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Revisiting Oxytocin through the Medium of Isonitriles

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Supporting Information

ABSTRACT: The reaction of thioamino acids and N-terminal peptides, mediated by hindered isonitriles and hydroxybenzotriazole, 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 dihydrooxytocin suggest a high level of preorganization in the latter, perhaps favoring oxidative folding.

Oxytocin (**1**) is a long-known, well-appreciated 9-mer peptide hormone that enables parturition and lactation in nursing mothers.[1,2] More recently, there has been a growing perception that the receptor-binding characteristics of non-apeptides, including **1**, 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 **1** 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 **1** 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 **1** itself has been synthesized several times,[6] the corresponding "dihydro" compound **2** apparently has not been characterized. We also hoped to reach characterizable **2** as part of our total synthesis effort. We would then be in a better position to understand the structural changes that must occur as a consequence of oxidative disulfide formation in the parent system, particularly in the analogues we would be synthesizing and evaluating.[7,8]

In addition to a clear basis for interest in **1** at various biological levels, we also viewed it as a compelling object molecule for testing the usefulness and scope of methodology we have recently been developing that involves 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 remarkably mild conditions*. The reaction has been shown to commence with an addition event that affords a thio-formimidate−carboxylate mixed anhydride (thio-FCMA) (**6**). The latter is apparently a very powerful acyl donor that provides an amide (**7**) upon reaction with a suitable amine.[12] While the thioacid−amine coupling can occur with no further additives, it was recently shown to be enhanced by the presence of hydroxybenzotriazole (HOBt).[13] It seems likely 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 **1** 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 the synthesis of our proposed analogues.

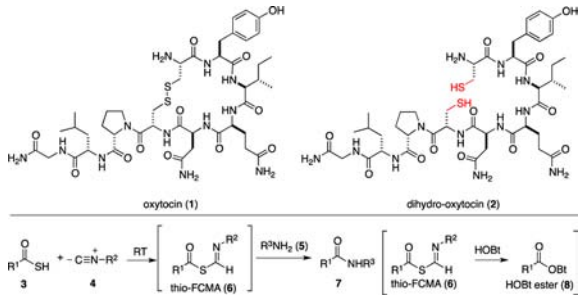

Figure 1. Oxytocin (**1**) and dihydrooxytocin (**2**).

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

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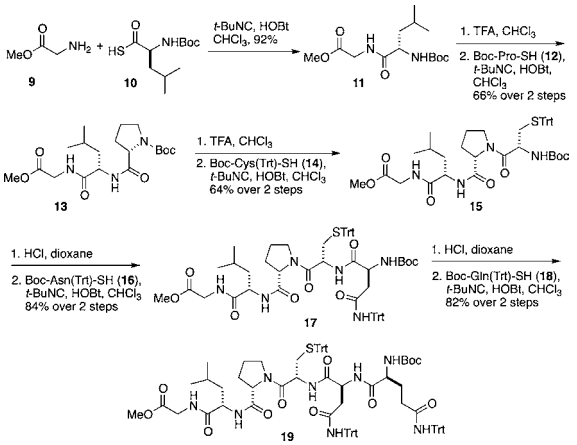
Scheme 1. Synthesis of Intermediate 19



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 **2** as a homogeneous entity. Finally, aeration of **2** in pH 7 aqueous solution furnished **1** in 63% isolated yield (Scheme 2). The 600 MHz ^{1}H NMR spectra of homogeneous **2** and **1** are provided for comparison (Figure 2). Their striking similarity in terms of both appearance and level of definition, 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 C→N elongation synthesis of **1**, we next investigated the applicability of isonitrile chemistry to enable 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 show that the thioacid/isonitrile/HOBt-mediated amidation can be used to provide coupling substrates for an NCL ligation. We particularly hoped to use our orthomercaptoaryl ester rearrangement (OMER) variation of classical NCL to generate the *o*-hydroxy-activated phenyl thioester.[16,17]

