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Peptide Synthesis in Aqueous Solution. V.[1] Properties and Reactivities of (*p*-Hydroxyphenyl)benzylmethylsulfonium Salts for Direct Benzyl Esterification of *N*-Acylpeptides[2]

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Some (*p*-hydroxyphenyl)benzylmethylsulfonium salts were prepared. These compounds generated a benzyl cation and converted not only *N*-acylamino acids but also *N*-acylpeptides into their corresponding benzyl esters without causing the racemization.

In a previous paper,[3] we reported that (*p*-hydroxyphenyl)dimethylsulfonium methyl sulfate (HODSP) was an excellent reagent for the preparation of water-soluble active esters. The most remarkable character of this compound is solubility in water, due to the presence of a counter anion in the molecule. Since the condensation reaction is carried out in aqueous solution, functional groups of the side chain can be left unprotected by controlling the pH of the reaction media. In order to show the usefulness of the water-soluble active ester method, FMRFamide (Phe–Met–Arg–Phe–NH_2) was synthesized.[3—5] During the synthesis of the peptide, the side chain of Arg was left unprotected and the condensation reaction proceeded without any problem.

Several kinds of water-soluble *N*-acylating reagents, such as Z–DSP, Boc–DSP, and Fmoc–DSP, were also prepared. Since these reagents are soluble in water, acylation of amino acids, peptides, and proteins is easily carried out in aqueous solution.[6] The usefulness of water-soluble acylating reagents is shown in the chemical modification of enzymes.[7—12] Enzymes thus modified by water-soluble *N*-acylating agents became very stable in organic solvents.

We then focused on developing another useful aspect of HODSP, as an esterification reagent. There are very few methods to convert peptides to peptide esters. The most efficient one is probably diazomethane.[13] However, diazomethane is not always suitable for esterification of peptides because of its high reactivity and limitation of solvents. Since an proper method to prepared peptide esters, of which functional groups on the side chains other than carboxyl groups were left unprotected, was required, the authors have been looking for a new esterification reagent. (*p*-Hydroxyphenyl)-benzylmethylsulfonium derivatives (HOBMX) were thought to be suitable compounds for this purpose. They easily generated benzyl cation, which converts amino acids and peptides into the corresponding benzyl esters.[14,15]

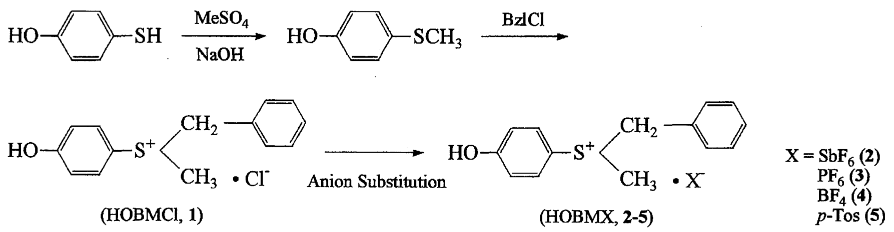
In order to confirm the usefulness of HOBMX, we carried out esterification reactions by HOBMX employing several conditions. In this paper, we will discuss optimal conditions for forming benzyl esters using HOBMX. We also determined X group for the most efficient esterification. Additionally, we applied an *N*-acylpeptide benzyl ester, which was obtained by the reaction of HOBMX, to the azide method. We will describe the usefulness of benzyl esters produced by HOBMX method for peptide fragment condensation as well.

Results and Discussion

Preparation of (*p*-Hydroxyphenyl)benzylmethylsulfonium Salts (HOBMX). Several kinds of (*p*-hydroxyphenyl)benzylmethylsulfonium salts (HOBMX) were prepared along the synthetic scheme indicated in Scheme 1. 4-Mercaptophenol was converted to 4-(methylthio)phenol by dimethyl sulfate. This product was treated with benzyl chloride to give (*p*-hydroxyphenyl)benzylmethylsulfonium chloride (**1**). Several (*p*-hydroxyphenyl)benzylmethylsulfonium salts were prepared by changing the counter anion.

Finding the Optimum Condition for the Reaction. First, the most suitable counter anion was searched for. *N*-Benzyloxycarbonylglycine was treated with several HOBMX. Conditions and yields are listed in Table 1. All HOBMX's gave significant yields. However, they produced some by-products along with HOBMCl in the case of using the other substrates such as Z–Phe–OH. Therefore, Cl^- was determined as the most suitable counter anion.

Benzyl esterification of Z–Gly–OH with HOBMCl was



Scheme 1. Preparation of (*p*-hydroxyphenyl)benzylmethylsulfonium salts (HOBMX, **1**—**5**).

Table 1. Effect of Counter Anion X^- in HOBMX Molecule on Direct Benzyl Esterification of Z–Gly–OH

Counter anion (X^-)	Yield (%)
Cl (**1**)	85
SbF_6 (**2**)	99
PF_6 (**3**)	99
BF_4 (**4**)	99
p-Tos (**5**)	95
Conditions	
Molar ratio of HOBMX to Z–Gly–OH	1.2 (mol/mol)
Base	Potassium carbonate
Solvent	DCM

then carried out in the presence of several bases. The results are shown in Table 2. The reaction proceeded in the presence of potassium carbonate with more satisfactory yield than in that of any other base.

Reaction of Z–Gly–OH by using HOBMCl in several solvents was performed. The results are shown in Table 3. The reactions in DCM and DMF proceeded with more satisfactory yield than those in any other solvent. DCM was determined as the most suitable solvent because of its easy workup.

