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Synthesis of a non-peptidic PET tracer designed for $\alpha_5\beta_1$ integrin receptor

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Arginine–glycine–aspartic acid (RGD)-containing peptides have been traditionally used as PET probes to noninvasively image angiogenesis, but recently, small selective molecules for $\alpha_5\beta_1$ integrin receptor have been developed with promising results. Sixty-one antagonists were screened, and *tert*-butyl (S)-3-(2-((3R,5S)-1-(3-(1-(2-fluoroethyl)-1H-1,2,3-triazol-4-yl)propanoyl)-5-((pyridin-2-ylamino)methyl)pyrrolidin-3-yloxy)acetamido)-2-(2,4,6-trimethylbenzamido)propanoate (FPMt) was selected for the development of a PET tracer to image the expression of $\alpha_5\beta_1$ integrin receptors. An alkynyl precursor (PMt) was initially synthesized in six steps, and its radiolabeling was performed according to the azide–alkyne copper(II)-catalyzed Huisgen's cycloaddition by using 1-azido-2-[^{18}F]fluoroethane ([^{18}F]12). Different reaction conditions between PMt and [^{18}F]12 were investigated, but all of them afforded [^{18}F]FPMt in 15 min with similar radiochemical yields (80–83%, decay corrected). Overall, the final radiopharmaceutical ([^{18}F]FPMt) was obtained after a synthesis time of 60–70 min in 42–44% decay-corrected radiochemical yield.

Keywords: $\alpha_5\beta_1$ integrin receptor; PET, 1-azide-2-[^{18}F]fluoroethane; click chemistry; peptido-mimetic

Introduction

Integrins are an extensive group of transmembrane cell adhesion receptors, composed by noncovalently linked α and β subunits.[1] They mediate cell migration and cell proliferation by the connection of the extracellular matrix with the intracellular cytoskeleton.[2] Activation of the integrins by ligand binding promotes cell proliferation, migration, and survival, but unligated or antagonized integrins may activate 'integrin-mediated death'.[3] Integrins are of crucial support for the formation of capillaries and angiogenesis in physiological and pathological processes,[4] and three of them ($\alpha_v\beta_3$, $\alpha_v\beta_5$, and $\alpha_5\beta_1$) have a prominent role for the development of a new vascular system.[5,6]

The involvement of these integrins in many diseases such as tumor, thrombosis, cardiovascular, and inflammatory diseases makes them an appealing target for the development of antiangiogenic therapies.[7,8] Of them, $\alpha_v\beta_3$ is the most targeted integrin, and several antagonists such as monoclonal antibodies[9,10] and arginine–glycine–aspartic acid (RGD)-based peptides[11] are currently in clinical trials.[7,12,13] Moreover, radiolabeled analogs of the RGD peptides have been evaluated in preclinical and clinical studies, as tracers, to visualize angiogenesis in growing tumors.[14,15]

""In the last years, integrin $\alpha_5\beta_1$ has gained great interest, not only because of its involvement in tumor angiogenesis but also for its fundamental role in brain angiogenesis.[16] Recently, several small molecules have been developed to selectively antagonize this integrin receptor.[17–19] The promising biological results obtained have enhanced the interest in non-α_v integrins as target for new therapies and for imaging to monitor tumor angiogenesis and neurovascular remodeling after ischemic stroke.[16,20]

In this manuscript, we advance a novel nonpeptidic PET tracer designed to image the $\alpha_5\beta_1$ integrin receptor. We report herein a reliable and reproducible procedure to radiolabel this pyrrolidine derivative that relies on the following: (i) the preparation and purification of a widely used radiolabeled synthon 1-azido-2-[^{18}F] fluoroethane and (ii) its subsequent conjugation to our alkynyl precursor (PMt) via the Huisgen's 1,3-dipolar cycloaddition.

Experimental

Reagents and instrumentation

All the solvents and reagents were purchased from Sigma-Aldrich and Fluka (Basel, Switzerland) or VWR (Nyon, Switzerland) and were used without further purification. Flash chromatography columns were performed on VWR silica gel (0.063–0.200 mm). Thin layer chromatography (TLC) was performed on precoated silica gel 60F_{254} plastic sheets from VWR. The compounds were monitored under ultraviolet (UV) light at 254 nm or by brief immersion in potassium permanganate solution. Radioactive spots

