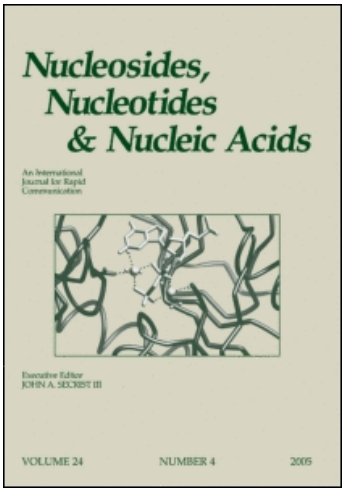


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis of Oligopeptides as Polynucleotide Analogs

Hiroki Umemiya[a]; Hiroyuki Kagechika[a]; Yuichi Hashimoto[b]; Koichi Shudo[a]
[a] Faculty of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan [b] Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo, Japan

To cite this Article Umemiya, Hiroki , Kagechika, Hiroyuki , Hashimoto, Yuichi and Shudo, Koichi(1996) 'Synthesis of Oligopeptides as Polynucleotide Analogs', Nucleosides, Nucleotides and Nucleic Acids, 15: 1, 465 — 475
To link to this Article: DOI: 10.1080/07328319608002398
URL: http://dx.doi.org/10.1080/07328319608002398

NUCLEOSIDES & NUCLEOTIDES, 15(1-3), 465-475 (1996)

SYNTHESIS OF OLIGOPEPTIDES AS POLYNUCLEOTIDE ANALOGS**

Hiroki UMEMIYA,[1] Hiroyuki KAGECHIKA,[1] Yuichi HASHIMOTO[2] and Koichi SHUDO[1]*
[1]Faculty of Pharmaceutical Sciences and [2]Institute of Molecular and Cellular Biosciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan

Abstract: Several dipeptides which have a uracil moiety in their side chains were designed as nucleotide analogs. Oligopeptides obtained from the dipeptides as monomer units were water-soluble, but exhibited no hypochromic effect with poly A or poly dA.

Agents that can sequence-specifically recognize nucleic acids have attracted much attention in connection with antisense/antigene methods.[1-3] Great efforts have been made to overcome the problems, such as penetration of the cell membrane and stability to enzymes, with natural polynucleotides which bind strongly to the complementary nucleic acids. Many modified polynucleotides have been synthesized, including modifications of the β-deoxyribose backbone, phosphodiester linkage and nucleic acid base itself, although these modifications have not generally been effective, and sometimes showed clear disadvantages. Based on the consideration that the sugar-phosphodiester backbone should be unnecessary for the specific recognition of the complementary nucleic acids, we previously designed an oligothymidine analog with a γ-amino acid backbone (compound **2**, Chart 1) which exhibited base-specific interaction with poly A.[4] Recently, other compounds without a sugar-phosphodiester backbone have been proposed as candidates for antisense agents. Nielsen *et al.* reported potent oligonucleotide analogs having *N*-(2-aminoethyl)glycine as the backbone unit.[5,6] In such cases, including our compound **2**, one of the disadvantages for application in antisense methods is the poor water-solubility of the compounds. [7] We therefore designed and synthesized new oligopeptides with uracil moieties in the side chain as candidate water-soluble polynucleotide analogs.

** This paper is dedicated to Dr. Yoshihisa Mizuno.

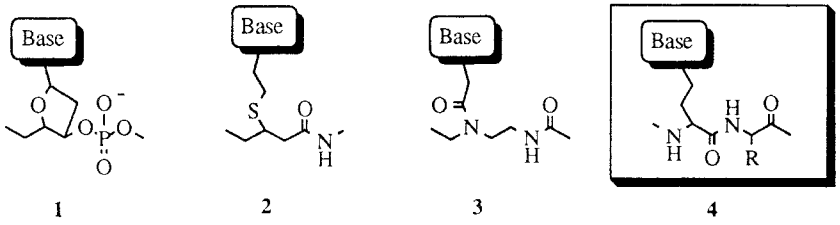
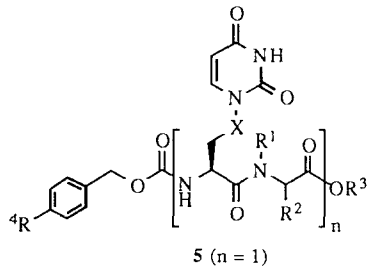


Chart 1

From a consideration of the structures of polynucleotide analogs synthesized by us (**2**)[4] and others (for example compound **3** by Nielsen *et al.*),[5,6] compared with those of natural nucleic acids (Chart 1, **1**), we designed the oligopeptide **4**. The monomer unit is composed of a dipeptide, that is, an amino acid having a nucleic acid base in the γ-position, connected with another amino acid as a spacer. The distance between the nucleic acid base and peptide linkage is three C–C bond lengths, similar to that of **1**. The physicochemical properties of **4** can be varied by modification of the R group in the spacer amino acid. Thus, we synthesized **5a** – **5e** (Chart 2) as monomer units for **4**. We chose uracil as the base, and glycine (**5a**), L-serine (**5b**), D-serine (**5c**), and *N*-methyl- L-serine (**5d**) as spacer amino acids. Serine has a polar hydroxy group which is expected to increase the hydrophilicity. The two serine enantiomers will give different conformations in the oligomer. *N*-Methylation of secondary amides generally favors *cis* conformation, as is found in many biologically active molecules.[8-10] The C_2 side chain of **5a** was replaced by a methylene-oxy group in **5e**. An intramolecular hydrogen bond between the side chain and backbone amide group is possible in **5e**, which may account for the extraordinary stability of the hybrid formation with nucleic acids.[11]

