

# Synthesis and Application of a Novel, Crystalline Phosphoramidite Monomer with Thiol Terminus, Suitable for the Synthesis of DNA Conjugates

Zoltán Kupihár,<sup>a</sup> Zoltán Schmel,<sup>b</sup> Zoltán Kele,<sup>a</sup>  
Botond Penke<sup>a</sup> and Lajos Kovács<sup>a,\*</sup>

<sup>a</sup>Department of Medicinal Chemistry, Dóm tér 8, University of Szeged, H-6720 Szeged, Hungary

<sup>b</sup>Department of Pharmaceutical Analysis, Somogyi B. u. 4, University of Szeged, H-6720 Szeged, Hungary

Received 7 September 2000; accepted 14 December 2000

**Abstract**—A new, crystalline 5'-thiol modifier phosphoramidite monomer (**3**), suitable for DNA synthesis, has been prepared. This monomer has been built into an oligonucleotide using the standard protocol. After cleavage, purification and removal of the trityl group with Ag<sup>+</sup>, a free 5'-thiol terminal oligonucleotide (**15**) has been obtained which was subsequently coupled to a cysteine derivative via a disulfide bridge to afford conjugate **16**. © 2001 Elsevier Science Ltd. All rights reserved.

## Introduction

Conjugates of synthetic nucleic acids are indispensable tools of modern molecular biology. These conjugates can be simple organic compounds (fluorescent dyes, biotin) and/or biopolymers (peptides, proteins). The synthesis of these conjugates implies the formation of a suitable functional group on the nucleic acid for coupling. This can be performed both in solution, when the corresponding nucleic acid is modified either chemically or enzymatically, and on solid phase, when the desired protected functional group is coupled to the terminus during oligonucleotide synthesis.

This latter method is applied most widely as it is easier to carry out and offers less side reactions. Among many others, a 5' terminal thiol group is one of the most popular groups. Coupling to this group can be performed by a reactive halogene-containing compound, such as derivatives of iodoacetic acid or another (active) thiol resulting in a thioether or disulfide, respectively. For solid-phase oligonucleotide synthesis, several 5'-thiol modifiers have been described previously.<sup>1–4</sup> These modifiers are mostly  $\alpha,\omega$ -thioalcohols containing three or six carbon atoms. The thiol group of these compounds is protected by trityl<sup>1,2</sup> or the base sensitive 2-(2,4-dinitrophenyl)ethyl (Dnpe)<sup>4</sup> groups and the hydroxyl function is converted to its methoxy-

morpholinophosphite (MMP),<sup>2</sup> 2-cyanoethyl-*N,N*-diisopropyl-phosphoramidite<sup>5</sup> or H-phosphonate<sup>1</sup> derivative. A high efficiency in incorporating these modifiers is due to the enhanced reactivity of P(III) compounds. The common drawback of these monomers, except the trityl protected propyl chain and MMP-containing compound, is that they can only be synthesized as oils, which makes further use less convenient. On the other hand, the use of the propyl chain-containing compound is restricted as the short spacer arm may result in low efficiency in further use of the DNA conjugates of peptides and proteins.<sup>6</sup>

In our experience, almost quantitative coupling can be achieved with the most widely applied and commercially available monomer **1**<sup>5</sup> (Fig. 1) if it is freshly prepared and purified while the older monomer (synthesized some months before) results in less efficient coupling. This is due to the difficult purification and easier decomposition of oils, and, in addition, amidites are sensitive to moisture, acidic environment and oxidation. Therefore, our aim was to develop a stable, crystalline amidite monomer and to demonstrate its utility in the preparation of an oligonucleotide conjugate.

## Results and Discussion

Purification of the modified oligonucleotides is easier if the 5'-thiol is protected by trityl rather than by Dnpe group because the former keeps its trityl group after

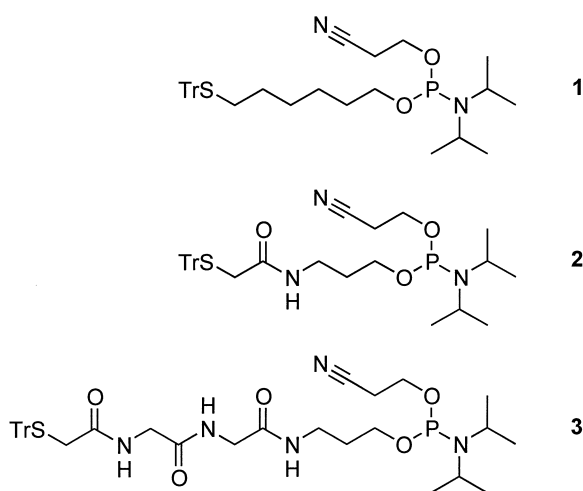
\*Corresponding author. Tel.: +36-62-54-5145; fax: +36-62-54-5971; e-mail: kupi@ovrisc.mdche.u-szeged.hu (Z. Kupihár), kovacs@ovrisc.mdche.u-szeged.hu (L. Kovács)

cleavage under standard condition with ammonia (this latter is commonly referred to as the 'trityl-on' method). The use of amidites compared to H-phosphonates is a more fortunate choice as subsequent oxidation after coupling requires milder conditions, ruling out the removal of trityl group, which can also take place under harsh oxidation conditions.<sup>7</sup> Therefore, we have applied phosphoramidite monomer **1** (Fig. 1) as a lead structure considering that incorporation of one or more amide bonds might result in a crystalline substance.

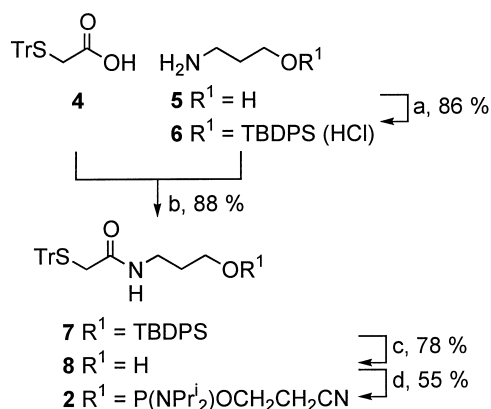
The first compound of choice has been a substance containing one amide bond (**2**, Fig. 1). The synthesis of this compound has been attempted first by coupling acid **4**<sup>8</sup> (Scheme 1) to 3-aminopropanol (**5**). In order to avoid coupling on the unprotected hydroxyl group of 3-aminopropanol, the less reactive DCC has been used as a coupling agent. Unfortunately, the resulting DCU could not be removed quantitatively even by repeated column chromatography. Therefore, we have performed the coupling reaction applying the TBDPS protecting group that has been stable during coupling and later upon Fmoc deprotection and its removal with TBAF has not affected the *S*-trityl group, and made the

identification of the products easier on TLC. Thus, amidite **2** has been obtained by coupling acid **4** and the protected amine **6** with HOBT/HBTU ( $\rightarrow$ **7**), subsequently desilylated with TBAF ( $\rightarrow$ **8**) and followed by phosphitylation using 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite<sup>9</sup> ( $\rightarrow$ **2**, Scheme 1). Unfortunately, although <sup>1</sup>H, <sup>13</sup>C and <sup>31</sup>P NMR characterization revealed the purity of compound **2**, it remained oil after several purifications by column chromatography followed by trituration with different solvents (light petroleum, diethyl ether or EtOAc).

