

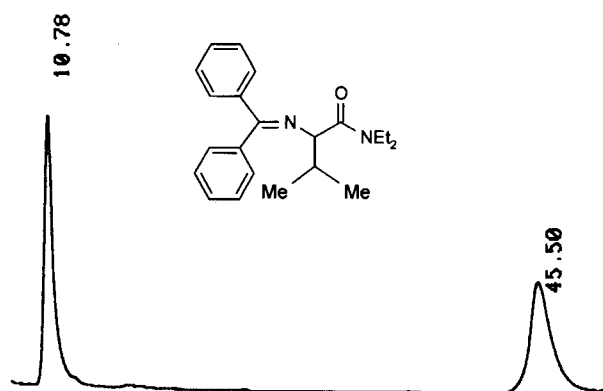


Figure 4. Separation of the enantiomers of valine N,N-diethylamide as benzophenone imine derivative; Mobile phase=10% 2-propanol in hexane (V/V); Flow rate=1 mL/min; UV 254 nm; Temperature ambient; Injection amount 10 nmol.

vatives (entries 4 and 8). It implies that a hydrogen bonding interaction between the carbonyl oxygen of the analyte and DNB N-H amide of CSP is essential for chiral recognition. On the other hand, the N-H of secondary amide derivative is considered to be detrimental to the chiral recognition, providing unnecessary achiral retention. The chromatogram illustrating the separation of the enantiomers of valine N,N-diethylamide as benzophenone imine derivative is presented in Figure 4. The chromatographic data with the study of CPK molecular models suggest a chiral recognition rationale which leads 1) simultaneous face-to-face and face-to-edge π - π interaction between the molecular cleft of the CSP and one phenyl group of benzophenone imine of the analyte and 2) a hydrogen bonding interaction between DNB N-H of the CSP and the carbonyl oxygen of the analyte.

In conclusion, a simple and convenient way of the enantioseparation of the benzophenone Schiff base derivatives of various amino acid ethyl and methyl esters on Whelk-O 1 CSP was described. The enantiomers of all examined amino

acid esters as their benzophenone imine derivatives were well resolved on Whelk-O 1 CSP. The (+)-(R)- or (+)-(2R, 3R)-enantiomers of the examined analytes are selectively retained on (R,R)-Whelk-O 1 CSP. Based on the observed chromatographic results and the study of CPK molecular models, a chiral recognition rationale consistent with observed elution orders was proposed. It is expected that Whelk-O 1 CSP will be useful for the resolution of other amino acid esters and amides as benzophenone imine derivatives.

References

1. Lee, W. *Anal. Lett.* **1997**, 30, 2791.
2. O'Donnell, M. J.; Boniece, J. M.; Earp, S. E. *Tetrahedron Lett.* **1978**, 30, 2641.
3. O'Donnell, M. J.; Polt, R. L. *J. Org. Chem.* **1982**, 47, 2663.
4. Benzophenone imine was purchased from Aldrich.
5. Whelk-O 1 CSP is commercially available from Regis Technologies (250 $\times$ 4.6 mm I.D., 5 μ m, Morton Grove, Illinois).
6. Pirkle, W. H.; Welch, C. J.; Lamm, B. *J. Org. Chem.* **1992**, 57, 3854.
7. Pirkle, W. H.; Welch, C. J.; Zych, A. J. *J. Chromatogr.* **1993**, 648, 101.
8. Pirkle, W. H.; Welch, C. J. *Tetrahedron Asym.* **1994**, 5, 777.
9. Pirkle, W. H.; Selness, S. R. *J. Org. Chem.* **1995**, 60, 3252.
10. Pirkle, W. H.; Koscho, M. E.; Wu, Z. *J. Chromatogr. A* **1996**, 726, 91.
11. Hyun, M. H.; Jin, J. S.; Lee, W. *Bull. Kor. Chem. Soc.* **1997**, 18, 336.
12. Pirkle, W. H.; Lee, W.; Welch, C. J. *Enantiomer* **1997**, 2, 423.
13. Lee, W.; Kim, B. *J. High Res. Chrom.* **1998**, 21, 189.
14. Pirkle, W. H.; Welch, C. J.; Wilson, S. R. *Chirality* **1994**, 6, 615.

