

Supplemental Data for STAR Methods:

Ubiquitin PhotoTrap (Ub^{PT}) uncovers Rpn1 as a ubiquitin-associating subunit of 26S proteasome complexes.

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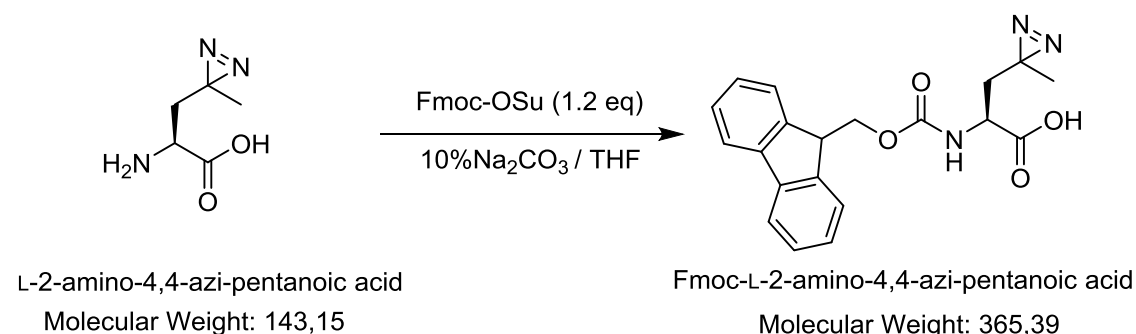
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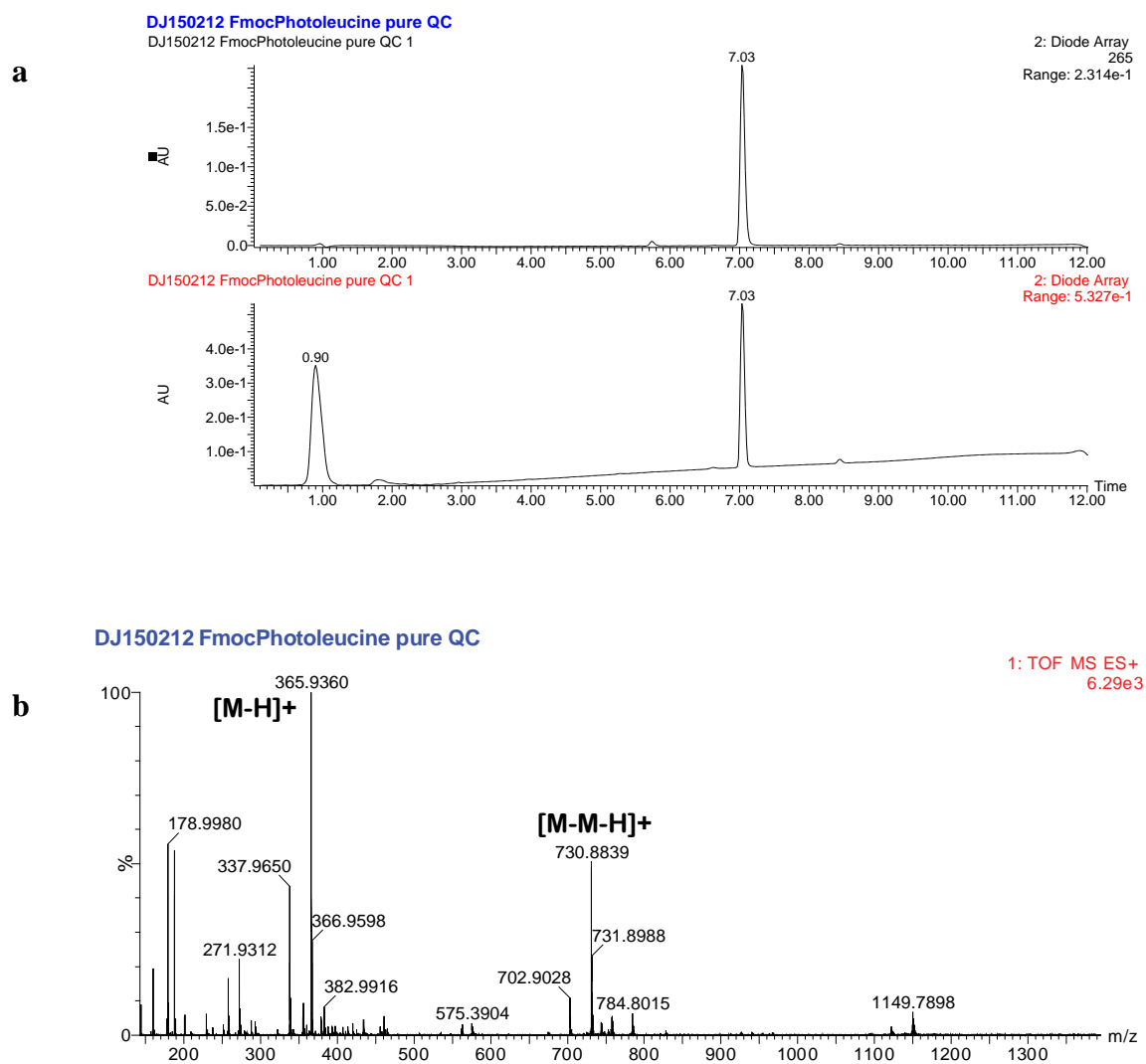
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CHEMICAL SCHEME 1. Fmoc-L-photoleucine

