Tetrahedron Letters,Vol.23,No.26,pp 2615-2618,1982 0040-4039/82/262615-04$03.00/0
Printed in Great Britain

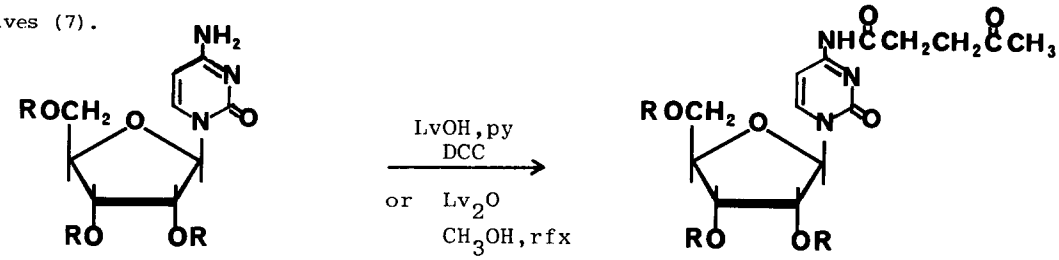
N-LEVULINATION OF NUCLEOSIDES

Kelvin K. Ogilvie*, Mona J. Nemer, Gholam H. Hakimelahi, Zbigniew A. Proba and Marc Lucas.
Department of Chemistry, McGill University, Montreal, Canada H3A 2K6

Summary - Procedures have been developed for the synthesis of the N-levulinyl derivatives of cytidine, adenosine and guanosine.

Recent advances in the synthesis of polynucleotides and their analogues have created the need for a protecting group for the amino function of nucleosides that can be removed under mild conditions. Generally the amino group of cytosine, adenine and guanine nucleosides is protected with an acyl group (1,2). These groups generally require strong base over extended periods for their removal (3). We have been exploring other protecting groups for amine protection. In this report we wish to describe the successful synthesis of the N-levulinyl derivatives of cytidine, adenosine and guanosine.

The levulinyl group was introduced to carbohydrates by Guthrie et al. (4) and extended to nucleosides for hydroxyl protection by Hassner et. al. (5). Van Boom has used the levulinyl group widely for 5'-hydroxyl protection during nucleotide synthesis (6). In all cases the strength of the group is its stability to general conditions used in nucleotide synthesis, yet its rapid selective removal. However, it has not been possible to easily obtain the N-levulinyl derivatives (7).



1, R=H
2, R=Si=TBDMS= $-\overset{|}{\underset{|}{Si}}-$

3, R=Si
4, R=H

In the case of cytidine we were able to use almost routine conditions for N-acylation. Thus treatment of trisilylcytidine (2, 8, 1g) in dioxane (50 ml) with levulinic acid (400 mg), DCC (2g) and dimethylaminopyridine (100 mg) for 15 h at 20°C led to the N-levulinyl derivative 3. The solution was collected by filtration, washed with 10% $NaHCO_3$ and evaporated to dryness. The residue was dissolved in hot hexane (25 ml) and on standing, pure white crystals of 3 were obtained (1.1 g, 95%, properties, see Table 1). Compound 3 on treatment with TBAF in THF (9) for 1 h was converted to N-levulinylcytidine (4). Compound 4 was isolated in 91% overall yield from 3 after passage through a DOWEX 50W-X8(Na^+form) exchange column followed by crystallization from water (properties, Table 1). Cytidine itself can be directly converted to the

