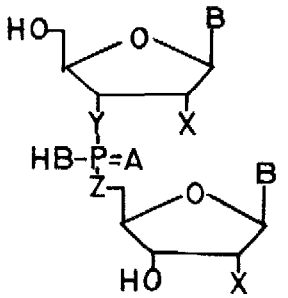
Tetrahedron Letters Vol. 21, pp 4149 - 4152 0040-4039/80/1022-4149$02.00/0

RIBONUCLEOTIDE ANALOGUES HAVING NOVEL INTERNUCLEOTIDE LINKAGES

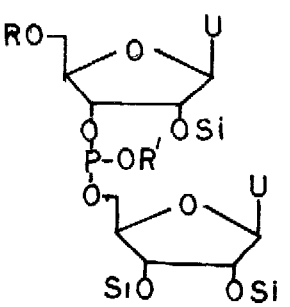
Mona J. Nemer and Kelvin K Ogilvie*
Department of Chemistry, McGill University, Montreal, Quebec, Canada H3A 2K6

The conversion of nucleoside phosphites into a number of novel nucleotide analogues is described

The synthesis of nucleotide analogues in which one or more of the internucleotide phosphate oxygens has been removed or replaced by another atom (eg N or S) has become of interest but has been limited to very few examples. The most common modification has been the replacement of a nucleoside-5' oxygen by a nitrogen (1, A=B=Y=O,Z=N,X=H or OH, 1-4) or a sulfur (1, A=B=Y=O,Z=S,X=H, 5,6). There have also been reports where the internucleotidic P=O has been replaced by P=S (1, Y=Z=B=O,A=S,X=H or OH, 7-9) Juodka (10) has reported on the attachment of amines, including amino acids, to the phosphate to form compounds of the type 1 (B=RN,A=Y=Z=O, X=OH). Some of these procedures have been difficult and involve low yields There are many more reports (see for example 11-17) on the modification of nucleoside monophosphates or cyclic phosphates. In this report, we wish to describe the synthesis of several new types of modified dinucleoside monophosphates by simple one-step and usually quantitative conversions of the easily prepared dinucleoside monophosphites.



1



2 a) R=Si, R'=TCE $SiU_P^{Si}(TCE)U_{Si}^{Si}$

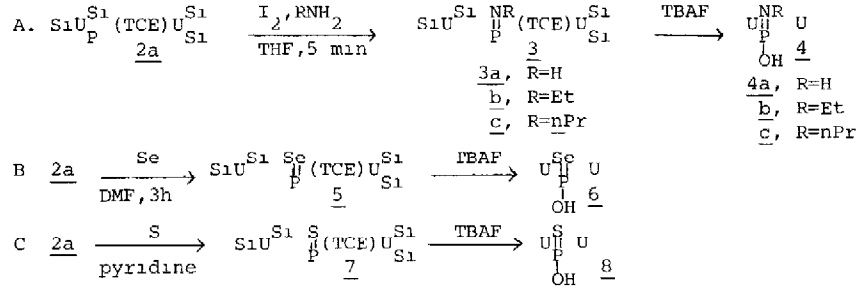
b) R=MMT, R'=TCE $MMTU_P^{Si}(TCE)U_{Si}^{Si}$

c) R=MMT, R'=CH_3 $MMTU_P^{Si}(CH_3)U_{Si}^{Si}$

The three dinucleoside monophosphites (2a-c) were prepared by the method described in the preceding article in this issue. All three compounds were characterized by iodine/water oxidation to the dinucleoside phosphate which was deprotected and in all cases gave UpU which was

completely degraded by snake venom, spleen and ribonuclease A enzyme

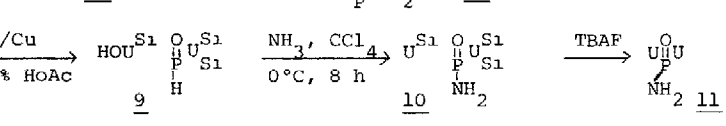
Compound 2a was subjected to the three different reaction conditions shown below (A - C)



The conversion of 2a to 8 employs the conditions previously described by Eckstein (7,8) for the synthesis of Ap(s)A except that in their procedures, the phosphite is not isolated prior to the thiation procedure. Reactions A and B describe the synthesis of completely new analogues of dinucleotides Reactions A are rapid and virtually quantitative The phosphite 2a is dissolved in THF (20 ml/mmole) and a solution containing iodine (200 mg/mmole) in 15 ml of THF RNH_2 (2 l) is added and the solution is stirred at room temperature for 5 min. Solvents are removed at reduced pressure and the residue dissolved in $CHCl_3$ which is washed with a dilute sodium bisulfite solution. Products are isolated in >90% yields by TLC. In the case of 2a where ammonia is used, the procedure involves bubbling NH_3 through a solution of 2a in THF and iodine for 10 min at 0°C.

For reaction B, to the nucleotide 2a dissolved in DMF (20 ml/mmole) selenium (200 mg/mmole) was added and the solution was stirred for 3 h at 20°C. The reaction mixture was centrifuged, solvents removed at reduced pressure, and products (5)isolated from TLC in >90% yields.

