Studies on the β-Turn of Peptides. II.[1] Syntheses and Conformational Properties of *N*-(2,4-Dinitrophenyl)tetrapeptide *p*-Nitroanilides Related to the β-Turn Part of Gramicidin S

Kazuki SATO,* Masao KAWAI, and Ukon NAGAI

Mitsubishi-Kasei Institute of Life Sciences, 11 Minamiooya, Machida, Tokyo 194

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N-(2,4-Dinitrophenyl)tetrapeptide *p*-nitroanilides related to the β-turn part of gramicidin S were synthesized and subjected to CD measurements. The β-turn preference of the tetrapeptide derivatives had correlation with the antibiotic activities of the gramicidin S analogs containing similar tetrapeptide sequences at their β-turn part. So, conformational preference of partial sequences of gramicidin S analogs seem to play a significant role for determining their biological activities.

Gramicidin S (GS) is a cyclic decapeptide antibiotic with the primary structure *cyclo*(–L-Val[1]–L-Orn[2]–L-Leu[3]–D-Phe[4]–L-Pro[5]–L-Val[1']–L-Orn[2']–L-Leu[3']–D-Phe[4']–L-Pro[5']–). Conformation of GS consists of the intramolecular antiparallel β-form with four hydrogen bonds between L-Val and L-Leu residues and two β-turns (type II') around the D-Phe–L-Pro sequences (Fig. 1).[2] The characteristic feature of the conformation is the orientation of side chains in which the charged L-Orn side chains are on one side and the hydrophobic L-Val and L-Leu side chains on the other side of the molecule. The conformation of GS is considered to be stabilized not only by the four intramolecular hydrogen bonds but also by the stable β-turn formed by D-Phe–L-Pro sequence. Izumiya *et al.* summarized the antibacterial activities of many GS analogs.[3] The activities of GS analogs were correlated to their conformations.[4] For example, the activity and the population of GS-type β-sheet conformer were in the order of GS≈[D-Ala[4,4']]-GS> [Gly[4,4']]-GS≫[L-Ala[4,4']]-GS≈0.[5] It is of interest to study β-turn preference of the amino acid sequences at the corner positions of GS analogs.

In the previous paper, we gave an outline of a new method to study the β-turn conformation of linear tetrapeptides.[1] *N*-(2,4-Dinitrophenyl)tetrapeptide *p*-nitroanilides (Dnp–tetrapeptide–pNA's)[6] exhibited characteristic CD spectra above 300 nm when they took β-turn conformations (Fig. 2). The Cotton effects were considered to be due to the interaction of the two terminal chromophores and the magnitude of the Cotton effects were shown to reflect the β-turn preference of the peptides.

This paper deals with the details of synthesis and conformational properties of Dnp–tetrapeptide–pNA's related to the β-turn part of GS and with the relation between the activity of GS analogs and the β-turn preference of their partial peptide sequences.

To study the effect of amino acid residues at 4,4'- and 5,5'-positions of GS on β-turn preference, we synthesized tetrapeptide derivatives having a general structure of Dnp–L-Leu–X–Y–L-Val–pNA (**7a—o**) for all the combinations of X=D-Ala, Gly, or L-Ala and Y=L-Pro, L-Leu, Gly, Sar, or D-Leu (Fig. 3). Boc–L-Pro–L-Val–pNA (**1a**) was prepared by EDC coupling of Boc–L-Pro–OH and H–L-Val–pNA, and deprotected with hydrogen chloride in formic acid, to afford H–L-Pro–L-Val–pNA·HCl (**2a**·HCl). Mixed anhydride coupling of Boc–L-Leu–OH and H–D-Ala–OEt afforded Boc–L-Leu–D-Ala–OEt (**3a**), which was converted into Boc–L-Leu–D-Ala–N_2H_3 (**4a**) with hydrazine hydrate. Compounds **2a** and **4a** were coupled by azide method to afford Boc–L-Leu–D-Ala–L-Pro–L-Val–pNA (**5a**), which was converted into the Dnp derivative, Dnp–L-Leu–D-Ala–L-Pro–L-Val–pNA (**7a**), by deprotection and subsequent treatment with 1-fluoro-2,4-dinitrobenzene. Homogeneities of the synthetic peptides were confirmed by thin-layer chromatography and elemental

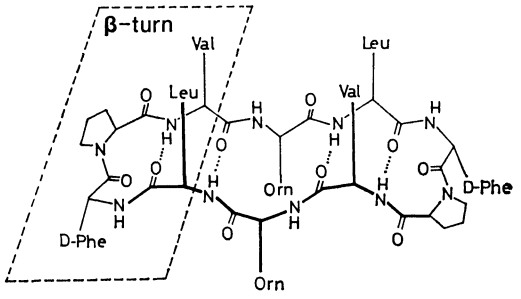

Fig. 1. β-Sheet conformation of gramicidin S.

