Tetrahedron Letters,Vol.24,No.29,pp 2953-2956,1983 0040-4039/83 $3.00 + .00
Printed in Great Britain

N^6(N-METHYL-2-PYRROLIDINE AMIDINE)DEOXYADENOSINE - A NEW DEOXYNUCLEOSIDE PROTECTING GROUP

L.J. McBride and M. H. Caruthers*

Department of Chemistry, University of Colorado, Boulder, CO 80309, USA

The synthesis and characterization of N^6(N-methyl-2-pyrrolidine amidine) 2'-deoxyadenosine is described. This amidine is significantly more stable toward acid catalyzed depurination than N-benzoyldeoxyadenosine, is easy to prepare and can be removed rapidly and quantitatively.

Oligodeoxynucleotides can be synthesized rapidly and efficiently on silica gel polymer supports using deoxymononucleotide phosphoramidites as synthons (1). An inherent problem associated with any polymer supported, cyclical synthesis is the stability of the growing deoxyoligonucleotide to repetitive exposure with various reagents used during the synthesis. One particularly troublesome problem has been acid catalyzed depurination of N-benzoyldeoxyadenosine during detritylation of the 5'-protecting group (2). Initially this problem was addressed by using a Lewis acid such as $ZnBr_2$ (3) where less depurination was observed at the expense of longer reaction times. More recently protic acids in mixed solvents such as nitromethane and methanol have been shown to depress depurination (4). An alternative solution is to change the protecting group on deoxyadenosine. Initial results utilizing the pthaloyl group have been shown to be useful in conjunction with the phosphate triester approach and highly reactive condensing agents (5). In this communication we report that certain amidine derivatives of deoxyadenosine are less susceptible to protic acid depurination than the N-benzoyl derivative. Moreover the ease of preparing amidines makes them quite attractive generally as potential nucleoside exocyclic amino protecting groups.

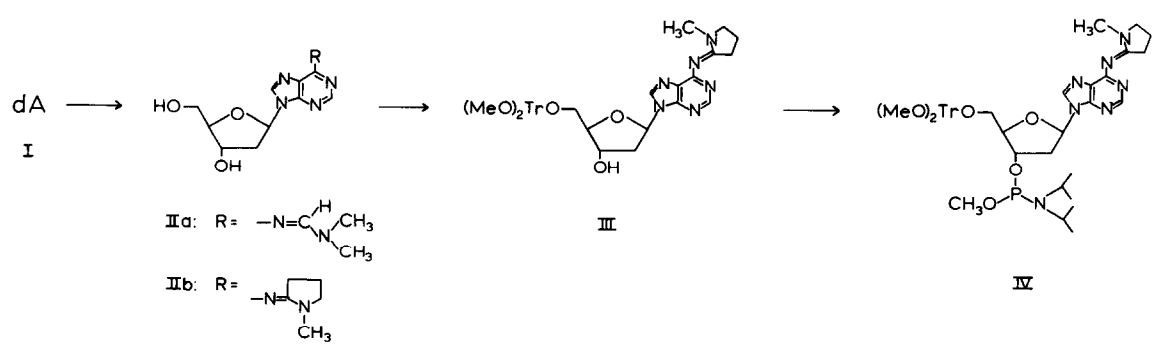

Figure 1. Outline of the Synthetic Route for Preparing Amidine Derivatives of Deoxyadenosine. $(MeO)_2Tr$ refers to the di-p-anisylphenylmethyl group.

Initially deoxyadenosine (I) was converted to the N,N-dimethylformamidine derivative (IIa) using dimethylformamide diethylacetal (6). Studies with a saturated solution of $ZnBr_2$ in nitromethane:methanol (95:5) and 3% trichloracetic acid in CH_2Cl_2 however demonstrated that IIa was rapidly hydrolyzed to I. Compound IIb was therefore prepared. To 2'-Deoxyadenosine (269 mg, 1.00 mmol), after coevaporation with 3 X 5 ml pyridine, was added dry pyridine (3 ml) and 1-methyl-2,2-diethoxypyrrolidine (7) (660 µl, 3.2 mmol). After 16 h at 25°C the solution was quenched with water (400 µl), concentrated to a viscous oil, dissolved in CH_2Cl_2:MeOH (95:5), and loaded on to a silica gel column (10 g) equilibrated with the same solvent. Compound IIb was eluted with CH_2Cl_2:MeOH (92:8) and concentrated to a white foam in 94% yield (330 mg, 0.94 mmole) as the monohydrate (8). λmax 301 mµ (H_2O, pH 5.1 and 11.2), $\varepsilon max = 22 \times 10^3$. The mass spectrum showed a parent peak at m/e = 332. ^1H NMR (d^6-DMSO) δ 1.96 (M, $C-CH_2-C$), δ 2.85 (M, $-N=C-CH_2-$), δ 3.05 (S, CH_3-N), and δ 3.48 (M, $-N-CH_2-$) were characteristic (9).

