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Synthesis and application of fluorescent Ras-proteins for live cell imaging**

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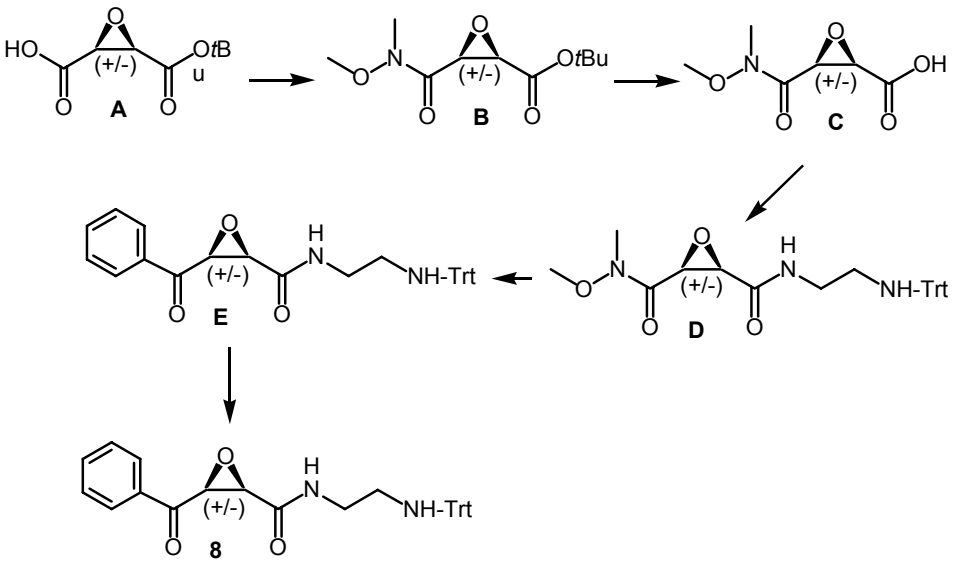
Experimental Section

General procedures: ^{1}H and ^{13}C spectra were recorded on Bruker AC-250, Bruker AM 400, Varian Mercury 400 and Bruker DRX-500 spectrometers. The signal of the residual protonated solvent ($CDCl_3$ or CD_3OD) was taken as reference (^{1}H: δ = 7.24 ($CHCl_3$) or 3.31 (CH_3OH), ^{13}C: δ = 77.0 ($CHCl_3$) or 49.0 (CH_3OH)). EI and FAB mass spectra were measured on a Finnigan MAT MS 70 Workstation (FAB: 3-nitrobenzylalcohol (NBA) as matrix).

Materials: Analytical chromatography was performed on E. Merck silica gel $60F_{254}$ coated plates. Flash chromatography was performed on Baker silica gel (40-65 μm). All solvents were distilled using standard procedures. Commercial reagents were used without further purification.

Compound **8** was synthesized according to the reaction Scheme below.



Cis-oxirane-2,3-dicarboxylic acid mono-*tert*-butyl ester **A** was prepared according to the literature [R. M. Demarinis, J. C. Boehn, J. V. Uri, J. R. Genarini, L. Phillips, G. L. Dunu, *J. Med. Chem.*, **1977**, *20*, 1164-1169]. N-(triphenylmethyl)ethylene diamine was prepared as described [J. W. Tilley, P. Levitan, R. W. Kierstead, M. Cohen, *J. Med. Chem.*, **1980**, *23*, 1387-1392].

3-(Methoxy-methyl-carbamoyl)-oxirane-2-carboxylic acid *tert*-butyl ester (B): To solution of **A** (11.7 g, 68.3 mmol), HBTU (28.5 g, 75.2 mmol), HOBt (11.5 g, 75.2 mmol) and DIPEA (22.9 g, 178 mmol) in dry methylene chloride (300 mL) and DMF (50mL) was added a solution of *N,O*-dimethylhydroxylamine hydrochloride (10 g, 102 mmol) in dry methylene chloride (150 mL) and DMF (25mL). The reaction mixture was left stirring at room temperature for 12 hours, diluted with ethyl acetate (200 mL) and extracted with 0.5 M HCl (2 x 100 mL), 1M $NaHCO_3$ (2 x

