Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Electronic Supplementary Information

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1. Experimental Procedures

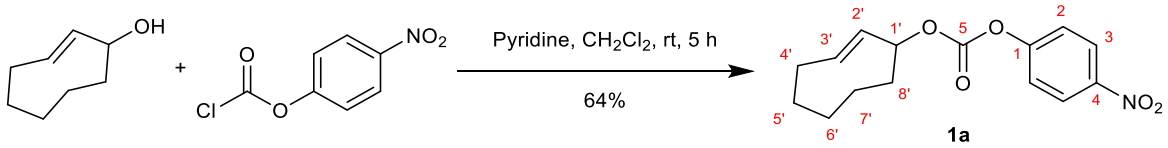
1.1 Synthesis

***General techniques**:* All non-aqueous reactions were performed in oven-dried glassware under argon atmosphere unless otherwise stated. Argon gas was pre-dried *via* passage through calcium chloride. Reaction vessels were heated using thermostatically controlled dry-syn blocks with the liquid level of the flask below that of the heating block. Reaction temperatures refer to the thermostat set point. All reagents were purchased from commercial sources and used without further purification unless otherwise stated. CH_2Cl_2, THF and Et_2O were purified either according to the method of Grubbs and Pangborn[1] or by distillation under an inert atmosphere (CH_2Cl_2, MeOH, toluene and MeCN were distilled from calcium hydride. THF and Et_2O were pre-dried over sodium wire then distilled from calcium hydride and lithium aluminium hydride). DIPEA was purified by distillation over calcium hydride and stored over 4 Å molecular sieves. Petroleum ether and EtOAc were distilled on site. 'Petrol' refers to the distillate of petroleum ether collected between 40–60 °C unless otherwise stated. Water used experimentally was deionised and prepared on site. Flash column chromatography was performed using silica gel 60 Å (40-63 µm) from Material Harvest. Analytical thin layer chromatography was performed using Merck Silica 42 gel 60 F254 1 mm glass plates and visualized by UV (254 nm) and/or by staining with potassium permanganate (KMnO4).

Trans-cyclooct-2-en-1-ol (TCO-OH) was purchased as the axial isomer from Sirius Fine Chemicals.

NMR spectra were recorded on Bruker 400-Avance III HD, Avance DPX-400, 400-QNP Cryoprobe or 500-DCH Cryoprobe spectrometers. Chemical shifts are reported in parts per million (ppm) and the spectra are calibrated to the residual solvent peak ^{1}H NMR: $CDCl_3$ δ 7.26 ppm; ^{13}C NMR: $CDCl_3$ δ 77.16 ppm. Multiplicities are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (double doublet) and so on. Coupling constants (*J*) are reported in hertz (Hz) to 1 decimal place using MestreNova for signal processing. The center of each peak is reported except for multiplet signals where a range of ppm values is given. Structural assignments are made with the aid of COSY, HSQC, and HMBC experiments, performed by the NMR Spectrometry Service, University of Cambridge. High-resolution mass spectra were performed by the Mass Spectrometry Service, Department of Chemistry, University of Cambridge using a Waters LCT Premier or a Waters Xevo G2-S spectrometer and ionized by ESI or ASAP.

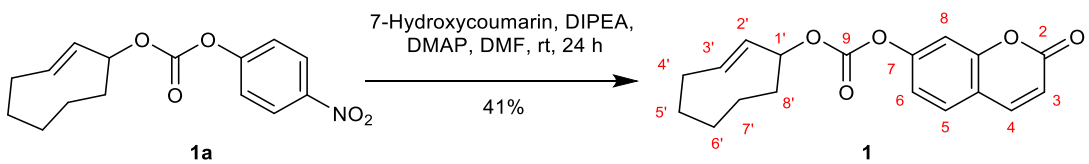
(2*E*)-Cyclooct-2'-en-1'-yl 4-nitrophenyl carbonate (1a)



According to the procedure by Lemke *et al.*,[2] (2*E*)-cyclooct-2-en-1-ol (axial isomer) (35 mg, 0.277 mmol, 1.0 equiv.) and pyridine (0.06 mL, 0.693 mmol, 2.5 equiv.) were dissolved in CH_2Cl_2 (3 mL). A solution of 4-nitrophenyl chloroformate (84 mg, 0.416 mmol, 1.5 equiv.) in CH_2Cl_2 (3 mL) was added and the reaction was stirred at rt for 5 h. The reaction was stopped by addition of $NH_4Cl_{(aq)}$ (sat. soln., 5 mL) and the phases were separated. The aqueous phase was extracted with CH_2Cl_2 (2 x 10 mL) and the combined organic extracts were washed with brine (10 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (5% EtOAc/Petrol) yielded **1a** as a white solid (49 mg, 0.168 mmol, 61%). The NMR data were in accordance with the previously reported literature data.[2]

R_f 0.17 (5% EtOAc/Petrol). **Mp** 85.3–86.5 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.48 – 8.19 (m, 2H, H3), 7.42 (d, *J* = 9.2 Hz, 2H, H2), 6.00 (ddd, *J* = 16.2, 11.1, 3.8 Hz, 1H, H3'), 5.58 (dd, *J* = 16.2, 2.3 Hz, 1H, H2'), 5.51 – 5.35 (m, 1H, H1'), 2.55 (dd, *J* = 10.8, 4.3 Hz, 1H, H4'), 2.30 – 2.17 (m, 1H, H8'), 2.17 – 2.01 (m, 2H, H4', H5'), 1.99 – 1.87 (m, 1H, H6'), 1.88 – 1.68 (m, 2H, H7', H8'), 1.63 – 1.50 (m, 1H, H5'), 1.31 – 1.12 (m, 1H, H7'), 0.86 (tdd, *J* = 12.9, 5.4, 3.2 Hz, 1H, H6'). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 155.6 (C1), 151.6 (C5), 145.3 (C4), 133.1 (C3'), 129.4 (C2'), 125.3 (C3), 121.8 (C2), 78.8 (C1'), 40.4 (C8'), 36.0 (C4'), 35.9 (C5'), 28.9 (C6'), 24.0 (C7'). **HRMS (**ASAP$^-$): *m/z* calc. for $C_{15}H_{17}NO_5$ $[M]^-$ 291.1107, found 291.1105, Δ -0.7 ppm.

(2*E*)-Cyclooct-2'-en-1'-yl 2-oxochromen-7-yl carbonate (1)

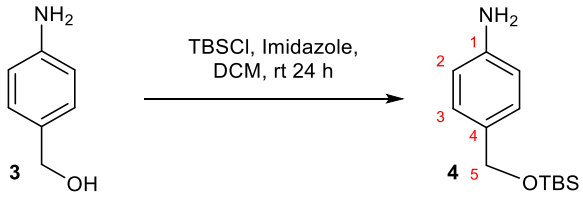


According to the modified procedure by Renslo *et al.*,[3] 7-hydroxycoumarin **2** (71 mg, 0.437 mmol, 2.6 equiv.), DIPEA (0.09 mL, 0.505 mmol, 3.0 equiv.) and DMAP (8 mg, 0.067 mmol, 0.4 equiv.) were added to a solution of (2*E*)-cyclooct-2-en-1-yl 4-nitrophenyl carbonate **1a** (49 mg, 0.168 mmol, 1.0 equiv.) in DMF (3 mL). The reaction mixture was stirred at

rt for 24 h then an additional portion of 7-hydroxycoumarin **2** (30 mg, 0.185 mmol, 1.1 equiv.) and DIPEA (0.03 mL, 0.185 mmol, 1.1 equiv.) was added. The reaction was stirred for a further 3 h then diluted with Et_2O (30 mL), washed with HCl (1M, 10 mL), NaOH (1M, 5 x 10 mL), brine (20 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification *via* flash column chromatography (20–25% EtOAc/Petrol) yielded **1** as a white solid (32 mg, 0.102 mmol, 61%).

R_f 0.30 (25% EtOAc/Petrol). **Mp** 105.1–106.7 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.69 (d, *J* = 9.5 Hz, 1H, H4), 7.49 (d, *J* = 8.5 Hz, 1H, H5), 7.23 (d, *J* = 2.3 Hz, 1H, H8), 7.16 (dd, *J* = 8.5, 2.3 Hz, 1H, H6), 6.40 (d, *J* = 9.5 Hz, 1H, H3), 5.98 (ddd, *J* = 16.2, 11.2, 3.8 Hz, 1H, H3'), 5.56 (dd, *J* = 16.2, 2.3 Hz, 1H, H2'), 5.46 – 5.40 (m, 1H, H1'), 2.53 (dd, *J* = 10.7, 4.3 Hz, 1H, H4'), 2.27 – 2.16 (m, 1H, H8'), 2.13 – 1.97 (m, 2H, H4', H5'), 1.92 (dddd, *J* = 15.1, 7.2, 3.5, 1.7 Hz, 1H, H6'), 1.85 – 1.65 (m, 2H, H7', H8'), 1.65 – 1.46 (m, 1H, H5'), 1.19 (dddd, *J* = 19.1, 15.0, 9.7, 1.7 Hz, 1H, H7'), 0.92 – 0.72 (m, 1H, H6'). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 160.3 (C2), 154.7 (C8a), 153.5 (C7), 151.9 (C9), 142.8 (C4) , 133.1 (C3'), 129.5 (C2'), 128.6 (C5), 117.8 (C6), 116.7 (C4a), 116.2 (C3), 109.9 (C8), 78.6 (C1'), 40.5 (C8'), 36.0 (C4'), 35.9 (C5'), 29.0 (C6'), 24.0 (C7'). **IR (**thin film, **ν_{max}**/cm^{-1}) 2929m, 2852w, 1759s, 1726s, 1706m, 1619m, 1567w, 1503w, 1453w, 1401m, 1277m. **HRMS (**ESI$^+$): *m/z* calc. for $C_{18}H_{18}O_5$ $[M+H]^+$ 315.1227, found 315.1220, Δ -2.1 ppm.

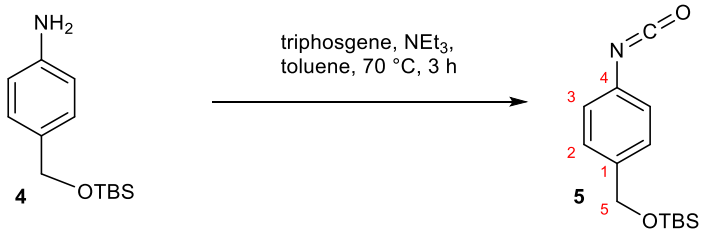
4-{[(tert-butyldimethylsilyl)oxy]methyl}aniline (4)



According to the procedure by Haynes *et al.*,[4] 4-aminobenzyl alcohol **3** (0.250 g, 2.03 mmol, 1.0 equiv.) and imidazole (0.152 g, 2.23 mmol, 1.1 equiv.) were dissolved in CH_2Cl_2 (5 mL). TBSCl (0.336 g, 2.23 mmol, 1.1 equiv.) was added and the reaction was stirred at rt for 24 h. The reaction was quenched with water (10 mL) and extracted with CH_2Cl_2 (4 x 10 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (40% EtOAc/Petrol) yielded **4** as a yellow liquid (0.373 g, 1.57 mmol, 77%). The NMR data were in accordance with reported data.[5]

R_f 0.76 (40% EtOAc/Petrol). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.18 – 7.06 (m, 2H, H3), 6.66 (d, *J* = 8.4 Hz, 2H, H2), 4.62 (s, 2H, H5), 3.61 (br s, 2H, NH_2), 0.92 (s, 9H, $OSi(CH_3)_2C(CH_3)_3$), 0.08 (s, 6H, $OSi(CH_3)_2C(CH_3)_3$).

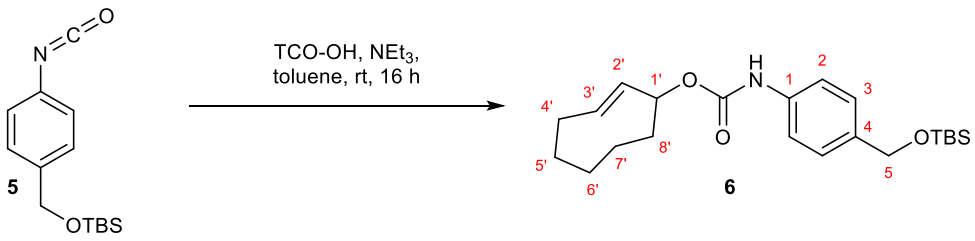
tert-butyl[(4-isocyanatophenyl)methoxy]dimethylsilane (5)



According to the procedure by Alaoui *et al.*,[6] amine **4** (50 mg, 0.211 mmol, 1.0 equiv.) was dissolved in toluene (5 mL). Triethylamine (0.03 mL, 0.232 mmol, 1.1 equiv.) and triphosgene (25 mg, 0.084 mmol, 0.4 equiv.) were added and the reaction mixture was heated to 70 °C for 3 h. The mixture was filtered, washed with toluene and concentrated *in vacuo* to give **5** as a colourless oil which was used directly in the next step. The NMR data were in accordance with reported data.[6]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 – 7.24 (m, 2H, H2/3), 7.05 (dd, *J* = 8.5, 2.0 Hz, 2H, H2/3), 4.71 (s, 2H, H5), 0.95 (d, *J* = 1.6 Hz, 9H, $OSi(CH_3)_2C(CH_3)_3$), 0.10 (d, *J* = 1.6 Hz, 6H, $OSi(CH_3)_2C(CH_3)_3$).

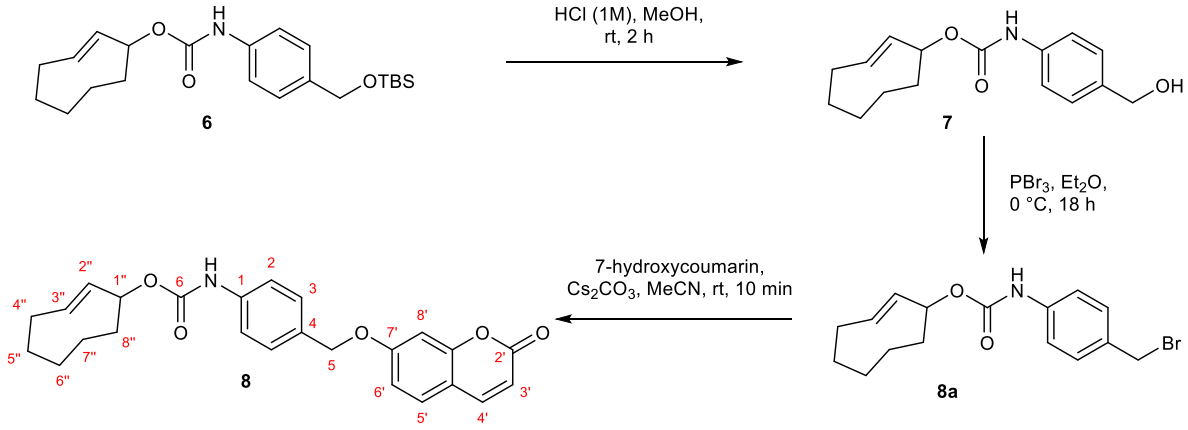
(2*E*)-cyclooct-2-en-1-yl N-(4-{[(tert-butyldimethylsilyl)oxy]methyl}phenyl)carbamate (6)



(2*E*)-Cyclooct-2-en-1-ol (axial isomer) (60 mg, 0.486 mmol, 1.0 equiv.) was dissolved in toluene (3 mL) and cooled to 0 °C. Triethylamine (0.07 mL, 0.535 mmol, 1.1 equiv.) was added followed by a solution of isocyanate **5** (166 mg, 0.632 mmol, 1.3 equiv.) in toluene (2 mL). The reaction was stirred at rt for 16 h then silica was added and all volatiles were removed *in vacuo*. Purification *via* flash column chromatography (2–10% EtOAc/Petrol) yielded **6** as a colourless oil (60 mg, 0.154 mmol, 24%).

R*f* 0.26 (10% EtOAc/Petrol). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.36 (d, *J* = 8.2 Hz, 2H, H2), 7.25 (s, 1H, H3 (masked by $CDCl_3$ peak)), 5.88 (td, *J* = 13.2, 11.1, 3.6 Hz, 1H, H3'), 5.57 (dd, *J* = 16.4, 2.5 Hz, 1H, H2'), 5.44 (s, 1H, H1'), 4.69 (s, 2H, H5), 2.52 – 2.42 (m, 1H, H4'), 2.20 – 2.10 (m, 1H, H8'), 2.09 – 1.95 (m, 2H, H4', H5'), 1.89 (dddt, *J* = 12.8, 8.9, 5.2, 1.7 Hz, 1H, H6'), 1.80 – 1.63 (m, 2H, H7', H8'), 1.53 – 1.43 (m, 1H, H5'), 1.12 (ddt, *J* = 14.7, 12.7, 8.0 Hz, 1H, H7'), 0.93 (s, 9H, $OSi(CH_3)_2C(CH_3)_3$), 0.89 – 0.77 (m, 1H, H6'), 0.09 (s, 6H, $OSi(CH_3)_2C(CH_3)_3$). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 152.7 (*C*=O), 136.7 (C4), 136.4 (C1), 132.1 (C3'), 131.0 (C2'), 126.9 (C3), 118.3 (C2), 74.4 (C1'), 64.7 (C5), 40.7 (C8'), 36.0 (C4'), 35.9 (C5'), 29.1 (C6'), 26.0 ($OSi(CH_3)_2C(CH_3)_3$), 24.2 (C7'), 18.4 ($OSi(CH_3)_2C(CH_3)_3$), -5.2 ($OSi(CH_3)_2C(CH_3)_3$). **HRMS (**ESI$^+$): *m/z* calc. for $C_{22}H_{35}O_3NSiNa$ [M+Na]+ 412.2278, found 412.2262, Δ –3.9 ppm.

(2''*E*)-cyclooct-2''-en-1''-yl N-(4-{[(2'-oxochromen-7'-yl)oxy]methyl}phenyl)carbamate (8) from route 1



According to the procedure by Hay *et al.*[7], **6** (60 mg, 0.154 mmol) was dissolved in MeOH (2 mL). HCl (1 M, 1 mL) was added and the reaction was stirred at rt for 1 h, until TLC showed complete consumption of the starting material. The reaction mixture was then poured into brine (5 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with water (10 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. ^{1}H NMR showed that the reaction was not complete, so the crude mixture was redissolved in MeOH (2 mL) and HCl (1 M, 1 mL), stirred at rt for 1 h and then worked up as described above to give **7** which was used crude in the following step assuming quantitative yield.

(2'*E*)-cyclooct-2'-en-1'-yl N-[4-(hydroxymethyl)phenyl]carbamate (7)

R*f* 0.11 (20% EtOAc/Petrol). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 8.0 Hz, 2H, H2), 7.33 – 7.28 (m, 2H, H3), 6.67 (s, 1H, N*H*), 5.88 (ddd, *J* = 15.8, 11.1, 3.7 Hz, 1H, H3'), 5.57 (dd, *J* = 16.4, 2.4 Hz, 1H, H2'), 5.44 (s, 1H, H1'), 4.64 (s, 2H, H5), 2.49 (dd, *J* = 10.5, 4.8 Hz, 1H, H4'), 2.14 (dd, *J* = 14.3, 5.2 Hz, 1H, H8'), 2.08 – 1.95 (m, 2H, H4', H5'), 1.95 – 1.83 (m, 1H, H6'), 1.81 – 1.63 (m, 2H, H7', H8'), 1.58 (d, *J* = 4.8 Hz, 1H, H5'), 1.10 (dd, *J* = 14.1, 6.1 Hz, 1H, H7'), 0.83 (ddd, *J* = 16.6, 9.8, 4.3 Hz, 1H, H6').

According to the procedure by Behnam *et al.*,[8] crude compound **7** was dissolved in Et_2O (5 mL) and cooled to 0 °C. PBr_3 (0.1 M in CH_2Cl_2, 0.12 mL, 0.8 equiv.) was added dropwise and the reaction was stirred at 0 °C for 18 h. The reaction mixture was then poured into $NaHCO_{3\,(aq)}$ (sat. soln., 10 mL) and extracted with Et_2O (3 x 10 mL). The combined organic layers were washed with water (20 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. 2 products were observed by TLC and ^{1}H NMR which correspond to the *cis*- and *trans*-isomers. Compound **8a** was used crude in the next step assuming quantitative yield.

(2'*E*)-cyclooct-2'-en-1'-yl N-[4-(bromomethyl)phenyl]carbamate (8a)

R_f 0.61, 0.7 (20% EtOAc/Petrol). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.42 – 7.30 (m, 4H), 6.69 (s, 0.4H, N*H trans*), 6.61 (s, 0.6H, N*H cis*), 5.93 – 5.82 (m, 0.3H), 5.74 – 5.61 (m, 0.6H), 5.62 – 5.57 (m, 0.2H), 5.57 – 5.48 (m, 0.6H), 5.44 (s, 0.3H), 4.48 (d, *J* = 1.6 Hz, 2H), 2.52 – 2.41 (m, 1H), 2.34 – 2.21 (m, 1H), 2.19 – 2.09 (m, 1H), 2.08 – 1.96 (m, 1H), 1.94 – 1.82 (m, 0.3H), 1.81 – 1.47 (m, 4H), 1.19 – 1.02 (m, 0.5H), 0.94 – 0.73 (m, 1H). NMR not assigned due to mixture of isomers.

