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Practical and efficient method for amino acid derivatives containing β-quaternary center: application toward synthesis of hepatitis C virus NS3 serine protease inhibitors

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Abstract—A practical and efficient route toward synthesis of amino acid derivatives containing β-quaternary center has been developed using diastereoselective Strecker reaction. The method was employed for preparation of >100 g of β-methylcyclohexyl glycine derivative, **21**. Incorporation of some of the hindered amino acid derivatives at the P3 position resulted in potent HCV NS3 serine protease inhibitors.

Hepatitis C virus (HCV) infection is a global health crisis leading to liver cirrhosis, hepatocellular carcinoma and liver failure in humans.[1] SCH 503034, a HCV NS3 serine protease inhibitor, discovered in our labs is currently undergoing clinical studies in humans.[2] The structure of 503034 is shown in Figure 1. The ketoamide moiety acts as the electrophile, trapping the active site serine. X-ray structure of the inhibitor bound to the protease revealed P1, P2, P3, and P3 cap moieties occupied the corresponding pockets on the enzyme, which resulted in the observed potency.

During the course of our studies leading to potent HCV NS3 serine protease inhibitors, we identified L-*tert*-leucine and L-cyclohexylglycine as suitable P3 residues. In an effort to further explore the P3 area, we decided to introduce β-methyl cyclohexyl (or β-methyl cycloalkyl) glycine, combination of the aforementioned amino acid derivatives containing β-quaternary center, at that position. Hence we turned our attention toward the synthesis of these hindered amino acids.

β-Quaternary centered amino acids are useful class of nonnatural amino acids that occur as components in pharmaceuticals and natural products.[3] Numerous methods are available for stereoselective α-amino acid synthesis. However, α-amino acids containing β-quaternary center are not trivial to synthesize.[4] We decided to employ the Strecker reaction for preparation of these nonproteinogenic hindered amino acid derivatives. Only few reports have appeared in the literature detailing use of Strecker protocol for construction of amino acids containing β-quaternary center.[5] Herein we describe a practical and efficient method for the diastereoselective synthesis of β-methylcycloalkyl glycine derivatives. Furthermore, P3 exploration with these nonnatural amino acids resulted in potent HCV NS3 serine protease inhibitors.

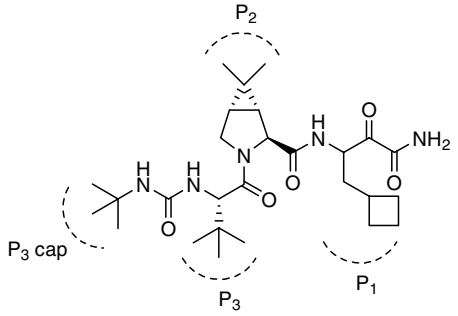

Figure 1. Structure of SCH 503034.

Aldehyde precursors for the diastereoselective Strecker reaction were prepared as shown in Scheme 1.

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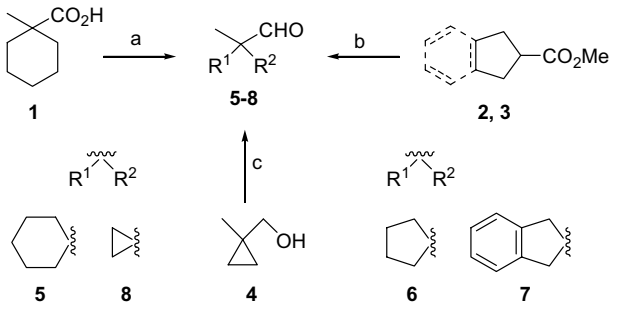
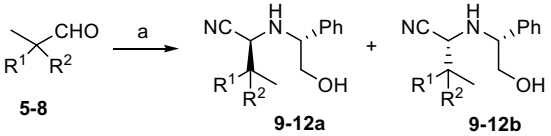


Scheme 1. Reagents and conditions: (a) (i) MeOH, concd HCl (cat.), reflux, 16 h; (ii) DIBAL or LAH, CH_2Cl_2, −78 °C to rt, 16 h; (iii) PCC or DM periodinane, CH_2Cl_2, 0 °C to rt, 16 h; (b) (i) LDA or KHMDS, MeI, THF, 0–10 °C, 2 h; then repeat step (a) (ii) and (iii); (c) repeat step (a) (iii).

