Application of a Unique Automated Synthesis System for Solution-phase Peptide Synthesis

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An automated synthesis system, which is suitable for repetitive syntheses using similar reaction procedures, was used to synthesize systematically a library of all possible dipeptides (25) and tripeptides (125) from 5 protected amino acids. The apparatus has also been applied to the automated synthesis of 10 fragment tripeptide derivatives that are constituents of the hormone PACAP-27. The measured molecular optical rotation values of the library of 125 tripeptides were found to correlate well with calculated values obtained by summation of the molecular optical rotation values for the constituent amino acids.

Key words computer-assisted automated synthesis system; fragment peptide synthesis; PACAP-27; solution-phase peptide synthesis; molecular optical rotation; least-squares method

Pharmaceutical chemists have to carry out routine tasks, such as optimizing reaction conditions and synthesizing many derivatives on the several hundred milligram scale for detecting biological activity at the early stage of screening. To aid chemists in these time-consuming and often tedious tasks, a considerable effort has gone into developing automated chemistry techniques. Perhaps the most well-known and successful example of automation in chemistry involves the solid-phase synthesis of peptides and nucleotides. Since Merrifield introduced the solid-phase method of peptide synthesis in 1963,[1] it has revolutionized the synthesis of these biomolecules and the methodology has proven equally suitable for the synthesis of nucleotides including DNA,[2] polysaccharides[3] and other molecules composed of modular building blocks. Solid-phase chemistry, organic synthesis, and an apparatus capable of multiple and simultaneous syntheses were recently combined to generate libraries of organic compounds known as "diversomers".[4]

In solid-phase synthesis, the amino acid forming the carboxy terminus of the peptide is first attached to an insoluble polymer. Through protecting manipulations and coupling reactions, successive amino acids are added in a stepwise fashion to the nascent peptide chain. After all the amino acids have been linked in the synthetic peptide, it is cleaved from the insoluble polymer and purified *via* standard chemical procedures. The automated synthesis of peptides and DNA is now a routine process and automated synthesizers dedicated to such solid-phase synthesis are commercially available. Purification of the products in solid-phase synthesis, however, is often difficult especially on a large scale and yields vary due to the changing physical characteristics of the polymers.

On the other hand, we have been developing automated work stations,[5,7] that employ solution-phase methodology. A series of basic procedures that are regularly used in general organic synthesis, such as the addition of reagents, control of reaction temperature and time, extraction of products, analysis by HPLC, product purification by column chromatography and so forth, can be performed by the automated synthesis apparatus. One of the characteristics of the automated synthesis apparatus is its high reproducibility, making it particularly suitable for the optimization of each reaction step and the synthesis of many derivatives using similar reaction procedures.

In this report, we describe the automated solution-phase synthesis of di- and tripeptide derivatives on a gram scale in a single operating cycle. The performance of the apparatus was demonstrated firstly with the synthesis of a library of all possible sequences of di- and tripeptide derivatives available from 5 different protected amino acids, and secondly with the synthesis of 10 tripeptide derivatives that are constituents of the hormone PACAP-27.[6]

The automated apparatus (MAVIS and TARO),[7] designed for repetitive syntheses using similar reaction procedures, were utilized to synthesize systematically 25 (5^2) dipeptides and 125 (5^3) tripeptide derivatives starting from 5 representative Boc-amino acids of different characters [Boc–Leu, Boc–Ser(Bzl), Boc–Glu(OcHex), Boc–Trp and Boc–Arg(Tos)].

Figure 1 shows the peptide sequence of PACAP-27, which has been well studied in connection with its effect on cAMP activity in pituitary cells.[6] The amino acid benzyl esters and dipeptide derivatives were prepared as intermediates and then the ten fragment tripeptide derivatives that constitute PACAP-27 were synthesized in 50—80% isolated yield.

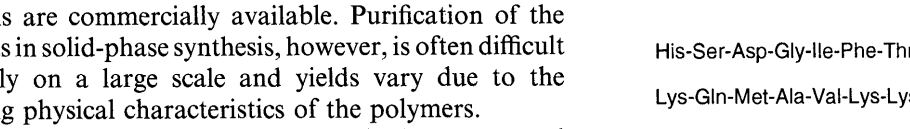

Fig. 1. Structure of PACAP-27

Results and Discussion

a) General Synthesis of Di- and Tripeptide Derivatives Organic chemists often carry out experiments according to synthetic flowcharts or reaction procedures. Similarly, the automated synthesis system is operated according to

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Table 1. Reaction Subroutine Pools for the Automated Synthesis Apparatus

Title	Function
START	First subroutine to input all required parameters
RS*x*-RF*y*	Measure volume of liquid in RS*x* in measuring tube, transport to RF*y* ($x=1$—6, $y=1$—3)
RR*x*-RF*y*	Transport liquid in RR*x* to RF*y* ($x=1$—3, $y=1$; $x=4$—6, $y=2$; $x=7$—9, $y=3$)
RF*x*-RF*y*	Transport liquid in RF*x* to RF*y* ($x=1$, $y=2$, 3; $x=2$, $y=1$, 3; $x=3$, $y=1$, 2)
RF*x*-PH	Transport liquid in RF*x* to PH ($x=1$—3)
PH-RF*x*	Transport liquid in PH to RF*x* ($x=1$—3)
RF*x*-SR*y*	Transport liquid in RF*x* to SR*y* ($x=1$—3, $y=2$, 3)
RF*x*-SF	Transport liquid in RF*x* to SF ($x=1$—3)
SF-RF*x*	Transport liquid in SF to RF*x* ($x=1$—3)
SF-SR*x*	Transport liquid in SF to SR*x* ($x=0$, 1)
SR*x*-SF	Transport liquid in SR*x* to SF ($x=0$, 1)
SF-F*x*-F*y*	Transport liquid in SF by half to RF*x* and RF*y* (x, y=combination of two out of 1, 2 and 3)
FRACT-RF*x*	Transport liquid in fraction tubes to RF*x* ($x=1$—3)
RF*x*-MIX	Stir vigorously ($x=1$—3)
RF*x*-STR-ON	Start stirring in RF*x* ($x=1$—3)
RF*x*-STR-OF	Stop stirring in RF*x* ($x=1$—3)
RF*x*-BUBB	Bubble in RF*x* ($x=1$—3)
SF-BUBB	Bubble in SF
PH-BUBB	Bubble in PH
RF*x*-REA-*n*	The *N*th reaction in RF*x* ($N=1$—3, $x=1$—3): monitoring available
RF*x*-CON-*n*	The *N*th concentration in RF*x* ($N=1$—3, $x=1$—3)
EXT*n*	The *N*th extraction ($N=1$—10)
RF*x*-CL-ON	Start cooling RF*x* ($x=1$, 2)
RF*x*-CL-OF	Stop cooling RF*x* ($x=1$, 2)
RF*x*-HT-ON	Start heating RF*x* ($x=1$—3)
RF*x*-HT-OF	Stop heating RF*x* ($x=1$—3)
A-LC-ON	Switch on monitoring HPLC system
A-LC-OF	Switch off monitoring HPLC system
DE-CO-ON	Switch on preparative HPLC system
DE-CO-OF	Switch off preparative HPLC system
HPLC	Inject solution in SR3 to HPLC, carry out column chromatography
PH-ADJ	Adjust pH of liquid in PH
MATU	Wait for 5 min
MATU15	Wait for 15 min
ALARM	Alarm and pause until user's interference
WASH	Clear screen for washing
MR-WASH	Wash MT1, MT2 and RR1—RR9 Washing solvent remains in RF1, RF2 and RF3
WS-RF*x*	Transport washing solvent to RF*x* *via* MT1
SF-DR	Drain liquid in SF
SR*x*-DR	Drain liquid in SR*x* ($x=0$—3)
RF*x*-DRY	Dry RF*x* ($x=1$—3)
RF-DRY	Dry RF1, RF2 and RF3
R-WASH	Wash specified RS

