

Electronic Supporting Information

Solid Phase Synthesis of Biocompatible *N*-Heterocyclic Carbene–Pd Catalysts Using a Sub-monomer Approach

Durgadas Cherukaraveedu, Paul T. Cowling, Gavin P. Birch, Mark Bradley* and Annamaria Lilienkampf*

EaStCHEM School of Chemistry, University of Edinburgh, Kings' Buildings, David Brewster Road, Edinburgh, EH9 3FJ, UK

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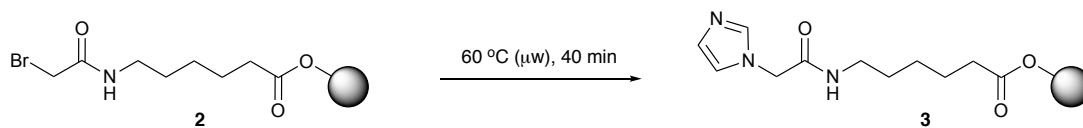
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1. Supporting Figures and Tables

Table S1. Optimisation of the on-resin alkylation of **2** with imidazole. All the reactions were done under μw heating (60 °C, 25W, 40 min) using a Biotage® Initiator⁺ SP Wave peptide synthesizer. At the end of the reaction, the compound was cleaved off the resin with 30% HFIP in DCM (45 min) for analysis by HPLC or ¹H NMR.



Entry	Imidazole conc.	Additive	Solvent	Conversion
1	1 M	–	DMF	< 50% ^a
2	1.5 M	1 eq. Et ₃ N	DMF	< 60% ^b
3	1.5 M	1 eq. Et ₃ N	DMSO	< 75% ^b
4	2 M	–	anhyd. DMSO	< 77% ^b
5	2 M	0.5 M AgNO ₃	anhyd. DMSO	> 97% ^a

^a Determined by HPLC. ^b Determined by ¹H NMR.

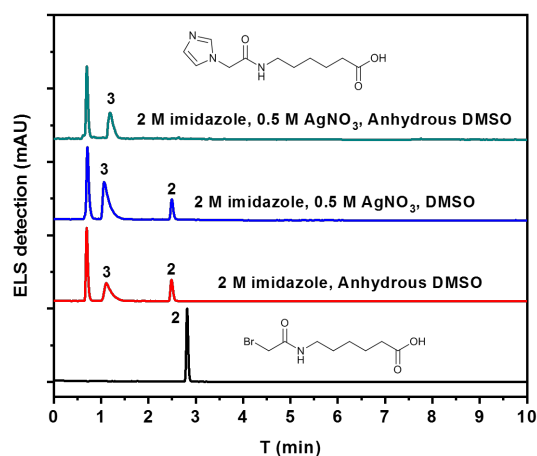
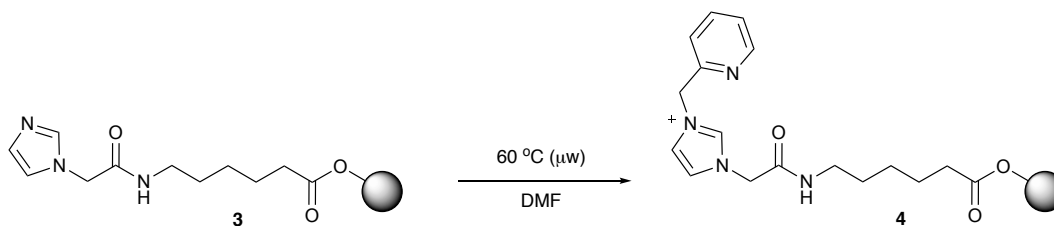


Figure S1. Normalised RP-HPLC chromatograms (detection by ELSD) of the alkylation of **2** after 40 min at 60 °C with μw irradiation from independent experiments are shown. Complete conversion to product **3** was observed when anhydrous DMSO was used as solvent with AgNO₃ as an additive. The peak at 0.8 min is associated with sample injection/solvent make-up.

Table S2. Optimisation of the on-resin alkylation of **3** with 2-(bromomethyl)pyridine hydrobromide (2-(BrCH₂)Py). All the reactions were done in DMF under μ w heating (60 °C, 25W) using a Biotage® Initiator⁺ SP Wave peptide synthesizer. At the end of the reaction, the compound was cleaved off the resin with 30% HFIP in DCM (45 min) for analysis by HPLC or ¹H NMR.



Entry	2-(BrCH ₂)Py conc.	Additives	Time	Conversion
1	1.5 M	2 eq. Et ₃ N	30 min	< 50% ^a
2	1.5 M	2 eq. Et ₃ N	60 min	< 70% ^a
3	1.5 M	3 eq. Et ₃ N	60 min	< 70% ^a
4	2 M	3 eq. Et ₃ N	90 min	< 80% ^b
5	2 M	3 eq. Et ₃ N, 0.5M AgNO ₃	90 min	> 96% ^a
6	1 M	1 M Et ₃ N, 0.5 M AgNO ₃	90 min	>97% ^a

^a Determined by HPLC. ^b Determined by ¹H NMR.

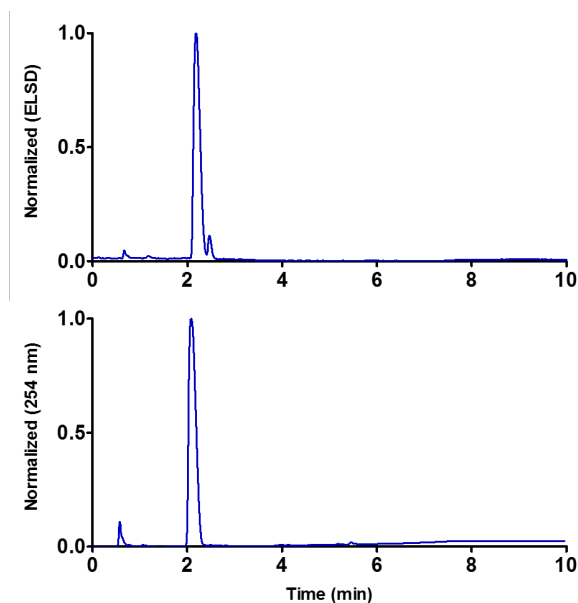


Figure S2. HPLC chromatograms of crude ligand **4** after cleavage off the resin (crude purity > 95%). Top: detection by ELSD. Below: detection at 254 nm.

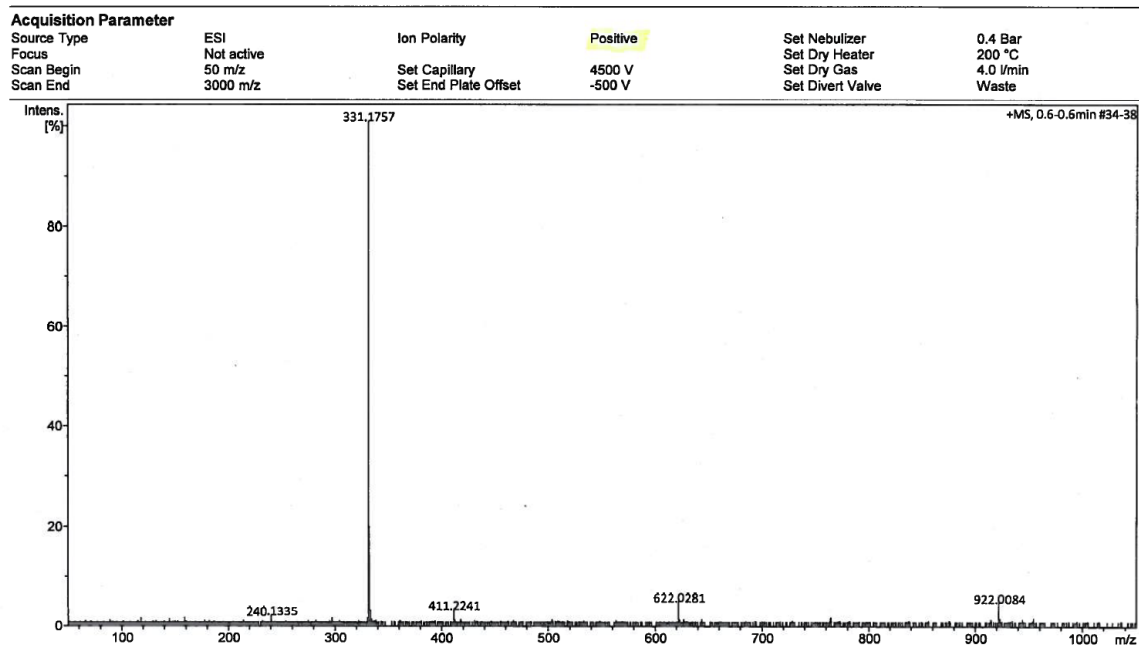


Figure S3. HRMS (ESI) spectra of crude ligand **4** (calculated 331.1765 for $C_{17}H_{23}N_4O_3$; found 331.1757).

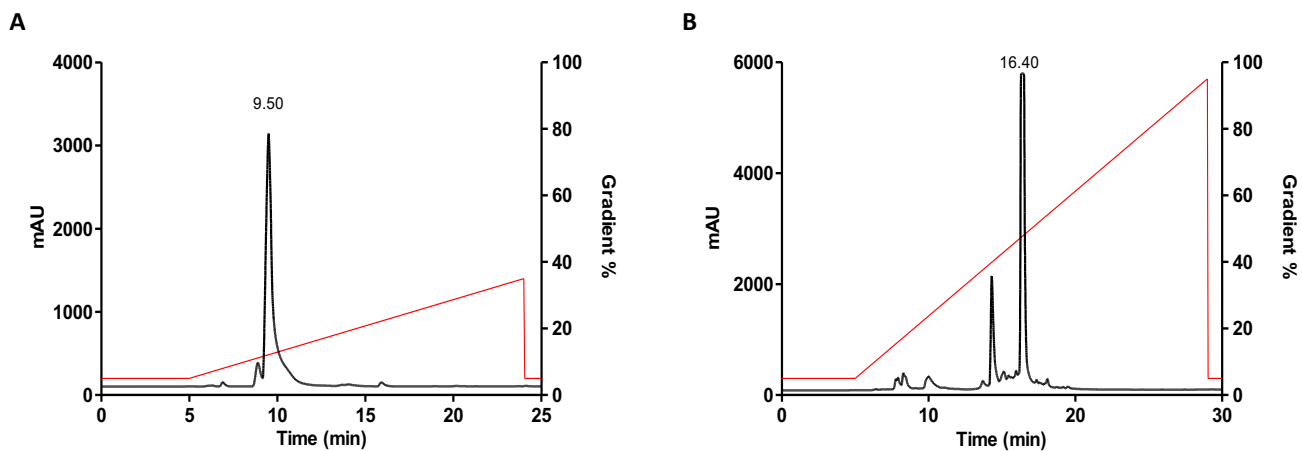


Figure S4. Purification of catalyst **8** by HPLC (detection at 254 nm). A) Semi-preparative HPLC trace of the crude ligand for catalyst **8** (t_R 9.50 min) before palladium loading. B) Semi-preparative HPLC trace of the crude catalyst **8** (t_R 16.40 min) showing the change in retention time upon palladium loading.

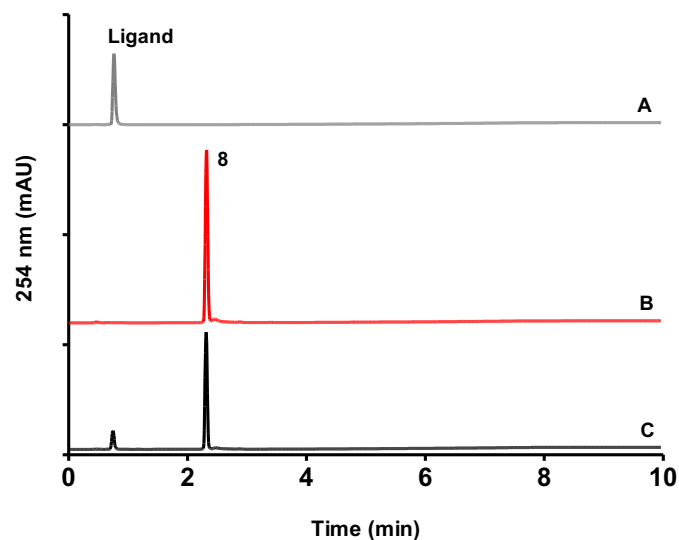


Figure S5. HPLC analysis (detection at 254 nm) of the ligand and catalyst **8**. A) The ligand after HPLC purification. B) Catalyst **8** after HPLC purification. C) The sample of catalyst **8** spiked with the ligand, showing the change in retention time upon palladium loading.

