Supplementary Material (ESI) for Chemical Communications

Reversible Michael addition of thiols as a new tool for dynamic combinatorial chemistry

Baolu Shi and Michael F. Greaney*

University of Edinburgh, School of Chemistry, Joseph Black Building, King's Buildings, West Mains Rd, Edinburgh EH9 3JJ, UK; E-mail: Michael.Greaney@ed.ac.uk

General

All reagents were purchased from commercial suppliers and used as received. Dichloromethane (DCM) was distilled from CaH_2. TLC was performed with Merck aluminium plates silica gel 60 F_{254}. Melting points were recorded on a Gallenkamp melting point instrument and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker DPX360 MHz NMR spectrometer unless otherwise specified. Chemical shifts δ are quoted in ppm relative to the $CDCl_3$ signal as reference. Coupling constants are given in Hz. Mass spectra were obtained from EPSRC National Mass Spectrometry Service Centre, University of Wales, Swansea.

Syntheses of amide derivatives of ethacrynic acid 4b-4f

Ethacrynic acid (151.6 mg, 0.5 mmol), HOBt·H_2O (84.2 mg, 0.55 mmol), EDCI (105.4 mg, 0.55 mmol) and DIPEA (95 µl, 0.55 mmol) were dissolved in DCM (10 mL) and the mixture stirred at room temperature for 10 minutes. The primary or secondary amine (0.75 mmol) and DIPEA (130 µl, 0.75 mmol) were then added and the reaction left to stir at room temperature overnight. The reaction was worked up in the usual fashion, followed by flash column chromatography with DCM/EtOAc mixtures to yield the amides **4b** – **4f** as white solids (17-40% yields).

1-[2,3-Dichloro-4-(2-oxo-2-piperidin-1-yl-ethoxy)-phenyl]-2-ethyl-propenone 4b

R_f = 0.4 (SiO_2, DCM:EtOAc 9:1); m.p. 79-80°C (DCM/EtOAc); ^1H NMR (360 MHz, $CDCl_3$) δ = 1.12 (3H, t, J = 7.4, CH_3), 1.51-1.63 (6H, m, $(CH_2)_3$), 2.44 (2H, q, J = 7.4, CH_2), 3.49-3.54 (4H, m, $N(CH_2)_2$), 4.80 (2H, s, OCH_2), 5.58 (1H, s, vinyl), 5.91 (1H, s, vinyl), 6.95 (1H, d, J = 8.6, ArH), 7.11 (1H, d, J = 8.6, ArH); ^{13}C NMR (91 MHz, $CDCl_3$) δ=12.2, 23.2, 24.2, 25.4, 26.4, 43.2, 46.4, 68.6, 110.5, 122.6, 126.9, 128.6, 131.1, 133.2, 150.0, 155.3, 164.7, 195.8; HRMS (ES+) calcd. for $C_{18}H_{25}Cl_2N_2O_3$ (MNH_4^+): 387.1237, found: 387.1242.

1-[2,3-Dichloro-4-(2-morpholin-4-yl-2-oxo-ethoxy)-phenyl]-2-ethyl-propenone 4c

R_f = 0.4 (SiO_2, DCM:EtOAc 6:4); m.p. 114-115°C (DCM/EtOAc); ^{1}H NMR (360 MHz, $CDCl_3$) δ=1.11 (3H, t, J = 7.4, CH_3), 2.44 (2H, q, J = 7.3, CH_2), 3.60-3.69 (8H, m, $N(CH_2CH_2)_2O$), 4.81 (2H, s, OCH_2), 5.57 (1H, s, vinyl), 5.93 (1H, s, vinyl), 6.95 (1H, d, J = 8.6, ArH), 7.13 (1H, d, J = 8.5, ArH); ^{13}C NMR (91 MHz, $CDCl_3$) δ= 12.2, 23.2, 42.4, 45.9, 66.6, 68.4, 110.5, 122.6, 126.9, 128.6, 131.3, 133.6, 150.0, 154.9, 165.1, 195.6; HRMS(ES+) calcd. for $C_{17}H_{23}Cl_2N_2O_4$ (MNH_4^+): 389.1029, found: 389.1033.

