1-Alkoxycarbonyl-3-bromoazetidin-2-ones as Potential Elastase Inhibitors

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Three 1-alkoxycarbonyl-3-bromoazetidin-2-ones have been prepared by reaction of (3*S*)-3-(*tert*-butoxycarbonyl)aminoazetidin-2-one with benzyl, trichloroethyl, and trifluoroethyl chloroformates followed by *t*Boc deprotection, diazotation of the exocyclic amino function and its substitution with potassium bromide. The 3-bromoazetidin-2-ones were obtained as racemic mixtures. Their hydroxide-catalyzed hydrolysis exclusively affords ring-opening products. Porcine pancreatic elastase (PPE) catalyzes the same reaction stereospecifically. Model building suggests that it is the (*R*) isomer that is enzymatically hydrolysed. The PPE-catalyzed hydrolysis is characterised by low k_{cat} and K_m values. Accordingly, these compounds behave as transient inhibitors of the enzyme.

Introduction

β-Lactam derivatives have been intensively investigated both to understand their chemical properties and because of their interest as inhibitors of enzymes. Analysis of their chemical reactivity has clarified their relationship with amides and shown that release of strain on ring opening does not lead, as initially expected, to a large increase in reactivity.[1] Bicyclic β-lactams derived from cephem or penam antibiotics and several β-lactam derivatives have been shown to efficiently inhibit the DD-peptidases involved in the crosslinking of the bacterial walls and sometimes also the β-lactamases used as a defense mechanism by bacteria. Synthetic β-lactam derivatives have also been developed to inhibit human leukocyte elastase, a serine protease whose uncontrolled degradative action on connective tissue has been implicated in several disease states (for reviews see refs.[2−4]).

Several *N*-activated monocyclic β-lactams act on elastase through a suicide inhibition mechanism:[5] The enzyme-catalyzed ring opening leads to the departure of substituents introduced in their structures and allows the unmasking of a reactive electrophilic function into the enzymatic cavity.[6] One family of inhibitors involves *N*-aryl-3-haloazetidin-2-ones featuring a latent quinoniminium methide function.[7−13] Another family is based on *N*-acylazetidin-2-ones bearing a good leaving group in the C-4 position (*O*-aryl); usually, the endocyclic nitrogen atom is part of a lipophilic urea function.[14−18] Processing of such β-lactams by the target enzyme creates a Schiff base that is susceptible to quenching by a nucleophilic residue of the active site. The reverse approach, i.e. to have a leaving group on N-1 and an electron-withdrawing group at the C-4 position, was not successfully developed.[4]

In this work, we investigate the hydroxide- and elastase-catalyzed hydrolysis of monocyclic β-lactams bearing an alkoxycarbonyl function on N-1, i.e. a substituent that could significantly increase the β-lactam carbonyl reactivity towards nucleophilic attack[19] while providing a potential leaving group. We address the following questions: Which of the β-lactam and urethane functions will be preferentially hydrolyzed? If ring opening occurs, will it lead to leaving-group expulsion? In the elastase active site, ring opening by nucleophilic attack of Ser-195 followed by expulsion of the leaving group could lead to a suicide inhibition by unmasking an electrophilic cumulene (e.g. an isocyanate function) susceptible to accept a nucleophile (His-57) from the enzyme (Scheme 1). Isoxazoline inhibitors of elastase fused to *N*-(alkoxysulfonyl)succinimide motifs have been postulated to follow such a mechanism in which the reactive isocyanate function results from a rapid Lossen rearrangement.[20]

To answer these questions, we selected the (3*R*)-1-alkoxycarbonyl-3-bromoazetidin-2-one family (Z = O; L = OR; X = Br). The choice of the C-3 substituent was based on the work of Wakselman's group,[13] who demonstrated that HLE was more efficiently inactivated with 3,3-dibromo- and (3*R*)-3-bromo-3-fluoroazetidinones rather than with 3,3-difluoro- and (3*S*)-3-bromo-3-fluoroazetidinones. Three potential leaving groups were considered; the benzyloxy-, trichloroethyloxy-, and trifluoroethyloxy groups.

