Studies on the β-Turn of Peptides. IV.[1] Effect of the Chain Length on the CD Spectra of *N*-(2,4-Dinitrophenyl)-(Gly)$_n$-D-Ala-L-Pro-(Gly)$_n$ *p*-Nitroanilides (n=1—3)

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N-(2,4-Dinitrophenyl)-(Gly)$_n$-D-Ala-L-Pro-(Gly)$_n$ *p*-nitroanilides (**7b**, n=2; **7c**, n=3) were synthesized in order to study the effect of chain length on their conformations. The CD spectra of both **7b** and **7c** showed similar patterns to that of **7a** (n=1), but their Cotton effects were much weaker. Therefore, both compounds seem still to have some populations of folded conformers because their Cotton effects near 350 nm were significantly larger than those of the tetrapeptide derivatives in random conformation. However, the population of the folded conformers must be quite small.

In the previous paper, we gave an outline of a new method to study the β-turn conformation of linear tetrapeptides.[2] *N*-(2,4-Dinitrophenyl)tetrapeptide *p*-nitroanilides (Dnp-tetrapeptide-pNA's)[3] exhibit characteristic CD spectra above 250 nm when they take β-turn conformations. The Cotton effects are considered to be due to exciton coupling of the transition moments in the two terminal chromophores. The magnitudes of the Cotton effects near 350 nm and 310 nm were shown to reflect well the β-turn preference of the tetrapeptides.

In this paper, we attempted to apply the method to larger peptides than tetrapeptides. CD spectrum of Dnp-Gly-D-Ala-L-Pro-Gly-pNA (**7a**) is known to show large Cotton effects at 350 nm and 310 nm, indicating that it takes β-turn conformation to a high extent.[2] So, in this work, two compounds with a general structure Dnp-(Gly)$_n$-D-Ala-L-Pro-(Gly)$_n$-pNA (**7b**, n=2; **7c**, n=3) were synthesized to study the effect of chain length on the CD spectra. If **7b** and **7c** also have high population of folded conformers with a β-turn at the D-Ala-L-Pro sequence, large Cotton effects are expected to be observed due to the approach of the Dnp and pNA chromophores (Fig. 1).

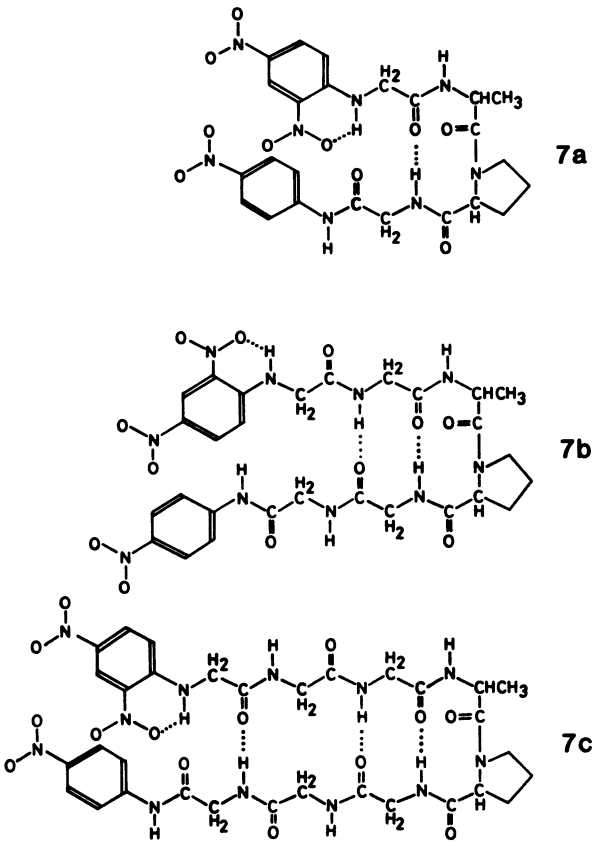

Fig. 1. Models of folded (β-sheet) conformers with a β turn at D-Ala-L-Pro sequence of Dnp-(Gly)-D-Ala-L-Pro-(Gly)$_n$-pNA (**7a**—**c**).

