

Supporting information:

## Controlling miRNA-like Off-target Effects of an siRNA with Nucleobase Modifications

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Table S1: Data Collection, Phasing, and Refinement Statistics

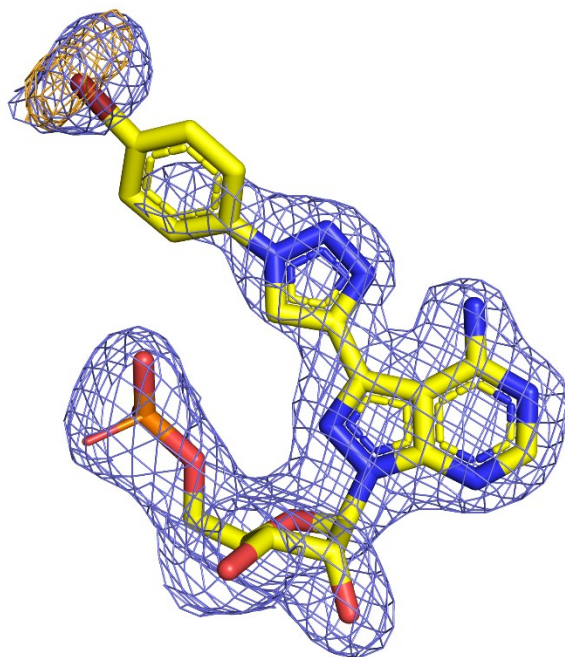
	Br <sup>-</sup> peak	Br <sup>-</sup> remote	<b>“High Resolution”</b>
X-Ray source	ALS 8.3.1	ALS 8.3.1	ALS 8.3.1
Wavelength	0.92025 Å	0.91157 Å	1.11583 Å
Space Group	C2	C2	C2
Unit Cell Parameters	a=74.74 Å b=43.16 Å c=48.88 Å β=120.64°	a=74.75 Å b=43.16 Å c=48.88 Å β=120.64°	a=74.64 Å b=43.15 Å c=48.98 Å β=120.46°
Resolution	1.75Å (1.78-1.75Å)	1.75Å (1.78-1.75Å)	1.65 Å (1.69-1.65 Å)
Number of reflections	359,400 (19,161)	358,539 (19,163)	93,686 (3,083)
Number Unique	13,619 (704)	13,630 (705)	15,214 (695)
Completeness (%)	99.9 (99.6)	99.9 (99.6)	92.9 (57.4)
Multiplicity	26.4 (27.2)	26.3 (27.2)	6.2 (4.4)
Mean I/σ(I)	21.4 (2.6)	21.3 (2.4)	22.5 (3.3)
R <sub>merge</sub> <sup>a</sup> (%)	5.3 (139.2)	5.3 (145.5)	2.8 (21.8)
Anomalous Completeness	99.9 (99.6)	99.9 (99.6)	
Anomalous Multiplicity	13.5 (13.5)	13.4 (13.8)	
Initial Figure of Merit		0.38	
Density Modified Figure of Merit		0.86	
<b><u>Refinement Statistics</u></b>			
Resolution (Å)			50-1.65
R-factor <sup>b</sup>			0.2167
R-free <sup>b</sup>			0.2558
RMS Bond Length (Å)			0.004
RMS Bond Angles (°)			1.97
Overall B Value (Å <sup>2</sup> )			59.8
PDBID			6BGB

<sup>a</sup> R<sub>merge</sub> =  $[\sum_h \sum_i |I_h - I_{hi}| / \sum_h \sum_i I_{hi}]$  where  $I_h$  is the mean of  $I_{hi}$  observations of reflection  $h$ . Numbers in parenthesis represent highest resolution shell.

