Azasulfonamidopeptides as Peptide Bond Hydrolysis Transition State Analogues. Part 2.[1] Potential HIV-1 Proteinase Inhibitor

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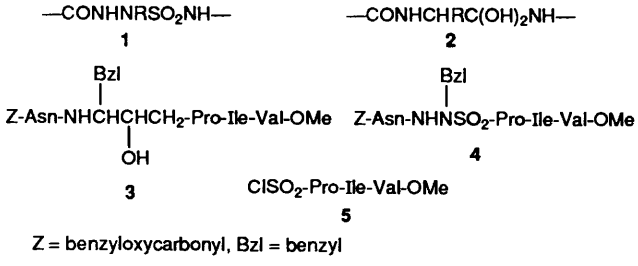
The synthesis of [*N*-benzyl-*N'*-(*N*$^{\alpha}$-benzyloxycarbonyl-L-asparaginyl)hydrazino]sulfonyl-L-prolyl-L-isoleucyl-L-valine methyl ester **4**, a potential HIV-1 proteinase inhibitor, is described.

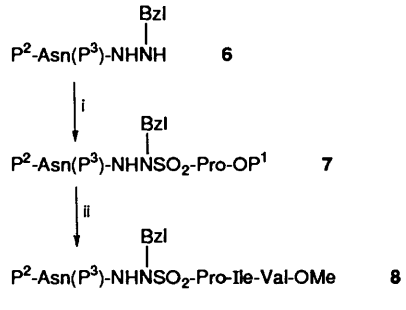
We recently defined feasible synthetic approaches to azasulfonamidopeptides **1**,[1] which are a novel class of analogues for the transition states (approximated as **2**) of peptide bond hydrolysis.

The replication of the human immunodeficiency virus (HIV) depends, *inter alia*, on a key proteinase, which may be its Achilles' heel. Much current effort is therefore being devoted to the design of inhibitors for this enzyme.[2] In particular, several groups have focussed on the scissile Phe–Pro bond in substrate sequences incorporating -Asn-Phe-Pro-Ile-Val-, replacing the Phe-Pro unit by a corresponding transition-state isostere and then working towards potent inhibitors by systematic variation of the rest of the assembly.[2–8] Modest inhibitory activity is shown by compound **3**,[7,8] for example, and from such a starting point the promising antiviral Ro 31-8959 was developed.[8] It therefore seemed worthwhile to synthesize the azasulfonamidopeptide **4** as a possible new lead compound.



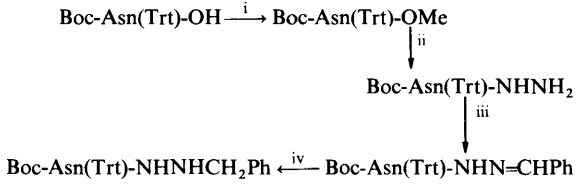
Results and Discussion

The first strategy to be explored for the synthesis of compound **4** was one in which the azasulfonamido unit would have been constructed at a late stage by reaction of a suitably protected asparagine hydrazide with the chlorosulfonyltripeptide ester **5**, but all attempts to prepare the sulfonyl chloride **5** from the corresponding tripeptide ester gave complex and intractable mixtures.



Scheme 1 *Reagents and conditions:* i, $ClSO_2$-Pro-OP^1; ii, remove P^1, activate, and couple with Ile-Val-OMe. *N.B.*: Groups P^1, P^2 and P^3 are protecting groups, not phosphate moieties.

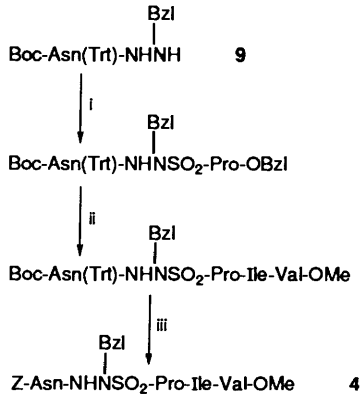
We therefore turned to an alternative in which backbone assembly would involve addition of the C-terminal dipeptide last, *i.e.* from compound **6** through dipeptide **7** to tetrapeptide **8**, Scheme 1. The choice of protecting group P^1 = Me was precluded by adverse experience[1] with the saponification of related models, and led to the choice P^1 = Bzl; the consequent need to hydrogenolyse the protecting group in turn prevented a direct approach with the desired benzyloxycarbonyl (Z) group of our target molecule **4** present *ab initio*, and imposed the choice P^2 = *tert*-butoxycarbonyl (Boc). Recorded experience with the preparation of hydrazides from asparagine derivatives is very sparse, and the implication was that side reactions would be problematic with an unprotected side-chain, so the choice P^3 = trityl (Trt) was made. The key intermediate **9** was prepared as outlined in Scheme 2. Several procedures were investigated for the final reduction, but only that of Calabretta, Gallina and Giordano[9] was successful, and gave compound **9** in good yield, albeit slightly contaminated with the hydrazone precursor **10**. It is noteworthy that, in our hands, the work-up following reduction required extended treatment with alkali to complete the decomposition of the complex cyanoborane adduct(s) formed.



