Supplementary Material Available: Experimental procedures, including the methods of enzyme preparation and inhibition assay, and ^1H NMR spectra for all new compounds, except for the unstable dioxophosphonate 7 (24 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Articles

Synthesis of the Hydroxyethylene Isostere of Leu-Val

Peter G. M. Wuts,* Allen R. Ritter, and Lynn E. Pruitt

Chemical Process Research and Development, Upjohn Co., Kalamazoo, Michigan 49001

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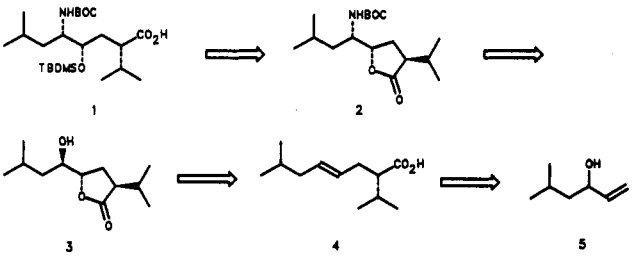
The hydroxyethylene isostere of the dipeptide leu-val was synthesized from isovaleryl aldehyde in nine steps in 15% overall yield without the use of chromatographic separations. A key finding is the ability of an amide to selectively direct an epoxidation in an acyclic system and that the selectivity is a function of the amide's size.

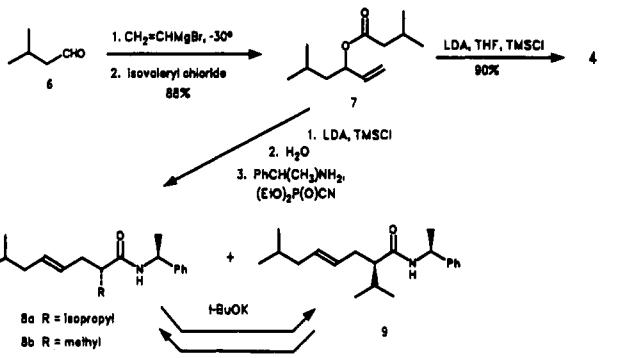
The dipeptide hydroxyethylene isostere of leu-val 1 is a representative of an important class of unnatural amino acids related to the natural amino acid statine that when incorporated into peptide substrates of proteolytic enzymes such as Renin impart pronounced inhibitory effects. The inhibitory action is believed to occur by mimicking the transition state for amide hydrolysis. More recently, the same strategy has been used to develop inhibitors of a key protease of the human immunodeficiency virus (HIV).[1]

Earlier, we described a synthesis of 1 utilizing leucine as a starting material and source of chirality.[2,3] We would now like to describe a new more economical approach that promises to be much more general, does not require a single chromatographic purification, does not pass through sensitive intermediates, and can easily be scaled up for commercial development. The strategic concept of the synthesis is outlined in Scheme I. The success of this approach lies in the ability to prepare the acid 4 with a stereogenic center at C-2 and to transfer that chirality to the centers at C-4 and C-5. Although a number of approaches for the preparation of acid 4 are readily envisioned, we chose to use the Ireland enolate Claisen rearrangement[4] for both economic reasons and its known ability to transfer chirality present in the allylic alcohol precursors.[5] The required ester 7 (Scheme II) is prepared in 88% distilled yield by the slow addition of 3-methylbutyraldehyde (6) to a slurry of vinylmagnesium bromide

Scheme I



Scheme II



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(2) Wuts, P. G. M.; Putt, S. R.; Ritter, A. R. *J. Org. Chem.* **1988**, *53*, 4503.

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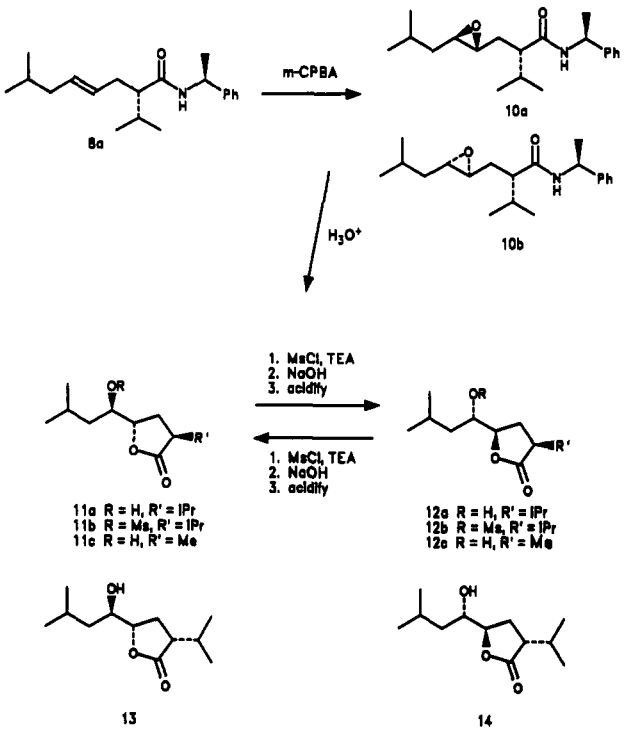
Scheme III