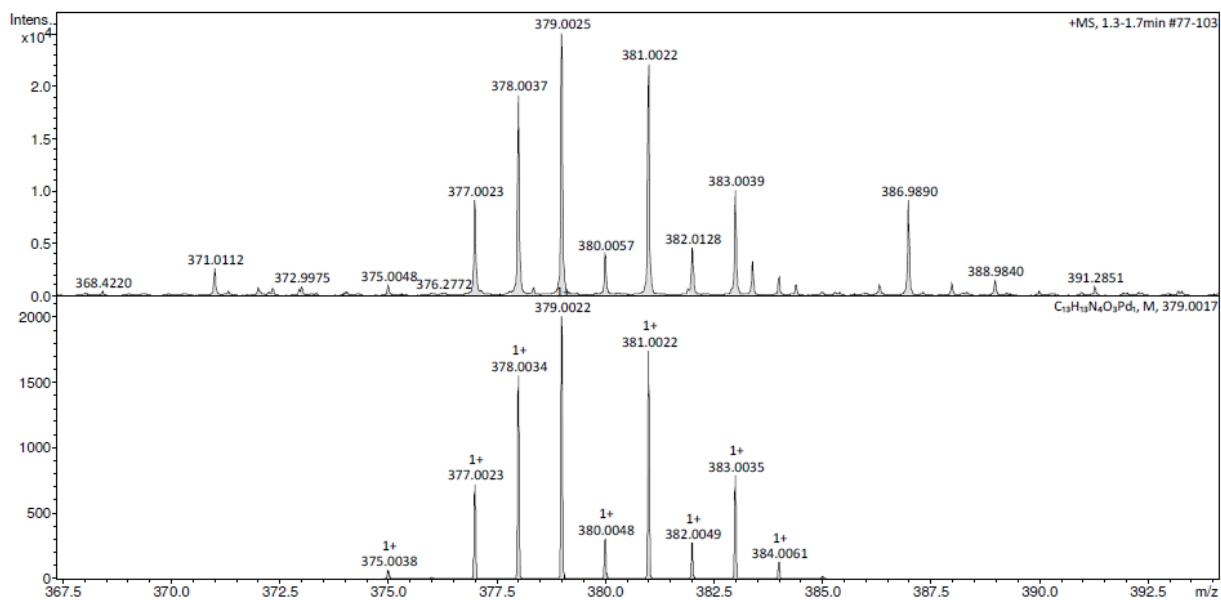
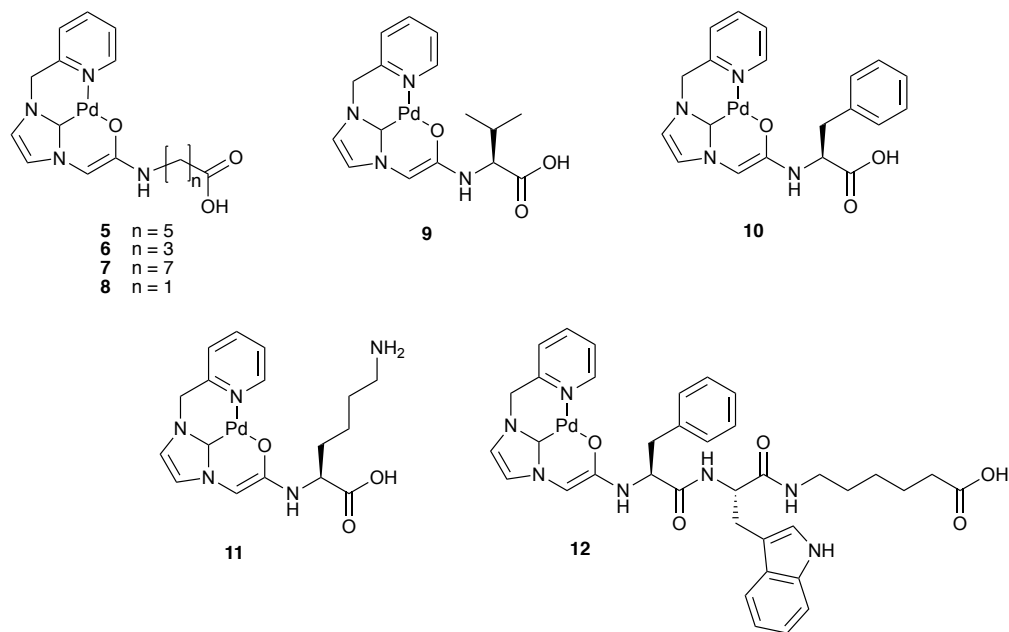


Figure S6. Measured (top) and predicted (bottom) HRMS (ESI) spectra for catalyst **8** (calculated 379.0017 for $C_{13}H_{13}N_4O_3Pd$; found 379.0025).

Table S3. Characterisation of the ligands and their corresponding NHC–Pd catalysts **5–12**.



Compd.	Ligand		Catalyst		
	Yield ^a (%)	Purity ^b (%)	Purity ^c (%)	Mass calcd.	Mass obs.
5	65	96	94	435.0643	435.0640
6	62	94	90	407.0330	407.0332
7	46	96	82	463.0963	463.0956
8	57	81	94	379.0017	379.0025
9	51	82	93	421.0486	421.0491
10	36	93	94	469.0486	469.0488
11	32	58	95	450.0752	450.0750
12	37	96	96	768.2136	768.2120

^a The ligand yields were calculated based on the resin loading and are reported without purification. ^b The crude ligand purities were determined by HPLC with detection at 254 nm. ^c NHC–Pd catalyst yields and purity after purification by semi-preparative HPLC.

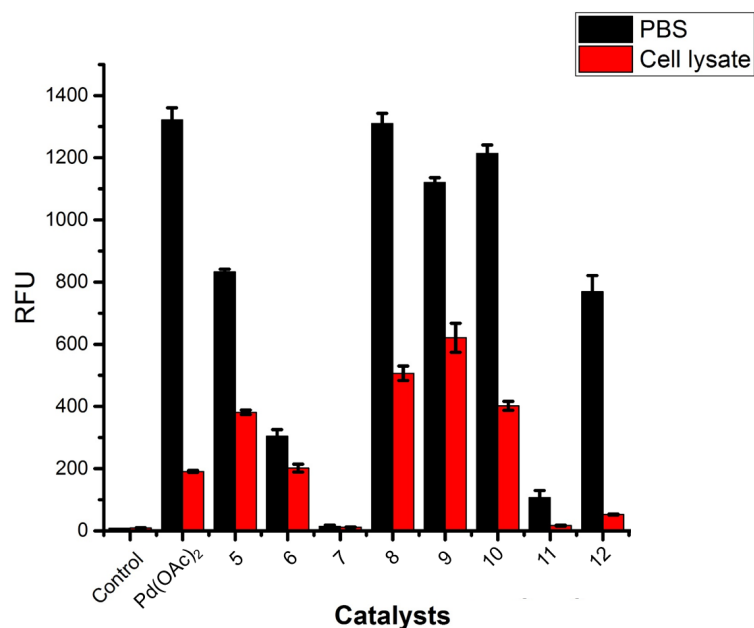


Figure S7. Increase in fluorescence after 20 h treatment of **DCF-1** (10 μ M) with NHC–Pd catalyst (0.8 mol%) or Pd(OAc)₂ (0.8 mol%) (normalised to the control without catalyst).

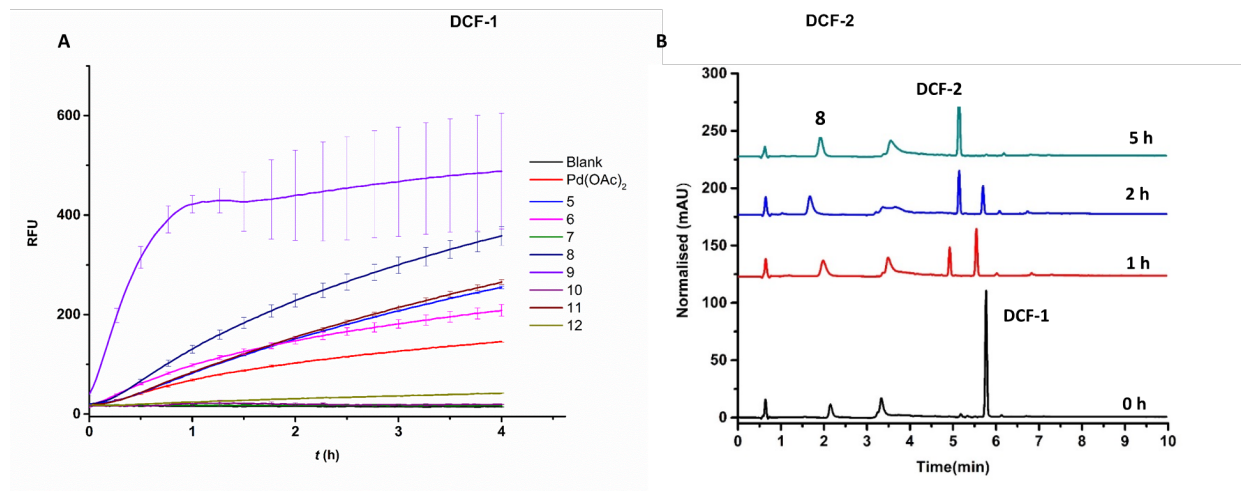
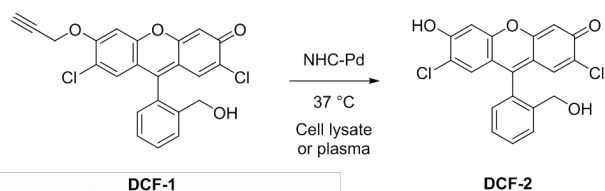


Figure S8. A) Fluorescence based screening of NHC–Pd catalysts 5–12 (0.8 mol%) for depropargylation of the probe **DCF-1** (10 μ M) in cell lysate (n = 3). B) The depropargylation of **DCF-1** (50 μ M) with catalyst **8** (2 mol%) in human plasma monitored by HPLC (detection at 282 nm) showed > 92% conversion over 5 h.

2. Experimental

2.1 Materials and Methods

Amino acids and 2-chlorotrityl polystyrene resin (100–200 mesh) were purchased from GL Biochem Ltd and NovaBiochem. All other chemicals were purchased from Sigma Aldrich and Acros and used without purification unless otherwise stated. A LIVE/DEAD™ Cell Imaging Kit (488/570) was purchased from Thermo Fischer Scientific. Dulbecco's phosphate buffered saline (PBS) with MgCl₂ and CaCl₂ (D8662) and human plasma containing 4% trisodium citrate (P9523) were purchased from Sigma-Aldrich. **Pro-5-FU**¹ and the fluorogenic probe **DCF-1**^{2,3} were synthesised according to published procedures.

Microwave-assisted reactions on resins were carried out in a Biotage Initiator+ SP Wave at 2.45 GHz. ¹H and ¹³C NMR spectra were recorded on an automated Bruker AVA 500 (500 and 126 MHz, respectively) or Bruker AVA 600 (600 and 151 MHz, respectively) in the indicated solvents at 298 K. Chemical shifts (δ) are quoted in ppm using the residual non-deuterated solvent (¹H NMR) or the deuterated solvent (¹³C NMR) as internal standards, and all coupling constants (J) were measured in Hertz (Hz). Resonances are specified as singlet (s), doublet (d), triplet (t), multiplet (m), broad singlet (br s) or aromatic (Ar).

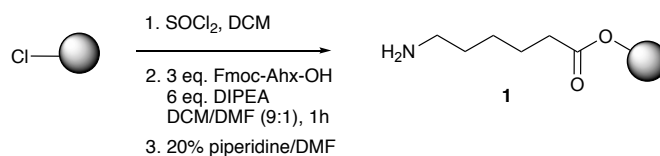
High Resolution Mass Spectra (HRMS) were performed on a Bruker microTOF2 spectrometer by direct infusion. Analytical RP-HPLC was performed using an Agilent Technologies 1100 modular HPLC system coupled to a Polymer Lab 1000 Evaporative Light Scattering Detector (ELSD) and multi-wavelength detector equipped with a Phenomenex Kinetex® XB-C18 100 Å LC Column (50 × 4.6 mm, 5 μm), eluting with a gradient of H₂O/formic acid (0.1%) to ACN/formic acid (0.1%) over 10 min, with a flow rate of 1 mL/min. Semi-preparative RP-HPLC was performed with an Agilent Zorbax Eclipse® 5μm XDB-C18 column (250 × 10 mm, 5 μm), eluting with a gradient of H₂O/formic acid (0.1%) to ACN/formic acid (0.1%) over 25 min, with a flow rate of 2 mL/min.

Fluorescent kinetic assays were monitored on a BioTek SynergyHT plate reader. Confocal images were obtained on a Leica SP5 confocal microscope and ImageJ was used for analysis.

2.2 Solid phase synthesis of NHC-Pd catalysts 5–12

Detailed protocol for the synthesis of catalyst **5** with a 6-aminohexanoic acid spacer.

Synthesis of Fmoc-Ahx-OH loaded 2-chlorotrityl-linker PS-resin **1**



426 mg of the 2-chlorotrityl chloride linker on polystyrene resin (loading 1 mmol/g according to the supplier, 100–200 mesh) was swollen in anhyd. DCM (5 mL) and subsequently re-activated with SOCl_2 (2.5 eq.) in anhyd. DCM (3.5 mL) under N_2 atm for 1 h. The resin was drained and washed with anhyd. DMF (3×5 mL) and anhyd. DCM (3×5 mL). Fmoc-Ahx-OH (451 mg, 3 eq., 0.4 M) in 3 mL of anhyd. DCM/DMF (9:1) was added to the resin, followed by DIPEA (444 μL , 6 eq.), and the reaction was stirred for 1 h. The resin was washed with anhyd. DMF (3×5 mL) and anhyd. DCM (3×5 mL). The resin was capped with a mixture of DCM/MeOH/DIPEA (8:1.5:0.5) for 2×15 min. The Fmoc deprotection was carried out using 20% piperidine in DMF (2×10 min) and the resin washed thoroughly with DMF and DCM.

