(100 MHz, $CDCl_3$) δ −5.5 (2C), 18.1, 19.6, 20.7, 24.3, 25.8 (3C), 26.2, 28.0 (3C), 28.4, 32.6, 38.1, 44.5, 47.6, 48.5, 52.8, 54.0 (t, J=27.3 Hz), 62.0, 64.9, 79.6, 119.6 (t, J=245.4 Hz), 125.2, 137.7 (t, J=26.5 Hz), 155.2, 161.9; ^{19}F NMR (376 $CDCl_3$) δ −108.1 (dt, J=248.3, 10.3 Hz), −110.8 (dt, J=248.3, 12.4 Hz). Anal. Calcd for $C_{29}H_{50}F_2N_2O_6SSi$: C, 56.10; H, 8.12; N, 4.51. Found: C, 56.20; H, 8.14; N, 4.45.

*4.20. (2R,5S,3Z)-5-[N-(tert-Butoxycarbonyl)amino]-8-(tert-butyldimethylsiloxy)-4-fluoro-2-(prop-2-enyl)oct-3-enoyl (S)-sultam (**30**)*

By use of a procedure similar to that described for the preparation of Boc–L-Val–L-Ala FADI derivative **15a**, the (*S*)-sultam derivative **29** (1.01 g, 1.62 mmol) was converted into the title compound **30** (1.03 g, 97% yield) as a colorless oil: $[\alpha]_D^{21}$ −71.9 (*c* 1.11, $CHCl_3$); IR (ATR): 3352 (NHCO), 3300 (NHCO), 1716 (CO), 1695 (CO), 1331 (NSO_2), 1166 (NSO_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.04 (s, 6H), 0.88 (s, 9H), 0.97 (s, 3H), 1.15 (s, 3H), 1.25–1.66 (m, 15H), 1.85–2.04 (m, 5H), 2.38–2.32 (m, 1H), 2.52–2.59 (m, 1H), 3.39–3.53 (m, 2H), 3.57–3.63 (m, 2H), 3.84–3.90 (m, 1H), 4.09–4.25 (m, 2H), 4.76 (d, J=9.0 Hz, 1H), 4.93–5.10 (m, 3H), 5.69–5.81 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.4 (2C), 18.2, 19.8, 20.7, 25.6, 25.9 (3C), 26.4, 28.2 (3C), 28.6 (m), 32.8, 38.3, 38.4, 40.7, 44.6, 47.6, 48.2, 51.5 (d, J=27.3 Hz), 53.0, 62.4, 65.1, 79.4, 103.3 (d, J=11.6 Hz), 117.7, 134.0, 154.8, 158.6 (d, J=263.2 Hz), 172.2; ^{19}F NMR (376 MHz, $CDCl_3$) δ −119.6 (dd, J=35.2, 20.7 Hz); HRMS (FAB), *m/z* calcd for $C_{32}H_{54}FN_2O_6SSi$ ($[M-H]^-$) 641.3461, found 641.3469.

*4.21. (2R,5S,3Z)-5-[N-(tert-Butoxycarbonyl)amino]-8-(tert-butyldimethylsiloxy)-2-[3-(tert-butyldimethylsiloxy)propyl]-4-fluorooct-3-enoyl (S)-sultam (**32**)*

To a solution of the (*S*)-sultam derivative **30** (929 mg, 1.45 mmol) in THF (30 mL) at 0 °C under argon was added dropwise $(Sia)_2BH$ in THF (0.49 M, 8.88 mL, 4.35 mmol), and the mixture was stirred at room temperature overnight. After being diluted with THF (60 mL), aqueous 50% H_2O_2 (1.15 mL) and 20% AcOK (1.45 mL) were added with additional stirring at room temperature for 2 h. The mixture was extracted with Et_2O and the extract was washed with saturated $Na_2S_2O_3$ and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel gave the corresponding alcohol. To a solution of the alcohol in CH_2Cl_2 (20 mL) at room temperature under argon was added imidazole (120 mg, 1.75 mmol) and TBSCl (240 mg, 1.60 mmol). After stirring for 1 h, the precipitate was filtrated off and the filtrate was concentrated under reduced pressure, followed by flash chromatography over silica gel to give the title compound **32** (930.7 mg, 83%) as a colorless oil: $[\alpha]_D^{23}$ −57.7 (*c* 1.32, $CHCl_3$); IR (ATR): 3375 (NHCO), 1702 (CO), 1336 (NSO_2), 1165 (NSO_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.02 (s, 6H), 0.04 (s, 6H), 0.87 (s, 9H), 0.88 (s, 9H), 0.97 (s, 3H), 1.15 (s, 3H), 1.32–1.72 (m, 18H), 1.84–1.95 (m, 4H), 2.04–2.06 (m, 2H), 3.40–3.50 (m, 2H), 3.56–3.64 (m, 4H), 3.85–3.88 (m, 1H), 4.04–4.21 (m, 2H), 4.75 (d, J=8.3 Hz, 1H), 4.97 (dd, J=36.4, 9.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.4 (2C), −5.3 (2C), 18.2, 18.3, 19.9, 20.8, 25.9 (3C), 26.0 (3C), 26.4, 28.3 (3C), 28.7, 28.8, 29.9, 30.7, 32.9, 38.4, 41.0, 44.6, 47.7, 48.3, 51.7 (d, J=27.6 Hz), 53.0, 62.5 (2C), 65.1, 79.5, 103.9 (d, J=12.0 Hz), 154.9, 158.7 (d, J=261.5 Hz), 173.0; ^{19}F NMR (470 MHz, $CDCl_3$) δ −120.7 (dd, J=36.4, 19.3 Hz); HRMS (FAB), *m/z* calcd for $C_{38}H_{70}FN_2O_7SSi_2$ ($[M-H]^-$) 773.4432, found 773.4454.

*4.22. (2R,5S,3Z)-5-[N-(tert-Butoxycarbonyl)amino]-8-[N-(tert-butoxycarbonyl)-N-(2-nitrophenylsulfonyl)amino]-2-{3-[N-(tert-butoxycarbonyl)-N-(2-nitrophenylsulfonyl)-amino]propyl}-4-fluorooct-3-enoyl (S)-sultam (**35**)*

To a solution of the bis-TBS ether **32** (259.7 mg, 0.335 mmol) in CH_3CN–H_2O (1:1, 6.7 mL) at 0 °C under argon was added aqueous H_2SiF_6 (3.28 N, 104 μL), and the mixture was stirred at room temperature for 1 h. After diluted with AcOEt (100 mL), the reaction mixture was washed with aqueous 5% K_2CO_3 and dried over $MgSO_4$. Concentration under reduced pressure gave the corresponding diol, which was used in the next step without purification. To a solution of the diol, PPh_3 (529.3 mg, 2.02 mmol) and NsNHBoc (606.8 mg, 2.01 mmol) in THF (6.7 mL), and a solution of DEAD in toluene (2.2 M, 913 μL, 2.01 mmol) were successively added at 0 °C under argon. After being stirred at room temperature overnight, concentration under reduced pressure followed by flash chromatography over silica gel gave the title compound **35** (320 mg, 85%) as a semisolid: $[\alpha]_D^{25}$ −35.9 (*c* 1.00, $CHCl_3$); IR (ATR): 3394 (NHCO), 1728 (CO), 1336 (NSO_2), 1152 (NSO_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.96 (s, 3H), 1.18 (s, 3H), 1.30–1.48 (m, 30H), 1.61–1.93 (m, 10H), 2.04–2.11 (m, 2H), 3.42–3.50 (m, 2H), 3.73–3.79 (m, 4H), 3.90–3.91 (m, 1H), 4.11–4.13 (m, 1H), 4.19–4.29 (m, 1H), 4.76 (d, J=9.0 Hz, 1H), 5.03 (dd, J=36.1, 9.2 Hz, 1H), 7.72–7.73 (m, 6H), 8.26–8.29 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.7, 20.7, 26.2, 26.3, 27.1, 27.6 (3C), 27.6 (3C), 28.1 (3C), 29.4, 31.0, 32.5, 38.2, 40.7, 44.4, 47.3, 47.3, 47.5, 48.2, 51.3 (d, J=28.8 Hz), 52.7, 64.9, 79.5, 84.7, 84.9, 103.7, 103.7, 124.1, 124.2, 131.6, 131.6, 132.7, 132.8, 133.2, 134.0, 134.1, 147.4 (2C), 150.1 (2C), 154.7, 158.5 (d, J=262.7 Hz), 172.2; ^{19}F NMR (470 MHz, $CDCl_3$) δ −119.1 to −119.0 (m); HRMS (FAB), *m/z* calcd for $C_{48}H_{66}FN_6O_{17}S_3$ ($[M-H]^-$) 1113.3636, found 1113.3624.

*4.23. (2R,5S,3Z)-5-[N-(9-Fluorenylmethoxycarbonyl)amino]-4-fluoro-8-[N-(2-nitrophenylsulfonyl)amino]-2-{3-[N-(2-nitrophenylsulfonyl)amino]propyl}oct-3-enoic acid (**36**)*

To a solution of the sultam **35** (376.2 mg, 0.337 mmol) and aqueous 50% H_2O_2 (119.6 μL, 1.75 mmol) in THF–H_2O (5:1, 6 mL) at 0 °C was added aqueous 1 N LiOH (670 μL, 0.67 mmol), and the mixture was stirred at room temperature

for 2 h. After being diluted with AcOEt (20 mL), the mixture was washed with 0.1 N HCl and dried over $MgSO_4$. Concentration under reduced pressure gave the corresponding acid, which was used in the next step without purification. To a solution of the acid in CH_2Cl_2 (15 mL) at 0 °C was added TFA (4 mL), and the mixture was stirred at room temperature for 0.5 h. Concentration under reduced pressure gave an oily residue, which was dissolved in MeCN–DMF–H_2O (10:9:1, 20 mL). Fmoc–OSu (159.2 mg, 0.472 mmol) and Et_3N (94 μL, 0.675 mmol) were added to the mixture at 0 °C, and the mixture was stirred at room temperature for 12 h. After being diluted with AcOEt (70 mL), the reaction mixture was washed with 1 N HCl and dried over dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel gave the title compound **36** (267.3 mg, 94% yield) as a semisolid: $[\alpha]_D^{23}$ −21.1 (*c* 1.05, $CHCl_3$); IR (ATR): 3347 (OH), 1709 (CO), 1341 (NSO_2), 1165 (NSO_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.43–1.81 (m, 8H), 3.04–3.10 (m, 4H), 3.38–3.41 (m, 1H), 4.17–4.19 (m, 2H), 4.34–4.43 (m, 2H), 4.86 (dd, *J*=35.8, 9.5 Hz, 1H), 5.08 (d, *J*=8.4 Hz, 1H), 5.50–5.54 (m, 2H), 7.27–7.39 (m, 4H), 7.56–7.57 (m, 2H), 7.68–7.71 (m, 4H), 7.74 (d, *J*=7.4 Hz, 2H), 7.78–7.81 (m, 2H), 8.07–8.10 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.7, 26.8, 28.7, 28.9, 39.7, 43.0, 43.1, 50.2, 51.7 (d, *J*=28.8 Hz), 66.8, 104.7 (d, *J*=13.2 Hz), 119.8 (2C), 124.9, 125.0, 125.1, 125.1, 127.0 (2C), 127.6 (2C), 130.8 (2C), 132.6, 132.7, 133.3, 133.5, 133.5, 141.1 (2C), 143.6, 143.7, 147.8 (2C), 155.8, 158.4 (d, *J*=261.5 Hz), 176.1; ^{19}F NMR (470 MHz, $CDCl_3$) δ −120.8 to −120.7 (m); HRMS (FAB), *m/z* calcd for $C_{38}H_{37}FN_5O_{12}S_2$ ($[M-H]^-$) 838.1870, found 838.1882.

4.24. *General procedure for synthesis of protected peptide resin*

Protected peptide resin was manually constructed by Fmoc-based solid-phase synthesis. *t*-Bu ether for D-Tyr was employed for side-chain protection. Fmoc deprotection was achieved by 20% piperidine in DMF (20 min). Fmoc-amino acids except for Fmoc–L-Orn(Ns)–ψ[(Z)-CF=CH]–L-Orn(Ns)–OH **36** were coupled by treatment with 3 equiv of reagents [Fmoc–amino acid, DIPCI, and HOBt·H_2O] to free amino acids in DMF for 1.5 h (for **39**, see the following).

4.24.1. *H–D-Tyr(t-Bu)–Orn(Ns)–ψ[(Z)-CF=CH]–Orn(Ns)–Nal–Gly–(2-Cl)Trt resin (39)*

Nal residue was coupled by general coupling protocol on H–Gly–(2-Cl)Trt resin (0.87 mmol/g, 114.9 mg, 0.100 mmol). Fmoc–Orn(Ns)–ψ[(Z)-CF=CH]–Orn(Ns)–OH **36** (92.2 mg, 0.110 mmol) was incorporated by treatment of DIPCI (34 μL, 0.220 mmol) and HOBt·H_2O (84.3 mg, 0.550 mmol) for 12 h. D-Tyr(*t*-Bu) was coupled by general coupling protocol to afford the title protected peptide resin **39**.