Scheme 2 *Reagents:* i, CH_2N_2; ii, NH_2NH_2; iii, PhCHO; iv, $NaBH_3CN$. Boc = *tert*-butoxycarbonyl; Trt = trityl

The elaboration of the hydrazine **9** into the target molecule **4** was accomplished as indicated in Scheme 3. In the last stage, attempts to remove both protecting groups simultaneously by treatment with trifluoroacetic acid (TFA) failed, the *N*-trityl group being left largely undisturbed even after an hour and a half at room temperature. Friede *et al.*[10] have also found that N-terminal *N*$^{\omega}$-tritylasparagine is unusually resistant to acidolytic deprotection, ascribing this to the retarding effect of the adjacent protonated α-amino group. This interpretation is supported by our experience, since treatment with TFA after replacement of the *N*$^{\alpha}$-*tert*-butoxycarbonyl (Boc) group with an *N*$^{\alpha}$-benzyloxycarbonyl (Z) group resulted in smooth detritylation.

Peptide **4** inhibited the activity of recombinant HIV-1 proteinase in a peptide-cleavage assay. The equilibrium constant for inhibition (K_i) of HIV-1 proteinase by peptide **4**



Scheme 3 *Reagents:* i, $ClSO_2$-Pro-OBzl; ii, H_2/Pd(C); then Ile-Val-OMe (from hydrogenolysis of Z-Ile-Val-OMe), DCCI–HOBt; iii, HCl–MeOH; then ZONSu; then TFA

was calculated to be 27.1 ± 7.7 μmol dm^{-3}. In comparison, Ro 31-8959 was shown to have a K_i-value of 11.1 ± 3.8 pmol dm^{-3} in parallel assays.

Experimental

Instrumental and general experimental details are as before.[1] NMR data are given only for compound **4**, but NMR spectra in full accord with the structures stated were recorded on all the compounds described, which, with the exception of the intermediate **9**, were all chromatographically pure in appropriate TLC systems.

N$^{\alpha}$-tert-*Butoxycarbonyl*-N$^{\omega}$-*triphenylmethyl*-L-*asparagine Hydrazide*.—A solution of *N*$^{\alpha}$-*tert*-butoxycarbonyl-*N*$^{\omega}$-triphenylmethyl-L-asparagine[11] (5.00 g, 10.5 mmol) in methanol (50 cm^3) was titrated to a residual yellow colour with a freshly prepared solution of diazomethane in diethyl ether. Excess of diazomethane was destroyed by dropwise addition of acetic acid. The ether was evaporated off, to give a solid. A solution of this crude methyl ester and hydrazine hydrate (2.5 cm^3, 50 mmol) in methanol (60 cm^3) was heated at 50 °C for 19 h. The solution was cooled and concentrated to ~30 cm^3. The precipitate was separated by filtration, washed successively with methanol (5 cm^3) and copious amounts of water, and dried *in vacuo* over phosphorus pentaoxide to give the *title hydrazide* (3.34 g, 65%) as a solid, m.p. 232–233 °C; $[\alpha]_D^{20}$ −17.1 (*c* 0.55, AcOH) (Found: C, 69.0; H, 6.55; N, 11.5. $C_{28}H_{32}N_4O_4$ requires C, 68.8; H, 6.6; N, 11.5%). In a separate preparation, the intermediate N$^{\alpha}$-tert-*butoxycarbonyl*-N$^{\omega}$-*triphenylmethyl*-L-*asparagine methyl ester* was isolated by dissolution of the crude material in the minimum amount of methanol and allowing the solution to evaporate slowly at ambient temperature to a small volume over a period of several days. The precipitate was collected, and dried *in vacuo* over P_2O_5 to give the *methyl ester* as a solid, m.p. 187–188 °C; $[\alpha]_D^{20}$ − 17.4 (*c* 0.54, MeOH) (Found: C, 71.1; H, 6.65; N, 5.65. $C_{29}H_{32}N_2O_5$ requires C, 71.3; H, 6.6; N, 5.7%).

