The Heteroselective Solvation of Protected Hexapeptides in a Variety of Mixed Solvents and the Criteria for the Choice of Mixed Solvents Effective for Peptide and Protein Synthesis[1)]

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Focusing our attention on both the van der Waals interactions between solvents and peptide side chains and the electron donor–acceptor interactions between mixed solvents, the solubility and conformation in a variety of mixed solvents were examined for protected hexapeptide fragments of *E. coli* ribosomal protein L7/L12. Although it was difficult to precisely evaluate the van der Waals interactions between solvents and peptide side chains, it was confirmed that the van der Waals interactions between solvents and peptide side chains were important for the solvation of protected peptides in mixed solvents. The electron donor–acceptor interactions between mixed solvents remarkably decreased the β-sheet-structure-disrupting potential of a variety of mixed solvents. In connection with the β-sheet-structure-stabilizing potential, $<SP_\beta>$, of protected peptides, it was also confirmed that the $<SP_\beta>$ value of protected peptides properly reflected their β-sheet-structure stability in a variety of mixed solvents. The criteria for the choice of mixed solvents effective in peptide and protein synthesis was led on the basis of both the heteroselective solvation mechanism in mixed solvents and the electron donor–acceptor interactions between mixed solvents. The significance of the present study in the choice of effective solvents for liquid- and solid-phases peptides synthesis is elaborately discussed.

In the study of the β-sheet-structure-disrupted behavior of Boc–Val–Gly–Phe–Gly–Leu–Ile–Leu_2–OBzl in a three component solvent system, it was elucidated that the electron donor–acceptor interaction between mixed solvents remarkably decreased the β-sheet-structure-disrupting potential of mixed solvents.[2,3)] The generality of the results obtained above was further confirmed by conformational analysis of a variety of resin-bound protected peptides in mixed solvents, although the concentrating efficacy of a cross-linked resin reinforced the β-sheet-structure stability of resin-bound peptides.[3—6)] On the basis of the results, the criteria for the choice of effective solvents for peptide and protein synthesis were proposed.

On the other hand, the solvation of protected peptides principally proceeds through intermolecular interactions between solvents and peptides, and the solvation mechanism in a single organic solvent could be explained by two types of intermolecular interactions, namely, intermolecular hydrogen bonding and van der Waals interactions.[7)] In liquid- and solid-phases peptide synthesis, sufficient solvation of peptide chains is essential for the smooth achievement of chain elongation, purification, and homogeneity assessment of growing peptides.[8—11)] Mixing of organic solvents often results in a remarkable increase in solubility and mixed solvents are commonly used in coupling reactions between large protected peptides.[12,13)] Especially, a mixture of DMF, NMP, DMSO, and HMPA has been the best solvent combination as far as we know.[2—4)] The term "heteroselective solvation" was coined for solvation of metal salts in aqueous solution.[17—19)] In this paper, heteroselective solvation is used for solvation of protected peptides in mixed solvents as shown in Fig. 1. It proceeds through both electron donor–acceptor interactions and van der Waals interactions in which the electron donor–acceptor interactions are orthogonal to the van der Waals interactions. The elucidation of the solvation mechanism of protected peptides in mixed solvents is expected to provide a basis for the choice of mixed solvents effective for synthesis.

In this study, focusing our attention on both the electron donor–acceptor interactions between mixed solvents and the van der Waals interactions between solvent and peptide side chains, we examine the solubility and conformation of protected peptides in a variety of mixed solvents and discuss the solvation mechanism of protected peptides. In addition, the choice of mixed solvents effective for peptide and protein synthesis is contemplated on the basis of the present study.

Experimental

Materials. The protected hexapeptides studied are those previously used for the estimation of the β-sheet-structure stability of protected peptides.[14)]

IR Absorption Spectra Measurements. IR absorption spectra measurements were carried out as described in a previous paper.[14)]

Results

The solubility and conformation in mixed solvents were examined for the following protected hexapeptides: Boc–Leu–Lys(Z)–Glu(OBzl)–Ala–Lys(Z)–Asp(OBzl)–OPac, **1**; Boc–Ala–Ala–Leu–Lys(Z)–Glu(OBzl)–Gly–OPac, **2**; Boc–Val–Glu(OBzl)–Ala–Ala–Glu(OBzl)–Glu(OBzl)–OPac, **3**; Boc–Val–Ala–Val–Ala–Ala–Gly–OPac, **4**; Boc–Ile–Ile–Glu(OBzl)–Ala–Val–Ala–OPac, **5**. They are fragments of *E. coli* ribosomal protein