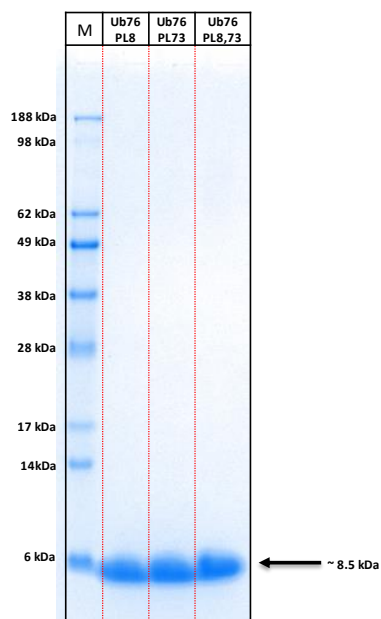


Chemical Scheme 2. HPLC-purified Fmoc protected L-photoleucine. **a** – Bottom - Diode Array chromatogram using LC-MS; Top - UV chromatogram at 265 nm; **b** - Spectrum of peak at 7.03 min. ESI-Mass [M+H] Expected: 366.39 / Found: 365.93

Time (in mins)	Solvent B (%)
0 ⇨ 5	5
5 ⇨ 7	5 ⇨ 25
7 ⇨ 22	25 ⇨ 55
22 ⇨ 24	55 ⇨ 95
24 ⇨ 27	95
27 ⇨ 27.5	95 ⇨ 5
27.5 ⇨ 30	5

Chemical Scheme 3. Gradient used in the HPLC purification of the ubiquitin mutants.

Analysis of purified ubiquitin incorporated with photoleucine

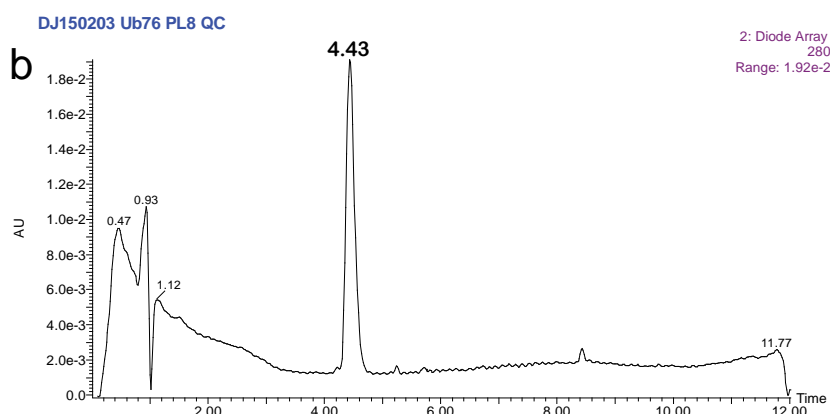


Chemical Scheme 4. Coomassie stained SDS-PAGE gel of ubiquitin with photoleucines incorporated at specific sites after purification by reversed phase HPLC. (M – Marker, prestained seeblue, invitrogen; Ub76 pL8 – ubiquitin with photoleucine at position 8; Ub76pL73 – ubiquitin with photoleucine at position 73; Ub76 pL8,73 – ubiquitin with photoleucine at positions 8 and 73)