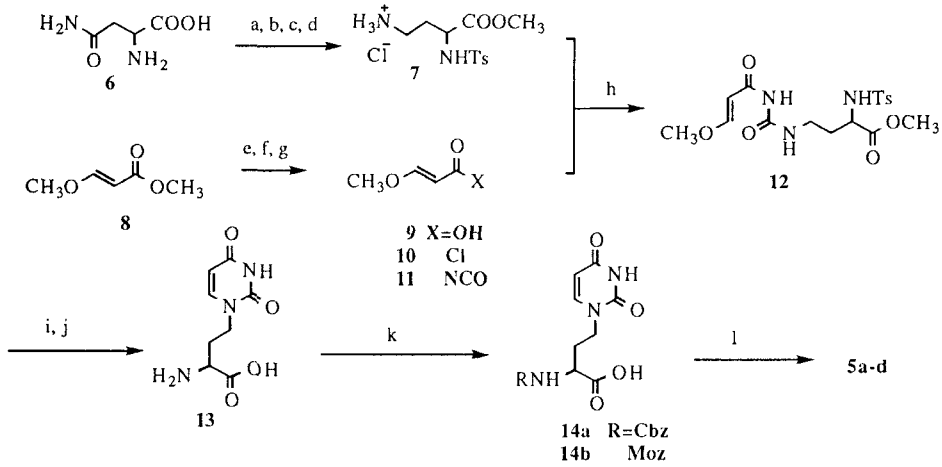
The synthetic route to the dipeptides (**5a–5d**) is shown in scheme 1. The α,γ-diaminoacid methyl ester **7** was prepared from L-asparagine in 4 steps.[12] The acyl isocyanate **11** was obtained from methyl methoxyacrylate **8** by the literature method with some modification.[13] The condensation of **7** with **11** gave a linear urea **12**, which was cyclized in alkaline solution, followed by deprotection in HBr–AcOH to give **13**.[14] After protection of the amino group of **13** with a benzyloxycarbonyl (Cbz) or *p*-methoxybenzyloxycarbonyl (Moz) group, **14** was condensed with a spacer amino acid moiety by the WSC-HONB method to give **5a**, **5b**, and **5c**. The *N*-methyl monomer **5d** was obtained through condensation of **14** and *N*-methyl-L-serine methyl ester.[15]



5 (n = 1)

	R^1	R^2	R^3	R^4	X
5a	H	H	C_2H_5	OCH_3	CH_2
5b	H	β-CH_2OH	CH_3	H	CH_2
5c	H	α-CH_2OH	CH_3	OCH_3	CH_2
5d	CH_3	β-CH_2OH	CH_3	OCH_3	CH_2
5e	H	H	C_2H_5	OCH_3	O

Chart 2

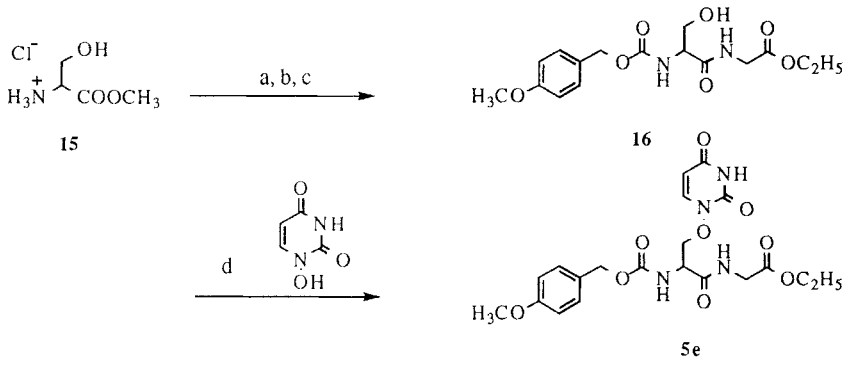


a) TsCl / $NaHCO_3$ / H_2O / Et_2O b) CH_3OH / HCl c) TsCl / Pyridine d) H_2 / PtO_2 / CH_3OH / HCl e) 2 N NaOH f) $SOCl_2$ / K_2CO_3 / THF g) AgOCN / Benzene h) Benzene-DMF / 90 °C i) 1N NaOH j) HBr / AcOH k) CbzCl / $NaHCO_3$ or MozON / H_2O / Dioxane / Et_3N l) HCl·$H_2NCH(R^1)COOR^2$ / WSC / HONB / DMF / Et_3N

Scheme 1

The synthetic route to **5e** is shown in Scheme 2. The coupling of 1-hydroxypyrimidine[16] with serine methyl ester under the Mitsunobu condition gave only the eliminated product. However, **15** was first converted to a dipeptide **16**, which reacted under the same conditions to give **5e** in 23% yield.

