Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Exploring the structural determinants of selective phosphopeptide recognition using bivalent metal-coordination complexes

Supporting Information

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Experimental

General Methods

Anhydrous solvents methanol, DMSO, DCM, THF and DMF were purchased from Sigma Aldrich and used directly from Sure-Seal bottles. Molecular sieves were activated by heating to 300 °C under vacuum overnight. All reactions were performed under an atmosphere of dry nitrogen in oven-dried glassware and were monitored for completeness by thin-layer chromatography (TLC) using silica gel (visualized by UV light, or developed by treatment with $KMnO_4$ stain or phosphomolybdic acid stain). ^{1}H and ^{13}C NMR spectra were recorded on Bruker 400 MHz and a Varian 500 MHz spectrometers in either $CDCl_3$, CD_3OD or D_6-DMSO. Chemical shifts (δ) are reported in parts per million after calibration to residual isotopic solvent. Coupling constants (*J*) are reported in Hz. Before biophysical testing, inhibitors were subjected to further purification by reversed-phase HPLC (rpHPLC). Analysis and purification by rpHPLC were performed using either Atlantis Prep T3 10 μm C18 (2) 250.0 x 19.0 mm column run at 20.00 mL/min (preparative) or a Microsorb-MV 300 A C18 250.0 mm x 4.6 mm column run at 1.00 mL/min (analytical), using gradient mixtures of (A) water with 0.1% TFA and (B) 10:1 acetonitrile/water with 0.1% TFA. Ligand purity was confirmed by analytical rpHPLC using

linear gradients from 100% A to 100% B, with changing solvent composition of either (I) 4.5% or (II) 1.5% per min after an initial 2 min of 100% A.

Computational Modeling of Zn(II)-BDPA Receptors

All computational modeling was performed by Dr. Steven Burger (Wayne State University). The simulation was done with the AMBER11 (http://www.citeulike.org/user/bmduggan/article/5692441) software. The bonded parameters for the Zn scaffold were obtained by using the para_freq program (http://pubs.acs.org/doi/abs/10.1021/ct2007742). Charges for the FAM and the Zn scaffold were obtained by restrained electrostatic potential (RESP) fitting using the density from a HF/6-31G* optimized structure. AMBERTOOLS 11 was used to do the RESP fitting and to assign GAFF parameters for the system. The two polypeptide strands were built and solvated in an explicit TIP3P water box. An initial 1ns equilibration phase was run with a 2.0 kcal/mol/A restraint on the distance between the N on the derivative group and the delta carbon on the glutamate. The constraint was removed and a further 5ns molecular dynamics was performed with a NPT ensemble at 298 K using a Langevin thermostat.

Determination of Receptor:Phosphopeptide Binding Constants via a Fluorescence Intensity Assay

Fluorescence intensity screens were conducted as previously described using Corning Black 384 well plates in a Tecan M1000 fluorometer with plate reader. Each well (of a 96 well plate) contained 10.0 nM phosphopeptide in 50.00 mM HEPES buffer (pH 7.3, 25ºC) and a variable concentration of receptor (1.0 nM to 100 μM). Z-depth and gain were both cell-optimized by the

system automatically. Concentrations exceeding 100 μM were not used in order to avoid fluorescence quenching due to collisional non-binding events. Each receptor was screened in triplicate, with data averaged to ensure accurate K_d values. Binding constants were obtained using non-linear logistic fitting in Origin 8.

Table S1: Receptor binding affinities (K_a M^{-1}) across the six Fam-G-labelled peptides

Receptor	pSGEGG	Error	pSGGEG	Error	pSGRGG	Error
1	92964	6371	109705	14434	24098	3370
2	171851	24107	80426	18806	42585	8751
3	1325190	119768	927859	95640	407113	30740
4	825621	41990	1110350	205112	540503	54908
5	530650	69581	630847	60093	109467	14564
6	3217190	309061	3762510	337491	949821	182056
7	3190710	89895	3137550	273276	1618670	94140

Receptor	pSGGRG	Error	pSGIGG	Error	pSGGIG	Error
1	11904	2978	106720	25455	104943	15646
2	50642	10397	91236	7612	54099	6895
3	477733	71456	1019320	96233	670637	143355
4	398259	23324			737278	205799
5	154206	13689	1088730	159866	630847	60093
6	755692	130798	660899	168399	2641100	273924
7	1117780	113786	2299960	294273	2064750	185193

Table S2: Receptor binding affinities (K_a M^{-1}) across the three Fam-G-labeled peptides

Receptor	pSDDDD	error	pSDLDL	error	pSRLRL	error
1	1.05E+06	2.47E+04	1.57E+06	5.33E+05	8.35E+05	2.07E+05
2	2.44E+06	2.69E+05	1.55E+06	1.93E+05	7.56E+05	9.00E+04
3	2.52E+06	4.22E+05	5.40E+06	1.78E+06	1.67E+06	1.13E+05
4	1.88E+06	1.64E+05	3.06E+06	7.16E+05	1.23E+06	1.88E+05
5	1.18E+07	2.61E+06	1.01E+07	1.85E+06	2.33E+06	8.34E+05
6	8.66E+06	3.10E+06	5.47E+06	2.22E+06	3.20E+06	6.69E+05
7	2.61E+07	6.64E+06	1.17E+07	3.24E+06	6.33E+06	8.22E+05

Isothermal Titration Calorimetry (ITC) Experiments

ITC experiments were used to measure the binding of the metal complexes to various substrates, and were performed at 25.0 ºC (298 K) using Microcal VP-ITC titration micro-calorimeter. In

order to minimize mixing heat effects caused by differences in solution composition, the substrates and receptor were both dissolved in freshly prepared HEPES buffer (± 5.0% DMSO) (50 mM, pH = 7.2) before each titration experiment. All solutions prior to experiments were degassed before being added to the calorimeter cell. The substrates, at a concentration of approximately 2 mM, were injected in 10 μL increments into the reaction cell (cell volume 1.5 mL) containing complex at a concentration of *ca* 0.10 mM, until there occurred a saturation of binding sites. A 250 μL injection syringe with 310–400 rpm stirring was used to give a series of 10.0 μL injections at 3.5-min intervals. Control experiments for heats of mixing and dilution were performed under identical conditions and used for data correction in subsequent analysis. Data acquisition and subsequent non-linear regression analysis were done in terms of a simple binding model using the Microcal ORIGIN software package.

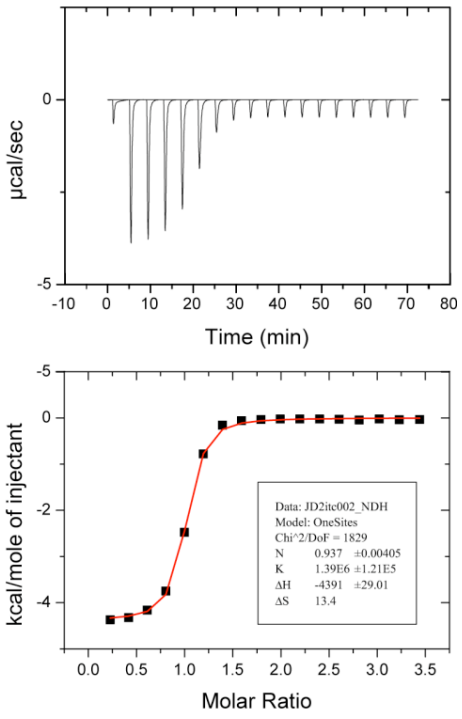


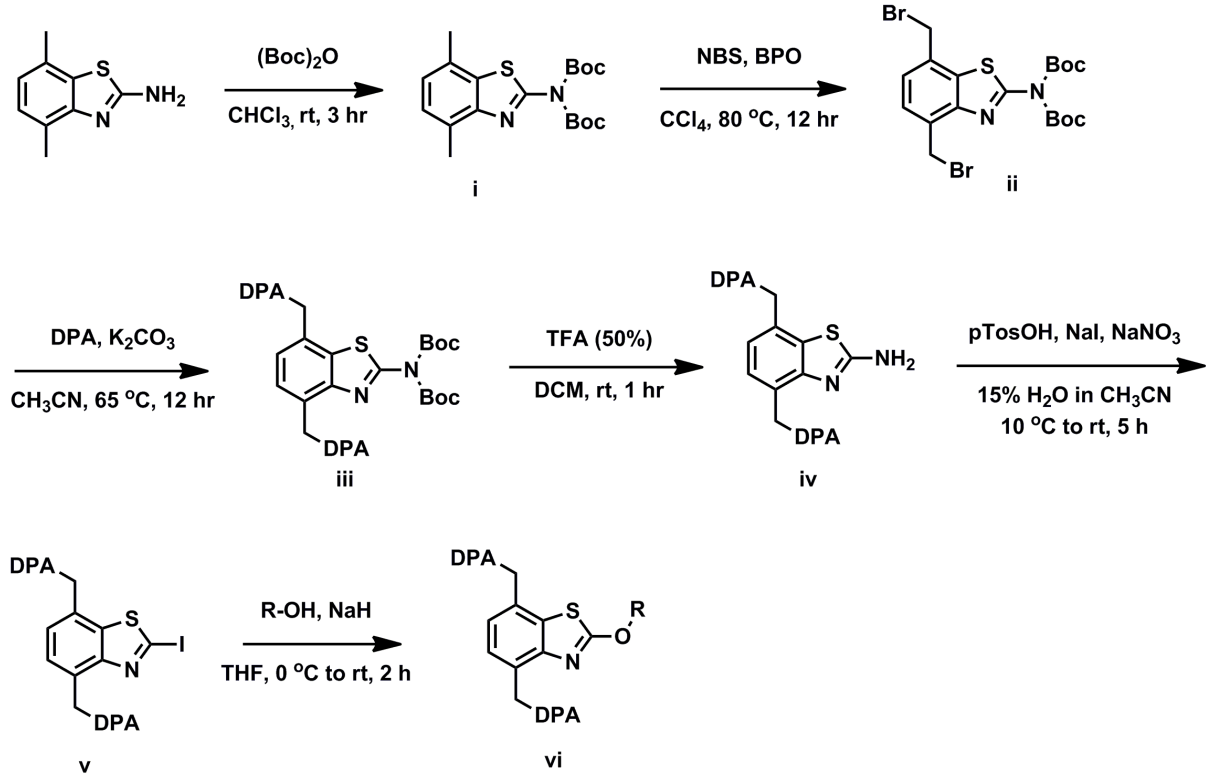
Figure 1 ITC trace showing a high affinity stoichiometric binding between **7** and FAM-GpSGEGG (K_a = 1.39 ± 0.12 x 10^6 M^{-1}). This affinity constant is consistent with the value calculated from the fluorescence intensity assay (K_a = 3.50 ± 0.11 x 10^6 M^{-1}).

Table S3: Summary of thermodynamic parameters obtained by ITC experiments for receptors **5** and **7**.