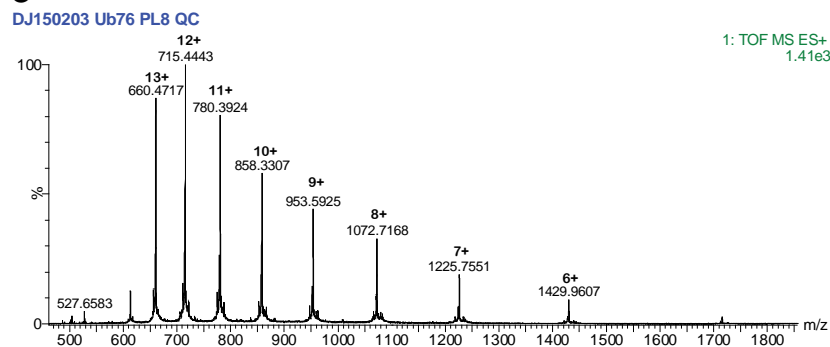
Conformation of Photo-leucine incorporated into position 8 of ubiquitin.

a

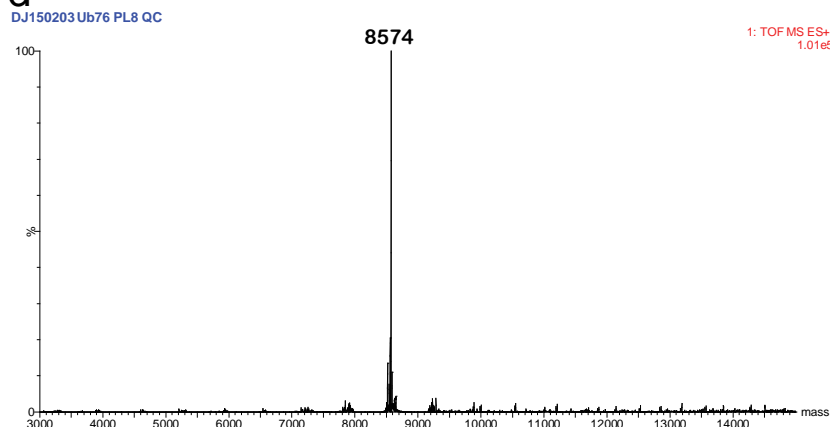
ID	Sequence	Mass (Da)	Length	6+	7+	8+	9+	10+	11+	12+	13+
Ub76	MQIFVK(T ^{PL})TGKTITLEVEPSDTIENVKAKIQDKEGIP	8575.9	76	1430.3	1226.1	1073.0	953.9	858.6	780.6	715.7	660.7
PL8	DQQLIFAGKQLEDGRTLSDYNIQKESTLHLVRLRGG										



