A convenient synthesis of a lymphocyte function-associated antigen-1 (LFA-1) antagonist of 'Compound 4'

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The lymphocyte function-associated antigen-1 (LFA-1) antagonist of 'Compound 4' was synthesised by a convenient route using cheap, commercially available starting materials and catalysts under mild reaction conditions and by easily handled reactions. The total yield in the preparation of 'Compound 4' was more than 38% *via* Sonogashira coupling of an iodide and an alkyne, reduction of the alkyne catalysed by Raney nickel and later steps involving hydrolysis of an ester, condensation of an acid and an amine and a final hydrolysis of an ester.

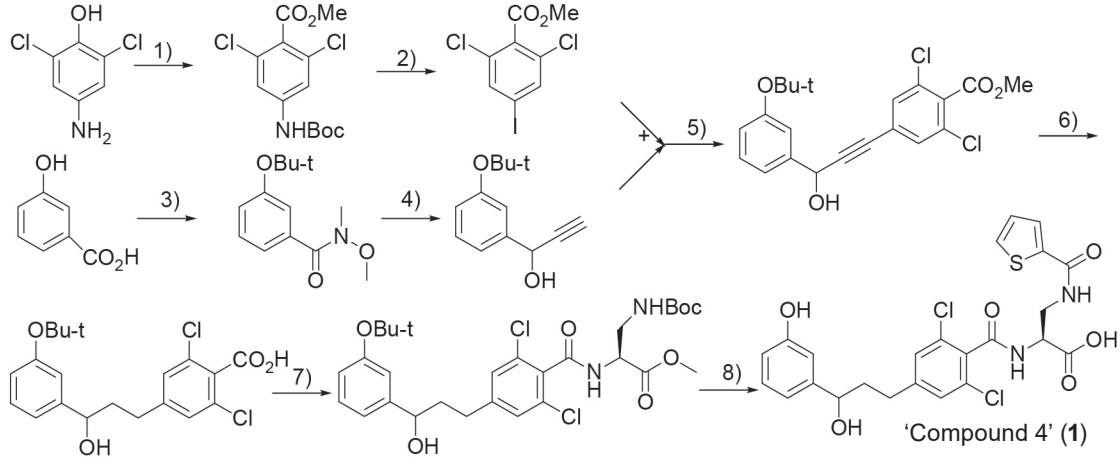
Keywords: lymphocyte function-associated antigen-1 (LFA-1), antagonist, 'Compound 4'

Lymphocyte function-associated antigen-1 (LFA-1), also known as integrin $\alpha_L\beta_2$, belongs to the β_2 integrin subfamily and is constitutively expressed on all leukocytes. LFA-1 stands in a low-affinity state in resting lymphocytes but provokes dramatic conformational changes during lymphocyte activation, resulting in enormous increases in the binding affinities of its ligands to intercellular adhesion molecules -1, -2 and -3 (ICAM-1, -2 and -3). It has been demonstrated that LFA-1 is the major integrin that mediates lymphocyte adhesion and activation, and regulation of LFA-1 activation is pivotal for controlling leukocyte trafficking and immune responses in both health and disease.[1,2] The interactions of LFA-1 and ICAM have been directly implicated in numerous inflammatory diseases, including graft rejection, dermatitis, psoriasis, asthma, inflammatory bowel disease, diabetes mellitus, rheumatoid arthritis, *etc*. Therefore, LFA-1 is an important pharmaceutical target for treating autoimmune and inflammatory diseases.[3–7] In addition to humanised antibodies (*e.g.* efalizumab[8,9]) to LFA-1 that block LFA-1's binding to the ligand ICAM-1 to treat autoimmune disease(s), small molecule antagonists of LFA-1 have been discovered and are in development.[10–15] The first LFA-1 small molecule antagonist of Lifitegrast was approved by the Food and Drug Administration in the USA for the treatment of dry eye disease in 2016.[16,17]

The titled compound (CAS 245466-70-0) (**1**), which was first reported by Genentech Inc. in a patent[18] of WO9949856A2 in 1999 and named as 'Compound 4' in some later references,[19–21] is an LFA-1 antagonist and a potential drug for treatment of autoimmune disease(s). The synthetic procedure (Scheme 1) for 'Compound 4' was only reported in a patent,[18] without, however, any reaction data or physicochemical properties. Moreover, relatively expensive starting materials and catalysts, such as the rhodium catalyst for hydrogenation, and difficult and complicated procedures, including pressured reactors for CO in the carbonylation step and the use of isobutylene in the phenol protection step, were applied, as depicted in Scheme 1. In this paper, we report our modified synthesis of 'Compound 4' and provide important characterisation both of it and of some key intermediates.

