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Supporting information for:

A clickable aminooxy probe for monitoring cellular ADP-ribosylation

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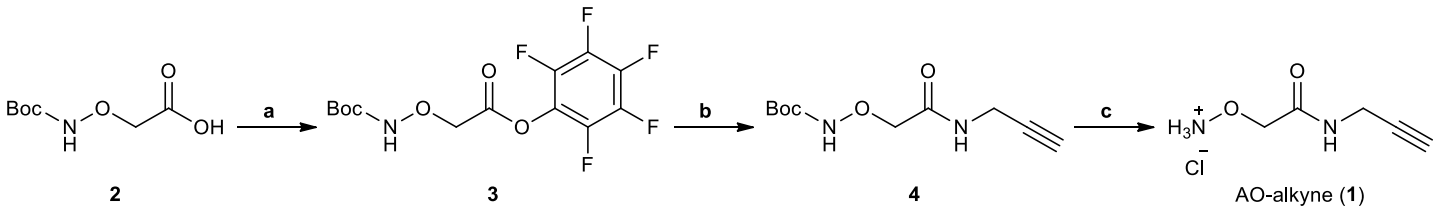
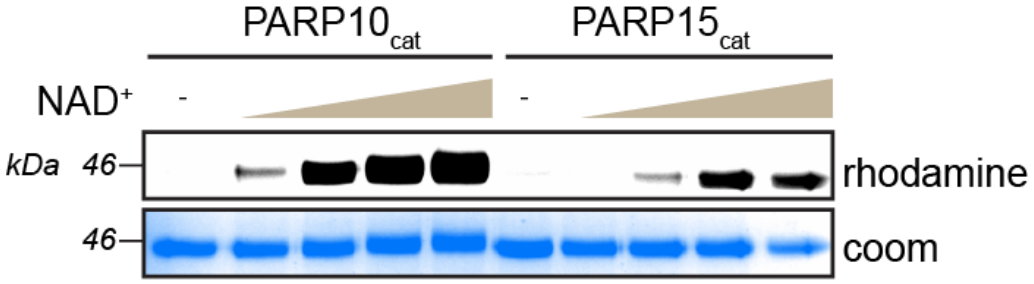
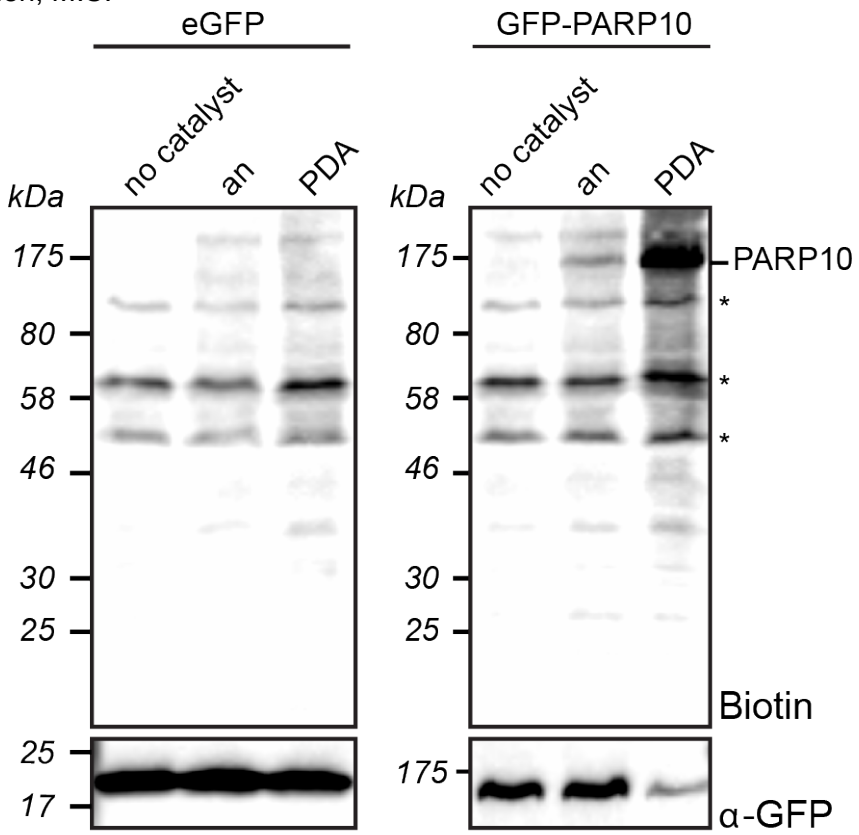


