SUPPORTING INFORMATION

Synthesis, Radio-Synthesis and in vitro Evaluation of Terminally Fluorinated Derivatives of HU-210 and HU-211 as Novel Candidate PET Tracers

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General Information

$^1$H (400.13 MHz), $^{13}$C (100.58 MHz) and $^{19}$F (376.45 MHz) NMR spectra were recorded on a Bruker ADVANCE III spectrometer. For $^1$H NMR spectra the solvent resonance was employed as the internal standard (CDCl$_3$ $\delta$ = 7.26, CD$_3$OD $\delta$ = 3.31). $^{13}$C NMR spectra were recorded with complete proton decoupling, and the solvent resonance was employed as the internal standard (CDCl$_3$, $\delta$ = 77.0, CD$_3$OD $\delta$ = 49.00). All chemical shifts ($\delta$) are expressed in parts per million and coupling constant ($J$) are given in Hertz. HPLC-MS experiments were performed on an Agilent Technologies 1200 Series HPLC system equipped with a DAD and a 6120 MS detector composed by an ESI ionization source and a Single Quadrupole mass selective detector. Specific optical rotation measurements were performed on an AA-65 Angular Scale automatic polarimeter (Optical Activity Limited) with a 1dm cell at the sodium D line. All reactions were carried out in oven- or flame-dried glassware under nitrogen atmosphere, unless stated otherwise. All commercially available reagents were used as received. Reactions were magnetically stirred and monitored by TLC on silica gel (60 F254 pre-coated glass plates, 0.25 mm thickness). Visualization was accomplished by irradiation with a UV lamp and/or staining with a ceric ammonium molybdate or KMnO$_4$ solution. Flash chromatography was performed on silica gel (60 Å, particle size 0.040–0.062 mm). Yields refer to chromatographically and spectroscopically pure compounds, unless stated otherwise.

HPLC analyses of radioactive compounds were performed using a Shimadzu HPLC system equipped with a SPD-M20A Prominence DAD UV detector and NaI radio-detector (Berthold Technologies). Semi-prep HPLC purification of radioactive compounds were performed using a lead shielded Shimadzu semiprep HPLC system equipped with a SPD-M20A Prominence DAD UV detector and NaI radio-detector (Berthold Technologies).

RadioTLC were performed using a Raytest miniGITA RadioTLC scanner. The dose calibrators used to measure doses were CAPINTEC CRC 15R and CAPINTEC CRC 15PET.

$[^{18}$F]-Fluoride was produced on a cyclotron by proton bombardment of 97% enriched $[^{18}$O]H$_2$O (Cambridge Isotope Laboratories, Inc.) by the $^{18}$O(p,n)$^{18}$F nuclear reaction. The silver target (1.1ml) was pressurised to 600psi and irradiated with 11MeV protons produced by the CTI/SIEMENS RDS-111 cyclotron at the John Mallard Scottish PET Centre in Aberdeen. Irradiation with a beam current of 29 $\mu$A for 7 minutes was typically used (3.9 GBq). At the end of bombardment (EOB) the target was unloaded within 5 min using argon gas.
(1R,5S)-2-[(2,2-dimethylpropoxy)methyl]-6,6-dimethylbicyclo[3.1.1]hept-2-ene (RM1)\(^1,2\)

To a solution of a commercially available (1R,5S)-myrtenol (5.0 g, 32.8 mmol, 1 eq, ≥95% ee) in DCM (30 mL) and pyridine (30 mL) at 0°C was added dropwise trimethylacetyl chloride (5 mL, 41.0 mmol, 1.3 eq). The reaction was stirred for 4 h at 0°C, diethyl ether (100 mL) was added and the mixture was washed with 10% aqueous hydrochloric acid, saturated aqueous bicarbonate solution, brine, dried (Na\(_2\)SO\(_4\)) and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/ EtOAc 1:1) to give compound RM1 (6.4 g, 83%) as a colorless oil. 

\[ [\alpha]_D^{27} = -30 \text{ (c = 1.6 CHCl}_3). \]