Compound III was prepared via a one flask procedure from I. Deoxyadenosine (107mg, 0.414 mmol) and 1-methyl-2,2-diethoxypyrrolidine (222 µl, 1.2 mmol) were allowed to react in 1.0 ml dry pyridine for 12 h. The reaction mixture was quenched with water (100 µl), concentrated in vacuo to a gum, and reconcentrated three additional times with dry pyridine to remove water. The gum was dissolved in dry pyridine (1.0 ml) and di-p-anisylphenylmethylchloride (162 mg, 0.48 mmol) was added. After 5 h at r.t., the reaction mixture was quenched with methanol (100 µl), and fractionated by chromatography on a silica gel column equilibrated with chloroform:pyridine (99:1). Compound III was eluted from the column with chloroform:methanol:pyridine (95:4:1) and then isolated by first concentrating the solution to a viscous oil followed by two coevaporations with toluene (2 ml each) and precipitation from CH_2Cl_2 into hexane:ethylether (3:1). The product was isolated as a white powder in 80% yield (210 mg, 0.33 mmol). ^1H-NMR ($CDCl_3$) δ 2.03 (M, $C-CH_2-C$), δ 2.94 (M, $-N=C-CH_2-$), δ 3.15 (S, $N-CH_3$), and δ 3.48 (M, $-N-CH_2-$) were characteristic.

Compound IV was prepared from III (90 mg, 0.14 mmol) and N,N-diisopropylaminomethoxychlorophosphine (42 µl, 0.21 mmol) using a published procedure (1). The yield was 95% (108 mg, 0.146 mmol). ^{31}P-NMR signals characteristic of a diastereoisomers of deoxynucleotide phosphoramidites were observed at -148.3 and -148.2 ppm in acetonitrile (relative to an external standard of 85% phosphoric acid in acetonitrile). The ^{31}P-NMR of IV (Figure 2), indicated that the product was 95% homogeneous (relative to phosphorous compounds) simply by aqueous extraction of the reaction mixture followed by precipitation (1). Further activation to the tetrazolide (10) at -126 ppm (11) was observed (^{31}P-NMR) by addition of tetrazole to the deoxynucleotide phosphoramidite in acetonitrile.

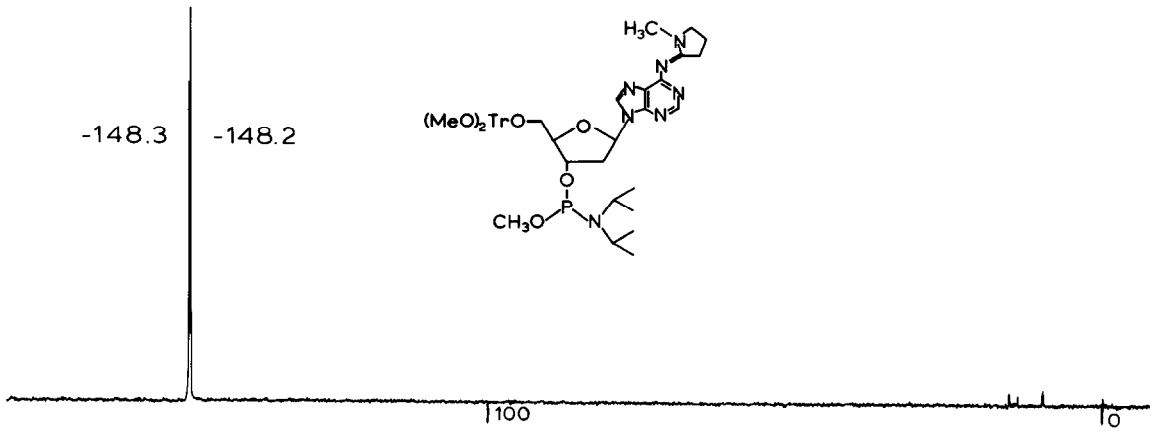

Figure 2. ^{31}P-NMR Spectrum of Compound IV. The spectrum is relative to 85% phosphoric acid in acetonitrile (15:85, v/v) as an external standard.

