

# ChemBioChem

Supporting Information

## **Development of the First Tritiated Tetrazine: Facilitating Tritiation of Proteins**

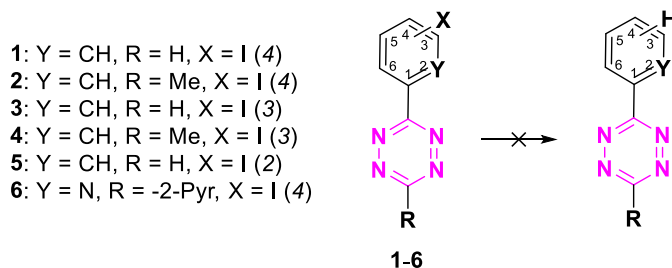
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## 2. Radiolabeling

**Table S1.** Reagents and conditions for Pd-catalyzed hydrodehalogenations of Tz **1-6**.



Entry	Reagent	Catalyst	H-source	Base/Acid	Solvent	Temperature	Time	Conversion	Ref
1	<b>1-6</b>	Pd/C	H <sub>2</sub> (g)	-	EtOH	R.T. to 100 °C	1h-3d	-	[1]
2	<b>1-6</b>	Pd/C	H <sub>2</sub> (g)	TEA	EtOH	R.T. to 100 °C	1h-3d	-	[1a, 2]
3	<b>1-6</b>	Pd/C	H <sub>2</sub> (g)	TEA	MeOH	R.T. to 100 °C	1h-3d	-	[1a, 2]
4	<b>1-6</b>	Pd/C	HCO <sub>2</sub> NH <sub>4</sub>	-	MeOH/THF	R.T. to 100 °C	1h-3d	-	[3]
5	<b>1,2,6</b>	Pd/C	H <sub>2</sub> (g)	NH <sub>4</sub> OH	MeOH	R.T. to 100 °C	1h-3d	-	[4]
6	<b>1,2,6</b>	Pd/C	H <sub>2</sub> (g)	Hunig's base	DMF	R.T. to 100 °C	1h-3d	-	[5]
7	<b>1,2,6</b>	Pd/C	H <sub>2</sub> (g)	-	THF	R.T. to 100 °C	1h-3d	-	-
8	<b>1-6</b>	PtO <sub>2</sub>	H <sub>2</sub> (g)	-	EtOH	R.T. to 100 °C	1h-3d	-	[1a, 6]
9	<b>1-6</b>	Pd(dba) <sub>2</sub>	H <sub>2</sub> (g)	-	MeOH	R.T. to 100 °C	1h-3d	-	-
10	<b>1-6</b>	Pd(dba) <sub>2</sub> , SiMes.HCl, KOMe	H <sub>2</sub> (g)	KOMe	dioxane	100 °C	1h-3d	-	[7]
11	<b>1-6</b>	Pd(PPh <sub>3</sub> ) <sub>4</sub>	H <sub>2</sub> (g)	HCO <sub>2</sub> Na	MeOH	R.T. to 100 °C	1h-3d	-	[8]
12	<b>1,2,6</b>	Pd(PPh <sub>3</sub> ) <sub>4</sub>	H <sub>2</sub> (g)	-	THF	R.T. to 100 °C	1h-3d	-	[8]
13	<b>1-6</b>	PdCl <sub>2</sub>	TMDS	-	H <sub>2</sub> O	R.T.	1h-3d	-	[9]

#### *General reduction entry 1*

To a solution of Tz **1-6** (10 mg) in ethanol (0.1 mL) was added Pd/C (10 wt.%, 1 mg) at argon atmosphere. Consequently, the reaction mixture was charged with hydrogen while the flask was connected to a vacuum line. The mixture was stirred at room temperature for 24 h and monitored frequently. After 24 hours the temperature was raised to 100 °C, and monitored for 48 h. For all 6 Tzs no reduction was observed, based on LC-MS, TLC and NMR after filtration of the Pd/C, evaporation of the solvent.

#### *General reduction entry 2*

To general reduction entry 1, 4 µL trimethylamine was added. For all 6 Tzs no reduction was observed, based on LC-MS, TLC and NMR after filtration of the Pd/C, evaporation of the solvent.

#### *General reduction entry 3*

To general reduction entry 1, instead of ethanol, methanol was used. Caution was taken that all glassware and stirring bars were vacuum oven dried before use, and glassware was purged 3 x with argon. For all 6 Tzs no reduction was observed, based on LC-MS, TLC and NMR after filtration of the Pd/C, evaporation of the solvent. Hence, the compounds

#### *General reduction entry 4*

To a solution of Tz **1-6** (10 mg) in ethanol (0.1 mL) was added 1 ml of a saturated solution of HCO<sub>2</sub>NH<sub>4</sub> (40-50 mg of HCO<sub>2</sub>NH<sub>4</sub> in MeOH/THF, 2/1). Subsequently, Pd/C (10 wt.%, 1 mg) was added to the flask at argon atmosphere. The mixture was stirred at room temperature for 24 h and monitored frequently. After 24 hours the temperature was raised to 150 °C, and monitored for 48 h. For all 6 Tzs no reduction was observed, based on LC-MS, TLC and NMR after filtration of the Pd/C and drying of the filtrate in vacuo.

#### *General reduction entry 5*

To general reduction entry 3, 40 µl of HCl in dioxane (8M) was added. For all 3 Tzs (**1, 2, 6**) no reduction was observed, based on LC-MS, TLC and NMR after filtration of the Pd/C, evaporation of the solvent.

#### *General reduction entry 6*

To general reduction entry 3, 4  $\mu\text{L}$  saturated  $\text{NaHCO}_3$  was added. THF was used as solvent instead of MeOH. For all 3 Tzs (**1**, **2**, **6**) no reduction was observed, based on LC-MS, TLC and NMR after filtration of the Pd/C, evaporation of the solvent.

*General reduction entry 7*

To general reduction entry 1, instead of ethanol, THF was used. For all 3 Tzs (**1**, **2**, **6**) no reduction was observed, based on LC-MS, TLC and NMR after filtration of the Pd/C, evaporation of the solvent.

*General reduction entry 8*

To general reduction entry 1, instead of Pd/C, 10 wt%  $\text{PtO}_2$  was used. For all 6 Tzs no reduction was observed, based on LC-MS, TLC and NMR after filtration of the  $\text{PtO}_2$ , evaporation of the solvent.

*General reduction entry 9*

To general reduction entry 1, instead of Pd/C, 10 wt%  $\text{Pd}(\text{dba})_2$  was used. For all 6 Tzs no reduction was observed, based on LC-MS, TLC and NMR after filtration of the  $\text{PtO}_2$ , evaporation of the solvent.

*General reduction entry 10*

To a solution of 10 mg (1 eq.) Tz **1-6** was added  $\text{Pd}(\text{dba})_2$  (0.02 eq.),  $\text{SiMe}_3\text{HCl}$  (0.02 eq.), KOMe (2 eq.), 100 mL of dioxane. Reaction vial was closed and purged with argon. Consequently, the reaction mixture was heated until 100  $^\circ\text{C}$ . For all 6 Tzs no reduction was observed, based on LC-MS, TLC and NMR after filtration of the  $\text{PtO}_2$ , evaporation of the solvent.

