Bioorganic & Medicinal Chemistry Letters 18 (2008) 6189–6193

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of novel, potent and bioavailable proline-urea based macrocyclic HCV NS3/4A protease inhibitors

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ARTICLE INFO

Article history:
Received 28 August 2008
Revised 29 September 2008
Accepted 1 October 2008
Available online 5 October 2008

Keywords:
Hepatitis C
Serine protease
Macrocycle
NS3

ABSTRACT

A novel series of P3-truncated macrocyclic HCV NS3/4A protease inhibitors containing a P2 proline-urea or carbamate scaffold was synthesized. Very potent inhibitors were obtained through the optimization of the macrocycle size, urea and proline substitution, and bioisosteric replacement of the P1 carboxylic acid moiety. Variation of the lipophilicity by introduction of small lipophilic substituents resulted in improved PK profiles, ultimately leading to compound **13Bh**, an extremely potent (K_i = 0.1 nM, EC_{50} = 4.5 nM) and selective (CC_{50} (Huh-7 cells) > 50 μM) inhibitor, displaying an excellent PK profile in rats characterized by an oral bioavailability of 54% and a high liver exposure after oral administration.

An estimated 170 million people are chronically infected by HCV, a major cause of acute hepatitis and chronic liver disease worldwide.[1] Only around 20% of newly infected individuals spontaneously clear the virus from their body, while in the majority of cases HCV persists as a chronic infection. Untreated HCV infection can ultimately progress to cirrhosis, hepatocellular carcinoma and liver failure, and is the major indication for liver transplants in western countries.[2] The current standard of care therapy consists of pegylated interferon-α and the antiviral drug ribavirin.[3] This treatment, indirectly targeting the virus, suffers from several drawbacks,[4] such as limited efficacy on genotype 1 infected patients, adverse events and poor tolerability often leading to treatment discontinuations. Consequently there is a unmet medical need for new therapies, with a better efficacy and an improved adverse event profile.

The virally encoded NS3/4A serine protease, which cleaves the viral polyprotein has been shown to be an essential component of the HCV life cycle.[5] Numerous medicinal chemistry programs have been initiated to find potent and selective inhibitors of this target.

Currently there are several NS3/4A protease inhibitors in clinical trials, which can be divided into two main classes in Figure 1: (i) the α-ketoamide electrophilic trap-containing inhibitors, examples being VX-950 (**1**, Telaprevir)[6] and SCH-503034 (**2**, Boceprevir)[7] and (ii) macrocyclic inhibitors exemplified by BILN-2061 (**3**).[8,9] Macrocyclic NS3/4A inhibitors are characterized by a hydrocarbon bridge linking the P1 side-chain to the P3 moiety, leading to a β-strand mimetic conformation. Other important features are the

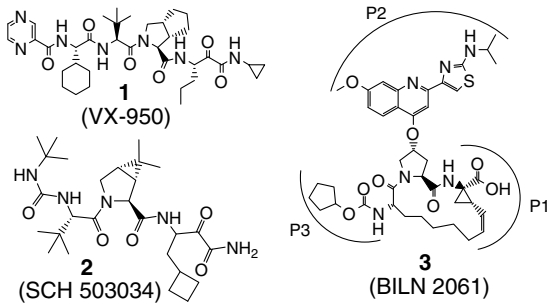

Figure 1. Structures of NS3/4A reference inhibitors.

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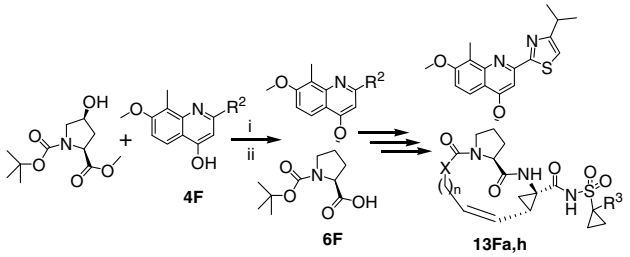
doi:10.1016/j.bmcl.2008.10.004

P1 carboxylic acid binding to the active site oxyanion hole, the P2 quinoline and the P3-capping group.[10]

Although SAR on this class of inhibitors have been extensively studied,[11] little is known about the variations of the P3-capping group in the macrocyclic series, whose presence was thought to be essential to obtain a good enzymatic and cellular potency. We have recently shown in a related macrocyclic series that adequate combinations of P3-truncated molecules with optimized P2 moieties and a P1 sulfonamide could lead to extremely potent and bioavailable compounds.[12] Now we report on our efforts to transfer our previous findings into a new series of P3-truncated inhibitors, leading to derivatives with an excellent antiviral and pharmacokinetic (PK) profile. Structure–activity relationships and PK properties of the most promising compounds are reported.

