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Nitromethane as a scavenger of acrylonitrile in the deprotection of synthetic oligonucleotides

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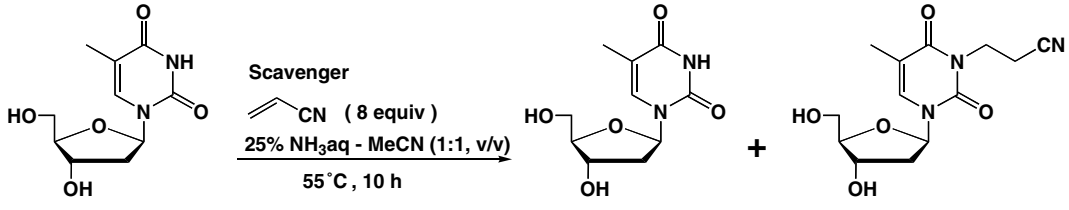
Abstract—A novel deprotection procedure for synthetic oligonucleotides was developed to prevent nucleobase alkylation. Acrylonitrile, a side product of the deprotection of a 2-cyanoethyl phosphate protecting group and which causes nucleobase alkylation, was found to be trapped by the addition of some acidic compounds, which generate a carbanion species under the conventional deprotection conditions using aqueous NH_3. The 2-cyanoethylation of thymidine was inhibited effectively in the presence of nitromethane.

Chemically synthesized oligonucleotides have become important for a wide range of applications such as biological research and oligonucleotide therapeutics. Currently, oligonucleotides are synthesized automatically by a stepwise coupling of phosphoramidite building blocks.[1] 2-Cyanoethyl is widely used as a protecting group of phosphates, which is removed under the basic conditions via a β-elimination mechanism.[2] However, acrylonitrile, a side product of the deprotection reaction, is a toxic compound and a potential carcinogen. In large-scale synthesis of oligonucleotides, their nucleobases, especially thymine moieties, are alkylated to some extent by acrylonitrile via the Michael-type addition.[3,4] In order to avoid the undesired side reaction, new procedures for the deprotection of synthetic oligodeoxyribonucleotides and their thiophosphate analogs have been reported. For example, a mixture of Et_3N and MeCN is used for the deprotection of 2-cyanoethyl thiophosphates on solid supports before ammonia treatment.[4] Moreover, new protecting groups for phosphates other than 2-cyanoethyl have been also reported such as 2-trialkylsilylethyl,[5] allyl,[6] and the protecting groups are removed via intramolecular ring formation processes.[3,7,8] In this study, we wish to report a new deprotection procedure for a 2-cyanoethyl group to prevent nucleobase-alkylation in the presence of an acidic compound as a scavenger of acrylonitrile.

A scavenger must react with acrylonitrile much faster than thymine or other nucleobases, and the resulting Michael adduct of acrylonitrile should be stable under basic conditions. If the Michael adduct degraded to regenerate acrylonitrile, the scavenger would not work effectively. As a model reaction for the deprotection of



Scheme 1. The simulation of the side reaction under the deprotection condition for oligonucleotide synthesis.

Keywords: Oligonucleotide; Chemical synthesis; 2-Cyanoethylation.
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Table 1. 2-Cyanoethylation of thymidine in the presence or absence of scavengers

Entry	Conditions		Ratio of N^3-(2-cyanoethyl)thymidine (%)[a]
	Base	Scavenger (8 equiv)	
1	25% NH_3aq–MeCN (1:1, v/v)	—	6.8
2[b]		Thymine	5.7
3		Trifluoroacetamide	4.6
4		1,3-Diphenylacetone	1.8
5		Nitromethane	0.4
6	0.25 M $MeNH_2$ in H_2O–EtOH–MeCN (1:1:6, v/v/v)	—	Not detected
7	0.25 M DBU in MeCN	—	*N*- and *O*-Cyanoethylation[c]
8		Nitromethane	0.5

[a] The ratios were estimated by HPLC.
[b] Thymine was not dissolved completely throughout the reaction.
[c] Thymidine was completely consumed during the reaction.