Results and discussion

We first prepared 2,6-dichloro-4-iodobenzene carboxylic acid methyl ester **2** from cheap 4-nitro-toluene modified from the references,[22,23] as depicted in Scheme 2. Then, 2,6-dichloro-4-nitrotoluene **3** was prepared in 77% yield in two steps of polychlorination of 4-nitro-toluene by trichloroisocyanuric acid (TCCA) or chlorine, followed by selective reduction of the chlorine group in the *ortho* position to the nitro group with copper and acetic acid as reducing agents in refluxing chlorobenzene. Oxidation of the methyl group in **3** to a carboxylic acid with potassium permanganate ($KMnO_4$) in a mixed solvent of *t*-butanol/water (v/v, 1/1) using the phase transfer catalyst (PTC) triethylbenzylammonium chloride (TEBAC) gave 2,6-dichloro-4-nitrobenzoic acid, and then conversion of the acid in methanol into the methyl ester **4** was carried out by catalysis using H_2SO_4 in more than 61% yield over two steps. After the nitro group in **4** was reduced to the amine by Raney nickel in methanol, the amine intermediate



Scheme 1 Reagents and conditions: 1) (a) Boc_2O, $NaHCO_3$; (b) Tf_2O, 2,6-lutidine; (c) CO, $Pd(OAc)_2$, dppp, TEA, MeOH, DMF; 2) (a) HCl, MeOH; (b) $NaNO_2$, H_2SO_4; (c) KI; 3) (a) MeONHMe-HCl, EDC, HOBt, DIPEA; (b) Isobutylene, H_2SO_4; 4) (a) DIBAL-H; (b) ethynyl magnesium bromide; 5) $Pd(Ph_3P)_2Cl_2$, CuI, TEA, EtOAc; 6) (a) 5% Rh/Al_2O_3; (b) LiI, pyridine; 7) β-Boc-diaminopropionic acid methyl ester, EDC, HOBt, DMF; 8) (a) TFA, DCM; (b) thiophene 2-carboxylic acid, EDC, HOBt, DMF; (c) LiOH, THF/H_2O (3/1).

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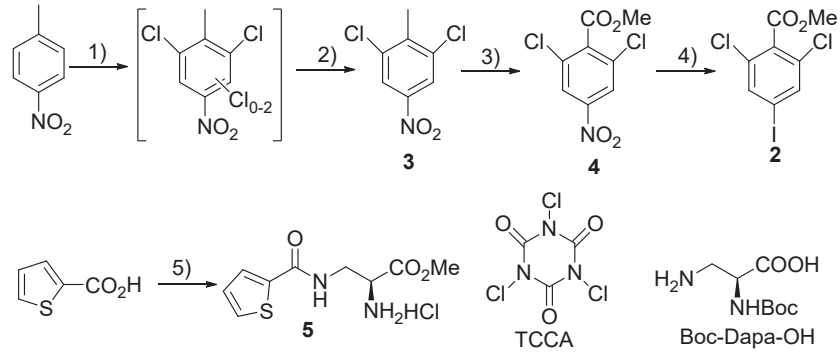
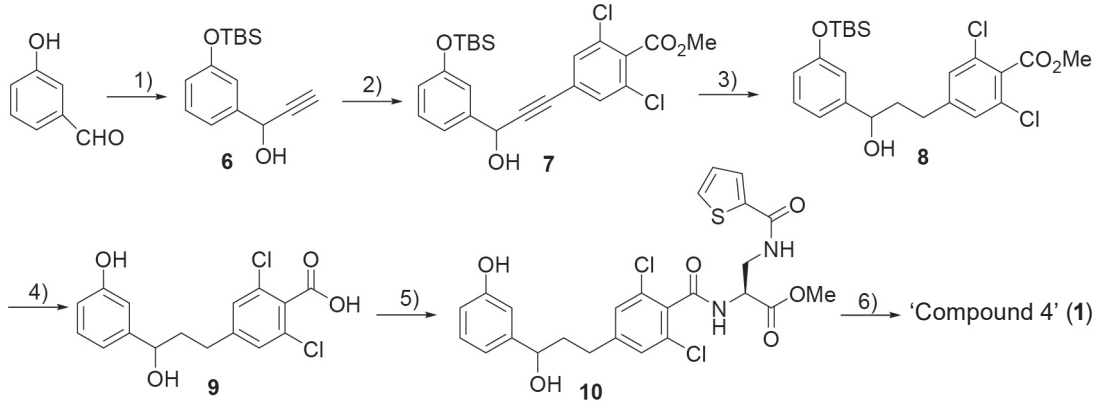


