A Mimic of Both a Torsionally-Distorted Peptide Ground State and the Transition State for Peptide Bond Hydrolysis: Synthesis of a Spiro[4.4]nonyl Derivative

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Because of their potential utility for catalyzing novel reactions with high substrate specificity, antibody catalysts have attracted much attention.[1,2] To date, however, no general method for eliciting antibody peptidases has been developed (although some notable successes have been reported.[3-6] Thus, we have been focusing on a new strategy: immunization with analogues that mimic not only the transition state for peptide bond hydrolysis but also a distorted peptide ground state.[7,8] After incorporation into longer peptides, these derivatives may elicit antibodies that catalyze sequence-specific peptide bond hydrolysis both by destabilizing the bound peptide substrate and by stabilizing the transition state.

We report here the synthesis of the spiro[4.4]nonane-containing dipeptide analogue 7-*trans*-amino-6-*trans*-hydroxyspiro[4.4]nonane-1-carboxylic acid, **16**, as a racemic mixture. (Although the analogue is diastereomerically pure, the relative stereochemistry at the carboxyl center has not yet been assigned.) This dipeptide analogue mimics both torsionally-strained glycyl-proline and glycyl-glycine as shown in Figure 1; in addition, the peptide bond has been replaced by a hydroxyethylene group, an effective transition state

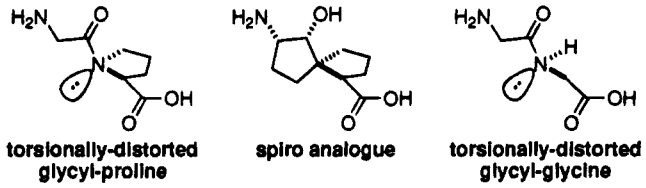


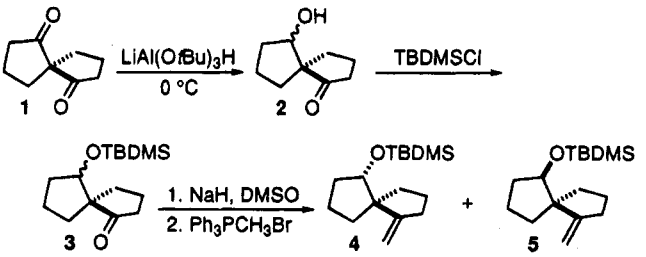
Figure 1. Alignment of analogue **16** with the corresponding dipeptides.

analogue for acyl group transfer.[9,10] Molecular modeling studies[11] indicate that in low-energy conformations of the *N*-acetylcarboxamide derivative of **16**, the peptide bond mimicked is significantly distorted from planarity (by an amount ranging from 58° to 80°). In preparation for immunization, **16** has been flanked by the amino acids D-tyrosine and D-phenylalanine, and the two diastereomeric products are separated. (D-Amino acids were used in this initial synthesis because of their enhanced immunogenicity.)

Results and Discussion

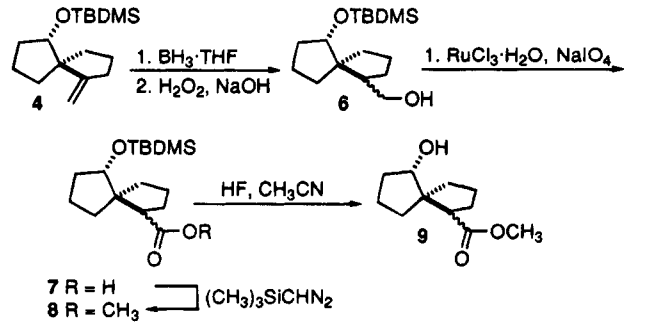
The critical step in the synthesis of the analogue **16** was the introduction of nitrogen functionality into the corresponding hydroxy ester, methyl 6-hydroxyspiro[4.4]-nonane-1-carboxylate **9**, via an intramolecular acylnitrene insertion reaction.[12] We have also utilized this approach in the synthesis of a series of [2.2.1]bicycloheptyl and cyclobutyl dipeptide analogues.[7,8] The hydroxy ester **9** was not a known compound, however, and was synthesized as outlined in Schemes 1 and 2.

Scheme 1



In a modification of a published procedure,[13] the slow addition of lithium (tri-*tert*-butoxyalumino)hydride to racemic spiro[4.4]nonane-1,6-dione (**1**) at 0 °C yielded a diastereomeric mixture of the hydroxy ketone **2**, with the *trans*-isomer predominating (approximately 70% by ^{1}H NMR). The alcohol functionality was then protected to give the *tert*-butyldimethylsilyl derivative **3**. Homologation to the alkenes **4** and **5**, which separated during chromatography on silica gel, was accomplished with methyltriphenylphosphonium bromide in sodium dimsylate.[14] (The *tert*-butyldimethylsilyl derivative **3** was unreactive with 2-(trimethylsilyl)-2-lithio-1,3-dithiane[15] and (methoxymethylene)triphenylphosphonium bromide/sodium dimsylate,[16] reagents that would have led to simultaneous homologation and oxidation.) Hydroboration[14] of the alkene **4** produced, as shown in Scheme 2,

