Stepwise Solid-Phase Synthesis of Peptide-Oligonucleotide Conjugates on New Solid Supports

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Dedicated to Prof. Dr. *Frank Seela* on the occasion of his 60th birthday

Several peptide-oligonucleotide and peptide-(oligonucleotide phosphorothioate) conjugates were synthesized on new solid supports. These supports are designed to link the 3′-terminus of an oligonucleotide to the C-end of a peptide *via* a phosphodiester or phosphorothioate bond in the process of stepwise solid-phase assembly.

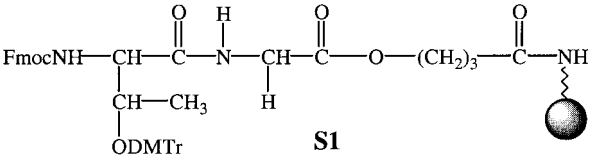
Introduction. – Covalent oligonucleotide-peptide conjugates have received considerable attention as new antisense agents that exhibit potentially enhanced cellular uptake [1–16]. The preparation of such conjugates has been based either on postsynthetic conjugation of oligonucleotides and peptides, both bearing appropriate reactive groups [1–6][8][9][11][12][14–16], or on stepwise solid-phase assembly [7][10][13]. All the postsynthetic conjugations described so far are associated with lengthy and troublesome procedures that include several intermediate purification steps. In contrast, stepwise solid-phase procedures to prepare oligonucleotide-peptide conjugates require usually only one purification step upon the final deprotection. However, the choice of permanent protecting groups of nucleoside and amino-acid monomers, employed in the stepwise solid-phase synthesis, remains the critical factor, which explains the relatively small number of publications on this subject [7][10][13]. Moreover, these papers describe the stepwise synthesis of conjugates, in which relatively short peptides and oligonucleotides with phosphodiester bonds are incorporated. No data on conjugates of peptides and relatively long oligonucleotide phosphorothioates (15- to 30-mers), obtained by this methodology, are available to our knowledge.

Here we describe the synthesis on three different solid supports, of several peptide-oligonucleotide and peptide-(oligonucleotide phosphorothioate) conjugates designed to link the 3′-terminus of an oligonucleotide to the C-end of a peptide *via* a phosphodiester or phosphorothioate bond in the process of the stepwise solid-phase assembly.

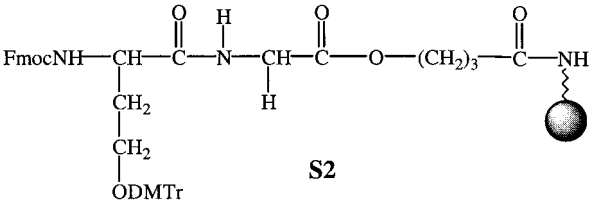
Results and Discussion. – *Preamble.* In the present work, our objective was to synthesize certain peptide-(oligonucleotide phosphorothioate) conjugates. In these, the 3′-terminus of the 15-mer oligonucleotide phosphorothioate should be linked to the C-terminus of a 16-mer membrane-permeable motif (**MPM**) – the hydrophobic region of the signal peptide sequence of the *Kaposi* fibroblast growth factor, known to interact

with lipid bilayers [17]. To achieve this goal, we had to prepare a suitable solid support, containing a (9*H*-fluoren-9-ylmethoxy)carbonyl(Fmoc)-protected amino function for easy standard peptide-chain elongation, a 4,4′-dimethoxytrityl($(MeO)_2Tr$)-blocked hydroxy group for standard oligonucleotide chain synthesis, and, finally, a base-labile bridge to a solid matrix, *i.e.* to a long chain alkylamino controlled pore glass (lcaa-CPG).

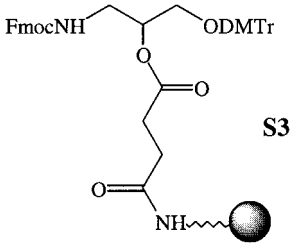
Solid Supports **S1** *and* **S2**. The structure of solid support **S1** seemed to fulfill the above mentioned requirements. The N-terminal L-threonine residue contained the necessary Fmoc-protected amino function and $(MeO)_2Tr$-blocked hydroxyl group. The C-terminal glycine residue served as an achiral linker between the L-threonine residue and 4-hydroxybutanoic acid, which was attached to lcaa-CPG. The ester bond of this linker could be easily cleaved under basic conditions.