c



d

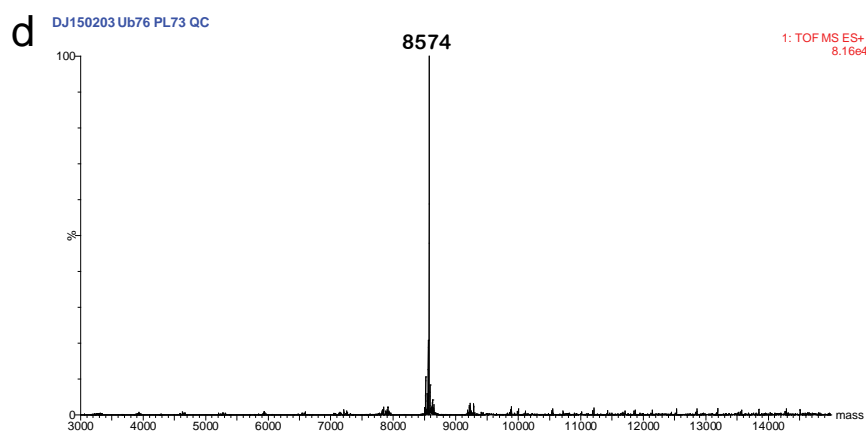
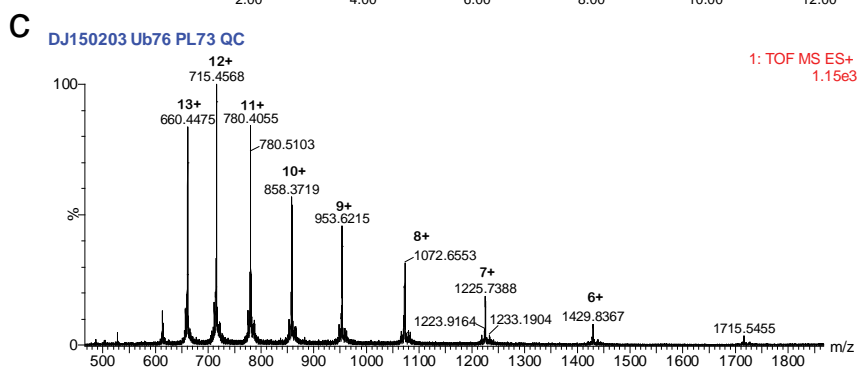
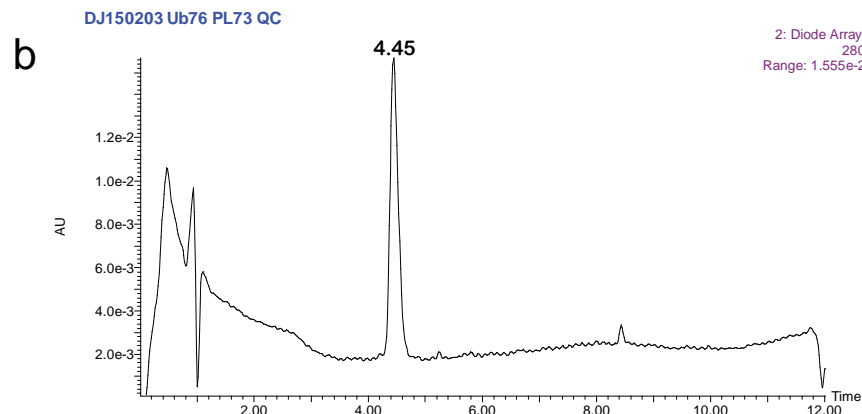


Chemical Scheme 5. Ubiquitin containing photoleucine at position 8. **a** – ESI-MS calculated mass; **b** - UV chromatogram (λ - 280 nm) using LC-MS; **c** - Spectrum of peak at 4.43 min; **d** - Deconvoluted mass of peak spectra found in **c**. ESI-Mass [M+H] Expected: 8576 / Found: 8574

Conformation of Photo-leucine incorporation at position 73 of Ub.

a

ID	Sequence	MW	Length	6+	7+	8+	9+	10+	11+	12+	13+
Ub76	MQIFVKTLTGKTTITLEVPSDTIENVKAKIQDKEGI										
PL73	PPDQQRLLIFAGKQLEDGRTLSDYNIQKESTLHLVLR(PL)RGG	8575.9	76	1430.3	1226.1	1073.0	953.9	858.6	780.6	715.7	660.7