Scheme 2 Reagents and conditions: 1) TCCA, H_2SO_4; 2) Cu powder, HOAc, chlorobenzene, reflux, 77% for two-step yield; 3) (a) $KMnO_4$, TEBAC; (b) H_2SO_4, MeOH; 4) (a) Raney Ni; (b) $NaNO_2$, diluted HCl; (c) KI; 5) (a) $SOCl_2$; (b) Boc-Dapa-OH, NaOH, THF/H_2O (3/1); (c) $SOCl_2$, MeOH.



Scheme 3 Reagents and conditions: 1) (a) TBSCl, imidazole; (b) ethynyl magnesium bromide; 2) **2**, $Pd(PPh_3)_2Cl_2$, CuI, TEA; 3) Raney Ni, H_2; 4) KOH, MeOH/H_2O (v/v, 1/1); 5) **5**, EDC, HOBt and DIPEA in DMF; 6) LiOH, THF/H_2O (3/1).

was forwarded by diazotisation with sodium nitrite and dilute hydrochloric acid, followed by treatment with KI, to give iodide **2** in 69% yield over three steps. The synthetic procedure to give iodide **2** from 4-nitro-toluene is more easily handled than the methods reported by Genentech Inc. and the starting materials are cheap and commercially available.

Synthesis of the key intermediate **5** began with 2-thiophenecarboxylic acid (Scheme 2) by the reaction of thiophene-2-carboxylic acid with thionyl chloride at 80 °C to yield the acyl chloride, which was then condensed with β-amine of Boc-Dapa-OH, followed by deprotection of Boc and esterification with thionyl chloride in methanol.

As shown in Scheme 3, protection of the phenol group in inexpensive 3-hydroxybenzaldehyde by *t*-butyldimethylsilyl (TBS), followed by Grignard reaction of the aldehyde with ethynyl magnesium bromide, provided the intermediate propargyl alcohol **6** in 81% yield over two steps. Coupling of the ethynyl intermediate **6** with iodide **2** by Sonogashira coupling with $Pd(PPh_3)_2Cl_2$, CuI, TEA gave the desired product **7** in a high yield of 91%. Instead of catalysis by the expensive 5% Rh/Al_2O_3 used in the Genentech methods, saturation of the ethynyl group was successfully fulfilled using much cheaper Raney nickel under a hydrogen atmosphere, yielding compound **8** cleanly in 91% yield, *i.e.* a higher yield than the Genentech method. Removal of the TBS protecting group and saponification with KOH were achieved simultaneously, to give the benzoic acid **9** in 89% yield. Finally, the condensation reaction of acid **9** and amine **5** was carried out smoothly using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), hydroxybenzotriazole (HOBt) and *N*,*N*-diisopropylethylamine (DIPEA) in dimethylformamide (DMF) to yield the amide intermediate **10** in 62% yield, which was hydrolysed with lithium hydroxide in THF/water (v/v, 3/1) to yield **1** in 85% yield. The total yield for preparation of 'Compound 4' (**1**) *via* Sonogashira coupling of the iodide **2** and the alkyne **6**, hydrogenation of the alkyne **7** catalysed by Raney nickel, hydrolysis of the ester **8**, condensation of the acid **9** and the amine **5**, and hydrolysis of ester **10** was more than 38%.

Conclusion

In summary, we have developed a new and convenient procedure for the synthesis of an LFA1 antagonist of 'Compound 4' (**1**) from cheap, commercially available starting materials (*e.g.* 4-nitrotoluene in this paper in contrast to 4-amine-2,6-dichlorophenol by Genentech Inc. and 3-hydroxybenzaldehyde in this paper in contrast to the 3-hydroxybenzoic acid used by Genentech). Also, Raney nickel catalyst was used in our procedure reported in this paper rather than the Rh/Al_2O_3 used by Genentech. With the advantages of mild reaction conditions, easily handled reagents and satisfactory yields, this synthesis of 'Compound 4' has potential to be used in drug R&D of 'Compound 4' and similar drug candidates.

