Supporting Information

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

Ayumu Niida[†], Kenji Tomita[†], Makiko Mizumoto[†], Hiroaki Tanigaki[†], Tomohiro Terada[‡], Shinya Oishi[†], Akira Otaka[†, §], Ken-ichi Inui[‡] and Nobutaka Fujii[*, †]

[†]*Graduate School of Pharmaceutical Sciences, Kyoto Univeraity*

[‡]*Department of Pharmacy, Kyoto University Hospital*

[§]*Graduate School of Pharmaceutical Science, The University of Tokushima*

*e-mail: nfujii@pharm.kyoto-u.ac.jp

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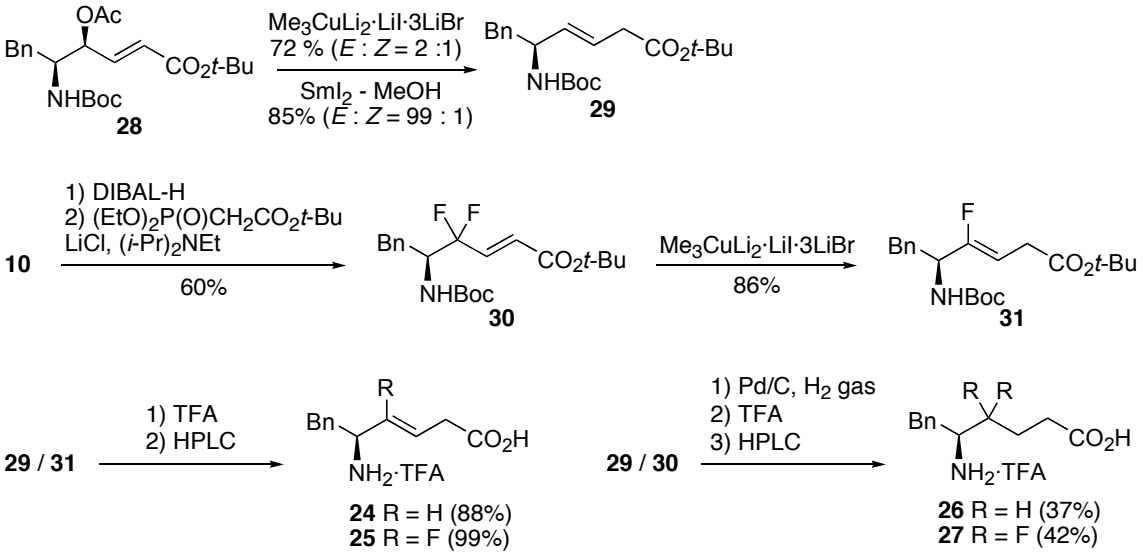
Synthesis of trans-amide type isosteres 24, 25 and their flexible analogs 26, 27.



Scheme S1.

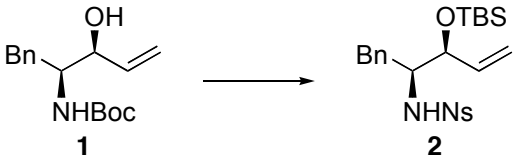
trans-Amide equivalents **24** and **25** were synthesized by procedures developed by us (Scheme S1). Organocopper-mediated reduction of the □-acetoxy-□,□-enoate **28** yielded (*E*)-□,□-enoate **29** with considerable amount of (*Z*)-isomer. Using SmI_2 - MeOH reduction system improved *E*-selectivity. Reduction of (*E*)-□,□-difuluoro-□,□-enoate **30** with Me_3CuLi_2·LiI·3LiBr gave (*Z*)-□-fluoro-□,□-enoate **31** exclusively. Deprotection of **29** and **31** by TFA followed by HPLC purification gave isosteres Phe-□[(*E*)-CH=CH]-Gly **24** and Phe-□[(*Z*)-CF=CH]-Gly **25** as *trans*-amide bond equivalents. Flexible analogs Phe-□[CH_2-CH_2]-Gly **26** or Phe-□[CF_2-CH_2]-Gly **27** was also synthesized by hydrogenation of **29** or **30** following deprotection and HPLC purification.

Experimental Procedures

General Methods.

^{1}H NMR spectra were recorded using a JEOL EX-270, JEOL AL-400 or a Bruker AM600 spectrometer. Chemical shifts are reported in δ (ppm) relative to TMS (in $CDCl_3$) or solvent peak (in D_2O, CD_3OD) as internal standard. ^{13}C NMR spectra were recorded using a JEOL AL-400 and referenced to the residual $CHCl_3$ or CH_3OH signal. ^{19}F NMR spectra were recorded using a JEOL AL-400 and referenced to the internal $CFCl_3$ (δ_F 0.00 ppm). Exact mass (HRMS) spectra were recorded on a JMS-HX/HX 110A mass spectrometer. Optical rotations were measured with a Horiba high-sensitive polarimeter SEPA-200 (Kyoto, Japan). Melting points were measured by a hot stage melting point apparatus and are uncorrected. For flash chromatographies, Wakosil C-300 was employed. For HPLC separations, a Cosmosil 5C18-ARII analytical (4.6 x 250 mm, flow rate 1 mL/min) column or a Cosmosil 5C18ARII preparative (20 x 250 mm, flow rate 10 mL/min) column was employed, and eluting products were detected by UV at 220 nm. A solvent system consisting of 0.1% TFA solution (v/v) and 0.1% TFA in CH_3CN (v/v) were used for HPLC elution.

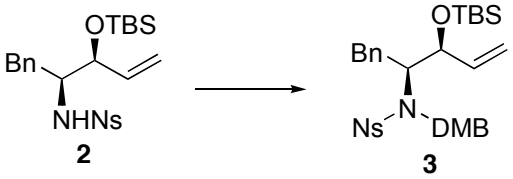
(3*S*,4*S*)-3-[(*tert*-Butyl)dimethylsiloxy]-4-[*N*-(2-nitrobenzenesulfonyl)amino]-5-phenylpent-1-en (2).



The allyl alcohol **1** (6.0 g, 6.13mmol) was dissolved in 4M HCl - Dioxane (30 mL), and the mixture was stirred for 1.5 h at room temperature. Concentration under reduced pressure gave an oily residue, which was dissolved in $CHCl_3$ (20 mL). 2,4,6-Collidine (5.98 mL, 45.3 mmol) and a solution of 2-nitrobenzenesulfonyl chloride (5.03 g, 22.7 mmol) in $CHCl_3$ (10 mL) were added to the above solution at 0 °C, and the mixture was stirred for 5 h at room temperature. Saturated citric acid (10 mL) was added to the mixture at 0 °C, and the whole was extracted with EtOAc. The extract was washed successively with saturated citric acid, brine, saturated $NaHCO_3$, and brine and dried over $MgSO_4$. Concentration under reduced pressure gave an oily residue, which was dissolved in CH_2Cl_2 (40 mL). 2,6-Lutidine (6.54 mL, 56.2 mmol) and TBSOTf (6.44 mL, 28.1 mmol) were added to the above solution at 0 °C, and the mixture was stirred overnight at room temperature. Saturated $NaHCO_3$ (15 mL) was added to the mixture at 0 °C,

and the whole was extracted with EtOAc. The extract was washed successively with saturated citric acid, brine, saturated $NaHCO_3$, and brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (6:1) gave the title compound **2** (9.24g, 89.5% yield) as colorless crystals: mp 77–79 °C; $[\alpha]^{28}_D$ +40.5 (c 1.06, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.06 (s, 3H), 0.13 (s, 3H), 0.97 (s, 9H), 2.58 (dd, *J* = 13.8, 9.2 Hz, 1H), 2.99 (dd, *J* = 13.8, 5.6 Hz, 1H), 3.63–3.75 (m, 1H), 4.25–4.33 (m, 1H), 5.02 (dt, *J* = 10.5, 1.3 Hz, 1H), 5.18 (dt, *J* = 17.1, 1.3 Hz, 1H), 5.70 (d, *J* = 7.6 Hz, 1H), 5.78 (ddd, *J* = 16.8, 10.2, 5.9 Hz, 1H), 6.95–7.05 (m, 5H), 7.45–7.80 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ –4.8, –4.2, 18.1, 25.8, 37.6, 62.0, 74.4, 116.8, 125.3, 126.5, 128.2, 129.0, 129.9, 132.6, 132.7, 135.0, 136.9, 137.5, 147.1. Anal. Calcd for $C_{23}H_{32}N_2O_5SSi$: C, 57.95; H, 6.77; N, 5.88. Found: C, 57.79; H, 6.61; N, 5.84.

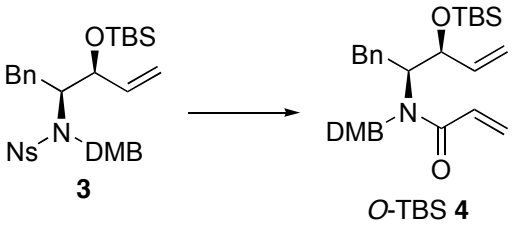
(3*S*,4*S*)-3-[(*tert*-Butyl)dimethylsiloxy]-4-[*N*-(2,4-dimethoxybenzyl)-*N*-(2-nitrobenzenesulfonyl)-amino]-5-phenylpent-1-en (3).



To a stirred solution containing the sulfonamide **2** (200 mg 0.419 mmol), 2,4-dimethoxybenzylalcohol (247 mg, 1.47 mmol) and PPh_3 (385 mg, 1.47 mmol) in THF (4 mL) was added dropwise a solution of DEAD in toluene (40% solution, 660 μL, 1.47 mmol) at 0 °C under argon, and the mixture was stirred overnight at room temperature. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (5:1) gave the title compound **3** (238 mg, 90.6% yield) as colorless oil: $[\alpha]^{28}_D$ –87.5 (c 0.38, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ –0.01 (s, 6H), 0.91 (s, 9H), 3.15 (d, *J* = 7.2 Hz, 2H), 3.67 (s, 3H), 3.81 (s, 3H), 4.23 (dd, *J* = 7.2, 3.3 Hz, 1H), 4.37 (dt, *J* = 7.2, 3.33 Hz, 1H), 4.65 (d, *J* = 15.8 Hz, 1H), 4.86 (d, *J* = 15.8 Hz, 1H), 4.95–5.10 (m, 2H), 5.83 (ddd, *J* = 17.1, 10.0, 7.3 Hz, 1H), 6.17 (d, *J* = 2.7 Hz, 1H), 6.32 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.14–7.58 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ –4.7, –3.4, 18.3, 26.1, 35.2, 45.2, 54.8, 55.4, 64.8, 66.6, 75.5, 97.7, 98.3, 103.6, 116.9, 117.4, 123.5, 126.1, 128.1, 129.2, 130.0, 130.9, 131.2, 131.6, 132.0, 135.0, 138.2, 138.5, 147.2, 157.9, 160.2; HRMS (FAB), *m*/*z* calcd for $C_{32}H_{43}N_2O_7SSi$ (MH^+) 627.2560, found 627.2575.