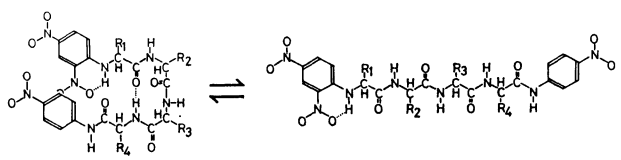

Fig. 2. Models of folded (β-turn) and non-folded conformers of a Dnp–tetrapeptide–pNA.

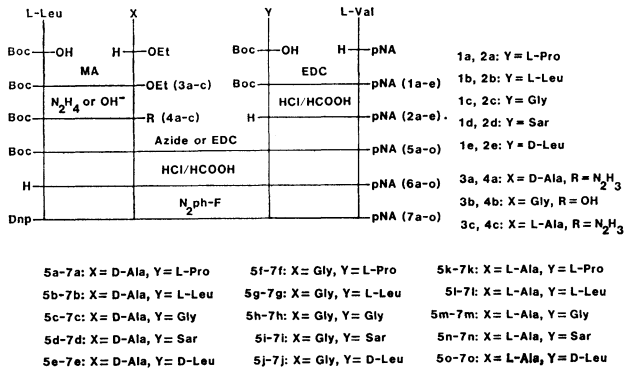

Fig. 3. Syntheses of Dnp–tetrapeptide–pNA's (**7a—o**).

analysis. Compounds **7b—o** were prepared by a similar manner as described for the synthesis of **7a** except for the steps of saponification of **3b** to **4b** and EDC coupling of **4b** and **2a—e** to afford **5f—j**.

Figures 4, 5, and 6 show CD spectra of synthetic peptides with a general structure of Dnp–L-Leu–D-Ala–Y–L-Val–pNA (**7a—e**), Dnp–L-Leu–Gly–Y–L-Val–pNA (**7f—j**), and Dnp–L-Leu–L-Ala–Y–L-Val–pNA (**7k—o**), respectively. From these figures it can be seen that the highest Cotton effects were observed

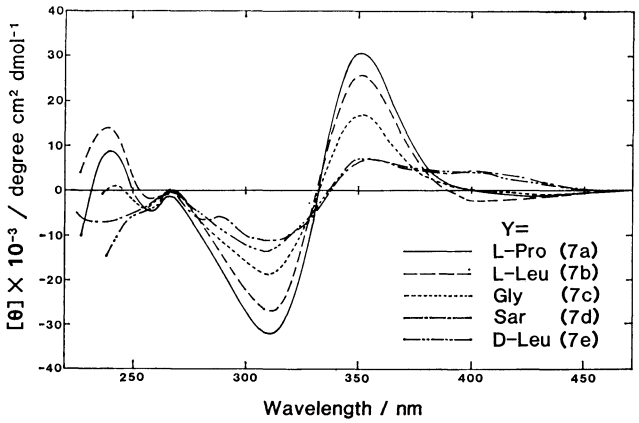

Fig. 4. CD spectra of Dnp–L-Leu–D-Ala–Y–L-Val–pNA's (**7a—e**) in MeOH.

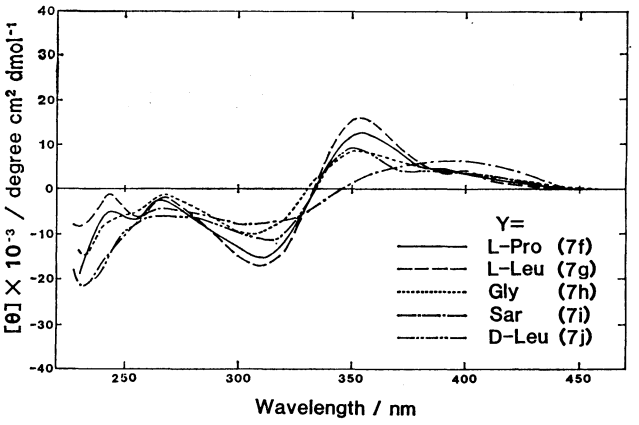

Fig. 5. CD spectra of Dnp–L-Leu–Gly–Y–L-Val–pNA's (**7f—j**) in MeOH.

