Bioluminescence, Photophysical, Computational and Molecular Docking Studies of a Rationally Designed Fully Conformationally Restricted Enamine Infraluciferin

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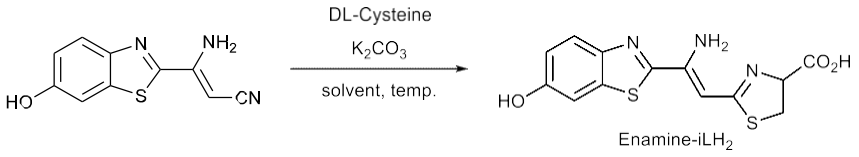
General Experimental

Anhydrous reactions were performed in flame dried glassware under a positive flow of nitrogen gas. Reaction temperatures of 0 ˚C was achieved by the use of an ice-water bath and of -78 ˚C by using a dry ice in acetone bath. All commercially available solvents and chemicals were used without further purification. The anhydrous solvents tetrahydrofuran (THF), dichloromethane (DCM) and toluene were obtained from the in-house drying solvent system. Thin layer chromatography (TLC) was carried out on Merck aluminum backed DC 60 F254 0.2 mm pre-coated plates. Visualization of the TLC was under ultraviolet light (254 nm or 364 nm). Flash column chromatography was prepared on Gedran silca gel 60, 40-63 μm.

The ^{1}H and ^{13}C NMR spectra were recorded on the following machines: Bruker Avance III 400, Bruker Avance III 600, and Bruker Acance Neo 700 spectrometer. All the NMR spectra were manipulated using MestReNova (Version 11.0). The chemical shife (δ) was reported in parts per million (ppm) and coupling constants are quoted in Hertz (Hz). Two-dimensional NMR (COSY, HSQC, HMBC and NOESY) were used to assist full assignment where required. A Perkin Elmer Spectrum 100 FT/IR with 100 μATR machine was used to collect IR spectra under thin film conditions or as a fine solid. Mass spectra were obtained on a ThermoMAT900 and an Accela LC-Finnigan LTQ instruments. Reverse phase HPLC was carried out with ZoRBAX 300SB-C18, 5 μm, 9.4*250 mm column with ASI-100 Automated Sample Injector. Computational study was conducted by Q-Chem 5.4, and visualized by Avogadro.

Optimising reaction conditions for the synthesis of Enamine-iLH$_2$ **13**



20 **13**

A solution of **20** (10 mg, 50 μmol), DL-cysteine (6.0 mg, 50 μmol), and anhydrous K_2CO_3 (6.2 mg, 50 μmol) in solvent (1 mL) were stirred at temp ◦C for 24 h under N_2. After the volatile materials were removed in vacuo, any crude product was purified by reverse phase C18 HPLC with gradient elution (5% Methanol：95% H_2O followed by 95% Methanol：5% H_2O) to give Enamine-iLH$_2$ **13**.

Table S 1 Screening Reaction Conditions for the synthesis of Enamine-iLH$_2$

Entry	Solvents (1.0 mL)	Temperature (°C)	Time (h)	Yields (%)
1	H_2O：MeOH = 1：2	rt	0.5	trace
2	H_2O：MeOH = 1：2	rt	24	trace
3	H_2O：MeOH = 1：2	80	0.5	trace
4	H_2O：MeOH = 1：2	80	24	trace
5	MeOH	50	24	trace
6	MeOH	60	24	trace
7	MeOH	70	24	trace
8	MeOH	80	24	trace
9	Dried MeOH	50	24	~6
10	Dried MeOH	60	24	~6
11	Dried MeOH	70	24	~10
12	Dried MeOH	80	24	20

Docking method via AutoDock Vina 10.1

Preparing the protein

Docking studies *via* AutoDock Vina 10.1 Preparing the protein using Chimera (UCFS Chimera version 1.13.1), the protein PpyLuc (from protein data bank accession number 4G36)[S1] was loaded, and any water molecules and other subunits deleted. The protein was then saved as a pdb file. The protein pdb file was opened using AutoDock Tools (version 1.5.6) and hydrogens added to the protein. The box size for ligand docking was set to cover the active site but not bias the results and saved as a pdbqt file.

Preparing the ligand

ChemDraw smiles were generated, and the energy of the ligand was minimised by using Avogadro (version 1.2.0). The ligand pdb file was converted into a pdbqt file via AutoDock Tools.

Using Autodock Vina (v.1.2.0)

A text file was created input all the parameters for docking:

receptor = protein file name.pdbqt

ligand = ligand file name.pdbqt

out = out.pdbqt

center_x = -58.854

center_y = 27.22

center_z = -49.47

size_x = 40

size_y = 54

size_z = 34

Centre x, y and z and the size parameters were for the docking box - the values that were recorded from Autodock tools. 'out = out.pdbqt' stored output files (different conformations and positions of the ligand). This was saved as the receptor and ligand pdbqt files. Then docking was performed *via* Vina through a terminal window. Vina was run and the binding modes results and energies were saved in a log file (log.txt).

Viewing the results

Docking results (binding modes) were viewed using Chimera by loading in the receptor pdb file and then out.pdb (all the output files). Nine possible binding modes were generated and ranked according to the affinity free energy. The scoring function used in Vina was derived using the PDBbind data set, in which the receptors were treated as rigid components, and the

ligands as flexible molecules with the number of active rotatable bonds ranging from 0 to 32. Vina uses a gradient optimization method in its local optimization procedure. The calculation of the gradient gives the optimization algorithm a “sense of direction” from a single evaluation. By using multithreading, Vina can further speed up the execution by taking advantage of multiple CPUs or CPU cores.

