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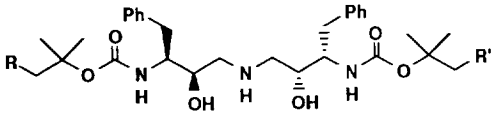
AMINODIOL HIV PROTEASE INHIBITORS. 2. 1,1-DIMETHYL-2-HYDROXYETHYL CARBAMATE DERIVATIVES WITH ENHANCED POTENCY

G. S. Bisacchi,* S. Ahmad, M. Alam, A. Ashfaq, J. Barrish, P. T. W. Cheng, J. Greytok, M. Hermsmeier, P.-F. Lin, Z. Merchant, M. Skoog, S. Spergel, R. Zahler

Bristol-Myers Squibb Pharmaceutical Research Institute
P. O. Box 4000, Princeton, N. J. 08543-4000

Abstract: A series of BOC-modified analogs of the aminodiol HIV protease inhibitor **BMS-182193** (**1**) was prepared and tested for inhibitory activity against the enzyme and the virus in cell culture. The hydroxy-modified analogs, **5** and **20**, showed enhanced potency against the protease and **5** was more active than **1** against the virus in cell culture.

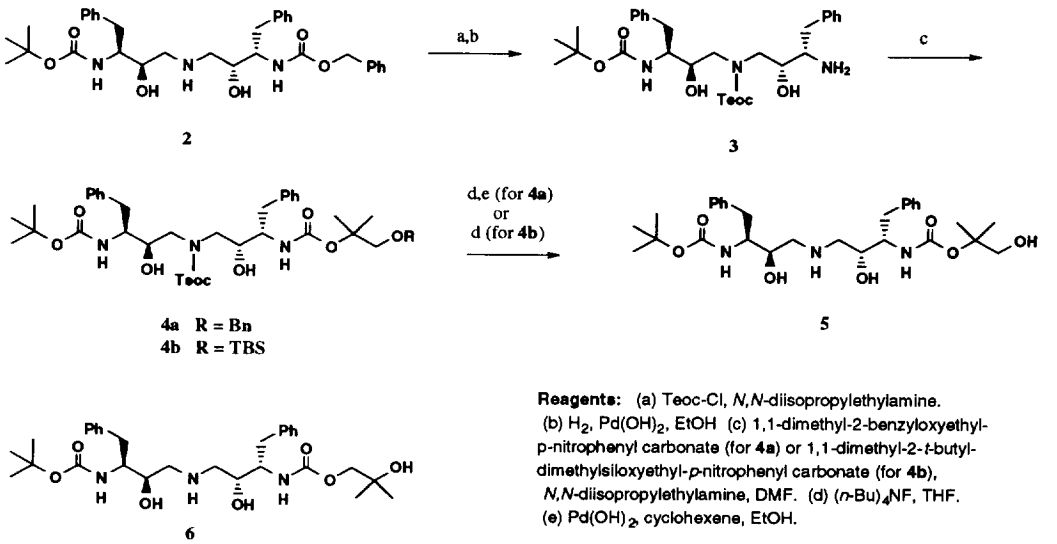
Recently we reported the discovery of a new class of selective HIV protease inhibitors which incorporates a C_2 symmetric aminodiol core as its key structural feature.[1] Members of this class, represented by the prototype compound **1**, display potent anti-HIV activity in cell culture. As part of our continuing investigation into this structural series, a number of modifications were made to the BOC group of compound **1** in order to probe additional potential hydrophobic and/or hydrogen bonding interactions with the protease. In particular it was found that compounds **5** and **20**, wherein one or both of the BOC groups of **1** is replaced by a 1,1-dimethyl-2-hydroxyethyl carbamate group, show enhanced potency against the enzyme compared to **1**. Compound **5** also displays a greater antiviral effect in cell culture than **1**.



