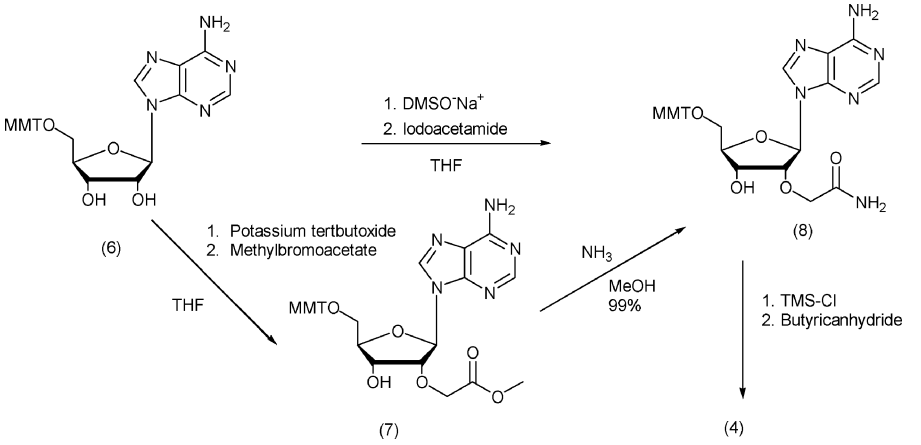


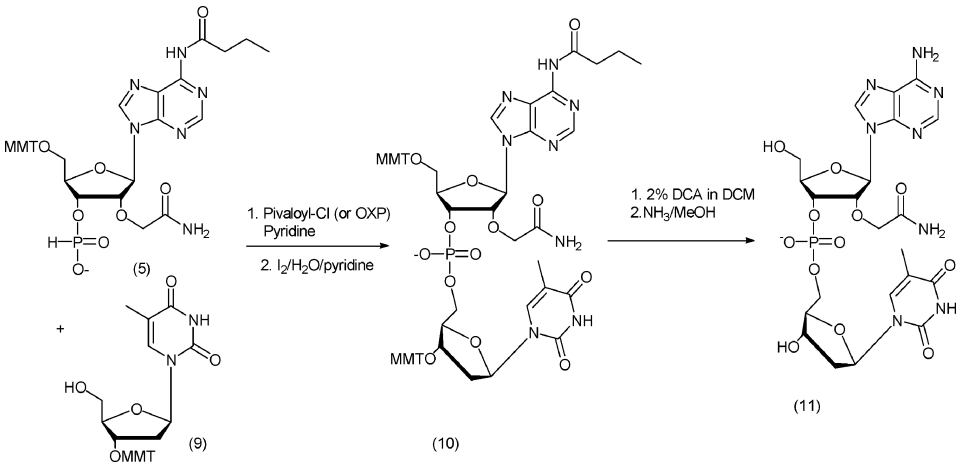
SCHEME 1 Route for the intial method for synthesis of the 2′-*O*-carbamoylmethyladenosine H-phosphonate building block **5**.

(**5**). The isomeric mixture of the 3′ and 2′-*O*-alkylated H-phosphonates, was separated by straight-phase HPLC.

In an alternative approach (Scheme 2), we started with 5′-*O*-monomethoxytrityladenosine **6** which was alkylated with iodoacetamide in THF using dimsylsodium as base. This gave the 2′ and 3′-*O*-carbamoylmethylated product with substantially higher selectivity for the 2′-alkylated product (2′:3′ ratio was about 9/1 as estimated from TLC) and although some unreacted material remained and some dialkylated product formed we achieved 39% isolated yield of (**8**) after chromatography. The same product was also produced in a one-pot procedure via the methyl



SCHEME 2 Improved synthesis route to the protected 2′-*O*-carbamoylmethyladenosine **4**.



SCHEME 3 Synthesis of the 2′-*O*-carbamoylmethyl containing ApT model dimer **11**.

ester (**7**) and subsequent ammonolysis to (**8**). Compound (**8**) was then separated from its isomers by column chromatography, base protected to give compound (**4**) and finally phosphonylated to (**5**).

In synthesis of the model ApT dimer (Scheme 3), (**5**) was first coupled to 3′-*O*-MMT-T (**9**) and the product was oxidized in situ to give the protected dimer (**10**). The coupling was performed using either pivaloyl chloride or bis(2-oxo-3-oxazolidinyl)phosphinic chloride (OXP), in both cases with virtually quantitative coupling. (**10**) was then detritylated and finally treated with NH_3 in methanol to give the desired model dimer (**11**). A simple method of synthesizing a 2′-*O*-carbamoylmethyladenosine H-phosphonate building block has been developed and a model dimer with carrying the 2′-*O*-carbamoylmethyl modification has been made. The nuclease resistance and chemical stablity of the dimer is under investigation.