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were detected with a Cyclone Phosphorimager from PerkinElmer (Waltham, Massachusetts). The radiochemical yields were determined by TLC analysis of reaction mixture samples taken at specified times. High-performance liquid chromatography (HPLC) was performed on an UltiMate 3000 Rapid Separation LC system (Dionex, Basel, Switzerland) with Chromeleon 6.8 software package. The UV detector was set at four different wavelengths (215 nm, 220 nm, 254 nm, and 280 nm). Radioactivity was detected with a NaI scintillation detector (Nuclear Interface, Munich, Germany) connected on the outflow of the UV detector. The HPLC column used for the purification of the product and the characterization of radioactive compounds against standards was a Phenomenex Gemini 5-μ C18 110-Å (250 × 4.60 mm) column operated at a flow rate of 1 mL/min using a gradient of 5% acetonitrile in water containing 0.1% trifluoroacetic acid (TFA) to CH_3CN/TFA (99.9:0.1) over 20 min. The radiochemical purities were determined by HPLC analyses. NMR data were recorded with a Varian Gemini 2000 NMR Spectrometer and Oxford 300 Nuclear Magnetic Instrument (Oxfordshire, UK). The samples were dissolved either in $CDCl_3$ or in acetone-d_6 (Cambridge Isotope Laboratories Inc., Burgdorf, Switzerland), and the data were processed by MestRe-C 4.8 software. Chemical shifts (δ) are expressed in ppm relative to the signals of the solvents and coupling constants (*J*) in Hz. Mass spectrometry (MS) analyses were performed using an ESI-MS instrument, API 150EX from AB/MDS (Sciex, Framingham, Massachusetts), or ES TQ-detector Aqui™ (Waters, Baden-Dättwil, Switzerland). High-resolution mass spectrometry (HRMS) spectra were recorded by ESI/nanoESI-IT Esquire 3000 plus (Bruker, Fällanden, Switzerland).

Chemistry

*Methyl (2S,4R)-4-hydroxy-1-(pent-4-ynoyl)pyrrolidine-2-carboxylate (**2**)*

Methyl-(2S,4R)-4-hydroxypyrrolidie-2-carboxylate (**1**) (1.00 g, 5.48 mmol), 4-pentynoic acid (0.54 g, 5.48 mmol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (1.26 g, 6.57 mmol), and 4-dimethylaminopyridine (DMAP) (1.00 g, 8.21 mmol) were dissolved in 20 mL of dichloromethane (DCM). Et_3N (0.20 mL) was added dropwise, and the reaction mixture was stirred at room temperature overnight. Then, the mixture was extracted with EtOAc (3 × 30 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel hexane/EtOAc (1:1) to obtain colorless oil. Yield: 90%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 4.42 (1H, d, J = 10.2 Hz), 4.39 (2H, t, J = 7.7 Hz), 4.21 (1H, bs), 3.66–3.59 (4H, m), 2.48–2.35 (4H, m), 2.16 (1H, t, J = 10.65 Hz), and 1.95–1.86 (2H, m). ^{13}C-NMR (300 MHz, $CDCl_3$) δ: 172.75, 170.35, 83.44, 70.53, 69.03, 57.77, 55.16, 52.59, 38.02, 33.69, and 14.17. MS (ESI): *m/z* 226.4 $[M+H]^+$.

*Methyl (2S,4R)-4-(2-tert-butoxy)-2-oxoethoxy)-1-(pent-4-ynoyl)pyrrolidine-2-carboxylate (**3**)*

NaH 60% in paraffin oil (0.31 g, 7.75 mmol), *tert*-butylbromoacetate (2.00 mL, 13.67 mmol), and *n*-$Bu_4N^+I^-$ (1.44 g, 3.90 mmol) were suspended under argon in 28 mL of dry THF. Then, **2** (1.10 g, 4.88 mmol) was dissolved in 2 mL of dry THF, slowly added to the reaction mixture, and stirred overnight. The solvent was removed under reduced pressure. The crude product was purified by chromatography on silica gel using EtOAc/hexane (2:3) to obtain light yellow oil. Yield: 75%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 4.53 (1H, t, J = 7.65 Hz), 4.28 (1H, q, J = 4.3 Hz), 3.96 (2H, d, J = 2.1 Hz), 3.77 (1H, s), 3.72 (2H, s), 3.65 (1H, dd, J_d = 1, J_d = 3 Hz), 2.57–2.50 (4H, m), 2.41–2.22 (1H, m), 2.16–2.03 (1H, m), 1.96 (1H, t, J = 2.4 Hz), 1.68 (1H, s), and 1.47 (9H, s). ^{13}C-NMR (300 MHz, $CDCl_3$) δ: 172.64, 169.77, 169.09, 83.24, 82.13, 78.35, 68.84, 68.56, 67.06, 57.51, 52.25, 34.66, 33.45, 28.09 (3C), and 13.85. MS (ESI): *m/z* 340.3 $[M+H]^+$.