1 R, R' = H BMS-182193
5 R = H, R' = OH
20 R, R' = OH

Our initial synthetic route leading to β-hydroxyethylcarbamate **5** is shown in Scheme 1. Protection of the central amino group of aminodiol **2**[1] with Teoc-Cl followed by removal of the CBZ group with H_2-$Pd(OH)_2$ afforded the primary amino compound **3** in 72% overall yield. Amine **3** was reacted with 1,1-dimethyl-2-benzyloxyethyl-*p*-nitrophenyl carbonate[2] in DMF in the presence of N,N'-diisopropylethylamine to provide **4a** in 55% yield. The Teoc group was removed using $(n\text{-Bu})_4NF$ at 50° in THF (70% yield). Finally, the benzyl group was removed under phase transfer hydrogenolysis conditions (cyclohexene, $Pd(OH)_2$, refluxing ethanol) to provide **5** in 32% yield. This unexpectedly low yield for the final debenzylation could not be improved by altering hydrogenolysis conditions (e.g. H_2, 10% Pd-C; H_2, $Pd(OH)_2$), and therefore a silyl protecting group was investigated as an alternative. Thus, reaction of amine **3** with 1,1-dimethyl-2-*t*-butyldimethylsiloxyethyl-*p*-nitrophenylcarbonate[2] afforded the *t*-butyldimethylsilyl-protected intermediate **4b**. However simultaneous removal of both Teoc and TBS groups by treatment of **4b** with $(n\text{-Bu})_4NF$ afforded a 93:7 mixture of desired **5** and its isomer **6**[3] (combined yield 80%) which was very difficult to separate by chromatography or crystallization. Ultimately the alternate synthetic methodology shown in Scheme 2 proved

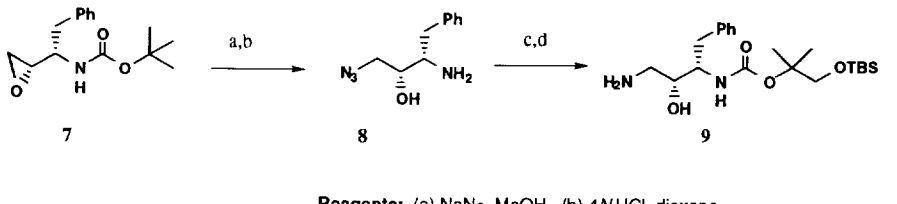
SCHEME 1



Reagents: (a) Teoc-Cl, *N,N*-diisopropylethylamine. (b) H_2, $Pd(OH)_2$, EtOH (c) 1,1-dimethyl-2-benzyloxyethyl-p-nitrophenyl carbonate (for **4a**) or 1,1-dimethyl-2-*t*-butyl-dimethylsiloxyethyl-*p*-nitrophenyl carbonate (for **4b**), *N,N*-diisopropylethylamine, DMF. (d) $(n\text{-Bu})_4NF$, THF. (e) $Pd(OH)_2$, cyclohexene, EtOH.

the most reliable for the preparation of **5** and related analogs. Thus epoxide **7**[1] was reacted with sodium azide followed by removal of the BOC group with 4*N* HCl to provide aminoazide **8**. Reaction of **8** with the activated silyl-protected carbonate followed by reduction of the azido group with H_2/Pd-C in MeOH afforded amine **9** (61% overall yield, four steps from epoxide **7**). Finally, reaction of **9** with epoxide **7** in DMF at 100°C followed by deprotection of the silyl group with HOAc-H_2O-THF 3:1:1 at room temperature afforded **5** in 59% overall yield. Compounds **10**-**19** (Tables 1 and 2) were prepared by methods analogous to those shown in Schemes 1 and 2.[5]

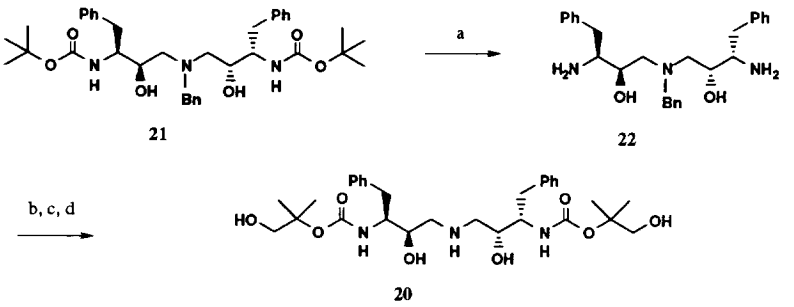
SCHEME 2



Reagents: (a) NaN_3, MeOH. (b) 4*N* HCl, dioxane. (c) 1,1-dimethyl-2-*t*-butyldimethylsiloxyethyl-*p*-nitrophenyl carbonate, *N,N*-diisopropylethylamine, DMF. (d) H_2, Pd-C, MeOH. (e) **7**, DMF, 100°C. (f) HOAc, H_2O, THF

Compound **20** was prepared as shown in Scheme 3. Thus, both BOC groups of N-benzyl protected aminodiol **21**[1] were removed with 4*N* HCl in dioxane to afford triamine **22**. Reaction of **22** with two equivalents of 1,1-dimethyl-2-*t*-butyldimethylsiloxyethyl-*p*-nitrophenyl carbonate followed by sequential deprotection of the N-benzyl (cyclohexene, $Pd(OH)_2$, refluxing ethanol) and TBS groups (HOAc-H_2O-THF 3:1:1) afforded **20** in 32% overall yield for 4 steps[5].

