Supporting Information

Design, Synthesis and SAR of C-3 Benzoic Acid, C-17 Triterpenoid Derivatives. Identification of the HIV-1 Maturation Inhibitor 4-((1R,3aS,5aR,5bR,7aR,11aS,11bR,13aR,13bR)-3a-((2-(1,1-dioxidothiomorpholino)ethyl)amino)-5a,5b,8,8,11a-pentamethyl-1-(prop-1-en-2-yl)-2,3,3a,4,5,5a,5b,6,7,7a,8,11,11a,11b,12,13,13a,13b-octadecahydro-1H-cyclopenta[a]chrysen-9-yl)benzoic acid (GSK3532795, BMS-955176)

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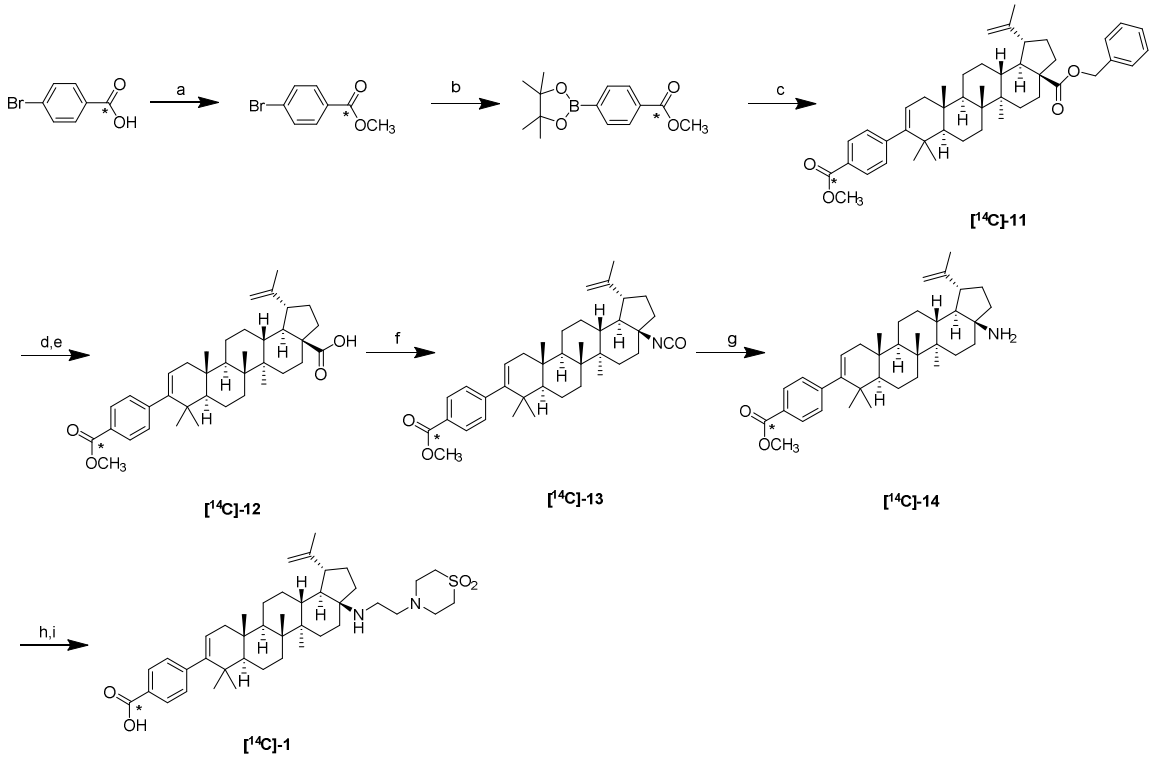
Scheme S1. Synthesis of carbon-14 labeled **1**.



a) MeOH, H_2SO_4, 68 °C; b) $Pd(OAc)_2$, KOAc, THF, bis(pinacolato)diboron, 1,3-bis(2,6-di-i-propylphenyl)-imidazolium chloride, 70°C; c) **10**, $Pd(PPh_3)_4$, Na_2CO_3 1,4-dioxane/H_2O 92 °C; d) *t*-$BuMe_2SiH$, $Pd(OAc)_2$, TEA, DCE, 60°C; e) TBAF/ H_2O, dioxane; rt; f) DPPA, TEA,dioxane, 80 °C g) 12M HCl, THF, 45 °C; h) 4-(2-chloroethyl)thiomorpholine 1,1-dioxide, K_3PO_4/KI, CH_3CN, 80 °C; i) 1,4-dioxane, NaOH, 78 °C

Experimental Procedures for the Synthesis of [^{14}C]-1.

Methyl 4-bromo-[^{14}C]-benzoate.

