Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 1179–1184

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Structure-based Design of Diaminopyranosides as a Novel Inhibitor Core Unit of HIV Proteases

Shinwa Kurihara, Takeshi Tsumuraya, and Ikuo Fujii*

Biomolecular Engineering Research Institute, 6-2-3 Furuedai, Suita, Osaka 565-0874, Japan

Received 27 January 1999; accepted 15 March 1999

Abstract : Novel HIV PR inhibitors, which contain a diaminopyranoside moiety as an inhibitor core unit, were designed based on the 3D structures of complexes of HIV-1 PR with transition-state mimics. These compounds were examined for their ability to inhibit the hydrolytic activity of a recombinant HIV-1 PR. © 1999 Elsevier Science Ltd. All rights reserved.

Human immunodeficiency virus proteases (HIV PR), which are responsible for viral maturation into infectious particles,[1] have become an attractive therapeutic target for acquired immunodeficiency syndrome (AIDS). Thus far, numerous examples of potent inhibitors of HIV-1 PR have been reported.[2] However, the daunting ability of the virus to rapidly generate resistant mutants[3] suggests that there is an ongoing need for new inhibitors. Our strategy for the development of HIV PR inhibitors has been based on the 3D structures of HIV-1 PR complexed with transition-state mimics, such as norstatine[4] and dihydroxy ethylene derivatives.[5] X-ray studies have revealed that the transition-state mimics bind to HIV-1 PR by virtue of two hydrogen bond interactions between the hydroxyl (or carbonyl) groups of the mimics and the catalytic aspartic acids (Asp25 and Asp125) of the active site, as shown in Figure 1a. Therefore, our initial attempt focused on altering the binding mode by replacing the hydrogen bond interactions with the electrostatic interactions (Figure 1b). Herein, we report the design and synthesis of HIV PR inhibitors containing a diaminopyranoside moiety as an inhibitor core unit.

The key to the design of diaminopyranoside **1** was the assumption that upon binding to HIV-1 PR, the two primary amino groups of **1** make electrostatic interactions with the two catalytic aspartic acids in the active site. In an unliganded form of the protease, the two aspartic acids are generally hydrogen-bonded to exist as the anion form and the acid form,[6] whereas, in a liganded form with the inhibitor it is most likely that the aspartic acids make salt bridges with the positively charged amino groups (Figure 1b). The electrostatic interactions would be more energetically favorable than the corresponding hydrogen-bond interactions.[7] For optimal interactions, the two amino groups were adapted to possess a dihedral angle of 60°, by using D-glucosamine as a template (Figure 1c). Thus, the alteration of a flexible and linear structure into a rigid and cyclic one with restricted conformations should provide a positive entropic effect. In addition, the two amino groups were accommodated in an axial-equatorial orientation to maintain the dihedral angle of 60° even in two flipping chair conformations of the 6-membered ring. In fact, the x-ray crystal structures of the transition-state mimics complexed with HIV-1 PR show that the dihedral angle of

0960-894X/99/$ - see front matter © 1999 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(99)00154-7

