Construction of Gold-based Imaging Probes for Mapping Drug Binding Sites on HIV-1 Envelope Glycoproteins

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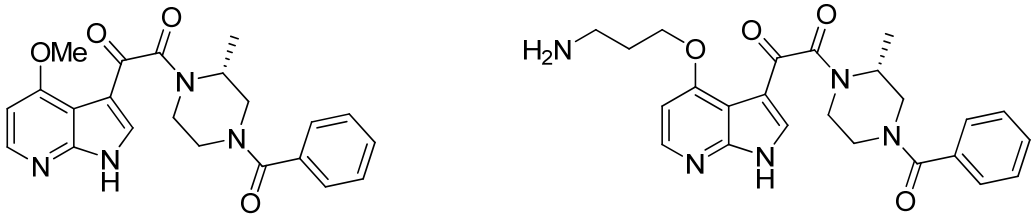
Background

The conserved CD4 binding site of the HIV-1 gp120 envelope glycoprotein is an attractive target for both vaccine and microbicide development as well as rational anti-viral drug design.[1] While atomic models for the structure of the core of gp120 monomer in the absence and presence of CD4-ligand are currently available, determination of the structure of the intact envelope glycoprotein trimer in a native context remains a significant goal in HIV structural biology.[2] Recent imaging efforts aimed at obtaining a 3D structure by way of electron tomography have yielded contradictory results and are ultimately hindered by insufficient resolution.[3] With the goal of further investigating the native structure, a derivative of the small molecule HIV-1 inhibitor BMS-378806 (**11**)[4] was synthesized and covalently labeled with 1.4nm Nanogold® and 0.8nm Undecagold reagents, respectively.[5] These electron-dense gold nanoparticle conjugates are intended to provide the improvement in resolution necessary to map the site of drug binding on the gp120 trimer.

Figure 1. The structures of BMS-378806 and 4-(3-aminopropoxy)-functionalized BMS-378806 **(11).**



BMS-378806 **11**

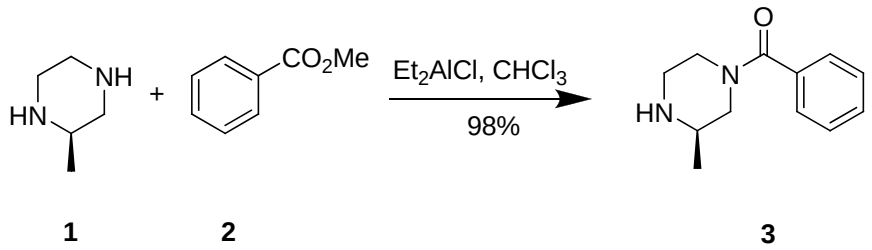
Chemistry

The syntheses of 3-aminopropoxy derivative **11**,[6] as well as the BMS-378806[4] were previously described by teams at the University of Maryland[6] and at Bristol-Myers Squibb[4], respectively. The piperazine derivative **3** was prepared from commercially available (*R*)-2-methylpiperazine **(1)** and methyl benzoate **(2)** (Scheme 1) according to the known procedure[4] with some modifications as noted in the experimental section. The synthetic pathway to a key intermediate **11**, 4-benzoyl-1-[4-(3-aminopropoxy)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)oxoacetyl]-2-(*R*)-methyl-piperazine, is shown in Scheme 2. Compound **5** was prepared by acylation of commercially available 7-azaindole **(4)** with methyl chlorooxoacetate in the presence of aluminum trichloride in good yield. Conversion of **5** to its potassium salt **6** was achieved using potassium carbonate in aqueous methanol. The intermediate **6** was then transformed to **7** by coupling with *N*-(benzoyl)-3-methylpiperazine **(3)** in the presence of 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT) and *N,N*-diisopropylethylamine (Hünig's base) in 88% yield. Oxidation of **7** with 70% *meta*-chloroperbenzoic acid (mCPBA) furnished the *N*-oxide **8**. Following the nitration procedure for **8** as described by Wang co-workers,[4] however, yielded a mixture of mono- and dinitro-compounds in a ratio of 1:1 (by LC/MS and NMR). The desired mono-derivative, 4-nitro *N*-oxide **9**, was obtained by adding fuming nitric acid dropwise to a solution of *N*-oxide **8** in trifluoroacetic acid (TFA) at 0°C followed by heating at 45-50°C for 4 hours (details are described in the experimental procedure). Furthermore, microwave heating was found to be a superior technique and very efficient in the *ipso*-substitution of nitrite **9** with 3-azido-1-propanol **(13)** in the presence of 60% sodium hydride in anhydrous dimethylformamide (DMF) (Scheme 3). This reaction provided 4-benzoyl-1-[4-(3-azidopropoxy)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)oxoacetyl]-2-(*R*)-methyl-piperazine **(10)** in good yield after subsequent removal of the *N*-oxide function with phosphorus trichloride in chloroform. Reduction of the azido group of **10** to a 3-aminopropyloxy moiety *via* catalytic hydrogenation with palladium afforded the key intermediate 4-benzoyl-1-[4-(3-aminopropoxy)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)oxoacetyl]-2-(*R*)-methyl-piperazine **(11)**[6] which was then used in labeling with gold nanoparticles. The NMR and mass spectra of **11** were consistent with its structure. Analytical RP-HPLC indicated its purity >99%.

