Tetrahedron Letters 49 (2008) 5144–5146

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Postsynthetic modification of DNA via threoninol on a solid support by means of allylic protection

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ARTICLE INFO

Article history:
Received 13 May 2008
Revised 17 June 2008
Accepted 20 June 2008
Available online 25 June 2008

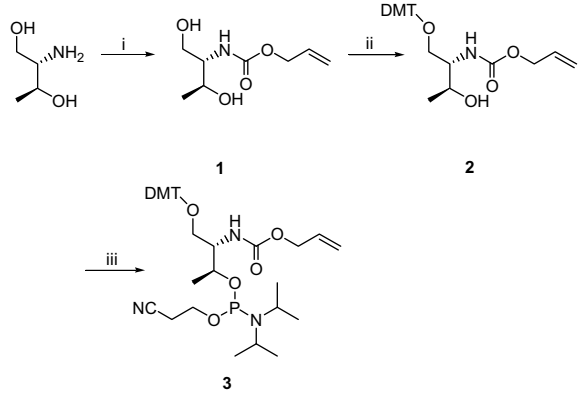
ABSTRACT

We have developed a facile but versatile method to introduce functional molecules into DNA on a CPG support. Threoninol, whose amino group was protected with an (allyloxy)carbonyl (Alloc) group, was introduced into DNA via the corresponding phosphoramidite monomer. After selective deprotection of the Alloc group by treatment with palladium(0), a dye with a carboxyl group could be introduced into the DNA on the CPG support through an amide bond.

Recent advances in phosphoramidite chemistry have enabled a wide variety of chemical modifications of oligonucleotides.[1] The growing field of DNA/RNA research requires further advances in synthetic chemistry for DNA modification. Postsynthetic modification methods on solid supports have been developed to facilitate the functionalization of DNA:[1c] oligonucleotides involving specifically masked functional groups or a designed convertible unit can be synthesized on an automated DNA synthesizer, followed by introduction of functional molecules on the solid support.[2,3] Postsynthetic modification is effective for the introduction of functional molecules that are hard to convert to phosphoramidite monomers. Furthermore, it should be also very effective in diversifying the functionality of modified DNA, since we do not have to synthesize each phosphoramidite precursor.

Recently, we found that D-threoninol is an excellent internucleosidic linker (base surrogate) for the introduction of functional molecules.[4] By tethering azobenzene or pyrene we successfully synthesized photo-responsive oligonucleotides, fluorescent probes, and molecular clusters.[4,5] These modified DNAs were synthesized via the corresponding phosphoramidite monomers. Our previous study on the optimization of azobenzene required the syntheses of as many as eight different monomers of azobenzene derivatives, which was highly time-consuming.[6] Although a postsynthetic approach is very effective in avoiding such problems, little research has been reported on facile methods for introducing functional molecules into oligonucleotides via amino groups on solid support, except for the photo-labile strategy.[2]

Allyl and (allyloxy)carbonyl (Alloc) groups are useful protectors for hydroxyl, carboxyl, and amino groups, and can be removed by a palladium(0)-catalyzed reaction under mild conditions.[7] Recently, Seitz et al. reported on the solid-phase modification of PNA with Alloc protection.[8] Here, we report a facile and versatile method to introduce functional molecules into oligonucleotides through amide bonds on a solid phase via an Alloc-protected phosphoramidite sub-monomer as illustrated in Schemes 1 and 2: the sub-monomer **3** is incorporated into DNA through the automated DNA synthesizer. The obtained controlled pore glass (CPG) support anchoring fully protected DNA is treated with palladium(0) to remove the Alloc group selectively, followed by the coupling with a functional molecule through an amide bond. The desired DNA is finally obtained by deprotection with aqueous ammonia solution. By this method, we can easily diversify the functionality of DNA. Furthermore, functional molecules that are difficult to convert to



Scheme 1. Synthesis of Alloc-protected phosphoramidite monomer. Reagents and conditions: (i) allyl chloroformate, triethylamine, THF, 0 °C, 15 min, then rt, 1.5 h, 97%; (ii) dimethoxytrityl chloride, DIPEA, pyridine, CH_2Cl_2, rt, 4.5 h, 79%; (iii) $(iPr_2N)_2PO(CH_2)_2CN$, 1*H*-tetrazole, acetonitrile, rt, 1.5 h, >99%.

