Fluorescent ligands targeting the intracellular allosteric binding site of the chemokine receptor CCR2

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**Synthesis and compound characterization**

2-(4-Chloro-2-((3,4-dichlorophenyl)sulfonamido)phenoxy)benzoic acid (1, SD-24)\(^1\)

![Chemical structure of SD-24](image)

The synthesis of SD-24 (1) was previously published by Peace et al.\(^1\) To provide experimental details and unpublished characterization data (\(^1^H\) NMR, \(^{13}^C\) NMR, HRMS), we have outlined the synthesis and the characterization data for this compound. An aqueous solution of LiOH (2 N, 8 mL) was added to a solution of methyl 2-(4-chloro-2-((3,4-dichlorophenyl)sulfonamido)phenoxy)benzoate (37, 660 mg, 1.35 mmol, 1.0 eq) dissolved in THF (10 mL). The reaction mixture was stirred for 4 h at ambient temperature. Then, THF was removed under reduced pressure and the aqueous residue was acidified with 2 N HCl until the carboxylic acid precipitated. The aqueous layer was extracted with ethyl acetate. The organic layer was subsequently washed with brine, dried over Na\(_2\)SO\(_4\), filtered, and the solvent was removed under reduced pressure. The title compound was obtained as a colorless solid (585 mg, 1.24 mmol, 92%). \(^1^H\) NMR (400 MHz, DMSO-\(d_6\)), \(\delta\) [ppm]: 13.09 (bs, 1H, -COOH), 10.56 (bs, 1H, -NHSO\(_3\)), 7.89 (d, \(J = 2.2\) Hz, 1H, benzene sulfonamide H-2), 7.80 (dd, \(J = 7.7, 1.8\) Hz, 1H, salicylate H-6), 7.74 (d, \(J = 8.4\) Hz, 1H, benzene sulfonamide H-5), 7.64 (dd, \(J = 8.4, 2.2\) Hz, 1H, benzene sulfonamide H-6), 7.46 (d, \(J = 2.6\) Hz, 1H, 4-chlorophenol H-3), 7.44-7.37 (m, 1H, salicylate H-4), 7.25 (dd, \(J = 7.7, 7.5\) 1.0 Hz, 1H, salicylate H-5), 7.18 (dd, \(J = 8.8, 2.6\) Hz, 1H, 4-chlorophenol H-5), 6.72 (dd, \(J = 8.8\) Hz, 1H, 4-chlorophenol H-6), 6.48 (dd, \(J = 8.2, 1.0\) Hz, 1H, salicylate H-3); \(^{13}^C\) NMR (151 MHz, DMSO-\(d_6\)), \(\delta\) [ppm]: 166.4 (C=O), 154.2 (salicylate C-2), 148.5 (4-chlorophenol C-1), 140.2 (benzene sulfonamide C-1), 136.1 (benzene sulfonamide C-4), 133.4 (salicylate C-4), 132.1 (benzene sulfonamide C-3), 131.5 (benzene sulfonamide C-5), 131.4 (salicylate C-6), 128.5 (4-chlorophenol C-2), 128.2 (benzene sulfonamide C-2), 127.0 (4-chlorophenol C-4), 126.5 (benzene sulfonamide C-6), 126.3 (4-chlorophenol C-5), 124.4 (4-chlorophenol C-3), 124.2 (salicylate C-5), 123.9 (salicylate C-1), 119.9 (salicylate C-3 & 4-chlorophenol C-6); LRMS (ESI\(^+\)) \(m/z\) 472 [M+H\(^+\)]; HRMS (ESI\(^+\)) \(m/z\) calc for C\(_{29}\)H\(_{23}\)Cl\(_3\)NO\(_5\): 471.9575 [M+H\(^+\)]; found: 471.9577; HPLC retention time: 20.32 min, >99% (M1). The attached \(^1^H\) and \(^{13}^C\) NMR spectra contains residual solvent signals (EIOAc).

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4-((5-Chloro-3-((4-chloro-3-(trifluoromethyl)phenyl)sulfonamido)pyridin-2-yl)oxy)-N-(2-(piperidin-1-yl)ethyl)benzamide (2)\(^2\)

![Chemical structure of compound 2](image)

The synthesis of compound 2 was previously published by Wang et al.\(^2\) To provide experimental details and unpublished characterization data (\(^1^H\) NMR, \(^{13}^C\) NMR), we have outlined the synthesis and the characterization data for this compound. 4-((5-Chloro-3-((4-chloro-3-(trifluoromethyl)phenyl)sulfonamido)pyridin-2-yl)oxy)benzoic acid (27, 20 mg, 39.4 µmol, 1.0 eq) was dissolved in DMF (500 µL) and cooled to 0 °C. TBTU (25 mg, 78.9 µmol, 2.0 eq) and DIPEA (10 mg, 78.9 µmol, 2.0 eq) were dissolved in DMF (500 µL) in a separate flask and added dropwise to the reaction flask. The reaction was stirred for 15 minutes before adding the 2-(piperidin-1-yl)ethylamine (1.0 eq). The reaction mixture was stirred for 30 minutes at ambient temperature, the solvent was evaporated and water was added to the residue. The aqueous layer was extracted with EIOAc (3 x 10 mL). The combined organic layer was washed with brine, dried over Na\(_2\)SO\(_4\), filtered, and the solvent was removed under reduced pressure. The residue was purified by preparative HPLC acetonitrile/water + 0.1% TFA, gradient 20-72% to obtain the TFA salt of the title compound as a colorless solid (18 mg, 24.6 µmol, 62%). \(R_t = 0.3\) (MeOH/dichloromethane 1:9); \(^1^H\) NMR (600 MHz, DMSO-\(d_6\)), \(\delta\) [ppm]:10.88 (bs, 1H, -NHSO\(_3\)), 9.08 (bs, 1H, piperidinium R\(^+\)NH\(^+\)), 8.68 (t, \(J = 5.6\) Hz, 1H, -NHCO\(_2\)), 8.19 (d, \(J = 2.2\) Hz, 1H, benzene sulfonamide H-2), 8.05 (d, \(J = 2.5\) Hz, 1H, pyridine H-6), 8.02 (dd, \(J = 8.4, 2.2\) Hz, 1H, benzene sulfonamide H-6), 7.93 (d, \(J = 2.5\) Hz, 1H, pyridine H-4), 7.89 (d, \(J = 8.4\) Hz, 1H, benzene sulfonamide H-5), 7.85-7.77 (m, 2H, benzamide H-2,6), 6.85-6.77 (m, 2H,
benzamide H-3.5, 3.64-3.60 (m, 2H, -CONH-CH2-CH2-), 3.56-3.54 (m, 2H, piperidinium -CH2-), 3.26-3.21 (m, 2H, -CONH-CH2-CH2-), 2.99-2.90 (m, 2H, piperidinium -CH2-), 2.57-2.43 (m, 2H, piperidinium -CH2-), 1.86-1.80 (m, 2H, piperidinium -CH2-), 1.74-1.58 (m, 3H, piperidinium -CH3-), 1.43-1.33 (m, 1H, piperidinium -CH2-); 13C NMR (151 MHz, DMSO-d6, δ [ppm]): 165.9 (-CONH-), 157.9 (q, J = 33.6 Hz, OOC-CH3), 155.3 (benzamide C-4), 154.9 (pyridine C-2), 142.8 (pyridine C-6), 139.6 (benzene sulfonamide C-1), 135.8 (pyridine C-4), 135.7 (benzene sulfonamide C-4), 133.0 (benzene sulfonamide C-5), 132.1 (benzene sulfonamide C-6), 130.2 (benzamide C-1), 128.8 (benzamide C-2,6), 127.5 (q, J = 31.7 Hz, benzene sulfonamide C-3), 126.0 (q, J = 5.2 Hz, benzene sulfonamide C-2), 125.3 (pyridine C-5), 121.9 (q, J = 273.7 Hz, -CF3), 121.7 (pyridine C-3), 120.2 (benzamide C-3,5), 55.0 (-CONH-CH2-CH2-), 52.3 (2C, piperidinium C-2,6), 34.2 (-CONH-CH2-CH2-), 22.5 (2C, piperidinium C-3,5), 21.2 (piperidinium C-4); LRMS (ESI+) m/z: 617 [M+H]+; HPLC retention time 17.44 min, >99%. The attached 1H and 13C NMR contain residual solvent signals (EtOAc).

4-Acetyl-1-(4-chloro-2-fluorophenyl)-5-cyclohexyl-3-hydroxy-1,5-dihydro-2H-pyrrol-2-one (4)3

![Structure](image)

Cyclohexane carbaldheyde (275 mg, 1.89 mmol, 1.0 eq), 4-chloro-2-fluorooaniline (215 mg, 1.89 mmol, 1.0 eq), and ethyl 2,4-dioxopentanate (300 mg, 1.89 mmol, 1.0 eq) were placed in a microwave tube and dissolved in acetic acid (4.5 mL). The reaction mixture was heated to 95 °C and stirred for 4 h. After complete conversion of the starting materials monitored by LC-MS, the acetic acid was removed under reduced pressure and the brown residue was triturated with Et2O. The colorless precipitate was separated by filtration and washed with Et2O. This step was performed several times to obtain the title compound as a colorless solid (91 mg, 0.26 mmol, 14%). 1H NMR (400 MHz, DMSO-d6, δ [ppm]): 12.30 (s, 1H, -OH), 7.70-7.57 (m, 2H, phenyl H-3,6), 7.39 (m, 1H, phenyl H-5), 4.91 (d, J = 1.9 Hz, 1H, pyrrole H-5), 2.44* (s, 3H, -CH3), 2.00-1.76 (m, 1H, cyclohexyl H-1), 1.65-1.32 (m, 5H, cyclohexyl -CH2-), 1.06-0.74 (m, 4H, cyclohexyl -CH2-), 0.54-0.38 (m, 1H, cyclohexyl -CH2-); 13C NMR (151 MHz, DMSO-d6, δ [ppm]): 193.2 (-CO-CH3), 164.46 (pyrrole C-3), 156.6 (d, J = 253.7 Hz, phenyl C-2), 152.5 (pyrrole C-2), 132.6 (d, J = 10.6 Hz, phenyl C-4), 129.5 (phenyl C-5), 125.24 (d, J = 3.0 Hz, phenyl C-6), 124.5 (d, J = 12.1 Hz, phenyl C-1), 119.7 (pyrrole C-4), 117.3 (d, J = 24.2 Hz, phenyl C-3), 62.7 (pyrrole C-5), 39.7* (s, cyclohexyl C-1), 30.6 (s, -CH3), 30.25 (cyclohexyl -CH2-), 26.4 (cyclohexyl -CH2-), 25.9 (cyclohexyl -CH2-), 25.8 (cyclohexyl -CH2-), 25.5 (cyclohexyl -CH2-); LRMS (ESI+) m/z: 352 [M+H]+; HRMS (ESI+): m/z calcd for C13H12ClFNO3: 352.1110 [M+H]+; found: 352.1106; HPLC retention time 19.83 min, 96.4% (M1). The obtained analytical data are in good agreement with literature values.3 The attached 1H NMR contains a residual solvent signal (CH2Cl2).

4-Acetyl-1-(4-bromo-2-fluorophenyl)-5-cyclohexyl-3-hydroxy-1,5-dihydro-2H-pyrrol-2-one (5)3

![Structure](image)

Cyclohexane carbaldehyde (71 mg, 0.63 mmol, 1.0 eq), 4-bromo-2-fluoroaniline (120 mg, 0.63 mmol, 1.0 eq), and ethyl 2,4-dioxopentanate (100 mg, 0.63 mmol, 1.0 eq) were placed in a microwave tube and dissolved in acetic acid (2.5 mL). The reaction mixture was heated to 95 °C and stirred for 4 h. After complete conversion of the starting materials monitored by LC-MS, the acetic acid was removed under reduced pressure and the brown residue was triturated with Et2O. The colorless precipitate was separated by filtration and washed with Et2O. This step was performed several times to obtain the title compound as a colorless solid (50 mg, 0.13 mmol, 20%). 1H NMR (400 MHz, DMSO-d6, δ [ppm]): 12.30 (s, 1H, -OH), 7.77 (dd, J = 10.2, 2.1 Hz, 1H, phenyl H-5), 7.60-7.47 (m, 2H, phenyl H-3,6), 4.91 (d, J = 1.9 Hz, 1H, pyrrole H-5), 2.44* (s, 3H, -CH3), 1.96-1.85 (m, 1H, cyclohexyl H-1), 1.66-1.29 (m, 5H, cyclohexyl -CH2-), 1.07-0.73 (m, 4H, cyclohexyl -CH2-), 0.54-0.40 (m, 1H, cyclohexyl -CH2-); 13C NMR (101 MHz, DMSO-d6, δ [ppm]): 193.2 (-CO-CH3), 164.5 (pyrrole C-3), 156.7 (d, J = 255.5 Hz, phenyl C-2), 152.7 (pyrrole C-2), 129.8 (phenyl C-5), 128.2 (d, J = 4.0 Hz, phenyl C-6), 125.0 (d, J = 11.0 Hz, phenyl C-1), 120.5 (d, J = 9.1 Hz, phenyl C-4), 120.1 (d, J = 23.2 Hz, phenyl C-3), 119.8 (pyrrole C-4), 62.6 (pyrrole C-5), 39.5* (cyclohexyl C-1), 30.6 (-CH3), 30.4 (cyclohexyl -CH2-), 26.5 (cyclohexyl -CH2-), 26.0 (cyclohexyl -CH2-), 25.9 (cyclohexyl -CH2-), 25.5 (cyclohexyl -CH2-); LRMS (ESI+) m/z: 398 [M+H]+; HRMS (ESI+): m/z
calcd for C_{24}H_{20}BrFNO_{3}^{+}: 396.0605 [M+H]^{+}; found: 396.0603; HPLC retention time 19.7 min, 96.8%. The obtained analytical data are in good agreement with literature values. The attached ¹H NMR contains a residual solvent signal (CH_{2}Cl_{2}).

4-(((1-((4-Chloro-2-fluorophenyl)-2-cyclohexyl-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-3-yl)-4-oxobutyl)-1H-1,2,3-triazol-4-yl)methyl)carbamoyl)-2-(6-(dimethylamino)-3-(dimethyliminio)-3H-xanthen-9-yl)benzoate (8)

4-((4-Azidobutanoyl)-1-(4-chloro-2-fluorophenyl)-5-cyclohexyl-3-hydroxy-1,5-dihydro-2H-pyrrol-2-one (19, 19 mg, 45.1 µmol, 1.0 eq) was dissolved in a water/tert-butanol (1.2 mL, 1:1) mixture. 6-TAMRA-alkyne (42, 21 mg, 45.1 µmol, 1.0 eq) dissolved in DMF (600 µL), tris(1-benzyl-1H-1,2,3-triazol-4-yl)methylamine (TBTA, 2.4 mg, 4.5 µmol, 0.1 eq) dissolved in DMF (400 µL), and an aqueous CuSO_{4} solution (0.1 M, 90 µL, 0.2 eq) were added. The reaction was started by the addition of an aqueous sodium ascorbate solution (0.1 M, 90 µL, 0.2 eq) and stirred for 6 h at ambient temperature. After complete conversion of the starting materials, solvents were removed under reduced pressure and the resulting crude product was purified by preparative HPLC (acetonitrile/water + 0.1% TFA, gradient 35-95%). The TFA salt of the title compound was obtained as a pink solid (17.0 mg, 17.0 µmol, 38%). ¹H NMR (600 MHz, DMSO-d_{6} [ppm]): 13.39 (s, 1H, -COOH), 12.32 (s, 1H, -OH), 9.28 (t, J = 5.6 Hz, 1H, -CH_{2}-NHCOO-), 8.32–8.23 (m, 2H, carbamoyl benzoate H-5, 6), 7.90 (s, 1H, carbamoyl benzoate H-3), 7.61–7.57 (m, 2H, phenyl H-3, 6), 7.41–7.35 (m, 1H, phenyl H-5), 7.11–6.90 (m, 6H, 3H-xanthene H-1,2,4,5,7,8), 4.91 (d, J = 1.9 Hz, 1H, pyrrole-H-5), 4.52 (d, J = 5.6 Hz, 2H, -CH_{2}-NHCOO-), 4.35 (t, J = 7.2 Hz, 2H, triazole-C_{2}-CH_{2}-), 3.24 (s, 12H, -N(CH_{3})_{2} & =N(CH_{3})_{2}), 2.92–2.79 (m, 2H, -CH_{2}-CH_{2}-CO), 2.12–2.04 (m, 2H, -CO-CH_{2}-CH_{2}-CH_{2}-triazole), 1.92–1.85 (m, 1H, cyclohexyl H-1), 1.61–1.30 (m, 3H, cyclohexyl -CH_{2}-), 1.03–0.73 (m, 4H, cyclohexyl -CH_{2}-), 0.51–0.42 (m, 1H, cyclohexyl -CH_{2}-); ²C NMR (151 MHz, DMSO-d_{6} [ppm]): 194.6 (-CO-CH_{2}-), 166.0 (-N=O), 164.4 (pyrrole C-3), 157.7 (q, J = 31.2 Hz, F_{2}C-C=O), 156.6 (d, J = 253.7 Hz, phenyl C-2), 152.4 (pyrrole C-2), 144.4 (triazole C-4), 132.6 (d, J = 10.6 Hz, phenyl C-4), 130.6 (3H-xanthene C-1,8), 129.5 (phenyl C-6), 129.1 (carbamoyl benzoate C-5), 125.3 (d, J = 3.0 Hz, phenyl C-5), 124.5 (d, J = 12.1 Hz, phenyl C-1), 123.0 (triazole C-5), 119.2 (pyrrole C-4), 117.3 (d, J = 24.2 Hz, phenyl C-3), 114.4 (3H-xanthene C-2,7), 96.3 (3H-xanthene C-4,5), 62.6 (pyrrole C-5), 48.7 (-triazole-C_{2}=CH_{2}-CH_{2}-), 40.5 (-N(CH_{3})_{2} & =N(CH_{3})_{2}), 39.7 (-cyclohexyl C-1), 39.0 (-C_{2}=CO-), 34.9 (-triazole-C_{2}=NHCOO-), 30.2 (cyclohexyl -CH_{2}-), 26.4 (cyclohexyl -CH_{2}-). 25.9 (cyclohexyl -CH_{2}-), 25.8 (cyclohexyl -CH_{3}-), 24.2 (-CH_{2}=CH_{2}-CH_{2}-), 18.5 (F). UV-Vis (λ_{max} (Ex) = 558 nm, λ_{max} (Em) = 630 nm, Stokes shift = 72 nm; LRMS (ESI^+) m/z: 888.7 [M+H]^+; HRMS (ESI^+) m/z: calcd for C_{24}H_{30}F_{2}ClN_{3}O_{4}^{+}: 888.3282 [M+H]^+; found: 888.3289; HPLC retention time 18.16 min, 95.9% (M1). The attached ¹H and ²C NMR contain residual solvent signals (MeOH).

4-(((1-(4-Bromo-2-fluorophenyl)-2-cyclohexyl-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-3-yl)-4-oxobutyl)-1H-1,2,3-triazol-4-yl)methyl)carbamoyl)-2-(6-(dimethylamino)-3-(dimethyliminio)-3H-xanthen-9-yl)benzoate (9)

4-((4-Azidobutanoyl)-1-(4-bromo-2-fluorophenyl)-5-cyclohexyl-3-hydroxy-1,5-dihydro-2H-pyrrol-2-one (20, 20 mg, 43.1 µmol, 1.0 eq) was dissolved in 1.2 mL of a water/tert-butanol (1:1) mixture. 6-TAMRA-alkyne (42, 20 mg, 43.1 µmol, 1.0 eq) dissolved in DMF (600 µL), tris(1-benzyl-1H-1,2,3-triazol-4-yl)methylamine (TBTA, 2.3 mg, 4.3 µmol, 0.1 eq)
dissolved in DMF (400 μL), and an aqueous CuSO₄ solution (0.1 M, 86 μL, 0.2 eq) were added. The reaction was started by the addition of 0.2 eq of an aqueous 0.1 M sodium ascorbate solution (86 μL) and stirred for 6 h at ambient temperature. After complete conversion of the starting materials, solvents were removed under reduced pressure and the residue was purified by preparative HPLC (acetone/2-propanol + 0.1% TFA, gradient 35-95%). The TFA salt of the title compound was obtained as a pink solid (10.0 mg, 9.6 μmol, 22%). ¹H NMR (600 MHz, DMSO-d₆ δ [ppm]): 13.40 (bs, 1H, -COOH), 12.37 (bs, 1H, -OH), 9.27 (t, J = 5.7 Hz, 1H, -NH(OH)), 8.26 (s, 2H, carbamoyl H-5,6), 8.01 (s, 1H, triazole H-5), 7.88 (s, 1H, carbamoyl H-3), 7.76 (dd, J = 10.1, 2.1 Hz, 1H, bromophenyl H-5), 7.60-7.46 (m, 2H, bromophenyl H-3,6), 7.12-6.81 (m, 6H, 3H-xanthene H-1,2,3,4,5,8), 4.90 (d, J = 1.9 Hz, 1H, pyrrole H-5), 4.51 (d, J = 5.6 Hz, 2H, -C₂=NH-CO₂), 4.35 (t, J = 7.2 Hz, 2H, -triazole-CH₂), 3.21 (s, 12H, -N(CH₃)₂ & =N(CH₃)₂), 2.97–2.82 (m, 2H, -CH₂-CO₂), 2.12-2.02 (m, 2H, -CH₂-C₆H₄-CH₂), 1.92–1.83 (m, 1H, cyclohexyl H-1), 1.61–1.63 (m, 5H, cyclohexyl -CH₂), 1.02–0.74 (m, 4H, cyclohexyl -CH₂), 0.52-0.41 (m, 1H, cyclohexyl -CH₂); ¹³C NMR (151 MHz, DMSO-d₆ δ [ppm]): 194.6 (CO-CH₂), 164.4 (pyrrole C-3), 157.7 (q, J = 31.3 Hz, OOC-CH₂), 156.6 (d, J = 255.2 Hz, phenyl C-2), 152.5 (pyrrole C-2), 144.4 (triazole C-4), 130.4 (3H-xanthene C-1,8), 129.1 (phenyl C-5), 128.2 (d, J = 3.4 Hz, phenyl C-6), 124.9 (d, J = 12.0 Hz, phenyl C-1), 123.0 (triazole C-5), 120.5 (d, J = 9.2 Hz, phenyl C-4), 120.1 (d, J = 23.5 Hz, phenyl C-3), 119.2 (pyrrole C-4), 96.5 (3H-xanthene C-4,5), 62.6 (pyrrole C-5), 48.7 (-triazole-CH₂-CH₂-CH₂), 40.4 (-N(CH₃)₂ & =N(CH₃)₂), 39.8 (cyclohexyl C-1), 38.9 (-CH₂-CO₂), 34.9 (-triazole-CH₂-NH), 30.2 (cyclohexyl -CH₂), 26.4 (cyclohexyl -CH₂), 25.9 (cyclohexyl -CH₂), 25.8 (cyclohexyl -CH₂), 24.2 (-CH₂-CH₂-CH₂); UV-Vis: λ_max (EtOH) = 556 nm, λ_max (MeOH) = 628 nm, Stokes shift = 72 nm; LRMS (ESI⁺) m/z: 934 [M⁺Br⁺]+; HRMS (ESI⁺): m/z: calcd for C₉₈H₆BrF₅O₆N₂: 466.6425 [M⁺Br⁺]+; found: 466.6420; HPLC retention time 18.24 min, 95.5% (M1). The attached ¹H and ¹³C NMR contain residual solvent signals (DMF).

a) the ¹³C NMR signals for OOC-CH₃, carbamoyl benzoate C-1,2,3,4,5,6, -COOH, -CONH, 3H-xanthene C-2,3,4a,6,7,8a,9,9a,10a could not be detected.

