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DEOXYNUCLEOSIDE PHOSPHORAMIDITES—A NEW CLASS OF KEY INTERMEDIATES FOR DEOXYPOLYNUCLEOTIDE SYNTHESIS

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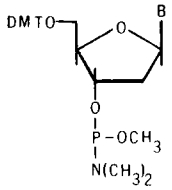
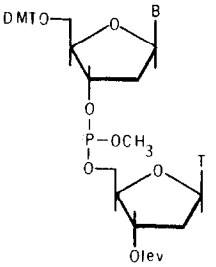
The development of a new class of nucleoside phosphites is described. These compounds are stable to normal laboratory conditions, are activated by mild acid treatment, and are observed to react essentially quantitatively with protected nucleosides.

A recent, key innovation in oligonucleotide synthesis was the introduction of the phosphite coupling approach by Letsinger and coworkers (1-3). This approach has been adapted to the syntheis of deoxyoligonucleotides (4-8), oligoribonucleotides (9-12), and nucleic acid analogs (13-15). Generally the approach involves the reaction of a suitably protected nucleoside, a bifunctional phosphitylating agent such as methoxydichlorophosphine, and a second protected nucleoside. Mild oxidation using iodine in tetrahydrofuran, lutidine and water generates the natural internucleotide bond. By varying the oxidation procedure, phosphorus analogs such as selenophosphates (14), imidophosphates (14) and thiophosphates (14, 15) can be generated. A serious limitation of this methodology, however, has been the instability of the reactive intermediates (nucleoside phosphomonochloridites or monotetrazolides) towards hydrolysis and air oxidation. This problem has been circumvented by either preparing the reactive species immediately prior to use or storing the active phosphite as a precipitate in hexanes at -20°C. We have recently solved this problem by synthesizing a new class of nucleoside phosphites that are easy to prepare by standard organochemical procedures, are stable under normal laboratory conditions to hydrolysis and air oxidation, and are stored as dry, stable powders. These key intermediates are N, N-dimethylaminophosphoramidites of the appropriately protected deoxynucleosides and are



Ia-d



IIIa-d

Ia, IIIa, B = 1-Thyminyl
Ib, IIIb, B = 1-(N-4-Benzoylcytosinyl)
Ic, IIIc, B = 9-(N-6-Benzoyladeninyl)
Id, IIId, B = 9-(N-2-Isobutyrylguaninyl)
lev = levulinyl
DMT = Di-*p*-anisylphenylmethyl

represented as compounds Ia-d. This communication outlines the synthesis, characterization, and reactivity of these phosphoramidites.

The synthesis of compounds Ia-d begins with the preparation of chloro-N, N-dimethylaminomethoxyphosphine [CH_3O P(Cl) $N(CH_3)_2$] which is used as a monofunctional phosphitylating agent. A 250 ml addition funnel was charged with 100 ml of precooled anhydrous ether (-78°C) and precooled (-78°C) anhydrous dimethylamine (45.9 g, 1.02 mol). The addition funnel was wrapped with aluminum foil containing dry ice in order to avoid evaporation of dimethylamine. This

solution was added dropwise at -15°C (ice-acetone bath) over 2 h to a mechanically stirred solution of methoxydichlorophosphine (16) (47.7 ml, 67.32 g., 0.51 mol) in 300 ml of anhydrous ether. The addition funnel was removed and the 1 ℓ, three-necked round bottom flask was stoppered with serum caps tightened with copper wire. The suspension was mechanically stirred for 2 h at room temperature. The suspension was filtered and the amine hydrochloride salt was washed with 500 ml anhydrous ether. The filtrate and washings were combined and ether was distilled at atmospheric pressure. The residue was distilled under reduced pressure. The product was collected at 40-42°C @ 13 mm Hg and was isolated in 71% yield (51.1 g, 0.36 mol). d^{25} = 1.115 g/ml. ^{31}P-N.M.R., δ = -179.5 ppm ($CDCl_3$) with respect to internal 5% v/v aqueous H_3PO_4 standard. ^{1}H-N.M.R. doublet at 3.8 and 3.6 ppm J_{P-H} = 14 Hz (3H, OCH_3) and two singlets at 2.8 and 2.6 ppm (6H, $N(CH_3)_2$). The mass spectrum showed a parent peak at m/e = 141.

