ESI for 'Fluorescent Molecular Logic Gates based on Photoinduced Electron Transfer (PET) Driven by a Combination of Atomic and Biomolecular Inputs' by G. D. Wright, C. Y. Yao, T. S. Moody and A. P. de Silva

***Editor's Note**: Due to the 2020 global COVID-19 pandemic, not all characterization spectra could be made available prior to publication. This ESI replaces that originally published on 5th May 2020. The characterisation data is now included.*

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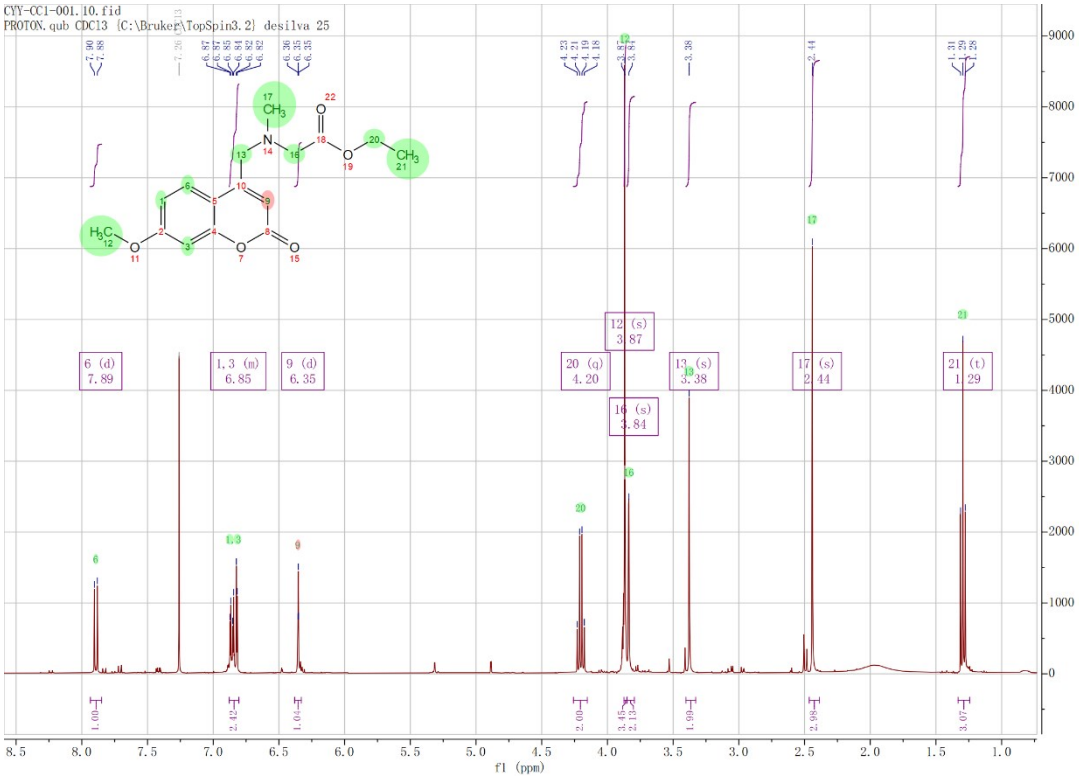
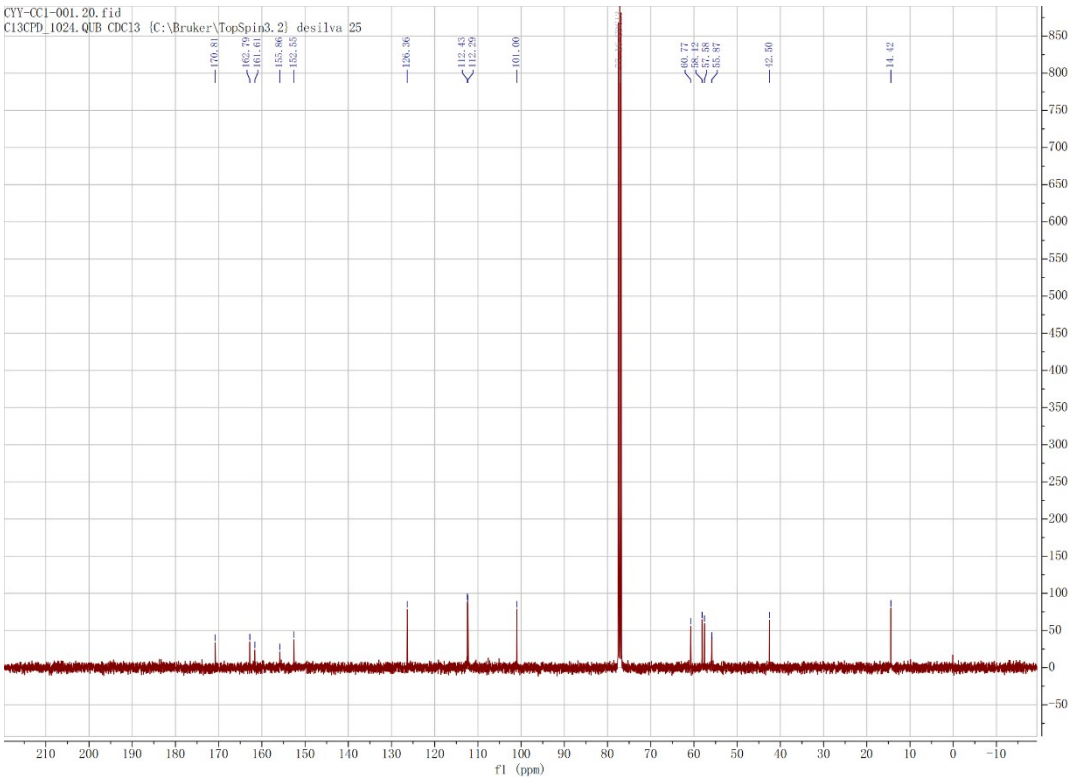
S1. Synthesis procedures and characterization details for logic gates 1, 3, 5 and 7.

Ethyl 2-(((7-methoxy-2-oxo-2*H*-chromen-4-yl)methyl)(methyl)amino)ethanoate, 1

To 4-(bromomethyl)-7-methoxycoumarin (1.0 g, 3.72 mmol) was added sarcosine ethyl ester hydrochloride (0.57 g, 3.74 mmol), K_2CO_3 (3.0 g, anhydrous) and dichloromethane (15 ml) and the mixture stirred at approximately 70 °C for 18 hours. The mixture was then washed with water (3 x 50 ml), the organic layer dried with $MgSO_4$ and the solvent removed under vacuum, affording a yellow oil.

Yield: 0.96 g (84 %)