	Ka (M^{-1}, ITC)		**Ka** (M^{-1}, FI)		**ΔH** (cal/mol)		**ΔS** (cal/mol K)	
	5	7	5	7	5	7	5	7
GEGG	8.36×10^{5}	1.45×10^{6}	5.31×10^{5}	3.19×10^{6}	-2973	-4901	17.1	11.8
GRGG	1.59×10^{4}	3.16×10^{5}	1.09×10^{5}	1.62×10^{6}	-1241	-2028	15.1	18.4
DLDL	3.90×10^{6}	-	1.01×10^{7}	-	-2193	-	22.8	-
DDDD	1.25×10^{7}	-	1.18×10^{7}	-	-2865	-	22.9	-

Synthesis and Characterization

Synthesis of ligands with O-coupled R-substituents



Scheme 1. Synthesis of O-coupled phosphopeptide receptors

Scheme 1.

Compound i. 4,7-dimethylbenzo[d]thiazol-2-amine (2.50 g, 14.00 mmol, 1.0 eq) was dissolved in DCM (140.0 mL) and stirred at 0 ºC for 3 h in the presence of Boc anhydride (6.72 g, 30.80 mmol, 2.2 eq), NMM (3.70 mL, 33.60 mmol, 2.4 eq) and catalytic DMAP. The solution was then diluted via the addition of DCM, and washed twice with water. Following drying and concentration *in vacuo*, the product was purified by flash chromatography (5:1 hexanes:EtOAc), to furnish **i** (4.70 g, 89 %): δ_H (400 MHz, $CDCl_3$) 1.59 (s, 18H, -$C(CH_3)_3$), 2.48 (s, 3H, -CH_3), 2.57 (s, 3H, -CH_3), 6.99 (d, *J* = 7.7 Hz, 1H, CH(Ar)), 7.13 (d, *J* = 7.7 Hz, 1H, CH(Ar)); δ_C (100 MHz, $CDCl_3$) 17.5, 20.5, 84.6, 124.2, 126.5, 128.3, 128.8, 133.1, 148.1, 149.4, 156.3; LRMS (ES+) *m/z* calculated for $C_{19}H_{27}N_2O_4S$ = [M+H] 379.2. Found 379.2.

Compound ii. **i** (1.92 g, 5.1 mmol, 1.0 eq), NBS (2.30 g, 11.7 mmol, 2.3 eq) and catalytic BPO were dissolved in anyhydrous carbon tetrachloride (50.0 mL), and refluxed under nitrogen overnight, after which TLC confirmed that the reaction had gone to completion. The product was diluted with DCM and washed several times with saturated sodium bicarbonate solution. Following drying and concentration *in vacuo*, the product was purified by flash chromatography (9:1 hexanes:EtOAc), to furnish **ii** (1.52 g, 55 %): δ_H (400 MHz, $CDCl_3$) 1.62 (s, 18H, -$C(CH_3)_3$), 4.64 (s, 2H, -CH_2Br), 4.89 (s, 2H, -CH_2Br), 7.24 (d, *J* = 7.8 Hz, 1H, CH (Ar)), 7.43 (d, *J* = 7.8 Hz, 1H, CH (Ar)) δ_C (400 MHz, $CDCl_3$) 27.6, 29.0, 31.6, 85.6, 124.5, 127.1, 130.1, 131.3, 132.8, 148.2, 149.0, 158.1; LRMS (ES+) *m/z* calculated for $C_{19}H_{25}Br_2N_2O_4S$ [M+H] 534.98. Found 535.0

N,N'-((2-aminobenzo[d]thiazole-4,7-diyl)bis(methylene))bis(1-(pyridin-2-yl)-N-(pyridin-2-ylmethyl)methanamine) (Compound iv). **ii** (1.00 g, 1.9 mmol, 1.0 eq), 2,2-dipicolylamine (726 µL, 4.0 mmol, 2.1 eq) and K_2CO_3 (0.80 g, 5.8 mmol, 3.0 eq) were dissolved in anhydrous

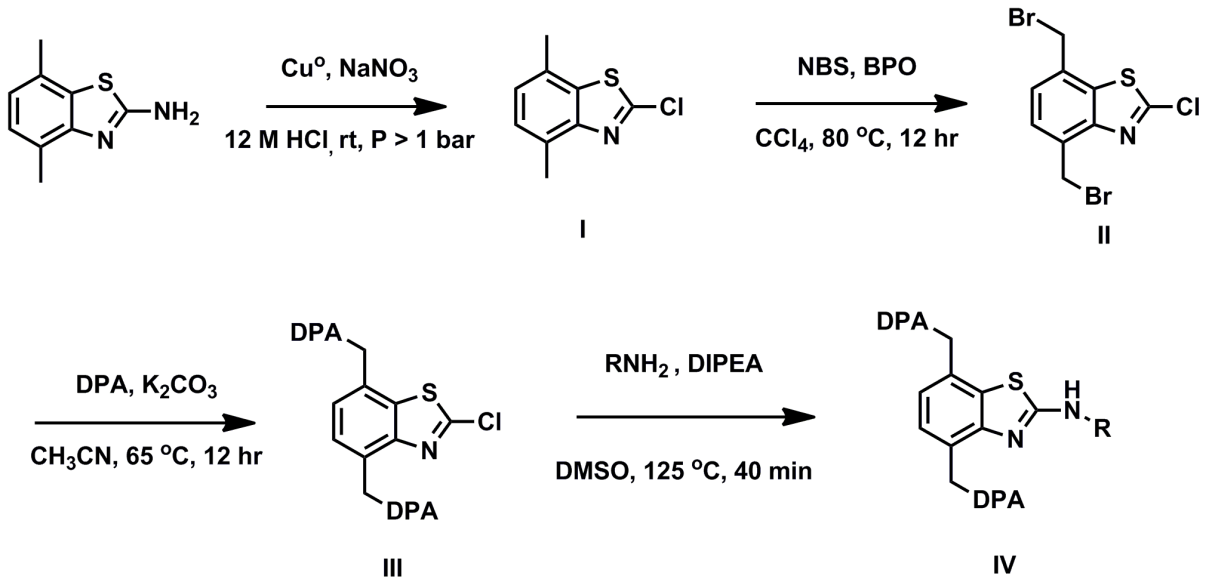
acetonitrile (20 mL). The solution was then heated in a microwave vial to 100 ^{o}C for a period of 23 min, after which TLC confirmed the reaction had gone to completion. The product was extracted several times into DCM from saturated sodium bicarbonate solution, after which the organic fraction was dried and concentrated in vacuo to yield a crude oily solid (**iii**). The crude was then taken up in 50.0 % (v/v) TFA in DCM, and stirred at room temperature for 1.0 h to deprotect the 2'-amino group, followed by removal of solvent in vacuo. Purification was carried out using flash chromatography (92% dichloromethane/7% methanol/1% NH_4OH, gradient), to furnish **iv** (0.66 g, 60 %): δ_H (400 MHz, MeOD) 3.79 (s, 2H, $PhCH_2N$-), 3.98 (s, 2H, $PhCH_2N$-), 4.22 (s, 4H, -NCH_2Pyr), 4.39 (s, 4H, -NCH_2Pyr), 7.01 (d, *J* = 7.7 Hz, 1H, CH(Ar)), 7.15 (d, *J* = 7.7 Hz, 1H, CH(Ar)), 7.66-7.76 (m, 4H, CH(Pyr)), 7.79 (t, *J* = 6.6 Hz, 2H, CH(Pyr)), 7.91 (d, *J* = 8.3 Hz, 2H, CH(Pyr)), 8.22 (t, *J* = 7.7 Hz, 2H, CH(Pyr)), 8.34 (t, *J* = 7.7 Hz, 2H, CH(Pyr)), 8.66 (d, *J* = 5.4 Hz, 2H, CH(Pyr)), 8.79 (d, *J* = 6.0 Hz, 2H, CH(Pyr)); δ_C (100 MHz, $CDCl_3$) 56.7, 57.0, 57.1, 58.2, 114.3, 117.2, 123.8, 124.1, 124.8, 125.3, 125.7, 126.2, 128.5, 130.9, 141.2, 142.3, 144.4, 144.7, 153.1, 153.5, 169.5; LRMS (ES+) m/z calculated for $C_{43}H_{49}N_8O_4$ [M+H] 773.35. Found 773.4.

N,N'-((2-iodobenzo[d]thiazole-4,7-diyl)bis(methylene))bis(1-(pyridin-2-yl)-N-(pyridin-2-ylmethyl)methanamine) (Compound v). iv (1.70 g, 3.0 mmol, 1.0 eq) and *p*-toluene sulfonic acid (2.88 g, 15.0 mmol, 5.0 eq) were dissolved in 15 % H_2O in acetonitrile (10 mL) at 15.0 ºC, after which $NaNO_3$ (0.42 g, 6.0 mmol, 2.0 eq) and NaI (1.00 g, 6.8 mmol, 2.3 eq) were added in one portion. The solution was then allowed to warm passively to room temperature. When the reaction was judged complete by TLC (~8 h), 0.35 g of sodium bisulfite was added to the solution. The solution was subsequently diluted with water and the product was extracted several times into dry ether. The organic layers were combined, dried and concentrated *in vacuo*. The

product **v** (1.02 g, 50 %) was isolated as an off-white powder after columning in a flat gradient of 92% dichloromethane/7% methanol/1% NH_4OH. δ_H (400 MHz, $CDCl_3$) 3.80 (s, 4H, -NCH_2Pyr), 3.86 (s, 2H, PhCH_2N-), 3.89 (s, 4H, -NCH_2Pyr), 4.23 (s, 2H, PhCH_2N-), 7.10 (t, *J* = 6.0 Hz, 2H, CH(Ar)), 7.14 (t, *J* = 5.6 Hz, 2H, CH(Pyr)), 7.49 (d, *J* = 8.2 Hz, 2H, CH(Pyr)) 7.57-7.71 (m, 8H, CH(Pyr)), 8.49 (d, *J* = 4.3 Hz, 2H, CH(Pyr)), 8.52 (d, *J* = 5.2 Hz, 2H, CH(Pyr)); δ_C (100 MHz, $CDCl_3$) 54.2, 57.9, 60.2, 121.8, 122.0, 122.6, 123.6, 125.5, 126.3, 131.3, 136.2, 136.3, 137.6, 148.8, 148.9, 153.6, 158.2; LRMS (ES+) *m/z* calculated for $C_{33}H_{31}IN_7S$ [M+H] 684.14. Found 684.2.

