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Supporting Information

Controlling Antibacterial Activity Exclusively with Visible Light: Introducing a Tetra-*ortho*-Chloro-Azobenzene Amino Acid

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1. General Information

All solvents and reagents used were purchased from commercial suppliers and used without further purification. ^{1}H-NMR spectra were obtained at room temperature on a Varian 400 MHz spectrometer. ^{13}C-NMR spectra were obtained at 100 MHz. All NMR spectra were processed using *MestReNova* NMR software. Chemical shifts are reported in parts per million (ppm) and coupling constants (*J*) reported in Hz. Splitting patterns are reported as follows: singlet (s), doublet (d), triplet (t), quadruplet (q), quintuplet (quint), doublet of doublets (dd), doublet of doublets of doublets (ddd), multiplet (m), etc. NMR signals were assigned using the appropriate 2D NMR experiments (*i.e.* HSQC and HMBC when necessary). UPLC analysis was performed on a Waters high class (PDA detector, simple manager FNT and Quaternary solvent manager) coupled to an electrospray ion source ESI-MS Micromass ZQ, using the MassLynx 4.1 software (Waters, Milford, MA) and a BEH C18 column (50 × 2.1 mm × 1.7 µm, Waters). The flow rate was 0.6 mL·min^{-1}, and MeCN (0.07% formic acid) and H_2O (0.1% formic acid) were used as solvents. TLC analysis was carried out on aluminium sheets coated with silica gel and visualised using potassium permanganate solution and/or UV light. Infra-red spectra were recorded as evaporated films or neat using FT/IR spectrometers. Melting points were measured on solids as obtained after chromatography. Mass spectra were obtained using positive or negative nanoelectrospray (nanoESI) and data was acquired and processed with Xcalibur software. Irradiation with red light (650 nm, 369 W·m^{-2}) was performed using a PAUL red LED lamp by GenIUL, S. L.

2. Solid Phase Peptide Synthesis (SPPS)

Peptides were obtained using either a manual synthesis approach or with an automated microwave-assisted synthesizer (Liberty Blue, CEM).

2.1. Manual Solid Phase Peptide Synthesis

Manual peptide elongation was performed in propylene syringes fitted with a frit for filtration using the Fmoc/*t*-Bu strategy. RinkAmide ChemMatrix resin (functionalisation 0.54 mmol·g^{-1}) was used to obtain *C*-terminal amides upon cleavage. The first amino acid was introduced by treating the resin with the appropriate Fmoc-amino acid (3.0 equiv), Oxyma (3.3 equiv) and DIC (3.3 equiv) in DMF (0.81 M solution of amino acid) during overnight gentle shaking. Subsequent couplings were performed treating the resin with the appropriate Fmoc-amino acids (2.3 equiv), Oxyma (2.5 equiv) and DIC (2.5 equiv) in DMF (0.81 M solution of amino acid) for 2 h. Photoswitches were introduced as regular amino acids using the same reaction conditions, but performing the coupling overnight. 20% piperidine in DMF was used to deprotect the temporary Fmoc protecting groups (2 × 1 min + 2 × 5 min treatments). After every elongation and deprotection the resin was washed with DMF (× 5) and CH_2Cl_2 (× 5). The success each of coupling was assessed performing the ninhydrin test.

In order to cleave the peptides, resins were treated with TFA/CH_2Cl_2 (95:5; 5 × 45 min treatments), then washed with CH_2Cl_2 (× 5), unless indicated otherwise. The collected cleavage solution was concerted under vacuum until all volatiles were removed and a nice flowing solid was obtained. Precipitation in cold ether was avoided due to the low recoveries caused by the high hydrophobicity of products.

2.2. Automated Microwave-Assisted Peptide Synthesis

Automated peptide synthesis was performed in a LibertyBlue peptide synthesizer (CEM) using the Fmoc/*t*-Bu strategy. RinkAmide ProTide resin (functionalisation 0.56 mmol·g^{-1}) was used to obtain *C*-terminal amides upon cleavage. Photoswitches were introduced manually as detailed above for the manual synthesis.

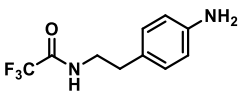
Peptides were cleaved from the resin as detailed above for manual synthesis.

2.3. Peptide Purification and Analysis

Peptides were purified in a Waters system with MassLynx 4.1 software, a 2707 binary gradient module, a 2767 manager collector, a 2998 photodiode array detector, and an Aeris 5 μm PEPTIDE XB-C18 column (250 × 21.2 mm). The flow rate was 16 mL·min^{-1} using MeCN (0.1% TFA) and H_2O (0.1% TFA). Purity was checked by analytical reverse-phase UPLC. UPLC-MS was used to confirm the identity of the compounds synthesized, and purity was assessed by UPLC. All peptides were obtained with high purity (> 95%) and stored lyophilized at −20 °C.

3. Synthesis: Experimental Details and Characterisation

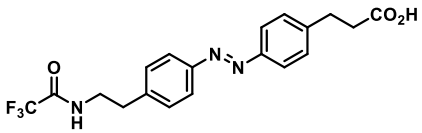
_N_-(4-Aminophenethyl)-2,2,2-trifluoroacetamide (6)



To a stirring solution of 4-(2-aminoethyl)aniline (3.96 mL, 30.0 mmol) in dichloromethane (150 mL) was added methyl trifluoroacetate (3.03 mL, 30.0 mmol). The resulting mixture was stirred under air at room temperature. After 18 h volatiles were removed under vacuum and the crude

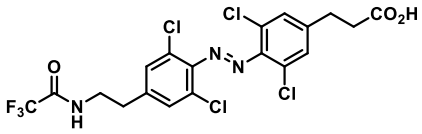
product was purified by silica column chromatography (CH_2Cl_2/MeOH, 100:0 to 98:2). The titles product was obtained as a yellow solid (6.13 g, 88%), mp (CH_2Cl_2): 91-93 ˚C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.77 (t, *J* = 6.6 Hz, ArC*H*$_2$CH$_2$NH, 2 H), 3.56 (q, *J* = 6.6 Hz, ArCH$_2$C*H*$_2$NH, 2 H), 3.64 (bs, ArN*H*$_2$, 2 H), 6.35 (bs, N*H*COCF$_3$, 1 H), 6.66 (d, *J* = 8.2 Hz, Ar*H*, 2 H), 6.97 (d, *J* = 8.2 Hz, Ar*H*, 2 H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 34.0 (Ar*C*H$_2$CH$_2$NH), 41.2 (ArCH$_2$*C*H$_2$NH), 115.5 (Ar*C*H), 115.8 (*J* = 286.0 Hz, NHCO*C*F$_3$), 127.2 (Ar*C*), 129.5 (Ar*C*H), 145.3 (Ar*C*), 157.1 (*J* = 36.5 Hz, NH*C*OCF$_3$) ppm; ^{19}F-NMR (376 MHz, $CDCl_3$) δ –76.1 ppm; IR ν_{max} (neat/cm^{-1}): 3328, 3065, 2948, 1712, 1627, 1559, 1519, 1208, 1158; HRMS calcd for $C_{10}H_{12}O_1N_2F_3$ [M+H]$^+$: 233.08962, found 233.08937.

(*E*)-3-(4-((4-(2-(2,2,2-Trifluoroacetamido)ethyl)phenyl)diazenyl)phenyl)propanoic acid (7)



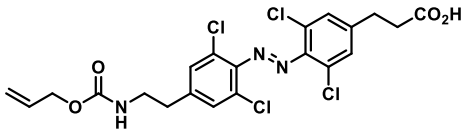
To a degassed solution of aqueous NH_4Cl (802 mg, 15.0 mmol in 9.7 mL H_2O) were added 2-methoxyethanol (77 mL) and 3-(4-nitrophenyl)propionic acid (**4**) (1.95 g, 10.0 mmol). The mixture was stirred under N_2 and cooled to 0 ºC before adding zinc powder (1.58 g, 25.0 mmol) portion wise over 15 minutes. The resulting mixture was stirred vigorously under N_2. After 17 h it was checked that no more zinc powder remained unreacted and the mixture was cooled to 0 ºC and a solution of $FeCl_3$ (4.87 g, 30.0 mmol) in a mixture of EtOH (59 mL) and H_2O (30.0 mL) was added over the course of 30 min. The mixture was stirred for a further 1.5 h at 0 ºC before the reaction mixture was diluted with H_2O (aprox. 100 mL) and extracted with Et_2O (4×200 mL). The organic layer was dried ($MgSO_4$) and concentrated under vacuum. The green solid residue was checked by ^{1}H-NMR to confirm clean full conversion into the nitroso intermediate. The crude nitroso compound was dissolved in AcOH (50 mL) and stirred at room temperature under a N_2 atmosphere before adding **6** (2.32 g, 10.0 mmol) portion wise. After 18 h volatiles were removed under vacuum and the crude product was triturated in as little MeOH as possible. The precipitate was filtered under vacuum, washed with small portions of cold MeOH and dried under vacuum. The title product was obtained as an orange solid (2.13 g, 54%), mp (MeOH): 248-250 ˚C. ^{1}H-NMR (400 MHz, DMSO-d$_6$) δ 2.60 (t, *J* = 7.6 Hz, ArCH$_2$C*H*$_2$CO$_2$H, 2 H), 2.86-2.96 (m, ArC*H*$_2$CH$_2$ × 2, 4 H), 3.49 (q, *J* = 6.2 Hz, ArCH$_2$C*H*$_2$NH, 2 H), 7.40-7.47 (m, Ar*H*, 4 H), 7.78-7.84 (m, Ar*H*, 4 H), 9.52 (t, *J* = 6.2 Hz, N*H*COCF$_3$, 1 H), 12.18 (bs, CO$_2$*H*, 1 H) ppm; ^{13}C-NMR (100 MHz, DMSO-d$_6$) δ 30.2 (Ar*C*H$_2$CH$_2$CO$_2$H), 33.9 (Ar*C*H$_2$CH$_2$NH), 34.8 (ArCH$_2$*C*H$_2$CO$_2$H), 40.3 (ArCH$_2$*C*H$_2$NH), 115.9 (*J* = 278.0 Hz, NHCO*C*F$_3$), 122.5 (Ar*C*H × 2), 129.3 (Ar*C*H), 129.7 (Ar*C*H), 142.4 (Ar*C*), 144.8 (Ar*C*), 150.4 (Ar*C*), 150.6 (Ar*C*), 156.1 (*J* = 35.8 Hz, NH*C*OCF$_3$), 173.6 (*C*O$_2$H) ppm; ^{19}F-NMR (376 MHz, DMSO-d$_6$) δ –74.4 ppm; IR ν_{max} (neat/cm^{-1}): 3299, 2922, 1698, 1561, 1299, 1163, 1106; HRMS calcd for $C_{19}H_{19}O_3N_3F_3$ [M+H]$^+$: 394.13730, found 394.13670.

(*E*)-3-(3,5-Dichloro-4-((2,6-dichloro-4-(2-(2,2,2-trifluoroacetamido)ethyl)phenyl)diazenyl)phenyl)propanoic acid (3, Tfa-CEBA-OH)



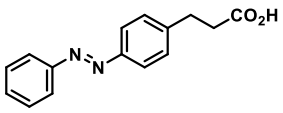
Previously reported conditions by Trauner and co-workers were re-optimised.[1] To a thick-wall glass reaction tube charged with **7** (1.75 g, 4.45 mmol), $Pd(OAc)_2$ (101 mg, 0.450 mmol) and NCS (3.56 g, 27.6 mmol) under N_2 was added acetic acid (89 mL) before the tube was sealed and the mixture stirred at 140 ºC. After 19 h the mixture was allowed to cool to room temperature. Volatiles were removed under vacuum and the residue was dissolved in EtOAc (300 mL). The solution was washed with H_2O (8 × 250 mL), dried ($MgSO_4$) and concentrated. The crude product was purified by silica column chromatography (hexane/EtOAc, 50:50 to 0:100). The title product was obtained as a red solid (2.00 g, 85%), mp (CH_2Cl_2): 189-192 ˚C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 2.65 (t, *J* = 7.6 Hz, $ArCH_2CH_2CO_2H$, 2 H), 2.85-2.94 (m, $ArCH_2CH_2$ × 2, 4 H), 3.51 (q, *J* = 6.2 Hz, $ArCH_2CH_2NH$, 2 H), 7.55 (s, Ar*H*, 2 H), 7.60 (s, Ar*H*, 2 H), 9.52 (t, *J* = 6.2 Hz, N*H*$COCF_3$, 1 H), 12.26 (bs, CO_2H, 1 H) ppm; ^{13}C-NMR (100 MHz, DMSO-d_6) δ 29.4 ($ArCH_2CH_2CO_2H$), 33.0 ($ArCH_2CH_2NH$), 34.2 ($ArCH_2CH_2CO_2H$), 39.8 ($ArCH_2CH_2NH$), 115.8 (*J* = 286.5 Hz, $NHCOCF_3$), 126.0 (Ar*C*Cl), 126.0 (Ar*C*Cl), 129.8 (Ar*C*H), 130.2 (Ar*C*H), 142.8 (Ar*C*), 144.5 (Ar*C*), 144.8 (Ar*C*), 145.2 (Ar*C*), 156.3 (*J* = 35.8 Hz, $NHCOCF_3$), 173.4 (CO_2H) ppm; ^{19}F-NMR (376 MHz, DMSO-d_6) δ –74.4 ppm; IR ν_{max} (neat/cm^{-1}): 3318, 2922, 1692, 1551, 1401, 1176, 1066; HRMS calcd for $C_{19}H_{15}O_3N_3Cl_4F_3$ $[M+H]^+$: 529.98141, found 529.98191.

(*E*)-3-(4-((4-(2-(((Allyloxy)carbonyl)amino)ethyl)-2,6-dichlorophenyl)diazenyl)-3,5-dichlorophenyl)propanoic acid (Alloc-CEBA-OH)



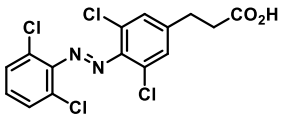
To a flask charged with a solution of **3** (122 mg, 0.230 mmol) in THF (0.12 mL) was added 10% aqueous NaOH (0.46 mL, 1.15 mmol). The resulting mixture was stirred at room temperature under air. After 20 min the mixture was cooled to 0 ºC before adding allyl chloroformate (30 μL, 0.276 mmol). The flask was sealed under air and the reaction mixture was stirred for 25 min at 0 ºC. The reaction was quenched with 2 M aqueous HCl (25 mL) and extracted with EtOAc (3 × 25 mL). The organic layer was washed with H_2O (3 × 25 mL), dried ($MgSO_4$) and concentrated under vacuum. Analysis of the crude material confirmed full conversion into the desired product and was used without further purification.

