Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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Supporting Information

Proteomic Searches Comparing Two (*R*)-Lacosamide Affinity Baits: An Electrophilic Arylisothiocyanate and a Photoactivated Arylazide Group

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Treatment of (*R*)-*N*-(4-azidobenzyl) 2-Acetamido-3-(prop-2-ynyloxy)propionamide ((*R*)-3) with TCEP. A 100 μM solution (10 mL) of (*R*)-*N*-(4-azidobenzyl) 2-acetamido-3-(prop-2-ynyloxy)propionamide ((*R*)-**3**) (0.32 mg, 1 μmol) in 5% CH_3CN aqueous 50 mM HEPES (pH 7.2) was treated with TCEP (1 mM) and then stirred at room temperature (15 – 60 min). The reaction solution was analyzed at various time intervals by TLC and HPLC using a photodiode array detector (210 – 340 nm). Samples (50 μL) were injected onto a μBondapak C-18 column (3.9 × 300 mm, Waters Corp. Part No. WAT027324) (Figure S1). A gradient mobile phase (0/100 CH_3CN/H_2O – 50/50 CH_3CN/H_2O) was employed for 30 min using a flow rate of 1 mL/min; new product: TLC R_f = 0.35 (1/7 acetone/EtOAc), HPLC t_R 9.7 min; (*R*)-**3**: TLC R_f = 0.50 (1/7 acetone/EtOAc), HPLC t_R 15.5 min.

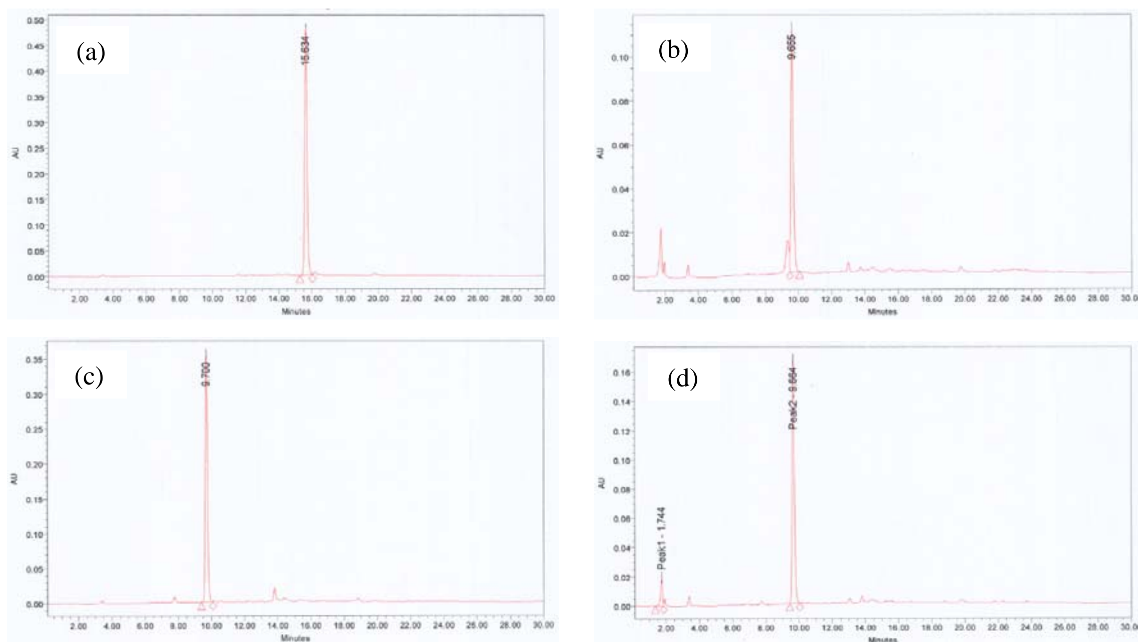

Figure S1. Treatment of (*R*)-*N*-(4-azidobenzyl) 2-acetamido-3-(prop-2-ynyloxy)propionamide ((*R*)-**3**) with TCEP (10 equiv): (a) (*R*)-**3;** (b) Reaction of (*R*)-**3** with TCEP after 30 min; (c) (*R*)-*N*-(4-aminobenzyl) 2-acetamido-3-(prop-2-ynyloxy)propionamide ((*R*)-**16**); (d) Co-injection of the reaction of (*R*)-**3** with TCEP after 30 min with (*R*)-**16**.