Final O-coupled ligands (Scheme 1) – Representative synthesis – Ligand 3. **v** (50.0 mg, 0.07 mmol, 1.0 eq) and was dissolved in 0.50 mL THF, and cooled over ice. In a second flask, phenol (16.0 μL, 0.14 mmol, 2.0 eq) was reacted with stoichiometric NaH (5.8 mg, 0.14 mmol, 2.0 eq) in 0.30 mL THF at room temperature for 5 min. The solution containing the alkoxide was then added in one portion to the solution containing **v**, and the reaction was monitored for 2 h by TLC. When the reaction was complete, the product was extracted into EtOAc and the combined organic fractions were washed with water. After drying and concentrating *in vacuo*, the crude product was purified by rpHPLC to furnish the final ligand **L3** in high yield (42.0 mg, 92 %) δ_H (400 MHz, $CDCl_3$) 3.64-4.70 (m, 12H, PhCH_2N- and –NCH_2Pyr), 7.09-7.19 (m, 4H, CH (Ar)), 7.21 (d, *J* = 8.0 Hz, 1H, CH (Ar)), 7.28-7.52 (m, 8H, CH (Ar)), 7.56-7.69 (m, 6H, CH (Ar)), 7.42-7.54 (m, 4H, CH (Ar)); δ_C (100 MHz, $CDCl_3$) 54.3, 58.6, 60.2, 60.3, 121.9, 121.3, 121.3, 122.4, 123.0, 125.7, 130.6, 136.0, 136.2, 148.8, 158.9, 160.1, 167.3; HRMS (ES+) *m/z* calcd for $C_{39}H_{37}N_7OS$ [M+2H] 325.6384. Found 325.6379.

Synthesis of ligands with N-coupled substituents



Scheme 2.

2-chloro-4,7-dimethylbenzo[*d*]thiazole (Compound I). 4,7-dimethylbenzo[d]thiazol-2-amine (2.00 g, 11.40 mmol, 1.0 eq) was dissolved in concentrated hydrochloric acid (27.00 mL) and cooled to at 0 ºC using an ice bath. To this solution, elemental copper (0.6 g, 9.00 mmol, 0.8 eq) was added in one portion. Large quantities of $NaNO_3$ were then added (in excess of 20.0 eq) in ~ 1.00 g portions, between which the reaction vessel was sealed using a rubber septum and hand strength. Significant pressures of noxious brown gas built up during this reaction. After the reaction was judged complete by TLC, the solution was neutralized using 1.0 M NaOH and the product was extracted several times into EtOAc. Following drying and concentration of the organic fractions *in vacuo*, the product was purified by flash chromatography on an extra-large column setup (8:1 hexanes:EtOAc), to furnish **I** (1.89 g, 85 %): δ_H (400 MHz, $CDCl_3$) 2.43 (s, 3H, -CH_3), 2.66 (s, 3H, -CH_3), 7.06 (d, J = 7.5 Hz, 1H, CH(Ar)), 7.16 (d, J = 7.5 Hz, 1H, CH(Ar)); δ_C (100 MHz, $CDCl_3$) 17.9, 20.9, 125.9, 127.4, 128.4, 130.1, 136.5, 150.0, 151.5; LRMS (ES+) *m/z* calculated for C_9H_9ClNS [M+H] 198.01. Found 198.1.

4,7-bis(bromomethyl)-2-chlorobenzo[*d*]thiazole (Compound II). **I** (1.89 g, 9.5 mmol, 1.0 eq), NBS (3.52 g, 19.8 mmol, 2.1 eq) and catalytic BPO were dissolved in anyhydrous carbon tetrachloride (94.0 mL), and refluxed under nitrogen overnight, after which TLC confirmed that the reaction had gone to completion. The product was diluted with DCM and washed several times with saturated sodium bicarbonate solution. Following drying and concentration *in vacuo*, the product was purified by flash chromatography (9:1 hexanes:EtOAc), to furnish **II** (1.30 g, 40 %): δ_H (400 MHz, $CDCl_3$) 4.6 (s, 2H, -CH_2Br), 4.94 (s, 2H, -CH_2Br), 7.40 (d, J = 7.7 Hz, 1H, CH(Ar)), 7.52 (d, J = 7.7 Hz, 1H, CH(Ar)); δ_C (100 MHz, $CDCl_3$) 28.3, 32.1, 126.2, 128.0, 131.2, 132.4, 141.1, 154.1, 170.1; HRMS (ES+) *m/z* calculated for $C_9H_7Br_2ClNS$ [M+H] 353.83590. Found 353.83545

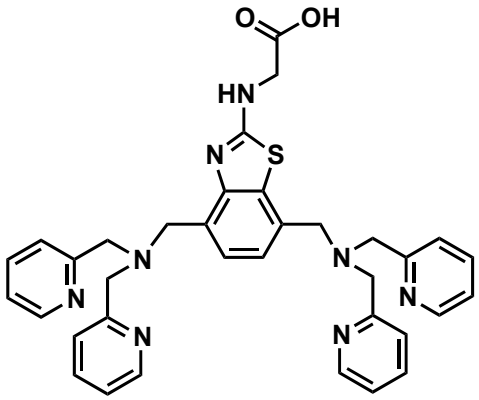
***N*,*N*'-((2-chlorobenzo[*d*]thiazole-4,7-diyl)bis(methylene))bis(1-(pyridin-2-yl)-*N*-(pyridin-2-ylmethyl)methanamine) (Compound III)**. **II** (0.26 g, 0.7 mmol, 1.0 eq), 2,2-dipicolylamine (278 μL, 1.5 mmol, 2.1 eq) and K_2CO_3 (0.31 g, 2.2 mmol, 3.0 eq) were dissolved in anhydrous acetonitrile (20 mL). The solution was then heated in a microwave vial to 100 ºC for a period of 23 min, after which TLC confirmed the reaction had gone to completion. The product was extracted several times into DCM from saturated sodium bicarbonate solution, after which the organic fraction was dried and concentrated *in vacuo* to yield a crude oily solid. Purification was carried out using silica gel chromatography on a large column (92% dichloromethane/7% methanol/1% NH_4OH, gradient), to furnish **III** (0.31 g, 71 %) in reasonable yield: δ_H (400 MHz, $CDCl_3$) 3.70 (s, 4H, -NCH_2Pyr), 3.77 (s, 2H, PhCH_2N-), 3.78 (s, 4H, -NCH_2Pyr), 4.10 (s, 2H, PhCH_2N-), 6.90-7.05 (m, 4H, CH(Ar)), 7.18 (d, J = 7.3 Hz, 1H, CH(Ar)), 7.38 (d, J = 8.1 Hz, 2H, CH(Ar)), 7.44-7.58 (m, 7H, CH(Ar)), 8.31-8.42 (m, 4H, CH(Ar)); δ_C (100 MHz, ($CDCl_3$) 54.0, 57.7, 60.1, 121.8, 122.1, 122.7, 123.7, 124.5, 125.6, 126.4, 126.6, 131.6, 131.8, 134.4,

136.3, 136.4, 148.6, 148.8, 150.4, 154.1, 158.0, 159.3; HRMS (ES+) calculated for $C_{33}H_{31}ClN_7S$ [M+H] 592.20502. Found 592.20384.

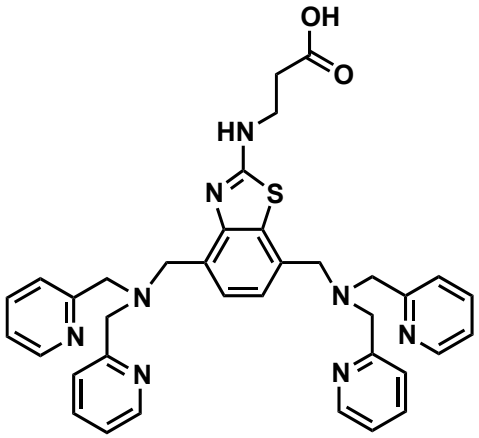
Final N-coupled ligands (Scheme 2) – Representative synthesis – Ligand 5. **III** (53.0 mg, 0.09 mmol, 1.0 eq), ethylene diamine (18 μL, 0.27 mmol, 3.0 eq) and DIPEA (47 μL, 0.27 mmol, 3.0 eq) were dissolved in 0.90 mL acetonitrile, and heated at 125 ºC in a microwave vial for 90 min. The solvent was then removed *in vacuo* and the crude product was purified by rpHPLC, to yield **L5** in high purity (50.0 mg, 90 %). δ_H (400 MHz, $CDCl_3$) 2.01 (s, 2H, -NH_2), 2.98 (t, *J* = 5.8 Hz, 2H, -C$\underline{H}_2$$NH_2$), 3.48 (t, *J* = 5.8, 2H, -NHCH_2-), 3.71 (s, 2H, PhCH_2N-), 3.76 (s, 4H, -NCH_2Pyr), 3.86 (s, 4H, -NCH_2Pyr), 4.04 (s, 2H, PhCH_2N-), 6.99-7.10 (m, 5H, CH (Ar)), 7.42 (d, *J* = 7.5 Hz, 1H, CH (Ar)), 7.49-7.67 (m, 8H, CH (Ar)), 8.48 (t, *J* = 4.9 Hz, 4H, CH (Ar)); δ_C (100 Hz, $CDCl_3$) 41.1, 54.4, 58.5, 60.2, 60.3, 121.6, 121.7,121.8, 122.4, 123.4, 125.8, 130.6, 136.1, 136.2, 148.7, 158.9, 160.1, 167.3; HRMS (ES+) *m/z* calculated for $C_{35}H_{39}N_9S$ [M+2H] 308.6519. Found 308.6529.

Preparation of all coordination complexes. Ligands were dissolved in anhydrous MeOH and zinc (II) triflate (2.0 eq) was added in one portion. The solution was allowed to stir overnight at room temperature. MeOH was then removed *in vacuo* and the resulting solid was washed twice with ether to remove any unreacted scaffold. The solid was then re-dissolved in a small volume of MeOH and filtered through National Scientific Target Syringe Filters (Cellulose Acetate Membrane) 4mm, 0.2 μm. Finally, the filtrate was diluted in distilled water and lyophilized to dryness.

