Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3615–3617

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

An Expedient Synthesis of N^{α}-Protected-L-tetrahydrofuranylglycine and Its Application in the Synthesis of Novel Substrate Based Inhibitors of HIV-1 Protease

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Received 26 July 2002; accepted 3 September 2002

Abstract—Z- and Fmoc-L-tetrahydrofuranylglycines have been obtained from L-vinylglycine through dipolar cycloaddition reaction, and its Fmoc derivative has been applied in the synthesis of modified S9 and S10 substrates of HIV-1 protease. These compounds mostly acted as strong inhibitors, rather than substrates, of the protease, probably due to the favourable interactions of the tetrahydrofuranylglycine moiety at the S_2 site.

The virally encoded HIV-1 protease is responsible for the post-translational modification of gag- and gag-pol polyproteins leading to the production of infectious virions. Ever since it was realized that the inhibition of this enzyme leads to non-infectious virions, this finding has generated a lot of interest as a means to control HIV replication and combat AIDS. It has thus resulted in six HIV protease inhibitors approved by FDA.

In 1993, the Merck group reported that the incorporation of tetrahydrofuranylglycine (THF-glycine/Tfg) as an Asn surrogate at the P_2 position of saquinavir led to remarkable improvement in the inhibitory potencies.[1] X-ray crystal structure of the protease–inhibitor complex revealed that the Asn side chain at P_2 of the inhibitor was within a hydrogen-bonding distance to the backbone NH groups of Asp 29 and Asp 30.[1] Thus, a cyclic ether in place of the carboxamide of Asn as the hydrogen bonding acceptor proved highly rewarding. This hydrogen-bonding design was also exploited in the generation of newer type of P_2 ligands such as the bis(tetrahydrofuranyl)carbamate.[2] The inhibitors containing THF-glycine at the P_2 position were highly effective against both HIV-1 and HIV-2 types making them very attractive from a resistance standpoint. However, the novel but long-winded synthesis reported by Merck remains the only option to synthesize this amino acid. Alternatives that give access to this important amino acid from inexpensive starting material and its use in solid-phase peptide synthesis (SPPS) would definitely be very well received. Here, we describe a novel and short synthesis of THF-glycine and its application in the synthesis of modified substrates for HIV-1 protease. In addition to this, we also outline a new strategy for the development of substrate-based HIV-1 protease inhibitors.

The retrosynthetic analysis of THF-glycine suggested that vinylglycine could act as the precursor. Previously, vinylglycine has been used for the construction of heterocyclic rings through cycloadditions.[3] For example, acivicin and β-hydroxyornithine have been synthesized through nitrone cycloaddition to vinylglycine. Thus, we decided to exploit the cycloaddition chemistry developed by Hosomi and co-workers for the construction of the THF ring.[4] Z-L-vinylglycine-OBn[3f] was subjected to the cycloaddition using the non-stabilized carbonyl ylide generated from bis(chloromethyl) ether (**CAUTION** *Very high carcinogenic activity has been reported for this compound*).[5] When the reaction was conducted at room temperature, only a 31% yield could be

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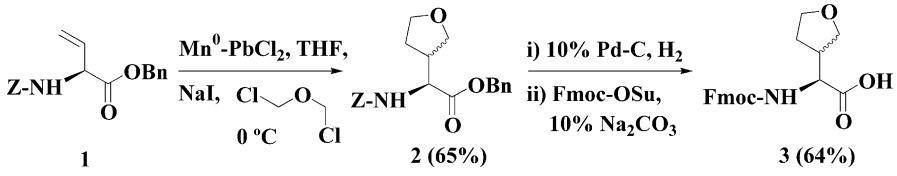
0960-894X/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(02)00781-3

obtained with the recovery of another 20% of the starting material mainly as the crotonate ester. We certainly found it helpful to cool the exothermic reaction, that ensues upon addition of the dipolaraphile and the dipole, with an ice-water bath as the reaction was cleaner with the cooling. Gratifyingly, **1** underwent 3 + 2 cycloaddition to give Z-L-THF-glycine-OBn (**2**) in 65% yield as a 57:43 mixture of diastereomers as determined by HPLC.[6] The presence of diastereomers was also clear from the ^{13}C NMR where all the signals had duplicated. All our efforts to separate the diastereomers through column chromatography or HPLC proved futile. To determine if the reaction conditions[6] lead to any racemization at the α-position, we subjected Z-L-Phe-OBn to the same conditions and then analyzed the recovered starting material using chiral HPLC. The recovered material had identical retention time (t_R) to that of Z-L-Phe-OBn. The protecting groups of **2** were removed through hydrogenolysis with 10% Pd-C and the resulting compound subsequently converted to its Fmoc protected form with Fmoc–OSu in 64% yield.[7,8] Thus, we could synthesize Fmoc-L-tetrahydrofuranylglycine (**3**) as a diastereomeric mixture in three straightforward steps (Scheme 1).