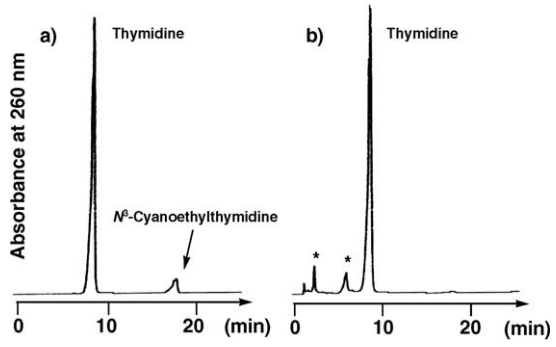

Figure 1. RP-HPLC analyses of the reaction mixtures of thymidine with acrylonitrile in 25% NH_3aq–MeCN (1:1, v/v). (a) without scavenger; (b) with nitromethane. *The Michael adducts, 4-nitrobutanenitrile and 4-nitroheptanedinitrile.[10]

synthetic oligonucleotides in a large scale, thymidine (0.1 mmol) was allowed to react with acrylonitrile (0.8 mmol) in 25% NH_3aq–MeCN (1:1, v/v, 1 ml). After adding a scavenger (0.8 mmol), the reaction mixture was heated to 55 °C for 10 h in a screw-capped vial (Scheme 1). The reaction mixture was then cooled to rt, and NH_3 was removed under reduced pressure. The aqueous layer was washed with ether and concentrated under reduced pressure. The crude mixture was analyzed by reverse-phase HPLC (Table 1 and Fig. 1). When the reaction was carried out in the absence of any scavenger, about 7% of N^3-(2-cyanoethyl)thymidine was detected by HPLC analysis (Fig. 1a). Then, we tested several kinds of acidic compounds as scavengers of acrylonitrile. The scavengers were selected on the basis of their pK_a values in DMSO.[9] At first, thymine (pK_a 14.1 for uracil in DMSO) was tested as a scavenger, but the 2-cyanoethylation of thymidine was not suppressed (entry 2). In this reaction, only 35% of thymine was cyanoethylated. Trifluoroacetamide (pK_a 17.2 in DMSO) was also ineffective as a scavenger for the N^3-alkylation (entry 3). In the presence of compounds bearing an acidic C–H, such as 1,3-diphenylacetone (pK_a 18.7 in DMSO) and nitromethane (pK_a 17.2 in DMSO), the N^3-alkylation was inhibited effectively (entries 4 and 5). Especially in the presence of nitromethane as a scavenger, only 0.4% of N^3-cyanoethylthymidine was detected (Fig. 1b). In spite of the fact that nitromethane and trifluoroacetamide have the same pK_a value, the efficiency of these scavengers was considerably different. These results indicate that the efficiency of the scavengers depends both on their acidity and the stability of the resulting Michael adducts.

When CH_3NH_2[11] was used as a base in the place of ammonia, N^3-alkylation was not observed (entry 6). In this case, excess CH_3NH_2 would act as a scavenger, because CH_3NH_2 is more reactive than ammonia with acrylonitrile. When 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was used as a strong non-nucleophilic base, thymidine was quickly consumed, and several peaks corresponding to the N^3-alkylated thymidine and N^3-,3′-*O*-, N^3-,5′-*O*- and N^3-,3′-*O*-,5′-*O*- alkylated products were observed (entry 7). In contrast, the addition of nitromethane to this system effectively reduced the formation of the alkylated products (entry 8).

In addition, by the GC–MS analysis of the reaction mixture of nitromethane and acrylonitrile in 25% NH_3aq–MeCN (1:3, v/v), the Michael adduct, 4-nitrobutanenitrile, was detected (*m*/*z* 115).

It was found that the addition of nitromethane did not affect the deprotection of 2-cyanoethyl phosphate protecting group and the nucleobase protecting groups for N^6-benzoyldeoxyadenosine, N^4-benzoyldeoxycytidine, and N^2-isobutylyldeoxyguanosine. Moreover no side reactions were observed in all the cases.

In conclusion, nitromethane was found to be effective as a scavenger of acrylonitrile to prevent nucleobase alkylation under basic conditions, which are similar to the deprotection conditions of synthetic oligonucleotides in large scale. The present deprotection procedure will be useful for other organic compounds involving protecting groups which have to be deprotected via β-elimination processes to produce reactive olefins.

