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Synthesis of [19, 35, 36-$^{13}C_3$]-labeled TAK779 as a molecular probe

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ABSTRACT

N,N-Dimethyl-*N*-[4-[[[2-(4-methylphenyl)-6,7-dihydro-5*H*-benzocyclohepten-8-yl]carbonyl]amino]benzyl]tetrahydro-2*H*-pyran-4-aminium chloride (TAK779) is a potent and selective non-peptide CCR5 antagonist. To use a site-specifically labeled form as a molecular probe, TAK779 containing ^{13}C at positions C19, 35, and 36 was produced. A commercially available [^{13}C]-methyl iodide was employed for the labeling. Starting from a known carboxylic acid segment containing no labeled carbon, the labeled TAK779 was constructed by the successive coupling of [^{13}C]-labeled tolyl boronic ester by the Suzuki–Miyaura reaction and a [^{13}C]-labeled aniline segment by amide bond formation.

1. Introduction

The β-chemokine receptor CCR5, a G protein-coupled seven-transmembrane domain receptor, acts as a major co-receptor for the fusion and entry of macrophase-tropic (M-tropic or R5) HIV-1 into host cells.[1] As a result, CCR5 is considered an attractive target for the inhibition of M-tropic HIV replication.[2] In 1999, *N,N*-dimethyl-*N*-[4-[[[2-(4-methylphenyl)-6,7-dihydro-5*H*-benzocyclohepten-8-yl] carbonyl]amino]benzyl]tetrahydro-2*H*-pyran-4-aminium chloride (**1**, TAK779) was reported as the first small molecule that acts as a potent and selective non-peptide CCR5 antagonist with a IC_{50} value of 1.4 nM.[3] TAK779 (**1**) inhibited the entry of HIV-1 into target cells by blocking the interaction between the gp120/CD4 complex and CCR5. Following the discovery of TAK779, other small molecule CCR5 antagonists with improved potency and/or pharmaceutical properties were also been described.[4,5]

The structural and molecular interactions of CCR5 inhibitors, including TAK779 with CCR5, however, are not clearly understood. Combining site-directed mutagenesis of CCR5 and molecular modeling, the interactions of small molecule inhibitors with CCR5 have been examined.[6–8] Although possible inhibitor-binding pockets of CCR5 have been proposed, additional experimental data are required to clarify the exact mode of binding. High resolution solid-state NMR spectroscopy can provide structural information on complex solids. By combining solid-state NMR spectroscopy and site-specific labeling with stable isotopes such as ^{13}C, ^{2}D, and/or ^{15}N, accurate inter-nuclear distances between the isotopic labels can be estimated. Thus, site-specifically labeled TAK779 would be a useful probe for examining the molecular interactions between TAK779 and CCR5 by solid-state NMR techniques. Here, we report the synthesis of 100% enriched [$^{13}C_3$]-labeled TAK779 (**2**), which contains ^{13}C isotopes at C-19, -35, and -36 (Fig. 1).

2. Results and discussion

In an energy-minimized form, TAK779 (**1**) adopts a bent conformation with the bend dividing a hydrophobic aromatic segment from a positively charged aniline segment. Previously, it was suggested that the two contrasting parts of TAK779 (**1**) interact with complementary binding pockets of CCR5.[7] To obtain experimental data regarding the proposed model, TAK779 containing ^{13}C isotopes at both ends, that is, positions C-19, -35, and -36, was selected as a probe (**2**) (Fig. 1).

For the convenient introduction of 100% ^{13}C-enriched isotope carbon, a commercially available [^{13}C]-methyl iodide was selected. Thus, the scheme for the synthesis of TAK779[3b] was modified to enable the introduction of methyl groups at C-19, -35, and -36 by [^{13}C]-methyl iodide. The backbone of the ^{13}C-labeled TAK779 (**2**) was constructed by successive coupling of the left-side boronate segment (**4**) and right-side aniline segment (**6**) to the central segment (**5**) (Scheme 1). The central carboxylic acid segment (**5**) containing no labeled carbon was prepared according to a published procedure[3b] starting with bromobenzene in eight steps without difficulty. For the synthesis of the [$^{13}CH_3$]-labeled boronyl toluene derivative (**4**), boronic acid (**7**) and [^{13}C]-methyl iodide

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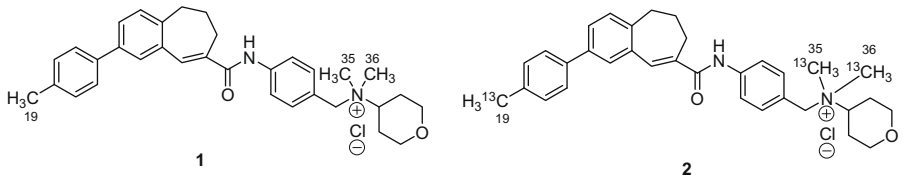
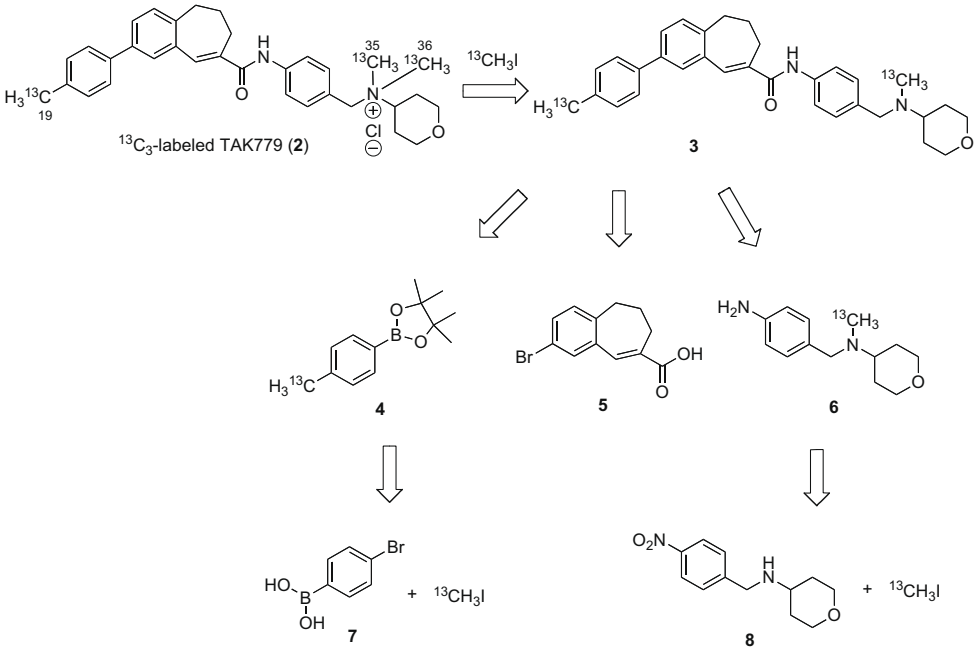


