Synthesis of Five-Membered Halo Enol Lactone Analogues of α-Amino Acids: Potential Protease Suicide Substrates

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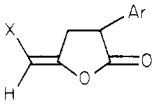
Aryl-substituted halo enol lactones can act as enzyme-activated irreversible inhibitors of serine proteases. We have developed synthetic routes to five-membered halo enol lactone analogues of α-amino acids, in order to extend these inactivators to more highly selective peptide substrate analogues. All of these methods involve the conversion of a propargyl-substituted amino acid derivative (i.e., a 4-pentynoic acid) into a 5(*E*)-halomethylidenetetrahydro-2-furanone by a halolactonization process. The parent system, 3-benzamido-5(*E*)-(iodomethylidene)-tetrahydro-2-furanone, a halo enol lactone analogue of glycine, was prepared from *N*-benzoylpropargylglycine, which itself was prepared by propargyl alkylation of diethyl benzamidomalonate. The phenylalanine analogue, 3-amino-3-benzyl-5(*E*)-(bromomethylidene)tetrahydro-2-furanone, was prepared from N-protected propargylphenylalanine derivatives, which were themselves prepared by three methods: an indirect propargyl alkylation of the 2-phenyloxazolone derived from phenylalanine by using the 2-bromo-2-propenyl group as a propargyl synthon, successive propargyl and benzyl alkylation of a STABASE-protected glycine derivative, and direct propargyl substitution of a benzylidine-protected phenylalanine. The phenylglycine analogue, 3-benzamido-3-phenyl-5-(*E*)-(bromomethylidene)tetrahydro-2-furanone, was prepared by propargyl alkylation of the 2-phenyloxazolone of phenylglycine, which itself was prepared from *N*-benzoylphenylglycine. These methods provide satisfactory approaches to several five-membered halo enol lactone analogues of amino acids.

The development of mechanism-based enzyme inactivators has attracted significant attention in recent years.[1] Utilizing its catalytic machinery, the target enzyme plays the essential role of unmasking a latent reactive functional group contained in the suicide substrate molecule, revealing a reactive, electrophilic species that may alkylate the enzyme. The potential for generating this reactive species exclusively within the active site of the enzyme imparts, in principal, a much higher degree of selectivity to these inactivators than that exhibited by conventional active-site-directed irreversible inhibitors. Thus, suicide inactivators have found utility in in vitro enzyme studies and in vivo biochemical investigations,[2] and several have shown promise as clinically useful drugs.[3]

Rando[4] proposed that a halo enol lactone such as I would, on acyl transfer to the active site hydroxyl group of a serine protease, release an α-halo ketone that could alkylate accessible nucleophilic residues (see Scheme I). Thus, lactone I might be a prototype for a series of effective mechanism-based protease inactivators. Rando's proposal prompted us to prepare several aryl-substituted five- and six-membered-ring halo enol lactones, **1** and **2**, as potential



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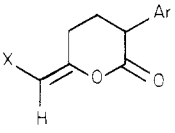

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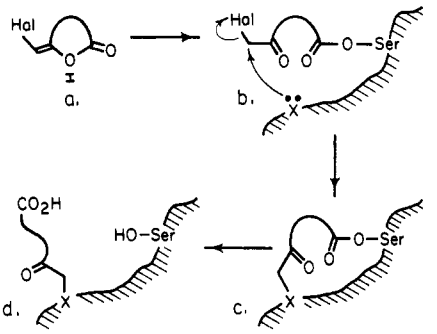
X = Br, I; Ar = Ph or α-Np

inactivators for chymotrypsin.[5] Our experimental results with these lactones support the concept proposed by Rando of mechanism-based inactivation.[6] In fact, the α-naphthyl-substituted lactones have proved to be extremely efficient chymotrypsin inactivators.[7]

In developing these halo enol lactones further as mechanism-based irreversible inactivators of proteases, we thought that we might be able to attain greater selectivity toward a specific protease by preparing analogues of oligopeptides (**3**) known to be highly selective substrates for this protease. These oligopeptide inactivators (**4**) would contain an α-amino halo enol lactone in place of the scissile amino acid unit and would have the remaining peptide chain appended to the α-amino group on the lactone. Thus, it was our desire to prepare halo enol lactone analogues that resemble amino acids and to study their binding and inactivating ability toward proteases. In this report we describe several synthetic approaches to five-membered halo enol lactone analogues of glycine, phenylalanine, and phenylglycine. Enzymatic studies with these lactones will be described elsewhere.

Scheme I



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(1) See for instance: (a) Seiler, N.; Jung, M. J.; Koch-Weser, J., Eds. "Enzyme-Activated Irreversible Inhibitors"; Elsevier/North Holland Biomedical Press: Amsterdam, 1978. (b) Rando, R. R. *Acc. Chem. Res.* **1975**, *8*, 281. (c) Bloch, K. In "The Enzymes", 3rd ed.; Boyer, P. Ed.; Academic Press: New York, 1971; Vol. 5. (d) Abeles, R. H.; Maycock, A. L. *Acc. Chem. Res.* **1976**, *9*, 313. (e) Walsh, C. *Horiz. Biochem. Biophys.* **1977**, *3*, 36.

(2) (a) Fisher, J.; Charnias, R.; Knowles, J. R. *Biochemistry* **1978**, *17*, 2180. (b) Charnias, R.; Fisher, J.; Knowles, J. R. *Ibid.* **1978**, *17*, 2185. (c) Wiseman, J.; Abeles, R. *Ibid.* **1979**, *18*, 427. (d) Prakash, N. J.; Schechter, P. J.; Mamont, P. S.; Grove, J.; Koch-Weser, J.; Sjoerdsma, A. *Life Sci.* **1980**, *26*, 181.

(3) (a) Metcalf, B. W. *Biochem. Pharmacol.* **1979**, *28*, 1705. (b) Massey, V.; Kamai, H.; Palmer, G.; and Elion, G. *J. Biol. Chem.* **1970**, *245*, 2387.

(4) Rando, R. R. *Science (Washington, D.C.)* **1974**, *185*, 320.

(5) Krafft, G. A.; Katzenellenbogen, J. A. *J. Am. Chem. Soc.* **1981**, *103*, 5459.

(6) Chakravarty, P. K.; Krafft, G. A.; Katzenellenbogen, J. A. *J. Biol. Chem.* **1982**, *257*, 610.

(7) Daniels, S.; Sofia, M. J.; Cooney, E.; Chakravarty, P. K.; Katzenellenbogen, J. A. *J. Biol. Chem.*, in press.

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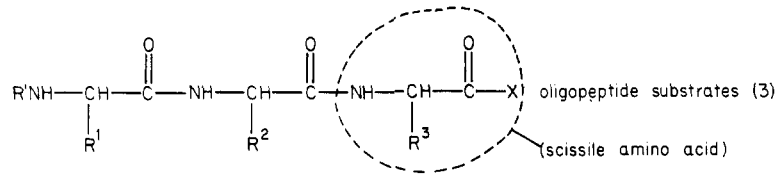
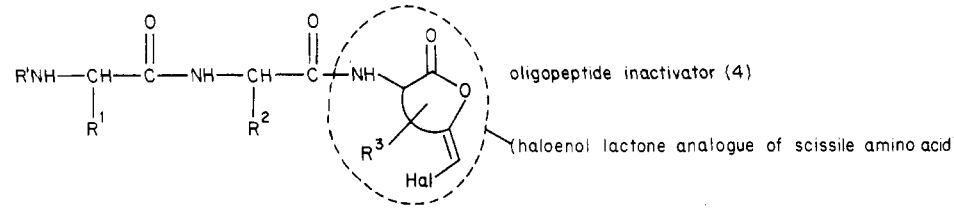
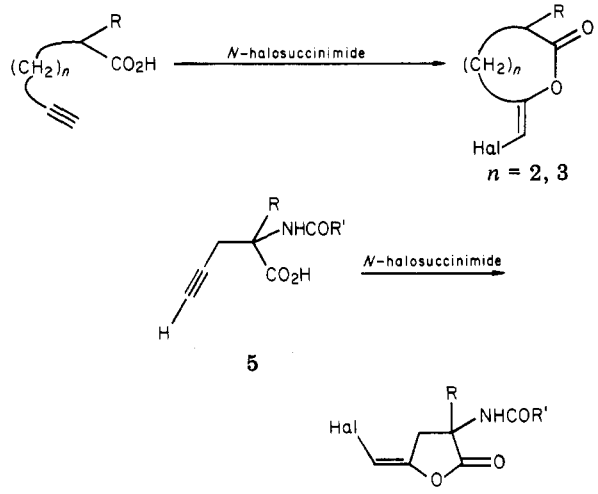


