SUPPORTING INFORMATION

For

β-Lactones as a New Class of Cysteine Proteinase Inhibitors: Inhibition of Hepatitis A Virus 3C Proteinase by N-Cbz-Serine β-Lactone

Manjinder S. Lall, Dean Karvellas and John C. Vederas*

Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

General Methods. Most general procedures and instrumentation have been previously described.[21,34] Isotopically labeled L-serine (3-^{13}C, 99%) was purchased from Cambridge Isotope Laboratories (Andover MA) and was used directly without further purification. Unlabeled N-Cbz-L-serine β-lactone (**1**) and the corresponding D-enantiomer **2** were prepared as previously described.[25a]

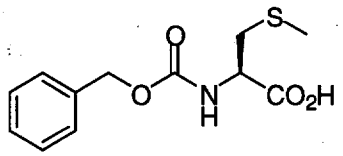
Enzyme Assays. Recombinant C24S HAV 3C proteinase was expressed in *Escherichia coli* and purified as reported previously.[35] Purity of the enzyme samples was greater than 90% as determined by SDS-PAGE analysis (data not shown). Proteinase concentrations were determined spectrophotometrically ε_{280} = 1.2 mg / mL. Enzyme was dialyzed against reaction buffer to remove DTT immediately prior to use. Cleavage reactions (700 µl) were performed at 25 ^{0}C in a solution containing 100 mM K_3PO_4 at pH 7.5, 2 mM EDTA, 0.1 mg / mL BSA, 10 µM fluorogenic substrate Dabcyl-GLRTQSFS-Edans (Bachem), 0.1 µM 3C proteinase and 1% DMF. Reactions were initiated by the addition of enzyme or substrate, depending on the kinetic assay performed. Fluorescence was continuously monitored by excitation at 336 nm and

emission at 472 nm at bandwidths of 3 nm with a Shimadzu RF-5301PC spectrofluorophotometer.[36] DMF, THF or DMSO, in which the substrate and inhibitors were dissolved, did not have a significant effect on the 3C proteinase activity when used at a concentration of 10% or less. For proteinase inhibition studies, rates were derived from the initial 3 min of the reaction, inhibitor stock solutions were prepared at 10 mM in DMF and serial dilutions made in DMF. At least five different inhibitor concentrations were examined along with the control sample containing no inhibitor under the conditions described above. The HAV 3C proteinase activity in the presence of the specified inhibitor was expressed as a percentage of that obtained from the respective control samples. For inhibitors displaying dose-dependent inhibition of the proteinase activity, IC_{50} values were determined from plots of the relative proteinase activity versus log inhibitor concentration. Time-dependent loss of enzyme activity was determined by the protocol of Silverman.[37] The rate of inactivation of β-lactone **1** was determined by the method of Kitz and Wilson.[27] The competitive inhibition constant (K_i) for β-lactone **2** was determined from a slope replot of the Dixon plot.[28] Under similar conditions, a slow-binding tetrapeptide aldehyde inhibitor[9] of HAV 3C has a Ki of 42 nM whereas a tetrapeptide fluoromethyl ketone inhibitor[11] shows irreversible inactivation with a second order rate constant of $3.3x10^2$ $M^{-1}s^{-1}$.

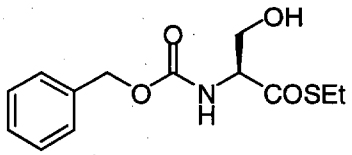
The sensitivity of inhibitors **1** and **2** to DTT was evaluated using reactions similar to those described in the previous paragraph, but with the addition of up to 500 μM DTT to the inhibitor-containing mixture followed by the addition of enzyme.

Dialysis experiments with **1** involved the preparation of two 0.1 μM enzyme solutions identical in all respects other than one contained inhibitor (100 μM of **1**) and the other solution

contained no inhibitor. The two solutions were independently assayed for initial enzyme activity and again after 8 hr dialysis using a Centriprep-10 (Amicon) centricon ultrafiltration unit.

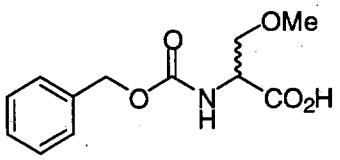


***N*-(Benzyloxycarbonyl)-*S*-methyl-L-cysteine (10).**[38] Reaction of *S*-methyl-L-cysteine (Aldrich) (1 g, 7.39 mmol) and benzyl chloroformate (1.16 mL, 8.15 mmol) in the usual procedure[39] gave **10** (1.76 g, 89%) as an oil: $[\alpha]_D^{26}$ -25.64^0 (*c* 44, MeOH); IR ($CHCl_3$ cast) 3311, 2920, 1718, 1586, 1215, 774, 697, 611 cm^{-1}; ^{1}H NMR (360 MHz, CD_3OD) δ 7.35 (m, 5H, Ph), 5.12 (br s, 2H, $PhCH_2$), 4.38 (dd, 1H, *J* = 8, 5 Hz, CH), 2.98 (dd, 1H, *J* = 14, 5 Hz, CH_2), 2.82 (dd, 1H, *J* = 14, 8 Hz, SCH_2), 2.11 (s, 3H, CH_3); ^{13}C NMR (75 MHz, CD_3CN) δ 172.3, 156.0, 136.9, 128.3, 127.7, 127.6, 65.4, 53.5, 34.8, 15.1; MS (ES) *m/z* (relative intensity) 270 (MH^+, 80%); Anal. Calcd for $C_{12}H_{15}NO_4S$: C, 53.51; H, 5.61; N, 5.20. Found: C, 53.11; H, 5.73; N, 5.36.

