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The Synthesis of Cyclobutanol-Containing Dipeptide Analogues

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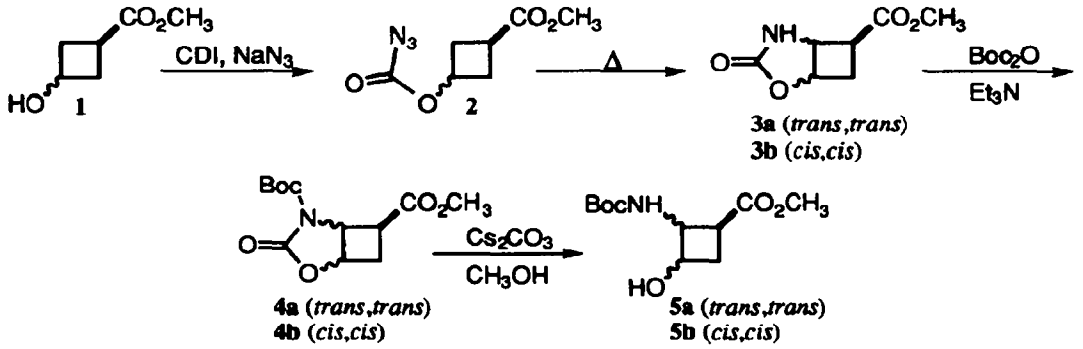
Abstract: The dipeptide analogue methyl *trans*-2-[(*t*-butoxycarbonyl)amino]-*trans*-3-hydroxycyclobutanecarboxylate (**5a**) and its *cis,cis*-isomer (**5b**) have been synthesized, and each has been incorporated into a longer peptide sequence.

Recently, immunization with transition state analogues has yielded a variety of antibody hydrolases,[1] but never an antibody peptidase. (Such antibodies have been obtained, however, by other, less general means.[2,3]) Thus, we have been concentrating on the synthesis of analogues that mimic, in addition to the transition state for peptide bond hydrolysis, a conformationally-distorted peptide ground state. Antibodies raised against these derivatives may catalyze the hydrolysis of the cognate peptide both by straining the scissile bond and by selectively stabilizing the transition state. As outlined in **Scheme 1**, we report here the synthesis of the cyclobutanol-containing dipeptide analogues **5a** and **5b** (as racemic mixtures).