Benzaldehyde N$^{\alpha}$-tert-*Butoxycarbonyl*-N$^{\omega}$-*triphenylmethyl*-L-*asparaginylhydrazone* **10**.—A solution of the above hydrazide (3.13 g, 6.4 mmol) and benzaldehyde (1.0 cm^3, 9.85 mmol) in methanol (50 cm^3) was heated at 50 °C for 12 h. After cooling of the mixture, the precipitate was separated by filtration, washed successively with methanol (1 cm^3) and diethyl ether (5 cm^3) and dried *in vacuo* to give the *title hydrazone* (0.55 g, 15%) as a solid. The mother liquor was evaporated under reduced pressure to give a solid, which was suspended in diethyl ether (150 cm^3). The suspension was stirred at ambient temperature for 2 h. The solution was filtered, and the solid was washed with copious amounts of diethyl ether and dried to give a further crop (2.30 g, 62%) of the *title hydrazone*. An analytical sample was prepared by allowing a solution in methanol to evaporate slowly at ambient temperature. The solid which separated was collected before evaporation was complete: m.p. 178–180 °C, $[\alpha]_D^{22}$ −7.1 (*c* 0.52, MeOH) (Found: C, 72.7; H, 6.3; N, 9.8. $C_{35}H_{36}N_4O_4$ requires C, 72.9; H, 6.3; N, 9.7%).

N-*Benzyl*-N′-(N$^{\alpha}$-tert-*Butoxycarbonyl*-N$^{\omega}$-*triphenylmethyl* L-*asparaginyl*)*hydrazine* **9**.—The procedure used was based on that reported by Calabretta *et al.*[9] A solution of toluene-*p*-sulfonic acid (PTSA) (1.00 g, 5.2 mmol) in dry tetrahydrofuran (THF) (8 cm^3) was added to a solution of sodium cyanoborohydride (0.38 g, 6.0 mmol),† the above hydrazone (2.68 g, 4.64 mmol) and Bromocresol Green indicator (~2 mg) in dry THF (8 cm^3) under nitrogen. The addition was controlled by indicator toning (orange to green). Care was taken to avoid an excess of acid. The addition took 15 h. The reaction was then quenched by addition of 1 mol dm^{-3} aq. sodium hydroxide (35 cm^3). The mixture was stirred for a further 2 h at ambient temperature, and was then extracted with ethyl acetate (2 × 50 cm^3). The organic extracts were combined washed successively with 5% aq. sodium hydrogen carbonate (3 × 40 cm^3) and water (2 × 20 cm^3), then was dried, and evaporated under reduced pressure to give the title *N*-benzylhydrazide in crude form (2.65 g) as a solid. All preparations were contaminated with a small amount (~5%) of the hydrazone which could not be removed by chromatography or reprecipitation [Found: *m*/*z*, FAB$^+$, 579 (<1%, MH$^+$). $C_{35}H_{38}N_4O_4$ requires M, 578].

[N-*Benzyl*-N′-(N$^{\alpha}$-tert-*Butoxycarbonyl*-N$^{\omega}$-*triphenylmethyl*-L-*asparaginyl*)*hydrazino*]*sulfonyl*-L-*proline benzyl Ester*.—A solution of the above crude *N*-benzylhydrazide (2.60 g, 4.5 mmol), *N*-chlorosulfonyl-L-proline benzyl ester (1.38 g, 4.5 mmol) and triethylamine (0.65 cm^3, 4.7 mmol) in dry dichloromethane (15 cm^3) was heated under reflux for 66 h. The solvent was removed under reduced pressure, and ethyl acetate (50 cm^3) was added. The mixture was washed successively with 0.5 mol dm^{-3} aq. citric acid (3 × 25 cm^3) and water (3 × 15 cm^3), dried, and evaporated under reduced pressure to give a foam. Flash chromatography (silica, dry loaded) with elution with (7:3) diethyl ether–light petroleum and combining of appropriate fractions gave chromatographically pure *title azasulfonamidopeptide* as a foam (2.11 g, 55%). For analysis, a portion was taken into ethanol and stored overnight at ambient temperature: the solid which separated was collected, and dried *in vacuo* over phosphorus pentaoxide to give a solid, m.p. 180–182 °C; $[\alpha]_D^{20}$ −44.2 (*c* 0.51, MeOH) [Found: C, 67.0; H, 6.1; N, 8.3; S, 3.5%, *m*/*z*, FAB$^+$, 868 (100%, MNa$^+$) and 846 (3%, MH$^+$). $C_{47}H_{51}N_5O_8S$ requires C, 66.7; H, 6.1; N, 8.3; S, 3.8%; M, 845].

