Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 605–609

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Controlled Drug Release: New Water-Soluble Prodrugs of an HIV Protease Inhibitor

Hikaru Matsumoto, Youhei Sohma, Tooru Kimura, Yoshio Hayashi and Yoshiaki Kiso*

Department of Medicinal Chemistry, Center for Frontier Research in Medicinal Science, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8412, Japan

Received 22 November 2000; accepted 6 January 2001

Abstract—We designed and synthesized a series of highly water-soluble prodrugs of an HIV protease inhibitor, KNI-727 (**1**), containing tandem-linked two auxiliary units, a solubilizing moiety and a self-cleavable spacer. Prodrugs with an ionized amino group at the solubilizing moiety exhibited a remarkable increase of water-solubility ($>10^4$ fold) compared to the parent drug **1**. These prodrugs released **1** not enzymatically, but chemically via an intramolecular cyclization-elimination reaction through an imide formation in physiological conditions. Diversified rates of parent drug release were observed when the chemical structure of both the solubilizing and the spacer moieties were modified. This new approach for water-soluble prodrugs will enable to control chemically the release of parent drug as well as to maintain high water-solubility.

Introduction

The sparing water-solubility of drugs often cause undesirable pharmaceutical properties such as erratic oral absorption profile, poor oral bioavailability and the limitation in the availability of both oral and parenteral dosage forms.[1] To date, there are many drugs possessing low aqueous solubility due to the inherent high lipophilicity, which ultimately reduces their therapeutic efficacy.[2–6] This undesirable limitation often occurs in many of the peptidomimetic drugs such as HIV protease (PR) inhibitors and renin inhibitors.[7–10] Thaisrivongs and co-workers[9,10] reported highly water-soluble phosphate prodrugs of HIV PR inhibitors with improved biological activity. Therefore, increasing the water-solubility of these drugs is of more concern.

One of the effective strategies to improve the solubility is to convert the water-insoluble compounds into prodrugs by covalently attaching an appropriate solubilizing moiety.[11] The critical requirement for the prodrug is to attach an solubilizing moiety to the parent drug in a bioreversible manner.[11] In our previous studies, we had reported 'double-drug' strategy, a new prodrug form of HIV PR inhibitor conjugated with a reverse transcriptase inhibitor by spontaneously cleavable linkers such as glutarylglycine and succinylglycine.[12] In physiological conditions, these linkers can release the parent compounds spontaneously via intramolecular cyclization through an imide formation.[12–15] Therefore, we considered these linkers to be useful as the solubilizing moiety, which could be attached to the parent drug.

The objective of the present study is to design water-soluble prodrugs by utilizing this spontaneously cleavable linker strategy. As a model compound, we selected a dipeptide-based HIV PR inhibitor with poor water-solubility, KNI-727 (**1**) (Fig. 1).[16,17] For the design of prodrugs, two auxiliary units, a solubilizing moiety and a self-cleavable spacer, were tandemly

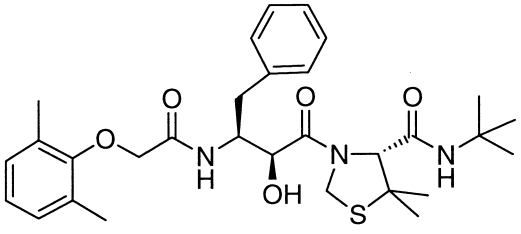


Figure 1. Structure of water-insoluble HIV protease inhibitor, **1** (KNI-727).

*Corresponding author. Tel.: +81-75-595-4635; fax: +81-75-591-9900; e-mail: kiso@mb.kyoto-phu.ac.jp

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00007-5

linked to the parent drug **1** (Fig. 2). As a spacer, we used substituted succinamide or glutaramide to link the parent drug and the solubilizing moiety. In physiological conditions (pH 7.4), we expect the spacer to spontaneously cyclize to the imide derivative and thereby release the parent drug.[12] The advantages of water-soluble prodrugs utilizing this type of spacers are as follows: (a) As prodrugs can release the parent drug by chemical reaction that is dependent on the cyclization tendency of the spacer, the rate of the parent drug release can be controlled by the modification of the structure of the spacer units. The controlled release prodrug strategy will be widely applicable to various kinds of drugs and dosage forms. (b) Various solubilizing moieties (e.g. acidic, basic or nonionizable) can be introduced on demand.