Scheme 2



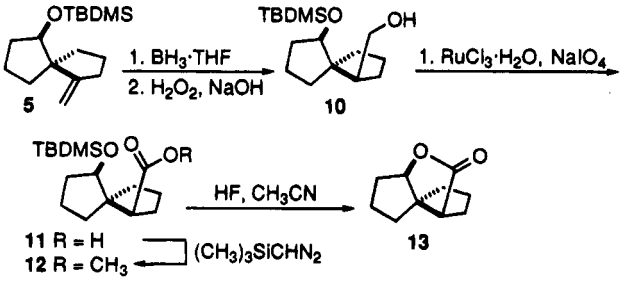
(1) Lerner, R. A.; Benkovic, S. J.; Schultz, P. G. *Science* **1991**, *252*, 659–667.
(2) Schultz, P. G.; Lerner, R. A. *Acc. Chem. Res.* **1993**, *26*, 391–395.
(3) Iverson, B. L.; Lerner, R. A. *Science* **1989**, *243*, 1184–1188.
(4) Gibbs, R. A.; Taylor, S.; Benkovic, S. J. *Science* **1992**, *258*, 803–805.
(5) Paul, S.; Mei, S.; Mody, R.; Tewary, H. K.; Massey, R. J.; Gianferrara, T.; Mehrotra, S.; Dreyer, T.; Meldal, M.; Tramontano, A. *J. Biol. Chem.* **1992**, *267*, 13142–13145.
(6) Martin, M. T.; Angeles, T. S.; Sugasawara, R.; Aman, N. I.; Napper, A. D.; Darslay, M. J.; Sanchez, R. I.; Booth, P.; Titmas, R. C. *J. Am. Chem. Soc.* **1994**, *116*, 6508–6512.
(7) Smith, R. A.; Yuan, P.; Weiner, D. P.; Dutton, C. R.; Hansen, D. E. *Appl. Biochem. Biotech.* **1994**, *47*, 329–343.
(8) Yuan, P.; Driscoll, M. R.; Raymond, S. J.; Hansen, D. E.; Blatchly, R. A. *Tetrahedron Lett.* **1994**, *35*, 6195–6198.
(9) Liotta, L. J.; Benkovic, P. A.; Miller, G. P.; Benkovic, S. J. *J. Am. Chem. Soc.* **1993**, *115*, 350–351.
(10) Wolfenden, R.; Radzicka, A. *Curr. Opin. Struct. Biol.* **1991**, *1*, 780–787.
(11) MM+, *HyperChem*, Autodesk, Inc., 1992.
(12) Lowe, G.; Swain, S. *J. Chem. Soc., Perkin Trans. 1* **1985**, 391–398.
(13) Carruthers, W.; Orridge, A. *J. Chem. Soc., Perkin Trans. 1* **1977**, 2411–2416.

0022-3263/95/1960-5360$09.00/0

the alcohol **6** in an epimeric ratio of 9:1, as determined by ^{1}H NMR. Ruthenium tetraoxide-catalyzed oxidation[17] of **6** afforded the carboxylic acid **7**, which was methylated with trimethylsilyl diazomethane ($TMSCHN_2$)[18] to yield the protected hydroxy ester **8**. Deprotection with hydrogen fluoride[19] gave the corresponding hydroxy ester **9**. The derivatives **7**–**9** were each also obtained as an approximately 9:1 ratio of epimers.

As shown in Scheme 3, we had initially attempted to elaborate the alkene **5** by the route above. (In this synthesis, we again began with an epimeric mixture of the hydroxy ketone **2**, enriched, however, in the *cis*-isomer. This mixture was obtained, as previously described,[13] by the monoreduction dione **1** with lithium (tri-*tert*-butoxyalumino)hydride at −30 °C, rather than 0 °C. In our hands, platinum oxide-catalyzed reduction of the dione **1**, which had been reported[20] to yield predominantly the *trans*-isomer, also yielded the *cis*-isomer as the major product.) Hydroboration of the alkene **5** yielded exclusively the alcohol **10**, which was oxidized to the acid **11**. Methylation yielded the ester **12**, which upon deprotection with hydrogen fluoride in acetonitrile gave the tricyclic lactone **13** as the sole product. Lactone formation established that the precursor **12** is the *cis,cis*-isomer; the protected hydroxyl substituent in alkene **5** must thus also be *cis*. Hence, this substituent in the epimeric alkene **4**, and in the hydroxy ester **9** derived from it, must be *trans*. Lactone **13** resisted hydrolytic and methanolytic opening and was not elaborated further.

Scheme 3



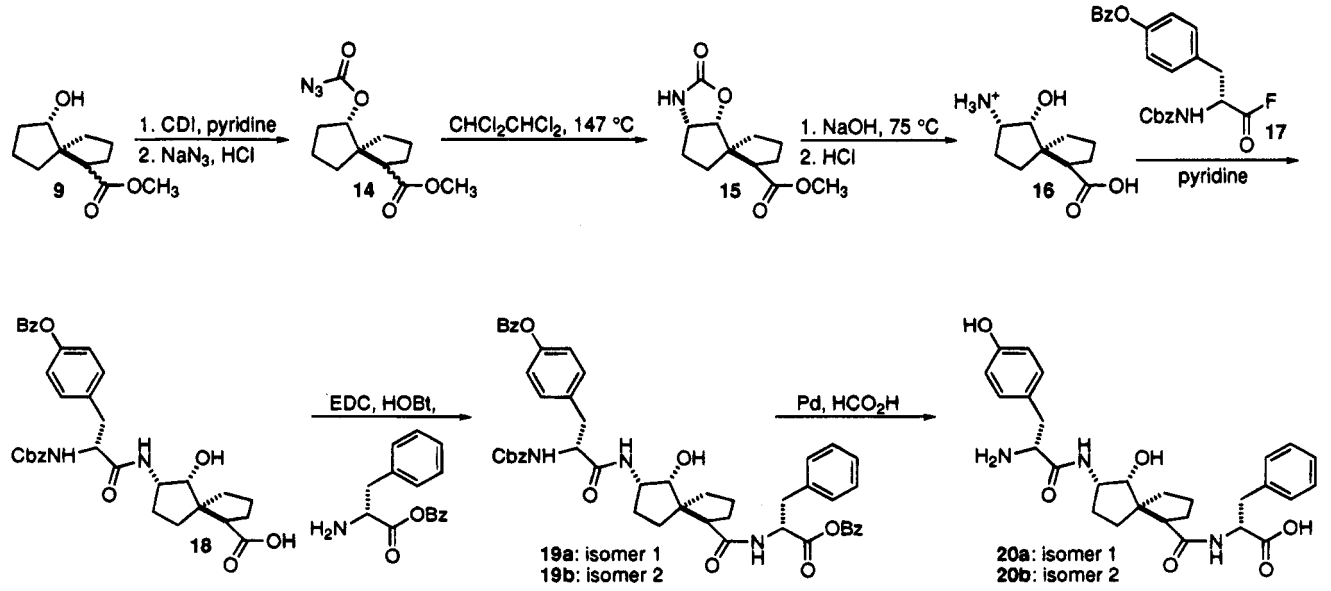
To complete the synthesis, therefore, we turned to the hydroxy ester **9**, which, as indicated in Scheme 4, was treated with 1,1′-carbonyldiimidazole (CDI), followed by sodium azide to generate the azidocarbonate **14**. This material was immediately refluxed in 1,1,2,2-tetrachloroethane (TCE)[21] to give the corresponding carbamate **15** in 54% yield. We have found the use of refluxing TCE preferable to thermolysis in methylene chloride, the method we had employed previously.[7,8] Only the carbamate product corresponding to the major stereoisomer was isolated in this step (again, the stereochemistry at the carboxyl center has not been assigned). Hydrolysis of the carbamate **15** in hot aqueous sodium hydroxide afforded the hydroxyamino acid **16**, which was directly coupled to the protected D-tyrosyl acid fluoride **17**[22] to yield the protected dipeptide **18**, as a mixture of diastereomers. This mixture was coupled to D-phenylalanine benzyl ester with 1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide (EDC)/1-hydroxybenzotriazole (HOBt) to yield the diastereomeric, protected peptide derivatives **19a** and **19b**, which separated during chromatography on silica gel. Deprotection by catalytic transfer hydrogenation with palladium black and formic acid yielded the diastereomeric peptide derivatives **20a** and **20b**, which have been conjugated to the carrier proteins keyhole limpet haemocyanin and bovine serum albumin in preparation for immunization.