Table 2. Extraction Module Subroutine Pools for the Automated Synthesis Apparatus

Title	Function
EX-START	Compulsory first subroutine
EXSLCT	Select extracting solvent, measure, transport to RF
EWSSLC	Select washing solvent, measure, transport to RF
ERS*x*-RF*y*	Measure volume of liquid in RS*x* im measuring tube, transport to RF*y* ($x=1$—6, $y=1$—3)
ERR*x*-RF*y*	Transport liquid in RR*x* to RF*y* ($x=1$—3, $y=1$; $x=4$—6, $y=2$; $x=7$—9, $y=3$)
ERF*x*-SF	Transport liquid in RF*x* to SF ($x=1$—3)
ESF-RF*x*	Transport liquid in SF to RF*x* ($x=1$—3)
ESF-SR*x*	Transport liquid in SF to SR*x* ($x=0$, 1)
ESR*x*-SF	Transport liquid in SR*x* to SF ($x=0$, 1)
ERF*x*-BUBB	Bubble in RF*x* ($x=1$—3)
ESF-BUBB	Bubble in SF
ERF*x*-MIX	Stir vigorously ($x=1$—3)
ESEP-SR*x*	Separate, transport bottom layer to SR*x* ($x=0$, 1)
ESF-DT-F*x*	Transport through drying tube to RF*x* ($x=1$—3)
ESF-DR	Drain liquid in SF
ESR*x*-DR	Drain liquid in SR*x* ($x=0$, 1)
EDT-SLCT	Select drying tube
EDT-RST	Reset drying tube

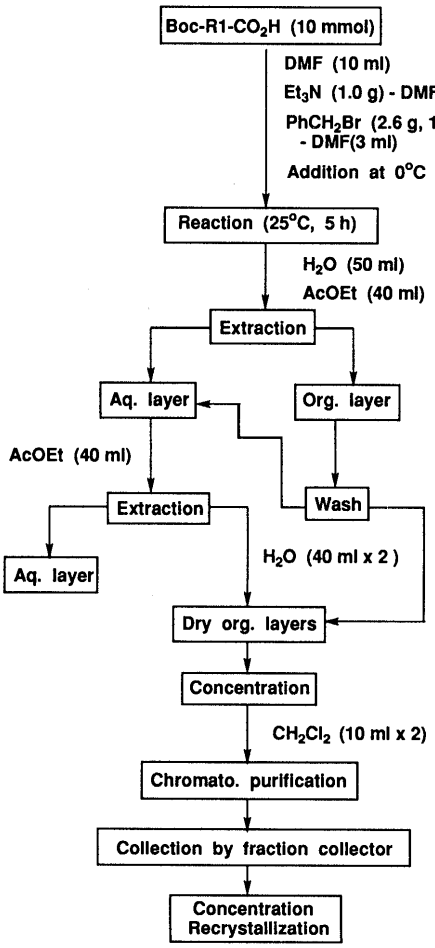

Fig. 2. Flowchart of a Benzylation Reaction

a series of procedures for reaction, extraction, washing, drying and so on, which are contained in subroutine programs. The user can "compose" a reaction program easily by drawing one by one from the reaction subroutine pool which contains about 120 subroutines (Table 1). For the reaction work-up procedure, the user can modify or create an extraction module by choosing from the extraction subroutine pool, which has about 60 subroutines (Table 2).

In oligopeptide synthesis, similar procedures are repeatedly carried out in the automated synthesis system. As an example, the flowchart for the benzylation reaction of a protected amino acid, synthesis of Boc–Leu–OBzl, is shown in Fig. 2. The automated synthesis system operates according to the chosen subroutine programs which are sequenced to follow the flowchart.

The peptide bond formation starts with activating the carboxy group of Boc–R2–CO_2H in one reaction vessel, while simultaneously removing the Boc-group from Boc–R1–OBzl in another reaction vessel. The flowchart for the

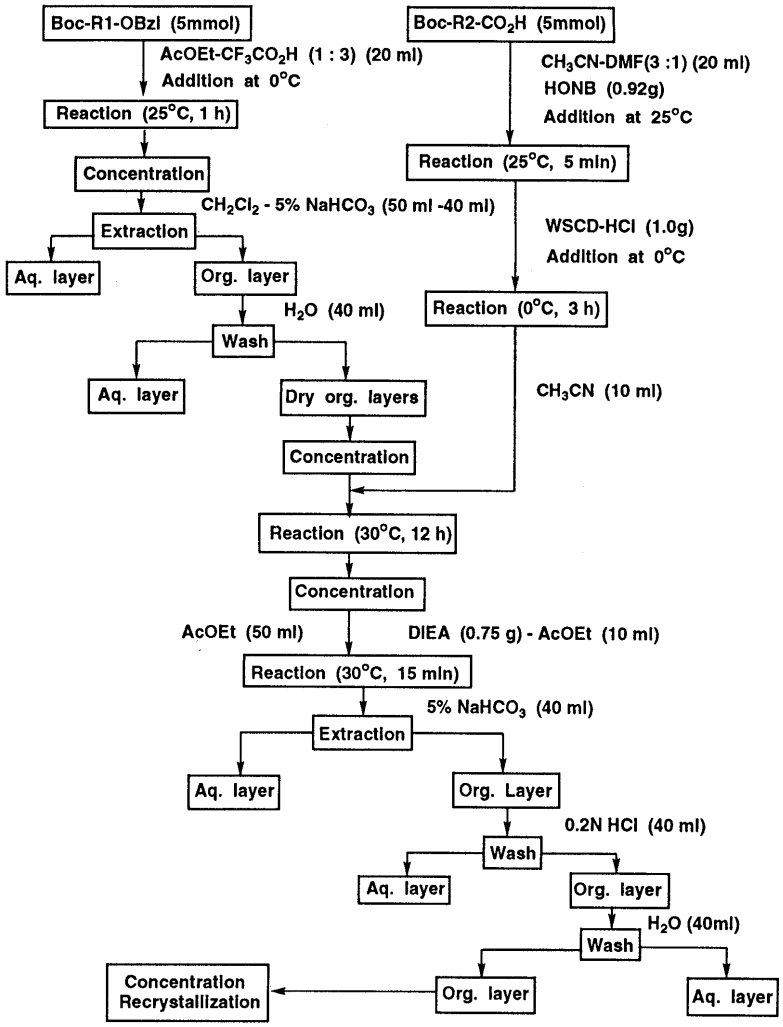

Fig. 3. Flowchart of a Peptide Synthesis

synthesis of a dipeptide derivative, Boc–Glu–Leu–OBzl is shown as an example in Fig. 3. Tripeptide derivatives were synthesized in a similar manner.

Melting points, yields, specific rotation values and elemental analyses of the 5 kinds of amino acid benzyl esters, 25 dipeptides and 125 tripeptides are presented in Tables 3, 4 and 5, respectively.

b) Fragment Synthesis of PACAP-27 As an application of the automated synthesis system, the ten tripeptide derivatives which make up PACAP-27 were synthesized. Benzyl esters and di- and tripeptide derivatives were synthesized in a similar manner to those described above. Yields, melting points, specific rotation values and elemental analyses for the compounds are shown in Table 6.

c) Additivity of the Molecular Rotation Values for the Tripeptide Derivatives Additivity of specific optical rotation values caused by multiple asymmetric carbons has been reported in the field of steroid chemistry.[8] When multiple asymmetric centers exist separately in a molecule, the additivity rule is reported to be generally valid, whereas it fails when the asymmetric centers exist close together. The validity of the additivity rule for the library of 125 tripeptide derivatives was thus investigated.