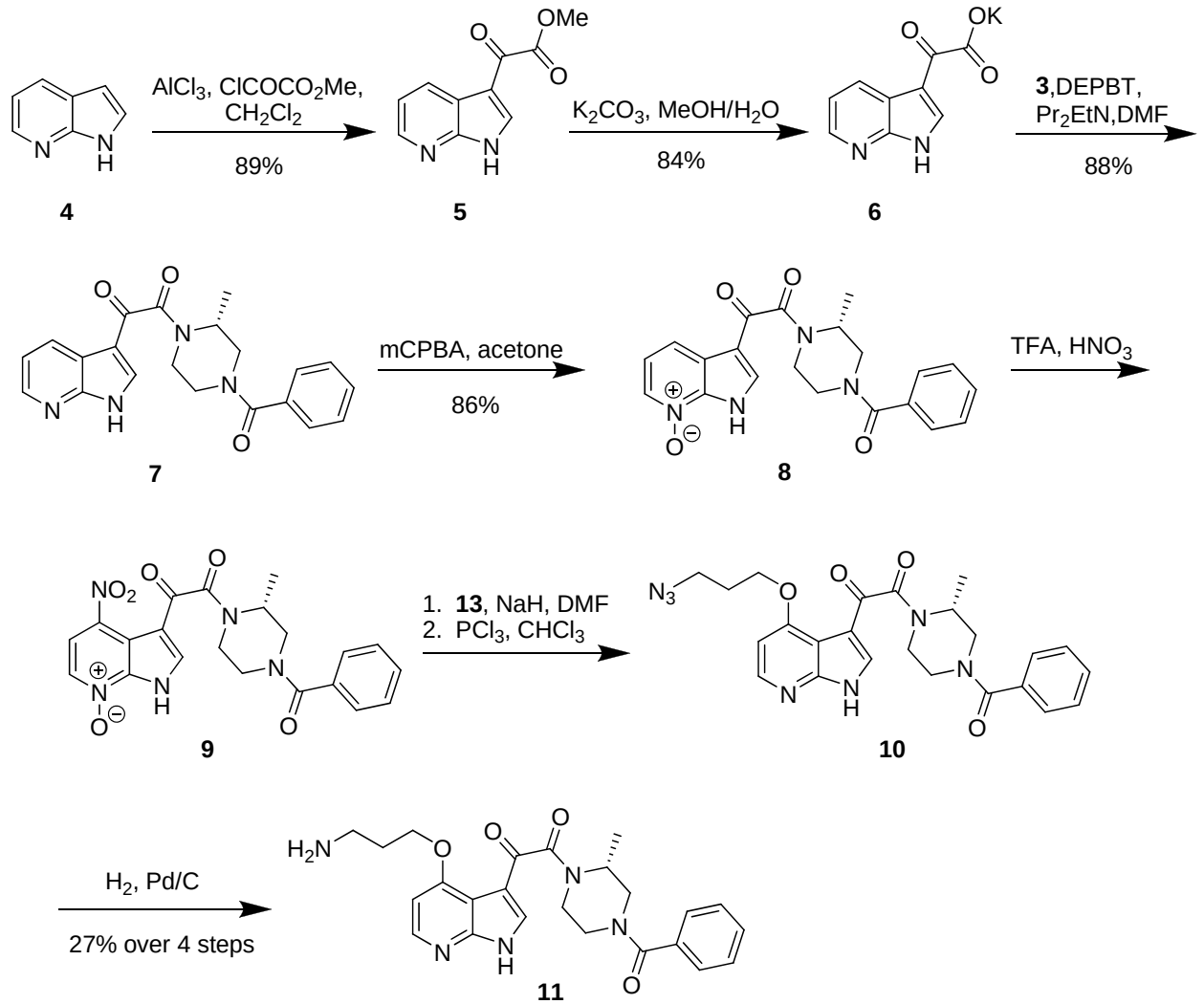
The synthesis of BMS-378806 was accomplished in two steps by removal of the *N*-oxide function of **9** using phosphorus trichloride followed by *ipso*-substitution of the resultant nitrite **14**