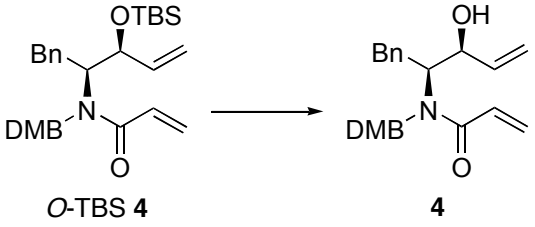
(3*S*,4*S*)-4-[*N*-Acryloyl-*N*-(2,4-dimethoxybenzyl)amino]-3-[(*tert*-butyl)dimethylsiloxy]-5-phenylpent-

1-en (*O*-TBS derivative of 4).



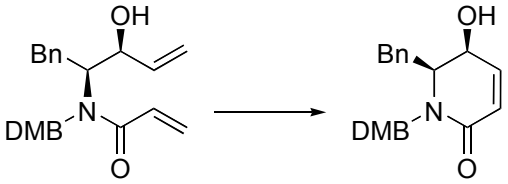
To a stirred solution of the *N*-DMB-sulfonamide **3** (159mg, 0.253 mmol) in DMF (1mL) was added $LiOH·H_2O$ (106 mg, 2.53 mmol) and $HSCH_2CO_2H$ (87.5 μL, 1.26 mmol) at 0 °C, and the mixture was stirred for 4 h at room temperature. The mixture was extracted with EtOAc. The extract was washed with saturated $NaHCO_3$ and dried over $MgSO_4$. Concentration under reduced pressure gave an oily residue, which was dissolved in CH_2Cl_2 (1.5 mL). Et_3N (176 μL, 1.26 mmol) and acryloyl chloride (82.1 μL, 1.01 mmol) were added dropwise to the above solution at – 20 °C, and the mixture was stirred for 1.5 h at 0 °C under argon. Saturated $NaHCO_3$ (2 mL) was added to the above mixture at 0 °C, and the whole was extracted with EtOAc. The extract was washed successively with saturated citric acid, brine, saturated $NaHCO_3$, and brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (6:1) gave the title compound (76.2mg, 60.7% yield) as colorless oil (rotamer mixture): $[\alpha]^{29}_{D}$ –67.7 (c 0.32, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$), major isomer at 320 K, δ 0.02 (s, 3H), 0.03 (s, 3H), 0.91 (s, 9H), 2.88 (dd, *J* = 14.5, 5.5 Hz, 1H), 3.12 (m, 1H), 3.71 (s, 3H), 3.75 (s, 3H), 3.83 (m, 1H), 4.42 (d, *J* = 17.8 Hz, 1H), 4.63 (m, 1H), 4.74 (d, *J* = 17.7 Hz, 1H), 5.14 (d, *J* = 10.3 Hz, 1H), 5.25 (d, *J* = 17.1 Hz, 1H), 5.49 (dd, *J* = 10.2, 1.7 Hz, 1H), 5.83 (ddd, *J* = 17.1, 10.3, 6.8 Hz, 1H), 6.16 (dd, *J* = 8.2, 1.6 Hz, 1H), 6.28–6.34 (m, 2H), 6.41 (dd, *J* = 16.0, 10.0 Hz, 1H), 6.54 (d, *J* = 8.2 Hz, 1H), 6.95–7.20 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ –4.9, –4.0, –3.6, 18.0, 25.7, 34.7, 54.7, 55.2, 75.4, 97.9, 103.2, 116.3, 125.7, 127.3, 128.0, 128.1, 128.3, 128.5, 128.8, 129.0, 129.3, 138.9, 139.3, 157.2, 159.7, 167.9; HRMS (FAB), *m/z* calcd for $C_{29}H_{42}NO_4Si$ (MH^+) 496.2883, found 496.2871.

(3*S*,4*S*)-4-[*N*-Acryloyl-*N*-(2,4-dimethoxybenzyl)amino]-5-phenylpent-1-en-3-ol (4).



The *O*-TBS-acrylamide *O*-TBS **4** (103 mg, 0.207 mmol) was dissolved in 1.0 M TBAF in THF (621 μL, 0.621 mmol), and the mixture was stirred for 9h at room temperature. The mixture was extracted with EtOAc. The extract was washed with brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (3:1) gave the title compound **4** (70.0mg, 88.6% yield) as colorless oil: $[\alpha]^{29}_D$ –65.2 (c 1.21, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$), δ2.92 (dd, *J* = 13.4, 7.2 Hz, 1H), 3.33 (dd, *J* = 13.3, 7.9 Hz, 1H), 3.47 (m, 1H), 3.74 (s, 3H), 3.81 (s, 3H), 3.96 (m, 1H), 4.04 (m, 1H), 4.30 (d, *J* = 15.6 Hz, 1H), 4.93 (d, *J* = 10.4 Hz, 1H), 5.23 (d, *J* = 17.0 Hz, 1H), 5.39 (m, 1H), 5.70 (dd, *J* = 10.5, 1.5 Hz, 1H), 6.35 (dd, *J* = 16.7, 1.9 Hz, 1H), 6.40–6.44 (m, 2H), 6.78 (dd, *J* = 15.3, 10.6 Hz, 1H), 6.92 (dd, *J* = 8.7 Hz, 1H), 7.00–7.27 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ29.6, 34.7, 54.8, 55.4, 72.4, 98.5, 103.8, 114.3, 116.1, 126.2, 127.6, 128.3, 129.3, 129.4, 131.0, 138.6, 138.9, 158.8, 161.1, 168.9; HRMS (FAB), *m/z* calcd for $C_{23}H_{28}NO_4$ (MH^+) 382.2018, found 382.2008.

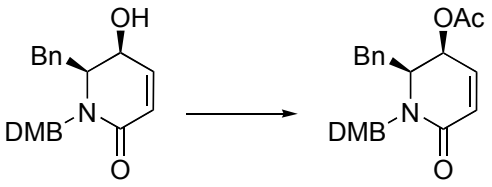
(5*S*,6*S*)-6-Benzyl-5,6-dihydro-1-(2,4-dimethoxy)benzyl-5-hydroxy-pyridin-2-one (5).



4 **5**

To a solution of the acrylamide **4** (163 mg, 0.427 mmol) in CH_2Cl_2 (3mL) was added Grubbs' catalyst 2nd generation (54.3 mg, 0.0640 mmol), and the mixture was stirred for 12 h at room temperature. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (1 : 1) gave the title compound **5** (111mg, 73.6% yield) as colorless crystals: mp 118–120 °C; $[\alpha]^{22}_D$ –10.8 (c 0.65, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ2.93 (dd, *J* = 13.4, 8.8 Hz, 1H) 3.07–3.17 (m, 1H), 3.17 (d, *J* = 14.6 Hz, 1H), 3.20–3.42 (m, 1H), 3.69 (s, 3H), 3.74 (s, 3H), 3.79 (m, 1H), 4.68 (m, 1H), 4.82 (d, *J* = 14.6 Hz, 1H), 5.81 (dd, *J* = 9.8, 2.2 Hz, 1H), 6.27–6.42 (m, 3H), 6.91 (d, *J* = 8.0 Hz, 1H), 7.10–7.34 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ33.0, 44.2, 55.0, 55.1, 61.8, 67.4, 76.6, 98.2, 103.8, 117.8, 123.1, 126.2, 128.3, 129.6, 130.8, 138.1, 143.6, 158.3, 160.1, 163.5; Anal. Calcd for $C_{21}H_{23}NO_4$: C, 71.37; H, 6.56; N, 3.96. Found: C, 71.33; H, 6.52; N, 3.96.

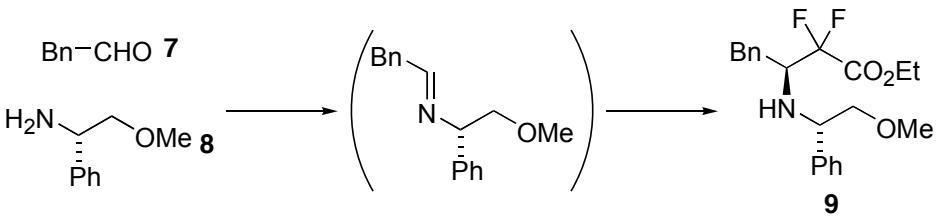
(5*S*,6*S*)-5-Acetoxy-6-benzyl-5,6-dihydro-1-[(2,4-dimethoxy)benzyl]pyridin-2-one (6).



5 **6**

To a stirred solution of the lactam **5** (545 mg, 1.54 mmol), pyridine (2.48 mL, 30.8 mmol) and DMAP (18.8 mg, 0.154 mmol) in $CHCl_3$ was added Ac_2O (1.45 mL, 15.4 mmol) at 0 °C, and the mixture was stirred for 1.5 h at 0 °C. H_2O (2 mL) was added to the above mixture at 0 °C, and the whole was extracted with EtOAc. The extract was washed successively with saturated citric acid, brine, saturated $NaHCO_3$, and brine, and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (1:1) gave the title compound (583mg, 95.5% yield) as colorless oil: $[\alpha]^{22}_D$ +18.1 (c 1.38, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ1.82 (s, 3H), 2.93 (dd, *J* = 13.9, 7.3 Hz, 1H), 3.00 (dd, *J* = 13.9, 6.6 Hz, 1H), 3.47 (d, *J* = 14.4 Hz, 1H), 3.77 (s, 3H), 3.82 (s, 3H), 4.20 (m, 1H), 4.78 (d, *J* = 14.4 Hz, 1H), 5.65 (dt, *J* = 6.3, 2.2 Hz, 1H), 5.93 (dd, *J* = 10.0, 2.4 Hz, 1H), 6.24 (dt, *J* = 10.0, 1.8 Hz, 1H), 6.37–6.46 (m, 2H), 7.05–7.33 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 20,5, 34.3, 44.7, 55.3, 58.8, 69.9, 98.2, 103.9, 117.6, 125.2, 126.2, 128.3, 129.1, 131.4, 137.8, 137.9, 158.3, 160.1, 162.4, 169.5; HRMS (FAB), *m/z* calcd for $C_{23}H_{26}NO_5$ (MH^+) 396.1811, found 396.1819.

Ethyl (3*S*)-2,2-Difluoro-3-{*N*-[(1*S*)-(2-methoxy-1-phenylethyl)]amino}-4-phenylbutanoate (9).