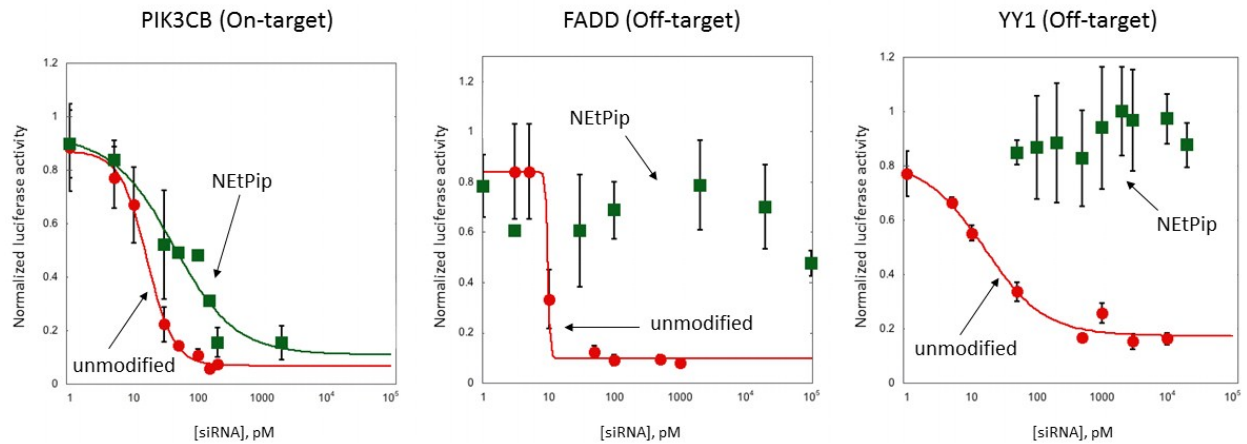
<sup>b</sup> R-factor and  $R_{free} = \sum ||F_{obs}| - |F_{calc}|| / \sum |F_{obs}| \times 100$  for 95% of recorded data (R-factor) or 5% of data ( $R_{free}$ ). Numbers in parenthesis represent highest resolution shell.

