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SUPPLEMENTARY INFORMATION

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A metabolite-derived protein modification integrates glycolysis with KEAP1–NRF2 signalling

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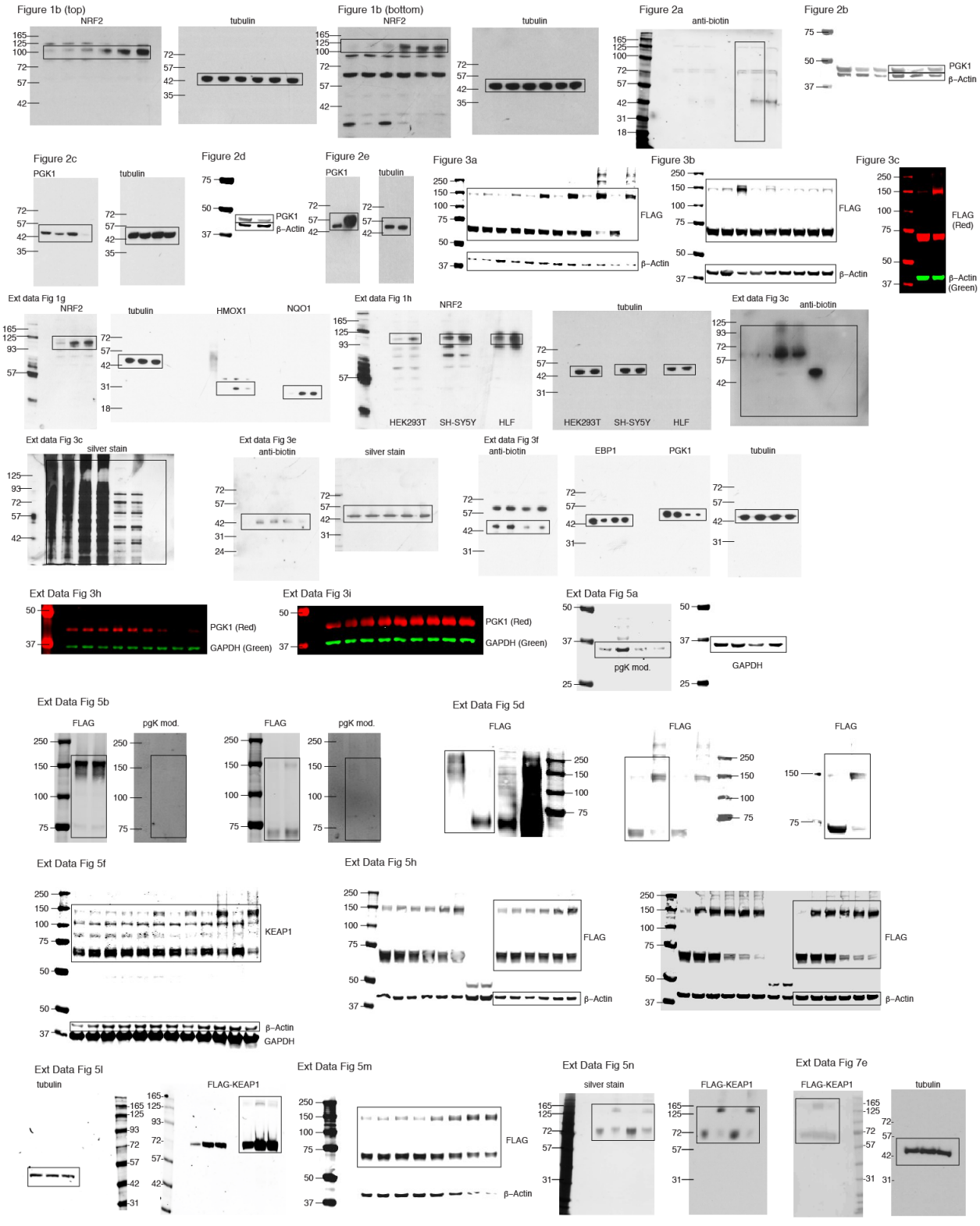
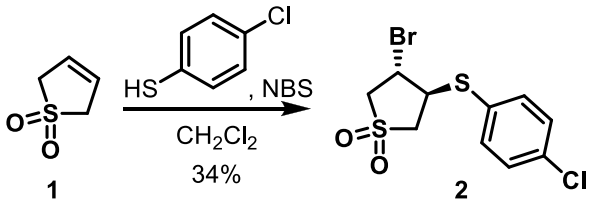


Supplementary Figure 1: Uncropped scans with size marker indications.

