SUPPORTING INFORMATION FOR

Microgram-Scale Testing of Reaction Conditions Using Nanoliter-Plugs in Microfluidics with Detection by MALDI-MS

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Contents

1. Instruments and materials
2. Methods
    a. Merging and Fabrication of Reagent Cartridge
    b. MALDI-MS
3. Characterization of merging
    a. Cross-contamination
    b. Merging ratio
4. Control experiments on reproducibility and quantitativity of MALDI-MS
5. MALDI-MS data for Figure 2
6. MALDI-MS data for Figure 3
7. No-loss handling of sub-microliter volume of solution
8. Synthesis of Ac_3-OUA, Ac_4-OUA, and Ac_5-OUA on 0.01 mmol scale
    a. General
    b. Preparation of ouabain 1, 2', 3', 4', 11, 19-hexaacetate (Ac_6-OUA)
    c. Preparation of ouabain 1, 11, 19-triacetate (Ac_3-OUA) via tris-deacetylation of Ac_6-OUA
    d. Preparation of ouabain 1, 4', 11, 19-tetraacetate (Ac_4-OUA) via bis-deacetylation of Ac_6-OUA
    e. Preparation of ouabain 1, 3', 4', 11, 19-pentaacetate (Ac_5-OUA) via mono-deacetylation of Ac_6-OUA
9. Solubility of organic reagent in FC-70 and other fluorinated solvent
10. Additional MALDI-MS data from screens
11. Representative MALDI-MS spectra

1. Instruments and materials

Syringe: Hamilton 1801RN 10μL

Syringe pump: Harvard Apparatus PHD 2000 Infusion pump

Manual microsyringe pump: Model 51222, Stoelting Co., Illinois

Tubing: Upchurch #1933, 150μm ID Teflon PFA HP PLUS tubing, 360mm OD

PEEK Tee: Upchurch #P-888 MicroTee for 360mm OD tubing, 0.006'' thru-holes

Two-way connector: Upchurch #P-772, MicroTight Union 0.006'' thru-hole for 360 mm OD tubing

Sleeve: Upchurch #F-376x, 395μm ID, for tubing size 340-380 μm

MALDI-MS: Voyager-DE Pro, Applied Biosystems

Data analysis software: Data Explorer^{TM} version 4.0, Applied Biosystems

MALDI plate: Applied Biosystems, MALDI Sample plate, SS, 100 well with circles, P/N: V700666

Proton nuclear magnetic resonance (^{1}H NMR): Bruker DRX-500 spectrometer

Carbon nuclear magnetic resonance (^{13}C NMR): Bruker DRX-400 spectrometer

IR spectra: Nicolet 20 SXB FT-IR spectrometer

FC-70 (carrier fluid): Acros Organics, #123782500.

Ouabain octahydrate: Acros Organics, #161730050

Other solvents and materials were purchased from Fisher Scientific, Acros Organics, Aldrich Inc., and other commercial suppliers, and were used without further purification unless otherwise noted.

2. Methods

a. Merging and Fabrication of Reagent Cartridge. A schematic for merging the cartridge with substrate solution is shown in the manuscript, Figure 1a. The PEEK Tee (P-888 MicroTee for 360mm OD tubing, 0.006'' thru-holes) and the tubing (Teflon PFA HP PLUS tubing, 150μm ID, 360mm OD) were purchased from Upchurch. The syringe pumps were Harvard Apparatus PHD 2000 Infusion pumps. The flow rates were 3.0 mL/min for the cartridge, and 1.5 mL/min for substrate, respectively. The tubing containing the plugs after merging was incubated at 0, 18, or 40 °C for predetermined period of time.

Procedures to fabricate cartridges of preformed reagent plugs were adopted from previous publication with further modification (*Angew. Chem. Int. Ed.* **2005**, *44*, 2520−2523). The Teflon tubing was connected to the needle of the syringe using a short piece of plastic sleeve (Upchurch, F-376x, 395 μm ID, for tubing size 340-380 μm) as the adaptor. The syringe and tubing were pre-filled with carrier fluid FC-70 (Acros Organics). The gas (air), carrier fluid, and solutions were aspirated into the Teflon tubing by pulling the syringe plunger; the movement of plunger was controlled with a manual micro syringe pump (model 51222, Stoelting Co., IL). The order of substances being aspirated is (in the parenthesis are the lengths of plunger movement): gas bubble (0.08 mm), carrier fluid (0.10 mm), buffer plug (0.20 mm), carrier fluid (0.10 mm), gas bubble (0.08 mm), the first reagent (0.09 mm), gas bubble (0.08 mm), buffer plug (0.20 mm) … and so on. A schematic of the cartridge is shown in Figure S2 A. Usually each cartridge contains 7~8 reagent plugs for best merging results.

b. MALDI-MS. Plugs were flown out of the tubing and deposited to the MALDI-sample plate. The flow was induced using a syringe filled with FC-70 carrier fluid. The syringe and tubing was connected via a 2-way connector (Upchurch, P-772, MicroTight Union 0.006'' thru-hole for 360 mm OD tubing). Movement of the tubing-end controlled the position of plug deposition on the plate. The washing buffer plugs were discarded. The reagent plug, gas bubble, and buffer plug could be recognized by naked eye or with the help of a stereoscope when necessary. It usually took ~2 minutes to deposit a cartridge containing 7~8 reaction trials.

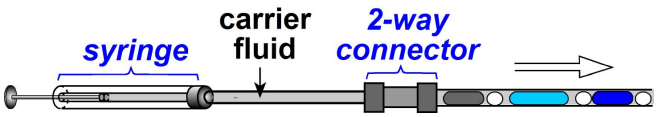


Figure S1. Schematic of flowing the plugs out of the tubing

The reactions were immediately quenched after depositing the plugs. The reaction with an amine was quenched by removal of amine *in vacuo* (0.3 mmHg, 10 min). Matrix solution (0.4 μL, 0.2 M 2, 5-dihydroxybenzoic acid and 0.2 M acetic acid in DMF) was deposited over each dried spot. For others, the reaction was quenched by adding 0.4 μL matrix solution to each plug immediately after deposition. After removal of the solvents *in vacuo*, MALDI-TOF-MS spectrum was acquired on Instrument Voyager-DE Pro, Applied Biosystems.

DMF was chosen as the solvent for matrix, because it has good solubility for both OUA and Ac_6-OUA. The same matrix solution was used for all the MALDI-MS experiments in this paper. The areas of peaks in MALDI-MS were measured with software Data Explorer™ 4.0 (Applied Biosystems). The threshold for peak detection was 1% of the base peak by area, and up to 2 isotope peaks were summed (M, M+1, M+2)

All spectra were obtained with the same instrument settings:

Instrument:	Voyager-DE Pro, Applied Biosystems		
Mode of operation:	Reflector	Extraction delay time	100 nsec
Extraction mode:	Delayed	Acquisition mass range	600-900 Da
Polarity:	Positive	Number of laser shots	100~150 shots
Acquisition control:	Manual	Laser intensity	1479 V
Accelerating voltage:	20000 V	Laser rep rate	3.0 Hz
Grid voltage:	68%	Calibration type	Default
Mirror voltage ratio:	1.12	Low mass gate	600 DA
Guide wire 0	0.05%	Timed ion selector	Off

3. Characterization of merging

a. Cross contamination. Cross contamination is reduced to < 1% by inserting one large blank buffer plug between every two reagent plugs (*Angew. Chem. Int. Ed.* **2005**, *44*, 2520 −2523)**.** A cartridge of alternating fluorescent and non-fluorescent (buffer) plugs, separated by one buffer plug, was fabricated (Figure S2). After merging with a non-fluorescent buffer stream, the fluorescence in each plug was measured. Cartridge fabrication and flow rates for merging were the same as described in the "merging" section. Fluorescence intensities were measured with a Leica DMI 6000 B microscope equipped with a digital camera (Hamamatsu, 1394 ORCA-ER). The exposure time was 500 milliseconds. A 5X objective and DAPI filter (#31000 DAPI/ Hoechst/ AMCA, Chroma Technology Corp.) were used. The fluorescent solution was 1.5 mg/mL solid dye (1, 3, 6, 8-pyrenetetrasulfonic acid tetrasodium salt 85%, Acros organics) dissolved in 1X PBS buffer prepared in 2:1 methanol:H_2O. The buffer plugs were 1X PBS buffer prepared in 2:1 methanol:H_2O.

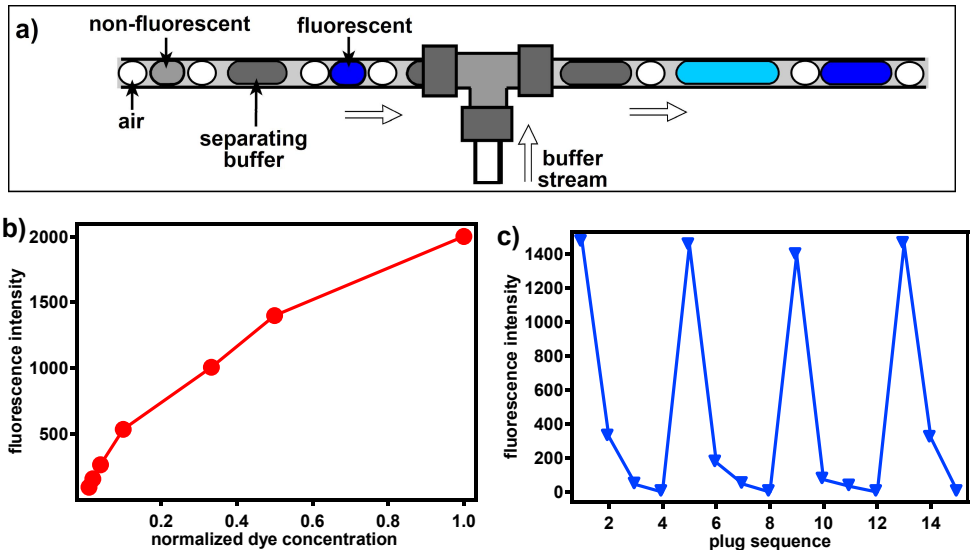


Figure S2. Characterization of the cross-contamination during merging: (Top) a schematic of cartridge and merging, (left) calibration curve of fluorescence intensity-normalized concentration, (right) Fluorescence intensities after merging. After merging, the originally non-fluorescent reagent plugs (plug 3, 7, 11, 15) became weakly fluorescent due to cross-contamination from the fluorescent plugs (plug 1, 5, 9, 13). The estimated cross-contamination from the neighboring reagent plugs was < 1% according to the fluorescence intensities.

b. Merging ratio. The volumetric ratio of the substrate and reagent solution combined during merging was measured from microphotographs of the plug after and before merging. For simplicity, the volume of a plug was approximated to be proportional to its length. The methods of photographing and image analysis were described in detail in previous publication (*Langmuir* **2003**, *19*, 9127-9133). The ratio measured from 34 plugs was $V_{reagent}/V_{substrate}$ = 1.3, with a standard deviation of 0.2. In scale-up reactions, a volumetric ratio of $V_{reagent}/V_{substrate}$ = 1.5 was used.