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AB&CR 3 Labeling of GST-CRMP2 in Mouse Lysate, Cycloaddition, and In-Gel Fluorescence Scanning. Utilizing the method described in the paper, lysate aliquots (150 μL, pH 7.8) were prepared after passage through a Nap-10 column. Overexpressed GST-CRMP2 (30 or 45 μg) was added to the lysate (150 μL) and the resulting mixture was divided into three equal aliquots. The aliquots (50 μL) were treated with either (*R*)-**3** or (*S*)-**3** (10 μM) without or with competing ligands ((*R*)-**1**, (*S*)-**1**) at 4 °C (10 min), and irradiated with 312 nm light (8 W, Spectroline EB-280C, Spectronics Corp., New York, USA) at 4 °C (2 min). The reaction mixtures were treated with TCEP (1 mM) for quenching of the remaining azide compound. The modified lysates were sequentially treated with rhodamine reporter tag (rhodamine-azide (Rho-N_3) (50 μM)), TBTA (100 μM) and $CuSO_4$ (1 mM). Samples were mixed by rotating using Roto-shake (8 rpm, Scientific Industries Inc., Model No. SI-1100, Bohemia, NY) at room temperature (1 h). Proteins were separated by SDS-PAGE after addition of 4× SDS-PAGE loading buffer and visualized by in-gel fluorescence using a typhoon 9400 scanner (Amersham Bioscience) with excitation at 532 nm and detection at 580 nm.

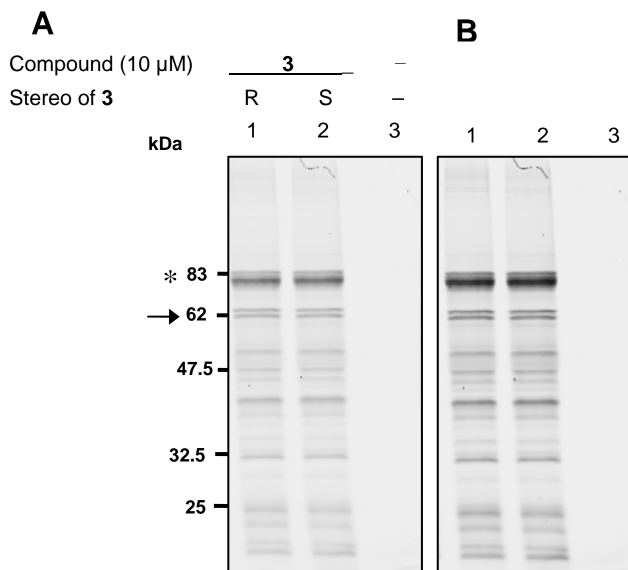

Figure S2. *In vitro* labeling of externally added GST-CRMP2 and endogenous CRMP2 (marked by an arrow) in mixture of mouse lysate and overexpressed GST-CRMP2 (marked by an asterisk).

Figure S2A; **3**-labeled proteins were detected by in-gel fluorescence scanning after Cu(I)-mediated cycloaddition with **6**. Figure S2B; All **3**-labeled proteins in Figure S2A were detected by higher intensity of in-gel fluorescence scanning.

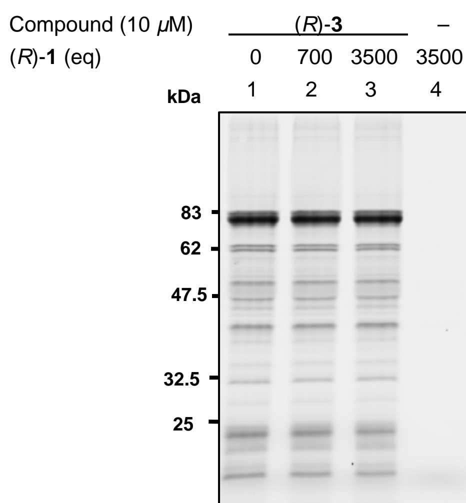

Figure S3. Competition experiments using (*R*)-**3** with excess (*R*)-**1** in the mouse lysate supplemented with overexpressed GST-CRMP2.