Commercially available acid **1** was converted to the corresponding methyl ester that was subsequently processed to aldehyde **5** via a two-step reduction/oxidation process. Aldehydes **6** and **7** were prepared from the respective methyl esters **2** and **3** by alkylation followed by the reduction/oxidation method. Aldehyde **8** was synthesized by oxidation of the commercial alcohol **4** using PCC.

The aforementioned aldehydes were then subjected to stereoselective Strecker reaction (Scheme 2). Thus, treatment of various aldehydes with *R*-2-phenyl glycinol in anhydrous chloroform at room temperature resulted in in situ formation of the corresponding imines. Addition of TMSCN to the cooled solution of the imines followed by slow warming to room temperature afforded the desired amino nitriles **9**–**12** with high diastereoselectivity. The required *S*-amino nitrile was obtained as the major product in all cases. Following completion of the reaction, part of the product mixture remained protected as the TMS–ether. The free alcohol could be easily regenerated by treatment with aqueous 3 N HCl. The diastereoselectivity and yield for the Strecker reaction with various aldehydes are listed in Table 1.[6]

The mixture of amino nitrile products described above were hydrolyzed to the respective methyl esters **13**–**16a/b** using anhydrous HCl/MeOH conditions. It should be noted that amino esters **13**–**16a/b** were easier to separate on silica gel column compared to their amino nitrile counterpart **9**–**12a/b.** Thus, the required major *S*-isomers **13**–**16a** was separated and the chiral auxiliary was removed under hydrogenation conditions, with concomitant protection of the liberated amino residue to give *N*-*t*-Boc intermediates **17**–**20**. Hydrolysis of the methyl ester using LiOH provided the expected N-protected amino acid derivatives **21**–**24**.



Scheme 2. Reagents and conditions: (a) *R*-2-Phenylglycinol (1 equiv), $CHCl_3$, rt, 2 h. Then add TMSCN (2 equiv), 0 °C to rt, 16 h.

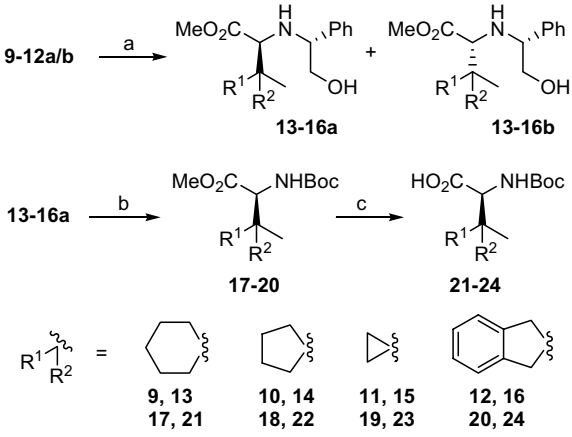
Table 1.

SM	Amino nitrile		Ratio **a:b**[a]	Yield[b] (%)
	a	**b**		
1	**9a**	**9b**	8:1	85
2	**10a**	**10b**	9:1	26
4	**11a**	**11b**	7:1	37
3	**12a**	**12b**	6:1	32

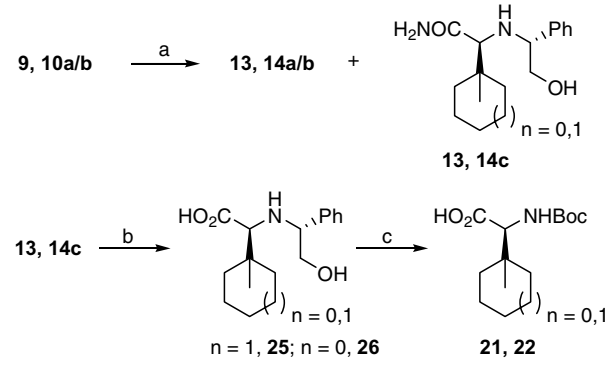
[a] Ratio determined from ^{1}H NMR of crude material after Strecker reaction or after hydrolysis to the amino methylester.

[b] Yield reported for spectroscopically pure product from corresponding SM.