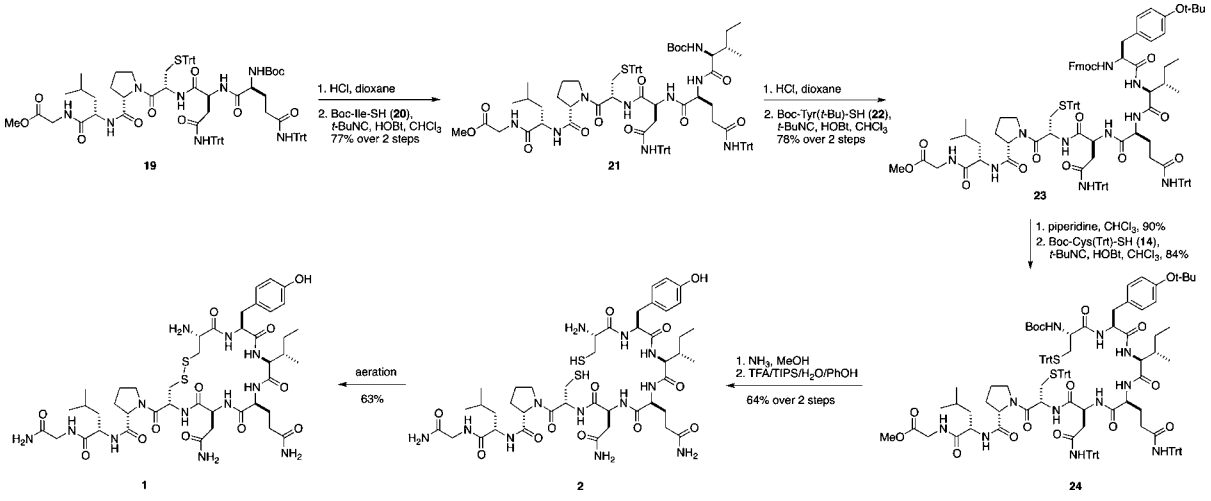
Accordingly, protected glutamine **26** was prepared from **25** in 87% yield by esterification (Scheme 3). Removal of the *t*-Boc group followed by coupling with isoleucine-derived thioacid **20** in the presence of *t*-BuNC and HOBt afforded dipeptide **27** in 83% yield. After elongation with thioacid **22** by isonitrile chemistry, the resulting adduct was treated with 20% piperidine/$CHCl_3$ solution to furnish tripeptide **28**. Elongation of **28** via isonitrile-mediated coupling of 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, H_2O) provided NCL ligation partner **32**. The complementary ligation element **33** was prepared from **15** via aminolysis followed by global deprotection to liberate the N-terminal cysteine.

The merger of **32** and **33** occurred smoothly in a pH 7.2 buffer solution, providing a ligation product that upon treatment with *O*-methylhydroxylamine[18] furnished **2** with no detected epimerization. As before, aeration of **2** gave **1**. Since this remarkably smooth chemistry gives rise to readily purifiable compounds, it was not surprising that the 600 MHz ^{1}H NMR spectra of **1** and **2** were identical to those obtained by the linear route described above.

We next evaluated the feasibility of a more risky proposition, namely, the possibility of using the thioacid/isonitrile/HOBt-mediated amidation reaction (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**; see Scheme 5) 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 the corresponding carboxylic acid **34** through use of tetrabutylammonium fluoride (TBAF) (61% over two steps from **28**). Coupling of **34** with aspartate-derived 9-fluorenylmethyl (Fm) thioester **35** followed by liberation of the thioacid afforded the required **36** in 70% yield.

Scheme 2. Synthesis of Oxytocin (1) and Dihydrooxytocin (2)



