

New, potent P1/P2-morpholinone-based HIV-protease inhibitors

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Abstract—We have developed efficient synthesis of morpholinone-based cyclic mimetics of the P1/P2 portion of the HIV-1 protease inhibitor Amprenavir. This effort led to discovery of allyl- and spiro-cyclopropyl—P2-substituted inhibitors **17** and **31**, both 500 times more potent than the parent inhibitor **1**. These results support morpholinones as novel mimetics of the P1/P2 portion of Amprenavir and potentially of other HIV-protease inhibitors, and thus provide a novel medicinal chemistry template for optimization toward more potent and drug-like inhibitors.

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Proteolytic cleavage of *gag* and *gag-pol* gene protein products by the virally encoded aspartyl protease (HIV-PR) is one of the key steps in the life cycle of HIV. Several HIV-protease inhibitors (PI), such as Amprenavir (Fig. 1), have been developed for use in Highly Active Antiretroviral Therapy (HAART), which is credited for a dramatic reduction in AIDS-related mortality and morbidity.¹ Nonetheless, the high pill burden generally required by the early HAART regimens is not conducive to patient compliance, and thus may result in the emergence of viral resistance. In order to address this issue, we have been involved in a research program directed toward PI prodrugs with improved solubility^{2,3} as well as next generation PIs with increased potency, by exploring heterocyclic mimetics for the P1/P2 portion of Amprenavir.^{4–9}

In the latter approach, we have explored cyclic lactams, cyclic sulfonamides, cyclic ureas, and cyclic sulfamates among other heterocyclic scaffolds,^{4–9} and in this letter we report our findings with the morpholinone ring system as a putative P1/P2 scaffold. Molecular modeling supported the design rationale toward compound **1**, obtained from (5*S*)-5-(phenylmethyl)-3-morpholinone (A = B = H) and the Amprenavir-like P1'/P2' scaffold (R1 in Fig. 1). This prototype molecule indeed exhibited moderate potency in the HIV-1 protease enzyme assay ($K_i = 1.0 \mu\text{M}$).¹⁰ Encouraged by this result, we

embarked on a medicinal chemistry program incorporating P2 substituents in the morpholinone scaffold in order to optimize the potency in this series.^{4,10}

To that end, we have explored a number of approaches toward mono-, di-, and spiro-substituted morpholinones in order to establish the SAR in this series. Herein, we disclose chemical routes toward analogues of **1**, which enabled the discovery of potent inhibitors, such as **17** and **31**.

Our initial target was the racemic 2-methyl-5-(phenylmethyl)-3-morpholinone, which was obtained according to the previously published method.¹⁰ Interestingly, we found that the Williamson cyclization of the diastereomeric mixture **2** resulted in the exclusive formation of a pure (2*S*,5*S*)-2-methyl-5-(benzyl)-morpholin-3-one **3** in 34% yield (Fig. 2).^{11–14} While thermodynamic equilibration leading to a single diastereomer cannot be ruled out as the mechanism leading to pure **3**, molecular modeling also supports a kinetic argument in that cyclization of the *S*,*R*-**2** diastereomer appeared energetically much more facile, thus enabling the formation of 2*S*,5*S*-**3**, while the cyclization of the *S*,*S*-**2** diastereomer appears disfavored, precluding the formation of 2*R*,5*S*-**3**.^{14,15}

Fragment **3** was next coupled to epoxide **4** in a fashion similar to one previously reported,^{4,10} yielding HIV-PR inhibitor **5** ($K_i = 38 \text{ nM}$, Table 1), which was 50 times more potent than the parent compound **1**.

We already reported that in the related 3,5-(P1/P2)-di-substituted pyrrolidone scaffold,⁴ the 3*S*,5*R*-isomers

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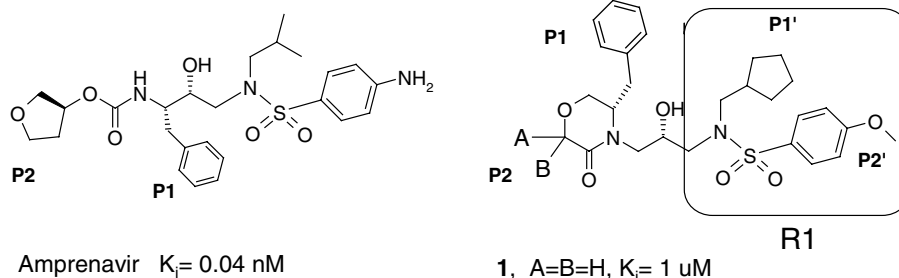


Figure 1. Structure of Amprenavir and its morpholinone P1/P2 mimetic-containing analogue **1**.