Results

Syntheses

The (3*S*)-3-(*tert*-butoxycarbonyl)aminoazetidin-2-one **1** was prepared in five steps from L-serine.[21] Reaction of **1** with lithium hexamethyldisilylazide (LiHMDS)[22−23] in THF at −78°C followed by addition of chloroformates **2**

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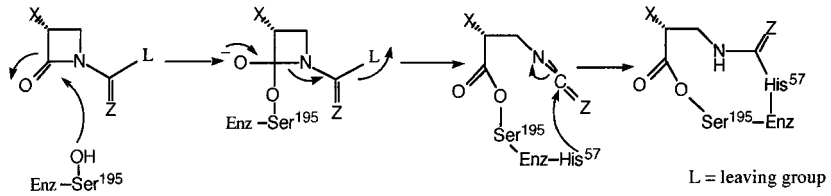


Scheme 1. Potential suicide mechanism of elastase inhibition

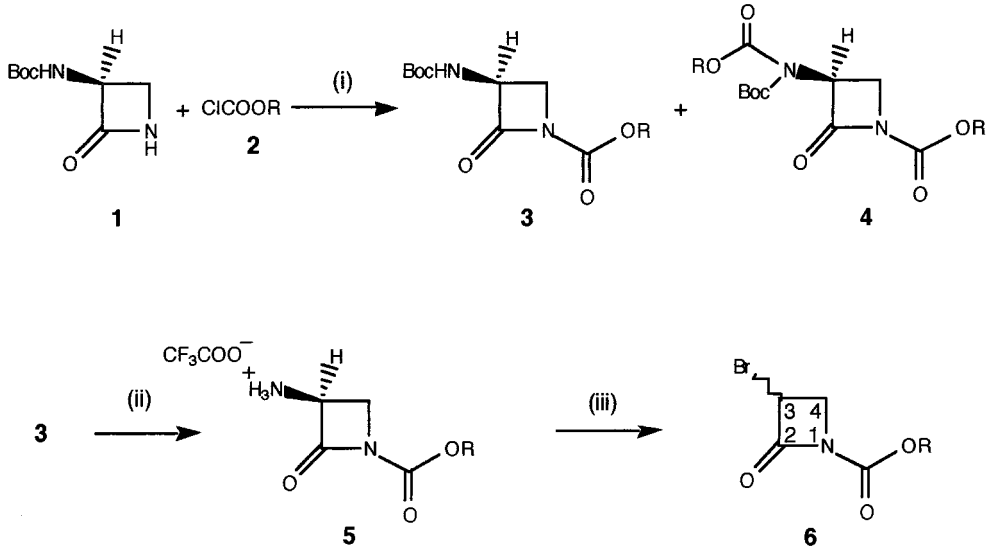
gave the *N*-alkoxycarbonyl derivatives **3**,[24−25] which could be contaminated by the double acylation products **4**. Compounds **3a** (R = CH_2Ph), **3b** (R = CH_2CCl_3) and **3c** (R = CH_2CF_3) were purified by chromatography on silica gel and characterized using standard spectroscopic techniques. Typical spectroscopic values are the IR carbonyl stretching near 1820 cm^{-1} for the activated azetidinone function, and the ^{1}H-NMR chemical shift near δ = 4.8 for 3-H. Treatment with trifluoroacetic acid furnished the amines **5**, which were directly treated with sodium nitrite and potassium bromide in aqueous sulfuric acid at 6°C.[26] As shown by GC analysis on a chiral capillary column, the 3-bromoazetidin-2-ones **6a−c** were obtained as racemic mixtures; racemization occurs presumably during the last step of the synthesis.

In the cases of 6-aminopenam[27] and 7-aminocephem antibiotics,[28] similar substitutions involving the corresponding 6- and 7-diazonium intermediates are stereoselective giving the (6*R*)-6- and (7*R*)-7-halo derivatives. This selectivity results from the steric control exercised by the (5*R*)-5- and (6*R*)-6-sulfur substituents. However, epimerization of the (6*R*)-6-bromopenicillanic acid in aqueous solution has been demonstrated to occur through an enolization process involving 6-H.[29] Accordingly, in the absence of induction due to a bulky C-3 substituent, the formation of racemic 1-alkoxycarbonyl-3-bromoazetidin-2-ones **6** is not surprising. The compounds **6a−c** were characterized in their ^{13}C-NMR spectra by signals at δ = 40 (C-3) and δ = 49 (C-4). In the ^{1}H-NMR spectra, the three cyclic protons gave a typical ABX pattern at δ = 3.7−3.8 (4-H), 4.1−4.2 (4′-H) and 4.8−4.9 (3-H).