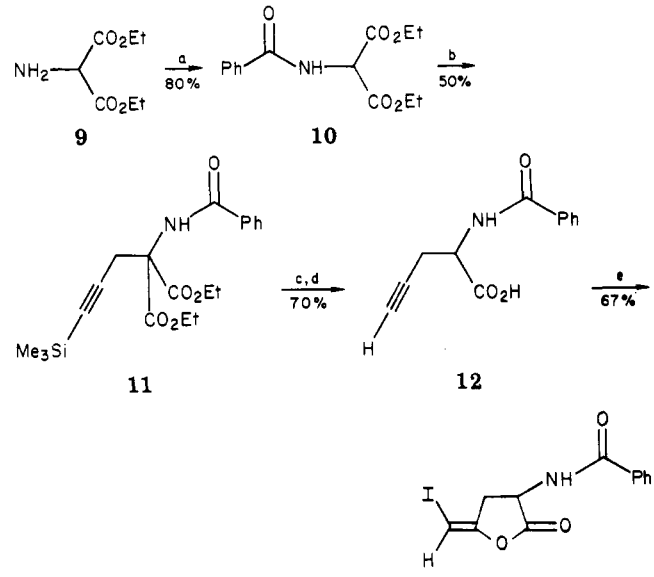


Scheme II



6, R = H (glycine analogue)
7, R = Benzyl (phenylalanine analogue)
8, R = Phenyl (phenylglycine analogue)

Scheme III[a]



[a] (a) Et_3N, PhCOCl, CH_2Cl_2; (b) NaH, THF, 3-(trimethylsilyl)propargyl bromide; (c) KOH, MeOH; (d) *m*-xylene, 135 °C; (e) *N*-iodosuccinimide, $KHCO_3$, CH_3CN.

Results and Discussion

In earlier publications, we demonstrated that both five- and six-membered halo enol lactones could be formed efficiently and stereoselectively by halolactonization of 4-pentynoic and 5-hexynoic acids, respectively, by utilizing *N*-halosuccinimides (Scheme II),[5,8] and it appeared reasonable to utilize this approach for the preparation of the three five-membered halo enol lactone systems, the glycine analogue **6**, the phenylalanine analogue **7**, and the phenylglycine analogue **8**. In each case an appropriate propargyl-substituted amino acid **5** is required as a precursor.

3-Benzamido-5(*E*)-(iodomethylidene)tetrahydro-2-furanone (Halo Enol Lactone Analogue of Glycine). The glycine analogue **13** was prepared according to the sequence shown in Scheme III. Diethyl benzamidomalonate,[9] prepared from diethyl aminomalonate, was alkylated with 3-(trimethylsilyl)propargyl bromide, prepared according to the method of Miller.[10] The silylated diester **11** was hydrolyzed to the free diacid by treatment with 10% aqueous KOH. Decarboxylation by refluxing in *m*-xylene gave the acid **12** as a white solid after purification by flash chromatography. Lactonization to give the halo enol lactone **13** was accomplished in 67% yield with *N*-iodosuccinimide in CH_3CN at 0 °C; *N*-bromosuccinimide gave many products.

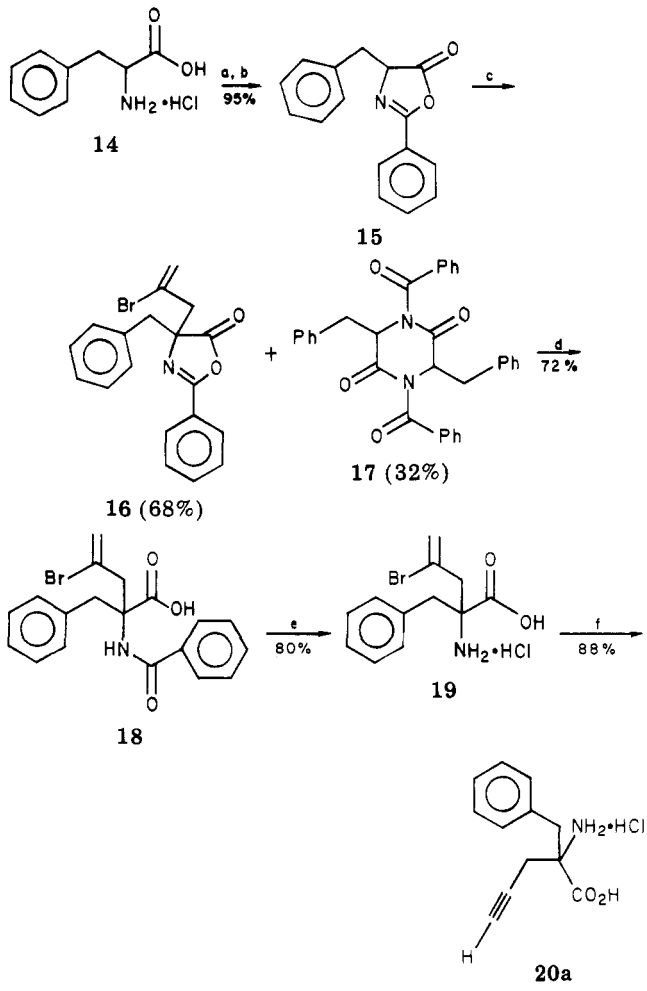
3-Amino- or 3-(Acylamino)-3-benzyl-5(*E*)-(bromomethylidene)tetrahydro-2-furanone (Halo Enol Lactone Analogue of Phenylalanine). Preparation of the 3-amino-3-benzyl lactones (phenylalanine analogues) requires the synthesis of α-propargylphenylalanine **20a** from which lactonization would proceed. We have used three approaches to prepare propargyl phenylalanine (Schemes IV–VI).

Steglich[11] and co-workers had previously reported the preparation of propargylphenylalanine by way of the 4-benzyl-2-phenyl-2-oxazolin-5-one (**15**). However, their use of a low-yield electrochemical cleavage of the oxazolone to circumvent α-acetonylphenylalanine formation during acidic cleavage of propargylated **15** appeared impractical for our purposes. To avoid these problems, we used 2,3-dibromo-1-propene as a propargyl precursor (Scheme IV).[12]

Reaction of 4-benzyl-2-phenyl oxazolone **15** with 2,3-dibromo-1-propene gave the alkylated product **16** in 68% yield. This reaction was run under high dilution with sequential addition of base and alkylating agent to minimize dioxopiperazine **17** formation. The oxazolone **16** was

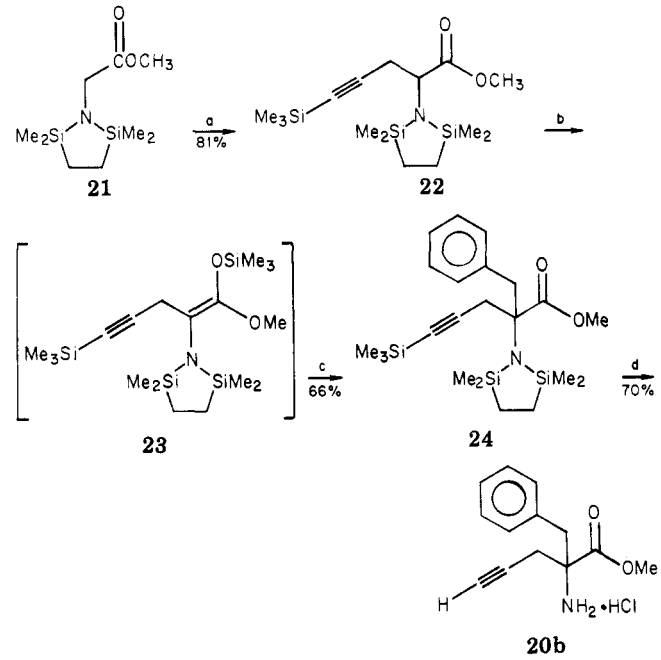
(8) For a recent review on halolactonization, see: Dowl, M. D.; Davies, D. I. *Chem. Soc. Rev.* **1979**, 171.
(9) Leukart, O.; Caviezel, M.; Eberle, A.; Escher, E.; Tun-kyi, A.; Schwyzer, R. *Helv. Chim. Acta* **1976**, *59*, 2181.
(10) Miller, R. B. *Synth. Commun.* **1972**, *2*, 267.
(11) Kübel, B.; Guber, P.; Hurmaus, R.; Steglich, W. *Chem. Ber.* **1979**, *112*, 128.
(12) Jacobs, T. In "Organic Reactions"; Adams, R., et al., Eds.; Wiley: New York, 1949; Vol. 5, Chapter 1.