The molecular optical rotation values of the tripeptide derivatives were calculated from those of the constituent amino acid derivatives. A program for calculating the molecular optical rotation values for each of the 125 tripeptide derivatives, (*e.g.* Boc–A3–A2–A1–OBzl), was made using values calculated by the least-squares method

Table 3. Melting Points, Yields, Specific Rotation Values and Elemental Analyses of Amino Acid Benzyl Esters

$$\text{Boc–A1–OH} \xrightarrow{\text{PhCH}_2\text{Br}} \text{Boc–A1–OBzl}$$

[A1: Leu, Ser(Bzl), Glu(OcHex), Trp, Arg(Tos)]

–A1–[a]	mp (°C)	Yield (%)	$[\alpha]_D$	*Anal.* Calcd (Found)		
				C	H	N
Leu	Oil	86	−37.8	67.26	8.47	4.36
				(67.28	8.45	4.48)
Ser	Oil	83	−20.5	68.55	7.06	3.63
				(68.50	7.08	3.50)
Glu	65.0—66.0	67	−22.0	65.85	7.93	3.34
				(65.78	8.04	3.41)
Trp	144.0—145.0	64	−8.4	70.03	6.64	7.10
				(70.03	6.62	7.12)
Arg	Amorphous	45	−13.5	57.90	6.61	10.80
				(57.61	6.79	10.59)

a) Ser, Glu and Arg are protected with Bzl, OcHex and Tos, respectively.

Table 4. Melting Points, Yields, Specific Rotation Values and Elemental Analyses of Dipeptide Derivatives

Boc–A1–OBzl $\xrightarrow{\text{Boc–A2–OH}}$ Boc–A2–A1–OBzl

[A2, A1: Leu, Ser(Bzl), Glu(OcHex), Trp, Arg(Tos)]

–A2–A1–[a]	mp (°C)	Yield (%)	$[\alpha]_D$	*Anal.* Calcd (Found)		
				C	H	N
Leu–Leu	92.0—92.8	73	−51.9	66.33	8.81	6.45
				(66.60	8.72	6.63)
Ser–Leu	Oil	96	−20.6	66.25	7.74	5.52
				(65.76	7.57	5.46)[b]
Glu–Leu	58.0—58.5	87	−33.6	65.39	8.33	5.26
				(65.46	8.36	5.14)
Trp–Leu	50.0—52.0	93	−29.0	68.62	7.35	8.28
				(68.44	7.43	8.11)
Arg–Leu	Amorphous	65	−22.5	58.93	7.18	11.08
				(58.94	7.26	11.16)
Leu–Ser	35.0—36.0	94	−25.5	67.45	7.68	5.62
				(67.32	7.60	5.46)
Ser–Ser	49.0—50.0	76	−4.4	68.31	6.81	4.98
				(68.33	6.72	4.94)
Glu–Ser	66.0—67.0	80	−15.6	66.42	7.43	4.69
				(66.50	7.39	4.72)
Trp–Ser	90.0—91.0	85	−13.7	69.33	6.52	7.35
				(69.30	6.25	7.13)
Arg–Ser	Amorphous	54	−8.7	60.41	6.52	10.06
				(60.26	6.55	9.94)
Leu–Glu	83.0—84.0	70	−34.1	65.39	8.33	5.26
				(65.22	8.37	5.42)
Ser–Glu	58.0—59.0	95	−12.4	65.42	7.43	4.69
				(66.66	7.43	4.96)
Glu–Glu	101.0—102.0	67	−21.6	64.74	7.99	4.44
				(64.53	8.04	4.41)
Trp–Glu	114.0—115.0	83	−19.0	67.42	7.16	6.94
				(67.21	7.13	6.98)
Arg–Glu	Amorphous	80	−13.8	58.52	7.09	9.48
				(58.85	7.10	9.63)[b]
Leu–Trp	92.0—93.0	56	−24.2	68.62	7.35	8.28
				(68.32	7.37	8.09)
Ser–Trp	146.0—147.0	73	−1.9	69.33	6.52	7.35
				(69.27	6.61	7.23)
Glu–Trp	106.0—108.0	63	−9.2	67.42	7.16	6.94
				(67.09	7.40	6.69)
Trp–Trp	Amorphous	71	−21.6	69.25	6.32	9.50
				(69.41	6.38	9.32)[b]
Arg–Trp	Amorphous	78	−4.5	59.82	6.41	11.63
				(59.73	6.22	11.67)[c]
Leu–Arg	Amorphous	84	−24.0	58.93	7.18	11.08
				(58.66	7.24	11.03)
Ser—Arg	Amorphous	88	−9.0	59.64	6.58	9.94
				(59.71	6.54	10.08)[b]
Glu–Arg	Amorphous	79	−15.5	58.52	7.09	9.48
				(58.64	7.02	9.52)[b]
Trp–Arg	110.0—111.0	29	−13.2	60.57	6.35	11.77
				(60.58	6.26	12.05)[b]
Arg–Arg	Amorphous	75	−8.8	54.46	6.37	13.37
				(54.18	6.29	13.21)[b]

a) Ser, Glu and Arg are protected with Bzl, OcHex and Tos, respectively. *b*) Estimated water content, 0.5 mol eq. *c*) Estimated water content, 1.0 mol eq.

from the molecular optical rotation values of the amino acid constituents (Table 7, 1—15).

Figure 4 shows the relationship between the calculated and measured molecular optical rotation values when only the amino acid constituents were considered for the calculation [the first three terms in the following equation (Eq. 1)]. Some scatter is seen for the derivatives that include tryptophan (W) components. In order to determine whether this scatter was due to partial racemization of the tripeptides containing W components, hydrolysis experiments (see Experimental) were carried out. However, no significant racemization was observed. We also carried out energy calculations for typical amino acid residues, with and without Boc, in order to estimate how the Boc substituent affects peptide conformations. Relative energies of side chain conformations were determined by systematically rotating χ^1 and χ^2 angles using DISCOVER (Biosym Technologies). Comparison of the resultant energy contour maps for unprotected and Boc-protected amino acids revealed that tryptophan and Boc-tryptophan have different energetic profiles for both χ^1 and χ^2 angles, whereas leucine and Boc-leucine have similar ones (Fig. 6). Hence, the Boc substituents are believed to change the rotational barriers of side chains in amino acids such as Boc-tryptophan, presumably by van der Waals interactions. When W exists in the tripeptide, we found it necessary to add an additional term, $[M]_{bond}$ (tryptophan interaction), to the correlation equation (Eq. 1).

$$[M]_D=[M]_{Boc\text{-}A3\text{-}}+[M]_{\text{-}A2\text{-}}+[M]_{\text{-}A1\text{-}OBzl}+[M]_{bond} \quad (1)$$

Tripeptides having W–W bonds ($P=17$; parameters 1—15, 34 and 59 in Table 7) or having at least one W bond ($P=33$), gave very good linear correlations as shown in the following equations. When 65 parameters including all bond–bond interactions (Table 7) were used, an excellent correlation was obtained (Fig. 5).

$P=15$; $y=0.8705x+21.2$ ($r=0.9297$, $\sigma=29.10$)

$P=17$; $y=0.9784x+7.11$ ($r=0.9661$, $\sigma=20.96$)

$P=33$; $y=0.9823x-1.84$ ($r=0.9883$, $\sigma=13.03$)

$P=65$; $y=0.9802x+3.85$ ($r=0.9936$, $\sigma=8.86$)

Conclusion

The solution-phase synthesis of various kinds of protected di- and tripeptide derivatives was performed using a computer-assisted automated synthesis system. A good relationship between the measured and calculated molecular optical rotation values based on additivity of the constituents was obtained for the tripeptide derivatives.

Experimental

Computer-assisted automated synthesis systems (MAVIS and TARO)[7] were used to synthesize oligopeptide derivatives in solution. Optical rotation values (in MeOH) were measured with a Nihon Bunko DIP-370 spectrometer. The least-squares calculations were carried out by using a Hewlet Packard MP-85 with a 7225B plotter. All melting points were taken with a Yanagimoto micro melting point apparatus and are uncorrected. NMR spectra were measured on a Varian Gemini-200 or a JEOL JNM-GX400 FT NMR spectrometer (in $CDCl_3$). Solvents were of special or first grade from Wako Pure Chemical Industries Ltd., and the starting amino acid derivatives were commercially available reagents from Peptide Institute Inc. Column chromatography was carried out on Micro Sphere Gel (DF-50-120A, Dokai Chem. Ind.) or Silica gel 60 (70—230 mesh, ASTM, Merck).