4-(((1-(2-(2-(4-Chlorophenyl)sulfonamido)phenoxo)benzamido)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazole-5-yl)methyl)carbamoyl)-2-(6-(dimethylamino)-3-(dimethyliminio)-3H-xanthen-9-yl)benzoate (10)
39.9 (0-CH$_3$-CH$_2$-NHCO$^-$), 34.9 (CONH-CH$_2$-triazole$^-$); UV-Vis: $\lambda_{\text{max}}$(Ex) = 556 nm, $\lambda_{\text{max}}$(Em) = 630 nm, Stokes shift = 74 nm; LRMS (ESI$^+$) m/z: 1095 [M+H$^+$]; HRMS (ESI$^+$): m/z calcd for C$_{50}$H$_{57}$Cl$_7$N$_{16}$O$_{18}$S$^2$: 548.1252 [M+2+H$^+$]+$^+$, found: 548.1257; HPLC retention time: 17.88 min, 95.7% (M1). The attached $^1$H and $^{13}$C NMR spectra contain residual solvent signals (MeOH).

$^a$the $^{13}$C NMR signals for -COOH, F$_3$C-COO$^-$, carbamoyl benzoate C-1,2,3,4,6, and 3H-xanthene C-3,4a,6,8a,9,9a,10a could not be detected.

4-(((1-(2-(2-(4-((3-chloro-2-(trifluoromethyl)phenyl)sulfonamido)phenoxy)benzamido)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl)carbamoyl)-2-(6-(dimethylamino)-3-(dimethyliminio)-3H-xanthen-9-yl)benzoate (11)

$^{13}$C NMR signals for $^1$H and $^2$H, benzene sulfonamide C-1,2,3,4,5,6,7,8 & 1H NMR (600 MHz, DMSO-d$_6$) $^\delta$ [(ppm)]; 7.76-7.73 (m, 2H, benzamide C-2,3), 7.45 (d, $J$ = 2.6 Hz, 1H, 4-chlorophenol H-3), 7.28 (dd, $J$ = 8.8, 2.6 Hz, 1H, 4-chlorophenol H-5), 7.01-6.77 (m, 7H, 3H-xanthene H-1,2,4,5,7,8 & 4-chlorophenol H-6), 6.82-6.53 (m, 2H, benzamide H-3,5), 4.49 (d, $J$ = 5.6 Hz, 2H, -triazole-CH$_2$-NHCO$^-$), 4.45 (t, $J$ = 5.3 Hz, 2H, -triazole-CH$_2$-CH$_2$-O$^-$), 3.78 (t, $J$ = 5.3 Hz, 2H, -CONH-CH$_2$-CH$_2$-O$^-$), 3.54-3.41 (m, 8H, -CONH-CH$_2$-CH$_2$-O$^-$), 3.19 (s, 12H, -N(CH$_3$)$_2$), 1.1C NMR$^a$ (151 MHz, DMSO-d$_6$) $^\delta$ [(ppm)]; 156.5 (CONH-CH$_2$-CH$_2$-O$^-$), 164.4 (triazole-CH$_2$-NHCO$^-$), 158.0 (benzamide C-4), 157.7 (q, $J$ = 31.7 Hz, OCC$^-$), 148.2 (4-chlorophenol C-1), 144.2 (triazole C-4), 139.6 (benzene sulfonamide C-1), 135.5 (benzene sulfonamide C-4), 132.8 (benzene sulfonamide C-5), 131.9 (benzene sulfonamide C-6), 129.4 (4-chlorophenol C-4), 129.1 (carbamoyl benzoate C-5), 129.0 (benzene sulfonamide C-6), 128.6 (4-chlorophenol C-2), 127.8 (benzamide C-1), 127.6 (4-chlorophenol C-3), 127.3 (q, $J$ = 31.8 Hz, benzene sulfonamide C-3), 127.1 (4-chlorophenol C-3), 125.8 (q, $J$ = 5.4 Hz, benzene sulfonamide C-2), 123.5 (triazole C-5), 121.9 (q, $J$ = 273.31 Hz, -CF$_3$), 121.0 (4-chlorophenol C-6), 116.9 (benzamide C-5), 96.6 (3H-xanthene C-4,5), 69.5, 69.4 (O-CH$_2$-CH$_2$-O$^-$), 68.9 (O-CH$_2$-CH$_2$-NHCO$^-$-triazole-CH$_2$-CH$_2$-O$^-$), 68.7 (O-CH$_2$-CH$_2$-NHCO$^-$-triazole-CH$_2$-CH$_2$-O$^-$), 49.2 (triazole-CH$_2$-CH$_2$-O$^-$), 40.3 (N-(CH$_3$)$_2$), 39.9 (O-CH$_2$-CH$_2$-NHCO$^-$), 34.9 (CONH-CH$_2$-triazole$^-$); UV-Vis: $\lambda_{\text{max}}$(Ex) = 558 nm, $\lambda_{\text{max}}$(Em) = 630 nm, Stokes shift = 72 nm; LRMS (ESI$^+$) m/z calcd for C$_{34}$H$_{35}$ClF$_3$N$_{18}$O$_{18}$S$^2$: 1129 [M+H$^+$]$^+$; HRMS (ESI$^+$): m/z calcd for C$_{34}$H$_{35}$ClF$_3$N$_{18}$O$_{18}$S$^2$: 1129.2694 [M+H$^+$]$^+$, found: 1129.2700; HPLC retention time 18.18 min, 98.0% (M1).

$^a$the $^{13}$C NMR signals for -COOH, OCC$^-$, carbamoyl benzoate C-1,2,3,4,6, and 3H-xanthene C-1,2,3,4a,6,7,8,9a,9b could not be detected.
4-(((1-(2-2-(2-4-(5-Chloro-3-( trifluoromethyl)phenyl)sulfonamido)pyridin-2-
ol)oxy)benzamido)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl(methyl)carbamoyle-2-(6-(dimethylamino)-3-(dimethyliminio)-3H-xanthen-9-yl)benzoate (12)

\[
\text{\begin{align*}
\text{N}-(2-(2-(2-Azidoethoxy)ethoxy)ethyl)-4-((5-chloro-3-(4-chloro-3-(trifluoromethyl)phenyl)sulfonamido)pyridin-2-
ol)oxy)benzamide (30, 28 mg, 42.2 \mu\text{mol, 1.0 eq}) and 6-TAMRA-alkyne (42, 20 mg, 42.2 \mu\text{mol, 1.0 eq}) were suspended in a water/fert-butanol mixture (1.2 mL, 1:1). Tris[1-benzyl-1H-1,2,3-triazol-4-yl]methyl]amine (1.03 mg, 0.1 eq) was dissolved in DMF (600 \mu\text{L}) and added to the reaction mixture. An aqueous CuSO\(_4\) solution (169 \mu\text{L}, 0.1 M, 0.4 eq) and an aqueous sodium ascorbate solution (338 \mu\text{L}, 0.1 M, 0.8 eq) were added in that order. The reaction mixture was stirred for 2 h at ambient temperature under a nitrogen atmosphere. Then, the solvents were removed under reduced pressure and the residue was purified by preparative HPLC (acetonitrile/water + 0.1% TFA, gradient 35-95%) to obtain the TFA salt of the title compound as a pink solid (12 mg, 9.6 \mu\text{mol, 23%}). \(^1\)H NMR (600 MHz, DMSO-\(d_6\)); 13.37 (s, 1H, -COOH), 10.86 (s, 1H, -SO\(_2\)NH\(_2\)), 9.31 (t, \(J = 5.6\) Hz, 1H, -NHCO-CH\(_2\)-triazole-), 8.43 (t, \(J = 5.8\) Hz, 1H, -CONH-CH\(_2\)-CH\(_2\)O-), 8.30-8.24 (m, 2H, carbamoyl benzoate H-5,6), 8.17 (d, \(J = 2.3\) Hz, 1H, benzene sulfonamide H-2), 8.03 (d, \(J = 2.5\) Hz, 1H, pyridine H-6), 8.01 (dd, \(J = 8.4, 2.3\) Hz, 1H, benzene sulfonamide H-6), 7.96 (s, 1H, triazole H-5), 7.92 (d, \(J = 2.5\) Hz, 1H, pyridine H-4), 7.90 (bs, 1H, carbamoyl H-3), 7.88 (d, \(J = 8.4\) Hz, 1H, benzene sulfonamide H-5), 7.80-7.75 (m, 2H, benzamide H-2,6), 7.07-6.89 (m, 6H, 3H-xanthene H-1,2,4,5,7,8), 6.77-6.73 (m, 2H, benzamide H-3,5), 4.51 (d, \(J = 5.6\) Hz, 2H, -triazole-CH\(_2\)-NHCO-), 4.46 (t, \(J = 5.3\) Hz, 2H, -O-CH\(_2\)-CH\(_2\)-triazole-), 3.78 (t, \(J = 5.3\) Hz, 2H, -O-CH\(_2\)-CH\(_2\)-triazole-), 3.51-3.40 (m, 6H, -O-CH\(_2\)-CH\(_2\)-O- & -CONH-CH\(_2\)-CH\(_2\)O-), 3.34 (q, \(J = 5.8\) Hz, 2H, -CONH-CH\(_2\)-CH\(_2\)O-), 3.25 (s, 12H, -N(CH\(_3\))\(_2\) & =N-(CH\(_3\))\(_2\)). \(^13\)C NMR\(^{ai}\) (151 MHz, DMSO-\(d_6\)); 165.9 (-COOH), 165.3 (-CONH-CH\(_2\)-CH\(_2\)O-), 164.4 (-triazole-CH\(_2\)-NHCO-), 157.8 (q, \(J = 33.8\) Hz, OOC-\(\text{CF}_3\)), 155.0 (benzamide C-4), 154.8 (pyridine C-2), 144.2 (triazole C-4), 142.8 (pyridine C-6), 139.6 (benzene sulfonamide C-1), 137.3 (benzene sulfonamide C-4), 135.7 (pyridine C-4), 133.2 (carbamoyl benzoate C-2), 133.0 (benzene sulfonamide C-5), 132.1 (benzene sulfonamide C-6), 131.1 (3H-xanthene C-1,8), 130.8 (benzamide C-1), 130.6 (carbamoyl benzoate C-3), 129.1 (carbamoyl benzoate C-5), 128.8 (carbamoyl benzoate C-6), 128.6 (benzamide C-2,6), 127.5 (q, \(J = 32.1\) Hz, benzene sulfonamide C-3), 126.0 (q, \(J = 5.3\) Hz, benzene sulfonamide C-2), 125.3 (pyridine C-5), 123.5 (triazole C-5), 121.9 (q, \(J = 273.6\) Hz, -CF\(_2\)), 121.7 (pyridine C-3), 120.0 (benzamide C-3,5), 114.4 (3H-xanthene C-2,7), 96.3 (3H-xanthene C-4,5), 69.5, 69.4 (-O-CH\(_2\)-CH\(_2\)-O-), 68.8 (-O-CH\(_2\)-CH\(_2\)-NHCO- & -triazole-CH\(_2\)-CH\(_2\)O-), 68.7 (-O-CH\(_2\)-CH\(_2\)-NHCO- & -triazole-CH\(_2\)-CH\(_2\)O-), 49.3 (-triazole-CH\(_2\)-CH\(_2\)O-), 40.5 (-N(CH\(_3\))\(_2\) & =N-(CH\(_3\))\(_2\)), 39.1 (-O-CH\(_2\)-CH\(_2\)-NHCO-), 34.9 (-CONH-CH\(_2\)-triazole-); UV-Vis: \(\lambda_{\text{max}}\) (Ex) = 554 nm, \(\lambda_{\text{max}}\) (Em) = 630 nm, Stokes shift = 76 nm; LRMS (ESI\(^+\)) \(m/z\) = 566 [M+2H\(^+\)]; HRMS (ESI\(^+\)): \(m/z\) calcd for C\(_{39}H\(_34\)F\(_2\)N\(_2\)O\(_8\)S\(_2\)H\(_5\): 565.6360 [M+2H\(^+\)]; found: 565.6363 [M+2H\(^+\)].

\(^{ai}\) the \(^13\)C NMR signals for OOC-\(\text{CF}_3\), carbamoylbenzoate C-1,4, and 3H-xanthene C-3,4a,6,8a,9,9a,10a could not be detected.

5-(((4-(4-Chloro-2-(3,4-dichlorophenyl)sulphonamido)phenoxy)benzamido)methyl)-1H-1,2,3-triazol-1-
yl)propyl)carbamoyle-2-(6-(dimethylamino)-3-(dimethyliminio)-3H-xanthen-9-yl)benzoate (13)

\[
\text{5-((4-(4-Chloro-2-(3,4-dichlorophenyl)sulphonamido)phenoxy)benzamido)methyl)-1H-1,2,3-triazol-1-
yl)propyl)carbamoyle-2-(6-(dimethylamino)-3-(dimethyliminio)-3H-xanthen-9-yl)benzoate (13)}
\]
The commercially available TAMRA-5-azide (10 mg, 19.5 µmol, 1.0 eq) and 4-(4-chloro-2-((3,4-dichlorophenyl)sulfonylamido)phenoxo)-N-(prop-2-yn-1-yl)benzamide (31, 11 mg, 22.0 µmol, 1.1 eq) were suspended in a water/tert-butanol mixture (1.2 mL, 1:1). Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA, 1.1 mg, 2.0 µmol, 0.1 eq) was dissolved in 600 µL of DMF and added to the reaction mixture. A CuSO₄ solution (32 µL, 0.1 M, 0.2 eq) and a sodium ascorbate solution (76 µL, 0.1 M, 0.4 eq) were added in that order. The reaction mixture was stirred for 6 h at ambient temperature under a nitrogen atmosphere. Then, solvents were removed under reduced pressure and the residue was purified by preparative HPLC (acetonitrile/water + 0.1% TFA, gradient 35-95%) to obtain the TFA salt of the title compound as a pink solid (10 mg, 8.80 µmol, 45%). ¹H NMR (600 MHz, DMSO-d₆ [ppm]): 13.38 (bs, 1H, -COOH), 10.53 (s, 1H, -SO₂NH₂), 8.98-8.89 (m, 2H, -CONH-CH₂-triazole - and -CONH-(CH₂)₂-O-), 8.67 (s, 1H, carboxamoyl benzote H-6), 8.33-8.23 (m, 1H, carboxamoyl benzote H-4), 8.03 (s, 1H, triazole H-5), 7.84 (d, J = 2.1 Hz, 1H, benzene sulfonamide H-2), 7.83-7.80 (m, 2H, benzylide H-2,6), 7.66 (d, J = 8.4 Hz, 1H, benzene sulfonamide H-5), 7.60 (dd, J = 8.4, 2.1 Hz, 1H, benzene sulfonamide H-6), 5.75-5.73 (m, 1H, carboxamoyl benzote H-3), 4.74 (d, J = 2.6 Hz, 1H, 4-chlorophenol H-3), 7.28 (dd, J = 8.8, 2.6 Hz, 1H, 4-chlorophenol H-5), 7.10-6.84 (m, 7H, 3H-xanthene H-1,2,4,5,7,8, 4-chlorophenol H-6), 6.69-6.63 (m, 2H, benzamide H-3,5), 4.50 (d, J = 5.5 Hz, 2H, triazole-(CH₂-N=CO)₂), 4.45 (t, J = 7.0 Hz, 2H, -CONH-CH₂-CH₂-CH₂-triazole-), 3.40-3.34* (m, 2H, -CONH-CH₂-CH₂-CH₂-triazole-), 3.24* (s, 12H, -N(CH₃)₂) for 13C NMR* (151 MHz, DMSO-d₆, δ [ppm]): 165.1 (CONH-CH₂-CH₂-O-), 164.8 (-CONH-CH₂-triazole-), 158.3 (benzamide C-4), 157.7 (q, J = 33.4 Hz, -OCO-CF₃), 147.7 (4-chlorophenol C-1), 145.0 (triazole C-4), 140.2 (benzamide sulfonamide C-1), 136.1 (benzamide sulfonamide C-4), 135.9 (carboxamoyl benzote C-2), 132.1 (benzene sulfonamide C-3), 131.4 (benzene sulfonamide C-5), 131.3 (carboxamoyl benzote C-4), 130.3 (3H-xanthene C-1,8 & carboxamoyl benzote C-3), 129.5* (carboxamoyl benzote C-6), 129.2 (benzamide C-3,5), 129.1 (benzamide C-1), 128.9 (4-chlorophenol C-4), 128.2 (benzene sulfonamide C-2), 128.0 (4-chlorophenol C-2), 127.4 (4-chlorophenol C-5), 126.6 (4-chlorophenol C-3), 126.5 (benzene sulfonamide C-6), 123.1 (triazole C-5), 121.4 (4-chlorophenol C-6), 116.7 (H-2,6), 114.6* (3H-xanthene C-2,7), 96.4 (3H-xanthene C-4,5), 47.3 (CONH-CH₂-CH₂-CH₂-triazole-), 40.4 (CONH-CH₂-CH₂-CH₂-triazole-), 36.8 (CONH-CH₂-CH₂-CH₂-triazole-), 34.9 (triazole-NHSO₂-), 29.8 (CONH-CH₂-CH₂-triazole-), UV-Vis: λ exceptionally high at 25-450 nm, 100% max at 556 nm, λ₅₅₆ (Em) = 556 nm, λ₅₅₆ (Em) = 556 nm; 530 nm, 600 nm, 700 nm; λmax (ESI-) m/z: 1021 [M⁺H⁺]; HRMS (ESI⁻): m/z calc'd for C₄₅H₃₅Cl₃N₃O₄S₂+: 511.1688 [M²⁻H⁺], found: 511.1678; HPLC retention time 18.00 min, 96.0% (M1).

a) the ¹³C NMR signals for carboxamoyl benzote C-1,5, -COOH, F₃C-COO-, and 3H-xanthene C-3,4a,6,8a,9a,10a could not be detected.

4-(2-(2-(4-(4-(5-Chloro-3-((4-chloro-3-(trifluoromethyl)phenyl)sulfonylamido)pyridin-2-yl)oxy)benzamido)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)ethoxy)carbamoyl)-2-(6-dimethylamino)-3-(dimethylaminio)-3H-xanthen-9-yl)benzote (14)
3.22° (s, 12H, -N(CH3)2 & =N-(CH3)2); 13C NMR41 (151 MHz, DMSO-d6 δ [ppm]): 165.2 (CONH-CH2-triazole), 164.6 (O-CH2-CH2-NHC=O), 157.9 (q, δ = 33.2 Hz, OOC-CF3), 155.2 (benzamide C-4), 154.8 (pyridine C-2), 144.9 (triazole C-4), 142.6 (pyridine C-6), 139.6 (benzene sulfonamide C-1), 135.7 (benzene sulfonamide C-4), 135.6 (pyridine C-4), 132.9 (benzene sulfonamide C-5), 132.1 (benzene sulfonamide C-6), 130.5 (benzamide C-1), 130.4 (3H-xanthene C-1, 2), 129.0 (carbamoyl benzoate C-3), 129.7 (benzene sulfonamide C-2), 127.5 (q, δ = 31.9 Hz, benzene sulfonamide C-3), 126.0 (q, δ = 5.2 Hz, benzene sulfonamide C-2), 125.3 (pyridine C-5), 123.2 (triazole C-5), 121.9 (q, δ = 273.8 Hz, CF3), 121.9 (pyridine C-3), 120.0 (benzene C-3, 5), 96.4 (3H-xanthene C-4, 5), 69.4, 69.4 (-O-CH2-CH2-O), 68.7, 68.7 (-O-CH2-CH2-NHC=O), 49.2 (-triazole-CH2-CH2-), 40.4 (-N-(CH3)2 & =N-(CH3)2), 39.8° (-O-CH2-CH2-NHC=O), 34.8 (-CONH-CH2-triazole); UV-Vis: λ max (Ex) = 558 nm, λ max (Em) = 611 nm, Stokes shift = 53 nm; LRMS (ESI)107 m/z: 566 [M+H]+; HRMS (ESI): m/z calc for C26H14ClF3N4O6S2+: 565.6360 [M+2H]+; found: 565.6363; HPLC retention time 19.53 min, 95.2% (M2). The attached 1H and 13C NMR contain residual solvent signals (MeOH).