4. Control experiments on reproducibility and quantitativity of MALDI-MS

We confirmed that there was a strong correlation between the fraction of peak area in MALDI-MS of the compound and its actual fraction in the sample. Measurements among samples with different salt additives were reproducible as long as the salt concentration is not higher than 0.1 M.

Solutions of varying OUA: Ac_6-OUA ratios were prepared in 3:2:5 ethanol:dioxane:H_2O, with different salt additives. 30 nL plugs of each solution were aspirated and deposited to the MALDI-MS sample plate. The methods of MALDI-MS were described in the previous section. The ratio of peak area in MALDI-MS spectra corresponding to OUA over the total peak area of OUA and Ac_6-OUA was plotted against the actual fraction of OUA (Figure S3).

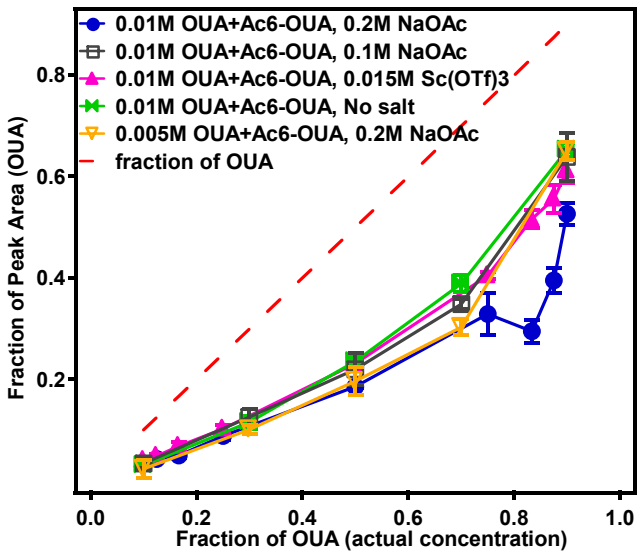


Figure S3. The fraction of peak area corresponding to OUA measured at different fractions of OUA. Each data point is an average of four parallel measurements. The error bar was +/- one standard deviation.

5. MALDI-MS data for Figure 2

Incubation conditions: 1 hour at 18 °C

Substrate: 0.01 M Ac_6-OUA in 1:1 ethanol:dioxane

Reagents: amine (neat), 0.03 M Lewis acid in 2:1 ethanol:H_2O, lipases saturated in 1:1 ethanol:H_2O, inorganic bases dissolved in 2:1 methanol:H_2O (except for 0.3 M Na_2CO_3 dissolved in H_2O).

Identity of Reagents

Amines		Lewis acids		Lipases		Inorganic bases	
#	Reagent	#	Reagent	#	Reagent	#	Reagent
1	*n*-hexylamine	15	$Yb(OTf)_3$	27	PPL	31	0.3M NaOPh
2	cyclohexanemethylamine	16	$Sc(OTf)_3$	28	PSL	32	0.1M NaOPh
3	cyclohexyllamine	17	$La(OTf)_3$	29	LPL	33	0.3M 4:1 PhONa:PhOH
4	2-methoxyethylamine	18	$In(OTf)_3$	30	CRL	34	0.3M 2:1 PhONa:PhOH
5	piperidine	19	$Cu(OTf)_2$			35	0.3M 1:1 PhONa:PhOH
6	morpholine	20	$Zn(OTf)_2$			36	0.3M 1:2 PhONa:PhOH
7	2-methylpiperidine	21	$CeCl_3$			37	0.3M 1:4 PhONa:PhOH
8	2-aminoethanol	22	$B(OH)_3$			38	0.3M Na_2CO_3
9	(*R*)-(-)-2-amino-1-propanol	23	$HfCl_4$			39	0.06M Na_2CO_3
10	2-amino-1-methylpropanol	24	$ZrCl_4$			40	0.3M NaOH
11	3-amino-1-propanol	25	$EuCl_3$			41	0.12M NaOH
12	1,2-diaminoethane	26	$FeCl_3$			42	0.05M NaOH
13	1,3-diamiopropane					43	0.02M NaOH
14	aniline					44	0.1M NaOAc

MALDI-MS Data for Figure 2

Abbreviations: Rg # – reagent number; sol: solvent

	Peak Area										
Rg #	OUA m/z 607.4	OUA + sol m/z 607.4+M	Ac_3-OUA m/z 733.5	Ac_3-OUA + sol m/z 733.5+M	Ac_4-OUA m/z 775.5	Ac_4-OUA + sol m/z 775.5+M	Ac_5-OUA m/z 817.5	Ac_5-OUA + sol m/z 817.5+M	Ac_6-OUA m/z 859.4	Ac_6-OUA + sol m/z 859.4+M	Fraction of Area Ac_3-OUA
1			4002		34755		113911		181065		0.01
2			0		16177		71493		163384		0.00
3			0		0		3236		69079		0.00
4					3353		22083		56227		0.00
5							7809		89786		0.00
6							12938		136433		0.00
8			45998		60689		32483		17942		0.29
9							7690		68172		0.00
10							7161		437838		0.00
11			54150		49621		39012		29114		0.32
12			47355		28290	17279 (M=60)	8690	15536 (M=60)	2414	4973 (M=60)	0.38
13			23993	6692 (M=74)	9656	8337 (M=74)	2671				0.47
18							3655		255209		0.00
20							21931		505610		0.00
21							4314		293797		0.00
26							7893		297937		0.00
31			118331	12451 (M=18)							0.90
32			486853	14459 (M=18)							0.97
33			419369		7786						0.98
34			1								1.00
35			289025		23025		10011		44421		0.79
36			362599		146160		60699		303801		0.42
37			88288		101171		84314		484752		0.12
38		22037 (M=18)	109635	13993 (M=18)	7811		10041		96860		0.42

39			1								1.00
40	23697	141522 (M=18)	15792	86581 (M=18)							0.06
41	24097	8206 (M=18)	397259	191932 (M=18)							0.64
42			378886	19401 (M=18)					49428		0.85
43			637352						223104		0.74
44							15714		789714		0.00
1			1524		16364		56627		82428		0.01
2			1775		11194		54409		153381		0.01
3							17523		619345		0.00
4					1019		29017		110513		0.00
5							12726		189699		0.00
6							10868		442256		0.00
8			17010		27058		14214		7452		0.26
9									37051		0.00
10							8863		237664		0.00
11			21824		41026		35208		26139		0.18
12			4626		4853		2059		749		0.38
13			70994		26479		4601				0.70
1			9963		58960		141894		211087		0.02
2					8477		16513		135927		0.00
3							31645		858206		0.00
4					5228		88436		296091		0.00
5							63964		749250		0.00
6							6816		269112		0.00
8			37945		37464		20811		5138		0.37
9							9845		88988		0.00
10							4737		327926		0.00
11			71812		96952		80360		34670		0.25
12			262105	61570 (M=60)	95567	102145 (M=60)	9219	124609 (M=60)		24019 (M=60)	0.39
13			295364	108166 (M=74)	80346		12633		2202		0.59
31			109128	7740							0.93

				(M=18)							
32			104008								1.00
33			169483						3307		0.98
34			229428								1.00
35			191323		19734		12452		44125		0.71
36			168516		53080		41504		153827		0.40
37			18803		59210		41887		131444		0.07
38			65585		4343		2045		46422		0.55
39			538819								1.00
40	63436	374103 (M=18)	5546								0.01
41	24598	10061 (M=18)	176793	132438 (M=18)	2849						0.51
31			179885	14155 (M=18)					3342		0.91
32			775031	20671 (M=18)	13426				5131		0.95
33			474145	6453 (M=18)					6188		0.97
34			741984		22103				6501		0.96
35			591904		81174		41980		164460		0.67
36			301648		144699		73630		376042		0.34
37			99485		150418		142912		771641		0.09
38			168755		9520		13097		189166		0.44
39			549489		5644						0.99
40	25750	176524 (M=18)	3154	20395 (M=18)							0.01
41			101153	58771 (M=18)	18792		26079		147814		0.29
42	5006		387281	19309 (M=18)	2528				22064		0.89
43			497543		22671		10317		67584		0.83
44							2520		464679		0.00

Reagents 7, 14, 15, 16, 17, 19, 22-25, and 27-30 showed no product peaks in MALDI-MS. Reagents 34 and 39 only had the peak for Ac_3-OUA.

