Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Direct Palladium-Mediated On-Resin Disulfide Formation from Allocam Protected Peptides

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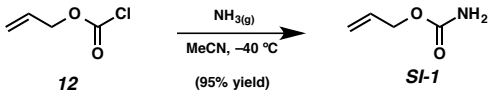
General Information. Unless otherwise specified, all commercially available reagents were purchased from Sigma-Aldrich and used without further purification. Anhydrous Et_2O, PhMe, *n*-hexane, MeCN, DMF, DMSO, CH_2Cl_2 were purchased from Fisher, THF was purchased from EMD, and PhH was purchased from Sigma-Aldrich. These were passed through a commercial solvent purification system (2 columns of alumina) and used without further drying. Triethylamine, diisopropylamine, pyridine, and Hünig's base were distilled over CaH_2 immediately prior to use. Unless otherwise noted, all reactions were performed in flame-dried glassware under 1 atm of pre-purified anhydrous N_2 or argon gas. ^1H NMR spectra and ^{13}C NMR spectra were recorded on a Varian Mercury-400 MHz or a Varian VNMRS-500MHz spectrometer with a multinuclear broadband probe at ambient temperature unless otherwise stated. Chemical shifts are reported in parts per million relative to residual solvent peaks (as established by Stoltz, et. al. in *Organometallics* **2010**, *29*, 2176). All ^{13}C spectra are recorded with complete proton decoupling. High-resolution mass spectral analyses were performed by the Lumigen Instrument Center, Wayne State University. All purifications were performed on SiliaFlash® P60 40-63μm (230-400 mesh) 60Å Irregular Silica Gels (cat. # R12030B) or on a Biotage Isolera IV flash purification system using SNAP cartridges (cat. # FSKO-1107-XXXX). Thin layer chromatography was performed using glass-backed SiliaPlate™ TLC Plates (cat. # TLG-R10011B-323) cut to the desired size then visualized with short-wave UV lamps and $KMnO_4$, CAM, PMA, or Anisaldehyde stains prepared according to standard recipes. All yields refer to chromatographically and spectroscopically pure products. IR data was obtained on a Varian/Digilab Excalibur 3100 High Resolution FT-IR, and optical rotation data was collected on a Perkin-Elmer 341 automated Polarimeter at the concentration noted.

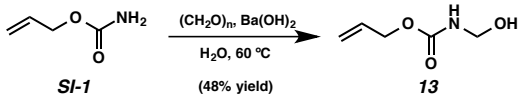
SPPS General Information. Solid-phase peptide synthesis was executed on a Biotage Isolera+ semi-automated synthesizer with microwave heating.

- *Reactor Vials [Vial size (Volume range allowed)]:* 2 mL reactor vial (0.8-1.1 mL), 5 mL reactor vial (1.6-3.2 mL), and 10 mL reactor vial (3.2-6.4 mL)
- *Swelling + Heat:* DMF was added and vortexed at 1200 RPM for 20 min at 70 ºC. The solvent was then removed over 1 min followed by two DMF washes (DMF was added and the suspension was vortexed at 600 RPM for 45 s, followed by the removal of solvent (over 2 m)).
- *Coupling:* A solution of Fmoc-aa-OH (5 equiv), HATU (4.9 equiv), and DIPEA (10 equiv) in DMF was made immediately prior to addition to the reaction vial containing the resin. Once the solution was added, the suspension was heated to 75 ºC (except for Fmoc-Cys(Allocam)-OH, which was heated to 50 ºC) for 5 min with a vortex rate of 1200 RPM. After the reaction, the solution was removed (over 2 m) and the resin was rinsed with DMF 4 times (after addition of DMF, the suspension was agitated at a vortex rate of 1200 RPM for 1 min, solvent removal was at a rate of 2 m).
- *Fmoc Removal (Deprotection):* The reactor vial was filled with 20% piperidine in DMF. The suspension was vortexed at 1200 RPM for 3 min at RT. The solvent is removed followed by addition of 20% piperidine in DMF. The suspension is vortexed again at 1200 RPM for 10 min at RT. The solvent was removed over 2 min, followed by 4 DMF washes (after addition of DMF, the suspension was agitated at a vortex rate of 1200 RPM for 1 min, solvent removal was at a rate of 2 min).
- *Wash*: DMF was added to the reaction vial and agitated at a vortex rate of 1200 RPM for 1 min. The solvent was removed over 1 min and repeated for a total of 4 times.

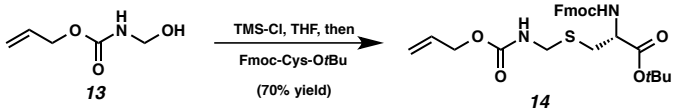
Experimental Procedures and Spectroscopic Data (Fmoc-Cys(Allocam)-OH)



Carbamate SI-1. To a cooled (–40 ºC) solution of allyl chloroformate (**12**, 10 mL, 94.084 mmol) in MeCN (125 mL, 0.8 M) was bubbled ammonia gas (excess) for 5 to 10 min. During this time, a white precipitate was observed (appearance of solution went from transparent to opaque white). The reaction was allowed to stir for 15 min. The solution was removed from the cooling bath and allowed to gradually come to ambient temperature (with needle ventilation). The reaction was monitored by TLC for the complete disappearance of starting material. If starting material was observed, the process of ammonia bubbling was repeated. Upon completion, the reaction solution was filtered and solvents were removed under reduced pressure conditions. The residue was then placed on high vacuum to remove any final traces of solvent. No further purification was necessary to isolate carbamate **SI-1** as a clear viscous liquid (9.20 g, 95% yield) R_f =0.50 (40% EtOAc/Hexanes). ^{1}H NMR matches previous literature.[1]



Hydroxymethylcarbamate 13. To a solution of allyl carbamate (**SI-1**, 4.0 g, 39.553 mmol) in water (66 mL, 0.6 M) was added paraformaldehyde (1.663 g, 55.374 mmol) and barium hydroxide (374 mg, 1.187 mmol). The reaction flask was then placed in an oil bath that was brought to 60 ºC. Though the solution does not reflux, a condenser is used during the reaction with a ventilation needle. This solution was allowed to stir overnight at 60 ºC. Thereafter, another portion of paraformaldehyde (1.663 g, 55.374 mmol) was added and allowed to stir until starting material disappeared, or until the consumption of starting material ceased. The reaction was monitored by TLC. Once the reaction was complete, the solution was diluted with water and extracted with ethyl acetate four times. The organic layers were collected, dried over sodium sulfate, and solvents removed. Flash column chromatography was preformed, and hydroxymethylcarbamate **13** was isolated as a white solid (2.43 g, 47% yield) R_f =0.50 (60% EtOAc/Hexanes). Byproduct fractions were also collected and consisted of starting material and bis-methylhydroxylated material. ^{1}H NMR matches previous literature. [1]



Fmoc-Cys(Allocam)-O*t*Bu (14). To a solution of allyl hydroxymethyl carbamate (**13**, 629 mg, 4.794 mmol) in THF (16 mL, 0.3 M) was added TMS-Cl (548 μL, 4.315 mmol, 0.9 equiv). The solution was allowed to stir for 10 mins. In a separate flask, Fmoc-Cys(H)-OtBu (1.915 g, 4.794 mmol, 1.0 equiv) was dissolved in THF (16 mL, 0.3 M), and this solution was transferred into the flask with the allyl hydroxymethyl carbamate. The reaction solution was allowed to stir overnight,

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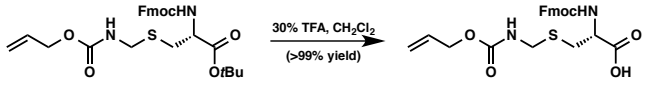
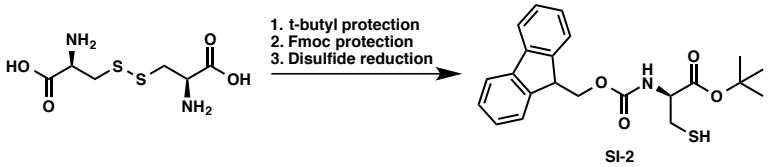
[1] Kimbonguila, A. M.; Merzouk, A.; Guibé; Loffet, A. *Tetrahedron* **1999**, *55*, 6931–6944.

and was monitored by TLC and mass spectrometry. Upon completion (as determined by the disappearance of Fmoc-Cys(H)-O*t*Bu, or both starting materials), the reaction solution was quenched with water. The aqueous layer was then extracted with ethyl acetate 3 times, and the organic layers were combined and dried over sodium sulfate. Flash column chromatography was performed, and pure Fmoc-Cys(Allocam)-O*t*Bu **14** was isolated as a fluffy looking, sticky solid (2.11 g, 86% yield) R_f =0.30 (30% EtOAc/Hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 7.5 Hz, 2H), 7.65 (d, *J* = 7.5 Hz, 2H), 7.44 (t, *J* = 7.5 Hz, 2H), 7.35 (t, *J* = 7.4 Hz, 2H), 6.03 – 5.87 (m, 1H), 5.28 (dd, *J* = 37.4, 13.7 Hz, 2H), 4.62 (d, *J* = 5.3 Hz, 2H), 4.55 (d, *J* = 6.8 Hz, 1H), 4.50 – 4.31 (m, 3H), 4.28 (t, *J* = 7.0 Hz, 1H), 3.09 (d, *J* = 38.9 Hz, 2H), 1.52 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.67, 155.89, 143.82, 143.74, 141.29, 132.51, 127.71, 127.06, 125.14, 119.98, 117.99, 83.08, 67.16, 65.99, 54.75, 47.12, 44.35, 33.82, 27.98.



14 *15*

Fmoc-Cys(Allocam)-OH (15). To a round bottom flask was added Fmoc-Cys(Allocam)-OtBu and DCM. To this was added slowly TFA until the composition of the solution was 30% TFA in DCM. Then the reaction mixture was stirred vigorously for 2 hours. Upon completion of the reaction, approximately half of the solvent was removed under low pressure. Then an equal volume of toluene to DCM was added to the flask, again, approximately half of the solvent was removed under low pressure. This procedure was performed 5 times in total, with the removal of all solvents in the final treatment. Thereafter, the remaining residue was dissolved in 1:1 acetonitrile:water and lyophilized to produce a white solid (2.62 g, 96% yield) R_f =0.20 (60% EtOAc/Hexanes). ^{1}H NMR matches previous literature.[1]



SI-2

Fmoc-Cys(H)-O*t*Bu (SI-2). Prepared according to known procedures,[2] with slight modifications below:

- **Preparation (R,R)-cystine bis-t-butyl ester**: Allowed L-cystine to stir in perchloric acid for 20-30 min before the addition of t-butyl acetate. Over the 72 hour time period the reaction mixture went from clear liquid, to a white thick slurry (almost solid).
- **Preparation N,N'-Bis(9-fluorenylmethoxycarbonyl)-(R,R)-cystine bis-t-butyl ester**: Upon dilution of the reaction mixture with ethyl acetate (during reaction work up) a white precipitate forms, which is filtered prior to the addition of potassium hydrogen sulfate. Instead of recrystallization flash chromatography was used for isolation (using an ethyl acetate/hexanes system), which gives the product as a white, fluffy, crystalline solid.
- **Preparation N-9-Fluorenylmethoxycarbonyl-(R)-cysteine t-butyl ester**: N,N'-Bis(9-fluorenylmethoxycarbonyl)-(R,R)-cystine bis-t-butyl ester, dry THF, and ultra pure water

