

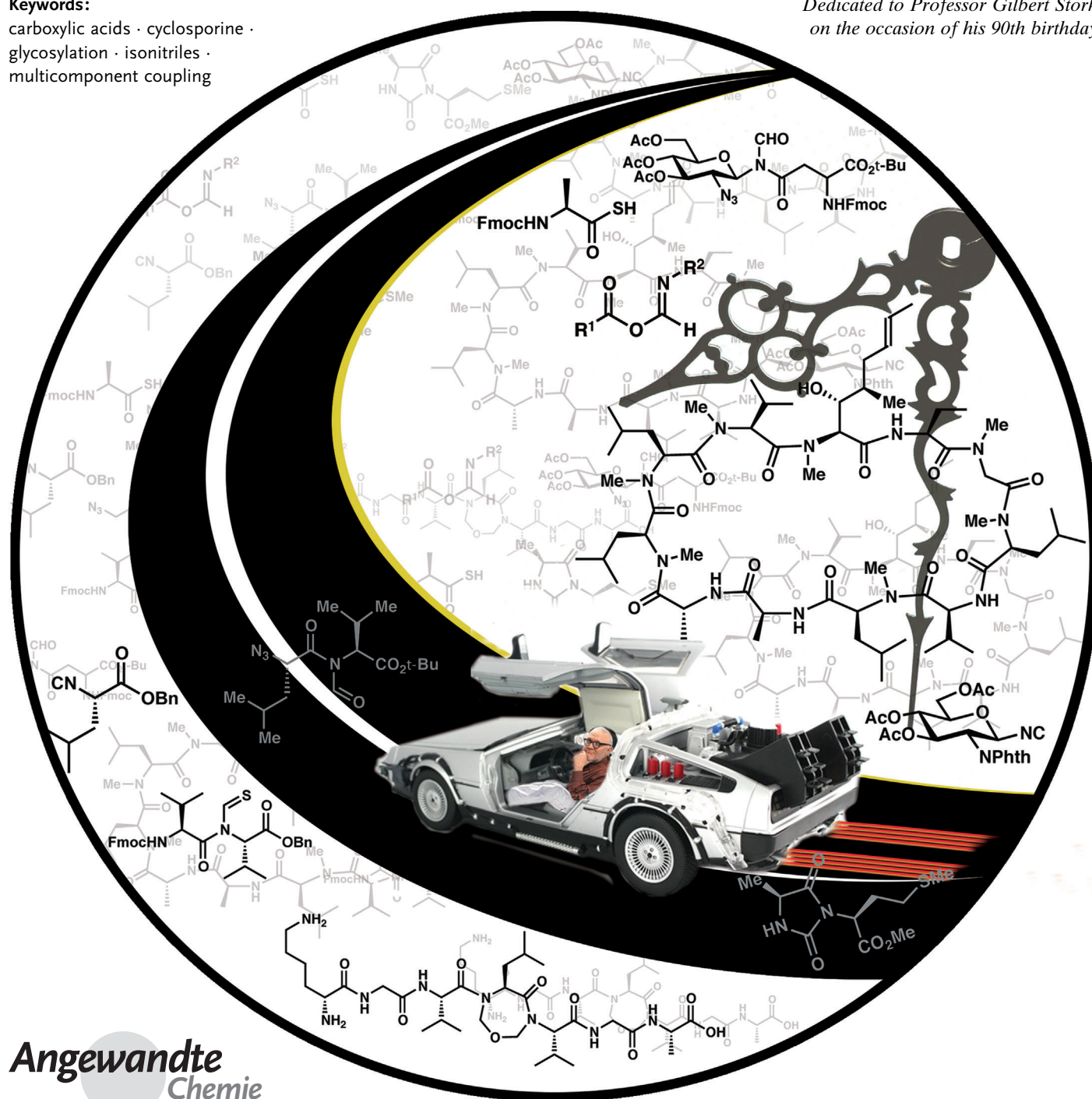
A Fascinating Journey into History: Exploration of the World of Isonitriles En Route to Complex Amides

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multicomponent coupling

*Dedicated to Professor Gilbert Stork
on the occasion of his 90th birthday*



We describe herein our recent explorations in the field of isonitrile chemistry. An array of broadly useful coupling methodologies has been developed for the formation of peptidyl and glycopeptidyl amide bonds. We further describe the application of these methods to the syntheses of complex systems, including the cyclic peptide cyclosporine A, constrained peptide systems, and heterocycles.