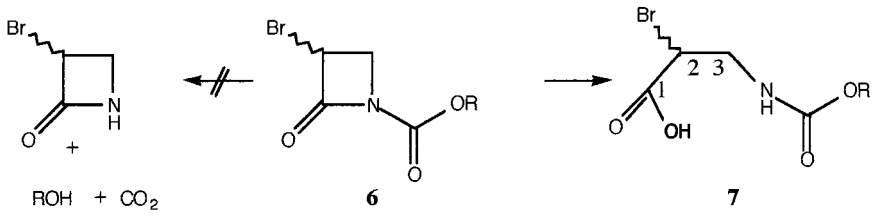
Chemical Hydrolysis of 6a−c

We have studied the chemical reactivity of compounds **6a−c** (Scheme 2) towards hydrolysis. The products of chemical hydrolysis were analyzed by NMR spectroscopy and mass spectrometry. Compounds **6a−c** were dissolved in D_2O (phosphate buffer pH = 7.5)/5% [D_6]DMSO and ^{1}H-NMR spectra were recorded as a function of time; after the disappearance of the starting material, the only products detected were **7a−c** resulting from β-lactam ring opening (Scheme 3); no cleavage of the urethane was observed. The hydrolysis products are characterized by an AB pattern at δ = 3.5−3.7 (3-H) and δ = 3.4−3.6 (3′-H), with a large geminal coupling constant *J* = 13−15 Hz. The methylene protons (3-H and 3′-H) split the resonance of the methine proton (2-H) to give a doublet of doublet at δ = 4.1−4.3. In the mass spectra of the crude mixture, peaks are observed at a molecular mass corresponding to the addition of water to the β-lactam ring.

The rates of hydrolysis have been monitored by UV spectrophotometry at 232 nm and the absorbance was found to



Scheme 2. Preparation of 1-alkoxycarbonyl-3-bromoazetidin-2-ones; reactions conditions: (i) LiHMDS, THF, −78°C, 30 min, then **2**, −78°C to 20°C, 1 h: **3a**: R = CH_2Ph, yield 89%, **3b**: R = CH_2CCl_3, yield 69%, **3c**: R = CH_2CF_3, yield 83%; (ii) CF_3COOH, 20°C, 30 min; (iii) KBr, 2.5 N H_2SO_4/EtOH, $NaNO_2$, 6−8°C, 3 h: **6a**: R = CH_2Ph, yield 80%, **6b**: R = CH_2CCl_3, yield 23%, **6c**: R = CH_2CF_3, yield 56%



Scheme 3. Hydrolysis product of 1-alkoxycarbonyl-3-bromoazetidin-2-ones

decrease by 40%. First-order rate constants (k_{obs}) have been measured at different pH values; buffer catalysis is negligible; plots of k_{obs} versus hydroxide ion concentration are linear, the following second-order rate constants are obtained: **6a**: $k_{OH^-} = (5.2 \pm 1.1) \times 10^2$ M^{-1}s^{-1}; **6c**: $k_{OH^-} = (1.0 \pm 0.2) \times 10^3$ M^{-1}s^{-1}. The rate constant increases when the pK_a of the alcohol constituent of the urethane function decreases: **6a**: pK_a = 15.2; **6c**: pK_a = 12.37.[30] These compounds appear to be less stable to chemical hydrolysis (ca. 80 times) than the 3-ethyl-4-*p*-carbophenoxy-substituted azetidinone described previously as a leukocyte elastase inhibitor.[31]

Elastase Inhibition and Enzymatic Hydrolysis

Compounds **6a−c** were first evaluated for their potential inhibitory effect on porcine pancreatic elastase (PPE). The rates of elastase-catalyzed hydrolysis of *N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide were measured ([E] = 2.10^{-7} M, [S] = 10^{-4} M, pH = 7.5) in the presence of various concentrations of **6a−c**. At [I] = 10^{-4} M, transient inhibitions were observed (percentage of inhibition: **6a**: 98.5%; **6b**: 97%; **6c**: 82%); at the relatively high enzyme concentration used, however, complete enzymatic activity was restored after 20 min. Plots of V/V_I, ratios of initial rates of hydrolysis in the absence and in the presence of inhibitor versus [I] gave the following inhibition constants: **6a**: $K_I = (1.5 \pm 0.1) \times 10^{-6}$ M; **6b**: $K_I = (3.0 \pm 0.36) \times 10^{-6}$ M; **6c**: $K_I = (2.4 \pm 0.4) \times 10^{-5}$ M.

The observation of a transient inhibition suggested that PPE catalyzes the hydrolysis of compounds **6a−c**, both the urethane and the β-lactam functions being potentially sensitive to enzymatic hydrolysis. ^{1}H-NMR analysis of the products of enzymatic hydrolysis of a $2{\cdot}10^{-2}$ M solution of **6a** in the presence of PPE ($6{\cdot}10^{-6}$ M) in D_2O/5% [D_6]DMSO showed that only ring opening occurs. When the course of the elastase-catalyzed hydrolysis of **6c** was monitored by UV spectrophotometry, a biphasic curve was observed (Figure 1): The rate of the fast reaction is sensitive to the enzyme concentration while the slow phase reflects the hydroxide-catalyzed reaction. Elastase catalyzes the hydrolysis of only one enantiomer of the racemic mixture of 3-bromoazetidin-2-one derivatives. This interpretation was confirmed by chiral GC analysis of the mixture during hydrolysis (Figure 1, insert). The following rate constants were extracted for the enzymatic reaction by curve fitting of the data: $k_{cat} = 6.2{\cdot}10^{-3}$ s^{-1} and $K_m = 1.1{\cdot}10^{-5}$ M. As for the fitting, the concentrations of the individual enantiomers are