*tert-Butyl 2-((3R,5S)-5-(hydroxymethyl)-1-(pent-4-ynoyl)pyrrolidin-3-yloxy) acetate (**4**)*

To a cooled solution of **3** (1.15 g, 3.39 mmol) in anhydrous THF/EtOH (65:35, 26 mL) at 0°C, LiCl (0.80 g, 18.87 mmol) and $NaBH_4$ (0.30 g, 7.93 mmol) were added. After 1 h, the ice bath was removed, and the reaction mixture was stirred overnight. Then, the reaction mixture was concentrated, and 20 mL of water was added. The aqueous layer was extracted with ethyl acetate (3 × 30 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated under vacuum. The crude product was purified by chromatography on silica gel using EtOAc/hexane (1:1) to obtain light yellow oil. Yield: 88%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 4.96 (1H, d, J = 8.1 Hz), 4.30 (1H, q, J = 8.5 Hz), 4.20 (1H, t, J = 1,8 Hz), 3.95 (2H, d, J = 5.4 Hz), 3.76–3.5 (4H, m), 2.62–2.47 (4H, m), 2.26–2.18 (1H, m), 1.73–1.64 (2H, m), and 1.46 (9H, s). ^{13}C-NMR (300 MHz, $CDCl_3$) δ: 172.07, 169.25, 82.08, 77.42, 77.21, 69.00, 68.70, 66.68, 60.13, 53.61, 34.12, 33.86, 28.07 (3C), and 14.86. MS (ESI): *m/z* 312.1 $[M+H]^+$.

*tert-Butyl 2-((3R,5S)-5-((tert-butyldimethylsilyl)oxy)methyl)-1-(pent-4-ynoyl)pyrrolidin-3-yl)oxy)acetate (**5**)*

4 (0.80 g, 2.57 mmol), imidazole (0.44 g, 6.42 mmol), and *tert*-butyldimethylsilyl chloride (0.43 g, 2.83 mmol) were dissolved in 15 mL of dimethylformamide (DMF) and stirred for 3 h at room temperature. The solvent was removed by evaporation, and 20 mL of EtOAc was added and extracted with water, saturated $NaHCO_3$, and dried over Na_2SO_4, then filtered. The solvent was removed by evaporation, and the product was purified by chromatography on silica gel using EtOAc to obtain colorless oil. Yield: 82%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 4.37–4.24 (2H, m), 3.96–3.89 (3H, m), 3.68–3.63 (1H, dd, J_d = 4.2, J_d = 6 Hz), 3.58–3.51 (2H, m), 2.59–2.41 (4H, m), 2.26–2.17 (1H, m), 2.07–1.95 (2H, m), 1.47 (9H, s), 0.85 (9H, s), and 0.04–0.01 (6H, m). ^{13}C-NMR (300 MHz, $CDCl_3$) δ: 169.40, 156.50, 81.88, 78.43, 78.35, 68.60, 67.08 (2C), 63.11, 57.73, 52.91, 33.91, 33.27, 28.21 (3C), 25.85 (3C), 14.06, and −5.50 (2C). MS (ESI): *m/z* 426.5 $[M+H]^+$.

*tert-Butyl (S)-3-(2-((3R,5S)-5-(hydroxymethyl)-1-(pent-4-ynoyl)pyrrolidin-3-yloxy)acetamido)-2-(2,4,6-trimethylbenzamido)propanoate (**6**)*

5 (0.10 g, 2.35 mmol) was dissolved in 25 mL of MeOH, and LiOH * H_2O (0.23 g, 9.40 mmol) was added. After stirring for 4 h at room temperature, NH_4Cl was added, and the solution was stirred for 10 min. The solid was filtered, the solvent was removed under vacuum, and then, 30 mL of EtOAc were added and extracted with water (2 × 30 mL). The organic layer was dried over Na_2SO_4 and filtered, and the solvent was removed under reduced pressure. The crude was dissolved in 2 mL of DMF; **9** (0.92 g, 2.99 mmol), EDC (0.54 g, 2.82 mmol), and DMAP (0.43 g, 3.53 mmol) were added; and the solution was stirred overnight at room temperature. The solvent was then removed under reduced pressure, 20 mL of ethyl acetate was added, and the organic layer was washed with saturated $NaHCO_3$ (2 × 20 mL) and brine (20 mL). The organic layer was dried with Na_2SO_4 and filtered, and the solvent removed under reduced pressure. The product was purified by column chromatography on silica gel using EtOAc/hexane (2:3) to obtain light brown oil. Yield: 40%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.39 (1H, td, J = 31.2 Hz), 6.79 (3H, s), 4.78–4.71 (1H, m), 4.20–4.09 (2H, m), 3.95–3.81 (8H, m), 2.57–2.31 (4H, m), 2.23–2.22 (9H, m), 2.14 (3H, s), 1.76–1.65 (1H, m), and 1.45 (9H, m). ^{13}C-NMR (300 MHz, $CDCl_3$) δ: 171.68, 171.24, 169.56, 168.60, 138.63, 133.88 (2C), 133.66, 128.15 (2C), 83.27, 77.90, 77.68, 68.76, 68.52, 67.91, 65.55, 59.48, 52.97, 42.26, 33.83, 33.36, 27.81 (3C), 20.94, 18.88 (2C), and 13.86. MS (ESI): *m/z* 544.7 $[M+H]^+$.