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\): 0.87 (s, 3 H), 1.17-1.22 (m, 2 H), 1.22 (s, 9 H), 1.31 (s, 3 H), 2.13 (dd, 2H, \(J = 1.3, 5.6\) Hz), 2.31 (q, 2H, \(J = 18.0\) Hz), 2.42 (dt, 2H, \(J = 5.6, 8.7\) Hz), 4.44 (ddd, 1H, \(J = 1.6, 3.2, 12.5\) Hz), 4.49 (ddd, 1H, \(J = 1.6, 3.2, 12.5\) Hz), 5.57 (dq, 1 H, \(J = 1.4, 4.4\) Hz).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\): 21.1, 26.1, 27.2 (x 3 C), 31.2, 31.4, 38.0, 38.8, 40.7, 43.5, 66.7, 121.0, 143.3, 178.1. 

MS (ESI) for C\(_{15}\)H\(_{25}\)O\(_2\): \(m/z\) calculated 237.2 [M+H]\(^+\); \(m/z\) found (relative intensity) 237.2 [M+H]\(^+\) (100).

(1R,5S)-4-[(2,2-dimethylpropoxy)methyl]-6,6-dimethylbicyclo[3.1.1]hept-3-en-2-one (RM2)\(^1,2\)

To a suspension of chromium trioxide (16.8 g, 168.0 mmol, 12 eq) in dry DCM (130 mL) at -20°C was added 3,5-dimethylpyrazole (16.1 g, 168.0 mmol, 12 eq) in small portions. After stirring for 15 min a solution of RM1 (3.3 g, 14.0 mmol, 1 eq) in dichloromethane (35 mL) was added and the mixture was stirred at -20°C for 4 h. Aqueous sodium hydroxide solution (5 M, 15 mL) was added and the mixture was stirred at 0°C for 1 h. The organic phase was separated, washed with 10% aqueous HCl, brine, dried (Na\(_2\)SO\(_4\)) and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/ EtOAc 8:2) to give compound RM2 (1.8 g, 50%) as a colorless oil. 

\[ [\alpha]_D^{27} = -150 \text{ (c = 1.4 CHCl}_3). \]

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\): 1.05 (s, 3 H), 1.27 (s, 9 H), 1.54 (s, 3 H), 2.16 (d, 1 H, \(J = 9.3\) Hz), 2.46 (td, 1 H, \(J = 1.4, 5.9\) Hz), 2.72 (td, 1 H, \(J = 1.7, 5.9\) Hz), 2.89 (dt, 2 H, \(J = 5.5, 9.3\) Hz), 4.71 (dd, 1H, \(J = 1.9, 16.6\) Hz), 4.76 (dd, 1H, \(J = 1.9, 16.6\) Hz), 5.89 (m, 1 H).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\): 22.0, 26.4, 27.1 (x 3 C), 38.7, 40.6, 45.3, 53.9, 58.0, 63.9, 119.1, 165.9, 172.2, 202.3.

S3
MS (ESI) for C₁₅H₂₂O₃: m/z calculated 251.2 [M+H]⁺; m/z found (relative intensity) 251.2 [M+H]⁺ (100).

(1R,5S)-4-[(2,2-dimethylpropoxy)methyl]-6,6-dimethylbicyclo[3.1.1]hept-3-en-2-ol (2a)¹,²
To a solution of ketone RM₂ (500 mg, 2.0 mmol, 1 eq) in dry THF (4 mL) at 0°C was added lithium tri-tert-butoxyaluminium hydride (508 mg, 2.0 mmol, 1 eq). The mixture was allowed to warm to room temperature and stirred for a further 4 h. The reaction was quenched with saturated aqueous ammonium chloride solution (5 mL) and extracted with diethyl ether. The combined organic extracts were dried (Na₂SO₄) and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/ EtOAc 8:2) to give alcohol 2a (454 mg, 90%, diastereomeric mixture) as a colorless oil.

Rᵣ 0.40 (Hexane/EtOAc 8:2).

[α]ᵢ₂⁰ : 0 (c = 1.0 CHCl₃).

¹H NMR (400 MHz, CDCl₃) δ: 1.11 (s, 3 H), 1.24 (s, 9 H), 1.38 (s, 3 H), 1.59 (bs, OH), 2.13 (t, 1 H, J = 5.2 Hz), 2.36 (ddd, 1 H, J = 2.6, 5.7, 8.1 Hz), 2.54 (dt, 1 H, J = 5.8, 9.2 Hz), 4.52 (m, 3 H), 5.68 (bs, 1 H).

