Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 4059–4063

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Inhibitors of Hepatitis C Virus NS3·4A Protease 1. Non-Charged Tetrapeptide Variants

Robert B. Perni,* Shawn D. Britt, John C. Court, Lawrence F. Courtney, David D. Deininger, Luc J. Farmer, Cynthia A. Gates, Scott L. Harbeson, Joseph L. Kim, James A. Landro, Rhonda B. Levin, Yu-Ping Luong, Ethan T. O'Malley, Janos Pitlik, B. Govinda Rao, Wayne C. Schairer, John A. Thomson, Roger D. Tung, John H. Van Drie and Yunyi Wei

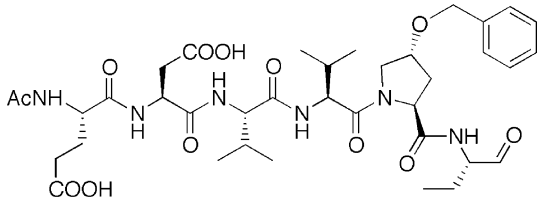
Vertex Pharmaceuticals Inc., 130 Waverly Street, Cambridge, MA 02139, USA

Received 13 June 2003; accepted 18 August 2003

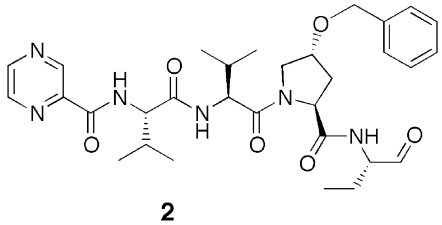
Abstract—Tetrapeptide-based peptidomimetic compounds have been shown to effectively inhibit the hepatitis C virus NS3·4A protease without the need of a charged functionality. An aldehyde is used as a prototype reversible electrophilic warhead. The SAR of the P_1 and P_2 inhibitor positions is discussed.

HCV infection has reached epidemic proportion worldwide and to date therapy options are limited and clinical results are often unsatisfactory. Prevalence of the disease and currently available treatments have been recently reviewed.[1] Inhibition of the hepatitis C virus NS3·4A protease has been an intense area of research since the mid-1990s and numerous groups have reported progress in the field.[2–4]

The shallow, hydrophobic, highly flexible binding pocket of the NS3·4A protein represents a formidable challenge to drug design and only recently has a compound been reported to enter human testing.[5] Inclusion of terminal charged groups (e.g., carboxylates) on either or both sides of the enzyme active site has proven to provide substantial biochemical potency. Indeed, product inhibition of the NS3·4A proteolytic reaction have formed the basis of many design efforts.[6] However, charged groups often result in compromising cellular penetration and in vivo pharmacokinetics.[7] Reversible covalent binding to the catalytic serine is an alternative to electrostatic binding that has been utilized in other serine protease inhibitor series.[8] A series of peptide aldehydes have recently been reported which exploits this approach, but these compounds require carboxylate residues to attain reasonable potency.[9] Deletion of the acidic residues at P_5 and P_6 results in a dramatic decrease in binding affinity. We herein report a series of potent tetrapeptide inhibitors that do not require carboxyl groups for binding potency and therefore offer improved potential for cell potency.



1



2

We and others have recently described the effects of truncating substrates and substrate-derived competitive inhibitors from the optimal recognition length of ten

*Corresponding author. Tel.: +1-617-444-6237; fax: +1-617-444-6766; e-mail: robert_perni@vrtx.com

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.08.050

amino acids down to a four to six residue length.[6b,10] Based on this work, we synthesized inhibitor **1** ($K_i = 0.89$ μM).[4] Removal of the P_5 and P_6 amino acids and capping with a neutral heterocycle provided **2**, which possesses reasonable inhibitory activity ($K_i = 12$ μM) as a prototype tetrapeptide inhibitor. This latter compound served as the basis for our subsequent optimization efforts.

