Solution-Phase Automated Synthesis of Tripeptide Derivatives

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An improved general method for automated synthesis of tripeptides was developed, in which methanesulfonic acid (MSA) was used in place of trifluoroacetic acid (TFA), thus making it possible to avoid, 1) corrosion of the apparatus by strong acid vapor, 2) formation of emulsions, and 3) use of the restricted solvent, dichloromethane. As an application of the automated synthesis apparatus, 216 fragment tripeptide derivatives were synthesized systematically using the MSA method, in excellent yield and with increased efficiency.

Key words automated synthesis; solution-phase peptide synthesis; fragment tripeptide; methanesulfonic acid method; trifluoroacetic acid method

As combinatorial chemistry has been rapidly expanding for solution-phase as well as solid-phase synthesis, several kinds of automated apparatus for both synthesis strategies have been developed.[1)] When chemists want to synthesize large numbers of compounds efficiently using automated apparatus, it is necessary to carefully plan how to apply the apparatus. Most reaction procedures were originally developed assuming manual operations, and it might not be suitable to use them directly for an automated synthesis. For successful automation, it is important to develop new reaction processes that are specially suited to automated synthesis apparatus.[2)] Recently, new methodologies, such as those using scavenger resins,[3)] cation pools or phase tags,[4)] have been developed for automation, and solution-phase laboratory automation is likely to become more and more important as a research tool.

In our laboratories, we have developed automated synthesis systems that can utilize a variety of reaction procedures, including those that have been developed for manual operation. For example, solid reagents can be automatically added to a vessel using ASRA,[5)] or a centrifugal separatory funnel can be used to separate emulsions in NEW-TACOS.[6)] However, another approach to automation involves developing new reaction procedures specially for automated synthesis apparatus, and recent emphasis has been made on increasing throughput by parallelisation.

In a previous report,[7)] we described the solution-phase automated synthesis of fragment peptide derivatives using our unique automated synthesis system. It was used to synthesize systematically a library of all possible tripeptides (125 derivatives) from 5 different kinds of protected amino acids [*tert*-Butoxycarbonyl-Leu (Boc-Leu), Boc-Ser(Bzl), Boc-Glu(O-cHex), Boc-Trp, Boc-Arg(Tos)]. In this report, we describe the automated synthesis of fragment tripeptides, using a new methanesulfonic acid (MSA) method, that has improved compatibility with our automated synthesis apparatus.

Results and Discussion

a) Improved Method for General Synthesis of Tripeptides Automated synthesis of fragment tripeptides was first carried out following the trifluoroacetic acid (TFA) method as shown in Fig. 1.[7)] It is a good general method for manual synthesis, but from the view point of application to the automated apparatus, it was found to have several demerits. One of these was the necessity to use TFA for the deprotection of the Boc group, and its subsequent vacuum evaporation, which caused extensive damage to the solenoid valves and tubings, resulting in leakages and expensive maintenance. Another problem was the use of dichloromethane for liquid–liquid extraction. The use of dichloromethane is restricted as it is harmful to the environment, and in addition, its use can lead to further problems due to emulsion formation, which may stop the automated process due to blockages in the drying tubes or the inability to separate phases. Also the parallel processing of the deprotection of the Boc group and the activation of the carboxyl group caused some problems. If the activated ester was left for long periods (*e.g.* 3 h), it decomposed and low yields were obtained. In addition, manual operations were needed during the process, such as when it was necessary to manually restart the process after the activated ester had been made in one vessel, to acylate the amine, which was deprotected in an another vessel. Considering these problems, we planned to change the reagent for the deprotection of the Boc group, from TFA to MSA, which would also allow the subsequent liquid–liquid extraction step to be replaced by a simple stoichiometrical neutralization. Dilute MSA has been reported deprotection of the Boc group in liquid-phase fragment condensation[8)] and in solid-phase peptide elongation,[9)] so we focused on how to effectively use it in the automated apparatus.

First we investigated the reaction conditions, and found that four mol eq of MSA, relative to the starting Boc-amino acid derivative, was required for deprotection. Dichloromethane, diisopropylether, methyl ethyl ketone, or acetonitrile could be used as the reaction solvent, but acetonitrile was chosen as it could also be used for the following acylation process. Reaction at 40 °C for 1 h gave complete deprotection, and then excess MSA could be stoichiometrically neutralized with diisopropylethylamine. Our new MSA method was used for the automated synthesis of several tripeptides and the results were compared to those obtained using the TFA method (see Table 1). It was clear that the MSA method gave higher yields because of the simplicity and reliablity of the process. The complete MSA method, as shown in Chart 1, was then used to synthesize 216 fragment tripeptides.

b) Synthesis of 216 Fragment Tripeptides Figure 2 shows the flowchart of a peptide synthesis performed following the MSA method, and Table 2 shows the subroutine sequence of the program.[7)] We planned to synthesize fragment

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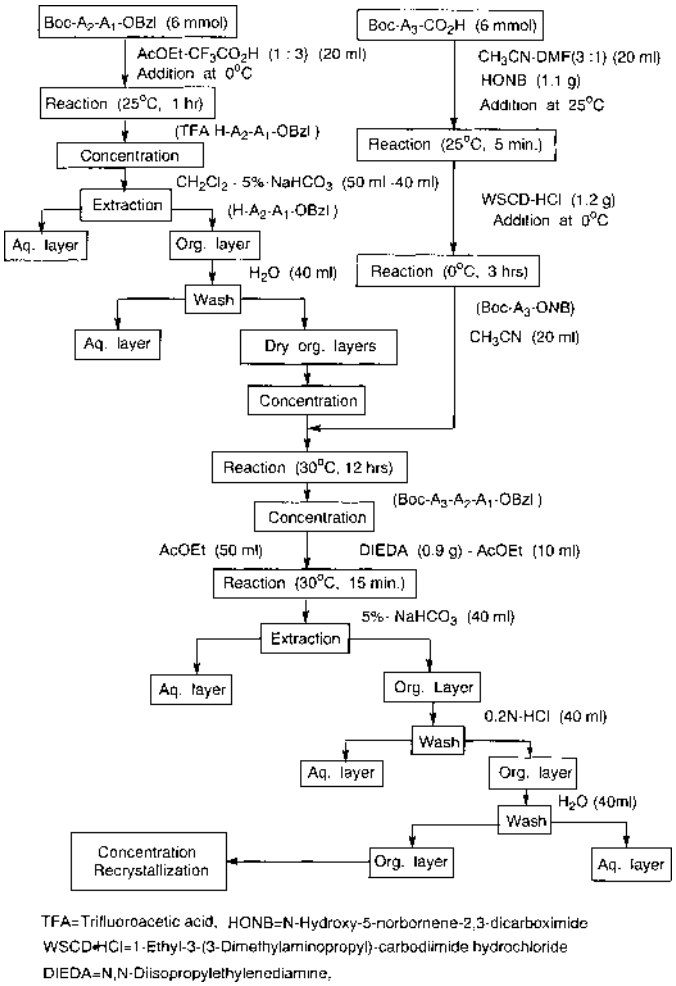


Fig. 1. Flowchart of a Peptide Synthesis by the TFA Method

Table 1. Comparison of Yields for the Synthesis of Tripeptide Derivatives (Boc-A_3–A_2–A_1-OBzl)

-A_3–A_2–A_1-[a)]	TFA method yield (%)	MSA method yield (%)
EEL	66	75
LRW	64	72
KWA	77	81
EWA	80	85
LES	71	92

a) A=Ala, E=Glu(O-cHex), L=Leu, R=Arg(Tos), W=Trp, K=Lys(Z).

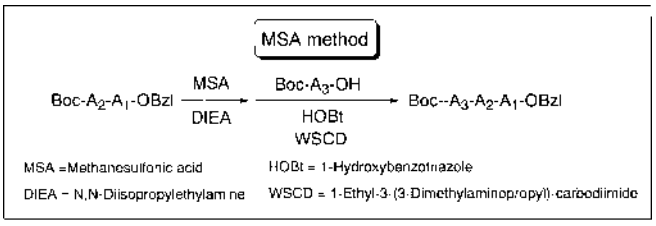


Chart 1. Schematic Diagram of the MSA Method

tripeptides which possessed a prolyl, tyrosyl or tryptophyl group in the center of the amino acid, and the other two amino acids were randomly selected from Boc-Ala, Boc-Leu, Boc-Ser(Bzl), Boc-Glu(O-cHex), Boc-Pro, Boc-Lys(Z), Boc-Tyr(Bzl), Boc-Trp, Boc-Arg(Tos), Boc-His(Bom). All yields are shown in Table 3, and physical data is shown in Table 4.