Scheme IV[a]



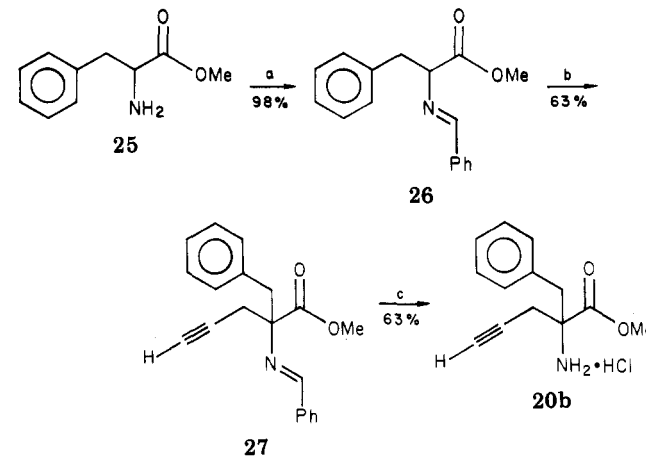
[a] (a) NaOH, C_7H_5OCl; (b) Ac_2O; (c) DMF, $(i\text{-Pr})_2NEt$, 2,3-dibromo-1-propene; (d) NaOH, THF; (e) 1:1 HCl(concn)/AcOH; (f) KOH/EtOH.

Scheme V[a]



[a] (a) THF, $(i\text{-Pr})_2NLi$, (trimethylsilyl)propargyl bromide; (b) $(Me_3Si)_2NLi$, Me_3SiCl; (c) $ZnBr_2$, CH_2Cl_2, $PhCH_2Br$; (d) HCl, MeOH.

Scheme VI[a]



[a] (a) Et_3N, PhCHO, EtOH; (b) $(i\text{-Pr})_2NLi$, 3-chloropropyne; (c) HCl, MeOH.

then cleaved under basic conditions to give the *N*-benzoyl amino acid **18** which was subsequently converted to the free amino acid **19** by heating in concentrated HCl in acetic acid.[11] No loss of the vinyl bromide substituent was observed during these deprotections. The unpurified amino acid **19** was subjected to basic elimination conditions, giving α-propargylphenylalanine **20a** in 88% yield after purification on Dowex cation-exchange resin (50W-X4).

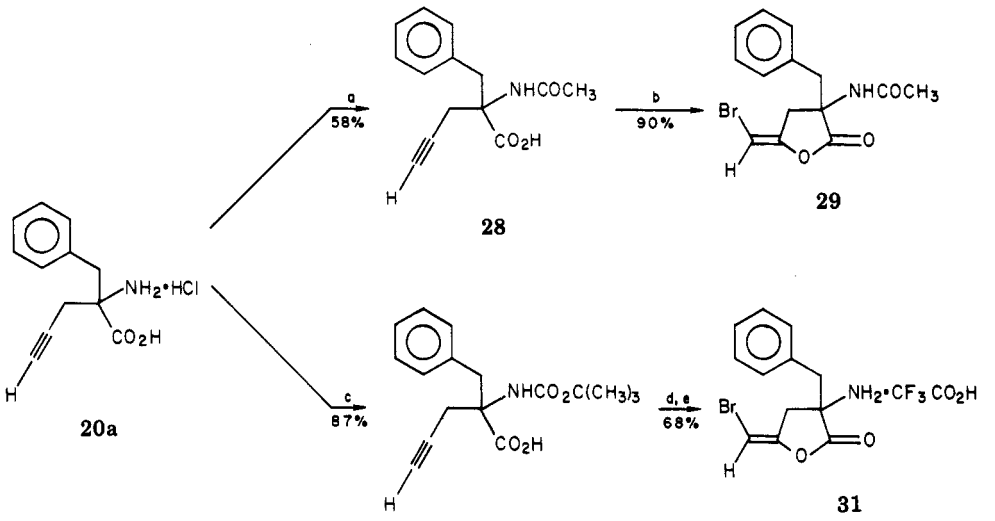
Alternative approaches to propargyl phenylalanine systems involving the selective alkylation of suitably protected α-amino acid derivatives are shown in Schemes V and VI. In the first sequence (Scheme V), methyl glycinate, converted into the STABASE[13] adduct **21** by its reaction with 1,1,4,4-tetramethyldichlorosilylethylene and triethylamine at 25 °C, was smoothly alkylated with 1-bromo-3-(trimethylsilyl)-2-propyne at −78 °C in the presence of lithium diisopropylamide, producing **22** in 81% yield. Initial attempts to alkylate **22** with benzyl bromide under a variety of nucleophilic conditions resulted in the formation of **24** in poor yields (10–25%). However, efficient alkylation under electrophilic conditions was achieved. The trimethylsilyl ketene acetal **23**, derived from **22** and chlorotrimethylsilane, was subjected to Friedel–Crafts-type alkylation[14] with benzyl bromide and $ZnBr_2$, producing **24** in 66% yield. In the final step, the silyl protecting groups were removed by aqueous acid, giving propargylphenylalanine methyl ester (**20b**).

Direct propargyl alkylation was accomplished according to an alternative scheme (Scheme VI) by using benzylidene protection of the amino function of phenylalanine methyl ester.[15] Thus, the protected derivative **26** was converted to the enolate by treatment with lithium diisopropylamide at −78 °C and alkylated with 1-chloro-2-propyne to give **27**. Deprotection in acid gave propargylphenylalanine methyl ester **20b**.

The preparations of the phenylalanine halo enol lactone analogues are shown in Scheme VII. N-Acetylation of α-propargylphenylalanine **20a** gave **28**, which upon halolactonization gave the *N*-acetyl lactone **29** in 52% yield. Attempts to lactonize α-propargylphenylalanine directly proved unsuccessful. Therefore, the amine was protected as the *tert*-butyl carbamate **30**. This compound was either

(13) Djuric, S.; Venit, J.; Magnus, P. *Tetrahedron Lett.* **1981**, *22*, 1787.
(14) Lion, C.; Dubois, J. E. *Tetrahedron* **1981**, *37*, 319.
(15) Stork, G.; Leong, Y.; Touzin, J. *J. Org. Chem.* **1976**, *41*, 3491.

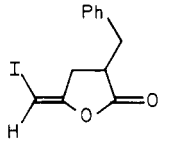
Scheme VII[a]



[a] (a) Ac_2O, Et_3N, DMAP; (b) NBS, $KHCO_3$, CH_2Cl_2, (c) Et_3N, CH_3CN, $((CH_3)_3CO_2)_2O$; (d) NBS, $KHCO_3$, CH_2Cl_2; (e) 25% TFA/CH_2Cl_2.

prepared directly from propargylphenylalanine **20a** or in two steps from the methyl ester **20b** by N-protection followed by methyl ester hydrolysis. Lactonization followed by cleavage of the carbamate with 25% TFA in CH_2Cl_2,[16] afforded the desired lactone **31** as the TFA salt in 60% yield from **20a** (Scheme VII).

3-Benzamido-3-phenyl-5(*E*)-(bromomethylidene)-tetrahydro-2-furanone (Halo Enol Lactone Analogue of Phenylglycine). From a preliminary study of another set of halo enol lactones, we noted that the α-benzyl-substituted lactone **32** is not an irreversible inactivator of



32

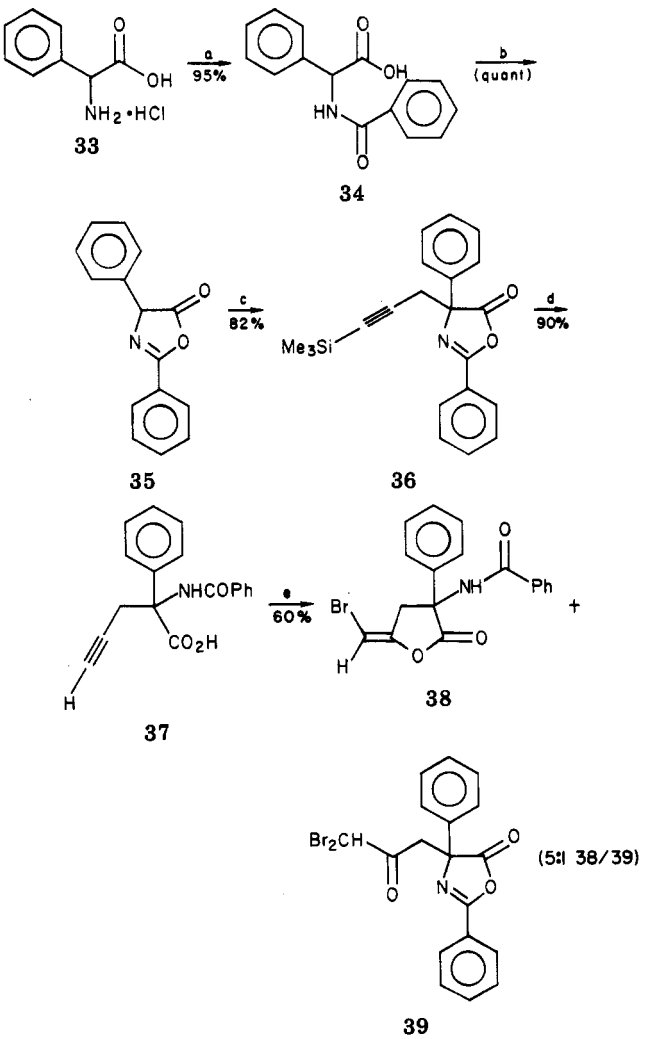
α-chymotrypsin but is a competitive inhibitor.[17] This contrasts with the α-phenyl-substituted lactones **1** which are good irreversible inactivators. Therefore, in order to be able to investigate the dependence of inactivation potency on the nature of the aromatic substituent, we prepared the phenylglycine analogue **38** (Scheme VIII).