4.24.2. *cyclo[–D-Tyr(t-Bu)–Orn(Ns)–ψ[(Z)-CF=CH]–Orn(Ns)–Nal–Gly–] (41)*

The protected peptide resin **39** (0.100 mmol) was subjected to HFIP–CH_2Cl_2 (3:7, 15 mL) treatment at room temperature for 2 h. After filtration of the residual resin, the filtrate was concentrated under reduced pressure to give a crude linear peptide. To a mixture of the linear peptide and $NaHCO_3$ (57.1 mg, 0.680 mmol) in DMF (41 mL) was added diphenylphosphoryl azide (DPPA, 87.9 μL, 0.408 mmol) at −40 °C. The mixture was stirred for 48 h with warming to room temperature and then filtered. The filtrate was concentrated under reduced pressure, followed by flash chromatography over silica gel with $CHCl_3$–MeOH (99:1) to give the title cyclic pseudopeptide **41** (68.3 mg, 64% yield) as a white powder: $[\alpha]_D^{25}$ −45.0 (*c* 0.565, DMSO); IR (ATR): 3288 (NHCO), 1644 (CO), 1340 (NSO_2), 1161 (NSO_2); ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.80–0.88 (m, 2H), 0.99–1.08 (m, 1H), 1.11–1.45 (m, 13H), 1.56–1.62 (m, 1H), 2.47–2.56 (m, 2H), 2.67–2.89 (m, 5H), 3.04–3.14 (m, 2H), 3.48 (dd, *J*=14.4, 3.9 Hz, 1H), 3.80 (dd, *J*=14.5, 6.9 Hz, 1H), 4.01 (br s, 1H), 4.34–4.39 (m, 2H), 4.74 (dd, *J*=37.8, 9.8 Hz, 1H), 6.77 (d, *J*=8.4 Hz, 2H), 7.02 (d, *J*=8.5 Hz, 2H), 7.30–7.33 (m, 3H), 7.61 (br s, 1H), 7.70–7.97 (m, 16H), 8.48 (d, *J*=8.2 Hz, 1H); HRMS (FAB), *m/z* calcd for $C_{51}H_{56}FN_8O_{13}S_2$ ($[M-H]^-$) 1071.3398, found 1071.3409.

4.24.3. *FCN001: cyclo(–D-Tyr–Arg–ψ[(Z)-CF=CH]–Arg–Nal–Gly–)·2TFA (25)*

The cyclic pseudopeptide **41** (34.4 mg, 0.0320 mmol) was treated with aqueous 95% TFA (3 mL) for 3 h. Concentration under reduced pressure gave an oily residue, which was used immediately in the next step without purification. To a solution of the crude mixture in DMF (5 mL) were added 2-mercaptoethanol (22.4 μL, 0.32 mmol) and DBU (193 μL, 1.56 mmol), and the mixture was stirred at 50 °C for 2.5 h. After concentration under reduced pressure, the residue was washed three times with Et_2O and treated with Et_3N (392 μL, 2.84 mmol) and 1*H*-pyrazole-1-carboxamidine hydrochloride (55.8 mg, 0.38 mmol) in DMF (5 mL). After concentration under reduced pressure, purification by preparative HPLC gave the ditrifluoroacetate of the title cyclic pseudopeptide **25** (2.4 mg, 8%) as colorless freeze-dried powder: $[\alpha]_D^{25}$ −24.9 (*c* 0.150, DMSO); IR (ATR): 3275, 3191, 3071, 2925, 2852, 1651, 1644, 1634, 1537, 1514, 1435, 1367, 1182, 1132; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.12–1.38 (m, 6H), 1.57–1.67 (m, 2H), 2.68 (dd, *J*=13.7, 7.8 Hz, 1H), 2.87 (dd, *J*=13.8, 7.2 Hz, 1H), 2.93–3.01 (m, 5H), 3.12–3.22 (m, 2H), 3.55 (dd, *J*=14.6, 4.2 Hz, 1H), 3.79 (dd, *J*=14.7, 6.7 Hz, 1H), 4.20 (br s, 1H), 4.33–4.41 (m, 2H), 4.92 (dd, *J*=38.2, 9.6 Hz, 1H), 6.63 (d, *J*=8.5 Hz, 2H), 6.67–7.55 (br m, 4H), 6.97 (d, *J*=8.5 Hz, 2H), 7.34–7.36 (m, 2H), 7.43–7.51 (m, 5H), 7.67 (s, 1H), 7.79–7.81 (m, 2H), 7.84–7.87 (m, 2H), 7.94–7.99 (m, 2H), 8.46 (d, *J*=8.1 Hz, 1H), 9.18 (br s, 1H); HRMS (FAB), *m/z* calcd for $C_{37}H_{48}FN_{10}O_5$ ($[M+H]^+$) 731.3793, found 731.3754.

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Supplementary data

Copies of ^{1}H NMR spectra for all new compounds; preparation and copies of CD spectra of methyl ester derivatives of **15a,b**, **16a,b**, and **18a,b**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.02.076.

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19. Although the exact mechanism of the described reductive defluorination is unclear at this stage, single electron transfer from an organocopper to the substrate is responsible for the observed results.[12d,14,15]
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JOC*Note*

Efficient Synthesis of Trifluoromethyl and Related Trisubstituted Alkene Dipeptide Isosteres by Palladium-Catalyzed Carbonylation of Amino Acid Derived Allylic Carbonates

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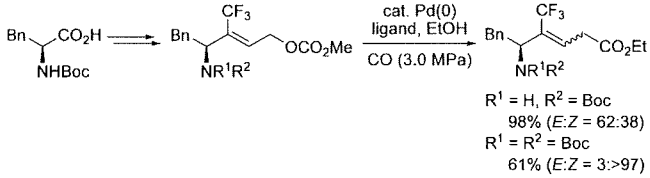

A novel stereoselective synthetic approach to (*Z*)-trifluoromethylalkene dipeptide isosteres (CF_3-ADIs) is described. Starting from readily available *N*-Boc-L-phenylalanine, Phe-Gly type CF_3-ADIs were obtained through palladium-catalyzed carbonylation of allylic carbonates under CO. While the reaction of *N*-Boc derivatives proceeds in excellent yields but lower stereoselectivity (*E*:*Z* = 62:38–43:57), the reaction of the *N*,*N*-diBoc derivative exclusively affords the desired (*Z*)-isomer in 61% yield. We also present a highly stereoselective synthesis of several Phe-Gly type trisubstituted alkene dipeptide isosteres by palladium-catalyzed carbonylation.

Peptides constitute attractive and useful drug leads because a large number of bioactive peptides have already been isolated and identified. However, peptidase-mediated digestion of peptides as well as lower membrane permeability of generally hydrophilic peptides decrease their bioavailability in clinical use. The backbone modification of amide bonds in bioactive peptides is one of the most promising approaches to solving these problems.[1] Among the known isosteric units, (*E*)-alkene dipeptide isosteres (EADIs, Figure 1) have been studied extensively because the (*E*)-carbon−carbon double bond closely resembles the planar structure of the parent amide bond.[2] Fluoroalkene dipeptide isosteres (FADIs) can be considered as more ideal surrogates than nonpolar EADIs due to the presence of a highly electronegative fluorine substituent. This substituent mimics a carbonyl oxygen atom and might contribute to both the

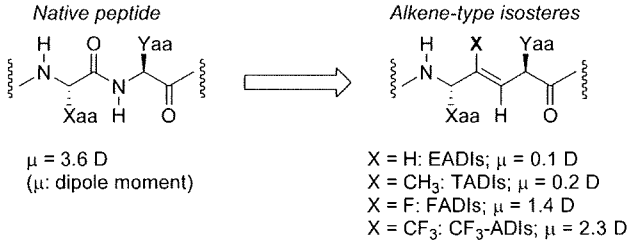

FIGURE 1. Structures of native peptides and corresponding alkene-type isosteres. Xaa, Yaa = Amino acid side chains.

electrostatic nature and the three-dimensional structure of bioisosteres.[3] However, our recent studies on stereoselective synthesis and evaluation of functionalized (*Z*)-FADIs have revealed that the (*Z*)-FADI of Phe-Gly showed lower binding affinity for peptide transporter PEPT1 compared with the corresponding EADI.[4] Moreover, when compared to the parent peptide, the EADI analogue of the GPR54 agonistic peptide expressed similar agonist activity,[5] whereas the (*Z*)-FADI analogue showed significantly lower potency. These results suggest that FADIs are not always effective as dipeptide mimetics, even though some examples of bioactive compounds containing FADIs have been reported.[3b,c,6]

We next turned our attention to trifluoromethylalkene dipeptide isosteres (CF_3-ADIs, Figure 1) that possess a dipole moment (2.3 D) closer to a native peptide bond (3.6 D) than do other alkene-type isosteres (FADI, 1.4 D; EADI, 0.1 D).[7] CF_3-ADIs could serve as more favorable dipeptide isosteres than FADIs due to the presence of fluorine atoms on the sp^3 carbon atoms.[8] Although several asymmetric syntheses of CF_3-ADIs have been reported,[7,9] stereoselective synthesis of optically pure Xaa-Gly

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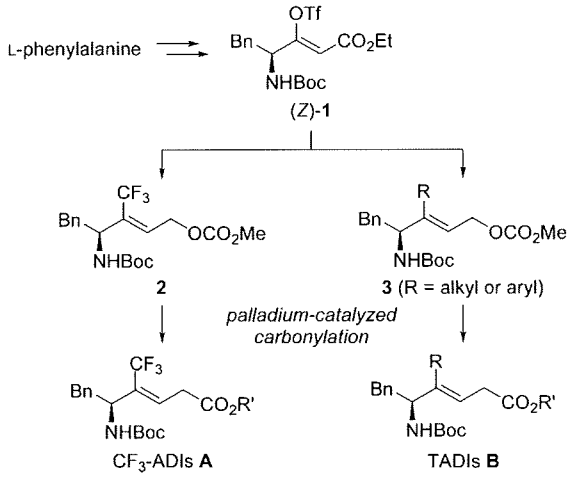
SCHEME 1. Palladium-Catalyzed Synthesis of CF_3-ADIs A and TADIs B via a Single Intermediate



type CF_3-ADIs by use of the reported methodologies would be extremely difficult because the construction of a stereogenic center at the δ-position relies upon the chirality of the α-carbon.

We proposed that palladium-catalyzed carbonylation of allylic carbonates **2**[10d] derived from α-amino acids would provide a general and convenient approach to enantiopure Xaa-Gly type CF_3-ADIs **A** (Scheme 1). This strategy could also be applied to facile synthesis of trisubstituted alkene dipeptide isosteres (TADIs) **B**, which are known as useful hydrolytically stable structural surrogates of dipeptides[2d] as well as potent β-turn promoters in acyclic sequences.[9,11] To evaluate the utility of CF_3-ADIs and TADIs, development of a simple and efficient methodology for the preparation of both isosteres by way of the same intermediate is highly desirable. Herein, we describe a novel synthetic approach to Phe-Gly type CF_3-ADIs **A** and TADIs **B** (R = *n*-Bu, Me, *i*-Pr, or Ph) by palladium-catalyzed carbonylation of allylic carbonates such as **2** and **3**. These approaches enable a facile synthesis of enantiomerically pure dipeptide isosteres by retention of the asymmetric centers of the starting amino acids.