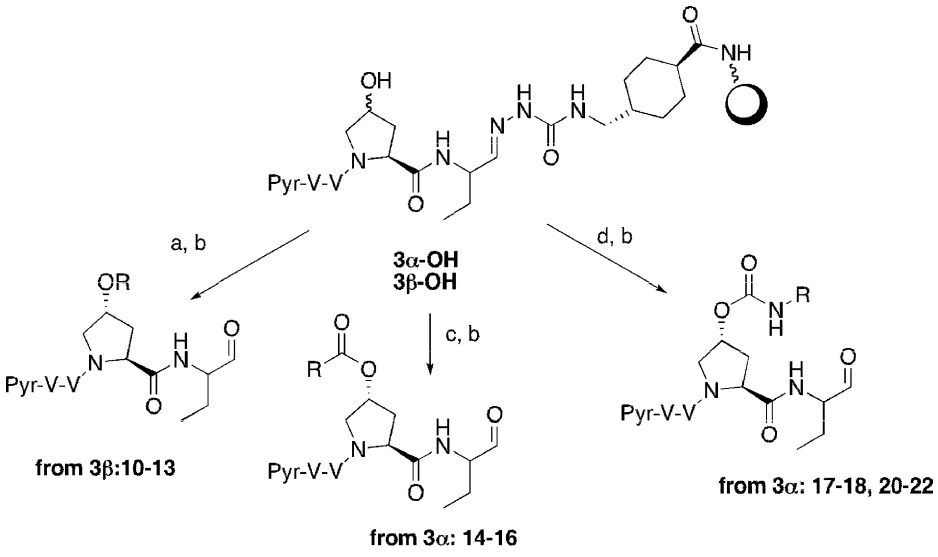
Compound **2** was prepared from commercially available 4-benzyloxyproline as we have previously reported.[4] A related method for the solid phase synthesis of peptidic aldehydes has been published by others.[11] P_2 derivatives were prepared via solid-phase methodology utilizing a semicarbazone linker. P_2 ethers, esters and carbamates were prepared as shown in Scheme 1 using standard methodology followed by removal of the solid support under acidic conditions.

Carbamate **19** and benzylic ethers **8** and **9** were prepared by preassembly of the P_2 group (**5a** and **5b**) via the chemistry shown in Scheme 2. The proline intermediates, **5a** and **5b**, were then elaborated to the complete tetrapeptide via standard peptide coupling methodology.

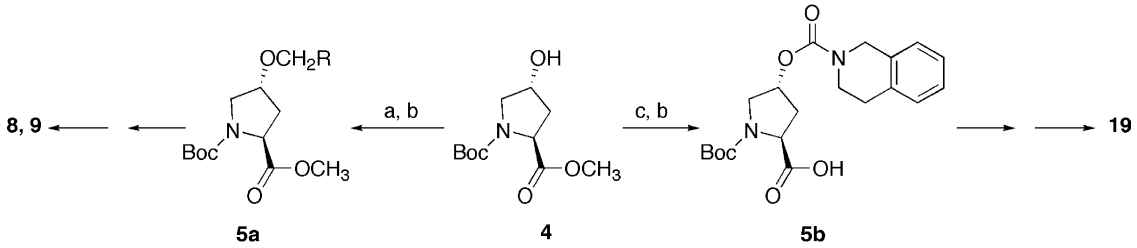
P_1 modified analogues of compound **2** were prepared as shown in Scheme 3. Tripeptide **7** was prepared via standard peptide coupling chemistry. The carboxylic acid **7** was coupled to the corresponding amino alcohol and subsequently oxidized with DMP to give the final products, **23**–**26**. The P_1 position was usually epimerized during the oxidation step.

Compounds were evaluated for enzyme-binding activity (K_i) via colorimetric monitoring of the hydrolysis of an HCV NS5A-pNA substrate.[10] The P_2 SAR (Table 1) resists any simple interpretation. In a related series of compounds (data not shown) L isomers were always more potent than D isomers consistent with the P_1 geometry of the natural substrate. Comparing compounds **2** and **8**, and compounds **14** and **15**, one may be tempted to infer that increased steric bulk and/or π-density improves affinity. However, the phenyl on compound **10** represents one of the smallest groups, yet yields potency comparable to the naphthyl. The orientation of the P_2 group clearly influences affinity, as evidenced by compounds **11** ($K_i = 2.1$ μM) and **12** ($K_i = 7.4$ μM), and compounds **15** ($K_i = 1.9$ μM) and **16** ($K_i = 0.4$ μM). It appears that this influence is due to interactions with the side chains forming the S_2 pocket, Arg181, Asp107, and His83, the latter two part of the catalytic triad. Further analysis of these interactions with structure-based methods should allow us to refine our understanding of them.

