Zinc-Dysprosium functionalized amyloid fibrils

Stavroula Sampani,[a] Youssra K. Al-Hilaly,[a,b] Sharali Malik,[c] Louise Serpell,[a*] and George E. Kostakis[a*]

[a]*School of Life Sciences, University of Sussex, Falmer, BN1 9QG, United Kingdom E-mail: L.c.serpell@sussex.ac.uk, G.Kostakis@sussex.ac.uk,*
[b]*Chemistry Department, College of Science, Mustansiriyah University, Baghdad, Iraq*
[c]*Institute of Nanotechnology, Karlsruhe Institute of Technology (KIT), Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany*

Experimental Procedures

Synthesis of the Coordination Cluster

The tetranuclear coordination cluster $[Zn^{II}_2Dy^{III}_2L_4(NO_3)_2(DMF)_2]$ (**1**) was synthesized following previous procedure.[1]

Peptide stock and working peptide solutions

The lyophilized peptide Acetyl-HYFNIF-NH_2 (1mg) (purchased from JPT peptide technologies, Germany), was incubated for one week in 0.5 ml Milli-Q H_2O to produce fibrils at a 2.38 mM stock solution **I**. Working peptide solutions $\mathbf{A^I}$-$\mathbf{D^I}$ with metal sources ($Zn(NO_3)_2.6H_2O$ and/or $Dy(NO_3)_3.5H_2O$ or Coordination Cluster) were freshly prepared by mixing the stock solution (100 µL) with 0.12 mM solutions of metal sources in methanol (90 µL) and incubated for 24 hours. Similarly, working peptide solutions $\mathbf{E^I}$ was prepared by mixing peptide stock solution (100 µL) with methanol (90 µL) and incubated for 24 hours (Table S1).

Transmission electron microscopy (TEM)

The working peptide solution (1.26 mM, 4 µL) was incubated for 1 min on a grid (400 mesh copper grid with Carbon/Formvar film from Agar Scientific), blotted and then washed with 4 µl of 0.22 µM filtered milli-Q water. Uranyl acetate (4 µL of 2% w/v) was placed on the grid once for one minute and then blotted and the grid was allowed to air-dry. TEM projection images were collected using a JEOL JEM1400-Plus Transmission Electron Microscope operated at 120 kV equipped with a Gatan OneView camera (4kx4k). Images were recorded at 25 fps with drift correction using GMS3.

UV-Vis and Circular dichroism (CD)

UV-Vis spectra were collected for samples $\mathbf{A^I}$ - $\mathbf{E^I}$. The peptide solution in H_2O-MeOH (1.26 mM), with and without metal sources, was monitored after one day incubation at 25 °C, using a Jasco J-715 spectropolarimeter with a Peltier temperature control system at 21 °C. Peptide samples (30 µl) were placed into 0.1-mm path length quartz cuvettes (Hellma), and scanned from 180-320 nm. The parameters were set as the following: a pitch of 0.1 nm, a scan speed 50 nm min^{-1}, response time 4 s, slit widths 1 nm and with standard sensitivity. Each set of data was collected in triplicate. Spectrum of the blanks, H_2O-MeOH with and without metal sources was subtracted from the readings. Spectra were converted to molar ellipticity per residue (MER).

NanoDrop UV-Vis spectroscopy

UV-Vis spectra were collected for samples $\mathbf{A^I}$-$\mathbf{E^I}$ and the coordination cluster $[Zn^{II}_2Dy^{III}_2L_4(NO_3)_2(DMF)_2]$ ($0.06x10^{-3}$ M in H_2O-MeOH) using NanoDrop spectrophotometry.