In summary, we have described the synthesis of a spiro[4.4]nonane-containing dipeptide analogue, which mimics both a torsionally-distorted peptide ground state and the transition state for peptide bond hydrolysis, and have coupled both the amino and carboxyl termini of this derivative to amino acids of the D-configuration.

Experimental Section

General. Melting points are uncorrected. ^{1}H and ^{13}C NMR data were run at 400 MHz and 100.6 MHz, respectively. *J* values are given in Hz. Spectra are referenced with respect to the solvent peak (δ_H = 7.26 ppm and δ_C = 77.0 ppm for $CDCl_3$; δ_H = 3.30 ppm and δ_C = 49.0 ppm for CD_3OD). High-resolution mass spectra (HRMS) were determined at the Harvard Chemistry Department Mass Spectrometry Facility. Analytic HPLC utilized an Alltech Econosphere C18 column (15 × 0.46 cm, 5 μm); preparative HPLC utilized a Waters μBondapack Phenyl Radial-Pak cartridge (10 × 2.5 cm, 10 μm). All elution solvents were CH_3CN/water mixtures containing 0.025% TFA. Column chromatography employed Aldrich silica gel 60 Å (200–400

Scheme 4



mesh), and analytical thin-layer chromatography was performed on Bake precoated silica gel plates (Si250F). Chemical reagents were obtained from Aldrich or Sigma unless otherwise noted. Anhydrous solvents, such as DMF and THF, were purchased from Aldrich in Sure/Seal bottles and used without further purification. When indicated, solutions were dried over anhydrous $MgSO_4$ and the solvent removed by evaporation under reduced pressure.

***cis*- and *trans*-6-Hydroxyspiro[4.4]nonan-1-one (2).** A solution of spiro[4.4]nonane-1,6-dione (1) (47 mg, 0.309 mmol) in 1 mL of dry THF was slowly added to a stirred solution of lithium (tri-*tert*-butoxyalumino)hydride (96 mg, 0.307 mmol) in 4 mL of dry THF under nitrogen at 0 °C. The solution was then warmed to room temperature and stirred for 3 h. The mixture was acidified with 5% acetic acid and extracted with ether (4 × 5 mL). The combined ether extracts were dried and the solvent removed. The residue was purified by chromatography on silica gel (1:1 EtOAc:hexanes, R_f 0.53) to afford **2** (37 mg, 78%) as a colorless oil; ^{1}H NMR showed the *trans*-isomer to be the major product (approximately 70%): IR (neat) 3446, 2957, 1733, 1161 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.16 (t, J = 6.8, 1H, *trans*-isomer), 4.01 (t, J = 3.9, 1H, *cis*-isomer), 3.37 (br s, 1H), 2.35−1.51 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 224.4, 224.0, 80.0, 59.7, 58.5, 38.3, 37.8, 35.3, 33.9, 33.7, 33.1, 32.8, 29.7, 20.8, 20.1, 19.0, 18.7; HRMS (M + NH_4)$^+$ calcd for $C_9H_{18}NO_2$ 172.2474, found 172.1330.

***cis*- and *trans*-6-((*tert*-Butyldimethylsilyl)oxy)spiro[4.4]nonan-1-one (3).** To a stirred solution of **2** (691 mg, 4.516 mmol) in 1.4 mL of dry DMF were added *tert*-butyldimethylsilyl chloride (817 mg, 5.420 mmol) and imidazole (768 mg, 11.281 mmol). The reaction mixture was stirred for 2 days at room temperature, and then 5 mL of water was added. The mixture was extracted with ether (4 × 8 mL), the combined organic layers were dried, and the solvent was removed. Chromatography on silica gel (6:1 EtOAc:hexanes, R_f 0.68) afforded **3** as a colorless oil (1.124 g, 93%): IR (neat) 2957, 2857, 1738, 1772, 1250, 1113, 837, 776 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.15 (app t, J = 7.3, 1H, *trans*-isomer), 3.97 (dd, J = 6.5, 7.7, 1H, *cis*-isomer), 2.33-1.32 (m, 12H), 0.83 (s, 9H, *trans*-isomer), 0.82 (s, 9H, *cis*-isomer), 0.01 (s, 3H, *cis*-isomer), −0.05 (s, 3H, *trans*-isomer), −0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 222.4, 219.5, 82.5, 77.2, 59.6, 58.1, 38.3, 38.2, 36.6, 34.0, 33.9, 33.3, 32.2, 30.0, 25.3, 20.7, 20.2, 19.3, 19.0, 17.5, 17.4, −5.1, −5.47, −5.50; HRMS (M + H)$^+$ calcd for $C_{15}H_{29}O_2Si$ 269.1937, found 269.1940.

