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Soluble α-Amino Acid Salts in Acetonitrile: Practical Technology for the Production of Some Dipeptides

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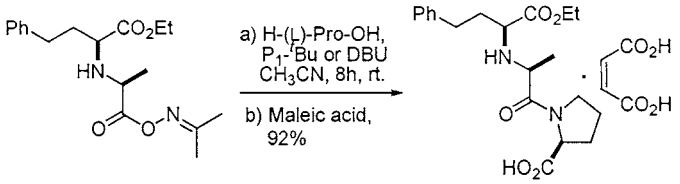
ABSTRACT



α-Amino acids are soluble in acetonitrile when treated with phosphazene bases. As a result, the protection/deprotection events that are usually required for peptide coupling reactions can be minimized. This is illustrated in the synthesis of the important angiotensin-converting enzyme (ACE) inhibitor enalapril.

Due to their versatility, amino acids have been extensively used in synthesis; furthermore, their activation, protection, and deprotection are well documented.[1] Although in peptide synthesis protection and deprotection are normally necessary to prevent autocondensation or secondary reactions, in certain cases, some of these additional steps might be avoided if amino acids were more soluble in organic media. Unfortunately, the zwitterionic character of α-amino acids renders this task quite difficult. Here we disclose a solution to this problem and its application to the synthesis of some dipeptides.

Typical procedures for the preparation of BOC- and Cbz-amino acids starting from the zwitterionic forms imply the use of sodium hydroxide and water to achieve initial solubilization. Next, the resulting N-protected amino acids, after carboxyl group activation, are coupled with another α-amino acid whose carboxyl group has been previously protected as an ester function. With this scheme in mind, it appears that the use of organic bases could give the same result in organic media but, more significantly, without the need to protect the second amino acid.

To check this hypothesis, the choice of the base was critical. As expected, conventional inorganic bases were completely inefficient; neutral organic bases were required that, on one hand, should be strong enough to remove the proton from the amino acid zwitterion and, on the other hand, should not be able to deprotonate the α-stereocenter of the resulting soluble salt, which would result in undesirable racemization.[2] Phosphazene bases (Schwesinger bases), whose synthesis and properties have been recently described, proved to be suitable.[3] These compounds have been proposed as an alternative to organometallic bases for the deprotonation of weakly acidic compounds.[4,5] They are strong bases (pk_{BH^+}

(1) Bodanszky, M. *Principles of Peptide Synthesis*; Springer-Verlag: Berlin, 1993. (b) Lloyd-Williams, P.; Albericio, F.; Giralt, E. *Chemical Approaches to the Synthesis of Peptides and Proteins*; CRC Press: New York, 1997.

(2) This goal can also be achieved by using common organic bases such as Hunnig's base, DBU, or tri-*n*-butylamine in *N*,*N*-dimethylformamide as the solvent, but under these conditions, only a few amino acids are solubilized.

(3) (a) Schwesinger, R. *Chimia* **1985**, *39*, 269−272. (b) Schwesinger, R. *Nachr. Chem. Technol. Lab.* **1990**, *38*, 1214−1226. (c) Schwesinger, R. In *Encyclopedia of Reagents for Organic Synthesis*; Paquette, L., Ed.; Wiley: New York, 1995; Vol. 6, p 4110. (d) Schwesinger, R.; Schlemper, H.; Hasefratz, C.; Willaredt, J. W.; Dambacher, T.; Breuer, T.; Ottaway, C.; Fletschinger, M.; Boele, J.; Fritz, H.; Putzas, D.; Rotter, H. W.; Bordwell, F. G.; Satish, A. V.; Ji, G.-Z.; Peters, E.-M.; Peters, K.; von Schnering, G. H.; Walz, L. *Liebigs Ann.* **1996**, 1055−1081.

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values between 33 and 42 on the acetonitrile scale), hindered, and kinetically very stable.[3d,6]

We first investigated the optimal conditions for solubilization and found that the nature of the base and the solvent was critical. The results with some representative (L)-amino acids revealed that the best conditions for solubilization were the use of P_1-^{t}Bu or P_2-Et as the base, albeit in particular cases 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and 2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphine (BEMP) were equally effective (Figure 1).