4-(((1-(2-(2-(2-(4-Chloro-2-((3,4-dichlorophenyl)sulfonamido)phenoxy)benzamido)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl)carbamoyl)-2-(4-(dimethylamino)-3-(dimethylaminolino)-3H-xanthen-9-yl)benzene (15)

TAMRA-6-alkyne (42, 12 mg, 25.6 µmol, 1.0 eq) and N-(2-(2-(azidoethoxy)ethoxy)ethyl)-2-(4-chloro-2-((3,4-dichlorophenyl)sulfonamido)phenoxy)benzamide (35, 18 mg, 28.2 µmol, 1.1 eq) were suspended in a water/tert-butanol mixture (1.2 mL, 1:1). Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]ammonium (1.3 mg, 2.5 µmol, 0.1 eq) was dissolved in 600 µL of DMF and added to the reaction. A CuSO4 solution (51 µL, 0.1 M, 0.2 eq) and a sodium ascorbate solution (102 µL, 0.1 M, 0.4 eq) were added in that order. The reaction mixture was stirred for 6 h at ambient temperature and under a nitrogen atmosphere. The solvents were removed under reduced pressure and the residue was purified by preparative HPLC (acetonitrile/water + 0.1% TFA, gradient 35-95%) to obtain the TFA salt of the title compound as a pink solid (12 mg, 9.90 µmol, 39%). 1H NMR (600 MHz, DMSO-d6 δ [ppm]): 13.36 (bs, 1H, Ar-COOH), 10.86 (bs, 1H, -NHSO2), 9.28 (t, δ = 5.6 Hz, 1H, -CONH-CH2-triazole), 8.45 (bs, 1H, -CONH-CH2-CH2-O), 8.25–8.17 (m, 2H, carbamoyl benzoate H-5, 6), 7.92 (s, 1H, triazole H-5), 7.83 (s, 1H, carbamoyl benzoate H-3), 7.74 (s, 1H, benzene sulfonamide H-2), 7.66 (d, δ = 8.4 Hz, 1H, benzene sulfonamide H-5), 7.61 (dd, δ = 7.6, 1.8 Hz, 1H, benzamide H-6), 7.53 (d, δ = 2.6 Hz, 1H, 4-chlorophenol H-3), 7.51 (dd, δ = 8.4, 2.2 Hz, 1H, benzene sulfonamide H-6), 7.24–7.14 (m, 3H, benzamide H-4, 5 & 4-chlorophenol H-5), 6.96–6.72 (m, 7H, 3H-xanthene H-1,2,4,5,7,8 & 4-chlorophenol H-6), 6.25 (dd, δ = 8.1, 1.2 Hz, 1H, benzamide H-4), 4.48 (d, δ = 5.6 Hz, 2H, -CONH-CH2-triazole), 4.43 (t, δ = 5.3 Hz, 2H, -triazole-CH2-CH2-O), 3.73 (t, δ = 5.3 Hz, 2H, -O-CH2-CH2-NHC=O), 3.44–3.36° (m, 8H, -triazole-CH2-CH2-O-CH2-CH2-O-CH2-CH2-NHC=O), 3.32–3.04 (bs, 12H, -N(CH3)2 & -N+(CH3)2); 13C NMR41 (151 MHz, DMSO-d6 δ [ppm]): 165.5 (CONH-CH2-CH2-O), 164.4 (CONH-CH2-triazole), 157.6 (q, δ = 33.2 Hz, OOC-CF3), 153.2 (benzamide C-2), 146.6 (4-chlorophenol C-1), 144.3 (triazole C-4), 139.8° (benzene sulfonamide C-1), 136.2 (benzene sulfonamide C-4), 132.2 (benzene sulfonamide C-5), 130.2 (benzamide C-4), 131.8 (benzamide C-4), 131.6 (benzene sulfonamide C-5), 130.2 (benzamide C-6), 129.1 (carbamoyl benzoate C-5), 128.4 (4-chlorophenol C-2), 128.2° (carbamoyl benzoate C-1), 128.0 (benzene sulfonamide C-2), 126.3 (benzene sulfonamide C-6), 126.3 (benzamide C-1), 126.0 (4-chlorophenol C-5), 123.8 (benzamide C-5), 123.4 (4-chlorophenol C-3, 2), 122.5° (carbamoyl benzoate C-3), 121.9 (4-chlorophenol C-6), 117.3 (benzamide C-3), 96.8 (3H-xanthene C-4, 5), 69.4, 69.3 (-O-CH2-CH2-O), 68.7 (-O-CH2-CH2-NHC=O), 68.7 (-triazole-CH2-CH2-O), 49.2 (triazole-CH2-CH2-O), 40.2 ((-N(CH3)2 & -N+(CH3)2), 39.1° (-O-CH2-CH2-NHC=O), 34.9° (CONH-CH2-triazole); UV-Vis: λ max (Ex) = 560 nm, λ max (Em) = 630 nm, Stokes shift = 70 nm; LRMS (ESI): m/z: 549 [M+2Cl]+, 1x [Cl]+/2+H]+; HRMS (ESI): m/z calc for C26H27ClN3O9S2+: 548.1522 [M+2x
35Cl, 1x [37Cl]2+ H]+; found: 548.1524; HPLC retention time 18.71 min, 99.0% (M1).

4 the 13C NMR signals for carbamoyl benzoate C-2,4,6, -COOH, F3C-COO; 3H-xanthene C-1,2,3,4a,6,7,8,8a,9,9a,10a, and 4-chlorophenol C-4 could not be detected.
5-((3-(4-((2-(4-Chloro-2-((3,4-dichlorophenyl)sulfonylamido)phenoxy)benzamido)propyl)carbamoyl)-2-(6-dimethylamino)-3-(dimethyliminio)-3H-xanthen-9-yl)propyl)carbamoyl)-2-(6-dimethylamino)-3-(dimethyliminio)-3H-xanthen-9-yl)benzoate (16)

Commercially available TAMRA-5-azide (10 mg, 19.5 µmol, 1.0 eq) and 2-(4-chloro-2-((3,4-dichlorophenyl)sulfonylamido)phenoxy)-N-(prop-2-yn-1-yl)benzamide (36, 12 mg, 21.5 µmol, 1.1 eq) were suspended in a water/tert-butanol mixture (1.2 mL, 1:1). Tris[(benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (11.1 mg, 19 µmol, 0.1 eq) was dissolved in 600 µL of DMF and added to the reaction mixture. A CuSO₄ solution (39 µL, 0.1 M, 0.2 eq) and a sodium ascorbate solution (78 µL, 0.1 M, 0.4 eq) were added in that order. The reaction mixture was stirred overnight at ambient temperature and under nitrogen atmosphere. Then, solvents were removed under reduced pressure and the residue was purified by preparative HPLC (acetonitrile/water + 0.1% TFA, gradient 35-95%) to obtain the TFA salt of the title compound as a pink solid (7 mg, 6.16 µmol, 32%). ¹H NMR (600 MHz, DMSO-d₆, δ [ppm]): 13.36 (bs, 1H, Ar-COOH), 10.80 (s, 1H, H-N), 8.94 (t, J = 5.5 Hz, 1H, (CH₂)₂-NHCO₂⁻), 8.90 (t, J = 5.8 Hz, 1H, -CONH-CH₂-triazole-), 8.71-8.66 (m, 1H, carbamoyl benzoate H-6), 8.29 (dd, J = 7.9, 1.8 Hz, 1H, carbamoyl benzoate H-4), 7.89 (s, 1H, triazole H-5), 7.76 (d, J = 2.2 Hz, 1H, benzene sulfonamide H-2), 7.68 (dd, J = 7.5, 1.9 Hz, 1H, benzamide H-6), 7.66 (d, J = 8.5 Hz, 1H, benzene sulfonamide C-5), 7.60-7.50 (m, 3H, benzene sulfonamide H-6, carbamoyl benzoate H-3, 4-chlorophenol H-3), 7.27-7.17 (m, 3H, 4-chlorophenol H-5, benzamide H-4.5), 7.05 (bs, 3H-xanthen H-1,2,7,8), 6.96 (bs, 2H, 3H-xanthen H-4,5), 6.90 (d, J = 8.7 Hz, 1H, 4-chlorophenol H-6), 6.20 (dd, J = 8.1, 1.2 Hz, 1H, benzamide H-3), 4.52 (d, J = 5.8 Hz, 2H, -triazole-CH₂-NHCO₂⁻), 4.42 (t, J = 7.0 Hz, 2H, -triazole-CH₂-CH₂CH₂-), 3.35 (q, J = 6.5 Hz, 2H, -CH₂-CH₂-NHCO₂⁻), 3.25 (s, 12H, -N(CH₃)₂ & =N-(CH₃)₃), 2.09 (p, J = 6.9 Hz, 2H, -CH₂-CH₂-N(CH₃)₂); ¹³C NMR (151 MHz, DMSO-d₆, δ [ppm]): 165.9 (Ar-COOH),165.3 ( -CONH-CH₂-triazole-), 164.8 ( -CONH-(CH₂)₂-), 157.8 (q, J = 32.8 Hz, OOC-F₃), 156.7 (3H-xanthen C-3, 3H-xanthen C-4, 10a), 156.5 (3H-xanthen C-3, 3H-xanthen C-4, 10a), 153.2 (benzamide C-2), 146.8 (4-chlorophenol C-1), 144.6 (triazole), 139.6 (benzamide C-1), 136.3 (benzene sulfonamide C-4), 135.9 (carbamoyl benzoate C-2), 132.3 (benzene sulfonamide C-3), 132.0 (benzamide C-4), 131.7 (benzene sulfonamide C-5), 131.2 (carbamoyl benzoate C-4) 130.5 (3H-xanthen C-1,8), 130.4 (carbamoyl benzoate C-3 & benzene sulfonamide C-6), 129.6 (carbamoyl benzoate C-6), 129.3 (4-chlorophenol C-4), 128.4 (4-chlorophenol C-2), 128.0 (benzene sulfonamide C-2), 126.4 (4-chlorophenol C-5), 126.4 (benzene sulfonamide C-6), 126.1 (benzamide C-1), 124.0 (4-chlorophenol C-3), 123.9 (benzamide C-3), 122.8 (triazole-CH₂, 121.8 (4-chlorophenol C-6), 117.3 (benzamid C-3), 114.6 (3H-xanthen C-2,7), 112.8 (3H-xanthen C-8,9a), 96.3 (3H-xanthen C-4,5), 47.3 (-CH₂-CH₂-triazole-), 40.5 (i-(N(CH₃)₂) & =N-(CH₃)₃), 36.8 ( -CONH-CH₂-CH₂-), 35.0 ( -CONH-CH₂-triazole-), 29.8 (-CH₂-CH₂-CH₂-); UV-Vis: λmax(Ex) = 556 nm, λmax(Em) = 630 nm, Stokes shift = 74 nm; LRMS (ESI⁺): m/z: 512 [M+2H⁺]; 379 [(M+H⁺)²⁺]; 351 [(M+H⁺)³⁺]; found: 511.1072; HPLC retention time 18.90 min, 95.3% (M1).

5-Azidopentan-2-one (17)₄

5-Chloropentan-2-one (1.0 g, 7.86 mmol, 1.0 eq) was dissolved in anhydrous DMSO (6 mL). NaN₃ (1.53 g, 23.6 mmol, 3.0 eq) was added and the reaction mixture was stirred at 70 °C for 24 h. After TLC confirmed the conversion of the starting material, the mixture was quenched with water and extracted with diethyl ether (3 x 25 mL). The combined organic layer was washed with water, brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude product was purified by automated column chromatography (EtOAc/isohexane, gradient: 0% to 60%, elution at 20%). The title compound was obtained as a yellowish oil (320 mg, 2.52 mmol, 31%). Rₙ = 0.4 (EtOAc/isohexane 2:8, stained with KMnO₄); ¹H NMR (400 MHz, DMSO-d₆, δ [ppm]): 3.31 (t, J = 7.0 Hz, 2H, -CH₂-N₃), 2.52-2.47 (m, 2H, H₂C-CO-CH₂-), 2.10 (s, 3H, -CH₃), 1.70 (p, J = 7.0 Hz, 2H, -CH₂-CH₂-CH₂-N₃). The obtained analytical data are in good agreement with literature values.₄

S10
Ethyl 7-azido-2,4-dioxoheptanoate (18)

\[
\text{O} \quad \text{O} \quad \text{N}_3
\]

5-Azidopentan-2-one (17, 255 mg, 2.01 mmol, 1.0 eq) and diethyl oxalate (293 mg, 2.01 mmol, 1.0 eq) were added slowly to a stirred, freshly prepared and -5°C cooled NaOEt solution (3 mL). After completion monitored by LC-MS and TLC, the reaction mixture was poured into ice cold water and acidified to pH 3-4. After extraction with EtOAc (3x) the combined organic layer was washed with water and brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified by automated column chromatography (EtOAc/isohexane, gradient: 0-30%). The title compound was obtained as a colorless oil (140 mg, 0.62 mmol, 30%). Rₙ = 0.5 (EtOAc/isohexane, 2:8); ¹H NMR (400 MHz, DMSO-d₆ δ [ppm]): 6.34 (s, 1H, -CO-CH₂-CO-), 4.26 (q, J = 7.1 Hz, 2H, -CH₂-CH₃), 3.36 (t, J = 6.9 Hz, 2H, -CO-CH₂-CH₂-), 2.68 (t, J = 7.1 Hz, 2H, -CH₂-N₃), 1.86–1.75 (m, 2H, -CH₂-CH₂-CH₂-), 1.27 (t, J = 7.1 Hz, 3H, -CH₃-CH₃). LRMS (ESI⁺) m/z (%): 228 [M+H⁺]. The compound was synthesized according to a procedure described by Kopp et al.⁵

4-(4-Azidobutanoyl)-1-(4-chloro-2-fluorophenyl)-5-cyclohexyl-3-hydroxy-1,5-dihydro-2H-pyrrol-2-one (19)

Cyclohexane carbaldehyde (25 mg, 0.22 mmol, 1.0 eq), 4-chloro-2-fluoroaniline (32 mg, 0.22 mmol, 1.0 eq), and ethyl 7-azido-2,4-dioxoheptanoate (18, 50 mg, 0.22 mmol, 1.0 eq) were placed in a microwave tube and dissolved in acetic acid (700 µL). The reaction mixture was heated to 95 °C and stirred for 4 h. After complete conversion of the starting materials monitored by LC-MS, the acetic acid was removed under reduced pressure and the brown residue was triturated with Et₂O. The colorless precipitate was separated by filtration and was washed with Et₂O. This step was performed several times to obtain the title compound (33 mg, 78 µmol, 36%) as a colorless solid. Rₙ = 0.5 (EtOAc/isohexane + AcOH 0.1% 3:7); ¹H NMR (600 MHz, DMSO-d₆ δ [ppm]): 12.34 (s, 1H, -OH), 7.67–7.59 (m, 2H, phenyl H-3,6), 7.42–7.38 (m, 1H, phenyl H-5), 4.94 (d, J = 1.9 Hz, 1H, pyrrole H-5), 3.38° (t, J = 6.9, 2.3 Hz, 1H, N₃-CH₂-), 2.93 (td, J = 7.2, 1.9 Hz, 2H, -CH₂-CO-), 1.94-1.87 (m, 1H, cyclohexyl H-1), 1.87-1.80 (m, 2H, -CH₂-CH₂-CH₂-), 1.65-1.33 (m, 5H, cyclohexyl -CH₂-), 1.07-0.70 (m, 4H, cyclohexyl -CH₂-); ¹³C NMR (100 MHz, DMSO-d₆ δ [ppm]): 194.9 (-CO-CH₂-), 164.5 (pyrrole C-3), 156.6 (d, J = 254.5 Hz, phenyl C-2), 152.5° (pyrrole C-2), 132.6 (d, J = 10.1 Hz, phenyl C-4), 129.6 (phenyl C-6), 125.3 (d, J = 4.0 Hz, phenyl C-5), 124.5 (d, J = 12.1 Hz, phenyl C-1), 119.3 (pyrrole C-4), 117.4 (d, J = 24.2 Hz, phenyl C-3), 62.7 (pyrrole C-5), 50.3 (N₃-CH₂-), 39.5° (cyclohexyl C-1), 39.1° (-CH₂-CO-), 30.4 (cyclohexyl -CH₂-), 26.5 (cyclohexyl -CH₂-), 26.0 (cyclohexyl -CH₂-), 25.9 (cyclohexyl -CH₂-), 25.5 (cyclohexyl -CH₂-), 22.8 (-CH₂-CH₂-CH₂-); LRMS (ESI⁺): m/z 421 [M+H⁺]; HRMS (ESI⁺): m/z calcd for C₂₉H₂₅F₃N₅O₂: 421.1437 [M+H⁺]; found: 421.1432.

4-(4-Azidobutanoyl)-1-(4-bromo-2-fluorophenyl)-5-cyclohexyl-3-hydroxy-1,5-dihydro-2H-pyrrol-2-one (20)

Cyclohexane carbaldehyde (25 mg, 0.22 mmol, 1.0 eq), 4-bromo-2-fluoroaniline (42 mg, 0.22 mmol, 1.0 eq), and the ethyl 7-azido-2,4-dioxoheptanoate (18, 50 mg, 0.22 mmol, 1.0 eq) were placed in a microwave tube and dissolved in acetic acid (1 mL). The reaction mixture was heated to 95 °C and stirred for 4 h. After complete conversion of the starting materials monitored by LC-MS, acetic acid was removed under reduced pressure and the brown residue was triturated with Et₂O. The colorless precipitate was separated by filtration and was washed with Et₂O. This step was performed several times to obtain the title compound (33 mg, 71 µmol, 32%) as a colorless solid. ¹H NMR (400 MHz, DMSO-d₆ δ [ppm]): 12.39 (s, 1H, -OH), 7.77 (dd, J = 10.2, 2.1 Hz, 1H, phenyl H-5), 7.65–7.44 (m, 2H, phenyl H-3,6), 4.93 (d, J = 1.9 Hz, 1H, pyrrole...
H-5). 3.39–3.29* (m, 2H, N$_2$H$_2$-CH$_2$-), 2.97–2.82 (m, 2H, -CH$_2$-CO$_2$-), 1.95–1.76 (m, 3H, cyclohexyl H-1 & -CH$_2$-CH$_2$-CH$_2$-), 1.66–1.28 (m, 5H, cyclohexyl -CH$_2$-), 1.05–0.70 (m, 4H, cyclohexyl -CH$_2$-), 0.54–0.39 (m, 1H, cyclohexyl -CH$_2$-); $^{13}$C NMR (101 MHz, DMSO-d$_6$ δ [ppm]): δ 194.9 (C=O), 164.4 (pyrrolone C-3); 156.7 (d, J = 255.5 Hz, phenyl C-2), 152.5 (pyrrolone C-2), 129.8 (phenyl C-5), 128.2 (d, J = 4.0 Hz, phenyl C-6), 124.9 (d, J = 12.1 Hz, phenyl C-1), 120.5 (d, J = 9.1 Hz, phenyl C-4), 120.1 (d, J = 23.2 Hz, phenyl C-3), 119.3 (pyrrolone C-4), 62.6 (pyrrolone C-5), 50.3 (N$_2$-CH$_2$-), 39.6* (cyclohexyl C-1), 39.0* (-CH$_2$-CO$_2$-), 30.4 (cyclohexyl -CH$_2$-), 26.5 (cyclohexyl -CH$_2$-), 26.0 (cyclohexyl -CH$_2$-), 25.9 (cyclohexyl -CH$_2$-), 25.5 (cyclohexyl -CH$_2$-), 22.8 (-CH$_2$-CH$_2$-CH$_2$-); LRMS (ESI+) m/z: 465 [M+H]+.

