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One Pot Synthesis of the Dinucleoside Phosphonate $GpCH_2U$

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Summary. The preparation of the dinucleoside phosphonate $GpCH_2U$, starting from the isosteric phosphonate analogue of uridine (**3**) and the guanosine derivative **5** is described.

Keywords. Dinucleoside phosphonate; Ribonuclease T1; Cocrystallization.

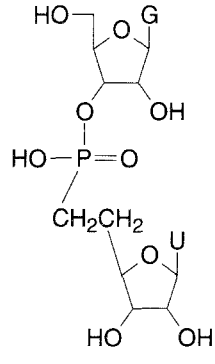
Eintopfsynthese des Dinucleosidphosphonats $GpCH_2U$

Zusammenfassung. Die Herstellung des Dinucleosidphosphonats $GpCH_2U$ aus dem isosteren Phosphonatanalogen von Uridin (**3**) und dem Guanosinderivat **5** wird beschrieben.

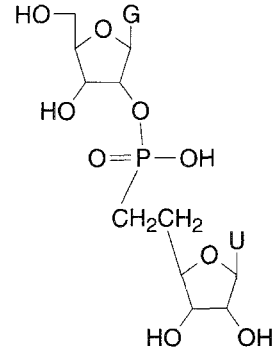
Introduction

Several years ago, *Moffatt* [1] and coworkers prepared the isosteric phosphonate analogues $UpCH_2U$ and $UpCH_2A$ in order to obtain substrate mimicking inhibitors for studying the mechanism of ribonuclease action. Here, the P–C bonds are expected to be inert towards enzymatic cleavage as opposed to the P–O bonds in natural DNA.

We have performed the synthesis of $GpCH_2U$ in the context of RNase T1 cocrystallization. Now, we report the one pot synthesis of $GpCH_2U$.



1
G = guanine



2
U = uracil

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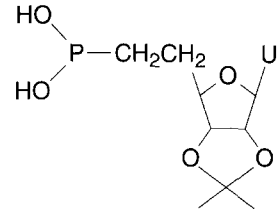
Results and Discussion

Initially, we attempted the synthesis of the title compound **1** using the phosphodiester method by coupling **3** [2–4] and derivative **4** [5] using *DCC* as coupling reagent in the presence of anhydrous pyridine, but no reaction occurred even after stirring for one week at room temperature under anhydrous conditions. Following this, we attempted to couple compounds **5** and **4** in dry pyridine in the presence of *MSNT*, but this was also unsuccessful. These failures were probably due to the steric hinderance in the region of the 3′ OH group of guanosine derivative **4**.



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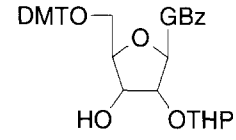

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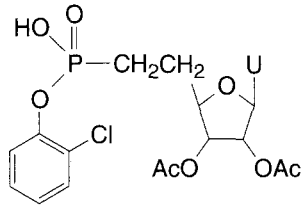
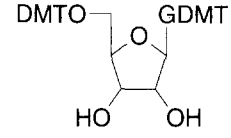


5



6

Consequently, we tried to run the reaction by coupling compound **3** with the guanosine derivative **6** [6] with the 2′ and 3′ hydroxyls free, using *DCC* as coupling reagent in the presence of dry pyridine and stirring under anhydrous conditions. The reaction was monitored by TLC and stopped after four days at room temperature by neutralization with tetraethylammonium bicarbonate (*TEAB*) solution and extraction with chloroform. The chloroform extract was evaporated to dryness and acidified with acetic acid. The two isomers **1** and **2** were separated using preparative HPLC [7], yielding 22% and 10% respectively.

Experimental

In a typical experiment, a solution of compound **3** (0.4 mmol) and 0.4 mmol of guanosine derivative **6** in anhydrous pyridine (5 ml) was treated with 4 equivalents of *DCC* and the reaction mixture was stirred under anhydrous conditions for four days at room temperature, followed by extraction with chloroform. The chloroform layer was evaporated to dryness and acidified with 50% acetic acid (10 ml). After complete cleavage of isopropylidene and *DMT* groups, the two isomers were separated by preparative HPLC (retention time of isomer **1**: 15.30 min; of isomer **2**: 14.92 min).

MS: $m/e = 588$ (M + 1), ^{1}H NMR: (D_2O, δ (ppm)): 8.09 (s, 1H, H-8), 7.73 (d, H-6, $J_{6,5} = 8.10$ Hz), 5.99 (d, 1H, H-1′$_G$, $J_{1',2'} = 5.70$ Hz), 5.91 (m, 2H, H-5 and H-1′$_U$), 4.85 (m, 1H, H-2′$_G$), 4.42 (m, 2H, H-2′$_U$ and H-3′$_G$), 4.10 (m, 1H, H-3′$_U$), 3.90 (m, 3H, H-4′$_G$, H-5′$_G$ and H-5″$_G$), 2.10–1.80 (m, 4H, H-5′, H-5″, H-6′, H-6″); ^{31}P NMR (D_2O, δ (ppm)): 29.91 (s). **2**: ^{1}H NMR (D_2O, δ (ppm)): 8.06 (s, H-8), 7.49 (d, 1H, H-6, $J_{6,5} = 8.10$ Hz), 6.05 (d, 1H, H-1′$_G$, $J_{1',2'} = 6.93$ Hz), 5.91 (d, 1H, H-5, $J_{5,6} = 8.10$ Hz), 5.77 (d, 1H, H-1′$_U$, $J_{1',2'} = 4.62$ Hz), 5.22 (m, 1H, H-2′$_G$), 4.50 (q, 1H, H-3′$_G$, $J_{3',4'} = 5.05$ Hz), 4.32 (m, 1H, H-4′$_G$), 4.21 (t,

1H, H-$2'_U$, $J_{2',3'} = 4.95$ Hz), 3.90 (m, 3H, H-$5'_G$, H-$5''_G$ and H-$3'_U$), 3.82 (m, 1H, H-$4'_U$), 1.71–1.31 (m, 4H, H-5′, H-5″, H-6′ and H-6″ of U); ^{31}P NMR (D_2O, δ (ppm)): 29.58 (s).

The conditions for HPLC were as follows: preparative C_{18} column VYDAC, packing 201 HS 1010, 25 cm length and 1 cm diametre. The system of elution was as illustrated in the following table (A = 0.1 *M* ammonium acetate, *pH* = 6; B = CH_3CN):

Time (min)	% A	% B
0	100	0
5	100	0
20	80.0	20
25	0.0	100

NMR spectra were recorded on a Bruker instrument at 250 MHz (^1H) and 32.37 MHz (^{31}P), *TMS* (internal) and 85% H_3PO_4 (external) were used as standards.

References

[1] Jones GH, Albrcht HP, Damodaran NP, Moffatt JG (1970) J Am Chem Soc **92**: 5510–5511
[2] Jones GH, Moffatt JG (1968) Am Chem Soc **90**: 5337–5338
[3] Albrecht HP, Jones GH, Moffatt JG (1984) Tetrahedron **40**: 79–85
[4] This compound was synthesised by the method of *Moffat* [2], but using the lithium salt of tetraethyl methylenediphosphonate instead of diphenyltriphenylphosphoranylidenemethyl phosphonate
[5] Haikal A (in press)
[6] Zegers I, Haikal A, Palmer R, Wyns L (1994) J Biol Chem **269**: 127–133

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