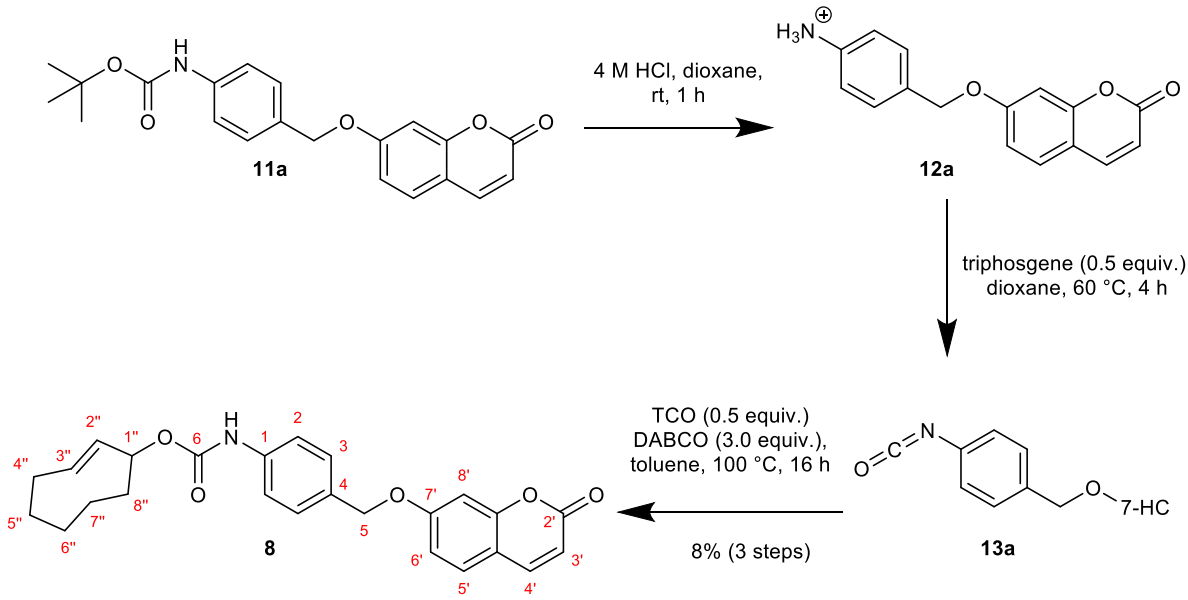
8a was dissolved in MeCN (2 mL) and added to a solution of 7-hydroxycoumarin **2** (19 mg, 0.116 mmol, 1.5 equiv.) and caesium carbonate (50 mg, 0.154 mmol, 2.0 equiv.) in MeCN (3 mL). The reaction mixture was stirred at rt for 10 min then the solvent was removed *in vacuo*. The residue was re-dissolved in CH_2Cl_2 (10 mL) and washed with water (5 mL), $NaHCO_{3\,(aq)}$ (sat. soln., 2 x 5 mL), water (5 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Purification *via* flash column chromatography (1% MeOH/ CH_2Cl_2) yielded impure product **8** as a white powder (3 mg) as a 3:7 mixture of *trans:cis*. The product was not pure and ^{1}H NMR is only shown to confirm the mixture of isomers. Full characterisation was obtained on the pure *trans*-isomer of **8** that was obtained during route 2 (see below).

(2''*E*)-cyclooct-2''-en-1''-yl N-(4-{[(2'-oxochromen-7'-yl)oxy]methyl}phenyl)carbamate (8)

R_f 0.43 (1% MeOH/ CH_2Cl_2). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 9.5 Hz, 1H, H4'), 7.44 (dd, *J* = 8.5, 6.8 Hz, 2H, H2), 7.36 (dt, *J* = 6.4, 2.3 Hz, 3H, H3, H5'), 6.92 – 6.84 (m, 2H, H6', H8'), 6.75 (s, 0.3H, N*H trans*), 6.65 (s, 0.7H, N*H cis*), 6.25 (d, *J* = 9.5 Hz, 1H, H3'), 5.88 (ddd, *J* = 15.8, 11.1, 3.7 Hz, 0.4H, H3'' *trans*), 5.71 (ddd, *J* = 10.9, 7.3, 1.8 Hz, 1.1H), 5.59 (t, *J* = 2.7 Hz, 0.2H), 5.53 (ddd, *J* = 10.9, 7.0, 1.3 Hz, 0.7H, H2'' or H3'' *cis*), 5.44 (s, 0.3H), 5.07 (d, *J* = 2.8 Hz, 2H, H5), 2.56 – 2.39 (m, 1H), 2.34 – 2.23 (m, 1H), 2.14 (ddt, *J* = 18.0, 7.7, 3.3 Hz, 1H), 2.02 (ddt, *J* = 14.6, 9.9, 4.8 Hz, 1H), 1.95 – 1.82 (m, 0.2H), 1.80 – 1.62 (m, 1H), 1.56 (q, *J* = 13.1, 11.8 Hz, 3H), 1.11 (td, *J* = 14.2, 6.0 Hz, 0.4H), 0.92 – 0.77 (m, 1H).

See figure S3 for a detailed image of the alkene region of the spectra.

(2''*E*)-cyclooct-2''-en-1''-yl N-(4-{[(2'-oxochromen-7'-yl)oxy]methyl}phenyl)carbamate (8) from route 2

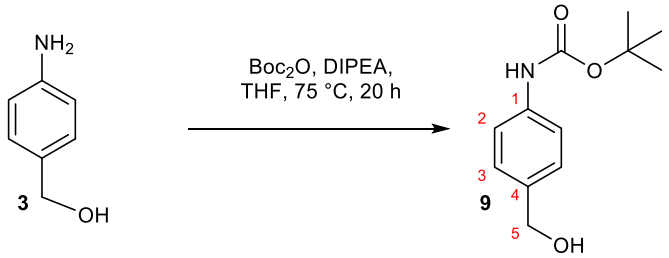


Boc protected aniline **11a** (183 mg, 0.499 mmol, 1.0 equiv.) was dissolved in 4 M HCl in dioxane (8 mL) and the reaction was stirred at rt for 1 h. The solvent was evaporated, then the residue was redissolved in dioxane (8 mL). Triphosgene (74 mg, 0.249 mmol, 0.5 equiv.) was added and the reaction was heated to 60 °C for 4 h before the mixture was concentrated *in vacuo*. The residue was dissolved in toluene (20 mL) before the addition of DABCO (168 mg, 1.50 mmol, 3.0 equiv.) followed by TCO-OH (31 mg, 0.250 mmol, 0.5 equiv.). The reaction was stirred at 100 °C for 16 h then cooled to rt. Purification *via* flash column chromatography (40% EtOAc/Petrol) then flash column chromatography (3% MeOH/ CH_2Cl_2) yielded **8** as a white solid (8 mg, 0.019 mmol, 8%).

R_f 0.40 (40% EtOAc/Petrol). **T_{decomp}** 139.7 °C. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.63 (dd, *J* = 9.5, 0.6 Hz, 1H, H4'), 7.45 (d, *J* = 8.3 Hz, 2H, H2), 7.39 – 7.34 (m, 3H, H3, H5'), 6.92 – 6.86 (m, 2H, H6', H8'), 6.75 – 6.70 (m, 1H, N*H*), 6.25 (d, *J* = 9.5 Hz, 1H, H3'), 5.88 (ddd, *J* = 15.8, 11.0, 3.6 Hz, 1H, H3''), 5.58 (dd, *J* = 16.4, 2.5 Hz, 1H, H2''), 5.44 (s, 1H, H1''), 5.07 (d, *J* = 3.5 Hz, 2H, H5), 2.49 (dd, *J* = 10.5, 5.1 Hz, 1H, H4''), 2.22 – 2.10 (m, 1H, H8''), 2.09 – 1.95 (m, 2H, H4'', H5''), 1.94 – 1.83 (m, 1H, H6''), 1.80 – 1.64 (m, 2H, H7'', H8''), 1.56 – 1.42 (m, 1H, H5''), 1.17 – 1.03 (m, 1H, H7''), 0.91 – 0.75 (m, 1H, H6''). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 162.0 (C7'), 161.3 (C2'), 156.0 (C8a'), 152.8 (C6), 143.5 (C4'), 138.3 (C1), 132.3 (C3''), 131.0 (C2''), 130.6 (C3), 128.9 (C5'), 128.8 (C4), 118.8 (C2), 113.4 (C6'), 113.4 (C3'), 112.9 (C4a'), 102.1 (C8'), 74.7 (C1''), 70.3 (C5), 40.8 (C8''), 36.2 (C4''), 36.1 (C5''), 29.2 (C6''), 24.4 (C7''). **IR (**thin film, **ν_{max}**/cm^{-1}) 2981w, 2924w,

2855w, 1723m, 1612m, 1528m, 1463w, 1350w, 1277m, 1227m, 1059m, 801m, 734m, 618s. **HRMS** (ESI$^+$): *m/z* calc. for $C_{25}H_{25}NO_5Na$ $[M+Na]^+$ 442.1625, found 442.1613, Δ −2.6 ppm.

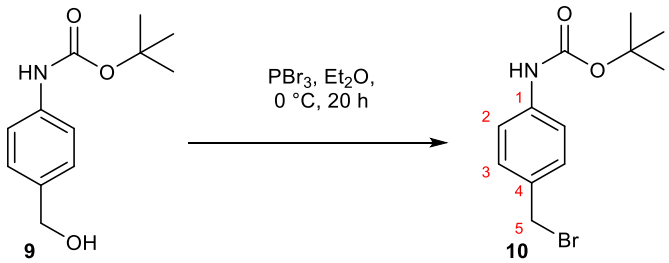
tert-butyl N-[4-(hydroxymethyl)phenyl]carbamate (9)



According to the procedure by Hong *et al.*[9], a solution of 4-aminobenzyl alcohol **3** (50 mg, 0.406 mmol, 1.0 equiv.), DIPEA (0.07 mL, 0.406 mmol, 1.0 equiv.) and Boc anhydride (89 mg, 0.408 mmol, 1.0 equiv.) in THF (4 mL) was heated to 75 °C for 20 h. The reaction was cooled to rt and the solvent was removed *in vacuo*. The residue was re-dissolved in EtOAc (10 mL), washed with HCl (0.1 M, 5 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Purification *via* flash column chromatography (50% EtOAc/Petrol) yielded **9** as white crystalline solid (87 mg, 0.390 mmol, 96%). The NMR data were in accordance with reported data.[9]

R_f 0.48 (50% EtOAc/Petrol). **T_{decomp}** 81.6–82.4 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.35 (d, *J* = 8.3 Hz, 2H, H2/3), 7.29 (d, *J* = 8.6 Hz, 2H, H2/3), 6.47 (s, 1H, N*H*), 4.63 (d, *J* = 5.2 Hz, 2H, H5), 1.52 (s, 7H, C(C*H*$_3$)$_3$).

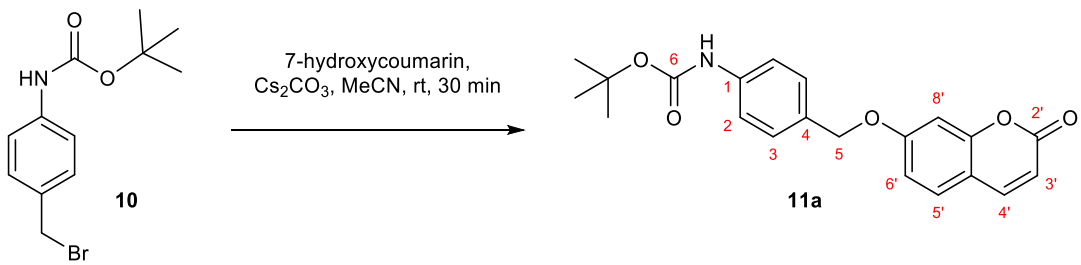
tert-butyl N-[4-(bromomethyl)phenyl]carbamate (10)



According to the procedure by Behnam *et al.*,[8] alcohol **9** (67 mg, 0.30 mmol, 1.0 equiv.) was dissolved in Et_2O (8 mL) and the solution was cooled to 0 °C. PBr_3 (1 M in CH_2Cl_2, 0.24 mL, 0.8 equiv.) was added dropwise and the reaction was stirred for 20 h at 0 °C. The reaction was poured into $NaHCO_{3\,(aq)}$ (sat. soln., 5 mL) and extracted with Et_2O (3 x 5 mL). The organic layers were washed with water (5 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. This gave **10** as a white solid which was sufficiently pure by ^{1}H NMR to proceed to the next step. The NMR data were in accordance with reported data.[8]

R_f 0.85 (30% EtOAc/Petrol). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.33 (d, *J* = 2.2 Hz, 4H, H2, H3), 6.49 (s, 1H, N*H*), 4.48 (d, *J* = 1.9 Hz, 2H, H5), 1.52 (d, *J* = 1.9 Hz, 9H, C(C*H*$_3$)$_3$).

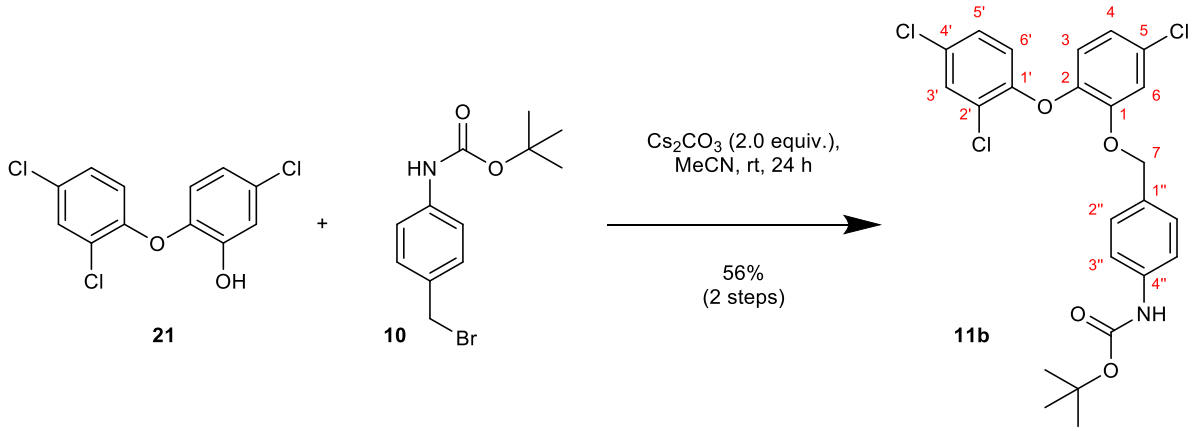
tert-butyl N-(4-{[(2'-oxochromen-7'-yl)oxy]methyl}phenyl)carbamate (11a)



A solution of bromide **10** (0.30 mmol, 1.0 equiv, assuming quantitative yield) in MeCN (3 mL) was added to a solution of 7-hydroxycoumarin **2** (73 mg, 0.45 mmol, 1.5 equiv.) and caesium carbonate (195 mg, 0.60 mmol, 2.0 equiv.) in MeCN (5 mL). The reaction was stirred at rt for 30 min then the solvent was removed *in vacuo*. The residue was re-dissolved in CH_2Cl_2 (10 mL), washed with water (3 x 10 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Purification *via* flash column chromatography (30% EtOAc/Petrol) yielded **11a** as a white solid (22 mg, 0.06 mmol, 20% over 2 steps).

R_f 0.28 (30% EtOAc/Petrol). **Mp** 187.5–188.3 °C. **^{1}H NMR** (500 MHz, $CDCl_3$) 7.63 (d, *J* = 9.5 Hz, 1H, H4'), 7.42 – 7.33 (m, 5H, H2, H3, H5'), 6.93 – 6.82 (m, 2H, H6', H8'), 6.50 (s, 1H, N*H*), 6.25 (d, *J* = 9.5 Hz, 1H, H3'), 5.07 (s, 2H, H5), 1.52 (s, 9H, C(C*H*$_3$)$_3$). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 161.9 (C7'), 161.2 (C2'), 155.8 (C8a'), 152.6 (C6), 143.4 (C4'), 138.6 (C1), 130.1 (C4), 128.7 (C5'), 128.6 (C3), 118.6 (C2), 113.3 (C6'), 113.2 (C3'), 112.7 (C4a'), 101.9 (C8'), 80.8 (*C*(CH$_3$)$_3$), 70.2 (C5), 28.3 (C(*C*H$_3$)$_3$). **IR (**thin film, ν_{max}/cm^{-1}) 3336m, 2987w, 1731m, 1699s, 1614s, 1598m, 1527s, 1453w, 1402m, 1385m, 1348m, 1315m, 1273m, 1228s, 1153s, 1002s, 815s. **HRMS (**ESI$^+$): *m/z* calc. for $C_{21}H_{21}NO_5Na$ [M+Na]$^+$ 390.1312, found 390.1302, Δ –2.7 ppm.

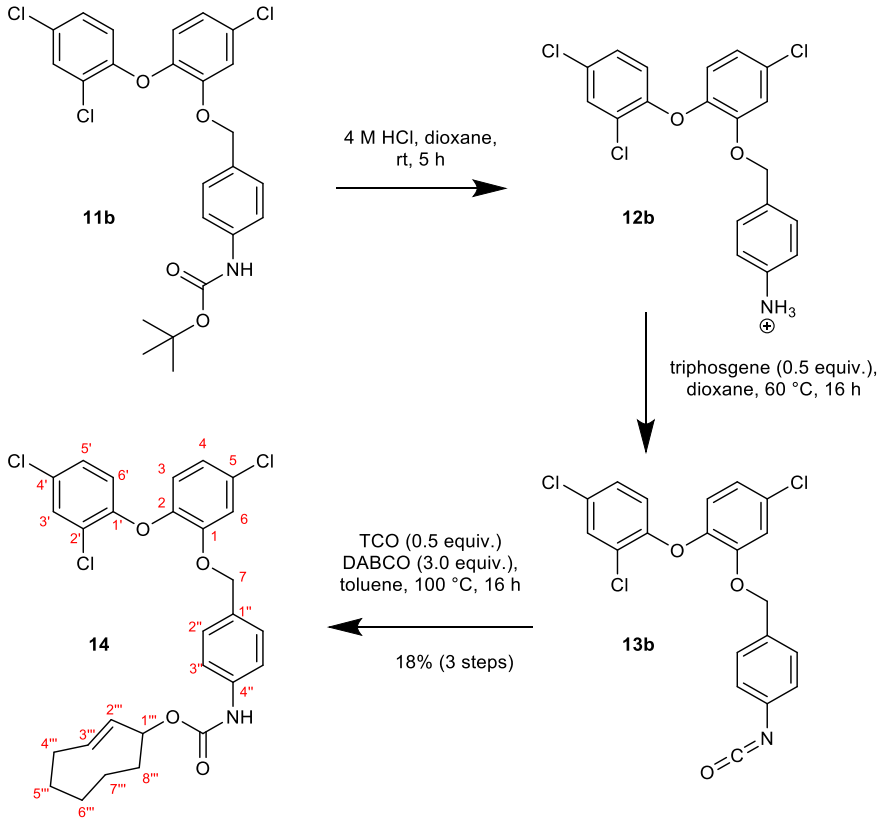
tert-butyl N-{4''-[5-chloro-2-(2',4'-dichlorophenoxy)phenoxymethyl]phenyl}carbamate (11b)



A solution of bromide **10** (0.560 mmol, 1.0 equiv, assuming quantitative yield) in MeCN (3 mL) was added to a solution of triclosan **21** (178 mg, 0.616 mmol, 1.1 equiv.) and caesium carbonate (365 mg, 1.12 mmol, 2.0 equiv.) in MeCN (2 mL). The reaction was stirred at rt for 24 min then the solvent was removed *in vacuo*. The residue was re-dissolved in CH_2Cl_2 (10 mL), washed with water (3 x 10 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Purification *via* flash column chromatography (10% EtOAc/Petrol) yielded **11b** as a white solid (176 mg, 0.356 mmol, 63%).

R_f 0.18 (10% EtOAc/Petrol). **Mp** 142.3–144.1 °C. **1H NMR** (400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 2.5 Hz, 1H, H3'), 7.30 (d, *J* = 8.4 Hz, 2H, H3''), 7.11 (d, *J* = 8.5 Hz, 2H, H2''), 7.09 – 7.06 (m, 1H, H5'), 7.01 (d, *J* = 1.8 Hz, 1H, H6), 6.94 – 6.92 (m, 2H, H3, H4), 6.64 (d, *J* = 8.8 Hz, 1H, H6'), 6.51 (s, 1H, N*H*), 4.98 (s, 2H, H7), 1.52 (s, 9H, C(C*H*$_3$)$_3$). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 152.8 (*C*=O), 152.5 (C1'), 150.5 (C1), 143.6 (C2'), 138.4 (C1''), 130.6 (C5), 130.4 (C4'), 130.3 (C3'), 128.2 (C2''), 128.1 (C4''), 127.7 (C5'), 124.7 (C2'), 122.2 (C3), 121.6 (C4), 118.5 (C3''), 118.2 (C6'), 115.9 (C6), 80.8 (*C*(CH$_3$)$_3$), 70.9 (C7), 28.5 (C(*C*H$_3$)$_3$). **IR (**thin film, ν_{max}/cm^{-1}) 3360m, 1695s, 1599m, 1528s, 1498s, 1471s, 1406m, 1308m, 1366m, 1312m, 1270m, 1227s, 1163s, 1056s, 910m, 841s. **HRMS (**ESI$^+$): *m/z* calc. for $C_{24}H_{22}NO_4{}^{35}Cl_3Na$ [M+Na]$^+$ 516.0507, found 516.0507,Δ 0.1 ppm.

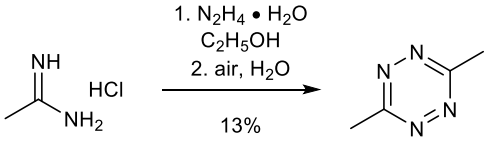
(2'''*E*)-cyclooct-2'''-en-1-yl N-{4''-[5-chloro-2-(2',4'-dichlorophenoxy)phenoxymethyl]phenyl}carbamate (14)



Boc protected aniline **11b** (150 mg, 0.303 mmol, 1.0 equiv.) was dissolved in 4 M HCl in dioxane (5 mL) and the reaction was stirred at rt for 15 h. The solvent was evaporated then the residue was redissolved in dioxane (4 mL). Triphosgene (45 mg, 0.152 mmol, 0.5 equiv.) was added and the reaction was heated to 60 °C for 5 h. The mixture was concentrated *in vacuo*. The residue was dissolved in toluene (5 mL) then DABCO (102 mg, 0.909 mmol, 3.0 equiv.) was added followed by TCO-OH (42 mg, 0.333 mmol, 1.1 equiv.) and the reaction was stirred at 100 °C for 19 h then cooled to rt. Purification *via* flash column chromatography (5% EtOAc/Petrol) then preparative TLC (70% CH_2Cl_2/petrol) yielded **14** as a white solid (9.7 mg, 0.0177 mmol, 18%).