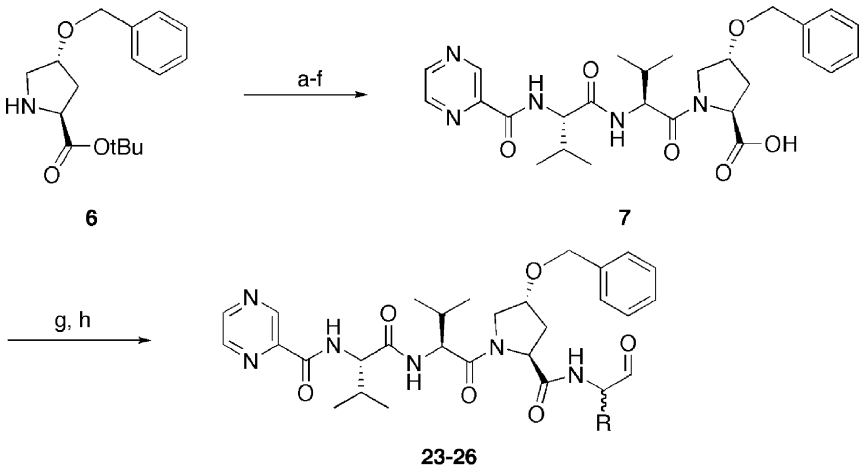
The linkage of the P_2 substituent to the proline also has an effect. In general, esters provide more potency relative to ether linked substituents. Unfortunately, esters were found to be too hydrolytically unstable to be use-



Scheme 1. (a) PPh_3, DEAD, ROH; (b) AcOH, THF, HCHO or 50% TFA/DCM; (c) RCOCl, *i*PrNEt$_2$, CH_2Cl_2 DCM or RCOOH, HOB*t*, HBTU, NMP; (d) RNCO or RCOOH, $(PhO)_2P(O)N_3$.

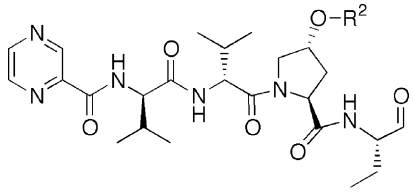


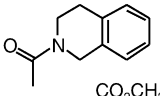
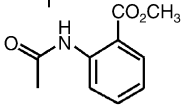
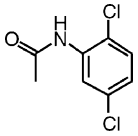
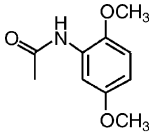
Scheme 2. (a) NaH, RCH_2Br or RCH_2Cl, DMF/THF; (b) LiOH, H_2O, THF; (c) carbonyldiimidazole, tetrahydroquinoline, CH_2Cl_2.



Scheme 3. (a) Fmoc-Val-NCA, iPr$_2$NEt, CH_2Cl_2; (b) Et_2NH, CH_3CN; (c) Fmoc-Val-NCA, iPr$_2$NEt, CH_2Cl_2; (d) Et_2NH, CH_3CN; (e) pyrazinecarboxylic acid, HOBt, EDC, iPr$_2$NEt, CH_2Cl_2; (f) 4 N HCl/dioxane; (g) amino alcohol, EDC, HOBt, CH_2Cl_2; (h) DMP, CH_2Cl_2 or Swern.

Table 1. Inhibition of the HCV NS3·4A protease enzyme by tetrapeptide P_2 variants



	R^2	P1 Stereochemistry[a]	K_i (μM)
2	$CH_2C_6H_5$	L	12
8	CH_2-1-Naphthyl	D,L	2.9
9	CH_2-2-Naphthyl	D,L	1.7
10	C_6H_5	D,L	3.9
11	1-Naphthyl	D,L	2.1
12	2-Naphthyl	L	7.4
13	8-Quinolinyl	D,L	> 50
14	COC_6H_5	D,L	5.8
15	CO-1-Naphthyl	D,L	1.9
16	CO-2-Naphthyl	D,L	0.40
17	$CONHC_6H_5$	L	22.3
18	CONH-1-Naphthyl	L	12.5
19		D,L	0.89
20		D,L	2.5
21		L	3.2
22		D,L	7.9

[a]Where indicated, single isomers were separated from a D,L mixture by HPLC.

ful beyond determining inhibition constants. Simple primary carbamates as an alternative to esters (e.g., **17**–**18** and **20**–**22**) were prepared and were found to be less potent relative to ester **16.** However, carbamates **20**–**22** were essentially equipotent to esters **14** and **15**. Electron donating, or electron withdrawing groups appear to have little influence on binding affinity. A naphthyl carbamate, **18**, however shows improved potency relative to the phenyl carbamate **17**.