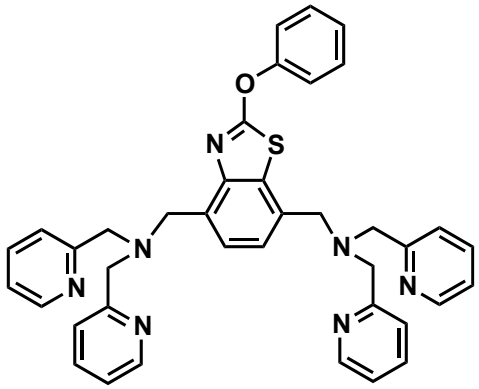
Characterization of Final Ligands 1-7



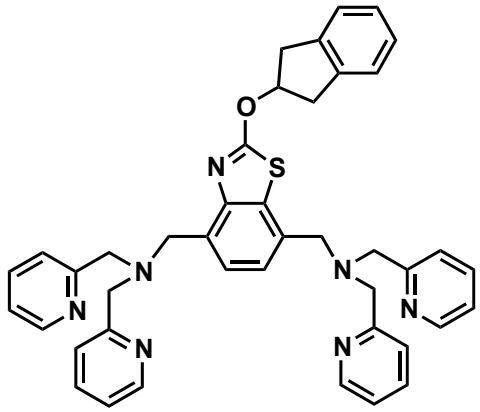
2-((4,7-bis((bis(pyridin-2-ylmethyl)amino)methyl)benzo[*d*]thiazol-2-yl)amino)acetic acid (L1). δ_H (400 MHz, MeOD) 3.78 (s, 2H, -NHC$\underline{H_2}$CO$_2$H-), 4.21 (s, 2H, -NCH$_2$(Ar)), 4.26 (s, 4H, -NCH$_2$Pyr), 4.38 (s, 2H, -NCH$_2$(Ar)), 4.42 (s, 4H, -NCH$_2$Pyr), 7.00 (d, *J* = 7.7 Hz, 1H, CH(Ar)), 7.12 (d, *J* = 7.8 Hz, 1H, Ar), 7.55 (t, *J* = 6.9 Hz, 2H, CH(Pyr)), 7.59 (d, *J* = 7.9 Hz, 2H, CH(Pyr)), 7.68 (t, *J* = 6.6 Hz, 2H, CH(Pyr)), 7.80 (d, *J* = 8.3 Hz, 2H, CH(Pyr)), 8.06 (t, *J* = 8.3 Hz, 2H, CH(Pyr)), 8.23 (t, *J* = 7.9 Hz, 2H, CH(Pyr)), 8.66 (d, *J* = 5.2 Hz, 2H, CH(Pyr)), 8.81 (d, *J* = 5.5 Hz, 2H, CH(Pyr)); δ_C (100 Hz, MeOD) 45.2, 56.5, 57.2, 57.5, 59.0, 122.8, 124.4, 124.8, 124.9, 126.3, 127.7, 129.7, 131.0, 141.7, 142.4, 144.1, 144.7, 150.3, 151.8, 153.6, 160.0, 160.4, 171.5; HRMS (ES+) m/z calculated for $C_{35}H_{34}N_8O_2S$ [M+H] 630.2505. Found 630.9350; HRMS (ES+) *m/z* calcd for $C_{35}H_{34}N_8O_2SZn$ [L1+Zn] 347.0902. Found 347.0906; IR (KBr, cm^{-1}) 3516, 3236, 2925, 1683, 1609, 1559, 1486, 1442, 1408, 1229, 1171, 1033, 765, 646, 578, 519.



3-((4,7-bis((bis(pyridin-2-ylmethyl)amino)methyl)benzo[*d*]thiazol-2-yl)amino)propanoic acid (L2). δ_H (400 MHz, MeOD) 2.83 (t, *J* = 6.7 Hz, 2H, -NHC<u>H</u>$_2$CH$_2$-), 3.88 (t, *J* = 6.6 Hz, 2H, -CH$_2$C<u>H</u>$_2$COOH); 3.91 (s, 2H, -NCH$_2$(Ar)), 4.19 (s, 4H, -NCH$_2$Pyr), 4.50 (s, 4H, -NCH$_2$Pyr), 4.64 (s, 2H, -NCH$_2$(Ar)), 7.05 (d, *J* = 8.3 Hz, 1H, CH(Ar)), 7.18 (d, *J* = 7.7 Hz, 1H, Ar), 7.39 (d, *J* = 7.1 Hz, 4H, CH(Pyr)), 7.49 (t, *J* = 6.4 Hz, 2H, CH(Pyr)), 7.59 (d, *J* = 8.3 Hz, 2H, CH(Pyr)), 7.83 (t, *J* = 7.7 Hz, 2H, CH(Pyr)), 8.02 (t, *J* = 8.3 Hz, 2H, CH(Pyr)), 8.63 (d, *J* = 5.1 Hz, 2H, CH(Pyr)), 8.72 (d, *J* = 5.8 Hz, 2H, CH(Pyr)); δ_C (100 Hz, MEOD) 37.3, 40.4, 56.6, 57.3, 57.5, 59.0, 77.9, 114.4, 117.3, 122.7, 124.2, 124.7, 126.0, 127.6, 129.4, 131.4, 140.1, 143.0, 143.4, 146.3, 151.2, 154.1, 168.2, 173.7; HRMS (ES+) *m/z* calcd for $C_{36}H_{38}N_8O_2S$ [M+2H] 323.1413. Found 323.1408; HRMS (ES+) *m/z* calcd for $C_{36}H_{36}N_8O_2SZn$ [L2+Zn] 354.0981. Found 354.0973; IR (KBr, cm^{-1}) 3519, 3214, 3019, 2895, 1681, 1611, 1562, 1441, 1384, 1263, 1202, 1032, 842, 802, 765.

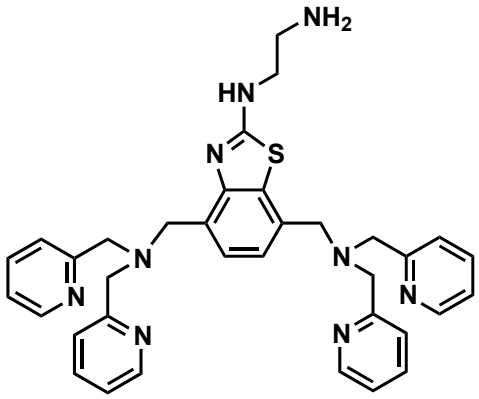


***N*,*N*'-((2-phenoxybenzo[*d*]thiazole-4,7-diyl)bis(methylene))bis(1-(pyridin-2-yl)-*N*-(pyridin-2-ylmethyl)methanamine) (L3).** δ_H (400 MHz, $CDCl_3$) 3.64-4.70 (m, 12H, $PhCH_2N$- and –NCH_2Pyr), 7.09-7.19 (m, 4H, CH (Ar)), 7.21 (d, *J* = 8.0 Hz, 1H, CH (Ar)), 7.28-7.52 (m, 8H, CH (Ar)), 7.56-7.69 (m, 6H, CH (Ar)), 7.42-7.54 (m, 4H, CH (Ar)); δ_C (100 MHz, MeOD) 54.4, 57.9, 59.6, 59.8, 120.4,122.0, 122.3, 123.1, 124.0, 124.2, 126.0, 127.0, 128.0, 130.0, 130.4, 131.5, 136.8, 137.0, 147.7, 147.9, 148.5, 150.6, 155.0, 158.0, 159.0, 172.9; HRMS (ES+) *m/z* calcd for $C_{39}H_{37}N_7OS$ [M+2H] 325.6384. Found 325.6379; HRMS (ES+) *m/z* calcd for $C_{39}H_{35}N_7OSZn$ [L3+Zn] 356.5952. Found 356.5941; IR (KBr, cm^{-1}) 3474, 2920, 2359, 1610, 1576, 1518, 1484, 1445, 1384, 1252, 1173, 1032, 825, 768.



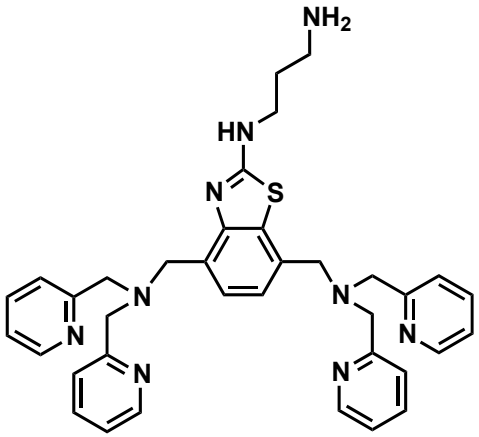
***N*,*N*'-((2-((2,3-dihydro-1*H*-inden-2-yl)oxy)benzo[*d*]thiazole-4,7-diyl)bis(methylene))bis(1-(pyridin-2-yl)-*N*-(pyridin-2-ylmethyl)methanamine) (L4).** δ_H (400 MHz, $CDCl_3$) 3.29 (dd, *J* =

17.3 and 3.3 Hz, 2H, >CHC$\underline{H_2}$-), 3.45 (dd, *J* = 18 and 5.2 Hz, 2H, >CHC$\underline{H_2}$-), 3.76 (s, 4H, –NCH_2Pyr), 3.77 (s, 2H, $PhCH_2N$-), 3.90 (s, 4H, –NCH_2Pyr), 4.14 (s, 2H, $PhCH_2N$-), 5.88-5.94 (m, 1H, -OCH<), 7.05-7.16 (m, 5H, CH(Ar)), 7.20-7.30 (m, 4H, CH(Ar)), 7.49 (d, *J* = 7.2, 1H, CH(Ar)), 7.55-7.70 (m, 8H, CH(Ar)), 8.48 (d, *J* = 5.2 Hz, 4H, CH(Ar)); δ_C (100 MHz, $CDCl_3$) 39.5, 42.5, 58.4, 60.2, 60.3, 82.3, 121.6, 121.9, 122.4, 123.5, 123.8, 124.6, 124.8, 126.4, 126.7, 136.2, 136.3, 140.3, 148.7, 148.8, 158.7, 160.1, 172.3; HRMS (ES+) *m/z* calcd for $C_{42}H_{41}N_7OS$ [M+2H] 345.6541. Found 346.6548; HRMS (ES+) *m/z* calcd for $C_{42}H_{39}N_7OSZn$ [L4+Zn] 376.6108. Found 376.6112; IR (KBr, cm^{-1}) 3475, 2923, 1610, 1575, 1485, 1445, 1250, 1173, 1032.

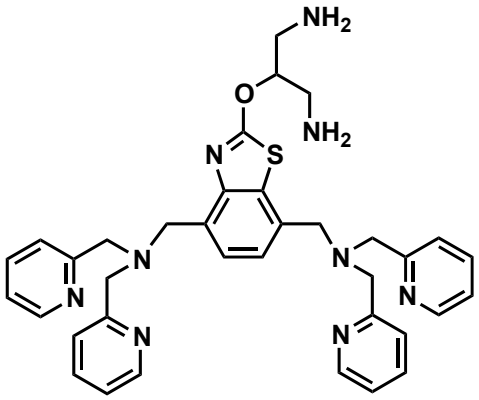


***N*^{1}-(4,7-bis((bis(pyridin-2-ylmethyl)amino)methyl)benzo[*d*]thiazol-2-yl)ethane-1,2-diamine (L5).** δ_H (400 MHz, $CDCl_3$) 2.01 (s, 2H, -NH_2), 2.98 (t, *J* = 5.8 Hz, 2H, -C$\underline{H_2}NH_2$), 3.48 (t, *J* = 5.8, 2H, -$NHCH_2$-), 3.71 (s, 2H, $PhCH_2N$-), 3.76 (s, 4H, -NCH_2Pyr), 3.86 (s, 4H, -NCH_2Pyr), 4.04 (s, 2H, $PhCH_2N$-), 6.99-7.10 (m, 5H, CH (Ar)), 7.42 (d, *J* = 7.5 Hz, 1H, CH (Ar)), 7.49-7.67 (m, 8H, CH (Ar)), 8.48 (t, *J* = 4.9 Hz, 4H, CH (Ar)); δ_C (100 MHz, $CDCl_3$) 41.1, 54.4, 58.5, 60.2, 60.3, 121.6, 121.7,121.8, 122.4, 123.4, 125.8, 130.6, 136.1, 136.2, 148.7, 158.9, 160.1, 167.3; HRMS (ES+) *m/z* calculated for $C_{35}H_{39}N_9S$ [M+2H] 308.6519. Found 308.6529; HRMS

(ES+) *m/z* calcd for $C_{35}H_{37}N_9SZn$ [L5+Zn] 339.6068. Found 339.6083; IR (KBr, cm^{-1}) 3506, 2923, 2360, 1609, 1560, 1484, 1444, 1384, 1251, 1171, 1033, 823, 766.