R_f 0.45 (70% CH_2Cl_2/Petrol). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.41 (dd, *J* = 2.6, 0.6 Hz, 1H, H3'), 7.35 (d, *J* = 8.2 Hz, 2H, H3''), 7.12 (d, *J* = 8.4 Hz, 2H, H2''), 7.08 (ddd, *J* = 8.8, 2.5, 0.6 Hz, 1H, H5'), 7.01 (dd, *J* = 1.9, 0.9 Hz, 1H, H6), 6.94 (dd, *J* = 2.6, 0.7 Hz, 2H, H3, H4), 6.72 – 6.61 (m, 2H, H6', N*H*), 5.87 (ddd, *J* = 15.7, 11.0, 3.7 Hz, 1H, H3'''), 5.57 (dd, *J* = 16.4, 2.4 Hz, 1H, H2'''), 5.43 (s, 1H, H1'''), 4.99 (s, 2H, H7), 2.48 (td, *J* = 8.8, 7.3, 4.0 Hz, 1H, H4'''), 2.19 – 2.10 (m, 1H, H8'''), 2.07 – 1.94 (m, 2H, H4''', H5'''), 1.94 – 1.83 (m, 1H, H6'''), 1.80 – 1.62 (m, 2H, H7''', H8'''), 1.48 (td, *J* = 12.6, 4.3 Hz, 1H, H5'''), 1.17 – 1.04 (m, 1H, H7'''), 0.91 – 0.76 (m, 1H, H6'''). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 152.6 (*C*=O), 152.4 (C1'), 150.4 (C1), 143.5 (C2'), 137.9 (C1''), 132.1 (C3'''), 130.9 (C2'''), 130.6 (C5), 130.4 (C4'), 130.2 (C3'), 128.1 (C2''), 128.0 (C4''), 127.6 (C5'), 124.6 (C2'), 122.0 (C3), 121.5 (C4), 118.5 (C3''), 118.0 (C6'), 115.8 (C6), 74.6 (C1'''), 70.8 (C7), 40.7 (C8'''), 36.0 (C4'''), 35.9 (C5'''), 29.1 (C6'''), 24.2 (C7'''). **IR (**thin film, ν_{max}/cm^{-1}) 2922m, 2853w, 1711m, 1599m, 1527m, 1494s, 1474s, 1407m, 1317m, 1227s, 1204m, 1075m. **HRMS (**ESI): *m/z* calc. for $C_{28}H_{26}N_1O_4{}^{35}Cl_3Na$ $[M+Na]^+$ 568.0820, found 568.0810, Δ -1.7 ppm.

dimethyl-1,2,4,5-tetrazine (15)



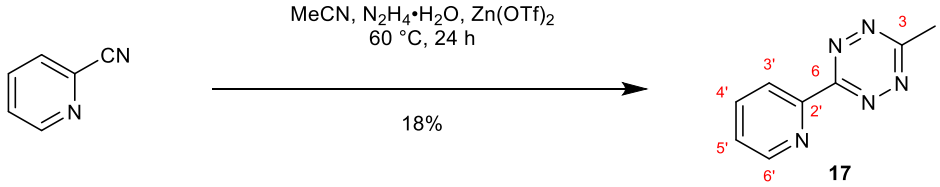
15

Acetamidine hydrochloride (2 g, 21.2 mmol, 1.0 equiv.) was dissolved in EtOH (10 mL) and $N_2H_4{\cdot}H_2O$ (4 mL, 4.0 equiv.) was added under argon. The mixture was allowed to stir at room temperature overnight. The solution was then diluted with H_2O (30 mL) and stirred vigorously in air. The aqueous layer was then extracted with CH_2Cl_2 until no pink colour remained. The organic layers were combined, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Purification *via* flash column chromatography (100% Et_2O) yielded **15** as a dark red solid (308mg, 2.80 mmol, 13%). The NMR data were in accordance with literature data.[10]

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.02 (s, 6H, CH_3).

bis(pyridin-2-yl)-1,2,4,5-tetrazine (16)
This reagent was purchased from Fluorochem.

3-Methyl-6-(pyridin-2'-yl)-1,2,4,5-tetrazine (17)

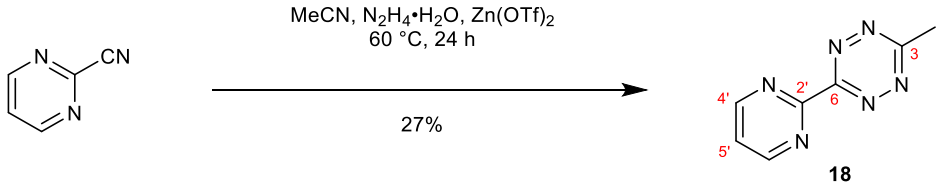


17

According to the procedure by Fan *et al.*,[11] 2-pyridinecarbonitrile (0.104 g, 1.0 mmol, 1.0 equiv.), MeCN (0.26 mL, 5.0 mmol, 5.0 equiv.), zinc trifluoromethane sulfonate (18 mg, 0.05 mmol, 5 mol%) and hydrazine monohydrate (0.24 mL, 5.0 mmol, 5.0 equiv.) were added to a Schlenk tube under a positive argon gas stream. The reaction mixture was stirred at 60 °C for 24 h under argon. The reaction was cooled to rt then sodium nitrite $_{(aq)}$ (1 M, 10 mL) was added. HCl (1 M) was added until the reaction mixture was pH 3. The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (10% acetone/CH_2Cl_2) yielded **17** as a pink solid (31 mg, 0.177 mmol, 18%). The NMR data were in accordance with literature data.[11]

Mp 92.2–96.4 °C.**^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.95 (ddd, *J* = 4.8, 1.8, 0.9 Hz, 1H, H6'), 8.64 (dt, *J* = 7.9, 1.1 Hz, 1H, H3'), 7.98 (td, *J* = 7.8, 1.7 Hz, 1H, H4'), 7.55 (ddd, *J* = 7.7, 4.8, 1.2 Hz, 1H, H5'), 3.16 (d, *J* = 0.8 Hz, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 168.1 (C3), 163.6 (C6), 150.9 (C6'), 150.3 (C2'), 137.4 (C4'), 126.3 (C5'), 123.9 (C3'), 21.4 (CH_3). **HRMS** (ESI^+): *m/z* calc. for $C_8H_7N_5$ $[M+H]^+$ 174.0774, found 174.0767, Δ -4.0 ppm.

3-Methyl-6-(pyrimidin-2'-yl)-1,2,4,5-tetrazine (18)



18

According to the procedure by Fan *et al.*,[11] 2-pyrimidinecarbonitrile (0.53 g, 5.0 mmol, 1.0 equiv.), MeCN (1.30 mL, 25 mmol, 5.0 equiv.), zinc trifluoromethane-sulfonate (91 mg, 0.25 mmol, 5 mol%) and hydrazine monohydrate (1.20 mL, 25 mmol, 5.0 equiv.) were added to a Schlenk tube under a positive argon gas stream. The reaction mixture was stirred at 60 °C for 24 h under argon. The reaction was cooled to rt then sodium nitrite $_{(aq)}$ (1 M, 50 mL) was added. HCl (1 M) was added until the reaction mixture was pH 3. The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash column chromatography (2–4% MeOH/CH_2Cl_2) followed by another flash column chromatography (10–16% acetone/CH_2Cl_2) yielded **18** as a pink solid (0.237 g, 1.36 mmol, 27%). The NMR data were in accordance with literature data.[11]

Mp 97.3–101.4 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.10 (d, *J* = 4.9 Hz, 2H, H4'), 7.57 (td, *J* = 4.8, 1.0 Hz, 1H, H5'), 3.20 (d, *J* = 1.0 Hz, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 168.7 (C3), 163.3 (C6), 159.6 (C2'), 158.4 (C4'), 122.5 (C5'), 21.5 (CH_3). **HRMS** (ESI^+): *m/z* calc. for $C_7H_6N_6$ $[M+H]^+$ 175.0727, found 175.0719, Δ -4.7 ppm.

1-[4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl]methanamine (19)
This reagent was purchased from SiChem (Sirius fine chemicals) as the formate salt.

4-(1,2,4,5-tetrazin-3-yl)benzoic acid (20)
This reagent was synthesized according to the previous report.[12]

1.2 Stability Studies

TCO-carbonate 1 in 50% H_2O/DMSO: A solution of **1** (100 μM, 50% H_2O/DMSO) was added to a 96 well plate (Greiner, black, clear bottomed) in technical triplets. The fluorescence intensity was measured over 2 h at 37 °C, at the same time as measuring the fluorescence intensity of the reaction of **1** with tetrazine. Readings were taken at 8 second intervals using a SpectraMax i3x plate reader (λ_{ex} = 325 nm, λ_{em} = 460 nm). This assay was repeated two independent times. Data were processed using GraphPad Prism (GraphPad Software, La Jolla, CA, USA).

TCO-carbonate 1 in PBS, cell media and plasma: 5 μL of **1** in DMSO (1 mM) was added to 95 μL PBS, DMEM cell culture media or 20% plasma in PBS in a 96 well plate (Greiner, black, clear bottomed) in technical triplets. The fluorescence intensity was measured over 2 h at 37 °C. Readings were taken at 8 second intervals using a SpectraMax i3x plate reader (λ_{ex} = 325 nm, λ_{em} = 460 nm). This assay was repeated two independent times. Data were processed and fitted to a one phase association using GraphPad Prism (GraphPad Software, La Jolla, CA, USA).

***TCO-carbamate benzyl ether 8 in PBS, cell media and plasma*:** 10 μL of **8** in DMSO (0.5 mM) was added to 90 μL PBS, DMEM cell culture media, 20% plasma in H_2O or LB media in a 96 well plate (Greiner, black, clear bottomed) in technical triplets. The fluorescence intensity was measured over 15 h at 37 °C. Readings were taken at 30 second intervals using a SpectraMax i3x plate reader (λ_{ex} = 325 nm, λ_{em} = 460 nm). This assay was repeated two independent times. Data were processed using GraphPad Prism (GraphPad Software, La Jolla, CA, USA).

***Tetrazines 15–20*:** 60 μL of tetrazines **15**–**20** (2 mM, 50% DMSO/H_2O) was added to a 96 well plate (Greiner, black, clear bottomed) in technical triplicates and the absorbance was monitored over 24 h at 37 °C. For tetrazine **20**, which was not completely soluble at this concentration, the stock solution was centrifuged and the supernatant was used for the experiment. Readings were taken every minute using a SpectraMax i3x plate reader (λ_{max} = 530 nm). This assay was repeated two independent times. Data were processed and fitted to a one phase decay using GraphPad Prism (GraphPad Software, La Jolla, CA, USA).

1.3 Decaging Studies

Preparation and use of external NMR standard to determine accurate concentrations of stock solutions: A 100 mM solution of benzoic acid in DMSO was prepared in a volumetric flask. A sample of this solution was diluted in D_6-DMSO to give a 10 mM solution which was then sealed inside a capillary tube. This external standard was then calibrated against stock solutions of 7-hydroxycoumarin at known concentrations (26.5–5.4 mM prepared by accurate dilution based on solvent weight). 7-hydroxycoumarin was chosen as the second reference compound as it contains peaks in the aromatic region of the ^{1}H NMR spectrum that do not overlap with the peaks from benzoic acid. Briefly, the coumarin sample and the capillary containing the external standard were placed in a medium-walled NMR tube (Wilmad, Precision, 400 MHz). The ^{1}H NMR spectra was recorded twice for each sample and the integration ratio of coumarin:benzoic acid was plotted against concentration. Importantly the relationship between concentration and integration ratio was shown to be solvent dependent. See figure S5.

The capillary was then added to NMR tubes containing stock solutions of compounds and the exact concentration of these stock solutions was determined by measuring the ratio of integration (compound:benzoic acid standard) and converting it to concentration using the fitting on the appropriate graph.

Decaging of 1 by monitoring the increase in fluorescence: Rate constants were determined under second order conditions. Stock solutions of **1** and **18** were prepared and the exact concentrations were determined by qNMR. Working solutions (200 μM in 50% H_2O/DMSO) were prepared by dilution. 40 μL these solutions were mixed together in a 96 well plate (Greiner, black, clear bottomed) in technical triplets to give a final reactant concentration of 100 μM. The fluorescence intensity was measured over 2 h at 37 °C with readings taken at 8 second intervals using a SpectraMax i3x plate reader (λ_{ex} = 325 nm, λ_{em} = 460 nm). An adhesive film (Bio-Rad) was used to prevent solvent evaporation. This assay was repeated two independent times. Data were processed and fitted to a one phase association using GraphPad Prism (GraphPad Software, La Jolla, CA, USA).

Decaging of 8 by monitoring the increase in fluorescence: Rate constants were determined under second order conditions. Stock solutions of **8** and **20** were prepared and the exact concentrations were determined by qNMR. Working solutions (200 μM in 50% H_2O/DMSO) were prepared by dilution. 40 μL these solutions were mixed together in a 96 well plate (Greiner, black, clear bottomed) in technical triplets to give a final reactant concentration of 100 μM. The fluorescence intensity was measured over 5 h at 37 °C with readings taken at 8 second intervals using a SpectraMax i3x plate reader (λ_{ex} = 325 nm, λ_{em} = 460 nm). An adhesive film (Bio-Rad) was used to prevent solvent evaporation. This assay was repeated two independent times. Data were processed and fitted to a one phase association using GraphPad Prism (GraphPad Software, La Jolla, CA, USA).

Quenching effect of the reaction mixture on the coumarin fluorescence: TCO-coumarin **8** and tetrazine **20** were mixed to give a final concentration of 100 µM in 50% H_2O/DMSO for both reagents. The reaction was incubated at 37 °C for 6 h. At various timepoints (0.5, 1, 2, 4, 6 h) an aliquot was taken and added to a solution of coumarin to give a final concentration of 50 µM in 50% H_2O/DMSO for coumarin **2**, TCO-coumarin **8** and tetrazine **20**. The fluorescent intensity was measured and compared to that of a sample containing only coumarin **2** (50 µM, 50% H_2O/DMSO). Readings were taken using a SpectraMax i3x plate reader (λ_{ex} = 325 nm, λ_{em} = 460 nm) and the assay was repeated 2 independent times. Data were processed using GraphPad Prism (GraphPad Software, La Jolla, CA, USA).

Yield of reaction of TCO-coumarin 8 with tetrazines 15–18 by HPLC: TCO-coumarin **8**, tetrazines **15**–**18** and the internal standard benzoic acid were mixed to give final reactant concentrations of 0.83 mM (**8** and **15**–**18**) and 0.67 mM (benzoic acid standard) in 50% H_2O/DMSO. The sample mixtures were incubated at 37 °C and analysed after 24 h. The HPLC/UV analysis was performed on a Dionex UltiMate 3000 UHPLC+ system (Thermo Scientific) with a Phenomenex Kinetex C18 Column (100 Å 5 µm, 50 mm x 4.6 mm). The mobile phase consisted of A: H_2O with 0.1% formic acid, and B: MeCN with 0.1% formic acid. The samples were eluted with a linear gradient (1–10 min 0–50% B, and 10–14 min 50–100% B, 0.50 mL/min) and detected at 220 nm.

Yield of reaction of TCO-coumarin 8 with tetrazine 19 by HPLC: TCO-coumarin **8**, tetrazine **19** and the internal standard benzoic acid were mixed to give final reactant concentrations of 0.83 mM (**8** and **19**) and 0.67 mM (benzoic acid standard)) in 50% H_2O/DMSO. The sample mixtures were incubated at 37 °C and analysed after 24 h. The HPLC/UV analysis was performed on a Dionex UltiMate 3000 UHPLC+ system (Thermo Scientific) with a Phenomenex Kinetex C18 Column (100 Å 5 µm, 50 mm x 4.6 mm). The mobile phase consisted of A: H_2O with 0.1% formic acid, and B: MeCN with 0.1% formic acid. The samples were eluted with a linear gradient (1–5 min 0–100% B, 0.80 mL/min) and detected at 220 nm.

Yield of reaction of TCO-coumarin 8 with tetrazine 20 by HPLC: TCO-coumarin **8**, tetrazine **20** and the internal standard benzoic acid were mixed to give final reactant concentrations of 0.83 mM (**8** and **20**) and 0.67 mM (benzoic acid standard)) in the appropriate solvent system. The conditons pH 9, 7.4, 6, 5.6, 4 and LB media refer to 50% DMSO/ corresponding buffer (NaPi pH 9, PBS pH 7.4, NaPi pH 6, acetate pH 5.6, acetate pH 4 and LB media). A minimum of 50% DMSO was required for solubility of the reactants. The sample mixtures were incubated at 37 °C and analysed after 24 h. The HPLC/UV analysis was performed on a Dionex UltiMate 3000 UHPLC+ system (Thermo Scientific) with a Phenomenex Kinetex C18 Column (100 Å 5 µm, 50 mm x 4.6 mm). The mobile phase consisted of A: H_2O with 0.1% formic acid, and B: MeCN with 0.1% formic acid. The samples were eluted with a linear gradient (1–5 min 0–50% B and 5–8 min 50–100% B, 0.80 mL/min) and detected at 220 nm.

Following reaction of TCO-coumarin 8 with tetrazine 20 by HPLC/FLD: TCO-coumarin **8** in DMSO (23 µL, 11.4 mM), tetrazine **20** in DMSO (23 µL, 11.4 mM) and the internal standard benzoic acid in MeCN (80 µL, 2 mM) were mixed to give final reactant concentrations of 0.83 mM (**8** and **20**) and 0.67 mM (benzoic acid standard). The reaction mixture was incubated at 37 °C and an aliquot was taken for analysis at 5 min, 1, 2, 3, 4 and 24 h. The HPLC/UV-FLD analysis was performed on a Dionex UltiMate 3000 UHPLC+ system (Thermo Scientific) coupled to a Dionex UltiMate 3000 fluorescence detector with a Phenomenex Kinetex C18 Column (100 Å 5 µm, 50 mm x 4.6 mm). The mobile phase consisted of A: H_2O with 0.1% formic acid, and B: MeCN with 0.1% formic acid. The samples were eluted with a linear gradient (1–5 min 0–50% B and 5–8 min 50–100% B, 0.80 mL/min) and detected at λ_{ex} = 325 nm, λ_{em} = 460 nm.

Second order rate constant of reaction of TCO-coumarin 8 with tetrazine 20: The second order rate constant was determined under second order conditions using stopped flow spectrometry. Stock solutions of **8** and **20** were prepared and the exact concentrations were determined by qNMR. Working solutions (1 mM in DMSO) were prepared by dilution. The rate was calculated in 100% DMSO due to the poor solubility of **8** at this concentration. Both reactant solutions were injected simultaneously in a 1:1 ratio into the reaction vessel to give a final concentration of 0.5 mM. The decrease in absorbance at 530 nm was monitored for 6 minutes at 25 °C. A calibration curve was prepared for concentrations of tetrazine **20** of 0.15–1 mM and the absorbance values were converted into concentrations. The curve of concentration *vs* time was fitted to a two phase decay using GraphPad Prism (GraphPad Software, La Jolla, CA, USA). This experiment was repeated two independent times.

Decaging yield of TCO-triclosan 14 by HPLC: TCO-triclosan **14**, tetrazine **20** and the internal standard benzoic acid were mixed to give final reactant concentrations of 0.83 mM (**14** and **20**) and 0.67 mM (benzoic acid standard) in 20% DMSO/MeCN. The reaction mixture was incubated at 37 °C and an aliquot was taken for analysis at 0.5, 1, 2, 4 and 24 h. The HPLC/UV analysis was performed on a Dionex UltiMate 3000 UHPLC+ system (Thermo Scientific) with a Phenomenex Kinetex C18 Column (100 Å 5 µm, 50 mm x 4.6 mm). The mobile phase consisted of A: H_2O with 0.1% formic acid, and B: MeCN with 0.1% formic acid. The samples were eluted with a linear gradient (1–26 min 0–50% B, 26–29 min 50–70% B and 29–32 min 70–100% B, 0.80 mL/min) and detected at 220 nm.

Decaging of 14 by LC-MS: TCO-triclosan **14** and tetrazine **20** were mixed to give final reactant concentrations of 0.83 mM in 20% DMSO/MeCN. The reaction mixture was incubated at 37 °C and analysed after 24 h. The LC-MS analysis was performed on a Waters SQ Detector 2 with a Waters Acquity UPLC H-Class system and a Waters Acquity UPLC BEH C18 column (1.7µm, 2.1 x 50 mm). The mobile phase consisted of A: H_2O with 0.1% formic acid, and B: MeCN with 0.1% formic acid. The samples were eluted with a linear gradient (0–20 min 0–100% B, 0.20 mL/min).

1.4 Live cell studies

Cell Culture: *E. Coli* BL21(DE3) cells (ThermoFisher Scientific, USA) were cultured in Luria Bertani (LB) broth (MP Biomedicals). Overnight cultures were prepared by inoculating 20 mL of LB broth and incubating at 37 °C, shaken at 180 rpm.

Cytotoxicity in E. Coli BL21(DE3) cells: Cytotoxicity of triclosan **21**, TCO-triclosan **14**, tetrazine **20**, TCO-triclosan **14** + **20** was assessed using a CellTiter-Blue Cell Viability Assay (ThermoFisher Scientific, USA). Overnight cultures were diluted and added to flat-bottom 96-well plates to give a density of 0.002 OD_{600} units (200 µL). Compounds (dissolved in DMSO) were added to wells in 6 replicates to give final concentrations of 0.05 µM, 0.1 µM, 0.2 µM, 0.4 µM, 0.6 µM, 1.0 µM for **21** and **14** and 0.5 µM, 1 µM, 2 µM, 4 µM, 6 µM, 10 µM for tetrazine **20**. Final concentration of DMSO in each well was ≤ 1%. The plates were incubated at 37 °C for 12 h. 100 µL from each well was transferred to a new plate, for use in the cell density assay. Cell viability was assessed by the addition of 20 µL CellTiter-Blue Reagent to the remaining 100 µL in the initial plate. After incubation for a further 2 h without shaking, the fluorescence was measured on a SpectraMax i3x plate reader ($\lambda_{ex.}$ = 570 nm, $\lambda_{em.}$ = 590 nm).