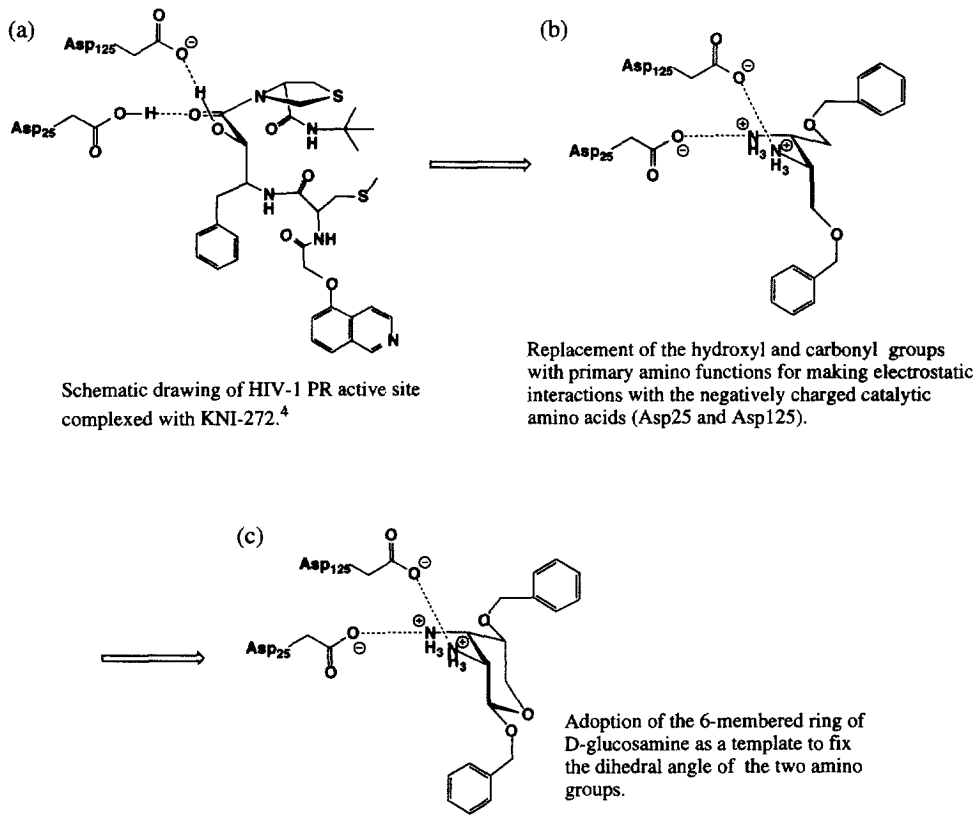
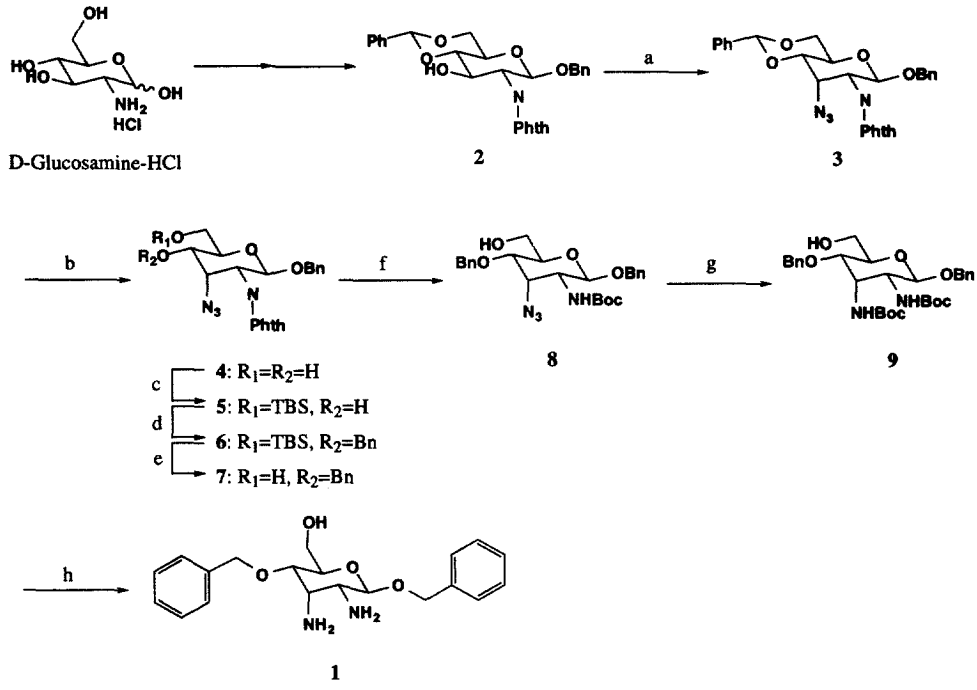


Figure 1. Strategy and steps involved in the design of diaminopyranosides as HIV PR inhibitors.

the two hydrogen-bonded groups, in most cases, is within the range from 40 to 70°.[5,8] Much effort has been made for developing the peptide-based inhibitors. However, it has been difficult to combine adequate potency with oral bioavailability, because peptide-based molecules are in general biologically unstable and rapidly metabolized.[9] Considering the major liabilities in this regard, the non-peptidic structure of **1** would be beneficial for the pharmacokinetic profiles.

