Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

69451 Weinheim, Germany

Synchronized Synthesis of Peptide-Based Macrocycles by Digital Microfluidics**

Mais J. Jebrail, Alphonsus H. C. Ng, Vishal Rai, Ryan Hili, Andrei K. Yudin, and Aaron R. Wheeler**

anie_201001604_sm_miscellaneous_information.pdf

Reagents and Materials

L-Isoleucine (Ile), L-methionine (Met), L-Proline (Pro), L-Threonine (Thr), L-Valine (Val), proline-leucine (ProLeu), *tert*-butyl isocyanide (^{t}BuNC), thiobenzoic acid (PhCOSH), trifluoroethanol (TFE), methanol, water, boric acid, 50% formic acid, and fluorinert FC-40, were purchased from Sigma Chemical (Oakville, ON). Deuterated Met (d_3-Met) was obtained from Cambridge Isotope Laboratories (Andover, MA). Aziridine aldehyde was synthesized using the method we reported previously.[1,2] In all experiments, organic solvents were HPLC grade and deionized (DI) water had a resistivity of 18 MO·cm at 25°C. Parylene C dimer was from Specialty Coating Systems (Indianapolis, IN), and Teflon-AF was from DuPont (Wilmington, DE).

Device Fabrication and Operation

Digital microfluidic devices were fabricated in the University of Toronto Emerging Communications Technology Institute (ECTI) cleanroom facility, using a transparent photomask printed at Norwood Graphics (Toronto, ON). Glass devices bearing patterned chromium electrodes were formed by photolithography and etching as described previously,[3,4] and were coated with 2.5 µm of Parylene-C and 50 nm of Teflon-AF. Parylene-C was applied using a vapor deposition instrument (Specialty Coating Systems), and Teflon-AF was spin-coated (1% wt/wt in Fluorinert FC-40, 2000 rpm, 60 s) followed by post-baking on a hot-plate (160 ºC, 10 min). The polymer coatings were removed from contact pads by gentle scraping with a scalpel to facilitate electrical contact for droplet actuation. In addition to patterned devices, unpatterned indium tin oxide (ITO) coated glass substrates (Delta Technologies Ltd, Stillwater, MN) were coated with Teflon-AF (50 nm, as above).

The device design featured an array of eighty-eight actuation electrodes (2.2 × 2.2 mm ea.) connected to ten reservoir electrodes (5 × 5 mm ea.), with inter-electrode gaps of 40 μm. Devices were assembled with an unpatterned ITO–glass top plate and a patterned bottom plate separated by a spacer formed from two pieces of double-sided tape (total spacer thickness 180 μm). Reagents were delivered to their respective reservoirs by simultaneously applying potential to reservoir and pipetting the reagent adjacent to the 180 μm gap between the bottom and top plates. Unit droplet and reservoir droplet volumes on these devices were ~900 nL and ~4.5 μL, respectively. To actuate droplets, driving potentials (70–100 V_{RMS}) were generated by amplifying the output of a function generator (Agilent Technologies, Santa Clara, CA) operating at 18 kHz. As described elsewhere,[3] droplets were sandwiched between the two plates and actuated by applying driving potentials between the top electrode (ground) and sequential electrodes on the bottom plate via the exposed contact pads. To ensure droplet actuation at all times, droplet must be larger than the footprint of home-electrode so that it overlaps its neighbouring electrode. Droplet actuation was monitored and recorded by a CCD camera mounted with a lens.

Mass Spectrometry

For analysis by mass spectrometry, reaction products were diluted into methanol containing 0.1% formic acid (~870 μM final concentration of products) and injected into an LTQ linear ion trap mass spectrometer (Thermo Fischer Scientific, Waltham, MA) operating in positive ion mode. Samples were delivered *via* a fused silica capillary transfer line (100 μm i.d.) mated to a New Objective Inc. (Woburn, MA) nanoelectrospray emitter (100 μm i.d. tapering to 30 μm i.d.). The samples were delivered at a flow rate of 1.5 μL min^{-1}, with an applied voltage of 1.7–

1.9 kV and capillary temperature of 170 ºC. Spectra were collected as an average of 50 acquisitions, and data shown here are representative of analysis of samples in triplicate.

Nuclear Magnetic Resonance

For analysis by nuclear magnetic resonance (NMR), concentrated solutions (~7 mM) of peptide-based macrocycle containing Met or its aziridine ring-opened derivative were prepared by re-suspending the reaction products synthesized in a single droplet by digital microfluidics in 12 µL MeOH-d_4. A Protasis CapNMR probe (Savoy, IL) was used for sample injection and ^{1}H-NMR spectra were generated using a Varian UnityPlus 500 MHz NMR spectrometer referenced to MeOH-d_4 (3.31 ppm). ^{1}H-NMR spectra were recorded with a spectral window of 10998 Hz, using a 2.4 µs pulse with 45505 real plus complex points acquired with 256 scans. For analysis of reaction products synthesized by macroscale, ^{1}H spectra were recorded on Varian Mercury 400 MHz spectrometers.