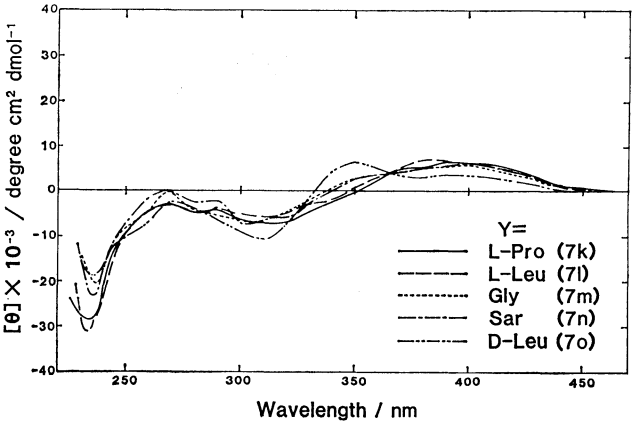

Fig. 6. CD spectra of Dnp–L-Leu–L-Ala–Y–L-Val–pNA's (**7k—o**) in MeOH.

in the case of X–Y=D-Ala–L-Pro or D-Ala–L-Leu. When Y is fixed to L-Pro or L-Leu, D-Ala at X position gives the highest CD band indicating strong β-turn preference of the sequence, Gly at X position gives weaker bands, and L-Ala gives negligibly small $[\theta]_{350}$-values suggesting their random conformations. The tendency is also kept though at a lower level when Y is fixed to Gly. Antibiotic activities of the GS analogs containing the tetrapeptide sequences with L-Pro at the Y position have good correlation with their β-turn preference described above. Though the synthesis of [D-Ala$^{4,4'}$, L-Leu$^{5,5'}$]-, [Gly$^{4,4'}$, L-Leu$^{5,5'}$]-, and [L-Ala$^{4,4'}$, L-Leu$^{5,5'}$]-GS have not been reported, we can predict that they would have the similar tendency of their conformations and antibiotic activities to that of [D-Ala$^{4,4'}$]-, [Gly$^{4,4'}$]-, and [L-Ala$^{4,4'}$]-GS.[5]

In the series of compounds with D-Ala at X position, both **7b** and **7c** with D-Ala–L-Leu and D-Ala–Gly sequences, respectively, also showed large Cotton effects as well as **7a**, but **7d** and **7e** showed very weak Cotton effects (Fig. 4). The L-Pro residue at 5,5′-positions of GS can be replaced by a variety of amino acids (Gly, L-Leu, L-Phe, Sar) without loss of activity.[3] So, high activities of [L-Leu$^{5,5'}$]-GS[7] and [Gly$^{5,5'}$]-GS[8] can be explained by the β-turn preference of their amino acid sequences at the corner positions. When Sar was at Y position, an exception was observed. Compounds **7d** with D-Ala–Sar sequence showed weak Cotton effects, though [Sar$^{5,5'}$]-GS was as active as GS.[9] The β-turn conformer in linear tetrapeptide **7d** would be decreased because of *cis-trans* isomerism of D-Ala–Sar bond. There has been no report about GS analogs having D-amino acid at 5,5′-positions, but D-D sequences at 4-5- and 4′-5′-positions are considered not to prefer the β-turn conformation and the analogs not to show any antibiotic activity since weak Cotton effects were observed in **7e** with D-Ala–D-Leu sequence. When the second residue X was L-Ala, all the derivatives (**7k—o**) showed weak Cotton effects (Fig. 6). This afford the reason why L-amino acid is not suitable for 4,4′-positions of GS, regardless of the configurations of the amino acid residues at 5,5′-positions. All the compounds with Gly at X position (**7f—j**) showed intermediate Cotton effects between the corresponding D-Ala (**7a—e**) and L-Ala (**7k—o**) compounds, respectively (Fig. 5), and the similar effect of substitution at the Y position on the CD spectra was observed.