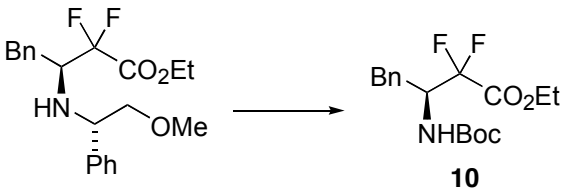


9

A solution of the aldehyde **7** (2.80 mL, 25.4 mmole) and the amine **8** (5.00g, 33.1 mmol) in THF (60 mL) was stirred at 0 °C for 4 h under argon in the presence of activated molecular sieves 3Å (12g). To the mixture were successively added a suspension of Wilkinson's catalyst (1.18g, 1.27mmole) in THF (62.5 mL), $BrCF_2CO_2Et$ (5.68 g, 27.9 mmol) and a solution of Et_2Zn in hexane (1.0 M, 102 mL, 102 mmol). After being stirred for 30 min at 0 °C, the reaction was quenched with saturated $NaHCO_3$. The mixture was filtered over Celite and the filtrate was extracted with EtOAc. The extract was washed with saturated $NaHCO_3$, and brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (10 : 1) gave the title compound **9** (5.01 g, 52.3%

yield) as colorless oil: $[\alpha]^{29}_{D}$ +27.2 (c 2.50, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.35 (t, J = 7.1 Hz, 3H), 2.09 (br, 1H), 2.53 (dd, J = 13.6, 10.4 Hz, 1H), 2.91 (dd, J = 13.6, 3.4 Hz, 1H), 3.20–3.34 (m, 3H), 3.29 (s, 3H), 4.07 (m, 1H), 4.45–4.40 (m, 2H), 6.77 (d, J = 6.8 Hz, 2H), 7.00–7.17 (m, 5H), 7.19–7.32 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8, 36.3 (t, J = 4.1 Hz), 58.5 (t, J = 22.3 Hz), 58.7, 59.7, 62.7, 77.7, 117.2 (t, J = 257 Hz), 126.4, 127.2, 127.6, 128.1, 128.2, 129.7, 137.5, 139.7, 164.0 (t, J = 32.3 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ –111.8 (ddd, J = 273, 258, 10.3 Hz, 2F); HRMS (FAB), m/z calcd for $C_{21}H_{26}NO_3F_2(MH^+)$ 378.4328, found 378.1875.

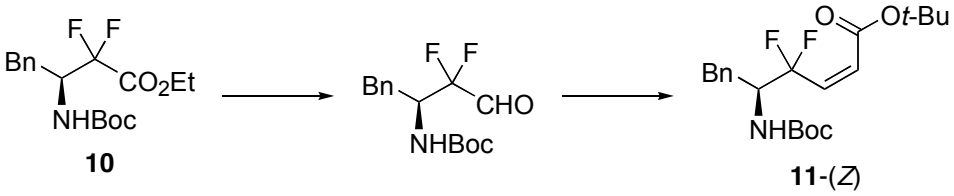
Ethyl (3*S*)-3-[*N*-(*tert*-Butoxycarbonyl)amino]-2,2-difluoro-4-phenylbutanoate (10).



9 **10**

To a solution of the ester **9** (5.00 g, 13.2 mmol) in EtOH were added 20% palladium hydroxide on carbon (5.00 g) and $(Boc)_2O$ (5.78 g, 26.5 mmol), and the suspension was stirred for 24 h under H_2 at room temperature. The mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. Flash chromatography over silica gel with *n*-hexane-EtOAc (20:1) gave the title compound **10** (3.48 g, 76.4% yield) as colorless crystals: mp 86–87 °C; $[\alpha]^{30}_{D}$ +42.6 (c 1.10, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28 (s, 9H) 1.32 (t, J = 7.3 Hz, 3H), 2.70 (dd, J = 14.4, 9.8 Hz, 1H), 3.14 (d, J = 14.4 Hz, 1H), 4.18–4.36 (m, 2H), 4.51–4.68 (m, 1H), 4.54–4.70 (m, 1H), 7.17–7.38 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8, 28.0, 32.4 (d, J = 323 Hz), 53.7 (dd, J = 27.1, 28.1 Hz), 63.1, 80.1, 114.5 (t, J = 254 Hz), 126.8, 128.5, 129.3, 135.8, 154.7, 163.1 (dd, J = 33.1, 31.4 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ –119.9 (ddd, J = 290, 259, 17.7 Hz, 1F), –112.3 (ddd, J = 530, 259, 6.8 Hz, 1F); Anal. Calcd for $C_{17}H_{23}NO_4F_2$: C, 59.46; H, 6.75; N, 4.08. Found: C, 59.65; H, 6.75; N, 4.05.

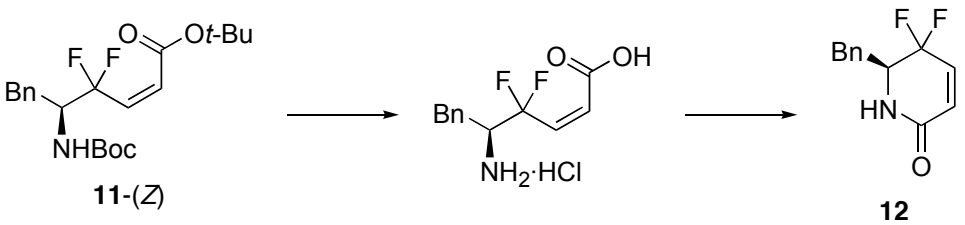
***tert*-Butyl (5*S*, 2*Z*)-5-[*N*-(*tert*-Butoxycarbonyl)amino]-4,4-difluoro-6-phenylhex-2-enoate [11-(*Z*)].**



To a solution of the ester **10** (300 mg, 0.874 mmol) in CH_2Cl_2 (8.7 mL) was added dropwise a solution of DIBAL-H in toluene (1.0 M, 1.31 mL, 1.31 mmol) at – 78 °C under argon, and the mixture was stirred for 30 min at – 78 °C. The reaction was quenched with saturated citric acid and extracted with Et_2O. The extract was washed with saturated citric acid and brine, and dried over $MgSO_4$. Concentration under reduced pressure gave an oily aldehyde, which was used immediately in the next step without purification.

To a stirred solution of $(o\text{-MePhO})_2P(O)CH_2CO_2t\text{-Bu}$ (294 mg, 0.853 mmol) in THF (5 mL) were added NaI (143 mg, 0.957 mmol) and DBU (130 □l, 0.870 mmol) at 0 °C under argon. After stirring for 10 min, a solution of the above aldehyde in THF (3.7 mL) was added to the mixture at – 78 °C, and the mixture was stirred for 2 h at 0 °C. The reaction was quenched with saturated NH_4Cl and extracted with EtOAc. The extract was washed with saturated citric acid, brine, 5% $NaHCO_3$, and brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (14:1) gave the title compound **11**-(*Z*) (250 mg, 71.9% yield) and **11**-(*E*) (**30**) (13.8 mg, 4.0% yield). **11**-(*Z*): mp 76 – 78 °C; $[\square]^{31}_D$ + 16.0 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) □1.29 (s, 9H) 1.52 (s, 9H), 2.71 (dd, *J* = 13.9, 10.5 Hz, 1H), 3.19 (dd, *J* = 14.3, 3.17 Hz, 1H), 4.47–4.68 (m, 1H), 4.79-4.94 (m, 1H), 5.88 (dd, *J* = 27.8, 12.9 Hz, 1H), 6.05 (dd, *J* = 12.7, 1.71 Hz, 1H), 7.17–7.33 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) □27.9, 28.1, 34.5, 55.6 (dd, *J* = 30.6, 24.0 Hz), 79.7, 82.3, 120.0 (t, *J* = 246 Hz), 126.5, 128.3, 129.3, 130.6 (t, *J* = 28.1 Hz), 136.7, 155.1, 164.3; ^{19}F NMR (376 MHz, $CDCl_3$) □–107.4 (ddt, *J* = 252, 172, 16.1 Hz, 1F), –100.7 (dd, *J* = 333, 252 Hz, 1F); Anal. Calcd for $C_{21}H_{29}NO_4F_2$: C, 63.46; H, 7.35; N, 3.52. Found: C, 63.16; H, 7.34; N, 3.51.

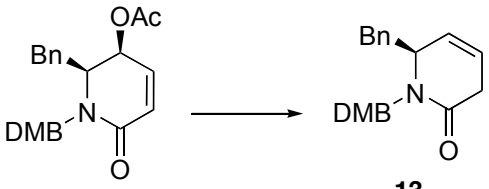
(6*S*)-6-Benzyl-5,5-difluoro-6-hydro-1*H*-pyridin-2-one (12).



The enoate **11**-(*Z*) (250 mg, 0.629 mmol) was dissolved in 4 M HCl-Dioxane (8 mL) at 0 °C, and the mixture was stirred for 1 h at room temperature. Concentration under reduced pressure gave an oily

residue, which dissolved in DMF (7 mL). To the above mixture were successively added (*i*-Pr)$_2$NEt (730 μL, 3.14 mmol), HOAt (428 mg, 3.14 mmol), and EDC (550 μl, 3.14 mmol) at 0 °C, and the mixture was stirred for 23 h at room temperature. After concentration under reduced pressure, the residue was extracted with EtOAc. The extract was washed with saturated citric acid, brine, 5% $NaHCO_3$, and brine, and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (4:1) gave the title compound **12** (122 mg, 86.8% yield) as colorless crystals: mp 89–90 °C; $[\alpha]^{32}_D$ +9.87 (c 1.54, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.81 (dd, *J* = 13.9, 11.2 Hz, 1H), 3.28 (dd, *J* = 13.9, 3.4 Hz, 1H), 3.95–4.08 (m, 1H), 5.53–5.69 (m, 1H), 6.18 (dd, *J* = 10.2, 2.2 Hz, 1H), 6.59 (ddd, *J* = 10.5, 7.1, 3.4 Hz, 1H), 7.20–7.42 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 33.5 (dd, *J* = 5.8, 1.7 Hz), 57.6 (dd, *J* = 32.3, 27.3 Hz), 115.0 (dd, *J* = 244, 236 Hz), 127.7, 129.2, 129.3, 130.3 (dd, *J* = 10.7, 9.9 Hz), 134.1 (dd, *J* = 31.4, 27.3 Hz), 134.6, 162.7; ^{19}F NMR (376 MHz, $CDCl_3$) δ –107.0 (d, *J* = 277 Hz, 1F), –105.1 (dd, *J* = 277, 18.6 Hz, 1F); Anal. Calcd for $C_{12}H_{11}NOF_2$: C, 64.57; H, 4.97; N, 6.27. Found: C, 64.68; H, 5.01; N, 6.26.

General Procedure for the Organocopper-mediated Reduction. Synthesis of (6*S*)-6-Benzyl-3,6-dihydro-1-(2,4-dimethoxy)benzyl-pyridin-2-one (13).