Scheme IV

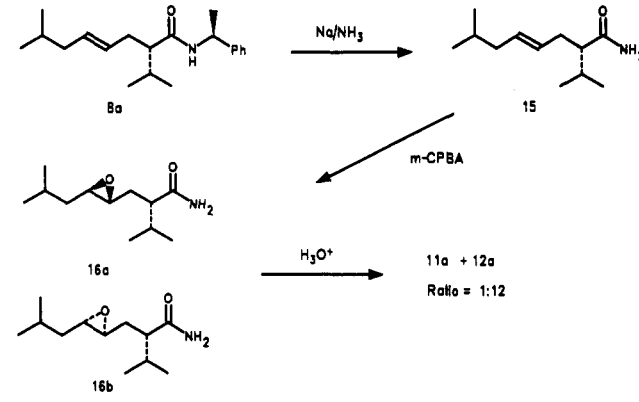


Table I. Effect of pH on the Stereochemistry of Epoxide Hydrolysis

pH[9]	0.4	2	3	4
reactn time (h)	8	8	8	8
completion	yes	no	no	no
isomer distribn[a,10]				
% (2S,4R,5S)-12a	82.5	60.1	40.2	39.1
% (2R,4R,5S)-14	3.7	18.2	29.2	30.9
% (2S,4S,5R)-11a	11.1	19.2	27.5	28.2
% (2R,4S,5R)-13	2.7	2.3	2.0	1.8
ratio 2S/2R	93.6/6.4	79.3/20.5	67.7/31.2	67.3/32.7

[a] The starting purity of the epoxy amide **10b** was 97% (2S).

in THF at −30 °C followed by the slow addition of 3-methylbutyryl chloride at −30 °C. Alternatively, but less efficiently (50% yield), the ester can be prepared from 2-methylpropylmagnesium bromide and acrolein followed by acylation. The low efficiency of the latter approach is due to the ease of polymerization of the acrolein. The Claisen rearrangement proceeds smoothly under the standard conditions to afford the racemic acid **4** in 90% yield. If the enolate is allowed to rearrange without TMSCl trapping, the yield is only 70%.[6] At this point we attempted to resolve the acid as the α-methylbenzylamine salt, but the resolution and the efficiency are quite poor. Conversion of the acid **4** to the diastereomeric amides **8a** and **9** followed by a selective crystallization affords amide **8a** with a de of 98% in yields ranging from 25 to 35%. Initially, the amides were prepared from the derived acid chloride but later it was discovered that the entire sequence of Claisen rearrangement and amide formation could be carried out in a single vessel. Thus, the Claisen rearrangement was performed normally, but upon completion enough water was added to hydrolyze the TMS ester. The mixture is treated with α-methylbenzyl amine and DEPC (diethylphosphoryl cyanide)[7] which results in clean conversion to the mixture of amides **8a** and **9**. After isolation with ethyl acetate the mixture is crystallized to afford amide **8a** selectively. Equilibration of the mother liquors to nearly a 1:1 mixture with *t*-BuOK in THF affords additional material after crystallization. Recrystallization of the two crops affords 32.4% amide **8a** with a de of 98.6%.

Having secured the stereogenic center at C-2, the next real problem in the synthesis was to transfer the asymmetry at C-2 to the prochiral centers at C-4 and C-5. Thus, when the amide is epoxidized with *m*-CPBA a 1:4 mixture of epoxides **10a** and **10b** is obtained. The epoxide stereochemistry relative to C-2 was never proven absolutely but is inferred from the derived lactones **11a** and **12a** after acid hydrolysis with the assumption that intramolecular assistance from the carbonyl occurs at C-4. Several attempts to improve the ratio by varying the oxidant or the conditions for the reaction were to no avail.

What is the controlling factor in the epoxidation? Based on the following set of results we believe that hydrogen bond directed epoxidation, controlled by the C-2 side chain, is responsible for the observed selectivity. When the bulky benzyl group of the amine is removed by a dissolving metal reduction and the resulting amide **15** is epoxidized a 1:12 ratio of epoxides **16a** and **16b** is obtained. Epoxidation of the diastereomeric amide **9** affords a 1:4 ratio of lactones **14** and **13** after acid-catalyzed hydrolysis. This indicates that the chirality of the amine has no effect. Furthermore, when the C-2 methyl derivative **8b** is epoxidized only a 1:2 ratio of lactones **11c** and **12c** is obtained after hydrolysis. A 1:2 ratio of lactones **11a** and **12a** (racemate) is also obtained upon epoxidation of the racemic acid **4**. Clearly the amide through its strong hydrogen-bonding capability is responsible for the observed selectivity.[8] We believe that this is the first example of an amide-directed epoxidation in an acyclic framework.

One thing that is not clear from these studies is the nature of the transition state in the epoxidation. This question has no satisfactory answer beyond the usual speculation. Model building, either electronic or manual, fails to provide any clear-cut bias for one transition structure or another due to the large number of degrees of freedom in several hypothetical transition structures.

Attention was next turned to the hydrolytic lactonization of the epoxy amides. As alluded to previously this reaction was pH dependent. Table I shows that as the pH is in-

(6) As a matter of procedure the TMSCl is stored over polyvinylpyridine to trap out HCl that is inevitably present. This procedure is far more efficient than using triethylamine and centrifuging to settle the TEA·HCl.