Cell density assay in E. Coli BL21(DE3) cells: Cytotoxicity of triclosan **21**, TCO-triclosan **14**, tetrazine **20**, TCO-triclosan **14** + **20** was assessed by measuring the OD_{600}. Overnight cultures were diluted and added to flat-bottom 96-well plates to give a density of 0.002 OD_{600} units (200 µL). Compounds (dissolved in DMSO) were added to wells in 6 replicates to give a final concentration of 1 µM for **21** and **14** and 10 µM for tetrazine **20**. Final concentration of DMSO in each well was ≤ 1%. The plates were incubated at 37 °C for 12 h. 100 µL from each well was transferred to a new plate and the absorbance was measured on a SpectraMax i3x plate reader ($\lambda_{.}$ = 600 nm). 100 µL LB broth was then added to each well, to generate a 2x dilution and the absorbance was measured again.

2. Results and Discussion

2.1 Kinetic and Stability Studies

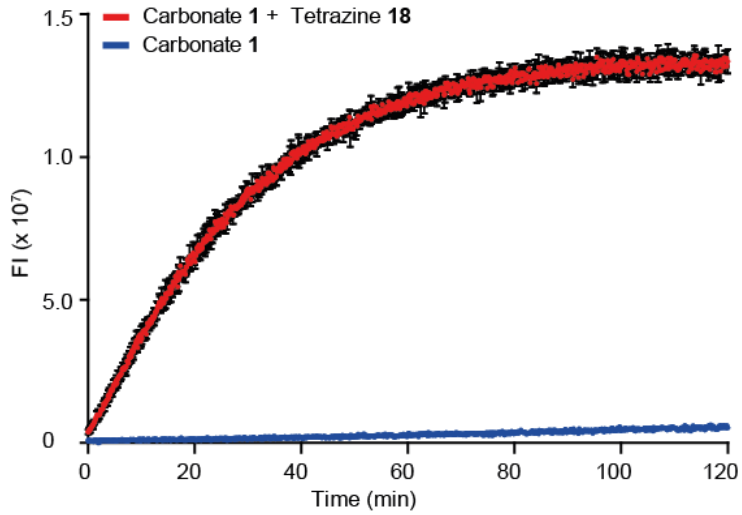


Figure S1: Stability of carbonate **1** in 50% H2O/DMSO monitored by following the increase in fluorescence (λ_{ex} = 320, λ_{em} = 465 nm) at the same time as the decaging reaction of **1** + **18**.

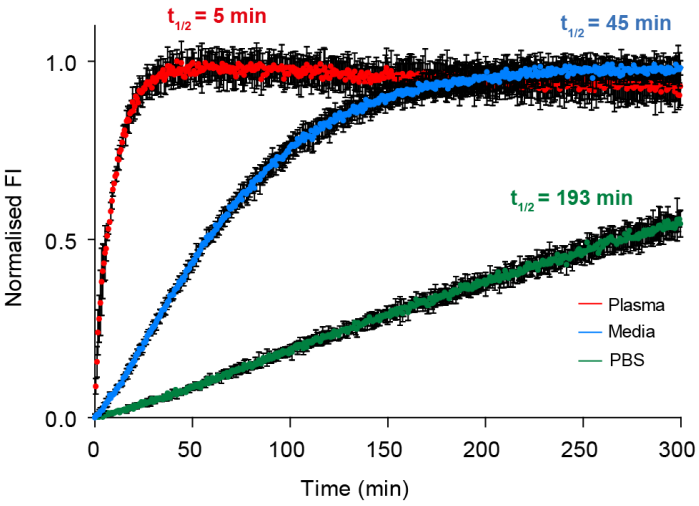


Figure S2: Stability of carbonate **1** in PBS, complete cell media (DMEM) and 20% plasma/PBS, followed by the increase in fluorescence (λ_{ex} = 320, λ_{em} = 465 nm). Data points and error bars shown.

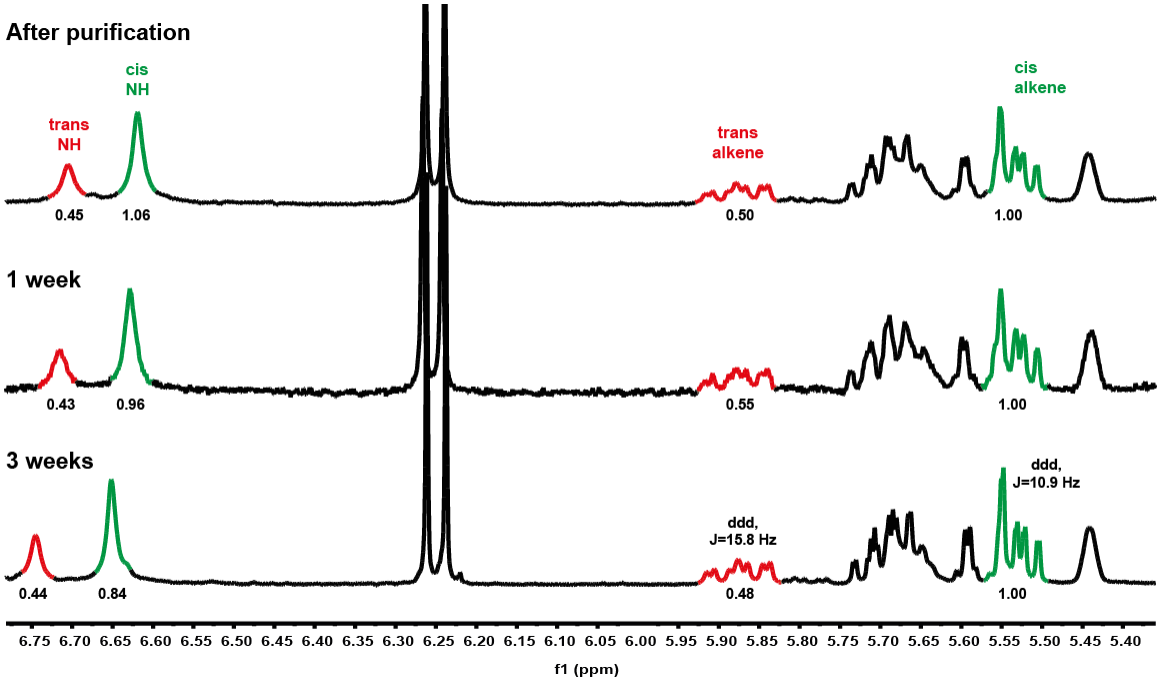


Figure S3: Alkene region of the ^{1}H NMR spectra of product **8** obtained from route 1 as a 7:3 mixture of *cis*:*trans*-isomers. The NMR sample was left in $CDCl_3$ in the light at rt and no further isomerisation occurred after 3 weeks.

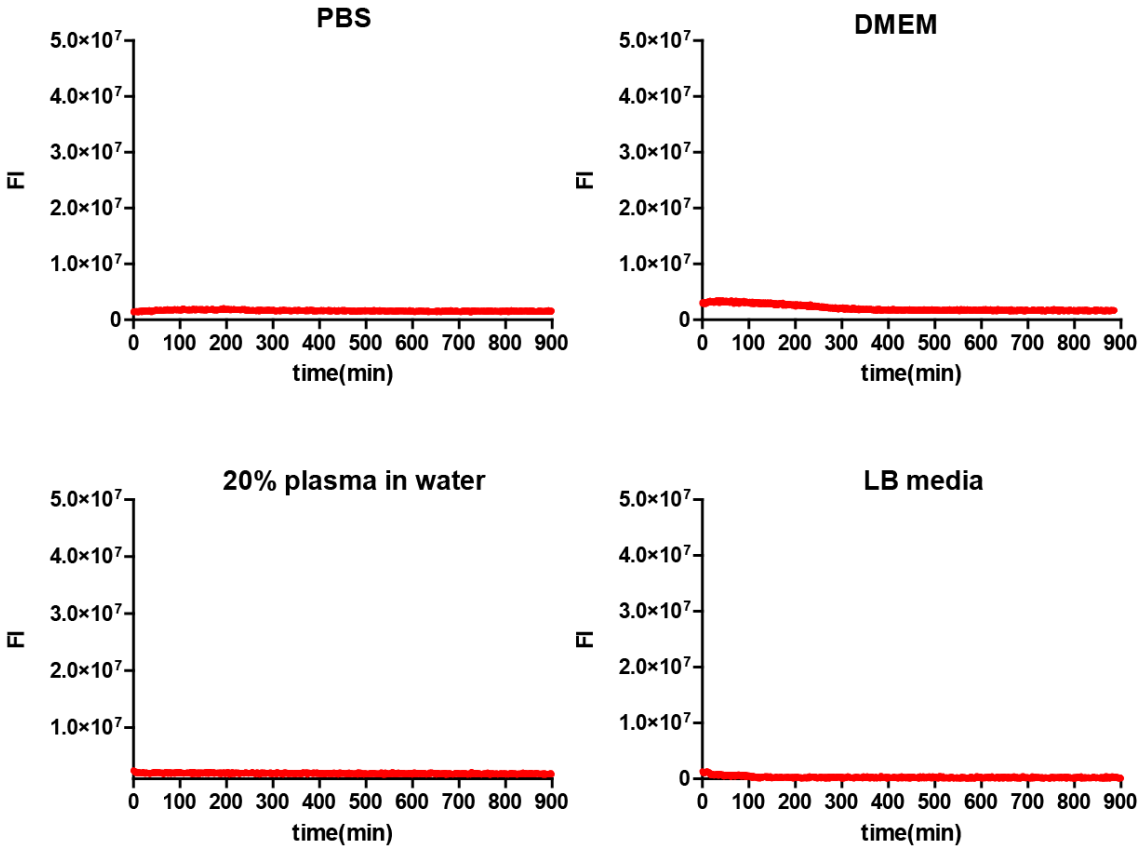


Figure S4: Stability of TCO-carbamate benzyl ether **8** in PBS, complete cell culture media (DMEM), LB media and 20% plasma in H_2O. Assessed by monitoring the fluorescence intensity (λ_{ex} = 325 nm, λ_{em} = 460 nm) over 15 h.

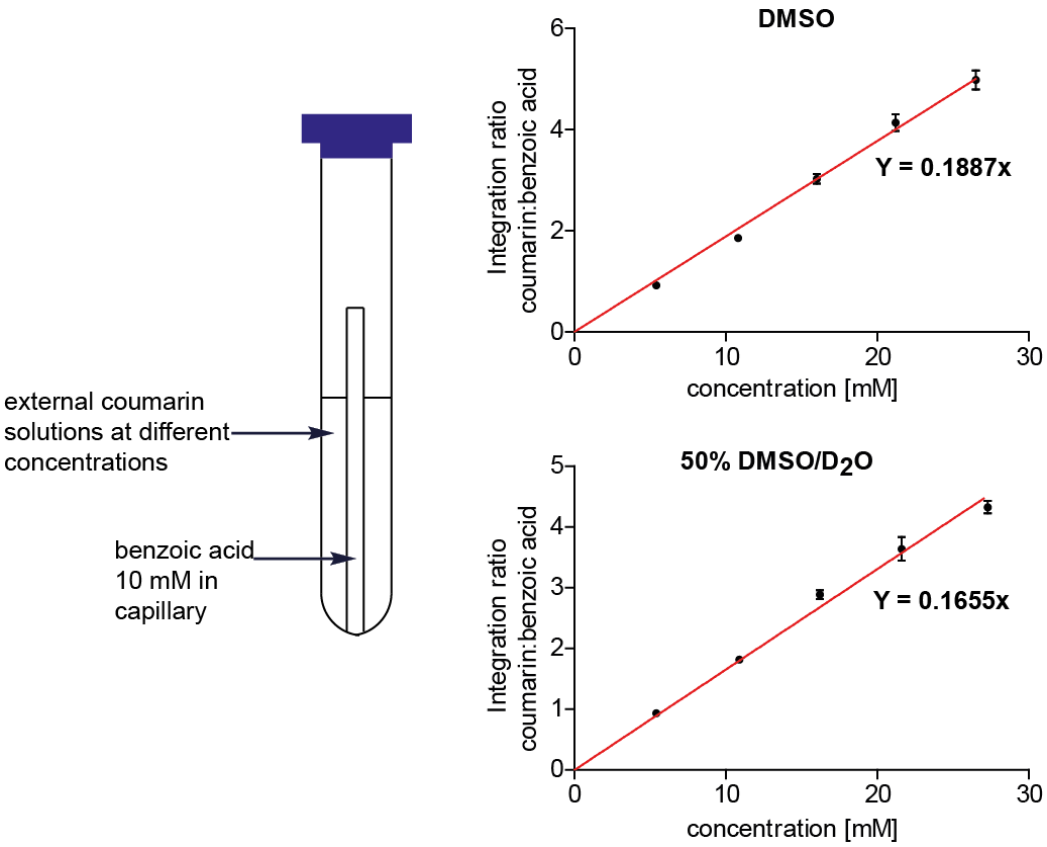


Figure S5: Quantitative NMR (qNMR) with benzoic acid as an external standard was used in order to accurately determine the concentration of stock solutions of each reagent. A sealed capillary tube containing 10 mM benzoic acid in D_6-DMSO as the external standard was calibrated against stock solutions of 7-hydroxycoumarin at known concentrations (26.5–5.4 mM prepared by accurate dilution based on solvent weight). The ^{1}H NMR spectra was recorded twice for each sample and the integration ratio of coumarin:benzoic acid was plotted against concentration. The capillary was then added to NMR tubes containing stock solutions of compounds and the exact concentration of these stock solutions was determined by measuring the ratio of integration (compound:benzoic acid standard) and converting it to concentration using the fitting on the appropriate graph.

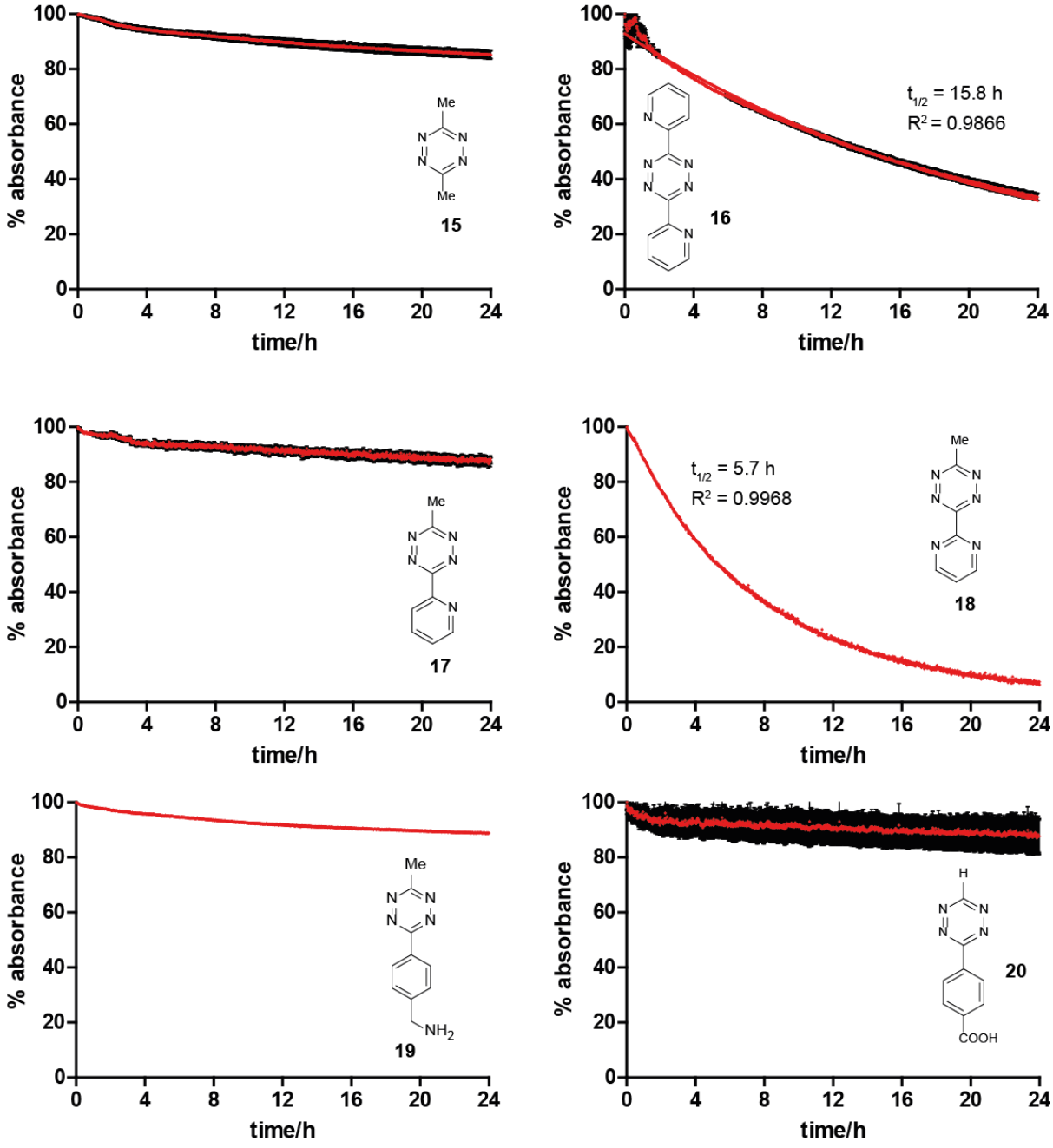


Figure S6: Stability of tetrazines **15**–**20** in 50% H_2O/DMSO assessed by monitoring the absorbance at 530 nm. The data for tetrazines **15**–**18** and **20** were originally reported in a previous paper.[13]

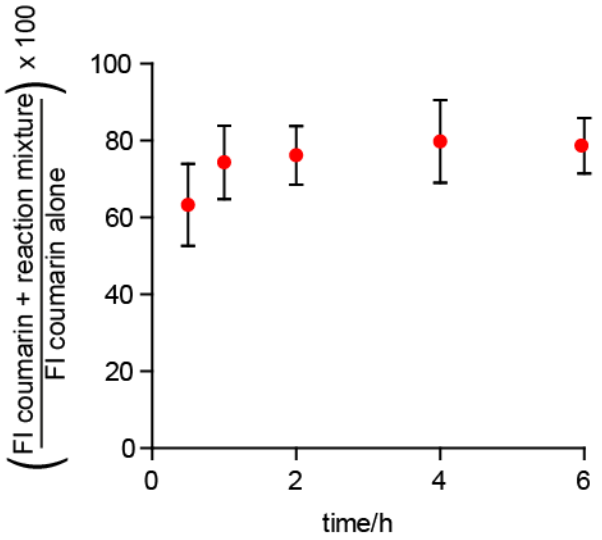


Figure S7: Quenching effect of the reaction mixture on the coumarin fluorescence in 50% H_2O/DMSO. An aliquot of the reaction mixture of **8** + **20** was added to a sample of 7-hydroxycoumarin **2** at various time points and the fluorescence intensity was recorded and compared to the fluorescence intensity of **2** alone.

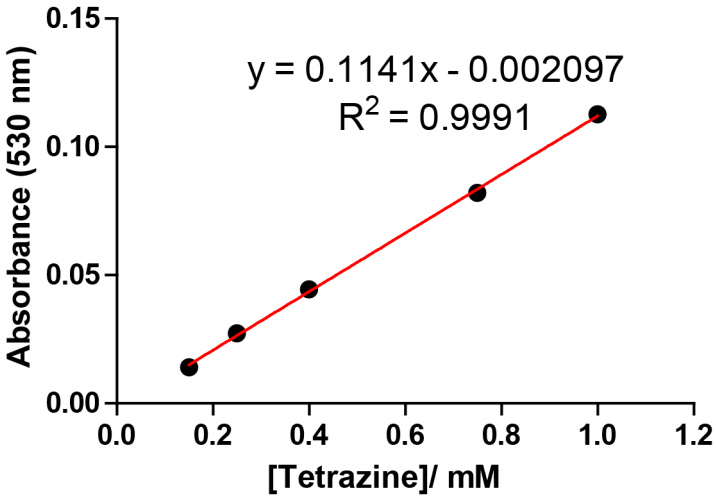


Figure S8: Stopped flow calibration curve. The absorbance at 530 nm was measured for known concentrations of stock tetrazine **20** (0.15–1 mM) and plotted against concentration of tetrazine **20**.

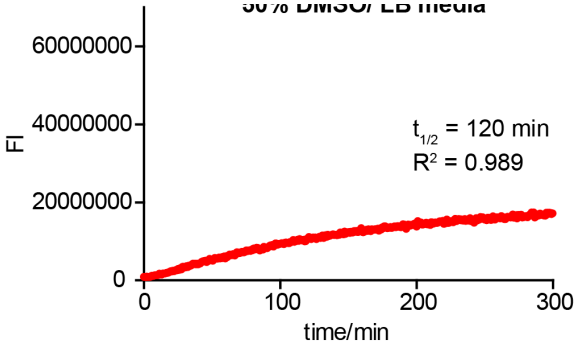


Figure S9: Reaction of **8** + **20** (final concentration 100 µM) in 50% DMSO/LB media. The fluorescence intensity was measured over 5 h at 37 °C (λ_{ex} = 320, λ_{em} = 465 nm).

