Disulfide-rich peptides

Optimizing and automating syntheses and regioselective formation of disulfide bonds

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31 October 2018

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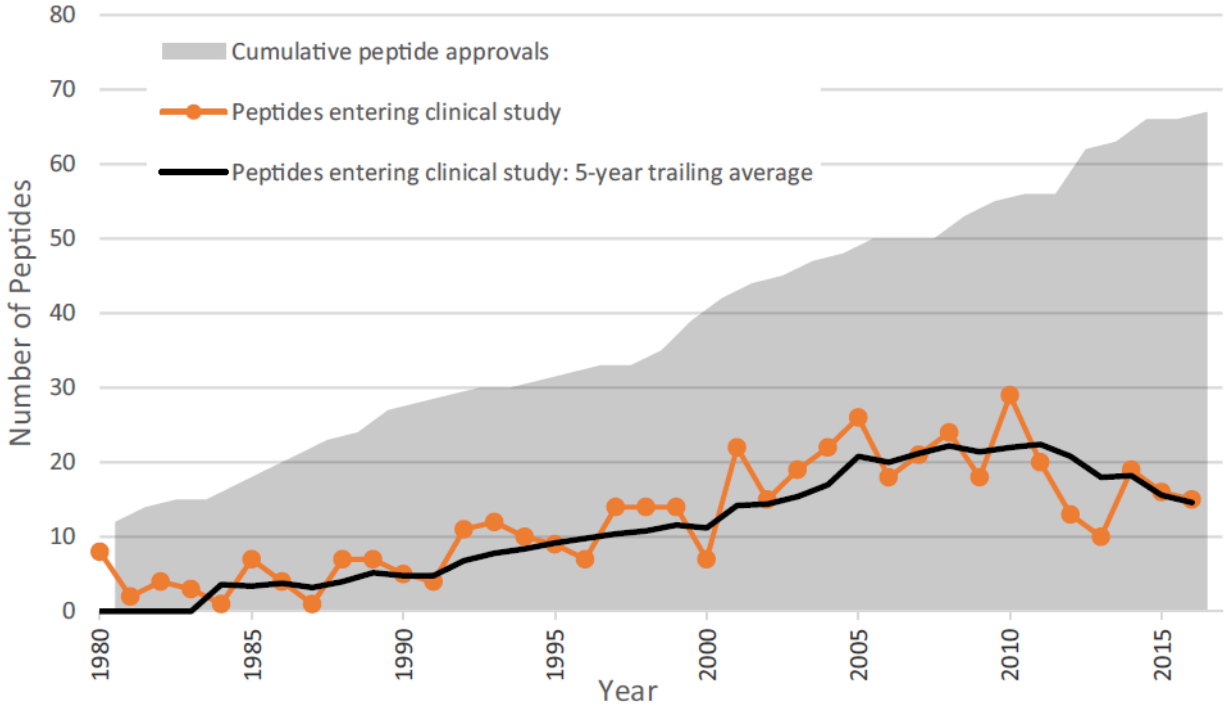
Peptide therapeutics continues to grow

Delivery and bioavailability still largest hurdles

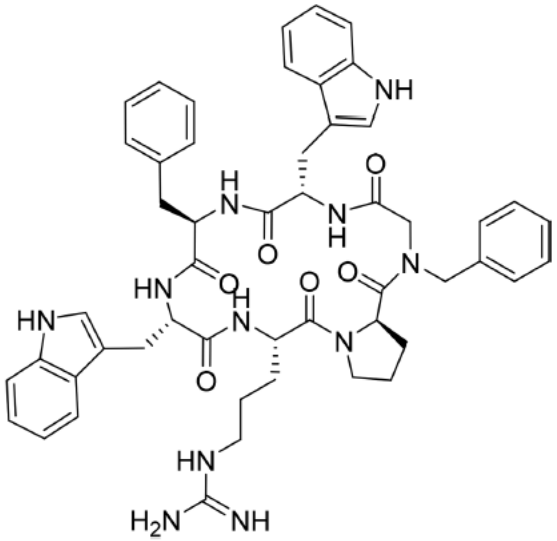
- Expecting approximately $50 bil market for peptide therapeutics by 2025
  - More than 60 approved therapeutics
  - >150 in active clinical trials

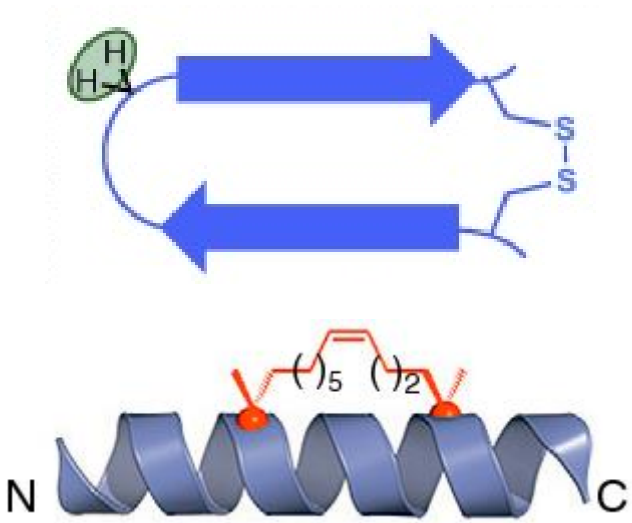


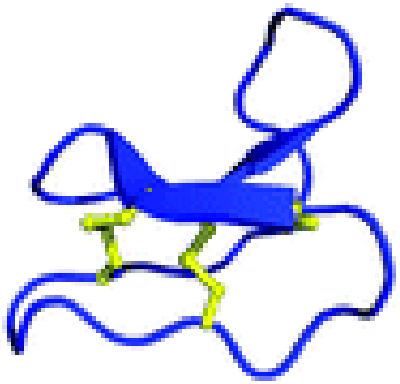
Lau, J. L. and Dunn, M. K. *BioOrg. and Med. Chem.* **2018**, *10*, 2700-2707.

Structural stabilization improves biological activity

- Head-to-tail cyclization reduces proteolytic degradation
- Secondary structure stabilization improves binding affinity
- Small macrocycles seem to be passively cell permeable
- Disulfide rich peptides present loop regions for binding