Diaminopyranoside **1** was synthesized as shown in scheme 1. Compound **2** was prepared from D-glucosamine hydrochloride according to the previous method.[10] Triflation of **2** with trifluoromethane sulfonic anhydride (Tf_2O), followed by treatment with sodium azide, gave **3** in 67% yield via 2 steps. Hydrolysis of **3** with 70% aqueous acetic acid gave **4** in 79% yield. The inversion of the stereochemistry at the C3-position of **4** was confirmed by the ^1H NMR spectrum, which showed coupling constants of $J = 3.3$ and 3.1 Hz (dd) for the proton at the C3-position.[11] Diol **4** was converted to **7** through 3 steps: 1) silylation of the primary alcohol at the C6-position with *tert*-butyldimethylsilyl chloride (87%), 2) benzylation of the secondary alcohol at the C4-position with benzyl bromide (68%), and 3) acidic hydrolysis of the silyl group

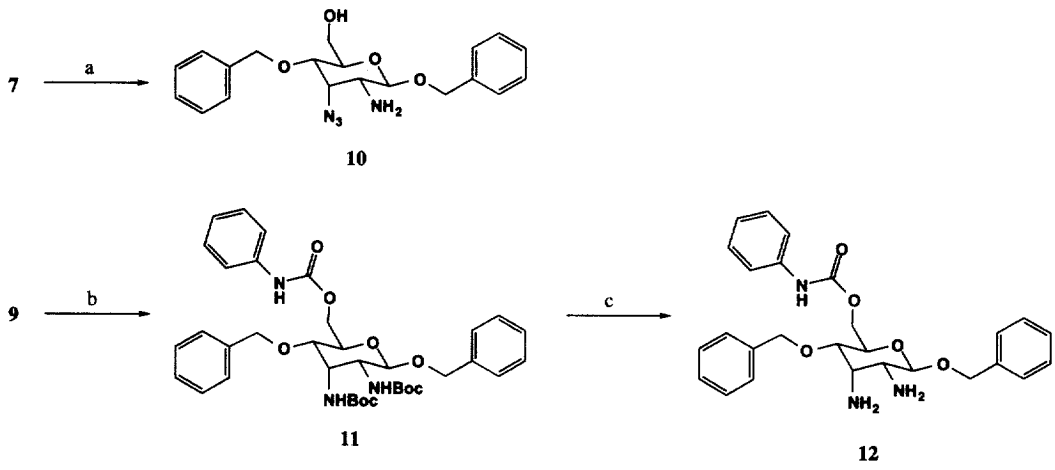


Scheme 1. Synthesis of compound **1**.

Reagents and conditions: (a) i) Tf_2O, pyridine, CH_2Cl_2, -15°C, ii) NaN_3, DMF, 75°C, 67% in 2 steps; (b) 70% aqueous acetic acid, 70°C, 79%; (c) TBSCl, imidazole, DMF, r.t., 87%; (d) BnBr, NaH, DMF, 0°C, 68%; (e) acetic acid-THF-H_2O (3:1:1), r.t., 98%; (f) i) hydrazine monohydrate, EtOH, 90°C, ii) di-*tert*-butyl dicarbonate (Boc_2O), EtOH, r.t., 89% in 2 steps; (g) i) Ph_3P, THF, reflux, ii) di-*tert*-butyl dicarbonate (Boc_2O), THF-H_2O, 50°C, 75% in 2 steps; (h) 4N HCl/1,4-dioxane, EtOH, r.t., 94%.

(98 %). Removal of the phthaloyl group with hydrazine monohydrate, followed by treatment with di-*tert*-butyl dicarbonate, afforded **8** (89% in 2 steps). After reduction of the azide with triphenyl phosphine, the resulting amine was treated with di-*tert*-butyl dicarbonate to yield **9** (75% in 2 steps). Finally, deprotection of the *tert*-butyloxycarbonyl groups of **9** with 4*N* HCl in 1,4-dioxane gave diaminopyranoside **1** in 94% yield.[12]