Alkylation of the phenylglycine oxazolone **35** with 3-(trimethylsilyl)-1-bromo-2-propyne gave the oxazolone **36** in 85% purified yield. The *N*-benzoyl amino acid **37** was then obtained by NaOH hydrolysis of the oxazolone. Bromolactonization of the amino acid **37** produced the desired lactone **38** and a side product, whose spectral data are consistent with structure **39**, in a 5:1 ratio. Product **39** arises from lactone **38** as evidenced by the formation of **39** when lactone **38** is subjected to the bromolactonization conditions.

Conclusion

We have developed several routes to α-propargyl-substituted aromatic amino acids and have cyclized them to the corresponding α-amino halo enol lactones. The utility of these lactones as suicide inactivators of α-chymotrypsin and other proteins will be described elsewhere.

Scheme VIII[a]



[a] (a) NaOH; PhCOCl; (b) Ac_2O; (c) $(i\text{-}Pr)_2NEt$, DMF, 3-(trimethylsilyl)propargyl bromide; (d) NaOH, THF; (e) NBS, $KHCO_3$, CH_3CN.

Experimental Section

General Methods. Analytical thin-layer chromatography was performed by using 0.25-mm silica gel glass-backed plates with

(16) Lundt, B.; Johansen, N.; Vølund, A.; Markussen, J. *Int. J. Pept. Protein Res.* **1978**, *12*, 258.
(17) Unpublished results from this laboratory.

F-254 indicator (Merck). Visualization was by ultraviolet light, iodine, or phosphomolybdic acid. All column chromatography was done by using the flash chromatography technique as described by Still.[18] The column packing was Woelm 32–63-μm silica gel.

Proton magnetic resonance (^{1}H NMR) spectra were recorded on Varian Associates spectrometers, Models EM-390 and HR-220; chemical shifts are reported in parts per million downfield from a tetramethylsilane internal standard (δ scale). Spectra were run in the locked mode unless otherwise specified. The ^{1}H NMR data are presented in the following form: δ value of signal (peak multiplicity, integrated number of protons, coupling constant (if applicable)). Carbon-13 magnetic resonance (^{13}C NMR) spectra were recorded on a Nicolet NT-360 spectrometer. Carbon-13 spectra chemical shifts are reported in parts per million downfield from a tetramethylsilane internal standard (δ scale). Carbon-13 spectra were run in both the decoupled and off-resonance modes, and the ^{13}C NMR data are presented in the following form: δ value of signal (peak multiplicity in off resonance (where important)). Infrared (IR) spectra were obtained with a Beckmann IR-12, a Perkin-Elmer Model 137, or a Nicolet 7000 FT IR spectrometer, and the data are presented as wavenumbers for important diagnostic absorptions. Low-resolution electron-impact mass spectra were obtained on a Varian Associates MAT CH-5 at 10 or 70 eV. Low-resolution field-desorption (FD) or fast-atom-bombardment (FAB) mass spectra were obtained on a Varian Associates MAT 731. Data are reported in the following form: m/z (intensity relative to base peak = 100). High-resolution electron-impact mass spectrometry (HRMS) for exact mass determination was performed on a Varian Associates MAT 731 mass spectrometer. Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by the Microanalytical Service Laboratory of the University of Illinois School of Chemical Sciences.

Chemicals were obtained from the following sources. Aldrich Chemical Co.: triethylamine, diisopropylethylamine, diisopropylamine, 2,3-dibromo-1-propene, *N*-bromosuccinimide (NBS), 4-(dimethylamino)pyridine (DMAP), di-*tert*-butyl dicarbonate, dimethylformamide, trimethylsilyl chloride, phenylalanine, phenylglycine. Alfa (Ventron): *n*-butyllithium in hexane, sodium hydride. Parish: *N*-iodosuccinimide (NIS).

Tetrahydrofuran (THF) was distilled from sodium–benzophenone prior to use. Diisopropylamine, diisopropylethylamine, dimethylformamide (DMF), and triethylamine were refluxed over calcium hydride and then distilled to ensure dryness. The organolithium reagents were titrated periodically to determine the organic base present by using either the double titration method[19] or the single titration method with 1,10-phenanthroline as indicator.[20]

The following compounds were prepared according to literature procedures: 4-benzyl-2-phenyl-2-oxazolin-5-one (**15**),[21,22] methyl 2-(2,2,5,5-tetramethyl-2,5-disilyl-1-azacyclopentyl)glycinate (**21**),[13] and *N*-benzoylphenylglycine (**34**).[21]

Diethyl Benzamidomalonate (10). A solution of diethyl aminomalonate (2.0 g, 9.4 mmol) in CH_2Cl_2 (25 mL) at 25 °C was stirred with Et_3N (3.94 mL, 28 mmol) for 10 min under an N_2 atmosphere. To this solution at 25 °C was added benzoyl chloride (1.3 g, 9.4 mmol). The reaction mixture was stirred for 1.5 h at 25 °C, diluted with CH_2Cl_2, and washed with 1.5 N HCl and H_2O. The CH_2Cl_2 extract was dried over $MgSO_4$ and filtered to give 3.65 g of a white solid which was crystallized from EtOAc and hexane to give 2.1 g (80%) of white crystals of **10**: mp 62–63 °C; IR ($CHCl_3$) 3418, 3009, 1755, 1740, 1668 cm^{-1}; NMR ($CDCl_3$) δ 7.87 (m, 2), 7.46 (m, 3), 7.15 (br s, 1), 5.35 (d, 1, J = 7.0 Hz), 4.30 (q, 4, J = 7.0 Hz), 1.33 (t, 6, J = 7.0 Hz); mass spectrum, m/z (relative intensity), 279 (1, M$^+$), 206 (7), 133 (1), 105 (100), 77 (25); HRMS, calcd for $C_{14}H_{17}O_5N$ m/z 279.1106, found m/z 279.1107.

Diethyl Benzamido[3-(trimethylsilyl)-2-propynyl]-malonate (11). To a solution of NaH (5.9 mmol) in THF (25 mL) at 25 °C under an N_2 atmosphere was added a THF solution (15 mL) of diethyl benzamidomalonate (1.5 g, 5.4 mmol). After the reaction mixture was stirred for 5 min at 25 °C a THF solution (10 mL) of 3-(trimethylsilyl)propargyl bromide was added. After the reaction mixture was stirred for 16 h at 25 °C, it was quenched with saturated NH_4Cl solution. The reaction mixture was then extracted with EtOAc and washed with H_2O. The organic extract was dried over $MgSO_4$ and filtered to give 1.4 g of oil after solvent removal. Purification by flash chromatography, eluting with 15% EtOAc/hexane, produced 1.0 g (50%) of the desired diester **11** as a colorless oil: IR ($CHCl_3$) 3420, 3120, 2280, 1740, 1665, 1600, 1590 cm^{-1}; NMR (unlocked $CDCl_3$) δ 7.80 (m, 2), 7.50 (m, 4), 4.28 (q, 4, J = 7.0 Hz), 3.40 (s, 2), 1.32 (t, 6, J = 7.0 Hz), 0.2 (s, 9); mass spectrum, m/z (relative intensity), 389 (4, M$^+$), 316 (39), 105 (100), 85 (8); HRMS, calcd for $C_{20}H_{27}O_5NSi$ m/z 389.1658, found m/z 389.1647.