Macroscale Synthesis

Peptide-based macrocycle containing methionine (Met) and its aziridine ring-opened derivative were synthesized using methods we reported previously.[2] In brief, for synthesis of peptide-based macrocycle containing Met, in a screw-cap vial equipped with a magnetic stirring bar was added Met (0.2 mmol) and 1 mL of TFE:H_2O (20:1) and stirred until homogeneous solution has been obtained. Aziridine aldehyde dimer (0.1 mmol) and *tert*-butyl isocyanide (0.2 mmol) were then added sequentially and the resulting mixture was stirred for 1-3 h. For aziridine ring-opened derivative, in a screw-cap vial equipped with a magnetic stirring bar was added peptide-based macrocycle containing Met (0.06 mmol). Solvent (CH_2Cl_2, 0.2 ml) and thiobenzoic acid (0.12 mmol) were then added sequentially and the resulting mixture was stirred for 1 h.

Reaction Progress Analysis

For reaction progress, the rate reaction of Met was analyzed. Two sets of five ~900 nL droplets containing Met (0.1 M) were dispensed on the same device. The first set of droplets were reacted with aziridine aldehyde and *tert*-butyl isocyanide (as above) for various periods of time (15, 30, 60 and 90 min), and the second set of control droplets were not reacted. After the reactions were completed, the top plate was removed and the two sets of droplets (reaction and controls) were allowed to dry. The solids were resolubilized in 100 µL methanol/water 50:50 v/v containing 250 µM deuterated Met (d_3-Met) as an internal standard, collected in a pipette, and evaluated by MS (as above). % conversion of Met reactant over time was determined by comparing the abundance ratio of the Met:d_3-Met peaks in the spectra of reacted samples to the abundance ratio of the Met:d_3-Met peaks in the spectra of the controls, and subtracting this ratio from 100%. Four replicate measurements were made for each sample and control. An analagous method (with volumes scaled appropriately) was used to evaluate reaction progress of macroscale synthesis.

References

[1] R. Hili, A. K. Yudin, *J. Am. Chem. Soc.* **2006**, *128*, 14772.
[2] R. Hili, V. Rai, A. K. Yudin, *J. Am. Chem. Soc.* **2010**, *132*, 2889.
[3] M. J. Jebrail, A. R. Wheeler, *Anal. Chem.* **2009**, *81*, 330.
[4] M. J. Jebrail, V. N. Luk, S. C. C. Shih, R. Fobel, A. H. C. Ng, H. Yang, S. L. S. Freire, A. R. Wheeler, *J Vis Exp* **2009**, *33*, DOI: 10.3791/1603.

Figure Captions

Figure S1: ESI-MS spectra generated from **(a)** pure reactant solution of isoleucine (Ile) (m/z 132), **(b)** on-chip synthesized peptide-based macrocycle containing Ile (m/z 324), and **(c)** on-chip synthesized aziridine ring-opened derivative (m/z 462).

Figure S2: ESI-MS spectra generated from **(a)** pure reactant solution of valine (Val) (m/z 118), **(b)** on-chip synthesized peptide-based macrocycle containing Val (m/z 310), and **c)** on-chip synthesized aziridine ring-opened derivative (m/z 448).

Figure S3: ESI-MS spectra generated from **(a)** pure reactant solution of proline (Pro) (m/z 116), **(b)** on-chip synthesized peptide-based macrocycle containing Pro (m/z 306), and **c)** aziridine ring-opened derivative (m/z 446).

Figure S4: ESI-MS spectra generated from **(a)** pure reactant solution of threonine (Thr) (m/z 120), **(b)** on-chip synthesized peptide-based macrocycle containing Thr (m/z 312), and **c)** on-chip synthesized aziridine ring-opened derivative (m/z 450).