***trans*-((*tert*-Butyldimethylsilyl)oxy)spiro[4.4]nonan-6-ene (4) and *cis*-((*tert*-Butyldimethylsilyl)oxy)spiro[4.4]non-6-ene (5).** Sodium hydride (24 mmol as a 60% dispersion in mineral oil) was washed with 50 mL of anhydrous ether under nitrogen, and 10 mL of dry DMSO was added. The mixture was heated at 70−75 °C for approximately 1 h until a clear dark-gray solution formed. The resulting solution was cooled to 0 °C, and methyltriphenylphosphonium bromide (9.0 g, 25.193 mmol) in 25 mL of dry DMSO was added. The resulting dark-red solution was stirred at room temperature for 15 min, and then **3** (680 mg, 2.537 mmol) in 5 mL of DMSO was added. The reaction mixture was stirred at 55 °C for 3 h, cooled, and diluted with water. The resulting mixture was extracted with EtOAc (4 × 40 mL), and the combined extracts were washed with brine and dried. The solvent was removed and the residue purified by chromatography on silica gel (hexanes, R_f **4** 0.39, R_f **5** 0.68) to afford **4** (316 mg, 47%) and **5** (160 mg, 24%) as colorless oils. **4**: IR (neat) 3077, 2955, 2856, 1646, 1472, 1256, 1122, 877, 836, 775 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.91 (app t, J = 2.0, 1 H), 4.77 (app t, J = 2.1, 1H), 3.98 (app t, J = 7.2, 1H), 2.36 (m, 2H), 2.12 (m, 1H), 1.96 (m, 1H), 1.64−1.38 (m, 8H), 0.90 (s, 9H), 0.04 (s, 3H), 0.02 (s, 3H); ^{13}C ($CDCl_3$) δ 159.6, 103.0, 80.5, 57.0, 37.7, 34.7, 33.0, 32.7, 25.9, 23.5, 20.1, 18.0, −4.6, −4.8; HRMS (M + H)$^+$ calcd for $C_{16}H_{31}O_6Si$ 267.2144, found 267.2149. **5**: IR (neat) 3085, 2954, 2856, 1653, 1472, 1254, 1113, 1057, 835, 773 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.97 (m, 1H), 4.88 (m, 1H), 3.66 (dd, J = 2.6, 5.0, 1H), 2.35 (m, 2H), 1.94 (m, 2H), 1.79 (m, 1H), 1.38−1.67 (m, 7H), 0.87 (s, 9H), −0.01 (s, 3H), −0.02 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 153.8, 107.6, 79.5, 57.7, 39.4, 36.4, 34.6, 32.8, 26.0, 23.0, 20.8, 18.2, −4.6, −4.8; HRMS (M + H)$^+$ calcd for $C_{16}H_{31}O_6Si$ 267.2144, found 267.2131.

***trans*-1-((*tert*-Butyldimethylsilyl)oxy)-*trans*-6-(hydroxymethyl)spiro[4.4]nonane and *trans*-1-((*tert*-Butyldimethylsilyl)oxy)-*cis*-6-(hydroxymethyl)spiro[4.4]nonane (6).** To a stirred solution of **4** (362 mg, 1.356 mmol) at 0 °C in 27 mL of dry THF under an atmosphere of nitrogen was added 1 M borane-THF complex (2.7 mL, 2.700 mmol). The reaction mixture was maintained at 0 °C for 30 min and then stirred at room temperature for another 30 min. The solution was cooled to 0 °C again. After successive additions of 3 N aqueous NaOH (4 mL) and 30% hydrogen peroxide (4 mL), the reaction mixture was stirred at 0 °C for 1 h. The solution was concentrated to half its volume by evaporation under reduced pressure and diluted with 10 mL of water. The solution was extracted with EtOAc (4 × 25 mL), and the combined extracts were washed with brine and dried. The solvent was removed and the residue purified by chromatography on silica gel (4:1 hexanes:EtOAc, R_f 0.53) to give **6** as a colorless, viscous oil (336 mg, 87%). ^{1}H NMR showed **6** to be a 9:1 ratio of diastereomers: IR (neat) 3347, 2957, 2862, 1463, 1256, 1109, 1027, 881, 836, 774 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.09 (app t, J = 7.70, 1H, major), 3.83 (app t, J = 7.3, 1H, minor), 3.62 (m, 2H, major), 3.48 (m, 2H, minor), 2.49 (br s, 1H), 1.90−1.32 (m, 13H), 0.88 (s, 9H), 0.08 (s, 3H, major), 0.06 (s, 3H, major), 0.03 (s, 3H, minor), 0.02 (s, 3H, minor); ^{13}C NMR ($CDCl_3$) δ 76.7, 76.1, 64.3, 64.0, 54.9, 49.3, 46.0, 36.9, 32.9, 31.9, 31.8, 30.8, 29.8, 29.1, 25.83, 25.80, 23.1, 22.7, 19.0, 17.9, −3.4, −4.6, −4.8, −5.0; HRMS (M + H)$^+$ calcd for $C_{16}H_{33}O_2Si$ 285.2250, found 285.2240.