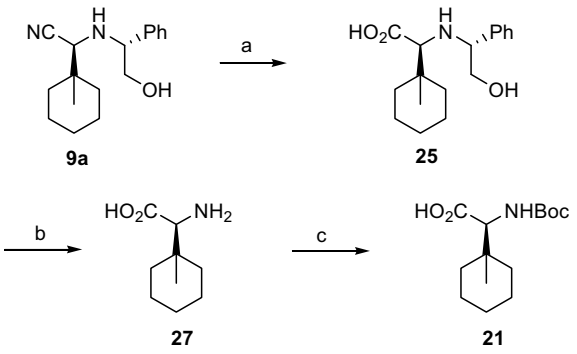
While the above described reaction sequence (Scheme 3) worked well on a small scale, conversion of the amino nitrile **9**, **10a/b** to amino ester **13**, **14a/b** were generally low on a larger scale (>20 mmol).[7] Careful analysis of the reaction mixture showed the presence of amino amide **13**, **14c**, resulting from partial hydrolysis of nitrile functionality. It was interesting to note that the amino amides **13**, **14c** were isolated (via crystallization or chromatography) as single diastereomer with *S*-stereochemistry at the α-center. These amino amides were also



Scheme 3. Reagents and conditions: (a) 6 M HCl/MeOH, 0 °C to rt, 16 h, add another portion of 6 M HCl/MeOH reflux, 16 h (43–45%); (b) H_2, 10% Pd/C or $Pd(OH)_2$, Boc_2O, EtOAc or MeOH, rt, 16 h (88–100%); (c) aq 1 M LiOH, THF, MeOH, rt, 4–48 h.



Scheme 4. Reagents and conditions: (a) Condition (a) as in Scheme 3 (**13c**—40%, **14c**—32%); (b) aq 6 M HCl, reflux, 16 h (91–100%); (c) (i) H_2, 10% Pd/C MeOH, rt, 16 h; (ii) Boc_2O, NaOH, dioxane/water, rt, 16 h.



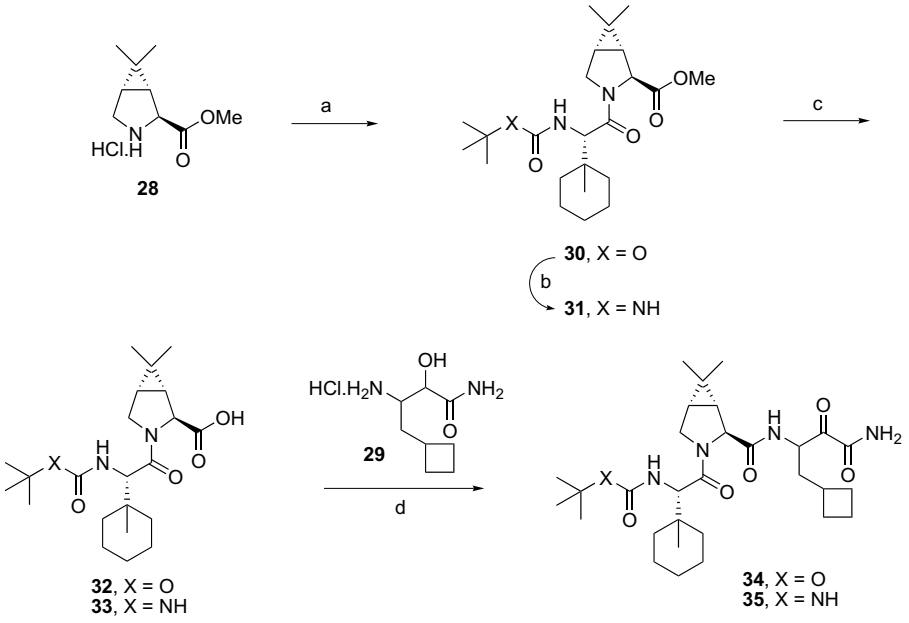
Scheme 5. Reagents and conditions: (a) (i) 6 M HCl/MeOH, 0 °C to rt, 3 d; (ii) aq 6 M HCl, reflux, 2 d; (b) H_2, 10% Pd/C MeOH, rt, 16 h; (c) Boc_2O, NaOH, dioxane/water, rt, 16 h (53% for three steps).

progressed further to the required N-protected amino acid derivatives **21**, **22** as shown in Scheme 4.