S1



S2



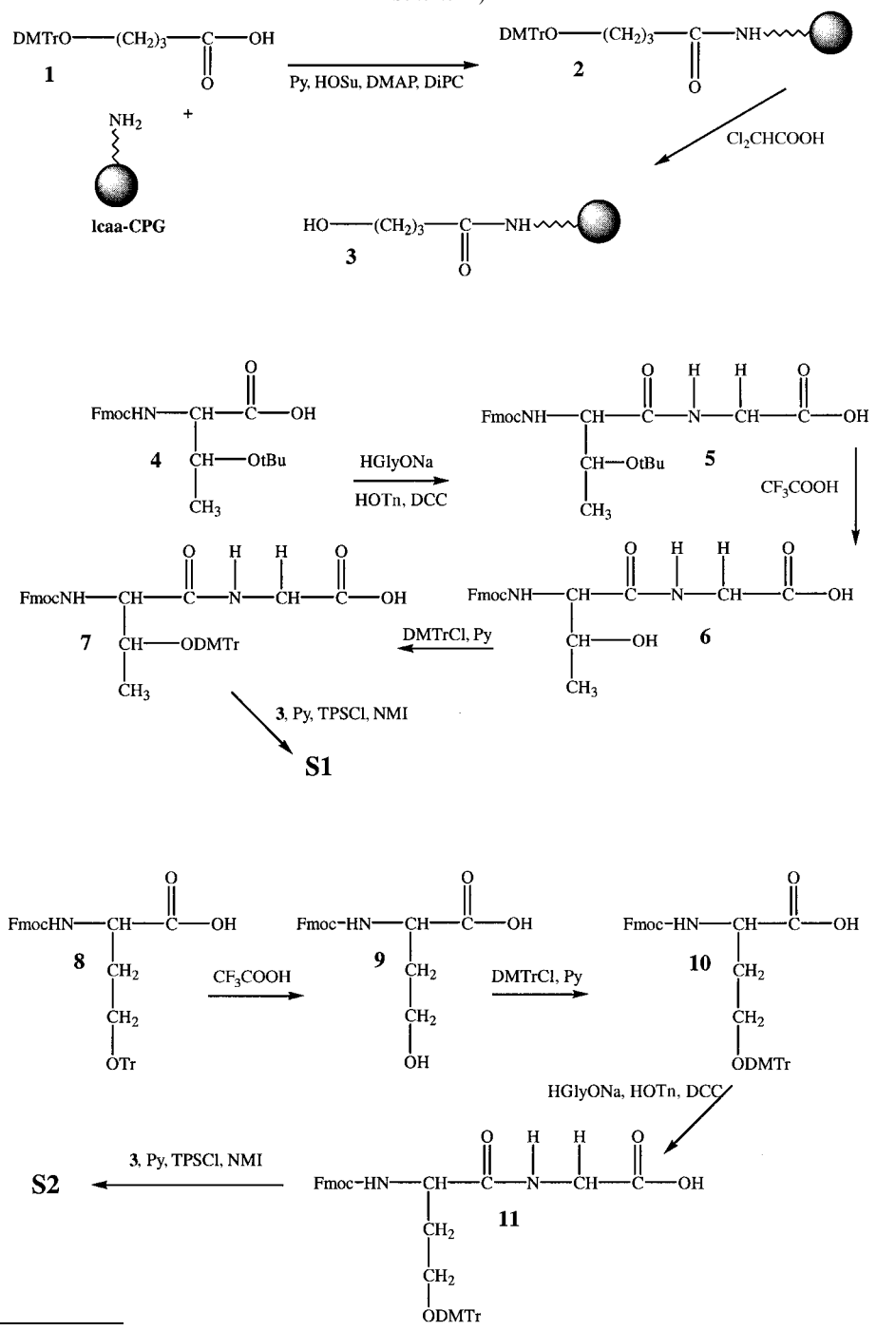
S3

DMTr = $(MeO)_2Tr$ = bis(4-methoxyphenyl)phenylmethyl
Fmoc = (9*H*-fluoren-9-ylmethoxy)carbonyl

Of certain concern to us was the possible β-elimination of the peptide residue linked to the oligonucleotide *via* the hydroxy group of L-threonine during the final basic cleavage and deprotection step. It was obvious that the substitution of the L-threonine for a L-homoserine residue in the structure of solid matrix **S2** would ensure the absence of this possible side reaction during the synthesis of desired conjugates.

The preparation of solid supports **S1** and **S2** is outlined in *Scheme 1*. First, lcaa-CPG was derivatized with 4-hydroxybutanoic acid to give matrix **3** *via* **1** and **2**. Two dipeptides, Fmoc-Thr[$(MeO)_2Tr$]-Gly-OH (**7**) and Fmoc-Hse[$(MeO)_2Tr$]-Gly-OH

Scheme 1 [a])