*General reduction entry 11*

To a solution of 10 mg (1 eq.) Tz **1-6** was added  $\text{Pd}(\text{PPh}_3)_4$  (0.10 eq.),  $\text{HCOONa}$  (0.2 eq.), 100 mL of MeOH. Reaction vial was closed and purged with argon. Consequently, the reaction mixture was heated until 100  $^\circ\text{C}$ . For all 11 Tzs no reduction was observed, based on LC-MS and TLC.

*General reduction entry 12*

To a solution of 10 mg (1 eq.) Tz **1-6** was added Pd(PPh<sub>3</sub>)<sub>4</sub> (0.10 eq.) in 100 mL of MeOH. Reaction vial was closed and purged with argon. Consequently, the reaction mixture was heated until 100 °C. Consequently, the reaction mixture was charged with hydrogen while the flask was connected to a vacuum line. The mixture was stirred at room temperature for 24 h and monitored frequently. After 24 hours the temperature was raised to 100 °C, and monitored for 48 h. For all 6 Tzs no reduction was observed, based on LC-MS and TLC.

#### *General reduction entry 13*

To a solution of Tz **1-6** (10 mg) in deionized water (0.1 mL) was added PdCl<sub>2</sub> (0.05 eq.). The reaction mixture was stirred for 10 min. To the reaction mixture was added TMDS (0.4 eq.), dropwise. The mixture was stirred at room temperature for 3 days and monitored frequently. For all 6 Tz's no reduction was observed, based on LC-MS and TLC.

#### *3-(4-propoxyphenyl)-1,2,4,5-tetrazine*

Tz **7** was subjected to general reduction entry 1, 2 and 4. No reduction was observed, based on LC-MS, TLC and NMR after filtration of the Pd/C, and evaporation of the solvent.

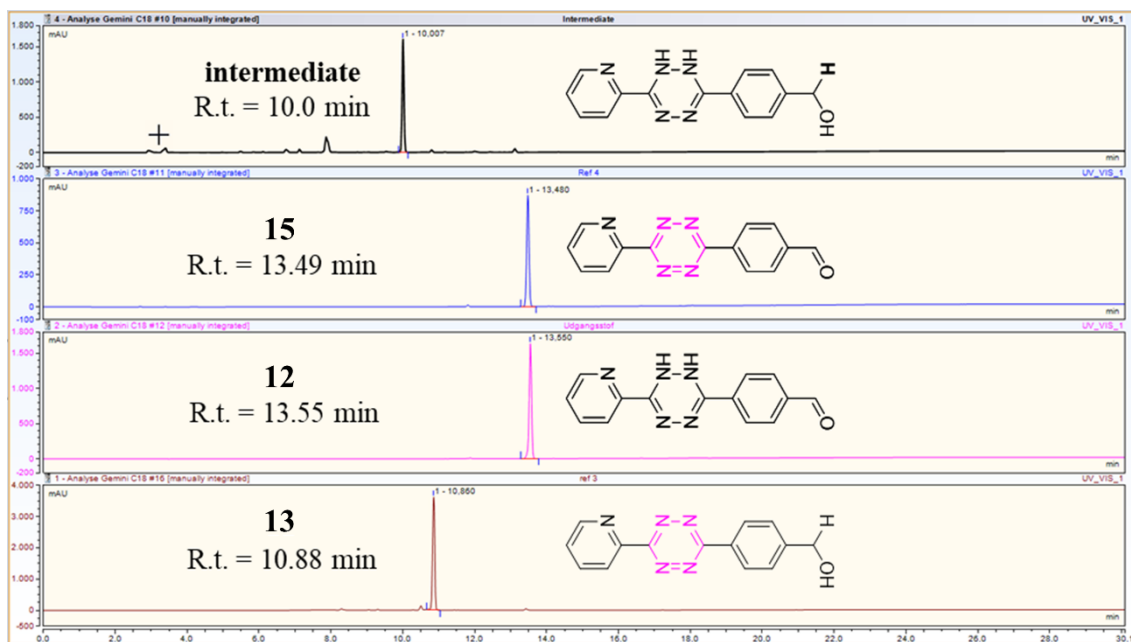
#### *3-(4-vinylphenyl)-1,2,4,5-tetrazine*

To **8** (50 mg, 0.27 mmol), dissolved in 2 mL MeOH was added 2.5 mg of Lindlar catalyst and 25 mg of quinoline. Hydrogen gas contained in a balloon was bubbled into the reaction mixture at room temperature for 3 days. Reaction was monitored by TLC and LC-MS, however after 5 days no conversion was observed yet.

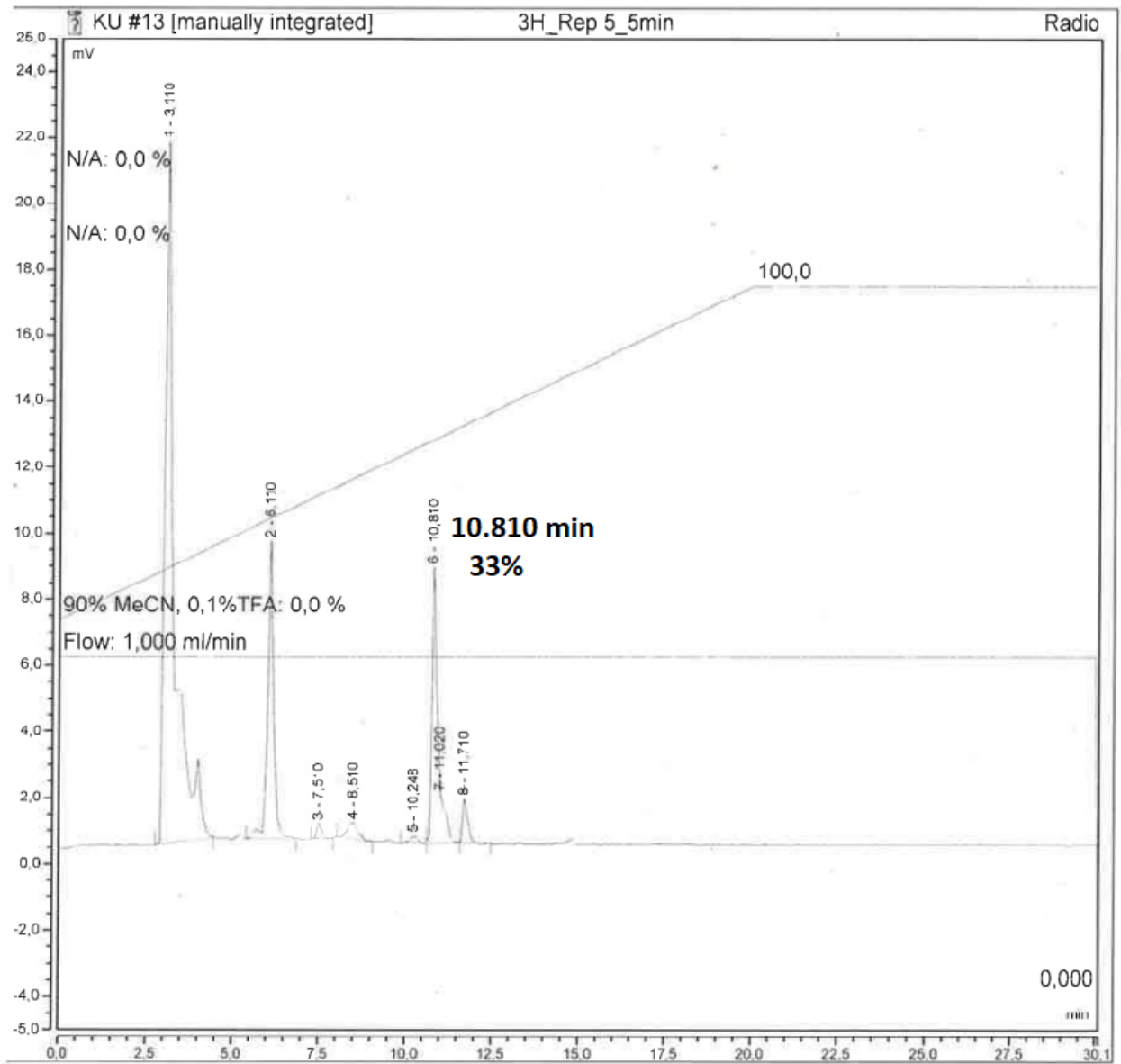
#### *3,6-di(pyridin-2-yl)-1,2-dihydro-1,2,4,5-tetrazine*