Although we were able to identify all the products of amino nitrile hydrolysis and maximize the yield of the required amino acid derivatives **21** and **22**, this method was not very practical on a large scale. Since we needed modified cyclohexylglycine derivative **21** in larger quantities, a practical and efficient route to this hindered amino acid was then investigated. Thus, our optimized conditions are shown in Scheme 5. After the diastereoselective Strecker reaction, the major *S*-amino nitrile **9a** was obtained via silica gel column chromatography. Amino nitrile **9a** was converted to acid **25** in a one-pot two-step process. Initial treatment with 6 M HCl in MeOH resulted in a mixture of amino ester and amino amide. The solvent was then removed and the residue was refluxed with aqueous 6 N HCl when the acid **25** precipitated out (on cooling the reaction mixture). Removal of the chiral auxiliary using catalytic hydrogenation condition followed by protection of the amino residue resulted in the desired compound **21** as a solid in 53% yield (for three steps).[8]

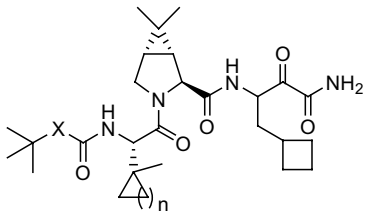
Having obtained the hindered amino acid derivatives, we then set out to incorporate those at the P3 position of NS3 serine protease inhibitors. Synthesis of inhibitors derived from **21** is shown in Scheme 6. Modified proline derivative **28**[9] was coupled to **21** under standard coupling conditions (HATU, DIPEA). Basic hydrolysis resulted in acid **32**, which was subsequently coupled with previously described intermediate **29**.[2] Oxidation under modified Moffatt protocol afforded the desired inhibitor **34**. Urea capped inhibitor **35** was obtained from **30** as follows: removal of *t*-Boc functionality followed by treatment with *t*-butylisocyanate provided dipeptide **31**. Further processing to inhibitor **35** followed procedures described in Scheme 6. Inhibitors **36**–**38** described in Table 2 were also prepared from the corresponding P3 amino acid derivatives, **22** and **23**, in a similar manner.

Targets **34**–**38** were then tested for HCV NS3 serine protease inhibition[10] using the continuous spectrophotometric assay described earlier[11] and the results are summarized in Table 2. Cyclization of two of the methyl groups of L-*tert*-leucine (see Fig. 1) to a cyclopropyl ring resulted in a drastic loss in potency as evidenced by the K_i^* value of inhibitors **37** and **38**, 700 nm and 280 nm, respectively. Increasing the ring size resulted in inhibitor **36** containing β-methyl cyclopentylglycine moiety at P3, with improved potency ($K_i^* = 23$ nm) compared to



Scheme 6. Reagents and conditions: (a) **21**, HATU (1.3 equiv), DIPEA (3 equiv), CH_2Cl_2/DMF, −20–0 °C, 16 h; (b) (i) 4 M HCl/dioxane, rt, 3 h; (ii) *tert*-butylisocyanate (1.3 equiv), DIPEA (2.5 equiv), CH_2Cl_2, 0 °C, 16 h (55%); (c) aq 1 M LiOH, THF, MeOH, rt, 4 h; (d) (i) **29**, HATU (1.3 equiv), DIPEA (3 equiv), CH_2Cl_2/DMF, −20–0 °C, 2 d; (ii) EDCI (10 equiv), dichloroacetic acid (5 equiv), DMSO/toluene, 0 °C to rt, 5 h (for **34**—46% after four steps, for **35**—59% after two steps).

Table 2.



Compd #	X	n	K_i^* (nm)	EC_{90}[a] (nM)
37	O	1	700	nt
38	NH	1	280	nt
36	NH	3	23	500
34	O	4	120	nt
35	NH	4	*13*	*400*

[a] nt = not tested.