Table 2. Effect of Base on Synthesis of Z–Gly–OBzl

Base	Yield (%)
NaOH	36
Na_2CO_3	43
$NaHCO_3$	15
KOH	81
K_2CO_3	85
MgO	18
Al_2O_3	15
$5MgO \cdot 2Al_2O_3$	28
$Mg_6Al_2(OH)_{16}CO_3$	12
$2MgO \cdot 6SiO_2$	14
TEA	13
NMM	32
DCHA	35
CHA	24
Py	By-product
Conditions	
Molar ratio of HOBMCl to Z–Gly–OH	1.2 (mol/mol)
Counter anion (X^-)	Cl^-
Solvent	DCM

Table 3. Effect of Solvent on Synthesis of Z–Gly–OBzl

Solvent	Yield (%)
MeOH	41
CH_3CN	65
THF	46
DCM	85
DMF	82
CH_3COCH_3	54
DCM–DMF	57
MeOH–H_2O	10
CH_3CN–H_2O	18
THF–H_2O	42
DCM–H_2O	47
DMF–H_2O	37
CH_3COCH_3–H_2O	32
H_2O	73
Conditions	
Molar ratio of HOBMCl to Z–Gly–OH	1.2 (mol/mol)
Counter anion (X^-)	Cl^-
Base	Potassium carbonate

The results of these model experiments indicate that direct benzyl esterification of HOBMCl is to be carried out in DCM in the presence of potassium carbonate.

Direct Benzyl Esterification of *N*-Acylamino Acids. Amino acids, which have functional groups on their side chains, such as His, Arg, Tyr or Trp, were also treated with HOBMCl. As shown in Table 4, all *N*-acylamino acids were converted into the corresponding benzyl esters in good yields according to Scheme 2, with the exception of Z–Cys–OH which was converted into *S*-benzyl-Cys. The result definitely indicates that HOBMCl is the more suitable reagent for benzyl esterification than conventional benzylating reagent such as benzyl chloride, because HOBMCl has the same reactivity as benzyl chloride while it is much easier to handle.

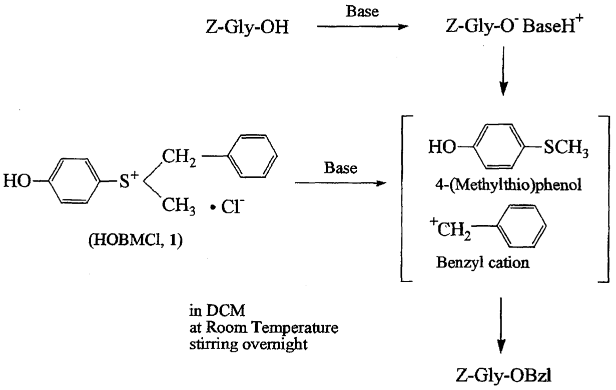
We then performed the reaction of Cys with HOBMX by changing the reactant condition in methanol–aqueous media to investigate the reactivity of the mercapto group of Cys with HOBMX. As shown in Table 5, HOBMCl converted Cys into *S*-benzyl-Cys in more satisfactory yield than any other HOBMX. When the reaction was carried out in the presence of several bases, NaOH and TEA were determined as proper bases. (*p*-Hydroxyphenyl)benzylmethylsulfonium

Table 4. Preparation of *N*-Acylamino Acid Benzyl Esters Using HOBMCl (**1**)

Substrate	Benzyl ester	Solvent	Yield (%)
Z–Gly–OH	(**6**)	DCM	85
Z–Met–OH	(**7**)	DCM	56
Boc–Arg(Tos)–OH	(**8**)	DCM–DMF	63
Z–Arg–OH·HCl	(**9**)	DCM	50
Z–Cys–OH	(**10**)	DCM	79[a]
Z–Glu–OH	(**11**)	DCM	77[b]
Z–Val–OH	(**12**)	DCM	76
Z–His–OH	(**13**)	DCM–DMF	41
Boc–Phe–OH	(**14**)	DCM	75
Boc–Tyr–OH	(**15**)	DCM	81
Boc–Tyr(Bzl)–OH	(**16**)	DMF	73
Boc–Trp–OH	(**17**)	DCM	70
Boc–Ser–OH	(**18**)	DCM	94
Boc–Asn–OH	(**19**)	DCM–DMF	87
Boc–Gln–OH	(**20**)	DCM–DMF	32
pGlu–OH	(**21**)	DCM–DMF	89

Conditions	
Molar ratio of HOBMCl to each *N*-acylamino acid	1.5 (mol/mol)
Base	Potassium carbonate

a) Z–Cys(SBzl)–OH. b) Dibenzyl ester.



Scheme 2. Mechanism of direct benzyl esterification using HOBMCl (**1**).

chloride yielded *S*-benzyl-Cys in good yield, at the same level as benzyl chloride.

Furthermore, we studied the reaction of HOBMX in aqueous media. As shown in Table 6, Z–Phe–OH was converted to the corresponding benzyl ester in a good yield even in aqueous media.

It was confirmed that HOBMX converts *N*-acylamino acids into the corresponding benzyl esters under mild conditions even though they have functional groups on the side chains other than Cys.

Table 5. Preparation of *S*-Benzyl-Cys Using HOBMX (**1**—**5**)

Counter anion (X^-)	Solvent	Base	Yield (%)
Cl (**1**)	MeOH–H_2O	NaOH	88
Cl (**1**)	MeOH–H_2O	TEA	86
Cl (**1**)	MeOH–H_2O	Na_2CO_3	67
Cl (**1**)	MeOH–H_2O	$NaHCO_3$	68
Cl (**1**)	MeOH–H_2O	K_2CO_3	63
Cl (**1**)	MeOH–H_2O	Py	7
SbF_6 (**2**)	MeOH–H_2O	NaOH	71
PF_6 (**3**)	MeOH–H_2O	NaOH	57
BF_4 (**4**)	MeOH–H_2O	NaOH	19
p-Tos (**5**)	MeOH–H_2O	NaOH	41
BzlCl	EtOH	NaOH	85

Condition	
Molar ratio of HOBMCl to Cys	1.2 (mol/mol)

Table 6. Preparation of Z–Phe–OBzl in Aqueous Solution Using HOBMCl (**1**)

Solvent	Base	Yield (%)
DCM	K_2CO_3	85
DCM	CS_2CO_3	77
DMF	K_2CO_3	81
MeOH	K_2CO_3	47
H_2O	K_2CO_3	73

Condition	
Molar ratio of HOBMCl to Z–Phe–OH	1.2 (mol/mol)

Direct Benzyl Esterification of *N*-Acylpeptides. Various kinds of *N*-acylpeptides (**20**—**37**) were treated with HOBMCl; those with no functional group on the side chains; those which have functional groups; and those with a branched *β*-carbon on C-terminus amino acid such as Ile and Val residue, in order to investigate how steric hindrance of side chains affects the reaction. *N*-Acylpeptides and *N*-acylamino acids were allowed to react with HOBMCl. Results are listed in Table 7. Most *N*-acylpeptides were converted into the corresponding peptide benzyl esters with good yield. Benzylation tended to proceed in lower yield when DMF was added to the reaction media to increase solubility of peptides. *N*-Acylpeptides which possessed a branched *β*-carbon on C-terminus amino acids also gave poor yields. It is suggested that the conformational hindrance between the side chain and benzyl cation prevents the *N*-acylpeptide from producing the corresponding benzyl ester.