Tz **9** was subjected to general reduction entry 1,2 and 4. No reduction was observed, based on LC-MS, TLC and NMR after filtration of the Pd/C, and evaporation of the solvent. Hence the product **14** or **12** were never obtained.

SI-2-1 HPLC Chromatograms of Precursor and References **12-15** plus dihydro-intermediate

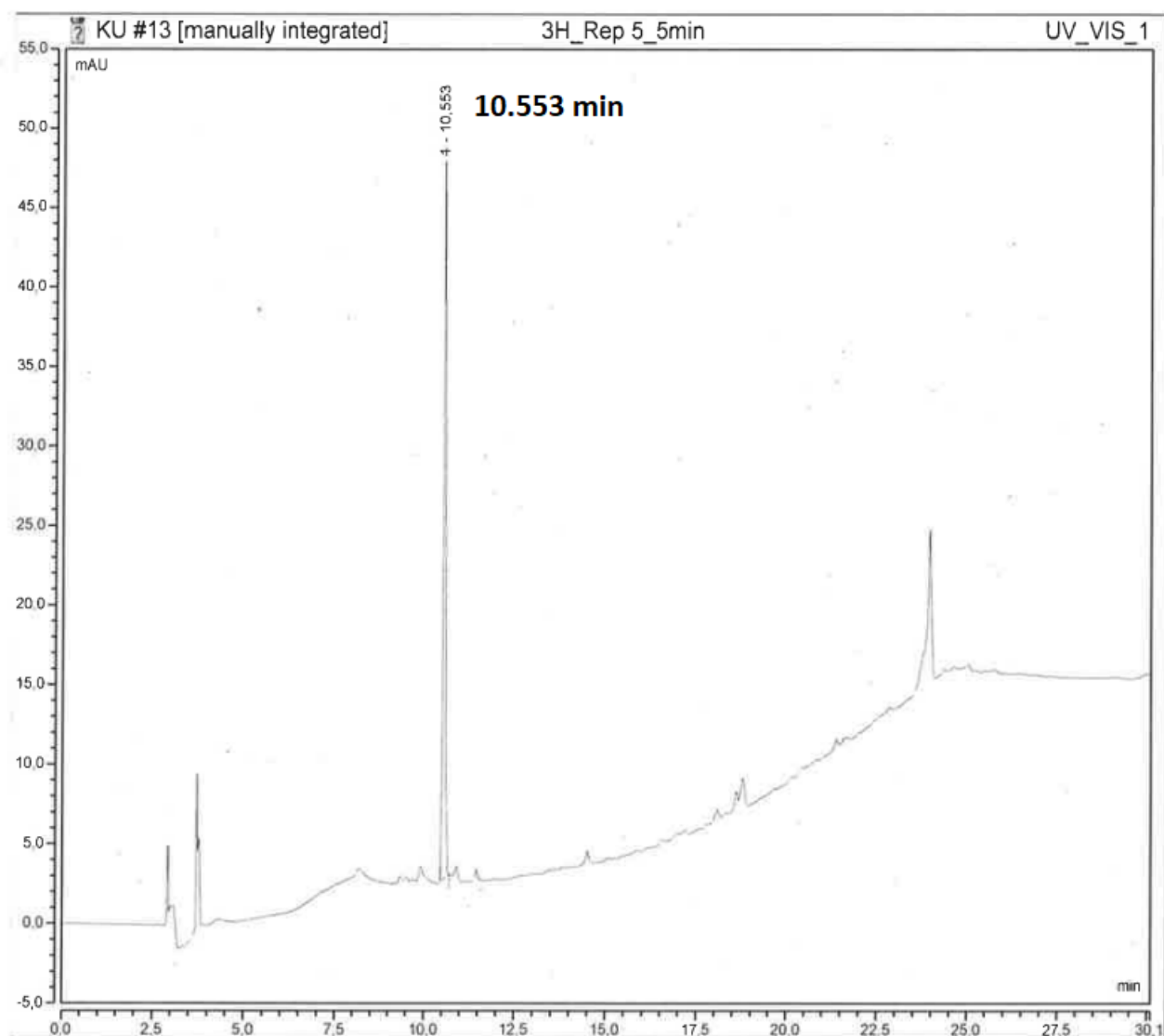


SI-2-2 Crude <sup>3</sup>H-Tz 16 Radio-HPLC Chromatogram



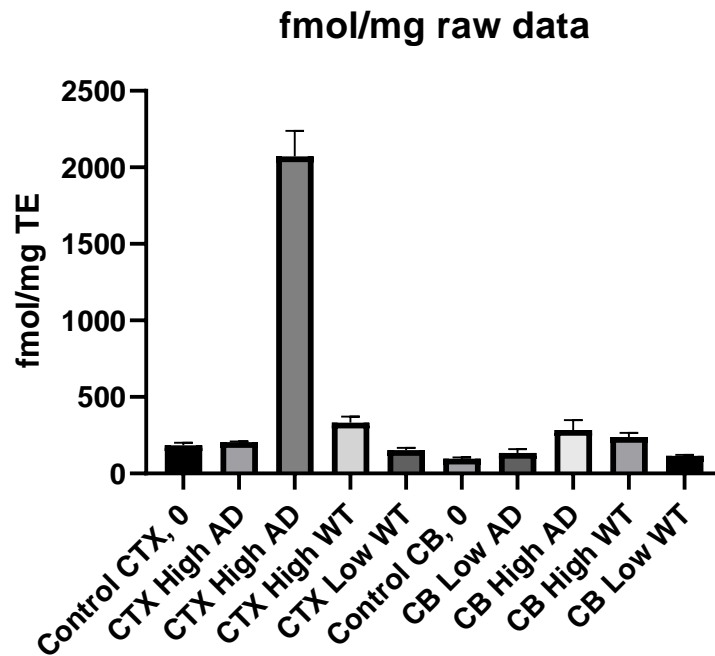