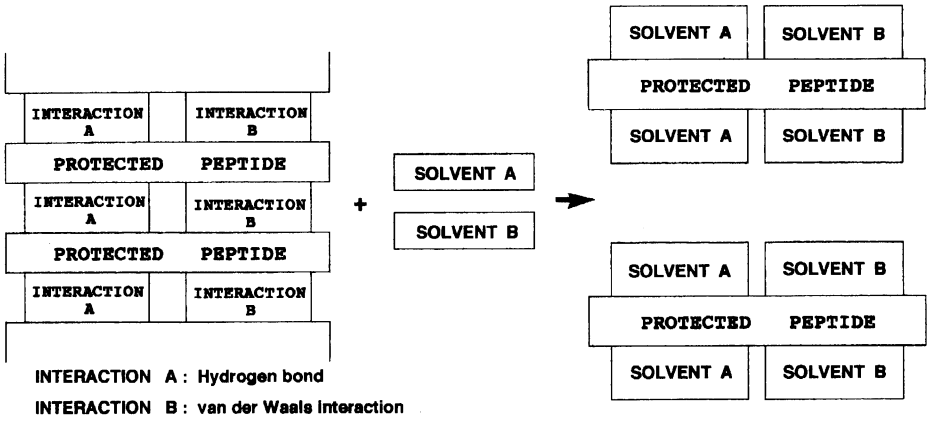

Fig. 1. Schematic view of the heteroselective solvation mechanism of protected peptides in mixed solvents. Solvent "A" participates in hydrogen bonding with peptide main chains and Solvent "B" participates in van der Waals interactions with peptie side chains.

Table 1. The Solubility [a] and Conformation [b] of Peptides **1**—**5** in a Mixture of TBPO with Benzene, CCl_4, Hexane, Et_2O, or Et_3N

		TBPO									
	$<SP_\beta>$	Benzene		CCl_4		Hexane		Et_2O		Et_3N	
LKEAKD	3.0	r	A	β	B	β	C	β(s)/r	B*	r	C
AALKEG	4.0	r	A	β(s)/r	B*	β(s)/r	C	β(s)/r	C	β(s)/r	C
VEAAEE	4.2	r	A	β(s)/r	B	β(s)/r	B*	r	B*	β/r	C
IIEAVA	4.8	β/r(s)	C	β/r(s)	C	β	C	β	C	β	C
VAVAAG	5.3	β	B	β/r	C	β	C	β	C	β	C

a) Solubility: A, completely soluble at room temperature; B, completely soluble at 80°C or refluxing temperature with no deposit after cooling to room temperature; B*, completely soluble at 80°C or refluxing temperature with deposit after cooling to room temperature; C, partially or nearly insoluble at 80°C or refluxing temperature. b) Conformation: β, β-sheet structure; r, random and/or α-helix structure; (s), small peak.

L7/L12.[14] The solubility (c=1.0 g·dl^{-1}) was divided into the following four classes: (A) completely soluble at room temperature, (B) completely soluble at 80°C or refluxing temperature with no deposit after cooling to room temperature, (B*) completely soluble at 80°C or refluxing temperature with deposit after cooling to room temperature, and (C) partially or nearly insoluble at 80°C or refluxing temperature.

Typical IR absorption spectra of protected peptides in mixed solvents are presented in Fig. 2. The bands around 3280 and 1630 cm^{-1} are assigned to a β-sheet structure, and those around 3420 and 1670 cm^{-1} to an unordered structure. The peaks around 3320 and 1660 cm^{-1} are mainly due to intramolecular hydrogen bonding.[15,16] In order to summarize the results of the IR absorption spectra of the peptide in Tables 1, 2, 3, 4, and 5, the IR absorption spectra of the peptides were classified into five groups. The first group (Fig. 2a) showed a strong band around 1630 cm^{-1} together with no or a weak shoulder band around 1670 cm^{-1}. They are referred to as β in the tables. The second group (Fig. 2b) exhibited a strong band around 1630 cm^{-1} and a medium shoulder band around 1670 cm^{-1} and are referred to as β/r(s), the third (Fig. 2c) had medium bands around both 1630 and 1670 cm^{-1} (β/r), and the fourth (Fig. 2d) had a medium shoulder band around 1630 cm^{-1} and a strong band around 1670 cm^{-1} (β(s)/r). The fifth group (Fig. 2e) showed a strong band around 1670 cm^{-1} accompanied by no or a weak shoulder band at 1630 cm^{-1} (r). Tables 1, 2, 3, 4, and 5 summarize the results of the solubility and conformation of the protected peptides in a variety of mixed solvents along with the $<SP_\beta>$ values of the peptides.