New Amino Acid Derivatives for the Synthesis of Pharmaceutical Peptides by Liquid Phase Method: Boc-Asp(OPse)-OH · DCHA and Boc-Glu(OPse)-OH · DCHA

Yeon Sun Lee*, Hyun Jin Lee, Pavel I. Pozdnyakov†, Vladimir V. Samukov†, and Hack Joo Kim*

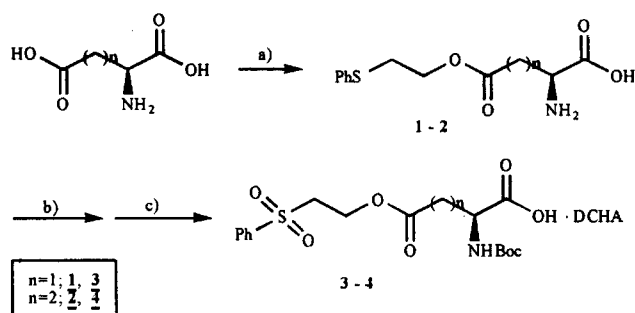
Research Institute, Hyundai Pharm. Ind. Co., Ltd., 213, Sosabon 1-dong, Sosa-gu, Bucheon 422-231, Korea

†State Research Center of Biotechnology and Virology Vector, Koltsovo, Novosibirsk Reg. 633159, Russia

Received March 9, 1998

The side carboxyls of aspartic acid (Asp) and glutamic acid (Glu) should be protected semipermanently and orthogonally to α -groups during peptide synthesis, since they have the possibility of branching a peptide chain *via* intramolecular or intermolecular reactions. Among various protecting

groups, benzyl group which can be removed by catalytic hydrogenolysis has been the most widely used in conjunction with *t*-butyloxycarbonyl (Boc) group. This group, however, has some drawbacks: instability under acidic condition for removal of the Boc group and difficulty of facile detach-

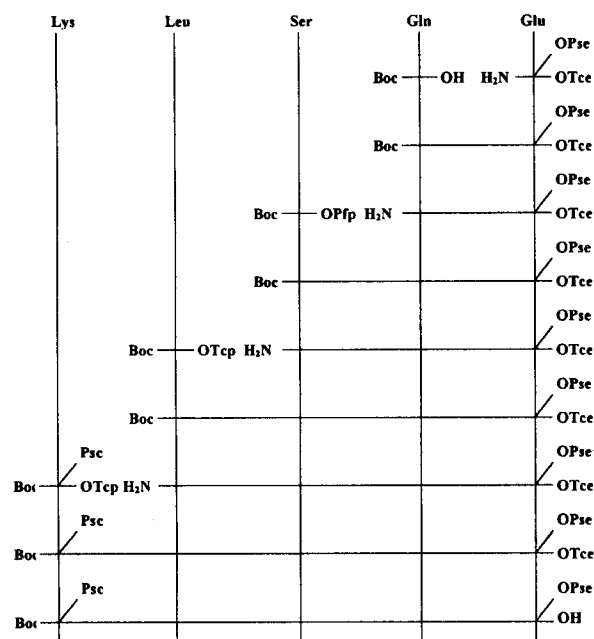


Scheme 1. Synthesis of Boc-Asp(OPse)-OH·DCHA (3) and Boc-Glu(OPse)-OH·DCHA (4). ^a H₂SO₄, 2-phenylthioethanol, dimethoxyethane, rt, ^b TEA, Boc₂O, DMF, 50 °C, ^c 0.3 M Na₂MoO₄, H₂O₂, acetone, dicyclohexylamine (DCHA), 50 °C.