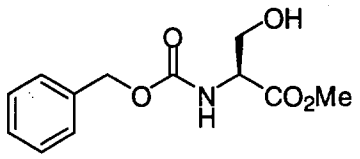


***N*-(Benzyloxycarbonyl)-L-serine ethyl thioester (11).** A solution of *N*-(benzyloxycarbonyl)-L-serine (1 g, 4.18 mmol) in CH_2Cl_2 (25mL) under argon at 0 ^{0}C was treated with triethylamine (0.7 mL, 5.01 mmol) and ethyl chloroformate (0.48 mL, 5.01 mmol) and stirred for 20 min. Upon the formation of a white precipitate was added ethanethiol (0.37 mL, 5.01 mmol) followed by an additional equivalent of triethylamine (0.7 mL, 5.01 mmol) the solution was stirred at 0 ^{0}C for an additional 30 min, the ice-bath was removed and stirring was continued overnight. To the reaction mixture was added CH_2Cl_2 (25mL), the solution was

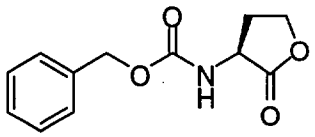
washed with 0.5 N HCL (2 x 10mL) and then saturated aqueous $NaHCO_3$ (10 mL) which gave an emulsion. The emulsion was filtered through a pad of Celite the organic layer was washed with brine (5mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification by flash chromatography (EtOAc : Hex, 1 : 4) gave the title compound **11** (77.1 mg, 7%) as a solid: mp 49-51 ^{0}C; $[\alpha]_D^{26}$ -26.75^0 (*c* 1.6 $CHCl_3$); IR ($CHCl_3$ cast) 3351, 2931, 1684, 1520, 1261, 1059, 737, 697 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.27 (m, 5H, Ph), 5.68 (br s, 1H, NH), 5.11 (s, 2H, $PhCH_2$), 4.42 (m, 1H, CH), 4.08 (dd, 1H, *J* = 11, 4 Hz, OCH_2); 3.79 (dd, 1H, *J* = 11, 4 Hz, OCH_2), 2.88 (q, 2H, *J* = 7 Hz, SCH_2), 1.83 (br s, 1H, OH), 1.21 (t, 3H, *J* = 7 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.0, 156.2, 136.1, 128.6, 128.3, 128.2, 67.4, 63.2, 62.4, 23.6, 14.3; HRMS (ES) Calcd for $C_{13}H_{17}NO_4S$ 284.0956, found 284.0955; Anal. Calcd for $C_{13}H_{17}NO_4S$: C, 55.10; H, 6.05; N, 4.94. Found: C, 54.91; H, 6.14; N, 4.90.



***N*-(Benzyloxycarbonyl)-*O*-methyl-DL-serine (13).**[40] Reaction of *O*-methyl-DL-serine (Sigma) (1 g, 8.39 mmol) and benzyl chloroformate (1.32 mL, 9.25 mmol) in the usual fashion[39] gave **13** (1.85 g, 87%) as a yellow oil: IR ($CHCl_3$ cast) 3313, 2936, 1723, 1521, 1213, 775, 698, 623 cm^{-1}; ^{1}H NMR (360 MHz, CD_3OD) δ 7.34 (m, 5H, Ph), 5.11 (s, 2H, $PhCH_2$), 4.37 (dd, 1H, *J* = 4, 4 Hz, CH), 3.76 (dd, 1H, *J* =10, 4 Hz, CH_2), 3.64 (dd, 1H, *J* = 10, 4 Hz, CH_2), 3.38 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $(CD_3)_2SO$) δ 171.7, 156.0, 136.9, 128.3, 127.8, 127.7, 71.4, 65.5, 58.2, 54.1; MS (ES) *m/z* (relative intensity) 254 (MH^+, 100%); Anal. Calcd for $C_{12}H_{15}NO_5$: C, 56.91; H, 5.97; N, 5.53. Found: C, 56.83; H, 6.07; N, 5.69.

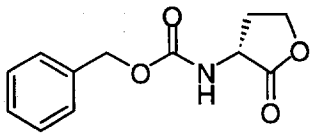


***N*-(Benzyloxycarbonyl)-L-serine methyl ester (12).**[41] This material was prepared from L-serine methyl ester hydrochloride (Aldrich) (1 g, 6.43 mmol) by the procedure of Greenstein and Winitz.[39] Purification by flash chromatography gave **12** (1.51 g, 71%) as a pale yellow oil: $[\alpha]_D^{26}$ +8.05^0 (*c* 14.7 $CHCl_3$); IR ($CHCl_3$ cast) 3373, 2955, 1722, 1527, 1214, 1062, 776, 698, 577 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.39 (m, 5H, Ph), 5.76 (br s, 1H, NH), 5.17 (s, 2H, PhCH_2), 4.48 (m, 1H, CH), 4.03 (dd, 1H, *J* = 11, 4 Hz, CH_2), 3.95 (dd, 1H, *J* = 11, 3 Hz, CH_2), 3.78 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 156.3, 136.1, 128.6, 128.3, 128.1, 67.3, 63.2, 56.1, 52.7; MS (ES) *m/z* (relative intensity) 254 (MH^+, 53%); Anal. Calcd for $C_{12}H_{15}NO_5$: C, 56.91; H, 5.97; N, 5.53. Found: C, 56.65; H, 6.01; N, 5.47.