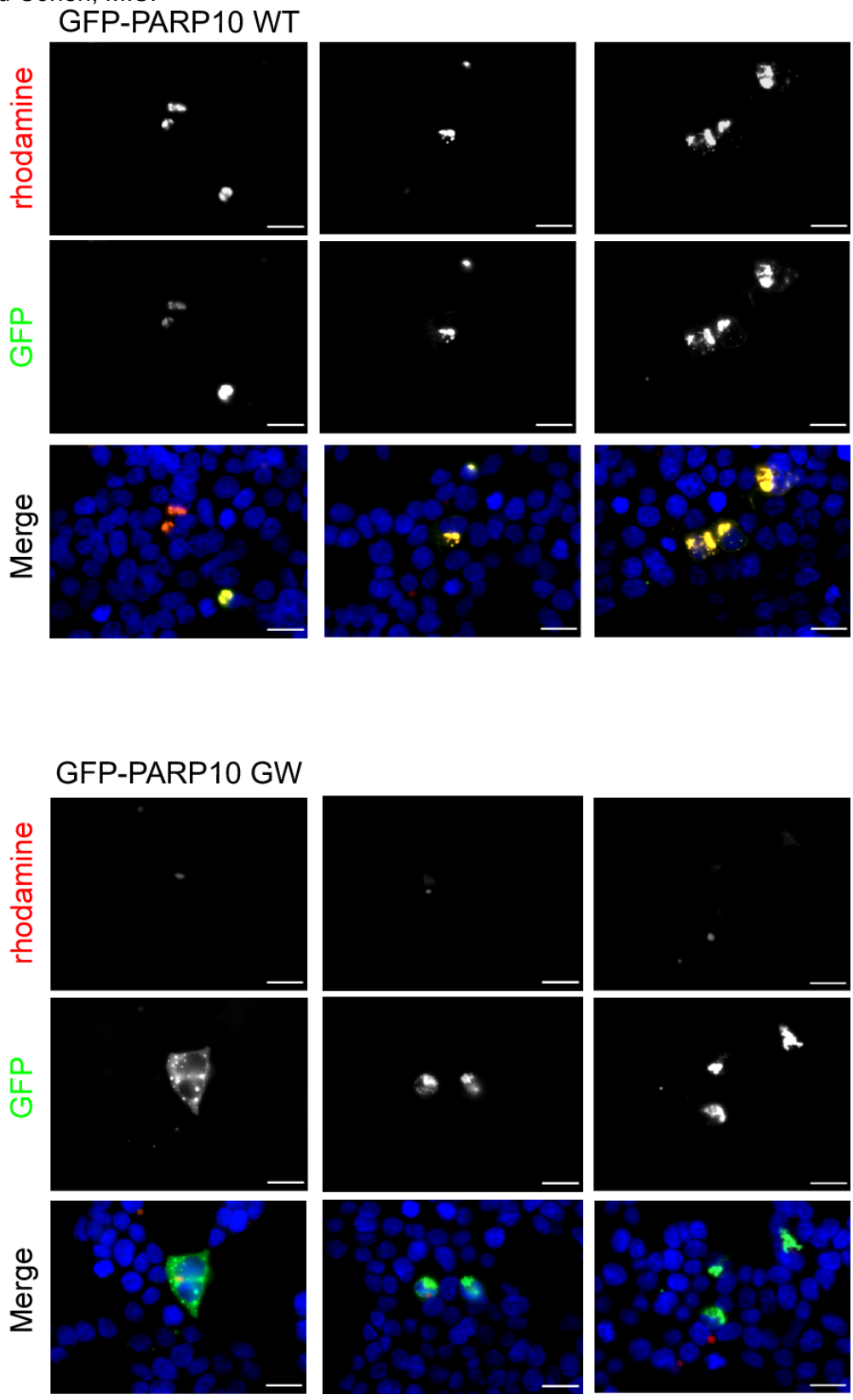
Supporting Scheme 1. Synthesis of AO-alkyne (**1**). *Reagents and conditions*: (**a**) TFA-OPfp, pyr, DCM, rt, 71%; (**b**) propargylamine, DIPEA, DCM, rt, 96%; (**c**) 4N HCl, dioxane, rt, 63%.



