Chemical synthesis of pharmaceutical peptide Syntocinon® (oxytocin) and a related analogue

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Abstract

Oxytocin is a natural hormone in the human body. It was the first synthesized peptide hormone, being synthesized in 1954 by Vincent du Vigneaud[1]. It is well known that oxytocin is involved in the process of labor during childbirth, particularly its role in inducing contractions[2]. This is possible due to oxytocin's ability to bind to the oxytocin receptor within the human body. Here we synthesized oxytocin and an analogue for the purpose of altering oxytocin's natural pharmacokinetics and pharmacodynamics. Oxytocin was synthesized using solid phase peptide synthesis and oxidized in a pH 8.0 100mM ammonium bicarbonate buffer with oxygen present in air to produce its cyclic form. Also using solid phase peptide synthesis, two moieties of the analogue were produced. These moieties were a trimer and a hexamer, which were linked together with an o-aminoanilide linker through native chemical ligation, yielding a novel oxytocin analogue. Synthesis of this analogue allows for future study of its binding efficiency to the oxytocin receptor and could yield a pharmaceutical oxytocin substitute.

Introduction - Oxytocin

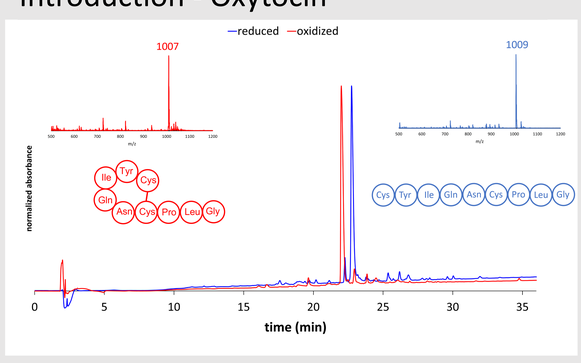

Figure 1. HPLC and MS of reduced and oxidized oxytocin. The retention time varies slightly between the oxidized and reduced forms.

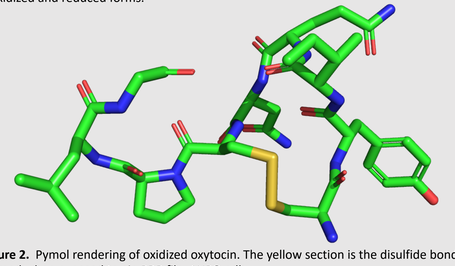

Figure 2. Pymol rendering of oxidized oxytocin. The yellow section is the disulfide bond formed when exposed to air. PDB file: 1NPO.pdb

