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Solid-phase synthesis of oligomers carrying several chromophore units linked by phosphodiester backbones

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Abstract—A method for the preparation of oligomers by linking chromophore units is described. Specifically, the synthesis of chromophore units having a protected-hydroxyl group and a phosphoramidite function is described, along with a method to link several units using solid-phase phosphite-triester protocols.

DNA-intercalating drugs are planar molecules formed by several fused aromatic rings that form stacks between DNA base pairs, thus reducing the opening and unwinding of the double helix. Each intercalating drug binds strongly to particular base pairs due to several interactions, ranging from van der Waals interactions to the formation of hydrogen bonds with adjacent nucleobases. Intercalating drugs have varying selectivity, which may be improved by linking several intercalating units. Various authors have described the synthesis of bis- or tris-intercalating drugs with promising activity and selectivity.[1,2] However, there is no general methods for the rapid synthesis of a small library of oligomers carrying DNA-intercalating compounds.

The linkage of one single DNA-intercalating drug to peptides and oligonucleotides has been reported.[3,4] Oligonucleotides carrying one DNA-intercalating drug have better hybridization properties, nuclease resistance and cellular uptake. Intercalating agents have also been introduced in peptide nucleic acids (PNA)[5] and peptides.[6] Recently Kool et al. described the preparation and fluorescent properties of oligomeric fluorophores based on C-glycoside backbones.[7,8]

In these two papers we describe the synthesis of oligomeric compounds containing several chromophore units using solid-phase protocols. This paper reports the synthesis of oligomers with phosphodiester linkages and the accompanying paper reports the synthesis of oligomers linked by amide bonds.

We chose the L-threoninol and (*R*)-3-amino-1,2-propandiol backbones for the oligomers with phosphodiester links for several reasons. They have the adequate length to hold the chromophore units between DNA base

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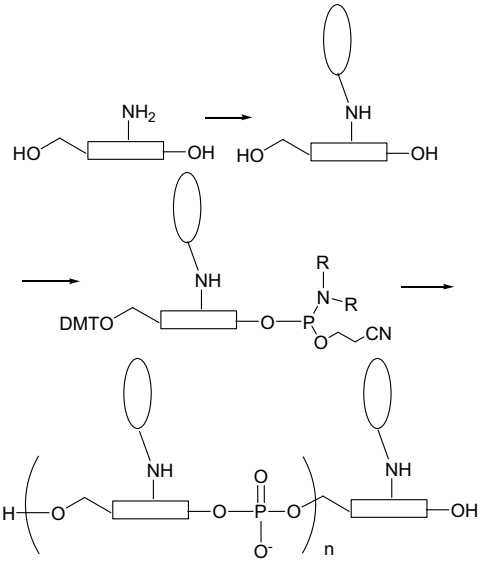
pairs. Both compounds can be obtained in enantiomerically pure form from commercial sources and they have two hydroxyl groups (one primary and the other secondary) and one amino group. The chromophore can be attached at the amino group position. Then, the primary hydroxyl group can be protected by the 4,4′-dimethoxytrityl (DMT) group. Finally the secondary alcohol will be used to prepare the phosphoramidite or the hemisuccinate derivatives needed for the assembly of oligomers by solid-phase methods (Scheme 1).