Our synthesis started from commercially available *N*-Boc-L-phenylalanine **4** as illustrated in Scheme 2. After conversion to β-keto ester **5** by the reaction with carbonyldiimidazole and ethyl acetate lithium enolate,[12] treatment with Tf_2O in the presence of $(i\text{-Pr})_2NH$ afforded the triflate **1** as a separable mixture of *E*- and *Z*-isomers (*E*:*Z* = 1:4). Next, we investigated the tributylstannylation of (*Z*)-**1** under various conditions. Representative results are shown in Table 1. While the reactions with bis(tributyltin) in the presence of a catalytic amount of $Pd(PPh_3)_2Cl_2$ or $PdCl_2[P(o\text{-tol})_3]_2$ gave a mixture of unidentified products (entries 1 and 2), the reaction with cyano Gilman reagent [$n\text{-Bu}_3\text{Sn}(n\text{-Bu})\text{Cu(CN)Li}_2$], a well-known stannylating reagent for cross-coupling reaction,[13] afforded the desired

SCHEME 2. Synthesis of Carbonate 2 Bearing a CF_3 Group

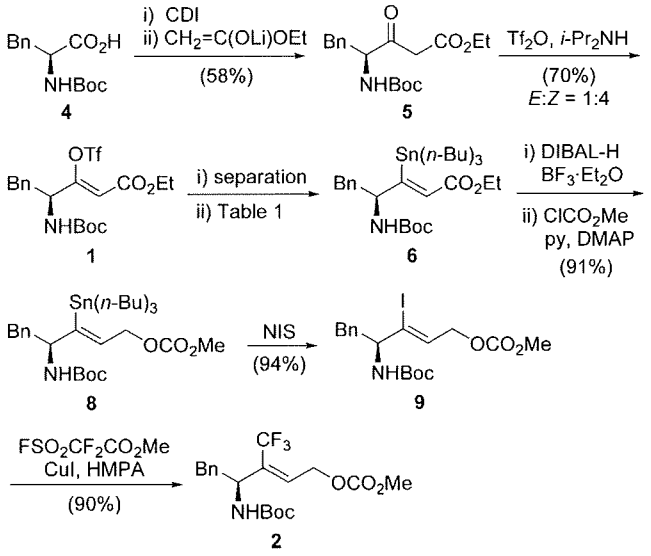
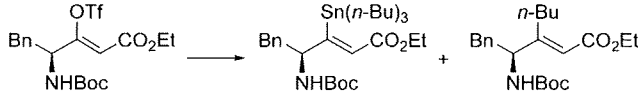


TABLE 1. Synthesis of Tributylstannylated Enoate 6 via Organocopper-Mediated Stannylation of (*Z*)-1[a]



entry	reagents	solvent	products (yield)	
1	$(n\text{-Bu}_3\text{Sn})_2$, $Pd(PPh_3)_2Cl_2$	THF	ND[b]	
2	$(n\text{-Bu}_3\text{Sn})_2$, $PdCl_2[P(o\text{-tol})_3]_2$	DMF	ND[b]	
3	$n\text{-Bu}_3\text{Sn}(n\text{-Bu})\text{Cu(CN)Li}_2$	THF	**6** (35%)	**7** (17%)
4	$n\text{-Bu}_3\text{SnCu(CN)Li}$[c]	THF	**6** (23%)	**7** (62%)
5	$n\text{-Bu}_3\text{SnCu(CN)Li}$[d]	THF	**6** (80%)	**7** (trace)

[a] Catalytic reactions (entries 1 and 2) were carried out with $(n\text{-Bu}_3\text{Sn})_2$ (1.2 equiv) in the presence of a palladium catalyst (5 mol %) and LiCl (6.0 equiv). [b] Not determined (a complex mixture of unidentified products was obtained). [c] Prepared by stirring at 0 °C for 10 min. [d] Prepared by stirring at 0 °C for 60 min.

product **6** in 35% yield along with 17% of the butylated enoate **7** (entry 3). The reaction with a lower-order cuprate [$n\text{-Bu}_3\text{SnCu(CN)Li}$] gave **7** as a major product (entry 4). In sharp contrast, the cyanocuprate with the same constituent of the reagents with a prolonged reaction time gave the desired stannylated product **6** in 80% yield (entry 5). Stannyl ester **6** was then reduced with DIBAL-H to the corresponding allylic alcohol, which was converted to the allylic carbonate **8** under standard conditions. After treatment of **8** with NIS,[9] the resulting vinyl iodide **9** was subjected to copper-mediated trifluoromethylation using $FSO_2CF_2CO_2Me/CuI$[14] as a trifluoromethyl anion equivalent to give the requisite allylic carbonate **2** containing a CF_3-alkene unit in high yield.[15]

Next, allylic carbonates **3** for the syntheses of several Phe-Gly type TADIs **B**, Boc-Phe-Ψ[(*E*)-CR=CH]-Gly-OR' (Scheme 1), were prepared from the key intermediate (*Z*)-**1** through

(11) Tyndall, J. D. A.; Pfeiffer, B.; Abbenante, G.; Fairlie, D. P. *Chem. Rev.* **2005**, *105*, 793–826.

(12) The ketone **5** with >95% ee (Chiralcel OD-RH, $MeCN/H_2O$ = 53:47) was obtained according to Hoffman's protocol: Hoffman, R.V.; Maslouh, N.; Cervantes-Lee, F *J. Org. Chem.* **2002**, *67*, 1045−1056. It should be noted that a prolonged reaction time for CDI treatment caused serious racemization.

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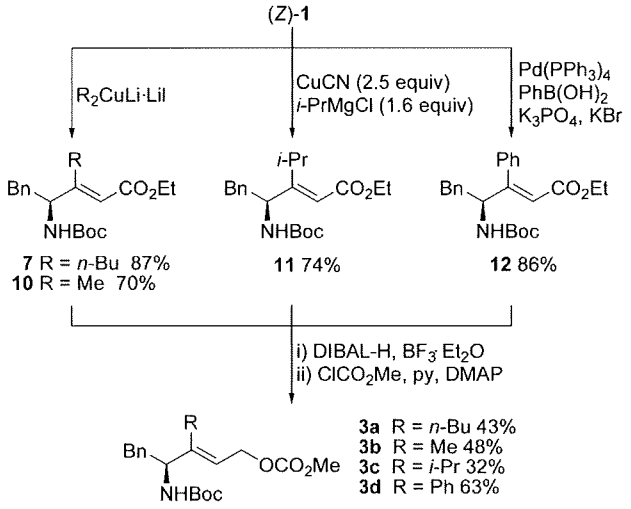
(15) The *E*/*Z* stereochemistry of the olefinic compounds was determined by NOE analyses. With the carbonates **2**, **3**, and **15** and (*Z*)-enoates **13**, **16**, and **17**, NOE correlations were observed between the olefinic proton and the proton on the carbon bearing a nitrogen atom. On the other hand, this correlation was not observed with the (*E*)-enoates **13**.

SCHEME 3. Synthesis of Carbonates 3a–d

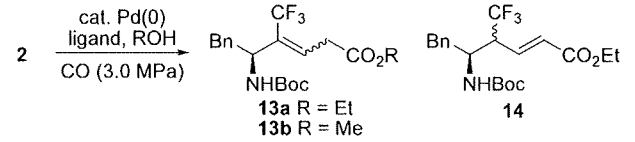


organocopper-mediated alkylation[16] or Pd-catalyzed Suzuki–Miyaura coupling[17] (Scheme 3). Organocopper-mediated alkylation of (*Z*)-**1** using Gilman reagents (n-$Bu_2CuLi \cdot LiI$ or $Me_2CuLi \cdot LiI$) at −78 °C proceeded smoothly to yield the desired n-butyl- and methyl-substituted enoates **7** and **10** in 87% and 70% yields, respectively. Although the reactions of (*Z*)-**1** with some typical isopropylcopper reagents [i-$Pr_2CuLi \cdot LiI$, i-PrCuI·MgCl, i-$Pr_2Cu(CN)Li_2$, or i-PrCu(CN)MgCl] afforded only a mixture of unidentified compounds, the reaction with the reagent prepared from i-PrMgCl (1.6 equiv) and a slight excess of copper cyanide (2.5 equiv) afforded the desired product **11** in 74% yield.[16] Phenylated enoate **12** was prepared in 86% yield by Suzuki–Miyaura coupling of (*Z*)-**1** using $PhB(OH)_2$ in the presence of a catalytic amount of $Pd(PPh_3)_4$.[17] The resulting substituted enoates **7** and **10**–**12** were converted to the allylic carbonates **3a**–**d** by a sequence of reactions similar to the preparation of **2** (Scheme 3).

Next, we investigated the palladium-catalyzed carbonylation of allylic carbonate **2** bearing a CF_3 group (Table 2).[10] Treatment of **2** with 10 mol % of $Pd_2(dba)_3 \cdot CHCl_3$ and 40 mol % of PPh_3 in EtOH at room temperature under 3.0 MPa of carbon monoxide afforded the desired β,γ-unsaturated ester **13a** in 60% yield with a low *E*/*Z* selectivity (entry 1).[15] Similarly, the reaction proceeded smoothly at 50 °C to give **13a** in 98% combined isolated yield (entry 2). In sharp contrast, when the reaction was carried out at 80 °C, a considerable amount of α,β-unsaturated ester **14** was obtained (26% yield, entry 3). Other palladium sources and ligands were ineffective at improving the *E*/*Z* selectivity (entries 4–7). The reaction in MeOH gave the desired product **13b** in 80% yield within 3 h (compare entries 8 vs 9), but the stereoselectivities were similarly low. Decreased loading of $Pd_2(dba)_3 \cdot CHCl_3$ (5 mol %) gave a comparable result, while a slightly lower yield of **13b** was obtained with 2.5 mol % of $Pd_2(dba)_3 \cdot CHCl_3$ (entries 9–11). Based on these observations, it was determined that the palladium-catalyzed carbonylation of **2** produces the desired esters in good yields but that improvement of *E*/*Z* selectivity is difficult.

It is worth noting that related palladium-catalyzed allylic carbonylation favors formation of trans-isomers due to the

TABLE 2. Palladium-Catalyzed Carbonylation of CF_3-Containing Allylic Carbonate 2



entry	Pd cat. (mol %)[a]	ligand (mol %)	ROH	temp. (°C)	time (h)	yield (%)[b] (*E*)-**13**	(*Z*)-**13**
1	10	PPh_3 (40)	EtOH	rt	12	26	34
2	10	PPh_3 (40)	EtOH	50	12	61	37
3[c]	10	PPh_3 (40)	EtOH	80	12	16	20
4	10	PCy_3 (40)	EtOH	50	12	ND[d]	
5	10	dppm (40)	EtOH	50	12	ND[d]	
6	10	dppe (40)	EtOH	50	12	20	5
7	10	dppp (40)	EtOH	50	12	12	14
8	10	PPh_3 (40)	EtOH	50	3	27	38
9	10	PPh_3 (40)	MeOH	50	3	32	48
10	5	PPh_3 (20)	MeOH	50	3	35	45
11	2.5	PPh_3 (10)	MeOH	50	3	29	25

[a] Mol % of $Pd_2(dba)_3 \cdot CHCl_3$. [b] Isolated yield. [c] α,β-Unsaturated compound **14** was obtained (26%). [d] Starting material was recovered.

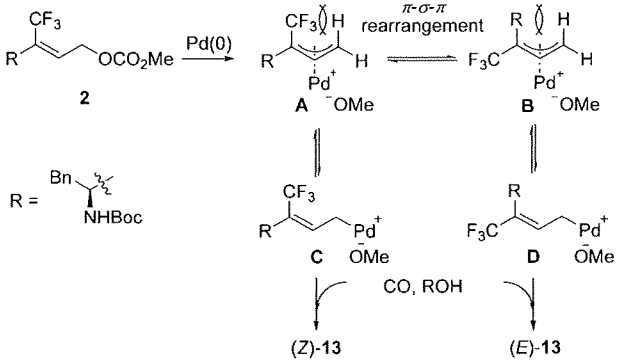


FIGURE 2. Plausible explanation for the observed stereochemical outcome with allylic carbonate **2**.

preference for *syn*-π-allylpalladium intermediates.[10b] The observed low *E*/*Z* selectivities with the carbonate **2** can be partly explained by the presence of a sterically demanding CF_3 group. Thus, oxidative addition of **2** to palladium(0) would give π-allylpalladium intermediate **A** via decarboxylation (Figure 2), which is in an equilibrium state with the π-complex **B** and σ-complexes **C** and **D** via π–σ–π rearrangement. Coordination of CO to the palladium atom of **C**, exchange of alkoxide, and reductive elimination would afford (*Z*)-**13**. On the other hand, carbonylation of **D** derived from **B** gives (*E*)-**13** in a similar manner. The bulky CF_3 group would partly destabilize the syn complex **A** because of the unfavorable 1,3-repulsion with a hydrogen atom thus leading to nonselective formation of (*Z*)- and (*E*)-**13**.

To overcome the effect of the CF_3 group and improve the stereoselectivity, we introduced another N-substituent to increase unfavorable 1,3-repulsion in the π-complex **B**, thereby destabilizing **B** and assisting the selective formation of the desired (*Z*)-**13** through the intermediate **A**. Accordingly, we prepared *N*,*N*-diprotected allylic carbonates **15a,b** from **2** by the standard protocols and investigated the palladium-catalyzed carbonylation reaction. As expected, the reaction proceeded smoothly to afford the desired (*Z*)-β,γ-enoates **16a,b** as single isomers in moderate yields (61% and 60%, respectively) (Scheme 4).