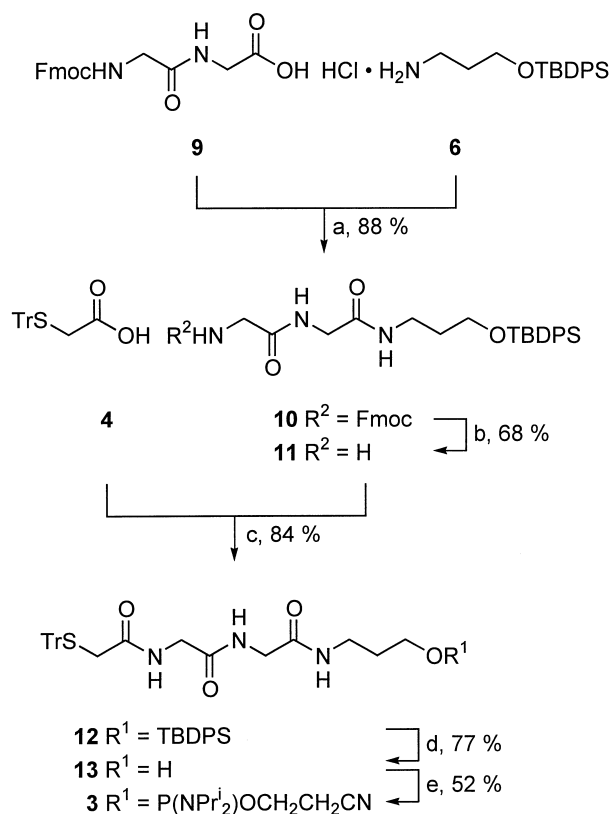
Therefore, we have considered the incorporation of further amide bonds into the spacer arm in order to improve its crystallization propensity. Thus, an additional Gly-Gly moiety has been built into the former amidite (**3**, Fig. 1). The multistep synthesis of this compound is illustrated in Scheme 2. Fmoc-Gly-Gly-OH (**9**) has been coupled to protected amine **6** to give compound **10**, then, after removal of the Fmoc group with diethylamine, acid **4** has been coupled to amine **11** applying HOBT/HBTU ( $\rightarrow$ **12**). After desilylation with TBAF ( $\rightarrow$ **13**), subsequent phosphitylation using 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite and purification by column chromatography followed by trituration with cold diethyl ether, the desired amidite monomer **3** has been obtained as a crystalline substance. It is noteworthy that phosphoramidites are sensitive to acids, nucleophilic attack and oxidation therefore their mass spectrometric characterization is



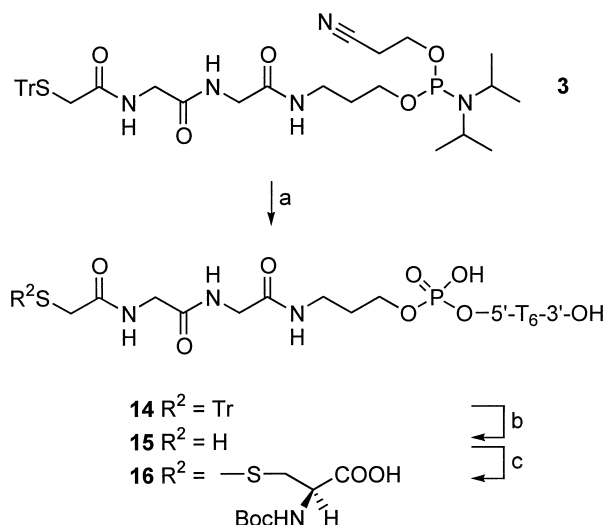
**Figure 1.** Phosphoramidite monomers with thiol terminus, amenable for the synthesis of DNA conjugates.



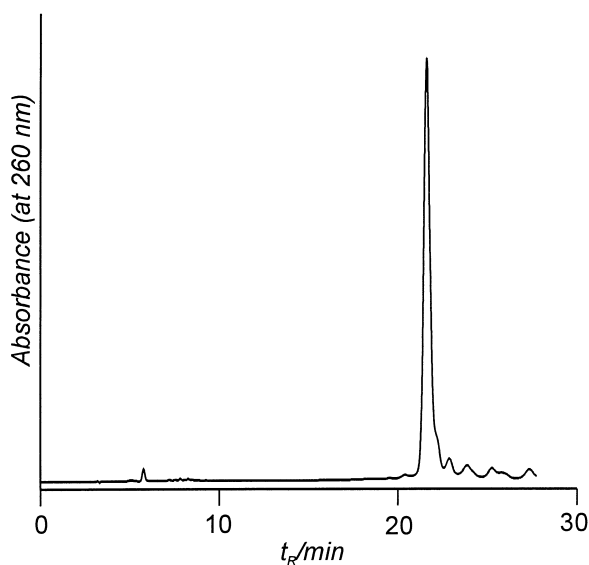
**Scheme 1.** Reagents and conditions: (a) TBDPS-Cl, DCM, TEA, rt, 1 h, then aq HCl; (b) HOBT, HBTU, DIPEA, DCM, rt, 1 h; (c) TBAF, THF, rt, 6 h; (d) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, DIPEA, abs DCM, rt, 1 h.



**Scheme 2.** Reagents and conditions: (a) HOBT, HBTU, DIPEA, THF, rt, 3 h; (b) diethylamine, DCM, rt, 16 h; (c) HOBT, HBTU, DIPEA, DCM, rt, 3 h; (d) TBAF, THF, rt, 6 h; (e) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, DIPEA, abs DCM, rt, 1 h.



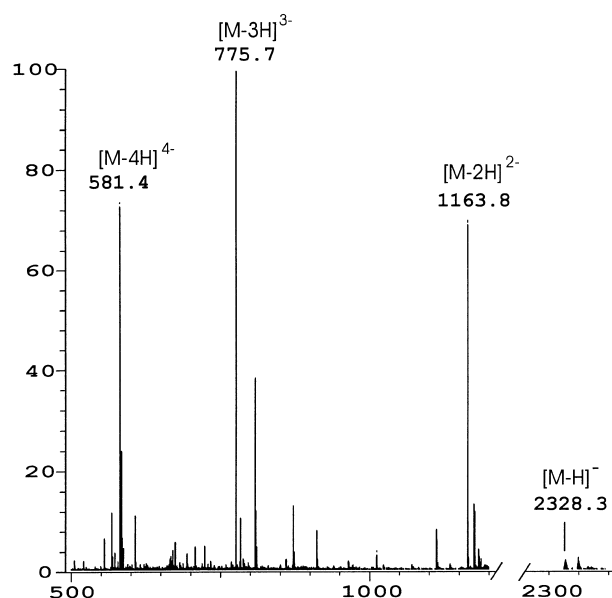
**Scheme 3.** Reagents and conditions: (a) (1) standard phosphoramidite coupling with CPG-bound 5'-HO-T<sub>6</sub> oligomer; (2) deprotection with aq cc. NH<sub>3</sub>, 50 °C, 16 h; (b) (1) AgNO<sub>3</sub>, 0.1 M TEAA, pH 7.0, 30 min; (2) DTT, 0.1 M TEAA, pH 7.0, 1 h; (c) *N*-Boc-L-Cys(Npys)-OH, 0.1 M TEAA, pH 7.0, rt, 16 h.