X-ray fibre diffraction

X-Ray fibre diffraction was collected for samples $\mathbf{A^I}$ and $\mathbf{E^I}$. Fibre were aligned by placing 10 µL between wax-tipped capillary tubes allowed to dry overnight as described previously.[3] The partially aligned samples were mounted on a goniometer head and a diffraction pattern was collected using a Rigaku rotating anode with Saturn CCD detector, using an oscillation of 0.5°. Exposure times were 30 or 60

seconds and the specimen to detector distance was 50 mm or 100 mm, respectively. Diffraction data were converted to TIFF format using Mosflm[2] and inspected using CLEARER.[4]

Tyr-fluorescence

The fibrils were resuspended by agitation. Fluorescence measurements were carried out on a Varian Cary Eclipse fluorimeter (Varian Ltd., Oxford, UK) using a 1 cm path length quartz cuvette (Starna, Essex, UK) and tyrosine fluorescence signal was monitored using an excitation wavelength of 280 nm and emission wavelength of 305 nm. Excitation and emission slits were set to 10nm and 5nm respectively, and the scan rate was set to 300 nm/min with 2.5 nm data intervals and an averaging time of 0.5 s. The photomultiplier tube detector voltage was set at 500 V.

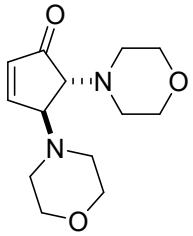
EDX

The element composition of sample $\mathbf{A^I}$ further investigated by Field Emission Scanning Electron Microscope with Energy Dispersion X-ray analysis (FESEM/EDX). The sample $\mathbf{A^I}$ suspended in Milli-Q H_2O - MeOH, was prepared by pipetting a drop onto a polished Si chip and leaving it to dry overnight at room temperature to afford a light yellow film with some "folds". EDX measurements were performed with a Zeiss Leo 1530 SEM operating at 20kV. The EDX were acquired using the "Point & ID" option in INCA software using an Oxford X-MaxN 50 detector. The EDX system (LINK ISIS 300, Oxford Corp.) is equipped with a high-resolution Ge detector (112 eV @ 5.9 keV). As the sample was placed on a polished Si chip, Si is excluded from the EDX analysis. The results are summarized in Table S2.

Monitoring catalysis

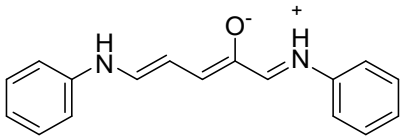
To determine the catalytic ability of the HYFNIF-Zn_2Dy_2 ($\mathbf{A^I}$), the procedure described below was applied to the synthesis of trans-4,5-diaminocyclopent-2-enones from 2-furaldehyde and primary or secondary amines. The solvent mixture - H_2O/MeOH - was evaporated (at 36◦C) from each peptide solution $\mathbf{A^I}$-$\mathbf{D^I}$, and the remaining precipitates (HYFNIF-Zn_2Dy_2, HYFNIF-Zn, HYFNIF-Dy, HYFNIF-Zn-Dy) were tested as catalysts. Three stock solutions, one of each substrate [S] in MeCN (2-furaldehyde (0.5 M), morpholine (1.2 M) and aniline (1.2 M)) were used to prepare fresh working solutions of [S] 4.8 mM for 2-furaldehyde and 10.6 mM for each amine in MeCN-MeOH. Specifically, MeCN (dry, 200 μL), MeOH (50 μL), furfural (1.2 μmol, 2.4 μL from the stock solution), amine (2.64 μmol, 2.4 μL from the stock solution) and the appropriate amount of catalyst (1 mol %), were added. ^{1}H NMR spectra of the products were recorded in Chloroform-*d* for the reactions of 2-Furaldehyde - Morpholine and 2-Furaldehyde - Aniline, catalysed by HYFNIF-Zn_2Dy_2. The latter, shows the co-existence of both (4S,5R)-4,5-bis(phenylamino)cyclopent-2-enone and (1E,2Z,4E)-5-(phenylamino)-1-(phenyliminio)penta-2,4-dien-2-olate in the solution.

trans-4,5-Dimorpholin-4-yl-cyclopent-2-enone



In air, MeCN (dry, 200 μL), MeOH (50 μL), furfural (1.2 μmol, 2.4 μL from the stock solution), morpholine (2.64 μmol, 2.4 μL from the stock solution) and the appropriate amount of catalyst (1 mol %), were added in a 2.5 mL capped vial equipped with a magnetic stir bar. The resultant mixture was stirred at room temperature for 24h. Chloroform-*d* was added in the reaction mixture and the crude mixture was analysed by ^{1}H NMR. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.61 (1H, dd, J = 6.0, 2.0 Hz), 6.24 (1H, dd, J = 6.0, 2.0Hz), 3.81 (1H, ddd, J = 3.0, 2.0 Hz), 3.71 (4H, t, J = 4.5 Hz), 3.67 (4H, t, J = 4.5 Hz), 3.29 (1H, d, J = 3.0 Hz), 2.86-2.82 (2H, m), 2.67-2.51 (6H, m). Yield=67%. (Figure S5)