Figure 1. TAK779 (**1**) and [$^{13}C_3$]-labeled TAK779 (**2**).



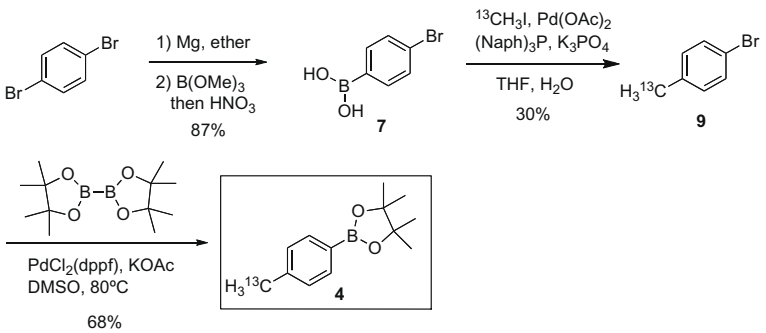
Scheme 1. Synthetic plan for [$^{13}C_3$]-labeled TAK779 (**2**).

were selected as the coupling components. The [$^{13}CH_3$]-labeled aniline derivative (**6**) was prepared via the N-methylation of amine (**8**) by [^{13}C]-methyl iodide.

In the preparation of ^{13}C-labeled *p*-tolylboronic acid ester (**4**), [$^{13}CH_3$]-labeled *p*-bromotoluene (**9**), instead of 4-methylphenylboronic acid employed in the original synthesis of non-labeled TAK779 (**1**), is required. Although the synthesis of [$^{13}CH_3$]-labeled *p*-bromotoluene (**9**) by zeolite-mediated bromination of [$^{13}CH_3$]–toluene has been reported by Kesling et al.,[9] separation of the product mixture containing *para/ortho* derivatives was not effectively achieved. In addition, with this route, very expensive [$^{13}CH_3$]-labeled toluene was necessary. To enable the use of readily available [^{13}C]-methyl iodide, a route starting from 4-dibromobenzene was selected (Scheme 2).

First, *p*-bromophenyl boronic acid (**7**) was synthesized by the reaction of trimethyl borate with the Grignard reagent prepared from 1,4-dibromobenzene in 87% yield, according to the method reported by Kabalka et al.[10] Successive palladium (0)-catalyzed cross coupling of **7** with [^{13}C]-methyl iodide proceeded at 25 °C under Gooβen's conditions.[11] Subsequent purification by careful distillation (bp = 58 °C, 20 mmHg) afforded [$^{13}CH_3$]-labeled *p*-bromotoluene (**9**) in 30 % yield. The spectral data of the resulting compound (**9**) was well consistent with those of the reported compound.[9] The labeled product (**9**) was then converted to the desired [$^{13}CH_3$]-labeled *p*-tolylboronate (**4**) in 68% yield by treatment with bis(pinacorato)diboron[12] in the presence of $PdCl_2$(dppf).

Previously in the synthesis of non-labeled TAK779 (**1**), the right-segment, the aniline derivative (**6**), was prepared by successive



Scheme 2. Synthesis of [$^{13}CH_3$]-*p*-tolylboronate (**4**).

reductive amination of 4-nitro-benzylamine using tetrahydro-4*H*-pyran-4-one and formaldehyde. In the present study, the stepwise introduction of substitution groups on the nitrogen atom, which enables the introduction of a labeled methyl group by [^{13}C]-methyl iodide, was employed. Thus, the desired [$^{13}CH_3$]-labeled aniline derivative (**6**) was produced starting with *N*,*N*-Boc(*o*-Nos)NH (**10**) (Scheme 3). The coupling of **10** with 4-tetrahydropyranyl alcohol by Fukuyama's protocol[13] gave the corresponding amine, which was then treated with TFA to provide nosyl amine (**11**) in 38% overall yield. The same procedure was repeated on **11** with *p*-nitro benzyl alcohol to obtain the nitro derivative (**12**) in 52% yield. After removal of the nosyl group of **12** in the presence of mercapto acetic acid and LiOH (62% yield), methylation of amine (**8**) with [^{13}C]-methyl iodide in the presence of NaH afforded [$^{13}CH_3$]-labeled *N*-methyl amine (**13**) in 72% yield. The coupling constant of the product was 133 Hz (doublet, 3H) in the ^{1}H NMR spectrum, which confirmed the successful ^{13}C labeling in **13**. Reduction of the nitro group of **13** gave the desired aniline (**6**) in 76% yield.