Results and Discussion

Syntheses of Peptides. Compounds **7b** and **7c** were synthesized by a similar manner to that described previously for **7a** (Fig. 2).[4] Boc-D-Ala-L-Pro-OH[5] and H-(Gly)$_2$-pNA·HCl (**2b**·HCl)[1] were coupled by EDC method to afford Boc-D-Ala-L-Pro-(Gly)$_2$-pNA (**3b**), which was treated with hydrogen chloride in formic acid. H-D-Ala-L-Pro-(Gly)$_2$-pNA·HCl (**4b**·HCl) obtained was coupled with Boc-(Gly)$_2$-OH[6] to afford Boc-(Gly)$_2$-D-Ala-L-Pro-(Gly)$_2$-pNA (**5b**), which was converted to the Dnp derivative, Dnp-(Gly)$_2$-D-Ala-L-Pro-(Gly)$_2$-pNA (**7b**), by deprotection and subsequent treatment with 1-fluoro-2,4-dinitrobenzene. Synthesis of **7c** was carried out similarly. Homogeneities of the peptides synthesized were confirmed by thin-layer chromatography and elemental analyses.

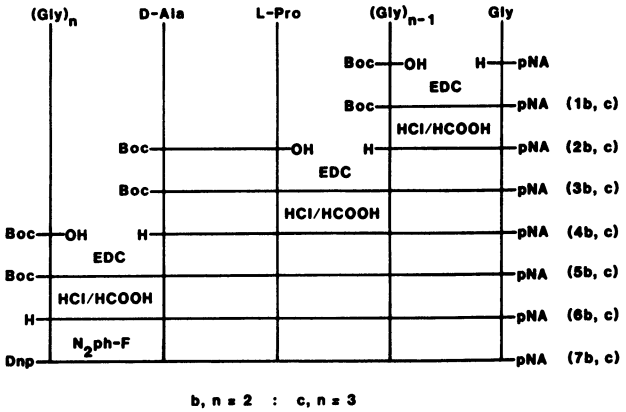

Fig. 2. Syntheses of Dnp-(Gly)$_n$-D-Ala-L-Pro-(Gly)$_n$-pNA (n=2, 3).

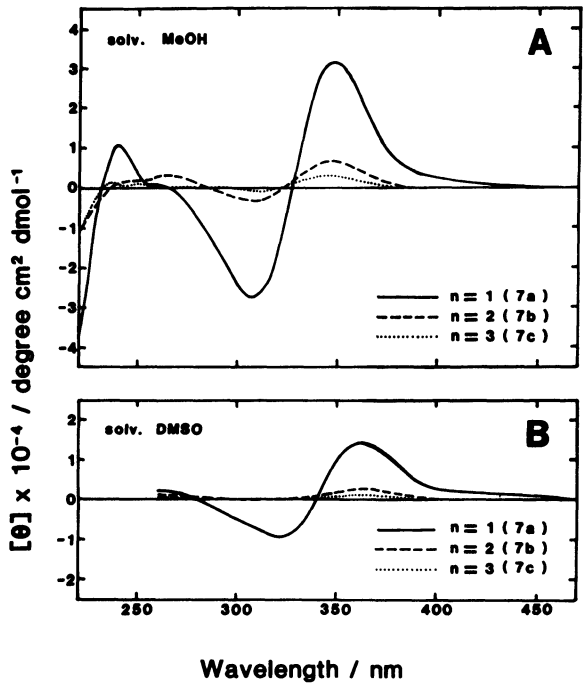

Fig. 3. CD spectra of Dnp–$(Gly)_n$–D-Ala–L-Pro–$(Gly)_n$–pNA (n=1—3).