Compounds **5a-c, e** were converted to the corresponding hexamers **17a-c, e** (Table 1). Typically, the protecting group at the terminal amino (Cbz or Moz group) or carboxyl group (ester group) was removed by treatment with TFA or alkaline, respectively. The condensation of *C*-free compound and *N*-free compound was performed by the WSC-HONB



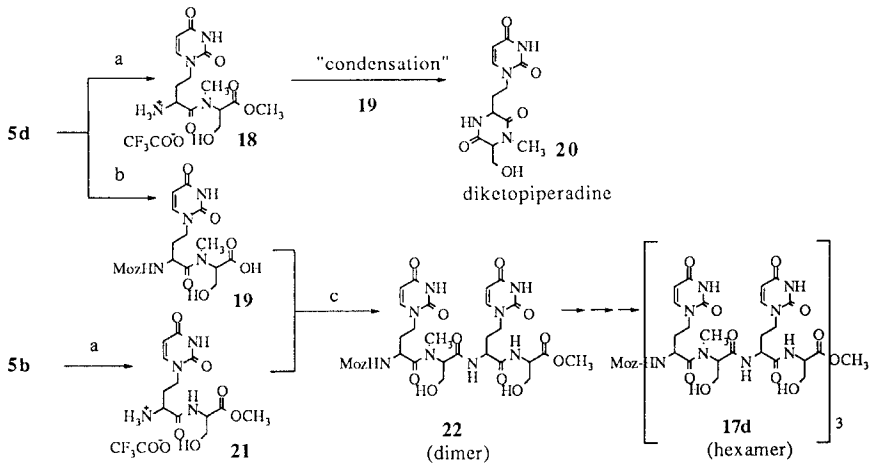
a) MozON / Et_3N / H_2O / Dioxane b) 0.5 N NaOH / CH_3OH c) GlyOEt·HCl / DPPA / DMF / Et_3N d) DEAD / PPh_3 / DMF

Scheme 2

method in DMF (yield 60–80%). The degree of epimerization was estimated to be less than 10% during the deprotection and condensation of **5b** or **5c**. In the case of **5d**, the *N*-free compound **18** readily cyclized to afford the 2,5-diketopiperadine **20** under the reaction condition used with **19** because of the *cis*-amide conformation of the *N*-methyl amide in **18**. Compound **19** was successfully condensed with **21**, which was obtained from **5b**, to give **22**. Therefore, we synthesized the hexamer **17d** (Scheme 3) from **22** as a dimer unit. The hexamers (**17a-e**) were each purified by HPLC (ODS; eluent: 20% CH_3CN-H_2O) and their structures were determined by NMR and FABMS (Table 1).

The solubility of the hexamers **17a-e** in water is high. The ^{1}H-NMR spectra indicated that *N*-methylated oligopeptides exist in equibrium between *cis* and *trans* conformations. The chemical shift of the amide proton in **5e** is at lower field, which indicates a hydrogen-bonding interaction with oxygen (X in Chart 2). Measurement of interaction of the hexamers with complementary polyadenylic acids was carried out by UV spectroscopy. Hexamers were mixed in various proportions with Poly dA or Poly A in 10 mM Tris-HCl buffer (pH 7.4) in the presence of 10 mM $MgCl_2$ or 1 M NaCl. However the hexamers showed no hypochromic effect on mixing with Poly dA or Poly A.

In conclusion, we synthesized several oligopeptides as oligonucleotide analogs. None of the oligomers interacted the interaction with complementary natural polynucleotide as judged from the UV spectra. These oligomers are water-soluble, and further structural modifications may afford hydrophilic antisense compounds.



a) TFA / $PhSCH_3$ b) 0.1N NaOH c) WSC / HONB / DMF / Et_3N

Scheme 3

Table 1 Properties of Synthesized Hexamers **17a-e**

Compd [a]	Monomer	HPLC (R_T) [min] [b]	FABMS $[M+H]^+$
17a	**5a**	14.8	1723
17b	**5b**	8.8	1859
17c	**5c**	7.5	1889
17d	**5b & 5d**	10.2	1953
17e	**5e**	24.4	1735

[a] Structures are shown in Chart 2 (n = 6) except **17d** (Scheme 3).

[b] Polygosil 60-$_5C_{18}$, eluent: 20% CH_3CN-H_2O

EXPERIMENTAL

General. Melting points were determined by using a Yanagimoto hot-stage melting point apparatus and are uncorrected. Elemental analyses were carried out in the Microanalytical Laboratory, Faculty of Pharmaceutical Sciences, University of Tokyo, and were within ±0.3% of the theoretical values. ^{1}H-NMR spectra were recorded on a JEOL JNM-GX400 (400 MHz) spectrometer. Chemical shifts are expressed in ppm relative to tetramethylsilane. FABMS spectra were taken with a JEOL JMS-SX102A MASS spectrometer.

Abbreviations: TsCl, toluene-*p*-sulfonyl chloride; HONB, *N*-hydroxy-5-norbornene-2,3-dicarboximide; DPPA, diphenylphosphoryl azide; WSC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; DEAD, diethyl azodicarboxylate; TFA, trifluoroacetic acid.