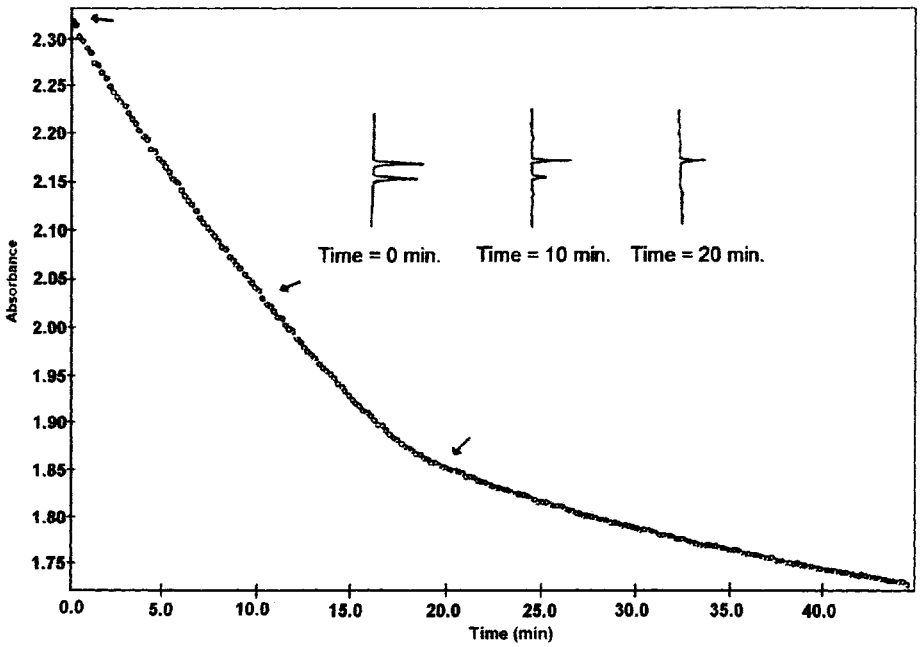


Figure 1. Elastase-catalyzed hydrolysis of **6c**; the absorbance of a $2{\cdot}10^{-4}$ M solution of **6c** was monitored in the presence of $1.9 \cdot 10^{-6}$ M PPE at 232 nm at pH = 7.67; inset: GC analysis on a Chrompack chiral-dex capillary column of the mixture during hydrolysis

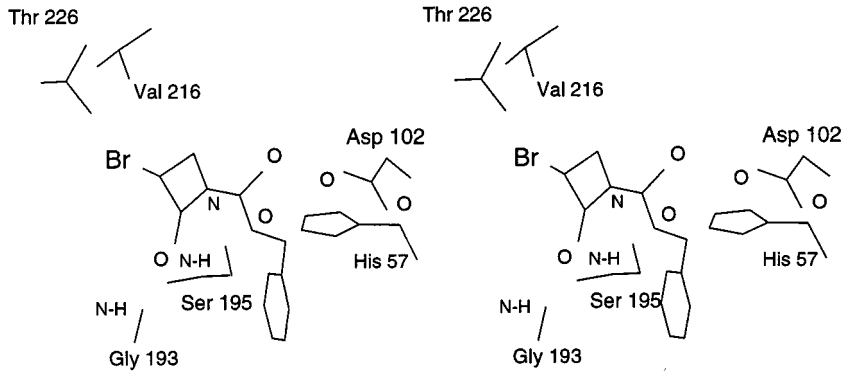


Figure 2. Stereodiagram representation of the docking of **6a** in the active site of PPE

used, the calculated K_m is the concentration of half saturation for the enantiomer whose hydrolysis is catalyzed by the enzyme. When the inhibition of anilide hydrolysis was measured, the K_I values were calculated on the basis of the sum of the (*R*) and (*S*) isomer concentrations. The ratio between K_I/K_m determined for **6c**, i.e. ca. 2, suggests that only one enantiomer is recognized by the enzyme. This hypothesis is confirmed by the fact that no inhibition of *p*-nitroanilide hydrolysis is observed when this substrate is added to solutions of **6a−c** in which the enzymatic hydrolysis of the recognized isomers was complete.

Docking of (*R*)- and (*S*)-**6a−c** in the PPE active site (3EST)[32] followed by optimization by molecular mechanics using AMBER[33] (Figure 2) showed that the (*R*) derivatives fit nicely with their bromine atom in the S-1 binding pocket and the β-lactam carbonyl group is suitably placed for nucleophilic attack by Ser-195 on the α face of the ring. The β-lactam oxygen atom is located in the oxy anion hole, the urethane carbonyl group is oriented towards His-57.