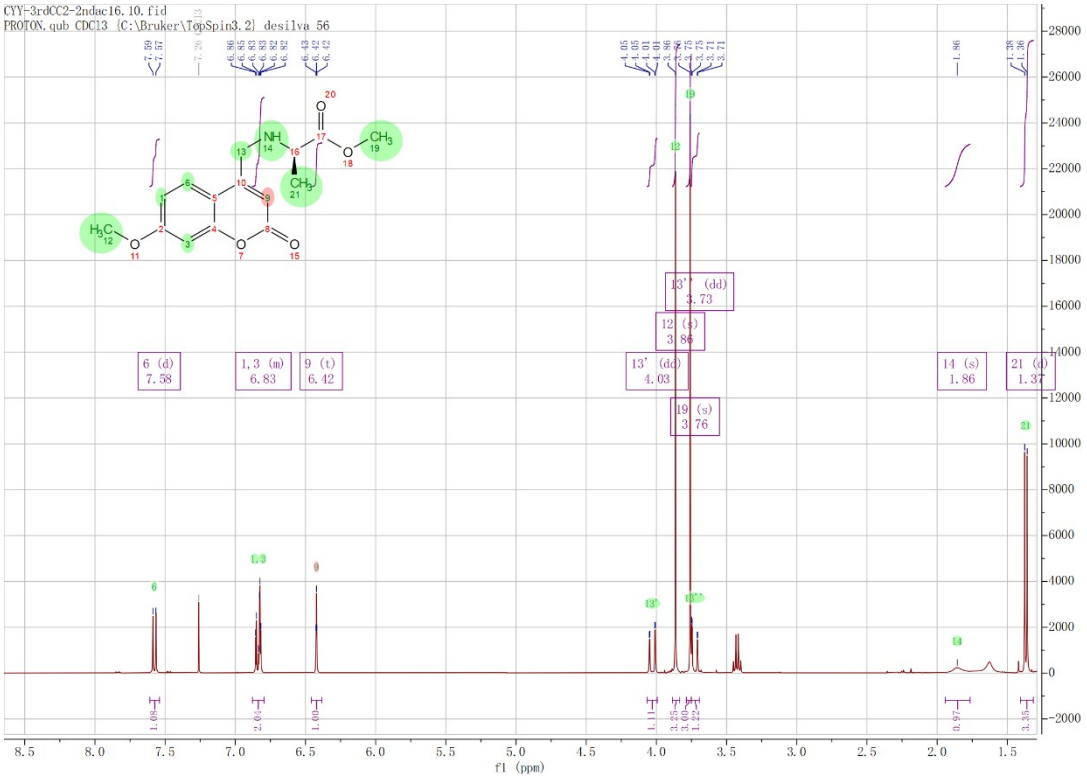


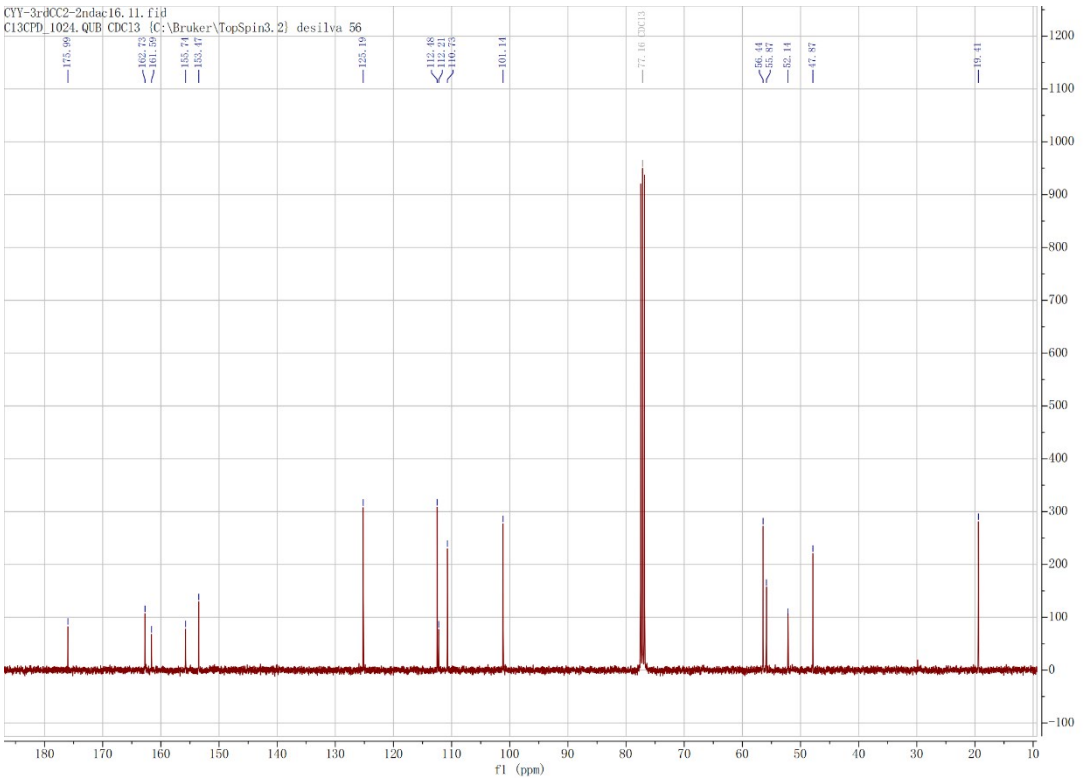


Main characterization details are given in the paper.
i.r. ν_{max} (KBr): 3084, 2984, 2944, 2904, 2871, 2849, 2039, 1724, 1613, 1557, 1513, 1456, 1445, 1409, 1390, 1348, 1284, 1264, 1207, 1150, 1135, 1071, 1023, 995, 979, 964, 920, 886, 862, 849, 834, 814, 754, 742, 707, 650, 632, 576, 538, 486, 462 cm^{-1}.

(*R*)-methyl 2-((7-methoxy-2-oxo-2*H*-chromen-4-yl)methylamino)propanoate, 3
A procedure similar to that for **1** was employed, but with D-alanine methyl ester hydrochloride, affording a yellow oil.

Yield: 0.082 g (78 %)





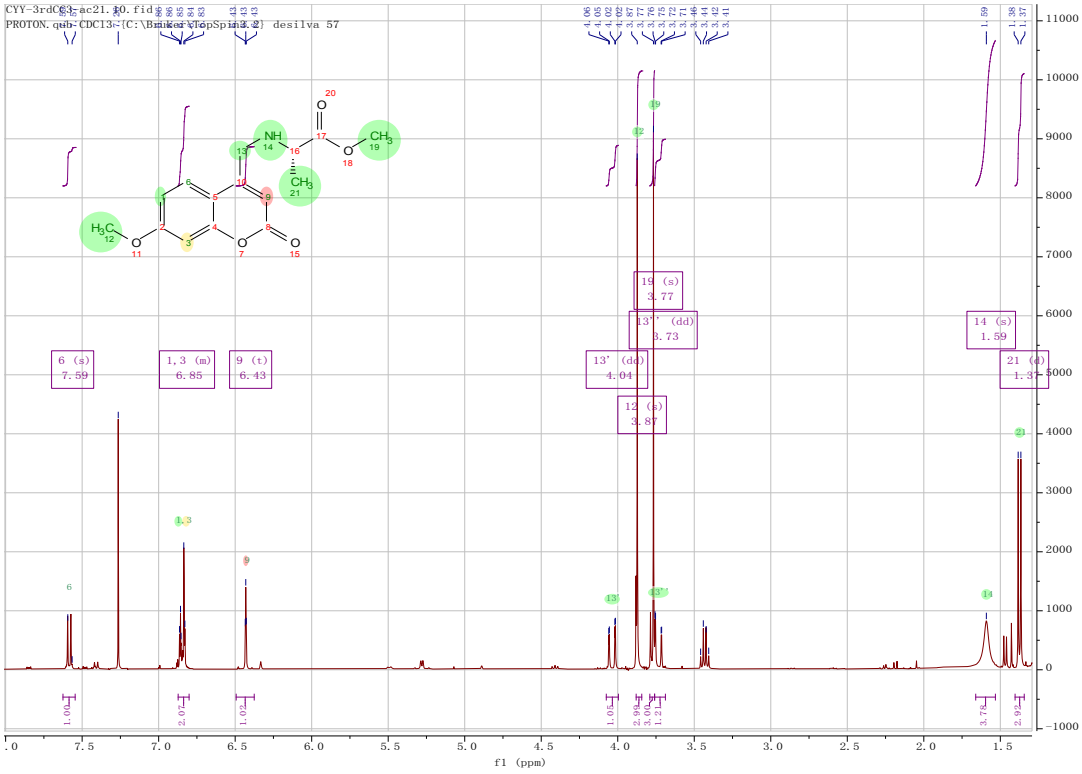
Main characterization details are given in the paper.