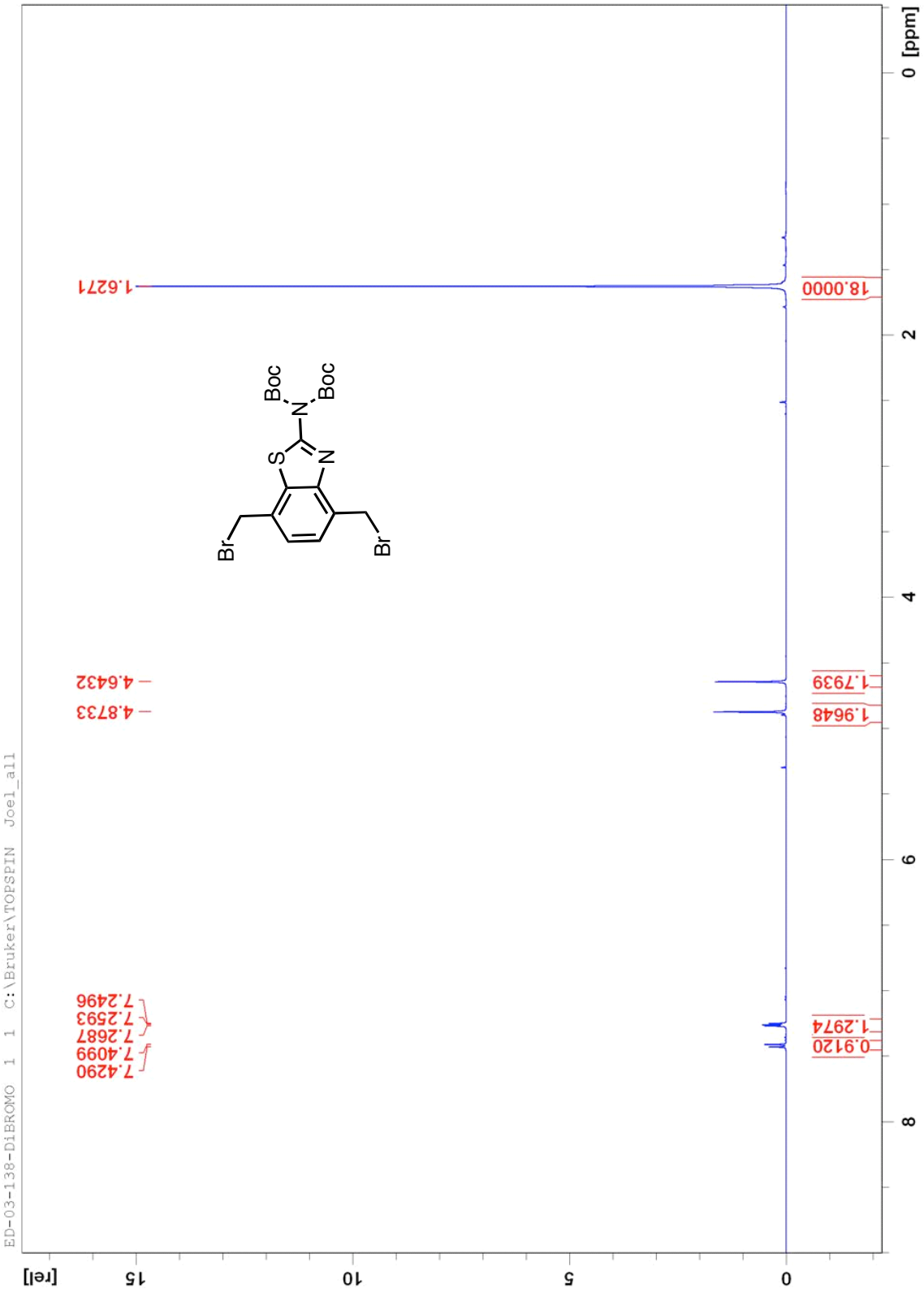


***N*^{1}-(4,7-bis((bis(pyridin-2-ylmethyl)amino)methyl)benzo[*d*]thiazol-2-yl)propane-1,3-diamine (L6).** δ_H (400 MHz, $CDCl_3$) 1.81 (p, *J* = 7.4 Hz, 2H, -$CH_2C\underline{H_2}CH_2$) 1.98 (s, 2H, -NH_2), 2.88 (t, *J* = 6.1 Hz, 2H, -$C\underline{H_2}NH_2$), 3.54 (t, *J* = 6.1, 2H, -$NHCH_2$-), 3.74 (s, 2H, $PhCH_2N$-), 3.79 (s, 4H, -NCH_2Pyr), 3.86 (s, 4H, -NCH_2Pyr), 4.04 (s, 2H, $PhCH_2N$-), 6.34 (s, 1H, NH), 6.99-7.13 (m, 5H, CH (Ar)), 7.44 (d, *J* = 7.7 Hz, 1H, CH (Ar)), 7.51-7.69 (m, 8H, CH (Ar)), 8.47 (t, *J* = 5.9 Hz, 4H, CH (Ar)); δ_C (100 MHz, $CDCl_3$) 32.2, 39.9, 54.5, 58.5, 60.2, 121.6, 121.7, 121.8, 122.5, 123.4, 125.8, 130.6, 136.1, 136.2, 148.7, 148.8, 159.0, 160.1, 163.5, 173.5; HRMS (ES+) *m/z* calcd for $C_{36}H_{41}N_9S$ [M+2H] 315.6598. Found 315.6601; HRMS (ES+) *m/z* calcd for $C_{36}H_{39}N_9SZn$ [L6+Zn] 346.6164. Found 346.6168; IR (KBr, cm^{-1}) 3519, 3204, 2923, 1608, 1561, 1485, 1444, 1411, 1383, 1261, 1169, 1101, 1032, 821, 769.

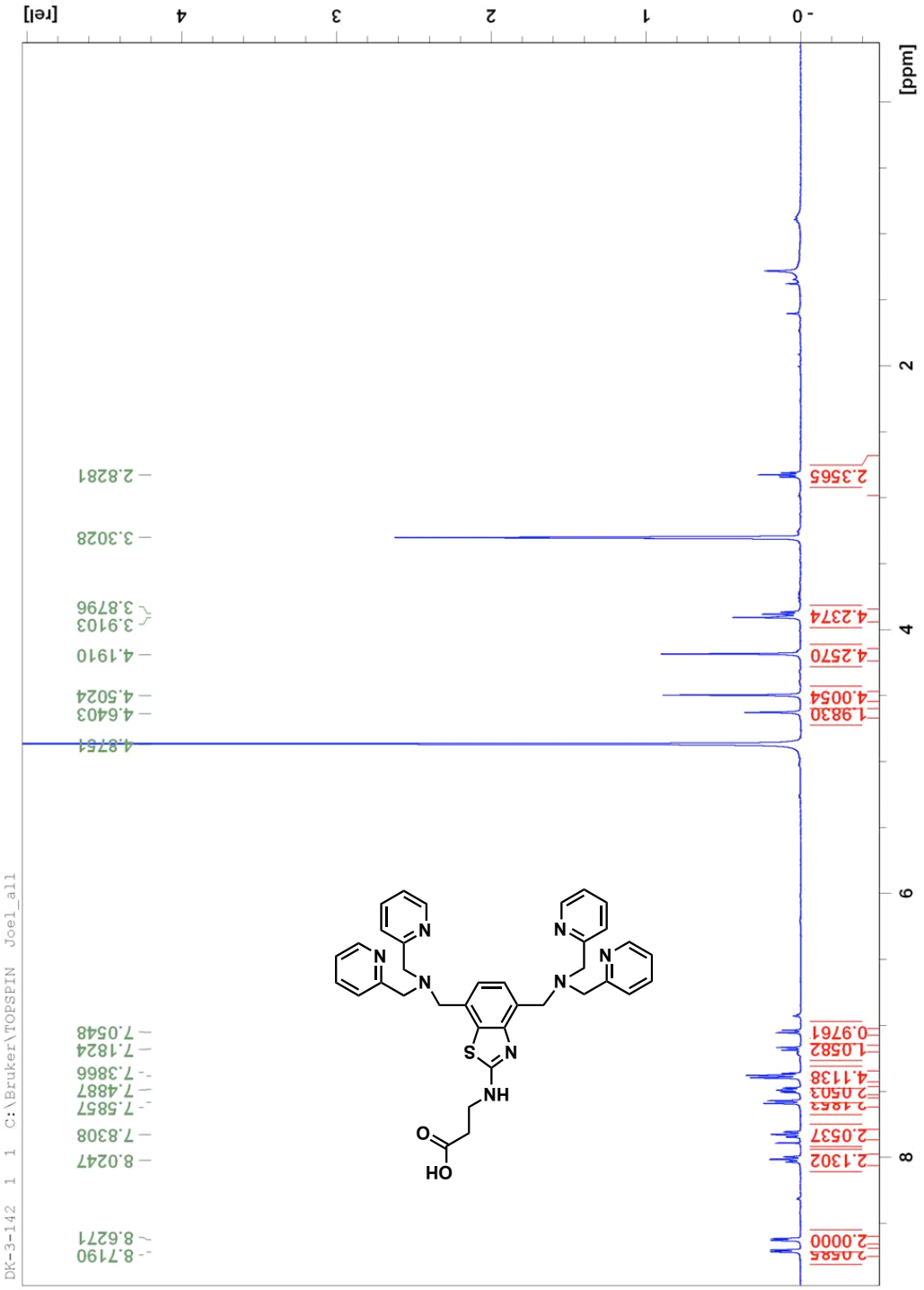


2-((4,7-bis((bis(pyridin-2-ylmethyl)amino)methyl)benzo[*d*]thiazol-2-yl)oxy)propane-1,3-diamine (L7). δ_H (400 MHz, $CDCl_3$) 2.96-3.44 (m, 4H, -C<u>H</u>$_2$$NH_2$) 3.53-4.16 (m, 12H, -$NCH_2$Pyr and $PhCH_2N$-), 4.67 (m, 1H, -OC<u>H</u>(CH_2NH_2) 6.92 (d, *J* = 8.9 Hz, 1H, CH (Ar)), 7.03-7.19 (m, 3H, CH (Ar)), 7.20-7.29 (m, 4H, CH (Ar)), 7.60-7.71 (m, 4H, CH (Ar)), 7.79 (t, *J* = 8.1 Hz, 2H, CH (Ar)), 8.51 (d, *J* = 5.7 Hz, 2H, CH (Ar)) , 8.74 (s, 2H, CH (Ar)), 9.79 (s, 2H, -NH_2); δ_C (100 MHz, $CDCl_3$) 44.2, 54.7, 58.5, 59.1, 59.7, 59.8, 71.2, 121.6, 122.0, 122.3, 123.3, 123.8, 126.7, 129.3, 129.4, 130.4, 131.9, 136.9, 147.7, 147.8, 151.7, 158.3, 159.4, 167.9; HRMS (ES+) *m/z* calcd for $C_{36}H_{41}N_9OS$ [M+2H] 323.6572. Found 323.6585; HRMS (ES+) *m/z* calcd for $C_{36}H_{39}N_9OSZn$ [L7+Zn] 354.6139. Found 354.6146; IR (KBr, cm^{-1}) 3483, 2923, 1609, 1560, 1443, 1384, 1262, 1172, 1033.