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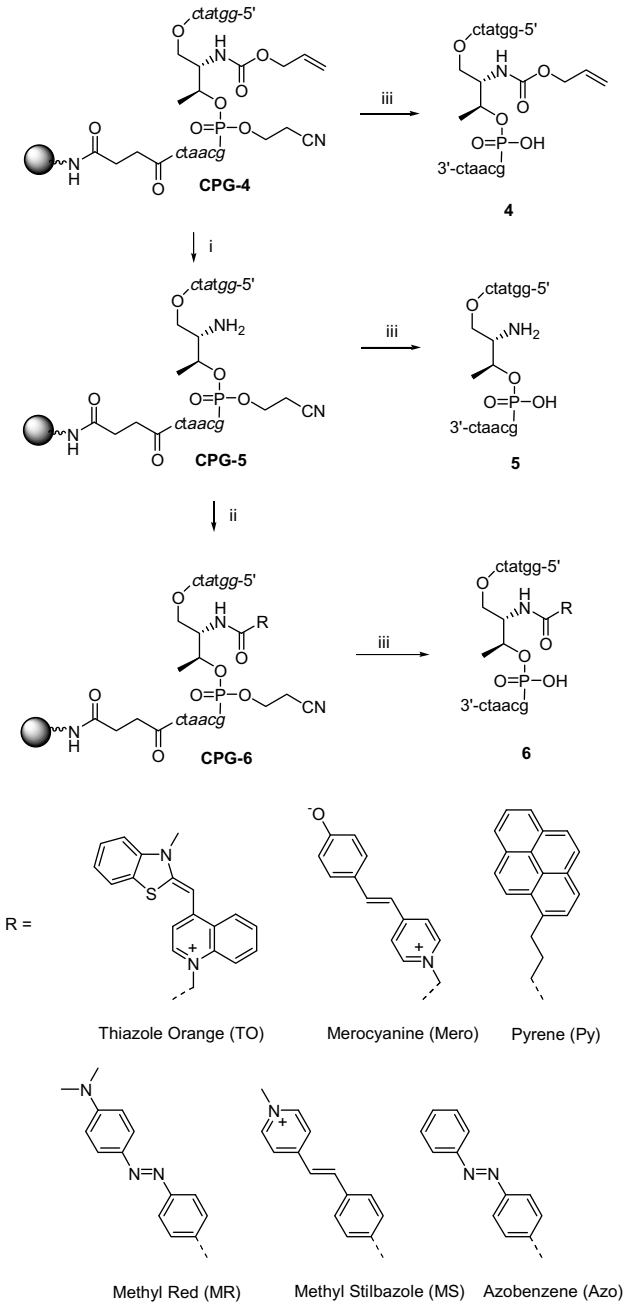
doi:10.1016/j.tetlet.2008.06.090



Scheme 2. Deprotection of Alloc and amide coupling. Reagents and conditions: (i) $Pd(P(Ph)_3)_4$, *N*-methylaniline, CH_2Cl_2, rt, 3 h; (ii) RCOOH, PyBOP, PPTS, *N*-methylmorpholine, DMF, rt 3 days; (iii) 25% aq ammonia, 50 °C, 12 h.

a phosphoramidite monomer can be incorporated into DNA via the single phosphoramidite precursor **3**.

