

N-Hydroxysuccinimidyl derivatization reagents, friends or foes? From synthesis to derivatization of amino compounds and their analysis using Liquid Chromatography tandem mass spectrometry

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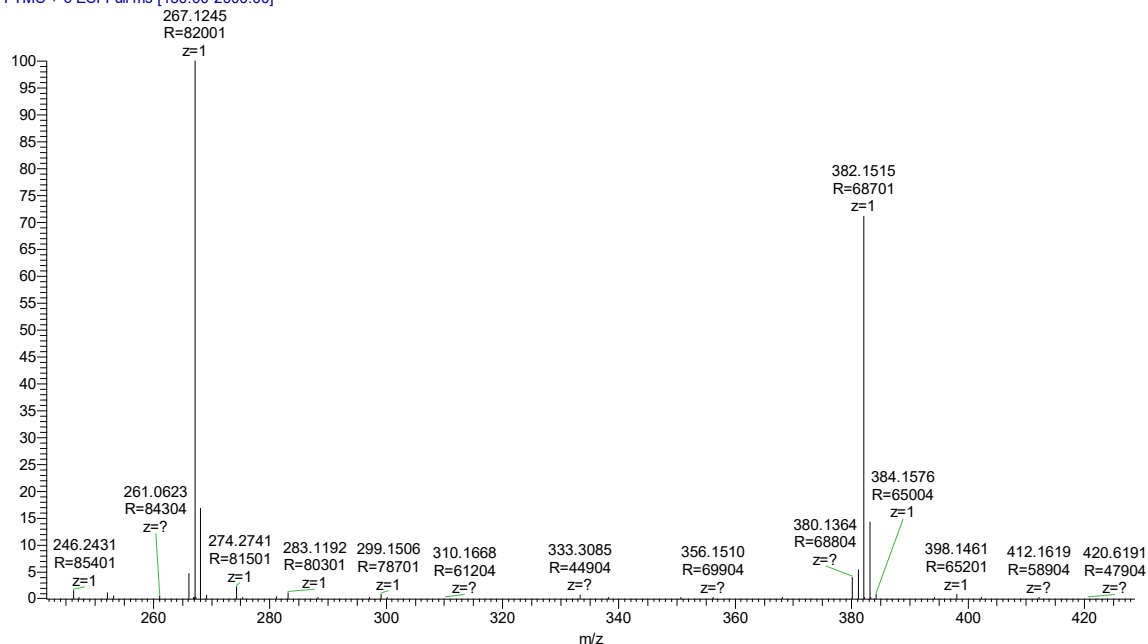
Ernesto Zapata and Nguyễn Kim Ngân Bùi made an equivalent contribution to this study.

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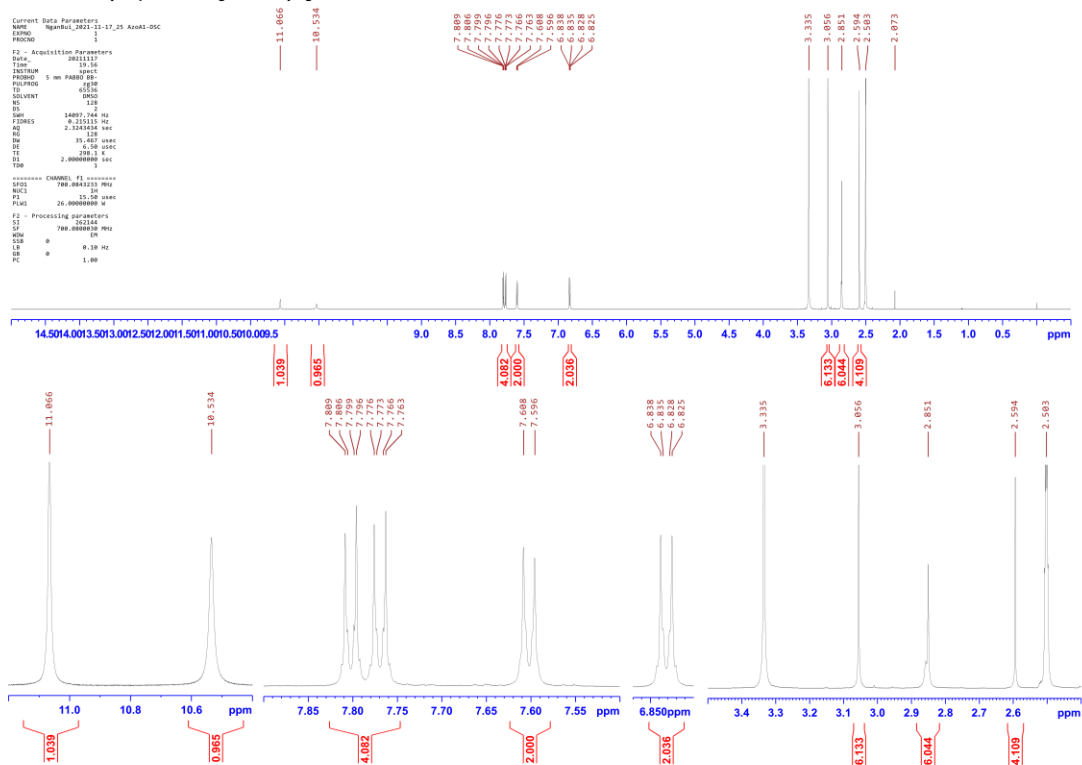
Supplementary material

S1. High resolution mass spectrum and ¹H NMR of AzoA.