6. MALDI-MS data for Figure 3

Reagent – cyclohexanemethylamine; Substrate – 0.01 M Ac_6-OUA in DMF; Temperature – 18 °C

t: time of incubation; A: Peak Area; A_f: fraction of peak area

t /hours	Ac_6-OUA		Ac_5-OUA		Ac_4-OUA		Ac_3-OUA	
	A	A_f	*A*	A_f	*A*	A_f	*A*	A_f
0	100	1.00	0	0.00	0	0.00	0	0.00
0.5	559091.3	0.78	149655	0.21	7280.42	0.01	0	0.00
0.5	1405384	0.74	467778.3	0.25	17209.05	0.01	0	0.00
1	439380.4	0.61	248663.7	0.35	30751.19	0.04	0	0.00
1	545513.6	0.54	401389.3	0.40	65167.47	0.06	0	0.00
2	401676.7	0.38	487773.7	0.46	168344.7	0.16	9071.67	0.01
3	368535.4	0.36	406391.8	0.39	227492.7	0.22	34755.72	0.03
3	584725.6	0.35	684105.3	0.41	358392.9	0.22	32311.6	0.02
3	451706.2	0.39	453611.6	0.39	234250.4	0.20	32143.17	0.03
3.5	492657.2	0.26	856115.3	0.46	470759.3	0.25	57083.5	0.03
5*	4617.43	0.06	13546.62	0.17	44006.06	0.55	17451.57	0.22
5*	32833.3	0.16	109768.9	0.52	54985.39	0.26	12097.14	0.06
7	70319.68	0.06	375731.6	0.33	518926.3	0.46	165402.1	0.15
9.5	10042.4	0.02	96522.69	0.23	238598.6	0.56	83427.05	0.19
16.5	0	0.00	43587.11	0.06	322326.5	0.46	341067.1	0.48

* These two data points clearly deviated from the curves. We assumed this deviation was due to operational mistake (e.g., amines reacted with CO_2 in air to produce considerable amount of non-volatile salt interfering with MALDI-MS), and discarded these points.

Reagent – 0.3 M PhONa:PhOH in 2:1 MeOH:H_2O; Substrate – 0.01 M Ac_6-OUA in 1:1 ethanol:dioxane; Temperature – 18 °C

t: time of incubation; A: Peak Area; A_f: fraction of peak area

T /hours	Ac_6-OUA		Ac_5-OUA		Ac_4-OUA		Ac_3-OUA	
	A	A_f	*A*	A_f	*A*	A_f	*A*	A_f
0	100	1.00	0	0.00	0	0.00	0	0.00
0.5	784605.8	0.67	149061.8	0.13	153294.5	0.13	84987.63	0.07
1	319810.1	0.43	84726.9	0.11	115482.6	0.15	231723	0.31
2	471303	0.26	135988	0.08	229701.9	0.13	970799.7	0.54
3.5	45557.42	0.07	10941.22	0.02	27402.22	0.04	535471.8	0.86

7. No-loss handling of sub-microliter volume of solution

The goal of this experiment is to demonstrate the ability to do screening starting with less than 10 μg of solid material. We did not have the equipment to accurately weigh 10 μg of solid material; Instead 0.8 μL 0.01 M Ac_6-OUA in DMF (containing 7 μg Ac_6-OUA) was dispensed. After drying the solution under vacuum (0.3 mmHg, 4 hours), we obtained ~ 7 μg of Ac_6-OUA solid to start with. The solid was re-dissolved in 0.8 μL of DMF, and 14 reagents (7 amines, # 1~ # 7; 7 inorganic bases, # 38 ~ # 44) were tested using this solution, with 3 hours of incubation at 18 °C. The data of peak areas were listed.

Abbreviations: Rg # – reagent number; sol: solvent.

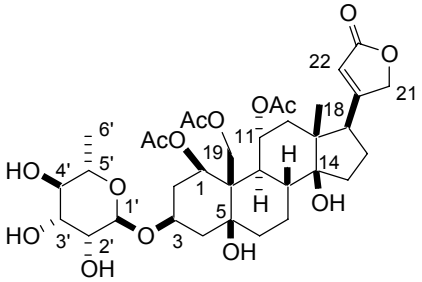
	Peak Area										
Rg #	**OUA m/z 607.4**	**OUA + sol m/z 607.4+M**	**Ac_3-OUA m/z 733.5**	**Ac_3-OUA + sol m/z 733.5+M**	**Ac_4-OUA m/z 775.5**	**Ac_4-OUA + sol m/z 775.5+M**	**Ac_5-OUA m/z 817.5**	**Ac_5-OUA + sol m/z 817.5+M**	**Ac_6-OUA m/z 859.4**	**Ac_6-OUA + sol m/z 859.4+M**	**Fraction of Area Ac_3-OUA**
1			107805		512468		704965		567513		0.06
2			7028		143714		505047		619317		0.01
3							120918		970393		0.00
4					65632		336137		319906		0.00
5					4891		136411		807338		0.00
6							76012		836912		0.00
7							5104		905540		0.00
38			340614		65051		22402		97789		0.65
39			721187		5401						0.99
40	39143	135096	35959	203382							0.087
41	50876	7596	513744	203675						4973	0.66
42			663373	39384	5248						0.94
43			667158		101798		26951		486834		0.52
44							13580		919856		0.00

8. Synthesis of Ac_3-OUA, Ac_4-OUA, and Ac_5-OUA on 0.01 mmol scale

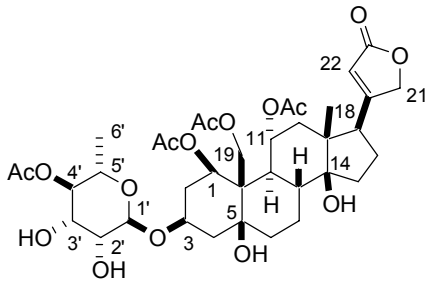
a. General. All the reactions were carried out in a glass vessel under air-sealed condition with continuous stirring using a magnetic stirring bar unless otherwise noted. Liquids and solutions were transferred via a pipette (Eppendorf) using a disposal pipette tip (Fisher Scientific). Analytical thin-layer chromatography was performed on a polystrene plate coated with 0.25 mm silica gel containing a fluorescent indicator (Whatman, #4410222). Thin layer chromatography plates were visualized by immersion in an acidic staining solution of CAM (prepared from $CeSO_4$, H_2SO_4, H_2O, and $(NH_4)_2MoO_4$) followed by heating on a hot plate. Organic solutions were concentrated by rotary evaporation at *c.a.* 30 mmHg. Flash column chromatography was performed on silica gel (ACROS, neutral, 0.060–0.200 mm) as described by Still et al. (*J. Org. Chem.* **1978**, *43*, 2923–2924). Chemical shift values of proton nuclear magnetic resonance (^{1}H NMR) spectra are reported in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to tetramethylsilane (δ 0.00). Chemical shifts for carbon nuclear magnetic resonance (^{13}C NMR) spectra are reported in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to the carbon resonance of CH_3CN (δ 118.26). Data are presented as: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, sext = sextet, sept = septet, m = multiplet and/or multiplet resonances, br = broad), coupling constant in hertz (Hz), and signal area integration in natural numbers, assignment (*italic*). Characteristic IR absorptions are reported in cm^{-1}.

b. Preparation of ouabain 1, 2', 3', 4', 11, 19-hexaacetate (Ac_6-OUA). Ouabain octahydrate (5.0 g, 6.86 mmol) was heated to reflux in acetic acid anhydride (150 mL) for 90 min and evaporated to dryness *in vacuo*. Purification of the crude product was purified by chromatography on silica gel (50 g, CH_2Cl_2 and then 2%, 5% EtOH in CH_2Cl_2) and then by recrystallization from Et_2O/CH_2Cl_2 to give the title compound in 71% yield with >99% purity (4.1 g, 4.90 mmol) (*Eur. J. Med. Chem.* **1994**, *29*, 799-804).

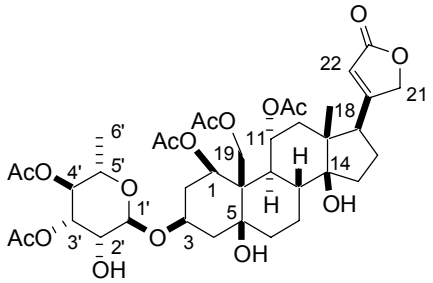
c. Preparation of ouabain 1, 11, 19-triacetate (Ac_3-OUA) via tris-deacetylation of Ac_6-OUA. Ac_6-OUA (83.7 mg, 0.01 mmol) was dissolved in 1:1 mixture of dioxane and EtOH (10 mL). 0.06 M Na_2CO_3 (in 2:1 MeOH:H_2O, 15 mL) was added at 18 °C, and then the reaction mixture turned to suspension. After 1 h, acetone (10 mL) was added and the reaction mixture was filtered. To the corresponding filtrate was added acetic acid (0.034 mL, 0.6 mmol) and then the solvents were removed *in vacuo*. The crude product was purified by chromatography on silica gel (8.0 g, CH_2Cl_2 and then 10%, 15% EtOH in CH_2Cl_2) to give the title compound in 85% yield with >98 % purity (60.3 mg, 0.085 mmol). R_f = 0.19 (15% EtOH in CH_2Cl_2); IR (neat) 3446 (br, OH), 2931, 1733 and 1717 and 1700 (C=O), 1456, 1437, 1367, 1249, 1037, 733, 668.; ^{1}H NMR (500 MHz, CD_3CN) δ 0.92 (s, 3H, 18- C*H*$_3$), 1.17 (d, *J* = 6.2 Hz, 3H, 6'-C*H*$_3$), 1.19–1.48 (m, 5H), 1.68–2.26 (m, 11H), 1.83, 1.93, 2.08 (s, 9H, 1, 11, 19-Ac), 2.58 (brs, 1H, 5-O*H*), 2.80 (dd, *J* = 5.4, 9.4 Hz, 1H, 17-C*H*), 3.24 (t, *J* = 9.4 Hz, 1H, 4'-C*H*), 3.44 (dq, *J* = 6.2, 9.3 Hz,, 1H, 5'-C*H*), 3.47 (dd, *J* = 3.4, 9.4 Hz, 1H, 3'-C*H*), 3.68 (dd, *J* = 1.8, 3.4 Hz, 1H, 2'-C*H*), 4.05 (s, 1H, 14-O*H*), 4.16 (brs, 1H, 3-C*H*), 4.28 (d, *J* = 11.8 Hz, 1H, 19-C*H*H), 4.81 and 4.91 (dd, *J* = 1.8, 18.2 Hz, 2H, 21-C*H*$_2$), 4.82 (brs, 1H, 1'-C*H*), 4.95 (d, *J* = 11.8 Hz, 1H, 19-CH*H*), 5.07 (dt, *J* = 4.3, 11.0 Hz, 1H, 11-C*H*), 5.84 (s, 1H, 22-C*H*), 5.95 (brt, *J* = 2.8 Hz, 1H, 1-C*H*). Signals of 2', 3', and 4'-O*H* were not found.; ^{13}C NMR (100 MHz, CD_3CN) δ 16.6, 17.9, 20.9, 21.6, 21.7, 23.7, 27.1, 30.2, 33.5, 34.3, 35.6, 40.7, 43.9, 45.3, 48.0, 50.3, 50.7, 61.8, 69.4, 70.9, 71.3 ,71.9, 72.2, 72.4, 73.4, 74.1, 74.2, 84.9, 98.4, 118.0, 170.6, 170.7, 171.5, 175.1, 175.7. NMR spectra are in agreement with that taken in CD_3OD (*Eur. J. Med. Chem.* **1994**, *29*, 799-804.).