Table 2. The Solubility [a] and Conformation [b] of Peptides **1**—**5** in a Mixture of Benzene with DMSO, TBPO, HFIP, or Phenol

		Benzene							
	$<SP_\beta>$	DMSO		TBPO		HFIP		Phenol	
LKEAKD	3.0	r	B	r	A	r	A	r	A
AALKEG	4.0	β/r	A	r	A	r	A	r	A
VEAAEE	4.2	r	A	r	A	r	A	r	A
IIEAVA	4.8	β	C	β/r(s)	C	r	C	r	C
VAVAAG	5.3	β	C	β	B	r	A	r	A

a) Solubility and b) Conformation, see Table 1.

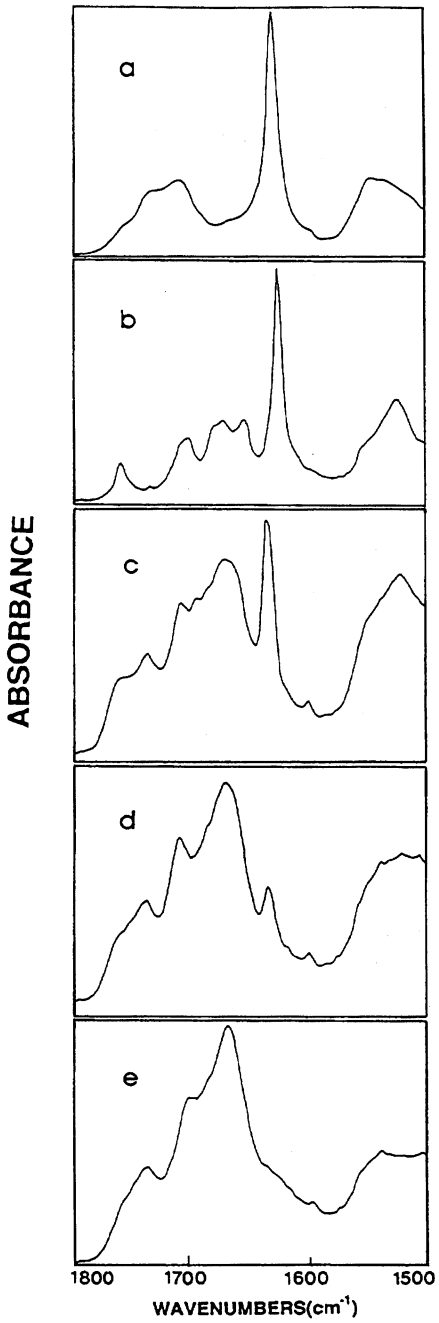

Fig. 2. The classification of typical IR absorption spectra in the amide I region of the peptides in Tables 1, 2, 3, 4, and 5. a) LKEAKD in THF/HFIP, β; b) LKEAKD in $CHCl_3$/TBPO, β/r(s); c) AALKEG in THF/HFIP, β/r; d) VEAAEE in $CHCl_3$, β(s)/r; e) LKEAKD in THF/DMSO, r.