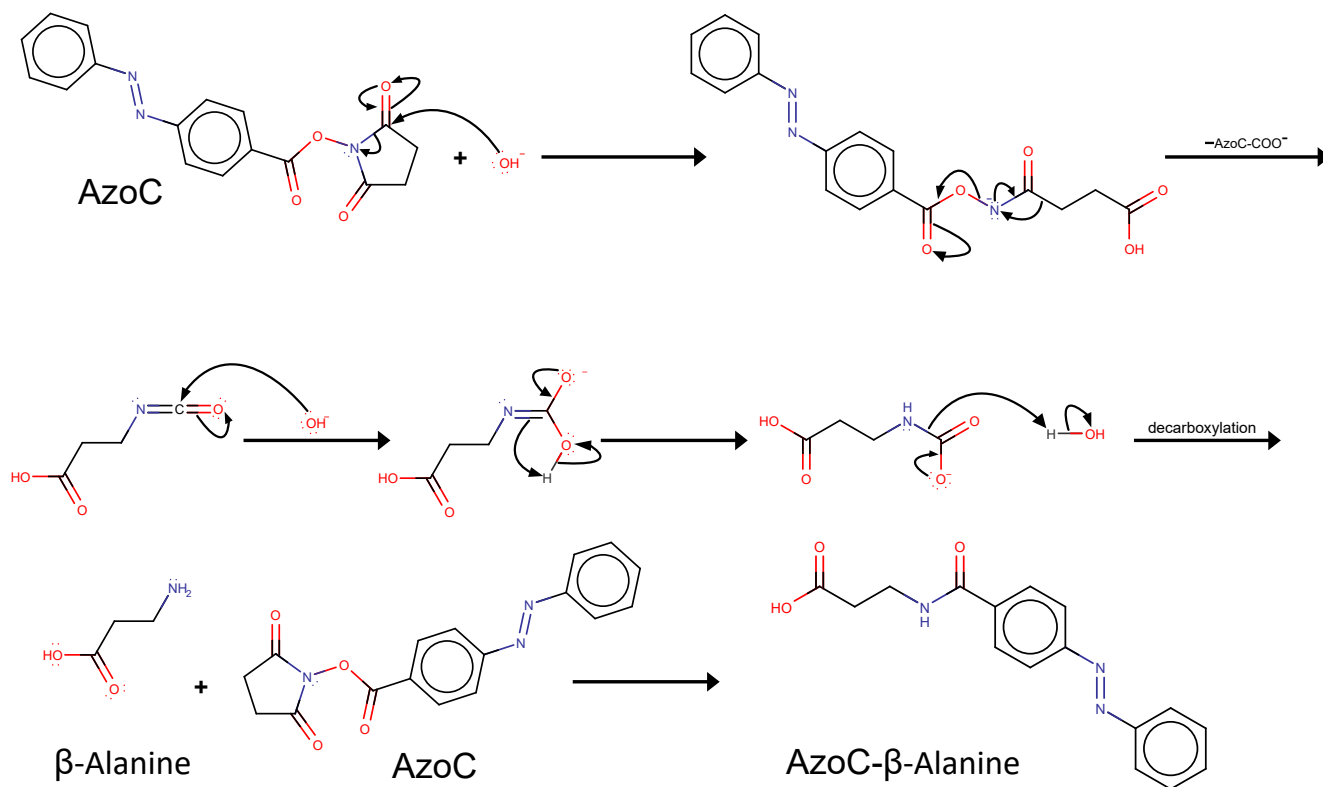
AzoA attempt 7 #167 RT: 2.37 AV: 1 NL: 1.17E8
T: FTMS + c ESI Full ms [150.00-2000.00]



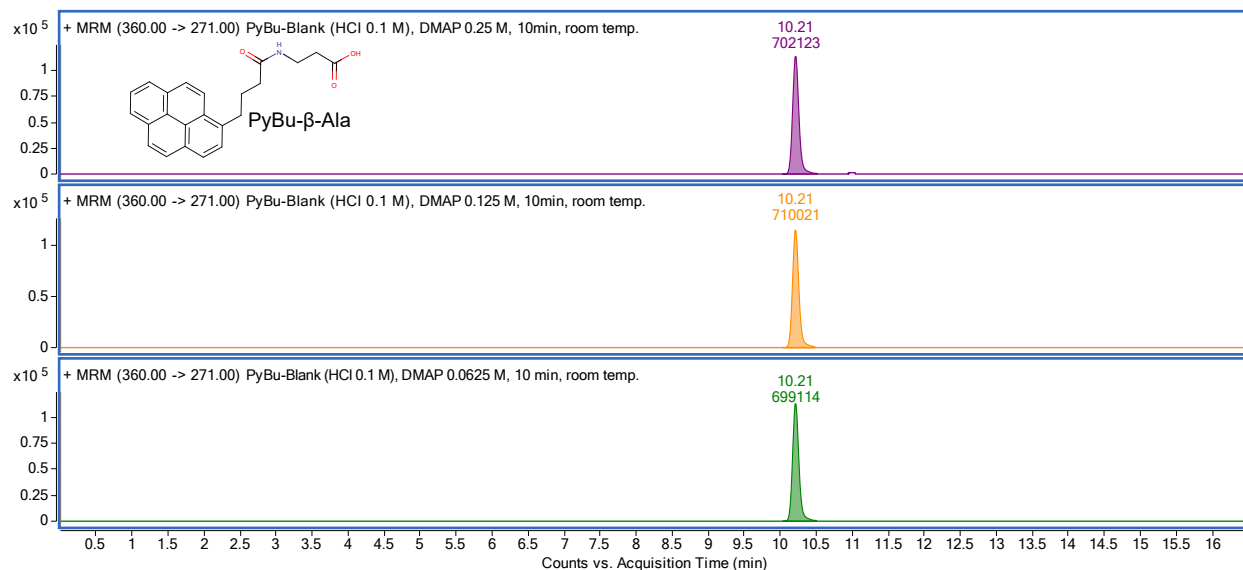
PROTON DMSO (C:\Spectra\data\NgaBui\mr) NgaBui 25