Although there are many ways to prepare amino acid esters, only diazomethane can be used for esterification of peptides. However, the diazomethane method is not always thought to be suitable for esterification of peptide fragments because of its high reactivity and limitation in use. On the other hand, HOBMCl is easy to handle and generates benzyl cation in the presence of a base. The most remarkable advantage of "HOBMCl method" is that HOBMCl has an ability to directly esterify *N*-acylpeptides under mild conditions. Development of the HOBMCl method contains a possibility that the peptide synthesis will become easier than any other conventional method. Azide coupling method via *N*-acylpeptide benzyl esters seems to be the best way to condense partially protected peptide fragments so that the number of synthetic steps were expected to decrease significantly.

Table 7. Preparation of *N*-Acylpeptide Benzyl Esters Using HOBMCl (**1**)

Substrate	Benzyl ester	Solvent	Yield(%)
Z–Phe–Gly–OH	(**22**)	DCM	83
Z–Val–Tyr–OH	(**23**)	DCM–DMF	46
Z–Pro–Leu–OH	(**24**)	DCM	85
Z–Gly–Ala–OH	(**25**)	DCM	57
Boc–Ala–Glu–OH	(**26**)	DCM–DMF	66[a]
Boc–Gly–Gly–OH	(**27**)	DCM	89
Boc–Ile–Val–OH	(**28**)	DCM	64
Boc–Pro–Phe–OH	(**29**)	DCM	79
Boc–Thr–Pro–OH	(**30**)	DCM	53
Boc–Gln–Pro–OH	(**31**)	DCM–DMF	92
Boc–Trp–Gly–OH	(**32**)	DCM	62
Z–Gly–His–OH	(**33**)	DCM	64
Boc–Ser–Ile–OH	(**34**)	DCM	74
Z–Lys(Z)–Pro–OH	(**35**)	DCM	91
Boc–Met–Pro–OH	(**36**)	DCM	71
Boc–Phe–Asp–OH	(**37**)	DCM	57[a]
pGlu–Phe–OH	(**38**)	DCM	80
Boc–Pro–Pro–Pro–OH	(**39**)	DCM	90
Boc–Val–Val–Val–Pro–Pro–OH	(**40**)	DCM–DMF	57

Conditions

Molar ratio of HOBMCl to each *N*-acylpeptide	1.5 (mol/mol)
Base	Potassium carbonate

[a] Dibenzyl ester.

This fact indicates that HOBMCl is a suitable esterification reagent to convert peptide fragments into the corresponding benzyl esters, while the functional groups on the side chain was left unprotected, with the exception of Cys, Lys/Orn, and Asp/Glu residue.

Racemization Test. To investigate whether this direct benzyl esterification causes racemization, the racemization test of Gly–L-Ala–L-Leu (**38**), which was prepared as shown in Fig. 1, was carried out according to the method of Izumiya et al.[16] As shown in Fig. 2, this method caused no evident racemization. This fact indicates a possibility that partially protected peptide benzyl esters, which were prepared by HOBMCl method, can be applied to azide coupling method as building blocks.

Application to Synthesis of Bitter Peptide Analog. In

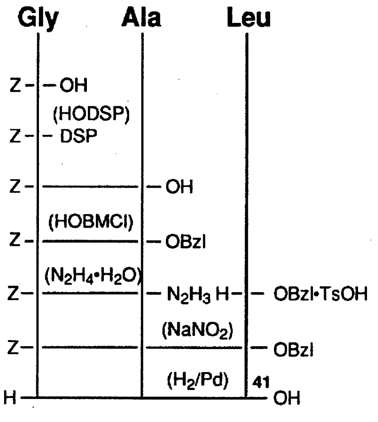
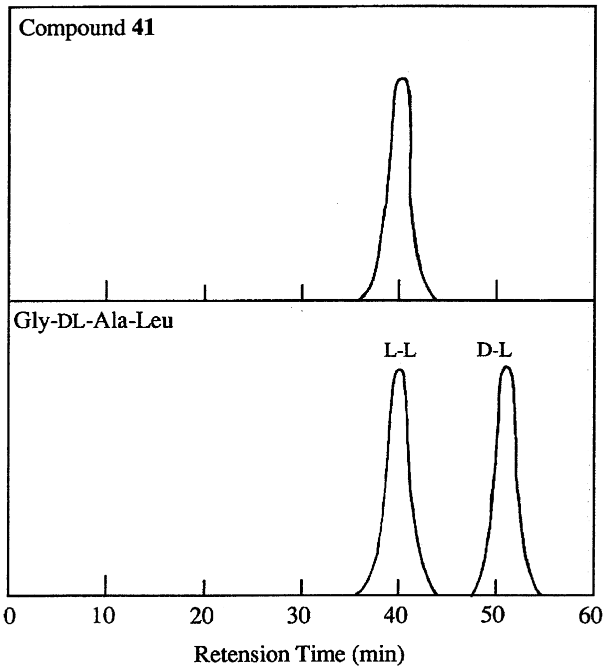


Fig. 1. Synthetic route for Gly–Ala–Leu (**41**).