Methyl *N*-tosyl-α,γ-diaminobutyrate hydrochloride (7). $NaHCO_3$ (10.0 g) was added to a solution of L-asparagine monohydrate (16.3 g, 108.7 mmol) in H_2O (150 ml), and the mixture was warmed. Another 10 g of $NaHCO_3$ was added and then TsCl (16.07 g, 84.3 mmol) in 100 ml of ether was added. After 8 h, TsCl (17.59 g, 92.3 mmol) in 100 ml of ether was added, and the mixture was stirred for 24 h. The mixture was cooled and acidified with concentrated HCl. After 2 h, the precipitates were collected, washed with ice-cold water, and recrystallized from water to give *N*-tosyl-L-asparagine (21.0 g, 68%). ***N*-Tosyl-L-asparagine**: colorless needles; ^{1}H-NMR (DMSO-d_6) δ 7.92 (d, 1 H, J = 9.2 Hz, Ts-NH), 7.66 (d, 2 H, J = 8.4 Hz), 7.34 (d, 2 H, J = 8.4 Hz), 7.32 (s, 1 H), 6.88 (s, 1 H), 4.05 (q, 1 H, J = 7.7 Hz, Hα), 2.45 (dd, 1 H, J = 7.3, 15.8 Hz), 2.37 (s, 3 H), 2.24 (dd, 1 H, J = 7.3, 15.8 Hz). *N*-Tosyl-L-asparagine (65.2 g, 228 mmol) was suspended in 5% HCl-CH_3OH (dry, 600 ml). The solution was set aside at room temperature overnight and concentrated under vacuum. The precipitates were collected, washed with cold methanol, and recrystallized from methanol to give *N*-tosyl-L-asparagine methyl ester (45.7 g, 67%). ***N*-Tosyl-L-asparagine methyl ester**: colorless needles; ^{1}H-NMR ($CDCl_3$) δ 7.76 (d, 2 H, J = 8.4 Hz), 7.74 (d, 2 H, J = 8.4 Hz), 5.87 (d, 1 H, J = 8.4 Hz, Ts-NH), 5.57 (s, 1 H), 5.38 (s, 1 H), 4.11 (m, 1 H, Hα), 3.59 (s, 3 H), 2.93 (dd, 1 H, J = 16.1, 4.4 Hz), 2.76 (dd, 1 H, J = 16.1, 4.4 Hz), 2.43 (s, 3 H). A solution of *N*-tosyl-L-asparagine methyl ester (6.02 g, 20.0 mmol) in dry pyridine (30 ml) was treated with TsCl (5.72 g, 30.0 mmol) in several portions over 5 min. The reaction mixture turned black, and was kept at 50 °C for 1 h. Pyridine was removed under vacuum and the residue was treated with water (100 ml) and set aside at -15 °C overnight. Water was added, and the mixture was extracted with AcOEt. The organic layer was dried over Na_2SO_4, and evaporated. The crude product was chromatographed on silica gel to afford methyl β-cyano-α-L-tosylaminopropionate (4.20 g, 74%). **Methyl β-cyano-α-L-tosylaminopropionate**: colorless needles (CH_3OH); mp 116-117 °C; ^{1}H-NMR ($CDCl_3$) δ 7.75 (d, 2 H, J = 8.4 Hz), 7.33 (d, 2 H, J = 7.7 Hz), 5.59 (d, 1 H, J = 7.0 Hz, Ts-NH), 4.17 (m, 1 H, Hα), 3.73 (s, 3 H), 2.90 (m, 2 H), 2.44 (s, 3 H); ^{13}C-NMR ($CDCl_3$) δ 168, 144, 136, 130, 127, 115, 54, 52, 23, 21; Anal calcd for $C_{12}H_{14}N_2O_4S$: C, 51.05; H, 5.00; N, 9.92. Found: C, 51.10; H, 4.96; N, 9.79. A solution of methyl β-cyano-α-L-tosylaminopropionate (4.85 g, 17.2 mmol) in 5% HCl–CH_3OH (150 ml) was hydrogenated over PtO_2 catalyst (976 mg). After the consumption of hydrogen had ceased, the catalyst was filtered off. The filtrate

was evaporated, and the residue was recrystallized from methanol-ether to give methyl *N*- tosyl-α,γ-diaminobutyrate hydrochloride (**7**, 5.31 g, 96%). **7**: colorless needles; mp 195-198 °C; ^{1}H-NMR (DMSO-d_6) δ 8.41 (brs, 1 H), 8.03 (brs, 3 H), 7.64 (d, 2 H, *J* = 8.1 Hz), 7.38 (d, 2 H, *J* = 8.1 Hz), 3.98 (m, 1 H, Hα), 3.36 (s, 3 H), 2.73 (m, 2 H), 2.38 (s, 3 H), 1.90 (m, 2 H); Anal calcd for $C_{12}H_{18}N_2O_4S$·HCl: C, 44.65; H, 5.93; N, 8.68. Found: C, 44.42; H, 5.93; N, 8.50.