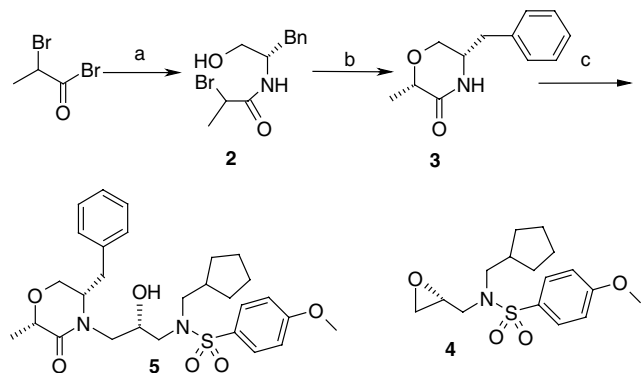


Figure 2. (a) DIEA, (*S*)-phenylalaninol; (b) NaH, DMF, 0 °C to rt, 1 h; 34% yield over two steps; (c) NaH, DMF, 0–80 °C, 1–3 h, **4**, 94%.

Table 1. Inhibitory potencies of final products in the HIV-PR assay¹⁸

Compound	K_i [nM]
1	1000
5	38
9	490
10	55
17	2
18	270
19	35
21	30
23	9
24	81
25	600
27	11
28	5800
29	49
31	2
35	23

(with *trans* rather than *cis* relationship of P1 and P2 substituents) were more potent in the HIV-PR enzyme assay.^{4,5} To explore such stereochemical preference in the morpholinone series described herein, in addition to Williamson chemistry yielding the 2*S*,5*S* (e.g., 2*S*,5*S*-**3**) diastereomer, we also needed to develop routes toward 2*R*,5*S* diastereomers. To this end, we explored the alkylation of *p*-methoxybenzyl-protected morpholinone **6** (Fig. 3) and of Boc-protected morpholinone **12** (Fig. 4). The alkylation of **6** with *m*-CN-benzyl bromide proceeded with a modest diastereoselectivity of 3:1, 2*R*,5*S*/2*S*,5*S*. The resulting mixture **7** was then deprotected and coupled with sulfate **8**, and after

chromatography, provided the individual 2*S*,5*S* and 2*R*,5*S* diastereomers **9** and **10**, respectively.¹¹

Interestingly, the 2*R*,5*S* diastereomer **10** turned out to be about 10 times more potent than 2*S*,5*R* isomer **9**, revealing that stereochemical preference also existed in the morpholinone scaffold (Table 1). Consequently, we explored other approaches to optically pure diastereomers or to easily separable mixtures of diastereomers. Thus, the alkylation of **12** with allyl iodide proceeded with a much higher diastereoselectivity (95:5%, Fig. 4), yielding essentially pure 2*R*,5*S*-**13**,^{4,11} and enabling facile entry into the desired diastereomer series.

We also examined the alkylation of advanced intermediate **14**, a TBDMS-protected product of the condensation of morpholinone **11**¹⁰ and epoxide **4**, with allyl iodide.^{10,16} Although the alkylation of **14** proceeded with modest diastereoselectivity, the resulting mixture of **15** (2*R*,5*S*-morpholin-3-one) and **16** (2*S*,5*S*-morpholin-3-one) was readily separable on silica gel, affording inhibitors **17** and **18** after treatment of **15** and **16** with 1 M TBAF in THF. Similarly, the alkylation of **14** with benzyl bromide also yielded a mixture of diastereomers, affording the 2*R*,5*S*-morpholin-3-one **19**, after chromatographic separation of the minor 2*S*,5*S* component (Fig. 4).¹¹ The stereochemical outcome of these alkylations is likely controlled by $A_{1,3}$ allylic strains in **12** and **14**.¹⁷