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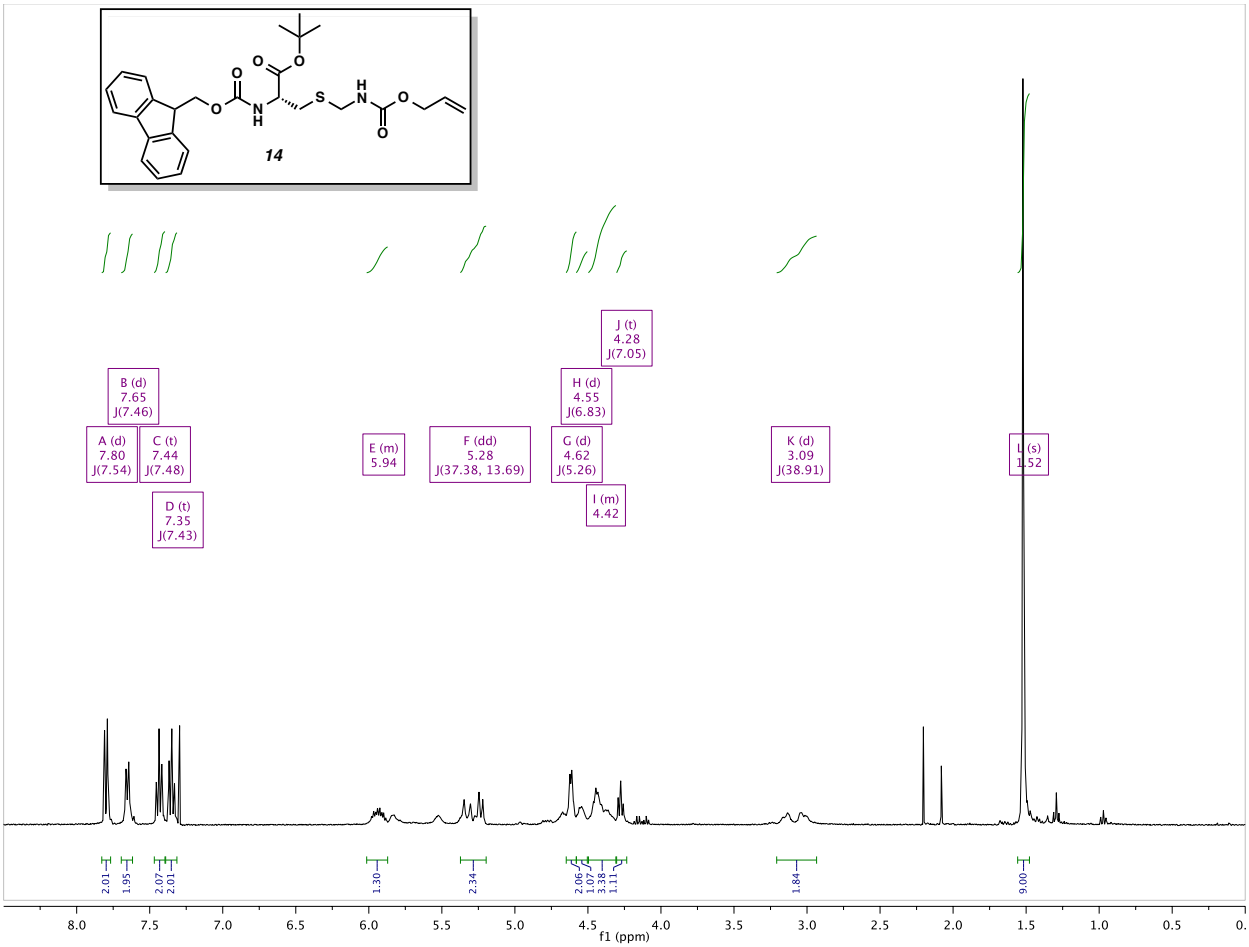
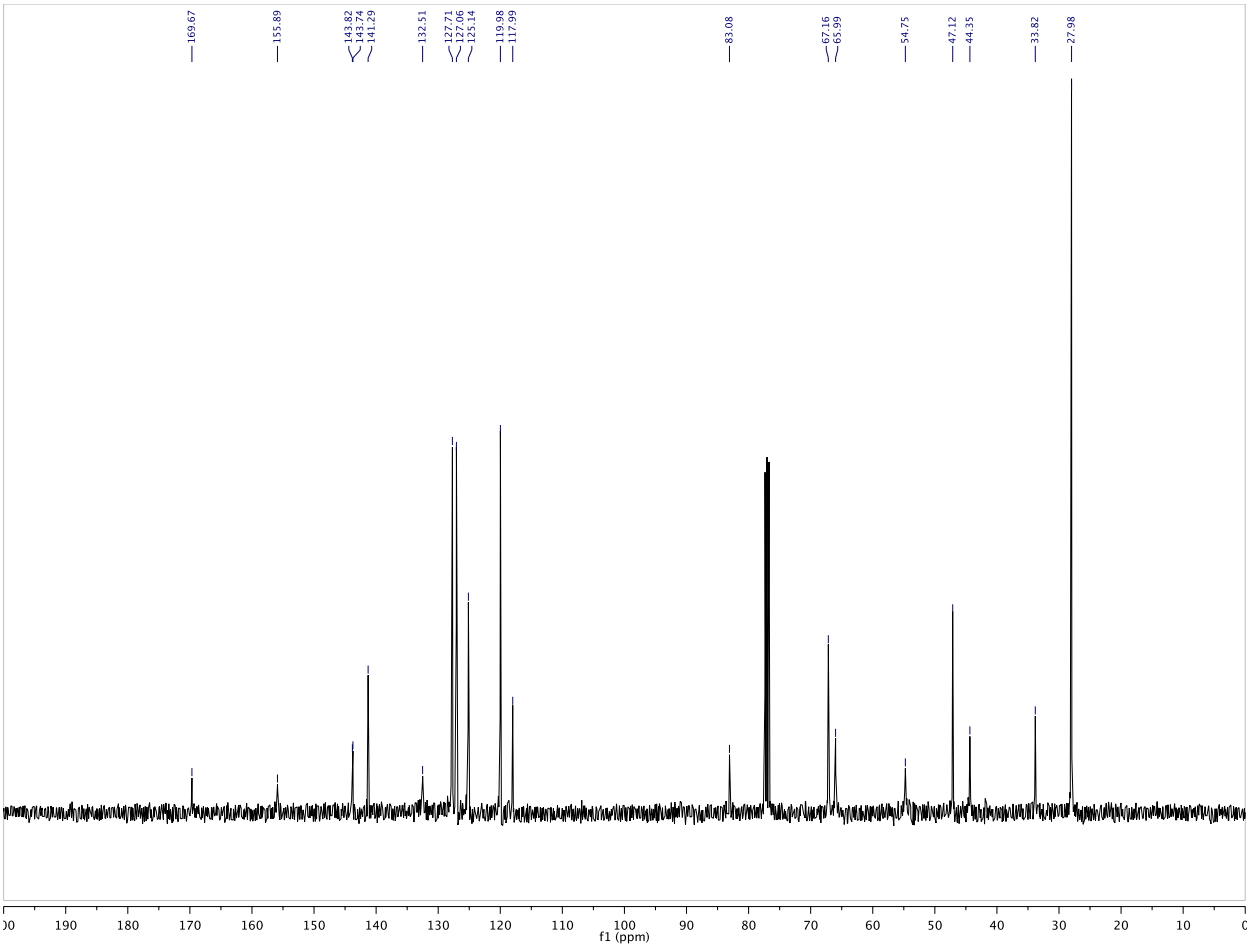
[2] Mustapa, M. F. M.; Harris, R.; Bulic-Subanovic, N.; Elliot, S. L.; Bregant, S.; Groussier, M. F. A.; Mould, J.; Schultz, D.; Chubb, N. A. L.; Gaffney, P. R. J.; Driscoll, P. C.; Tabor, A. B. *J. Org. Chem.* **2003**, *68*, 8185–8192.

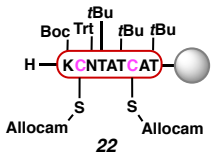
were all combined in a flame dried flask, and argon was bubbled through the solution while stirring for at least 30 min prior to the addition of tributylphosphine. Column chromatography was performed using ethyl acetate/hexanes, and the product was isolated as a sticky, but fluffy looking, white crystalline solid. Storage in benzene (with known concentration) for later use was appropriate. A non-sticky, fluffy, white solid can be isolated after dissolving in 1:1 MeCN:H_2O and lyophilizing.

^{1}H, ^{13}C NMR Spectra




169.67
155.89
143.82
143.74
141.29
132.51
127.71
127.06
125.14
119.98
117.99
83.08
67.16
65.99
54.75
47.12
44.35
33.82
27.98
f1 (ppm)

Experimental Procedures and Spectroscopic Data (IAPP and Oxytocin)



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Synthesis of $IAPP_{1-9}$ (22). The synthesis was done using a semi-automated peptide synthesizer, in a 10-mL reaction vial. First, the resin was swelled followed by deprotection. Then, each amino acid was added accordingly followed by coupling and deprotection steps. Finally, the resin + peptide was washed with DCM and Methanol. The conditions used for each step are given below (R1 = round 1, R2 = round 2):

	Swelling	Deprotection	Coupling	Wash (4x)	Final washes (3x each wash)		
Reaction time / min	20	3.00(R1) + 10.00(R2)	5	1	1	0.75	0.75
Temperature/ ºC	70	RT	75	RT	RT	RT	RT
Vortex (rpm)	1200	1200	1200	1200	1200	600	600
Reagent used	DMF	20% Piperidine in DMF	AA+HATU+DMF	DMF	DCM	MeOH	DCM
Volume of the reagent/ mL	4	3.00(R1) + 3.00(R2)	3	3	3	6	6

**For Cys(Allocam) coupling, Temp = 50 ºC

MS Analysis:
About 10 mg of the final product (resin bound) was cleaved from the resin using 300 μl of the cleavage mixture (1:1:3 TFE:AcOH:DCM) for 30 min and the filtrate was analyzed by MS to confirm the desired product formation.

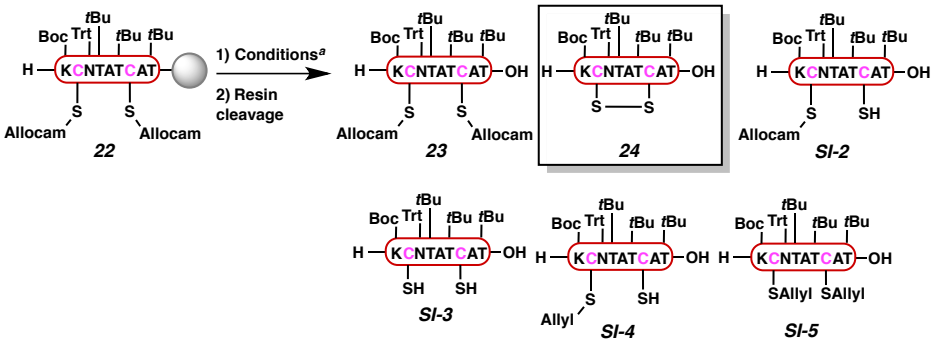
Analysis of final loading of the peptide:
Fmoc Deprotection – From the resulting resin, 10 mg was weighed out and swelled in DMF in the peptide synthesizer. Then, 1.00 mL of 20% piperidine in DMF was added and it was allowed to vortex at 1200 RPM in the peptide synthesizer at room temperature for 3 min. After 3 min, the filtrate was collected into a separate tube, another 1.00 mL of 20% piperidine in DMF was added to it and allowed to vortex again at 1200 RPM in the peptide synthesizer at room temperature for 10 min. The resulting filtrate was collected to the same tube and the deprotected resin was washed with DMF (4 x 1.75 mL).

Sample preparation for UV analysis – From the collected ”deprotection solution” above, 100 μl was transferred in to an Eppendorf tube and it was diluted with 900 μl of DMF.

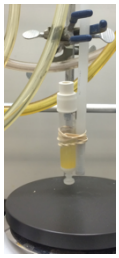
The resulting sample was used to get the absorbance at 301 nm versus a DMF blank using a UV spectrometer with 1 cm cuvette.