To address the hydrolytic instability of the ester groups, the tetrahydroisoquinolinyl (THIQ) carbamate **19** was synthesized. The carbamate nitrogen is incorporated into the ring system of **19** to mimic ester **16** in terms of overall steric demand. Figure 1 shows the X-ray structures of **16** and **19** in identical orientations. While the scaffold itself binds identically in both cases, including the OC=O link, the rings of the naphthyl and THIQ do not. The THIQ rings of **19** are rotated approximately 180°. Additionally, the puckered aliphatic THIQ ring tilts the rings away from the space occupied by the naphthyl group of **16**. The low RMS deviation for the molecule excluding the bicyclic rings (RMSD = 0.3 Å, maximum interatom variability 0.6 Å) implies that the S_2 pocket accommodates the different orientations but does not force the orientation on them. If the protein required the change in conformation of the P_2 ring systems it would likely disturb the scaffold backbone as well. Inhibitor **19** did show improved binding relative to primary carbamates and similar potency to **16** (K_i = 0.89 μM vs K_i = 0.40 μM, respectively).

The S_1 specificity pocket is defined by the Leu135, Phe154, and Ala157 side chains and allows only for the inclusion of relatively small, preferably hydrophobic P_1 substituents. This is the specificity pocket[9] that provides excellent selectivity versus the clotting cascade enzymes such as thrombin, kallikrein and factor Xa all of which require basic substitution at P_1. The consensus sequence for all *trans* cleavage sites, NS4A-4B, NS4B-5A and NS5A-5B incorporates a cysteine at P_1. The incorporation of an electrophilic warhead in an inhibitor is

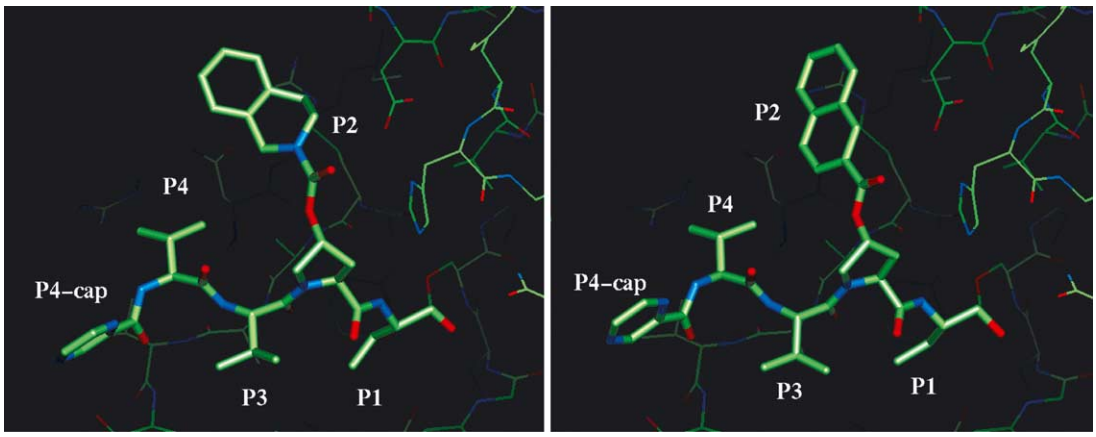
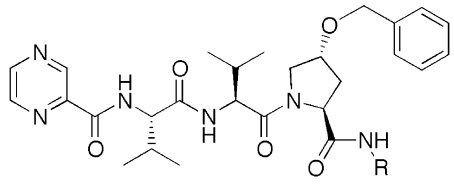
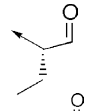
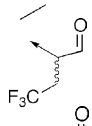
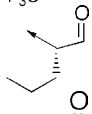
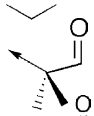
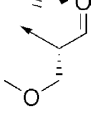


Figure 1. X-ray comparison of **16** (left, resolution 2.9 Å) and **19** (right, resolution 2.8 Å).