[N-*Benzyl*-N′-(N$^{\alpha}$-tert-*Butoxycarbonyl*-N$^{\omega}$-*triphenylmethyl*-L-*asparaginyl*)*hydrazino*]*sulfonyl*-L-*prolyl*-L-*isoleucyl*-L-*valine Methyl Ester*.—A saturated solution of hydrogen chloride in methanol (1 cm^3) was added to a solution of benzyloxycarbonyl-L-isoleucyl-L-valine methyl ester[12] (0.189 g, 0.5 mmol) in methanol (5 cm^3). The mixture was stirred with 10% palladium on charcoal (0.02 g) in hydrogen for 30 min, filtered through Celite, and evaporated under reduced pressure to give the crude dipeptide ester hydrochloride as a solid. A solution of the immediately preceding benzyl ester (0.421 g, 0.50 mmol) in a mixture of dimethylformamide (DMF) (10.5 cm^3) and ethanol (3.5 cm^3) was stirred with 10% palladium on charcoal (0.04 g) in

† The *weight* ration of sodium cyanoborohydride to PTSA used was the same as that used by Calabretta *et al.*, although this is not the same as the *mole* ration stated by them.

hydrogen for 1 h. The solution was filtered through Celite and diluted with ethyl acetate (50 cm^3). The mixture was washed successively with 0.5 mol dm^{-3} aq. citric acid (3 × 15 cm^3), water (3 × 20 cm^3) and brine (10 cm^3). Drying and evaporation under reduced pressure gave the crude acid as an oil. Dicyclohexylcarbodiimide (DCCI) (0.112 g, 0.54 mmol) was added to a mixture of the crude acid and the crude dipeptide ester hydrochloride with triethylamine (~0.1 cm^3) and 1-hydroxybenzotriazole (HOBt) (0.082 g, 0.53 mmol) in DMF (8 cm^3). The solution was stirred overnight at ambient temperature, filtered, diluted with ethyl acetate (30 cm^3), and washed successively with 0.5 mol dm^{-3} aq. citric acid (3 × 20 cm^3), 5% aq. sodium hydrogen carbonate (2 × 20 cm^3) and brine (2 × 20 cm^3). The solution was dried and evaporated to give a light brown foam. Flash chromatography (silica, dry loaded) with elution with a gradient from diethyl ether–light petroleum (3:2 to 100% Et_2O) gave the title azasulfonamidopeptide derivative (0.284 g, 58%) as a foam [Found: *m/z*, FAB^+, 1004 (100%, MNa^+). $C_{52}H_{67}N_7O_{10}S$ requires M, 981].

[N-*Benzyl*-N′-(N$^{\alpha}$-*benzyloxycarbonyl*-N$^{\omega}$-*triphenylmethyl*-L-*asparaginyl*)*hydrazino*]*sulfonyl*-L-*prolyl*-L-*isoleucyl*-L-*valine Methyl Ester*.—A saturated solution of hydrogen chloride in methanol (1 cm^3) was added to a stirred solution of the above *tert*-butoxycarbonyl derivative (0.183 g, 0.19 mmol) in methanol (1 cm^3). The solution was stirred at ambient temperature for 1 h. The methanol was removed under reduced pressure, and fresh methanol was added and evaporated (3 × 5 cm^3) to give an oil. *N*-(Benzyloxycarbonyloxy)succinimide (ZONSu) (0.054 g, 0.22 mmol) was added to a stirred solution of the preceding oil and triethylamine (25 mm^3, 0.18 mmol) in a mixture of THF (5 cm^3) and water (0.5 cm^3). The mixture was stirred at ambient temperature overnight and then the solvents were evaporated off. Ethyl acetate (20 cm^3) was added and the mixture was washed with water (3 × 10 cm^3), dried, and evaporated to give an oil. Flash chromatography and elution with chloroform gave the title azasulfonamidopeptide derivative (0.164 g, 85%) as a glass [Found: *m/z*, FAB^+, 1038 (100%, MNa^+). $C_{55}H_{65}N_7O_{10}S$ requires M, 1015].