Conditions

Column	AA pack Na
Temperature	60 °C
Eluent	0.1M Citrate Buffer
pH	3.25

Fig. 2. Racemization test.

the last stage, the HOBMCl method was applied to the synthesis of the D-Phe analog of C-terminus octapeptide of β-casein in order to demonstrate the usefulness of this direct esterification using HOBMCl (Fig. 3).[17] N-Acyldipeptide acid, Boc–Pro–D-Phe–OH (**42**) was converted to the corresponding hydrazide (**44**) and its C-terminus carboxyl group was activated to condense with H–Pro–Ile–Ile–Val–OBzl·HCl (**45**). The physicochemical properties of synthetic intermediates before the azide coupling reaction are listed in Table 8. Azide coupling reaction of **44** with **45** was carried out, the coupling reaction proceeded in good yields and gave **46** without any problem in chemical purity. These results indicated that an azide coupling method via HOBMCl benzyl esterification "HOBMCl-Azide Coupling Method" could be developed as a new facile peptide synthesis.

Consequently, N-acylpeptides, which possess several kinds of functional groups on the side chains, could be converted directly into the corresponding benzyl esters without causing racemization by HOBMCl in aqueous solution as well as in organic solvent. Any N-acylpeptide could be directly esterified by HOBMCl method, and we could succeed in expanding the applicable range of the azide method. In general, azide coupling method used to be carried out via N-acylpeptide methyl or ethyl ester. However, we proved that a partially protected peptide benzyl ester, which was prepared by HOBMCl, can be applied to azide method without any problem. We believe that this newly developed esterification reagent is a powerful tool for not only the direct protection of carboxyl groups on the synthetic intermediates in peptide synthesis but also the preparation of a biologically active peptide/protein and the chemical modification of enzymes without causing any conformational changing.

Experimental

General. All the melting points are uncorrected. Thin layer chromatography was carried out on Merck silica gel G with the solvent systems: Rf_1, 1-buthanol–acetic acid–pyridine–water (4 : 1 : 1 : 2, v/v); Rf_2, chloroform–methanol (5 : 1, v/v). Spots of materials possessing a free amino group on a thin layer plate were detected by spraying ninhydrin. In the case of the compounds whose amino groups were protected by Z or Boc groups, spots were detected by spraying 25% HBr in acetic acid and then ninhydrin. Infrared spectra were recorded on a Hitachi 269-30 infrared spectrometer. ^{1}H NMR spectra were measured on a Hitachi R-24B (60 MHz) spectrometer and JEOL GSX-270 (270 MHz) spectrometer. The chemical shifts are given in δ (ppm) scale with tetramethylsilane as an internal standard. The optical rotations were measured on a Union PM-101 polarimeter. HPLC analysis was performed by using JASCO TRI ROTER-V and UVDEC-100V apparatus.

Preparation of (p-Hydroxyphenyl)benzylmethylsulfonium Salts. (p-Hydroxyphenyl)benzylmethylsulfonium Chloride (HOBMCl, 1). A mixture of benzyl chloride (7.60 g, 60 mmol) and (p-hydroxyphenyl)methyl sulfide (7.01 g, 50 mmol) in methanol (15 ml) was stirred at 35 °C for 3 d. Thereafter, acetone (15 ml) was poured into the reaction mixture, and the precipitates were collected by filtration. By washing with acetone (50 ml), the white crystals were dried in vacuo to afford (p-hydroxyphenyl)-benzylmethylsulfonium chloride. Yield 85%; mp 109—111 °C. $\nu_{\max}^{\mathrm{KBr}}$: 3450, 3045, 2950, 1600, 1580, 1500, 1290, 840, 515 cm^{-1}. ^{1}H NMR (CD_3OD) $\delta = 3.27$ (s, 3H, CH_3), 4.65—5.20 (m, 2H, benzyl CH_2), 6.90—7.72 (m, 9H, ArH). Found: C, 62.95; H, 5.69%.

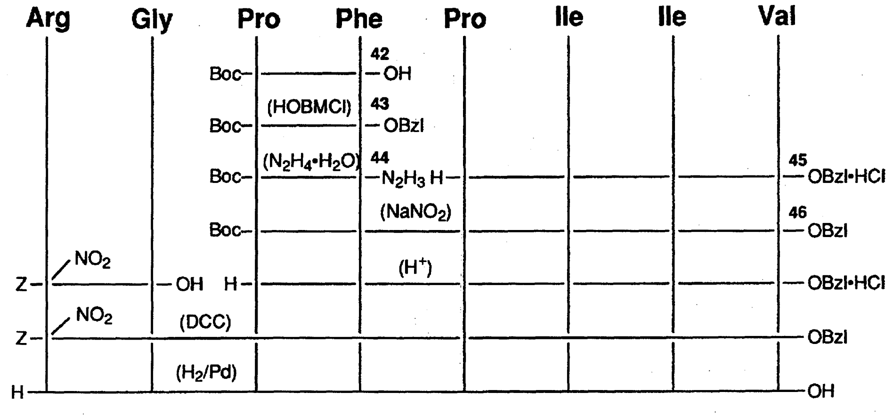


Fig. 3. Synthetic route for intermediate of C-terminus octapeptide analog of β-casein.

Table 8. Analytical Data of Intermediates of C-Terminus Octapeptide Analog of β-Casein

Compound	Yield	Mp	Rf_1/Rf_2	$[\alpha]_D^{20}$ (c 1, DMF)	Elemental analysis Found (Calcd) (%)		
	%	°C			C	H	N
Boc–Pro–D-Phe–OBzl (**43**)	53	oil	0.86/0.91	—	68.42, (69.00,	7.12, 7.13,	5.97 6.19)
Boc–Pro–D-Phe–N_2H_3 (**44**)	77	154—156	0.81/0.80	−14.0	60.29, (60.62,	7.51, 7.50,	14.84 14.88)
Boc–Pro–D-Phe–Ile–Ile–Val–OBzl (**46**)	78	115—118	0.95/0.91	−55.0	65.28, (65.88,	8.03, 8.06,	9.64 9.60)

Calcd for $C_{14}H_{15}OSCl$: C, 63.03; H, 5.67%.