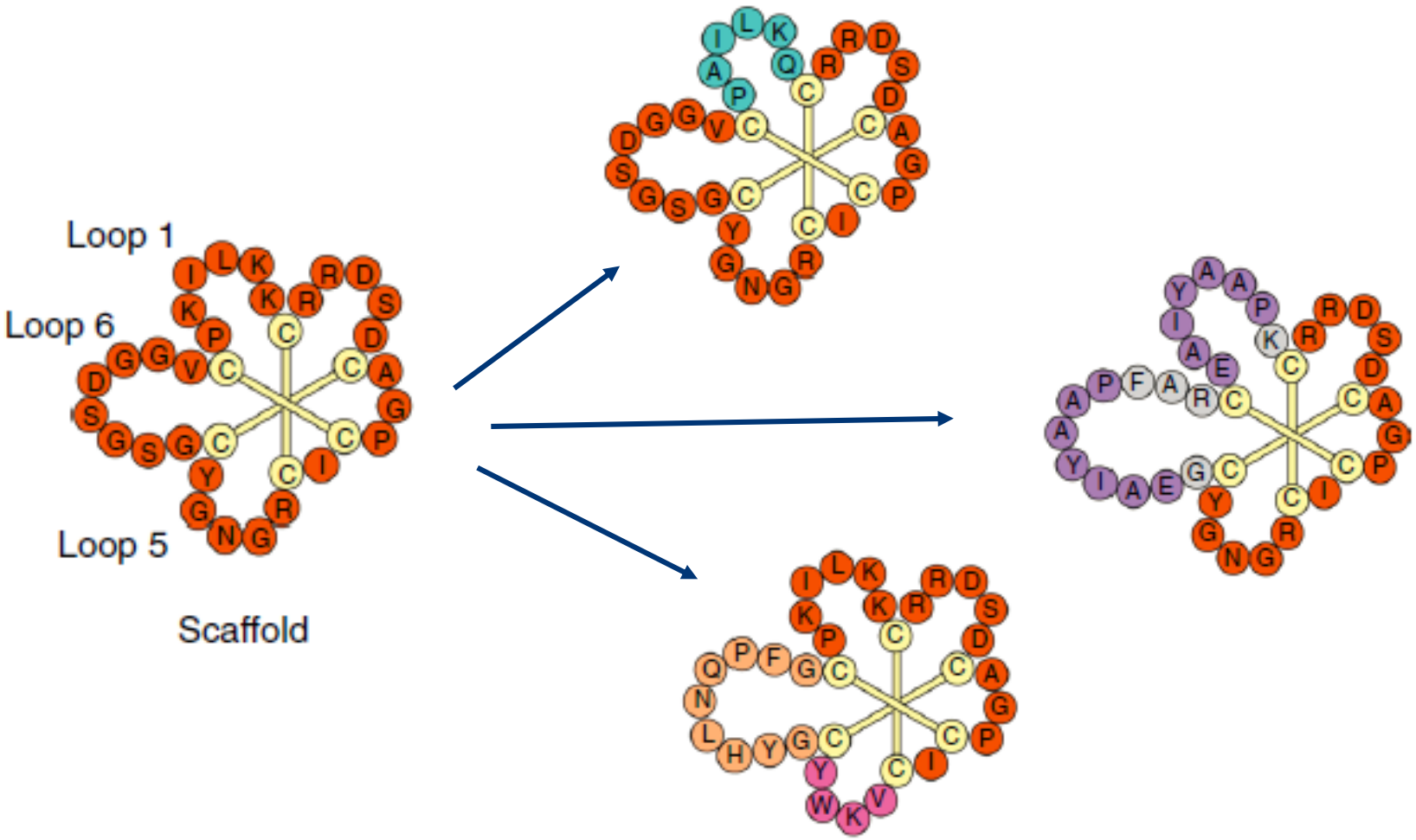






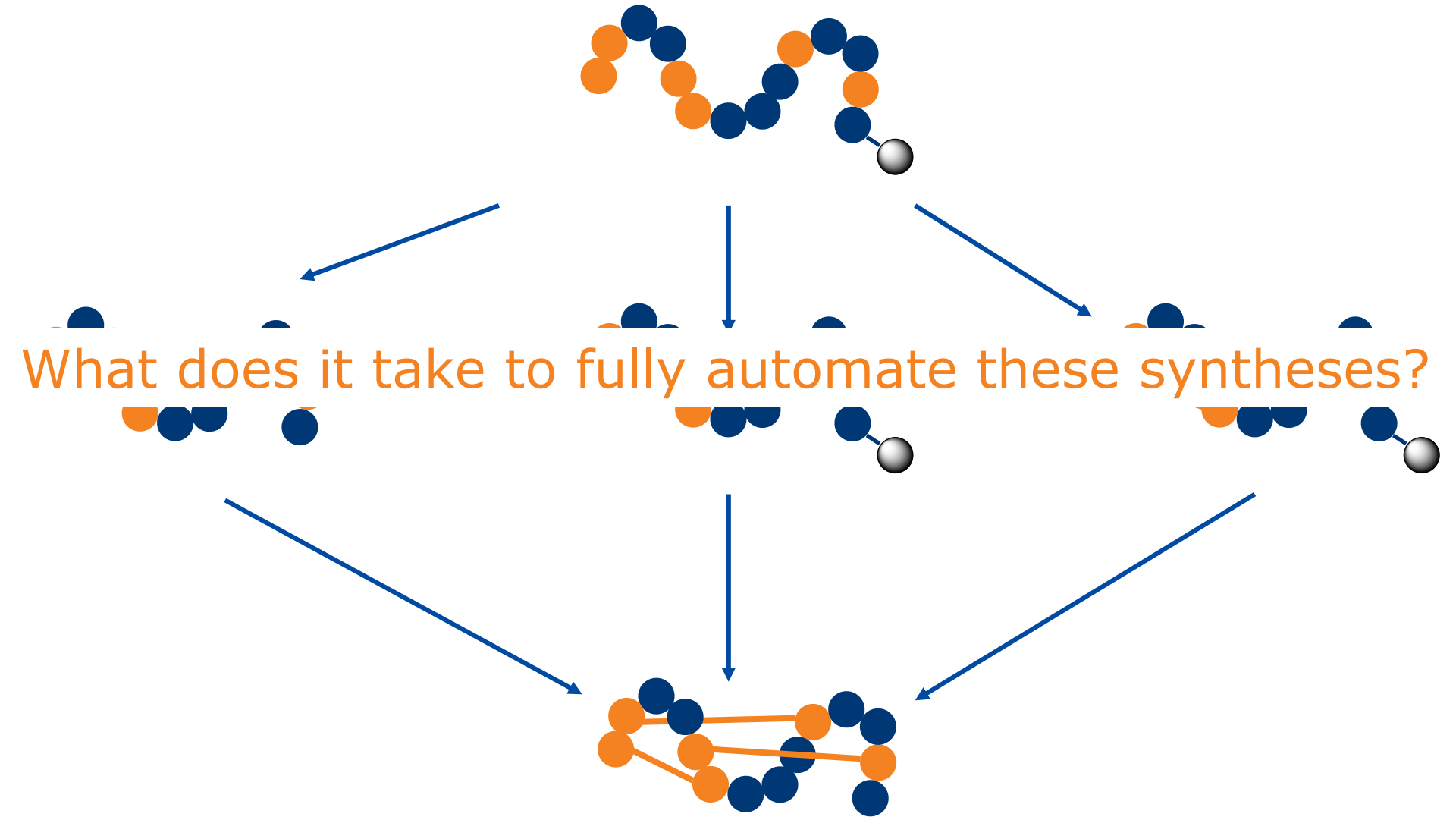
Boehm, M. et al. *J. Med. Chem* **2017**, *60*, 9653-9663.
Sarnowski, M. P. et al, *Bio. and Med. Chem.* **2018**, *26*, 1162-1166.
Verdine, G. L. and Hilinski, G. J. *Drug Discov Today Tech.* **2012**, *9*, e1-e70.
Cascales, L. and Craik, D. J. *Org. Biomol. Chem.* **2010**, *8*, 5035-5047.

Disulfide Rich peptides as structural scaffolds



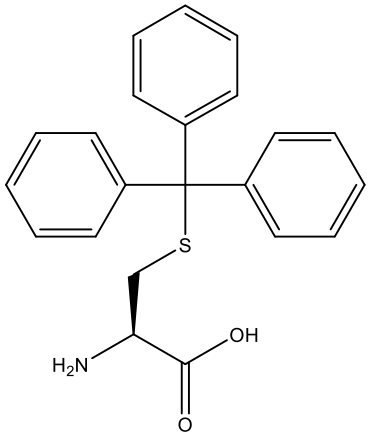
Wang, C. and Craik, D. J. *Nat. Chem. Bio.* **2018**, *14* 417-427.

