Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 3601–3605

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

1,2,5,6-Tetra-*O*-benzyl-D-mannitol Derivatives as Novel HIV Protease Inhibitors

Abderrahim Bouzide, Gilles Sauvé,*,† Guy Sévigny and Jocelyn Yelle*

Pharmacor Inc., 535 West, Cartier blvd., Laval, Quebec, Canada H7V 3S8

Received 28 February 2003; accepted 16 May 2003

Abstract—The synthesis and structure–activity relationships of HIV protease inhibitors derived from carbohydrate alditols are discussed. We disclose a new series of 1,2,5,6-tetra-*O*-alkyl-D-mannitol exhibiting sub-micromolar activity against HIV-protease. This series of inhibitors are non-nitrogen containing HIV-protease inhibitors and they are readily prepared in a few chemical steps from inexpensive commercially available starting materials.

The human immunodeficiency virus (HIV) has been identified as the etiologic agent of acquired immunodeficiency syndrome (AIDS).[1] The *pol* gene of the human immunodeficiency virus (HIV) encodes the aspartic protease which mediates proteolytic processing of the *gag* and the *gag pol* viral gene products, liberating functional enzymes and structural proteins which are essential for the formation of the mature, infectious virus.[2] Inactivation of the aspartic protease leads to the formation of noninfectious virions.[3] As a result the HIV protease has become one of the major targets for therapeutic intervention in AIDS and in HIV infection.[4] Despite the success of the FDA-approved HIV protease inhibitors saquinavir,[5] ritonavir,[6] indinavir,[7] nelfinavir,[8] amprenavir[9] and lopinavir,[10] there is an urgent need for new and improved HIV protease inhibitors due to increasing viral resistance, a matter that is now of great concern.[11] The high cost of synthesis is today a barrier to the widespread use of the currently approved protease inhibitors, notably in the less developed countries. It is thus important to develop new improved protease inhibitors accessible at low cost.[12] Moreover the challenge today is to discover orally bioavailable, long half-life compounds with activity against several protease resistant strains.

The carbohydrates have been extensively used in the synthesis of HIV-protease inhibitors. Their low cost and appropriate stereochemistry made them a starting material of choice in various syntheses.[13] Herein we report the synthesis of a new class of HIV-protease inhibitors of the general structure **1** (Chart 1), prepared readily from carbohydrate alditols. These compounds have been evaluated in an enzyme assay and their structure–activity relationships (SAR) have been investigated.

The 1,2,5,6-tetra-*O*-benzyl-D-mannitol **1a** was easily prepared from D-mannitol (Scheme 1). Conversion of D-mannitol to the acetonide **2** was achieved according to the literature.[14] Treatment of **2** with benzyl bromide in the presence of NaH in DMF afforded the corresponding fully benzylated intermediate, which under the action of HCl in MeOH produced **1a**. Compounds **1b**–**1e** were prepared similarly in good yields, ranging from 65 to 80%.

1,2,5,6-Tetra-*O*-benzyl-L-mannitol **3**, 1,2,5,6-tetra-*O*-benzyl-dulcitol **4** and 1,2,5,6-tetra-*O*-benzyl-D-sorbitol **5** (Chart 2) were prepared respectively from L-mannitol, dulcitol and D-sorbitol following the same steps as in Scheme 1.

Preparation of diol **8**, corresponding to compound **1a** having one of the hydroxyls of the central diol inverted was achieved in a four-step process. Monoprotection of the diol **1a** using silver oxide[15] in the presence of *p*-methoxybenzyl chloride (PMBCl) in dichloromethane furnished **6** in 72% yield. Oxidation of **6** with pyridinium chlorochromate to the corresponding ketone, followed by reduction with sodium borohydride in methanol, gave the desired epimer **7**, along with **6** in

*Corresponding authors. Tel.: +1-514-496-2943; fax: +1-450-973-8404; e-mail: jyelle@procynbiopharm.com
†Present address: INRS-Institut Armand-Frappier, 531, des Prairies Blvd, Building 27, Office D-103, Laval, Quebec, Canada H7V1B7.