100 mL) and finally with brine (2 x 100 ml). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuo. Purification of the resulting oil by flash chromatography using *c*-hexane/ ethyl acetate (1:1) as eluent obtained 10.4 g (44.8 mmol, 65%) of the desired product **B** as a yellowish oil. R_f 0.77 (ethyl acetate/methanol (5:1)); ^{1}H NMR (400 MHz, CD_3OD): δ = 1.36 (s, 9H, CCH_3); 3.11 (s, 3H, NCH_3); 3.68 (s, 3H, OCH_3); 3.65-3.70 (m, 1H, CH epox); 3.84 (m, 1H, CH epox); MS (FAB, 3-NBA): m/z: calcd for $[M+H]^+$: 232.1107; found: 232.1088; $C_{10}H_{17}NO_5$ (231.2).

3-(Methoxy-methyl-carbamoyl)-oxirane-2-carboxylic acid (C): To solution of **B** (8.66 g, 37.5 mmol) and TFA (37.5 ml) in methylene chloride (100 mL) was added triethylsilane (15.0 ml, 93.5 mmol). The mixture was left stirring at room temperature for 2 hours. The solvent was removed by evaporation in vacuo under repeated addition of toluene for azeotropic removal of the acid. Purification of the resulting oil by flash chromatography using *c*-hexane/ ethyl acetate (1:1) as eluent obtained 4.80 g (25.7 mmol, 69%) of the desired product **C** as a yellowish oil. R_f 0.05 (ethyl acetate/methanol (10:1)); ^{1}H NMR (400 MHz, CD_3OD): δ = 3.25 (s, 3H, NCH_3); 3.78 (s, 3H, OCH_3); 3.81 (d, J = 5.0 Hz, 1H, CH epox); 4.14 (d, J = 5.0 Hz, 1H, CH epox); ^{13}C NMR (125.8 MHz, CD_3OD): δ = 32.8 (NCH_3); 50.2 (CH epox); 53.6 (CH epox); 62.4 (OCH_3); 166.2 (CONHR); 168.9

(CO_2H); MS (FAB, 3-NBA): m/z: calcd for $[M+H]^+$: 176.0481; found: 176.0575; $C_6H_9NO_5$ (175.1).

Oxirane-2,3-dicarboxylic acid 2-(methoxy-methyl-amide) 3-{[2-(trityl-amino)-ethyl]-amide} (D): To solution of **C** (650 mg, 3.72 mmol) and *N*-(triphenylmethyl)ethylenediamine[69] (1.10 g, 3.72 mmol) in dry methylene chloride (20 mL) was added under an argon atmosphere HOBt (753 mg, 5.58 mmol), followed by EDC (856 mg, 4.46 mmol). The reaction mixture was left stirring at room temperature for 12 hours, diluted with ethyl acetate (50 mL) and extracted with 0.5 M HCl (2 x 10 mL), 1M $NaHCO_3$ (2 x 10 mL) and finally with brine (2 x 10 ml). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuo. Purification of the resulting oil by flash chromatography using a gradient from *c*-hexane/ ethyl acetate (3:1) to *c*-hexane/ ethyl acetate (1:1) as eluent obtained 801 mg (1.74 mol, 47%) of the desired product **D** as a yellowish oil. R_f 0.20 (c-hexane/ethyl acetate (1:1)); ^1H NMR (400 MHz, $CDCl_3$): δ = 2.21 (t, J = 4.8 Hz, 2H, $C\underline{H}_2NH$); 2.90 (s, 3H, NCH_3); 3.24 (m, 1H, $C\underline{H}_{2a}NH$); 3.48 (m, 1H, $C\underline{H}_{2b}NH$); 3.61 (s, 3H, OCH_3); 3.73 (d, J = 4.2 Hz, 1H, CH epox); 4.06 (d, J = 4.2 Hz, 1H, CH epox); 6.80 (t, J = 4.8 Hz, 1H, NH); 7.14 (t, J = 5.8 Hz, 3H, CH Trt); 7.24 (t, J = 6.0 Hz, 6H, CH Trt); 7.46 (d, J = 6.0 Hz, 6H, CH Trt); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ = 32.4 (NCH_3); 40.0 (CH_2NH); 43.2 (CH_2NH); 54.1 (CH epox); 54.8 (CH epox); 62.1 (OCH_3); 70.9 ($\underline{C}Ph_3$ Trt); 126.4 (CH Trt); 127.9 (CH Trt);

128.7 (CH Trt); 146.1 (C_q Trt); 166.2 (CONHR); 171.2 (CONHR); MS (FAB, 3-NBA): m/z: calcd for $[M+Na]^+$: 482.2056; found: 482.2031; $C_{27}H_{29}N_3O_5$ (459.5).