d. Preparation of ouabain 1, 4', 11, 19-tetraacetate (Ac_4-OUA) via bis-deacetylation of Ac_6-OUA. Ac_6-OUA (83.7 mg, 0.01 mmol) was dissolved in DMF (10 mL). Cyclohexanemethylamine was added at 18 °C. After 11 h, excess amine and DMF were removed *in vacuo* (0.3 mmHg, 2 h). The crude product was purified by chromatography on silica gel (12 g, CH_2Cl_2 and then 7%, 10% EtOH in CH_2Cl_2) to give a mixture of three regioisomers of ouabain tetraacetate (1, 2' or 3' or 4', 11, 19) in 47% yield (35.7 mg, 0.047 mmol). Further purification by chromatography on silica gel gave the title compound in 19% yield with >95% purity (14.9 mg, 0.019 mmol). R_f = 0.39 (15% EtOH in CH_2Cl_2); IR (neat) 3524 (br, OH), 2938, 1734 and 1718 and 1700 (C=O), 1456, 1437, 1373, 1246, 1037.; ^{1}H NMR (500 MHz, CD_3CN) δ 0.92 (s, 3H, 18- C*H*$_3$), 1.08 (d, *J* = 6.3 Hz, 3H, 6'-C*H*$_3$), 1.18–1.49 (m, 5H), 1.69–2.26 (m, 11H), 1.83, 1.93, 2.03, 2.08 (s, 12H, 1, 4', 11, 19-Ac), 2.56 (s, 1H, 5-O*H*), 2.80 (dd, *J* = 5.4, 9.4 Hz, 1H, 17-C*H*), 3.24 (d, *J* = 6.7 Hz, 1H, 3'-O*H*), 3.34 (d, *J* = 4.3 Hz, 1H, 2'-O*H*), 3.64 (dq, *J* = 6.3, 9.8 Hz,, 1H, 5'-C*H*), 3.63-3.67 (m, 1H, 3'-C*H*), 3.72-3.75 (m, 1H, 2'-C*H*), 3.99 (s, 1H, 14-O*H*), 4.17 (brs, 1H, 3-C*H*), 4.29 (d, *J* = 11.8 Hz, 1H, 19-C*H*H), 4.76 (t, *J* = 9.8 Hz, 1H, 4'-C*H*), 4.81 and 4.91 (dd, *J* = 1.8, 18.2 Hz, 2H, 21-C*H*$_2$), 4.86 (brs, 1H, 1'-C*H*), 4.95 (d, *J* = 11.8 Hz, 1H, 19-CH*H*), 5.07 (dt, *J* = 4.3, 11.0 Hz, 1H, 11-C*H*), 5.84 (s, 1H, 22-C*H*), 5.96 (brt, *J* = 2.8 Hz, 1H, 1-C*H*); ^{13}C NMR (100 MHz, CD_3CN) δ 16.5, 17.6, 20.9, 21.1, 21.5, 21.7, 23.7, 27.1, 30.2, 33.5, 34.5, 35.6, 40.6, 43.8, 45.2, 48.0, 50.2, 50.6, 61.8, 67.3, 69.8, 71.3,71.7, 71.8, 72.3, 74.0, 74.2, 74.7, 84.8, 98.6, 118.0, 170.6, 170.6, 171.4, 171.5, 175.1, 175.7.



e. Preparation of ouabain 1, 3', 4', 11, 19-pentaacetate (Ac_5-OUA) via mono-deacetylation of Ac_6-OUA. Ac_6-OUA (83.7 mg, 0.01 mmol) was dissolved in DMF (10 mL). *N*-Hexylamine (15 mL) was added at 18 °C. After 3 h, excess amine and DMF were removed *in vacuo* (0.3 mmHg, 2 h). The crude product was purified by chromatography on silica gel (12 g, CH_2Cl_2 and then 4%, 6% EtOH in CH_2Cl_2) to give a mixture of three regioisomers of ouabain triacetate {1, (2', 3') or (2', 4') or (3', 4'), 11, 19} in 39% yield (30.9 mg, 0.039 mmol). Further purification by chromatography on silica gel gave the title compound in 25% yield with >97% purity (20.1 mg, 0.025 mmol). R_f = 0.51 (15% EtOH in CH_2Cl_2); IR (neat) 3477 (br, OH), 2938, 1743 and 1728 and 1711 (C=O), 1441, 1369, 1247, 1040.; ^{1}H NMR (500 MHz, CD_3CN) δ 0.91 (s, 3H, 18- C*H*$_3$), 1.16 (d, *J* = 6.2 Hz, 3H, 6'-C*H*$_3$), 1.18–1.48 (m, 5H), 1.68–2.28 (m, 11H), 1.83, 1.94, 1.95, 1.99, 2.08 (s, 15H, 1, 3', 4', 11, 19-Ac), 2.55 (s, 1H, 5-O*H*), 2.79 (dd, *J* = 5.4, 9.4 Hz, 1H, 17-C*H*), 3.54 (d, *J* = 5.6 Hz, 1H, 2'-O*H*), 3.72 (dq, *J* = 6.2, 8.8 Hz,, 1H, 5'-C*H*), 3.86–3.88 (m, 1H, 2'-C*H*), 3.96 (s, 1H, 14-O*H*), 4.18 (brs, 1H, 3-C*H*), 4.30 (d, *J* = 11.8 Hz, 1H, 19-C*H*H), 4.81 and 4.90 (dd, *J* = 1.7, 18.2 Hz, 2H, 21-C*H*$_2$), 4.87 (d, *J* = 1.6 Hz, 1H, 1'-C*H*), 4.92 (d, *J* = 11.8 Hz, 1H, 19-CH*H*), 4.93–5.00 (m, 2H, 3' and 4'-C*H*), 5.05 (dt, *J* = 4.3, 11.0 Hz, 1H, 11-C*H*), 5.83 (s, 1H, 22-C*H*), 5.96 (brt, *J* = 2.8 Hz, 1H, 1-C*H*); ^{13}C NMR (100 MHz, CD_3CN) δ 16.6, 17.6, 20.9 (2C), 21.0, 21.4, 21.7, 23.6, 27.1, 30.3, 33.5, 34.2, 35.6, 40.7, 43.9, 45.2, 48.0, 50.3, 50.6, 61.7, 67.6, 69.4, 71.3, 71.3, 71.5, 72.3 (2C), 74.0, 74.2, 84.8, 98.2, 118.0, 170.6, 170.9 (2C), 171.0, 171.5, 175.0, 175.7.



9. Solubility of organic reagent in FC-70 and other fluorinated solvent.

Solubilities of *n*-hexylamine, heptane, dioxane, and ethanol in FC-3283 (viscosity = 0.75 cSt, 3M), perfluoroperhydrophenthrene (PFP, Alfa Aesar), FC-70 (viscosity = 14 cSt, Acros Organics), Krytox GPX 103 (viscosity = 80 cSt, Dupont) have been determined by ^{1}H NMR analysis using 1H, 1H, 2H, 2H-perfluorooctan-1-ol as an internal standard. From these results, we have chosen FC-70 as a carrier fluid because of its low miscibility with organic reagents and moderate viscosity.

n-Hexylamine: 9.2 mg/mL in FC-3283, 8.0 mg/mL in PFP,
5.2 mg/mL in FC-70, 3.8 mg/mL in Krytox GPX 103

Heptane: 28 mg/mL in FC-70

Dioxane: 9.4 mg/mL in FC-70

Ethanol: 0.19 mg/mL in FC-70

10. Additional MALDI-MS data from screens

In addition to the conditions used in large-scale reactions, there were 2 reagents found promising for hydrolysis with high selectivity for Ac_5-OUA or Ac_4-OUA, as shown in Figure S4.