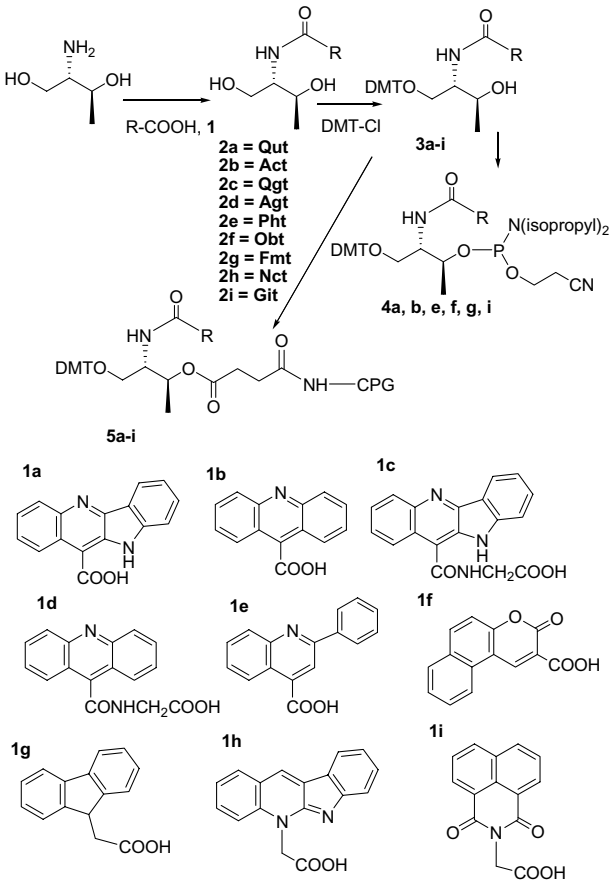
The threoninol molecule has been used to introduce several units of Methyl Red moieties in oligonucleotides[9] and the 3-amino-1,2-propandiol backbone has been used to introduce a phenanthridinium unit into oligonucleotides.[10] Recently aminopropyl nucleic acids with a 3-amino-1,2-propanediol backbone have been described.[11]

Threoninol derivatives of general formula **2a**–**d** can be prepared according to the process summarized in Scheme 2. As monomeric units we chose several heterocyclic derivatives carrying a carboxylic acid function: 10*H*-indolo[3,2-*d*]quinoline (**1a**), acridine (**1b**), phenylquinoline (**1e**), 3-oxo-3*H*-benzo[*f*]chromene (**1f**), and fluorenylmethyl (**1g**), neocriptolepine (**1h**), and benzo[*de*]-1,3-diketoisoquinoline (**1i**). In order to increase the distance between the intercalating drug and the threoninol linker molecule, and to give more flexibility to the units a glycine unit was added in compounds **1a** and **1b** by reaction of the corresponding carboxyl derivative with glycine methyl ester and subsequent hydrolysis of the methyl ester intermediate to yield derivatives **1c**–**d**.

L-Threoninol was reacted with carboxyl derivatives (**1a**–**i**) using diisopropylcarbodiimide and 1-hydroxybenzotriazole, yielding compounds **2a**–**i**. The threoninol derivatives (**2a**–**i**) were reacted with dimethoxytrityl (DMT) chloride in pyridine to yield the DMT derivatives (**3a**–**i**). The DMT derivatives (**3a**–**i**) were reacted with chloro-*N*,*N*-diisopropylamino-*O*-2-cyanoethoxy phosphine to yield phosphoramidites **4a**, **b**, **e**, **f**, **g**, and **i** or loaded to controlled pore glass (CPG) solid supports using the succinyl linkage (**5a**–**i**).



Scheme 1. General preparation of the monomers.

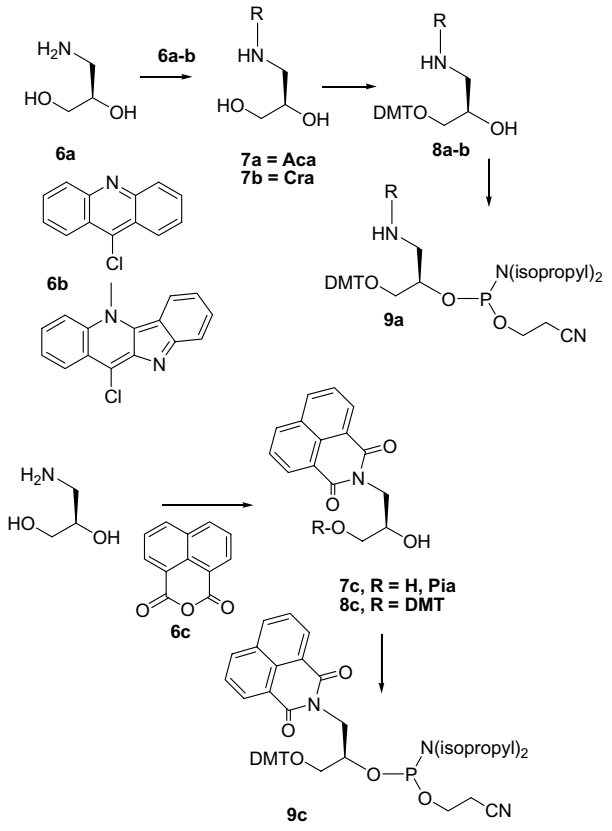


Scheme 2. Synthesis of threoninol derivatives.