In the series of tripeptide derivatives with a prolyl group in the center, *cis–trans* isomers, relative to the proline ring, were obtained, especially when the prolyl group was between two bulky amino acid residues, such as Tyr(Bzl) and Glu(O-cHex). ^{1}H- and ^{13}C-NMR spectra were measured, and the cross coupling peaks between the major and minor components were observed in nuclear Overhauser effect spectroscopy (NOESY) spectra (Tables 5, 6). In the case of the tripeptide derivatives containing a histidyl group, extremely low isolated yields were observed because of partial salt formation during work-up with 0.2 N HCl, as shown in Fig. 2. Therefore the work-up procedure was changed for the synthesis of later derivatives (*cf.* Table 3, APH-RPH, HPW, HPR, HKA-HKR) to increase the yields. The series of tripeptides which possess a tyrosyl group in the center were found to have poor solubility, especially for the lysyl–tyrosyl derivatives, which were poorly soluble even in dimethylformamide (DMF). Precipitates were often formed during synthesis, and these caused considerable trouble in the automated synthesis apparatus, but the problem was overcome by dissolving the DIEA reagent in a greater volume of DMF. In tripeptide derivatives having a tryptophyl group, other than at the N-terminal position, a substituted indole by-product (5—10%) was formed during removal of the Boc group. Anisole, thioanisole, or *m*-cresol was added to the reaction mixture to

Table 2. Subroutine Sequence for the MSA Method

Program name MSA1				
1. START	2. RF1-ST-ON	3. RR2-RF1	4. RF1-LF-UP	5. REA1 (40c, 60 m)
6. RF1-T-ON (0c)	7. RR3-RF1	8. RF1-T-OF	9. RF2-LF-UP	10. RF2-T-ON (0c)
11. RF2-ST-ON	12. RF1-RF2	13. RS6-RF1	14. RF1-MIX	15. RF1-RF2
16. RF1-ST-OF	17. RF1-LF-DN	18. RR4-RF2	19. RF2-T-OF	20. REA2 (25c, 600 m)
21. CON2 (40c, 60 m)	22. RF2-LF-DN	23. RS4-RF2	24. RS2-RF2	25. RF2-MIX
26. BKEXT	27. RF2-ST-OF	28. ALARM	29. SR1-DR	30. BKWASH
31. BKWASH	32. RF1-LF-UP	33. RF2-LF-UP	34. RF1-DRY	35. RF2-DRY
36. RF1-LF-DN	37. RF2-LF-DN	38. END		
BKEXT				
1. BKSTART	2. RF2-SF	3. SEP-SR1	4. RS3-RF2	5. RF2-MIX
6. RF2-SF	7. SF-BUBB	8. SEP-SR0	9. RS1-RF2	10. RF2-MIX
11. RF2-SF	12. SF-BUBB	13. SEP-SR1	14. SF-RF3	15. RS4-RF2
16. RF2-MIX	17. RF2-SF	18. SF-BUBB	19. SF-RF3	20. SR0-DR
21. BKEND				
BKWASH				
1. BKSTART	2. WS-RR2	3. WS-RR3	4. WS-RR4	5. WS-RF1
6. WS-RF2	7. RF1-BUBB	8. RF2-BUBB	9. RF2-SF	10. SF-BUBB
11. SF-SR1	12. SR1-BUBB	13. SR1-DR	14. RF1-RF2	15. RF2-SF
16. SF-RF3	17. BKEND			

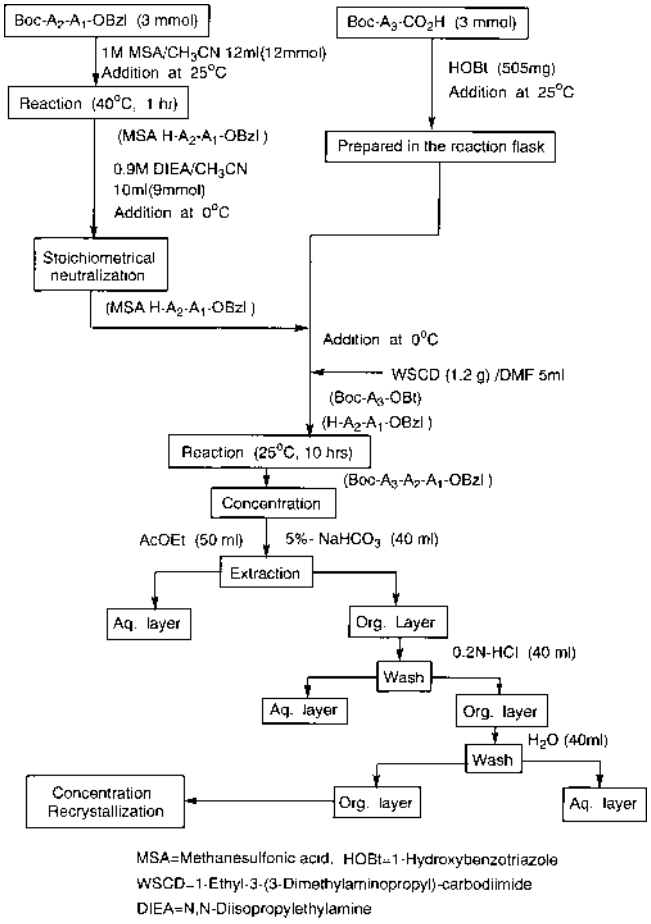


Fig. 2. Flowchart of a Peptide Synthesis by the MSA Method

prevent by-product formation for the synthesis of *SYW-HYW* tripeptides. Typically, yields were increased by the addition of *m*-cresol from an average 64.5% to 74.7%. (*cf.* Table 3, tripeptides in italics.) Acid-labile side chain deprotection reactions, such as those of benzyl or benzyloxycarbonyl protecting groups by MSA,[8,9] were checked by measuring representative NMR spectra of the separated tripeptides. Elemental analysis (Table 4) and ^{1}H-NMR (Table 7) showed that no deprotected byproducts were formed for the tripeptides containing Lys(Z) or Ser(Bzl) or Tyr(Bzl) residues. The advantages of the MSA method, found during the synthesis of the tripeptide series, are summarized in Table 8.

Table 3. PercentageYields of Tripeptides

	LPA	SPA	EPA	KPA	YPA	WPA	RPA	HPA
	54	77	61	71	78	47	57	40
APL		SPL	EPL	KPL	YPL	WPL	RPL	HPL
92		100	94	57	86	81	79	38
APS	LPS		EPS	KPS	YPS	WPS	RPS	HPS
84	83		83	68	84	83	81	24
APE	LPE	SPE		KPE	YPE	WPE	RPE	HPE
74	89	94		84	73	93	85	33
APK	LPK	SPK	EPK		YPK	WPK	RPK	HPK
74	84	91	82		31	63	63	29
APY	LPY	SPY	EPY	KPY		WPY	RPY	HPY
59	51	37	52	86		82	77	34
APW	LPW	SPW	EPW	KPW	YPW		RPW	HPW
47	71	77	59	28	42		64	55
APR	LPR	SPR	EPR	KPR	YPR	WPR		HPR
86	84	82	64	85	80	38		52
APH	LPH	SPH	EPH	KPH	YPH	WPH	RPH	
79	84	64	73	72	87	79	69	

	LKA	SKA	EKA	PKA	YKA	WKA	RKA	HKA
	95	91	85	83	84	86	78	89
AKL		SKL	EKL	PKL	YKL	WKL	RKL	HKL
87		91	83	64	86	71	91	69
AKS	LKS		EKS	PKS	YKS	WKS	RKS	HKS
84	88		75	77	77	81	84	64
AKE	LKE	SKE		PKE	YKE	WKE	RKE	HKE
95	88	76		68	70	84	89	55
AKP	LKP	SKP	EKP		YKP	WKP	RKP	HKP
80	77	85	86		80	89	60	80
AKY	LKY	SKY	EKY	PKY		WKY	RKY	HKY
92	90	97	88	84		89	87	86
AKW	LKW	SKW	EKW	PKW	YKW		RKW	HKW
55	66	71	61	75	80		86	79
AKR	LKR	SKR	EKR	PKR	YKR	WKR		HKR
86	89	82	76	48	85	80		68
AKH	LKH	SKH	EKH	PKH	YKH	WKH	RKH	
85	51	70	83	91	86	82	76	

