Electronic Supplementary Information

Click Chemistry by Microcontact Printing on Self-Assembled Monolayers: A Structure-Reactivity Study by Fluorescence Microscopy

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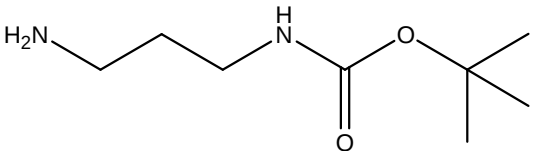
Synthesis of ink molecules

All chemicals were purchased from ACROS, exceptions are marked. First the synthesis of the amine compounds of all inks are described, the labelling with fluorophores is described in a separate section. The following compounds have been synthesized according to literature procedures and are therefore not described in detail here:

- 6-Amino-1-hexyne (**1**) - D. I. Rozkiewicz, D. Jancewski, W. Verboom, B. J. Ravoo and D. N. Reinhoudt, *Angew. Chem. Int. Ed.* 2006, **45**, 5292
- Furylethylamine (**6**) – F. A. Marques, D.C. Silva, E.P. Wendler, C.L. Wosch, Q.B. Cass and F. Batigalhia, *Lett. Org. Chem.*, 2007, **4**, 155-157.
- 5-Amino-1,3-pentadiene (**7**) – J. Linder, A.J. Blake and C.J. Moody, *Org. Biomol. Chem.*, 2008, **6**, 3908-3916.
- Cyclopentadienylethylamine (**8**) – L. Li, S. Han, Q. Li, Z. Chen and Z. Pang, *Eur. J. Inorg. Chem.*, 2007, 5127-5137.

1 Alkyne inks for 1,3-dipolar cycloaddition

1.1 Propiolic acid 3-amino-propylcarbamide (2)

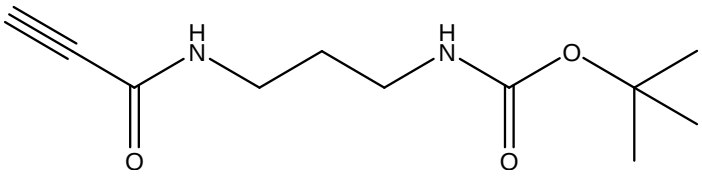


A solution of 7.4 g (100 mmol) of 1,3-diaminopropane in 50 ml chloroform was cooled to 0°C. Then 4.36 g (20 mmol) of Boc_20 in chloroform were added slowly. After stirring overnight at room temperature the solvent was evaporated and the resulting oil dissolved in ethylacetate. After washing three time with half saturated brine and drying the solvent was evaporated resulting in the desired product (yield: 45 %).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.06 (NH_2), 3.13 (m, 2H, $CH_2CH_2NH_2$), 2.69 (t, 2H, CH_2CH_2NHR), 1.55 (qn, 2H, $CH_2CH_2CH_2$), 1.37 (s, 9H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.16 (NH*C*OOR), 77.50 (*C*H_2NH_2), 60.35 (CH_2CH_2NHR), 39.56 (O*C*$(CH_3)_3$), 33.30 ($CH_2CH_2CH_2$), 28.37 (CH_3).

MS (ES+) $[C_8H_{18}N_2O_2+H]^+$: m/z = 175.1455 (cal. 175.1441).

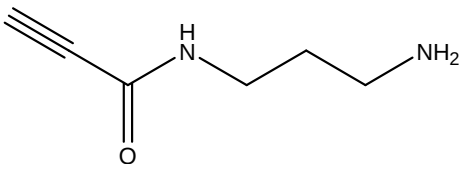


To a mixture of 700 mg (10 mmol) of propiolic acid in 50 ml DCM 2.06 g (10 mmol) of DCC were added. After cooling to 0°C 1.74 g of monoprotected diaminopropane dissolved in 30 ml DCM were added and the mixture was stirred for 10 h at room temperature. After filtration and evaporation of the solvent the residual oil was dissolved in 100 ml diethylether. Filtration and evaporation yielded the desired product (81%).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.82 (br, 1H, CON*H*CH_2), 4.79 (br, 1H, CH_2N*H*COO), 3.27 (m, 2H, CONHC*H*$_2$), 3.13 (m, 2H, CH_2C*H*$_2$NHCOO), 2.73 (s, 1H, 1H, *H*C≡C), 1.58 (m, 2H, CH_2C*H*$_2$$CH_2$), 1.38 (s, 9H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.40 (HCC*C*ONR), 152.92 (NH*C*OOR), 77.61 (CON*C*$H_2$$CH_2$), 74.76 (HC*C*CONR), 64.01 (H*C*CCONR), 60.23 (CH_2*C*H_2NHR), 38.45 (O*C*(CH_3)$_3$), 31.89 (CH_2*C*$H_2$$CH_2$), 28.35 ($CH_3$).

MS (ES+) $[C_{11}H_{18}N_2O_3+Na]^+$: m/z = 249.1214 (cal. 249.1210)