Next, in order to study the contribution of L-Val$^{1,1'}$ and L-Leu$^{3,3'}$ residues of GS to the β-turn conformation, we synthesized Dnp–Gly–D-Ala–L-Pro–Gly–pNA (**12**), in which both of L-Leu and L-Val residues of **7a** were replaced with Gly residues. Figures 7 and 8 show the synthetic scheme and CD spectrum of **12**, respectively. Cotton effect of **12** was as strong as that of **7a**, indicating that **12** took the β-turn conformation to a high extent. The result agreed well with the fact that both L-Leu and L-Val residues had no significant role for stabilizing the conformation of GS but contributed to the activity of GS with the hidrophobic side chains.[3] For example, [L-Ala$^{1,1',3,3'}$]-GS had similar conformation to that of

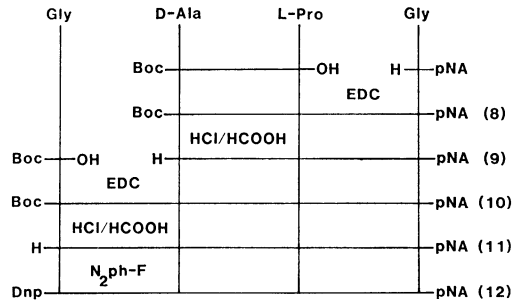

Fig. 7. Synthesis of Dnp–Gly–D-Ala–L-Pro–Gly–pNA (**12**).

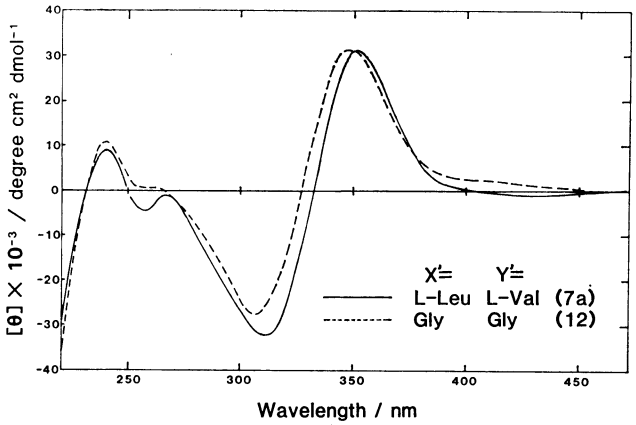

Fig. 8. CD spectra of Dnp–X'–D-Ala–L-Pro–Y'–pNA's (**7a** and **12**) in MeOH.

GS but had no antibiotic activity.[10]

Conformational properties of a number of tetrapeptides related to the corner position sequence of GS analogs were analyzed by means of CD spectra of their Dnp-pNA derivatives. From the results, following conclusion can be drawn: (i) D-Amino acids are suitable choice for the 4,4'-position residues. They can be replaced by Gly, but L-amino acids are not allowed for β-turn conformation at the corner. (ii) L-Pro seems the best choice of the 5,5'-residues for β-turn. It can be replaced by other L-amino acid, but D-amino acids are not allowed. (iii) The terminal L-Leu and L-Val residues of the tetrapeptide sequence seem to have no significant effect for stabilizing β-turn in the series of compounds. As a whole, structure-activity relationship of GS analogs concerning the 4,4'- and 5,5'-positions can be explained largely on the basis of β-turn preference of the tetrapeptide sequences at the corner positions.

Experimental

Synthesis of Peptides. All the melting points were measured on a Yanagimoto micro melting point apparatus and 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. Yields, physical constants, and the results of elemental analyses of the synthetic peptides are summarized in Table 1.

*Boc-L-Pro-L-Val-pNA (**1a**).* To a chilled solution of Boc-L-Pro-OH (1.08 g, 5 mmol) and H-L-Val-pNA (1.19 g, 5 mmol) in DMF (20 ml) was added EDC·HCl (0.96 g, 5 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h and at room temperature overnight, evaporated *in vacuo*, and the residue was dissolved in EtOAc. 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 and petroleum ether, and the product was recrystallized from EtOH–ether–petroleum ether; yield, 1.76 g (81%).

Compounds **1b**—**e** were prepared by a similar manner to that described for **1a**.