Using the labeled segments above, [$^{13}C_3$]-labeled TAK779 (**2**) was synthesized via two routes (routes (a) and (b), in Scheme 4). The left segment (**4**) was introduced to the central segment (**5**) first in route (a), whereas the right segment (**6**) was introduced first in route (b). With route (a), Suzuki–Miyaura coupling of [^{13}C]-labeled boronate (**4**) and phenyl bromide (**5**) was conducted in the presence of potassium carbonate and a catalytic amount of $PdCl_2$(dppf) to obtain [^{13}C]-labeled methyl ester (**14**) in 64% yield. After saponification of **14**, the resulting [^{13}C]-labeled carboxylic acid (**15**) was treated with oxalyl chloride in the presence of a catalytic amount of DMF. The addition of [^{13}C]-labeled aniline (**6**) and triethylamine to the mixture gave the desired [^{13}C]-labeled amide (**3**) in 74% yield. With route (b), coupling of carboxylic acid (**16**) and [^{13}C]-labeled aniline (**6**) afforded [^{13}C]-labeled amide (**17**) in 47% yield, and a subsequent cross-coupling reaction gave [$^{13}C_2$]-labeled amide (**3**) in 36% yield. Thus, the synthesis via route (a) resulted in a better yield than that via route (b). Finally, treatment of [$^{13}C_2$]-labeled amide (**3**) with [^{13}C]-methyl iodide in DMF and subsequent counter ion exchange gave the desired [$^{13}C_3$]-labeled TAK779 (**2**) in 70% yield. The product was confirmed to have the expected molecular weight by MALDI TOF-MS. The expected large coupling constants of the methyl protons of the tolyl group (126 Hz, doublet, 3H) and quaternary ammonium group (144 Hz, doublet, 6H) were observed in the ^{1}H NMR spectrum of **2**. In the ^{13}C NMR spectrum, extremely strong signals from three methyl carbons were also observed as expected. These spectral data clearly support a successful ^{13}C labeling in **2**.

In conclusion, [$^{13}C_3$]-labeled TAK779 (**2**) was produced by introducing 100% ^{13}C-enriched isotope carbon at C-19, -35, and -36. For convenience, the readily available [^{13}C]-methyl iodide was employed, and the method used to synthesize non-labeled TAK779[3b] was modified to enable the efficient use of [^{13}C] methyl iodide. Studies on molecular interactions between the synthesized labeled-TAK779 and CCR5 using solid-state NMR techniques are now underway and will be reported in due course.

3. Experimental

3.1. General

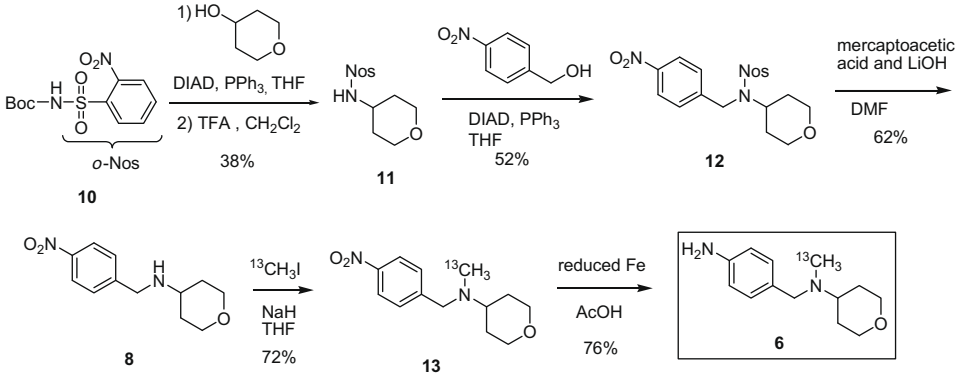
[^{13}C]-Methyl iodide was purchased from ISOTEC. All manipulations were conducted under an inert atmosphere (N_2). All solvents were of reagent grade. THF was distilled from sodium and benzophenone ketyl. CH_2Cl_2 was distilled from CaH_2. All commercial reagents were of the highest purity available. Analytical TLC was performed on silica gel (60 F-254, Plates 0.25 mm). Column chromatography was carried out on Wakogel 60 (particle size, 0.063–0.200 mm) or Dowex 1 × 8 (Cl^- form: particle size, 100–200 mesh). ^{1}H (300 MHz), and ^{13}C (75 MHz) NMR spectra were recorded on a Bruker AM-300. Chemical shifts are expressed in ppm relative to TMS (0 ppm) or $CHCl_3$ (7.28 ppm for ^{1}H and 77.0 ppm for ^{13}C) or MeOH (3.30 ppm for ^{1}H and 49.0 ppm for ^{13}C). IR spectra were obtained on a HORIBA FREEXACT-II FT-710 spectrometer. Low-resolution mass spectra (LRMS) and high-resolution mass spectra (HRMS) were obtained on either a JEOL JMS-HX-211A or a JMS-HX-110A (EI or FAB) or a Bruker Autoflex-II (MALDI-TOF).

3.2. [$^{13}CH_3$]-*p*-Bromotoluene (9)