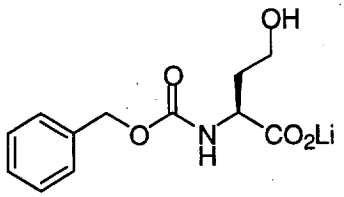
***N*-(Benzyloxycarbonyl)-L-homoserine γ-lactone (5).**[42] To a suspension of L-homoserine lactone hydrochloride (Sigma) (0.1 g, 0.73 mmol) in CH_2Cl_2 (20 mL) at 0 ^{0}C was added triethylamine (0.2 mL, 1.44 mmol) and then benzyl chloroformate (0.125 mL, 0.88 mmol) dropwise. The reaction mixture was vigorously stirred for 3 hrs. The solution was washed with saturated aqueous $NaHCO_3$ (2 x 20 mL) followed by H_2O (10 mL), dried over $MgSO_4$ and evaporated *in vacuo* to give crude product as an off-white solid. Recrystallization from chloroform-hexane gave the title compound **5** (40.0 mg, 23%) as a white solid: mp 118-120 ^{0}C; $[\alpha]_D^{26}$ -1.05^0 (*c* 2.85 $CHCl_3$); IR ($CHCl_3$ cast) 3329, 2949, 1777, 1692, 1543, 1298, 1074, 778, 693 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.38 (m, 5H, Ph), 5.39 (br s, 1H, NH), 5.13 (s, 2H, PhCH_2),

4.42 (m, 2H, $OC\underline{H}_2$), 4.22 (m, 1H, C<u>H</u>), 2.79 (m, 1H, $C\underline{H}_2$), 2.21 (dddd, 1H, J = 12, 12, 12, 9 Hz, $C\underline{H}_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.9, 158.1, 135.9, 128.6, 128.4, 128.2, 67.4, 65.8, 50.6, 30.6; HRMS (EI) Calcd for $C_{12}H_{13}NO_4$ 235.08446, found 235.08453; Anal. Calcd for $C_{12}H_{13}NO_4$: C, 61.27; H, 5.57; N, 5.95. Found: C, 60.85; H, 5.25; N, 5.92.

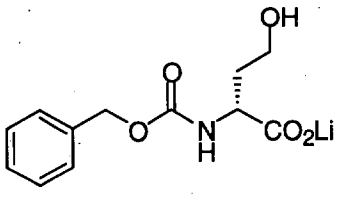


***N*-(Benzyloxycarbonyl)-D-homoserine δ-lactone (6).**[42] To a suspension of $NaHCO_3$ (0.5 g, 5.95 mmol) in THF (2.5 mL) and water (5 mL) at room temperature was carefully added D-homoserine (Sigma) (0.25 g, 2.09 mmol). After the cessation of gas evolution, benzyl chloroformate (0.375 mL, 2.71 mmol) was added dropwise over 30 min and stirring was continued for a further 1hr. The reaction mixture was then washed with Et_2O (2 x 2.5 mL), and acidified to pH 2 by careful addition of 1 N HCl. The slurry was extracted with EtOAc (2 x 2.5 mL) and the organic phases were pooled. The combined EtOAc layer was washed with 1 N HCl (2 x 2.5 mL) and then brine (2.5 mL), dried over $MgSO_4$ and evaporated *in vacuo* to give crude δ-lactone **6** plus *N*-(benzyloxycarbonyl)-D-homoserine as a white solid. This material was heated under reflux with a soxhlet containing CaH_2 for 4hrs. The solution was evaporated *in vacuo*, redissolved in EtOAc (5 mL), washed with saturated aqueous $NaHCO_3$ followed by brine (2.5 mL), dried over $MgSO_4$ and evaporated *in vacuo* to give **6** (0.1 g, 50%) as a white solid: mp 118-120 °C; $[\alpha]_D^{26}$ +1.750 (c 2.6 $CHCl_3$); IR ($CHCl_3$ cast) 3327, 2940, 1777, 1693, 1542, 1298, 1073, 741, 693 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.38 (m, 5H, <u>Ph</u>), 5.39 (br s, 1H, N<u>H</u>), 5.13 (s, 2H, $PhC\underline{H}_2$), 4.42 (m, 2H, $OC\underline{H}_2$), 4.22 (m, 1H, C<u>H</u>), 2.79 (m, 1H, $C\underline{H}_2$), 2.21 (dddd, 1H, J = 12, 12, 12, 9 Hz, $C\underline{H}_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.9, 156.1, 135.9, 128.6, 128.3, 128.2, 67.4,

65.8, 50.5, 30.3; HRMS (EI) Calcd for $C_{12}H_{13}NO_4$ 235.08446, found 235.08462; Anal. Calcd for $C_{12}H_{13}NO_4$: C, 61.27; H, 5.57; N, 5.95. Found: C, 61.20; H, 5.49 N, 5.85.

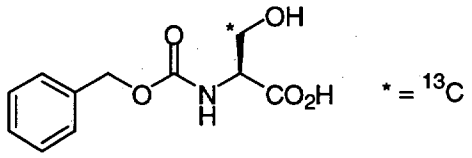


***N*-(Benzyloxycarbonyl)-L-homoserine lithium salt (7).** A solution of *N*-(benzyloxycarbonyl)-L-homoserine δ-lactone **5** (50.0 mg, 0.197 mmol) suspended in THF / H_2O (20 mL, 1 : 1) was treated with LiOH (8.3 mg, 0.197 mmol). The reaction mixture was stirred at room temperature for 2 hrs. The solution was evaporated *in vacuo* to give a white solid, this material was redissolved in H_2O (10 mL) and extracted with ether (2 x 5 mL). The aqueous layer was evaporated *in vacuo* to afford the salt **7** (20.0 mg, 40%) as a pale yellow oil: $[\alpha]_D^{26}$ -8.54^0 (*c* 4.7 H_2O); IR (MeOH cast) 3316, 2955, 1694, 1599, 1538, 1258, 1061, 697 cm^{-1}; ^{1}H NMR (360 MHz, D_2O) δ 7.28 (m, 5H, Ph), 4.99 (d, 1H, *J* = 12 Hz, PhCH_2), 4.93 (d, 1H, *J* = 12 Hz, PhCH_2), 3.87 (dd, 2H, *J* = 9, 4 Hz, CH), 3.48 (m, 2H, OCH_2), 1.88 (m, 1H, CH_2), 1.67 (m, 1H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 180.4, 158.8, 137.4, 129.6, 129.1, 1298.5, 67.7, 59.4, 54.6, 34.9; MS (ES) *m/z* (relative intensity) 260 (MH^+, 100%).