*H-L-Pro-L-Val-pNA·HCl (**2a**·HCl).* Compound **1a** (434 mg, 1 mmol) was dissolved in 0.1 M (1 M = 1 mol dm^{-3}) hydrogen chloride in formic acid (15 ml). The solution was allowed to stand at room temperature for 30 min and evaporated *in vacuo* to leave an oil which was crystallized by addition of ether; yield, 345 mg (93%).

Compounds **2b**—**e** were prepared by a similar manner to that described for **2a**.

*Boc-L-Leu-D-Ala-OEt (**3a**).* To a chilled solution of Boc-L-Leu-OH (2.31 g, 10 mmol) and TEA (1.4 ml, 10 mmol) in THF (20 ml) was added isobutyl chloroformate (1.31 ml, 10 mmol) at −15 °C. After 10 min, a chilled solution of H-D-Ala-OEt·HCl (1.54 g, 10 mmol) and TEA (1.4 ml, 10 mmol) in $CHCl_3$ (20 ml) was added. The reaction mixture was treated by a similar manner to that described for the preparation of **1a**; yield, 2.58 g (78%).

Boc-L-Leu-Gly-OEt (**3b**)[11] and Boc-L-Leu-L-Ala-OEt (**3c**)[11] were prepared as described for **3a**; yield, 85 and 81%, respectively.

*Boc-L-Leu-D-Ala-N_2H_3 (**4a**).* A solution of **3a** (1.65 g, 5 mmol) and hydrazine hydrate (4.85 ml, 100 mmol) in DMF (10 ml) was allowed to stand at room temperature for 3 d. The solution was evaporated and the residue was dissolved in $CHCl_3$ (100 ml). The solution was washed with water, dried (Na_2SO_4), and evaporated. The residue was solidified by addition of ether, and recrystallized from MeOH–ether; yield, 1.33 g (84%).

*Boc-L-Leu-Gly-OH (**4b**).* A solution of **3b** (1.58 g, 5 mmol) in a mixture of MeOH (20 ml) and 1 M NaOH (6 ml) was allowed to stand at room temperature for 1 h. After the addition of water (50 ml), the solution was concentrated at low temperature and acidified with 10% citric acid. The oily product was extracted with EtOAc and the solution was washed with H_2O, dried (Na_2SO_4), and evaporated. The residue was solidified by addition of ether and petroleum ether, and recrystallized from MeOH–ether–petroleum ether; yield, 1.38 g (96%).

Compound **4c** was prepared as described for **4a**.

*Boc-L-Leu-D-Ala-L-Pro-L-Val-pNA (**5a**).* To a solution of **4a** (158 mg, 0.5 mmol) in DMF (2 ml) were added 2 M hydrogen chloride in EtOAc (0.75 ml) and isopentyl nitrite (0.071 ml, 0.5 mmol) at −60 °C. After being left to stand at −20 °C for 20 min, the solution was cooled again to −60 °C and neutralized with TEA (0.21 ml, 1.5 mmol). To the mixture was added a chilled solution of **2a**·HCl (196 mg, 0.5 mmol) and TEA (0.07 ml, 0.5 mmol) in DMF (2 ml). The reaction mixture was stirred at 0 °C for 4 d and then 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 and petroleum ether. The crude product (287 mg, 93%) dissolved in MeOH (2 ml) was applied to a column (3 × 170 cm) of Sephadex LH-20 and eluted with MeOH.

