Supporting Information

For

Mechanistic Evaluation of Bioorthogonal Decaging with *trans*-Cyclooctene: The Effect of Fluorine Substituents on Aryl Azide Reactivity and Decaging from the 1,2,3-Triazoline

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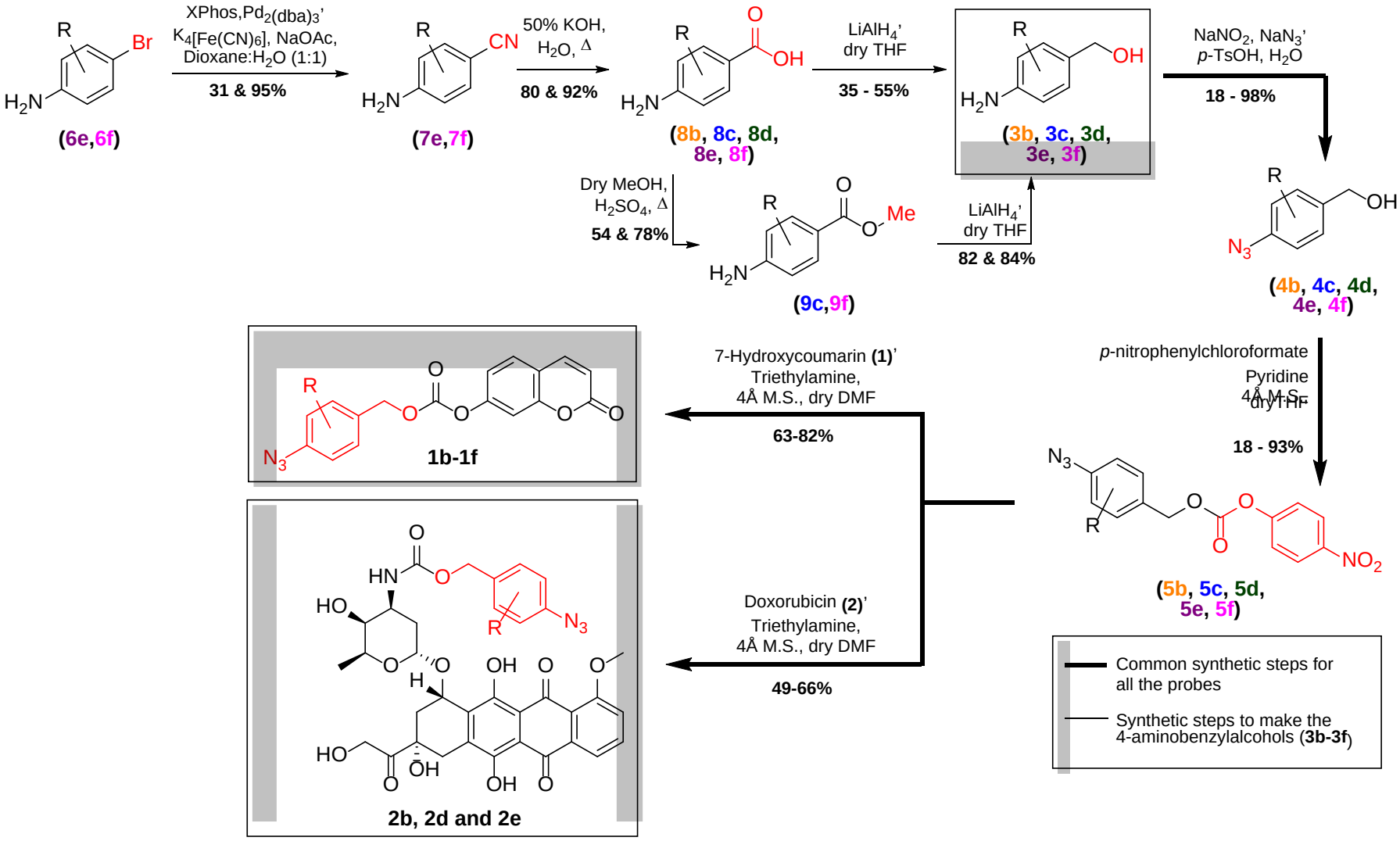
1. General Experimental

Unless otherwise stated, all chemical reagents and solvents were purchased from commercial sources and used without further purification. Doxorubicin hydrochloride salt was purchased from AK Scientific, California and Lancrix, Shanghai. 5-Hydroxy-1-cyclooctene (*cis*-cyclooct-4-enol) was purchased from Carbosynth Limited, UK. Silver nitrate-impregnated silica gel was purchased from Sigma-Aldrich and Silicycle (SiliaBond® Silver Nitrate, 40-63μm, 60Å). Powdered molecular sieves were activated in an oven at 180 °C overnight before use and then stored at 110 °C. All other reagents were purchased from Sigma-Aldrich or AK Scientific. Reaction solvents were purchased dry from Sigma-Aldrich, Thermo Fischer Scientific or Merck. Thin layer chromatography was performed on 0.2 mm aluminium-backed silica gel plates 60 F_{254}, and visualized with UV light (λ = 254 nm) or basic $KMnO_4$ dip. Flash column chromatography was carried out using 40-63 μm silica gel, with AR or liquid chromatography grade solvents. ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz or 500 MHz Varian MR spectrometer. Chemical shifts are reported as δ in parts per million (ppm) and coupling constants are reported as *J* values in Hz. High resolution electrospray ionization mass spectra were recorded on a microTOF_Q mass spectrometer. Photochemical reactions were performed in a Southern New England Ultraviolet Company Rayonet® reactor model RPR-100, equipped with eight RPR-2537 Å lamps. Reactions were performed in either the RQV-118 or RQV-218 quartz reaction vessels supplied by Southern New England Ultraviolet Company.

Fluorescence data were recorded on a Hitachi F-7000 Fluorescence Spectrofluorometer. Excitation was at 360 nm and emission monitored in the range of 400 – 650 nm (λ_{max} = 455 nm). Default settings were selected for other parameters.

HPLC was performed using either an Agilent 1200 system or Shimadzu 20 series system, equipped with a Phenomenex Synergi 4 μm Fusion-RP 80A (150 x 4.6 mm) column, and a photodiode array detector. The applied mobile phases used for kinetic studies and purity determinations were: A, *aq* H_2O + 0.1% formic acid; and B, *aq* MeCN + 0.1% formic acid. Flow speed was 1 mL/min and injection volumes were 20 μl or 50 μl. Gradient mobile phase, 80% H_2O/20% MeCN with 0.1% formic acid to 100% MeCN with 0.1% formic acid in 10 minutes, 5 minutes at 100% MeCN with 0.1% formic acid, returning to starting conditions by 20 minutes.

2. Synthesis



Scheme S1. Overview of the synthetic protocol employed to synthesize **1b-1f**, **2b, 2d,** and **2e**.

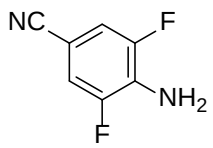
The synthesis for **1a** and **2a** has been reported in our previous work.[1]

General procedure-I: Synthesis of 4-Aminobenzonitriles

(Used for **7e** and **7f**)

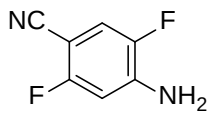
2-Dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (XPhos) and $Pd_2(dba)_3$, were dissolved in freshly distilled 1,4-dioxane. The mixture was incubated for 3 min in an oil bath pre-heated to 120 °C. Simultaneously, to a pressure tube equipped with a rubber septum, $K_4(Fe(CN)_6).3H_2O$ and the required aryl bromide were added under N_2 (flask was evacuated and backfilled with N_2; this cycle was repeated three times). Following which, the contents from the incubated mixture and NaOAc dissolved in degassed water were added to the screw-top pressure tube under N_2. Subsequently, the screw-top test tube was sealed with a PTFE bushing. The tube was placed in an oil bath pre-heated to 100 °C and stirred for 3 h. A clear, yellow solution was observed upon initial stirring. During the course of the reaction, a greenish-yellow precipitate formed on the walls of the reaction vessel. After stirring the reaction mixture at 100 °C for 3 h, it was then cooled to 25 °C. The contents of the test tube were transferred to a separating funnel using EtOAc and brine, and the organic layer was separated from the aqueous layer, concentrated *in vacuo* and purified using column chromatography.

4-Amino-3,5-difluorobenzonitrile (7e):



Synthesized according to **general procedure-I**. In a screw-top pressure tube equipped with a magnetic stir bar, XPhos (9.2 mg, 19.3 µmol), $Pd_2(dba)_3$ (17.6 mg, 0.0193 µmol) $K_4[Fe(CN)_6]\cdot 3H_2O$ (1.02 g, 2.40 mmol), the aryl halide **6e** (1.0 g, 4.80 mmol) and NaOAc (98.6 mg, 1.20 mmol) were reacted in a mixture of 8 mL dioxane and 8 mL H_2O. The crude sample was subjected to silica gel flash column chromatography (30% EtOAc : Hexane to 50% EtOAc : Hexane), providing **7e** as a pale yellow powder (0.20 mg, 31%) which was spectroscopically similar to that reported in the literature.[2] ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.15 (dd, J = 6.0, 2.4 Hz, 2H), 4.27 (brs, 2H).

4-Amino-2,5-difluorobenzonitrile (7f):



Synthesized according to **general procedure-I**. In a screw-top pressure tube equipped with a magnetic stir bar, XPhos (18.3 mg, 38.6 µmol), $Pd_2(dba)_3$ (35.2 mg, 0.0386 µmol) $K_4[Fe(CN)_6]\cdot 3H_2O$ (2.04 g, 4.80 mmol), the aryl halide **6f** (1.0 g, 4.80 mmol) and NaOAc (197.2 mg, 2.40 mmol) were reacted in a mixture of 2 mL dioxane and 2 mL H_2O. The crude sample was subjected to silica gel

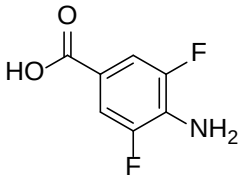
flash column chromatography (100% Hexane to 20% EtOAc : Hexane), providing **7f** as a pale yellow powder (0.20 mg, 95%) which was spectroscopically similar to that reported in the literature.[2] ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.15 (dd, *J* = 10.0, 5.6 Hz, 1H), 6.53 (dd, *J* = 10.0, 7.2 Hz, 1H), 4.46 (brs, 2H).

General procedure-II: Synthesis of 4-aminobenzoic acids

(Used for **8e** and **8f**)

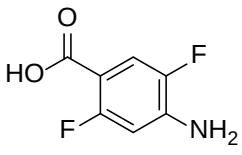
4-Aminobenzonitrile was dissolved in a 50% solution of potassium hydroxide. The reaction mixture was heated to reflux (105 °C) overnight. The progress of the reaction was monitored *via* TLC analysis and upon complete conversion, the reaction mixture was acidified with 2 M HCl. The acidification led to the formation of a yellow precipitate. The yellow precipitate was extracted out using EtOAc and the organic layer was washed with water and brine then dried ($MgSO_4$) and concentrated *in vacuo*. The crude mixture was taken forward without any further purification.

4-Amino-3,5-difluorobenzoic acid (8e):



Synthesized according to **general procedure-II**. 0.80 g (4.62 mmol) of the 4-amino-3,5-difluorobenzonitrile **7e** was dissolved in 50% solution of KOH and refluxed for 19 h. The resulting reaction mixture was acidified with 2 M HCl, following which the organics were extracted with EtOAc and washed with water (3 × 20 mL) and brine (3 × 20 mL). Drying ($MgSO_4$) and concentration of the organic fractions *in vacuo* provided **8e** as a yellow powder (0.72 g, 80%) which was spectroscopically similar to that reported in the literature.[3] ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.66 (s, 1H), 7.39 (dd, *J* = 7.0, 2.6 Hz, 2H), 3.38 (s, 2H).

4-Amino-2,5-difluorobenzonitrile (8f):



Synthesized according to **general procedure-II**. 0.505 g (2.91 mmol) of the 4-amino-2,5-difluorobenzonitrile **7f** was dissolved in 50% solution of KOH and refluxed for 14 h. The resulting reaction mixture was acidified with 2 M HCl, following which the organics were extracted with EtOAc and washed with water (3 × 20 mL) and brine (3 × 20 mL). Drying ($MgSO_4$) and concentration of the organic fractions *in vacuo* provided **8f** as a yellow powder (0.52 g, 92%). ^{1}H NMR (Acetone-d_6, 400 MHz) δ 10.75 (s, 1H), 7.50 (dd, *J* = 12.0, 6.8 Hz, 1H), 6.60 (dd, *J* = 12.4, 7.2 Hz, 1H). ^{13}C NMR (Acetone-d_6, 100 MHz) δ 206.4 (d, $J_{C\text{-}F}$ = 0.9 Hz), 163.9 (dd, $J_{C\text{-}F}$ = 4.0, 2.0 Hz), 159.9 (dd, $J_{C\text{-}F}$ = 251.8, 1.2 Hz), 146.1 (dd, $J_{C\text{-}F}$ = 233.2, 1.7

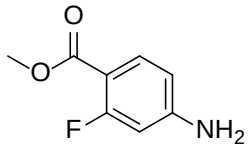
Hz), 142.9 (dd, $J_{C\text{-}F}$ = 15.1, 12.8 Hz), 117.3 (dd, $J_{C\text{-}F}$ = 21.6, 3.5 Hz), 102.3 (dd, $J_{C\text{-}F}$ = 28.5, 4.4 Hz).

General procedure- III to synthesize 4-aminobenzoates

(Used for **9c** and **9f**)

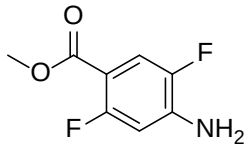
4-Aminobenzoic acid was dissolved in dry methanol and conc. H_2SO_4 was added to the solution under N_2. The reaction mixture was heated at reflux (65 °C) overnight. The progress of the reaction was monitored *via* TLC analysis and upon complete conversion of the 4-aminobenzoic acid to the title compound the reaction mixture was concentrated down to approx. 10% of total methanol volume. The resulting mixture was then diluted in an excess of H_2O and extracted with EtOAc. After which, it was washed with $NaHCO_3$, brine, dried using $MgSO_4$ and concentrated *in vacuo*. The crude mixture was taken forward without any further purification.

Methyl-4-amino-2-fluorobenzoate (9c):



Synthesized according to **general procedure-III**. Benzoic acid **8c** (0.1 g, 0.64 mmol) in methanol (3 mL) and H_2SO_4 (0.2 mL) under nitrogen was heated at reflux for 3 h. Following the work-up procedure as described above, concentration of the organic fractions *in vacuo* provided **9c** as a pale white solid (85.0 mg, 78%), which was spectroscopically similar to that previously reported.[4] ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.77 (appt, *J* = 8.4 Hz, 1H), 6.42 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.34 (dd, *J* = 12.8, 2.3 Hz, 1H), 3.87 (s, 3H).

Methyl-4-amino-2,5-difluorobenzoate (9f):



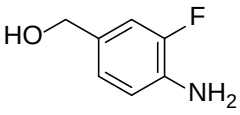
Synthesized according to **general procedure-III**. Benzoic acid **9c** (0.50 g, 2.89 mmol) in methanol (3 mL) and H_2SO_4 (0.2 mL) under nitrogen was heated at reflux for 3 h. The work-up procedure as described above and concentration of the organic fractions *in vacuo* provided **9f** as a pale white solid (0.46 g, 54%), which was spectroscopically similar to that previously reported.[5] ^{1}H NMR (CD_3OD, 400 MHz) δ 7.48 (dd, *J* = 11.9, 6.6 Hz, 1H), 6.52 (dd, *J* = 12.6, 7.2 Hz, 1H), 3.85 (s, 3H).

General procedure-IV: Synthesis of 4-aminobenzyl alcohols from the corresponding 4-aminobenzoic acid/ 4-aminobenzoate ester derivatives

(Used for the synthesis of **3b, 3c, 3d, 3e** and **3f**)

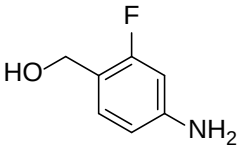
A stirred dispersion of $LiAlH_4$ in dry THF was prepared under N_2 and carried out at 0 °C on ice. To this solution was added dropwise the corresponding 4-aminobenzoic acid/ 4-aminobenzoate ester derivative dissolved in dry THF. The reaction mixture was warmed up to 25 °C and subjected to stirring overnight. The progress of the reaction was monitored by TLC-analysis and once complete, the reaction mixture was quenched by slow addition of saturated aqueous Na_2SO_4 solution at 0 °C on ice. The resulting residue was dissolved in excess water and extracted into EtOAc. Following which, the organic fractions were dried using $MgSO_4$ and concentrated *in vacuo* to result in a residue which was subjected to flash silica gel column chromatography to afford the desired 4-aminobenzyl alcohol.

(4-Amino-3-fluorophenyl)methanol (3b):



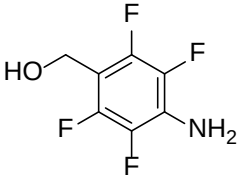
Synthesized according to **general procedure-IV**. Benzoic acid **8b** (1.42 g, 9.16 mmol) was reacted with $LiAlH_4$ (698.3 mg, 18.38 mmol) to form a crude residue which upon work-up and silica gel flash column chromatography (20% EtOAc:hexane), resulted in 0.531 g (35%) of the title compound as a dark brown amorphous solid which was spectroscopically similar to that reported in literature.[6] ^{1}H NMR (DMSO-d_6, 400 MHz) δ 6.91 (dd, J = 12.4, 2.0 Hz, 1H), 6.81 (dd, J = 8.0, 2.0 Hz, 1H), 6.70 (m, 1H), 4.31 (s, 2H).

(4-Amino-2-fluorophenyl)methanol (3c):



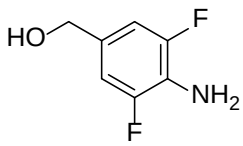
Synthesized according to **general procedure-IV**. Benzoate ester **9c** (266.0 mg, 1.57 mmol) was reacted with $LiAlH_4$ (119.3 mg, 3.14 mmol) to form a crude residue which upon work-up and silica gel flash column chromatography with 20% EtOAc:hexane, resulted in 186.0 mg (84%) of the title compound as a dark brown amorphous solid which was spectroscopically similar to that reported in literature.[7] ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.13 (dd, J = 8.4 Hz, 1H), 6.43 (dd, J = 8.0, 2.4 Hz, 1H), 6.38 (m, 1H), 4.61 (s, 2H).

(4-Amino-2,3,5,6-tetrafluorophenyl)methanol (3d):



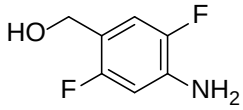
Synthesized according to **general procedure-IV**. Benzoic acid **8d** (1.84 g, 8.79 mmol) was reacted with $LiAlH_4$ (667.8 mg, 17.59 mmol) to form a crude residue which upon work-up and silica gel flash column chromatography using a small plug of silica gel (60% EtOAc:hexane) resulted in 0.95 g (55%) of the title compound as a pale brown amorphous solid which was spectroscopically similar to that reported in literature.[8] ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.73 (t, J = 1.6 Hz, 2H; CH_2).

(4-Amino-3,5-difluorophenyl)methanol (3e):



Synthesized according to **general procedure-IV**. Benzoic acid **8e** (0.8 g, 4.62 mmol) was reacted with $LiAlH_4$ (350.7 mg, 9.24 mmol) to form a crude residue which upon work-up and silica gel flash column chromatography with 15% EtOAc:hexane resulted in 402.0 mg (55%) of the title compound as a beige colored amorphous solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.85 (m, 2H), 4.57 (s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) 151.9 (dd, $J_{C\text{-}F}$ = 239.7, 8.0 Hz), 130.2 (dd, $J_{C\text{-}F}$ = 7.4, 7.4 Hz), 123.0 (d, $J_{C\text{-}F}$ = 16.3 Hz), 109.6 (dd, $J_{C\text{-}F}$ = 14.9, 7.2 Hz), 64.3.

(4-Amino-2,5-difluorophenyl)methanol (3f):



Synthesized according to **general procedure-IV**. Benzoate ester **9f** (450.0 mg, 2.40 mmol) was reacted with $LiAlH_4$ (182.6 mg, 4.80 mmol) to form a crude residue which upon work-up and silica gel flash column chromatography with 15% EtOAc:hexane resulted in 370.0 mg (82%) of the title compound as a dark brown amorphous soild which was spectroscopically similar to that reported in the literature.[9] ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.01 (dd, J = 10.8, 6.4 Hz, 1H), 6.48 (dd, J = 10.8, 7.6 Hz, 1H), 4.60 (s, 2H).

General procedure-V: Synthesis of the 4-azidobenzyl alcohols from the corresponding 4-aminobenzyl alcohols

(Used for linkers corresponding to **4b, 4c, 4d, 4e,** and **4f**)

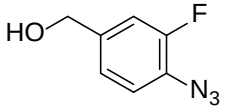
Procedure (A): 4-Aminobenzyl alcohol was dissolved in 5M HCl and cooled to 0 °C on ice, followed by a dropwise addition of $NaNO_2$ solution in water. The mixture was allowed to stir for 30 min at 0 °C, upon which time NaN_3 was added in portions. The reaction mixture was then stirred at 0 °C for 2 hours. The reaction mixture was then adjusted to a pH of ~8 using saturated $NaHCO_3$, and extracted with EtOAc. The combined organic layers were washed with water, dried using $MgSO_4$ and concentrated *in vacuo* (temperature to be maintained at less than 30 °C). The residue was finally purified by flash silica gel column chromatography (whenever required) to obtain the required product.

Procedure (B): 4-Aminobenzyl alcohol and *p*-toluenesulfonic acid was dissolved in water and cooled to 0 °C on ice, followed by a portion-wise addition of NaN_3. The reaction was allowed to stir for 10 min at 0 °C, upon which time $NaNO_2$ was added in portions over 20 min. The reaction mixture was stirred at 0 °C for 10 mins. The reaction mixture was adjusted to a pH of ~8 using saturated $NaHCO_3$, and extracted with EtOAc. The combined organic

layers were washed with water, dried using $MgSO_4$ and concentrated *in vacuo* (temperature to be maintained at less than 30 °C). The crude residue was purified by flash silica gel column chromatography (whenever required) to obtain the required product.

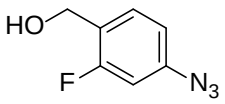
Procedure (C): 4-Aminobenzyl alcohol and *p*-toluenesulfonic acid was dissolved in water and cooled to 0 °C on ice, followed by a dropwise addition of $NaNO_2$ solution in water. The reaction was allowed to stir for 30 min (sufficient time for the 4-aminobenzyl alcohol to completely convert into the corresponding diazonium salt). The conversion was monitored using thin layered chromatography (TLC) analysis. Upon significant conversion, NaN_3 was added to the reaction mixture in portions over 10 min. The reaction mixture was then subjected to a work-up where the non-polar products formed (based on TLC analysis) were extracted using EtOAc. Following which the aqueous layer was added back to the round-bottomed flask and equimolar amounts of azide was added to the reaction mixture. This process was repeated thrice after every 20 min. The combined organic layers were washed with water, dried using $MgSO_4$ and concentrated *in vacuo* (temperature to be maintained at less than 30 °C). The residue was finally purified by flash silica gel column chromatography (whenever required) to obtain the required product.

(4-Azido-3-fluorophenyl)methanol (4b):



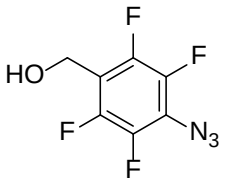
Synthesized according to **general procedure-V (A)**. The benzyl alcohol **3b** (195.5 mg, 1.38 mmol) was reacted with 5M HCl (5 mL), $NaNO_2$ (105.1 mg, 1.52 mmol), NaN_3 (360.1 mg, 5.54 mmol) in water for 2 h. Purification using silica gel column chromatography with 15% EtOAc:hexane, resulted in brown needle shaped solid (205.0 mg, 88%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.15 (m, 1H), 7.11 (m, 1H), 7.06 (m, 1H), 4.68 (s, 2H).

(4-Azido-2-fluorophenyl)methanol (4c):



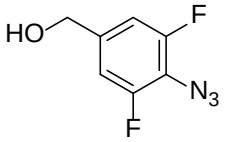
Synthesized according to **general procedure-V (C)**. The benzyl alcohol **3c** (186.0 mg, 1.31 mmol) was reacted with *p*-toluenesulfonic acid (2.25 g, 11.86 mmol), $NaNO_2$ (363.6 mg, 5.27 mmol) and NaN_3 (137.1 mg, 2.10 mmol) to result in the title compound as pale brown solid (125.2 mg, 57%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.38 (appt, J = 8.1 Hz, 1H), 6.82 (dd, J = 8.2, 2.2 Hz, 1H), 6.72 (dd, J = 10.6, 2.2 Hz, 1H), 4.67 (s, 2H), 2.32 (brs, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.0 (d, $J_{C\text{-}F}$ = 246.8 Hz), 141.1 (d, $J_{C\text{-}F}$ = 10.1 Hz), 130.4 (d, $J_{C\text{-}F}$ = 5.8 Hz), 124.4 (d, $J_{C\text{-}F}$ = 15.2 Hz), 114.7 (d, $J_{C\text{-}F}$ = 3.4 Hz), 106.5 ($J_{C\text{-}F}$ = 25. Hz), 58.7 (d, $J_{C\text{-}F}$ = 3.9 Hz).