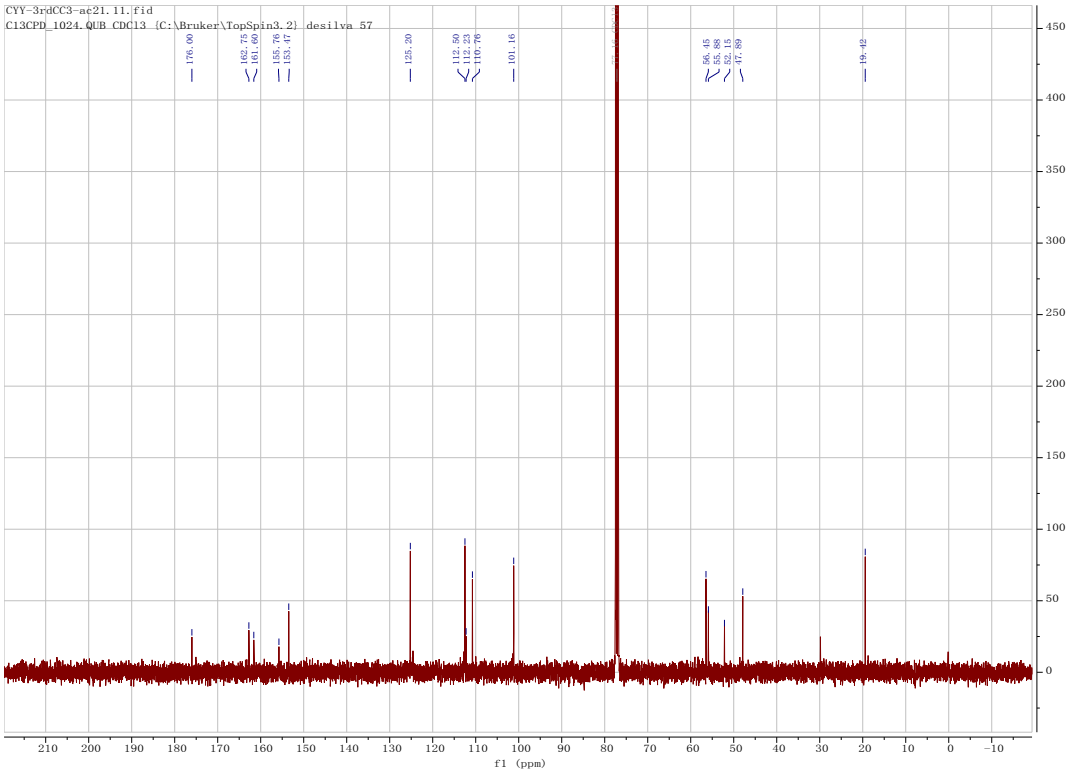
i.r. ν_{max} (KBr): 3080, 2952, 2843, 2361, 1724, 1615, 1558, 1513, 1456, 1394, 1348, 1283, 1265, 1209, 1150, 1074, 1023, 984, 851 cm^{-1}.

(*S*)-methyl 2-((7-methoxy-2-oxo-2*H*-chromen-4-yl)methylamino)propanoate, 5

A procedure similar to that for **1** was employed, but with L-alanine methyl ester hydrochloride, affording a yellow oil.

Yield: 0.073 g (69 %)





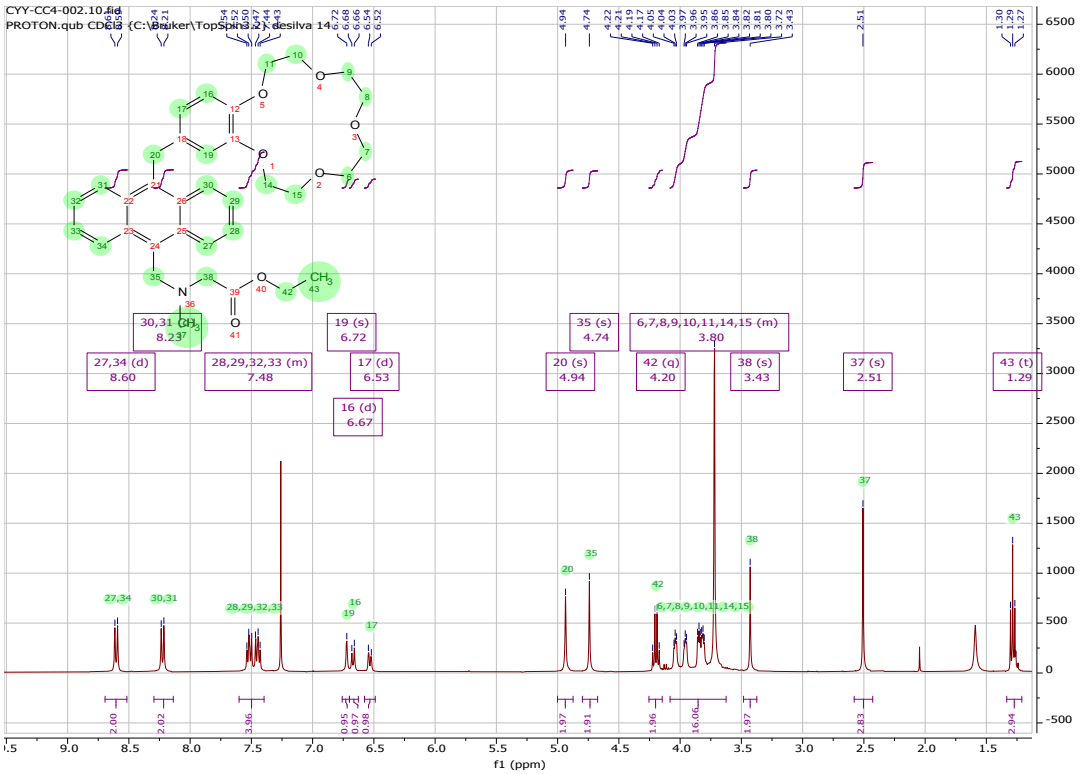
Main characterization details are given in the paper.

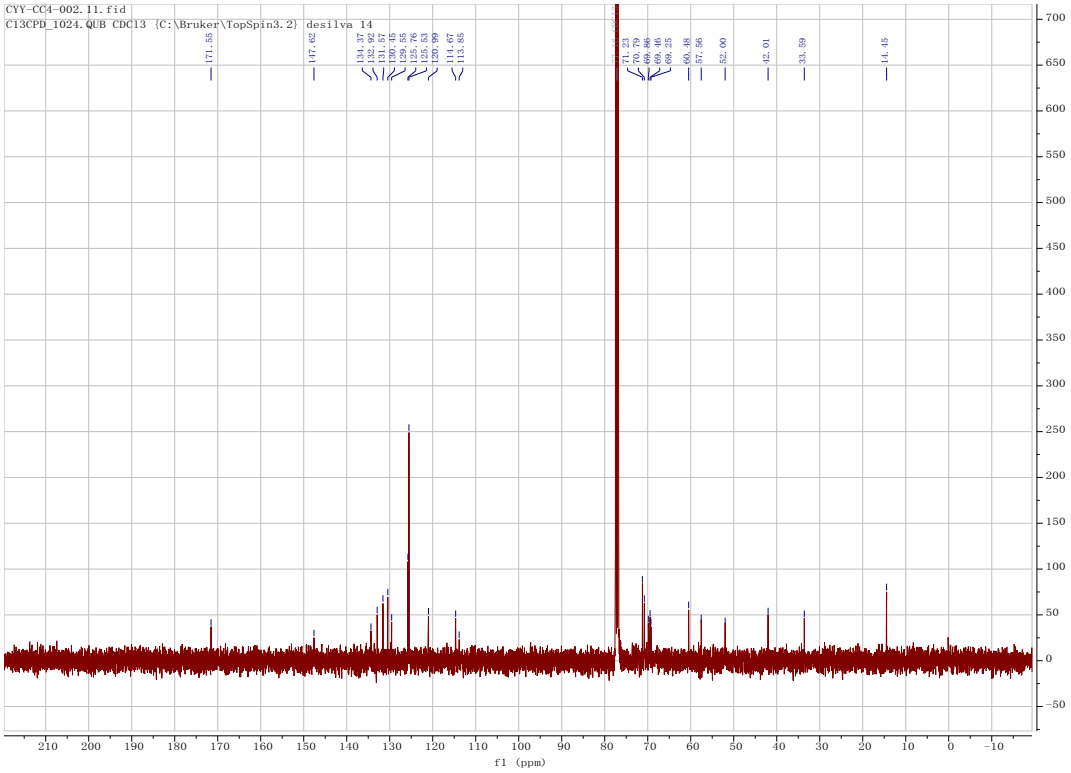
i.r. ν_{max} (KBr): 3082, 2952, 2843, 2361, 1724, 1615, 1558, 1514, 1456, 1394, 1349, 1283, 1265, 1209, 1150, 1078, 1023, 985, 850 cm^{-1}.

