Supporting Information
© Georg Thieme Verlag KG Stuttgart · New York 2009
A novel approach to preparation of peptide-oligonucleotide conjugates (POCs)

Renata Kaczmarek, Janina Baraniak and Wojciech J. Stec
Department of Bioorganic Chemistry, Center of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, 90-363 Łódź, Poland
baraniak@bio.cbmm.lodz.pl

$^{31}$P NMR spectra were measured on a 200 MHz spectrometer. $^{31}$P NMR shift values were assigned relative to $\text{H}_3\text{PO}_4$ as an external standard. Analytical thin-layer chromatography (TLC) was performed on precoated (0.25 mm thickness) glass plates (E.Merck, silica gel 60 F-254). Column chromatography was performed using Kieselgel-60 230-400 mesh silica gel (E.Merck). All nonvolatile compounds were routinely dried under high vacuum. Anhydrous solvents and other liquid reagents were transferred by syringe. Acetonitrile was distilled from $\text{P}_2\text{O}_5$ after being refluxed for several hours, and stored over CaH$_2$. Pyridine was distilled from CaH$_2$ after being refluxed for several hours, and stored over CaH$_2$. Other solvents were dried according to known methods and distilled prior to use. 2-Chloro-1,3,2-oxathiaphospholane was prepared as previously reported.

---

P NMR spectra were measured on a 200 MHz spectrometer. $^{31}$P NMR shift values were assigned relative to $\text{H}_3\text{PO}_4$ as an external standard. Analytical thin-layer chromatography (TLC) was performed on precoated (0.25 mm thickness) glass plates (E.Merck, silica gel 60 F-254). Column chromatography was performed using Kieselgel-60 230-400 mesh silica gel (E.Merck). All nonvolatile compounds were routinely dried under high vacuum. Anhydrous solvents and other liquid reagents were transferred by syringe. Acetonitrile was distilled from $\text{P}_2\text{O}_5$ after being refluxed for several hours, and stored over CaH$_2$. Pyridine was distilled from CaH$_2$ after being refluxed for several hours, and stored over CaH$_2$. Other solvents were dried according to known methods and distilled prior to use. 2-Chloro-1,3,2-oxathiaphospholane was prepared as previously reported.

To a solution of L-alanyl-N-methyl-L-alanyl-L-alanine methyl ester (6) (1mmol, 259mg) in dry pyridine (5ml) was added elemental sulphur (0.5mmol, 128mg) followed by dropwise addition of 2-chloro-1,3,2-oxathiaphospholane (2) (1mmol, 142mg). The reaction mixture was stirred at room temperature for 12 h. Then the solvent was removed in vacuo and the residue was triturated with acetonitrile (10ml). Undissolved sulfur was filtered and the filtrate was condensed in vacuo. The residue was dissolved in 3 ml of chloroform and applied to a silica gel column (2.5x18 cm). The column was eluted with methanol in chloroform (0→12%). Appropriate fractions were combined and evaporated in vacuo to give desired compounds 7 in 71% yield [\(^{31}\)P NMR (CD\(_3\)OD) \(\delta\) 97.60, 97.40 ppm, FAB-MS m/z (M-1) 396].

Synthesis of p-nitrophenyl 3-[6-(4,4′-dimethoxytrityloxy)-hexylcarbamoyl]propanoate (9) was performed according to the procedure described by Halambris et al. 3

Synthesis of 3-[6-(4,4′-dimethoxytrityloxy)-hexylcarbamoyl]propanoyl-N-(L-leucyl)glicylglycine methyl ester (11).

To a solution of p-nitrophenyl 3-[6-(4,4′-dimethoxytrityloxy)-hexylcarbamoyl]propanoate (9) (0.5mmol, 320mg) in DMF (3ml) was added methyl ester of L-leucyl-glicylglycine (10) (0.34mmol, 100 mg) and DMAP (0.74mmol, 90mg). The reaction mixture was stirred at room temperature for 48 h. Then the solvent was removed in vacuo and the residue was dissolved in 2ml of chloroform and applied to a silica gel column (2.5x18 cm). The column was eluted with methanol in chloroform (0→5%). Appropriate fractions were combined and evaporated in vacuo to give desired compounds 11 in 39% yield [FAB-MS m/z (M-1) 759].

Synthesis of 3-(hexylcarbamoyl)propanoyl-N-(L-leucyl)glicylglycine methyl ester (12).

To a solution of compound 11 (0.1mmol, 76mg) in CH\(_2\)Cl\(_2\) (3ml) was added p-toluenesulfonic acid (1mmol, 190 mg). The reaction mixture was stirred at room temperature for 3 h. Then pyridine (0.8ml) was added and the solvents were removed in vacuo. The desired compound 12 was isolated by column chromatography [SiO\(_2\), eluent: methanol in chloroform (O→10%)] in 61% yield [FAB-MS m/z (M-1) 457].

Synthesis of 3-[6-O-(2-thiono-1,3,2-oxathiaphospholanyloxy)-hexylcarbamoyl] propanoyl-N-(L-leucyl)glicylglycine methyl ester (13).

To a solution of compound 12 (0.1mmol, 46mg) in dry pyridine (2ml) was added elemental sulphur (0.5mmol, 128mg) followed by dropwise addition of 2-chloro-1,3,2-oxathiaphospholane (2) (0.1mmol, 14mg). The reaction mixture was stirred at room temperature for 12 h. Then the solvent was removed in vacuo and the residue was triturated with acetonitrile (10ml). Undissolved sulfur was filtered and the filtrate was condensed in vacuo. The residue was dissolved in 1 ml of chloroform and applied to a silica gel column (2.5x18 cm). The column was eluted with methanol in chloroform (0→5%). Appropriate fractions were combined and evaporated in vacuo to give desired compounds 13 in 90% yield [\(^{31}\)P NMR (CD\(_3\)OD) \(\delta\) 104.5 ppm, FAB-MS m/z (M-1) 595].
**Solid phase synthesis of compounds 8 and 14**

The synthesis of resin bound tetra-thymidine phosphorothioate (4) was carried out manually by the syringe technique on 1μmol scale according to the oxathiaphospholane methodology. After oligonucleotide chain assembly the conjugation reaction was performed using compound 7 or 13 (20μmol), which was dissolved in CH$_3$CN (100μl) followed by addition of DBU (50 μmol). This mixture was applied to a syringe with compound 4. Entirety was shaken for 15 min. Then the cleavage from the support was performed by means of ammonia for 2 h. The yields were calculated from the DMT cation assay. Compounds 8 and 14 were purified by means of HPLC using Econosil C-18 column (4.6x250 mm),1.0 mL/min flow rate; buffer A, 0.1 M TEAB pH 7.5; buffer B, 40% CH$_3$CN in 0.1 M TEAB; gradient from 0→60% B over 30 min, 60% over 5 min, 60%→0% over 5 min. Yield was calculated based on A$_{260}$ units of starting crude oligonucleotide and conjugate.

**References**


Figure S1. MALDI-MS analysis of compound 8

![MALDI-MS analysis of compound 8](image)

Figure S2. HPLC analysis of compound 8

![HPLC analysis of compound 8](image)
Figure S2. HPLC analysis of compound 8
Figure S3. MALDI-MS analysis of compound 14

Figure S4. HPLC analysis of compound 14
Figure S4. HPLC analysis of compound 14