The inhibitory potency of **1** against HIV-1 PR was examined by an intramolecular fluorescence resonance energy transfer (RET) assay.[13] The hydrolysis of a fluorogenic substrate, 4-(4-dimethyl amino phenyl azo) benzoic acid (DABCYL)-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-5-[(2-aminoethyl) amino] naphthalene-1-sulfonic acid (EDANS) (Bachem, M-1865), by a recombinant HIV-1 PR at 25°C was monitored by fluorescence (excitation 360 nm/emission 460 nm). The reaction was carried out with 20 μM substrate at pH 5.5 in a buffer containing 50 mM NaOAc, 1.0 M NaCl, 1 mg/ml BSA, 1.0 mM EDTA, and 10% DMSO.[14] As a result, diaminopyranoside **1** was found to inhibit the hydrolytic activity of HIV-1 PR with a 50% inhibitory concentration (IC_{50}) of 205 ± 8 μM. To examine whether the inhibitory potency of **1** is due to the diamine moiety, we prepared azide **10**,[15] which lacked the amino function at the C3-position



Scheme 2. Syntheses of compounds **10** and **12**.
Reagents and conditions: (a) hydrazine monohydrate, EtOH, 90°C, 88%; (b) phenyl isocyanate, DMF, r.t., 77%; (c) 4N HCl/1,4-dioxane, EtOH, r.t., 86%.

(Scheme 2). As expected, the activity of azide **10** was much lower (IC_{50} > 1 mM) than that of **1**. This suggested the crucial role of the charged diamino functionality in the inhibition. As compared with previously reported potent inhibitors, which have resulted from enormous efforts concerning structure-activity relationships, the inhibitory activity of **1** was considerably low. However, this was not surprising, because **1** was imperfect in the set of common pharmacophores observed as potent inhibitors. Diaminopyranoside **1** has only P1/P1' substituents, whereas the inhibitors of cyclic urea reported by the DuPont group have four hydrophobic groups as the P1/P1' and P2/P2' substituents, and an urea group as a mimic for the structural water observed in HIV-1 PR/inhibitor complexes.[5c] In fact, derivative **12**,[16] in which an additional phenyl ring was attached to the C6-position for a P2 or P2' substituent, showed an increased inhibitory potency with a IC_{50} of 28 ± 3 μM (Scheme 2). These results suggest that the diaminopyranoside moiety is a promising inhibitor core unit for HIV-1 PR. Further structural optimizations of **1** will provide a new design direction for the discovery of more potent HIV-1 PR inhibitors as potential therapeutic agents for the treatment of HIV infection.

In this work, we have demonstrated that diaminopyranosides are able to act as an inhibitor core unit for HIV proteases. In addition, we propose diaminopyranoside **1** as a potent antigen for the generation of catalytic antibodies. Given the potency of **1** for making electrostatic interactions with the catalytic aspartic acids of HIV-1 PR, it is expected that immunization with **1** can elicit two aspartic acids working in concert in catalysis, to generate catalytic antibodies such as aspartic proteases. This is now under investigation, and the results will be reported elsewhere.

Acknowledgments:

We are grateful to Dr. Y. Ozawa and Miss A. Sugiyama, of Biological Research Laboratories, Sankyo Co. Ltd., Japan, for measuring the inhibitory activities.

References and notes:

1. Debouck, C.; Metcalf, B. W. *DrugDev. Res.* **1990**, *21*, 1.
2. (a) Martin, J. A. *Antiviral Res.* **1992**, *17*, 265. (b) Wlodawer, A.; Erickson, J. W. *Annu. Rev. Biochem.* **1993**, *62*, 543. (c) Wlodawer, A.; Vondrasek, J. *Annu. Rev. Biophys. Biomol. Struct.* **1998**, *27*, 249. (d) Rich, D. H. Inhibitors of Aspartic Proteinases. In *Proteinase Inhibitors*; Barrett, A. J., Salvesen, G., Eds.; Elsevier Science: New York, 1986; pp 179-218.
3. (a) Jacobsen, H.; Yasargil, K.; Winslow, D. L.; Craig, J. C.; Krohn, A.; Duncan, I. B.; Mous, J. *Virology* **1995**, *206*, 527. (b) Markowitz, M. M.; Mo, H.; Kempf, D. J.; Norbeck, D. W.; Bhat, T. N.; Erickson, J. W.; Ho, D. *J. Virol.* **1995**, *69*, 701. (c) Condra, J. H.; Schlelf, W. A.; Blahy, O. M.; Gabryelski, L. J.; Graham, D. J.; Quintero, J. C.; Rhodes, A.; Robbins, H. L.; Roth, E.; Shivaprakash, M.; Titus, D.; Yang, T.; Teppler, H.; Squires, K. E.; Deutsch, P. J.; Emini, E. A. *Nature* **1995**, *374*, 569. (d) Ridky, T.; Leis, J. *J. Biol. Chem.* **1995**, *270*, 29621.
4. (a) Mimoto, T.; Imai, J.; Kisanuki, S.; Enomoto, H.; Hattori, N.; Akaji, K.; Kiso, Y. *Chem. Pharm. Bull.* **1992**, *40*, 2251.(b) Baldwin, E. T.; Bhat, T. N.; Gulnik, S.; Liu, B.; Topol, I. A.; Kiso, Y.; Mimoto, T.; Mitsuya, H.; Erickson, J. W. *Structure* **1995**, *3*, 581.
5. (a) Backbro, K.; Lowgren, S.; Osterlund, K.; Atepo, J.; Unge, T. *J. Med. Chem.* **1997**, *40*, 898. (b) Jadhav, P. K.; Ala, P.; Woerner, F. J.; Chang, C.- H.; Garber, S. S.; Anton, E. D.; Bacheler, L. T. *ibid.* **1997**, *40*, 181. (c) Lam, P. Y. S.; Ru, Y.; Jadhav, P. K.; Aldrich, P. E.; DeLucca, G. V.; Eyermann, C. J.; Chang, C-H.; Emmett, G.; Holler, E. R.; Daneker, W. F.; Li, L.; Confalone, P. N.; McHugh, R. J.; Han, Q.; Li, R.; Markwalder, J. A.; Seitz, S. P.; Sharpe, T. R.; Bacheler, L. T.; Rayner, M. M.; Klabe, R. M.; Shum, L.; Winslow, D. L.; Kornhauser, D. M.; Jackson, D. A.; Erickson-Viitanen, S.; Hodge, C. N. *ibid.* **1996**, *39*, 3514. (d) Sham, H. L.; Zhao, C.; Stewart, K. D.; Betebenner, D. A.; Lin, S.; Park, C. H.; Kong, X-P.; Rosenbrook, W. Jr.; Herrin, T.; Madigan, D.; Vasavanonda, S.; Lyons, N.; Molla, A.; Saldivar, A.; Marsh, K. C.; McDonald, E.; Wideburg, N. E.; Denissen, J. F.; Robins, T.; Kempf, D. J.; Plattner, J. J.; Norbeck, D. W. *ibid.* **1996**, *39*, 392. (e) Hosur, M. V.; Bhat, T. N.; Kempf, D. J.; Baldwin, E. T.; Liu, B.; Gulnik, S.; Wideburg, N. E.; Norbeck, D. W.; Appelt, K.; Erickson, J. W. *J. Am. Chem. Soc.* **1994**, *116*, 847. (f) Thanki, N.; Rao, J. K. M.; Foundling, S. I.; Howe, W. J.; Moon, J. B.; Hui, J. O.; Tomasselli, A. G.; Heinrikson, R. L.; Thaisrivongs, S.; Wlodawer, A. *Protein Science* **1992**, *1*, 1061.
6. Fersht, A. The proteases. In *Enzyme structure and mechanism* (second edition); W. H. Freeman and Company: New York, 1985; pp 422-426.
7. (a) Fersht, A. *ibid.* pp 293-310. (b) *Intermolecular & Surface Forces* (second edition), Israelachvili, J.; Academic Press Ltd.: London, 1991; pp 27-35.
8. The Protein Data Bank (Brookhaven National Laboratories) was accessed for the 3D structural information of the following accession codes: 1ajv, 1ajx, 1pro, 1qbs, 1dmp, 1hvr, 1qbr, 1qbu, 1qbt (cyclic inhibitors); 1hpx, 1hvi, 1hvl, 1hvk, 1hiv (acyclic inhibitors).

9. Plattner, J. J.; Norbeck, D. W. Obstacles to drug development from peptide leads. In *Drug Discovery Technologies*; Clark, R., Moos, W. H., Eds.; Ellis Horwood Ltd.:Chichester, 1990; pp 92-126.