Experimental

^1H and ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer at 400 and 101 MHz, respectively, in CD_3OD and DMSO-d_6 ($(CD_3)_2SO$) as indicated. Coupling constants (*J*) are expressed in hertz (Hz) and chemical shifts (δ) are reported in parts per million (ppm) relative to the solvent. High- resolution mass spectra (MS) with electrospray ionisation (ESI) were recorded on an Applied Biosystems Q-STAR Elite ESI-LC-MS mass spectrometer. Unless otherwise noted, chemical materials were obtained from commercial suppliers and were used without further purification. Melting points were measured using a YRT-3 melting point apparatus (Shanghai,

China) and are uncorrected. Optical rotations were recorded on an Autopol III Polarimeter (Rudolph Research Analytical, USA).

1,3-Dichloro-2-methyl-5-nitrobenzene (**3**)[22,23]

To a solution of 4-nitrotoluene (8.2 g, 60 mmol) in concentrated sulfuric acid (50 mL) was added TCCA (13.9 g, 60 mmol). The mixture was then stirred at 60 °C for 20 h. After cooling, the reaction mixture was poured into ice water (200 mL) and filtered with diatomite and the filter cake was washed with methyl *t*-butyl ether (100 mL). The organic layer of the filtrate was separated and the aqueous layer was extracted with methyl *t*-butyl ether (2 × 50 mL). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate and concentrated *in vacuo* to give a crude product (13.0 g) as a yellow solid.

A mixture of crude product, copper powder (12 g, 188 mmol) and chlorobenzene (15 mL) was stirred at 25 °C for 5 min. Acetic acid (10 mL) was then added, and the mixture was refluxed for 20 h. After cooling, the mixture was filtered with diatomite and the filter cake was washed with toluene (100 mL). The organic layer was washed with brine, dried over anhydrous sodium sulfate and then concentrated *in vacuo*. The residue was purified by silica gel column chromatography with petroleum ether as eluent to yield **3** as a light yellow solid; yield 9.5 g) (77%) for two steps; m.p. 60–62 °C (recrystallised from petroleum ether) (lit.[23] 59–62 °C); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.28 (s, 2H), 2.51 (s, 3H).

Methyl 2,6-dichloro-4-nitrobenzoate (**4**)[24]

The mixture of **3** (8.0 g, 38.8 mmol), *t*-butanol (100 mL), water (100 mL) and TEBAC (442 mg, 1.94 mmol) was heated to reflux and then potassium permanganate ($KMnO_4$) (40.0 g, 252 mmol) was added to the mixture in 5 g portions over a period of 4 h. After full addition of $KMnO_4$ to the reaction, the mixture was refluxed for a further 20 h. The resulting mixture was cooled to r.t., filtered with diatomite and the filter cake washed with ethyl acetate (100 mL). The filtrate was adjusted to pH 3 with 1 N hydrochloric acid, and the product was extracted with ethyl acetate (3 × 50 mL). The combined organic phases were dried over anhydrous sodium sulfate and concentrated *in vacuo* to give 2,6-dichloro-4-nitrobenzoic acid (5.8 g) as a light yellow solid, which was used for the next step without further purification; m.p. 170–173 °C (recrystallised from ethyl acetate) (lit.[24] 175.4–176.4 °C); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.41 (s, 2H).

To a solution of 2,6-dichloro-4-nitrobenzoic acid (5.8 g, 24.6 mmol) in MeOH (100 mL), H_2SO_4 (2.0 mL) was added. The mixture was stirred at 80 °C for 20 h. After cooling, the mixture was treated with ice-cooled saturated $NaHCO_3$ solution and extracted with ethyl acetate (2 × 20 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography with petroleum ether/ethyl acetate (v/v, 100/1) as eluent to give **4** (6.0 g, 62% yield over two steps) as a white solid; m.p. 119–122 °C (recrystallised from petroleum ether) (lit.[24] 121.5–123.5 °C); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.48 (s, 2H), 3.99 (s, 3H).