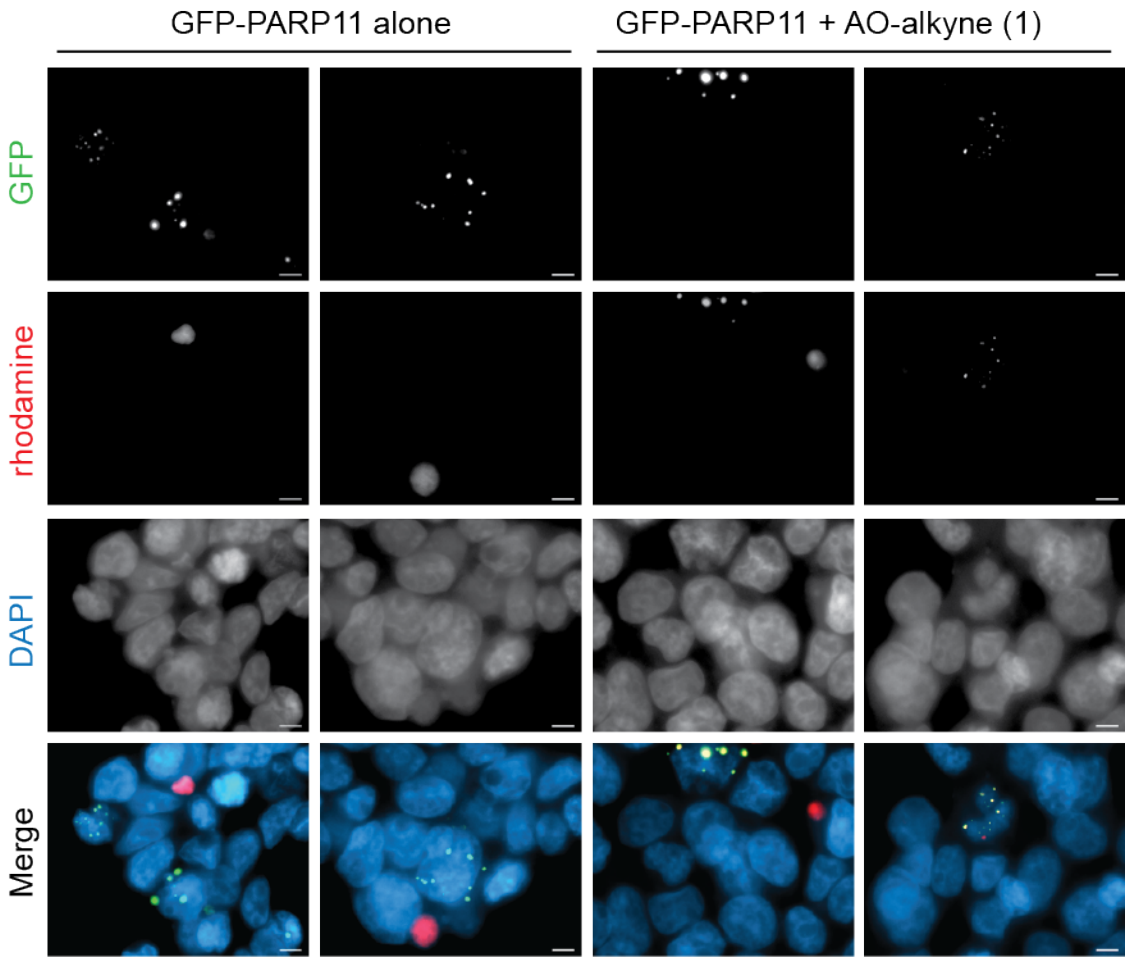
Supporting Figure 1. $PARP10_{cat}$ and $PARP15_{cat}$-mediated ADP-ribosylation of SRPK2 *in vitro* is detected by **1** following click conjugation with rhodamine-azide. SRPK2 was ADP-ribosylated by $PARP10_{cat}$ or $PARP15_{cat}$ in the presence of NAD^+ (0, 1, 10, 50, or 100 μM) at 30 °C for 1h, followed by labeling with **1** (100 μM) at pH 5 with PDA (10 mM) and subsequent click conjugation with rhodamine-azide (100 μM).



Supporting Figure 2. Labeling of ADP-ribosylated GFP-PARP10 by **1** is most efficient with PDA catalysis. HEK 293T cells overexpressing either GFP-PARP10 WT and eGFP (negative control) were treated with **1** (100 μM) and PDA (10 mM) for 1 h at 37 °C. Lysates were subjected to click conjugation with biotin-N_3 (100 μM) and analyzed via immunoblot. Detection with an α-GFP antibody serves as a loading control. Bands marked with an asterisk represent endogenous biotinylated proteins. PDA = *p*-phenylenediamine, an = aniline.



