Pergamon

Tetrahedron Letters 42 (2001) 3669–3672

TETRAHEDRON LETTERS

A novel linker for the solid-phase synthesis of a library of 3′-thiophosphorylated dinucleotides

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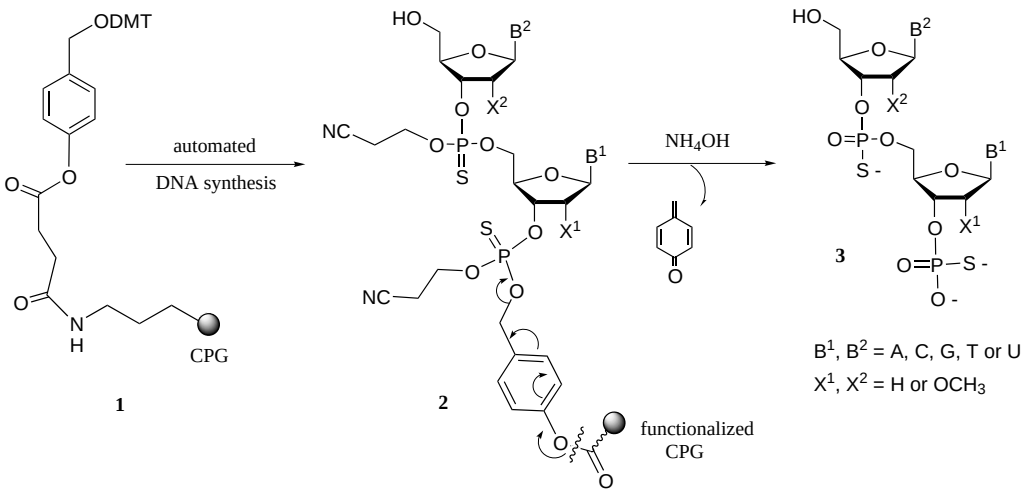
Received 26 February 2001; accepted 29 March 2001

Abstract—The preparation of a controlled-pore-glass (CPG) support carrying a novel linker is described. This support was compatible with the established phosphoramidite method of solid-phase oligonucleotide synthesis. The use of this linker for the synthesis of a library of 3′-thiophosphorylated dinucleotides is described. © 2001 Elsevier Science Ltd. All rights reserved.

Combinatorial libraries of small molecules engineered to participate in non-covalent interactions that mimic those existing between proteins or nucleic acids are a valuable source of biologically relevant chemical diversity for drug discovery. We have reported a nucleic acid-based (NAB™) scaffold as a logical template for library synthesis that could provide functional group and topological diversity through backbone, sugar, and nucleobase modifications.[1] Expanding on this theme, we envisioned the synthesis and evaluation of a nucleotide library that carry 3′-thiophosphate linkages as potential antivirals.[1] Furthermore, nucleotides and oligonucleotides bearing a 3′-thiophosphate moiety can serve as useful intermediates in the synthesis of circularly shaped molecules.[2] Besides serving as '3′-end capping' that protects oligonucleotides against nuclease-mediated degradation, the 3′-thiophosphate group can also be used to site-specifically attach reporter groups to oligonucleotides.[3,4]

Several procedures have been reported that allow incorporation of phosphate or thiophosphate groups at the 3′-terminus in an oligonucleotide.[4] We considered a CPG-based solid-support that could be derivatized with an inexpensive linker that: (a) would withstand the synthesis conditions employed in DNA synthesis and



Scheme 1. Solid-phase synthesis of 3′-phosphorylated dinucleoside phosphorothioates.