The synthesis of the 3-amino-1,2-propandiol derivatives is shown in Scheme 3. The R isomer of 3-amino-1,2-propandiol was reacted with the chloro acridine derivative (**6a**) or the chloro indoloquinoline derivative (**6b**), yielding the corresponding diol derivatives **7a**–**b**. In addition, anhydride **6c** was reacted with 3-amino-1,2-propandiol to yield diol **7c**. The resulting diols (**7a**–**c**) were reacted with DMT chloride in pyridine to yield DMT derivatives **8a**–**c**, followed by the reaction with chloro-*N*,*N*-diisopropylamino-*O*-2-cyanoethoxy phosphine to yield phosphoramidites **9a** and **c**.

In order to synthesize oligomers using solid-phase protocols, DMT derivatives of the chromophores described above were coupled to CPG supports using the succinyl linker as described elsewhere.[12] For this purpose, the DMT-derivatives described above were reacted with succinic anhydride, followed by coupling of the resulting hemisuccinates with amino functionalized CPG yielding glass beads loaded with the appropriate chromophores.

The phosphoramidites described above were assembled into strands of up to 6 units (Table 1) using the appro-



Scheme 3. Synthesis of 3-amino-1,2-propandiol derivatives.

priate solid supports (1–4 μmol scale). We used a three-letter code for each monomer unit. The first two letters of the code define the heterocyclic moiety (see Schemes 2 and 3) and the last letter indicates the backbone (**t** for threoninol and **a** for 3-amino-1,2-propandiol).

Standard phosphoramidite chemistry was used.[13] This consists of cycles of four chemical reactions: (1) removal of the DMT group with 3% trichloroacetic acid in dichloromethane; (2) phosphoramidite coupling using 5-fold excess of phosphoramidite and 20-fold excess of tetrazole; (3) capping with acetic anhydride and *N*-methylimidazole; and (4) oxidation of phosphite to phosphate with 0.01 M iodine in tetrahydrofurane/pyridine/water. Coupling yields were between 80% and 95%. For the synthesis of the dimer containing a phosphorothioate linkage (1-Act-ps-Cra-3) the oxidizing solution was replaced by a solution of 3*H*-1,2-benzodithiol-3-one 1,1-dioxide[14] in acetonitrile.

After the assembly of the sequences, supports were treated with conc. aqueous ammonia to yield the desired unprotected oligomers. Good yields and purities were obtained during the synthesis of oligomers with L-threoninol backbone as reported in Figure 1.

Some oligomers decomposed in hot ammonia solutions, for example, oligomers carrying the chromene ring (**1f**, **Obt**) and those with the propan-1,2-diol backbone carrying acridine (**7a**, **Aca**) and benzo[*d*,*e*]-1,3-diketoisoquinoline (**7c**, **Pia**). Alternatively oligomers with the propan-1,2-diol backbone were deprotected either with (1) a 0.5 M 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) solution in pyridine, or (2) NH_3 in methanol at room temperature for 2–4 h. In both cases yields were very low. Best results were obtained using an acid labile linker. In this strategy 4-trityloxybutanoic acid was reacted with polystyrene loaded with Rink-amide linker.[15] The