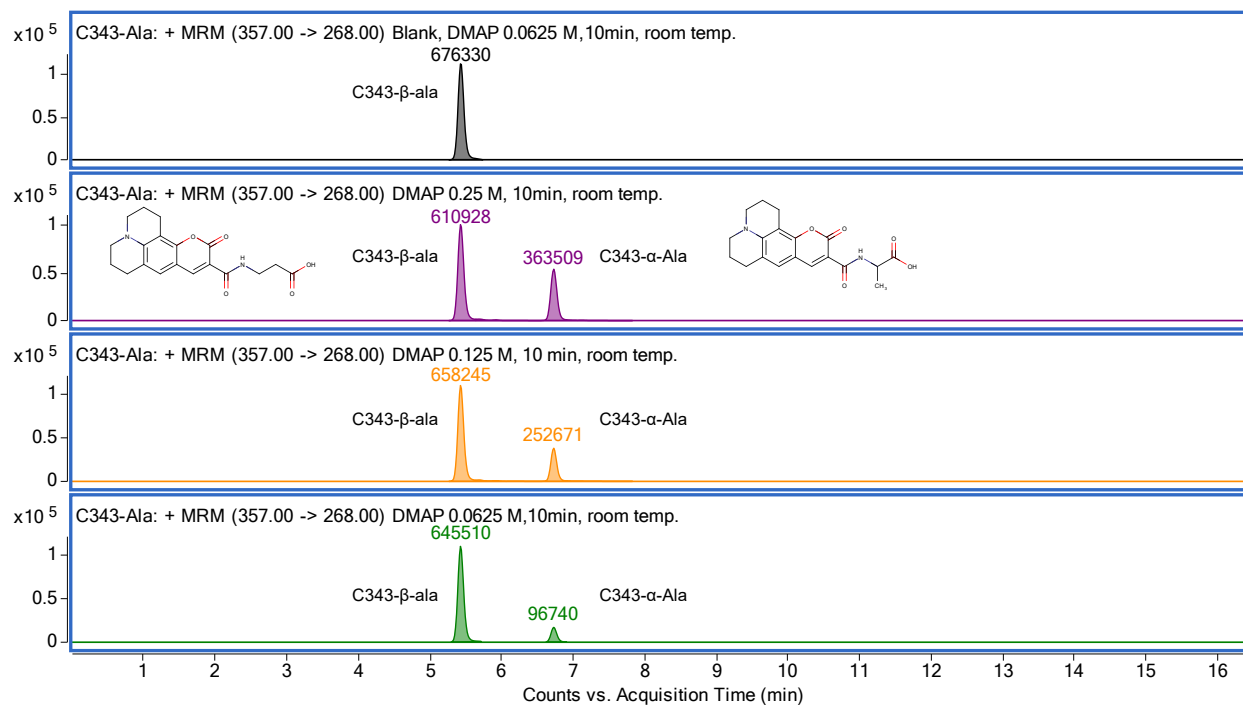
S2. Tentative mechanism of Lossen rearrangement of AzoC