Ethyl 2-(methyl((10-((2,3,5,6,8,9,11,12-octahydrobenzo[*b*][1,4,7,10,13]pentaoxacyclopentadecin-15-yl)methyl)anthracen-9-yl)methyl)amino)ethanoate, 7

15-{[10-(Bromomethyl)-9-anthryl]methyl}-2,3,5,6,8,9,11,12-octahydro-1,4,7,10,13-benzopentaoxacyclopentadecine[S1] (0.23 g, 0.415 mmol), sarcosine ethyl ester hydrochloride (0.0635 g, 0.415 mmol) and potassium carbonate (3 g, anhydrous) were refluxed in CH_2Cl_2 (10 ml) for 24 hours. Potassium carbonate was then filtered off and washed with CH_2Cl_2. The solvent was evaporated off producing a yellow oil.

Yield: 0.22 g (89 %)





Main characterization details are given in the paper.

i.r. ν_{max} (KBr): 3436, 3065, 2918, 2865, 2361, 1729, 1671, 1608, 1587, 1559, 1514, 1447, 1424, 1358, 1272, 1253, 1234, 1187, 1138, 1071, 1051, 970, 940, 854, 808, 787, 760, 734, 663, 602, 576 cm^{-1}.

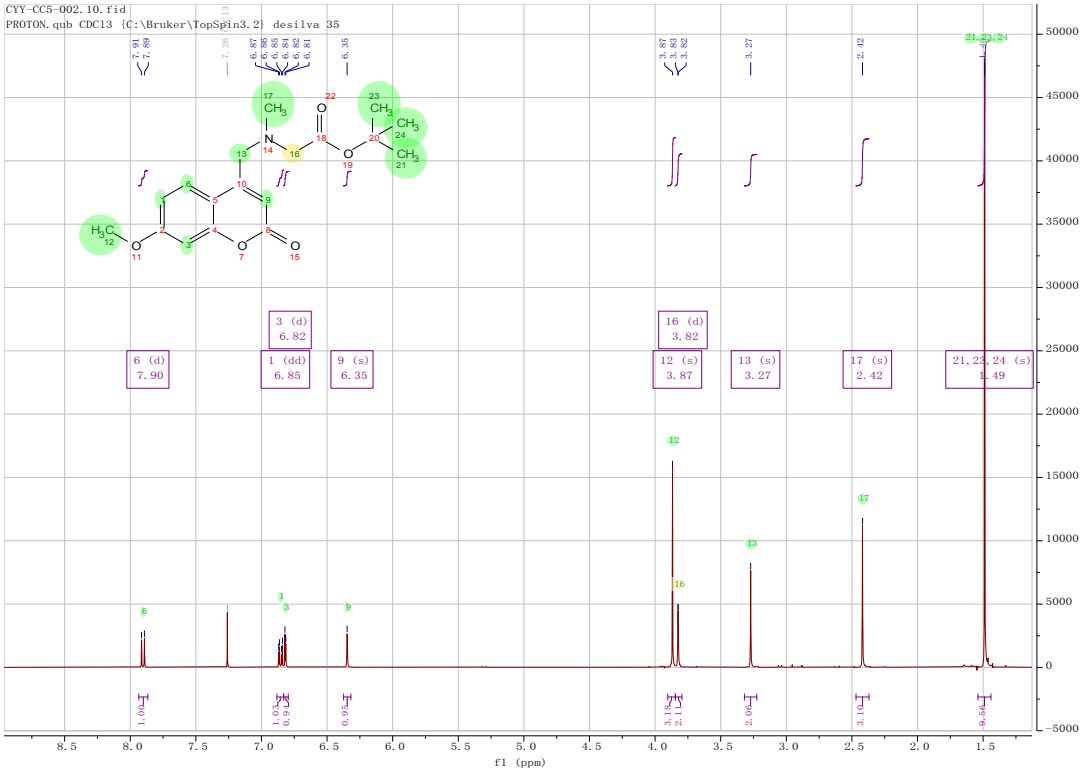
S2. Synthesis procedures and characterization details for hydrolysis products 2, 6 and 8, and intermediates 9 and 10

tert-Butyl 2-(((7-methoxy-2-oxo-2*H*-chromen-4-yl)methyl)(methyl)amino)ethanoate, 9

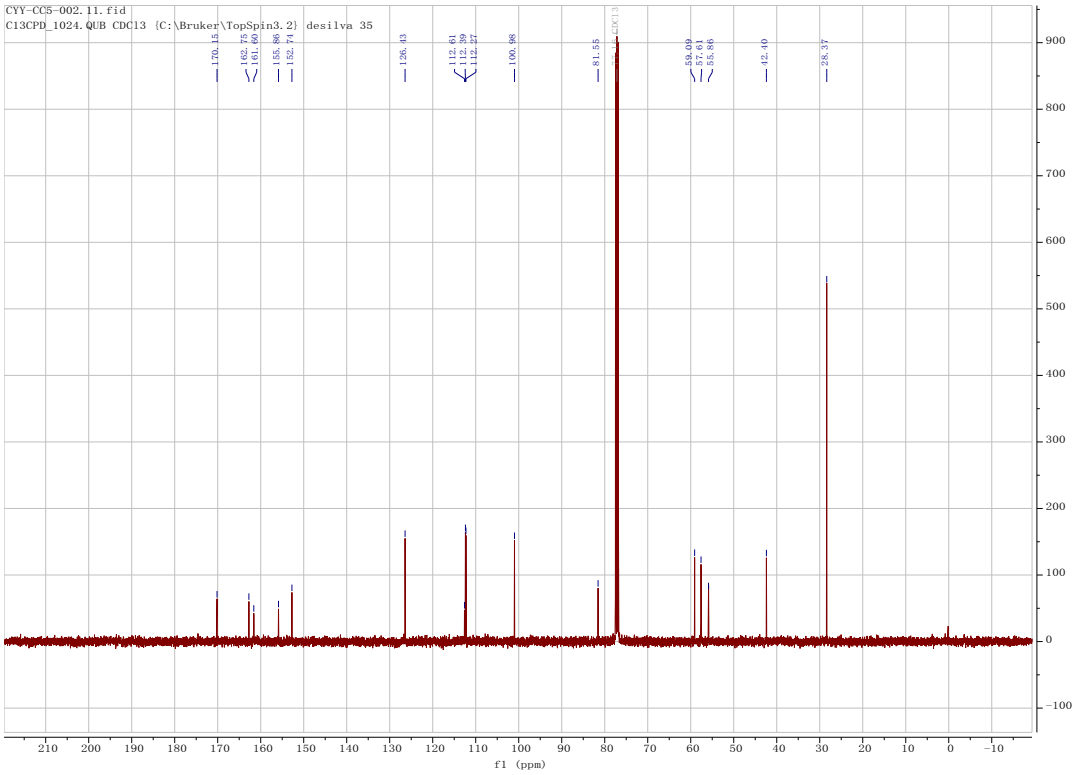
A procedure similar to that for **1** was employed, but with sarcosine *tert*-butyl ester hydrochloride, affording a yellow oil.

Yield: 1.13 g (91 %)

Found:	$C_{18}H_{24}NO_5$	$[M+H^+]$ 334.1654
Required:	$C_{18}H_{24}NO_5$	$[M+H^+]$ 334.0535



^{1}H NMR ($CDCl_3$): δ1.49 (s, 9H, C($C\underline{H}_3$)$_3$), 2.42 (s, 3H, N$C\underline{H}_3$), 3.28 (s, 2H, Ar$C\underline{H}_2$N), 3.83 (s, 2H, N$C\underline{H}_2$CO), 3.87 (s, 3H, O$C\underline{H}_3$), 6.35 (s, 1H, C$\underline{H}$CO), 6.82 (d, 1H, Ar$\underline{H}$, J=3 Hz), 6.86 (dd, 1H, Ar$\underline{H}$, J=3, 9 Hz), 7.91 (d, 1H, Ar$\underline{H}$, J=9 Hz).



^{13}C NMR ($CDCl_3$): δ28.2, 30.9, 42.2, 55.7, 100.8, 112.3, 126.3, 155.7, 161.5, 162.6, 169.9, 206.9.