{2-[2,3-Dichloro-4-(2-ethyl-acryloyl)-phenoxy]-acetylamino}-acetic acid methyl ester 4d

R_f = 0.4 (SiO_2, DCM:EtOAc 8:2); m.p. 92-93°C (DCM/EtOAc); ^{1}H NMR (360 MHz, $CDCl_3$) δ=1.09 (3H, t, J = 7.3, CH_3), 2.41 (2H, q, J = 7.3, CH_2), 3.73 (3H, s, $C(O)OCH_3$), 4.11 (2H, d, J = 5.3, CH_2), 4.57 (2H, s, OCH_2), 5.53 (1H, s, vinyl), 5.90 (1H, s, vinyl), 6.81 (1H, d, J = 8.5, ArH), 7.13 (1H, d, J = 8.5, ArH); ^{13}C NMR (91 MHz, $CDCl_3$) δ= 12.2, 23.2, 40.7, 52.4, 67.9, 110.7, 122.9, 127.0, 128.7, 131.4, 134.1, 150.0, 154.3, 166.9, 169.5, 195.5; HRMS(ES+) calcd. for $C_{16}H_{21}Cl_2N_2O_5$ (MNH_4^+): 391.0822, found: 391.0827.

2-[2,3-Dichloro-4-(2-ethyl-acryloyl)-phenoxy]-*N*-(2,2,2-trifluoro-ethyl)-acetamide 4e

R_f = 0.5 (SiO_2, DCM:EtOAc 6:4); m.p. 110-111°C (DCM/EtOAc); ^{1}H NMR (360 MHz, $CDCl_3$) δ= 1.13 (3H, t, J = 7.6, CH_3), 2.45 (2H, q, J = 7.6, CH_2), 4.04 (2H, m, NCH_2CF_3), 4.64 (2H, s, OCH_2), 5.57 (1H, s, vinyl), 5.95 (1H, s, vinyl), 6.85 (1H, d, J = 8.5, ArH), 7.18 (1H, d, J = 8.5, ArH). ^{13}C NMR (91 MHz, $CDCl_3$) δ= 12.2, 23.2, 40.1 (q, J = 34.6, CH_2CF_3), 67.9, 110.9, 122.9, 123.6 (q, J = 279.4, CH_2CF_3), 127.1, 128.8, 131.4, 134.3, 150.0, 154.0, 167.1, 195.4; HRMS(EI+) calcd. for $C_{15}H_{14}Cl_2F_3NO_3$ (M^+): 383.0297, found: 383.0297.

1-[2,3-Dichloro-4-(2-oxo-2-pyrrolidin-1-yl-ethoxy)-phenyl]-2-ethyl-propenone 4f

R_f = 0.6 (SiO_2, DCM:EtOAc 9:1); m.p. 119-120°C (DCM/EtOAc); ^{1}H NMR (360 MHz, $CDCl_3$) δ= 1.11 (3H, t, J = 7.6, CH_3), 1.80-2.00 (4H, m, CH_2CH_2), 2.43 (2H, q, J = 7.6, CH_2), 3.46-3.56 (4H, m, $N(CH_2)_2$), 4.74 (2H, s, OCH_2), 5.59 (1H, s, vinyl), 5.91 (1H, s, vinyl), 6.92 (1H, d, J = 8.6, ArH), 7.11 (1H, d, J = 8.6, ArH); ^{13}C NMR (91 MHz, $CDCl_3$) δ=12.2, 23.2, 23.6, 26.1, 46.0, 46.2, 68.9, 110.7, 122.6, 126.9, 128.6, 131.1, 133.3, 149.9, 155.4, 165.1, 195.8; HRMS(ES+) calcd. for $C_{17}H_{23}Cl_2N_2O_3$ (MNH_4^+): 373.1080; found: 373.1081.

Synthesis of GSH-EA adduct 5a

To a stirred solution of ethacrynic acid (151.6 mg, 0.5 mmol) in aqueous DMF (2:1 H_2O:DMF, 30 mL) was added glutathione (230.5 mg, 0.75 mmol) and aqueous NaOH (7.5 mL, 0.1M). The

reaction was allowed to stir at r.t. and monitored by HPLC at regular intervals. Using a pH meter, the pH was maintained at *ca.* 8 through occasional titration of aq. NaOH solution.

After completion, the pH value was adjusted to 6 through the addition of 0.1 M aq. HCl. The mixture was concentrated *in vacuo* to produce the adduct **5a** as a white solid. ^{1}H NMR analysis (600 MHz, DMSO) indicated a 1:1 mixture of diastereoisomers.[1]

Generation of dynamic combinatorial library (DCL)

An aqueous solution of reduced glutathione (GSH) (10 mM, 200 μl), aqueous NaOH (10 mM, 200 μl) and the five ethacrynic acid derivatives **4a** – **4e** (5x4 μl, 0.1 M in DMSO) were added to aqueous DMSO (1:1 DMSO: H_2O, 2 mL). The reaction was allowed to stir at r.t. and monitored by HPLC at regular intervals. Using a pH meter, the pH was maintained at *ca.* 8 through occasional titration of aq. NaOH solution.