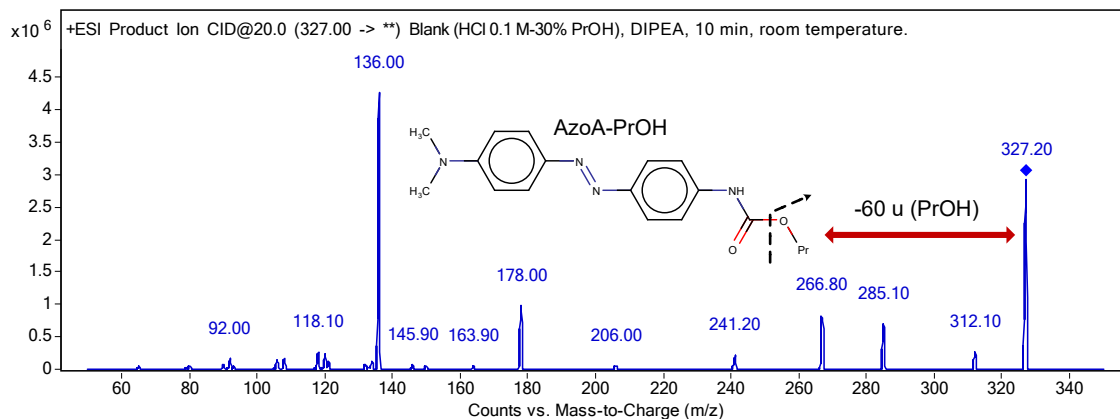
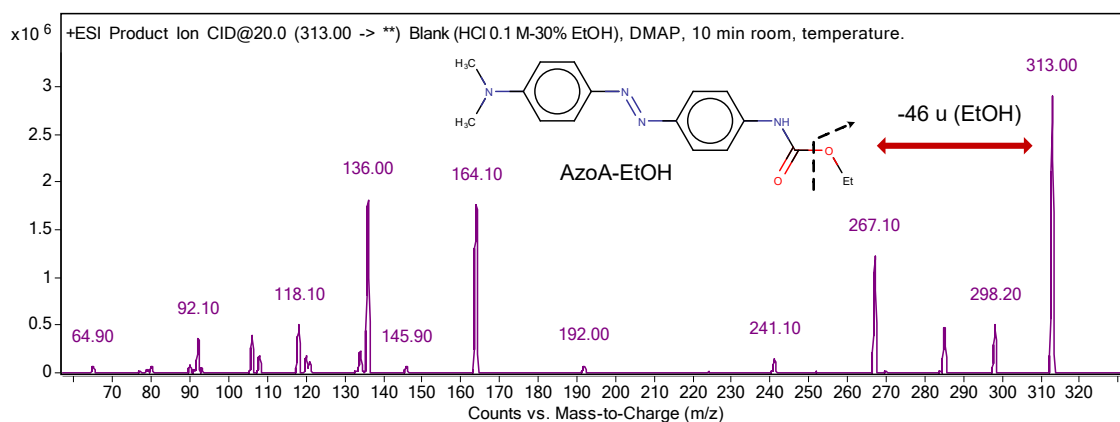
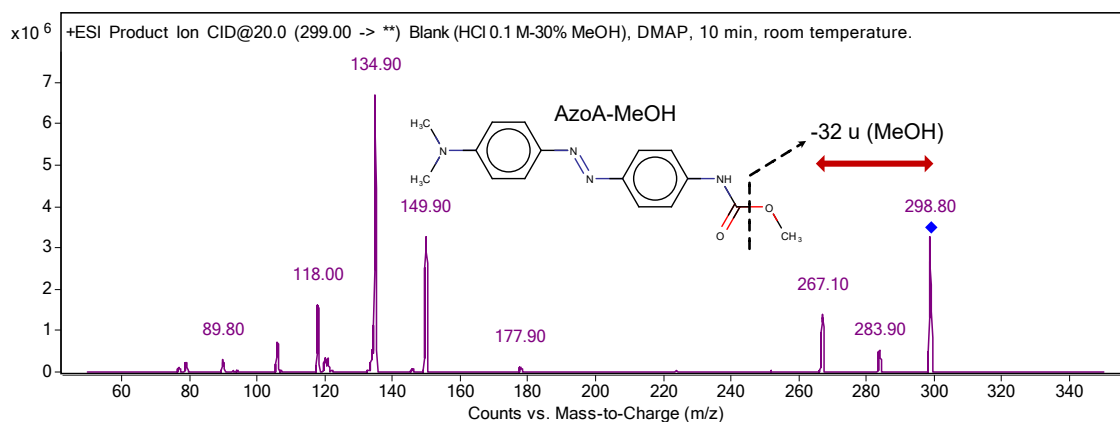
S3. Blank 1 solution ($0.1 \text{ mol L}^{-1} \text{ HCl}$) derivatized with PyBu-NHS in presence of DMAP at different concentrations (0.25 , 0.125 , and $0.0625 \text{ mol L}^{-1}$). Peak size of β -Ala derivative (Lossen rearrangement product) is almost constant regardless of the concentration of the base.