Keywords: solid-support; 3′-thiophosphorylation; combinatorial chemistry; automated synthesis; dinucleotides.
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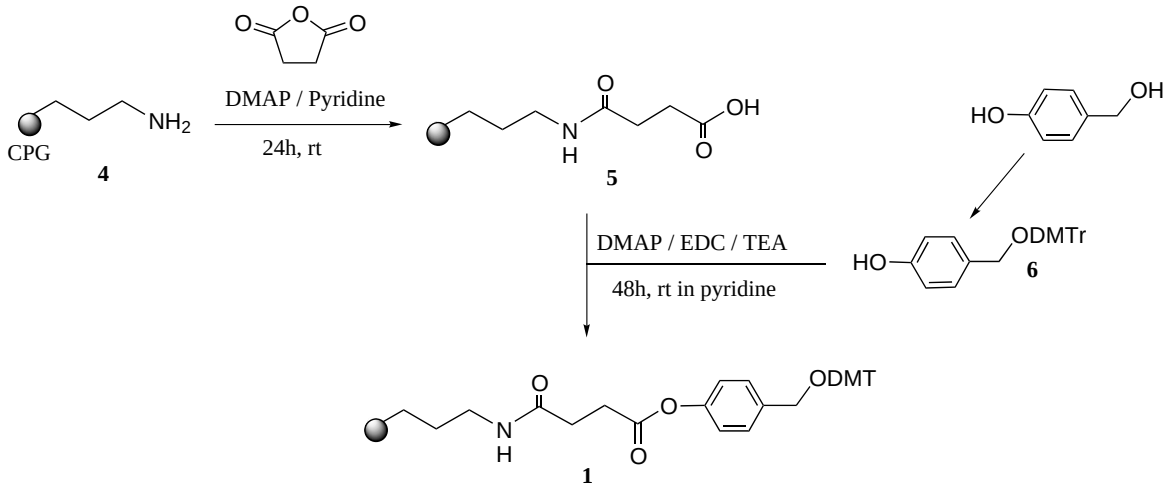
PII: S0040-4039(01)00550-0

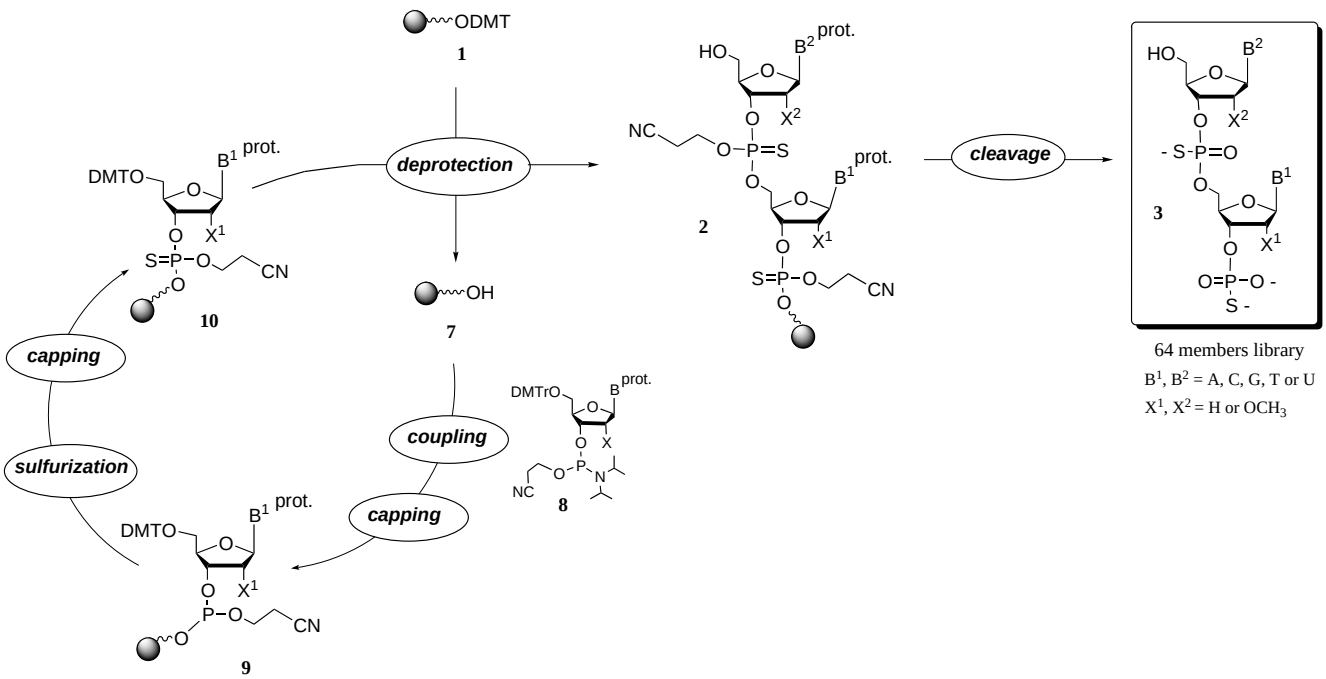
(b) would be susceptible to the aqueous ammonia deprotection/cleavage to release the 3′-thiophosphorylated compounds. Solid-supports with an appropriate tether have been reported by Guzaev[4b] and Markiewicz[4h] but their applicability for combinatorial synthesis has not been explored. We investigated an alternative support based on acyloxyaryl ester chemistry that had been previously employed for the development of oligonucleotide prodrugs.[5] Reported herein is the use of the acyloxyaryl group as a novel universal linker tethered to CPG (**1**) that is amenable to the synthesis of 3′-thiophosphorylated nucleotides. This linker was employed in the solid-phase synthesis of a library of 3′-thiophosphorylated dinucleotides **3** (Scheme 1).