0960-894X/$ - see front matter © 2003 Published by Elsevier Ltd.
doi:10.1016/S0960-894X(03)00677-2

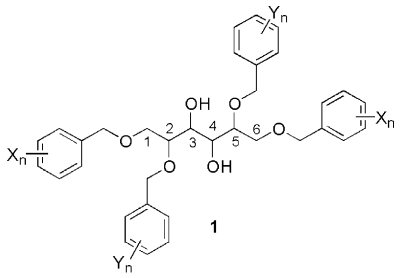
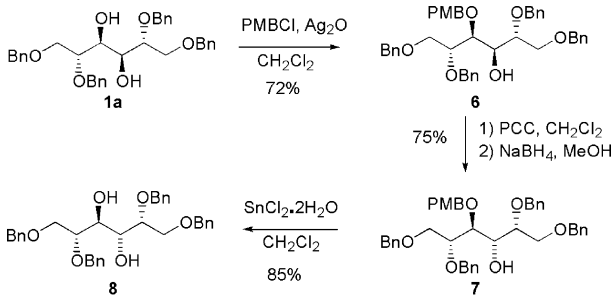


Chart 1. 1,2,5,6-Tetra-*O*-alkyl-alditol.



Scheme 2. Inversion of the C-3 hydroxyl of **1a**.

75% total yield (2:1 ratio in favour of **7**). $SnCl_2$ mediated selective deprotection of PMB protecting group[16] affording **8** in 85% yield (Scheme 2).

Compound **1f** was prepared from **2** according to Scheme 3. Selective *O*-benzylation of the primary hydroxyls was carried out in the presence of dibutyl tin oxide at reflux of toluene, with azeotropic removal of water. Treatment of the resulting dibutyl stannylene with benzyl bromide in the presence of cesium fluoride afforded **9** in 82% yield.[17] Alkylation of the remaining secondary hydroxyls was achieved as described previously in Scheme 1, to produce **10** in 88% yield. Removal of the isopropylidene group under acidic conditions gave **1f** in 95% yield. Compounds **1g**–**1q**, **1u**–**1ff**, and **1hh**–**1jj** were prepared similarly in yields ranging from 55 to 70%. Compounds **1r** and **1gg** were prepared respectively from **1p** and **1ee** by reduction in the presence of $LiAlH_4$. Basic hydrolysis of **1o** afforded **1t**, which under the action of $LiAlH_4$ produced **1s**.

In the synthesis of **13**, the diepoxide **11** (Scheme 4), readily available from D-mannitol in 42% yield,[18] was heated with phenol in DMF at 110 °C in the presence of potassium carbonate to give diol **12** in 88% yield. Di-*O*-benzylation of **12** in the presence of NaH in DMF, followed by the hydrolysis of the isopropylidene group in the presence of 3 N HCl produced **13** in 96% yield.

Compound **15** was prepared by ring opening of the diepoxide **12** with PhMgBr in the presence of $CuBr{\cdot}SMe_2$, to afford diol **14** in 86% yield. Dibenzylation of **14** and hydrolysis of the isopropylidene group afforded **15** in 95% yield.

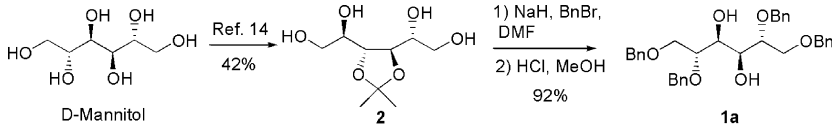
In the synthesis of the monohydroxylated compound **17**, diol **1a** was reacted with thiocarbonyldiimidazole, to give the cyclic thiocarbonate **16** in 86% yield. Slow addition of tributyltin hydride and 2,2′-azobis(isobutyronitrile)[19] to a solution of **16** in toluene at reflux afforded **17** in 66% yield (Scheme 5).

HIV-Protease Inhibition

The IC_{50} values of the synthetic compounds targeting the HIV protease were determined with a fluorometric assay according to the Matayoshi assay[20] and are reported in Tables 1 and 2.