A Range of Strategies for Folding



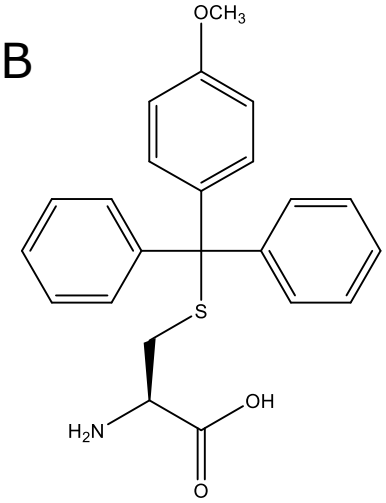
Suite of orthogonally protected Cys

A



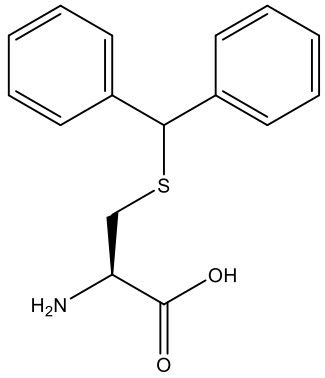
Cys(Trt)-OH

B



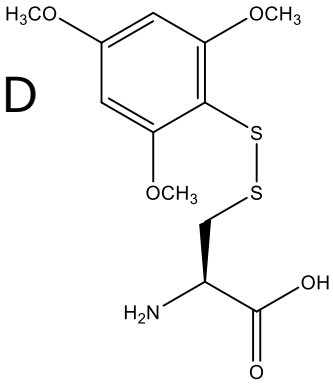
Cys(Mmt)-OH

C



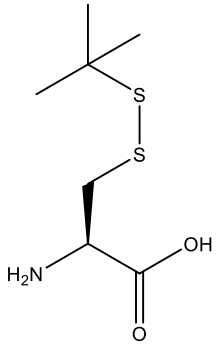
Cys(Dpm)-OH

D



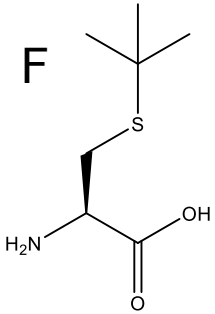
Cys(STmp)-OH

E



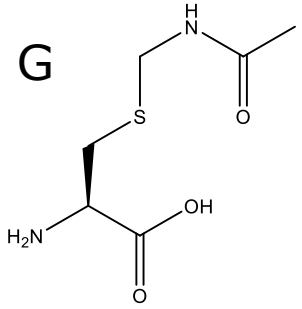
Cys(StBu)-OH

F



Cys(tBu)-OH

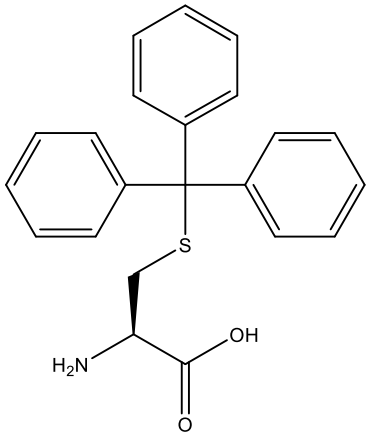
G



Cys(Acm)-OH

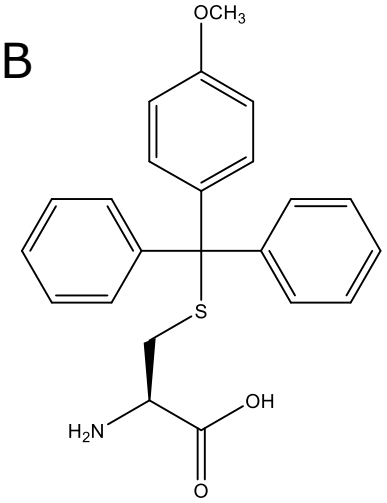
Suite of orthogonally protected Cys

A



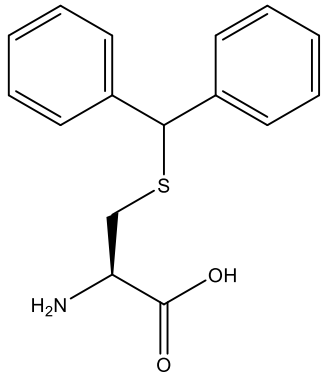
Cys(Trt)-OH

B



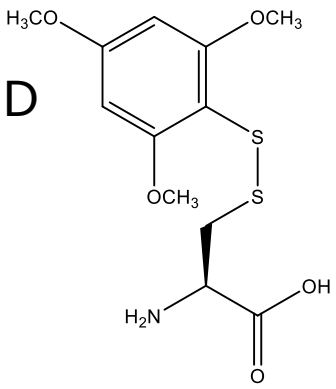
Cys(Mmt)-OH

C



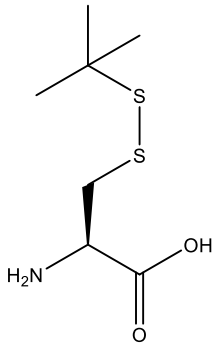
Cys(Dpm)-OH

D



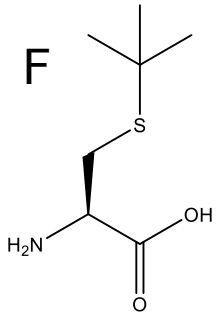
Cys(STmp)-OH

E



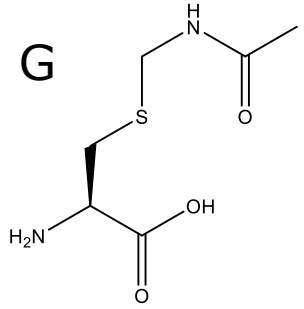
Cys(StBu)-OH

F



Cys(tBu)-OH

G



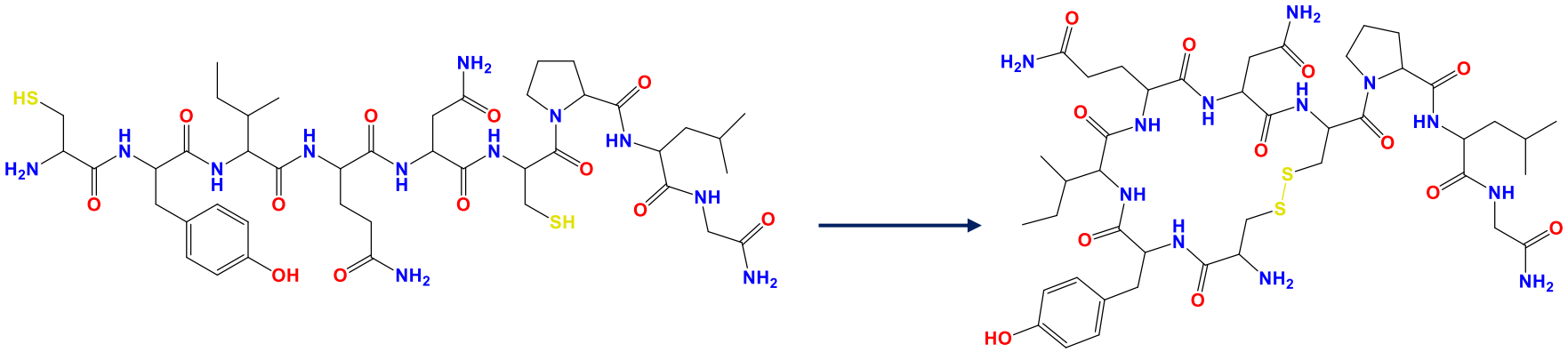
Cys(Acm)-OH

Where to start?
Managing instrumentation specifications

- Instruments perform tasks differently than you do manually
  - Volume limitations
  - Mixing mechanisms
  - Scaling?
- What we need:
  - Cysteine oxidation conditions
  - Mmt removal conditions
  - STmp removal conditions
  - Acm removal conditions
  - Model systems to evaluate efficacy
- Concerns:
  - Gentle oxidation to prevent disulfide shuffling
  - Efficient protecting group removal

Optimizing disulfide bond formation

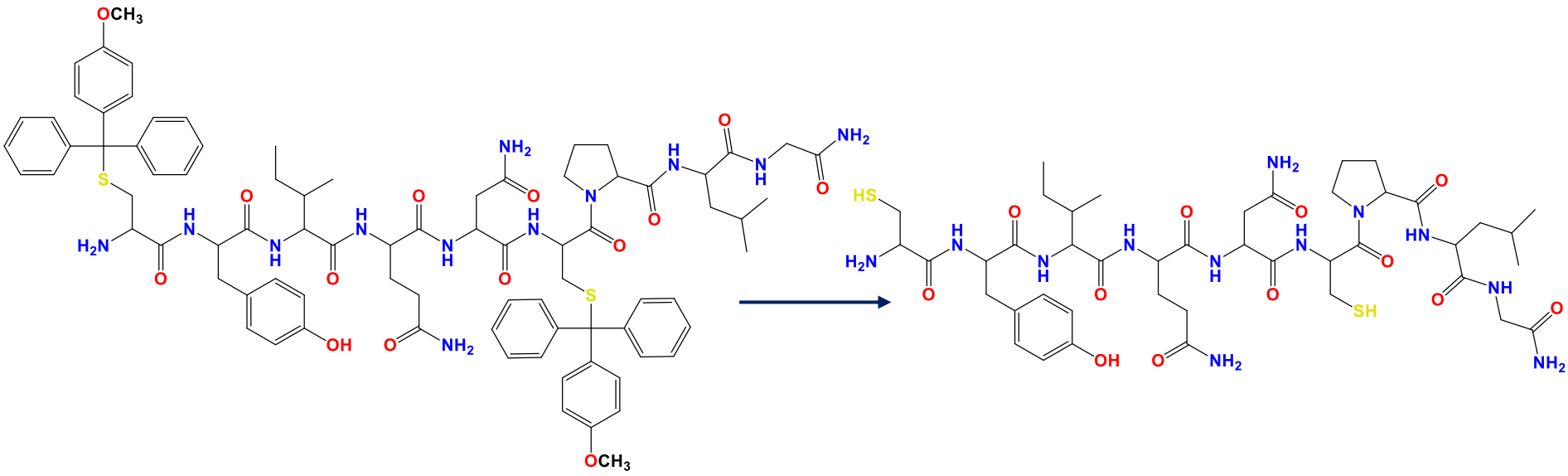
Reagents	Equivalents	Temperature	Time (min)	Percent completion
NH_3/H_2O_2	2/1.2	r.t.	30	50
NH_3/H_2O_2	4/2.4	r.t	30	50
NCS	2	r.t	15	100
NCS	4	r.t.	5	100
NCS	2	50 °C	5	100
NCS	1	50 °C	5	100



Postma, T. M. and Albericio, F. *Org. Let.* **2012**, 15, 616-619.

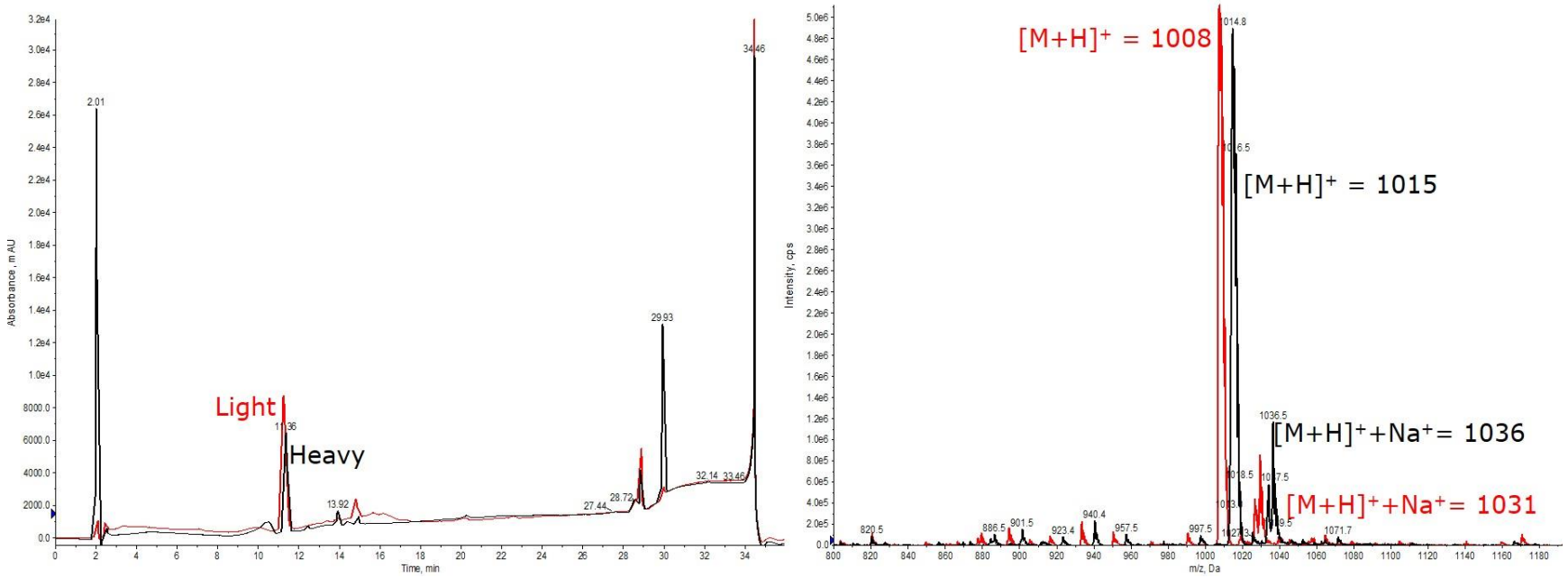
Mmt removal efficiency varies with scale

Scale (mmol)	TFA in DCM + 5% TIPS	Volume (mL)	Time (min)	Attempts
0.025	2% TFA	1.5	20	2
0.235	2% TFA	4.5	20	4
0.4	2% TFA	9	30	6