SI-2-3 Crude <sup>3</sup>H-Tz 16 UV-HPLC Chromatogram

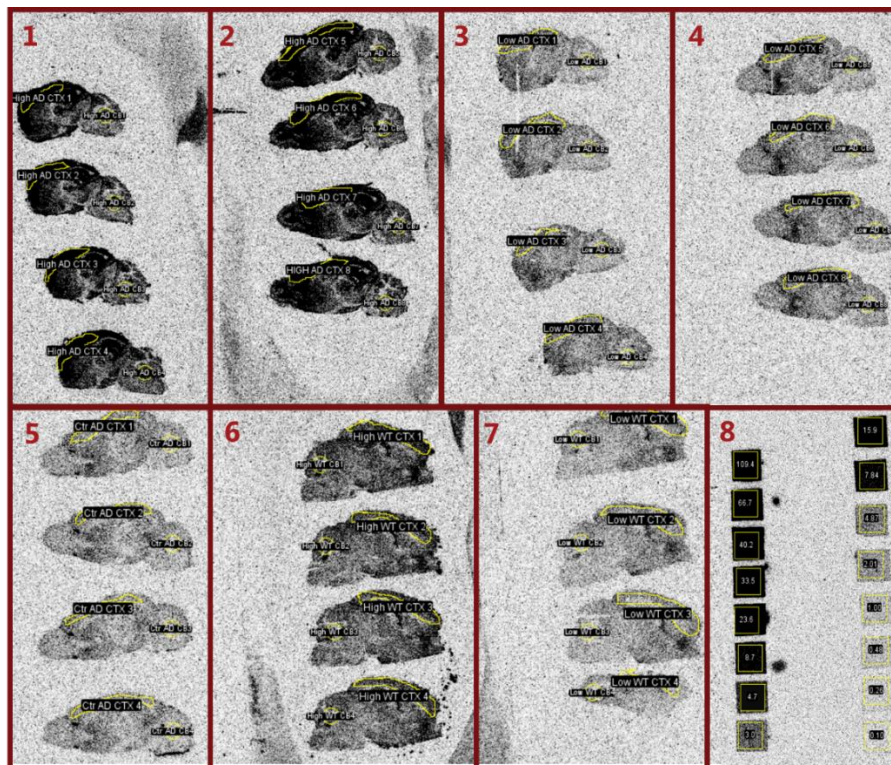


### 3. Pretargeted Autoradiography

SI-3-1 Raw data (fmol/mg) autoradiography. Calculated in GraphPad Prism, standard deviation calculated by ANOVA.



SI-3-2 Autoradiographic image as obtained in Image J



1: AD High 2: AD High 3: AD low 4: AD Low 5: AD Control 6: WT High 7: WT Low

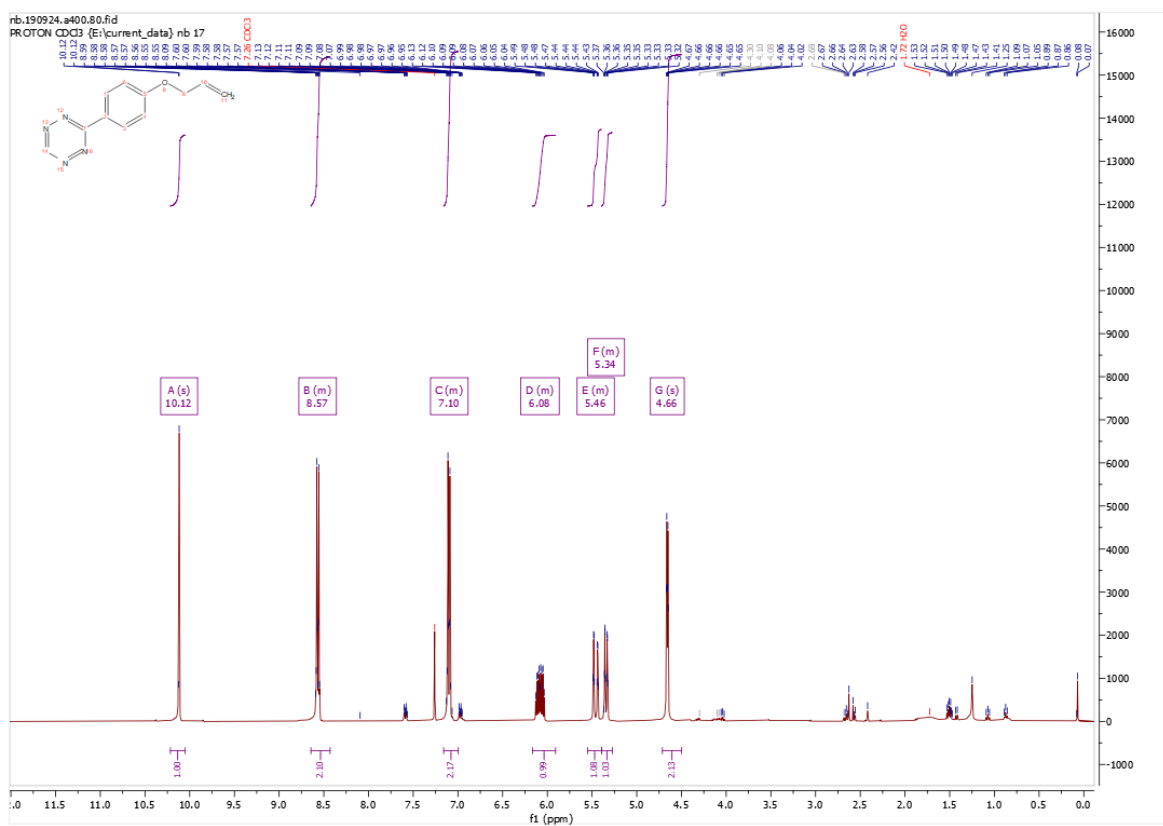
SI-3-3 Raw Data of pretargeting autoradiography  $^3\text{H-Tz 16}$  as calculated by Image J.

