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Diastereocontrolled Synthesis of Dinucleoside Phosphorothioates Using a Novel Class of Activators, Dialkyl(cyanomethyl)ammonium Tetrafluoroborates

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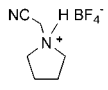
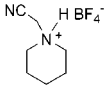
Recent studies have shown that the properties of oligodeoxyribonucleoside phosphorothioates (PS-ODNs) are affected by the chirality of the phosphorus atoms.[1] However, the PS-ODNs, synthesized by the conventional phosphoramidite method,[2] are mixtures of diastereomers.[3] Thus, stereoselective syntheses of PS-ODNs have been extensively studied.[4] Among them, Stec et al.[4a] and Beaucage et al.[4b] have reported the fully *P*-stereocontrolled synthesis of PS-ODNs. However, in their methods, the diastereopure monomers had to be separated from a mixture of diastereomers by troublesome column chromatography.

The phosphoramidite methods utilizing amino alcohols as chiral auxiliaries have been reported in recent years.[5] The advantage of these methods is that the diastereopure monomers can be obtained diastereoselectively from the appropriate enantiopure amino alcohols. Despite this advantage, the condensation reactions are more or less nonstereospecific. The nonstereospecificity would be attributed to the repetitive attack of a nucleophilic activator, such as 1*H*-tetrazole, to the phosphorus atom.

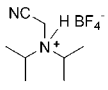
Under these circumstances, we tried to develop a new activator, which did not generate a nucleophilic anion species, with the expectation that such an activator would preferentially promote a nucleophilic substitution at the phosphorus atom of a protonated phosphoramidite by the hydroxy group of a nucleoside, while depressing the repetitive attack of the activator to the phosphorus atom, to give the corresponding dinucleoside phosphite without any loss of the enantiopurity of the phosphorus atom. To develop such an activator, the activator would have appropriate proton-donating ability to the nitrogen of a phosphoramidite and contain a highly less nucleophilic counteranion. On the basis of this consideration we developed a new class of activators, dialkyl(cyanomethyl)-ammonium tetrafluoroborates **1a**−**c**. The activators **1a**−**c** could be easily obtained from the corresponding amines and tetrafluoroboric acid etherate.



1a



1b

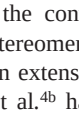
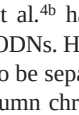
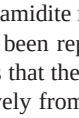
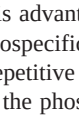


1c

In the present study, we synthesized diastereopure nucleoside 3′-phosphoramidites **3** by using appropriate enantiopure amino alcohols, (*S*)- and (*R*)-2-methylamino-1-phenylethanol[6] (**2a**,**b**), (1*R*,2*S*)-ephedrine (**2c**), and (1*R*,2*S*)-2-methylamino-1,2-diphenylethanol[7] (**2d**) as the components of the oxazaphospholidine rings. Compounds **3a**−**d** were synthesized by applying the method in the literature with some modifications.[5a]

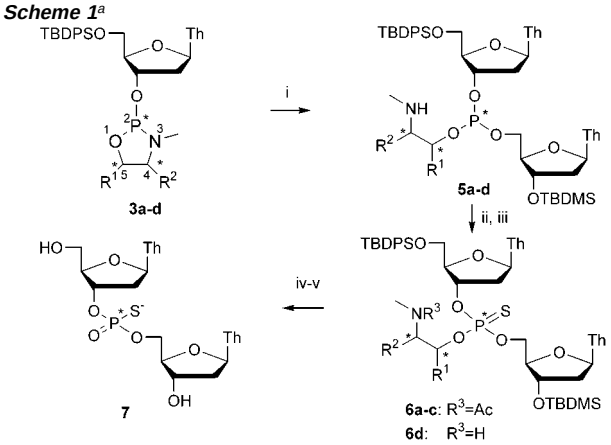
Diastereopure nucleoside 3′-phosphoramidites **3a**−**d** thus obtained were allowed to condense with 3′-*O*-(*tert*-butyldimethylsilyl)-thymidine (**4a**, 1 equiv) in the presence of **1a**−**c** (2 equiv) in CH_3CN-CD_3CN (4:1, v/v), and the reactions were monitored by ^{31}P NMR (Table 1).The reaction time required for completion was affected by the steric and electronic factors of the R^2 group in the oxazaphospholidine ring. The condensations of **3a**,**b** (R^2 = H) with **4a** proceeded quickly and completed within 5 min. On the other hand, the condensations of **3c** (R^2 = Me) and **3d** (R^2 = Ph) with **4a** required 15 min and 5 h, respectively, to complete. These results indicate that it is important for a rapid condensation to diminish the steric hindrance and electron-withdrawing character of the R^2 group.