Although we found that the alkylation of **12** could provide a single diastereomer, we decided that a more practical route toward diastereomerically pure 2*R*,5*S* morpholin-3-one-based inhibitors (Fig. 5) would be to utilize the easily available and scalable 2*R*,5*S* intermediate **15**. Accordingly, inhibitor **21** incorporating the 2-hydroxyethane as P2 was obtained in several steps from alcohol **20**. The conversion of **20** to mesylate **22** followed by the treatment of the latter with lithium hexamethyldisilazide and deprotection with 1 M TBAF in tetrahydrofuran yielded novel spiro-cyclopropyl analogue **23**. Furthermore, mesylate **22** was also converted to azide **24**, and the latter next reduced to 2-aminoethyl P2-substituted inhibitor **25**. Finally, **22** was converted to nitrile **26**, which served as a convenient intermediate toward inhibitors **27–29** (Fig. 5).

A more than 100-fold increase in potency against the HIV-1 PR¹⁸ was observed in the case of spiro-cyclopropyl inhibitor **23** versus reference compound **1** (Table 1).

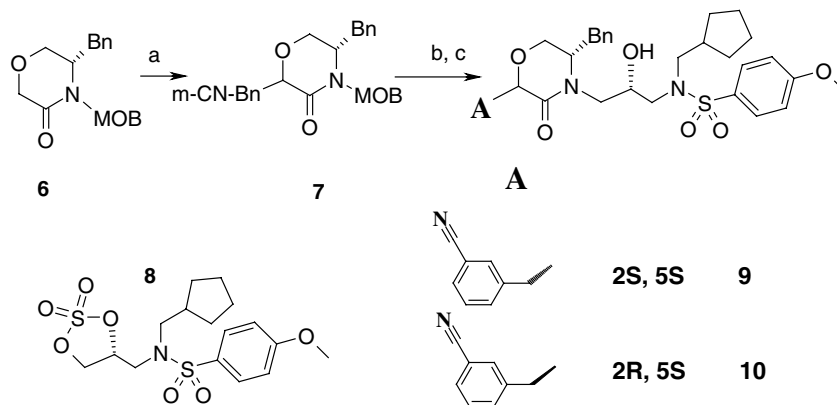


Figure 3. (a) LHMDS, -78°C ; *m*-CN-benzyl bromide; 74%; (b) CAN in MeCN/water, 25%; (c) P4-phosphazene, 1 equiv, -20°C , THF, cyclic sulfate **8**, 1 N aq sulfuric acid, 93% (cumulative for both diastereomers **9** and **10**).

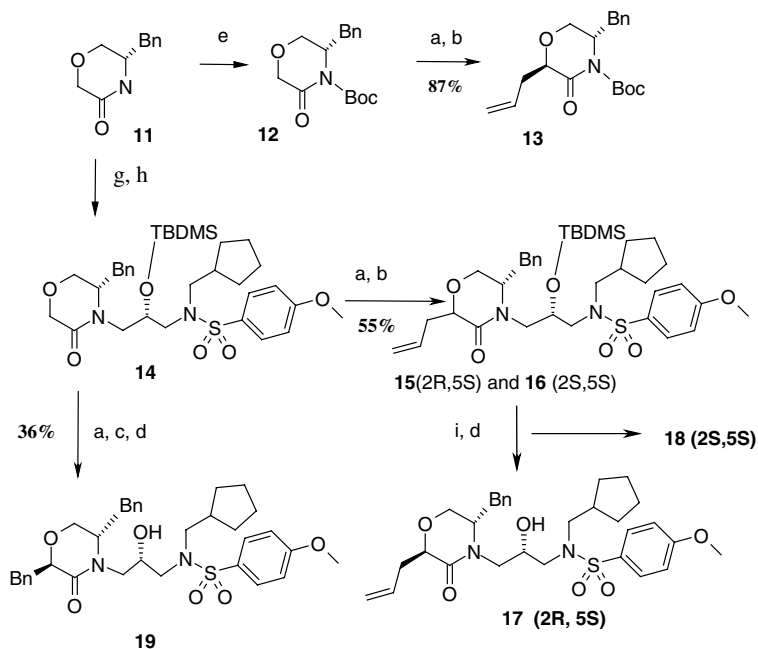


Figure 4. (a) LHMDS, -78°C ; (b) allyl iodide, 1.5 h at -78°C ; (c) BnBr; (d) 1 M TBAF in THF and chromatography, 85–95%; (e) Boc₂O, DMAP, CH₃CN, 96%; (g) NaH, **4**, DMF, 80°C ; (h) TBDMS-triflate, 79%, two steps; (i) silica gel chromatography.