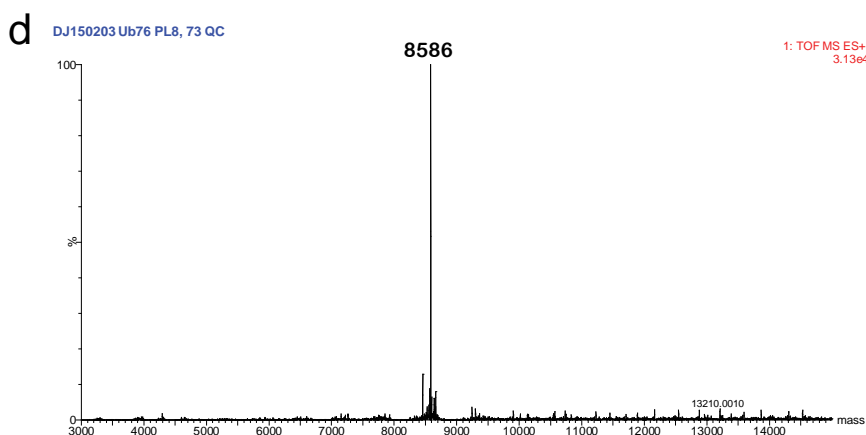
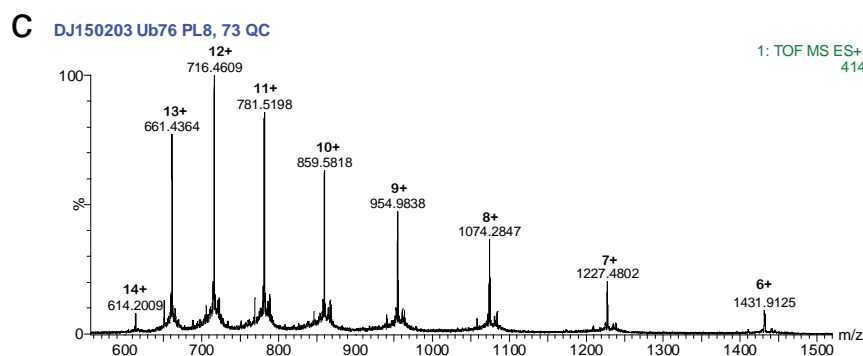
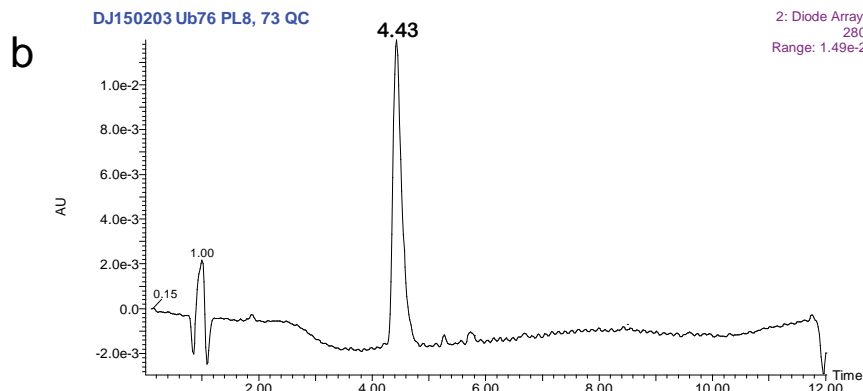


Chemical Scheme 6. Ubiquitin containing photoleucine at position 73. **a** – ESI-MS calculated mass; **b** - UV chromatogram (λ - 280 nm) using LC-MS; **c** - Spectrum of peak at 4.45 min; **d** - Deconvoluted mass of peak spectra found in **c**. ESI-Mass [M+H]⁺ Expected: 8576 / Found: 8574

