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A Practical Synthesis of (*S*) 3-*tert*-Butoxycarbonylamino-2-oxo-2,3,4,5-tetrahydro-1,5-benzodiazepine-1-acetic Acid Methyl Ester as a Conformationally Restricted Dipeptido-Mimetic for Caspase-1 (ICE) Inhibitors

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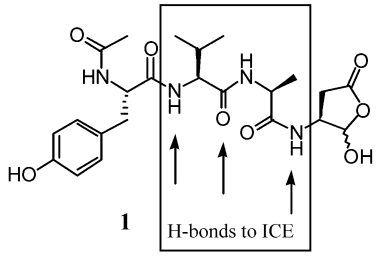
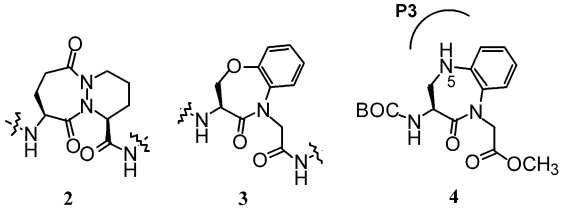
Abstract—A simple and versatile method for the synthesis of (*S*) 3-*tert*-butoxycarbonylamino-2-oxo-2,3,4,5-tetrahydro-1,5-benzodiazepine-1-acetic acid methyl ester (**4**), a dipeptide mimetic, has been developed. The regioselective functionalization of the N1 and N5 ring nitrogens and the C3 amino group is demonstrated in the synthesis of an interleukin-1β converting enzyme inhibitor **13**.

The development of small molecule inhibitors of caspases as therapeutics has been a target of intense research. Interleukin-1β converting enzyme (ICE, caspase-1) a member of the caspase[1] family activates interleukin-1β (IL-1β) and IL-18, important mediators of inflammation and infectious diseases.[2] The tetra-peptide **1**,[3] a potent ICE inhibitor, blocks the release of mature IL-1β from human whole blood stimulated with heat-killed *Staphylococcus aureus* with an IC_{50} of 4 μM.[4] Recently, we reported that prophylactic intravenous (iv) treatment with peptide-based irreversible ICE inhibitors in a collagen-induced mouse model of arthritis significantly delayed the onset of inflammation and reduced its severity.[5] Compound **1** was found to be poorly suited for therapeutic uses, as is the case with many peptide derived agents. We and others have directed our efforts towards overcoming these shortcomings.[6–11] For instance, pyridazinodiazepine ICE inhibitors (**2**) show good oral bioavailability in rodents and dogs[7,11] and were reported to be efficacious when orally dosed in a collagen-induced arthritis model in mice.[11] An ICE inhibitor has been reported containing a benzoxazepine scaffold (**3**).[7] In this paper, we report the synthesis of (*S*) 3-*tert*-butoxycarbonylamino-2-oxo-2,3,4,5-tetrahydro-1,5-benzodiazepine-1-acetic acid methyl ester (**4**), a novel dipeptide mimetic of the Val-Ala moiety in the tetra-peptide ICE inhibitor **1**. The key design criteria of **4** were: (1) preservation of the key interactions of the inhibitor **1** with ICE, particularly the hydrogen bonding pattern observed in the crystal structure of ICE/**1**,[12] (2) ease of synthesis of the optically active scaffold, and (3) synthetic versatility of the scaffold, specifically for access to P3 of the ICE inhibitor binding site via the N5 ring nitrogen of the benzodiazepine ring.



1



2 **3** **4**

The syntheses of related enantiomerically pure benzazepinones,[13] 1,5-benzoxazepinones,[14] and 1,5-benzothiazepinones[15] have been reported and utilized as dipeptide mimetics.[16] However, reported syntheses of enantiomerically pure 3-amino-1, 5-benzodiazepinones are