(*E*)-3-(4-(Phenyldiazenyl)phenyl)propanoic acid (S1)



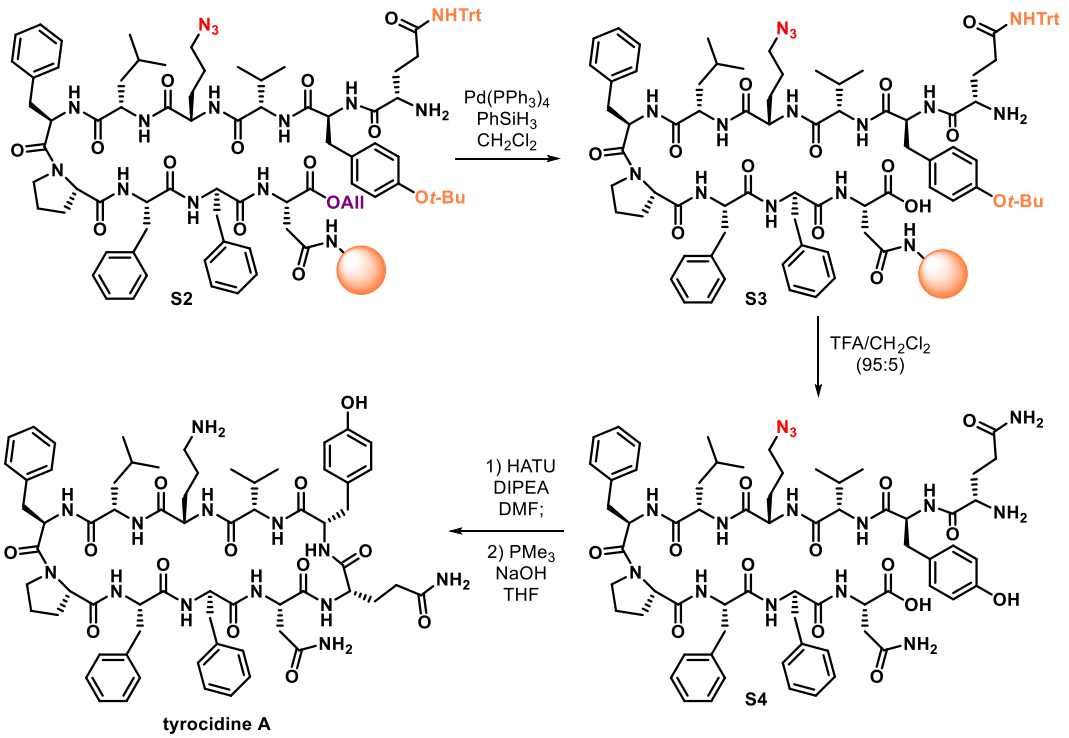
To a degassed solution of aqueous NH_4Cl (401 mg, 7.50 mmol in 9.0 mL H_2O) were added 2-methoxyethanol (38 mL) and 3-(4-nitrophenyl)propionic acid (976 mg, 10.0 mmol). The mixture was stirred under N_2 and cooled to 0 ºC before adding zinc powder (792 mg, 12.5 mmol) portion wise over 15 minutes. The resulting mixture was stirred vigorously under N_2. After 17 h it was checked that no more zinc powder remained unreacted and the mixture was cooled to 0 ºC and a solution of $FeCl_3$ (2.43 g, 15.0 mmol) in a mixture of EtOH (29 mL) and H_2O (15.0 mL) was added over the course of 30 min. The mixture was stirred for a further 1.5 h at 0 ºC before the reaction mixture was diluted with H_2O (aprox. 50 mL) and extracted with Et_2O (4×100 mL). The organic layer was dried ($MgSO_4$) and concentrated under vacuum. The green solid residue was checked by ^{1}H-NMR to confirm clean full conversion into the nitroso intermediate. The crude nitroso compound was dissolved in AcOH (25 mL) and stirred at room temperature under a N_2 atmosphere before adding aniline (456 mg, 5.00 mmol) dropwise. After 22 h volatiles were removed under vacuum and the crude product was purified by silica column chromatography (hexane/EtOAc, 70:30). The title product was obtained as an orange solid (550 mg, 43%), mp (CH_2Cl_2): 149-152 ˚C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.76 (t, *J* = 7.6 Hz, $CH_2CH_2CO_2H$, 2 H), 3.06 (t, *J* = 7.6 Hz, $CH_2CH_2CO_2H$, 2 H), 7.38 (d, *J* = 8.4 Hz, Ar*H*, 2 H), 7.44-7.56 (m, Ar*H*, 3 H), 7.87 (d, *J* = 8.4 Hz, Ar*H*, 2 H), 7.90-7.93 (m, Ar*H*, 2 H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 30.5 ($CH_2CH_2CO_2H$), 35.1 ($CH_2CH_2CO_2H$), 122.8 (Ar*C*H), 123.1 (Ar*C*H), 129.0 (Ar*C*H), 129.1 (Ar*C*H), 130.9 (Ar*C*H), 143.5 (Ar*C*), 151.4 (Ar*C*), 152.7 (Ar*C*), 177.4 (CO_2H) ppm; IR ν_{max} (neat/cm^{-1}): 2921, 1694, 1435, 1400, 1300, 1247, 1222, 1151; HRMS calcd for $C_{15}H_{15}O_2N_2$ $[M+H]^+$: 255.11280, found 255.11299.

(*E*)-3-(3,5-Dichloro-4-((2,6-dichlorophenyl)diazenyl)phenyl)propanoic acid (12)



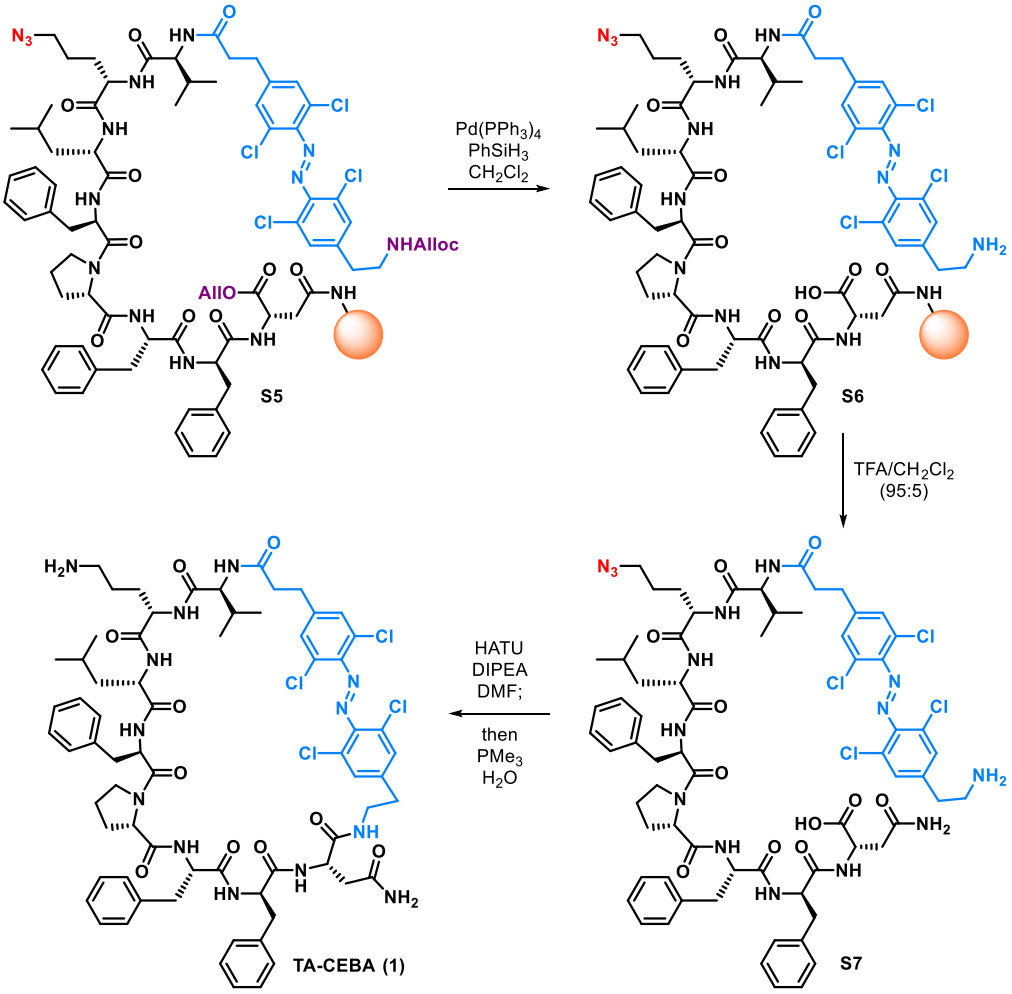
To a thick-wall glass reaction tube charged with **S1** (400 mg, 1.57 mmol), $Pd(OAc)_2$ (35 mg, 0.157 mmol) and NCS (1.26 g, 9.44 mmol) under N_2 was added acetic acid (63 mL) before the tube was sealed and the mixture stirred at 140 ºC. After 23 h the mixture was allowed to cool to room temperature. Volatiles were removed under vacuum and the residue was dissolved in EtOAc (100 mL). The solution was washed with H_2O (8 × 100 mL), dried ($MgSO_4$) and concentrated. The crude product was purified by silica column chromatography (EtOAc). The title product was obtained as a red solid (520 mg, 84%), mp (CH_2Cl_2): decomposes above 150 ˚C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.75 (t , *J* = 7.6 Hz, $CH_2CH_2CO_2H$, 2 H), 3.00 (t , *J* = 7.6 Hz, $CH_2CH_2CO_2H$, 2 H), 7.26 (dd, *J* = 8.4, 8.0 Hz, Ar*H*, 1 H), 7.34 (s, Ar*H*, 2 H), 7.46 (d, *J* = 8.0 Hz, Ar*H*, 2 H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 29.7 ($CH_2CH_2CO_2H$), 34.5 ($CH_2CH_2CO_2H$), 127.2 (Ar*C*Cl), 127.6 (Ar*C*Cl), 129.4 (Ar*C*H), 129.4 (Ar*C*H), 129.5 (Ar*C*H), 142.9 (Ar*C*), 145.8 (Ar*C*), 147.8 (Ar*C*), 176.7 (CO_2H) ppm; IR ν_{max} (neat/cm^{-1}): 2921, 2852, 1698, 1432, 1402, 1303, 1216; HRMS calcd for $C_{15}H_{11}O_2N_2Cl_4$ $[M+H]^+$: 390.95691, found 390.95758.

Tyrocidine A (TA)



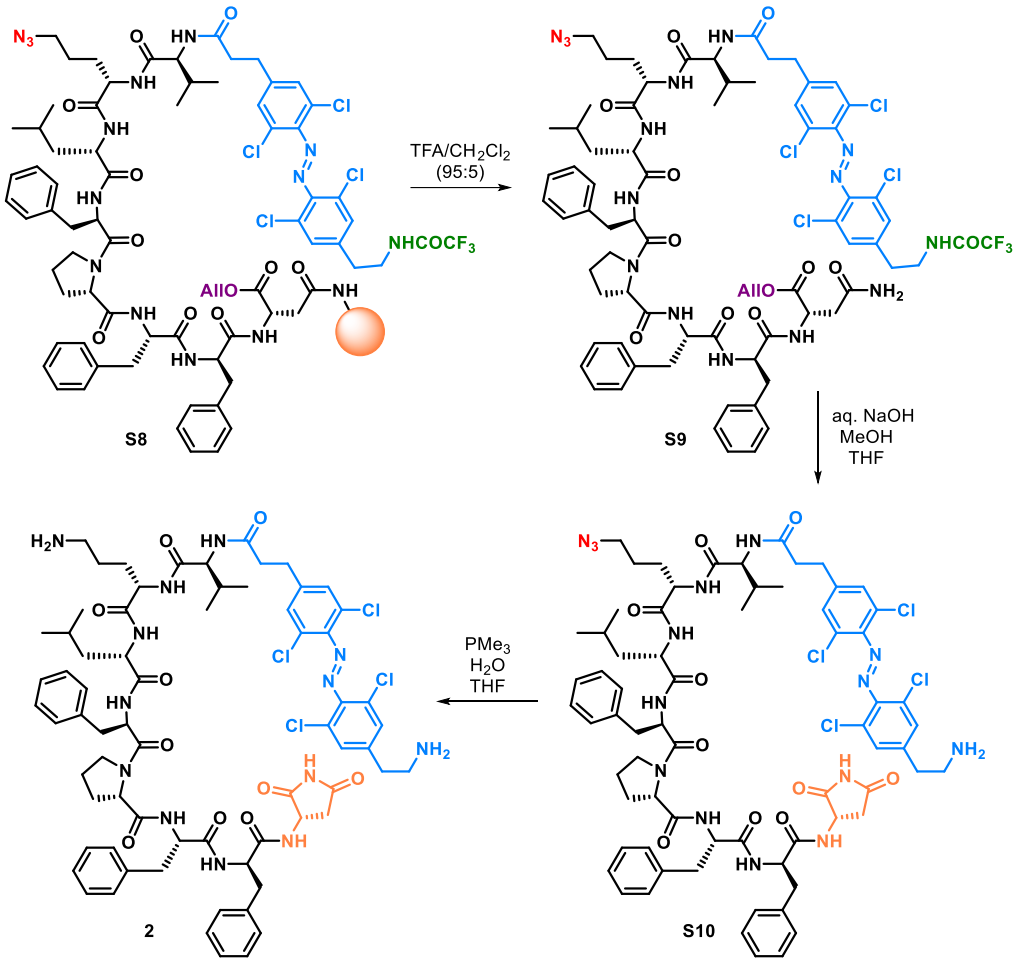
Linear precursor of tyrocidine A was obtained using automated microwave-assisted solid phase peptide synthesis (see above). The protected linear peptide **S2** (0.100 mmol) was treated with a solution of $Pd(PPh_3)_4$ (12 mg, 0.010 mmol) and $PhSiH_3$ (247 μL, 2.00 mmol) in CH_2Cl_2 (2.0 mL) at room temperature under air for 15 min. The resulting *N*- and *C*-termini deprotected peptide **S3** was cleaved from the resin using TFA/CH_2Cl_2 (95:5, 5 × 1 h) and then concentrated under reduced pressure. The crude product **S4** was dissolved in DMF (5.3 mL) before adding HATU (20 mg, 0.053 mmol) and diisopropylethylamine (28 μL, 0.160 mmol). The resulting mixture was stirred at room temperature under air. After 16 h the reaction mixture was cooled in an ice bath and 1.0 M PMe_3 in THF (133 μL, 0.133 mmol) and 5% aqueous NaOH (300 μL) were added. The resulting mixture was allowed to warm to room temperature. After 2 h, volatiles were removed and the residue was purified by preparative HPLC. Tyrocidine A (**TA**) (14 mg, 11%), was obtained as a white solid. HRMS calcd for $C_{66}H_{89}O_{13}N_{13}$ $[M+2H]^{+2}$: 635.83459, found 635.83450.