Ub with Photo-leucine in positions 8, 73

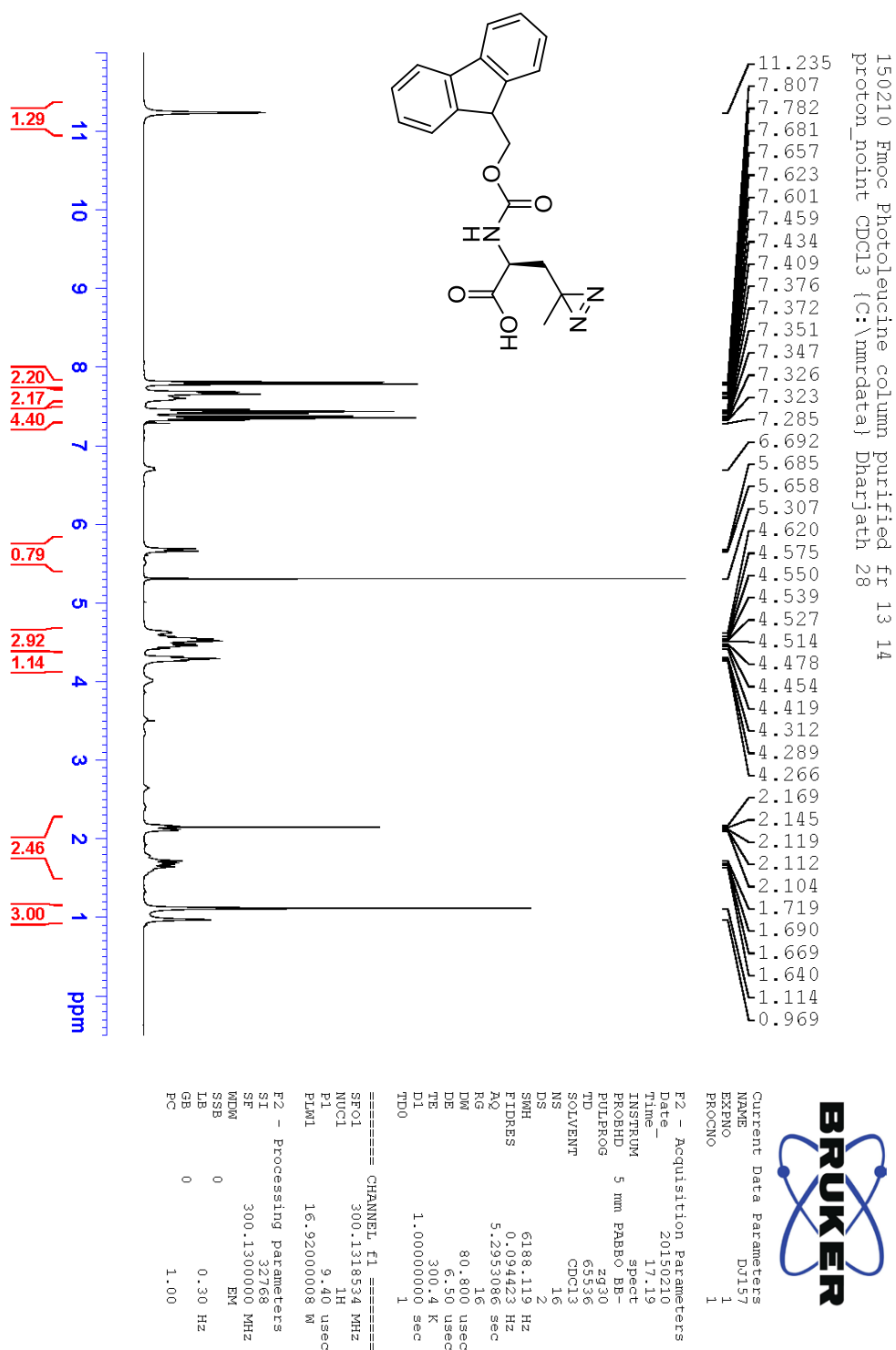
a

ID	Sequence	MW	Length	6+	7+	8+	9+	10+	11+	12+	13+	14+
Ub76	MQIFVK(T ^{PL})TGKTTITLEVEPSDTIENVKAKIQDKEG	8587.9	76	1432.3	1227.8	1074.5	955.2	859.8	781.7	716.7	661.6	614.4
PL8, 73	PPDQQRLLIFAGKQLEDGRTLSDYNIQKESTLHLVLR(T ^{PL})RGG											