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Glutathione (GSH) Effect on Cu(I)-mediated Cycloaddition. Mouse brain lysate (1 mL, 50 mM HEPES buffer (pH 7.4)) was passed through a Nap-10 column (GE Healthcare) to exchange buffer to an aqueous 50 mM HEPES buffer (pH 7.8). Lysate aliquots (200 *μ*L of 2.0 mg/mL protein in 50 mM HEPES buffer (pH 7.8)) were treated with (*R*)-**3** (10 *μ*M) at 4 °C (10 min) and irradiated with 312 nm light (8 W, Spectroline EB-280C, Spectronics Corp., New York, USA) at 4 °C (5 min). The reaction mixtures were treated with TCEP (1 mM, room temperature, 20 min) for quenching of the remaining azide compound. The modified lysate was divided into 4 equal aliquots and treated with 0, 0.1, 0.5 and 2.5 mM of GSH, respectively. Samples were sequentially treated with rhodamine reporter tag (rhodamine-azide (Rho-N_3) (50 *μ*M)), TBTA (100 *μ*M) and $CuSO_4$ (1 mM) and shaken using Roto-shake (8 rpm, Scientific Industries Inc., Model No. SI-1100, Bohemia, NY) at room temperature (1 h). Proteins were separated by SDS-PAGE after addition of 4× SDS-PAGE loading buffer and visualized by in-gel fluorescence using a typhoon 9400 scanner (Amersham Bioscience) with excitation at 532 nm and detection at 580 nm.

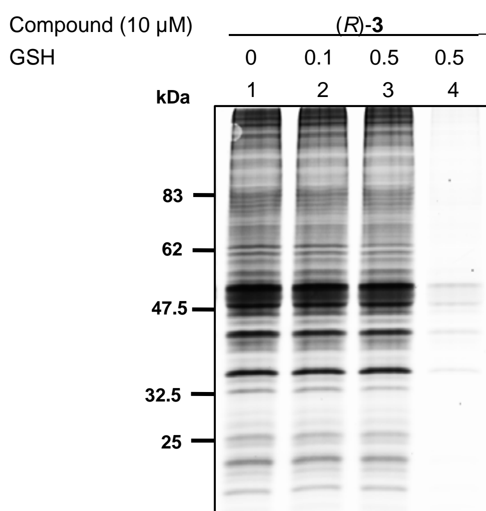

Figure S4. Glutathione (GSH) effect on Cu(I)-mediated cycloaddition.

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Table S1. Relative intensities of CRMP2 and other proteins in Figure 2.