Methyl 4-(4-chloro-2-nitrobenzoyl)benzoate (21)$^6$

![Chemical structure](image)

Cs$_2$CO$_3$ (3.71 g, 11.38 mmol, 2.0 eq) was added to a solution of 4-chloro-1-fluoro-2-nitrobenzene (1.00 g, 5.69 mmol, 1.0 eq) and methyl 4-hydroxybenzoate (1.70 g, 11.38 mmol, 2.0 eq) dissolved in DMF (7 mL). The reaction mixture was stirred for 2 h at 65 °C. Then, the reaction mixture was cooled down to ambient temperature and diluted with 5 mL of dichloromethane (5 mL). The organic layer was washed twice with water, brine, dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (EtOAc/isohexane, gradient 5:17). The title compound was obtained as a yellow solid (1.65 g, 5.37 mmol, 2.0 eq). Analytical data are in good agreement with literature values.$^6$

Methyl 4-((5-chloro-3-nitropyridin-2-yl)oxy)benzoate (22)$^7$

![Chemical structure](image)

A mixture of 2,5-dichloro-3-nitropyridine (1.00 g, 5.18 mmol, 1.0 eq), methyl 4-hydroxybenzoate (1.58 g, 10.36 mmol, 2.0 eq) and K$_2$CO$_3$ (1.43 g, 10.36 mmol, 2.0 eq) was heated to 60°C in anhydrous DMF (5 mL) for 2 h under a nitrogen atmosphere. After completion, the reaction was diluted with dichloromethane. The organic layer was separated and the aqueous layer was washed with dichloromethane. Then, the combined organic layer was washed with water and brine, dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. After evaporation of the solvents, the crude residue was purified by flash chromatography (EtOAc/isohexane, gradient 5:40%) to obtain the title compound as a colorless solid (1.34 g, 4.35 mmol, 84%). Analytical data are in good agreement with literature values.$^7$
Methyl 4-(2-amino-4-chlorophenoxy)benzoate (23)\(^6\)

\[
\text{H}_2\text{N} \quad \text{Cl} \\
\text{O} \quad \text{C} \\
\text{O} \quad \text{N} \\
\text{H}
\]

Methyl 4-(4-chloro-2-nitrophenoxy)benzoate (21, 1.4 g, 4.55 mmol, 1.0 eq) and SnCl\(_2\) x H\(_2\)O (4.77 g, 21.13 mmol, 5.0 eq) were dissolved in ethanol (100 mL) and stirred for 6 h. Then, the solvent was removed under reduced pressure and 1 M NaOH was added to the residue while stirring. The resulting suspension was extracted with EtOAc twice and the combined organic layer was dried over Na\(_2\)SO\(_4\), filtered, and concentrated under reduced pressure reduced. The title compound was obtained as a yellow solid (1.21 g, 4.3 mmol, 95%) and was used for the subsequent reaction without further purification. \(R_t = 0.45\) (EtOAc/isohexane, 2:8); \(^1\)H NMR (400 MHz, DMSO-d\(_6\) \(\delta\) [ppm]): 7.96–7.89 (m, 2H, methyl benzoate H-3,5), 6.98–6.94 (m, 2H, methyl benzoate H-2,6), 6.89 (d, \(J = 8.5\) Hz, 1H, 2-amino-4-chlorophenyl H-6), 6.85 (d, \(J = 2.6\) Hz, 1H, 2-amino-4-chlorophenyl H-3), 6.56 (dd, \(J = 8.5, 2.6\) Hz, 1H, 2-amino-4-chlorophenyl H-5), 5.36 (bs, 2H, -NH\(_2\)), 3.81 (s, 3H, -CH\(_3\)). \(^{13}\)C NMR (101 MHz, DMSO-d\(_6\) \(\delta\) [ppm]): 165.8 (-COOCH\(_3\)), 161.5 (methyl benzoate C-4), 142.5 (2-amino-4-chlorophenyl C-2), 138.8 (2-amino-4-chlorophenyl C-1), 131.4 (methyl benzoate C-2,6), 129.9 (2-amino-4-chlorophenyl C-4), 123.4 (methyl benzoate C-1), 122.9 (2-amino-4-chlorophenyl C-5), 116.0 (methyl benzoate C-3,5), 115.5 (2-amino-4-chlorophenyl C-6), 114.9 (2-amino-4-chlorophenyl C-3), 52.0 (-CH\(_3\)). LRMS (ESI\(^+\)) m/z: 278 [M+H]. The attached \(^1\)H NMR spectra contains residual solvent signals (EtOAc). The obtained analytical data are in good agreement with literature values.\(^6\)

Methyl 4-((3-amino-5-chloropyridin-2-yl)oxy)benzoate (24)\(^7\)

\[
\text{Cl} \quad \text{NH}_2 \\
\text{Cl} \\
\text{O} \\
\text{C}
\]

Methyl 4-((5-chloro-3-nitropyridin-2-yl)oxy)benzoate (22, 1.00 g, 3.25 mmol, 1.0 eq) was dissolved in EtOH (25 mL) and SnCl\(_2\) x H\(_2\)O (5.86 g, 25.98 mmol, 8.0 eq) was added in portions. The reaction mixture was heated to 60 °C overnight. Then, the ethanol was removed under reduced pressure and the residue was treated with 1N NaOH. The aqueous layer was extracted with EtOAc (3x 25 mL). The combined organic layer was washed with water, brine, dried over Na\(_2\)SO\(_4\), filtered, and concentrated under reduced pressure. The title compound was obtained as a yellow solid (770 mg, 2.77 mmol, 85%) and was used without further purification. \(^1\)H NMR (400 MHz, DMSO-d\(_6\) \(\delta\) [ppm]): 8.00–7.94 (m, 2H, benzoate H-2,6), 7.34 (d, \(J = 2.4\) Hz, 1H, pyridine H-6), 7.22–7.17 (m, 2H, benzoate H-3,5), 7.13 (d, \(J = 2.4\) Hz, 1H, pyridine H-4), 5.73 (s, 2H, -NH\(_2\)), 3.84 (s, 3H, -CH\(_3\)). \(^{13}\)C NMR (101 MHz, DMSO-d\(_6\) \(\delta\) [ppm]): 165.7 (C=O), 158.6 (benzoate C-4), 148.0 (pyridine C-2), 135.8 (pyridine C-5), 131.0 (benzoate C-2,6), 129.9 (pyridine C-6), 126.8 (pyridine C-3), 124.9 (benzoate C-1), 120.2 (pyridine C-4), 119.9 (benzoate C-3,5), 52.1 (-CH\(_3\)). LRMS (ESI\(^+\)) m/z: 279 [M+H]. The attached \(^1\)H NMR spectra contains residual solvent signals (MeOH). Analytical data are in good agreement with literature values.\(^7\)

4-(4-Chloro-2-((3,4-dichlorophenyl)sulfonamido)phenoxy)benzoic acid (25)\(^6\)

\[
\text{O} \quad \text{S} \\
\text{Cl} \quad \text{Cl} \\
\text{O} \\
\text{H}
\]

S13
Under vigorous stirring, an aqueous solution of LiOH (2 N, 15 mL) was added to a solution of methyl 4-(4-chloro-2-((3,4-dichlorophenyl)sulfonamido)phenoxy)benzoate (38, 1.00 g, 2.06 mmol, 1.0 eq) dissolved in THF (14 mL). The reaction mixture was stirred at ambient temperature for 4 h. Then, the organic solvent was removed under reduced pressure and the residual aqueous layer was acidified with 2 N HCl until the carboxylic acid precipitated. The aqueous layer was extracted with EtOAc twice. The combined organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The title compound was obtained as a colorless solid (950 mg, 2.01 mmol, 97%) and was used without further purification for the subsequent reactions. For analytical and biological testing 25 was purified by preparative HPLC (acetonitrile/water (0.1% TFA): gradient 20-90%). ¹H NMR (400 MHz, DMSO-d₆ δ [ppm]): 12.80 (bs, 1H, -OH), 10.59 (s, 1H, -SO₂NH₂), 7.83–7.79 (m, 3H, benzene sulfonamide H-3,5,8), 7.63 (d, J = 5.4 Hz, 1H, benzene sulfonamide H-2), 7.63 (d, J = 8.5 Hz, 7.60 Hz, 1H, benzene sulfonamide H-5), 7.57 (dd, J = 8.4, 2.1 Hz, 1H benzene sulfonamide H-6), 7.50 (d, J = 2.6 Hz, 1H, 4-chlorophenol H-3), 7.32 (dd, J = 8.8, 2.6 Hz, 1H, 4-chlorophenol H-5), 7.03 (d, J = 8.8 Hz, 1H, 4-chlorophenol H-6), 6.66–6.56 (m, 2H, benzene acid H-2,6); ¹³C NMR (151 MHz, DMSO-d₆ δ [ppm]): 166.6 (C=O), 159.8 (benzene acid C-4), 147.0 (4-chlorophenol C-1), 140.1 (benzene sulfonamide C-1), 136.1 (benzene sulfonamide C-4), 132.1 (benzene sulfonamide C-3), 131.5 (benzene sulfonamide C-5), 131.3 (benzene acid C-3,5), 129.3 (4-chlorophenol C-4), 128.6 (4-chlorophenol C-2), 124.1 (benzene acid C-1), 122.4 (4-chlorophenol C-6), 116.3 (benzene acid C-2,6); LRMS (ESI⁺) m/z: 494 [M+Na⁺]; HRMS (ESI⁺): m/z calcd for C₂₁H₂₁Cl₂N₂NaO₂S²: 493.9394 [M+Na⁺], found: 493.9396; The obtained analytical data are in good agreement with literature values.⁶

4-(4-Chloro-2-((4-chloro-3-(trifluoromethyl)phenyl)sulfonamido)phenoxy)benzoic acid (26)⁶

![Structure of 4-(4-Chloro-2-((4-chloro-3-(trifluoromethyl)phenyl)sulfonamido)phenoxy)benzoic acid](image)

4-Chloro-3-(trifluoromethyl)benzenesulfonic acid (602 mg, 2.16 mmol, 1.2 eq) and anhydrous pyridine (171 mg, 2.16 mmol, 1.0 eq) were added to a solution of methyl 4-(2-amino-4-chlorophenoxo)benzoate (23, 500 mg, 1.80 mmol, 1.0 eq) in anhydrous CH₂Cl₂ (12 mL) under a nitrogen atmosphere. The reaction mixture was stirred for 4 h at 50 °C. Then, the reaction was quenched with water, the aqueous layer was extracted with CH₂Cl₂ twice and the combined organic layer was washed with brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The slight yellow residue was purified by automated flash chromatography (EtOAc/isohexane, gradient 5-15%) to obtain 550 mg of the methyl benzoate intermediate (39, 1.06 mmol, 58%). LRMS (ESI⁺) m/z: 542 [M+Na⁺]. Under vigorous stirring, an aqueous solution of LiOH (2 N, 10 mL) was added to the methyl benzoate intermediate (500 mg, 0.97 mmol, 1.0 eq) dissolved in THF (10 mL). The reaction mixture was stirred for 4 h at ambient temperature. Then, THF was removed under reduced pressure and the aqueous layer was acidified with 2 N HCl until the carboxylic acid precipitated. The acidified aqueous layer was extracted with EtOAc twice, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The title compound was obtained as a tan solid (447 mg, 0.89 mmol, 91%) and was used for the subsequent reaction without further purification. ¹H NMR (400 MHz, DMSO-d₆ δ [ppm]): 12.76 (bs, 1H, -COOH), 10.68 (s, 1H, -NH₂SO₂⁻), 8.01 (d, J = 2.3 Hz, 1H, benzene sulfonamide H-2), 7.88 (dd, J = 8.5, 2.3 Hz, 1H, benzene sulfonamide H-6), 7.81–7.75 (m, 2H, benzene acid H-3,5), 7.73 (d, J = 8.5 Hz, 1H, benzene sulfonamide H-5), 7.51 (d, J = 2.6 Hz, 1H, 4-chlorophenol H-3), 7.32 (dd, J = 8.8, 2.6 Hz, 1H, 4-chlorophenol H-5), 7.00 (d, J = 8.8 Hz, 1H, 4-chlorophenol H-6), 6.60–6.52 (m, 2H, benzene acid H-2,6); ¹³C NMR (101 MHz, DMSO-d₆ δ [ppm]): 166.6 (C=O), 159.7 (benzene acid C-4), 147.3 (4-chlorophenol C-1), 133.9 (benzene sulfonamide C-1), 135.5 (benzene sulfonamide C-4), 132.9 (benzene sulfonamide C-3), 131.9 (benzene sulfonamide C-6), 131.3 (benzene acid C-3,5), 128.5 (4-chlorophenol C-2), 127.7 (4-chlorophenol C-3), 127.3 (q, J = 31.8 Hz, benzene sulfonamide C-3), 127.3 (4-chlorophenol C-3), 125.7 (q, J = 5.4 Hz, benzene sulfonamide C-2), 125.5 (benzene acid C-1), 122.2 (4-chlorophenol C-6), 121.9 (q, J = 273.7 Hz, -C¼F), 116.4 (benzene acid C-2,6); LRMS (ESI⁺) m/z: 528 [M+Na⁺]; HRMS (ESI⁺): m/z calcd for C₂₀H₁₅Cl₂F₃N₂NaO₂S²: 505.9838 [M+H⁺], found: 505.9838. The attached ¹H and ¹³C NMR spectra contains residual solvent signals (EtOAc, DMF). The obtained analytical data are in good agreement with literature values.⁶
4-((5-Chloro-3-((4-chloro-3-(trifluoromethyl)phenyl)sulfonamido)pyridin-2-yl)oxy)benzoic acid (27)

The synthesis compound 27 was previously reported by Wang et al. To provide experimental details and unpublished characterization data (1H NMR, 13C NMR, HRMS), we have outlined the synthesis and the characterization data for this compound. Methyl 4-((5-chloro-3-((4-chloro-3-(trifluoromethyl)phenyl)sulfonamido)pyridin-2-yl)oxy)benzoate (40, 700 mg, 1.35 mmol, 1.0 eq) was dissolved in a H2O/THF mixture (1:1). LiOH (310 g, 10.0 mmol, 10 eq) was added and the reaction mixture was stirred overnight at 30 °C. After completion, the organic solvent was removed under reduced pressure and the residual aqueous layer was washed with EtOAc. Then, the aqueous layer was acidified with 2 N HCl and extracted with EtOAc (3 x 20 mL). The combined organic layer was washed with water, dried over Na2SO4, filtered, concentrated under reduced pressure. The residue was purified by flash chromatography (MeOH/CH2Cl2, gradient from 2-10%) to obtain the title compound as a colorless solid (649 mg, 1.28 mmol, 95%). For biological testing the compound was further purified by preparative HPLC (acetonitrile/water + 0.1% TFA, gradient from 20-85%). Rf = 0.2 (MeOH/CH2Cl2 1:9); 1H NMR (600 MHz, DMSO-d6 δ [ppm]): 12.92 (bs, 1H, -COOH), 10.91 (s, 1H, -NHSO2-), 8.15 (d, J = 2.3 Hz, 1H, benzene sulfonamide H-2), 8.11 (d, J = 2.5 Hz, 1H, pyridine H-6), 7.99 (dd, J = 8.4, 2.3 Hz, 1H, benzene sulfonamide H-6), 7.97 (d, J = 2.5 Hz, 1H, pyridine H-4), 7.87 – 7.84 (m, 3H, benzoic acid H-2,6 & benzene sulfonamide H-5), 6.78 – 6.72 (m, 2H, benzoic acid H-3,5); 13C NMR (101 MHz, DMSO-d6 δ [pppm]): 166.57 (-COOH), 156.6 (benzoic acid C-4), 154.6 (pyridine C-2), 143.1 (pyridine C-6), 139.5 (benzene sulfonamide C-1), 136.2 (pyridine C-4), 135.7 (benzene sulfonamide C-4), 133.0 (benzene sulfonamide C-5), 132.1 (benzene sulfonamide C-6), 130.9 (benzoic acid C-2,6), 127.5 (q, J = 31.8 Hz, benzene sulfonamide C-3), 126.9 (benzoic acid C-1), 126.0 (q, J = 5.4 Hz, benzene sulfonamide C-2), 125.8 (pyridine C-5), 122.1 (pyridine C-3) 121.9 (q, J = 274.7 Hz, -C3F7), 119.8 (benzoic acid C-3,5); LRMS (ESI⁺) m/z: 507 [M+H⁺]. HRMS (ESI⁺): m/z calcd for C21H14F3Cl2N4O4S3S+H⁺: 506.9791 [M+H⁺], found: 506.9793. HPLC retention time 18.93 min, 97.9% (M1). The attached 1H NMR and 13C spectra contains residual solvent signals (acetonitrile).

N-(2-(2-(2-Azidoethoxy)ethoxy)ethyl)-4-(4-chloro-2-((3,4-dichlorophenyl)sulfonamido)phenoxy)benzamide (28)

Under dry conditions, HOBr (29 mg, 0.212 mmol, 1.1 eq), EDC x 5HCl (41 mg, 0.212 mmol, 1.1 eq), and DIPEA (72 mg, 2.9 eq) were added to a solution of 4-(4-chloro-2-((3,4-dichlorophenyl)sulfonamido)phenoxy)benzoic acid (25, 100 mg, 0.212 mmol, 1.1 eq) dissolved in anhydrous DMF (1 mL). The mixture was stirred for 1 h, then 2-(2-(2-azidoethoxy)ethoxy)ethan-1-amine (41, 33 mg, 0.192 mmol, 1.0 eq) was added. The solution was stirred overnight at ambient temperature. Then, volatiles were removed under reduced pressure and the residue was dissolved in dichloromethane. The organic layer was washed with 1 N NaOH, 1 N HCl, and brine, dried over Na2SO4, filtered, and concentrated under reduced pressure. The crude title compound was obtained as a pale yellow solid (91 mg, 0.144 mmol, 75%) and was used for the subsequent reaction without further purification. 1H NMR (400 MHz, DMSO-d6 δ [ppm]): 10.56 (bs, 1H, -NHSO2-), 8.42 (t, J = 5.6 Hz, 1H, -CONH-), 7.84 (d, J = 2.1 Hz, 1H, benzene sulfonamide H-2), 7.79–7.75 (m, 2H, benzamide H-3,5), 7.67 (d, J = 8.4 Hz, 1H, benzene sulfonamide H-5), 7.61–7.57 (m, 1H, benzene sulfonamide H-6), 7.45 (d, J = 2.6 Hz, 1H, 4-chlorophenol H-3), 7.26 (dd, J = 8.8, 2.6 Hz, 1H, 4-chlorophenol H-5), 6.92 (d, J = 8.8 Hz, 1H, 4-chlorophenol H-6), 6.67–6.62 (m, 2H, benzamide H-2,6), 3.62–3.50 (m, 8H, -CH2-), 3.43–3.34 * (m, 4H, -CH2-); 13C NMR (101 MHz, DMSO-d6 δ [ppm]): 165.2 (C=O), 158.24 (benzamide C-4), 147.7 (4-chlorophenol C-1), 140.4 (benzene sulfonamide C-1) 136.0 (benzene sulfonamide C-4), 132.1 (benzene sulfonamide C-3), 131.6 (benzene sulfonamide C-5), 131.5 (benzene sulfonamide C-6), 129.3 (4-chlorophenol C-4), 129.1 (benzamide C-2,6), 128.2 (benzene sulfonamide C-)}
C-2), 128.0 (benzamide C-1), 127.2 (4-chlorophenol C-3), 126.6 (4-chlorophenol C-5), 121.4 (4-chlorophenol C-6), 116.7 (benzamide C-3.5), 69.7 (-O-CH$_2$-CH$_2$-O), 69.3 (N$_2$-CH$_2$-CH$_2$), 69.0 (-O-CH$_2$-CH$_2$-NHCO), 50.0 (N$_2$-CH$_2$), 40.0 (-O-CH$_2$-CH$_2$-NHCO); LRMS (ESI$^+$) m/z: 628 [M+H]$^+$. The attached $^1$H and $^{13}$C NMR spectra contains residual solvent signals (CH$_3$Cl, DMF).

a) the $^{13}$C NMR signals for 4-chlorophenol C-2 could not be detected.

\[\text{N}(2-(2-(2-Azidoethoxy)ethoxy)ethyl)-4-(4-chloro-2-((4-chloro-3-(trifluoromethyl)phenyl)sulfonamido)phenoxy)benzamide (29)\]

\[\text{O} \quad \text{Cl} \quad \text{CF}_3 \quad \text{Cl} \quad \text{N}\]

\[\text{O} \quad \text{S} \quad \text{N}\]

\[\text{H} \quad \text{O} \quad \text{O} \quad \text{N}_3\]

4-(4-Chloro-2-((4-chloro-3-(trifluoromethyl)phenyl)sulfonamido)phenoxy)benzoic acid (26.100 mg, 198 µmol, 1 eq) was placed in a reaction flask together with HOBt (27 mg, 198 µmol, 1.0 eq), EDC x HCl (38 mg, 198 µmol, 1.0 eq), and DIPEA (77 mg, 0.59 mmol, 3.0 eq) dissolved in anhydrous DMF. The reaction mixture was stirred at ambient temperature for 1 h. Then, 2-(2-(2-azidoethoxy)ethoxy)ethylamine (41, 35 mg, 198 µmol, 1.0 eq) was added to the reaction mixture. After 5 h, the DMF was removed under reduced pressure and the crude residue was treated with an aqueous saturated NH$_4$Cl solution. The aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layer was washed with 2 N NaOH, then dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The crude product (70 mg, 106 µmol, 53% yield) was used for the next reaction without further purification. $R_f = 0.4$ (EtOAc/isohexane, 15:85). $^1$H NMR (400 MHz, DMSO-d$_6$) δ 10.65 (s, 1H, -NHCO), 8.62 (t, $J = 5.6$ Hz, 1H, -NHCO$-$), 8.06 (d, $J = 2.2$ Hz, 1H, benzene sulfonamide H-2), 7.91 (dd, $J = 8.4$, 2.2 Hz, 1H, benzene sulfonamide H-6), 7.81-7.74 (m, 3H, benzamide H-2,6,8 & benzene sulfonamide H-5), 7.46 (d, $J = 2.6$ Hz, 1H, 4-chlorophenol H-3), 7.29 (dd, $J = 8.8$, 2.6 Hz, 1H, 4-chlorophenol H-5), 6.99 (d, $J = 8.8$ Hz, 1H, 4-chlorophenol H-6), 6.63-6.54 (m, 2H, benzamide H-3,5), 3.66-3.36 (m, 4H, -CH$_2$), 3.45-3.36 (m, 4H, -CH$_2$). $^{13}$C NMR$^R$ (101 MHz, DMSO-d$_6$ δ ppm): 165.2 (C=O), 158.0 (benzamide C-4), 148.2 (4-chlorophenol C-1), 139.6 (benzene sulfonamide C-1), 135.5 (benzene sulfonamide C-4), 132.9 (benzene sulfonamide C-5), 131.9 (benzene sulfonamide C-6), 129.5 (4-chlorophenol C-4), 129.1 (benzamide C-2,6), 128.6 (benzamide C-1), 127.9 (4-chlorophenol C-2), 127.7 (4-chlorophenol C-5), 127.3 (q, $J = 30.1$ Hz, benzene sulfonamide C-3), 127.2 (4-chlorophenol C-3), 125.8 (q, $J = 5.4$ Hz, benzene sulfonamide C-2), 121.9 (q, $J = 274.72$ Hz, benzene sulfonamide -CF$_3$), 121.1 (4-chlorophenol C-6), 116.9 (benzamide C-3,5), 69.7 (-O-CH$_2$-CH$_2$), 69.3 (-O-CH$_2$-CH$_2$-NHCO- or N$_2$-CH$_2$-CH$_2$), 69.0 (-O-CH$_2$-CH$_2$-NHCO- or N$_2$-CH$_2$-CH$_2$-O), 50.0 (-O-CH$_2$-CH$_2$), 39.2 (-O-CH$_2$-CH$_2$-NHCO). LRMS (ESI$^+$) m/z: 662 [M+H]$^+$. HRMS (ESI$^+$): m/z calcd for C$_{33}$H$_{24}$Cl$_3$F$_3$N$_3$O$_6^+$: 662.0849 [M+H]$^+$, found: 662.0852.