Next, synthesis of TADIs having a n-Bu, Me, i-Pr, or Ph group on the double bond was investigated. The carbonylation

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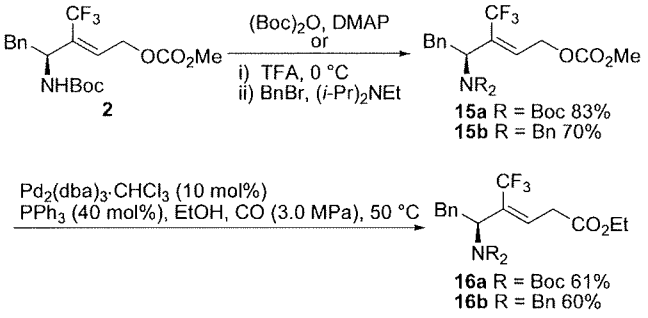
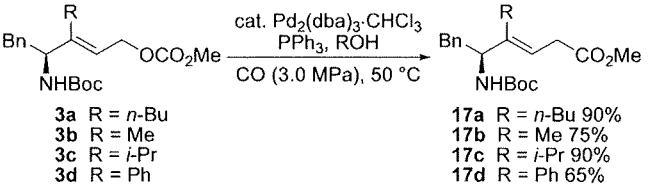
(17) (a) Oh-e, T.; Miyaura, N.; Suzuki, A. *J. Org. Chem.* **1993**, *58*, 2201–2208. (b) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457–2483.

SCHEME 4. Carbonylation of *N,N*-Diprotected Allylic Carbonates 15



SCHEME 5. Pd-Catalyzed Carbonylation of Allylic Carbonates 3a–d

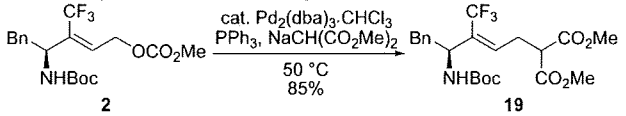


reaction of **3a–d** under the same conditions as those with **2** afforded the desired β,γ-unsaturated esters **17a–d** in good yields (Scheme 5). In sharp contrast to the reaction of CF_3 derivatives **2** (Table 2), all the *N*-Boc-TADIs, including **17c** having a bulky *i*-Pr group, were obtained as the sole isolable stereoisomer. These results suggest that the CF_3 group would also exert some interesting effects on the formation of (*E*)-**13**, not only as a sterically congested substituent (Figure 2).[18]

Finally, the esters (*Z*)-**13b** and **16a** were converted to the desired deprotected CF_3-ADI **18** by treatment under acidic conditions in 85% and 95% yields, respectively (Scheme 6).[19]

In summary, we have developed a novel synthetic route which is applicable to the general synthesis of CF_3-ADIs and TADIs starting from commercially available *N*-Boc-amino acids. This methodology is useful in that the chiral center at the δ-position of isosteres is derived from α-amino acids, enabling facile evaluation of CF_3-ADIs and several TADIs as peptidomimetics.

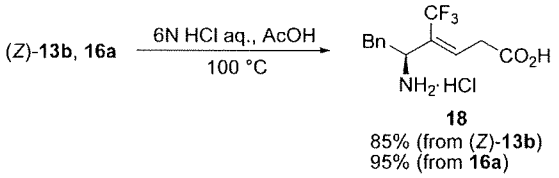
(18) Nucleophilic substitution of allylic carbonate **2** with sodium malonate gave the (*Z*)-γ,δ-malonate **19** as a single isomer.



Other examples of a CF_3-containing π-allylpalladium complex with a malonate anion have been reported to proceed with retention of configuration. See: (a) Hanzawa, Y.; Ishizawa, S.; Kobayashi, Y. *Chem. Pharm. Bull.* **1988**, *36*, 4209–4212. (b) Hanzawa, Y.; Ishizawa, S.; Kobayashi, Y.; Taguchi, T. *Chem. Pharm. Bull.* **1990**, *38*, 1104–1106. These results as well as the significantly higher stereoselectivity observed with the *i*-Pr derivative **3c** suggest other important influences of the CF_3 group in this reaction system containing carbon monoxide.

(19) Cragoe, E. J., Jr.; Gould, N. P.; Woltersdorf, O. W., Jr.; Ziegler, C.; Bourke, R. S.; Nelson, L. R.; Kimelberg, H. K.; Waldman, J. B.; Popp, A. J.; Sedransk, N. *J. Med. Chem.* **1982**, *25*, 567–579.

SCHEME 6. Conversion to the Desired CF_3-ADI 18 by Deprotection



Further studies including synthesis and evaluation of bioactive peptides with these isosteres as well as EADIs are now in progress.

Experimental Section

General Procedure for Palladium-Catalyzed Carbonylation of Allyl Carbonates: $Pd_2(dba)_3 \cdot CHCl_3$ (12.8 mg, 0.0124 mmol), PPh_3 (13.1 mg, 0.050 mmol), and EtOH (5 mL) were introduced to a 100 mL stainless steel pressure bottle containing the carbonate **2** (50 mg, 0.124 mmol). After evacuating, 3.0 MPa of CO gas was introduced at room temperature, and the mixture was stirred at 50 °C for 12 h. After purging, the mixture was concentrated under reduced pressure followed by flash chromatography over silica gel with *n*-hexane–AcOEt (7:1) to give (*Z*)-**13a** (18.3 mg, 37% yield) and (*E*)-**13a** (30.4 mg, 61% yield).

Compound (*Z*)-**13a**: pale yellow solid; mp 84.0–84.5 °C; $[\alpha]^{24}_D$ –10.4 (*c* 0.930, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 1.25 (t, J = 7.1 Hz, 3H), 1.36 (s, 9H), 2.82 (m, 1H), 3.02 (dd, J = 13.6, 5.0 Hz, 1H), 3.28–3.36 (m, 2H), 4.15 (q, J = 7.1 Hz, 2H), 4.42–4.71 (br, 2H), 6.12 (t, J = 7.1 Hz, 1H), 7.13–7.33 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ14.1, 28.2 (3C), 33.2, 40.6, 53.5, 61.1, 79.9, 125.1, 126.8, 128.5 (2C), 128.6, 129.3 (2C), 130.4, 136.5, 154.5, 170.1; ^{19}F NMR (376 MHz, $CFCl_3$) δ –59.0 (3F); HRMS (FAB) calcd for $C_{20}H_{27}F_3NO_4$ (MH^+) 402.1892; found 402.1895.

Compound (*E*)-**13a**: pale yellow oil; $[\alpha]^{24}_D$ +47.1 (*c* 0.950, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 1.23 (t, J = 6.8 Hz, 3H), 1.37 (s, 9H), 2.65–2.75 (m, 1H), 2.88–3.02 (br, 2H), 3.18–3.28 (m, 1H), 4.10 (q, J = 6.8 Hz, 2H), 4.82 (br s, 2H), 6.45 (t, J = 6.0 Hz, 1H), 7.18–7.33 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ14.1, 28.2 (3C), 32.4, 40.1, 49.8, 61.1, 79.9, 126.9, 128.4, 128.6 (2C), 129.0, 129.3 (2C), 136.8, 143.3, 154.9, 169.8; ^{19}F NMR (376 MHz, $CFCl_3$) δ –61.6 (3F); HRMS (FAB) calcd for $C_{20}H_{27}F_3NO_4$ (MH^+) 402.1892; found 402.1894.

Acknowledgment. This research was supported in part by the 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 Health Science Foundation, and Targeted Proteins Research Program. E.I., T.N., A.N., and K.T. are grateful for the JSPS Research Fellowships for Young Scientists.

Supporting Information Available: Experimental procedures and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Facile Synthesis of Fluoroalkenes by Palladium-Catalyzed Reductive Defluorination of Allylic *gem*-Difluorides

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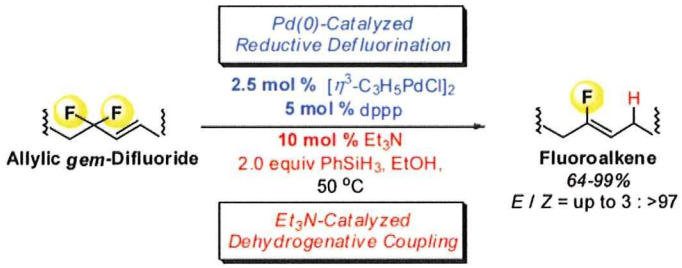
Received July 13, 2007

ABSTRACT



Chemo- and stereoselective synthesis of fluoroalkenes was achieved in excellent yields via Pd-catalyzed C–F bond activation. In this transformation, Et_3N plays a crucial role to produce reactive hydride species such as $Ph(EtO)SiH_2$ and $Ph(EtO)_2SiH$ by promoting dehydrogenative coupling. The reaction proceeds efficiently at 50 °C with a variety of substrates and is also useful for the synthesis of fluoroalkene peptidomimetics.

Although the development of catalytic reactions involving C−F bond activation represents a great challenge in organic chemistry,[1] only a few examples of Pd-catalyzed C−F bond activation have been reported to date.[2] Recently, several groups have disclosed cross-coupling reactions of alkyl or aryl fluorides through Pd-catalyzed C−F bond activation.[3] One example of Pd-catalyzed allylic C−F bond activation is the hydrogenolysis of allyl fluorides in the presence of Pd/C,[2a] which provides a facile method for the replacement of fluorine by hydrogen atom under mild conditions. A main drawback of this transformation exists in the chemoselectivity issue: the reaction always gives a mixture of two products, one formed by replacement of the fluorine by a hydrogen atom followed by saturation of the double bond, and the other resulting from the simple hydrogenation of the double bond. On the basis of these pioneering works, we envisioned that the reaction of allylic *gem*-difluoride **1** with a homogeneous palladium catalyst in the presence of appropriate additives having an affinity to fluorine could promote the elimination of fluorine, leading to the generation of a fluorinated π-allyl palladium intermediate **2**. By a chemoselective reaction with an appropriate nucleophile, this intermediate is expected to be transformed to (*Z*)-fluoroalkene **3**,[4] which constitutes an

(1) Recent reviews for activation and functionalization of C−F bonds, see: (a) Kiplinger, J. L.; Richmond, T. G.; Osterberg, C. E. *Chem. Rev.* **1994**, *94*, 373−431. (b) Burdeniuc, J.; Jedlicka, B.; Crabtree, R. H. *Chem. Ber./Recl.* **1997**, *130*, 145−154. (c) Murai, S. *Activation of Unreactive Bonds and Organic Synthesis*; Springer: New York, 1999; pp 243−269. (d) Hiyama, T. *Organofluorine Compounds Chemistry and Applications*; Springer: New York, 2000.

(2) Pd-catalyzed C−F bond activation: (a) Hudlicky, M. *J. Fluorine Chem.* **1989**, *44*, 345−359. (b) Hintermann, L.; Läng, F.; Maire, P.; Togni, A. *Eur. J. Inorg. Chem.* **2006**, 1397−1412. (c) Torrens, H. *Coord. Chem. Rev.* **2005**, *249*, 1957−1985. (d) Ichikawa, J.; Nadano, R.; Ito, N. *Chem. Commun.* **2005**, 4425−4427.

(3) Pd-catalyzed cross coupling via C−F bond activation: (a) Widdowson, D. A.; Wilhelm, R. *Chem. Commun.* **1999**, 2211−2212. (b) Wilhelm, R.; Widdowson, D. A. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3808−3813. (c) Widdowson, D. A.; Wilhelm, R. *Chem. Commun.* **2003**, 578−579. (d) Mi, Kim, Y.; Yu, S. *J. Am. Chem. Soc.* **2003**, *125*, 1696−1697. (e) Terao, J.; Ikumi, A.; Kuniyasu, H.; Kambe, N. *J. Am. Chem. Soc.* **2003**, *125*, 5646−5647.

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important class of molecules such as peptide isosteres,[5a–d] enzyme inhibitors,[5e] and liquid-crystalline materials.[5f]

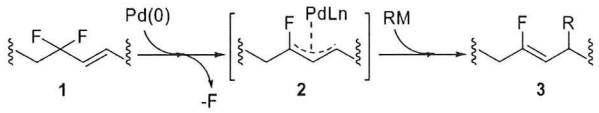
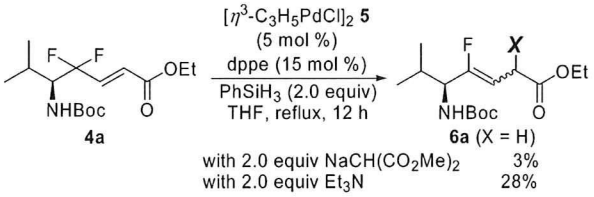


Figure 1. Synthesis of fluoroalkene via Pd catalysis.

Herein, we present a general catalytic system for the facile synthesis of fluoroalkene skeleta from readily available allylic difluorides by Pd-catalyzed reductive defluorination with phenylsilane. Some insight into the mechanistic aspect of this transformation is also described.