HPLC conditions: column Luna 5 μ C18(2) 30 × 4.60 mm, flow rate 2 mL min^{-1}, wavelength 254 nm, temperature 23 °C, gradient H_2O/MeCN (0.01% TFA) 95:5 over first 2 min, then 80:20 to 60:40 over 6 min, 60:40 to 5:95 over next 4 min.

[1] Van Iersel M. L. P. S.; van Lipzig, M. M. H.; Rietjens, I. M. C. M.; Vervoort, J.; van Bladeren P. J. *FEBS Lett.*, **1998**, *441*, 153-157.

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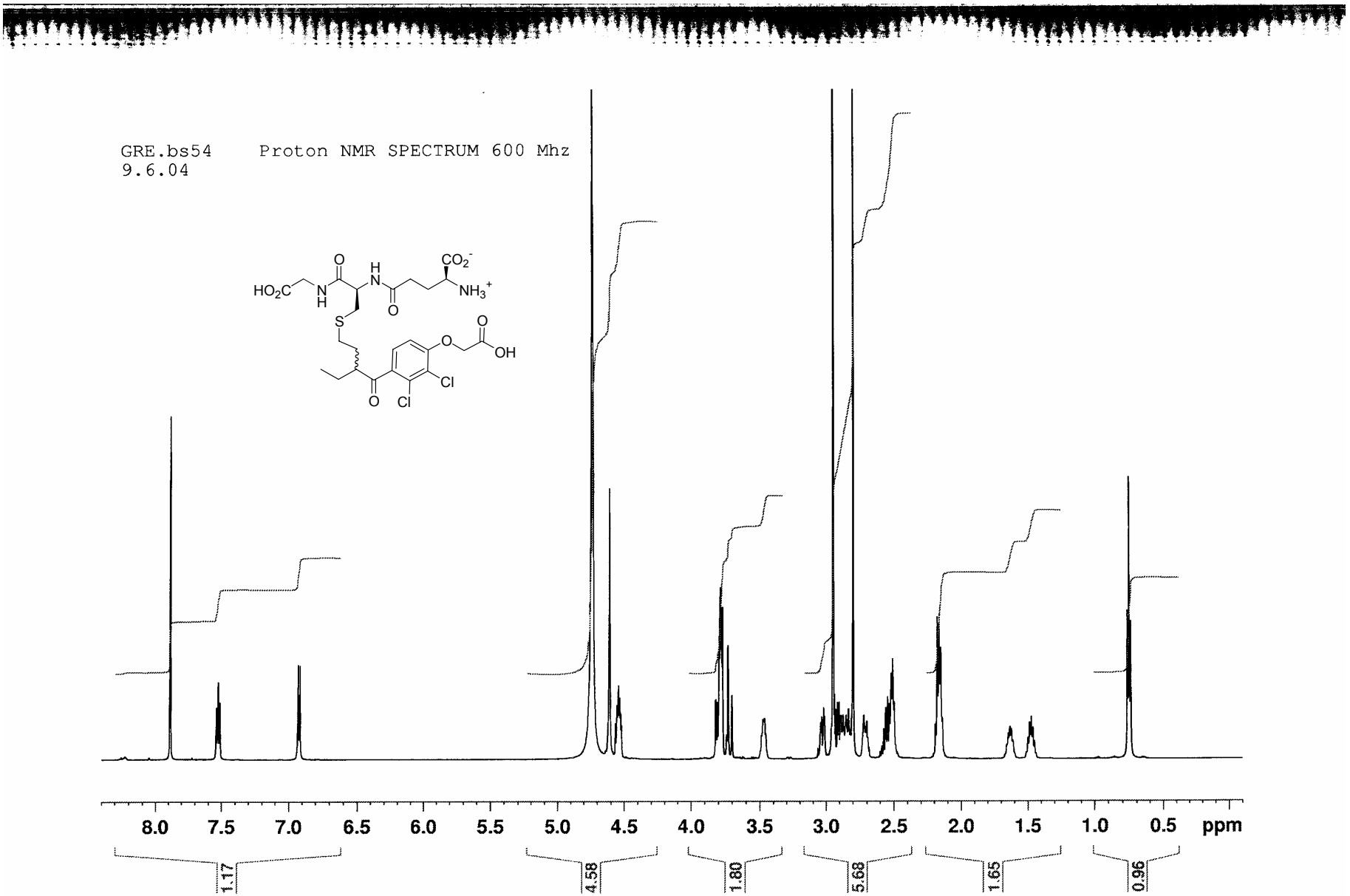

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