This prompted synthetic explorations of spiro-cyclic morpholinones such as **30**,^{12,13} which afforded potent inhibitor **31** (Fig. 6, $K_i = 1.7\text{ nM}$).¹⁹

A somewhat different synthetic strategy was developed toward the spiro-tetrahydropyran-based morpholinone **33**,²⁰ obtained in 45% yield by alkylation of intermediate **6**^{10,11} with bis-*O*-iodoethane. The only major byproduct of this reaction, the cross-linked derivative **32**, could be easily separated and a subsequent deprotection of **33** and coupling with sulfate **8** yielded potent inhibitor **35** (Table 1).

In summary, we developed efficient chemistry toward substituted and stereochemically defined analogues of **1**. In particular, advanced intermediate **15** enabled facile and convergent syntheses of several 2*R*,5*S*-morpholin-3-ones (Table 1). Analogues **17** and **31** were

found 500-fold, while analogues **23** and **27** 100-fold more potent in comparison to reference compound **1**.¹⁰ Consistent with the knowledge of the inhibitor binding site in the HIV protease, we also found out that polar P2 residues, such as a primary amine in **28**, substantially reduced the inhibitory potency. In contrast, the isosteric inhibitor **21** (OH in place of NH₂) maintained relatively high inhibitory potency (Table 1). We thus conclude that 2*R*,5*S*-disubstituted morpholin-3-ones, especially those with small and non-polar P2-substituents, can be suitable mimetics of the P1/P2 portion of Amprenavir²¹ and conceivably of other HIV-1 protease inhibitors. These findings support the rationale for additional explorations in the morpholinone scaffold with the goal of further improving the potency and potentially fine-tuning drug-like properties in this class of inhibitors. This work will be reported in due course.

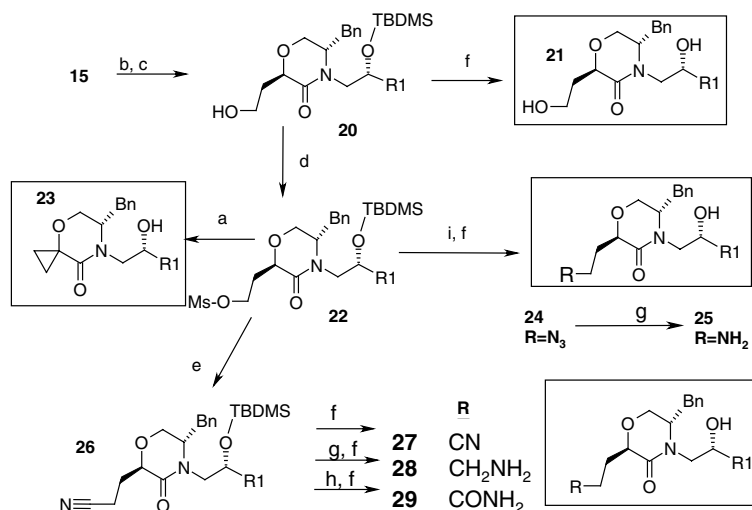


Figure 5. (a) LHMDS, $-78\text{ }^{\circ}\text{C}$, 90%; (b) OsO_4 , NaClO_4 , 86%; (c) NaBH_4 in MeOH (titration), 93%; (d) MsCl , DIEA, 73%; (e) NaCN , DMSO, rt, overnight; 81%; (f) 1 M TBAF in THF, 85–95%; (g) $\text{H}_2/\text{Ra-Ni}$, 56–67%; (h) urea hydrogen peroxide, 2 h, rt, pH = 10, 82%; (i) NaN_3 , DMF, $80\text{ }^{\circ}\text{C}$, 91%.