Fully automated oxytocin synthesis

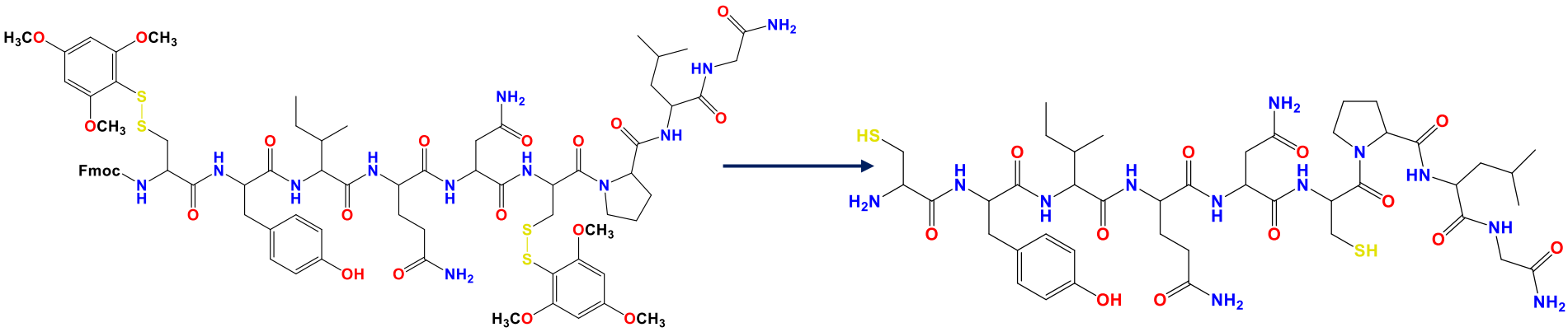
Incorporating Fmoc-Cys(Mmt)-OH and ^{C13,N15}Leu



Isotopically-labeled oxytocin prepared with fully automated synthesis and on-resin oxidation in 90% crude purity

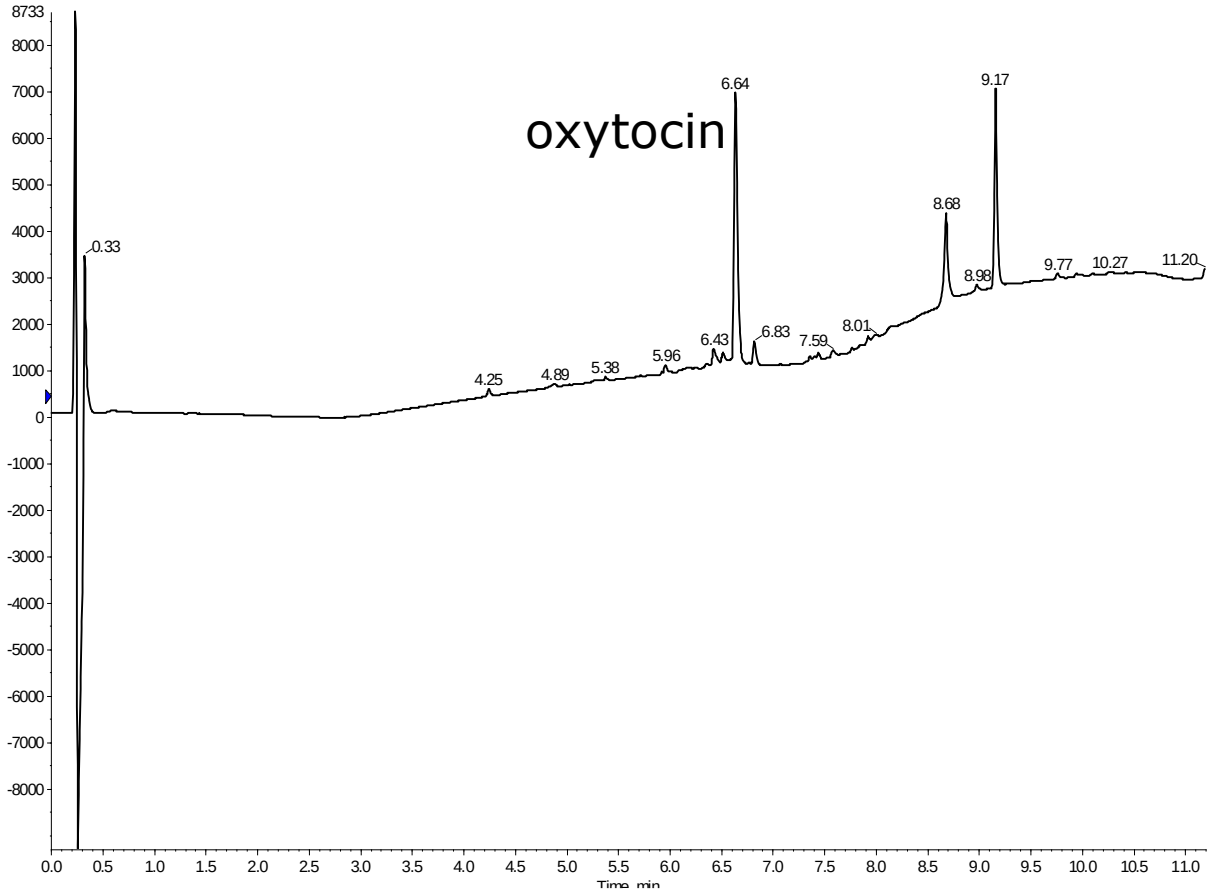
Optimizing STmp removal

Reagent volume (mL)	Reaction time (min)	Reaction temperature	Iterations
4.5	5	r.t.	3
3	5	r.t.	3
1	5	r.t.	3
0.5	5	r.t.	3
0.25	5	r.t.	3



Fully automated oxytocin

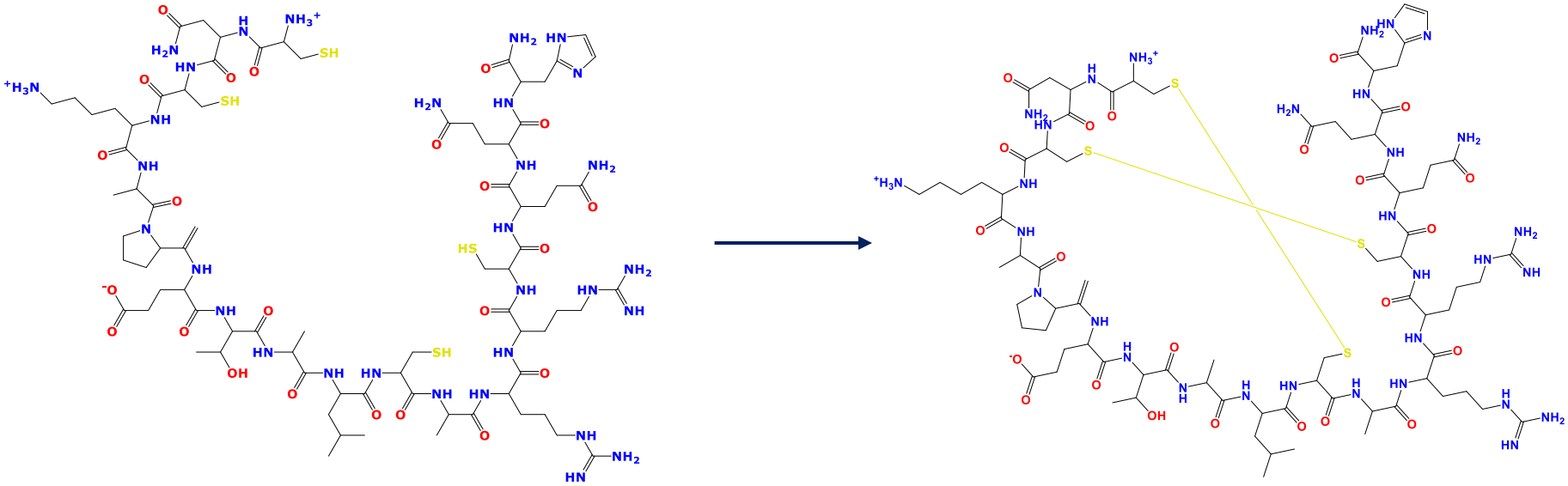
Incorporating Fmoc-Cys(STmp)-OH and optimized NCS-mediated oxidation