The actual procedure for the preparation of the sub-monomer **3** is shown in Scheme 1: D-threoninol was reacted with allyl chloroformate in THF in the presence of triethylamine at 0 °C to afford compound **1**. Then its primary hydroxyl group was protected with dimethoxytrityl group (compound **2**), followed by the conversion to phosphoramidite monomer **3**, which was then incorporated into DNA on an automated DNA synthesizer. The coupling efficiency of the obtained sub-monomer **3** was as high as those of conventional phosphoramidite monomers, as estimated from the coloration of the released trityl cation. We synthesized 13-mer DNA involving Alloc-protected D-threoninol residues on CPG support (50 nm pore size, **CPG-4** in Scheme 2), with which optimum conditions for the sufficient removal of Alloc group were determined. The CPG **4** binding 0.45 μmol of DNA was put in a polyethylene syringe equipped with a filter, and was exposed to the mixed solution of $Pd(PPh_3)_4$ and *N*-methylaniline as a scavenger in CH_2Cl_2 or THF (0.5 mL) for 3 h at a constant temperature. After the CPG-supported material was washed with 0.1 M *N*,*N*-diethyldithiocarbamate solution to remove contaminated palladium, it was cleaved from the CPG support and deprotected by treatment with 25% ammonia solution, followed by Poly-Pak treatment to evaluate the removal efficiency from the results of HPLC analysis.[4] Since the Alloc group was not removed at all under the deprotection conditions (ammonia treatment), the removal efficiency could be estimated from the ratio of the peak area of **5** to that of **4** (see Supplementary Fig. 1). We first applied the conditions reported by Hayakawa et al. (Table 1, entry 1): **CPG-4** was exposed with 3 equiv amounts of $Pd(P(Ph)_3)_4$ to Alloc group in THF at 50 °C for 3 h in the presence of a large excess (1000 equiv/Alloc) of *N*-methylaniline as a scavenger.[7] However, only 38% was found to be removed. We tried several conditions to raise the removal efficiency and found that the amount of the catalyst $Pd(P(Ph)_3)_4$ was crucial. As can be seen from entries 4 and 5 in Table 1, addition of 10 equiv amounts (5.2 mg of $Pd(PPh_3)_4$ under the present reaction conditions employed) improved the efficiency (see Supplementary Fig. 1 for the actual HPLC profile). Assumedly, concentration of $Pd(P(Ph)_3)_4$ rather than its absolute amount in the reaction mixture affected the removal efficiency.

After optimizing the conditions for the removal of the Alloc group as above, we next tried to introduce a dye with a carboxyl group on **CPG-5** through an amide bond (**CPG-6**). Here, we used thiazole orange (TO: see Scheme 2) as a target because TO, which is an effective fluorophore in detecting oligonucleotides sequence specifically,[8,9] is rather difficult to convert to a phosphoramidite monomer. Since TO hardly dissolved in DMF, pyridinium *para*-toluenesulfonate (PPTS) was added to the reaction mixture as an acidic additive.[8] After the coupling reaction, the DNA was cleaved from the support to evaluate the coupling efficiency by HPLC analysis. Among the coupling reagents we tried, PyBOP most efficiently accelerated the coupling compared with other reagents. An increase in the amount of PyBOP from 100 to 250 equiv/NH_2 raised the coupling efficiency from 28% to 60% (compare entry 5 with 6 in Table 2). The efficiency after three days coupling became 77% (see Fig. 1 for the HPLC profile).[10] Thus, we could attain the introduction of a dye through threoninol on a CPG-support through an amide bond. By this method, even two TO molecules could be efficiently incorporated into DNA. We also tried another conventional postsynthetic modification:[11] fully deprotected DNA **5** was reacted with *N*-hydroxysuccinimidyl (NHS) ester of merocyanine (Mero in Scheme 2) in aqueous solution under several reaction conditions according to the literature.[12] However, since decomposition of this activated ester proceeded much faster, the target DNA **6** could not be obtained by all means (see Supplementary data).

Table 1
Removal of Alloc group from **CPG-4** by palladium(0)-catalyzed allyl transfer[a]

Entry	$Pd(PPh_3)_4$ (equiv/Alloc)	Solvent	Temp (°C)	Removal efficiency[b] (%)
1	3	THF	50	38
2	3	THF	25	13
3	3	CH_2Cl_2	25	24
4	3	CH_2Cl_2	35	70
5	10	CH_2Cl_2	35	>90

[a] *N*-Methylaniline (1000 equiv/Alloc) was used as an allyl scavenger, and deprotecting reaction was carried out for 3 h.
[b] The removal efficiency was evaluated by reversed phase HPLC analysis from the peak areas of **4** and **5**.