In an initial study, we investigated the Pd-catalyzed allylic alkylation[6] of γ,γ-difluoro-α,β-enoate **4a**, which can be readily prepared from isobutyl aldehyde[7] by modifying Honda's protocol.[8] Various additives were screened for the reaction of enoate **4a** with dimethyl sodiomalonate in the presence of a catalytic amount of $[\eta^3\text{-}C_3H_5PdCl]_2$ (**5**) and dppe. Although TMSCl, Et_4Si, $(EtO)_4Si$, or Me_3Al did not promote the desired defluorination reaction, we found that, when using $PhSiH_3$, a small amount of reductive defluorinated product **6a** was obtained (3%), without forming alkylated products **6b** [X = $CH(CO_2Me)_2$] (Scheme 1). Since

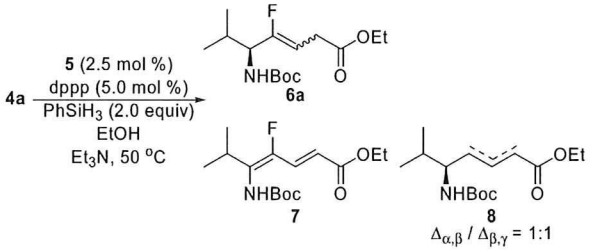
Scheme 1. Pd-Catalyzed Allylic Alkylation



the reduced product **6a** was not detected without using dimethyl sodiomalonate, we postulated that basicity of sodium malonate plays an important role in this reaction. Therefore, we tested the reaction in the presence of triethylamine instead of sodium dimethyl malonate to obtain **6a** in increased yields (28%).

After screening of the reaction conditions, we were pleased to find that the combination of dppp and EtOH at 50 °C afforded the expected products **6a** in up to 96% yield (Table 1, entry 1). However, a small amount of undesired diene **7**,

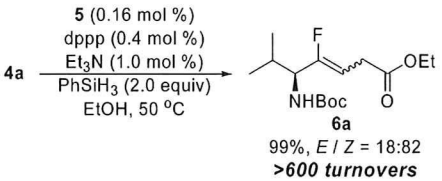
Table 1. Effect of the Amount of Et_3N[a]



entry	Et_3N [equiv]	yield of **6a**[b] [%]	*E*:*Z*[c]
1	2.0	<96	20:80
2	1.0	99	17:83
3	0.5	99	15:85
4	0.1	99	9:91
5	0.01	87	6:94

[a] Reactions were carried out with **4a** (0.13 mmol), $PhSiH_3$ (0.25 mmol), Et_3N, $[\eta^3\text{-}C_3H_5PdCl]_2$ **5** (2.5 mol %), and dppp (5.0 mol %) in EtOH (2.5 mL) at 50 °C for 2 h. [b] Yields of isolated products. [c] The ratio of *E*/*Z* isomer was determined by ^1H NMR spectroscopy.

presumably produced by Et_3N-assisted β-hydride elimination of a plausible intermediate of the type **2**, was observed in an irreproducible fashion (<10%). Therefore, we performed the reaction with 1.0 equiv of Et_3N to obtain the desired defluorinated products in 99% yield without the formation of the β-elimination product **7** (entry 2). Unexpectedly, the reduction of Et_3N to a catalytic amount improves the *E*/*Z* selectivity (entries 3–5). Of particular interest is the formation of a small amount of the bis-defluorinated product **8**, which was obtained in 8% yield when using 1 mol % of Et_3N.[9] Finally, the reaction can be conducted in quantitative conversion at catalyst loadings as low as 0.16 mol % (eq 1).



(1)

With these results in hand, we examined the scope of this reaction with readily available and synthetically useful

(4) For a recent example of the fluoroalkene synthesis: (a) Yoshida, M.; Komata, A.; Hara, S. *Tetrahedron* **2006**, *62*, 8636–8645 and references cited therein.

(5) (a) Dutheuil, G.; Couve-Bonnaire, S.; Pannecoucke, X. *Angew. Chem., Int. Ed. Engl.* **2007**, *46*, 1290–1292. (b) Narumi, T.; Niida, A.; Tomita, K.; Oishi, S.; Otaka, A.; Ohno, H.; Fujii, N. *Chem. Commun.* **2006**, 4720–4722. (c) Nakamura, Y.; Okada, M.; Sato, A.; Horikawa, H.; Koura, M.; Saito, A.; Taguchi, T. *Tetrahedron* **2005**, *61*, 5741–5753. (d) Allmendinger, T.; Felder, E.; Hungerbühler, E. *Tetrahedron Lett.* **1990**, *31*, 7301–7304. (e) Bey, P.; McCarthy, J. R.; McDonald, I. A. *ACS Symp. Ser.* **1991**, *456*, 105–133 and references cited therein. (f) Yokokoji, O.; Shimizu, T.; Kumai, S. JP 08040952, 1996 [*Chem. Abstr.* **1996**, *124*, 316586].

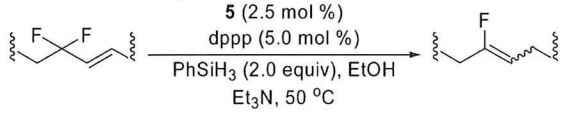
(6) For review, see: (a) Tsuji, J. *Palladium Reagents and Catalysis, Innovations in Organic Synthesis*; Wiley: New York, 1995. (b) Trost, B. M.; Van Vranken, D. L. *Chem. Rev.* **1996**, *96*, 395–422. (c) Johannsen, M.; Jorgensen, K. A. *Chem. Rev.* **1998**, *98*, 1689–1708. (d) Paquin, J.-F.; Lautens M. In *Comprehensive Asymmetric Catalysis*; Jacobsen, E. N., Pfaltz, A., Yamamoto, H., Eds.; Springer-Verlag: Berlin, Germany, 2004: Vol. 2, pp 73–95 and references cited therein. (e) Trost, B. M.; Crawley, M. L. *Chem. Rev.* **2003**, *103*, 2921–2943.

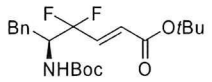
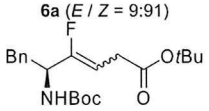
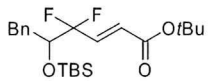
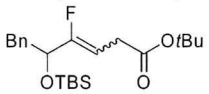
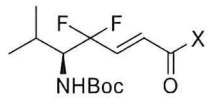
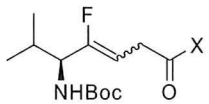
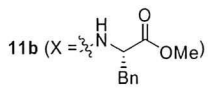
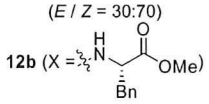
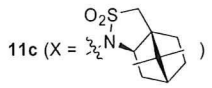
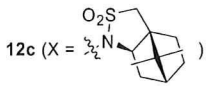
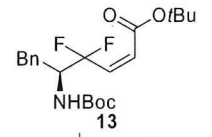
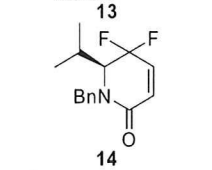
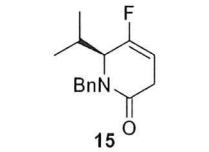
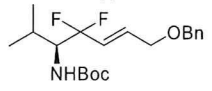
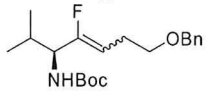
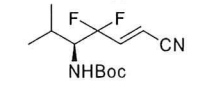
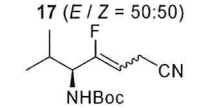
(7) (a) Otaka, A.; Watanabe, J.; Yukimasa, A.; Sasaki, Y.; Watanabe, H.; Kinoshita, T.; Oishi, S.; Tamamura, H.; Fujii, N. *J. Org. Chem.* **2004**, *69*, 1634–1645 and references cited therein.

(8) Honda, T.; Wakabayashi, H.; Kanai, K. *Chem. Pharm. Bull.* **2002**, *50*, 307–308.

(9) Formation of defluorinated product **8** could be rationalized by reaction of π-allyl Pd intermediate by hydride at the fluorinated carbon to give allyl fluoride followed by re-reductive defluorination.

Table 2. Pd- and Et_3N-Catalyzed Reductive Defluorination[a]



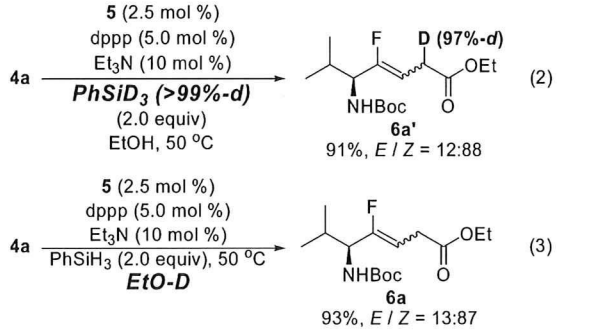
entry	substrate	product(s) (*E*:*Z*)[b]	yield [%][c]
1	**4a**	**6a** (*E* / *Z* = 9:91)	99
2	 **4b**	 **6b** (*E* / *Z* = 18:82)	97
3	 **9**	 **10** (*E* / *Z* = 3:>97)	91
4	 **11a** (X = NH_2)	 **12a** (X = NH_2) (*E* / *Z* = 30:70)	64
5	 **11b**	 **12b** (*E* / *Z* = 21:79)	97
6	 **11c**	 **12c** (*E* / *Z* = 3:>97)	76[d]
7	 **13**	**6c** (*E* / *Z* = 26:74)	99
8	 **14**	 **15**	99
9	 **16**	 **17** (*E* / *Z* = 50:50)	77
10	 **18**	 **19** (*E* / *Z* = 14:86)	73

[a] All reactions were carried out with allylic *gem*-difluoride (1.0 equiv), $PhSiH_3$ (2.0 equiv), Et_3N (10 mol %), $[\eta^3\text{-}C_3H_5PdCl]_2$ **5** (2.5 mol %), and dppp (5.0 mol %) in EtOH at 50 °C for 2 h. [b] Yields of isolated products. [c] The ratio of *E*/*Z* isomer was determined by ^1H NMR spectroscopy. [d] A trace amount of starting material was detected by ^1H NMR spectroscopy.

substrates possessing various functional groups (Table 2). In all cases, the reaction was completely chemoselective, and good to excellent yields of fluoroalkenes were obtained with modest to high selectivity. *N*-Boc amide, esters, and substituents such as alkyl and siloxy groups introduced at the δ-carbon did not affect the reaction (entries 1−3). Furthermore, amides, including a peptide, **11a**−**c** (entries 4−6), (*Z*)-enoate **13** (entry 7), and lactam **14** (entry 8), can be employed to give the desired fluoroalkenes **12a**−**c**, **6c**, and **15**. The applicability of this reaction to the substrates without a conjugated carbonyl moiety such as benzyl ether **16** and nitrile **18** (entries 9 and 10) clearly demonstrates an advantage of this reaction over the known related reduction using a single-electron donor,[7a] which is limited to α,β-unsaturated carbonyl compounds.

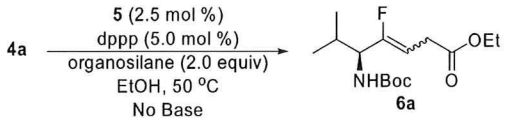
To gain some insight into the mechanism of this transformation, we examined isotopic labeling experiments (Scheme 2). The reaction with $PhSiD_3$ in EtOH induced deuterium

Scheme 2. Isotopic Labeling Experiments



incorporation at the α-position (97% -*d*) (eq 2). On the other hand, the reaction performed in EtO−D with $PhSiH_3$ promoted no deuterium incorporation (eq 3), suggesting that the introduced hydrogen originates from $PhSiH_3$. Furthermore, to determine the hydride species, we performed the reaction with $PhSiH_3$, $Ph(EtO)SiH_2$, and $Ph(EtO)_2SiH$ in the absence of Et_3N (Table 3). While no reaction was observed

Table 3. Investigation of the Hydride Species[a]



entry	organosilane	yield of **6a**[b] [%]	*E*:*Z*[c]
1	$PhSiH_3$	*d*	
2	$Ph(EtO)SiH_2$	83	13:87
3	$Ph(EtO)_2SiH$	70	45:55

[a] Reactions were carried out with **4a** (0.13 mmol), organosilane (0.25 mmol), $[\eta^3\text{-}C_3H_5PdCl]_2$ **5** (2.5 mol %), and dppp (5.0 mol %) in EtOH (2.5 mL) at 50 °C for 2 h. [b] Yields of isolated products. [c] The ratio of *E*/*Z* isomers was determined by ^1H NMR spectroscopy. [d] No reaction was observed.

with $PhSiH_3$ (entry 1), the reactions with $Ph(EtO)SiH_2$ and $Ph(EtO)_2SiH$ proceeded smoothly to provide the desired defluorinated products **6a** (entries 2 and 3). Therefore, these alkoxysilanes would be considered as the actual reactive species. On the basis of these results and Buchwald's observation,[10] Et_3N plays a crucial role for the generation of these active hydride sources such as $Ph(EtO)SiH_2$ and $Ph(EtO)_2SiH$ by promoting catalytic dehydrogenative coupling of $PhSiH_3$ with EtOH.[11] Once those active species have

been generated, they could work both as reducing agents to generate Pd^0 complexes and as hydride sources.