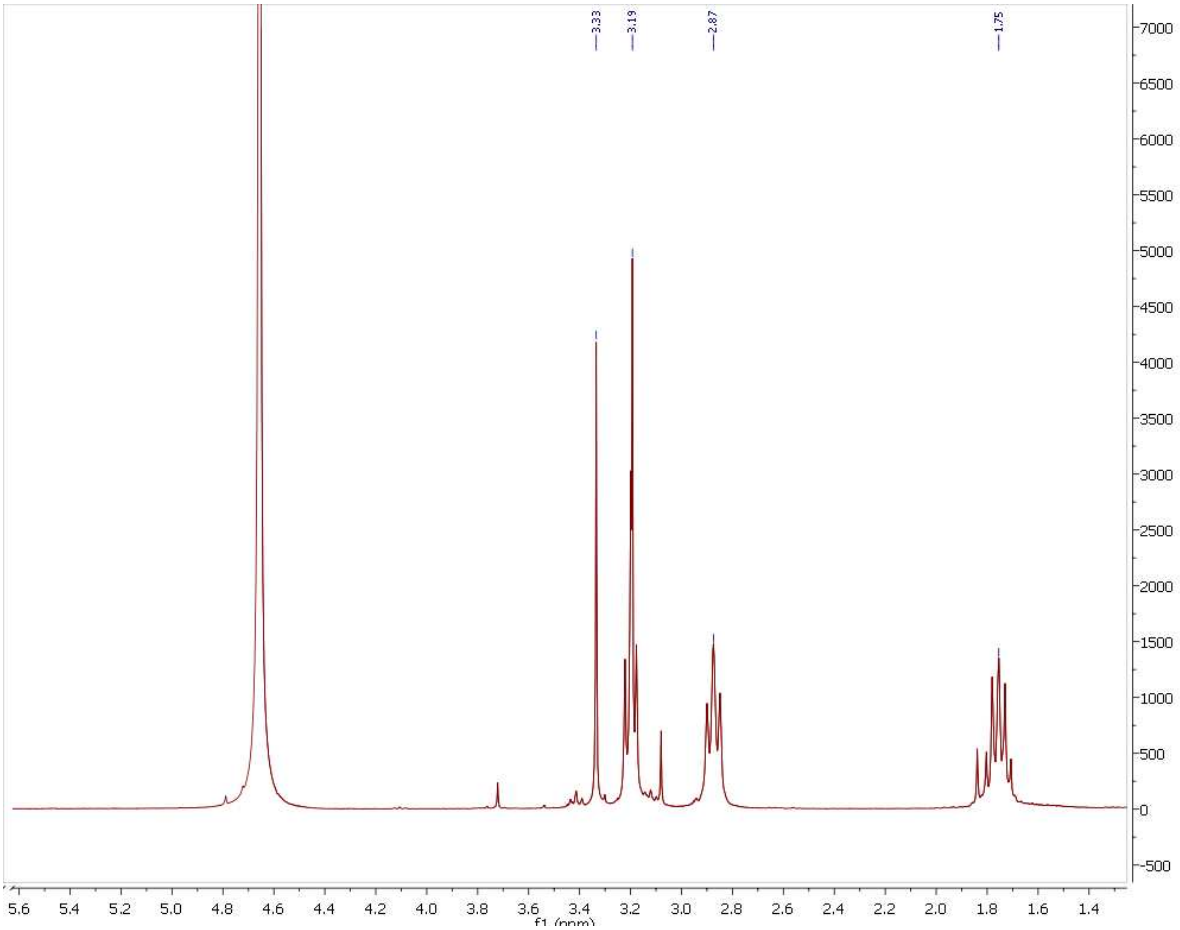


To deprotect the amino function 0.5 g (2,2 mmol) of the protected ester were dissolved in 10 ml DCM and 4.4 ml of trifluoric acid were added. After stirring for 20 h at room temperature the solvent was evaporated. To remove residual TFA methanol was added and evaporated in vacuum. This procedure was repeated three times (yield: 95%).

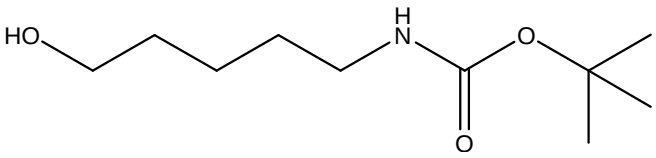
^{1}H NMR (300 MHz, DMSO): δ = 3.33 (s, 1H, *H*C≡C), 3.19 (t, 2H, CONHC*H*$_2$), 2.87 (t, 2H, CH_2C*H*$_2$$NH_2$), 1.75 (qn, 2H, CH_2C*H*$_2$$CH_2$).

^{13}C NMR (75 MHz, DMSO): δ = 158.64 (HCC*C*ONR), 78.05 (CON*C*$H_2$$CH_2$), 75.78 (HC*C*CONR), 63.25 (H*C*CCOOR), 36.71 (CH_2*C*$H_2$$NH_2$), 26.88 ($CH_2$*C*$H_2$$CH_2$).

MS (ES+) $[C_6H_{10}N_2O+H]^+$: m/z = 127.0879 (cal. 127.0866)



1.2 Propiolic acid 5-amino-propylester (3)

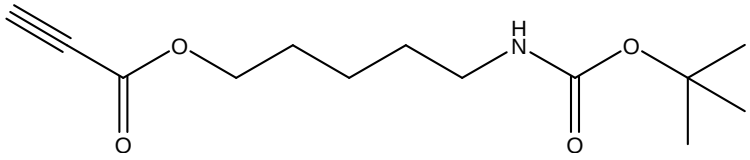


1.37 g (13,3 mmol) of 5-aminopentanol in a mixture of 28 ml methanol and 4 ml triethylamine was stirred for 10 min at 0°C (in an ice bath). A solution of 4.36 g (20 mmol) of Boc_2O in 10 ml methanol was added dropwise within 10 min. After stirring overnight the solvents were removed with an evaporator. The resulting oil was dissolved in DCM and washed with water. The organic phase was dried with $MgSO_4$. The protected amine was obtained in 88 % yield after evaporation of the solvent.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.23 (s, 1H, OH), 3.58 (t, 2H, CH_2OH), 3.05 (m, 2H, CH_2CH_2NHR), 1.56 – 1.43 (m, 6H, $CH_2(CH_2)_3CH_2$), 1.37 (s, 9H, CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 156.10 (NH*C*OOR), 77.02 (*C*H_2OH), 62.66 (CH_2CH_2NHR), 36.97 (O*C*$(CH_3)_3$), 32.24, 29.85, 22.89 ($CH_2CH_2CH_2$), 28.41 (CH_3).

MS (ES+) $[C_{10}H_{21}NO_3Na]^+$: m/z = 226.1415 (cal. 226.1414)

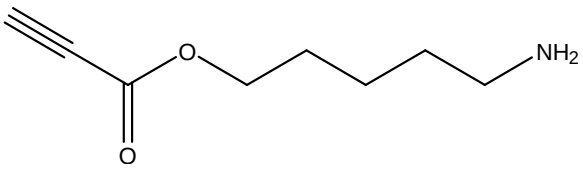


2.0 g (9.9 mmol) of the Boc-protected 5-aminopentanol were dissolved in 100 ml ethylacetate and 2.13 g (10,3 mmol) of dicyclohexylcarbodiimide (DCC) and 0.12 g (0,99 mmol) DMAP were added. After cooling to 0°C under argon atmosphere 0.821 g (11.7 mmol) propiolic acid dissolved in 10 ml ethylacetate were added dropwise within 4 h. The mixture was stirred overnight, filtered, washed with diethylether, saturated $NaHCO_3$ and saturated NaCl. After drying with $MgSO_4$ evaporation of the solvent yielded the protected ester. The crude product was purified by flash chromatography (ethylacetate-pentane 1:1), final yield was 78 %.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.52 (br, NH_2), 4.13 (t, 2H, COOCH_2CH$_2$), 3.06 (br, 2H, CH$_2$CH_2NHR), 2.83 (s, 1H, *H*C≡C), 1.66 – 1.43 (m, 6H, CH$_2$(CH_2)$_3$CH$_2$), 1.37 (s, 9H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 157.23 (HCC*C*OOR), 152.78 (NH*C*OOR), 77.03 (COO*C*H$_2$CH$_2$), 74.68 (HC*C*COOR), 66.14 (H*C*CCOOR), 62.17 (CH$_2$*C*H$_2$NHR), 40.33 (O*C*(CH$_3$)$_3$), 32.64, 29.68, 23.04 (CH$_2$*C*H$_2$CH$_2$), 28.40 (CH_3).

MS (ES+) [$C_{13}H_{21}NO_4$+Na]$^+$: m/z = 278.1368 (cal. 278.1363).