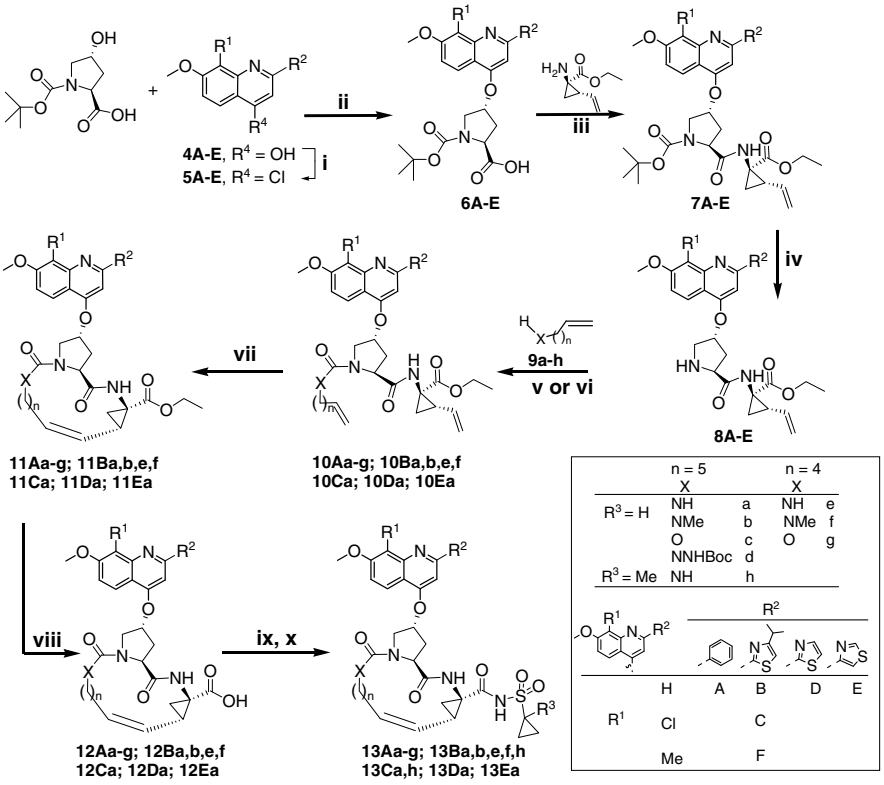
The synthesis of the target proline-urea and carbamate products **13** is outlined in Schemes 1 and 2. The P2 quinoline substituents **4A–E** were introduced at the first step of the synthesis, in very good yields (75–100%), via a nucleophilic aromatic substitution of the corresponding 4-chloroquinolines **5A–E** by Boc-*trans*-4-hydroxy-L-proline. The P1 cyclopropyl amino acid was then coupled to the P2 proline cores **6A–E** using a standard HATU-mediated peptide coupling procedure. After removal of the Boc protecting group under standard conditions (TFA/DCM), the proline intermediates **8A–E** were either treated with phosgene, to afford the corresponding chloroimidate derivatives, which were subsequently reacted with an alkenamine in the presence of sodium bicarbonate to afford ureas **10Aa,b,d,e,f, 10Ba,b,e,f, 10Ca, 10Da** and **10Ea** in good yields (i.e., 80%). Alternatively a pre-activated paranitrophenylcarbonate hydroxyalkene was used to give the corresponding carbamate derivatives **10Ac** and **10Ag**. Then, the 14- and 15-membered macrocyclic ring esters **11** were obtained in good yields



Scheme 2. Reactions and conditions: (i) DIAD, pyridyldiphenylphosphine, THF, −15 °C to rt; (ii) LiOH, THF/MeOH/H_2O, rt.

(68–87%) by ring-closing olefin metathesis of the open precursors **10** using Hoveyda–Grubbs 1st generation catalyst.[13] Hydrolysis of the ethyl esters **11** provided the corresponding acids **12** in quantitative yields. Finally, in situ activation of the carboxylic acids **12** to the non-isolated oxazolidinone intermediates followed by reaction with cyclopropylsulfonamide or methylcyclopropylsulfonamide, led to the desired target products **13**. Noteworthy the low yield observed in this last reaction (30% yield after recrystallization) could be improved by protecting the NH of the urea with a paramethoxybenzyl group, which can be introduced at the urea formation stage. Although this pathway involved one more step at the end of the synthesis (removal of the paramethoxybenzyl group in TFA), overall yields were much higher with 95% for the reaction with sulfonamide and almost quantitative yield for the deprotection.