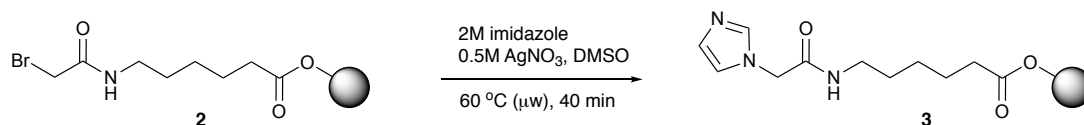
Coupling to resin **1**



Resin **1** (426 mg) was loaded into a 10 mL SP wave reaction cartridge. 1 M DIC (469 μL , 3 mmol) was added to 2M bromoacetic acid (886 mg, 6.5 mmol) in anhyd. DMF (3 mL) and, after mixing, the solution was added to the resin. The reaction was heated at 60 °C (μw) for 20 min. The resin was washed thoroughly with DMF (5×2 mL) and DCM (5×2 mL). For compound characterisation, a small portion of the resin was treated with 30% HFIP in DCM for 45 min. The liquid was collected and the resin was rinsed with a small portion of the cleavage solution and the solvent evaporated to dryness *in vacuo*.

^1H NMR (500 MHz, Methanol- d_4) δ 3.81 (s, 2H), 3.21 (t, $J = 7.0$ Hz, 2H), 2.30 (t, $J = 7.4$ Hz, 2H), 1.59 (m, 4H), 1.43–1.33 (m, 2H). ^{13}C NMR (126 MHz, Methanol- d_4) δ 176.1, 168.0, 39.3, 33.4, 28.4, 27.4, 26.0, 24.3.

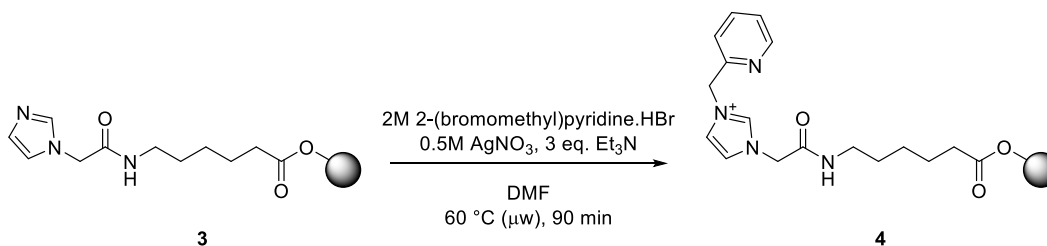
Alkylation of resin 2



Resin **2** (400 mg) was loaded into a 10 mL SP wave reaction cartridge. AgNO₃ (253 mg, 1.5 mmol) in DMSO (1 mL) was added to a 2 M solution of imidazole (408 mg, 6 mmol) in DMSO (2 mL). The solution was added to the resin and the reaction was heated at 60 °C (μw) for 40 min. The resin was washed with DMF (5 × 5 mL) and DCM (5 × 5 mL). For compound characterisation, a small portion of the resin was treated with 30% HFIP in DCM for 45 min. The liquid was collected and the resin was rinsed with a small portion of the cleavage solution and the solvents evaporated to dryness *in vacuo*.

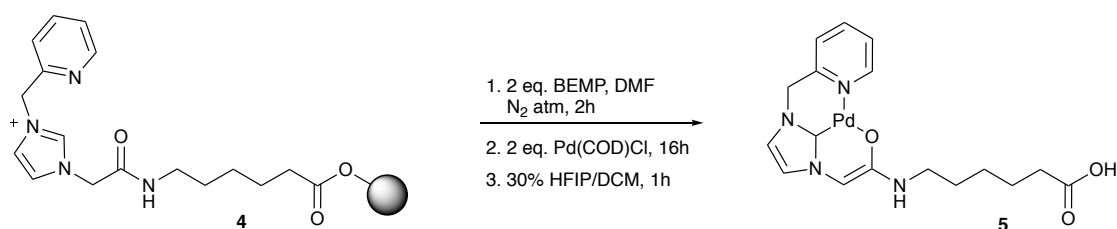
¹H NMR (500 MHz, Methanol-*d*₄) δ 7.72 (s, 1H), 7.13 (s, 1H), 7.01 (s, 1H), 4.73 (s, 2H), 3.28 – 3.20 (m, 2H), 2.32 – 2.23 (m, 2H), 1.68 – 1.49 (m, 4H), 1.43 – 1.34 (m, 2H). ¹³C NMR (126 MHz, Methanol-*d*₄) δ 169.1, 166.5, 139.4, 128.7, 124.6, 121.8, 52.0, 40.4, 29.9, 27.5, 25.9.

Alkylation of resin 3



Resin **3** (380 mg) was loaded into a 10 mL SP wave cartridge. To 2-(bromomethyl) pyridine hydrobromide salt (750 mg, 3 mmol) and Et₃N (550 μL, 4 mmol) in anhyd. DMF (2.25 mL), AgNO₃ (253 mg, 1.5 mmol) in anhyd. DMF (0.75 mL) was added, and the solution was mixed thoroughly and gently heated until homogeneous solution was obtained. This solution was added to the resin and heated at 60 °C (μw) for 90 min. The resin was washed with 20% piperidine in DMF (3 × 5 mL), DMF (5 × 5 mL) and DCM (5 × 5 mL), and dried. The resin was transferred to a clean SP wave cartridge ensuring that no black precipitate (silver oxide or silver nanoparticles) remained. For compound characterisation, a small portion of the resin was treated with 30% HFIP in DCM for 45 min. The liquid was collected and the resin was rinsed with a small portion of the cleavage solution and the solvents evaporated to dryness *in vacuo*. See section 2.3 for ligand characterisation.

Palladium loading on resin bound ligand **4** and cleavage off the resin



Dry resin **4** (100 mg) was placed under vacuum and flushed with N₂. Anhyd. DMF (200 μ L) was added to the resin, followed by 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BEMP) (2.5 equiv., 0.5 mmol), and the reaction mixture was stirred 45 min at room temperature under N₂ atm. Pd(COD)Cl₂ (1.5 equiv. 0.3 mmol) in DMF (100 μ L) was added and the reaction was stirred for 16 h. The resin was washed with DMF (5 \times 3 mL) and DCM (5 \times 3 mL). The catalyst was cleaved from the resin with 5% TFA in DCM for 30 min. The filtrate was collected, evaporated under a flow of N₂ and purified by semi-preparative HPLC.

Synthesis of the catalyst library

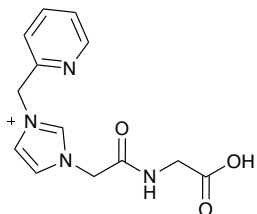
The reactions were done on a \sim 0.25 mmol scale (\sim 250 mg resin). For the synthesis of catalysts **6–12**, the appropriate *N*-Fmoc protected amino acids/spacers were loaded onto the 2-chlorotrityl chloride linker and the ligand built as described above for catalyst **5**. For catalyst **12** with the Phe-Trp-Ahx sequence, the amino acid couplings were carried out as follows. Fmoc-protected amino acid (3 eq., 0.25 M) in DMF was stirred at rt with DIC (3 eq. 0.25 M) for 5 min, followed by addition of Oxyma (3 eq. 0.25 M) and stirred for additional 10 min. The activated Fmoc-amino acid was added to the resin and coupling reactions were carried out at 60 $^{\circ}$ C for 20 min under μ w irradiation. The resin was washed with DMF (3 \times 5 mL), DCM (3 \times 5 mL) and MeOH (3 \times 5 mL). Next, the pre-swollen resin (in DCM) was treated with 20% piperidine in DMF (2 \times 10 min). The resin washed thoroughly with DMF (3 \times 5 mL) and DCM (3 \times 5 mL).

2.3 Characterisation of the ligands for catalysts **5–12**

For compound characterisation, a small sample of the ligand was cleaved off the resin with 30% HFIP prior to palladium loading. The analytical samples were purified by semi-preparative HPLC.

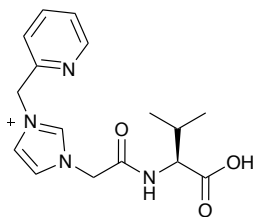
2H), 2.23 (t, $J = 7.4$ Hz, 2H), 1.65 – 1.57 (m, 2H), 1.57 – 1.50 (m, 2H), 1.40 – 1.30 (m, 6H). ^{13}C NMR (126 MHz, Methanol- d_4) δ 179.9, 169.8, 154.3, 151.1, 139.2, 125.3, 125.1, 124.1, 123.9, 54.9, 52.0, 40.8, 36.7, 30.2, 30.1, 29.9, 27.6, 26.6. HRMS (ESI) calculated 359.2078 for $\text{C}_{19}\text{H}_{27}\text{N}_4\text{O}_3$; found 359.2078; HPLC t_{R} 3.5 min (254 nm).

Ligand for catalyst 8



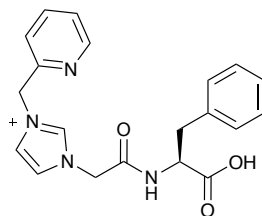
^1H NMR (600 MHz, Methanol- d_4) δ 9.19 – 9.13 (m, 1H), 8.58 (ddd, $J = 4.9, 1.8, 0.9$ Hz, 1H), 7.89 (td, $J = 7.7, 1.8$ Hz, 1H), 7.69 (t, $J = 1.9$ Hz, 1H), 7.64 (t, $J = 1.8$ Hz, 1H), 7.51 (d, $J = 7.8$ Hz, 1H), 7.42 (ddd, $J = 7.7, 4.9, 1.1$ Hz, 1H), 5.57 (s, 2H), 5.10 (s, 2H), 3.94 (s, 2H). ^{13}C NMR (151 MHz, Methanol- d_4) δ 167.0, 154.2, 151.0, 139.6, 139.2, 125.3, 125.2, 124.1, 123.8, 54.9, 51.9, 42.6. HRMS (ESI) calculated 276.1217 for $\text{C}_{13}\text{H}_{15}\text{N}_4\text{O}_3$; found 276.1209; HPLC t_{R} 0.7 min (254 nm).

Ligand for catalyst 9



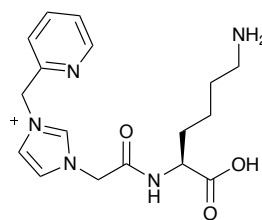
^1H NMR (500 MHz, Methanol- d_4) δ 8.57 (d, $J = 5.0$ Hz, 1H), 7.92 – 7.85 (m, 1H), 7.67 (d, $J = 1.6$ Hz, 1H), 7.63 (d, $J = 1.5$ Hz, 1H), 7.50 (d, $J = 7.7$ Hz, 1H), 7.44 – 7.38 (m, 1H), 5.56 (s, 2H), 5.15 – 5.04 (m, 2H), 4.25 (d, $J = 4.7$ Hz, 1H), 2.31 – 2.21 (m, 1H), 0.96 (dd, $J = 7.3, 1.2$ Hz, 6H). ^{13}C NMR (126 MHz, Methanol- d_4) δ 177.7, 169.2, 154.3, 151.0, 139.2, 125.3, 125.1, 124.1, 123.7, 61.7, 54.9, 52.1, 32.0, 20.1, 18.0. HRMS (ESI) calculated 318.1686 for $\text{C}_{16}\text{H}_{22}\text{N}_4\text{O}_3$; found 318.1686; HPLC t_{R} 2.7 min (254 nm).

Ligand for catalyst 10



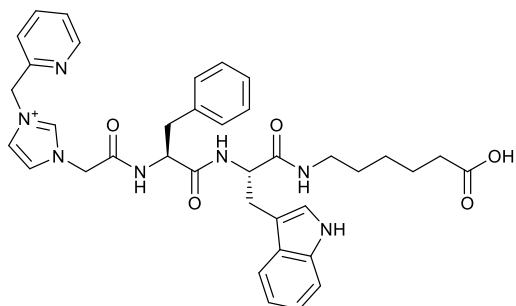
^1H NMR (500 MHz, Methanol- d_4) δ 8.60 – 8.53 (m, 1H), 7.88 (td, J = 7.7, 1.8 Hz, 1H), 7.63 (d, J = 2.0 Hz, 1H), 7.51 – 7.46 (m, 2H), 7.41 (dd, J = 7.6, 4.9 Hz, 1H), 7.28 – 7.21 (m, 4H), 7.20 – 7.12 (m, 1H), 5.54 (s, 2H), 4.99 (d, J = 16.2 Hz, 1H), 4.88 (d, J = 16.4 Hz, 1H), 4.55 (dd, J = 9.3, 4.3 Hz, 1H), 3.29 – 3.27 (m, 1H), 2.92 (dd, J = 14.0, 9.3 Hz, 1H). ^{13}C NMR (126 MHz, Methanol- d_4) δ 177.6, 169.1, 154.2, 151.0, 139.7, 139.2, 130.4, 129.2, 127.4, 125.3, 124.9, 124.1, 123.7, 57.8, 54.9, 52.1, 39.3. HRMS (ESI) calculated 366.1686 for $\text{C}_{20}\text{H}_{22}\text{N}_4\text{O}_3$; found 366.1669; HPLC t_{R} 3.6 min (254 nm).