Table 2. Inhibition of the HCV NS3·4A protease enzyme by tetrapeptide P_1variants

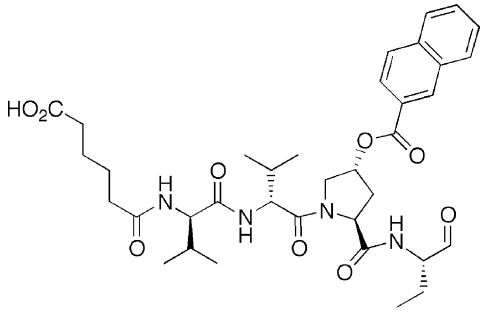


	R[a]	K_i (μM)
2		12
23		3.1
24		3.8
25		>50
26		>50

[a]Compounds are single P_1 isomers except for **23** which was prepared as mixture of epimers which were not separated.

consequently incompatible with a cysteine residue at P_1 due to intermolecular and possibly intramolecular reactivity. Table 2 summarizes the P_1 SAR for a series of small substituents replacing the cysteine side chain with non-reactive groups. Replacing the cysteine sulfur with a carbon atom results in an ethyl (amino butyric acid derivative, e.g, **2**) P_1 side chain, The ethyl P_1 side-chain while an effective cysteine surrogate is not the optimal group for the S_1 subsite. Increasing the hydrophobicity of the side-chain in the form of trifluoroethyl (**23** as an epimeric P_1 mixture) or propyl (norvaline, **24**) substituents resulted in more potent inhibitors. Though expensive, 4,4,4-trifluoro-2-aminobutyric acid is commercially available as a racemate. Norvaline is commercially available as single isomer. Given that the trifluoroethyl group provides no significant advantage over the *n*-propyl group, norvaline was chosen as the P_1 substituent with which to pursue optimization of the scaffold.[12] Not surprisingly the geminal substitution (**25**) at P_1 significantly reduces binding affinity while the introduction of an oxygen atom (**26**) also is substantially detrimental to activity. These data are consistent with those reported for charged, non covalent P_1 termini.[13,14]

Incorporation of a single charged P_4 terminus afforded **26**[15] ($K_i = 0.11$ μm), which demonstrated improved potency relative to the bis-carboxylate (P_5 and P_6) **1** ($K_i = 0.89$ μM). Lengthening or shortening the chain length has little effect on potency (data not shown).



26

In summary, using an aldehyde as a prototype electrophilic covalent warhead, a series of P_1 and P_2 analogues were prepared as an initial step in the optimization of a tetrapeptide HCV NS3·4A protease inhibitor scaffold. Small non-polar substituents at P_1 were found to be optimal, consistent with results previously obtained with non-covalent peptidic inhibitors.[13,14] Large hydrophobic substituents appended to the 4-position of a proline residue at P_2 in an appropriate vector were found to provide substantial binding recognition by the protease, resulting in the discovery of a sub-micromolar tetrapeptide inhibitor. We believe this important class of inhibitors offers great potential for the treatment of HCV infection in humans.