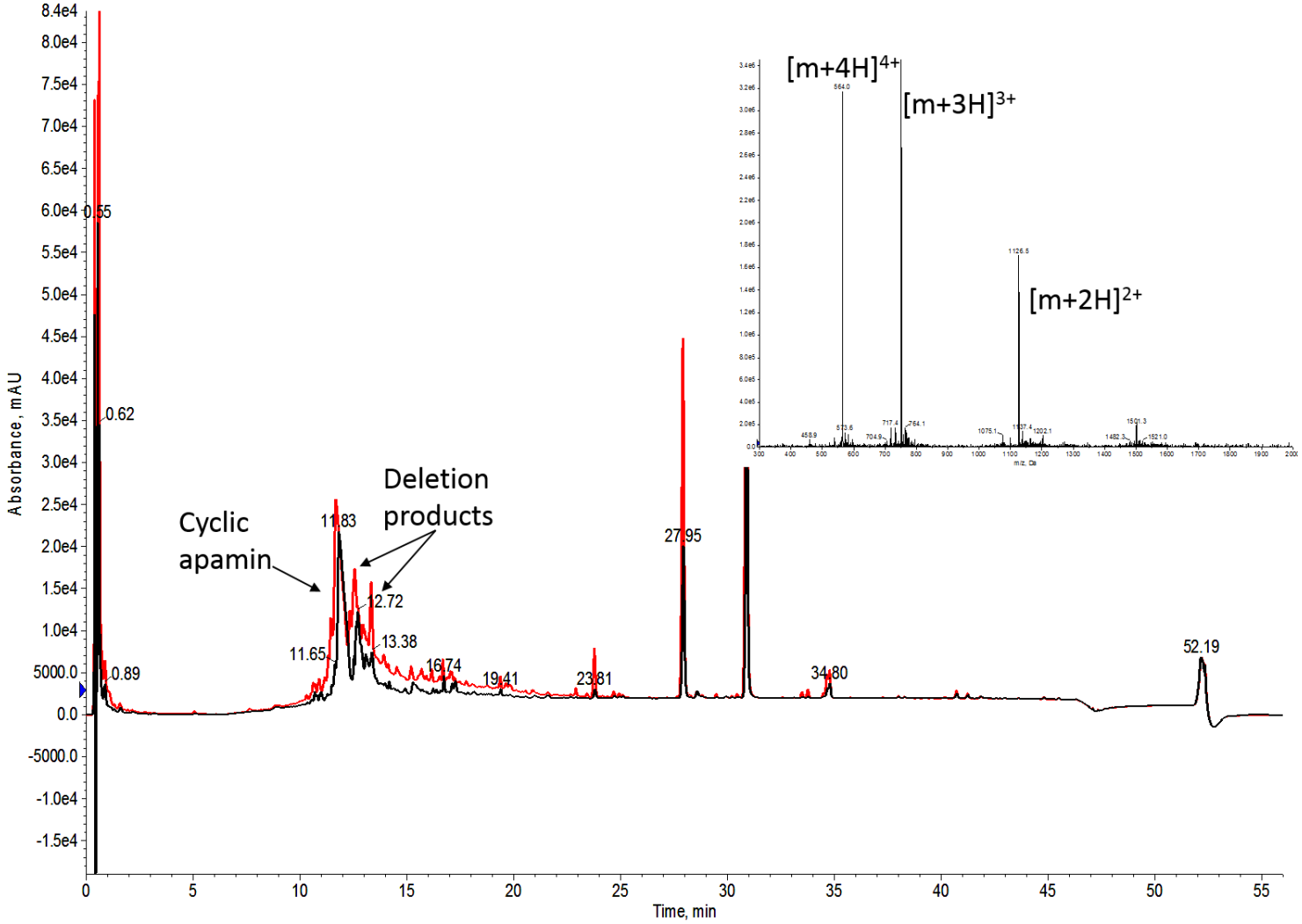
Automated synthesis and on-resin disulfide bond formation in >83% crude purity

Optimizing Acm removal with concomitant Cys oxidation

Experiment	Time (min)	I_2 equivalents (mmol)
1	60	15
2	45	15
3	30	15
4	60	10
5	60	5
6	60	2.5

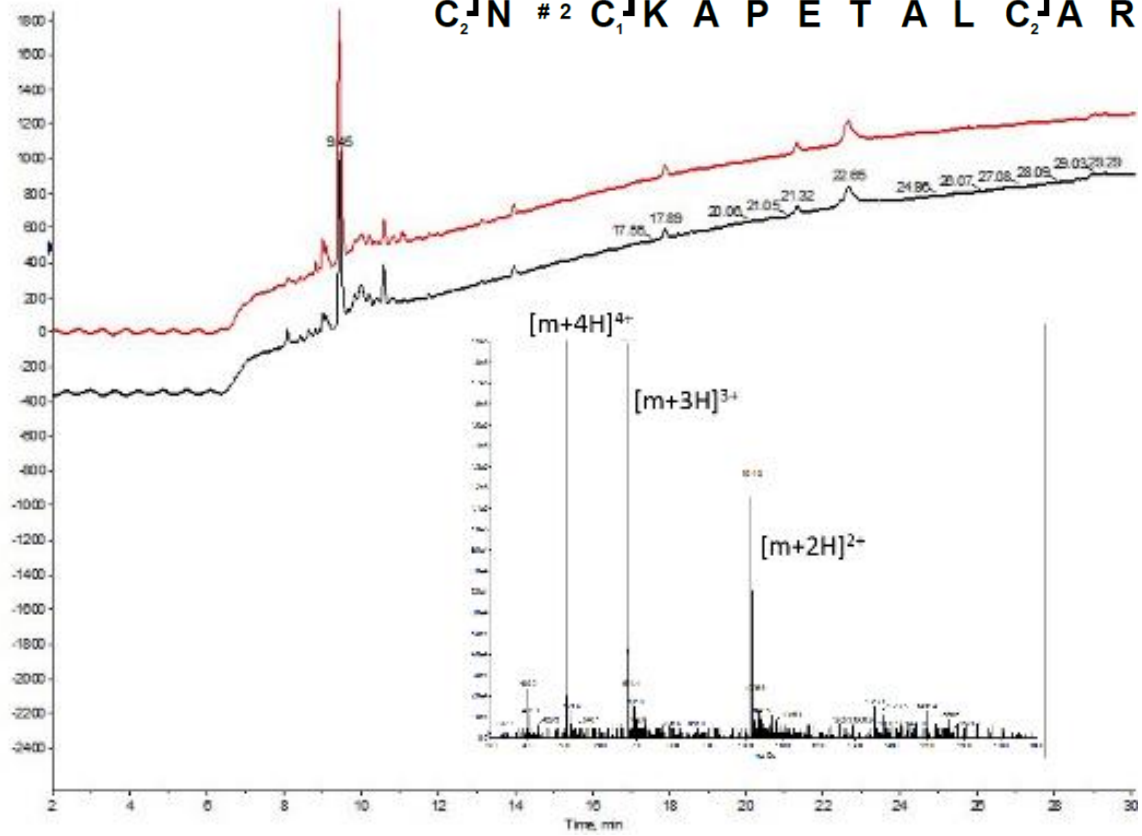


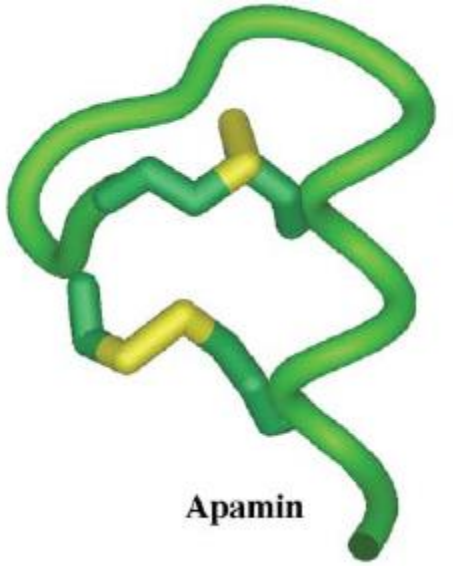
All conditions yielded desired product as majority species



Increasing complexity

Does the order of disulfide bond formation matter?



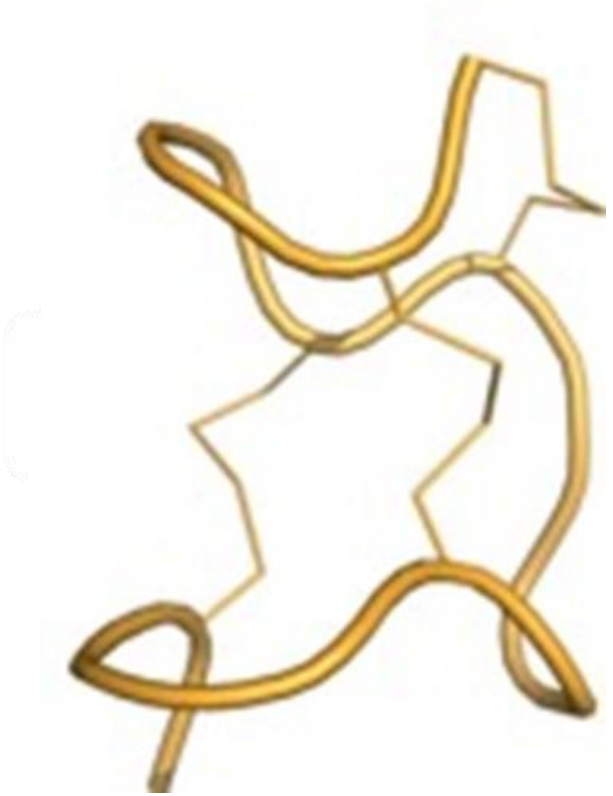


Pease, J. H. B. and Wemmer, D. E. *Biochemistry* **1988**, *27*, 8491-8498.