*tert-Butyl (S)-3-(2-((3R,5S)-1-pent-4-ynoyl-5-((pyridin-2-ylamino)methyl) pyrrolidin-3-yloxy)acetamido)-2-(2,4,6-trimethylbenzamido)propanoate (PMt, **7**)*

6 (0.11 g, 0.22 mmol) and Et_3N (0.46 mL) were dissolved in 4.6 mL of DCM/dimethyl sulfoxide (DMSO) (75:25) and cooled at 0°C. SO_3-pyridine complex (0.43 g, 2.67 mmol) was added, and the reaction mixture was stirred for 2 h. The DCM fraction was removed under reduced pressure. The remaining DMSO fraction was diluted in 30 mL of ethyl acetate. The organic layer was washed with H_2O (30 ml) and saturated $NaHCO_3$ solution (2 × 20 mL). The organic layer was dried over Na_2SO_4 and filtered, and the solvent was removed under reduced pressure. The crude obtained (0.14 mg, 0.26 mmol), 2-aminopyridine (0.24 g, 2.58 mmol), and $Ti(OiPr)_4$ (0.96 mL, 4.96 mmol) were dissolved in 1.8 mL of 1,2-dichloroethane. After stirring overnight at room temperature, $NaBH(OAc)_3$ (1.5 g, 0.71 mmol) was added, and the reaction was stirred for 4

additional hours. Saturated $NaHCO_3$ solution (2.70 mL) was added, and the mixture was stirred for 1 h. It was then extracted with DCM, dried with Na_2SO_4, and the solvent removed by evaporation. Light brown oil was obtained in 67% yield. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.09–8.02 (2H, m), 7.40 (1H, dd, J_d = 1.8, J_d = 8.1 Hz), 7.35–7.30 (2H, t, J = 7.8 Hz), 6.80 (1H, s), 6.63–6.53 (2H, m), 6.44 (2H, td, J_t = 8.4, J_d = 16.5 Hz), 6.01 (1H, bs), 5.09–4.99 (1H, m), 4.90–4.78 (1H, m), 4.71–4.52 (2H, m), 4.28 (1H, t, J = 4.5 Hz), 4.05–3.58 (5H, m), 2.49–1.94 (13H, m), 1.47 (2H, s), 1.28–1.19 (6H, m), and 0.87 (1H, bs). ^{13}C-NMR (300 MHz, $CDCl_3$) δ: 172.14, 171.10, 169.57, 168.78, 158.28, 157.79, 157.44, 147.79, 137.64, 133.96 (2C), 128.15 (2C), 113.79, 108.53, 83.27, 83.09, 78.12, 77.34, 68.76, 68.17, 62.95, 60.57, 52.79, 42.09, 33.64, 30.79, 27.82 (3C), 20.95, 18.95 (2C), and 13.96. HRMS (ESI) for $C_{34}H_{43}N_5O_6$ $[M+H]^+$: calculated *m/z* 618.3286 and found *m/z* 618.3282.

*tert-Butyl (S)-3-(2-((3R,5S)-1-(3-(1-(2-fluoroethyl)-1H-1,2,3-triazol-4-yl) propanoyl)-5-((pyridin-2-ylamino)methyl)pyrrolidin-3-yloxy)acetamido)-2-(2,4,6-trimethylbenzamido)propanoate (FPMt, **8**)*

7 (10 mg, 0.16 mmol) was dissolved in 1 mL of water/THF, and **12** (62% m/m in DMF, 20 μL, 7.60 mmol) was added. Sodium ascorbate (0.5 mL, 2.1 M) and $CuSO_4$ (0.5 mL, 0.7 M) were slowly added, and the reaction was stirred at room temperature for 2 h. The crude was filtered, and the product was purified by C-18 chromatography to obtain a light brown solid. Yield: 45%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 9.33 (1H, d, J = 3.9 Hz), 7.75–7.63 (3H, m), 6.82 (2H, s), 4.92 (1H, t, J = 4.7 Hz), 4.76–4.68 (4H, m), 4.64–4.21 (2H, m), 4.01–3.59 (7H, m), 2.96–2.70 (7H, m), 2.24 (9H, d, J = 5.7 Hz), 1.48 (9H, s), and 0.88 (2H, t, J = 5.6 Hz). ^{13}C-NMR (300 MHz, $CDCl_3$) δ: 171.35, 169.75, 169.41, 168.76, 161.61, 155.39, 149.30, 146.83, 138.85, 134.06 (2C), 133.42, 128.30 (2C), 126.55, 116.39, 102.89, 83.52, 81.51 (d, J = 171.3 Hz), 80.39, 78.82, 68.24, 62.86, 52.94, 52.64, 50.26, 42.55, 33.06, 31.23, 29.68, 27.95 (3C), 21.07, and 19.04 (2C). MS (APPI$^+$) for $C_{36}H_{47}FN_8O_6$: *m/z* 707.00 $[M+H]^+$.