ment, etc. As an alternative to the combination, 9-fluorenylmethyloxycarbonyl (Fmoc) protected amino acids were investigated,¹ but this method also had the disadvantage of lengthy and complicate preparation process. In an attempt to overcome these problems, we were going to introduce base-labile 2-(phenylsulphonyl)ethyl (Pse) group to the side carboxyl functions of Asp and Glu, since the substituted 2-sulphonyl ethyl group had been reported to be useful for the α -carboxyl group protection.²

New amino acid derivatives 3 and 4³ were easily prepared by the direct esterification of the side carboxyl group with 2-phenylthioethanol,⁴ followed by the protection of α -amino group with Boc₂O, and the subsequent hydrogen peroxide oxidation in moderate yields (Scheme 1). These derivatives showed high purity and crystallinity, and also good solubility after desalting.

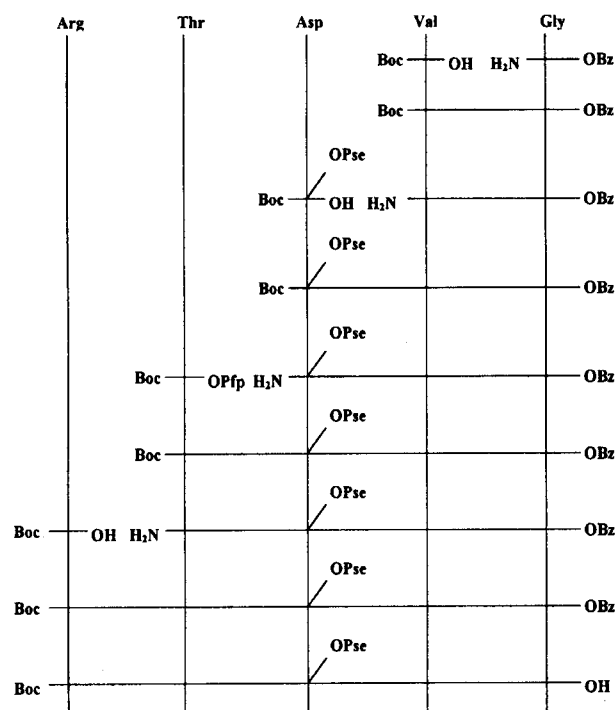
In order to evaluate the usefulness of 3 and 4, key frag-



Tce: trichloroethyl, Pfp: pentafluorophenyl

Tcp: trichlorophenyl, Psc: 2-(Phenylsulphonyl)ethyloxycarbonyl

Scheme 2. Synthesis of Boc-Lys(Psc)-Leu-Ser-Gln-Glu(OPse)-OH.



Scheme 3. Synthesis of Boc-Arg-Thr-Asp(OPse)-Val-Gly-OH.

ments for the preparation of calcitonin (salmon, chicken, and eel calcitonin) having hypocalcemic potency, Boc-Lys(Psc)-Leu-Ser-Gln-Glu(OPse)-OH (5) and Boc-Arg-Thr-Asp(OPse)-Val-Gly-OH (6), were synthesized in solution (Scheme 2, 3). Acylations were carried out by preactivation with DCC/HOBt or activated esters of appropriate N^{Boc}-amino acids, and deprotections of the Boc groups were carried out with TFA or HCl/AcOH, as usual. At the completion of chain-lengthening, C-terminal protecting groups were removed by catalytic hydrogenation, and the resulting solid recrystallized to give high quality fragments 5 and 6 in 58% and 62% yields, respectively. With the aid of stable Pse group at the chain-lengthening step, the whole synthetic process developed without any serious side reaction. The intermediate peptides retained good crystallinity to be easily purified. After completion of the peptide chain (1-32) by the fragment condensation,⁵ the Pse group was readily removed by piperidine in a short period of time, and the resulting peptide could be simply purified by preparative RP-HPLC to give biologically active calcitonin.⁶

From this work, it has been demonstrated that the Pse-protected amino acid derivatives (3 and 4) can be well applied to Boc-liquid phase peptide synthesis. Further studies including synthetic application of these derivatives to pharmaceutical peptides are in progress.