with sodium methoxide (Scheme 4). Reduction of the *N*-oxide functionality of **9** before installation of the methoxy group proved to be more efficient. Two sets of signals were observed for several protons in the ^{1}H and ^{13}C NMR spectra of compounds **8, 10** and **11**. In particular, a doubling of signals was observed for carbonyl carbons and for some of the aromatic carbons which we ascribed to the existence of rotamers in the solution.

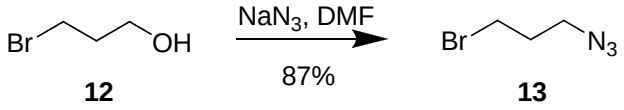
Scheme 1. Synthesis of piperazine (**3)**



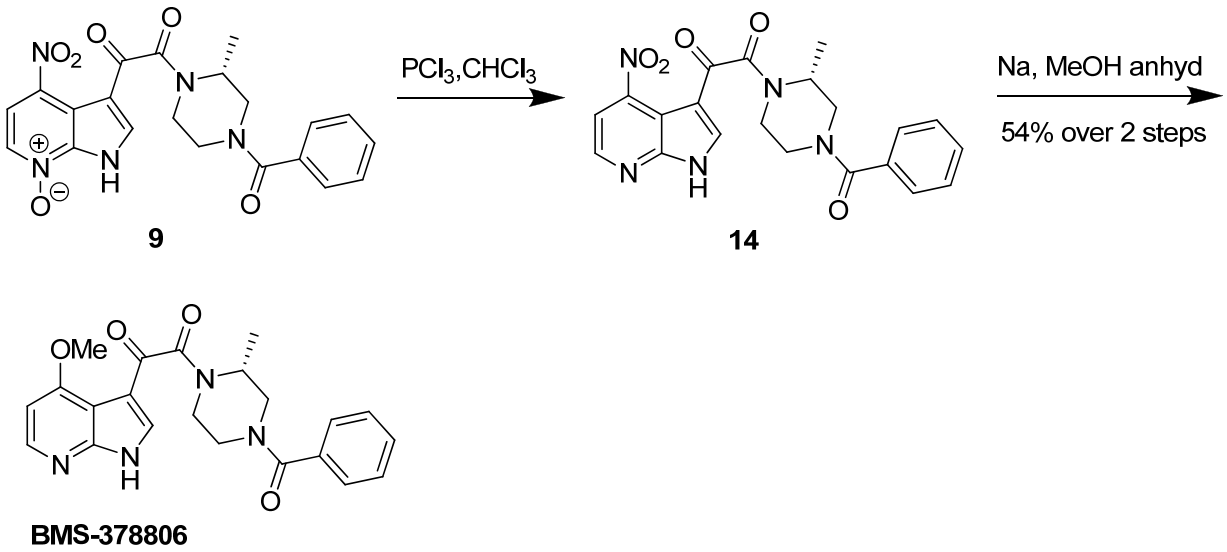
Scheme 2. Synthesis of 4-(3-aminopropoxy)-functionalized BMS-378806 **(11)**