High AD CTX 1		0.040	24,635
HIGH AD CTX 2		0.039	25,641
HIGH AD CTX 3		0.053	24,940
HIGH AD CTX 4		0.056	21,291
HIGH AD CTX 5		0.059	24,460
HIGH AD CTX 6		0.071	24,488
HIGH AD CTX 7		0.058	28,652
HIGH AD CTX 8		0.054	24,466
LOW AD CTX 1		0.058	2,363
LOW AD CTX 2		0.059	2,469
LOW AD CTX 3		0.030	2,526
LOW AD CTX 4		0.059	2,544
LOW AD CTX 5		0.065	2,484
LOW AD CTX 6		0.069	2,404
LOW AD CTX 7		0.055	2,441
LOW AD CTX 8		0.071	2,406
CTR AD CTX 1		0.047	2,292
CTR AD CTX 2		0.050	2,389
CTR AD CTX 3		0.035	2,237
CTR AD CTX 4		0.064	1,962
HIGH WT CTX 1		0.071	4,688
HIGH WT CTX 2		0.060	3,874
HIGH WT CTX 3		0.066	3,645
HIGH WT CTX 4		0.076	3,745
LOW WT CTX 1		0.097	2,049
LOW WT CTW 2		0.089	1,836
LOW WT CTX 3		0.106	1,836
LOW WT CTX 4		0.041	1,584
High AD CB 1		0.018	3,338
HIGH AD CB 2		0.018	3,973
HIGH AD CB 3		0.018	2,992
HIGH AD CB 4		0.018	4,935
HIGH AD CB 5		0.018	3,132
HIGH AD CB 6		0.018	2,630
HIGH AD CB 7		0.017	3,584

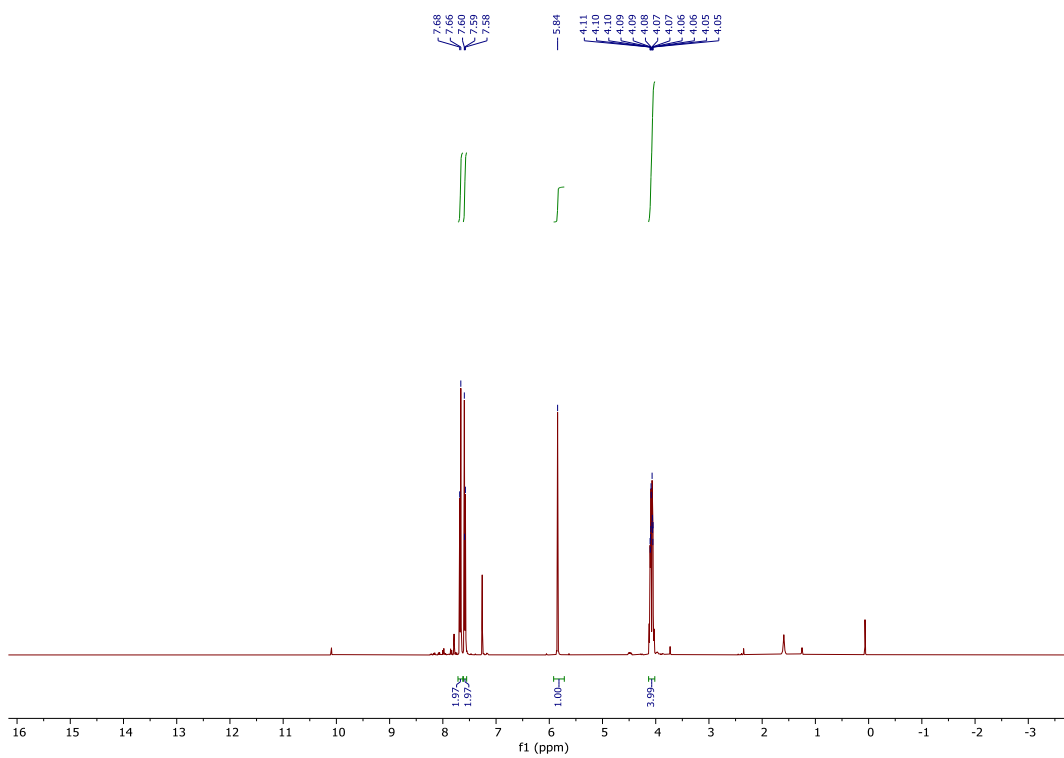
<b>HIGH AD CB 8</b>	<b>0.018</b>	<b>2,753</b>
<b>LOW AD CB 1</b>	<b>0.018</b>	<b>1,777</b>
<b>LOW AD CB 2</b>	<b>0.018</b>	<b>1,917</b>
<b>LOW AD CB 3</b>	<b>0.018</b>	<b>1,918</b>
<b>LOW AD CB 4</b>	<b>0.018</b>	<b>1,147</b>
<b>LOW AD CB 5</b>	<b>0.018</b>	<b>1,378</b>
<b>LOW AD CB 6</b>	<b>0.018</b>	<b>1,416</b>
<b>LOW AD CB 7</b>	<b>0.018</b>	<b>1,709</b>
<b>LOW AD CB 8</b>	<b>0.018</b>	<b>1,700</b>
<b>CTR AD CB 1</b>	<b>0.018</b>	<b>1,266</b>
<b>CTR AD CB 2</b>	<b>0.018</b>	<b>1,103</b>
<b>CTR AD CB 3</b>	<b>0.018</b>	<b>1,226</b>
<b>CTR AD CB 4</b>	<b>0.018</b>	<b>1,090</b>
<b>HIGH WT CB 1</b>	<b>0.018</b>	<b>3,304</b>
<b>HIGH WT CB 2</b>	<b>0.018</b>	<b>2,914</b>
<b>HIGH WT CB 3</b>	<b>0.018</b>	<b>2,676</b>
<b>HIGH WT CB 4</b>	<b>0.018</b>	<b>2,533</b>
<b>LOW WT CB 1</b>	<b>0.018</b>	<b>1,431</b>
<b>LOW WT CB 2</b>	<b>0.018</b>	<b>1,312</b>
<b>LOW WT CB 3</b>	<b>0.018</b>	<b>1,475</b>
<b>LOW WT CB 4</b>	<b>0.018</b>	<b>1,374</b>