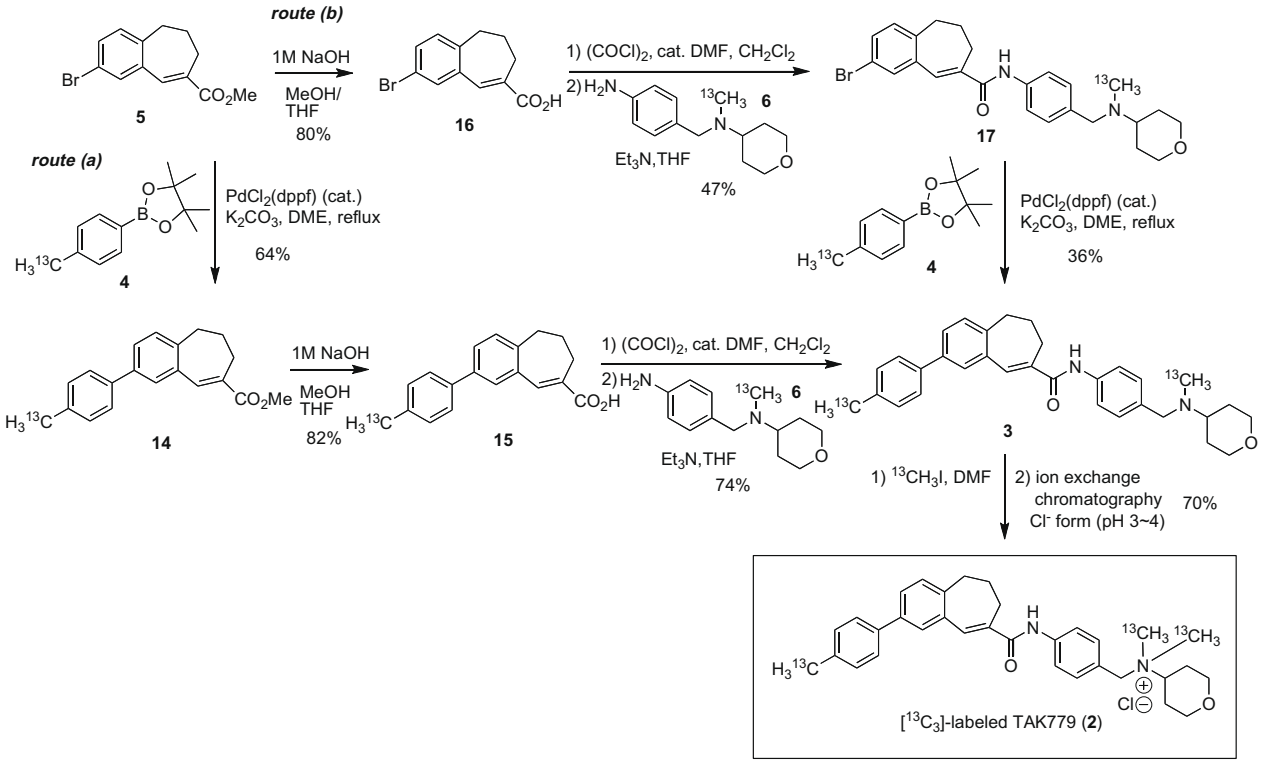
To a solution of palladium acetate (56 mg, 0.249 mmol), trinaphthylphosphine (205 mg, 0.498 mmol), and potassium phosphate (4.11 g, 19.9 mmol) in THF (20 ml) were added [^{13}C]-methyl iodide (2.00 g, 14.9 mmol), *p*-bromophenylboronic acid (**7**) (2.00 g, 9.96 mmol), and H_2O (0.350 ml, 19.9 mmol). The reaction mixture was stirred at room temperature for 12 h, poured into water (10 ml), and extracted with hexane. The combined organic layers were dried over $MgSO_4$, and filtered through a plug of silica, and the product was carefully distilled to give [$^{13}CH_3$]-*p*-bromotoluene (**9**) (516 mg, 2.99 mmol, 30%) as a white solid, bp = 58 °C (20 mmHg), mp = 16 °C. ^{1}H NMR ($CDCl_3$) δ: 2.32 (3H, d, J = 126.6 Hz), 7.06 (2H, dd, J = 8.4, 4.5 Hz), 7.38 (2H, d, J = 8.4 Hz). ^{13}C NMR ($CDCl_3$) δ: 21.0 (^{13}C), 119.1, 130.9 (d, J = 3.1 Hz), 131.3, 136.7 (d, J = 44.2 Hz). The spectral data were well consistent with those reported by Kesling et al.[9]

3.3. [$^{13}CH_3$]-*p*-(*B*-Pinacolate-boronyl)toluene (4)

To a solution of [1,1′-bis(diphenylphosphino)-ferrocene]dichloropalladium (II) (22 mg, 0.030 mmol), KOAc (294 mg, 3.00 mmol), and bis(pinacolate)diboron (279 mg, 1.10 mmol) in DMSO (6 ml)



Scheme 3. Synthesis of [$^{13}CH_3$]-aniline derivative (**6**).



Scheme 4. Synthesis of [$^{13}C_3$]-labeled TAK779 (**2**).

was added [$^{13}CH_3$]-*p*-bromotoluene (**9**) (202 mg, 1.18 mmol). After being stirred at 80 °C for 12 h, the product was extracted with benzene, washed with water, dried over $MgSO_4$ and evaporated in vacuo. The residue was purified by column chromatography (hexane/AcOEt = 20:1) to give **4** (150 mg, 0.684 mmol, 68%) as a colorless oil. IR (film) ν_{max} cm^{-1}: 2978, 1606, 1362, 1146, 1018, 962, 656. ^{1}H NMR ($CDCl_3$) δ: 1.38 (12H, s), 2.39 (3H, d, J = 126.3 Hz), 7.21 (2H, dd, J = 7.5, 4.5 Hz), 7.73 (2H, d, J = 7.5 Hz). ^{13}C NMR ($CDCl_3$) δ: 21.7 (^{13}C), 24.9, 83.6, 128.5 (d, J = 2.3 Hz), 134.8 (d, J = 3.8 Hz), 141.3 (d, J = 40.5 Hz). HRCIMS (M)$^+$ calcd for $^{12}C_{12}{}^{13}C_1H_{20}O_2B_1$: 220.1556, found: 220.1597.

3.4. *N*-Nosyl-*N*-(tetrahydropyran-4-yl)amine (11)

To a solution of Boc(Nos)NH (1.78 g, 5.90 mmol) and 4-tetrahydropyranyl alcohol (402 mg, 3.94 mmol) in THF (10 ml) were added triphenyl phosphine (1.55 g, 5.90 mmol) and diisopropyl azodicarboxylate (1.14 ml, 5.90 mmol) at room temperature. After being stirred for 2 h, the mixture was concentrated in vacuo. To a solution of the crude product (937 mg) in CH_2Cl_2 (2 ml) was added TFA (2 ml) at room temperature. After being stirred for 2 h, the mixture was concentrated in vacuo and AcOEt and H_2O were added to the residue. The organic layer was washed successively with water and brine, dried over $MgSO_4$, and evaporated in vacuo. The crude product was purified by column chromatography (hexane/AcOEt = 2:1) to give **11** (430 mg, 1.50 mmol, 38%) as a white solid, mp = 156–160 °C. IR (film) ν_{max} cm^{-1}: 3265, 3097, 2956, 2858, 1541, 1362, 1340, 1167, 1086, 742. ^{1}H NMR ($CDCl_3$) δ: 1.51–1.66 (2H, m), 1.78–1.83 (2H, m), 3.40 (2H, td, J = 11.7, 2.4 Hz), 3.58 (1H, m), 3.90 (2H, dt, J = 12.0, 3.9 Hz), 5.36 (1H, d, J = 7.5 Hz), 7.77 (2H, m), 7.88 (1H, m), 8.19 (1H, m). ^{13}C NMR ($CDCl_3$) δ: 34.9, 51.5, 67.6, 125.7, 125.9, 130.6, 131.4, 133.5, 133.9, 134.6, 134.9, 135.8, 137.6, 149.2, 149.5. HRFABMS (M+H)$^+$ calcd for $C_{11}H_{15}N_2O_5S$: 287.0702, found: 287.0707.