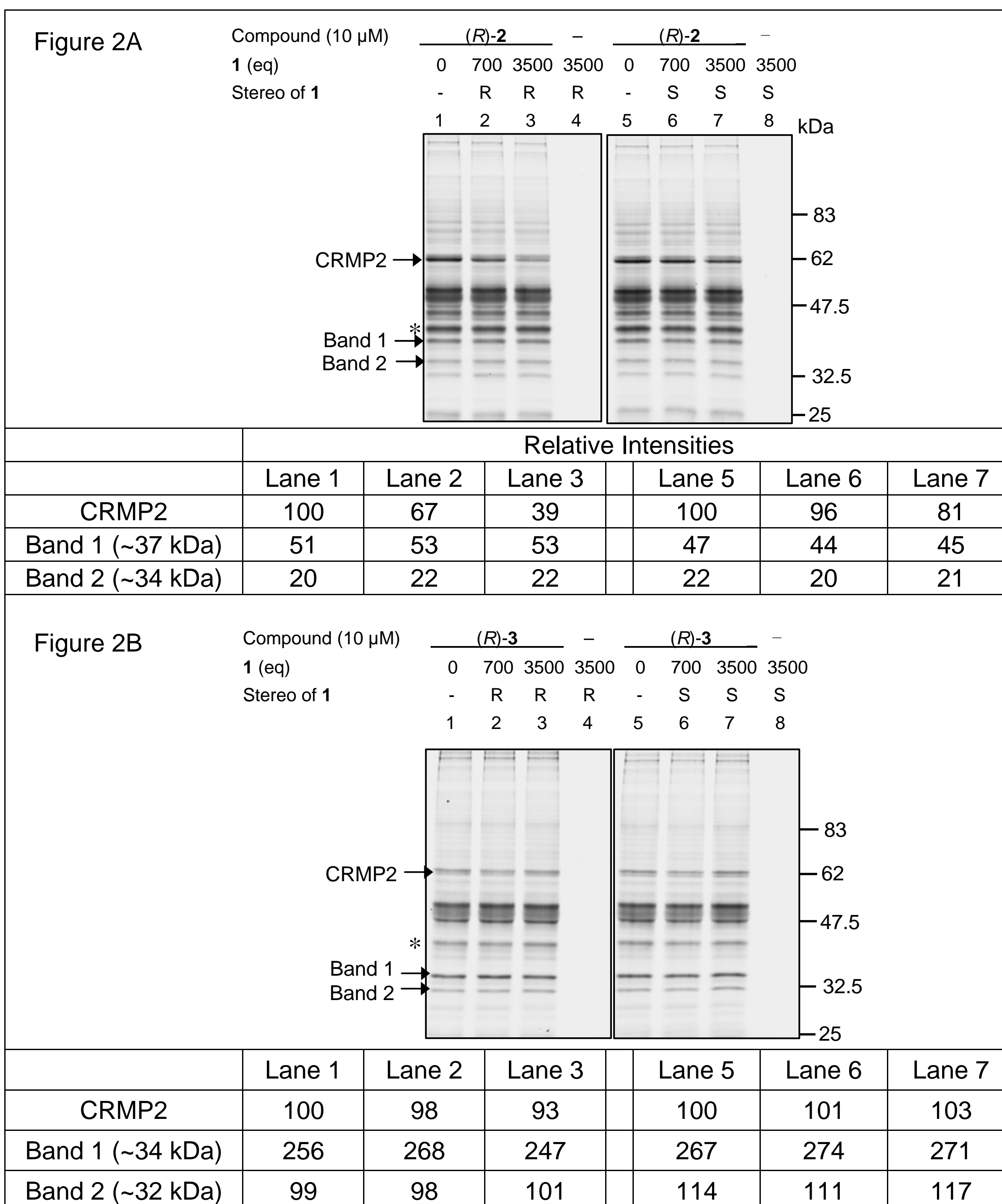

Figure 2A

	Relative Intensities						
	Lane 1	Lane 2	Lane 3		Lane 5	Lane 6	Lane 7
CRMP2	100	67	39		100	96	81
Band 1 (~37 kDa)	51	53	53		47	44	45
Band 2 (~34 kDa)	20	22	22		22	20	21

Figure 2B

	Lane 1	Lane 2	Lane 3		Lane 5	Lane 6	Lane 7
CRMP2	100	98	93		100	101	103
Band 1 (~34 kDa)	256	268	247		267	274	271
Band 2 (~32 kDa)	99	98	101		114	111	117

All relative intensities are calculated after normalization using an internal standard protein band (~40 kDa, asterisk). CRMP2 band in the first lane of each gel is given a value of 100.

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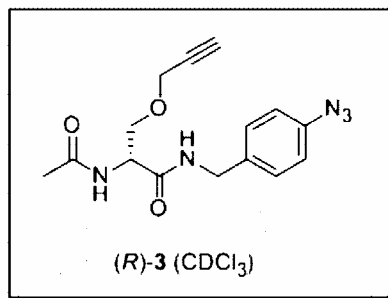

(*R*)-**3** ($CDCl_3$)