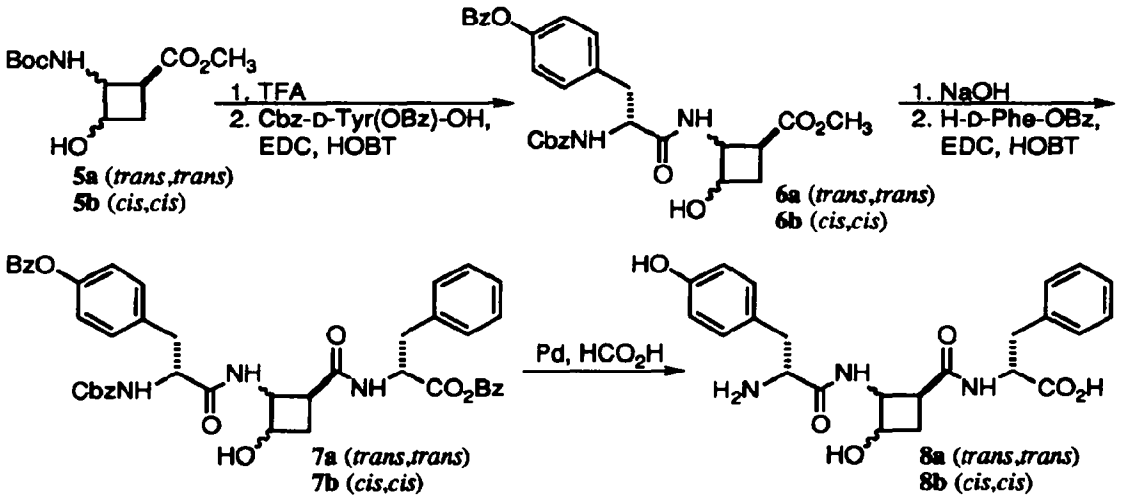
Scheme 1

The derivatives **5a** and **5b** are designed to mimic a ring-strained glycyl-glycine moiety; in addition, the peptide bond has been replaced by the hydroxyethylene isostere. Although tetrahedral-phosphorus derivatives are perhaps the best analogues of the transition state(s) of ester and amide hydrolysis, their incorporation into four-membered rings remains a synthetic challenge. Nonetheless, the hydroxyethylene group has also been demonstrated to be an effective transition state mimic. For example, in a recent study of an antibody raised against a bifunctional transition state analogue, Liotta *et al.*[4] have estimated that a phosphinate group was only four-to-seven times more efficacious in eliciting hydrolytic activity than was a hydroxyethylene group. Note also that hydroxyethylene derivatives are potent inhibitors of many aspartyl proteases.[5]

The key step in the synthesis of **5a** and **5b** was the thermolysis of a mixture of the *cis*- and *trans*-azidoformates **2** to yield methyl *trans*-4-aza-*trans*-2-oxa-3-oxo[3.2.0]bicycloheptane-6-carboxylate (**3a**) and its *cis,cis*-isomer (**3b**), which separated during chromatography on silica gel. (The *trans,trans* and *cis,cis* designations are relative to the fixed 6-carbomethoxy group.) Acyl nitrene insertion had been previously utilized by Lowe and Swain[6] in their synthesis of a 1-oxabisnorpenicillin G analogue. Furthermore, we have

also used this strategy to synthesize two norbornyl dipeptide analogues,[7] and overall, the method appears remarkably general. The yield of the two carbamates from a mixture of the *cis*- and *trans*-hydroxyesters **1** (synthesized as described by Wiberg *et al.*[8]) was 45%. The relative configurations of **3a** and **3b** were assigned from the multiplicities of the H6 resonances in the ^{1}H NMR spectra. (Presumably, each is the *cis*-fused bicycloheptane derivative.) Although no general trend exists for *cis*- and *trans*-couplings in cyclobutyl systems, the Karplus relationship can be applied.[9] Molecular mechanics calculations (MM+[10]) indicate that the dihedral angles $H_{vicinal}$-C-C-H6 in the *trans,trans*-isomer are 131°, 2°, and -129°, while the corresponding dihedral angles in the *cis,cis*-isomer are -5°, 5°, and 135°. (These values are for the enantiomers of absolute configuration *R* at C6.) For isomer **3a**, the resonance for H6 is an overlapping doublet of doublet of doublets, with coupling constants of 2.7, 6.5, and 9.7 Hz, while for isomer **3b**, the resonance for H6 is an apparent doublet of triplets, with coupling constants of 5.9 and 9.5 Hz, respectively. We thus assigned **3a**, which overall has smaller coupling constants, as the *trans,trans*-isomer, and **3b** as the *cis,cis*-isomer.

The carbamate functionality in **3a** and **3b** was cleaved using the methodology of Ishizuka and Kunieda[11] to generate the (*t*-butoxycarbonyl)amino hydroxyesters **5a** and **5b**, which were elaborated into the peptide derivatives H-D-tyrosine-[dipeptide analogue]-D-phenylalanine-OH, **8a** and **8b**, as shown in **Scheme 2**. (Amino acids of the D-configuration were chosen so as to generate a more immunogenic hapten,[12] and the diastereomeric mixtures of products were not separated.) Due to the presence of the flanking amino-acid residues, the antibodies obtained against **8a** and **8b** should be sequence specific. Conveniently, protection of the free hydroxyl group in **5a** and **5b** prior to the peptide coupling reactions proved unnecessary.



Scheme 2

In conclusion, we have synthesized two ring-strained glycyl-glycine analogues and have incorporated each into a longer peptide sequence.

EXPERIMENTAL

General: ^{1}H and ^{13}C NMR data were obtained on JEOL GXS-400 spectrometer. Infrared spectra were recorded on a Perkin-Elmer 1600 series FT-IR spectrometer. High-resolution mass spectra (HRMS) were determined by the Harvard Chemistry Department Mass Spectrometry Facility. Elemental analyses were performed by the Microanalysis Laboratory at the University of Massachusetts. Procedures are given only for the elaboration of carbamate **3a** into the peptide derivative **8a**. Reaction conditions for **3b** are identical.