Alternatively the more electron-rich 8-methylquinoline derivative **4F** was introduced via a Mitsunobu reaction[14] with Boc-*cis*-hydroxy-L-proline to yield the intermediate **6F** in 85% yield. The



Scheme 1. Reactions and conditions: (i) $POCl_3$ reflux, 95% yield; (ii) DMSO, KO*t*-Bu, then 4-chloroquinoline derivative **5A–E**, rt; (iii) HATU, diisopropylethylamine, DMF, 0–20 °C; (iv) TFA, CH_2Cl_2, 20 °C; (v) phosgene 1.9 M in toluene, then **9a,b,d,e,f**, $NaHCO_3$, DCM; (vi) a—hydroxyalkenyl derivative **9c,g**, 4-nitrophenylchloroformate, pyridine (3 equiv), DCM, 0 °C to rt, 73%; b—triethylamine, DCM, rt, 12 h; (vii) Hoveyda–Grubbs 1st generation catalyst, dichloroethane, 70 °C, 20 h; (viii) LiOH, THF/MeOH/H_2O, rt, 48 h; (ix) CDI, THF, reflux; (x) cyclopropylsulfonamide or methylcyclopropylsulfonamide, DBU, THF, 50 °C.

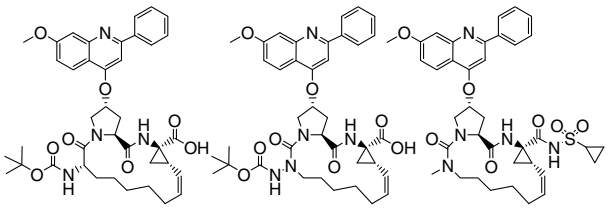
cleavage of the methylester to obtain the carboxylic acid **6F** was subsequently achieved under classical conditions. Compounds **13Fa** and **13Fh** were then obtained using the procedure depicted in Scheme 1.

The compounds described herein were tested in an enzymatic assay against the full length NS3/4A protease as described earlier[15] and their potency, expressed as rough K_i value,[16,17] is listed in Table 1. The cell-based anti-HCV activities (EC_{50}) were determined in the Huh-7 replicon cell line containing the subgenomic bicistronic HCV replicon clone ET with a luciferase read out[18] and compared to the toxicity measured in MT4 and Huh-7 cell lines.

Although the replacement of the C-α in the known inhibitor **14**[11] by a nitrogen as exemplified by compound **12Ad** resulted in a decrease in both enzymatic and cellular potency (K_i = 600 nM, EC_{50} > 10 μM), previous work from our laboratory on trisubstituted cyclopentane dicarboxylic acid as a novel P2 mimetic[12] prompted us to synthesize derivative **12Ab** (Fig. 2). To our satisfaction, this strategy was indeed applicable to this new proline-urea series, and completely restored the enzymatic potency (compare **14** to **12Ab**). Thus, shortening the P3-capping group from Boc-hydrazine to a *N*-methyl moiety in combination with a carboxylic acid isostere provided **12Ab** with a similar enzymatic potency to the reference **14**, however with a decreased cellular potency.

Preliminary analysis performed on a limited subset of *N*-substituted prolines bearing a 2-phenylquinoline (compounds **13Aa–c,e–g**) revealed that both the nature of the substitution, carbamate or urea and the ring size dramatically influence the potency of the compounds. Although the *N*-methylurea **13Ab** in the 15-membered macrocycle series was found much less potent than the corresponding carbamate **13Ac**, the opposite observation was made in the 14-membered series (compare **13Af** with **13Ag**). However, the NH proline-ureas **13Aa** and **13Ae** were constantly found to be the most potent compounds in the cell-based assay in both the 14- and 15-membered ring series with EC_{50} values of 14 and 15 nM, respectively. Noteworthy, a high shift between the enzymatic potency and replicon activity was observed with the two carbamates **13Ac** and **13Ag**, which could be attributed to a lower cell membrane permeability. The same trend was observed with the isopropylthiazole quinolines **13Ba,b,e,f**, with the NH-carbamate **13Ba** and **13Be** being the most active compounds of this subseries.

Having three different potent series in hand, we decided to evaluate their in vivo PK properties in male Sprague–Dawley rats in order to further prioritize and optimize these subseries.