*2-Fluoroethyl 4-methylbenzenesulfonate (**11**)*

Fluoroethanol (**10**) (1.00 mL, 30.25 mmol) was stirred overnight with *p*-toluenesulfonyl chloride (4.80 g, 51.20 mmol) and 7 mL of Et_3N in 15 mL of DCM. The solvent was removed under reduced pressure. Then, 20 mL of EtOAc was added and the mixture extracted with saturated solution of $NaHCO_3$ (2 × 20 mL). The organic layer was dried over Na_2SO_4 and filtered, and the solvent was removed under reduced pressure. The crude obtained was purified by column chromatography on silica gel using ethyl acetate/hexane (1:5). Brown oil was obtained with a yield of 78%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.79 (2H, d, J = 8.1 Hz), 7.35 (2H, dd, J_d = 3.9, J_d = 8.4 Hz), 4.13 (2H, td. J_t = 2, J_d = 5.1 Hz), 3.46 (2H, t, J = 4.95 Hz), and 2.43 (3H, s). ^{13}C-NMR (300 MHz, $CDCl_3$) δ: 145.19, 132.32, 129.89 (2C), 127.81 (2C), 68.07, 49.43, and 21.56. MS (ES$^+$): *m/z* 219 $[M+H]^+$.

*1-Azido-2-fluoroethane (**12**)*

11 (1.52 g, 7.09 mmol) was dissolved in 3.5 mL of DMF, and NaN_3 (1.34 g, 20.60 mmol) was added. The mixture was stirred overnight at 80°C. The product was purified by azeotropic distillation giving a DMF solution of **12** (62% *m/m*). ^{1}H-NMR (300 MHz, $CDCl_3$, in DMF solution) δ: 4.56 (2H, dt, J_t = 4.2, J_d = 38.1 Hz) and 3.49 (2H, d, J = 27.9 Hz). ^{13}C-NMR (300 MHz, $CDCl_3$, extrapolated form a DMF solution) δ: 99.90 and 82.14 (d, J = 170 Hz). HRMS (ESI): *m/z* 90.0462 $[M+H]^+$.

Radiochemistry

^{18}F-Fluoride production

No-carrier-added fluorine-18 was produced according to the nuclear reaction ^{18}O(p,n)^{18}F by irradiation (10 min at 10 μA) of 2 mL of highly enriched (>97%) [^{18}O]water (Marshall Isotopes, Tel-Aviv, Israel) by a proton beam using a Cyclone-18/9 cyclotron (IBA, Louvain-la-Neuve, Belgium). The initial radioactivity of [^{18}F]fluoride was estimated to be around 5 GBq.

*1-Azido-2-[^{18}F]fluoroethane ([^{18}F]**12**)*

The produced carrier-free [^{18}F]fluoride was trapped on a single use light-QMA anion-exchange column (Waters, Baden-Dättwil, Switzerland), and the excess of [^{18}O]water was removed. Trapped [^{18}F]fluoride was eluted from the column with 2.5 mL of a CH_3CN/H_2O solution (6:1) of K_2CO_3 (3.5 mg, 25 μmol) and Kryptofix [2.2.2] (K_{222}; 20 mg, 53 μmol) and transferred into the reactor. This solution was then dried by consecutive heating at 85 and 110°C under a stream of argon. After cooling the reactor to 40°C, a solution of 2-azidoethyl-4-methylbenzenesulfonate (**13**) (2 mg, 9 μmol) in CH_3CN (350 μL) was added to the dry K[^{18}F]F$^-$/K_{222} complex, and the reaction mixture was stirred at 80°C for 15 min. Then, 1-azido-2-[^{18}F]fluoroethane ([^{18}F]**12**) was purified by distillation at 130°C for 5 min and collected into a vial containing 150 μL of CH_3CN. The radiochemical purity of this intermediate was estimated to be above 98% (Figure 1), and the radiochemical yield was 64% (decay corrected).

*tert-Butyl (S)-3-(2-((3R,5S)-1-(3-(1-(2-[^{18}F]fluoroethyl)-1H-1,2,3-triazol-4-yl)propanoyl)-5-((pyridin-2-ylamino)methyl)pyrrolidin-3-yloxy) acetamido)-2-(2,4,6-trimethylbenzamido)propanoate ([^{18}F]FPMt, [^{18}F]**8**)*