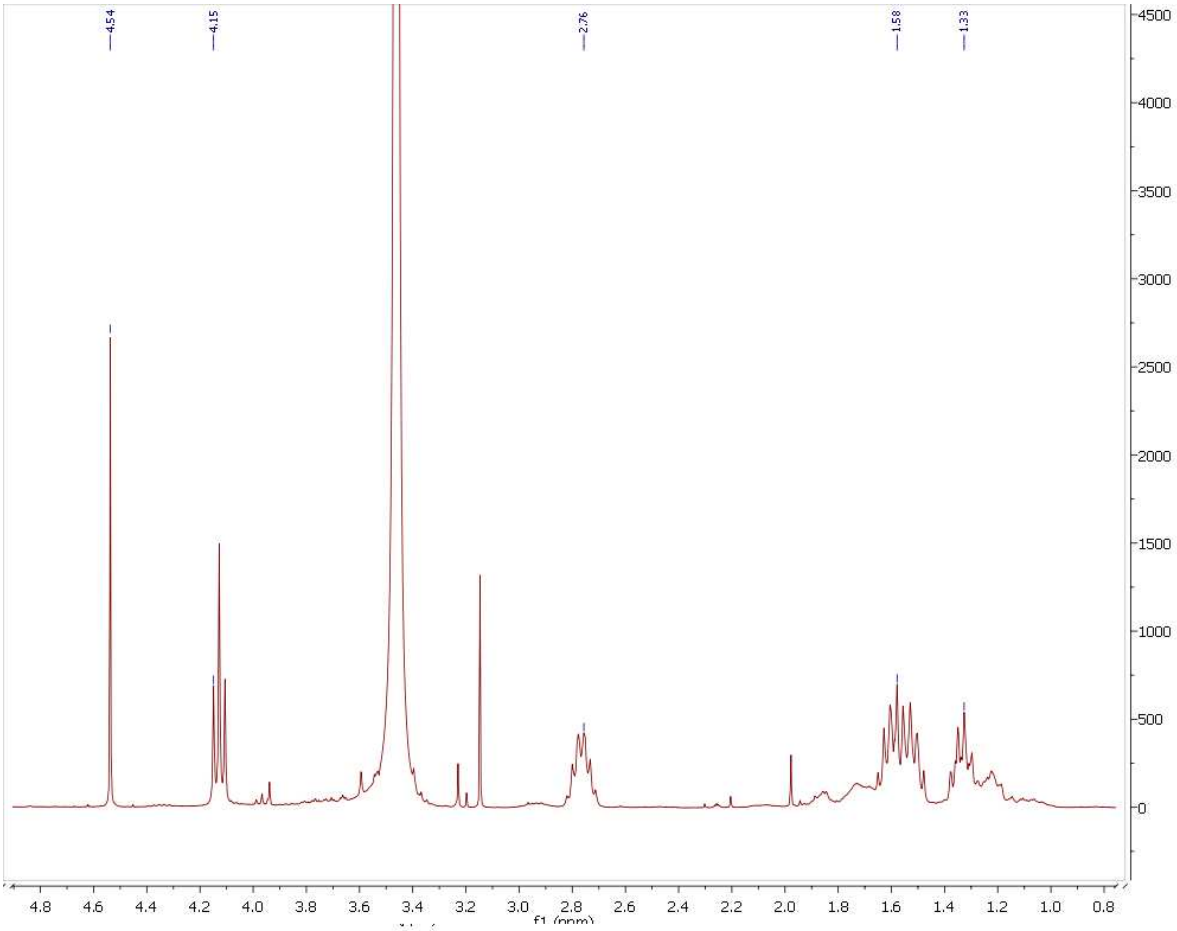


To deprotect the amino function 0.5 g (1.96 mmol) of the protected ester were dissolved in 10 ml DCM and 3.92 ml of trifluoric acid were added. After stirring for 20 h at room temperature the solvent was evaporated. To remove residual TFA methanol was added and evaporated in vacuum. This procedure was repeated three times (yield: 92 %).

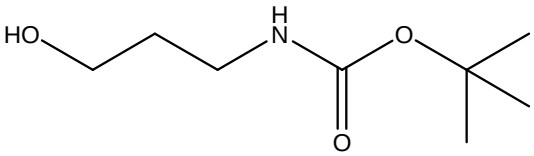
^{1}H NMR (300 MHz, DMSO): δ = 4.54 (s, 1H, *H*C≡C), 4.15 (t, 2H, COOCH_2CH$_2$), 2.76 (m, 2H, CH$_2$CH_2NH$_2$), 1.58 (m, 4H, CH$_2$CH_2CH$_2$CH_2CH$_2$), 1.33 (q, 2H, (CH$_2$)$_2$CH_2(CH$_2$)$_2$).

^{13}C NMR (75 MHz, DMSO): δ = 158.16 (HCC*C*OOR), 78.82 (COO*C*H$_2$CH$_2$), 74.69 (HC*C*COOR), 65.63 (H*C*CCOOR), 39.62 (CH$_2$*C*H$_2$NH$_2$), 33.63, 26.45, 22.11 (CH$_2$*C*H$_2$CH$_2$).

MS (ES+) [$C_8H_{13}NO_2$+H]$^+$: m/z = 156.1029 (cal. 156.1019).



1.3 Propiolic acid 3-amino-propylester (4)

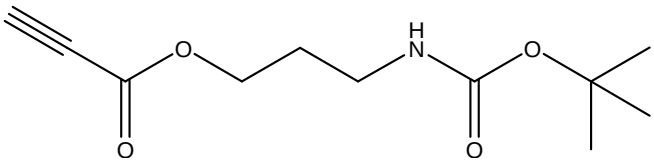


1.0 g (13,3 mmol) of 3-aminopropanol in a mixture of 28 ml methanol and 4 ml triethylamine was stirred for 10 min at 0°C (in an ice bath). A solution of 4.36 g (20 mmol) of Boc_2O in 10 ml methanol was added dropwise within 10 min. After stirring overnight the solvents were removed with an evaporator. The resulting oil was dissolved in DCM and washed with water. The organic phase was dried with $MgSO_4$. The protected amine was obtained after evaporation of the solvent (yield: 93 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.24 (s, 1H, OH), 4.90 (br, 1H, NH), 3.58 (m, 2H, C*H*$_2$OH), 3.20 (m, 2H, CH_2CH_2NHR), 1.60 (qn, 2H, $CH_2CH_2CH_2$), 1.37 (s, 9H, CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 157.13 (NH*C*OOR), 77.05 (*C*H_2OH), 59.22 (CH_2CH_2NHR), 36.94 (O*C*$(CH_3)_3$), 32.77 ($CH_2CH_2CH_2$), 28.34 (CH_3).

MS (ES+) $[C_8H_{17}NO_3+Na]^+$: m/z = 198.1106 (cal. 198.1101).

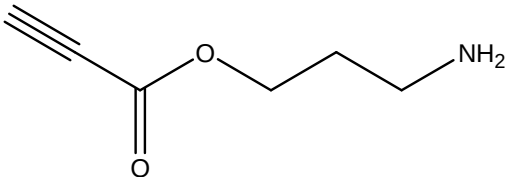


2.0 g (11 mmol) of the Boc-protected 3-aminopropanol was dissolved in 110 ml ethylacetate and 2.48 g (12 mmol) of dicyclohexylcarbodiimide (DCC) and 0.128 g (1.1 mmol) DMAP were added. After cooling to 0°C under argon atmosphere 0.945 g (13,5 mmol) propiolic acid dissolved in 10 ml ethylacetate were added dropwise within 4 h. The mixture was stirred overnight, filtered, washed with diethylether, saturated $NaHCO_3$ and saturated NaCl. After drying with $MgSO_4$ evaporation of the solvent yielded the protected ester. The crude product was purified by flash chromatography (ethylacetate-pentane 1:1), final yield was 85 %.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.19 (t, 2H, COOC*H*$_2$CH$_2$), 3.16 (br, 2H, CH$_2$C*H*$_2$NHR), 2.85 (s, 1H, *H*C≡C), 1.80 (qn, 2H, CH$_2$C*H*$_2$CH$_2$), 1.37 (s, 9H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.90 (HCC*C*OOR), 152.73 (NH*C*OOR), 77.03 (COO*C*H$_2$CH$_2$), 74.96 (HC*C*COOR), 63.78 (H*C*CCOOR), 60.40 (CH$_2$*C*H$_2$NHR), 37.20 (O*C*(CH$_3$)$_3$), 32.62 (CH$_2$*C*H$_2$CH$_2$), 28.36 (CH_3).