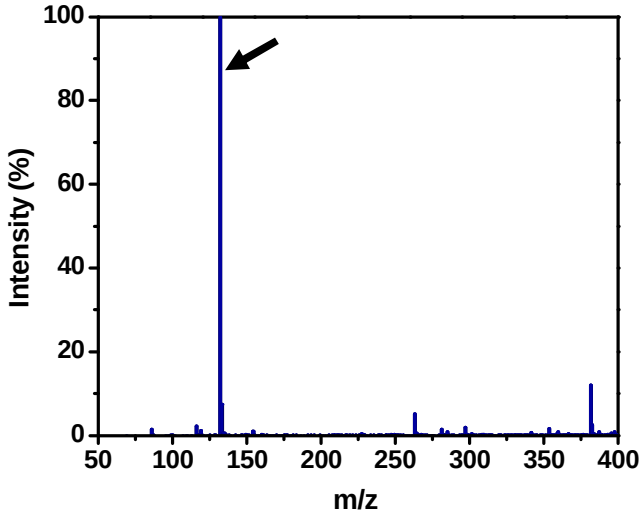
Figure S5: ESI-MS spectra of **(a)** macroscale synthesized peptide-based macrocycle containing Met (m/z 342) and **(b)** macroscale synthesized aziridine ring-opened derivative (m/z 480). **(c)** Macroscale reaction progress as **%** conversion of Met over time. Each data point represents the mean ± S.D. of 4 samples.

Figure S6: NMR spectra generated from **(a)** on-chip and **(b)** macroscale synthesized cyclic peptide-Met in MeOH-d4. Below are the chemical shifts of protons

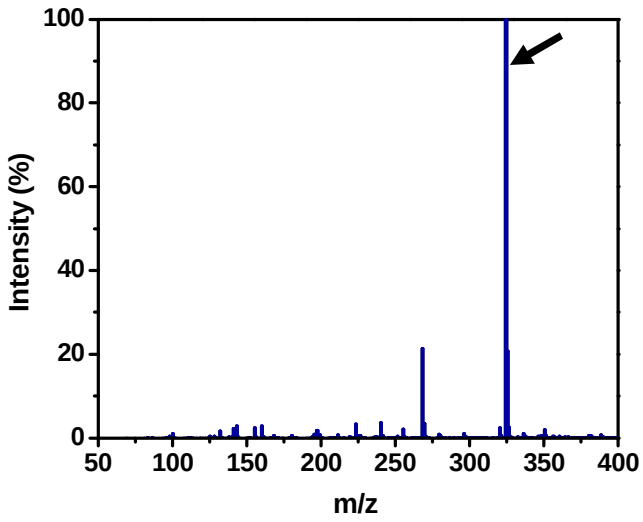
Figure S7: NMR spectra generated from **(a)** on-chip and **(b)** macroscale synthesized ring opened cyclic peptide-Met in MeOH-d4. Below are the chemical shifts of protons.

Isoleucine (Ile)

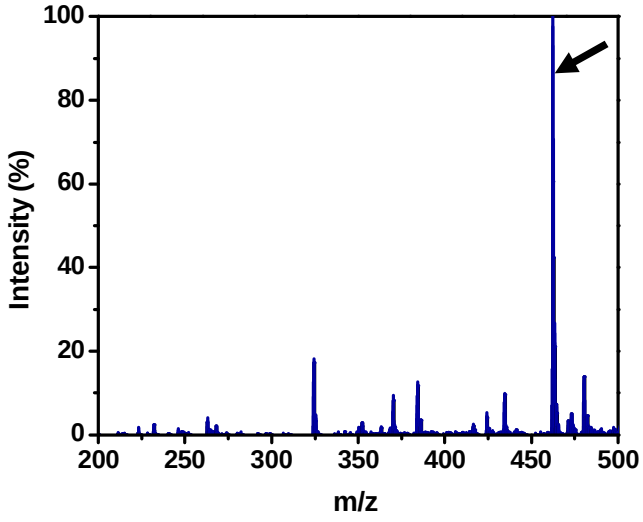
Figure S1 (a)



(b)

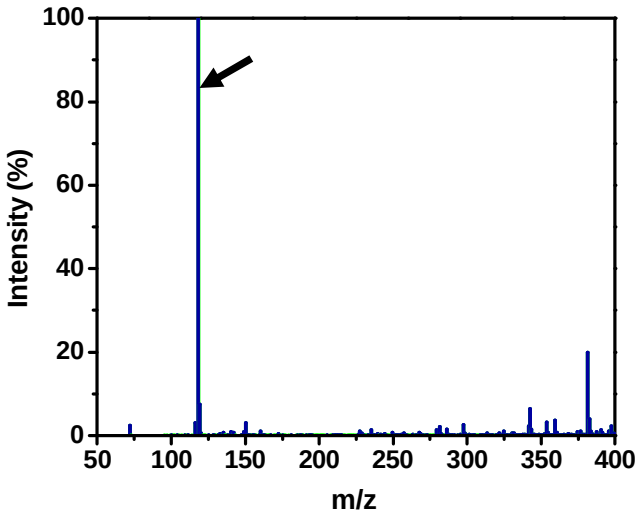


(c)