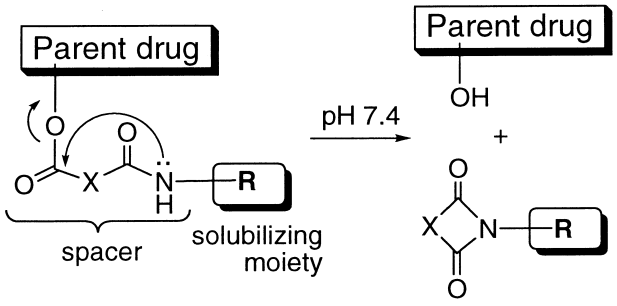


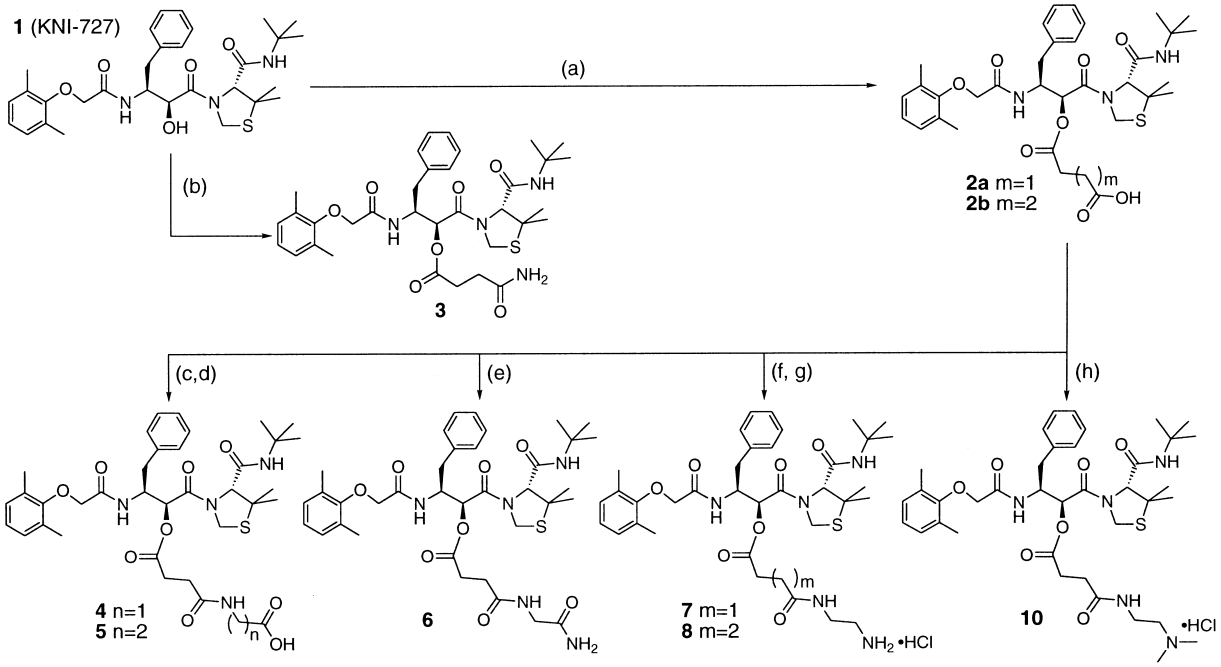
Figure 2. General structure of water-soluble prodrug, and its intramolecular cyclization-elimination reaction releasing the parent drug and an imide fragment.

Based on these premises, we synthesized water-soluble prodrugs containing various solubilizing moieties and spontaneously cleavable spacers. These prodrugs were examined for their water-solubility and the rate of drug release. The prodrug strategy employed in this paper allowed us to control the drug release rate by altering the chemical structure of the auxiliary as well as to increase the water-solubility.