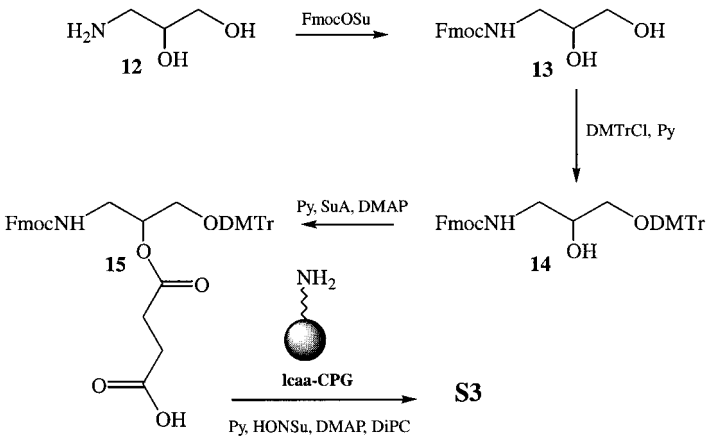
[a]) DMTr = $(MeO)_2Tr$ = bis(4-methoxyphenyl)phenylmethyl; Fmoc = (9*H*-fluoren-9-ylmethoxy)carbonyl; HOSu = *N*-hydroxysuccinimide; DMAP = 4-(dimethylamino)pyridine; DiPC = diisopropylcarbodiimide; HOTn = *N*-hydroxy-8,9,10-trinorbornane-2,3-dicarboximide; DCC = dicyclohexylcarbodiimide; TPSCl = 2,4,6-triisobenzenesulfonyl chloride; NMI = 1-methyl-1*H*-imidazole.

(**11**), were synthesized in solution following routine procedures (from **4** *via* **5** and **6**, and from **8** *via* **9** and **10**, resp.), and finally linked to the matrix **3** [18] to give solid supports **S1** and **S2**. The resulting **S1** and **S2** contained 11.6 and 11.3 μmol, respectively, of $(MeO)_2Tr$ groups per g of solid support [19].

Solid Support **S3**. Stepwise solid-phase assembly on supports **S1** and **S2** would lead to conjugates, incorporating the desired peptide, extended by two amino-acid residues (-Thr-Gly-, **S1**; -Hse-Gly-, **S2**) at the C-terminus. This may change the structure of some peptide moieties and, possibly, influence the cellular uptake of certain conjugates. Therefore, it seemed reasonable to design a solid support which would lead to a simple neutral linker bridging the peptide and oligonucleotide parts of conjugates.

Solid support **S3** was prepared starting from readily available (±)-3-amino-propane-1,2-diol (**12**) (*Scheme 2*). After protection of the amino function with the Fmoc group (→**13**) and of the primary alcohol function with the $(MeO)_2Tr$ group, compound **14** was linked to the lcaa-CPG by a succinate bridge between the secondary alcohol function (see **15**) and the amino group of the support [19]. The resulting **S3** contained 36.0 μmol of $(MeO)_2Tr$ groups per g of solid support [19].

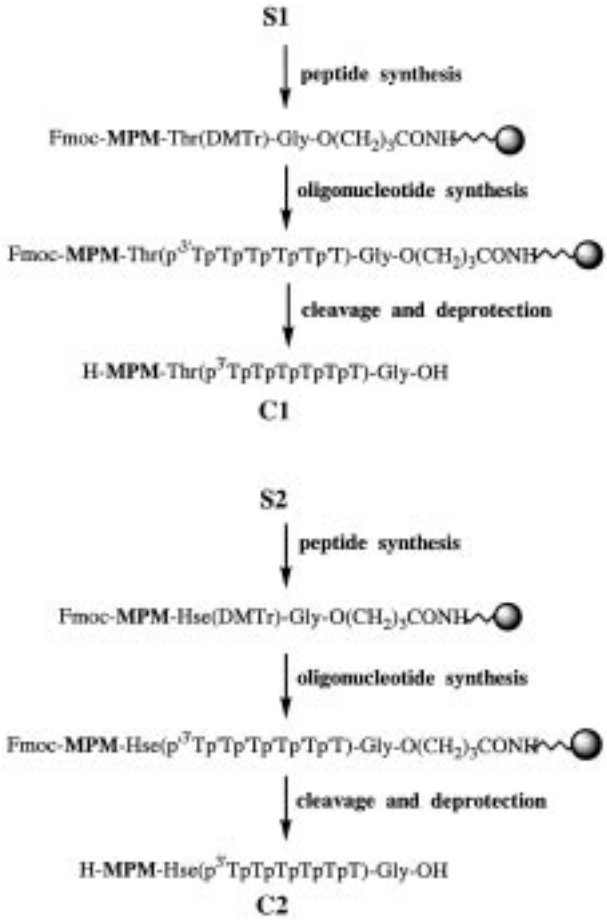
Scheme 2[a])



[a]) For abbreviations, see *Scheme 1*. SuA = Succinic anhydride.

