21.2 (c 1.0, 1 N HCl); HPLC (C-18, ODS hypersil, 5 μm, 4.6×200 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 °C; $[\alpha]_D^{20}$ +17.7 (c 1.0, 0.5 N HCl); HPLC (C-18, ODS hypersil, 5 μm, 4.6×200 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·DCHA (**3**) yield 95%; mp 142-144 °C; $[\alpha]_D^{20}$ −5.4 (c 1.0, 10% AcOH); HPLC (C-18, ODS hypersil, 5 μm, 4.6×200 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 (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·DCHA (**4**) yield 92%; mp 155-157 °C; $[\alpha]_D^{20}$ −5.8 (c 1.0, 10% AcOH); HPLC (C-18, ODS hypersil, 5 μm, 4.6×200 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 (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).

4. 2-Phenylthioethanol was obtained quantitatively by the reaction of phenylsulfide with 2-chloroethanol and appropriate base.
5. Synthetic route will be discussed elsewhere.
6. 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

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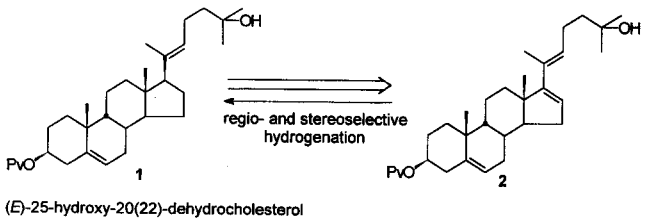
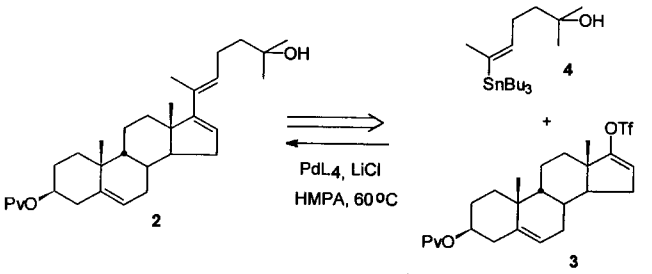
The development of versatile methods for synthesizing steroid side chains has been spurred by the significant dependence of the biological activities of various steroids such as insect hormones,[1] corticosteroids,[2] brassinosteroids[3] and many important vitamin D metabolites[4] on structural features 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 routes[6] 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 systems[7] *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 coupling[8] between **3** and **4** in the presence of lithium chloride (5 eq) and tetrakis (triphenylphosphine)palladium (5 mol%) in HMPA at 60 °C for 4 hr in 70% yield (Scheme 2) and a high level of stereocontrol at C-17 center was achieved as desired during subsequent regio- and stereoselective diimide reduction of the diene compound (Scheme 5).