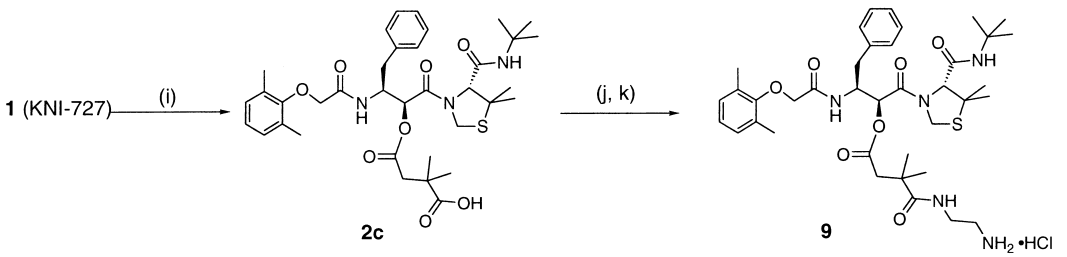
Results and Discussion

Chemistry

The synthesis of these prodrugs is summarized in Schemes 1 and 2. KNI-727 (**1**) was coupled with



Scheme 1. Reagents and conditions: (a) succinic anhydride or glutaric anhydride, DCHA, THF–ether (1:2); (b) $NH_2COCH_2CH_2COOH$, EDC·HCl, DMAP, DMF; (c) HCl·H-Gly-OBu^t or HCl·H-β-Ala-OBu^t, Et_3N, BOP, HOBt, DMF; (d) TFA; (e) HCl·H-Gly-NH_2, EDC·HCl, Et_3N, HOBt, DMF; (f) Boc-$NH(CH_2)_2NH_2$, EDC·HCl, HOBt, DMF; (g) 4 N HCl–dioxane; (h) $(Me)_2NCH_2CH_2NH_2$, EDC·HCl, HOBt, DMF.



Scheme 2. Reagents and conditions: (i) 2,2-dimethylsuccinic anhydride, DCHA, THF–ether (1:2); (j) Boc-$NH(CH_2)_2NH_2$, EDC·HCl, HOBt, DMF; (k) 4 N HCl–dioxane.

succinic anhydride in THF–ether in the presence of dicyclohexylamine (DCHA) to yield the half-ester **2a**.[12] Compound **2b** was prepared from glutaric anhydride in a similar manner. Compound **3** was prepared by coupling of **1** with succinamic acid using 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC·HCl) in the presence of dimethylaminopyridine (DMAP). Condensation of **2a** and *tert*-butyl ester of glycine or β-alanine by use of benzotriazole-1-yloxy-tris (dimethylamino) phosphonium hexafluorophosphate/1-hydroxybenzotriazole (BOP/HOBt) in the presence of triethylamine, and following deprotection by trifluoroacetic acid (TFA) afforded **4** and **5**. Compound **6** was obtained by coupling **2a** with glycine amide using EDC/HOBt. Compounds **7** and **8** were prepared by coupling **2a** or **2b** and *N*-Boc-ethylenediamine by the use of EDC/HOBt followed by the deprotection of the Boc group under acidic conditions.

Compound **9** was prepared as shown in Scheme 2. The treatment of **1** with 2,2-dimethylsuccinic anhydride in the presence of DCHA afforded the half ester **2c**. Condensation of **2c** and *N*-Boc-ethylenediamine followed by the deprotection of Boc group afforded **9**. Compound **10** was prepared by coupling **2a** with *N,N*-dimethylethylenediamine by the use of EDC/HOBt. All the crude products were purified and converted to corresponding hydrochloride salts by HPLC using binary solvent system (linear gradient of CH_3CN in 12 mM aqueous HCl), and after lyophilization the obtained amorphous products were used for the evaluation.

Water-solubility of prodrugs

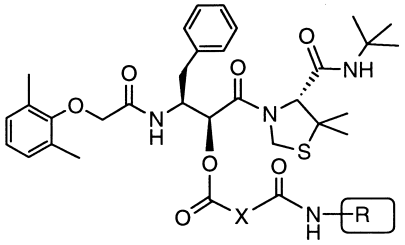
Water-solubility of the prodrugs was determined and compared to the parent drug, **1** (Table 1).[18] Compounds **3**, **4** and **6** showed a moderate increase in water-solubility. On the other hand, prodrugs **7**–**10** containing the amine structures, which were hydrochloride salts, drastically increased water-solubility more than 5100-fold. Among these prodrugs, **10** showed the highest solubility with the value of 15450-fold (171.5 mg/mL) compared to **1** (0.011 mg/mL).