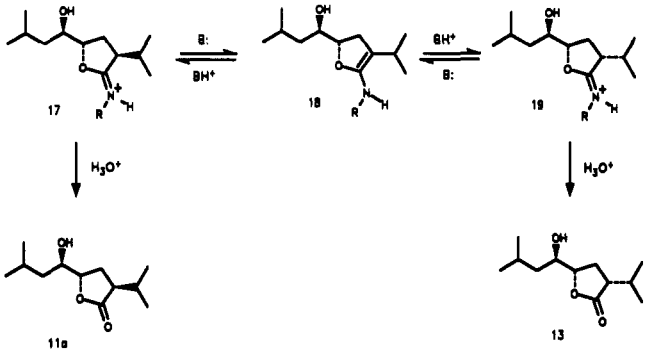
(7) Shiori, T.; Yokoyama, Y.; Kasai, Y.; Yamada, S. *Tetrahedron* **1976**, *32*, 2211.

(8) For a previous case of an amide-directed epoxidation see: Mohamadi, F.; Specs, M. M. *Tetrahedron Lett.* **1989**, *30*, 1309.

(9) The pH was determined with a standardized pH probe in the rapidly stirred biphasic reaction mixture.

(10) A 60-m Durawax-DX1 capillary column was used to separate the isomers, 210–240 °C at 4 °C/min.

Scheme V



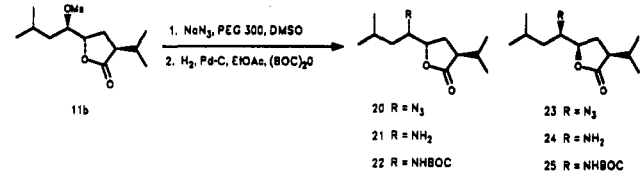
creased from 0.4 to 4 increasing quantities of the lactone **14** are being formed as a result of C-2 isomerization. From the table it is also noteworthy that a substantial amount of the lactone **11a** is formed as the pH is increased. That competition for the 5-position by the carbonyl or external water is now competing with C-4 opening is the only reasonable explanation for the observed stereochemical diversion, but it is not clear how pH would control the relative rates of C-4 versus C-5 opening of the epoxide. Isomerization at C-2 is readily explained upon examination of Scheme V. As the pH is increased the relative basicity is increased, and as a result the loss of a proton from the intermediate iminium ion **17** to form **18** becomes competitive with addition of water. Of course, once deprotonation has occurred the stereochemistry at C-2 is also lost, especially since reprotonation of **18** is not expected to be particularly selective, thus returning the desired (2*S*)-lactone **11a** as well as the (2*R*)-lactone **14**. That the molarity of the acid effects the hydrolysis of the epoxides was quite surprising. When the molarity is increased the rate of hydrolysis decreases but the 2*S*:2*R* ratio remains constant. This might be attributed to a decrease in the effective molarity of water in the organic phase and would explain the reduced rate if addition of water to the iminium ion is the rate-determining step in the reaction. From these results it is clear that the pH must be maintained at about 0.4 in order to prevent C-2 isomerization.

Since the sense of the epoxidation is enantiomeric with the desired form, a means of achieving inversion was required. Earlier, in connection with an alternate route to Leuψ(CH_2CHOH)Val we developed a procedure for the inversion of the related (2*S*,4*S*,5*S*)-lactone to its (2*S*,4*R*,5*R*)-isomer (not shown).[11] The mixture of lactones **11a** and **12a** is converted to the mesylate, at which point they are also much easier to separate by chromatography if desired. The major mesylate **12b** is converted to the lactone **11a** by treatment with aqueous NaOH in acetonitrile to hydrolyze the lactone and form a transient epoxide. Acidification of the mixture gives the desired lactone **11a** with complete inversion at the 4 and 5 positions. In practice, the two lactones are not separated, but the 1:4 or 1:12 mixture is inverted and then carried on to the BOC aminolactones where the desired isomer **22** is easily crystallized from the undesired isomer **25**.

The remaining steps of the synthesis are straightforward. The mesylate is treated with NaN_3 in DMSO to afford azide **20** which is directly reduced to the amine **21** with Pd/C in ethanol at 45 psi. Filtration of the mixture to remove the catalyst and concentration affords the crude amine **21** which is treated directly in THF with aqueous $NaHCO_3$ and $(BOC)_2O$ at rt overnight to afford lactone **22**. The azide can also be reduced in the presence of

(11) Wuts, P. G. M. Unpublished results.

Scheme VI



$(BOC)_2O$ in ethyl acetate to afford the lactone **22** directly.[12] When the entire sequence is performed without purification of intermediates and omitting the dissolving metal reduction the overall yield for the chromatographically free sequence is 15% after crystallization of amide **22** from amide **25**.

Experimental Section

3-(5-Methyl-1-hexenyl) 3-Methylbutyrate (7). A 3-L flask fitted with addition funnel, nitrogen inlet, and mechanical stirrer was charged with 800 mL of vinylmagnesium bromide in THF (1.0 M) and 400 mL of THF. The mixture was cooled to −30 °C, and the addition of 86.0 mL (0.80 mol) of isovaleraldehyde in 400 mL of THF was begun, keeping the temperature below −30 °C. When the addition was complete the mixture was stirred for 0.5 h at −30 °C, and then 107.0 mL (0.88 mol) of isovaleryl chloride was slowly added, keeping the temperature between −30 and −25 °C. When the addition was complete the mixture was warmed to 0 °C and quenched with 400 mL of water. The aqueous layer was washed twice with 200 mL of MTBE (methyl *tert*-butyl ether). The combined organic layers were washed successively with 50 mL of saturated ammonium chloride, 50 mL of saturated sodium bicarbonate, and 100 mL of brine. The organic layer was dried over sodium sulfate, concentrated, and distilled to afford 139.7 g (88.0% yield) of **7**. Bp: 47–50 °C (0.70 Torr). ^1H NMR ($CDCl_3$): 5.78 (m, 1 H), 5.22 (m, 3 H), 2.2 (m), 0.95 (d, J = 7 Hz, 6 H), 0.90 (d, J = 7 Hz, 6 H) ppm. IR (film): 3070, 1730, 1640, 1460, 1360, 1285, 1245, 980, 1160, 1010, 985, 980 cm^{-1}.