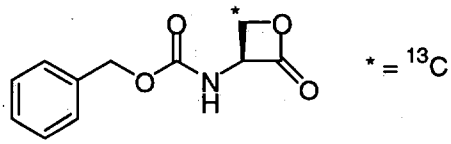


***N*-(Benzyloxycarbonyl)-D-homoserine lithium salt (8).** Reaction of *N*-(benzyloxycarbonyl)-D-homoserine γ-lactone **6** (0.10 g, 0.394 mmol) and LiOH (17.0 mg, 0.394 mmol) as described for **7** afforded the salt **8** (40.0 mg, 40%) as a pale yellow oil: $[\alpha]_D^{26}$ +10.21^0 (*c* 30.3 H_2O); IR (MeOH cast) 3378, 2956, 1695, 1598, 1538, 1261, 1060, 697 cm^{-1}; ^{1}H NMR (360

MHz, D_2O) δ 7.28 (m, 5H, Ph), 4.98 (d, 1H, J = 12 Hz, PhCH_2), 4.92 (d, 1H, J = 12 Hz, PhCH_2), 3.88 (dd, 2H, J = 9, 4 Hz, CH), 3.49 (m, 2H, OCH_2), 1.88 (m, 1H, CH_2), 1.67 (m, 1H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 180.4, 158.7, 137.4, 129.6, 129.1, 1298.5, 67.7, 59.4, 54.6, 34.9; MS (ES) *m/z* (relative intensity) 260 (MH^+, 100%).



***N*-(Benzyloxycarbonyl)-L-serine, 3-^{13}C (3(β-^{13}C)).**[43] Reaction of L-serine (3-^{13}C, 99%) (0.15 g, 1.41 mmol) and benzyl chloroformate (0.22 mL, 1.56 mmol) by the usual procedure[39] gave **3**(β-^{13}C) (0.18 g, 52%) as a white solid: $[\alpha]_D^{26}$ +7.29^0 (*c* 2.5 CH_3CN); IR (uscope) 3317, 3028, 2950, 1748, 1690, 1533, 1247, 1056, 1018, 749, 696 cm^{-1}; ^{1}H NMR (360 MHz, CD_3OD) δ 7.35 (m, 5H, Ph), 5.09 (br s, 2H, PhCH_2), 4.28 (dd, 1H, J = 8, 4 Hz, CH), 3.85 (dddd, 2H, $^1J_{C\text{-}H}$ = 145 Hz, J = 11, 8, 4 Hz, CH_2); ^{13}C NMR (75 MHz, CD_3CN) δ 172.3, 157.2, 138.1, 129.4, 128.9, 128.7, 67.8, 62.7, 56.7; MS (ES) *m/z* (relative intensity) 263 (MNa^+, 47%).



***N*-(Benzyloxycarbonyl)-L-serine β-lactone, 3-^{13}C (1(β-^{13}C)).** Cyclization of *N*–(benzyloxycarbonyl)-D-serine, 3-^{13}C **3**(β-^{13}C) (0.16 g, 0.67 mmol) by Mitsunobu procedure[25a] gave **1**(β-^{13}C) (45.4 mg, 31%) as a white solid: $[\alpha]_D^{26}$ -6.45^0 (*c* 1.6, $CHCl_3$); IR (uscope) 3366, 1842, 1686, 1532, 1268, 751, 701 cm^{-1}; ^{1}H NMR (360 MHz, CD_2Cl_2) δ 7.38 (m, 5H, Ph), 5.54 (br s, 1H, NH), 5.14 (br s, 2H, PhCH_2), 5.06 (m, 1H, CH), 4.43 (dm, 2H, $^1J_{C\text{-}H}$ = 160 Hz, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.5, 155.2, 135.5, 128.7, 128.6, 128.4, 67.9, 66.4, 59.5; HRMS (EI) Calcd for $C_{10}H_{11}NO_4$^{13}C 222.07216, found 222.07213.

Rate of Hydrolysis of β-Lactone 1 in Phosphate Buffer. Assuming pseudo-first order kinetics, the hydrolysis of **1** was followed by FT-IR with a Nicolet Magna 750 FT-IR instrument using a 0.0294 mm IR-Trans 4 cell (Kodak, polycrystalline ZnS). A solution containing 100 mM K_3PO_4 pH 7.5, 2 mM EDTA, 20 mM **1**, and 20% DMF was prepared, an aliquot was removed and placed in the IR cell at 22 ^{0}C and the disappearance of the β-lactone carbonyl stretch (1830 cm^{-1}) was monitored over a 1 h period.

Mass Spectrometry of HAV 3C-1 Inhibitor Complex. HAV 3C proteinase was dialyzed against a solution containing 2 mM EDTA and 100 mM K_3PO_4 at pH 7.5 to remove DTT using a Centriprep-10 (Amicon) centricon ultrafiltration unit. Dialyzed HAV 3C proteinase (0.3 mM) was incubated with (10 equivalents) of **1** and 1% DMF at 25 ^{0}C for 1 h with mixing. The HAV 3C-**1** complex was then dialyzed against H_2O for 1h using a Centriprep-10 (Amicon) centricon ultrafiltration unit, to a volume of approximately 300 µl. In addition, a control parallel experiment was performed on the enzyme alone without inhibitor **1**. Mass spectrometric analysis was performed by positive mode electrospray ionization on a Micromass ZabSpec Hybrid Sector-TOF. The liquid carrier used was a 0.5% solution of formic acid in acetonitrile : water (1 : 1), infused into the electrospray source by means of a Harvard syringe pump at a flow rate of 10 µl / min. An aliquot of the sample to be analyzed was dissolved in 0.5% solution of formic acid in acetonitrile : water (1 : 1) and introduced *via* a 1 µl-loop-injector. Prepurified nitrogen was used as a spray pneumatic aid and filtered air as the bath gas, heated at *ca.* 60 ^{0}C. The low resolution mass spectra were acquired by full scan with the magnet from 300 to 3000 daltons, at a rate of 5 sec / decade. The obtained data, corresponding to a series of multiple charged ions,

were processed (smoothed, peak detection, production of centroid spectra, series calculation and data transformation) to produce average molecular weights. Data acquisition and processing was achieved by using the OPUS software package on a Digital Alpha station with VMS operating system.