The requisite support-bound acyloxyaryl derivative **1** was prepared as shown in Scheme 2. Thus, treatment of the aminoalkyl-CPG **4** with succinic anhydride led to the succinylated-CPG **5**. Reaction of the derivatized CPG **5** with the alcohol **6** gave the support-bound derivative **1**. The loading of the linker on the support was in the range of 60–90 μmol/g, based upon the 4,4′-dimethoxytrityl cation assay.

With the solid-support **1** in hand, the synthesis of library **3** was initiated in an automated DNA synthesizer on a 15 μmol scale (DMT-off), using two cycles of the standard DNA synthesis protocol that employed phosphoramidite chemistry[6] (Scheme 3). Thus, the deprotection of the 4′,4′-dimethoxytrityl group from **1**,



Scheme 2. Synthesis of the universal solid-support **1**.



Scheme 3. Library assembly. Deprotection: 5% dichloroacetic acid in dichloromethane; coupling: 1*H*-tetrazole in acetonitrile; capping: acetic anhydride and *N*-methylimidazole in THF; sulfurization: 3*H*-1,2-benzodithiole-3-one-1,1-doxiode in CH_3CN; cleavage: 28% aq. NH_4OH, 55°C, overnight.

followed by coupling with the first nucleoside phosphoramidite **8** yielded the phosphite triester **9** (B = A, C, G, T or U; X = H or OCH_3). Oxidative sulfurization of **9** with 3*H*-benzodithiole-3-one-1,1-dioxide[7] led to the thiophosphotriester **10**, which was carried through a second round of synthesis cycle to give the support-bound dinucleotide **2**.

The cleavage of the solid-support and the deprotection of nucleobases and thiophosphate groups were conducted in a single step by treatment with concentrated ammonium hydroxide. The mechanism of cleavage of the acyloxyaryl group is postulated in Scheme 1.

A 64-member library **3** representing 3′-psXY dimer sequences (X,Y = dA, dC, dG, dT, 2′-OMe-rA, 2′-OMe-rC, 2′-OMe-rG or 2′-OMe-rU) was prepared using this approach. Purification of the crude library members was achieved by weak anion-exchange resin (DEAE-5PW) followed by a desalting on C_{18} column. These sequential steps removed the by-products such as benzylamide, isobutyrylamide resulting from the base deprotection, as well as the unreacted 3′-thiophosphate monomer **7**. The purity of the resulting library members, determined by HPLC, ranged between 85 and 99%. The average yield of the library members was 50–55%. ^{31}P, ^{1}H NMR, and MS analysis of selected library members confirmed their identity. There was no evidence of any base modification on the compounds.