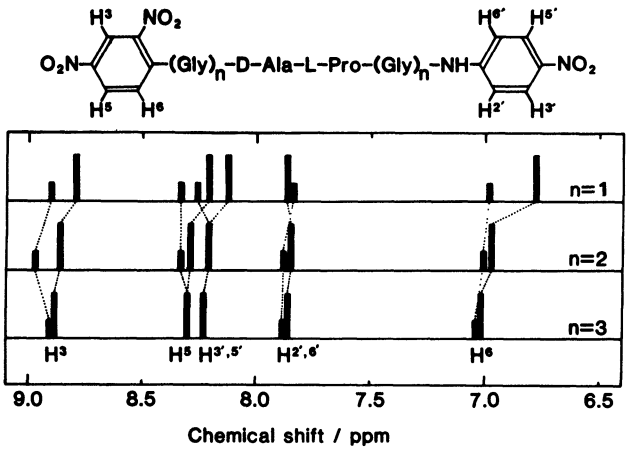

Fig. 4. Chemical shifts of aromatic protons of Dnp–$(Gly)_n$–D-Ala–L-Pro–$(Gly)_n$–pNA (n=1—3) in DMSO-d_6 at 23 °C. The large and the small bars show the major and the minor conformers with *trans* and *cis* D-Ala–L-Pro bonds, respectively.

CD Spectra Due to the Interaction between Dnp and pNA Chromophores. CD spectra of **7b** and **7c** measured in MeOH solution are represented in Fig. 3A together with that of **7a**. Both showed a similar pattern to that of **7a**, but the magnitudes were much smaller than that of **7a**. Between the two, **7b** showed a little larger Cotton effects than **7c**. Figure 3B represents the spectra measured in DMSO solution, and both showed similar spectra but of weaker strength than those in MeOH. Decrease of Cotton effects was observed also in **7a**, but the relative strengths of Cotton effects of **7b** and **7c** to those of **7a** were not so much affected by the change of solvents. Such decrease of Cotton effects as mentioned above suggested that the populations of β-turn conformer of **7a**—**c** decreased in DMSO solution as compared with those in MeOH solution, since it had been ascertained that the solvent dependence of Cotton effects were in good accordance with that of the populations of β-turn conformers using a model peptide (Dnp–L-Leu–D-Ala–L-Pro–L-Val–pNA) related to the β-turn part of gramicidin S,[2] which showed larger Cotton effects in MeOH solution than in DMSO solution as well as **7a**—**c**. MeOH is considered to be suitable for β-turn conformation of hexa- or octapeptides as well as for those of tetrapeptides. UV absorption spectra of **7a**—**c** showed maxima at 326 nm in MeOH solution and at 343 nm in DMSO solution as well as those of other peptides reported previously.[2,4] This red-shift of UV maxima corresponded well to that of CD maxima as expected from the fact that the CD spectra of Dnp–peptide–pNA's were considered to be due to the exciton coupling of the transition moments in two terminal chromophores. These results indicated that there was still a little interaction between the two terminal chromophores of **7b** and **7c**, and that they contained some populations of folded conformers, because, in the case of Dnp–L-Leu–L-Ala–L-Pro–L-Val–pNA and other tetrapeptide derivatives which take random conformation, no CD bands are observed near 350 nm. From comparison of the CD spectra, **7b** is considered to have a little higher population of β-turn conformers than **7c**, and the population was higher in MeOH solution than in DMSO.

Higher-field Shifts of Aromatic Proton Chemical Shifts Due to the Ring-current Effects. ^{1}H NMR spectra of **7a**—**c** were measured in DMSO-d_6 solution. All the spectra showed two sets of resonances for most of the protons due to *cis-trans* isomerism for the D-Ala–L-Pro bonds. In all the peptides, the populations of *trans* isomers were estimated to about 70% according to the relative peak area of the same proton. Figure 4 shows that aromatic protons of the *trans* isomer in **7a** were shifted apparently to higher field than those of the *cis* isomer because of ring-current effects, indicating the proximity of the two terminal chromophores. The *trans* isomers of **7b** and **7c** also showed similar higher-field shifts, but the shifts were much smaller than that of **7a**. Between the two, **7b** showed a little higher shift than **7c**. The *cis* isomers of **7a**—**c** are considered to take unordered conformations and consequently to have little contribution to the CD spectra of **7a**—**c**, since they showed similar chemical shifts of aromatic and some other protons for the three compounds irrespective of the chain length. In contrast, the *trans* conformers of **7a**—**c** showed large chain-length dependences of chemical shifts for many of the protons, indicating that their *trans* conformations depended on chain length. The *trans* isomers of **7b** and **7c** are considered still to have a little population of folded conformers which cause proximities of the two terminal chromophores. Between the two, **7b** is considered to have a little higher population of folded conformers than **7c**.