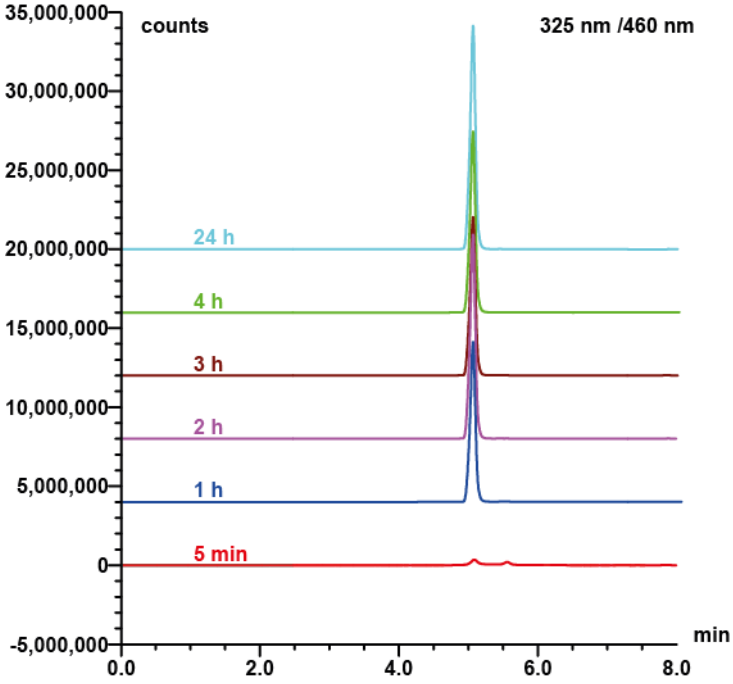


Figure S10: Following the reaction of TCO-coumarin **8** with tetrazine **20** by HPLC/FLD (λ_{ex} = 325 nm, λ_{em} = 460 nm). The reaction was analysed at the following times: 5 min, 1 h, 2 h, 3 h, 4 h, 24 h.

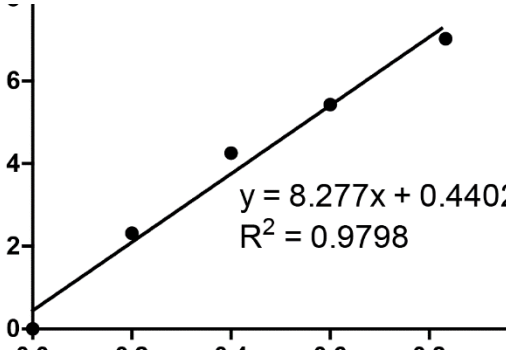


Figure S11: Calibration curve for detection of 7-hydroxycoumarin **2** by HPLC. Mixtures of **2** at known concentrations (0.2–0.83 mM) and internal standard benzoic acid (0.67 mM) were analysed by HPLC/UV and the ratio of areas of the UV peaks at 220 nm were plotted against concentration of **2**.

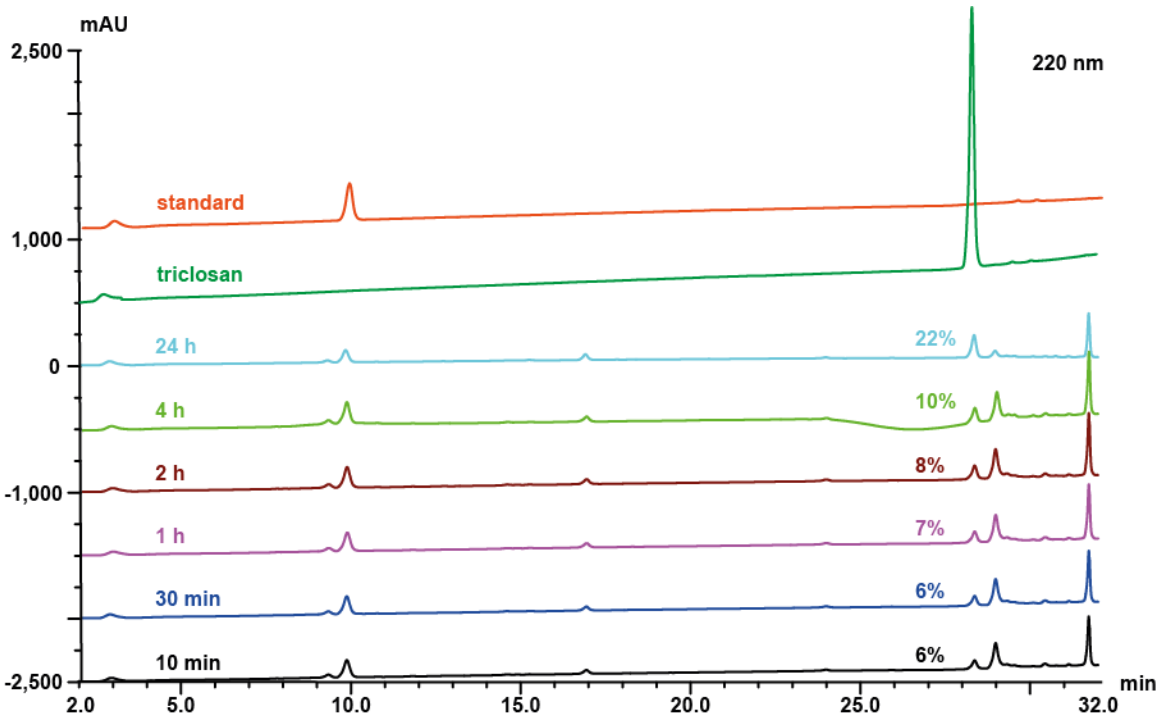


Figure S12: Decaging yield for the reaction of TCO-triclosan **14** with tetrazine **20** by HPLC analysis with an internal standard (benzoic acid).

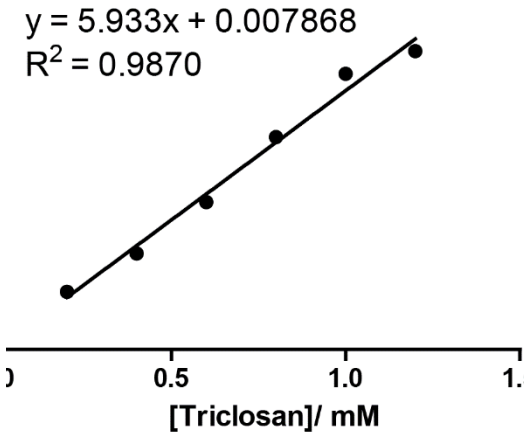


Figure S13: Calibration curve for detection of triclosan **21** by HPLC. Mixtures of **21** at known concentrations (0.2–1.2 mM) and internal standard benzoic acid (0.67 mM) were analysed by HPLC/UV and the ratio of areas of the UV peaks at 220 nm were plotted against concentration of **21**.

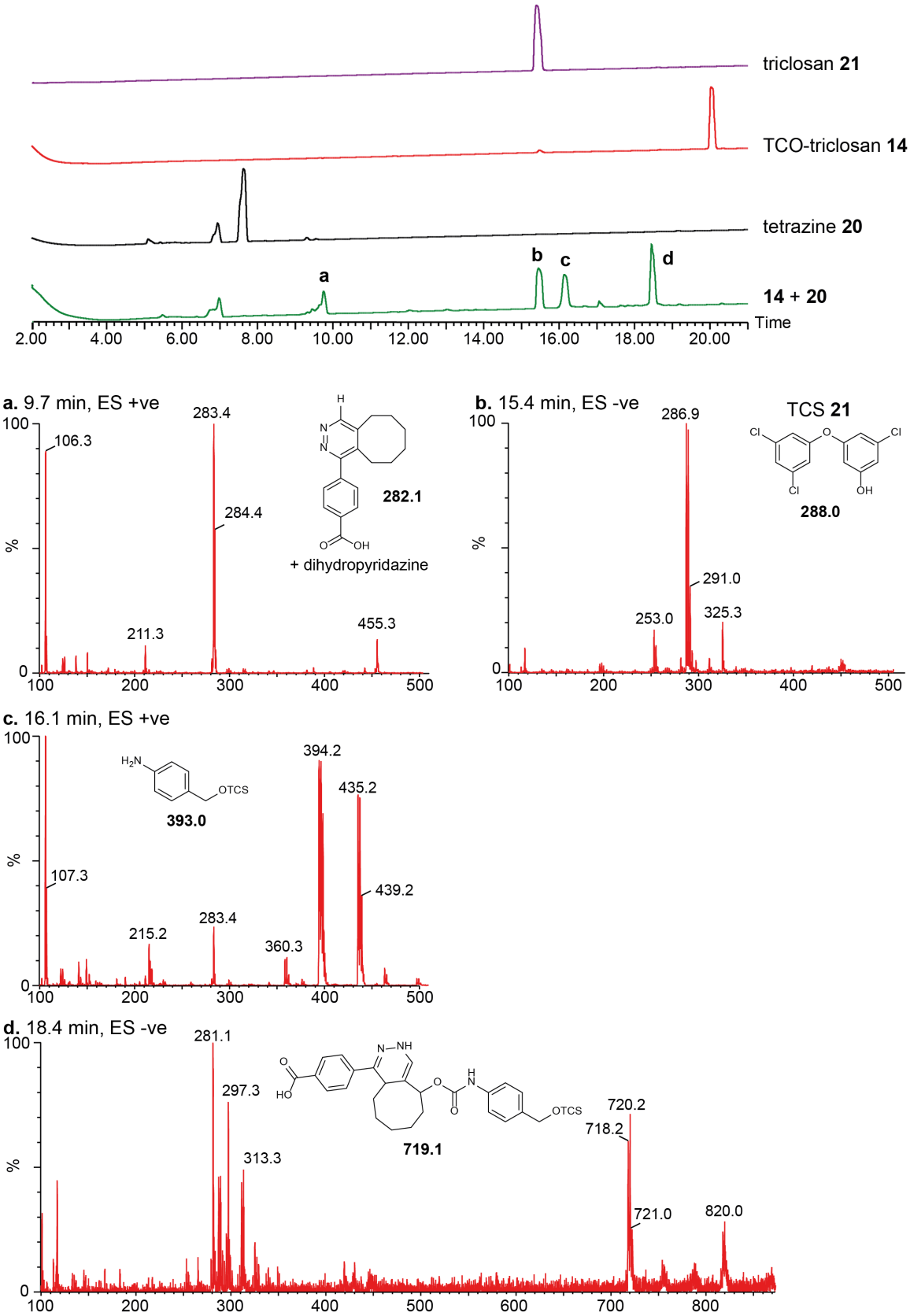


Figure S14: LCMS analysis of reaction mixture of TCO-triclosan **14** and tetrazine **20** after 24 h. Triclosan **21** and the pyridazine by-product were observed. Detection of intermediates confirms the proposed mechanism of decaging.

2.2 Live Cell Studies

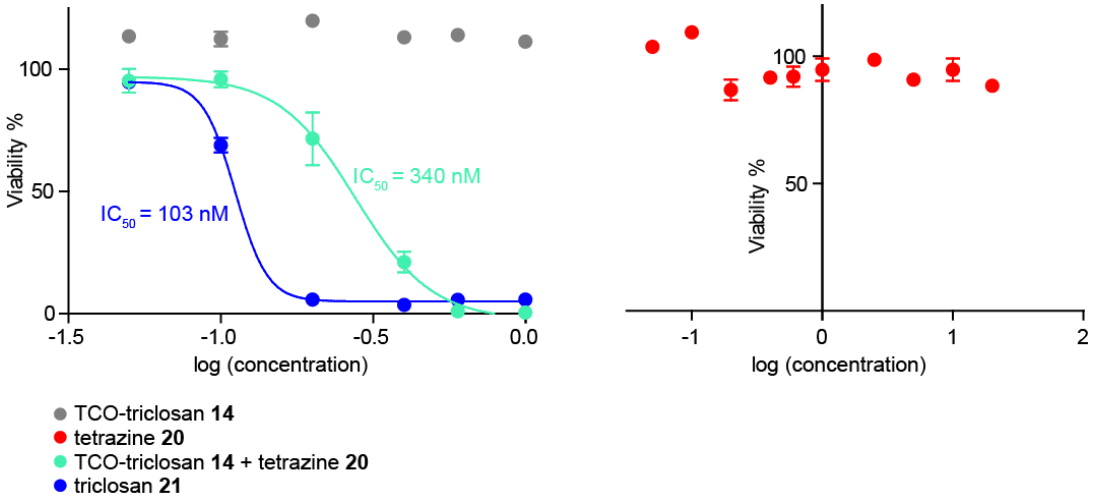


Figure S15: Toxicity of all compounds assessed by the CellTiter-Blue assay. Tetrazine **20** and prodrug TCO-triclosan **14** proved to be non-toxic at all concentrations tested (0.05–1 µM for **14** and 0.5–10 µM for **20**).

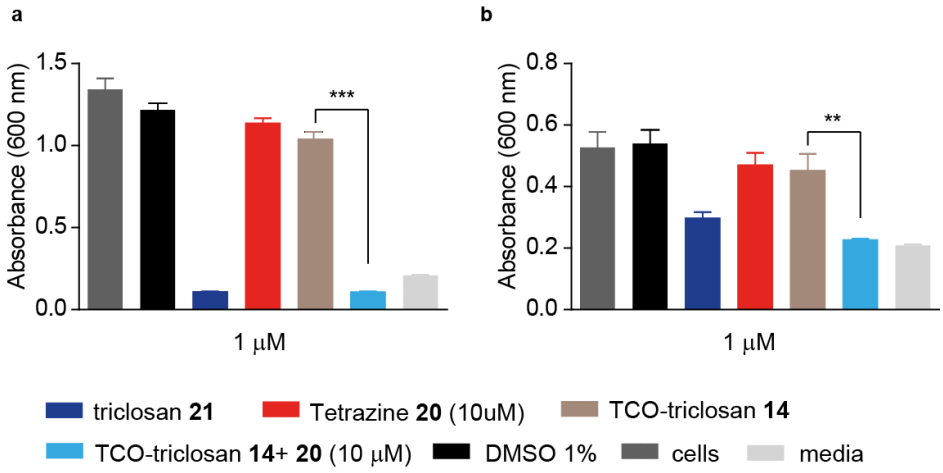
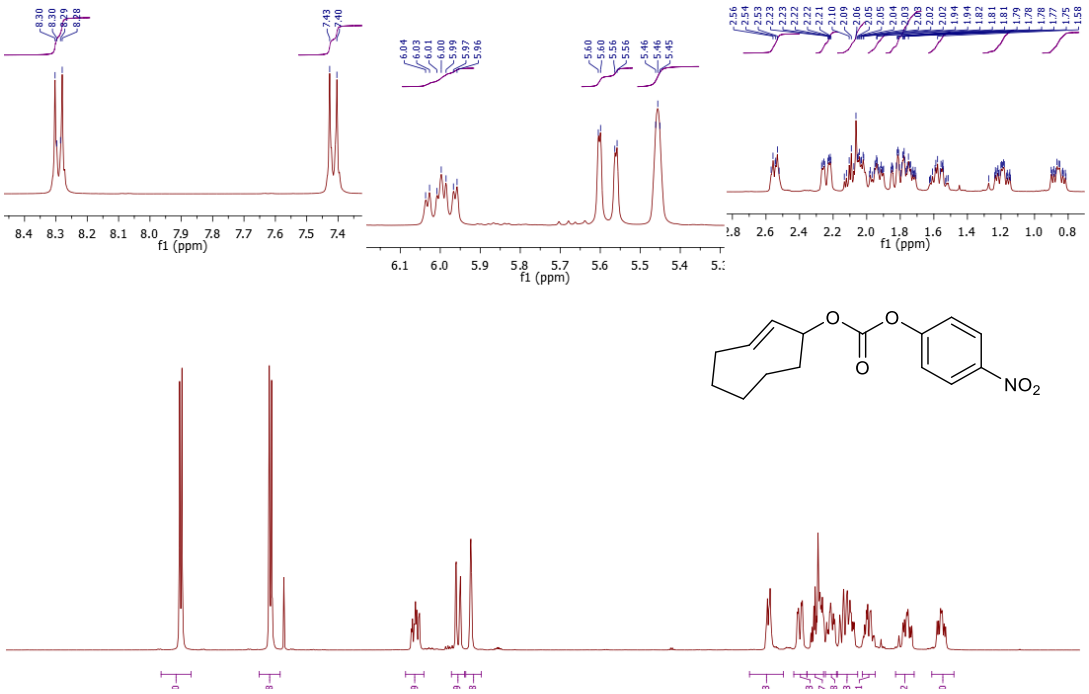


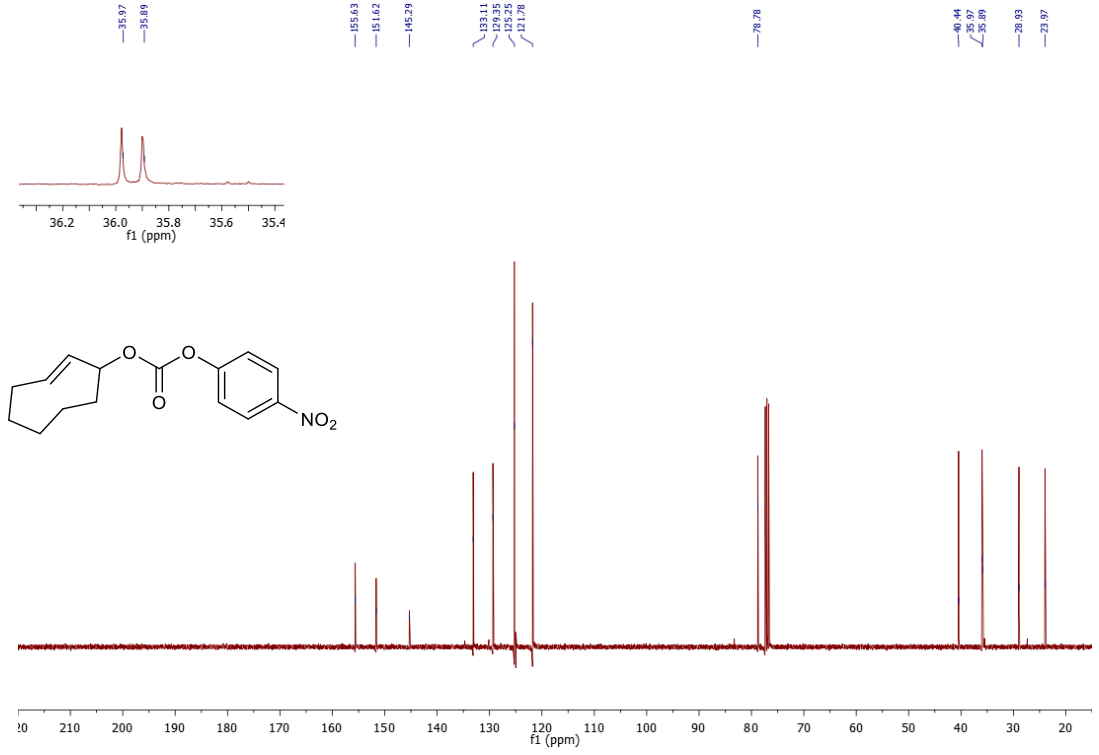
Figure S16: Cell density assay. **a.** Cytotoxicity of triclosan **21**, TCO-triclosan **14**, tetrazine **20**, TCO-triclosan **14** + **20** was assessed by measuring the OD_{600}. **b**. The OD_{600} was measured a second time after 2 x dilution. Statistical analysis was performed on prism using a one-way ANOVA. ** (P≤0.01), *** (P≤0.001).

3. NMR spectra

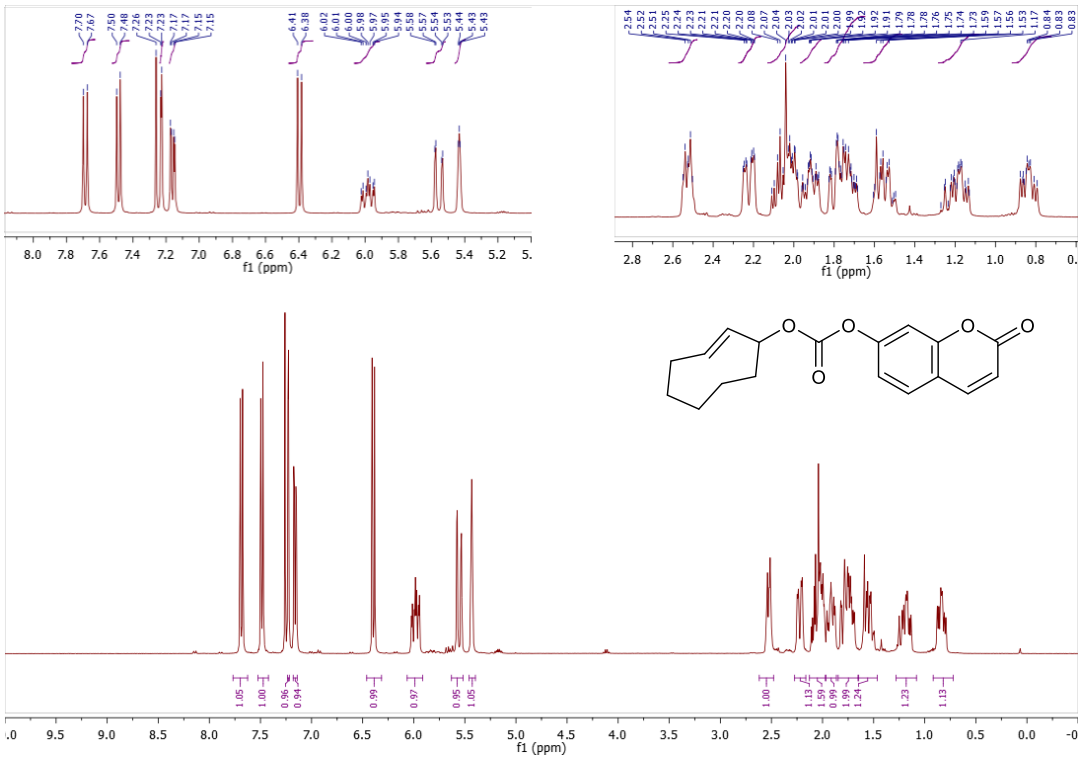
^{1}H NMR (400 MHz, $CDCl_3$) of (2*E*)-Cyclooct-2'-en-1'-yl 4-nitrophenyl carbonate **1a**