Comparison of Supports **S1**, **S2**, *and* **S3** *for Synthesis of* **MPM**-*(oligo-T_6) Conjugates.* To investigate the usefulness of solid supports **S1**, **S2**, and **S3** in the stepwise solid-phase synthesis of peptide-oligonucleotide conjugates, the **MPM** sequence (-Ala-Ala-Val-Ala-Leu-Leu-Pro-Ala-Val-Leu-Leu-Ala-Leu-Leu-Ala-Pro-) was assembled on a *Milligen-9050* peptide synthesizer, by standard protocols based on Fmoc/2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU)/triethylamine (TEA) chemistry (*Schemes 3* and *4*). Columns were removed from the peptide synthesizer. Portions of derivatized supports, containing 1 μmol of $(MeO)_2Tr$ groups were packed into oligonucleotide-synthesizer columns and installed on a *PE-Applied Biosystems-392* DNA synthesizer. Standard phosphoramidite chemistry and recommended protocols (1.0-μmol scale) were employed to assemble the oligo-T_6 sequence (*Schemes 3* and *4*). The assembled conjugates were first cleaved and deprotected with

Scheme 3[a])



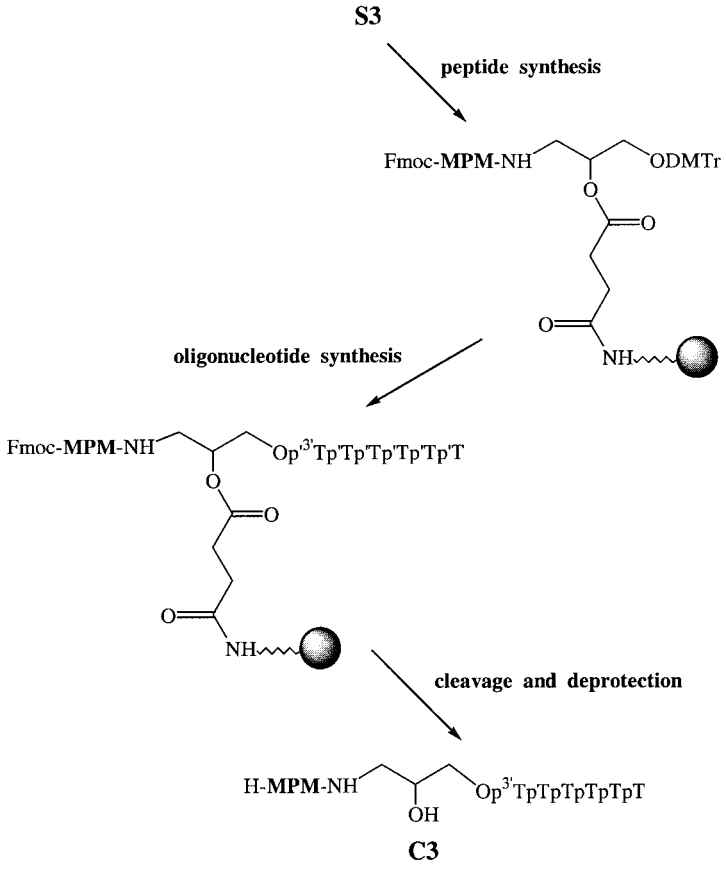
[a]) **MPM** = 16-mer membrane-permeable motif; p′ = 2-cyanoethyl-protected internucleosidic phosphotriester unit; p = phosphodiester moiety; for Fmoc and DMTr, see *Scheme 1*.

conc. aqueous NH_3 solution at 25° for 2 h, and the composition of the reaction mixtures was investigated by reversed-phase and ion-exchange HPLC.

In the case of the reaction mixture derived from support **S1**, both HPLC techniques revealed the 21% content of conjugate **C1** (see *Table*) and *ca*. 10% of oligo-T_6. The presence of oligo-T_6 may be explained by a β-elimination of the peptide residue linked to the oligonucleotide *via* the hydroxy group of L-threonine during the final basic cleavage and deprotection step. This phenomenon considerably limits the use of support **S1** for preparation of conjugates.

In contrast, HPLC analysis of the mixtures derived from supports **S2** and **S3** revealed higher contents of conjugate **C2** and **C3** (34 and 45%, resp.; see *Table*). As expected, no oligo-T_6 was detected in either case. Thus, matrices **S2** and **S3** appear to

Scheme 4[a])



[a]) **MPM** = 16-mer membrane-permeable motif; p′ = 2-cyanoethyl-protected internucleosidic phosphotriester unit; p = phosphodiester moiety; for Fmoc and DMTr, see *Scheme 1*.