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References and notes

1. (a) Matteucci, M. D.; Caruthers, M. D. *J. Am. Chem. Soc.* **1981**, *103*, 3185–3191; (b) Beaucarge, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859–1862.
2. (a) Sinha, N. D.; Biernat, J.; Köster, H. *Tetrahedron Lett.* **1983**, *24*, 5843–5846; (b) Sinha, N. D.; Biernat, J.; McManus, J.; Köster, H. *Nucleic Acids Res.* **1984**, *12*, 4539–4557.
3. Wilk, A.; Grajikowski, A.; Phillips, L. R.; Beaucage, S. L. *J. Org. Chem.* **1999**, *64*, 7515–7522.
4. Capaldi, D. C.; Gaus, H.; Kortz, A. H.; Arnold, J.; Carty, R. L.; Moore, M. N.; Scozzari, A. N.; Lowery, K.; Cole, D. L.; Ravikumar, V. T. *Org. Process Res. Dev.* **2003**, *7*, 832–838.
5. (a) Wada, T.; Sekine, M. *Tetrahedron Lett.* **1994**, *35*, 757–760; (b) Kortz, A. H.; Wheeler, P.; Ravikumar, V. T. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2406–2409; (c) Ravikumar, V. T. *Synth. Commun.* **1995**, *25*, 2161–2164; (d) Ravikumar, V. T.; Cole, D. L. *Gene* **1994**, *149*, 157–161; (e) Ravikumar, V. T.; Wyrzykiewicz, T. K.; Cole, D. L. *Tetrahedron* **1994**, *50*, 9225–9266; (f) Ravikumar, V. T.; Sasmor, H.; Cole, D. L. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2637–2640; (g) Dagland, G. T.; Meyer, A.; Imbach, J.-L.; Movan, F. *Eur. J. Org. Chem.* **2003**, 2327–2335.
6. (a) Bergmann, F.; Kueng, E.; Iaiza, P.; Bannwarth, W. *Tetrahedron* **1995**, *25*, 6971–6976; (b) Hayakawa, Y.; Hirose, M.; Hayakawa, M.; Noyori, R. *J. Org. Chem.* **1995**, *60*, 925–930; (c) Hayakawa, Y.; Wakabayashi, S.; Kato, H.; Noyori, R. *J. Am. Chem. Soc.* **1990**, *112*, 1691–1696; (d) Hayakawa, Y.; Uchiyama, M.; Kato, H.; Noyori, R. *Tetrahedron Lett.* **1985**, *39*, 6505–6508; (e) Bogdan, F.; Chow, C. S. *Tetrahedron Lett.* **1998**, *39*, 1897–1900; (f) Manoharan, M.; Lu, Y.; Casper, M. D.; Just, G. *Org. Lett.* **2000**, *2*, 243–246.
7. (a) Guzaev, A. P.; Manoharan, M. *Tetrahedron Lett.* **2000**, *41*, 5623–5626; (b) Guzaev, A. P.; Manoharan, M. *J. Am. Chem. Soc.* **2001**, *123*, 783–793.
8. (a) Wilk, A.; Srinivasachar, K.; Beaucage, S. L. *J. Org. Chem.* **1997**, *62*, 6712–6713; (b) Grajikowski, A.; Wilk, A.; Chimielewski, M. K.; Phillips, L. R.; Beaucage, S. L. *Org. Lett.* **2001**, *3*, 1287–1290; (c) Wilk, A.; Chimielewski, M. K.; Grajikowski, A.; Phillips, L. R.; Beaucage, S. L. *J. Org. Chem.* **2002**, *67*, 6430–6438; (d) Ciesilak, J.; Grajkowski, A.; Livengood, V.; Beaucage, S. L. *J. Org. Chem.* **2004**, *69*, 2509–2515; (e) Cieslak, J.; Beaucage, S. L. *J. Org. Chem.* **2003**, *68*, 10123–10129.
9. Bordwell, F. G. *Acc. Chem. Res.* **1988**, *21*, 463–469.
10. Danilenko, V. M.; Tishkov, A. A.; Ioffe, S. L.; Lyapkalo, I. M.; Strelenko, Y. A.; Tartakovsky, V. A. *Synthesis* **2002**, *5*, 635–647.
11. There is a limitation in the use of CH_3NH_2 for the deprotection of oligonucleotides. Only acetyl can be used as a protecting group for the cytosine base. If a stable acyl group is used as an N^4-protecting group, nucleophilic attack occurs to the C-4 carbon by CH_3NH_2 before the acyl group is removed, and the cytosine base is converted into N^4-methylcytosine. Reddy, M. P.; Hanna, N. B.; Farooqui, F. *Tetrahedron Lett.* **1994**, *35*, 4311–4314.