2-Benzamido-4-pentynoic Acid (12). A solution of the diester **11** (0.85 g, 2.2 mmol) in methanol (26 mL) was stirred with 10% aqueous KOH (17 mL) at 25 °C for 2 h. Removal of the methanol under vacuum and addition of 50 mL of water were followed by acidification of the water solution to pH 1 with 6 N HCl. Extraction of the aqueous solution with EtOAc followed by drying over $MgSO_4$, filtration, and solvent removal produced the diacid as a white solid. Without further purification the diacid was subjected to decarboxylation conditions (*m*-xylene, 135 °C, 1.5 h) to give 0.31 g of a white solid. Purification by flash chromatography, eluting with 1% AcOH/9% MeOH/90% CH_2Cl_2, gave 0.26 g (70%) of the acid **12**: mp 127–128.5 °C; IR (KBr) 3700–2200 (br), 1720, 1645 cm^{-1}; NMR ($CDCl_3$ + Me_2SO-d_6) δ 7.83 (m, 2), 7.45 (m, 4), 4.75 (dd, 1, J = 12.0, 6.0 Hz), 2.88 (dd, 2, J = 5.0, 2.0 Hz), 2.15 (t, 1, J = 2.0 Hz); mass spectrum, m/z (relative intensity), 217 (7, M$^+$), 178 (3), 172 (7), 105 (100), 77 (48), 39 (5); HRMS, calcd for $C_{12}H_{11}O_3N$ m/z 217.0739, found m/z 217.0723.

3-Benzamido-5(*E*)-(iodomethylidene)tetrahydro-2-furanone (13). To a solution of the acid **12** (0.058 g, 0.27 mmol) in CH_3CN (15 mL) at 0 °C under an N_2 atmosphere were added NIS (0.066 g, 0.3 mmol) and $KHCO_3$ (0.027 g, 0.27 mmol) sequentially. The reaction mixture was stirred for 78 min at 0 °C. After being quenched at 0 °C with a 5% aqueous solution of $Na_2S_2O_3$, the reaction mixture was extracted with EtOAc. The organic extract was dried over Na_2SO_4 and filtered to give 0.10 g of a solid after solvent removal. Purification by crystallization (EtOAc/hexane) gave 0.061 g (67%) of the lactone **13**: mp 187–188 °C; IR (KBr) 1820, 1660, 1629, 1580, 1535 cm^{-1}; NMR ($CDCl_3$ + Me_2SO-d_6) δ 9.23 (br d, 1, J = 9.0 Hz), 7.90 (m, 2), 7.50 (m, 3), 5.90 (t, 1, J = 2.0 Hz), 4.78 (m, 1), 3.15 (m, 2); mass spectrum, m/z (relative intensity) 343 (5, M$^+$), 216 (7), 127 (1), 106 (8), 105 (100), 77 (42); HRMS, calcd for $C_{12}H_{10}O_3IN$ m/z 342.9708, found m/z 342.9700.

4-Benzyl-2-phenyl-2-oxazolin-5-one (15). The precursor of the oxazolone, *N*-benzoylphenylalanine, was prepared from phenylalanine by using the Schotten–Baumann procedure as described by Greenstein:[21] mp 187–188 °C (lit.[21] mp 187 °C) IR (KBr) 3340 (NH), 1760 (C=O), 1640 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 7.78 (d, 1, J = 1.5 Hz), 7.69 (d, 1, J = 3.0 Hz), 7.44–7.30 (m, 3), 7.24 (s, 5), 4.90 (m, 1), 3.35 (dd, 1, J = 7, 18 Hz), 3.18 (dd, 1, J = 7, 18 Hz). Anal. Calcd for $C_{16}H_{15}O_3N$: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.50; H, 5.63; N, 5.12.

The oxazolone **15** was prepared according to the procedure as described by Greenstein[21] and Mohr.[22] Crystallization of the solid (acetone–hexane) afforded 7.68 g (82%) of the oxazolone **15**: mp 71–74 °C (lit.[22] mp 71 °C); IR (KBr) 1820 (C=O), 1650 cm^{-1}; NMR ($CDCl_3$) δ 7.91 (d, 1, J = 1.5 Hz), 7.75 (d, 1, J = 2.8 Hz), 7.38 (d, 3, J = 7 Hz), 7.2 (s, 5), 4.6 (dd, 1, J = 6, 6 Hz, 3.33 (dd, 1, J = 6, 17 Hz), 3.10 (dd, 1, J = 6, 17 Hz).

4-Benzyl-2-phenyl-4-(2-bromo-2-propenyl)-2-oxazolin-5-one (16). 4-Benzyl-2-phenyl-2-oxazolin-5-one (**15**; 1.0 g, 3.98 mmol) was dissolved in 25 mL of dry DMF, and (*i*-Pr)$_2$NEt (0.62 g, 4.8 mmol) and 2,3-dibromo-1-propene (1.19 g, 6.0 mmol) were added sequentially. This solution was stirred for 4 days at room temperature under N_2 atmosphere. The reaction was then quenched with 10 mL of water, and the mixture was extracted twice with 50 mL of ethyl acetate. The ethyl acetate was removed under vacuum, leaving an oil which was dissolved in $CHCl_3$, washed twice

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with water to remove any residual DMF, and then dried over Na_2SO_4. After filtration, the $CHCl_3$ was removed in vacuo to give an orange oil which was purified by silica gel flash chromatography, eluting with 1:4 acetone/hexane, to give 0.93 g (68%) of the desired alkylated oxazolone **16**: IR ($CHCl_3$) 1815, 1655, 1625 cm^{-1}; NMR ($CDCl_3$) δ 7.85 (d, 1, J = 1.5 Hz), 7.78 (d, 1, J = 3.0 Hz), 7.5–7.3 (3 H, aromatic), 7.15 (s, 5), 5.70 (d, 1, J = 1.5 Hz), 5.50 (d, 1, J = 1.5 Hz), 3.20 (s, 4); mass spectrum, m/z (relative intensity), 369, 371 (9, M^+), 290 (2), 278, 280 (3), 250 (4), 105 (72), 91 (100). Anal. Calcd for $C_{19}H_{16}O_2BrN$: C, 61.79; H, 4.33; N, 3.97. Found: C, 62.00; H, 4.20; N, 3.86.

A side product, the dioxopiperazine **17**, was also isolated by the flash chromatography: IR (KBr) 1820, 1750, 1685 cm^{-1}; NMR (CDCl) δ 7.7–7.0 (m, 20), 5.42 (t, 2, J = 4 Hz), 3.2 (d, 2, J = 4 Hz), 3.12 (d, 2, J = 4 Hz); mass spectrum, m/e (relative intensity), 502 (17, M^+), 387 (4), 382 (4), 251 (20), 105 (100), 91 (61), 77 (100).

α-(2-Bromo-2-propenyl)-*N*-benzoylphenylalanine (18). 4-Benzyl-2-phenyl-4-(2-bromo-2-propenyl)-2-oxazolin-5-one (**16**; 1.26 g, 3.4 mmol) in THF (32 mL) was stirred with 2 N NaOH (11.2 mL) under a drying tube for 24 h, after which time the reaction mixture was diluted with 8 mL of water and chilled in an ice bath. The mixture was acidified with concentrated HCl and extracted three times with CH_2Cl_2. The extract was dried over $MgSO_4$ and filtered through a pad of Celite, furnishing a yellow solid after solvent removal. Recrystallization from ethanol and water gave 0.95 g (72%) of the amino acid derivative **18**: mp 172–174 °C; IR ($CHCl_3$) 3400, 3350–2500, 1725, 1650, 1625 cm^{-1}; NMR ($CDCl_3$) δ 9.80 (s, 1), 7.70 (d, 1, J = 1.5 Hz), 7.60 (d, 1, J = 3.0 Hz), 7.5–7.3 (m, 3), 7.17 (s, 5), 5.74 (s, 1), 5.47 (d, 1, J = 1.5 Hz), 4.1 (d, 1, J = 14.4 Hz), 4.05 (d, 1, J = 13.2 Hz), 3.20 (d, 1, J = 14.4 Hz), 3.13 (d, 1, J = 13.2 Hz); mass spectrum (FAB), m/z (relative intensity) 388, 389 (20, $M^+ + 1$), 344 (3), 310 (13), 269 (5), 121 (3), 105 (100), 91 (22).

α-(2-Bromo-2-propenyl)phenylalanine (19). A solution of 1:1 concentrated HCl/glacial acetic acid (30.75 mL) was added to the solid α-(2-bromo-2-propenyl)-*N*-benzoylphenylalanine (**18**; 0.93 g, 2 mmol). This mixture was stirred at 100 °C under N_2 atmosphere for 16 h. The reaction mixture was cooled, and all volatiles were removed in vacuo, leaving a brown residue which was stirred with ether to remove any traces of acetic acid or benzoyl chloride. The remaining oily residue was dried under vacuum to a tan powder, the amino acid **19**, which was not further purified: 80% yield; IR (KBr) 3700–2400, 1710, 1625 cm^{-1}; NMR (CD_3CN) δ 7.20 (s, 5), 6.03 (d, 1, J = 2.0 Hz), 5.6 (d, 1, J = 2.0 Hz), 3.35 (s, 2), 3.28 (s, 2); mass spectrum (FAB), m/z (relative intensity) 283, 285 (97, $M^+ + 1$), 206 (50), 204 (15), 164 (50), 91 (100).