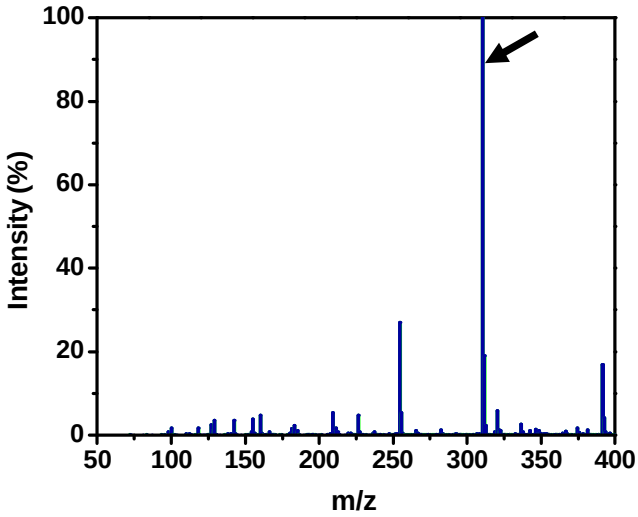


Valine (Val)

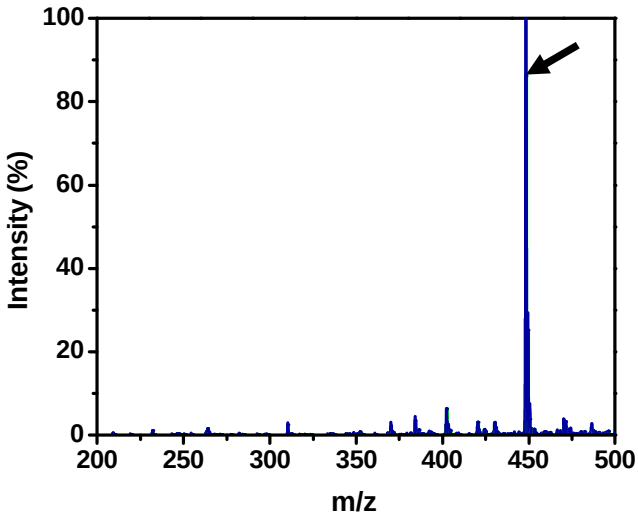
Figure S2 (a)



(b)

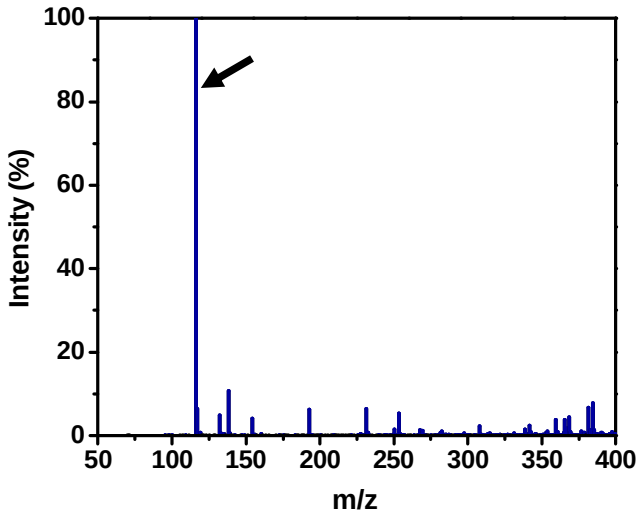


(c)

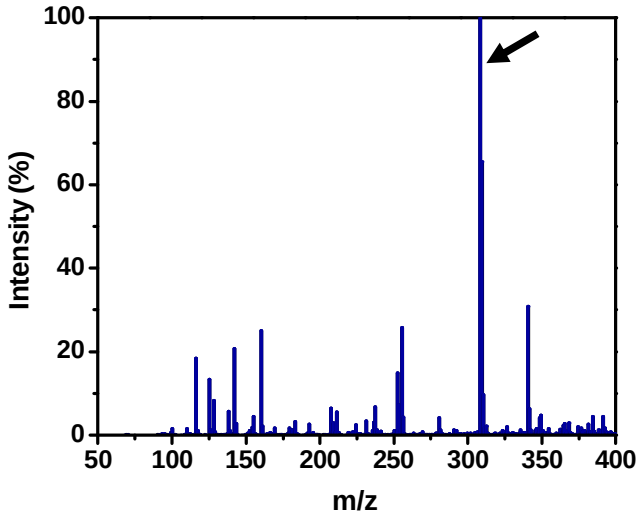


Proline (Pro)

Figure S3 (a)



(b)



(c)

