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Selective and Controlled Hydrolysis of Chloropeptin I. HIV-1 Integrase Activity of Fragments

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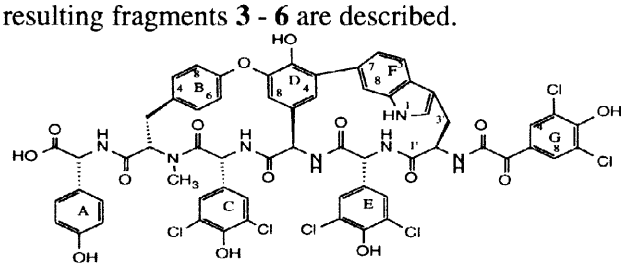
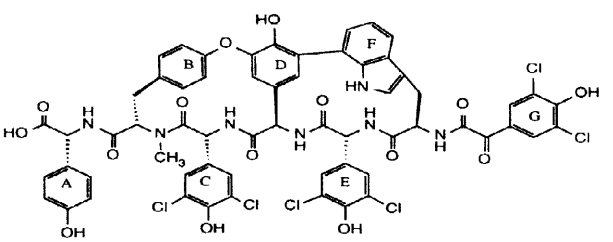
Abstract: Selective amide bond cleavages of chloropeptin I were accomplished using trifluoroacetic acid (TFA), and a mixture of acetic acid, hydrochloric acid and thioglycolic acid. The hydrolysis products maintaining the bicyclic core retained HIV-1 integrase inhibitory activity similar to that of the chloropeptin I. The hydrolysis products and their HIV-1 integrase activities are described.

Complestatin (**1**)[1] and chloropeptin I (**2**), bicyclo hexapeptides comprised entirely of aromatic amino acids, have been characterized as equipotent inhibitors of HIV-1 gp120 glycoprotein CD4 binding (IC_{50} = 2.0 μM).[2] Recently, the non-enzymatic acid catalyzed rearrangement of complestatin to chloropeptin I[3] and the HIV-1 integrase and antiviral inhibitory activities of **1** and **2** were reported.[4] In order to define the minimal pharmacophore required for antiviral activity, the selective hydrolysis of chloropeptin I was investigated. Herein the conditions for the selective hydrolysis of chloropeptin I and the HIV-1 integrase inhibitory activities of the resulting fragments **3** - **6** are described.



1: Complestatin



2: Chloropeptin I

The lack of aqueous solubility and the presence of the labile amino acid tryptophan present a significant challenge for selective hydrolysis of chloropeptin I. For example, heating **2** in aqueous mineral acid solutions led to only unreacted **2**, probably due to the lack of solubility. However, when **2** was heated in carefully selected compositions of aqueous mineral acids containing either organic acids or water miscible organic solvents in the presence of the anti-oxidant thioglycolic acid, successful hydrolysis reactions were observed. The conditions for the chemoselective generation of chloropeptin I fragments **3** - **6** are summarized below.

(I) Cleavage of the terminal hydroxyphenyl glycine (A): Heating of chloropeptin I in neat TFA at 60 °C for 18-20 h produced *exclusively* pentapeptide (**3**). This hydrolytic procedure represents a new mild method for the selective cleavage of the terminal hydroxyphenyl glycine in the presence of three additional hydroxyphenyl glycines.

(II) Cleavage of the hydroxyphenyl glycine (A) and the keto-acid termini (G): The hydrolysis of **2** in a 5:3:2 mixture of AcOH: 6N HCl: thioglycolic acid at 110-120 °C for 2-4 h in a sealed tube under an inert environment afforded mainly pentapeptide (**4**) *via* pentapeptide (**3**).