(4*E*)-2-(2-Propyl)-7-methyl-4-octenoic Acid (4). A flask equipped with a mechanical stirrer, thermometer, and addition funnel was charged with 250 mL of THF and 21.7 mL (0.16 mol) of diisopropylamine. The solution was cooled to −70 °C, and 62 mL (2.5 M, 0.16 mol) of BuLi was added, causing a temperature rise to −50 °C. After 5 min a solution of 28.77 g (0.15 mol) of the ester **7** in 50 mL of THF was added over 10 min, keeping the temperature below −50 °C. When the addition was complete the solution was stirred for 5 min and treated with 26.4 mL (0.16 mol) of TMSCl. The solution was slowly warmed to 60 °C with a steam bath and maintained at that temperature for 15 min. The solution was cooled to 0 °C and treated with 50 mL of 4 M NaOH and 100 mL of water. The combined aqueous layers were acidified with concd. HCl and extracted with MTBE (methyl *tert*-butyl ether) (2 × 100 mL). The combined organic layers were dried over $MgSO_4$ and concentrated to give 26.0 g (93% yield) of a viscous clear oil. IR (film): 1702, 1465, 1435, 1410, 1385, 1366, 1280, 1220, 1165, 967 cm^{-1}. ^1H NMR ($CDCl_3$): 5.40 (m, vinyl H, 2H), 2.23 (m, allylic H, 2 H), 1.85 (t, allylic H, J = 6 Hz, 2 H), 0.96 (d, isopropyl, J = 6 Hz, 6 H), 0.83 (d, isopropyl, J = 6 Hz, 6 H) ppm. ^{13}C NMR ($CDCl_3$): 181.9, 131.6, 127.9, 53.0, 41.9, 32.6, 30.0, 28.4, 22.2, 22.1, 20.2, 20.1 ppm. MS (EI, direct probe): 55, 69, 87, 97, 109, 137, 155, 180, 198. CI (methane): 181, 199, 216, 227, 239.

Amides 8a and 9 from Claisen. A 3-L Morton flask fitted with mechanical sirrer, nitrogen inlet, thermometer, and addition funnel was charged with 850 mL of THF and diisopropylamine (51.6 g, 73.0 mL, 0.51 mol) and cooled to −30 °C. A solution of BuLi in hexane (319.0 mL, 0.51 mol) was then slowly added, keeping the temperature at −30 °C. When the addition was complete the ester **7** (85.0 g, 0.43 mol) was added over 45 min at −30 °C. The mixture was stirred for 0.5 h, and then TMSCl (65 mL, 0.51 mol) was added over 0.5 h. The solution was heated to 60 °C for 2 h and then allowed to cool to room temperature,

(12) Saito, S.; Nakajima, H.; Inaba, M.; Moriwake, T. *Tetrahedron Lett.* **1989**, *30*, 837.

treated with 68 mL of water, and stirred for 1.0 h. The solution was cooled to 10 °C and treated with α-methylbenzylamine (62.3 g, 0.52 mol) followed by DEPC (143.0 g, 133.0 mL, 0.88 mol). The mixture was stirred at rt overnight and then poured into a mixture of 500 mL of EtOAc and 250 mL of water. The phases were separated, and the aqueous phase was washed with EtOAc (2 × 200 mL). The combined organic layers were washed with 100 mL of brine and concentrated to an oil. The oil was taken up in 500 mL of heptane and slowly cooled to 0 °C and stirred for 2 h. The crystals were filtered to afford an 80:20 mixture of the diastereomeric amides **8a** and **9**. The mother liquors were concentrated and treated with 46.5 g of *t*-BuOK in 800 mL of THF at 50 °C for 5 h. The mixture was poured into water and the amide mixture isolated with ethyl acetate. A second crop of amide was crystallized from heptane to afford a 73:27 mixture of amides. This was combined with the first crop and crystallized from MTBE to afford 41.8 g (32.4% yield from the ester) of the desired amide with a diastereomeric purity of 98.6%. For amide **8a**: mp 107–109 °C. IR ($CDCl_3$): 3420, 3310, 1700, 1490, 1440, 1360, 1205, 1180, 1160, 1120, 1060, 1015, 965 cm^{-1}. ^1H NMR ($CDCl_3$): 7.25 (m, 5 H), 6.15 (d, J = 8 Hz, NH), 5.4 (m, 2 H), 5.15 (q, J = 7.4 Hz, 1 H), 2.25 (m, 2 H), 1.80 (m, 4 H), 1,57 (sep, J = 6.7 Hz, 1 H), 1.43 (d, J = 7 Hz, 3 H), 0.86 (d, J = 6.6 Hz, 6 H), 0.90 (d, J = 6.7, 3 H), 0.84 (d, J = 6.2 Hz, 3 H) ppm. ^{13}C NMR ($CDCl_3$): 173.77, 143.44, 130.98, 128.58, 128.34, 126.96, 126.15, 55.19, 48.15, 41.89, 33.33, 30.34, 28.21, 22.20, 21.59, 20.74, 20.21 ppm. Anal. Calcd for $C_{20}H_{31}NO$: C, 79.68; H, 10.37; N, 4.65. Found: C, 79.62, H, 10.62; N, 4.88.