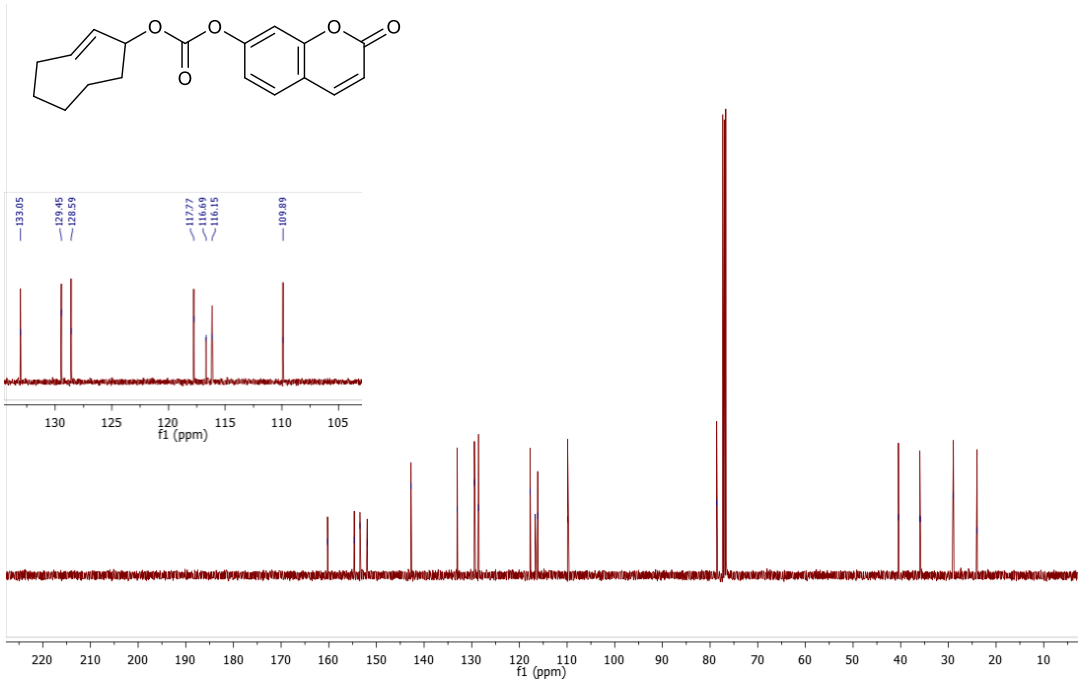
^{13}C NMR (101 MHz, $CDCl_3$) of (2*E*)-Cyclooct-2'-en-1'-yl 4-nitrophenyl carbonate **1a**



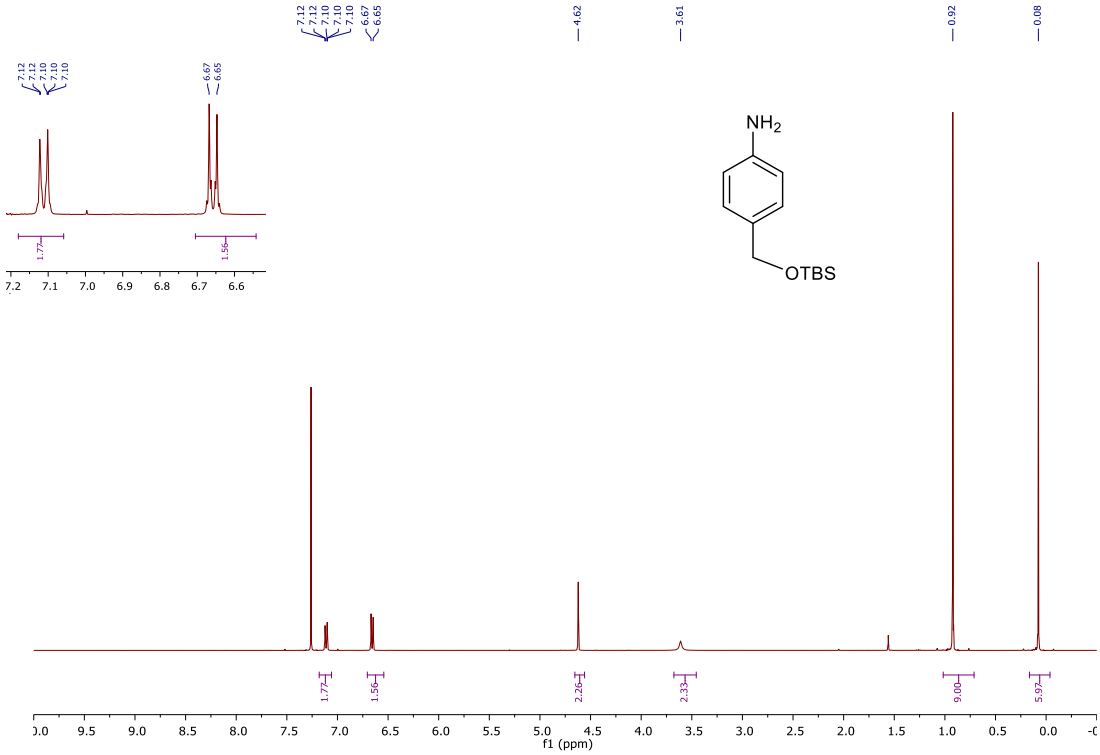
^{1}H NMR (400 MHz, $CDCl_3$) of (2*E*)-Cyclooct-2'-en-1'-yl 2-oxochromen-7-yl carbonate **1**



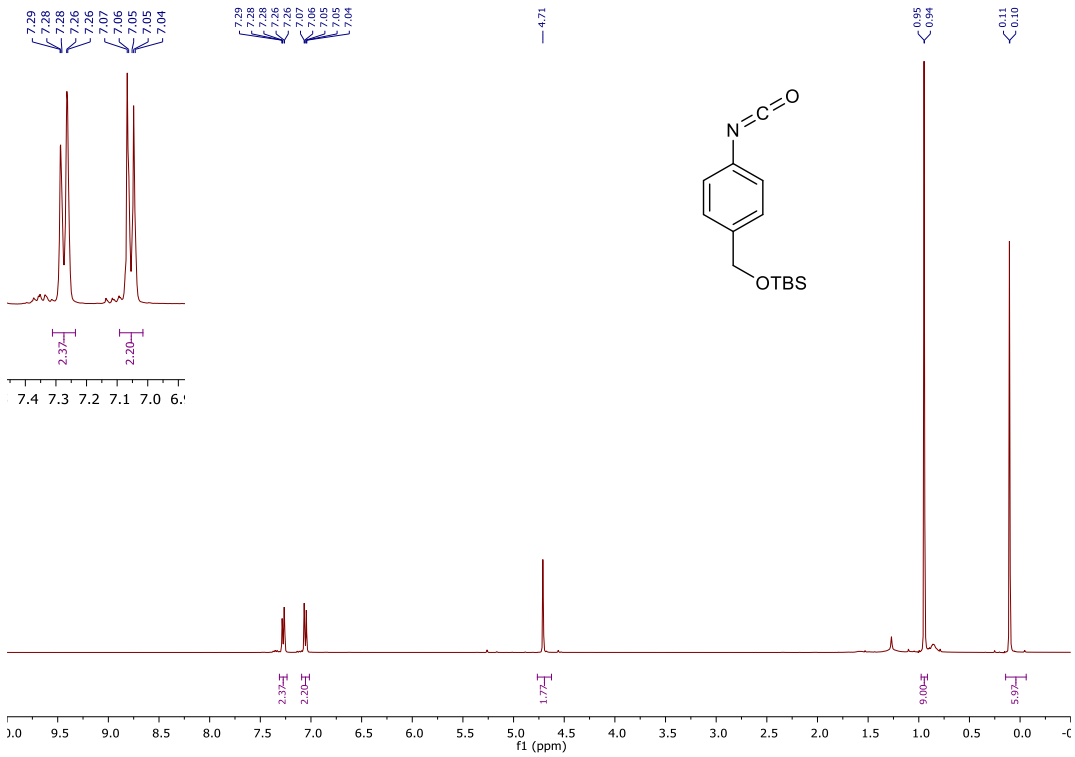
^{13}C NMR (101 MHz, $CDCl_3$) of (2*E*)-Cyclooct-2'-en-1'-yl 2-oxochromen-7-yl carbonate **1**



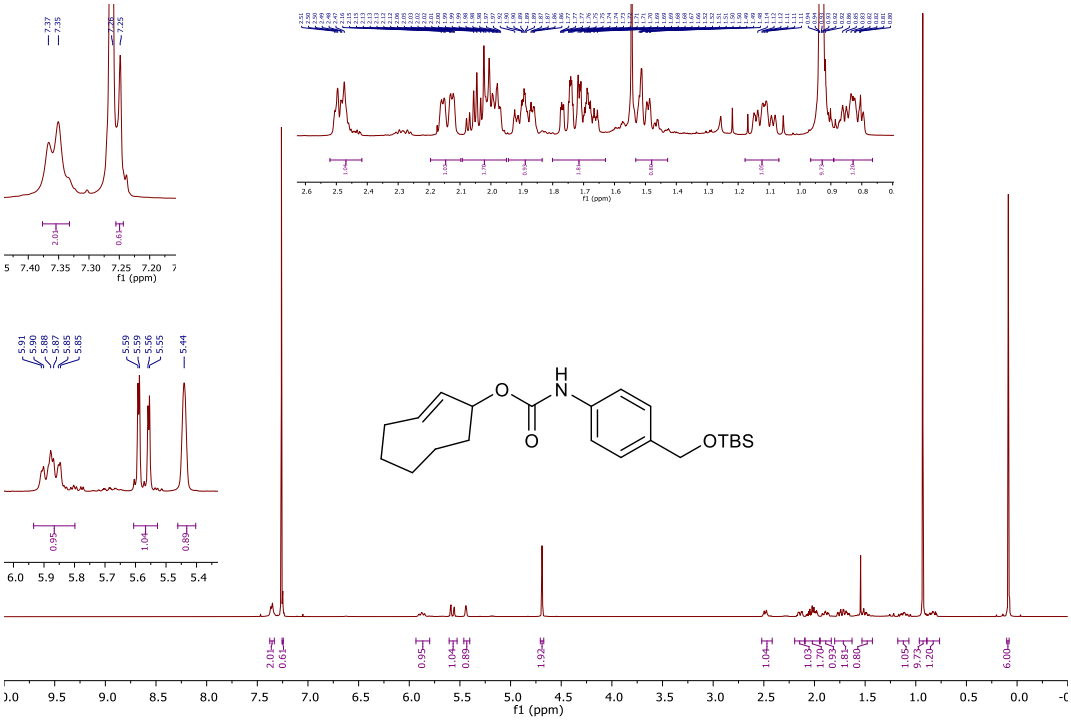
^{1}H NMR (400 MHz, $CDCl_3$) of 4-{[(tert-butyldimethylsilyl)oxy]methyl}aniline **4**



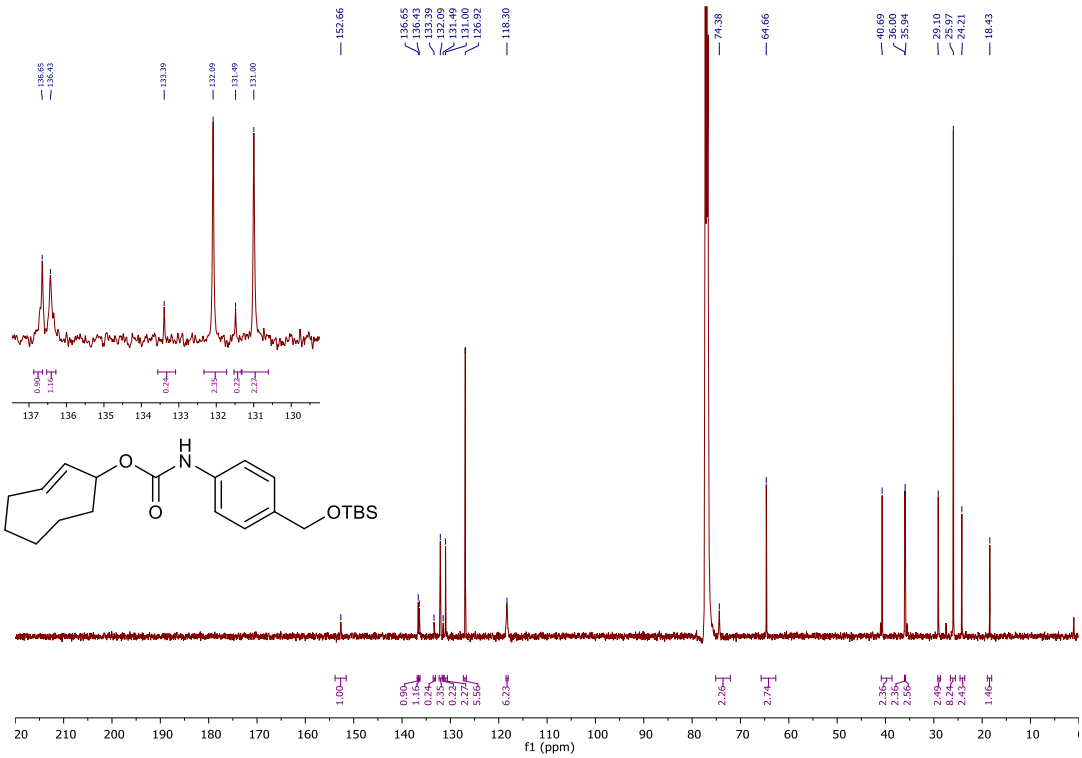
^{13}C NMR (101 MHz, $CDCl_3$) of tert-butyl[(4-isocyanatophenyl)methoxy]dimethylsilane **5**



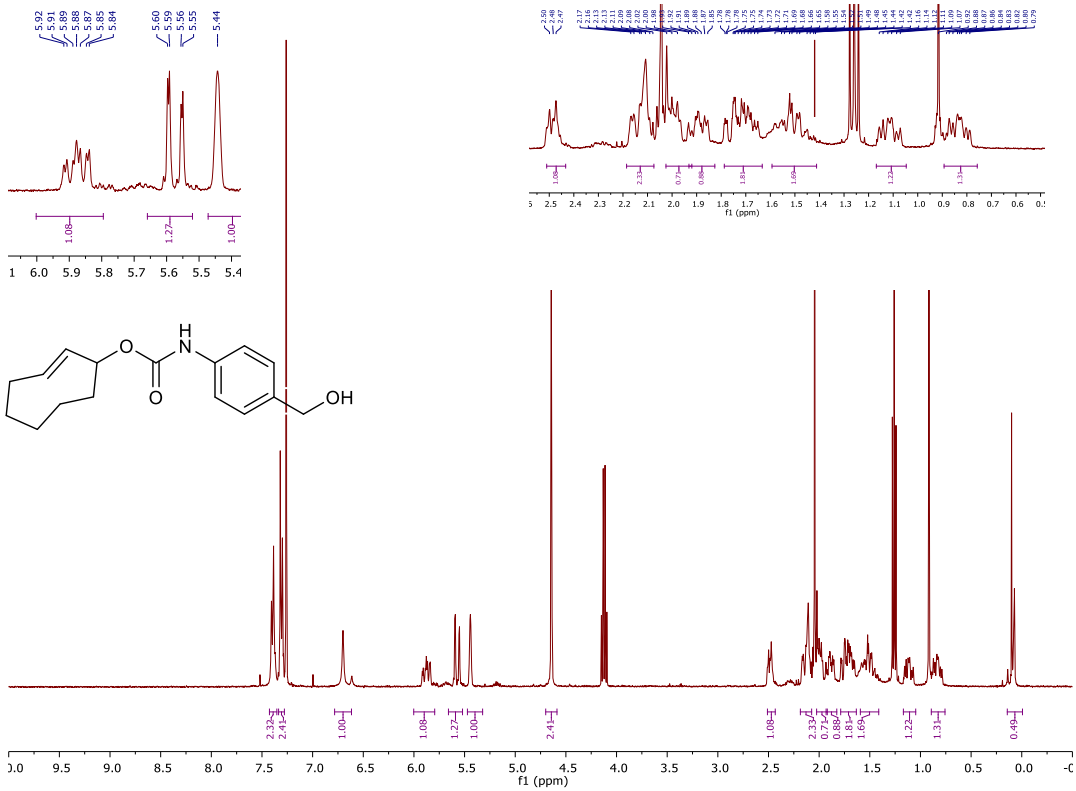
^{1}H NMR (500 MHz, $CDCl_3$) of (2*E*)-cyclooct-2-en-1-yl N-(4-{[(tert-butyldimethylsilyl)oxy]methyl}phenyl)carbamate **6**



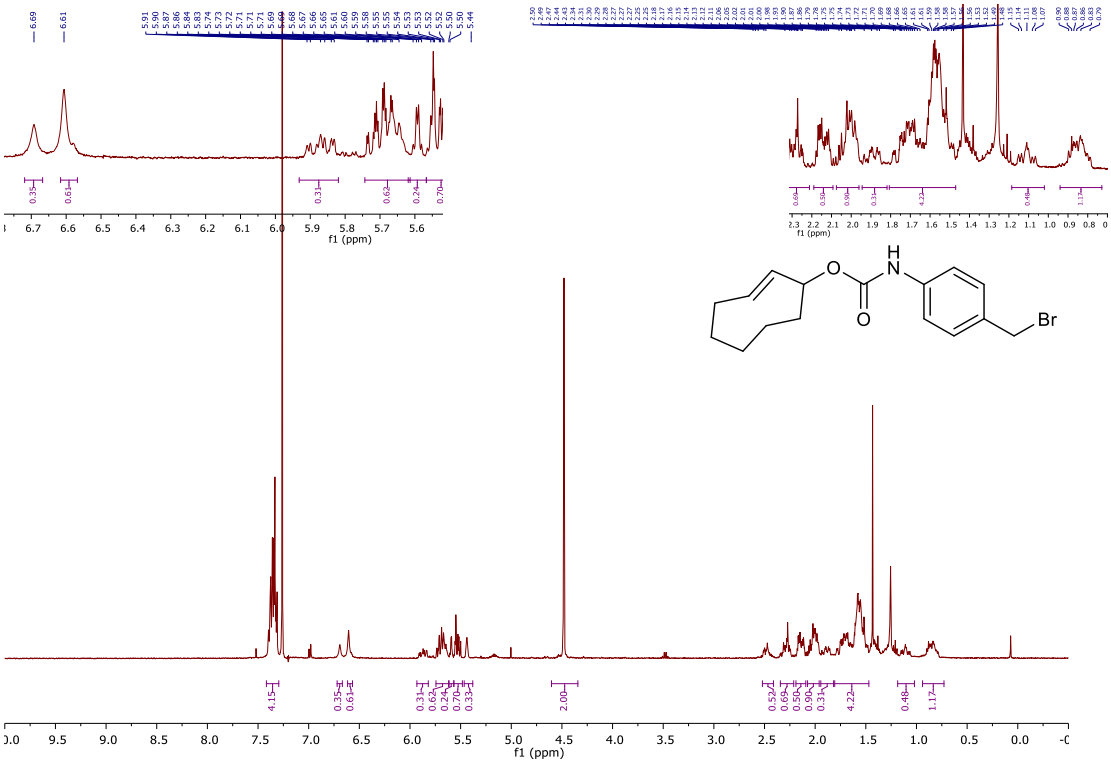
^{13}C NMR (126 MHz, $CDCl_3$) of (2*E*)-cyclooct-2-en-1-yl N-(4-{[(tert-butyldimethylsilyl)oxy]methyl}phenyl)carbamate **6**



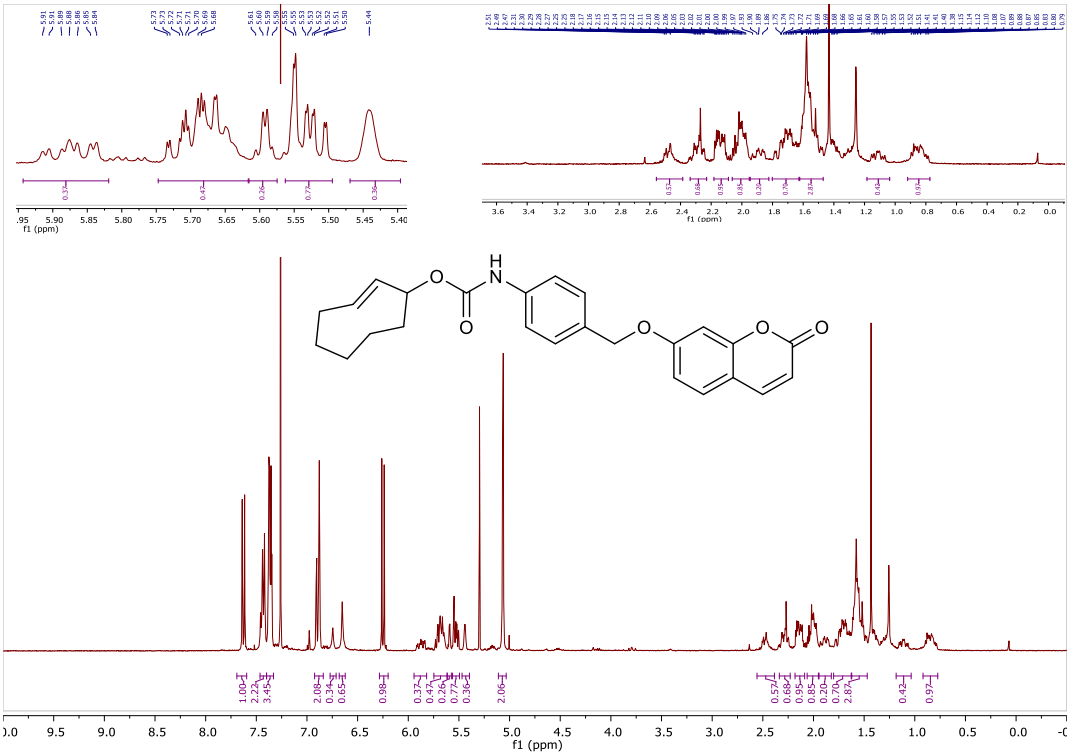
Crude ^{1}H NMR (400 MHz, $CDCl_3$) of (2'*E*)-cyclooct-2'-en-1'-yl N-[4-(hydroxymethyl)phenyl]carbamate **7**