Methyl 2,6-dichloro-4-iodobenzoate (**2**)[22]

To a solution of **4** (6.0 g, 23.8 mmol) in degassed methanol (100 mL) was added wet Raney nickel 1.4 g (23.8 mmol). One balloonful of hydrogen was passed through the solution, and the mixture was stirred for 10 h under a hydrogen atmosphere at r.t. After the reaction was finished, the mixture was filtered through diatomite and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography with petroleum ether/ethyl acetate (v/v, 10/1) as eluent to give methyl 4-amino-2,6-dichlorobenzoate (4.7 g) as a white solid; m.p. 121–123 °C (recrystallised from petroleum ether); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 6.61 (s, 2H), 6.10 (s, 2H), 3.81 (s, 3H).

To a suspension of methyl 4-amino-2,6-dichlorobenzoate (4.0 g, 18.2 mmol) in 2 N hydrochloric acid solution (80 mL), an aqueous solution (10 mL) of sodium nitrite (2.5 g, 36.4 mmol) was added dropwise below 0 °C, and the resulting mixture was stirred for 2 h at 0–5 °C. After a solution of potassium iodide (16 g, 96.8 mmol) in water (50 mL) was slowly added to the mixture, the resulting mixture was stirred for 3 h at r.t. and then poured into water (100 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with 10% aqueous solution of sodium thiosulfate and brine, dried over anhydrous sodium sulfate and then concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography with petroleum ether/ethyl acetate (v/v, 100/1) as eluent to give **2** (4.62 g, 69 % for three-step yield) as a colourless oil; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.03 (s, 2H), 3.91 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 164.7, 136.8, 132.8, 131.5, 98.0, 53.8; HRMS (ESI): *m/z* 352.8603 [M+Na]$^+$; Calcd for $C_8H_5O_2NaCl_2I$: 352.8609.

Methyl (S)-2-((t-butoxycarbonyl)amino)-3-(thiophene-2-carboxamido) propanoate (**5**)

Thionyl chloride (6 mL) and a catalytic amount of DMF were added to a solution of thiophene-2-carboxylic acid (1.13 g, 8.8 mmol) in 1,2-dichloroethane (20 mL). The mixture was refluxed for 2 h before cooling to r.t. and the resulting mixture was concentrated *in vacuo*. The residue was dissolved in anhydrous tetrahydrofuran (THF) (5 mL) and then added to the mixture of Boc-DAPA-OH (lit.[25] $[\alpha]_D^{25} = -14.5$ (*c* 3, H_2O)) (1.5 g, 7.35 mmol) and potassium hydroxide (880 mg, 22.05 mmol) in THF/water (15 mL/5 mL) at 0 °C. The reaction mixture was stirred for 1 h at r.t. and 1 N hydrochloric acid was added to adjust the pH to 3. The product was extracted with ethyl acetate (3 × 30 mL), and the combined organic layer was dried over anhydrous sodium sulfate, concentrated *in vacuo* to give (*S*)-2-((*t*-butoxy carbonyl) amino)-3-(thiophene-2-carboxamido)propanoic acid (2.25 g) as a white solid, which was used for the next step without further purification; m.p. 78–80 °C (recrystallised from ethyl acetate); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 12.63 (s, 1H), 8.52 (t, *J* = 5.7 Hz, 1H), 7.76 (d, *J* = 4.9 Hz, 1H), 7.71 (d, *J* = 3.6 Hz, 1H), 7.14 (dd, *J* = 5.1, 3.7 Hz, 1H), 7.07 (d, *J* = 8.2 Hz, 1H), 4.15 (m, 1H), 3.56 (m, 2H), 1.36 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 172.7, 162.0, 155.8, 140.0, 131.4, 128.8, 128.4, 78.8, 54.0, 40.9, 28.6.

To a solution of (*S*)-2-((*t*-butoxy carbonyl)amino)-3-(thiophene-2-carboxamido)propanoic acid (2.0 g, 6.36 mmol) in methanol (20 mL) was added thionyl chloride (4.6 ml, 63.62 mmol) and the reaction was refluxed for 10 h. After cooling to r.t., the mixture was quenched with saturated $NaHCO_3$ solution, extracted with ethyl acetate (30 mL) and washed with brine. The organic layer was dried over anhydrous sodium sulfate and then concentrated under depressed pressure. The crude residue was purified by silica gel column chromatography with dichloromethane/methanol (v/v, 50/1) as the eluent to give intermediate **5** (1.68 g, 97% yield for two steps) as a white solid; m.p. 128–130 °C (recrystallised from dichloromethane); $[\alpha]_D^{25} = -1.4$ (*c* 1, CH_3OH); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 9.06 (t, *J* = 6.1 Hz, 1H), 8.79 (s, 3H), 7.94 (dd, *J* = 3.7, 1.2 Hz, 1H), 7.80 (dd, *J* = 5.0, 1.2 Hz, 1H), 7.17 (dd, *J* = 5.0, 3.7 Hz, 1H), 4.18 (t, *J* = 5.6 Hz, 1H), 3.82–3.72 (m, 2H), 3.71 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 168.7, 162.3, 139.5, 131.8, 129.6, 128.4, 53.4, 52.6, 40.8; HRMS (ESI): *m/z* 229.0644 [M+H]$^+$; Calcd for $C_9H_{13}N_2O_3S$: 229.0641.