***trans*-6-((*tert*-Butyldimethylsilyl)oxy)spiro[4.4]nonane-*trans*-1-carboxylic Acid and *trans*-6-((*tert*-Butyldimethylsilyl)oxy)spiro[4.4]nonane-*cis*-1-carboxylic Acid (7).** To a solution of **6** (210 mg, 0.739 mmol) and sodium periodate (514 mg, 2.403 mmol) in a mixture of 1.5 mL of CCl_4, 1.5 mL of CH_3CN, and 2.25 mL of water was added ruthenium trichloride hydrate (4 mg, 0.019 mmol). The reaction mixture was stirred vigorously for 3 h at room temperature, and 10 mL of CH_2Cl_2 was added. The upper aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL), the combined organic extracts were dried, and the solvent was removed. The resulting residue was diluted with 120 mL of ether and the solution filtered through a small silica gel column to remove the ruthenium trichloride. The filtrate was dried and the solvent removed to afford **7** (203 mg, 92%) as a viscous, colorless oil: IR (neat) 3500−2450, 2956, 2857, 1703, 1699, 1471, 1422, 1250, 1112, 837, 775 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 12.05 (br s, 1H), 4.05 (app t, J = 6.0, 1H), 2.69 (app t, J = 8.6, 1H, minor), 2.52 (app t, J = 7.7, 1H, major), 2.12−2.20 (m, 1H), 2.06−1.38 (m, 11H), 0.88 (s, 9H), 0.03 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 182.0, 76.5, 58.0, 51.8, 36.6, 33.8, 32.6, 28.7, 25.8, 23.1, 20.0, 17.9, −4.1, −5.1; HRMS (M − H)$^-$ calcd for $C_{16}H_{29}O_3Si$ 297.1886, found 297.1891.

Methyl *trans*-6-((*tert*-Butyldimethylsilyl)oxy)spiro[4.4]nonane-*trans*-1-carboxylate and Methyl *trans*-6-((*tert*-Butyldimethylsilyl)oxy)spiro[4.4]nonane-*cis*-1-carboxylate (8). To a stirred solution of **7** (157 mg, 0.524 mmol) in a mixture of 3.5 mL of hexanes and 1.0 mL of CH_3OH was added trimethylsilyl diazomethane (500 μL, 2M solution in hexanes) at room temperature. The mixture was stirred for 1 h at room temperature, and the solvents were removed to give **8** as a colorless liquid (164 mg, 100%): R_f 0.64 (6:1 hexanes:EtOAc); IR (neat) 2955, 2857, 1732, 1463, 1434, 1257, 1157, 1111, 837, 775 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.96 (app t, J = 7.7, 1H, minor), 3.84 (app t, J = 5.3, 1H, major), 3.63 (s, 3H), 2.62 (app t, J = 8.5, 1H, minor), 2.46 (app t, J = 7.7, 1H, major), 2.14−1.24 (m, 12H), 0.87 (s, 9H, minor), 0.84 (s, 9H, major), 0.04 (s, 3H, minor), 0.02 (s, 3H, minor), 0.00 (s, 3H, major), −0.01 (s, 3H, major); ^{13}C NMR ($CDCl_3$) δ 175.3, 76.7, 58.2, 51.8, 51.1, 36.5, 33.9, 33.1,

(14) Piers, E.; Britton, R. W.; Geraghty, M. B.; Keziere, R. J.; Kido, F. *Can. J. Chem.* **1975**, *53*, 2838−2848.
(15) Jones, P. F.; Lappert, M. F. *J. Chem. Soc., Chem. Commun.* **1972**, 526.
(16) Welch, S. C.; Gruber, J. M.; Morrison, P. A. *J. Org. Chem.* **1985**, *50*, 2676−2681 and references therein.
(17) Carlsen, P. H.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936−3938.
(18) Hashimoto, N.; Aoyama, T.; Shioiri, T. *Chem. Pharm. Bull.* **1981**, *29*, 1475−1478.
(19) Newton, R. F.; Reynolds, D. P. *Tetrahedron Lett.* **1979**, *20*, 3981−3882.
(20) Hardegger, B. E.; Maeder, E.; Semarne, H. M.; Cram, D. J. *J. Am. Chem. Soc.* **1959**, *81*, 2729−2737.
(21) Meth-Cohn, O. *Acc. Chem. Res.* **1987**, *20*, 18−27.
(22) Carpino, L. A.; Mansour, E.-S. M. E.; Sadat-Aalaee, D. *J. Org. Chem.* **1991**, *56*, 2611−2615.

28.7, 25.8, 23.0, 20.2, 17.9, −4.2, −5.1; HRMS (M + H)$^+$ calcd for $C_{17}H_{33}O_3Si$ 313.2199, found 313.2205.

Methyl *trans*-6-Hydroxyspiro[4.4]nonane-*trans*-1-carboxylate and Methyl *trans*-6-Hydroxyspiro[4.4]nonane-*cis*-1-carboxylate (9). To a stirred solution of **8** (210 mg, 0.673 mmol) in 3 mL of CH_3CN at 0 °C were added two drops of 50% of hydrogen fluoride. The reaction was stirred at room temperature for 2 h, and 5 mL of water was added. The mixture was extracted with CH_2Cl_2 (4 × 10 mL), and the combined extracts were dried. The solvent was removed to afford **9** as a colorless, viscous oil (130 mg, 98%): R_f 0.53 (major), 0.44 (minor) (2:1 hexanes:EtOAc); IR (neat) 3503, 2957, 2872, 1731, 1436, 1289, 1160 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.94 (app t, J = 7.3, 1H, minor), 3.78 (app t, J = 7.6, 1H, major), 3.61 (d, J = 1.2, 3H, major), 3.58 (d, J = 1.2, 3H, minor), 2.61 (app t, J = 8.3, 1H, minor), 2.50 (app t, J = 7.5, 1H, major), 2.07−1.37 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 177.3, 76.3, 56.3, 52.2, 51.5, 37.5, 31.3, 30.6, 30.3, 23.8, 18.7; HRMS (M + H)$^+$ calcd for $C_{11}H_{19}O_3$ 199.2701, found 199.1344.