For amide **9**. Mp: 100–103 °C. IR ($CDCl_3$): 3410, 3310, 1700, 1440, 1442, 1360, 1205, 1180, 1160, 1120, 1015, 965 cm^{-1}. ^1H NMR ($CDCl_3$): 7.5 (m, 5 H), 6.15 (d, J = 8.1 Hz), 5.25 (ABq split into dt, $\Delta\nu$ = 61.4 Hz, J = 6.1, 15.3 Hz, 2 H), 5.27 (q, J = 7.3 Hz, 1 H), 2.22 (m, 2 H), 1.80 (m, 4 H), 1.51 (sep, J = 6.7 Hz, 1 H), 1.43 (d, J = 6.9 Hz, 3 H), 0.94 (d, J = 6.3 Hz, 6 H), 0.81 (d, J = 6.5 Hz) ppm. ^{13}C NMR ($CDCl_3$): 173.71, 143.32, 130.93, 128.59, 128.31, 126.94, 126.15, 55.31, 47.98, 41.77, 33.34, 30.36, 28.18, 22.18, 22.10, 21.53, 20.64, 20.30 ppm. Anal. Calcd for $C_{20}H_{31}NO$: C, 79.68; H, 10.37; N, 4.65. Found: C, 79.69, H, 10.45; N, 4.71.

Epoxides 10a and 10b. A solution of 40.0 g of the amide **8a** in 400 mL of methylene chloride at −5 °C was treated with 43.0 g (199.3 mmol, 80% pure) of *m*-CPBA. The mixture was stirred at −5 °C for 5 h, washed with 10% KOH (100 mL, 200 mL), NH_4OH (2 × 90 mL, 15%), and brine (100 mL), dried over sodium sulfate, and concentrated. A sample was recrystallized 3× from EtOAc/Hept to afford material for analysis. ^{13}C NMR ($CDCl_3$): major diastereomer 173.48, 143.34, 128.37, 127.04, 126.11, 58.55, 57.47, 57.04, 51.33, 48.48, 41.03, 32.52, 31.89, 30.78, 26.19, 22.78, 22.33, 21.59, 20.60, 19.94 ppm. $[\alpha]_D$ = −98° (c = 0.96, $CHCl_3$). Mp: = 109–110 °C. Anal. Calcd for $C_{20}H_{31}NO_2$: C, 75.67; H, 9.84; N, 4.41. Found: C, 75.63; H, 9.44; N, 4.63. The minor isomer could not be separated chromatographically.

(2*S*,4*R*,5*S*)- and (2*S*,4*S*,5*R*)-4,5-Dihydroxy-2-isopropyl-7-methyloctanoic Acid, γ-Lactone (12a and 11a). A mixture of 169.0 mmol of the crude epoxides **10a** and **10b**, 650 mL of THF, and 350 mL of 1 M H_2SO_4 was heated to reflux until TLC showed the reaction to be complete. After the two-phase mixture was cooled to rt the lactones were isolated with 300 mL of MTBE. The organic phase is washed with 15% NH_4OH and brine and dried over Na_2SO_4. Concentration of the solution affords a 92.5% yield of the lactones in a 4.17:1 ratio. (2*S*,4*R*,5*S*)-Lactone **12a**. Mp: 61–63 °C. $[\alpha]_D$ = 7° ($CHCl_3$). IR (mull): 3435.2 2956.9, 2927.9, 1738.8, 1467.8, 1454.3, 1406.1, 1383.9, 1369.5, 1355.0, 1342.5, 1250.8, 1222.9, 1206.5, 1190.1, 1154.4, 1010.7, 984.7, 978.9, 951.9, 947.0, 844.8 cm^{-1}. ^{13}C NMR ($CDCl_3$): 179.45, 81.57, 69.91, 45.88, 40.96, 29.08, 24.43, 23.51, 23.09, 21.81, 20.37, 18.30 ppm. Anal. Calcd for $C_{12}H_{22}O_3$: C, 67.26, H, 10.34. Found: C, 67.42, H, 10.43. See below for spectroscopic data for the (2*S*,4*S*,5*R*)-lactone **11a**.