In conclusion, we have described the synthesis of a 64-member 3′-thiophosphorylated dinucleotide library using a CPG bearing a novel linker. The method is general and could be applied to the synthesis of other small molecules with a thiophosphate group. With some modifications, the synthesis of 3′-phosphorylated libraries could also be accomplished. Other applications of this solid-support are still in process in our laboratory. These studies, as well as the results of the antiviral evaluation of our libraries against specific viral targets, will be presented elsewhere.

Experimental procedures

Synthesis of the solid-support 1

To a solution of 4-hydroxybenzyl alcohol (1.24 g, 10 mmol) in anhydrous pyridine (30 mL) was added 4,4′-dimethoxytritylchloride (3.38 g, 10 mmol). The mixture was stirred overnight. Pyridine was evaporated under reduced vacuum. Dichloromethane (20 mL) was added, the organic layer was washed with water (3×10 mL) and dried over anhydrous $MgSO_4$. After evaporation of the solvent, the residue was purified by flash chromatography on silica gel (hexane:ethyl acetate = 2:1) to give 3.89 g of product **6** as a yellow solid in 89% yield. ^{1}H NMR ($CDCl_3$, 500 MHz, δ ppm) 3.79 (s, $OC\underline{H}_3$, 6H), 4.07 (s, $OC\underline{H}_2Ar$, 2H), 4.72 (br, OH, 1H), 6.80 (d, J = 8.5 Hz, H-Ar-OH, 2H), 6.83 (d, J = 8.7 Hz, H-Ar-OCH_3, 4H), 7.22 (d, J = 7.6 Hz, H-Ar, 1H), 7.24 (d, J = 8.5 Hz, H-Ar-OH, 2H), 7.29 (t, J = 7.6 Hz, H-Ar, 2H), 7.40 (d, J = 8.7 Hz, H-Ar-OCH_3, 4H), 7.49 (d, J = 7.6 Hz, H-Ar, 2H).

Ether **6** was employed in the synthesis of the solid-support **1** as follows:

(a) Functionalization of the aminopropyl-CPG **4**: Succinic anhydride (20 mmol, 2 g) and DMAP (2 mmol, 244 mg) were added to oven-dried aminopropyl-CPG **4** (10 g) with 80 mL of anhydrous pyridine. The mixture was shaken at room temperature for 24 h. The succinylated CPG **5** was filtered and washed with pyridine (2×50 mL) and dichloromethane (3×50 mL).

(b) Anchorage of the linker: Aralkyl ether **6** (2 mmol, 872 mg) and DMAP (2 mmol, 244 mg) were coevaporated twice with anhydrous pyridine and dried overnight under high vacuum. To this mixture, dissolved in anhydrous pyridine (100 mL), were added the succinylated CPG **5**, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (20 mmol, 3.8 g), and triethylamine (0.8 mL) and then shaken for 48 h.

(c) Capping steps: Pentachlorophenol (5 mmol, 1.35 g) was then added to the mixture which was shaken for an additional 15 h. Piperidine (50 mL) was then added and stirring was applied for only 5 minutes before collecting the resin by filtration and washing with dichloromethane (4×100 mL) and ether (2×100 mL). The dry solid-support was mixed with Cap A (THF/Ac_2O, 9:1) (50 mL) and Cap B (10% *N*-methylimidazole in THF/pyridine, 8:1) (50 mL) for 1 h. CPG was collected by filtration, washed with dichloromethane (3×200 mL) and ether (2×200 mL), and dried overnight under high vacuum prior to use in synthesis.

(d) 4,4′-Dimethoxytrityl cation assay procedure: To a solution of 14 mg of dry solid-support **1** in dichloromethane (70 mL) was added 0.2 mL of perchloric acid. The mixture was slowly stirred for 20 minutes. UV absorbance was measured at 503 nm and the loading was determined.