Discussion

Both chemical and enzymatic hydrolyses afford β-lactam ring-opening products without alkoxide expulsion. In the pH range investigated, the chemical hydrolysis is a hydroxide-catalyzed process with a negatively charged transition state. These conditions are particularly favorable for expulsion of an alkoxide leaving group before protonation of the nitrogen atom. As the hydrolysis product contains an intact urethane unit, the alkoxide expulsion appears to be too slow to generate an isocyanate function.

The 3-bromoazetidin-2-one derivatives described in this work block the active site of PPE with apparent inhibition constants in the micromolar range. The expected suicide inhibition was not observed, instead PPE was shown to catalyze the hydrolysis of these compounds. As this hydrolysis presumably occurs by an acyl-enzyme mechanism, the inhibitory effect is likely due to the fact that they act as slowly deacylating substrates,[34] characterized by low k_{cat} and K_m values. Suicide inhibition frequently arises from a minor side pathway; its efficiency is then measured from the ratio between suicide and turnover events. At the end of the hydrolysis, the enzymatic activity was fully restored within experimental error. This suggests that no cumulene function is generated within the active site during hydrolysis. A strategy to favor cumulene formation would be to incorporate less basic alkoxides in the urethane function but, unfortunately, the 3-bromoazetidin-2-one derivatives become then too ustable (results not shown).

When oriented in the PPE active site as described above, and as observed in the crystallographic structure of the complex formed between the PPE and a β-lactam inhibitor complex,[35] the (*R*) derivative **6a** fits easily in the enzyme cavity (Figure 2). The carbonyl oxygen atom of the β-lactam ring is in hydrogen-bonding interactions with the backbone NH groups of Ser-195 and Gly-193, which create the oxyanion hole. The esterified carboxylic group of the inhibitor is located about 4 Å from the His-57 imidazole ring. The presence of the ester group is important as it prevents an electrostatic interaction that would otherwise occur between the His side-chain and a free carboxylate group, thus perturbating the catalytic mechanism. The phenyl group of **6a** protrudes outside the active site. With the (*R*) derivative, the Br substituent is positioned in the S-1 cavity of the serine protease bordered by the side chains of Val-216 and Thr-226, which leave room for favorable interactions only with a small side chain like a Br atom. By contrast, with the (*S*) derivative the Br atom is oriented towards Cys-191 and the disulfide bridge it forms with Cys-220, this induces less favourable enzyme-ligand interactions. Replacement of the phenyl CH_2 group of **6a** by the more bulky CF_3 group in **6c** could lead to a weak steric hindrance with the C42−C58 S−S bridge or modify the electronic environment in the vicinity of the oxy anion hole (Gly-193 NH), but these effects would nevertheless be small.

Experimental Section

Porcine pancreatic elastase (type 1) and *N*-succinyl-L-alanyl-L-alanyl-L-alanyl-*p*-nitroanilide were purchased from Sigma Chemical Co. The other reagents were purchased from Acros chimica, Aldrich, and Fluka. Tetrahydrofuran (THF) was dried with sodium/benzophenone, then distilled. Dichloromethane and hexane were dried with phosphorous pentoxide and distilled. Ethyl acetate was distilled. − Column chromatography was carried out with silica gel 60 (70−230 mesh ASTM) supplied by Merck. Analytical thin-layer

chromatography was performed on silica gel 60 plates F254 (Merck, 0.2 mm thick), on which the compounds were detected with UV light, I_2 and ninhydrin. − The optical rotations (± 0.1°) were determined with a Perkin−Elmer 241 MC polarimeter. − The IR spectra were recorded with a Perkin−Elmer 1710 instrument (Fourier Transform Infra Red Spectrometer), only the most significant absorption bands being reported. − The ^{1}H- and ^{13}C-NMR spectra were obtained with a Bruker AM-500 spectrometer; chemical shifts are reported in ppm (δ) downfield from internal TMS. − The UV spectra were recorded with a Varian Cary 210 apparatus. − The mass spectra were obtained with a Finnigan MAT TSQ-70 instrument. The high resolution mass spectra were obtained at the University of Mons, Belgium (Prof. R. Flammang). − The microanalyses were performed at the University College of London, U.K. (Dr. A. Stones). − Melting points were determined with an Electrothermal microscope and are uncorrected. − The gas chromatography (GC) analyses were recorded using a Varian Aerograph 1400 (detector FID, nitrogen) equipped with a chrompack chiraldex (25 m × 0.25 mm) capillary column and connected to a Hitachi D-2000 integrator.