Table S 2 Docking results of Enamine-iLH$_2$-DLSA **27** with PpyLuc

Mode Ra ıkings	Affinity (Kcal/mol)	Distance from best mode rmsd l.b.	rmsd u.b.
1	-10.1	0.000	0.000
2	-10.0	12.999	15.175
3	-10.0	12.395	15.812
4	-9.9	5.294	9.985
5	-9.7	14.206	15.999
6	-9.5	4.343	9.525
7	-9.2	5.564	10.722
8	-9.2	5.817	10.057
9	-9.2	13.538	16.887

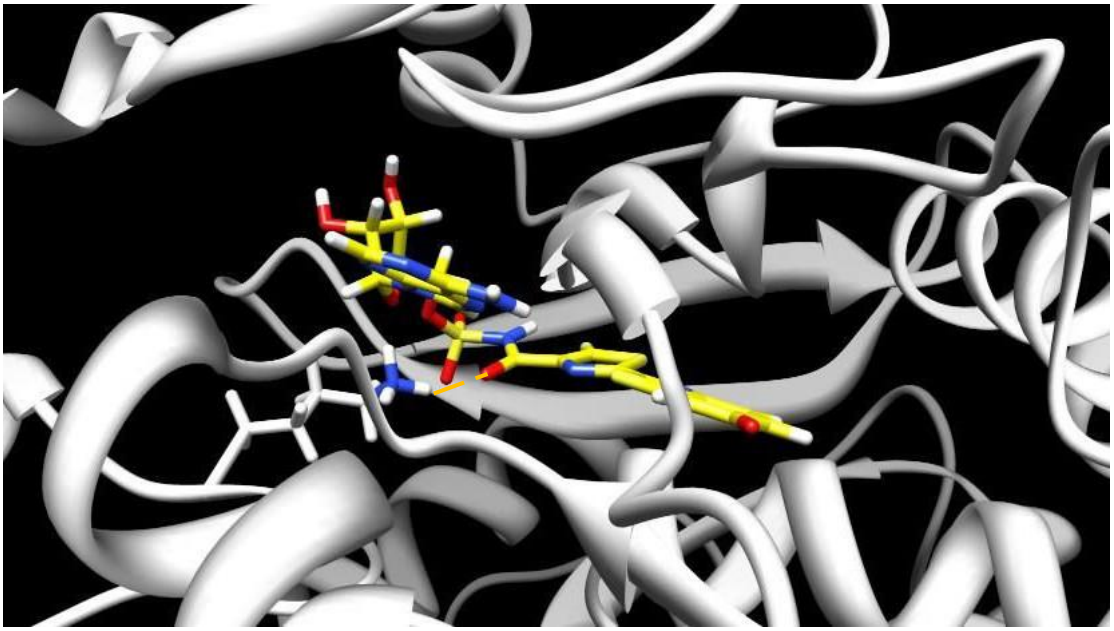


Figure S 1 Cocrystal structure of LH$_2$-DLSA **25** with PpyLuc [S1]

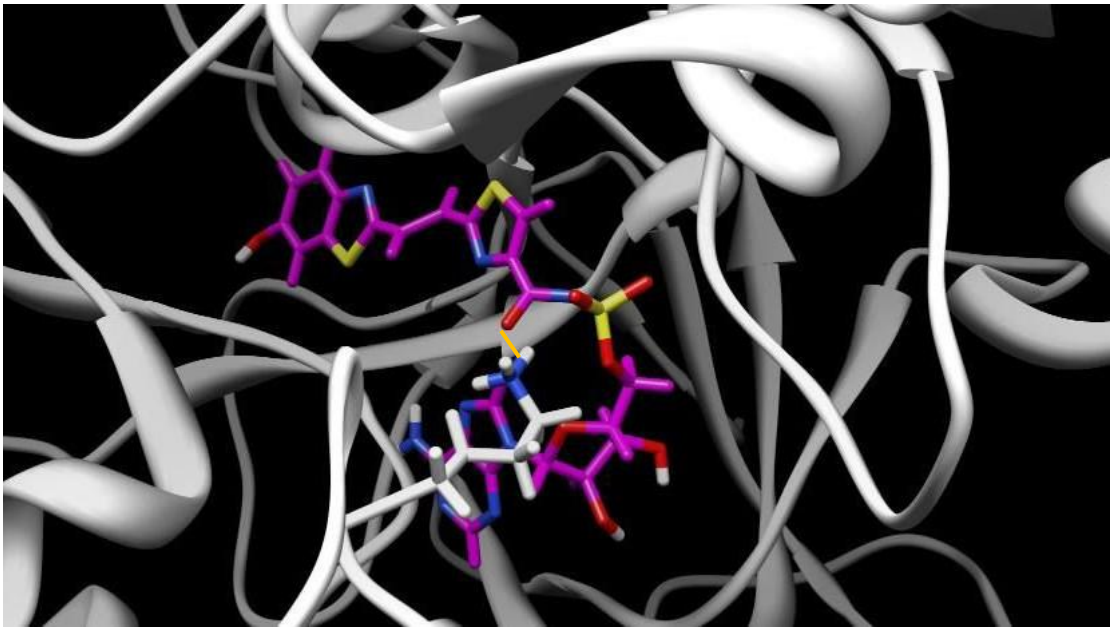


Figure S 2 Cocrystal structure of iLH_2-DLSA **26** with PpyLuc [S2]