EXPERIMENTAL

2′-*O*-Carbamoylmethyl-5′-*O*-Monomethoxytrityladenosine (8)

5′-*O*-monomethoxytrityl-adenosine (0.45 g, 0.83 mmol) was coevaporated with 30 ml dry MeCN and then 30 ml dry THF. The material was dissolved in distilled THF (50 ml) and flushed with dry N_2 gas. Dimsyl sodium (1.00 mmol, 0.5 ml) was slowly added to the mixture. The mixture was stirred for 30 minutes and then iodoacetamide (0.186 g, 1.00 mmol) was added. After 2 hours the solvent was evaporated and purified by column chromatography (CH_2Cl_2/MeOH 13:1 + 0.05% triethylamine). The yield of the 2′-*O*-alkylated isomer was 39% and the 3′ was 2%. ^{1}HNMR ($CDCl_3$): 3.35–3.55 (m, 2H, 5H′); 3.65 (s, 3H, O-Me trityl); 4.15 (d, 2H, CH_2-acetamid); 4.28 (d, 1H, 4H′); 4.50 (m, 1H, 3H′); 4.55 (m, 1H, 2H′); 6.18 (d, 1H, H1′);

6.80 (d, 2H, trityl); 7.15–7.40 (m, 12H, trityl); 8.00 (s, 1H, H2-base); 8.20 (s, 1H, H8-base). ES-TOF: m/z calculated for $C_{32}H_{32}N_6O_6$ $[M+H]^+$, 597.6; found, 597.4.

6-N-Butyryl-2′-*O*-Carbamoylmethyl-5′-*O*-Monomethoxytrityladenosine 3′-H-Phophonate Triethylammonium Salt (5)

Imidazole (0.761 g, 11.29 mmol) was suspended in (30 ml) dry $CH_2Cl_2$and cooled down to –100°C. PCl_3(0.505 g, 0.322 ml, 3.68 mmol) and distilled triethylamine (1.195 g, 1.64 ml, 11.81 mmol) was added dropwise under vigorous stirring. The mixture was stirred at –10°C for 30 minutes and then cooled down to −78°C. The protected nucleoside (0.70 g, 1.05 mmol) dissolved in CH_2Cl_2 (13 ml) was added during 30 minutes. The mixture was stirred at −78°C for 1 hour and extracted with triethylammoniun acetate pH 7.5 (2*30 ml). The organic layer was dried over $MgSO_4$ and evaporated. The crude product was purified by column chromatography on silica CH_2Cl_2/MeOH (6:1 + 0.5% triethylamine) that gave a total yield of 88%. ^{1}HNMR+COSY ($CDCl_3$):1.05–1.10 (t, 3H, CH_3-Bu); 1.75–1.85 (six, 2H, CH_2-Bu); 2.80–2.90 (t, 2H, CH_2-Bu); 3.40–3,60 (m, 2H, H5′); 4.78 (s, 3H, CH_3-O-trityl); 4.10 (d, 2H, CH_2-acetamid); 4.40 (d, 1H, H4′); 4.80 (t, 1H, H3′); 5.05–5.10 (m, 1H, H2′); 6.26 (d, 1H, H1′); 8.15 (s, 1H, H2); 8.60 (s, 1H, H8); 6.15 and 7.75 (two singlets, P-H). ES-TOF: m/z calculated for $C_{36}H_{38}N_6O_9P$ $[M+H]^+$, 730.7; found, 730.8.

2′-*O*-Carbomoylmethyladenosine 3′-(Thymidine 5′-Phosphate) (11)

The protected dimer **10** was synthesized from **5** (.200 g, 0.236 mmol) and **9** (0.134 mg, 0.259 mmol) using a standard procedure involving coupling and in situ oxidation with iodine in pyridine/water.[5] After the final removal of solvent in this procedure, crude **10** was detritylated by dissolving it in 8 ml 1% trifluoroacetic acid (TFA) in dichloromethane (DCM). After 2 hours the solution was evaporated under reduced pressure and then coevaporated with MeOH (2 × 10 ml). The residue was then directly subjected to ammonolysis in sat. MeOH/ NH_3 (10 ml). After standing overnight the solution was concentrated and the residue was triturated repeatedly with diethylether an finally dried under reduce pressure to give 70 mg of the model dimer (**11**). The dimer was then additionally purified by reversed phase HPLC on a Reprosil C-18AQ column (10 μm, 250 × 22 mm) using a 45-minute linear gradient from 50 mM triethylammonum acetate (TEAA, *aq*, pH 6.5) to 50 mM TEAA in 50% CH_3CN and a flow rate of 10 ml/minute Retention time for **11** was 26.3 minute. Collected fractions were lyophilized. ^{1}H-NMR (assignments by COSY) (D_2O);1.60 (s, 3H, CH_3-Tym); 2.20 (m,

2H, H2′-tym); 3.70–3.90 (m, 2H, H5′-Ad); 3.90–4.10 (m, 2H, H4′-Ad and H4′-Tym); 4.20 (d, 2H, CH_2-carbomoyl); 4.40 (m, 2H, H3′-Ad and H3′-Tym); 4.70 (m, 1H, H2′-Ad); 6.10 (m, 2H, H1′-Ad and H1′-Tym); 7.50 (s, 1H, H5-Tym); 8.10 (s, 1H, H2-Ad); 8.25 (s, 1H, H8-Ad). ES-TOF: m/z calculated for $C_{22}H_{28}N_8O_{12}P$ [M], 627.1564; found, 627.1552.

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