^{1}H / ^{13}C HMQC Spectroscopy of Model Compounds, 1(β-^{13}C) , 10-13, HAV 3C and HAV 3C-1(β-^{13}C) Inhibitor Complex. Solutions of individual compounds **1**(β-^{13}C) and **10-13** contained 1.2 mM of the model compound, 6% DMSO-d_6 in 20 mM K_3PO_4 / D_2O at pD 7.5 to give a total volume of 700 μL. Due to significant hydrolysis in buffer solution at pD 7.5, during the HMQC NMR acquisition of **1**(β-^{13}C), the NMR sample solution was altered to contain 1.2 mM of **1**(β-^{13}C), 6% DMSO-d_6 in D_2O at pD 5.0 (adjusted with 20% solution of DCl; D = 99.5%) to give a total volume of 700 μL. Prior to use, DTT was removed from the enzyme preparation by dialysis with a Centriprep-10 (Amicon) centricon ultrafiltration unit with 20 mM Na_3PO_4 / D_2O at pD 7.5. The resulting enzyme solution (1.2 mM) was inactivated with inhibitor **1**(β-^{13}C) (1.2 mM) and 1% THF-d_8. Model compounds **1**(β-^{13}C) and **10-13**, HAV 3C alone and the HAV 3C-**1**(β-^{13}C) enzyme inhibitor complex were analyzed by HMQC NMR using an Inova 600 Varian instrument, see Table S1. The parameters for model compound **1**(β-^{13}C): temperature: 27 ^{0}C, solvent: D_2O, number of transients: 4, number of increments: 512, number of data point: 2368, acquisition time: 0.247 sec, sweep width in F2: 4801.9 Hz, sweep width in F1: 30172.3 Hz, see Figure S1. The parameters for the HAV 3C-**1**(β-^{13}C) enzyme inhibitor complex: temperature: 27 ^{0}C, solvent: D_2O, number of transients: 98, number of increments: 512, number of data point: 2368, acquisition time: 0.247 sec, sweep width in F2: 4801.9 Hz, sweep width in

F1: 30172.3 Hz, see Figure S1. Solvent presaturation was used for 1.2 sec, and ^{1}H, ^{13}C decoupling was applied. The chemical shifts were referenced to 1% external acetone.

Additional References

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Caption for Figure S1: ^{1}H / ^{13}C HMQC spectra of **1**(β-^{13}C) inhibitor alone (top spectra) and in complex with HAV 3C proteinase; 1.2 mM HAV 3C / 1.2 mM **1**(β-^{13}C), (bottom spectra). The spectra were acquired at 600 MHz, 25 ^{0}C in D_2O at pD 5.0 and 20 mM Na_3PO_4 / D_2O at pD 7.5, respectively. Cross peak (A) shows the proton-carbon correlation of the unreacted inhibitor **1**(β-^{13}C) and peak (B) is the cross peak for the β-thioalkylated adduct, i.e., HAV 3C-**1**(β-^{13}C) enzyme inhibitor complex.

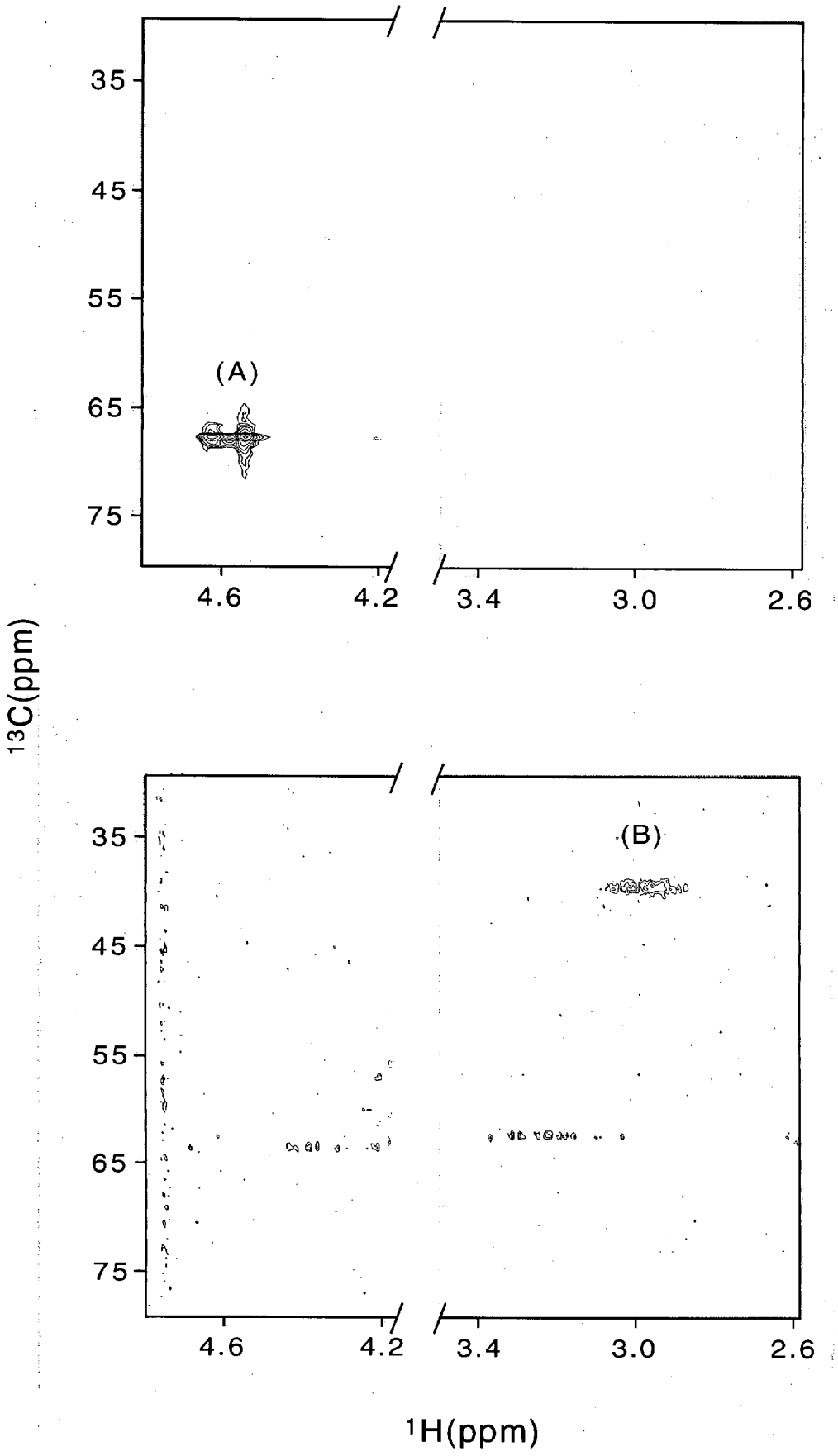
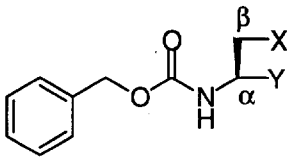