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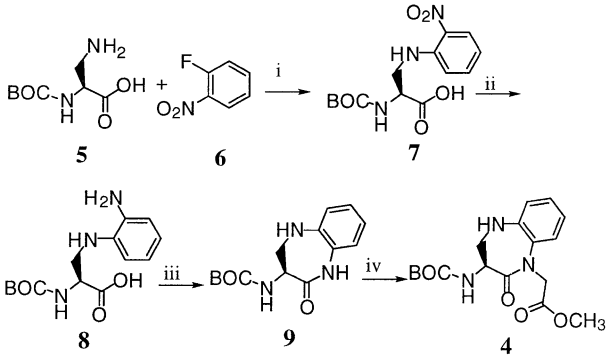
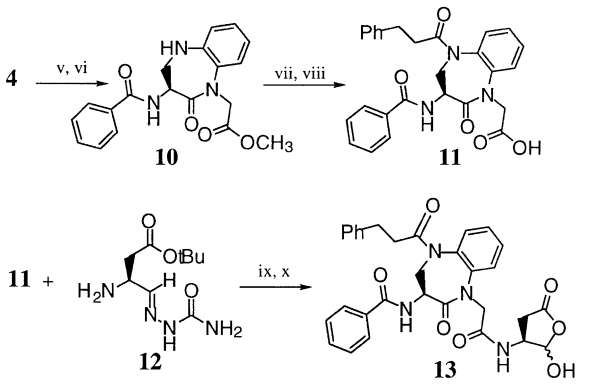
impractical and lengthy and require a late stage resolution.[17] Herein, we describe a short, high yielding, and versatile synthesis **4** from BOC-(L)-2,3-diaminopropionic acid (Scheme 1). The utility of **4** as a versatile dipeptidomimetic is demonstrated in the synthesis of **13**, a potent ICE inhibitor (Scheme 2).

The aromatic nucleophilic substitution reaction of 1-fluoro-2-nitrobenzene (**6**) and BOC-(L)-2,3-diaminoproprionic acid (**5**) in the presence of $NaHCO_3$ in DMF afforded product **7**.[18] Reduction of the nitro group by catalytic hydrogenolysis followed by water-soluble carbodiimide mediated cyclization gave **9** in good yield.[19] Regioselective alkylation of the lactam nitrogen in the presence of bis(trimethylsilyl)amide afforded the desired intermediate **4**.[20]

Deprotection of the *N*-*tert*-butoxycarbonyl group was achieved via anhydrous HCl to afford the amine hydrochloride salt that was coupled with benzoic acid to afford the *N*-benzoyl derivative **10**. Acylation with 3-phenylpropionyl chloride followed by base hydrolysis of the methyl ester gave the acetic acid derivative **11.** Reaction of semicarbazone **12** with the acid **11** followed by deprotection[21] afforded the desired aldehyde **13** (Scheme 2). Proton NMR of the penultimate hydrazone indicated the presence of only one diastereomer and confirmed by the synthesis of the *R* isomer of **4** from BOC-(*R*)-2,3-diaminopropionic acid. The K_i's of **13** and its C(3) amino group diastereomer are 90 and 850 nM, respectively.[22]



Scheme 1. Reagents and conditions: (i) $NaHCO_3$, DMF, 70 °C, 83%; (ii) 10% Pd/C, H_2 (1 atm.); (iii) EDC, DMF, 5–20 °C, 71% for steps (ii) and (iii); (iv) LHMDS (1.1 equiv), $BrCH_2CO_2CH_3$, THF, 90%.



Scheme 2. Reagents and conditions: (v) HCl (g), EtOAc; (vi) benzoic acid, HOBT, EDC, 65% for steps (v) and (vi); (vii) 3-phenylpropionyl chloride, Et_3N, CH_2Cl_2, 83%; (viii) 2 N NaOH, quant; (ix) HOBT, EDC; (x) (a) 25% TFA, CH_2Cl_2; (b) MeOH/AcOH/37% aq formaldehyde (5:1:1), 20 °C.

In conclusion, we have described an efficient four-step synthesis of (*S*) 3-*tert*-butoxycarbonylamino-2-oxo-2,3,4,5-tetrahydro-1,5-benzodiazepine-1-acetic acid methyl ester (**4**), from BOC-(L)-2,3-diaminopropionic acid (**5**) in >50% overall yield. We have demonstrated the regioselective functionalization of N(1), N(5), and the C(3) nitrogens and utilized this dipeptide mimetic in **13**, a potent ICE inhibitor. The advantage of scaffold **4** over previously reported scaffolds is that it allows for the broad exploration of the P3, thus providing an opportunity to improve potency and physical properties of compounds in this class.

