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# Solid-Phase Synthesis of Oligonucleotides Containing a Bipyridine Ligand at the 3′-3′ Inversion of Polarity Site

Aldo Galeone, Luciano Mayol,* Giorgia Oliviero, Daniela Rigano and Michela Varra

*Dip. di Chimica delle Sostanze Naturali, Univ. di Napoli "Federico II", Via D. Montesano, 49 I-80131 Napoli, Italy*

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**Abstract**—The preparation of a solid support useful for the synthesis of oligonucleotides with a 3′-3′ inversion of polarity, via a linker containing a chelating molecule, namely 2,2′-bipyridine, is described. 

## Introduction

There is a growing interest in design and synthesis of newly modified oligonucleotides (ODNs) as potential drugs in anticancer or antiviral therapy.[1,2] Such molecules, indeed, may be very efficient tools in the selective inhibition of gene expression, both in the antisense approach, where the target is an mRNA, and in the antigene strategy, through the formation of a triple helix complex (triplex) with a selected double-stranded DNA sequence.[2–6] In the latter strategy, one of the major restrictions is that a stable triplex, via Hoogsteen triads formation, can be envisaged under physiological conditions only for relatively long (15–17 bases) homopurine tracts within the same strand of a double helical DNA fragment and such a requirement is rarely met in biologically important regions of DNA. To recognize a wider number of DNA sequences, a possible solution is the use of ODNs containing a 3′-3′ inversion of polarity, able to target $(purine)_m(pyrimidine)_n$ sequences by hybridization of the adjacent purine blocks on alternate strands and by switching strand at the junction between the oligopurine and the oligopyrimidine domains.[7–9] From a chemical point of view, the 3′-3′ inversion can be fulfilled by a suitable linker capable of crossing the major groove and whose properties can be, in addition, exploited to supply the oligonucleotide with useful characteristics. For example, the 3′-3′ linker may incorporate an intercalating agent or a major groove ligand in order to improve the hybridization between the probe ODN and the target duplex.

In this frame, we have designed and prepared a solid-phase support useful for the synthesis of oligonucleotides with 3′-3′ inversion containing a chelating agent, namely a bipyridine moiety. Transition metal complexes—containing oligonucleotides (ODNs)[10,11]—represent a topic in constant growth. Such conjugates are involved in the study of electron transfer processes[12–14] as well as in the development of artificial nucleases characterized by a high sequence-specificity and efficiency.[15,16] In fact, a metal centre tethered to a particular sequence could enable the complex to oxidatively modify or cleave a nucleic acid target.

## Chemistry

The synthetic route (see Scheme 1) used for the preparation of oligomers with a 3′-3′ inversion containing a bipyridine moiety is based on a three-functionalized molecule (2-amino-1,3-propandiol) that allows: (i) anchorage to the polymeric support (Tentagel-$NH_2$); (ii) linkage to the metal complexing unit (2,2′-dipyridine); (iii) oligonucleotide chain assembly.

2-Amino-1,3-propandiol (**1**) is protected at the amino function and at one of the two hydroxyls by 9-fluorenylmethoxy-carbonyl (Fmoc) and 4,4′-dimethoxytrityl protecting groups, respectively (derivatives **2** and **3**).[17,18] Subsequently, the Fmoc group is removed by piperidine thus giving derivative **4**[19] which, in turn, is reacted with the pentafluorophenolic ester of 2,2′-bipyridine-4,4′-dicarboxylic acid (**6**)[20] affording derivative **7**.[21] Compound **6** represents the activated chelating molecule and is obtained by reaction of commercially available 2,2′-bipyridine-4,4′-dicarboxylic acid (**5**) with the pentafluorophenolic ester of