**Figure 2.** HPLC chromatogram of purified oligonucleotide **14**.

troublesome. Their analysis is further hampered by the presence of (substituted) trityl groups, common protective groups in oligonucleotide chemistry, which afford very intensive signals in the mass spectrum. These difficulties have been successfully eliminated by the application of our recently developed nanoelectrospray methodology employing lithium chloride in acetonitrile solution.<sup>10</sup> We have extensively applied this novel method during the course of the synthesis.

Next, amidite monomer **3** has been coupled to a homothymidine hexamer (Scheme 3, →**14**) using an automatic DNA synthesizer. As the monomer is highly soluble in acetonitrile, its coupling was very similar to



**Figure 3.** Nano-ESI mass spectrum of conjugate **14**. Calcd  $M_r$ , 2330.5 (monoisotopic).

the general protocol with the only difference being at the oxidation step. There are contradictory data in the literature as if iodine removes the *S*-trityl group,<sup>2,7</sup> therefore a 'BuOOH/THF/water solution, applied successfully in the phosphorylation of peptides,<sup>11</sup> has been used for oxidation instead of iodine. Subsequent cleavage with aqueous NH<sub>3</sub> and purification after synthesis has been carried out in the usual manner. According to LC–MS analyses, only 1–2% of homoT hexamer lacking the linker residue has been found. The HPLC profile and nano-ESI mass spectrum of oligonucleotide **14**, purified on a Poly-Pak<sup>TM</sup> cartridge according to a modified procedure, are presented in Figs 2 and 3. Removal of the trityl group (→**15**) was carried out by AgNO<sub>3</sub> followed by DTT treatment.<sup>2</sup> According to HPLC and MS analyses, this method can be applied with the same efficiency as for the previously used monomer **1**.

In order to test the new monomer, the free thiol-containing oligonucleotide **15** has been coupled to a protected cysteine. To form this asymmetric disulfide bridge, the 3-nitro-2-pyridinesulfenyl (*S*-Npys) activated<sup>12</sup> cysteine derivative *N*-Boc-L-Cys(Npys)-OH has been used. According to LC–MS analysis, the oligonucleotide has been converted to the corresponding disulfide **16** quantitatively.

## Conclusion

We have described the synthesis and application of a novel, crystalline 5'-thiol modifier phosphoramidite monomer (**3**) for oligonucleotide synthesis. Although the multistep synthesis described herein is a lengthy one, compared to that of the generally used monomer (**1**), it employs standard steps with good yields and the

following use of the compound is more convenient. NMR purity control revealed that the new compound is stable for more than half a year without detectable trace of decomposition or oxidation if stored at  $-20^{\circ}\text{C}$  under argon. Furthermore,  $t\text{BuOOH}$ -mediated oxidation, previously employed in peptide phosphorylation, has been successfully applied to the P(III)→P(V) transformation in the solid phase oligonucleotide synthesis in the presence of a thioether group. The applicability of amidite **3** was proved by incorporating it into DNA which was then coupled, via a disulfide bridge, to a cysteine derivative to yield a cysteine–DNA conjugate.

## Experimental

### General

The following abbreviations are employed: controlled pore glass support (CPG); *N,N*-diisopropylethylamine (DIPEA); dichloromethane (DCM); *N,N'*-dicyclohexylurea (DCU); 1,4-dithio-DL-threitol (DTT); 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU); *N*-hydroxybenzotriazole (HOBT); triethylamine (TEA); triethylammonium acetate (TEAA); tetrabutylammonium fluoride (TBAF); *tert*-butyldiphenylsilyl chloride (TBDPS-Cl). The 5'-*O*-dimethoxytrityl-protected thymidine phosphoramidite monomer for oligonucleotide synthesis was obtained from ChemGenes Corporation (Ashland, MA, USA). Other chemicals were purchased from Aldrich, Fluka or Bachem. 2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite was prepared using the procedure of Sinha et al.<sup>9</sup> Amidite **1**<sup>5</sup> was synthesized by direct tritylation of 6-mercaptohexanol<sup>13</sup> followed by phosphorylation.<sup>9</sup> Acids **4** and **9** have been prepared first by Biilman and Due<sup>8</sup> and Carpino and Han,<sup>15</sup> respectively, without characterization except the mp and elementary analysis. Oligonucleotide conjugate **14** was synthesized on an Expedite 8909 nucleic acid synthesizer on a 1  $\mu\text{mol}$  scale. Cleavage of the oligonucleotide from the CPG-support was performed by cc. aq  $\text{NH}_3$  for 16 h at  $50^{\circ}\text{C}$ . The crude cleavage mixture was purified on 50 mg Poly-Pak<sup>TM</sup> (Glen Research, Sterling, VA, USA) cartridge using a slightly modified eluent profile (1) washing with acetonitrile (5 mL), (2) conditioning with 1.0 M TEAA (5 mL), (3) sample application, (4) washing with 3% (w/v) aq  $\text{NH}_3$  (5 mL) then with deionized water (10 mL), (5) elution with 30% (v/v) aq acetonitrile<sup>16</sup> and lyophilized. The quantity of the resulting oligonucleotide was determined by using UV absorbance at 260 nm, its purity was checked by HPLC and characterized by nano-ESI mass spectrometry. Purification, detritylation and conjugation of oligonucleotides were performed in aq 0.1 M TEAA buffer (pH 7.0). HPLC chromatography was performed on a HP1050 instrument with the following conditions: Rutin RP column C18, 300 Å, 250×4 mm (supplier: BST Ltd., Budapest, Hungary); detection at 260 nm; flow rate: 1.0 mL/min; eluents, A: 0.1 M aq TEAA (pH 7.0), B: 0.1 M aq TEAA (pH 7.0)–acetonitrile 2:8; gradient: 10–50% B in A in 40 min. Anhydrous solvents were prepared as described.<sup>17</sup> Light petroleum

refers to the fraction with distillation range  $40\text{--}60^{\circ}\text{C}$ . Organic solutions were dried using magnesium sulfate and evaporated in Büchi rotary evaporators. TLC: Kieselgel 60  $\text{F}_{254}$  (Merck), visualization: UV light and ninhydrin solution for amines,  $\text{IN}_3$  solution for S(II) and P(III) compounds. Column chromatography: Kieselgel 60 (0.063–0.200 mm, Merck). Amidites (**1–3**) were purified on Merck TLC silica gel 60 H (mean particle size 15  $\mu\text{m}$ ) absorbent.<sup>18,19</sup> Mp: Electrothermal IA 8103 apparatus. Elemental analysis: Perkin-Elmer CHNS analyzer model 2400; no attempt was made to analyze syrupy substances. NMR: Bruker Avance DRX 500 spectrometer ( $^1\text{H}$ : 500.13 MHz;  $^{13}\text{C}$ : 125.76 MHz,  $^{31}\text{P}$ : 202.50 MHz),  $\text{CDCl}_3$  or  $\text{DMSO}-d_6$  solutions,  $\delta$  (ppm), *J* (Hz). Spectral patterns: s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet; br, broad. For the 2D experiments (HMQC, HMBC, COSY), the standard Bruker software packages (INV4GSSW, INV4GSLRNDWS, COSYGS) were applied. Mass spectrometry: Finnigan MAT TSQ 7000, electrospray (ESI) or nanoelectrospray<sup>20</sup> for analysis of oligonucleotides (**14–16**), phosphoramidites (**1–3**) and highly apolar compounds (**7**, **8**, **10**, **12**). Calculated molecular weights for oligonucleotides **14–16** are given for the most intensive monoisotopic peak.