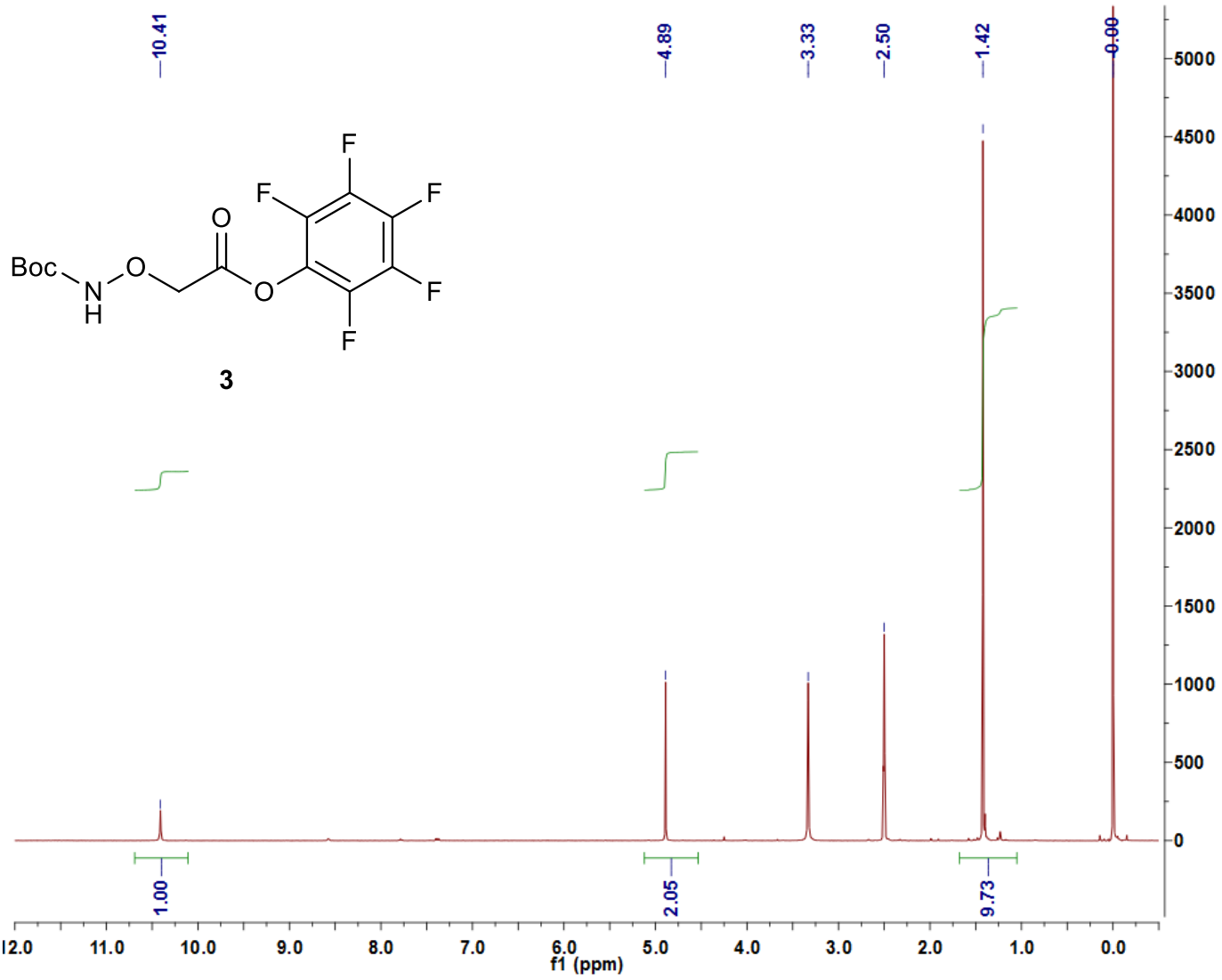
Supporting Figure 3. Additional representative images for detection of PARP10 activity in cells with **1**. HEK 293T cells expressing either GFP-PARP10 WT or GFP-PARP10 GW (catalytically dead) were treated with **1** (100 μM) and PDA (10 mM) for 1 h at 37 °C. After fixation, permeabilization, and blocking, the cells were subjected to click conjugation with rhodamine-azide (1 μM) for 30 min. Scale bar corresponds to 20 μm.