The resin was then subjected to a cleavage mixture of TFE:AcOH:DCM (1:1:3, 300 μ℘) for 3 x 15 min. The filtrates were collected and blown down with a stream of nitrogen gas. The concentrated solution was analyzed directly (with no lyophilization) by analytical HPLC-MS using a Hypersil GOLD column (5 μL, 150 x 4.6 mm) at a flow rate of 1 mL/min with 20–80% MeCN/H_2O gradient for 60 min.

m/z ES calc'd for Allocam IAPP (**23**) $[(C_{80}H_{121}N_{13}O_{20}S_2)+1]^+$: 1648.83; observed: **1648.70**; m/z ES calc'd for $[(C_{70}H_{105}N_{11}O_{16}S_2 + 2)/2]^+$: 824.92; observed: **835.90**.



General Procedure for Allocam Cleavage/Oxidation for IAPP$_{1\text{-}9}$ (Table 1). IAPP$_{1\text{-}9}$ on the stated resin (10 mg, 2.0 μmol) was swelled in DMF (800 μL) on the Biotage Isolera+ synthesizer. After removing excess DMF, the cartridge was removed from the synthesizer and capped on the bottom. A solution of the stated catalyst in 5% AcOH/DMSO solution (250 μL) was added, and then the stated amount of $PhSiH_3$ (if relevant) and NMM were added. The cartridge was capped on the top, attached to a magnetic stirbar retriever using a rubber band (see image), and agitated for the stated amount of time under an atmosphere of ambient air. The reaction was filtered then transferred back to the synthesizer, where it was washed successively: 1) 750 μL of DMF was added and vortexed 2 min, then filtered (3 total times), 2) 750 μL of CH_2Cl_2 was added and vortexed 2 min, then filtered (3 total times), 3) 800 μL of 0.2 M sodium diethyldithiocarbamate in DMF was added and vortexed 15 min, then filtered (3 total times). *Note: resin changes from dark orange/brownish to yellowish/tan. 4) 750 μL of DMF was added and vortexed 2 min, then filtered (5 total times), 5) 750 μL of CH_2Cl_2 was added and vortexed 2 min. The resin was then subjected to a cleavage mixture of TFE:AcOH:DCM (1:1:3, 300 μ℘) for 3 x 15 min (not on synthesizer). The filtrates were collected and concentrated with a stream of nitrogen gas. The concentrated solution was analyzed directly (with no lyophilization) by analytical HPLC-MS using a Hypersil GOLD column (5 μL, 150 x 4.6 mm) at a flow rate of 1 mL/min with 20–80% MeCN/H_2O gradient for 60 min. The possible expected masses are:



m/z ES calc'd for Allocam IAPP (**23**) $[(C_{80}H_{121}N_{13}O_{20}S_2)+1]^+$: 1648.83; observed: **1648.70**; m/z ES calc'd for $[(C_{70}H_{105}N_{11}O_{16}S_2 + 2)/2]^+$: 824.92; observed: **835.90**.

m/z ES calc'd for IAPP Disulfide (**24**) $[(C_{70}H_{105}N_{11}O_{16}S_2)+1]^+$: 1420.72; observed: **1421.05**; *m/z* ES calc'd for $[(C_{70}H_{105}N_{11}O_{16}S_2 + 2)/2]^+$: 710.86; observed: **711.100**.

m/z ES calc'd for 1-Allocam (**SI-2**) $[(C_{70}H_{105}N_{11}O_{16}S_2)+1]^+$: 1535.78; observed: **1535.00**; *m/z* ES calc'd for $[(C_{70}H_{105}N_{11}O_{16}S_2 + 2)/2]^+$: 768.39; observed: **767.85**.

m/z ES calc'd for free thiols (**SI-3**) $[(C_{70}H_{105}N_{11}O_{16}S_2)+1]^+$: 1422.73; observed: **1422.25**; *m/z* ES calc'd for $[(C_{70}H_{105}N_{11}O_{16}S_2 + 2)/2]^+$: 711.86; observed: **712.10**.

m/z ES calc'd for 1 allyl (**SI-4**) $[(C_{70}H_{105}N_{11}O_{16}S_2)+1]^+$: 1462.77; observed: **1463.15**; *m/z* ES calc'd for $[(C_{70}H_{105}N_{11}O_{16}S_2 + 2)/2]^+$: 731.88; observed: **732.20**.

m/z ES calc'd for 2 allyls (**SI-5**) $[(C_{70}H_{105}N_{11}O_{16}S_2)+1]^+$: 1502.80; observed: **1502.25**; *m/z* ES calc'd for $[(C_{70}H_{105}N_{11}O_{16}S_2 + 2)/2]^+$: 751.90; observed: **752.15**.

Table 1, entry 1. The general procedure was followed. The relevant quantities are: $Pd(PPh_3)_4$ (11.6 mg, 10 μmol), NMM (6.3 μL, 58 μmol). The reaction mixture was agitated in a closed vial at room temperature for 2 hours.

Table 1, entry 2. The general procedure was followed. The relevant quantities are: $Pd(PPh_3)_4$ (11.6 mg, 10 μmol), NMM (25 μL, 227 μmol). The reaction mixture was agitated in a closed vial at room temperature for 2 hours.

Table 1, entry 3. The general procedure was followed. The relevant quantities are: $Pd(PPh_3)_4$ (16.2 mg, 14 μmol), NMM (25 μL, 227 μmol). The reaction mixture was agitated in a closed vial at room temperature for 2 hours.

Table 1, entry 4. The general procedure was followed. The relevant quantities are: $Pd(dppf)Cl_2$ (11.4 mg, 14 μmol), NMM (25 μL, 227 μmol). The reaction mixture was agitated in a closed vial at room temperature for 2 hours.

Table 1, entry 5. The general procedure was followed. The relevant quantities are: $Pd(PPh_3)_4$ (11.4 mg, 14 μmol), $PhSiH_3$ (5 μL, 40 μmol), NMM (25 μL, 227 μmol). The reaction mixture was agitated in a closed vial at room temperature for 2 hours.

Table 1, entry 6. The general procedure was followed. The relevant quantities are: $Pd(OAc)_2$ (3.1 mg, 14 μmol), NMM (25 μL, 227 μmol). The reaction mixture was agitated in a closed vial at room temperature for 2 hours.

Table 1, entry 7. The general procedure was followed. The relevant quantities are: $Pd(OAc)_2$ (2.2 mg, 10 μmol), NMM (25 μL, 227 μmol). The reaction mixture was agitated in a closed vial at room temperature for 4 hours.

Table 1, entry 8. The general procedure was followed. The relevant quantities are: $Pd(OAc)_2$ (0.45 mg, 2 μmol), NMM (25 μL, 227 μmol). The reaction mixture was agitated in a closed vial at room temperature for 4 hours.

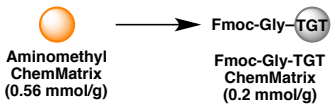
Table 1, entry 9. The general procedure was followed. The relevant quantities are: $Pd(OAc)_2$ (0.67 mg, 3 μmol), NMM (25 μL, 227 μmol). The reaction mixture was agitated in a closed vial at room temperature for 4 hours.

Table 1, entry 10. The general procedure was followed. The relevant quantities are: $Pd(OAc)_2$ (0.67 mg, 3 μmol), NMM (7.5 μL, 68.4 μmol). The reaction mixture was agitated in a closed vial at room temperature for 4 hours.

Table 1, entry 11. The general procedure was followed. The relevant quantities are: $Pd(OAc)_2$ (0.34 mg, 1.5 μmol), NMM (7.5 μL, 68.4 μmol). The reaction mixture was agitated in a closed vial at room temperature for 4 hours.

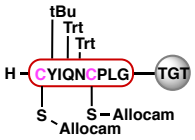
Table 1, entry 12. The general procedure was followed. The relevant quantities are: $Pd(OAc)_2$ (0.45 mg, 2 μmol), NMM (7.5 μL, 68.4 μmol). The reaction mixture was agitated in a closed vial at room temperature for 4 hours.

Table 1, entry 13. The general procedure was followed. The relevant quantities are: $Pd(OAc)_2$ (0.67 mg, 3 μmol), NMM (7.5 μL, 68.4 μmol). The reaction mixture was agitated in a closed vial at room temperature for 2 hours.



TGT-ChemMatrix Resin. The coupling of 4-(diphenylhydroxy methyl)benzoic acid on to the Aminomethyl ChemMatrix resin was done using the semi-automated peptide synthesizer, in a 10-mL reaction vial. The general procedure was followed. The relevant quantities are: Aminomethyl ChemMatrix resin (200 mg, 0.112 mmol), 4-(diphenylhydroxy methyl)benzoic acid (170.4 mg, 0.56 mmol), HATU (212.9 mg, 0.56 mmol), and DIPEA (195 μl, 1.12 mmol). After the coupling, the reaction vial with the resin was taken out from the synthesizer and 25% acetic anhydride in DMF (3 mL) was added. It was agitated for 5 min and then DIPEA (30 μl, 0.168 mmol) was added and agitated for another 30 min. After the reaction, the solution was removed and the resin was rinsed with DMF 4 times. Next, 1M NaOH (3 mL) was added to the resin and agitated for 12 h. At the end of the reaction, the solution was removed followed by 4 DMF washes. To the resulting resin in a close-sealed vial, 2% $SOCl_2$ in DCM (3 mL) was added and agitated for 12 h. Finally, the solution was removed and the resin was rinsed with DCM 5 times and 2% DIPEA in DCM 3 times.

Fmoc-Gly-TGT-ChemMatrix. The resin was swelled in DMF and a solution of the desired Fmoc-Gly-OH (5 equiv) with DIPEA (6 equiv) in DMF was added. Then the resulting reaction vial was agitated at RT for 12 h. After the reaction, the solution was drained out and the resin was rinsed with DMF, DCM and MeOH (3 times each). Finally, the resin was dried in the lyophilizer for 12 h.



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Protected Oxytocin-TGT-ChemMatrix (25). The synthesis was done using a semi-automated peptide synthesizer, in a 10-mL reaction vial. First, the resin (**Fmoc-Gly-TGT-ChemMatrix**) was swelled followed by deprotection. Then, each amino acid was added accordingly followed by coupling and deprotection steps. Both Fmoc-Pro-OH and Fmoc-Cys(Allocam)-OH were double-coupled in this synthesis. Finally, the peptide-bound resin was washed with DCM and methanol. The conditions used for each step are given below (R1 = round 1, R2 = round 2):

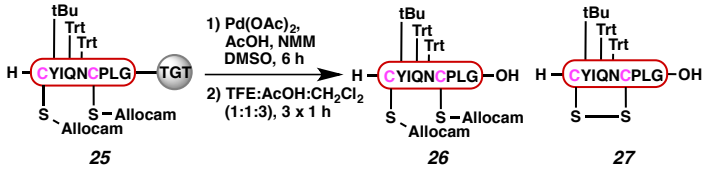
	Swelling	Deprotection	Coupling	Wash (4x)	Final washes (3x each wash)		
Reaction time / min	20	3.00(R1) + 10.00(R2)	5	1	1	0.75	0.75
Temperature/ ºC	70	RT	75	RT	RT	RT	RT
Vortex (rpm)	1200	1200	1200	1200	1200	600	600
Reagent used	DMF	20% Piperidine in DMF	AA+HATU+DMF	DMF	DCM	MeOH	DCM
Volume of the reagent/ mL	4	3.00(R1) + 3.00(R2)	3	3	3	6	6

**For Cys(Allocam) coupling, Temp = 50 ºC

MS Analysis of Protected Oxytocin-TGT-ChemMatrix (26): 10 mg of the final product (resin bound) was cleaved from the resin using 300 μl of the cleavage mixture (1:1:3 TFE:AcOH:DCM)

for 30 min and the filtrate was analyzed directly (with no lyophilization) by analytical HPLC-MS using a Phenomenex column (5 μL, 250 x 4.6 mm) at a flow rate of 1 mL/min with 5–95% MeCN/H_2O gradient for 30 min.