¹³C NMR (100 MHz, CDCl₃) δ: 22.9, 26.7, 27.2 (x 3 C), 35.6, 38.8, 38.9, 44.0, 48.3, 65.8, 72.0, 122.3, 145.1, 178.2.

MS (ESI) for C₁₅H₂₄O₃: m/z calculated 253.2 [M+H]⁺; m/z found (relative intensity) 253.2 [M+H]⁺ (100).

(+)-(1S)-Myrtenol⁰,³
A solution of selenium dioxide (2.4g, 22.0 mmol, 0.6 eq) in ethanol (36 mL) was added over a period of 1h to (+)-α-pinene (5.0 g, 36.7 mmol, 1 eq, ≥99% ee). The mixture was heated to 75°C and was stirred for 2 h. The mixture was cooled, the precipitate of selenium was filtered off, and the filtrate was evaporated under reduced pressure to remove volatile products. The crude mixture was added under stirring to a solution of NaBH₄ in (138.8 mg, 3.67 mmol, 0.1 eq) in ethanol (70 mL) and the mixture was stirred for 1 h. A saturated aqueous solution of NH₄Cl was added and the mixture was extracted with diethyl ether (3 x 50 mL). The organic phase was washed with water, dried over Na₂SO₄, and the solvent was evaporated under reduced pressure. The resulting (+)-(1S)-Myrtenol was used crude, without further purification.

MS (ESI) for C₁₀H₁₆O: m/z calculated 153.1 [M+H]⁺; m/z found (relative intensity) 153.1 [M+H]⁺ (100).
[(1S,5R)-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl]methyl 2,2-dimethylpropanoate (SM1)\(^{1,2}\)

To a solution of crude (+)-(1S)-Myrtenol (1 g, 6.6 mmol, 1 eq) in DCM (6 mL) and pyridine (6 mL) at 0°C was added dropwise trimethylacetyl chloride (1 mL, 8.2 mmol, 1.3 eq). The reaction was stirred for 4 h at 0°C, diethyl ether (20 mL) was added and the mixture was washed with 10% aqueous hydrochloric acid, saturated aqueous bicarbonate solution, brine, dried (Na\(_2\)SO\(_4\)) and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/ EtOAc 1:1) to give compound SM1 (935 mg, 60%) as a colorless oil.

R\(_f\) 0.40 (Hexane/EtOAc 1:1).

\([\alpha]_D^{27}: +31\) (c = 1.6 CHCl\(_3\)).

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\): 0.82 (s, 3 H), 1.19 (m, 2 H), 1.17 (s, 9 H), 1.26 (s, 3 H), 2.20 (dd, 2H, \(J = 1.3, 5.6\) Hz), 2.33 (q, 2H, \(J = 18.0\) Hz), 2.38 (dt, 2H, \(J = 5.6, 8.7\) Hz), 4.39 (ddd, 1H, \(J = 1.6, 3.2, 12.5\) Hz), 4.45 (ddd, 1H, \(J = 1.6, 3.2, 12.5\) Hz), 5.52 (dq, 1H, \(J = 1.4, 4.4\) Hz).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\): 21.1, 26.1, 27.2 (x 3 C), 31.2, 31.4, 38.0, 38.8, 40.7, 43.5, 66.7, 121.0, 143.3, 178.1.

MS (ESI) for C\(_{15}\)H\(_{25}\)O\(_2\): \(m/z\) calculated 237.2 [M+H]+; \(m/z\) found (relative intensity) 237.2 [M+H]+ (100).

[(1S,5R)-6,6-dimethyl-4-oxobicyclo[3.1.1]hept-2-en-2-yl]methyl 2,2-dimethylpropanoate (SM2)\(^{1,2}\)

To a suspension of chromium trioxide (2.4 g, 24.0 mmol, 12 eq) in dry DCM (20 mL) at -20°C was added 3,5-dimethylpyrazole (2.3 g, 24.0 mmol, 12 eq) in small portions. After stirring for 15 min a solution of SM1 (500 mg, 2.0 mmol, 1 eq) in dichloromethane (5 mL) was added and the mixture was stirred at -20°C for 4 h. Aqueous sodium hydroxide solution (5 M, 5 mL) was added and the mixture was stirred at 0°C for 1 h. The organic phase was separated, washed with 10% aqueous HCl, brine, dried (Na\(_2\)SO\(_4\)) and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/ EtOAc 8:2) to give compound SM2 (240 mg, 48%) as a colorless oil.