1. Introduction

In recent years, our laboratory has been concerned with the development of improved methods for the synthesis of polypeptides and glycopeptides of biological importance. As a result of methodological advances from many quarters,^[1] including our own,^[2] it is now possible to gain access to complex glycopeptides and even modestly sized glycoproteins through purely synthetic means. Drawing inspiration and insights from established principles of mechanistic chemistry, we continue to pursue fresh ideas and strategies for the efficient formation of amide bonds housed in complex “biologic” settings. Along these lines, we recently developed a novel mechanistic platform, with potentially far-reaching applications, which exploits the rather unique reactivity of the isonitrile functional group.^[3] This Review is offered to summarize the body of advances that have been realized by revisiting the chemistry of isonitriles.

We begin with a few introductory observations. The impressive growth in the capacity of chemists to synthesize complex structures has arisen less from exceptional new insights into abstract issues of strategy than from explosive developments in enabling synthetic methodology. It can hardly be disputed that the major driving force in the growth of increasingly powerful new reactions in synthesis, has been the recognition of the potential of mechanistically inspired organometallic chemistry to allow for bond constructions that were previously difficult to achieve. This review will deal with what we regard as a significant advance in enabling methodology, pursued from a dramatically different type of perspective. What we describe below was initially undertaken to feed our collective curiosities about a class of compounds, namely isonitriles, which date from the early history of systematized organic chemistry.^[4]

As will be seen, the subject of isonitriles is a very old one in organic and organometallic chemistry. Moreover, isonitriles are of considerable contemporary interest with respect to their tendencies in cycloaddition and insertion reactions, as well as in reactions with various “standalone” and Lewis acid-activated electrophiles. It is not our intention here to provide a review of the chemistry of isonitriles.^[3] Rather, what we offer the reader is a perspective of how our particular research laboratory became interested in isonitriles, and a travelogue describing our own personal journey. Moreover, our story focuses strictly on recently reported uses of isonitrile chemistry with respect to amide bond formation. These, now logical, reactions might well have been discovered a century ago but, in fact, were not (at least as judged by published information which survived scrutiny). That these advances were still ripe for discovery in 2006, arose from a failure of

earlier work to address some simple core questions. Curiously, earlier chemistry in this subfield had been posed in a more complex form. In retrospect, it seems that the now-obvious chemistry we describe below was obscured by the higher complexity-level at which the questions had been posed (as well as by several serious, if well intentioned, errors at the level of structure). The reader is urged to peruse our primary publications, provided below, for the epistemology which governed these isonitrile–amide bond related discoveries.

Before launching into our exploration of isonitriles, it is perhaps appropriate to begin by noting that the nitrogen counterpart of an acylium ion is called a nitrilium ion. In principle, two obvious routes for the generation of nitrilium ions present themselves. One begins with N-protonation (or N-alkylation) of a nitrile. The most celebrated reaction of this type, which well reflects the highly exploitable electrophilicity of nitrilium ions, is the Ritter Reaction.^[5] Another obvious possible route to a nitrilium ion, though less well appreciated, would be initiated by the carbon of an isonitrile attacking an electrophile. Two notable examples of this mode of reactivity