Table S1. Model β–carbon chemical shifts.



entry	X	Y	β-carbon (ppm)
1(β-^{13}C)*[a]	O———	CO	68
10[b]	SMe	CO_2H	37
11[b]	OH	COSEt	64
12[b]	OH	CO_2Me	62
13[b,c]	OMe	CO_2H	73

* = ^{13}C-Labeled at β-position. ^{1}H / ^{13}C HMQC

Conditions: [a] D_2O at pD 5.0, 6% DMSO-d_6. [b] 20mM Na_2PO_4 / D_2O at pD 7.5, 6% DMSO-d_6.

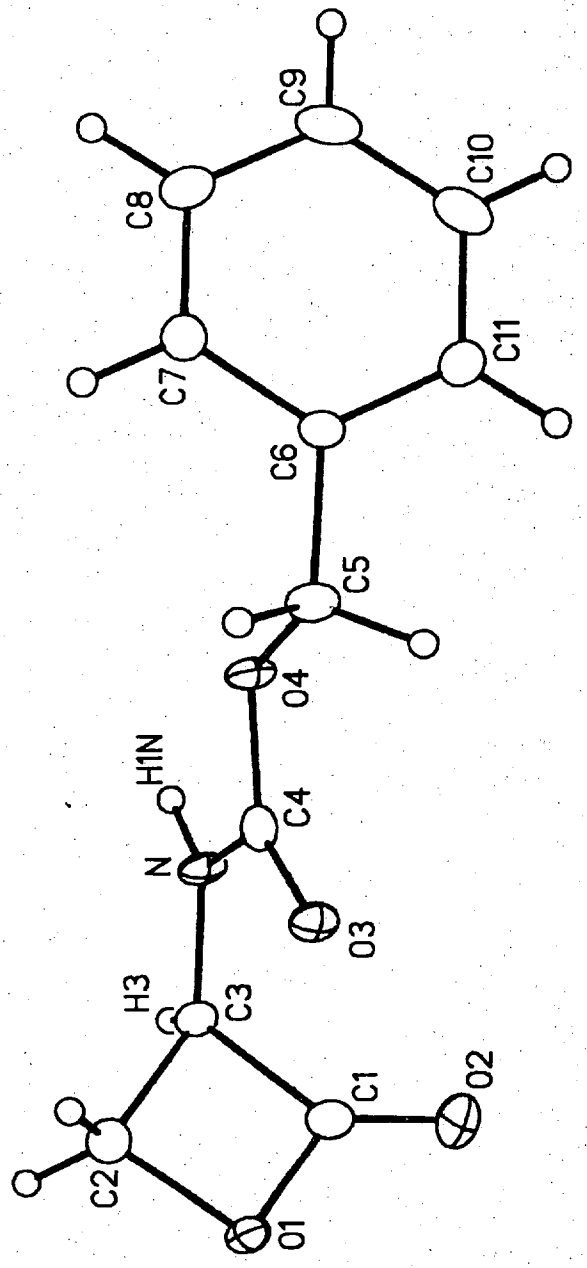
[c] Racemic.

Supporting Information

X-Ray Structural Information for β-Lactone 2

Compound 2: 4(*R*)-Benzyloxycarbonylamino-1-oxacyclobutan-2-one
Formula: $C_{11}H_{11}NO_4$

Figure 1. Perspective view of the 4(R)-benzyloxycarbonylamino-1-oxacyclobutan-2-one molecule/ion showing the atom labeling scheme. Non-hydrogen atoms are represented by Gaussian ellipsoids at the 20% probability level. Hydrogen atoms are shown with arbitrarily small thermal parameters.