R\(_f\) 0.42 (Hexane/EtOAc 8:2).

\([\alpha]_D^{27}: +152\) (c = 1.5 CHCl\(_3\)).

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\): 1.05 (s, 3 H), 1.27 (s, 9 H), 1.54 (s, 3 H), 2.15 (d, 1 H, \(J = 9.3\) Hz), 2.46 (td, 1 H, \(J = 1.4, 5.9\) Hz), 2.72 (td, 1 H, \(J = 1.7, 5.9\) Hz), 2.89 (dt, 2 H, \(J = 5.5, 9.3\) Hz), 4.72 (dd, 1H, \(J = 1.9, 16.6\) Hz), 4.75 (dd, 1H, \(J = 1.9, 16.6\) Hz), 5.89 (m, 1 H).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\): 22.0, 26.4, 27.1 (x 3 C), 38.7, 40.6, 45.3, 53.9, 58.0, 63.9, 119.1, 165.9, 172.2, 202.3.

MS (ESI) for C\(_{15}\)H\(_{22}\)O\(_3\): \(m/z\) calculated 251.2 [M+H]+; \(m/z\) found (relative intensity) 251.2 [M+H]+ (100).

[(1S,5R)-4-hydroxy-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl]methyl 2,2-dimethylpropanoate (2b)

To a solution of ketone SM2 (250 mg, 1.0 mmol, 1 eq) in dry THF (2 mL) at 0°C was added lithium tri-tert-butoxyaluminium hydride (254 mg, 1.0 mmol, 1 eq). The mixture was allowed to warm to room temperature and stirred for a further 4 h. The reaction was quenched with saturated aqueous ammonium chloride solution (2.5 mL) and extracted with diethyl ether. The combined organic extracts were dried (Na\(_2\)SO\(_4\)) and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/ EtOAc 8:2) to give alcohol 2b (229 mg, 91%, diastereomeric mixture) as a colorless oil.
(1R,5S)-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl]methyl acetate (RM3)

To a solution of a commercially available (1R,5S)-myrtenol (2.0 g, 13.16 mmol, 1 eq, ≥95% ee) in DCM 10 mL was added dropwise acetic anhydride (1.37 mL, 14.47 mmol, 1.1 eq), Et₃N (7.3 mL, 52.63 mmol, 4 eq) and DMAP (112 mg, 0.92 mmol, 0.07 eq). The reaction was stirred for 2 h at room temperature. The mixture was washed with 10% aqueous hydrochloric acid, saturated aqueous bicarbonate solution, brine, dried (Na₂SO₄) and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/ EtOAc 1:1) to give compound RM3 (2.1 g, 80%) as a colorless oil.

\[ R_f 0.41 \text{ (Hexane/EtOAc 1:1).} \]

\[ [\alpha]_{D}^{27} 0 \text{ (c = 1.2 CHCl}_3). \]

\[ ^1H \text{ NMR (400 MHz, CDCl}_3) \delta: 1.03 (s, 3 H), 1.16 (s, 9 H), 1.31 (s, 3 H), 2.05 (t, 1 H, } J = 5.2 \text{ Hz), 2.27 (ddd, 1 H, } J = 2.6, 5.7, 8.1 \text{ Hz), 2.37 (bs, OH), 2.44 (dt, 1 H, } J = 5.8, 9.2 \text{ Hz), 4.40-4.43 (m, 3 H), 5.61 (bs, 1 H).} \]

\[ ^13C \text{ NMR (100 MHz, CDCl}_3) \delta: 22.9, 26.7, 27.2 (x 3 C), 35.6, 38.8, 38.9, 44.0, 48.3, 65.8, 72.0, 122.3, 145.1, 178.2. \]

MS (ESI) for C₁₅H₂₄O₃: m/z calculated 253.2 [M+H]⁺; m/z found (relative intensity) 253.2 [M+H]⁺ (100).