Disintegration rate of prodrugs

Disintegration profile of the prodrugs in phosphate buffered saline (PBS, pH 7.4) at 37 °C was determined by HPLC analysis.[19] In all the cases, prodrugs completely released the parent drug. A typical HPLC chromatogram (using **7** as an example) and a kinetic profile for the disappearance of the prodrug and the appearance of parent drug **1** are shown in Figure 3 and 4, respectively. Prodrug **7** (Rt = 12 min) released **1** (Rt = 18 min) distinctly with $t_{1/2}$ of 21 min. The imide derivative that is the other disintegration product from **7** was invisible, because it was undetectable at 230 nm in HPLC analysis. Kinetic analysis of the disintegration showed that **1** was released following pseudo-first-order kinetics, consistent with an intramolecular reaction as shown in Figure 2.

In order to confirm the disintegration mechanism of the prodrugs, compound **11** containing succinylbenzylamide was synthesized, and the cleavage products of **11** in PBS (pH 7.4, 37 °C) were analyzed by HPLC and mass spectrometry. Two new peaks appeared, which were assigned as parent drug **1** and succinimide derivative. In the same condition, hemisuccinate **2a** was stable for at least 48 h. These results suggested that the regeneration of the parent compound was not due to the simple hydrolysis of the ester bond but intramolecular cyclization-elimination reaction through the imide formation. The stability of the prodrugs to the enzymatic

Table 1. Water-solubility and disintegration rate of prodrugs



Compound	X	R	Water-solubility		$t_{1/2}$[a] (min)
			mg/mL	Relative	
1	—	—	11.1×10^{-3}	1.0	—
3	$-CH_2CH_2-$	H	89.5×10^{-3}	8.1	960
4	$-CH_2CH_2-$	$-CH_2COOH$	73.5×10^{-3}	6.6	1122
5	$-CH_2CH_2-$	$-(CH_2)_2COOH$	N.D.[b]	N.D.[b]	1008
6	$-CH_2CH_2-$	$-CH_2CONH_2$	210×10^{-3}	18.9	20
7	$-CH_2CH_2-$	$-(CH_2)_2NH_2\cdot HCl$	115.4	10,400	21
8	$-CH_2CH_2CH_2-$	$-(CH_2)_2NH_2\cdot HCl$	56.9	5,130	1680
9	$-CH_2C(Me)_2-$	$-(CH_2)_2NH_2\cdot HCl$	136.7	12,320	< 1
10	$-CH_2CH_2-$	$-(CH_2)_2N(CH_3)_2\cdot HCl$	171.5	15,450	11
11	$-CH_2CH_2-$	$-CH_2C_6H_5$	$< 11.1\times10^{-3}$	—	528

[a] $t_{1/2}$ is the time required for 50% release of parent drug (**1**) at 37 °C in phosphate buffered saline (pH 7.4).
[b] Not determined.

hydrolysis was examined using carboxyesterase (porcine liver esterase, E.C. 3.1.1.1.). Consequently, the prodrugs exhibited resistance towards esterase hydrolysis, and the disintegration profile was almost the same as seen in the case of PBS (pH 7.4).

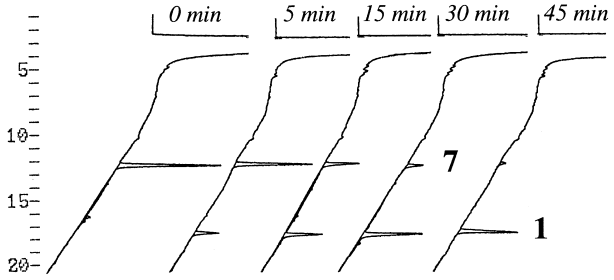


Figure 3. Typical HPLC chromatograms of the disintegration of prodrug **7** in PBS (pH 7.4, 37 °C), at 0, 5, 15, 30 and 45 min. The retention times of **1** (KNI-727) and prodrug **7** were 18 and 12 min, respectively.