Further increasing complexity

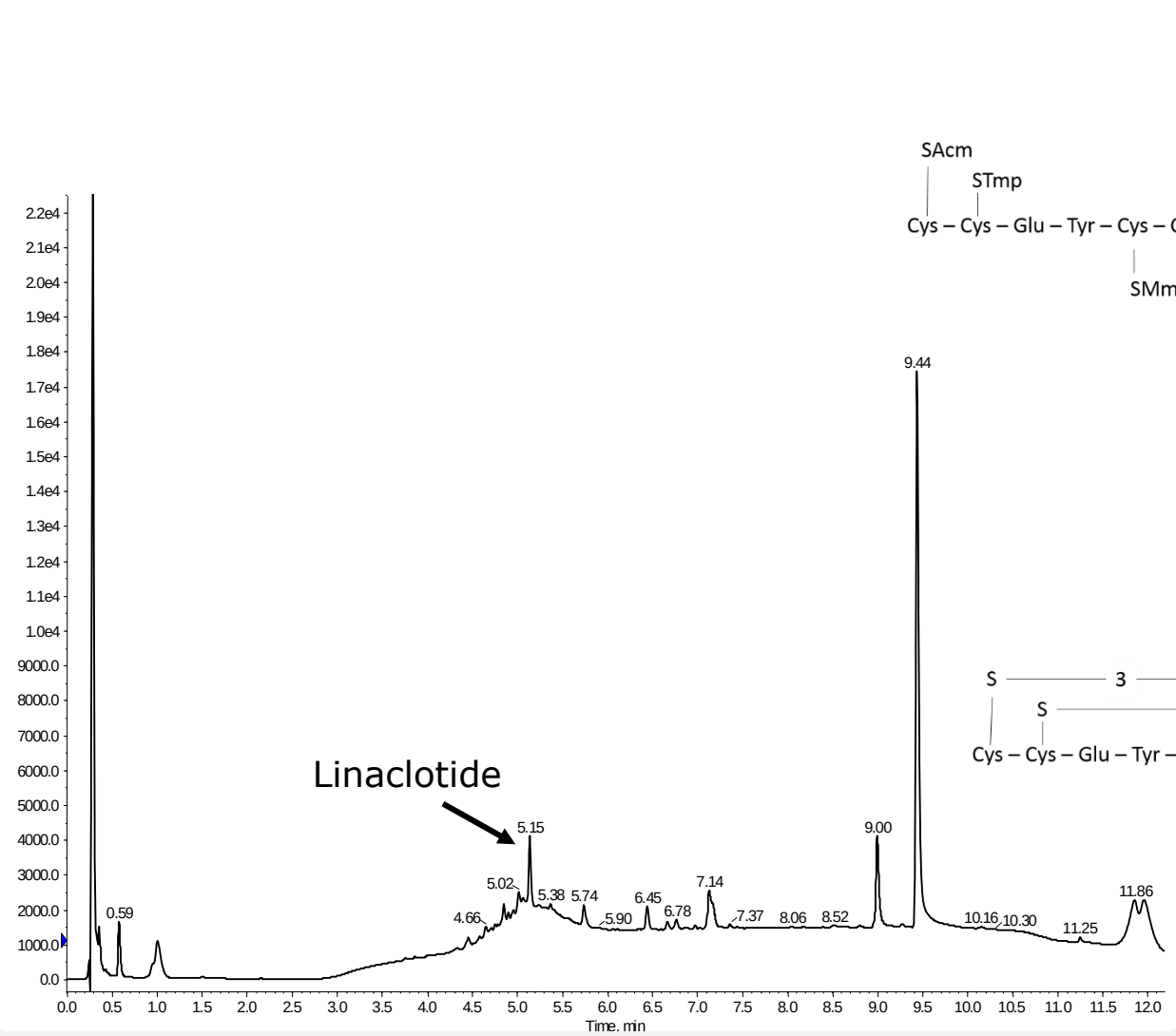
Synthesizing Linaclotide

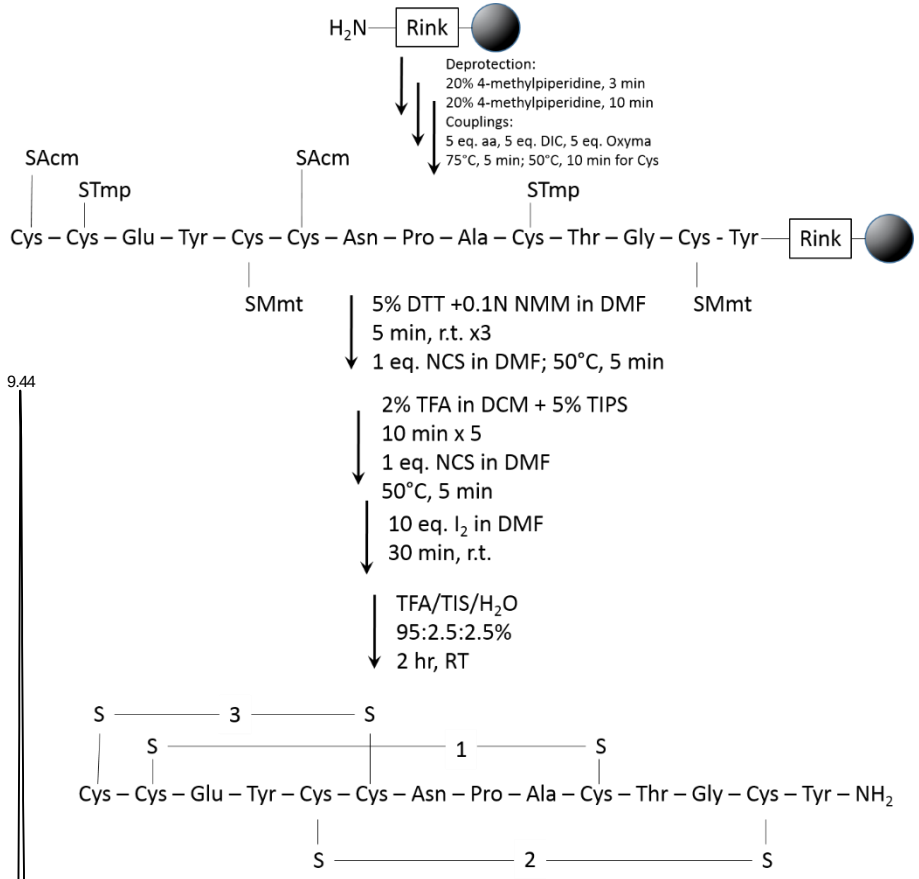
- Linaclotide
  - FDA approved therapeutic
  - 14 amino acids
  - 3 disulfide bonds
  - 43% Cys content
- Goal: automate synthesis and regioselective disulfide bond formation on-resin using orthogonal protecting groups



Further increasing complexity

What order should the disulfide bonds be formed?





Further increasing complexity

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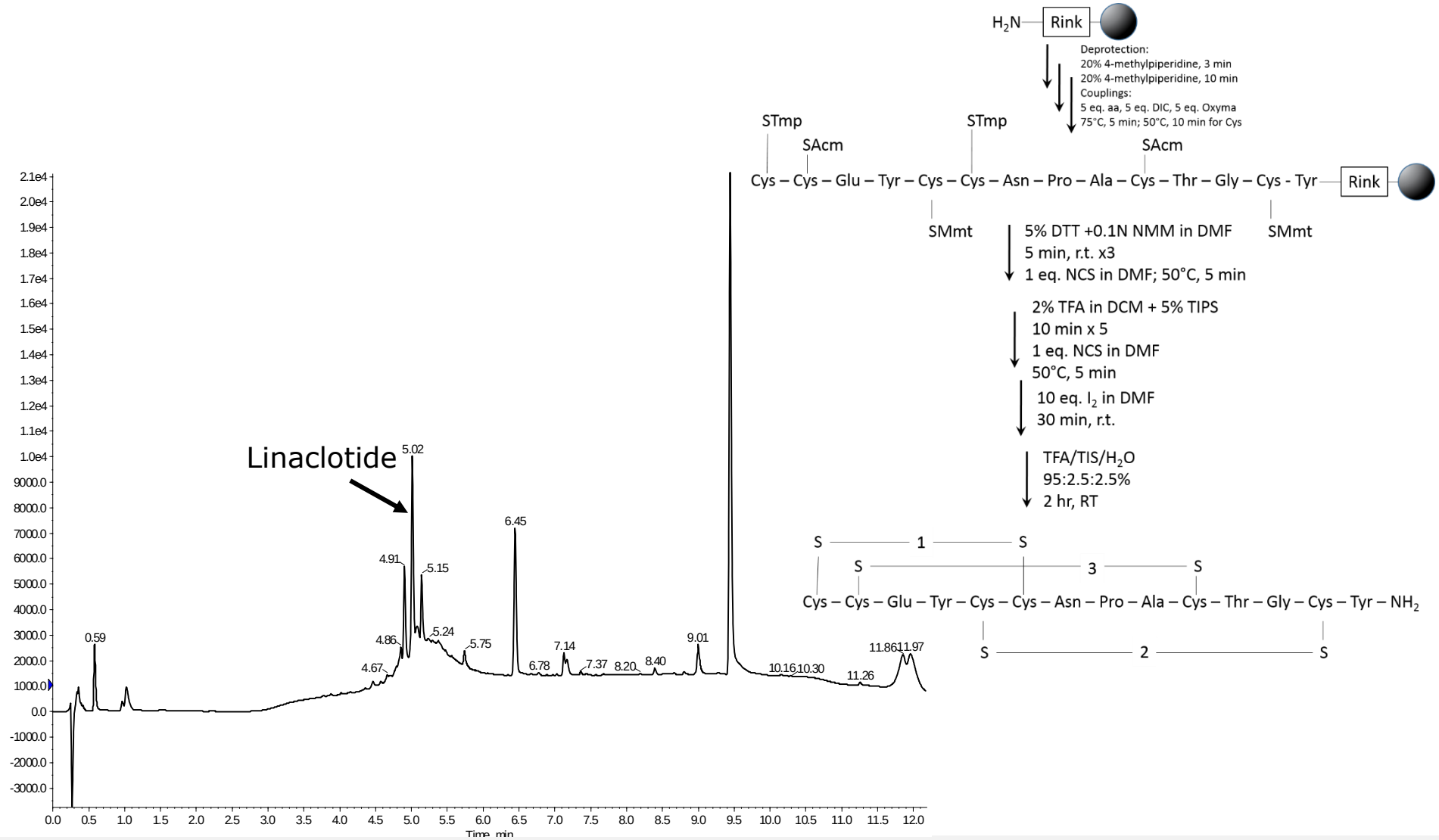