(1E,2Z,4E)-5-(phenylamino)-1-(phenyliminio)penta-2,4-dien-2-olate



In air, MeCN (dry, 200 µL), MeOH (50 µL), furfural (1.2 µmol, 2.4 µL from the stock solution), morpholine (2.64 µmol, 2.4 µL from the stock solution) and the appropriate amount of catalyst (1 mol %), were added in a 2.5 mL capped vial equipped with a magnetic stirbar. The resultant mixture was stirred at room temperature for 24h. Methanol-d_4 was added in the reaction mixture and the crude mixture was analysed by ^{1}H NMR. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.25 (s, 1H), 7.58 (s, 1H), 7.33 – 7.35 (m, 2H), 7.21 – 7.09 (m, 6H), 6.94 (d, J = 3.4 Hz, 1H), 6.76 – 6.64 (m, 4H), 6.52 (s, 1H). The ^{1}H NMR spectrum shows the (1E,2Z,4E)-5-(phenylamino)-1-(phenyliminio)penta-2,4-dien-2-olate (**2b**) as the main product, with the co-existence of trace amounts of the corresponding difunctionalised cyclopentenone (4S,5R)-4,5-bis(phenylamino)cyclopent-2-enone in the solution. Yield=84%. (Figure S6)

Tables

Table S1. Peptide working solutions by stock solution **I**.

Peptide working solutions	Metal source	Molarity of HYFNIF (x10^{-3} mol/L) [a]	Molarity of metal source (x10^{-3} mol/L) [b]	Catalyst ID
A^{I}	$[Zn^{II}_2Dy^{III}_2L_4(NO_3)_2(DMF)_2]$ (**1**)	1.26	0.06	HYFNIF-Zn_2Dy_2
B^{I}	$Zn(NO_3)_2.6H_2O$	1.26	0.06	HYFNIF-Zn
C^{I}	$Dy(NO_3)_3.5H_2O$	1.26	0.06	HYFNIF-Dy
D^{I}	$Zn(NO_3)_2.6H_2O$ and $Dy(NO_3)_3.5H_2O$	1.26	0.06	HYFNIF-Zn-Dy
E^{I}	none	1.26	0.00	HYFNIF

[a] Molarity of HYFNIF (1.26 mM) in Milli-Q H_2O-MeOH. [b] Molarity of the coordination cluster and the metal salts (0.06 mM) in Milli-Q H_2O-MeOH.

Table S2. FESEM/EDX analysis showing the material composition of the film.

Sample	C	N	O	Na	Zn[a]	Dy[a]
A^{I}	60.22 ± 0.55 wt%, 65.31 At%	22.01 ± 0.64 wt%, 20.47 At%	17.24 ± 0.33 wt%, 14.03 At%	0.27 ± 0.04 wt%, 0.15 At%	0.19 ± 0.06 wt%, 0.04 At%	0.08 ± 0.08 wt%, 0.01 At%

[a] Another area shows 0.19 ± 0.09 wt%, 0.04 At% for Zn and 0.14 ± 0.12 wt%, 0.01 At% for Dy.