Ligand for catalyst 11



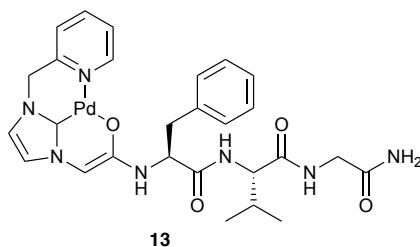
^1H NMR (500 MHz, Methanol- d_4) δ 9.18 – 9.15 (m, 1H), 8.60 – 8.55 (m, 1H), 7.89 (td, J = 7.7, 1.8 Hz, 1H), 7.70 (t, J = 1.8 Hz, 1H), 7.63 (t, J = 1.8 Hz, 1H), 7.53 – 7.50 (m, 1H), 7.43 – 7.40 (m, 1H), 5.58 (s, 2H), 5.12 (s, 2H), 4.46 – 4.39 (m, 1H), 2.93 (t, J = 7.7 Hz, 2H), 2.01 – 1.90 (m, 1H), 1.80 (dt, J = 13.5, 7.5 Hz, 1H), 1.74 – 1.65 (m, 2H), 1.58 – 1.47 (m, 2H). ^{13}C NMR (126 MHz, Methanol- d_4) δ 175.0, 166.8, 154.2, 151.0, 139.6, 139.2, 125.3, 125.1, 124.2, 123.9, 54.9, 54.0, 51.8, 40.4, 32.2, 28.0, 23.8. HRMS (ESI) calculated 346.1873 for $\text{C}_{17}\text{H}_{24}\text{N}_5\text{O}_3$; found 346.1858; HPLC 0.6 min (254 nm).

Ligand for catalyst 12



^1H NMR (500 MHz, Methanol- d_4) δ 8.56 – 8.54 (m, 1H), 7.86 (td, $J = 7.7, 1.8$ Hz, 1H), 7.65 (d, $J = 2.0$ Hz, 1H), 7.55 (d, $J = 7.9$ Hz, 1H), 7.49 (d, $J = 7.8$ Hz, 1H), 7.40 (d, $J = 2.0$ Hz, 1H), 7.40 – 7.37 (m, 1H), 7.35 – 7.32 (m, 1H), 7.28 – 7.16 (m, 5H), 7.11 – 7.05 (m, 2H), 7.04 – 6.99 (m, 1H), 5.54 (s, 2H), 4.92 (d, $J = 3.7$ Hz, 2H), 4.64 – 4.60 (m, 1H), 4.59 – 4.55 (m, 1H), 3.22 – 3.15 (m, 1H), 3.12 (dd, $J = 14.5, 6.2$ Hz, 2H), 3.08 – 2.93 (m, 2H), 2.88 (dd, $J = 14.0, 9.2$ Hz, 1H), 2.15 (t, $J = 7.4$ Hz, 2H), 1.49 (m, 2H), 1.30 – 1.21 (m, 2H), 1.10 (dt, $J = 10.3, 5.4$ Hz, 2H). ^{13}C NMR (126 MHz, Methanol- d_4) δ 180.5, 173.2, 172.7, 169.9, 154.1, 151.1, 139.2, 138.0, 138.0, 130.3, 129.6, 128.8, 127.9, 125.3, 124.9, 124.7, 124.1, 123.8, 122.5, 119.8, 119.4, 112.4, 110.7, 56.7, 55.9, 54.9, 40.3, 38.6, 37.1, 29.7, 29.1, 27.5, 26.5. HRMS (ESI) calculated 665.3310 for $\text{C}_{37}\text{H}_{43}\text{N}_7\text{O}_5$; found 665.3320; HPLC t_R 4.4 min (254 nm).

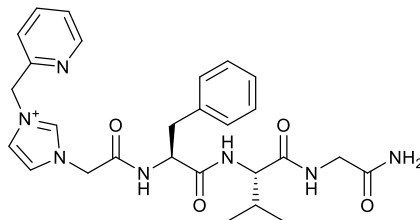
2.4 Catalyst synthesis on a Rink-amide functionalised resin



Catalyst **13** was synthesised on Rink-amide functionalised polystyrene resin to demonstrate the adaptability of the synthetic protocol. Fmoc-Rink Amide linker (3 eq. 0.75 M) and DIC (3 eq. 0.75 M) were stirred in DMF for 5 min, followed by addition of Oxyma (3 eq. 0.75 M) and stirring for an additional 10 min. This solution was added to 300 mg of preswollen aminomethyl polystyrene resin (loading 0.745 mmol/g, 200–400 mesh) and the mixture shaken at room temperature for 2 h. The resin was washed with DMF (3 \times 5 mL), DCM (3 \times 5 mL) and MeOH (3 \times 5 mL). Following this, the Fmoc group was deprotected with 20% piperidine in DMF (2 \times 10 min) and the resin was washed with DMF (3 \times 5 mL) and DCM (3 \times 5 mL). Subsequent amino acids were coupled (Fmoc-Val-OH and Fmoc-Phe-OH) and the NHC-palladium ligand synthesised on resin as described for **5**. For compound characterisation, a small portion of the resin was treated with 95:5 TFA/water for 90 min. The cleavage solution was collected by filtration

and evaporated to dryness *in vacuo* (see ligand analysis below). Pd was loaded onto the resin bound ligand and the catalyst cleaved from the resin using 95:5 TFA/water for 2 h. HRMS ESI-MS calculated 624.1545 for C₂₇H₃₂N₇O₄Pd; found 624.1556; HPLC; *t*_R 3.5 min (254 nm).

Ligand for catalyst 13



¹H NMR (500 MHz, Methanol-*d*₄) δ 9.10 (t, *J* = 1.6 Hz, 1H), 8.61 (ddd, *J* = 4.9, 1.8, 1.0 Hz, 1H), 8.27 (d, *J* = 7.7 Hz, 1H), 7.93 (td, *J* = 7.7, 1.8 Hz, 1H), 7.70 (t, *J* = 1.8 Hz, 1H), 7.53 (t, *J* = 1.8 Hz, 1H), 7.46 (ddd, *J* = 7.7, 4.9, 1.1 Hz, 1H), 7.34–7.30 (m, 4H), 7.27 (dd, *J* = 4.8, 3.8 Hz, 1H), 5.59 (s, 2H), 5.08 (d, *J* = 16.5 Hz, 1H), 5.01 (d, *J* = 16.6 Hz, 1H), 4.77 (dd, *J* = 9.4, 5.4 Hz, 1H), 4.20 (d, *J* = 2.4 Hz, 1H), 3.95 (dd, *J* = 16.9, 6.2 Hz, 1H), 3.79 (dd, *J* = 16.9, 5.5 Hz, 1H), 3.24 (dd, *J* = 14.0, 5.4 Hz, 1H), 2.96 (dd, *J* = 14.0, 9.4 Hz, 1H), 2.15–2.10 (m, 1H), 1.00 (d, *J* = 1.8 Hz, 3H), 0.98 (d, *J* = 1.8 Hz, 3H). ¹³C NMR (126 MHz, Methanol-*d*₄) δ 210.0, 173.9, 173.8, 166.7, 154.2, 151.0, 139.4, 139.2, 138.1, 130.3, 129.57, 127.9, 125.3, 125.1, 124.2, 123.9, 60.9, 56.5, 55.0, 51.8, 43.1, 38.9, 31.7, 19.7, 18.8. HRMS (ESI) calculated 520.2667 for C₂₇H₃₄N₇O₄; found 520.2673; HPLC; *t*_R 3.3 min (254 nm).

2.5 Catalyst screening

Stock solutions (20 μM) of the probe **DCF-1²** were prepared in 5% acetonitrile either in PBS or in MCF-7 cell lysate. The catalyst solutions were freshly prepared either in PBS or in MCF-7 lysate. The screenings were carried out on black 96-well plates. 50 μL of the probe **DCF-1** (to give a working concentration of 10 μM) was added per well, followed by 50 μL of the catalyst solution (0.8 mol%) and the increase in fluorescence ($\lambda_{\text{Ex/Em}}$ 485/520 nm) at 37 °C recorded over time (4 h or 12 h) on a plate reader (*n* = 3). The relative increase in fluorescence was compared with the blank and the control experiment (PBS or cell lysate).

2.6 Stability of Catalysts

Catalysts **8**, **9** and **12** (~ 1 mg/mL) were stored in H₂O/ACN (9:1) at 4 °C for 2 months. Following this, the samples were analysed by analytical HPLC.

2.7 Prodrug activation in MCF-7 cells

MCF-7 cells (from the European Collection of Authenticated Cell Cultures (ECACC)) were grown in Dulbecco's modified eagle medium (DMEM) supplemented with 10% FBS, 1% penicillin/streptomycin and 200 nM L-glutamine in a humidified incubator at 37 °C with 5% CO₂. Cells were cultured in T-75 flasks (Corning) to ≥ 80% confluence. Cells were harvested with trypsin/EDTA (Gibco.)

The MCF-7 cells (8×10^3 cells/well) were seeded on 96-well plates and grown to ~60% confluence overnight. Control cells were treated with 100 μM of **5-FU** (with 1% DMSO), 100 μM **Pro-5-FU** (with 1% DMSO), and 10 μM and 50 μM of catalyst **8**. The cells for prodrug activation were treated first with 100 μM of **Pro-5-FU** for 1 day and followed by addition of 10 mol% (Pd) of catalyst **8**, and the cells were incubated for 4 days under standard cell culture conditions. The cells were washed with PBS, followed by the addition of the MTT reagent and incubated for 3 h. The formazan crystals formed were dissolved in MTT dissolving solution (isopropanol and Triton-X 100 (9:1) acidified with conc. HCl) and the absorbance measured on a plate reader at 570 nm. The results were compared with untreated cells and expressed as percentage of cell viability.

2.8 Prodrug activation in MCF-7 spheroids

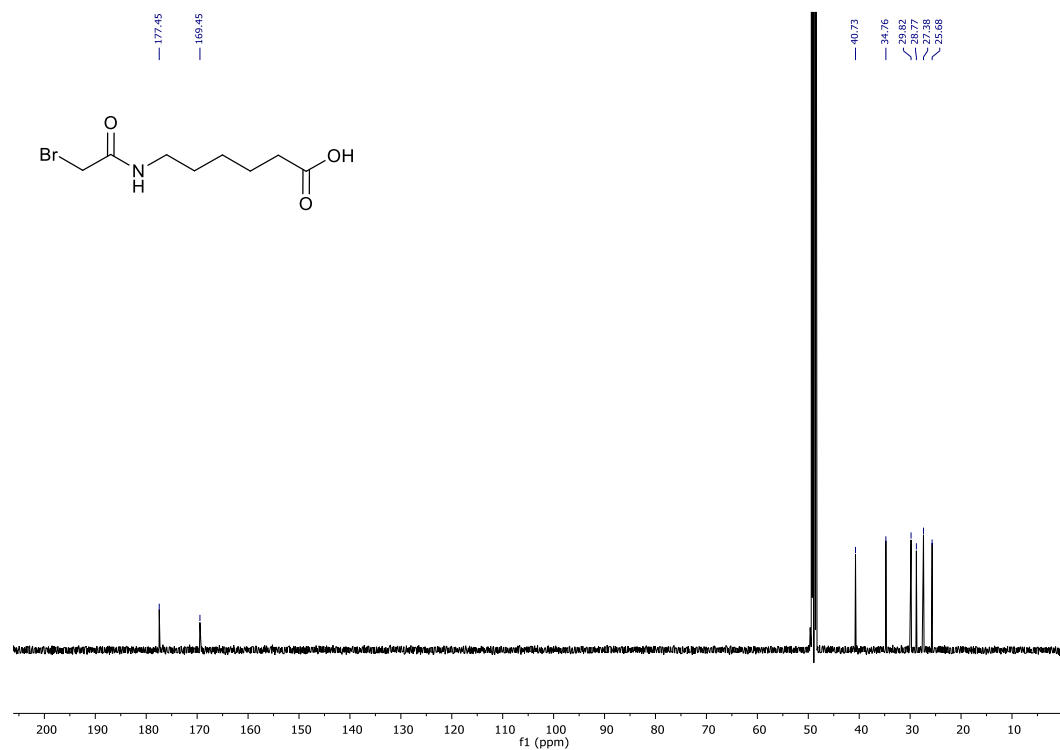
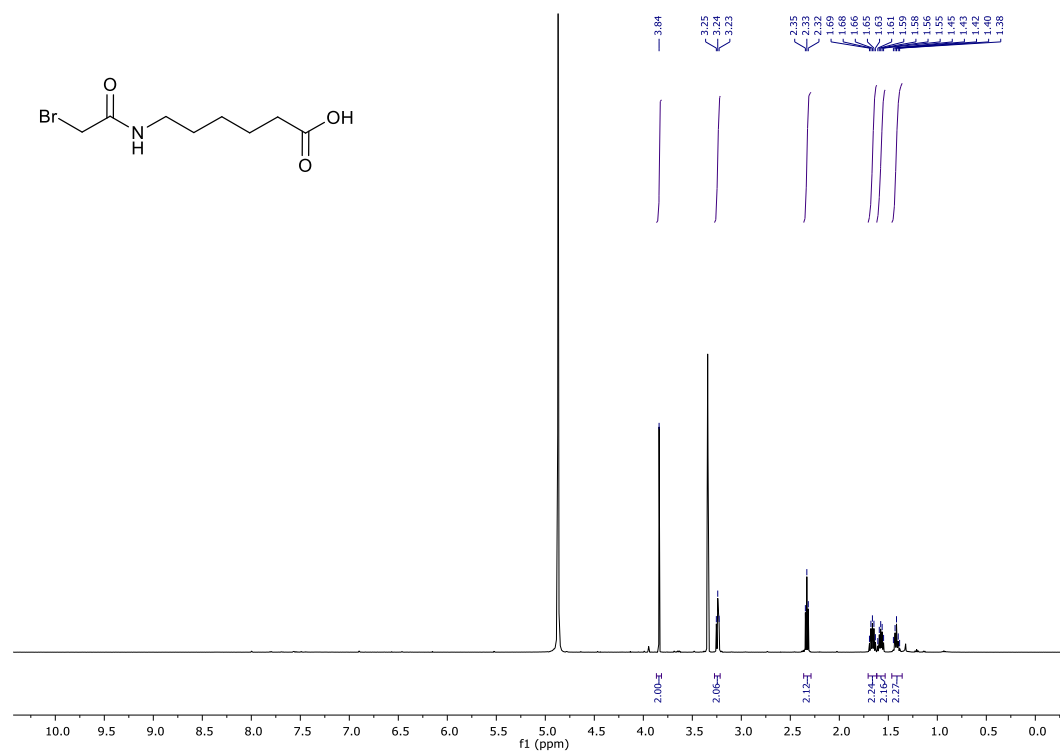
MCF-7 cells (ECACC) were added into black 96-well Corning Ultra low attachment surface coated microplates (~2000 cells/well) to generate the multicellular spheroids. The media was changed daily until spheroids formed (approximately 750 μm diameter). The spheroids were treated with 100 μM of **Pro-5-FU** and 10 mol% of the catalyst **8** on the well plate, and incubated for 4 days. The control spheroids were treated with catalyst **8** (10 mol%), or **5-FU** (100 μM) or **Pro-5-FU** (100 μM). The spheroids were washed thoroughly with PBS and stained for cell viability with the LIVE/DEAD™ Cell Imaging Kit (488/570) according to manufacturer's instructions, and imaged with a confocal microscope.