(1R,5S)-6,6-dimethyl-4-oxobicyclo[3.1.1]hept-2-en-2-yl]methyl acetate (RM4)

To a solution of RM3 (1.0 g, 5.11 mmol, 1 eq, in n-butyl butanoate (10 mL) at -20°C diacetoxyiodobenzene (4.8 g, 15.3 mmol, 3 eq) and K₂CO₃ (350 mg, 2.5 mmol, 0.5 eq) were added. The mixture was vigorously stirred and a solution of tert-butyl hydroperoxide (3.6 mL, 18.4 mmol, 4 eq) was added dropwise over 30 minutes. The resulting solution was stirred for 8 hours at -20°C then was allowed to warm to room temperature, filtered to remove the solid and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/ EtOAc 1:1) to give compound RM4 (319 mg, 30%) as a colorless oil.

\[ R_f 0.38 \text{ (Hexane/EtOAc 8:2).} \]
$\alpha_D^{27}$: -146 ($c = 1.0 \text{ CHCl}_3$).

$^1$H NMR (400 MHz, CDCl$_3$) δ: 1.03 (s, 3 H), 1.53 (s, 3 H), 2.13 (m, 1 H), 2.15 (s, 3 H), 2.36 (td, 1 H, $J = 1.3, 5.9$ Hz), 2.71 (td, 1 H, $J = 1.7, 5.9$ Hz), 2.88 (dt, 1 H, $J = 5.5, 9.3$ Hz), 4.69 (dd, 1H, $J = 1.9, 16.6$ Hz), 4.78 (dd, 1H, $J = 1.9, 16.6$ Hz), 5.88 (m, 1 H).

$^{13}$C NMR (100 MHz, CDCl$_3$) δ: 20.5, 21.9, 26.4, 40.7, 45.3, 54.1, 58.0, 64.0, 119.3, 165.7, 170.0, 202.7.

MS (ESI) for C$_{12}$H$_{16}$O$_3$: $m/z$ calculated 209.1 [M+H]$^+$; $m/z$ found (relative intensity) 209.1 [M+H]$^+$ (100).

(1R,5S)-4-hydroxy-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-ylmethyl acetate(2c)$^{1,2}$

To a solution of ketone R$M^4$ (305 mg, 1.46 mmol, 1 eq) in dry THF (6 mL) at 0°C was added lithium tri-tert-butoxyaluminium hydride (599 mg, 2.2 mmol, 1.5 eq). The mixture was allowed to warm to room temperature and stirred for a further 4 h. The reaction was quenched with saturated aqueous ammonium chloride solution (5 mL) and extracted with diethyl ether. The combined organic extracts were dried (Na$_2$SO$_4$) and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/ EtOAc 8:2) to give alcohol $2c$ (158 mg, 75%, diastereomeric mixture) as a colorless oil.

$R_f$ 0.35 (Hexane/EtOAc 7:3).

$\alpha_D^{27}$: 0 ($c = 1.1 \text{ CHCl}_3$).

$^1$H NMR (400 MHz, CDCl$_3$) δ: 1.05 (s, 3 H), 1.34 (m, 1 H), 1.36 (s, 3 H), 1.76 (bs, OH), 2.06 (s, 3H) 2.10 (m, 1 H), 2.31 (m, 1H), 2.50 (ddd, 1 H, $J = 5.4, 6.2, 9.2$ Hz), 4.49 (m, 3 H), 5.65 (bs, 1 H).

$^{13}$C NMR (100 MHz, CDCl$_3$) δ: 20.9, 22.7, 26.7, 35.8, 38.9, 44.1, 48.4, 65.8, 73.0, 122.3, 145.1, 178.8.

MS (ESI) for C$_{12}$H$_{19}$O$_3$: $m/z$ calculated 210.1 [M+H]$^+$; $m/z$ found (relative intensity) 210.1 [M+H]$^+$ (100).

Synthesis of 1: Unsuccessful Approaches

The first retrosynthetic approach to the fluorinated building block 1 (Scheme S1) was initially based on two steps: the insertion of the alkyl side chain (A) and the introduction of the gem-dimethyl group (B).