β-Methoxyacryloyl chloride (10). Methyl 3-methoxyacrylate (**8**, 92 g, 0.79 mol) was heated at 100 °C with 2 N sodium hydroxide (240 ml) for 2 h. After cooling, the mixture was acidified to give β-ethoxyacrylic acid (**9**) as precipitates (37.4 g, 46%). **9**:^{1}H-NMR ($CDCl_3$) δ 7.72 (d, 1 H, *J* = 12 Hz), 5.18 (d, 1 H, *J* = 12 Hz), 3.37 (s 3 H). A mixture of **9** (37 g, 0.36 mol) and K_2CO_3 (24 g, 0.17 mol) was refluxed with thionyl chloride (64 g, 0.54 mol) in THF (500 ml) for 3 h. After removal of the solvent, the product was distilled under reduced pressure to give β-methoxyacryloyl chloride (**10**, 30.22 g, 62%). **10**: colorless oil; bp 66-74 °C (14 mmHg); ^{1}H-NMR ($CDCl_3$) δ 7.82 (d, 1 H, *J* = 12.8 Hz), 5.53 (d, 1 H, *J* = 12.5 Hz), 3.82 (s, 3 H).

Methyl 4-(β-methoxyacrylamidocarbonylamino)-1-(tosylamino)butyrate (12). Silver cyanate (2.58 g, 17.22 mmol, dried at 100 °C under vacuum before use) was added to a solution of **10** (1.487 g, 12.3 mmol) in dry benzene (freshly distilled, 15 ml), and the whole was stirred at room temperature for 2 h. The precipitates were carefully filtered off, and the filtrate (crude **11**) was added to a solution of **7** (1.98 g, 6.15 mmol) in 50 ml of benzene-dimethylformamide (4:1). The mixture was heated at 90 °C for 2 h, then evaporated, and the crude product was chromatographed on silica gel, (eluent: AcOEt:*n*-hexane=3:1). The product was recrystallized from AcOEt to give **12** (1.15 g, 45%). **12:** colorless prisms; ^{1}H-NMR (DMSO-d_6) δ 10.04 (s, 1 H, CONHCO), 8.40 (t, 1 H, *J* = 6.0 Hz, CONHCH$_2$), 8.30 (d, 1 H, *J* = 8.8 Hz, Ts-NH), 7.62 (d, 2 H, *J* = 8.1 Hz), 7.59 (d, 1 H, *J* = 12.5 Hz), 7.36 (d, 2 H, *J* = 8.1 Hz), 5.51 (d, 1 H, *J* = 12.1 Hz), 3.78 (q, 1 H, *J* = 7.0 Hz, Hα), 3.68 (s, 3 H), 3.38 (s, 3 H), 3.10 (m, 2 H), 2.37 (s, 3 H), 1.73 (m, 2H).

L-1-Amino-3-(1-uracilyl) butyric acid (13). Compound **12** (3.818 g, 9.244 mmol) was dissolved in 50 ml of 1 N NaOH (50 ml) and the solution was stirred at 65 °C for 3 h. The aqueous layer was washed with ether, acidified with concentrated HCl, and extracted with AcOEt. The organic layer was dried over Na_2SO_4, and evaporated. The crude product (3.32 g) was purified by ODS column chromatography, and then recrystallized from water to give L-1-tosylamino-3-(1-uracilyl)butyric acid (1.42 g, 42%). **L-1-Tosylamino-3-(1-uracilyl)butyric acid**: colorless prisms (water); mp 210 °C; ^{1}H-NMR (DMSO-d_6) δ 11.22 (brd, 1 H), 8.16 (d, 1 H, *J* = 8.8 Hz, Ts-NH), 7.65 (d, 2 H, *J* = 8.4 Hz), 7.41 (d, 1 H, *J* = 7.7 Hz), 7.35 (d, 2 H, *J* = 8.4 Hz), 5.49 (dd, 1 H, *J* = 8.1, 2.2 Hz), 3.64 (m, 3 H), 2.37 (s, 3 H), 1.86 (m, 2 H). L-1-Tosylamino-3-(1-uracilyl)butyric acid

(400 mg, 1.34 mmol) was treated with 30% HBr-AcOH (10 ml) for 28 h at 40 °C in a glass bomb. Ether was added to the solution to yield a white precipitate. The resulting solids were collected, washed with ether, and dried under vacuum to give **13** (117 mg, 41%). **13**:colorless powder; ^{1}H-NMR (D_2O) δ 7.65 (d, 1 H, J = 7.7 Hz), 5.83 (d, 1 H, J = 7.7 Hz), 4.00 (m, 3 H), 2.30 (m, 2 H).