\[\text{N}(2-(2-Azidoethoxy)ethoxy)ethyl)-4-(5-chloro-3-((4-chloro-3-(trifluoromethyl)phenyl)sulfonamido)pyridin-2-yl)oxy)benzamide (30)\]

\[\text{O} \quad \text{Cl} \quad \text{CF}_3 \quad \text{Cl} \quad \text{N}\]

\[\text{O} \quad \text{S} \quad \text{N}\]

\[\text{H} \quad \text{O} \quad \text{O} \quad \text{N}_3\]

TBTU (25 mg, 78.9 µmol, 2.0 eq) and DIPEA (25 mg, 197.0 µmol, 5.0 eq) were added to a solution of 27 (27, 20 mg, 39.5 µmol, 1.0 eq) dissolved in anhydrous DMF (1 mL). The mixture was stirred for 15 min at ambient temperature. Then, 2-(2-azidoethoxy)ethoxy)ethylamine (41, 5.2 mg, 47.3 µmol, 1.2 eq) was added. The solution was stirred for 2 h at ambient temperature. Afterwards, the DMF was removed under reduced pressure and the residue was dissolved in dichloromethane. The organic layer was washed with aq. NH$_4$Cl solution and brine, dried over Na$_2$SO$_4$, filtered and
concentrated under reduced pressure. The title compound was obtained as a pale yellow solid (11 mg, 16.6 µmol, 42%) and used for the subsequent reaction without further purification. \( R = 0.4 \) (MeOH/CH₂Cl₂ 2%); \(^{1}H\) NMR (400 MHz, DMSO-d₆ δ [ppm]): 10.88 (s, 1H, -NH₂SO₃), 8.48 (t, J = 5.6 Hz, 1H, -CONH-C₆H₄-CH₂-OH), 8.18 (d, J = 2.3 Hz, 1H, benzene sulfonamide H-2), 8.04 (d, J = 2.4 Hz, 1H, pyridine H-6), 8.00 (dd, J = 8.4, 2.3 Hz, 1H, benzene sulfonamide H-6), 7.93 (d, J = 2.4 Hz, 1H, pyridine H-4), 7.89 (d, J = 8.4 Hz, 1H, benzene sulfonamide H-5), 7.82–7.73 (m, 2H, benzoic acid H-2,6), 6.81–6.72 (m, 2H, benzoic acid H-3,5), 3.61–3.51 (m, 8H, -CH₂-), 3.45–3.33 (m, 4H, -CH₂-); \(^{13}C\) NMR\(^{a)}\) (151 MHz, DMSO-d₆ δ [ppm]): 165.3 (-C=O), 155.04 (benzamide C-4), 154.8 (pyridine C-2), 142.7 (pyridine C-6), 139.7 (benzene sulfonamide C-1), 135.6 (benzene sulfonamide C-4), 135.5 (pyridine C-4), 132.9 (benzene sulfonamide C-5), 132.1 (benzene sulfonamide C-6), 130.8 (benzamide C-1), 128.6 (benzamide C-2,6), 127.5 (q, J = 31.8 Hz, benzene sulfonamide C-3), 126.0 (q, J = 5.4 Hz, benzene sulfonamide C-2), 125.3 (pyridine C-5), 121.9 (q, J = 273.7 Hz, -CF₃), 119.9 (benzamide C-3,5), 69.6 (-O-CH₂-C₆H₄-O-), 69.2 (-O-CH₂-C₆H₄-NHCO- or N₂-CH₂-C₆H₄-O-), 68.9 (-O-CH₂-C₆H₄-NHCO- or N₂-CH₂-C₆H₄-O-), 50.1 (-CH₂-N₂), 39.2 (-O-CH₂-C₆H₄-NHCO-); LRMS (ESI⁺) m/z: 685 [M+Na]⁺.

\( a)\) the \(^{13}C\) NMR signals for pyridine C-3 could not be detected.

4-(4-Chloro-2-((3,4-dichlorophenyl)sulfonamido)phenoxy)-N-(prop-2-yn-1-yl)benzamide (31)

\[
\begin{align*}
\text{Cl} & \quad \text{Cl} \\
\text{NH} & \quad \text{S} \\
\text{O} & \quad \text{Cl}
\end{align*}
\]

Under dry conditions, HOBr (9 mg, 63.0 µmol, 1.1 eq), EDC x 5HCl (12 mg, 63.0 µmol, 1.1 eq), and DIPEA (23 mg, 0.183 mmol, 2.9 eq) were added to a solution of 4-(4-chloro-2-((3,4-dichlorophenyl)sulfonamido)phenoxy)benzoic acid (25, 30 mg, 63.0 µmol, 1.1 eq) dissolved in anhydrous DMF (1 mL). The reaction was stirred for 1 h at ambient temperature. Then, propargyl amine (3.5 mg, 57.0 µmol, 1.0 eq) was added. The resulting reaction mixture was stirred for further 2 h at ambient temperature. Subsequently, DMF was removed under reduced pressure and the residue was dissolved with dichloromethane. The organic layer was washed with 1 N NaOH, 1 N HCl, and brine in that order. The organic layer was dried over NaSO₄, filtered, and concentrated under reduced pressure. The title compound was obtained as a colorless solid (20 mg, 0.039 mmol, 68%) and was used without further purification for the subsequent reaction. \(^{1}H\) NMR (400 MHz, DMSO-d₆, δ [ppm]): 10.56 (s, 1H, -NH₂SO₃), 8.84 (t, J = 5.6 Hz, 1H, -NHCO-), 7.84 (d, J = 2.1 Hz, 1H, benzene sulfonamide H-2), 7.81–7.75 (m, 2H, benzamide H-3,5), 7.66 (d, J = 8.4 Hz, 1H, benzene sulfonamide H-5), 7.59 (dd, J = 8.4, 2.1 Hz, 1H, benzamide H-3,5), 7.46 (d, J = 2.6 Hz, 1H, 4-chlorophenol H-3), 7.29 (dd, J = 8.8, 2.6 Hz, 1H, 4-chlorophenol H-5), 6.95 (d, J = 8.8 Hz, 1H, 4-chlorophenol H-6), 6.68–6.61 (m, 2H, benzamide H-2,6), 4.04 (dd, J = 5.6, 2.5 Hz, 2H, -CONH-C₆H₄-), 3.13 (t, J = 2.5 Hz, 1H, HC≡C-); \(^{13}C\) NMR\(^{a)}\) (101 MHz, DMSO-d₆ δ [ppm]): 164.9 (C=O), 158.5 (benzamide C-4), 147.6 (4-chlorophenol C-1), 136.0 (benzene sulfonamide C-4), 132.1 (benzene sulfonamide C-3), 131.5 (benzene sulfonamide C-5), 129.2 (benzoic acid C-3,5), 128.7 (4-chlorophenol C-4), 128.2 (benzene sulfonamide C-2), 128.1 (benzamide C-1), 127.3 (4-chlorophenol C-5), 126.7 (benzene sulfonamide C-6), 126.5 (4-chlorophenol C-3), 121.6 (4-chlorophenol C-6), 116.6 (benzamide C-2,6), 72.8 (HC≡C-C₆H₄-), 28.5 (HC≡C-C₆H₄-); LRMS (ESI⁺) m/z: 509 [M+H]⁺; HRMS (ESI⁺): m/z calcd for C₉₂H₇₁N₂O₂S₂: 508.9891 [M+H]+, found: 508.9888. The attached \(^{1}H\) and \(^{13}C\) NMR spectra contains residual solvent signals (DMF).

\( a)\) the \(^{13}C\) NMR signals for 4-chlorophenol C-2, benzene sulfonamide C-1, and HC≡C-C₆H₄- could not be detected.
4-((5-Chloro-3-((4-chloro-3-(trifluoromethyl)phenyl)sulfonamido)pyridin-2-yl)oxy)-N-(prop-2-yn-1-yl)benzamide (32)

4-((5-Chloro-3-((4-chloro-3-(trifluoromethyl)phenyl)sulfonamido)pyridin-2-yl)oxy)benzoic acid (27, 50 mg, 428.2 µmol, 2.0 eq) was dissolved in DMF (500 µL) and cooled to 0 °C. TBTU (48 mg, 148.5 µmol, 1.5 eq) and DIPEA (19 mg, 148.5 µmol, 1.5 eq) were dissolved in DMF (500 µL) in a separate flask and added dropwise to the reaction flask. The reaction was stirred for 30 minutes before adding the propargyl amine (6.5 mg, 118.8 µmol, 1.2 eq) to the reaction mixture. After stirring for 1 h at ambient temperature, the solvent was evaporated and water was added to the residue. The aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layer was washed with brine, dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The residue was purified by automated flash column chromatography (MeOH/dichloromethane: gradient 0-7%) to obtain the title compound as a yellowish solid (53 mg, 97.4 µmol, 98%). R₆ = 0.2 (MeOH/dichloromethane 1:9); 'H NMR (400 MHz, DMSO-d₆ [ppm]): 10.89 (s, 1H, -NHSO₂), 8.90 (t, J = 5.6 Hz, 1H, -NHCO-), 8.17 (d, J = 2.2 Hz, 1H, benzene sulfonamide H-2), 8.06 (d, J = 2.4 Hz, 1H, pyridine H-6), 8.00 (dd, J = 8.4, 2.2 Hz, 1H, benzene sulfonamide H-6), 7.94 (d, J = 2.4 Hz, 1H, pyridine H-4), 7.88 (d, J = 8.4 Hz, 1H, benzene sulfonamide H-5), 7.85-7.78 (m, 2H, benzamide H-2, 6), 6.80-6.74 (m, 2H, benzamide H-3, 5), 4.04 (dd, J = 5.6, 2.5 Hz, 2H, -CH₂-C=CH₂), 3.13 (t, J = 2.5 Hz, 1H, -CH₂-C=CH₂); ¹³C NMR (101 MHz, DMSO-d₆ [ppm]): 165.0 (CONH, -CONH), 155.3 (benzamide C-4), 154.8 (pyidine C-2), 142.9 (pyidine C-6), 139.6 (benzene sulfonamide C-1), 135.9 (pyidine C-4), 135.7 (benzene sulfonamide C-4), 133.0 (benzene sulfonamide C-5), 132.1 (benzene sulfonamide C-6), 130.2 (benzamide C-1), 128.8 (benzamide C-2, 6), 127.5 (q, J = 31.7 Hz, benzene sulfonamide C-3), 126.0 (q, J = 5.3 Hz, benzene sulfonamide C-2), 125.4 (pyidine C-5), 122.0 (q, J = 273.7, -CF₃), 121.9 (pyidine C-3), 120.0 (benzamide C-3, 5), 81.3 (C=CH), 72.9 (-CONH-CH₂), 28.5 (-CONH-CH₂); LRMS (ESI⁺) m/z: 544 [M+H⁺]; HRMS (ESI⁺): m/z calcd for C₂₂H₁₃Cl₅F₂N₂O₅S⁺: 544.0107 [M+H⁺]; found: 544.0109.

Methyl 2-(4-chloro-2-nitrophenoxy)benzoate (33)

Cs₂CO₃ (1.85 g, 5.7 mmol, 2.0 eq) was added to a solution of 4-chloro-1-fluoro-2-nitrobenzene (500 mg, 2.85 mmol, 1.0 eq) and methyl 2-hydroxybenzoate (867 mg, 5.7 mmol, 2.0 eq) dissolved in DMF (5 mL). The resulting mixture was stirred for 2 h at 60 °C. Then, the reaction mixture was cooled down to room temperature and diluted with 5 mL of dichloromethane. The organic layer was washed twice with water, dried over Na₂SO₄, and volatiles were removed under reduced pressure. The crude product (1.20 g) was purified by automated flash chromatography (EtOAc/isohexane: gradient 2-20%). The title compound was obtained as a yellow solid (770 mg, 2.52 mmol, 88%). R₆ = 0.3 (EtOAc/isohexane 2:8); 'H NMR (400 MHz, DMSO-d₆ [ppm]): 8.21 (d, J = 2.7 Hz, 1H, 2-nitro-4-chlorophenyl H-3), 7.96 (dd, J = 7.8, 1.8 Hz, 1H, methyl salicylate H-6), 7.73 (ddd, J = 8.2, 7.8, 1.8 Hz, 1H, methyl salicylate H-4), 7.67 (dd, J = 9.0, 2.7 Hz, 1H, 2-nitro-4-chlorophenyl H-5), 7.45 (ddd, J = 7.8, 7.8, 1.0 Hz, 1H, methyl salicylate H-5), 7.32 (dd, J = 8.2, 1.0 Hz, 1H, methyl salicylate H-3), 6.88 (d, J = 9.0 Hz, 1H, 2-nitro-4-chlorophenyl H-6), 3.68 (s, 3H, -CH₃); ¹³C NMR (101 MHz, DMSO-d₆ [ppm]): 164.4 (C=O), 153.1 (methyl salicylate C-2), 149.4 (2-nitro-4-chlorophenyl C-1), 140.3 (2-nitro-4-chlorophenyl C-2), 134.9 (methyl salicylate C-4), 134.5 (2-nitro-4-chlorophenyl C-5), 132.1 (methyl salicylate C-6), 126.4 (2-nitro-4-chlorophenyl C-4), 126.1 (methyl salicylate C-5), 125.2 (2-nitro-4-chlorophenyl C-3), 122.7 (methyl salicylate C-1), 122.5 (methyl salicylate C-3), 119.8 (2-nitro-4-chlorophenyl C-6), 52.3 (-CH₃). LRMS (ESI⁺) m/z: 330 [M+N⁺]. The obtained analytical data are in good agreement with literature values.⁸
Methyl 2-(4-chloro-2-nitrophenoxy)benzoate (33)

Methyl 2-(4-chloro-2-nitrophenoxy)benzoate (33, 700 mg, 2.28 mmol, 1.0 eq) was dissolved in ethanol (40 mL). The reaction was cooled down to 0 °C and SnCl₂ x H₂O (2.57 g, 11.40 mmol, 5.0 eq) was added while stirring. The mixture was stirred at room temperature for 3 h until full conversion of starting material was detected by means of TLC and LC-MS. The solvent was removed under reduced pressure and the residue was dissolved in water, cooled down to -4 °C and neutralized with a sat. Na₂CO₃ solution. The aqueous layer was extracted with ethyl acetate three times. The combined organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The title compound was obtained in an amount of 530 mg (1.91 mmol, 84%) as a pale yellow oil, which was used without further purification. Rf = 0.36 (EtOAc/isohexane 1:9); ¹H NMR (400 MHz, DMSO-d₆, δ [ppm]): 7.79 (dd, J = 7.7, 1.8 Hz, 1H, methyl salicylate H-6), 7.51 (dd, J = 8.4, 7.5, 1.8 Hz, 1H, methyl salicylate H-4), 7.18 (ddd, J = 7.7, 7.5, 0.9 Hz, 1H, methyl salicylate H-5), 6.85 (dd, J = 8.4, 0.9 Hz, 1H, methyl salicylate H-3), 6.82 (d, J = 2.6 Hz, 1H, 2-amino-4-chlorophenyl H-3), 6.74 (d, J = 8.5 Hz, 1H, 2-amino-4-chlorophenyl H-6), 6.53 (dd, J = 8.5, 2.6 Hz, 1H, 2-amino-4-chlorophenyl H-5), 5.34 (bs, 2H, -NH₂), 3.79 (s, 3H, -CH₃); ¹³C NMR (101 MHz, DMSO-d₆, δ [ppm]): 165.9 (C=O), 156.0 (methyl salicylate C-2), 141.8 (2-amino-4-chlorophenyl C-2), 140.7 (2-amino-4-chlorophenyl C-1), 133.8 (methyl salicylate C-4), 131.2 (methyl salicylate C-6), 128.8 (2-amino-4-chlorophenyl C-4), 122.8 (methyl salicylate C-5), 121.5 (methyl salicylate C-1), 121.2 (methyl salicylate C-3), 117.6 (2-amino-4-chlorophenyl C-5), 115.3 (2-amino-4-chlorophenyl C-6), 114.6 (2-amino-4-chlorophenyl C-3), 52.2 (-CH₃); LRMS (ESI⁺) m/z: 278.0 [M+H]⁺. The obtained analytical data are in good agreement with literature values.⁸

N-(2-(2-(2-Azidoethoxy)ethoxy)ethyl)-2-(4-chloro-2-((3,4-dichlorophenyl)sulfonamido)phenoxy)benzamide (35)

Under dry conditions, HOBT (29 mg, 0.212 mmol, 1.1 eq), EDC x HCl (41 mg, 0.212 mmol, 1.1 eq), and DIPEA (72 mg, 2.9 eq) were added to a solution of 2-(4-chloro-2-((3,4-dichlorophenyl)sulfonamido)phenoxy)benzoic acid (1 (SD-24), 100 mg, 0.212 mmol, 1.1 eq) dissolved in anhydrous DMF (1 mL). The reaction mixture was stirred for 1 h at ambient temperature. Then, 2-(2-(2-azidoethoxy)ethoxy)ethan-1-amine (41, 33 mg, 0.192 mmol, 1.0 eq) was added and the reaction mixture was stirred overnight at ambient temperature. Subsequently, volatiles were removed under reduced pressure and the residue was dissolved in dichloromethane. The organic layer was washed with 1 N NaOH, 1 N HCl, and brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude title compound was obtained as a pale yellow solid (90 mg, 0.157 mmol, 82%) and was used for the subsequent reaction without further purification.

¹H NMR (600 MHz, DMSO-d₆, δ [ppm]): 10.84 (s, 1H, -NH₂CO₂⁻), 8.42 (s, 1H, -NHCO⁻), 7.77 (d, J = 2.1, 1H, benzene sulfonamide H-2), 7.69 (d, J = 8.4 Hz, 1H, benzene sulfonamide H-5), 7.64 (dd, J = 7.5, 1.9 Hz, 1H, benzamide H-6), 7.57-7.53 (m, 2H, 4-chlorophenol H-3 & benzene sulfonamide H-6), 7.27-7.15 (m, 3H, benzamide H-4, 4-chlorophenol H-5), 6.91 (d, J = 8.8 Hz, 1H, 1H, 4-chlorophenol H-6), 6.25 (d, J = 7.3 Hz, 1H, benzamide H-3), 3.55 (t, J = 5.5, 2H, N₂CH₂-CH₂-CH₂-NH₂), 3.40 (q, J = 5.7 Hz, 2H, -CH₂-NHCO⁻), 3.35° (t, J = 5.5 Hz, 2H, N₂CH₂-CH₂-CH₂-NH₂); ¹³C NMR (101 MHz, DMSO-d₆, δ [ppm]): 165.5 (C=O), 153.2 (benzamide C-2), 146.7 (4-chlorophenol C-1), 139.6 (benzene sulfonamide C-1), 136.4 (benzene sulfonamide C-4), 132.4 (benzene sulfonamide C-6), 131.9 (benzamide C-4), 131.7 (benzene sulfonamide C-5), 130.3 (benzamide C-6), 129.4 (4-chlorophenol C-4), 128.5 (4-chlorophenol C-5), 128.1 (benzene sulfonamide C-6), 126.3 (benzamide C-1), 126.3 (4-chlorophenol C-5), 124.0 (benzamide C-5), 123.7 (4-chlorophenol C-3), 121.9 (4-chlorophenol C-6), 117.4 (benzamide C-3), 69.6, 69.6 (O-CH₂-CH₂-O₂⁻), 69.3 (N₂CH₂-CH₂-), 68.8 (O-CH₂-CH₂-CH₂-N₂), 50.0 (N₂CH₂-), 39.2° (O-CH₂-CH₂-CH₂-N₂); LRMS (ESI⁺) m/z: 630 [M+2Cl⁻]⁺, 1 x [37Cl⁻]⁺; HRMS (ESI⁺): m/z calcd for C₂₂H₂₂Cl₂N₂O₂S⁺: 628.0586 [M+37Cl⁻]+H⁺, found 628.0589. 
2-(4-Chloro-2-((3,4-dichlorophenyl)sulfonamido)phenoxy)-N-(prop-2-yn-1-yl)benzamide (36)

![Chemical Structure](image)

Under dry conditions, HOBr (14 mg, 0.106 mmol, 1.1 eq), EDC x HCl (20 mg, 0.106 mmol, 1.1 eq), and DIPEA (36 mg, 2.9 eq) were added to a solution of 2-(4-chloro-2-((3,4-dichlorophenyl)sulfonamido)phenoxy)benzoic acid (1 SD-24), 50 mg, 0.106 mmol, 1.1 eq) dissolved in anhydrous DMF (1 mL). The reaction mixture was stirred for 1 h. Then, propargyl amine (5.3 mg, 0.096 mmol, 1 eq) was added. The resulting reaction mixture was stirred for further 2 h. Afterwards, the DMF was removed under reduced pressure and the residue was dissolved in dichloromethane. The organic layer was washed with 1 N NaOH, 1 N HCl, and brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude product was purified by automated flash chromatography (EtOAc/isohexane, gradient: 0-50%). The title compound was obtained as a colorless solid (50 mg, 0.098 mmol, 92%). R₂ = 0.2 (EtOAc/isohexane 2:8); ¹H NMR (400 MHz, DMSO-d₆, δ [ppm]): 10.75 (s, 1H, -SO₂NH-), 8.73 (bt, J = 5.7 Hz, 1H, -CH₂-NHCO-), 7.78 (s, 1H, benzene sulfonyl H-2), 7.70–7.62 (m, 2H, benzamide H-6 & benzene sulfonylamine H-5), 7.58–7.52 (m, 2H, benzene sulfonyl H-6 & benzamide H-4), 7.28–7.12 (m, 3H, 4-chlorophenol H-3, 5, benzamide H-5), 6.89 (d, J = 8.0 Hz, 1H, 4-chlorophenol H-6), 6.13 (d, J = 7.8 Hz, 1H, benzamide H-3), 4.05 (dd, J = 5.7, 2.5 Hz, 2H, HC≡CH₂-NHCO-), 3.13 (t, J = 2.5 Hz, 1H, HC≡C=CH₂); ¹³C NMR (101 MHz, DMSO-d₆, δ [ppm]): 164.9 (C=O), 153.3 (benzamide C-2), 146.8 (4-chlorophenol C-1), 139.7 (benzene sulfonylamine C-1), 136.4 (benzene sulfonyl C-4), 132.4 (benzene sulfonyl C-3), 132.1 (benzamide C-4), 131.7 (benzene sulfonyl C-5), 130.5 (benzene sulfonyl C-6), 129.3 (4-chlorophenol C-3), 128.5 (4-chlorophenol C-2), 128.1 (benzene sulfonyl C-2), 126.6 (benzene sulfonyl C-5), 125.6 (benzamide C-1), 124.4 (benzamide C-5), 123.9 (4-chlorophenol C-3), 121.9 (4-chlorophenol C-6), 117.2 (benzamide C-3), 73.2 (HC≡C-), 28.6 (-CH₂-NH-); LRMS (ESI⁺) m/z: 509 [M+H⁺]; HRMS (ESI⁺): m/z calcd for C₃₀H₂₇Cl₂N₃O₆S₂: 508.9891 [M+H⁺]; found: 508.9898. The attached ¹H and ¹³C NMR spectra contain residual solvent signals (DMF).