MS (ES+) $[C_{11}H_{17}NO_4+Na]^+$: m/z = 250.1046 (cal. 250.1050)

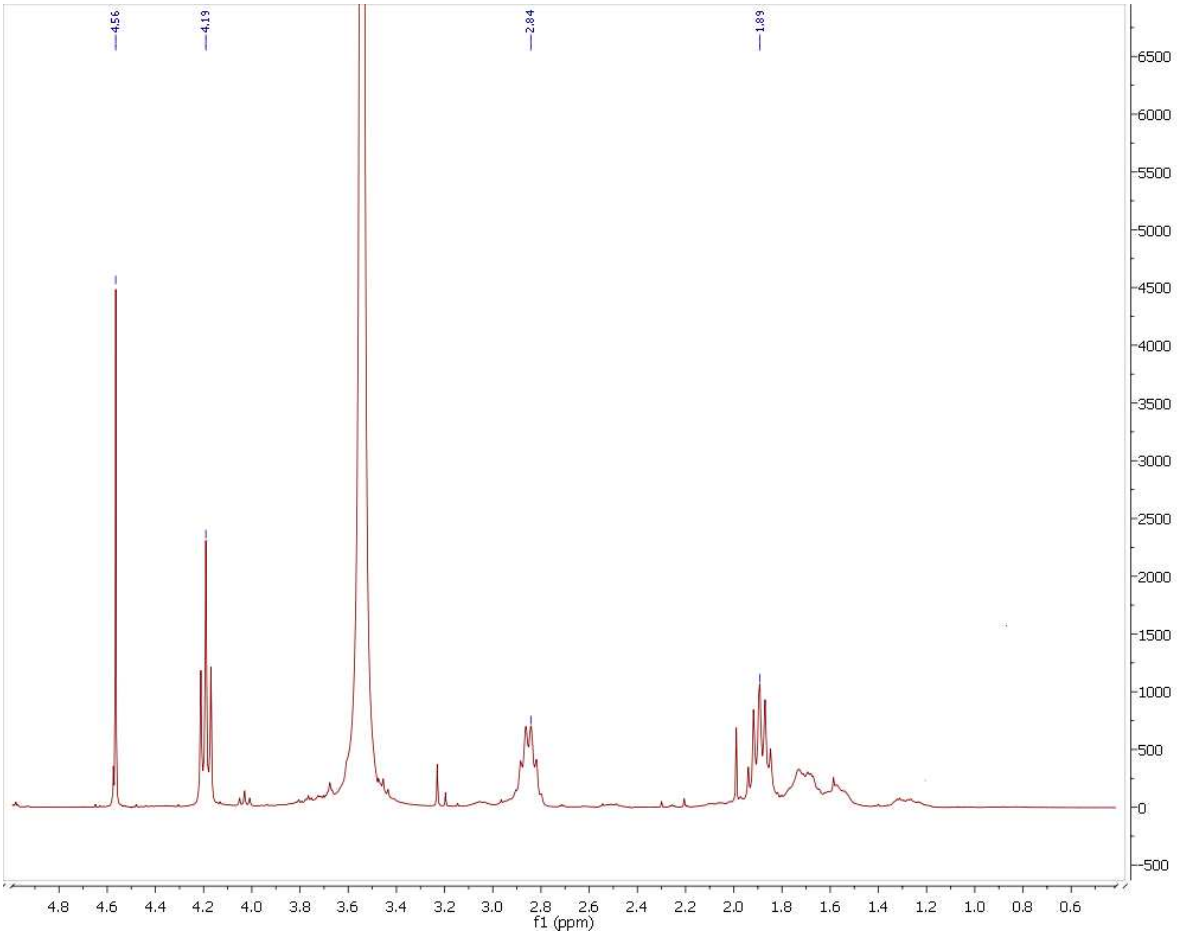


To deprotect the amino function 0.5 g (2.2 mmol) of the protected ester were dissolved in 10 ml DCM and 4.4 ml of trifluoric acid were added. After stirring for 20 h at room temperature the solvent was evaporated. To remove residual TFA methanol was added and evaporated in vacuum. This procedure was carried out three times. The final yield was 89 %.

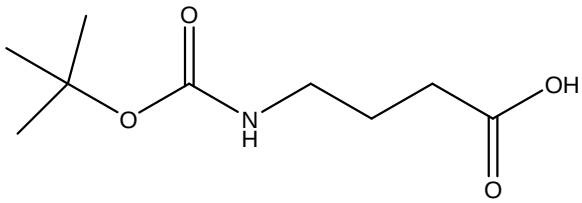
^{1}H NMR (300 MHz, DMSO): δ = 4.56 (s, 1H, *H*C≡C), 4.19 (t, 2H, COOC*H*$_2$CH$_2$), 2.84 (m, 2H, CH$_2$C*H*$_2$NH$_2$), 1.89 (qn, 2H, CH$_2$C*H*$_2$CH$_2$).

^{13}C NMR (75 MHz, DMSO): δ = 158.13 (HCC*C*OOR), 79.09 (COO*C*H$_2$CH$_2$), 74.60 (HC*C*COOR), 63.10 (H*C*CCOOR), 39.64 (CH$_2$*C*H$_2$NH$_2$), 25.85 (CH$_2$*C*H$_2$CH$_2$).

MS (ES+) $[C_6H_9NO_2+H]^+$: m/z = 128.0716 (cal. 128.0706).



1.4 6-Aminohex-1-yn-3-one (5)

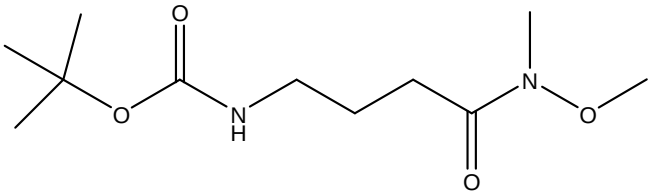


4.0 g 4-aminobutyric acid were dissolved in 20 ml methanol and 23.6 g Et_3N and 17.78 g Boc_2O were added. The mixture was stirred at 60°C overnight. After evaporation of the solvent the remaining solid was taken in saturated $NaHCO_3$ solution and extracted with pentane. The aqueous phase was slightly acidified (~pH 2) and extracted with ethylacetate. The combined organic phases were dried over $MgSO_4$ and the solvent was removed, yielding in 7.45 g of the pure product.

^{1}H NMR (300 MHz, DMSO): δ = 3.16 (t, 2H, CH_2CH_2NHR), 2.34 (t, 2H, $COCH_2CH_2$), 1.87 (qn, 2H, $CH_2CH_2CH_2$), 1.38 (s, 9H, CH_3).

