Published in final edited form as:

J Am Chem Soc. 2012 August 15; 134(32): 13244–13247. doi:10.1021/ja3063452.

Revisiting Oxytocin Through the Medium of Isonitriles

Ting Wang[†] and Samuel J. Danishefsky^{†,‡,*}

[†]Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, NY 10065

[‡]Department of Chemistry, Columbia University, Havemeyer Hall, 3000 Broadway, New York, NY 10027

Abstract

The reaction of thioamino acids and N-terminal peptides, mediated by hindered isonitriles and HOBt, gives rise to peptide bonds. In one pathway, oxytocin was synthesized by eight such reiterative amidations. In another stereospecific track, oxytocin was constructed by native chemical ligation, wherein the two building blocks were assembled by thioacid amine amidation. The NMR spectra of oxytocin and dihydro-oxytoxin suggest a high level of pre-organization in the latter, perhaps favoring oxidative folding.

Oxytocin is a long-known, well-appreciated 9-mer peptide hormone which enables parturition and lactation in nursing mothers.^{1,2} More recently, there has been a growing perception that the receptor-binding characteristics of nonapeptides, including oxytocin, are of considerable moment in other neuropathological phenomena, including autism. Indeed, some encouraging effects, including indications of memory enhancement and improved social associations, have been demonstrated in autistic subjects following inhalation of oxytocin, in the context of an organized clinical trial.^{3,4} It has been argued that a breakdown in the bioprocessing of polypeptidic precursors en route to oxytocin may be implicated in provoking and maintaining the autistic state.⁵ Accordingly, it seemed to us worthwhile to revisit the oxytocin manifold – first from a chemical perspective, and then for the purpose of promoting improved biological function via wisely selected oxytocin-inspired congeners. Though oxytocin (**1**) itself has been synthesized several times,⁶ the corresponding “dihydro” bis cysteinyl compound, **2**, had apparently not been characterized. We also hoped to reach characterizable **2** as part of our total synthesis effort. We could then be in a better position to understand the structural changes which must occur as a consequence of oxidative disulfide formation in the parent system, and particularly in analogs we would be synthesizing and evaluating.^{7,8}

In addition to a clear basis for interest in oxytocin at various biological levels, we also viewed it as a compelling object molecule for testing the usefulness and scope of methodology which we have recently been developing, involving the use of isonitriles in the chemical synthesis of amide bonds, including those in peptidic contexts.⁹ The reaction of particular interest for us, drawn from our menu of new isonitrile chemistry, involves coupling of a thioacid (**3**) with an amine (**5**) in the presence of a hindered isonitrile (**4**). As we have shown,^{10,11} this three-component reaction gives rise to an amide linkage *under*

Corresponding Author: s-danishefsky@ski.mskcc.org.

No competing financial interests have been declared.

ASSOCIATED CONTENT

General experimental procedures, including spectroscopic and analytical data for new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

remarkably mild conditions. Evidence has been provided that the reaction commences with an addition event, which gives rise to a thio-FCMA (**6**). The latter is apparently a very powerful acyl donor and, upon reaction with a suitable amine, provides an amide (**7**).¹² While the thioacid-amine coupling can occur with no further additives, it was recently shown to be enhanced by the presence of HOBt.¹³ It seems not unlikely that under these HOBt conditions, the reaction progresses from the thio-FCMA through an HOBt ester (**8**), en route to amide **7**. (Figure 1) Thus, we viewed oxytocin as an attractive target to test the scope of the hindered isonitrile/HOBt-mediated amidation process, hopeful that information gleaned from the parent target system could be applied to synthesizing our proposed analogs.