Discussion

The classification of organic solvents in a previous paper was practically explained by their AN and DN and suggested that the hydrogen bonding ability of solvents was much more important in the solvation of peptide bonds in protected peptides than their van der Waals interaction ability.[7] Nevertheless, the insolubility of protected peptides in H_2O strongly indicated that the van der Waals interactions between organic solvents and peptide side chains were also important as well. The favorable results of the solubility and conformation of peptides **1**—**5** in a mixture of TBPO with benzene, CCl_4, hexane, Et_2O, or Et_3N (first group solvents in the classification of organic solvents) also suggest that the van der Waals interactions are important for the solvation of protected peptides since mixing of TBPO with a first group solvent does not change the hydrogen-bonding ability of TBPO. A mixture of TBPO with benzene or CCl_4 has a high solvating potential for protected peptides, while that of TBPO with hexane, Et_2O, and Et_3N has a low potential. This fact indicates that the van der Waals interaction ability of benzene and CCl_4 is larger than that of hexane, Et_2O, and Et_3N and that the solvation mechanism of protected peptides in mixed solvents can be explained by two types

Table 3. The Solubility [a] and Conformation [b] of Peptides **1**—**5** in a Mixture of Dioxane with DMSO, TBPO, HFIP, or Phenol

		Dioxane							
	<SP_β>	DMSO		TBPO		HFIP		Phenol	
LKEAKD	3.0	r	A	r	A	β	B	r	B
AALKEG	4.0	r	A	r	A	β/r(s)	B	β(s)/r	B
VEAAEE	4.2	r	A	r	A	β(s)/r	A	β(s)/r	A
IIEAVA	4.8	β/r	C	r	C	β/r(s)	C	β	C
VAVAAG	5.3	β	B	β/s	B	β	C	β	B

a) Solubility and b) Conformation, see Table 1.

Table 4. The Solubility [a] and Conformation [b] of Peptides **1**—**5** in a Mixture of THF with DMSO, TBPO, HFIP, or Phenol

		THF							
	<SP_β>	DMSO		TBPO		HFIP		Phenol	
LKEAKD	3.0	r	A	r	A	β	B	β/r(s)	A
AALKEG	4.0	r	A	r	A	β/r	B	β/r(s)	A
VEAAEE	4.2	r	A	r	A	β/r	B	r	A
IIEAVA	4.8	r	C	r	C	β	C	β	C
VAVAAG	5.3	r	C	r	C	β	C	β	C

a) Solubility and b) Conformation, see Table 1.

Table 5. The Solubility [a] and Conformation [b] of Peptides **1**—**5** in a Mixture of $CHCl_3$ with DMSO, TBPO, HFIP, or Phenol

		$CHCl_3$							
	<SP_β>	DMSO		TBPO		HFIP		Phenol	
LKEAKD	3.0	r	A	β/r(s)	B	r	A	r	A
AALKEG	4.0	r	B	r	B	r	A	r	A
VEAAEE	4.2	β(s)/r	A	β(s)/r	B	r	A	r	A
IIEAVA	4.8	β(s)/r	C	β/r(s)	C	r	A	r	A
VAVAAG	5.3	β	C	β	C	r	A	r	A

a) Solubility and b) Conformation, see Table 1.

of intermolecular interactions, namely, intermolecular hydrogen bonding (N–H···O=P) and van der Waals interactions (between peptide side chains and benzene or CCl_4). In fact, IR absorption spectra of typical peptides in a mixture of TBPO and benzene (Fig. 2) have no bands around 3280 or 1630 cm^{-1}, indicating that they are free from a β-sheet structure in the mixture. The hydrogen-bonding interaction between the peptide main chain and the solvent occurs through the electron donor–acceptor interactions, so that the interactions can be regarded as orthogonal to the van der Waals interactions. Thus, the solvation of protected peptides in mixed solvents appears to proceed heteroselectively as depicted in Fig. 1. The term "heteroselective solvation" was used in the solvation mechanism of metal salts in mixed solvents[17—19] and, in this case, "heteroselective" means that hydrogen bonding and van der Waals interactions occur independently.

The heteroselective solvation mechanism also suitably explains the fact that some deca- to tetradecapeptides are soluble neither in TFE nor HFIP, but are easily soluble in a mixture of CH_2Cl_2 and TFE or HFIP (9/1, v/v).[3,5,20] In the mixture, peptide side chains are solvated to CH_2Cl_2 by van der Waals interactions and peptide C=O groups are solvated to TFE and HFIP by hydrogen bonding (C=O···H–O). In addition, a mixture of DMF, NMP, DMSO, and HMPA is commonly used for coupling reactions between large protected peptides. A mixture of these solvents does not change their hydrogen-bonding ability. Hence, the remarkable increase in the solubility in the mixed solvent is due to the van der Waals interactions between peptide side chains and the solvents. In fact, a significant difference in the ability to form van der Waals interactions with hydrophobic helical peptides was observed among DMF, NMP, DMSO, and HMPA.[6] The order of their solvating potentials for hydrophobic helical peptides is as follows: HMPA > NMP > DMF > DMSO. The helical peptides examined have hydrocarbon substitutes as peptide side chains and the order is in harmony with the fact that hexane is miscible with HMPA in all proportions and with DMSO in limited ones.[3,6]