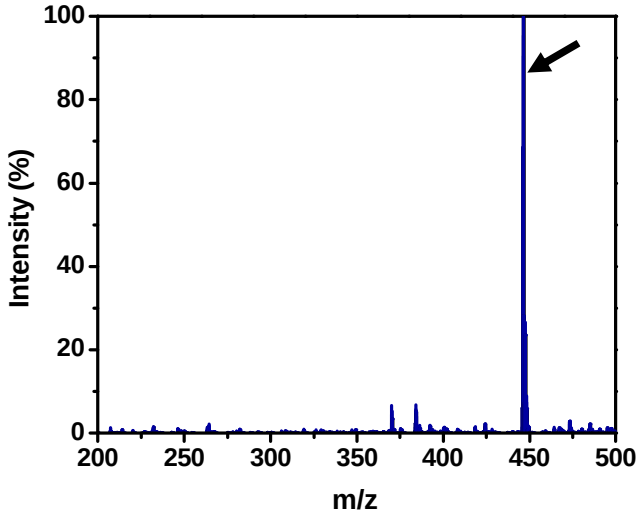


Figure S4 (a)

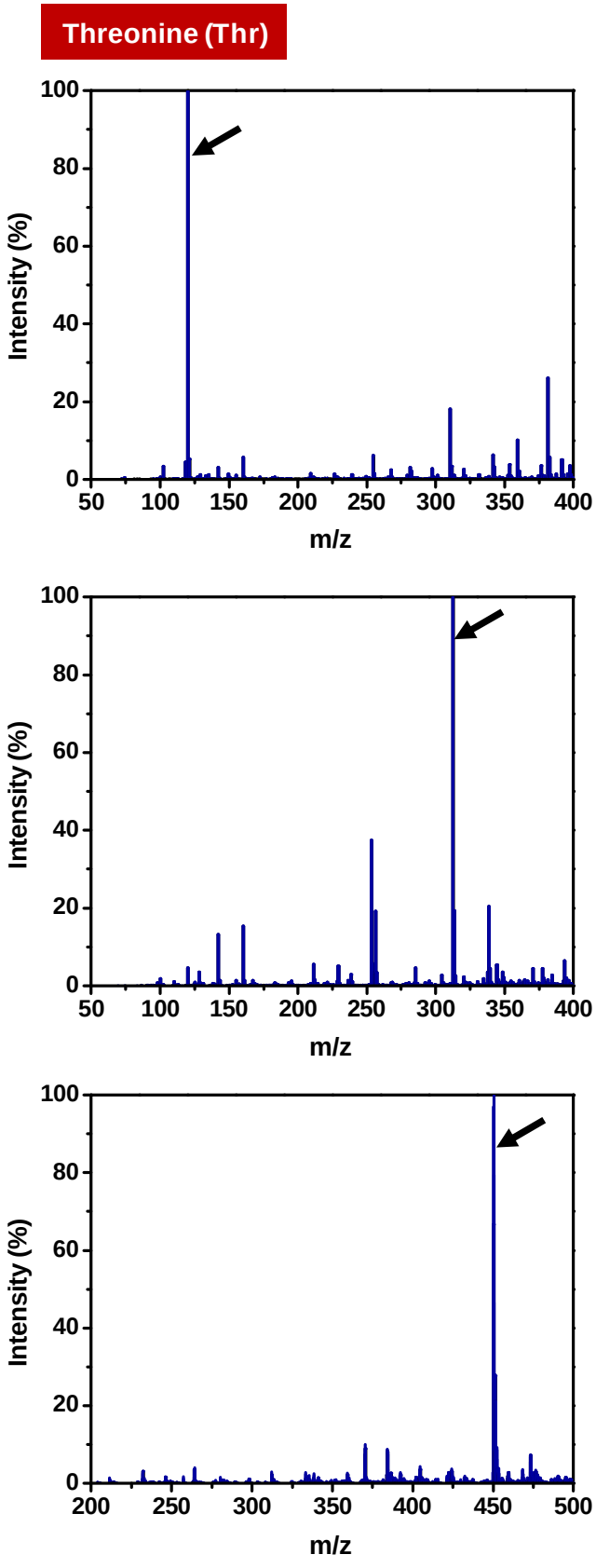


Figure S5 (a)