Study on the Possibility of Racemization Each of three derivatives (0.3 mg) containing at least one W component (WWL, SWS and LWE) was weighed in a glass tube, then 6 N HCl containing 4% thioglycolic

Table 5. Melting Points, Yields, Specific Rotation Values and Elemental Analyses of Tripeptide Derivatives

$$\text{Boc–A2–A1–OBzl} \xrightarrow{\text{Boc–A3–OH}} \text{Boc–A3–A2–A1–OBzl}$$

[A3, A2, A1: Leu, Ser(Bzl), Glu(OcHex), Trp, Arg(Tos)]

–A3–A2–A1–[a]	mp (°C)	Yield (%)	$[\alpha]_D$	Anal. Calcd			Found		
				C	H	N	C	H	N
Leu–Leu–Leu	140.5—141.5	77	−69.5	65.79	9.02	7.67	65.70	9.04	7.60
Ser–Leu–Leu	89.0—89.5	59	−41.4	66.75	8.07	6.87	66.70	8.19	6.82
Glu–Leu–Leu	106.0—107.0	68	−51.5	65.09	8.58	6.51	65.03	8.68	6.45
Trp–Leu–Leu	104.0—105.0	84	−49.2	67.72	7.79	9.03	68.00	7.84	9.22
Arg–Leu–Leu	Amorphous	72	−36.5	58.94	7.62	11.15	59.01	7.56	11.42[b]
Leu–Ser–Leu	110.0—110.8	57	−35.5	66.75	8.07	6.87	66.46	8.05	6.86
Ser–Ser–Leu	79.0—80.0	65	−12.3	67.54	7.31	6.22	67.45	7.35	6.29
Glu–Ser–Leu	105.0—106.0	76	−24.0	65.99	7.81	5.92	66.22	7.80	5.95
Tro–Ser–Leu	100.0—102.0	87	−22.6	68.40	7.06	8.18	68.11	7.05	7.98
Arg–Ser–Leu	Amorphous	74	−16.8	60.20	7.02	10.27	60.25	7.08	10.13[b]
Leu–Glu–Leu	98.0—102.0	63	−47.3	65.09	8.58	6.51	65.00	8.45	6.40
Ser–Glu–Leu	114.6—115.5	72	−26.0	65.99	7.81	5.92	66.04	7.54	5.83
Glu–Glu–Leu	100.0—101.0	66	−34.2	64.58	8.26	5.65	64.60	8.42	5.54
Trp–Glu–Leu	145.0—146.5	69	−33.1	66.83	7.57	7.79	66.72	7.46	7.79
Arg–Glu–Leu	Amorphous	78	−24.9	59.20	7.45	9.86	59.22	7.31	9.73[b]
Leu–Trp–Leu	156.0—157.0	71	−49.1	67.72	7.79	9.03	67.66	7.66	9.15
Ser–Trp–Leu	140.0—141.0	62	−32.5	68.40	7.06	8.18	68.26	7.15	8.28
Glu–Trp–Leu	144.0—145.0	69	−34.4	66.83	7.57	7.79	66.76	7.53	7.70
Trp–Trp–Leu	115.0—116.0	81	−54.0	68.36	6.88	9.96	68.36	7.17	9.68[b]
Arg–Trp–Leu	Amorphous	69	−27.5	61.67	6.78	11.99	61.44	6.93	11.76
Leu–Arg–Leu	Amorphous	85	−32.2	58.94	7.62	11.15	59.17	7.50	11.16[b]
Ser–Arg–Leu	Amorphous	82	−20.3	60.20	7.02	10.27	60.11	7.03	10.53[b]
Glu–Arg–Leu	Amorphous	87	−25.3	59.26	7.52	10.11	59.07	7.44	9.97
Trp–Arg–Leu	Amorphous	68	−24.7	61.00	6.83	11.86	61.29	6.85	11.70[b]
Arg–Arg–Leu	Amorphous	74	−19.0	56.09	6.74	13.38	55.88	6.87	13.11
Leu–Leu–Ser	103.0—104.0	71	−44.9	66.75	8.07	6.87	66.82	8.21	6.81
Ser–Leu–Ser	59.0—60.0	70	−23.5	67.54	7.31	6.22	67.47	7.33	6.29
Glu–Leu–Ser	76.0—77.0	70	−33.2	65.99	7.81	5.92	66.04	7.57	5.99
Trp–Leu–Ser	107.0—108.0	63	−31.8	68.40	7.06	8.18	68.23	7.00	8.12
Arg–Leu–Ser	Amorphous	75	−23.3	60.20	7.02	10.27	60.38	6.92	10.53[b]
Leu–Ser–Ser	100.0—101.0	75	−17.7	67.54	7.31	6.22	67.48	7.45	6.13
Ser–Ser–Ser	89.0—90.0	62	−0.3	68.18	6.68	5.68	68.12	6.53	5.60
Glu–Ser–Ser	92.0—93.0	71	−10.7	66.73	7.16	5.43	66.62	7.38	5.32
Trp–Ser–Ser	143.0—144.0	72	−12.0	68.97	6.46	7.48	68.96	6.53	7.43
Arg–Ser–Ser	Amorphous	73	−5.7	61.35	6.41	9.54	61.64	6.54	9.48[b]
Leu–Glu–Ser	72.0—74.0	71	−29.4	65.99	7.81	5.92	65.83	7.85	5.86
Ser–Glu–Ser	48.0—49.0	72	−14.5	66.73	7.16	5.43	66.56	7.17	5.54
Glu–Glu–Ser	93.0—94.0	73	−22.5	65.41	7.61	5.20	65.17	7.60	5.42
Trp–Glu–Ser	72.0—73.0	72	−20.4	67.50	6.95	7.16	67.42	6.96	7.21
Arg–Glu–Ser	109.0—110.0	68	−14.5	60.91	6.89	9.26	60.97	6.85	9.24
Leu–Trp–Ser	126.0—128.0	65	−27.5	68.40	7.06	8.18	68.56	7.07	8.34
Ser–Trp–Ser	63.0—64.0	67	−13.6	68.46	6.57	7.60	68.63	6.53	7.44
Glu–Trp–Ser	140.0—141.0	62	−17.4	67.50	6.95	7.16	67.43	6.95	7.08
Trp–Trp–Ser	92.0—93.0	66	−35.2	68.91	6.31	9.13	69.13	6.28	9.09[b]
Arg–Trp–Ser	Amorphous	61	−13.7	62.64	6.28	11.12	62.58	6.49	10.75
Leu–Arg–Ser	Amorphous	79	−21.9	60.87	6.98	10.39	60.65	7.16	10.23
Ser–Arg–Ser	Amorphous	74	−10.3	61.28	6.51	9.53	61.58	6.52	9.57[b]
Glu–Arg–Ser	Amorphous	75	−16.8	60.31	6.93	9.17	60.05	6.87	8.95[b]
Trp–Arg–Ser	Amorphous	78	−15.4	62.01	6.33	11.00	61.88	6.31	10.59[b]
Arg–Arg–Ser	Amorphous	81	−9.8	57.30	6.31	12.53	57.19	6.44	12.42
Leu–Leu–Glu	135.0—136.0	73	−50.8	65.09	8.58	6.51	65.02	8.58	6.80
Ser–Leu–Glu	Amorphous	69	−28.3	65.99	7.81	5.92	65.75	7.98	5.98
Glu–Leu–Glu	129.0—130.0	75	−38.1	64.58	8.26	5.65	64.45	8.17	5.65
Trp–Leu–Glu	156.0—157.0	52	−37.2	66.83	7.57	7.79	66.83	7.58	7.87
Arg–Leu–Glu	Amorphous	75	−26.8	59.20	7.45	9.86	59.29	7.36	10.11[b]
Leu–Ser–Glu	Amorphous	75	−24.3	65.99	7.81	5.92	65.83	7.84	5.83
Ser–Ser–Glu	97.0—98.0	72	−6.8	66.73	7.16	5.43	66.65	7.28	5.22
Glu–Ser–Glu	81.0—82.0	73	−16.3	65.41	7.61	5.20	65.17	7.53	5.30
Trp–Ser–Glu	103.0—105.0	73	−14.7	67.50	6.95	7.16	67.52	6.76	7.07
Arg–Ser–Glu	Amorphous	73	−10.8	60.91	6.89	9.26	60.87	6.94	9.35
Leu–Glu–Glu	114.0—115.0	77	−35.2	64.58	8.26	5.65	64.62	8.46	5.95
Ser–Glu–Glu	98.0—99.0	71	−18.1	65.41	7.61	5.20	65.37	7.67	5.11