^{13}C NMR (75 MHz, DMSO): δ = 177.13 (*C*OOH), 152,75 (NH*C*OOR), 80.65 (O*C*$(CH_3)_3$), 41.22 ($CH_2CH_2NH_2$), 31.02 ($COOCH_2CH_2$), 28.29 (CH_3), 24.91 ($CH_2CH_2CH_2$).

MS (ES+) $[C_9H_{17}NO_4+H]^+$: m/z = 204.251 (cal. 204.248)

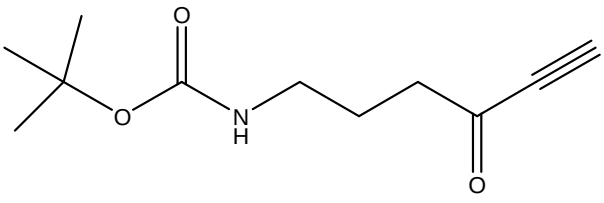


6.09 g of the Boc-protected aminobutyric acid in 50 ml of dry THF are cooled to -15°C. 3.28 ml 4-methylmorpholin, 3.88 ml isobutylchloroformate and a mixture of 2.83 g N,O-dimethylhydroxylamine and 4.57 ml Et_3N in 60 ml DMF are added in this order. Stirring was continued at -15°C for 30 min., then at room temperature overnight. The solvents were evaporated, the remaining solid was taken in 200 ml ethylacetate. Washing was performed with two times 1M H_3PO_4 first, then three times sat. $NaHCO_3$ solution. After drying with $MgSO_4$ and evaporating the solvent the crude product was cleaned by flash chromatography (pentane:EtOAc, 2:1).

^{1}H NMR (300 MHz, DMSO): δ = 3.88 (s, 3H, NOC*H*$_3$), 3.41 (s, 3H, (CO)N(C*H*$_3$)O), 3.12 (t, 2H, CH_2CH_2NHR), 2.37 (t, 2H, COC*H*$_2$$CH_2$), 1.85 (qn, 2H, $CH_2CH_2CH_2$), 1.38 (s, 9H, CH_3).

^{13}C NMR (75 MHz, DMSO): δ = 171.13 (CH_2*C*ON), 152,71 (NH*C*OOR), 80.67 (O*C*(CH_3)$_3$), 57.58 (NOCH_3), 41.22 (CH_2*C*H_2NH_2), 32.87 (CON(*C*H_3)O), 31.02 (COO*C*H_2CH_2), 28.29 (CH_3), 24.91 (CH_2*C*H_2CH_2).

MS (ES+) [$C_{11}H_{22}N_2O_4$+H]$^+$: m/z = 247.312 (cal. 247.317)

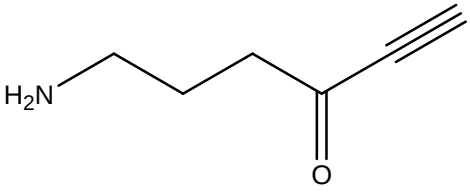


2.0 g of the previous compound in 100 ml dry Et_2O were cooled to -78°C. 50 ml of 0.5 M ethylene magnesium bromide in THF were added and the mixture was stirred at -78°C for additional 2 h and then at room temperature overnight. The mixture was then poured into a mixture of 150 ml Et_2O , 300 ml 1M KH_2PO_4 and ice. The aqueous was extracted with Et_2O and the combined organic phases were washed two times with 1M KH_2PO_4, two times with sat. $NaHCO_3$ and two times with brine. After drying with $MgSO_4$ and evaporating the solvent the crude product was cleaned by flash chromatography (pentane:EtOAc, 2:1).

^{1}H NMR (300 MHz, DMSO): δ = 3.58 (s, 1H, COCCH), 3.13 (t, 2H, CH_2C*H*$_2$NHR), 2.51 (t, 2H, COC*H*$_2$$CH_2$), 1.84 (qn, 2H, CH_2C*H*$_2$$CH_2$), 1.38 (s, 9H, CH_3).

^{13}C NMR (75 MHz, DMSO): δ = 187.97 (*C*OCCH), 152,71 (NH*C*OOR), 82.66 (COC*C*H) 80,91 (CO*C*CH), 80.63 (O*C*(CH_3)$_3$), 46.55 (COO*C*H_2CH_2), 41.22 (CH_2*C*H_2NH_2), 28.29 (CH_3), 24.91 (CH_2*C*H_2CH_2).

MS (ES+) [$C_{11}H_{17}NO_3$+H]$^+$: m/z = 212.272 (cal. 212.271)