α-(2-Propynyl)phenylalanine Hydrochloride (20a). The solid α-(2-bromo-2-propenyl)phenylalanine (**19**; 0.90 g, 3.2 mmol) was stirred with 6 mL of a saturated solution of KOH in absolute ethanol at 80 °C under an N_2 atmosphere for 3.5 h. The reaction mixture was allowed to cool to 25 °C and was then acidified with 6 N HCl to give a white solid (KBr), which was removed by filtration leaving a yellow solution. This solution was evaporated to dryness, giving a brown solid which was purified by ion-exchange chromatography, using Dowex 50W-4X (H^+) cation-exchange resin and eluting with H_2O, with 3 N HCl, and finally with 6 N HCl. The amino acid **20a** (0.55 g, 88%) eluted with the 6 N HCl wash: R_f 0.55 (in butanol/AcOH/H_2O, 7:2:2); mp 72–74 °C; IR (film) 3880–2400, 1725, 1600, 1500 cm^{-1}; NMR (CD_3CN) δ 7.67 (br s, 3), 7.37 (s, 5), 3.40 (s, 2), 3.03 (d, 2, J = 3.0 Hz), 2.57 (t, 1, J = 3.0 Hz); mass spectrum (FD), m/e (relative intensity) 293 (4, M^+), 204 (100), 159 (50). Anal. Calcd for $C_{12}H_{14}O_2NCl \cdot {}^1/_4H_2O$: C, 59.26; H, 5.96. Found: C, 59.66; H, 6.25.

Methyl 2-(2,2,5,5-Tetramethyl-2,5-disila-1-azacyclopentyl)-5-(trimethylsilyl)-4-pentynoate (22). *n*-BuLi (2.65 mL, 2.45 M in hexane) was added dropwise to a solution of diisopropylamine (0.99 mL) in dry THF (15 mL) at −10 °C. The mixture was stirred at −10 °C for 15 min and was then cooled to −78 °C. A solution of **21**[13] (1.5 g, 6.5 mmol) in dry THF (10 mL) was then added dropwise over a period of 30 min. The resulting orange solution was stirred for an additional 30 min at −78 °C, and a solution of 3-(trimethylsilyl)propargyl bromide (1.49 g, 7.8 mmol) in a mixture of dry THF (5 mL) and HMPA (1 mL) was then added. After being stirred at −78 °C for 1 h, the reaction mixture was slowly warmed to 25 °C and stirred at that temperature for 1 h. The reaction was quenched with the addition of saturated NaH_2PO_4 and extracted with ether, the ether phase was washed with saturated NaCl and dried ($MgSO_4$), and the ether was removed in vacuo to give a yellow oil which was purified by distillation: 1.8 g (81%); bp 91–94 °C (0.02 mmHg); NMR ($CDCl_3$) δ 3.85 (t, 1, J = 11 Hz), 3.71 (s, 3), 2.7 (d, 2, J = 11 Hz), 0.65 (s, 4), 0.15 (s, 12), 0.05 (s, 9).

Methyl 2-Benzyl-2-(2,2,5,5-tetramethyl-2,5-disila-1-azacyclopentyl)-5-(trimethylsilyl)-4-pentynoate (24). A solution of *n*-BuLi (0.40 mL, 2.47 M) was added at −20 °C to a solution of hexamethyldisilazane (0.21 mL, 1 mmol) in THF (2 mL). After being stirred at that temperature for an additional 30 min, the solution was cooled to −78 °C, and a solution of **22** (0.34 g, 1 mmol) in THF (5 mL) was added, with stirring being continued for 1 h at −78 °C. Chlorotrimethylsilane (0.13 mL, 1 mmol) was added to the reaction mixture at −78 °C, and the colorless reaction mixture was stirred at 25 °C for 30 min. After filtration under N_2 to remove LiCl, evaporation of the solvent in vacuo gave the corresponding trimethylsilyl ketene acetal **23** as an oil. The NMR spectra of this crude product was consistent with the structure **23**.

Without further purification, **23** was dissolved in dry dichloromethane (5 mL); freshly distilled benzyl bromide (0.15 mL, 1.2 mmol) was added, followed by the addition of anhydrous zinc bromide (22.5 mg, 0.01 mmol). The mixture was stirred at 25 °C for 24 h under N_2. After this period, the reaction was quenched with saturated NaH_2PO_4, and the organic phase was washed with saturated NaCl, dried ($MgSO_4$), and evaporated to give the crude product (0.38 g) as an oil which was purified by distillation: 0.29 g (66%); bp 118–120 °C (0.02 mmHg); NMR ($CDCl_3$) δ 7.32 (s, 5), 3.70 (s, 3), 2.84 (s, 2), 2.62 (s, 2), 0.60 (s, 4), 0.15 (s, 12), 0.1 (s, 9); HRMS, calcd for $C_{22}H_{37}NO_2Si_3$ m/z 433.5610, found m/z 433.5880.

***N*-Benzylidenephenylalanine Methyl Ester (26).** A mixture of phenylalanine methyl ester hydrochloride (2.15 g, 10 mmol), freshly distilled benzaldehyde (1.1 g, 10 mmol), and triethylamine (1.4 mL, 10 mmol) in absolute ethanol (20 mL) were stirred at 25 °C for 24 h. The solvent was removed in vacuo, and the residue was redissolved in absolute ethanol (10 mL) and evaporated in vacuo. The residue was dissolved in dry ether (25 mL) and filtered. Removal of ether in vacuo gave the crude product as an oil, which was purified by distillation: 2.6 g (98%); bp 161–162 °C (1 mmHg); NMR ($CDCl_3$) δ 7.80 (s, 1), 7.58 (dd, 2, J = 8 Hz, J = 4.5 Hz), 7.28 (m, 3), 7.10 (s, 5), 4.12 (dd, 1, J = 10 Hz, J = 6 Hz), 3.66 (s, 3), 3.20 (2, AB q, $\Delta\nu$ = 0.57, J = 17 Hz, upfield portion a doublet, J = 9 Hz; downfield doublet, J = 6 Hz); Anal. Calcd for $C_{17}H_{17}NO_2$: C, 76.38; H, 6.41; N, 5.24. Found: C, 76.19; H, 6.62; N, 5.32.

Methyl 2-Benzyl-2-(benzylidenamino)-4-pentynoate (27). A solution of **26** (1.7 g, 6.36 mmol) in THF (10 mL) was added slowly at −78 °C to a solution of lithium hexamethyldisilylamide, generated in 10 mL of THF from hexamethyl disilazane (1.46 mL) and *n*-BuLi (2.5 mL of a 2.46 M solution in hexane) at −20 °C. The resulting orange solution was stirred at −78 °C for 1.5 h, and 1-chloro-2-propyne (0.48 g, 6.5 mmol) was then added. The mixture was stirred at −78 °C for 30 min and then slowly warmed to room temperature. After 2 h at room temperature, the reaction was quenched with saturated NH_4Cl and extracted with ether. The extract was washed with water, dried ($MgSO_4$), and evaporated in vacuo. The crude product, an oil, was purified by distillation: 1.2 g (63%); bp 135–138 °C (0.05 mmHg); NMR ($CDCl_3$) δ 8.20 (s, 1), 7.72 (m, 2), 7.25 (m, 8), 3.65 (s, 3), 3.40 (s, 2), 2.75 (d, 2, J = 3 Hz), 2.12 (t, 1, J = 3 Hz); HRMS, calcd for $C_{20}H_{19}NO_2$ m/z 305.3790, found m/z 305.3715.

Methyl 2-Amino-2-benzyl-4-pentynoate Hydrochloride (20b). Method A. A solution of **27** (1.0 g, 3.28 mmol) in methanol (10 mL) was mixed with 3 N aqueous HCl (10 mL) and stirred at 25 °C for 24 h. Methanol was removed in vacuo, and the aqueous phase, after extraction with ether, was loaded on a Dowex 50W-X4 (H^+) (12 cm × 2.5 cm) column. After initial elution with water, the column was eluted with 6 N HCl, which upon evaporation in vacuo gave **20b**: 0.58 g (70%); mp 193–194 °C (after recrystallization from methanol–dry ether); NMR (Me_2SO-d_6 + CD_3CN) δ 7.80–8.10 (br m, 3, NH_3^+), 7.30 (s, 5), 3.76 (s, 3), 3.35 (s, 2), 3.00 (d, 2, J = 3 Hz), 2.58 (t, 1, J = 3 Hz); mass spectrum (FD), m/z 218 (MH^+). Anal. Calcd for $C_{13}H_{16}NO_2Cl$: C, 61.54;

H, 3.95; N, 5.52. Found: C, 61.47, H, 4.14; N, 5.41.