Table 1. Condensations of Nucleoside 3′-Phosphoramidites **3a**−**d** with **4a** in the Presence of **1a**−**c**

phosphoramidite[a]	activator	reaction time	*R*p-**5** : *S*p-**5**[b]
3a 	**1a**	< 5 min	>99:1 (140.1)
	1b		98:2 (140.2, 138.4)
	1c		28:72 (140.1, 138.4)
3b 	**1a**	< 5 min	4:96 (138.1, 140.1)
	1b		4:96 (138.1, 140.0)
	1c		45:55 (138.1, 140.0)
3c 	**1a**	< 15 min	5:95 (137.4, 139.8)
	1b		4:96 (137.4, 139.8)
	1c		74:26 (137.4, 139.7)
3d 	**1a**	5 h	7:93 (138.0, 140.4)
	1b		12:88 (138.0, 140.5)
	1c		76:24 (138.0, 140.4)

[a] R = 5′-*O*-(*tert*-butyldiphenylsilyl)thymidin-3′-yl. [b] The chemical shifts of ^{31}P NMR are given in parentheses.

Scheme 1[a]



[a] Reagents and conditions: (i) 3′-*O*-(TBDMS)thymidine (**4a**), activating agent **1a**−**c**, CH_3CN-CD_3CN (4:1, v/v), room temperature; (ii) Ac_2O, pyridine; (iii) Beaucage reagent; (iv) DBU; (v) $Et_3N{\cdot}3HF$.

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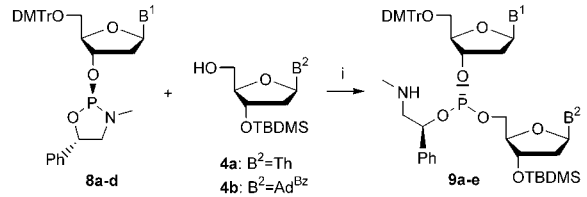
Scheme 2[a]



[a] Reagents and conditions: (i) activating agent **1a**, CH_3CN-CD_3CN (4:1, v/v), room temperature, <5 min.

Table 2. Condensations of 5′-*O*-(DMTr)nucleoside 3′-Phosphoramidites **8a**−**d** with **4** in the Presence of **1a**

phosphoramidite	B^1	B^2	phosphite	***Rp*-9**:**Sp-9**[a]
8a	Th	Th	**9a**	>99:1 (139.6)
8b	Ad^{Bz}	Th	**9b**	98:2 (139.4, 138.8)
8c	Cy^{Bz}	Th	**9c**	>99:1 (138.9)
8d[b]	Gu^{Pa}	Th	**9d**[b]	98:2 (141.2, 139.6)
8a	Th	Ad^{Bz}	**9e**	98:2 (142.5, 142.3)

[a] The chemical shifts of ^{31}P NMR are given in parentheses. [b] Gu^{Pa} = 2-*N*-phenylacetylguanin-9-yl.

The diastereoselectivity of the condensations varied with the activators; in the cases of **1a**,**b**, the condensations resulted in good to excellent diastereoselectivity, especially, the condensation of **3a** with **4a** in the presence of **1a** gave only one diastereoisomer of the corresponding phosphite **5a**.[9] The result is in contrast with the fact that the condensation proceeded very slowly with low diastereoselectivity when a conventional activator, 1*H*-tetrazole, was used in place of **1**. The diastereoselectivity changed dramatically, when **1c** was used; **1c** rather promoted the formation of the other diastereomer, although the reason is still unclear.

The resultant phosphites **5** were subjected to sulfurize by the Beaucage reagent.[10] The sulfurization of **5d** proceeded smoothly by using the Beaucage reagent. In the cases of **5a**−**c**, however, the formation of some unidentified byproducts was detected by ^{31}P NMR analyses; these byproducts would arise from some reaction of the secondary amino groups in **5a**−**c** with the Beaucage reagent and/or its residue. On the basis of the consideration, the amino groups were acetylated before sulfurization. This acetylation eliminated the undesired side reaction.

After sulfurization, the chiral auxiliary in **6a**−**c** was removed by treatment with 10 equiv of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) at 50 °C to give 5′-*O*- and 3′-*O*-silylated dithymidine phosphorothioate within 30 min.[11,12] Finally, the 5′-*O*- and 3′-*O*-silyl groups were removed by treatment with $Et_3N\cdot 3HF$,[13] and purification by reverse-phase column chromatography gave fully deprotected dithymidine phosphorothioate (**7**) in good total yield.[14] The reverse-phase HPLC analysis showed that the diastereomer ratio was preserved during the deprotection steps; the phosphorothioate **7**, obtained from **3a** upon activating with **1a**, was almost diastereopure (*R*p:*S*p = 99:1).[9,15]

Next, the most effective activating agent **1a** was applied to the reactions of 5′-*O*-(DMTr)nucleoside 3′-phosphoramidites (**8a**−**d**) with nucleosides **4a**,**b** (Scheme 2). It was noteworthy that no cleavage of the 5′-*O*-DMTr group during the reactions was detected by TLC analyses. In all cases, the ^{31}P NMR analyses showed that the reactions proceeded quickly with excellent diastereoselectivity (Table 2).[9]