The relative effects of N^6-protecting groups on the stability of the glycoside bond were compared in 1 M trichloroacetic acid ($CH_3OH:H_2O$, 1:1) at 23°C. Aliquots were removed periodically, quenched with aqueous KOH, and analyzed by thin layer chromatography on silica gel. The half-life of compound IIb (1 h) was three times the half-life of N-benzoyldeoxyadenosine (20 min).

Removal of the N-methylpyrrolidine amidine protecting group can be accomplished via several routes. Based on HPLC analysis (12), deprotection to deoxyadenosine using 28% aqueous ammonia was 95% complete after 48 h at 60°C in a sealed ampoule. A comparable experiment with N-benzoyldeoxyadenosine was 99.8% complete. The most satisfactory procedure appears to be deprotection with a solution of ethylenediamine:phenol:H_2O (10:40:4, v/w/v)(13). Based on HPLC analysis, IIb was converted completely to I within 6 h at 40°C.

These results using N-methyl-2-pyrrolidine amidine as a protecting group are quite encouraging. Synthesis of the N-protected, 5'-trityl derivative (III) can be completed via a one flask reaction and in very high overall yield. The nucleotide phosphoramidites of this derivative can be prepared using standard procedures and then activated with tetrazole. Finally the amino protecting group can be removed rapidly and efficiently using a solution of ethylenediamine:phenol. We therefore anticipate that this derivative can be used without any difficulty in oligodeoxynucleotide synthesis procedures.

Acknowledgements

This is paper XII on Nucleotide Chemistry. Paper XI is E. F. Fisher and M. H. Caruthers, Nucl. Acids Res. 11, 1589-1599 (1983). This work was supported by NIH (GM25680). L. McBride acknowledges an Upjohn Graduate Fellowship in Nucleic Acid Research. We wish to thank Martin Ashley for recording the NMR spectra.

References

1. S. L. Beaucage and M. H. Caruthers, Tetrahedron Letters 22, 1859-1862 (1981); L. J. McBride and M. H. Caruthers, Tetrahedron Letters 24, 245-248 (1983).
2. H. Schaller and H. G. Khorana, J. Am. Chem. Soc. 85, 3828-3835 (1963).
3. M. D. Matteucci and M. H. Caruthers, Tetrahedron Letters 21, 3243-3246 (1980); V. Kohli, H. Blocker and H. Koster, Tetrahedron Letters 21, 2683-2686 (1980).
4. T. Tanaka and R. L. Letsinger, Nucleic Acids Res. 10, 3249-3260 (1982).
5. A. Kume, M. Sekine and T. Hata, Tetrahedron Letters 23, 4365-4368 (1982).
6. J. Zemlicka, S. Chladek, A. Holy and J. Smrt, Collect. Czech. Chem. 31, 3198-3211 (1966).
7. H. Meerwein, P. Borner, O. Fuchs, H. J. Sasse, H. Schrodt and J. Spille, Chem. Ber. 89, 2060-2079 (1956).
8. Anal. calcd for $C_{15}H_{22}O_4N_6$: C, 51.43; H, 6.33; N, 23.97. Found C, 52.06; H,6.07; N, 23.99.
9. ^{1}H and ^{31}P-NMR were recorded on a Bruker WM-250.
10. M. D. Matteucci and M. H. Caruthers, J. Am. Chem. Soc. 103, 3185-3191 (1981); J. L. Fourrey and D. J. Shire, Tetrahedron Letters 22, 729-732 (1981).
11. S. L. Beaucage and M. H. Caruthers, in preparation.
12. H-J. Fritz, R. Belagaje, E. L. Brown, R. H. Fritz, R. A. Jones, R. G. Lees, and H. G. Khorana, Biochemistry 17, 1257-1267 (1978).
13. R. W. Barnett and R. L. Letsinger, Tetrahedron Letters 22, 991-994 (1981).

(Received in USA 15 March 1983)