1-(3-((t-Butyldimethylsilyl)oxy)phenyl)prop-2-yn-1-ol (**6**)[26]

m-Hydroxybenzaldehyde (1.0 g, 8.2 mmol) and imidazole (3.0 g, 24.6 mmol) in dry dichloromethane (50 mL) were treated with *t*-butyldimethylsilyl chloride (TBSCl) (5.0 g, 73.7 mmol) at r.t., and the reaction mixture was stirred for a further 10 h at r.t. Water (100 mL) was added to the mixture, which was then extracted with ethyl acetate (50 mL). The organic layer was washed with brine and dried over anhydrous sodium sulfate. After removing the solvent *in vacuo*, the residue was purified by silica gel column chromatography with petroleum ether as the eluent to give 3-((*t*-butyldimethylsilyl)oxy) benzaldehyde (5.6 g, 96%) as a colourless oil; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 9.96 (s, 1H), 7.53 (m, 1H), 7.49 (t, *J* = 7.6 Hz, 1H), 7.33 (dd, *J* = 2.6, 1.4 Hz, 1H), 7.19 (m, 1H), 0.95 (s, 9H), 0.21 (s, 6H).

Ethynyl magnesium bromide (0.5 M, THF, 30 mL, 15 mmol) was added dropwise to a solution of 3-((*t*-butyldimethylsilyl)oxy)benzaldehyde (3.0 g, 12.7 mmol) in THF (50 mL) at 0 °C under a nitrogen atmosphere. After the mixture was stirred for 12 h at 30 °C, an aqueous solution of ammonium

chloride was added to quench the reaction. The product was extracted with ethyl acetate (50 mL), washed with brine and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the crude residue was purified by silica gel column chromatography with petroleum ether/ethyl acetate (v/v, 70/1) to give **6** (2.8 g, 81% yield over two steps) as a light yellow oil; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 7.23 (t, *J* = 7.8 Hz, 1H),7.04 (d, *J* = 7.6 Hz, 1H), 6.95 (t, *J* = 2.1 Hz, 1H), 6.76 (dd, *J* = 8.0, 1.9 Hz, 1H), 6.02 (d, *J* = 6.1 Hz, 1H), 5.30 (dd, *J* = 6.2, 2.4 Hz, 1H), 3.48 (d, *J* = 2.3 Hz, 1H), 0.95 (s, 9H), 0.19 (s,6H); ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 155.4, 144.1, 129.8, 119.9, 119.3, 118.2, 85.9, 76.2, 62.5, 26.0, 18.4, −4.1; HRMS (ESI): *m/z* 285.1280 [M+Na]$^+$; Calcd for $C_{15}H_{22}O_2NaSi$: 285.0378.

*Methyl-4-(3-(3-((*t*-butyldimethylsilyl)oxy)phenyl)-3-hydroxyprop-1-yn-1-yl)-2,6-dichlorobenzoate* (**7**)