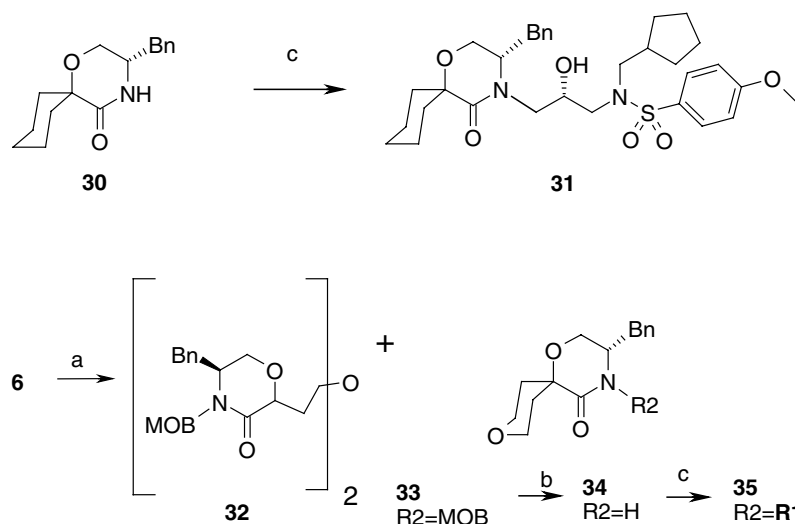


Figure 6. (a) LHMDS, $-78\text{ }^{\circ}\text{C}$, $(\text{I}-\text{CH}_2-\text{CH}_2)_2\text{O}$, 45% of **33**; (b) CAN, overnight, 33%; (c) P4-phosphazene, 1 equiv at $-20\text{ }^{\circ}\text{C}$, THF, cyclic sulfate **8** then 1 N aq sulfuric acid, 31–45%.

References and notes

- Moyle, G. J. *Expert Opin. Invest. Drugs* **1998**, 7, 1313.
- Kazmierski, W. M.; Bevans, P.; Furfine, E.; Spaltenstein, A.; Yang, H. *Bioorg. Med. Chem. Lett.* **2003**, 13, 2523.
- Kazmierski, W. M.; Bevans, P.; Furfine, E.; Porter, D.; Spaltenstein, A.; Yang, H. 223rd ACS National Meeting, Orlando, FL, United States, April 7–11, 2002, Abstract MEDI-160, 2002.
- Spaltenstein, A.; Cleary, D.; Bock, B.; Kazmierski, W.; Furfine, E.; Wright, L.; Hazen, R.; Andrews, W.; Almond, M.; Tung, R.; Salituro, F. *Bioorg. Med. Chem. Lett.* **2000**, 10, 1159.
- Kazmierski, W. M.; Andrews, W.; Furfine, E.; Spaltenstein, A.; Wright, L. L. *Bioorg. Med. Chem. Lett.* **2004**, 14, 5689.
- Kazmierski, W. M.; Furfine, E.; Spaltenstein, A.; Wright, L. L. *Bioorg. Med. Chem. Lett.* **2002**, 12, 3431.
- Kazmierski, W. M.; Furfine, E.; Gray-Nunez, Y.; Spaltenstein, A.; Wright, L. L. *Bioorg. Med. Chem. Lett.* **2004**, 14, 5685.
- Kazmierski, W.; Andrews, W.; Baker, C.; Bock, B.; Cleary, D.; Furfine, E.; Gray-Nunez, Y.; Hale, M.; Hazen, R.; Salituro, F.; Schairer, W.; Spaltenstein, A.; Tung, R.; Wright, L. Book of Abstracts, 219th ACS National Meeting, San Francisco, CA, March 26–30, 2000, Abstract MEDI-126, 2000.
- Kazmierski, W.; Furfine, E.; Spaltenstein, A.; Wright, L. In *Peptides 2002*, Proceedings of the European Peptide Symposium, 27th, Sorrento, Italy, August 31–September 6, 2002; Benedetti, E., Pedone, C., Eds.; pp 972–973.
- Salituro, F. G.; Baker, C. T.; Court, J. J.; Deininger, D. D.; Kim, E. E.; Li, B.; Novak, P. M.; Rao, B. G.; Pazhanisamy, S.; Porter, M. D.; Schairer, W. C.; Tung, R. D. *Bioorg. Med. Chem. Lett.* **1998**, 8, 3637.
- Stereochemical assignments were confirmed with NOE ^1H NMR spectroscopy experiments.