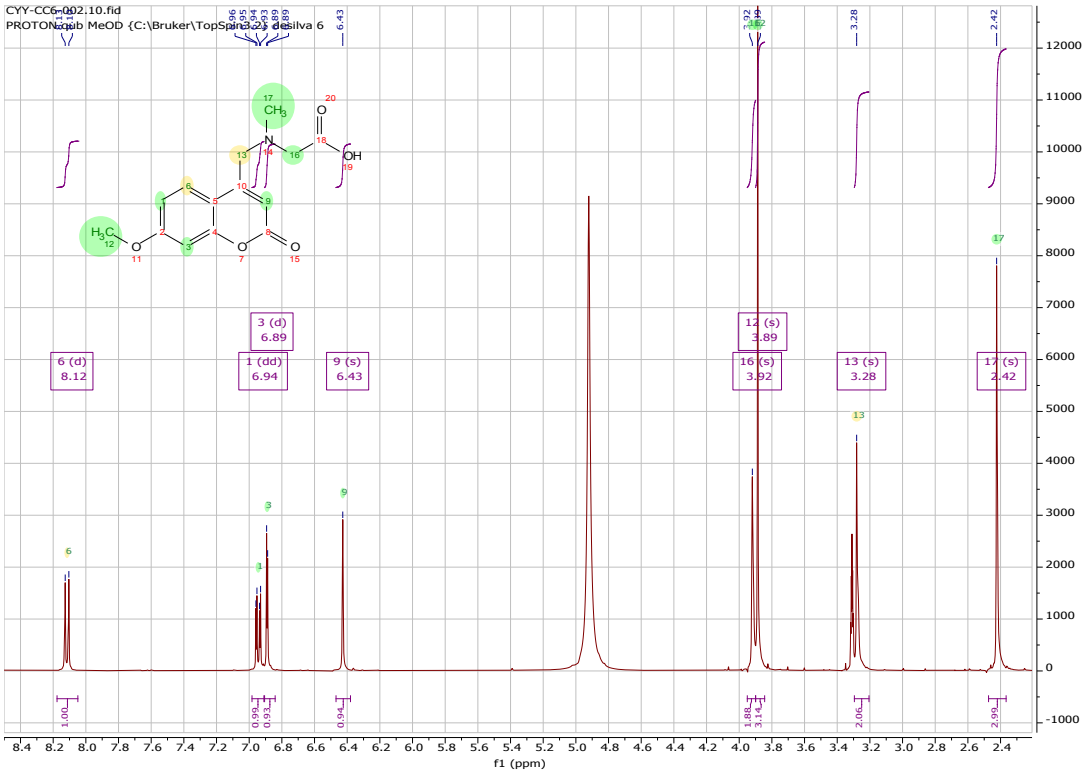
i.r. ν_{max} (KBr): 2980, 2361, 1724, 1616, 1558, 1513, 1457, 1395, 1348, 1292, 1209, 1149, 1069, 1026, 840 cm^{-1}.

2-(((7-Methoxy-2-oxo-2*H*-chromen-4-yl)methyl)(methyl)amino)ethanoic acid, 2

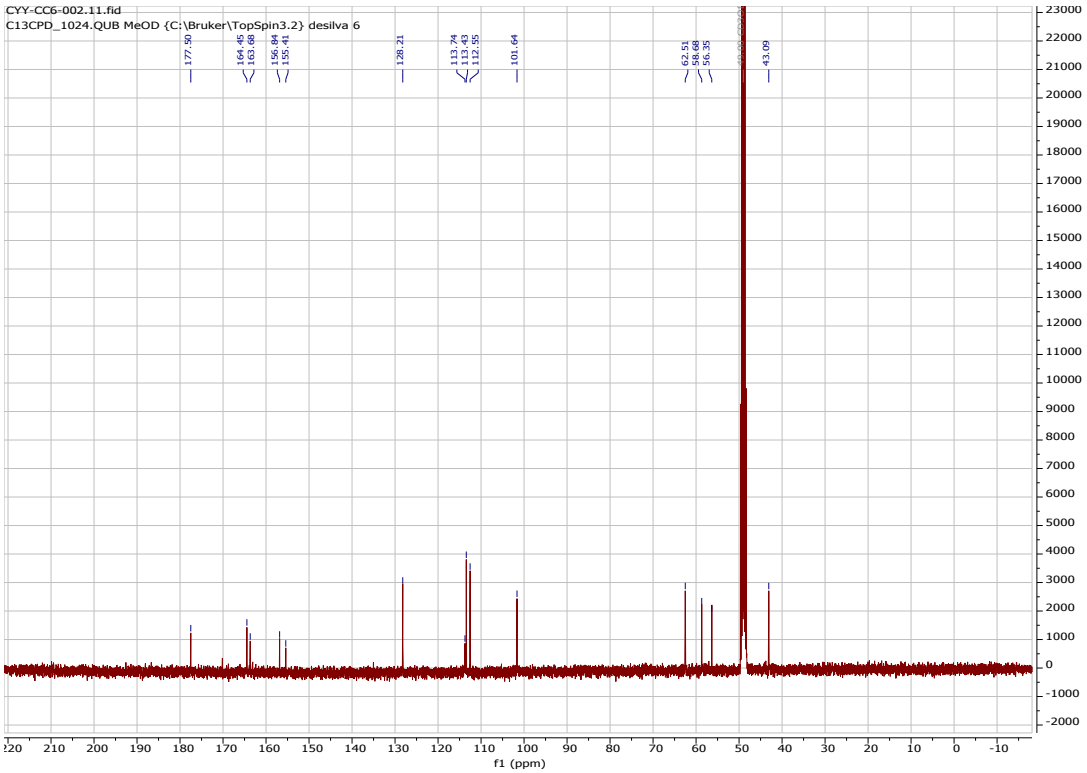
To **9** (0.604 g, 1.81 mmol) was added formic acid (15 ml) in a 50 ml round-bottom flask and the solution heated at 60 °C for 6 hours while stirring. The solution was then allowed to cool and then placed in the fridge for 72 hours. The solvent was then removed on the rotary evaporator and approximately 5 ml H_2O was added, followed by several drops of aqueous NH_3 (0.2 M), causing a precipitate to form. The round-bottom flask was again placed in the fridge, for 24 hours. The solution was then filtered using a glass-sintered funnel and filter paper, affording an off-white solid.

Yield: 0.379 g (76 %)

Found:	$C_{14}H_{16}NO_5$	$[M+H^+]$ 278.1033
Required:	$C_{14}H_{16}NO_5$	$[M+H^+]$ 278.1028



^{1}H NMR ($CDCl_3$): δ2.47 (s, 3H, NC<u>H</u>$_3$), 3.43 (s, 2H, ArC<u>H</u>$_2$N), 3.80 (s, 3H, OC<u>H</u>$_3$), 4.03 (s, 2H, NC<u>H</u>$_2$CO), 6.34 (s, 1H, C<u>H</u>CO), 6.84 (d, 1H, Ar<u>H</u>, J=3 Hz), 6.87 (dd, 1H, Ar<u>H</u>, J=9, 3 Hz), 7.99 (d, 1H, Ar<u>H</u>, J=9 Hz).



^{13}C NMR ($CDCl_3$): δ28.6, 42.6, 56.1, 57.7, 59.1, 82.0, 101.2, 112.7, 112.8, 126.7, 152.7, 156.1, 161.9, 163.0, 170.2,

i.r. ν_{max} (KBr): 3032, 3016, 2952, 2852, 1723, 1619, 1560, 1521, 1475, 1456, 1404, 1384, 1349, 1303, 1283, 1213, 1182, 1156, 1122, 1070, 1028, 987, 967, 950, 904, 879, 862, 848.