3. References

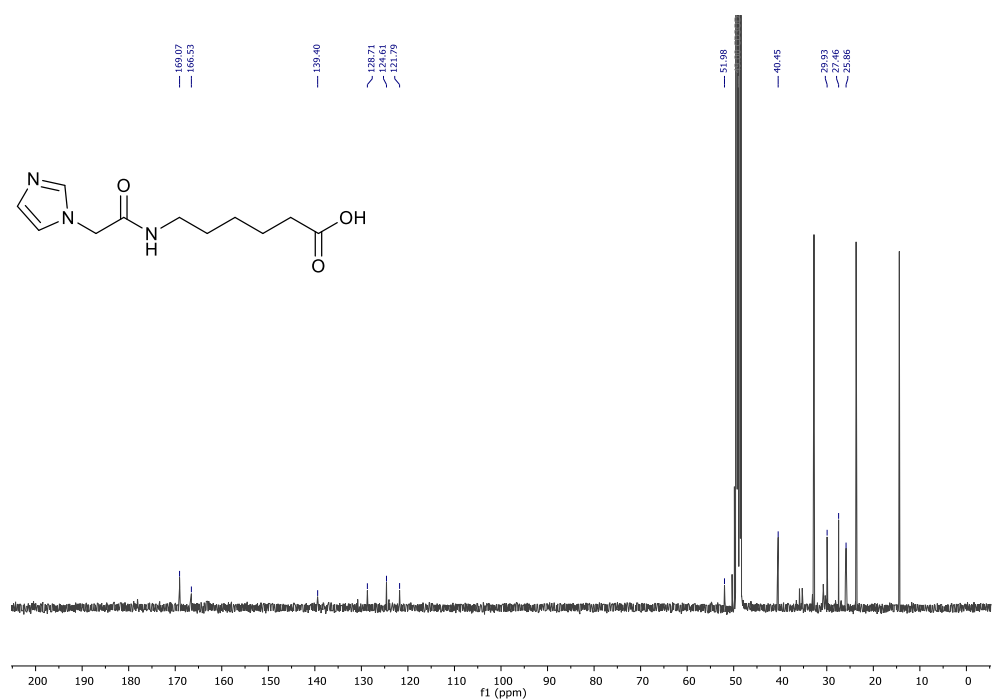
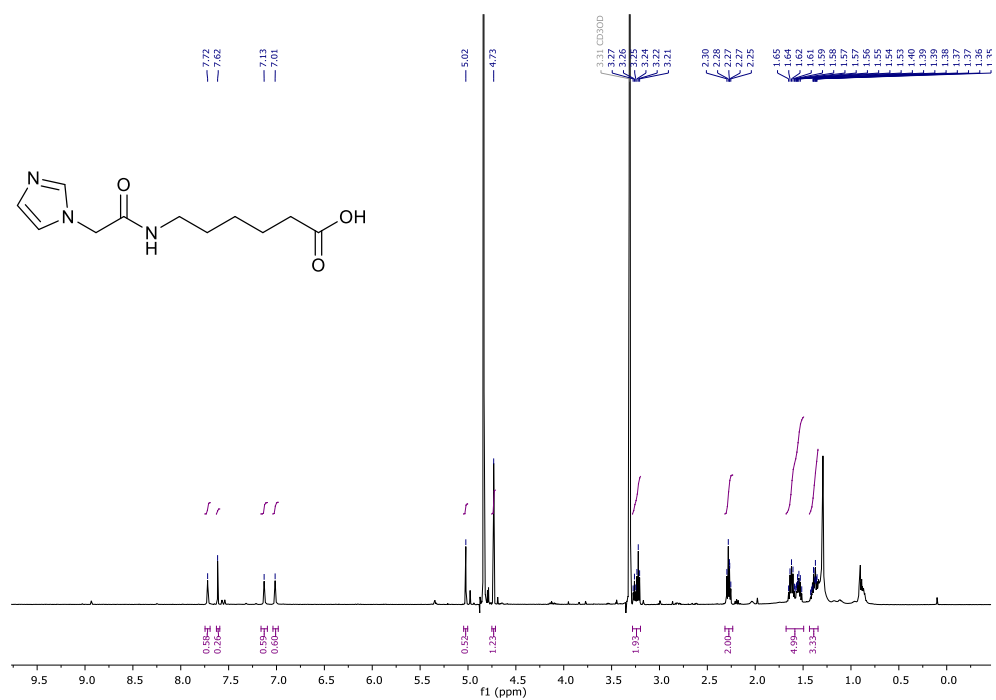
1. J. T. Weiss, J. C. Dawson, K. G. Macleod, W. Rybski, C. Fraser, C. Torres-Sanchez, E. E. Patton, M. Bradley, N. O. Carragher and A. Unciti-Broceta, *Nat Commun*, **2014**, 5, 3277.
2. M. Santra, S. K. Ko, I. Shin and K. H. Ahn, *Chem Commun*, **2010**, 46, 3964-3966.
3. F. Song, A. L. Garner and K. Koide, *J Am Chem Soc*, **2007**, 129, 12354-12355.

4. NMR Spectra

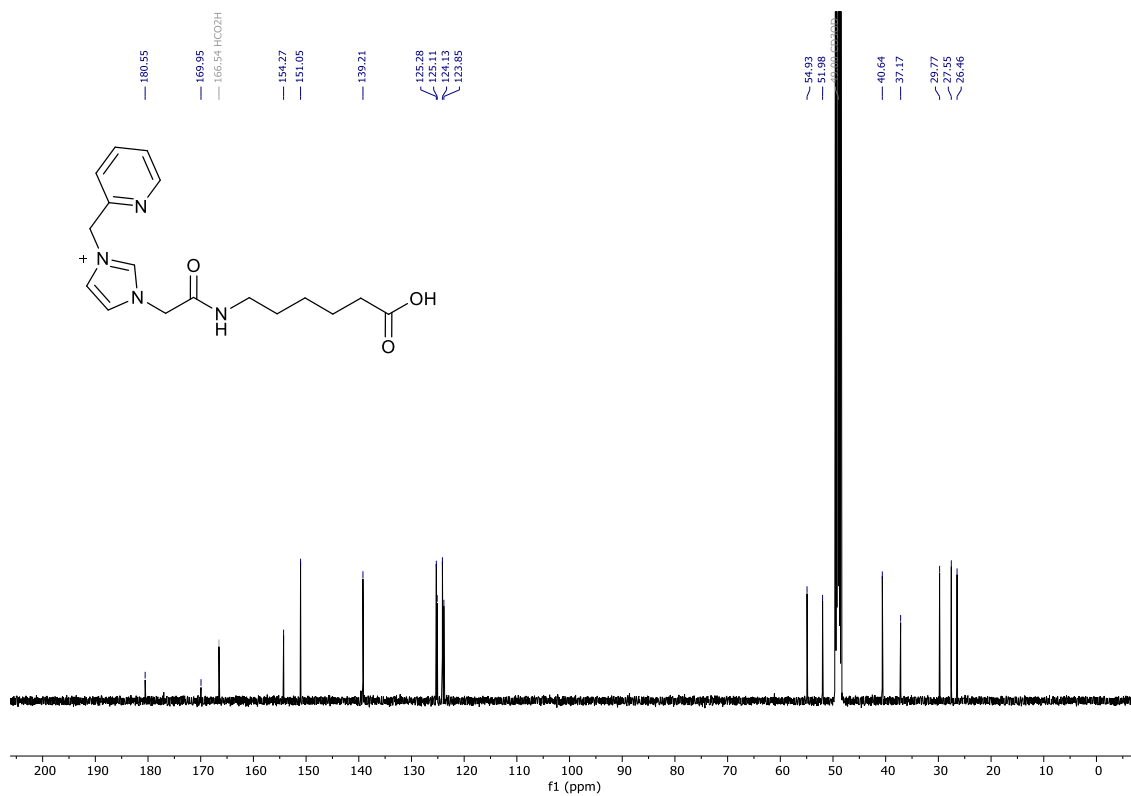
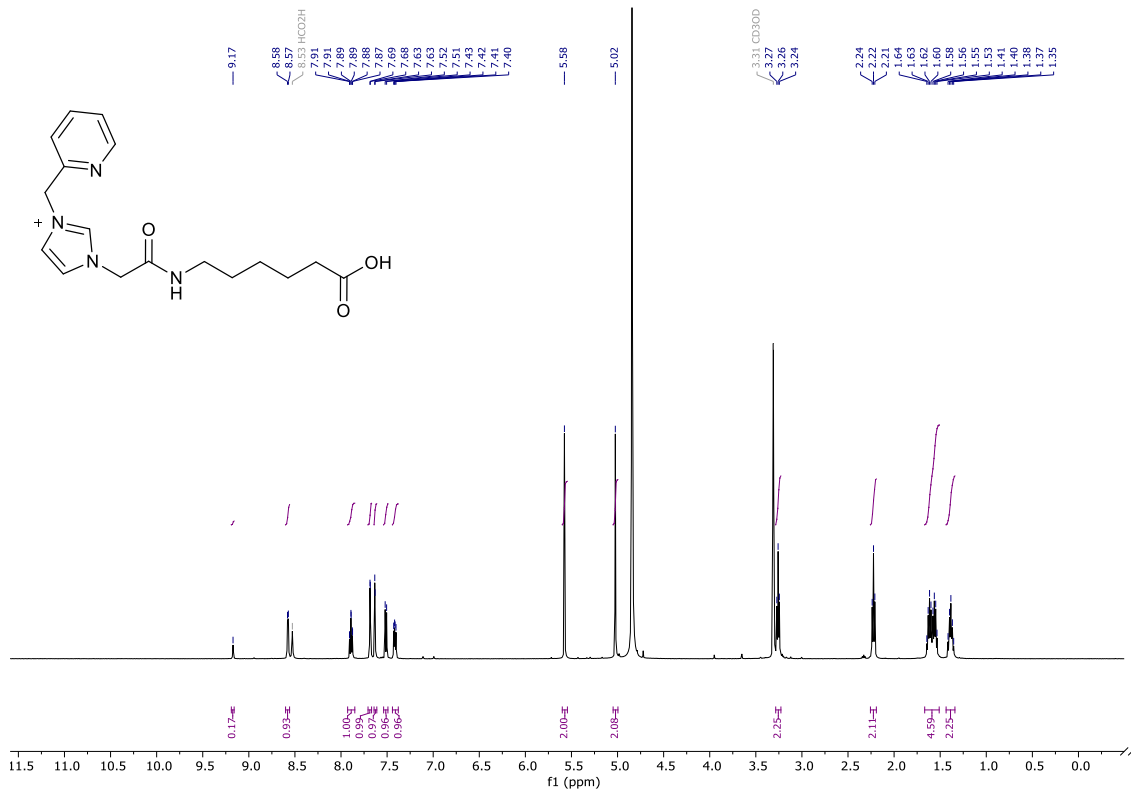
^1H NMR and ^{13}C NMR spectra of crude **2** in CD_3OD (isolated from the resin).