Table 5. (continued)

–A3–A2–A1–[a]	mp (°C)	Yield (%)	$[\alpha]_D$	Anal. Calcd C	Calcd H	Calcd N	Found C	Found H	Found N
Glu–Glu–Glu	88.0—89.0	73	−25.1	64.19	8.02	4.99	64.07	7.85	4.90
Trp–Glu–Glu	149.0—150.0	69	−22.8	66.16	7.40	6.86	66.09	7.32	6.85
Arg–Glu–Glu	Amorphous	71	−17.2	59.41	7.32	8.84	59.63	7.20	8.77[b]
Leu–Trp–Glu	143.0—145.0	71	−36.6	66.83	7.57	7.79	66.63	7.43	7.64
Ser–Trp–Glu	55.0—56.0	62	−25.5	67.50	6.95	7.16	67.55	6.82	7.10
Glu–Trp–Glu	93.0—95.0	64	−26.4	66.16	7.40	6.86	66.05	7.40	6.78
Trp–Trp–Glu	102.0—103.0	71	−48.5	68.25	6.75	8.84	68.05	6.71	8.73
Arg–Trp–Glu	Amorphous	69	−17.4	61.02	6.76	10.60	61.07	6.84	10.54[b]
Leu–Arg–Glu	Amorphous	81	−24.7	59.20	7.45	9.86	59.39	7.41	9.78[b]
Ser–Arg–Glu	Amorphous	79	−12.3	60.91	6.89	9.26	60.64	6.90	9.29
Glu–Arg–Glu	Amorphous	75	−18.2	59.98	7.28	8.93	59.72	7.28	9.37
Trp–Arg–Glu	Amorphous	77	−14.4	61.02	6.76	10.60	61.05	6.73	10.65[b]
Arg–Arg–Glu	Amorphous	66	−12.2	56.09	6.72	12.01	56.06	6.83	11.80[b]
Leu–Leu–Trp	100.0—101.0	46	−40.7	67.72	7.79	9.03	67.56	7.58	8.99
Ser–Leu–Trp	Amorphous	65	−23.0	68.40	7.06	8.18	68.22	7.09	7.92
Glu–Leu–Trp	159.0—160.0	68	−32.4	66.83	7.57	7.79	66.86	7.70	7.87
Trp–Leu–Trp	86.0—89.0	60	−26.2	68.36	6.88	9.96	68.78	7.00	9.76[b]
Arg–Leu–Trp	Amorphous	69	−22.2	61.00	6.83	11.86	61.02	6.93	11.41[b]
Leu–Ser–Trp	Amorphous	57	−12.8	68.40	7.06	8.18	68.17	7.09	8.01
Ser–Ser–Trp	Amorphous	74	−0.08	68.15	6.52	7.39	68.37	6.45	7.53[b]
Glu–Ser–Trp	111.0—112.0	56	−7.6	67.50	6.95	7.16	67.14	6.91	7.23
Trp–Ser–Trp	Amorphous	68	−6.7	69.73	6.25	9.24	69.49	6.34	9.50
Arg–Ser–Trp	Amorphous	59	−2.6	62.01	6.33	11.00	61.92	6.35	10.66[b]
Leu–Glu–Trp	Amorphous	58	−21.3	66.83	7.57	7.79	66.51	7.61	7.96
Ser–Glu–Trp	Amorphous	58	−11.2	66.73	7.00	7.07	66.98	6.96	6.94[b]
Glu–Glu–Trp	139.0—141.0	62	−18.3	66.16	7.40	6.86	65.94	7.50	6.73
Trp–Glu–Trp	Amorphous	62	−11.8	67.48	6.80	8.74	67.65	6.85	8.69[b]
Arg–Glu–Trp	Amorphous	80	−8.3	61.62	6.71	10.70	61.71	6.84	10.36
Leu–Trp–Trp	93.0—94.0	63	−25.7	69.24	6.83	10.09	69.12	6.74	9.84
Ser–Trp–Trp	Amorphous	47	−17.3	68.91	6.31	9.13	69.04	6.33	9.17[b]
Glu–Trp–Trp	149.0—150.0	53	−19.5	68.25	6.75	8.84	68.17	6.76	8.73
Trp–Trp–Trp	Amorphous	50	−39.3	69.66	6.11	10.83	69.37	6.07	11.11[b]
Arg–Trp–Trp	Amorphous	46	−16.0	62.10	6.21	12.33	62.30	6.25	12.54[c]
Leu–Arg–Trp	Amorphous	64	−17.6	59.70	6.92	11.60	59.61	6.88	11.66[d]
Ser–Arg–Trp	Amorphous	72	−7.0	61.39	6.38	10.89	61.51	6.14	10.94[c]
Glu–Arg–Trp	Amorphous	68	−14.2	61.02	6.76	10.60	60.84	6.77	10.28[b]
Trp–Arg–Trp	Amorphous	72	−9.3	62.10	6.21	12.33	61.90	6.08	12.07[c]
Arg–Arg–Trp	Amorphous	74	−7.3	56.96	6.24	13.56	57.20	6.21	13.30[c]
Leu–Leu–Arg	Amorphous	70	−37.8	58.94	7.62	11.15	59.26	7.52	11.13[b]
Ser–Leu–Arg	Amorphous	80	−21.2	60.87	6.98	10.39	60.96	7.02	10.66
Glu–Leu–Arg	Amorphous	81	−29.0	59.20	7.45	9.86	59.41	7.42	10.19[b]
Trp–Leu–Arg	Amorphous	73	−27.2	61.67	6.78	11.99	61.37	6.75	12.24
Arg–Leu–Arg	Amorphous	74	−21.2	55.56	6.78	13.25	55.74	6.77	13.40[b]
Leu–Ser–Arg	Amorphous	78	−18.8	60.87	6.98	10.39	60.70	7.03	10.16
Ser–Ser–Arg	Amorphous	76	−5.7	61.91	6.47	9.63	61.70	6.53	9.36
Glu–Ser–Arg	Amorphous	71	−13.5	60.58	6.52	9.21	60.44	6.96	9.02[b]
Trp–Ser–Arg	Amorphous	73	−13.2	62.01	6.33	11.00	62.00	6.29	11.09[b]
Arg–Ser–Arg	Amorphous	74	−6.2	56.79	6.35	12.42	57.04	6.40	11.96[b]
Leu–Glu–Arg	Amorphous	80	−27.2	59.20	7.45	9.86	59.38	7.46	9.86[b]
Ser–Glu–Arg	Amorphous	83	−14.1	60.38	6.83	9.18	60.46	6.95	9.09[b]
Glu–Glu–Arg	Amorphous	74	−19.8	59.98	7.28	8.93	59.80	7.21	9.43
Trp–Glu–Arg	Amorphous	70	−18.1	61.02	6.76	10.60	60.87	6.76	10.43[b]
Arg–Glu–Arg	Amorphous	76	−14.2	56.58	6.69	12.12	56.72	6.86	12.52
Leu–Trp–Arg	Amorphous	75	−26.7	61.00	6.83	11.86	61.08	6.82	11.55[b]
Ser–Trp–Arg	Amorphous	73	−13.6	62.01	6.33	11.00	61.99	6.39	10.83[b]
Glu–Trp–Arg	Amorphous	73	−17.2	61.02	6.76	10.60	60.82	6.74	10.43[b]
Trp–Trp–Arg	Amorphous	73	−38.7	62.72	6.16	12.45	62.53	6.22	12.35[b]
Arg–Trp–Arg	Amorphous	66	−15.1	57.46	6.20	13.68	57.37	6.25	13.47[b]
Leu–Arg–Arg	Amorphous	86	−19.3	55.56	6.78	13.25	55.35	6.74	13.24[b]
Ser–Arg–Arg	Amorphous	78	−9.2	56.79	6.35	12.42	56.74	6.33	12.32[b]
Glu–Arg–Arg	Amorphous	72	−15.0	56.09	6.72	12.01	55.89	6.68	12.01[b]
Trp–Arg–Arg	Amorphous	72	−13.4	56.96	6.24	13.56	56.99	6.14	13.58[c]
Arg–Arg–Arg	Amorphous	49	−9.0	52.93	6.27	14.52	52.86	6.18	14.32[c]