*Corresponding author. Fax: +39-81-678552; e-mail: mayoll@unina.it

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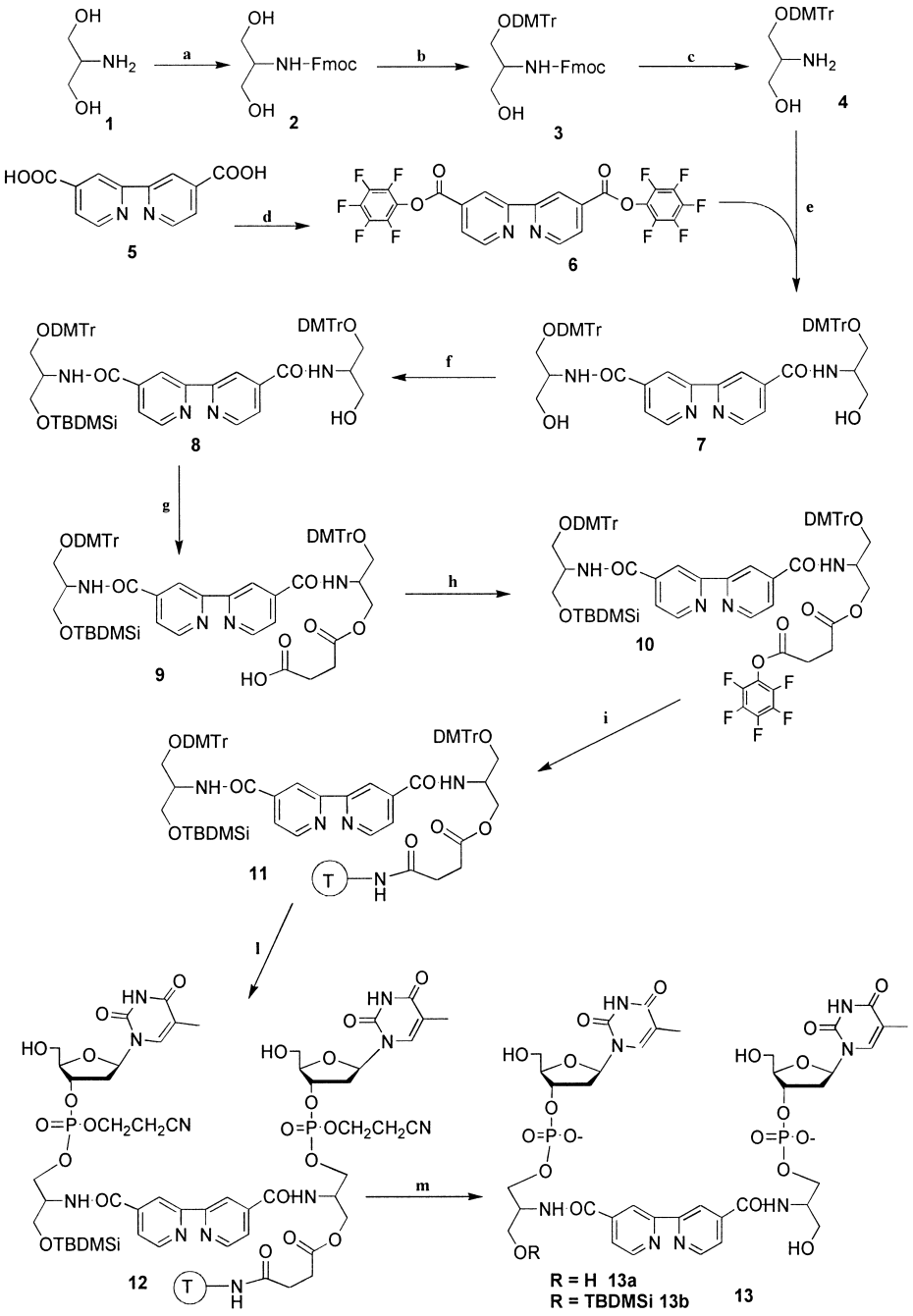
the trifluoroacetic acid. Derivative **7** is turned into compound **10,** the building block to be linked to the solid support, by treatment, in sequence, with: (i) *t*-butyl-dimethyl-silyl-chloride[22] in order to protect one of the two hydroxyl-groups; (ii) succinic anhydride[23] to obtain a carboxylic function useful for the anchorage to the solid support; (iii) the pentafluorophenolic ester of the trifluoroacetic acid for the activation of the carboxylic function.[24] Finally, **10** is anchored to the resin (Tentagel-$NH_2$),[25] to give the functionalized support (**11**) whose loading (88 μmol/g on average) is estimated by spectroscopic measurement of 4,4′-dimethoxytriphenylmethyl cation released by acidic treatment on a weighed sample of the support.

The so obtained Tentagel resin **11** can be used for the assembly of 3′-3′ inverted symmetric sequence by standard phosphoramidite chemistry.

## Results and Discussion

The functionalized support **11** was subjected to one coupling cycle in an automated DNA synthesizer, using




**Scheme 1.** (a) Fmoc-*N*-hydroxysuccinimide ester in dry DMF; (b) 4,4′-dimethoxytrityl chloride, 4-(dimethylamino)pyridine in dry Py; (c) piperidine in dry DMF; (d) pentafluorophenolic ester of trifluoroacetic acid ($CF_3COOC_6F_5$) in dry DMF; (e) dry DMF; (f) *t*-butyldimethylsilylchloride (TBDMSiCl), imidazole, in dry DMF; (g) succinic anhydride, 4-(dimethylamino)pyridine, in dry Py; (h) pentafluorophenolic ester of trifluoroacetic acid ($CF_3COOC_6F_5$) in dry DMF; (i) tentagel-$NH_2$ in dry DMF; (l) coupling cycle and trityl groups removal; (m) 25% aqueous $NH_3$.