Dipeptide 5b. A suspension of **13** (117 mg, 0.55 mmol) in water (5 ml) was treated with sodium hydrogen carbonate (92 mg, 1.10 mmol). A solution of 0.53 ml (1.10 mmol) of 35% carbobenzoxy chloride in toluene was then added at 0 °C with vigorous stirring. The mixture was stirred for 2 h, washed with ether, acidified with hydrochloric acid and extracted with AcOEt. The organic layer was washed with water, dried over Na_2SO_4 and evaporated to give **14a** (118 mg, 99%). **14a**: ^{1}H-NMR (DMSO-d_6) δ 11.23 (d, 1 H, J = 2.2 Hz, CONHCO), 7.68 (d, 1 H, J = 8.4 Hz), 7.52 (d, 1 H, J = 8.4 Hz), 7.38 (m, 5 H), 5.52 (dd, 1 H, J = 8.1, 2.2 Hz), 5.04 (s, 2 H, PhCH_2), 3.96 (m, 1 H), 3.71 (m, 2 H), 2.00 (m, 2 H). Et_3N (24 mg, 0.24 mmol) was added to a mixture of **14a** (35 mg, 0.10 mmol), L-serine methyl ester hydrochloride (19 mg, 0.12 mmol), HONB (21.5 mg, 0.12 mmol) and WSC (23 mg, 0.12 mmol) in DMF (2 ml) with stirring at 0 °C. The solution was stirred for 1 h at 0 °C, then for 18 h at room temperature. After concentration, the residue was extracted with AcOEt. The organic layer was washed with brine saturated with $NaHCO_3$, dried over Na_2SO_4 and evaporated. The crude product was purified by silica gel column chromatography (eluent: CH_2Cl_2 : CH_3OH=20:1) to give **5b** (36.2 mg, 78%). **5b**: colorless solid; mp 83-85 °C; ^{1}H-NMR ($CDCl_3$) δ 9.87 (s, 1 H), 8.04 (d, 1 H, J = 7.0 Hz), 7.47 (d, 1 H, J = 8.4 Hz, NH), 7.34 (m, 5 H, Ph), 5.91 (d, 1 H, J = 7.7 Hz, NH), 5.69 (d, 1 H, J = 7.0 Hz), 5.11 (s, 2 H, PhCH_2), 4.58 (m, 1 H), 4.30 (m, 1 H), 3.98 (m, 2 H), 3.77 (s, 3 H), 3.75 (m, 2 H), 2.20 (m, 3 H); FABMass calcd for $C_{20}H_{24}N_4O_8$ $[M+H]^+$ 449.17, Found 449.22. Dipeptides **5a**, **5c** and **5d** were also synthesized according to the method described above. **5a**: ^{1}H-NMR ($CDCl_3$) δ 8.69 (brs, 1 H, NH), 7.40 (brt, 1 H, NH), 7.30 (m, 3 H, Ph & NH), 6.88 (d, 2 H, J = 8.8 Hz, Ph), 5.79 (d, 1 H, J = 8.1 Hz), 5.72 (d, 1 H, J = 7.7 Hz), 5.04 (s, 2 H, PhCH_2), 4.30 (m, 1 H), 4.20 (q, 2 H, J = 7.3 Hz, OCH_2CH$_3$), 4.12 (m, 2 H), 3.94 (dd, 1 H, J = 18.3, 5.5 Hz), 3.81 (s, 3 H, OCH_3), 3.64 (m, 1 H), 2.15 (m, 2 H), 1.27 (t, 3 H, J = 7.0 Hz, OCH_2CH_3). **5c**: ^{1}H-NMR (CD_3OD) δ 7.56 (d, 1 H, J = 7.3 Hz), 7.41 (d, 2 H, J = 8.4 Hz, Ph), 6.99 (d, 2 H, J = 8.4 Hz, Ph), 5.69 (d, 1 H, J = 7.7 Hz), 5.13 (m, 2 H), 4.58 (m, 1 H), 4.35 (m, 1 H), 3.95 (m, 2 H), 3.88 (s, 3 H, OCH_3), 3.82 (s, 3 H, OCH_3), 3.75 (m, 2 H), 2.30 (m, 1 H), 2.10 (m, 1 H). **5d**: ^{1}H-NMR (DMSO-d_6, 90 °C) δ 10.87 (brs, 1 H, NH), 7.47 (d, 1 H, J = 7.7 Hz), 7.29 (d, 2 H, J = 8.4 Hz, Ph), 7.04 (s, 1 H), 6.92 (d, 2 H, J = 8.8 Hz, Ph), 5.49 (d, 1 H, J = 8.1 Hz), 4.98 (s, 2 H, PhCH_2), 4.84 (m, 1 H), 4.56 (m, 1 H), 3.76 (m, 4 H), 3.77 (s, 3 H, OCH_3), 2.88 (m, 3 H, N-CH_3), 2.03 (m, 1 H), 1.87 (m, 1 H).