	14	**12Ad**	**12Ab**
IC_{50}	11 nM	600 nM	10 nM
EC_{50}	77 nM	> 10,000 nM	4500 nM

Figure 2. NS3/4A inhibitors.

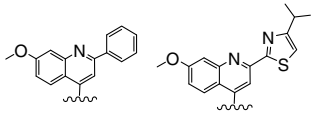
Pharmacokinetic parameters of compounds **13Ba, 13Be** and **13Bf**, obtained after a single intravenous (2 mg/kg) or an oral (10 mg/kg) administration using 50% PEG-400 in water as vehicle are summarized in Table 2. After oral administration, compounds **13Ba** and **13Bf** exhibited promising oral bioavailabilities of 20% and 37%, respectively, together with good C_{max} values (resp. 0.95 μM and 0.73 μM, respectively), unlike compound **13Be** whose bioavailability was very poor (1.3%) caused by limited absorption. These values are in line with results from the Caco-2 assay that was used as a prediction tool for absorption rate. Indeed with a Papp (A–B) of 3×10^{-6} cm/s, **13Be** was predicted to be poorly absorbed.

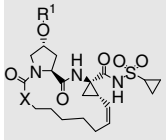
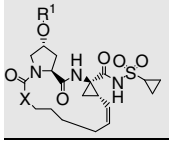
Table 2
Mean plasma levels (n = 2) and pharmacokinetic parameters of macrocycles **13Ba**, **13Bf** and **13Be** after a single intravenous (2 mg/kg) and oral administration (10 mg/kg) in male Sprague–Dawley rats

		Compound		
		13Ba	**13Bf**	**13Be**
iv (2 mg/kg, n = 2)	Cl (L/h/kg)	0.55	1.65	0.94
	Vz (L/kg)	1.46	4.50	1.60
	AUC (μM h)	7.09	1.65	3.00
Oral (10 mg/kg, n = 2)	AUC (μM h)	6.97	3.12	0.19
	C_{max} (μM)	0.95	0.73	0.026
	T_{max} (h)	1.25	1.25	2
	$T_{1/2}$	5.4	2.55	3.8
	F (%)	20	37	1.3
	Liver/plasma ratio	29*	12**	9.5*

* At 6 h.
** At 8 h.

Table 1
Effect of the macrocycle size and carbamate/urea substitution on enzymatic and cellular potency



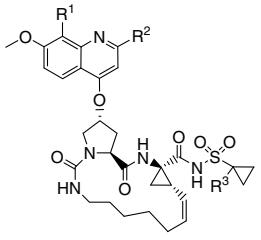
Structure	X	Compound	K_i (nM)	EC_{50} (nM)	Compound	K_i (nM)	EC_{50} (nM)
	NMe	**13Ab**	10	4500	**13Bb**	12	700
	O	**13Ac**	2.4	460	—	—	—
	NH	**13Aa**	0.24	15	**13Ba**	0.2	5
	NMe	**13Af**	0.4	44	**13Bf**	0.45	26
	O	**13Ag**	0.35	62	—	—	—
	NH	**13Ae**	1.5	14	**13Be**	0.5	5

Values obtained for compounds **13Ba** and **13Bf**, just higher than 10×10^{-6} cm/s, suggested a better absorption. The 15-membered ring compound **13Ba** had a low clearance associated with a low volume of distribution, and a 4-fold higher AUC after IV dosing compared to the 14-membered ring NH compound **13Be**.

Since HCV replicates in hepatocytes, achieving a high concentration in the liver is of prime importance for an anti-HCV drug. Thus, the ratios between liver and plasma concentrations were calculated at 6 or 8 h post-dose and showed that all three compounds had good liver exposure. The liver to plasma ratios were about 10 for the two 14-membered ring molecules, and 30 for the 15-membered ring inhibitor. Taken together with the good bioavailability and high potency, these ratios led us to select the 15-membered ring NH series for further optimization. Our challenge was mainly the optimization of the permeability of our lead compound **13Ba** to increase in vivo exposure, while maintaining the other parameters (i.e., $EC_{50} < 10$ nM and low clearance). Based on promising overall properties of the lead compound **13Ba**, we focused our efforts on the 7-methoxyquinoline moiety substituted with a thiazole as P2 substituent and embarked on subtle changes such as variations on the thiazole ring, introduction of a small lipophilic substituent at the position 8 of the quinoline, and introduction of a methyl group on the sulfonamide moiety. Enzymatic and cellular potency, Caco-2 permeability and metabolic stability in rat liver microsomes of the new derivatives were evaluated and are displayed in Table 3.