# 1. NMR Spectra

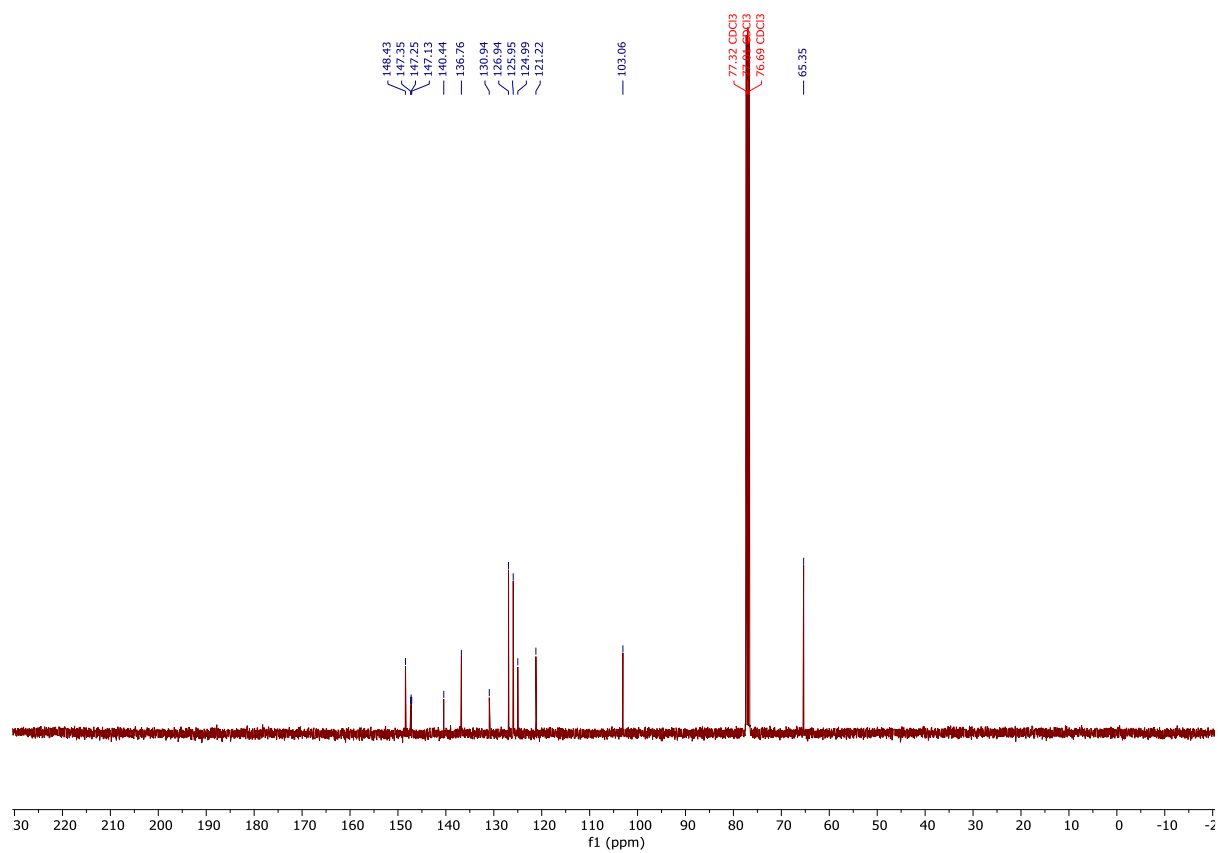
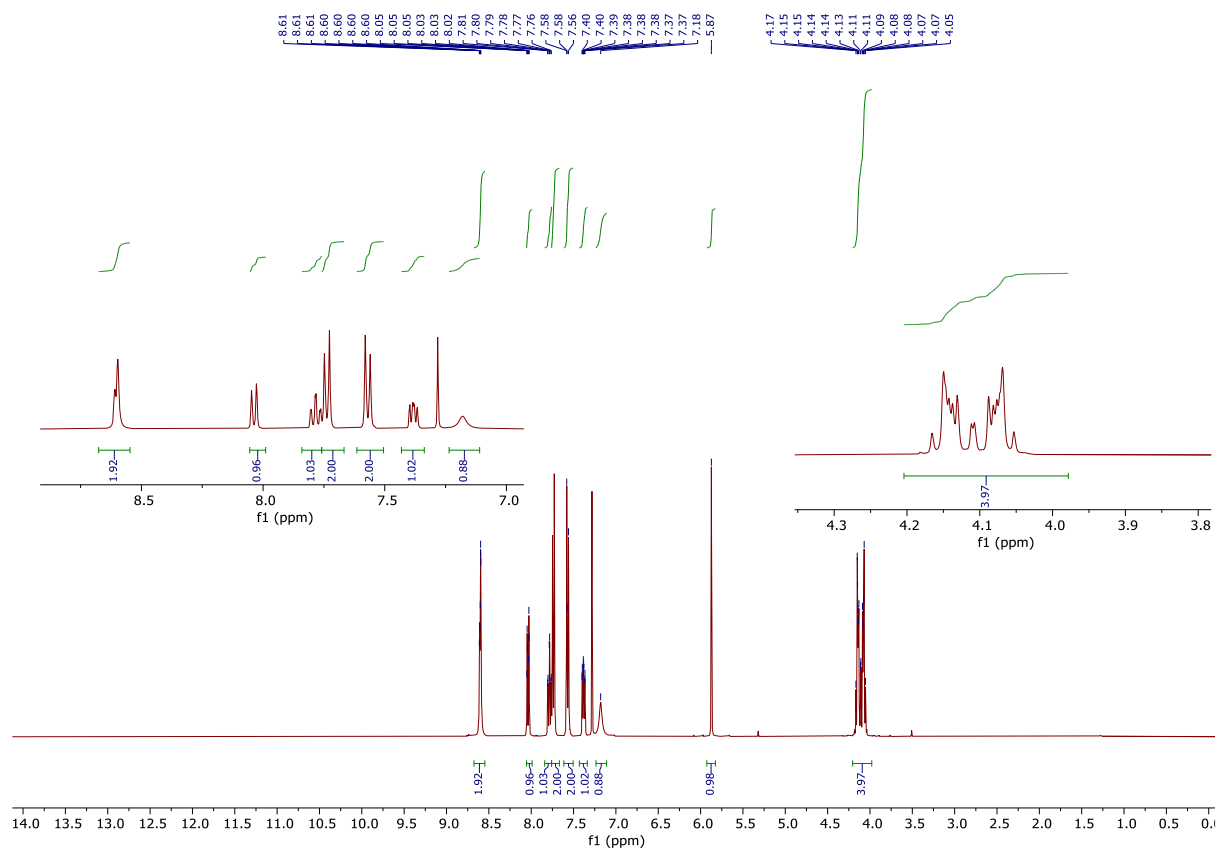
## 3-(4-(allyloxy)phenyl)-1,2,4,5-tetrazine (7)