These results could explain the dependence of the chemical yield and stereoselectivity on the amount of Et_3N in Table 1. A catalytic amount of Et_3N would generate the reactive alkoxysilanes in an appropriate rate through dehydrogenative coupling, while the excess of Et_3N considerably accelerates this process, which would consume reactive species to cause undesired side reactions.

In summary, we have developed a novel general method for the synthesis of fluoroalkenes under mild conditions utilizing Pd-catalyzed reductive defluorination. This is an unparalleled example of a highly effective catalytic synthesis of a fluoroalkene skeleton, including peptidomimetics. Mechanistic study has proven that Et_3N promotes the dehydrogenative coupling of $PhSiH_3$ with EtOH to produce reactive species.

Acknowledgment. This research was supported in part by the 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.

Supporting Information Available: Representative procedures and spectral and analytical data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL701627V

(10) Involvement of alkoxysilane accounts for the titanium-catalyzed hydrosilylation of imine with $PhSiH_3$, see: Verdaguer, X.; Lange, U. E. W.; Reding, M. T.; Buchwald, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 6784−6785.

(11) For examples of base-catalyzed dehydrogenative coupling of silanes with alcohols, see: (a) Lukevics, E.; Dzintara, M. *J. Organomet. Chem.* **1984**, *271*, 307−317. (b) Gilman, H.; Dunn, G. H.; Hartzfeld, H.; Smith, A. G. *J. Am. Chem. Soc.* **1955**, *77*, 1287−1288. (c) Bazant, V.; Chvalovsky, V.; Rathousky J. In *Organosilicon Compounds*; Publishing House of the Czechoslovak Academy of Science: Prague, Czechoslovakia, 1965: pp 54−56.

Multicomponent Reactions

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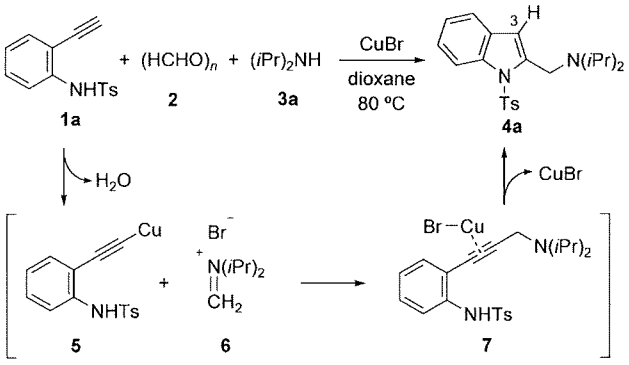
Direct Synthesis of 2-(Aminomethyl)indoles through Copper(I)-Catalyzed Domino Three-Component Coupling and Cyclization Reactions**

Hiroaki Ohno, Yusuke Ohta, Shinya Oishi, and Nobutaka Fujii**

The indole nucleus is a prominent structural motif found in numerous natural products and synthetic compounds with important biological activities, and thus considerable attention has been directed toward general, flexible, and selective methods for the synthesis of highly functionalized indole derivatives.[1] Among the functionalized indoles, the 2-(aminomethyl)indole motif is a key structure that exists in several biologically active compounds,[2] including calindol.[3] Most of the synthetic routes to 2-(aminomethyl)indoles rely upon functionalized indoles such as indole-2-carboxylic acid or its derivatives as the starting materials,[2-4] which limit the structure of the target molecules that can be readily synthesized.

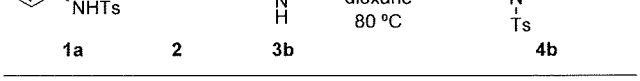
One current important area of modern synthetic chemistry is the development of efficient practical methods that minimize the requisite reagents, solvents, cost, time, and separation processes for the desired transformation and also minimize the formation of waste.[5] While the multicomponent reaction (MCR) approach is recognized as a powerful method toward this end, a catalytic domino reaction including a MCR would be more attractive to achieve this goal. During the course of our efforts directed toward the development of useful transformations of allenic compounds,[6,7] we found that treatment of N-protected ethynylaniline **1a** with paraformaldehyde (**2**) and diisopropylamine (**3a**) in the presence of copper(I) bromide (Crabbé conditions)[8] gave 2-(aminomethyl)indole derivative **4a** in 92% yield (Scheme 1) without formation of the expected [2-(*N*-tosylamino)phenyl]allene. This reaction would proceed presumably through a Mannich-type MCR followed by formation of the indole ring from the plausible intermediate **7**. This is the first example of the formation of an indole ring system by a three-component reaction without producing any salts as by-products, although the synthesis of indole derivatives by catalytic domino three-component reactions including Sonogashira-type cross-coupling of dihalobenzenes[9] and haloanilines[10] were recently reported.[11] Herein we present a copper(I)-catalyzed domino three-component coupling–cyclization reaction of ethynylaniline derivatives with high atom economy to produce 2-(aminomethyl)indoles, with water as the waste product. Construction of polycyclic indole derivatives through this domino reaction and palladium-catalyzed C–H functionalization is also presented.



Scheme 1. Domino three-component coupling-indole formation. Ts = toluene-4-sulfonyl.

By modifying the original reaction conditions shown in Scheme 1 (CuBr, 1.0 equiv; $(HCHO)_n$, 2 equiv; and diisopropylamine, 3 equiv), we investigated the three-component formation of an indole ring under various reaction conditions (Table 1). To reduce the requisite amount of the amine

Table 1: Optimization of reaction conditions for the reaction with ethynylaniline **1a** and piperidine (**3b**).[a]



Entry	CuBr [mol%]	$(HCHO)_n$ [equiv]	Additive [equiv]	t [h]	Yield [%][b]
1	100	2.0	Et_3N [2.0]	0.25	71
2	10	2.0	Et_3N [2.0]	0.25	84
3	1	2.0	Et_3N [2.0]	0.25	92[c]
4	1	2.0	none	0.25	87
5	1	1.5	none	1	75
6	1	1.1	none	12	70

[a] Unless otherwise stated, reactions were carried out with **1** (0.18 mmol), $(HCHO)_n$ **2** (equivalents are shown in the Table), and piperidine (**3b**, 1.1 equiv) in 1,4-dioxane at 80 °C. [b] Yields of isolated products. [c] The reaction was conducted on a 1.25-mmol scale.

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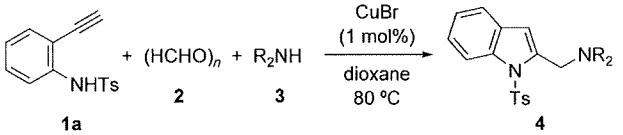
component **3**, the reaction was first examined in the presence of Et_3N (2 equiv) using 1.1 equivalents of piperidine **3b**, which gave the expected indole **4b** in 71% yield (Table 1, entry 1). A catalytic reaction using 10 or 1 mol% of CuBr is also possible, and gives rise to **4b** in better yields (84–92%, Table 1, entries 2 and 3). In contrast, the reaction in the absence of any copper salt led to recovery of the starting material. The addition of Et_3N is not essential (entry 4), although the yield of **4b** was slightly decreased (87%). This result can be rationalized by the plausible mechanism depicted in Scheme 1, in which the sulfonamide proton is efficiently transferred to the 3-position of the indole nucleus. A decreased loading of $(HCHO)_n$ (1.5 or 1.1 equivalents) is also tolerated in this transformation, and leads to **4b** in yields of 75 and 70%, respectively (Table 1, entries 5 and 6); however, a longer reaction time (1–12 h) was required.

Next, the reaction of **1a** with various amines **3a–e** under the optimized conditions (Table 1, entry 4) was investigated. The results are summarized in Table 2. The reaction with the bulky diisopropylamine (**3a**, 1.1 equiv) and a catalytic amount of CuBr (1 mol%) resulted in **4a** in 81% yield. As well as piperidine (**3b**; Table 2, entry 2), pyrrolidine (**3c**) was also a good amine component in this reaction (89%, Table 2, entry 3). When a volatile amine such as diethylamine (**3d**) was used, the reaction proceeded well (89%) if it was used in increased amount (2 equiv, Table 2, entry 4). Secondary amines with removable benzyl groups **3e** also afforded the desired 2-[(*N*,*N*-dibenzylamino)methyl]indole **4e** in good yield (78%; Table 2, entry 5).

Table 2: Reaction with various amines.[a]



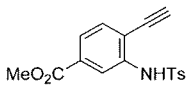
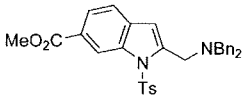
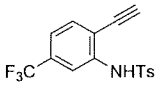
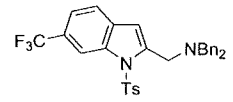
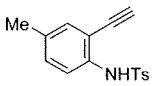
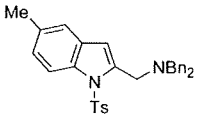
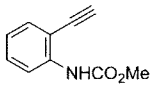
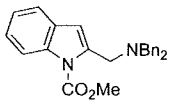
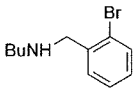
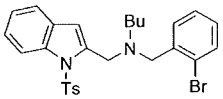
1a 2 3 4

Entry	Amine **3**	*t* [h]	Product	R	Yield [%][b]
1	iPr_2NH (**3a**)	0.25	**4a**	*i*Pr	81
2	piperidine (**3b**)	0.25	**4b**	$R_2=(CH_2)_5$	87
3	pyrrolidine (**3c**)	0.25	**4c**	$R_2=(CH_2)_4$	89
4	Et_2NH (**3d**)[c]	0.25	**4d**	Et	89
5	Bn_2NH (**3e**)	2	**4e**	Bn	78

[a] Unless otherwise stated, reactions were carried out with **1a** (0.18 mmol), $(HCHO)_n$ **2** (2.0 equiv), amine **3** (1.1 equiv), and CuBr (1 mol%) in 1,4-dioxane at 80°C. [b] Yields of isolated products. [c] 2 equivalents of amine **3d** were used because of its volatility.

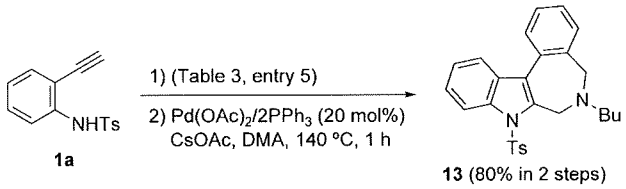
The reaction of various substituted components was then investigated. Anilines **1b** and **1c** bearing an electron-withdrawing methoxycarbonyl or trifluoromethyl group at the 3-position were allowed to react with dibenzylamine in the presence of a copper catalyst (1 mol%) to afford indoles **8** (79% yield) and **9** (61% yield), respectively (Table 3, entries 1 and 2). The reaction of **1d**, which has an electron-donating methyl group, also showed sufficient reactivity and gave indole **10** in 78% yield (Table 3, entry 3). This three-component cyclization is also applicable to the synthesis of 2-(aminomethyl)indoles having nitrogen-protecting groups other than the tosyl group: for example, *N*-(methoxycarbonyl)indole derivative **11** was prepared in 88% yield starting from ethynylaniline **1e** (Table 3, entry 4). The reaction is also possible with unsymmetrical secondary amines with functional groups: amine **3f** yielded indole **12** with a bromine atom on the benzene ring in 80% yield (Table 3, entry 5) without causing any undesired side reactions.

Table 3: Reaction of variously substituted components.[a]

Entry	Ethynylaniline	Amine	conditions	Product (yield)[b]
1	 **1b**	Bn_2NH, **3e**	80°C, 5 h	 **8** (79%)
2	 **1c**	Bn_2NH, **3e**	80°C, 3 h	 **9** (61%)
3	 **1d**	Bn_2NH, **3e**	80°C, 5 h then reflux, 1 h	 **10** (78%)
4	 **1e**	 **3b**	80°C, 15 min	 **11** (88%)
5	**1a**	 **3f**	80°C, 3 h then reflux, 1 h	 **12** (80%)

[a] All reactions were carried out with **1** (0.18 mmol), $(HCHO)_n$ **2** (2.0 equiv), amine **3** (1.1 equiv), and CuBr (1 mol%) in 1,4-dioxane. [b] Yields of isolated products.