(4-Azido-2,3,5,6-tetrafluorophenyl)methanol (4d):



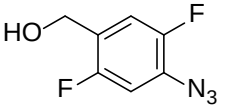
Synthesized according to **general procedure-V (B)**. The benzyl alcohol **3d** (100.0 mg, 0.51 mmol) was reacted with *p*-toluenesulfonic acid (877.3 mg, 4.61 mmol), $NaNO_2$ (141.4 mg, 2.05 mmol) and NaN_3 (49.9 mg, 0.76 mmol) in water for 10 min to result in brown solid (109.8 mg, 97.0%) which was spectroscopically similar to that reported in literature.[10] ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.80 (d, J = 6.6 Hz, 2H).

(4-Azido-3,5-difluorophenyl)methanol (4e):



Synthesized according to **general procedure-V (B)**. The benzyl alcohol **3e** (400.0 mg, 2.48 mmol) was reacted with *p*-toluenesulfonic acid (4.30 g, 22.63 mmol), $NaNO_2$ (694.0 mg, 10.04 mmol) and NaN_3 (245.2 mg, 3.77 mmol) in water for 10 min to result in the title compound as brown solid (457.2, 98%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.93 (m, 2H), 4.64 (s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.5 (dd, $J_{C\text{-}F}$ = 249.0, 4.9 Hz), 139.0 (dd, $J_{C\text{-}F}$ = 7.9, 7.9 Hz), 110.0 (m, $J_{C\text{-}F}$), 63.4 (dd, $J_{C\text{-}F}$ = 1.9, 1.9 Hz).

(4-Azido-2,5-difluorophenyl)methanol (4f):



Synthesized according to **general procedure-V (C)**. The benzyl alcohol **3f** (350.0 mg, 2.20 mmol) was reacted with *p*-toluenesulfonic acid (3.76 g, 19.8 mmol), $NaNO_2$ (607.3 mg, 8.80 mmol) and NaN_3 (214.5 mg, 3.30 mmol) to result in the title compound as brown solid (73.0 mg, 18%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.17 (dd, J = 11.0, 6.4 Hz, 1H), 6.74 (dd, J = 9.7, 6.7 Hz, 1H), 4.66 (s, 2H), 2.47 (brs, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.9 (dd, $J_{C\text{-}F}$ = 242.9, 2.6 Hz), 151.2 (dd, $J_{C\text{-}F}$ = 244.2, 2.8 Hz), 127.9 (dd, $J_{C\text{-}F}$ = 13.2, 10.2 Hz), 125.3 (dd, $J_{C\text{-}F}$ = 17.3, 6.1 Hz), 116.4 (dd, $J_{C\text{-}F}$ = 21.9, 5.9 Hz), 108.0 (dd, $J_{C\text{-}F}$ = 27.2, 1.8 Hz), 58.1 (dd, $J_{C\text{-}F}$ = 3.7, 0.9 Hz).

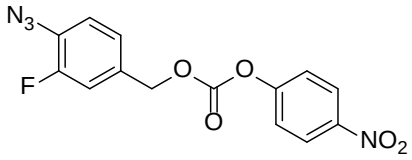
General procedure-VI: Synthesis of the 4-azidobenzyl-4'-nitrophenyl carbonates

(Used for **5b, 5c, 5d, 5e** and **5f**)

Pyridine was added to a solution of *p*-nitrophenyl chloroformate in dry THF (a; mL). The solution was cooled to 0 °C and a solution of 4-azidobenzyl alcohol in dry THF (b; mL) was added dropwise over 15 min. The resulting solution was allowed to warm to 25 °C and stirred in the dark for 72 h under nitrogen, after which time TLC analysis indicated that the reaction was complete. The THF was removed *in vacuo*, and the crude residue was re-dissolved in EtOAc. The organic fraction was washed with water, brine, dried using $MgSO_4$

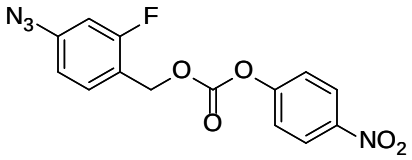
and concentrated *in vacuo* (with the temperature maintained below 30 °C). The crude residue was subjected to flash silica gel column chromatography to afford the title compound.

(4-Azido-3-fluorophenyl)methyl-4'-nitrophenyl carbonate (5b):



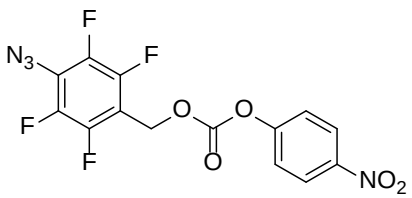
Synthesized according to **general procedure-VI**. The azidobenzyl alcohol **4b** (167.1 mg, 0.50 mmol) was reacted with *p*-nitrophenyl chloroformate (256.1 mg, 1.27 mmol), and pyridine (0.15 mL, 2.54 mmol) in THF (5 mL(a) + 15 mL(b)) to result in a brown colored crude residue, which upon silica gel flash column chromatography (10% EtOAc:hexane), resulted in a pale yellow solid (155.8 mg, 49%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.29 (d, J = 9.2 Hz, 2H), 7.39 (d, J = 9.2 Hz, 2H), 7.23 (m, 1H), 7.21 (d, J = 2.1 Hz, 1H), 7.11 (s, J = 8.3 Hz, 1H), 5.24 (s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.3, 154.7 (d, $J_{C\text{-}F}$ = 249.1 Hz), 152.3, 145.5, 132.2 (d, $J_{C\text{-}F}$ = 6.7 Hz), 128.8 (d, $J_{C\text{-}F}$ = 10.9 Hz), 125.32, 125.15 (d, $J_{C\text{-}F}$ = 3.6 Hz), 121.70, 121.30 (d, $J_{C\text{-}F}$ = 1.6 Hz), 117.0 (d, $J_{C\text{-}F}$ = 78.8 Hz), 69.5 (d, $J_{C\text{-}F}$ = 1.5 Hz).

(4-Azido-2-fluorophenyl)methyl-4'-nitrophenyl carbonate (5c):



Synthesized according to **general procedure-VI**. The azidobenzyl alcohol **4c** (125.0 mg, 0.74 mmol) was reacted with *p*-nitrophenyl chloroformate (225.9 mg, 1.12 mmol), and pyridine (0.30 mL, 3.73 mmol) in THF (5 mL(a) + 15 mL (b)) to result in a crude residue, which upon silica gel flash column chromatography (10% EtOAc:hexane) resulted in a pale white solid (140.0 mg, 56%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.27 (d, J = 9.2 Hz, 2H), 7.46 (appt, J = 8.0 Hz, 1H), 7.39 (d, J = 9.2 Hz, 2H), 6.88 (d, J = 8.0 Hz, 1H), 6.81(d, J = 10.0 Hz, 1H), 5.33 (s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.8 (d, $J_{C\text{-}F}$ = 250.3 Hz), 155.4, 152.3, 145.4, 143.2 (d, $J_{C\text{-}F}$ = 10.2 Hz), 132.4 (d, $J_{C\text{-}F}$ = 9.8 Hz), 125.3, 121.7, 118.0 (d, $J_{C\text{-}F}$ = 14.9 Hz), 114.9 (d, $J_{C\text{-}F}$ = 3.4 Hz), 106.9 (d, $J_{C\text{-}F}$ = 24.9 Hz), 64.3 (d, $J_{C\text{-}F}$ = 3.7 Hz).

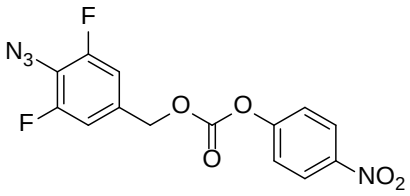
(4-Azido-2,3,5,6-tetrafluorophenyl)methyl-4'-nitrophenyl carbonate (5d):



Synthesized according to **general procedure-VI**. The azido benzyl alcohol **4d** (320.0 mg, 1.91 mmol) was reacted with *p*-nitrophenyl chloroformate (469.9 mg, 2.33 mmol), and pyridine (0.62 mL, 3.73 mmol) in THF (5 mL(a) + 15 mL (b)) to result in a crude residue, which upon silica gel flash column chromatography (10% EtOAc:hexane) resulted in a pale yellow solid (684.0 mg, 93%). ^{1}H NMR ($CDCl_3$, 400 MHz)

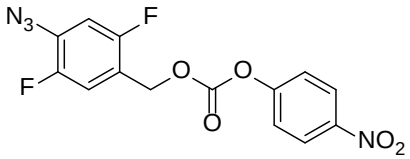
δ 8.30 (d, J = 9.2 Hz, 2H), 7.41 (d, J = 9.2 Hz, 2H), 5.41 (s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.2, 152.0, 145.69 (m, $J_{C\text{-}F}$), 145.56, 140.5 (m, $J_{C\text{-}F}$), 125.3, 121.90 (m, $J_{C\text{-}F}$), 121.65, 108.0 (dd, $J_{C\text{-}F}$ = 17.3, 17.3 Hz), 57.6.

(4-Azido-3,5-difluorophenyl)methyl-4'-nitrophenyl carbonate (5e):



Synthesized according to **general procedure-VI**. The azidobenzyl alcohol **4e** (732.0 mg, 3.95 mmol) was reacted with *p*-nitrophenyl chloroformate (1.19 g, 5.93 mmol), and pyridine (1.59 mL, 19.70 mmol) in THF (5 mL(a) + 20 mL (b)) to result in a crude residue, which upon silica gel flash column chromatography (10% EtOAc:hexane) resulted in a pale white solid (250.0 mg, 18%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.28 (d, J = 9.2 Hz, 2H), 7.39 (d, J = 9.2 Hz, 2H), 7.03 (d, J = 8.3 Hz, 2H), 5.21 (s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.5 (dd, $J_{C\text{-}F}$ = 250.1, 5.1 Hz), 155.2, 155.0, 152.2, 131.7 (dd, $J_{C\text{-}F}$ = 8.6, 8.6 Hz), 125.3, 121.6, 118.0 (dd, $J_{C\text{-}F}$ = 14.1, 14.1 Hz), 112.1 (m, $J_{C\text{-}F}$), 68.9 (dd, $J_{C\text{-}F}$ = 1.9, 1.9 Hz).

(4-Azido-2,5-difluorophenyl)methyl-4'-nitrophenyl carbonate (5f):



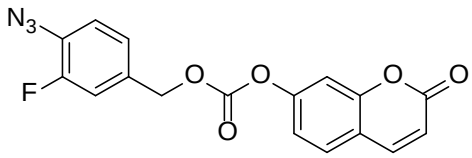
Synthesized according to **general procedure-VI**. The azidobenzyl alcohol **4f** (73.0 mg, 0.40 mmol) was reacted with *p*-nitrophenyl chloroformate (119.4 mg, 0.59 mmol), and pyridine (0.16 mL, 1.97 mmol) in THF (5 mL(a) + 15 mL (b)) to result in a crude residue, which upon silica gel flash column chromatography (10% EtOAc:hexane) resulted in a creamy white solid (88.8 mg, 64%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.28 (d, J = 9.3 Hz, 2H), 7.39 (d, J = 9.3 Hz, 2H), 7.24 (dd, J = 10.4, 6.4 Hz, 1H), 6.84 (dd, J = 9.5, 6.7 Hz, 1H), 5.29 (s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.9 (dd, $J_{C\text{-}F}$ = 246.5, 2.7 Hz), 155.2, 152.2, 151.0 (dd, $J_{C\text{-}F}$ = 245.2, 2.9 Hz), 145.5, 130.2 (dd, $J_{C\text{-}F}$ = 12.9, 10.2 Hz), 125.3, 121.7, 118.50 (dd, $J_{C\text{-}F}$ = 17.2, 6.7 Hz), 118.27 (dd, $J_{C\text{-}F}$ = 21.8, 4.6 Hz), 108.5 (dd, $J_{C\text{-}F}$ = 27.0, 1.6 Hz), 63.6 (dd, $J_{C\text{-}F}$ = 3.4, 1.0 Hz).

General procedure-VII: Synthesis of the coumarin pro-probes 1b-1f

7-hydroxycoumarin **1** and triethylamine were added to a solution of 4-azidobenzyl-4-nitrophenyl carbonate in dry THF. The reaction mixture was stirred in the dark at 25 °C under an atmosphere of nitrogen for 18-24 h. After which time, THF was diluted using water and extracted with EtOAc. The combined organic fractions were washed with water, brine, dried ($MgSO_4$), and concentrated *in vacuo* (temperature maintained below 30 °C).

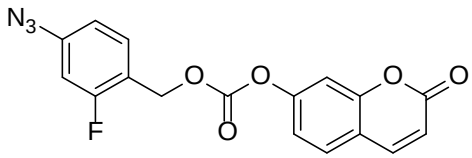
The crude residue was subjected to flash silica gel column chromatography to provide the title compound. In some cases, small amounts of 4-nitrophenol co-eluted with the product, but was removed by rinsing with ice-cold methanol (Note: product is partially soluble in methanol).

(4-Azido-3-fluorophenyl)methyl 2-oxo-2H-chromen-7-yl carbonate (1b):



Synthesized according to **general procedure-VII**. The azidobenzyl-4-nitrophenyl carbonate **5b** (50.0 mg, 0.15 mmol) was reacted with a mixture of 7-hydroxycoumarin **1** (48.8 mg, 0.30 mmol), triethylamine (83 µL, 0.60 mmol) and 4 Å molecular sieves (50 mg) in DMF (2 mL) for 18 h to result in a crude residue, which upon flash column chromatography using silica gel (25% EtOAc:hexane) resulted in a white solid (33.6 mg, 63%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.70 (d, J = 9.6 Hz, 1H), 7.51 (d, J = 8.8 Hz, 1H), 7.24 – 7.23 (m, 2H) (one proton corresponds to the azido-aryl ring and the other corresponds to the aryl ring on 7-hydroxycoumarin), 7.21 (s, 1H), 7.15 (dd, J = 8.4, 2.0 Hz, 1H), 7.11 (m, 1H), 6.42 (d, J = 9.6 Hz, 1H), 5.24 (s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.1, 154.68 (d, $J_{C\text{-}F}$ = 249.3 Hz), 154.61, 153.1, 152.6, 142.6, 132.3 (d, $J_{C\text{-}F}$ = 6.6 Hz), 128.675, 128.683 (d, $J_{C\text{-}F}$ = 11.0 Hz), 125.1 (d, $J_{C\text{-}F}$ = 3.6 Hz), 121.3 (d, $J_{C\text{-}F}$ = 1.7 Hz), 117.55, 117.11, 116.91 (d, $J_{C\text{-}F}$ = 2.1 Hz), 116.34, 109.8, 69.4 (d, $J_{C\text{-}F}$ = 1.6 Hz). HRMS (ESI+) calculated for $C_{17}H_{10}FN_3O_5Na$: 378.0497, found: 378.0495.

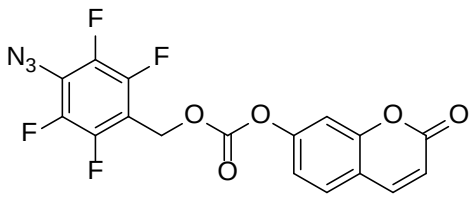
(4-Azido-2-fluorophenyl)methyl 2-oxo-2H-chromen-7-yl carbonate (1c):



Synthesized according to **general procedure-VII**. The azidobenzyl-4-nitrophenyl carbonate **5c** (50.0 mg, 0.15 mmol) was reacted with a mixture of 7-hydroxycoumarin **1** (48.8 mg, 0.30 mmol), triethylamine (84 µL, 0.60 mmol) and 4 Å molecular sieves (50 mg) in DMF (4 mL) for 20 h to result in a crude residue, which upon flash column chromatography using silica gel (25% EtOAc:hexane) resulted in a white solid (44.0 mg, 82%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.70 (d, J = 9.2 Hz, 1H), 7.50 (d, J = 8.4 Hz, 1H), 7.46 (d, J = 8.4 Hz, 1H), 7.24 (d, J = 2.4 Hz, 1H), 7.15 (dd, J = 8.8, 2.4 Hz, 1H), 6.89 (dd, J = 10.4, 2.0 Hz 1H), 6.82 (dd, J = 10.4, 2.0 Hz, 1H), 6.42 (d, J = 9.6 Hz, 1H), 5.33 (d, J = 0.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.8 (d, $J_{C\text{-}F}$ = 250.1 Hz), 160.2, 154.6, 153.2, 152.6, 143.1 (d, $J_{C\text{-}F}$ = 10.2 Hz), 142.7, 132.4 (d, $J_{C\text{-}F}$ = 4.8 Hz), 128.7, 118.1 (d, $J_{C\text{-}F}$ = 14.9 Hz), 117.6, 116.9, 116.3, 114.9 (d, $J_{C\text{-}F}$ = 3.4 Hz),

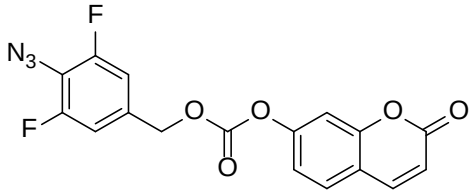
109.9, 106.0 (d, J_{C-F} = 24.9 Hz), 64.0 (d, J_{C-F} = 3.6 Hz). HRMS (ESI+) calculated for $C_{17}H_{10}FN_3O_5Na$: 378.0497, found: 378.0464.

(4-Azido-2,3,5,6-tetrafluorophenyl)methyl 2-oxo-2H-chromen-7-yl carbonate (1d):



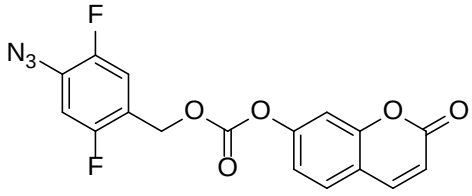
Synthesized according to **general procedure-VII**. The azidobenzyl-4-nitrophenyl carbonate **5d** (40.0 mg, 0.10 mmol) was reacted with a mixture of 7-hydroxycoumarin **1** (41.9 mg, 0.26 mmol), triethylamine (72 µL, 0.52 mmol) and 4 Å molecular sieves (50 mg) in DMF (5 mL) for 20 h to result in a crude residue, which upon flash column chromatography using silica gel (15% EtOAc:hexane) resulted in a white solid (27.8 mg, 66%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.70 (dd, *J* = 9.5 Hz, 1H), 7.51 (d, *J* = 8.5 Hz, 1H), 7.24 (d, *J* = 2.0 Hz, 1H), 7.16 (dd, *J* = 8.5, 2.0 Hz, 1H), 6.43 (d, *J* = 9.5 Hz, 1H), 5.40 (s, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 160.1, 154.6, 153.0, 152.3, 145.7 (m, J_{C-F}), 142.6, 140.4 (m, J_{C-F}), 128.7, 121.8 (m, J_{C-F}), 117.48, 117.0, 116.5, 109.8, 108.2 (m, J_{C-F}), 57.5 (m, J_{C-F}). HRMS (ESI+) calculated for $C_{17}H_7F_4N_3O_5Na$: 432.0214, found: 432.0199.

(4-Azido-3,5-difluorophenyl)methyl 2-oxo-2H-chromen-7-yl carbonate (1e):



Synthesized according to **general procedure-VII**. The azidobenzyl-4-nitrophenyl carbonate **5e** (40.0 mg, 0.11 mmol) was reacted with a mixture of 7-hydroxycoumarin **1** (46.3 mg, 0.29 mmol), triethylamine (79 µL, 0.57 mmol) and 4 Å molecular sieves (100 mg) in DMF (5 mL) for 24 h to result in a crude residue, which upon flash column chromatography using silica gel (25% EtOAc:hexane) resulted in a pale white solid (23.0 mg, 74%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.71 (d, *J* = 9.2 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.24 (d, *J* = 2.4 Hz, 1H), 7.15 (dd, *J* = 8.4, 2.4 Hz, 1H), 7.04 (m, 2H), 6.43 (d, *J* = 9.6 Hz, 1H), 5.21 (s, 2H).^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.1, 155.5 (dd, J_{C-F} = 250.2, 4.9 Hz), 154.6, 154.3, 153.1, 152.5, 142.6, 131.8 (dd, J_{C-F} = 8.6, 8.6 Hz), 128.7, 117.5, 116.98, 112.2 (m, J_{C-F}), 109.96 (m, J_{C-F}), 109.85, 68.8 (dd, J_{C-F} = 1.9, 1.9 Hz). HRMS (ESI+) calculated for $C_{17}H_9F_2N_3O_5Na$: 396.0402, found: 396.0380.

(4-Azido-2,5-difluorophenyl)methyl 2H-chromen-7-yl carbonate (1f):



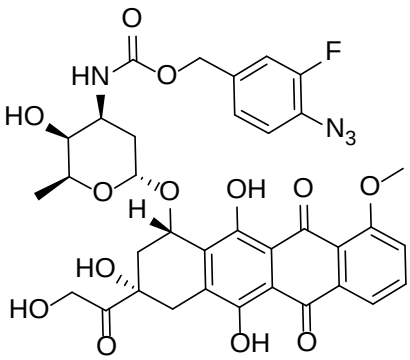
Synthesized according to **general procedure-VII**. The azidobenzyl-4-nitrophenyl carbonate **5f** (40.0 mg, 0.11

mmol) was reacted with a mixture of 7-hydroxycoumarin **1** (46.3 mg, 0.29 mmol), triethylamine (79 µL, 0.57 mmol) and 4 Å molecular sieves (100 mg) in DMF (5 mL) for 24 h to result in a crude residue, which upon flash column chromatography using silica gel (20% EtOAc:hexane) resulted in a creamy white solid (36.0 mg, 72%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.70 (d, *J* = 9.6 Hz, 1H), 7.51 (d, *J* = 8.8 Hz, 1H), 7.26 (m, 1H), 7.24 (d, *J* = 2.4 Hz, 1H), 7.16 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.85 (dd, *J* = 9.2, 6.4 Hz, 1H), 6.42 (d, *J* = 9.6 Hz, 1H), 5.29 (d, *J* = 1.2 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.11, 156.91 (dd, $J_{C\text{-}F}$ = 246.5, 2.6 Hz), 154.6, 153.1, 152.5, 151.0 (dd, $J_{C\text{-}F}$ = 245.3, 3.1 Hz), 142.6, 130.2 (dd, $J_{C\text{-}F}$ = 13.0, 10.3 Hz), 128.7, 118.76 (d, $J_{C\text{-}F}$ = 17.1, 6.6 Hz), 118.30 (dd, $J_{C\text{-}F}$ = 21.9, 4.7 Hz), 117.5, 116.9, 116.4, 109.8, 108.5 (dd, $J_{C\text{-}F}$ = 27.0, 1.7 Hz), 63.5 (dd, $J_{C\text{-}F}$ = 3.6, 1.0 Hz). HRMS (ESI+) calculated for $C_{17}H_9F_2N_3O_5Na$: 396.0402, found: 396.0401.

General procedure-VIII: Synthesis of the doxorubicin prodrugs 2b, 2d and 2e

To a solution of 4-azidobenzyl-4-nitrophenyl carbonate in dry DMF was added 4 Å molecular sieves and triethylamine. The solution was stirred at 25 °C for 10 min before a solution of doxorubicin hydrochloride salt **2** in dry DMF containing triethylamine was added. The solution was stirred under nitrogen at 25 °C for 19-24 h, after which time TLC analysis (5% methanol: DCM) indicated that all of the 4-azidobenzyl-4-nitrophenyl carbonate had been consumed. The reaction was diluted with water and extracted with EtOAc. The combined organic extracts were washed with saturated $NaHCO_3$, water, brine, dried ($MgSO_4$), and concentrated *in vacuo* (temperature maintained below 30 °C). The crude red residue was subjected to flash silica gel column chromatography, to provide the title compound as a dark red solid.