Intramolecular Hydrogen Bondings and β-Turn Conformations. Temperature dependences of chemical shifts of amide proton resonances are useful for distinguishing between "exposed" and "intramolecularly hydrogen-

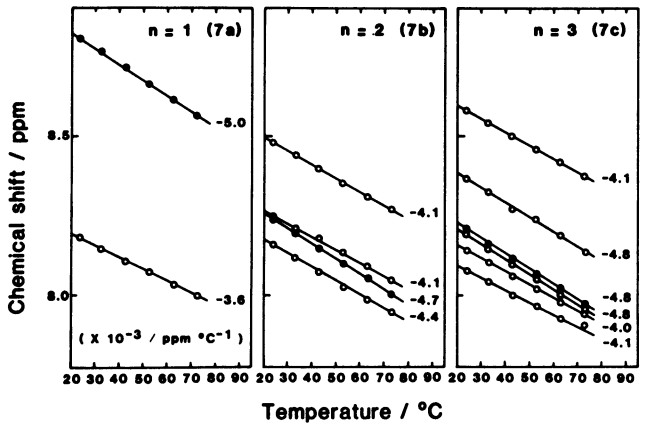

Fig. 5. Temperature dependences of chemical shifts of N^aH protons in major conformer of Dnp-$(Gly)_n$-D-Ala-L-Pro-$(Gly)_n$-pNA (n=1—3) in DMSO-d_6. The Gly^1-NH protons are omitted. ●: D-Ala-NH, ○: Gly-NH.

bonded" amide proton;[7] exposed protons exhibited larger temperature dependences than hydrogen-bonded amide protons. The temperature dependences of the chemical shifts of NH protons of **7a**—**c** were studied in DMSO-d_6 solution. Figure 5 shows the results for NH protons of the *trans* isomers in **7a**—**c**, where Gly^1-NH protons were omitted because they were not amide protons and also exhibited constantly small temperature dependence due to hydrogen bonding with the *o*-nitro group in the Dnp chromophore. The Gly^4-NH proton in **7a** showed smaller temperature dependences than the D-Ala^2-NH proton, indicating the presence of β-turn conformer with 4→1 hydrogen bond concerned by the Gly^4-NH proton as shown in Fig. 1. In the case of **7b** and **7c**, the differences in temperature dependences of Gly-NH protons and D-Ala-NH proton were quite small, but still a little differences were observed; the D-Ala-NH proton always showed the largest temperature dependence of all the NH protons, and the numbers of the Gly-NH protons with smaller dependences (-4.0——-4.1×10^{-3} ppm $°C^{-1}$) were in good accordance with the numbers of the Gly-NH protons involved in hydrogen bonding in the models of folded conformers of **7b** and **7c** shown in Fig. 1. These results suggested that both **7b** and **7c** had a little population of folded conformers, though they were quite lower levels as compared with that of **7a**. The Gly^4-NH proton of *cis* isomer in **7a**, which separated well from other resonances, showed large temperature dependence (-5.2×10^{-3} ppm $°C^{-1}$) as well as that of the D-Ala^2-NH proton (-5.3×10^{-3} ppm $°C^{-1}$), indicating that the *cis* isomer of **7a** took unordered conformation as described in the preceding section. In the case of **7b** and **7c**, the NMR spectra were too complex to study the temperature dependences of all the NH protons of minor (*cis*) isomers. Most of the protons other than amide ones of **7a**—**c** showed little temperature dependences, suggesting that the conformations of **7a**—**c** did not change significantly in the temperature range of these experiments.