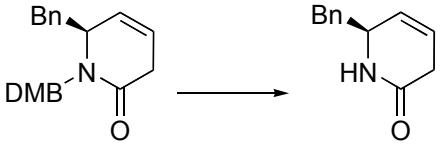


6 **13**

To a suspension of CuI (1.95g, 10.2 mmol) in THF (25mL) was added dropwise a solution of MeLi LiBr complex in Et_2O (1.5 M, 20.4 mL, 30.7 mmol) at – 78 °C under argon, and the mixture was stirred for 10 min at 0 °C. To the solution of organocopper reagent was added dropwise a solution of the acetate **6** (1.01 g, 2.55 mmol) in THF (11 mL) at – 78 °C, and the mixture was stirred for 30 min at – 78 °C. The reaction was quenched at – 78 °C by addition of a 1:1 saturated NH_4Cl-28%NH_4OH solution (30 mL) with additional stirring at room temperature for 30 min. The mixture was extracted with Et_2O and the extract was washed with H_2O and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (1:1) gave the title compound **13** (761 mg, 88.4% yield) as colorless oil: $[\alpha]^{26}_D$ +50.4 (c 3.07, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.17 (ddd, *J* = 21.2, 5.2, 2.8 Hz, 1H), 2.72 (ddd, *J* = 21.6, 4.4, 2.0 Hz, 1H), 2.88 (dd, *J* = 13.2, 3.2 Hz, 1H), 2.95 (dd, *J* = 13.2, 6.8 Hz, 1H), 3.78 (s, 3H), 3.84 (s, 3H), 4.11 (m, 1H), 4.27 (d, *J* = 14.8 Hz, 1H), 5.32 (d, *J* = 14.8 Hz, 1H),

5.60 (ddd, *J* = 9.6, 4.4, 2.8 Hz, 1H), 5.67 (ddd, *J* = 9.6, 4.4, 2.0 Hz, 1H), 6.40–6.50 (m, 2H), 7.02–7.30 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) 32.4, 39.5, 40.8, 55.3, 55.4, 57.8, 98.2, 104.3, 117.4, 123.4, 125.4, 126.4, 127.9, 129.8, 130.1, 135.9, 158.3, 159.9, 168.4; HRMS (FAB), *m*/*z* calcd for $C_{21}H_{24}NO_3$ (MH^+) 338.1756, found 338.1760.

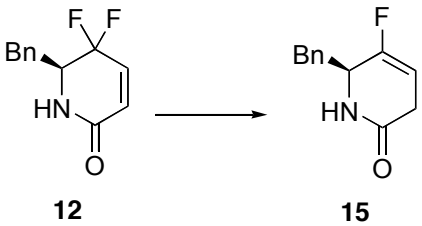
(6*S*)-6-Benzyl-3,6-dihydro-1*H*-pyridin-2-one (14).



13 **14**

The lactam **13** (223 mg, 0.661 mmol) was dissolved in TFA (10 mL), and the mixture was stirred overnight. Concentration under reduced pressure gave an oily residue, which was extracted with EtOAc. The extract was washed with saturated $NaHCO_3$ and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (1:3) gave the title compound **14** (88.2 mg, 71% yield) as colorless crystals: mp 89–90 °C; $[]^{26}_D$ +17.0 (c 1.54, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 2.64 (dd, *J* = 13.2, 8.1 Hz, 1H), 2.63-2.84 (m, 2H), 2.87 (dd, J = 13.4, 5.4 Hz, 1H), 4.13–4.22 (m, 1H), 5.61–5.72 (m, 2H), 6.10–6.29 (br, 1H), 7.06–7.29 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) 31.2, 43.7, 55.1, 122.2, 124.8, 127.0, 128.7, 129.5, 136.3, 169.5; Anal. Calcd for $C_{12}H_{13}NO$: C, 76.98; H, 7.00; N, 7.48. Found: C, 76.75; H, 7.01; N, 7.31.

(6*S*)-6-Benzyl-3,6-dihydro-5-fluoro-1*H*-pyridin-2-one (15).

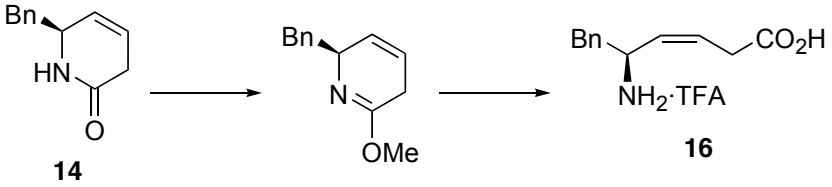


12 **15**

By use of a procedure similar to that described for organocopper-mediated reduction of the acetate **6**, -difluoro-,-unsaturated lactam **12** (600 mg, 2.69 mmol) was converted into the title compound **15** (506 mg, 91.9% yield) as colorless crystals: mp 158–159 °C; $[]^{32}_D$ –15.14 (c 1.16, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 2.55 (ddd, *J* = 21.7, 7.8, 4.4 Hz, 1H), 2.79 (ddd, *J* = 21.7, 8.8, 4.4 Hz, 1H), 2.86 (dd, *J* = 13.9, 6.8 Hz, 1H), 3.12 (dd, *J* = 13.7, 3.7 Hz, 1H), 4.26–4.37 (m, 1H), 5.23 (dt, *J* = 14.6, 3.7 Hz 1H),

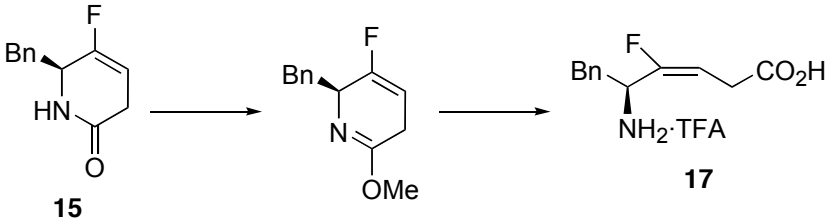
6.74–6.95 (m, 1H), 7.17–7.41 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) 29.0 (d, *J* = 8.3 Hz), 40.1, 53.6 (d, *J* = 35.6 Hz), 98.9 (d, *J* = 17.4 Hz), 127.2, 128.6, 129.9, 134.9, 154.1 (d, *J* = 251 Hz), 169.2; ^{19}F NMR (376 MHz, $CDCl_3$) –120.3 (m, 1F); Anal. Calcd for $C_{12}H_{12}NOF$: C, 70.23; H, 5.89; N, 6.82. Found: C, 70.47; H, 5.99; N, 6.68.

(5*S*, 3*Z*)-5-Amino-6-phenylhex-3-enoic acid trifluoroacetate (16).



To a stirred solution of the lactam **14** (5 mg, 0.0267 mmol) in CH_2Cl_2 (0.25 mL) was added 2,4,6-collidine (3.53 l, 0.0267 mmol) and $Me_3O{\cdot}BF_4$ (19.8 mg, 0.0267 mmol). After stirring for 20 h at room temperature, 1 M aqueous HCl (1.5 mL) was added to the abave mixture. After stirring for 48hr, H_2O (3mL) was added to the mixture, and the whole was purified by preparative HPLC (13% CH_3CN in H_2O containing 0.1 % TFA) followed by freeze-drying gave the title compound **16** as mono TFA salt (colorless oil, 54.1 mg, 36.1% yield): $[]^{24}_{D}$ –31.9 (c 0.138, MeOH); ^{1}H NMR (400 MHz, D_2O) 2.61 (dd, *J* = 17.8, 8.1 Hz, 1H), 2.78 (dd, *J* = 12.9, 9.8 Hz, 1H), 2.87 (dd, *J* = 17.6, 7.3 Hz, 1H), 3.04 (dd, *J* = 13.2, 5.1 Hz, 1H), 4.16–4.26 (m, 1H), 5.48 (td, *J* = 10.5, 1.5 Hz, 1H), 5.72 (dt, *J* = 11.0, 7.4 Hz, 1H), 7.13–7.7.30 (m, 5H); ^{13}C NMR (100 MHz, CD_3OD) 33.3, 40.5, 51.3, 124.6 (q, *J* = 269 Hz), 128.1, 128.4, 129.8, 130.2, 130.6, 136.6, 164.0 (q, *J* = 33.1 Hz), 174.2; ^{19}F NMR (376 MHz, CD_3OD) –75.4 (s, 3F); HRMS (FAB), *m*/*z* calcd for $C_{12}H_{16}NO_2$ (MH^+) 206.1181, found 206.1186.

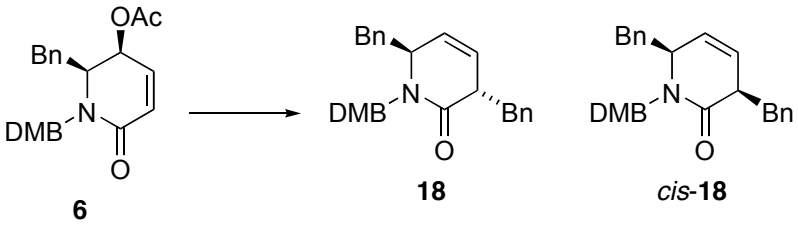
(5*S*, 3*E*)-5-Amino-4-fluoro-6-phenylhex-3-enoic acid trifluoroacetate (17).



By use of a procedure similar to that described for the preparation of the (*Z*)-alkene dipeptide isostere **16**, the lactam **15** (5 mg, 0.0244 mmol) was converted into the title compound **17** as mono TFA salt (white powder, 6.5 mg, 79.1% yield): $[]^{23}_{D}$ +13.2 (c 0.075, MeOH); ^{1}H NMR (400 MHz, D_2O) 2.47 (dd, *J* =

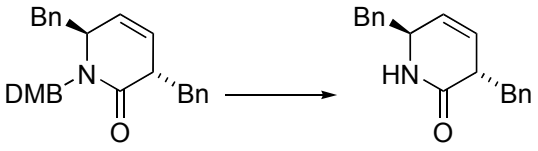
18.1, 9.0 Hz, 1H), 2.77 (dd, *J* = 18.1, 7.3 Hz, 1H), 2.98 (dd, *J* = 13.2, 10.7 Hz, 1H), 3.09 (dd, *J* = 13.2, 5.4 Hz, 1H), 4.39 (ddd, *J* = 27.6, 10.5, 5.4 Hz, 1H), 5.41 (dt, *J* = 20.7, 8.1 Hz, 1H), 7.15–7.29 (m, 5H); ^{13}C NMR (100 MHz, CD_3OD) 30.4 (d, *J* = 9.0 Hz), 37.1, 51.0 (d, *J* = 25.7 Hz), 107.6 (d, *J* = 23.8 Hz), 118.2 (q, *J* = 295 Hz), 128.7, 129.9, 130.4, 136.0, 155.0 (d, *J* = 249 Hz), 163.1 (q, *J* = 34.7 Hz), 173.6 (d, *J* = 2.5 Hz); ^{19}F NMR (376 MHz, CD_3OD) –122.4 (dd, *J* = 27.4, 20.7 Hz, 1F), –75.3 (s, 3F); HRMS (FAB), *m/z* calcd for $C_{12}H_{15}FNO_2$ (MH^+) 224.1087, found 224.1084.

General Procedure for the Organocopper-mediated one-pot reduction-alkylation. Synthesis of (3*S*, 6*S*)-3,6-Dibenzyl-3,6-dihydro-1-(2,4-dimethoxy)benzyl-pyridin-2-one (18).



