

# Unequivocal Synthesis of (*Z*)-Alkene and (*E*)-Fluoroalkene Dipeptide Isosteres To Probe Structural Requirements of the Peptide Transporter PEPT1

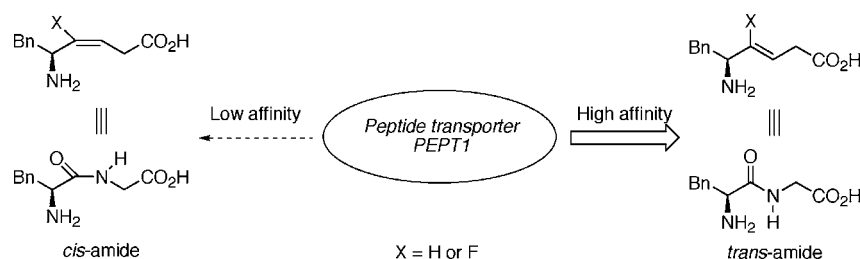
Ayumu Niida,<sup>†</sup> Kenji Tomita,<sup>†</sup> Makiko Mizumoto,<sup>†</sup> Hiroaki Tanigaki,<sup>†</sup> Tomohiro Terada,<sup>‡</sup> Shinya Oishi,<sup>†</sup> Akira Otaka,<sup>†,§</sup> Ken-ichi Inui,<sup>‡</sup> and Nobutaka Fujii<sup>\*,†</sup>

Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan, Department of Pharmacy, Kyoto University Hospital, Sakyo-ku, Kyoto 606-8507, Japan, and Graduate School of Pharmaceutical Sciences, The University of Tokushima, Tokushima 770-8505, Japan

nfujii@pharm.kyoto-u.ac.jp

Received November 17, 2005

## ABSTRACT



Described is a novel synthetic route for dipeptide isosteres containing (*Z*)-alkene and (*E*)-fluoroalkene units as *cis*-amide bond equivalents via organocopper-mediated reduction of  $\gamma$ -acetoxo- or  $\gamma,\gamma$ -difluoro- $\alpha,\beta$ -unsaturated- $\delta$ -lactams. The synthesized isosteres were evaluated in terms of their affinities for the peptide transporter PEPT1. *trans*-Amide isosteres tended to possess higher affinities for PEPT1 as compared to the corresponding *cis*-amide bond equivalents.

In postgenomic drug discovery research, the rapid elucidation of structural requirements of the ligands for newly identified drug targets (e.g., GPCRs, enzymes, transporters, etc.) is strongly needed in the arena of medicinal chemistry.<sup>1</sup> Many protein drug targets interact with proteinic or peptidic ligands. Therefore, development of peptidomimetic small molecules is important for investigating criteria for the mutual molecular recognition.<sup>2</sup> Alkene-type dipeptide isosteres represent potential amide bond mimetics (Figure 1).<sup>3</sup> Fluoroalkene dipeptide isosteres were designed as electrostatically favor-

able mimetics as compared to simple alkene isosteres.<sup>4</sup> These isosteres have structural similarities with the parent peptides

(2) (a) Burgess, K. *Acc. Chem. Res.* **2001**, *34*, 826. (b) Bursavich M. G.; Rich, D. H. *J. Med. Chem.* **2002**, *45*, 541. (c) Hruby, V. J. *J. Med. Chem.* **2003**, *46*, 4215.

(3) (a) Oishi, S.; Kamano, T.; Niida, A.; Odagaki, Y.; Hamanaka, N.; Yamamoto, M.; Ajito, K.; Tamamura, H.; Otaka, A.; Fujii, N. *J. Org. Chem.* **2002**, *67*, 6162. (b) Wipf, P.; Xiao, J. *Org. Lett.* **2005**, *7*, 103. (c) Xiao, J.; Weisblum, B.; Wipf, P. *J. Am. Chem. Soc.* **2005**, *127*, 5742.