	LYA	SYA	EYA	PYA	KYA	WYA	RYA	HYA
	85	81	85	85	84	80	98	84
AYL		SYL	EYL	PYL	KYL	WYL	RYL	HYL
95		93	80	93	86	80	92	85
AYS	LYS		EYS	PYS	KYS	WYS	RYS	HYS
90	87		91	82	84	88	96	72
AYE	LYE	SYE		PYE	KYE	WYE	RYE	HYE
74	72	85		92	38	76	75	98
AYP	LYP	SYP	EYP		KYP	WYP	RYP	HYP
80	93	89	72		64	100	83	81
AYK	LYK	SYK	EYK	PYK		WYK	RYK	HYK
91	76	75	85	87		94	60	81
AYW	LYW	*SYW*	*EYW*	*PYW*	*KYW*		*RYW*	*HYW*
77	68	80	82	77	78		77	54
AYR	LYR	SYR	EYR	PYR	KYR	WYR		HYR
77	89	86	64	81	92	90		83
AYH	LYH	SYH	EYH	PYH	KYH	WYH	RYH	
76	70	85	79	69	70	82	85	

Table 4. Physical Data of Tripeptide Derivatives (Boc-A_3–A_2–A_1-OBzl)

A_3–A_2–A_1	mp (°C)	$[\alpha]_D$	*Anal.* Calcd (Found)
Glu–Pro–Ala	Amorphous	−85.1	C; 62.87 (62.84) H; 7.74 (7.89) N; 7.10 (6.99)
His–Pro–Ala	Amorphous	−71.2	C; 63.54 (63.40) H; 6.90 (6.97) N; 10.90 (11.03)
Lys–Pro–Ala	Amorphous	−78.6	C; 63.49 (63.32) H; 7.29 (7.50) N; 8.71 (8.52)
Leu–Pro–Ala	Amorphous	−118.3	C; 63.78 (63.69) H; 8.03 (8.09) N; 8.58 (8.63)
Arg–Pro–Ala	Amorphous	−63.1	C; 56.60 (56.42) H; 6.84 (6.44) N; 11.92 (11.74)
Ser–Pro–Ala	Oil	−71.7	C; 65.08 (64.88) H; 7.10 (7.29) N; 7.59 (7.54)
Trp–Pro–Ala	217.0—218.0	−75.3	C; 65.65 (65.85) H; 6.84 (6.82) N; 9.88 (9.95)
Tyr–Pro–Ala	73.0—75.0	−56	C; 68.66 (68.69) H; 6.88 (6.73) N; 6.67 (6.76)
Ala–Pro–Glu	Amorphous	−86.9	C; 62.87 (62.81) H; 7.74 (7.75) N; 7.10 (6.99)
His–Pro–Glu	Amorphous	−49.3	C; 64.43 (64.67) H; 7.21 (7.21) N; 8.95 (8.98)
Lys–Pro–Glu	Oil	−52.7	C; 64.76 (64.62) H; 7.51 (7.58) N; 7.19 (7.22)
Leu–Pro–Glu	Oil	−80.8	C; 63.93 (64.05) H; 8.21 (8.27) N; 6.58 (6.42)
Arg–Pro–Glu	Amorphous	−43.1	C; 59.55 (59.65) H; 7.07 (7.18) N; 10.16 (10.13)
Ser–Pro–Glu	Oil	−47.2	C; 64.94 (64.96) H; 7.46 (7.55) N; 5.98 (6.01)
Trp–Pro–Glu	Amorphous	−62	C; 66.31 (66.27) H; 7.19 (7.13) N; 7.93 (8.03)
Tyr–Pro–Glu	Amorphous	−35.6	C; 68.64 (68.52) H; 7.20 (7.11) N; 5.46 (5.68)
Ala–Pro–His	Amorphous	−73.4	C; 63.69 (63.54) H; 6.86 (6.90) N; 10.86 (10.90)
Glu–Pro–His	Amorphous	−47.4	C; 63.67 (63.85) H; 7.10 (7.18) N; 9.05 (8.93)
Lys–Pro–His	Amorphous	−42.3	C; 63.78 (63.79) H; 6.96 (6.98) N; 9.92 (10.19)
Leu–Pro–His	Amorphous	−66.4	C; 65.14 (65.09) H; 7.44 (7.35) N; 10.55 (10.43)
Arg–Pro–His	149.0—151.0	−36.5	C; 60.53 (60.23) H; 6.47 (6.64) N; 12.83 (12.80)
Ser–Pro–His	Amorphous	−41.8	C; 65.76 (65.85) H; 6.73 (6.61) N; 9.35 (9.29)
Trp–Pro–His	Amorphous	−59.8	C; 65.02 (64.73) H; 6.63 (6.25) N; 10.83 (10.73)
Tyr–Pro–His	Amorphous	−31.8	C; 68.73 (68.79) H; 6.58 (6.45) N; 8.53 (8.60)
Ala–Pro–Lys	92.0—93.0	−81.2	C; 63.93 (63.76) H; 7.26 (7.26) N; 8.77 (8.78)
Glu–Pro–Lys	Amorphous	−52.5	C; 64.76 (64.40) H; 7.51 (7.51) N; 7.19 (7.13)
His–Pro–Lys	Amorphous	−44	C; 64.81 (64.53) H; 6.89 (6.94) N; 10.08 (10.12)
Leu–Pro–Lys	Amorphous	−70.6	C; 65.27 (64.97) H; 7.70 (7.74) N; 8.23 (8.45)
Arg–Pro–Lys	Amorphous	−39.1	C; 59.88 (59.84) H; 6.80 (7.05) N; 11.11 (10.90)
Ser–Pro–Lys	92.0—93.0	−44.5	C; 66.11 (66.25) H; 7.04 (6.86) N; 7.52 (7.72)
Trp–Pro–Lys	117.0—118.0	−53.8	C; 66.91 (66.85) H; 6.82 (6.84) N; 9.29 (9.34)
Tyr–Pro–Lys	138.0—139.0	−30.5	C; 68.76 (68.65) H; 6.88 (6.95) N; 6.82 (6.86)