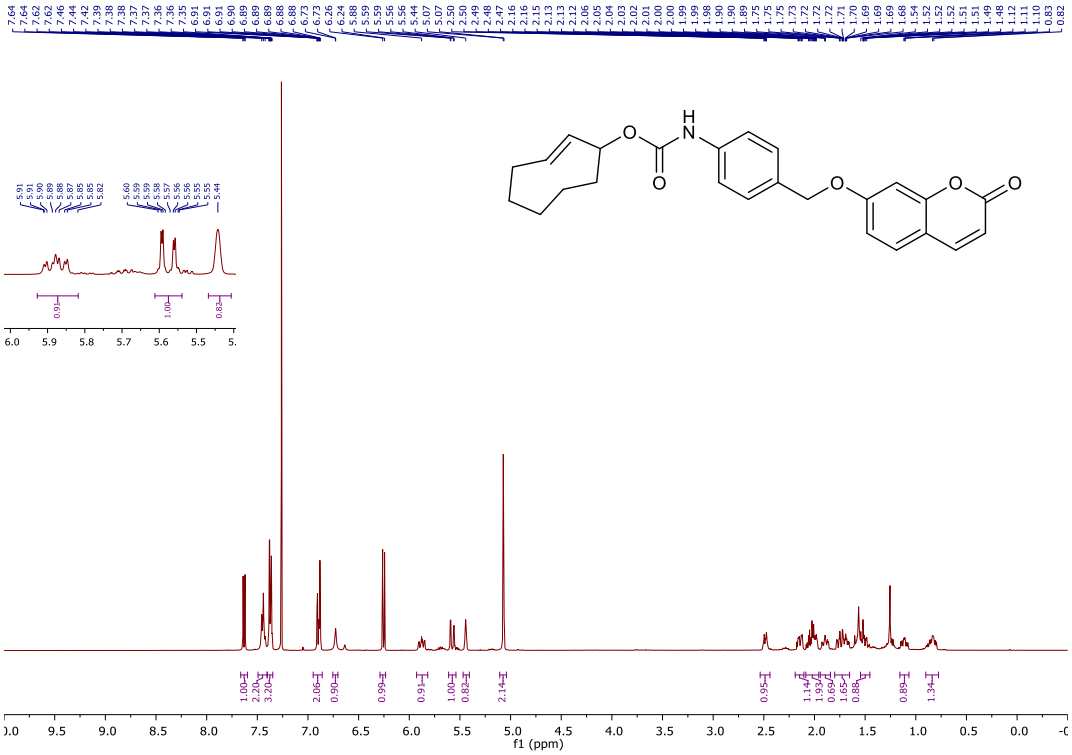
^{1}H NMR (400 MHz, $CDCl_3$) of (2'*E*)-cyclooct-2'-en-1'-yl N-[4-(bromomethyl)phenyl]carbamate **8a**



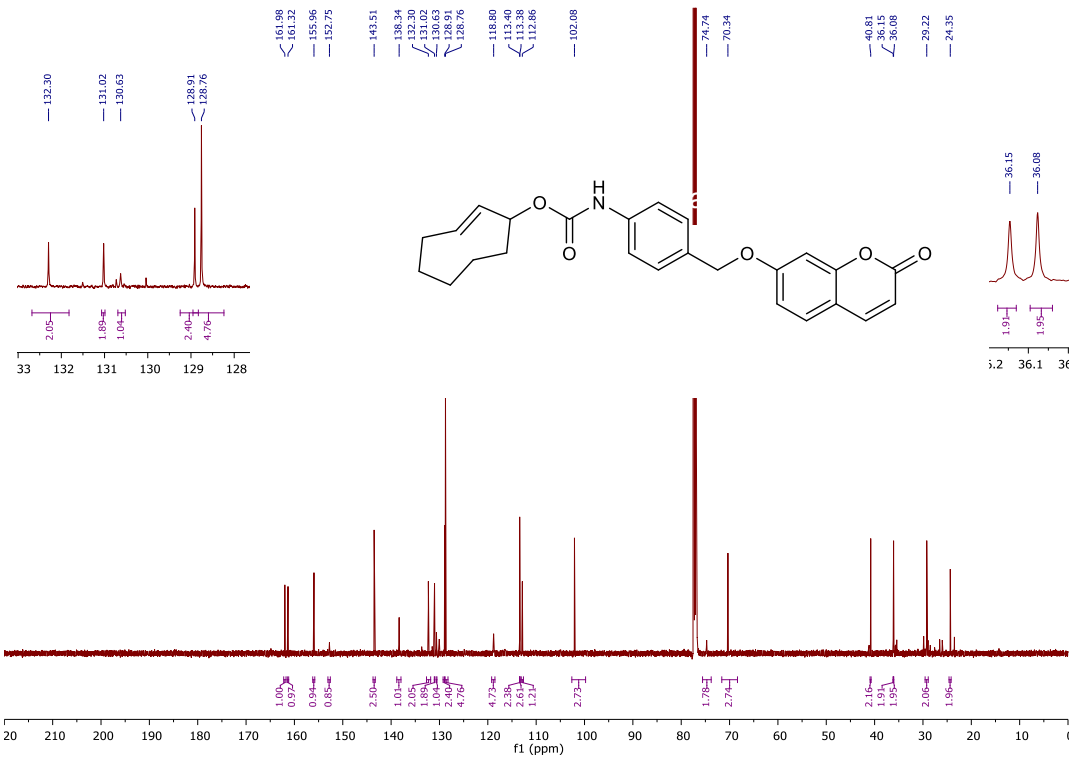
Crude ^{1}H NMR (400 MHz, $CDCl_3$) of (2''*E*)-cyclooct-2''-en-1''-yl N-(4-{[(2'-oxochromen-7'-yl)oxy]methyl}phenyl)carbamate **8** from route 1



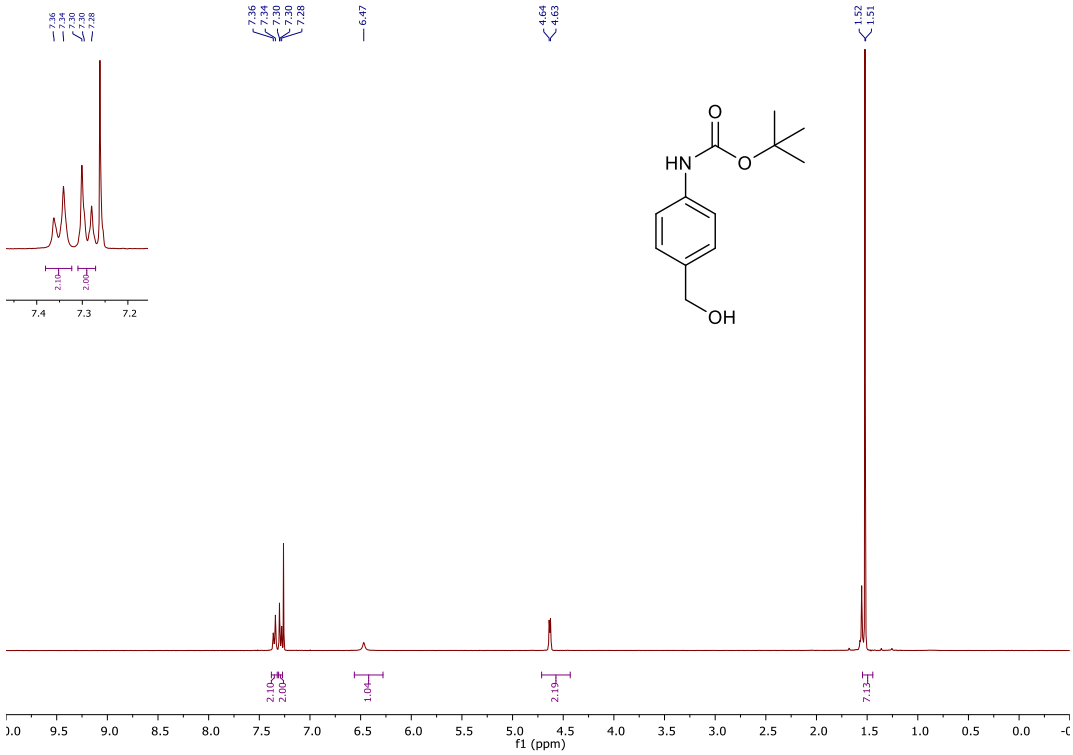
^{1}H NMR (500 MHz, $CDCl_3$) of (2''*E*)-cyclooct-2''-en-1''-yl N-(4-{[(2'-oxochromen-7'-yl)oxy]methyl}phenyl)carbamate **8** from route 2



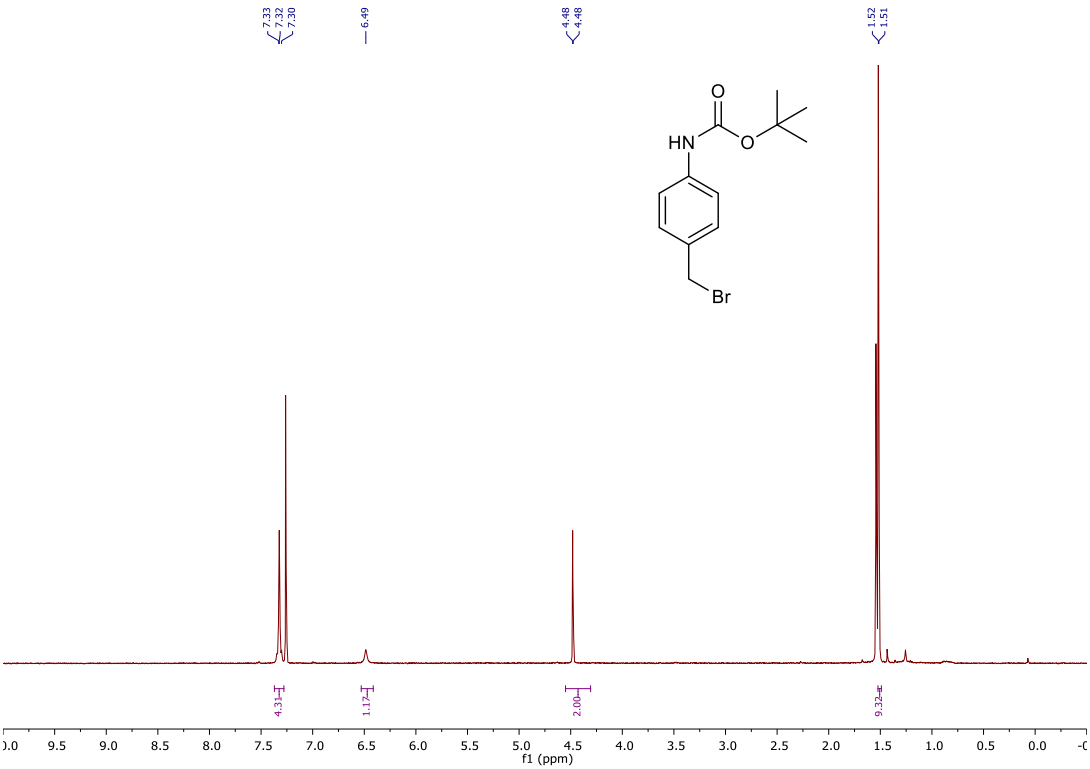
^{13}C NMR (126 MHz, $CDCl_3$) of (2''*E*)-cyclooct-2''-en-1''-yl N-(4-{[(2'-oxochromen-7'-yl)oxy]methyl}phenyl)carbamate **8** from route 2



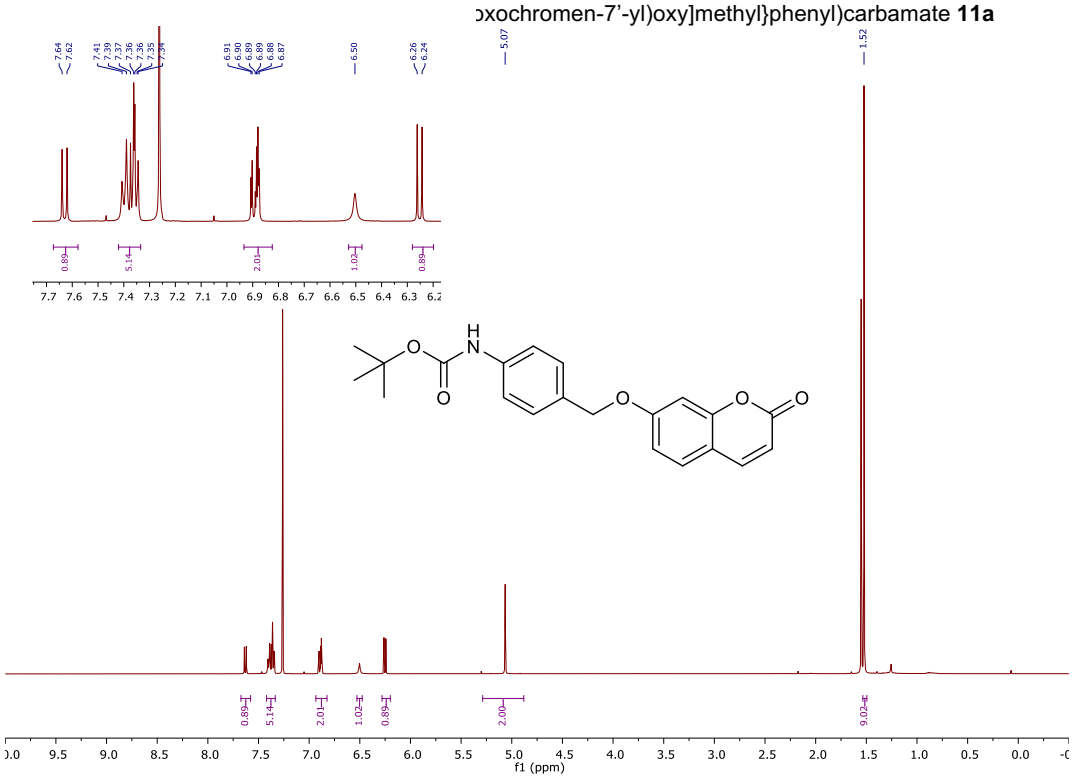
^{1}H NMR (400 MHz, $CDCl_3$) of tert-butyl N-[4-(hydroxymethyl)phenyl]carbamate **9**



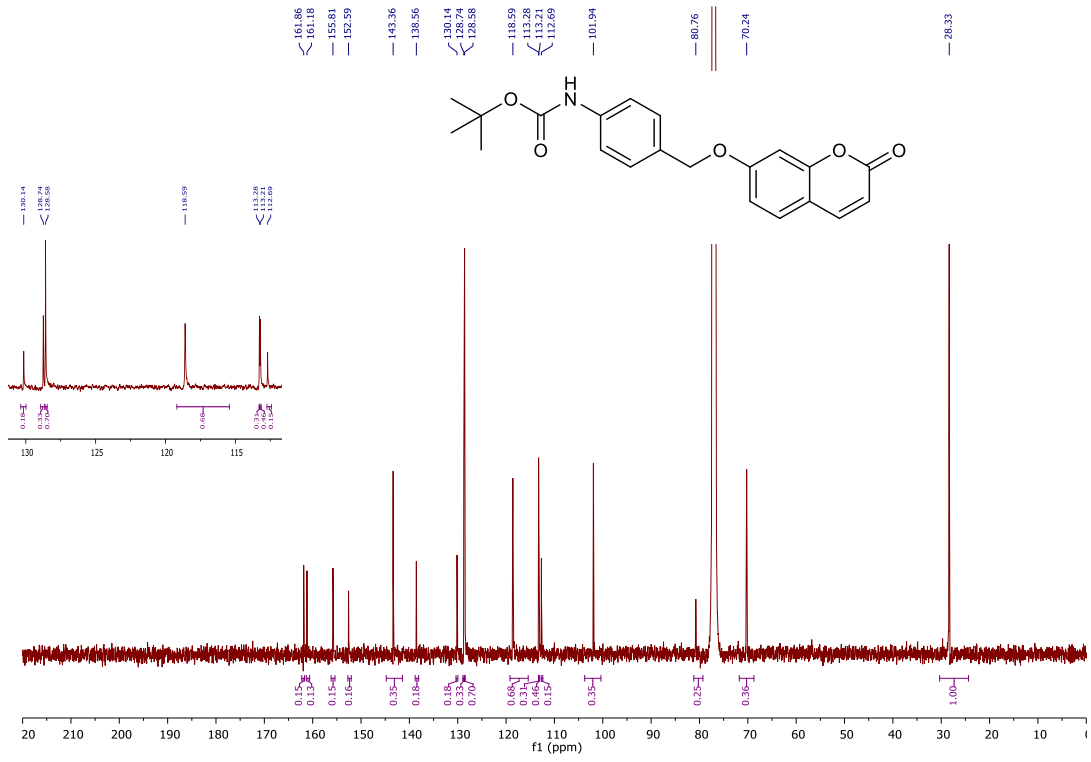
^{1}H NMR (400 MHz, $CDCl_3$) of tert-butyl N-[4-(bromomethyl)phenyl]carbamate **10**



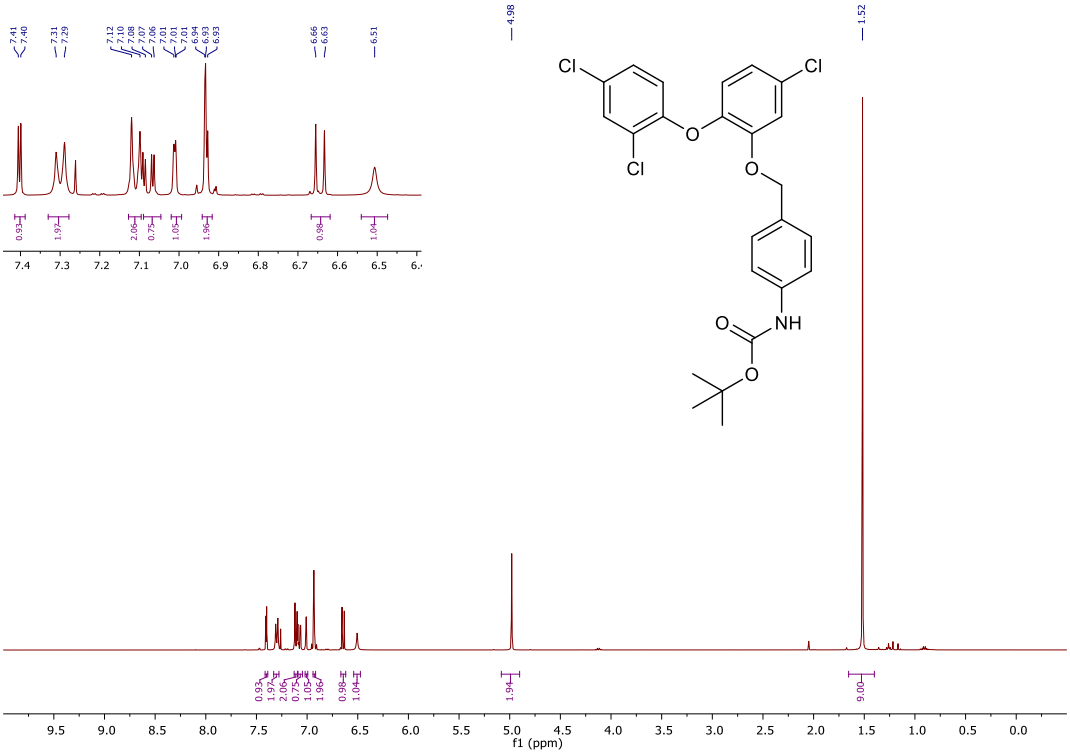
ɔxochromen-7'-yl)oxy]methyl}phenyl)carbamate **11a**



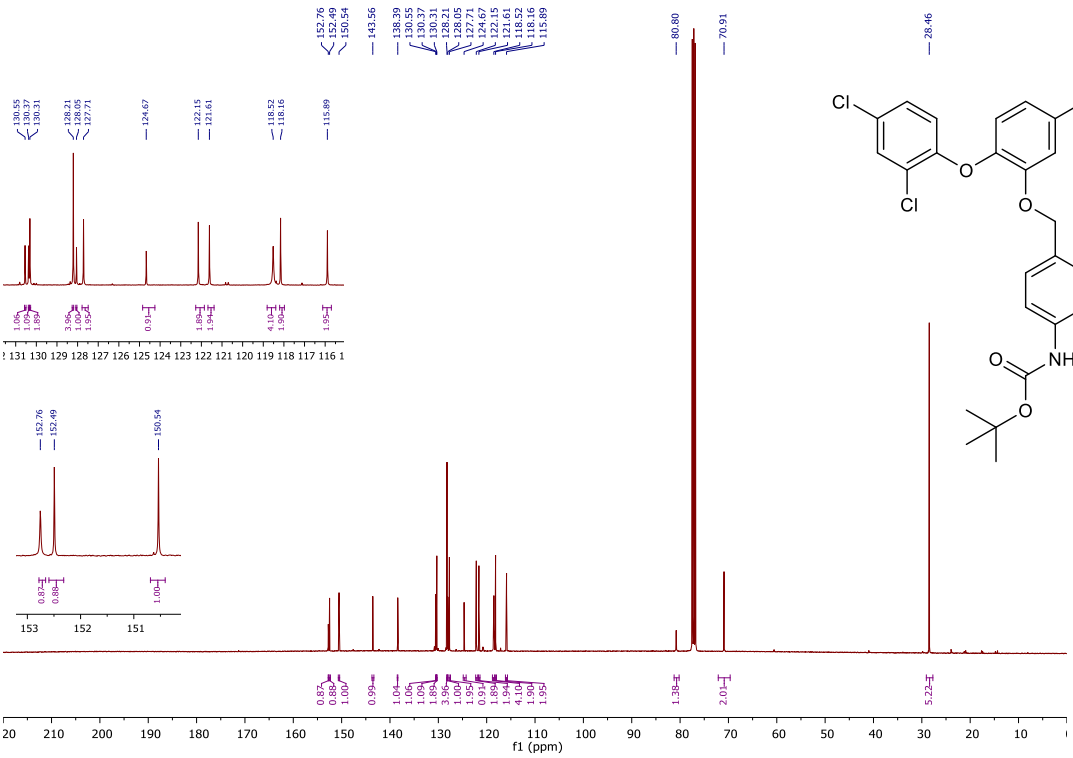
^{13}C NMR (126 MHz, $CDCl_3$) of tert-butyl N-(4-{[(2'-oxochromen-7'-yl)oxy]methyl}phenyl)carbamate **11a**