A mixture of [^{14}C]4-bromobenzoic acid (195 mg, 0.96 mmol) was dissolved in MeOH (8 mL) and H_2SO_4 (0.05 mL, 0.96 mmol) was added. The reaction mixture was heated at 68 °C for 18 h. After cooling to rt, the mixture was concentrated under reduced pressure to remove the MeOH. The crude material was dissolved in H_2O (10 mL), neutralized with 5% $NaHCO_3$ and extracted with CH_2Cl_2 (3 x 25 mL). The combined CH_2Cl_2 extracts were concentrated under reduced pressure and the resulting solids dried under reduced pressure overnight. The HPLC radiochemical purity was determined to be 99%. ^{1}H-NMR (400 MHz, *$CDCl_3$*) δ 7.95 - 7.87 (m, 2H), 7.62 - 7.54 (m, 2H), 3.93 (s, 3H). Methyl 4-bromo-[^{14}C]benzoate (101 mg, 99 % yield) was isolated as a white solid.

Methyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[^{14}C]-benzoate.

A mixture of [^{14}C]methyl 4-bromobenzoate (101 mg, 0.47 mmol), methyl 4-bromobenzoate (100 mg, 0.47 mmol) bis(pinacolato)diboron (330 mg, 1.3 mmol), 1,3-bis(2,6-di-i-propylphenyl)imidazolium chloride (11.8 mg, 0.03 mmol), $Pd(OAc)_2$ (12.5 mg, 0.06 mmol) and KOAc (228 mg, 2.3 mmol) in THF (20 mL) was combined in a flask that was flushed with N_2 and heated at 70 °C. After 6 h, the reaction mixture was cooled to rt, filtered through a plug of Celite which was rinsed with EtOAc. The filtrate was diluted with EtOAc (25 mL) and extracted with H_2O (2 x 25 mL) and 250 mL of saturated NaCl. The EtOAc extracts were concentrated under reduced pressure to yield methyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[^{14}C]benzoate (291 mg, 97 % yield) as a pale yellow solid. ^{1}H-NMR (400 MHz, *$CDCl_3$*) δ 8.05 - 8.00 (m, 2H), 7.90 -

7.84 (m, *J*=8.3 Hz, 2H), 3.92 (s, 3H), 1.36 (s, 13H). The HPLC radiochemical purity was determined to be 88%. Material was used in the next step without additional purification.

[^{14}C]-(1*R*,3a*S*,5a*R*,5b*R*,7a*R*,11a*S*,11b*R*,13b*R*)-benzyl 9-(4-(methoxycarbonyl)phenyl)-5a,5b,8,8,11a-pentamethyl-1-(prop-1-en-2-yl)-2,3,3a,4,5,5a,5b,6,7,7a,8,11,11a,11b,12,13,13a,13b-octadecahydro-1*H*-cyclopenta[a]chrysene-3a-carboxylate ([^{14}C]-11).

To methyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-[^{14}C]benzoate (291 mg, 0.93), methyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (61 mg, 0.23 mmol) and **10** (627 mg, 0.93mmol) was added 1,4-dioxane (8 mL) and H_2O (2 mL). The mixture was stirred under an argon atmosphere for ten minutes (all solids went into solution). A solution of Na_2CO_3 (295 mg, 2.78 mmol) in H_2O (2 mL) was added and the mixture was degassed with argon for 5 minutes. $Pd(PPh_3)_4$ (32 mg, 0.03 mmol) was added, the reaction mixture was degassed with argon for 5 minutes and then heated to 92°C under an argon atmosphere. After 10 h, the mixture was cooled to rt and diluted with EtOAc (325 mL) and H_2O (25 mL). The mixture was filtered through a plug of Celite to remove any solids. The filtrate layers were separated and the aqueous layer extracted with EtOAc (30 mL). The combined EtOAc extracts were concentrated under reduced pressure. The residue was adsorbed on silica gel and the product purified by silica gel flash chromatography using 3% EtOAc in hexanes as eluent to give **[^{14}C]-11** (357 mg, 58 % yield) as a white solid. ^{1}H-NMR (400 MHz, *$CDCl_3$*) δ 7.92 (d, *J*=8.5 Hz, 2H), 7.40 - 7.36 (m, 3H), 7.19 (d,*J*=8.3 Hz, 2H), 5.28 (d, *J*=4.5 Hz, 1H), 5.20 - 5.05 (m, 2H), 4.74 (d, *J*=2.0 Hz, 1H), 4.60 (s, 1H), 3.94 - 3.86 (m, 3H), 2.28 (s, 2H), 2.09 (d, *J*=10.8 Hz, 1H), 1.96 - 1.85 (m, 2H), 1.72 - 1.66 (m, 4H), 1.63 (s, 1H), 1.57 (s, 4H), 1.52 - 1.33 (m, 9H),

1.33 - 1.23 (m, 4H), 1.19 (d, *J*=15.3 Hz, 2H), 0.96 (d, *J*=9.5 Hz, 6H), 0.91 (d, *J*=2.8 Hz, 6H), 0.82 (s, 3H).