To a suspension of CuI (48.3 mg, 0.254 mmol) in THF (1 mL) was added dropwise a solution of MeLi LiBr complex in Et_2O (1.48 M, 516 L, 0.764 mmol) at – 78 °C under argon, and the mixture was stirred for 10 min at 0 °C. To the solution of organocopper reagent was added dropwise a solution of the acetate **6** (50.4 mg, 0.127 mmol) in THF (1 mL) at – 78 °C, and the mixture was stirred for 20 min at – 78 °C. To the above mixture was added dropwise Bn-Br (120 L, 1.01 mmol), and the mixture was stirred for 3 h at – 78 °C. The reaction was quenched at – 78 °C by addition of a 1:1 saturated NH_4Cl-28%NH_4OH solution (2 mL) with additional stirring at room temperature for 30 min. The mixture was extracted with Et_2O and the extract was washed with H_2O and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (2:1) gave the title compound **18** (29.2 mg, 53.7% yield) as colorless oil and (3*R*)-isomer (12.8 mg, 23.5% yield). Compound **18**: $[]^{23}_D$ +77.9 (c 0.68, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 2.42–2.50 (m, 1H), 2.87 (dd, *J* = 13.6, 8.5 Hz, 1H), 2.87–3.00 (m, 2H), 3.18 (dd, *J* = 13.6, 4.4 Hz, 1H), 3.79 (s, 3H), 3.81 (s, 3H), 3.95–4.05 (m, 1H), 4.27 (d, *J* = 14.8 Hz, 1H), 5.33 (d, *J* = 15.1 Hz, 1H), 5.49–5.60 (m, 2H), 6.37 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.44 (d, *J* = 2.2 Hz, 1H), 6.88 (d, *J* = 8.3 Hz, 1H), 7.00–7.12 (m, 4H), 7.13–7.27 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) 37.3, 39.3, 40.8, 41.5, 55.3, 57.8, 98.2, 104.4, 117.5, 125.2, 125.9, 126.5, 127.2, 128.0, 128.1, 129.5, 129.8, 129.9, 136.2, 139.2, 158.4, 160.0, 170.5; HRMS (FAB), *m/z* calcd for $C_{28}H_{30}NO_3$ (MH^+) 428.2226, found 428.2219.

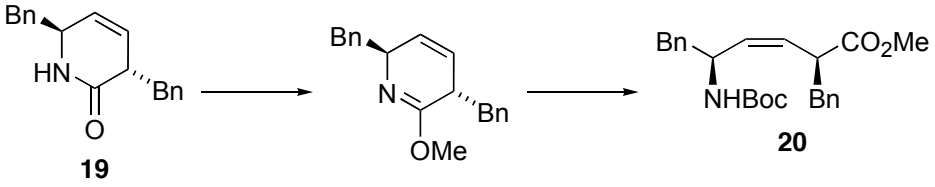
(3*S*, 6*S*)-3,6-Dibenzyl-3,6-dihydro-1*H*-pyridin-2-one (19).



18 **19**

By use of a procedure similar to that described for the DMB group deprotection of lactam **13**, -substituted lactam **18** (40.2 mg, 0.0940 mmol) was converted into the title compoumd **19** (26.1 mg, 99.9% yield) as colorless oil: $[\alpha]^{22}_{D}$ –20.8 (c 0.33, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 2.61 (dd, *J* = 13.4, 8.5 Hz, 1H), 2.85 (dd, *J* = 13.4, 5,4 Hz, 1H), 2.91 (dd, *J* = 13.2, 5.4 Hz, 1H), 3.01–3.11 (m, 1H), 3.13 (dd, *J* = 13.2, 4.1 Hz, 1H), 3.84–3.98 (m, 1H), 5.57–5.70 (m, 2H), 5.98 (br, 1H), 7.08–7.34 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) 38.5, 42.1, 43.5, 54.6, 125.1, 125.8, 126.3, 127.0, 128.1, 128.7, 129.3, 129.5, 136.2, 138.1, 171.4; HRMS (FAB), *m*/*z* calcd for $C_{19}H_{20}NO$ (MH^+) 278.1545, found 278.1541.

Methyl (2*S*, 5*S*, 3*Z*)-2-Benzyl-5-[*N*-(*tert*-butoxycarbonyl)amino]-6-phenylhex-3-enoate (20).

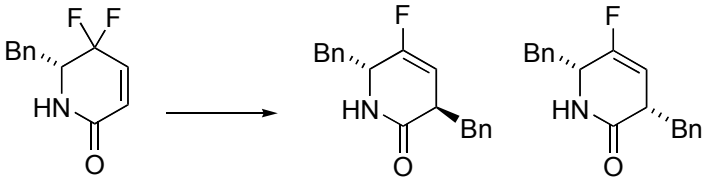


19 **20**

To a solution of lactam **19** (71 mg, 0.257 mmol) in CH_2Cl_2 (3.5 mL) was added $Me_3O{\cdot}BF_3$ (189 mg, 1.28 mmol) under argon and the mixture was stirred for 3.5 h at room temperature. Reaction was quenched by solid $NaHCO_3$. After removal of $NaHCO_3$ by filtration, the filtrate was concentrated under reduced pressure. The resulting residue was dissolved in the mixture of 0.5 M HCl (4 mL) and THF (1 mL). After stirring for 24 h at room temperature, the mixture was concentrated under reduced pressure. The resulting residue was dissolved in DMF (3 mL). DIEPA (89.5 l, 0.514 mmol) and $(Boc)_2O$ was successively added to the above solution at 0 °C. After stirring for 3h at room temperature, the mixture was extracted with EtOAc. The extract was washed with saturated citric acid, brine, saturated $NaHCO_3$, and brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (8:1) gave the title compound **20** (42.1 mg, 40.0 % yield) as colorless oil with -epimerized (2*R*)-product (14.0 mg, 13.3 %). Compound **20**: $[\alpha]^{25}_{D}$ +61.3 (c 1.06, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 1.42 (s, 9H), 2.74 (dd, *J* = 13.4, 6.8 Hz, 1H), 2.81 (dd, *J* = 13.4, 6.8 Hz, 1H), 2.80–2.92 (m, 1H), 3.03 (dd, *J* = 13.7, 8.1 Hz, 1H), 3.51 (s, 3H), 3.72 (br, 1H), 4.27 (br, 1H), 4.56 (br,

1H), 5.35 (t, *J* = 9.8 Hz, 1H), 5.47 (t, *J* = 10.3 Hz, 1H), 7.11–7.31 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) 28.3, 38.7, 41.4, 46.1, 51.7, 79.3, 126.3, 128.2, 128.9, 129.1, 129.5, 132.1, 138.4, 154.8, 173.5; HRMS (FAB), *m/z* calcd for $C_{25}H_{32}NO_4$ (MH^+) 410.2331, found 410.2317.

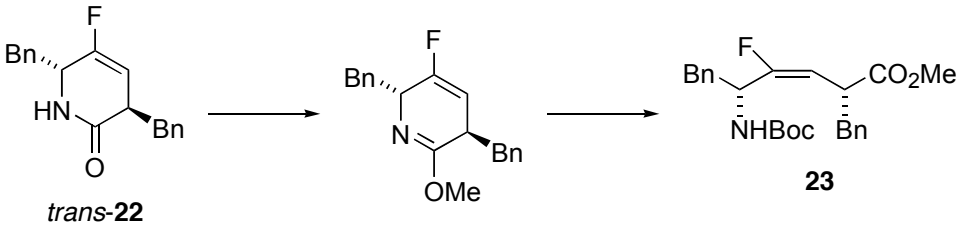
(3*R*,6*R*)-3,6-Dibenzyl-5-fluoro-3,6-dihydro-1*H*-pyridin-2-one (*trans*-22).



21 *trans*-**22** *cis*-**22**

By use of a procedure similar to that described for organocopper-mediated one-pot reduction-alkylation of lactam **6** for the synthesis of -substituted lactam **18**, -difluoro-lactam **21** (enantiomer of lactam **12**, 49.0 mg, 0.219 mmol) was converted into the title compoumd *trans*-**22** (18.1 mg, 28% yield) and *cis*-**22** (17.4 mg, 27% yield). *trans*-**22** (colorless oil): $[]_D^{28}$ –60.0 (*c* 0.050, $CHCl_3$); ^{1}H NMR (400MHz, $CDCl_3$) 2.69 (dd, *J* = 13.9, 8.5Hz, 1H), 2.83–2.88 (m, 1H), 3.00–3.03 (m, 1H), 3.10–3.28 (m, 2H), 4.03–4.05 (m, 1H), 5.15 (dd, *J* = 15.6, 3.4Hz, 1H), 5.60 (br, 1H), 7.12–7.35 (m, 10H); ^{13}C NMR (100MHz, $CDCl_3$) 38.9, 40.3, 50.9, 53.5 (d, *J* = 35.6 Hz), 102.2 (d, *J* = 15.7 Hz), 126.6, 127.3, 128.4, 128.9, 129.5, 130.5, 134.9, 137.6, 154.4 (d, *J* = 252 Hz), 170.5; ^{19}F NMR (376 MHz, $CDCl_3$) –120.2 (dt, *J* = 15.5, 4.6 Hz, 1F); HRMS (FAB) *m/z,* calcd. for $C_{19}H_{19}FNO$ (MH^+) 296.1451; found: 296.1446. *cis*-**22** (colorless oil): $[]_D^{28}$ +37.6 (*c* 0.062, $CHCl_3$); ^{1}H NMR (400MHz, $CDCl_3$) 1.89–1.94 (m, 1H), 2.43–2.48 (m, 1H), 2.83–2.96 (m, 2H), 3.20–3.24 (m, 1H), 4.08–4.09 (m, 1H), 5.15 (dd, *J* = 15.4, 4.4Hz, 1H), 5.86 (br, 1H), 7.04-7.38 (m, 10H); ^{13}C NMR (100MHz, $CDCl_3$) 39.2, 40.4, 41.3 (d, *J* = 8.3 Hz), 53.8 (d, *J* = 36.4 Hz), 102.1 (d, *J* = 14.0 Hz), 126.7, 127.3, 128.3, 128.7, 129.6, 129.7, 129.9, 135.3, 137.4, 154.5 (d, *J* = 251 Hz), 170.5; ^{19}F NMR (376 MHz, $CDCl_3$) –119.7 (d, *J* = 15.2 Hz, 1F); HRMS (FAB) *m/z,* calcd. for $C_{19}H_{19}FNO$ (MH^+) 296.1451; found: 296.1449.

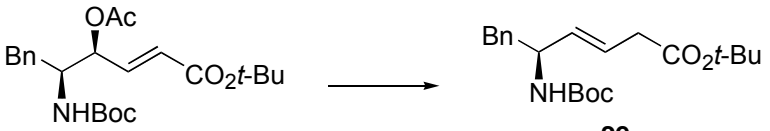
Methyl (2*R*,5*R*,3*E*)-2-Benzyl-5-[*N*-(*tert*-butoxycarbonyl)amino]-4-fluoro-6-phenyl-hex-3-enoate (23).



trans-**22** **23**

By use of procedure similar to that described for the preparation of the isostere **20**, the lactam *trans*-**22** (13 mg, 0.044 mmol) was converted into the title compound **23** (7.0 mg, 37% yield) and □-epimerized (2*R*)-product (1 mg, 5.3% yield). Compound **23** (colorless oil): $[\square]_D^{28}$ +23.8 (*c* 0.056, $CHCl_3$); ^{1}H NMR (400MHz, $CDCl_3$) □ 1.43 (s, 9H), 2.78–2.95 (m, 4H), 3.31–3.45 (m, 1H), 3.39 (s, 3H), 4.69 (br, 1H), 4.76 (m, 1H), 5.13 (dd, *J* = 20.5, 10.9 Hz, 1H), 6.93–7.29 (m, 10H); ^{13}C NMR (100MHz, $CDCl_3$) □ 28.3, 38.2, 43.9 (d, *J* = 8.3 Hz), 49.6 (d, *J* = 26.4 Hz), 51.7, 106.8 (d, *J* = 23.2 Hz), 126.6, 128.4, 129.1, 130.9, 136.5, 138.0, 154.7, 159.5, 173.0; ^{19}F NMR (376 MHz, $CDCl_3$) □ –120.2 (dd, *J* = 26.9, 21.8 Hz, 1F); HRMS (FAB) *m/z,* calcd. for $C_{25}H_{31}FNO_4$ (MH^+) 428.2237; found: 428.2232.