Although the lead compound **1a** has shown a moderate activity against HIV protease (Table 1), we decided to pursue this project for the following reasons: (1) **1a** is very stable and non-nitrogen containing HIV-protease inhibitor,[21] (2) **1a** is prepared in only two steps from the commercially available 3,4-*O*-isopropylidene-D-mannitol, and (3) contrary to other HIV protease inhibitors, **1a** does not contain any carbonyl or sulfonyl group to interact with Il50 and Il50′.[22]

The activity of **1a** is attributed to the hydrophobic interactions induced by the four benzylic groups into the subsites S1, S2, S1′, and S2′, and to the interaction of the (3*S*,4*S*) central diol with the Asp25 and Asp25′. No activity was observed below a concentration of 50 μM when one OH group of the central diol was epimerized (**8**) or deoxygenated (**17**) (Table 1). The (*R*,*R*) stereochemistry at C-2 and C-5 was critical for the activity of **1a**, for example, 1,2,5,6-tetra-*O*-benzyl-D-sorbitol **5**



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

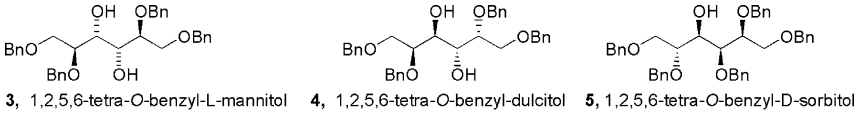
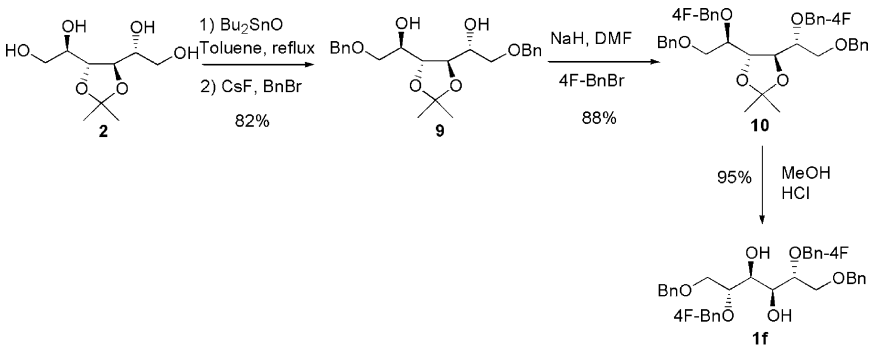
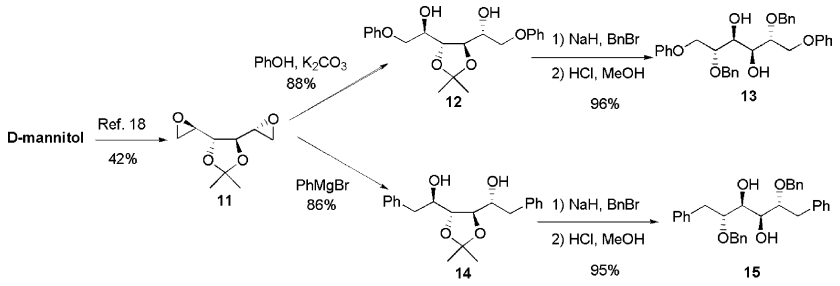


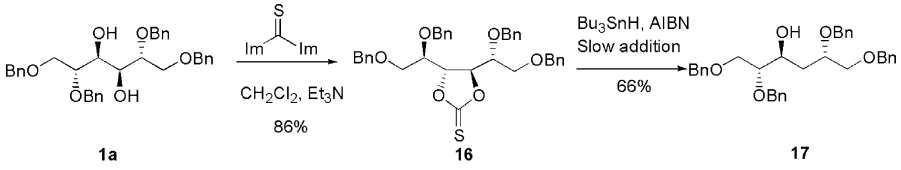
Chart 2. Other diastereoisomers of **1a**.



Scheme 3. Synthesis of compound **1f**.



Scheme 4. Synthesis of compounds **13** and **15**.



Scheme 5. Monodeoxygenation of diol **1a**.