(*E*)-25-hydroxy-20(22)-dehydrocholesterol

Scheme 1

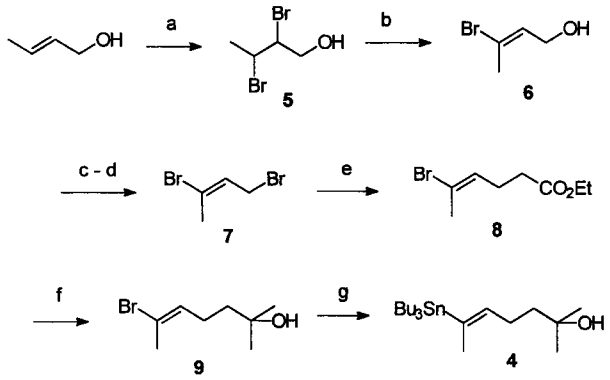


Scheme 2

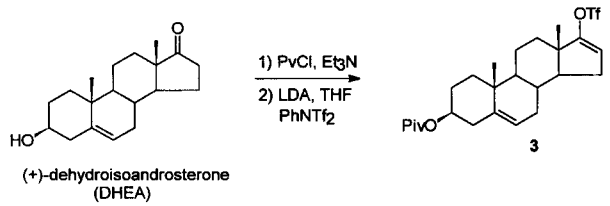
The (*E*)-vinyl stannyl unit was prepared in **7** steps from *t*-crotyl alcohol (Scheme 3). Stereoselective dehydrobromination of dibromo compound **5** obtained by addition of bromine to *t*-crotyl alcohol yielded 3-bromocrotyl alcohol[9] with (*E*)-configuration in 40% overall yield. The hydroxy function of **6** was converted into the bromide **7** *via* a formation of the mesylate (75%). Four-carbon homologation to ethylacetate using **7** was made via enolate chemistry[10] to provide vinyl ester **8** (81%). Addition of excess amount of methylmagnesium bromide to **8** gave vinyl bromide **9** (73%) which was then converted into the vinyl stannyl derivative **4** (71%) by the reaction of **9** with tributyltin lithium.[11]

Next, protected (+)-dehydroisoandrosterone was transformed into its enol triflate **3** (95%) by trapping the lithium enolate of with N-phenyltrifluoromethanesulfonimide[12] (Scheme 4).

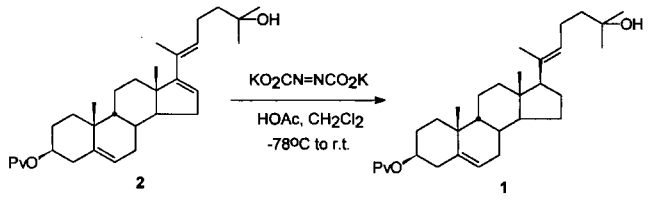
Regio- and stereoselective hydrogenation on the conjugated steroid system **2** to give (*E*)-25-hydroxy-20(22)-dehydrocholesterol (**1**) was achieved by diimide reduction[13] at low temperature in 92% yield (Scheme 5). Its structure was determined from its high resolution NMR data which showed characteristic features of the (*E*)-20(22)-dehydrocholesterol systems having singlet C-18 methyl proton with the



Scheme 3. [a] Br_2, CCl_4; −20 °C. [b] LDA (2.3 eq.), HMPA (0.5 eq.), THF, −78 °C. [c] MsCl, Et_3N, 0 °C. [d] LiBr, THF, 0 °C. [e] AcOEt; LHMDS (1.2 eq.), HMPA (0.5 eq.), THF, −78 °C. [f] MeMgBr (3.3 eq.), THF, −40 °C to r.t.. [g] Bu_3SnLi (2 eq.), THF, −20 °C to 0 °C.



Scheme 4



Scheme 5

chemical shift observed at the high field region (0.545 ppm)[14] in ^1H NMR spectrum along with ^{13}C NMR data.[15]

In summary, we described a novel and concise synthetic pathway to (*E*)-25-hydroxy-20(22)-dehydrocholesterol from 17-ketosteroid *via* stereoselective diimide reduction of 16,20-diene steroid obtained by palladium-mediated coupling of steroid enol triflate and vinyl stannyl moiety of side chain unit.