Under a nitrogen atmosphere, $Pd(PPh_3)_2Cl_2$ (393 mg, 0.56 mmol), cuprous iodide (212 mg, 1.13 mmol) and trimethylamine (7.9 ml, 56.7 mmol) were added to a solution of **2** (3.75g, 11.3 mmol) and **6** (3.0 g, 11.4 mmol) in ethyl acetate (50 mL). The mixture was stirred for 10 h at r.t. and then an aqueous solution of $NaHCO_3$ was added. The product was extracted with ethyl acetate (50 mL), and the organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography with petroleum ether/ethyl acetate (v/v, 50/1) as eluent to give **7** (4.8 g, 91%) as a light brown oil; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 7.68 (s, 2H), 7.27 (t, *J* = 7.8 Hz, 1H), 7.14 (d, *J* = 7.6 Hz, 1H), 7.01 (t, *J* = 2.2 Hz, 1H), 6.80 (dd, *J* = 8.0, 1.9 Hz, 1H), 6.30 (d, *J* = 6.1 Hz, 1H), 5.60 (d, *J* = 6.1 Hz, 1H), 3.92 (s, 3H), 0.95 (s, 9H), 0.19 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 164.6, 155.6, 143.5, 133.2,131.3, 131.1, 130.0, 126.6, 120.1, 119.7, 118.4, 95.9, 81.5, 63.0, 53.9, 26.0, 18.4, −4.1; HRMS (ESI): *m/z* 487.0869 [M+Na]$^+$; Calcd for $C_{23}H_{26}Cl_2O_4NaSi$: 486.0875.

*Methyl-4-(3-(3-((*t*-butyldimethylsilyl)oxy)phenyl)-3-hydroxy-propyl)-2,6-dichloro-benzoate* (**8**)

To a solution of **7** (3.5 g, 7.5 mmol) in degassed methanol (100 mL) was added wet Raney nickel (440 mg, 7.5 mmol), and then one balloonful of hydrogen was passed through the solution. The mixture was stirred for 7 h under a hydrogen atmosphere at r.t. to complete hydrogenation, and then the mixture was filtered through diatomite and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography with petroleum ether/ethyl acetate (v/v, 50/1) as eluent to give **8** (3.2 g, 91% yield) as a light brown oil; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 7.40 (s, 2H), 7.18 (t, *J* = 7.8 Hz, 1H), 6.91 (d, *J* = 7.7 Hz, 1H), 6.82 (t, *J* = 2.1 Hz, 1H), 6.69 (dd, *J* = 8.0, 1.7 Hz, 1H), 5.32 (d, *J* = 4.6 Hz, 1H), 4.53–4.41 (m, 1H), 3.89 (s, 3H), 2.80–2.54 (m, 2H), 1.86 (m, 2H), 0.94 (s, 9H), 0.17 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 165.2, 155.4, 148.2, 147.9, 130.7, 130.67, 129.5, 128.6, 119.4, 118.5, 117.7, 71.6, 53.6, 40.5, 31.4, 26.0, 18.4, −4.0; HRMS (ESI): *m/z* 491.1183 [M+Na]$^+$; Calcd for $C_{23}H_{30}Cl_2O_4NaSi$: 491.1188.

2,6-Dichloro-4-(3-hydroxy-3-(3-hydroxyphenyl)propyl)benzoic acid (**9**)

Compound **8** (1.0 g, 2.1 mmol) was dissolved in methanol (10 mL) and water (10 mL), and potassium hydroxide (358 mg, 6.4 mmol) was added and the reaction mixture was stirred at r.t. for 20 h. After completion of the reaction, 1 N hydrochloric acid was added to adjust the pH to 3–4. The mixture was extracted with ethyl acetate (3 × 20 mL), and the combined organic layer was dried over anhydrous sodium sulfate, concentrated *in vacuo* to give **9** (650 mg, 89%) as a light brown oil, which was used for the next step without further purification; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 9.28 (s, 1H), 7.38 (s, 2H), 7.09 (t, *J* = 7.8 Hz, 1H), 6.75 (t, *J* = 2.1 Hz, 1H), 6.72 (d, *J* = 7.7 Hz, 1H), δ 6.60 (dd, *J* = 8.0, 1.6 Hz, 1H), 5.25 (d, *J* = 4.3 Hz, 1H), 4.40 (m, 1H), 2.66 (m, 2H), 1.84 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 166.0, 157.6, 147.9, 146.9, 132.5, 130.2, 129.4, 128.4, 116.9, 114.1, 113.1, 71.8, 39.4, 31.3.