Table. *Yields, HPLC Retention Times* t_R, *and Measured (ESI-MS) and Calculated Average Molecular Masses of the Synthesized Conjugates*

	Content after cleavage and deprotection [%]	Isolated yield [%]	Reversed-phase HPLC t_R [min]	Ion-exchange HPLC t_R [min]	Calculated mass	Measured mass ± s.d. ($n = 6$)
C1	21	12.1	22.12	8.02	3499.3	3498.6 ± 0.4
C2	34	29.2	20.10	6.84	3499.3	3498.4 ± 0.7
C3	45	36.5	20.24	7.01	3414.2	3414.8 ± 0.3
C4	22	17.5	18.72	17.27	6502.0	6502.3 ± 0.5
C5	30	26.4	18.95	17.22	6416.9	6416.3 ± 1.5
O1[a])	–	–	7.12	10.28	–	–
O2[b])	–	–	12.03	22.60	–	–

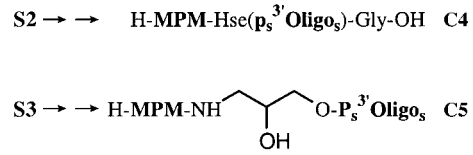
[a]) **O1** = 5′-TpTpTpTpTpT-3′. [b]) **O2** = 5′-$Tp_sGp_sGp_sCp_sGp_sTp_sCp_sTp_sTp_sCp_sCp_sAp_sTp_sTp_sT$-3′.

perform well in the stepwise solid-phase synthesis of peptide-oligonucleotide conjugates.

All three conjugates **C1**, **C2**, and **C3** of oligo-T_6 were isolated by ion-exchange HPLC and finally desalted (for isolated yields, see *Table*).

Peptide-(Oligonucleotide Phosphorothioate) Conjugates on Solid Supports **S2** *and* **S3**. The next question was whether our supports **S2** and **S3** are suitable for the stepwise synthesis of conjugates of **MPM** peptide and a 15-mer oligonucleotide phosphorothioate. Conjugates **C4** and **C5** were assembled analogously to the conjugates **C1**–**C3** of oligo-T_6 by the protocol recommended for phosphorothioates (*Scheme 5*). HPLC Analysis of mixtures derived from **S2** and **S3** showed reasonable contents of conjugates **C4** and **C5**, respectively (22% and 30%, resp.; see *Table*). In the ion-exchange chromatogram of the **C5** sample, after treatment with conc. aqueous NH_3 solution at 55° for 5 h, the product peak at t_R 17.22 min (see *Fig.*) corresponds to conjugate **C5**, as demonstrated by electrospray-ionization mass spectrometry (see *Table*). Compounds **C4** and **C5** were isolated by ion-exchange HPLC and finally desalted (for isolated yields, see *Table*).

Scheme 5[a])



[a]) **MPM** = 16-mer membrane-permeable motif; **Oligo**$_s$ = oligonucleotide phosphorothioate 5′-$Tp_sTp_sTp_sAp_sCp_sCp_sTp_sTp_sCp_sTp_sGp_sCp_sGp_sGp_sT$-3′

Hence, supports **S2** and **S3** may be successfully employed in the stepwise solid-phase synthesis of peptide-(oligonucleotide phosphorothioate) conjugates. It is noteworthy that both supports are relatively easy to prepare. While synthesis of matrix **S2** requires the use of the expensive L-homoserine monomer, preparation of **S3** employs the readily available (±)-3-aminopropane-1,2-diol (**12**). Moreover, unlike compounds derived from **S2**, conjugates synthesized on **S3** do not incorporate two additional amino-acid residues but contain only a simple neutral linker bridging the peptide and oligonucleotide moieties. Additionally, synthesis on solid support **S3** brings about higher yields of desired compounds (see *Table*). Thus, matrix **S3** appears to be the solid support of choice for the stepwise solid-phase synthesis of peptide-oligonucleotide conjugates.

Characterization of Peptide-Oligonucleotide Conjugates. Reversed-phase and ion-exchange HPLC retention times of the compounds synthesized are given in the *Table.* The purity of all conjugates, as assessed from reversed-phase HPLC, was higher than 95%. The final characterization was achieved by electrospray-ionization mass spectrometry (ESI-MS), which has proven to be a powerful method for the characterization of peptide-oligonucleotide conjugates [16]. The measured and calculated average molecular masses of the compounds reported here were in excellent agreement (see *Table*), the difference between the calculated and measured M_r being always less than 0.05%.

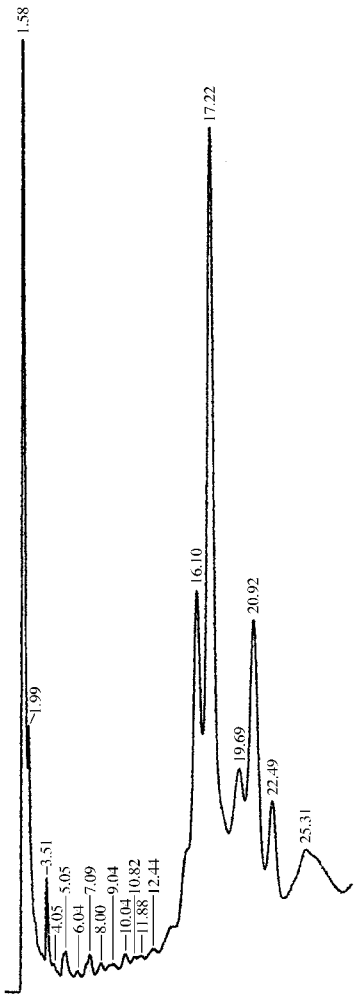


Figure. *Ion-exchange HPLC traces of the* **C5** *sample, synthesized on support* **S3**, *after treatment with conc. aqueous* NH_3 *solution at 55° for 5 h*