(2*S*,4*R*,5*S*)- and (2*S*,4*S*,5*R*)-4-Hydroxy-5-[(methanesulfonyl)oxy]-2-isopropyl-7-methyloctanoic Acid, γ-Lactone (12b and 11b). Methanesulfonyl chloride (1.08 mL, 14 mmol) was slowly added to a solution of 2.69 g (12.6 mmol) of the hydroxy lactones, 2.09 mL (15 mmol) of triethylamine, and 15 mL of CH_2Cl_2 at −20 °C. After the addition was complete the mixture was stirred for an additional 10 min and then poured into water. The mesylate was isolated with MTBE (3 × 50 mL). The combined organic layers were dried over $MgSO_4$ and concentrated. The crude product was chromatographed on silica gel with 30% isopropyl acetate/hexane to give three fractions, a total of 3.37 g (91.5% yield). The nonpolar fraction is the undesired (2*S*,4*R*,5*S*)-lactone **12b** (1.29 g). ^1H NMR ($CDCl_3$): 4.95 (m, 1 H), 4.56 (m, 1 H), 3.04 (s, 3 H), 2.65 (m, 1 H), 2.31 (m, 1 H), 2.15 (m, 2 H), 1.75 (m, 2 H), 1.03 (d, J = 7 Hz, 3 H), 0.99 (d, J = 6.5 Hz, 3 H), 0.98 (d, J = 6.4 Hz, 3 H), 0.95 (d, J = 6.7 Hz, 3 H) ppm. ^{13}C NMR ($CDCl_3$): 177.80, 81.32, 77.91, 45.32, 39.84, 38.61, 29.26, 24.17, 23.71, 22.95, 22.05, 20.25, 18.36 ppm. IR (neat): 2940, 1770, 1460, 1345, 1170, 1026, 965, 915, 890, 780 cm^{-1}. There was obtained 1.80 g of mixed fractions. The desired (2*S*,4*S*,5*R*)-lactone **11b** was eluted last (280 mg). ^1H NMR ($CDCl_3$): 5.06 (m, 1 H), 4.46 (m, 1 H), 3.05 (s, 3 H), 2.67 (m, 1 H), 2.21 (m, 2 H), 2.06 (m, 1 H), 1.85 (m, 1 H), 1.70 (m, 1 H), 1.35 (m, 1 H), 1.06 (d, J = 6.8 Hz, 3 H), 0.99 (d, J = 6.6 Hz, 6 H), 0.96 (d, J = 6.9 Hz, 3 H) ppm. ^{13}C NMR ($CDCl_3$): 176.61, 79.83, 77.53, 46.04, 39.51, 39.03, 27.59, 24.08, 23.88, 23.13, 21.75, 20.43, 18.15 ppm. IR (neat): 2940, 1770, 1460, 1395, 1195, 1170, 1140, 1030, 970, 920, 895, 782, 750, 710 cm^{-1}.

Mesylate Inversion: (2*S*,4*S*,5*R*)-4,5-Dihydroxy-2-isopropyl-7-methyloctanoic Acid, γ-Lactone (11a). A solution of the mesylate **12b** (1.72g, 5.90 mmol) in 13 mL of CH_3CN and 5 mL of water was treated with 0.93 g of KOH and stirred overnight at room temperature. The biphasic solution was poured into water and the lactone isolated with MTBE (2 × 75 mL). The organic layers were dried over $MgSO_4$ and concentrated to afford a viscous oil. A sample was purified for analysis by chromatography on silica gel with 15% EtOAc/cyclohexane to afford 1.15 g (91.5% yield) of the lactone after Kugelrohr distillation. The lactone **11a** crystallized upon standing. Recrystallization from heptane gave material with mp 70.5–72.5 °C. $[\alpha]_D$ = −30° ($CDCl_3$, c = 0.93). IR (neat): 3430, 2940, 1760, 1460, 1360, 1330, 1270, 1195, 1180, 1162, 1145, 1075, 1035, 1005, 970, 940 cm^{-1}. ^1H NMR ($CDCl_3$): 0.925 (d, J = 7.0 Hz, 6 H), 0.96 (d, J = 6.6 Hz, 3 H), 1.01 (d, J = 4.1 Hz, 3 H), 1.05 (m, 1 H), 1.40 (m, 1 H), 1.85 (m, 1 H), 2.08 (m, 1 H), 2.2 (m, 1 H), 2.6 (m, 1 H), 2.70 (d, J = 3.3 Hz, OH), 4.04 (m, 1 H), 4.25 (m, 1 H). ^{13}C NMR ($CDCl_3$): 178.0, 80.74, 68.65, 46.61, 40.53, 27.71, 24.47, 23.49, 23.10, 21.84, 20.61, 18.21 ppm. Anal. Calcd for $C_{12}H_{22}O_3$: C, 67.26, H, 10.34. Found: C, 67.31, H, 10.51.

(2*S*,4*R*,5*R*)-2-(2-Propyl)-4-hydroxy-5-azido-7-methyloctanoic Acid, γ-Lactone (23). A solution of 1.30 g (4.46 mmol) of mesylate **12b** in 12 mL of DMSO was treated with 1.45 g (22.3 mmol) of NaN_3 and 2.50 mL of PEG 300 and was heated to 70 °C for 22 h. The solution was cooled to rt and poured into water. The azide was isolated with MTBE. The organic layers were dried over $MgSO_4$ and concentrated to afford a viscous oil which was chromatographed on silica gel with 15% EtOAc/cyclohexane to afford 890 mg (83.5% yield) of a clear viscous oil. For preparative purposes this chromatography was omitted. ^{13}C NMR ($CDCl_3$): 176.83, 79.71, 62.33, 46.43, 38.90, 27.62, 26.99, 24.83, 23.21, 21.54, 20.55, 18.12 ppm. ^1H NMR ($CDCl_3$): 4.33 (dt, J = 5.6, 10.3 Hz, 1 H), 3.32 (dt, J = 10.3, 4.5 Hz, 1 H), 2.22 (m, 1 H), 1.89 (m, 2 H), 1.63 (m, 1 H), 1.33 (m, 1 H) ppm. IR (film): 2940, 2100, 1770, 1460, 1380, 1360, 1260, 1160, 1030, 967, 910 cm^{-1}. Anal. Calcd for $C_{12}H_{21}N_3O_2$: C, 60.23; H, 8.84; N, 17.56. Found: C, 60.87; H, 8.98; N, 17.77.