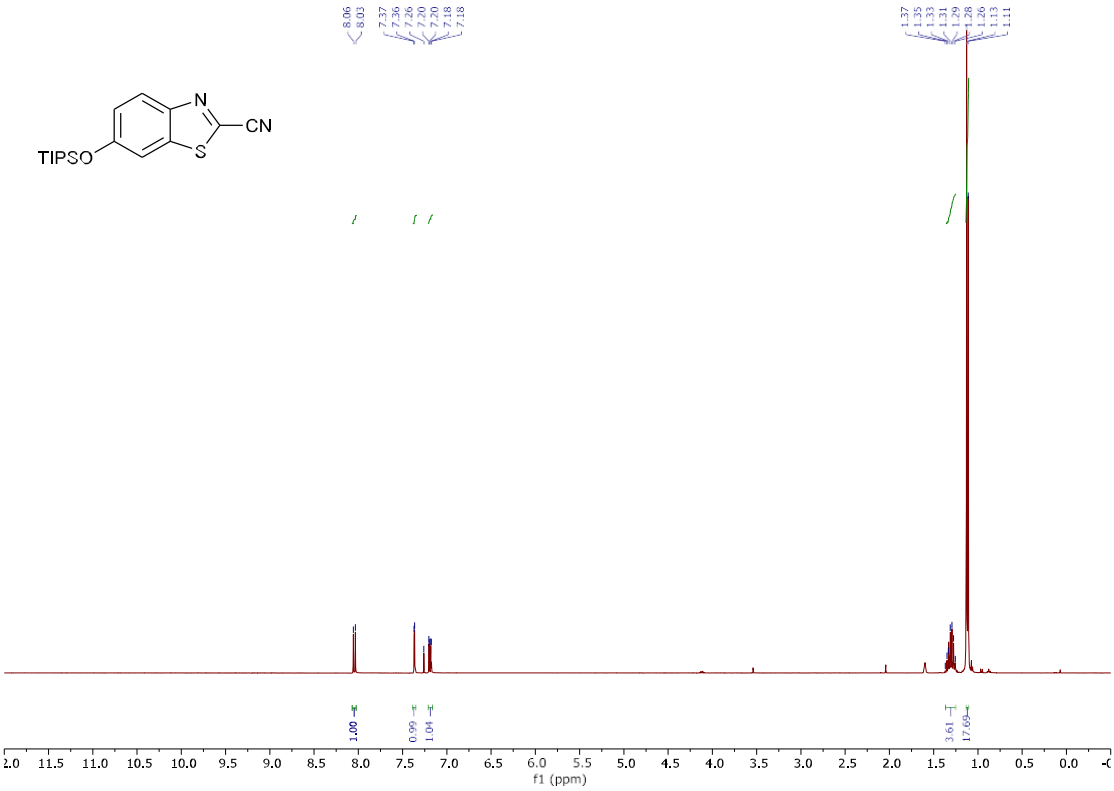
References

S1 J. A. Sundlov, D. M. Fontaine, T. L. Southworth, B. R. Branchini and A. M. Gulick, *Biochemistry*, 2012, **51**, 6493–6495.

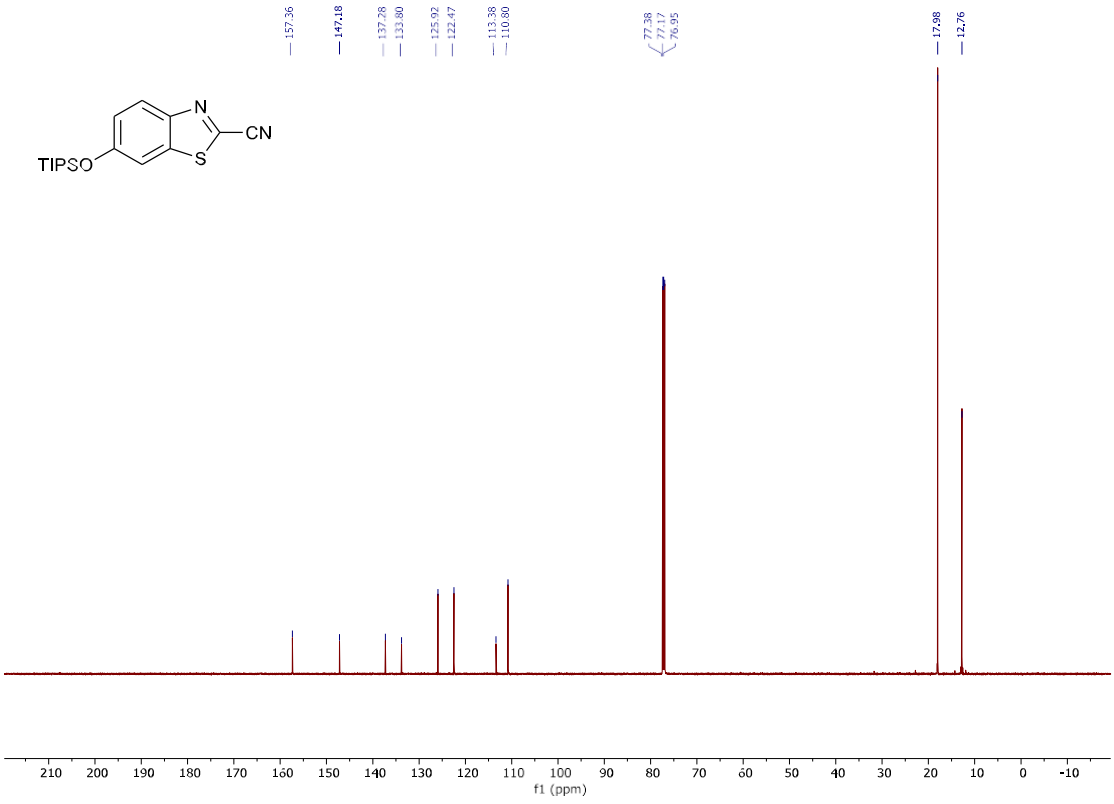
S2 C. L. Stowe, T. A. Burley, H. Allan, M. Vinci, G. Kramer-Marek, D. M. Ciobota, G. N. Parkinson, T. L. Southworth, G. Agliardi, A. Hotblack, M. F. Lythgoe, B. R. Branchini, T. L. Kalber, J. C. Anderson and M. A. Pule, *Elife*, 2019, **8**, 1–22.