The polycyclic indole is an important core framework for biologically active compounds.[12] Therefore, the development of an efficient method for the construction of this framework is strongly required.[13] We expected that the newly developed three-component reaction for the formation of an indole ring would serve as an extremely useful synthetic route to this class of compounds. We thus investigated the construction of polycyclic indole skeletons by a sequential three-component reaction leading to the formation of an indole ring followed by a palladium-catalyzed functionalization of a C–H bond. As shown in Scheme 2, the formation of an indole ring system



Scheme 2. Construction of a polycyclic indole structure by a three-component reaction and palladium-catalyzed cyclization. DMA = dimethylacetamide.

from N-protected ethynylaniline **1a** (Table 3, entry 5) and, after purification, subsequent palladium-catalyzed cyclization of the resulting 2-(aminomethyl)indole **12** gave the desired dihydrobenzoazepine-fused indole **13** in 80% yield over the two steps.

In conclusion, we have developed a novel domino three-component coupling reaction for the synthesis of 2-(aminomethyl)indoles and polycyclic indole derivatives. This study has resulted in the first catalytic multicomponent construction of an indole ring that produces water as the only theoretical by-product. This domino reaction, in which two carbon–nitrogen bonds and one carbon–carbon bond are formed, is synthetically useful since functionalized 2-(aminomethyl)indoles and their polycyclic derivatives can be obtained directly from readily available N-protected ethynylanilines.

Experimental Section

General procedure for three-component formation of an indole: Piperidine (**3b**; 20.0 μL, 0.20 mmol) was added at room temperature under Ar to a stirred suspension of N-tosylated ethynylaniline **1a** (50 mg, 0.18 mmol), paraformaldehyde (**2**; 11.1 mg, 0.37 mmol), and CuBr (0.26 mg, 0.0018 mmol) in dioxane. After stirring the mixture for 15 min at 80 °C, it was concentrated under reduced pressure and purified by column chromatography over silica gel with hexane/EtOAc (5:1) as the eluent to afford the desired product **4b** (59.2 mg, 87%) as a colorless oil: IR: $\tilde{\nu}$ = 1367 (NSO_2), 1173 cm^{-1} (NSO_2); ^1H NMR (400 MHz, $CDCl_3$): δ = 1.42–1.49 (m, 2H, CH_2), 1.50–1.58 (m, 4H, 2 × CH_2), 2.33 (s, 3H, Ph*Me*), 2.44-2.54 (m, 4H, 2 × NCH_2), 3.84 (s, 2H, 1′-CH_2), 6.54 (s, 1H, 3-H), 7.18 (d, J = 8.4 Hz, 2H, Ar), 7.25 (ddd, J = 7.4, 7.4, 1.1 Hz, 1H, Ar), 7.17–7.20 (m, 1H, Ar), 7.44 (d, J = 7.4 Hz, 1H, Ar), 8.03 (d, J = 8.4 Hz, 2H, Ar), 8.07 ppm (d, J = 8.3 Hz, 1H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.5, 24.3, 25.9 (2C), 54.6, 56.1 (2C), 111.2, 114.5, 120.4, 123.2, 124.0, 127.2 (2C), 129.0, 129.4 (2C), 136.5, 137.0, 138.4, 144.4 ppm; MS (FAB) *m/z* (%): 369 (100); HRMS (FAB) calcd for $C_{21}H_{25}N_2O_2S$ [M+H$^+$]: 369.1637; found: 369.1632.

Synthesis of polycyclic indole **13** by palladium-catalyzed functionalization of the C–H bond: $Pd(OAc)_2$ (4.3 mg, 0.019 mmol), PPh_3 (10 mg, 0.038 mmol), and CsOAc (36 mg, 0.19 mmol) were added to a stirred solution of **12** (50 mg, 0.095 mmol) at room temperature under Ar. After stirring the mixture for 1 h at 140 °C, it was concentrated under reduced pressure and purified by column chromatography over silica gel with hexane/AcOEt (4:1) as the eluent to afford the desired product **13** (42 mg, quant.) as a colorless oil: IR: $\tilde{\nu}$ = 1374 (NSO_2), 1174 cm^{-1} (NSO_2); ^1H NMR (400 MHz, $CDCl_3$): δ = 0.98 (t, J = 7.2 Hz, 3H, CH_3), 1.39–1.49 (m, 2H, CH_3CH_2), 1.61–1.68 (m, 2H, $CH_2CH_2CH_2$), 2.30 (s, 3H, Ph*Me*), 2.67 (t, J = 7.2 Hz, 2H, NCH_2CH_2), 3.42 (s, 2H, NCH_2), 4.05 (s, 2H, NCH_2), 7.16 (d, J = 8.8 Hz, 2H, Ar), 7.23–7.45 (m, 5H, Ar), 7.67 (d, J = 6.1 Hz, 1H, Ar), 7.73 (d, J = 7.6 Hz, 1H, Ar), 7.83 (d, J = 8.4 Hz, 2H, Ar), 8.57 ppm (d, J = 8.4 Hz, 1H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.1, 20.6, 21.5, 30.1, 47.9, 55.8, 56.2, 115.6, 119.3, 123.8, 124.0, 124.8, 126.7 (2C), 127.2, 127.4, 127.5, 128.0, 129.7 (2C), 130.6, 134.4, 135.4 (2C), 137.0, 137.0, 144.7 ppm; MS (FAB) *m/z* (%): 445 (100); HRMS (FAB) calcd for $C_{27}H_{29}N_2O_2S$ [MH$^+$]: 445.1950; found: 445.1952.

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The Novel CXCR4 Antagonist KRH-3955 Is an Orally Bioavailable and Extremely Potent Inhibitor of Human Immunodeficiency Virus Type 1 Infection: Comparative Studies with AMD3100▽

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The previously reported CXCR4 antagonist KRH-1636 was a potent and selective inhibitor of CXCR4-using (X4) human immunodeficiency virus type 1 (HIV-1) but could not be further developed as an anti-HIV-1 agent because of its poor oral bioavailability. Newly developed KRH-3955 is a KRH-1636 derivative that is bioavailable when administered orally with much more potent anti-HIV-1 activity than AMD3100 and KRH-1636. The compound very potently inhibits the replication of X4 HIV-1, including clinical isolates in activated peripheral blood mononuclear cells from different donors. It is also active against recombinant X4 HIV-1 containing resistance mutations in reverse transcriptase and protease and envelope with enfuvirtide resistance mutations. KRH-3955 inhibits both SDF-1α binding to CXCR4 and Ca^{2+} signaling through the receptor. KRH-3955 inhibits the binding of anti-CXCR4 monoclonal antibodies that recognize the first, second, or third extracellular loop of CXCR4. The compound shows an oral bioavailability of 25.6% in rats, and its oral administration blocks X4 HIV-1 replication in the human peripheral blood lymphocyte-severe combined immunodeficiency mouse system. Thus, KRH-3955 is a new promising agent for HIV-1 infection and AIDS.

The chemokine receptors CXCR4 and CCR5 serve as major coreceptors of human immunodeficiency virus type 1 (HIV-1), along with CD4 as a primary receptor for virus entry (2, 15, 18, 19). SDF-1α, which is a ligand for CXCR4, blocks the infection of CXCR4-utilizing X4 HIV-1 strains (7, 34). On the other hand, ligands for CCR5 such as RANTES inhibit CCR5-utilizing R5 HIV-1 (10). These findings made chemokines, chemokine derivatives, or small-molecule inhibitors of chemokine receptors attractive candidates as a new class of anti-HIV-1 agents. Many CCR5 antagonists have been developed as anti-HIV-1 drugs. These include TAK-779 (Takeda Pharmaceutical Company) (5), TAK-652 (6), TAK-220 (45), SCH-C (Schering-Plough) (43), SCH-D (vicriviroc) (42), GW873140 (aplaviroc; Ono Pharmaceutical/Glaxo Smith Kline) (28), and UK-427,857 (maraviroc; Pfizer Inc.) (17). Of these, maraviroc was approved by the U.S. FDA in 2007 for the treatment of R5 HIV-1 in treatment-experienced adult patients, combined with other antiretroviral treatment. Several classes of CXCR4 antagonists have also been reported. The bicyclam AMD3100 showed antivirus activity against many X4 and some R5X4 HIV strains in peripheral blood mononuclear cells (PBMCs) but not against R5 strains (16, 40). The pharmacokinetics and antiviral activity of this compound were also evaluated in humans (21, 22). T22, [Tyr-5,12, Lys-7]polyphemusin II, which is an 18-mer peptide derived from horseshoe crab blood cells, was reported to specifically inhibit X4 HIV-1 strains (30). Studies on the pharmacophore of T140 (a derivative of T22) led to the identification of cyclic pentapeptides (46).

In 2003, we reported that KRH-1636 is a potent and selective CXCR4 antagonist and inhibitor of X4 HIV-1 (23). Although the compound was absorbed efficiently from the rat duodenum, it has poor oral bioavailability. Continuous efforts to find more potent CXCR4 antagonists that are bioavailable when administered orally allowed us to develop KRH-3955 by a combination of chemical modification of the lead compound and biological assays. In this report, we describe the results of a preclinical evaluation of KRH-3955, including its in vitro anti-HIV-1 activity, its in vivo efficacy in the human peripheral blood lymphocyte (hu-PBL)-severe combined immunodeficiency (SCID) mouse model, and its pharmacokinetics in rats in comparison with those of AMD3100.

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MATERIALS AND METHODS

Compounds. The synthesis and purification of KRH-3955, *N,N*-dipropyl-*N'*-[4-({[(1*H*-imidazol-2-yl)methyl][(1-methyl-1*H*-imidazol-2-yl)methyl]amino}methyl)benzyl]-*N'*-methylbutane-1,4-diamine tri-(2*R*,3*R*)-tartrate, were carried out by Kureha Corporation. The chemical structure of KRH-3955 is shown in Fig. 1. The CXCR4 antagonist AMD3100 and zidovudine (AZT) were obtained from Sigma. Saquinavir was obtained

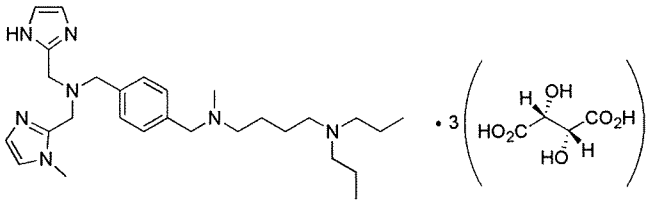

FIG. 1. Chemical structure of KRH-3955.

from the NIH AIDS Research and Reference Reagent Program, NIAID, Bethesda, MD. AMD070 and SCH-D were synthesized at Kureha Corporation.

Cells. Molt-4 no. 8 cells (24) were maintained in RPMI 1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO) and antibiotics (50 ng/ml penicillin, 50 ng/ml streptomycin, and 100 ng/ml neomycin; Invitrogen), which is referred to as RPMI medium. Chemokine receptor-expressing human embryonic kidney 293 (HEK293) cells (ATCC CRL-1573) and Chinese hamster ovary (CHO) cells (ATCC CCL-61) were maintained in minimal essential medium or F-12 (Invitrogen) supplemented with 10% fetal bovine serum and antibiotics (50 ng/ml penicillin, 50 ng/ml streptomycin, and 100 ng/ml neomycin). PBMCs from HIV-1-seronegative healthy donors were isolated by Ficoll-Hypaque density gradient (Lymphosepal; IBL, Gunma, Japan) centrifugation (31) and grown in RPMI medium supplemented with recombinant human interleukin-2 (rhIL-2; Roche, Mannheim, Germany) at 50 U/ml.

Viruses. Viral stocks of HIV-1_{NL4-3}, HIV-1_{JR-CSF}, and HIV-$1_{89.6}$ were each produced in the 293T cell line by transfection with HIV-1 molecular clone plasmids pNL4-3 (1), pYK-JRCSF (25), and p89.6 (11), respectively, by the calcium phosphate method. The 50% tissue culture infective dose was determined by an end-point assay with PBMC cultures activated with immobilized anti-CD3 monoclonal antibody (MAb) (33, 51). Subtype B HIV-1 primary isolates 92HT593, 92HT599 (N. Hasley), and 91US005 (B. Hahn) and AZT-resistant HIV-1 (A018) (D. D. Richman) (26) were obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. These clinical isolates were propagated in the activated PBMCs prepared as described above.

Anti-HIV-1 assays. Human PBMCs activated with immobilized anti-CD3 MAb (OKT-3; ATCC, Manassas, VA) in RPMI medium for 3 days were infected with various HIV-1 strains, including primary clinical isolates, at a multiplicity of infection of 0.001. After 3 h of adsorption, the cells were washed and cultured in RPMI medium supplemented with rhIL-2 (50 U/ml) in the presence or absence of the test compounds. Amounts of HIV-1 capsid (p24) antigen produced in the culture supernatants were measured by an enzyme-linked immunosorbent assay kit (ZeptoMetrix Corp., Buffalo, NY) 7 to 10 days after infection. The cytotoxicities of the compounds were tested on the basis of the viability and proliferation of the activated PBMCs, as determined with Cell Proliferation Kit II (XTT) from Roche (36).