The key intermediates Ia-d were prepared by the following procedure. 5'-O-Di-*p*-anisyl phenylmethyl nucleoside (1 mmol) was dissolved in 3 ml of dry, acid free chloroform and diisopropylethylamine (4 mmol) in a 10 ml reaction vessel preflushed with dry nitrogen. $[CH_3OP(Cl)N(CH_3)_2]$ (2 mmol) was added dropwise (30-60 sec) by syringe to the solution under nitrogen at room temperature. After 15 min the solution was transferred with 35 ml of ethyl acetate into a 125 ml separatory funnel. The solution was extracted four times with an aqueous, saturated solution of NaCl (80 ml). The organic phase was dried over anhydrous Na_2SO_4 and evaporated to a foam under reduced pressure. The foam was dissolved with toluene (10 ml) (Id was dissolved with 10 ml of ethyl acetate) and the solution was added dropwise to 50 ml of cold hexanes (-78°C) with vigorous stirring. The cold suspension was filtered and the white powder was washed with 75 ml of cold hexanes (-78°C). The white powder was dried under reduced pressure and stored under nitrogen. Isolated yields of compounds Ia-d were 90-94% (see Table I).

TABLE I

COMPOUND	δ-^{31}P (ppm) (Acetone-d_6)	δ-^{31}P (ppm) ($CDCl_3$)	ISOLATED YIELD (%)
Ia	-146.0, -145.4	-147.7, -146.8	93, 95*
Ib	-146.3, -145.5	-148.0, -147.0	92, 95*
Ic	-146.1, -145.8	-147.4, -147.3	90, 98*
Id	-145.9, -145.7	-147.7, -147.2	90, 98*
IIIa	-139.6, -138.9	-140.8, -139.9	97**
IIIb	-139.6, -139.0	-140.6, -140.0	94**
IIIc	-139.7, -138.9	-141.0, -139.9	97**
IIId	-140.3, -140.2	-143.6, -141.9	93**

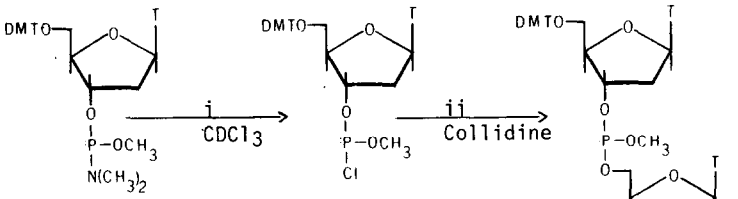
*Estimated purity from ^{31}P-N.M.R.

**Estimated yield from ^{31}P-N.M.R.

The purity of the products was checked by ^{31}P-N.M.R. Additionally, when analyzed by ^{31}P-N.M.R., these compounds were stable for at least a month when stored at room temperature under nitrogen. Furthermore, no significant amount of 3'-3' dinucleoside phosphite was detected by ^{31}P-N.M.R. (less than 4%). The low content of the 3'-3' dinucleoside phosphite was expected and represents a significant improvement over the original phosphite coupling procedure where a considerable amount of the unwanted 3'-3' dinucleoside phosphite was unavoidable (1-3, 9).