Table 1. IC_{50} of tetra-*O*-benzyl-alditols

No.	IC_{50}, μM
1a	2.4
3	> 50
4	> 50
5	> 50
8	> 50
13	> 50
15	> 50
17	> 50

where the *R* stereochemistry at C-2 was inverted to *S* did not show any activity at a concentration below 50 μM. The compounds 1,2,5,6-tetra-*O*-benzyl-L-mannitol **3** and 1,2,5,6-tetra-*O*-benzyl-dulcitol **4** were inactive (Table 1).

The benzyloxy groups are essential for the activity of **1a** as it was substantiated by the low activity of compound **13** lacking the methylene groups and the low activity of compound **15** lacking CH_2O groups. Consequently, one can conclude that shortening these groups may prevent the aromatic group to reach the appropriate subsite.

Substitution of all the benzyl groups at the *para*-positions with CH_3, CF_3, F and CN groups (Table 2, **1b**–**1e**) did not show any improvement.[23] Interestingly, substitution of the benzyl groups at positions 2 and 5 with *para*-fluoro-benzyl group (**1f**) showed an encouraging sub-micromolar activity. Replacement of the *para*-fluoro with *para*-chloro (**1g**) or *para*-bromo (**1h**) gave less active inhibitors, and no gain in activity was noticed when the fluoro group was switched from *para* position to *meta* (**1i**) or *ortho* position (**1j**). On the other hand, substitution of the benzyl groups at positions 1 and 6 with *ortho*-fluoro-benzyl groups (**1w**) resulted in a good enhancement in activity (3-fold more active than **1a**) Furthermore, the inhibition is increased when both *ortho* positions are occupied by the fluoro group as in the case of **1x** which is 8-fold more active than **1a**. As we anticipated, the combination of *para*-fluoro-benzyl at positions 2, 5 and *ortho*-fluoro-benzyl at positions 1,6 leading to inhibitor **1jj** permitted the best IC_{50}.

In order to minimize the hydrophobic character of the inhibitors and improve their bioavailability, hydrophilic groups such as esters (**1p** and **1ee**) carboxylic acids (**1q** and **1ff**), carboxamides (**1s**), alcohols (**1r** and **1gg**) and amines (**1t**) were added on the aromatic groups. These derivatives present variable activity.

A new series of HIV-protease inhibitors were readily prepared in few steps from inexpensive alditols. Although these inhibitors do not contain a carbonyl or sulfonyl group to interact with Il50 and Il50′ via a water

Table 2. IC_{50} of tetra-*O*-benzyl-D-mannitol derivatives

No.	X_n	Y_n	IC_{50}, μM[a]
1a	H	H	2.4
1b	4-CH_3	4-CH_3	7.0
1c	4-CF_3	4-CF_3	6.0
1d	4-F	4-F	1.6
1e	4-CN	4-CN	3.1
1f	H	4-F	0.7
1g	H	4-Cl	2.0
1h	H	4-Br	2.1
1i	H	3-F	1.8
1j	H	2-F	3.7
1k	H	2,6-F_2	6.4
1l	H	2,4-F_2	2.6
1m	H	4-CF_3	10.0
1n	H	4-CH_3	3.7
1o	H	4-CN	5.4
1p	H	4-CO_2Me	8.4
1q	H	4-CO_2H	26
1r	H	4CH_2OH	10
1s	H	4-CH_2NH_2	27
1t	H	4-$CONH_2$	20
1u	4-F	H	2.5
1v	3-F	H	1.5
1w	2-F	H	0.6
1x	2,6-F_2	H	0.33
1y	2,4-F_2	H	4.2
1z	4-Br	H	6.0
1aa	4-Cl	H	5.2
1bb	4-CF_3	H	5.0
1cc	4-CH_3	H	3.0
1dd	4-CN	H	4.3
1ee	4-CO_2Me	H	2.0
1ff	4-CO_2H	H	5.0
1gg	4-CH_2OH	H	1.2
1hh	2-F	2-CH_3	0.8
1ii	2,6-F_2	4-F	0.33
1jj	2-F	4-F	0.20

[a]All values are average of at least two experiments.

molecule, some of them exhibited sub-micromolar IC_{50}'s. The mode of interaction between these inhibitors and the enzyme is still unknown in the absence of an X-ray structure. Further improvement will be published in due course.