^{1}H and ^{13}C NMR, and IR spectra for new compounds

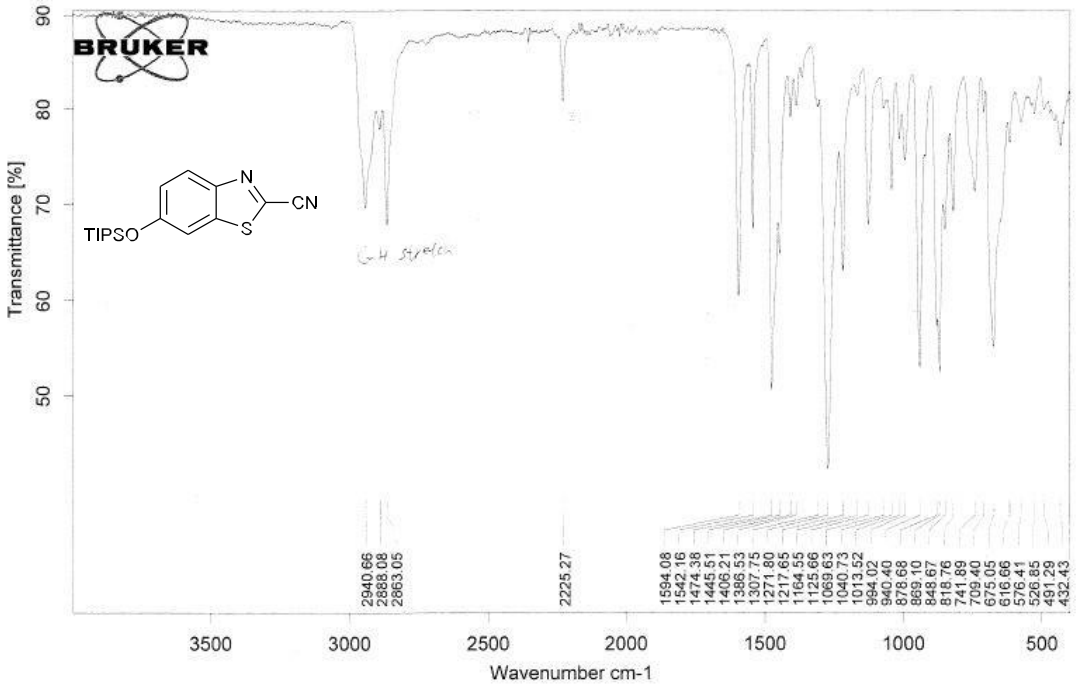
^{1}H NMR (400 MHz, $CDCl_3$): 6-(triisopropylsilyloxy)benzo[*d*]thiazole-2-carbonitrile (**18**)



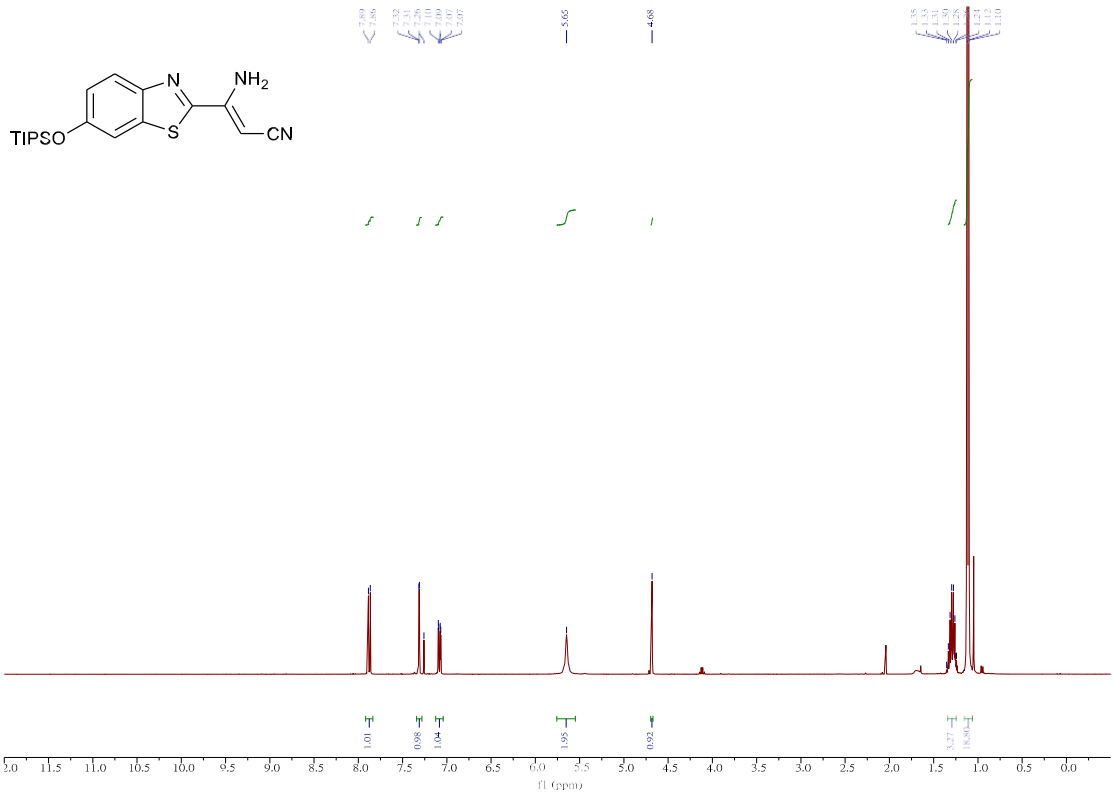
^{13}C NMR (150 MHz, $CDCl_3$): 6-(triisopropylsilyloxy)benzo[*d*]thiazole-2-carbonitrile (**18**)