Ala–Pro–Leu	Oil	−115.5	C; 63.32 (63.37) H; 8.05 (8.12) N; 8.52 (8.54)
Glu–Pro–Leu	Oil	−77.7	C; 64.29 (64.23) H; 8.19 (8.21) N; 6.62 (6.53)
His–Pro–Leu	Amorphous	−66.4	C; 64.89 (64.71) H; 7.36 (7.30) N; 10.23 (10.25)
Lys–Pro–Leu	Amorphous	−71.4	C; 64.84 (64.72) H; 7.72 (7.79) N; 8.18 (8.19)
Arg–Pro–Leu	Amorphous	−58.2	C; 59.32 (59.08) H; 7.19 (7.44) N; 11.53 (11.34)
Ser–Pro–Leu	Oil	−66.4	C; 66.03 (65.99) H; 7.64 (7.66) N; 7.00 (6.89)
Trp–Pro–Leu	173.0—175.0	−82.3	C; 67.53 (67.55) H; 7.33 (7.19) N; 9.26 (9.40)
Tyr–Pro–Leu	Oil	−52.2	C; 69.26 (69.16) H; 7.38 (7.11) N; 6.21 (6.27)
Ala–Pro–Arg	Amorphous	−70.5	C; 56.96 (57.04) H; 6.81 (6.92) N; 12.08 (11.92)
Glu–Pro–Arg	Amorphous	−44.3	C; 58.90 (59.14) H; 7.11 (7.17) N; 10.05 (10.27)
His–Pro–Arg	Amorphous	−36.1	C; 58.72 (58.98) H; 6.61 (6.56) N; 12.45 (12.19)
Lys–Pro–Arg	Amorphous	−42.4	C; 59.58 (59.49) H; 6.82 (6.73) N; 11.05 (10.96)
Leu–Pro–Arg	Amorphous	−60.6	C; 58.60 (58.86) H; 7.24 (7.47) N; 11.39 (11.54)
Ser–Pro–Arg	Amorphous	−37	C; 59.91 (59.95) H; 6.66 (6.75) N; 10.48 (10.54)
Trp–Pro–Arg	Amorphous	−46.5	C; 60.06 (60.00) H; 6.51 (6.44) N; 11.96 (11.57)
Tyr–Pro–Arg	Amorphous	−27.1	C; 62.92 (62.71) H; 6.54 (6.31) N; 9.57 (9.54)
Ala–Pro–Ser	Oil	−86.4	C; 64.04 (63.82) H; 7.17 (7.10) N; 7.47 (7.39)
Glu–Pro–Ser	Oil	−57.2	C; 64.94 (65.01) H; 7.46 (7.43) N; 5.98 (5.88)
His–Pro–Ser	Amorphous	−46	C; 64.98 (65.06) H; 6.78 (6.62) N; 9.24 (9.26)
Lys–Pro–Ser	94.0—95.0	−54.2	C; 66.11 (66.05) H; 7.04 (7.09) N; 7.52 (7.55)
Leu–Pro–Ser	Oil	−76	C; 65.54 (65.56) H; 7.67 (7.58) N; 6.95 (6.97)
Arg–Pro–Ser	Oil	−42.2	C; 60.59 (60.37) H; 6.61 (6.65) N; 10.60 (10.52)
Trp–Pro–Ser	Amorphous	−50.7	C; 67.79 (67.86) H; 6.66 (6.70) N; 8.32 (8.26)
Tyr–Pro–Ser	Oil	−31.8	C; 69.34 (69.54) H; 6.77 (6.78) N; 5.64 (5.83)
Ala–Pro–Trp	Amorphous	−75.1	C; 65.65 (65.87) H; 6.84 (7.07) N; 9.88 (9.60)
Glu–Pro–Trp	Amorphous	−49.4	C; 66.65 (66.64) H; 7.17 (7.39) N; 7.97 (7.95)
His–Pro–Trp	Amorphous	−36.2	C; 67.36 (67.24) H; 6.46 (6.63) N; 11.22 (10.94)
Lys–Pro–Trp	Amorphous	−47.3	C; 66.52 (66.57) H; 6.84 (6.81) N; 9.23 (9.27)
Leu–Pro–Trp	Amorphous	−65.6	C; 67.03 (67.01) H; 7.36 (7.57) N; 9.20 (9.07)
Arg–Pro–Trp	Amorphous	−34.6	C; 60.06 (60.28) H; 6.52 (6.22) N; 11.96 (11.66)
Ser–Pro–Trp	Amorphous	−41.9	C; 67.79 (67.86) H; 6.66 (6.72) N; 8.32 (8.36)
Tyr–Pro–Trp	Amorphous	−25.9	C; 70.10 (69.84) H; 6.55 (6.71) N; 7.43 (7.39)
Ala–Pro–Tyr	84.0—86.0	−70.3	C; 68.66 (68.48) H; 6.88 (6.71) N; 6.67 (6.75)
Glu–Pro–Tyr	66.0—68.0	−46.6	C; 68.64 (68.75) H; 7.20 (7.30) N; 5.46 (5.39)
His–Pro–Tyr	Amorphous	−33.1	C; 68.80 (68.74) H; 6.57 (6.53) N; 8.54 (8.55)
Lys–Pro–Tyr	131.0—132.0	−41.9	C; 68.76 (68.52) H; 6.88 (6.96) N; 6.82 (6.93)
Leu–Pro–Tyr	57.0—58.0	−62.4	C; 69.72 (69.64) H; 7.35 (7.21) N; 6.25 (6.37)
Arg–Pro–Tyr	Amorphous	−32.4	C; 63.58 (63.51) H; 6.49 (6.69) N; 9.67 (9.61)
Ser–Pro–Tyr	75.0—77.0	−39.8	C; 70.18 (70.35) H; 6.71 (6.42) N; 5.71 (5.62)
Trp–Pro–Tyr	Amorphous	−47.9	C; 70.52 (70.58) H; 6.52 (6.39) N; 7.48 (7.48)
Glu–Lys–Ala	84.0—86.0	−33.1	C; 63.81 (63.68) H; 7.50 (7.53) N; 7.44 (7.59)