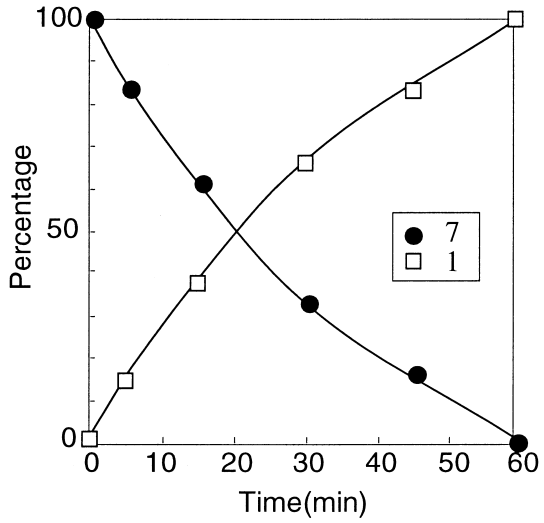


Figure 4. Typical kinetic profile showing the disappearance of prodrug **7** and the appearance of parent drug **1** in PBS, pH 7.4 at 37 °C.

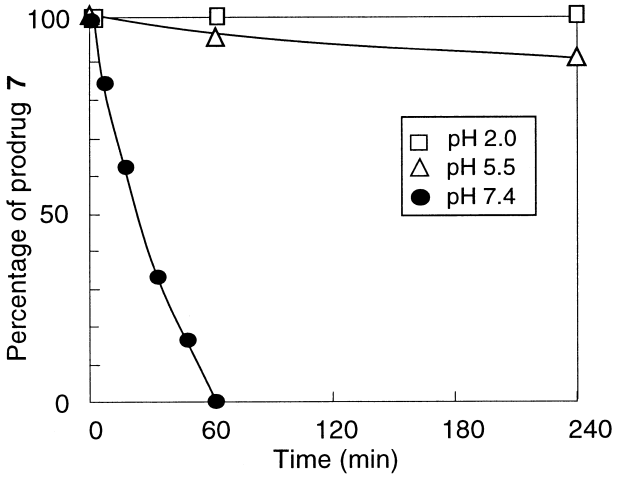


Figure 5. Typical kinetic profile showing the disintegration of prodrug **7** in buffer at various pH (at 37 °C). The buffers used were as follows: pH 2.0, glycine–HCl buffer; pH 5.5 and 7.4, phosphate buffered saline.

Interestingly, the disintegration rates of prodrugs were affected by the structure of both spacer and solubilizing moiety. Prodrug **7** containing succinyl moiety as the spacer released **1** much faster ($t_{1/2}=21$ min) than **8** containing glutaryl group ($t_{1/2}=28$ h). This is attributed to the lower energy for cyclization reaction of **7** in the formation of the five-membered cyclic imide.[15] The geminal methyl substitution of the succinyl moiety drastically increased the disintegration rate of the prodrug. The prodrug **9** released **1** completely within 5 min. This acceleration of the intramolecular cyclization rate is attributed to a unique interlocking of the geminal methyl group, which produces a severe conformation restriction (*gem* effect).[20–25]

Prodrug **4** and **5** containing the carboxyl group as a solubilizing moiety (R) released **1** much slower ($t_{1/2}=18.7$ h and 16.8 h, respectively) than **7**. When the carboxyl group of **4** was converted to carboxamide, resulting prodrug **6** disintegrated at an almost similar rate ($t_{1/2}=20$ min) as **7**. The stability of **4** and **5** is probably due to the presence of a negative charge in the same part of the molecule, which may reduce the nucleophilicity of amide nitrogen and inhibit the cyclization reaction.[14,15] Prodrug **10** containing *N,N*-dimethylethylenediamide disintegrated about two times faster ($t_{1/2}=11$ min) than **7**, whereas **3** containing succinamide disintegrated more slowly ($t_{1/2}=16$ h).