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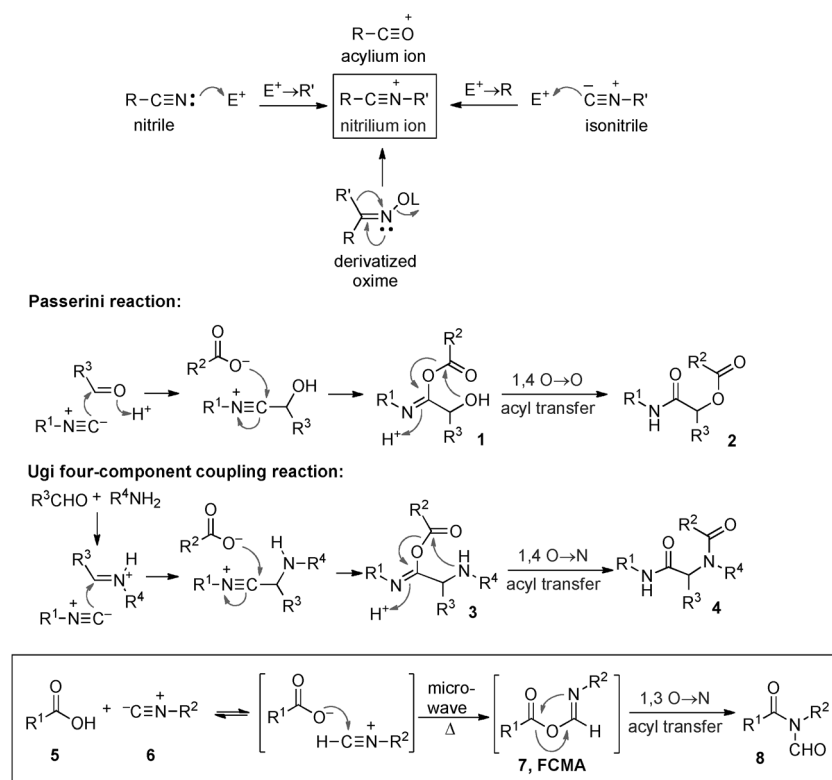
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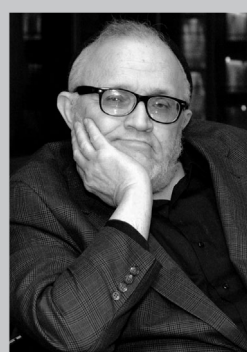
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were devised by Passerini^[6] and Ugi (Scheme 1).^[7] Each of these transformations provides access to the mechanistic equivalent of a nitrilium ion through electrophilic functionalization of the carbon atom of an isonitrile. For the sake of completeness, we also note the emergence of nitrilium character in the Beckmann rearrangement of activated oximes.^[8] However, this route was not pursued in our study.



Scheme 1. Synthesis of nitrilium ion; Passerini reaction and Ugi reaction; Type Ia coupling of isonitriles and carboxylic acids.



Samuel J. Danishefsky received his B.S. degree at Yeshiva University. He did his graduate research under Professor Peter Yates and then joined the laboratory of Professor Gilbert Stork at Columbia University as an NIH Postdoctoral Associate. His first academic position was at the University of Pittsburgh, where he joined as Assistant Professor in 1963. He was promoted to Associate Professor, Professor, and University Professor. In January 1980, he moved to Yale University and was named Eugene Higgins Professor in 1981. Appointed by President A. Bartlett Giamatti as Chairman of the Department of Chemistry, he served until 1987. He became Sterling Professor at Yale in 1990 and in 1993 moved back to New York, where he is currently Centennial Professor of Chemistry at Columbia University and the Eugene Kettering Chair and Head of the Laboratory of Bioorganic Chemistry at Memorial Sloan-Kettering Cancer Center. In 1996, he shared the Wolf Prize in Chemistry with Professor Gilbert Stork. In 2006, he was the recipient of the Franklin Medal in Chemistry, the Bristol Myers Squibb Lifetime Achievement Award in Chemistry, and the National Academy of Sciences Award in the Chemical Sciences.