Compound ii



3-((4,7-bis((bis(pyridin-2-ylmethyl)amino)methyl)benzo[d]thiazol-2-yl)amino)propanoic acid (L2).



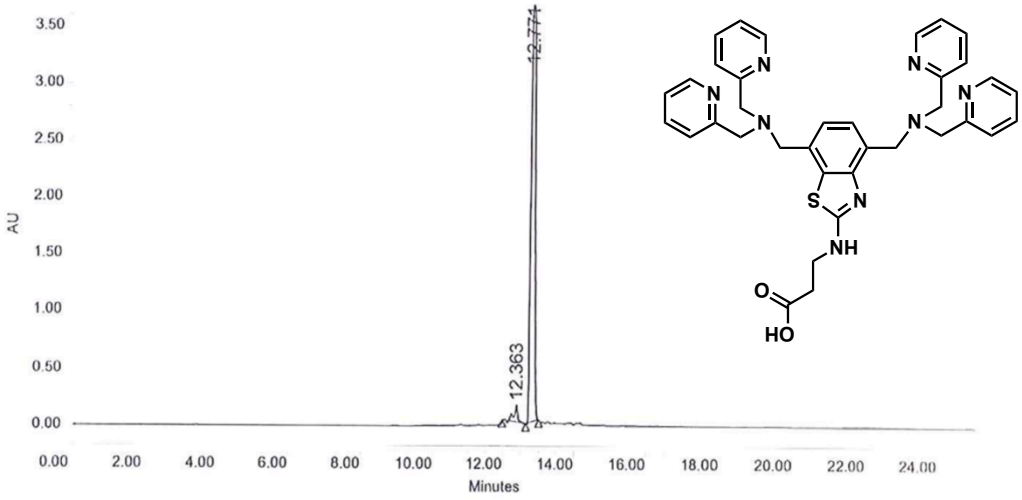
L2

Project Name: GunningP
Reported by User: PatrickGunning

Breeze

SAMPLE INFORMATION

Sample Name:	JD-9-007	Acquired By:	PatrickGunning
Sample Type:	Unknown	Date Acquired:	3/17/2012 12:13:41 PM
Vial:	59	Acq. Method:	Anal_MeOH_Joel2_254nm
Injection #:	1	Date Processed:	3/21/2012 9:08:02 AM
Injection Volume:	50.00 ul	Channel Name:	2487Channel 1
Run Time:	25.00 Minutes	Sample Set Name:	JOEL_HPLC_SCAFFOLDS



	RT (min)	Area (V*sec)	% Area	Height (V)	% Height
1	12.363	1385842	4.33	153998	4.02
2	12.771	30584995	95.67	3676041	95.98

3-((4,7-bis((bis(pyridin-2ylmethyl)amino) methyl)benzo[d]thiazol-2-yl)amino)propanoic acid (L2)

Report Method: Untitled Printed 9:52:34 AM 4/16/2012 Page: 1 of 1

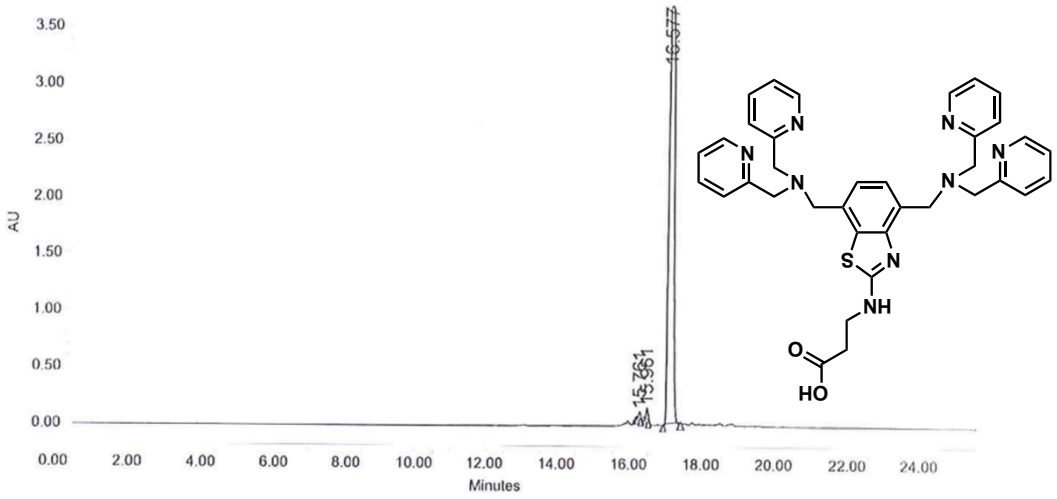
L2

Project Name: GunningP
Reported by User: PatrickGunning

Breeze

SAMPLE INFORMATION

Sample Name:	JD-9-007	Acquired By:	PatrickGunning
Sample Type:	Unknown	Date Acquired:	3/17/2012 11:34:04 AM
Vial:	59	Acq. Method:	Anal_MeOH_Joel_254nm
Injection #:	1	Date Processed:	3/21/2012 9:08:19 AM
Injection Volume:	50.00 ul	Channel Name:	2487Channel 1
Run Time:	25.00 Minutes	Sample Set Name:	JOEL_HPLC_SCAFFOLDS

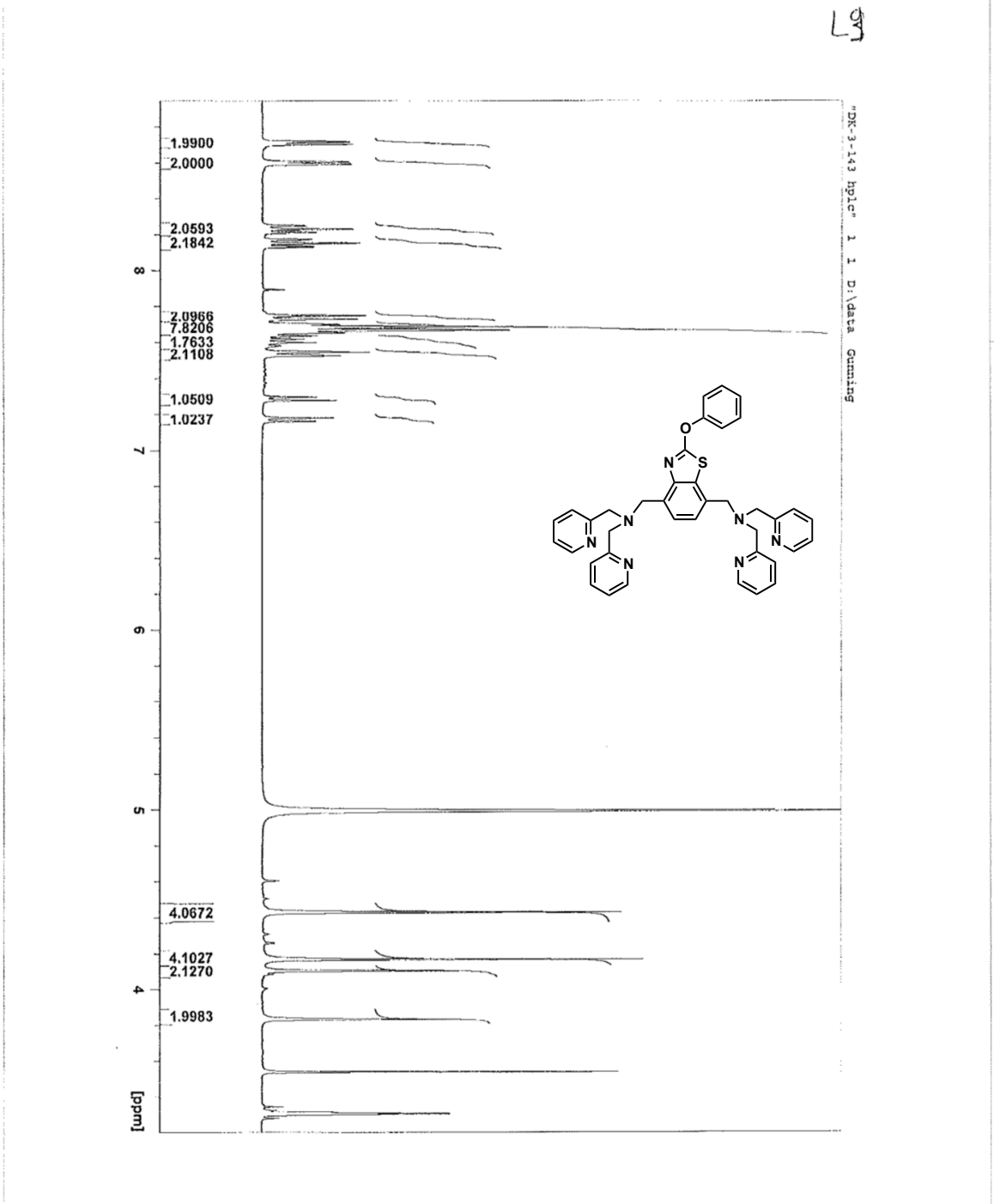


	RT (min)	Area (V*sec)	% Area	Height (V)	% Height
1	15.761	236261	0.61	58824	1.53
2	15.961	360788	0.94	96805	2.51
3	16.577	37851267	98.45	3700154	95.96

3-((4,7-bis((bis(pyridin-2ylmethyl)amino) methyl)benzo[d]thiazol-2-yl)amino)propanoic acid (L2)

Report Method: Untitled Printed 9:51:50 AM 4/16/2012 Page: 1 of 1

N,N'-((2-phenoxybenzo[d]thiazole-4,7-diyl)bis(methylene))bis(1-(pyridin-2-yl)-N-(pyridin-2-ylmethyl)methanamine) (L3).