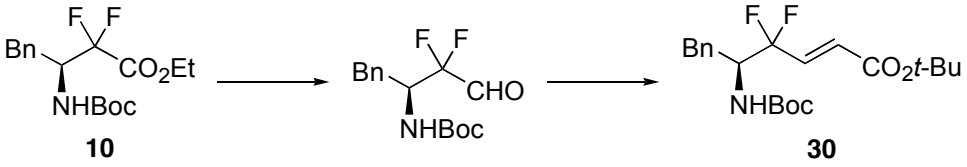
***tert*-Butyl (5*S*, 3*E*)-5-[*N*-(*tert*-Butoxycarbonyl)amino]-6-phenylhex-3-enoate (29).**



28 **29**

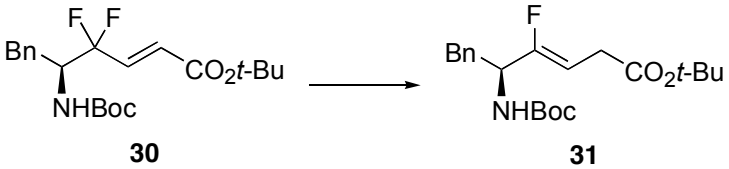
To a suspension of Sm powder (300 mg, 2.00 mmol) in THF (4 mL) was added a solution of CH_2I_2 (80.4 mL, 1.00 mmol) in THF (6 mL) at room temperature under argon. The mixture was stirred for 1h. To a solution of enoate **28** (82.4 mg, 0.196 mmol), which prepared by precedent procedure (*J. Org. Chem.*, **2002**, 67, 2162-6173), in THF-EtOH (2:1, 3.75 mL) was added the above solution of SmI_2 (0.1M, 5.89 mL, 0.589 mmol) at 0 °C under argon. After 20 min, the reaction mixture was quenched with saturated NH_4Cl and extracted with Et_2O. The extract was washed with saturated NH_4Cl and brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with EtOAc-*n*-hexane (1:8) gave the title compound **29** (59.9 mg, 84.5% yield) as colorless oil: $[\square]^{29}_D$ +4.76 (c 1.05, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) □1.39 (s, 9H), 1.43 (s, 9H), 2,83 (d, *J* = 6.4 Hz, 2H), 2.94 (d, *J* = 6.0 Hz, 2H), 4.40 (br, 1H), 4.51 (br, 1H), 5.52 (dd, *J* = 15.6, 4.8 Hz, 1H), 5.63 (dt, *J* = 15.6, 6.8 Hz, 1H), 7.12–7.33 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) □28.0, 28.3, 38.9, 41.6, 79.3, 80.6, 123.2, 126.3, 128.2, 129.5, 133.3, 137.4, 155.1, 170.8; HRMS (FAB), *m/z* calcd for $C_{21}H_{32}NO_4$ (MH^+) 362.2331, found 362.2335.

***tert*-Butyl (5*S*, 2*E*)-5-[*N*-(*tert*-Butoxycarbonyl)amino]-4,4-difluoro-6-phenylhex-2-enoate (30).**



By use of a similar procedure for the preparation of the enoate **11**, the ester **10** (1.0 g, 2.91 mmol) was converted into the corresponding aldehyde. To a stirred suspension of LiCl (173 mg, 4.08 mmol) in CH_3CN (10 mL) under argon were added $(EtO)_2P(O)CH_2CO_2t$-Bu (1.03 g, 4.08 mmol) and $(i\text{-Pr})_2NEt$ (710 μL, 4.08 mmol) at 0 °C. After 20 min, the above aldehyde in CH_3CN (10 mL) was added to the mixture at 0 °C, and the mixture was stirred for 6 h at 0 °C. The reaction was quenched by saturated NH_4Cl at 0 °C, and extracted with EtOAc. The extract was washed with saturated citric acid, brine, 5% $NaHCO_3$, and brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (9:1) gave the title compound **30** (**11**-(*E*)) (690 mg, 59.6% yield) as colorless crystals: mp 124–125 °C; $[\alpha]^{32}_D$ +17.9 (c 1.24, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29 (s, 9H), 1.48 (s, 9H), 2.67 (dd, *J* = 13.7, 11.0 Hz, 1H), 3.17 (dd, *J* = 14.4, 3.7 Hz, 1H), 4.25–4.44 (m, 1H), 4.46–4.59 (m, 1H), 6.25 (d, *J* = 15.6 Hz, 1H), 6.74 (ddd, *J* = 15.9, 12.9, 11.0 Hz, 1H), 7.14–7.35 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.9, 28.1, 34.3, 55.2 (t, *J* = 28.1 Hz), 80.1, 81.6, 119.7 (t, *J* = 247 Hz), 126.7, 128.2, 128.5, 129.1, 135.9, 136.3 (t, *J* = 26.4 Hz), 154.9, 163.9; ^{19}F NMR (376 MHz, $CDCl_3$) δ –112.8 (ddt, *J* = 707, 249, 14.6 Hz, 1F), –105.7 (dd, *J* = 541, 249 Hz, 1F); Anal. Calcd for $C_{21}H_{29}NO_4F_2$: C, 63.46; H, 7.35; N, 3.52. Found: C, 63.44; H, 7.28; N, 3.56.

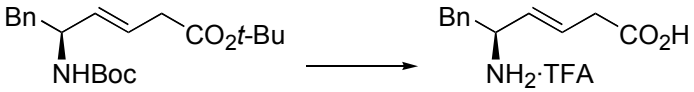
***tert*-Butyl (5*S*, 3*Z*)-5-[*N*-(*tert*-Butoxycarbonyl)amino]-4-fluoro-6-phenylhex-3-enoate (31).**



By use of a procedure similar to that described for organocopper-mediated reduction of the acetate **6**, β,β-difluoro-α,β-unsaturated enoate **30** (500 mg, 1.26 mmol) was converted into the title compoumd **31** (410 mg, 85.9% yield) as colorless crystals: mp 76–77 °C; $[\alpha]^{32}_D$ +5.51 (c 1.40, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (s, 9H) 1.42 (s, 9H), 2.85–3.08 (m, 4H), 4.38–4.58 (m, 1H), 4.62–4.76 (m, 1H), 4.86 (dt, *J*

= 36.8, 7.1 Hz, 1H), 7.14–7.32 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.0, 28.2, 30.4 (d, *J* = 4.9 Hz), 38.6, 52.5 (d, *J* = 27.3 Hz), 79.8, 80.8, 99.8 (d, *J* = 12.4 Hz), 126.7, 128.4, 129.3, 136.5, 154.7, 158.2 (d, *J* = 259 Hz), 170.0 (d, *J* = 2.5 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ –120.2 (dd, *J* = 36.9, 16.2 Hz, 1F); Anal. Calcd for $C_{21}H_{30}NO_4F$: C, 66.47; H, 7.97; N, 3.69. Found: C, 66.41; H, 8.13; N, 3.63.

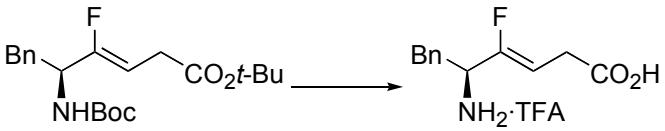
(5*S*, 3*E*)-5-Amino-6-phenylhex-3-enoic acid trifluoroacetate (24).



29 **24**

To a solution of enoate **29** (51 mg, 0.141 mmol) in CH_2Cl_2 (1.1 mL) was added TFA (220 μL), and the mixture was stirred for 2.0 h at room temperature. Et_2O (2 mL) was added and evaporated three times. The crude mixture was dissolved in H_2O and purification by preparative HPLC followed by freeze-drying gave the title compound **24** as TFA salt (39.5 mg, 87.7 %): $[\alpha]^{30}_D$ +11.4 (c 1.098, $CHCl_3$); ^{1}H NMR (400 MHz, D_2O) δ 2.85 (dd, *J* = 13.6, 8.2 Hz, 1H), 2.91 (dd, *J* = 13.6, 6.6 Hz, 1H), 2.96 (dd, *J* = 6.0, 4.6 Hz, 2H), 3.91 (q, *J* = 7.4 Hz, 1H), 5.51 (dd, *J* = 15.6, 8.0 Hz, 1H), 5.62 (dt, *J* = 15.6, 6.4 Hz, 1H), 7.07–7.28 (m, 5H); ^{13}C NMR (100 MHz, CD_3OD) δ 38.2, 40.5, 48.3, 49.6, 55.7, 128.4, 129.7, 129.9, 130.6, 131.1, 136.7, 162.2 (m), 174.7; ^{19}F NMR (376 MHz, CD_3OD) δ –75.6 (s, 3F); HRMS (FAB), *m*/*z* calcd for $C_{12}H_{16}NO_2$ (MH^+) 206.1181, found 206.1177.

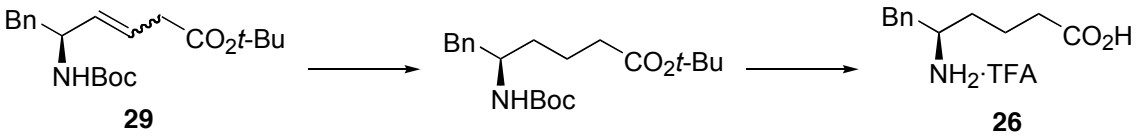
(5*S*, 3*Z*)-5-Amino-4-fluoro-6-phenylhex-3-enoic acid trifluoroacetate (25).