3-Benzoyl-oxirane-2-carboxylic acid [2-(trityl-amino)-ethyl]-amide (E) Compound **E** was prepared from **D** (104 mg, 226 μmol) and phenyllithium (2 *M* solution in toluene, 283 μl, 566 μmol). To solution of phenyllithium (2 *M* solution in toluene, 284 μl, 568 μmol) in dry diethyl ether (5 mL) was added **D** (227 μmol) at -78°C. The mixture was left stirring at −78°C for 2 h and quenched with 1M ammonium chloride solution (5 mL), diluted with methylene chloride and extracted with brine (2 x 100 ml). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuo. Purification of the resulting oil by flash chromatography using a gradient from *c*-hexane/ ethyl acetate (10:1) to *c*-hexane/ ethyl acetate (2:1) as eluent obtained 57 mg (120 μmol, 53%) of the desired product **E** as a yellowish oil. R_f 0.20 (c-hexane/ethyl acetate (4:1)); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.13 (m, 2H, C<u>H</u>$_2$NH); 3.25 (m, 1H, C<u>H</u>$_2$NH); 3.97 (d, *J* = 6.2 Hz, 1H, CH epox); 4.44 (d, *J* = 6.2 Hz, 1H, CH epox); 6.71 (t, *J* = 4.8 Hz, 1H, NH); 7.16 (t, *J* = 5.8 Hz, 3H, CH Trt); 7.24 (t, *J* = 6.0 Hz, 6H, CH Trt); 7.35-7.45 (m, 2H, CH Ph); 7.42 (d, *J* = 6.0 Hz, 6H, CH Trt); 7.61 (t, *J* = 7.6 Hz, 1H, CH Ph); 7.81 (t, *J* = 8.2 Hz, 2H, CH Ph); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ = 40.2 (CH_2NH); 43.0 (CH_2NH); 56.2 (CH epox);

58.1 (CH epox); 71.0 (C(Ph)$_3$); 126.5; 128.1; 128.6; 128.8; 129.3 (3*CH Trt, 3*CH Ph); 134.8 (C_q Ph); 146.1 (C_q Trt); 164.9 (CONHR); 191.7 (COR); MS (FAB, 3-NBA): m/z: calcd for $[M+H]^+$: 477.2100; found: 477.2145; $C_{31}H_{28}N_2O_3$ (476.6).

3-Benzoyl-oxirane-2-carboxylic acid (2-amino-ethyl)-amide (8): To a solution of **E** (30 mg, 63 μmol) in methylene chloride (10 mL) under an argon atmosphere was added TFA (100 µl). The reaction mixture was left stirring for 10 minutes and after addition of methanol (1 mL) the solvent was removed by evaporation in vacuo under repeated addition of toluene for azeotropic removal of the acid. Purification of the resulting oil by flash chromatography using a gradient from *c*-hexane/ ethyl acetate (1:1) to methanol as eluent obtained 10 mg (43 μmol, 68%) of the desired product **8** as a yellowish oil. R_f 0.20 (methanol); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.87-2.95 (m, 2H, CH_2NH); 3.08-3.15 (m, 2H, CH_2NH); 3.98 (d, *J* = 5.1 Hz, 1H, CH epox); 4.46 (d, *J* =5.1 Hz, 1H, CH epox); 7.32-7.38 (m, 2H, CH Ph); 7.60 (t, *J* = 7.6 Hz, 1H, CH Ph); 7.91 (d, *J* = 8.2 Hz, 2H, CH Ph); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ = 36.8 (CH_2NH); 39.4 (CH_2NH); 55.7 (CH epox); 60.8 (CH epox); 127.1; 128.7; 129.3 (CH Ph); 135.2 (C_q Ph); 167.0 (CONHR); 192.9 (COR); MS (FAB, 3-NBA): m/z: calcd for $[M+Na]^+$: 257.0902; found: 257.0886; $C_{12}H_{14}N_2O_3$ (234.3).