12. Fritch, P. C.; Kazmierski, W. M. *Synthesis* **1999**, *1*, 112.
13. Fritch, P. C.; Kazmierski, W. M. In *Peptidomimetics Protocols*; Kazmierski, W. M., Ed.; Humana Press: Totowa, NJ, 1998; pp 281–292.
14. TenBrink, R. E.; Pals, D. T.; Harris, D. W.; Johnson, G. A. *J. Med. Chem.* **1988**, *31*, 671.
15. Breton, P.; Monsigny, M.; Mayer, R. *Int. J. Pept. Protein Res.* **1990**, *35*, 346.
16. Baker, C. T.; Salituro, F. G.; Court, J. J.; Deininger, D. D.; Kim, E. E.; Li, B.; Novak, P. M.; Rao, B. G.; Pazhanisamy, S.; Schairer, W. C.; Tung, R. D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3631.
17. Norman, B. H.; Kroin, J. S. *Tetrahedron Lett.* **1995**, *36*, 4151.
18. Toth, M. V.; Marshall, G. R. *Int. J. Peptide Protein Res.* **1990**, *36*, 544.
19. Inhibitor **31** was synthesized from 0.159 g of **30**,^{12,13} 4.4 mg of NaH, and 0.20 g of **4**,¹⁰ yielding 111 mg of **31** (yield 31%). Compound **31** ¹H NMR (CDCl₃, 300 MHz) δ 7.52 (d, 2H), 7.30 (m, 5H), 6.95 (d, 2H), 4.05 (m, 1H), 3.87 (3H, s), 3.60 (m, 2H), 3.16 (m, 4H), 3.0 (m, 4H), 2.18 (1H, m), 1.97 (m, 2H), 1.60 (m, 14H), 1.23 (m, 4H).
20. Compound **33** ¹H NMR: (CDCl₃, 300 MHz) δ 7.82 (m, 4H, ar), 7.18 (m, 3H, ar), 6.87 (d, J = 8.5 Hz, 2H, ar), 5.36 (d, J = 14.7 Hz, 1H, N–CH), 3.82 (m, 8H, OCH₃+N–CH+2 $\times$ O–CH₂), 3.62 (app. d, $J_{\text{app.}}$ = 12.1, 1H, O–CH₂–CH α), 3.54 (app. d, $J_{\text{app.}}$ = 12.1 Hz, 1H, O–CH₂–CH α), 3.28 (m, 1H, CH– α), 3.00 (m, 2H, CH– β), 2.42 (m, 1H, Cq–CH), 2.25 (m, 1H, Cq–CH), 1.78 (app. d, $J_{\text{app.}}$ = 14 Hz, 1H, Cq–CH), 1.64 (app. d, $J_{\text{app.}}$ = 13.1 Hz, 1H, Cq–CH). Compound **33** ¹³C NMR (CDCl₃, 75.42 MHz): 134.3 (ar), 126.3 (ar), 125.6 (ar), 123.7 (ar), 111.1 (ar), 75.5 (Cq), 59.5 (CH₂), 57.4 (CH₂), 53.3 (O–CH₃), 52.2 (CH_a), 44.3 (CH₂), 34.0 (CH₂), 32.0 (CH₂), 27.3 (CH₂). Compound **34** ¹H NMR (CDCl₃, 300 MHz) δ 7.25 (m, 5H, ar), 5.43 (s, 1H, NH), 3.78 (m, 7H, CH α +3 $\times$ O–CH₂), 2.95 (dd, J = 6.0, 13.6 Hz, 1H, CH–CH₂–phenyl), 2.74 (dd, J = 8.4, 13.6 Hz, 1H, CH–CH₂–phenyl), 2.25 (m, 2H, spiro-cyclic CH₂), 1.74 (m, apparent ddd J = 2.1, 8.1, 16.0 Hz, 2H, spiro-cyclic CH₂).
21. Kim, E. E.; Baker, C. T.; Dwyer, M. D.; Murcko, M. A.; Rao, B. G.; Tung, R. D.; Navia, M. A. *J. Am. Chem. Soc.* **1995**, *117*, 1181.