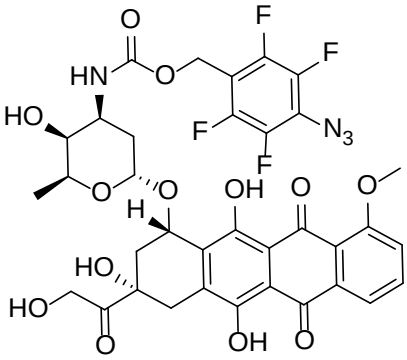
(4-Azido-3-fluorophenyl)methyl-doxorubicin carbamate (2b):



Synthesized according to **general procedure-VIII**. To a solution of the azidobenzyl-4-nitrophenyl carbonate **5b** (20.0 mg, 0.06 mmol) and triethylamine (16 µL, 0.12 mmol) in DMF (1 mL) was added a mixture of doxorubicin.HCl **2** (41.9 mg, 0.07 mmol) and triethylamine (16 µL, 0.12 mmol) in DMF (2 mL), and the resulting mixture stirred for 20 h at 25 °C. Following work-up, the resultant crude residue was subjected to flash silica gel column chromatography (5% MeOH: EtOAc), providing the title compound as a dark red solid (28.4 mg, 66%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 13.92 (s, 1H; Ar-OH), 13.15 (s, 1H; Ar-OH), 7.99 (dd, *J* = 8.0, 1.0 Hz, 1H), 7.77

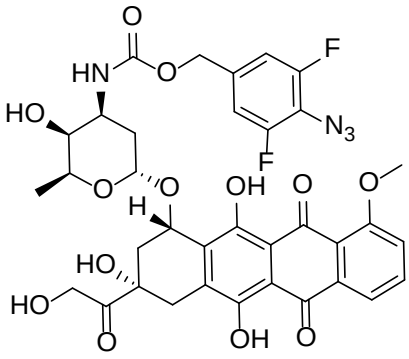
(dd, J = 8.0, 8.0 Hz, 1H), 7.38 (d, J = 8.0 Hz, 1H), 7.04 (m, 1H), 7.02 (m, 1H), 6.97 (m, 1H), 5.49 (d, J = 4.0 Hz, 1H), 5.26 (m, 2H), 5.24 (br s, 1H), 4.94 (m, J_{AB}, 2H), 4.75 (d, J = 2.0 Hz, 1H), 4.53 (s, 1H), 4.14 (m, 1H), 4.06 (s, 3H), 3.86 (m, 1H), 3.66 (s, 1H), 3.21 (dd, 18.5, 2.0 Hz, 1H), 2.92 (d, J_{AB} = 16.0 Hz, 1H), 2.32 (m, 1H), 2.17 (s, 1H), 2.16 (dd, J = 15.0, 4.0 Hz, 1H), 1.87 (dd, J = 13.5, 5.5 Hz, 1H), 1.79 (dt, J = 13.0, 4.0 Hz, 1H), 1.29 (d, J = 6.5 Hz, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 213.7, 187.0, 186.6, 161.0, 156.1, 155.2, 154.4 (d, J_{C-F} = 277.3 Hz), 135.8, 135.4, 134.7 (d, J_{C-F} = 8.6 Hz), 133.50, 133.45, 127.5 (d, J_{C-F} = 14.1 Hz), 124.4 (d, J_{C-F} = 3.4 Hz), 120.9 (d, J_{C-F} = 2.0 Hz), 120.7, 119.8, 118.4, 116.3 (d, J_{C-F} = 24.3 Hz), 111.51, 111.33, 100.7, 76.6, 69.7, 69.5, 67.24, 65.5, 65.4, 56.6, 47.0, 35.6, 33.9, 30.1, 29.7, 16.8. HRMS (ESI+) calculated for $C_{35}H_{33}FN_4O_{13}Na$: 759.1920, found: 759.1892.

(4-Azido-2,3,5,6-tetrafluorophenyl)methyl-doxorubicin carbamate (2d):



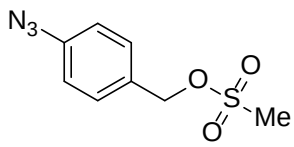
Synthesized according to **general procedure-VIII**. To a solution of the azidobenzyl-4-nitrophenyl carbonate **5d** (16.6 mg, 0.04 mmol) and triethylamine (12 µL, 0.09 mmol) in DMF (2 mL) was added a mixture of doxorubicin.HCl **2** (30.0 mg, 0.05 mmol), triethylamine (12 µL, 0.09 mmol) and 4 Å molecular sieves (100 mg) in DMF (2 mL), and the resulting mixture stirred for 22 h at 25 °C. Following work-up, the resultant crude residue was subjected to flash silica gel column chromatography (5% MeOH: EtOAc), providing the title compound as a dark red solid (31.0 mg, 62%). Note: ^{13}C NMR spectrum showed the presence of possible *syn*-/*anti*- carbamate isomers (see Figure S20, peak doubling at δ 69.8, 69.7, 69.42, 69.36, 56.54, 56.49, and 16.74, 16.72 ppm). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 13.97 (s, 1H; Ar-OH), 13.24 (s, 1H; Ar-OH), 8.04 (d, J = 7.6 Hz, 1H), 7.79 (dd, J = 8.0, 8.0 Hz, 1H), 7.40 (d, J = 8.4 Hz, 1H), 5.50 (d, J = 3.6 Hz, 1H), 5.31 (s, 1H), 5.29 (br s, 1H), 5.17 (m, 1H), 5.12 (s, 2H), 4.76 (m, J_{AB}, 2H), 4.50 (s, 1H), 4.14 (m, 1H), 4.09 (s, 3H), 3.86 (br s, 1H), 3.66 (br s, 1H), 3.28 (d, 18.4, 1H), 2.99 (m, 1H), 2.33 (d, J = 14.8 Hz, 1H), 2.18 (dd, J = 14.8, 4.0 Hz, 1H), 1.94 (m, 2H), 1.77 (dt, J = 13.2, 4.0 Hz, 1H), 1.29 (d, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 213.7, 186.7, 186.2, 160.9, 156.1, 155.4, 154.7, 145.4 (m, J_{C-F}), 140.2 (m, J_{C-F}), 135.7, 135.2, 133.5, 133.4, 120.5, 119.7, 118.4, 111.3, 111.20, 110.1 (m, J_{C-F}), 100.8, 76.5, 69.8, 69.7, 69.42, 69.36, 67.2, 65.5, 56.54, 56.49, 53.8 (m, J_{CF}), 47.2, 35.5, 33.7, 30.0, 16.74, 16.72. HRMS (ESI+) calculated for $C_{35}H_{30}F_4N_4O_{13}Na$: 813.1683, found: 813.1677.

(4-Azido-3,5-difluorophenyl)methyl-doxorubicin carbamate (2e):



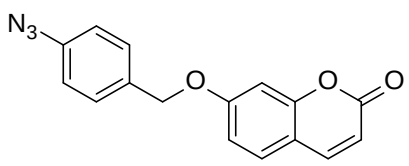
Synthesized according to **general procedure-VIII**. To a solution of the azidobenzyl-4-nitrophenyl carbonate **5e** (50.0 mg, 0.14 mmol) and triethylamine (40 µL, 0.29 mmol) in DMF (2 mL) was added a mixture of doxorubicin.HCl **2** (99.3 mg, 0.17 mmol), triethylamine (40 µL, 0.29 mmol) and 4 Å molecular sieves (100 mg) in DMF (2 mL), and the resulting mixture stirred for 24 h at 25 °C. Following work-up, the resultant crude residue was subjected to flash silica gel column chromatography (5% MeOH: EtOAc), providing the title compound as a dark red solid (53.1 mg, 49%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 13.94 (s, 1H; Ar-OH), 13.17 (s, 1H; Ar-OH), 8.01 (d, J_{AB} = 7.5 Hz, 1H), 7.78 (dd, *J* = 8.0, 8.0 Hz, 1H), 7.39 (dd, *J* = 8.5, 1.0 Hz, 1H), 6.86 (d, *J* = 8.5 Hz, 2H), 5.50 (d, *J* = 4.0 Hz, 1H), 5.28 (d, *J* = 8.5 Hz, 1H), 5.26 (br s, 1H), 4.92 (m, 2H), 4.75 (m, 1H), 4.14 (m, 1H), 4.08 (s, 3H), 3.86 (br s, 1H), 3.67 (s, 1H), 3.23 (d, *J* = 18.5 Hz, 1H), 2.94 (d, J_{AB} = 19.0 Hz, 1H), 2.33 (d, *J* = 14.5 Hz, 1H), 2.17 (dd, *J* = 14.5, 4.0 Hz, 1H), 1.89 (dd, *J* = 13.5, 5.0, 1H), 1.79 (dt, *J* = 13.5, 3.5 Hz, 1H), 1.30 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 213.7, 186.97, 186.53, 161.0, 156.0, 155.40 (dd, $J_{C\text{-}F}$ = 249.5, 5.0 Hz), 155.50, 155.0, 135.8, 135.4, 134.3 (t, $J_{C\text{-}F}$ = 8.1 Hz), 133.51, 133.42, 120.7, 119.8, 118.4, 116.8 (m, $J_{C\text{-}F}$), 111.43 (m, 2 × C), 100.7, 76.6, 69.8, 69.5, 67.2, 65.5, 64.9, 56.62, 56.60, 47.1, 35.6, 33.9, 30.1, 16.8. HRMS (ESI+) calculated for $C_{35}H_{32}F_2N_4O_{13}Na$: 777.1826, found: 777.1758.

4-Azidobenzyl mesylate (10a):



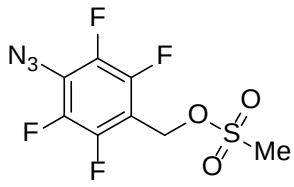
To a solution of 4-azidobenzyl alcohol **3a** (0.21 g, 1.3 mmol) dissolved in DCM (12 mL), was added triethylamine (0.28 mL, 2.0 mmol) and the mixture was allowed to cool on ice. Mesyl chloride (0.30 mL, 4.0 mmol) was then added dropwise to the reaction mixture and left to stir under N_2 (at 25 °C) for 20 h. After which time, TLC analysis indicated that all of **3a** had been consumed. The reaction mixture was then diluted with DCM (50 mL) and washed with water (3 × 50 mL), brine (1 × 50 mL), dried ($MgSO_4$) and concentrated *in vacuo* to provide **10a** as a dark brown coloured oil (0.283 g), which was used in the next synthetic step without further purification.

7-((4-azidobenzyl)oxy)-2H-chromen-2-one (11a):



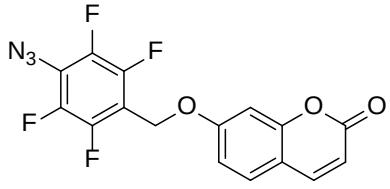
To a solution of the crude azidobenzyl mesylate **10a** (0.100 g, 0.44 mmol) dissolved in MeCN (2 mL) was dropwise added a mixture of 7-hydroxycoumarin **1** (0.134 g, 0.83 mmol) and potassium carbonate (0.190 g, 1.37 mmol) in MeCN (8 mL). The resulting mixture was then allowed to stir in the dark (at 25 °C) under nitrogen for five days. After which time, TLC analysis indicated that all of the **10a** had been consumed. Following which, the reaction mixture was concentrated and the crude residue was dissolved in EtOAc (20 mL) and washed with water (3 × 50 mL), brine (1 × 50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting crude residue was purified by silica gel flash column chromatography (30% EtOAc:hexane) to provide **11a** as a pale-brown crystalline solid (53.0 mg, 41%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.63 (d, *J* = 9.4 Hz, 1H), 7.42 (d, *J* = 8.5 Hz, 2H), 7.38 (d, *J* = 8.5 Hz, 1H), 7.08 – 7.04 (m, 2H), 6.90 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.87 (d, *J* = 2.4 Hz, 1H), 6.26 (d, *J* = 9.5 Hz, 1H), 5.09 (s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.8, 161.2, 155.9, 143.5, 140.4, 132.5, 129.3, 129.0, 119.5, 113.5, 113.3, 113.0, 102.0, 70.1. HRMS (ESI+) calculated for $C_{16}H_{11}N_3NaO_3$: 316.0693, found: 316.0666.

4-Azidotetrafluorobenzyl mesylate (10d):



To a solution of 4-azidobenzyl alcohol **3d** (65.0 mg, 0.29 mmol) dissolved in DCM (4 mL), was added triethylamine (0.06 mL, 0.44 mmol) and the mixture was allowed to cool on ice. Mesyl chloride (0.04 mL, 0.44 mmol) was then added dropwise to the reaction mixture and left to stir under N_2 (at 25 °C) for 24 h. After which time, TLC analysis indicated that all of **3d** had been consumed. The reaction mixture was then diluted with DCM (20 mL) and washed with water (3 × 20 mL), brine (1 × 20 mL), dried ($MgSO_4$) and concentrated *in vacuo* to provide **10d** as a brown coloured oil (54.0 mg), which was used in the next synthetic step without further purification.

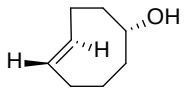
7-((4-azido-2,3,5,6-tetrafluorobenzyl)oxy)-2H-chromen-2-one (11d):



To a solution of the crude azidobenzyl mesylate **10d** (54.0 mg, 0.18 mmol) dissolved in MeCN (2 mL) was dropwise added a mixture of 7-hydroxycoumarin **1** (41.0 mg, 0.25 mmol) and potassium carbonate (49.0 mg, 0.35 mmol) in MeCN (4 mL). The resulting mixture was then allowed to stir in dark (at 25 °C) under nitrogen for five days. After which time, TLC analysis indicated that all of **10d** had been

consumed. Following which, the reaction mixture was concentrated and the crude residue was dissolved in EtOAc (20 mL) and washed with water (3 × 50 mL), brine (1 × 50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting crude residue was purified by silica gel flash column chromatography (20% EtOAc:hexane) to provide **11d** as a white crystalline solid (48.0 mg, 72%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.65 (dd, *J* = 9.5, 0.7 Hz, 1H), 7.41 (d, *J* = 8.5 Hz, 1H), 6.92 – 6.86 (m, 2H), 6.29 (d, *J* = 9.5 Hz, 1H), 5.17 (t, *J* = 1.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 160.90, 160.85, 155.7, 145.6 (m, $J_{C\text{-}F}$), 143.1, 140.4 (m, $J_{C\text{-}F}$), 129.4, 121.3 (m, $J_{C\text{-}F}$), 113.8, 113.3, 112.8, 109.4 (m, $J_{C\text{-}F}$), 101.9, 57.9 (m, $J_{C\text{-}F}$). HRMS (ESI+) calculated for $C_{16}H_8F_4N_3O_3$: 366.0496, found: 366.0517.

Synthesis of *trans*-Cyclooct-4-enol (12; major/equatorial isomer):



major-**12**
-OH equitorial

trans-Cyclooct-4-enol (TCO) was synthesized using a modified literature procedure.[11, 12] To a 250 mL quartz reaction vessel was added *cis*-cyclooct-4-enol (0.75 g, 5.94 mmol), methyl benzoate (2.56 g, 17.8 mmol) and diethyl ether (200 mL). The reaction mixture was placed in a Rayonet photoreactor and irradiated for 30 min at 254 nm. The solution was removed from the photoreactor and passed through a silver nitrate ($AgNO_3$) impregnated silica column (maintained in dark with aluminium foil), washed with one column volume of diethyl ether and the filtrate re-subjected to the photoreactor for a further 30 min. The process was repeated for a total of six times. On the final run, the $AgNO_3$-impregnated silica gel was washed with DCM (2 x column volume), diethyl ether (2 x column volume), ethyl acetate (1 x column volume), and the silica gel allowed to dry under a flow of air pressure. The dry silica gel was then removed from the column and concentrated ammonium hydroxide (28%) was added (20 mL). The slurry was stirred vigorously for 5 min. Diethyl ether (20 mL) was added and stirred vigorously for a further 5 min, and then the diethyl ether was decanted from the $AgNO_3$ silica gel. The silica gel was washed with additional diethyl ether (2 x 20 mL), or until no more *trans*-cyclooct-4-enol was observed on the TLC ($KMnO_4$). The combined diethyl ether extracts were washed with water (3 x 30 mL), brine (3 x 30 mL), dried ($MgSO_4$) and concentrated *in vacuo* to provide a pure mixture of *trans*-cyclooct-4-enol (1.34:1 mixture of diastereomers) as a clear viscous liquid (0.365 g, 49%). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 135.0, 134.3, 133.0, 132.8, 77.7, 67.4, 44.6, 43.0, 41.1, 34.3, 34.1, 34.0, 32.6, 31.2, 29.3, 27.7.

The mixture of diastereomers was subjected to flash silica gel column chromatography (10% diethyl ether: pentane) providing the minor isomer as a clear viscous liquid (160 mg, 21%).[11] ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.62-5.51 (m, 2H); 4.05-4.02 (m, 1H); 2.40-2.32 (m,

1H); 2.28-2.18 (m, 2H); 2.17-2.06 (m, 2H); 1.92-1.71 (m, 3H); 1.70-1.61 (m, 1H); 1.33 (br s, 1H); 1.30-1.23 (m, 1H). Continued elution with 20% diethyl ether: pentane provided the major isomer as a clear viscous liquid (195 mg, 26%).[11] ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.61-5.53 (m, 1H); 5.42-5.34 (m, 1H); 3.48-3.43 (m, 1H); 2.37-2.23 (m, 3H); 2.00-1.88 (m, 4H); 1.72-1.51 (m, 3H); 1.32 (br s, 1H).

3. Results for *in vitro* pro-probe activation and kinetics study

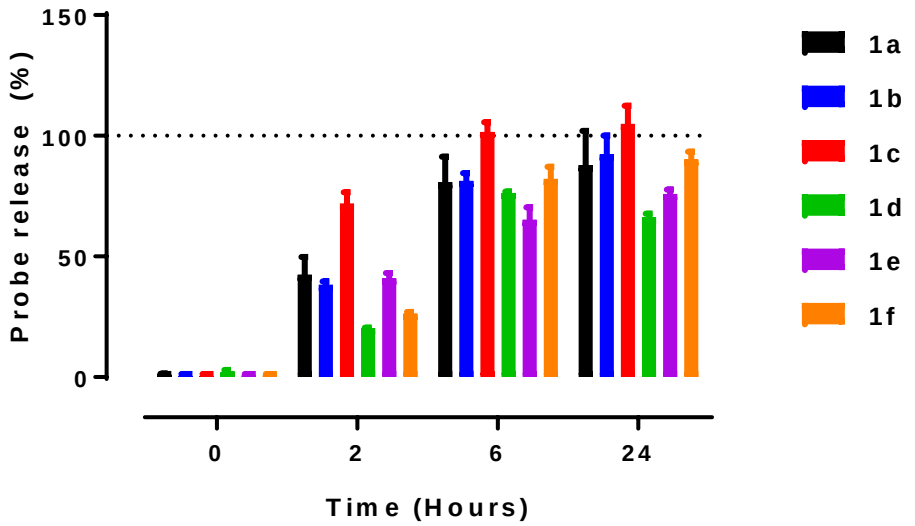


Figure S1a. Release of 7-hydroxycoumarin **1** from **1a-1f** in PBS:MeCN (1:1), measured by fluorescence (Ex. 360, Em. 455 nm). Error represented as ± SD (n = 3). Results for **1a** taken from our previous study.[1]

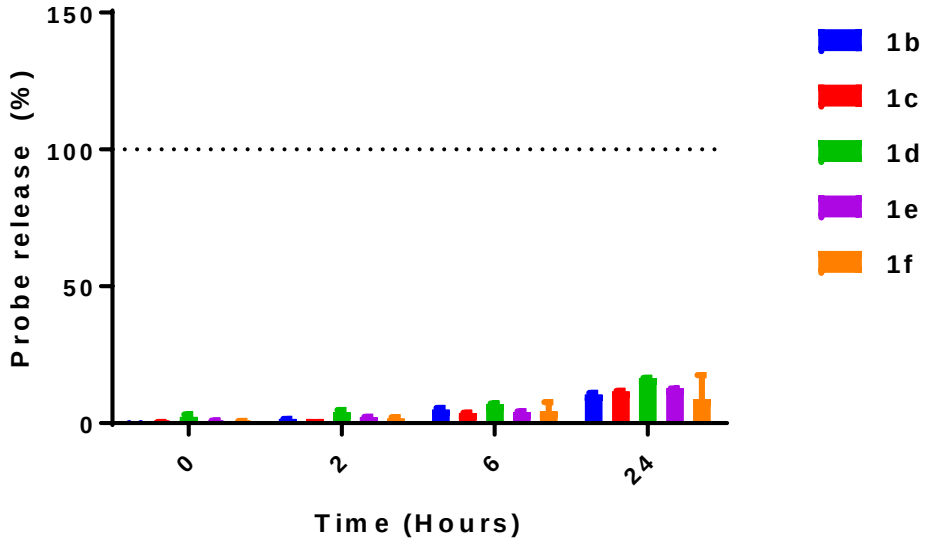


Figure S1b. Background hydrolysis of **1b-1f** in PBS:MeCN (1:1), measured by fluorescence (Ex. 360, Em. 455 nm). Error represented as ± SD (n = 3).

4. HPLC Kinetic Experiments: 1,3-Dipolar Cycloaddition

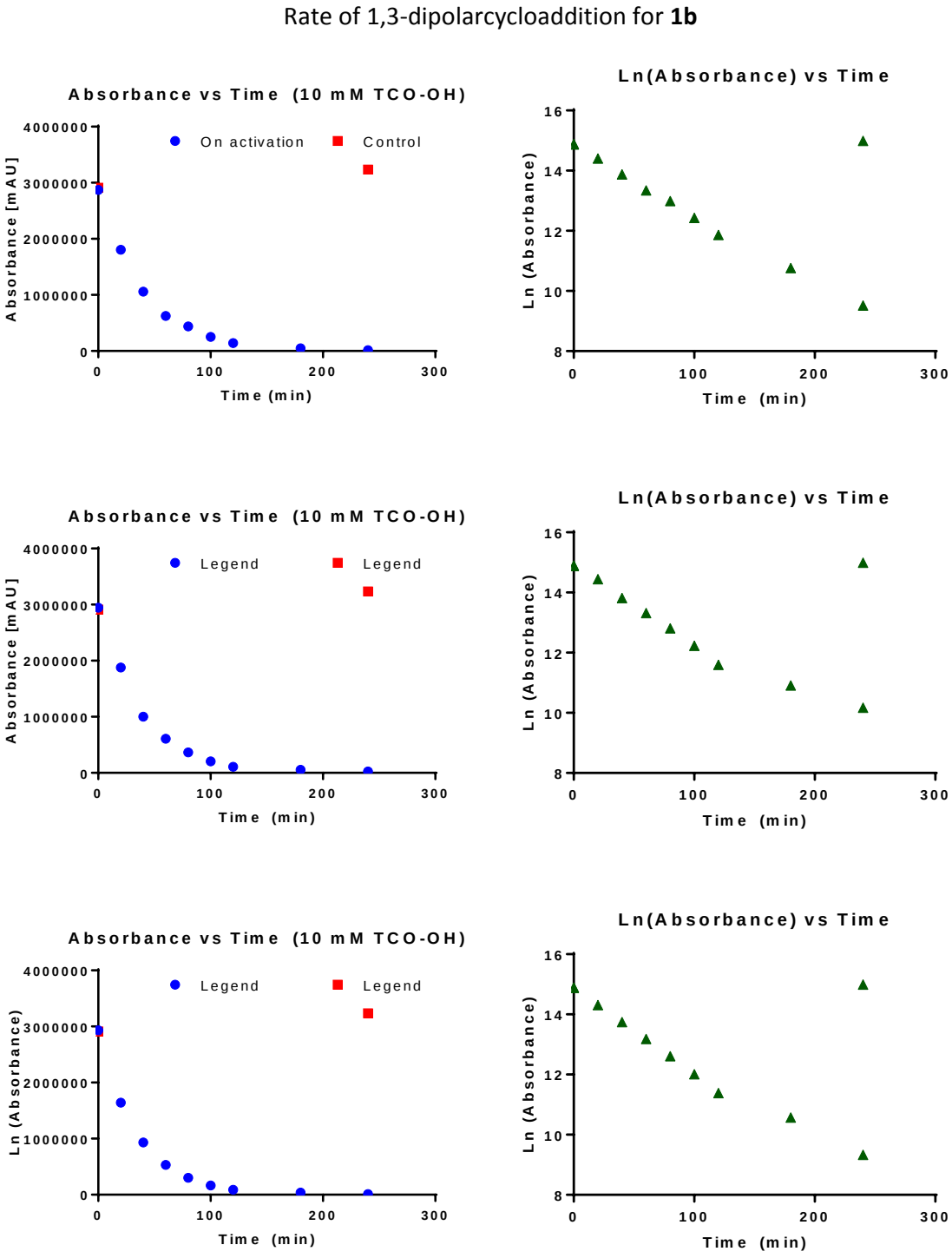


Figure S2a. Triplicate *pseudo* first-order kinetic data obtained for reaction of azido-coumarin probe **1b** (0.5 mM) and TCO **12** (20 mM). Second-order rate constant calculated as: 0.035 $M^{-1}s^{-1}$ ± 0.004 $M^{-1}s^{-1}$ (n = 3).