[^{14}C]-(1*R*,3a*S*,5a*R*,5b*R*,7a*R*,11a*S*,11b*R*,13a*R*,13b*R*)-9-(4-(methoxycarbonyl)phenyl)-5a,5b,8,8,11a-pentamethyl-1-(prop-1-en-2-yl)-2,3,3a,4,5,5a,5b,6,7,7a,8,11,11a,11b,12,13,13a,13b-octadecahydro-1*H*-cyclopenta[a]chrysene-3a-carboxylic acid ([^{14}C]-12).

To a solution of **[^{14}C]-11** (357 mg, 0.54 mmol) in CH_2Cl_2 (5 mL) was added Et_3N (0.12 mL, 0.86 mmol), *tert*-butyldimethylsilane (0.18 mL, 1.08 mmol) and $Pd(OAc)_2$ (30 mg, 0.13 mmol). The reaction mixture was degassed with argon for 5 minutes and then heated to 60 °C under an argon atmosphere. After 2.5 h, the mixture was cooled to rt, filtered through Celite to remove the catalyst and the solids washed with CH_2Cl_2 (25 mL). The CH_2Cl_2 filtrate was concentrated under reduced pressure and the residue dissolved in 1,4-dioxane (10 mL) and 75 wt% tetrabutylammonium fluoride in H_2O (0.0.3 mL, 0.81 mmol) was added. After stirring overnight at rt, the mixture was partitioned with CH_2Cl_2 (20 mL), 1N HCl (5 mL) and H_2O (20 mL). The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (20 mL). The combined CH_2Cl_2 extracts were concentrated under reduced pressure to give **[^{14}C]-12** (334 mg, 108 % yield) as a white solid. ^{1}H-NMR (400 MHz, *DMSO-d6*) δ 12.10 (s, 1H), 7.88 (d, *J*=8.5 Hz, 2H), 7.24 (d, *J*=8.3 Hz, 2H), 5.24 (d, *J*=4.5 Hz, 1H), 4.70 (d, *J*=2.0 Hz, 1H), 4.57 (s, 1H), 3.84 (s, 3H), 3.33 (s, 2H), 2.97 (td, *J*=10.6, 4.9 Hz, 1H), 2.35 - 2.22 (m, 1H), 2.16 - 2.01 (m, 2H), 1.88 - 1.75 (m, 2H), 1.75 - 1.62 (m, 5H), 1.62 - 1.48 (m, 2H), 1.48 - 1.30 (m, 9H), 1.29 - 1.13 (m, 4H), 1.01 - 0.91 (m, 10H), 0.91 - 0.82 (m, 6H).

[^{14}C]-methyl 4-((1*R*,3a*S*,5a*R*,5b*R*,7a*R*,11a*S*,11b*R*,13a*R*,13b*R*)-3a-isocyanato-5a,5b,8,8,11a-pentamethyl-1-(prop-1-en-2-yl)-2,3,3a,4,5,5a,5b,6,7,7a,8,11,11a,11b,12,13,13a,13b-octadecahydro-1*H*-cyclopenta[a]chrysen-9-yl)benzoate, ([^{14}C]-13).

To a suspension of **[^{14}C]-12** (334 mg, 0.54 mmol) in 1,4-dioxane (6 mL) was added Et_3N (0.1 mL, 0.70 mmol) followed by diphenylphosphoryl azide (0.14 mL, 0.64 mmol). The resulting mixture was heated with stirring at 100 °C for 6 h, cooled to rt and the mixture partitioned with 25 mL of EtOAc and 1N NaOH (10 mL). The layers were separated, the aqueous phase was extracted with EtOAc (25 mL) and the combined EtOAc extracts were concentrated under reduced pressure. The residue was dried under vacuum for 2 h to yield **[^{14}C]-13** (307 mg, 0.54 mmol) as a pale yellow solid. This material was used as-is in the next step.

[^{14}C]-Methyl 4-((1*R*,3a*S*,5a*R*,5b*R*,7a*R*,11a*S*,11b*R*,13a*R*,13b*R*)-3a-amino-5a,5b,8,8,11a-pentamethyl-1-(prop-1-en-2-yl)-2,3,3a,4,5,5a,5b,6,7,7a,8,11,11a,11b,12,13,13a,13b-octadecahydro-1*H*-cyclopenta[a]chrysen-9-yl)benzoate ([^{14}C]-14).