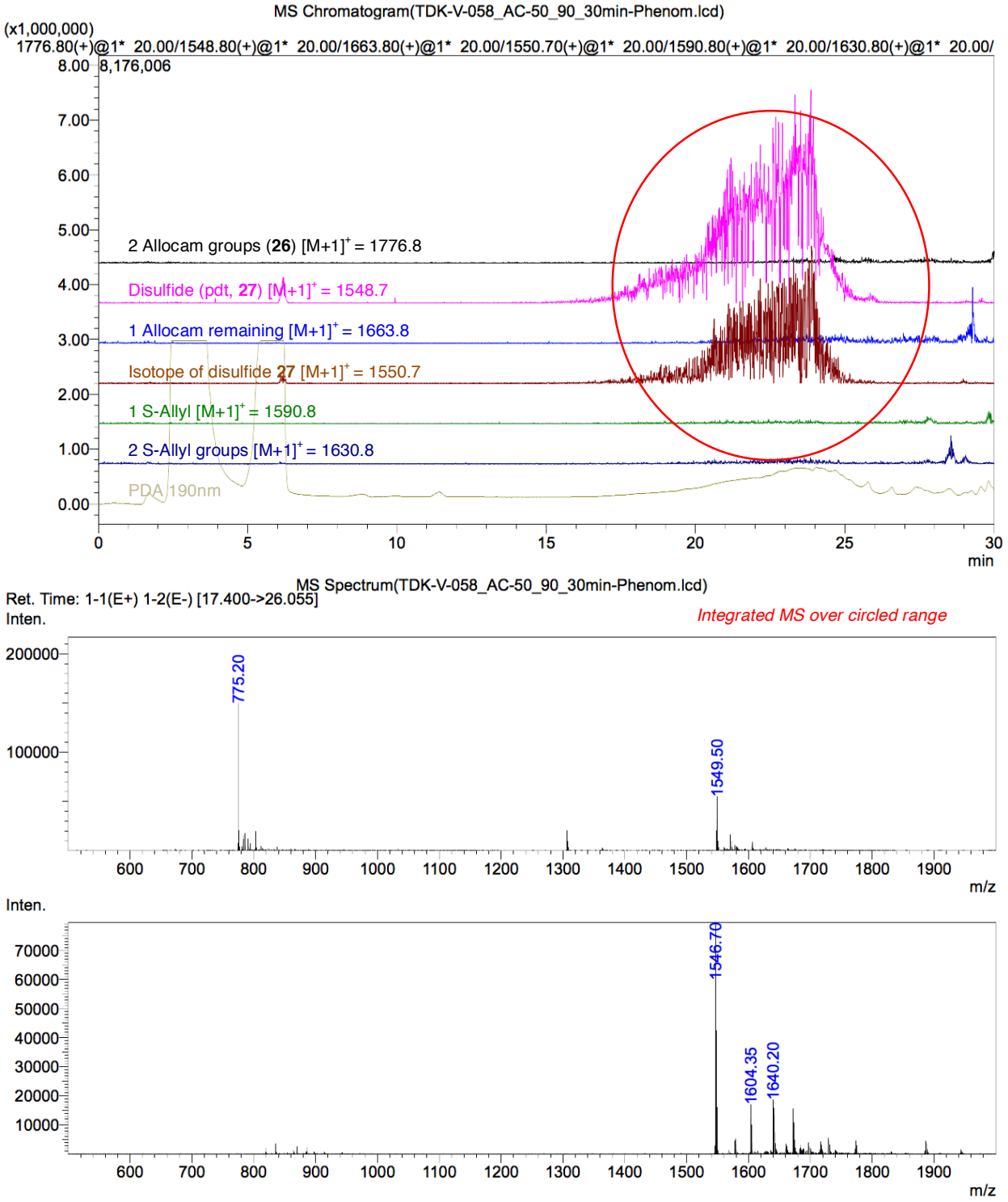
Analysis of final loading of the peptide:
Fmoc Deprotection – From the resin-bound peptide **25**, 10 mg was weighed out and swelled in DMF in the peptide synthesizer. Then, 1.00 mL of 20% piperidine in DMF was added and it was allowed to vortex at 1200 RPM in the peptide synthesizer at room temperature for 3 min. After 3 min, the filtrate was collected into a separate tube, another 1.00 mL of 20% piperidine in DMF was added to it and allowed to vortex again at 1200 RPM in the peptide synthesizer at room temperature for 10 min. The resulting filtrate was collected to the same tube and the deprotected resin was washed with DMF (4 x 1.75 mL).
Sample preparation for UV analysis – From the collected "deprotection solution" above, 100 μl was transferred in to an Eppendorf tube and it was diluted with 900 μl of DMF. The resulting solution was used to get the absorbance at 301 nm versus a DMF blank using a UV spectrometer with 1 cm cuvette. Absorbance at 301 nm: 0.509; calc'd final loading: 0.13 mmol/g.



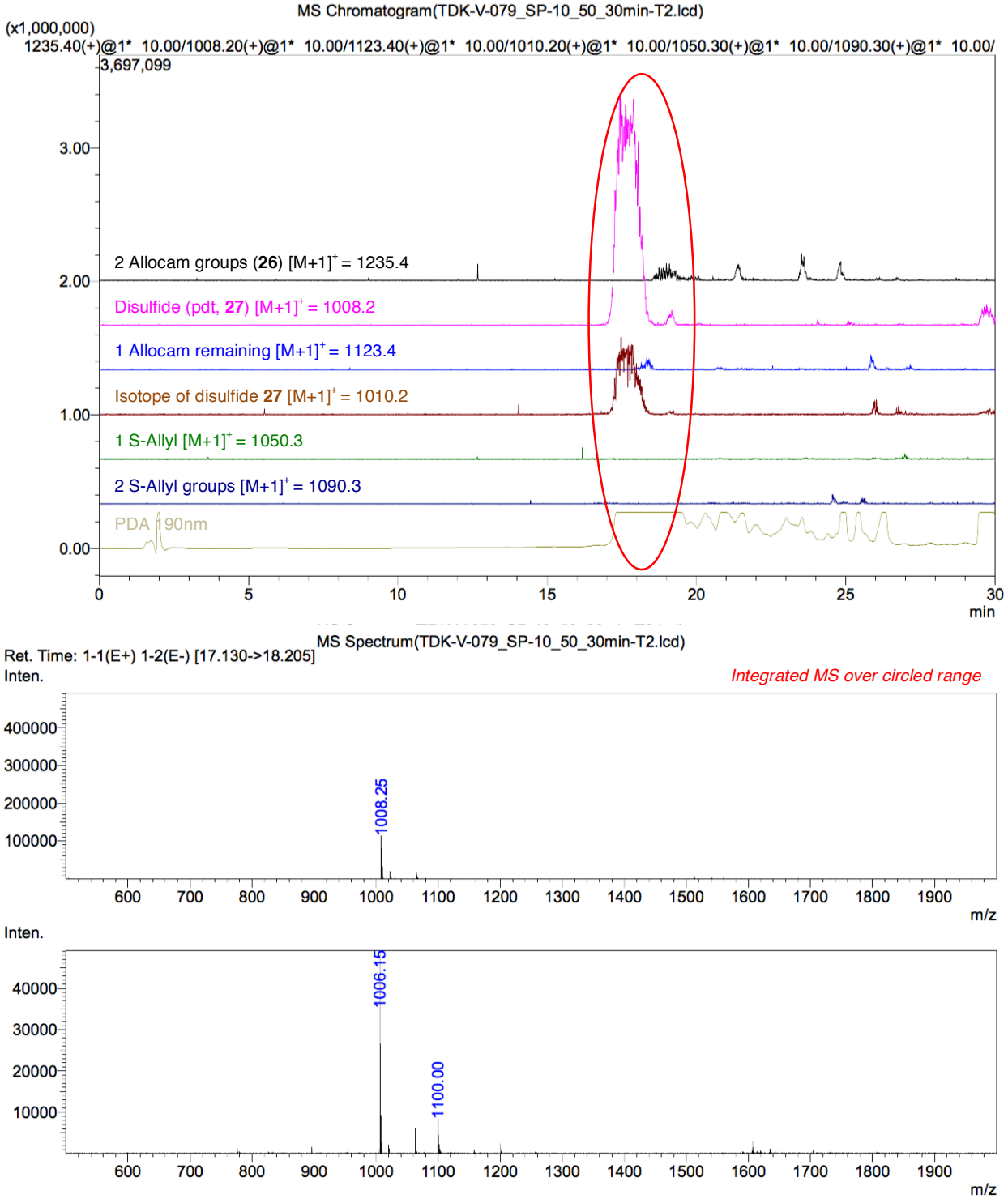
25 *26* *27*

Optimized Conditions for On-Resin Folding of Oxytocin-OH (25 → 27). The IAPP standard protocol was followed. The relevant quantities are: $Pd(OAc)_2$ (1.34 mg, 6 μmol, 3 equiv), NMM (7.5 μL, 68.4 μmol). The reaction mixture was agitated in a closed vial at room temperature for 6 hours. The resin was then subjected to a cleavage mixture of TFE:AcOH:DCM (1:1:3, 300 μl) for 3 x 1 h. The filtrates were collected and analyzed directly (with no lyophilization) by analytical HPLC-MS using a Phenomenex column (5 μL, 250 x 4.6 mm) at a flow rate of 1 mL/min with 50–90% MeCN/H_2O gradient for 30 min. The side chain protecting groups were then cleaved with 95:2.5:2.5 TFA:TIPS:water, the peptide was crashed out with cold ether, redissolved in 1:1 MeCN/H_2O, and lyophilized. The resulting white solid was purified on a Hypersil GOLD column (5 μL, 150 x 10 mm) at a flow rate 5 mL/min with 10–50% MeCN/H_2O gradient for 30 min. The purified product was analyzed by analytical HPLC-MS using a Phenomenex column (5 μL, 250 x 4.6 mm) at a flow rate of 1 mL/min with 10–50% MeCN/H_2O gradient for 30 min.

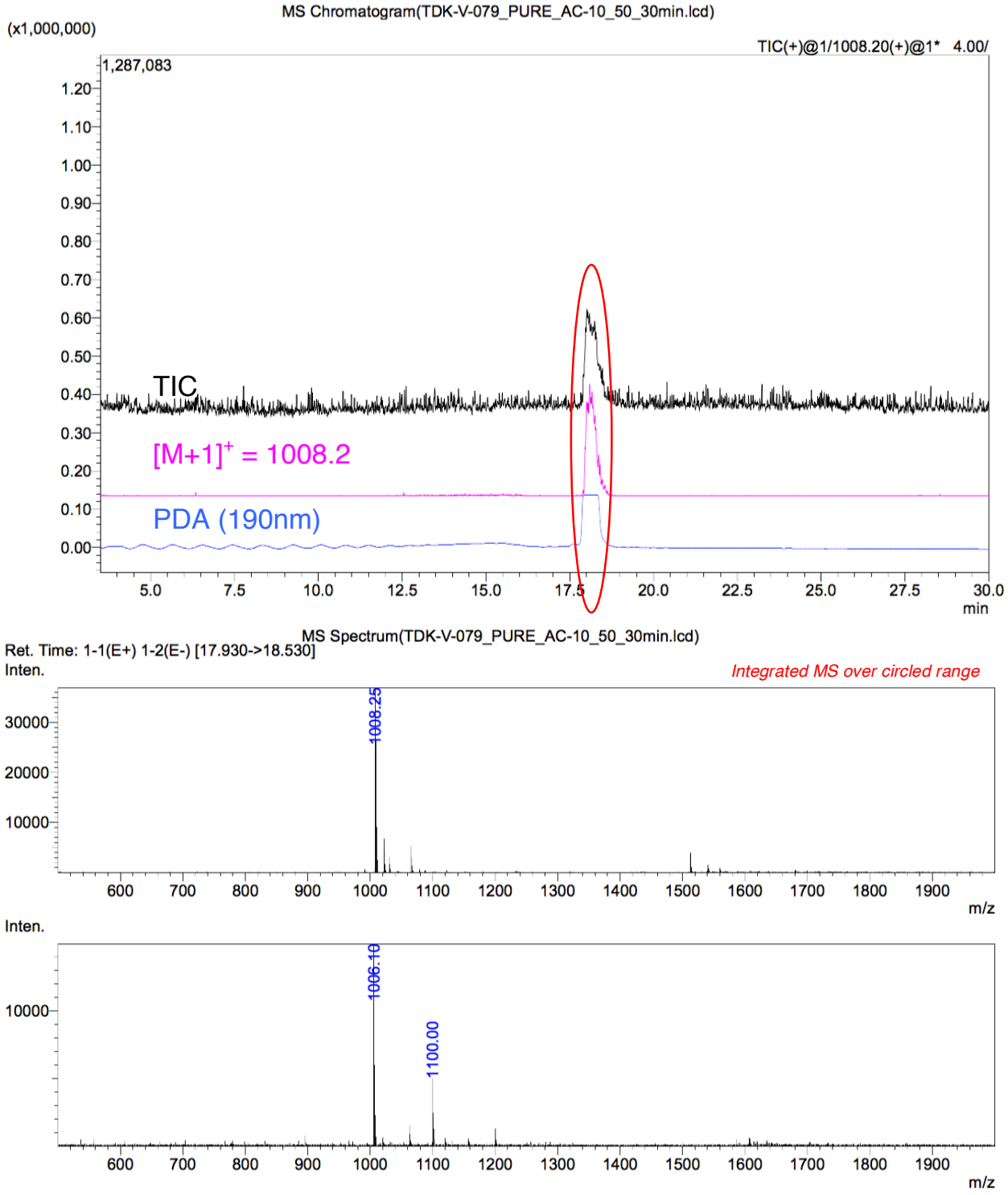
Crude LCMS data of protected oxytocin-OH (27)