(3*S*)-1-Alkoxycarbonyl-3-(*tert*-butyloxycarbonyl)aminoazetidin-2-ones (3): To a solution of lithium bis(trimethylsilyl)amide (1 M solution in THF, 1 equiv.) in dry THF (2.5 mL/mmol), cooled at −78°C under argon, was added (3*S*)-3-(*tert*-butyloxycarbonyl)aminoazetidin-2-one (**1**) (1 equiv.), dissolved in THF (1 mL/mmol). The mixture was stirred for 30 min at −78°C, then chloroformate **2** (1 equiv.) was added with a syringe through a rubber stopper. Stirring was continued for 1 h at −78°C and a further 45 min at room temperature. After addition of water, the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine, dried with Na_2SO_4, and concentrated under vacuum. The crude product was purified by column chromatography on silica gel with CH_2Cl_2/EtOAc as eluent. If necessary, the product could be precipitated from hexane.

1-Benzyloxycarbonyl Compound 3a: This compound was obtained from 450 mg (2.4 mmol) of **1** by treatment with 2.4 mL (2.4 mmol) of 1 M LiHMDS solution and 0.337 mL (2.4 mmol) of benzyl chloroformate (**2a**), in 7 mL of THF. Yield: 687 mg (89%); CH_2Cl_2/EtOAc (60:40, v/v); R_F = 0.73; m.p. 148.1°C; $[\alpha]_D$ = −1.9 (*c* = 1 g/100 mL, CH_2Cl_2). − IR (KBr): $\tilde{\nu}$ = 3366, 2977, 1816, 1713 cm^{-1}. − ^{1}H NMR (500 MHz, [D$_6$]acetone): δ = 1.42 (s, 9 H), 3.71 (dd, *J* = 6.4 Hz and 3.73 Hz, 1 H), 3.91 (dd, *J* = 6.4 Hz and 5.8 Hz, 1 H), 4.85 (ddd, *J* = 7.9 Hz, 3.73 Hz and 5.8 Hz, 1 H), 5.25 (s, 2 H), 6.88 (d, *J* = 7.9 Hz, 1 H), 7.25−7.5 (m, 5 H). − ^{13}C NMR (125 MHz, [D$_6$]acetone): δ = 28.38, 46.77, 58.06, 68.05, 80.18, 128.92, 129.07, 129.3, 136.74, 149.86, 155.75, 165.8. − C16H20N2O5·H2O (338): calcd. C 56.80, H 5.92, N 8.28; found C 56.94, H 5.96, N 7.96. − HRMS (FAB$^+$); *m*/*z*: 321.14504 [M + 1] (calcd. for $C_{16}H_{21}N_2O_5$: 321.14283).

1-Trichloroethyloxycarbonyl Compound 3b: This compound was obtained from 500 mg (2.69 mmol) of **1** by treatment with 2.75 mL (2.7 mmol) of 1 M LiHMDS solution and 0.4 mL (2.7 mmol) of 2,2,2-trichloroethyl chloroformate (**2b**) in 8 mL of THF. Yield: 670 mg (69%); CH_2Cl_2/EtOAc (95:5, v/v); R_F = 0.84; m.p. 133.2−133.4°C; $[\alpha]_D$ = +4.7 (*c* = 1 g/100 mL, CH_2Cl_2). − IR (KBr): $\tilde{\nu}$ = 1822, 1711, 1330, 1155, 1022, 719 cm^{-1}. − ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.44 (s, 9 H), 3.86 (dd, *J* = 6.7 Hz and 4.5 Hz, 1 H), 3.98 (dd, *J* = 6.7 Hz and 6.7 Hz, 1 H), 4.76 (ddd, *J* = 6.7 Hz, 4.5 Hz and 6.4 Hz, 1 H), 4.82 (s, 2 H), 5.16 (d, *J* = 6.4 Hz, 1 H). − ^{13}C NMR (125 MHz, $CDCl_3$): δ = 28.09, 46.98, 57.80, 74.98, 81.19, 94.18, 147.25, 154.52, 164.07. − MS (FAB$^-$); *m*/*z*: 359, 361, 363, and 365 [M − 1]. − $C_{11}H_{15}Cl_3O_5N_2$ (361.6): calcd. C 36.53, H 4.18, N 7.74; found C 36.24, H 3.90, N 7.39.