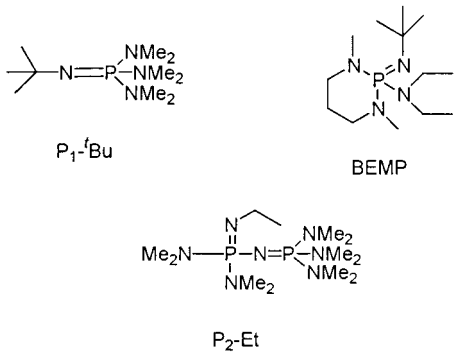


Figure 1. Representative phosphazene bases for α-amino acid solubilization in organic media.

Acetonitrile was the most effective solvent; halogenated solvents such as methylene chloride and dichloroethane were unsuitable because of the presence of competitive reactions. Other common organic solvents such as diethyl ether, dioxane, tetrahydrofuran, and toluene were also ineffective.

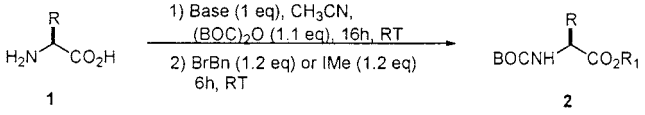
To check the extent of racemization during this solubilization process, several representative α-amino acid phosphazene salts were submitted to treatment with $(BOC)_2O$ (1.1 equiv) at room temperature overnight, followed by one-pot esterification with methyl iodide or benzyl bromide. For comparative purposes, the racemic products were also prepared following the same sequence. Importantly, comparison of the HPLC chromatograms of the racemic and the optically active products revealed that no racemization takes place during the solubilization process.

(4) For recent applications of these and related bases in α-amino acid chemistry, see the following. For the perbenzylation and perallylation of some N-protected peptides: (a) Pietzonka, T.; Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1481. (b) Seebach, D.; Beck, A. K.; Studer, A. In *Modern Synthetic Methods*; Ernst, B., Leumann, C. H., Eds.; Verlag: Basel, 1955; pp 3−178. For the alkylation of glycine derivatives, see: (c) O'Donnell, M. J.; Zhou, C.; Scott, W. L. *J. Am. Chem. Soc.* **1996**, *18*, 6070. (d) O'Donnell, M. J.; Delgado, F.; Pottorf, R. S. *Tetrahedron* **1999**, *55*, 6347.

(5) (a) Legrand, O. *Synlett* **1999**, 752. (b) Mandani, H. T.; Hartley, R. C.; *Tetrahedron Lett.* **2000**, *41*, 747. (c) Clark, A. J.; Al-Faiyz, Y. S. S.; Patel, D.; Broadhurst, M. J. *Tetrahedron Lett.* **2001**, *42*, 2007. (d) Palomo, C.; Oiarbide, M.; López, R.; Gómez-Bengoa, E. *Tetrahedron Lett.* **2000**, *41*, 1283. (e) Kraus, G. A.; Zhang, N.; Verkade, J. G.; Nagarajan, M.; Kisanga, P. B. *Org. Lett.* **2000**, *2*, 2409. (f) Baxendale, I. R.; Lee, A.-L.; Ley, S. V. *Synlett* **2001**, 1482. (g) Fruchart, J.-S.; Gras-Masse, H.; Melnyk, O. *Tetrahedron Lett.* **2001**, *42*, 9153. (h) Solladié-Cavallo, A.; Liptaj, T.; Schmidtt, M.; Solgadi, A. *Tetrahedron Lett.* **2002**, *43*, 415.

(6) For the basicity of phosphazene bases in tetrahydrofuran, see: Rodima, T.; Kaljurand, I.; Pchl, A.; Mäemets, V.; Leito, I.; Koppel, I. A. *J. Org. Chem.* **2002**, *67*, 1873.