Further increasing complexity

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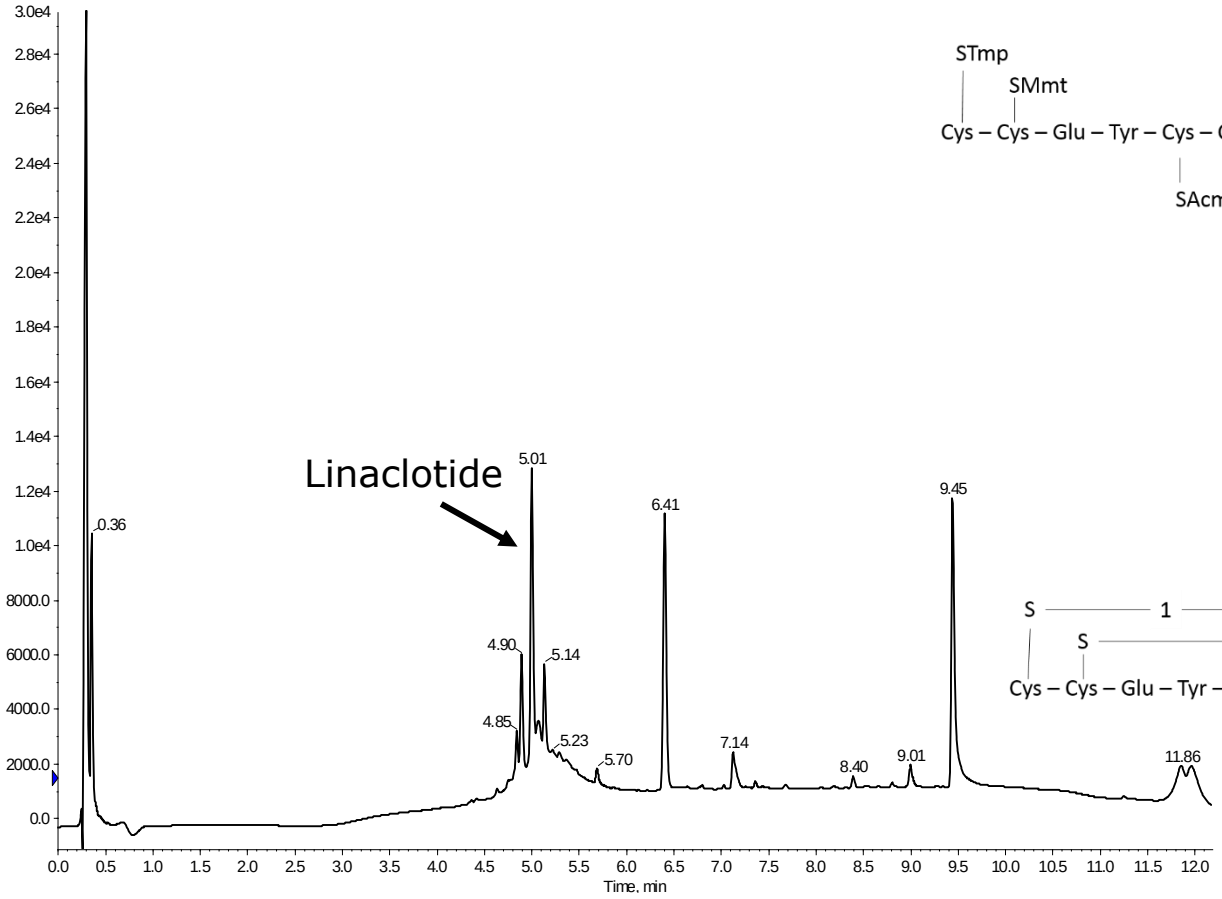

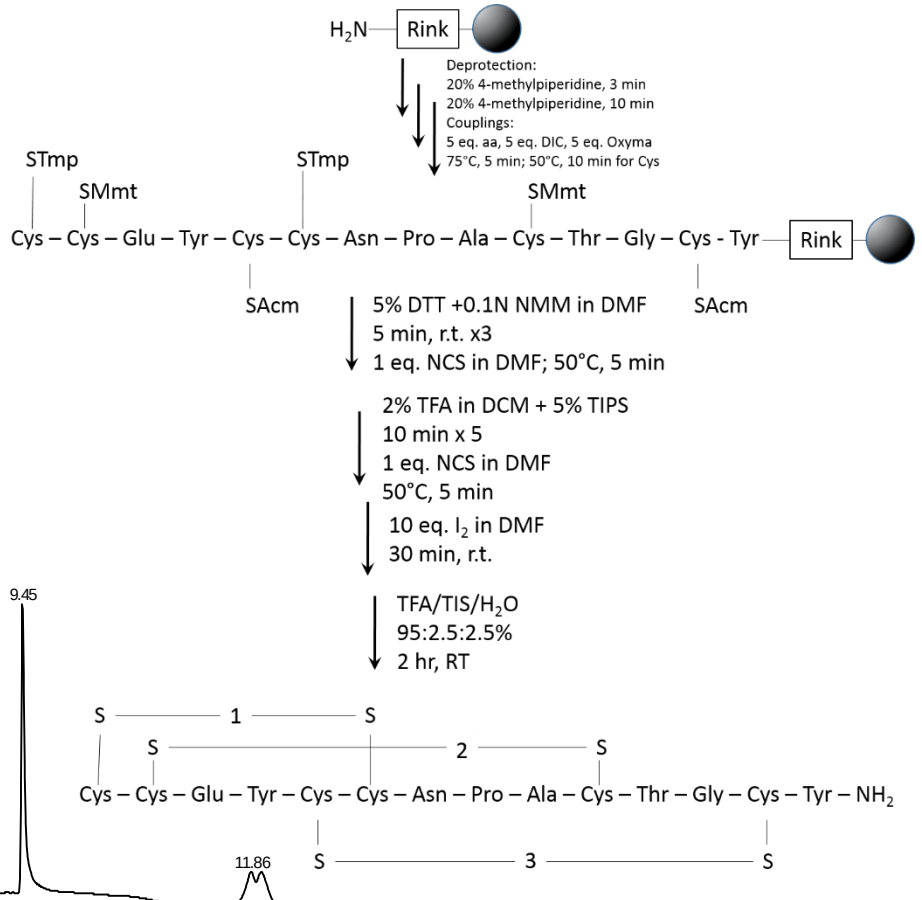

Gongora-Benitez, M et al. *Biopolymers*, **2011**, *96*, 69-80.

Vast structural diversity causes challenges for synthesis, production

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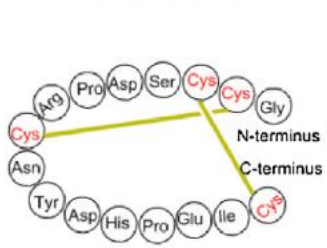
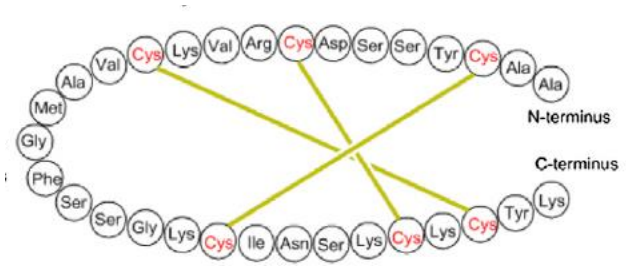
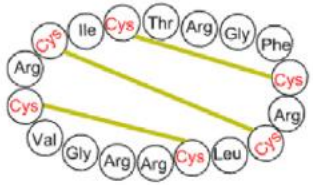
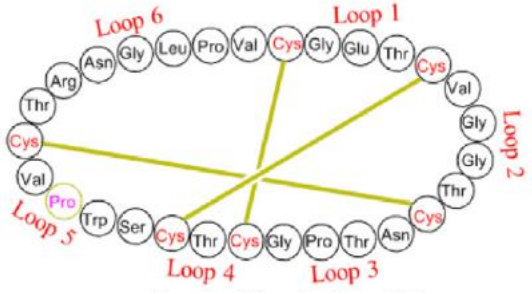
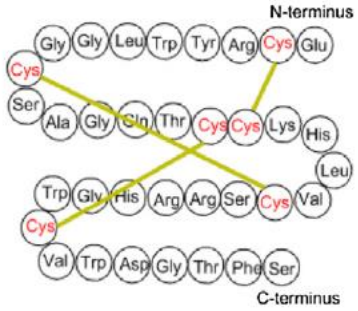