Aliquots (50 μL) of the previous solution of 1-azido-2-[^{18}F]fluoroethane ([^{18}F]**12**) were treated with *tert*-butyl (*S*)-3-(2-((3R,5S)-1-pent-4-ynoyl-5-((pyridin-2-ylamino)methyl)pyrrolidin-3-yloxy)acetamido)-2-(2,4,6-trimethyl benzamido)propanoate (**7**) (3 mg, 4.85 μmol or 6 mg, 9.70 μmol), $CuSO_4$ (50 μL, 0.35 M or 1.05 M), and sodium ascorbate (50 μL, 0.7 M or 2.1 M) in a mixture of CH_3CN/H_2O (1:1, *v/v*). The reaction mixtures were stirred for 15 min at room temperature and filtered, and then, [^{18}F]**8** was purified by HPLC under the conditions previously described (Figure 2). Radiochemical yields between 80% and 83% (decay corrected) were obtained for the click chemistry reactions. Typically, [^{18}F]**8** was prepared in 60–70 min from the end of bombardment (EOB), and the radiochemical yield was estimated to be 42–44% (decay corrected). Identity of the final product was confirmed by comparing its HPLC mobility with the retention time of the nonradioactive standard under the same analytical conditions used to characterize [^{18}F]**12**. [^{18}F]**8** and **8** had similar retention time of 13.2 and 13.1 min, respectively, versus 9.7 and 11.3 min for the starting materials [^{18}F]**12** and **7** under the same elution conditions. The radiochemical purity of [^{18}F]**8** was estimated to be above 95%, and its specific activity, determined by using on-line measurements of radioactivity and UV absorption, was >1.3 GBq/μmol.

Results and discussion

Chemistry

Based on docking studies, a pyrrolidine pharmacophore has been reported to yield selective ligands for integrin $\alpha_5\beta_1$. Indeed, the 2-aminopyridine and 2,4,6-trimethyl benzoic acid moieties

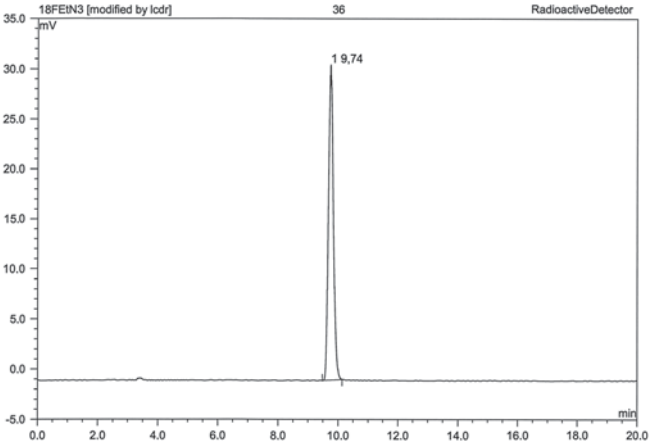


Figure 1. HPLC radiochromatogram of [^{18}F]**12**.