(*p*- Hydroxyphenyl)benzylmethylsulfonium Hexafluoroantimonate (HOBMSbF$_6$, 2): A mixture of (*p*-hydroxyphenyl)benzylmethylsulfonium chloride (2.67 g, 10 mmol) and $KSbF_6$ (2.75 g, 10 mmol) in methanol (16 ml) was stirred at room temperature for 2 h. The reaction mixture was concentrated by evaporation and ethyl acetate (50 ml) was added to the residue. The precipitates were filtrated off and the organic layer was washed three times with water in order to remove KCl. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was evaporated. The oily residue was triturated with chloroform–hexane and the formed crystals were collected by decantation. Yield 84%; mp 114—116 °C. $\nu_{\mathrm{max}}^{\mathrm{KBr}}$: 3400, 3100, 1600, 1580, 1500, 1290, 840, 660 cm^{-1}. ^{1}H NMR (CD_3OCD_3) $\delta = 3.50$ (s, 3H, CH_3–), 4.84—5.35 (dd, 2H, –CH_2–), 7.07—7.93 (m, 9H, –C_6H_4–, –C_6H_5), 9.75 (s, 1H, HO–). Found: C, 36.00; H, 3.21%. Calcd for $C_{14}H_{15}OSSbF_6$: C, 35.81; H, 3.15%.

(*p*-Hydroxyphenyl)benzylmethylsulfonium Hexafluorophosphate (HOBMPF$_6$, 3): A mixture of (*p*-hydroxyphenyl)benzylmethylsulfonium chloride (2.67 g, 10 mmol) and KPF_6 (1.84 g, 10 mmol) in methanol–water (25 ml/25 ml) was stirred at room temperature for 2 h. The reaction mixture was concentrated by evaporation and ethyl acetate (20 ml) was added to the residue. The precipitates which formed were filtered off and the filtrate was washed three times with water in order to remove KCl. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was evaporated. The oily residue was triturated with hexane and the crystals which formed were separated by decantation. Yield 86%; mp 138—140 °C. $\nu_{\mathrm{max}}^{\mathrm{KBr}}$: 3420, 3050, 1600, 1580, 1500, 1290, 860, 560, 520 cm^{-1}. ^{1}H NMR (CD_3COCD_3) $\delta = 3.40$ (s, 3H, CH_3–), 4.80—5.27 (dd, 2H, –CH_2–), 7.06—7.90 (m, 9H, –C_6H_4–, C_6H_5–). Found: C, 44.67; H, 4.04%. Calcd for $C_{14}H_{15}OSPF_6$; C, 44.69; H, 4.02%.

(*p*- Hydroxyphenyl)benzylmethylsulfonium Tetrafluoroborate (HOBMBF$_4$, 4): A mixture of (*p*-hydroxyphenyl)benzylmethylsulfonium chloride (2.67 g, 10 mmol) and $NaBF_4$ (1.10 g, 10 mmol) in methanol–water (20 ml/5 ml) was stirred at room temperature for 2 h. The reaction mixture was concentrated by evaporation and ethyl acetate (20 ml) was added to the residue. After removal of the precipitates which formed, the filtrate was washed three times with water in order to remove KCl. The obtained white crystals were purified by recrystalization from ethanol–methanol. Yield 44%; mp 137—138 °C. $\nu_{\mathrm{max}}^{\mathrm{KBr}}$: 3400, 3050, 1600, 1580, 1500, 1290, 1120, 1080, 1040 cm^{-1}. ^{1}H NMR (CD_3COCD_3) $\delta = 3.43$ (s, 3H, CH_3–), 4.83—5.32 (dd, 2H, –CH_2–), 7.91 (m, 9H, –C_6H_4–, C_6H_5–), 9.70 (s, 1H, HO–). Found: C, 52.87; H, 4.83%. Calcd for $C_{14}H_{15}OSBF_4$: C, 52.86; H, 4.75%.

(*p*- Hydroxyphenyl)benzylmethylsulfonium *p*- Toluenesulfonate (HOBM*p*-Tos, 5): A mixture of benzyl 4-hydroxyphenyl sulfide (2.16 g, 10 mmol) and methyl *p*-toluenesulfonate (1.86 g, 10 mmol) in acetonitrile (20 ml) was stirred at 80 °C for 5 h. The reaction mixture was concentrated by evaporation and ethyl acetate (50 ml) was added to the residue. The precipitated material was collected by filtration. After washing with ethyl acetate, the white crystals were dried in vacuo to afford (*p*-hydroxyphenyl)benzylmethylsulfonium *p*-toluenesulfonate. Yield 85%; mp 105—110 °C. $\nu_{\mathrm{max}}^{\mathrm{KBr}}$: 1600, 1585, 1500, 1457, 1382, 1290, 1221, 1179, 1122, 1087, 1034, 1011, 836, 817, 777, 687, 565, 518 cm^{-1}. ^{1}H NMR (CD_3OCD_3) $\delta = 2.35$ (s, 3H, –C_6H_4–CH_3), 3.23 (s, 3H, –S–CH_3), 4.82 (dd, 2H, $J_1 = 52.7$ Hz, $J_2 = 12.5$ Hz, benzyl CH_2), 7.17–7.23 (m, 2H, two protons of benzyl C_6H_5), 7.28 (dd, 4H, $J_1 = 167.2$ Hz, $J_2 = 9.0$ Hz, HO–C_6H_4–), 7.29—7.38 (m, 3H, three protons of benzyl C_6H_5), 7.45 (dd, 4H, $J_1 = 146.7$ Hz, $J_2 = 8.3$ Hz, CH_3–C_6H_4–). Found: C, 62.01; H, 5.52%. Calcd for $C_{21}H_{22}O_4S_2$: C, 62.66; H, 5.51%.