TABLE 1. YIELDS AND ANALYTICAL DATA OF SYNTHETIC PEPTIDES

Compound	Yield %	Mp θ_m/°C	$[\alpha]_D^{22}$ a) °	Found(%) C	Found(%) H	Found(%) N	Calcd(%) C	Calcd(%) H	Calcd(%) N	R_f^1	R_f^2	R_f^3
1a	81	94—96	−113.2	58.22	6.66	12.72	58.05	6.96	12.90	0.91	0.52	
1b	73	88—90	−61.6	58.66	7.13	12.30	58.65	7.61	12.44	0.94	0.54	
1c	75	175—177	−45.8	55.01	6.55	14.15	54.80	6.60	14.21	0.81	0.41	
1d	76	83—86	−81.1	55.45	7.29	14.09	55.87	6.91	13.72	0.96	0.57	
1e	68	168—170	−44.8	58.92	7.28	12.00	58.65	7.61	12.44	0.89	0.51	
2a·HCl	93	138—139	−60.4	50.33	6.08	14.42	50.59b)	6.37b)	14.75b)	0.49	0	0.76
2b·HCl	97	168—169	−1.6	51.50	6.92	13.83	51.57b)	7.13b)	14.16b)	0.65	0	0.84
2c·HCl	100	155—156	+14.0	47.29	5.55	16.73	47.21	5.79	16.94	0.03	0	0.61
2d·HCl	88	135—136	−32.8	47.84	5.86	15.60	47.52b)	6.27b)	15.83b)	0.35	0	0.72
2e·HCl	88	157—158	−48.8	51.92	6.99	14.59	52.17c)	7.08c)	14.32c)	0.62	0	0.85
3a	78	82—84	+4.2	58.62	9.42	8.62	58.16	9.15	8.48	0.96	0.78	
4a	84	96—98	+5.0	53.43	8.91	17.54	53.13	8.92	17.71	0.52	0.11	
4b	96	121—122	−26.3	54.36	7.97	9.63	54.15	8.39	9.72	0.17	0.11	
4c	80	171—172	−46.6	53.15	9.01	17.71	53.13	8.92	17.71	0.52	0.11	
5a	80	102—103	−55.5	58.15	7.77	13.28	58.23	7.49	13.58	0.83	0.42	
5b	75	218—219	−38.5	58.84	7.84	12.84	58.65	7.94	13.24	0.77	0.34	
5c	67	206—207	−31.4	55.78	7.32	14.43	56.04	7.32	14.53	0.77	0.31	
5d	78	118—120	−52.3	56.50	7.55	13.85	56.74	7.48	14.18	0.73	0.26	
5e	90	243—245	−20.4	57.07	8.37	12.67	57.03d)	8.03d)	12.88d)	0.81	0.52	
5f	67	124—126	−74.6	57.40	7.34	13.77	57.60	7.33	13.90	0.75	0.42	
5g	67	222—234	−64.8	58.17	7.77	13.34	58.04	7.79	13.54	0.75	0.28	
5h	61	142—143	−47.0	55.41	6.88	14.91	55.31	7.14	14.88	0.81	0.31	
5i	70	126—127	−77.4	55.77	7.49	14.33	56.04	7.32	14.53	0.65	0.30	
5j	63	187—188	−52.8	57.91	7.87	13.51	58.05	7.79	13.54	0.77	0.53	
5k	40	123—125	−134.8	58.09	6.93	13.37	58.23	7.49	13.58	0.77	0.32	
5l	63	241—242	−32.2e)	58.82	8.04	12.99	58.65	7.94	13.24	0.87	0.52	
5m	56	191—192	−52.0	55.95	7.32	14.58	56.04	7.32	14.53	0.72	0.24	
5n	68	123—125	−69.9	56.52	7.64	14.08	56.74	7.48	14.18	0.83	0.30	
5o	66	220—222	−43.8	58.93	7.94	13.25	58.65	7.94	13.24	0.77	0.41	
7a	67	126—128	+17.4	54.54	5.67	16.12	54.37	5.89	16.39	0.91	0.35	
7b	78	292—293	−57.1	55.32	6.10	15.94	54.87	6.33	15.99	0.91	0.33	
7c	87	215—216	+2.8e)	51.45	5.44	17.14	51.44b)	5.70b)	17.14b)	0.64	0.22	
7d	78	205—207	+18.6	52.59	5.61	16.72	52.89	5.82	17.02	0.89	0.28	
7e	70	283—284	+2.4e)	54.57	6.11	15.83	54.87	6.33	15.99	0.88	0.35	
7f	76	233—235	−2.2e)	52.95	5.63	16.43	53.01b)	5.78b)	16.49b)	0.79	0.36	
7g	90	284—285	+23.4e)	54.17	6.15	16.25	54.22	6.16	16.32	0.87	0.36	
7h	84	260—262	+12.4e)	51.41	5.34	17.72	51.43	5.43	17.77	0.68	0.14	
7i	80	223—225	+13.0e)	51.07	5.77	16.92	50.74d)	5.78d)	16.91d)	0.63	0.31	
7j	82	137—140	−7.8	54.34	6.22	16.12	54.22	6.16	16.32	0.84	0.40	
7k	76	135—136	−42.5	54.24	5.68	16.21	54.37	5.89	16.39	0.94	0.35	
7l	80	>300	+10.9	55.00	6.01	15.99	54.87	6.33	15.99	0.92	0.30	
7m	93	>300	+21.8e)	52.27	5.68	17.29	52.16	5.63	17.39	0.64	0.22	
7n	58	127—129	+8.0	53.01	5.59	16.82	52.89	5.82	17.02	0.90	0.30	
7o	66	251—253	+5.6e)	54.72	6.34	15.87	54.87	6.33	15.99	0.89	0.34	
8	77	231—233	+79.8	54.63	6.08	15.14	54.42	6.31	15.11	0.79	0.46	
9·HCl	73	203—206	−20.8	48.03	5.28	17.54	48.06	5.55	17.52	0.12	0	0.59
10	67	125—126	+37.0	52.76	6.03	15.81	53.07	6.20	16.14	0.69	0.25	
12	72	276—278	+51.8e)	48.98	4.43	18.83	49.15	4.47	19.10	0.56	0.13	