Method B. A solution of **24** (0.15 g) in methanol (5 mL) was treated with 3 N HCl (5 mL) at 25 °C for 24 h. The workup and purification, as described in method A, gave **20b** (48 mg, 63%).

***N*-Acetyl-α-propargylphenylalanine (28).** A DMF (3 mL) solution of α-propargylphenylalanine (**20a**; 0.10 g, 4.2 mmol) was stirred with Et_3N (0.13 g, 1.3 mmol), Ac_2O (0.09 g, 8.4 mmol), and 4-(dimethylamino)pyridine (0.005 g, 0.13 mmol) at 25 °C under a nitrogen atmosphere for 24 h. The reaction mixture was evaporated to dryness followed by acidification with 3 N HCl. The residue was dissolved in CH_2Cl_2 and washed with water. The organic extract was then dried over Na_2SO_4 and filtered to give a solid, which was crystallized from EtOH/water to give 0.057 g of the *N*-acetyl amino acid **28**: mp 164–166 °C; IR (KBr) 3020, 1725, 1655 cm^{-1}; NMR ($CDCl_3$) δ 7.17 (m, 5), 6.73 (br s, 1), 3.08 (s, 2), 2.73 (s, 1), 2.69 (s, 1), 2.00 (s, 3); mass spectrum *m/z* (relative intensity) 245 (6, M^+), 206 (14), 200 (2), 201 (3), 186 (15), 158 (12), 154 (11), 142 (15), 115 (10), 91 (63); HRMS, calcd for $C_{14}H_{15}O_3N$ *m/z* 245.1052, found *m/z* 245.1053.

3-(Acetylamino)-3-benzyl-5(*E*)-(bromomethylidene)-tetrahydro-2-furanone (29). *N*-Bromosuccinimide (0.025 g, 0.14 mmol) and $KHCO_3$ (0.014 g, 0.14 mmol) were added to a solution of *N*-acetyl-α-propargylphenylalanine (**28**; 0.033 g, 0.14 mmol) in CH_2Cl_2 (5 mL) under an N_2 atmosphere at 25 °C. The reaction mixture was stirred for 30 min at 25 °C and was then quenched with 5% aqueous $Na_2S_2O_3$ solution. After being washed with a $Na_2S_2O_3$ solution and water, the CH_2Cl_2 extract was dried over Na_2SO_4. The resulting solid was purified by flash chromatography, eluting with 25% EtOAc/CH_2Cl_2. The lactone **29** (90%) was then crystallized from Et_2O/hexane: mp 149–150 °C; IR (KBr) 1825, 1790, 1675, 1655 cm^{-1}; NMR ($CDCl_3$) δ 7.25 (m, 5), 6.38 (br s, 1), 6.63 (t, 1, *J* = 2.0 Hz), 3.10 (m, 4), 2.03 (s, 3); mass spectrum, *m/z* (relative intensity) 323, 325 (3, M^+ and $M^+ + 1$), 264 (3), 255, 253 (4), 232, 234 (17), 91 (72), 43 (100); HRMS, calcd for $C_{14}H_{14}O_3NBr$ *m/z* 323.0157, found *m/z* 323.0153.

***N*-(*tert*-Butoxycarbonyl)-α-propargylphenylalanine (30).** α-Propargylphenylalanine (**20a**; 0.1 g, 0.42 mmol) was stirred in CH_3CN (3 mL) under an N_2 atmosphere at 25 °C. To this mixture at 25 °C was added Et_3N (0.167 mL, 0.84 mmol), followed by di-*tert*-butyldicarbonate (0.11 g, 0.50 mmol). The mixture was stirred at 25 °C for 17 h. It was then evaporated to dryness, leaving a white solid, which was suspended in water and acidified with citric acid at 0 °C. This solution was then extracted with three portions of EtOAc. An oil (0.111 g) was obtained after the extracts were dried over $MgSO_4$, and the solvent was removed. Purification by flash chromatography, eluting with 92% CH_2Cl_2/7% MeOH/1% AcOH, gave 0.10 g (87%) of *N*-(*tert*-butoxycarbonyl)-α-propargylphenylalanine (**30**): mp 138–140 °C; IR ($CHCl_3$) 3380, 3225, 2930, 1700 (br), 1480 cm^{-1}; NMR ($CDCl_3$) δ 8.27 (br s, 1), 7.22 (s, 5), 5.35 (br s, 1), 3.20 (m, 4), 2.08 (t, 1, *J* = 2.0 Hz), 1.48 (s, 9); mass spectrum (FAB), *m/z* (relative intensity) 304 (100, $M^+ + 1$), 202 (40), 187 (14), 186 (21).

3-Amino-3-benzyl-5(*E*)-(bromomethylidene)tetrahydro-2-furanone (31). *N*-(*tert*-butoxycarbonyl)-α-propargylphenylalanine (**30**; 0.1 g, 0.33 mmol) in CH_2Cl_2 (5 mL) was stirred with NBS (0.059 g, 0.33 mmol) and $KHCO_3$ (0.033 g, 0.33 mmol) under a nitrogen atmosphere at 25 °C for 1 h. The reaction mixture was then diluted with CH_2Cl_2 and washed with 5% $Na_2S_2O_3$ solution, with saturated NaCl solution, and with H_2O. The organic extract was dried over Na_2SO_4. After filtration and solvent removal, 0.12 g (98%) of a white solid remained: NMR ($CDCl_3$) δ 7.30 (m, 5), 5.67 (t, 1, *J* = 2.0 Hz), 5.13 (br s, 1), 3.23 (m, 2), 3.03 (m, 2), 1.54 (br s, 9). Without further purification, this solid was stirred with 25% TFA in CH_2Cl_2 at 0 °C for 3.5 h and then at 25 °C for 1.5 h under nitrogen. The reaction mixture was evaporated to dryness, leaving a white solid which was crystallized from EtOAc/hexane to give the α-aminofuranone **31** (0.086 g, 68% from **30**) as the TFA salt: mp 174–175 °C; IR (KBr) 3460 (br), 2980 (br), 1810, 1685, 1670 cm^{-1}; NMR (CD_3CN) δ 7.21 (m, 5), 5.74 (t, 1, *J* = 2.0 Hz), 3.23 (s, 2), 3.20 (m, 2); mass spectrum (FAB), *m/z* (relative intensity) 281, 283 (100, M^+, $M^+ + 1$), 267 (15), 265 (15), 202 (10), 92 (45), 91 (100). Anal. Calcd for $C_{14}H_{13}NO_4BrF_3$: C, 42.53; H, 3.29; N, 3.54; Br, 20.00; F, 14.43. Found: C, 42.32; H, 3.14; N, 3.63; Br, 19.96; F, 14.60.

***N*-Benzoylphenylglycine (34).** Phenylglycine (5.0 g, 33 mmol) was stirred in 20 mL of 2 N NaOH at 0 °C. To this solution was added 4.63 g (33 mmol) of benzoyl chloride with concomitant addition of 2 N NaOH to keep reaction solution basic. The reaction mixture was then warmed to 25 °C and stirred for 10 min followed by acidification with concentrated HCl to give a white precipitate. The total mixture was kept at −5 °C for 2.5 h, after which a solid was collected, washed with cold water, and air-dried. This solid was then boiled in CCl_4 and filtered while warm. The remaining solid was dried and crystallized from EtOH/H_2O to give 8.0 g (95%) of the white solid *N*-benzoylphenylglycine (**34**): mp 176–177 °C; IR (KBr) 3700–2500, 1725, 1738, 1578 cm^{-1}; NMR ($CDCl_3$ + Me_2SO-d_6) δ 7.88 (m, 2), 7.43 (m, 9), 5.73 (d, 1); mass spectrum, *m/z* (relative intensity) 255 (1.4 M^+), 210 (11), 105 (100), 77 (54); HRMS, calcd for $C_{15}H_{13}O_3N$ *m/z* 255.0895, found *m/z* 255.0889.