C9
C10
C8
C11
C7
C6
C5
O4
H1N
C4
N
O3
H3
C3
C1
O2
C2
O1

List of Tables

Table 1. Crystallographic Experimental Details

A. Crystal Data	
formula	$C_{11}H_{11}NO_4$
formula weight	221.21
crystal dimensions (mm)	0.40 x 0.04 x 0.02
crystal system	orthorhombic
space group	$P2_12_12_1$ (No. 19)[a]
unit cell parameters[b]	
a (Å)	4.9803 (8)
b (Å)	8.6965 (14)
c (Å)	24.377 (4)
V (Å^3)	1055.8 (3)
Z	4
ρcalcd (g cm^{-3})	1.392
μ (mm^{-1})	0.107
B. Data Collection and Refinement Conditions	
diffractometer	Bruker P4/RA/SMART 1000 CCD[c]
radiation (λ[Å])	graphite-monochromated Mo Kα (0.71073)
temperature (°C)	–80
scan type	ϕ rotations (0.3°) / ω scans (0.3°) (20 s exposures)
data collection 2θ limit (deg)	51.40
total data collected	5599 (-6 ≤ *h* ≤ 5, -10 ≤ *k* ≤ 10, -29 ≤ *l* ≤ 29)
independent reflections	1987
number of observations (*NO*)	935 ($F_o^2 \geq 2\sigma(F_o^2)$)
structure solution method	direct methods (*SHELXS–86*[d])
refinement method	full-matrix least-squares on F^2 (*SHELXL–93*[e])
absorption correction method	*SADABS*
range of transmission factors	0.9979–0.9584
data/restraints/parameters	1987 [$F_o^2 \geq -3\sigma(F_o^2)$] / 0 / 145
Flack absolute structure parameter[f]	-1(2)
goodness-of-fit (*S*)[g]	0.794 [$F_o^2 \geq -3\sigma(F_o^2)$]
final *R* indices[h]	
$F_o^2 > 2\sigma(F_o^2)$	**R_1 = 0.0464,** wR_2 = 0.0647
all data	R_1 = 0.1335, **wR_2 = 0.0808**
largest difference peak and hole	0.184 and –0.193 e Å^{-3}

[a]Although the compound is a single stereoisomer and crystallizes in a chiral space group, the X-ray results can only be used to determine the relative stereochemistry. The absolute stereochemistry is deduced from the known absolute stereochemistry of its precursor. See also footnote *f*.

[b]Obtained from least-squares refinement of 1473 centered reflections.

(continued)

Table 1. Crystallographic Experimental Details (continued)

[c]Programs for diffractometer operation, data collection, data reduction and absorption correction were those supplied by Bruker.

[d]Sheldrick, G. M. *Acta Crystallogr.* **1990**, *A46*, 467–473.

[e]Sheldrick, G. M. *SHELXL-93*. Program for crystal structure determination. University of Göttingen, Germany, 1993. Refinement on F_o^2 for all reflections (all of these having $F_o^2 \geq -3\sigma(F_o^2)$). Weighted *R*-factors wR_2 and all goodnesses of fit *S* are based on F_o^2; conventional *R*-factors R_1 are based on F_o, with F_o set to zero for negative F_o^2. The observed criterion of $F_o^2 > 2\sigma(F_o^2)$ is used only for calculating R_1, and is not relevant to the choice of reflections for refinement. *R*-factors based on F_o^2 are statistically about twice as large as those based on F_o, and *R*-factors based on ALL data will be even larger.

[f]Flack, H. D. *Acta Crystallogr.* **1983**, *A39*, 876–881. The Flack parameter will refine to a value near zero if the structure is in the correct configuration and will refine to a value near one for the inverted configuration. In this case, the large negative value of the Flack parameter and the very large magnitude of its estimated standard deviation imply that the absolute configuration cannot be inferred from diffraction results alone.

[g]$S = [\Sigma w(F_o^2 - F_c^2)^2/(n-p)]^{1/2}$ (n = number of data; p = number of parameters varied; $w = [\sigma^2(F_o^2) + (0.0176P)^2]^{-1}$ where $P = [\mathrm{Max}(F_o^2, 0) + 2F_c^2]/3$).

[h]$R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^4)]^{1/2}$.

Table 2. Atomic Coordinates and Equivalent Isotropic Displacement Parameters

Atom	*x*	*y*	*z*	U_{eq}, Å^2
O1	0.2481(5)	0.0974(3)	0.00166(9)	0.0391(7)*
O2	0.2339(6)	0.1768(3)	-0.08719(10)	0.0603(9)*
O3	0.2603(5)	-0.1971(3)	-0.07908(8)	0.0319(6)*
O4	0.6172(4)	-0.3084(3)	-0.12234(9)	0.0320(7)*
N	0.6795(5)	-0.0978(3)	-0.07392(10)	0.0322(8)*
C1	0.3435(8)	0.1133(5)	-0.05075(17)	0.0372(11)*
C2	0.4734(8)	-0.0028(5)	0.01728(14)	0.0480(12)*
C3	0.6040(7)	0.0270(4)	-0.03893(13)	0.0314(10)*
C4	0.4962(8)	-0.2021(5)	-0.09085(14)	0.0285(10)*
C5	0.4428(7)	-0.4303(4)	-0.14372(14)	0.0341(10)*
C6	0.6053(7)	-0.5141(4)	-0.18611(13)	0.0271(9)*
C7	0.7980(8)	-0.6195(4)	-0.16988(15)	0.0350(11)*
C8	0.9655(8)	-0.6881(5)	-0.20692(16)	0.0454(12)*
C9	0.9362(8)	-0.6564(5)	-0.26223(18)	0.0488(13)*
C10	0.7431(9)	-0.5542(4)	-0.27940(14)	0.0455(12)*
C11	0.5774(7)	-0.4816(4)	-0.24118(14)	0.0368(11)*

Anisotropically-refined atoms are marked with an asterisk (*). The form of the anisotropic displacement parameter is: $\exp[-2\pi^2(h^2a^{*2}U_{11} + k^2b^{*2}U_{22} + l^2c^{*2}U_{33} + 2klb^*c^*U_{23} + 2hla^*c^*U_{13} + 2hka^*b^*U_{12})]$.