## 4-(1,3-dioxolan-2-yl)benzonitrile (10)

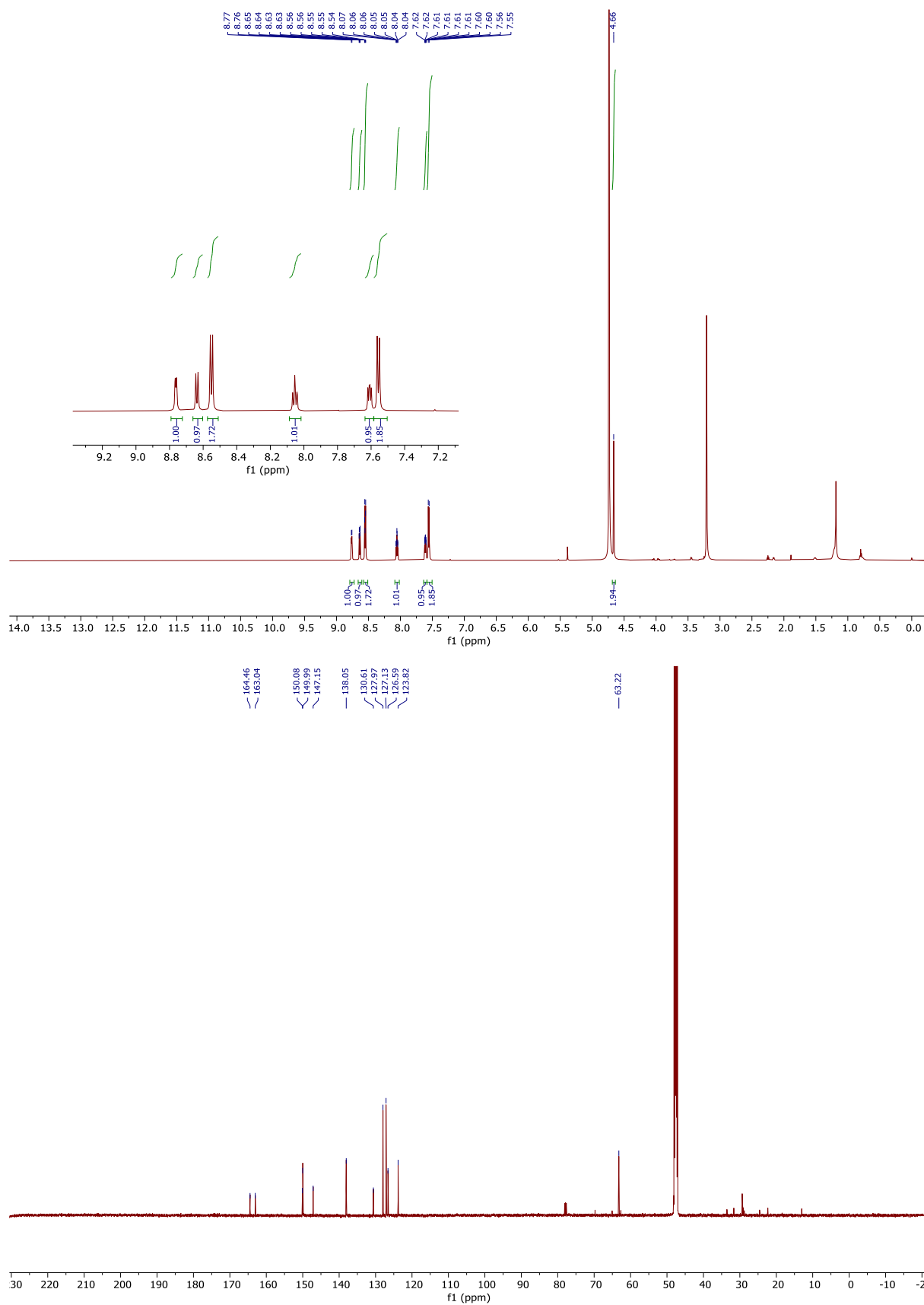


**3-(4-(1,3-dioxolan-2-yl)phenyl)-6-(pyridin-2-yl)-1,4-dihydro-1,2,4,5-tetrazine (11)**



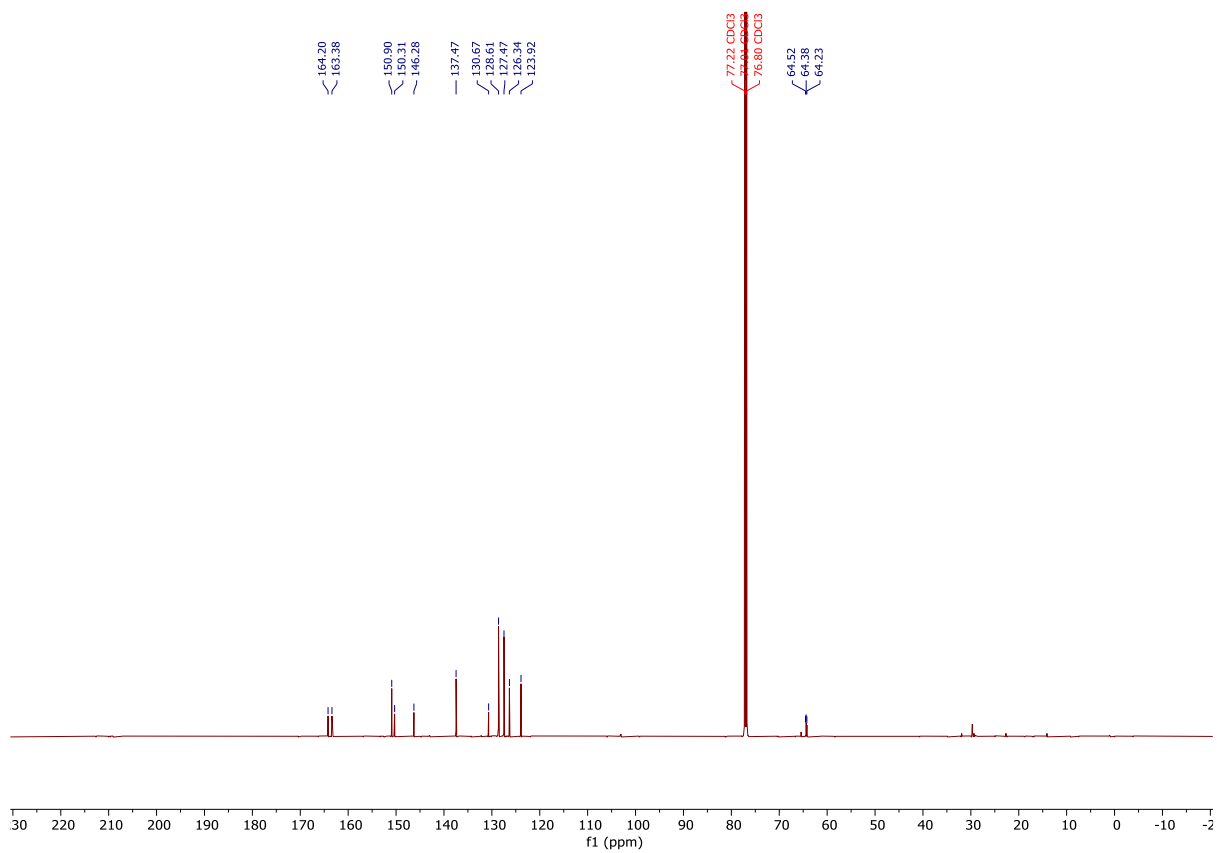
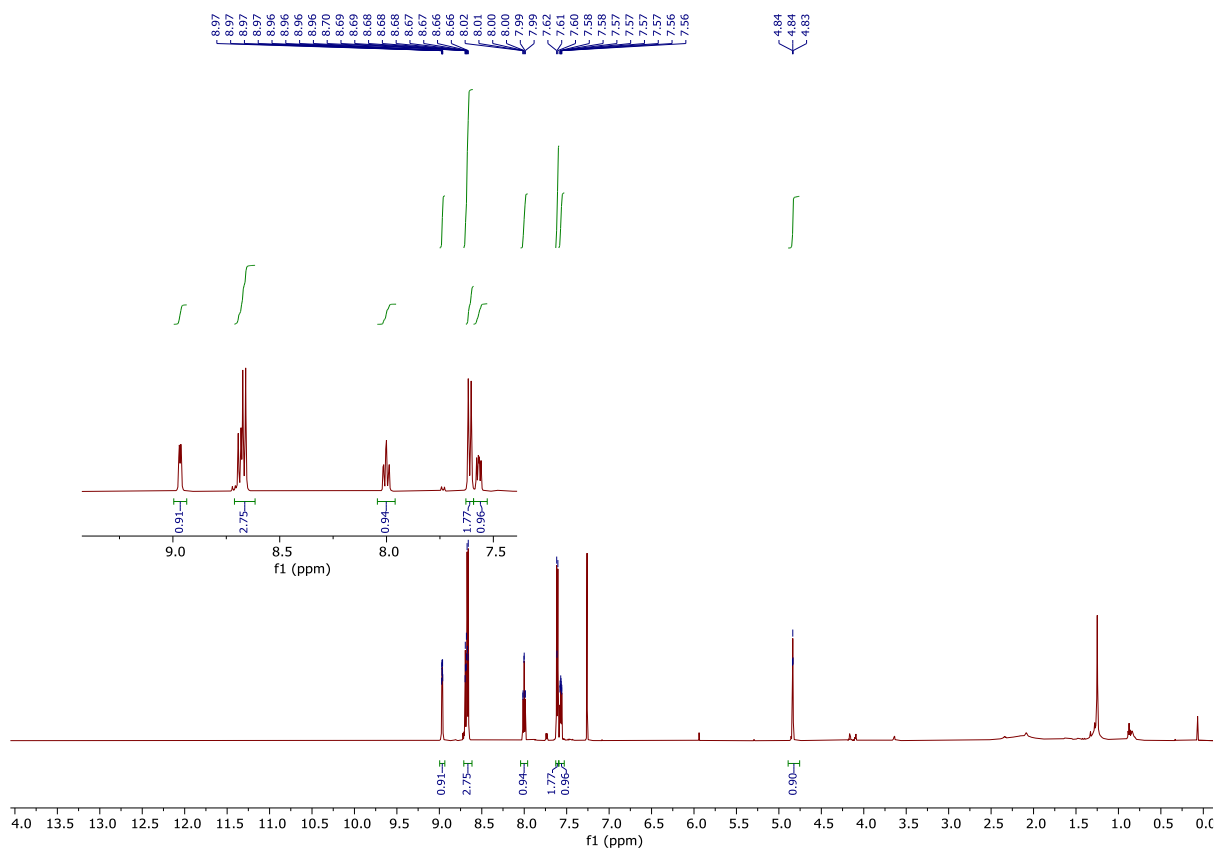