Table 2
Incorporation of a dye to amine of threoninol residue of **CPG-5**[a]

Entry	Dye	Conditions[b]	Coupling efficiency[c] (%)
1	TO	DCC/NHS (100 equiv), 1 day	7.3
2	TO	DCC/HOAt (100 equiv), 1 day	1.3
3	TO	DCC/HOBt (100 equiv), 1 day	3.0
4	TO	HATU (100 equiv), DMF, 1 day	6.4
5	TO	PyBOP (100 equiv), DMF, 1 day	28
6	TO	PyBOP (250 equiv), DMF, 1 day	60
7	TO	PyBOP (250 equiv), DMF, 3 days	77
8	Mero	PyBOP (250 equiv), DMF, 3 days	44
9	Py	PyBOP (250 equiv), DMF, 3 days	>90
10	MR	PyBOP (250 equiv), DMF, 3 days	73
11	MS	PyBOP (250 equiv), DMF, 3 days	50
12	Azo	PyBOP (250 equiv), DMF, 3 days	82

[a] The coupling reaction was conducted in DMF in the presence of PPTS and *N*-methylmorpholine at rt.
[b] Molar ratio of the added coupling reagent with respect to Alloc group on CPG support.
[c] The coupling efficiency was evaluated by reversed phase HPLC analysis from the peak areas of **5** and **6** tethering corresponding dye.

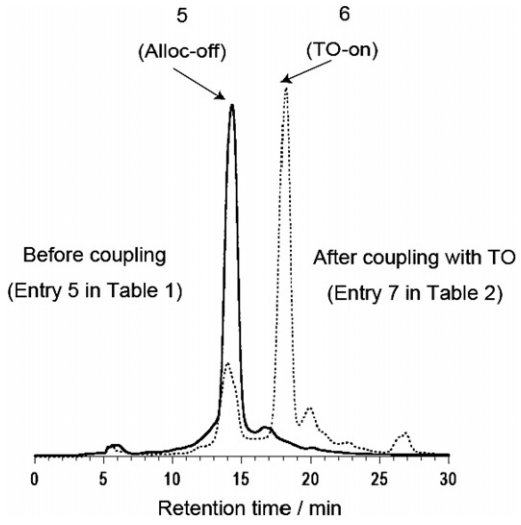


Fig. 1. HPLC profiles of the modified DNAs before (solid line) and after (dotted line) coupling with thiazole orange monitored at 260 nm. The peaks at 14 and 18.5 min correspond to the modified DNAs without (compound **5** in Scheme 2) and with thiazole orange (compound **6** conjugated with thiazole orange in Scheme 2) at threoninol residue, respectively. These peaks were characterized by MALDI-TOFMS after being fractionally collected. Only the peak at 18.5 min had orange color due to the attached TO molecule. HPLC conditions: Reversed phase HPLC (Merck LiChrospher 100 RP-18(e) column) with acetonitrile/water containing 50 mM ammonium formate (pH7.0) as mobile phase with the linear gradient 7.5–17.5% acetonitrile/water (30 min, 0.5 mL/min).

Besides thiazole orange, all the dyes we tried in this study were successfully incorporated into DNA on CPG support with a reasonable efficiency as shown in Table 2. By this method, we could easily diversify the functionality of DNA with a single sub-monomer **3** by varying the functional molecules attached. It should be noted that merocyanine was difficult to introduce into DNA via conventional phosphoramidite chemistry. Even merocyanine that was hard to be tethered to DNA through a conventional coupling in solution could be incorporated into DNA with reasonable yield (Table 2, entry 8). This is one of the merits of the present postsynthetic modification on CPG support. As the present amide coupling proceeds in DMF in which activated ester does not decompose, simply elongating reaction time can raise coupling efficiency. In addition, we can further elongate natural nucleotides and the threoninol units on **CPG-6** with a DNA synthesizer because protecting groups on nucleotides and phosphorus are intact. Accordingly, second dye that is also hard to convert to phosphoramidite monomer can be incorporated by the subsequent deprotection of Alloc group and amide coupling on CPG.[13] This method could be also applied to the modification of 3-amino-1,2-propanediol as well as threoninol, demonstrating the versatility of the present Allylic protection method.