a) *c* 1, MeOH, unless otherwise stated. b) ½ H_2O. c) ¼ H_2O. d) H_2O. e) *c* 1, DMF.

The fractions with the desired product detected by UV absorption and TLC were collected and evaporated, and the oily residue was solidified by addition of ether and petroleum ether and recrystallized from MeOH–ether–petrolum ether; yield, 248 mg (80%).

Compounds **5b**—**e** and **5k**—**o** were prepared as described for **5a**.

Boc-L-*Leu*-*Gly*-L-*Pro*-L-*Val*-*pNA* (**5f**). This compound was prepared from **4b** (144 mg, 0.5 mmol) and **2a** (167 mg, 0.5 mmol) as described for **1a**; yield, 202 mg

(67%).

Compounds **5g—j** were prepared as described for **5f**.

H-L-Leu-D-Ala-L-Pro-L-Val-pNA·HCl (6a·HCl). Compound **5a** (118 mg, 0.2 mmol) was dissolved in 0.1 M hydrogen chloride in formic acid (3 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; yield was quantitative.

Compounds **6b—o** were prepared as described for **6a**.

Dnp-L-Leu-D-Ala-L-Pro-L-Val-pNA (7a). To a solution of **6a**·HCl (106 mg, 0.2 mmol) and TEA (0.084 ml, 0.6 mmol) in DMF (2 ml) was added N_2ph-F (74 mg, 0.4 mmol). The reaction mixture was stirred at room temperature for 3 h and evaporated *in vacuo*. 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 desired product was eluted with a mixture of $CHCl_3$ and MeOH (5: 1, v/v). The fractions containing the desired product were evaporated to leave an oil, which was crystallized by addition of ether and recrystallized from MeOH-ether; yield, 91 mg (67%).

Compounds **7b—o** were prepared as described for **7a**.

Boc-D-Ala-L-Pro-Gly-pNA (8). This was prepared from Boc-D-Ala-L-Pro-OH[5] (573 mg, 2 mmol) and H-Gly-pNA (390 mg, 2 mmol) as described for **1a**; yield, 713 mg (77%).

H-D-Ala-L-Pro-Gly-pNA·HCl (9·HCl). This was prepared from **8** (463 mg, 1 mmol) as described for **2a**; yield, 292 mg (73%).

Boc-Gly-D-Ala-L-Pro-Gly-pNA (10). This was prepared from Boc-Gly-OH (88 mg, 0.5 mmol) and **9**·HCl (200 mg, 0.5 mmol) as described for **1a**; yield, 174 mg (67%).

H-Gly-D-Ala-L-Pro-Gly-pNA·HCl (11·HCl). This was prepared from **10** (104 mg, 0.2 mmol) as described for **6a**·HCl; yield was quantitative; R_f^1 0.12, R_f^3 0.68.

Dnp-Gly-D-Ala-L-Pro-Gly-pNA (12). This was prepared from **11**·HCl (91 mg, 0.2 mmol) as described for **7a**; yield, 84 mg (72%).

CD Measurements. CD spectra were recorded on a JASCO spectropolarimeter model J-20 or J-40 in a 0.5 mM MeOH solution at room temperature (23±2 °C) unless otherwise stated.

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6) 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; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; N_2ph-F, 1-fluoro-2,4-dinitrobenzene; TEA, triethylamine; MA, mixed anhydride; TLC, thin-layer chromatography; GS, gramicidin S.

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