W: Water
X: Impurity
S: Solvent

S 7.266

2.035

X

7 6 5 4 3 2 1 ppm

2.73 2.00 0.97 0.99 1.06 2.04 2.15 1.07 1.07 1.00 3.12

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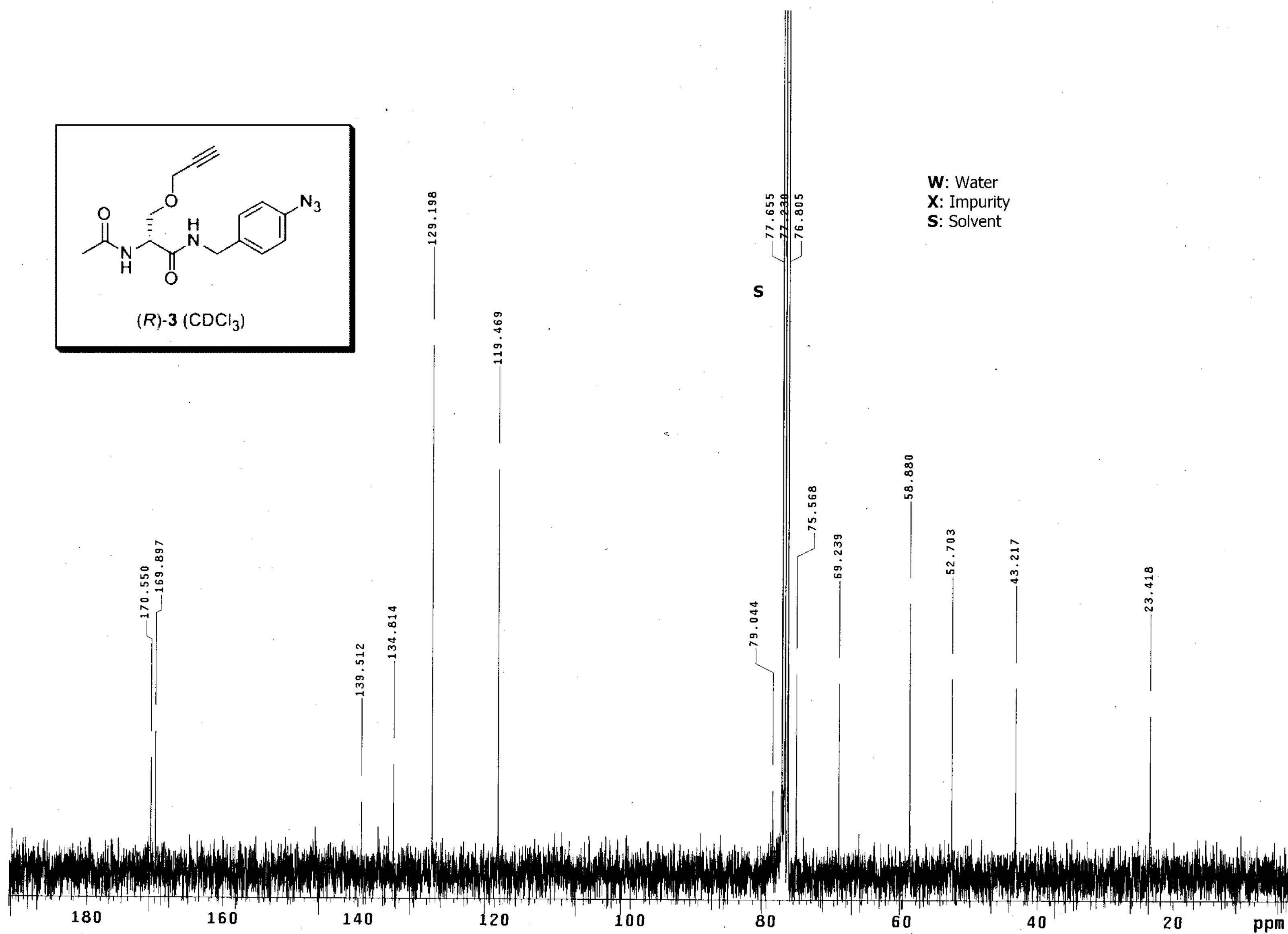

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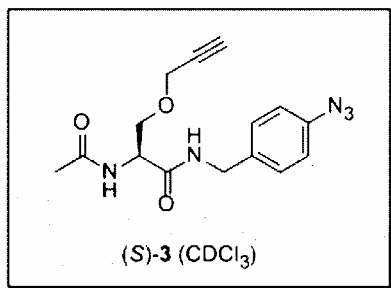

W: Water
X: Impurity
S: Solvent

7.268
2.029
X

7 6 5 4 3 2 1 ppm

2.30 1.97 1.04 1.01 0.97 2.00 2.08 1.09 1.06 1.00 3.10

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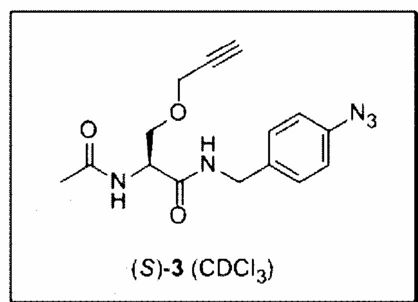

170.580
169.912
139.481
134.814
129.183
119.447
79.051
77.647
77.230
76.805
75.553
69.277
58.857
52.710
43.194
23.387

S

W: Water
X: Impurity
S: Solvent

160 140 120 100 80 60 40 20 ppm