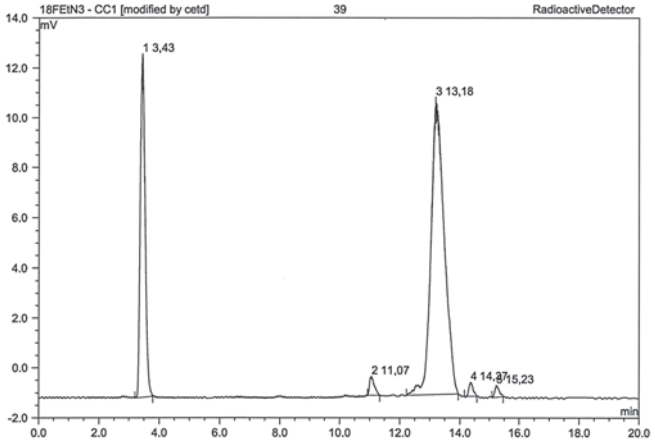
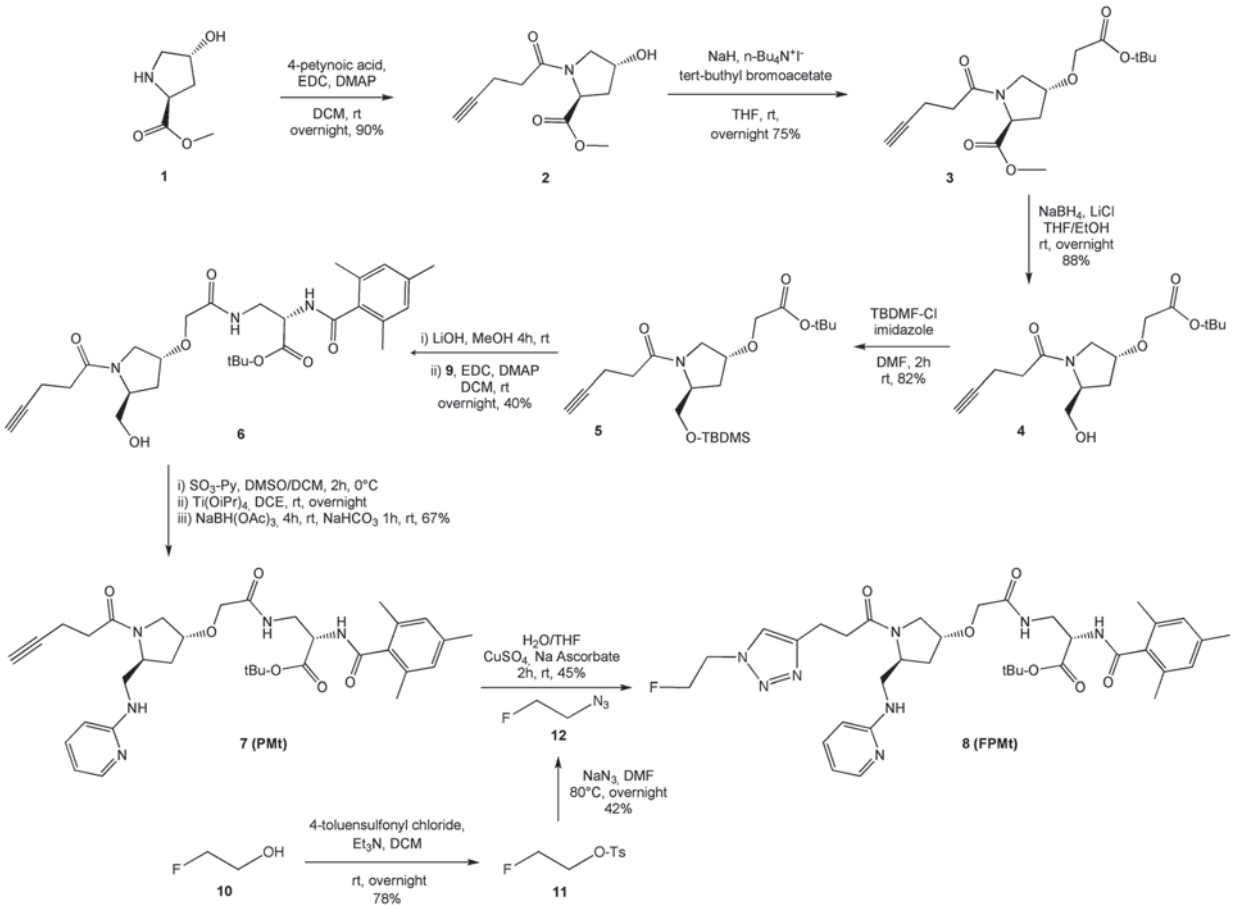


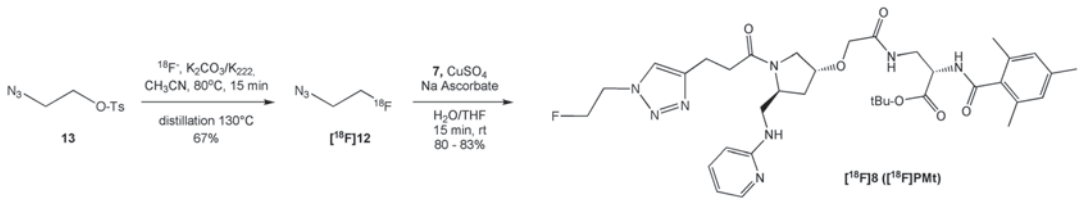
Figure 2. HPLC radiochromatogram of [^{18}F]FPMt ([^{18}F]**8**). No traces of [^{18}F]**12** were found. The peak at 3.4 min corresponds to an unidentified impurity.

divided by an appropriate linker determine the selectivity for $\alpha_5\beta_1$ integrin receptor.[21,22] The 2-aminopyridine interacts with the (α_5)-Phe 187 on the (α_5)β-propeller domain, while the mesitylene group enables π–π interaction with (β_1)-Tyr 127 placed in a pocket on the β_1 subunit, which is not available on the $\alpha_v\beta_3$ surface.[21] *tert*-Butyl (*S*)-3-(2-((3R,5S)-1-(3-(1-(2-fluoroethyl)-1H-1,2,3-triazol-4-yl)propanoyl)-5-((pyridin-2-ylamino)methyl)pyrrolidin-3-yloxy)acetamido)-2-(2,4,6-trimethylbenzamido)propanoate (FPMt, **8**) has been identified, among 61 $\alpha_5\beta_1$ antagonists, as the most appropriate molecule for the development of a ^{18}F-labeled $\alpha_5\beta_1$ radioligand. FPMt has a structure closely related to the selective $\alpha_5\beta_1$ antagonist JSM6427.[17] A fluorinated substituent is incorporated on the pyrrolidine nitrogen in order to not interfere with the binding of this peptidomimetic molecule with $\alpha_5\beta_1$ integrin receptor. Synthesis of **7** (Scheme 1) was adapted from the synthesis of JSM6427, as reported by Stragies *et al*.