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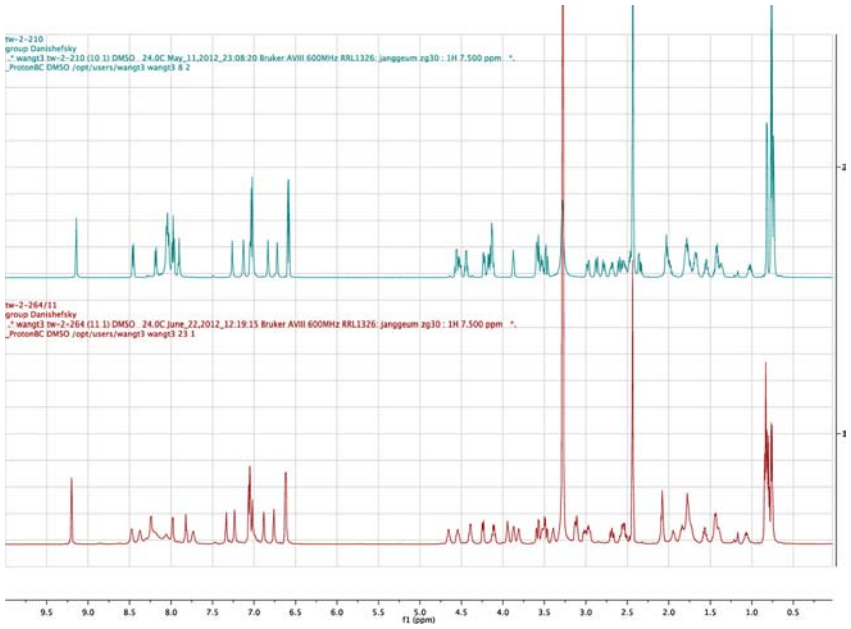
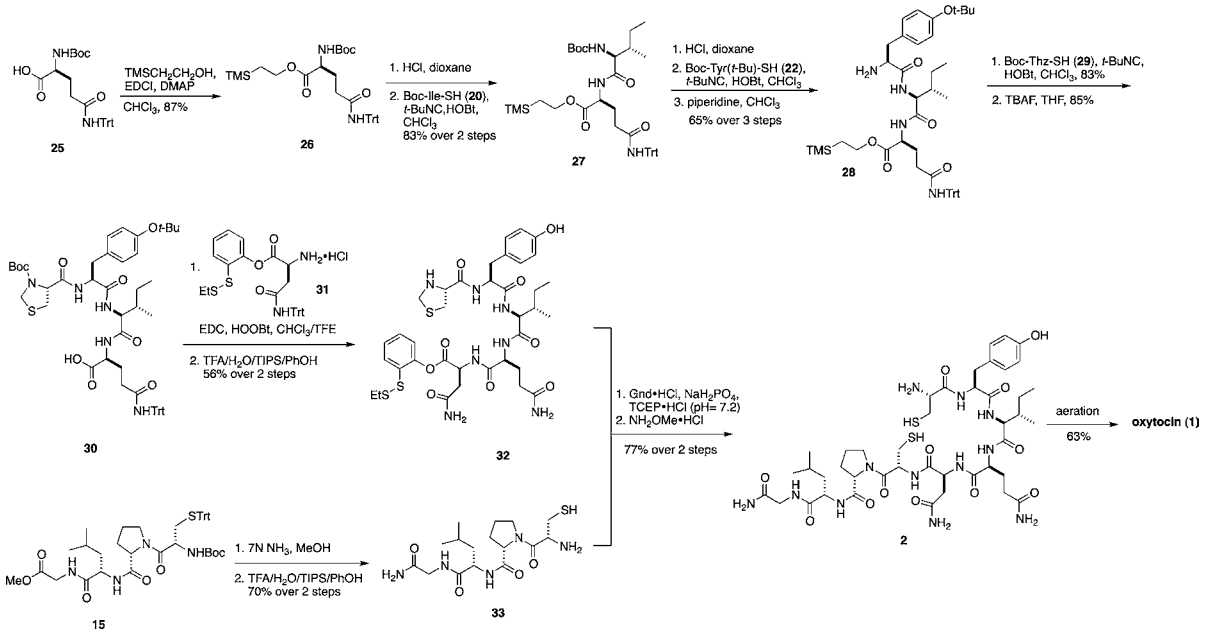


Figure 2. ^{1}H NMR spectra of **1** (red) and **2** (blue).

Scheme 3. Synthesis of 1 through NCL



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, **24** was accompanied by significant amounts of its D-aspartate-containing epimer **38**, presumably formed by the mechanism shown in Scheme 5. Even after significant attempts to optimize the stereointegrity in the coupling step, the best **24**:**38** ratio we obtained was only 6:1. While the feasibility of suppressing 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 was iterated eight times to produce homogeneous dihydrooxytocin (**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 600 MHz ^{1}H spectra of **2** and **1** suggests that oxidative folding could well be facilitated by considerable preorganization. Studies involving the extension of isonitrile chemistry to more automatable settings in polypeptide synthesis as well as its application to the facilitation of structure–activity

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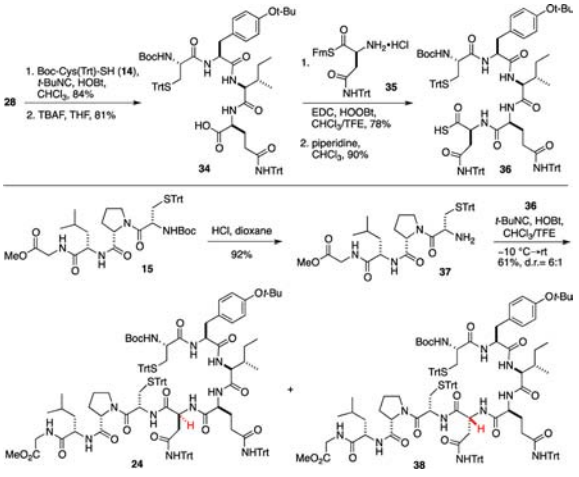
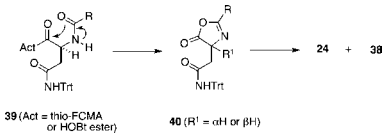
Scheme 4. Attempted Synthesis of 1 through Isonitrile/HOBt-Mediated Couplings



Scheme 5. Epimerization Mechanism



relationship (SAR) studies of **1** and related behavior-modifying congeners are in progress.

■ ASSOCIATED CONTENT

Supporting Information

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.

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Notes

The authors declare no competing financial interest.

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