Photoswitchable cyclic peptide 1



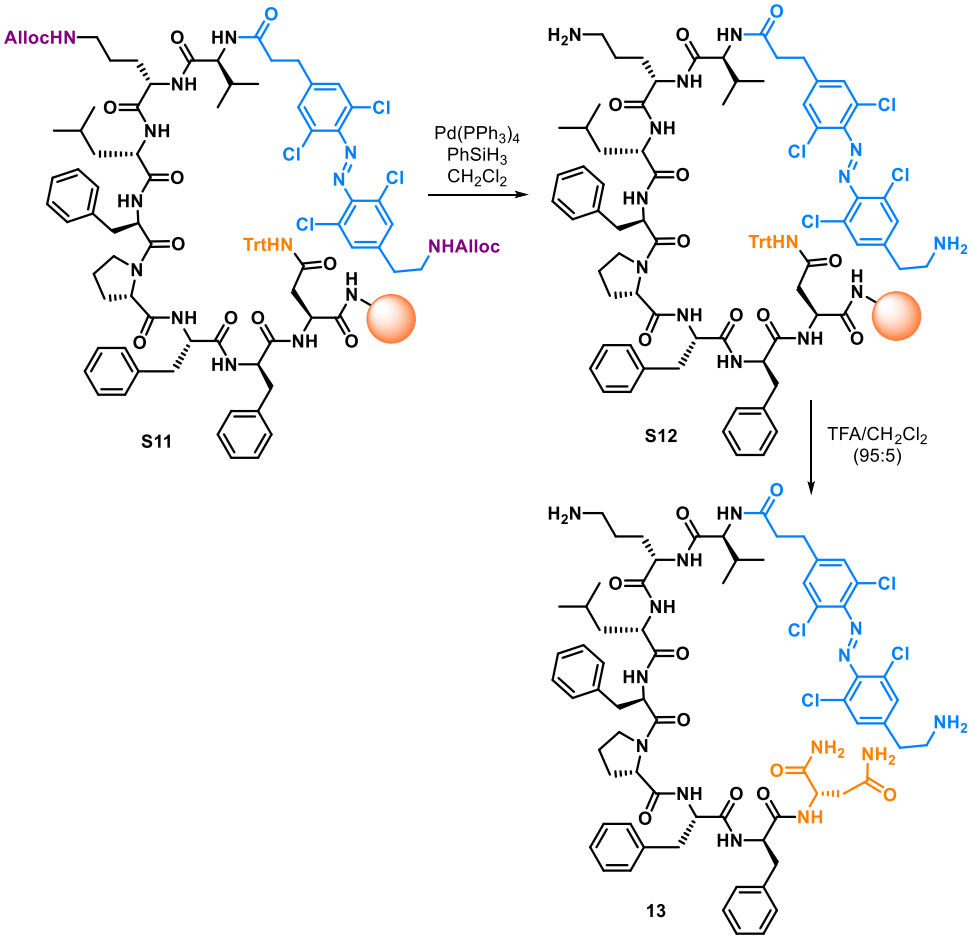
Linear precursor **S5** was obtained using manual solid phase peptide synthesis (see above). Resin-bound **S5** (0.100 mmol) was treated with a solution of $Pd(PPh_3)_4$ (12 mg, 0.010 mmol) and $PhSiH_3$ (247 µL, 2.0 mmol) in CH_2Cl_2 (2.0 mL) at room temperature under air for 15 min. The resulting *N*- and *C*-termini deprotected peptide **S6** was cleaved from the resin using TFA/CH_2Cl_2 (95:5, 5 × 1 h) and then concentrated under reduced pressure. Crude **S7** (0.100 mmol) was dissolved in DMF (20 mL) before adding HATU (76 mg, 0.200 mmol) and diisopropylethylamine (107 µL, 0.600 mmol). The resulting mixture was stirred at room temperature under air. After 16 h the mixture was cooled in an ice bath before adding 1.0 M PMe_3 in THF (0.5 mL, 0.500 mmol) and H_2O (480 µL). The resulting mixture was allowed to warm to room temperature. After 5 h, volatiles were removed and the residue was purified by preparative HPLC. TA-CEBA (**1**) (2 mg, 1.5% overall), was obtained as an orange solid. HRMS calcd for $C_{69}H_{84}O_{10}N_{13}Cl_4$ $[M+H]^+$: 1394.52127, found 1394.52254.

Photoswitchable linear peptide 2



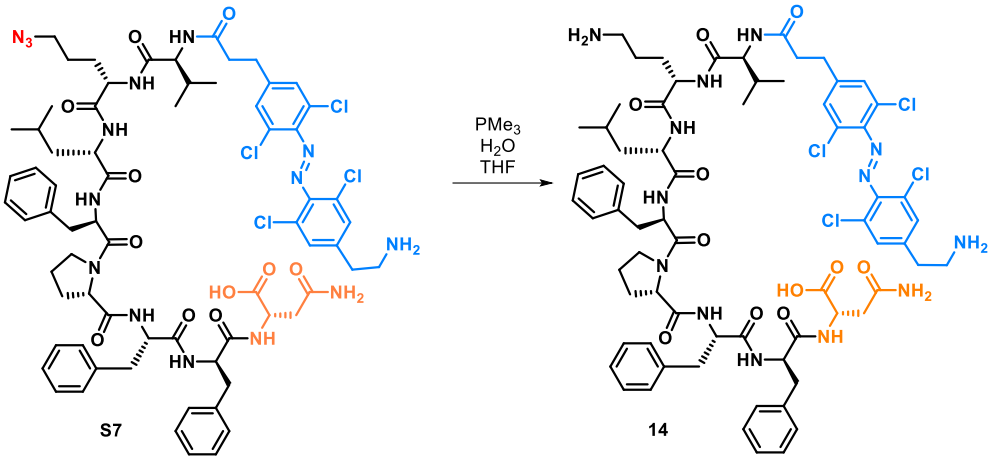
Linear precursor **S8** was obtained using automated microwave-assisted solid phase peptide synthesis (see above). Resin-bound **S8** (0.126 mmol) was treated with TFA/CH_2Cl_2 (95:5, 5 × 1 h). Volatiles were removed under vacuum to obtain crude **S9** as an orange solid. The crude was dissolved in THF (21 mL) and MeOH (14 mL) and cooled to 0 ºC before adding 2.5% aqueous NaOH (7 mL). The mixture was allowed to warm to room temperature and stirred for 3 h. The reaction mixture was diluted with brine (200 mL) and extracted with EtOAc (4 × 150 mL). The organic layer was dried ($MgSO_4$) and concentrated to obtain crude **S10** as an orange solid. A portion of crude **S10** (30 mg, 0.021 mmol) was dissolved in THF (590 µL) and cooled to 0 ºC before adding 1.0 M PMe_3 in THF (63 µL, 0.063 mmol) and H_2O (100 µL). The resulting mixture was allowed to warm to room temperature. After 1.5 h, volatiles were removed and the residue was purified by preparative HPLC. **2** was obtained as an orange solid (6.3 mg, 21% for the last step). HRMS calcd for $C_{69}H_{85}O_{10}N_{13}Cl_4$ $[M+2H]^{2+}$: 697.76428, found 697.76402.

Photoswitchable linear peptide 13



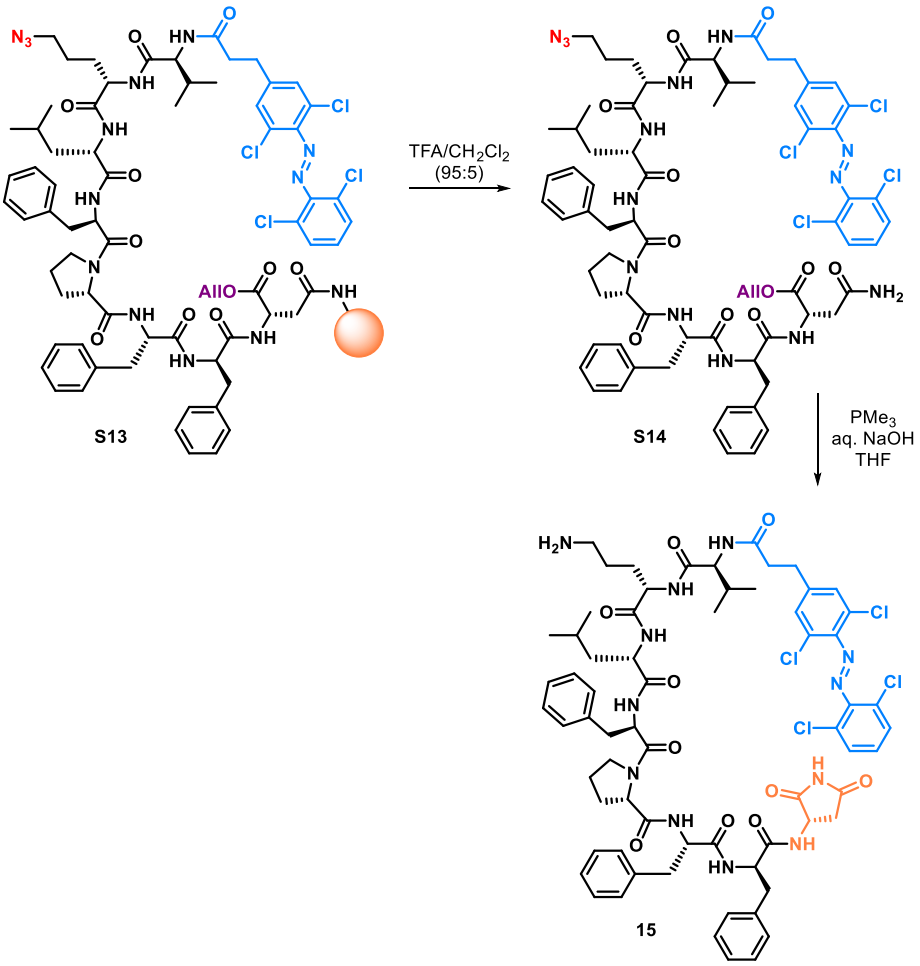
Linear precursor **S11** was obtained using automated microwave-assisted solid phase peptide synthesis (see above). Resin-bound **S11** (0.100 mmol) was treated with a solution of $Pd(PPh_3)_4$ (12 mg, 0.010 mmol) and $PhSiH_3$ (247 μL, 2.0 mmol) in CH_2Cl_2 (2.0 mL) at room temperature under air (2 × 15 min). The resulting *N*- and *C*-termini deprotected peptide **S12** was cleaved from the resin using TFA/CH_2Cl_2 (95:5, 5 × 1 h) and then concentrated under reduced pressure. The crude was added onto cold *t*-BuOMe and the supernatant was decanted after centrifugation to recover the crude cleaved peptide as an orange precipitate. Purification by preparative HPLC yielded **13** as an orange solid (14.0 mg, 10% overall). HRMS calcd for $C_{69}H_{87}O_{10}N_{14}Cl_4$ $[M+H]^+$: 1411.54782, found 1411.54756.

Photoswitchable linear peptide 14



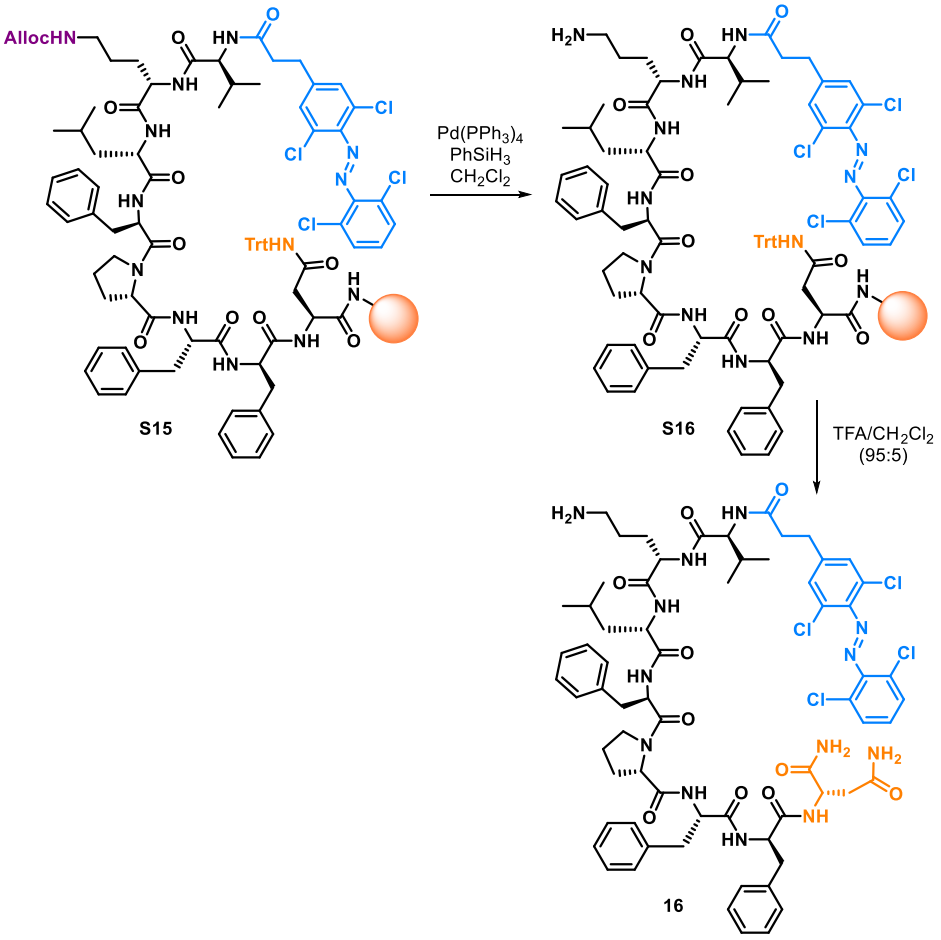
To a stirring solution of crude **S7** (50 mg, see synthesis of **1** above) in THF (960 µL) at 0 ºC were added 1.0 M PMe_3 in THF (104 µL, 0.104 mmol) and H_2O (165 µL). The resulting mixture was allowed to warm to room temperature. After 5 h volatiles were removed and the crude product was purified by preparative HPLC. **14** was obtained as an orange solid (2.5 mg, 5% overall). HRMS calcd for $C_{69}H_{86}O_{11}N_{13}Cl_4$ $[M+H]^+$: 1412.53184, found 1412.3296.