Acknowledgements

The authors would like to thank Mr. Patrick Soucy for the biological evaluation of the molecules, and Dr. Gervais Bérubé for revising the manuscript.

References and Notes

1. (a) Barré-Sinoussi, F.; Chermann, J. C.; Rey, F.; Nugeyre, M. T.; Chamaret, S.; Gruest, J.; Dauguet, C.; Axler-Blin, C.; Vézinet-Brun, F.; Rouzioux, C.; Rozenbaum, W.; Montagnier, L. *Science* **1983**, *220*, 868. (b) Gallo, R. C.; Salhuddin, S. Z.; Popovic, M.; Shearer, G. M.; Kaplan, M.; Haynes, B. F.; Palker, T. J.; Redfield, R.; Oleske, J.; Safaia, B.; White, G.; Foster, P.; Markham, P. D. *Science* **1984**, *224*, 550.
2. McQuade, T. J.; Tomaselli, A. G.; Liu, L.; Karacostas, V.; Moss, B.; Sawyer, T. K.; Heinrikson, R. L.; Tarpley, W. G. A. *Science* **1990**, *247*, 454.
3. Kohl, N. E.; Emini, E. A.; Schleif, W. A.; Davis, L. J.; Heimbach, J. C.; Dixon, R. A. F.; Scolnick, E. M.; Siga, I. S. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6612.
4. (a) Babine, R. E.; Bender, S. L. *Chem. Rev.* **1997**, *97*, 1359. (b) Leung, D.; Abbenate, G.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 305.
5. Roberts, N. A.; Martin, J. A.; Kinchington, D.; Broadhurst, A. V.; Craig, J. C.; Duncun, I. B.; Galpin, S. A.; Handa, B. K.; Kay, J.; Krohn, A.; Redchaw, S.; Ritchie, A. J.; Taylor, D. L.; Thomas, G. J.; Machin, P. J. *Science* **1990**, *248*, 358.
6. Kempf, D. J.; Marsh, K. C.; Denissen, J. F.; McDonald, E.; Vasavanonda, S.; Flentge, C. A.; Green, B. E.; Fino, L.; Park, C. H.; Kong, X. P.; Wideburg, N. E.; Saldivar, A.; Ruiz, L.; Kati, W. M.; Sham, H. L.; Robbins, T.; Stewart, K. D.; Hsu, A.; Plattner, J. J.; Leonard, J. M.; Norbek, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 2484.
7. Vacca, J. P.; Dorsey, B. D.; Schleif, W. A.; Levin, R. B.; McDaniel, S. L.; Darke, P. L.; Zugay, J.; Quintero, J. C.; Blahy, O. M.; Roth, E.; Sardana, V. V.; Schlacach, A. J.; Graham, P. I.; Condra, J. H.; Gotlib, L.; Holloway, M K.; Lin, J.; Chen, I. W.; Vastag, K.; Ostovic, D.; Anderson, P. S.; Emini, E. A.; Huff, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4096.
8. Kaldor, S. W.; Kalish, V. J.; Davies, J. F., II; Shetty, B. V.; Fritz, J. F.; Appelt, K.; Burgess, J. A.; Campanal, K. M.; Chirgadze, N. Y.; Clawsson, D. K.; Dressman, B. A.; Hach, S. D.; Khalil, D. A.; Kosa, M. B.; Lubbehusen, P. P.; Muesing, M. A.; Patick, A. K.; Reich, S. H.; Sue, K. S.; Tatlock, J. H. *J. Med. Chem.* **1997**, *40*, 3979.
9. Kim, E. E.; Baker, C. T.; Dwyer, M. D.; Murcko, M. A.; Rao, B. J.; Tung, R. D.; Navia, M. A. *J. Am. Chem. Soc.* **1995**, *117*, 1181.
10. Stoll, V.; Qin, W.; Stewart, K. D.; Jakob, C.; Park, C.; Walter, K.; Simmer, R. L.; Hlfrich, R.; Bussier, D.; Kao, J.; Kempf, D.; Sham, H. L.; Borbeck, D. W. *Bioorg. Med. Chem.* **2002**, *10*, 2803.