Correlation between CD Spectra and β-Turn Conformations. The characteristic CD spectra of Dnp-peptide-pNA's are considered to be due to the exciton coupling of the transition moments in the two terminal chromophores. The relative strength of Cotton effects of **7a**—**c** shown in Fig. 3 were in good accordance with the populations of β-turn conformers in **7a**—**c** estimated from NMR measurements. All of the results with both CD and NMR spectra indicate that the interaction between the two chromophores decreases rapidly as length of the peptide chain increases, however, some populations of β-turn conformers are considered still to be present in both compounds (**7b** and **7c**) examined. Between the two, **7b** is considered to have a little higher population of β-turn conformers than **7c**.

Experimental

Syntheses of Peptides. All the melting points were measured on a Yanagimoto micro melting point apparatus and were uncorrected. TLC's were carried out on Merck silica gel 60 F_{254} plates with the following solvent systems: R_f^1, $CHCl_3$–MeOH (5 : 1, v/v); R_f^2, $CHCl_3$–MeOH–AcOH (95 : 5 : 1, v/v); R_f^3, *n*-BuOH–AcOH–pyridine–H_2O (4 : 1 : 1 : 2, v/v). Optical rotations were measured on an Union automatic polarimeter PM-201.

*Boc-(Gly_3)-pNA (**1c**).* To a chilled solution of Boc-$(Gly)_2$-OH[6] (465 mg, 2 mmol) and H-Gly-pNA (390 mg, 2 mmol) in DMF (8 ml) was added EDC·HCl (383 mg, 2 mmol). The mixture was stirred at 0 °C for 1 h and at room temperature overnight and evaporated *in vacuo*. Addition of water to the residue gave a solid, which was collected by filtration and washed successively with 10% citric acid, 4% $NaHCO_3$, and water. After being dried *in vacuo* over P_2O_5, the product was recrystallized from DMF–ether; yield, 562 mg (69%), mp 235—237 °C; R_f^1 0.44, R_f^2 0.10, R_f^3 0.75.

Found: C, 49.93; H, 5.71; N, 17.11%. Calcd for $C_{17}H_{23}O_7N_5$: C, 49.87; H, 5.66; N, 17.11%.

*H-$(Gly)_3$-pNA·HCl (**2c**·HCl).* Compound **1c** (491 mg, 1.2 mmol) was dissolved in 0.1 M (1 M=1 mol dm^{-3}) hydrogen chloride in formic acid (18 ml). The solution was allowed to stand at room temperature for 30 min and evaporated to leave an oil, which was crystallized by addition of ether; yield, 426 mg (100%); mp 238—240 °C; R_f^3 0.51.

Found: C, 41.47; H, 4.74; N, 20.17%. Calcd for $C_{12}H_{16}O_5N_5Cl$: C, 41.68; H, 4.66; N, 20.26%.

*Boc-D-Ala-L-Pro-$(Gly)_2$-pNA (**3b**).* To a chilled solution of Boc-D-Ala-L-Pro-OH[5] (287 mg, 1 mmol), H-$(Gly)_2$-pNA·HCl[1] (289 mg, 1 mmol), and TEA (0.14 ml, 1 mmol) in DMF (6 ml) was added EDC·HCl (192 mg, 1 mmol), and the mixture was stirred at 0 °C for 1 h and at room temperature overnight and evaporated. The residue was dissolved in EtOAc, and the solution was washed successively with 10% citric acid, 4% $NaHCO_3$, and water, dried (Na_2SO_4), and evaporated. The residue was solidified by addition of ether. The crude product dissolved in MeOH (2 ml) was applied to a column (3×170 cm) of Sephadex LH-20 and eluted with MeOH. The fractions containing the desired product detected with UV absorption and TLC were collected and evaporated, and the residue was solidified by addition of ether and recrystallized from EtOH–ether; yield, 215 mg (42%); mp 197—200 °C; $[\alpha]_D^{22}$ −2.1° (*c* 1, MeOH); R_f^1 0.77, R_f^2 0.28, R_f^3 0.75.