β-methyl cyclopropyl P3 compound **38**. Incorporation of the six-membered ring, β-methyl cyclohexylglycine, at P3 position afforded inhibitors **34** and **35**. While the *tert*-butyl carbamate capped inhibitor **34** was less potent, the *tert*-butyl urea capped compound **35** exhibited the best binding ($K_i^* = 13$ nm) and cellular potency (replicon $EC_{90} = 400$ nM).[12] Previously, we discovered interesting synergy between the P3 cap and P3 moiety.[2] Thus, for P3 L-cyclohexylglycine containing inhibitors, *tert*-butyl *carbamate* cap provided better potency. On the other hand, for P3 L-*tert*-leucine containing inhibitors, *tert*-butyl *urea* cap was optimal, which resulted in discovery of SCH 503034. In our present study, from the potency data (Table 2) it was clear that inhibitors with β-methyl cycloalklglycine P3 moiety exhibited synergy similar to those containing *tert*-leucine P3. Thus, urea capped targets were more potent than their respective carbamate capped compounds.

X-ray crystal structure of the inhibitor **35** bound to the protease is shown in Figure 2. The core interactions of **35** with the protease resembled those of our previous inhibitors.[2] Interestingly, the quaternary methyl group at P3 was in hydrophobic contact with the enzyme, while the cyclohexyl residue was solvent exposed.

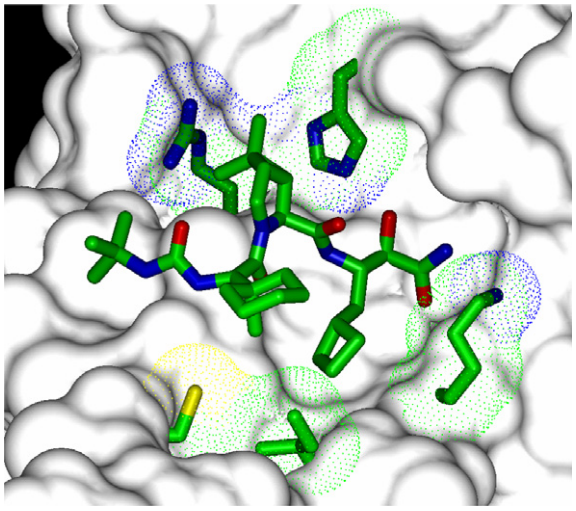


Figure 2. X-ray structure of **35** bound to the protease.

In summary we have developed a practical and efficient diastereoselective route toward amino acids containing β-quaternary center using Strecker chemistry. Some of the hindered amino acids prepared were evaluated as P3 surrogates resulting in potent HCV NS3 serine protease inhibitors. From SAR studies, β-methyl cycloalkylglycine residue containing inhibitors exhibited P3 cap/P3 synergy similar to earlier P3 *tert*-leucine containing inhibitors. Inhibitor **35**, with β-methyl cyclohexylglycine moiety at P3 and urea cap afforded the best binding and cellular replicon potency, similar to SCH 503034 that is currently undergoing human clinical studies.