Table S2: Mass Spectrometry Data for Modified RNAs

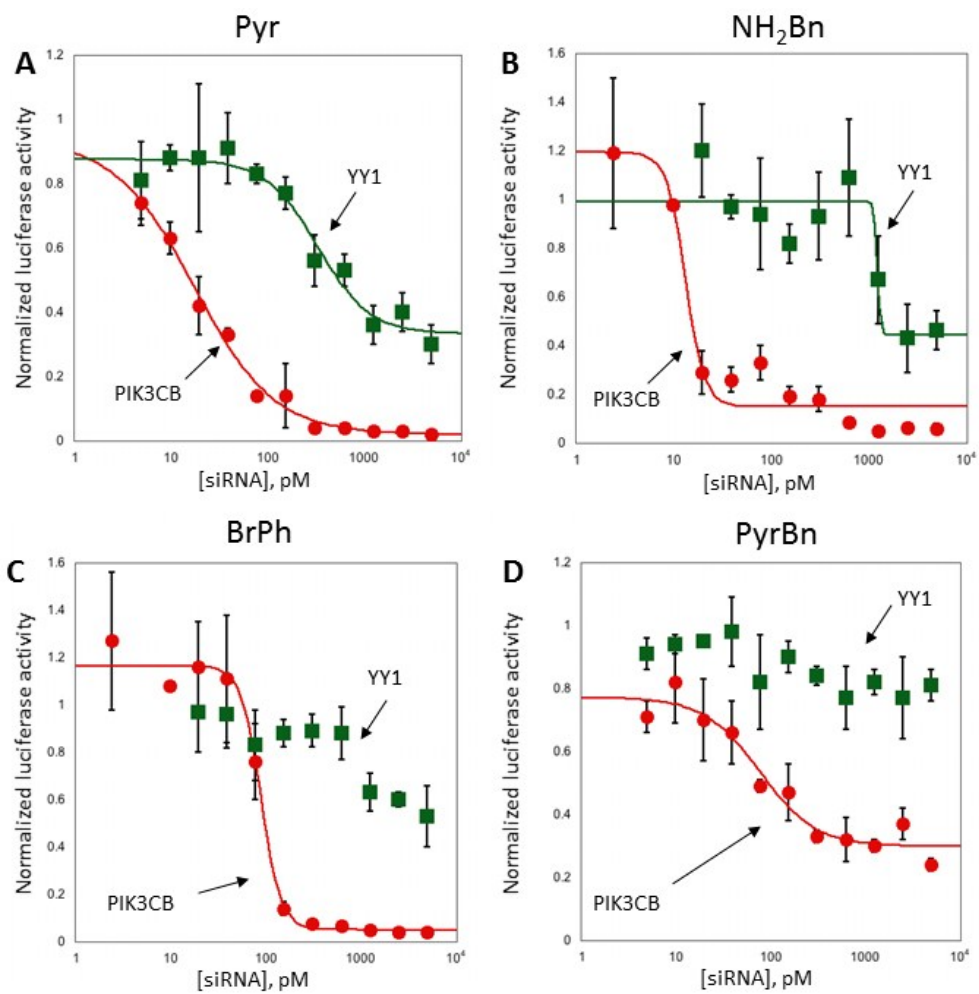
<b>16mer crystallization triazoles (5'-GCAGXCUUAAGUCUGC-3')</b>		
	<u>Calculated</u>	<u>Observed</u>
<b>BrPh:</b>	5301.0	5303.8
<b>12mer TM triazoles (5'-CAUUA<del>X</del>GGUGGG-3')</b>		
	<u>Calculated</u>	<u>Observed</u>
<b>PyrBn:</b>	4109.4	4110.8
<b>NH<sub>2</sub>Bn:</b>	4046.4	4048.3
<b>Pyr:</b>	4018.4	4020.3
<b>BrPh:</b>	4095.3	4098.2
<b>21 mer PIK3CB g6 triazoles (5'-p-AUAGGXUUCAUAUUAGGAGAU-3')</b>		
	<u>Calculated</u>	<u>Observed</u>
<b>PyrBn:</b>	7076.1	7080.1
<b>NH<sub>2</sub>Bn:</b>	7013.1	7005.9
<b>Pyr:</b>	6985.1	6980.6
<b>BrPh:</b>	7061.9	7060.7