Crude LCMS of deprotected oxytocin-OH (28)

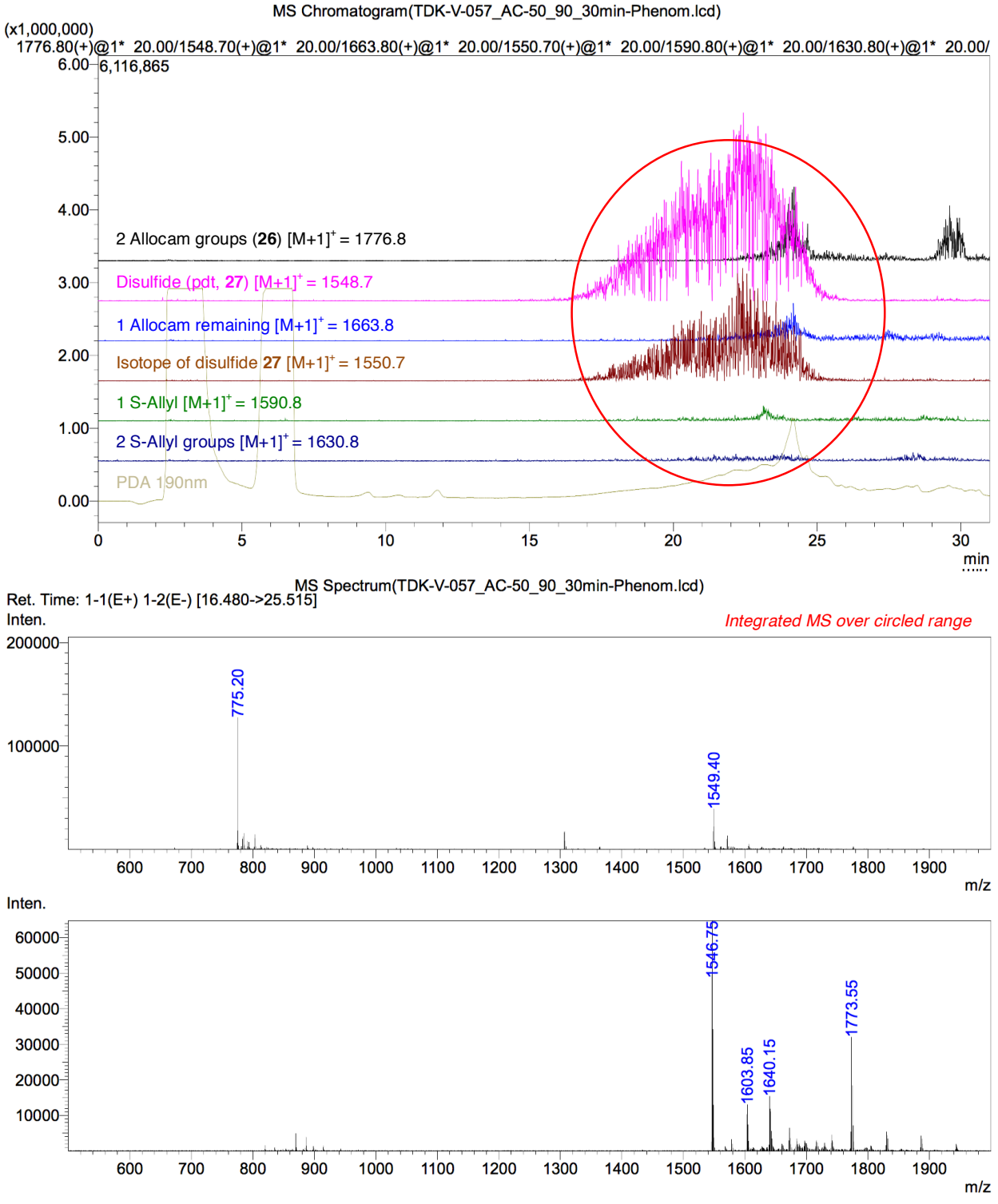


Pure LCMS of oxytocin-OH (28)

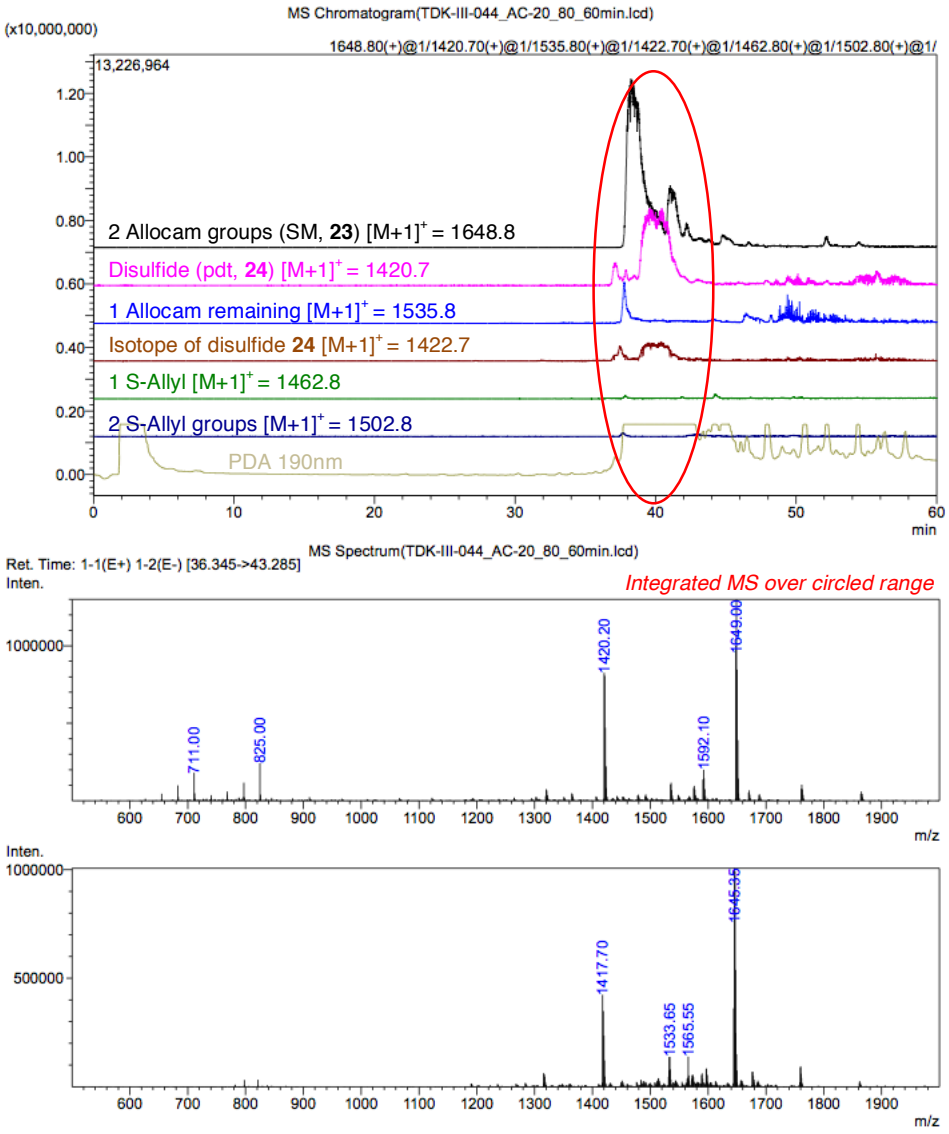


Partial Reactivity in On-Resin Folding of Oxytocin-OH (27). The IAPP standard protocol was followed. The relevant quantities are: $Pd(OAc)_2$ (0.67 mg, 3 μmol, 1.5 equiv), NMM (7.5 μL, 68.4 μmol). The reaction mixture was agitated in a closed vial at room temperature for 6 hours.

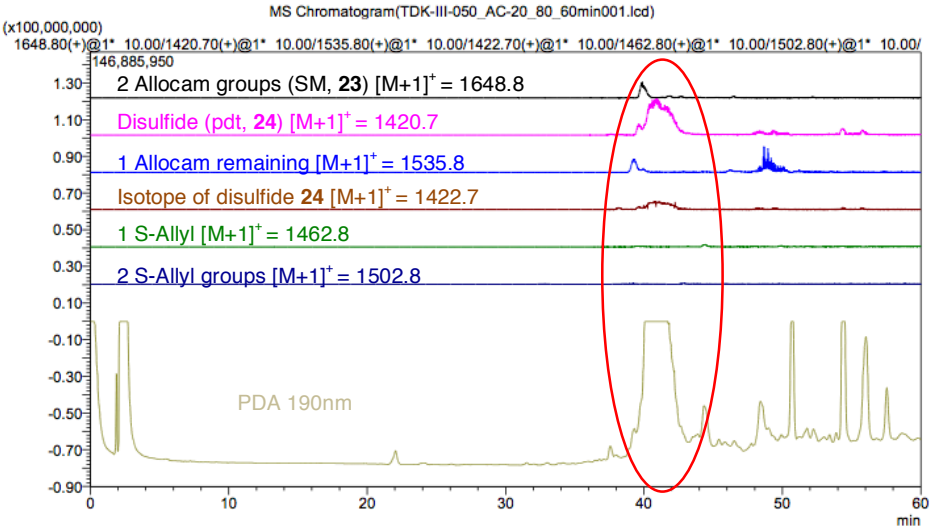
The resin was then subjected to a cleavage mixture of TFE:AcOH:DCM (1:1:3, 300 μL) for 30 min. The filtrates were collected analyzed directly (with no lyophilization) by analytical HPLC-MS using a Phenomenex column (5 μL, 250 x 4.6 mm) at a flow rate of 1 mL/min with 50–90% MeCN/H_2O gradient for 30 min. The crude LCMS trace below shows residual **26** and byproducts.