Methyl Spiro-*trans*-1-oxa-2-oxo-*trans*-3-azabicyclo[3.3.0]-octane-7,2′-cyclopentane-1′-carboxylate (15). To a solution of **9** (125 mg, 0.631 mmol) in 2 mL of benzene were added 1,1′-carbonyldiimidazole (205 mg, 1.264 mmol) and pyridine (0.153 mL, 1.892 mmol). The reaction mixture was stirred at room temperature for 3 h, and then 10 mL of EtOAc was added. The resulting solution was washed quickly with brine (3 × 5 mL), the organic phase dried, and the solvent removed to give a clear oil. To this oil were added 3 mL of dry DMF and sodium azide (205 mg, 3.153 mmol). The reaction medium was then acidified to approximately pH 4 with concentrated HCl and stirred at room temperature overnight. Fifteen mL of brine was added, and the aqueous layer was extracted with EtOAc (4 × 20 mL). The combined organic layers were washed with brine (2 × 10 mL) and dried and the solvent removed to afford the azidoformate **14** as a colorless oil (156 mg, yield 92%): ^{1}H NMR ($CDCl_3$) δ 4.98 (dd, J = 5.6, 6.8, 1H, minor), 4.88 (dd, J = 4.8, 6.7, 1H, major), 3.60 (s, 3H), 2.61 (app t, J = 8.3, 1H), 2.48 (dd, J = 6.4, 7.6, 1H), 2.10−1.40 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 174.7, 156.6, 83.2, 56.8, 51.6, 51.4, 36.6, 31.9, 30.4, 28.3, 22.7, 19.7. A solution of **14** (103 mg, 0.386 mmol) in 30 mL of 1,1,2,2-tetrachloroethane (TCE) was added to an additional 220 mL of refluxing TCE. The solution ws refluxed for 45 min, and the solvent was removed to yield a brown oil, which was purified by chromatography on silica gel (EtOAc, R_f 0.53) to afford **15** as a waxy solid (50 mg, 54%): IR (film) 3277, 2956, 2874, 1766−1716, 1435, 1252, 1162, 1047 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.33 (br s, 1H), 4.84 (d, J = 7.1, 1H), 4.20 (app t, J = 6.1, 1H), 3.67 (s, 3H), 2.51 (app t, J = 8.2, 1H), 2.19−1.59 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 175.2, 159.6, 84.7, 58.8, 57.0, 52.3, 51.7, 34.7, 33.6, 32.4, 29.5, 22.9; HRMS (M + H)$^+$ calcd for $C_{12}H_{18}NO_4$ 240.1236, found 240.1235.

***N*-(Carbobenzyloxy)-*O*-benzyl-D-tyrosinecarboxylic Acid Fluoride (17).** To a solution of Cbz-D-Tyr(OBz)-H [BACHEM Bioscience, Inc.] (1.62 g, 4.000 mmol) in CH_2Cl_2 (10 mL) under argon at −10 °C was added pyridine (0.324 mL, 4.007 mmol) and cyanuric fluoride (Fluka Chemie AG) (1.08 g, 7.997 mmol). After 75 min the reaction was quenched by the addition of crushed ice, and an additional 20 mL of CH_2Cl_2 was added. The organic layer was then removed, and the aqueous layer extracted with CH_2Cl_2 (10 mL). The combined organic layers were washed with ice-cold water (10 mL), dried, and the solvent removed. The yellow residue was recrystallized from hexanes to afford **17** (1.08 g, 72%) as a white solid (mp 79−80 °C): IR ($CHCl_3$) 3431, 3034, 1845, 1723, 1512, 1244; ^{1}H NMR ($CDCl_3$) δ 7.42−7.30 (m, 10 H), 7.10−7.07 (d, J = 8.0 Hz, 2 H), 6.96−6.93 (d, J = 8.0 Hz, 2 H), 5.14 (s, broad, 1 H), 5.12 (s, 2 H), 5.02 (s, 2 H), 4.81−4.75 (m, 1 H), 3.14−3.10 (d, J = 5.8 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 161.9 (d, $^1J_{C-F}$ = 363.6 Hz), 158.4, 155.5, 136.8, 135.7, 130.4, 128.6, 128.3, 128.1, 128.0, 127.5, 126.3, 115.4, 70.0, 67.4, 53.8 (d, $^2J_{C-F}$ = 58.1 Hz), 36.0; HRMS (M + Na)$^+$ calcd for $C_{24}H_{22}O_4$-NF 407.1533, found 407.1535.

***N*-(*N*-(Benzyloxycarbonyl)-*O*-benzyl-D-tyrosyl)-7-*trans*-amino-6-*trans*-hydroxyspiro[4.4]nonane-1-carboxylic Acid (as a Mixture of Diastereomers) (18).** A solution of **15** (19 mg, 0.079 mmol) in 0.4 M aqueous sodium hydroxide (2 mL) was heated at 75 °C for 18 h. After cooling, the reaction mixture was neutralized with 1 N HCl. The solvent was removed and the residue taken up in acetone. The slurry was filtered to remove sodium chloride and the solvent removed to yield the deprotected derivative **16** (approximately 100% by ^{1}H NMR): ^{1}H NMR (CD_3OD) δ 3.97 (δ, J = 4.9, 1H), 3.70 (m, 1H), 2.49 (app t, J = 5.5, 1H), 2.18−1.43 (m, 10H); ^{13}C NMR (CD3OD) δ 179.2, 75.3, 59.5, 54.5, 54.1, 35.9, 34.4, 29.6, 27.7, 23.5. To a stirred solution of the derivative **16** in 2 mL of dry DMF at 0 °C under nitrogen was added pyridine (15 μL, 0.185 mmol). The acid fluoride **17** (35 mg, 0.086 mmol) in 0.5 mL of dry DMF was then added dropwise. The solution was stirred at 0 °C and the reaction monitored by analytical HPLC (CH_3CN 45% for 2 min; 45−70% over 28 min; 70−100% over 5 min, flow rate: 1 mL/min; t_R = 14.67 and 15.23 min for the two diastereomers). After 30 min, the solvent was removed and the residue dissolved in a minimum of CH_3CN/water. The products were purified by preparative HPLC (CH_3CN: 45% for 2 min; 45−60% over 30 min; 60−100% over 10 min; flow rate: 7 mL/min) to yield **18** (25 mg, 54%) as a mixture of diastereomers: ^{1}H NMR (CD_3OD) δ 7.42−7.22 (m, 10H), 7.12 (d, J = 7.6, 2H), 6.89 (d, J = 7.6, 2H), 5.02 (s, 4H), 4.32−4.15 (m, 2H), 3.83 (dd, J = 5.0, 25.5, 1H), 3.04 (ddd, J = 5.9, 13.9, 27.8, 1H), 2.78 (m, 1H), 2.48 (dd, J = 6.6, 14.7, 1H), 2.13 (m, 2H), 1.94 (m, 2H), 1.79 (m, 1H), 1.61−1.41 (m, 5H); ^{13}C NMR (CD_3OD) δ 179.6, 174.2, 156.0, 139.6, 132.2, 131.7, 131.5, 130.3, 130.6, 130.2, 129.7, 129.6, 129.5, 129.3, 116.7, 77.5, 71.8, 68.3, 59.8, 59.7, 55.0, 54.9, 54.6, 39.2, 38.6, 37.0, 35.4, 30.3, 30.1, 24.2; HRMS (M − H)$^-$ calcd for $C_{34}H_{37}N_2O_7$ 585.2601, found 585.2609.