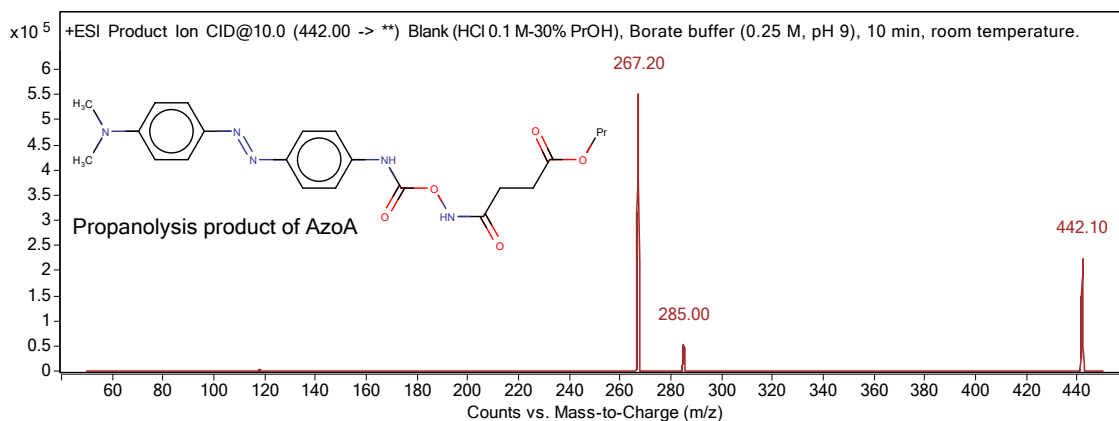
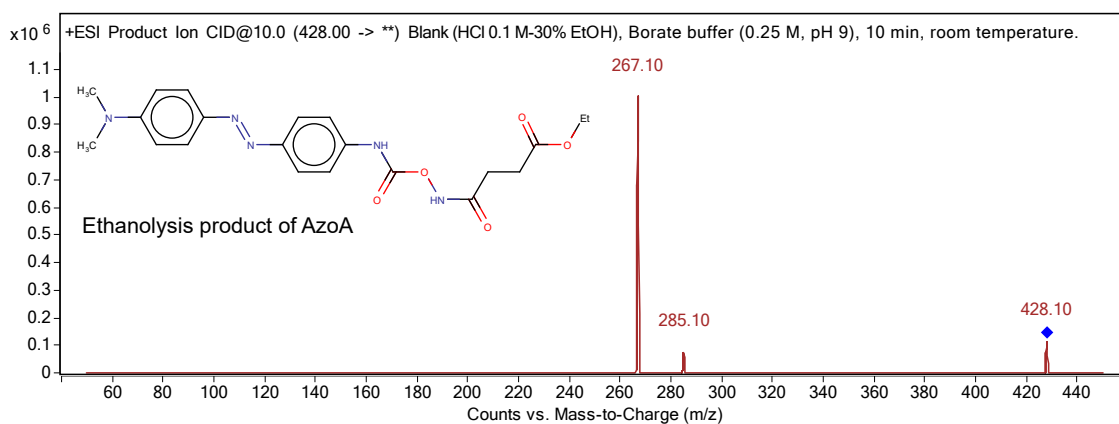
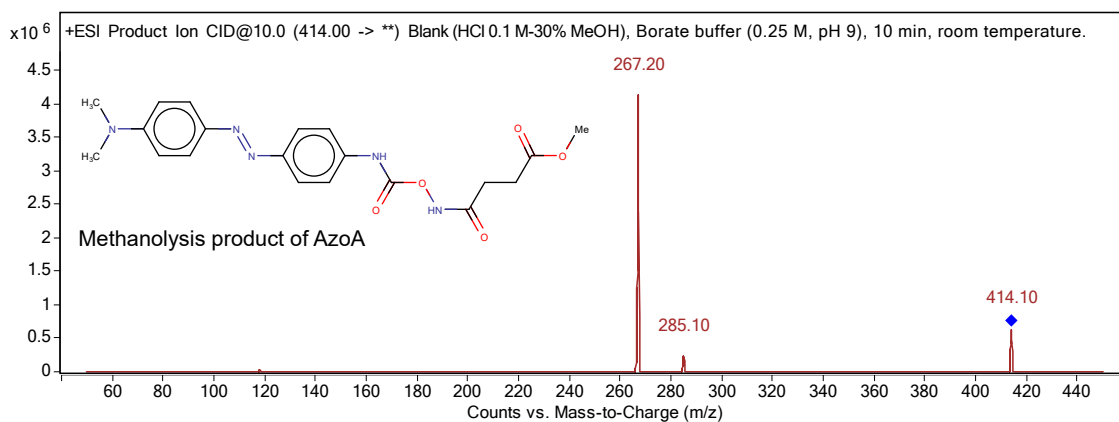
S4. Solution of α -Ala (2 mmol L^{-1} in $0.1 \text{ mol L}^{-1} \text{ HCl}$) and blank 1 ($0.1 \text{ mol L}^{-1} \text{ HCl}$) derivatized with C343-NHS using DMAP at different concentrations (0.25 , 0.125 , and $0.0625 \text{ mol L}^{-1}$). Peak size of β -Ala derivative (Lossen rearrangement product) is almost constant regardless of the concentration of the base, but peak area of the α -Ala derivative decreases as the concentration of DMAP decreases.



S5. Product ion scan mass spectra of transcarbamation products of AzoA with MeOH (299 m/z), EtOH (313 m/z) and PrOH (327 m/z).



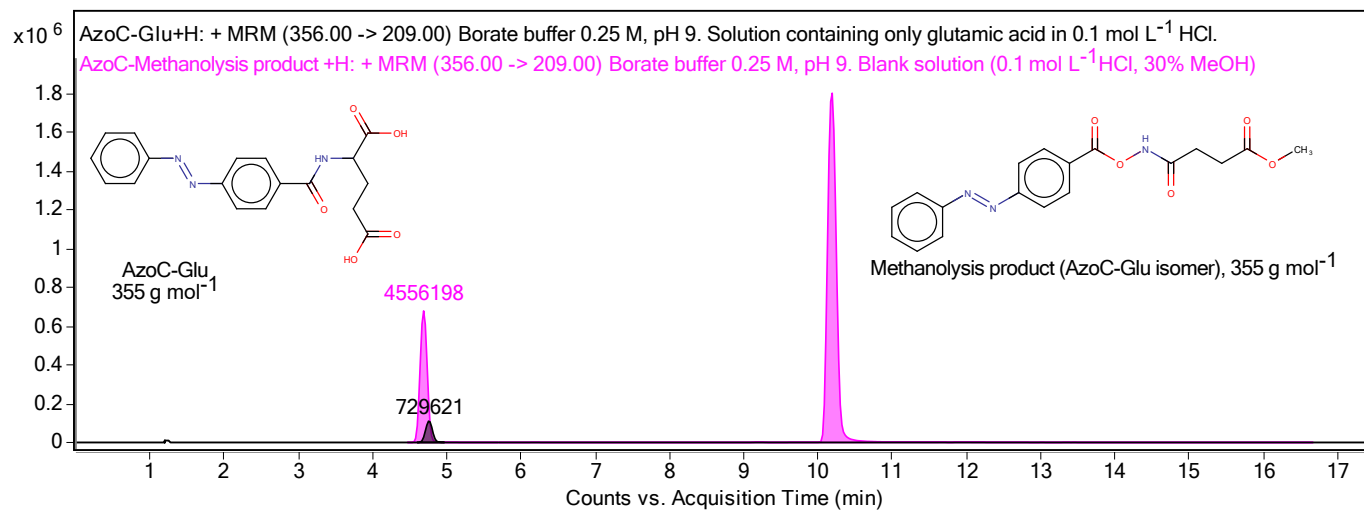
S6. Product ion scan mass spectra of alcoholysis over succinimide ring of AzoA with MeOH (414 m/z), EtOH (428 m/z) and PrOH (442 m/z).