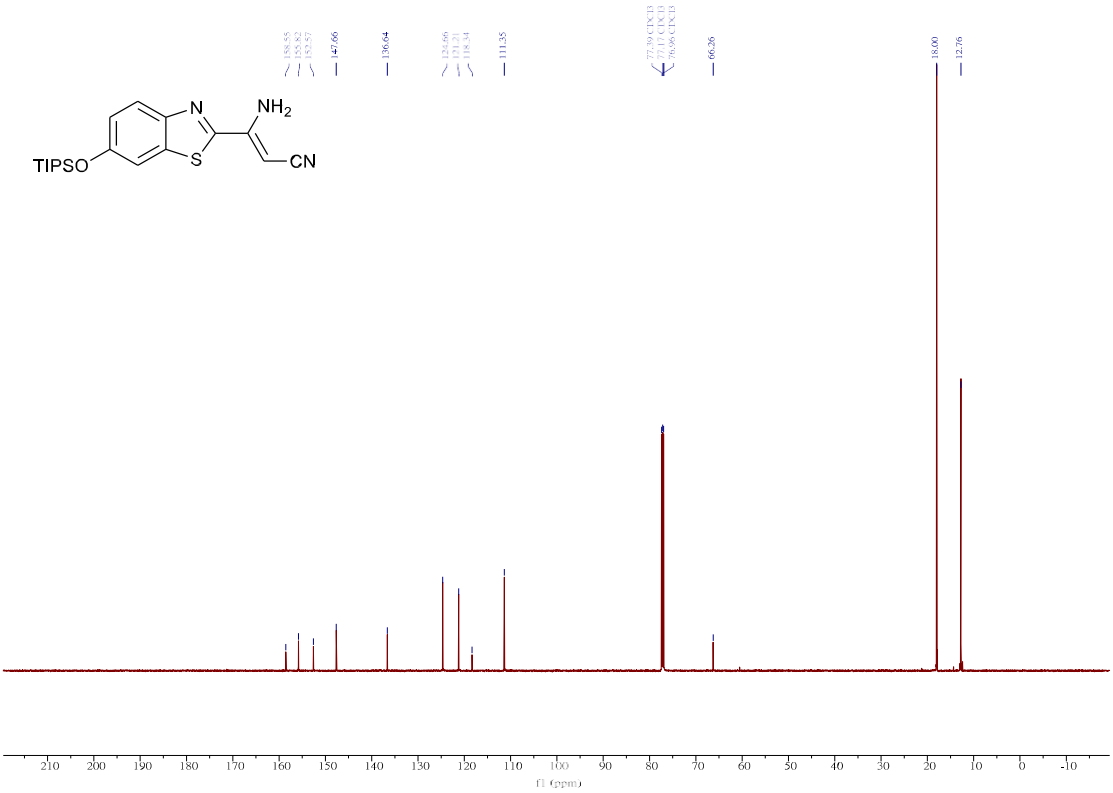
FTIR: 6-(triisopropylsilyloxy)benzo[*d*]thiazole-2-carbonitrile (**18**)