Photoswitchable linear peptide 15



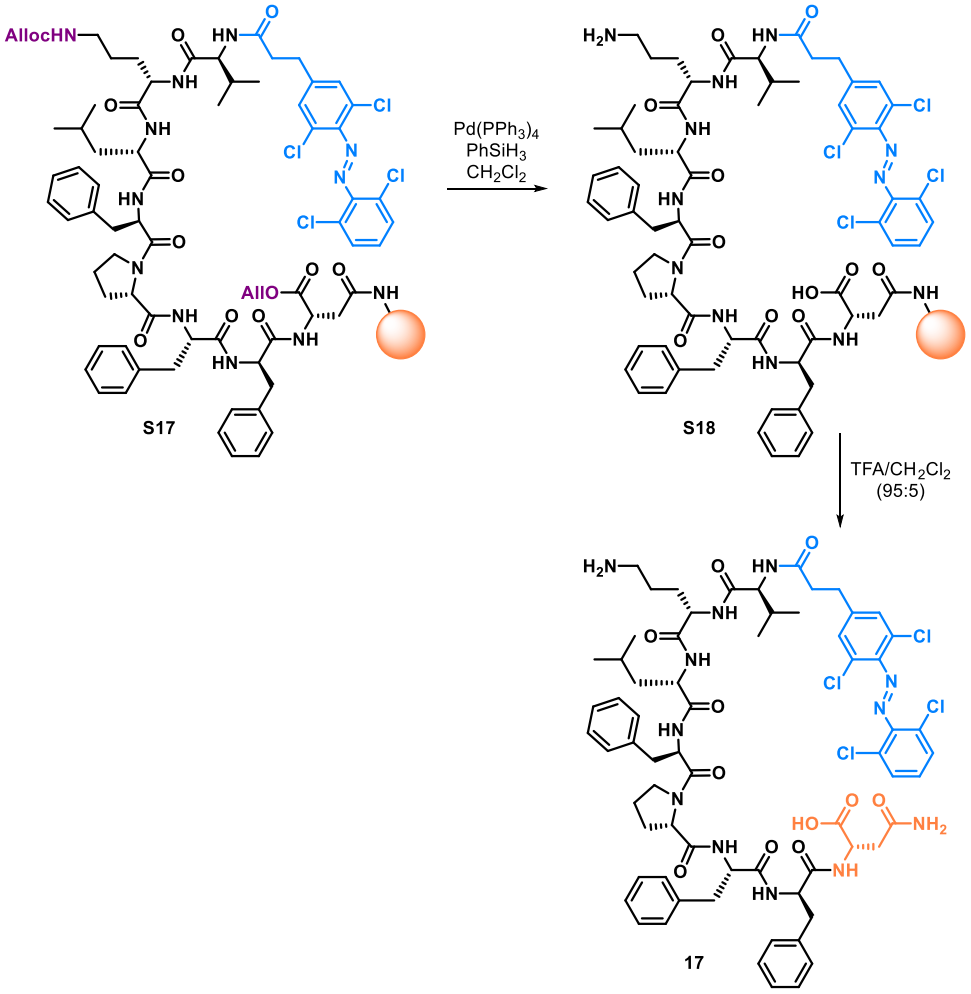
Linear precursor **S13** was obtained using automated microwave-assisted solid phase peptide synthesis (see above). Resin-bound **S13** was treated with TFA/CH_2Cl_2 (95:5, 5 × 1 h). Volatiles were removed under vacuum to obtain crude **S14** as an orange solid. A portion of crude **S14** (60 mg, 0.0417 mmol) was dissolved in THF and cooled in an ice bath before adding 2.5% aqueous NaOH (0.2 mL) and 1.0 M PMe_3 in THF (125 µL, 0.125 mmol). The resulting mixture was allowed to warm to room temperature. After stirring for 2 h, volatiles were removed and the residue was purified by preparative HPLC. **15** was obtained as an orange solid (7 mg, 12% overall). HRMS calcd for $C_{67}H_{79}O_{10}N_{12}Cl_4$ $[M+H]^+$: 1351.47907, found 1351.47997.

Photoswitchable linear peptide 16



Linear precursor **S15** was obtained using automated microwave-assisted solid phase peptide synthesis (see above). Resin-bound **S15** (0.100 mmol) was treated with a solution of $Pd(PPh_3)_4$ (12 mg, 0.010 mmol) and $PhSiH_3$ (247 μL, 2.0 mmol) in CH_2Cl_2 (2.0 mL) at room temperature under air (2 × 15 min). The resulting *N*- and *C*-termini deprotected peptide **S16** was cleaved from the resin using TFA/CH_2Cl_2 (95:5, 5 × 1 h) and then concentrated under reduced pressure. The crude product was purified by preparative HPLC to yield **16** as an orange solid (39 mg, 28% overall). HRMS calcd for $C_{67}H_{82}O_{10}N_{13}Cl_4$ $[M+H]^+$: 1368.50562, found 1368.50559.

Photoswitchable linear peptide 17

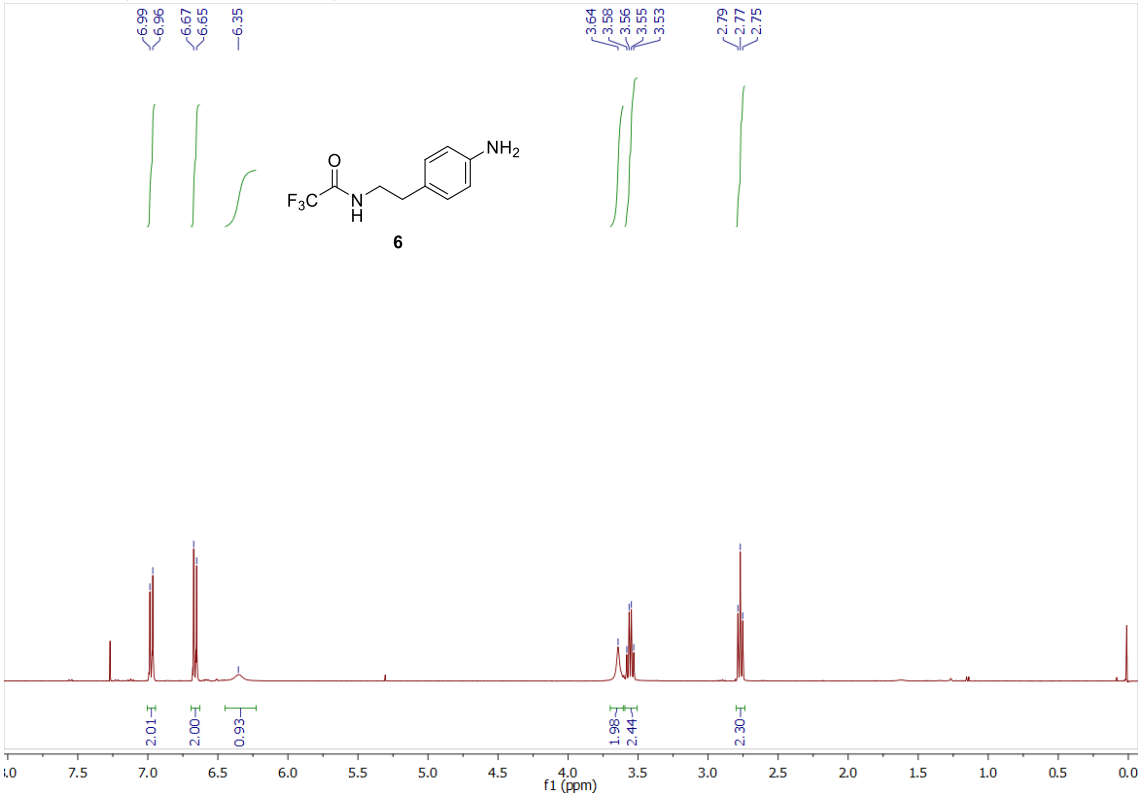


Linear precursor **S17** was obtained using automated microwave-assisted solid phase peptide synthesis (see above). Resin-bound **S17** (0.100 mmol) was treated with a solution of $Pd(PPh_3)_4$ (12 mg, 0.010 mmol) and $PhSiH_3$ (247 μL, 2.0 mmol) in CH_2Cl_2 (2.0 mL) at room temperature under air (2 × 15 min). The resulting *N*- and *C*-termini deprotected peptide **S18** was cleaved from the resin using TFA/CH_2Cl_2 (95:5, 5 × 1 h) and then concentrated under reduced pressure. The crude product was purified by preparative HPLC to yield **17** as an orange solid (29 mg, 21% overall). HRMS calcd for $C_{67}H_{81}O_{11}N_{12}Cl_4$ $[M+H]^+$: 1369.48964, found 1369.49025.

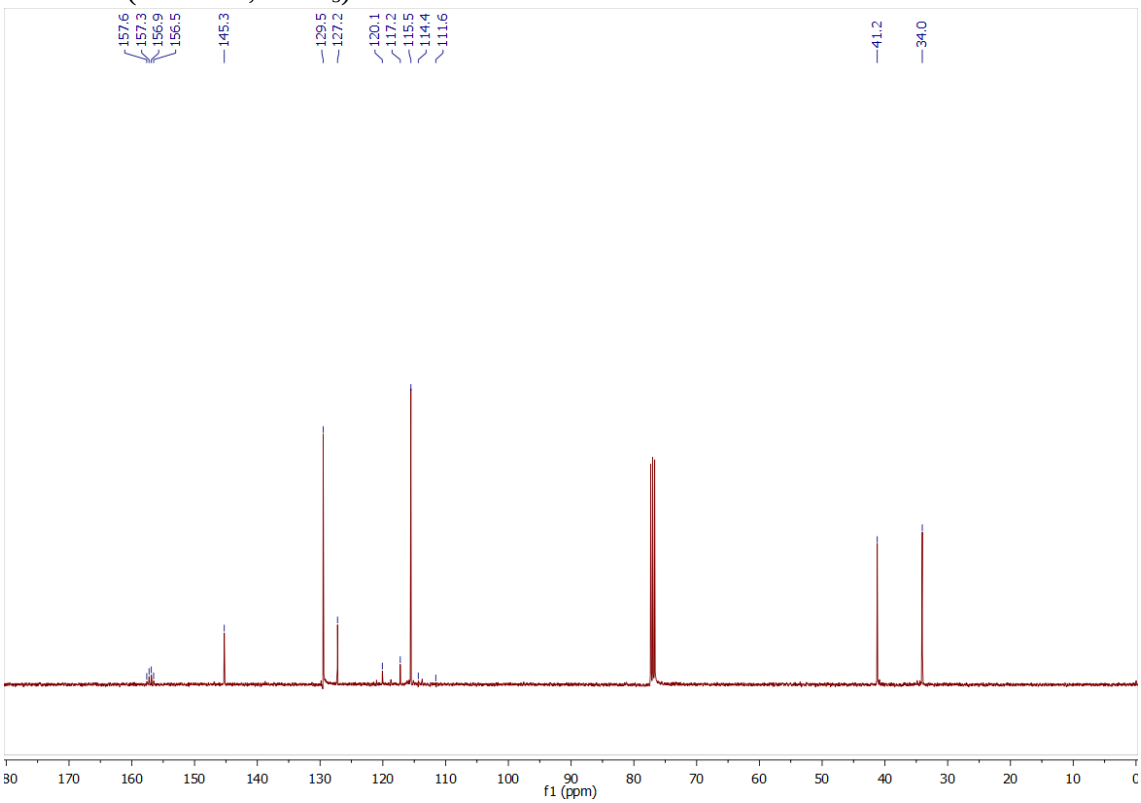
4. NMR Spectra

N-(4-Aminophenethyl)-2,2,2-trifluoroacetamide (6)

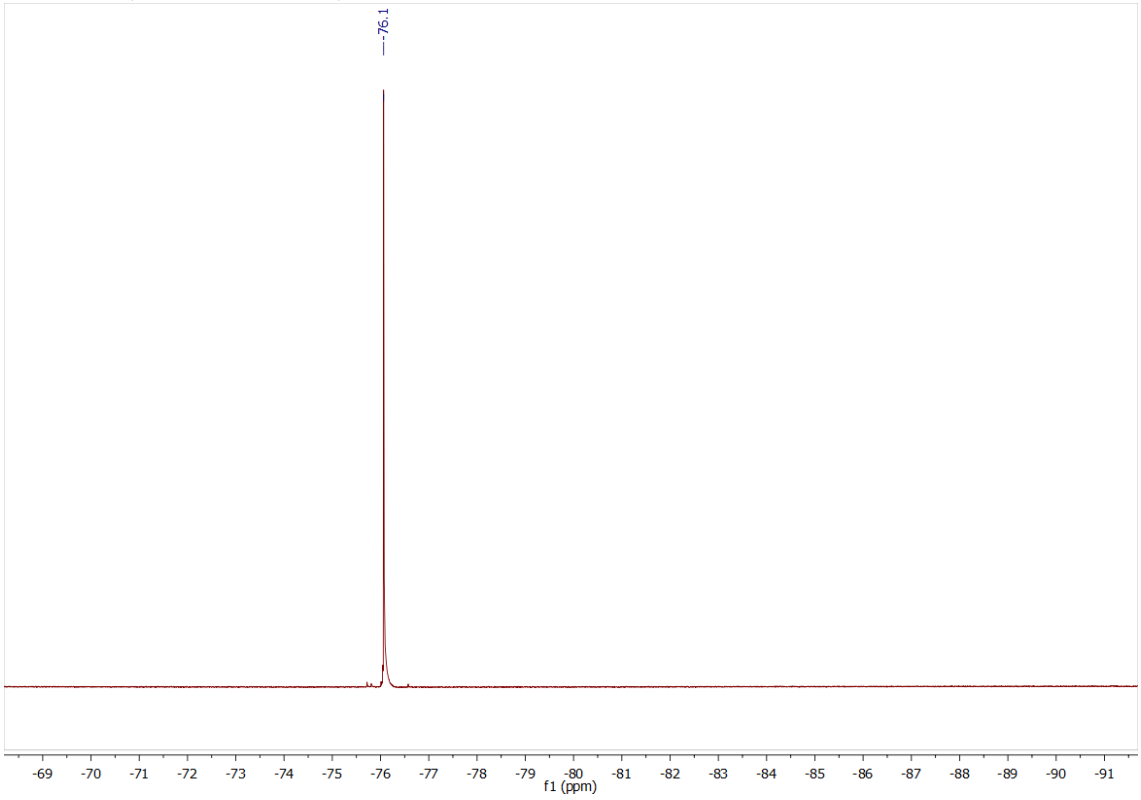
^{1}H-NMR (400 MHz, $CDCl_3$)



^{13}C-NMR (100 MHz, $CDCl_3$)