Figures

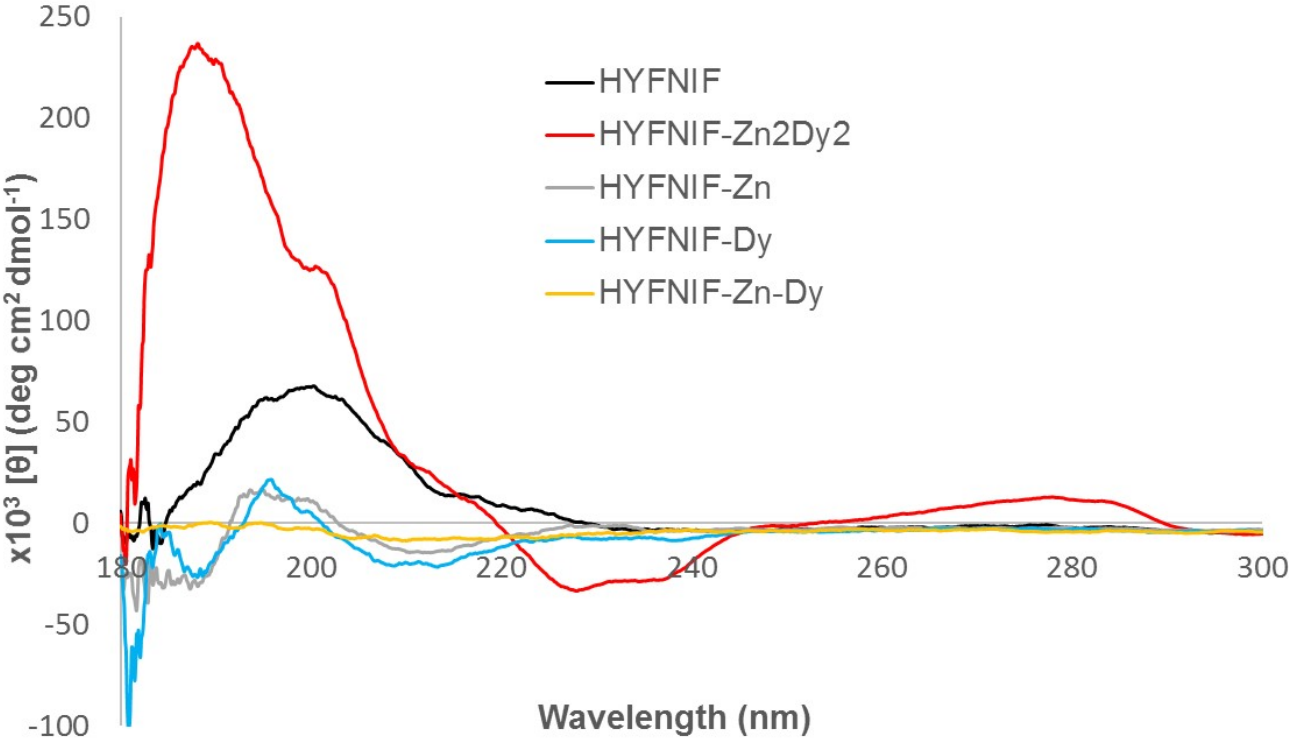


Figure S1. CD spectra of $1.26x10^{-3}$ M HYFNIF untreated (black) and treated with metal sources: Zn_2Dy_2 (red), $Zn(NO_3)_2.6H_2O$ (grey), $Dy(NO_3)_3.6H_2O$ (blue), $Zn(NO_3)_2.6H_2O$-$Dy(NO_3)_3.6H_2O$ (yellow), in Milli-Q H_2O-MeOH and incubated for 24 h at room temperature. The CD signals reveal HYFNIF fibrils treated with Zn_2Dy_2 maintain the β-sheet secondary structure with significantly enhanced contribution from Tyrosine residues at 230 and 275 nm.