Assembly of the library 3

The library synthesis was performed on a 15 μmol scale using the standard automated DNA synthesis protocol (DMT-off) used for the oligonucleoside phosphorothioate assembly. After synthesis, the CPG was dried in the column and transferred to 5 mL safe-sealed polypropylene tubes and treated with ammonium hydroxide (28%, 4 mL, 55°C, overnight). After cooling, the solution was concentrated and analyzed by HPLC.[1] The samples showing more than 85% purity were submitted to ethyl acetate extraction (2×1 mL), while the others were purified by anion-exchange resin (DEAE-5PW, gradient of 0.5 M NaCl in H_2O from 0 to 40%) followed by desalting on reverse-phase column (C_{18}, Buffer A: H_2O, Buffer B: 20%CH_3CN in H_2O). In both cases, the residual organic solvents were evaporated before filtration through a 0.2 μm filter. Each product appeared as a white foam after lyophilization. ^{31}P NMR analysis of selected members revealed clear sig-

nals corresponding to the triester phosphorothioate (doublet ca. δ 58 and 61 ppm) and diester phosphorothioate (single peak ca. δ 47 ppm). ^{1}H NMR and MS were also in agreement with the expected structures.

Acknowledgements

We thank Julie Ann Cabana for help with the manuscript.

References

1. Jin, Y.; Roland, A.; Zhou, W.; Fauchon, M.; Lyaku, J.; Iyer, R. P. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 1921–1924.
2. (a) Prakash, G.; Kool, E. T. *J. Am. Chem. Soc*. **1992**, *114*, 3523–3527; (b) Wang, S.; Kool, E. T. *Nucleic Acids Res*. **1994**, *22*, 2326–2333; (c) Dolinnaya, N. G.; Blumenfeld, M.; Merenkova, I. N.; Oretskaya, T. S.; Krynetskaya, N. F.; Ivanovskaya, M. G.; Vasseur, M.; Shabarova, Z. A. *Nucleic Acids Res*. **1993**, *21*, 5403–5407.
3. For a review see: Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 1925–1963.
4. For examples of CPG-based 3′-phosphorylation methods, see: (a) McMinn, D. L.; Hirsch, R.; Greenberg, M. M. *Tetrahedron Lett*. **1998**, *39*, 4155–4158; (b) Guzaev, A.; Lönnberg, H. *Tetrahedron Lett*. **1997**, *38*, 3989–3992; (c) Dell'Aquila, C.; Imbach, J.-L.; Rayner, B. *Tetrahedron Lett*. **1997**, *38*, 5289–5292; (d) Kumar, P.; Gupta, K. C.; Rosh, R.; Seliger, H. *Chem. Lett*. **1997**, 1231; (e) Gryaznov, S. M., Letsinger, R. L. *Tetrahedron Lett*. **1992**, *33*, 4127–4128; (f) Asseline, U.; Bonfils, E.; Kurfurst, R.; Chassignol, M.; Roig, V.; Thuong, N. T. *Tetrahedron* **1992**, *48*, 1233–1254; (g) Kumar, P.; Bose, N. K.; Gupta, K. C. *Tetrahedron Lett*. **1991**, *32*, 967–970; (h) Markiewicz, W. T.; Wyrzykiewicz, T. K. *Nucleic Acids Res*. **1989**, *17*, 7149–7158; (i) Asseline, U.; Thuong, N. T. *Tetrahedron Lett*. **1989**, *30*, 2521–2524; (j) Felder, E.; Schwyzer, R.; Charubala, R.; Pfleiderer, W.; Schulz, B. *Tetrahedron Lett*. **1984**, *25*, 3967–3970.
5. Iyer, R. P.; Yu, D.; Devlin, T.; Ho, N.-H.; Agrawal, S. *Bioorg. Chem. Lett*. **1996**, *6*, 1917–1922.
6. Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett*. **1981**, *22*, 1859–1862. For a review, see: Beaucage S. L.; Iyer, R. P. *Tetrahedron* **1992**, *48*, 2223–2311.
7. Iyer, R. P.; Egan, W.; Regan, J. B.; Beaucage, S. L. *J. Am. Chem. Soc*. **1990**, *112*, 1253–1254.