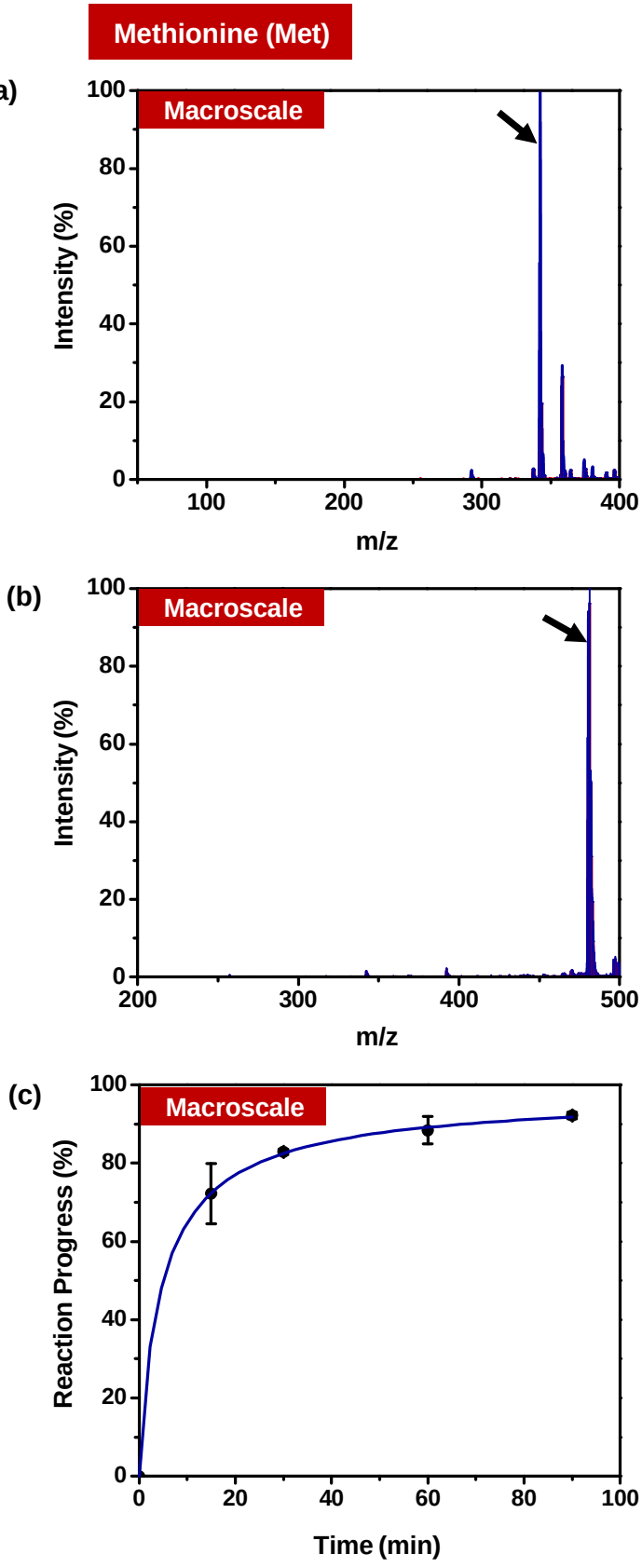
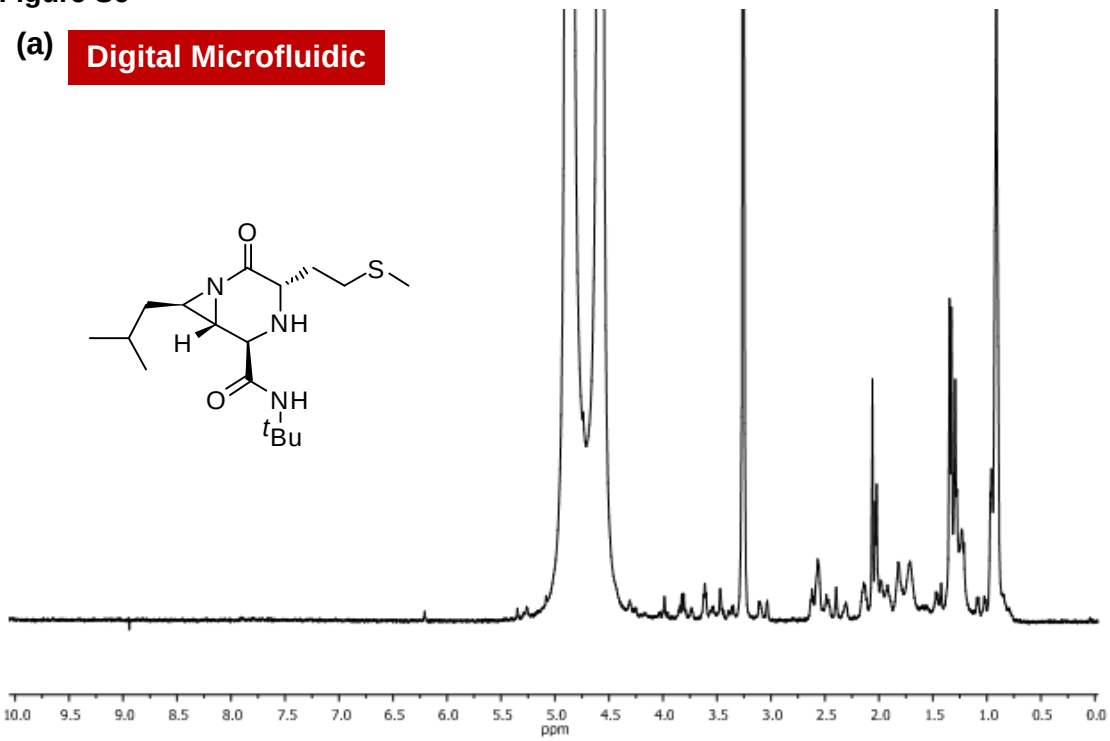