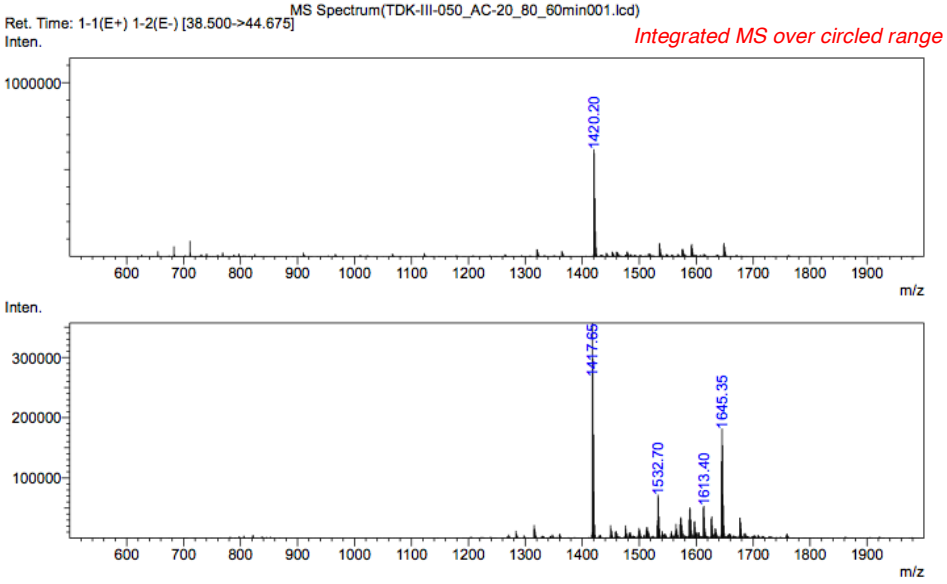


LCMS Data for Table 1 – entry 1



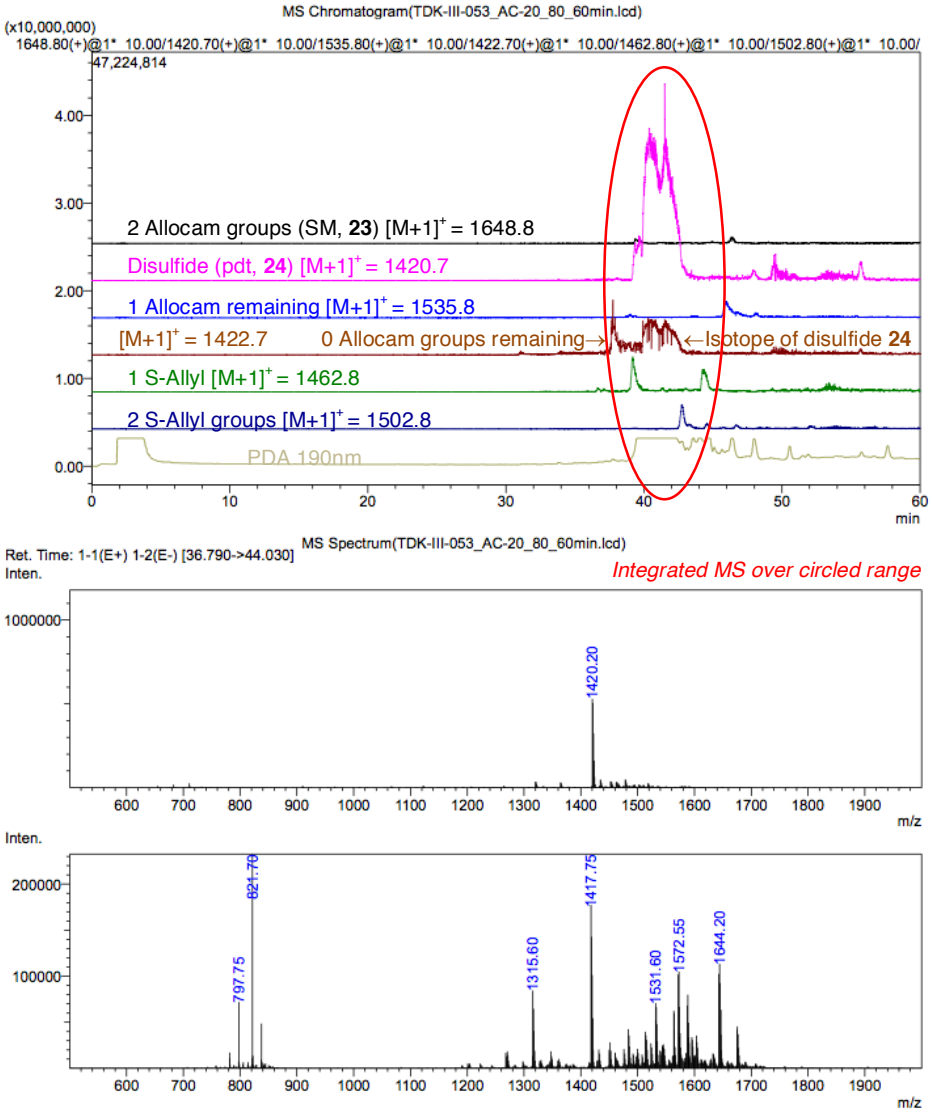
LCMS Data for Table 1 – entry 2



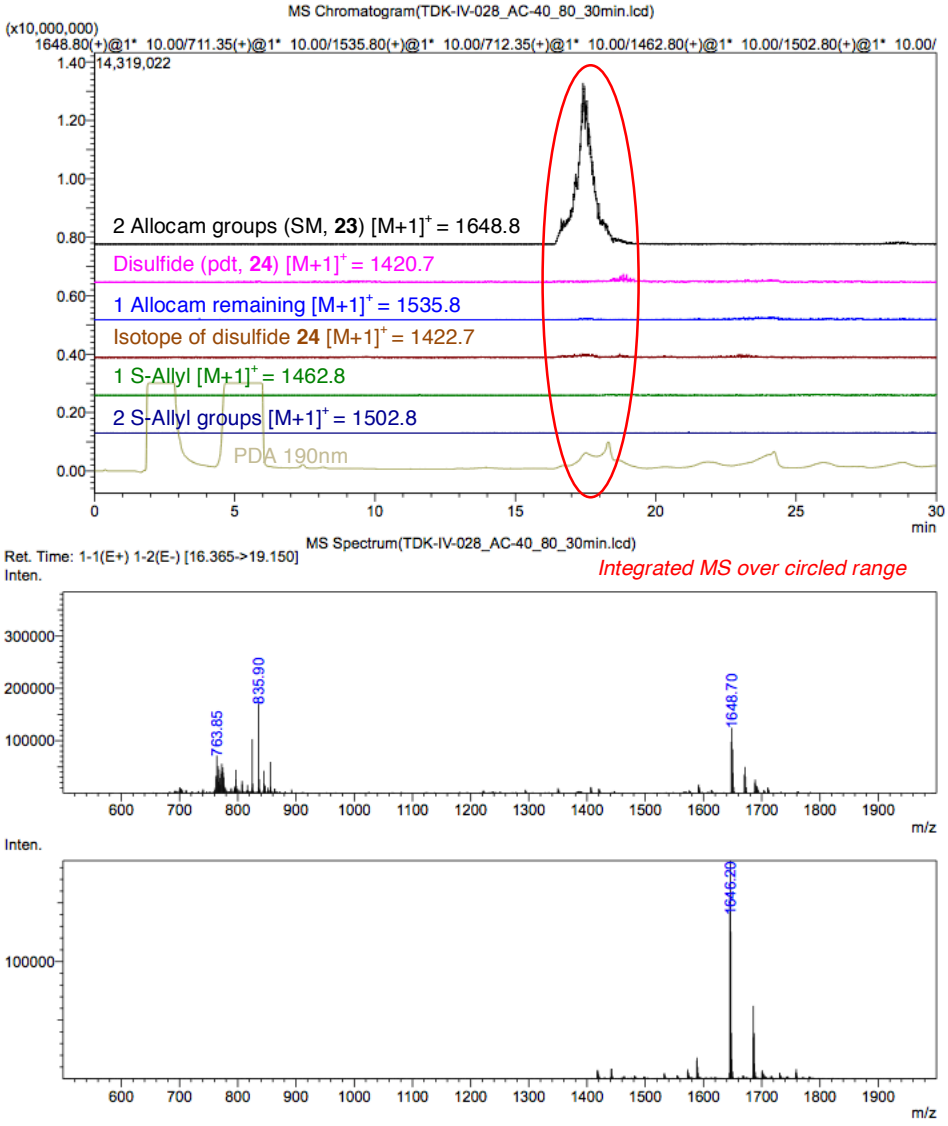


Integrated MS over circled range

LCMS Data for Table 1 – entry 3



LCMS Data for Table 1 – entry 4



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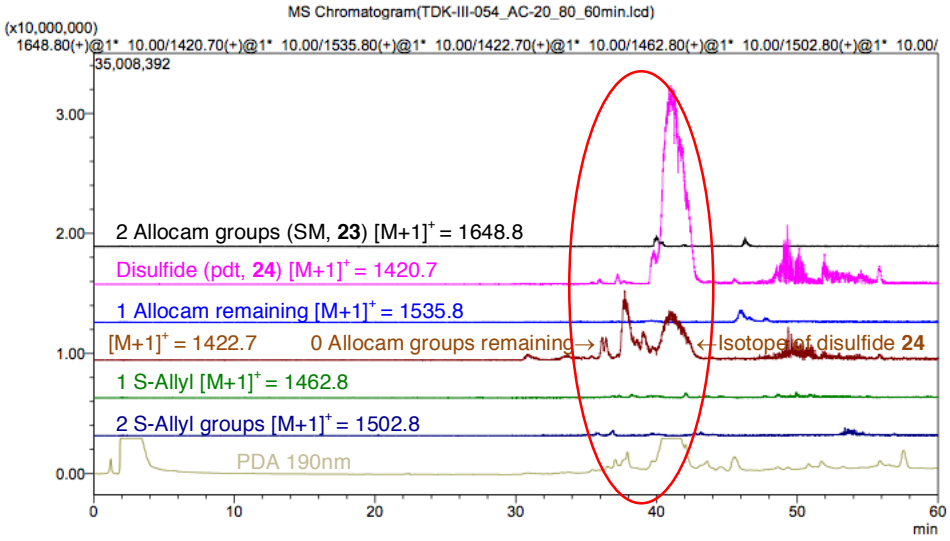