10. (a) Lemieux, R. U.; Takeda, T.; Chung, B. Y. *ACS Symp. Ser.* **1976**, *39*, 90. (b) Ogawa, T.; Nakabayashi, S. *Carbohydr. Res.* **1981**, *97*, 81. (c) Campos- Valdes, M. T.; Marino-Albernas, J. R.; Verez-Bencomo, V. *J. Carbohydr. Chem.* **1987**, *6*, 509.

11. Analytical data for compound **4**: $[\alpha]_D^{25}$ -32.0° (c 0.56, $CHCl_3$); IR (film) 3450, 2105, 1779, 1715 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ 7.88-7.85 (m, 2H), 7.77-7.74 (m, 2H), 7.24 (s, 5H), 5.99 (d, *J*=8.4Hz, 1H), 4.84 (d, *J*=11.1Hz, 1H), 4.66 (d, *J*=11.1Hz, 1H), 4.26 (dd, *J*=3.1, 3.3Hz, 1H), 4.21 (dd, *J*=3.1, 8.4Hz, 1H), 4.07 (m, 1H), 3.96-3.81 (m, 3H), 2.82 (m, 1H), 2.06 (m, 1H); FAB HRMS *m/z* calcd for (M+Na) $C_{21}H_{20}O_6N_4Na$ 447.1281, found 447.1270

12. Analytical data for compound **1**: $[\alpha]_D^{25}$ -39.1° (c 0.66, MeOH); IR (film) 3400, 2932, 2888 cm^{-1}; ^1H NMR (300MHz, CD_3OD): δ 7.45-7.28 (m, 10H), 5.06 (d, *J*=5.4Hz, 1H), 4.94 (m, 1H), 4.74-4.63 (m, 3H), 4.09-4.05 (m, 2H), 4.00 (m, 1H), 3.84 (dd, *J*=5.4, 11.6Hz, 1H), 3.78 (dd, *J*=5.0, 11.6Hz, 1H), 3.53 (dd, *J*=3.7, 5.4Hz, 1H); FAB HRMS *m/z* calcd for (M+H) $C_{20}H_{27}O_4N_2$ 359.1971, found 359.1984

13. Matayoshi, E. D.; Wang, G. T.; Krafft, G. A.; Erickson, J. *Science* **1990**, *247*, 954.

14. The inhibition assay was accomplished with the condition that the final concentration of DMSO was 10%, due to the insolubility of the compounds. The obtained values were corrected against the background, to give the IC_{50} values described in this paper.

15. Analytical data for compound **10**: $[\alpha]_D^{25}$ -23.8° (c 0.57, MeOH); IR (film) 3400, 2900, 2112 cm^{-1}; ^1H NMR (300MHz, CD_3OD): δ 7.41-7.28 (m, 10H), 4.93 (d, *J*=11.3Hz, 1H), 4.79-4.72 (m, 2H), 4.65 (d, *J*=11.1Hz, 1H), 4.62 (d, *J*=11.3Hz, 1H), 4.46 (m, 1H), 3.94-3.88 (m, 2H), 3.82 (m, 1H), 3.75 (dd, *J*=4.3, 11.8Hz, 1H), 3.23 (dd, *J*=3.4, 8.2Hz, 1H); FAB HRMS *m/z* calcd for (M+H) $C_{20}H_{25}O_4N_4$ 385.1876, found 385.1878

16. Analytical data for compound **12**: $[\alpha]_D^{25}$ -15.8° (c 0.51, MeOH); IR (film) 2928, 2890, 1684, 1601, 1541 cm^{-1}; ^1H NMR (300MHz, CD_3OD): δ 7.45-7.24 (m, 14H), 7.05 (m, 1H), 5.05 (d, *J*=5.6Hz, 1H), 4.91 (d, *J*=11.6Hz, 1H), 4.68-4.65 (m, 3H), 4.48 (dd, *J*=4.6, 11.2Hz, 1H), 4.35-4.26 (m, 2H), 4.10 (m, 1H), 3.90 (m, 1H), 3.47 (m, 1H); FAB HRMS *m/z* calcd for (M+H) $C_{27}H_{32}O_5N_3$ 478.2342, found 478.2348