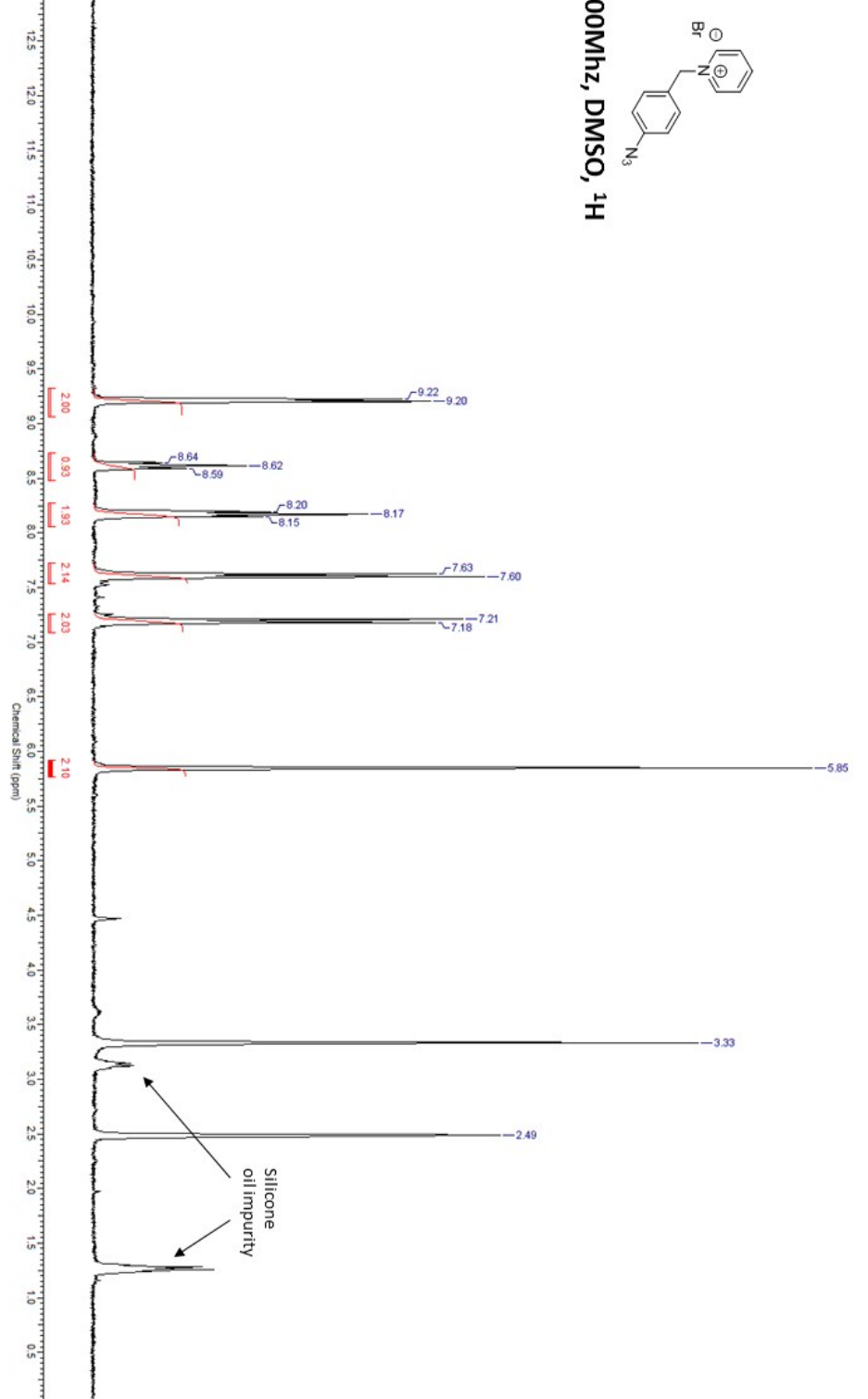
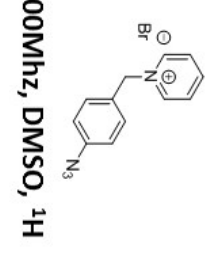
**Figure S1:** The overlaid 2Fo-Fc electron density (blue) for the 16 mer BrPh triazole modification at  $6\sigma$ , along with the anomalous difference electron density (orange) confirming location of bromine atom.