Found: C, 52.96; H, 5.88; N, 16.39%. Calcd for $C_{23}H_{32}O_8N_6$: C, 53.07; H, 6.20; N, 16.15%.

*Boc-D-Ala-L-Pro-$(Gly)_3$-pNA (**3c**).* This compound

was prepared from Boc-D-Ala-L-Pro-OH[5] (287 mg, 1 mmol) and **1c**·HCl (346 mg, 1 mmol) as described for **3b**; yield, 284 mg (48%); mp 112—115 °C; $[\alpha]_D^{22}$ +5.8° (*c* 1, MeOH); R_f^1 0.63, R_f^2 0.12, R_f^3 0.74.

Found: C, 51.62; H, 6.14; N, 16.77%. Calcd for $C_{25}H_{35}O_9N_7$: C, 51.98; H, 6.11; N, 16.98%.

*H-D-Ala-L-Pro-(Gly)$_2$-pNA·HCl (**4b**·HCl).* Compound **3b** (182 mg, 0.35 mmol) was dissolved in 0.1 M hydrogen chloride in formic acid (4.2 ml). The solution was allowed to stand at room temperature for 30 min and evaporated to leave an oil. The product was used for the next reaction without further treatment; the yield was quantitative; R_f^3 0.61.

*H-D-Ala-L-Pro-(Gly)$_3$-pNA·HCl (**4c**·HCl).* Compound **3c** (104 mg, 0.18 mmol) was treated with 0.1 M hydrogen chloride in formic acid (2.7 ml) as described for **4b**·HCl. The product was used for the next reaction without further treatment; the yield was quantitative; R_f^3 0.42.

*Boc-(Gly)$_2$-D-Ala-L-Pro-(Gly)$_2$-pNA (**5b**).* This compound was prepared from Boc-(Gly)$_2$-OH[6] (81 mg, 0.35 mmol) and **4b**·HCl (134 mg, 0.35 mmol) as described for **3b** except that the column chromatography with Sephadex LH-20 was not used. The crude product was recrystallized from MeOH–ether; yield, 66 mg (30%); mp 133—136 °C; $[\alpha]_D^{22}$ +0.4° (*c* 1, MeOH); R_f^1 0.52, R_f^2 0.03, R_f^3 0.73.

Found: C, 50.13; H, 5.99; N, 17.32%. Calcd for $C_{27}H_{38}O_{10}N_8 \cdot 1/2\ H_2O$: C, 50.38; H, 6.11; N, 17.41%.

*Boc-(Gly)$_3$-D-Ala-L-Pro-(Gly)$_3$-pNA (**5c**).* To a chilled solution of Boc-(Gly)$_3$-OH[8] (52 mg, 0.18 mmol), **4c**·HCl (93 mg, 0.18 mmol), and TEA (0.025 ml, 0.18 mmol) in DMF (2 ml) were added DCC (37 mg, 0.18 mmol) and HOBt (49 mg, 0.36 mmol), and the reaction mixture was stirred at 0 °C for 1 h and at room temperature overnight. Insoluble materials were removed by filtration, and the filtrate was evaporated. The residue was dissolved in MeOH (3 ml), applied to a column (3×170 cm) of Sephadex LH-20, and eluted with MeOH. The fractions containing the desired product were collected and evaporated, and the residue was crystallized from EtOH–ether, and collected by filtration. The product dissolved in a mixture (3 ml) of $CHCl_3$–MeOH (5 : 1) was applied to a column (2.5×20 cm) of silica gel 60 (Merck) and eluted with the same solvent. The fractions with the desired product were collected and evaporated, and the residue was crystallized from MeOH–ether; yield, 82 mg (61%); mp 141—143 °C; $[\alpha]_D^{22}$ +11.8° (*c* 1, MeOH); R_f^1 0.17, R_f^3 0.64.

Found: C, 48.71; H, 5.80; N, 18.44%. Calcd for $C_{13}H_{44}O_{12}N_{10} \cdot H_2O$: C, 48.56; H, 6.04; N, 18.27%.