(2*S*,4*S*,5*S*)-2-(2-Propyl)-4-hydroxy-5-azido-7-methyloctanoic Acid, γ-Lactone (20). A solution of 2.59 mmol of mesylate **11b** in 8.0 mL of DMSO was treated with 1.3 g of NaN_3 and 1.75 mL of PEG 300. The slurry was heated to 70 °C for 24.5 h, cooled to room temperature, and then poured into water. The crude azide was isolated with MTBE. The organic layers were dried over $MgSO_4$ and concentrated to an oil. A sample was chromatographed on silica gel with 20% EtOAc/cyclohexane for analysis to afford 456 mg (66% yield) of the azide **20**. $[\alpha]_D$ = 30°. ^{13}C NMR ($CDCl_3$): 177.8, 79.3, 63.1, 45.0, 38.8, 28.9, 26.7, 24.6, 22.8, 21.6, 20.1, 18.2 ppm. ^1H NMR ($CDCl_3$): δ 4.38–4.48 (m, 1 H), 3.32–3.42 (m, 1 H), 2.64–2.74 (m, 1 H), 2.06–2.24 (m, 3 H), 1.76–1.94 (m, 1 H), 1.58–1.72 (m, 1 H), 1.36–1.48 (m, 1 H), 0.92–1.08 (m, 12 H) ppm. IR (neat): azido absorbance 2100 cm^{-1}.

Hydrogenation–BOC Formation: (2*S*,4*R*,5*R*)-2-(2-Propyl)-4-hydroxy-5-[(*tert*-butyloxycarbonyl)amino]-7-methyloctanoic Acid, γ-Lactone (25). A flask charged with 720 mg (3.0 mmol) of the azide **23**, 700 mg (3.2 mmol) of $(BOC)_2O$, 10 mL of EtOAc, and 75 mg of 5% Pd–C was placed in an at-

mospheric hydrogenation apparatus and subjected to hydrogen overnight. The catalyst was removed by filtration through solka floc and the filtrate concentrated to afford a solid which was crystallized from EtOAc/Hept to afford 747 mg (80% yield) of the lactone **25**. ^{13}C NMR ($CDCl_3$): 177.99, 156.04, 79.91, 79.56, 50.201, 46.46, 42.47, 28.36, 27.50, 25.94, 24.86, 23.05, 21.93, 20.58, 17.94 ppm. ^1H NMR ($CDCl_3$): 4.50 (d, J = 9.9 Hz, NH), 4.46 (dd, J = 6.2, 9.3 Hz, 1 H), 3.85 (td, J = 10.2, 4.5 Hz, 1 H), 2.6 (m, 1 H), 2.15 (m, 2 H), 1.85 (m, 1 H), 1.60 (m, 3 H), 1.43 (s, 9 H), 1.01 (d, J = 6.9 Hz, 3 H), 0.93 (d, J = 6.3 Hz, 6 H), 0.89 (d, J = 6.7 Hz, 3 H) ppm.

(2*S*,4*S*,5*S*)-2-(2-Propyl)-4-hydroxy-5-[(*tert*-butyloxycarbonyl)amino]-7-methyloctanoic Acid, γ-Lactone (22). A mixture of 457 mg (1.91 mmol) of the azide **20**, 525 mg of $(BOC)_2O$, and a catalytic amount of Pd–C in 10 mL of EtOAc was hydrogenated at 40 psi for 2 h. The catalyst was removed by filtration through solka floc and the product crystallized upon concentration to afford 607 mg (quantitative) of amide **22**. Recrystallization from EtOAc/Hept gave 407 mg of amide **22** along with 200 mg of mother liquor which crystallized upon concentration. $[\alpha]_D$ = −41° (c = 1, ethanol). Mp: 144.5–146 °C. ^1H NMR ($CDCl_3$): 4.55 (d, J = 9.5 Hz, NH), 4.45 (t, J = 6.1 Hz), 3.86 (bm), 2.58 (bm), 2.0–2.35 (m), 1.48 (s, 9 H), 0.96 (d, J = 5.6 Hz, 3 H), 0.94 (m, 9 H) ppm. ^{13}C NMR ($CDCl_3$): 179.0, 156.08, 80.74, 79.54, 51.68, 45.66, 41.77, 29.07, 28.21, 26.25, 24.67, 22.99, 21.77, 20.27, 18.32 ppm. IR (film): 3427, 3320, 1754, 1667, 1677, 1524, 1275, 1200, 1164, 1060, 1035, 675 cm^{-1}. Anal. Calcd for $C_{17}H_{31}NO_4$: C, 65.14, H, 9.97, N, 4.47. Found: C, 65.26, H, 9.92, N, 4.34.