The oxytocin directed synthesis program began by coupling of glycine methyl ester **9** and the leucine-derived thio-acid **10**. Indeed, under mediation by *t*-BuNC and HOBt, dipeptide **11** was obtained in 92% yield. The latter was converted to tripeptide **13** in 66% yield over two steps, through *t*-Boc cleavage and subsequent isonitrile-mediated amidation with thioacid **12**. Cleavage of the *t*-Boc protecting group of **13**, followed by coupling the resulting N-terminus with **14** afforded tetrapeptide **15** in 64% yield over two steps. The isonitrile-mediated “peptide homologation” strategy was then demonstrated to be compatible with a suitable asparagine derivative (**16**), providing pentapeptide **17** in 84% yield. Similarly, following cleavage of the carbamate linkage of **17**, the resulting N-terminus served to amidate glutamine-derived thioacid **18** to afford hexapeptide **19** in 82% yield over the two steps (Scheme 1).

Our journey then progressed through the same two step coupling-deprotection elongation process, using, in sequence, isoleucine-derived thioacid **20**, tyrosine-derived thioacid **22** and cysteine-derived thioacid **14**, in 77%, 78%, 84% yields, respectively, thereby affording **24**. Conversion of the C-terminal methyl ester to the corresponding primary amide followed by global deprotection provided the linear nonapeptide **2**. Happily, the chemistry described above allowed us to characterize “dihydro-oxytocin” **2** as a homogeneous entity. Finally, aeration of **2** in pH=7 aqueous solution furnished oxytocin **1** in 63% isolated yield (Scheme 2). The 600 MHz ¹H spectra of homogeneous **2** and **1** are provided for comparison (Figure 2). Their striking similarity, both in terms of levels of definition, and in appearance, suggests significant preorganization of **2** en route to **1**.¹⁴

Having demonstrated the reach of isonitrile mediated thioacid amidation by the sequential, step-by-step C3N elongation synthesis of oxytocin, we next investigated the applicability of isonitrile chemistry to the enablement of a more convergent strategy. With the goal of avoiding epimerization, we first took recourse to the native chemical ligation (NCL) logic of Kent and associates.¹⁵ One of the highlights of the Kent chemistry is its extraordinary level of stereointegrity (epimerization avoidance) in the key amide-forming ligation step. We hoped to demonstrate that the thioacid/isonitrile/HOBt-mediated amidation can be used to provide coupling substrates for an NCL ligation. We particularly hoped to exploit our orthomercaptoaryl ester rearrangement (OMER) variation of the classical NCL to generate the O-hydroxy activated phenyl thioester.^{16,17}

Accordingly, protected glutamine **26** was prepared from **25** by esterification in 87% yield (Scheme 3). Removal of the *t*-Boc group followed by coupling with isoleucine-derived thioacid (**20**) in the presence of *t*-BuNC and HOBt afforded dipeptide **27** in 83% yield. After elongation with thioacid **22** by isonitrile chemistry, the resulting adduct was subjected to 20% piperidine/CHCl₃ solution to furnish tripeptide **28**. Elongation of **28** via isonitrile-mediated coupling of cysteine-derived thioacid (**29**) and tripeptide **28**, followed by deprotection of the trimethylsilylethyl ester, afforded tetrapeptide **30**. Coupling of **30** with asparagine derivative **31** bearing the required C-terminal OMER^{16,17} machinery, followed

by global deprotection, using cocktail B(trifluoroacetic acid, phenol, triisopropylsilane, H₂O) provided NCL ligation partner **32**. The complementary ligation element, **33**, was prepared in two steps from **15** via aminolysis followed by global deprotection to liberate the N-terminal cysteine.

Merger of **32** and **33** occurred smoothly in a pH 7.2 buffer solution, providing a ligation product, which, when treated with *O*-methylhydroxylamine,¹⁸ furnished (the previously synthesized) **2** with no detected epimerization. As before, aeration of **2** resulted in oxytocin **1**. Since this remarkably smooth chemistry gives rise to readily purifiable compounds, it was not surprising that the discerning high field (600 MHz) spectra of **1** and **2** were in each case identical with those obtained by the linear route described above.