Dipeptide 5e. Triethylamine (126 mg, 1.245 mmol) was added to a mixture of *N*-Moz-serine (133.9 mg, 0.498 mmol), glycine ethyl ester hydrochloride (83mg, 0.597 mmol), DPPA (164 mg, 0.597 mmol) and HONB (116 mg 0.65 mmol) in DMF (5 ml) at 0 °C. The mixture was stirred for 16 h, concentrated and extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4 and evaporated. The crude product was purified by silica gel column chromatography (eluent: AcOEt:*n*-hexane=2:1) to give **16** (112.7 mg, 64%). **16**: ^{1}H-NMR ($CDCl_3$) δ 7.30 (d, 2 H, J = 8.8 Hz, Ph), 7.00 (brs, 1 H, NH), 6.89 (d, 2 H, J = 8.4 Hz, Ph), 5.78 (brd, 1 H), 5.09 (d, 1 H, J = 12.1 Hz, PhC$\underline{H}_2$), 5.05 (d, 1 H, J = 12.1 Hz, PhC$\underline{H}_2$), 4.28 (brs, 1 H), 4.21 (q, 2 H, J = 7.3 Hz, OC$\underline{H}_2$CH$_3$), 4.09 (m, 1 H), 4.04 (m, 2 H), 3.81 (s, 3 H, OCH_3), 3.67 (m, 1 H), 3.12 (brs, 1 H), 1.28 (t, 3 H, J = 7.3 Hz, OCH$_2$C$\underline{H}_3$). DEAD (96 mg, 0.55 mmol) was added to a mixture of 1-hydroxypyrimidine (29 mg, 0.18 mmol), **16** (64.9 mg, 0.18 mmol) and PPh_3 (144 mg, 0.55 mmol) in DMF (1 ml), and the mixture was stirred for 15 h. After removal of the solvent under vacuum, the residue was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated. The crude product was purified by silica gel column chromatography (eluent: CH_2Cl_2:CH_3OH=20:1) to give **5e** (21.3 mg, 23%). **5e**: ^{1}H-NMR ($CDCl_3$) δ 9.10 (brs, 1 H, NH), 7.64 (brt, 1 H, Gly-N$\underline{H}$), 7.49 (d, 1 H, J = 8.4 Hz, NH), 7.30 (d, 2 H, J = 8.4 Hz, Ph), 6.88 (d, 2 H, J = 8.8 Hz, Ph), 6.39 (d, 1 H, J = 8.8 Hz), 5.63 (d, 1 H, J = 8.1 Hz), 5.09 (d, 1 H, J = 12.1 Hz, PhC$\underline{H}_2$), 5.05 (d, 1 H, J = 12.1 Hz, PhC$\underline{H}_2$), 4.59 (brm, 2 H), 4.26 (q, 1 H, J = 5.1 Hz), 4.20 (q, 2 H, J = 7.3 Hz), 4.03 (m, 2 H), 3.81 (s, 3 H), 1.27 (t, 3 H, J = 7.0 Hz, OCH$_2$C$\underline{H}_3$).

Deprotection of 5d (19). A solution of **5d** (80 mg, 0.16 mmol) in H_2O-CH_3OH (3ml, 2ml) was stirred at 0 °C, then 0.5 N NaOH (0.97 ml, 0.48 mmol) was added. After 2 h, 0.5 ml of 1 N HCl was added, and the CH_3OH was removed in vacuum. The product was purified by ODS column chromatography (eluent: CH_3CN:H_2O=1:1). The yield after freeze-drying in vacuum was 65.7 mg (86%) **19**: ^{1}H-NMR (DMSO-d_6) δ 11.24 (m, 1 H, NH), 7.52 (m, 1 H, NH), 7.29 (m, 2 H, Ph), 6.93 (m, 2 H, Ph) 5.52 (m, 1 H), 4.94 (m, 2 H, PhC$\underline{H}_2$), 4.80 (m, 1 H), 4.50 (m, 1 H), 3.78 (m, 2 H), 3.74 (s, 3 H, OCH_3), 3.66 (m, 2 H), 2.00 (m, 1H), 1.75 (m, 1 H).

Tetrapeptide (22). Compound **5b** (38.8 mg, 0.081 mmol) was stirred in TFA (2 ml) in the presence of 0.5 M thioanisole at 0 °C for 1 h. After evaporation of TFA in vacuo, the residue was washed with ether several times. The powder **21** thus obtained, was dried over KOH pellets in vacuum, and then used for the next condensation. Compound **21** (0.144 mmol) was dissolved in DMF (4 ml), together with **19** (70 mg, 0.146 mmol), HONB (32.7 mg, 0.182 mmol), WSC (35.3 mg, 0.184 mmol) and *N*-methylmorpholine (46.6 mg, 0.461 mmol). The mixture was stirred overnight at 0 °C, then concentrated, and the product was purified on an ODS column chromatography (eluent: 1:3=CH_3CN:H_2O).

Freeze-drying afforded **22** as a powder (46.2 mg, 41%). **22**: ^{1}H-NMR (CD_3OD) δ 7.61 (m, 2 H), 7.36 (m, 2 H), 6.98 (m, 2 H), 5.75 (m, 2 H), 5.11 (m, 2 H), 4.71 (m, 2 H), 4.60 (m, 2 H), 3.98 (m, 14 H), 3.15 (s, 3 H), 2.33 (m, 2 H), 2.08 (m, 2 H); FABMass calcd for $C_{33}H_{42}N_8O_{14}$ $[M+H]^+$ 775.29, Found $[M+H]^+$ 775, $[M+Na]^+$ 797, $[M+K]^+$ 813.