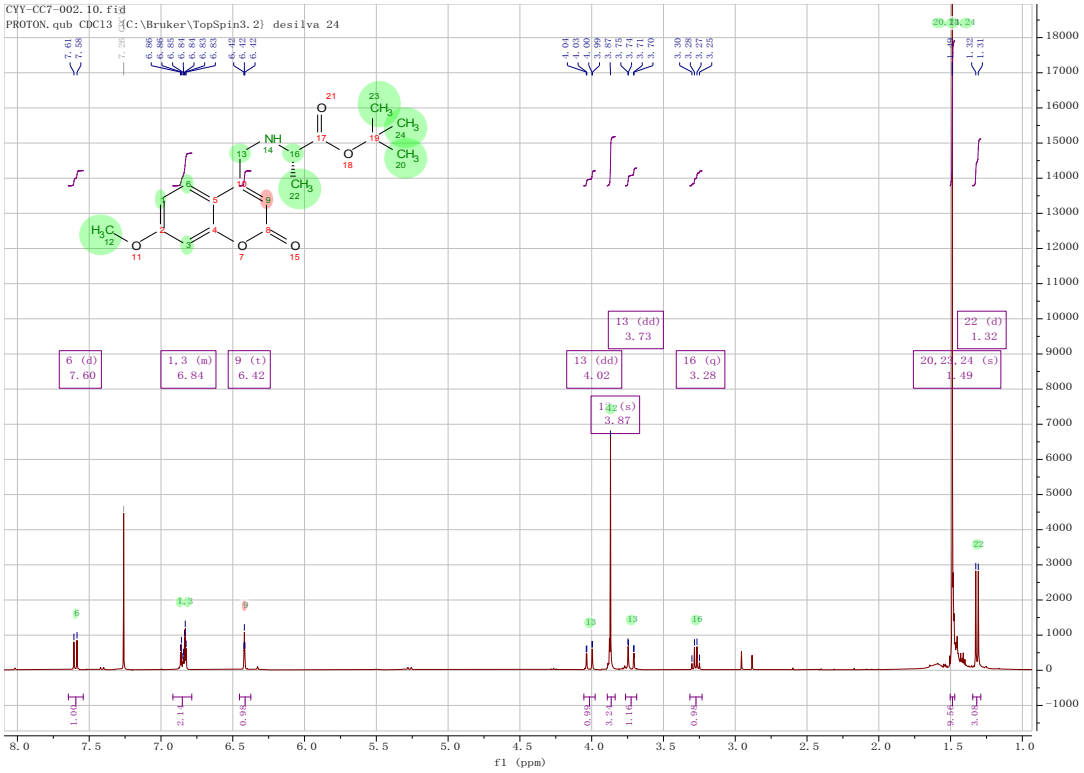
Hydrolysis product **4** could not be prepared conveniently since D-alanine *tert*-butyl ester hydrochloride was not commercially available at a reasonable price at the time.

(*S*)-*tert*-butyl 2-((7-methoxy-2-oxo-2*H*-chromen-4-yl)methylamino)propanoate, 10

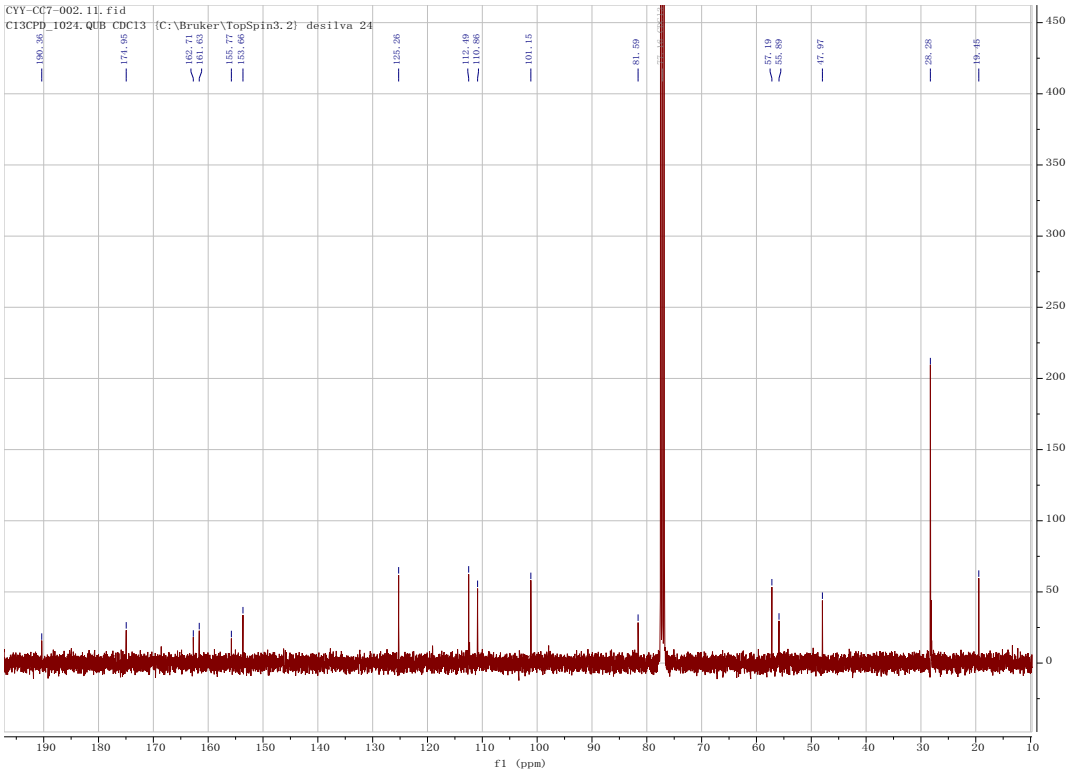
A procedure similar to that for **1** was employed, but with L-alanine *tert*-butyl ester hydrochloride, affording a yellow solid.

Yield: 0.607 g (97 %)

Found:	$C_{18}H_{24}NO_5$	$[M+H^+]$ 334.1667
Required:	$C_{18}H_{24}NO_5$	$[M+H^+]$ 334.1668



^{1}H NMR ($CDCl_3$): δ1.46 (d, 3H, C(C<u>H</u>$_3$)H, J=7 Hz), 1.51 (s, 9H, C(C<u>H</u>$_3$)$_3$), 3.28 (q, 1H, C(CH$_3$)<u>H</u>, J=7 Hz), 3.87 (dd, 2H, C<u>H</u>$_2$NH, J=16, 88 Hz), 3.87 (s, 3H, OC<u>H</u>$_3$), 3.89 (s, 1H, N<u>H</u>), 6.38 (s, 1H, C<u>H</u>CO), 6.82 (m, 1H, Ar<u>H</u>), 6.89 (m, 1H, Ar<u>H</u>), 7.62 (d, 1H, Ar<u>H</u>, J=9 Hz).



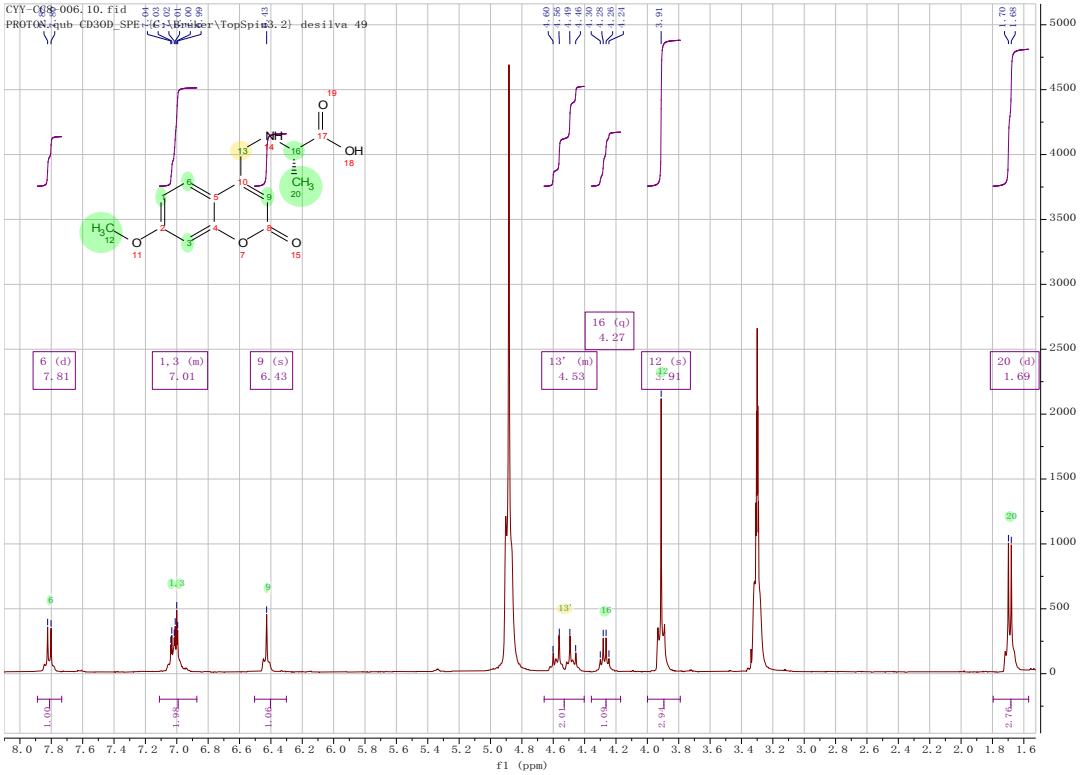
^{13}C NMR ($CDCl_3$): δ16.9, 28.4, 48.1, 56.2, 57.4, 101.4, 111.1, 112.7, 125.5, 153.9, 155.9, 161.9, 163.0, 175.1.