Table 1. Oligomers prepared in this study

Compound	MS (expected)	MS (found)	HPLC, retention time (min)
1-Act-p-Qut-3[a]	721.7	766.5 [M+2Na$^+$]	12.7
1-Qut-p-Qut-3[a]	760.7	761.3	14.4
1-Act-p-Qut-p-Qut-3[a]	1133.0	1139.8	15.3
1-Act-(p-Act)$_5$-3[a]	2171.8	2172.3	11.6
1-Act-p-Cra-3[a]	693.6	694.6	13.0
1-Act-ps-Cra-3[a]	709.6	708.4	15.0, 15.5 (2 isomers)
1-Pht-p-Act-3[a]	708.7	707.8	14.5
1-Pht-p-Pht-3[a]	734.7	733.7	19.1
1-Act-p-Pht-3[a]	708.7	707.6	14.9
1-Pht-p-Qut-3[a]	747.7	746.6	16.8
1-Act-p-Qut-p-Agt-3[a]	1151.0	1152.2	12.3
1-Fmt-p-Fmt-3[a]	684	707 [M+Na$^+$]	18.8
1-Qut-p-Fmt-3[a]	722	780.5 [M+Na$^+$+K$^+$]	17.9
1-Qut-p-Cra-2[a]	732.7	733	14.4
1-Qut-p-Qut-p-Qut-3[a]	1172.0	1238 [M+3Na$^+$]	15.3
1-Act-p-Act-p-Act-3[a]	1154.9	1077.1 [M+Na$^+$]	11.3
1-Act-p-Qut-p-Act-3[a]	1093.9	1093.9	11.3
1-Act-p-Qut-p-Nct-3[a]	1146.0	1146	16.8
1-Pia-p-Pia-p-ba[b]	769.5	792.4	11.6
1-Aca-p-Aca-p-ba[b]	763.6	764.6	5.8
1-Obt-p-Obt-p-ba[b]	881.6	880.1	13.8
1-Git-p-Git-p-ba[b]	911	934.3 [M+Na$^+$]	12.7
1-Git-p-Obt-p-ba[b]	896	919 [M+Na$^+$]	14.1

p, phosphate; ps, phosphorothioate; ba, $(CH_2)_3CONH_2$.
[a] Deprotection using ammonia.
[b] Deprotection using trifluoroacetic acid.

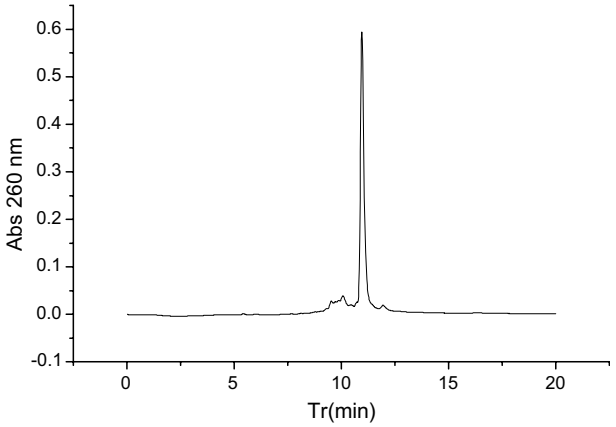


Figure 1. HPLC chromatogram of hexamer 1-Act-(p-Act)$_5$-3 after ammonia deprotection.

resulting support was used for the assembly of the polymers using phosphoramidite derivatives (**4f**, **9a**, **9c**). After the assembly of the sequences, supports were treated with 0.5 M DBU solution, washed in acetonitrile and treated with a solution containing 95% trifluoroacetic acid in water. Oligomers with the propan-1,2-diol backbone (**7a** and **7c**) and the chromene ring (**1f**) were obtained in excellent yields under these conditions.

Drugs that bind nucleic acids may show preference for certain sequences. One of the methods to analyze the sequence specificity of DNA-binding drugs is the competition dialysis.[16] In this method different nucleic acid structures are dialyzed against a common ligand solution. Figure 2 shows the competitive dialysis experiment for the monomer Act and the trimer Act-p-Act-p-Act with oligonucleotide sequences representing alternated or consecutive A-T or G-C duplex sequences. While the monomer has little variation on the affinity for each model duplex, the trimer has a clear affinity for contiguous A-T sequence.

The excitation and emission wavelengths of the new compounds were measured (see Supporting information). Optimal excitation wavelengths ranged from 247 to 433 nm and the corresponding emission wavelengths ranged from 395 to 499 nm. The phenylquinoline derivatives had the lowest emission wavelengths.