SCHEME 3



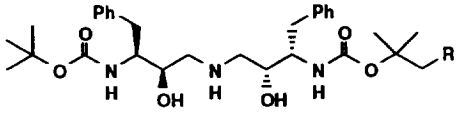
Reagents: (a) 4*N* HCl, dioxane. (b) 1,1-dimethyl-2-*t*-butyldimethylsiloxyethyl-*p*-nitrophenyl carbonate, *N,N*-diisopropylethylamine, DMF. (c) $Pd(OH)_2$, cyclohexene, EtOH. (d) HOAc, H_2O, THF

From Table 1 it can be seen that the 1,1-dimethyl-2-hydroxyethyl carbonate moiety provides a 4-fold increase in potency against HIV-1 protease (compound **5**; IC_{50} = 35nM) compared to the corresponding BOC analog (compound **1**; IC_{50} = 125nM).[6] Extending the distance of the hydroxy group of **5** away from the aminediol core results in less potent compounds (**10**; IC_{50} = 200nM and **11**; IC_{50} = 1,100nM). Replacing the hydroxy moiety of **5** with a methoxy group results in a substantial loss in potency (**12**; IC_{50} = 420nM) suggesting that the hydroxy group of **5** may be donating a hydrogen bond to the enzyme. However, other hydrogen bond donating groups did not effectively substitute for a hydroxy group: the formamido analog **13** (IC_{50} = 1500nM) was much less potent than even the parent unsubstituted BOC derivative **1**, while an amino analog **14** was totally inactive (IC_{50} = >10,000 nM) undoubtedly due to its cationic character at the assay pH (5.5[1]).

The compounds in Table 2 examine the effect of various steric modifications to the key β-hydroxyethylcarbamate pharmacophore as well as the effect of additional β-hydroxyethyl groups within the same molecule. Diastereomeric β-methyl carbamates **15** and **16** (IC_{50}'s = 160nM and 105nM respectively) as well as the α-cyclobutyl analog **17** (IC_{50} = 100nM) demonstrate that increasing the steric and/or hydrophobic environment around the β-hydroxyethyl group results in loss of potency compared to **5**. In the same manner, a decrease in hydrophobicity by the elimination of the two α-methyl groups of the carbamate in **5** also results in a less potent analog (**18**; IC_{50} = 230nM). Compound **19** (IC_{50} = 200nM) incorporating two hydroxyethyl moieties on the same carbamate is also less potent than the parent monohydroxyethyl carbamate **5**. These results suggest that the hydrophobic S_2 pocket into which the carbamate alkyl moiety fits accommodates a group very close in size to an isopentyl. In contrast, the C_2 symmetric bis-hydroxyethyl carbamate **20** (IC_{50} = 16nM) displays twice the potency compared to compound **5**, which suggests that each hydroxy group of the 1,1-dimethyl-2-hydroxyethyl carbonate moiety of compound **20** may be contributing to binding to the enzyme.

The co-crystal structure of compound **1** complexed to HIV-1 protease has recently been determined[8,9] and that structure provides insights into the possible hydrogen bond interaction(s) of the hydroxy group of **5** with the enzyme. The crystal structure showed the BOC *t*-butyl groups to reside symmetrically in the S_2 sites of the protease. Constrained molecular dynamics calculations[10] were carried out allowing the 1,1-dimethyl-2-hydroxyethyl carbamate moiety of **5** to freely rotate within one S_2 pocket while maintaining the original positions of the enzyme as well as the remainder of the structure of aminediol **5**. The lowest energy

Table 1. Structure and Inhibitory Potencies of Substituted BOC Derivatives



compd	R	IC_{50}[a] (nM)	ED_{50}[b,c](nM)
1	**-H**	125	80[d]
5	**-OH**	35	38[e]
10	**$-CH_2OH$**	200	175
11	**$-(CH_2)_2OH$**	1100	650
12	**$-OCH_3$**	420	1035
13	**-NHCHO**	1500	610
14	**$-NH_2$**	>10,000	nd[f]