Removal of the isopropyl group of compound **13Ba** led to the less lipophilic analogue **13Da**, with a 2 log difference in calculated Alog*P* value. This drop of lipophilicity impacted the Caco-2 permeability which became very low (0.4×10^{-6} cm/s). Replacement of the 2-thiazole moiety by its isomer 4-thiazole **13Ea** led to an important decrease of potency ($EC_{50} = 98$ nM). Interestingly, the introduction of a methyl or chlorine on the position 8 of the quinoline provided compounds with a slightly better potency and a significantly improved metabolic stability in RLM. Thus compounds **13Fa** and **13Ca** were only 23% and 45% metabolized in rat liver microsomes. Furthermore, the introduction of a methyl on the P1 sulfonamide moiety also further enhanced metabolic stability leading to the very stable compound **13Ch**, which was only 21% metabolized in RLM. The permeability for these more lipophilic analogues remained high to very high in Caco-2 experiments. From this list of highly potent, permeable and stable analogues, **13Fh** and **13Bh** mainly differing by their lipophilicity were chosen for rat PK evaluation. Compound **13Fh** was found to have a moderate

Table 3
Potency and in vitro ADME parameters of lead optimization compounds



Compound	R^1	R^2	R^3	K_i (nM)	EC_{50} (nM)	Papp (AB)[a] $\times 10^{-6}$ cm/s	RLM[b] (% metabolized)	Alog*P*	*F*[c] (%)
13Ba	H		H	0.28	5	10	93	4.13	20
13Da	H		H	0.3	11	0.4	—	2.72	—
13Ea	H		H	—	98	—	—	2.74	—
13Bh	H		Me	0.1	4.5	30	89	4.34	54
13Fa	Me		H	0.05	1.7	9	23	4.62	—
13Ca	Cl		H	0.1	2.2	22	45	4.80	—
13Fh	Me		Me	0.2	6.8	—	—	4.82	11
13Ch	Cl		Me	0.1	4	29	21	5.00	—

[a] A–B apparent permeability coefficient (Papp) measured in Caco-2 cells.
[b] Rat liver microsomes (RLM) stability measured by the % of metabolized product after 15 min at 37 °C in the presence of 5 μM of test compound using 1 mg of protein/mL.
[c] Bioavailability after a single intravenous (2 mg/kg) and oral administration (10 mg/kg) in male Sprague–Dawley rats.

bioavailability (11%), slightly worse than the lead compound **13Ba**. The higher volume of distribution (Vz = 3.5 L/kg), probably resulting from an increased and high lipophilicity, resulted in a low C_{max} (0.38 μM) and the clearance remained low but was not better (Cl = 0.78 L/h/kg) despite a better in vitro metabolic stability. Overall compound **13Bh** was found to possess the desired properties in terms of permeability (highest Papp (AB) value), potency (single digit nanomolar) and metabolic stability (slightly better than the lead). These properties translated in excellent PK parameters: a very good C_{max} (1.71 μM), low volume of distribution, low clearance (0.59 L/h/kg) and excellent bioavailability (54%) associated with a very good liver exposure (the liver/plasma ratio at 6 h after oral dosing is 105).

In conclusion, we have developed a new series of P3-truncated proline-urea based macrocyclic NS3/4A protease inhibitors. Early rat PK evaluation and a good correlation between in vivo and in vitro permeability allowed us to select, with a limited set of derivatives, the promising compound **13Bh** which combines a high potency (EC_{50} = 4.5 nM) and no cytotoxicity (selectivity index-Huh-7 cells > 10,000) with excellent PK properties and high liver exposure. These data warrant further characterization of this novel preclinical candidate.