Table 3. Selected Interatomic Distances (Å)

Atom1	Atom2	Distance	Atom1	Atom2	Distance
O1	C1	1.370(4)	C2	C3	1.538(4)
O1	C2	1.471(4)	C5	C6	1.501(4)
O2	C1	1.180(4)	C6	C7	1.385(5)
O3	C4	1.210(4)	C6	C11	1.379(4)
O4	C4	1.345(4)	C7	C8	1.366(5)
O4	C5	1.466(4)	C8	C9	1.384(5)
N	C3	1.430(4)	C9	C10	1.375(5)
N	C4	1.351(4)	C10	C11	1.395(5)
C1	C3	1.526(5)			

Table 4. Selected Interatomic Angles (deg)

Atom1	Atom2	Atom3	Angle	Atom1	Atom2	Atom3	Angle
C1	O1	C2	92.1(3)	O3	C4	N	124.0(4)
C4	O4	C5	115.8(3)	O4	C4	N	109.5(3)
C3	N	C4	120.9(3)	O4	C5	C6	106.0(3)
O1	C1	O2	126.1(4)	C5	C6	C7	119.9(3)
O1	C1	C3	94.0(3)	C5	C6	C11	121.1(3)
O2	C1	C3	139.9(4)	C7	C6	C11	118.9(3)
O1	C2	C3	89.6(3)	C6	C7	C8	121.5(4)
N	C3	C1	118.9(3)	C7	C8	C9	119.5(4)
N	C3	C2	121.0(3)	C8	C9	C10	120.0(4)
C1	C3	C2	83.8(3)	C9	C10	C11	120.2(3)
O3	C4	O4	126.5(4)	C6	C11	C10	119.9(4)

Table 5. Torsional Angles (deg)

Atom1	Atom2	Atom3	Atom4	Angle	Atom1	Atom2	Atom3	Atom4	Angle
C2	O1	C1	O2	174.3(4)	O2	C1	C3	C2	-174.6(6)
C2	O1	C1	C3	-6.1(3)	O1	C2	C3	N	-125.6(3)
C1	O1	C2	C3	6.1(3)	O1	C2	C3	C1	-5.5(3)
C5	O4	C4	O3	-0.6(5)	O4	C5	C6	C7	78.2(4)
C5	O4	C4	N	179.8(3)	O4	C5	C6	C11	-98.0(4)
C4	O4	C5	C6	168.6(3)	C5	C6	C7	C8	-174.3(4)
C4	N	C3	C1	-42.9(5)	C11	C6	C7	C8	2.0(6)
C4	N	C3	C2	57.9(4)	C5	C6	C11	C10	175.9(3)
C3	N	C4	O3	1.4(5)	C7	C6	C11	C10	-0.3(6)
C3	N	C4	O4	-179.0(3)	C6	C7	C8	C9	-2.5(6)
O1	C1	C3	N	128.0(3)	C7	C8	C9	C10	1.3(6)
O1	C1	C3	C2	5.9(3)	C8	C9	C10	C11	0.4(6)
O2	C1	C3	N	-52.6(7)	C9	C10	C11	C6	-0.8(6)

Table 6. Anisotropic Displacement Parameters (U_{ij}, Å^2)

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O1	0.0299(16)	0.0478(18)	0.0396(14)	-0.0095(14)	-0.0005(14)	0.0120(16)
O2	0.066(2)	0.058(2)	0.0567(18)	0.0073(18)	-0.0122(18)	0.022(2)
O3	0.0189(14)	0.0379(16)	0.0389(14)	-0.0079(14)	0.0058(14)-	0.0034(15)
O4	0.0213(15)	0.0316(17)	0.0430(15)	-0.0159(14)	0.0053(13)	0.0017(14)
N	0.013(2)	0.037(2)	0.0458(19)	-0.0167(18)	0.0032(15)	0.0002(16)
C1	0.037(3)	0.030(3)	0.045(3)	-0.016(2)	-0.003(2)	-0.001(2)
C2	0.045(3)	0.058(3)	0.041(2)	-0.010(3)	-0.008(2)	0.016(3)
C3	0.020(2)	0.036(3)	0.038(2)	-0.013(2)	-0.0036(18)	0.001(2)
C4	0.032(2)	0.026(3)	0.027(2)	0.004(2)	-0.005(2)	-0.003(2)
C5	0.027(2)	0.032(3)	0.043(2)	-0.010(2)	0.007(2)	-0.004(2)
C6	0.026(2)	0.023(2)	0.032(2)	-0.008(2)	0.0016(18)-	0.004(2)
C7	0.036(3)	0.032(3)	0.037(2)	-0.001(2)	-0.002(2)	-0.002(2)
C8	0.038(3)	0.036(3)	0.062(3)	-0.016(3)	-0.007(3)	0.005(2)
C9	0.037(3)	0.048(3)	0.061(3)	-0.030(3)	0.009(2)	-0.011(3)
C10	0.063(3)	0.039(3)	0.034(2)	-0.007(2)	0.009(3)	-0.017(3)
C11	0.035(3)	0.031(3)	0.044(2)	0.000(2)	-0.003(2)	0.001(2)

The form of the anisotropic displacement parameter is:
$\exp[-2\pi^2(h^2a^{*2}U_{11} + k^2b^{*2}U_{22} + l^2c^{*2}U_{33} + 2klb^*c^*U_{23} + 2hla^*c^*U_{13} + 2hka^*b^*U_{12})]$

Table 7. Derived Atomic Coordinates and Displacement Parameters for Hydrogen Atoms

Atom	x	y	z	U_{eq}, Å^2
H1N	0.8476	-0.1067	-0.0846	0.039
H2A	0.4212	-0.1113	0.0232	0.058
H2B	0.5797	0.0367	0.0486	0.058

H3	0.7563	0.1011	-0.0351	0.038
H5A	0.2792	-0.3857	-0.1604	0.041
H5B	0.3885	-0.5011	-0.1139	0.041
H7	0.8142	-0.6447	-0.1321	0.042
H8	1.1010	-0.7571	-0.1948	0.054
H9	1.0494	-0.7052	-0.2883	0.059
H10	0.7225	-0.5329	-0.3174	0.055
H11	0.4457	-0.4100	-0.2531	0.044