Oligopeptides (hexamers **17a-e**) were synthesized from **5a-e** according to the method described above. All the peptides were purified by column chromatography (ODS) and / or HPLC (Polygosil 60-$_5C_{18}$ 7.6 x 250 mm, Table 1), and their structures were confirmed by ^{1}H-NMR and mass spectrometry. **17a**: ^{1}H-NMR (D_2O) δ 7.53 (m, 6 H), 7.32 (d, 2 H, J = 9 Hz), 6.96 (d, 2 H, J = 8 Hz), 5.80 (m. 6 H), 5.12 (d, 1 H, J = 12 Hz, PhC$\underline{H}_2$), 5.03 (d, 1 H, J = 12 Hz, PhC$\underline{H}_2$), 4.45 (m, 5 H), 4.25 (m, 1 H), 4.22 (q, 2 H, J = 7 Hz, OC$\underline{H}_2$CH$_3$), 3.93 (m, 24 H), 3.84 (s, 3 H, OCH$_3$), 2.31 (m, 6 H), 2.16 (m, 6 H), 1.27 (t, 3 H, J = 7 Hz, OCH$_2$C$\underline{H}_3$). **17b**: ^{1}H-NMR (D_2O) δ 7.45 (m, 5 H), 7.30 (m, 6 H), 5.68 (m, 6 H), 5.05 (m, 2 H), 4.35 (m, 12 H), 3.80 (m, 24 H), 3.70 (s, 3 H, OCH$_3$), 2.22 (m, 6 H), 2.02 (m, 6 H). **17c**: ^{1}H-NMR (D_2O) δ 7.45 (m, 5 H), 7.27 (m, 1 H), 7.16 (d, 2 H, J = 7 Hz), 6.79 (d, 2 H, J = 7 Hz), 5.69 (m, 5 H), 5.60 (m, 1 H), 5.06 (d, 1 H, J = 14 Hz, PhC$\underline{H}_2$), 4.85 (d, 1 H, J = 14 Hz, PhC$\underline{H}_2$), 4.35 (m, 12 H), 3.82 (m, 18 H), 3.69 (s, 3 H, OCH$_3$), 3.67 (s, 3 H, OCH$_3$), 3.60 (m, 6 H), 2.22 (m, 6 H), 2.02 (m, 6 H). **17d**: ^{1}H-NMR (D_2O) δ 7.53 (m, 5 H), 7.35 (m, 1 H), 7.18 (m, 2 H), 6.84 (m, 2 H), 5.73 (m, 6 H), 5.05 (m, 1 H), 4.85 (m, 1 H), 4.40 (m, 12 H), 3.80 (m, 18 H), 3.26 (m, 2 H), 3.10 (m, 5 H), 2.80 (m, 2 H), 2.20 (m, 6 H), 2.00 (m, 6 H). **17e**: ^{1}H-NMR (D_2O) δ 7.69 (m, 6 H), 7.28 (d, 2 H, J = 7 Hz), 6.88 (d, 2 H, J = 7 Hz), 5.60 (m, 6 H), 5.02 (m, 2 H), 4.48 (m, 13 H), 4.20 (m, 2 H), 4.15 (m, 2 H), 4.00 (m, 12 H), 3.70 (s, 3 H, OCH$_3$), 1.22 (m, 3 H). FABMS data for **17a-e** are summarized in Table 1.

REFERENCES

1. Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543-584.
2. Hélène, C.; Toulmé, J-J. *Biochem. Biophys. Acta* **1990**, *1049*, 99-125.
3. Thuong, N. T.; Hélène, C. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 666-690.
4. Hashimoto, Y.; Shudo, K. *Life Chem. Rep.* **1988**, *6*, 231-265.
5. Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497-1500.
6. Egholm, M.; Buchardt, O.; Nielsen, P. E.; Berg, R. H. *J. Amer. Chem. Soc.* **1992**, *114*, 1895-1897.
7. Wada, T.; Nakamori, M.; Mochizuki, E.; Inaki, Y. *Nucleic Acids Symp. Ser.* **1994**, *31*, 135-136.
8. Yamaguchi, K.; Shudo, K. *J. Agric. Food Chem.* **1991**, *39*, 793-796.

9. Itai, A.; Toriumi, Y.; Saito, S.; Kagechika, H.; Shudo, K. *J. Amer. Chem. Soc.* **1992**, *114*, 10649-10650.
10. Shimasaki, H.; Kagechika, H.; Fukasawa, H.; Kawachi, E.; Shudo, K. *Chem. Pharm. Bull.* **1995,** *43*, 100-107.
11. Bruice, T.C. *Proc. Natl. Acad. Sci.* **1993**, *90*, 7518-7522.
12. Zaoral, M.; Rudinger, J. *Collection Czchoslov Chem. Commun.* **1959**, *24*, 1993-2012.
13. Shaw, G.; Warrener, R. N. *J. Chem. Soc.* **1958**, 153-156.
14. The optical purity of **13** was confirmed by the conversion of **13** into **5b / c**. Each compound contained less than 1% of the diastereomer as judged from the ^{1}H-NMR spectrum.
15. Grieco, P. A.; Bahsas, A. *J. Org. Chem.* **1987**, *52*, 5746-5749.
16. Parkin, A. *J. Chem. Soc. Perkin Trans 1* **1991**, 2983-2990.