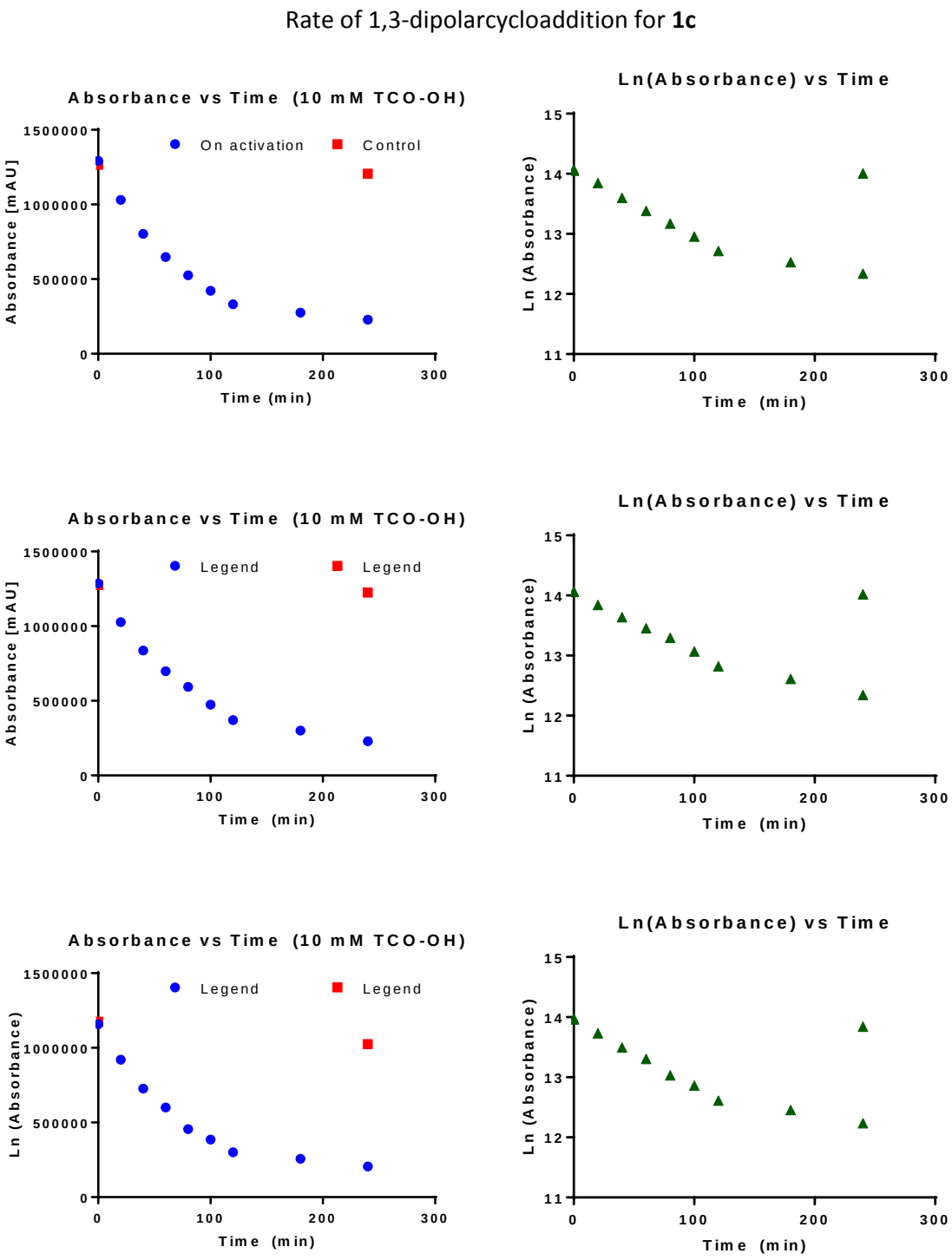


Figure S2b. Triplicate *pseudo* first-order kinetic data obtained for reaction of azido-coumarin probe **1c** (0.5 mM) and TCO **12** (10 mM). Second-order rate constant calculated as: 0.036 $M^{-1}s^{-1}$ ± 0.006 $M^{-1}s^{-1}$ (n = 3).

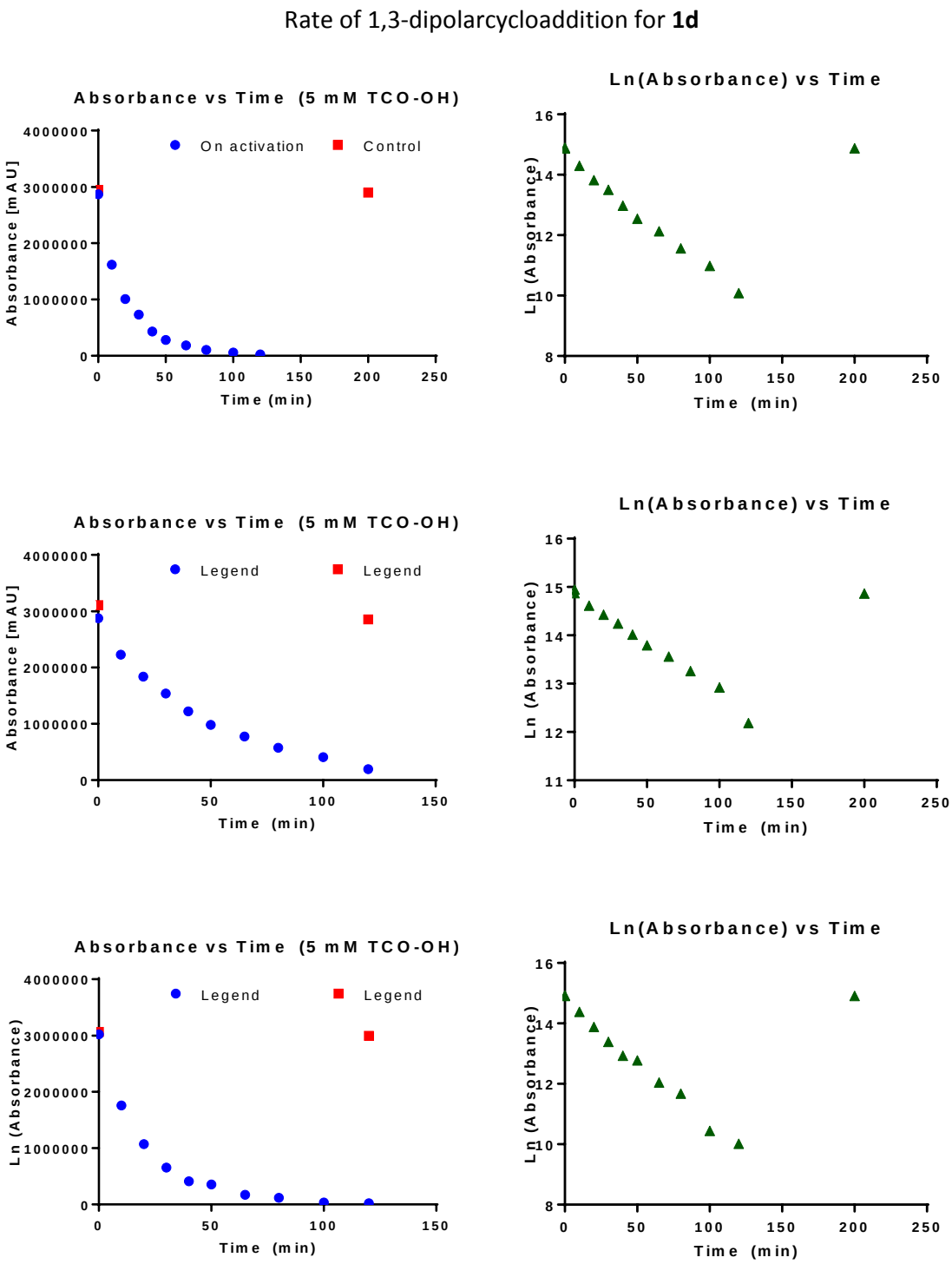


Figure S2c. Triplicate *pseudo* first-order kinetic data obtained for reaction of azido-coumarin probe **1d** (0.5 mM) and TCO **12** (5 mM). *Note:* To enable more data points for rate calculation, the HPLC run was stopped at 10 min and the next sample injected at initial mobile phase concentration (See section S1). Second-order rate constant calculated as: 0.110 $M^{-1}s^{-1}$ ± 0.036 $M^{-1}s^{-1}$ (n = 3).

Rate of 1,3-dipolarcycloaddition for **1e**

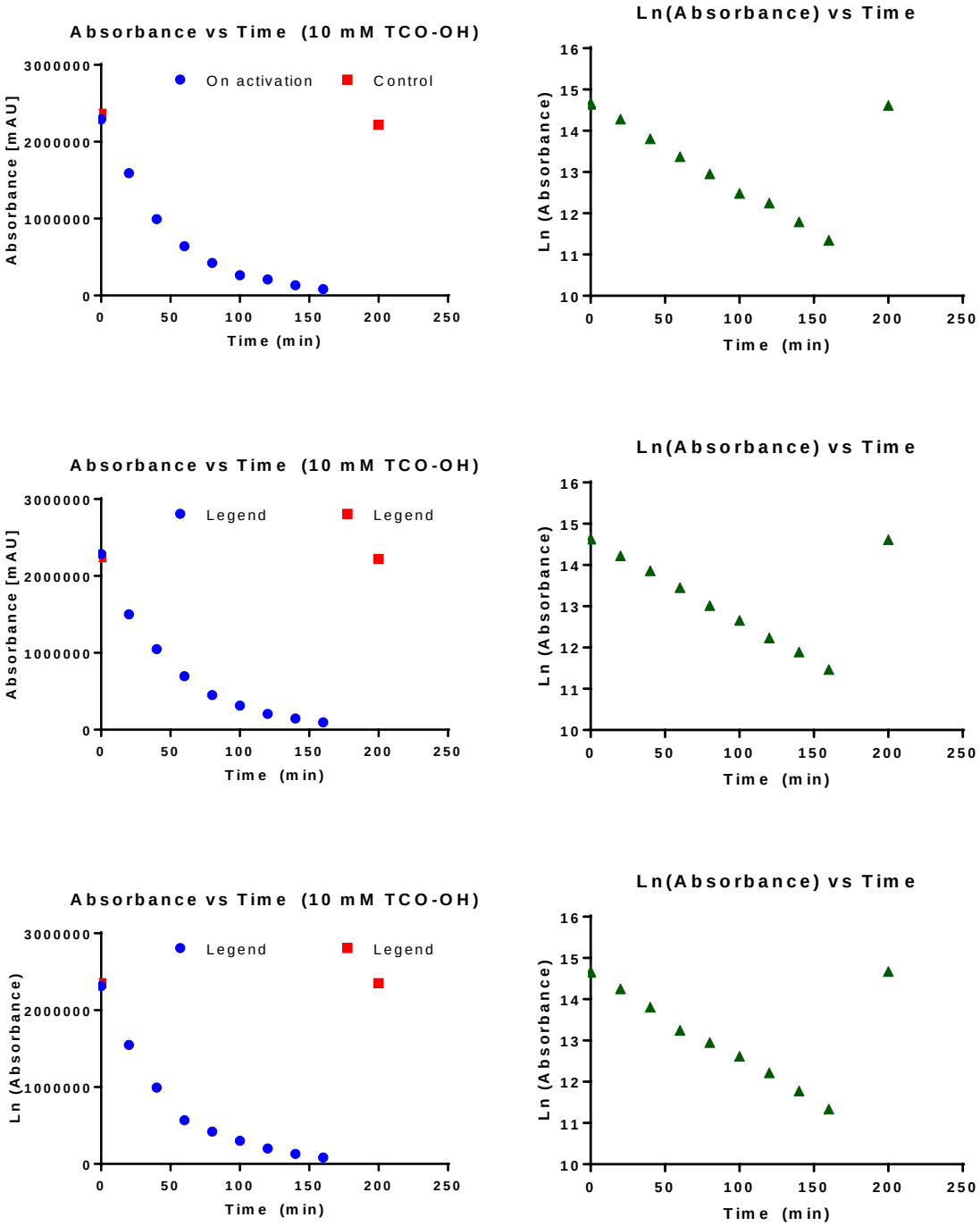


Figure S2d. Triplicate *pseudo* first-order kinetic data obtained for reaction of azido-coumarin probe **1e** (0.5 mM) and TCO **12** (10 mM). Second-order rate constant calculated as: 0.067 $M^{-1}s^{-1}$ ± 0.001 $M^{-1}s^{-1}$ (n = 3).

Rate of 1,3-dipolarcycloaddition for **1f**

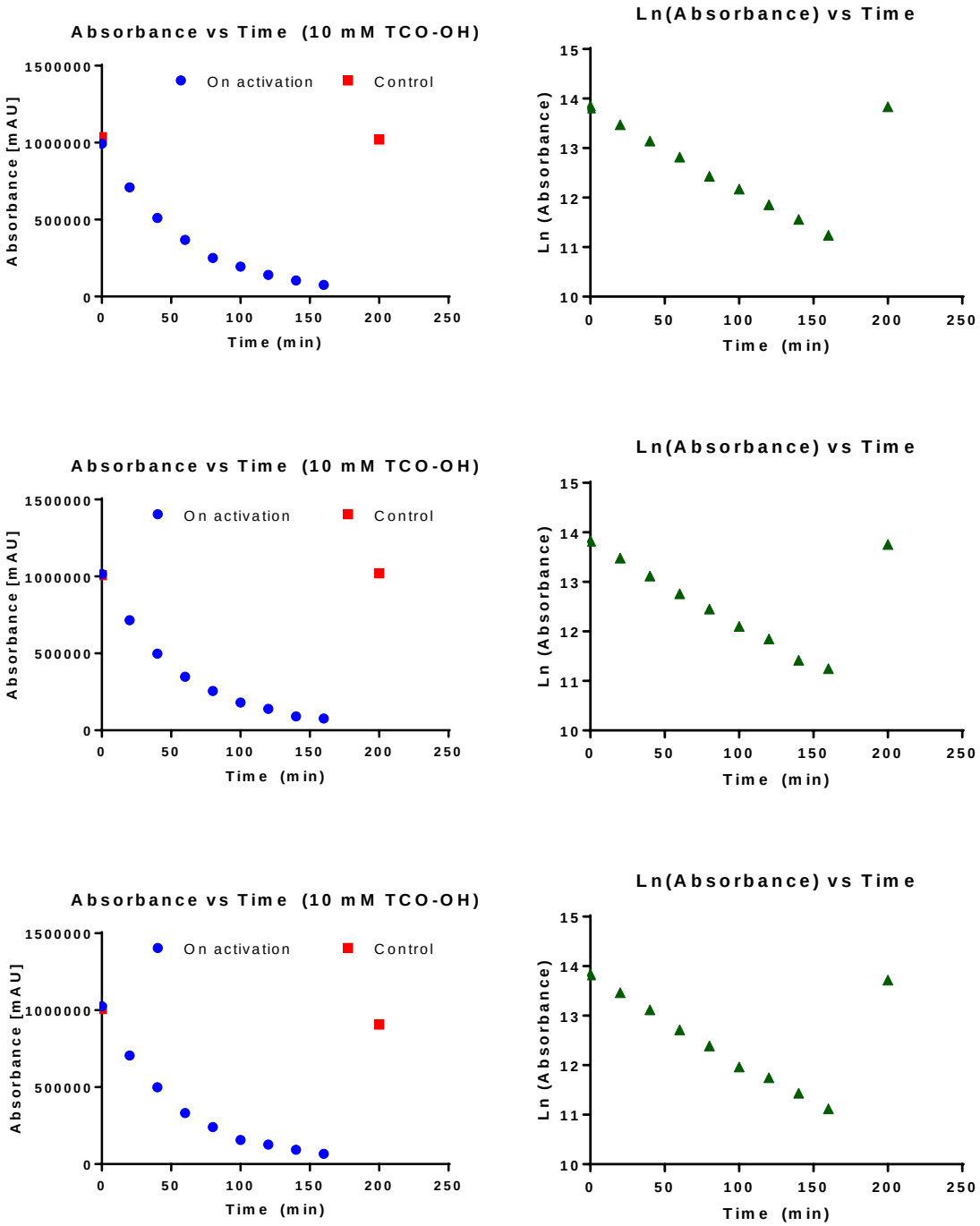


Figure S2e. Triplicate *pseudo* first-order kinetic data obtained for reaction of azido-coumarin probe **1f** (0.5 mM) and TCO **12** (10 mM). Second-order rate constant calculated as: 0.055 $M^{-1}s^{-1}$ ± 0.002 $M^{-1}s^{-1}$ (n = 3).

5. Spectrofluorometry Kinetic Experiments:

Triazoline and Imine Degradation: Cycloaddition between probe **1a-1f** and **12** monitored by ^{1}H NMR spectroscopy in MeCN-d_3.

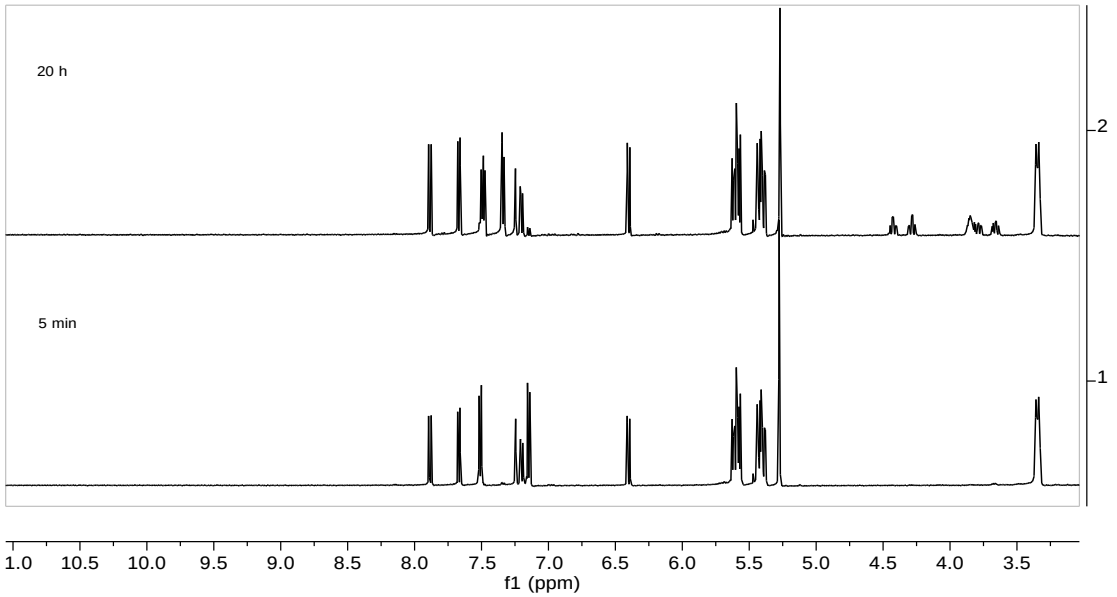


Figure S3a. Full ^{1}H NMR spectrum monitoring the 1,3-dipolar cycloaddition between probe **1a** + TCO **12** in 100% MeCN-d_3

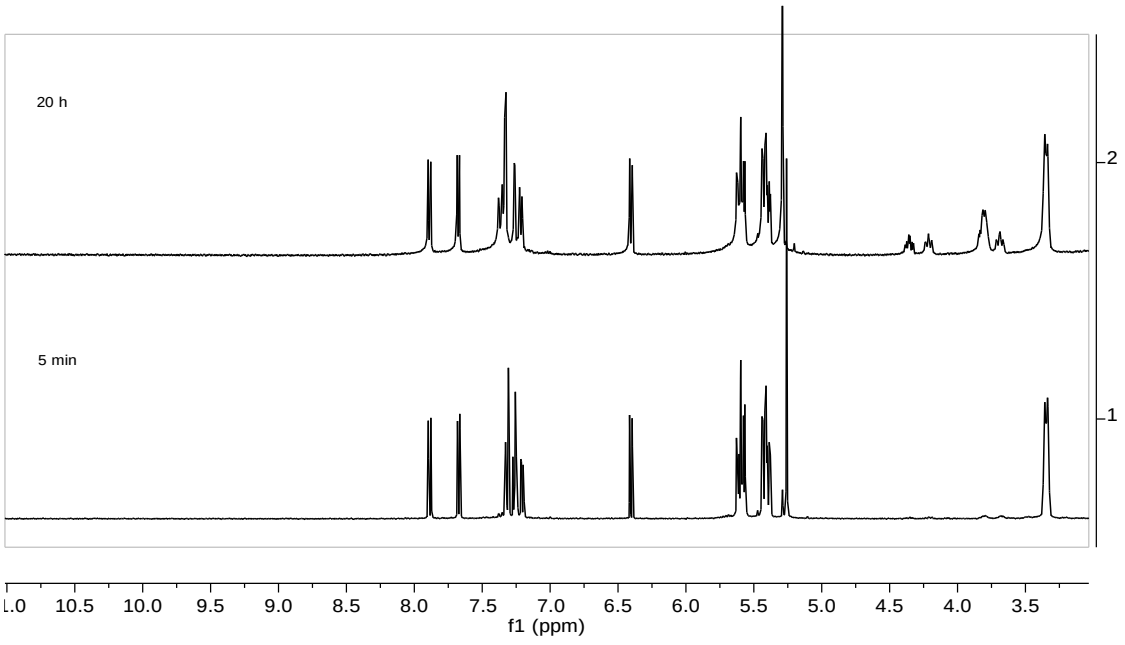


Figure S3b. Full ^{1}H NMR spectrum monitoring the 1,3-dipolar cycloaddition between probe **1b** + TCO **12** in 100% MeCN-d_3

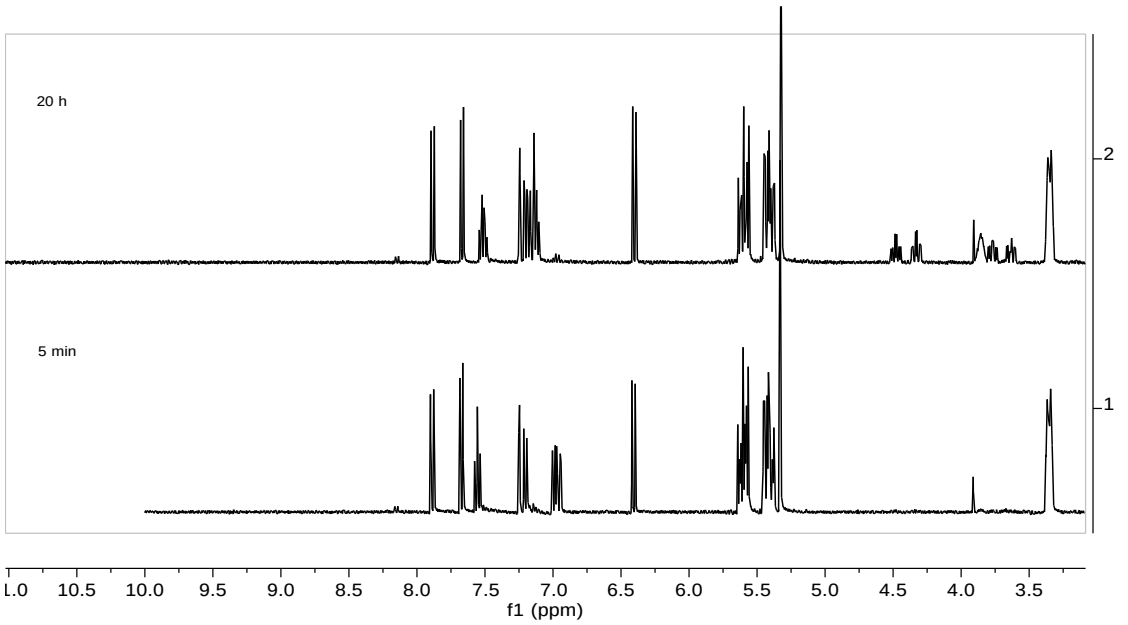


Figure S3c. Full ^{1}H NMR spectrum monitoring the 1,3-dipolar cycloaddition between probe **1c** + TCO **12** in 100% MeCN-d_3

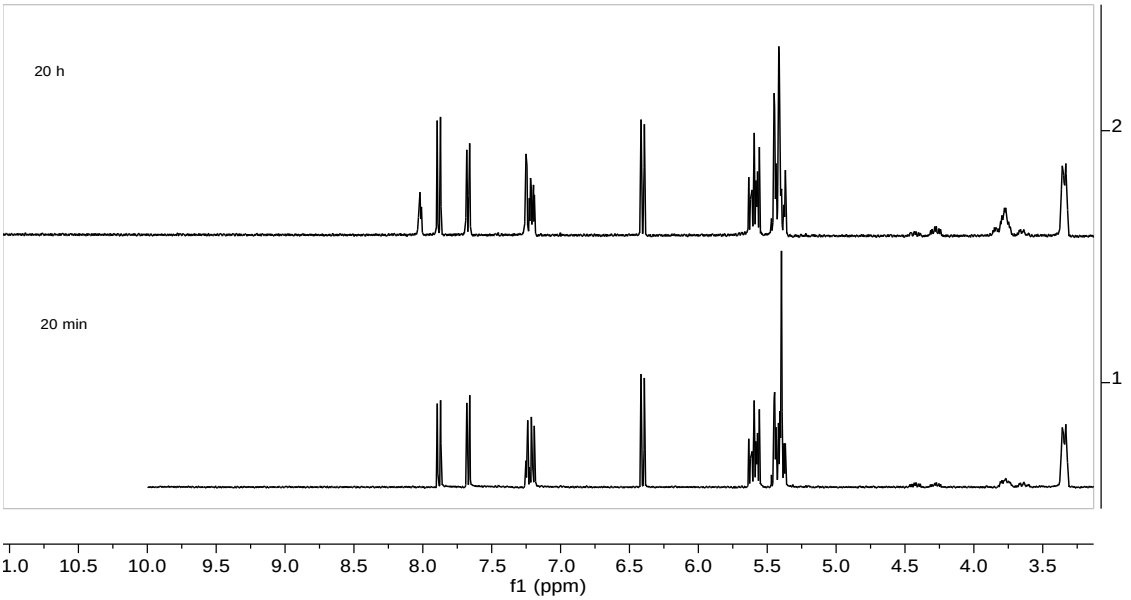


Figure S3d. Full ^{1}H NMR spectrum monitoring the 1,3-dipolar cycloaddition between probe **1d** + TCO **12** in 100% MeCN-d_3