Susceptibility of multidrug-resistant HIV-1 to CXCR4 antagonists was also measured by using recombinant viruses in a single replication cycle assay (9, 49). HIV-1 resistance test vectors (RTVs) contain the entire protease (PR) coding region and the reverse transcriptase (RT) coding region, from amino acid 1 to amino acid 305, amplified from patient plasma and a luciferase expression cassette inserted in the *env* region. The RTVs in this study contain patient-derived PR and RT sequences that possess mutations associated with resistance to PR, RT, or both PR and RT. Env-pseudotyped viruses were produced by cotransfecting 293 cells with RTV plasmids and expression vectors encoding the Env protein of well-characterized X4-tropic laboratory strain HXB2, NL4-3, or NL4-3 containing the Q40H enfuvirtide (T20) resistance mutation introduced by site-direct mutagenesis. The virus stocks were harvested 2 days after transfection and used to infect U87 $CD4^+$ cells (kind gifted from N. Landau, NYU School of Medicine) expressing CXCR4 in 96-well plates, with serial dilutions of CXCR4 antagonists. Target cells were lysed, and luciferase activity was measured to assess virus replication in the presence and absence of inhibitors. Drug concentrations required to inhibit virus replication by 50% (IC_{50}) were calculated.

Immunofluorescence. Molt-4 cells or CXCR4-expressing HEK293 cells were treated with various concentrations of KRH-3955 or AMD3100 in RPMI medium or phosphate-buffered saline containing 1% bovine serum albumin and 0.05% NaN_3 (fluorescence-activated cell sorting [FACS] buffer). In washing experiments, cells were washed with RPMI medium or FACS buffer. The cells were Fc blocked with 2 mg/ml normal human immunoglobulin G (IgG) in FACS buffer and then stained directly with mouse MAbs 12G5-phycoerythrin (PE) and 44717-PE (R&D Systems, Inc., Minneapolis, MN) or rat MAb A145-fluorescein isothiocyanate (FITC) and indirectly with MAb A80. The A145 and A80 MAbs were produced in ascitic fluid of BALB/c nude mice, and IgG fractions were obtained from ascitic fluid by gel filtration chromatography with Superdex G200 (Amersham Pharmacia). Goat anti-rat IgG (heavy and light chains) labeled with FITC was purchased from American Corlex (47). After washing, the cells were analyzed on a FACScalibur (BD Biosciences, San Jose, CA) flow cytometer with CellQuest software (BD Biosciences).

DNA construction and transfection. Chemokine receptor-expressing CHO cells were generated as reported previously (23). Human CXCR4 cDNA was cloned into the pcDNA3.1 vector. Mutations were introduced by using the QuikChange II site-directed mutagenesis kit (Stratagene, La Jolla, CA). All constructs were verified by DNA sequencing and transfected into 293 cells by using the Lipofectamine reagent (Invitrogen) (48). Stable transfectants were selected in the presence of 400 μg/ml G418 (Invitrogen). The COOH-terminal intracellular domain of CXCR4 (residues 308 to 352) was deleted in all mutants and the wild type. This deletion has no influence on HIV-1 infection or on SDF-1α binding and signaling but abolishes ligand-induced endocytosis (3).

Ligand-binding assays. Chemokine receptor-expressing CHO cells (5×10^6/0.2 ml per well) were cultured in a 24-well microtiter plate. After 24 h of incubation at 37°C, the culture medium was replaced with binding buffer (RPMI medium supplemented with 0.1% bovine serum albumin). Binding reactions were performed on ice in the presence of ^{125}I-labeled chemokines (final concentration of 100 pmol/liter; PeproTech Inc., Rocky Hill, NJ) and various concentrations of test compounds. After washing away of unbound ligand, cell-associated radioactivity was counted with a scintillation counter as described previously (23).

CXCR4-mediated Ca^{2+} signaling. Fura2-acetoxymethyl ester (Dojindo Laboratories, Kumamoto, Japan)-loaded CXCR4-expressing CHO cells were incubated in the absence or presence of various concentrations of KRH-3955 or AMD3100. Changes in intracellular Ca^{2+} levels in response to SDF-1α (1 μg/ml) were determined by using a fluorescence spectrophotometer as described previously (30).

Detection of KRH-3955 in blood after oral administration. The plasma concentration-time profile of R-176211 (distilled water was used as a vehicle), the free form of KRH-3955, was examined after a single oral administration of KRH-3955 at a dose of 10 mg/kg or intravenous administration at a dose of 10 mg/kg to male Sprague-Dawley rats (CLEA, Kanagawa, Japan). R-176211 in plasma was measured by liquid chromatography-tandem mass spectrometry. Pharmacokinetic parameters were calculated by using WinNonlin Professional (ver. 3.1; Pharsight Co.).

Infection of hu-PBL–SCID mice. Two groups of C.B-17 SCID mice (CLEA, Kanagawa, Japan) were administered a single dose of either KRH-3955 or tartrate (2% glucose solution was used as the vehicle) as a control orally (p.o.) and fed for 2 weeks. These mice were then engrafted with human PBMCs (1×10^7 cells/animal intraperitoneally [i.p.]) and after 1 day were infected i.p. with 1,000 infective units of X4 HIV-1_{NL4-3}. IL-4 (2 μg per animal) was administrated i.p. on days 0 and 1 after PBMC engraftment to enhance X4 HIV-1 infection. After 7 days, human lymphocytes were collected from the peritoneal cavities and spleens of the infected mice and cultured in vitro for 4 days in RPMI medium supplemented with 20 U/ml rhIL-2. HIV-1 infection was monitored by measuring p24 levels in the culture supernatant. We used a selected donor whose PBMCs could be engrafted at an efficiency of >80% in C.B-17 SCID mice. Usually, 5×10^5 to 10×10^5 human $CD4^+$ T cells can be recovered from each hu-PBL–SCID mouse. Mice with no or low recovery of human $CD4^+$ T cells at the time of analysis were omitted. For ex vivo cultures, we used a quarter of the cells recovered from a mouse. The protocols for the care and use of the hu-PBL–SCID mice were approved by the Committee on Animal Research of the University of the Ryukyus before initiation of the present study.

RESULTS

Anti-HIV-1 activities of KRH-3955 in activated PBMCs. The inhibitory activity of KRH-3955 against X4 HIV-1 (NL4-3), R5X4 HIV-1 (89.6), and R5 HIV-1 (JR-CSF) was examined in activated human PBMCs from two different donors. KRH-3955 inhibited the replication of both X4 and R5X4 HIV-1 in activated PBMCs with 50% effective concentrations (EC_{50}) of 0.3 to 1.0 nM but did not affect R5 HIV-1 replication, even at concentration of up to 200 nM (Table 1). In contrast, the CCR5 antagonist SCH-D (vicriviroc) inhibited R5 HIV-1 rep-

TABLE 1. Anti-HIV-1 activity of KRH-3955 in activated PBMCs[a]

Virus	Donor	EC_{50} (nM)[b]					
		KRH-3955	AMD3100	AMD070	SCH-D	AZT	SQV
NL4-3	A	1.1	41	35	>1,000	11	9.0
X4	B	0.33	15	15	>1,000	8.0	29
89.6	A	0.38	44	55	>1,000	7.4	9.9
R5X4	B	ND[c]	ND	ND	ND	ND	ND
JR-CSF	A	>200	>200	>200	0.37	0.96	2.6
R5	B	>200	>200	>200	1.2	6.2	8.0
A018H (X4) (pre-AZT)	C	1.4	38	ND	ND	1.9	ND
A018G (X4) (post-AZT)	C	1.3	32	ND	ND	87,000	ND

[a] PBMCs from two different donors were used in each assay. Anti-HIV-1 activity was determined by measuring the p24 antigen level in culture supernatants.
[b] Assays were carried out in triplicate wells. The average of two to four experiments is shown.
[c] ND, not determined.

lication but inhibited neither X4 nor R5X4 HIV-1 replication (Table 1). The anti-HIV activity of KRH-3955 against the 89.6 virus from donor B was not determined because the virus did not replicate enough for calculation of the anti-HIV activity of KRH-3955 and other drugs. Notably, the anti-HIV-1 activity of KRH-3955 was much higher than that of AMD3100, a well-known X4 HIV-1 inhibitor, or AMD070, the other X4 inhibitor that is bioavailable when administered orally. KRH-3955 also inhibited the replication of clinical isolates of X4 HIV-1 (92HT599) and R5X4 HIV-1 (92HT593) with EC_{50} ranging from 4.0 to 4.2 nM (data not shown). Although both KRH-3955 and AMD3100 were effective against at least some R5X4 HIV-1 strains in activated PBMCs, neither KRH-3955 nor AMD3100 inhibited the infection of CD4/CCR5 cells by R5 or R5X4 HIV-1, even at a concentration of 1,660 nM (data not shown). Importantly, the 50% cytotoxic concentration of KRH-3955 in activated PBMCs (donor A) was 57 μM, giving a high therapeutic index (51,818) in the case of NL4-3 infection, which was higher than that of AZT (8,000 in the case of donor A). These results indicate that the compound is a selective inhibitor of HIV-1 that can utilize CXCR4 as a coreceptor. Since a CXCR4 antagonist should be used in combination with a CCR5 antagonist in a clinical setting, we next examined whether the combined use of both antagonists efficiently blocks mixed infection with X4 and R5 HIV-1. Combination of KRH-3955 and SCH-D at 4 plus 4 nM and 20 plus 20 nM blocked the replication of 50:50 mixtures of NL4-3 and JR-CSF by 91 and 96%, respectively (data not shown). Thus, KRH-3955 is a highly potent and selective inhibitor of X4 HIV-1.

Anti-HIV-1 activities of KRH-3955 in activated PBMCs from different donors. It has been observed that the anti-HIV-1 activity of compounds in PBMCs varies from donor to donor. Therefore, the anti-HIV-1 activity of KRH-3955 against X4 HIV-1 was examined in activated PBMCs from eight different donors. The levels of p24 antigen in NL4-3-infected cultures ranged from 17 to 120 ng/ml (Table 2). KRH-3955 inhibited the replication of NL4-3 with EC_{50} ranging from 0.23 to 1.3 nM and with EC_{90} ranging from 2.7 to 3.5 nM (Table 2), demonstrating that the anti-HIV-1 activity of KRH-3955 was independent of the PBMC donor.

Anti-HIV-1 activities of KRH-3955 against drug-resistant HIV-1 strains. To further assess the efficacy of KRH-3955, we used a single-cycle assay to evaluate the activity of KRH-3955 against a panel of recombinant viruses that express an X4-tropic envelope protein (HXB2) but contain PR and RT sequences containing a wide variety of mutations associated with resistance to PR inhibitors (PIs), nucleoside RT inhibitors (NRTIs), and non-NRTIs (NNRTIs). This assessment was also performed with recombinant viruses that express an X4-tropic envelope protein (NL4-3) that contains the Q40H mutation and displays resistance to T20 (an entry inhibitor). The results of these experiments demonstrate that both KRH-3955 and AMD3100 inhibited the infection of CD4/CXCR4 cells by these recombinant drug-resistant viruses, including viruses resistant to PIs, NRTIs, or NNRTIs; multidrug-resistant viruses; and T20-resistant viruses (Table 3). We also observed that KRH-3955 inhibited the replication of A018G, a highly AZT-resistant strain, in activated PBMCs with an EC_{50} of 1.3 nM (Table 1).

KRH-3955 selectively inhibits ligand binding to CXCR4. To investigate whether KRH-3955 specifically blocks ligand binding to CXCR4, the inhibitory effect of the compound on chemokine binding to CHO cells expressing CXCR4, CXCR1, CCR2b, CCR3, CCR4, or CCR5 was determined. KRH-3955 efficiently inhibited SDF-1α binding to CXCR4 in a dose-dependent manner (Fig. 2 and 3b), and the IC_{50} for SDF-1α binding was 0.61 nM, which is similar to its EC_{50} against HIV-1. Similar results were obtained when we used a Molt-4 T cell line as the CXCR4-expressing target cell (Fig. 3a). Interestingly, the inhibitory activity of AMD3100 against SDF-1α binding was much weaker than its anti-HIV-1 activity (Fig. 3), suggesting that the binding sites of these two compounds are different. In contrast, the compound did not affect the binding

TABLE 2. Anti-HIV-1 activity of KRH-3955 against NL4-3 infection of PBMCs from eight different donors

Donor	p24 level (ng/ml)	EC_{50} (nM)	EC_{90} (nM)
1	31	1.30	3.2
2	25	1.20	3.2
3	17	1.20	3.3
4	40	0.70	2.9
5	120	0.77	2.9
6	58	1.50	3.5
7	49	0.23	2.7
8	53	1.00	3.0
Mean ± SD	49 ± 32	0.99 ± 0.40	3.1 ± 0.30