S7. Retention time, precursor ion and product ions (CE 10V unless other way stated) of the different side products found when the blank solutions were derivatized with the different reagents based on azobenzene.

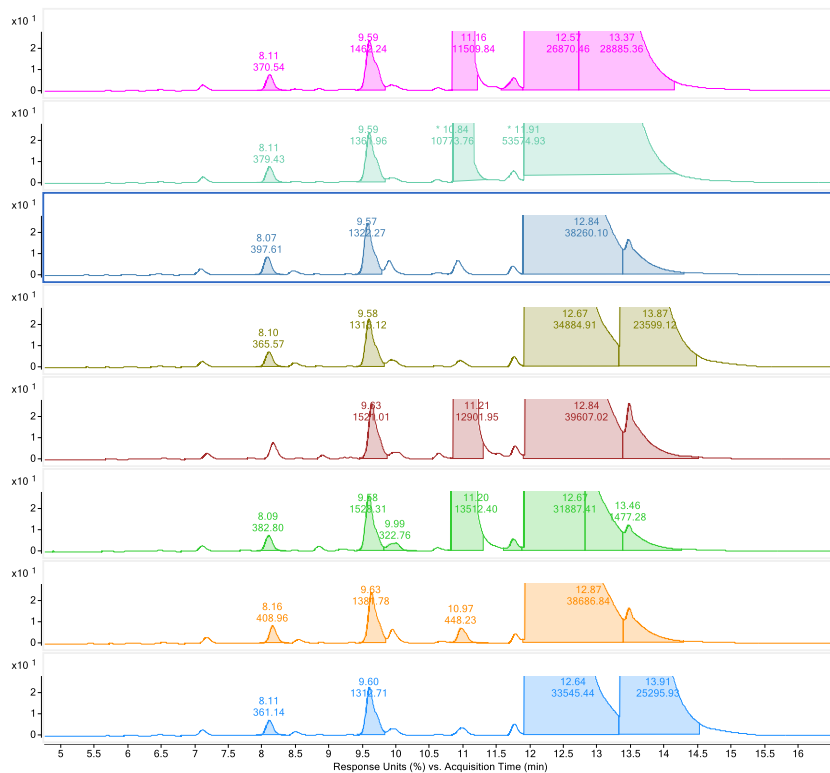
Reagent	Side product	Retention time (min)	Precursor ion (m/z)	Product ion (m/z)
AzoA	Alkaline hydrolysis (hydroxamate ester)	6.4	400	267
	Methanolysis	9.2	414	267
	Ethanolysis	10.6	428	267
	Propanolysis	11.8	442	267,285
	Transcarbamation (with MeOH) ¹	11.1	299	267,150,135
	Transcarbamation (with EtOH) ¹	12.3	313	285,267,164
	Transcarbamation (with PrOH) ¹	12.9	327	285,267,136
	Lossen rearrangement product (β -Alanine derivative) ²	6.1	356	267
AzoB	Alkaline hydrolysis (hydroxamate ester)	6.1	357	224
	Methanolysis	9	371	224
	Ethanolysis	10.4	385	224
	Propanolysis	11.8	399	224
	Transcarbamation (with MeOH)	11	256	224,77
	Transcarbamation (with EtOH) ¹	12.2	270	242,224,77
	Transcarbamation (with PrOH) ¹	12.9	284	242,224,77
	Lossen rearrangement product (β -Alanine derivative) ²	6.4	313	224
AzoC ³	Alkaline hydrolysis (hydroxamate ester)	2.9 and 4.8	342	209
	Methanolysis	5.1 and 10.4	356	209,115
	Ethanolysis	6.9 and 11.6	370	209,129
	Propanolysis	9.3 and 12.4	384	209, 101
	Transesterification (with MeOH) ¹	8 and 12.7	241	135,77
	Transesterification (with EtOH) ¹	10.4 and 13.2	255	227, 77
	Transesterification (with PrOH) ¹	10.3	269	227, 210, 77
	Lossen rearrangement product (β -Alanine derivative)	1.7 and 6	298	209
AzoD	Alkaline hydrolysis (hydroxamate ester)	8.2	385	270, 252
	Methanolysis	10.7	399	270,252
	Ethanolysis	11.9	413	270, 252
	Propanolysis	12.4	427	270, 252
	Transesterification (with MeOH)	12.8	284	135, 103
	Transesterification (with EtOH)	13.3	298	270,121
	Transesterification (with PrOH) ⁴	10.7 and 13.7	312	270,163,121
	Lossen rearrangement product (β -Alanine derivative)	6.5	341	252
¹ CE 20 V				
² β -ala derivative was present in a very small amount, see main text section 3.4.				
³ AzoC produced 2 peaks for almost every derivative because of E/Z isomerism, see main text section 3.5.				
⁴ two peaks were observed in product ion scan, possibly because of E/Z isomerism				