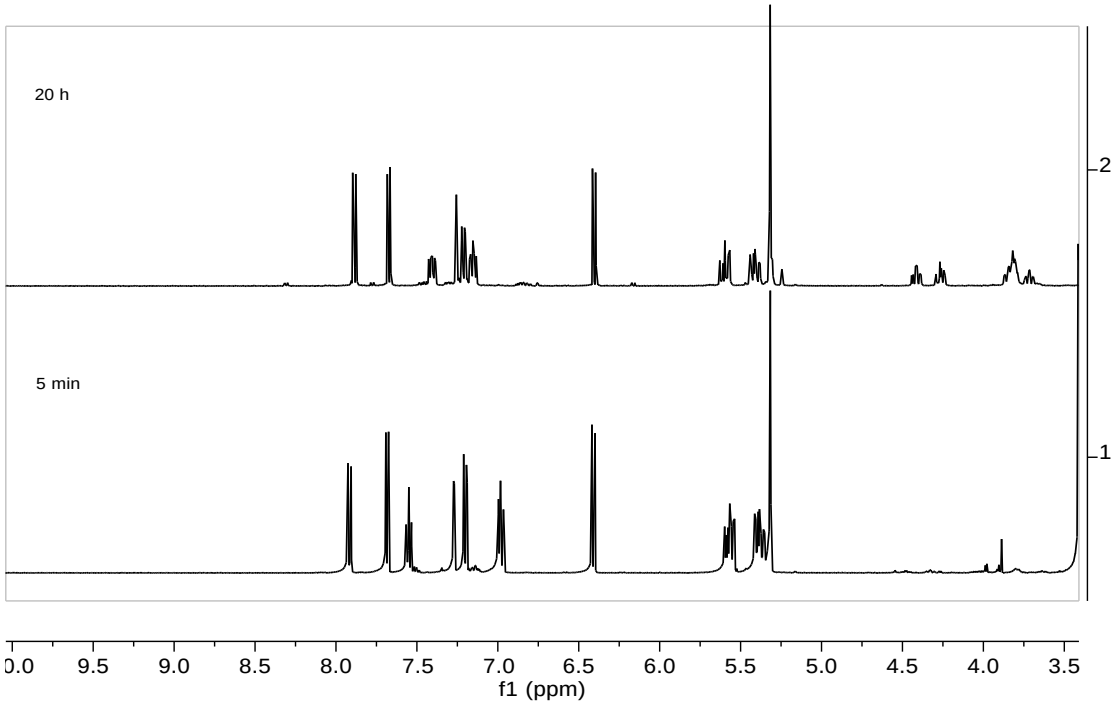


Figure S3e. Full ^{1}H NMR spectrum monitoring the 1,3-dipolar cycloaddition between probe **1e** + TCO **12** in 100% MeCN-d_3

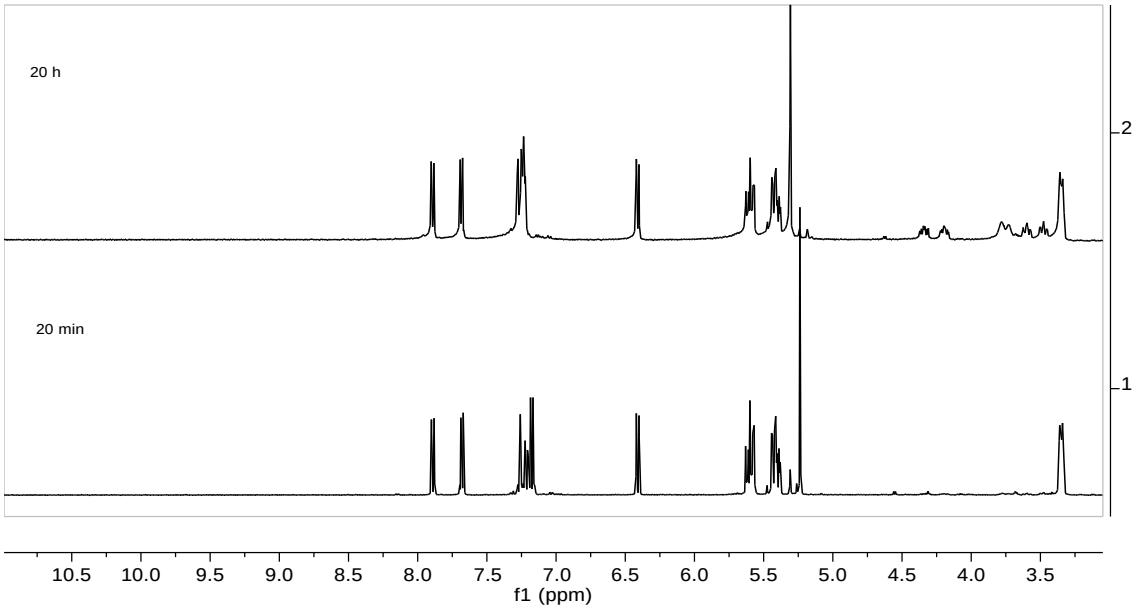


Figure S3f. Full ^{1}H NMR spectrum monitoring the 1,3-dipolar cycloaddition between probe **1f** + TCO **12** in 100% MeCN-d_3

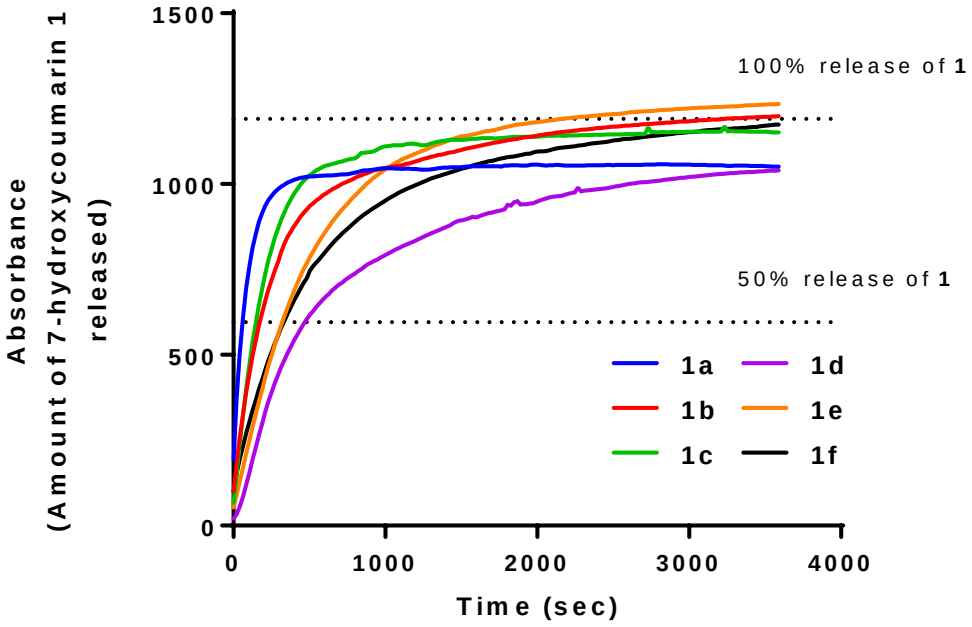


Figure S4. Average of the release of 7-hydroxycoumarin **1** from triazoline corresponding to **1a-1f** monitored by spectrofluorometry (Ex. 360, Em. 455 nm). Experiments run in triplicate from one NMR sample.

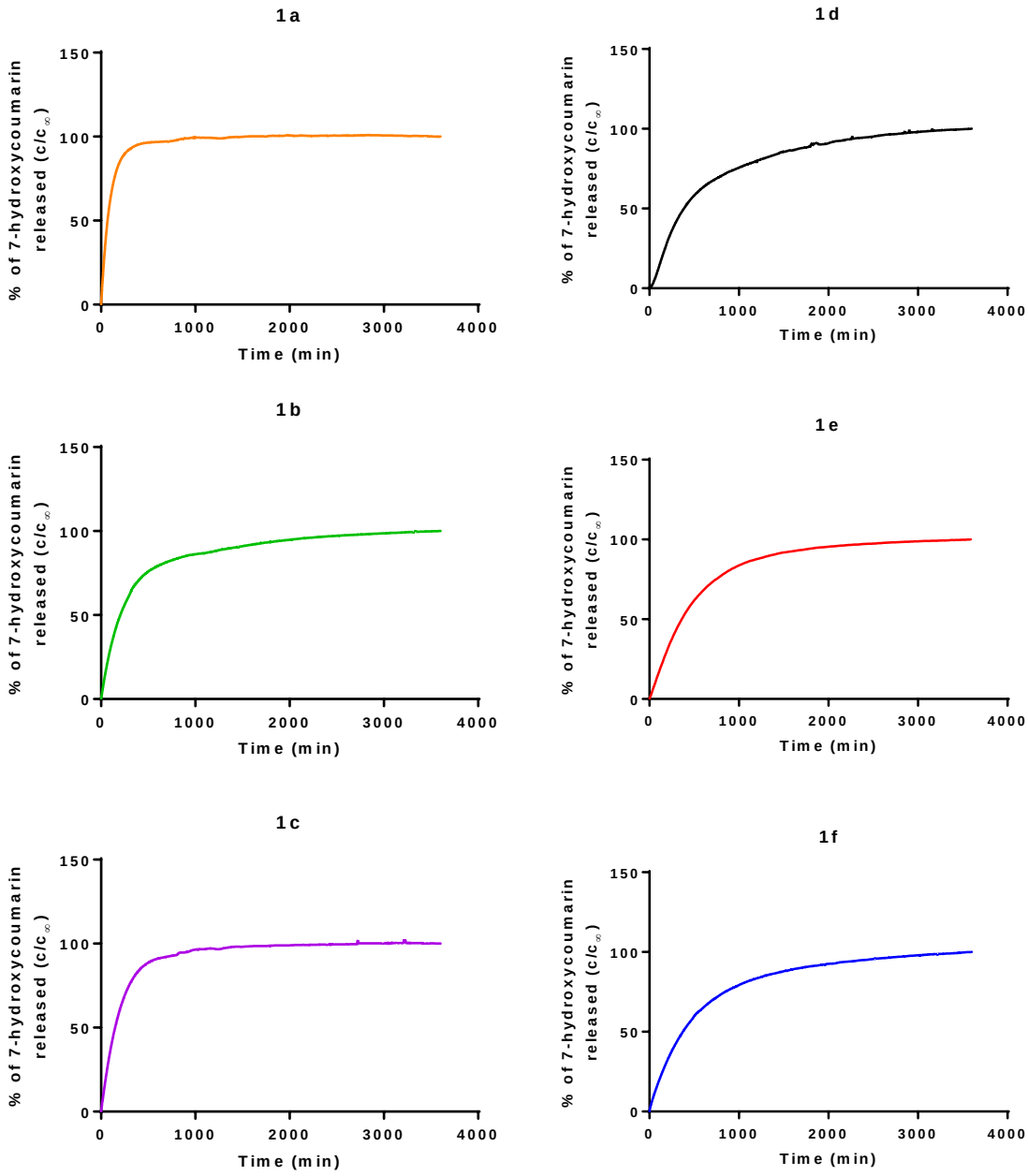


Figure S5. Normalised release (to 100%) of 7-hydroxycoumarin **1** from triazoline corresponding to **1a-1f** monitored by spectrofluorometry (Ex. 360, Em. 455 nm). Experiments run in triplicate.

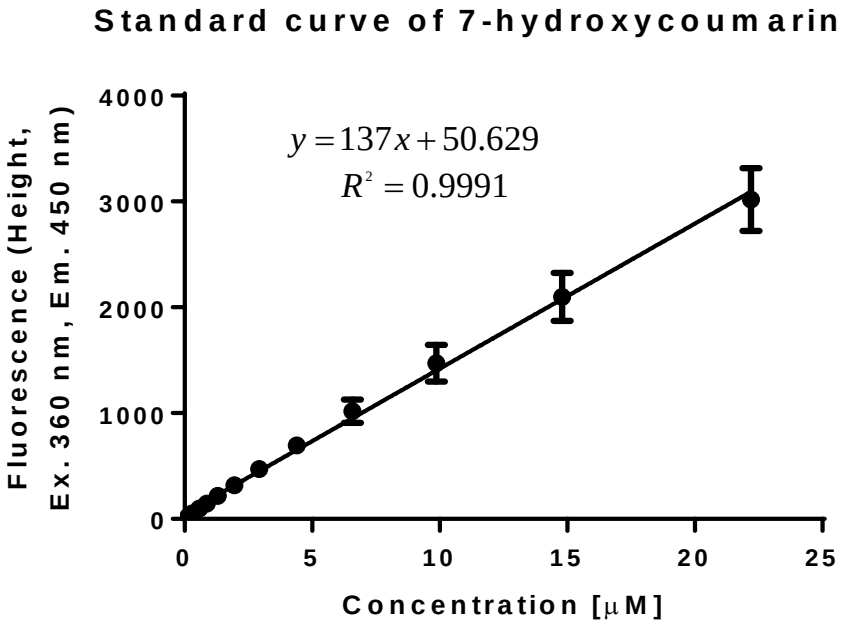


Figure S6. Standard curve of 7-hydroxycoumarin **1** measured at an Ex. of 360 nm and an Em. of 455 nm (height). Error bars represent ± SD (n = 3). Fluorescence expected at 100% release of drug from an 8.33 μM solution is 1191.8 units.

6. Computational Analysis

Solvent effects: The effects of using an implicit solvent model on the reaction energetics of triazoline degradation were investigated by calculating the free energies of the reactant and product *in vacuo* and then reoptimizing using the SMD solvent model as implemented in Gaussian09. For all linkers, the reaction free energy was found to change by less than 3 kJ mol^{-1} (Table S1).

Table S1. Free energy of reaction, (ΔG, in kJ mol^{-1}) of 1,2,3-triazoline degradation calculated *in vacuo* and with an implicit water solvent.

Entry	ΔG*(in vacuo)*	ΔG(water)
18a	126	129
18b	123	128
18c	124	123
18d	112	112
18e	119	121
18f	120	121

Transition state for triazoline degradation: Initial structures were generated using a Monte Carlo conformer distribution search performed in Spartan '14[13] with a Merck molecular force field (MMFF06).[14] This was performed only for **18e** and other molecules were built from this one. 10,000 conformers were searched and the lowest energy conformer was retained for further calculation. This conformer was found to account for either a very high proportion of the population at 298 K (>96 %) or, in the case of other low-lying conformers, the structures were very similar near the triazoline and varied only further out on the probe.

All subsequent calculations were performed using density functional theory (DFT) with the B3LYP functional and 6-31G+(d) basis set within the Gaussian09 program suite. Note that all calculations are performed *in vacuo*. In the first part of the study, geometry optimization of the non-substituted linker was performed. These correspond to finding the most stable arrangement of the atoms. In Gaussian, the default convergence criteria was used both for single point energy evaluation and in optimization. For problematic cases, the keywords **scf=QC and Integral(UltraFineGrid)** was added to the root section of the input file. Frequency calculations were performed to confirm that a minimum has been found in the geometry optimization rather than a higher order stationary point. The existence of (3N–6) real frequencies confirmed that a minimum had been located (the reactant of the model

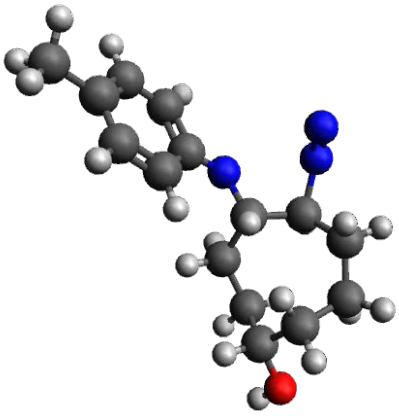
triazoline degradation reaction). This processed was repeated for the non-substituted linker with the triazoline group broken (the product of triazoline degradation).

The next step was to perform a constrained optimization/relaxed surface scan through the NNN angle of the possible triazoline breakdown. Initial attempts resulted in cyclooctane ring opening, which was overcome by also fixing the C-C bond on the triazoline. The resulting energy points were plotted and a likely transition state (TS) guess was identified as the highest point along this reaction coordinate (Figure S8). After obtaining a good guess of the TS a QST3 calculation to refine the TS structure was performed with the optimised reactant, product and the TS guess structure via the keywords **opt=(calcfc,qst3)** (all geometrical constraints lifted. The resulting structure was confirmed as a TS by a frequency calculation; the existence of one imaginary frequency indicates a TS has been found (first-order saddle point). Subsequently, an intrinsic reaction coordinate (IRC) from the TS in both the forward and backward directions was performed to ensure that the TS obtained indeed connected the reactant and product i.e. indicates that the correct TS for the conversion of reactants to products has been obtained. The keyword that was used in the root section was:
irc=(forward,stepsize=2,maxpoints=250,maxcycles=200,calcfc).

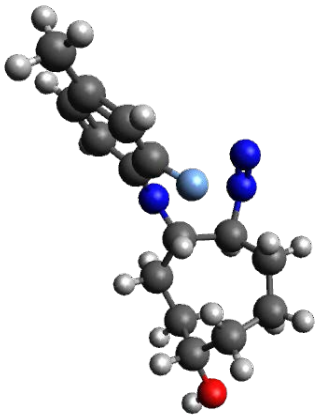
Relative rates: The rates of reaction were expressed using the Eyring equation as follows:

$$k = \frac{k_B T}{h} e^{-\frac{G_a}{k_B T}}$$

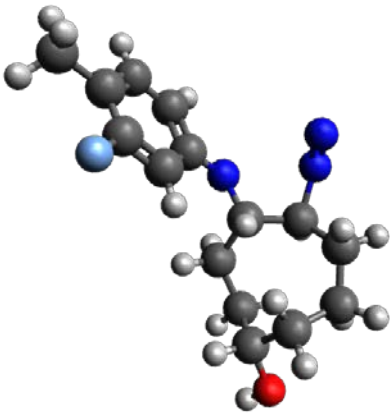
where k_B is the Boltzmann constant, h is Planck's constant, T=298 K and G_a is the free energy of activation, defined as $G_{transition\ state}$-$G_{reactant}$.



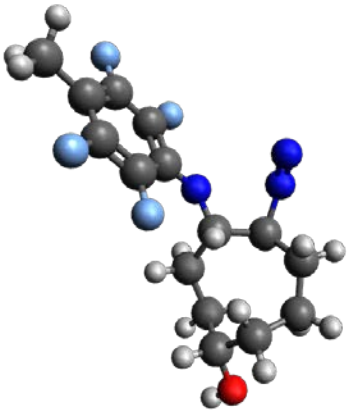
18a(transition state)



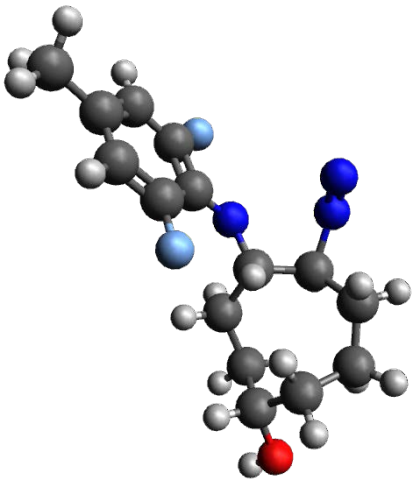
18b(transition state)



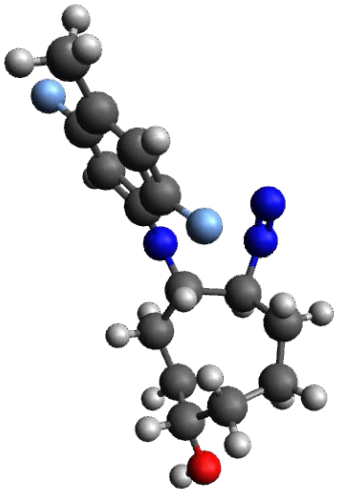
18c(transition state)



18d(transition state)



18e(transition state)



18f(transition state)

Figure S7. Structures of the transition states that are identified *via* elementary reaction modelling for the 1,2,3-triazoline degradation

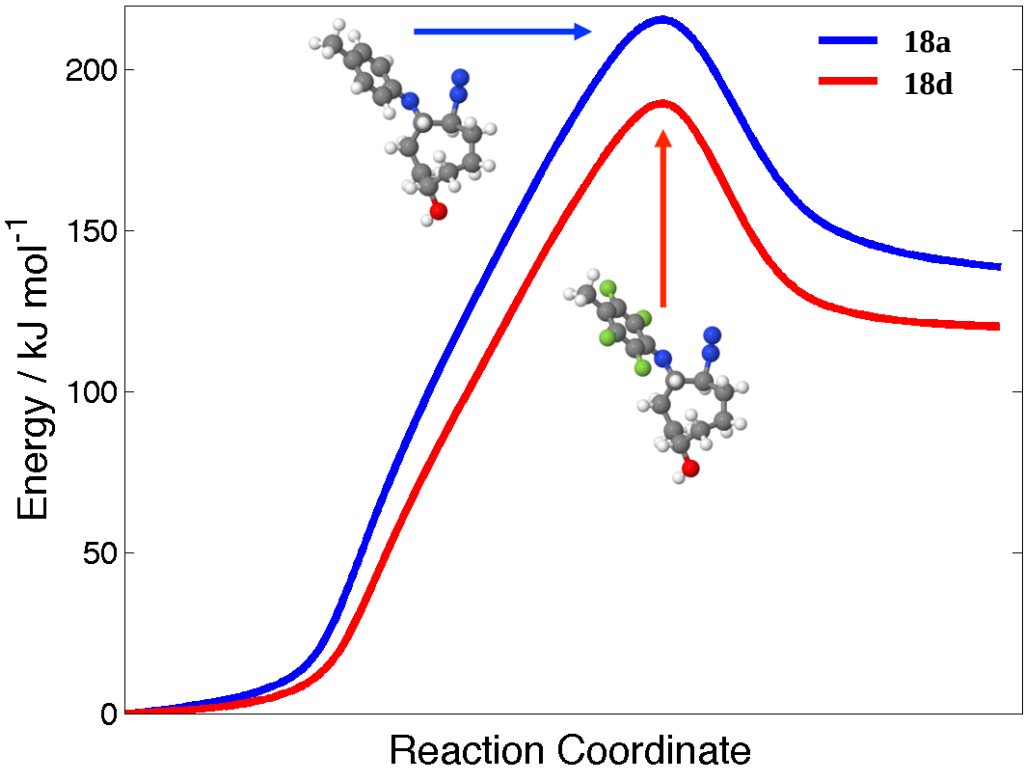


Figure S8. Reaction coordinate diagram of the triazoline degradation of **18a** and **18d** where the highest point indicates the energy of the transition state (structure pictured). The difference in transition state energies of **18a** and **18d** of 26 kJ mol^{-1}, when converted to a free energy difference of 25 kJ mol^{-1} leads to a 27,000-fold increase in the relative kinetics of the triazoline degradation of **18d** in comparision to **18a**.

Table S2. Coordinates and energies of transition states of elementary reaction modelling 1,2,3-triazoline degradation

(A) **18a**

SCF energy: -823.611542510 a.u.

C	-0.049890	0.157164	0.025232
C	0.099140	0.009009	3.061782
C	1.635195	-0.123284	3.100883
C	1.349235	0.685875	-0.325108
C	-0.447169	1.260187	2.341707
C	-0.947617	1.028619	0.910902
C	2.400396	-0.304885	1.781820
C	2.452251	0.884821	0.772817
N	3.896353	-0.516375	2.201358
N	4.640080	0.128993	2.780581

N	3.708154	0.897961	0.083767
C	4.272942	2.073132	-0.269002
C	5.703784	4.435282	-1.083721
C	3.732416	3.385359	-0.083669
C	5.551252	2.005841	-0.914369
C	6.239004	3.145508	-1.288968
C	4.436950	4.516685	-0.484452
C	6.461060	5.668583	-1.511096
O	-2.236439	0.402829	1.055020
H	0.012216	-0.844679	0.467936
H	-0.572383	0.016384	-0.933931
H	-0.361920	-0.896720	2.650373
H	-0.216287	0.041844	4.112005
H	1.882185	-0.979759	3.741150
H	2.054224	0.763786	3.596884
H	1.793166	-0.001368	-1.054456
H	1.264300	1.652813	-0.838457
H	-1.301914	1.659640	2.899450
H	0.309408	2.053904	2.336468
H	-1.079796	2.011705	0.430125
H	2.157488	-1.237052	1.268484
H	2.258636	1.802150	1.350333
H	7.214837	3.044332	-1.762166
H	3.985693	5.496476	-0.333675
H	7.430072	5.745231	-0.998762
H	6.671600	5.661428	-2.589534
H	5.896510	6.581007	-1.289690
H	-2.583783	0.188466	0.174627
H	5.971525	1.018211	-1.083480
H	2.746813	3.517536	0.352726

(B) **18b**

SCF energy: -922.852492265 a.u.