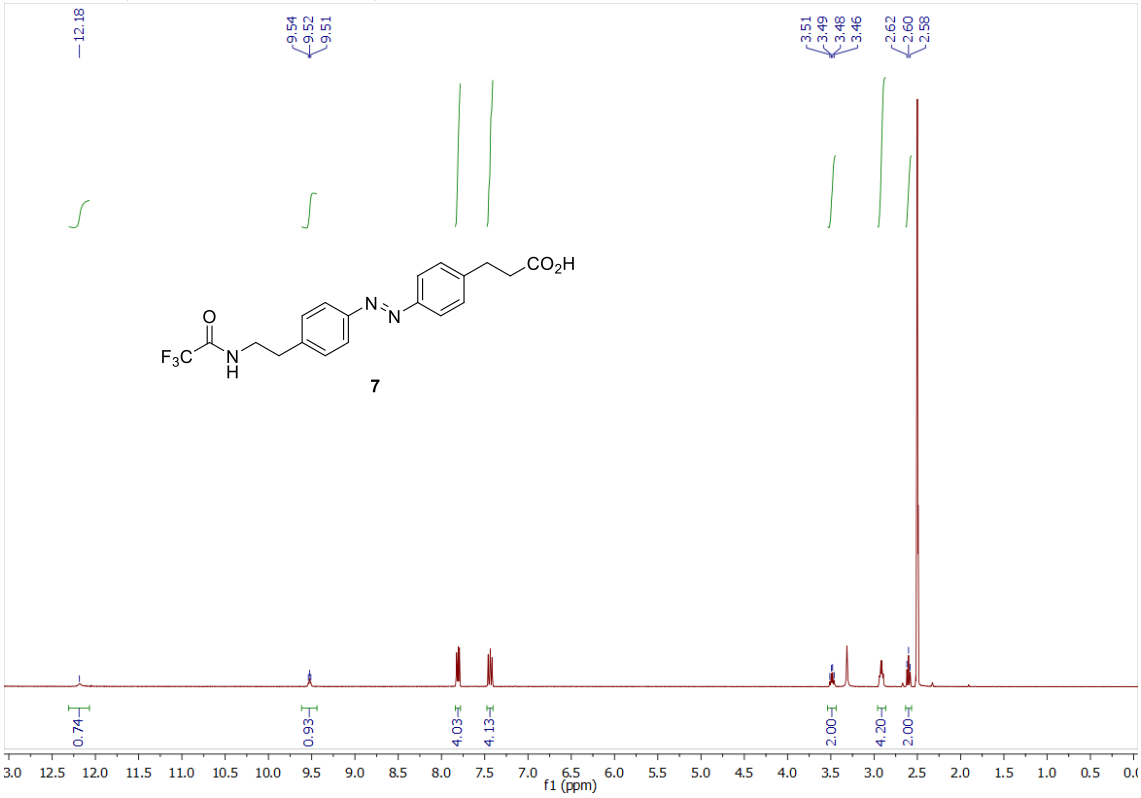


^{19}F-NMR (376 MHz, $CDCl_3$)

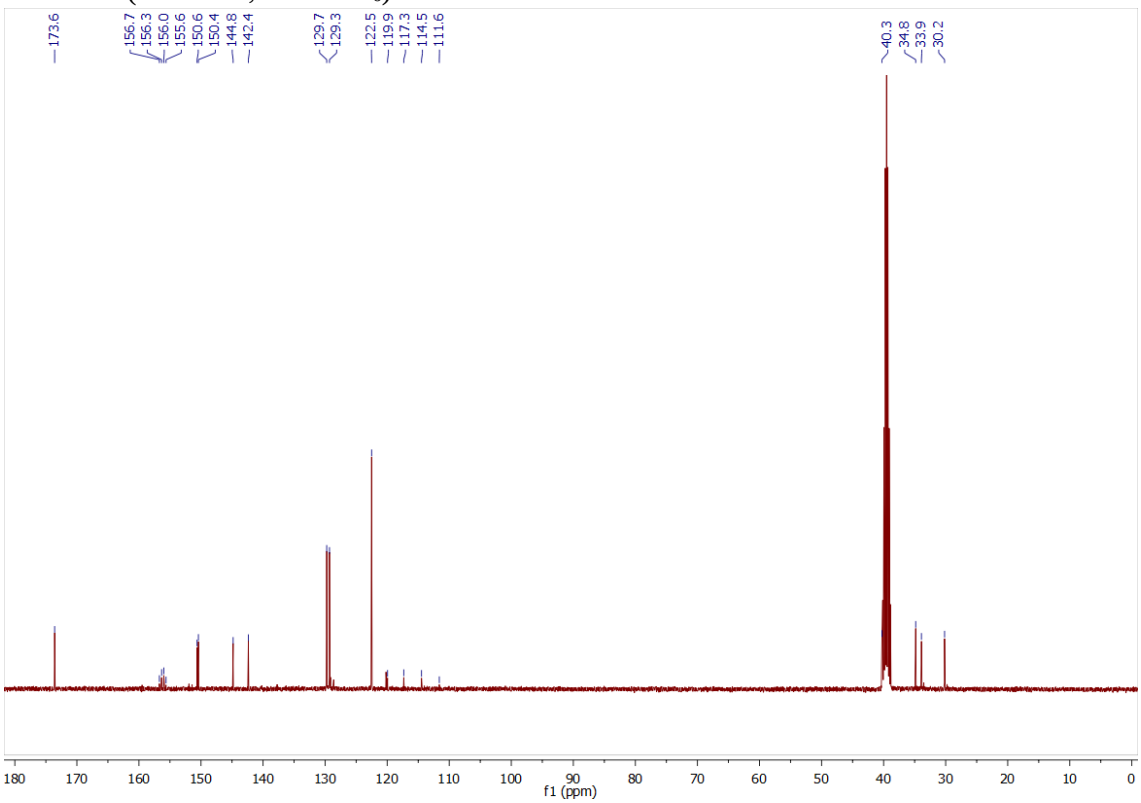


(*E*)-3-(4-((4-(2-(2,2,2-Trifluoroacetamido)ethyl)phenyl)diazenyl)phenyl)propanoic acid (7)

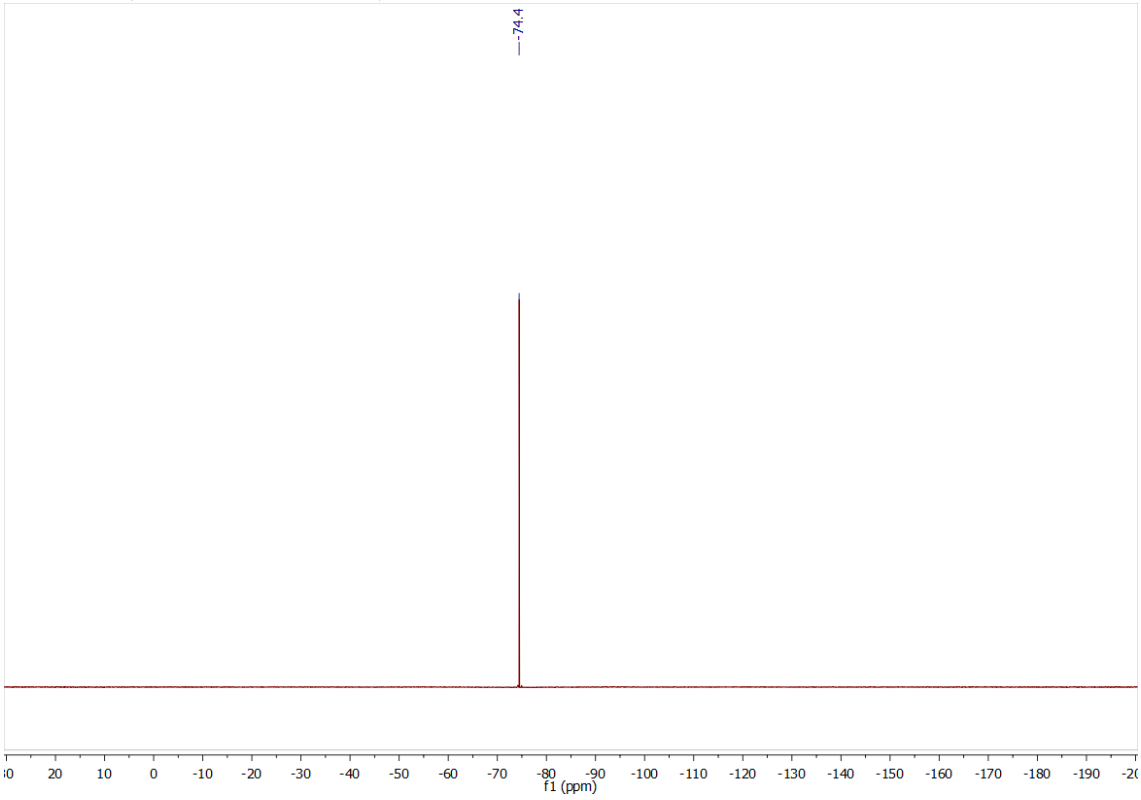
^{1}H-NMR (400 MHz, DMSO-d$_6$)



^{13}C-NMR (100 MHz, DMSO-d$_6$)

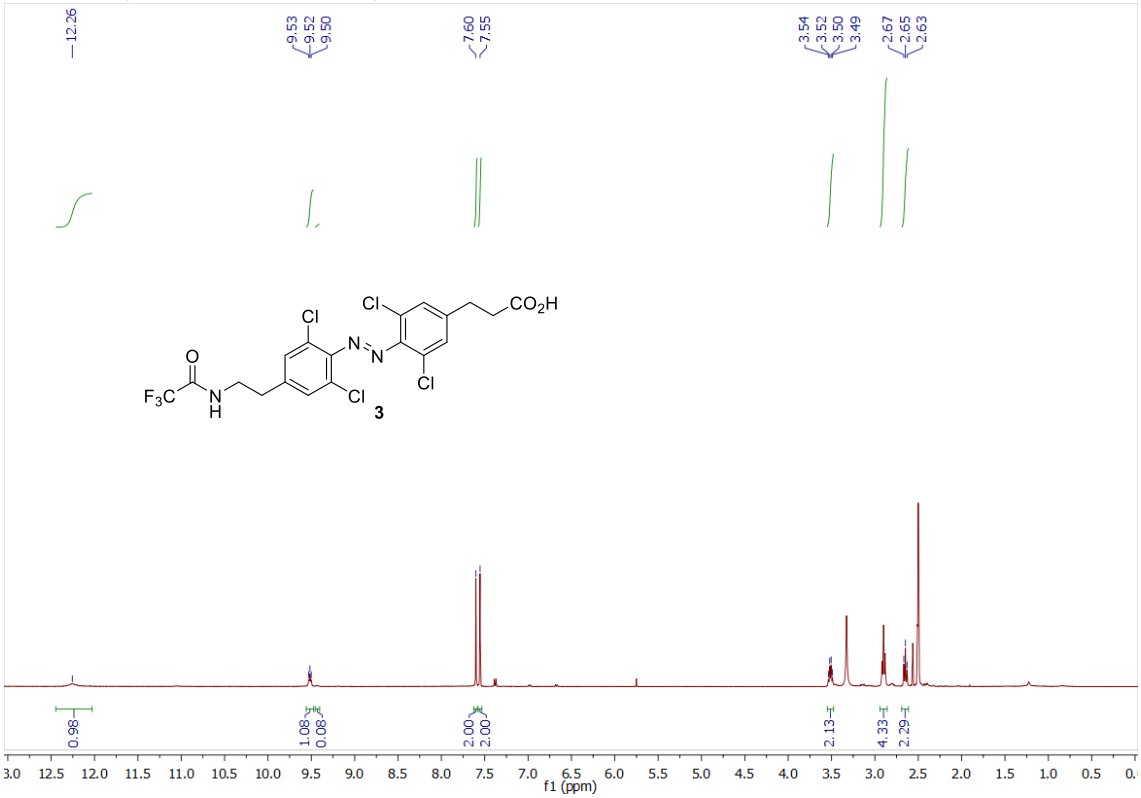


^{19}F-NMR (376 MHz, DMSO-d_6)

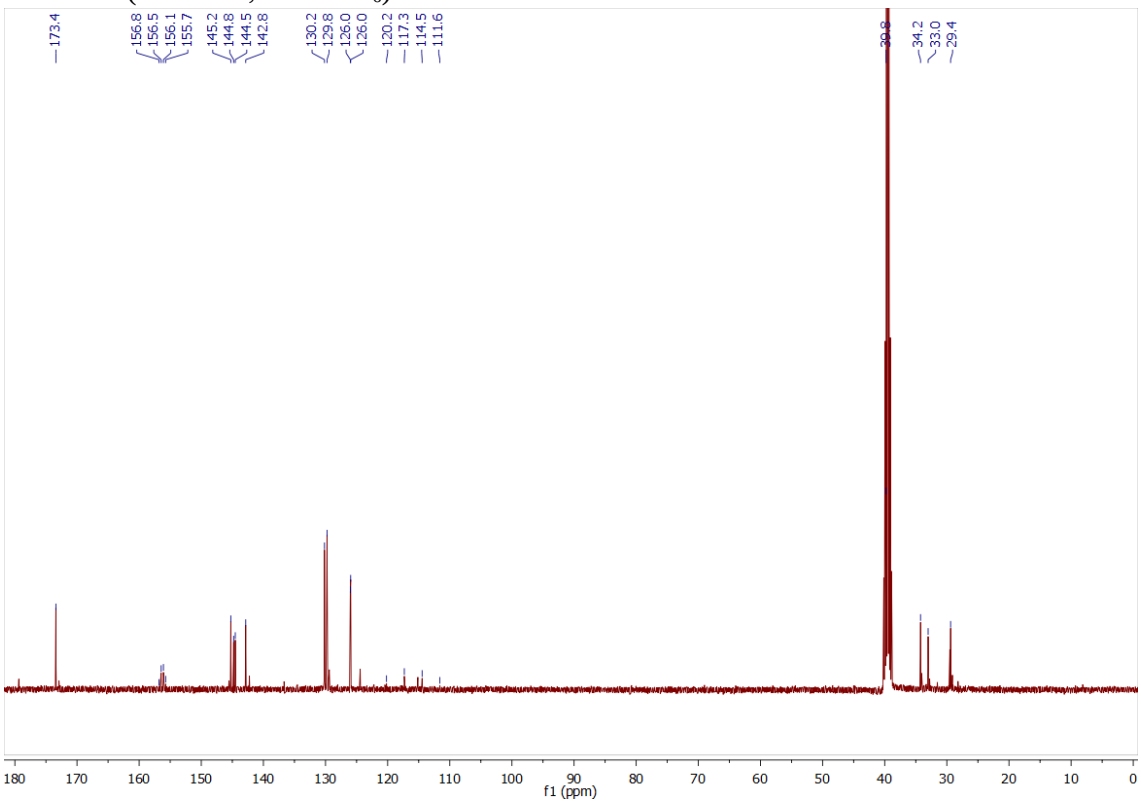


(*E*)-3-(3,5-Dichloro-4-((2,6-dichloro-4-(2-(2,2,2-trifluoroacetamido)ethyl)phenyl)diazenyl)phenyl)propanoic acid (3)