Direct Benzyl Esterification of *N*-Acylamino Acids (6—21) and *N*-Acylpeptides (22—40). Preparation of Z–Gly–OBzl (6): Z–Gly–OH (0.63 g, 3 mmol) and potassium carbonate (0.41 g, 3 mmol) were dissolved in DCM (6 ml), and the mixture was stirred at room temperature. After 10 min, HOBMCl (1.20 g, 4.5 mmol) was added to the solution and it was stirred for 8 h. Inorganic salts in the reaction mixture were filtered off and the filtrate was evaporated in vacuo. The residue was dissolved in toluene and washed with 0.5 M NaOH (1 M = 1 mol dm^{-3}) aqueous solution and then with water in order to remove the by-product, 4-(methylthio)phenol. The organic layer was dried over anhydrous sodium sulfate and the filtrate was evaporated in vacuo. The residue was crystallized with the aid of petroleum ether. Yield 0.76 g (85%), Rf$_1$ 0.89; Rf$_2$ 0.95; mp 72—74 °C; $[\alpha]_{\mathrm{D}}^{20}$ 0° (*c* 1, DMF). Found: C, 68.24; H, 5.78; N, 4.64%. Calcd for $C_{17}H_{17}O_4N$: C, 68.22; H, 5.72; N, 4.68%.

Z–Met–OBzl (7): Rf$_1$ 0.95; Rf$_2$ 0.95; mp 85—87 °C; $[\alpha]_{\mathrm{D}}^{20}$ −17° (*c* 1, DMF). Found: C, 64.01; H, 6.23; N, 4.21%. Calcd for $C_{20}H_{23}O_4NS$: C, 63.98; H, 6.17; N, 4.26%.

Boc–Arg(Tos)–OBzl (8): Rf$_1$ 0.89; Rf$_2$ 0.79; mp 70—73 °C; $[\alpha]_{\mathrm{D}}^{20}$ −9° (*c* 1, DMF). Found: C, 58.03; H, 6.68; N, 10.67%. Calcd for $C_{25}H_{34}O_6N_4S$: C, 57.90; H, 6.61; N, 10.80%.

Z–Arg–OBzl (9): Rf$_1$ 0.87; Rf$_2$ 0.82; hygroscopic powder.

Z–Cys(SBzl)–OH (10): Rf$_1$ 0.90; Rf$_2$ 0.86; mp 93—94 °C; $[\alpha]_{\mathrm{D}}^{20}$ −24° (*c* 1, DMF). Found: C, 62.65; H, 5.61; N, 4.01%. Calcd for $C_{18}H_{19}O_4NS$: C, 62.59; H, 5.54; N, 4.06%.

Z–Glu(OBzl)–OBzl (11): Rf$_1$ 0.97; Rf$_2$ 0.97; mp 95—96 °C; $[\alpha]_{\mathrm{D}}^{20}$ −12° (*c* 1, AcOH). Found: C, 70.35; H, 5.97; N, 2.98%. Calcd for $C_{27}H_{27}O_6N$: C, 70.27; H, 5.90; N, 3.03%.

Z–Val–OBzl (12): Rf$_1$ 0.95; Rf$_2$ 0.95; mp 76—79 °C; $[\alpha]_{\mathrm{D}}^{20}$ −6° (*c* 1, DMF). Found: C, 70.43; H, 6.84; N, 4.02%. Calcd for $C_{20}H_{23}O_4N$: C, 70.36; H, 6.79; N, 4.10%.

Z–His–OBzl (13): Rf$_1$ 0.81; Rf$_2$ 0.91; mp 76—78 °C; $[\alpha]_{\mathrm{D}}^{20}$ −4° (*c* 1, DMF). Found: C, 66.54; H, 5.63; N, 10.94%. Calcd for $C_{21}H_{21}O_4N_3$: C, 66.48; H, 5.58; N, 11.07%.

Boc–Phe–OBzl (14): Rf$_1$ 0.95; Rf$_2$ 0.95; mp 63—64 °C; $[\alpha]_{\mathrm{D}}^{20}$ −22° (*c* 1, DMF). Found: C, 71.05; H, 7.15; N, 3.85%. Calcd for $C_{21}H_{25}O_4N$: C, 70.96; H, 7.09; N, 3.94%.

Z–Tyr–OBzl (15): Rf$_1$ 0.97; Rf$_2$ 0.97; mp 94—96 °C; $[\alpha]_{\mathrm{D}}^{20}$ −20° (*c* 1, DMF). Found: C, 68.03; H, 6.85; N, 3.68%. Calcd for $C_{21}H_{25}O_5N$: C, 67.91; H, 6.78; N, 3.77%.

Boc–Tyr(Bzl)–OBzl (16): Rf$_1$ 0.97; Rf$_2$ 0.97; mp 74—75 °C; $[\alpha]_{\mathrm{D}}^{20}$ −8° (*c* 1, DMF). Found: C, 73.04; H, 6.82; N, 2.98%. Calcd for $C_{28}H_{31}O_5N$: C, 72.86; H, 6.77; N, 3.03%.

Boc–Trp–OBzl (17): Rf$_1$ 0.95; Rf$_2$ 0.93; mp 137—139 °C; $[\alpha]_{\mathrm{D}}^{20}$ −10° (*c* 1, MeOH). Found: C, 70.14; H, 6.71; N, 7.04%. Calcd for $C_{23}H_{26}O_4N_2$: C, 70.03; H, 6.64; N, 7.10%.

Boc–Ser–OBzl (18): Rf$_1$ 0.90; Rf$_2$ 0.88; mp 59—60 °C; $[\alpha]_{\mathrm{D}}^{20}$ −19° (*c* 1, MeOH). Found: C, 61.07; H, 7.24; N, 4.68%. Calcd for $C_{15}H_{21}O_5N$: C, 61.00; H, 7.17; N, 4.74%.

Boc–Asn–OBzl (19): Rf$_1$ 0.87; Rf$_2$ 0.92; mp 80—82 °C; $[\alpha]_{\mathrm{D}}^{20}$ −24° (*c* 1, DMF). Found: C, 59.73; H, 6.93; N, 8.60%. Calcd for $C_{16}H_{22}O_5N_2$: C, 59.62; H, 6.88; N, 8.69%.

Boc–Gln–OBzl (20): Rf$_1$ 0.89; Rf$_2$ 0.94; mp 82—84 °C; $[\alpha]_{\mathrm{D}}^{20}$ −4° (*c* 1, DMF). Found: C, 60.74; H, 7.27; N, 8.24%. Calcd for $C_{17}H_{24}O_5N_2$: C, 60.70; H, 7.19; N, 8.33%.

pGlu–OBzl (21): Rf$_1$ 0.96; Rf$_2$ 0.96; mp 84—87 °C; $[\alpha]_{\mathrm{D}}^{20}$ −5° (*c* 1, DMF). Found: C, 53.75; H, 5.34; N, 5.13%. Calcd for $C_{12}H_{14}O_4N$: C, 53.73; H, 5.26; N, 5.22%.