Methyl *trans*-4-aza-*trans*-2-oxa-3-oxo[3.2.0]bicycloheptane-6-carboxylate (3a) and its *cis,cis*-isomer (3b). To a mixture of *cis*- and *trans*-methyl 3-hydroxycyclobutanecarboxylate (100 mg, 0.769 mmol)

in 12.5 mL of benzene was added pyridine (0.15 mL, 1.87 mmol) and 1,1-carbonyldiimidazole (CDI) (141 mg, 0.870 mmol). The solution was stirred under argon at room temperature for 1.5 h, and additional CDI (70 mg, 0.432 mmol) added. The resulting solution was stirred for 1 h and washed with brine (5 mL). The solvent was removed, and the residue dissolved in 26 mL of DMF. Sodium azide (250 mg, 3.85 mmol) and 10 drops of concentrated HCl were added, and the solution was stirred under argon for 2 h at room temperature. 400 mL of water was then added, and the aqueous layer extracted with diethyl ether (4 x 100 mL). The organic layer was dried, and the solvent removed. The product was purified by chromatography on silica gel (3:1 hexanes:EtOAc, R_f 0.66) to afford a mixture of the *cis*- and *trans*-azidoformates **2** (114 mg, 0.573 mmol), which was dissolved in 100 mL of CH_2Cl_2 and thermolyzed in a sealed glass tube for 30 min at 130 °C. The solvent was removed, and the resulting yellow oil purified by chromatography on silica gel (3:1 EtOAc:hexanes, R_f **3a** 0.44, R_f **3b** 0.33) to afford **3a** (32 mg, 0.187 mmol, 24%) as a white solid (mp 71-75 °C) and **3b** (27 mg, 0.158 mmol, 21%) as a white solid (mp 127-131 °C). **3a**: IR (film) 3231, 1736 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.69-2.78 (m, 2H), 3.24-3.29 (overlapping ddd, 2.7, 6.5, 9.7 Hz, 1H), 3.75 (s, 3H), 4.45-4.58 (m, 1H), 5.14 (app dt, J = 3.9, 6.6 Hz, 1H), 6.87 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 172.8, 160.1, 74.6, 55.0, 52.3, 45.2, 32.0; Anal. calcd for $C_7H_9NO_4$: C, 49.12; H, 5.30; N, 8.19; found: C, 48.69; H, 5.12; N, 7.85. **3b**: IR (film) 3253, 1738 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.75-2.80 (m, 2H), 3.34 (app dt, J = 5.9, 9.5 Hz, 1H), 3.75 (s, 3H), 4.57 (app tt, J = 1.7, 6.3 Hz, 1H), 5.00-5.04 (m, 1H), 6.70 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 171.0, 160.3, 71.7, 55.3, 52.2, 40.5, 32.0; Anal. calcd for $C_7H_9NO_4$: C, 49.12; H, 5.30; N, 8.19; found: C, 48.84; H, 5.16; N, 8.10.

Methyl *N*-(*t*-butoxycarbonyl)-*trans*-4-aza-*trans*-2-oxa-3-oxo[3.2.0]bicycloheptane-6-carboxylate (4a). To a solution of **3a** (32 mg, 0.187 mmol) in 5 mL of THF was added di-*t*-butyl dicarbonate [$(Boc)_2O$] (80 mg, 0.366 mmol), triethylamine (36 mg, 0.356 mmol), and dimethylaminopyridine (7 mg, 0.057 mmol) at room temperature. The solution was stirred for 12 h, and the solvent removed. The residue was dissolved in 15 mL EtOAc, washed with HCl (1N, 1 x 5 mL) and brine (4 x 5 mL), dried, and the solvent removed to afford **4a** (47 mg, 0.173 mmol, 93%) as a white solid (mp 87-93 °C): IR (film) 2981, 1828, 1732 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.52 (s, 9H), 2.65-2.68 (m, 1H), 2.73-2.76 (m, 1H), 3.29-3.32 (m, 1H), 3.76 (s, 3H), 4.79 (m, 1H), 5.01 (app dt, J = 3.9, 6.8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 172.4, 152.5, 148.3, 84.3, 70.4, 57.2, 52.3, 43.7, 31.5, 27.9; HRMS $[M + Na]^+$ calcd for $C_{12}H_{17}NO_6Na$ 294.0954, found 294.0949.