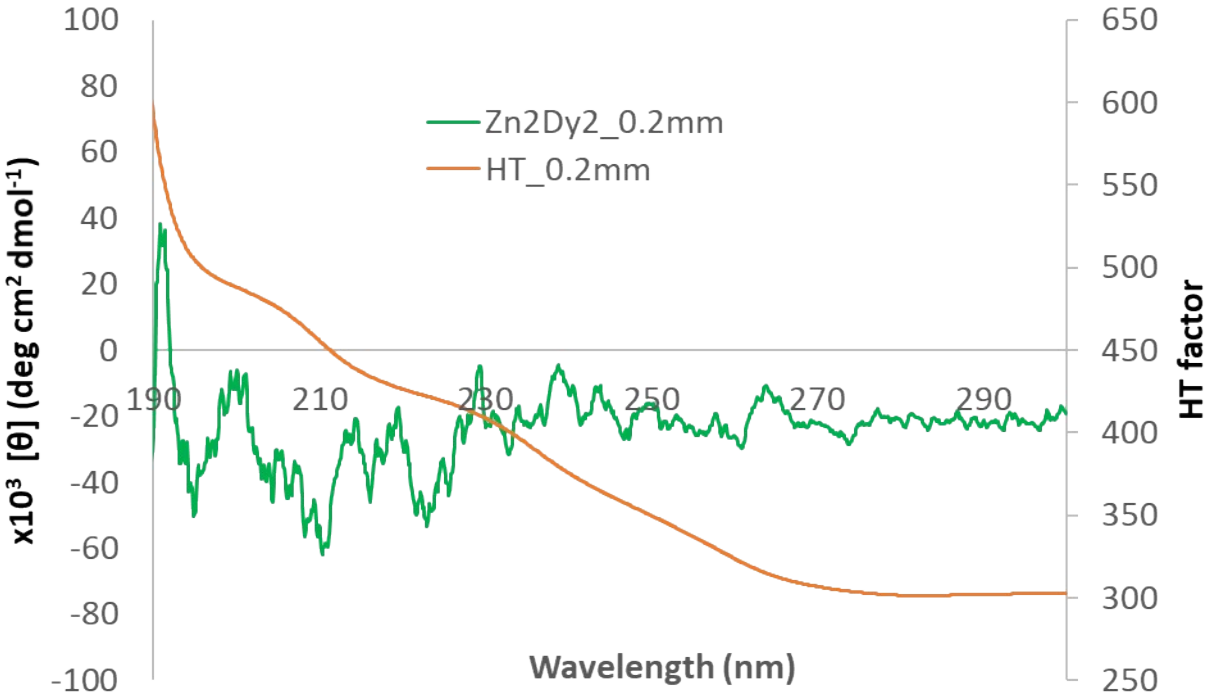


Figure S2. CD spectrum of the Coordination Cluster Zn_2Dy_2 shows no significant peaks.