3.5. 4-[*N*-Nosyl-*N*-(tetrahydropyran-4-yl)aminomethyl]-nitrobenzene (12)

To a solution of **11** (1.87 g, 6.53 mmol) and *p*-nitrobenzyl alcohol (1.00 g, 6.53 mmol) in THF (10 ml) were added triphenyl phosphine (2.57 g, 9.80 mmol) and diisopropyl azodicarboxylate (1.90 g, 9.80 mmol) at room temperature. After being stirred for 2 h, the mixture was concentrated in vacuo and the residue was purified by column chromatography ($CHCl_3$/MeOH = 95:5) to give **12** (1.43 g, 3.39 mmol, 52%) as a colorless solid: mp = 128–130 °C. IR (film) ν_{max} cm^{-1}: 3084, 2964, 1545, 1522, 1346, 1161, 872, 742, 723. ^{1}H NMR ($CDCl_3$) δ: 1.58–1.70 (4H, m), 3.39 (2H, td, J = 11.4, 3.9 Hz), 3.91 (2H, dd, J = 10.2, 2.7 Hz), 4.12 (1H, m), 4.66 (2H, s), 7.53 (2H, d, J = 9.0 Hz), 7.58–7.74 (3H, m), 7.96 (1H, dd, J = 7.8, 1.2 Hz), 8.13 (2H, d, J = 8.7 Hz). ^{13}C NMR ($CDCl_3$) δ: 31.7, 47.0, 55.8, 67.2, 123.6, 124.4, 128.2, 131.1, 131.8, 133.6, 133.9, 145.6, 147.3, 147.7, HRFABMS (M+H)$^+$ calcd for $C_{18}H_{20}N_3O_3S_1$: 422.1022, found: 422.1018.

3.6. 4-[*N*-(Tetrahydropyran-4-yl)aminomethyl]-nitrobenzene (8)

To a solution of **12** (1.43 g, 3.39 mmol) in DMF (5 ml) were added mercaptoacetic acid (0.707 ml, 10.2 mmol) and LiOH (853 mg, 20.3 mmol) at room temperature. The mixture was stirred for 2 h, and AcOEt and water were added. The organic layer was separated and the aqueous layer was extracted with AcOEt. The combined organic layer was washed successively with water and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by column chromatography ($CHCl_3$/MeOH = 95:5) to give **8** (495 mg, 2.10 mmol, 62%) as a colorless oil. IR (film) ν_{max} cm^{-1}: 3317, 2943, 2844, 1602, 1516, 1342, 1090, 1012, 860, 739. ^{1}H NMR ($CDCl_3$) δ: 1.38–1.51 (4H, m), 1.88 (2H, d, J = 12.6 Hz), 2.73 (1H, m), 3.39 (2H, td, J = 11.7, 2.1 Hz), 3.95 (3H, s), 4.00 (1H,

br s), 7.53 (2H, d, J = 9.0 Hz), 8.17 (2H, dd, J = 9.0, 1.2 Hz). ^{13}C NMR ($CDCl_3$) δ: 33.7, 49.7, 53.4, 66.6, 123.5, 128.5, 146.9, 148.6. HREIMS $(M+H)^+$ calcd for $C_{12}H_{16}N_2O_3$: 236.1161, found: 236.1137.

3.7. 4-[*N*-[$^{13}CH_3$]-Methyl-*N*-(tetrahydropyran-4-yl)aminomethyl]-nitrobenzene (13)

To a solution of **8** (250 mg, 1.06 mmol) in THF (2 ml) was added NaH (60% in oil; 80 mg, 2.03 mmol) at 0 °C. The mixture was stirred for 10 min, and [^{13}C]-methyl iodide (120 μl, 1.95 mmol) was added. The mixture was stirred at room temperature for 12 h. It was then poured into water (2 ml) and the product was extracted with AcOEt. The organic layer was washed with saturated NH_4Cl aq and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by column chromatography ($CHCl_3$/MeOH = 98:2) to give **13** (190 mg, 763 μmol, 72%) as a colorless oil. IR (film) ν_{max} cm^{-1}: 2949, 2843, 1599, 1520, 1346, 1140, 1009, 741. ^{1}H NMR ($CDCl_3$) δ: 1.60–1.78 (4H, m), 2.21 (3H, d, J = 133.2 Hz), 2.65 (1H, m), 3.38 (2H, t, J = 11.7 Hz), 3.68 (2H, d, J = 5.7 Hz), 4.04 (2H, dd, J = 11.1, 3.3 Hz), 7.51 (2H, d, J = 8.1 Hz), 8.16 (2H, d, J = 8.1 Hz). ^{13}C NMR ($CDCl_3$) δ: 29.3, 37.7 (^{13}C), 57.1, 60.1, 67.5, 123.5, 129.0, 147.0, 148.2. HREIMS $(M)^+$ calcd for $^{12}C_{12}{}^{13}C_1H_{18}N_2O_3$: 251.1317, found: 251.1345.