Methyl *trans*-2-[(*t*-butoxycarbonyl)amino]-*trans*-3-hydroxycyclobutanecarboxylate (5a). To a solution of **4a** (52 mg, 0.192 mmol) in 3 mL of methanol was added cesium carbonate (11 mg, 0.034 mmol) at room temperature. The reaction mixture was stirred for 2 h. After neutralization with citric acid, the product was extracted with chloroform (5 x 5 mL). The solution was dried, and the solvent removed to afford **5a** (44 mg, 0.180 mmol, 94%): IR (film) 3383, 2977, 1716, 1520 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (s, 9H), 2.05 (t, J = 11.7 Hz, 1H), 2.29-2.34 (m, 1H), 3.25 (br s, 1H), 3.70 (s, 3H), 4.25 (m, 1H), 4.51 (app t, J = 5.5 Hz, 1H), 5.27 (br d, J = 6.8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 173.8, 155.4, 80.1, 68.5, 52.1, 51.9, 43.8, 29.7, 28.3; HRMS $[M + Na]^+$ calcd for $C_{11}H_{19}NO_5Na$ 268.1161, found 268.1150.

Methyl *N*-[(*N*-benzyloxycarbonyl-*O*-benzyl)-D-tyrosyl]-*trans*-2-amino-*trans*-3-hydroxycyclobutanecarboxylate (6a) (as a mixture of diastereomers). To a solution of **5a** (22 mg, 0.090 mmol) in 1 mL of CH_2Cl_2 under nitrogen was added 1 ampule of trifluoroacetic acid (1 mL). The mixture was stirred for 2 h at room temperature, and the solvent removed to yield the trifluoroacetate salt (23 mg, 0.089 mmol, 99%). The TFA salt (14.2 mg, 0.055 mmol), 1-hydroxybenzotriazole (HOBT) (18 mg, 0.133 mmol), (*N*-benzyloxycarbonyl-*O*-benzyl)-D-tyrosine (22 mg, 0.055 mmol) and triethylamine (20 μL, 0.119 mmol) were dissolved in a mixture of 1 mL of DMF and 4 mL of CH_2Cl_2. The solution was stirred under nitrogen at 0 °C, and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (21 mg, 0.110 mmol) was added. The resulting solution was stirred overnight at room temperature, the solvent removed, and the residue purified by chromatography on silica gel (1:1 CH_2Cl_2:EtOAc, R_f 0.46) to afford **6a** (24 mg, 0.045 mmol, 82%) as a waxy solid: IR (film) 3306, 2950, 1732, 1653, 1512 cm^{-1}; ^1H NMR($CDCl_3$) δ 1.95 (app t, J = 11.2 Hz, 1H), 2.23-2.32 (m, 1H), 2.87-3.15 (m, 3H), 3.67 (s, 3H), 4.25-4.54 (m, 3H), 5.01-5.08 (m, 4H), 5.40 (br s, 0.5H), 5.59 (br s,

0.5H), 6.36 (br s, 0.5 H), 6.58 (br s, 0.5 H) 6.87-6.91 (m, 2H), 7.09 (d, J = 6.8, 2H), 7.26-7.42 (m, 10H); ^{13}C NMR ($CDCl_3$): δ 174.5, 172.1, 171.7, 158.8, 137.7, 136.8, 131.2, 129.3, 128.8, 128.1, 115.8, 70.4, 68.3, 67.6, 56.8, 52.3, 50.8, 43.7, 38.3, 29.8; HRMS $[M + Na]^+$ calcd for $C_{30}H_{32}N_2O_7Na$ 555.2107, found 555.2114.