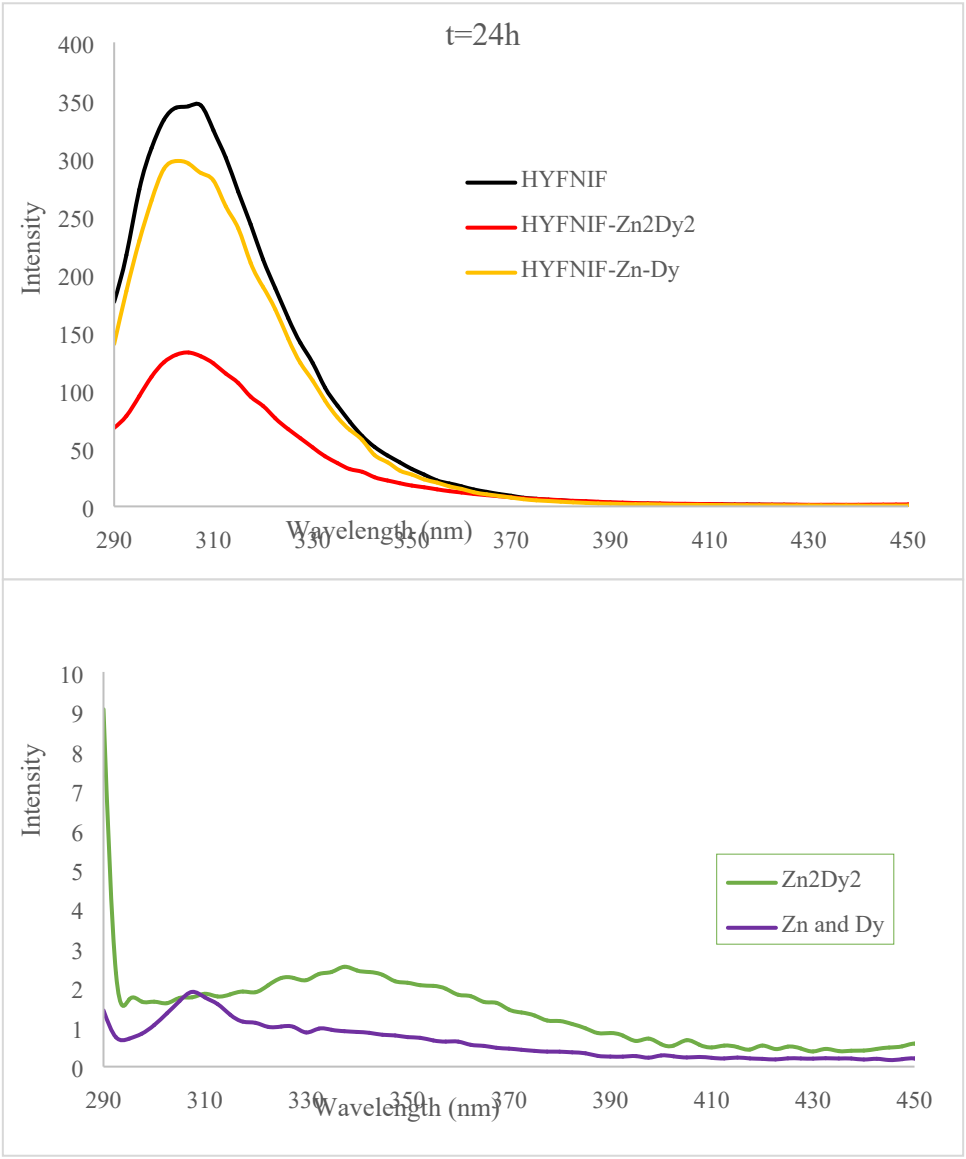


Figure S3. Tyr-fluorescence plots for fibrils (upper) and **Zn_2Dy_2** and metal salts (lower)

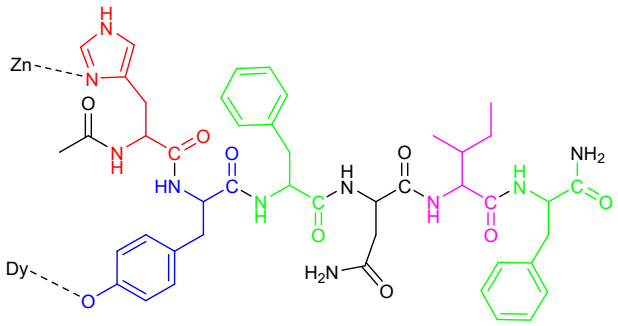


Figure S4. Possible coordination mode(s) of the fibril.

[1] H NMR Spectra for the purified Compounds employing HYFNIF-Zn_2Dy_2 as catalyst.