Table 1

Properties of N-Levulinyl* Nucleosides

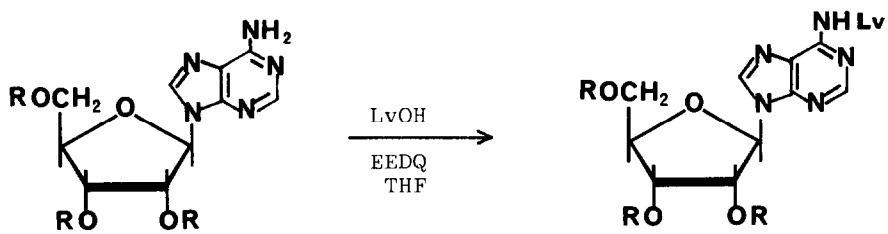
Compound	mp °C	λmax,nm (EtOH)	R_f+
TriSiC^{Lv} (3)	174-176	249,302	0.79[a]
^{Lv}C (4)	158-160	246,302	0.22[b]
MMTC^{Lv} (11)	140-146	233(sh),304	0.31[a]
MMT$C^{Lv}_{Si\ OH}$ (12)	132-136	232(sh),303	0.61[a]
MMT$C^{Lv\ OH}_{Si}$ (13)	106-109	234(sh),303	0.40[a]
$^{Lv}C^{Si}_{Si}$ (14)	213-215	249,300	0.50[a]
TriSiA^{Lv} (6)	85-87	271	0.87[a]
^{Lv}A (7)	180-183	270	0.12[a]
TriSiG^{Lv} (9)	95-98	283,257	0.77[c]
^{Lv}G (10)	241-245(dec)	285,259	0.06[c]

*New compounds had satisfactory elemental analyses. In the PMR, the CH_3-C(=O)- protons appear as a singlet at δ = 2.16 ppm(TMS) for C and between 2.3 and 2.4 for A and G derivatives.

+Solvents:(a) $CHCl_3$:EtOH(9:1) (b) $CHCl_3$:EtOH(4:1) (c) acetone:$CHCl_3$(9:1)

N-levulinylcytidine by a procedure identical to that for selective N-benzoylation (10). By this method levulinic anhydride is added portionwise over a 6 h period to cytidine in refluxing methanol. After standing overnight at 20°C the product 4 was collected by filtration. The filtrate was concentrated and retreated with levulinic anhydride at reflux for 6h as before. The total yield of 4 by this direct procedure was 70%.

The above procedure which works so well for cytidine does not work at all for adenosine and guanosine. After evaluating several alternatives we found that trisilyladenosine 5 could be N-levulinated in 80% yield using EEDQ (N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline).



5, R = Si

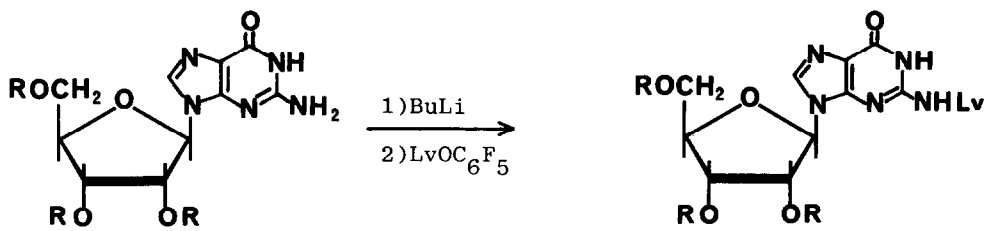
6, R=Si

7, R=H

In a typical experiment trisilyladenosine (5, 2mmole,9) was dissolved in THF(40 ml); levulinic acid (20 mmole) and EEDQ (24 mmole) were added and the solution was stirred at room temperature for 90 min followed by refluxing for 6 h. The solvent was evaporated and the residue was dissolved in ether (50 ml). The resulting solution was washed with dilute HCl (5%) followed by washing with water, dried, and evaporated. The residue was chromatographed on silica gel (short column,

4 x 10 cm silica, impurities eluted first with CH_2Cl_2 and the product collected in $CHCl_3$:EtOAc (1:1)). The product 6 was obtained in 80% yield (Table 1). The silyl groups were easily removed from 6 using TBAF in THF to give N-levulinyladenosine (7, 60%, Table 1).

The above procedure also worked well for cytidine. Compound 2 was converted into 3 in 95% yield using the above EEDQ procedure. However, the procedure did not work at all for guanosine.