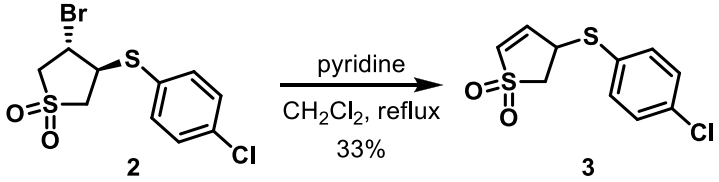
Supplementary Methods

Synthesis of CBR-470-1



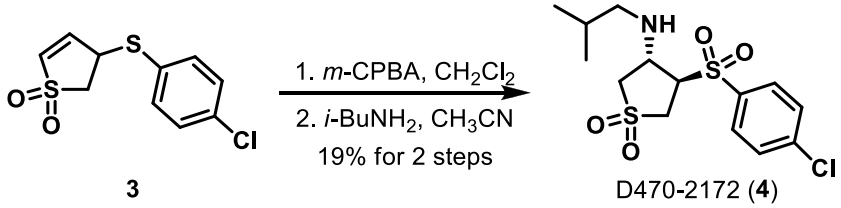
1 2

To a stirred solution of ***N***-bromosuccinimide (3.15 g, 17.7 mmol) in CH_2Cl_2 (25 mL) was added dropwise a solution of 4-chlorothiophenol (2.56 g, 17.7 mmol). After stirred for 30 min, to the reaction mixture was added dropwise a solution of 3-sulfolene **1** (2.09 g, 17.7 mmol). After stirring for 2 h, the reaction mixture was quenched with H_2O. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were dried over $MgSO_4$ and concentrated ***in vacuo***. The residue was purified by flash column chromatography on silica gel (EtOAc : ***n***-hexane = 1 : 4) to afford 2.04 g (34%) of **2** as white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.45 (d, 2H, ***J*** = 8.5 Hz), 7.37 (d, 2H, ***J*** = 8.5 Hz), 4.31 (q, 1H, ***J*** = 7.0 Hz), 4.21 (q, 1H, ***J*** = 7.3 Hz), 3.93 (q, 1H, ***J*** = 7.0 Hz), 3.70 (q, 1H, ***J*** = 7.1 Hz), 3.49 (q, 1H, ***J*** = 6.9 Hz), 3.12 (q, 1H, ***J*** = 7.1 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 135.9, 135.5, 130.0, 128.5, 59.3, 55.8, 51.7, 43.5; LR-MS (ESI+) ***m***/***z*** 340 (M + H^+); HR-MS (ESI+) calculated for $C_{10}H_{11}BrClO_2S_2$ (M + H^+) 340.9067; found 340.9068.



2 3

To a stirred solution of **2** (2.04 g, 5.94 mmol) in CH_2Cl_2 (15 mL) was added pyridine (1.20 mL, 14.9 mmol). After stirring for 3 h at 70 ℃, the reaction mixture was cooled to room temperature and quenched with saturated ***aq.*** NH_4Cl. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were dried over $MgSO_4$ and concentrated ***in vacuo***. The residue was purified by flash column chromatography on silica gel (EtOAc : ***n***-hexane = 1 : 3) to afford 513 mg (33%) of **3** as white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.39 (d, 2H, ***J*** = 8.6 Hz), 7.33 (d, 2H, ***J*** = 8.6 Hz), 6.67 (m, 2H), 4.43 (m, 1H), 3.63 (q, 1H, ***J*** = 7.4 Hz), 3.19 (q, 1H, ***J*** = 6.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 139.3, 135.9, 135.5, 133.2, 129.9, 128.9, 54.3, 45.0; LR-MS (ESI+) ***m***/***z*** 260 (M + H^+); HR-MS (ESI+) calculated for $C_{10}H_{10}ClO_2S_2$ (M + H^+) 260.9805; found 260.9807.