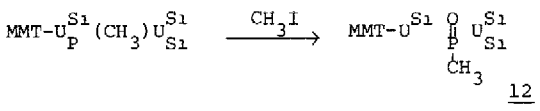
The phosphoramidate analogues 10 and 11 were obtained by the following sequence Compound 2b was first treated with Zn/Cu in DMF followed by 80% HoAc to produce 9 Compound 9 on treatment with ammonia in CCl_4 at 0°C for 8 h gave a quantitative conversion to 10. The silyl groups were removed from 10 with TBAF to give $U_p^O(NH_2)U$ (11) as the sole product



There are two major features to the procedures described here. Firstly, virtually quantitative yields of products are obtained in the synthetic steps and all the protecting groups can be removed in one step with TBAF. For example, 5 and 7 yield 6 and 8 respectively on treatment with TBAF for 30 min. A small amount of chain cleavage (<10%) is observed when neutral species such as 5 and 7 are treated directly with TBAF as reported for the normal phosphate triesters (18).

Finally, we wish to describe the conversion of 2c into compound 12. Compound 2c was stirred overnight with methyl iodide (10 ml/mmole) at 50°C Solvents were evaporated

to leave the product 12 (mp 118-122°C, $R_f^{Et_2O}$ 0.28 ($R_f^{Et_2O}$ of 2c is 0 37)) in quantitative yield



12

Compounds 4, 6, 8, and 11 were treated with spleen, snake venom and Ribonuclease A under conditions which completely degrade UpU. None of the compounds 4, 6, 8, and 11 were degraded by spleen. U(O)(NH$_2$)U was completely degraded by both snake venom and Ribonuclease A Compounds 4c, 6, and 8 all consist of two stereoisomers as determined by ^{31}P nmr In each case, one of the stereoisomers (high R_f isomer) is degraded by both snake venom and Ribonuclease A. The other isomer is unaffected This has previously been reported for the thiophosphate analogues(8,9) in which the R_p configuration has been assigned to the stereoisomer which is degraded by snake venom The current work is the first report of such observations with seleno and amino compounds (U(Se)(OH)U and U(NPr)(OH)U)

All of the phosphate derivatives were characterized by ^{31}P nmr as listed in the Table below

Table

Compound	^{31}P	Compound	^{31}P
SiU$_p^{Si}$(TCE)U$_{Si}^{Si}$(2a)	-136 7, -136 3	MMTU^{Si}(O)(CH$_3$)U$_{Si}^{Si}$(12)	-30.84, -29.95
SiU^{Si}(O)(TCE)U$_{Si}^{Si}$	+3.08, +3 50	U(O)(H)U	-6 85
SiU^{Si}(S)(TCE)U$_{Si}^{Si}$(7)	-62.2, -61 5	U(O)(OH)U	-1 60
SiU^{Si}(Se)(TCE)U$_{Si}^{Si}$(5)	-67.6, -67.0	U(S)(OH)U (8)	-57 17, -56.02
SiU^{Si}(NH)(TCE)U$_{Si}^{Si}$(3a)	+5 48, +5 90	U(Se)(OH)U (6)	-51 68, -50 90
SiU^{Si}(NEt)(TCE)U$_{Si}^{Si}$(3b)	-13.26, -13.72	U(O)(NH$_2$)U (11, 4a)	-0 50
SiU^{Si}(NPr)(TCE)U$_{Si}^{Si}$(3c)	-14 86, -14 08	U(NPr)(OH)U (4c)	-11 89, -11.48
MMTU$_p^{Si}$(CH$_3$)U$_{Si}^{Si}$ (2c)	-138.5, -138.1		
MMTU^{Si}(O)(CH$_3$)U$_{Si}^{Si}$	+4 57, +5.02		

Acknowledgement

We wish to acknowledge financial support from NSERCC, FCAC, and McGill University We wish particularly to acknowledge our debt to Elva Heyge for the ^{31}P spectra

References

1. B Jastorff and H Hettler, Chem Ber , 102, 4119 (1969).
2 R L. Letsinger and W S. Mungall, J Org Chem , 35, 3800 (1970).
3 R.L Letsinger and G A Heavner, Tetrahedron Lett , 147 (1975).
4. A.V Azhayev, A A. Krayevsky and J. Smrt, Coll. Czech Chem Comm , 44, 792 (1979)
5. A.F Cook, J Am Chem. Soc , 92, 190 (1970)
6. J. Kresse, K.L. Nagpal, J Nagyvary and J T. Uchic, Nucleic Acids Res , 2, 1 (1975)
7. F. Eckstein, Tetrahedron Lett , 1157 (1967)
8. P.M J Burgers and F Eckstein, ibid , 3835 (1978)
9 J.F Mallier and S.J Benkovic, ibid., 1121 (1980).
10. B.A Juodka and J Smrt, Coll. Czech Chem Comm , 39, 963 (1974) and references therein
11 M. Sekine and T Hafa, Tetrahedron Lett , 801 (1979)
12 G Baschang and V Kvita, Angew. Chemie int ed , 12, 70 (1973)
13 A. Simoncsits and J. Tomasz, Nucleic Acids Res., 2, 1223 (1975)
14 K C. Tsou and K F Yip, Life Sciences, 13, 1505 (1973)
15. S. Battka and J Tomasz, Tetrahedron Lett., 35, 2909 (1979)
16 R B Meyer, D A. Shuman and R K Robins, Tetrahedron Lett., 269 (1973)
17 G S. Bajwa and W.G Bentrude, ibid , 421 (1978).
18 K K. Ogilvie and S L Beaucage, Nuc. Acids Res. 7, 805-823 (1979)

(Received in USA 7 May 1980)