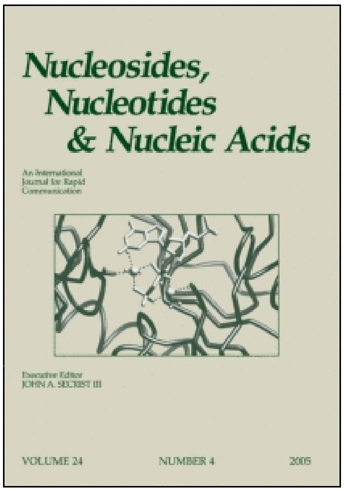

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Vasulinga T. Ravikumar[a]; Zacharia S. Cheruvallath[a]; Douglas L. Cole[a]
[a] Isis Pharmaceuticals, Carlsbad, CA

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4-CYANO-2-BUTENYL GROUP : A NEW TYPE OF PROTECTING GROUP IN OLIGONUCLEOTIDE SYNTHESIS VIA PHOSPHORAMIDITE APPROACH

Vasulinga T. Ravikumar,* Zacharia S. Cheruvallath & Douglas L. Cole
Isis Pharmaceuticals
2292 Faraday Avenue, Carlsbad, CA 92008

Abstract: 4-Cyano-2-butenyl (CB) is a new type of protecting group for the internucleotidic bonds in the synthesis of oligodeoxyribonucleotides by the phosphoramidite approach. This group is stable to acidic conditions and can be removed under mild conditions by a δ-elimination pathway using aqueous ammonium hydroxide.

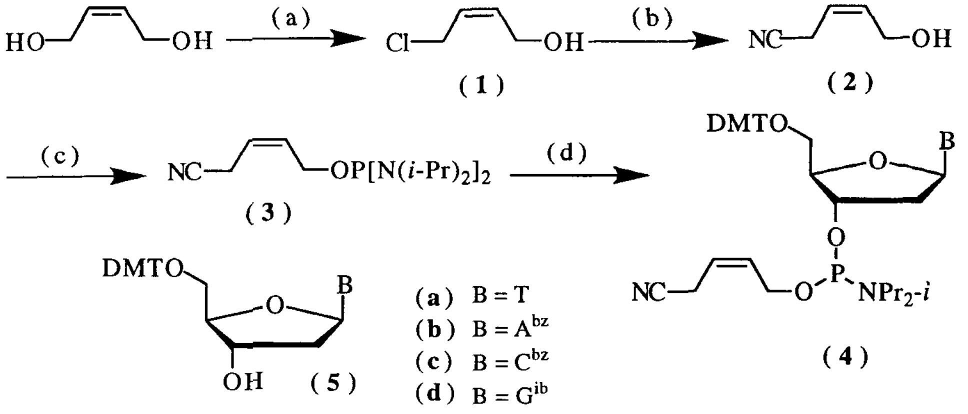
Oligonucleotides complementary to selected regions of mRNA may inhibit biosynthesis of those proteins encoded or controlled by genetic information included in these particular parts of mRNA.[1] This antisense principle has inspired several groups to extend their work beyond the synthesis of modified oligonucleotides to investigation of the potential of small nucleic acid sequences as therapeutic agents.[2] Uniformly modified oligodeoxyribonucleoside phosphorothioates have been the first class of compounds to reach the clinic. Recent animal data and clinical findings demonstrate that this new class of therapeutics works.[3] Hence there is a demand for synthesizing large quantities of these drugs.[4] In order to develop cost-effective synthetic processes, issues related to fast and efficient synthesis, automation, scalability and product purification are being investigated with renewed attention. Among the currently reported phosphate protecting groups such as 2-cyanoethyl,[5] 2,2,2-trichloro-1,1-dimethylethyl,[6] *p*-nitrophenylethyl,[7] and allyl[8] groups, only the 2-cyanoethyl group meets most of the demands. However, the 2-cyanoethyl protected deoxyribonucleoside phosphoramidite monomers are very expensive even on multi-klogram scales. It is thus of prime importance to develop efficient, low-cost phosphoramidite monomers containing new protecting groups.

The phosphate proctecting groups reported so far can be broadly classified into groups removable by a) β-elimination b) β-fragmentation c) direct displacement d) hydrogenation, and e) other methods. In this report we wish to report a new group removable by δ-elimination and that a) the easily accessible reagent bis[*N,N*-diisopropylamino]-4-cyano-2-butenyloxyphosphine (**3**) has excellent phosphitylating properties and b) the cyanobutenyl (CB) group for protecting internucleotide linkage can be removed under mild conditions using aqueous ammonium hydroxide.

The phosphoramidites were synthesized according to Scheme **1**. Monohalogenation of 2-butene-1,4-diol with thionyl chloride (1.1 equiv.) and pyridine (1.1 equiv.) in anhydrous ether afforded 1-chloro-4-hydroxy-2-butene (**1**)[9] in nearly quantitative yield. Displacement of the chloro by cyano group was effectively achieved by reacting with potassium cyanide (6 equiv.) and sodium iodide (0.025 equiv.) in anhydrous acetonitrile at room temperature to afford the desired product (**2**) as a colorless liquid (b.p. 89-92°C/2 mm). Treatment of 4-cyano-2-butene-1-ol (**2**) with bis(diisopropylamino)chlorophosphine[10] in ether at 0°C afforded directly the phosphitylating agent (**3**) in almost quantitative yield. Subsequent treatment of phosphine (**3**) without purification with 5'-*O*-DMT nucleosides afforded the phosphoramidites (**4a-d**) in 75-85% yields.

