

Appendix C

Custom Edman Degradation

To allow for resolution of artificial azide-containing amino acids by Edman degradation, the **Pulsed-Liquid cLC extended** method was utilized (Figure C.1A) on the model 494 Procise cLC sequencing system (Applied BioSystems, Foster City, CA). It includes a modified gradient, **Normal 1 cLC extended** (Figure C.1B), and a flask cycle extended by 5 min (**Flask Normal extended**, Figure C.2).

The chromatograms corresponding to elution of Az2, Az4, Az6 and Az8 following Edman degradation are shown in Figure C.3 and demonstrate a 6-min retention time increase for every two methylene units added to the azidoalkyl side chain. Fmoc-Az2-OH was synthesized according to literature protocol,¹ while Fmoc-Az6-OH was synthesized from 1,6-dibromohexane according to Scheme 2.1.

(A)

Cycle #	Cartridge Cycle		Flask Cycle	Gradient
Default	Cart-PL 6mmGFF cLC		Flask Normal extended	Normal 1 extended
1	None		Prepare Pump cLC	Prepare Pump cLC
2	None		Flask Blank cLC	Normal 1 extended
3	Cart Begin cLC		Flask Standard cLC	Normal 1 extended

(B)

Time	%B	uL/min	Event	Cum. Volume A	Cum. Volume B
0.0	8	40	12	0.00	0.00
0.4	12	40	1	14.40	1.60
4.0	20	40	1	135.36	24.64
22.0	45	40	1	621.36	258.64
34.0	60	40	1	849.36	510.64
35.0	90	40	1	859.36	540.64
39.0	90	60	0	883.36	756.64
40.0	50	20	0	889.36	770.64

Figure C.1. (A) **Pulsed-Liquid cLC extended** method and (B) **Normal 1 cLC extended** gradient.

Cycle or Procedure :

Step	Function Name	Fn #	Value	Global	El. Tin
51	Load Position	226	0	<input type="checkbox"/>	31:16
52	Bubble Flask	212	5	<input type="checkbox"/>	31:21
53	Empty Flask	215	20	<input type="checkbox"/>	31:41
54	Del S4, Flask	171	10	<input type="checkbox"/>	31:51
55	Dry Flask	213	10	<input type="checkbox"/>	32:01
56	Bubble Flask	212	5	<input type="checkbox"/>	32:06
57	Flush Flask/Injector	222	40	<input type="checkbox"/>	32:46
58	Flush Injector	221	20	<input type="checkbox"/>	33:06
59	Wait	257	850	<input type="checkbox"/>	47:16
60	Wait	257	360	<input type="checkbox"/>	53:16
61	End	259	0	<input type="checkbox"/>	53:16

Figure C.2. Final steps of **Flask Normal extended** flask cycle.

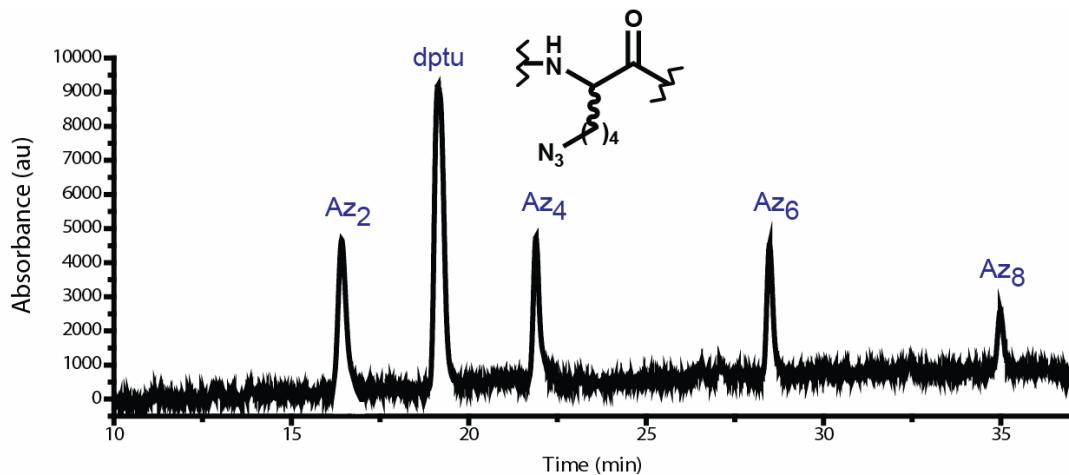


Figure C.3. Edman traces for artificial azide-containing amino acids.

REFERENCES

1. Roice, M.; Johannsen, I.; Meldal, M. *QSAR Comb. Sci.* **2004**, 23, 662–673.