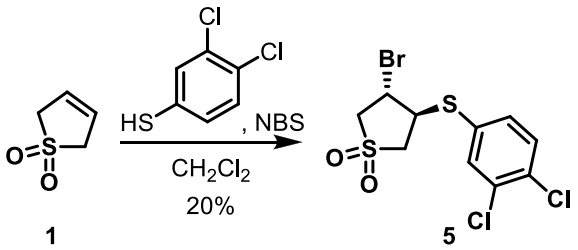


3 D470-2172 (**4**)

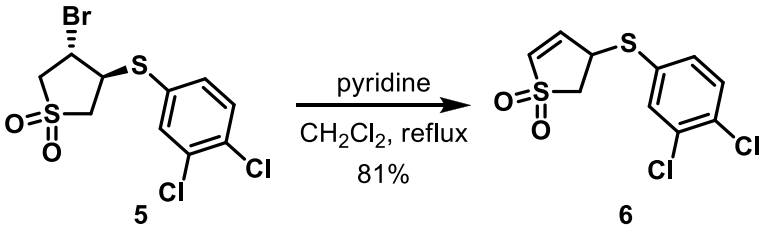
To a stirred solution of **3** (6.01 g, 23.0 mmol) in CH_2Cl_2 (100 mL) was added ***m***-CPBA (14.2 g, 57.5 mmol, 70~75%). After stirring for 3 h, the reaction mixture was filtered and quenched with

saturated ***aq.*** $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were dried over $MgSO_4$ and concentrated ***in vacuo***. The crude mixture was used for next step without further purification. To a stirred solution of the sulfone in CH_3CN (50 mL) was added ***i***-butylamine (2.29 mL, 23.0 mmol). After stirring for 2 h, the reaction mixture was concentrated ***in vacuo***. The residue was purified by preparative HPLC and lyophilized to afford 1.60 g (19%) of CBR-470-1 (D470-2172, **4**) as white solid: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.97 (d, 2H, ***J*** = 8.6 Hz), 7.76 (d, 2H, ***J*** = 8.6 Hz), 4.30 (m, 1H), 3.64 (m, 3H), 3.37 (m, 1H), 3.13 (q, 1H, ***J*** = 6.9 Hz), 2.16 (m, 1H), 2.05 (m, 1H), 1.87 (q, 1H, ***J*** = 7.5 Hz), 1.33 (m, 1H), 0.69 (d, 3H, ***J*** = 2.4 Hz), 0.67 (d, 3H, ***J*** = 2.5 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 139.6, 136.6, 130.7, 129.5, 63.5, 55.2, 55.1, 54.4, 49.5, 27.9, 20.3; LR-MS (ESI+) ***m/z*** 366 (M + H^+); HR-MS (ESI+) calculated for $C_{14}H_{21}ClNO_4S_2$ (M + H^+) 366.0595; found 366.0595.