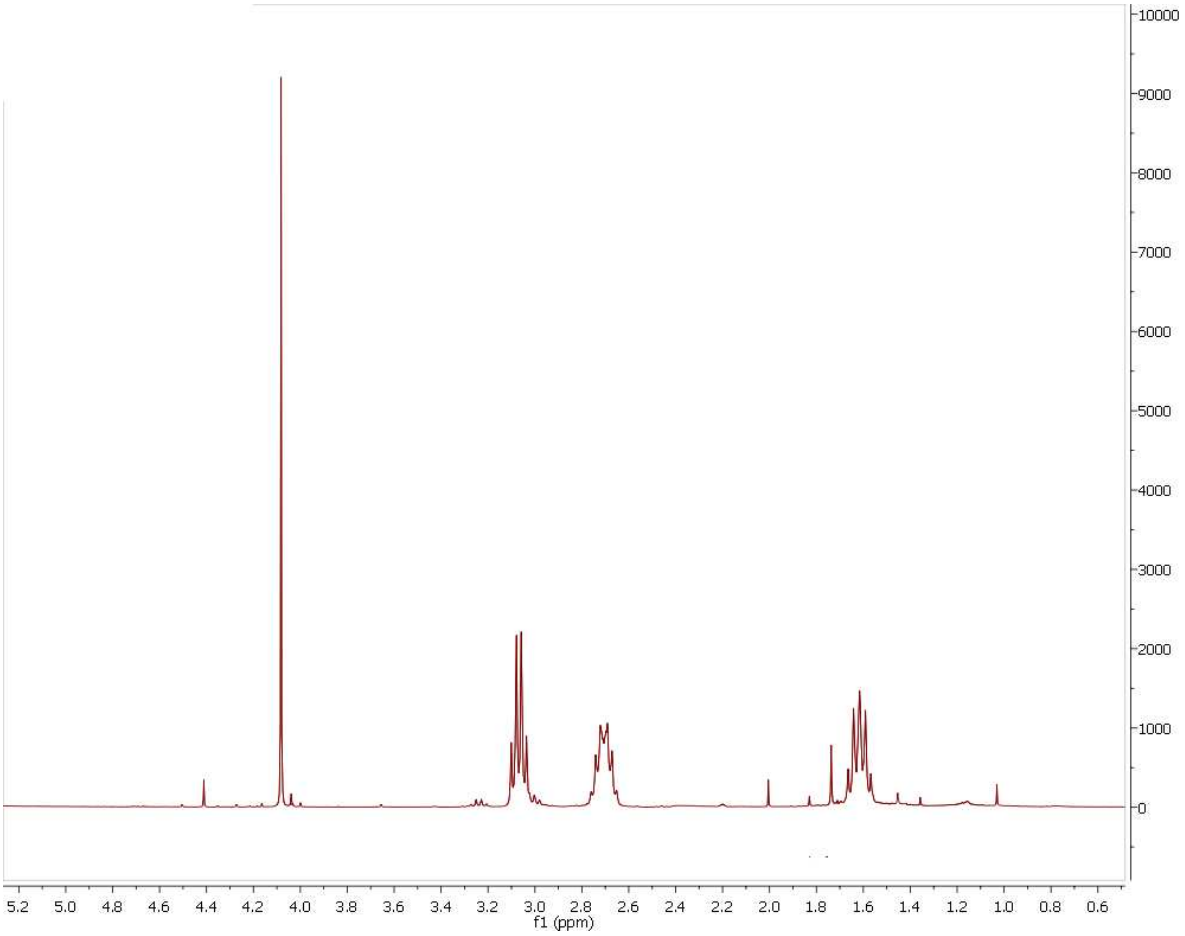


Deprotection of the amine function was carried out in DCM (10ml/mmol) with TFA (2ml/mmol). It was found that with longer treatment the compound decomposes. The highest yield was obtained when stirred at room temperature for 2 h. The solvent was evaporated. To remove residual TFA methanol was added and evaporated again to dryness. This procedure was carried out three times.

^{1}H NMR (300 MHz, DMSO): δ = 4.09 (s, 1H, COCCH), 3.08 (m, 2H, COCH_2CH$_2$), 2.70 (m, 2H, CH$_2$CH_2NH$_2$), 1.59 (qn, 2H, CH$_2$CH_2CH$_2$).

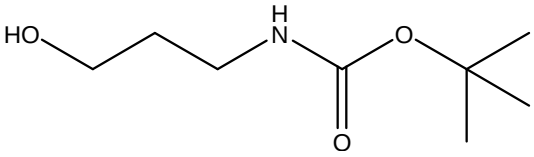
^{13}C NMR (75 MHz, DMSO): δ = 187.98 (*C*OCCH), 82.67 (COC*C*H) 80,92 (CO*C*CH), 46.37 (COO*C*H$_2$CH$_2$), 41.03 (CH$_2$*C*H$_2$NH$_2$), 28.63 (CH$_2$*C*H$_2$CH$_2$).

MS (ES+) [C$_6$H$_9$NO+Na]$^+$: m/z = 134.135 (cal. 134.134)



2 Inks for thiol-ene reaction

2.1 Acrylic acid 3-amino-propylester (12)

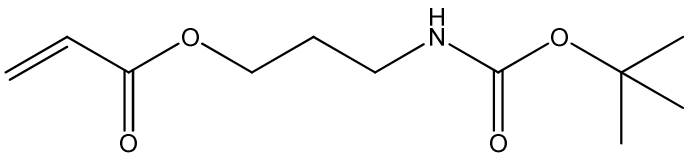


1.0 g (13,3 mmol) of 3-aminopropanol in a mixture of 28 ml methanol and 4 ml triethylamine was stirred for 10 min at 0°C (in an ice bath). A solution of 4.36 g (20 mmol) of Boc_2O in 10 ml methanol was added dropwise within 10 min. After stirring overnight the solvents were removed with an evaporator. The resulting oil was dissolved in DCM and washed with water. The organic phase was dried with $MgSO_4$. The protected amine was obtained after evaporation of the solvent (yield: 93 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.24 (s, 1H, OH), 4.90 (br, 1H, NH), 3.58 (m, 2H, C*H*$_2$OH), 3.20 (m, 2H, CH_2CH_2NHR), 1.60 (qn, 2H, $CH_2CH_2CH_2$), 1.37 (s, 9H, CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 157.13 (NH*C*OOR), 77.05 (*C*H_2OH), 59.22 (CH_2CH_2NHR), 36.94 (O*C*$(CH_3)_3$), 32.77 ($CH_2CH_2CH_2$), 28.34 (CH_3).

MS (ES+) $[C_8H_{17}NO_3+Na]^+$: m/z = 198.1106 (cal. 198.1101).

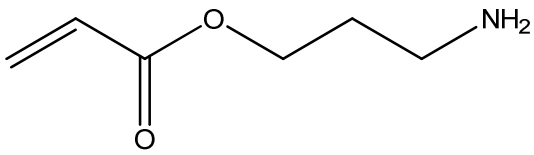


2.0 g (11 mmol) of the Boc-protected 3-aminopropanol was dissolved in 110 ml ethylacetate and 2.48 g (12 mmol) of dicyclohexylcarbodiimide (DCC) and 0.128 g (1.1 mmol) DMAP were added. After cooling to 0°C under argon atmosphere 0.972 g (13.5 mmol) acrylic acid dissolved in 10 ml ethylacetate were added dropwise within 4 h. The mixture was stirred overnight, filtered, washed with diethylether, saturated $NaHCO_3$ and saturated NaCl. After drying with $MgSO_4$ evaporation of the solvent yielded the protected ester. The crude product was purified by flash chromatography (ethylacetate-pentane 1:1), final yield was 93 %.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.36 (dd, 1H, COCH=C*H*$_2$), 6.11 (dd, 1H, COCH=C*H*$_2$), 6.06 (dd, 1H, COC*H*=CH_2), 4.19 (t, 2H, COOC*H*$_2$$CH_2$), 3.16 (br, 2H, CH_2C*H*$_2$NHR), 1.80 (qn, 2H, CH_2C*H*$_2$$CH_2$), 1.37 (s, 9H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.91 (H_2CCH*C*OOR), 152.77 (NH*C*OOR), 130.96 (H_2C*C*HCOOR), 127.78 (H_2*C*CHCOOR), 77.12 (COO*C*$H_2$$CH_2$), 60.58 ($CH_2$*C*$H_2$NHR), 37.20 (O*C*$(CH_3)_3$), 32.62 ($CH_2$*C*$H_2$$CH_2$), 28.36 ($CH_3$).

MS (ES+) $[C_{11}H_{19}NO_4+Na]^+$: m/z = 252.1156 (cal. 252.1162)