Scheme S1 First retrosynthetic approach to 1

Step A: insertion of the alkyl side chain

In our first synthetic attempt (Scheme S2) the commercially available 3,5-dimethoxybenzoyl chloride A1 was transformed into the corresponding Weinreb amide A2 which was directly subjected to the reaction with the appropriate Grignard reagent (prepared from the 6-tert-butyldimethylsilyl hexyl bromide). Unfortunately, despite the different conditions tried, we were unable to isolate the desired ketone A3 whereas we obtained only the by-product A4 derived from the undesired Wurtz coupling.5

Scheme S2 Reagents and conditions: (i) N-methoxymethylamine hydrochloride, TEA, DCM, 0°C, on, 90%, (ii) 6-tert-butyldimethylsilyl hexyl bromide, Mg(0), THF, 0°C, 6 h.

We, therefore, changed strategy, planning to synthesize the saturated ketone A5 (Scheme S3) and then to hydrogenate it into the target compound A3. We explored both the Sonogashira reaction and the direct addition of terminal alkyne. Unfortunately, the palladium catalyzed cross coupling between the acetyl chloride A6 and the TBDMS-protected alkyne A7 gave unsatisfactory yields (20%) while the direct alkylation of the Weinreb amide A2 with the same alkyne A7 by means of nBuLi, occurred in good yield (80%).
**Scheme S3** Reagents and conditions: (i) CuI (2%), PdCl$_2$(PPh$_3$)$_2$ (0.4%), TEA, rt, 12h, 20%; (ii) nBuLi, THF, -78 °C, 3h, 84%; (iii) 10% Pd, EtOAc, H$_2$, rt, 2h, 95%.

**Step B: insertion of the gem-dimethyl group**

In order to insert the gem-dimethyl moiety in A3 to give the target B1 we opted for the Reetz reaction, inspired by the various successful examples present in the literature on similar substrates (**Scheme S4**). Unfortunately, A3 proved to be unsuitable for this procedure.

**Scheme S4** Reagents and conditions: (i) TiCl$_4$, Zn(CH$_3$)$_2$, DCM, -30 °C to rt, 6 h (and plenty of other different conditions).

In fact, despite countless attempts using different reaction temperatures, organozinc reagents, solvents, Lewis acids and reaction times, we were able to isolate only complex mixtures of products and the undesired alcohol B2. We then decided to change the protecting group on the terminal OH trying both the benzyl and the PMP group that are known to tolerate acidic conditions$^6$ (**Scheme S5**).
**BENZYL PROTECTION**

\[ \text{B2} \xrightarrow{i} \text{Ph} \xrightarrow{\text{OCl}_2} \text{NH} \xrightarrow{\text{B3}} \text{B4} \]

**PMP PROTECTION**

\[ \text{B5} \xrightarrow{\text{ii}} \text{B6} \xrightarrow{\text{iii}} \text{B7} \xrightarrow{\text{iv}} \text{B8} \]

Scheme S5: Reagents and conditions: (i) CF₃SO₂H, DCM, rt, 3h, 90%; (ii) 4-methoxyphenol, PPh₃, DIAD, THF, rt, 2h, 70%; (iii) nBuLi, THF, -78 °C, 3h, 85%; (iv) 10% Pd, EtOAc, H₂, rt, 3h, 95%.

The benzyl group was introduced by means of the reaction between alcohol B2 and the freshly synthesised benzyl trichloroacetimidate B3, to give compound B4. The PMP group was introduced on the alcohol B5 by means of the Mitsunobu reaction, subsequently, the resulting alkyne B6, was subjected to the coupling with the Weinreb amide A2 in order to give ketone B7 which was hydrogenated to provide the saturated compound B8. Unfortunately, even in this case, the Reetz reaction both using B4 and B8 failed and the alcohol B2 was the only isolated product.

At this point we decided to invert the synthetic pathways (Scheme S6), introducing the terminal fluorine atom before the gem-dimethyl group, in order to perform the Reetz reaction on the fluorinated derivative B9. Even if the fluorination reaction occurred in excellent yields and we were able to obtain compound B9 effectively, the subsequent Reetz reaction once again did not work. Even in this scenario, performing the Reetz reaction under different conditions did not lead to the formation of the desired molecule B10 but only to a complex mixture of cyclic and linear products.