2,4-Diphenyl-2-oxazolin-5-one (35). This material was prepared from *N*-benzoylphenylglycine (**34**) by the method used to prepare oxazolone **15**. Oxazolone **35** was formed in quantitative yield and was crystallized from Et_2O/hexane: mp 98–100 °C; IR ($CHCl_3$) 3975, 1825, 1640, 1600, 1575 cm^{-1}; NMR ($CDCl_3$) δ 8.10 (m, 2), 7.50 (m, 3), 7.43 (s, 5), 5.50 (s, 1); mass spectrum, *m/z* (relative intensity), 237 (15, M^+), 193 (92), 105 (100), 77 (76); HRMS, calcd for $C_{15}H_{11}O_2N$ *m/z* 237.0789, found *m/z* 237.0787.

2,4-Diphenyl-4-[3-(trimethylsilyl)-2-propynyl]-2-oxazolin-5-one (36). 2,4-Diphenyl-2-oxazolin-5-one (**35**; 0.5 g, 2.2 mmol) was stirred in dry DMF (15 mL) at room temperature under a nitrogen atmosphere. To this stirred solution were added sequentially (*i*-Pr)$_2$NEt (0.344 g, 2.66 mmol) and 1-bromo-3-(trimethylsilyl)-2-propyne (0.43 g, 2.65 mmol) at 25 °C. This reaction mixture was stirred for 1 h at 25 °C and then quenched with 50 mL of water. The resulting mixture was extracted with EtOAc, and the extracts were washed twice with water, dried over $MgSO_4$, and filtered. A yellow oil that remained after solvent removal was purified by flash chromatography, eluting with 2% EtOAc/hexane, to give 0.643 g (82%) of a clear oil: IR ($CHCl_3$) 2950, 2160, 1830, 1650, 1600, 1575 cm^{-1}; NMR ($CDCl_3$) 8.10 (m, 2), 7.54 (m, 8), 3.21 (d, 1, *J* = 16.5 Hz), 2.97 (d, 1, *J* = 16.5 Hz); mass spectrum, *m/z* (relative intensity), 347 (2, M^+), 236 (100), 111 (18), 105 (23); HRMS, calcd for $C_{21}H_{21}O_2NSi$ *m/z* 347.1341, found *m/z* 347.1340.

α-Propargyl-*N*-benzoylphenylglycine (37). The oxazolone **36** (0.26 g, 0.75 mmol) was dissolved in THF (5 mL), and 2.3 mL of 2 N NaOH was added at 25 °C. After this mixture was stirred for 2 h, the reaction vessel was placed in an ice bath and acidified to pH 1 by the addition of concentrated HCl. The resulting mixture was extracted three times with CH_2Cl_2, and the organic extract was dried over $MgSO_4$ and filtered to give, after solvent removal, 0.25 g of a white solid. The solid was crystallized from Et_2O/hexane to give the amino acid **37**: 0.2 g (90%); mp 171–172.5 °C, IR (KBr) 3700–3100, 3360, 3310, 1755, 1625, 1600, 1580 cm^{-1}; NMR ($CDCl_3$ + Me_2SO-d_6) δ 7.90 (m, 2), 7.43 (m, 9), 3.73 (AB q, Δν = 30.5 Hz, *J* = 16.5 Hz, split into a doublet, *J* = 2.0 Hz), 2.00 (t, 1, *J* = 2.0 Hz), mass spectrum, *m/z* (relative intensity), 293 (7.47, M^+), 254 (21), 248 (22), 188 (2), 173 (12), 172 (92), 105 (100), 77 (100); HRMS, calcd for $C_{18}H_{15}O_3N$ *m/z* 293.1051, found *m/z* 293.1042.

3-Benzamido-3-phenyl-5(*E*)-(bromomethylidene)tetrahydro-2-furanone (38). The *N*-benzoyl amino acid **37** (0.12 g, 0.41 mmol) in CH_3CN (5 mL) was stirred at 25 °C with NBS (0.087 g, 0.49 mmol) and solid $KHCO_3$ (0.049 g, 0.49 mmol). After 25 min at 25 °C the reaction was quenched with 5% aqueous $Na_2S_2O_3$. The resulting mixture was extracted with three portions of CH_2Cl_2. The organic extract was dried over Na_2SO_4, filtered, and evaporated to dryness. The resulting white solid, showing two components by TLC (1:4 EtOAc/hexane), was purified by flash chromatography, eluting with 30% EtOAc/hexane. The more polar material [0.105 g; R_f 0.16 (1:4 EtOAc/hexane)], crystallized from EtOAc/hexane, was the furanone **38**: 50% yield; mp 164–165.5 °C; IR ($CHCl_3$) 3350, 2950, 1800, 1665, 1600, 1575 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.77 (m, 2), 7.48 (m, 8), 6.70 (br s, 1), 6.03 (t, 1, *J* = 2.0 Hz), 3.69 (d, 2, *J* = 2.0 Hz); ^{13}C NMR ($CDCl_3$) δ (multiplicity in off resonance) 172.023 (s), 167.005 (s), 149.402 (s), 136.676, 132.568, 132.486, 129.766, 129.728, 128.800, 127.213, 125.623, 86.250 (d), 62.821 (s), 38.308 (t); mass spectrum, *m/z* (relative intensity), 371, 373 (0.5, M^+), 293 (17), 252 (8), 105 (100), 77 (60); HRMS, calcd for $C_{18}H_{14}O_3NBr$ *m/z* 371.0157, found *m/z* 371.0157.

The less polar product [0.02 g (9%); R_f 0.34 (1:4 EtOAc/hexane)] was crystallized from Et_2O and hexane and was identified as the oxazolone **39**: mp 152.5–153.5 °C; IR ($CHCl_3$) 2950, 1825, 1820, 1650, 1585, 1255 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.05 (m, 2), 7.67 (m, 2), 7.41 (m, 6), 5.95 (s, 1), 3.11 (d, 1, J = 12 Hz), 2.50 (d, 1, J = 12 Hz); ^{13}C NMR δ (multiplicity in off resonance) 169.244 (s), 157.195 (s), 135.437, 132.339, 130.432, 128.825, 128.675, 128.448, 127.762, 126.973, 102.736 (s), 64.770 (s), 39.304 (d), 39.278 (t); mass spectrum, m/z (relative intensity) 327, 329 (13, 11), 326, 328 (63, 63), 250 (10), 221 (3), 171, 173, 175 (1:2:1), 120 (2), 105 (96); mass spectrum (FAB), m/z 449, 451, 453 (7, 14, 7), 448, 450, 452 (24, 48, 24). Anal. Calcd for $C_{18}H_{13}O_3NBr_2$: C, 48.10; H, 2.89; N, 3.12; Br, 35.20. Found: C, 47.87; H, 2.80; N, 3.00; Br, 35.64.

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Spatane Diterpenoids from the Tropical Marine Algae *Spatoglossum schmittii* and *Spatoglossum howleii* (Dictyotaceae)

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Three new diterpenoids of the spatane class, spatol (1), the triol **2**, and a triacetate derivative of **3**, have been isolated from the tropical brown algae *Spatoglossum schmittii* and *Spatoglossum howleii*. The diol **4** and the tetraol **5**, metabolites previously isolated from the related alga *Stoechospermum marginatum*, were also components of these algae. Spatol was found to inhibit synchronous cell division in the fertilized sea urchin egg assay and to inhibit human cancer cell cleavage in vitro in the 1–5 μg/mL range.

Preliminary pharmacological investigations of extracts of brown marine algae of the family Dictyotaceae have illustrated considerable antibacterial,[2-9] antiviral,[2,4,9] and cytotoxic activities.[2,10,11] In several subsequent studies the active compounds have been isolated and shown to be novel new diterpenoids or metabolites of mixed biosynthesis.[12-19] Our investigations of this family of marine algae have emphasized the isolation of biologically active metabolites from new genera not yet investigated, and in a recent paper, we described the structure of a new cytotoxic diterpenoid, spatol (1), from extracts of the brown algae *Spatoglossum schmittii*.[19] In this paper we report, in full, on the isolation of spatol and several related spatane diterpenoids from two *Spatoglossum* species, *S. schmittii* and *S. howleii*, both of which are common algae of the Galapagos Islands.

Spatoglossum schmittii Taylor and *S. howleii* (Setchell and Gardner)[20] were found in luxuriant growth along the western coast of Isla Isabella, Galapagos Islands (Archipelago de Colon) in February 1978, and the freshly collected algae were immediately preserved in 2-propanol. Subsequent chloroform-methanol extraction of each species, followed by silica chromatography of the condensed extracts, yielded mixtures rich in the spatane diterpenoids **1–5**. The extract of *S. schmitii* yielded diterpenoids **1**, **2**, and **4**, while the *S. howleii* extract yielded diterpenoids **2–5**. The diterpenoids **1–3** were recognized as new modifications of the spatane ring system, while **4** and **5** were identical with two compounds earlier isolated from the related alga *Stoechospermum marginatum* from the Indian Ocean.[21] Recently, several spatane and sec-

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