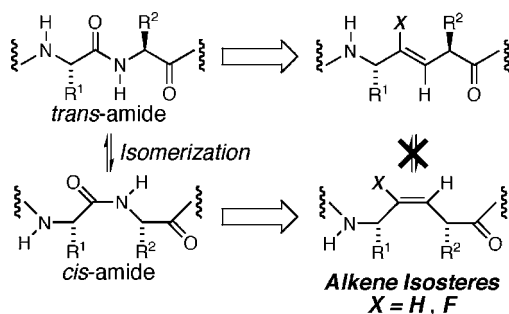
(4) (a) Abraham, R. J.; Ellison, S. L. R.; Schonholzer, P.; Thomas, W. A. *Tetrahedron* **1986**, *42*, 2101. (b) Allmendinger, T.; Furet, P.; Hungerbühler, E.; *Tetrahedron Lett.* **1990**, *31*, 7297. (c) Allmendinger, T.; Furet, P.; Hungerbühler, E.; *Tetrahedron Lett.* **1990**, *31*, 7301. (d) Otaka, A.; Watanabe, J.; Yukimasa, A.; Sasaki, Y.; Watanabe, H.; Kinoshita, T.; Oishi, S.; Tamamura, H.; Fujii, N. *J. Org. Chem.* **2004**, *69*, 1634. (e) V. d. Veken, P.; Senten, K.; Kertész, I.; D. Meester, I.; Lambeir, A.-M.; Maes, M.-B.; Scharpé, S.; Haemers, A.; Augustyns, K. *J. Med. Chem.* **2005**, *48*, 1768. (f) Nakamura, Y.; Okada, M.; Sato, A.; Horikawa, H.; Koura, M.; Saito, A.; Taguchi, T. *Tetrahedron* **2005**, *61*, 5741.

<sup>†</sup> Graduate School of Pharmaceutical Sciences, Kyoto University.

<sup>‡</sup> Kyoto University Hospital.

<sup>§</sup> The University of Tokushima.

(1) (a) Drews, J. *Science* **2000**, *287*, 1960. (b) Klabunde, T.; Hessler, G. *ChemBioChem* **2002**, *3*, 928. (c) Tyndall, J. D. A.; Pfeiffer, B.; Abbenante, G.; Fairlie, D. P. *Chem. Rev.* **2005**, *105*, 793.



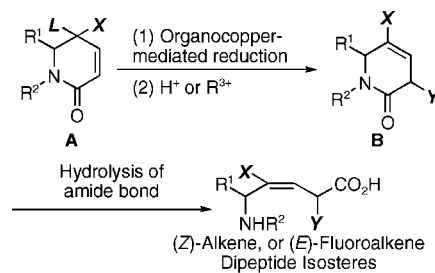
**Figure 1.** *Cis/trans* equilibrium of peptide bond and the corresponding alkene- or fluoroalkene isosteres.

and resist enzymatic degradation. Peptide bonds exist in *cis/trans* equilibrium, while alkene isosteres serve as defined *trans*-amide or *cis*-amide equivalents, which do not isomerize to each other. *Cis/trans* isomerization of peptide bonds (especially Xaa-Pro sequences) in several bioactive peptides tends to play an important role in their conformations and biological activities.<sup>5</sup> Therefore, alkene and fluoroalkene isosteres might be promising tools for conformational analysis of bioactive peptides and proteins.<sup>6</sup> We have been engaged in the development of synthetic methodologies for (*E*)-alkene or (*Z*)-fluoroalkene dipeptide isosteres as *trans*-amide bond equivalents utilizing organocopper reagents or SmI<sub>2</sub>. However, the lack of efficient synthetic methodologies for the preparation of (*Z*)-alkene or (*E*)-fluoroalkene dipeptide isosteres as *cis*-amide bond equivalents has limited an extensive application of alkene and fluoroalkene isosteres in the analysis of amide bond geometries in bioactive peptides and proteins. In this paper, we describe a new synthetic approach for the preparation of (*Z*)-alkene or (*E*)-fluoroalkene dipeptide isosteres. We also include the application of these isosteres to probe structural requirements of the peptide transporter PEPT1.

Our synthetic routes for the preparation of (*Z*)-alkene and (*E*)-fluoroalkene isosteres are depicted in Scheme 1. We envisioned key synthetic intermediates **B** would be synthesized by organocopper-mediated reduction of lactam **A** with predominant formation of  $\beta,\gamma$ -(*Z*)-alkenes or (*E*)-fluoroalkenes as *cis*-amide equivalents. This strategy could be expanded into consecutive one-pot reduction/ $\alpha$ -alkylation methodologies for the synthesis of structurally diverse  $\alpha$ -alkylated (*Z*)-alkene and (*E*)-fluoroalkene dipeptide isosteres.<sup>7</sup> First, we synthesized  $\gamma$ -acetoxy- or  $\gamma,\gamma$ -difluoro- $\alpha,\beta$ -unsaturated lactams and examined the organocopper-mediated reduction of these substrates to confirm whether this approach was applicable to the synthesis of *cis*-amide bond isosteres.