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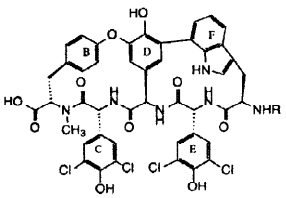
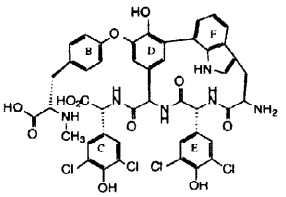
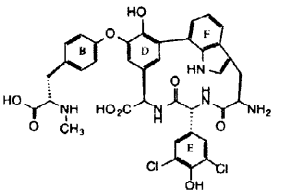
(III) Cleavage of the amide bond(s) of the biaryl ether ring: Prolonged heating (10-24 h) of the reaction (**II**) led initially to the ring opened product **5** which eventually produced compound **6** as a major product. The rate of formation of compound **6** increased by heating the reaction at 140 °C. The time course of the hydrolysis reaction at 125 °C for 24 h followed by heating at 140 °C was monitored by HPLC (graph below). (*R*)-Hydroxyphenyl glycine and (*R*)-3,5-dichloro-4-hydroxyphenyl glycine were also isolated.



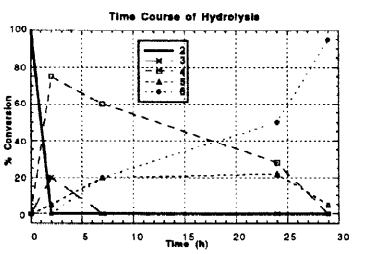
3: R = subunit G
4: R = H



5



6



These reactions can also be performed with other proportions of solvents including the substitution of AcOH with propionic acid, DME or other high boiling ethers. However, the 5:3:2 composition of AcOH: 6N HCl :thioglycolic acid produced optimum results.

Structural studies of fragments: The compounds were purified by preparative HPLC[5] and structures were determined by NMR and mass spectral analysis. The bicyclic peptides **3** and **4** each exhibited two sets of peaks in the HPLC chromatograms. These separable peaks correspond to interconvertible isomers deriving from the *cis -trans* isomerization of tertiary N-methyl amide group. The isomerization appears to occur after the cleavage of the terminal hydroxyphenyl glycine (A) residue. The ^{1}H NMR spectra of both bicyclic compounds in DMSO-d_6 also indicated the presence of a ~1:1 mixture of two isomers. Complete coalescence to a single isomer was not observed upon heating the sample up to 150 °C. The ^{1}H NMR spectrum of the bicyclic compound **3** in THF-d_8 showed a similar population (~1:1) of the two isomers but the spectrum of the compound **4** in THF-d_8 was complex due to the presence of multiple isomeric populations. However, the addition of excess of LiCl significantly simplified and sharpened the ^{1}H NMR spectra and one major isomeric population emerged in the solutions of both samples.[6] The cleavage of the amide bond(s) within the biaryl ether ring was expected to relieve the restricted rotation of the aromatic ring of the tyrosine (B) unit and would eliminate the magnetic anisotropy of the aromatic protons (H - 5, 9 and H - 6, 8). This phenomenon was observed in the ^{1}H NMR spectra of ring opened peptides **5** and **6** which showed two proton doublets, each for two equivalent aromatic protons of the tyrosine. The H-8 of the central dihydroxyphenyl glycine (D) shifted downfield by about 0.5 - 1.0 ppm in compounds **5** - **6** due to cessation of the ring current effect of the tyrosine unit.

HIV-1 Integrase activity: The pentapeptides **3** and **4** show IC_{50} values of 0.3 - 0.5 μM and 3 - 10 μM, respectively, in HIV-1 integrase coupled and strand transfer assays,[7] which were comparable to chloropeptin I (IC_{50}= 0.4 and 5 μM).[4] The ring opened peptides **5** and **6** were significantly less potent in both assays.

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4. For isolation, structure and details of HIV-1 integrase activity see Jayasuriya, H. J. et al manuscript in preparation.
5. HPLC condition, Zorbax RX C-8 (4.6 X 250 mm), CH_3CN + H_2O (both containing 0.1% TFA), gradient elution with 10% CH_3CN to 30% CH_3CN in 20 min followed by 80% CH_3CN in another 20 min +0.1% TFA, 1mL/min, retention times (in min), **2** (37.04), **3** (38.04, 38.55), **4** (31.58, 32.83), **5** (27.78), and **6** (21.57).
6. Similar observations have been reported (a) Kock, M.; Kessler, H.; Seebach, D.; Thaler, A. *J. Am. Chem. Soc.* **1992**, *114*, 2676. (b) Boger, D. L.; Patane, M. A.; Zhou, J. *J. Amer. Chem. Soc.* **1995**, *117*, 7357.
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