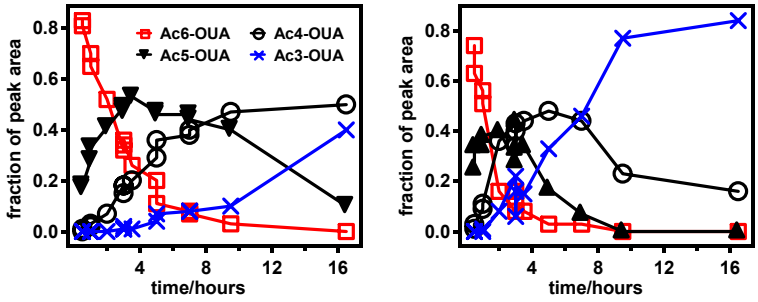


Figure S4 The relative concentrations of species (approximated as fraction of peak areas in MALDI-MS) during the hydrolysis of Ac_6-OUA at 18°C. (Left): Reagent – 2-methoxyethylamine; Substrate – 0.01 M Ac_6-OUA in DMF; (Right): Reagent – *n*-hexylamine; Substrate – 0.01 M Ac_6-OUA in DMF.

Other conditions tested in the screenings, but not used in large-scale reactions were listed below.

Abbreviations: *t*: time of incubation; *A*: Peak Area; A_f: fraction of peak area

Substrate – 0.01 M Ac_6-OUA in 1:1 ethanol:acetonitrile; Incubation time – 1 hour; Temperature – 18 °C

reagent	Ac_6-OUA		Ac_5-OUA		Ac_4-OUA		Ac_3-OUA	
	A	A_f	*A*	A_f	*A*	A_f	*A*	A_f
n-hexylamine	1104911	0.63	525729.2	0.30	113763.5	0.06	8054.38	0.00
cyclohexanemethylamine	1276650	0.81	279026.1	0.18	23867.77	0.02	0	0.00
cyclohexyllamine	1281474	0.96	51374.61	0.04	0	0.00	0	0.00
2-methoxyethylamine	1185156	0.92	102360.2	0.08	0	0.00	0	0.00
piperidine	814293.2	0.97	24881.68	0.03	0	0.00	0	0.00
morpholine	331247.9	0.90	32078.47	0.09	4187.14	0.01	0	0.00
2-methylpiperidine	1295816	0.99	14356.83	0.01	0	0.00	0	0.00
2-aminoethanol	4292.9	0.03	20480.24	0.13	48028.71	0.31	84374.15	0.54
(*R*)-(-)-2-amino-1-propanol	331247.9	0.89	37492.98	0.10	4187.14	0.01	0	0.00
2-amino-1-methylpropanol	453852.7	0.97	14231.75	0.03	0	0.00	0	0.00

3-amino-1-propanol	109308	0.11	295052.1	0.31	353792.7	0.37	209008.1	0.22
1,2-diaminoethane	3181.86	0.03	12974.78	0.11	36216.91	0.30	68085.91	0.57
1,3-diamiopropane	2091.44	0.01	8175.29	0.05	54643.85	0.31	109094.4	0.63
aniline	no reaction							

Substrate – 0.01 M Ac_6-OUA in DMF; Incubation time – 1 hour; Temperature – 18 °C

A: Peak Area; A_f: fraction of peak area

Reagent	Ac_6-OUA m/z 859.4		Ac_5-OUA m/z 817.5		Ac_4-OUA m/z 775.5		Ac_3-OUA m/z 733.5	
	A	A_f	*A*	A_f	*A*	A_f	*A*	A_f
n-hexylamine	574642.4	0.51	433709.5	0.38	124842	0.11	3912.6	0.00
cyclohexanemethylamine	545513.6	0.54	401389.3	0.40	65167.47	0.06	0	0.00
cyclohexyllamine	1022661	0.97	30073.07	0.03	0	0.00	0	0.00
2-methoxyethylamine	438934.9	0.70	172131.9	0.28	14857.14	0.02	0	0.00
piperidine	311084.1	0.96	12946.73	0.04	0	0.00	0	0.00
morpholine	220649.7	0.97	5944.58	0.03	0	0.00	0	0.00
2-methylpiperidine	No reaction							
2-aminoethanol	55281.7	0.12	169227.4	0.36	166145.2	0.36	76836.87	0.16
(*R*)-(-)-2-amino-1-propanol	38723.73	0.56	22550.02	0.33	6235.86	0.09	1345.4	0.02
2-amino-1-methylpropanol	702607	0.96	26787.05	0.04	0	0.00	0	0.00
3-amino-1-propanol	5071.85	0.15	11500.05	0.34	10898.68	0.32	6845.58	0.20
1,2-diaminoethane	1612.24	0.03	8652.25	0.16	20601.14	0.39	22475.91	0.42
1,3-diamiopropane	8972.38	0.04	17366.58	0.09	21034.46	0.10	155032.3	0.77
aniline	No reaction							

Reagent – *n*-hexylamine; Substrate – 0.01 M Ac_6-OUA in 1:1 ethanol:acetonitrile; Temperature – 18 °C. *A*: Peak Area; A_f: fraction of peak area

t /hours	Ac_6-OUA		Ac_5-OUA		Ac_4-OUA		Ac_3-OUA	
	A	A_f	*A*	A_f	*A*	A_f	*A*	A_f
1	1104911	0.63	525729.2	0.30	113763.5	0.06	8054.38	0.00
2	1186236	0.50	743060.4	0.31	368955.1	0.15	87687.55	0.04
3.5	347535.1	0.31	242853.4	0.22	292076.9	0.26	228184.3	0.21
5	197004.5	0.24	192523.8	0.24	232545.1	0.28	194379.8	0.24
7	195388.7	0.24	192523.8	0.24	232545.1	0.29	194379.8	0.24

13	47511.08	0.07	53161.71	0.08	103408.5	0.16	460129	0.69

Reagent – cyclohexanemethylamine; Substrate – 0.01 M Ac_6-OUA in 1:1 ethanol:acetonitrile; Temperature – 18 °C. *A*: Peak Area; A_f: fraction of peak area

t /hours	Ac_6-OUA		Ac_5-OUA		Ac_4-OUA		Ac_3-OUA	
	A	A_f	*A*	A_f	*A*	A_f	*A*	A_f
1	1276650	0.81	279026.1	0.18	23867.77	0.02	0	0.00
2	1495602	0.51	952833.8	0.33	392361.4	0.13	68838.92	0.02
3.5	827248	0.55	406851.8	0.27	217290.7	0.14	56190.79	0.04
5	389181.5	0.45	247252	0.28	169819.2	0.19	67088.35	0.08
7	205272	0.38	134390.3	0.25	125980.6	0.23	74671.92	0.14
13	132318.3	0.14	130615.6	0.14	256366	0.27	414562.7	0.44

Reagent – 2-methoxyethylamine; Substrate – 0.01 M Ac_6-OUA in 1:1 ethanol:acetonitrile; Temperature – 18 °C. *A*: Peak Area; A_f: fraction of peak area

t /hours	Ac_6-OUA		Ac_5-OUA		Ac_4-OUA		Ac_3-OUA	
	A	A_f	*A*	A_f	*A*	A_f	*A*	A_f
1	1185156	0.92	102360.2	0.08	0	0.00	0	0.00
2	1315956	0.62	661100.7	0.31	150472.2	0.07	10429.73	0.00
3.5	430485.9	0.55	241958.8	0.31	94002.75	0.12	20788.47	0.03
5	360383	0.42	307163.2	0.36	154407.3	0.18	32615.22	0.04
7	286476.2	0.43	198146	0.30	145561.6	0.22	38912.43	0.06
13	269601	0.20	289501.6	0.22	366573.5	0.27	414562.7	0.31

Reagent – 0.3 M 1:4 PhONa:PhOH in 2:1 MeOH:H_2O; Substrate – 0.01 M Ac_6-OUA in 1:1 ethanol:dioxane; Temperature – 18 °C. *A*: Peak Area; A_f: fraction of peak area

t /hours	Ac_6-OUA		Ac_5-OUA		Ac_4-OUA		Ac_3-OUA	
	A	A_f	*A*	A_f	*A*	A_f	*A*	A_f
0.5	1023792	0.80	157933.8	0.12	82473.33	0.06	22691.05	0.02
1	316904	0.60	78940.59	0.15	83624.69	0.16	49388.35	0.09
2	797686.9	0.48	202115.6	0.12	282736.6	0.17	364377.9	0.22
3.5	422816.4	0.36	106398.5	0.09	174546.3	0.15	465323.5	0.40

Reagent – 0.3 M PhONa in 2:1 MeOH:H_2O; Substrate – 0.01 M Ac_6-OUA in 1:1 ethanol:dioxane; Temperature – 18 °C. *A*: Peak Area; A_f: fraction of peak area

t /hours	Ac_6-OUA		Ac_5-OUA		Ac_4-OUA		Ac_3-OUA		Ac_3-OUA+H_2O	
	A	A_f	*A*	A_f	*A*	A_f	*A*	A_f	*A*	A_f
0.5	0	1.00	0	0.00	0	0.00	0	0.00	16277.59	0.05
1	4860.41	0.00	0	0.00	3267.01	0.01	296767.4	0.94	40087.2	0.13
2	8641.32	0.02	0	0.00	3132.87	0.01	271091.2	0.85	32918.7	0.13
3.5	0	0.04	0	0.00	1791.98	0.01	206190.7	0.83	24871.4	0.11

Reagent – 0.1 M PhONa in 2:1 MeOH:H_2O; Substrate – 0.01 M Ac_6-OUA in 1:1 ethanol:dioxane; Temperature – 18 °C. *A*: Peak Area; A_f: fraction of peak area

t /hours	Ac_6-OUA		Ac_5-OUA		Ac_4-OUA		Ac_3-OUA		Ac_3-OUA+H_2O	
	A	A_f	*A*	A_f	*A*	A_f	*A*	A_f	*A*	A_f
0.5	0	0.00	0	0.00	2500.62	0.01	336891	0.99	0	0.00
1	4012.7	0.01	0	0.00	9899.79	0.02	599881.5	0.96	10130.73	0.02
2	0	0.00	0	0.00	7790.33	0.01	792065.3	0.93	47754.09	0.06
3.5	0	0.00	0	0.00	5919.98	0.01	637219.6	0.93	39666.2	0.06