References

- Al-obeidi, F.; Sanderson, D. G.; Hruby, V. J. *Int. J. Peptide Protein Res.* **1990**, 35, 215.
- (a) Hardy, P. M.; Rydon, H. N.; Thompson, R. C. *Tetrahedron Letters* **1968**, 21, 2525. (b) Joaquina, M.; Amaral, S. A. *J. Chem. Soc(c)* **1969**, 2496.
- H-Asp(OPte)-OH (1) yield 54%; mp 215-217 °C; [α]_D²⁰ +

21.2 (c 1.0, 1 N HCl); HPLC (C-18, ODS hypersil, 5 μ m, 4.6 $\times$ 200 mm; gradient 0% to 100% of acetonitrile (0.1% TFA) in 45 min; 1 mL/min; 216 nm) R_T 17.6 min; IR (KBr, cm^{-1}) 3123, 2379, 2306, 1739, 1636, 1416, 1343, 1135, 730, 688; ^1H NMR (D_2O , DCl) δ 7.10 (d, $J=7.6$ Hz, 2H), 7.03 (t, $J=7.7$ Hz, 2H), 6.95 (t, $J=7.3$ Hz, 1H), 3.98-4.02 (m, 3H), 2.89 (t, $J=6.2$ Hz, 2H), 2.70 (abq, $J_1=18.2$ Hz, $J_2=5.5$ Hz, 2H) H-Glu(OPte)-OH (2) yield 55%; mp 182-183 $^\circ\text{C}$; $[\alpha]_D^{20} +17.7$ (c 1.0, 0.5 N HCl); HPLC (C-18, ODS hypersil, 5 μ m, 4.6 $\times$ 200 mm; gradient 0% to 100% of acetonitrile (0.1% TFA) in 45 min; 1 mL/min; 216 nm) R_T 18.7 min; IR (KBr, cm^{-1}) 2955, 1726, 1586, 1508, 1421, 1202, 733; ^1H NMR (D_2O , DCl) δ 7.10 (d, $J=7.5$ Hz, 2H), 7.03 (t, $J=7.6$ Hz, 2H), 6.96 (t, $J=7.3$ Hz, 1H), 3.98 (t, $J=6.3$ Hz, 2H), 3.83 (t, $J=6.8$ Hz, 1H), 2.90 (t, $J=6.3$ Hz, 2H), 2.20-2.24 (m, 2H), 1.89 (q, $J=7.6$ Hz, 2H) Boc-Asp(OPse)-OH $\cdot$ DCHA (3) yield 95%; mp 142-144 $^\circ\text{C}$; $[\alpha]_D^{20} -5.4$ (c 1.0, 10% AcOH); HPLC (C-18, ODS hypersil, 5 μ m, 4.6 $\times$ 200 mm; gradient 0% to 100% of acetonitrile (0.1% TFA) in 45 min; 1 mL/min; 216 nm) R_T 26.5 min; IR (KBr, cm^{-1}) 3397, 2937, 2866, 1740, 1708, 1584, 1489, 1397, 1318, 1149; ^1H NMR ($\text{DMSO}-d_6$) δ 7.90 (d, $J=7.6$ Hz, 2H),