† the ¹³C NMR signal for HC≡C=CH₂ could not be detected.

Methyl 2-(4-chloro-2-((3,4-dichlorophenyl)sulfonamido)phenoxy)benzoate (37)

![Chemical Structure](image)

3,4-Dichlorobenzensulfonyl chloride (446 mg, 1.82 mmol, 1.2 eq) and anhydrous pyridine (144 mg, 1.82 mmol, 1.2 eq) were added to a mixture of methyl 2-(2-amino-4-chlorophenoxo)benzoate (34, 450 mg, 1.62 mmol, 1.0 eq) and anhydrous CH₂Cl₂ (5 mL). The reaction mixture was stirred for 5 h at 45 °C. Then, the reaction was quenched with water and the aqueous layer was extracted with CH₂Cl₂ twice, dried over Na₂SO₄, and volatiles were removed under reduced pressure. The crude product was purified by automated flash chromatography (EtOAc/isohexane: gradient 5-20%). The title compound was obtained as a colorless solid (750 mg, 1.54 mmol, 95%). R₂ = 0.10 (EtOAc/isohexane 1:9); ¹H NMR (600 MHz, DMSO-d₆, δ [ppm]): 10.47 (s, 1H, -NHCO-), 7.92 (d, J = 2.2 Hz, 1H, benzene sulfonylamine H-2), 7.81 (dd, J = 7.7, 1.8 Hz, 1H, methyl salicylate H-6), 7.75 (d, J = 8.4 Hz, 1H, benzene sulfonylamine H-5), 7.67 (dd, J = 8.4, 2.2 Hz, 1H, benzene sulfonylamine H-6), 7.49 (ddd, J = 8.2, 7.5, 1.8 Hz, 1H, methyl salicylate H-4), 7.44 (d, J = 2.6 Hz, 1H, 4-chlorophenol H-3), 7.28 (ddd, J = 7.7, 7.5, 0.9 Hz, 1H, methyl salicylate H-5), 7.18 (dd, J = 8.8, 2.6 Hz, 1H, 4-chlorophenol H-5), 6.64 (d, J = 8.8 Hz, 1H, 4-chlorophenol H-6), 6.57 (dd, J = 8.2, 0.9 Hz, 1H, methyl salicylate H-3), 3.69 (s, 3H, -CH₃); ¹³C NMR (101 MHz, DMSO-d₆, δ [ppm]): 165.0 (C=O), 154.0 (methyl salicylate C-2), 149.1 (4-chlorophenol C-1), 140.5 (benzene sulfonylamine C-1), 136.0 (benzene sulfonyl C-4), 134.0 (methyl salicylate C-4), 132.1 (benzene sulfonylamine C-3), 131.5 (methyl salicylate C-2) 131.4 (benzene sulfonylamine C-5), 128.3 (benzene sulfonylamine C-2), 128.0 (4-chlorophenol C-2), 126.7 (4-chlorophenol C-4), 126.7 (benzene sulfonylamine C-6), 126.4 (4-chlorophenol C-5), 125.1 (4-chlorophenol C-3), 124.6 (methyl salicylate C-5), 122.9 (methyl salicylate C-1), 120.4 (methyl salicylate C-3), 119.0 (4-chlorophenol C-6), 52.1 (-CH₃); LRMS (ESI⁺) m/z: 488 [M+2Cl + 1 x Cl₂] + H⁺. The attached ¹H and ¹³C NMR spectra contain residual solvent signals (EtOAc). The obtained analytical data are in good agreement with literature values.⁸
Methyl 4-(4-chloro-2-(((3,4-dichlorophenyl)sulfonamido)phenoxy)benzoate (38)\(^6\)

\[
\begin{align*}
\text{O} & \text{S} \\
\text{Cl} & \text{Cl} \\
\text{N} & \text{H} \\
\text{O} & \text{O} \\
\end{align*}
\]

3,4-Dichlorobenzensulfonyl chloride (1.27 g, 5.18 mmol, 1.2 eq) and anhydrous pyridine (0.41 g, 5.18 mmol, 1.2 eq) were added to a stirred solution of methyl 4-(2-amino-4-chlorophenox)benzoate (23, 1.2 g, 4.32 mmol, 1.0 eq) in anhydrous CH\(_2\)Cl\(_2\) (12 mL) under a nitrogen atmosphere. The reaction mixture was stirred for 5 h at 50 °C. Then, the reaction was quenched with water, the aqueous layer was extracted with CH\(_2\)Cl\(_2\) twice and the combined organic layer was washed with brine, dried over Na\(_2\)SO\(_4\), filtered, and concentrated under reduced pressure. The crude slight yellow powder was purified by automated flash chromatography (EtOAc/isohexane, gradient 5-15%) to obtain the title compound as a pale yellow solid (1.50 g, 3.09 mmol, 71%). \(R_f = 0.6\) (EtOAc/isohexane, 3:17); \(^1\)H NMR (400 MHz, DMSO-\(d_6\), ppm): 10.59 (s, 1H, -NH), 7.83–7.77 (m, 2H, methyl benzoate H-3,5), 7.76 (d, J = 2.0 Hz, 1H, benzene sulfonamide H-2), 7.60 (d, J = 8.4 Hz, 1H, benzene sulfonamide H-5), 7.56–7.51 (m, 2H, benzene sulfonamide H-6 & 4-chlorophenol H-3), 7.33 (dd, J = 8.8, 2.6 Hz, 1H, 4-chlorophenol H-5), 7.07 (d, J = 8.8 Hz, 1H, 4-chlorophenol H-6), 6.66–6.61 (m, 2H, methyl benzoate H 2,6), 3.83 (s, 3H, -CH\(_3\)): \(^{13}\)C NMR (101 MHz, DMSO-\(d_6\), ppm): 165.5 (C=O), 160.3 (methyl benzoate C-4), 146.6 (4-chlorophenol C-1), 140.1 (benzene sulfonamide C-1), 136.1 (benzene sulfonamide C-4), 132.1 (benzene sulfonamide C-3), 131.5 (benzene sulfonamide C-5), 131.1 (methyl benzoate C-3,5), 129.5 (4-chlorophenol C-4), 128.9 (4-chlorophenol C-2), 128.1 (benzene sulfonamide C-2), 127.5 (4-chlorophenol C-5), 126.9 (4-chlorophenol C-3), 126.4 (benzene sulfonamide C-6), 124.1 (methyl benzoate C-1), 122.8 (4-chlorophenol C-6), 116.2 (methyl benzoate C-2,6), 52.0 (-CH\(_3\)); LRMS (ESI\(^+\)) m/z: 486 [M+H\(^+\)]. The obtained analytical data are in good agreement with literature values.\(^6\)

Methyl 4-(((5-chloro-3-(((3,4-dichlorophenyl)sulfonamido)phenoxy)benzoate (40)

\[
\begin{align*}
\text{O} & \text{S} \\
\text{Cl} & \text{Cl} \\
\text{N} & \text{H} \\
\text{O} & \text{O} \\
\end{align*}
\]

Methyl 4-(((3-amino-5-chloropyridin-2-yl)oxy)benzoate (24, 650 mg, 2.34 mmol, 1.0 eq) and 4-chloro-3-((trifluoromethyl)phenyl)sulfenyl chloride (783 mg, 2.81 mmol, 1.2 eq) were dissolved in anhydrous pyridine (5 mL) and heated to 95 °C for 4 h under a nitrogen atmosphere. After completion, the mixture was cooled to ambient temperature, quenched with water, and extracted with CH\(_2\)Cl\(_2\) (3 x 15 mL). The combined organic layer was washed with brine, dried over Na\(_2\)SO\(_4\), filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc/isohexane, gradient 5-40%) to obtain the title compound as a yellow solid (706 mg, 1.36 mmol, 58%). \(R_f = 0.2\) (EtOAc/isohexane, 2:8); \(^1\)H NMR (400 MHz, DMSO-\(d_6\), ppm): 10.92 (s, 1H, -NH\(_2\)), 8.14 (d, J = 2.2 Hz, 1H, benzene sulfonamide H-2), 8.11 (d, J = 2.5 Hz, 1H, pyridine H-6), 8.00–7.93 (m, 2H, benzene sulfonamide H-6, pyridine H-4), 7.91–7.85 (m, 2H, benzene H-2,6), 7.83 (d, J = 8.4 Hz, 1H, benzene sulfonamide H-5), 6.85–6.71 (m, 2H, benzene H-3,5), 3.85 (s, 3H, -CH\(_3\)): \(^{13}\)C NMR (151 MHz, DMSO-\(d_6\), ppm): 165.5 (-COO-CH\(_3\)), 157.0 (benzene C-4), 145.4 (pyridine C-2), 143.1 (pyridine C-6), 139.5 (benzene sulfonamide C-1), 136.1 (pyridine C-4), 135.7 (benzene sulfonamide C-4), 133.0 (benzene sulfonamide C-5), 132.0 (benzene sulfonamide C-6), 130.8 (benzene C-2,6), 127.5 (q, J = 31.9 Hz, benzene sulfonamide C-3), 125.9 (q, J = 5.53 Hz, benzene sulfonamide C-2), 125.9 (benzene C-1), 125.7 (pyridine C-5), 122.2 (pyridine C-3), 121.9 (q, J = 273.8 Hz, -CF\(_3\)), 119.8 (benzene C-3,5), 52.1 (-CH\(_3\)); LRMS (ESI\(^+\)) m/z: 521 [M+H\(^+\)].
2-(2-(Azidoethoxy)ethoxy)ethan-1-amine (41, azido-PEG₂-amine)⁹

\[
\begin{align*}
\text{N}_2 & \text{o} \text{H} & \text{O} & \text{O} & \text{NH}_2
\end{align*}
\]

To a stirred solution of 1,2-bis(2-azidoethoxy)ethane (57, 500 mg, 2.49 mmol, 1.0 eq) in EtOAc/1N HCl (1:1), a solution of PPh₃ (0.721 g, 2.75 mmol, 1.1 eq) dissolved in EtOAc (2.5 mL) was added over a period of 1.5 h. The reaction was stirred overnight at ambient temperature. Both layers were separated and the organic layer was extracted with 4 N HCl (20 mL). The combined aqueous layer was washed with TBME and CH₂Cl₂. Then, the aqueous layer was basified to pH 14 by the addition of sodium hydroxide pellets and was then extracted with TBME. The organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure to yield the title compound as a colorless oil (170 mg, 0.98 mmol, 40%).

The crude product was used for the subsequent reaction without further purification. ¹H NMR (400 MHz, DMSO-d₆): δ [ppm]: 3.63–3.49 (m, 6H, -O-CH₂-CH₂-O- & N₂=CH₂-CH₂-N₂), 3.41–3.34 (m, 4H, -OH-CH₂-CH₂-N₂), 2.64 (t, J = 5.8 Hz, 2H, N₂-CH₂-), 1.45 (bs, 2H, -NH₂); ¹³C NMR (101 MHz, DMSO-d₆): δ [ppm]: 73.1 (-O-CH₂-CH₂-N₂), 69.7, 69.6, 69.3 (N₂-CH₂-CH₂-O-CH₂-CH₂-), 50.0 (N₂-CH₂-), 41.4 (-CH₂-N₂H₂); LRMS (ESI⁺) m/z: 175.12 [M+H⁺]. The obtained analytical data are in good agreement with literature values.⁹

2-(6-(Dimethylamino)-3-(dimethyliminio)-3H-xanthene-9-yl)-4-(prop-2-yn-1-ylcarbamoyl)benzoate (42, 6-TAMRA-alkyne)¹⁰

6-Carboxy-tetramethylrhodamin was synthesized following the procedure described by Mudd et al.¹¹ 6-Carboxy-tetramethylrhodamin (50 mg, 0.12 mmol, 1.0 eq) was dissolved in DMF (1 mL) and cooled to 0 °C. TBTU (58 mg, 0.18 mmol, 1.5 eq) was dissolved in DMF (0.5 mL), DIPEA (23 mg, 0.18 mmol, 1.5 eq) was added and the mixture was added dropwise to the solution of 6-carboxy-tetramethylrhodamin. The mixture was stirred at 0 °C for 15 minutes before adding propargyl amine (7.9 mg, 0.14 mmol, 1.2 eq). The reaction mixture was stirred for further 2 h at ambient temperature. After evaporation of the volatile components the crude product was purified by flash chromatography (MeOH/dichloromethane: gradient 0.5-5% (MeOH + 2.5% NEt₃) to obtain the title compound (33 mg, 70.7 µmol, 59%) as a green solid; ¹H NMR (600 MHz, DMSO-d₆): δ [ppm]: 13.36 (s, 1H, -COOH), 9.21 (t, J = 5.5 Hz, 1H, -NHCO-), 8.30 (d, J = 8.2 Hz, 1H, carbamoyl benzoate H-6), 8.24 (dd, J = 8.2, 1.8 Hz, 1H, carbamoyl benzoate H-5), 7.93–7.85 (m, 1H, carbamoyl benzoate H-3), 7.13–6.75 (m, 6H, 3H-xanthene H-1,2,4,5,7,8), 4.07 (dd, J = 5.5, 2.5 Hz, 2H, -CONH-C=N-C≡CH), 3.26* (s, 1H, -N(CH)₂) & =N(-CH)₂), 3.14 (t, J = 2.5 Hz, 1H, -CH₂=C≡CH). ¹³C NMR (151 MHz, DMSO-d₆): δ [ppm]: 168.2 (-COOH), 164.3 (-CONH-), 152.9 (carbamoyl benzoate C-4), 152.2 (3H-xanthene C-4a,10a or 3H-xanthene C-3,6), 152.0 (3H-xanthene C-4a,10a or 3H-xanthene C-3,6), 139.7 (carbamoyl benzoate C-2), 129.3 (carbamoyl benzoate C-5), 128.8 (carbamoyl benzoate C-1), 128.5 (3H-xanthene C-1,8), 124.8 (carbamoyl benzoate C-6), 122.4 (carbamoyl benzoate C-3), 109.1 (3H-xanthene C-2,7), 105.6 (3H-xanthene C-8a,9a), 98.0 (3H-xanthene C-4,5), 84.8* (3H-xanthene C-9), 80.7 (H(C≡C=O), 73.1 (H(C≡C), 39.9 (N(CH)₂) & =N(-CH)₂), 28.6 (-CONH-CH₂-); LRMS (ESI⁺) m/z: 468 [M+H⁺]. The obtained analytical data are in good agreement with literature values.¹⁰
6-Carboxy-tetramethylrhodamin (50.0 mg, 0.12 mmol, 1.0 eq) was dissolved in DMF (1 mL) and cooled to 0°C. TBTU (58 mg, 0.18 mmol, 1.5 eq) was dissolved in DMF (0.5 mL). DIPEA was added (23 mg, 0.18 mmol, 1.5 eq) and the mixture was added dropwise. The reaction mixture was stirred for 15 minutes at 0 °C before azido-PEG2-amine (41, 22 mg, 0.13 mmol, 1.1 eq) was added. The reaction mixture was stirred for further 2 h at ambient temperature. After evaporation of the volatile components the crude product was purified by automated flash chromatography (methanol:CH2Cl2:gradient 1-10% MeOH + 0.1% NEt3) to obtain the title compound (30 mg, 51.2 µmol, 43%) as a pink solid; 1H NMR (400 MHz, DMSO-d6 δ [ppm]): 8.76 (t, J = 5.5 Hz, 1H, -NHCO-), 8.16 (d, J = 8.1 Hz, 1H, carbamoyl benzoate H-5), 8.06 (d, J = 8.1 Hz, 1H, carbamoyl benzoate H-6), 7.65 (s, 1H, carbamoyl benzoate H-3), 6.55-6.46 (m, 6H, 3'H-xanthene H-1,2,4,5,7,8), 3.55-3.45 (m, 8H & N2-CH2-CH2-O-CH2-CH2-O-CH2-CH2-NHCO-), 3.40-3.31 (m, 2H, -O-CH2-CH2-NHCO-), 3.31-3.25 (m, 2H, N2-CH2-), 2.95 (d, J = 6.4 Hz, 12H, -N(OH)2 & =N(N(CH3)2); 13C NMR (101 MHz, DMSO-d6 δ [ppm]): 168.3 (-COO), 164.6 (-NHCO-), 152.9 (carbamoyl benzoate C-4), 152.2 (3'H-xanthene C-4a,10a or C-3,6), 152.0 (3'H-xanthene C-4a,10a or C-3,6), 140.4 (carbamoyl benzoate C-2), 129.2 (carbamoyl benzoate C-5), 128.6 (carbamoyl benzoate C-1), 128.5 (3'H-xanthene C-1,8), 124.7 (carbamoyl benzoate C-6), 122.3 (carbamoyl benzoate C-3), 109.1 (3'H-xanthene C-2,7), 105.6 (3'H-xanthene C-8a,9a), 98.0 (3'H-xanthene C-4,5), 69.6, 69.5 (-O-CH2-CH2-NHCO- or -O-CH2-CH2-N3), 69.2 (-O-CH2-CH2-NHCO- or -O-CH2-CH2-N3), 49.9 (N2-CH2), 39.9 (-O-CH2-CH2-NHCO-), 39.8° (-N(C2H5)2 & =N(N(CH3)2); LRMS (ESI+) m/z: 587 [M+H+].

4-((Chloro-3-(4-chloro-3-(trifluoromethyl)phenyl)sulfonyl)amido)pyridin-2-yl)(oxy)-N-((1-(2-methoxyethyl)-1H-
1,2,3-triazol-4-y)methyl)benzamide (44)
calcd for C_{82}H_{18}ClF_{9}N_{2}O_{3}S: 645.0696 [M+H]^+; found: 645.0698; HPLC retention time 18.54 min, 98.5% (M1). The attached ^1H and ^13C NMR contains a residual solvent signal (MeOH).

4-Chloro-N-(5-chloropyridin-3-yl)-3-(trifluoromethyl)benzenesulfonyamide (45)

![Chemical structure of 4-Chloro-N-(5-chloropyridin-3-yl)-3-(trifluoromethyl)benzenesulfonyamide (45)](image)

4-Chloro-3-(trifluoromethyl)benzenesulfonyl chloride (120 mg, 0.43 mmol, 1.1 eq) was slowly added at 0°C under nitrogen atmosphere to 5-chloropyridin-3-amine (50 mg, 0.39 mmol, 1.0 eq) dissolved in anhydrous pyridine. The reaction mixture was stirred for 3 h at ambient temperature. Then, volatiles were removed under reduced pressure. The resulting residue was treated with aq. NaHCl solution and extracted with dichloromethane (3 x 20 mL). The combined organic layer was washed with brine, dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The residue was purified by automated column chromatography (EtOAc/isohexane: gradient 5-60%) to obtain the title compound as a colorless solid (116 mg, 0.31 mmol, 80%). $R_f$ = 0.5 (EtOAc/isohexane 2:8); $^1$H NMR (400 MHz, DMSO-d$_6$, δ [ppm]): 11.07 (bs, 1H, -NHSO$_2$-), 8.39 (d, $J$ = 2.2 Hz, 1H, pyridine H-6), 8.26 (d, $J$ = 2.2 Hz, 1H, pyridine H-2), 8.12 (d, $J$ = 2.3 Hz, 1H, benzene sulfonamide H-2), 8.05 (dd, $J$ = 8.5, 2.3 Hz, 1H benzene sulfonamide H-6), 7.97 (d, $J$ = 8.5 Hz, 1H, benzene sulfonamide H-5), 7.61 (t, $J$ = 2.2 Hz, 1H, pyridine H-4). $^{13}$C NMR (101 MHz, DMSO-d$_6$, δ [ppm]): 144.4 (pyridine C-6), 140.2 (pyridine C-2), 138.3 (benzene sulfonamide C-1), 136.1 (benzene sulfonamide C-4), 134.6 (pyridine C-3), 133.5 (benzene sulfonamide C-5), 132.3 (benzene sulfonamide C-6), 131.0 (pyridine C-5), 127.8 (q, $J$ = 32.3 Hz, benzene sulfonamide C-3), 127.3 (pyridine C-4), 125.9 (q, $J$ = 5.3 Hz, benzene sulfonamide C-2), 122.0 (d, $J$ = 274.7 Hz, -CF$_3$). LRMS (ESI$^+$) m/z: 371 [M+H]$^+$; HRMS: m/z calcd for C$_{82}$H$_{18}$ClF$_{9}$N$_2$O$_3$S$: 370.9630 [M+H]$^+$; found 370.9631 [M+H]$^+$; HPLC retention time 18.98 min, >99% (M1).

