Tetrahedron Letters No. 32, pp 2779 - 2782, 1975. Pergamon Press. Printed in Great Britain.

2,2,2-TRIBROMOETHYL PHOSPHOROMORPHOLINOCHLORIDATE: A CONVENIENT REAGENT FOR THE SYNTHESIS OF RIBONUCLEOSIDE MONO-, DI- AND TRI-PHOSPHATES.

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(Received in UK 6 June 1975; accepted for publication 26 June 1975)

It is well recognized that ribonucleoside 5'-phosphoromorpholidates are of considerable importance as intermediates in the synthesis of ribonucleoside di- and tri-phosphates.[2] The only draw-back associated with the above method is that the synthesis of the morpholidates requires an easy availability or accessibility of the corresponding ribonucleoside 5'-phosphates. This limitation[3] will certainly be true for modified ribonucleotides.

We now wish to report that phosphorylation of some modified ribonucleosides of adenosine (IIa,b,c)with 2,2,2-tribromoethyl phosphoromorpholinochloridate I affords the phosphotriesters of ribonucleosides IIIa,b,c (R = $-CH_2CBr_3$), which after the selective removal of the 2,2,2-tribromoethyl protecting group, with Cu/Zn couple, give directly the ribonucleoside 5'- phosphoromorpholidates IIIa,b,c (R = H) in excellent yields.

The monofunctional reagent I can be easily prepared[4] from the reaction of 2,2,2-tribromoethyl phosphorodichloridate[5] and morpholine in dry ether. Work-up of the reaction product and recrystallization from cyclohexane/n-pentane gave the crystalline product[6], m.p. 79°, yield 80%.

The phosphotriester derivatives IIIa,b,c (R = $-CH_2CBr_3$) were prepared by allowing the modified nucleosides IIa,b,c (1 mmole) and I (2 mmole) to react together in anhydrous pyridine solution at 20°. Work-up of the products after 48 hr. and fractionation by Short Column Chromatography[7] gave IIIa,b,c which were isolated as homogeneous (t.l.c.) colourless solids in 75-80% yields.

N.m.r. spectroscopy of IIIa,b,c in dry $DMSO_{d6}$ showed, inter alia, two doublets in the ratio 1 : 1; however, addition of D_2O led to the complete disappearance of these two doublets. These findings are indicative for the presence of the two hydroxyl protons of the cis-diol system. Furthermore, treatment of nucleotides IIIa,b,c with Cu/Zn couple[8] in dry DMF during 10 min at 20° and filtration of the excess Cu/Zn gave the nucleoside phosphoromorpholidates IIIa,b, c (R = H), which after acidic deblocking (pH = 2, 0.01 N HCL) during 2 hr. at 20° followed by neutralization with aqueous ammonia (pH = 9) and purification by Sephadex G-25, afforded the nucleotides IVa,b,c in nearly quantitative yields.

The homogeneity and identity of these compounds was established by t.l.c. (MN-cellulose), high pressure liquid chromatography (HPLC) and n.m.r. spectroscopy.

The above results clearly show that the monofunctional phosphorylating agent I, apart from being an effective phosphorylating agent, has the added advantage of being a selective phosphorylating agent.

The availability of the phosphoromorpholidates IIIa,b,c allowed the synthesis of ribonucleoside di- and tri-phosphates to be undertaken.

As expected, the morpholidates were, without isolation, smoothly converted into the corresponding di- (Va,b,c) and tri-phosphates (VIa,b,c) by treatment with the mono- and bis(tri-n-butylammonium) salts of phosphoric and pyrophosphoric acid, respectively.

For example, N^6-(Δ^2-isopentenyl)adenosine 5'-triphosphate (VIa) was obtained when the morpholidate IIIa (0.1 mmole) in dry DMF (2 ml) was treated with bis(tri-n-butylammonium) pyrophosphate (0.5 mmole) in dry DMF (2 ml) at 20° for 3 hr. under the exclusion of moisture. The reaction product was concentrated under reduced pressure and, after treatment with Dowex 50 (ammonium form), purified by chromatography on DEAE cellulose (25 x 2 cm, column) using linear gradient elution (0.0 - 0.3 M) with Et_3NHCO_3 solution. The appropriate fractions were pooled and iPATP (VIa) was obtained in 82% yield.

N^6-Methyl- and N^6,N^6-dimethyladenosine 5'-triphosphates (VIb,c) were obtained similarly in 84% and 81% yields, respectively.

In the above experiments, when mono(tri-n-butylammonium)phosphate salt (0.6 mmole) in dry pyridine (4 ml) was used instead of the pyrophosphate in dry DMF, the corresponding modified nucleoside diphosphates (Va,b,c) could be easily isolated in 70%, 72% and 75% yields, respectively.