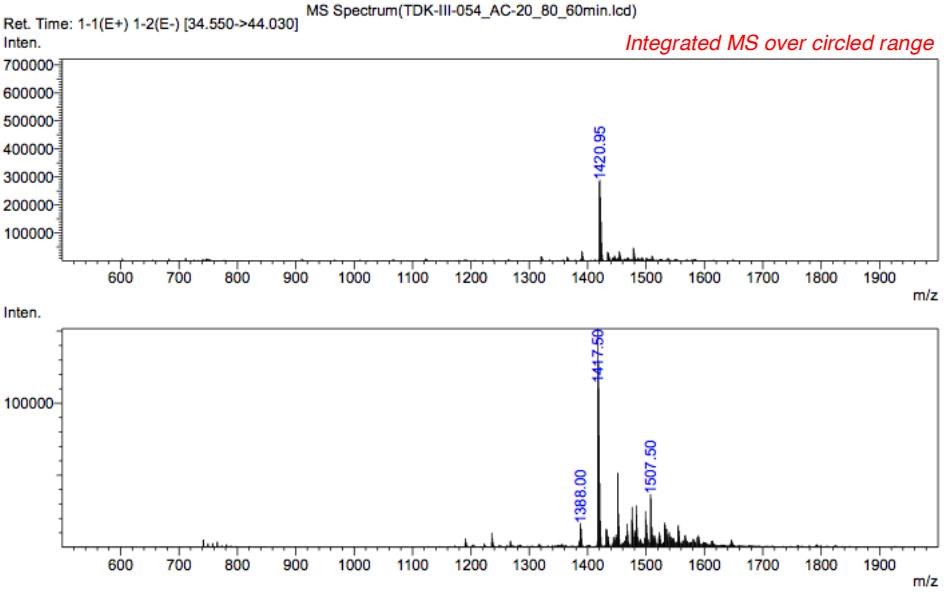

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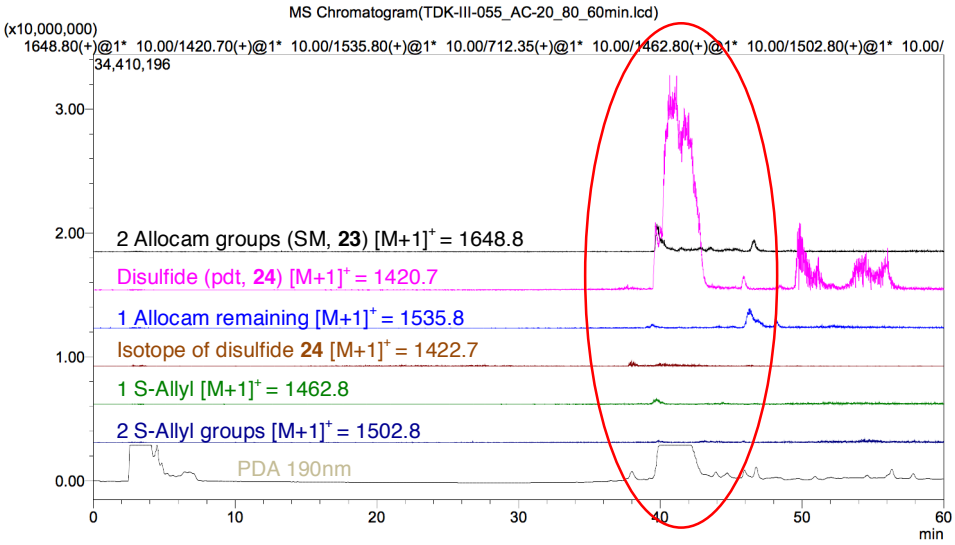
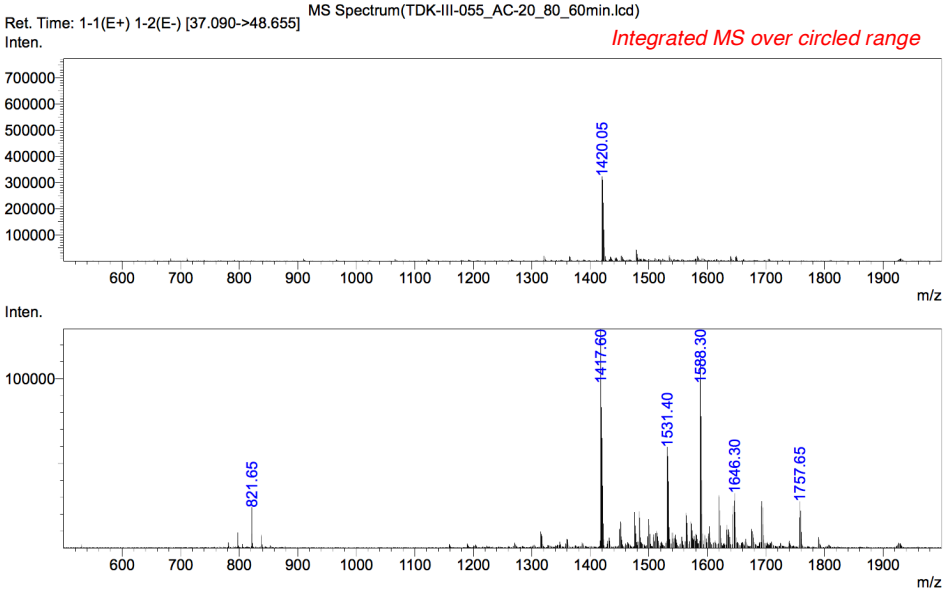
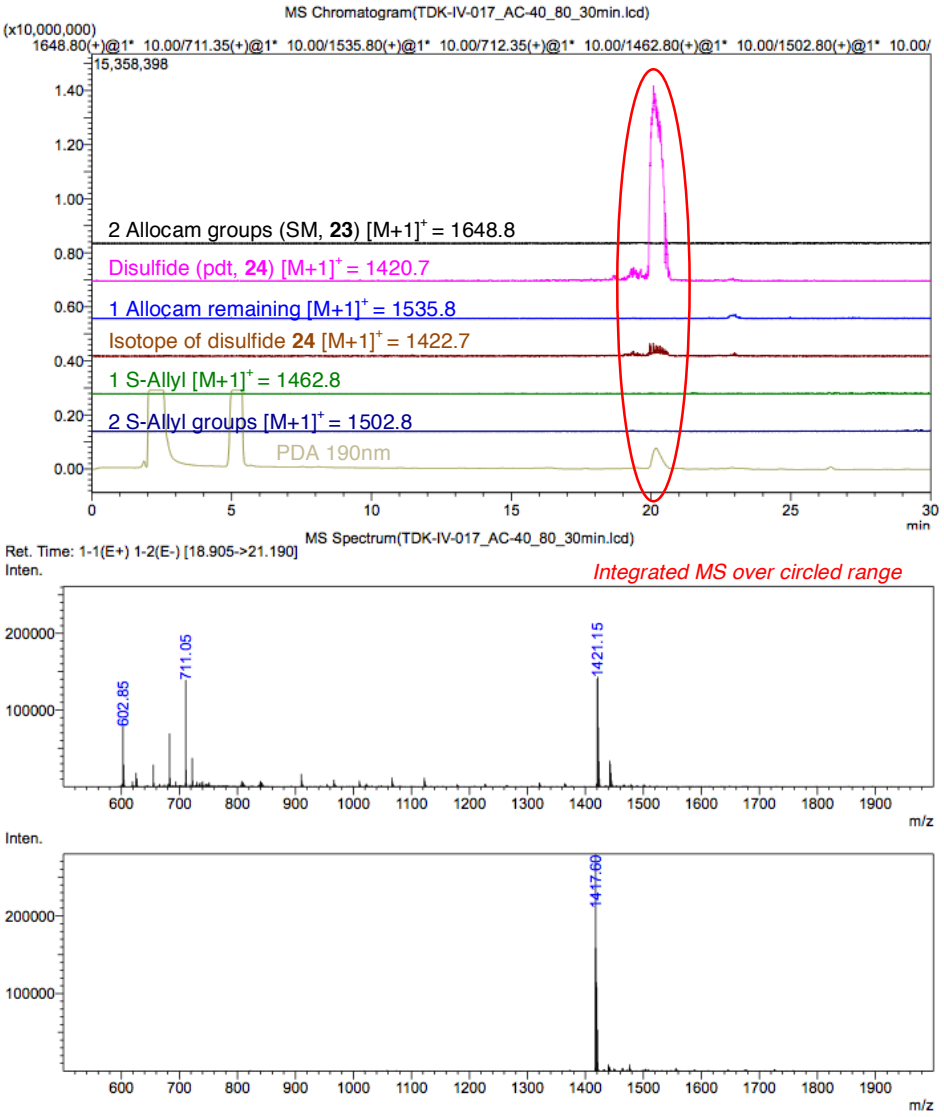
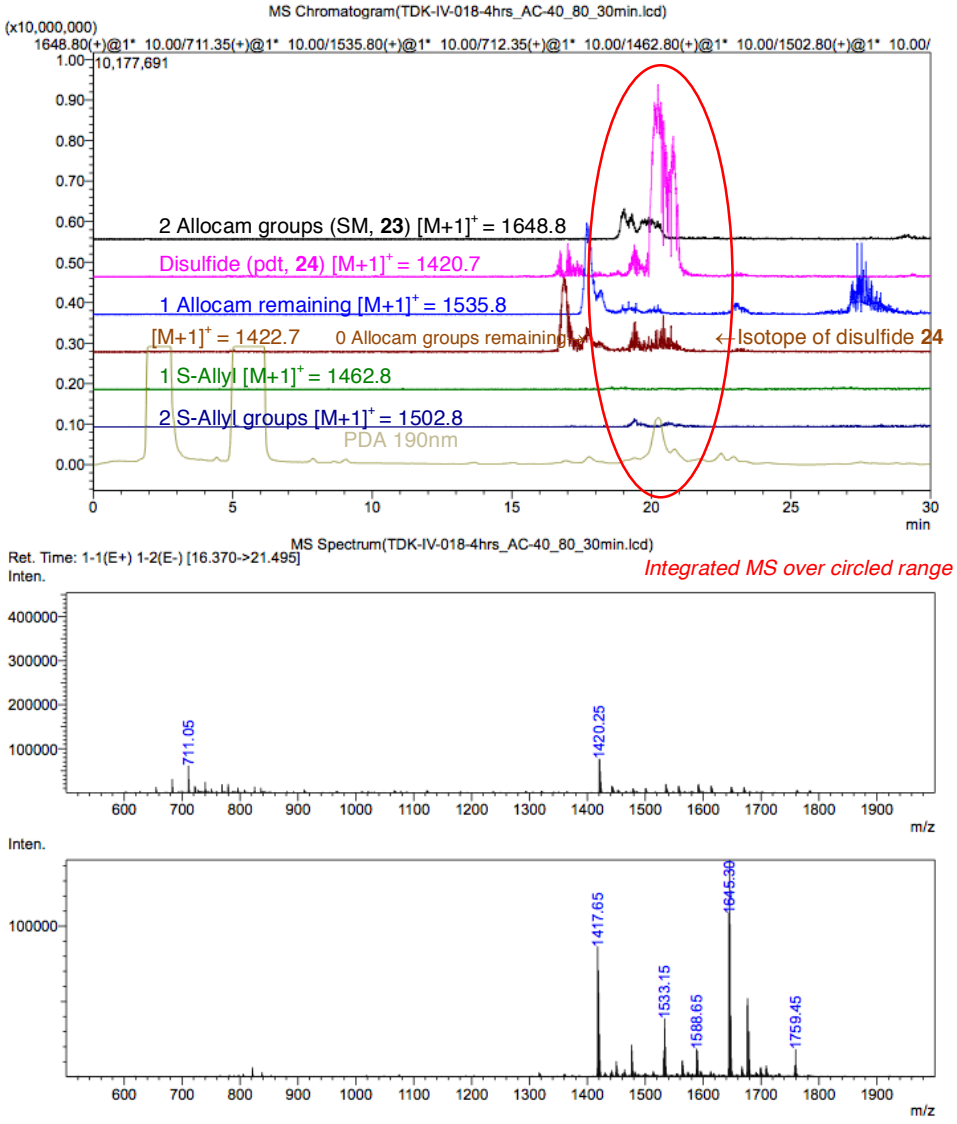