Table 1. Conservation of the Chiral Integrity of Representative α-L-Amino Acids during the Solubilization Process with Schwesinger Bases



1 2

			compound **2**	
amino acid, **1**	R	base	R_1	%L:%D[a]
Gly	−H	P_2-Et		
Phe	$-CH_2Ph$	P_1-^{t}Bu	Me	≥99%:≤1%
Phg	−Ph	P_1-^{t}Bu	Bn	≥99%:≤1%
Ser	$-CH_2OH$	P_2-Et	Bn	≥99%:≤1%
Pro	$-(CH_2)_3-$	P_1-^{t}Bu	Bn	≥99%:≤1%
Met	$-(CH_2)_2SMe$	P_1-^{t}Bu	Bn	≥99%:≤1%
Tyr	$-CH_2$-*p*-OH−Ph	P_2-Et	Bn	≥99%:≤1%
Glu[b]	$-(CH_2)_2CO_2H$	P_1-^{t}Bu	Bn	≥99%:≤1%

[a] Determined by analytical HPLC (Chiralcel OD 250 × 4.6 mm, hex: ^{i}PrOH, flow rate = 0.5 mL/min, UV detection at 254 nm). [b] Both acid groups were benzylated using 2 equiv of base and 2.4 equiv of BnBr.

As shown in Table 1, α-amino acids with functional groups are also tolerated, and even phenylglycine, an amino acid very prone to racemization, does not racemize (Figure 2) under the conditions examined. From these data, it is likely

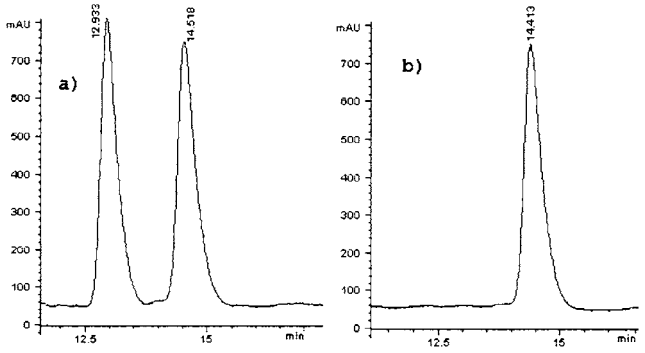


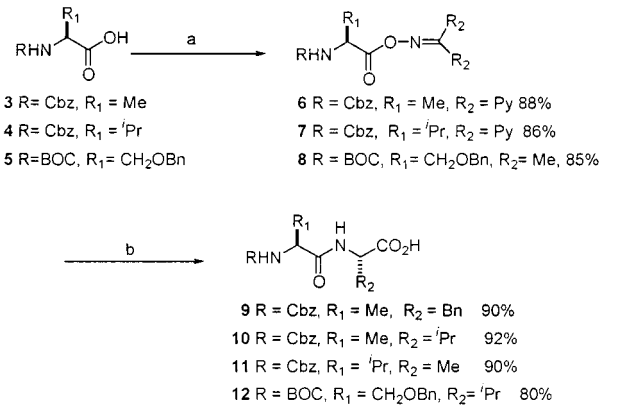
Figure 2. (a) HPLC chromatogram of BOC-(±)-Phg-OBn (retention times = 12.93 and 14.51 min). (b) HPLC chromatogram of BOC-(L)-Phg-OBn (retention time = 14.41 min.). Conditions: Chiralcel OD, 250 × 4.6 mm, flow rate = 0.5 mL/min, 95:5 hex: ^{i}PrOH, UV detection at 254 nm.

that other α-amino acids will be equally solubilized without racemization.[7] For the amino acids shown in Table 1, DBU was ineffective except for proline. When treated with BEMP in the same solvent, only phenylalanine and proline gave clear solutions. Likewise, P_1-^{t}Bu was not suitable to solubilize glycine, serine, and tyrosine. In these cases, P_2-Et was the most effective.

(7) Other amino acids examined were L-Ile, L-Lys, L-*tert*-Leu, L-Thr, and L-Trp. In these cases, either P_1-^{t}Bu or P_2-Et was an effective base for obtaining clean solutions in acetonitrile within about 5 min. For L-Asn and L-His, only P_2-Et was effective. In all of these instances, the extent of racemization was not checked.

To confirm the feasibility of this methodology, we next focused on the preparation of several dipeptides. For this purpose, the reaction between ketoxime esters[8] of α-(L)-amino acids and unprotected α-(L)-amino acids in acetonitrile was explored in the presence of a phosphazene base (Scheme 1).