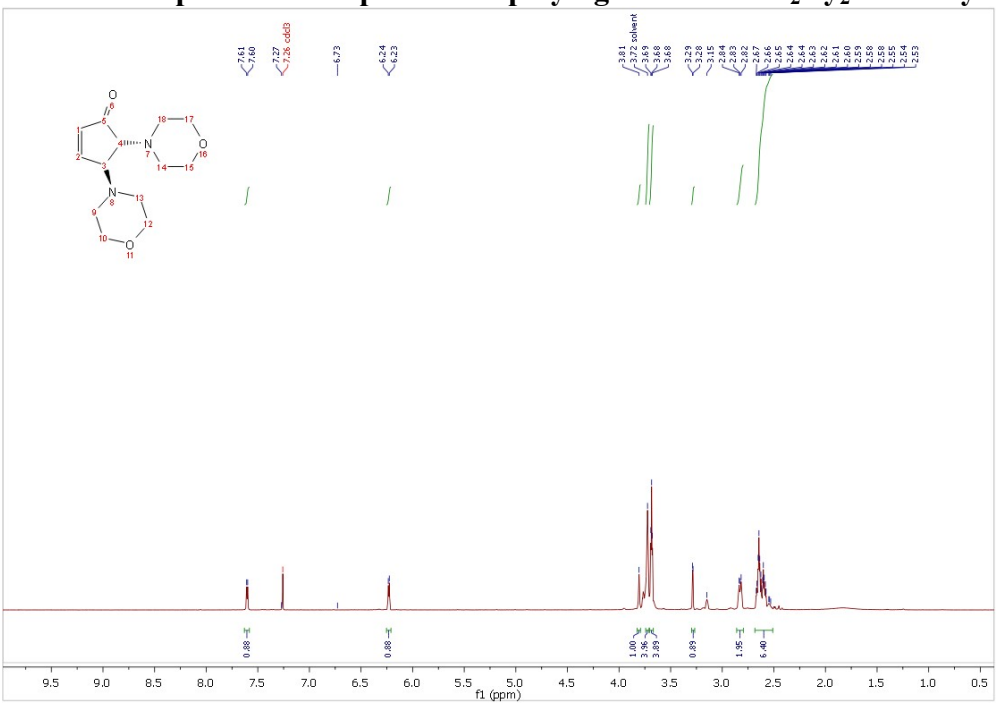


Figure S5. HYFNIF-Zn_2Dy_2 (1 mol%) was employed as catalyst for the reaction of furfural and morpholine.

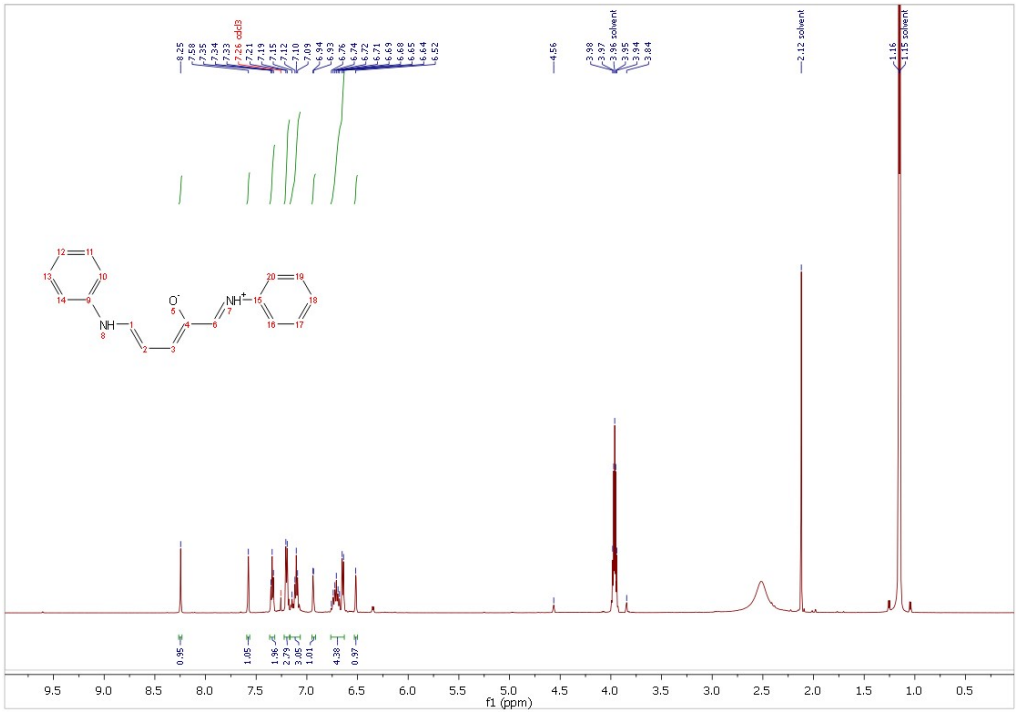


Figure S6. HYFNIF-Zn_2Dy_2 (1 mol%) was employed as catalyst for the reaction of furfural and aniline to afford the corresponding Stenhouse salt.

References

1 K. Griffiths, P. Kumar, G. R. Akien, N. F. Chilton, A. Abdul-Sada, G. J. Tizzard, S. J. Coles and G. E. Kostakis, *Chem. Commun.*, 2016, **52**, 7866–7869.

2 O. S. Makin, P Sikorski and L. C. Serpell, *J. Appl. Cryst.*, 2007, **40**, 966-972.

3 M. D. Winn, *J Synchrotron Radiat,* 2003, **10**, 23-5.

4 D. M. Vadukul, Y. K. Al-Hilaly and L. C. Serpell, *Methods Mol Biol*, 2019, **1873**, 109-122.