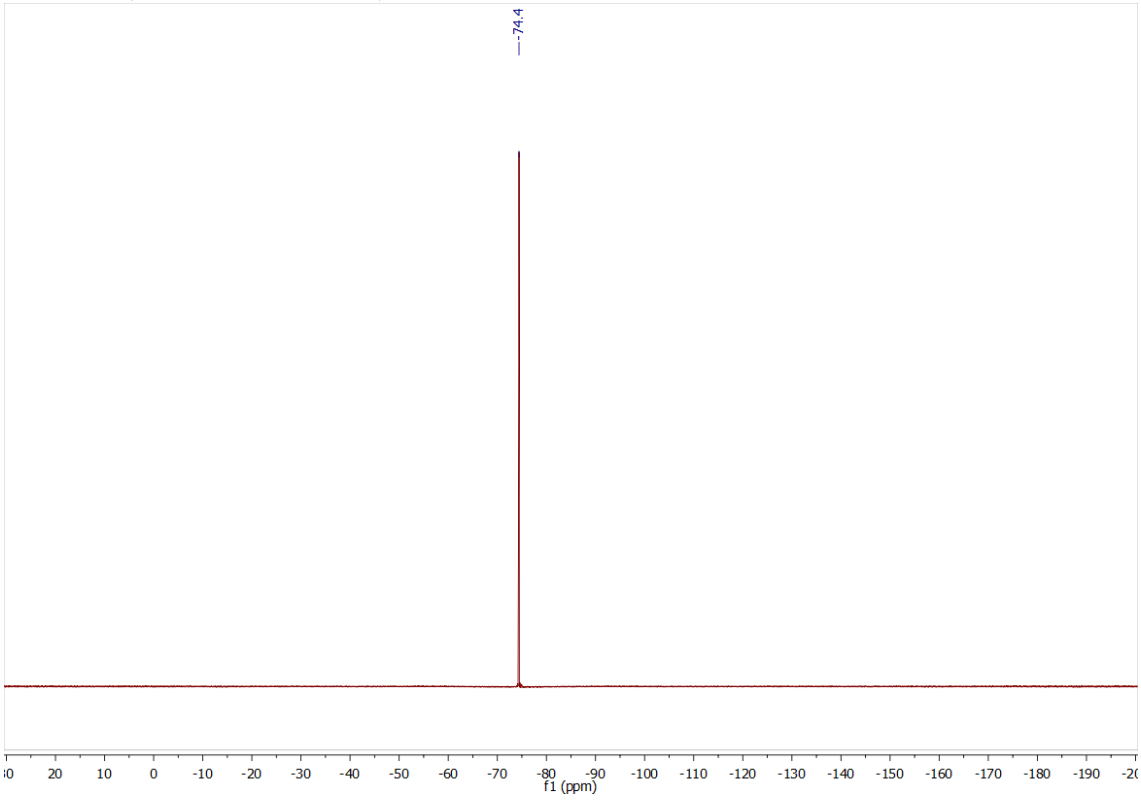
^{1}H-NMR (400 MHz, DMSO-d$_6$)



^{13}C-NMR (100 MHz, DMSO-d$_6$)

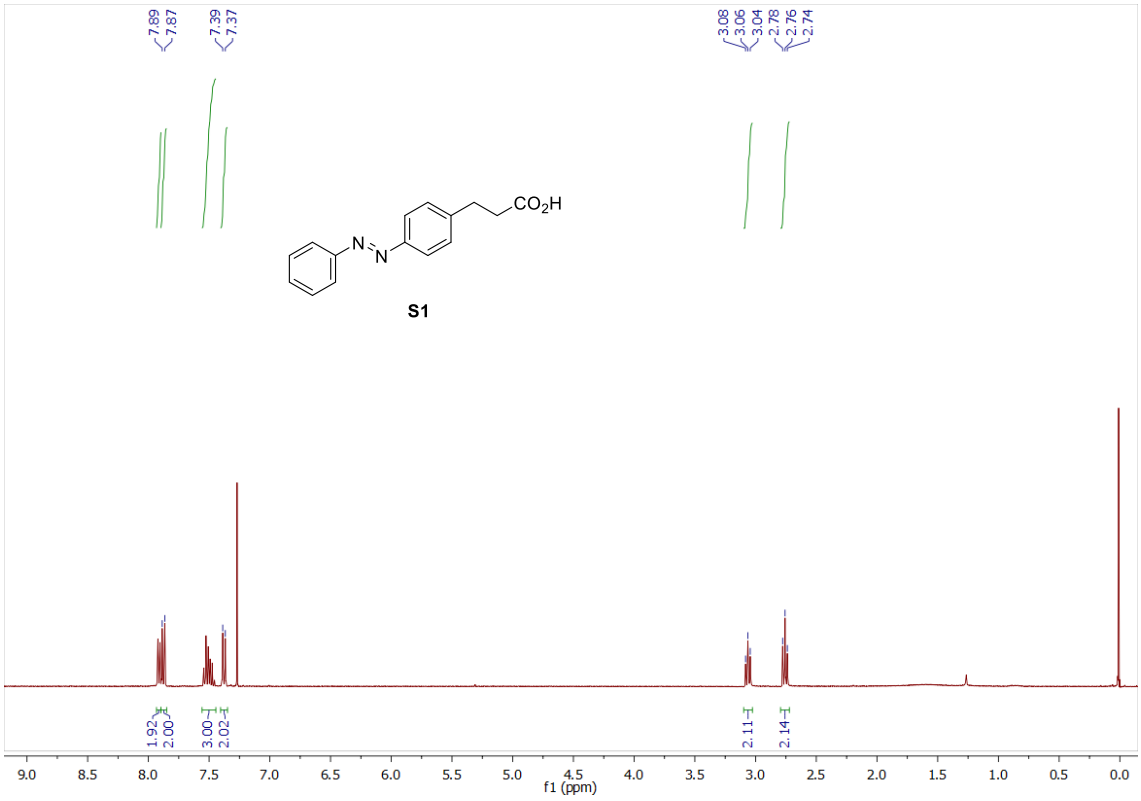


^{19}F-NMR (376 MHz, DMSO-d_6)

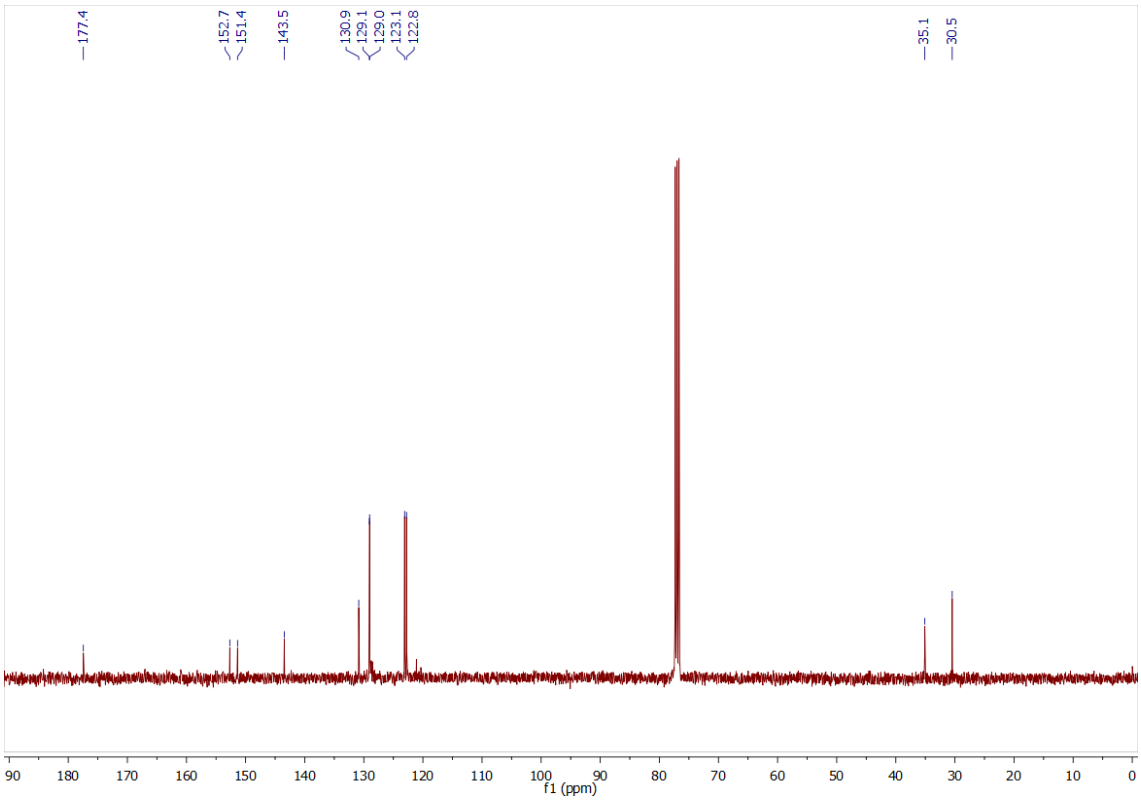


(*E*)-3-(4-(Phenyldiazenyl)phenyl)propanoic acid (S1)

^{1}H-NMR (400 MHz, $CDCl_3$)

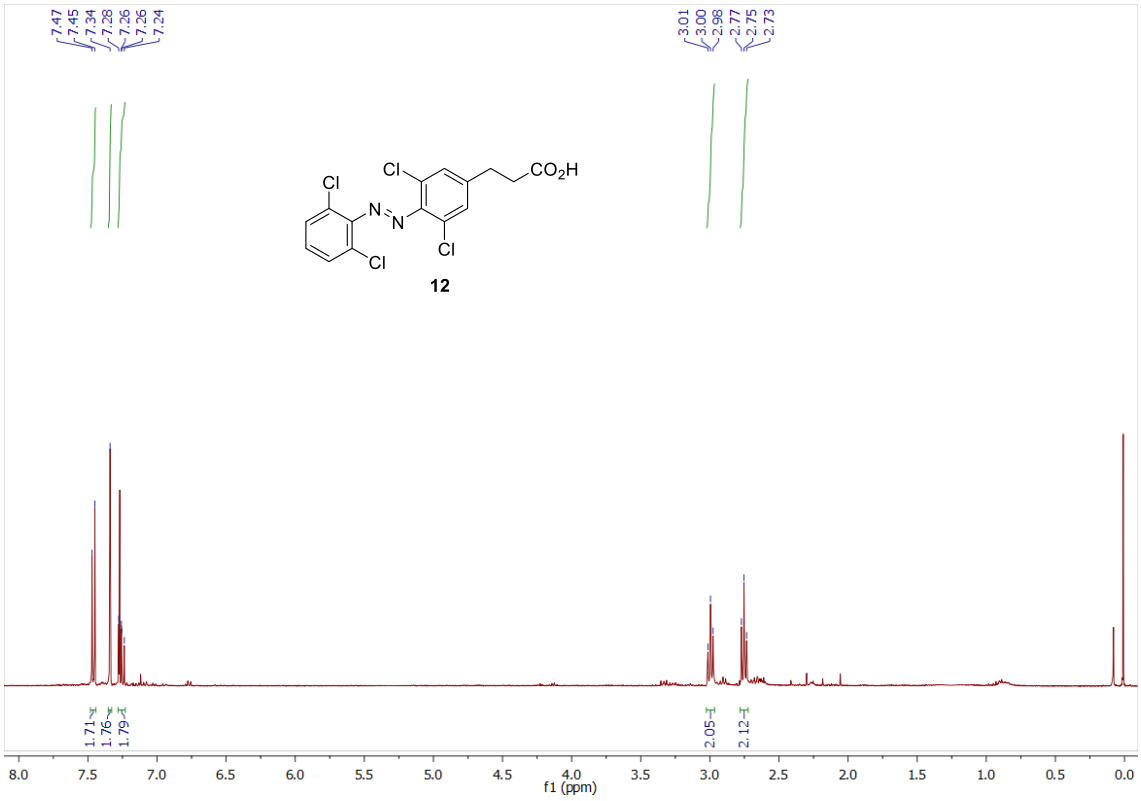


^{13}C-NMR (400 MHz, $CDCl_3$)

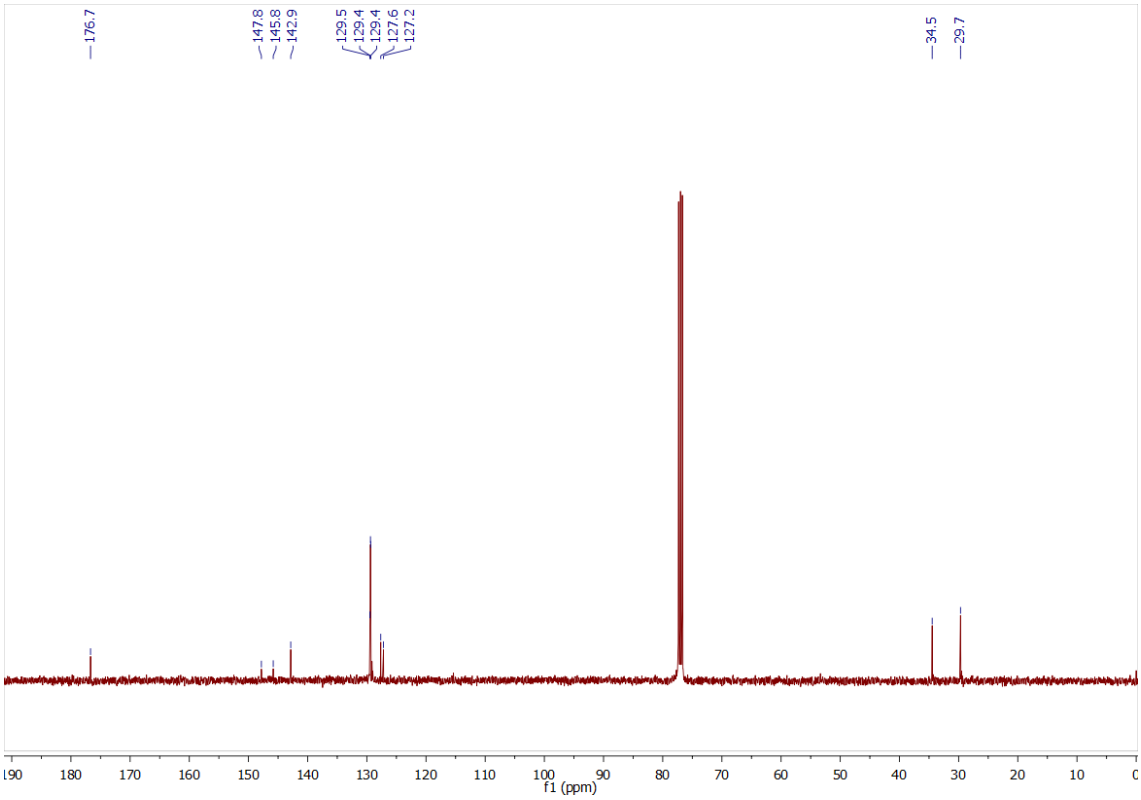


(*E*)-3-(3,5-Dichloro-4-((2,6-dichlorophenyl)diazenyl)phenyl)propanoic acid (12)