Z-Gly-Phe-OBzl (22): Rf$_1$ 0.96; Rf$_2$ 0.93; mp 130—131 °C; $[\alpha]^{20}_{\mathrm{D}}$ $-12°$ (*c* 1, DMF). Found: C, 69.98; H, 5.93; N, 6.33%. Calcd for $C_{26}H_{26}O_5N_2$: C, 69.94; H, 5.87%; N, 6.27%.

Z-Val-Tyr-OBzl (23): Rf$_1$ 0.96; Rf$_2$ 0.92; mp 169—172 °C; $[\alpha]^{20}_{\mathrm{D}}$ $-5°$ (*c* 1, DMF). Found: C, 69.27; H, 6.56; N, 5.33%. Calcd for $C_{29}H_{32}O_6N_2$: C, 69.03; H, 6.39; N, 5.55%.

Z-Pro-Leu-OBzl (24): Rf$_1$ 0.91; Rf$_2$ 0.95; mp 69—72 °C; $[\alpha]^{20}_{\mathrm{D}}$ $-46°$ (*c* 1, DMF). Found: C, 68.85; H, 7.33; N, 6.20%. Calcd for $C_{26}H_{32}O_5N_2$: C, 69.00; H, 7.13; N, 6.19%.

Z-Gly-Ala-OBzl (25): Rf$_1$ 0.86; Rf$_2$ 0.86; mp 76—78 °C; $[\alpha]^{20}_{\mathrm{D}}$ $-15°$ (*c* 1, DMF). Found: C, 65.21; H, 6.22; N, 7.59%. Calcd for $C_{20}H_{22}O_5N_2$: C, 64.85; H, 5.99; N, 7.56%.

Boc-Ala-Glu(OBzl)-OBzl (26): Rf$_1$ 0.97; Rf$_2$ 0.92; mp 55—58 °C; $[\alpha]^{20}_{\mathrm{D}}$ $-20°$ (*c* 1, DMF). Found: C, 65.10; H, 7.09; N, 5.77%. Calcd for $C_{27}H_{34}O_7N_2$: C, 65.04; H, 6.87; N, 5.62%.

Boc-Gly-Gly-OBzl (27): Rf$_1$ 0.93; Rf$_2$ 0.86; mp 87—90 °C; $[\alpha]^{20}_{\mathrm{D}}$ 0° (*c* 1, DMF). Found: C, 59.27; H, 7.05; N, 8.55%. Calcd for $C_{16}H_{22}N_5O_2$: C, 59.61; H, 6.88; N, 8.69%.

Boc-Ile-Val-OBzl (28): Rf$_1$ 0.97; Rf$_2$ 0.93; mp 89—91 °C; $[\alpha]^{20}_{\mathrm{D}}$ $-52°$ (*c* 1, DMF). Found: C, 65.63; H, 8.89; N, 6.40%. Calcd for $C_{23}H_{36}N_5O_2$: C, 65.69; H, 8.63; N, 6.66%.

Boc-Pro-Phe-OBzl (29): Rf$_1$ 0.94; Rf$_2$ 0.95; mp 69—72 °C; $[\alpha]^{20}_{\mathrm{D}}$ $-41°$ (*c* 1, DMF). Found: C, 68.94; H, 7.26; N, 6.19%. Calcd for $C_{26}H_{32}O_5N_2$: C, 69.00; H, 7.13; N, 6.19%.

Boc-Thr-Pro-OBzl (30): Rf$_1$ 0.95; Rf$_2$ 0.91; mp 102—105 °C; $[\alpha]^{20}_{\mathrm{D}}$ $-54°$ (*c* 1, DMF). Found: C, 62.08; H, 7.38; N, 6.89%. Calcd for $C_{21}H_{30}O_6N_2$: C, 62.05; H, 7.44; N, 6.89%.

Boc-Gln-Pro-OBzl (31): Rf$_1$ 0.92; Rf$_2$ 0.90; mp 129—131 °C; $[\alpha]^{20}_{\mathrm{D}}$ $-60°$ (*c* 1, DMF). Found: C, 60.85; H, 7.41; N, 9.66%. Calcd for $C_{22}H_{31}O_6N_3$: C, 60.95; H, 7.21; N, 9.69%.

Boc-Trp-Gly-OBzl (32): Rf$_1$ 0.97; Rf$_2$ 0.95; hygroscopic powder.

Z-Gly-His-OBzl (33): Rf$_1$ 0.93; Rf$_2$ 0.93; mp 63—66 °C; $[\alpha]^{20}_{\mathrm{D}}$ $-25°$ (*c* 1, DMF). Found: C, 63.32; H, 5.58; N, 12.86%. Calcd for $C_{23}H_{24}O_5N_4$: C, 63.29; H, 5.54; N, 12.84%.

Boc-Ser-Ile-OBzl (34): Rf$_1$ 0.93; Rf$_2$ 0.91; mp 72—75 °C; $[\alpha]^{20}_{\mathrm{D}}$ $-18°$ (*c* 1, DMF). Found: C, 61.91; H, 7.95; N, 7.01%. Calcd for $C_{21}H_{32}O_6N_2$: C, 61.74; H, 7.90; N, 6.86%.

Z-Lys(Z)-Pro-OBzl (35): Rf$_1$ 0.90; Rf$_2$ 0.86; oily form.

Boc-Met-Pro-OBzl (36): Rf$_1$ 0.96; Rf$_2$ 0.90; oily form.