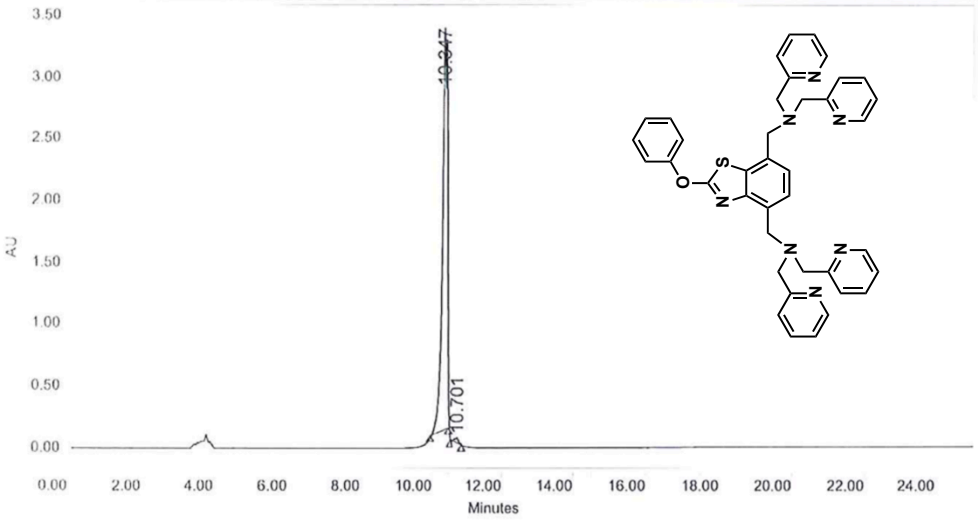
L3

Project Name: GunningP
Reported by User: PatrickGunning

Breeze

SAMPLE INFORMATION

Sample Name:	JD-7-024	Acquired By:	PatrickGunning
Sample Type:	Unknown	Date Acquired:	3/20/2012 10:15:34 AM
Vial:	64	Acq. Method:	Anal_MeOH_Joel_254nm
Injection #:	1	Date Processed:	4/16/2012 12:06:10 PM
Injection Volume:	50.00 ul	Channel Name:	2487Channel 1
Run Time:	25.00 Minutes	Sample Set Name:	JOEL_HPLC_SCAFFOLDS



	RT (min)	Area (V*sec)	% Area	Height (V)	% Height
1	10.347	32275682	98.95	3267270	98.68
2	10.701	341048	1.05	43701	1.32

N,N'-((2-phenoxybenzo[d]thiazole-4,7-diyl)bis(methylene))bis(1-(pyridin-2-yl)-N-(pyridin-2-ylmethyl)methanamine) (L3).

Report Method: Untitled Printed 12:06:16 PM/16/2012 Page: 1 of 1

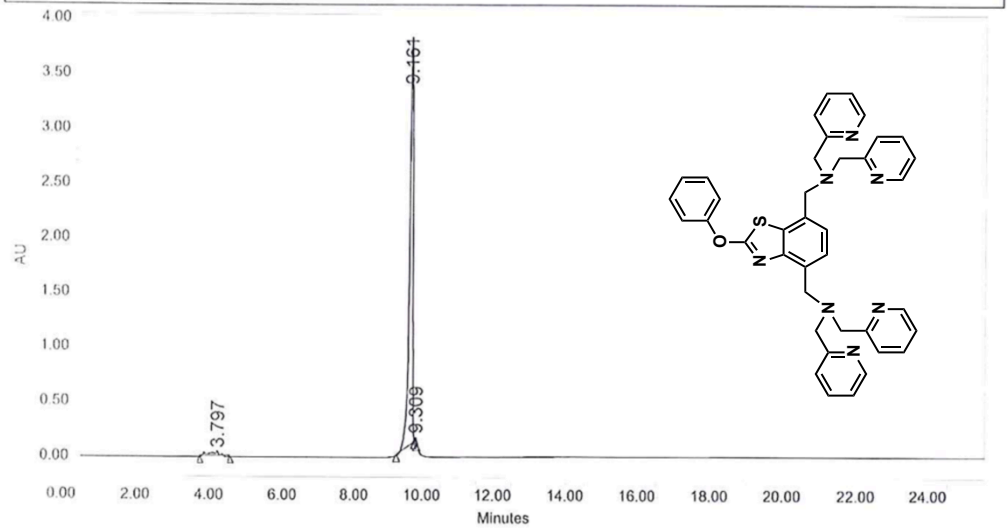
LB

'Breeze

Project Name: GunningP
Reported by User: PatrickGunning

SAMPLE INFORMATION

Sample Name:	JD-7-024	Acquired By:	PatrickGunning
Sample Type:	Unknown	Date Acquired:	3/20/2012 10:55:14 AM
Vial:	64	Acq. Method:	Anal_MeOH_Joel2_254nm
Injection #:	1	Date Processed:	4/16/2012 10:02:19 AM
Injection Volume:	50.00 ul	Channel Name:	2487Channel 1
Run Time:	25.00 Minutes	Sample Set Name:	JOEL_HPLC_SCAFFOLDS

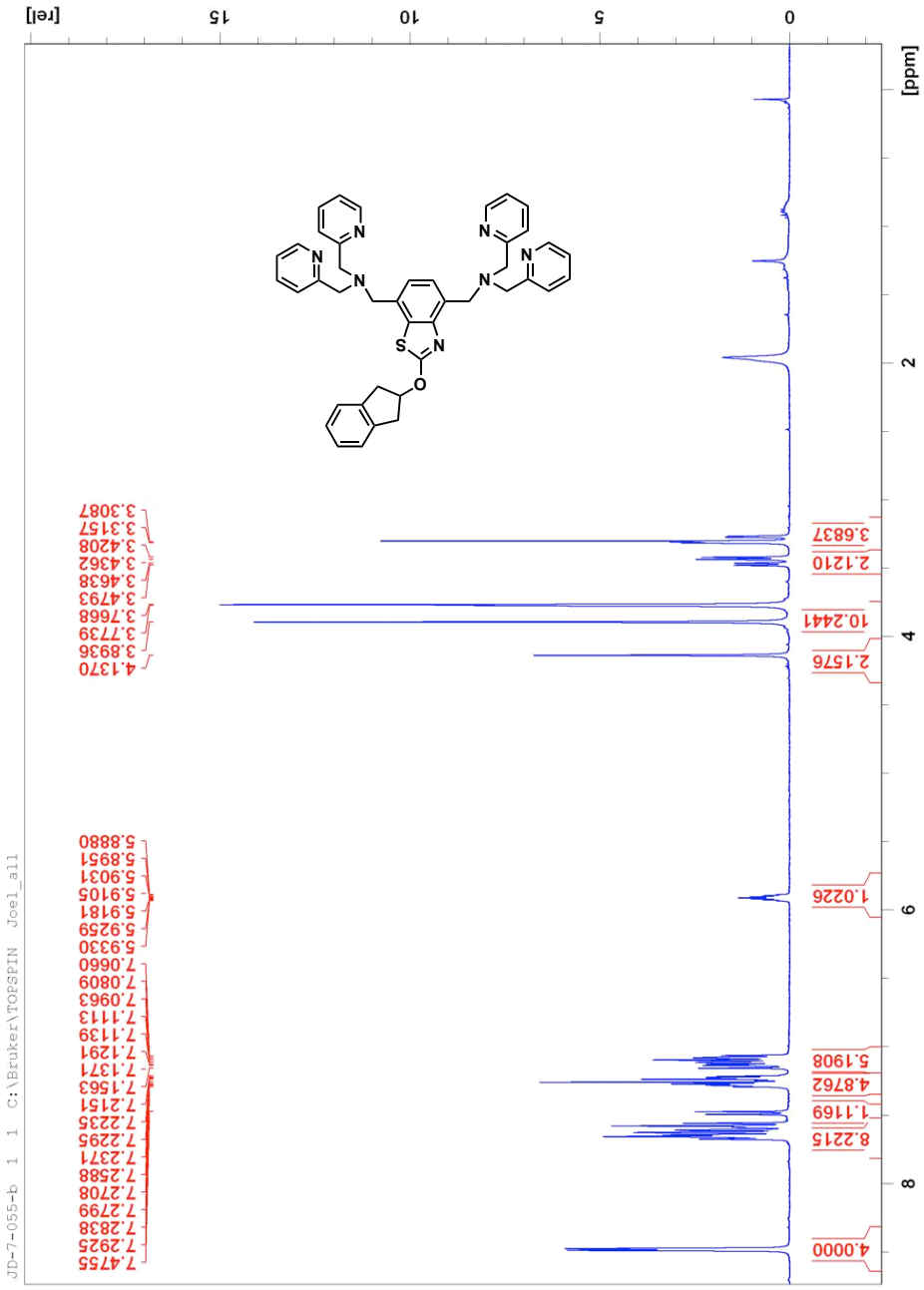


	RT (min)	Area (V*sec)	% Area	Height (V)	% Height
1	3.797	990040	3.98	48088	1.26
2	9.161	23747833	95.51	3735318	97.50
3	9.309	127377	0.51	47615	1.24