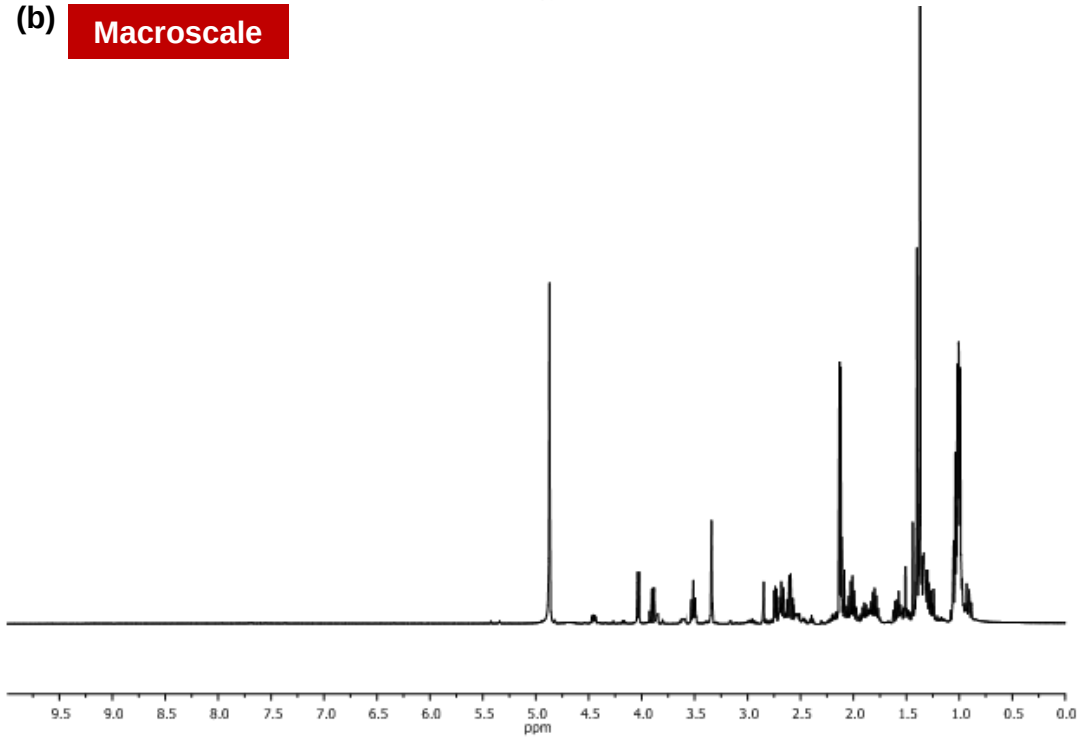