3.8. 4-[*N*-[$^{13}CH_3$]-Methyl-*N*-(tetrahydropyran-4-yl)aminomethyl]-aniline (6)

To a solution of **13** (190 mg, 763 μmol) in acetic acid (1 ml) was added reduced iron (100 mg, 1.79 mmol), and the mixture was stirred at room temperature for 10 h. The solvent was evaporated in vacuo, and AcOEt was added to the residue. The precipitate was removed by filtration and the filtrate was washed successively with 1 M NaOH, water and brine, dried over $MgSO_4$ and evaporated in vacuo to give **6** (156 mg, 720 μmol, 76%) as a colorless oil. IR (film) ν_{max} cm^{-1}: 3350, 3222, 2956, 2852, 1612, 1520, 1398, 1292, 1086, 1011, 752. ^{1}H NMR ($CDCl_3$) δ: 1.65–1.85 (4H, m), 2.31 (3H, d, J = 129.3 Hz), 2.89 (1H, m), 3.34 (2H, t, J = 11.7 Hz), 3.68 (2H, d, J = 4.5 Hz), 4.02 (2H, dd, J = 11.1, 3.0 Hz), 5.68 (2H, br s), 6.63 (2H, dd, J = 8.1, 1.5 Hz), 7.11 (2H, dd, J = 8.1, 1.2 Hz). ^{13}C NMR ($CDCl_3$) δ: 28.1, 35.7 (^{13}C), 56.5, 58.7, 67.0, 114.9, 125.0, 130.8, 146.1. HRFABMS $(M+H)^+$ calcd for $^{12}C_{12}{}^{13}C_1H_{21}N_2O_1$: 222.1654, found: 222.1680.

3.9. Methyl 2-(4-[$^{13}CH_3$]-methylphenyl)-6,7-dihydro-5H-benzocycloheptene-8-carboxylate (14)

To a solution of bromo ester (**5**) (30 mg, 107 μmol), [$^{13}CH_3$]-*p*-(*B*-pinacolate-boronyl)–toluene (**4**) (40 mg, 183 μmol), and, K_2CO_3 (10 mg) in DME (2 ml) was added $PdCl_2$(dppf) (10 mg, 8.65 μmol). The mixture was refluxed overnight under an argon atmosphere, and AcOEt was added. The organic layer was washed successively with water and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by column chromatography (hexane/AcOEt = 2:1) to give **14** (20 mg, 68 μmol, 64%) as a colorless oil. IR (film) ν_{max} cm^{-1}: 2929, 2858, 1238, 1186, 810. ^{1}H NMR ($CDCl_3$) δ: 2.10 (2H, m), 2.42 (3H, d, J = 126.3 Hz), 2.69 (2H, t, J = 6.9 Hz), 2.88 (2H, m), 3.85 (3H, s), 7.24 (2H, m), 7.44–7.62 (5H, m), 7.80 (1H, br s). ^{13}C NMR ($CDCl_3$) δ: 21.1 (^{13}C), 27.6, 30.3, 34.9, 52.1, 55.4, 126.7, 126.8, 126.9, 127.2, 127.3, 127.4, 128.8, 129.5, 129.85, 129.89, 131.3, 131.5, 132.6, 132.7, 134.6, 136.8, 137.4, 137.5, 139.1, 139.2, 139.6, 139.7, 140.5, 141.8, 142.1, 169.1. HREIMS $(M)^+$ calcd for $^{12}C_{19}{}^{13}C_1H_{20}O_2$: 293.1463, found: 293.1501.

3.10. 2-(4-[$^{13}CH_3$]-Methylphenyl)-6,7-dihydro-5H-benzocycloheptene-8-carboxylic acid (15)

A solution of **14** (9 mg, 30.7 μmol) and 1 M NaOH (0.20 ml) in MeOH (0.20 ml) and THF (0.20 ml) was stirred at room temperature for 5 h. The mixture was concentrated in vacuo and extracted with AcOEt after being acidified using 1 M HCl. The organic layer was washed successively with water and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by column chromatography ($CHCl_3$/MeOH = 9:1) to give **15** (7 mg, 25.1 μmol, 82%) as a colorless prism, mp 138–139 °C. IR (film) ν_{max} cm^{-1}: 3022, 2925, 2854, 1680, 1421, 1290, 1273, 1254, 808. ^{1}H NMR ($CDCl_3$) δ: 2.08 (2H, m), 2.40 (3H, d, J = 126.3 Hz), 2.69 (2H, t, J = 6.6 Hz), 7.23 (2H, m), 7.42–7.50 (3H, m), 7.54–7.61 (2H, m), 7.90 (1H, br s). ^{13}C NMR ($CDCl_3$) δ: 20.9 (^{13}C), 27.2, 30.1, 34.8, 126.6, 127.2, 129.4, 129.8, 131.3, 132.2, 134.4, 137.0, 137.4, 139.0, 140.6, 141.9, 171.7. HREIMS $(M)^+$ calcd for $^{12}C_{18}{}^{13}C_1H_{18}O_2$: 279.1307, found: 279.1356.

3.11. 2-Bromo-6,7-dihydro-5*H*-benzocycloheptene-8-carboxylic acid (16)

A solution of **5** (1.00 g, 3.57 mmol) and 1 M NaOH (5 ml) in MeOH (5 ml) and THF (10 ml) was stirred at room temperature for 5 h. The mixture was concentrated in vacuo and extracted with AcOEt after being acidified using 1 M HCl. The organic layer was washed successively with water and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by column chromatography ($CHCl_3$/MeOH = 9:1) to give **16** (760 mg, 2.86 mmol, 80%) as a colorless prism, mp = 213–214 °C. IR (KBr) ν_{max} cm^{-1}: 2931, 2634, 1668, 1421, 1292, 1201, 916, 872. ^{1}H NMR (CD_3OD) δ: 2.02 (2H, m), 2.58 (2H, td, J = 6.6, 0.9 Hz), 2.76 (2H, m), 7.08 (1H, d, J = 8.1 Hz), 7.34 (1H, dd, J = 8.1, 2.1 Hz), 7.44 (1H, d, J = 1.8 Hz), 7.62 (1H, s). ^{13}C NMR (CD_3OD) δ: 29.0, 30.1, 35.3, 120.6, 132.2, 132.5, 135.6, 135.7, 138.0, 139.0, 143.4, 171.4. HRFABMS $(M-H)^+$ calcd for $C_{12}H_{10}O_2Br$: 264.9864, found: 264.9860.