Scheme S6: Reagents and conditions: (i) DAST, THF, 2h, 84%; (ii) TiCl₄, Zn(CH₃)₂, DCM, -30 °C to rt, 6 h (and plenty of other different conditions).

**Conclusion**

Due to the inability to introduce the geminal dimethyl function employing the Reetz reaction we decided to change our retrosynthetic approach as described in the main paper.

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Chiral HPLC Purification of (-)-(R,R)-HU-210F and (+)-(S,S)-HU-211F

The determination of the enantiomeric purity of the cannabinoids mimics (-)-(R,R)-HU-210F and (+)-(S,S)-HU-211F was performed by HPLC. The compounds were first injected separately into the HPLC, then together as a mixture, in order to establish the retention time of each enantiomer and confirm the separation of the two peaks and thus the efficiency of the analytical method.

The analytical HPLC was performed on an amylose-based analytical chiral column ChiralPak AD-I, 250x4.6mm, 5μm particle size (Daicel Ltd), as mobile phase a mixture of Hexane : IPA (96%-4% v/v) was used, in isocratic condition and with 1mL/min of flow rate (as reported previously for the separation of HU-210 and HU-211). The analytical HPLC analyses showed that compound (-)-(R,R)-HU-210F has a retention time of 40.2 minutes and an ee of about 97:3 while compound (+)-(S,S)-HU-211F has a retention time of 27.5 minutes and an ee of about 99:1. The enantiomeric excess calculated by HPLC are consistent with the enantiomeric purity of the commercially available starting materials: for (-)-(R,R)-HU-210F we started from (1R,5S)-myrtenol ≥95% ee and for (+)-(S,S)-HU-211F we started from (+)-α-pinene ≥99 % ee.

In order to perform the biological tests we decided to purify both the enantiomers via HPLC. The HPLC separations were performed on an amylose-based Semi-prep. chiral column ChiralPak AD-I, 250x20mm, 5μm particle size (Daicel Ltd), as mobile phase a mixture of Hexane : IPA (92%-8% v/v) was used, in isocratic condition and with 5mL/min of flow rate. Using the Semi-prep. conditions (-)-(R,R)-HU-210F showed a retention time of 14.2 minutes, while (+)-(S,S)-HU-211F 10.6 minutes. After the separation, an HPLC spectra of the two compounds (and of a mixture of them) were recorded to confirm theirs enantiopurity.

Radio synthesis of (-)-(R,R)-[¹⁸F]HU210

The radiosynthesis of [¹⁸F]HU210F was carried out using a remotely controlled synthesis module Eckert&Ziegler ModularLab synthesizer (Figure S1). Prior to the EOB, vial PO4 was filled with KH₂PO₄ aqueous solution (0.5 mL, 10 mg/mL), and vial K222 was filled with Kryptofix 2.2.2 (15 mg) in dry acetonitrile (1 mL). Vial ACN was filled with 1 mL of dry acetonitrile and vial PREC was filled with precursor 14b (7.7 mg) dissolved in 1 mL of dry acetonitrile.

After the EOB [¹⁸F]-fluoride was unloaded and transferred with argon gas to the synthesis module, which was contained in a lead shielded hot cell. The [¹⁸F]-fluoride was subsequently isolated using a CHROMAFIX anion exchange cartridge (Macherey Nagel, Germany) and then eluted into the reaction vessel with the dipotassium hydrogen orthophosphate solution (vial PO4). The solution in vial K222 was added to the reaction vessel and the azeotropic mixture of water and acetonitrile was evaporated under vacuum using a stream of helium. The content of the vial ACN was then added and the mixture was evaporated under vacuum using a stream of helium to ensure formation of the dried complex [K/K222]¹⁸F. Precursor 14b in vial PREC was then added and the reaction mixture was heated at 100°C for 15 minutes to produce the fluorinated desired product [¹⁸F]HU210 (Radiochemical conversion by RadioHPLC of 6%, Figure S2).
HPLC purification of the crude mixture was attempted. An aliquot (100 μL, 120 MBq) of the crude mixture was purified by semipreparative HPLC (Phenomenex Luna C18(2) column 10x250 mm 100Å 5 μm) using a linear gradient reported in Table S1, affording 4.65 Mbq of [18F]HU210; the collected peak was contaminated by approx. 20% of unreacted [18F]fluoride (Figure S3, black trace).