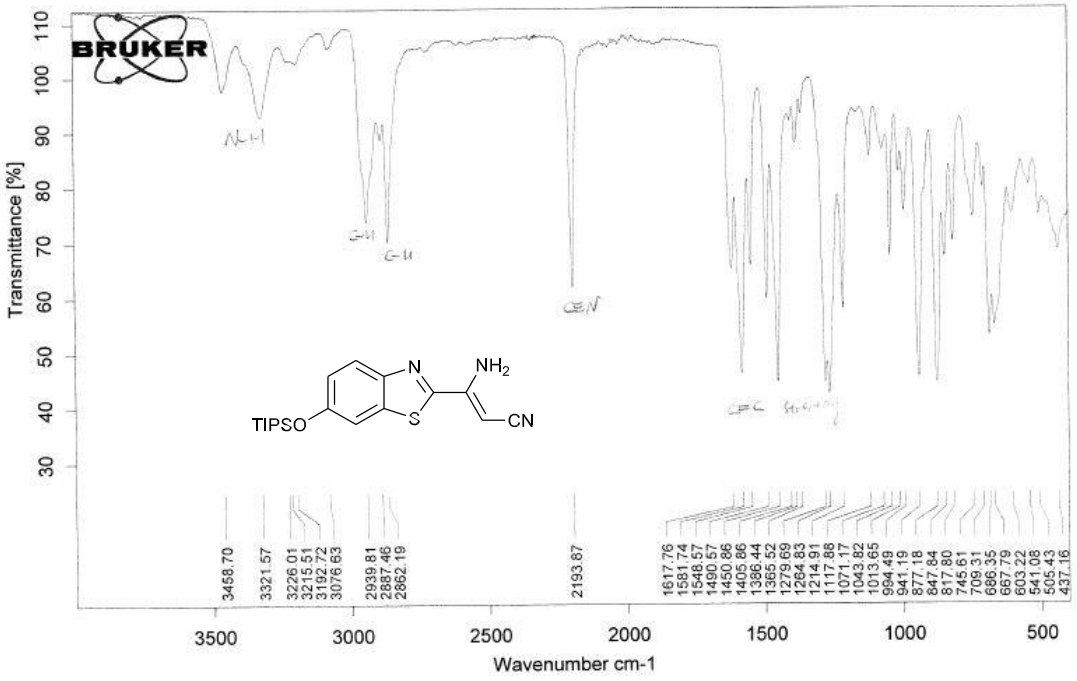
^{1}H NMR (400 MHz, $CDCl_3$): (*Z*)-3-amino-3-(6-(triisopropylsilyloxy)benzo[d]thiazol-2-yl)acrylonitrile (**19**)



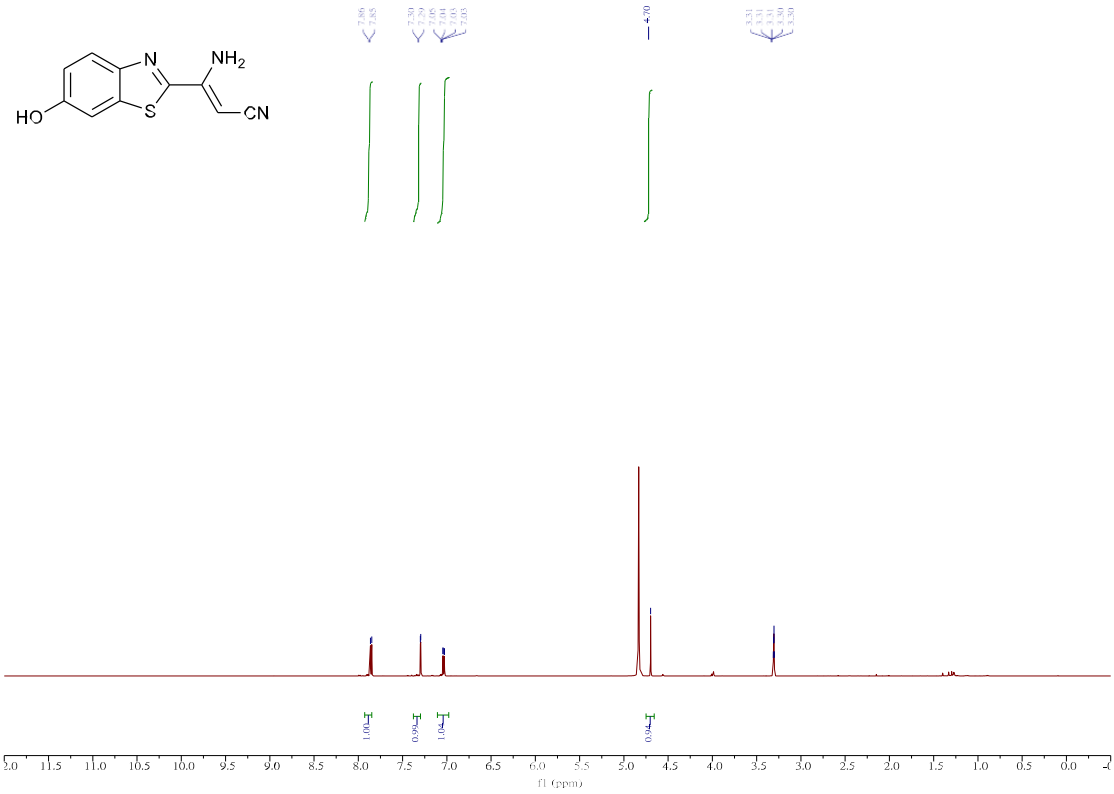
^{13}C NMR (150 MHz, $CDCl_3$): (*Z*)-3-amino-3-(6-(triisopropylsilyloxy)benzo[d]thiazol-2-yl)acrylonitrile (**19**)