We have observed that mild acidic conditions can be used to activate Ia-d toward formation

of phosphite internucleotide bonds. These investigations were prompted by earlier research showing that aminophosphines can be protonated and therefore activated by acidic species (17-24). This activation process was initially monitored by ^{31}P-N.M.R. Thus, when N, N-dimethylaniline hydrochloride (1 mmol) in 0.5 ml of dry $CDCl_3$ was added at room temperature under nitrogen to Ia (0.5 mmol, -147.7 and -146.8 ppm) in 2 ml of dry, acid free $CDCl_3$ in a 10 mm N.M.R. tube, the chlorophosphite II (-167.2 ppm) was obtained in quantitative yield. Addition of 1.2 molar equivalents of 3'-O-levulinylthymidine (25) to the chlorophosphite II led to an essentially quantitative conversion to the dinucleoside phosphite IIIa (-140.8 and -139.9 ppm).



Ia II IIIa

i, N, N-Dimethylaniline hydrochloride
ii, 3'-O-Levulinylthymidine

Evidence supporting the assignment of the active chlorophosphite II to the peak at -167.2 was independently obtained by reacting 5'-O-Di-*p*-anisylphenylmethylthymidine with excess methoxydichlorophosphine (-181.6 ppm) in the presence of collidine in $CDCl_3$. The major reaction product as monitored by ^{31}P-N.M.R. was localized at -167.2 ppm.

Of the various weak acids investigated as potential activating agents, 1H-tetrazole fulfills all requirements. The compound is a non-hygroscopic, commercially available solid that can be easily purified and dried in one step by sublimation at 110°C @ 0.05 mm Hg. Activation by 1H-tetrazole was also monitored by ^{31}P-N.M.R. Thus, Ia (0.5 mmol) and 3'-O-levulinylthymidine (0.6 mmol) were placed in a 10 mm N.M.R. tube and sublimed 1H-tetrazole (1.5 mmol) in 2.5 ml of dry acetonitrile-d_3 was added under nitrogen atmosphere. The ^{31}P-N.M.R. spectrum was immediately recorded and displayed a quantitative yield of IIIa. Similar results were also obtained when Ib, Ic and Id were reacted with 3'-O-levulinylthymidine. The appropriate chemical shifts of compounds Ia-d and IIIa-d with respect to internal 5% v/v aqueous H_3PO_4 standard are reported in Table I. Complete physical and analytical properties of these compounds will be reported elsewhere.

The applicability of these reagents to the synthesis of deoxyoligonucleotides on polymer supports was also tested. Trial experiments were completed by condensing compounds Ia-d with N-2-isobutyryldeoxyguanosine attached covalently to silica gel. Thus, N-2-isobutyryldeoxyguanosine (1 μmole) covalently attached to silica gel (20 mg) at the 3'-position, Ia (10 μmole), and 1H-tetrazole (50 μmole in 0.1 ml dry acetonitrile) were shaken for 20 min and the reaction was then quenched with aqueous lutidine. The same reaction sequence was completed with Ib, Ic and Id. After the usual oxidation and deprotection procedures (8), d(TpG), d(CpG), d(ApG) and d(GpG) were obtained in 100%, 98%, 94%, and 93% yield respectively (measured spectrometrically from the dimethoxytrityl cation using an extinction of 7 X 10^4 at 498 nm). These dinucleotides were completely degraded by snake venom phosphodiesterase and the appropriate nucleosides and

nucleotides were obtained in the proper ratios (monitored via high pressure liquid chromatography analysis of snake venom phosphodiesterase hydrolysates).

The N, N-dimethylamino phosphines Ia-d therefore display tremendous potential in oligodeoxynucleotide synthesis. These compounds are easy to prepare and are stable to normal laboratory conditions. They are readily activated via protonation and condense with appropriate nucleosides to form internucleotide bonds in very high yields.