Scheme 1[a]



[a] Reaction conditions: (a) ketone oxime (1 equiv), CH_2Cl_2, EDC (1.4 equiv), DMAP (cat.), 2 h, rt. (b) H-L-AA-OH (1.2 equiv), P_1-^{t}Bu (1.2 equiv) for **9**, **10**, and **12** or P_2-Et (1.2 equiv) for **11**, CH_3CN, rt, 5 min for **6** and **7**, 4 h for **8**.

The ketoxime esters were easily obtained from Cbz-(L)-alanine **3**, Cbz-(L)-valine **4**, or the O-protected BOC-(L)-serine **5** by treatment with di-2-pyridyl ketone oxime or acetone oxime (1 equiv) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) (1.4 equiv) in dichloromethane in the presence of a catalytic amount of 4-*N*,*N*-(dimethylamino)pyridine (DMAP) (85−88% yields). Isolated oxime esters **6**−**8** were reacted with free α-amino acids (L)-phenylalanine, (L)-valine, and (L)-alanine in acetonitrile in the presence of a phosphazene base to afford the expected dipeptides **9**−**12** in excellent yields (80−92%). The general protocol consisted of the addition of 1.2 equiv of the phosphazene base (P_1-^{t}Bu or P_2-Et) to a suspension of the amino acid in dry acetonitrile under a nitrogen atmosphere. Usually, after 5−10 min of stirring at room temperature, complete solubilization of the starting amino acid was observed. This solution was added at once to a solution of the oxime ester in acetonitrile cooled to 0 °C. After the solution was stirred for 5 min at room temperature (4 h for the acetone oxime esters), TLC showed no presence of the starting di-2-pyridyl ketone oxime ester and the formation of the free di-2-pyridyl ketone oxime. Acidic workup followed by purification of the resulting crude products by column chromatography and/or crystallization in dichloromethane/hexane afforded the pure dipeptides.[9] In these instances, the di-2-pyridyl ketone oxime can easily be recovered in 80−85% yields.[10]

The potential of the methodology for the preparation of *N*-alkyl α-amino acid dipeptides was also evaluated. To this end, we focused on the dipeptide product enalapril, which belongs to a class of dipeptides of major significance for controlling hypertension and congestive heart failure.[11] The N-substituted ethyl (*S*)-2-amino-4-phenyl-butyrate moiety is common to these angiotensin-converting enzyme (ACE) inhibitors, as exemplified in the therapeutic agents quinapril, trandolapril, and moexipril (Figure 3).

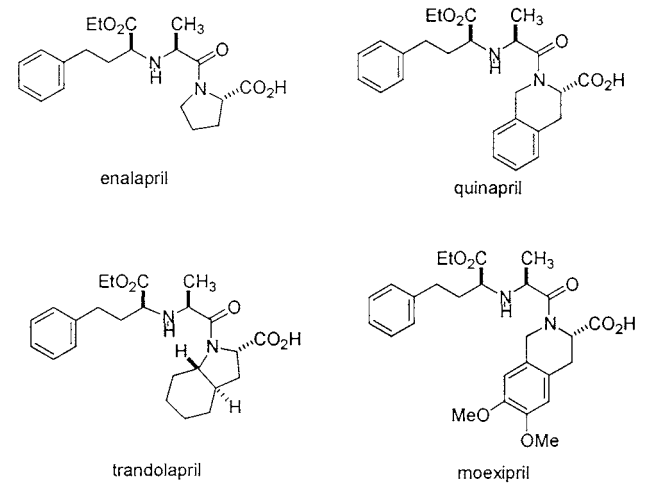


Figure 3. Representative ACE inhibitors characterized by the presence of the N-substituted-(*S*)-2-amino-ethyl-4-phenylbutyrate moiety.

As illustrated in Scheme 2, compound **13**, which is readily accessible in bulk from ethyl 2-oxo-4-phenylbutyrate[12] or ethyl-2-hydroxy-4-phenylbutyrate,[13] was converted into the acetoxime ester **14** over three steps (95% yield).[14] This compound, upon treatment with a solution of the phosphazene salt of (L)-proline in acetonitrile as a solvent, gave enalapril, which was isolated as maleate salt **15** in 92% over the last two steps. In this case, substitution of P_1-^{t}Bu for DBU

(8) (a) Fujino, M.; Nishimura, O. *Chem. Pharm. Bull.* **1969**, *17*, 1937. (b) Miyasaka, T.; Noguchi, S. *Chem. Lett.* **1985**, 701.