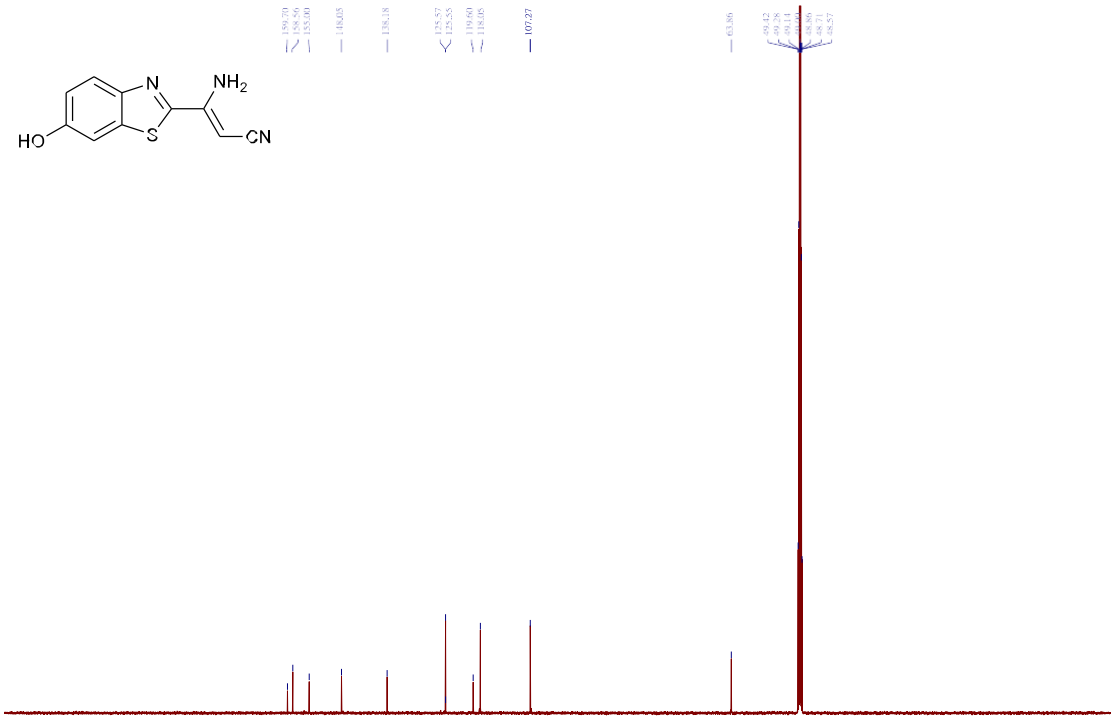
FTIR: (*Z*)-3-amino-3-(6-(triisopropylsilyloxy)benzo[d]thiazol-2-yl)acrylonitrile (**19**)



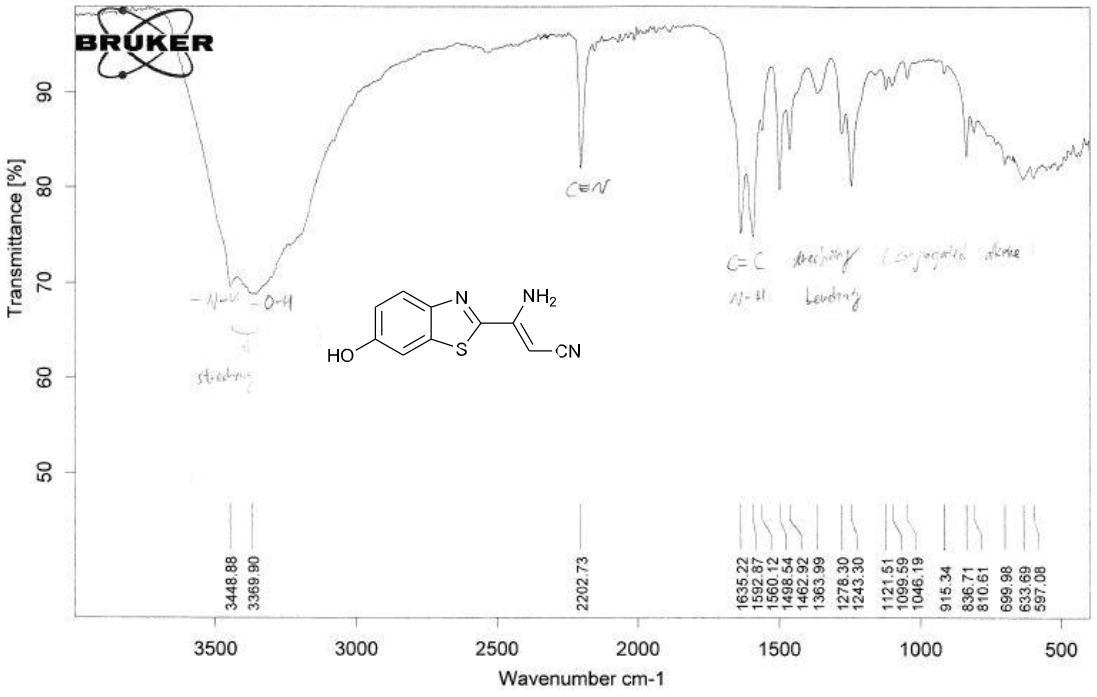
^{1}H NMR (600 MHz, CD_3OD): (*Z*)-3-amino-3-(6-hydroxybenzo[d]thiazol-2-yl)acrylonitrile (**20**)