Affiliations and Acknowledgments

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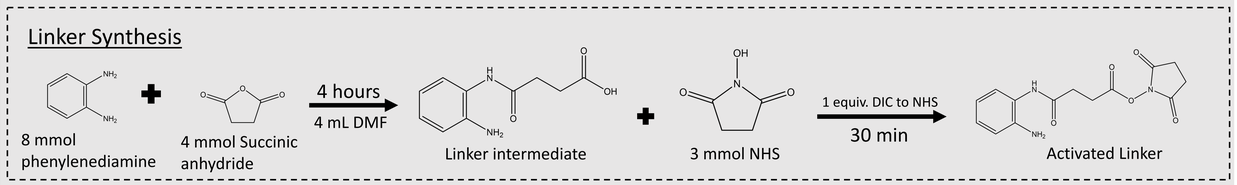
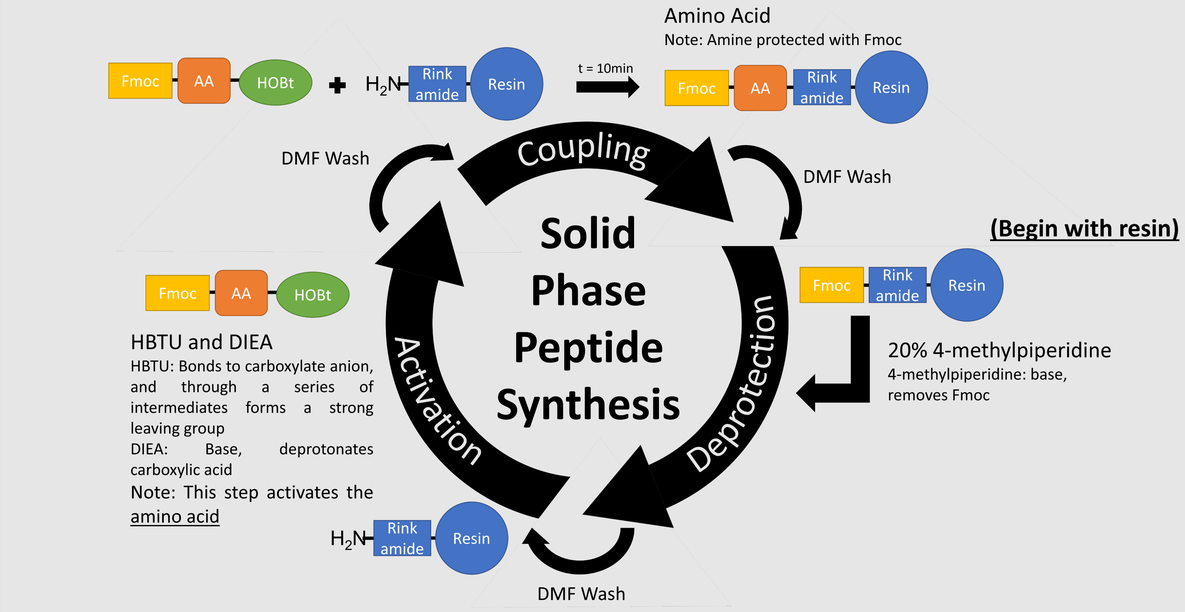
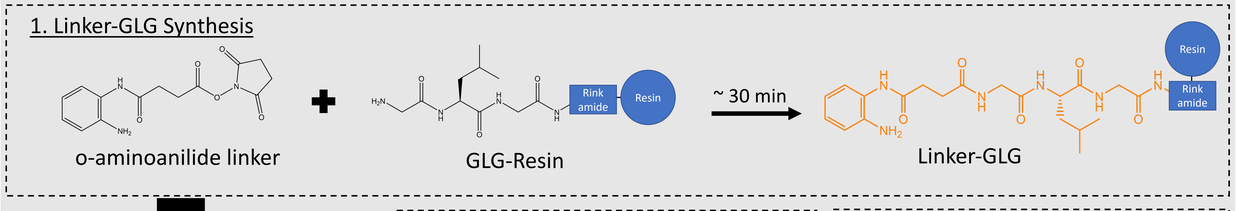
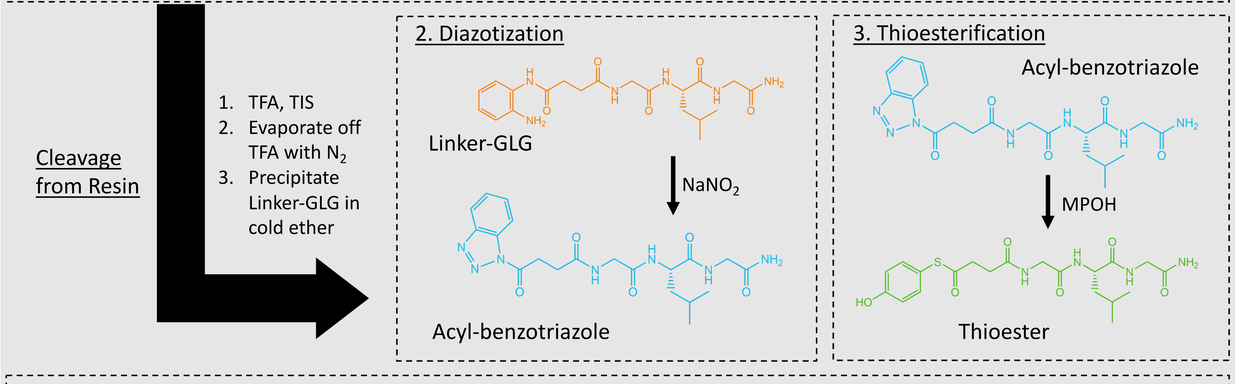
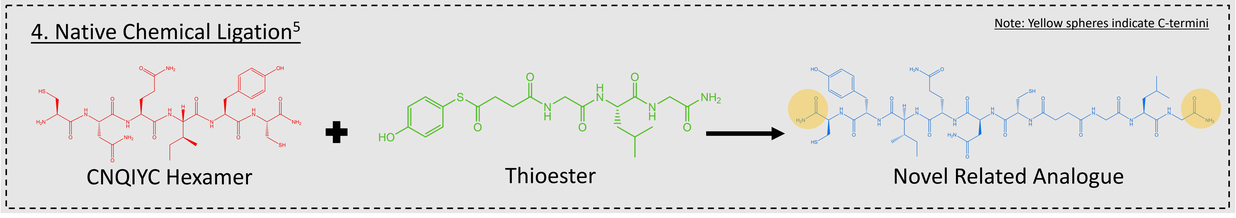
Methods – Solid Phase Peptide Synthesis and linker formation