[N-*Benzyl*-N′-(N$^{\alpha}$-*benzyloxycarbonyl*-L-*asparaginyl*)*hydrazino*]*sulfonyl*-L-*prolyl*-L-*isoleucyl*-L-*valine Methyl Ester* **4**.—A solution of the above triphenylmethyl derivative (81 mg, 0.080 mmol) in TFA (1.5 cm^3) was stirred at ambient temperature for 90 min. Methanol (5 cm^3) was added and the solvents were evaporated off. The resulting yellow oil was partitioned between chloroform (5 cm^3) and 5% aq. sodium hydrogen carbonate (excess). The organic layer was separated, dried and evaporated to give an oil. Flash chromatography and elution with a gradient from chloroform–methanol (49:1) to chloroform–methanol (24:1) gave the title azasulfonamidopeptide derivative (0.050 g, 81%) as a glass, δ_H($CDCl_3$; 500 MHz) 0.90–0.94 [12 H, m, CH(*Me*)$_2$, CH(*Me*)CH_2*Me*], 1.11–1.17 [1 H, m, CH(Me)C*H*HMe], 1.47–1.52 [1 H, m, CH(Me)CH*H*Me], 1.83–1.88 [1 H, m, C*H*Me_2 or C*H*(Me)CH_2Me], 1.96–2.02 [1 H, m, C*H*(Me)CH_2Me or C*H*$(Me)_2$], 2.08–2.15 (3 H, m, C$^{\alpha}$HC*H*HCH_2), 2.19–2.27 (1 H, m, C$^{\alpha}$HCH*H*CH_2), 2.52–2.78 (2 H, ABX, J_{AB} 16, J_{AX} 4, J_{BX} 7.5, C$^{\alpha}$HCH_2CO), 3.57–3.64 (2 H, m, Pro NCH_2), 3.70 (3 H, s, OMe), 4.33–4.37 (2 H, m, 2 × C$^{\alpha}$H), 4.41–4.43 (1 H, m, C$^{\alpha}$H), 4.52–4.56 (1 H, m, C$^{\alpha}$H), 4.65 and 4.68 (2 H, ABq, J_{AB} 14, NCH_2Ph), 5.05 and 5.09 (2 H, ABq, J_{AB} 12, OCH_2Ph), 5.99 (2 H, br, NH_2), 6.06–6.20 (1 H, br, Asn N$^{\alpha}$H), 6.83 (1 H, d, *J* 8.5, Ile or Val N$^{\alpha}$H), 7.20 (1 H, d, *J* 9, Val or Ile N$^{\alpha}$H), 7.30–7.39 (10 H, m, ArH) and 8.44 (1 H, s, NHN); δ_C($CDCl_3$; 125.75 MHz) 11.4 (CH_3), 15.7 (CH_3), 18.4 (br, CH_3), 18.9 (CH_3), 24.5 (CH_2), 29.6 (CH_2), 30.4 (CH), 31.5 (CH_2), 36.4 (br, CH), 50.3 (br, CH_2), 52.0 (OMe), 56.3 (CH_2), 58.2 (br, CH), 62.9 (CH), 67.2 (OCH_2), 127.9–129.5 (aryl CH), 134.3 and 135.9 (aryl *C*C), 156.0 (urethane C=O), 170.3 (C=O), 171.1 (C=O) and 172.6 (br, C=O) [Found: *m/z*, FAB^+, 796 (< 1%, MNa^+) and 774 (1%, MH^+). $C_{36}H_{51}N_7O_{10}S$ requires M, 773].

Cleavage-inhibition Assays.—Cleavage-inhibition assays were performed using recombinant HIV-1 proteinase (MRC AIDS Directed Project; ADP 630) and a synthetic peptide substrate incorporating the reporter residue *p*-nitro-L-phenylalanine [Phe(NO_2)] at the P1′ position relative to the scissile bond. The peptide substrate was synthesized using a Zinsser solid-phase synthesizer and standard Fmoc chemistry: it had the sequence Lys-Ala-Arg-Val-Leu-Phe(NO_2)-Glu-Ala-Met, analogous to a native HIV-1 *gag* maturation site. Peptide hydrolysis was assayed using a continuous spectrophotometric assay.[13] K_i-Values were calculated at five concentrations of peptide **4** in equilibrium with proteinase.

Acknowledgements

Ro 31-8959 was kindly provided by Roche Products Ltd., Herts. We thank the SERC for the studentship held by T. J. C.

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Paper 4/01288G
Received 3rd *March* 1994
Accepted 15th *March* 1994