1-Trifluoroethyloxycarbonyl Compound 3c: This compound was obtained from 1 g (5.37 mmol) of **1** by treatment with 5.4 mL (5.4 mmol) of 1 M solution of LiHMDS and 1.3 g (8 mmol, 1.5 equiv.) of 2,2,2-trifluoroethyl chloroformate (**2c**)[36] in 15 mL of THF. Yield: 1.394 g (83%); CH_2Cl_2/EtOAc (80:20, v/v); R_F = 0.63; m.p. 138.2−138.4°C; $[\alpha]_D$ = +6.5 (*c* = 1 g/100 mL, CH_2Cl_2). − IR (KBr): $\tilde{\nu}$ = 1818, 1747, 1735, 1522, 1172, 762 cm^{-1}. − ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 1.37 (s, 9 H), 3.57 (dd, *J* = 6.4 Hz and 3.95 Hz, 1 H), 3 82 (dd, *J* = 6.4 Hz and 6.7 Hz, 1 H), 4.75 (ddd, *J* = 6.7 Hz, 3.95 Hz and 8.22 Hz, 1 H), 4.88 (qAB, *J* = 8.85 Hz, 2 H), 7.66 (d, *J* = 8.22 Hz, 1 H). − ^{13}C NMR (125 MHz, $CDCl_3$): δ = 28.08, 46.78, 57.77, 61.59, 81.28, 122.33 (*C*F$_3$), 147.31, 154.43, 164.04. − MS (FAB$^+$); *m*/*z*: 313 [M + 1]. − $C_{11}H_{15}F_3N_2O_5$ (312): calcd. C 42.31, H 4.84, N 9.01; found C 42.34, H 4.61, N 8.85.

(3*S*)-1-Benzyloxycarbonyl-3-[(benzyloxycarbonyl)(*tert*-butyloxycarbonyl)amino]azetidin-2-one (4a): In the chromatographic purification of **3a**, the first fraction contained **4a**: yield < 10%; R_F = 0.94; m.p. 101.1°C. − IR (KBr): $\tilde{\nu}$ = 3040, 2977, 1817, 1729 cm^{-1}. − ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.41 (s, 9 H), 3.72 (dd, *J* = 6.6 Hz and 4.04 Hz, 1 H), 3.85 (dd, *J* = 6.6 Hz and 6.6 Hz, 1 H), 5.23 (s, 2 H), 5.24 (s, 2 H), 5.59 (dd, *J* = 6.6 Hz and 4.04 Hz, 1 H), 7.3−7.45 (m, 10 H). − ^{13}C NMR (125 MHz, $CDCl_3$): δ = 27.55, 45.14, 59.38, 68.06, 69.50, 85.16, 128.27, 128.53, 134.36, 134.84, 149.05, 149.95, 152.56, 162.93. − MS (FAB$^-$); *m*/*z*: 453 [M − 1] ($C_{24}H_{26}N_2O_7$).

(3*S*)-1-Alkoxycarbonyl-3-aminoazetidin-2-ones 5: Boc deprotection of **3a−c** was performed by dissolution in trifluoroacetic acid (2 mL/100 mg of **3**). After 30 min at 20°C, the solvent was evaporated under vacuum and the crude amine (as the trifluoroacetate salt) was precipitated from ether. Yield: 90−100%.

1-Alkoxycarbonyl-3-bromoazetidin-2-ones 6: To 2.5 N aqueous H_2SO_4 (1 mL), cooled at 5°C, were successively added, with stirring, 3-aminoazetidin-2-one (**5**) (0.15 mmol, 1 equiv.), KBr (5 equiv.), and ethanol (1 mL/0.3 mmol of **5**). A solution of $NaNO_2$ (1.5 equiv.) in water (1 mL/0.5 mmol) was added dropwise within 1 h, and the mixture was stirred at 6−8°C for a further 3−4 h. The mixture was extracted with $CHCl_3$ (several times) and the organic layers were washed with cold brine, dried with Na_2SO_4 and concentrated under vacuum. The product was purified by column chromatography on silica gel with CH_2Cl_2 as eluent.

1-Benzyloxycarbonyl Compound 6a: This compound was obtained from 164 mg (0.49 mmol) of **5a**, 300 mg (2.5 mmol) of KBr, and 54 mg (0.68 mmol) of $NaNO_2$. Yield: 112 mg (80%); R_F = 0.36; m.p. 46.5°C. − IR (KBr): $\tilde{\nu}$ = 1816, 1733, 1122, 954, 764 cm^{-1}. − ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.69 (dd, *J* = 7.82 Hz and 2.94 Hz, 1 H), 4.11 (dd, *J* = 7.82 Hz and 5.86 Hz, 1 H), 4.75 (dd, *J* = 5.86 Hz and 2.94 Hz, 1 H), 5.21 (s, 2 H), 7.35 (sharp m, 5 H). − ^{13}C NMR (125 MHz, $CDCl_3$): δ = 40.34, 48.86, 68.57, 128.37, 128.58, 128.66, 134.44, 148.56, 160.25. − MS (EI); *m*/*z*: 285 and 283 [M]. − HRMS (EI); *m*/*z*: 282.98416 [M] (calcd. for $C_{11}H_{10}BrNO_3$: 282.98440).