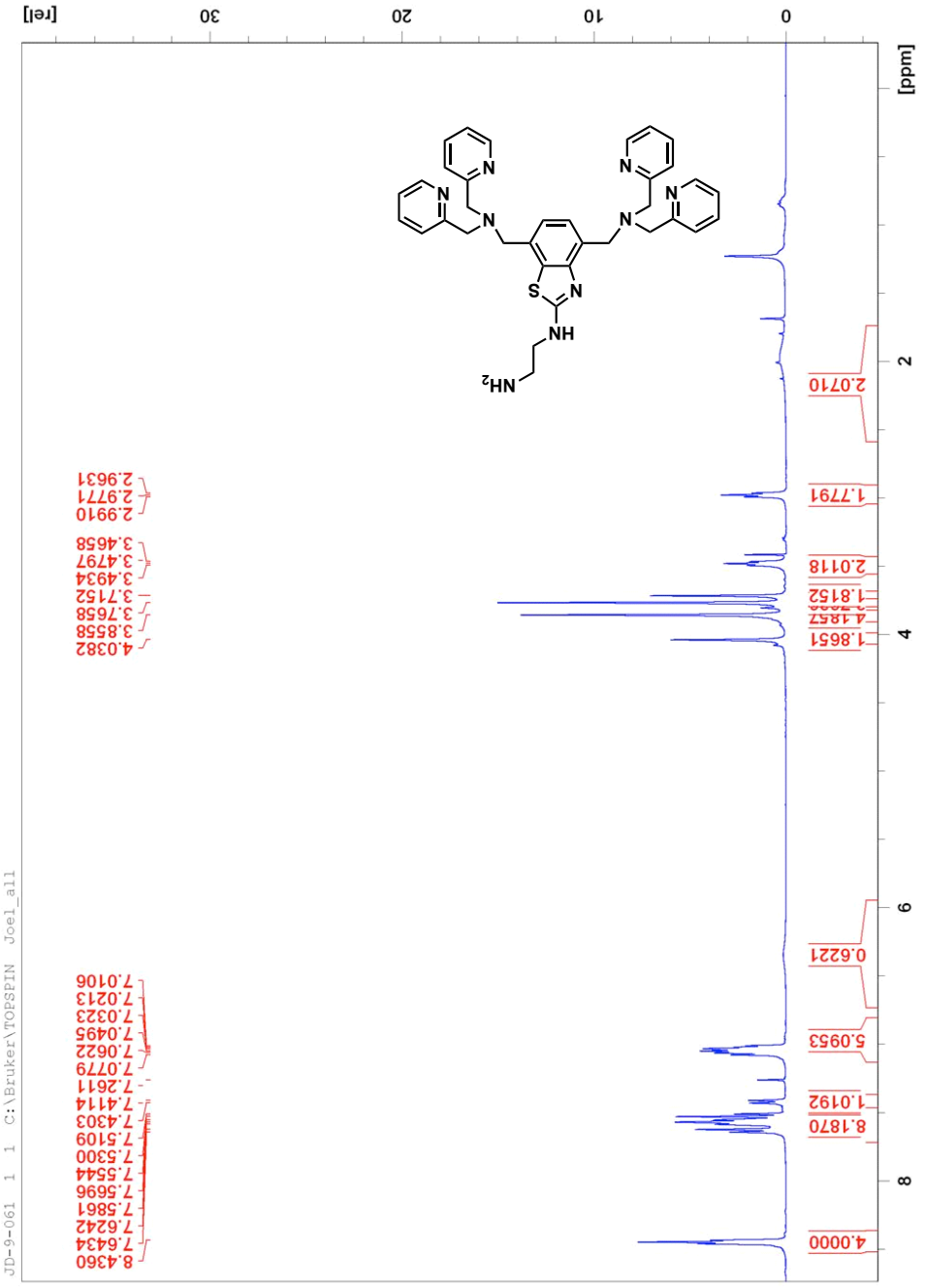
N,N'-((2-phenoxybenzo[d]thiazole-4,7-diyl)bis(methylene))bis(1-(pyridin-2-yl)-N-(pyridin-2-ylmethyl)methanamine) (L3).

Report Method: Untitled Printed 10:02:46 AM/16/2012 Page: 1 of 1

N,N'-((2-((2,3-dihydro-1H-inden-2-yl)oxy)benzo[d]thiazole-4,7-diyl)bis(methylene))bis(1-(pyridin-2-yl)-N-(pyridin-2-ylmethyl)methanamine) (L4



N1-(4,7-bis((bis(pyridin-2-ylmethyl)amino)methyl)benzo[d]thiazol-2-yl)ethane-1,2-diamine (L5).



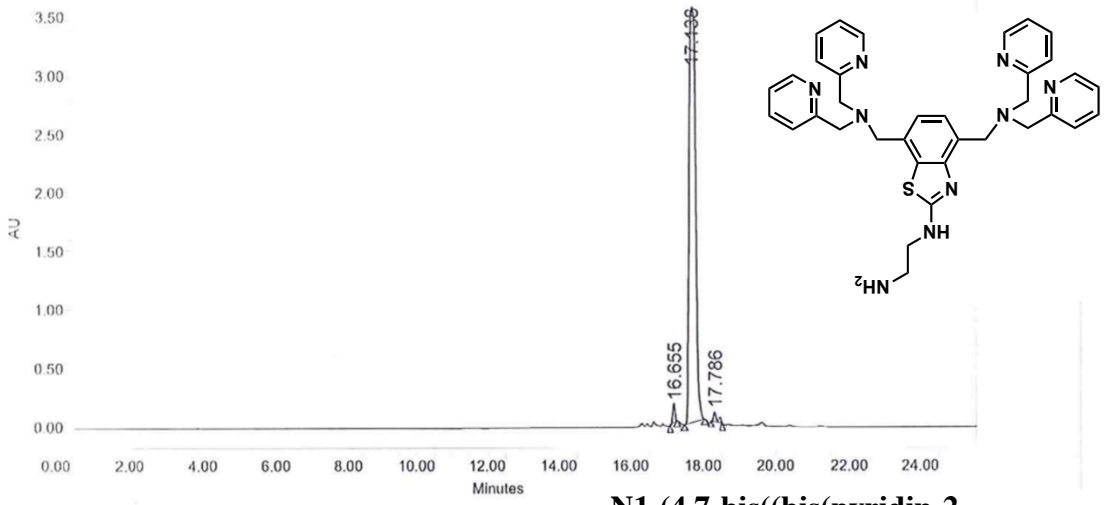
L15

Project Name: GunningP
Reported by User: PatrickGunning

Breeze

SAMPLE INFORMATION

Sample Name:	JD-9-061	Acquired By:	PatrickGunning
Sample Type:	Unknown	Date Acquired:	3/17/2012 12:53:22 PM
Vial:	60	Acq. Method:	Anal_MeOH_Joel_254nm
Injection #:	1	Date Processed:	3/21/2012 9:07:39 AM
Injection Volume:	50.00 ul	Channel Name:	2487Channel 1
Run Time:	25.00 Minutes	Sample Set Name:	JOEL_HPLC_SCAFFOLDS



	RT (min)	Area (V*sec)	% Area	Height (V)	% Height
1	16.655	759182	1.63	173765	4.55
2	17.138	45142128	96.90	3559459	93.22
3	17.786	684533	1.47	85286	2.23

N1-(4,7-bis((bis(pyridin-2-ylmethyl)amino)methyl)benzo[d]thiazol-2-yl)ethane-1,2-diamine (L5).

Report Method: Untitled Printed 9:53:09 AM 4/16/2012 Page: 1 of 1

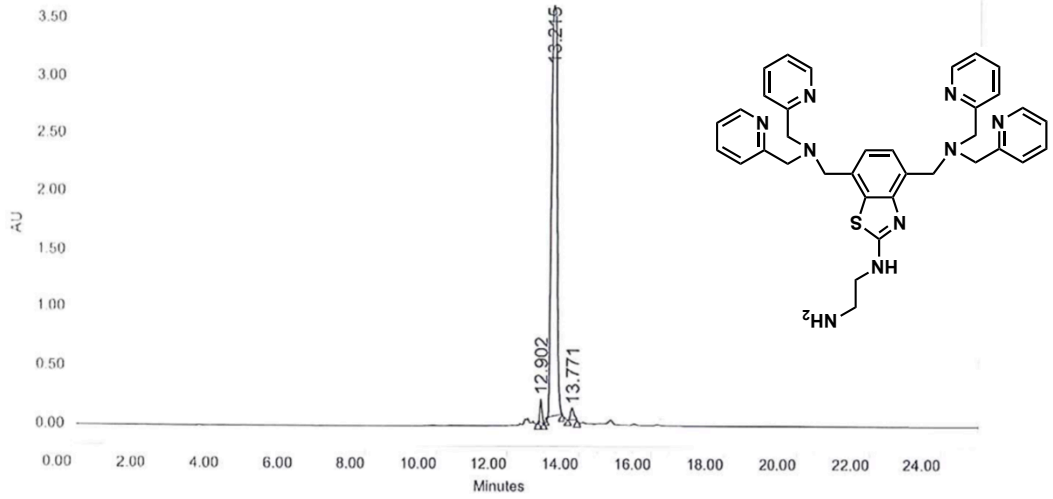
L5

Breeze

Project Name: GunningP
Reported by User: PatrickGunning

SAMPLE INFORMATION

Sample Name:	JD-9-061	Acquired By:	PatrickGunning
Sample Type:	Unknown	Date Acquired:	3/17/2012 1:32:59 PM
Vial:	60	Acq. Method:	Anal_MeOH_Joel2_254nm
Injection #:	1	Date Processed:	3/21/2012 9:07:11 AM
Injection Volume:	50.00 ul	Channel Name:	2487Channel 1
Run Time:	25.00 Minutes	Sample Set Name:	JOEL_HPLC_SCAFFOLDS



	RT (min)	Area (V*sec)	% Area	Height (V)	% Height
1	12.902	821405	2.03	200704	5.18
2	13.215	38879013	96.08	3563562	92.04
3	13.771	765272	1.89	107617	2.78

N1-(4,7-bis((bis(pyridin-2-ylmethyl)amino)methyl)benzo[d]thiazol-2-yl)ethane-1,2-diamine (L5).

Report Method: Untitled Printed 9:53:55 AM 4/16/2012 Page: 1 of 1

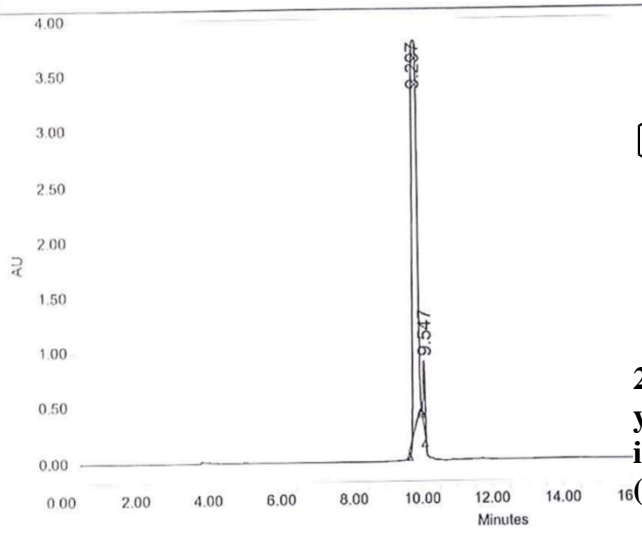
LA

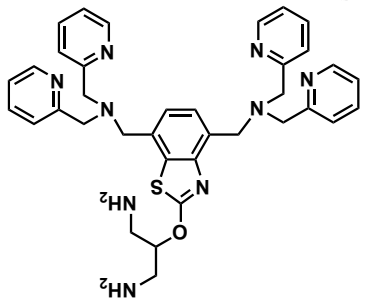
Breeze

Project Name: GunningP
Reported by User: PatrickGunning

SAMPLE INFORMATION

Sample Name:	jd-7-055	Acquired By:	PatrickGunning
Sample Type:	Unknown	Date Acquired:	3/20/2012 8:03:36 PM
Vial:	71	Acq. Method:	Anal_MeOH_Joel2_254nm
Injection #:	1	Date Processed:	3/21/2012 8:39:44 AM
Injection Volume:	50.00 ul	Channel Name:	2487Channel 1
Run Time:	25.00 Minutes	Sample Set Name:	JOEL_HPLC_SCAFFOLDS





2-((4,7-bis((bis(pyridin-2-ylmethyl)amino)methyl)benzo[d]thiazol-2-yl)oxy)propane-1,3-diamine (L7).

	RT (min)	Area (V*sec)	% Area	Height (V)	% Height
1	9.297	34975277	95.07	3569366	85.75
2	9.547	1813430	4.93	593326	14.25

Report Method: Untitled Printed 10:08:12 AM/16/2012 Page: 1 of 1