**S-Trityl-2-mercaptoacetic acid (4).** Trityl chloride (6.13 g, 22 mmol, 1.1 equiv) was dissolved in toluene (50 mL), then TEA (3.07 mL, 22 mmol, 1.1 equiv) and 2-mercaptoacetic acid (1.40 mL, 20 mmol) were added at rt. After 3 h, the reaction mixture was evaporated, dissolved in DCM (100 mL) and extracted with water ( $2\times 100\text{ mL}$ ). The organic phase was dried and concentrated in vacuo, the residue was crystallized from toluene to afford the product as white needles (4.95 g, 74%). Mp  $163\text{--}164^{\circ}\text{C}$  (lit.<sup>8</sup> mp.  $162.5\text{--}163^{\circ}\text{C}$ );  $R_f$  0.43 (DCM:MeOH 9:1); (found: C, 75.1; H, 5.2; S, 9.4. Calcd for  $\text{C}_{21}\text{H}_{18}\text{O}_2\text{S}$ : C, 75.4; H, 5.4; S, 9.6);  $\delta_{\text{H}}$  (500 MHz,  $\text{DMSO}-d_6$ ) 2.89 (2 H, s,  $\text{CH}_2$ ), 7.22–7.26 (3 H, m, arom. CH), 7.30–7.36 (12 H, m, arom. CH), 12.74 (1 H, br s, COOH);  $\delta_{\text{C}}$  (125 MHz,  $\text{DMSO}-d_6$ ) 34.8 ( $\text{CH}_2$ ), 66.4 ( $\text{C}_q$ ), 127.0, 128.2, 129.1 (arom. CHs); 144.0 (arom.  $\text{C}_q$ ); 170.3 (COOH) *m/z* (ESI (EtOH)): 333.1 ( $(\text{M}-\text{H})^-$ , 84%), 667.1 ( $(2\text{M}-\text{H})^-$ , 100%).

**O-tert-Butyldiphenylsilyl-3-aminopropanol hydrochloride (6).** 3-Aminopropanol (**5**, 3.80 mL, 50.0 mmol, 2.5 equiv) was dissolved in DCM (50 mL), then TBDPS-Cl (5.20 mL, 20 mmol) in DCM (10 mL) was added dropwise during 15 min at  $0^{\circ}\text{C}$  then TEA (4.20 mL, 30 mmol) was added. After stirring at rt for 1 h, the reaction mixture was extracted with 10% (w/v) aq  $\text{NaHSO}_4$  ( $2\times 50\text{ mL}$ ) and satd aq  $\text{NaHCO}_3$  ( $2\times 50\text{ mL}$ ). The organic phase was concentrated in vacuo, the residue was dissolved in methanol (100 mL), the pH of the solution was adjusted to 6 with 1 M aq HCl and evaporated to dryness followed by repeated co-evaporation with acetonitrile ( $2\times 50\text{ mL}$ ). The residue was crystallized from DCM/diethyl ether to afford the product as white needles (6.02 g, 86%). Mp  $165\text{--}166^{\circ}\text{C}$  (subl. from  $130^{\circ}\text{C}$ );  $R_f$  0.63 (DCM:MeOH:TEA 70:30:5); (Found: C, 64.95; H, 7.9; N, 4.1. Calcd for  $\text{C}_{19}\text{H}_{28}\text{ClNOSi}$ : C, 65.2; H, 8.1; N, 4.0%);  $\delta_{\text{H}}$  (500 MHz,  $\text{CDCl}_3$ ) 1.03 [9H,

s, C(CH<sub>3</sub>)<sub>3</sub>, 2.01 (2 H, m, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 3.20 (2 H, t,  $J=7.5$ , NCH<sub>2</sub>), 3.74 (2 H, t,  $J=5.5$ , CH<sub>2</sub>O), 7.39 (6 H, m, arom. CH), 7.63 (4 H, m, arom. CH) 8.39 (3 H, br s, NH<sub>3</sub><sup>+</sup>);  $\delta_C$  (125 MHz, CDCl<sub>3</sub>) 19.8 (C(CH<sub>3</sub>)<sub>3</sub>), 27.6 (CH<sub>3</sub>), 30.9 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 38.7 (NCH<sub>2</sub>), 61.9 (CH<sub>2</sub>O), 128.5, 130.5, 136.2 (arom. CHs); 133.7 (arom. C<sub>q</sub>);  $m/z$  (ESI (aq MeOH)): 314 ((M+H)<sup>+</sup>, 100%). The free amine has been mentioned by Kende and Mendoza<sup>21</sup> but not described in detail.

***N*-(*S*-Trityl-2-mercaptoacetyl)-*O*-*tert*-butyldiphenylsilyl-3-aminopropanol (7).** Acid **4** (2.68 g, 8 mmol, 2 equiv), HOBT (1.22 g, 8 mmol, 2 equiv) and HBTU (3.03 g, 8 mmol, 2 equiv) were dissolved in DCM (30 mL), hydrochloride **6** (1.40 g, 4 mmol) and DIPEA (2.10 mL, 12 mmol, 3 equiv) in DCM (20 mL) were added dropwise at rt. The resulting solution was stirred at rt for 1 h. The reaction mixture was extracted with 10% (w/v) aq NaHSO<sub>4</sub> (2×50 mL) and 10% (w/v) aq Na<sub>2</sub>CO<sub>3</sub> (2×50 mL), then the organic layer was dried and concentrated. The crude product was purified by column chromatography using DCM as eluent and crystallized from methanol to give a white powder (2.21 g, 88%). Mp 117–118 °C;  $R_f$  0.77 (DCM:MeOH 9:1); (found: C, 76.4; H, 7.1; N, 2.3; S, 5.1. Calcd for C<sub>40</sub>H<sub>43</sub>NO<sub>2</sub>SSi: C, 76.3; H, 6.9; N, 2.2; S, 5.2%);  $\delta_H$  (500 MHz, CDCl<sub>3</sub>) 1.03 (9H, s, C(CH<sub>3</sub>)<sub>3</sub>), 1.58 (2 H, m, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 3.05 (2 H, s, SCH<sub>2</sub>), 3.11 (2 H, m, NCH<sub>2</sub>), 3.60 (2 H, t,  $J=5.7$  Hz, CH<sub>2</sub>O), 6.02 (1 H, br s, NH), 7.21 (3 H, m, arom. CH), 7.27 (6 H, m, arom. CH), 7.34 (4 H, m, arom. CH), 7.39 (8 H, m, arom. CH), 7.62 (4 H, m, arom. CH);  $\delta_C$  (125 MHz, CDCl<sub>3</sub>) 19.9 (C(CH<sub>3</sub>)<sub>3</sub>), 27.6 (CH<sub>3</sub>), 32.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 36.7 (SCH<sub>2</sub>), 37.8 (NCH<sub>2</sub>), 62.4 (CH<sub>2</sub>O), 68.4 (Ph<sub>3</sub>C), 127.7, 128.4, 128.8, 130.2, 130.4, 136.2 (arom. CHs), 134.3, 144.8 (arom. C<sub>q</sub>s), 168.5 (CONH);  $m/z$  (nano-ESI (toluene)): 630 ((M+H)<sup>+</sup>, 3%), 243 (Tr<sup>+</sup>, 100).