The observed differences of the drug release rate suggest that there are at least three factors affecting the kinetics of cyclization reaction of the auxiliary, that is: (1) energy for cyclic imide formation; (2) conformational restriction caused by '*gem* effect'; and (3) the presence of negative charge in the same residue. Consequently, this type of prodrug enables to control the rate of drug release through chemical modification of the auxiliary.

The disintegration rate of **7** was examined by HPLC at various pH (at 37 °C). The rate was pH dependent. A typical kinetic profile showing the disintegration of **7** at various pH is shown in Figure 5. At pH 5.5, prodrug **7** released **1** slowly compared to the case of pH 7.4. At pH 2.0, prodrug **7** was stable for at least 48 h. Even prodrug **9**, which possessed the shortest half-life (< 1.0 min) at pH 7.4, was stable for at least 2 weeks at pH 2.0. The good water-solubility and smooth conversion of prodrug to the parent drug in physiological conditions (pH 7.4) would expect to be suitable for intravenous administration, and the sufficient chemical stability at low pH will allow the routine handling and storage.[26]

Conclusion

We designed and synthesized new water-soluble prodrugs with controlled release of the parent drug. All the prodrugs had increased water-solubility, and among them, prodrug **10** containing an amino group had a drastic increase of the water-solubility, that is more than 15000-fold compared to that of the parent drug. These prodrugs released the parent drug not enzymatically but chemically via intramolecular cyclization through imide

formation, and the rate of parent drug release can be controlled by: (1) chemical modification of the spacer and the solubilizing moiety; and (2) altering the pH of the media. We suggested that this prodrug strategy would be useful to increase the water-solubility as well as to control the rate of parent drug release, and be applicable to various water-insoluble drugs containing a hydroxyl group.

Acknowledgements

This research was supported in part by the Frontier Research Program of the Ministry of Education, Science and Culture of Japan, and grants from the Ministry of Education, Science and Culture of Japan and Japan Health Science Foundation. We thank Dr. C. V. Ramesh for his help in preparing the manuscript.