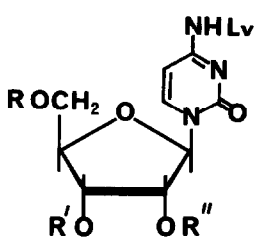
8

9, R=Si

10, R=H

In the guanosine case only very severe conditions resulted in N-levulination. Trisilylguanosine (8, 1 mmole, 8) was dissolved in 5 ml of dry THF. n-Butyllithium (2 mmole) was added at -20°C and the solution was then allowed to warm to room temperature over 30 min. This solution was added to the pentafluorophenyl ester of levulinic acid (prepared according to ref. 11, 10 mmole) dissolved in THF (5 ml). The system was stirred at room temperature for 30 h. A 1% solution of NH_4Cl (20 ml) was added. The products were extracted into chloroform which was dried over $MgSO_4$ and evaporated. The residue was first passed through a short column of silica eluting successively with CH_2Cl_2, $CHCl_3$ and ethyl acetate. A mixture of 8 and 9 was eluted with ethyl acetate. This mixture was separated on thick layer plates using acetone: $CHCl_3$(6:4). Compound 9 was obtained in 20% yield (Table 1). Compound 9 was converted into N-levulinylguanosine (10) in 60% yield using TBAF in THF.

The N-levulinyl group can be readily removed from all of the compounds described herein using the standard hydrazine solution (0.5N hydrazine hydrate in pyridine-acetic acid (4:1)), in 5 to 15 min. The N-levulinyl group is stable to all of the general derivatization techniques used to prepare protected nucleosides for nucleotide synthesis. This is illustrated by the synthesis of the cytidine derivatives 11-14 using standard technique (Table 1). These compounds have been used successfully in nucleotide synthesis (12).



11, R=MMT, R'=R''=H

12, R=MMT, R'=H, R''=Si

13, R=MMT, R'=Si, R''=H

14, R=H, R'=R''=Si

This manuscript describes the successful synthesis of the N-levulinyl derivatives of cytidine, adenosine and guanosine. The levulinyl group shows excellent promise for use in this capacity in nucleotide synthesis

Acknowledgement

We gratefully acknowledge financial assistance from the NSERCC and FCAC granting agencies.

References

1. K.L. Agarwal, A. Yamazaki, P.J. Cashion and H.G. Khorana, Angew. Chemie Int. Ed., 11, 451 (1972).
2. H. Kössel and H. Seliger, Progress in The Chemistry of Natural Products, 32, 297 (1975).
3. H. Weber and H.G. Khorana, J. Mol. Biol., 72, 219 (1972).
4. D. Guthrie, T.J. Lucas and R. Khan, Carbohydrate Res., 33, 391 (1974).
5. A. Hassner, G. Strand, M. Rubinstein and A. Patchornik, J. Am. Chem. Soc., 97, 1614 (1975).
6. J.A.J. den Hartog, G. Wille and J.H. van Boom, Recl. Trav. Chim. Pays-Bas, 100, 320 (1981).
7. K.K. Ogilvie and M.J. Nemer, Can. J. Chem., 58, 1389 (1980).
8. K.K. Ogilvie, A.L. Schifman and C.L. Penney, ibid., 57, 2230 (1978).
9. K.K. Ogilvie, S.L. Beaucage, A.L. Schifman, N.Y. Theriault and K.L. Sadana, ibid, 56, 2768 (1978).
10. T. Neilson and E.S. Werstiuk, ibid., 49, 493 (1971).
11. J. Igolen and C. Morin, J. Org. Chem., 45, 4802 (1980).
12. unpublished results.
13. The carbonyl absorptions from the levulinyl group occur at 1700 cm^{-1} and 1720 cm^{-1} for the amide and ketone respectively for 6 and at 1679 cm^{-1} and 1700 cm^{-1} in 7. These are typical values.

(Received in USA 4 February 1982)