Scheme 3. Synthesis of 3-azido-1-propanol **(13)**

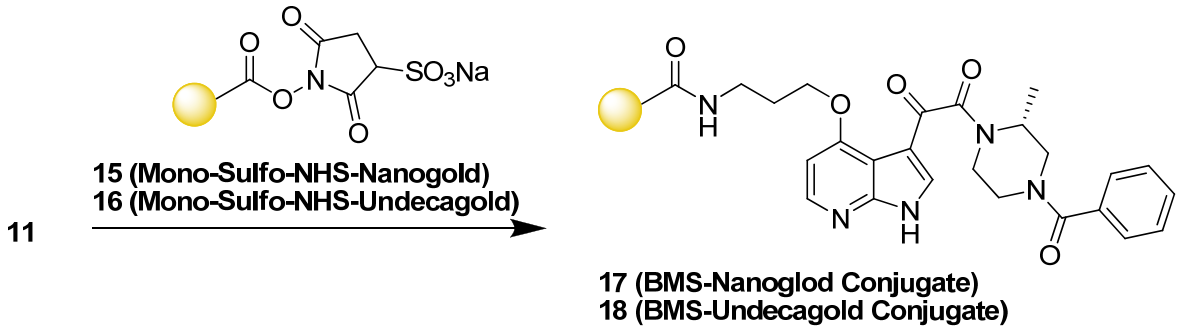


Scheme 4. Synthesis of **BMS-378806**



The synthesis of the BMS gold conjugates is summarized in Scheme 5.

Scheme 5. Synthesis of BMS-Nano and Undecagold Conjugates **(17** and **18)**



Experimental

General. Thin-layer chromatography (TLC) analyses were carried out on Analtech (Newark, DE) silica gel GHLF 0.25 mm plates using UV and iodine detection. Melting points were

determined in open glass capillaries on an Electrothermal MEL-TEMP 3.0 apparatus. ESI- and APCI-MS mass spectrometry was performed on an Agilent 1200 Series Mass Spectrometer. LC-MS analyses were completed on an Agilent 1200 Series Mass Spectrometer with an Eclipse XDB-C18 column, 2.1 x 50 mm, using the following method: initial gradient (95% A, 5% B) run to gradient (65% B, 35% A) over 10 min., and then to final gradient (95% B, 5% A), hold for 2 min. (95% B, 5% A). Solvent A: 0.1% TFA in water; Solvent B: 0.1% TFA in acetonitrile. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian spectrometer at 400 MHz and 100 MHz respectively. Chemical shifts are reported in ppm using tetramethylsilane (TMS) as internal standard. Microwave synthesis was carried out on an Initiator 2.2 Biotage instrument. Flash chromatography was carried out on a Teledyne Isco Combi*Flash*® Companion® instrument with UV detection at 254 nm. Preparative HPLC was performed on an Agilent 1200 Series instrument using an Agilent Prep-C18 column, 21.2-100 mm. The analytical HPLC analysis was performed on a Beckman instrument equipped with a UV System Gold 168 detector using an Agilent Extend-C18 column, 4.6 x 250 mm. The following purification methods were used in the HPLC analyses: Method I: initial gradient (95% A, 5% B) run to final gradient (95% B, 5% A) over 20 min., hold for 2 min. (95% B, 5% A). Solvent A: 2 mM ammonium acetate in deionized water; Solvent B: acetonitrile; Method II: initial gradient (95% A, 5% B) run to a gradient (50% A, 50% B) over 12 min. and then to gradient (5% A, 95% B) over 2 min., hold for 2 min. Solvent A: 0.1% TFA in water; Solvent B: 0.1% TFA in acetonitrile. Analytical HPLC analyses of the gold conjugated BMS-378806 derivatives **17** and **18** were also performed on a Beckman instrument equipped with a UV System Gold 168 detector, using a ZORBAX GF-450 column, 9.4 x 250mm. An isocratic method was used with an eluent of 0.02M phosphate buffered saline, pH 6.5. Dialyses were performed with Slide-a-Lyzer 3.5kDa MWCO Dialysis Cassettes from Pierce (Rockford, IL). 1.4nm Mono-Sulfo-NHS-Nanogold® and 0.8nm Mono-Sulfo-NHS-undecagold were purchased from Nanoprobes Incorporated (Yaphank, NY). All other starting materials and reagents were commercially available from Sigma-Aldrich and were used without further purification, unless otherwise noted.