*H-(Gly)$_2$-D-Ala-L-Pro-(Gly)$_2$-pNA·HCl (**6b**·HCl).* Compound **5b** (19 mg, 0.03 mmol) was treated with 0.1 M hydrogen chloride in formic acid (0.6 ml) as described for **4b**·HCl. The product was used for the next reaction without further treatment; the yield was quantitative; R_f^3 0.39.

*H-(Gly)$_3$-D-Ala-L-Pro-(Gly)$_3$-pNA·HCl (**6c**·HCl).* Compound **5c** (52 mg, 0.07 mmol) was treated with 0.1 M hydrogen chloride in formic acid (0.9 ml) as described for **4b**·HCl. The product was used for the next reaction without further treatment; the yield was quantitative; R_f^3 0.36.

*Dnp-(Gly)$_2$-D-Ala-L-Pro-(Gly)$_2$-pNA (**7b**).* To a solution of **6b**·HCl (17 mg, 0.03 mmol) and TEA (0.013 ml, 0.09 mmol) in DMF (1 ml) was added N_2ph–F (11 mg, 0.06 mmol), and the reaction mixture was stirred at room temperature for 3 h and evaporated. The residue dissolved in $CHCl_3$ (5 ml) was applied to a column (1.8×20 cm) of silica gel 60 (Merck), and the column was washed with $CHCl_3$. The product was eluted with the mixture of $CHCl_3$–MeOH (5 : 1, v/v). The fractions containing the desired product were evaporated to leave crystals, which were recrystallized from DMF–ether; yield, 18 mg (86%); mp 145—148 °C; $[\alpha]_D^{22}$ −15.0° (*c* 0.5, DMF); R_f^1 0.46, R_f^2 0.03, R_f^3 0.63.

Found: C, 47.48; H, 4.79; N, 19.46%. Calcd for $C_{28}H_{32}O_{12}N_{10} \cdot 1/2\ H_2O$: C, 47.39; H, 4.69; N, 19.74%.

*Dnp-(Gly)$_3$-D-Ala-L-Pro-(Gly)$_3$-pNA (**7c**).* Compound **6c**·HCl (48 mg, 0.07 mmol) was treated with N_2ph–F (26 mg, 0.14 mmol) as described for **7b**; yield, 46 mg (81%); mp 176—178 °C; $[\alpha]_D^{22}$ −1.6° (*c* 0.5, DMF); R_f^1 0.15, R_f^3 0.63.

Found: C, 44.94; H, 4.59; N, 19.72%. Calcd for $C_{32}H_{38}O_{14}N_{12} \cdot 2H_2O$: C, 45.17; H, 4.97; N, 19.76%.

CD Measurements. CD spectra were recorded on a JASCO spectropolarimeter model J-20 or J-40 in a 0.1 mM solution at room temperature (23±2 °C).

^1H NMR Measurements. ^1H NMR spectra were recorded on a Bruker WH-270 spectrometer equipped with a Nicolet-1180 computer and with a Bruker B-ST-100/700 temperature control unit. The spectra were measured at 23±0.5 °C if not mentioned otherwise. Chemical shifts were measured from the internal standard of DSS.[3] The spectra were assigned by spin-decoupling, H–D exchange, and saturation transfer methods.

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References

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2) Part I of this series: K. Sato, M. Kawai, and U. Nagai, *Biopolymers*, **20**, 1921 (1981).

3) The abbreviations used in this paper are those recommended by IUPAC-IUB: *J. Biol. Chem.*, **247**, 977 (1972). Additional abbreviations: Boc, *t*-butoxycarbonyl; Dnp, 2,4-dinitrophenyl; pNA, *p*-nitroanilide; DCC, dicyclohexylcarbodiimide; DSS, sodium 2,2-dimethyl-2-silapentane-5-sulfonate; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; N_2ph–F, 1-fluoro-2,4-dinitrobenzene; TEA, triethylamine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; TLC, thin-layer chromatography.

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