[a]Concentration needed to inhibit cleavage of V-S-Q-N-(b-naphthylalanine)-P-I-V by 50%.[1] [b]Concentration needed to inhibit virus replication by 50% as determined by an XTT endpoint;[1] average of two determinations (n=2) unless otherwise noted. [c]Cytotoxicity[1] was ≥10,000 nM for all compounds. [d](n=45). [e](n=6). [f]Not determined.

conformation which was found is shown in Figure 1[11]. As seen in the Figure, the carbamate hydroxy group of **5** donates a hydrogen bond to the backbone carbonyl of Asp 30 and accepts a hydrogen bond from the Asp 30 -NH. This model provides possible explanations for the diminished activities of several analogs related to **5**. Methoxy analog **10** does not provide the hydrogen-bond donation component of **5** and the added methyl group may be sterically disadvantageous. While the -NH moiety of the formamide analog **13** should be able to provide both H-bond donating and accepting capability similar to **5**, the formyl group is probably too

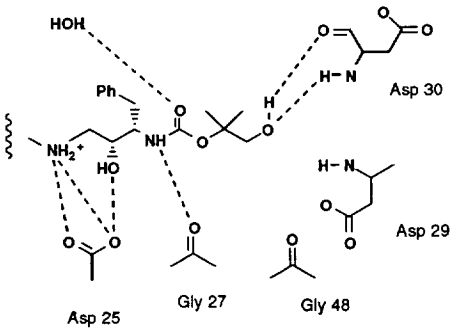
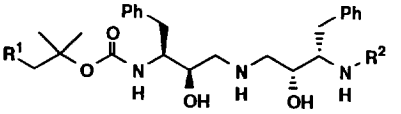
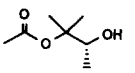
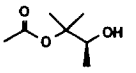
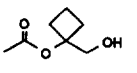
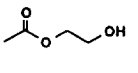
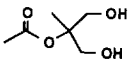
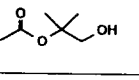


Figure 1. Proposed binding mode of aminodiol 5

Table 2. Structure and Inhibitory Potencies of β-Hydroxyethyl Carbamates Related to Compound 5[a]



compd	R^1	R^2	IC_{50} (nM)	ED_{50} (nM)[b]
15	-H		160	320
16	-H		105	35[c]
17	-H		100	155
18	-H		230	280
19	-H		200	nd[d]
20	-OH		16	843[e]

[a]See Table 1 for definitions. [b]Cytotoxicity was >10,000 nM for all compounds. [c](n = 4). [d]not determined; see footnote 14. [e](n = 3)

encumbering in the S_2 pocket to allow these interactions. Hydroxy homologs **11** and **12** likely position the hydroxyl group into orientations not favorable for hydrogen bonding with Asp 30.

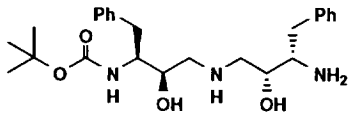
Most of the compounds of Tables 1 and 2 were tested for their ability to inhibit the replication of the HIV-1 virus in cell culture. It can be seen that the anti-HIV activity of **5** in cell culture is twice that of the prototype compound **1**. As can be readily observed, the measured ED_{50} values are generally quite close to the IC_{50} values for enzyme inhibition as had been noted previously for other members of the aminediol class[1]. One notable exception however is the bis-hydroxyethyl carbamate **20** wherein the cell culture ED_{50} value is 53-fold greater than the IC_{50} value. One likely explanation is that the greater polarity of **20** compared to the other analogs impedes its passage across the lipophilic cell membrane.

Hydroxycarbamate **5** is also potent against HIV-2 in cell culture (ED_{50} = 103nM, n = 4; strain CBL-20). Additionally, the aqueous solubility of hydroxycarbamate **5** is substantially improved compared to **1**. In pH 7 HEPES buffer, the solubility of **1** is 5μM whereas the solubility of **5** is increased 20-fold (100μM).

In conclusion, substitution of one or both of the BOC groups of aminodiol **1** with a 1,1-dimethyl-2-hydroxyethyl carbamate group affords compounds **5** and **20**, respectively, which are more potent inhibitors of HIV protease than **1**. Additionally, **5** shows improved activity against HIV-1 in cell culture and is more (20X) water-soluble than aminodiol **1**.