References and Notes

1. Tam, S.-L.; Pause, A.; Shi, Y.; Sonenberg, N. *Nature Rev.* **2002**, *1*, 867. McHutchison, J. G.; Patel, K. *Hepatology* **2002**, *36*, S245.
2. (a) Narjes, F.; Koch, U.; Steinkühler, C. *Exp. Op. Inves. New Drugs* **2003**, *12*, 153. Perni, R. B.; Kwong, A. D. In *Progress in Medicinal Chemistry*, King F. D., Oxford, A. W., Eds. Elsevier Science B.V.: Amsterdam, 2002; Volume 39, p 215
3. Recent representative peptide inhibitor papers: (a) Llinas-Brunet, M.; Bailey, M.; Fazal, G.; Ghiro, E.; Gorys, V.; Goulet, S.; Halmos, T.; Maurice, R.; Poirier, M.; Poupart, M.-A.; Rancourt, J.; Thibeault, D.; Wernic, D.; Lamarre, D. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2267. (b) Han, W.; Hu, Z.; Jiang, X.; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 711. (c) Johansson, A.; Poliakov, A.; Akerblom, E.; Lindeberg, G.; Winiwarter, S.; Samuelsson, B.; Danielson, U. H.; Hallberg, A. *Bioorg. Med. Chem.* **2002**, *12*, 3915. (d) Nizi, E.; Koch, U.; Ponzi, S.; Matassa, V. G.; Gardelli, C. *Bioorg. Med, Chem. Lett.* **2002**, *12*, 3325.
4. Tung, R. D.; Harbeson, S. L.; Deininger, D. D.; Murcko, M. A.; Bhisetti, G. R.; Farmer, L. J. PCT WO 98/17679, **1998**.
5. Lamarre, D.; Bailey, M.; Bolger, G.; Cameron, D.; Cartier, M.; Faucher, A.-M.; Goudreau, N.; Kukolj, G.; Lagace, L.; Pause, A.; Rancourt, J.; Thibeault, D.; Tsantrizos, Y.; Llinas-Brunet, M. *Hepatology* **2002**, *36*, 92A (Abstract #464). Hinrichsen, H.; Benhamou, Y.; Reiser, M.; Sentjens, R.; Wedemeyer, H.; Calleja, J. L.; Forns, X.; Cronlein, J.; Nehmiz, G.; Steinmann, G. *Hepatology* **2002**, *36*, 379A (Abstract #866).
6. Product based inhibitors: (a) Steinkühler, C.; Basiol, G.; Brunetti, M.; Urbani, A.; Koch, U.; Cortese, R.; Pessi, A.; De Francesco, R. *Biochemistry* **1998**, *37*, 8899. (b) Ingallinella, P.; Altamura, S.; Bianchi, E.; Taliani, M.; Ingenito, R.; Cortese, R.; De Francesco, R.; Steinkühler, C.; Pessi, A. *Biochemistry* **1998**, *37*, 8906.
7. For a recent example of this phenomenon see: Larsen, S. D.; Stevens, F. C.; Lindberg, T. J.; Bodnar, P. M.; O'Sullivan, T. J.; Schostarez, H. J.; Palazuk, B. J.; Bleasdale, J. E. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 971.
8. Leung, D.; Abbenante, G.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 305.
9. Attwood, M. R.; Bennet, J. M.; Campbell, A. D.; Canning, G. G. M.; Carr, M. G.; Conway, E.; Dunsdon, R. M.; Greening, J. R.; Jones, P. S.; Kay, P. B.; Handa, B. K.; Hurst, D. N.; Jennings, N. S.; Jordon, S.; Keech, E.; O'Brien, M. A.; Overton, H. A.; King-Underwood, J.; Raynham, T. M. *Antiviral Chem. Chemother.* **1999**, *10*, 259.
10. Landro, J. A.; Raybuck, S. A.; Luong, Y. P. C.; O'Malley, E. T.; Harbeson, S. L.; Morgenstern, K. A.; Rao, B. G.; Livingston, D. *Biochemistry* **1997**, *36*, 9340. Details of the hexapeptide to tetrapeptide scaffold truncation are the subject of a manuscript currently in preparation.
11. Ede, N. J.; Eagle, S. N.; Wickham, G.; Bray, A. M.; Warnes, B.; Shoemaker, K.; Rosenberg, S. *J. Pept. Sci.* **2000**, *6*, 11.
12. Further optimization of the tetrapeptide scaffold described in this paper is the subject of manuscripts currently in preparation.
13. Llinas-Brunet, M.; Bailey, M.; Deziel, R.; Fazal, G.; Gorys, V.; Goulet, S.; Halmos, T.; Maurice, R.; Poirier, M.; Poupart, M.-A.; Rancourt, J.; Thibeault, D.; Wernic, D.; Lamarre, D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2719.
14. For recent reports of novel P1 substituents see: (a) Tsantrizos, Y.; Bolger, G.; Bonneau, P.; Cameron, D. R.; Goudreau, N.; Kukolj, G.; LaPlante, S. R.; Llina-Brunet, M.; Nar, H.; Lamarre, D. *Angew. Chem. Int. Ed.* **2003**, *42*, 1355. (b) Poupart, M.-A.; Cameron, D. R.; Chabot, C.; Ghiro, E.; Goudreau, N.; Goulet, S.; Poirier, M.; Tzantrizos, Y. *J. Org. Chem.* **2001**, *66*, 4743. (c) Narjes, F.; Koehler, K. F.; Koch, U.; Gerlach, B.; Colarusso, S.; Steinküler, C.; Brunetti, M.; Altamura, S.; DeFrancesco, R.; Matassa, V. G. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 701.
15. Compound **26** was prepared according to the route shown in Scheme 1. The terminal carboxylate was introduced into the scaffold by using the unprotected dicarboxylic acid in large excess under standard coupling conditions.