The investigation of the solubility and conformation of protected peptides in a variety of mixed solvents also clarifies the influence of the electron donor–acceptor interactions between mixed solvents on their solvating potential for protected peptides. The solubilizing potential of benzene (a first group solvent)[7] for protected peptides is extremely low due to its poor electron donor–acceptor interactions (AN 8.2, DN 0.1).[21] Thus, the electron donor–acceptor interaction between benzene and a mixed solvent can not be expected. Table 2 summarizes the results of the solubility and conformation of peptides **1**—**5** in a mixture of benzene with DMSO, TBPO, or phenol. In fact, HFIP (AN 88) and phenol (AN 70) function as strong electron-acceptor solvents in benzene and IR absorption spectra of the peptides in a mixture of benzene and HFIP (Table 2) have no bands around 3280 or 1630 cm^{-1}, indicating that they are free from a β-sheet structure in this mixture. In addition, DMSO (DN 29.8) and TBPO (DN 40) function as electron-donor solvents in benzene. The existence of a β-sheet structure in a mixture of benzene with DMSO or TBPO is due to the high stability of the β-sheet structure of peptides **4** and **5**, which have $<SP_\beta>$ values of 4.8 and 5.3, respectively. In fact, they exist in a mixture of β-sheet and unordered structures even in DMSO alone. The properties of CCl_4 (AN 8.6, DN ca. 0) resemble those of benzene and the results in a mixture of CCl_4 with DMSO, TBPO, HFIP, or phenol are similar to those obtained above in benzene.

The solubilizing potential of dioxane for protected peptides is rather low and it is classified in the second group. The AN and DN of dioxane are 10.8 and 14.8, respectively, and electron donor–acceptor interactions with strong electron-acceptor solvents such as HFIP and phenol are expected. In fact, the results in a mixture of dioxane with HFIP or phenol are in contrast to those obtained in a mixture of benzene with HFIP or phenol. In the mixtures, hydrogen bonding between the oxygen of dioxane and the hydroxyl groups of HFIP and phenol occurs through electron donor–acceptor interactions and the β-sheet-structure-disrupting potential of the mixed solvents decreases remarkably (Table 3). THF is classified in the fourth group and its AN and DN are 8.0 and 20.0, respectively. Similar to the results obtained in a mixture of dioxane with HFIP or phenol, electron donor–acceptor interactions clearly occur in a mixture of THF with HFIP or phenol (Table 4). On the other hand, DMSO and TBPO effectively function as electron-donor solvents in dioxane and THF. It is noteworthy that peptides **4** and **5** have an unordered structure in a mixture of THF with DMSO. Since the van der Waals interaction ability of DMSO and TBPO is rather low, the heteroselective solvation mechanism of protected peptides in a mixture of THF with DMSO or TBPO is reliable. In mixed solvents, peptide side chains are solvated mostly to THF by van der Waals interactions and peptide N–H groups are solvated predominantly to the O=S and O=P groups by hydrogen bonding.

CH_2Cl_2 and $CHCl_3$ are also classified in the fourth group and their solubilizing potential is similar to that of THF. The AN and DN of CH_2Cl_2 are 20.4 and nearly zero, respectively, and those of $CHCl_3$ are 23.1 and nearly zero, respectively. For both solvents, electron donor–acceptor interactions are expected with strong electron-donor solvents such as DMSO and TBPO. Practically, in many cases, a β-sheet structure is observed in a mixture of $CHCl_3$ with DMSO or TBPO (Table 5). This is in contrast to the results obtained in a mixture of THF with DMSO or TBPO. The electron donor–acceptor interactions between CH_2Cl_2 and DMSO in this study are not as clear as those observed

for resin-bound peptides in a previous paper.[2] The difference is clearly due to the concentrating efficacy of a cross-linked resin. On the other hand, HFIP and phenol effectively function as electron-acceptor solvents in CH_2Cl_2 and $CHCl_3$.