Methyl-(2S)-2-(2,6-dichloro-4-(3-hydroxy-3-(3-hydroxyphenyl) propyl)benzamido)-3-(thiophene-2-carboxamido)propanoate (**10**)

To a solution of **9** (600 mg, 1.76 mmol) in DMF (20 mL) at r.t., EDC (674 mg, 3.52 mmol), HOBt (356 mg, 2.64 mmol), **5** (465 mg, 1.76 mmol) and DIPEA (1.56 ml, 8.79 mmol) were added. After stirring for 24 h at r.t., the mixture was diluted with ethyl acetate (50 mL) and washed with brine. The organic layer was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with dichloromethane/methanol (v/v, 50/1) as eluent to give **10** (605 mg, 62% yield) as a white solid; m.p. 68–70 °C (recrystallised from ethyl acetate); $[\alpha]_D^{25}$ = −20.0 (*c* 1, CH_3OH); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 9.29 (s, 1H), 9.12 (d, *J* = 7.7 Hz,1H), 8.53 (t, *J* = 5.8 Hz, 1H), 7.77 (dd, *J* = 5.0, 1.1 Hz, 1H), 7.69 (dd, *J* = 3.7, 1.2 Hz, 1H), 7.34 (s, 2H), 7.16 (dd, *J* = 5.0, 3.8 Hz, 1H), 7.10 (t, *J* = 7.8 Hz, 1H), 6.76 (t, *J* = 2.1 Hz, 1H), 6.73 (d, *J* = 7.7 Hz, 1H), δ 6.61 (dd, *J* = 8.0, 1.7 Hz, 1H), 5.25 (d, *J* = 4.4 Hz, 1H), 4.74 (m, 1H), 4.41 (m, 1H), 3.65 (s, 3H), 3.60 (dd, *J* =13.3, 6.8 Hz, 2H), 2.74–2.56 (m, 2H), 1.84 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 170.7, 164.2, 161.9, 157.7, 147.9, 146.7, 139.9, 133.7, 131.44, 131.41, 129.4, 128.8, 128.4, 128.3, 116.9, 114.1, 113.1, 71.7, 52.5, 52.1, 40.8, 39.4, 31.3.

(2S)-2-(2,6-Dichloro-4-(3-hydroxy-3-(3-hydroxyphenyl)propyl) benzamido)-3-(thiophene-2-carboxamido) propanoic acid 'Compound 4' (**1**)

A solution of **10** (605 mg, 1.1 mmol) in THF/water (15 mL/5 mL) was treated with lithium hydroxide (78 mg, 3.39 mmol). After the mixture was stirred for 10 h at r.t., 1 N hydrochloric acid was added to adjust the pH to 3, and then the product was extracted with ethyl acetate (3 × 30 mL), dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with ethyl acetate/methanol (v/v, 30/1) as eluent to give **1** (510 mg, 85%) as a white solid; m.p. 109–111 °C (recrystallised from methyl *t*-butyl ether); $[\alpha]_D^{25}$ = −17.3 (*c* 1, CH_3OH); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 12.84 (s, 1H), 9.29 (s, 1H), 8.97 (d, *J* = 8.1 Hz, 1H), 8.47 (t, *J* = 5.7 Hz, 1H), 7.76 (dd, *J* = 5.0, 0.9 Hz, 1H), 7.70 (dd, *J* = 3.7, 1.3 Hz, 1H), 7.33 (s, 2H), 7.16 (dd, *J* = 5.0, 3.7 Hz, 1H), 7.11 (t, *J* = 7.8 Hz, 1H), 6.77 (t, *J* = 2.1 Hz, 1H), 6.74 (d, *J* = 7.3 Hz, 1H), 6.62 (dd, *J* = 8.0, 1.8 Hz, 1H), 5.25 (d, *J* = 4.5 Hz, 1H), 4.73 (m, 1H), 4.41 (m, 1H), 3.72–3.55 (m, 2H), 2.75–2.56 (m, 2H), 1.85 (m, 2H); ^{13}C NMR (101 MHz, CD_3OD): δ (ppm) 171.1, 166.1, 163.4, 157.2, 146.4, 146.2, 138.5, 133.0, 131.7, 130.5, 129.0, 128.6, 127.8, 127.4, 116.8, 113.9, 112.4, 72.4, 52.2, 40.7, 39.8, 31.0; HRMS (ESI): *m/z* 537.0651 [M+H]$^+$; Calcd for $C_{24}H_{23}N_2O_6SCl_2$: 537.0654.

Acknowledgements

This work was supported in part by the National Natural Science Foundation of China (30973621) and Six Major Talents of Jiangsu Province of China (2014).

Electronic supplementary information

The supplementary data associated with this paper can be found at:

http://ingentaconnect.com/content/stl/jcr/2018/00000042/00000005/art00013

Received 2 April 2018; accepted 9 May 2018
Paper 1805354
https://doi.org/10.3184/174751918X15272594754006
Published online: 30 May 2018

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