^{1}H-NMR (400 MHz, $CDCl_3$)



^{13}C-NMR (400 MHz, $CDCl_3$)



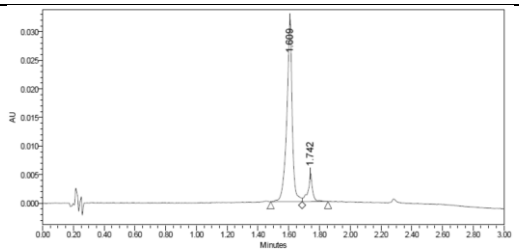
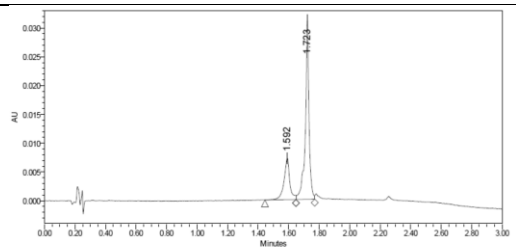
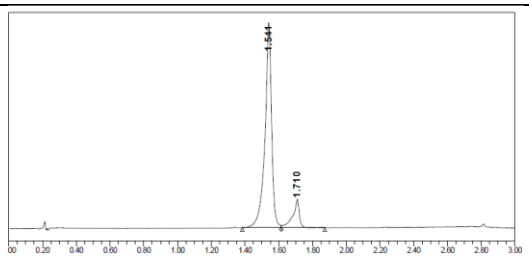
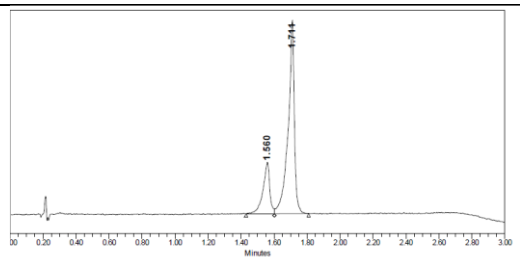
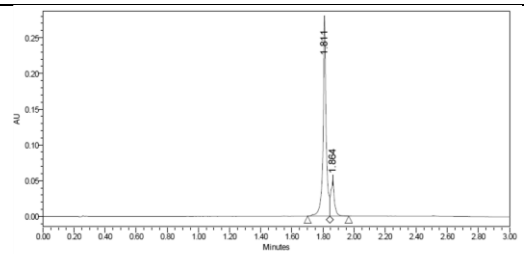
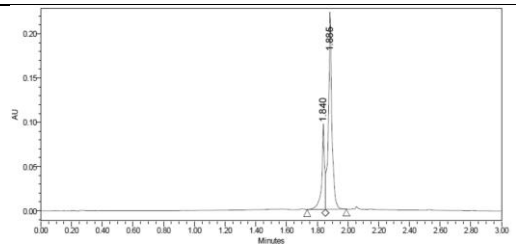
5. Photoisomerisations

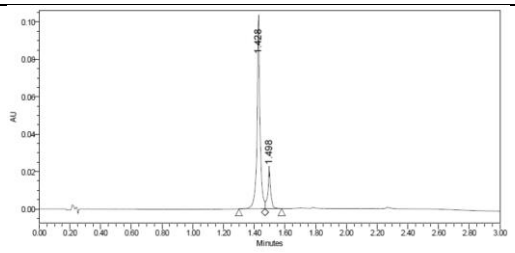
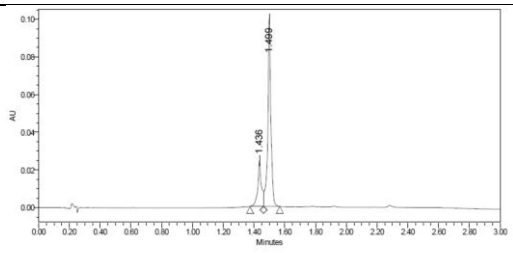
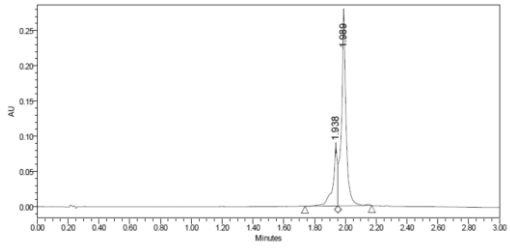
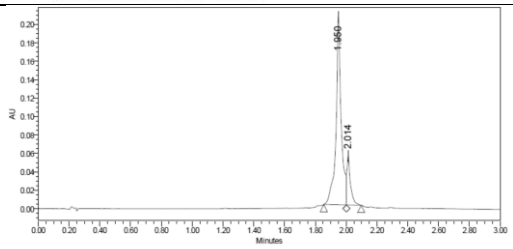
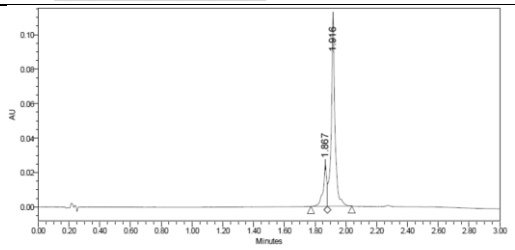
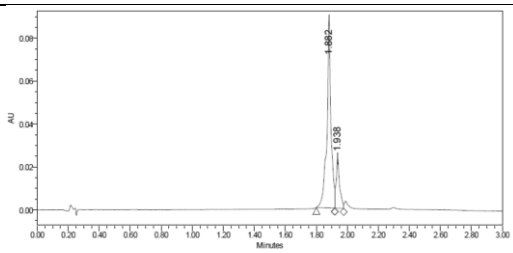
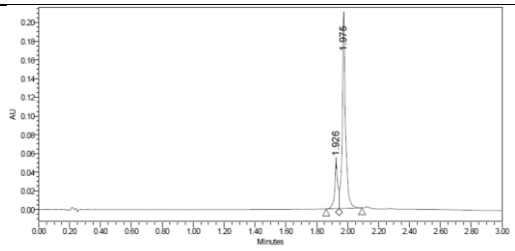
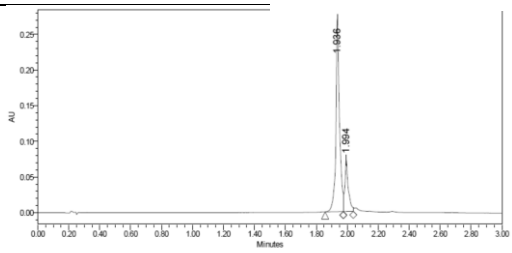
5.1. Photostationary States

Diluted solutions (*ca.* 0.3 mg·mL^{-1} in MeOH) of all compounds were analysed by UPLC after isolation. These samples were then irradiated under 650 nm for 50 min before being analysed again by UPLC. MeCN (0.07% formic acid) and H_2O (0.1% formic acid) were used as solvents in the indicated gradients and detecting at 300 nm. After irradiation, samples were handled in the dark until the analysis was complete.

Samples prepared for in vitro assays (254 µg·mL^{-1} in 10% DMSO aqueous solution) were photoisomerised in the same conditions and analysed as detailed above and the isomer ratios observed were the same.

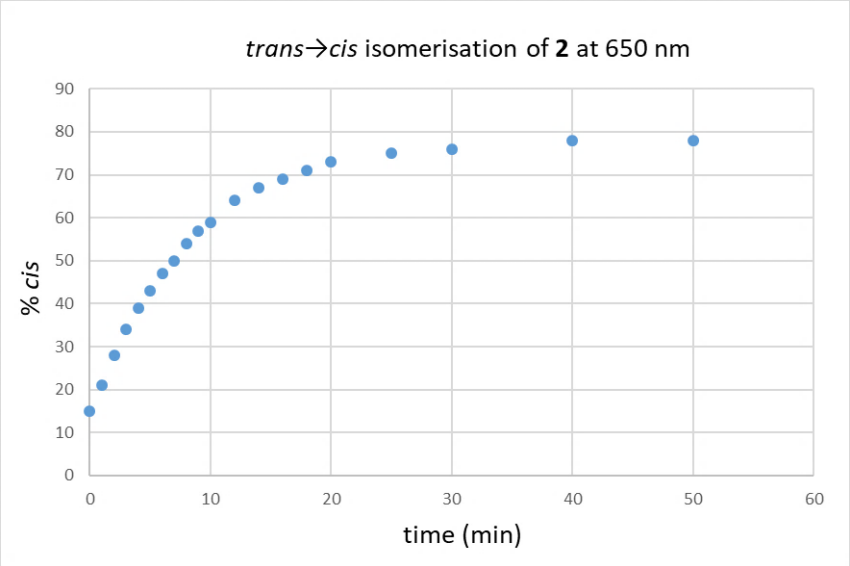
Table S1. UPLC chromatograms of photostationary states of **TA** photoswitchable analogues.

Compound	Non-irradiated	Irradiated (650 nm for 50 min)
1	 Peak Results Name RT Area % Area Height Amount Units 1 1.609 77816 88.68 31901 2 1.742 9935 11.32 4916 **Gradient:** H_2O/MeCN (70:30 to 0:100)	 Peak Results Name RT Area % Area Height Amount Units 1 1.592 18715 26.50 7168 2 1.723 51894 73.50 31093 **Gradient:** H_2O/MeCN (70:30 to 0:100)
2	 RT Area % Area Height 1 1.541 187316 88.04 68448 2 1.710 25440 11.96 9451 **Gradient:** H_2O/MeCN (60:40 to 30:70)	 RT Area % Area Height 1 1.560 14272 22.36 5006 2 1.711 49559 77.64 18799 **Gradient:** H_2O/MeCN (60:40 to 30:70)
13	 Peak Results Name RT Area % Area Height Amount Units 1 1.811 382669 82.72 273324 2 1.864 79941 17.28 49967 **Gradient:** H_2O/MeCN (100:0 to 0:100)	 Peak Results Name RT Area % Area Height Amount Units 1 1.840 118143 25.35 90736 2 1.885 347926 74.65 216707 **Gradient:** H_2O/MeCN (100:0 to 0:100)

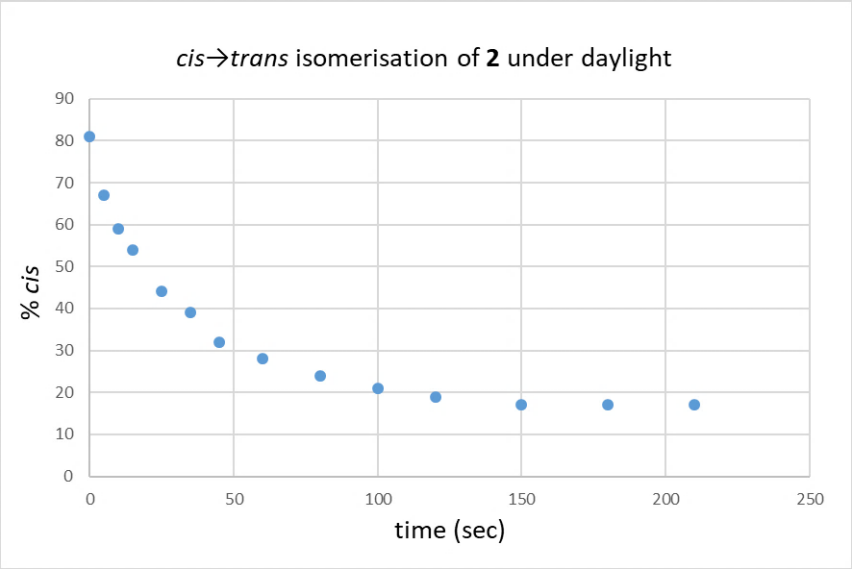
14	 **Peak Results** Name RT Area % Area Height Amount Units 1 1.428 139632 83.67 100938 2 1.498 27247 16.33 19667 **Gradient:** H_2O/MeCN (70:30 to 0:100)	 **Peak Results** Name RT Area % Area Height Amount Units 1 1.436 38593 21.86 24267 2 1.499 137973 78.14 99580 **Gradient:** H_2O/MeCN (70:30 to 0:100)
15	 **Peak Results** Name RT Area % Area Height Amount Units 1 1.938 171953 23.22 82671 2 1.989 568577 76.78 271679 **Gradient:** H_2O/MeCN (70:30 to 0:100)	 **Peak Results** Name RT Area % Area Height Amount Units 1 1.950 480811 84.79 204112 2 2.014 86245 15.21 53211 **Gradient:** H_2O/MeCN (70:30 to 0:100)
16	 **Peak Results** Name RT Area % Area Height Amount Units 1 1.867 38951 17.39 24116 2 1.916 185098 82.61 109549 **Gradient:** H_2O/MeCN (70:30 to 0:100)	 **Peak Results** Name RT Area % Area Height Amount Units 1 1.882 160441 81.21 87421 2 1.938 37130 18.79 23231 **Gradient:** H_2O/MeCN (70:30 to 0:100)
17	 **Peak Results** Name RT Area % Area Height Amount Units 1 1.926 68012 18.19 48961 2 1.975 305828 81.81 204959 **Gradient:** H_2O/MeCN (70:30 to 0:100)	 **Peak Results** Name RT Area % Area Height Amount Units 1 1.936 438253 79.35 269751 2 1.994 114026 20.65 71947 **Gradient:** H_2O/MeCN (70:30 to 0:100)

5.2. Isomerisation Kinetic Studies

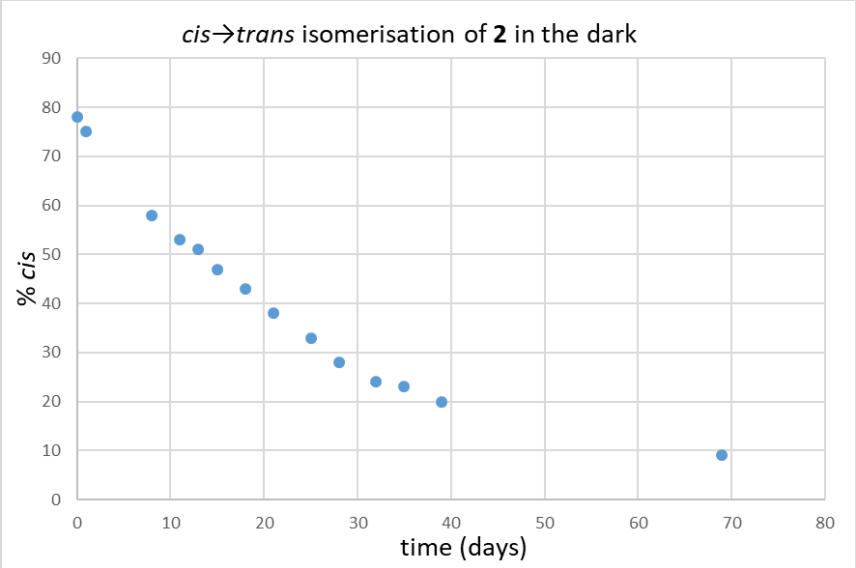
The kinetic profiles for both *trans*→*cis* and *cis*→*trans* isomerisations of **2** were recorded. A solution of compound **2** (*ca*. 0.3 mg·mL^{-1} in MeOH) was loaded to a quartz glass cells (Suprasil, Hellma) of 1 mm path length and irradiated at 650 nm during the required time intervals. While keeping a dark environment, a drop of this solution was taken after each period of irradiation and further diluted in MeOH for UPLC analysis, detecting at 300 nm. This process was repeated until a plateau was reached.