31 **25**

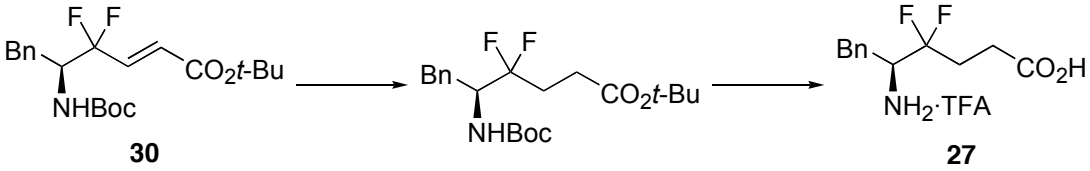
By use of a procedure similar to that described for the preparation of the (*E*)-alkene dipeptide isostere **24**, the enoate **31** (108 mg, 0.287 mmol) was converted into the title compound **25** (96.7 mg, 99.9% yield) as mono TFA salt: $[\alpha]^{27}_D$ –31.6 (c 2.461, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 2.95–3.11 (m, 4H), 4.12 (dt, *J* = 23.2, 7.4 Hz, 1H), 5.00 (dt, *J* = 36.6, 7.4 Hz, 1H), 7.13–7.32 (m, 5H); ^{13}C NMR (100 MHz, CD_3OD) δ 29.8, 37.2, 54.9 (d, *J* = 28.1 Hz), 106.9 (d, *J* = 10.8 Hz), 118.3 (q, *J* = 291 Hz), 128.6, 129.9, 130.4, 136.1, 155.1 (d, *J* = 257 Hz), 163.1 (q, *J* = 33.9 Hz), 173.4; ^{19}F NMR (376 MHz, CD_3OD) δ –124.4 (dd, *J* = 36.3, 23.7 Hz, 1F), –75.4 (s, 3F); HRMS (FAB), *m*/*z* calcd for $C_{12}H_{15}FNO_2$ (MH^+) 224.1087, found 224.1092.

(5*S*)-5-Amino-6-phenylhexanoic acid trifluoroacetate (26).



To the solution of the enoate **29** (*E*-, *Z*-mixture, 1.00g, 2.77 mmol) in EtOAc (28 mL) was added 10 % Pd/C (28 mg), and the mixture was stirred overnight under H_2 at room temperature. The mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. Flash chromatography over silica gel with *n*-hexane-EtOAc (20:1) gave the reduced product in quantitatively yield. By use of a procedure similar to that described for the preparation of the (*E*)-alkene dipeptide isostere **24**, the resulting reduced product (300 mg, 0.83 mmol) was converted into the title compound **26** (97.8 mg, 36.9% yield) as mono TFA salt: $[\alpha]^{27}_{D}$ –7.47 (c 0.82, $CHCl_3$); ^{1}H NMR (400 MHz, D_2O) δ 1.48–1.70 (m, 4H), 2.23–2.33 (m, 2H), 2.77 (dd, *J* = 14.2, 7.8 Hz, 1H), 2.93 (dd, *J* = 14.4, 6.3 Hz, 1H), 3.37–3.49 (m, 1H), 7.15–7.35 (m, 5H); ^{13}C NMR (100 MHz, CD_3OD) δ 21.5, 32.8, 34.2, 39.8, 54.0, 118.2 (q, *J* = 295 Hz), 128.4, 130.0, 130.4, 137.1, 163 (q, *J* = 34.7 Hz), 176.8; ^{19}F NMR (376 MHz, CD_3OD) δ –75.4 (s, 3F); HRMS (FAB), *m*/*z* calcd for $C_{12}H_{18}NO_2$ (MH^+) 208.1338, found 208.1337.

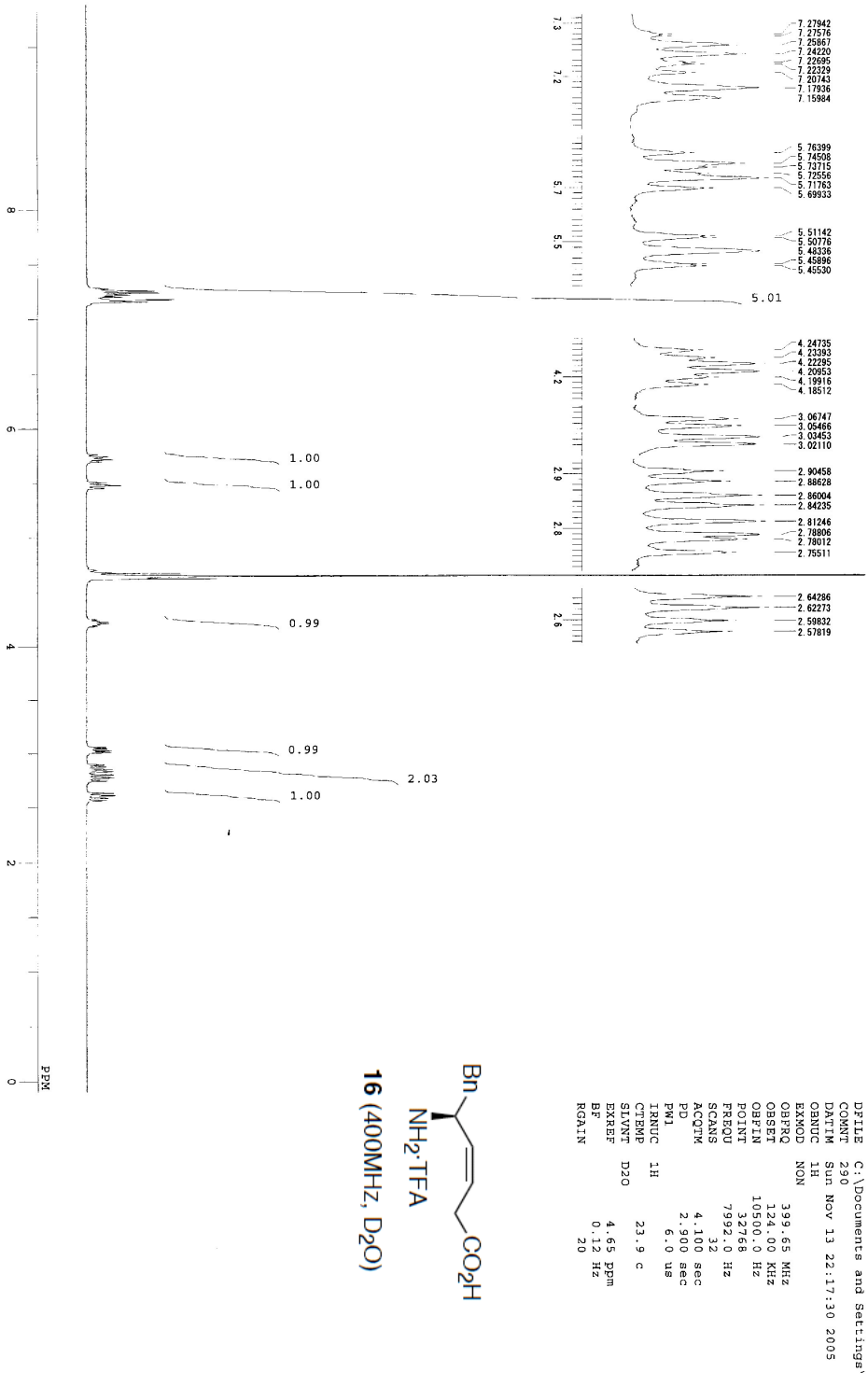
(5*S*)-5-Amino-4,4-difluoro-6-phenylhexanoic acid trifluoroacetate (27).

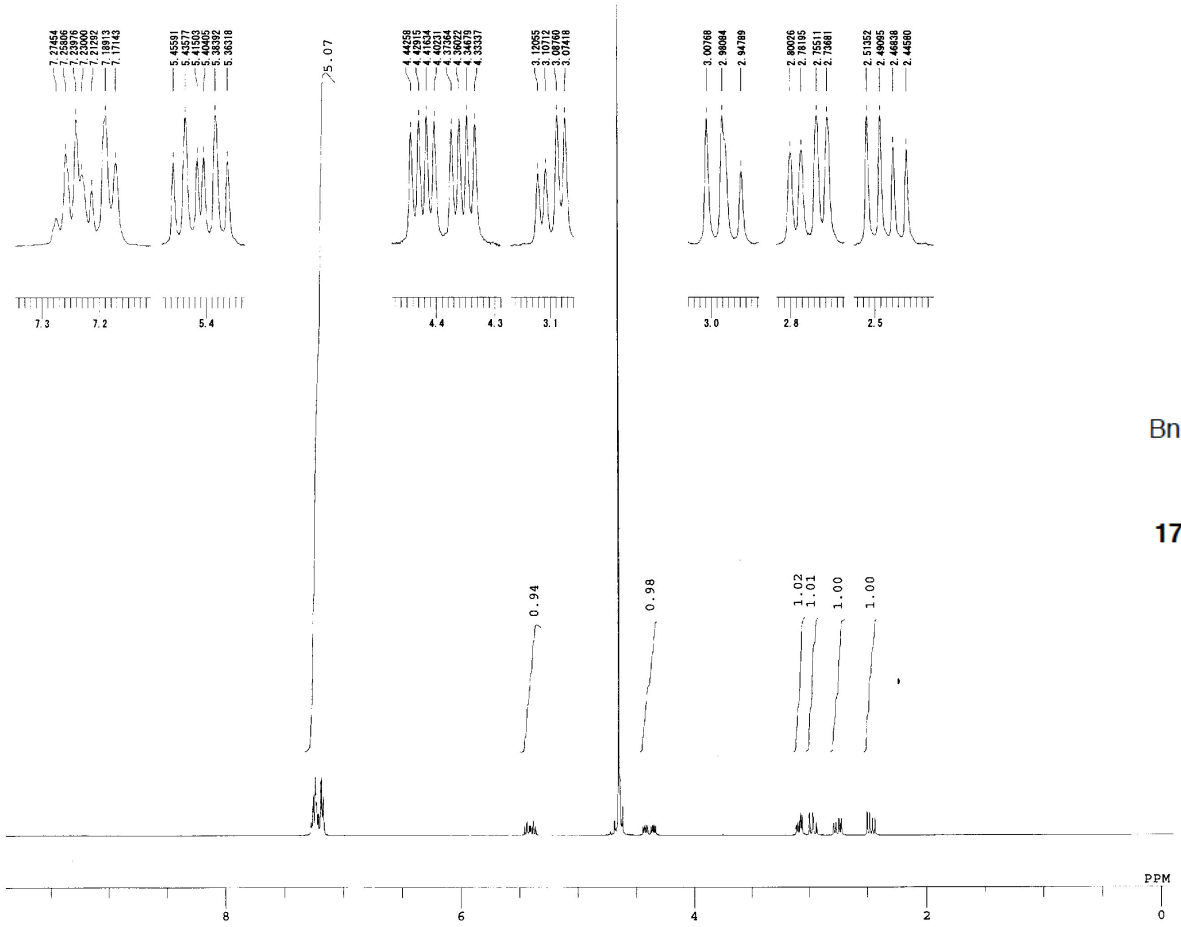


By use of a procedure similar to that described for the preparation of the isostere **26**, the ester **30** (500 mg, 1.26 mmol) was converted into the reduced product (460 mg, 91.5% yield). The resulting reduced product (120 mg, 0.300 mmol) was converted to the title compound **27** (44.9 mg, 41.8% yield) by a procedure similar to that described for the preparation of the (*E*)-alkene dipeptide isostere **24**. mono TFA salt **27**: $[\alpha]^{30}_{D}$ +31.1 (c 0.643, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 2.22–2.45 (m, 2H), 2.56 (td, *J* = 7.1, 2.0 Hz, 1H), 2.90 (dd, *J* = 14.6, 9.8 Hz, 1H), 3.27–3.36 (m,1H), 3.99–4.12 (m, 1H), 7.30–7.43 (m, 5H); ^{13}C NMR (100 MHz, CD_3OD) δ 27.0 (t, *J* = 4.9 Hz), 30.0 (t, *J* = 23.6 Hz), 34.7, 57.3 (t, *J* = 24.4 Hz), 126.0 (t, *J* = 246 Hz), 130.4, 130.6, 135.4, 163.2 (m), 175.2; ^{19}F NMR (376 MHz, CD_3OD) δ –111.5 (m, 1F), –104.0 (m, 1F), –75.5 (s, 3F); HRMS (FAB), *m*/*z* calcd for $C_{12}H_{16}F_2NO_2$ (MH^+) 244.1149, found

244.1140.