The $<SP_\beta>$ values of the protected peptides in Tables 1, 2, 3, 4, and 5 are defined as the arithmetic average of the β-sheet-structure-stabilizing potentials, SP_β, of the amino acid residues composing a protected peptide and represents the stability of the β-sheet structure of a protected peptide in organic solvents.[22] The results in this study also indicate that, even in a variety of mixed solvents, the $<SP_\beta>$ values of protected peptides clearly reflect their β-sheet-structure stability.

The solvation mechanism in solid-phase peptide synthesis is essentially the same as the one in liquid-phase peptide synthesis and, in both syntheses, the choice of effective solvents is an important factor in the achievement of peptide synthesis, namely, chain elongation, purification, homogeneity assessment, and final deprotection of growing peptides. The present study clearly shows that the consideration of both the heteroselective solvation mechanism and the electron donor–acceptor interactions between mixed solvents is a benefit to the choice of mixed solvents effective for solid- and liquid-phases peptide syntheses. Accordingly, criteria for the choice of mixed solvents can be derived as follows: (1) Mixed solvents should be chosen to be suitable for heteroselective solvation. In the solvation mechanism, one part of the mixed solvents participates in van der Waals interactions with peptide side chains and the other part of the mixed solvents participates in hydrogen bonding with peptide main chains. (2) The combination of electron-donor solvents with other electron-donor solvents or electron-acceptor solvents with other electron-acceptor solvents retains the hydrogen-bonding ability and is suitable for peptide synthesis. (3) The combination of an electron-donor solvent and an electron-acceptor solvent decreases the hydrogen-bonding ability and is not suitable for the solvation of protected peptides, but may be suitable for the precipitation of protected peptides from solution. The concept of heteroselective separation can also be applied to the separation mechanism of HPLC analysis.

Based on the criteria for the choice of mixed solvents, we can determine mixed solvents effective for each step in peptide synthesis. In a previous paper,[6] we found a mixture of CH_2Cl_2 and TFE to be effective for coupling reactions between protected large peptides which were insoluble in DMF, NMP, DMSO, and HMPA. The present study indicates that a mixture of THF and TBPO is superior to that of CH_2Cl_2 and TFE since TFE has a high nucleophilicity and actually reacts with activated carboxyl components. In solid-phase peptide synthesis, the addition of HFIP was also recommended for the completion of coupling reactions of difficult sequences.[4,5] For the removal of the Boc group, the combination of TFA with CH_2Cl_2 is commonly used and it is reasonable for the heteroselective solvation mechanism. Liquid NH_3 (DN 59) is expected to have a high solubilizing potential for protected peptides. Practically, it is commonly used as a solvent for final deprotection of peptides by hydrogenolysis with sodium metal.[23] When the solubility of peptides in NH_3 is poor, the addition of THF may be effective to bring about an increase in the solubility. Hydrogenolysis using Pd/C as a catalyst is also often used for final deprotection of peptides.[24] Halogen compounds prevent hydrogenolysis so effectively that the use of *m*-cresol (AN ca. 70) as a solvent is expected to further the hydrogenolysis successfully without the trouble of insolubility of peptides. The AN of HF, TFMS, and MS is over 100 and actually they are good solvents for protected peptides. Final deprotection is commonly carried out in these solvents. The criteria for the choice of mixed solvents can also be successfully applied to the purification process and homogeneity assessment.

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References

1) The abbreviations for amino acids are recommended by the IUPAC-IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.*, **247**, 977 (1972). Amino acid symbols except for Gly denote the L-configuration. Additional abbreviations used are the following: Et_3N, triethylamine; Et_2O, ethylether; THF, tetrahydrofuran; DMF, *N,N*-dimethylformamide; NMP, *N*-methyl-2-pyrrolidinone; DMSO, dimethyl sulfoxide; TBPO, tributylphosphine oxide; TFE, 2, 2, 2- trifluoroethanol; HFIP, 1, 1, 1, 3, 3, 3- hexafluoro- 2-propanol; HMPA, hexamethylphosphoric triamide; HF, hydrofluoric acid; TFMS, trifluoromethanesulfonic acid; MS, methanesulfonic acid; Pd/C, palladium carbon; Boc, *t*-butoxycarbonyl; Pac, phenacyl; Bzl, benzyl; Z, benzyloxycarbonyl; IR, infrared.

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