3,4-Dichloro-N-phenylbenzenesulfonyamide (46)$^{12}$

![Chemical structure of 3,4-Dichloro-N-phenylbenzenesulfonyamide (46)](image)

The synthesis of 46 was previously published by Cremlyn et al.$^{12}$ To provide experimental details and unpublished characterization data ($^1$H NMR, $^{13}$C NMR, LRMS), we have outlined the synthesis and the characterization data for this compound. 3,4-Dichloro benzene sulfonyl chloride (316 mg, 1.29 mmol, 1.2 eq) was slowly added at 0 °C under a nitrogen atmosphere to aniline (100 mg, 1.08 mmol, 1.0 eq) dissolved in anhydrous pyridine. The reaction mixture was stirred for 1 h at ambient temperature. Then, volatiles were removed under reduced pressure and the resulting residue was treated with aq. NH$_4$Cl solution and extracted with dichloromethane (3 x 20 mL). The combined organic layer was washed with brine, dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The yellow residue was purified by automated flash column chromatography (EtOAc/isohexane: gradient 0-20%) to obtain the title compound as a colorless solid (320 mg, 1.06 mmol, 98%). $R_f$ = 0.4 (EtOAc/isohexane 3:17); $^1$H NMR (400 MHz, DMSO-d$_6$, δ [ppm]): 10.43 (s, 1H, -NHSO$_2$-), 7.90 (d, $J$ = 2.1 Hz, 1H, benzene sulfonamide H-2), 7.84 (d, $J$ = 8.4 Hz, 1H, benzene sulfonamide H-5), 7.66 (dd, $J$ = 8.4, 2.2 Hz, 1H, benzene sulfonamide H-6), 7.31–7.21 (m, 2H, phenyl H-3,5), 7.09 (d, $J$ = 7.7 Hz, 3H, phenyl H-2,4,6). $^{13}$C NMR (101 MHz, DMSO-d$_6$, δ [ppm]): 139.7 (benzene sulfonamide C-1), 137.0 (phenyl C-1), 136.1 (benzene sulfonamide C-4), 132.1 (benzene sulfonamide C-3), 131.8 (benzene sulfonamide C-5), 129.4 (phenyl C-3,5), 128.4 (benzene sulfonamide C-2), 126.8 (benzene sulfonamide C-6), 124.9 (phenyl C-4), 120.8 (phenyl C-2,6). LRMS (ESI$^+$) m/z: 324 [M+Na]$^+$; HPLC retention time 19.18 min, >99% (M1). The attached $^1$H and $^{13}$C NMR contains a residual solvent signal (EtOAc, CH$_2$Cl$_2$).
**N-(5-Chloropyridin-3-yl)benzenesulfonamide (47)**

![structure_formula](image)

Benzenesulfonfyl chloride (152 mg, 0.86 mmol, 1.1 eq) was slowly added at 0 °C under a nitrogen atmosphere to 5-chloropyridine-3-amine (100 mg, 0.78 mmol, 1.0 eq) dissolved in anhydrous pyridine. The reaction mixture was stirred for 2 h at ambient temperature. Then, volatiles were removed under reduced pressure and the resulting residue was treated with aq. NH₄Cl solution and extracted with dichloromethane (3 x 10 mL). The combined organic layer was washed with brine, dried over MgSO₄, filtered, and concentrated under reduced pressure. The yellow residue was purified by automated flash column chromatography (methanol/CH₂Cl₂: gradient 0-5%) to obtain the title compound as a colorless solid (168 mg, 0.63 mmol, 81%). Rf = 0.3 (methanol/CH₂Cl₂ 1:20); ¹H NMR (400 MHz, DMSO-d₆, δ [ppm]): 10.92 (s, 1H, -NH₂), 8.32 (dd, J = 2.2, 0.5 Hz, 1H, pyridine H-6), 8.24 (dd, J = 2.2, 0.5 Hz, 1H, pyridine H-2), 7.66–7.77 (m, 2H, benzene sulfonamide H-2,6), 7.70–7.63 (m, 1H, benzene sulfonamide H-4), 7.62–7.57 (m, 2H, benzene sulfonamide H-3,5), 7.55 (dd, J = 2.2, 2.2 Hz, 1H, pyridine H-4). ¹³C NMR (101 MHz, DMSO-d₆, δ [ppm]): 143.5 (pyridine C-6), 139.4 (pyridine C-2), 138.7 (benzene sulfonamide C-1), 134.5 (pyridine C-3), 133.6 (benzene sulfonamide C-4), 130.9 (pyridine C-5), 129.7 (benzene sulfonamide C-3,5), 126.7 (benzene sulfonamide C-2,4), 126.1 (pyridine C-4); LRMS (ESI⁺) m/z: 269 [M+H⁺]; HRMS: m/z calcld for C₁₁H₈ClN₂O₂S: 269.0146, found: 269.0147; HPLC retention time 16.27 min, 99.9% (M1).

**N-Phenylbenzenesulfonamide (48)**

![structure_formula](image)

Benzenesulfonfyl chloride (418 mg, 2.40 mmol, 1.1 eq) was slowly added at 0 °C under a nitrogen atmosphere to aniline (200 mg, 2.15 mmol, 1.0 eq) dissolved in anhydrous pyridine. The reaction mixture was stirred for 3 h at ambient temperature. Then, volatiles were removed under reduced pressure and the resulting residue was treated with aq. NH₄Cl solution and extracted with dichloromethane (3 x 20 mL). The combined organic layer was washed with brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The yellow residue was purified by automated flash column chromatography (EtOAc/isohexane: gradient 0-30%) to obtain the title compound as a colorless solid (490 mg, 2.10 mmol, 98%). Rf = 0.5 (EtOAc/isohexane 3:1); ¹H NMR (400 MHz, DMSO-d₆, δ [ppm]): 10.29 (s, 1H, -NH₂), 7.78–7.71 (m, 2H, benzene sulfonamide H-2,6), 7.65–7.57 (m, 1H, benzene sulfonamide H-4), 7.57–7.49 (m, 2H, phenyl H-3,5), 7.26–7.16 (m, 2H, benzene sulfonamide H-3,5), 7.11–7.05 (m, 2H, phenyl H-2,6), 7.05–6.96 (m, 1H, phenyl H-4). ¹³C NMR (151 MHz, DMSO-d₆, δ [ppm]): 139.5 (benzene sulfonamide C-1), 137.6 (phenyl C-1), 132.8 (benzene sulfonamide C-4), 129.2 (phenyl C-3,5), 129.1 (benzene sulfonamide C-3,5), 126.6 (benzene sulfonamide C-2,6), 124.1 (phenyl C-4), 120.1 (phenyl C-2,6). LRMS (ESI⁺) m/z: 256 [M+Na⁺]; HPLC retention time 17.00 min, 98.5% (M1). The obtained analytical data are in good agreement with literature values.¹³

**2-((5-Chloropyridin-2-yl)oxy)benzoic acid (49)**

![structure_formula](image)

The synthesis of compound 49 was previously published by Munro et al.¹⁴ To provide experimental details and unpublished characterization data (¹H NMR, ¹³C NMR, HRMS), we have outlined the synthesis and the characterization data for this compound. In a dry reaction flask and under a nitrogen atmosphere, 5-chloro-2-fluoropyridine (100 mg, 0.77 mmol, 1.0 eq), methyl 2-hydroxybenzoate (175 mg, 1.15 mmol, 1.5 eq), and Cs₂CO₃ (500 mg, 1.54 mmol, 2.0 eq) were dissolved in anhydrous DMF (5 mL). The mixture was stirred for 16 h at 60 °C. Then, the reaction was quenched with water and extracted with EtOAc (3x 20 mL). The combined organic layer was washed with brine, then dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude residue was purified by automated flash chromatography (EtOAc/isohexane: 5-40%) to obtain the methyl benzoate intermediate (30 mg, 0.114 mmol, 15%). Subsequently, 30 mg (114 μmol) of the intermediate were dissolved in THF/water (2:1) and LiOH (10 eq) was added. The reaction mixture was stirred overnight at 40°C. Then, volatiles were removed under reduced pressure and the residue was acidified with 2N HCl. The acidified aqueous layer was extracted with EtOAc (3x 20 mL). The combined organic layer was washed with brine (20 mL), dried over Na₂SO₄, filtered, and volatiles were removed under reduced pressure. The residue was purified...
by preparative HPLC (acetonitrile/water with 0.1% trifluoroacetic acid: gradient 35-75%). The title compound was obtained as a colorless solid (19 mg, 76.3 µmol, 67%, yield over two steps 10%). R<sub>f</sub> = 0.6 (EtOAc/isohexane 1:9); <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)<sup>δ</sup> [ppm]: 12.84 (bs, 1H, -COOH), 8.09 (dd, J = 2.3, 0.6 Hz, 1H, pyridine H-6), 7.96–7.85 (m, 2H, pyridine H-4, benzoic acid H-6), 7.64 (ddd, J = 8.1, 7.5, 1.8 Hz, 1H, benzoic acid H-4), 7.36 (ddd, J = 7.5, 7.5, 1.2 Hz, 1H, benzoic acid H-5), 7.24 (d, J = 8.1, 1.2 Hz, 1H, benzoic acid H-3), 7.09 (dd, J = 8.8, 0.6 Hz, 1H, pyridine H-3); <sup>13</sup>C NMR (101 MHz, DMSO-d<sub>6</sub>)<sup>δ</sup> [ppm]: 166.3 (COOH), 162.6 (pyridine C-2), 152.7 (benzoic acid C-2), 145.4 (pyridine C-6), 140.1 (pyridine C-4), 134.2 (benzoic acid C-4 or C-6), 131.9 (benzoic acid C-4 or C-6), 125.9 (benzoic acid C-5), 125.5 (pyridine C-5 or benzoic acid C-1), 125.0 (pyridine C-5 or benzoic acid C-1), 124.5 (benzoic acid C-3), 113.1 (pyridine C-3). LRMS (ESI) <sup>m/z</sup>: 250 [M+H]<sup>+</sup>; HRMS: <sup>m/z</sup> calcld for C<sub>13</sub>H<sub>17</sub>NClO<sub>3</sub>: 250.0265 [M+H]<sup>+</sup>, found 250.0264; HPLC retention time 16.13 min, 96.7% (M1).

4-Chloro-3-(trifluoromethyl)benzenesulfonylamide (50)<sup>15</sup>

4-Chloro-3-(trifluoromethyl)benzenesulfonyl chloride (50 mg, 179.2 µmol, 1.0 eq) was dissolved in ethyl acetate (400 µL) and added to an ice-cooled aqueous solution of ammonia (25% (m/m), 600 µL). The reaction mixture was stirred at ambient temperature for 1 h. After completion, water (5 mL) was added and the mixture was acidified with aqueous HCl (1 M), followed by an extraction with EtOAc (4 x 10 mL). The combined organic layer was washed with brine (10 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (EtOAc/isohexane: gradient 5-50%) to obtain the title compound as a colorless solid (40 mg, 154.5 µmol, 86%). R<sub>f</sub> = 0.32, (EtOAc/isohexane 2:8); <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)<sup>δ</sup> [ppm]: 8.22 (d, J = 2.2 Hz, 1H, H-2), 8.09 (dd, J = 8.4, 2.2 Hz, 1H, H-5), 7.98 (d, J = 8.4 Hz, 1H, H-6), 7.68 (s, 2H, -NH<sub>2</sub>). <sup>13</sup>C NMR (101 MHz, DMSO-d<sub>6</sub>)<sup>δ</sup> [ppm]: 143.5 (C-1), 134.4 (C-4), 132.9 (C-5 or C-6), 131.3 (C-5 or C-6), 127.2 (q, J = 31.6 Hz, C-3), 125.2 (q, J = 5.4 Hz, C-2), 122.3 (q, J = 273.7 Hz, -CF<sub>3</sub>). LRMS (ESI) <sup>m/z</sup>: 282 [M+Na]<sup>+</sup>; HRMS: <sup>m/z</sup> calcld for C<sub>13</sub>H<sub>15</sub>ClF<sub>3</sub>NO<sub>2</sub>: 259.9754 [M+H]<sup>+</sup>, found 259.9753; HPLC retention time 16.50 min, 95.5% (M1). Analytical data are in good agreement with literature values.<sup>15</sup>

4-((5-Chloropyridin-2-yl)oxy)benzoic acid (51)

The synthesis of compound 51 was already published by Wu et al.<sup>16</sup> To provide experimental details and unpublished characterization data (<sup>1</sup>H NMR, <sup>13</sup>C NMR, HRMS), we have outlined the synthesis and the characterization data for this compound.

In a dried reaction flask and under a nitrogen atmosphere, 5-chloro-2-fluoropyridine (100 mg, 0.77 mmol, 1.0 eq), methyl 4-hydroxybenzoate (175 mg, 1.15 mmol, 1.5 eq), and Cs<sub>2</sub>CO<sub>3</sub> (500 mg, 1.54 mmol, 2.0 eq) were dissolved in anhydrous DMF (5 mL). The mixture was stirred for 16 h at 60 °C. Then, the reaction was quenched with water and extracted with EtOAc (3x 20 mL). The combined organic layer was washed with brine, then dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the volatiles were removed under reduced pressure. The crude residue was purified by automated flash chromatography (EtOAc/isohexane 5:40%) to obtain the methyl benzoate intermediate (93 mg, 0.35 mmol, 46%). Subsequently, 60 mg (0.23 mmol) of the intermediate were dissolved in THF/water (2:1) and LiOH (10 eq) was added. The reaction mixture was stirred overnight at 40 °C. Then, volatiles were removed under reduced pressure and the residue was acidified with 2N HCl. The acidified aqueous layer was extracted with EtOAc (3x 20 mL). Afterwards, the combined organic layer was washed with brine (20 mL) and volatiles were removed under reduced pressure. The crude product was purified by preparative HPLC (acetonitrile/water with 0.1% trifluoroacetic acid: gradient 35-75%) to obtain the title compound as a colorless solid (34 mg, 93.7 µmol, 40%, yield over two steps 12%). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)<sup>δ</sup> [ppm]: 12.94 (bs, 1H, -COOH), 8.25 (d, J = 2.7 Hz, 1H, pyridine H-6), 8.02 (dd, J = 8.7, 2.7 Hz, 1H, pyridine H-4), 8.00–7.96 (m, 2H, benzoic acid H-2,6), 7.27–7.21 (m, 2H, benzoic acid H-3,5), 7.20 (d, J = 8.7 Hz, 1H, pyridine H-3); <sup>13</sup>C NMR (101 MHz, DMSO-d<sub>6</sub>)<sup>δ</sup> [ppm]: 166.7 (-COOH), 161.0 (pyridine C-2), 157.5 (benzoic acid C-4), 145.8 (pyridine C-6), 140.3 (pyridine C-4), 131.3 (benzoic acid C-2,6), 127.1 (benzoic acid C-1 or pyridine C-5), 126.0 (benzoic acid C-1 or pyridine C-5), 120.7 (benzoic acid C-3,5), 113.8 (pyridine C-3). LRMS (ESI) <sup>m/z</sup>: 250 [M+H]<sup>+</sup>; HRMS: <sup>m/z</sup> calcld for C<sub>13</sub>H<sub>17</sub>NClO<sub>3</sub>: 250.0265 [M+H]<sup>+</sup>, found 250.0264; HPLC retention time 16.71 min, >99% (M1).

S26
(Ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl)bis(4-methylbenzenesulfonate) (56)\(^{17}\)

\[
\text{TsO} - \overset{\text{O}}{\text{O}} - \overset{\text{O}}{\text{O}} - \text{Ts}
\]

TsCl (6.29 g, 0.033 mol, 2.2 eq) was added in portions to a mixture of triethylene glycol (2.24 g, 0.015 mol, 1.0 eq) and NEt\(_3\) (3.79 g, 0.038 mol, 2.5 eq) in acetonitrile (50 mL) at 0 °C. The reaction mixture was stirred for 4 h at ambient temperature under a nitrogen atmosphere. Then, the reaction was quenched by the addition of saturated aq. NH\(_4\)Cl solution (20 mL). The organic solvent was removed under reduced pressure and the aqueous layer was extracted with EtOAc. The organic layer was concentrated under reduced pressure. The residue was purified by automated flash chromatography (TBME/isohexane, gradient: 12-100%). The title compound was obtained as a colorless solid (4.6 g, 0.01 mol, 66%). \(R_f = 0.2\) (TBME/isohexane 1:1); \(^1\)H NMR (400 MHz, DMSO-d\(_6\) \(\delta\) [ppm]): 7.83–7.71 (m, 4H, tosylate H-2,6), 7.78–7.41 (m, 4H, tosylate H-3,5), 4.11–4.06 (m, 4H, TsO-C\(\text{H}_2\)-CH\(\text{2}\)-O\(-\)), 3.56–3.50 (m, 4H, TsO-\(\text{CH}_2\)-CH\(\text{2}\)-O\(-\)), 3.40–3.34 (m, 4H, -O-\(\text{CH}_2\)-CH\(\text{2}\)-O\(-\)), 2.42 (s, 6H, -CH\(\text{3}\)). LRMS (ESI\(^+\)) m/z: 459 [M+H]^+.

The obtained analytical data are in good agreement with literature values.\(^{17}\)

1,2-Bis(2-azidoethoxy)ethane (57)\(^{17}\)

(Ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl)bis(4-methylbenzenesulfonate) (56, 2.86 mg, 6.24 mmol, 1.0 eq) was dissolved in DMF (40 mL) and set under a nitrogen atmosphere. NaN\(_3\) (1.22 mg, 18.72 mmol, 3.0 eq) was added in one portion and the reaction mixture was heated for 4 h at 60 °C and stirred overnight at ambient temperature. Then, the solvent was evaporated to dryness. Water (40 mL) was added to the residue and the aqueous layer was extracted with EtOAc twice. The combined organic layer was washed with brine, dried over Na\(_2\)SO\(_4\), filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (TBME/isohexane: gradient 12-100%) to give the title compound as a pale-yellow oil (1.00 g, 0.50 mmol, 80%). \(R_f = 0.5\) (TBME/isohexane: 1:1, KmnO\(_4\) staining); \(^1\)H NMR (400 MHz, DMSO-\(d_6\) \(\delta\) [ppm]): 3.63–3.59 (m, 4H, N\(_3\)-CH\(\text{2}\)-CH\(\text{2}\)-), 3.58–3.50 (m, 4H, -O-CH\(\text{2}\)-CH\(\text{2}\)-O\(-\)), 3.41–3.37 (m, 4H, N\(_3\)-C\(\text{H}_2\)-); \(^{13}\)C NMR (101 MHz, DMSO-\(d_6\) \(\delta\) [ppm]): 69.7, 69.3 (N\(_3\)-CH\(\text{2}\)-CH\(\text{2}\)- \& -O-CH\(\text{2}\)-CH\(\text{2}\)-O\(-\)), 50.0 (N\(_3\)-CH\(\text{2}\)-); LRMS (ESI\(^+\)) m/z: 223 [M+Na]^+. The obtained analytical data are in good agreement with literature values.\(^\text{17}\)
Figure S1. Identification of suitable linker fragments and linker positions for the design of fluorescently labeled CCR2 ligands based on the scaffolds of SD-24 (1) or CCR2-RA (4). A) Chemical structures and docking scores of designed CCR2 ligand-linker-conjugates docked into the crystal structure of the CCR2 with the co-crystallized allosteric inhibitor 4-[R] (PDB ID: 5T1A). The reported intracellular allosteric CCR2 antagonist CCR2-RA-[R] was used as a reference compound for the docking study. The design of ligand-linker-conjugates is based on the reported (for CCR2-RA) or predicted (for SD-24, and analogues) binding modes of the intracellular CCR2 antagonists, available SAR data, and the synthetic accessibility of the final fluorescent ligands. For comparability, the overall lengths of the linkers were restricted to 5-6 atoms. B) The predicted binding modes of 6-[R] (cyan, left) and 7 (yellow, right) exemplify that the distal methyl groups at the triazole moieties are solvent exposed.
Figure S2. Development of a cell-free NanoBRET-based binding assay for CCR2. A) Schematic representation of the genetic constructs used in the course of assay development. B) Emission spectra of different CCR2-Nluc constructs using membrane preparations from HEK293T cells expressing the respective Nluc-labeled 3xHA-CCR2 fusion proteins (CCR2_Nluc = red, CCR2_GSSG_Nluc = blue). C) Spectral properties of fluorescent ligands (8-16). Absorption and fluorescence emission spectra of the fluorescent ligands (1000 μM) are represented as solid lines. D) Expression level of different 3xHA-CCR2-Nluc constructs detected via ELISA normalized to the expression wild-type 3xHA-CCR2 (CCR2_wt). Bar diagram representing the mean values ± SEM (n = 6) with each test performed in quadruplicate. The experiment indicates that all CCR2-Nluc constructs are well-expressed in HEK293T cells. E) Representative NanoBRET assay windows for 14, generated with membrane preparations from HEK293T cells expressing different CCR2-Nluc constructs. Tests were performed in triplicate. The signals for non-specific binding (full displacement of 14, red triangles) were detected in the presence of 14 (650 nM) and 4 (10 μM), whereas the signals obtained for the vehicle controls represent total binding of 14 at a concentration of 650 nM (blue circles). As the application of CCR2_GSSG_Nluc resulted in a large and robust assay window, we used this construct for further investigations. F) Representative binding curves (specific binding) of saturation binding experiments with fluorescent ligands 8-16 using a membrane preparation from HEK293T cells expressing the CCR2_GSSG_Nluc construct. Experiments were performed in triplicate. G) Representative binding curves (total, specific, and non-specific binding) for 14 using membrane preparation from HEK293T cells expressing the CCR2_GSSG_Nluc construct. The experiments were performed in triplicate. The netBRET signal was calculated as the difference between total BRET and the signal obtained in the absence of a fluorescent ligand. H) Zoomed-in view of the first 10 minutes of the association curve of shown in Figure 2C. I) 14 is stable under assay conditions (aqueous assay buffer: 50 mM NaH2PO4, 50 mM KH2PO4, 1 mg/mL saponin, 5% FBS, pH 7.4, 37°C. Analysis performed by analytical HPLC method M1 (detection at 560 nm, see Materials and Methods section for experimental details). J) Representative competition binding curves form single experiments for SD-24 (1; green) and CCR2-RA (4; blue), 27 (purple), and 44 (red) obtained with 14 (650 nM) and CCR2_GSSG_Nluc membranes (mean ± SEM, triplicate measurement).
Figure S3. The fluorescent ligand 14 selectively binds to CCR2 but not to the related chemokine receptors CCR1 and CCR5. A) Expression levels of different 3xHA-CCR1-Nluc constructs in HEK293T cells detected via ELISA and normalized to the expression wild-type 3xHA-CCR1_wt. Bar diagram representing the mean values ± SEM (n = 3) with each test performed in triplicate. The experiment indicates that all CCR1-Nluc constructs are well-expressed in HEK293T cells. B) Expression levels of different 3xHA-CCR5-Nluc constructs in HEK293T cells detected via ELISA and normalized to the expression wild-type 3xHA-CCR5_wt. Bar diagram representing the mean values ± SEM (n = 3) with each test performed in triplicate. The experiment indicates that all CCR5-Nluc constructs are well-expressed in HEK293T cells. C) Emission spectra of different CCR1-Nluc constructs expressed in HEK293T cells using membrane preparations from HEK293T cells expressing the CCR1_Nluc construct (CCR1_Nluc = red, CCR1_GSSG_Nluc = blue). D) Emission spectra of different CCR5-Nluc constructs expressed in HEK293T cells using membrane preparations from HEK293T cells expressing the CCR5_Nluc construct (CCR5_Nluc = red, CCR5_GSSG_Nluc = blue). E) Representative saturation binding curves (total, specific, and non-specific binding) for 14 using membrane preparations from HEK293T cells expressing the CCR1_Nluc construct. The experiments were performed in triplicate (n=3). F) Representative saturation binding curves (total, specific, and non-specific binding) for 14 using membrane preparations from HEK293T cells expressing the CCR5_Nluc construct. The experiments were performed in triplicate (n=3). G) Comparison of representative specific binding curves of the fluorescent ligand 14 binding to membrane preparations from HEK293T cells expressing the respective C-terminally Nluc-tagged chemokine receptor (CCR1, CCR2, CCR5). See Figure S2G for representative binding curves (total, specific, and non-specific binding) of 14 binding to membrane preparations from HEK293T cells expressing the C-terminally Nluc-tagged CCR2.
Figure S4. A) Competition binding curves for the literature compounds 2 (pink) and 5 (olive green) obtained with 14 (650 nM) and CCR2_GSSG_Nluc membranes (mean ± SEM, triplicate measurement, n = 3). B) Representative competition binding curves form single experiments for 2 (pink) and 5 (olive green) obtained with 14 (650 nM) and CCR2_GSSG_Nluc membranes (mean ± SEM, triplicate measurement). For chemical structures and reported affinity data see Chart1.
Figure S5. Application of 14 for kinetic competitive binding studies in a cell-free environment. The graph on the left of each sub-panel shows representative kinetic competitive binding curves for 14 (650 nM) competing with different concentrations of the respective competitor for binding to CCR2_GSSG_Nluc membranes. The table on the right of each sub-panel shows the obtained dissociation rate constants (koff) and the residence times (\( t_R \)).