**Table S1.** Linear gradient for Radio-HPLC analysis and purification

<table>
<thead>
<tr>
<th>Time [min]</th>
<th>% H2O</th>
<th>% ACN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Identity of the product was confirmed by comparison with cold reference (Figure S3). (–)-(R,R)-[18F]HU210 production was achieved using a non-optimised procedure.

**Figure S2.** Radio-HPLC chromatogram of crude mixture ([18F]HU210 Rt=14.546 min)

Since precursor 14b (Rt 16.5 min) and desired product (Rt 14.5 min) had a good separation, HPLC purification of the crude mixture was attempted. An aliquot (100 μL, 120 MBq) of the crude mixture was purified by semipreparative HPLC (Phenomenex Luna C18(2) column 10x250 mm 100Å 5 μm, 5 mL/min) using a linear gradient reported in Table S1, affording 4.65 Mbq of [18F]HU210; the collected peak was contaminated by approx. 20% of unreacted [18F]fluoride (Figure S3, black trace).

**Figure S3.** Radio-HPLC chromatogram of purified [18F]HU210 Rt=14.570 (black trace) and cold reference HU210F Rt 14.600 min (red trace)
$^1$H NMR of RM1
$^{13}$C NMR of $RM1$
$^1$H NMR of RM2
$^1$H NMR of 2a
$^{13}$C NMR of 2a
$^1$H NMR of SM1
$^{13}$C NMR of SM1
$^1$H NMR of SM2
$^{13}$C NMR of SM2
$^1$H NMR of 2b
$^{13}$C NMR of 2b
$^1$H NMR of RM3
$^{13}\text{C NMR of RM3}$
$^1$H NMR of RM4
$^{13}$C NMR of RM4
$^1$H NMR of 2c
$^{13}$C NMR of 2c
$^1$H NMR of 4
$^{13}$C NMR of 5
$^1$H NMR of 7
$^{13}$C NMR of 7
$^1$H NMR of 8
$^{13}$C NMR of 8
$^1$H NMR of 9
$^{13}$C NMR of 9
$^1$H NMR of 1
$^{19}$F NMR of 1
$^{13}$C NMR of 1
$^1$H NMR of 10a
$^{19}$F NMR of 10a
$^{13}$C NMR of 10a
$^1$H NMR of (-)-(R,R)-HU210F
$^{19}$F NMR of (-)-(R,R)-HU210F
$^{13}$C NMR of (-)-(R,R)-HU210F
\text{\textsuperscript{1}H NMR of 10b}
$^{19}$F NMR of 10b
$^{13}$C NMR of 10b
$^1$H NMR of (+)-(S,S)-HU211F
$^{19}$F NMR of (+)-(S,S)-HU211F
$^{13}$C NMR of (+)-(S,S)-HU211F
$^1$H NMR of 11
$^{13}$C NMR of 11
$^1H$ NMR of 12
$^{13}$C NMR of 12
$^1$H NMR of 13
$^{13}$C NMR of 13
$^1$H NMR of 14a
$^{13}$C NMR of 14a
$^1$H NMR of 14b
$^{13}$C NMR of 14b
ChiralPak AD-I, 250x4.6mm, 5µm; Hexane : IPA (96:4); isocratic condition; 1mL/min.
ChiralPak AD-I, 250x4.6mm, 5μm; Hexane : IPA (96:4); isocratic condition; 1mL/min.
ChiralPak AD-I, 250x4.6mm, 5μm; Hexane : IPA (96:4); isocratic condition; 1mL/min.
ChiralPak AD-I Semi-prep. 250x20mm, 5μm; Hexane : IPA (92:8); isocratic condition; 5mL/min.
ChiralPak AD-I Semi-prep. 250x20mm, 5μm; Hexane : IPA (92:8); isocratic condition; 5mL/min.
ChiralPak AD-I Semi-prep. 250x20mm, 5µm; Hexane : IPA (92:8); isocratic condition; 5mL/min.