Results

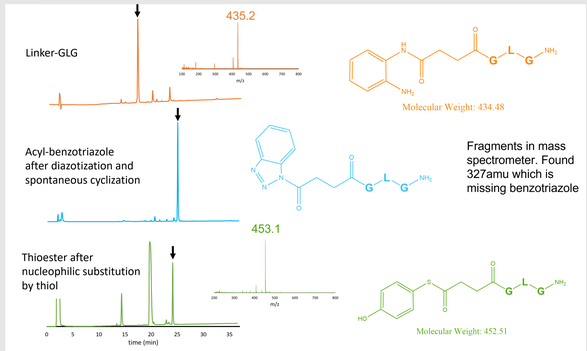


Figure 3. Product of steps 1-3. HPLC and MS of Linker-GLG, acyl-benzotriazole, and thioester.

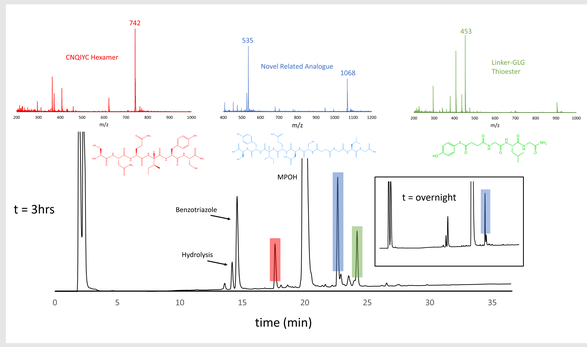


Figure 4. Product of step 4. HPLC of native chemical ligation of CNQIYC peptide to Linker-GLG peptide.

6M GuHCl 0.2M PO_4 pH 3 = low pH buffer | 6M GuHCl 0.2M PO_4 pH 8 = high pH buffer

Procedure for small scale NCL

1. Dissolve ortho-aminoanilide peptide at 20 mM in low pH buffer
2. Make 0.5 M $NaNO_2$ in low pH buffer
3. Place both in -20 ºC freezer for 5 minutes
4. Add 5 eq. $NaNO_2$ to o-aminoanilide, let react in freezer for 5 min
5. Add equal volume 200 mM 4-Mercaptophenol in high pH buffer, let stand at room temp 30 min
6. Add solution to 1 eq. dry (lyophilized) N-terminal cysteine peptide
7. Filter through 0.2 µm PES syringe filter, adjust pH to near neutral with NaOH, TCEP reduce, HPLC
   - Warning, do not adjust the pH too high, as hydrolysis happens rapidly, final pH 6.9-7.2

Pharmaceutical Conclusion

Oxytocin analogues are useful drug candidates[4]. Figure 5. shows the concentration of inositol monophosphate increasing as the concentration of oxytocin increases. The oxytocin analogue synthesized in this research could vary from oxytocin in how it moves through the body[4]. The study of this process is known as pharmacokinetics.

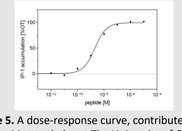


Figure 5. A dose-response curve, contributed by Markus Muttenthaler at The University of Queensland.

Novel Chemistry Conclusion

Formation of new method for conjugation of peptides using N-terminal o-aminoanilides:

- Inexpensive, commercially available reagents
- Relatively short amount of time consumed (5 hrs Linker-GLG, 2 hrs Hexamer, 1 day ligation)
- Hexamer successfully coupled to Linker-GLG, entirely novel ligation

Future Work:

- Collect oxidized material
- Send for bioassays

Work Cited

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