Guibé et al. reported a similar but inherently different convergent approach to the synthesis of (*Z*)-alkene isosteres

**Scheme 1.** Synthetic Route for (*Z*)-Alkene and (*E*)-Fluoroalkene Dipeptide Isosteres



L: leaving group (OAc or F). X: H or F. Y: H or R<sup>3</sup>.

via 3,6-dihydropyridin-2-ones, in which the  $\beta,\gamma$ -(*Z*)-alkene unit was constructed by Grubbs' RCM after condensation of chiral allylamines with chiral vinyl acetic acids.<sup>8</sup> The present method provides a new entity for the synthesis of (*Z*)-alkene isosteres in a divergent fashion. That is complementary to their method as well as our alternative method based on organocopper-mediated *anti*-S<sub>N</sub>2' reaction.<sup>9</sup> It is noteworthy that to our knowledge, this is the first unequivocal synthesis of (*E*)-fluoroalkene dipeptide isosteres.

Substrates for the organocopper-mediated reduction were synthesized by the sequence of reactions shown in Scheme 2. Synthesis of acetate **6** started from a known phenylalanine derivative **1**.<sup>9</sup> Conversion of the *N*-protecting group of **1** to *N*-Ns (Ns = 2-nitrobenzenesulfonyl)<sup>10</sup> followed by *O*-protection with a TBS group gave *N*-Ns amide derivative **2**. Treatment of **2** with DMB (2,4-dimethoxybenzyl) alcohol under Mitsunobu conditions afforded the *N*-DMB sulfonamide **3**. After removal of the *N*-Ns group of **3**, acylation of the resulting secondary amine followed by *O*-TBS deprotection gave the acrylamide derivative **4**. RCM reaction of **4** with Grubbs' ruthenium catalyst<sup>11</sup> proceeded smoothly at room temperature to yield the  $\gamma$ -hydroxy- $\alpha,\beta$ -unsaturated  $\delta$ -lactam **5**. Lactam **5** was converted to acetate **6** by Ac<sub>2</sub>O treatment in the presence of pyridine.

$\gamma,\gamma$ -Difluoro- $\alpha,\beta$ -unsaturated  $\delta$ -lactam **12** was synthesized from the  $\beta$ -amino ester **10**, which was prepared from phenylacetaldehyde **7** and the chiral amine **8** via rhodium catalyzed diastereoselective Reformatsky–Honda reaction.<sup>4d,12</sup> After DIBAL-H treatment of **10**, (*Z*)-selective Horner–Wadsworth–Emmons reaction<sup>13</sup> of the resulting aldehyde gave (*Z*)-enoate **11** in 72% yield with a concomitant formation of small amount of (*E*)-isomer (4%). After deprotection of the Boc and *t*-Bu groups of **11** using 4 M HCl in dioxane, cyclization with EDC gave the desired lactam **12**.

(8) Boucard, V.; S.-Dorizon, H.; Guibé, F. *Tetrahedron* **2002**, *58*, 7275.

(9) Niida, A.; Oishi, S.; Sasaki, Y.; Mizumoto, M.; Tamamura, H.; Fujii, N.; Otaka, A. *Tetrahedron Lett.* **2005**, *46*, 4183.

(10) Fukuyama, T.; Jow, C.-K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373.

(11) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953.

(12) Honda, T.; Wakabayashi, H.; Kanai, K. *Chem. Pharm. Bull.* **2002**, *50*, 307.