a) Ser, Glu and Arg are protected with Bzl, OcHex and Tos, respectively. *b*) Estimated water content, 0.5 mol eq. *c*) Estimated water content, 1.0 mol eq. *d*) Estimated water content, 1.5 mol eq.

Table 6. Melting Points, Yields, Specific Rotation Values and Elemental Analyses of Amino Acid Benzyl Esters, Dipeptide and Tripeptide Derivatives

$$\text{Boc–A1–OH} \xrightarrow{PhCH_2Br} \text{Boc–A1–OBzl} \xrightarrow{\text{Boc–A2–OH}} \text{Boc–A2–A1–OBzl} \xrightarrow{\text{Boc–A3–OH}} \text{Boc–A3–A2–A1–OBzl}$$

[A3, A2, A1: Phe, Ile, Gly, Ser(Bzl), Asp(OcHex), Thr(Bzl), Arg(Tos), Lys(Z), Tyr(Bzl), Ala, Met, Gln, Val, Leu, His(Bom)]

Product[a]	mp (°C)	$[\alpha]_D$	Yield (%)	*Anal.* Calcd			Found		
				C	H	N	C	H	N
Boc–Phe–OBzl	68.4—69.2	−14.1	30	70.96	7.09	3.94	71.23	7.17	4.15
Boc–Ile–Phe–OBzl	135.2—135.8	−33.5	64	69.21	7.74	5.98	69.35	7.54	6.23
Boc–Gly–Ile–Phe–OBzl	128.5—129.5	−30.8	76	66.27	7.48	7.99	66.30	7.61	7.81
Boc–Ser–OBzl	Oil	−20.5	40	68.55	7.06	3.63	68.50	7.08	3.50
Boc–Asp–Ser–OBzl	65.8—66.6	−15.3	67	65.96	7.24	4.81	65.86	7.23	4.76
Boc–Thr–Asp–Ser–OBzl	68.0—70.0	−6.4	55	66.74	7.16	5.43	66.52	6.95	5.51
Boc–Arg–OBzl	Amorphous	−13.5	45	57.90	6.61	10.80	57.61	6.79	10.59
Boc–Ser–Arg–OBzl	Amorphous	−9.0	21	59.64	6.58	9.94	59.71	6.54	10.08[b]
Boc–Tyr–Ser–Arg–OBzl	Amorphous	−4.7	79	63.93	6.42	8.77	64.11	6.36	8.84[b]
Boc–Lys–OBzl	54.6—55.2	−22.0	47	66.36	7.28	5.95	66.46	7.21	6.11
Boc–Arg–Lys–OBzl	Amorphous	−11.6	81	59.30	6.76	10.64	59.55	6.81	10.74[b]
Boc–Tyr–Arg–Lys–OBzl	Amorphous	−5.6	65	63.87	6.53	9.48	63.68	6.75	9.61
Boc–Ala–OBzl	Oil	−42.3	56	64.50	7.58	5.01	64.38	7.63	4.97
Boc–Met–Ala–OBzl	Oil	−35.6	85	58.51	7.37	6.82	58.25	7.48	6.82
Boc–Gln–Met–Ala–OBzl	183.5—184.3	−44.4	75	55.74	7.11	10.40	55.48	7.08	10.26
Boc–Lys–Lys–OBzl	84.0—86.3	−19.3	82	65.56	7.15	7.64	65.31	7.18	7.75
Boc–Val–Lys–Lys–OBzl	157.0—158.7	−32.0	77	64.96	7.39	8.42	65.01	7.11	8.64
Boc–Leu–Ala–OBzl	Oil	−48.4	76	64.26	8.22	7.14	63.97	8.15	7.05
Boc–Tyr–Leu–Ala–OBzl	163.5—165.0	−32.9	81	68.82	7.34	6.51	68.57	7.33	6.56
Boc–Leu–OBzl	Oil	−37.8	65	67.26	8.47	4.36	66.90	8.50	4.64
Boc–Val–Leu–OBzl	90.0—90.6	−55.2	57	65.69	8.63	6.66	65.70	8.61	6.87
Boc–Ala–Val–Leu–OBzl	Amorphous	−76.9	75	63.52	8.41	8.55	63.46	8.17	8.62
Boc–Gly–OBzl	73.4—74.6	0.0	54	63.38	7.22	5.28	63.58	7.21	5.50
Boc–Asp–Gly–OBzl	Oil	−12.7	79	62.32	7.41	6.06	62.20	7.39	6.30
Boc–Ser–Asp–Gly–OBzl	Oil	−7.6	74	63.83	7.09	6.57	63.53	7.19	6.46[b]
Boc–Asp–OBzl	65.8—66.3	−18.8	55	65.17	7.71	3.45	65.04	7.76	3.48
Boc–Ser–Asp–OBzl	83.0—83.8	−5.9	78	65.96	7.27	4.81	66.04	7.53	4.73
Boc–His–Ser–Asp–OBzl	125.0—126.0	−9.3	74	65.78	6.84	8.34	65.66	6.84	8.32

a) Ser, Asp, Thr, Arg, Lys, Tyr and His are protected with Bzl, OcHex, Bzl, Tos, Z, Bzl and Bom, respectively. *b*) Estimated water content, 0.5 mol eq.

Table 7. Molecular Optical Rotation Parameters of the Amino Acid Constituents (1—15) and Their Peptide Bonds (16—65) for the Tripeptide Derivatives (Boc–A3–A2–A1–OBzl)

Entry	Component[a]	Unit (°)	Entry	Component[a]	Unit (°)	Entry	Component[a]	Unit (°)	Entry	Component[a]	Unit (°)
1	3L	−56.445	18	3EL	−12.157	35	3RW	18.764	52	1SE	1.023
2	3S	41.987	19	3WL	27.266	36	3LR	−1.818	53	1EE	1.535
3	3E	−20.380	20	3RL	−4.830	37	3SR	−12.695	54	1WE	−11.492
4	3W	−32.875	21	3LS	−7.620	38	3ER	−10.502	55	1RE	12.012
5	3R	22.717	22	3SS	6.095	39	3WR	27.641	56	1LW	−7.879
6	2L	−129.840	23	3ES	−7.393	40	3RR	−5.327	57	1SW	3.524
7	2S	17.833	24	3WS	12.967	41	1LL	−6.473	58	1EW	15.918
8	2E	−64.338	25	3RS	−11.206	42	1SL	4.411	59	1WW	−17.070
9	2W	−99.617	26	3LE	−9.150	43	1EL	−1.579	60	1RW	−4.796
10	2R	−30.957	27	3SE	−7.537	44	1WL	−0.654	61	1LR	9.782
11	1L	−164.430	28	3EE	−9.705	45	1RL	4.050	62	1SR	−12.695
12	1S	−75.133	29	3WE	30.628	46	1LS	−1.460	63	1ER	−3.342
13	1E	−122.420	30	3RE	−2.172	47	1SS	−1.265	64	1WR	3.601
14	1W	−55.346	31	3LW	25.921	48	1ES	−10.713	65	1RR	0.913
15	1R	−95.858	32	3SW	5.524	49	1WS	22.807			
16	3LL	−14.113	33	3EW	40.598	50	1RS	−15.966			
17	3SL	3.291	34	3WW	−101.710	51	1LE	−0.110			

a) For example, 3L, 3SL and 1SL represent A3=Leu, A3–A2=Ser–Leu and A2–A1=Ser–Leu, respectively, in the expression Boc–A3–A2–A1–OBzl. Calculation of the molecular optical rotation value of Boc–R–L–S–OBzl is as follows: 1S (−75.133)+2L (−129.840)+3R (22.717)+3RL (−4.830)+1LS (−1.460)=−188.6 (*cf.* obs.=−190.6).