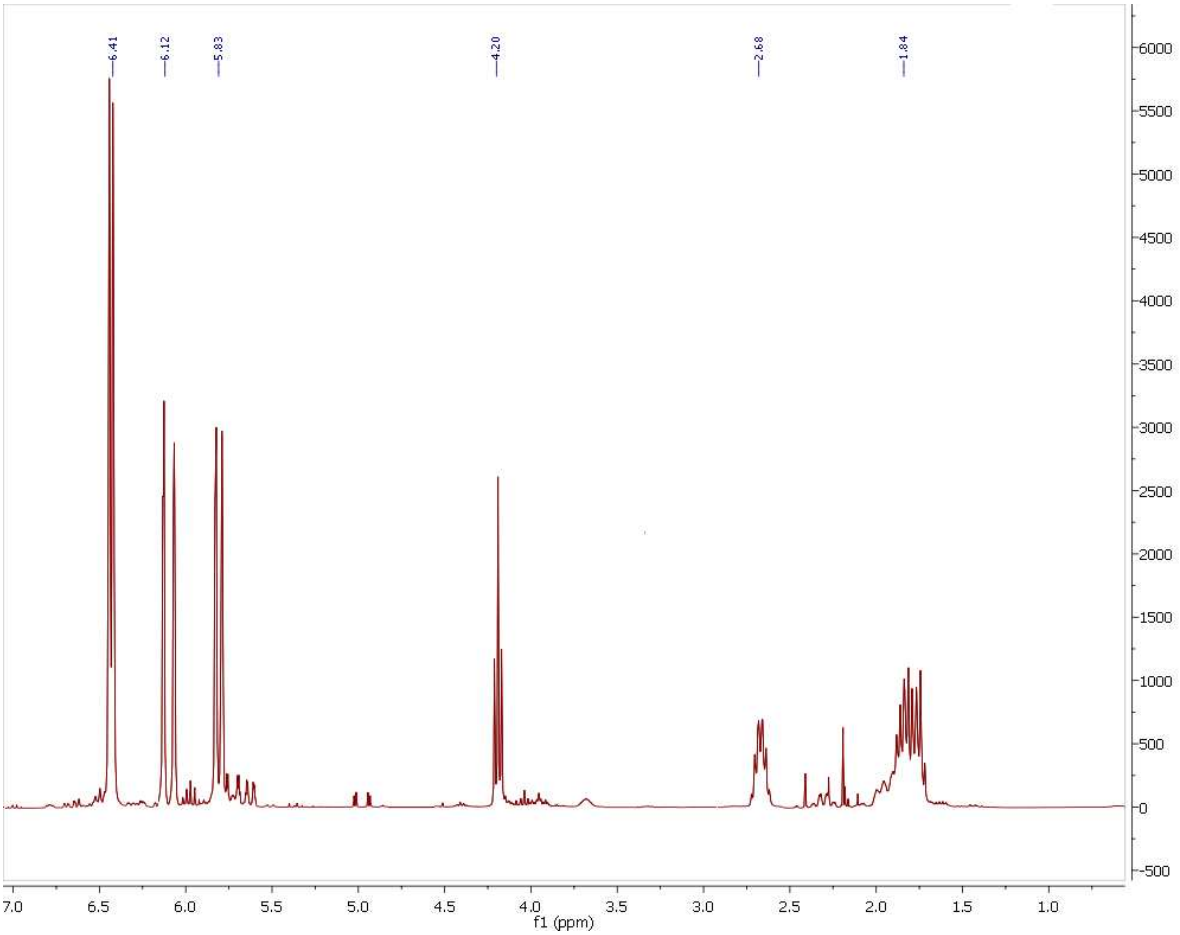


To deprotect the amino function 0.5 g (2,2 mmol) of the protected ester were dissolved in 10 ml DCM and 4.4 ml of trifluoric acid were added. After stirring for 20 h at room temperature the solvent was evaporated. To remove residual TFA methanol was added and evaporated in vacuum. This procedure was carried out three times. The final yield was 84 %.

^{1}H NMR (300 MHz, DMSO): δ = 6.41 (d, 1H, COCH=CH_2), 6.12 (dd, 1H, COCH=CH_2), 5.83 (d, 1H, COCH=CH_2), 4.20 (t, 2H, COOCH_2CH_2), 2.68 (m, 2H, CH_2CH_2NH_2), 1.84 (qn, 2H, CH_2CH_2CH_2).

^{13}C NMR (75 MHz, DMSO): δ = 158.21 (H$_2$CCH*C*OOR), 130.96 (H$_2$C*C*HCOOR), 127.78 (H$_2$*C*CHCOOR), 79.09 (COO*C*H$_2$CH$_2$), 39.64 (CH$_2$*C*H$_2$NH$_2$), 25.85 (CH$_2$*C*H$_2$CH$_2$).

MS (ES+) [$C_6H_{11}NO_2$+H]$^+$: m/z = 130.0778 (cal. 130.0773).



2.2 Cysteamine ink (13)

150 mg of Cystamine was labelled with lissamine rhodamine B sulfonyl chloride following the procedure in 3.1. The resulting disulfide was cleaved with a small amount of dithiotreitol

and catalytic amounts of DMAP in DMF by stirring overnight. The crude product was purified by column chromatography (eluent: acetonitrile-chloroform-methanol, 10:3:2) to obtain 30 mg of the desired product (23%).

MS (ES+) $[C_{29}H_{35}N_3O_6S_3+Na]^+$: m/z = 639.1502 (cal. 639.1507)

2.3 Aminothiophenol ink (14)

5 g of silica gel were given into a 100ml round bottom flask, 2.5 mL water were added through a septum, followed by the addition of 25 mL DCM. A solution of 500 mg 4-aminothiophenol (4 mmol) in 5 mL DCM was added through a syringe and a solution of 0.649 g bromine (4.1mmol) in 3.5 mL DCM was added dropwise. The addition of bromine was stopped as soon as the solution turned brownish. The reaction mixture was filtered and the filter was washed with 60 mL DCM. The solvent was removed and the crude disulfide was labeled with lissamine rhodamine B sulfonyl chloride following the procedure in 3.1. The resulting product was treated with a small amount of dithiotreitol and catalytic amounts of DMAP in DMF by stirring overnight. The crude product was purified by column chromatography (eluent: acetonitrile-chloroform-methanol, 10:3:2) to obtain 33 mg (0.05 mmol; 25 %) of the desired product.

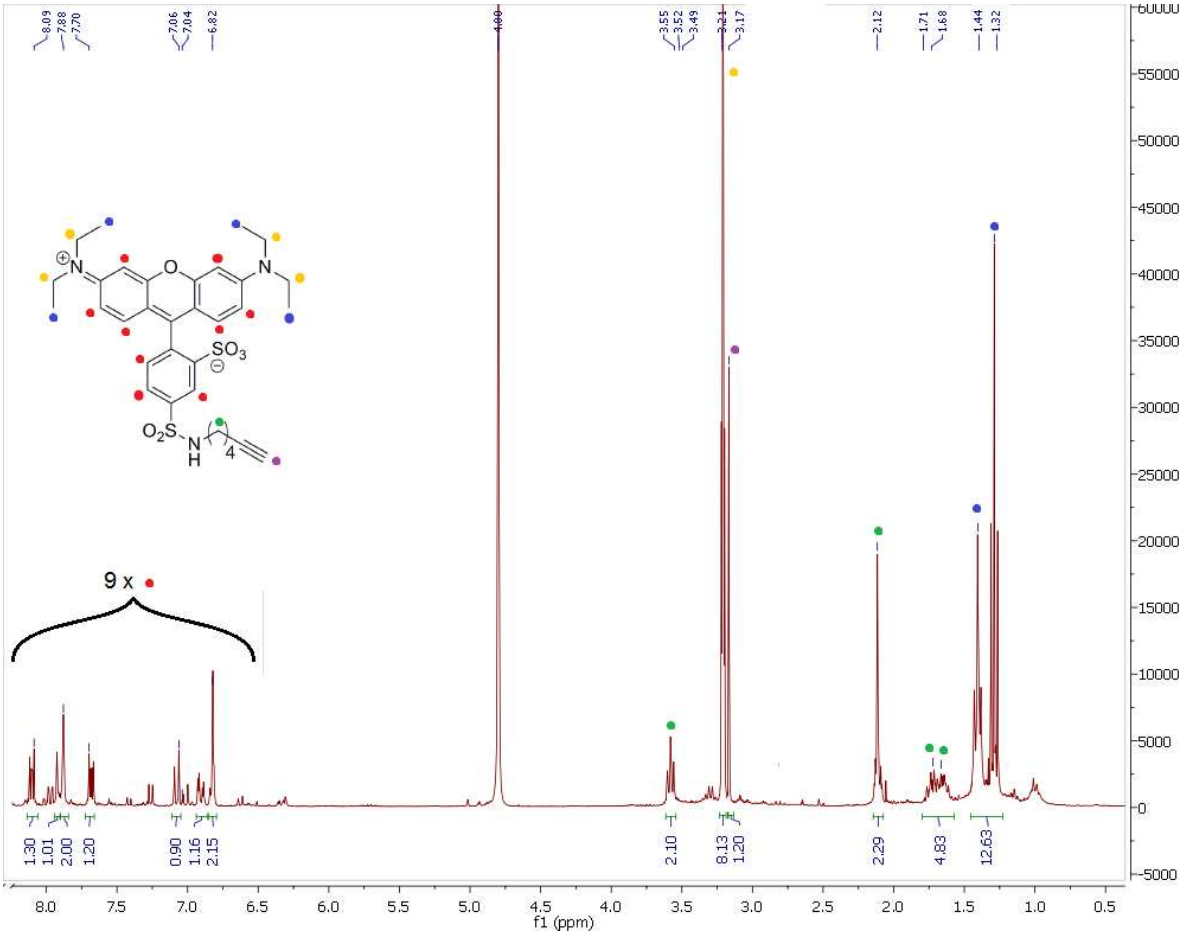
MS (ES+) $[C_{33}H_{34}N_3O_6S_3+Na]^+$: m/z = 687.1503 (cal. 687.1499)