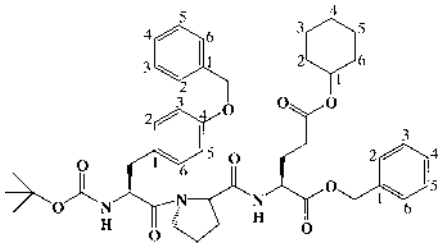
Table 4. (Continued.)

A_3–A_2–A_1	mp (°C)	$[\alpha]_D$	*Anal.* Calcd (Found)
His–Lys–Ala	Amorphous	−28.7	C; 64.65 (64.45) H; 6.81 (6.93) N; 10.52 (10.71)
Leu–Lys–Ala	161.0—162.0	−44	C; 64.20 (64.12) H; 7.70 (7.46) N; 8.56 (8.80)
Pro–Lys–Ala	73.0—75.0	−63	C; 63.93 (63.72) H; 7.26 (7.38) N; 8.77 (8.73)
Arg–Lys–Ala	Amorphous	−24.4	C; 58.59 (58.78) H; 6.79 (6.97) N; 11.39 (11.56)
Ser–Lys–Ala	126.0—128.0	−25.4	C; 65.16 (65.13) H; 7.01 (6.86) N; 7.79 (7.88)
Trp–Lys–Ala	107.5—108.5	−32.7	C; 66.01 (65.82) H; 6.79 (6.81) N; 9.62 (9.74)
Tyr–Lys–Ala	125.0—127.0	−20.2	C; 67.99 (67.80) H; 6.85 (6.78) N; 7.05 (7.04)
Ala–Lys–Glu	85.0—87.0	−33.8	C; 63.81 (63.63) H; 7.50 (7.40) N; 7.44 (7.52)
His–Lys–Glu	129.0—130.0	−20.8	C; 65.03 (65.23) H; 7.34 (7.08) N; 8.75 (8.95)
Leu–Lys–Glu	129.0—130.0	−32.3	C; 64.97 (64.96) H; 7.86 (7.89) N; 7.05 (7.22)
Pro–Lys–Glu	97.0—98.0	−49.5	C; 64.76 (64.73) H; 7.51 (7.50) N; 7.19 (7.22)
Arg–Lys–Glu	Amorphous	−16.4	C; 59.98 (59.81) H; 7.05 (7.25) N; 9.79 (10.12)
Ser–Lys–Glu	Amorphous	−17.5	C; 65.54 (65.72) H; 7.16 (7.27) N; 6.71 (6.52)
Trp–Lys–Glu	136.0—138.0	−22.8	C; 66.42 (66.23) H; 7.08 (6.88) N; 8.07 (8.14)
Tyr–Lys–Glu	139.0—140.0	−12.6	C; 68.07 (67.84) H; 7.11 (6.91) N; 5.99 (6.07)
Ala–Lys–His	Amorphous	−28.7	C; 63.75 (63.88) H; 6.89 (7.16) N; 10.34 (10.53)
Glu–Lys–His	Amorphous	−18.2	C; 64.00 (63.99) H; 7.16 (7.20) N; 8.78 (8.75)
Leu–Lys–His	Amorphous	−24.2	C; 64.32 (64.48) H; 7.27 (7.29) N; 9.78 (9.75)
Pro–Lys–His	Amorphous	−39.1	C; 64.12 (64.21) H; 6.94 (7.21) N; 9.97 (9.95)
Arg–Lys–His	Amorphous	−18.5	C; 61.31 (60.79) H; 6.50 (6.55) N; 12.14 (12.04)
Ser–Lys–His	87.0—88.0	−16.7	C; 64.43 (64.57) H; 6.81 (6.69) N; 9.02 (9.32)
Trp–Lys–His	163.0—165.0	−18	C; 66.69 (66.65) H; 6.53 (6.56) N; 10.67 (10.69)
Tyr–Lys–His	139.0—140.0	−8.9	C; 66.72 (66.89) H; 6.70 (6.58) N; 8.34 (8.57)
Ala–Lys–Leu	147.0—149.0	−46.4	C; 64.20 (64.06) H; 7.70 (7.66) N; 8.56 (8.47)
Glu–Lys–Leu	113.0—114.0	−35	C; 64.97 (64.79) H; 7.86 (7.74) N; 7.05 (7.09)
His–Lys–Leu	98.0—100.0	−25.9	C; 65.35 (65.34) H; 7.21 (7.22) N; 9.94 (10.02)
Pro–Lys–Leu	Amorphous	−61.6	C; 64.42 (64.45) H; 7.74 (7.78) N; 8.12 (8.07)
Arg–Lys–Leu	Amorphous	−24.5	C; 59.78 (59.85) H; 7.12 (7.14) N; 11.05 (10.86)
Ser–Lys–Leu	110.0—113.0	−25	C; 66.30 (66.24) H; 7.42 (7.37) N; 7.36 (7.38)
Trp–Lys–Leu	143.0—144.0	−35.2	C; 67.08 (67.00) H; 7.20 (7.19) N; 9.10 (9.11)
Tyr–Lys–Leu	123.0—125.0	−21.5	C; 68.88 (68.63) H; 7.23 (7.10) N; 6.69 (6.77)
Ala–Lys–Pro	Amorphous	−70.8	C; 63.45 (63.49) H; 7.10 (7.46) N; 8.97 (8.81)
Glu–Lys–Pro	Amorphous	−55.2	C; 64.02 (64.16) H; 7.55 (7.52) N; 7.11 (6.97)
His–Lys–Pro	Amorphous	−45.7	C; 64.12 (63.99) H; 6.94 (6.93) N; 9.97 (9.81)
Leu–Lys–Pro	Amorphous	−68.8	C; 64.84 (64.71) H; 7.72 (7.74) N; 8.18 (8.04)
Arg–Lys–Pro	Amorphous	−42.6	C; 59.28 (59.18) H; 6.84 (6.81) N; 11.00 (10.87)
Ser–Lys–Pro	Amorphous	−52.6	C; 66.11 (65.82) H; 7.04 (7.18) N; 7.52 (7.58)
Trp–Lys–Pro	Amorphous	−53.5	C; 66.52 (66.34) H; 6.84 (6.85) N; 9.23 (9.33)
Tyr–Lys–Pro	Amorphous	−37.7	C; 68.39 (68.17) H; 6.90 (6.82) N; 6.79 (6.90)
Ala–Lys–Arg	Amorphous	−28.8	C; 58.59 (58.88) H; 6.79 (6.57) N; 11.39 (11.44)
Glu–Lys–Arg	Amorphous	−19.4	C; 59.98 (60.11) H; 7.05 (7.06) N; 9.79 (9.90)
His–Lys–Arg	134.0—136.0	−15.6	C; 61.31 (61.05) H; 6.50 (6.31) N; 12.14 (12.43)
Leu–Lys–Arg	Amorphous	−25.4	C; 59.85 (60.10) H; 7.14 (6.99) N; 10.86 (10.96)
Pro–Lys–Arg	Amorphous	−39.1	C; 59.58 (59.51) H; 6.82 (6.90) N; 11.05 (10.92)
Ser–Lys–Arg	Amorphous	−15.3	C; 60.51 (60.85) H; 6.76 (6.67) N; 10.40 (10.14)
Trp–Lys–Arg	Amorphous	−18.5	C; 61.83 (62.09) H; 6.74 (6.46) N; 11.48 (11.59)
Tyr–Lys–Arg	101.0—103.0	−8.7	C; 63.60 (63.42) H; 6.55 (6.42) N; 9.44 (9.44)
Ala–Lys–Ser	140.0—141.0	−29.5	C; 65.16 (65.05) H; 7.01 (7.03) N; 7.79 (7.90)
Glu–Lys–Ser	118.0—119.0	−20.3	C; 65.72 (65.62) H; 7.27 (7.29) N; 6.52 (6.57)
His–Lys–Ser	147.0—148.0	−17.7	C; 66.22 (66.35) H; 6.46 (6.68) N; 9.42 (9.29)
Leu–Lys–Ser	124.0—126.0	−28	C; 66.30 (66.20) H; 7.42 (7.36) N; 7.36 (7.46)
Pro–Lys–Ser	92.0—94.0	−45.1	C; 66.11 (66.01) H; 7.04 (7.02) N; 7.52 (7.59)
Arg–Lys–Ser	87.0—89.0	−13.5	C; 60.85 (61.07) H; 6.67 (6.54) N; 10.14 (10.04)
Trp–Lys–Ser	128.0—129.0	−21.7	C; 67.69 (67.81) H; 6.65 (6.54) N; 8.40 (8.38)
Tyr–Lys–Ser	138.0—139.0	−9.5	C; 69.31 (69.51) H; 6.71 (6.65) N; 6.22 (6.43)
Ala–Lys–Trp	93.0—94.0	−23.3	C; 66.01 (66.05) H; 6.79 (6.83) N; 9.62 (9.53)
Glu–Lys–Trp	126.0—128.0	−16.6	C; 66.42 (66.39) H; 7.08 (6.86) N; 8.07 (8.09)
His–Lys–Trp	155.0—158.0	−15.3	C; 65.09 (65.17) H; 6.64 (6.42) N; 10.42 (10.43)
Leu–Lys–Trp	Amorphous	−21.1	C; 66.30 (66.46) H; 7.25 (7.27) N; 8.99 (9.07)
Pro–Lys–Trp	Amorphous	−41.6	C; 66.52 (66.48) H; 6.84 (6.71) N; 9.23 (9.19)
Arg–Lys–Trp	Amorphous	−9.8	C; 60.96 (60.77) H; 6.55 (6.53) N; 11.37 (11.20)
Ser–Lys–Trp	Amorphous	−10	C; 67.69 (67.49) H; 6.65 (6.48) N; 8.40 (8.39)
Tyr–Lys–Trp	154.0—156.0	−4.7	C; 69.95 (69.81) H; 6.53 (6.49) N; 7.70 (7.73)
Ala–Lys–Tyr	144.0—145.0	−26.7	C; 67.99 (68.06) H; 6.85 (6.78) N; 7.05 (7.06)
Glu–Lys–Tyr	115.0—117.0	−19.7	C; 68.07 (68.06) H; 7.11 (6.88) N; 5.99 (6.06)
His–Lys–Tyr	141.0—143.0	−16.7	C; 68.81 (68.55) H; 6.45 (6.57) N; 8.39 (8.57)
Leu–Lys–Tyr	135.5—136.5	−23.6	C; 68.88 (68.87) H; 7.23 (7.21) N; 6.69 (6.80)
Pro–Lys–Tyr	111.0—112.0	−40.7	C; 68.76 (68.52) H; 6.88 (6.61) N; 6.82 (6.98)
Arg–Lys–Tyr	Amorphous	−12.3	C; 63.32 (63.13) H; 6.57 (6.42) N; 9.40 (9.44)
Ser–Lys–Tyr	104.0—105.0	−13.9	C; 69.31 (69.06) H; 6.71 (6.59) N; 6.22 (6.38)
Trp–Lys–Tyr	124.0—125.0	−18.8	C; 69.26 (69.47) H; 6.58 (6.43) N; 7.62 (7.84)
Glu–Tyr–Ala	111.0—113.0	−24.9	C; 67.81 (67.90) H; 7.18 (7.08) N; 5.65 (5.80)
His–Tyr–Ala	149.5—150.5	−24.3	C; 68.42 (68.35) H; 6.51 (6.25) N; 8.87 (8.88)

Table 4. (Continued.)