S8. Methanolysis of AzoC (AzoC-Glu isomer) and its impact to the identification and quantification of glutamic acid. MRM Chromatogram of blank solution containing MeOH after derivatization with AzoC, two peaks with the transition $356 \rightarrow 209$ m/z appear at 4.7 and 10.1 minutes, so called “fast” and “slow” peaks [ref 30 in main text]. “Fast” peak overlaps with the real derivative of glutamic acid.

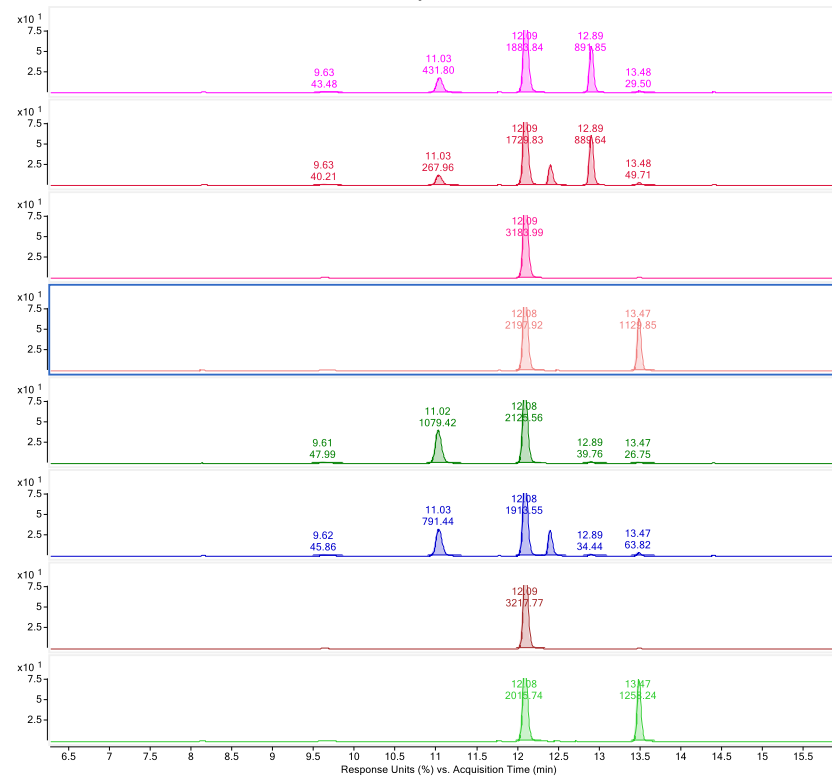


S9. FLD (Excitation 341 nm and Emission 394 nm) and UV (341 nm) chromatograms of PyBU derivatization

- A. RT10m 025MBo9 PyBu Blk HCl
- B. RT10m 025MBo9 PyBu MeOH
- C. RT10m 025MDMAP PyBu Blk HCl
- D. RT10m 025MDMAP PyBu MeOH