The purity and structure of the nucleoside di- and tri-phosphates were confirmed by t.l.c. (MN-cellulose), high-pressure liquid chromatography (HPLC), u.v. spectroscopy, ^1H- and ^{31}P n.m.r. spectroscopy.

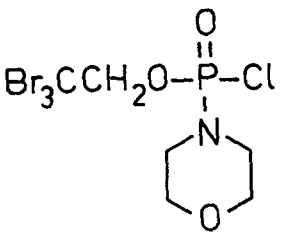
Another noteworthy feature of the phosphotriester derivatives IIIa,b,c was found in the possibility to convert them directly into the nucleoside diphosphates Va,b,c by treatment with Zn-dust in pyridine solution containing mono(tri-n-butylammonium)phosphates.

In a typical experiment; phosphotriester IIIb (1 mmole) was added to a stirred solution of dry pyridine (15 ml) containing finely devided Zn (0.1,g.) and mono(tri-n-butylammonium)-phosphate (12 mmole), under the exclusion of moisture. After 2 days at 20°, the reaction mixture was centrifugated,, the supernatant was co-evaporated several times with water (3 x 15 ml) and finally purified by DEAE cellulose. Pure Vb was obtained in 68% yield.

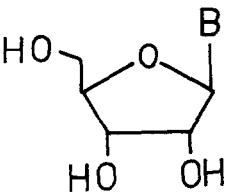
In the same way the diphosphates Va and Vb were prepared in 65% and 70% yields, respectively.

In conclusion, the present work amply demonstrates that monofunctional phosphorylating agent I meets the following criteria: (a) stable and crystalline compound, (b) easy available, (c) effective phosphorylating agent, (d) selective for the primary hydroxyl in the presence of unprotected secondary hydroxyls, (e) nature of its protective groups opens the possibility for the synthesis of mono-, di- and tri-phosphates from only one intermediate, (f) protective groups are stable under mild basic and acidic conditions.[9]

The scope of the reaction is presently under investigation and a full report will be published shortly.



I

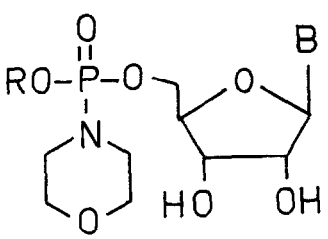


II

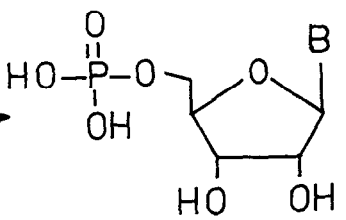
a; B = N^6-(Δ^2-Isopentenyl)adenin-9-yl

b; B = N^6,N^6-Dimethyladenin-9-yl

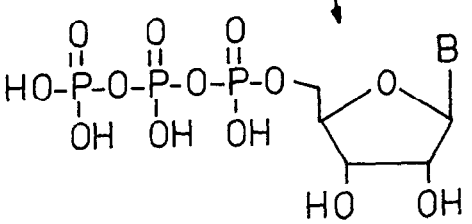
c; B = N^6-Methyladenin-9-yl



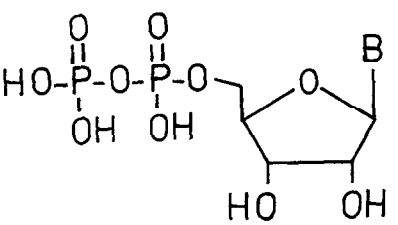
IIIa,b,c



IVa,b,c



VIa,b,c



Va,b,c

Reagents i, Cu/Zn, H^+; ii, Cu/Zn, $H_2PO_4^-$; iii, Cu/Zn, $H_2P_2O_7^-$

ACKNOWLEDGMENT.

One of us (R.C.) thanks the Netherlands Organization for Advancement of Pure Research (ZWO) for financial support.

FOOTNOTES AND REFERENCES

1. To whom enquires should be addressed.

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3. The same limitation also holds for the synthesis of di and tri-phosphates involving triesterified pyrophosphate intermediates. A.M.Michelson, J.Chem.Soc.,137,3655(1959); F.Cramer and R.Wittman, Angew. Chem., 72,628(1960); T.Hata, M.Sekine and K.Furusawa, J.C.S. Chem.Comm.,196(1975).

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6. In the same way the crystalline 2,2,2-trichloroethyl phosphoromorpholinochloridate was prepared. Satisfactory analytical data were obtained for both compounds.

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9. The phosphotriesters IIIa,b,c when subjected to basic or acidic conditions (aqueous ammonia 5.3 M, or hydrochloric acid, pH = 2) for 2 days at 20°, were recovered quantitatively. See also; H.A.C.Montgomery and J.H.Turnbull, J.Chem.Soc., 1963(1958).