***N*-(*S*-Trityl-2-mercaptoacetyl)-3-aminopropanol (8).** Compound **7** (2.00 g, 3.2 mmol) dissolved in THF (10 mL) was allowed to react with 1 M TBAF (in THF, 6.4 mL, 6.4 mmol, 2 equiv) for 6 h at rt. The reaction mixture was concentrated in vacuo and purified by column chromatography (1–4% (v/v) MeOH in DCM). The collected fractions were concentrated and the residue was crystallized from EtOAc and light petroleum to give the alcohol **8** as a white powder (0.98 g, 78%). Mp 128–129 °C;  $R_f$  0.42 (DCM:MeOH 9:1); (found: C, 73.5; H, 6.2; N, 3.65; S, 8.3. Calcd for C<sub>24</sub>H<sub>25</sub>NO<sub>2</sub>S: C, 73.6; H, 6.4; N, 3.6; S, 8.2%);  $\delta_H$  (500 MHz, CDCl<sub>3</sub>) 1.51 (2 H, m, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.87 (1 H, t,  $J=6.0$ , OH), 3.10 (2 H, m, NCH<sub>2</sub>), 3.15 (2 H, s, SCH<sub>2</sub>), 3.45 (2 H, m, CH<sub>2</sub>O), 6.28 (1 H, br s, NH), 7.23 (3 H, m, arom. CH), 7.29 (6 H, m, arom. CH), 7.41 (6 H, m, arom. CH);  $\delta_C$  (125 MHz, CDCl<sub>3</sub>) 32.7 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 36.4 (SCH<sub>2</sub>), 37.0 (NCH<sub>2</sub>), 59.7 (CH<sub>2</sub>O), 68.7 (Ph<sub>3</sub>C), 127.8, 128.9, 130.1 (arom. CHs), 144.7 (arom. C<sub>q</sub>), 170.0 (CONH);  $m/z$  (nano-ESI (MeOH/LiCl)) 398 ((M+Li)<sup>+</sup>, 100%), 243 (Tr<sup>+</sup>, 90).

***O*-(*N*-(*S*-Trityl-2-mercaptoacetyl)-3-aminopropyl)-*O'*-2-cyanoethyl-*N'*,*N'*-diisopropylphosphoramidite (2).** Alcohol **8** (0.78 g, 2 mmol) was dissolved in abs DCM (10 mL),

DIPEA (0.52 g, 3 mmol, 1.5 equiv) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.67 mL, 3 mmol, 1.5 equiv) were added. After 1 h stirring MeOH (0.1 mL, 2.5 mmol), then after 5 min DCM (40 mL) were added and the mixture was extracted with satd. aq NaHCO<sub>3</sub> (2×50 mL), dried and concentrated. The residue was purified by column chromatography using a light petroleum:DCM:TEA 95:5:10–70:30:10 eluent to give the phosphoramidite **2** (0.65 g, 55%) as a colorless oil.  $R_f$  0.36 (light petroleum:DCM:TEA 75:25:10);  $\delta_H$  (500 MHz, CDCl<sub>3</sub>) 1.16 (12 H, m, 4×CH<sub>3</sub>), 1.66 (2 H, m, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.57 (2 H, t,  $J=6.4$ , CH<sub>2</sub>CN), 3.09 (4 H, m, SCH<sub>2</sub>+NCH<sub>2</sub>CH<sub>2</sub>), 3.52–3.65 (4 H, m, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O+2×CH), 3.70–3.85 (2 H, m, CH<sub>2</sub>CH<sub>2</sub>CN), 6.22 (1 H, br s, NH), 7.23 (3 H, m, arom. CH), 7.29 (6 H, m, arom. CH), 7.40 (6 H, m, arom. CH);  $\delta_C$  (125 MHz, CDCl<sub>3</sub>) 20.7 (d,  $^3J_{C,P}=6.8$ , CH<sub>2</sub>CN), 25.0 (d,  $^3J_{C,P}=7.0$ , CH<sub>3</sub>), 30.9 (d,  $^3J_{C,P}=6.9$ , CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 36.5 (SCH<sub>2</sub>), 37.5 (NCH<sub>2</sub>), 43.5 (d,  $^2J_{C,P}=12.3$ , CH), 58.8 (d,  $^2J_{C,P}=19.4$ , CH<sub>2</sub>CH<sub>2</sub>CN), 61.8 (d,  $^2J_{C,P}=17.5$ , CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 68.5 (Ph<sub>3</sub>C), 118.0 (CN), 127.5, 128.5, 129.9 (arom. CHs), 144.5 (arom. C<sub>q</sub>), 168.3 (CONH);  $\delta_P$  (200 MHz, CDCl<sub>3</sub>, H-decoupling) 149.01;  $m/z$  (nano-ESI (MeCN/LiCl)): 614 ((M+Na)<sup>+</sup>, 5%), 598 ((M+Li)<sup>+</sup>, 100), 243 (Tr<sup>+</sup>, 5).