An alkynyl function was introduced into compound **2** by coupling of 4-pentynoic acid to the 4-hydroxyproline methyl ester (**1**) to allow subsequent attachment of the radiolabeled azido synthon to the alkynyl precursor **7** by the copper(II)-catalyzed Huisgen's cycloaddition. Conversion of the secondary alcohol into alcoholate and Williamson ether synthesis with *tert*-butyl bromoacetate provided intermediate **3** in 60% yield. Deprotection and concomitant reduction of the methyl ester in position 2 with sodium borohydride in the presence of lithium chloride yielded intermediate **4**. Protection of the primary alcohol of **4** as *tert*-butyldimethylsilyl ether afforded compound **5** in 82% yield. The *tert*-butyl group was then selectively removed with lithium hydroxide in methanol, and the resulting carboxylic acid underwent an amidation by treatment with *tert*-butyl (*S*)-3-amino-2-(2,4,6-trimethylbenzamido)propanoate (**9**) in presence of 4-dimethylaminopyridine and the coupling reagent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide. Deprotection in position 2 occurred simultaneously to afford the primary alcohol **6** in 40% yield. Compound **9** was synthesized in two steps starting from commercially available asparagine *tert*-butyl ester as previously described.[17] Treatment of **6** with the SO_3-pyridine complex to oxidize the primary alcohol into aldehyde, followed



Scheme 1. Synthesis of FPMt (**8**).



Scheme 2. Radiosynthesis of [^{18}F]FPMt ([^{18}F]**8**).

by the reductive amidation of the aldehyde with 2-aminopyridine in the presence of $Ti(OiPr)_4$ and $NaBH(OAc)_3$, gave the alkynyl precursor **7** in 67% yield. Our optimized synthetic approach allowed us to obtain the alkynyl pyrrolidine derivative **7** in seven steps, whereas the strategy described by Stragies provided similar analogs in nine steps. Moreover, **7** was obtained with an overall satisfactory yield of 45%.

1-azido-2-fluoroetanol (**12**) was synthesized in two steps. Activation of the hydroxyl group of 2-fluoroethanol (**10**) by a tosylation, followed by a nucleophilic substitution with NaN_3, afforded **12**, which was recovered by distillation and obtained as DMF solution (62% m/m of **12**) in 42% yield.[23,24] Finally, the Cu(II)-catalyzed Huisgen's 1,3-dipolar cycloaddition between **7** and the azido synthon **12** gave the final peptidomimetic molecule **8** (FPMt) in only 2 h with 45% yield.[25] The time of reaction and the yield obtained suggested that this strategy could be applied for the development of an ^{18}F-labeled analog.

Radiochemistry

Nucleophilic ^{18}F-fluorination of 2-azidoethyl-4-methylbenzenesulfonate (**13**) was carried out in anhydrous CH_3CN with a standard K[^{18}F]F$^-$/Kryptofix complex. The reaction was conducted in 15 min at 80°C, and 1-azido-2-[^{18}F]fluoroethane ([^{18}F]**12**) was isolated by distillation at 130°C into a vial containing anhydrous CH_3CN (Scheme 2).[24] [^{18}F]**12** was obtained in 30 min from the EOB with a radiochemical yield of 64% (decay corrected) in accordance with the data reported in literature[24,26] (Figure 1). The copper(II)-catalyzed Huisgen's cycloaddition with the alkynyl pyrrolidine **7** was performed at room temperature in CH_3CN/H_2O in the presence of $CuSO_4$ and sodium ascorbate (Scheme 2). The catalytic effect on the kinetic of the click reaction was studied on aliquot of [^{18}F]**12** by modulating the concentrations of **7**, $CuSO_4$, and sodium ascorbate. Good radiochemical yields between 80 and 83% (decay corrected) were obtained, and [^{18}F]**12** was completely consumed during the reaction (Figure 2). No influence of the concentration of the reagents was observed on the reaction yield. The radiolabeling method was reproducible, robust, and provided the final ^{18}F-labeled putative $\alpha_5\beta_1$ ligand ([^{18}F]**8**) in two steps with an overall synthesis time of 60–70 min from EOB and 42–44% radiochemical yield (decay corrected).

Conclusions

Herein, we report the synthesis of [^{18}F]FPMt ([^{18}F]**8**), a nonpeptidic radiopharmaceutical for PET imaging with a structure derived from a selective antagonist of $\alpha_5\beta_1$ integrin receptor.[17] Our alkynyl precursor **7** was successfully synthesized, and all the steps were optimized to provide good yields. 1-azido-2-fluoroethane (**12**) was chosen as synthon for the development of the PET tracer, and it was clicked to **7** following the standard Cu catalyzed alkyne-azide click reaction. Future studies will be aimed at determining the affinity and specificity of [^{18}F]FPMt for integrin $\alpha_5\beta_1$ and demonstrating how useful this imaging agent may be in assessing angiogenesis in tumors and monitoring therapy.

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Conflict of Interest

The authors did not report any conflict of interest.

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