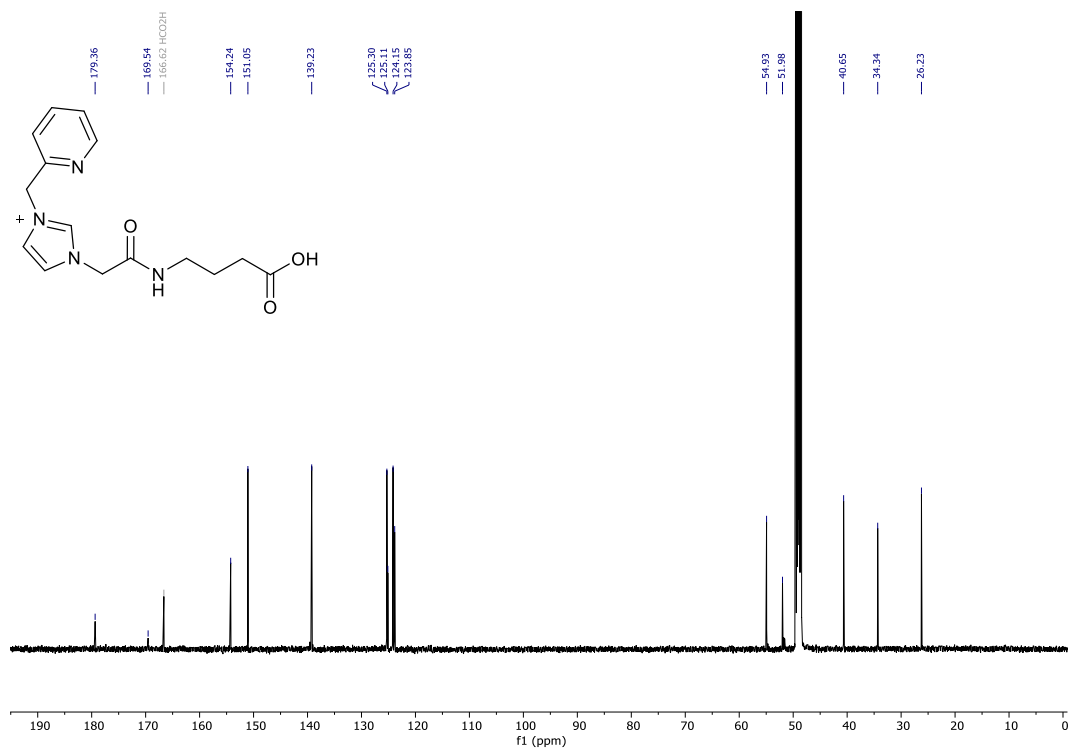
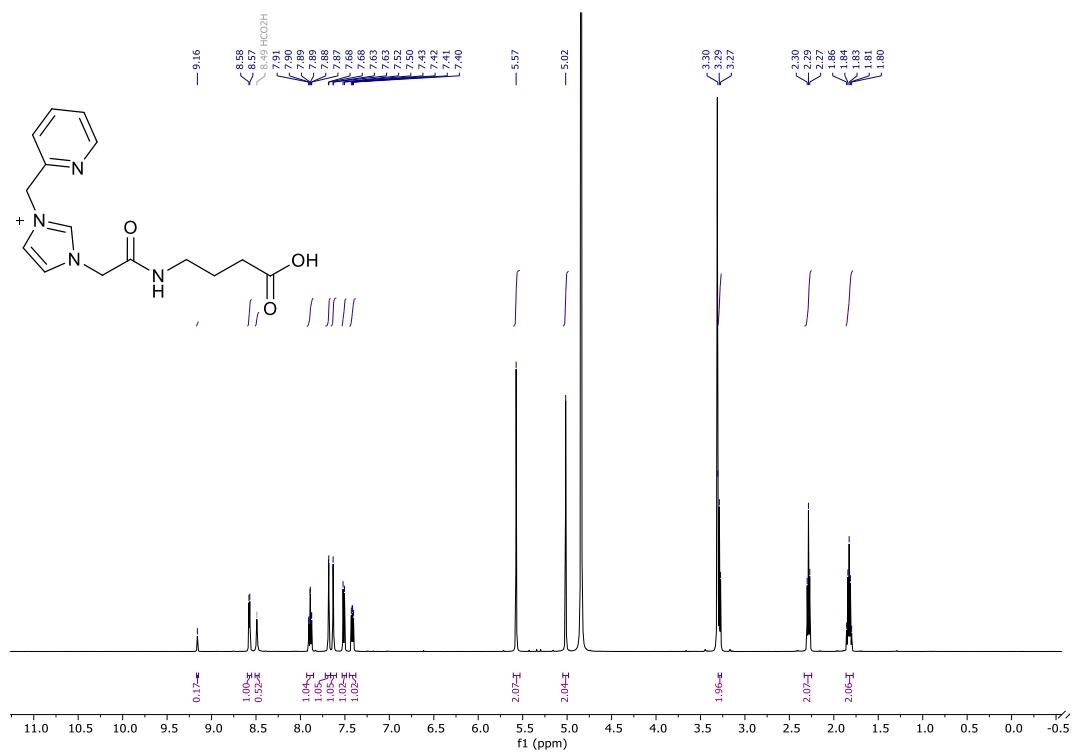
^1H NMR and ^{13}C NMR of crude **3** (entry 5 in Table S1) in CD_3OD (isolated from the resin).



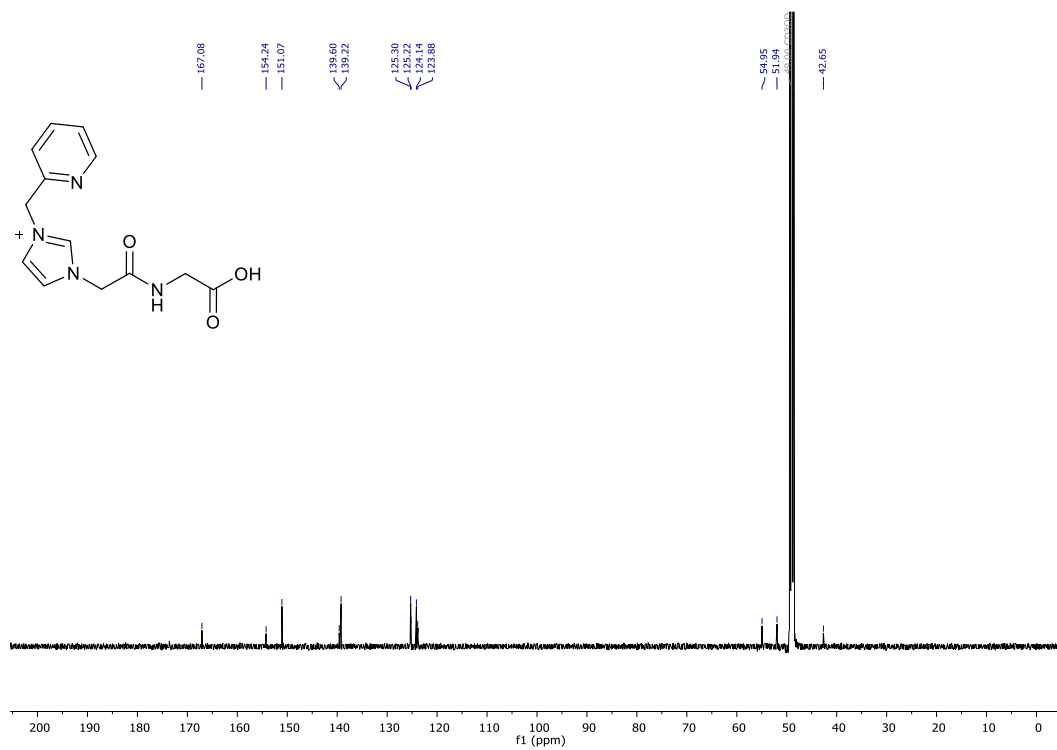
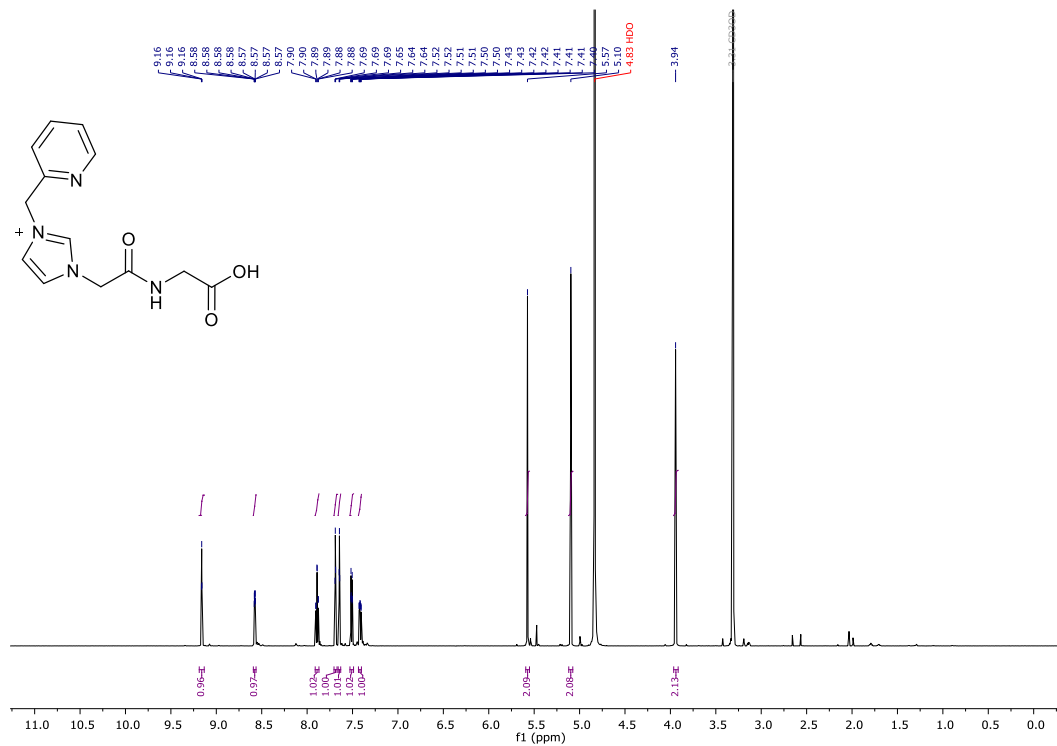
^1H and ^{13}C NMR spectra of purified ligand **4** (as the formate salt) in CD_3OD .