Na/NH_3 Reduction of Amide. The amide **8a** (5.0 g, 16.6 mmole) was dissolved in 20 mL of butylamine and 50 mL of liquid ammonia at −33 °C. Sodium was added slowly so as to maintain the blue color. After 5.5 h the GC showed the reaction to be essentially complete, and NH_4Cl was added and the ammonia allowed to evaporate. The product was isolated with MTBE after the reaction mixture was poured into water. The organic extracts were dried over Na_2SO_4 and concentrated. The GC/MS indicates that some overreduction (4.7%) has taken place because of the presence of an impurity with a m/e = 303. The yields varied from 78 to 89%. The crude product was crystallized from EtOAc/Hept to afford amide **15** melting at 116–119 °C. $[\alpha]_D$ = −13° (c = 0.8, $CHCl_3$). ^1H NMR ($CDCl_3$): 5.42 (m, 2 H), 2.24 (t, J = 6.6 Hz, 2 H), 1.85 (m, 4 H), 1.57 (septet, J = 6.7 Hz, 1 H), 0.98 (d, J = 6.5 Hz, 3 H), 0.96 (d, J = 6.3 Hz, 3 H), 0.86 (d, J = 6.7 Hz, 6 H) ppm. ^{13}C NMR ($CDCl_3$): 177.88, 131.41, 128.42, 54.52, 41.97, 33.20, 30.23, 28.37, 22.28, 20.64, 20.33 ppm. IR: 3510, 3990, 2940, 1670, 1580, 1460, 1375, 1240, 990 cm^{-1}. Anal. Calcd for $C_{12}H_{23}NO$: C, 73.045, H, 11.75, N, 7.099. Found: C, 72.93, H, 11.57, N, 7.22.

Epoxides 16a and 16b. A solution of 100 mg (0.5 mmol) of the amide **15** in 5 mL of CH_2Cl_2 was cooled to 0 °C and treated with solid KOAc and 0.33 g (1.5 mmol) of *m*-CPBA. The mixture was stirred at 0 °C for 6 h and then allowed to warm to rt and stir overnight. The reaction mixture was quenched with aqueous $NaHSO_3$ and the product isolated with MTBE. The organic layers were dried over Na_2SO_4 and concentrated to afford the epoxide which was shown by GC to be a 12:1 mixture of diastereomers. The epoxide **16b** can be upgraded by crystallization from MTBE. $[\alpha]_D$ = −62° (c = 0.9, $CHCl_3$). Mp: 145–148 °C. ^1H NMR ($CDCl_3$): 6.21, (bs, NH), 6.10, (bs, NH), 2.75 (td, J = 5.9, 2.2 Hz, 1 H), 2.70 (dt, J = 7.3, 2.8 Hz, 1 H), 2.10 (m, 2 H), 1.40 (m, 4 H), 0.97 (d, J = 4.3 Hz, 3 H), 0.95 (d, J = 3.5 Hz, 3 H), 0.94 (d, J = 8.9 Hz, 6 H) ppm. ^{13}C NMR ($CDCl_3$): 177.22, 58.75, 57.08, 50.64, 41.00, 32.47, 30.60, 26.25, 22.80, 22.36, 20.57, 20.03 ppm. IR: 3510, 2890, 2940, 1710, 1680, 1510, 1360 cm^{-1}. Anal. Calcd for $C_{12}H_{23}NO_2$: C, 67.57, H, 10.87, N, 6.57. Found: C, 67.44, H, 10.59, N, 6.75.

Hydrolysis of Epoxy Amide 16b. A solution of 0.935 g of crystallized epoxide in 10 mL of dioxane and 5 mL of 1 M H_2SO_4 was heated to reflux for 2 h. After being cooled to room temperature the reaction mixture was poured into water and the lactone isolated with MTBE. Drying (Na_2SO_4) and concentration of the organic layers afforded 1.0 g of lactone **12a** which crystallized upon standing. GC shows it to be a 95.8/3.3 mixture of the (2*S*,4*R*,5*S*)/(2*S*,4*S*,5*R*) isomers.

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Stachybotrins A and B: Novel Bioactive Metabolites from a Brackish Water Isolate of the Fungus *Stachybotrys* sp.

Xuemin Xu,[1] Florecita S. de Guzman, and James B. Gloer*,[2]

Department of Chemistry, University of Iowa, Iowa City, Iowa 52242

Carol A. Shearer

Department of Plant Biology, University of Illinois, Urbana, Illinois 61801

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Stachybotrins A and B (**1** and **2**), two new aromatic alkaloids with antibacterial and antifungal activity, have been isolated from an aquatic isolate of a new species of the genus *Stachybotrys* (CS-710-1). Compounds **1** and **2** were obtained from ethyl acetate extracts of liquid cultures by preparative TLC. The structures were determined primarily by analysis of HMBC, HMQC, COSY, and NOESY experiments.

In the course of our investigations of marine and aquatic fungi as sources of novel biologically active secondary metabolites,[3-6] we examined an isolate of *Stachybotrys* sp. (CS-710-1) collected from brackish water in Florida. This isolate differs significantly from previously known members of the genus *Stachybotrys* and has been established as a representative of a new species.[7] Compounds previously reported from other *Stachybotrys* spp. include trichothecene mycotoxins (satratoxins),[8,9] sterols,[9] and a novel spirobenzofuran that exhibits effects on the com-

(1) Visiting Scientist from Sichuan Institute of Chinese Materia Medica, Chongqing, P.R. China.

(2) Alfred P. Sloan Foundation Fellow (1990–92) and NIH Research Career Development Awardee (CA01571; 1990–95).

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