Acknowledgements

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References

1. R. L. Letsinger, J. L. Finnan, G. A. Heavner, W. B. Lunsford, *J. Am. Chem. Soc.* 97, 3278-3279 (1975).
2. R. L. Letsinger and W. B. Lunsford, *J. Am. Chem. Soc.* 98, 3655-3661 (1976).
3. R. L. Letsinger, J. L. Finnan, S. A. Jacobs, B. A. Juodka and A. K. Varshney, *Proc. Int. Conf. Transfer RNA*, Poznan, Poland, October 1976, pp 145-155.
4. M. D. Matteucci and M. H. Caruthers, *Tetrahedron Lett.*, 21, 719-722 (1980).
5. J. L. Finnan, A. Varshney and R. L. Letsinger, *Nucl. Acids Res. Symposium Series No. 7*, 133-145 (1980).
6. M. H. Caruthers, *Proc. of IUPAC International Symposium on Macromolecules*, Florence, Italy, September 1980, Pergamon Press, Oxford, England.
7. M. H. Caruthers, S. L. Beaucage, J. W. Efcavitch, E. F. Fisher, M. D. Matteucci and Y. Stabinsky, *Nucl. Acids Res. Symposium Series No. 7*, 215-223 (1980).
8. M. D. Matteucci and M. H. Caruthers, *J. Am. Chem. Soc.*, in press.
9. G. W. Daub and E. E. van Tamelen, *J. Am. Chem. Soc.* 99, 3526-3528 (1977).
10. K. K. Ogilvie and M. J. Nemer, *Can. J. Chem.* 58, 1389-1397 (1980) and references therein. See also K. K. Ogilvie, N. Y. Theriault, J. M. Seifert, R. T. Pon and M. J. Nemer, *Can J. Chem.* 58, 2686-2693 (1980).
11. K. K. Ogilvie, M. J. Nemer, N. Theriault, R. Pon and J. M. Seifert, *Nucl. Acids Res. Symposium Series No. 7*, 147-150 (1980).
12. K. K. Ogilvie and M. J. Nemer, *Tetrahedron Lett.* 21, 4159-4162 (1980).
13. B. P. Melnick, J. L. Finnan and R. L. Letsinger, *J. Org. Chem.* 45, 2715-2716 (1980).
14. K. K. Ogilvie and M. J. Nemer, *Tetrahedron Lett.* 21, 4145-4148 (1980); ibid., 4149-4152 (1980); ibid., 4153-4154 (1980).
15. P. M. J. Burgers and F. Eckstein, *Tetrahedron Lett.*, 3835-3838 (1978).
16. D. R. Martin and P. J. Pizzolato, *J. Am. Chem. Soc.* 72, 4584-4586 (1950).
17. H. Nöth and H. J. Vetter, *Chem. Ber.* 96, 1109-1118 (1963).
18. E. E. Nifant'ev, N. L. Ivanova and N. K. Bliznyuk, *Zh. Obschei Khim.* 36, 765 (1966).
19. E. E. Nifant'ev, N. L. Ivanova and I. V. Fursenko, *Zh. Obschei Khim.* 39, 854-856 (1969).
20. V. N. Eliseenkov, A. N. Pudovik, S. G. Fattakhov and N. A. Serkina, *Zh. Obschei Khim.* 40, 498 (1970).
21. V. P. Evdakov, V. P. Beketov and V. I. Svergun, *Zh. Obschei Khim.* 43, 55-59 (1973).
22. A. N. Pudovik, E. S. Batyeva, E. N. Ofitserov and V. A. Al'fonsov, *Zh. Obschei. Khim.* 45, 2338-2339 (1975).
23. E. S. Batyeva, V. A. Al'fonsov, G. U. Zamaletdinova and A. N. Pudovik, *Zh. Obschei. Khim.* 46, 2204-2207 (1976).
24. V. A. Al'fonsov, V. A. Kharlamov, E. S. Batyeva, T. Kh. Gazizov and A. N. Pudovik, *Iz. Akad. Nauk. SSSR. Ser. Khim.*, 2124-2126 (1977).
25. A. Hassner, G. Strand, M. Rubinstein, A. Patchornik, *J. Am. Chem. Soc.* 97, 1614-1615 (1975).

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