Boc-Phe-Asp(OBzl)-OBzl (37): Rf$_1$ 0.93; Rf$_2$ 0.93; mp 99—101 °C; $[\alpha]^{20}_{\mathrm{D}}$ $-15°$ (*c* 1, DMF). Found: C, 68.51; H, 6.64; N, 4.98%. Calcd for $C_{32}H_{36}O_7N_2$: C, 68.56; H, 6.47; N, 5.00%.

pGlu-Phe-OBzl (38): Rf$_1$ 0.78; Rf$_2$ 0.83; mp 102—104 °C; $[\alpha]^{20}_{\mathrm{D}}$ $-9°$ (*c* 1, DMF). Found: C, 65.69; H, 6.02; N, 7.28%. Calcd for $C_{21}H_{23}O_5N_2$: C, 65.78; H, 6.05; N, 7.31%.

Boc-Pro-Pro-OBzl (39): Rf$_1$ 0.84; Rf$_2$ 0.85; hygroscopic powder.

Boc-Val-Val-Val-Pro-Pro-OBzl (40): Rf$_1$ 0.93; Rf$_2$ 0.85; mp 150—153 °C; $[\alpha]^{20}_{\mathrm{D}}$ $-171°$ (*c* 1, DMF). Found: C, 63.55; H, 8.22; N, 10.14%. Calcd for $C_{37}H_{57}O_8N_5$: C, 63.48; H, 8.22; N, 10.01%.

Preparation of *S*-Benzyl Cysteine. Conventional Method: H-Cys-OH·HCl (1.57 g, 10 mmol) and 2 M NaOH (20 ml) were dissolved in water (50 ml). Benzyl bromide (2.57 g, 15 mmol) was added, and the ice-cooled reaction mixture was stirred. The reaction mixture was adjusted to pH 5 with acetic acid. The precipitate was filtered off and the filtrate cake was washed with water and 2 M NaOH–ethanol. The rough crystals were recrystallized from dilute ammonia water containing ethanol and neutralized by dilute hydrogenchloric acid; mp 210—212 °C; $[\alpha]^{20}_{\mathrm{D}}$ 26° (*c* 1, 1 M NaOH).

HOBMCl Method: H-Cys-OH·HCl (0.47 g, 3 mmol) and base (3 mmol) were dissolved in aqueous–methanol media (6 ml), and stirred at room temperature. After 10 min, HOBMCl (1.20 g, 4.5 mmol) was added to the solution and it was stirred for 8 h. Inorganic salts in the reaction mixture were filtered off and the filtrate was evaporated in vacuo. The residue was dissolved in toluene and washed with 0.5 M NaOH aqueous solution and then with water in order to remove the by-product, 4-(methylthio)phenol. The organic layer was dried over anhydrous sodium sulfate and the filtrate was evaporated in vacuo. The residue was crystallized by the aid of petroleum ether; mp 211—213 °C; $[\alpha]^{20}_{\mathrm{D}}$ 26° (*c* 1, 1 M NaOH).

Racemization Test. A model peptide, Gly-L-Ala-L-Leu (**41**), was prepared as shown in Fig. 1. Amino acid analysis of hydrogenated peptide showed that the coupling based on the azide method via benzyl ester prepared by the use of HOBMCl produced only the L-L isomer. A chromatographic detection of this diastereometric tripeptide was carried out with HPLC (column: AA pack Na; eluent: 0.1 M citrate buffer, pH 3.25, 60 °C). The detection of the tripeptide was performed by OPA detection system. Authentic samples of Gly-L-Ala-L-Leu and Gly-D-Ala-L-Leu were prepared by the usual manner, as shown in Fig. 2.

Application to Synthesis of Boc-Pro-D-Phe-Pro-Ile-Ile-Val-OBzl. Boc-Pro-D-Phe-OBzl (43): Compound **43** was prepared from **42** by using HOBMCl, as described in the preparation of **5**.

Boc-Pro-D-Phe-N_2H_3 (44): To a solution of **43** (4.53 g, 10 mmol) in methanol (20 ml) was added hydrazine hydrate (2.5 ml, 50 mmol). The mixture was left at room temperature for 2 d and evaporated. The resulting crystalline residue was recrystallized from ether–petroleum ether. It was recrystallized from methanol–ether–petroleum ether.

Boc-Pro-D-Phe-Pro-Ile-Ile-Val-OBzl (46): Compound **44** (1.51 g, 4 mmol) was dissolved in 1 M HCl (10 ml) and acetic acid (27 ml), and cooled at −15 °C. To this solution was added $NaNO_2$ (0.30 g, 4.4 mmol). The solution was allowed to stand at −15 °C for 5 min; chilled water was added, and the solution was stirred at the same temperature for 30 min. The solution was extracted with EtOAc, the organic layer was washed with chilled 10% sodium hydrogencarbonate, dried over anhydrous sodium sulfate, and filtered. The filtrate was added to a precooled solution of H-Pro-Ile-Ile-Val-OBzl·HCl (**45**) (2.27 g, 4 mmol) in DMF (40 ml). The mixture solution was stirred below 0 °C for 3 d and evaporated. The residue was dissolved in EtOAc, washed with 4% sodium hydrogencarbonate, 4% potassium hydrogensulfate and water, dried over anhydrous sodium sulfate, and evaporated. The oily residue was crystallized from ether-petroleum ether.

The physicochemical properties of the synthetic intermediates in the azide coupling reaction described above are listed in Table 8.

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References

1) This is one part of studies on the water-soluble active ester method for peptide synthesis. This previous studies (Part I, II, III, and IV) appeared in this journal. See Refs. 3, 4, 5, and 6.

2) Abbreviations used are according to IUPAC-IUB Commissions, [*Eur. J. Biochem.*, **138**, 9 (1984)]. Amino acid symbols except glycine denoted L-configuration. Additional abbreviations: Boc, *t*-butoxycarbonyl; Z, benzyloxycarbonyl; HODSP, (*p*-hydroxyphen-

yl)dimethylsulfonium sulfate; HOBMCl, (*p*-hydroxyphenyl)benzylmethylsulfonium chloride; Bzl, benzyl; TEA, triethylamine; Py, pyridine; DMF, *N*, *N*-dimethylformamide; DCM, dichloromethane; OPA, *o*-phthalaldehyde; *p*-Tos, *p*-toluenesulfonic acid; NMM, *N*-methylmorpholine; DCHA, dicyclohexylamine; CHA, cychlohexylamine.

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