NMR charts of representative compounds



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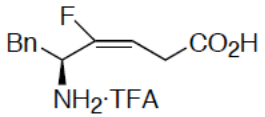

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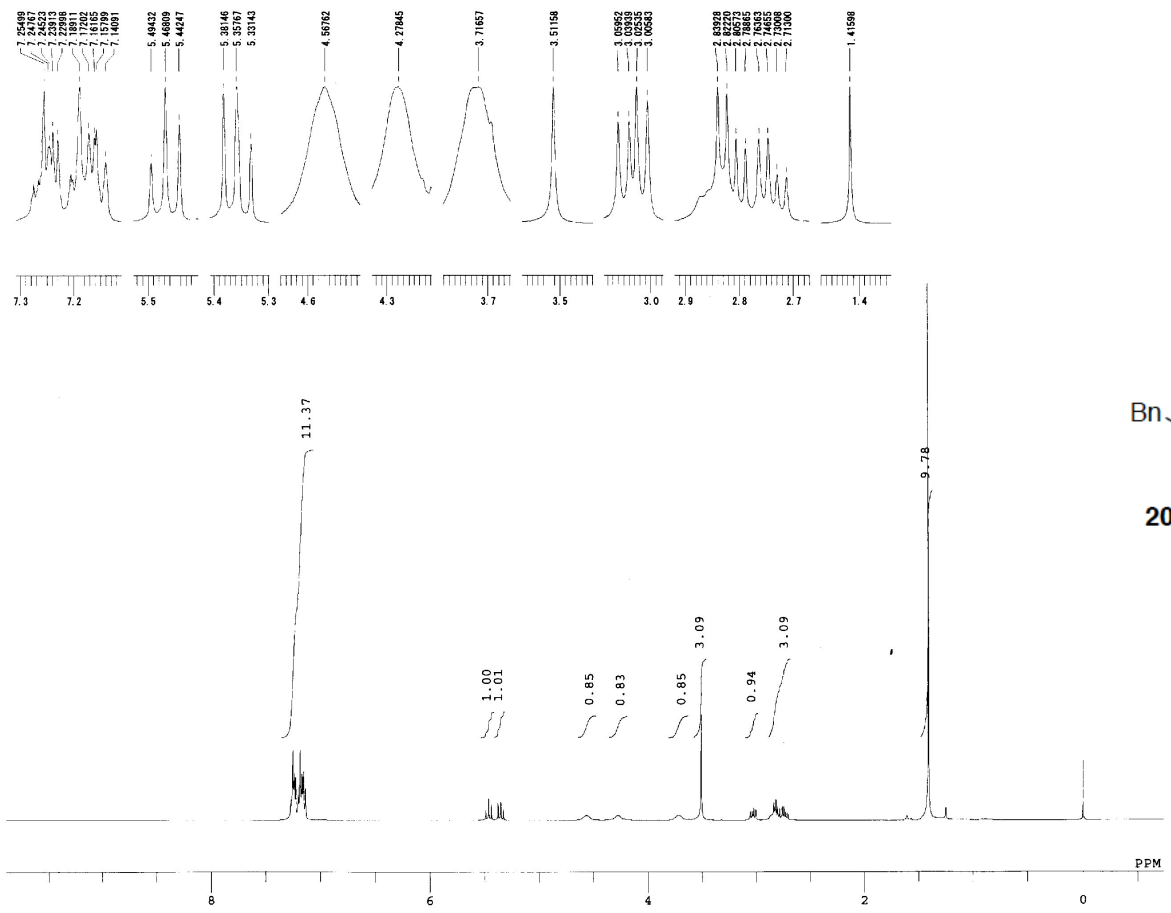


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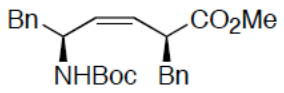

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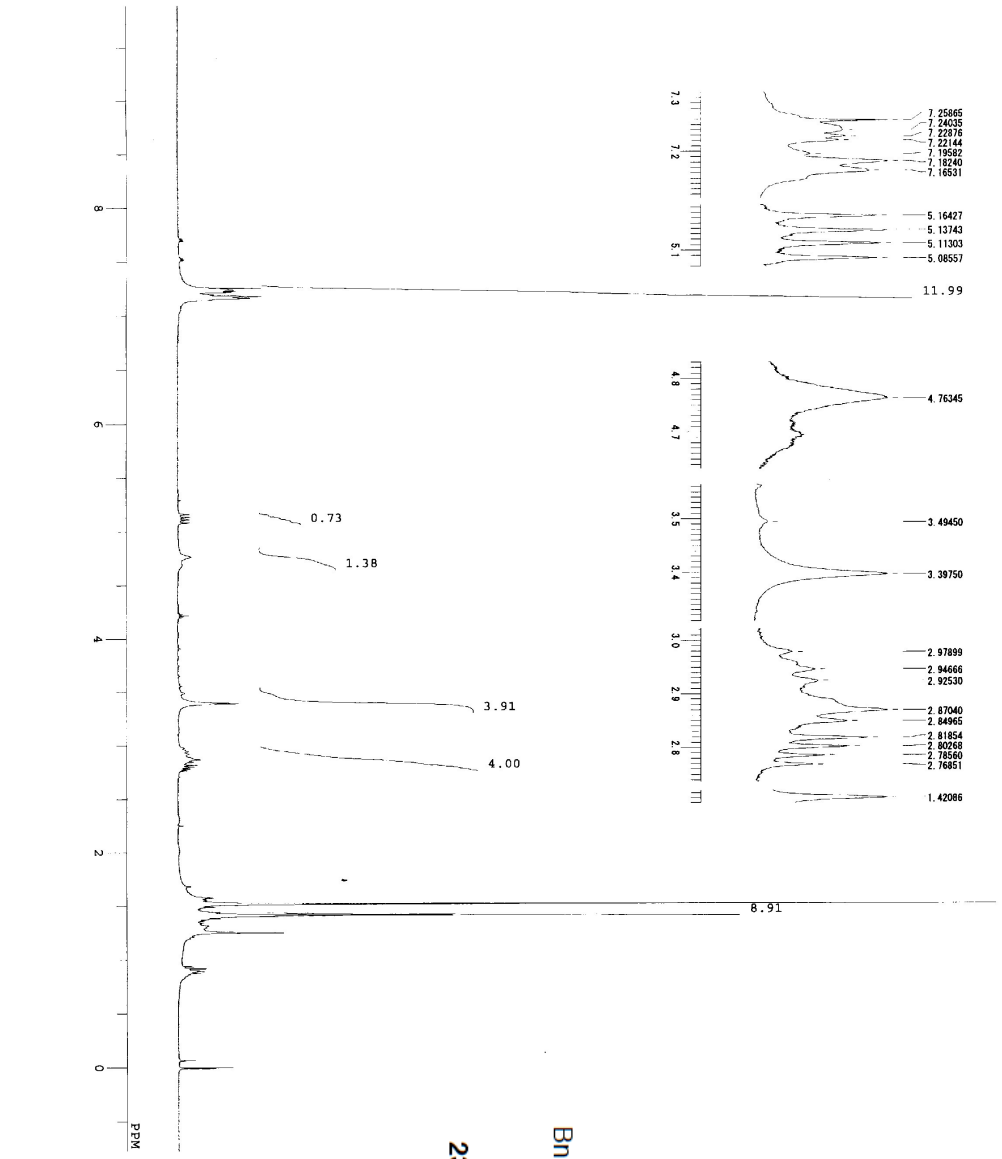

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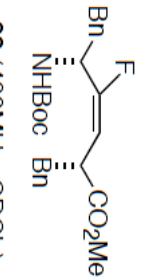

23 (400MHz, $CDCl_3$)

Biological Assay

Cell Culture. Caco-2 cells (American Type Culture Collection (ATCC CRL-1392)) were cultured as described previously (Terada et al., 1999). For uptake studies, cells were seeded on 24-well cluster plates, given fresh medium every 2-4 days and were used on the 15th day for uptake studies.

Uptake Studies by Cell Monolayers. Uptake studies using [^{3}H]glycylsarcosine (Gly-Sar) (PerkinElmer) were performed as previously reported (Terada et al., 1997; Terada et al., 1999). Briefly, Caco-2 cells were preincubated with incubation medium (pH 7.4) for 10 min at 37 °C. After the medium was removed, the cells were incubated with incubation medium (pH 6.0) containing [^{3}H]Gly-Sar (25 □M) for 15 min at 37 °C. Thereafter, monolayers were rapidly washed by ice-cold of incubation medium (pH 7.4), and were solubilized by 1N NaOH. The radioactivity of the solubilized cells was determined by liquid scintillation counting. The protein content of the solubilized cells was determined by the method of Bradford, using a Bio-Rad Protein Assay Kit (Bio-Rad, Richmond, CA).

Refs.

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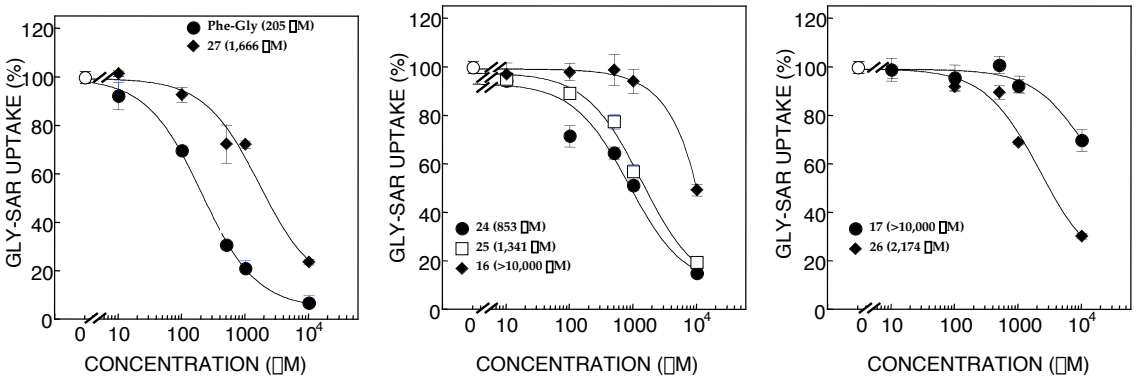
Effect of various Phe-Gly derivatives on [^{3}H]Gly-Sar uptake by Caco-2 cells



Caco-2 cells (day 16) , 24-well plate
[^{3}H]Gly-Sar (pH 6.0), 25 □M, 15 min uptake