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References

1. Nes, W. R.; McKean, M. L. *Biochemistry of Steroids and Other Isopentanoids*; University Park Press: Baltimore, MD, 1977.
2. Ciattini, P. G.; Morera, E.; Ortar, G. *Tetrahedron Lett.* **1990**, *31*, 1889 and see references therein.
3. Yokota, T.; Mori, K. In *Molecular Structure and Biological Activity of Steroids*: Bohl, M.; Duax, W. L. Eds.; CRC Press: Boca Raton, Florida, 1992 and see references therein.
4. Zhu, G.-D.; Okamura, W. H. *Chem. Rev.* **1995**, *95*, 1877 and see references therein.
5. For a review on the synthesis of steroid side chains, see: (a) Piatak, D. M.; Wicha, J. *Chem Rev.* **1978**, *78*, 199. (b) Redpath, J.; Zeelen, F. *J. Chem. Soc. Rev.* **1983**, *12*, 75. For more recent examples in this area, see: (c) Midland, M. M.; Kwon, Y. C. *Tetrahedron Lett.* **1985**, *26*, 5021. (d) Koreeda, M.; George, I. A. *J. Am. Chem. Soc.* **1986**, *108*, 8098. (e) Hanekamp, J. C.; Roohuizen, R. B.; Bos, H. J. T.; Brandsma, L. *Tetrahedron* **1992**, *24*, 5151. (f) Fall, Y.; Torneiro, M.; Castedo, L.; Mourino, A. *Tetrahedron Lett.* **1992**, *33*, 6683. (g) Sestelo, J. P.; Mascarenas, J. L.; Castedo, L.; Mourino, A. *J. Org. Chem.* **1993**, *58*, 118. (h) Mikami, K.; Kishino, H.; Loh, T.-P. *J. Chem. Soc. Chem. Commun.* **1994**, 495. (i) Houston, T. A.; Tanaka, Y.; Koreeda, M. *J. Org. Chem.* **1993**, *58*, 4287. (j) Yamamoto, K.; Takahashi, J.; Hamano, K.; Yamada, S.; Yamaguchi, K.; DeLuca, H. F. *J. Org. Chem.* **1993**, *58*, 2530. (k) Craig, A. S.; Norman, A. W.; Okamura, W. H. *J. Org. Chem.* **1992**, *57*, 4374. (l) Wilson, S. R.; Davey, A. E.; Guazzaroni, M. E. *J. Org. Chem.* **1992**, *57*, 2007. (m) Marczak, S.; Wicha, J. *Tetrahedron Lett.* **1993**, *34*, 6627.
6. For examples of the synthesis of steroid side chain to 17-keto steroids: (a) Midland, M. M.; Kwon, Y. C. *Tetrahedron Lett.* **1982**, *23*, 2077. (b) Midland, M. M.; Kwon, Y. C. *J. Org. Chem.* **1981**, *46*, 299. (c) Trost, B. M.; Verhoeven, T. R. *J. Am. Chem. Soc.* **1978**, *100*, 3435. (d) Batcho, A. D.; Berger, D. E.; Uskokovic.; Snider, B. B. *J. Am. Chem. Soc.* **1981**, *103*, 1294. (e) Krubiner, A. M.; Oliveto, E. P. C. *J. Org. Chem.* **1966**, *31*, 24.
7. For examples of 20(22)-dehydrocholesterols: (a) Midland, M. M.; Kwon, Y. C. *J. Am. Chem. Soc.* **1983**, *105*, 3725. (b) Schmit, J. P.; Piraux, M.; Pilette, J. F. *J. Org. Chem.* **1975**, *40*, 1586. (c) McMorris, T. C.; Schow, S. R. *J. Org. Chem.* **1976**, *41*, 3759. (d) Nes, W. R.; Varkey, T. E.; Krevitz, K. *J. Am. Chem. Soc.* **1977**, *99*, 260. (e) Bannai, K.; Morisaki, M.; Ikekawa, N. *J. Chem. Soc.*