Methionine (Met)
Macroscale
Intensity (%)
m/z
(b)
Macroscale
Intensity (%)
m/z
(c)
Macroscale
Reaction Progress (%)
Time (min)

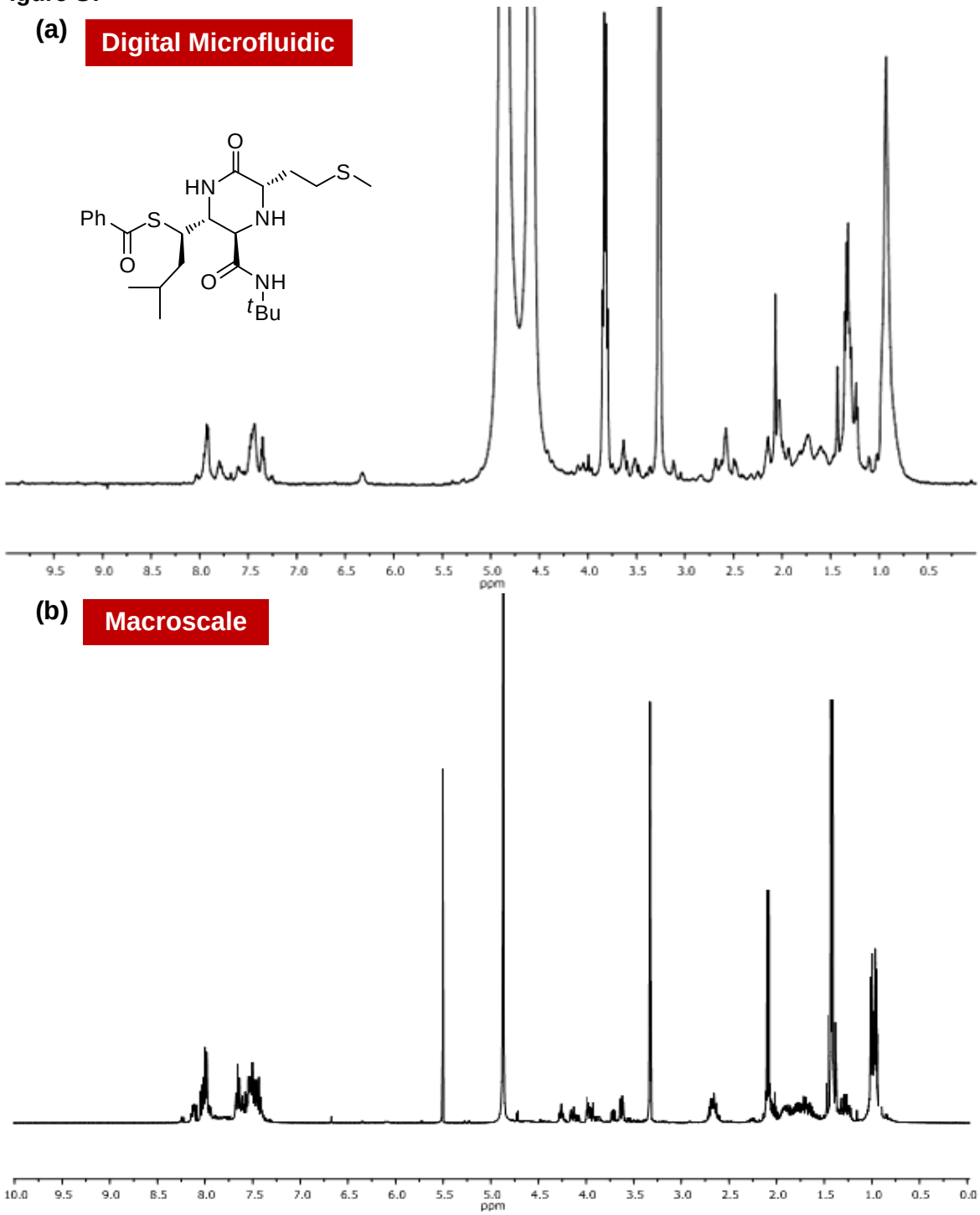
Figure S6





^{1}H NMR d: 4.42 (ddd, *J* = 10.0, 4.3, 1.9 Hz, 1H), 4.00 (d, *J* = 5.9 Hz, 1H), 3.58 (dd, *J* = 7.7, 4.1 Hz, 1H), 3.48 (dd, *J* = 8.6, 6.6 Hz, 1H), 2.71 (dd, *J* = 5.9, 3.7 Hz, 2H), 2.10 (s, 3H), 1.98 (ddd, *J* = 10.4, 8.0, 4.7 Hz, 2H), 1.87 (m, 2H), 1.77 (m, 1H), 1.34 (s, 9H), 0.98 (d, *J* = 5.1 Hz, 3H), 0.97 (d, *J* = 5.0 Hz, 3H) ppm.

Figure S7



^{1}H NMR d: 8.09 (m, 2H), 7.58 (m, 2H), 7.41 (dd, *J* = 8.0, 7.5 Hz, 1H), 4.70 (d, *J* = 6.7 Hz, 1H), 4.23 (ddd, *J* = 11.5, 5.8, 2.6 Hz, 1H), 4.13 (t, *J* = 3.4 Hz, 2 H), 4.10 (t, *J* = 3.5 Hz, 2 H), 3.60 (dd, *J* = 10.2, 3.8 Hz, 1H), 2.63 (m, 1H), 2.05 (s, 3H), 1.89 (m, 2H), 1.74 (m, 1H), 1.39 (s, 9H), 0.98 (d, *J* = 6.4 Hz, 3H), 0.93 (d, *J* = 6.3 Hz, 3H) ppm.