Conclusion. – We designed, synthesized, and tested new solid supports to prepare peptide-oligonucleotide and peptide-(oligonucleotide phosphorothioate) conjugates. These supports allow the linking of the 3′-terminus of an oligonucleotide to the C-end of a 16-mer peptide *via* a phosphodiester or phosphorothioate bond in the process of a stepwise solid-phase assembly. All conjugates obtained were thoroughly characterized by ESI-MS.

We thank Miss *Unni Tengvall* for ESI-MS experiments. Financial support from the *Technology Development Center of Finland* is gratefully acknowledged.

Experimental Part

General. Matrix 4-Hydroxy-N-*(lcaa-CPG)butanamide.* To a soln. of 4-hydroxybutanoic acid sodium salt (0.63 g, 5 mmol) in dry pyridine (50 ml), 4,4′-dimethoxytrityl chloride ($(MeO)_2TrCl$; 2.05 g, 6 mmol) was added.

The mixture was stirred for 4 h at 20° and the reaction finally quenched by addition of ice. The resulting soln. was evaporated and the residue dissolved in AcOEt (50 ml). The soln. was washed with 5% $NaHCO_3$ soln. and H_2O and evaporated, the residue dissolved in MeOH/pyridine/H_2O 1:1:1 (60 ml), and the soln. passed through a *Dowex 50* (Py^+) column (3 × 15 cm). The resulting soln. was evaporated, co-evaporated with dry pyridine (3 × 10 ml), and finally dried: 2.1 g of *4-[bis(4-methoxyphenyl)phenylmethoxy]butanoic acid* (**1**). Pale yellow oil which was used further without any purification.

To a soln. of crude **1** (0.7 g) in dry pyridine (Py; 10 ml), *N*-hydroxysuccinimide (HOSu; 0.14 g, 1 mmol) and 4-(dimethylamino)pyridine (DMAP; 24 mg, 0.2 mmol) were added, and the soln. was treated with 2.0 g of lcaa-CPG (2.0 g). Diisopropylcarbodiimide (DiPC; 0.4 ml, 2 mmol) was added and the mixture shaken overnight. The CPG derivative was filtered off, washed twice with dry pyridine, and treated with Ac_2O /pyridine/1-methyl-1*H*-imidazole (NMI) 1:5:1 (21 ml) for 30 min. The solid matrix was filtered off, washed with pyridine, tetrahydrofuran, and Et_2O, and dried: *4-[bis(4-methoxyphenyl)phenylmethoxy]*-N-*(lcaa-CPG)butanamide* (**2**), containing 16.1 μmol of $(MeO)_2Tr$ groups per g of matrix.

Matrix **2** (1 g) was treated with 2.5% $Cl_2CHCOOH$ in CH_2Cl_2 (10 ml). After 10 min at 20°, the matrix was filtered off, washed with CH_2Cl_2, tetrahydrofuran, and Et_2O, and dried to give **3**.

Fmoc-Thr-Gly-OH (**6**). To a soln. of Fmoc-Thr(*t*Bu)-OH (**4**, 2.0 g, 4.8 mmol) and *N*-hydroxy-8,9,10-trinorborn-5-ene-2,3-dicarboximide (= *N*-hydroxybicyclo[2.2.1]hept-2-ene-2,3-dicarboximide; HOTn, 0.9 g, 5 mmol) in MeCN/DMF 4:1 (25 ml) at −20°, a soln. of dicyclohexylcarbodiimide (DCC; 1.05 g, 5 mmol) in MeCN (5 ml) was added, and the mixture was left overnight at +4°. The precipitate was filtered off, the filtrate evaporated, and the residue dissolved in MeCN/DMF (20 ml) and mixed with glycine (0.38 g, 5 mmol) in a 1M NaOH soln. in MeCN/DMF 1:2 (15 ml). The mixture was stirred overnight and then evaporated, the residue dissolved in AcOEt (50 ml), the soln. washed with 2% H_2SO_4 soln. and H_2O and evaporated, and the residue crystallized from hexane. The precipitate was washed with hexane (2 × 20 ml), dried, and treated with CF_3COOH (20 ml) for 30 min. The CF_3COOH was evaporated and the product crystallized from hexane/AcOEt 1:1. The precipitate was washed with hexane and dried: 1.65 g (86%) of (**6**). White crystals. M.p. 82–83°. ^{1}H-NMR ($CDCl_3$): 1.12 (*d*, *J* = 6.1, *Me*CH_2); 3.91–4.44 (*m*, CHC*H*$_2$OCO (Fmoc), C*H*CH_2OCO (Fmoc), CHCHCO (Thr), MeC*H*CH(Thr), NHC*H*$_2$COOH(Gly)); 6.31 (br. *m*, NH); 6.56 (br. *m*, NH); 7.22–7.71 (*m*, 8 arom. H).