Reagent – 0.3 M 4:1 PhONa:PhOH in 2:1 MeOH:H_2O; Substrate – 0.01 M Ac_6-OUA in 1:1 ethanol:dioxane; Temperature – 18 °C. *A*: Peak Area; A_f: fraction of peak area

t /hours	Ac_6-OUA		Ac_5-OUA		Ac_4-OUA		Ac_3-OUA		Ac_3-OUA+H_2O	
	A	A_f	*A*	A_f	*A*	A_f	*A*	A_f	*A*	A_f
0.5	20512.19	0.06	0	0.00	7792.91	0.02	303036	0.91	2241.85	0.01
1	0	0.00	0	0.00	2736.74	0.01	262696.3	0.98	2154.16	0.01
2	0	0.00	0	0.00	12516.28	0.00	2929269	0.98	33544.28	0.01
3.5	0	0.00	0	0.00	0	0.00	368181.1	0.96	13373.1	0.04

Reagent – 0.3 M 2:1 PhONa:PhOH in 2:1 MeOH:H_2O; Substrate – 0.01 M Ac_6-OUA in 1:1 ethanol:dioxane; Temperature – 18 °C. *A*: Peak Area; A_f: fraction of peak area

t /hours	Ac_6-OUA		Ac_5-OUA		Ac_4-OUA		Ac_3-OUA		Ac_3-OUA+H_2O	
	A	A_f	*A*	A_f	*A*	A_f	*A*	A_f	*A*	A_f
0.5	57401.13	0.19	13492.71	0.04	2252.29	0.01	225525.7	0.76	1838	0.01
1	6084.7	0.02	0	0.00	8693.97	0.02	377640.8	0.96	0	0.00
2	0	0.00	0	0.00	4334.28	0.01	643065.6	0.97	15184.55	0.02
3.5	0	0.00	0	0.00	2188.73	0.01	306436.9	0.96	9374.61	0.03

Reagent – 0.3 M 1:1 PhONa:PhOH in 2:1 MeOH:H_2O; Substrate – 0.01 M Ac_6-OUA in 1:1 ethanol:dioxane; Temperature – 18 °C. *A*: Peak Area; A_f: fraction of peak area

t /hours	Ac_6-OUA		Ac_5-OUA		Ac_4-OUA		Ac_3-OUA	
	A	A_f	*A*	A_f	*A*	A_f	*A*	A_f
0.5	342661	0.47	59052.73	0.08	118931.9	0.16	206950.3	0.28
1	91276.48	0.16	32317.25	0.06	50955.67	0.09	380521.9	0.69
2	4688.45	0.01	3790.06	0.01	12975.47	0.02	507293.4	0.96
3.5	0	0.00	0	0.00	8477.93	0.02	538348.9	0.98

Reagent – 0.3 M Na_2CO_3 in H_2O; Substrate – 0.01 M Ac_6-OUA in 1:1 ethanol:dioxane; Temperature – 18 °C. *A*: Peak Area; A_f: fraction of peak area

t /hours	Ac_6-OUA		Ac_5-OUA		Ac_4-OUA		Ac_3-OUA	
	A	A_f	*A*	A_f	*A*	A_f	*A*	A_f
0.5	146374.3	0.51	13045.94	0.05	16566.13	0.06	113693.7	0.39
1	137931.2	0.62	6507.07	0.03	4997.09	0.02	72244.81	0.33
2	127799.1	0.27	12281.52	0.03	11052.79	0.02	324934.5	0.68

Reagent – 0.06 M Na_2C0_3 in 2:1 MeOH:H_2O; Substrate – 0.01 M Ac_6-OUA in 1:1 ethanol:dioxane; Temperature – 18 °C. *A*: Peak Area; A_f: fraction of peak area

t /hours	Ac_6-OUA		Ac_5-OUA		Ac_4-OUA		Ac_3-OUA		Ac_3-OUA+H_2O	
	A	A_f	*A*	A_f	*A*	A_f	*A*	A_f	*A*	A_f
0.5	0	0.00	0	0.00	18113.59	0.03	563157.6	0.97	8519.23	0.01
1	0	0.00	0	0.00	5008.67	0.02	230956.8	0.95	6564.75	0.03
2	0	0.00	0	0.00	4221.45	0.01	278232.2	0.95	11643.3	0.04
3.5	0	0.00	0	0.00	1296.48	0.01	157564.7	0.93	10774.09	0.06

Reagent – 0.3 M NaOH in 2:1 MeOH:H_2O; Substrate – 0.01 M Ac_6-OUA in 1:1 ethanol:dioxane; Temperature – 18 °C. *A*: Peak Area; A_f: fraction of peak area

t /hours	Ac_6-OUA		Ac_3-OUA		Ac_3-OUA+H_2O		OUA		OUA+H2O	
	A	A_f	*A*	A_f	*A*	A_f	*A*	A_f	*A*	A_f
0.5	0	0.00	22479.73	0.13	38256.6	0.21	22224.2	0.12	95670.03	0.54
1	0	0.00	7903.15	0.06	15925.73	0.11	30425.24	0.22	86463.59	0.61
2	0	0.00	7192.84	0.02	55868.51	0.17	37886.95	0.12	220993.6	0.69
3.5	0	0.00	3862.3	0.02	18003.76	0.09	21691.04	0.11	148745	0.77

Reagent – 0.12 M NaOH in 2:1 MeOH:H_2O; Substrate – 0.01 M Ac_6-OUA in 1:1 ethanol:dioxane; Temperature – 18 °C. *A*: Peak Area; A_f: fraction of peak area

t /hours	Ac_6-OUA		Ac_3-OUA		Ac_3-OUA+H_2O		OUA		OUA+H2O	
	A	A_f	*A*	A_f	*A*	A_f	*A*	A_f	*A*	A_f
0.5	0	0.00	358174.6	0.77	94783.99	0.20	14763.42	0.03	0	0.00
1	0	0.00	300002.2	0.64	133185.2	0.29	23297.84	0.05	10276.58	0.02
2	0	0.00	518960.9	0.47	435825.7	0.40	84791.36	0.08	59145.04	0.05
3.5	0	0.00	156599.4	0.26	311166.1	0.52	61230.84	0.10	66136.37	0.11

Reagent – 0.05 M NaOH in 2:1 MeOH:H_2O; Substrate – 0.01 M Ac_6-OUA in 1:1 ethanol:dioxane; Temperature – 18 °C. *A*: Peak Area; A_f: fraction of peak area

t /hours	Ac_6-OUA		Ac_5-OUA		Ac_4-OUA		Ac_3-OUA		Ac_3-OUA+H_2O		OUA	
	A	A_f	*A*	A_f	*A*	*A*	A_f	*A*	A_f	A_f	*A*	A_f
0.5	324027.9	0.37	60824.15	0.07	51927.02	0.06	418132.4	0.48	18032.67	0.02	6095.49	0.01
1	547696.9	0.42	70263.15	0.05	30708.26	0.02	588123.2	0.46	35081.97	0.03	16999.67	0.01
2	363310.9	0.55	17322.23	0.03	2399.66	0.00	223876.3	0.34	39822.55	0.06	7824.13	0.01
3.5	212686.6	0.63	9332.71	0.03	0	0.00	92038.69	0.27	23925.71	0.07	1664.64	0.00

Reagent – 0.02 M NaOH in 2:1 MeOH:H_2O; Substrate – 0.01 M Ac_6-OUA in 1:1 ethanol:dioxane; Temperature – 18 °C. *A*: Peak Area; A_f: fraction of peak area

t /hours	Ac_6-OUA		Ac_5-OUA		Ac_4-OUA		Ac_3-OUA		Ac_3-OUA+H_2O	
	A	A_f	*A*	A_f	*A*	A_f	*A*	A_f	*A*	A_f
0.5	453475.1	0.60	5961.39	0.01	17427.31	0.02	273075.1	0.36	7415.66	0.01
1	531674.3	0.50	23426.24	0.02	17751.51	0.02	445568.8	0.42	35081.97	0.03
2	562350.8	0.60	17164.56	0.02	5044.03	0.01	347173.4	0.37	0	0.00
3.5	306050.1	0.56	10722.5	0.02	6357.73	0.01	220197.7	0.40	3195.9	0.01

Reagent – 0.1 M NaOAc in 2:1 MeOH:H_2O; Substrate – 0.01 M Ac_6-OUA in 1:1 ethanol:dioxane; Temperature – 18 °C.

No reaction happened with incubation time of 0.5, 1.0, 2.0 and 3.5 hours.