3 Labelling with fluorophores

3.1 Lissamine rhodamine B

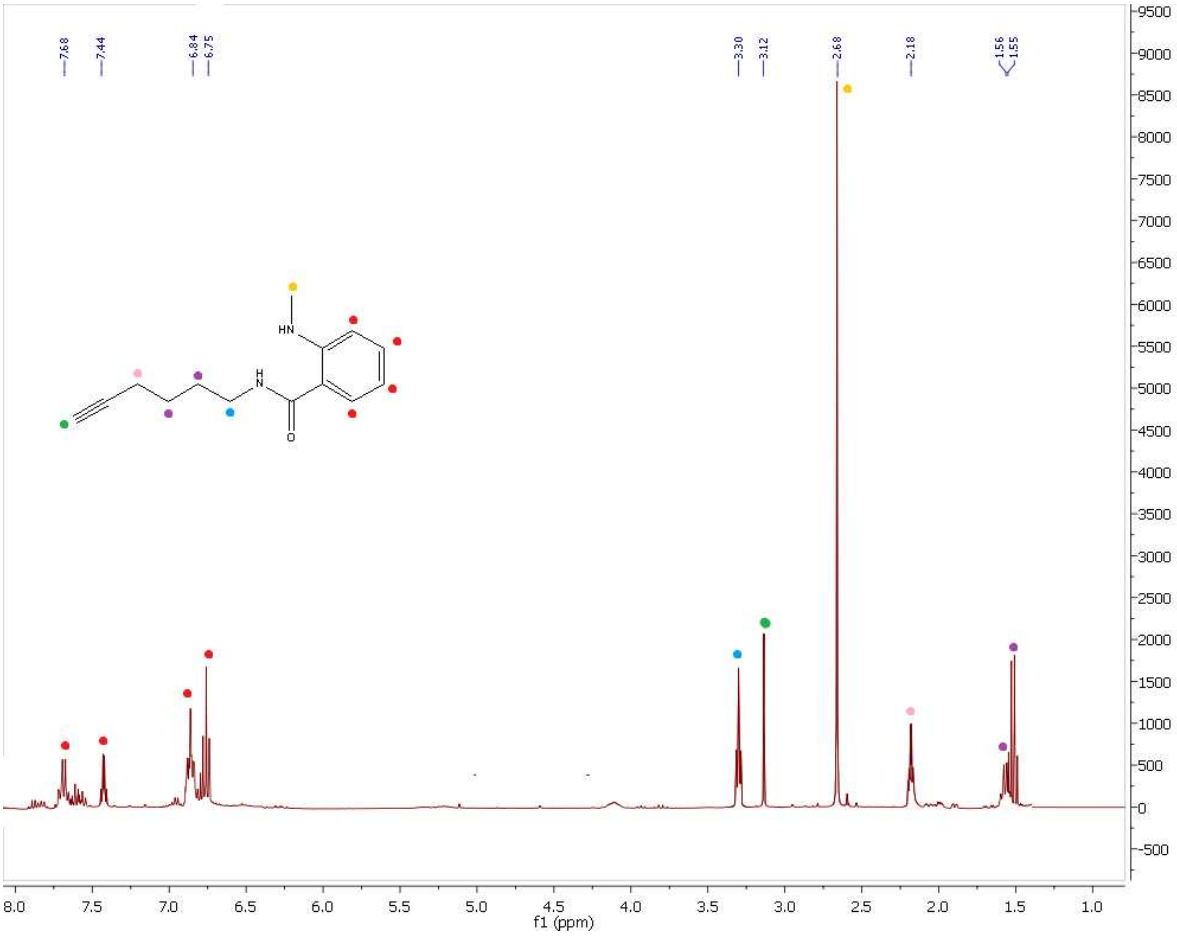
0.15 g of the amine was added to a solution of lissamine rhodamine B sulfonyl chloride (~100 mg) in 30 ml of dry DMF. After adding 0.1 ml triethylamine the mixture was stirred for 3 h at room temperature and then at -20°C for another 16 h (temperature changes of +/- 10°C were accepted). 3 ml of 1 M NaOH and 20 ml of ethanol were added. After evaporating the solvents a 2:1 mixture of dry THF and ethanol was added, the remaining solid residue was filtered off and evaporation of the solvent yielded the desired product in 70-80% yield.

All labelled inks were analyzed by MS. The NMR spectrum of 6-aminohexyne labelled with lissamine rhodamine B is shown below.



3.2 Anthranylic acid

The amine (~100mg) is dissolved in DMF, triethylamine (1 equiv.) and N-methylanthranylic acid anhydride (1equiv.) are added under dry conditions and in argon atmosphere. The mixture is stirred for 3 h at room temperature, then another 4 h at 60°C (reflux). The mixture is filtered, the solvent is removed under reduced pressure and the residue is taken up in ethyl acetate. The organic phase is washed with water (3 x 10 mL), the combined aqueous phases re-extracted with ethyl acetate (3 x 10mL) and the combined organic phases dried on $MgSO_4$. Removal of the solvent yields the raw product that is purified by column chromatography (silica gel, eluent: $CHCl_3$/pentane, 9:1). All labelled inks are analysed by MS and NMR spectroscopy. The NMR spectrum of 6-aminohexyne labelled with anthranylic acid is shown below.


7.68
7.44
6.84
6.75
3.30
3.12
2.68
2.18
1.56
1.55
HN
H
N
O
f1 (ppm)