In conclusion, a facile and versatile postsynthetic modification method was developed with Allylic protection. This method allowed diversification of the functionality of DNA via threoninol with a single sub-monomer. By using this method, modification of other functional nucleotides involving amino groups such as 2′-amino-2′-deoxyuridine is expected. We are now investigating the spectroscopic properties of the Mero- and TO-tethered DNAs.

Acknowledgments

This work was supported by Core Research for Evolution Science and Technology (CREST), Japan Science and Technology Agency (JST). Partial supports by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan and The Mitsubishi Foundation (for H.A.) are also acknowledged.

Supplementary data

Experimental procedures for the preparation of compounds **1**–**6**, analytical data for **1**, **2**, **4**–**6**, HPLC profile of Alloc-deprotection (Table 1, entry 5), and reaction conditions of the conventional coupling in solution with Mero. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.06.090.

References and notes

1. (a) Tawarada, R.; Seio, K.; Sekine, M. *J. Org. Chem.* **2008**, *73*, 383–390; (b) Oka, N.; Wada, T.; Saigo, K. *J. Am. Chem. Soc.* **2003**, *125*, 8307–8317; (c) Virta, P.; Katajisto, J.; Niittymäki, T.; Lönnberg, H. *Tetrahedron* **2003**, *59*, 5137–5174 and references cited therein.
2. Hwang, J.-T.; Greenberg, M. M. *J. Org. Chem.* **2001**, *66*, 363–369.
3. (a) Shinozuka, K.; Kohgo, S.; Ozaki, H.; Sawai, H. *Chem. Commun.* **2000**, 59–60; (b) Ozaki, H.; Momiyama, S.; Yokotsuka, K.; Sawai, H. *Tetrahedron Lett.* **2001**, *42*, 677–680.
4. Asanuma, H.; Liang, X. G.; Nishioka, H.; Matsunaga, D.; Liu, M.; Komiyama, M. *Nat. Protocol.* **2007**, *2*, 203–212 and references cited therein.
5. (a) Asanuma, H.; Shirasuka, K.; Takarada, T.; Kashida, H.; Komiyama, M. *J. Am. Chem. Soc.* **2003**, *125*, 2217–2223; (b) Kashida, H.; Asanuma, H.; Komiyama, M. *Chem. Commun.* **2006**, 2768–2770.
6. Nishioka, H.; Liang, X. G.; Kashida, H.; Asanuma, H. *Chem. Commun.* **2007**, 4354–4356.
7. Hayakawa, Y.; Wakabayashi, S.; Kato, H.; Noyori, R. *J. Am. Chem. Soc.* **1990**, *112*, 1691–1696.
8. Jarikote, D. V.; Köhler, O.; Socher, E.; Seitz, O. *Eur. J. Org. Chem.* **2005**, 3187–3195.
9. Constantin, T. P.; Silvia, G. L.; Robertson, K. L.; Hamilton, T. P.; Fague, K.; Waggoner, A. S.; Armitage, B. A. *Org. Lett.* **2008**, *10*, 1561–1564.
10. Dimethoxytrityl (DMT) group was gradually cleaved from the DNA anchored on CPG under these conditions.
11. (a) Reynolds, M. A.; Beck, T. A.; Hogrefe, R. I.; McCaffrey, A.; Arnold, L. J., Jr.; Vaghefi, M. M. *Bioconjugate Chem.* **1992**, *3*, 366–374; (b) Fukui, K.; Morimoto, M.; Segawa, H.; Tanaka, K.; Shimidzu, T. *Bioconjugate Chem.* **1996**, *7*, 349–355.
12. (a) Ami, T.; Fujimoto, K. *Sci. Technol. Adv. Mater.* **2006**, *7*, 249–254; (b) Viladkar, S. M. *Tetrahedron* **2002**, *58*, 495–502.
13. Introduction of two different dyes to threoninols at different positions in DNA is very difficult with the conventional coupling with activated ester in solution.