***N*-(benzoyl)-3-methylpiperazine (3).**[4] To a stirred solution of (*R*)-2-methylpiperazine **(1)** (10.0 g, 0.1 mol) in dry CH_2Cl_2 (400 mL) was added a solution of 1.0 M Et_2AlCl in hexanes (100 mL, 0.1 mmol) under an atmosphere of argon at room temperature. The reaction mixture was allowed

to stir for 15 min before adding a solution of methyl benzoate **(2)** (12.4 mL, 0.1 mmol) in dry CH_2Cl_2 (60 mL). The stirring was continued for 2 days. The reaction mixture was quenched with 2N NaOH (200 mL) at 0°C and allowed to warm to room temperature. The aqueous layer was extracted with CH_2Cl_2 (3 x 125 mL). The combined organic layers were washed with brine (250 mL), dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure to afford a crude product **3** (20 g, 98%) as a pale yellow oil which was stored under argon at 4°C and used in the next step of the synthesis without further purification. ^{1}H-NMR (400 MHz, DMSO-d_6): :δ 7.34-7.45 (m, 5H), 4.29 (bs, 1H), 3.37 (bs, 1H), 2.37-3.02 (m, 5H), 0.83-1.00 (m, 3H); ^{13}C-NMR (100 MHz, CD_3OD): δ 171.12, 135.75, 129.96, 128.63, 126.89, 54.42, 50.92, 50.47, 45.48, 44.79, 42.09, 17.69; APCI MS: *m*/z: 205.3 $(M+H)^+$.

Methyl *α*-oxo-1*H*-pyrrolo[2,3-*b*]pyridin-3-acetate (5).[4] A solution of 7-azaindole **(4)** (3 g, 25.4 mmol) in dry CH_2Cl_2 (100 mL) was added dropwise to a stirred suspension of $AlCl_3$ (10 g, 75.0 mmol) in dry CH_2Cl_2 (250 mL) under an atmosphere of argon at room temperature and stirring was continued for 1 hour. A solution of methyl chlorooxoacetate (9.19 g, 75.0 mmol) in dry CH_2Cl_2 (60 mL) was added dropwise and the reaction mixture was stirred for an additional 8 hours at room temperature. The suspension was cautiously poured onto water and ice (350 mL) and stirred for 1.5 hours. The mixture was brought to pH 6-6.5 by addition of saturated $NaHCO_3$ (aq) and the aqueous layer was extracted with CH_2Cl_2 (4 x 200 mL). The combined organic layers were washed with brine (300 mL), dried over anhydrous $MgSO_4$, and evaporated in vacuo to give 4.6 g (89%) of **5** as a pale yellow solid, mp 172.5-173.2°C. The product **6** was stored under an atmosphere of argon at 4°C and used in the next step of the synthesis without further purification. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.61 (bs, 1H), 8.49 (dd, 1H, J = 7.99 and 1.59 Hz), 8.42 (dd, 1H, J = 4.79 and 1.59 Hz), 7.36 (dd, 1H, J = 7.99 and 4.79 Hz), 3.93 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 179.38, 163.97, 149.76, 145.85, 139.49, 130.27, 119.67, 118.59, 111.81, 53.38. APCI MS *m*/z: 205.1 $(M+H)^+$.

Potassium *α*-oxo-1*H*-pyrrolo[2,3-*b*]pyridine-3-acetate (6).[4] Methyl *α*-oxo-1*H*-pyrrolo[2,3-*b*]pyridine-3-acetate **(5)** (4.6 g, 22.5 mmol) and K_2CO_3 (6.2 g, 45 mmol) were dissolved in MeOH:water (1:1) (40 mL). After stirring for 8 hours, the suspension was filtered off, washed with a cold mixture of EtOH:water (1:1) (6 mL) and dried under vacuum to a constant weight to

give 4.3 g (84%) of **6** as a white solid which was carried over to the next step without further purification. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.43 (dd, 1H, *J* = 7.82 and 1.56 Hz), 8.27 (dd, 1H, *J* = 4.70 and 1.56 Hz), 8.09 (s, 1H), 7.18 (dd, 1H, *J* = 7.82 and 4.70 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 169.49, 149.01, 143.65, 135.10, 128.89, 118.12, 117.25, 113.11.