thymidine phosphoramidite as a building block, to verify its resistance to the reagents and conditions usually employed in DNA solid-phase synthesis. After treatment with dichloroacetic acid to remove the trityl groups, the resin underwent a mild treatment[26] with ammonia to detach the nucleotidic material and to deprotect the phosphate groups, as well. Crude material was purified by reversed phase HPLC thus affording two main products, which were shown to be, by ESI-MS analyses, the completely deprotected dimer 5′-thymine-3′-dipyridine-3′-thymine-5′ (**13a**) and the same molecule still carrying the *t*-butyldimethylsilyl group (**13b**). As expected, the latter compound could be turned into the former one by treatment with tetrabutylammonium fluoride.[27] The same functionalized support has been exploited for the preparation of a number of conjugates of the type 5′-ODN-3′-dipyridine-3′-ODN-5′, whose hybridization properties towards specific double-stranded DNA targets are under investigation in order to verify whether the linker allows the molecule to efficiently cross the major groove without causing severe distortions of the triple helix. Particularly, we prepared successfully oligonucleotides 5′-$T_8$-3′-DiPy-3′-$T_8$-5′ (**I**) and 5′-$(CT)_4$-3′-DiPy-3′-$(TC)_4$-5′ (**II**).[28]

The solid-phase support we have prepared allows the synthesis of conjugates containing a ligand at the 3′-3′ inversion of polarity site which, to the best of our knowledge, represents the first example of such modified oligonucleotides. The metal-complexing molecule, namely 2,2′-bipyridine, was chosen on the basis of its well-known coordination properties.[29] The syntheses of similar supports based on other linkers characterized by different flexibility, are currently in progress.

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17. Procedure for the synthesis of **2**: 1 g (10.9 mmol) of 2-amino-1,3-propandiol (**1**) and 4.85 g (14.4 mmol) of 9-fluorenylmethyl-*N*-succinimidylcarbonate (Fmoc-OSu) are dissolved in dry *N*,*N*-dimethylformamide (25 mL) and stirred for 24 h. The reaction is monitored by TLC ($CHCl_3$/$CH_3OH$ 95:5). Crude material is purified by extraction with $H_2O$ (2%MeOH)/*n*-hexane. The aqueous phase is evaporated to dryness recovering 3.1 g of product (90% yield). $^1H$ NMR (500 MHz, $CD_3OD$): δ 7.82 (2H, d, $J=7.5$ Hz), 7.70 (2H, d, $J=7.5$ Hz), 7.42 (2H, t, $J=7.5$ Hz), 7.34 (2H, t, $J=7.5$ Hz), 4.39 (2H, d, $J=6.8$ Hz), 4.24 (1H, t, $J=6.8$ Hz), 4.25–3.55 (5H, m).
18. Procedure for the synthesis of **3**: 3 g (9.6 mmol) of 2-[(9-fluorenylmethoxy-carbonyl)amino]-1,3-propandiol are dried by coevaporations (×3) with dry pyridine and then dissolved in the same solvent (20 mL). 2.27 g (6.7 mmol) of 4,4′-dimethoxytrityl chloride and a catalytic amount of dimethylamino pyridine (50 mg, 0.4 mmol) are added to the solution. After stirring for 2 h, the reaction is quenched with methanol and evaporated to dryness. The product is purified on a silica gel column eluted with *n*-hexane/ethyl acetate 1:1. Yield: 3.5 g (59%). $^1H$ NMR (500 MHz, $CDCl_3$): δ 7.78 (2H, d, $J=7.5$ Hz), 7.61 (2H, t, $J=7.5$ Hz), 7.42–7.20 (13H, m), 6.80 (4H, d, $J=7.2$ Hz), 4.42 (2H, d, $J=6.8$ Hz), 4.19 (1H, t, $J=6.8$ Hz), 3.90–3.61 (11H, m).
19. Procedure for the synthesis of **4**: 3.5 g (5.7 mmol) of 1-(2,2′-dimethoxytriphenylmethoxy)-2-[(9-fluorenylmethoxy-carbonyl)amino]-propan-3-ol are treated with a freshly prepared solution of 10% piperidine in dry *N*,*N*-dimethylformamide (15 mL). After stirring for 3 h, the reaction mixture is evaporated to dryness and purified on a silica gel column eluted with increasing amounts of $CH_3OH$ in $CHCl_3$ (1% Py). Yield: 2.2 g (98%). $^1H$ NMR (500 MHz, $CDCl_3$): δ 7.51–7.20 (9H, m), 6.96 (4H, d, $J=7.2$ Hz), 3.80 (6H, s) 3.65 (1H, m), 3.50 (1H, m), 3.19 (1H, m), 3.10 (1H, m), 3.65 (1H, m).
20. Procedure for the synthesis of **6**: 400 mg (1.6 mmol) of 2,2′-bipyridine-4,4′-dicarboxylic acid (**5**) are dried in vacuum and dissolved in dry *N*,*N*-dimethylformamide (15 mL) to which a few drops of pyridine have been added. Then, the pentafluorophenolic ester of trifluoroacetic acid (780 μL, 3.8 mmol) is added and the solution kept overnight under stirring at room temperature. The reaction is monitored by TLC ($CHCl_3$/$CH_3OH$ 8:2). The mixture is diluted with ethyl acetate and rinsed with HCl 0.1 N (×3) and, successively, with 5% sodium bicarbonate (×3). The organic phase is evaporated to dryness to give **6** in 96% yield.
21. Procedure for the synthesis of **7**: 631 mg (1.09 mmol) of the pentafluorophenolic ester of 2,2′-bipyridine-4,4′-dicarboxylic acid (**6**) are dissolved in dry *N*,*N*-dimethylformamide (10 mL) containing a few drops of dry pyridine. 2.2 g (5.6 mmol, 8 equiv) of derivative **4** are added under stirring and the solution is kept at room temperature overnight. The reaction is monitored by TLC ($CHCl_3$/$CH_3OH$ 92:8). The solvent is removed under reduced pressure and the product