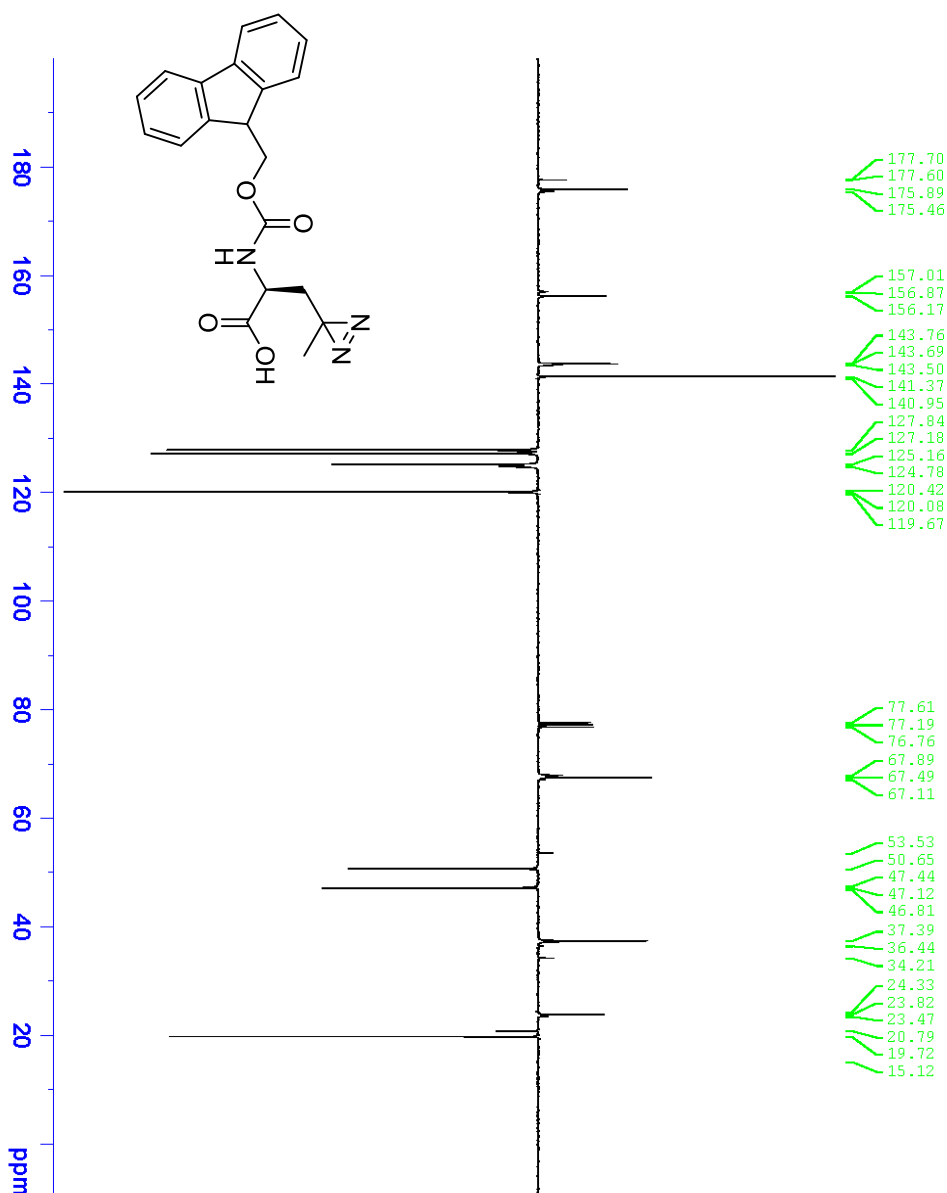
Chemical Scheme 7. Ubiquitin containing photoleucine at position 8 and 73. **a** – ESI-MS calculated mass; **b** - UV chromatogram (λ - 280 nm) using LC-MS; **c** - Spectrum of peak at 4.43 min; **d** - Deconvoluted mass of peak spectra found in **c**. ESI-Mass [M+H]⁺ Expected: 8588 / Found: 8586

Purity of Photo-leucine



Chemical Scheme 8. 1D¹H-NMR spectrum of photo-leucine

150210 Fmoc Photoleucine column purified fr 13 14
C13APT CDCl3 {C:\nmrdata\ Dharjath 28



Current Data Parameters
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EXPNO 4
PROCNO 1

F2 - Acquisition Parameters
Date_ 20150211
Time_ 9.13
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PULPROG jmod
TD 65536
SOLVENT CDCl3
NS 2833
DS 4
SWH 18028.846 Hz
FIDRES 0.275098 Hz
AQ 1.8175317 sec
RG 2050
DM 27.733 usec
DE 6.50 usec
TE 300.4 K
CNS2 145.0000000
CNST11 1.0000000
D1 2.00000000 sec
D20 0.00689655 sec
TD0 1

===== CHANNEL f1 =====
SFO1 75.4752953 MHz
NUC1 13C
P1 10.00 usec
P2 20.00 usec
PL1 29.1959915 W

===== CHANNEL f2 =====
SFO2 300.1312005 MHz
NUC2 1H
CPDPRG12 waltz16
PCPD2 90.00 usec
PLM2 16.92000008 W
PLM12 0.18457000 W

F2 - Processing parameters
SI 32768
SF 75.4677490 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

Chemical Scheme 9. ^{13}C -NMR spectrum of photo-leucine