i.r. ν_{max} (KBr): 2978, 2935, 1726, 1615, 1558, 1514, 1456, 1394, 1369, 1350, 1291, 1264, 1211, 1152, 1058, 1023, 990, 849 cm^{-1}.

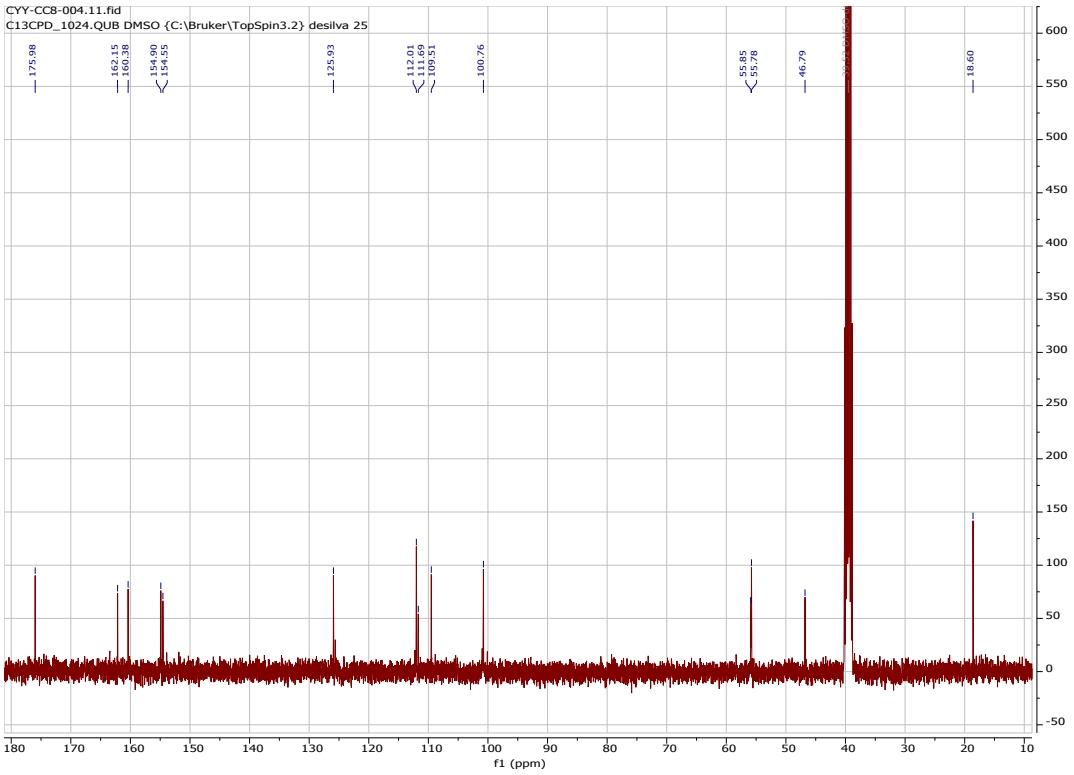
(*S*)-2-((7-methoxy-2-oxo-2*H*-chromen-4-yl)methylamino)propanoic acid, 6

To **10** (0.2713 g, 0.814 mmol) was added formic acid (6 ml) and the solution heated at 60 °C while stirring for 6 hours. The solvent was then removed and precipitation encouraged by the addition of aqueous NH_3 (0.2 M), affording an off-white solid.

Yield: 0.0964 g (43 %)



^{1}H NMR ($CDCl_3$): δ1.58 (d, 3H, C(C<u>H</u>$_3$)H, J=7 Hz), 3.88 (s, 3H, OC<u>H</u>$_3$), 3.89 (s, 2H, C<u>H</u>$_2$NH), 4.40 (q, 1H, C(CH$_3$)<u>H</u>, J=7 Hz), 6.43 (s, 1H, Ar<u>H</u>), 6.87 (m, 1H, Ar<u>H</u>), 6.91 (m, 1H, Ar<u>H</u>), 7.53 (m, 1H, Ar<u>H</u>).



^{13}C NMR ($CDCl_3$): δ16.9, 53.9, 56.3, 101.5, 110.2, 111.5, 112.9, 113.1, 125.2, 150.8, 155.7, 161.5, 163.4, 174.1.

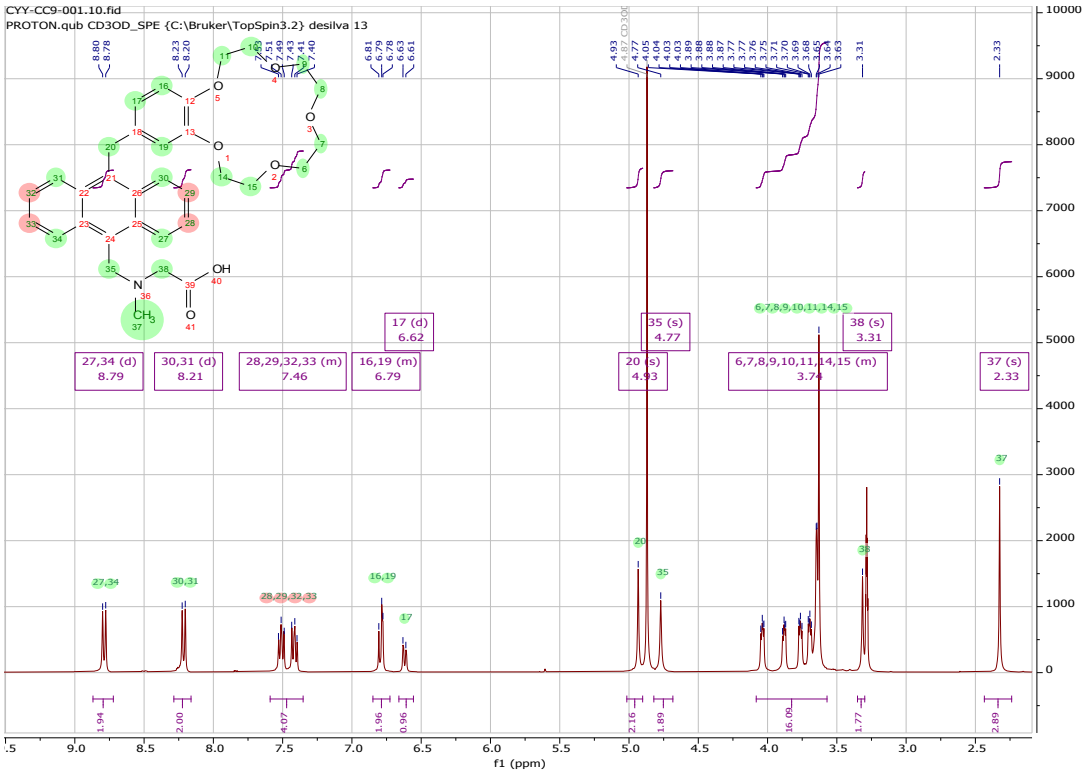
i.r. ν_{max} (KBr): 3138, 1725, 1604, 1400, 1349, 1290, 1212, 1139, 1075, 1026, 1005, 984, 851, 763, 702 cm^{-1}.