(9) Data of compounds: **9**, $[\alpha]^{25}_D$ +4.2 (*c* 1.3, Cl_2CH_2); **10**, $[\alpha]^{25}_D$ −20.0 (*c* 0.8, MeOH); **11**, $[\alpha]^{25}_D$ +4.6 (*c* 0.5, DMF); **12**, $[\alpha]^{25}_D$ +8.8 (*c* 0.84, Cl_2CH_2). The diastereomeric excess for each compound **9**−**12** (≥ 99%) was determined by comparison of the analytical HPLC chromatograms of the methyl ester crude dipeptides with those obtained by coupling the first amino acid with the racemic mixture of the second. For details, see Supporting Information.

(10) The reaction was quenched by the addition of 1 N HCl, and extractions with Cl_2CH_2 yielded the crude dipeptides, which were later purified. Treatment of the aqueous phase with a buffer solution (pH = 7), followed by extraction with dichloromethane and subsequent elimination of the solvent under vacuum, afforded the di-2-pyridyl ketone oxime. For details, see Supporting Information.

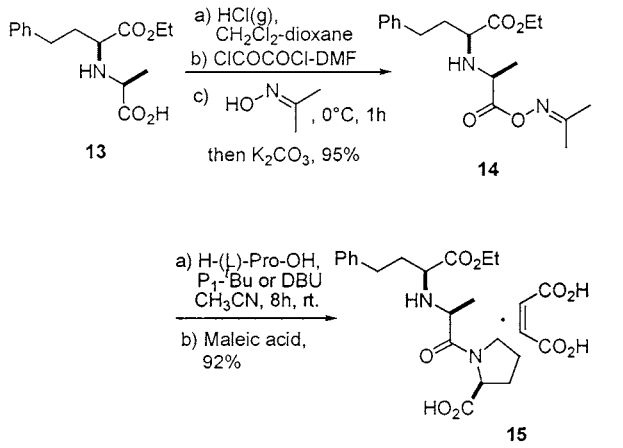
(11) For a comprehensive review on ACE inhibitors, see: (a) Wyvratt, M. J.; Patchett, A. A. *Med. Res. Rev.* **1985**, *7*, 483. (b) *Angiotensin Converting Enzyme Inhibitors*; Kostis, J. B., Defelice, E. A., Eds.; Alan R. Liss, Inc.: 1987. (c) Adang, A. E. P.; Hermkens, P. H. H.; Linders, J. T. M.; Ottenheijm, H. C. J.; van Staveren, C. J. *Recl. Trav. Chim. Pays-Bas* **1994**, *13*, 63. (d) For a survey of ACE inhibitors, see: Natoff, I. L.; Redshaw, S. *Drugs of The Future* **1987**, *12*, 475.

(12) Flynn, G. A.; Beight, D. W. *Tetrahedron Lett.* **1988**, *29*, 423.

(13) Urbach, H.; Henning, R. *Tetrahedron Lett.* **1984**, *25*, 1143.

(14) This compound can be stored at room temperature without decomposition for at least 2 months. Colorless oil; $[\alpha]_D^{25}$ −24.1 (*c* 2.0, Cl_2CH_2). Analytical HPLC: Lichrosorb Si 60 (5 μm), 70:30 AcOEt:hex, retention time = 4.94 min, UV detection at 254 nm.

Scheme 2



provided the same result. Remarkably, neither the first nor the second amino acid residue required amino or carboxyl group protection, respectively, for the coupling reaction to proceed efficiently. Therefore, other ACE inhibitors should also be readily available from this simple technology.

In conclusion, the above results illustrate that the key solubility issue of α-amino acids in organic media can be now addressed efficiently by the use of Schwesinger bases. This finding may be of great utility not only in peptide synthesis but also in other reactions where α-amino acids are involved.

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Supporting Information Available: Experimental procedures and spectroscopic data for compounds **9**−**12** and **14** and HPLC chromatograms of the compounds prepared. This material is available free of charge via the Internet at http://pubs.acs.org.

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