***N*-9-Fluorenylmethoxycarbonyl-glycyl-glycine (9).** Glycyl-glycine (6.60 g, 50 mmol, 1.25 equiv) and Na<sub>2</sub>CO<sub>3</sub> (15.0 g, 142 mmol) were dissolved in dioxane:water 2:1 (450 mL), then Fmoc chloride (10.35 g, 40 mmol) in dioxane (200 mL) was added dropwise over 1 h.<sup>14</sup> The reaction mixture was stirred overnight at rt, then it was evaporated. The resulting solid was triturated with 10% aq NaHSO<sub>4</sub> (400 mL), filtered, washed with water and dried. The residue was crystallized from EtOAc and light petroleum to give the product (**9**) as a white powder (10.91 g, 77%). Mp 178–179 °C (lit.<sup>15</sup> mp. 176–177 °C (MeCN));  $R_f$  0.52 (BuOH:AcOH:H<sub>2</sub>O 4:1:1); (found: C, 64.35; H, 4.9; N, 7.7. Calcd for C<sub>19</sub>H<sub>18</sub>N<sub>2</sub>O<sub>5</sub>: C, 64.4; H, 5.1; N, 7.9%);  $\delta_H$  (500 MHz, DMSO-*d*<sub>6</sub>) 3.73 (2 H, d,  $J=4.9$ , NHCH<sub>2</sub>), 3.83 (2 H, d,  $J=4.6$ , NHCH<sub>2</sub>), 4.25 (1 H, t,  $J=5.9$ , CH<sub>2</sub>CH), 4.32 (2 H, d,  $J=6.1$ , CH<sub>2</sub>CH), 7.33 (2 H, m, arom. CH), 7.41 (2 H, m, arom. CH), 7.64 (1 H, br s, NH), 7.73 (2 H, m, arom. CH), 7.87 (2 H, m, arom. CH), 8.21 (1 H, br s, NH), 12.68 (1 H, br s, COOH);  $\delta_C$  (125 MHz, DMSO-*d*<sub>6</sub>) 40.7, 43.4 (2×CH<sub>2</sub>CO), 46.7 (CHCH<sub>2</sub>), 65.9 (CHCH<sub>2</sub>), 120.2, 125.4, 127.2, 127.7 (arom. CHs), 140.8, 143.9 (arom. C<sub>q</sub>s), 156.6 (OCONH), 169.7 (CH<sub>2</sub>CONH); 171.3 (COOH)  $m/z$  (ESI (aq MeOH)): 355.2 ((M+H)<sup>+</sup>, 100%), 377.1 ((M+Na)<sup>+</sup>, 26%), 709.1 ((2M+H)<sup>+</sup>, 23%).

***N*-(*N'*-9-Fluorenylmethoxycarbonyl-glycyl-glycyl)-*O*-*tert*-butyldiphenylsilyl-3-aminopropanol (10)**

Fmoc-Gly-Gly-OH (**9**, 4.25 g, 12 mmol, 1.5 equiv), HOBT (1.84 g, 12 mmol, 1.5 equiv) and HBTU (4.55 g, 12 mmol, 1.5 equiv) were dissolved in THF (100 mL) then hydrochloride **6** (2.80 g, 8 mmol) and DIPEA (3.48 mL, 20 mmol, 2.5 equiv) in THF (30 mL) were added dropwise at rt. After 3 h at rt, the reaction mixture was evaporated, the residue was dissolved in

EtOAc (100 mL) and extracted with 10% (w/v) aq  $\text{Na}_2\text{CO}_3$  (3×100 mL) and 10% (w/v) aq  $\text{NaHSO}_4$  (2×100 mL). The organic phase was dried, concentrated and the residue was crystallized from methanol to afford the product as white crystals (4.57 g, 88%). Mp 70–71 °C;  $R_f$  0.53 (DCM–MeOH 9:1); (found C, 70.2; H, 6.45; N, 6.4. Calc. for  $\text{C}_{38}\text{H}_{43}\text{N}_3\text{O}_5\text{Si}$ : C, 70.25; H, 6.7; N, 6.5%);  $\delta_{\text{H}}$  (500 MHz,  $\text{CDCl}_3$ ) 1.06 (9 H, s,  $\text{C}(\text{CH}_3)_3$ ), 1.74 (2 H, m,  $\text{CH}_2\text{CH}_2\text{CH}_2$ ), 3.38 (2 H, m,  $\text{NCH}_2\text{CH}_2$ ), 3.73 (2 H, t,  $J=5.6$ ,  $\text{CH}_2\text{O}$ ), 3.79 (2 H, d,  $J=4.9$ ,  $\text{NHCH}_2\text{CO}$ ), 3.85 (2 H, d,  $J=5.0$ ,  $\text{NHCH}_2\text{CO}$ ), 4.20 (1 H, t,  $J=6.8$ ,  $\text{CH}_2\text{CH}$ ), 4.41 (2 H, d,  $J=6.8$ ,  $\text{CH}_2\text{CH}$ ), 5.56 (1 H, br s, NH), 6.21 (1 H, br s, NH), 6.73 (1 H, t,  $J=5.0$ , NH), 7.29 (2 H, m, arom. CH), 7.40 (8 H, m, arom. CH), 7.56 (2 H, m, arom. CH), 7.64 (4 H, m, arom. CH), 7.74 (2 H, m, arom. CH);  $\delta_{\text{C}}$  (125 MHz,  $\text{CDCl}_3$ ) 19.9 ( $\text{C}(\text{CH}_3)_3$ ), 27.6 ( $\text{CH}_3$ ), 32.1 ( $\text{CH}_2\text{CH}_2\text{CH}_2$ ), 38.3 ( $\text{NCH}_2\text{CH}_2$ ), 43.5, 45.1 ( $2\times\text{CH}_2\text{CONH}$ ), 47.8 (CH), 63.0 ( $\text{CH}_2\text{O}$ ), 67.8 ( $\text{CHCH}_2$ ), 120.7, 125.7, 127.8, 128.4, 128.5, 130.6, 136.2 (arom. CHs), 134.1, 142.0, 144.4 (arom.  $\text{C}_q$ s), 157.4 ( $\text{OCONH}$ ), 168.8, 169.9 ( $2\times\text{CH}_2\text{CONH}$ );  $m/z$  (nano-ESI (toluene)) 672 ( $(\text{M}+\text{Na})^+$ , 100%), 650 ( $(\text{M}+\text{H})^+$ , 2).

**N-Glycyl-glycyl-O-tert-butylidiphenylsilyl-3-aminopropanol (11).** Compound **10** (3.90 g, 6 mmol) and diethylamine (6.23 mL, 60 mmol, 10 equiv) in DCM (50 mL) were allowed to react for 16 h at rt, then the mixture was evaporated and purified by column chromatography. The column was washed with DCM and 50% (v/v) MeOH in DCM, then the product was eluted with MeOH. Attempted crystallization from petroleum ether, EtOAc or MeOH invariably resulted in a pale yellow oil (1.74 g, 68%).  $R_f$  0.25 (DCM:MeOH 7:3);  $\delta_{\text{H}}$  (500 MHz,  $\text{CDCl}_3$ ) 1.04 (9 H, s,  $\text{C}(\text{CH}_3)_3$ ), 1.72 (2 H, m,  $\text{CH}_2\text{CH}_2\text{CH}_2$ ), 3.35 (2 H, m,  $\text{NCH}_2\text{CH}_2$ ), 3.52 (2 H, d,  $J=6.1$ ,  $\text{NCH}_2\text{CO}$ ), 3.70 (2 H, t,  $J=5.7$ ,  $\text{CH}_2\text{O}$ ), 3.82 (2 H, s,  $\text{NCH}_2\text{CO}$ ), 6.42 (1 H, t,  $J=5.3$ , NH), 7.41 (7 H, m, arom. CH + NH), 7.62 (4 H, m, arom. CH) 7.74 (2 H, br s,  $\text{NH}_2$ );  $\delta_{\text{C}}$  (125 MHz,  $\text{CDCl}_3$ ) 19.9 ( $\text{C}(\text{CH}_3)_3$ ), 27.6 ( $\text{CH}_3$ ), 32.3 ( $\text{CH}_2\text{CH}_2\text{CH}_2$ ), 38.1 ( $\text{NCH}_2\text{CH}_2$ ), 43.5, 45.2 ( $2\times\text{CH}_2\text{CONH}$ ), 62.9 ( $\text{CH}_2\text{O}$ ), 128.5, 130.5, 136.2 (arom. CHs), 134.2 (arom.  $\text{C}_q$ ), 169.4, 173.7 ( $2\times\text{CH}_2\text{CONH}$ );  $m/z$  (ESI (aq MeOH)), 450 ( $(\text{M}+\text{Na})^+$ , 5%), 428 ( $(\text{M}+\text{H})^+$ , 100).