C	3.07640	-1.35850	-0.81670
C	3.43390	1.18940	0.83410
C	2.17120	1.87160	0.26850
C	1.55660	-1.37380	-1.05180
C	3.22520	-0.18680	1.50100
C	3.57640	-1.40160	0.63240
C	1.42890	1.17960	-0.89370
C	0.65110	-0.19390	-0.57150
N	0.37860	2.14260	-1.35570
N	-0.56290	2.63830	-0.94510
N	-0.58080	-0.33650	-1.26580
C	-1.78000	-0.34550	-0.63020
C	-4.44700	-0.49700	0.50490
C	-2.12860	0.17470	0.64990
C	-2.89070	-0.91830	-1.32600
C	-4.16410	-0.99160	-0.78290
C	-3.39270	0.10770	1.20590

C	-5.82070	-0.62390	1.11750
O	5.01520	-1.47290	0.64370
H	3.55020	-0.50700	-1.32160
H	3.47430	-2.24930	-1.32680
H	4.21600	1.12400	0.06830
H	3.82390	1.88620	1.58620
H	2.45920	2.87380	-0.07270
H	1.44010	2.01070	1.07590
H	1.37480	-1.45990	-2.12950
H	1.12850	-2.27930	-0.60310
H	3.86280	-0.25910	2.38950
H	2.19380	-0.28140	1.85930
H	3.17080	-2.30090	1.12400
H	2.06370	1.03410	-1.76920
H	0.54930	-0.20690	0.51870
H	-4.96260	-1.44800	-1.36490
H	-3.55050	0.55040	2.18660
H	-5.95390	-1.59810	1.61000
H	-5.99670	0.14890	1.87440
H	-6.60630	-0.53710	0.35770
H	5.29920	-2.20340	0.07150
H	-2.68550	-1.30740	-2.31930
F	-1.17660	0.87640	1.35040

(C) **18c**

SCF energy: -922.856297808 a.u.

C	-0.005407	0.039677	0.005026
C	0.049310	0.090118	3.047382
C	1.586177	0.005321	3.143912
C	1.390359	0.582323	-0.338443
C	-0.506137	1.277069	2.231940
C	-0.952833	0.942243	0.803697
C	2.397458	-0.241773	1.862336
C	2.453081	0.884052	0.774829
N	3.872539	-0.383686	2.335104
N	4.608043	0.296557	2.879640
N	3.731029	0.888080	0.132190
C	4.301878	2.054699	-0.235323
C	5.748771	4.417276	-1.067657
C	3.733876	3.364008	-0.113307
C	5.608537	1.985344	-0.817967
C	6.297054	3.125774	-1.197482
C	4.460285	4.463709	-0.524804
C	6.472564	5.666204	-1.495909
O	-2.229348	0.292201	0.947450
H	0.065363	-0.929826	0.513725
H	-0.492640	-0.176952	-0.958297
H	-0.372831	-0.853211	2.681571
H	-0.300241	0.180645	4.083134
H	1.834442	-0.799225	3.847730

H	1.964743	0.935583	3.590543
H	1.874894	-0.137568	-1.007987
H	1.295605	1.511308	-0.915883
H	-1.389286	1.685593	2.736066
H	0.228381	2.090479	2.201246
H	-1.092706	1.888974	0.257109
H	2.190230	-1.209451	1.402042
H	2.214836	1.828587	1.288360
H	5.916672	6.214508	-2.267656
H	6.615262	6.361125	-0.658124
H	7.458939	5.418013	-1.901240
H	-2.549619	0.026393	0.070816
H	6.051958	1.001127	-0.935009
H	2.734291	3.530208	0.272824
H	7.294654	3.027134	-1.621158
F	3.869505	5.694003	-0.400532

(D) **18d**

SCF energy: -1220.56028169 a.u.

C	-3.23280	-0.53660	1.63130
C	-4.34080	0.37750	-1.04990
C	-3.16240	1.32220	-1.36060
C	-1.72620	-0.30710	1.43900
C	-3.96720	-1.09750	-0.79190
C	-3.93820	-1.51250	0.68300
C	-2.10860	1.56920	-0.26340
C	-1.15140	0.38970	0.15620
N	-1.16420	2.61420	-0.86090
N	-0.43510	2.73900	-1.71930
N	0.12290	0.92990	0.52070
C	1.28690	0.32060	0.25110
C	3.98480	-0.76780	-0.13790
C	1.55310	-0.92860	-0.39100
C	2.48380	0.98320	0.68680
C	3.74670	0.46340	0.48890
C	2.83290	-1.43660	-0.55760
C	5.37680	-1.30760	-0.33100
O	-5.31890	-1.64520	1.06830
H	-3.77960	0.41500	1.62720
H	-3.35180	-0.92760	2.65340
H	-4.94740	0.76780	-0.22460
H	-4.98790	0.42940	-1.93400
H	-3.57780	2.29750	-1.64330
H	-2.62710	0.94110	-2.24120
H	-1.37000	0.30370	2.27630
H	-1.19410	-1.26390	1.51150
H	-4.70200	-1.75010	-1.27650
H	-3.00120	-1.33260	-1.25410
H	-3.44910	-2.49720	0.75180
H	-2.51310	2.06550	0.62060

H	-1.13090	-0.30860	-0.68590
H	5.34830	-2.28110	-0.82500
H	5.98440	-0.62870	-0.94210
H	5.89330	-1.42310	0.62970
H	-5.36420	-1.89300	2.00540
F	2.95200	-2.64420	-1.16710
F	0.51680	-1.69780	-0.83830
F	2.36610	2.17360	1.30900
F	4.81340	1.17680	0.93010

(E) **18e**

SCF energy: -1022.09071366 a.u.

C	-0.218337	-0.150800	0.143476
C	0.106875	0.167130	3.147366
C	1.646082	0.081121	3.117071
C	1.138268	0.355343	-0.370417
C	-0.516609	1.283248	2.282844
C	-1.093359	0.823289	0.939496
C	2.345058	-0.279650	1.794684
C	2.299685	0.748804	0.609673
N	3.847784	-0.371262	2.152536
N	4.641618	0.345391	2.542881
N	3.511967	0.652827	-0.147717
C	4.122399	1.712758	-0.700573
C	5.675020	3.786158	-2.047073
C	3.777807	3.100564	-0.708831
C	5.325798	1.470024	-1.448529
C	6.076221	2.437554	-2.081369
C	4.496952	4.092261	-1.351517
C	6.494097	4.859092	-2.721246
O	-2.348824	0.193533	1.258134
F	5.753505	0.186425	-1.519419
F	2.629525	3.493522	-0.064867
H	-0.098283	-1.071834	0.727458
H	-0.788451	-0.450405	-0.749609
H	-0.344290	-0.801597	2.903145
H	-0.150297	0.348998	4.198069
H	1.956696	-0.660937	3.863474
H	2.059301	1.045119	3.445753
H	1.562547	-0.420546	-1.017905
H	0.985806	1.234660	-1.008818
H	-1.347416	1.746789	2.826883
H	0.214967	2.080746	2.107693
H	-1.286435	1.717401	0.325194
H	2.110071	-1.285379	1.441575
H	2.113111	1.733185	1.048788
H	6.976468	2.132200	-2.607857
H	4.125320	5.112004	-1.297687
H	5.956135	5.812593	-2.747852
H	7.443919	5.030036	-2.195434

H	6.744223	4.585250	-3.754162
H	-2.744724	-0.151921	0.442297

(F) **18f**

SCF energy: -1022.09515975 a.u.

C	3.26750	-1.57900	-0.52070
C	3.79250	1.26490	0.46270
C	2.51970	1.84970	-0.18170
C	1.73750	-1.59190	-0.66830
C	3.58310	0.09360	1.44580
C	3.85090	-1.30330	0.87030
C	1.69460	0.93560	-1.11590
C	0.88530	-0.30100	-0.44940
N	0.66410	1.80300	-1.73970
N	-0.25260	2.42420	-1.48190
N	-0.37300	-0.55600	-1.05130
C	-1.54900	-0.33170	-0.41230
C	-4.19050	-0.02420	0.77960
C	-1.83100	0.53070	0.68650
C	-2.70110	-1.02040	-0.90370
C	-3.93780	-0.85750	-0.31880
C	-3.07960	0.68450	1.26070
C	-5.56520	0.10350	1.38120
O	5.28530	-1.42480	0.82500
H	3.73380	-0.88920	-1.23580
H	3.61150	-2.57890	-0.82680
H	4.52440	0.98790	-0.30520
H	4.24720	2.10170	1.00700
H	2.81520	2.73420	-0.75970
H	1.83980	2.19850	0.60650
H	1.49170	-1.93060	-1.68130
H	1.31310	-2.34440	0.00880
H	4.26760	0.20570	2.29430
H	2.57120	0.12690	1.86590
H	3.45130	-2.04610	1.57980
H	2.27830	0.55800	-1.95680
H	0.83140	-0.04780	0.61550
H	-5.56740	0.84550	2.18610
H	-6.30560	0.40990	0.63180
H	-5.91460	-0.85010	1.79810
H	5.51770	-2.28340	0.43730
H	-3.18890	1.38430	2.08510
H	-2.57540	-1.69060	-1.74720
F	-0.82500	1.34880	1.14680
F	-4.99220	-1.56130	-0.83450

Molecular electrostatic potential (MEP) at nucleus: Calculating the MEP at the nuclei can give useful comparisons between systems with similar chemical framework. The value and the sign of the MEP gives information about how electropositive a

region is. Therefore, the more negative the MEP on the nucleus, the larger the pKa. Note that MEP values can only be compared within atoms of a particular type.

Liu and Pedersen used a database of calculated MEP values and experimental pKa values to derive an empirical relationship between the two quantities.[15] A database of 154 primary, secondary and tertiary amines were considered and the following relationship was obtained:

$$pKa = -145.53 \times MEP@N - 2669.8$$

where "MEP@N" indicates the MEP at the N nucleus of each amine/aniline. An R^2 of 0.89 was obtained indicating a good linear fit. The pKa derived from our calculated MEPs are given in Table S3 where the 95 % confidence interval is estimated based on the data used to derive the empirical relationship.

Table S3. Predicted pKa values and computational data (including the 95% CI) for the molecular electrostatic potential at the nitrogen nucleus (MEP@N) of the imine corresponding to probes **1a-1f**

Imine generated from the reaction of TCO **12** and	**MEP@N**	**Predicted pKa**	**95 % CI**
1a	-18.404451	8.5	2.1
1b	-18.399703	7.8	2.1
1c	-18.398024	7.6	2.1
1d	-18.380875	5.1	2.1
1e	-18.392915	6.8	2.1
1f	-18.393313	6.9	2.1

Note that the reason for a relatively large 95% CI is the method used for predicting the pKa which can also be explained by the wide spread of data about the line of best fit in Liu's work.[15]

7. $^{1}H/^{19}F$ NMR analysis of the benzyl ether analogues 11a and 11d

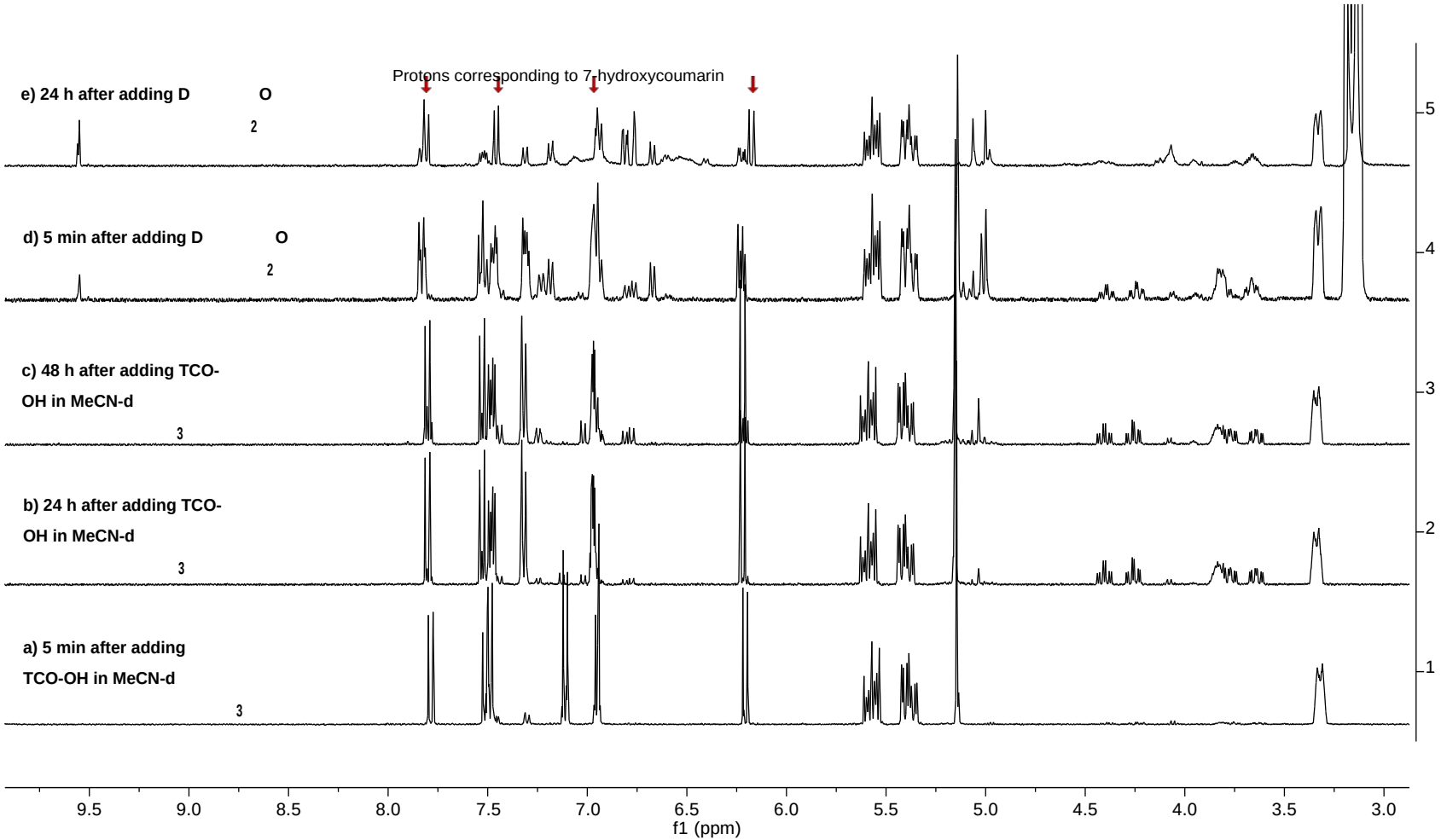


Figure S9a. ^{1}H NMR experiments for reaction of TCO **12** with **11a** a) t = 5 min (MeCN-d_3) b) t = 24 h (MeCN-d_3) c) t = 48 h (MeCN-d_3) d) t = 5 min after adding 10% D_2O e) t = 24 h after adding 10% D_2O.

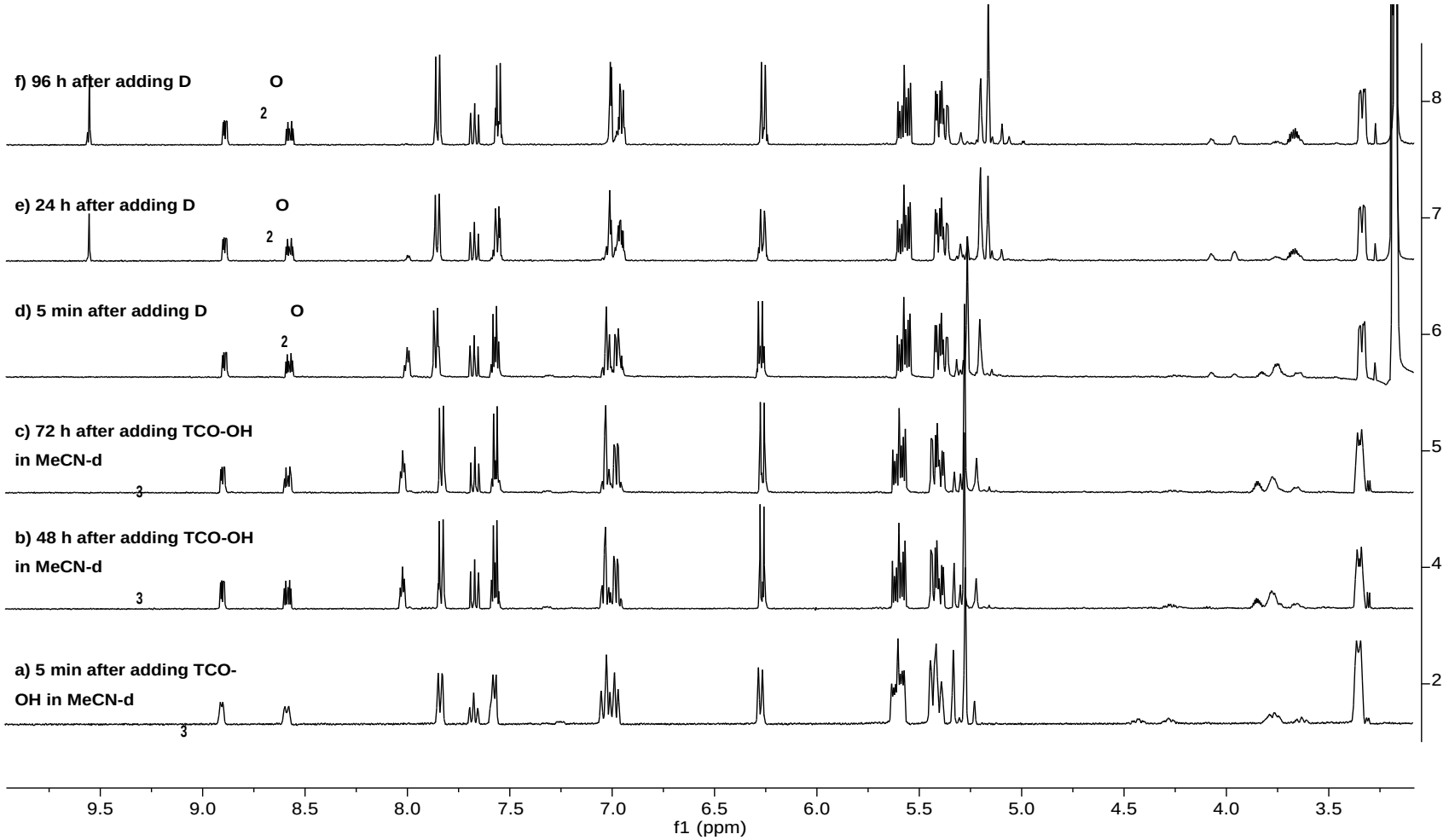


Figure S9b. ^{1}H NMR product distribution experiments (containing internal standard: 1-fluoro-2,4-dinitrobenzene) for reaction of TCO **12** with **11d** a) t = 5 min (MeCN-d_3) b) t = 48 h (MeCN-d_3) c) t = 72 h (MeCN-d_3) d) t = 5 min after adding 10% D_2O e) t = 24 h after adding 10% D_2O f) t = 96 h after adding 10% D_2O.

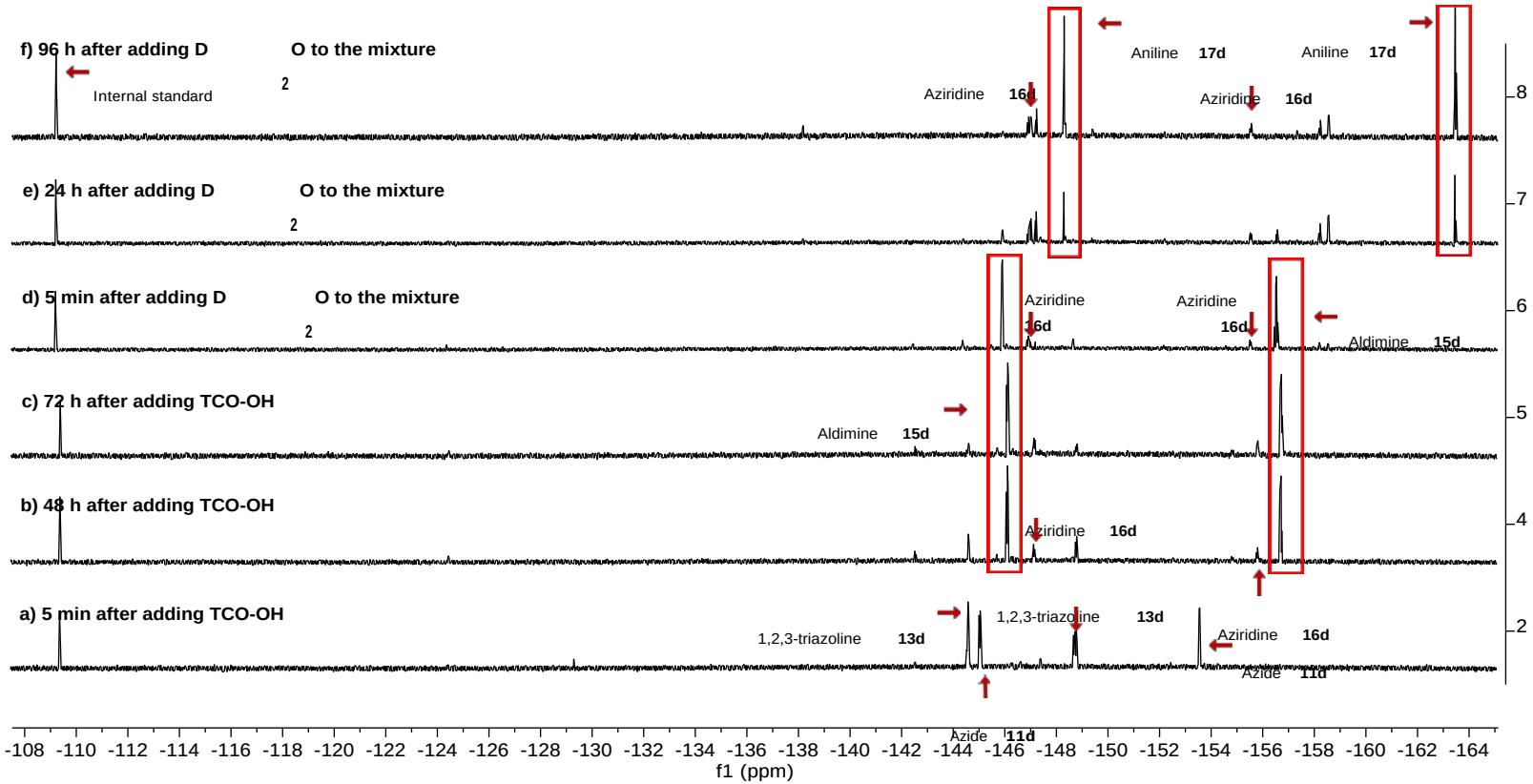


Figure S9c. ^{19}F NMR product distribution experiments (containing internal standard: 1-fluoro-2,4-dinitrobenzene) for reaction of TCO **12** with **11d** a) t = 5 min (MeCN-d_3) b) t = 48 h (MeCN-d_3) c) t = 72 h (MeCN-d_3) d) t = 5 min after adding 10% D_2O e) t = 24 h after adding 10% D_2O f) t = 96 h after adding 10% D_2O.

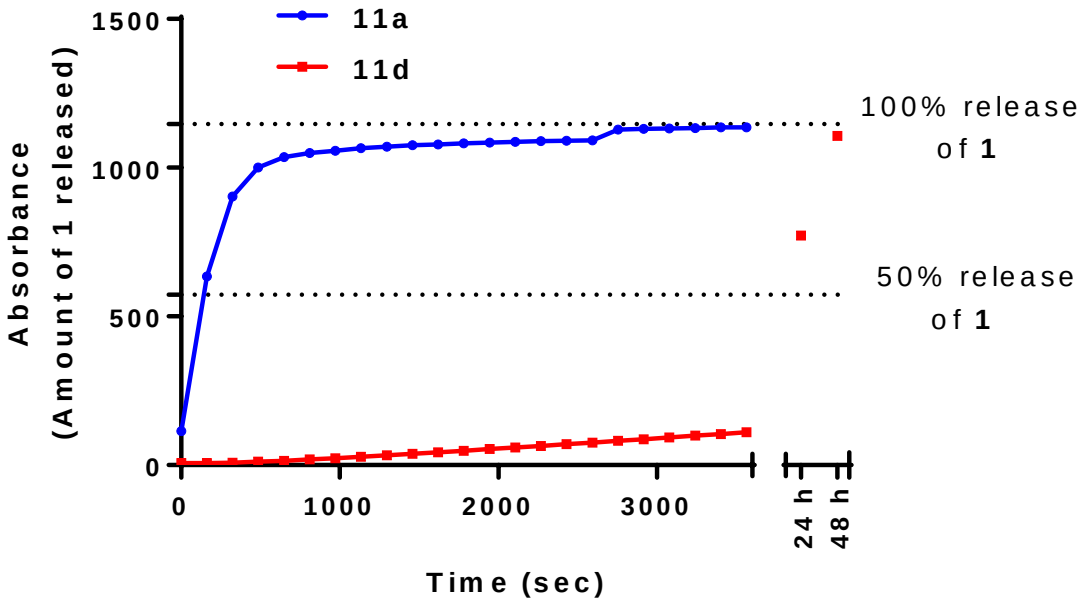


Figure S9d. Average release of 7-hydroxycoumarin **1** from triazoline **13a** and triazoline/imine **13d/15d** (corresponding to **11a** and **11d**) monitored by spectrofluorometry (ex. 360, em. 455). Experiments run in triplicate (**13a**) or duplicates (**13d**/**15d**) from one NMR sample.