11. (a) Molla, A.; Granneman, G. R.; Sun, E.; Kempf, D. *J. Antiviral Res.* **1998**, 1. (b) Flexner, C. J. *N. Engl. J. Med.* **1998**, *338*, 1281.
12. Sham, H. L.; Chen, X. *Exp. Opin. Invest. Drugs* **1996**, *5*, 977.
13. (a) See for example: Ghosh, A. K.; Mckee, S. P.; Thompson, W. J. *Tetrahedron Lett.* **1991**, *32*, 5729. (b) Chenara, B.; Boehm, J. C.; Dreyer, G. B. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 219. (c) Jadhav, P. K.; Man, H. W. *Tetrahedron Lett.* **1996**, *37*, 1153. (d) Zuccarello, G.; Bouzide, A.; Kvarnström, I.; Niklasson, G.; Svensson, S. C. T.; Brisander, M.; Danielsson, H.; Nillroth, U.; Karlén, A.; Hallberg, A.; Classon, B.; Samuelsson, B. *J. Org. Chem.* **1998**, *63*, 4898. (e) Chakraborty, T. K.; Ghosh, S.; Raman, Rao, M. H. V.; Kunwar, A. C.; Cho, H.; Ghosh, A. K. *Tetrahedron Lett.* **2000**, *41*, 10121. (f) Pyring, D.; Lindberg, J.; Rosenquist, A.; Zuccarello, G.; Kvarnström, I.; Zhang, H.; Vrang, L.; Unge, T.; Classon, B.; Hallberg, A.; Samuelsson, B. *J. Med. Chem.* **2001**, *44*, 3083. (g) Murphy, P. V.; O'Brien, J. L.; Goret-Feret, L. J.; Smith, A. B., III *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1763.
14. Morpain, C.; Tisserand, M. *J. Chem. Soc., Perkin Trans. 1* **1979**, 1379. Acetonide **2** is also commercially available.
15. Bouzide, A.; Sauvé, G. *Tetrahedron Lett.* **1997**, *38*, 5945.
16. Bouzide, A.; Sauvé, G. *Synlett* **1997**, 1153.
17. David, S.; Hanessian, S. *Tetrahedron* **1985**, *41*, 643.
18. Le Merrer, Y.; Duréault, A.; Greck, C.; Micas-Languin, D.; Gravier, C.; Depezay, J. C. *Heterocycles* **1987**, *25*, 541.
19. Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1574.
20. Matayoshi, E. D.; Wang, G. T.; Kraft, G. A.; Erickson, J. *Science* **1990**, *247*, 957.
21. (a) For other non-nitrogen containing HIV-protease inhibitors, see: Thaisrivong, S.; Tomich, P. K.; watenpaugh, K. D.; Chong, K.-T.; Howe, W. J.; Yang, C, P.; Strohbach, J. W.; Turner, S. R.; McGrath, J. P.; Bohanon, M. J.; Lyn, J. C.;

Mulichak, A. M.; Spinelli, P. A.; Hinshaw, R. R.; Pagano, P. J; Moon, J. B.; Ruwart, W. J.; Wilkinson, K. F.; Ruch, B. D.; Zipp, G. L.; Dalga, R. J.; Schwende, F. J.; Howard, G. M.; Padburry, G. E.; Toth, L. N.; Zhao, Z.; Koeplinger, K. A.; Kakuk, T. J.; Cole, S. L.; Zaya, R. M.; Piper, R. C.; Jeffrey, P. *J. Med. Chem.* **1994**, *37*, 3200. (b) Fan, X.; Flentke, G. R.; Rich, D. H. *J. Am. Chem. Soc.* **1998**, *120*, 8893.

22. Fullerene containing OH function was reported to inhibit HIV-Pr with a sub-micromolar K_i, see: Friedman, S. H.; Ganaphati, P. S.; Rubin, Y.; Kenyon, G. L. *J. Med. Chem.* **1998**, *41*, 2424. For other moderate inhibitors, see: Mahler, M. E.; Palmer, M. J. *Synlett* **1997**, 193.

23. Substitution at positions *ortho* and *meta* did not show any improvement as well.