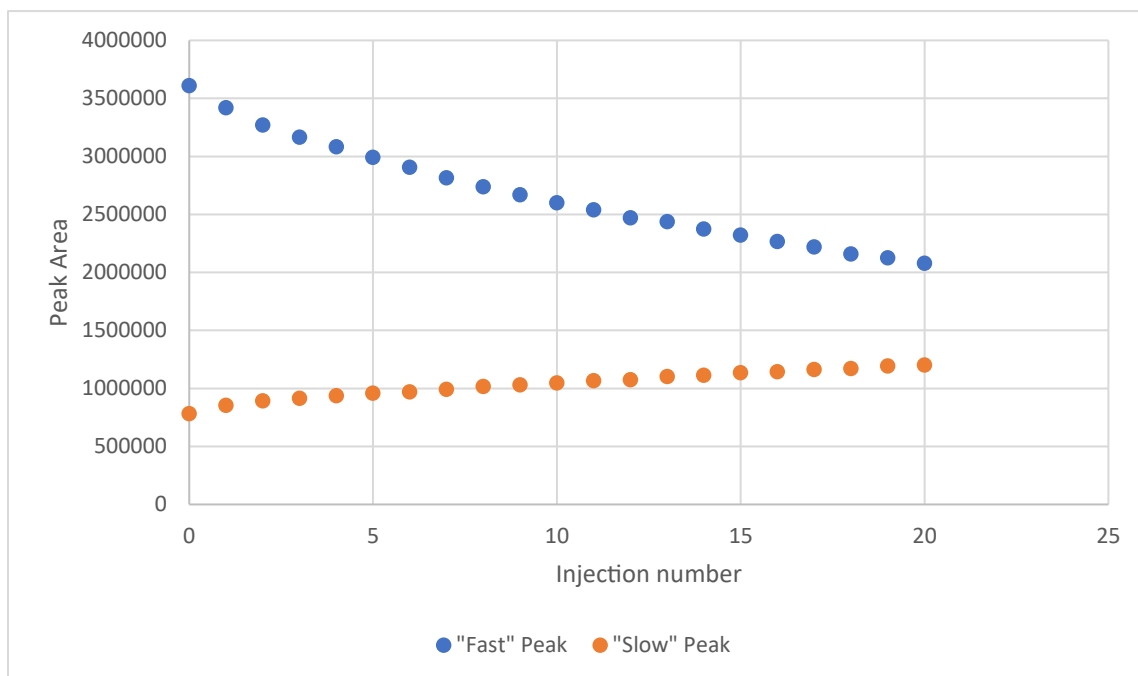


- E. 50C10m 025MBo9 PyBu Blk HCl
- F. 50C10m 025MBo9 PyBu MeOH
- G. 50C10m 025MDMAP PyBu Blk HCl
- H. 50C10m 025MDMAP PyBu MeOH



S10. Peak areas of successive injections of an irradiated solution of AzoC; Injection "0" is the measurement right after UV irradiation. Plot shows the trend of the peak areas of both peaks over time.

Injection number	Acquisition time	Peak Area	
		"Fast" Peak	"Slow" Peak
0	22:05	3612097	784581
1	22:51	3420840	856843
2	23:17	3272572	893086
3	23:38	3166454	916090
4	23:59	3083672	939560
5	00:20	2994200	959248
6	00:41	2907891	972686
7	01:02	2817834	994067
8	01:23	2739044	1018229
9	01:44	2671065	1031316
10	02:05	2602694	1049028
11	02:26	2540077	1068137
12	02:47	2470827	1077401
13	03:08	2438704	1103257
14	03:29	2376974	1116318
15	03:50	2321946	1135897
16	04:11	2266737	1145997
17	04:32	2221169	1164033
18	04:53	2159117	1173885
19	05:14	2128633	1195782
20	05:35	2079841	1202974



S11. The ratio of side product's peak areas (obtained from MRM measurements) using different buffers and temperatures.

		Replacing Borate with DMAP				Replacing DMAP with TEA			
		At RT	Ratio-times	At 50°C	Ratio-times	At RT	Ratio-times	At 50°C	Ratio-times
Alkaline hydrolysis over NHS ring (hydroxamate ester, isomer of aspartic acid derivative) Path 1 in figure 5	AzoA-ASP isomer	decreased	117.2	decreased	497.9	increased	12.9	increased	9.6
	AzoB-ASP isomer	decreased	759.2	decreased	155.0	increased	8.6	decreased	7.9
	AzoC-ASP isomer 2	decreased	5219.3	decreased	5129.6	increased	4724.3	increased	8543.1
	AzoD-ASP isomer	decreased	65.8	decreased	384.2	increased	165.5	increased	258.8
Lossen rearrangement of hydroxamate ester Path 1 in figure 5	AzoA-β-ALA	increased	1.2	increased	1.7	decreased	3.8	decreased	4.0
	AzoB--β-ALA	increased	3.3	decreased	1.2	decreased	15.9	decreased	10.5
	AzoC-β-ALA 2	decreased	3.2	decreased	75.4	increased	20.9	increased	103.5
	AzoD-β-ALA	decreased	1.3	decreased	59.0	increased	8.0	increased	21.3
Methanolysis product (isomer of glutamic acid derivative) Path 1 in figure 5	AzoA-GLU isomer	decreased	16435.7	decreased	1479.6	increased	416.8	increased	3.2
	AzoB--GLU isomer	decreased	2921.9	decreased	881.3	increased	4.0	increased	3.0
	AzoC-GLU isomer 2	decreased	2634.8	decreased	1440.3	increased	2734.8	increased	2599.5
	AzoD-GLU isomer	decreased	42.4	decreased	85.8	increased	87.6	increased	185.2
Transcarbamation product (with MeOH) Path 2 in figure 5	AzoA-OMe	increased	1.7	increased	1.7	decreased	3.9	decreased	1.7
	AzoB--OMe	increased	2.7	increased	2.7	decreased	5.7	decreased	5.0
	AzoC-OMe-2β-	increased	3.8	increased	3.2	decreased	6.3	decreased	1.1
	AzoD-OMe	increased	14.1	increased	3.7	decreased	5.9	decreased	3.1
Alkaline hydrolysis Of NHS carbamates Path 2 in figure 5	AzoA-NH2	decreased	1.2	decreased	1.3	decreased	18.6	decreased	18.7
	AzoB--NH2	increased	1.6	increased	1.0	decreased	130.0	decreased	116.9
	AzoA-urea	increased	14.3	increased	13.9	decreased	8.9	decreased	8.5
	AzoB--urea	increased	2.4	increased	4.1	increased	6.2	increased	5.1
Alkaline hydrolysis of NHS esters Path 2 in figure 5	AzoC-COOH 2	increased	2.4	increased	2.3	decreased	1.8	increased	2.1
	AzoD-COOH	decreased	1.4	increased	2.3	decreased	2.0	increased	2.5
	AzoC 2	decreased	351.7	decreased	58.9	increased	102.9	increased	250.7
	AzoD	decreased	2009.1	decreased	494.7	increased	1992.4	increased	1139.0