3.12. *N*-[4-[*N*-[$^{13}CH_3$]-Methyl-*N*-(4-tetrahydropyranyl)aminomethyl]-phenyl]-2-bromo-6,7-dihydro-5*H*-benzocycloheptene-8-carboxamide (17)

To a solution of **16** (35 mg, 131 μmol) in CH_2Cl_2 (0.50 ml) were added oxalyl chloride (40 ml, 316 μmol) and DMF (1 drop) on ice. The mixture was stirred for 2 h at room temperature. The solvent was evaporated in vacuo. The solution of the residue in THF (0.50 ml) was added dropwise to a solution of **6** (30 mg, 135 μmol) and triethylamine (55 μl, 400 μmol) in THF (0.50 ml) on ice and the reaction mixture was stirred for 2 h at room temperature. The solvent was evaporated in vacuo, and AcOEt and water were added. The organic layer was washed successively with water and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by column chromatography ($CHCl_3$/MeOH = 19:1) to give **17** (29 mg, 61.8 μmol, 47%) as a colorless oil. IR (film) ν_{max} cm^{-1}: 3294, 2943, 2844, 1653, 1599, 1516, 1408, 1246, 1140, 844, 816, 756. ^{1}H NMR ($CDCl_3$) δ: 1.63–1.81 (4H, m), 2.11 (2H, m), 2.23 (3H, d, J = 133.2 Hz), 2.68 (2H, t, J = 6.6 Hz), 2.79 (2H, t, J = 5.7 Hz), 3.38 (2H, t, J = 11.1 Hz), 3.60 (2H, d, J = 5.1 Hz), 4.03 (2H, m), 7.04 (1H, d, J = 8.1 Hz), 7.22 (1H, s), 7.29 (1H, d, J = 8.1 Hz), 7.32 (2H, d, J = 6.9 Hz), 7.41 (1H, s), 7.56 (2H, d, J = 6.9 Hz), 7.76 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 27.9, 29.1, 30.2, 34.2, 37.3 (^{13}C), 57.2, 59.5, 67.6, 119.6, 120.1, 129.4, 130.9, 131.0, 132.5, 134.2, 135.6, 136.5, 136.8, 139.3, 141.2, 167.7. HRFABMS $(M+H)^+$ calcd for $^{12}C_{24}{}^{13}C_1H_{30}N_2O_2Br$: 470.1491, found: 470.1507.

3.13. *N*-[4-[*N*-[$^{13}CH_3$]-Methyl-*N*-(4-tetrahydropyranyl)aminomethyl]-phenyl]-2-(4-[$^{13}CH_3$]-methylphenyl)-6,7-dihydro-5*H*-benzocycloheptene-8-carboxamide (3)

To a solution of **15** (110 mg, 394 μmol) in CH_2Cl_2 (3 ml) were added oxalyl chloride (300 μl, 2.37 mmol) and DMF (1 drop) on

ice. The mixture was stirred for 2 h at room temperature. The solvent was evaporated in vacuo. A solution of the residue in THF (1.5 ml) was added dropwise to a solution of **6** (156 mg, 702 μmol) and triethylamine (300 μl, 2.00 mmol) in THF (3 ml) on ice and the reaction mixture was stirred for 2 h at room temperature. The solvent was evaporated in vacuo, and then AcOEt and H_2O were added. The organic layer was washed successively with water and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by column chromatography ($CHCl_3$/MeOH = 19:1) to give **3** (141 mg, 292 μmol, 74%) as a colorless oil. IR (film) ν_{max} cm^{-1}: 3294, 3020, 2925, 2845, 2781, 1651, 1597, 1516, 1406, 1315, 1244, 1138, 810, 754, ^1H NMR ($CDCl_3$) δ: 1.60–1.77 (4H, m), 2.17–2.30 (2H, m), 2.26 (3H, d, J = 133 Hz, $^{13}CH_3N$), 2.42 (3H, d, J = 126 Hz, $^{13}CH_3Ph$), 2.45 (1H, m), 2.75 (2H, t, J = 6.3 Hz), 2.91 (2H, m), 3.39 (2H, td, J = 11.7, 2.7 Hz), 3.62 (2H, d, J = 4.2 Hz), 4.06 (2H, dd, J = 10.2, 3.0 Hz), 7.24–7.36 (6H, m), 7.44–7.63 (6H, m), ^{13}C NMR ($CDCl_3$) δ: 21.1 ($^{13}CH_3Ph$), 28.0, 29.2, 30.4, 34.6, 37.5 ($^{13}CH_3N$), 57.3, 59.5, 67.7, 120.0, 126.7, 126.8, 129.3, 129.5, 129.8, 130.6, 134.3, 134.7, 135.9, 136.9, 137.1, 137.5, 138.0, 139.1, 141.2, 168.1, HRFABMS $(M+H)^+$ calcd for $^{12}C_{30}{}^{13}C_2H_{37}N_2O_2$: 483.2855, found: 483.2924.

The coupling reaction of **17** with **4** using the same procedure as above afforded **3** (36% yield; the spectroscopic data (IR, ^1H NMR, ^{13}C NMR, and MS) were identical as above).

3.14. *N*,*N*-[$^{13}CH_3$,$^{13}CH_3$]-Dimethyl-*N*-[4[[[2-(4-[$^{13}CH_3$]-methylphenyl)-6,7-dihydro-5*H*-benzocycloheptan-8-yl]carbonyl]amino]benzyl]tetrahydro-2*H*-pyran-4-aminium chloride, TAK779 (2)