The applicability of phosphoramidites (**4**) were demonstrated by the synthesis of four hetero-dimers d(TpsC), d(CpsT), d(GpsA) and d(ApsG) (yields >99%) on solid support. Sulfurization was effected using 3H-1,2-benzodithiole-3-one 1,1-dioxide (Beaucage reagent).[11] The compounds were identified by comparison with the same hetero-dimers independently synthesized using cyanoethyl protection on the phosphate backbone.

Next, we synthesized a mixed sequence. Oligodeoxyribonucleoside phosphorothioate S-d(GCC-CAA-GCT-GGC-ATC-CGT-CA) was chosen as an example. This sequence is targeted for suppression of ICAM-1 expression[12] and is in Phase II clinical trials for treatment of renal transplant rejection, rheumatoid arthritis, psoriasis, Crohn's disease and ulcerative colitis. The synthesis was carried out on a 1 μmole scale using an ABI Synthesizer. Standard coupling conditions and concentration of amidite (0.2 M solution in acetonitrile) were used during the synthesis. The overall coupling efficiency was found to be >99% as determined by the usual spectrophotometric quantitation of released *p,p'*-dimethoxytriphenylmethyl cation. At the end of synthesis, the oligomer was deprotected by treating with aqueous ammonium hydroxide at room temperature for 1 h, and then at 60°C for 20



Scheme 1: a) SOCl$_2$, Py, ether, 0°C (95%); b) KCN, NaI, CH$_3$CN, rt (87%); c) ClP[*i*-Pr$_2$N]$_2$, ether, d) (**5**), tetrazole, CH$_2$Cl$_2$, rt.

h. The crude oligomer was purified by reversed-phase HPLC and characterized by capillary gel electrophoresis and electrospray mass spectrum. The compound was identical with the oligomer independently synthesized using cyanoethyl protection on the phosphate backbone.

In summary, the CB is a suitable group for internucleotide phosphate protection.[13] Further applications of the CB group in oligonucleotide synthesis are in progress.

References and Notes

1. Crooke, S. T. *Therapeutic Applications of Oligonucleotides*; Crooke, S. T., Ed.; R.G. Landes Company: Austin, **1995** and references cited therein.
2. Knorre, G. K.; Vlassov, V. V.; Zarytova, V. F.; Lebedev, A. V.; Federova, O. S. *Design and Targeted Reactions of Oligonucleotide Derivatives*; Knorre, G. K.; Vlassov, V. V.; Zarytova, V. F.; Lebedev, A. V.; Federova, O. S., Ed.; CRC Press: Boca Raton, **1994**.
3. (a) Agrawal, S.; Zhang, X.; Lu, Z.; Zhao, H.; Tamburin, J. M.; Yan, J.; Cai, H.; Diasio, R. B.; Habus, I.; Jiang, Z.; Iyer, R. P.; Yu, D.; Zhang, R. *Biochem. Pharmacol.* **1995**, *50*, 571-576. (b) Crooke, S. T.; Grillone, L. R.; Tendolkar, A.; Garrett, A.; Fraktin, M. J.; Leeds, J.; Barr, W. H. *Biochem. Pharmacol.* **1994**, *56*, 641-646.
4. It is roughly estimated that for a systemic dosing for treatment of a chronic

disease about 700 to 900 kilograms per year of drug is needed to meet the present demand.

5. (a) Sinha, N. D.; Biernat, J.; Köster, H. *Tetrahedron Lett.* **1983**, *24*, 5843-5846. (b) Lyttle, M. H.; Wright, P. B.; Sinha, N. D.; Bain, J. D.; Chamberlin, A. R. *J. Org. Chem.* **1991**, *56*, 4608-4615.
6. Letsinger, R. L.; Groody, E. P.; Lander, N.; Tanaka, T. *Tetrahedron* **1984**, *40*, 137-143 and references cited therein.
7. (a) Schwarz, M. W.; Pfleiderer, W. *Tetrahedron Lett.* **1984**, *25*, 5513-5516. (b) Hamamoto, S.; Takaku, H. *Chem. Lett.* **1986**, 1401-1404.
8. (a) Bergmann, F.; Kueng, E.; Iaiza, P.; Bannwarth, W. *Tetrahedron* **1995**, *51*, 6971-6976. (b) Hayakawa, Y.; Wakabayashi, S.; Kato, H.; Noyori, R. *J. Am. Chem. Soc.* **1990**, *112*, 1691-1696.
9. Cologne, J.; Poilane, G. *Bull. Soc. Chim. France,* **1955**, 953-955.
10. Commercially available from Aldrich Chemical Co.
11. (a) Iyer, R. P.; Philips, L. R.; Egan, W.; Regan, J. B.; Beaucage, S. L. *J. Org. Chem.* **1990**, *55*, 4693-4699. (b) Iyer, R. P.; Egan, W.; Regan, J. B.; Beaucage, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 1253-1254.
12. Stepkowski, S. M.; Tu, Y.; Condon, T. P.; Bennett, C. F. *J. Immunol.*, **1994**, 5336-5346.
13. We calculated the cost of manufacturing cyanoethyl and CB phosphoramidites at kilogram scales and found that the latter could be produced at approximately 60% of the cost of cyanoethyl amidite synthons.