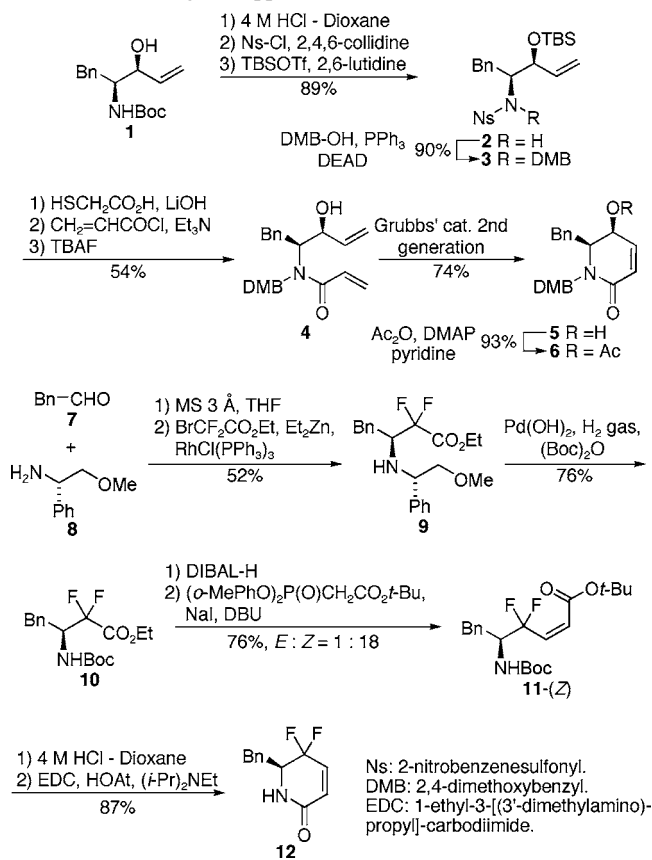
(13) Ando, K.; Oishi, T.; Hiram, M.; Ohno, H.; Ibuka, T. *J. Org. Chem.* **2000**, *65*, 4745.

(5) Dugave, C.; Demange, L. *Chem. Rev.* **2003**, *103*, 2475.

(6) Wang, X. J.; Xu, B.; Mullins, A. B.; Neiler, F. K.; Eitzkorn, F. A. *J. Am. Chem. Soc.* **2004**, *126*, 15533.

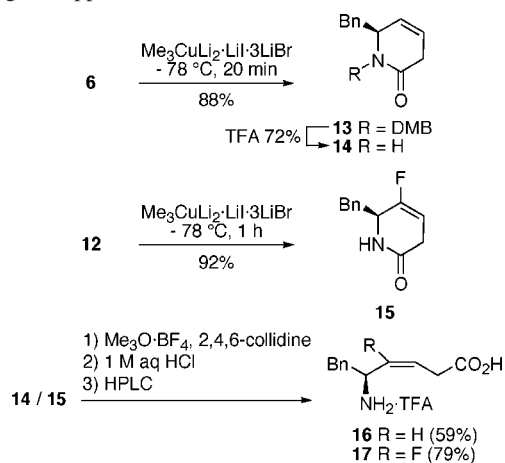
(7) Otaka, A.; Watanabe, H.; Yukimasa, A.; Oishi, S.; Tamamura, H.; Fujii, N. *Tetrahedron Lett.* **2001**, *42*, 5443, and references cited therein.

**Scheme 2.** Synthesis of Requisite Substrates for Organocopper-Mediated Reduction



Next we examined the organocopper-mediated reduction of lactams **6** and **12** (Scheme 3). The reaction of acetate **6** with Me<sub>3</sub>CuLi<sub>2</sub>·LiI·3LiBr<sup>14</sup> proceeded smoothly at -78 °C to yield the β,γ-unsaturated lactam **13** in a good yield (88%). The DMB group of lactam **13** was easily removed using TFA. Treatment of difluorolactam **12** with Me<sub>3</sub>CuLi<sub>2</sub>·LiI·

**Scheme 3.** Synthesis of Phe-Gly Type (*Z*)-Alkene- and (*E*)-Fluoroalkene Dipeptide Isosteres via Organocopper-Mediated Reduction of Lactams **6** and **12**

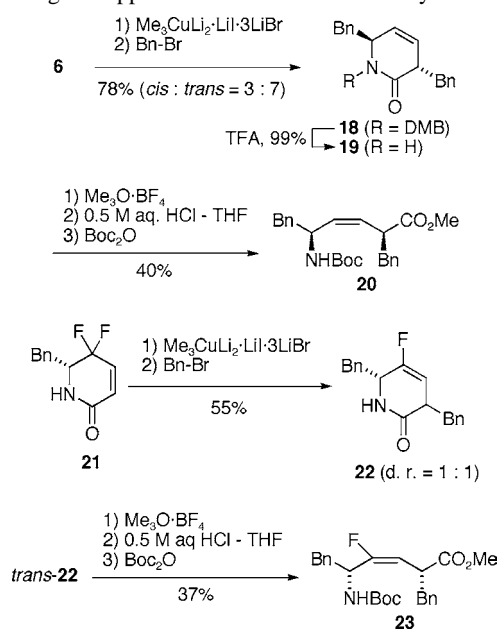


3LiBr also gave the desired reduction product **15** in excellent yield (92%).