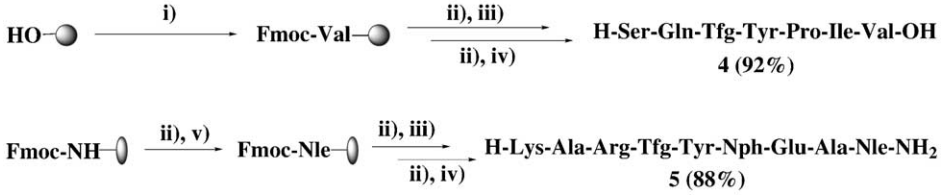
Next, we turned our attention to its use in the synthesis of substrates for HIV-1 protease. A short sequence of the S9 (H-Ser-Gln-Asn-Tyr-Pro-Ile-Val-OH) and S10 (H-Lys-Ala-Arg-Val-Tyr-Nph-Glu-Ala-Nle-NH_2) substrates of the protease derived from the gag protein fragment was chosen and Asn and Val at P_2 of the respective substrates were replaced with THF-glycine.[9] The modified substrates **4** and **5** were synthesized by SPPS on Wang resin and Rink amide AM resin, respectively (Scheme 2).[10] The preparation of the protected peptides on the resin could be completed without any hitch, and the peptides were cleaved from the resin using a cocktail solution of TFA/thioanisole/*m*-cresol/water (92.5:2.5:2.5:2.5; 2.5 h for the modified S9 substrate and 1 day for the modified S10 substrate). After the usual work-up procedure, the peptides were obtained as white powder in very good yields (92% for **4** and 88% for **5**). Efforts to separate the diastereomers were not successful at this stage too. The purity was checked by RP-HPLC and its structure was confirmed by HRMS.[11]

The peptides were then subjected to HIV protease assay.[9] The modified S9 substrate **4** was not cleaved by the protease and the modified S10 substrate **5** was cleaved very weakly. However, these compounds **4** and **5** were found to show the HIV protease inhibitory activity with values of 82 and 27%, respectively, at a 50 nM concentration, for the cleavage of original S10 substrate. The strong inhibitory activity observed for **4** indicated that its function was completely reversed from a substrate to an inhibitor by the incorporation of THF-glycine. It is likely that the introduction of THF-glycine into the P_2 site of peptide leads to tight interactions of the peptide with the protease and changes the conformation of the scissile amide bond at the active site cleft of the protease thereby preventing the action of the catalytic machinery. It is, however, not clear at this point of time as to which of the two diastereomers is responsible for the inhibitory activity. Thus, the introduction of THF-glycine at the P_2 site of the substrates represents a new strategy for the development of newer inhibitors of HIV-1 protease and other aspartic proteases that require Asn or Val at the P_2 position.

In conclusion, we have developed a new synthesis for N^{α}-protected THF-glycine and applied it in the synthesis of modified substrates for HIV-1 protease. These compounds, however, acted as substrate-based inhibitors of the HIV-1 protease, probably due to the favorable interactions at the S_2 site. We are sure that the synthesis of N^{α}-protected THF-glycine and its use in SPPS will prove to be very valuable to medicinal and peptide chemists alike.



Scheme 1.



Scheme 2. Reagents and conditions: (i) Fmoc-Val-OH, DIC, DMAP, DMF; (ii) 20% piperidine/DMF, 20 min; (iii) Fmoc-AA-OH, HOBt, DIC, DMF; (iv) TFA/thioanisole/*m*-cresol/H_2O (92.5/2.5/2.5/2.5); (v) Fmoc-Nle-OH, DIC, DMAP, DMF. Tfg = tetrahydrofuranylglycine; Nph = *p*-nitrophenylalanine; Nle = norleucine; ● = Wang resin; ◐ = Rink amide AM resin.