We next evaluated the feasibility of a more risky proposition, i.e. the possibility of using the thioacid/isonitrile/HOBt-mediated amidation reaction (see Figure 1) *for the ligation step, itself*. The question was whether “stereointegrity” at the C-terminus of the acyl donor could be maintained even when the thioacid is placed at the terminus of a peptide. It is well perceived that activated acyl donors corresponding to C-terminal peptides can be vulnerable to apparent oxazolone formation, accompanied by epimerization of the α-C-terminal stereocenter (cf **39**→**40**) prior to ligation.^{19–21} We hoped to probe this question in the context of C-terminal thioacid **36**.

Toward this end, tripeptide **28** was coupled with Boc-Cys(Trt)-SH (**14**) in the presence of *t*-BuNC and HOBt (Scheme 4). The ester linkage of the resulting adduct was converted to its corresponding carboxylic acid **34** through use of TBAF (61% over the two steps from **28**). Coupling of **34** with aspartate derived Fm thioester **35** followed by liberation of the thioacid afforded the required **36** in 70% yield.

The N-terminal cysteine coupling partner **37** was readily retrieved from its previously described protected form **15**, as shown. Isonitrile/HOBt mediated ligation of **36** and **37** occurred reasonably well, leading to the previously characterized **24** in 61% yield. However, the desired **24** was accompanied by significant amounts of its D-aspartate-containing epimer, **38**. Even after significant attempts at optimization of stereointegrity in the coupling step, the best ratio of **24**:**38** we obtained was only 6:1. While the feasibility of suppression of epimerization in such isonitrile-mediated thioacid ligations may well be sequence dependent, stereointegrity in this type of ligation is clearly not yet a solved problem.

In summary, the amidation of thiol acids derived from a wide range of potential single amino acids, with amines (including those from a variety of amino acids and N-terminal peptides) has been demonstrated. This central reaction has been iterated eight times to produce homogeneous dihydro (bis cysteinyl) oxytocin (**2**). This intermediate was oxidatively cyclized to afford oxytocin (**1**). The same type of isonitrile/HOBt mediated chemistry was used to prepare subunits to achieve an NCL type ligation using the OMER^{16,17} method to generate the required C-terminal activated thioester. Throughout these reactions, using Boc protected single amino thioacids, there was no indication of racemization in the acyl donor. However, epimerization was noted with a C-terminal thioacid of pentapeptide **36**.

Comparison of the high field (600 Mz) H' spectra of **2** and **1** suggests that oxidative folding could well be facilitated by considerable pre-organization. Studies involving the extension of isonitrile chemistry to more automatable settings in polypeptide synthesis, as well as its application to the facilitation of SAR studies of oxytocin and related behavior modifying congeners are in progress.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding Sources

This research was supported by NIH grant CA103823 (S.J.D.).