*Solid Support {*O-*[Bis(4-methoxyphenyl)phenylmethyl]*-N-*[(9*H*-*fluoren-9-ylmethoxy)carbonyl]*-L-*threonyl]glycine 4-[(lcaa-CPG)amino]-4-oxobutyl Ester* (**S1**). A soln. of **6** (1.6 g, 4 mmol) in 1M NaOH/pyridine 1:5 (25 ml) was evaporated and the residue dried by co-evaporation with pyridine and dissolved in dry pyridine (50 ml). $(MeO)_2TrCl$ (1.7 g, 5 mmol) was then added, the mixture stirred for 24 h at 20°, the reaction quenched by addition of ice, the mixture evaporated, and the residue dissolved in AcOEt (70 ml). The soln. was washed with 5% $NaHCO_3$ soln. and H_2O and evaporated and the residue dried under vacuum. The sodium salt of **7** (0.73 g, 1 mmol) was dissolved in MeOH/pyridine/H_2O 1:1:1 (60 ml), passed through a *Dowex 50* (Py^+) (3 × 15 cm), the soln. evaporated, and the residue dried by evaporation with pyridine and finally dissolved in dry pyridine (4 ml). Matrix **3** (1 g), 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl; 280 mg, 0.9 mmol), and 1-methyl-1*H*-imidazol (NMI; 80 μl, 0.9 mmol) were added, and the mixture was shaken overnight at 20°. Modified CPG was filtered off, washed twice with dry pyridine, and treated with Ac_2O/pyridine/NMI 1:5:1 (21 ml). After 30 min of shaking, support **S1** was filtered off, washed with pyridine, tetrahydrofuran, and Et_2O, and dried. The resulting **S1** contained 11.6 μmol of $(MeO)_2Tr$ groups per g of solid support.

Fmoc-Hse[(MeO)$_2$Tr]-Gly-OH (**11**). To a soln. of Fmoc-Hse(Tr)-OH (**8**; 2.8 g, 5 mmol) and *N*-hydroxysuccinimide (0.63 g, 55 mmol) in MeCN/DMF 4:1 (25 ml) at −20°, a soln. of dicyclohexylcarbodiimide (1.05 g, 5 mmol) in MeCN (5 ml) was added, and the mixture was left overnight at +4°. The precipitate was filtered off, the filtrate evaporated, and the residue dissolved in DMF (20 ml) and mixed with a soln. of glycine (0.38 g, 5 mmol) in 1M NaOH/DMF 1:2 (15 ml). The mixture was stirred overnight and then evaporated. The residue was dissolved in AcOEt (50 ml), the soln. washed with 2% H_2SO_4 soln. and H_2O and evaporated, and the residue crystallized from hexane. The precipitate was washed with hexane (2 × 20 ml), dried, and treated with 2% CF_3COOH in CH_2Cl_2 (25 ml) for 30 min, concentrated to 5 ml, and finally diluted with ^{i}Pr$_2$O (50 ml). The precipitate was washed with hexane (2 × 20 ml) and dried. A portion of the precipitate (1.6 g) was dried by co-evaporation with pyridine (2 × 25 ml) and dissolved in dry pyridine (50 ml), and $(MeO)_2TrCl$ (1.7 g, 5 mmol) was added. After stirring for 24 h at 20°, the reaction was quenched by addition of ice, the mixture evaporated and the residue dissolved in AcOEt (70 ml). The soln. was washed with 5% $NaHCO_3$ soln. and H_2O and evaporated and the residue crystallized from hexane/Et_2O: 2.34 g (87.3%) of **11**. M.p. 89–90°. ^{1}H-NMR ($CDCl_3$): 2.04 (br. *m*, CHC*H*$_2$C*H*$_2$(Hse)); 3.19 (br, *m*, CHC*H*$_2$C*H*$_2$(Hse)); 3.66 (*s*, 2 MeO); 3.71–4.27

(*m*, CHCH_2OCO (Fmoc), CHCH_2OCO (Fmoc), C*H*CH_2CH_2(Hse), NHCH_2COOH(Gly)); 6.28 (br. *m*, NH); 6.75–7.67 (*m*, 21 arom. H); 8.59 (br. *m*, NH).