Next, we carried out the hydrolysis of the amide bond in lactams **14** and **15** to accomplish the synthesis of the *cis*-amide bond isosteres. Lactams **14** and **15** were converted to lactim ethers using Me<sub>3</sub>O·BF<sub>4</sub>. Hydrolysis of the lactim ethers<sup>15</sup> under acidic conditions followed by HPLC purification using 0.1% TFA aqueous MeCN gave Phe-Gly type (*Z*)-alkene dipeptide isostere (Phe-ψ[(*Z*)-CH=CH]-Gly **16**) and (*E*)-fluoroalkene dipeptide isostere (Phe-ψ[(*E*)-CF=CH]-Gly **17**),<sup>16</sup> respectively as TFA salts.

The above organocopper-mediated reduction is applicable to consecutive one-pot α-alkylation (Scheme 4). After

**Scheme 4.** Synthesis of α-Substituted (*Z*)-Alkene and (*E*)-Fluoroalkene Dipeptide Isosteres Utilizing Organocopper-Mediated Reduction–Alkylation



reduction of lactam **6** with Me<sub>3</sub>CuLi<sub>2</sub>·LiI·3LiBr, the resulting metal enolate was trapped by Bn-Br to yield the *trans*-α-substituted diketopiperazine mimetic **18** as a main product. After deprotection of the DMB group using TFA, the resulting lactam **19** was subjected to ring-opening followed by *N*-Boc protection to yield Boc-*L*-Phe-ψ[(*Z*)-CH=CH]-*D*-Phe-OMe **20** in 40% yield with a small amount of α-epimerized product (13%). Boc-*D*-Phe-ψ[(*E*)-CF=CH]-*L*-Phe-OMe **23** was also synthesized from lactam **21** by a procedure

(14) Single electron transfer (SET) mechanism has been proposed as one of the plausible mechanisms of organocopper-mediated reduction. The electron-transfer potency of Me<sub>3</sub>CuLi<sub>2</sub> was proved to be higher than that of the corresponding Gilman-type reagent such as Me<sub>2</sub>CuLi. See: Chounan, Y.; Horino, H.; Ibuka, T.; Yamamoto, Y. *Bull. Chem. Soc. Jpn.* **1997**, *70*, 1953.

(15) Schöllkopf, U.; Hartwig, W.; Pospischil, K.-H.; Kehne, H. *Synthesis* **1981**, 966.

(16) Coupling constants of **17** and **23** (<sup>3</sup>J<sub>HF</sub> = 20.7 and 20.5 Hz, respectively) are consistent with those of α-fluorovinyl groups possessing a (*E*)-configuration (<sup>3</sup>J<sub>Htrans</sub> = 18–22 Hz). See ref 4b.

similar to that for the synthesis of isostere **20**.<sup>17</sup> Precise stereocontrol and introduction of other functional groups at the  $\alpha$ -position are under investigation.

Next, we investigated whether the di/tri-peptide transporter, PEPT1 recognized synthetic Phe-Gly type isosteres as substrates. PEPT1 is a membrane protein which has 12 transmembrane domains and mediates intestinal uptake of not only di-/tripeptides but also several drugs structurally related to small peptides such as  $\beta$ -lactam antibiotics.<sup>18</sup> Structure–activity relationship studies of various substrates for PEPT1 have been carried out in order to apply this transporter to develop orally bio-available drugs. However precise recognition mechanisms have not been elucidated. We envisioned that alkene dipeptide isosteres would be useful tools for analysis of recognition mechanisms of PEPT1 because of their structural similarity to parent dipeptides. We also expected that the potency of dipeptide isosteres as amide bond mimetics could be evaluated by use of the PEPT1 dipeptide transport system.