[^{14}C]-13 (307 mg, 0.54 mmol) was dissolved into dry THF (8 mL) and 12M HCl (2.6 mL, 31.0 mmol) was added. The mixture was stirred at rt for 18 h. After drying overnight under vacuum, the gummy solid was azeotroped with EtOH (2 x 10 mL) and finally with Et_2O (10 mL). The residue was dissolved in EtOH (15 mL) and the solution was concentrated under reduced pressure. The resulting solids were dried under vacuum to give **[^{14}C]-14** (497 mg, 94% yield). The HPLC radiochemical purity was determined to be 96.6%. ^{1}H-NMR (400 MHz, *$CDCl_3$*) δ 7.93 (d, *J*=8.3 Hz, 2H), 7.20 (d, *J*=8.3 Hz,

2H), 5.29 (dd, *J*=6.3, 1.8 Hz, 1H), 4.73 (s, 1H), 4.61 (s, 1H), 3.91 (s, 3H), 2.56 (d, *J*=5.0 Hz, 1H), 2.11 (dd, *J*=17.3, 6.5 Hz, 1H), 1.78 - 1.65 (m, 7H), 1.63 - 1.45 (m, 9H), 1.45 - 1.35 (m, 6H), 1.35 - 1.19 (m, 5H), 1.19 - 1.05 (m, 5H), 0.99 (s, 6H), 0.93 (s, 6H).

[^{14}C]-4-((1*R*,3a*S*,5a*R*,5b*R*,7a*R*,11a*S*,11b*R*,13a*R*,13b*R*)-3a-((2-(1,1-dioxidothiomorpholino)ethyl)amino)-5a,5b,8,8,11a-pentamethyl-1-(prop-1-en-2-yl)-2,3,3a,4,5,5a,5b,6,7,7a,8,11,11a,11b,12,13,13a,13b-octadecahydro-1*H*-cyclopenta[a]chrysen-9-yl)benzoic acid hydrochloride ([^{14}C]-1)

A suspension of **[^{14}C]-14** (497 mg, 0.50 mmol), 4-(2-chloroethyl)thiomorpholine 1,1-dioxide (265 mg, 1.34 mmol), K_3PO_4 (456 mg, 2.15mmol) and KI (248 mg, 1.49 mmol) in CH_3CN (20 mL) in a pressure flask was heated at 130 °C. After 48 h, the mixture was cooled to rt and treated with H_2O (25 mL) and 5% $NaHCO_3$ (10 mL). The mixture was extracted with CH_2Cl_2 (3 x 25 mL) and the combined extracts concentrated under reduced pressure to yield the intermediate methyl ester of **[^{14}C]-1**. The crude ester was purified by flash chromatography on silica gel using 20% acetone in CH_2Cl_2. Fractions containing the desired methyl ester of **[^{14}C]-1** were pooled and concentrated under reduced pressure to give the methyl ester of **[^{14}C]-1** (243 mg, 63% yield). To a solution of the methyl ester of **[^{14}C]-1** in dioxane (2 mL) was added 10M NaOH (0.69 mL, 6.9 mmol). The solution was heated at 78°C for 11h, cooled to rt and concentrated under reduced pressure to yield crude **[^{14}C]-1**. The crude product was purified by preparative HPLC (method 8) using UV detection at 254 nm. The pooled collections of the peak at 20 min were concentrated under reduced pressure to dryness. The resulting solids were dried under vacuum to afford **[^{14}C]-1** (120 mg, 47.6 % yield) as a pale yellow solid. The HPLC radiochemical purity was determined to be 99%. ^{1}H-NMR (400 MHz, *DMSO-d6*)

δ 12.86 (br. s., 1H), 7.86 (d, *J*=8.3 Hz, 2H), 7.22 (d, *J*=8.3 Hz, 2H), 6.91 (br. s., 1H), 5.25 (d, *J*=4.5 Hz, 1H), 4.78 (s, 1H), 4.68 (s, 1H), 3.34 (s, 10H), 3.25 - 3.05 (m, 10H), 2.99 (d, *J*=14.1 Hz, 1H), 2.80 (d, *J*=11.3 Hz, 2H), 2.36 - 2.31 (m, 1H), 2.13 - 1.83 (m, 7H), 1.77 - 1.65 (m, 7H), 1.62 (br. s, 1H), 1.57 (br. s, 1H), 1.45 (d, *J*=7.5 Hz, 6H), 1.34 (d, *J*=18.3 Hz, 3H), 1.23 (br. s., 5H), 1.14 (br. s., 1H), 1.09 (s, 4H), 1.03 (t, *J*=3.0 Hz, 4H), 1.00 - 0.94 (m, 3H), 0.93 - 0.82 (m, 7H). The specific activity was determined to be 22.7 uCi/mg (16.5 mCi/mmol, 2.7 mCi).

Specific activity was determined gravimetrically. A weighed sample was dissolved into methanol. An accurate volume was diluted with scintillation cocktail and counted on a Perkin-Elmer 2900 Tricard