Acknowledgements

This research was supported in part by the Frontier Research Program of the Ministry of Education, Science and Culture of Japan, and the Japan Health Science Foundation. We wish to profusely thank Professor A. Hosomi for providing us the procedure for 3+2 cycloaddition. We would also like to sincerely thank Ms. H. Tsukamoto and Ms. S. Shibakawa for HIV protease assay, and Ms. T. Tsushima and Ms. K. Oda for mass spectrometry.

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6. To a flame-dried flask was added Mn (52 mmol, 2.85 g) under argon. The flask was repeatedly heated with a flame under reduced pressure and then cooled under argon. To this flask was added 12 mL of THF followed by $PbCl_2$ (1.04 mmol, 0.29 g). The contents of the flask were vigorously stirred for 1 h. Then **1** (4.33 mmol, 1.41 g), NaI (34.7 mmol, 5.2 g) and bis(chloromethyl) ether (17.32 mmol, 1.97 g) were successively added and stirred. An exothermic reaction ensued immediately and the contents of the flask were cooled with an ice-water bath. The reaction was stirred for 6 h and quenched with EtOAc–water. The layers were separated and the aqueous phase was re-extracted with EtOAc thrice, drying over $MgSO_4$ followed by purification using column chromatography (eluent Hexane/EtOAc 3:1) yielded **2** as a colourless oil in 65% yield. HPLC (YMC-Pack, ODS-AP, 250×10 mm I.D.) (t_R = 26.04 min, 26.38 min; Elution was by a gradient of 25–55% B in 30 min; solvent A = 0.1% TFA in water; solvent B = acetonitrile; Flow rate = 0.9 mL/min; λ = 254 nm). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 (s, 10H); 5.56 (br s, 1H); 5.52–5.09 (m, 4H, PhC*H2*); 4.42 (dd, *J* = 8.1 Hz, 7 Hz, α C*H*); 3.88–3.72 (m, 3H); 3.68–3.60 (m, 1H); 2.69–2.60 (m, 1H, β CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 171.3, 156.2, 156.1, 136.0, 135.0, 128.5, 128.49, 128.4, 128.3, 128.28, 128.2, 128.1, 128.04, 128.01, 69.6, 69.3, 67.9, 67.7, 67.2, 67.1, 56.1, 55.2, 41.8, 41.2, 28.6, 27.9. HRMS (EI–MS$^+$) calcd for $C_{21}H_{23}NO_5$ (M^+) 369.1576; found *m*/*z* 369.1567.

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8. Selected spectroscopic data for **3** : Amorphous white powder (mp = 158 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, *J* = 7.5 Hz, 2H); 7.56 (d, *J* = 6.6 Hz, 2H); 7.38 (t, *J* = 7.5 Hz, 2H); 7.29 (t, *J* = 7.2 Hz, 2H); 5.62 (d, *J* = 5.4 Hz, 1H, NH); 4.40 (d, *J* = 5.1 Hz, 2H, OC*H2*); 4.20 (t, *J* = 6.6 Hz, 1H, FluorenylC*H*); 3.9–3.82 (br s, 1H); 3.73–3.65 (br m, 3H); 2.75 (br s, 1H); 2.11–1.80 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.1, 156.5, 143.7, 141.3, 127.7, 127.1, 125.0, 120.0, 69.5, 68.1, 67.2, 56.0, 47.1, 41.6, 28.7. HRMS (EI–MS$^+$) calcd for $C_{21}H_{21}NO_5$ (M^+) 367.1419; found *m*/*z* 367.1408.

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11. (a) Data for **4**: HPLC (t_R = 5.17 min, 5.24 min; Elution was by a gradient of 20–60% B in 40 min; Flow rate = 0.9 mL/min; λ = 254 nm).[12] HR-MS (FAB$^+$) calcd for $C_{39}H_{61}N_8O_{12}$ ($M+H^+$) 833.4409; found *m*/*z* 833.4418. (b) Data for **5** : HPLC (t_R = 16.60 min, 16.74 min. Elution was by a gradient of 0–100% B in 40 min; flow rate = 0.9 mL/min; λ = 230 nm).[12] HRMS (FAB$^+$ calcd for $C_{53}H_{82}O_{15}N_{15}$ ($M+H^+$) 1168.3023; found *m*/*z* 1168.6115.

12. Solvents A and B are the same as in ref 6.