Synthesis of CBR-470-2 and related analogs

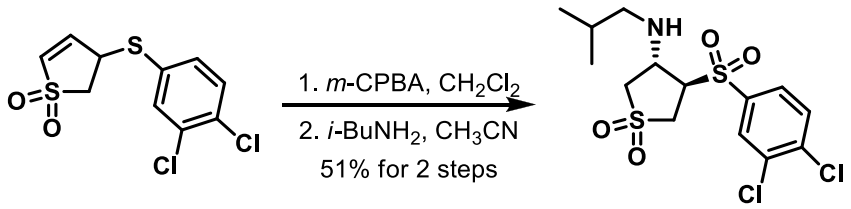


To a stirred solution of ***N***-bromosuccinimide (2.26 g, 12.7 mmol) in CH_2Cl_2 (20 mL) was added dropwise a solution of 3,4-dichlorobenzenethiol (1.62 mL, 12.7 mmol). After stirred for 30 min, to the reaction mixture was added dropwise a solution of 3-sulfolene **1** (1.50 g, 12.7 mmol). After stirring for 2 h, the reaction mixture was quenched with H_2O. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were dried over $MgSO_4$ and concentrated ***in vacuo***. The residue was purified by flash column chromatography on silica gel (EtOAc : ***n***-hexane = 1 : 5) to afford 974 mg (20%) of **5** as white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.59 (d, 1H, ***J*** = 2.1 Hz), 7.46 (d, 1H, ***J*** = 8.3 Hz), 7.33 (dd, 1H, ***J*** = 8.3, 2.1 Hz), 4.34 (q, 1H, ***J*** = 7.0 Hz), 4.03 (q, 1H, ***J*** = 7.3 Hz), 3.93 (q, 1H, ***J*** = 7.1 Hz), 3.73 (q, 1H, ***J*** = 7.1 Hz), 3.50 (q, 1H, ***J*** = 7.0 Hz), 3.14 (q, 1H, ***J*** = 7.1 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 135.3, 134.2, 133.8, 133.0, 131.6, 130.4, 59.4, 55.9, 51.9, 43.4; LR-MS (ESI+) ***m/z*** 374 (M + H^+); HR-MS (ESI+) calculated for $C_{10}H_{10}BrCl_2O_2S_2$ (M + H^+) 374.8677; found 374.8679.



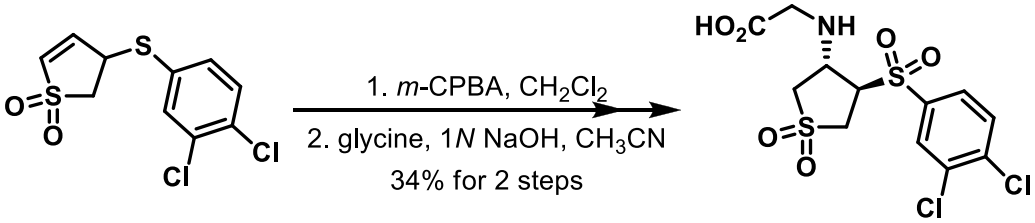
To a stirred solution of **5** (487 mg, 1.29 mmol) in CH_2Cl_2 (10 mL) was added pyridine (0.260 mL, 3.23 mmol). After stirring for 1 h at 70 ℃, the reaction mixture was cooled to rt and quenched with saturated ***aq.*** NH_4Cl. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were dried over $MgSO_4$ and concentrated ***in vacuo***. The residue was purified by flash column chromatography on silica gel (EtOAc : ***n***-hexane = 1 : 2) to afford 310 mg (81%) of **6** as white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.55 (d, 1H, ***J*** = 2.1 Hz), 7.44 (d, 1H, ***J*** = 8.3 Hz), 7.28 (dd, 1H, ***J*** = 8.4, 2.2 Hz), 6.70 (m, 2H), 4.47 (m, 1H), 3.66 (dd, 1H, ***J*** = 14.1, 8.2 Hz), 3.21 (dd,

1H, ***J*** = 14.1, 4.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.8, 135.2, 134.1, 133.7, 133.6, 132.9, 131.4, 130.7, 54.4, 45.0; LR-MS (ESI+) ***m*/*z*** 294 (M + H^+); HR-MS (ESI+) calculated for $C_{10}H_9Cl_2O_2S_2$ (M + H^+) 294.9416; found 294.9416.