**N-(S-Trityl-2-mercaptoacetyl-glycyl-glycyl)-O-tert-butylidiphenylsilyl-3-aminopropanol (12).** Acid **4** (2.20 g, 6.6 mmol, 2 equiv), HOBt (1.01 g, 6.6 mmol, 2 equiv), HBTU (2.50 g, 6.6 mmol, 2 equiv) and DIPEA (1.15 mL, 6.6 mmol, 2 equiv) were dissolved in DCM (100 mL), then the solution of amine **11** (1.40 g, 3.3 mmol) in DCM (10 mL) was added dropwise. After stirring at rt for 3 h, the mixture was successively extracted with 10% (w/v) aq  $\text{NaHSO}_4$  (100 mL) then with 10% (w/v) aq  $\text{NaHCO}_3$  (2×100 mL), the organic phase was dried, concentrated and purified by column chromatography (0–10% (v/v) MeOH in DCM). The collected fractions were evaporated and the resulting oil was triturated and crystallized from MeOH to give the product as a white powder (2.06 g, 84%). Mp 138–139 °C (sinters from 127 °C);  $R_f$  0.48 (DCM:MeOH 9:1); (found C, 71.2; H, 6.7; N, 5.8; S, 4.2. Calcd for

$\text{C}_{44}\text{H}_{49}\text{N}_3\text{O}_4\text{SSi}$ : C, 71.0; H, 6.65; N, 5.65; S, 4.3%);  $\delta_{\text{H}}$  (500 MHz,  $\text{CDCl}_3$ ) 1.06 (9 H, s,  $\text{C}(\text{CH}_3)_3$ ), 1.72 (2 H, m,  $\text{CH}_2\text{CH}_2\text{CH}_2$ ), 3.13 (2 H, s,  $\text{SCH}_2$ ), 3.36 (2 H, m,  $\text{NCH}_2\text{CH}_2$ ), 3.56 (2 H, d,  $J=5.2$ ,  $\text{NHCH}_2\text{CO}$ ), 3.72 (4 H, m,  $\text{CH}_2\text{O}+\text{NHCH}_2\text{CO}$ ), 6.17 (1 H, t,  $J=5.1$ , NH), 6.41 (1 H, t,  $J=5.1$ , NH), 6.49 (1 H, t,  $J=5.4$ , NH), 7.21 (3 H, m, arom. CH), 7.27 (6 H, m, arom. CH), 7.40 (12 H, m, arom. CH), 7.64 (4 H, m, arom. CH);  $\delta_{\text{C}}$  (125 MHz,  $\text{CDCl}_3$ ) 19.9 ( $\text{C}(\text{CH}_3)_3$ ), 27.6 ( $\text{CH}_3$ ), 32.2 ( $\text{CH}_2\text{CH}_2\text{CH}_2$ ), 36.3 ( $\text{SCH}_2$ ), 38.2 ( $\text{NCH}_2\text{CH}_2$ ), 43.5, 44.1 ( $2\times\text{CH}_2\text{CONH}$ ), 62.9 ( $\text{CH}_2\text{O}$ ), 68.5 ( $\text{Ph}_3\text{C}$ ), 127.7, 128.5, 128.9, 130.2, 130.5, 136.2 (arom. CHs), 134.1, 144.6 (arom.  $\text{C}_q$ s); 168.7, 169.1, 169.6 ( $3\times\text{CONHs}$ );  $m/z$  (nano-ESI (MeCN)) 766 ( $(\text{M}+\text{Na})^+$ , 100%), 243 ( $\text{Tr}^+$ , 95).

**N-(S-Trityl-2-mercaptoacetyl-glycyl-glycyl)-3-aminopropanol (13).** The solution of compound **12** (2.00 g, 2.7 mmol) in THF (20 mL) and 1 M TBAF (in THF, 5.4 mL, 5.4 mmol, 2 equiv) were allowed to react at rt for 6 h, then the reaction mixture was concentrated and purified by column chromatography (0–20% (v/v) MeOH in DCM). The collected fractions were evaporated and the resulting white foam was crystallized from toluene/DCM to afford the alcohol **13** as a white solid (1.05 g, 77%). Mp 139–140 °C (sinters from 115 °C);  $R_f$  0.24 (DCM:MeOH 9:1); (Found C, 66.3; H, 6.0; N, 8.5; S, 6.35. Calc. for  $\text{C}_{28}\text{H}_{31}\text{N}_3\text{O}_4\text{S}$ : C, 66.5; H, 6.2; N, 8.3; S, 6.3%);  $\delta_{\text{H}}$  (500 MHz,  $\text{CDCl}_3$ ) 1.65 (2 H, m,  $\text{CH}_2\text{CH}_2\text{CH}_2$ ), 3.15 (2 H, s,  $\text{SCH}_2$ ), 3.30 (1 H, br s, OH), 3.35 (2 H, m,  $\text{NCH}_2\text{CH}_2$ ), 3.56 (2 H, d,  $J=5.4$ ,  $\text{NHCH}_2\text{CO}$ ), 3.59 (2 H, m,  $\text{CH}_2\text{CH}_2\text{O}$ ), 3.85 (2 H, d,  $J=5.8$ ,  $\text{NHCH}_2\text{CO}$ ), 6.69 (1 H, t,  $J=5.3$ , NH), 6.86 (1 H, t,  $J=5.8$ , NH), 6.95 (1 H, t,  $J=5.6$ , NH), 7.23 (3 H, m, arom. CH), 7.29 (6 H, m, arom. CH), 7.42 (6 H, m, arom. CH);  $\delta_{\text{C}}$  (125 MHz,  $\text{CDCl}_3$ ) 32.3 ( $\text{CH}_2\text{CH}_2\text{CH}_2$ ), 36.3 ( $\text{SCH}_2$ ), 37.5 ( $\text{NCH}_2\text{CH}_2$ ), 43.8, 44.5 ( $2\times\text{CH}_2\text{CONH}$ ), 60.5 ( $\text{CH}_2\text{O}$ ), 68.7 ( $\text{Ph}_3\text{C}$ ), 127.8, 128.6, 130.2 (arom. CHs); 144.5 (arom.  $\text{C}_q$ s), 169.5, 170.0, 170.5 ( $3\times\text{CONH}$ );  $m/z$  (ESI (MeOH)) 528 ( $(\text{M}+\text{Na})^+$ , 40%), 506 ( $(\text{M}+\text{H})^+$ , 40), 243 ( $\text{Tr}^+$ , 100).