A_3–A_2–A_1	mp (°C)	$[\alpha]_D$	*Anal.* Calcd (Found.)
Lys–Tyr–Ala	152.0—155.0	−22.1	C; 67.99 (67.79) H; 6.85 (6.61) N; 7.05 (7.14)
Leu–Tyr–Ala	135.0—137.0	−34.4	C; 68.82 (68.74) H; 7.34 (7.15) N; 6.51 (6.67)
Pro–Tyr–Ala	Oil	−44.6	C; 68.17 (68.25) H; 6.91 (7.01) N; 6.63 (6.74)
Arg–Tyr–Ala	Amorphous	−16.2	C; 62.69 (62.41) H; 6.46 (6.37) N; 9.97 (10.11)
Ser–Tyr–Ala	97.0—99.0	−16	C; 69.37 (69.16) H; 6.67 (6.58) N; 5.92 (6.07)
Trp–Tyr–Ala	162.0—164.0	−32.3	C; 70.18 (70.08) H; 6.45 (6.31) N; 7.79 (7.90)
Ala–Tyr–Glu	99.0—101.0	−25.4	C; 67.81 (67.94) H; 7.18 (7.02) N; 5.65 (5.71)
His–Tyr–Glu	88.0—90.0	−17.1	C; 67.14 (67.32) H; 6.91 (6.93) N; 7.39 (7.43)
Lys–Tyr–Glu	145.0—147.0	−16.1	C; 68.07 (67.97) H; 7.11 (6.92) N; 5.99 (6.08)
Leu–Tyr–Glu	155.0—156.0	−24.2	C; 68.77 (68.53) H; 7.57 (7.49) N; 5.35 (5.40)
Pro–Tyr–Glu	106.0—107.0	−35.5	C; 68.64 (68.59) H; 7.20 (7.01) N; 5.46 (5.58)
Arg–Tyr–Glu	105.0—106.0	−10.6	C; 63.52 (63.43) H; 6.77 (6.70) N; 8.55 (8.62)
Ser–Tyr–Glu	128.0—129.0	−11.7	C; 69.24 (69.16) H; 7.00 (6.82) N; 4.94 (5.07)
Trp–Tyr–Glu	122.0—123.0	−22.7	C; 69.91 (69.83) H; 6.81 (6.79) N; 6.52 (6.63)
Ala–Tyr–His	96.0—97.0	−18.3	C; 68.42 (68.10) H; 6.51 (6.43) N; 8.87 (8.89)
Glu–Tyr–His	123.0—124.0	−12.5	C; 66.79 (66.94) H; 7.06 (6.76) N; 7.21 (7.53)
Lys–Tyr–His	160.0—163.0	−10.6	C; 67.93 (67.97) H; 6.62 (6.33) N; 8.49 (8.54)
Leu–Tyr–His	118.0—119.0	−16.2	C; 69.29 (69.02) H; 6.91 (6.67) N; 8.42 (8.55)
Pro–Tyr–His	Amorphous	−27.4	C; 69.18 (68.89) H; 6.55 (6.50) N; 8.58 (8.68)
Arg–Tyr–His	Amorphous	−7.5	C; 62.54 (62.56) H; 6.39 (6.04) N; 10.61 (10.80)
Ser–Tyr–His	94.0—95.0	−7.4	C; 69.35 (69.30) H; 6.44 (6.28) N; 7.78 (7.97)
Trp–Tyr–His	Amorphous	−17.1	C; 68.30 (68.34) H; 6.38 (6.29) N; 9.02 (9.16)
Ala–Tyr–Lys	189.0—190.0	−22.3	C; 67.99 (67.98) H; 6.85 (6.62) N; 7.05 (7.08)
Glu–Tyr–Lys	136.0—137.0	−17.4	C; 68.07 (67.95) H; 7.11 (6.81) N; 5.99 (6.06)
His–Tyr–Lys	98.0—99.0	−17.9	C; 67.32 (67.54) H; 6.66 (6.50) N; 8.41 (8.50)
Leu–Tyr–Lys	157.0—158.0	−23.9	C; 68.88 (68.83) H; 7.23 (7.15) N; 6.69 (6.75)
Pro–Tyr–Lys	112.0—113.0	−31.9	C; 68.76 (68.55) H; 6.88 (6.70) N; 6.82 (6.90)
Arg–Tyr–Lys	164.0—165.0	−10.7	C; 63.87 (63.65) H; 6.53 (6.40) N; 9.48 (9.55)
Ser–Tyr–Lys	162.0—163.0	−11.5	C; 69.31 (69.17) H; 6.71 (6.47) N; 6.22 (6.24)
Trp–Tyr–Lys	145.0—149.0	−21.4	C; 69.95 (69.70) H; 6.53 (6.61) N; 7.70 (7.77)
Ala–Tyr–Leu	121.0—122.0	−34.1	C; 68.82 (68.86) H; 7.34 (7.11) N; 6.51 (6.59)
Glu–Tyr–Leu	120.0—121.0	−24.8	C; 68.77 (68.66) H; 7.57 (7.44) N; 5.35 (5.59)
His–Tyr–Leu	145.0—146.0	−24.3	C; 68.55 (68.73) H; 6.95 (6.90) N; 8.33 (8.51)
Lys–Tyr–Leu	160.0—161.0	−23.9	C; 68.88 (68.87) H; 7.23 (7.05) N; 6.69 (6.85)
Pro–Tyr–Leu	Amorphous	−46	C; 69.72 (69.45) H; 7.35 (7.20) N; 6.25 (6.20)
Arg–Tyr–Leu	Amorphous	−17.9	C; 63.14 (63.32) H; 6.88 (6.77) N; 9.40 (9.60)
Ser–Tyr–Leu	105.0—107.0	−17.4	C; 70.28 (70.22) H; 7.10 (6.98) N; 5.59 (5.62)
Trp–Tyr–Leu	150.0—151.0	−34.9	C; 71.03 (71.01) H; 6.89 (6.87) N; 7.36 (7.47)
Ala–Tyr–Pro	Amorphous	−55	C; 68.17 (68.21) H; 6.91 (6.86) N; 6.63 (6.78)
Glu–Tyr–Pro	Amorphous	−43.5	C; 68.21 (68.31) H; 7.22 (7.13) N; 5.43 (5.63)
His–Tyr–Pro	Amorphous	−39.5	C; 68.43 (68.44) H; 6.60 (6.65) N; 8.49 (8.79)
Lys–Tyr–Pro	Amorphous	−38.6	C; 68.39 (68.26) H; 6.90 (6.73) N; 6.79 (6.97)
Leu–Tyr–Pro	Amorphous	−54.9	C; 69.72 (69.45) H; 7.35 (7.17) N; 6,25 (6.37)
Arg–Tyr–Pro	Amorphous	−30.4	C; 63.58 (63.30) H; 6.49 (6.43) N; 9.67 (9.77)
Ser–Tyr–Pro	Amorphous	−38	C; 69.34 (69.61) H; 6.77 (6.55) N; 5.64 (5.68)
Trp–Tyr–Pro	Amorphous	−46.3	C; 70.10 (70.24) H; 6.55 (6.55) N; 7.43 (7.54)
Ala–Tyr–Arg	Amorphous	−18.1	C; 62.03 (61.78) H; 6.51 (6.51) N; 9.86 (9.93)
Glu–Tyr–Arg	Amorphous	−12.1	C; 63.52 (63.36) H; 6.77 (6.71) N; 8.55 (8.63)
His–Tyr–Arg	Amorphous	−10.6	C; 63.63 (63.62) H; 6.31 (6.25) N; 10.79 (10.81)
Lys–Tyr–Arg	Amorphous	−12.1	C; 63.32 (63.48) H; 6.57 (6.45) N; 9.40 (9.45)
Leu–Tyr–Arg	82.0—84.0	−18	C; 63.78 (63.60) H; 6.83 (6.82) N; 9.50 (9.57)
Pro–Tyr–Arg	Amorphous	−25.3	C; 63.58 (63.27) H; 6.49 (6.60) N; 9.67 (9.84)
Ser–Tyr–Arg	99.0—101.0	−7.2	C; 64.54 (64.38) H; 6.37 (6.51) N; 8.85 (8.94)
Trp–Tyr–Arg	Amorphous	−16.9	C; 63.98 (64.22) H; 6.30 (6.14) N; 10.04 (9.97)
Ala–Tyr–Ser	140.0—142.0	−16.8	C; 69.37 (69.30) H; 6.67 (6.59) N; 5.92 (5.94)
Glu–Tyr–Ser	120.0—121.0	−14.2	C; 69.24 (69.06) H; 7.00 (7.11) N; 4.94 (4.95)
His–Tyr–Ser	129.0—131.0	−12.3	C; 69.70 (69.47) H; 6.41 (6.38) N; 7.82 (7.83)
Lys–Tyr–Ser	158.0—159.0	−10	C; 69.31 (69.17) H; 6.71 (6.72) N; 6.22 (6.22)
Leu–Tyr–Ser	149.0—150.0	−17.7	C; 70.28 (70.17) H; 7.10 (6.80) N; 5.59 (5.68)
Pro–Tyr–Ser	Amorphous	−27.4	C; 70.18 (70.09) H; 6.71 (6.83) N; 5.71 (5.73)
Arg–Tyr–Ser	Amorphous	−6.9	C; 64.29 (64.14) H; 6.39 (6.28) N; 8.82 (8.89)
Trp–Tyr–Ser	150.0—151.0	−20.1	C; 71.34 (71.14) H; 6.35 (6.31) N; 6.79 (6.84)
Ala–Tyr–Trp	Amorphous	−12.3	C; 69.31 (69.60) H; 6.51 (6.53) N; 7.70 (7.77)
Glu–Tyr–Trp	125.0—126.0	−7.2	C; 69.55 (69.55) H; 6.83 (6.74) N; 6.49 (6.55)
His–Tyr–Trp	192.0—193.0	−10	C; 69.64 (69.93) H; 6.29 (6.20) N; 9.19 (9.30)
Lys–Tyr–Trp	148.0—150.0	−6	C; 69.95 (69.85) H; 6.53 (6.48) N; 7.70 (7.68)
Leu–Tyr–Trp	Amorphous	−10.9	C; 70.86 (70.68) H; 6.90 (6.84) N; 7.35 (7.33)
Pro–Tyr–Trp	Amorphous	−27.1	C; 70.10 (69.86) H; 6.55 (6.52) N; 7.43 (7.47)
Arg–Tyr–Trp	Amorphous	−4.3	C; 63.40 (63.34) H; 6.34 (6.16) N; 9.95 (9.83)
Ser–Tyr–Trp	Amorphous	−4.2	C; 70.57 (70.89) H; 6.41 (6.30) N; 6.72 (6.80)