Chem., Perkin Trans. I **1976**, 2116. (f) Koreeda, M.; Koizumi, N.; Teicher, B. A. *J. Chem. Soc. Chem. Commun.* **1976**, 1035.
8. (a) Scott, W. J.; Stille, J. K. *J. Am. Chem. Soc.* **1986**, *108*, 3033. (b) Scott, W. J.; Crisp, G. T.; Stille, J. K. *J. Am. Chem. Soc.* **1984**, *106*, 4630. (c) Mitchell, T. N. *Synthesis* **1992**, 803.
9. Corey, E. J.; Bock, M. G.; Kozikowski, A. P.; Rama Rao, A. v.; Flyod, D.; Lipschutz, B. *Tetrahedron Lett.* **1978**, *19*, 1051.
10. Cregge, R. J.; Herrmann, J. C.; Lee, C. S.; Richman, J. E.; Schlessinger, R. H. *Tetrahedron Lett.* **1973**, *14*, 2425.
11. Newcomb, M.; Smith, M. G. *J. Organometal. Chem.* **1982**, *228*, 61.
12. McMurry, J. E.; Scott, W. J. *Tetrahedron Lett.* **1983**, *24*, 979.
13. (a) Adam, W.; Eggelte, H. J. *J. Org. Chem.* **1977**, *42*, 3987. (b) Grieco, P. A.; Lis, R.; Zelle, R. E.; Finn, J. *J. Am. Chem. Soc.* **1986**, *108*, 5908.
14. For a detailed discussion on the chemical shift of the 18-CH_3 group of (*E*)-20 (22)-dehydro compounds, see reference **7b** and **7c**.
15. Spectral data for **8**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.82 (t, *J*=7.45 Hz, 1H), 4.14 (q, *J*=7.14 Hz, 2H), 2.32 (m, 4H), 2.24 (s, 3H), 1.27 (t, *J*=7.16 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.48, 129.98, 120.78, 60.48, 33.39, 31.54, 23.27, 14.16; IR (neat, cm^{-1}): 2982, 1740. **9**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.82 (t, *J*=7.45 Hz, 1H), 2.12 (m, 2H), 1.55 (m, 2H), 1.23 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.48, 129.98, 120.78, 60.48, 33.39, 31.54, 23.27, 14.16; IR (neat, cm^{-1}): 3386, 2971, 1653. **4**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.51 (t, *J*=6.59 Hz, 1H), 2.19 (m, 2H), 1.85 (s, 3H) 1.24 (s, 6H), 0.83-1.64 (series of m, 29H); IR (neat, cm^{-1}): 3385, 2965, 1653. **2**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.58 (m, 2H), 5.38 (d, *J*=4.17 Hz, 1H), 4.52 (m, 1H), 2.33-1.23 (series of m, 21H), 1.79 (s, 3H), 1.25 (s, 6H), 1.18 (s, 9H), 1.06 (s, 3H), 0.96 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 178.03, 156.22, 140.09, 131.00, 126.21, 125.24, 122.29, 73.47, 71.07, 57.54, 50.19, 46.69, 43.50, 38.60, 38.00, 36.84, 36.71, 36.03, 31.52, 30.94, 30.27, 29.26, 27.62, 27.14, 23.44, 21.02, 19.26, 16.28, 15.31; IR (KBr, cm^{-1}): 3366, 2967, 1724, 1684, 1654, 1479, 1458. **1**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.37 (m, 1H), 5.21 (m, 1H), 4.56 (m, 1H), 2.31-1.18 (series of m, 24H), 1.65 (s, 3H), 1.24 (s, 6H), 1.18 (s, 9H), 1.02 (s, 3H), 0.545 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 178.04, 139.85, 134.89, 125.16, 122.41, 73.51, 71.13, 58.80, 56.18, 50.22, 43.73, 43.47, 38.61, 38.00, 37.00, 36.67, 32.14, 31.84, 29.24, 27.65, 27.15, 24.68, 24.27, 23.11, 21.01, 19.39, 17.86, 12.91.

Structural Confirmation of Ginsenosides By Fragmentation Pattern Using Tandem Mass Spectrometry

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Ginseng has long been used as a tonic, health promoter in Asian countries including Korea and China.[1,2] Its pharmacological effect as an anti-cancer drug has been known to the general public since 1987.[3] In recent years, many scientific studies have reported that the biological activities of ginseng are due to its active components, saponins.[4,5] Korean ginseng (*Panax ginseng*) contains a series of ginseng saponins called ginsenosides containing a large quantity of steroid glycosides.[6] Novel analytical techniques revealed the structures of major ginsenosides, Rb_1, Rb_2, Rc, Rd, Re, Rf and Rg (Figure 1).[7,8] Fast-growing market of ginseng products and their well-accepted pharmacological effects have greatly prompted us to pursue research on the isolation and structure determination of ginsenosides.

There are several analytical methods applicable for the identification of ginsenosides in ginseng extracts.[9] Fast atom bombardment mass spectrometry (FAB-MS) has been generally used for structural analysis of steroid glycosides containing two or more sugar units.[10] In addition, electrospray ionization mass spectrometry (ESI-MS) has been rapidly become an alternative method for the analysis of biomolecules.[11] Tandem mass spectrometric technique has especially been useful in the structure determination of biomolecules.[12] An important point of tandem mass spectrometry (MS/MS) is the ability to produce fragment ions from selected MS-1 ions.[13] Herein, we report our result on the first study of the fragmentation patterns of ginsenosides by tandem mass spectrometry. These fragmentation patterns can help us explain not only the structure of the biomolecule, but also its elemental composition.[14]

Ginsenosides were extracted from commercially available Korean Ginseng by the Folch-Suzuki partition method.[15,16] The compositions and structures of ginsenosides in ginseng have been investigated by means of high performance thin layer chromatography (HPTLC), high performance liquid chromatography (HPLC), nuclear magnetic resonance