**(4-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)phenyl)methanol (13)**

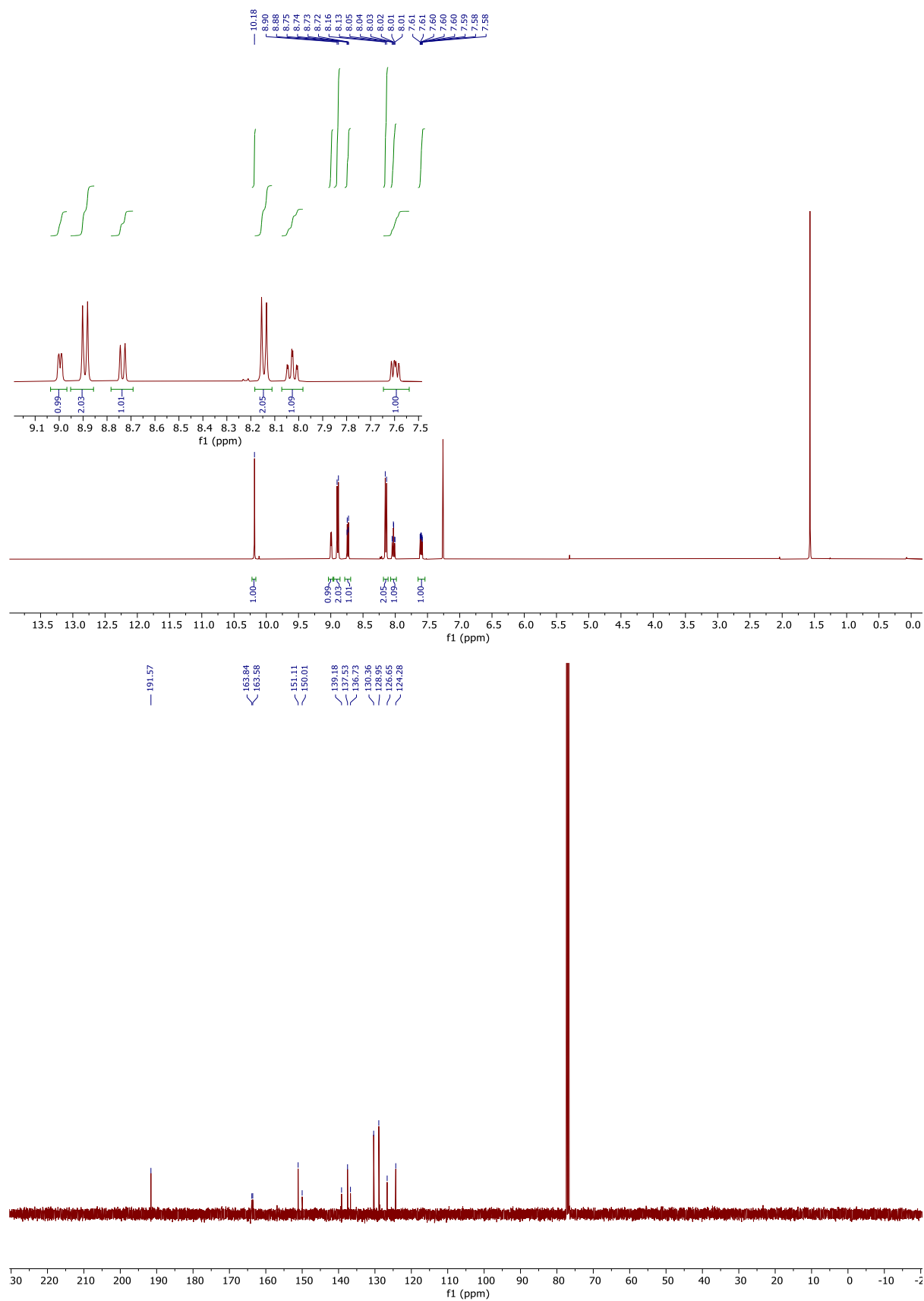




**(4-(6-(Pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)phenyl)methan-d-ol (14)**



**4-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)benzaldehyde (15)**



## References

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