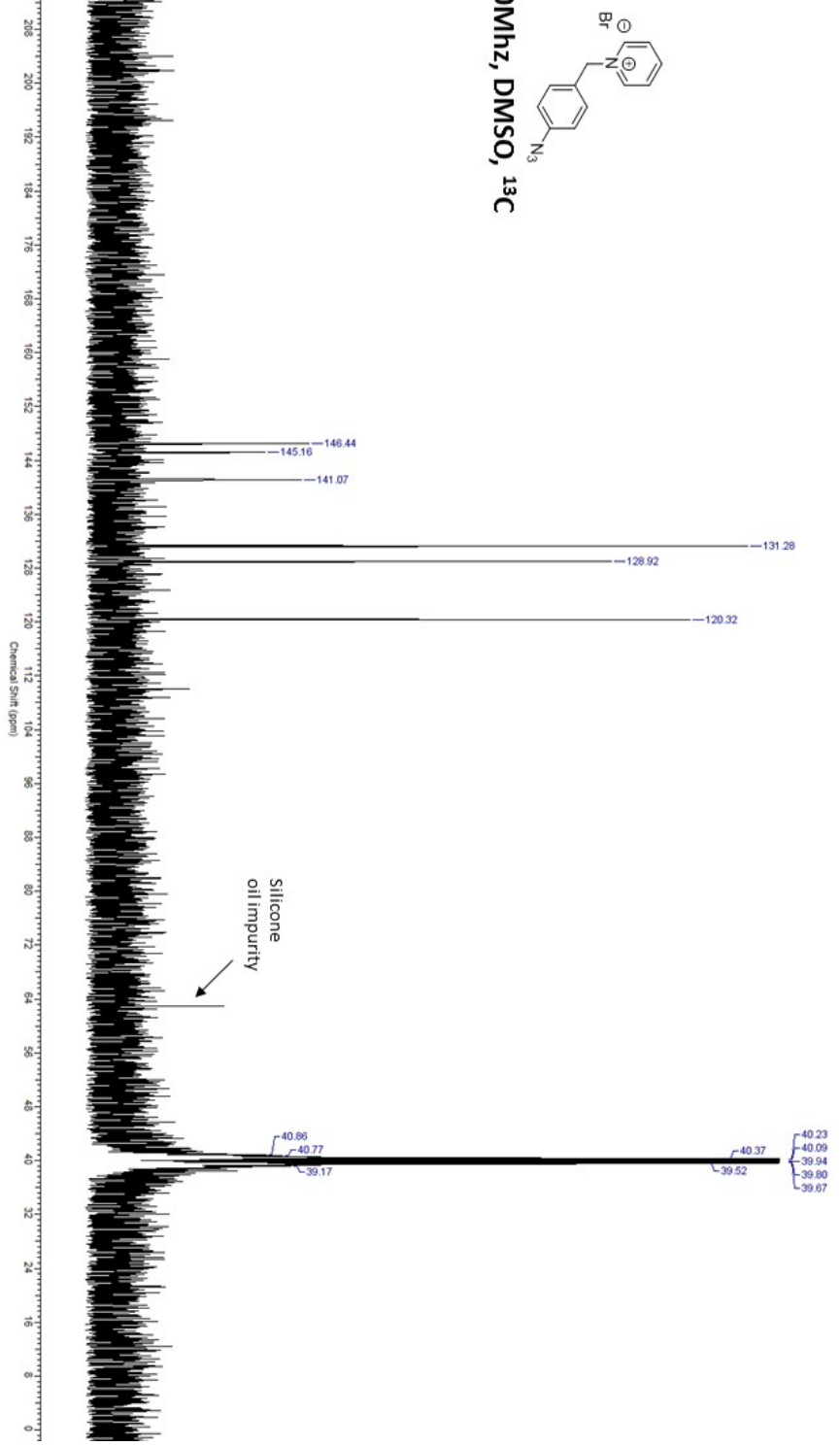
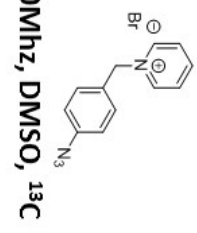


**Figure S2:** Dual Luciferase Assay  $IC_{50}$  data for the NETPip g6 modified PIK3CB siRNA compared to the unmodified guide strand. For PIK3CB on-target knockdown ( $IC_{50} = 15 \pm 5$  pM (unmodified);  $IC_{50} = 44 \pm 18$  pM (modified)). For FADD off-target knockdown ( $IC_{50} = 8 \pm 3$  pM (unmodified)). For YY1 off-target knockdown  $IC_{50} = 15 \pm 5$  pM (unmodified). No measurable  $IC_{50}$  could be measured for FADD or YY1 when the siRNA was modified at position 6 with NETPip.

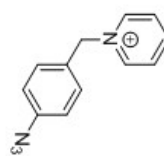


**Figure S3** Luciferase assay IC<sub>50</sub> curves for the Pyr (A), NH<sub>2</sub>Bn (B), BrPh (C), and PyrBn (D) guide position 6 modifications against the PIK3CB on-target (red) and YY1 off-target (green) sequences. All measurable IC<sub>50</sub> data can be found in the main text in Table 1. All IC<sub>50</sub> measurements were carried out to 5 nM.