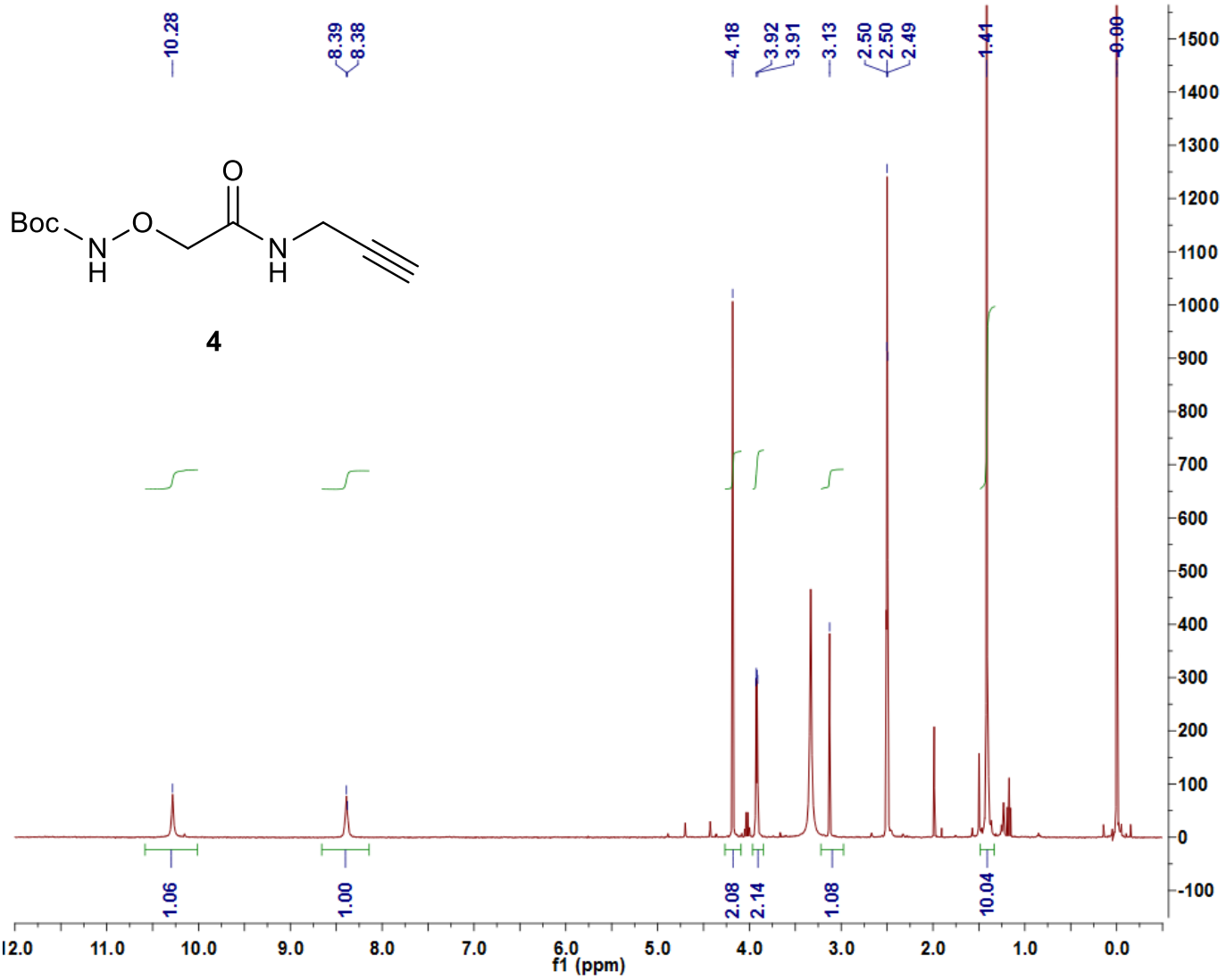
Supporting Figure 4. Detection of PARP11 activity in cells with **1**. HEK 293T cells expressing GFP-PARP11 WT were treated with or without **1** (100 μM) and PDA (10 mM) for 1 h at 37 °C. After fixation, permeabilization, and blocking, the cells were subjected to click conjugation with rhodamine-azide (1 μM) for 30 min. Scale bar corresponds to 5 μm.