To elucidate the mechanism for the present diasteroselective condensation, ab initio molecular orbital calculations were carried out for *N*-protonated (2*S*,5*R*)-2-methoxy-3-methyl-5-phenyl-1,3,2-oxazaphospholidine (**10**) as a model of *N*-protonated cyclic phosphoramidite intermediates.[9] The optimized geometry and the LUMO of **10**, obtained at the HF/6-31G* level, indicate that there exist two possible directions for the nucleophilic attack at the phosphorus atom. The hydroxy group of a deoxyribonucleoside would preferentially attack at the phosphorus atom from the backside of the protonated nitrogen atom via an *in-line* mechanism with the inversion of the *P*-configuration to give the corresponding phosphite triester as a major product. On the contrary, the nucleophilic attack of the hydroxy group from the front side of the protonated nitrogen atom would give rise to the product with the retention of the *P*-configuration. The later process is, however, inherently interrupted because of the steric hindrance of the *N*-methyl group. Thus, the dithymidine phosphorothioate would be diastereoselectively obtained through an *in-line* mechanism.

In summary, a new class of activators, dialkyl(cyanomethyl)-ammonium tetrafluoroborates **1**, were found to be effective for the stereospecific internucleotidic bond formation of diastereopure nucleoside 3′-cyclic phosphoramidites **3a**−**d** and **8a**−**d** with deoxyribonucleosides. It was also found that both the rate of condensation and diastereoselectivity were affected by the steric and electronic factors of the substituent at the 4-position of the oxazaphospholidine ring in **3a**−**d**. The solid-phase synthesis of *P*-stereodefined PS-ODNs by the present approach is now in progress.

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Supporting Information Available: ^1H, ^{13}C, and ^{31}P NMR spectra of **3a**−**d**, **7** and **8a**−**d**; ^{31}P NMR spectra of the mixtures obtained by the reaction of **3a** with **4a** in the presence of **1a** or **1c**, and those of **8a**−**d** with **4a**,**b** in the presence of **1a**, reverse-phase HPLC chromatograms of **7**, the optimized geometry and the LUMO of **10** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Yu, D.; Kandimalla, E. R.; Roskey, A.; Zhao, Q.; Chen, L.; Chen, J.; Agrawal, S. *Bioorg. Med. Chem.* **2000**, *8*, 275−284 and references therein.
(2) For a review, see: Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1992**, *48*, 2223−2311.
(3) Stec, W. J.; Wilk, A. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 709−722 and references therein.
(4) (a) Stec, W. J.; Karwowski, B.; Boczkowska, M.; Guga, P.; Koziolkiewicz, M.; Sochacki, M.; Wieczorek, M. W.; Blaszczyk, J. *J. Am. Chem. Soc.* **1998**, *120*, 7156−7167. (b) Wilk, A.; Grajkowski, A.; Phillips, L. R.; Beaucage, S. L. *J. Am. Chem. Soc.* **2000**, *122*, 2149−2156. (c) Lu, Y.; Just, G. *Angew. Chem., Int. Ed.* **2000**, *39*, 4521−4524.
(5) (a) Iyer, R. P.; Yu, D.; Ho, N.-H.; Tan, W.; Agrawal, S. *Tetrahedron: Asymmetry* **1995**, *6*, 1051−1054. (b) Iyer, R. P.; Guo, M.-J.; Yu, D.; Agrawal, S. *Tetrahedron Lett.* **1998**, *39*, 2491−2494. (c) Lu, Y.; Just, G. *Tetrahedron* **2001**, *57*, 1677−1687.
(6) Coote, S. J.; Davies, S. G.; Middlemiss, D.; Naylor, A. *J. Chem. Soc., Perkin Trans. 1* **1989**, *12*, 2223−2228.
(7) Enantiopure (1*R*,2*S*)-**2d** was prepared from (1*R*,2*S*)-2-amino-1,2-diphenylethanol.[8]
(8) (a) Saigo, K.; Ogawa, S.; Kikuchi, S.; Kasahara, A.; Nohira, H. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 1568−1573. (b) Saigo, K.; Sugiura, I.; Shida, I.; Tachibana, K.; Hasegawa, M. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 2915−2916.
(9) See the Supporting Information.
(10) Iyer, R. P.; Egan, W.; Regan, J. B.; Beaucage, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 1253−1254.
(11) The chiral auxiliary of **6d**, which was not acetylated, was removed within 4 h.
(12) We presumed that the oxygen atom of the enolate, generated from the *N*-acetyl group, attacked the benzyl carbon in **6a**,**b** to release the chiral auxiliary as 2-methylene-3-methyl-5-phenyl-1,3-oxazolidine.
(13) Gasparutto, D.; Livache, T.; Bazin, H.; Duplaa, A.-M.; Guy, A.; Khorlin, A.; Molko, D.; Roget, A.; Teoule, R. *Nucleic Acids Res.* **1992**, *20*, 5159−5166.
(14) Isolated total yields of **7** were 74 and 68% from **3a** and **3b**, respectively (5 steps).
(15) (*R*p)-TpsT eluted faster than (*S*p)-TpsT in a reverse-phase HPLC. Wada, T.; Kobayashi, N.; Mori, T.; Sekine, M. *Nucleosides Nucleotides* **1998**, *17* (1−3), 351−364.

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