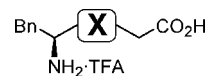
The bioactivities of synthetic Phe-Gly isosteres for PEPT1 were determined by the inhibition of [<sup>3</sup>H]Gly-Sar uptake in PEPT1-expressing Caco-2 cell in comparison with *trans*-amide type isosteres, **24**, **25**, and other related compounds (see the Supporting Information attached). Inhibition constants ( $K_i$ ) of parent dipeptide Phe-Gly and its isosteres are shown in Table 1. *trans*-Amide equivalents **24** and **25** possessed good affinity for PEPT1 corresponding to the parent dipeptide ( $K_i$ : Phe-Gly, 0.205 mM; **24**, 0.853 mM; **25**, 1.34 mM). It is of note that affinities of the *cis*-amide equivalents **16** and **17** for PEPT1 were more than 10 times weaker than those of *trans*-isomers. These data suggest that PEPT1 predominantly recognizes *trans*-amide conformations of dipeptides. This is in good accordance with the previous report by Brandsch et al., in which PEPT1 recognized *trans*-conformation of Ala- $\psi$ [CS–N]-Pro.<sup>19</sup> Conformationally flexible analogues **26** and **27** retained moderate affinity in comparison with *cis*-amide equivalents. Presumably, analogues **26** and **27** could exist as *trans*-amide-like conformers, which were favorable for the interaction with PEPT1, due to their flexibility. Contrary to our expectation, an increase of affinity by the introduction of fluoroalkene unit was not

(17) In <sup>1</sup>H NMR experiments,  $\alpha$ -protons (position-3) of 3,6-*trans* isomers such as **18** or *trans*-**22** appeared upfield from the corresponding  $\alpha$ -protons of 3,6-*cis* isomers. See ref 9.

(18) (a) Våbenø, J.; Lejon, T.; Nielsen, C. U.; Steffansen, B.; Chen, W.; Ouyang, H.; Borchardt, R. T.; Luthman, K. *J. Med. Chem.* **2004**, *47*, 1060. (b) Våbenø, J.; Nielsen, C. U.; Ingebrigtsen, T.; Lejon, T.; Steffansen, B.; Luthman, K. *J. Med. Chem.* **2004**, *47*, 4755. (c) Terada, T.; Inui, K. *Curr. Drug Metab.* **2004**, *5*, 85. (d) Biegel, A.; Gebauer, S.; Hartrodt, B.; Brandsh, M.; Neubert, K.; Thondorf, I. *J. Med. Chem.* **2005**, *48*, 4410.

(19) Brandsh, M.; Thuncke, F.; Küllertz, G.; Schutkowski, M.; Fischer, G.; Neubert, K. *J. Biol. Chem.* **1998**, *273*, 3861.

**Table 1.**  $K_i$  Values of Phe-Gly and Various Isosteres Based on Inhibition of [<sup>3</sup>H]Gly-Sar Uptake by PEPT1 in Caco-2 Cell



compd	X	$K_i$ (mM)
Phe-Gly	–CO–NH–	0.205
<b>16</b>	– $\psi$ [( <i>Z</i> )-CH=CH]–	>10.0
<b>17</b>	– $\psi$ [( <i>E</i> )-CF=CH]–	>10.0
<b>24</b>	– $\psi$ [( <i>E</i> )-CH=CH]–	0.853
<b>25</b>	– $\psi$ [( <i>Z</i> )-CF=CH]–	1.34
<b>26</b>	– $\psi$ [CH <sub>2</sub> -CH <sub>2</sub> ]–	2.17
<b>27</b>	– $\psi$ [CF <sub>2</sub> -CH <sub>2</sub> ]–	1.67

observed (**24** vs **25**). Further investigation is required for verification of the effect of fluorine as a carbonyl oxygen mimic.

In conclusion, we presented a novel unambiguous synthetic route for the syntheses of (*Z*)-alkene and (*E*)-fluoroalkene dipeptide isosteres as *cis*-amide bond mimetics via organo-copper-mediated reduction of  $\gamma$ -acetoxy- or  $\gamma,\gamma$ -difluoro- $\alpha,\beta$ -unsaturated  $\delta$ -lactams. We also carried out comparative studies of affinities for peptide transporter PEPT1 between the *cis*-amide mimetics and the corresponding *trans*-amide isosteres, and found that peptide transporter PEPT1 predominantly recognizes *trans*-amide bond conformations in dipeptides. Synthetic studies on various  $\alpha$ -substituted (*E*)-fluoroalkene isosteres and further structure–activity-relationship studies on dipeptide mimetics for PEPT1 are currently proceeding.

**Acknowledgment.** We thank Dr. Terrence R. Burke, Jr., NCI, NIH, for proofreading this manuscript. This research was supported in part by 21st Century COE Program “Knowledge Information Infrastructure for Genome Science”, a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan, the Japan Society for the Promotion of Science (JSPS), and the Japan Health Science Foundation. A.N. is grateful for Research Fellowships from the JSPS for Young Scientists.

**Supporting Information Available:** Synthesis of compounds **24**–**27**. Experimental procedures and spectral data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

OL052781K