A) Kinetic competitive binding studies with SD-24 (1, n=3 (for each concentration), triplicate measurement, mean ± SEM).

B) Kinetic competitive binding studies with CCR2-RA (4, n=3 for 25 nM and 100 nM, n=5 for 50 nM, triplicate measurement, mean ± SEM).

C) Kinetic competitive binding studies with 27 (n=3 (for each concentration), triplicate measurement, mean ± SEM).

D) Kinetic competitive binding studies with 44 (n=3 for 100 nM and 400 nM, n=4 for 200 nM, triplicate measurement, mean ± SEM).
Figure S6. Application of the NanoBRET-based CCR2 binding assay for fragment screening. DMSO tolerance of the NanoBRET-based CCR2 binding assay was studied, as fragment screening is usually associated with high compound concentrations and thus high amounts of DMSO as a co-solvent. A) $K_i$ values for 4, competing with 14 (650 nM) for binding to membrane preparations from HEK293T cells expressing the CCR2_GSSG_Nluc construct, were detected at different concentrations of DMSO. Bar diagram (mean ± SEM, n = 3, triplicate measurements) indicates that the assay setup tolerates even high DMSO concentrations. Statistics (One-Way ANOVA, followed by Tukey's multiple comparisons test): ns (not significant) indicates P value > 0.05, all compared pairwise to buffer control. B) Influence of the DMSO concentration on the assay window. Representative NanoBRET assay windows for 14, generated with membrane preparations from HEK293T cells expressing the CCR2_GSSG_Nluc construct. Tests were performed in triplicate. The signals for non-specific binding (full displacement of 14, red triangles) were detected in the presence of 14 (650 nM) and 4 (10 μM), whereas the signals obtained for the vehicle controls represent total binding of 14 at a concentration of 650 nM (blue circles).
Figure S7. Establishment of a cellular NanoBRET-based CCR2 binding assay. A) Representative NanoBRET assay windows for 14, generated with live HEK293T cells transiently expressing different CCR2-Nluc constructs. Tests were performed at least in quadruplicate (n ≥ 4). The signals for non-specific binding (full displacement of 14, red triangles) were detected in the presence of 14 (1000 nM) and 4 (10 μM), whereas the signals obtained for the vehicle controls represent total binding of 14 at a concentration of 1000 nM (blue circles). As the application of CCR2_Nluc resulted in a larger and more robust assay window than CCR2_GSSG_Nluc, we used this construct for further investigations. B) Representative saturation binding curves (total, specific, and non-specific binding) for 14 using live HEK293T cells transiently expressing the CCR2_Nluc construct. The experiments were performed with each condition in quadruplicate. The netBRET signal was calculated as the difference between total BRET and the signal obtained in the absence of a fluorescent ligand. C) Representative competition binding curves (mean ± SEM, quadruplicate measurement) from single experiments for SD-24 (1; green), CCR2-RA (4; blue), 27 (purple), and 44 (red) obtained with 14 (1000 nM) and live HEK293T cells expressing CCR2_Nluc.
Figure S8. Application of 14 for kinetic binding studies in live cells. Representative association (left) and dissociation (right) curves with 14 (1000 nM) and live HEK293T cells expressing CCR2_Nluc (mean ± SEM, triplicate measurement, n = 3).
Supplementary Schemes

**Scheme S1.** Synthesis of linker fragment 41. Reagents and conditions: a) TsCl, triethylamine, acetonitrile, 0°C to rt., 4 h, 66% yield; b) NaN₃, DMF, 60 °C, 4 h, then rt., overnight, 80% yield; c) PPh₃, EtOAc/1N HCl (1:1), rt., overnight, 40% yield.

**Scheme S2.** Synthesis of 44. Reagents and conditions: a) 1-Azido-2-methoxyethane, 0.1 M CuSO₄ solution, 0.1 M sodium ascorbate solution, TBTA, water/tBuOH/DMF mixture (1:1:1 (v/v)), rt., 4 h, 36% yield.
Supplementary NMR spectra

NMR spectra for compounds 1-2, 4-5, 8-38, 40-51, and 56-57 can be found on the following pages.
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$^{13}$C NMR (151 MHz, DMSO-$d_6$) for compound 1 (SD-24)
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$^1$H NMR (400 MHz, DMSO-$d_6$) for compound 4 (CCR2-RA)
$^{13}$C NMR (151 MHz, DMSO-$d_6$) for compound 4 (CCR2-RA)
$^1$H NMR (400 MHz, DMSO-$d_6$) for compound 5
\( ^{13}\text{C} \text{NMR (101 MHz, DMSO-d}_6 \text{)} \text{ for compound 5} \)
$^1\text{H NMR} (600 \text{ MHz}, \text{DMSO-}d_6)$ for compound 8
$^{13}$C NMR (151 MHz, DMSO-$d_6$) for compound 8

C NMR ($151$ MHz, DMSO-$d_6$) for compound 8
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C
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Use Multiplier & Dilution Factor with ISTDs

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<td>95.6834</td>
</tr>
<tr>
<td>2</td>
<td>17.876</td>
<td>VV</td>
<td>0.1082</td>
<td>1.51396e4</td>
<td>2203.35645</td>
<td>95.6834</td>
<td>95.6834</td>
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</table>

Totals: 1.58226e4 2313.00413
HPLC chromatogram of 11:

---

### Area Percent Report
---

Sorted By: Signal
Multiplier: 1.0000
Dilution: 1.0000
Use Multiplier & Dilution Factor with ISTDs

**Signal 2: DAD1 B, Sig=254,16 Ref=360,100**

<table>
<thead>
<tr>
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<th>RetTime</th>
<th>Type</th>
<th>Width</th>
<th>Area</th>
<th>Height</th>
<th>Area %</th>
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<tbody>
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<td>[min]</td>
<td>[mAU s]</td>
<td>[mAU]</td>
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<tr>
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<td>---------</td>
<td>------</td>
<td>-------</td>
<td>--------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>1</td>
<td>17.946</td>
<td>BV</td>
<td>0.0915</td>
<td>15.65226</td>
<td>2.48304</td>
<td>2.0094</td>
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<td>18.177</td>
<td>VB</td>
<td>0.0974</td>
<td>763.29266</td>
<td>118.11423</td>
<td>97.9906</td>
</tr>
</tbody>
</table>

Totals: 778.94492 120.59727
HPLC chromatogram of 12:

Blank run for consideration of baseline fluctuation:

---

Area Percent Report
---

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 2: DAD1 B, Sig=254,16 Ref=360,100

<table>
<thead>
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<th>Peak RetTime</th>
<th>Type</th>
<th>Width</th>
<th>Area</th>
<th>Height</th>
<th>Area %</th>
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<tbody>
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<td>[min]</td>
<td>[mAU*s]</td>
<td>[mAU]</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>19.201</td>
<td>0.1084</td>
<td>485.83728</td>
<td>68.81979</td>
<td>96.3018</td>
</tr>
<tr>
<td>2</td>
<td>19.504</td>
<td>0.1343</td>
<td>18.65728</td>
<td>1.97293</td>
<td>3.6982</td>
</tr>
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Totals : 504.49456 70.79272
HPLC chromatogram of 13:

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 2: DAD1 B, Sig=254,16 Ref=360,100

<table>
<thead>
<tr>
<th>Peak RetTime</th>
<th>Type</th>
<th>Width [min]</th>
<th>Area [mAU*]</th>
<th>Height [mAU]</th>
<th>Area [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BV</td>
<td>0.0928</td>
<td>26.24905</td>
<td>4.32777</td>
<td>3.9287</td>
</tr>
<tr>
<td>2</td>
<td>WV</td>
<td>0.0833</td>
<td>641.88531</td>
<td>118.23199</td>
<td>96.0713</td>
</tr>
</tbody>
</table>

Totals : 668.13438 122.55977
HPLC chromatogram of 14:

---

Area Percent Report
---

Sorted By :  Signal  
Multiplier :  1.0000  
Dilution :  1.0000  
Use Multiplier & Dilution Factor with ISTDs  
---

Signal 2: DAD1 B, Sig=254,16 Ref=360,100
---

<table>
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<tr>
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<th>Width [min]</th>
<th>Area [mAU's]</th>
<th>Height [mAU]</th>
<th>Area %</th>
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<td>19.376</td>
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<td>21.81118</td>
<td>4.76461</td>
<td>1.2448</td>
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<tr>
<td>2</td>
<td>19.532</td>
<td>0.0954</td>
<td>1668.91223</td>
<td>265.28125</td>
<td>95.2493</td>
</tr>
<tr>
<td>3</td>
<td>20.804</td>
<td>0.2475</td>
<td>43.42868</td>
<td>2.26694</td>
<td>2.4786</td>
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<tr>
<td>4</td>
<td>21.842</td>
<td>0.0817</td>
<td>7.24757</td>
<td>1.37002</td>
<td>0.4136</td>
</tr>
<tr>
<td>5</td>
<td>22.211</td>
<td>0.0947</td>
<td>10.75052</td>
<td>1.67957</td>
<td>0.6136</td>
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</table>

Totals : 1752.15117  275.36238
HPLC chromatogram of 15:

---

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 2: DAD1 B, Sig=254,16 Ref=360,100

<table>
<thead>
<tr>
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<th>Type</th>
<th>Width [min]</th>
<th>Area [mAU's]</th>
<th>Height [mAU]</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18.448</td>
<td>BV</td>
<td>0.0908</td>
<td>28.60690</td>
<td>4.85028</td>
<td>1.0375</td>
</tr>
<tr>
<td>2</td>
<td>18.705</td>
<td>VB</td>
<td>0.0969</td>
<td>2728.76855</td>
<td>436.83655</td>
<td>98.9625</td>
</tr>
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</table>

Totals : 2757.37546 441.68683
HPLC chromatogram of 16:

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 2: DAD1 B, Sig=254,16 Ref=360,100

<table>
<thead>
<tr>
<th>Peak</th>
<th>RetTime</th>
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<th>Width</th>
<th>Area</th>
<th>Height</th>
<th>Area</th>
<th>%</th>
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</thead>
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<tr>
<td>1</td>
<td>17.997</td>
<td>BB</td>
<td>0.1511</td>
<td>32.84683</td>
<td>3.21811</td>
<td>0.9249</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>18.522</td>
<td>BV</td>
<td>0.1657</td>
<td>128.13908</td>
<td>10.12140</td>
<td>3.6081</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>18.888</td>
<td>WV</td>
<td>0.0937</td>
<td>3382.82568</td>
<td>550.47278</td>
<td>95.2519</td>
<td></td>
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<tr>
<td>4</td>
<td>20.711</td>
<td>BB</td>
<td>0.0933</td>
<td>7.64233</td>
<td>1.25127</td>
<td>0.2152</td>
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</tr>
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</table>

Totals : 3551.45393 565.06356
HPLC chromatogram of 27:

![HPLC Chromatogram](image)

---

### Area Percent Report

**Sorted By**: Signal  
**Multiplier**: 1.0000  
**Dilution**: 1.0000

Use Multiplier & Dilution Factor with ISTDs

**Signal 2: DAD1 B, Sig=254,16 Ref=360,100**

<table>
<thead>
<tr>
<th>#</th>
<th>RetTime</th>
<th>Type</th>
<th>Width</th>
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<th>Height</th>
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<td>1</td>
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<td>0.1301</td>
<td>5.91223</td>
<td>7.57305e-1</td>
<td>2.1474</td>
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<td></td>
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<tr>
<td>2</td>
<td>18.934</td>
<td>BB</td>
<td>0.0852</td>
<td>269.41013</td>
<td>48.24266</td>
<td>97.8526</td>
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</table>

**Totals**: 275.32236 48.99997
HPLC chromatogram of 44:

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 2: DAD1 B, Sig=254,16 Ref=360,100

<table>
<thead>
<tr>
<th>#</th>
<th>RetTime</th>
<th>Type</th>
<th>Width</th>
<th>Area</th>
<th>Height</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.231</td>
<td>BV</td>
<td>0.1247</td>
<td>9.90397</td>
<td>1.21967</td>
<td>0.0913</td>
</tr>
<tr>
<td>2</td>
<td>17.415</td>
<td>VB</td>
<td>0.1083</td>
<td>36.73307</td>
<td>2.77711</td>
<td>0.3386</td>
</tr>
<tr>
<td>3</td>
<td>17.981</td>
<td>BV</td>
<td>0.1378</td>
<td>52.66885</td>
<td>5.41979</td>
<td>0.4874</td>
</tr>
<tr>
<td>4</td>
<td>18.114</td>
<td>VV</td>
<td>0.1449</td>
<td>60.21329</td>
<td>6.00769</td>
<td>0.5551</td>
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<tr>
<td>5</td>
<td>18.542</td>
<td>VB</td>
<td>0.1081</td>
<td>1.06880e4</td>
<td>1519.00586</td>
<td>98.5277</td>
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Totals : 1.08477e4 1534.43012

S147
HPLC chromatogram of 45:

```
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 2: DAD1 B, Sig=254,16 Ref=360,100

<table>
<thead>
<tr>
<th>#</th>
<th>RetTime</th>
<th>Type</th>
<th>Width [min]</th>
<th>Area [mAU*s]</th>
<th>Height [mAU]</th>
<th>Area %</th>
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<tbody>
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<td>1</td>
<td>17.390</td>
<td>BB</td>
<td>0.1312</td>
<td>10.90251</td>
<td>1.20873</td>
<td>0.0668</td>
</tr>
<tr>
<td>2</td>
<td>18.983</td>
<td>BB</td>
<td>0.1189</td>
<td>1.631113e+4</td>
<td>2094.26489</td>
<td>99.8734</td>
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<tr>
<td>3</td>
<td>23.069</td>
<td>BB</td>
<td>0.0927</td>
<td>9.77054</td>
<td>1.66024</td>
<td>0.0598</td>
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Totals : 1.63319e+4 2097.13386
```
HPLC chromatogram of 46:

Area Percent Report

<table>
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<th>Sorted By</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Signal</td>
<td>1.0000</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

Use Multiplier & Dilution Factor with ISTDs

Signal 2: DAD1 B, Sig=254.16 Ref=360,100

<table>
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<th>Width [min]</th>
<th>Area [mAU*s]</th>
<th>Height [mAU]</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
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<td>19.182</td>
<td>0.0896</td>
<td>613.34119</td>
<td>105.65668</td>
</tr>
</tbody>
</table>

Totals: 613.34119 105.65668
HPLC chromatogram of 47:

---

Area Percent Report
---

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 2: DAD1 B, Sig=254,16 Ref=360,100

<table>
<thead>
<tr>
<th>Peak RetTime Type</th>
<th>Width</th>
<th>Area</th>
<th>Height</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>#</td>
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<td>[min]</td>
<td>[mAU*s]</td>
<td>[mAU]</td>
</tr>
<tr>
<td>1</td>
<td>16.267</td>
<td>0.0875</td>
<td>1825.23303</td>
<td>315.46375</td>
</tr>
<tr>
<td>2</td>
<td>17.977</td>
<td>0.1293</td>
<td>19.31583</td>
<td>2.26950</td>
</tr>
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</table>

Totals : 1844.54886 317.73324
---
HPLC chromatogram of 48:

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 2: DAD1 B, Sig=254.16 Ref=360.100

<table>
<thead>
<tr>
<th>Peak</th>
<th>RetTime</th>
<th>Type</th>
<th>Width [min]</th>
<th>Area [mAU]</th>
<th>Height [mAU]</th>
<th>Area %</th>
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<td>BB</td>
<td>0.1263</td>
<td>4680.47656</td>
<td>533.65668</td>
<td>98.4525</td>
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<tr>
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<td>17.989</td>
<td>BB</td>
<td>0.1606</td>
<td>51.23030</td>
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<td>1.0776</td>
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<td>3</td>
<td>19.541</td>
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<td>0.1064</td>
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<td>0.2346</td>
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</table>

Totals: 4754.04552 541.27517
HPLC chromatogram of 49:

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 2: DAD1 B, Sig=254.16 Ref=360.100

<table>
<thead>
<tr>
<th>Peak</th>
<th>RetTime</th>
<th>Type</th>
<th>Width [min]</th>
<th>Area [mAU's]</th>
<th>Height [mAU]</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
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<td>BB</td>
<td>0.0888</td>
<td>6.25298</td>
<td>1.09156</td>
<td>0.1128</td>
</tr>
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<td>16.126</td>
<td>VB</td>
<td>0.0879</td>
<td>5362.54492</td>
<td>949.46124</td>
<td>96.7400</td>
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<td>16.713</td>
<td>BB</td>
<td>0.0857</td>
<td>39.23572</td>
<td>6.96253</td>
<td>0.7078</td>
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<td>4</td>
<td>17.339</td>
<td>BB</td>
<td>0.1505</td>
<td>14.47635</td>
<td>1.45011</td>
<td>0.2612</td>
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<tr>
<td>5</td>
<td>18.707</td>
<td>BB</td>
<td>0.0928</td>
<td>98.17499</td>
<td>16.65887</td>
<td>1.7711</td>
</tr>
<tr>
<td>6</td>
<td>20.592</td>
<td>VB</td>
<td>0.1146</td>
<td>15.16417</td>
<td>2.09219</td>
<td>0.2736</td>
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<td>7</td>
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<td>BB</td>
<td>0.1072</td>
<td>7.40446</td>
<td>1.03860</td>
<td>0.1336</td>
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</table>

Totals:  5543.25358  978.75510
HPLC chromatogram of 50:

```
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution   : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 2: DADl B, Sig=254,16 Ref=360,100

<table>
<thead>
<tr>
<th>#</th>
<th>RetTime</th>
<th>Type</th>
<th>Width</th>
<th>Area</th>
<th>Height</th>
<th>Area</th>
<th>%</th>
</tr>
</thead>
<tbody>
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<td>234.00641</td>
<td>95.5313</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>18.050</td>
<td>MM</td>
<td>0.275</td>
<td>111.05128</td>
<td>6.73058</td>
<td>4.4687</td>
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</table>

Totals : 2485.10377 240.73699
```
HPLC chromatogram of 51:

![HPLC Chromatogram](image)

Area Percent Report

Sorted By: Signal
Multiplier: 1.0000
Dilution: 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 2: DAD1 B, Sig=254,16 Ref=360,100

<table>
<thead>
<tr>
<th>Peak RetTime</th>
<th>Type</th>
<th>Width [min]</th>
<th>Area [mAU*s]</th>
<th>Height [mAU]</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 16.707 BB</td>
<td></td>
<td>0.0879 3107.87695</td>
<td>550.33850</td>
<td>99.4847</td>
<td></td>
</tr>
<tr>
<td>2 17.335 BB</td>
<td></td>
<td>0.1656 16.09649</td>
<td>1.42734</td>
<td>0.5153</td>
<td></td>
</tr>
</tbody>
</table>

Totals : 3123.97345 551.76584
HPLC chromatogram of stability tests with 14:

**Incubation time = 0 hours**

**Incubation time = 1 hour**

**Incubation time = 2 hours**

**Incubation time = 3 hours**

**Incubation time = 4 hours**
Supplementary References