7.74 (t, $J=7.4$ Hz, 2H), 6.79 (t, $J=7.3$ Hz, 1H), 3.76 (t, $J=6.2$ Hz, 2H), 3.66 (t, $J=7.6$ Hz, 1H), 2.73 (t, $J=6.2$ Hz, 2H), 2.58 (t, $J=6.1$ Hz, 2H), 1.46-1.58 (m, 2H), 0.92-1.07 (m, 2H) Boc-Glu(OPse)-OH $\cdot$ DCHA (4) yield 92%; mp 155-157 $^\circ\text{C}$; $[\alpha]_D^{20} -5.8$ (c 1.0, 10% AcOH); HPLC (C-18, ODS hypersil, 5 μ m, 4.6 $\times$ 200 mm; gradient 0% to 100% of acetonitrile (0.1% TFA) in 45 min; 1 mL/min; 216 nm) R_T 21.5 min; IR (KBr, cm^{-1}) 2938, 2857, 1733, 1701, 1637, 1560, 1449, 1399, 1297, 1141, 1085, 727, 686; ^1H NMR ($\text{DMSO}-d_6$) δ 7.89 (d, $J=7.4$ Hz, 2H), 7.74 (d, $J=7.4$ Hz, 1H), 7.66 (t, $J=7.6$ Hz, 2H), 6.06 (d, $J=5.8$ Hz, 1H), 4.24 (t, $J=7.1$ Hz, 2H), 3.69 (t, $J=7.1$ Hz, 2H), 3.55 (m, 1H), 2.92 (m, 2H), 1.56-2.08 (m, 14H), 1.37 (s, 9H), 1.06-1.27 (m, 10H).

- 2-Phenylthioethanol was obtained quantitatively by the reaction of phenylsulfide with 2-chloroethanol and appropriate base.
- Synthetic route will be discussed elsewhere.
- The purified salmon calcitonin showed high hypocalcemic potency of about 4000 IU/mg measured in rats by MRC method. The other calcitonins (eel, chicken) are being synthesized.

A New Approach to Steroid Side Chain: Synthesis and Stereoselective Reduction of 16(17), 20(22)-Diene System

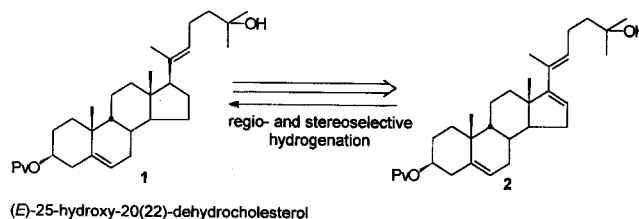
Choon Sup Ra* and Soon Ran Chung

Department of Chemistry and Institute of Materials Chemistry, Yeungnam University, Kyongsan 712-742, Korea
Received March 10, 1998

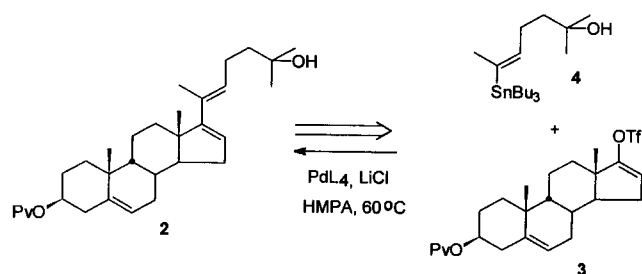
The development of versatile methods for synthesizing steroid side chains has been spurred by the significant dependence of the biological activities of various steroids such as insect hormones,¹ corticosteroids,² brassinosteroids³ and many important vitamin D metabolites⁴ on structural features of the side chains. Intense efforts have been made toward new methods for affording structural variations to the steroid side chain unit by many research groups.^{4,5} Among approaches developed in this area the synthetic route to construct side chains onto tetracyclic 17-ketosteroids has been one of the most attractive and versatile paths. Conventional routes⁶ employing this strategy adopt a multi-step operation of sequential attachments of several fragments to the existing functionality.

Herein we present a novel route to (*E*)-20(22)-dehydrocholesterol systems⁷ via 16,20-diene steroid compound. Scheme 1 and 2 illustrate an efficient approach to a new (*E*)-20(22)-dehydrocholesterol compound 1 in 3 steps from 17-ketosteroid utilizing this strategy. The diene intermediate 2 was prepared by palladium-mediated coupling⁸ between 3 and 4 in the presence of lithium chloride (5 eq) and tetrakis(triphenylphosphine)palladium (5 mol%) in HMPA at 60 $^\circ\text{C}$ for 4 hr in 70% yield (Scheme 2) and a high level of stereocontrol at C-17 center was achieved as desired during sub-

sequent regio- and stereoselective diimide reduction of the diene compound (Scheme 5).



Scheme 1



Scheme 2