6 ARE-HY-010 (**7**)

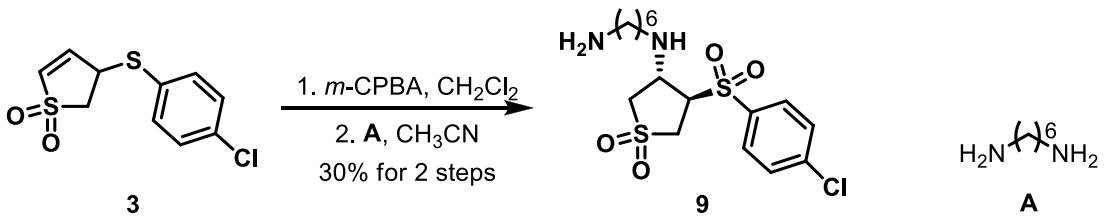
To a stirred solution of **6** (310 mg, 1.05 mmol) in CH_2Cl_2 (10 mL) was added ***m***-CPBA (648 mg, 2.63 mmol, 70~75%). After stirring for 1 h, the reaction mixture was filtered and quenched with saturated ***aq.*** $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were dried over $MgSO_4$ and concentrated ***in vacuo***. The crude mixture was used for next step without further purification. To a stirred solution of the sulfone in CH_3CN (10 mL) was added ***i***-butylamine (0.104 mL, 1.05 mmol). After stirring for 1 h, the reaction mixture was concentrated ***in vacuo***. The residue was purified by preparative HPLC and lyophilized to afford 215 g (51%) of ARE-HY-010 (**7**) as white solid: ^{1}H NMR (DMSO-***d***$_6$, 400 MHz) δ 8.21 (d, 1H, ***J*** = 2.0 Hz), 7.96 (d, 2H, ***J*** = 8.4 Hz), 7.90 (dd, 1H, ***J*** = 8.4, 2.1 Hz), 4.38 (m, 1H), 3.66 (m, 3H), 3.42 (dd, 1H, ***J*** = 14.1, 9.0 Hz), 3.12 (q, 1H, ***J*** = 6.1 Hz), 2.17 (m, 1H), 2.03 (m, 1H), 1.87 (m, 1H), 1.31 (m, 1H), 0.67 (d, 3H, ***J*** = 3.9 Hz), 0.65 (d, 3H, ***J*** = 3.9 Hz); ^{13}C NMR (DMSO-***d***$_6$, 100 MHz) δ 138.5, 137.6, 132.4, 131.6, 130.6, 128.7, 63.5, 55.4, 55.1, 54.4, 49.4, 28.0, 20.3; LR-MS (ESI+) ***m*/*z*** 400 (M + H^+); HR-MS (ESI+) calculated for $C_{14}H_{20}Cl_2NO_4S_2$ (M + H^+) 400.0205; found 400.0204.



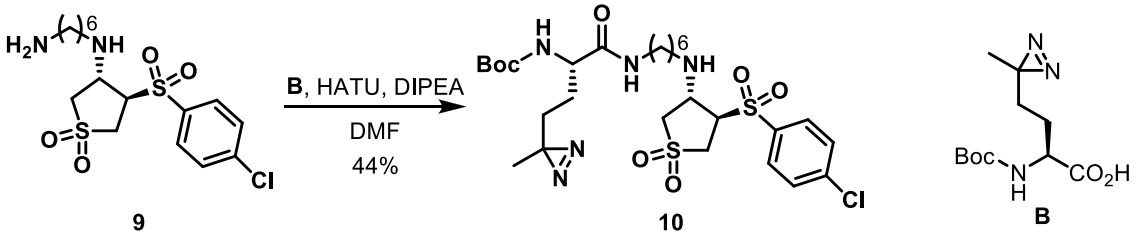
6 ARE-HY-049 (**8**)