For *cis*→*trans* isomerisation, a solution of compound **2** (*ca*. 0.3 mg·mL^{-1} in MeOH) was loaded to a quartz glass cells (Suprasil, Hellma) of 1 mm path length and isomerised to its *cis* form under 650 nm irradiation for 40 min. The loaded cell was kept in a fully opaque box and exposed to daylight during the required time intervals. Analysis of the solution was performed as above.



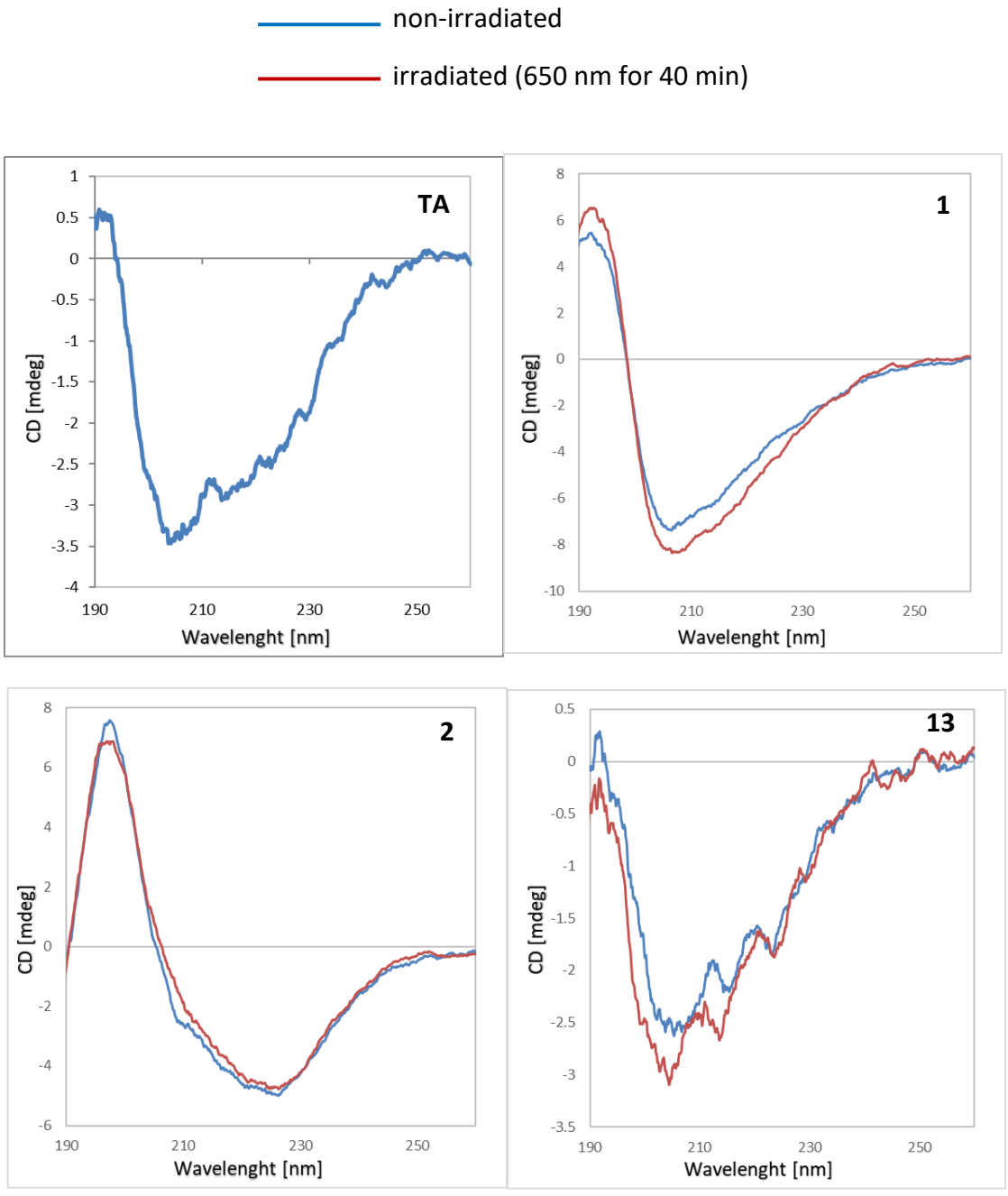
cis→*trans* isomerisation in the dark was assessed by analysing a solution of the *cis* form of **2**, which was carefully kept in the dark and analysed by UPLC as indicated above. Given the extremely slow isomerisation rate in the dark, it was not possible to calculate a precise half-life under these conditions.

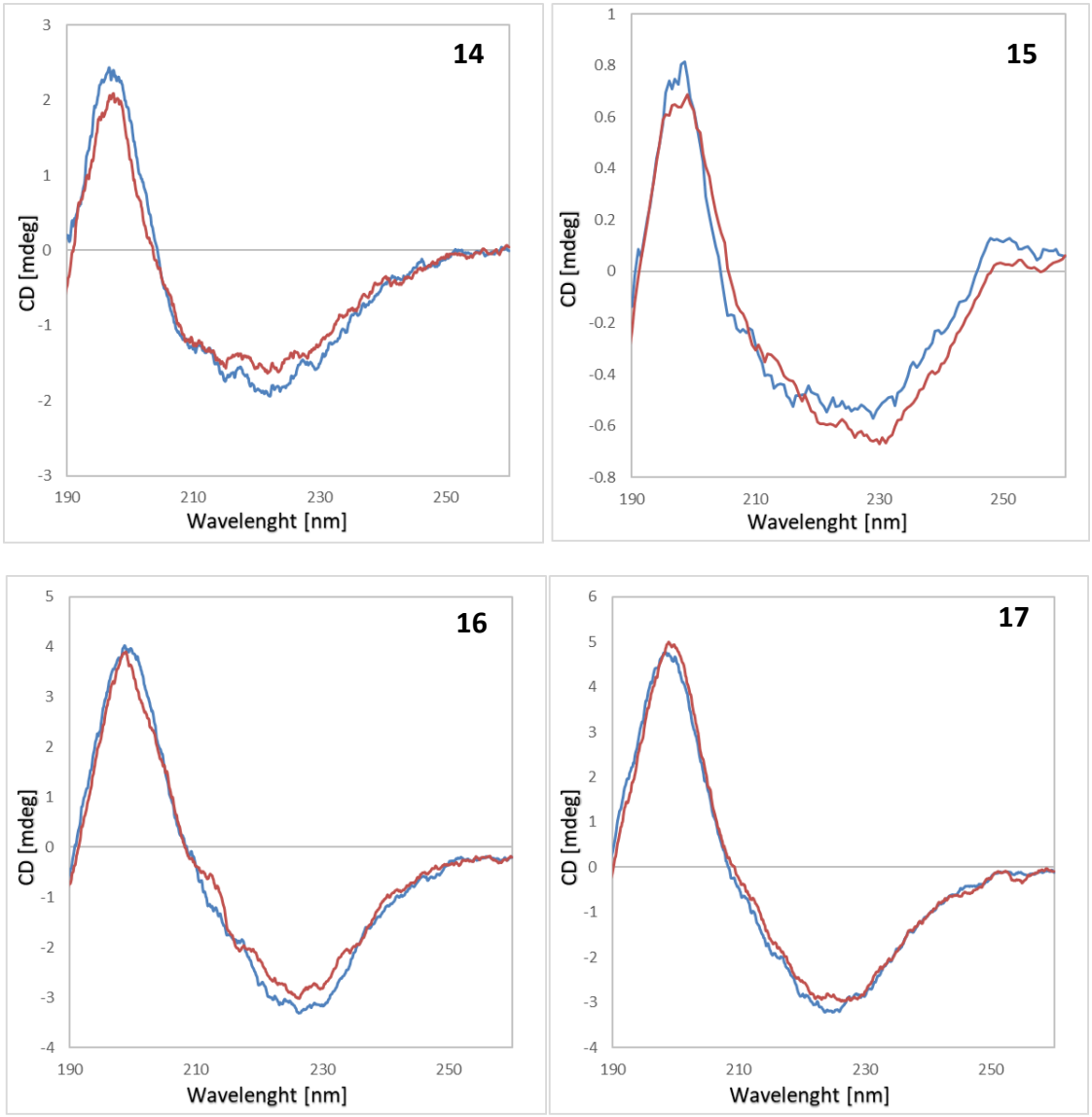


For other analogues, including linear analogue **1**, the observed kinetic profiles were virtually the same as those recorded for **2**.

6. Circular Dichroism

CD spectra were recorded on a Jasco J-815 spectropolarimeter. Measurements were performed on samples at a concentration of 50 $\mu g \cdot mL^{-1}$ in MeOH/H_2O (95:5) in quartz glass cells (Suprasil, Hellma) of 1 mm path length. The chamber was kept under constant N_2 flow. Spectra were recorded between 260 nm and 190 nm at 20 °C. The shown CD spectra for each sample are the average of three runs at a scan rate of 20 $nm \cdot min^{-1}$, 8 s response time at 0.1 nm intervals and 1 nm bandwidth with the blank already subtracted.





The recorded CD spectrum of **TA** matched the one reported for the monomeric species in water.[2]

7. *In vitro* Studies

7.1. Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) value is defined as the smallest concentration of an antibiotic that inhibits the macroscopic growth of a microorganism after standard incubation (18-22h). All MIC values obtained for each strain screened (triplicates) ranged +/- 2-fold difference.

Bacterial strain samples were prepared by streaking and incubating the bacterial strains in Columbia Agar plates (containing 5% of sheep blood) overnight at 37 ºC. Polypropylene-round bottom 96-well plates were used and 50 µL of Mueller-Hinton Broth were added to each well of the plate used (100 µL for negative controls). 50 µL of the antimicrobial agent (254 $\mu g \cdot mL^{-1}$ in 10% DMSO aqueous solution) were added to the first well of each row from which serial dilutions were made.

Inoculums were prepared by diluting 0.5 McFarland solutions from each bacterial strain in Mueller-Hinton broth.

Plates were then inoculated with 50 µL of Mueller-Hinton bacterial inoculums. Finally, plates were covered with film before overnight incubation (18 to 22 h) at 37 ºC. For assays with the *cis*-form of the compounds, plates already covered with film were irradiated with 650 nm light for 40 min prior to covering them with aluminium foil and incubation. MIC values were determined as the concentration of the first well in which no observable growth was appreciated.

Two biological replicates, including two technical replicates, per strain were done in the MIC assays for the analogues.

Table S2. *In vitro* antibacterial activities of **TA** and its photoswitchable analogues against Gram-positive and Gram-negative bacteria.[a]

	TA		**1**		**2**		**13**		**14**		**15**		**16**		**17**	
	NoIrr	Irr[b]	NoIrr	Irr[b]	NoIrr	Irr[b]	NoIrr	Irr[b]	NoIrr	Irr[b]	NoIrr	Irr[b]	NoIrr	Irr[b]	NoIrr	Irr[b]
Staphylococcus aureus ATCC 25923[c]	8	8	8	8	8	8	4	4	8	8	>64	64	64	32	>64	>64
Bacillus subtilis[c]	4	4	4	8	2	2	1	2	4	16	>64	>64	64	64	>64	>64
Staphylococcus epidermidis F652012[c]	8	8	8	8	4	4	4	4	8	8	>64	32	64	64	64	64
Streptococcus pyogenes 016[c]	4	4	4	4	4	4	4	4	8	4	>64	64	**>64**	**16**	**>64**	**16**
Enterococcus faecium VancoR[c]	4	4	8	8	4	4	4	4	8	8	64	64	32	32	64	64
Pseudomonas aeruginosa ATCC 27853[d]	>64	>64	>64	>64	>64	>64	>64	64	>64	>64	>64	>64	>64	>64	>64	>64
Escherichia coli ATCC 25922[d]	>64	>64	>64	>64	>64	64	>64	32	>64	>64	>64	>64	>64	>64	>64	>64
Acinetobacter baumannii ATCC 19606[d]	16	16	>64	>64	**64**	**8**	8	8	>64	>64	>64	>64	>64	>64	>64	>64
Acinetobacter baumannii CR17[d]	32	16	>64	>64	>64	32	16	8	>64	>64	>64	>64	>64	>64	>64	>64
Acinetobacter nosocomialis 256[d]	4	4	2	4	4	8	2	4	4	4	32	32	16	16	64	64

[a] Measured as minimum inhibitory concentration (MIC) in µg mL^{-1}. [b] Plates were irradiated at 650 nm for 40 min before incubation in the dark. [c] Gram-positive strain. [d] Gram-negative strain.

7.2. Haemolysis

A commercially available fresh human blood sample was used to obtain a 50% haematocrit solution (1:1 PBS 1X) by 3 consecutive washes with PBS 1X and centrifugation at 2,600 rpm for 10 minutes. From this, a 4% erythrocyte solution was prepared with PBS 1X and left on ice until used.

Analogue solutions (254 $\mu g \cdot mL^{-1}$ in 10% DMSO aqueous solution) were prepared and stored in ice or at 4 ºC until used.

In a polypropylene 96-well plate, 50 μL PBS 1X were added to each well of the plate but for the 1.0% TX-100 controls. Then, serial dilutions were performed with 50 μL of the analogues as in broth microdilution assays for MIC. When necessary, plates were irradiated for 40 min under 650 nm at this point and further manipulation was performed in the dark. Then, 50 μL of 4% RBC were added to all wells of the plate (including controls). These were gently re-suspended when dispensing the solution.

Plates were incubated for 4 h at 37 ºC and then centrifuged at 1500 rpm 5 min. From the plates, 80 μL of the supernatant of each well were carefully transferred to a flat bottom polystyrene 96-well plate. For this, the plate was gently tilted and the pipette tips were inserted in the wells without touching the RBC pellets. Plates were read in an EPOCH spectrophotometer at 450 nm.

Table S3. Haemolysis IC50 values.

	IC50 ($\mu g \cdot mL^{-1}$)	
	NoIrr	**Irr[b]**
TA	25	24
1	21	26
2	32	15
13	36	13
14	25	33
15	39	33
16	23	25
17	133	173

[b] Plates were irradiated at 650 nm for 40 min and further manipulated in the dark.

8. References

[1] D. B. Konrad, J. A. Frank, D. Trauner, *Chem. Eur. J.* **2016**, *22*, 4364.

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