Supporting Compound Characterization:

Compound **3**:

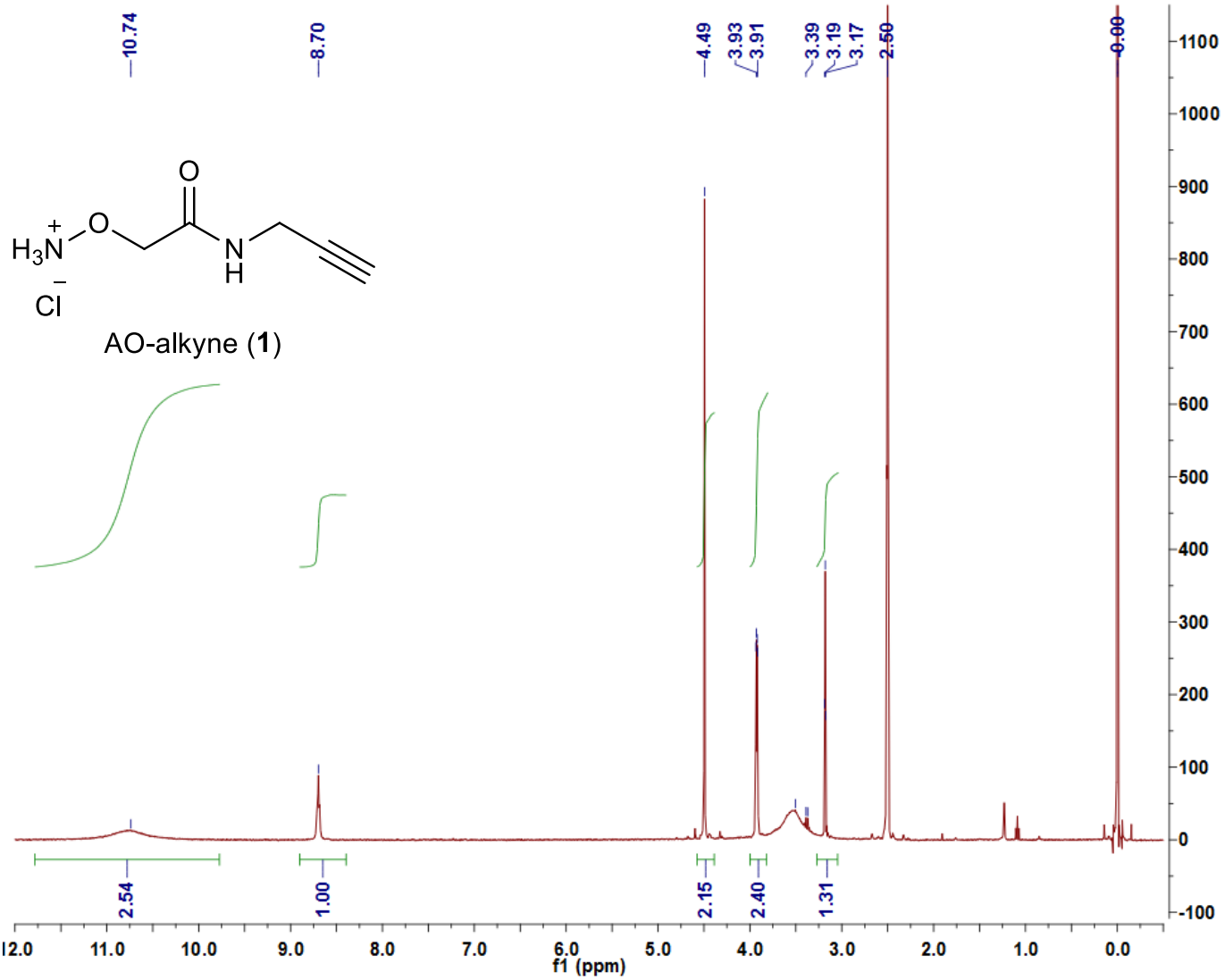


Compound **4**:


10.28
8.39
8.38
4.18
3.92
3.91
3.13
2.50
2.50
2.49
1.41
0.00
Boc
N
H
O
O
N
H
1.06
1.00
2.08
2.14
1.08
10.04
f1 (ppm)

4

AO-alkyne (**1**):


10.74
8.70
4.49
3.93
3.91
3.39
3.19
3.17
2.50
0.00
O
H3N
O
N
H
Cl
AO-alkyne (1)
2.54
1.00
2.15
2.40
1.31
f1 (ppm)