4-Benzoyl-2-(*R*)-methyl-1-[(oxo-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)acetyl]piperazine (7).[4] Potassium *α*-oxo-1*H*-pyrrolo[2,3-*b*]pyridine-3-acetate **(6)** (4.2 g, 18.4 mmol), *N*-benzoyl-3-(*R*)-methylpiperazine **(3)** (3.76 g, 18.4 mmol), (DEPBT) (5.5 g, 18.4 mmol) and *N,N*-diisopropylethylamine (6.4 mL, 36.8 mmol) were combined in anhydrous DMF (80 mL) and the mixture was stirred under an atmosphere of argon at room temperature for 6 hours. The reaction mixture was poured onto water and ice (250 mL) and stirred for 1 hour. The aqueous layer was extracted with CH_2Cl_2 (4 x 125 mL). The combined organic layers were washed with brine (150 mL), dried over anhydrous $MgSO_4$, and concentrated under vacuum. The crude product was purified by flash column chromatography, eluting with a mixture of CH_2Cl_2 and MeOH to afford 4-benzoyl-2-(*R*)-methyl-1-[(oxo-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)acetyl]piperazine **(7)** (6.1 g, 88%). ^{1}H-NMR (400 MHz, CD_3OD): δ 8.56 (bs, 1H), 8.36 (bs, 1H), 8.27 (s, 0.5H), 8.22 (s, 0.5H), 7.45 (bs, 5H), 7.33 (bs, 1H), 3.00-4.80 (m, 7H), 1.15-1.45 (b, 3H); ^{13}C-NMR (100 MHz, CD_3OD): δ 185.6, 171.56, 166.39, 149.79, 144.16, 136.75, 134.86, 131.02, 130.29, 128.34, 126.46, 118.76, 118.20, 111.92, 60.89, 50.13, 45.25, 35.45, 15.11, 13.67. ESI MS *m*/z: $(M+H)^+$: 377.30.

4-Benzoyl-2-(*R*)-methyl-1-[(7-oxido-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)oxoacetyl]piperazine (8).[4] To a solution of **7** (4 g, 10.63 mmol) in acetone (60 mL) was added 70% *meta*-chloroperbenzoic acid (mCPBA) (3 g, 17.4 mmol) at room temperature. After stirring for 1.5 hours, a white solid was collected by filtration and dried under vacuum to afford **8** (3.6 g, 86%), mp. 267-270°C (with decomposition). The crude compound **8** was used without further purification. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.37 (d, 1H, *J* = 12.6 Hz), 8.28 (d, 1H, *J* = 10.3 Hz), 8.07 (d, 1H, *J*= 7.45 Hz), 7.46 (s, 5H), 7.29 (m, 1H), 4.57-2.80 (m, 7H), 1.10-1.36 (b, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 186.85, 186.70, 170.76, 165.70, 165.64, 140.15, 137.57, 137.38, 136.19, 134.15, 130.35, 129.19, 127.62, 127.61, 122.26, 120.59, 120.54, 114.29, 114.25, 50.09, 44.95, 16.52, 15.45.

4-Benzoyl-2-(*R*)-methyl-1-[(4-nitro-7-oxido-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)oxoacetyl]piperazine (9).[4] To a solution of **8** (1.2 g, 3.06 mmol) in TFA (3 mL) was cautiously added dropwise fuming nitric acid (1.4 mL) at 0°C and the temperature was allowed to warm to room temperature for 1 hour. The resulted mixture was then heated at 45-50°C for 4 hours. After cooling, the reaction mixture was cautiously poured onto water and ice (20 mL) and extracted with $CHCl_3$ (4 x 30 mL). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and evaporated to give a crude product **9** (1.4 g) as yellow foam which was carried to the next step without further purification. ^{1}H-NMR (400 MHz, CD_3OD): δ 8.40-8.44 (m, 2H), 7.89 (t, 1H, J = 6.99 Hz), 7.48 (b, 5H), 4.64-3.20 (m, 7H), 1.10-1.50 (m, 3H); LC retention time: 7.86 minutes, purity: 90%; ESI MS *m*/z: 438.20 $(M+H)^+$.