purified by a silica gel column eluted with increasing amounts of $CH_3OH$ in $CHCl_3$ (1% Py). Yield: 0.92 g (85%). ESI-MS: 993.4 $m/z$ $[M-H]^-$.
22. Procedure for the synthesis of **8**: 1.1 g (1.1 mmol) of derivative **7** are dried by coevaporations (×3) with dry pyridine and then dissolved in dry *N*,*N*-dimethylformamide (10 mL) containing a few drops of pyridine. 100 mg (1.5 mmol) of imidazole and 100 mg (0.7 mmol) of *t*-butyldimethylsilyl chloride are added and the solution is kept overnight at room temperature under stirring. The reaction is monitored by TLC ($CHCl_3/CH_3OH$ 95:5). The product is purified on a silica gel column eluted with increasing amounts of $CH_3OH$ in $CHCl_3$ (1% Py). Yield: 700 mg (58%).
23. Procedure for the synthesis of **9**: A mixture of 700 mg (0.6 mmol) of derivative **8** and 80 mg of dimethylamino pyridine (0.6 mmol) is dried by coevaporations (×3) with dry pyridine and then dissolved in this solvent (8 mL). After addition of 240 mg (2.4 mmol) of succinic anhydride the reaction is kept overnight at room temperature under stirring. The reaction is monitored by TLC ($CHCl_3$/acetone 6:4). The product is purified on a silica gel column eluted with increasing amounts of $CH_3OH$ in $CHCl_3$ (1% Py), to give 704 mg of product (97% yield). ESI-MS: 1231.7 $m/z$ $[M+Na]^+$.
24. Procedure for the synthesis of **10**: 740 mg (0.6 mmol) of derivative **9** are dissolved in dry *N*,*N*-dimethylformamide (5 mL). 0.5 mL (0.85 mmol) of the pentafluorophenolic ester of trifluoroacetic acid and 150 μL of dry pyridine were added. The reaction is stirred at room temperature overnight and monitored by TLC (*n*-hexane/ethyl acetate 1:1). Product is used in the next step without any purification.
25. Attachment of derivative **10** to the resin: 65 mg of Tentagel-$NH_2$ are washed with $CHCl_3$ (×3), MeOH (×3), acetone (×3) and dried under vacuum. The resin is then washed with dry $CH_2Cl_2$ and allowed to swell at 50 °C for 1 h. Finally it is washed with dry Py and dried under vacuum, once again, for 3 h. Derivative **10** (210 mg) dissolved in dry DMF (1% dry Py) (1 mL) is added to the resin and the suspension is kept 24 h in an oscillating vessel. The resulting polymer is washed with dry DMF (×2) and $CHCl_3$ (1% dry Py) (×2) and dried under vacuum.
26. Mild conditions (25% aqueous $NH_3$, 1.5 h, room temperature) are required in order to prevent hydrolysis of the amide linkage between the bipyridine unit and 1,3-dihydroxy-2-amino moiety. In the case of the synthesis of C-containing sequences the more labile *t*-butylphenoxyacetyl (tBPA) protecting group for the exocyclic amino group of cytosine has been used.
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