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18. **(2*S*)-2-*tert*-Butoxycarbonylamino-3-(2-nitrophenyl-amino)-propionic acid** (**7**). (2*S*)-2-*tert*-Butoxycarbonylamino-3-amino-propionic acid (**5**; 10 g, 49 mmol), 2-fluoronitrobenzene (**6**; 5.7 mL, 54 mmol), and $NaHCO_3$ (8.25 g, 98 mmol) were stirred in DMF (100 mL) at 70 °C under a N_2 atmosphere. After 18 h, the reaction was subjected to aqueous workup to give an orange amorphous solid (yield 12.64 g, 83%) which was used without further purification: ^{1}H NMR (CD_3OD) δ 8.15–8.10 (1H, d), 7.54–7.48 (1H, t), 7.13–7.08 (1H, d), 6.73–6.65 (1H, t), 4.45–4.35 (1H, m), 3.9–3.8 (1H, dd), 3.65–3.55 (1H, dd), 1.45 (9H, s).
19. **(2*S*)-2-*tert*-Butoxycarbonylamino-3-(2-aminophenyl-amino) propionic acid** (**8**). (2*S*)-2-*tert*-Butoxycarbonylamino-3-(2-nitrophenyl-amino)propionic acid (**7**; 12.65 g) and palladium on carbon (10% by weight) was taken into 100 mL of methanol and stirred under hydrogen (1 atm) for 4 h. The catalyst was removed by filtration through Celite and the solvent evaporated in vacuo to afford 11.95 g of the product as a brown solid that was used without further purification: ^{1}H NMR (CD_3OD) δ 6.75–6.70 (3H, m), 6.65–6.58 (1H, m), 4.35–4.30 (1H, m), 3.6–3.38 (2H, m), 1.45 (9H, s).
(3*S*)-3-*tert*-Butoxycarbonylamino-2-oxo-2,3,4,5-tetrahydro-1*H*-1,5-benzo-diazepine (**9**). 1-(3-Dimethyl-aminopropyl)-3-ethyl-carbodiimide hydrochloride (8.45 g, 44.5 mmol) was added to a cooled (0 °C) solution of (2*S*)-2-*tert*-butoxycarbonylamino-3-(2-aminophenylamino)-propionic acid (**8**; 11.95 g, 40.5 mmol) in 100 mL of DMF and stirred for 18 h. Aqueous workup and purification by flash chromatography (SiO_2, 30% ethyl acetate/hexane eluant) gave 8 g (71%) of the product: ^{1}H NMR ($CDCl_3$) δ 7.78 (1H, s), 7.02–6.95 (1H, m), 6.88–6.82 (1H, m), 6.82–6.78 (1H, m), 6.75–6.70 (1H, m), 5.8–5.7 (1H, d), 4.55–4.45 (1H, m), 3.95 (1H, s), 3.9–3.82 (1H, m), 3.48–3.40 (1H, m), 1.45 (9H, s).
20. **(*S*)-(3-*tert*-Butoxycarbonylamino-2-oxo-2,3,4,5-tetrahydro-1,5-benzodiazepine-1-acetic acid methyl ester** (**4**). A 1.0 M solution of lithium bis(trimethylsilyl)amide (3.4 mL, 3.4 mmol) in THF was added dropwise to a −78 °C solution of **9** (0.94 g, 3.38 mmol) in 20 mL of anhyd THF and stirred for 30 min. Methyl bromoacetate (0.44 mL, 4 mmol) was added dropwise to the reaction mixture then warmed to room temperature. The reaction was diluted with 100 mL of ethyl acetate and washed with 0.3 N $KHSO_4$ (50 mL), water (2×50 mL), and brine. The combined organic layers were dried over anhyd Na_2SO_4, filtered, and evaporated in vacuo to afford a gum that was purified by flash chromatography (SiO_2) eluting with 3:7 EtOAc/Hex to afford 0.98 g (83%) desired product: ^{1}H NMR (CD_3OD) δ 7.15–7.07 (2H, m), 6.98–6.94 (1H, m), 6.88–6.84 (1H, d), 5.62–5.55 (1H, d), 4.71–4.65 (1H, d), 4.65–4.6 (1H, m), 4.33–4.27 (1H, d), 3.96–3.90 (1H, m), 3.78 (3H, s), 3.44–3.37 (1H, m), 1.4 (9H, s).
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