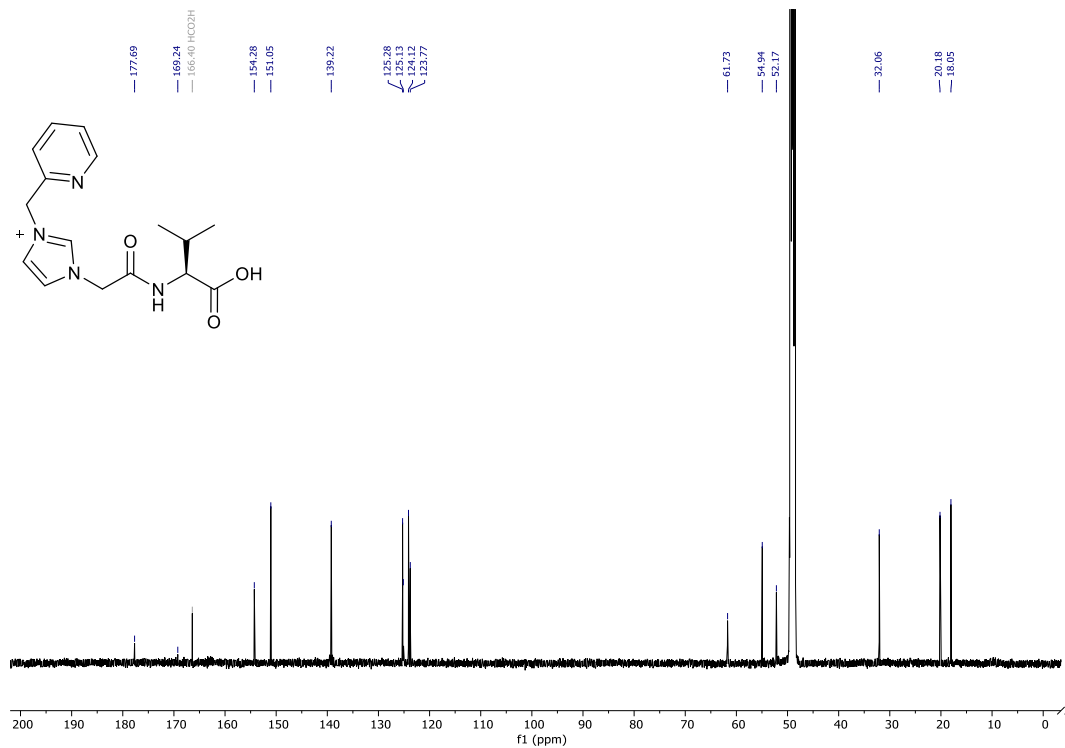
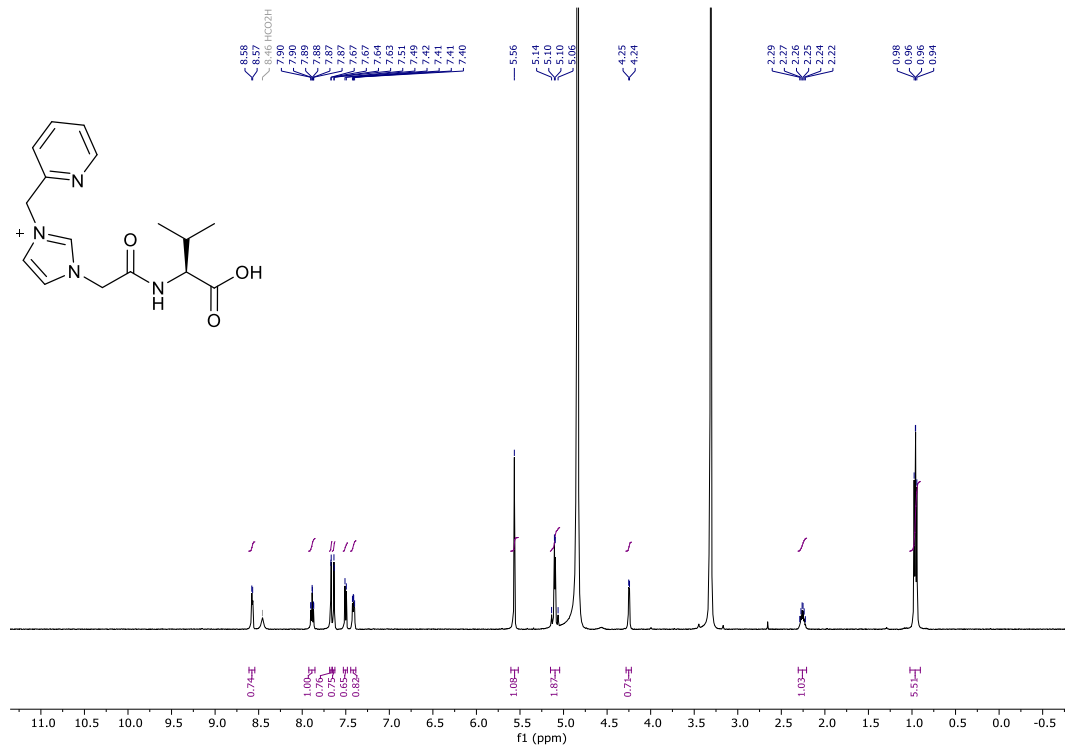
^1H and ^{13}C NMR spectra of purified ligand for catalyst **6** (as the formate salt) in CD_3OD .



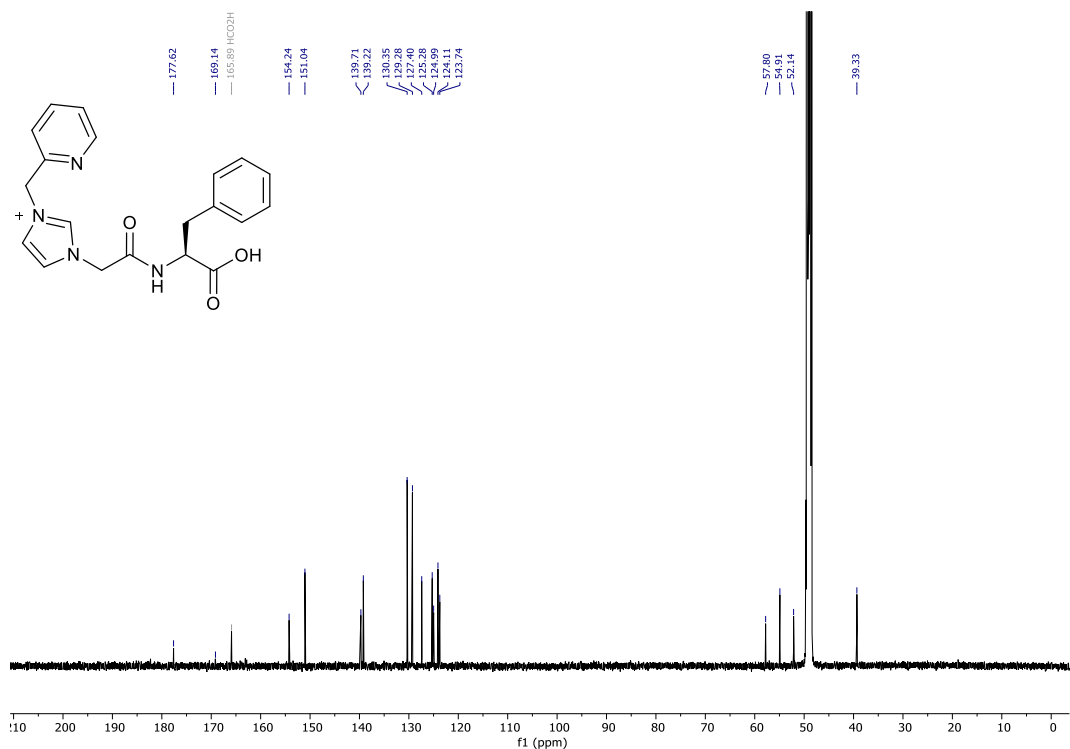
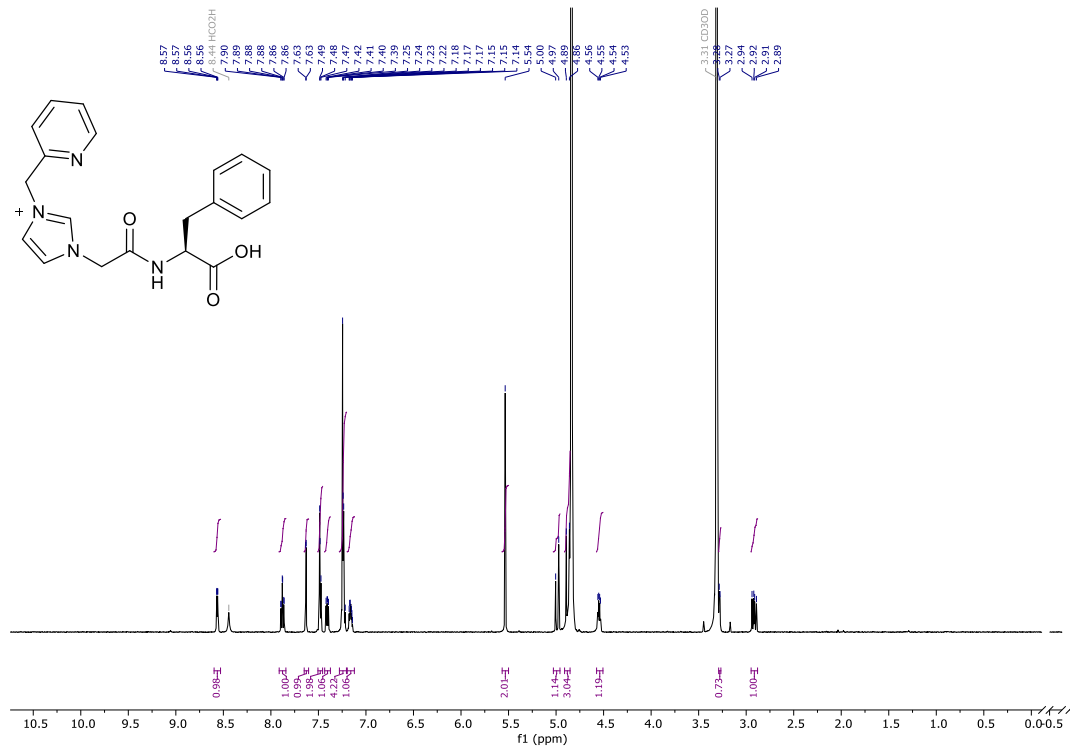
^1H and ^{13}C NMR spectra of purified ligand for catalyst **8** in CD_3OD .



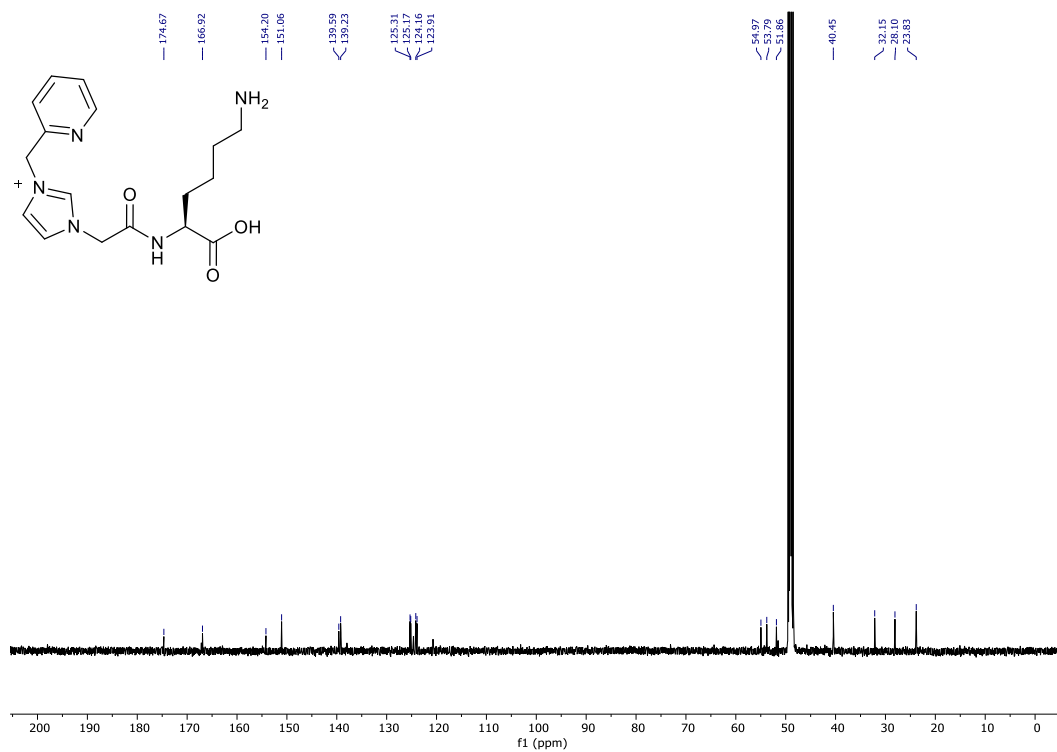
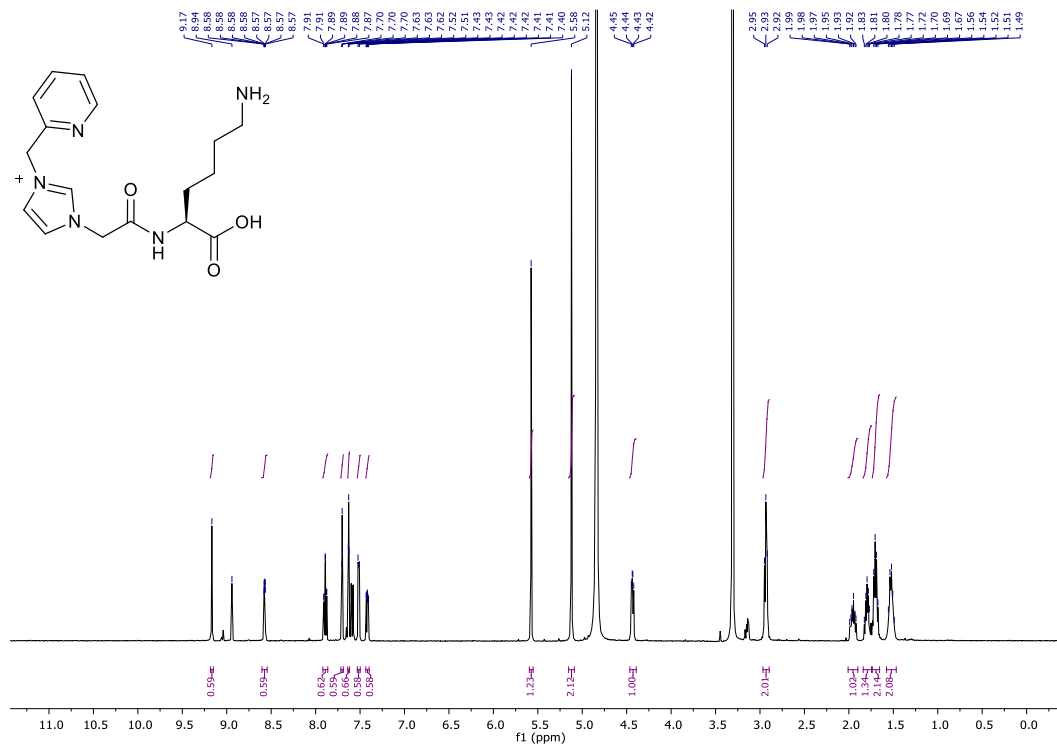
^1H and ^{13}C NMR spectra of purified ligand for catalyst **9** (as the formate salt) in CD_3OD .