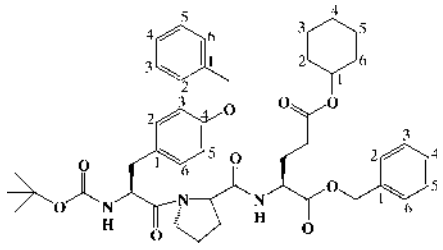
Table 5. ^{1}H Chemical Shifts of Boc-Tyr(Bzl)–Pro–Glu(O-c-Hex)-OBzl



Kind of proton		Major	Minor (δ)	Kind of proton		Major	Minor (δ)
Tyr	NH	5.50	5.22	Boc	CH_3	1.39	1.38
	αH	4.61	4.15	Bzl	CH_2	5.00	5.00
	βH	2.83, 2.98	2.69, 2.77			5.14, 5.18	5.00, 5.11
	Ring 2,6	7.16	6.97		Ring	7.26—7.41	
	Ring 3,5	6.89	6.85	c–Hex	1	4.70	4.73
Pro	αH	4.48	3.45		2,6	1.1—1.8	
	βH	1.88, 2.15	1.06, 2.06		3,5		
	γH	1.84, 1.98	1.44, 1.59		4	1.15, 1.45	1.30, 1.64
	δH	3.19, 3.56	3.42				
Glu	NH	7.47	8.28				
	αH	4.62	4.55				
	βH	2.13, 2.00	2.11, 2.34				
	γH	2.35	2.34				

Solvent: $CDCl_3$, major : minor = 2 : 1 at 22 °C. ^{3}J = 8.5,[1] 4.9,[2] 7.6,[3] 8.3 Hz.[4]

Table 6. ^{13}C Chemical Shifts of Boc–Tyr(Bzl)–Pro–Glu(O-c-Hex)-OBzl



Kind of carbon		Major	Minor	Kind of carbon		Major	Minor
Tyr	Cα	53.42	54.25	Boc	CH_3	28.30	28.30
	Cβ	38.30	37.64		C	79.50	80.16
	Ring 1	128.55	128.58		C=O	157.68	157.91
	Ring 2,6	130.55	130.38	Bzl	CH_2	69.84, 67.08	69.80, 66.92
	Ring 3,5	114.76	115.22		Ring 1	135.34, 137.06	135.56, 136.80
	Ring 4	155.16	155.75		Ring 2—6	128.24 128.45 128.53	128.18 128.34
Pro	Cα	59.96	60.58				
	Cβ	27.66	30.49				
	Cγ	25.05	21.89	c–Hex	1	72.84	72.74
	Cδ	47.28	46.50		2, 6	31.47	31.50
Glu	Cα	51.83	52.21		3, 5	23.63	23.63
	Cβ	27.27	25.63		4	25.26	25.30
	Cγ	30.39	31.16				
C=O		171.00 171.52 171.98	171.10 171.64 171.89				

Solvent: $CDCl_3$, major : minor = 2 : 1 at 22 °C.

Conclusion

An improved MSA method was developed for automated synthesis of peptides, in which it was possible to avoid 1) corrosion of the apparatus, 2) formation of emulsions, and 3) use of dichloromethane. By this method, we synthesized 216 fragment tripeptide derivatives in excellent yield. We have hitherto paid much attention to developing the hardware of our automated apparatus in order to apply it to a wide variety of standard reaction procedures. However, developing new chemical processes, or modifying established manual ones, is also very important for us to increase the scope, efficiency and reliability of automated synthesis.

Table 7. ^{1}H–NMR Spectrum Data of Tripeptide Derivatives (Boc-A_3–A_2–A_1-OBzl)