acid (0.1 ml) was added and the mixture was heated at 110 °C for 24 h. After evaporation of the solvent, the residue was treated with distilled water (0.2 ml) to obtain a sample for analysis. Amino acid analysis for each sample was carried out with a Hitachi HPLC system (L-6200) with standard D-tryptophan (retention time =47.8 min) and L-tryptophan (retention time =43.7 min) under the following conditions: column, SUMICHIRAL OA-5000; column size, 4.6×150 mm; column temperature, 40 °C; mobile phase, 3 mM copper sulfate aqueous solution

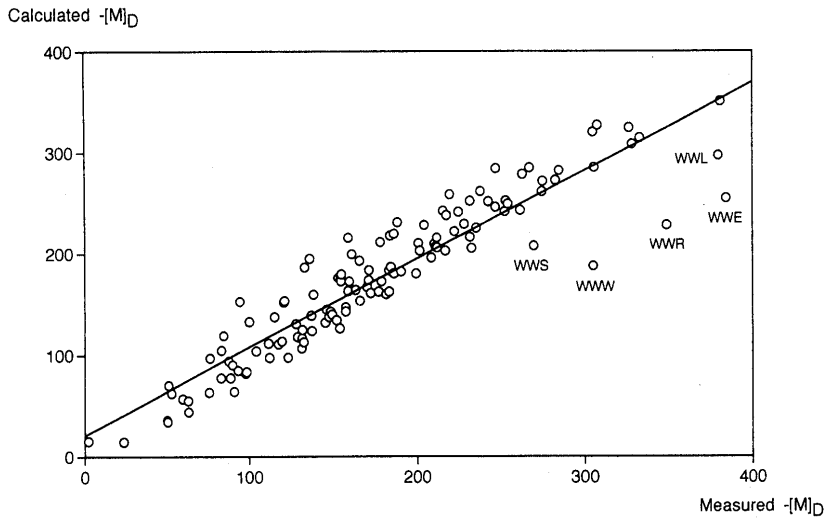

Fig. 4. Relationship between Measured and Calculated Molecular Rotations for Tripeptide Derivatives, Boc–A3–A2–A1–OBzl
$[M]_D^{cal.} = [M]_{Boc\text{-}A3\text{-}} + [M]_{\text{-}A2\text{-}} + [M]_{\text{-}A1\text{-}OBzl.}$ $y = 0.8705x + 21.2$, $r = 0.9297$, $\sigma = 29.1$, $P = 15$.

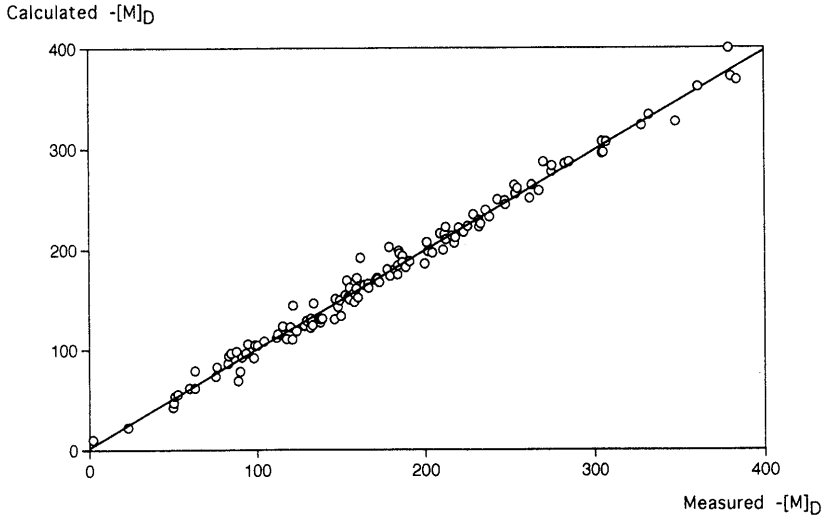

Fig. 5. Relationship between Measured and Calculate Molecular Rotations for Tripeptide Derivatives, Boc-A3-A2-A1-Bzl
$[M]_D^{cal.} = [M]_{Boc\text{-}A3\text{-}} + [M]_{\text{-}A2\text{-}} + [M]_{\text{-}A1\text{-}OBzl} + [M]_{Bond}$. $y = 0.9802x + 3.85$, $r = 0.9936$, $\sigma = 8.86$, $P = 65$.

containing 3% MeOH; flow rate, 1.0 ml/min; analytical wavelength, UV (254 nm); sample injection volume, 10 μl. The content of tryptophan in the three tripeptide derivatives was always less than 1%.

Synthesis of Boc–Leu–OBzl The procedure for the benzylation reaction of Boc–Leu is described below as a typical example. Tables 1 and 2 list the subroutine program titles together with a brief description. Titles are given in square brackets below. Conditions for the benzylation reaction (reaction time, temperature, and other information) were input in the subroutine [START-1]. To the solution of Boc–Leu (4.3 g) in *N*,*N*-dimethylformamide (DMF) (10 ml) was added NEt_3 (1.0 g) in DMF (4 ml) with cooling and stirring [RF2-CL-ON, RF2-ST-ON, RR5-RF2]. To the above solution was added benzyl bromide (2.6 g) in DMF (3 ml) [RR6-RF2]. Cooling and stirring were stopped [RF2-CL-OF, RF2-ST-OF], and then the reaction mixture was stirred at room temperature for 5 h [RF2-REA-1]. Water (50 ml) and ethyl acetate (40 ml) were added to the solution with stirring [RF2-ST-ON, RS1-RF2, RS4-RF2], and the solution was stirred vigorously to extract the products [RF2-ST-OF, RF2-MIX]. The mixture was transferred to the separation funnel and the separated aqueous layer was re-extracted with ethyl acetate (40 ml). The combined organic layer was washed with water (40 ml × 2) and then transported to another reaction flask (RF1) through a drying tube filled with anhydrous sodium sulfate. The separation funnel and flow lines were washed with ethyl acetate (40 ml) and the washings were transferred to RF1 [EXT1(PS-EXT1)]. The HPLC pump and the detector were switched on [DE-CO-ON], and then the ethyl acetate was evaporated under reduced pressure [RF1-CON-1]. The residue was dissolved in CH_2Cl_2 (10 ml × 2) and transferred to the reservoir for chromatography [RF1-ST-ON, RR1-RF1, MATU, RF1-ST-OF, RF1-SR3, RF1-ST-ON, RR2-RF1, MATU, RF1-ST-OF, RF1-SR3]. The solution was automatically injected onto a silica gel chromatography column, and the eluates (eluent, CH_2Cl_2) were collected using a fraction collector [HPLC]. After switching off the HPLC pump and the detector [DE-CO-OF], the reaction flasks and lines were washed and dried (subroutines following [WASH] and then [END]). The fractions were taken out of the apparatus, and the eluates were concentrated to afford Boc–Leu–OBzl (86%). *Anal.* Calcd for $C_{18}H_{27}NO_4$: C, 67.26; H, 8.48; N, 4.36. Found : C, 67.28; H, 8.45; N, 4.48. $[\alpha]_D$ −37.8° (MeOH, $c = 1.205$, 20 °C). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.923 and 0.915 (each 3H, each d, $J = 6.4$ Hz, Me), 1.43 (9H, s, *tert*-Bu), 1.49 and 1.61 (2H, each m, CH_2), 1.68 (1H, m, –CH–), 4.36 (1H, br dt, $J = 8.3$, 5.1 Hz, –CH–), 4.94 (1H, d, $J = 8.3$ Hz, –NH–), 5.13 and 5.18 (2H, each d, $J = 12.6$ Hz, CH_2), 7.29—7.38 (5H, m, aromatic-H).