References and notes

1. Shepard, C. W.; Alter, M. J. *Lancet* **2005**, *5*, 524.
2. Willems, M.; Metselaar, H. J.; Tilanus, H. W.; Schalm, S. W.; De Man, R. A. *Transpl. Int.* **2002**, *15*, 61.
3. (a) Strader, D. B.; Wright, T.; Thomas, D. L.; Seeff, L. B. *Hepatology* **2004**, *39*, 1147; (b) Dixit, N. M.; Layden-Almer, J. E.; Layden, T. J.; Perelson, A. S. *Nature* **2004**, *432*, 922; (c) Fried, M. W. et al *N. Engl. J. Med.* **2002**, *347*, 975.
4. Fried, M. W.; Peter, J.; Hoots, K.; Gaglio, P. J.; Talbut, D.; Davis, P. C.; Key, N. S.; White, G. C.; Lindblad, L.; Rickles, F. R.; Abshire, T. C. *Hepatology* **2002**, *36*, 967.
5. Bartenschlager, R.; Lohmann, V. *J. Gen. Virol.* **2000**, *81*, 1631.
6. Lin, C.; Kwong, A. D.; Perni, R. B. *Infect. Disord. Drug Targets* **2006**, *6*, 3.
7. Venkatraman, S.; Bogen, S. L.; Arasappan, A.; Bennett, F.; Chen, K.; Jao, E.; Liu, Y.-T.; Lovey, R.; Hendrata, S.; Huang, Y.; Pan, W.; Parekh, T.; Pinto, P.; Popov, V.; Pike, R.; Ruan, S.; Santhanam, B.; Vibulbhan, B.; Wu, W.; Yang, W.; Kong, J.; Liang, X.; Wong, J.; Liu, R.; Butkiewicz, N.; Chase, R.; Hart, A.; 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.; Piwinski, J.; Girijavallabhan, V.; Njoroge, F. G. *J. Med. Chem.* **2006**, *49*, 6074.
8. Llinàs-Brunet, M.; Bailey, M. D.; Bolger, G.; Brochu, C.; Faucher, A.-M.; Ferland, J. M.; Garneau, M.; Ghiro, E.; Gorys, V.; Grand-Maître, C.; Halmos, T.; Lapeyre-Paquette, N.; Liard, F.; Poirier, M.; Rhéaume, M.; Tsantrizos, Y. S.; Lamarre, D. *J. Med. Chem.* **2004**, *47*, 1605.
9. Lamarre, D.; Anderson, P. C.; Bailey, M.; Beaulieu, P.; Bolger, G.; Bonneau, P.; Boes, M.; Cameron, D. R.; Cartier, M.; Cordingley, M. G.; Faucher, A.-M.; Goudreau, N.; Kawai, S. H.; Kukolj, G.; Lagace, L.; LaPlante, S. R.; Narjes, H.; Poupart, M.-A.; Rancourt, J.; Sentjens, R. E.; St. George, R.; Simoneau, B.; Steinmann, G.; Thibeault, D.; Tsantrizos, Y. S.; Weldon, S. M.; Yong, C.-L.; Llinas-Brunet, M. *Nature* **2003**, *426*, 186.
10. Tsantrizos, Y. S.; Bolger, G.; Bonneau, P.; Cameron, D. R.; Goudreau, N.; Kukolj, G.; LaPlante, S. R.; Llinas-Brunet, M.; Nar, H.; Lamarre, D. *Angew. Chem.* **2003**, *42*, 1356.
11. Goudreau, N.; Llinas-Brunet, M. *Expert Opin. Investig. Drugs* **2005**, *14*, 1129.
12. Bäck, M.; Johansson, P.-O.; Wångsell, F.; Thorstensson, F.; Kvarnström, I.; Ayesa Alvarez, S.; Wähling, H.; Pelcman, M.; Jansson, K.; Lindström, S.; Wallberg, H.; Classon, B.; Rydergård, C.; Vrang, L.; Hamelink, E.; Hallberg, A.; Rosenquist, Å.; Samuelsson, B. *Bioorg. Med. Chem.* **2007**, *15*, 7184.
13. Poirier, M.; Aubry, N.; Boucher, C.; Ferland, J.-M.; LaPlante, S.; Tsantrizos, Y. S. *J. Org. Chem.* **2005**, *70*, 10765.
14. Mitsunobu, O. *Synthesis* **1981**, 1.
15. Poliakov, A.; Hubatsch, I.; Schuman, C. F.; Stenberg, G. *Protein Expr. Purif.* **2002**, *25*, 363.
16. Johansson, P.-O.; Bäck, M.; Kvarnström, I.; Vrang, L.; Hamelink, E.; Hallberg, A.; Rosenquist, Å.; Samuelsson, B. *Bioorg. Med. Chem.* **2006**, *14*, 5136.
17. The enzyme inhibition assay was performed by Professor Pie Zhen Tao at the Department of Virology, Institute of Medicinal, Technology, Beijing, China.
18. Lohmann, V.; Körner, F.; Koch, J.-O.; Herian, U.; Theilmann, L.; Bartenschlager, R. *J. Virol.* **2003**, *77*, 3007.