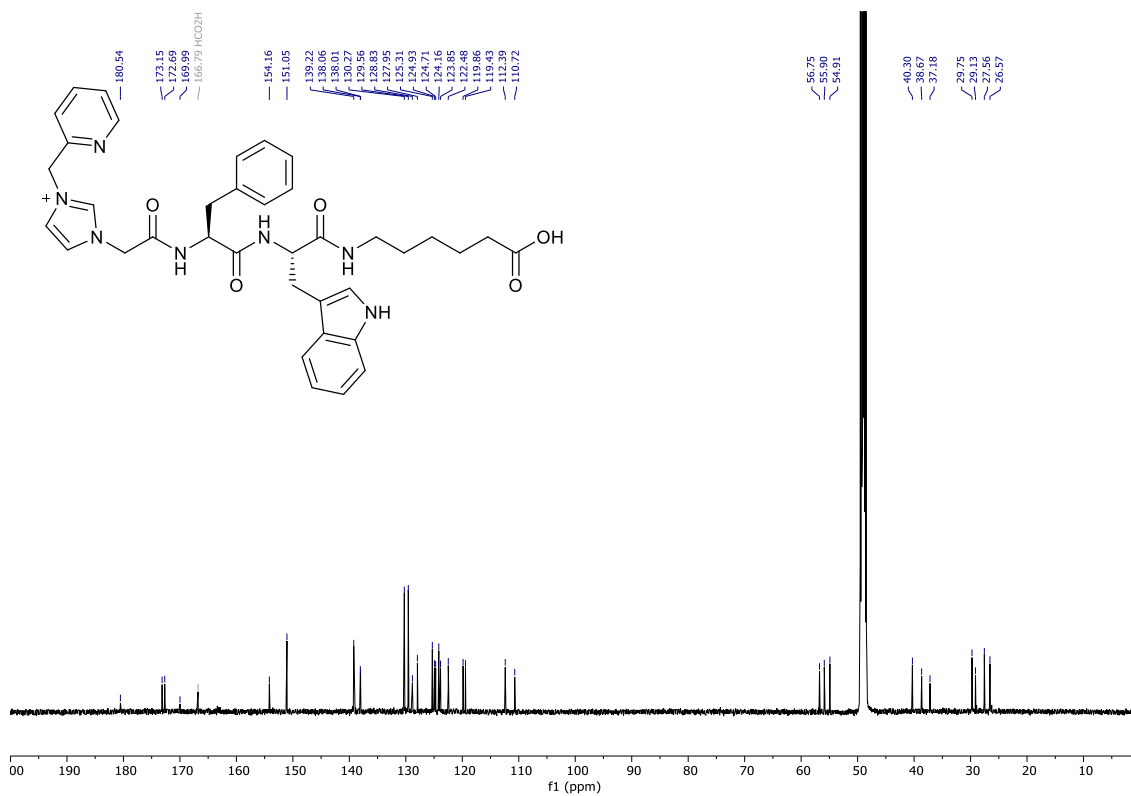
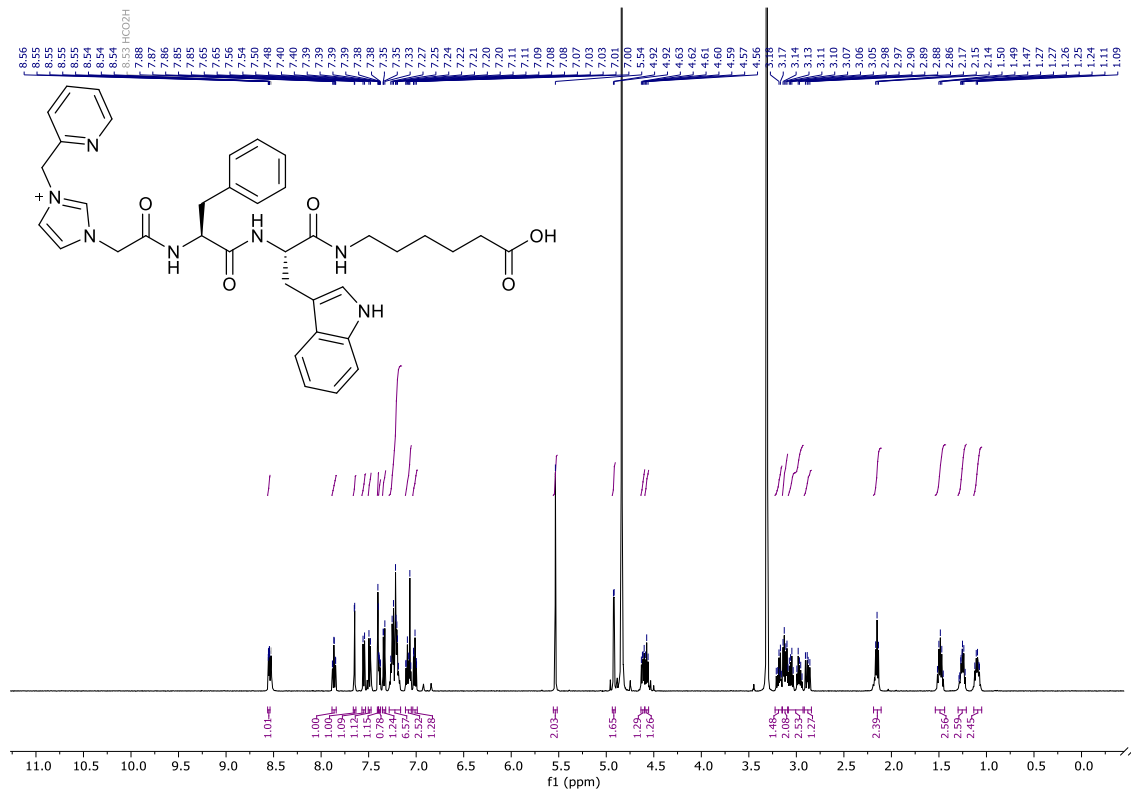
¹H and ¹³C NMR spectra of purified ligand for catalyst **10** (as the formate salt) in CD₃OD.



^1H and ^{13}C NMR spectra of ligand for catalyst **11** in CD_3OD .

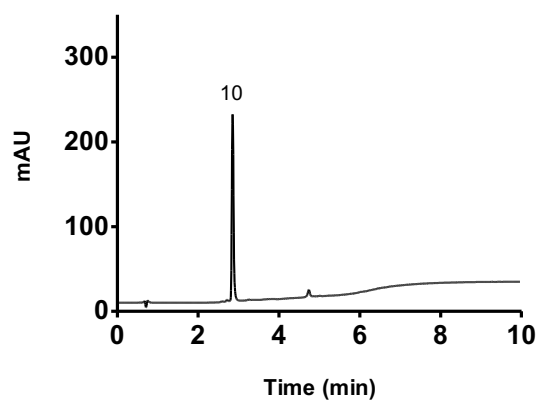
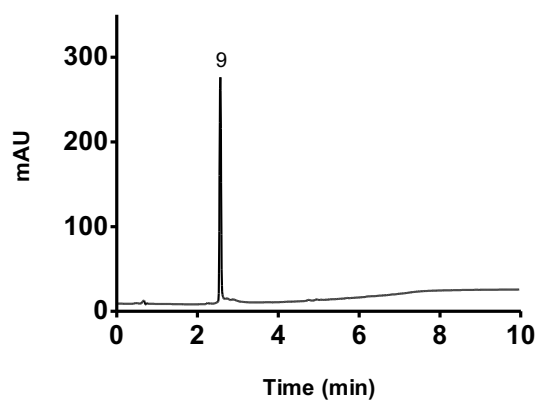
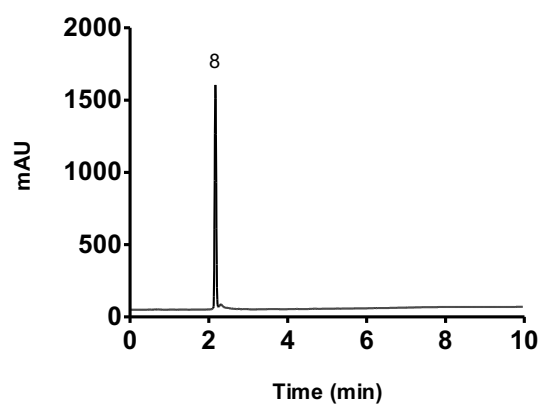
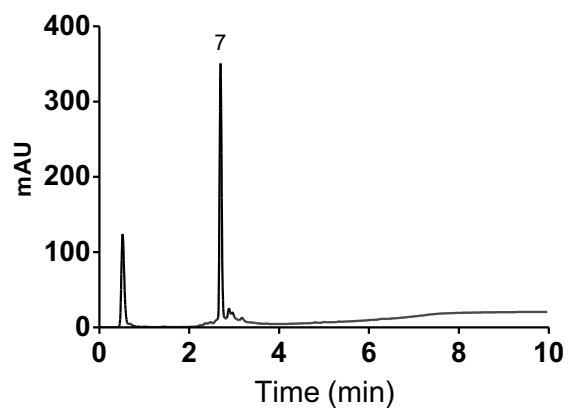
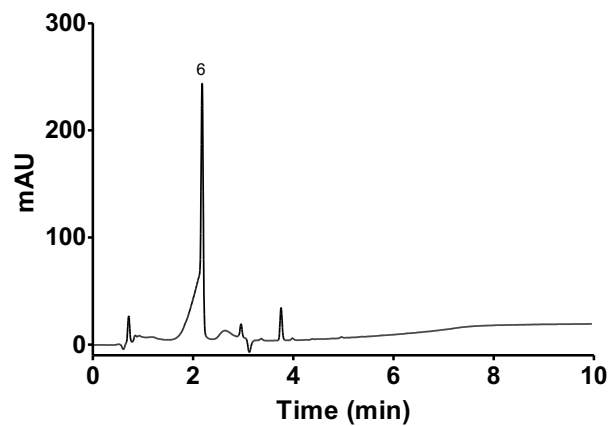
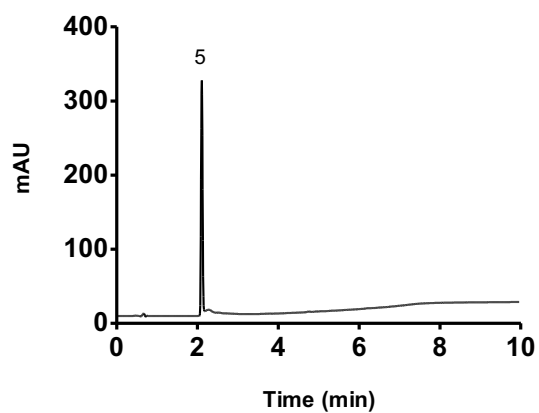


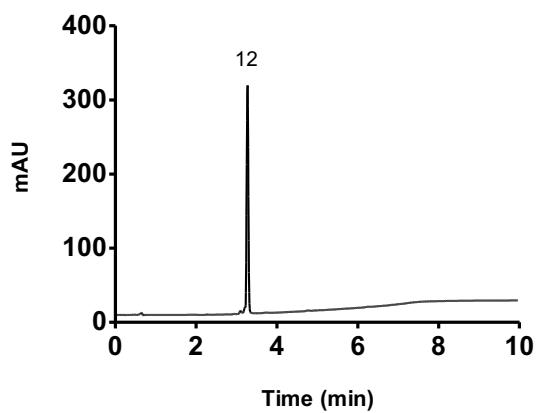
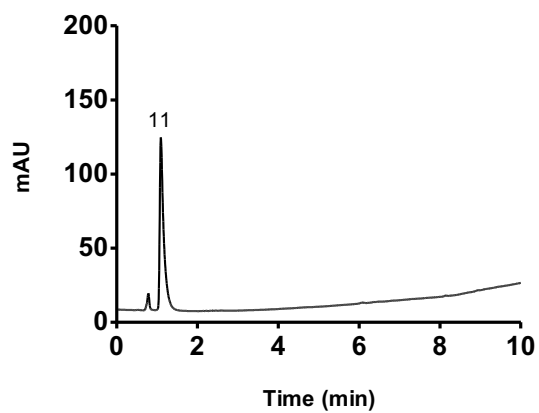
^1H and ^{13}C NMR spectra of purified ligand for catalyst **12** (as the formate salt) in CD_3OD .



5. HPLC traces of the catalysts

HPLC traces for the Pd catalysts 5–12 at 254 nm.





The following HPLC traces were used to confirm the stability of Pd catalysts **8**, **9**, **12** at 254 nm. Catalysts **8**, **9** and **12** were stored at 4 °C for 1 month in a solution of H₂O/ACN and no degradation was observed.