General Procedure of Peptide Bond Formation The procedure for peptide bond formation between Boc–Glu(OcHex) and Boc-Leu-OBzl is described as a typical example. Tables 1 and 2 list the subroutine program titles with a brief description. Conditions for the reaction (reaction time, temperature, and other information) were input in the subroutine [START-1]. In the reaction flask 1 (RF1), Boc–Glu(OcHex)

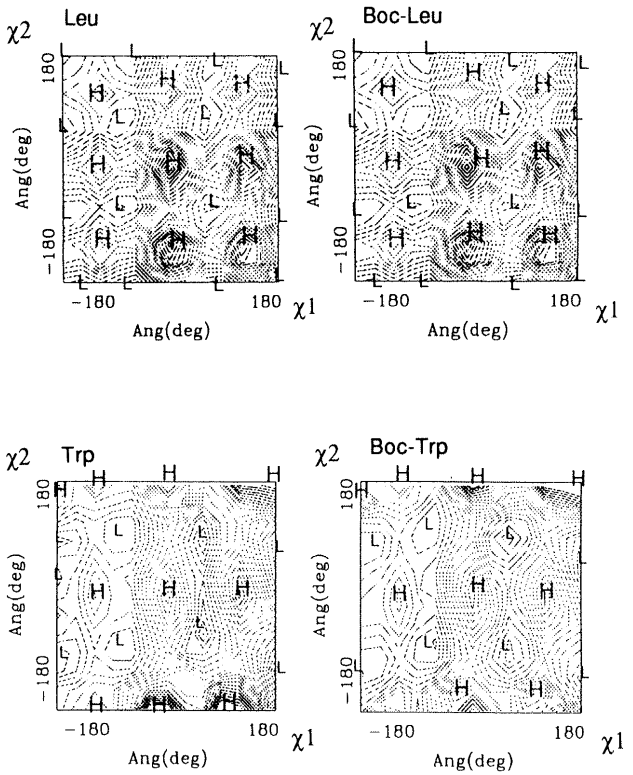

Fig. 6. Energy Contour Maps for Unprotected and Boc-Protected Leucine and Tryptophan

(1.65 g) and *N*-hydroxy-5-norbornene-2,3-dicarboximide (HONB, 0.92 g) were dissolved in CH_3CN–DMF (15 ml–5 ml) at room temperature [RF1-ST-ON, ALARM]. The solution was cooled to 0 °C [RF1-CL-ON], and then WSCD·HCl (1.0 g) was added manually at 0 °C. The solution was stirred at 0 °C for 1 h [RF1-ST-OF, ALARM, RF1-CL-OF, RF1-REA-1]. As the stirring of the solution in RF1 was continued [RF1-ST-ON], trifluoroacetic acid (TFA, 15 ml) was added to the solution of Boc–Leu–OBzl (1.6 g) in ethyl acetate (5 ml) in reaction flask 2 (RF2) at 0 °C [RF2-CL-ON, ALARM]. Then the reaction mixtures in RF2 and RF1 were stirred at room temperature and 0°C, respectively [RF2-CL-OF, RF1-CL-ON]. After 1 h, TFA in RF2 was evaporated under reduced pressure at 40 °C [RF2-CON-1]. The residue was dissolved in CH_2Cl_2 (50 ml) [RF2-ST-ON, RS5-RF2], 5% aqueous $NaHCO_3$ (40 ml) was added [RS2-RF2], and the solution was stirred vigorously [RF2-ST-OF, RF2-MIX]. The mixture was transferred to the separation funnel and the separated aqueous layer was re-extracted with CH_2Cl_2 (40 ml). The combined organic layer was washed with water (40 ml) and then transported to another reaction flask (RF3) through a drying tube filled with anhydrous sodium sulfate. The separation funnel and flow lines were washed with CH_2Cl_2 (40 ml) and the washings transferred to RF3 [EXT1(RF2-XL-RF3)]. The solvent was evaporated under reduced pressure at 40 °C [RF3-CON-2], and the residue was dissolved in CH_3CN (10 ml) [RF3-ST-ON, RS3-RF3]. The solution in RF1 was then transferred to RF3 [RF1-CL-OF, RF1-ST-OF, RF1-RF3], followed by washing with CH3CN (10ml) [RS3-RF1, RF1-MIX, RF1-RF3]. As the reaction mixture was stirred at room temperature, RF1 was washed and dried [WS-RF1, RF1-BUBB, RF1-SF, SF-BUBB, SF-DR, RF1-DRY]. The reaction mixture in RF3 was stirred for another 10 h at 30 °C [RF3-ST-OF, RF3-REA-3]. The excess solvent was removed under reduced pressure at 40 °C [RF3-CON-3], and the residue was dissolved in ethyl acetate (50 ml) [RF3-ST-ON, RS4-RF3]. *N,N*-Diisopropylethylenediamine (0.75 g) in ethyl acetate (10 ml) was added to the solution in RF3 at room temperature [RR7-RF3], and the mixture was stirred for a period from 15 min to several hours [ALARM]. A 5% aqueous $NaHCO_3$ solution (40 ml) was added [RS2-RF3], and then the mixture was transferred to the separation funnel and the separated organic layer was washed with 0.2 N HCl (40 ml) followed by water (40 ml). The organic layer was transported to RF1 through a drying tube filled with anhydrous sodium sulfate, and then the separation funnel and flow lines were washed with ethyl acetate (40 ml), which was transferred to RF1 [EXT2(PEP-EXT2)]. The solution in RF1 was kept under cooling until removed manually [RF1-CL-ON, ALARM]. The reaction flasks and lines were washed and dried [all subroutines following WASH]. The excess solvent was evaporated, the residue was loaded onto a silica gel column (eluent, AcOEt/*n*-hexane = 3/1), and the eluates were concentrated to give crystals. Recrystallization from AcOEt/*n*-hexane (1/20) gave Boc–Glu(OcHex)–Leu–OBzl (87.0%) as white crystals, mp 58.0—58.5 °C. *Anal.* Calcd for $C_{29}H_{44}N_2O_7$: C, 65.39; H, 8.33; N, 5.26. Found : C, 65.46; H, 8.36; N, 5.14. $[\alpha]_D$ −33.6° (MeOH, c = 0.365, 20 °C). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.910 and 0.907 (each 3H, each d, J = 6.1 Hz, Me), 1.43 (9H, s, *tert*-Bu), 1.56 and 1.65 (2H, m, CH_2), 1.65 (1H, m, –CH–), 1.90 and 2.08 (2H, m, CH_2), 4.16 (1H, br dt, J = 7.1, 6.1 Hz, –CH–), 4.63 (1H, ddd, J = 7.3, 8.3, 4.9 Hz, –CH–), 4.77 (1H, m, –CH–cyclohexyl), 5.14 and 5.17 (2H, each d, J = 12.2 Hz, $-CH_2$–Ph), 5.24 (1H, d, J = 7.1 Hz, –NH–), 6.72 (1H, d, J = 7.3 Hz, –NH–), 7.30—7.39 (5H, m, aromatic-H). In the case of synthesizing oligopeptides containing tryptophan, alkylation of the side chain might occur in the deprotecting step using trifluoroacetic acid. However, all the products were isolated as pure materials (see Tables 3—6) by silica gel column chromatography.

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