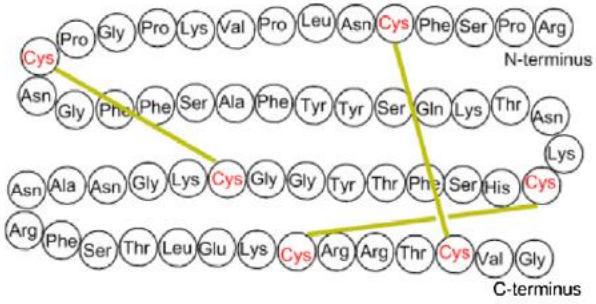




Disulfide Rich Peptides



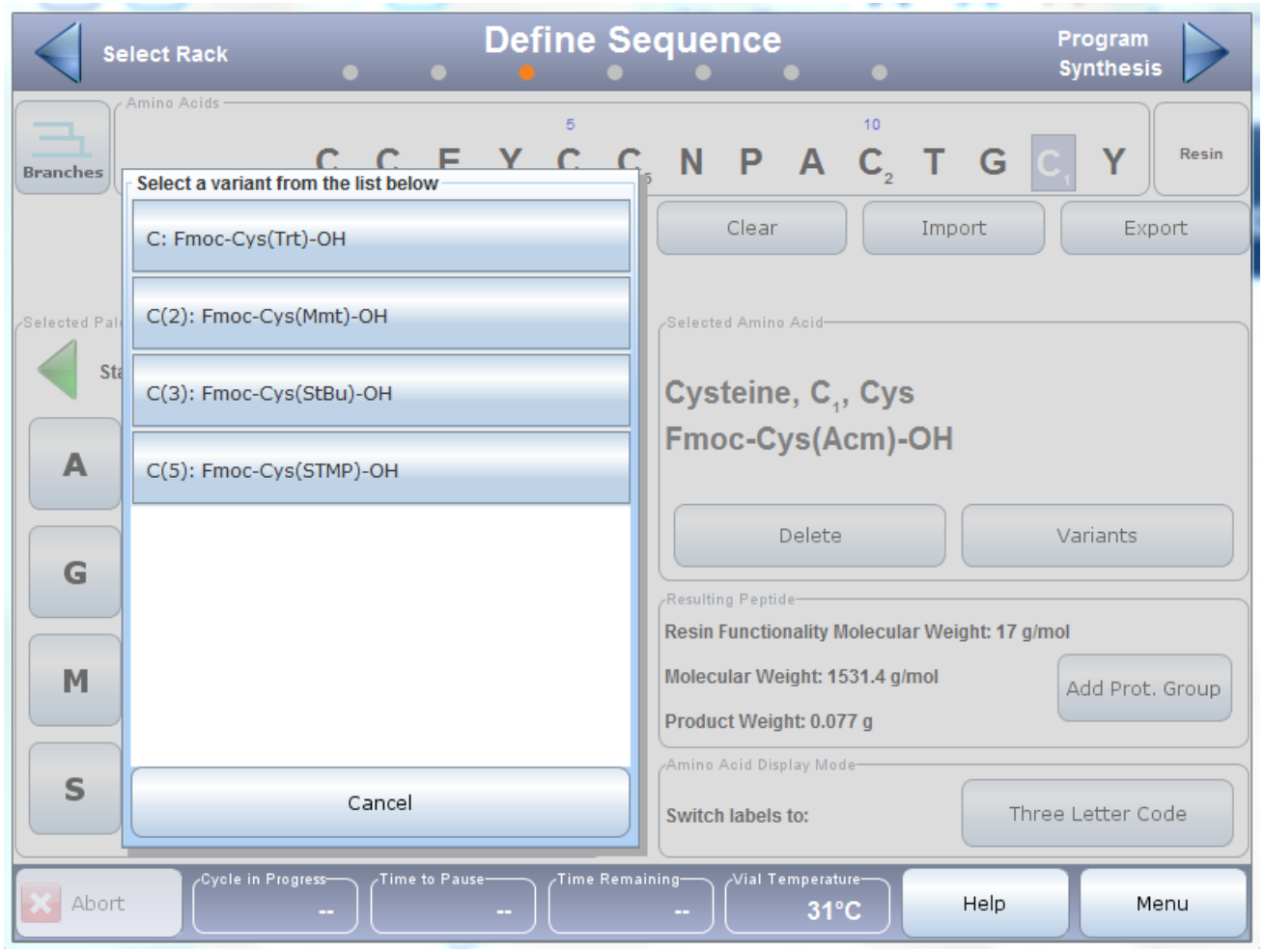




Fang, G.-M. et al. *Chin. Chem. Lett.* **2018**, *29*, 1022-1042.

Simplifying synthesis with smart software

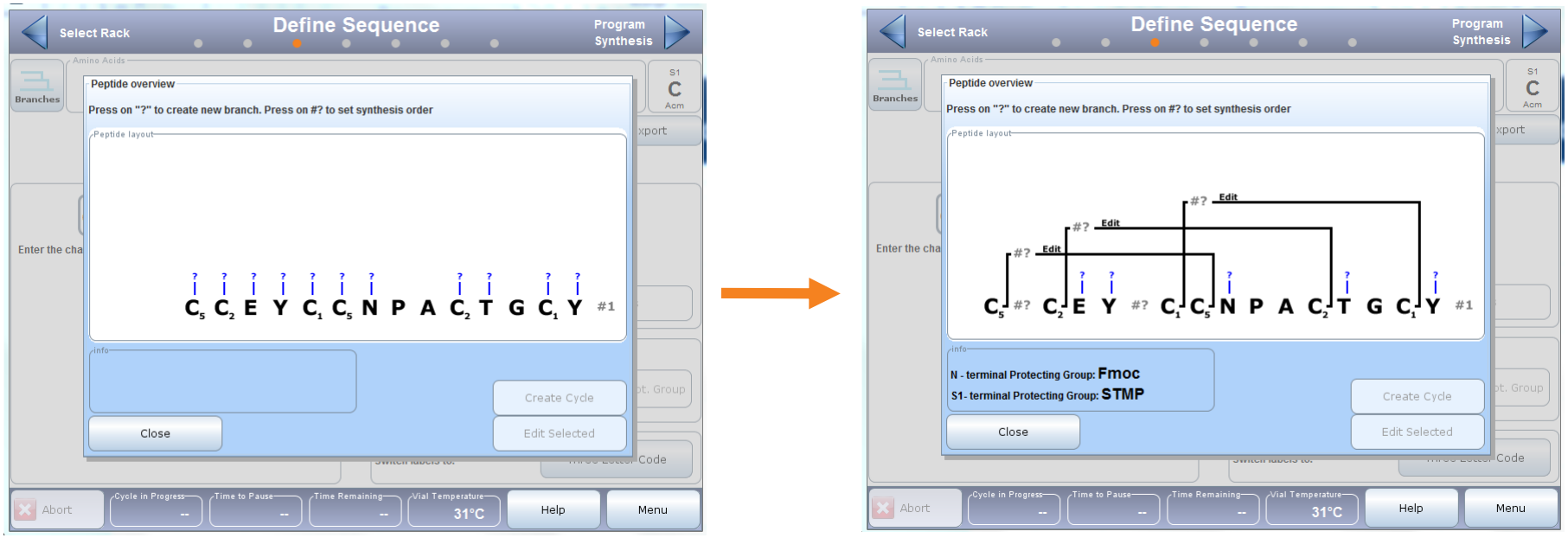
Directly visualize and specifically program everything



Easily match orthogonally protected Cys pairs with the Variants palette

Simplifying synthesis with smart software

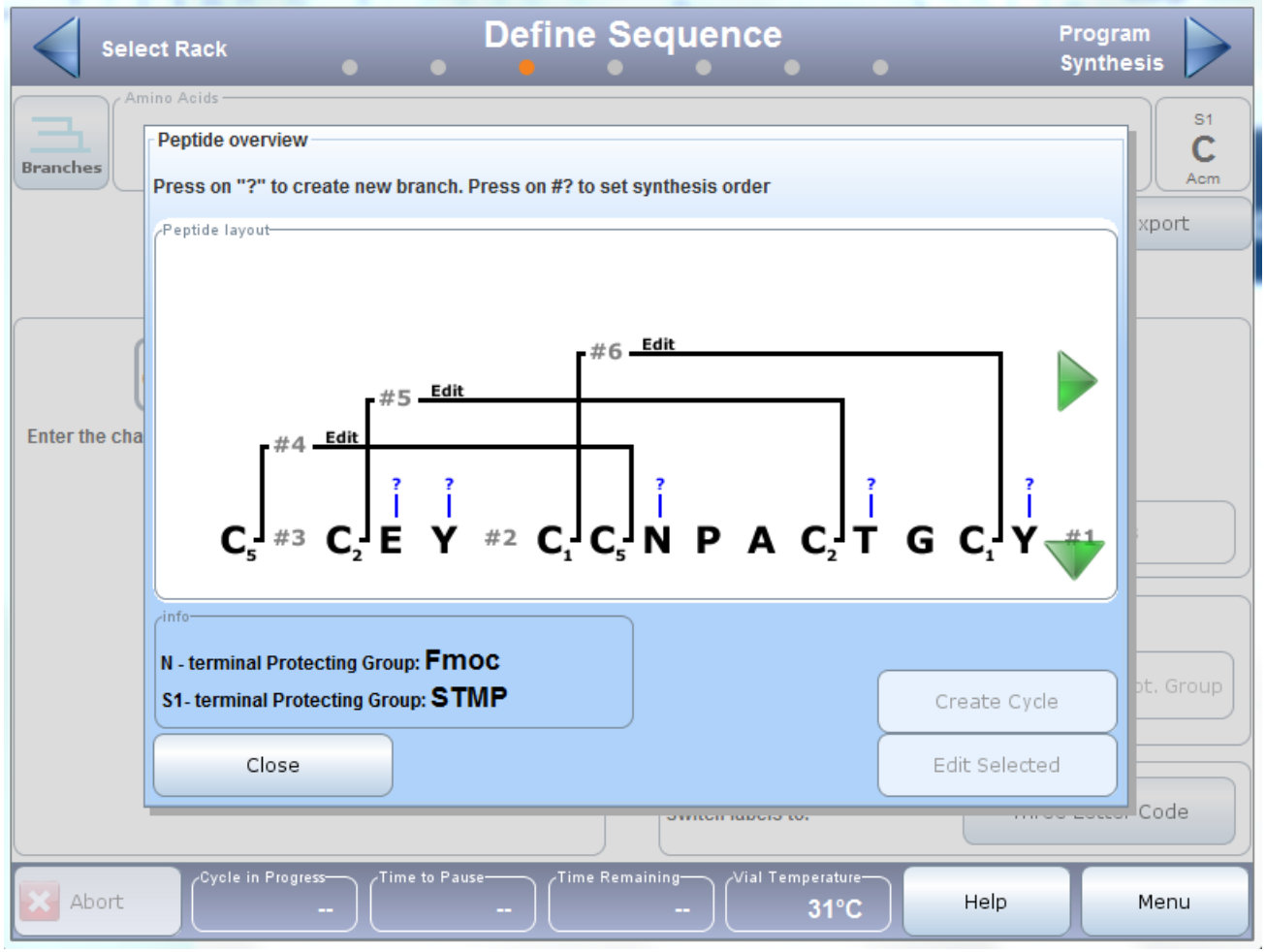
Directly visualize and specifically program cyclizations



Readily assign and *visualize* disulfide bond connectivity

Simplifying synthesis with smart software

Directly visualize and specifically program cyclizations



Simply assign order in which synthesis will occur

Simplifying synthesis with smart software

Directly visualize and specifically program cyclizations

Assign each reagent position wherever you want

Conclusions

- Successfully optimized *automated* orthogonal protecting group removal
- Successfully optimized on-resin disulfide bond chemistry with different reagents
- Successfully applied these optimized strategies to *automate* synthesis of complex, disulfide rich apamin and linaclotide peptides
- Highlighted smart software simplicity for automating syntheses of complex peptides like these and potentially others

Questions?

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