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Beaucage S L & Caruthers M H. Deoxynucleoside phosphoramidites—a new class of key intermediates for deoxypolynucleotide synthesis. *Tetrahedron Lett.* **22**:1859-62, 1981.
[Department of Chemistry, University of Colorado, Boulder, CO]

This paper describes the preparation and characterization of deoxynucleoside phosphoramidites. The usefulness of these intermediates in solid-phase DNA synthesis upon activation by weak proton donors is also presented. [The *SCI*® indicates that this paper has been cited in over 265 publications.]

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Upon graduation from McGill University in 1978, I joined Marvin Caruthers's group at the University of Colorado as a postdoctoral fellow. At that time, my project involved site-directed mutagenesis of the SV40 T-antigen binding sites. Synthetic DNA required for the project was prepared using the phosphite coupling method of Letsinger and coworkers.[1,2] Their approach, involving the use of deoxynucleoside chlorophosphites as intermediates, permitted unprecedented kinetics in the formation of internucleotidic linkages, but despite its revolutionary features, the methodology had limitations. These were the instability of the deoxynucleotide chlorophosphite synthons, their high reactivity towards moisture (which meant they had to be stored and handled under inert gas), and the generation of large amounts of unwanted 3'-3'-dinucleoside monophosphites. Such complications clearly compromised automation of the procedure.

We reasoned that replacing a chlorine atom of the phosphorodichloridite by a dimethylamino group would generate a monofunctional phosphitylating agent that would, upon reaction with properly protected deoxynucleosides, eliminate the formation of undesired 3'-3'-dinucleoside monophosphites and afford high yields of the deoxynucleoside phosphoramidites. Because these intermediates lack functional groups susceptible to hydrolysis, we also anticipated that their isolation and purification would be easier than with the deoxynucleoside chlorophosphites. Initially, our hypotheses were difficult to support because preliminary results on silica gel thin-layer chromatography appeared unsatisfactory. We rationalized that silica gel might have been acidic enough to activate the deoxynucleoside phosphoramidites and generate inconsistent data.

The use of ^{31}P NMR was the crucial step in the development of the deoxynucleoside phosphoramidite chemistry since it was free of these problems. The ^{31}P NMR data rapidly corroborated our theories. As anticipated, the deoxynucleoside phosphoramidites were efficiently prepared and subsequently isolated as stable amorphous solids. These intermediates were also shown by ^{31}P NMR to be easily activated by weak acids such as 1H-tetrazole. When activated deoxynucleoside phosphoramidites were then added to a deoxynucleoside attached covalently to a polymeric support, condensation to form a dinucleotide was complete in less than one minute. The efficacy of the deoxynucleoside phosphoramidites in solid-phase DNA synthesis became history.

Our *Tetrahedron Letters* paper is highly cited probably because, after further refinements,[3-5] the deoxynucleoside phosphoramidite methodology became "the chemistry that catapulted automated DNA synthesis into many laboratories"[6] and contributed, along with DNA sequencing and other basic developments in recombinant DNA technology, to many recent discoveries in molecular biology and genetics.

1. **Letsinger R L, Finnan J L, Heavner G A & Lunsford W B.** Phosphite coupling procedure for generating internucleotide links. *J. Amer. Chem. Soc.* 97:3278-9, 1975. (Cited 50 times.)
2. **Letsinger R L & Lunsford W B.** Synthesis of thymidine oligonucleotides by phosphite triester intermediates. *J. Amer. Chem. Soc.* 98:3655-61, 1976. (Cited 125 times.)
3. **McBride L J & Caruthers M H.** An investigation of several deoxynucleoside phosphoramidites useful for synthesizing deoxyoligonucleotides. *Tetrahedron Lett.* 24:245-8, 1983.
4. **Adams S P, Kavka K S, Wykes E J, Holder S B & Galluppi G R.** Hindered dialkylamino nucleoside phosphite reagents in the synthesis of two DNA 51-mers. *J. Amer. Chem. Soc.* 105:661-3, 1983.
5. **Sinha N D, Biernat J, McManus J & Köster H.** Polymer support oligonucleotide synthesis. XVIII: use of β-cyanoethyl-N,N-dialkylamino-N-morpholino phosphoramidite of deoxynucleosides for the synthesis of DNA fragments simplifying deprotection and isolation of the final product. *Nucl. Acid. Res.* 12:4539-57, 1984.
6. **Kaplan B E.** The automated synthesis of oligodeoxynucleotides. *Trends Biotech.* 3:253-6, 1985.

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