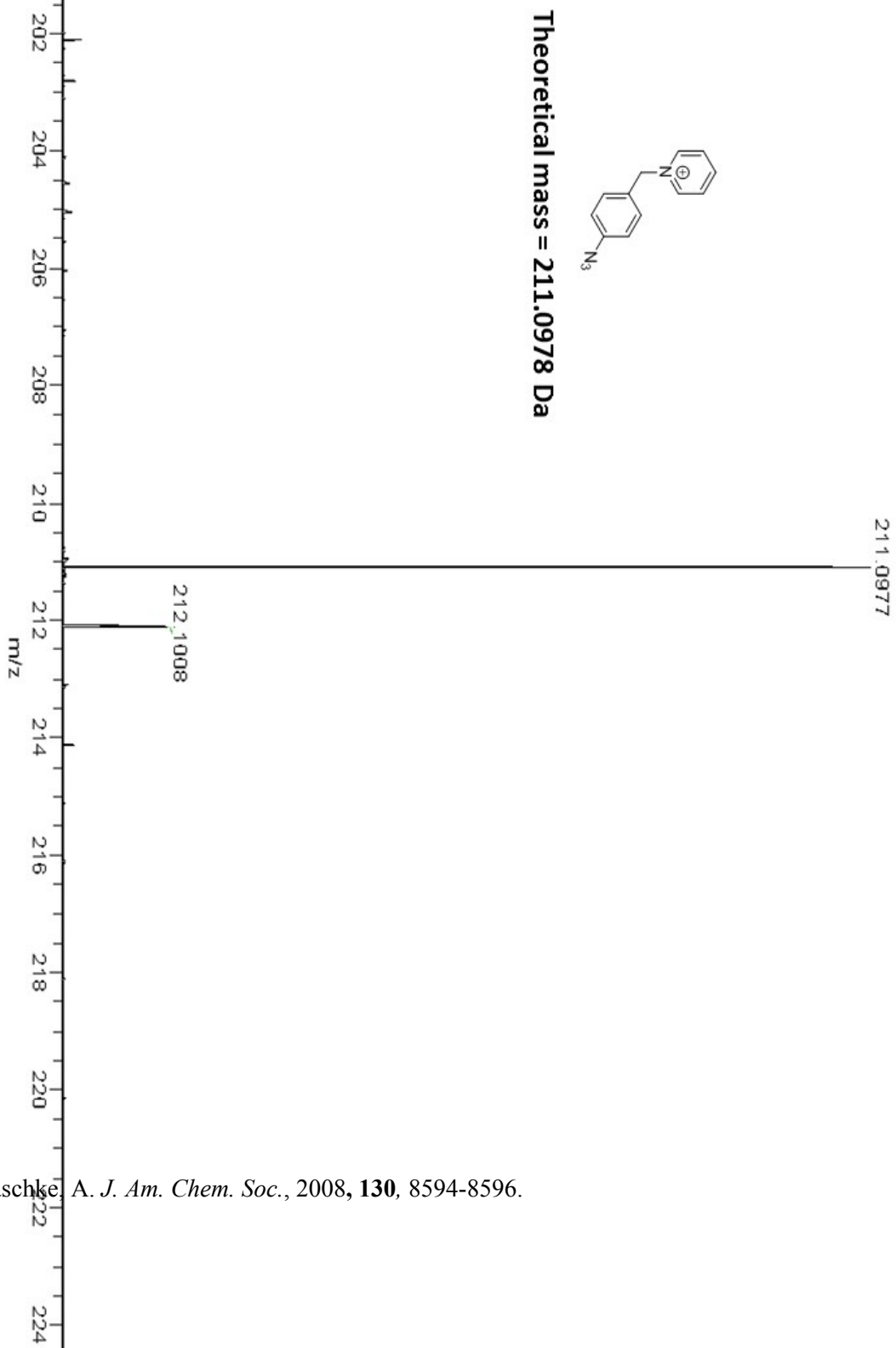








Theoretical mass = 211.0978 Da



### Reference

- (1) Wombacher, R.; Jaschke, A. *J. Am. Chem. Soc.*, 2008, **130**, 8594-8596.