***N*-[*N*-(benzyloxycarbonyl)-*O*-(benzyl)-D-tyrosyl]-*trans*-2-amino-*trans*-3-hydroxylcyclobutanecarboxoyl-D-phenylalanine benzyl ester (7a)** (as a mixture of diastereomers). To a solution of **6a** (13 mg, 0.024 mmol) in 1.5 mL of acetone was added 100 μL of 1N NaOH. The reaction mixture was stirred at room temperature for 4 h, and the solvent removed. To the residue was added 100 μL of 1N HCl and 2 mL of water, and the resulting solution was extracted with EtOAc (5 x 4 mL). The organic phases were combined, dried, and the solvent removed to give the free acid (8.5 mg, 0.016 mmol, 67%). To a mixture of the free acid (15 mg, 0.029 mmol), D-phenylalanine benzyl ester (7.6 mg, 0.029 mmol) and HOBT (8 mg, 0.058 mmol) in 3 mL of dry THF at 0 °C was added EDC (12 mg, 0.058 mmol) under nitrogen. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 3 h. The solvent was removed, and the residue was purified by chromatography on silica gel (4:1 EtOAc:CH_2Cl_2 , R_f 0.59) to afford **7a** (15 mg, 0.020 mmol, 69%) as a white solid: IR (film) 3303, 2947, 1734, 1653, 1512 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.92 (app t, J = 11.2 Hz, 1H), 2.23-2.40 (m, 1H), 2.86-3.25 (m, 3H), 3.96-3.98 (m, 1H), 4.10-4.40 (m, 2H), 4.82-4.86 (m, 2H), 5.01-5.12 (m, 6H), 6.56 (br d, J = 5.9 Hz, 0.5H), 6.65 (br d, J = 6.8 Hz, 0.5H), 6.84 (d, J = 8.8 Hz, 1H), 6.90 (d, J = 8.8 Hz, 1H), 6.99 (d, J = 8.8 Hz, 1H), 7.10 (d, 7.8 Hz, 1H), 7.17-7.43 (m, 20H), 8.05 (br d, J = 7.8 Hz, 0.5H), 8.24 (br d, J = 7.8 Hz, 0.5H); ^{13}C NMR ($CDCl_3$) δ 172.8, 172.6, 171.6, 157.9, 136.9, 136.5, 135.4, 130.4, 129.5, 129.2, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.5, 127.4, 126.8, 115.2, 70.0, 67.2, 66.9, 65.4, 65.0, 56.2, 53.8, 53.7, 51.0, 45.6, 45.3, 38.0, 37.8, 37.7, 30.0; HRMS $[M + Na]^+$ calcd for $C_{45}H_{45}N_3O_8Na$ 778.3104, found 778.3096.

***N*-(D-tyrosyl)-*trans*-2-amino-*trans*-3-hydroxylcyclobutanecarboxoyl-D-phenylalanine (8a)** (as a mixture of diastereomers). The a solution of **7a** (13 mg, 0.017 mmol) in 1 mL of 10% formic acid/THF was added palladium black (14 mg in 1 mL of water). The reaction mixture was stirred at room temperature for 30 min, at which point HPLC showed quantitative conversion to deprotected product (Alltech Econosphere C18, 5 micron, 15 x 0.46 cm; elution: 20% CH_3CN/water/0.1% TFA for 3 min, followed by a gradient of 20% CH_3CN/water/0.1% TFA to CH_3CN/0.1% TFA over 17 min, flow rate 1 mL/min; t_r = 5.9 min). The solvent was removed to afford **8a** as a waxy solid: IR (film): 3204, 3067, 1673 and 1439 cm^{-1}; ^1H NMR (DMF-d_6): δ 1.94-2.23 (m, 2H), 2.95-3.74 (m, 6H), 4.26-4.66 (m, 3H), 6.75-6.81 (m, 2H), 7.17-7.32 (m, 9H); ^{13}C NMR (DMF-d_6): δ 173.6, 170.5, 167.6, 67.4, 44.3, 138.8, 131.5, 130.0, 128.9, 127.1, 126.1, 115.9, 55.5, 54.7, 44.3, 38.0, 37.2; HRMS $[M + Na]^+$ calcd for $C_{23}H_{27}N_3O_6Na$ 464.1797, found 464.1808.

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