4-Benzoyl-1-[4-(3-azidopropoxy)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)oxoacetyl]-2-(*R*)-methyl-piperazine (10).[6] To a stirred suspension of NaH (0.55 g, 13.75 mmol, 60% in mineral oil) in anhydrous DMF (9 mL) in a microwave vial (20 mL) was added a solution of 3-azido-1-propanol **(13)** (1.39 g, 13.75 mmol) in dry DMF (3 mL) under an atmosphere of argon at room temperature. The mixture was stirred for 40 min before adding a solution of crude 4-benzoyl-2-(*R*)-methyl-1-[(4-nitro-7-oxido-1*H*-pyrrolo[2,3-b]pyridin-3-yl)oxoacetyl]piperazine **(9)** (1 g) in anhydrous DMF (8 mL). The resultant mixture was then placed in the microwave and irradiated for 15 min at 80°C. The reaction was quenched with MeOH (5 mL) and concentrated under vacuum. The residue was dissolved in $CHCl_3$ (10 mL), and PCl_3 (1.5 mL) was added dropwise. The mixture was allowed to stir at room temperature for 1 hour. The mixture was brought to pH 6-6.5 by addition of 5% NaOH at 0°C and the aqueous layer was extracted with $CHCl_3$ (4 x 20 mL). The combined organic layers were dried and evaporated under vacuum yielding a crude product **10** (1 g) which was used in the next step without further purification. ^{1}H-NMR (400 MHz, CD_3OD): δ 8.21 (d, 1H, J = 4.8 Hz), 8.12 (s, 0.5H), 8.04 (s, 0.5H), 7.43 (bs, 5H), 6.85 (s, 1H), 2.98-4.80 (m, 7H) 2.13(m, 2H), 1.12-1.40 (b, 3H); ^{13}C-NMR (100 MHz, CD_3OD): δ 185.06, 184.91, 172.30, 167.63, 167.53, 160.91, 160.85, 151.36, 147.14, 147.09, 144.98, 136.82, 136.77, 135.14, 130.18, 128.67, 126.96, 113.32, 113.30, 107.32, 101.53, 65.24, 65.21, 50.57, 47.89, 28.35, 15.08, 14.05. ESI MS *m*/z: 476.5 $(M+H)^+$.

4-Benzoyl-1-[4-(3-aminopropoxy)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)oxoacetyl]-2-(*R*)-methyl-piperazine (11).[6] A solution of **10** (1 g) in MeOH (50 mL) and TFA (0.5 mL) containing 10% Pd/C (0.2 g) was hydrogenated at 40 psi in a Parr hydrogenator for 4 hours. The mixture was filtered through a pad of Celite, washed with MeOH (3 x 10 mL), and evaporated. The crude product **11** was purified by flash column chromatography eluting with a mixture of $CHCl_3$ and 9% NH_4OH in MeOH to afford 0.37 g (27% over four steps from **8**) of **11** as pale yellow oil. Compound **11** was further purified by preparative reverse-phase HPLC (Method I). Fractions containing the product were combined and lyophilized to give pure 4-benzoyl-1-[4-(3-aminopropoxy)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)oxoacetyl]-2-(*R*)-methyl-piperazine **(11)** as its acetate salt. Compound **11** was stored under an atmosphere of argon at -20°C until use. ^{1}H-NMR (400 MHz, CD_3OD): δ 8.28 (bd, 1H, J = 5.19 Hz), 8.22 (s, 0.5H), 8.14 (s, 0.5H), 7.48 (b, 5H), 6.95 (d, 1H, J = 4.15 Hz), 3.00-4.84 (m, 11H), 2.29 (bs, 2H), 1.14-1.40 (b, 3H); ^{13}C-NMR (100 MHz, CD_3OD): δ 185.57, 185.46, 172.18, 167.31, 167.19, 160.55, 160.54, 151.71, 147.39, 138.50, 138.39, 135.08, 130.26, 128.71, 126.96, 113.10, 107.04, 101.80, 67.77, 67.72, 50.64, 38.38, 38.34, 26.59, 22.38, 15.07, 13.99. Analytical HPLC (Method I) indicates a purity >99%, retention time: 11.85 minutes; ESI MS *m*/z: 450.20 $(M+H)^+$.

3-Azido-1-propanol (13).[6] Sodium azide (20 g, 307 mmol) was added to a solution of 3-bromo-1-propanol **(12)** (6.7 mL, 76 mmol) in DMF (11 mL) under an atmosphere of argon at room temperature. After heating at 90°C in a pressure tube for 26 hours, the reaction mixture was cooled to room temperature, the excess sodium azide was filtered, and the filtrate was poured onto water and ice (80 mL). The mixture was extracted with CH_2Cl_2 (3 x 40 mL). The combined organic layers were washed with brine, dried and evaporated. The crude product was purified by distillation (ca. 84-86°C/0.5 mmHg) affording 6.68 g (87%) of **13** as a colorless oil which was stored under an atmosphere of argon at 4°C. ^{1}H-NMR (400 MHz, CD_3OD): δ 3.75 (t, 2H, J = 5.79 Hz), 3.46 (t, 2H, J = 6.59 Hz), 1.82-1.88 (m, 2H); ^{13}C-NMR (100 MHz, CD_3OD): δ 58.54, 48.03, 31.52.