2-(Methyl((10-((2,3,5,6,8,9,11,12-octahydrobenzo[*b*][1,4,7,10,13]pentaoxacyclopentadecin-15-yl)methyl)anthracen-9-yl)methyl)amino)ethanoic acid, 8

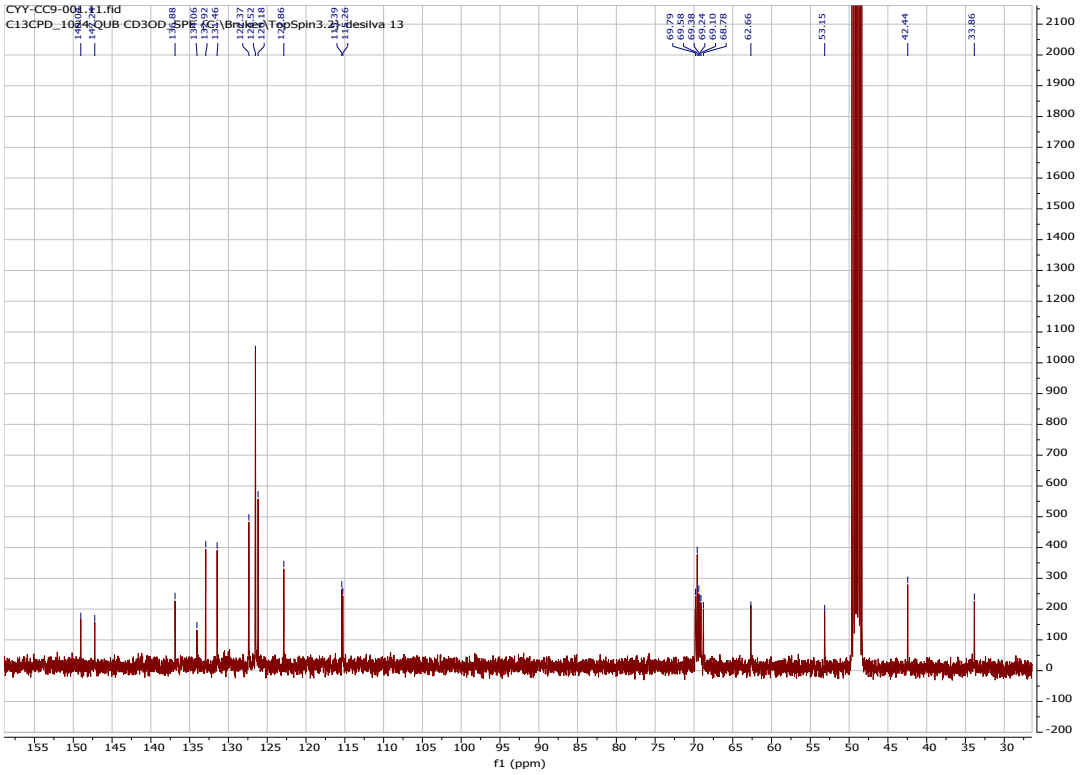
7 (0.176 g, 0.3 mmol) was dissolved in THF (10 ml). A solution of NaOH (0.24 g, 6 mmol) in MeOH:H_2O (1:1, v/v) was added. The solution was left to stir overnight. The solvent was then removed by evaporation. The yellow solid was dissolved in water and neutralised with acetic acid producing a yellow precipitate, which was filtered off.

Yield: 0.109 g (65 %)

Found:	$C_{33}H_{37}NO_7Na$	$[M+Na^+]$ 582.2486
Required:	$C_{33}H_{37}NO_7Na$	$[M+Na^+]$ 582.2468



^{1}H NMR (MeOD-d^{4}): δ2.75 (s, 3H, NC$\underline{H}_3$), 3.5-3.9 (m, 16H, C$\underline{H}_2$O), 3.65 (s, 2H, NC$\underline{H}_2$), 4.78 (s, 2H, AnthC$\underline{H}_2$N), 4.91 (s, 2H, AnthC$\underline{H}_2$Ar), 6.35 (d, 1H, Ar$\underline{H}$, J=8 Hz), 6.55 (d, 1H, Ar$\underline{H}$, J=8 Hz), 6.75 (s, 1H, Ar$\underline{H}$), 7.49 (dt, 4H, Anth$\underline{H}$, J=7 Hz), 7.64 (dt, 2H, Anth$\underline{H}$, J=7 Hz), 8.3 (d, 2H, Anth$\underline{H}$, J=9 Hz).



^{13}C NMR (MeOD-d^{4}): 38.7, 46.1, 48.7, 55.7, 58.0, 65.7, 74.2, 74.9, 75.7, 76.0, 119.7, 119.9, 125.8, 126.2, 135.9, 137.5, 139.3, 142.8, 153.0, 154.6, 175.9.

i.r. ν_{max} (KBr): 3035, 2867, 2361, 1633, 1511, 1451, 1424, 1360, 1271, 1252, 1137, 1055, 978, 938, 850, 763, 735, 692, 668, 603, 540 cm^{-1}.

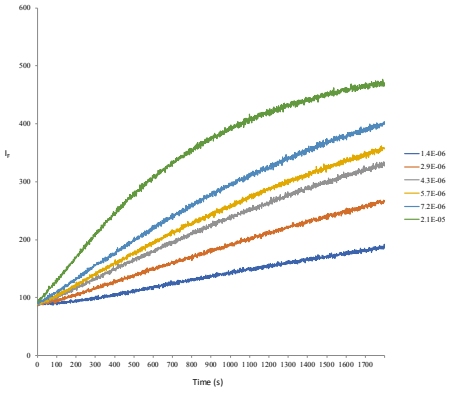
S3. Conditions and analytical procedures of enzyme screen

1 (91.0 mg, 0.30 mmol) was suspended in methyl *tert*-butyl ether (8 ml) and 200 μl of this suspension was added to 1 ml phosphate buffer (0.1 M) along with a 10 mg of enzyme in a small vial. The vials were then shaken overnight at room temperature. The samples were then put in the fridge for 4 nights. 700 μl acetonitrile was then added to each vial, and the contents centrifuged, causing the protein to separate from the liquid. The mother liquor (150 μl) was then removed, placed into an HPLC vial, which was then filled with MeCN (approx. 1 ml). This was then analysed by reversed phase HPLC carried out on a C18 column. A mobile phase with 5 % to 95 % solvent B in solvent A was run over 13 minutes, in which solvent A consisted of 0.1 % trifluoroacetic acid (TFA) in Milli Q® water and solvent B consisted of 0.1 % TFA in acetonitrile.

After having run the enzyme screen, the absorbance of the samples at 326 nm was reduced to 0.1 by dilution with pH 7 phosphate buffer, and their total fluorescence emission measured. The expected result was that those samples that showed a high conversion from the ester to the acid would be more fluorescent than those that did not. There is a linear relationship between the HPLC yield of **2** and the intensity of fluorescence. So the fluorescence method could be used to examine the activity of a particular enzyme.

S4. Time-dependent fluorescence during enzymatic hydrolysis

As **1** was hydrolysed to **2** by CALB, the fluorescence (λ_{exc} = 326 nm and λ_{em} = 415 nm) increased gradually. The example below shows the effect of varying the CALB concentration.



S5. Standards for fluorescence quantum yields

The reference compounds used were (anthracen-9-ylmethyl)diethylamine[S2] for the anthracene compounds **7**-**8** and 4-methyl-7-methoxycoumarin[S3] for the coumarin compounds **1**-**6**.

References

S1. A. P. de Silva, H. Q. N. Gunaratne and C. P. McCoy, *J. Am. Chem. Soc*. 1997, **119**, 7891.
S2. R. A. Bissell, E. Calle, A. P. de Silva, S. A. de Silva, H. Q. N. Gunaratne, J. L. Habib-Jiwan, S. L. A. Peiris, R. A. D. D. Rupasinghe, T. K. S. D. Samarasinghe, K. R. A. S. Sandanayake and J. -P. Soumillion, *J. Chem. Soc. Perkin Trans. 2* 1992, 1559.
S3. A. P. de Silva, H. Q. N. Gunaratne, P. L. M. Lynch, A. L. Patty and G. L. Spence, *J. Chem. Soc. Perkin Trans. 2* 1993, 1611.