References

1. Dale HH. *J Physiol.* 1906; 34:163–206. [PubMed: 16992821]
2. Lee H, Macbeth AH, Pagani JH, Young WS 3rd. *Progress in Neurobiology.* 2009; 88:127–151. [PubMed: 19482229]
3. Witt DM, Winslow JT, Insel TR. *Pharmacol Biochem Behav.* 1992; 43:855–861. [PubMed: 1448480]
4. Andari E, Duhamel J, Zalla T, Herbrecht E, Leboyer M, Sirigu A. *Proc Natl Acad Sci U S A.* 2010; 107:4389–4394. [PubMed: 20160081]
5. Green L, Fein D, Modahl C, Feinstein C, Waterhouse L, Morris M. *Biol Psychiatry.* 2001; 50:609–613. [PubMed: 11690596]
6. (a) Du Vigneaud V, Ressler C, Swan JM, Roberts CW, Katsoyannis PG, Gordon S. *J Am Chem Soc.* 1953; 75:4879–4880. (b) Du Vigneaud V, Ressler C, Swan JM, Roberts CW, Katsoyannis PG. *J Am Chem Soc.* 1954; 76:3115–3121. (c) Bodanszky M, Du Vigneaud V. *J Am Chem Soc.* 1959; 81:2504–2507. (d) Bodanszky M, Du Vigneaud V. *Nature.* 1959; 183:1324–1325. [PubMed: 13657100] (e) Ives DAJ. *Can J Chem.* 1968; 46:2318–2320.
7. For early SAR studies regarding to obstetrical applications see: Katsoyannis PG. *J Am Chem Soc.* 1957; 79:109–111. Hope DB, Du Vigneaud V. *J Am Chem Soc.* 1963; 85:3686–3688. Takashima H, Du Vigneaud V. *J Am Chem Soc.* 1970; 92:2501–2504. [PubMed: 5438020] Hruby VJ, Ferger MF, Du Vigneaud V. *J Am Chem Soc.* 1971; 93:5539–5542. [PubMed: 5165679] Manning M, Coy E, Sawyer WH. *Biochemistry.* 1970; 9:3925–3929. [PubMed: 5533681] Manning M, Sawyer WH. *nature.* 1970; 227:715–716. [PubMed: 5432071] Ferger MF, Jones WC Jr, Dyckes DF, Du Vigneaud V. *J Am Chem Soc.* 1972; 94:982–984. [PubMed: 5061145] Manning M, Coy EJ. *J Med Chem.* 1973; 16:463–466. [PubMed: 4718457] Manning M, Balaspiri L. *J Med Chem.* 1973; 16:975–978. [PubMed: 4745513] Grzonka Z, Glass JD, Schwartz IL, Walter R. *J Med Chem.* 1974; 17:1294–1298. [PubMed: 4427327] Lundell EO, Ferger MF. *J Med Chem.* 1975; 18:1045–1047. [PubMed: 1159688] Manning M, Balaspiri L, Moehring J. *J Med Chem.* 1976; 19:842–845. [PubMed: 950656] Walter R, Skala G, Smith CW. *J Am Chem Soc.* 1978; 100:972–973. Grzonka Z, Lammek B, Kasprzykowski F, Gazis D, Schwartz IL. *J Med Chem.* 1983; 26:555–559. [PubMed: 6187921] Bélec L, Slaninova J, Lubell WD. *J Med Chem.* 2000; 43:1448–1455. [PubMed: 10780900] Yuan Z, Blomberg D, Sethsom I, Brickmann K, Ekholm K, Johansson B, Nilsson A, Kihlberg J. *J Med Chem.* 2002; 45:2512–2519. [PubMed: 12036359] Wittelsberger A, Patiny L, Slaninova J, Barberies C, Mutter M. *J Med Chem.* 2005; 48:6553–6562. [PubMed: 16220972] Kowalczyk W, Prah A, Derdowska I, Soboleski D, Olejnik J, Zabrocki J, Borovicková L, Slaninová J, Lammek B. *J Med Chem.* 2006; 49:2016–2021. [PubMed: 16539389]
8. For carbon instead of sulfur bridge oxytocin derivatives see: Stymiest JL, Mitchell BF, Wong S, Vederas JC. *Org Lett.* 2003; 5:47–49. [PubMed: 12509887] Stymiest JL, Mitchell BF, Wong S, Vederas JC. *J Org Chem.* 2005; 70:7799–7809. [PubMed: 16277299] Muttenthaler M, Andersson A, de Araujo AD, Dekan Z, Lewis RJ, Alewood PF. *J Med Chem.* 2010; 53:8585–8596. [PubMed: 21117646]
9. Wilson RM, Stockdill JL, Wu X, Li X, Vadola P, Park PK, Wang P, Danishefsky SJ. *Angew Chem Int Ed.* 2012; 51:2834–2848.
10. Rao Y, Li X, Danishefsky SJ. *J Am Chem Soc.* 2009; 131:12924–12926. [PubMed: 19697932]