4-Benzoyl-2-(*R*)-methyl-1-[(4-nitro-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)oxoacetyl]piperazine (14). To a solution of **9** (356 mg, 0.8 mmol) in $CHCl_3$ (20 mL) was added PCl_3 (2.5 mL) dropwise, followed by stirring at room temperature for 1.5 hours. The reaction mixture was then

cautiously poured onto water and ice (20 mL) and extracted with $CHCl_3$ (3 x 20 mL). The combined organic layers were washed with brine, dried and evaporated to give crude product **14** (320 mg) as yellow foam which was used in the next step without further purification. ^{1}H-NMR (400 MHz, CD_3OD): δ 8.58 (dd, 1H, *J* = 5.14 and 4.81 Hz), 8.50 (d, 1H, *J* = 14.53 Hz), 7.64 (t, 1H, *J* = 3.59 Hz), 7.46 (b, 5H), 3.18-4.80 (m, 7H), 1.18-1.40 (m, 3H); LC-MS (APCI) *m*/z: 422.30 $(M+H)^+$; LC-MS retention time: 5.07 min.

4-Benzoyl-1-[(4-methoxy-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)oxoacetyl]-2-(*R*)-methylpiperazine (BMS-378806).[4] The crude compound **14** was dissolved in anhydrous MeOH (15 mL) under an atmosphere of argon. To that solution was added Na metal (575 mg, 25 mmol) in several portions at 0°C. The ice bath was removed and the mixture was heated to 60°C and stirred for 9 hours. The reaction mixture was cooled to room temperature and cautiously poured onto water and ice (20 mL). The solution was brought to pH 2-3 by addition of 5N HCl and extracted with $CHCl_3$ (3 x 20 mL). The combined organic layers were washed with brine, dried and evaporated. The crude product BMS-378806 (305 mg) was purified by HPLC (Method II) followed by crystallization from ethyl acetate yielding 176 mg (54% over two steps from **9**) as white crystals, mp 184.5-185.4°C. ^{1}H-NMR (400 MHz, CD_3OD): δ 8.29 (b, 1H), 8.18 (d, 1H, *J* = 28.76 Hz), 7.46 (b, 1H), 6.97 (d, 1H, *J* = 5.48 Hz), 3.04-4.80 (m, 7H), 4.06 (s, 3H), 1.10-1.50 (m, 3H); ^{13}C-NMR (100 MHz, CD_3OD): δ 185.49, 172.38, 167.44, 161.97, 150.21, 146.12, 135.89, 135.81, 135.15, 130.17, 128.65, 126.95, 113.31, 101.80, 55.48, 55.40, 50.51, 48.01, 14.86, 14.09; LCMS (APCI) *m*/z: 407.20 $(M+H)^+$; LC-MS retention time: 3.40 min. The purity was found to be ≥98.5% by analytical HPLC.

Gold labeled conjugates 17 and 18. The gold nanoparticle reagents **15** and **16** were reconstituted in 750 μL deionized H_2O. To these solutions was added a solution of **11** (0.300mg, 600nmol) in 200 μL deionized H_2O. The mixtures were allowed to react at room temperature for approximately 2 hrs. Using a Slide-a-Lyzer® cassette (3.5kDa MWCO) solutions were dialyzed to remove excess **11**. Dialyses were run overnight at room temperature against 1L of 0.02M phosphate buffered saline (PBS) at pH 6.5. Buffers were exchanged twice over the next day to ensure complete removal of excess **11**. The purified products were analyzed by size-exclusion HPLC using a 9.4 x 250mm Zorbax GF-450 column. A flow rate of 1 mL min^{-1} and eluent of

0.02M PBS buffer was used in the analytical procedure. The gold nanoparticle-labeled conjugates **17** and **18** were stored in 0.02M PBS buffer, pH 6.5, at -20°C.

References

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