**O-(N-(S-Trityl-2-mercaptoacetyl-glycyl-glycyl)-3-aminopropyl)-O'-2-cyanoethyl-N',N'-diisopropylphosphoramidite (3).** Alcohol **13** (0.80 g, 1.6 mmol) was dissolved in abs. DCM (10 mL), then DIPEA (0.42 mL, 2.4 mmol, 1.5 equiv) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.54 mL, 2.4 mmol, 1.5 equiv) were added dropwise under stirring. After 1 h, MeOH (0.10 mL, 2.5 mmol) was added and stirring was continued for 5 min, then the solution was diluted with DCM (40 mL) and the reaction mixture was extracted with satd aq  $\text{NaHCO}_3$  (2×50 mL). The organic phase was dried, concentrated and purified by column chromatography (10% (v/v) TEA in DCM). The collected fractions were evaporated and the resulting colorless oil was dried by repeated coevaporation with acetonitrile (2×25 mL) to leave a white foam that formed a white solid after trituration with dry diethyl ether (0.59 g, 52%). Mp 116–118 °C (sinters from 100 °C);  $R_f$  0.21 (DCM:EtOAc:TEA 45:45:10); (found C, 62.8; H, 6.7; N, 9.8; S, 4.5. Calc. for  $\text{C}_{37}\text{H}_{48}\text{N}_5\text{O}_5\text{PS}$ : C, 63.0; H, 6.85; N, 9.9; S,

4.5%);  $\delta_{\text{H}}$  (500 MHz,  $\text{CDCl}_3$ ) 1.18 (12 H, m,  $4 \times \text{CH}_3$ ), 1.82 (2 H, m,  $\text{CH}_2\text{CH}_2\text{CH}_2$ ), 2.63 (2 H, t,  $J=6.2$ ,  $\text{CH}_2\text{CN}$ ), 3.17 (2 H, s,  $\text{SCH}_2$ ), 3.36 (2 H, m,  $\text{NCH}_2\text{CH}_2$ ), 3.53–3.63 (4 H, m,  $\text{NCH}_2\text{CO} + 2 \times \text{CH}$ ), 3.65–3.80 (3 H, m,  $\text{CH}_2\text{CH}_2\text{CH}_2\text{O} + 1/2 \text{OCH}_2\text{CH}_2\text{CN}$ ), 3.83–3.90 (3 H, m,  $1/2 \text{OCH}_2\text{CH}_2\text{CN} + \text{NCH}_2\text{CO}$ ), 6.51 (1 H, br s, NH), 6.60 (1 H, t,  $J=5.0$ , NH), 6.65 (1 H, t,  $J=5.3$ , NH), 7.23 (3 H, m, arom. CH), 7.29 (6 H, m, arom. CH), 7.42 (6 H, m, arom. CH);  $\delta_{\text{C}}$  (125 MHz,  $\text{CDCl}_3$ ) 21.2 (d,  $^3J_{\text{C,P}}$  6.7,  $\text{CH}_2\text{CN}$ ), 25.3 (d,  $^3J_{\text{C,P}}$  7.0,  $\text{CH}_3$ ), 25.4 (d,  $^3J_{\text{C,P}}$  6.8,  $\text{CH}_3$ ), 31.2 (d,  $^3J_{\text{C,P}}$  7.3,  $\text{CH}_2\text{CH}_2\text{CH}_2$ ), 36.4 ( $\text{SCH}_2$ ), 37.8 ( $\text{NCH}_2\text{CH}_2$ ), 43.6, 44.3 ( $2 \times \text{CH}_2\text{CONH}$ ), 43.9 (d,  $^2J_{\text{C,P}}$  12.6, CH), 58.8 (d,  $^2J_{\text{C,P}}$  19.5,  $\text{CH}_2\text{CH}_2\text{CN}$ ), 62.5 (d,  $^2J_{\text{C,P}}$  17.2,  $\text{CH}_2\text{CH}_2\text{CH}_2\text{O}$ ), 68.6 ( $\text{Ph}_3\text{C}$ ), 118.8 (CN), 127.8, 128.9, 130.2 (arom. CHs), 144.6 (arom.  $\text{C}_q$ ), 169.1, 169.3, 169.8 ( $3 \times \text{CONH}$ );  $\delta_{\text{P}}$  (200 MHz,  $\text{CDCl}_3$ , H-decoupling) 148.96;  $m/z$  (nano-ESI (MeCN/LiCl)) 744 ( $(\text{M} + \text{K})^+$ , 76%), 728 ( $(\text{M} + \text{Na})^+$ , 100), 712 ( $(\text{M} + \text{Li})^+$ , 25), 243 ( $\text{Tr}^+$ , 18).

**Oligonucleotide 15.** The solution of oligonucleotide **14** (50 nmol) in 0.1 M TEAA (100  $\mu\text{L}$ ) was allowed to react with aq 0.1 M  $\text{AgNO}_3$  (5  $\mu\text{L}$ , 500 nmol, 10 equiv) at rt for 1 h. Aq 0.1 M DTT solution (7.5  $\mu\text{L}$ , 750 nmol, 15 equiv) was added and after 30 min the precipitate was centrifuged, the supernatant was removed, the precipitate was washed with 0.1 M TEAA ( $3 \times 50 \mu\text{L}$ ). The combined aq phases were extracted with EtOAc ( $3 \times 200 \mu\text{L}$ ) to remove excess DTT. The resulting aq solution containing the oligonucleotide **15** was purified by HPLC, lyophilized and analysed by mass spectrometry.  $m/z$  (Nano-ESI (aq MeCN)) 694.5 ( $(\text{M} - 3\text{H})^{3-}$ , 50%), 520.6 ( $(\text{M} - 4\text{H})^{4-}$ , 100), 416.2 ( $(\text{M} - 5\text{H})^{5-}$ , 20),  $\text{C}_{69}\text{H}_{95}\text{N}_{15}\text{O}_{46}\text{SP}_6$  requires 2087.4.

**Conjugation of *N*-Boc-L-Cys(Npys)-OH to oligonucleotide 15 (oligonucleotide 16).** The solution of oligonucleotide **15** (30 nmol) in 0.1 M TEAA (50  $\mu\text{L}$ ) was allowed to react with *N*-Boc-L-Cys(Npys)-OH (90 nmol, 3 equiv) in 0.1M TEAA (10  $\mu\text{L}$ ) at rt for 16 h. 3-Nitro-2-thiopyridone was removed by ethereal extraction ( $3 \times 100 \mu\text{L}$ ). The resulting aq solution containing the oligonucleotide **16** was purified by HPLC, lyophilized and analysed by mass spectrometry.  $m/z$  (Nano-ESI (aq MeCN)) 2305.8 ( $(\text{M} - \text{H})^-$ , 2%), 1152.5 ( $(\text{M} - 2\text{H})^{2-}$ , 80), 767.9 ( $(\text{M} - 3\text{H})^{3-}$ , 100), 575.5 ( $(\text{M} - 4\text{H})^{4-}$ , 10),  $\text{C}_{77}\text{H}_{109}\text{N}_{16}\text{O}_{50}\text{S}_2\text{P}_6$  requires 2307.4.

## Acknowledgements

This research has been supported by grants OTKA No. T 030135 and FKFP No. 0597/1999.

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