***N*-(*N*-(Benzyloxycarbonyl)-*O*-benzyl-D-tyrosyl)-*trans*-7-amino-*trans*-6-hydroxyspiro[4.4]nonane-1-carbonyl-D-phenylalanine Benzyl Ester (Separated into Diastereomers 19a and 19b).** To a stirred solution of **18** (24 mg, 0.041 mmol), 1-hydroxybenzotriazole (10 mg, 0.074 mmol), and H-D-Phe-OBz (11 mg, 0.043 mmol) in 4 mL of dry THF at 0 °C under nitrogen was added 1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide·HCl (14 mg, 0.073 mmol). (H-D-Phe-OBz was prepared by partitioning H-D-Phe-OBz·*p*-tosylate [BACHEM Biosciences, Inc.] between 5% sodium bicarbonate and EtOAc. The organic layer was separated and dried, and the solvent was removed.) The resulting solution was stirred at 0 °C for 1 h and then overnight at room temperature. The solvent was removed and the residue purified by chromatography on silica gel (2:1 EtOAc:hexanes, R_f **19a** 0.55, R_f **19b** 0.42) to afford **19a** (14 mg, 41%) and **19b** (14 mg, 41%). **19a**: ^{1}H NMR ($CDCl_3$) δ 7.43−7.24 (m, 12H), 7.10−7.04 (m, 5H), 6.87 (d, J = 4.7, 2H), 6.44 (d, J = 4.6, 1H), 6.00 (d, J = 8.1, 1H), 5.31 (d, J = 7.6, 1H), 5.17 (q, J = 12.0, 2H), 5.08 (d, J = 4.6, 2H), 5.01 (s, 2H), 4.96 (dt, J = 5.9, 7.8, 1H), 4.35 (m, 1H), 3.90 (m, 1H), 3.42 (d, J = 7.8, 1H), 3.17 (dd, J = 5.6, 13.9, 1H), 3.00 (m, 3H), 2.24 (app t, J = 7.6, 1H), 2.07 (m, 1H), 1.88 (m, 2H), 1.71 (m, 2H), 1.48 (m, 3H), 1.15 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 175.6, 171.6, 170.4, 157.9, 155.7, 137.0, 136.3, 135.6, 134.9, 130.4, 130.4, 130.3, 129.2, 128.7, 128.63, 128.56, 128.51, 128.2, 128.1, 128.0, 128.0, 128.0, 127.9, 127.5, 127.4, 127.3, 115.0, 76.6, 74.0, 70.0, 67.5, 66.9, 55.9, 55.4, 52.9, 51.3, 38.0, 36.6, 31.3, 30.0, 24.2; HMRS (M + Na)$^+$ calcd for $C_{60}H_{53}N_3O_8Na$ 846.3730, found 846.3705. **19b**: ^{1}H NMR ($CDCl_3$) δ 7.43−7.21 (m, 13H), 7.12−6.96 (m, 4H), 6.89 (d, J = 8.6, 2H), 6.33 (d, J = 4.9, 1H), 6.00 (d, J = 7.6, 1H), 5.16 (q, J = 12.0, 2H), 5.08 (s, 2H), 5.01 (s, 2H), 4.86 (dt, J = 7.3, 6.0, 1H), 4.31 (m, 1H), 3.98 (m, 1H), 3.83 (d, J = 7.8, 1H), 3.16−2.93 (m, 4H), 2.23 (app. t, J = 7.3, 1H), 2.04 (m, 1H), 1.74 (m, 4H), 1.43−1.21 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 176.0, 171.3, 170.3, 157.8, 155.6, 137.0, 135.4, 134.9, 130.4, 129.2, 128.7, 128.61, 128.56, 128.50, 128.12, 128.09, 128.01, 127.96, 127.5, 127.4, 127.2, 114.9, 73.9, 69.9, 67.5, 66.8, 56.0, 55.4, 53.4, 53.0, 51.5, 37.4, 36.8, 31.4, 30.7, 24.1; HRMS (M + Na)$^+$ calcd for $C_{60}H_{53}N_3O_8Na$ 846.3730, found 846.3713.