1-Trichloroethyloxycarbonyl Compound 6b: This compound was obtained from 345 mg (0.92 mmol) of **5b**, 568 mg (4.77 mmol) of KBr and 100 mg (1.43 mmol) of $NaNO_2$. Yield: 70 mg (23%); R_F = 0.9; m.p. 70.3−70.6°C. − IR (KBr): $\tilde{\nu}$ = 1802, 1741, 1339, 1127, 814, 773 cm^{-1}. − ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.82 (dd, *J* = 7.96 Hz and 3.06 Hz, 1 H), 4.25 (dd, *J* = 7.96 Hz and 6.01 Hz, 1 H), 4.81 (s, 2 H), 4.88 (dd, *J* = 6.01 Hz and 3.06 Hz, 1 H). − ^{13}C NMR (125 MHz, $CDCl_3$): δ = 40.55, 49.19, 75.08, 93.85 (*C*Cl$_3$), 146.75, 160.12. − MS (FAB$^+$); *m*/*z*: 332, 330, 328, 326, and 324 [M

+ 1]. − $C_6H_5BrCl_3NO_3$ (325.5): calcd. C 22.15, H 1.55, N 4.30; found C 22.50, H 1.51, N 4.28.

1-Trifluoroethoxycarbonyl Compound 6c: This compound was obtained from 98 mg (0.3 mmol) of **5c**, 186 mg (1.5 mmol) of KBr and 33.5 mg (0.45 mmol) of $NaNO_2$. Yield: 49 mg (55.9%); R_F = 0.9; oil. − IR (film): $\tilde{\nu}$ = 1830, 1751, 1350, 1126, 839, 759 cm^{-1}. − ^1H NMR (500 MHz, $CDCl_3$): δ = 3.78 (dd, *J* = 7.97 Hz and 3.05 Hz, 1 H), 4.21 (dd, *J* = 7.97 Hz and 5.97 Hz, 1 H), 4.56 (sharp q, AB, *J* = 8.02 Hz, 2 H), 4.85 (dd, *J* = 5.97 Hz and 3.05 Hz, 1 H). − ^{13}C NMR (125 MHz, $CDCl_3$): δ = 40.50, 49.16, 61.89, 122.16 (*C*F_3), 145.87, 159.96. − MS (FAB^+); *m/z*: 276 and 278 [M + 1]. − $C_6H_5BrF_3NO_3$ (276): calcd. C 26.11, H 1.82, N 5.07; found C 26.19, H 2.15, N 4.85.

Chemical Hydrolysis: For product analyses, $2\cdot10^{-2}$ M solutions of **6a−c** in [D_6]DMSO were added to a phosphate buffer in D_2O (pH = 7.5; final concentration of 10^{-3} M); ^1H-NMR spectra (500 MHz) were recorded every 30 min (the hydrolysis of **6a** was completed within 1.5 h).

3-[(Benzyloxycarbonyl)amino]-2-bromopropionic Acid (7a): δ = 3.38 (dd, *J* = 7.3 Hz and 14.6 Hz, 1 H), 3.47 (dd, *J* = 6.1 Hz and 14.6 Hz, 1 H), 4.12 (dd, *J* = 6.1 Hz and 7.3 Hz, 1 H).

2-Bromo-3-[(trichloroethoxycarbonyl)amino]propionic Acid (7b): δ = 3.59 (dd, *J* = 6.4 Hz and 13.7 Hz, 1 H), 3.69 (dd, *J* = 6.4 Hz and 13.7 Hz, 1 H), 4.30 (t, *J* = 6.4 Hz, 1 H).

2-Bromo-3-[(trifluoroethoxycarbonyl)amino]propionic Acid (7c): δ = 3.58 (dd, *J* = 6.4 Hz and 13.9 Hz, 1 H), 3.68 (dd, *J* = 6.4 Hz and 13.9 Hz, 1 H), 4.30 (t, *J* = 6.4 Hz, 1 H).

For kinetic analyses, spectra of $1\cdot10^{-3}$ M solutions of **6a−c** in 0.025 M phosphate or borate buffers (pH = 7.2−8.75) containing 5% DMSO were recorded between 220 and 400 nm. The disappearance of the 234-nm band associated with the β-lactam was used to obtain first-order rate constants.

Assay of Elastase: Solutions of **6a−c** in *N*-methylpyrrolidone were diluted twenty-fold into a solution of elastase in 100 mM TRIS buffer at pH = 7.5. Samples (3 μL) from these incubation mixtures were mixed with 300 μL of 0.3 mM *N*-succinyl-L-alanyl-L-alanyl-L-alanyl-*p*-nitroanilide (K_m = 2.4 mM[37]). The appearance of *p*-nitroaniline was observed by recording with a Cary 210 the absorbance change at 410 nm (Δε = 8480 $M^{-1}cm^{-1}$) for 10 min.

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