11. (a) Wu X, Stockdill JL, Wang P, Danishefsky SJ. *J Am Chem Soc.* 2010; 132:4098–4100. [PubMed: 20199071] (b) Wu X, Stockdill JL, Park PK, Danishefsky SJ. *J Am Chem Soc.* 2012; 134:2378–2384. [PubMed: 22280518]
12. For the first suggestion of a FCMA in the O-acid series see: Li X, Danishefsky SJ. *J Am Chem Soc.* 2008; 130:5446–5448. [PubMed: 18370392] Li X, Yuan Y, Berkowitz WF, Todaro LJ, Danishefsky SJ. *J Am Chem Soc.* 2008; 130:13222–13224. [PubMed: 18783224] Li X, Yuan Y, Kan C, Danishefsky SJ. *J Am Chem Soc.* 2008; 130:13225–13227. [PubMed: 18783225] Hou J, Ajami D, Rebek J Jr. *J Am Chem Soc.* 2008; 130:7810–7811. [PubMed: 18507459] Restorp P, Rebek J Jr. *J Am Chem Soc.* 2008; 130:11850–11851. [PubMed: 18698780]
13. Wang P, Danishefsky SJ. *J Am Chem Soc.* 2010; 132:17045–17051. [PubMed: 21049949]
14. (a) Kato T, Endo S, Fujiwara T, Nagayama KJ. *Biomol NMR.* 1993; 3:653–673. (b) Johnson LF, Schwartz IL, Walter R. *Proc Natl Acad Sci U S A.* 1969; 64:1269–1275. [PubMed: 5271751] (c) Fischman AJ, Live DH, Wyssbrod HR, Agosta WC, Cowburn D. *J Am Chem Soc.* 1980; 102:2533–2539. (d) Cowburn D, Live DH, Fischman AJ, Agosta WC. *J Am Chem Soc.* 1983; 105:7435–7442.
15. (a) Dawson PE, Muir TW, Clark-Lewis I, Kent SBH. *Science.* 1994; 266:776–779. [PubMed: 7973629] (b) Bang D, Pentelute BL, Kent SBH. *Angew Chem Int Ed.* 2006; 45:3985–3988. (c) Torbeev VY, Kent SBH. *Angew Chem Int Ed.* 2007; 46:1667–1770. (d) Durek T, Torbeev VY, Kent SBH. *Proc Natl Acad Sci U S A.* 2007; 104:4846–4851. [PubMed: 17360367]
16. Warren JD, Miller JS, Keding SJ, Danishefsky SJ. *J Am Chem Soc.* 2004; 126:6576–6578. [PubMed: 15161285]
17. (a) Brailsford JA, Danishefsky SJ. *Proc Natl Acad Sci U S A.* 2012; 109:7196–7201. [PubMed: 22499784] (b) Chen G, Warren JD, Chen J, Wu B, Wan Q, Danishefsky SJ. *J Am Chem Soc.* 2006; 128:7460–7462. [PubMed: 16756298]
18. Villain M, Vizzavona J, Rose K. *Chem Biol.* 2001; 8:673–679. [PubMed: 11451668]
19. Goodman M, McGahren WJ. *J Am Chem Soc.* 1966; 88:3887–3888. [PubMed: 5916382]
20. (a) Kemp DS, Bernstein Z, Rebek J Jr. *J Am Chem Soc.* 1970; 92:4756–4757. [PubMed: 5428884] (b) Kemp DS, Rebek J Jr. *J Am Chem Soc.* 1970; 92:5792–5793. [PubMed: 5458746]
21. Benoiton NL, Lee YC, Steinaur R, Chen FMF. *Int J Peptide Protein Res.* 1992; 40:559–566. [PubMed: 1286941]

Figure 1.
Oxytocin (1) and dihydro-oxytocin (2).

Figure 2.
¹H NMR Spectra of oxytocin (**1**), in red, and dihydro-oxytocin (**2**), in blue.

Scheme 1.
Synthesis of intermediate **19**.

Scheme 2.
Synthesis of oxytocin (1) and dihydro-oxytocin (2).

Scheme 3.
Synthesis of oxytocin (**1**) through NCL.

Scheme 4.

Attempted synthesis of **1** through isonitrile/HOBt-mediated couplings.

Scheme 5.

Mechanism of epimerization of activated thio-FCMA or HOBT ester.