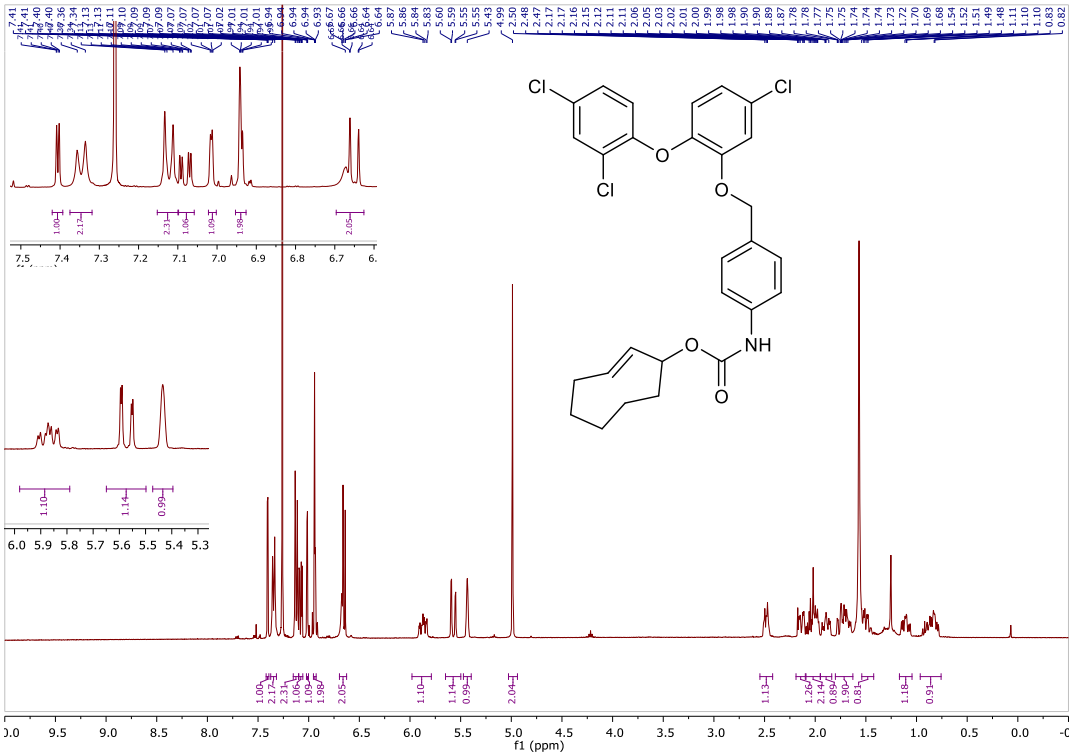
^{1}H NMR (400 M[illegible]hloro-2-(2',4'-dichlorophenoxy)phenoxymethyl]phenyl}carbamate **11b**



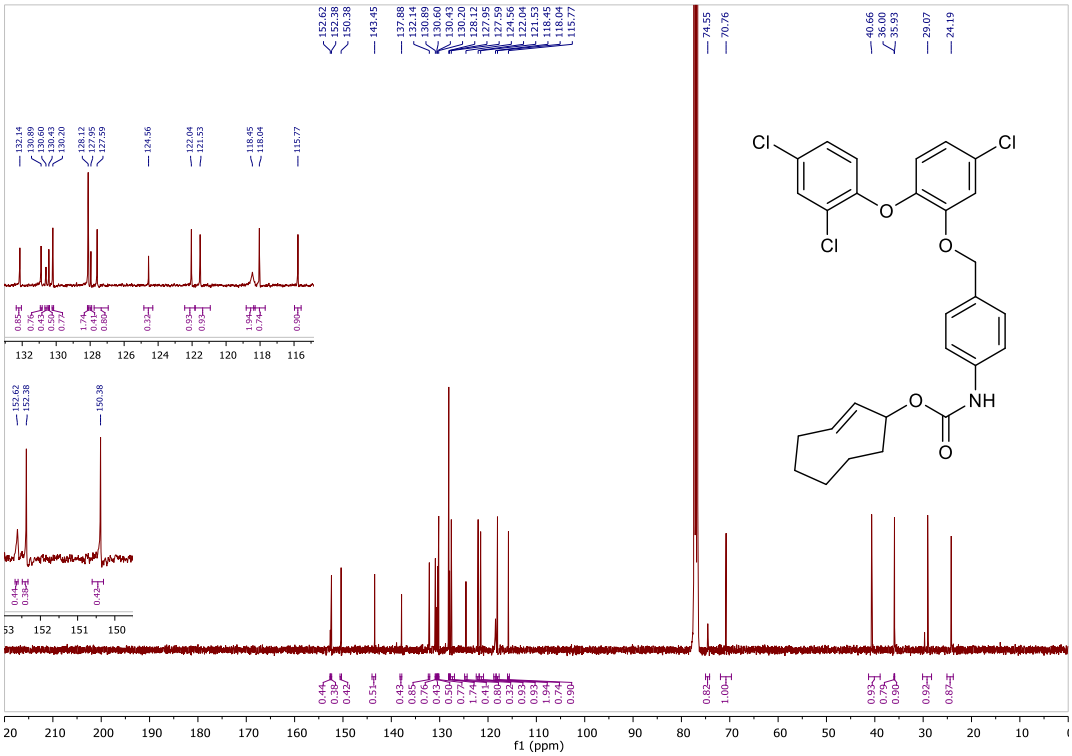
^{13}C NMR (101 MHz, $CDCl_3$) of tert-butyl N-{4''-[5-chloro-2-(2',4'-dichlorophenoxy)phenoxymethyl]phenyl}carbamate **11b**



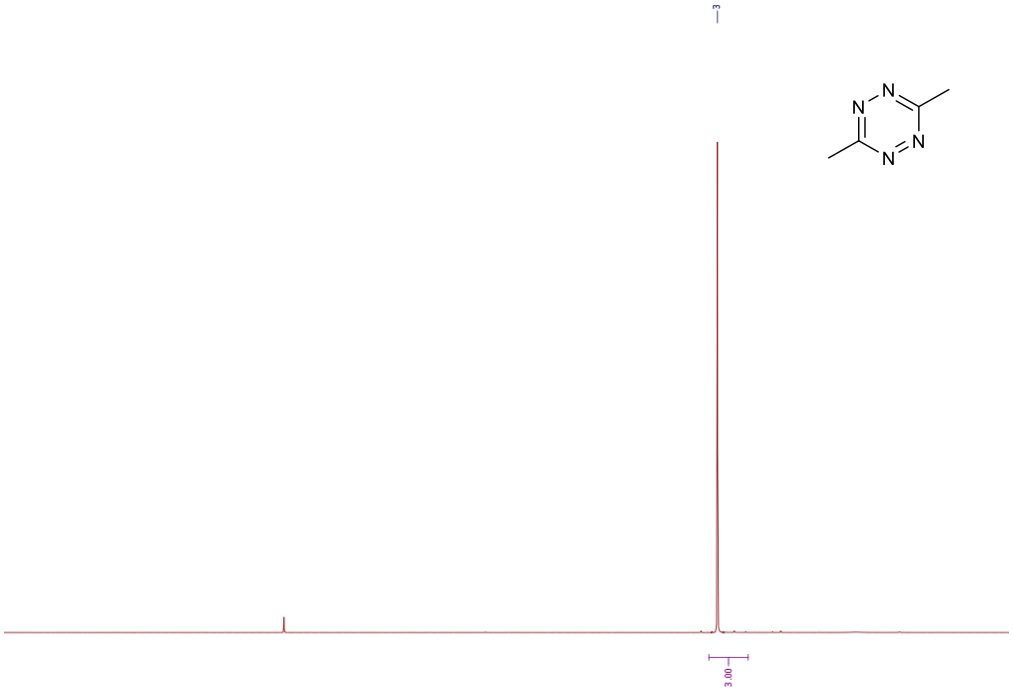
^{1}H NMR (400 MHz, $CDCl_3$) of
(2'''*E*)-cyclooct-2'''-en-1-yl N-{4''-[5-chloro-2-(2',4'-dichlorophenoxy)phenoxymethyl]phenyl}carbamate **14**



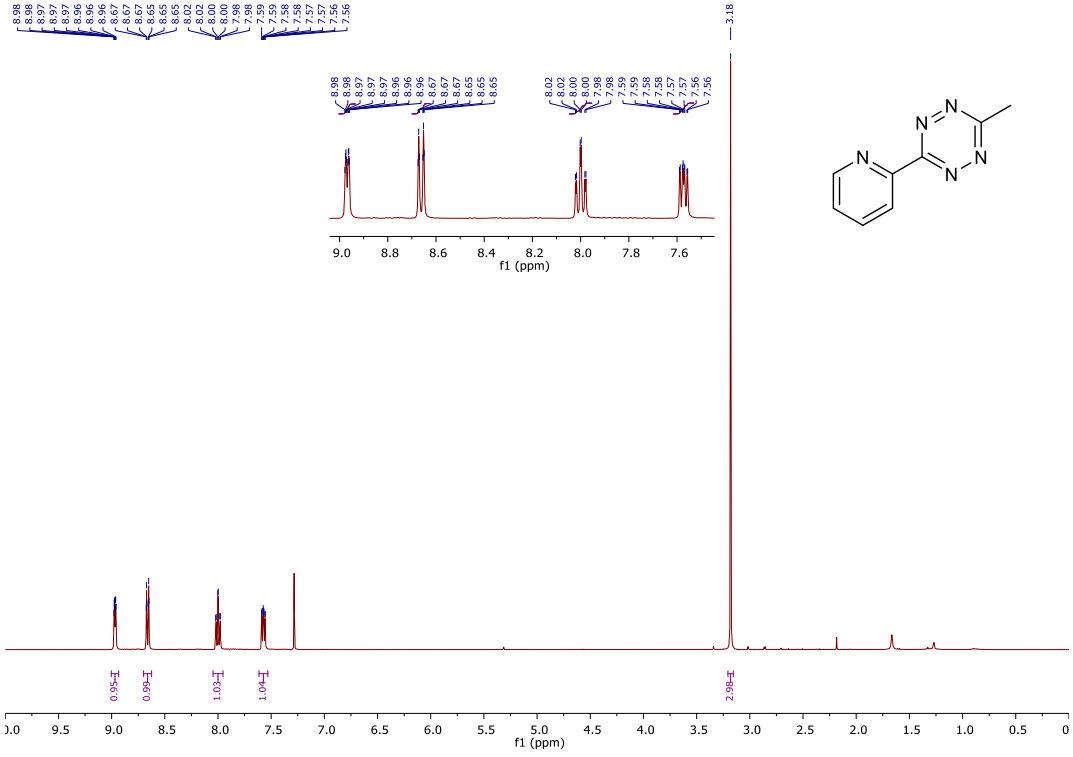
^{13}C NMR (101 MHz, $CDCl_3$) of
(2'''*E*)-cyclooct-2'''-en-1-yl N-{4''-[5-chloro-2-(2',4'-dichlorophenoxy)phenoxymethyl]phenyl}carbamate **14**



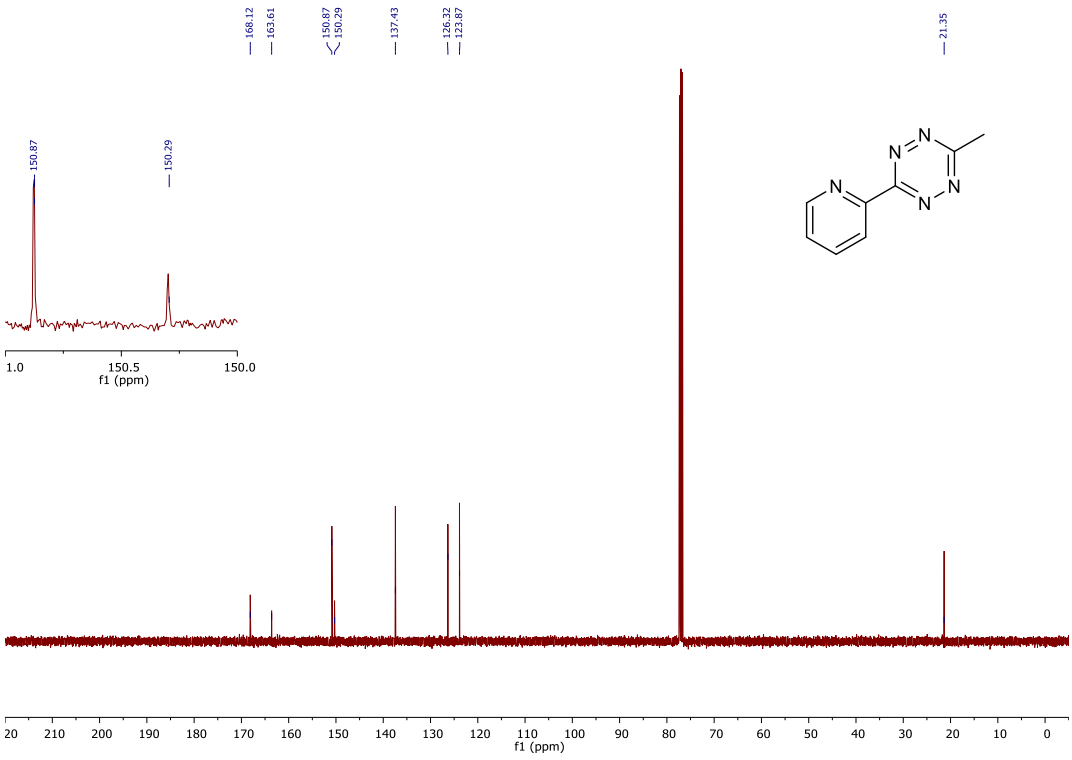
^{1}H NMR (400 MHz, $CDCl_3$) of dimethyl-1,2,4,5-tetrazine **15**



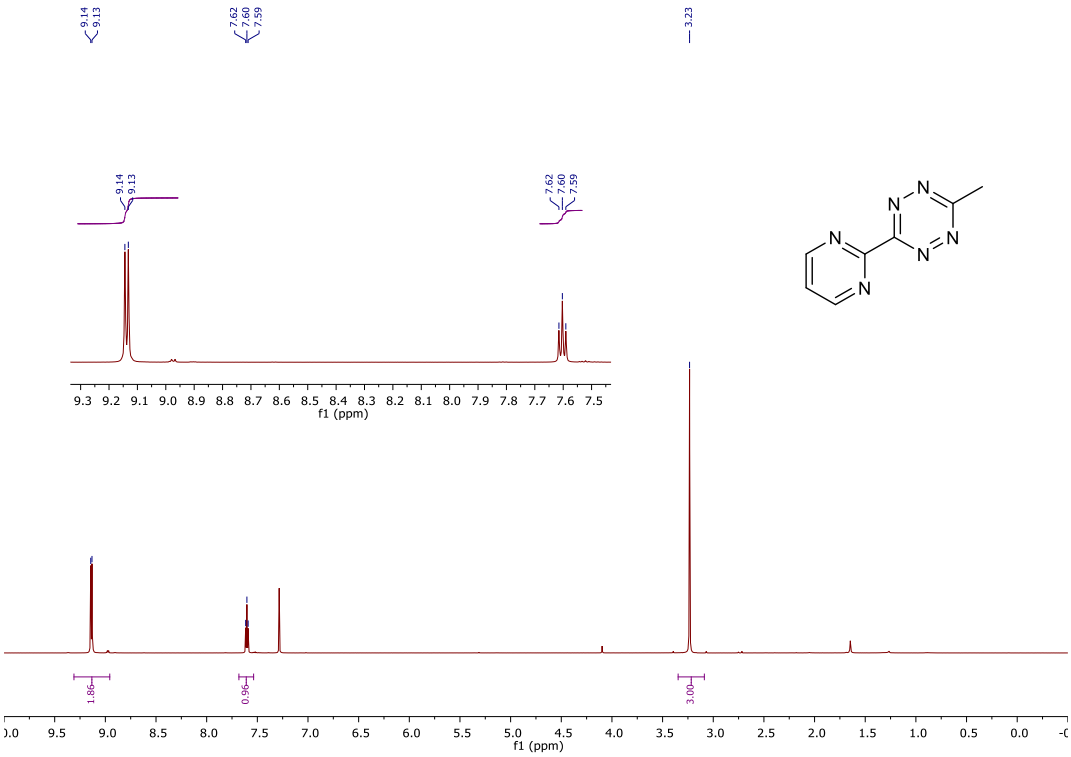
^{1}H NMR (400 MHz, $CDCl_3$) of 3-Methyl-6-(pyridin-2'-yl)-1,2,4,5-tetrazine **17**



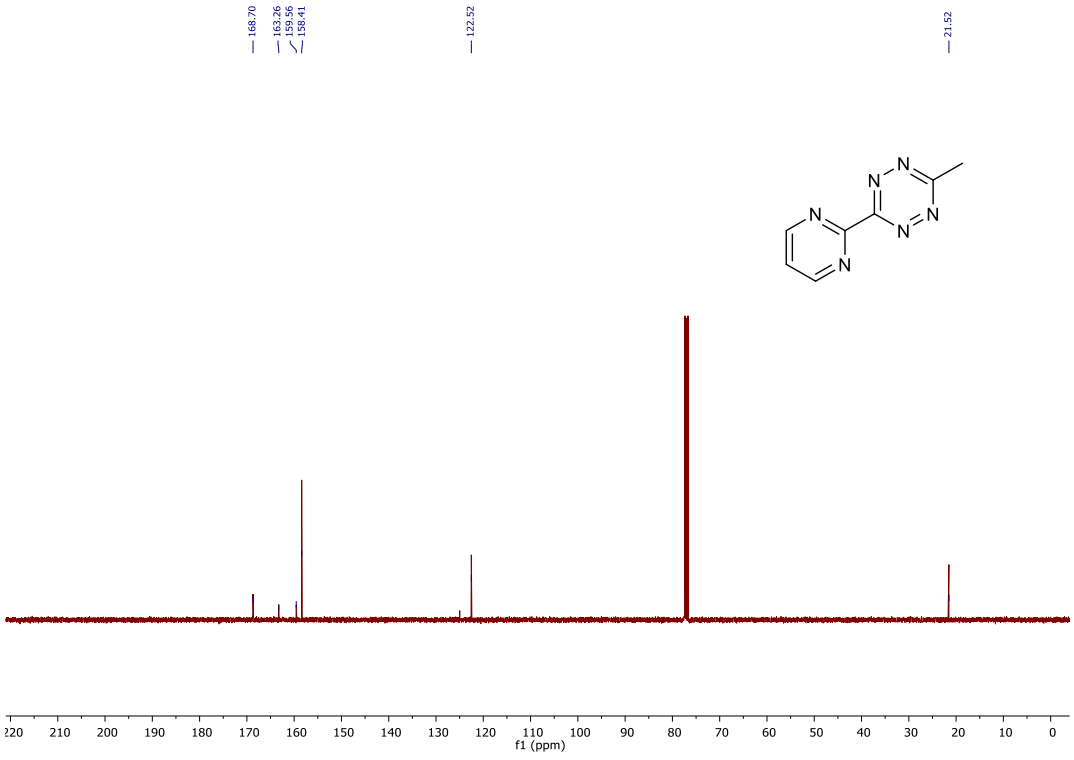
^{13}C NMR (101 MHz, $CDCl_3$) of 3-Methyl-6-(pyridin-2'-yl)-1,2,4,5-tetrazine **17**



^{1}H NMR (400 MHz, $CDCl_3$) of 3-Methyl-6-(pyrimidin-2'-yl)-1,2,4,5-tetrazine **18**



^{13}C NMR (101 MHz, $CDCl_3$) of 3-Methyl-6-(pyrimidin-2'-yl)-1,2,4,5-tetrazine **18**



4. References

1 A. B. Pangborn, M. A. Giardello, R. H. Grubbs, R. K. Rosen and F. J. Timmers, *Organometallics*, 1996, **15**, 1518–1520.

2 E. Lemke, C. Schultz, T. PlassIvana, I. Nikic, J-E. HoffmannIker and V. Aramburu. US Pat., 2016/0340297 A1, 2016.

3 A. R. Renslo, E. M. W. Lauterwasser, S. D. Fontaine, B. B. Spangler, J. A. Wells, WO Pat., WO 2015123595 A1, 2015.

4 K. M. Haynes, N. Abdali, V. Jhawar, H. I. Zgurskaya, J. M. Parks, A. T. Green, J. Baudry, V. V. Rybenkov, J. C. Smith and J. K. Walker, *J. Med. Chem.*, 2017, **60**, 6205–6219.

5 S. H. Morimoto, T. Ohshima, Y. Shimizu, M. Noshita, Y. Mukai and H. Morimoto, *Chem. Commun.*, 2014, **50**, 12623–12625.

6 A. El Alaoui, F. Schmidt, C. Monneret and J.-C. Florent, *J. Org, Chem.,* 2006, **71**, 9628–9636.

7 M. P. Hay, W. R. Wilson and W. A. Denny, *Bioorg. Med. Chem.*, 2005, **13**, 4043–4055.

8 M. A. M. Behnam, D. Graf, R. Bartenschlager, D. P. Zlotos and C. D. Klein, *J. Med. Chem.*, 2015, **58**, 9354–9370.

9 H. Zhao, P. Reddy and M. B. Rubio, US Pat., 8 367 065, 2013.

10 R. M. Versteegen, R. Rossin, W. Ten Hoeve, H. M. Janssen and M. S. Robillard, *Angew. Chemie Int. Ed.*, 2013, **52**, 14112–14116.

11 X. Fan, Y. Ge, F. Lin, Y. Yang, G. Zhang, W. S. C. Ngai, Z. Lin, S. Zheng, J. Wang, J. Zhao, J. Li and P. R. Chen, *Angew. Chemie Int. Ed.*, 2016, **55**, 14046–14050.

12 E. Jiménez-Moreno, Z. Guo, B. L. Oliveira, I. S. Albuquerque, A. Kitowski, A. Guerreiro, O. Boutureira, T. Rodrigues, G. Jiménez-Osés and G. J. L. Bernardes, *Angew. Chemie Int. Ed.*, 2017, **56**, 243–247.

13 S. Davies, L. Qiao, B. Oliveira, C. Navo, G. Jiménez-Osés and G. J. L. Bernardes, *ChemBioChem*, 2019, DOI:10.1002/cbic.201900098.