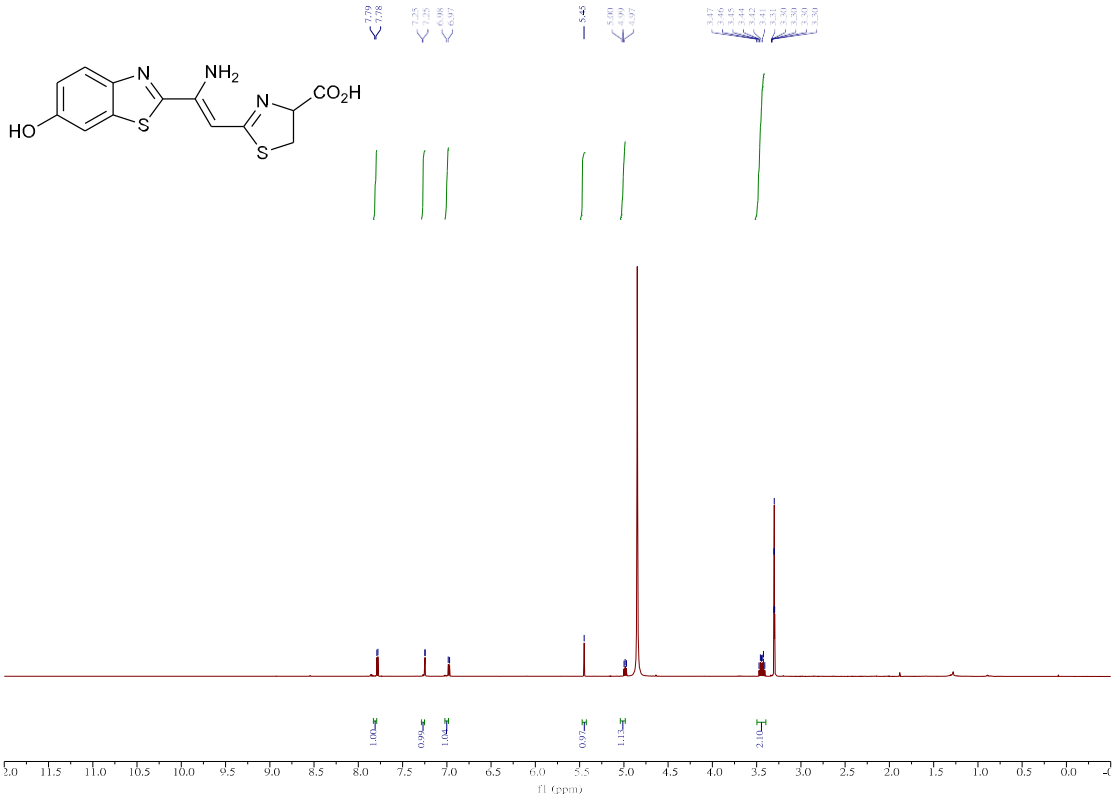
^{13}C NMR (150 MHz, CD_3OD): (*Z*)-3-amino-3-(6-hydroxybenzo[d]thiazol-2-yl)acrylonitrile (**20**)



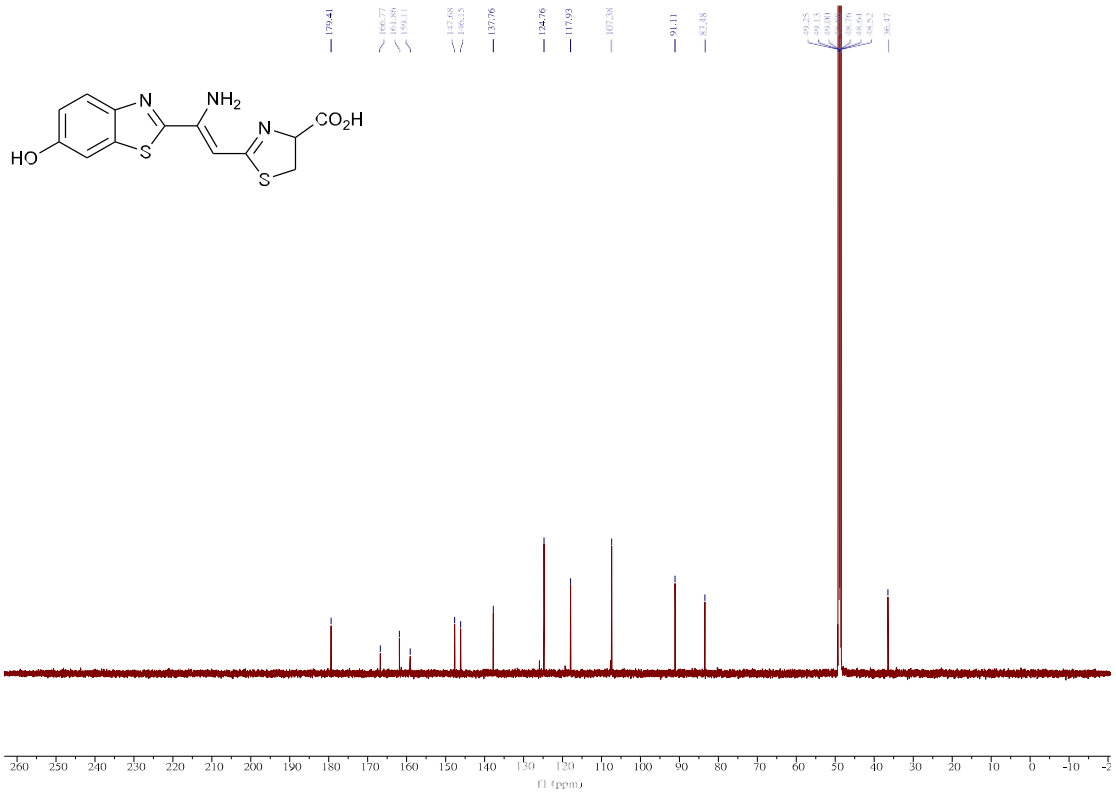
FTIR: (*Z*)-3-amino-3-(6-hydroxybenzo[d]thiazol-2-yl)acrylonitrile (**20**)



^{1}H NMR (700 MHz, CD_3OD): Enamine-iLH_2 **13**



^{13}C NMR (175 MHz, CD_3OD): Enamine-iLH_2 **13**



FTIR: Enamine-iLH_2 **13**