8. Cell Culture and Proliferation Assay

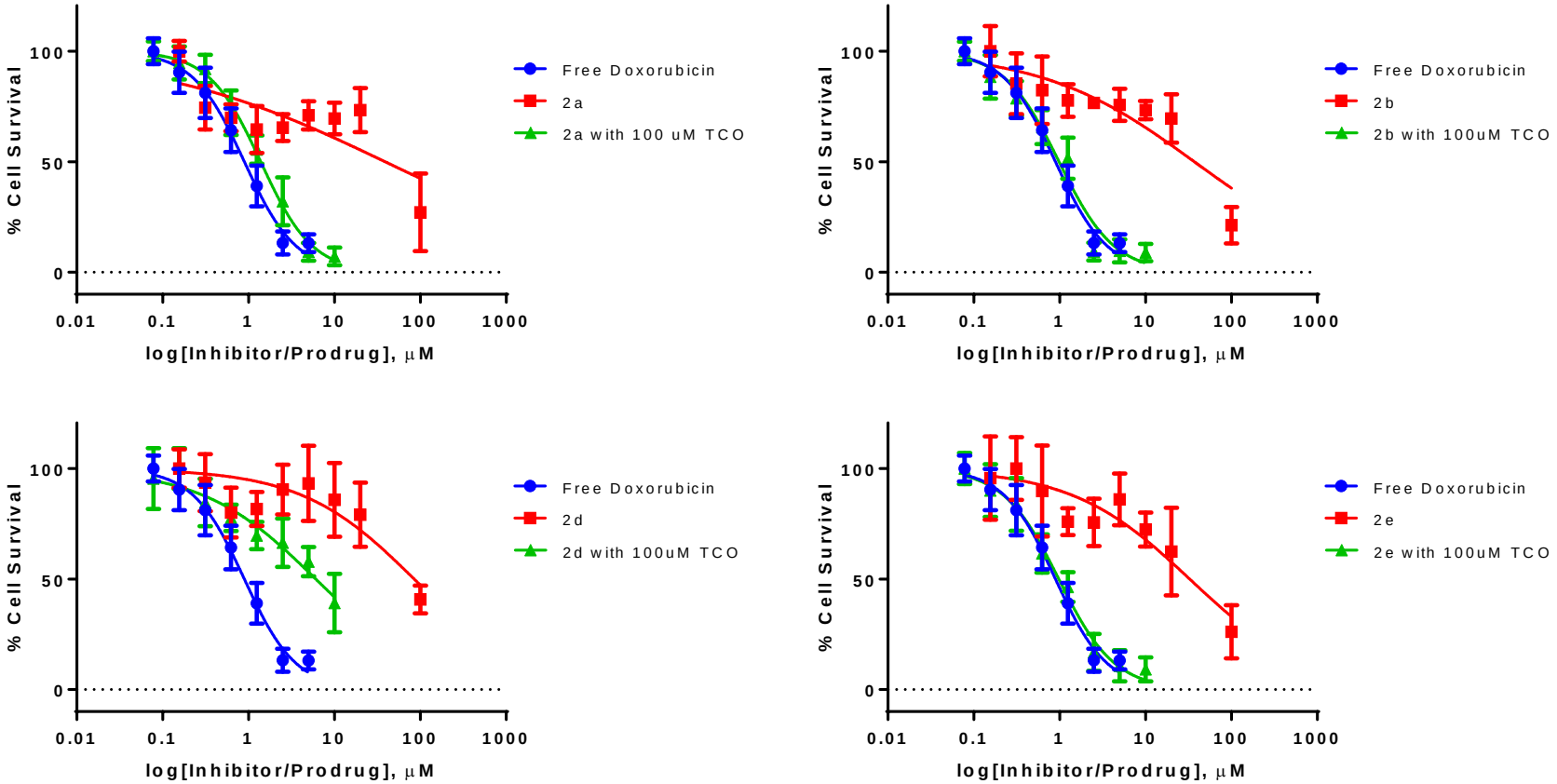


Figure S10. Cytotoxicity assay against the B16-OVA murine melanoma cell line. Error bars represent ±SD (n ≥ 3)

9. Final Compound NMR Spectra and Purity data of 1b-1f, 2b, 2d, and 2e:

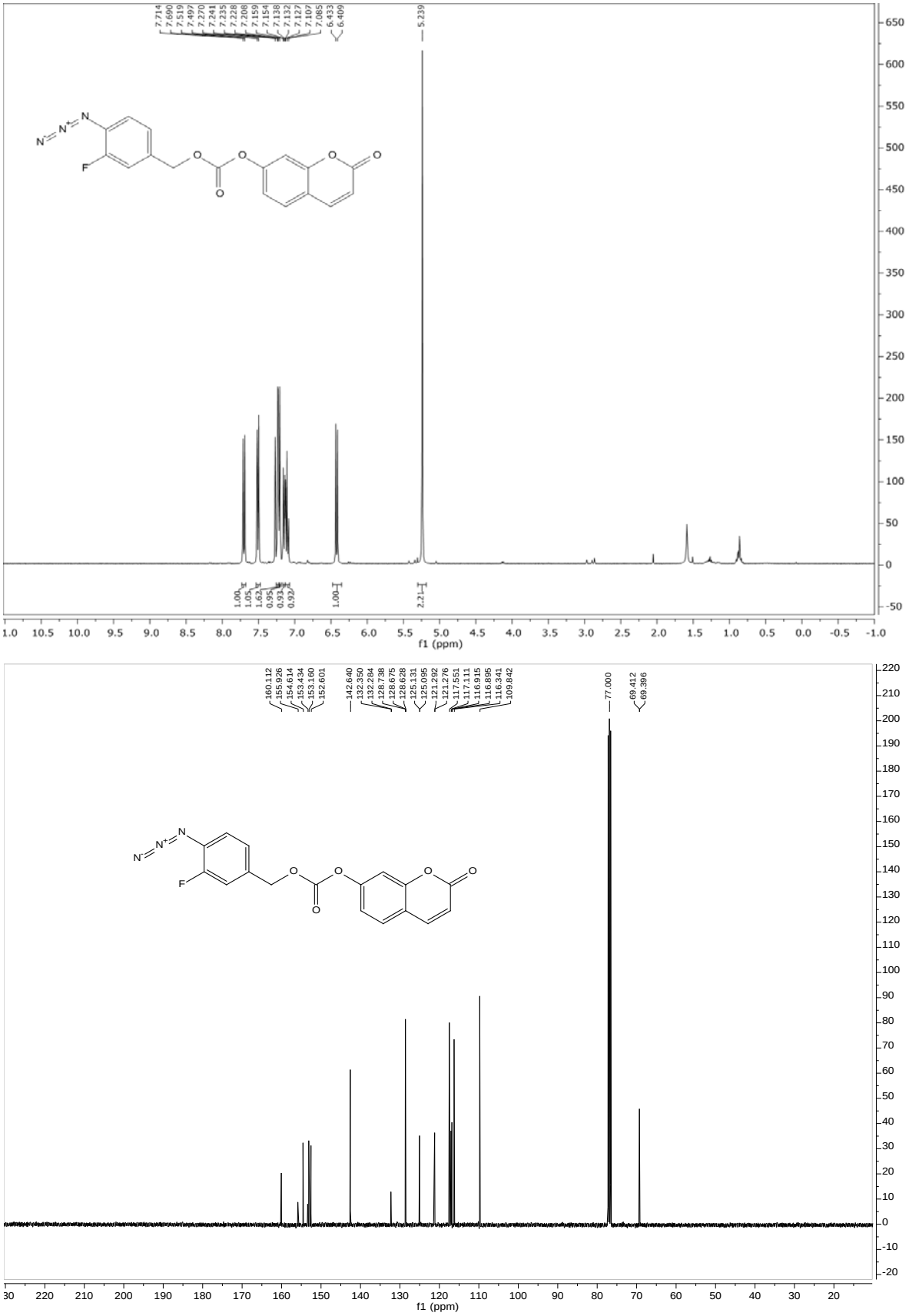


Figure S11. ^{1}H and ^{13}C NMR Spectrum of coumarin carbonate pro-probe **1b**

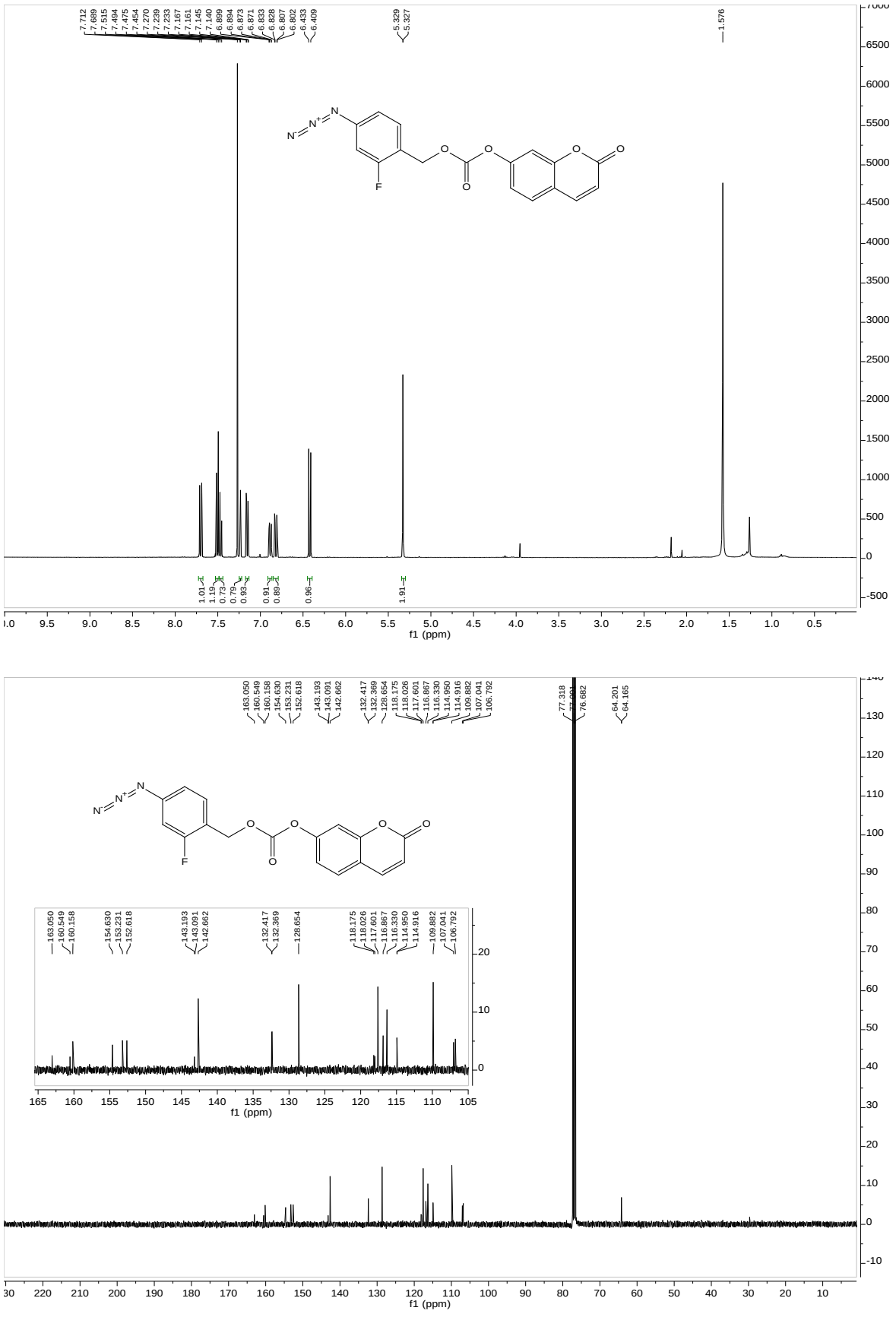


Figure S12. ^{1}H and ^{13}C NMR Spectrum of coumarin carbonate pro-probe **1c**

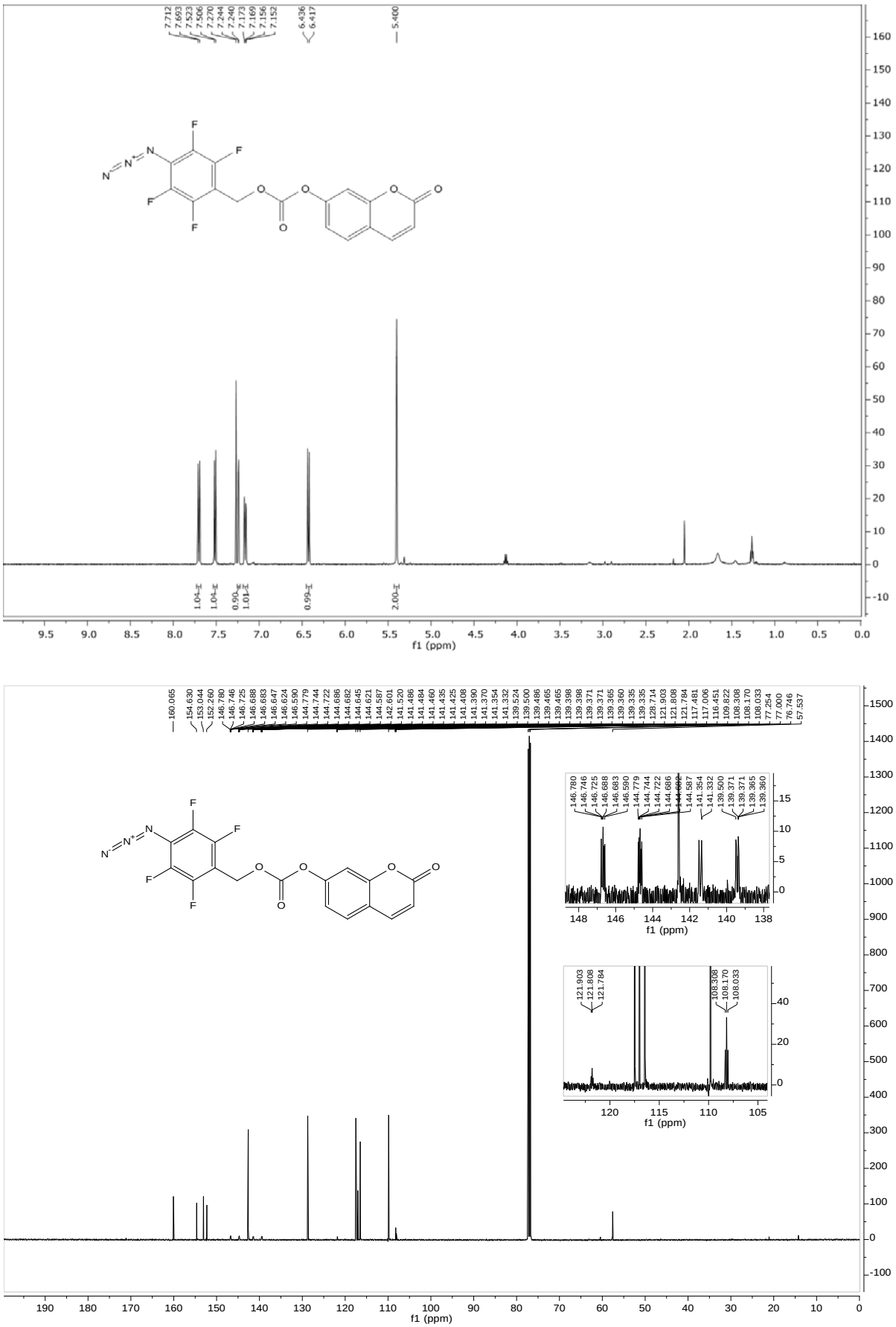


Figure S13. ^{1}H and ^{13}C NMR Spectrum of coumarin carbonate pro-probe **1d**

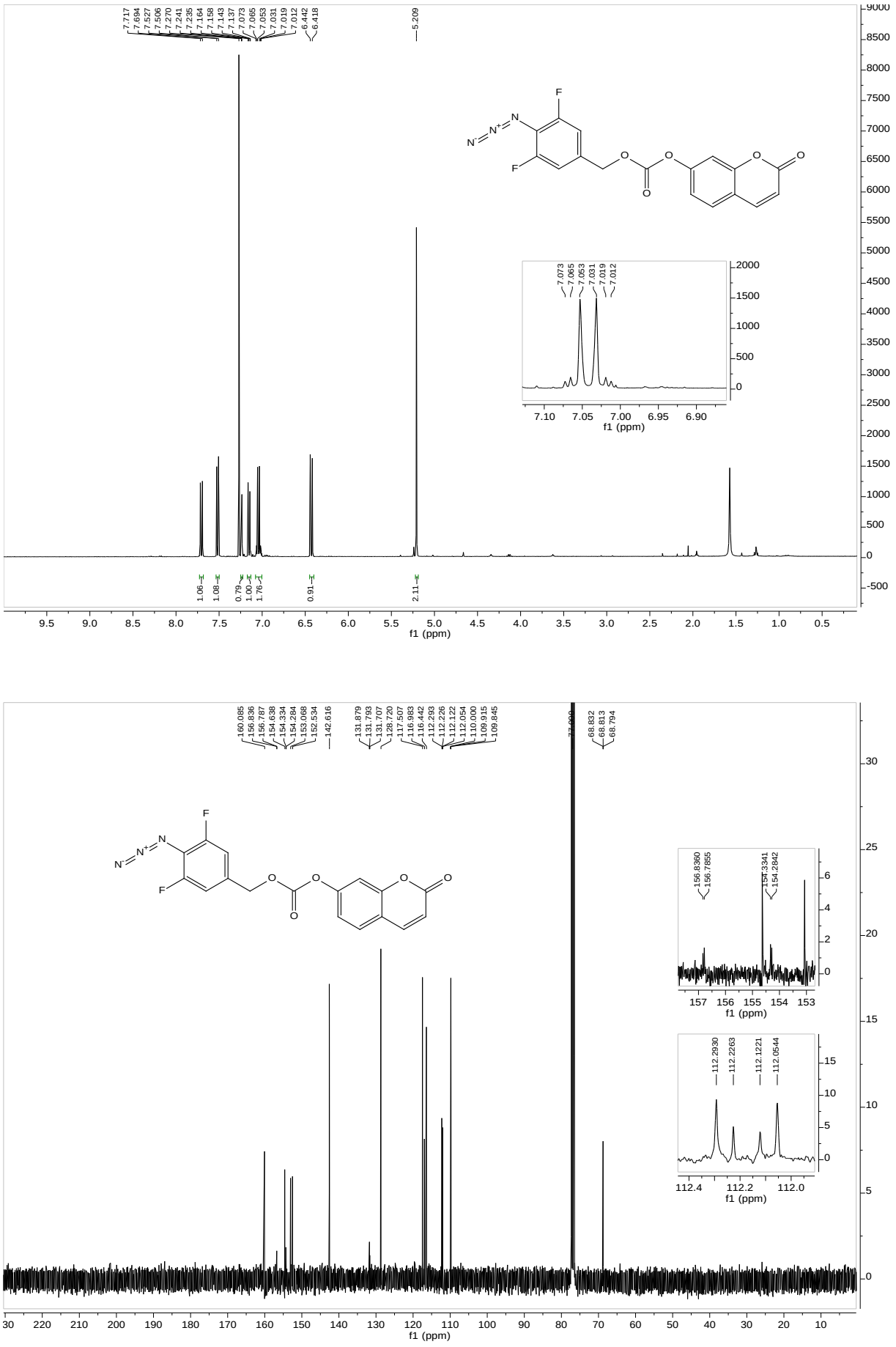


Figure S14. ^{1}H and ^{13}C NMR Spectrum of coumarin carbonate pro-probe **1e**

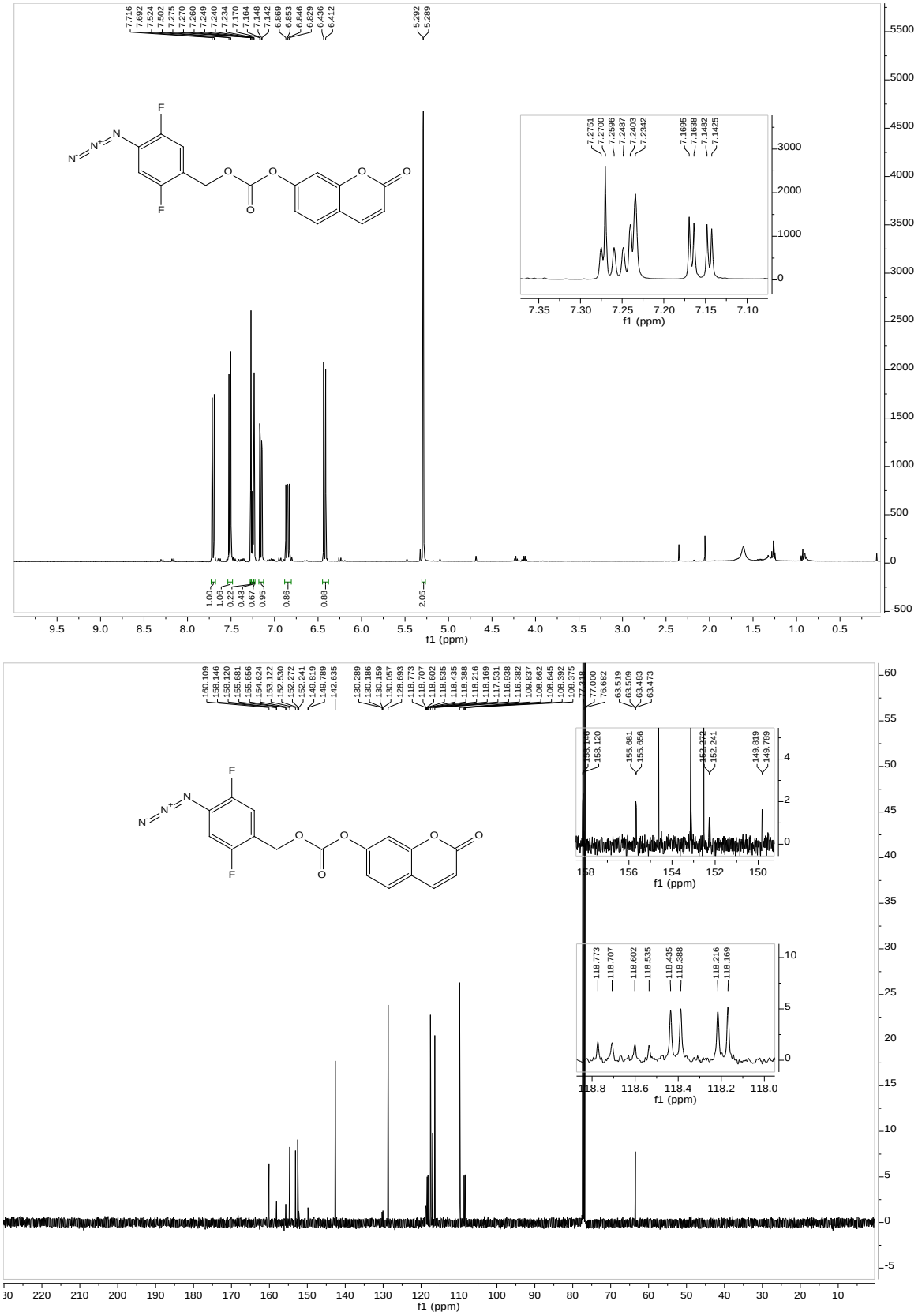


Figure S15. ^{1}H and ^{13}C NMR Spectrum of coumarin carbonate pro-probe **1f**

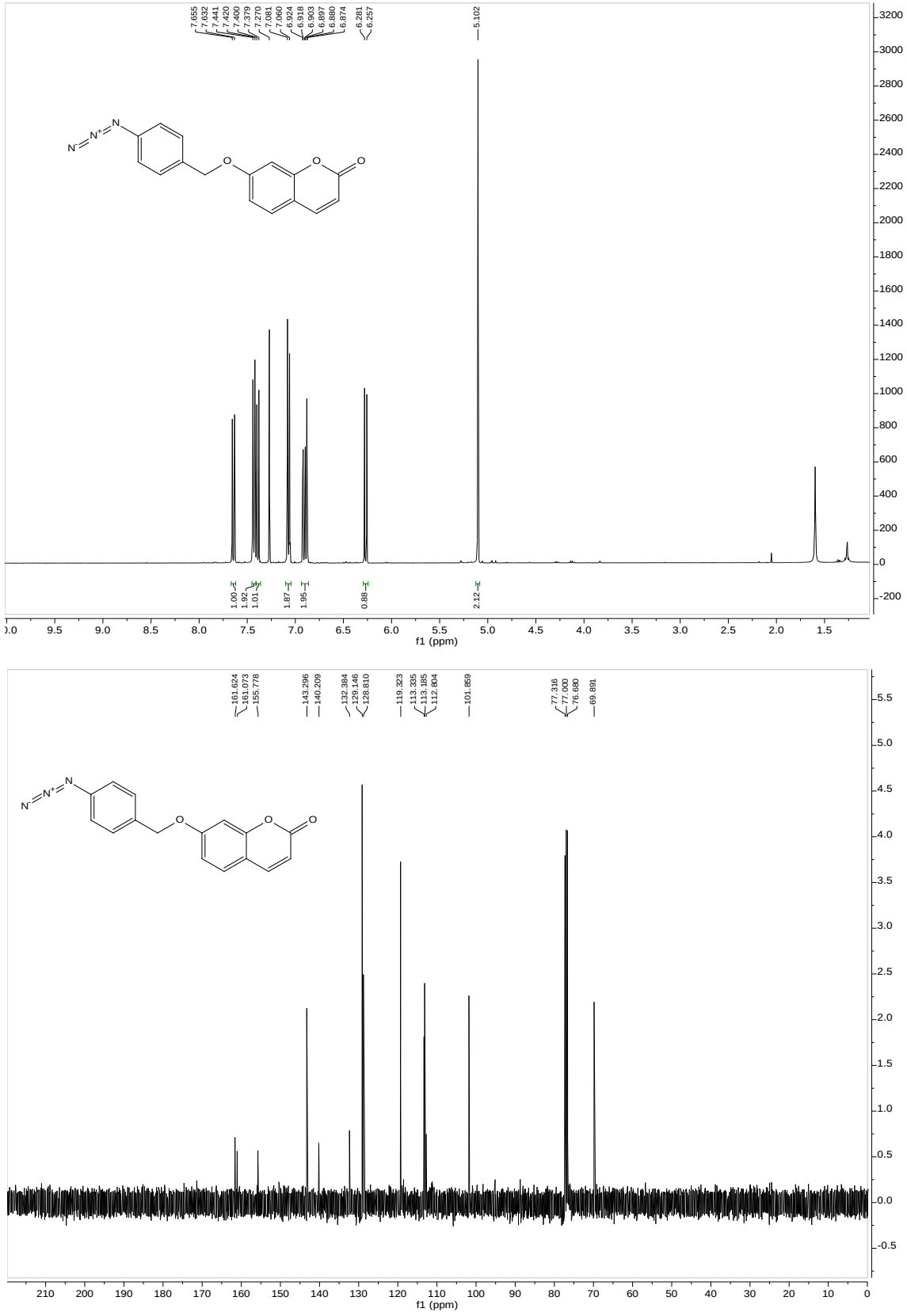


Figure S16. ^{1}H and ^{13}C NMR Spectrum of coumarin carbonate pro-probe **11a**