Acknowledgements

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References and notes

1. (a) Cohen, J. *Science* **1999**, *285*, 26; (b) Houghten, M. In *Virology*; Fields, B. N., Knipe, D. M., Howley, P. M., Eds.; Raven Press: New York, 1996; pp 1035–1058; (c) Cuthbert, J. A. *Clin. Microbiol. Rev.* **1994**, *7*, 505.
2. Venkatraman, S.; Bogen, S. L.; Arasappan, A.; Bennett, F.; Chen, K.; Jao, E.; Liu, Y.-T.; Lovey, R. G.; Hendrata, S.; Huang, Y.; Pan, W.; Parekh, T.; Pinto, P.; Popov, V.; Pike, R. E.; Ruan, S.; Santhanam, B.; Vibulbhan, B.; Wu, W.; Yang, W.; Kong, J.; Liang, X.; Wong, J.; Liu, R.; Butkiewicz, N.; Chase, R.; Hart, H.; Agrawal, S.; Ingravallo, P.; Pichardo, J.; Kong, R.; Baroudy, B.; Malcolm, B.; Guo, Z.; Prongay, A.; Madison, V.; Broske, L.; Cui, X.; Cheng, K.-C.; Hsieh, T. Y.; Brisson, J.-M.; Prelusky, D.; Korfmacher, W.; White, R.; Bogdanowich-Knipp, S.; Pavlovsky, A.; Bradley, P.; Saksena, A. K.; Ganguly, A. K.; Piwinski, J.; Girijavallabhan, V.; Njoroge, F. G. *J. Med. Chem.* **2006**, *49*, 6074.
3. (a) Magnin, D. R.; Robl, J. A.; Sulsky, R. B.; Augeri, D. J.; Huang, Y.; Simpkins, L. M.; Taunk, P. C.; Betebenner, D. A.; Robertson, J. G.; Abboa-Offei, B. E.; Wang, A.; Cap, M.; Xin, L.; Tao, L.; Sitkoff, D. F.; Malley, M. F.; Gougoutas, J. Z.; Khanna, A.; Huang, Q.; Han, S.-P.; Parker, R. A.; Hamann, L. G. *J. Med. Chem.* **2004**, *47*, 2587; (b) Zask, A.; Birnberg, G.; Cheung, K.; Kaplan, J.; Niu, C.; Norton, E.; Suayan, R.; Yamashita, A.; Cole, D.; Tang, Z.; Krishnamurthy, G.; Williamson, R.; Khafizova, G.; Musto, S.; Hernandez, R.; Annable, T.; Yang, X.; Discafani, C.; Beyer, C.; Greenberger, L. M.; Loganzo, F.; Ayral-Kaloustian, S. *J. Med. Chem.* **2004**, *47*, 4774.
4. (a) Kazmaier, U. *J. Org. Chem.* **1996**, *61*, 3694; (b) Hasegawa, M.; Taniyama, D.; Tomioka, K. *Tetrahedron* **2000**, *56*, 10153; (c) Chowdari, N. S.; Suri, J. T.; Barbas, C. F. *Org. Lett.* **2004**, *6*, 2507.
5. (a) Chakraborty, T. K.; Hussain, K. A.; Reddy, G. V. *Tetrahedron* **1995**, *51*, 9179; (b) Pellicciari, R.; Costantino, G.; Giovagnoni, E.; Mattoli, L.; Brabet, I.; Pin, J.-P. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1569; (c) Reddy, R.; Jaquith, J. B.; Neelagiri, V. R.; Saleh-Hanna, S.; Durst, T.

Org. Lett. **2002**, *4*, 695; (d) Noe, M. C.; Natarajan, V.; Snow, S. L.; Mitchell, P. G.; Lopresti-Morrow, L.; Reeves, L. M.; Yocum, S. A.; Carty, T. J.; Barberia, J. A.; Sweeny, F. J.; Liras, J. L.; Vaughn, M.; Hardink, J. R.; Hawkins, J. M.; Tokar, C. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2808.

6. *Selected spectroscopic data*: For minor isomer **9b**—^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.93 (s, 3H), 1.15–1.52 (m, 10H), 3.06 (s, 1H), 3.30 (s, 3H), 3.57–3.67 (m, 3 H), 7.21–7.35 (m, 5H); for major isomer **9a**—^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.93 (s, 3H), 1.11–1.40 (m, 10H), 2.95 (s, 1H), 3.51–3.60 (m, 2H), 3.64–3.70 (m, 1H), 3.70 (s, 3H), 7.27–7.33 (m, 5H).
7. Only **9**, **10a/b** were subjected to larger scale (>20 mmol) hydrolysis since we required those amino acid derivatives for SAR studies.
8. The optimized procedure was employed for large scale (~100 g) synthesis of **21** starting from >100 g of aldehyde **5**.
9. Mamai, A.; Zhang, R.; Natarajan, A.; Madalengoitia, J. S. *J. Org. Chem.* **2001**, *66*, 455.
10. The HCV NS3 Serine protease inhibitory value is provided as K_i^*. For a definition of K_i^* and discussion, see Morrison, J. F., Walsh, C. T., in *Adv. Enzymol. Relat. Areas Mol. Biol.*, Meister, A., Ed.; 1988, Vol. 61, pp 201–301.
11. Zhang, R.; Beyer, B. M.; Durkin, J.; Ingram, R.; Njoroge, F. G.; Windsor, W. T.; Malcolm, B. A. *Anal. Biochem.* **1999**, *270*, 268. For the present study, the substrate Ac-DTEDVVP(Nva)-O-PAP was employed.
12. For the replicon assay, see: Lohmann, V.; Korner, F.; Koch, J.-O.; Herian, U.; Theilmann, L.; Bartenschlager, R. *Science* **1999**, *285*, 110.