A_3–A_2–A_1	Solvent	Chemical shift (200 MHz) : δ (ppm)
Lys–Pro–Leu	DMSO–d_6	0.82 (3H, d, J=6.2 Hz), 0.89 (3H, d, J=6.2 Hz), 1.37 (9H, s), 1.47—1.89 (16H, m), 3.00 (2H, d, J=3.4 Hz), 3.37—3.70 (2H, m), 4.01—4.39 (2H, m), 5.02 (2H, s), 5.01 (2H, d, J=2.6 Hz), 6.90 (1H, d, J=7.8 Hz), 7.25—7.45 (10H, m), 8.24 (1H, d, J=7.8 Hz)
Lys–Pro–Ser	$CDCl_3$	1.26—2.21 (18H, m), 3.12 (2H, d, J=5.4 Hz), 3.54—3.74 (4H, m), 4.41 (2H, d, J=8.6 Hz), 4.50—4.88 (3H, m), 5.05 (2H, s), 5.14 (2H, d, J=10.4 Hz), 5.21—5.48 (2H,m), 6.96 (1H, d, J=8.4 Hz), 7.19—7.42 (15H, m)
Tyr–Pro–Lys	$CDCl_3$	1.25—1.49 (15H, m), 1.88—2.15 (2H, m), 2.68—2.98 (2H, m), 3.10—3.75 (3H, m), 4.34—4.68 (3H, m), 5.01 (2H, s), 5.05—5.15 (2H,m), 5.17 (2H, d, J=4.2 Hz), 5.59 (1H, d, J=8.4 Hz), 5.81 (1H, s), 6.75—7.10 (5H, m), 7.30—7.44 (16H, m)
Trp–Pro–Lys	$CDCl_3$	1.23—1.51 (15H, m), 1.78—2.18 (4H, m), 2.95—3.65 (6H, m), 4.39—4.41 (1H, m), 4.49—4.61 (1H, m), 4.71—4.85 (1H, m), 5.05—5.25 (4H,m), 5.58—5.75 (1H, m), 6.89—7.20 (16H, m), 7.68 (1H, d, J=4.2 Hz), 8.56 (1H, s)
Ser–Lys–Pro	$CDCl_3$	1.43 (9H, s), 1.48—1.74 (6H, m), 1.88—2.28 (4H, m), 3.11 (2H, d, J=5.4 Hz), 3.53—3.78 (4H, m), 3.92—3.98 (1H, m), 4.21—4.35 (1H, m), 4.50 (2H, s), 4.57 (1H, d, J=4.6 Hz), 4.75 (1H, d, J=4.6 Hz), 5.07 (2H, s), 5.10—5.45 (3H, m), 7.30—7.44 (15H, m)
Arg–Lys–Glu	$CDCl_3$	1.23—1.56 (24H, m), 1.78—2.02 (6H, m), 2.25—2.40 (3H, m), 3.05—3.25 (4H, m), 4.28—4.41 (1H, m), 4.45—4.61 (1H, m), 4.62—4.78 (1H, m), 5.06 (2H, s), 5.08 (2H, d, J=4.8 Hz), 5.21—5.32 (1H, m), 5.45—5.53 (1H, d, J=4.8 Hz), 6.38 (2H, br s), 7.20 (2H, d, J=6.2 Hz), 7.32 (12H, s), 7.75 (2H, d, J=6.2 Hz)
Trp–Lys–Pro	$CDCl_3$	1.23—1.56 (4H, m), 1.43 (9H, s), 1.84—2.23 (4H, m), 2.96—3.20 (3H, m), 3.36—3.62 (3H, m), 4.35—4.41 (1H, s), 4.43 (1H, d, J=4.8 Hz), 4.50—4.66 (1H, m), 5.04 (2H, m), 5.11—5.18 (2H, d, J=4.8 Hz), 5.12 (2H, s), 6.42 (1H, d, J=4.8 Hz), 6.72—6.86 (1H, d, J=6.2 Hz), 7.04—7.39 (13H, m), 7.57 (1H, d, J=7.2 Hz), 9.12 (1H, s)
Ala–Lys–His	$CDCl_3$	1.27 (3H, d, J=4.6 Hz), 1.15—1.78 (6H,m), 1.42 (9H, s), 3.18 (2H, t, J=4.4 Hz), 4.11 (1H, t, J=4.8 Hz), 4.33 (1H, t, J=4.8 Hz), 4.37 (2H, s), 4.90 (1H, q, J=4.8 Hz), 5.08 (2H, s), 5.10 (2H, s), 5.02—5.30 (2H, m), 5.21 (2H, s), 6.78 (1H, s), 6.78 (1H, d, J=4.2 Hz), 7.22—7.42 (16H, m)
Leu–Tyr–Pro	$CDCl_3$	0.90 (6H, d, J=5.2 Hz), 1.43 (9H, s), 1.48—2.23 (8H, m), 2.80—3.21 (3H, m), 3.45—3.58 (1H, m), 4.48—4.58 (2H, m), 4.61—4.92 (2H, m), 5.02 (2H, s), 5.01—5.15 (1H, m), 5.20 (2H, s), 6.72 (1H, d, J=4.8 Hz), 6.85 (2H, d, J=7.8 Hz), 7.14 (2H, d, J=7.8 Hz), 7.28—7.48 (10H, m), 6.78 (1H, d, J=4.2 Hz), 7.22—7.42 (16H, m)
Glu–Tyr–Trp	$CDCl_3$	1.21—1.85 (4H, m), 1.42 (9H, s), 2.22—2.34 (2H, m), 2.94 (2H, d, J=6.6 Hz), 3.33 (2H, d, J=6.6 Hz), 3.92—4.08 (1H, m), 4.51—4.96 (3H, m), 5.00 (2H, s), 5.06 (2H, s), 6.39—6.43 (1H, m), 6.61 (1H, d, J=8.4 Hz), 6.71 (1H, s), 6.82 (2H, d, J=8.4 Hz), 7.06 (2H, d, J=8.4 Hz), 7.28—7.46 (12H, m), 8.31 (1H, br s)
Ser–Tyr–His	$CDCl_3$	1.43 (9H, s), 2.98 (2H, d, J=6.6 Hz), 3.08 (2H, d, J=6.6 Hz), 3.61—3.78 (1H, m), 3.82—3.98 (1H, m), 4.21—4.40 (1H, m), 4.39 (2H, s), 4.55 (2H, s), 4.58—4.70 (1H, m), 4.82—4.95 (1H, m) 5.02 (2H, s), 5.15 (2H, s), 5.25 (2H, s), 5.39 (1H, d, J=4.8 Hz), 6.77—7.04 (7H, m), 7.31—7.51 (21H, m)
His–Tyr–Lys	$CDCl_3$	1.15—1.85 (6H, m), 1.39 (9H, s), 2.80—3.20 (6H, m), 4.32—4.58 (3H, m), 4.47 (2H, s), 4.97 (2H, s), 5.08 (2H, s), 5.12 (2H, s), 5.19 (2H, brs), 5.29—5.41 (1H, m), 5.85—5.99 (1H, s), 6.35 (1H, d, J=8.4 Hz), 6.55 (1H, m), 6.82 (2H, d, J=8.6 Hz), 6.99 (2H, d, J=8.6 Hz), 7.27—7.46 (23H, m)

Table 8. Improvement and Merits of the MSA Method

Improvement	Effect (Merit)
Avoid use of trifluoroacetic acid (volatile, corrosive acid)	Easy handling and less damage to the apparatus
Avoid use of dichloromethane	Friendly to the environment
Decrease of the number of extractions and drying processes	Increased reliability (*ca.* 90%→*ca.* 99%)
Generation of the free amine and activated ester at the same time	Higher yield (70.1→77.4%)
Easy set up and simpler process	Higher throughput

Experimental

Computer-controlled automated synthesis systems (TAFT, EASY, ASTRO)[10] were used to synthesize oligopeptide derivatives in solution. Optical rotation values (in MeOH) were measured with a Nihon Bunko DIP-370 spectrometer. 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 JOEL 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 Silica Gel 60 (70—230 mesh, ASTM, Merck). The starting dipeptides (27 kinds) were synthesized in our large-scale automated synthesis apparatus (FUTOSHI).[11] Typical synthesis for one dipeptide, Boc-Pro–Ala-OBzl, was as follows: In a 2 l three-necked flask, MSA (98 g) in acetonitrile (600 ml) was added to Boc-Ala-OBzl (112 g, 400 mmol) in acetonitrile (400 ml) at room temperature and the reaction mixture was stirred for 1 h. After the reaction mixture was cooled to 0 °C, diisopropylethylamine (78.6 g, 600 mmol) was added, and then Boc-Pro (86.1 g, 400 mmol), HOBt (67.4 g, 440 mmol), and WSCD (68.4 g, 440 mmol) in acetonitrile (100 ml)–DMF (200 ml) was added. After the reaction mixture was stirred for 16 h, solvents were evaporated-off, and the residue was extracted from ethyl acetate–5% sodium hydrogen carbonate, and washed with 0.2 N hydrochloric acid and water. The organic layer was dried, and evaporated, and recrystallized from diisopropylether affording 124.6 g (83%) of the expected product.

General Procedure of Peptide Bond Formation The procedure for peptide bond formation between Boc-Leu and Boc-Pro–Lys(Z)-OBzl is described as a typical example. Table 2 lists the subroutine sequence by which the apparatus was controlled. In the reaction flask1 (RF1), to a powder of Boc-Pro–Lys(Z)-OBzl (1.70 g, 3.0 mmol) was added MSA in acetonitrile (12 ml, 12 mmol) from reagent reservoir 2 (RR2) [RF1-ST-ON, RR2-RF1]. Then the reaction mixture was stirred at 40 °C for 1 h [RF1-LF-UP, REA1 (40c, 60 m)]. The solution was cooled to 0 °C, and ethyldiisopropylamine in

acetonitrile (10 ml, 9 mmol) was added from RR3 to neutralize the excess acid [RF1-T-ON (0c), RR3-RF1]. After the neutralization, the reaction mixture was transferred to RF2 where Boc-Leu (0.75 g, 3 mmol) and HOBt (505 mg, 3.3 mmol) were stored at 0 °C [RF1-T-OF, RF2-LF-UP, RF2-T-ON (0c), RF2-ST-ON, RF1-RF2, RS6-RF1, RF1-MIX, RF1-RF2, RF1-ST-OF, RF1-LF-DN]. Then water-soluble carbodiimide (1.2 g) in DMF (5 ml) was added from RR4 [RR4-RF2, RF2-ST-OF] before the reaction mixture was stirred at room temperature for 10 h [REA2 (25c, 600 m)]. After the reaction was completed, the solvents were removed under vacuum for 1 h [CON2 (40c, 60 m)], and extracted with ethyl acetate–5% sodium hydrogen carbonate, followed by the usual work-up [RF2-LF-DN, RS4-RF2, RS2-RF2, RF2-MIX, BKEXT, RF2-ST-OF]. The collection flask containing the expected tripeptide was taken from the apparatus, and chromatographed on silica gel (eluent; hexane–AcOEt (2 : 1)) to give the expected product as an amorphous solid (1.72 g, 84%).

Acknowledgements We wish to thank Drs. Masahiko Fujino and Tunehiko Fukuda for their helpful advice. We also express our thanks to Mrs. Mika Murabayashi for measuring NMR spectra.

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