To a stirred solution of **6** (1.05 g, 3.54 mmol) in CH_2Cl_2 (20 mL) was added ***m***-CPBA (2.18 mg, 8.85 mmol, 70~75%). After stirring for 2 h, the reaction mixture was filtered and quenched with saturated ***aq.*** $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were dried over $MgSO_4$ and concentrated ***in vacuo***. The crude mixture was used for next step without further purification. To a stirred solution of the sulfone in CH_3CN (20 mL) were added glycine (0.104 mL, 1.05 mmol) and 1***N*** NaOH (2.0 mL). After stirring for 1 h, the reaction mixture was concentrated ***in vacuo***. The residue was purified by preparative HPLC and lyophilized to afford 483 mg (34%) of ARE-HY-049 (CBR-470-2) (**8**) as white solid: ^{1}H NMR (DMSO-***d***$_6$, 400 MHz) δ 8.24 (s, 1H), 7.93 (s, 1H), 7.89 (m, 2H), 7.71 (d, 1H, ***J*** = 8.9 Hz), 7.55 (t, 1H, ***J*** = 5.4 Hz), 4.48 (q, 1H, ***J*** = 8.2 Hz), 3.89 (q, 1H, ***J*** = 6.9 Hz), 3.62 (m, 2H), 3.41 (dd, 1H, ***J*** = 14.6, 8.2 Hz), 2.22 (m, 1H); LR-MS (ESI+) ***m*/*z*** 401 (M + H^+); HR-MS (ESI+) calculated for $C_{12}H_{14}Cl_2NO_6S_2$ (M + H^+) 401.9634; found 401.9637.

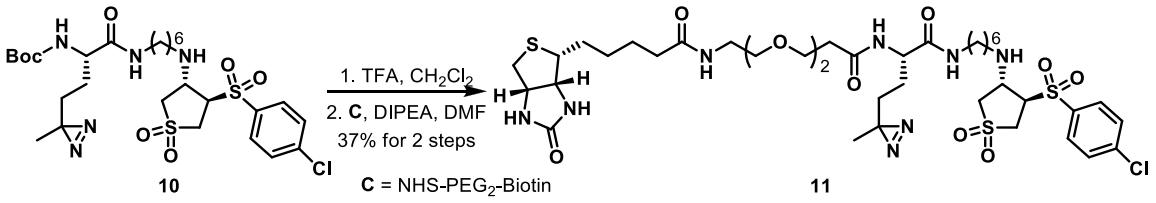
Synthesis of CBR-470-PAP



To a stirred solution of **3** (76.3 mg, 0.293 mmol) in CH_2Cl_2 (5 mL) was added ***m***-CPBA (288 mg, 1.17 mmol, 70~75%). After stirring for 1 h, the reaction mixture was filtered and quenched with saturated ***aq.*** $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were dried over $MgSO_4$ and concentrated ***in vacuo***. The crude mixture was used for next step without further purification. To a stirred solution of the sulfone in CH_3CN (5 mL) was added 1,6-hexanediamine **A** (0.115 mL, 0.879 mmol). After stirring for 1 h, the reaction mixture was concentrated ***in vacuo***. The residue was purified by preparative HPLC and lyophilized to afford 35.9 mg (30%) of **9** as pale yellow solid: LR-MS (ESI+) ***m***/***z*** 409 ($M + H^+$).



To a stirred solution of **9** (17.5 mg, 42.8 μmol) and **B** (11.0 mg, 42.8 μmol) in DMF (2 mL) were added HATU (32.5 mg, 85.6 μmol) and DIPEA (29.8 μL, 0.171 mmol). After stirring for 24 h, the reaction mixture was concentrated ***in vacuo***. The residue was purified by preparative HPLC and lyophilized to afford 12.2 mg (44%) of **10** as white solid: LR-MS (ESI+) ***m***/***z*** 648 ($M + H^+$).



To a solution of **10** (5.2 mg, 6.98 μmol) in CH_2Cl_2 (1 mL) was added TFA (0.5 mL). After stirring for 6 h, the residue was concentrated ***in vacuo*** and used for next step without further purification. To a stirred solution of the amine in DMF (1 mL) were added NHS-PEG_2-Biotin **C** (3.49 mg, 6.98 μmol) and DIPEA (4.86 μL, 27.9 μmol). After stirring for 6 h, the reaction mixture was concentrated ***in vacuo***. The residue was purified by preparative HPLC and lyophilized to afford 2.41 mg (37%) of **11** as white solid: LR-MS (ESI+) ***m***/***z*** 933 ($M + H^+$).