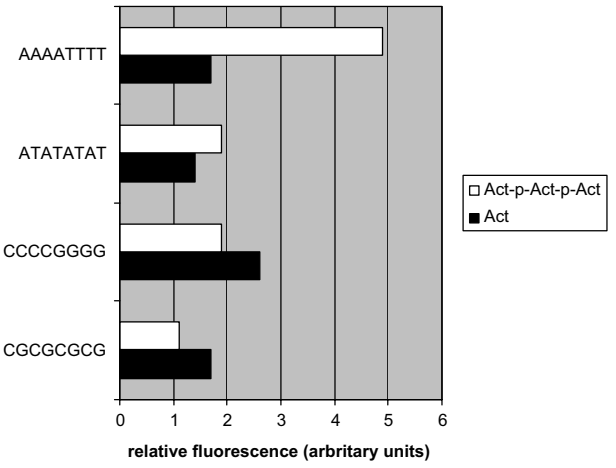


Figure 2. Competitive dialysis of monomer Act and trimer 1-Act-p-Act-p-Act-3 (see experimental conditions in Supplementary material).

In summary, we report the synthesis of several fused heterocyclic derivatives functionalized with threoninol and propan-1,2-diol that are useful for the rapid synthesis of oligomers of more than 6 units linked through phosphodiester or phosphorothioate bonds. The resulting oligomers are water soluble. The binding properties of these molecules are under investigation. Preliminary results show that the presence of the negatively charged phosphodiester backbone does not compromise the binding to DNA. Finally, the derivatives described in this work can also be used to introduce fluorescent and intercalating agents into synthetic DNA and RNA.[3,4]

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.02.074.

References and notes

1. Arya, D. P.; Willis, B. *J. Am. Chem. Soc.* **2003**, *125*, 12398.
2. Fechter, E.; Olenyuk, B.; Dervan, P. B. *Angew. Chem. Int. Ed.* **2004**, *43*, 3591.
3. Asseline, U.; Thuong, N. T.; Hélène, C. *New J. Chem.* **1997**, *212*, 5.
4. Christensen, U. B.; Pedersen, E. B. *Nucleic Acids Res.* **2002**, *30*, 4918.
5. Shiraishi, T.; Nielsen, P. E. *Nucleic Acids Res.* **2004**, *32*, 4893.
6. Carlson, C. B.; Beal, P. A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1979.
7. Cuppoletti, A.; Cho, Y.; Park, J. S.; Strässler, C.; Kool, E. T. *Bioconjug. Chem.* **2005**, *16*, 528.
8. Wilson, J. N.; Gao, J.; Kool, E. T. *Tetrahedron* **2007**, *63*, 3427.
9. Asanuma, H.; Shirasuka, K.; Takarada, T.; Kashida, ; Komiyama, M. *J. Am. Chem. Soc.* **2003**, *125*, 2217.
10. Huber, R.; Amann, N.; Wagenknecht, H. A. *J. Org. Chem.* **2004**, *69*, 744.
11. Zhou, D.; Froeyen, M.; Rozenski, J.; Van Aerschot, A.; Herdewijn, P. *Chem. Biodivers* **2007**, *4*, 740.
12. Pon, R. T. In *Methods in Molecular Biology Vol. 20: Protocols for Oligonucleotides and Analogs*; Agrawal, S.,

Ed.; Humana Press Inc.: Totowa, NJ, USA, 1993; pp 465–496.
13. Caruthers, M. H.; Barone, A. D.; Beaucage, S. L.; Dodds, D. R.; Fischer, E. F.; McBride, L. J.; Matteucci, M.; Stabinsky, Z.; Tang, J. Y. *Methods Enzymol.* **1987**, *154*, 287.
14. Iyer, R. P.; Phillips, L. R.; Egan, W.; Regan, J.; Beacauge, S. *J. Org. Chem.* **1990**, *55*, 4693.
15. Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787.
16. Ren, J.; Chaires, J. B. *Biochemistry* **1999**, *38*, 16067.