*Solid Support {O-[Bis(4-methoxyphenyl)phenylmethyl]-*N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*L*-homoseryl}glycine 4-[(lcaa-CGP)amino]-4-oxobutyl Ester* (**S2**). Attachment of **11** to the matrix **3** was performed as described for **S1**. The resulting **S2** contained 11.3 μmol of $(MeO)_2$Tr groups per g of solid support.

*(±)-1-[Bis(4-methoxyphenyl)phenylmethoxy]-3-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}propan-2-ol* (**14**). (±)-3-Aminopropane-1,2-diol (**12**, 0.77 ml, 10 mmol) was dissolved in DMF (30 ml), and *N*-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]oxy}succinimide (FmocOSu; 3.37 g, 10 mmol) was added. After 1 h stirring, the mixture was evaporated, the residue dissolved in AcOEt (100 ml), the soln. washed with 2% H_2SO_4 soln. and H_2O and concentrated to 15 ml, and the product precipitated with hexane. The precipitate was washed with hexane (2 × 30 ml) and dried to give **13**. To a soln. of **13** in dry pyridine (25 ml), a soln. of $(MeO)_2$TrCl (3.4 g, 10 mmol) in dry dioxane (10 ml) was added dropwise, and the mixture was stirred overnight at 20°. After evaporation, the product was purified by FC (silica gel, 0 → 30% AcOEt/hexane + 0.1% of pyridine): 4.7 g (76.5%) of **14**. White foam. ^{1}H-NMR ($CDCl_3$): 3.11–3.49 (*m*, NHCH_2CH, CHCH_2O); 3.76 (*s*, 2 MeO); 3.87 (br. *m*, CH_2CHCH_2); 4.19 (*t*, *J* = 7.0, C*H*CH_2OCO (Fmoc)); 4.36 (*d*, *J* = 7.0, CHCH_2OCO (Fmoc)); 5.01 (br. *m*, OH); 6.82 (*d*, *J* = 8.9, 4 arom. H); 7.30 (*d*, *J* = 8.9, 4 arom. H); 7.21–7.77 (*m*, 14 arom. H, NH).

*Solid Support 4-[(lcaa-CGP)amino]-4-oxobutanoic Acid 2-[Bis(4-methylphenyl)phenylmethoxy]-1-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}ethyl Ester* (**S3**). Compound **14** was linked to the lcaa-CPG (2.0 g) by a succinate bridge as described in [19]. The resulting **S3** contained 36.0 μmol of $(MeO)_2$Tr groups per g of solid support.

Peptide Synthesis. Fmoc-L-amino acids for peptide synthesis were purchased from *Novabiochem*. The synthesis of peptide on all solid supports was performed with a *Milligen-9050* peptide synthesizer by standard protocols based on Fmoc/TBTU/TEA chemistry.

Oligonucleotide Synthesis. The oligo-T_6 and oligonucleotide phosphorothioate conjugates were prepared on the **MPM**-derivatized solid supports **S1**, **S2**, or **S3** with a *PE-Applied Biosystems-392* DNA synthesizer on a 1-μM scale. 2′-Deoxyribonucleoside 3′-phosphoramidites and 'Fast sulfurizing reagent' were products of *Glen Research*.

Analysis and Isolation of Peptide-Oligonucleotide Conjugates. Cleavage and deprotection of conjugates **C1**–**C5** was achieved with 32% aq. NH_3 soln. at 25° for 2 h or at 55° for 5 h. After cleavage, all conjugates were analyzed by reversed-phase HPLC (*Genesis C4*, 300 Å, 4 μ, 4.0 × 150 mm column (*Jones*); buffer *A*, 0.05M (EtNH)OAc; buffer *B*, 0.05M (Et_3NH)OAc in 80% MeCN/H_2O; linear gradient from 0 → 100% *B* in 30 min, flow rate 1 ml/min) and by ion-exchange HPLC (*PolyWax LP* 300 Å, 5 μ, 4.6 × 100 mm column (*Poly LP*)) with a NH_4SO_4 gradient (buffer *A*, 0.05M KH_2PO_4 in 50% formamide/H_2O, pH 5.6; buffer *B*, buffer *A* + 0.6M NH_4SO_4, pH 5.6; linear gradient 0 → 30% *B* in 30 min, flow rate 1 ml/min) for conjugates **C1**–**C3** or with a NaBr gradient (buffer *A*, 0.05M KH_2PO_4 in 50% formamide/H_2O, pH 6.3; buffer *B*, buffer *A* + 1.5M sodium bromide, pH 6.3; linear gradient 30 → 100% *B* in 30 min, flow rate 1 ml/min) for conjugates **C4** and **C5**. Substances **C1**–**C5** were isolated by ion-exchange HPLC on prep. scale as described above and finally desalted.

Electrospray-Ionization Mass Spectrometry. Mass spectra were acquired and molecular masses were reconstructed as reported earlier [16].

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