***N*-(D-Tyrosyl)-*trans*-7-amino-*trans*-6-hydroxyspiro[4.4]-nonane-1-carbonyl-D-phenylalanine (Diastereomer 20a).** To a solution of **19a** (30 mg, 0.036 mmol) in 1.5 mL of 10% formic acid in THF was added palladium black (32 mg in 1.5 mL of water). The reaction mixture was stirred for 20 min, at which time analytical HPLC showed quantitative conversion to product (CH_3CN: 45% for 2 min, 45−70% over 28 min, 70−100% over 10 min, flow rate: 1 mL/min; t_r = 6.44 min). The catalyst was removed by filtration and the solvent removed to afford the pure peptide derivative **20a** (20 mg, 100%): ^{1}H NMR (CD_3OD) δ 8.17 (s, 1H), 7.29−7.03 (m, 7H), 6.78−6.68 (m, 2H), 4.68 (m, 1H), 4.16−3.47 (m, 3H), 3.42 (m, 1H), 3.20 (m, 1H), 2.95 (m, 2H), 2.40 (m, 1H), 2.08−1.24 (m, 10H); ^{13}C NMR (CD_3OD) 177.2, 169.1, 165.8, 158.2, 138.8, 131.6, 130.4, 129.5, 127.8, 126.2, 116.9, 116.8,

75.7, 58.1, 55.8, 55.5, 55.2, 53.6, 38.7, 38.1, 36.4, 34.1, 30.8, 29.7, 24.3; HRMS (M + H)$^+$ calcd for $C_{28}H_{36}N_3O_6$ 510.2604, found 510.2608. **Diasteromer 20b** was prepared from **19b** exactly as described above: HPLC t_r = 8.62 min; ^{1}H NMR (CD_3OD) δ 8.24 (s, 1H), 7.27–7.12 (m, 5H), 7.07 (d, J = 8.1, 2H), 6.74 (d, J = 8.3, 2H), 4.59 (dd, J = 4.6, 9.6, 1H), 4.16 (m, 1H), 4.04 (m, 1H), 3.96 (d, J = 5.9, 1H), 3.22 (m, 1H), 2.98–2.86 (m, 3H), 2.35 (m, 1H), 1.96 (m, 2H), 1.74 (m, 2H), 1.55 (m, 3H), 1.44–1.17 (m, 3H); ^{13}C NMR (CD_3OD) δ 177.3, 169.4, 166.6, 158.2, 139.2, 131.6, 130.3, 129.3, 127.5, 126.3, 116.9, 75.8, 58.6, 58.0, 55.8, 55.1, 53.9, 53.6, 38.8, 37.9, 36.6, 34.1, 33.2, 30.0, 29.2, 24.0; HRMS (M + H)$^+$ calcd for $C_{28}H_{36}N_3O_6$ 510.2604, found 510.2618.

Acknowledgment. We are grateful to the National Institutes of Health (GM-39758) and IGEN, Inc., Rockville, MD, for support. Acknowledgement is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research. This research was supported in part by a grant from the Amherst College Faculty Research Award Program. D.E.H. is a National Science Foundation Presidential Young Investigator (MCB-8958239) and a Camille and Henry Dreyfus Teacher-Scholar.

Supporting Information Available: ^{1}H-NMR spectra for compounds **2–9**, **15**, and **17–20b** (15 pages). This material is contained in 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.

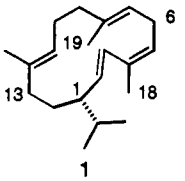
JO950216X

Additions and Corrections

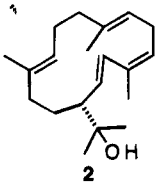
Vol. 60, 1995

Alexey V. Vorobjev,* Makhmut M. Shakirov, Victor A. Raldugin, and Clayton H. Heathcock. Conformational Analysis of the 10- and 13-Hydroxy Derivatives of Cembrene.

Page 64, column 1. Structures **1–10** should be replaced by the following structures:



1



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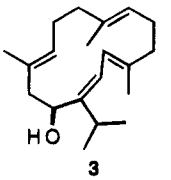
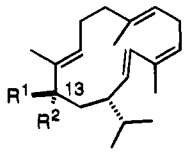
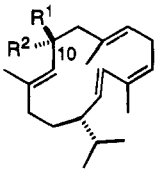


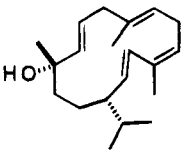
3



4: R^1 = OH, R^2 = H
5: R^1 = H, R^2 = OH
6: R^1 = H, R^2 = OAc



7: R^1 = OH, R^2 = H
8: R^1 = H, R^2 = OH
9: R^1 + R^2 = O



10

JO9540110

David P. Kelly,* Martin G. Banwell, John H. Ryan, James R. Phyland, and Jason R. Quick. ^{13}C–^{1}H Coupling Constants in Carbocations. 8. Application of the ΔJ Equation to Tertiary Dicyclopropylcarbinyl Cations: The Methyl Dicyclopropylcarbinyl, (1α,3β,5β,7α)-2-Methyltricyclo[5.1.0.0^{3,5}]-octan-2-yl, (1α,3α,5α,7α)-2-Methyltricyclo[5.1.0.0^{3,5}]octan-2-yl, and 3-Methyltetracyclo[3.3.1.0^{2,8}.0^{4,6}]nonan-3-yl (Triasteryl) Cations.

Page 1654. The data for compound **20** in Table 1 should read as follows: **20**[h] −110 43.9 (d, 179) 263.3 (s) 43.9 (d, 179) 40.7 (t, 169)[c] 74.2 (d, 171) 21.3 (t, 130) 38.0 (q, 130)

[h]Chemical shifts from internal CD_2Cl_2 taken as 52.8 ppm.

JO9540108

Dieter Seebach,* Robert Dahinden, Roger E. Marti, Albert K. Beck, Dietmar A. Plattner, and Florian N. M. Kühnle. On the Ti-TADDOLate-Catalyzed Diels–Alder Addition of 3-Butenoyl-1,3-oxazolidin-2-one to Cyclopentadiene. General Features of Ti-BINOLate- and Ti-TADDOLate-Mediated Reactions.

Page 1788. The correct receipt date for this manuscript is October 19, 1994.

JO9540099