References and Notes

1. Anderson, B. D.; Flora, K. P. In *The Practice of Medicinal Chemistry*; Wermuth, C. G., Ed.; Academic Press: London, 1996; pp.739–754.
2. Fleisher, D.; Bong, R.; Stewart, B. M. *Adv. Drug Delivery Rev*. **1996**, *19*, 115.
3. Lin, A. J.; Klayman, D. L.; Milhous, W. *J. Med. Chem*. **1987**, *30*, 2147.
4. Stella, V. J. *Adv. Drug Delivery Rev*. **1996**, *19*, 311.
5. Damen, E. W. P.; Wiegerinck, P. H. G.; Braamer, L.; Sperling, D.; Vos, D.; Scheeren, H. W. *Bioorg. Med. Chem*. **2000**, *8*, 427.
6. Leppanen, J.; Huuskonen, J.; Savolainen, J.; Nevalainen, T.; Taipale, H.; Vepsalainen, J.; Gynther, J.; Jarvinen, T. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 1967.
7. Bock, M. G.; Dipardo, R. M.; Evans, B. E.; Freidinger, R. M.; Rittle, K. E.; Oayne, L. S.; Boger, J.; Whitter, W. L.; LaMont, B. I.; Ulm, E. H.; Blaine, E. H.; Schorn, T. W.; Verber, D. F. *J. Med. Chem*. **1988**, *31*, 1918.
8. Rosenberg, S. H.; Woods, K. W.; Sham, H. L.; Kleinert, H. D.; Martine, D. L.; Stein, H.; Cohen, J.; Egan, D. A.; Bopp, B.; Merits, L.; Garren, K. W.; Hoffmann, D. J.; Plattner, J. J. *J. Med. Chem*. **1990**, *33*, 1962.
9. Chong, K.-T.; Ruwart, M. J.; Hinshaw, R. R.; Wilkinson, K. F.; Rush, B. D.; Yancey, M. F.; Strohbach, J. W.; Thaisrivongs, S. *J. Med. Chem*. **1993**, *36*, 2575.
10. Thaisrivongs, S.; Strohbach, J. W. *Biopolymers* **1999**, *51*, 51.
11. Wermuth, C. G. In *The Practice of Medicinal Chemistry*; Wermuth, C. G., Ed., Academic Press: London, 1996; pp 755–776.
12. Matsumoto, H.; Hamawaki, T.; Ota, H.; Kimura, T.; Goto, T.; Sano, K.; Hayashi, Y.; Kiso, Y. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 1227.
13. Tamamura, H.; Ishihara, T.; Oyake, H.; Imai, M.; Otaka, A.; Ibuka, T.; Arakaki, R.; Nakashima, H.; Murakami, T.; Waki, M.; Matsumoto, A.; Yamamoto, N.; Fuji, N. *J. Chem. Soc., Perkin Trans. 1* **1998**, 495.
14. Bodanszky, M.; Kwei, J. Z. *Int. J. Peptide Protein Res*. **1978**, *12*, 69.
15. Bodanszky, M. In *Principles of Peptide Synthesis*; Hafner, K. et al., Ed., Springer-Verlag: Berlin, 1984; pp 173–176.
16. Mimoto, T.; Kato, R.; Takaku, H.; Nojima, S.; Terashima, K.; Misawa, S.; Fukazawa, T.; Ueno, T.; Sato, H.; Shintani, M.; Kiso, Y.; Hayashi, H. *J. Med. Chem*. **1999**, *42*, 1789.
17. Kiso, Y.; Yamaguchi, S.; Matsumoto, H.; Mimoto, T.; Kato, R.; Nojima, S.; Takaku, H.; Fukazawa, T.; Kimura, T.; Akaji, K. *Arch. Pharm. Pharm. Med. Chem*. **1998**, *331*, 87.
18. Each prodrug was suspended in water, and then sonicated for 15 min at 25 °C. After filtering through a membrane filter (0.45 μm), the concentration of the prodrug was measured by HPLC.
19. To 10 mL of phosphate buffered saline (PBS, pH 7.4) was added 0.1 mL of prodrug solution (0.5 mM in dimethylsulfoxide) and the mixture was incubated at 37 °C. At different points of time, an aliquot of the sample was directly analyzed by HPLC. HPLC was performed using a C18 reverse phase column with a linear gradient of 40–100% acetonitrile in 0.1% aqueous TFA over 30 min, detected at UV 230 nm.
20. (a) Evidence for this effect was collected by Thorpe, J. F. and Ingold, C. K. in the period 1915–1930, see: Beesley, R. M.; Ingold, C. K.; Thorpe, J. F. *J. Chem. Soc*. **1915**, *107*, 1080. For a summary of pertinent references, see: Bordwell, F. G.; Osborne, C. E.; Chapman, R. D. *J. Am. Chem. Soc*. **1959**, *81*, 2698. (b) Smith, S. W.; Newman, M. S. *J. Am. Chem. Soc*. **1968**, *90*, 1249.
21. Bruce, T. C.; Bradbury, W. C. *J. Am. Chem. Soc*. **1965**, *87*, 4838.
22. Bruce, T. C.; Bradbury, W. C. *J. Am. Chem. Soc*. **1965**, *87*, 4846.
23. Bruce, T. C.; Bradbury, W. C. *J. Am. Chem. Soc*. **1965**, *87*, 4851.
24. Matsumoto, H.; Matsuda, T.; Nakata, S.; Mitoguchi, T.; Kimura, T.; Hayashi, Y.; Kiso, Y. *Bioorg. Med. Chem*., in press.
25. Hu, L.; Liu, B.; Hacking, D. R. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 797.
26. Hale, J. J.; Mills, S. G.; MacCoss, M.; Dorn, C. P.; Finke, P. E.; Budhu, R. J.; Reamer, R. A.; Huskey, S.-E. W.; Luffer-Atlas, D.; Dean, B. J.; McGowan, E. M.; Feeney, W. P.; Chiu, S.-H. L.; Cascieri, M. A.; Chicchi, G. G.; Kurtz, M. M.; Sadowski, S.; Ber, E.; Tattersall, F. D.; Rupniak, N. M. J.; Williams, A. R.; Rycroft, W.; Hargreaves, R.; Metzger, J. M.; MacIntyre, D. E. *J. Med. Chem*. **2000**, *43*, 1234.