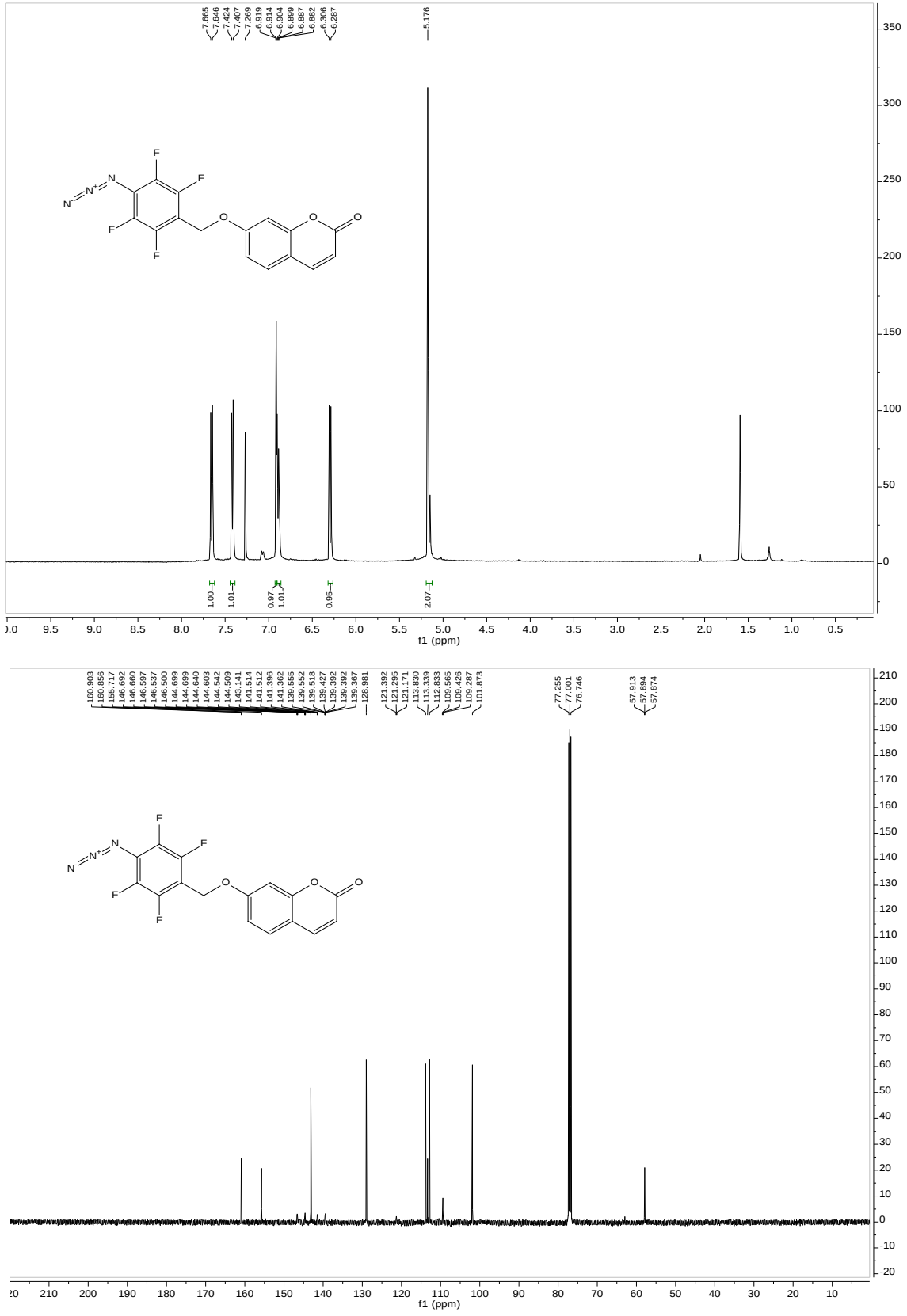


Figure S17. ^{1}H and ^{13}C NMR Spectrum of coumarin carbonate pro-probe **11d**

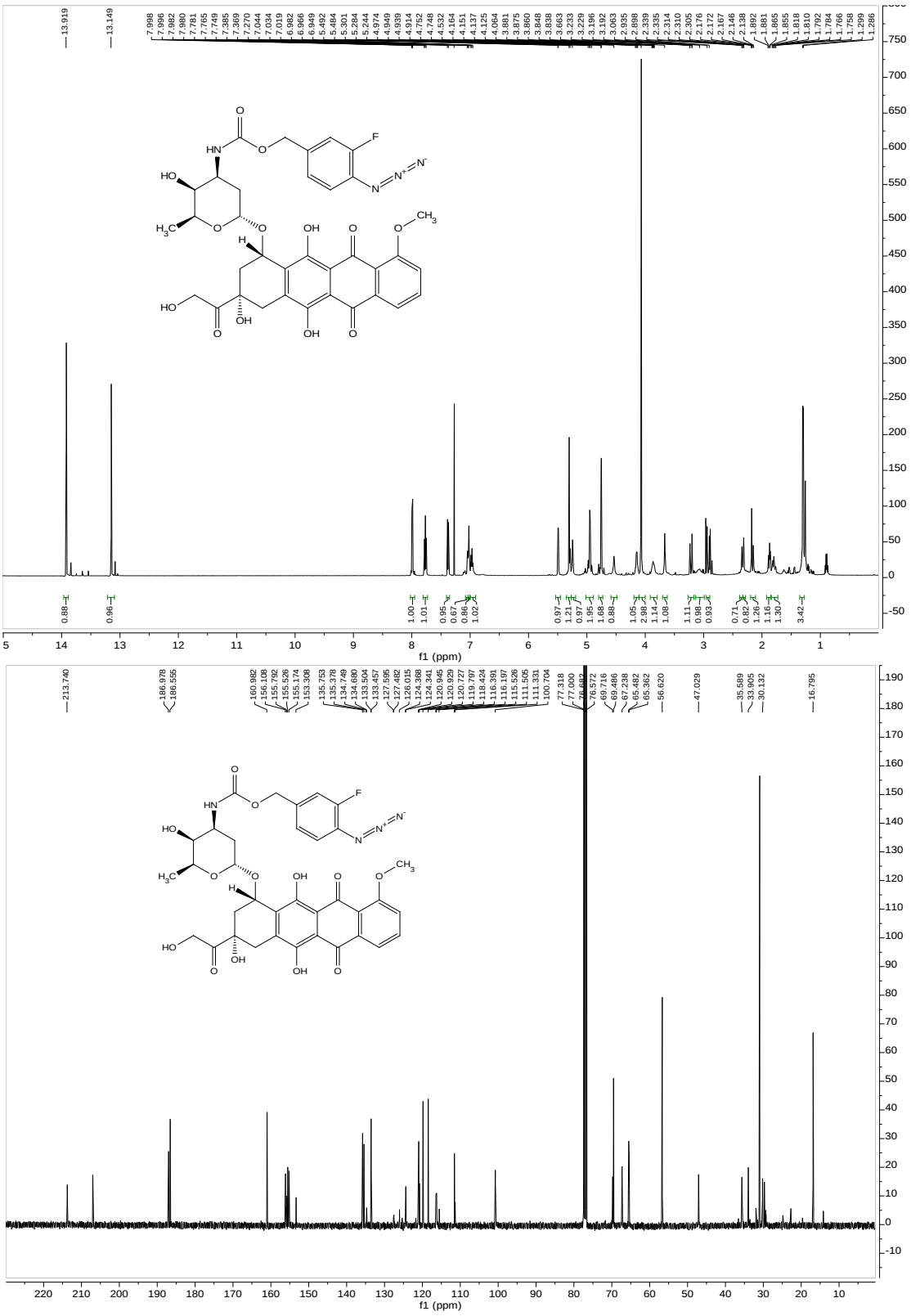


Figure S18. ^{1}H and ^{13}C NMR Spectrum of doxorubicin carbamate prodrug **2b**, (peaks at δ 207.0 and 30.89 ppm in ^{13}C correspond to residual acetone)

HPLC trace of 2b

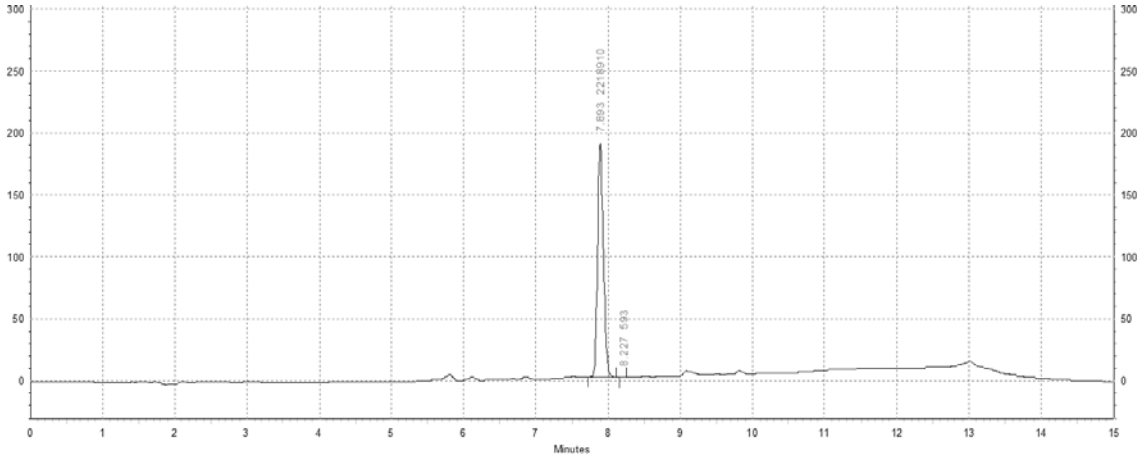


Figure S19. HPLC traces of the doxorubicin prodrug **2b** showing its purity

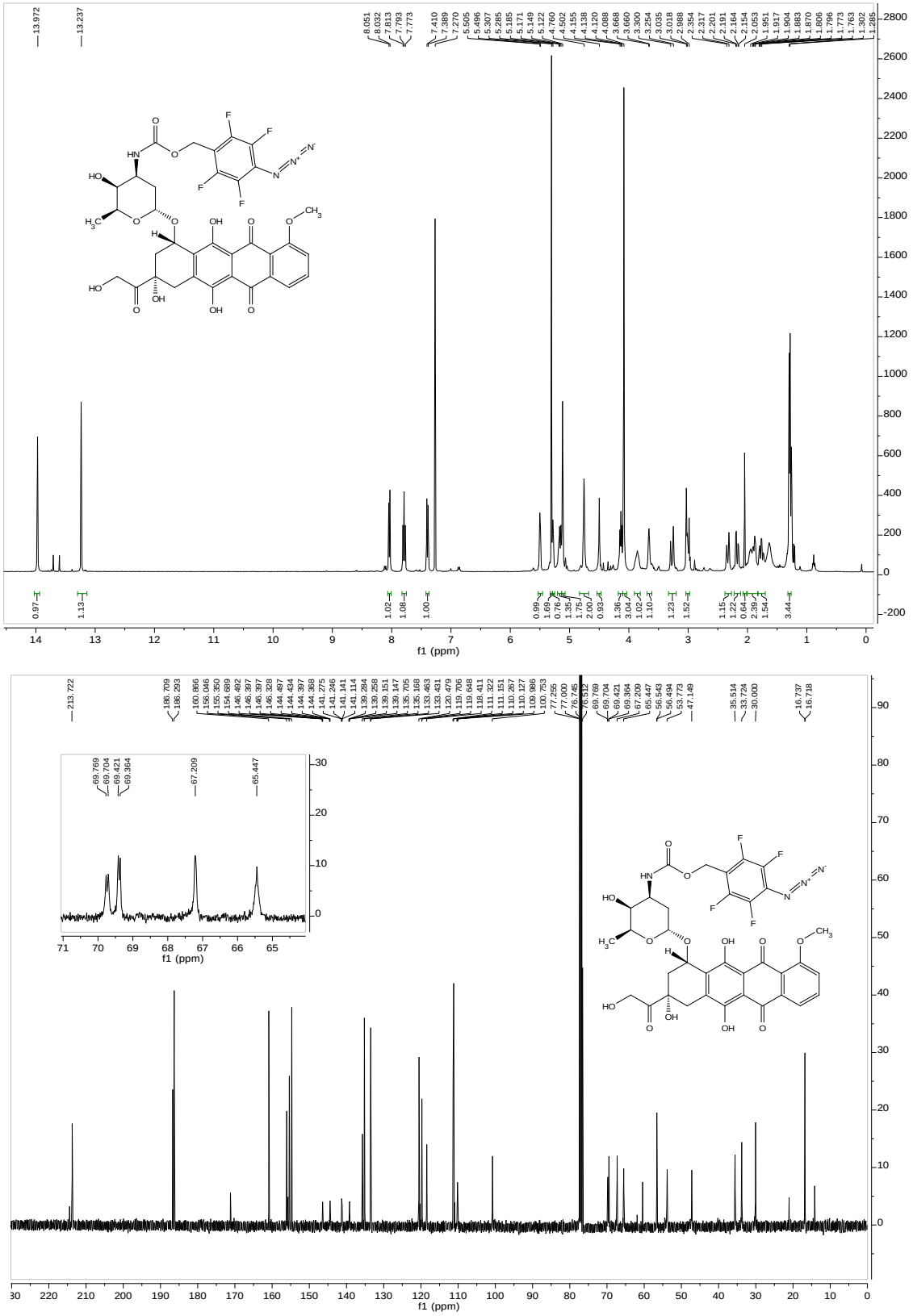


Figure S20. ^{1}H and ^{13}C NMR Spectrum of doxorubicin carbamate prodrug **2d.** Residual DCM (δ 5.30 ppm) and EtOAc (δ 2.05 and 1.26 ppm) peaks were found in ^{1}H NMR and residual EtOAc peaks were observed in ^{13}C NMR (δ 171.0, 60.5, 20.1 and 14.2 ppm) spectrum.

HPLC trace of 2d

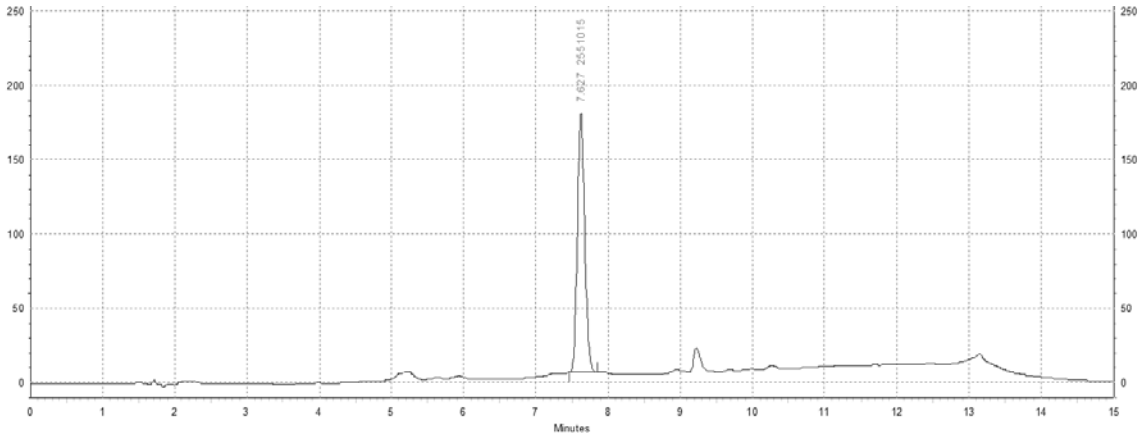


Figure S21. HPLC traces of the doxorubicin prodrug **2d** showing its purity

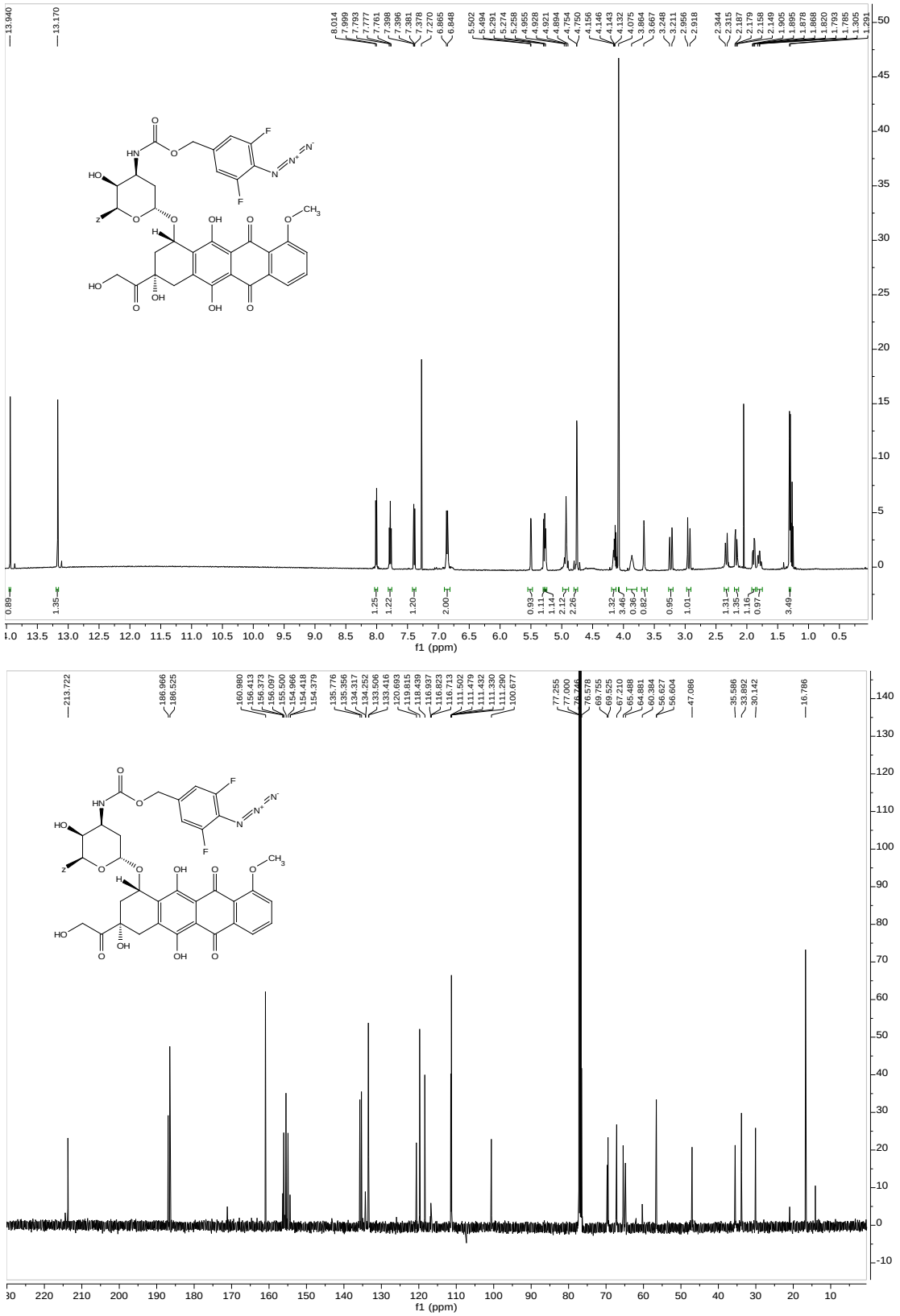


Figure S22. ^{1}H and ^{13}C NMR Spectrum of doxorubicin carbamate prodrug **2e.** EtOAc peaks were found in both ^{1}H (δ 2.05 and 1.26 ppm) and ^{13}C NMR (δ 171.0, 60.5, 20.1 and 14.2 ppm) spectra.

HPLC trace of 2e

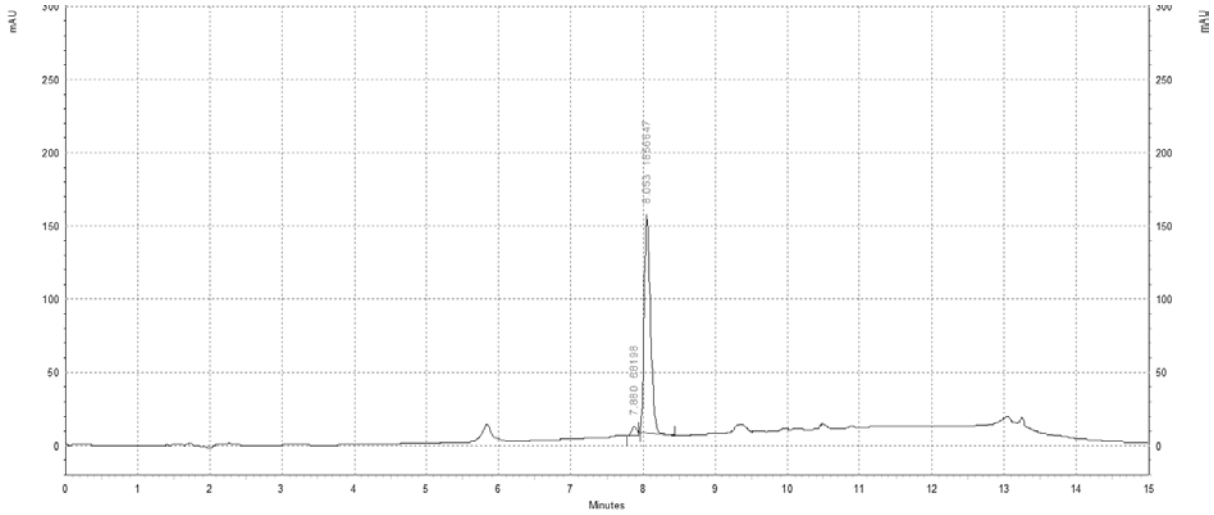


Figure S23. HPLC traces of the doxorubicin prodrug **2e** showing its purity

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