References and Notes

1) Barrish, J. C..; Gordon, E.; Alam, M.; Lin, P.-F.; Bisacchi, G. S.; Chen, P.; Cheng, P. T. W.; Fritz, A. W.; Greytok, J. A.; Hermsmeier, M. A.; Humphreys, W. G.; Lis, K. A.; Marella, M. A.; Merchant, Z.; Mitt, T.; Morrison, R. A.; Obermeier, M. T.; Pluscec, J.; Skoog, M.; Slusarchyk, W. A.; Spergel, S. H.; Stevenson, J. M.; Sun, C.-Q.; Sundeen, J. E.; Taunk, P.; Tino, J. A.; Warrack, B. M.; Colonno, R.; Zahler, R. *J. Med. Chem.*, **1994**, *37*, 1758.
2) 1,1-Dimethyl-2-benzyloxyethyl-p-nitrophenyl carbonate and 1,1-dimethyl-2-t-butyldimethylsiloxyethyl-p-nitrophenylcarbonate were prepared by reaction of p-nitrophenylchloroformate with, respectively, 1,1-dimethyl-2-benzyloxyethanol and 1,1-dimethyl-2-t-butyldimethylsiloxyethanol.
3) Compound **6** likely arises via a 5-membered ring internal rearrangement of the 1,1-dimethyl-1,4-dioxyethyl moiety of **5** promoted by the highly basic environment of the TBAF deprotection conditions. See Ref. 4 for an example of a similar rearrangement.
4) Sklavounos, C.; Goldman, I. M.; Kuhla, D. E. *J. Org. Chem.*, **1980**, *35*, 4239.
5) Proton and carbon NMR, infra red and mass spectra are consistent with assigned structures.
6) Interestingly, the 1,1-dimethyl-2-hydroxyethyl carbamate group has not been employed as a pharmacophore in medicinal chemistry until very recently: see Ref. 7.
7) (a) Carr, J. T.; DeMarsh, L. P.; Dreyer, B. G.; Fenwick, E. A.; World Pat. Appl. WO 93/02057, 1993 (b) Treiber, L. R.; Lingham, R. B.; Arison, B. H.; Colweil, L. F., Jr.; Dezeny, G.; Kohl, N. E. Eur. Pat. Appl. EP 480624, 1992.
8) Thanki, N.; Wlodawer, A.; Hermsmeier, M.; Barrish, J.; Tino, J., unpublished results. Details will be reported in a separate paper.
9) The X-ray structure of enzyme-bound BMS-182193 (**1**) differed slightly from the structure predicted from modelling (Ref. 1). In the X-ray structure, the enzyme-bound water molecule donates hydrogen bonds symmetrically to each BOC carbonyl of **1**. Also, each hydroxy group of **1** forms a hydrogen bond symmetrically with each catalytic aspartate residue.
10) The molecular dynamics simulation used the GB/SA solvent model for water (Ref. 12) along with the Kollman united atom AMBER force field (Ref. 13) as implemented in the BatchMin V4.0 program. The -NH-BOC portion of the inhibitor as well as 11 amino acids which form the S_2 pocket were allowed to freely move (the Cα of those amino acids, the remainder of the inhibitor and a shell of surrounding amino acids where included in the simulation but were held fixed.) Ten picoseconds of molecular dynamics at 300 degrees followed by 10 picoseconds ramping the temperature down to 100 degrees were carried out on 8 different orientations of the hydroxy boc group. The final energy of each orientation was taken after 500 steps of energy minimization.
11) Two other, slightly higher energy binding modes between the enzyme and the hydroxy of the 1,1-dimethyl-2-hydroxyethyl carbamate group were found using the above described modelling methodology. One of these involved H-bond donations from each backbone NH of Asp 29 and Asp 30 and the other involved a H-bond donation from the hydroxy to the carbonyl of Gly 48. Besides being higher in energy than the interaction mode depicted in Figure 1, these alternate modes were less effective at rationalizing the observed SAR for analogs **10** and **13**.
12) Still W.C.; Tempczyk, A.; Hawley, R.C.; Hendrickson, T. *J. Am. Chem . Soc.* **1990**, *112* , 6127.
13) Weiner, S.J.; Kollman, P.A.; Case, D. A.; Singh, U.C.; Ghio, C.; Alagona, G.; Profeta, S.; Weiner, P. *J. Am. Chem . Soc.* **1984**, *106* , 765.
14) Diol **19** was not assayed in cell culture due to its instability at the pH of the assay ($t_{1/2}$ = 18hr; pH 7.2; determined by analytical HPLC). The major decomposition product is compound **23** resulting from loss of the bis-hydroxymethylcarbamate moiety. Compound **23** was shown separately to be essentially devoid of activity against the protease (IC_{50} > 10,000nM).



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