Reagent: 0.3 M NaOH, aqueous; Substrate: 0.03 M Ac_6-OUA in 7:3 ethanol:dioxane

Conditions		Peak Height					Fraction of Peak Height				
T /°C	*time* /h	OUA+H_2O m/z 625.4	OUA m/z 607	Ac-OUA +H_2O m/z 667.4	Ac_3-OUA +H_2O m/z 751.4	Ac_3-OUA m/z 733.5	OUA +H_2O m/z 625.4	OUA m/z 607	Ac-OUA +H_2O m/z 667.4	Ac_3-OUA +H_2O m/z 751.4	Ac_3-OUA m/z 733.5
40	2	100	16	10	85	15	0.44	0.07	0.04	0.38	0.07
40	2	77	18	10	100	23	0.34	0.08	0.04	0.44	0.10
40	6	78	24		100	17	0.36	0.11	0.00	0.46	0.08
40	14	100	10		75	13	0.51	0.05	0.00	0.38	0.07
40	14	100	18	5	62		0.54	0.10	0.03	0.34	0.00
18	2	100	10	5	99	12	0.44	0.04	0.02	0.44	0.05
18	6	62	7	6	100	21	0.32	0.04	0.03	0.51	0.11
18	14	51	7.5		100	15	0.29	0.04	0.00	0.58	0.09

Reagent: 0.06 M Na_2CO_3, aqueous; Substrate: 0.03 M Ac_6-OUA in 7:3 ethanol:dioxane

Conditions		Peak Height					Fraction of Peak Height				
T /°C	*time* /h	OUA m/z 607	Ac_3-OUA +H_2O m/z 751.4	Ac_3-OUA m/z 733.5	Ac_4-OUA m/z 775.5	Ac_6-OUA m/z 859.5	OUA m/z 607	Ac_3-OUA +H_2O m/z 751.4	Ac_3-OUA m/z 733.5	Ac_4-OUA m/z 775.5	Ac_6-OUA m/z 859.5
40	2		7	100	6	0	0.00	0.06	0.88	0.05	0.00
40	2		18	100	6	0	0.00	0.15	0.81	0.05	0.00
40	6		7	100	5	0	0.00	0.06	0.89	0.04	0.00
18	2			100	23	27	0.00	0.00	0.67	0.15	0.18
18	6			100	8	0	0.00	0.00	0.93	0.07	0.00
18	14			100	20	0	0.00	0.00	0.83	0.17	0.00

Reagent: 0.1 M NaOPh, aqueous; Substrate: 0.03 M Ac_6-OUA in 7:3 ethanol:dioxane

Conditions		Peak Height				Fraction of Peak Height			
T /°C	*time* /h	OUA m/z 607	Ac_3-OUA +H_2O m/z 751.4	Ac_3-OUA m/z 733.5	Ac_4-OUA m/z 775.5	OUA m/z 607	Ac_3-OUA +H_2O m/z 751.4	Ac_3-OUA m/z 733.5	Ac_4-OUA m/z 775.5
40	2		25	100		0.00	0.20	0.80	0.00

40	2		23	100	5	0.00	0.18	0.78	0.04
40	6	5	40	100		0.03	0.28	0.69	0.00
40	14	12	76	100	10	0.06	0.38	0.51	0.05
18	2		25	100	13	0.00	0.18	0.72	0.09
18	6		36	100	15	0.00	0.24	0.66	0.10
18	14		37	100	13	0.00	0.25	0.67	0.09

Reagent: *n*-hexylamine; Substrate: 0.03 M Ac_6-OUA in 7:3 ethanol:dioxane

Conditions		Peak Height				Fraction of Peak Height			
T /°C	*time* /h	Ac_6-OUA m/z 859.5	Ac_5-OUA m/z 859.5	Ac_4-OUA m/z 775.5	Ac_3-OUA m/z 733.5	Ac_6-OUA m/z 859.5	Ac_5-OUA m/z 859.5	Ac_4-OUA m/z 775.5	Ac_3-OUA m/z 733.5
0	2	100	45			0.69	0.31	0.00	0.00
0	6	100	68	15		0.55	0.37	0.08	0.00
0	6	85	100	40	9	0.36	0.43	0.17	0.04
0	16	60	100	85	34	0.22	0.36	0.30	0.12
18	1	100	22	0	0	0.82	0.18	0.00	0.00
18	2	100	49	11		0.63	0.31	0.07	0.00
18	6	50	100	85	28	0.19	0.38	0.32	0.11
18	14			65	100	0.00	0.00	0.39	0.61

Reagent: cyclohexanemethylamine; Substrate: 0.03 M Ac_6-OUA in 7:3 ethanol:dioxane

Conditions		Peak Height				Fraction of Peak Height			
T /°C	*time* /h	Ac_6-OUA m/z 859.5	Ac_5-OUA m/z 859.5	Ac_4-OUA m/z 775.5	Ac_3-OUA m/z 733.5	Ac_6-OUA m/z 859.5	Ac_5-OUA m/z 859.5	Ac_4-OUA m/z 775.5	Ac_3-OUA m/z 733.5
0	2	100	34	0	0	0.75	0.25	0.00	0.00
0	6	100	37	9		0.68	0.25	0.06	0.00
0	6	100	56	12		0.60	0.33	0.07	0.00
0	16	100	66	32	15	0.47	0.31	0.15	0.07
18	1	100	22	0	0	0.82	0.18	0.00	0.00
18	2	100	65	21		0.54	0.35	0.11	0.00
18	6	80	100	60	10	0.32	0.40	0.24	0.04
18	14	20	50	100	62	0.09	0.22	0.43	0.27

Reagent: 2-methoxyethylamine; Substrate: 0.03 M Ac_6-OUA in 7:3 ethanol:dioxane

Conditions		Peak Height			Fraction of Peak Height		
T /°C	*time* /h	Ac_6-OUA m/z 859.5	Ac_5-OUA m/z 859.5	Ac_4-OUA m/z 775.5	Ac_6-OUA m/z 859.5	Ac_5-OUA m/z 859.5	Ac_4-OUA m/z 775.5
0	2	100	12		0.89	0.11	0.00
0	6	100	67	11	0.56	0.38	0.06
0	6	100	49	4	0.65	0.32	0.03
0	16	100	72	22	0.52	0.37	0.11
18	1	100	10	0	0.91	0.09	0.00
18	2	100	48	4	0.66	0.32	0.03
18	6	100	77	20	0.51	0.39	0.10
18	14	60	100	52	0.28	0.47	0.25

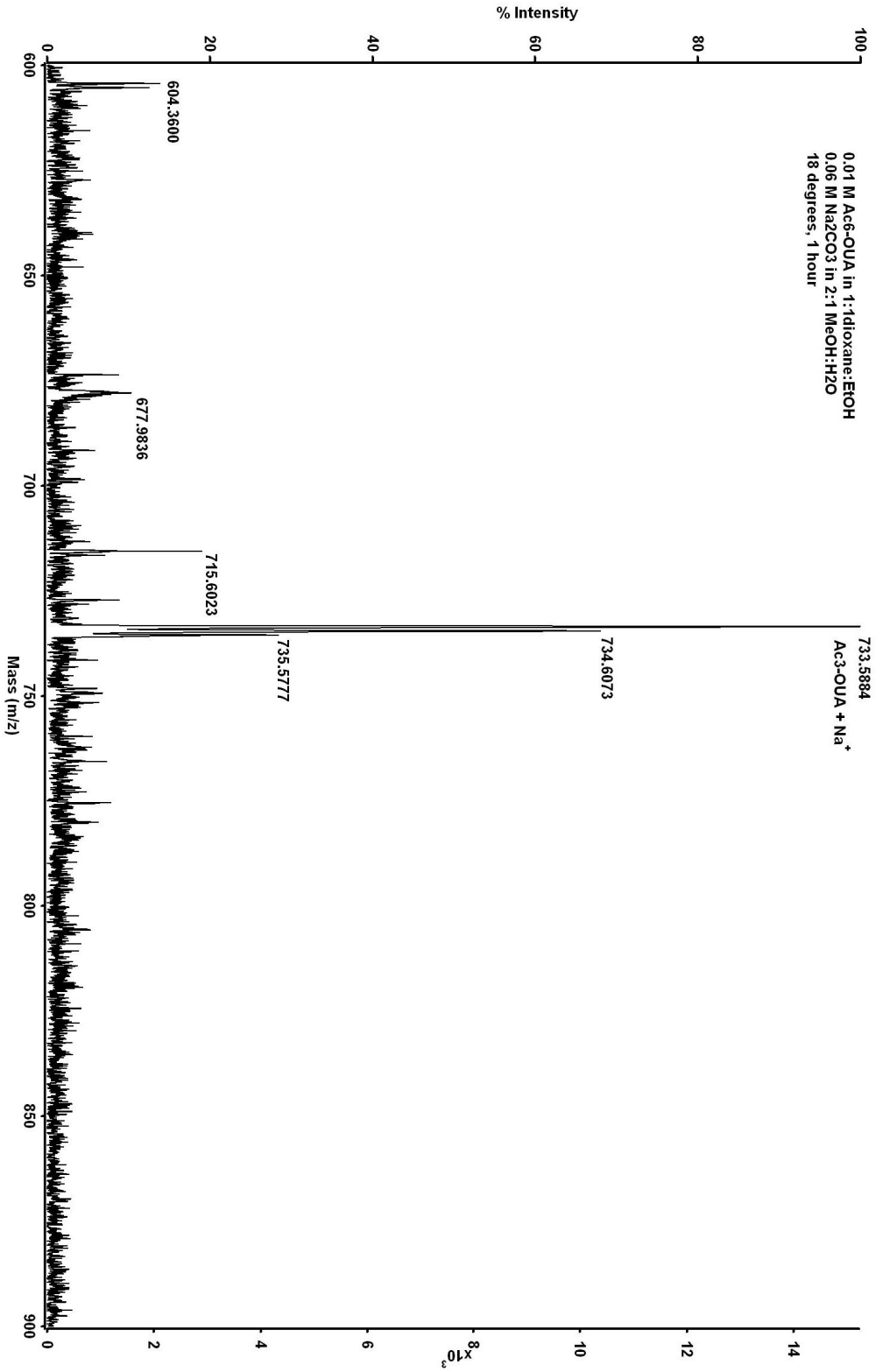
Reagent: cyclohexylamine; Substrate: 0.03 M Ac_6-OUA in 7:3 ethanol:dioxane