A solution of **3** (9 mg, 18.6 μmol) and [^{13}C]-methyl iodide (22 mg, 350 μmol) in DMF (1 ml) was stirred for 12 h at room temperature. The solvent was evaporated in vacuo, and AcOEt was added to the residue. The precipitate was filtered, washed with AcOEt and MeOH, and recrystallized from EtOH and AcOEt to give an iodide salt (9 mg, 14.4 μmol) as a red prism. A solution of the iodide salt (9 mg, 14.4 μmol) in MeOH and water was subjected to ion-exchange column chromatography (Dowex, 100–200 mesh, Cl^- form) to give [19, 35, 36-$^{13}C_3$]-labeled TAK779 (**2**) (7 mg, 13.1 μmol, 70%) as a colorless prism. IR (film) ν_{max} cm^{-1}: 3394, 3014, 2964, 2931, 2860, 2767, 2445, 1655, 1595, 1520, 1319, 1248, 1084, 847, 812, 756, ^1H NMR (CD_3OD) δ: 2.01 (2H, m), 2.16 (2H, m), 2.27 (2H, d, J = 12.0 Hz), 2.39 (3H, d, J = 126 Hz, $^{13}CH_3Ph$), 2.68 (2H, t, J = 6.0 Hz), 2.92 (2H, m), 2.99 (6H, dd, J = 144, 3.6 Hz, $(^{13}CH_3)_2N$), 3.52 (2H, t, J = 13.8 Hz), 3.76 (1H, m), 4.17 (2H, dd, J = 11.1, 3.6 Hz), 4.56 (2H, br s), 7.26 (2H, dd, J = 7.8, 4.5 Hz), 7.28 (1H, d, J = 8.1 Hz), 7.45 (1H, s), 7.48 (1H, dd, J = 5.7, 2.1 Hz), 7.53 (2H, d, J = 8.1 Hz), 7.59 (2H, d, J = 6.9 Hz), 7.61 (1H, s), 7.88 (2H, d, J = 8.7 Hz), 8.00 (1H, br s), ^{13}C NMR (CD_3OD) δ: 19.1 ($^{13}CH_3Ph$), 26.1, 27.7, 29.1, 33.4, 45.54 ($^{13}CH_3N$), 45.59 ($^{13}CH_3N$), 45.64 ($^{13}CH_3N$), 57.1, 63.9, 65.5, 68.5, 70.2, 71.2, 120.2, 121.9, 125.5, 125.7, 125.8, 126.6, 126.7, 127.4, 128.7, 128.5, 129.3, 132.8, 134.1, 134.3, 136.3, 136.9, 137.1, 138.5, 140.6, 140.7, 169.7, MALDI-TOFMS $(M-Cl)^+$ calcd for $^{12}C_{30}{}^{13}C_3H_{40}N_2O_2$: 498.301, found: 498.164.

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References and notes

1. (a) Choe, H.; Farzan, M.; Sun, Y.; Sullivan, N.; Rollins, B.; Ponath, P. D.; Wu, L.; MacKay, C. R.; LaRosa, G.; Newman, G.; Gerard, W.; Gerard, N.; Sodroski, J. *Cell* **1996**, *85*, 1135–1148; (b) Doranz, B. J.; Rucker, J.; Smyth, Y.; Samson, R. J.; Peiper, S. C.; Parmentier, M.; Collman, R. G.; Doms, R. W. *Cell* **1996**, *85*, 1149–1158.
2. (a) De Clercq, E. *Biochim. Biophys. Acta* **2002**, *1587*, 258–275; (b) LaBranche, C. C.; Galasso, G.; Moore, J. P.; Bolognesi, D. P.; Hirsch, M. S.; Hammer, S. M. *Antiviral. Res.* **2001**, *50*, 95–115.
3. (a) Baba, M.; Nishimura, O.; Kanzaki, N.; Okamoto, M.; Sawada, H.; Iizawa, Y.; Shiraishi, M.; Aramaki, Y.; Okonogi, K.; Ogawa, Y.; Meguro, K.; Fujino, M. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 5698–5703; (b) Shiraishi, M.; Aramaki, Y.; Seto, M.; Imoto, H.; Nishikawa, Y.; Kanzaki, N.; Okamoto, M.; Sawada, H.; Nishimura, O.; Baba, M.; Fujino, M. *J. Med. Chem.* **2000**, *43*, 2049–2063; (c) Ikemoto, T.; Nishiguchi, A.; Mitsudera, H.; Wakimasu, M.; Tomimatsu, K. *Tetrahedron* **2001**, *57*, 1525–1529.
4. Seibert, C.; Sakmar, T. P. *Curr. Pharm. Des.* **2004**, *10*, 2041–2062.
5. (a) Kazmierski, W.; Bifulco, N.; Yang, H.; Boone, L.; DeAnda, F.; Watson, C.; Kenakin, T. *Bioorg. Med. Chem.* **2003**, *11*, 2663–2676; (b) Zhao, Q.; Ma, L.; Jiang, S.; Lu, H.; Liu, S.; He, Y.; Strick, N.; Neamati, N.; Debnath, K. *Virology* **2005**, *339*, 213–225.
6. Dragic, T.; Trkola, A.; Thompson, D. A. D.; Cormier, E. G.; Kajumo, F. A.; Maxwell, E.; Lin, S. W.; Ying, W.; Smith, S. O.; Sakmar, T. P.; Moore, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5639–5644.
7. Seibert, C.; Ying, W.; Gavrilov, S.; Tsamis, F.; Kuhmann, S. E.; Palani, A.; Tagat, J. R.; Clader, J. W.; McCombie, S. W.; Baroudy, B. M.; Smith, S. O.; Dragic, T.; Moore, J. P.; Sakmar, T. P. *Virology* **2006**, *349*, 41–54.
8. Maeda, K.; Das, D.; Ogata-Aoki, H.; Nakata, H.; Miyakawa, T.; Tojo, Y.; Norman, R.; Takaoka, Y.; Ding, J.; Arnold, G. F.; Arnold, E.; Mitsuya, H. *J. Biol. Chem.* **2006**, *281*, 12688–12698.
9. Kesling, B. D.; Söderberg, B. C.; Gullion, T. *J. Labelled Compd. Radiopharm.* **2000**, *43*, 1059–1068.
10. Kabalka, G. W.; Sastry, U.; Sastry, K. A. R. *J. Organometal. Chem.* **1983**, *259*, 269–274.
11. Gooβen, L. J. *Appl. Organometal. Chem.* **2004**, *18*, 602–604.
12. Ishiyama, T.; Murata, M.; Miyaura, N. *J. Org. Chem.* **1995**, *60*, 7508–7510.
13. (a) Kan, T.; Fukuyama, T. *J. Syn. Org. Chem. Jpn.* **2001**, *59*, 779; (b) Kan, T.; Fukuyama, T. *Chem. Commun.* **2004**, 35; (c) Fukuyama, T.; Jow, C.-K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373–6376.