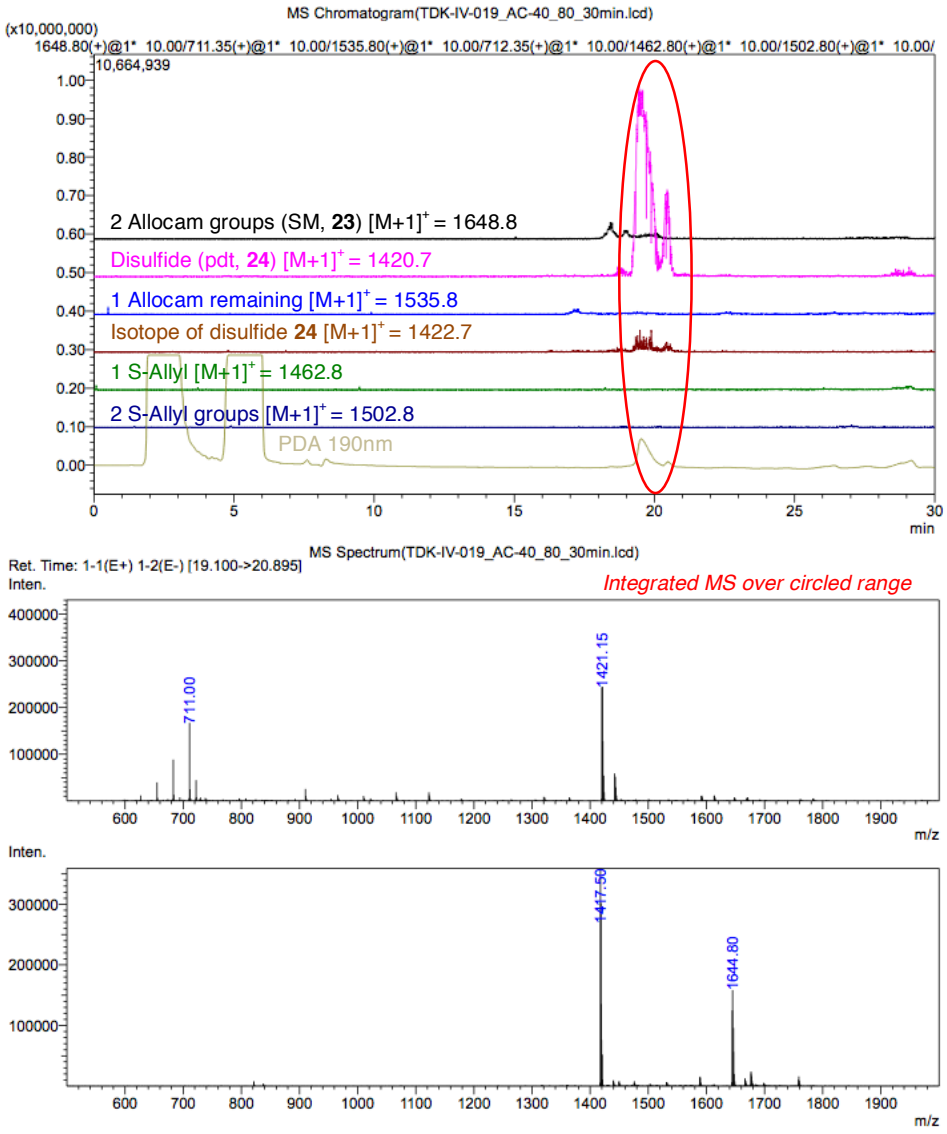
LCMS Data for Table 1 – entry 7



LCMS Data for Table 1 – entry 8



LCMS Data for Table 1 – entry 9



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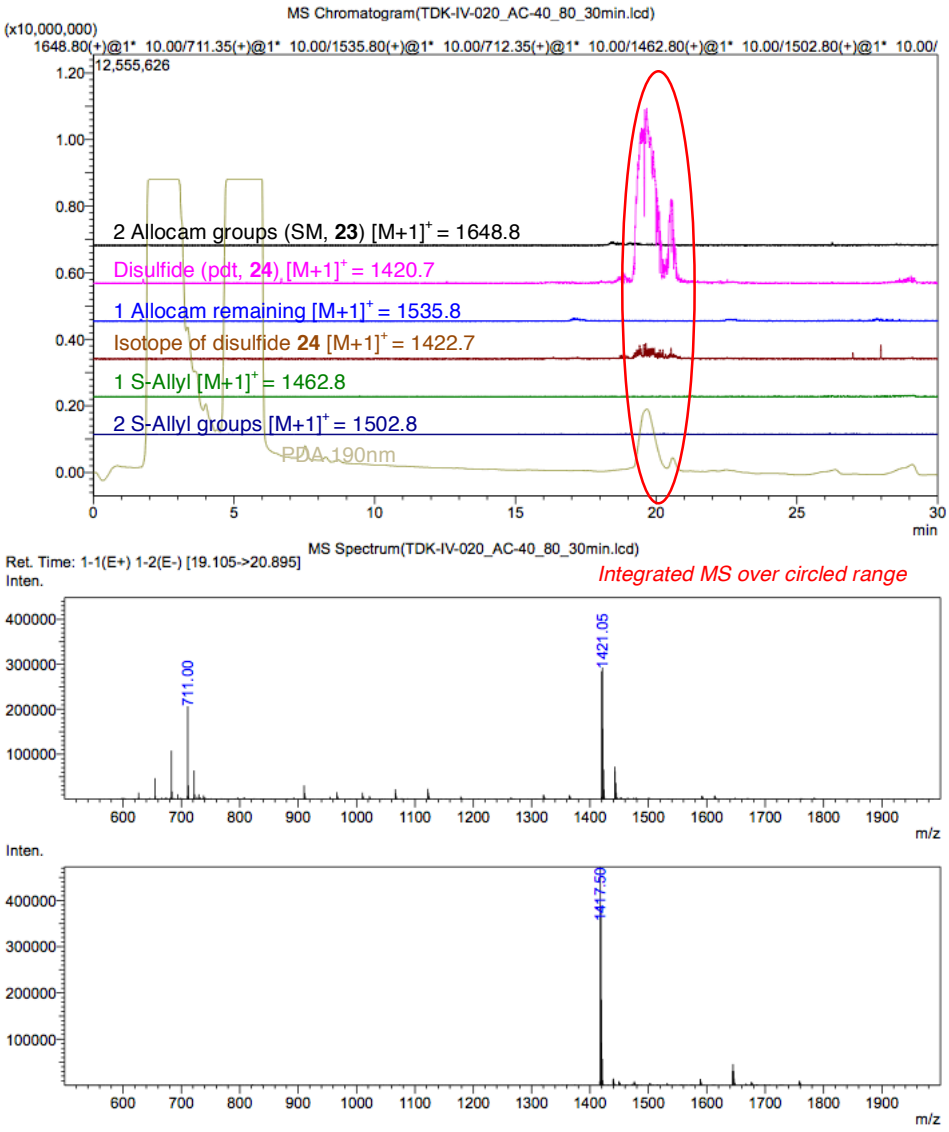
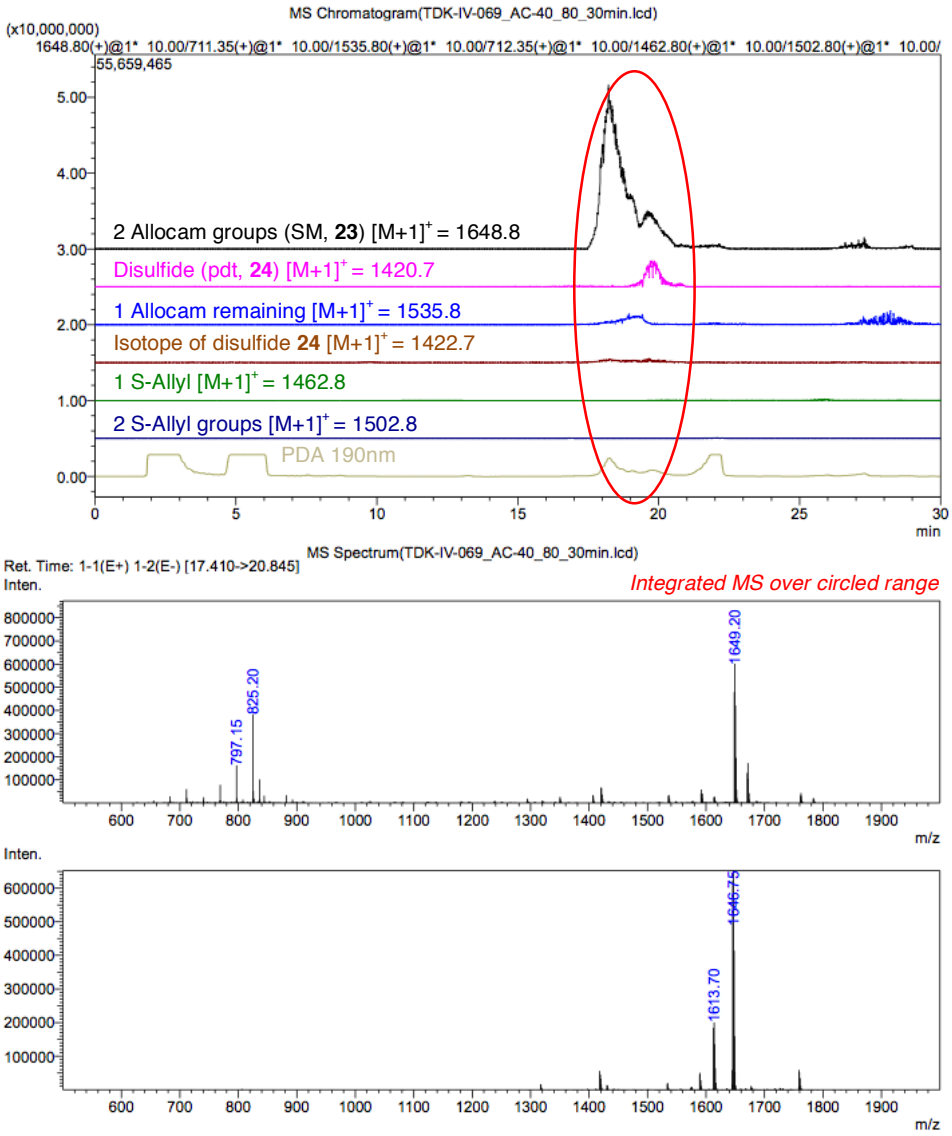
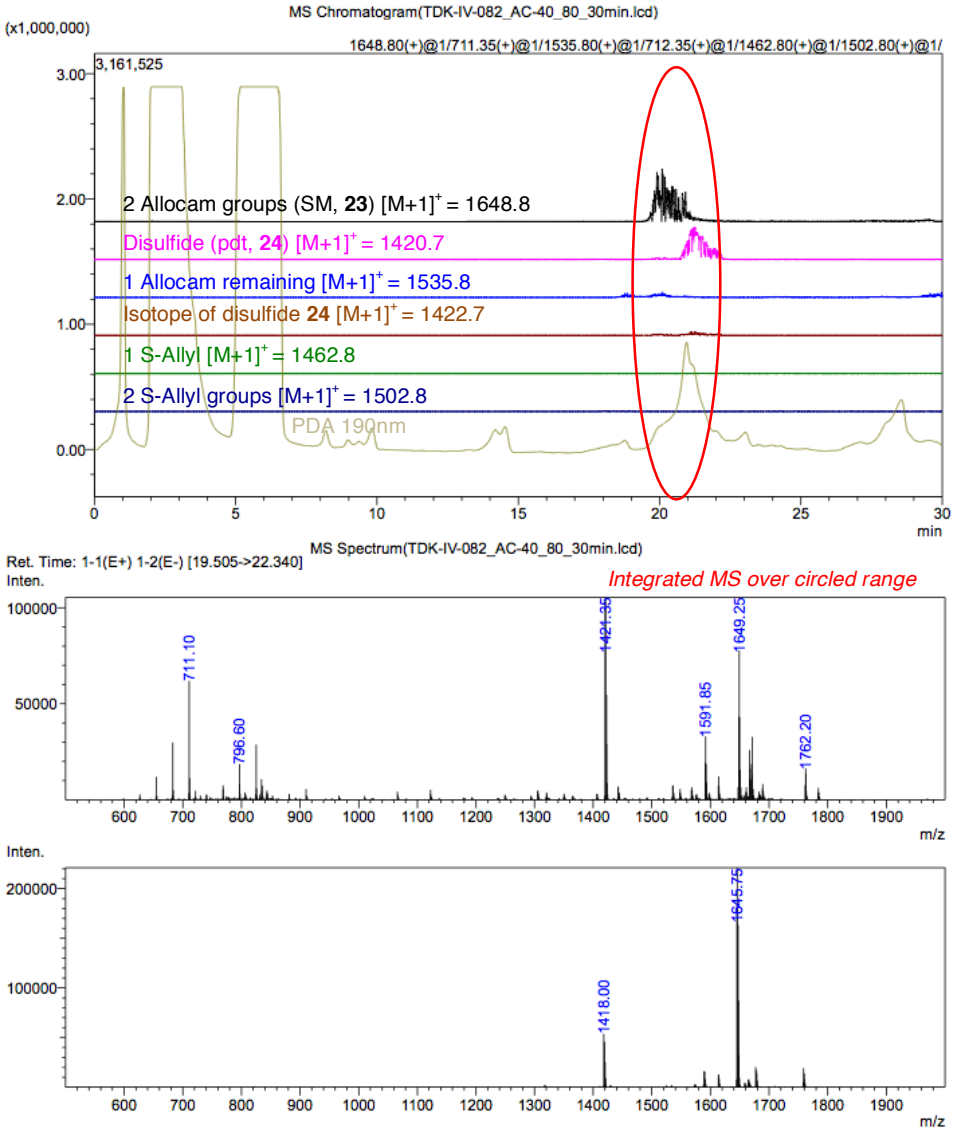


LCMS Data for Table 1 – entry 11



LCMS Data for Table 1 – entry 12



LCMS Data for Table 1 – entry 13