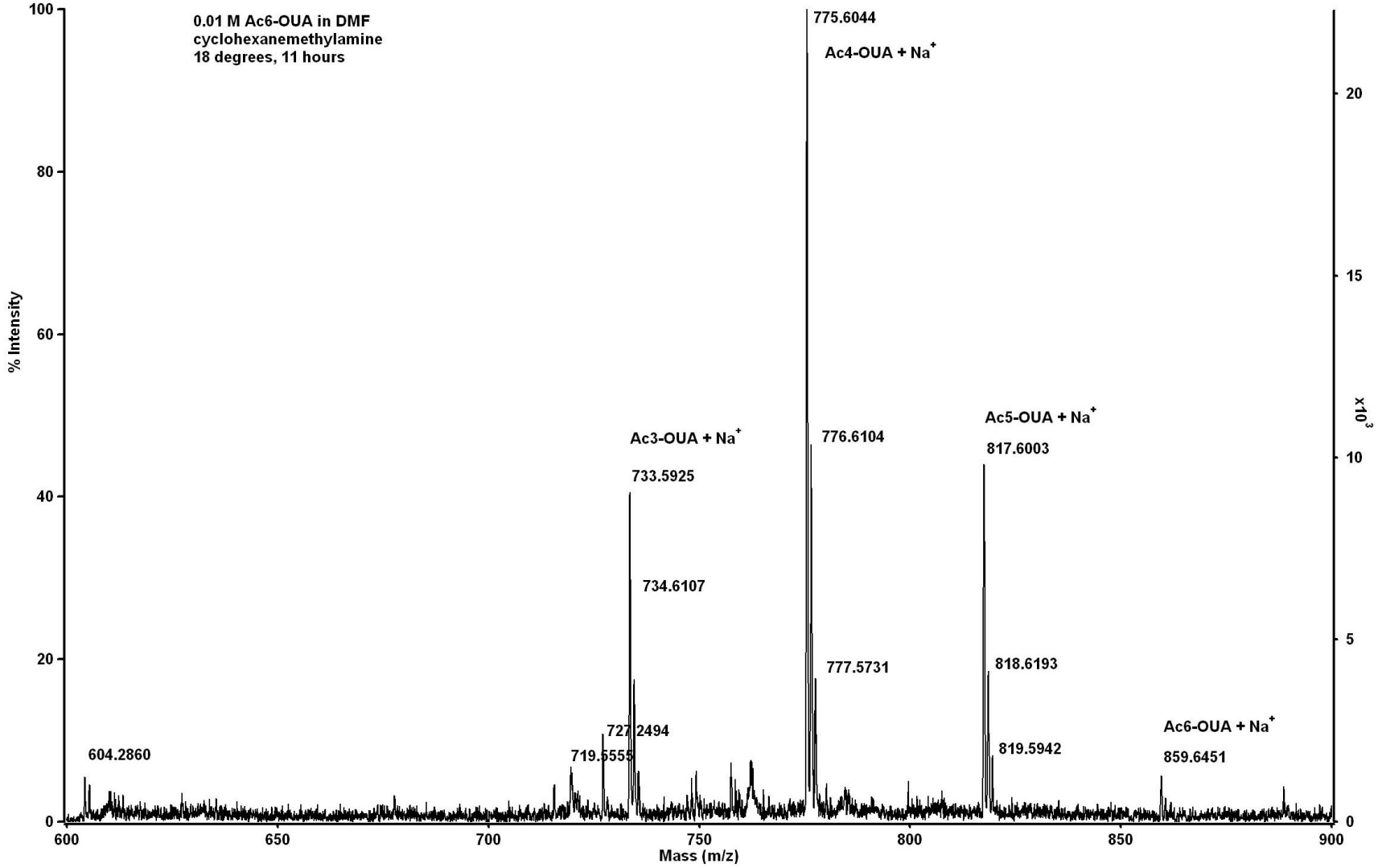
Conditions		Peak Height		Fraction of Peak Height	
T /°C	*time* /h	Ac_6-OUA m/z 859.5	Ac_5-OUA m/z 859.5	Ac_6-OUA m/z 859.5	Ac_5-OUA m/z 859.5
0	2	100		1.00	0.00
0	6	99	9	0.92	0.08
0	6	100		1.00	0.00
0	16	100	8	0.93	0.07
18	1	100	0	1.00	0.00
18	2	100	0	1.00	0.00
18	6	99	9	0.92	0.08
18	14	100	48	0.68	0.32

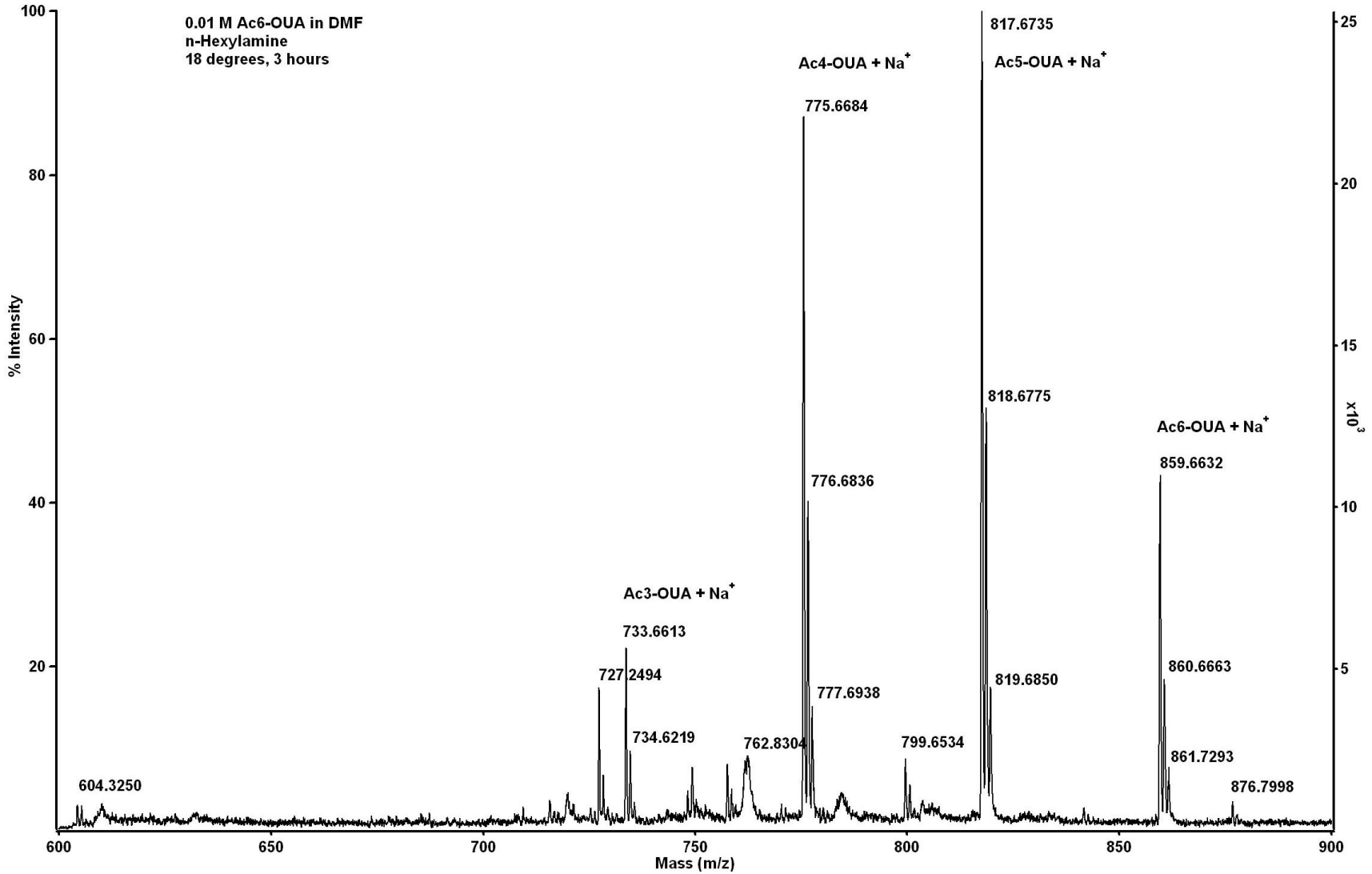
Reagent: 2-amino-1-propanol; Substrate: 0.03 M Ac_6-OUA in 7:3 ethanol:dioxane

Conditions		Peak Height			Fraction of Peak Height		
T /°C	*time* /h	Ac_6-OUA m/z 859.5	Ac_5-OUA m/z 859.5	Ac_4-OUA m/z 775.5	Ac_6-OUA m/z 859.5	Ac_5-OUA m/z 859.5	Ac_4-OUA m/z 775.5
0	2	95	16	0	0.86	0.14	0.00
0	6	98	18		0.84	0.16	0.00
0	6	99	18		0.85	0.15	0.00
18	2	100	20		0.83	0.17	0.00
18	6	97	38		0.72	0.28	0.00
18	14	97	34	17	0.66	0.23	0.11

11. Representative MALDI-MS spectra (the unmodified data were re-plotted using software Igor Pro, version 4.0.3.0, WaveMetrics Inc.)




0.01 M Ac6-OUA in DMF
cyclohexanemethylamine
18 degrees, 11 hours
775.6044
Ac4-OUA + Na+
Ac3-OUA + Na+
733.5925
734.6107
776.6104
777.5731
Ac5-OUA + Na+
817.6003
818.6193
819.5942
Ac6-OUA + Na+
859.6451
604.2860
719.5555
727.2494
% Intensity
Mass (m/z)
x10^3


0.01 M Ac6-OUA in DMF
n-Hexylamine
18 degrees, 3 hours
% Intensity
Mass (m/z)
x10³
Ac4-OUA + Na+
Ac5-OUA + Na+
Ac6-OUA + Na+
Ac3-OUA + Na+
817.6735
775.6684
818.6775
776.6836
859.6632
733.6613
727.2494
860.6663
819.6850
777.6938
734.6219
762.8304
799.6534
861.7293
604.3250
876.7998