In examining the literature pertinent to the feasibility of isonitriles as precursors of nitrilium ions, we were surprised to find a paucity of indications concerning an obvious possibility, namely, a simple two-component reaction of isonitriles with carboxylic acids. With the exception of a few isolated and seemingly curious entries (see below), this obvious question had not been properly addressed. It seemed to us (a priori)

that a carboxylic acid (**5**) might initially combine with an isonitrile (**6**), even in the absence of a catalyst or a basic additive, to generate a high-energy formimidate carboxylate mixed anhydride (**7**), which we came to describe as a FCMA. The formation of FCMA **7** may occur either through a step-wise process, commencing with protonation of the isonitrile by the carboxylic acid (see Scheme 1) or, alternatively, through direct insertion of the isonitrile into the carboxylic O–H bond through an Alder–ene like mechanism.^[9] In any event, we envisioned that this FCMA intermediate might subsequently undergo spontaneous rearrangement to generate an N-formyl amide adduct of the type **8**.^[10] Clearly, we were drawing precedent from the [1,4]-O→O transfer step in the Passerini process (**1**→**2**) and from the [1,4]-O→N acyl transfer step in the Ugi cascade (**3**→**4**). However, what we were contemplating was something rather more venturesome, that is, a [1,3]-O→N acyl transfer step (**7**→**8**). Furthermore, given the absence of external acidic or basic agents in our projected reaction, epimerization issues that often compromise the value of amide bond-forming reactions could well be attenuated.

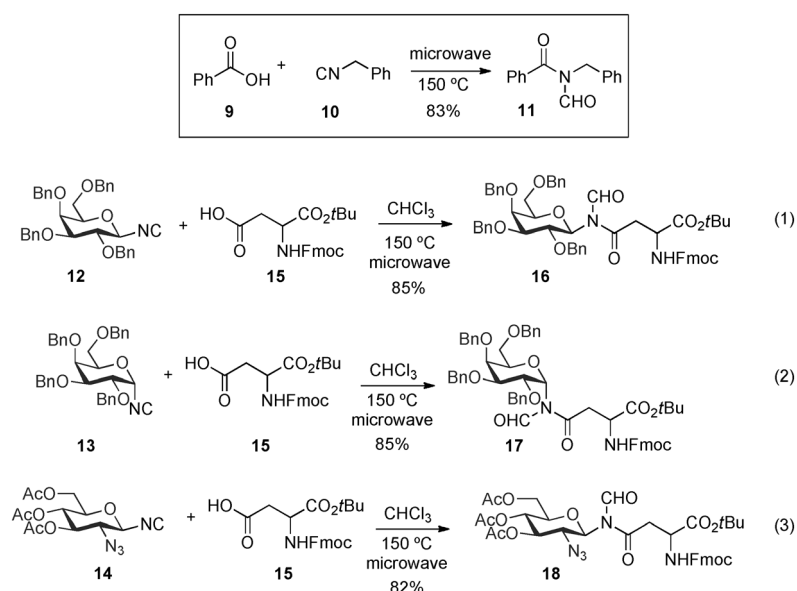
As will be shown, we have successfully reduced these concepts to practice.

Through extension of the original premises, an array of broadly useful coupling methodologies has been developed for the formation of peptidyl and glycopeptidyl amide bonds. We review herein these recent advances and provide applications of emerging methodologies to help tackle complex synthetic challenges.

2. Isonitriles and Carboxylic Acids: Coupling Types

2.1. Type Ia Coupling of Isonitriles and Carboxylic Acids

We first explored the concept of 1:1 isonitrile-carboxylic acid coupling in the context of benzoic acid (**9**) and benzyl isonitrile (**10**). Although no product was observed at room temperature, the desired coupling did occur under thermally induced microwave irradiation conditions (ca 130 °C), to provide N-formyl amide **11** in 83 % yield (Scheme 2). In order to classify the variants of isonitrile-based coupling reactions that have subsequently been developed in our laboratory, we assign the generic term “Type Ia”^[11] isonitrile-based coupling



Scheme 2. Assembly of glycosylamino acids through Type Ia coupling.

to the original carboxylic acid/isonitrile form of the transformation. We next demonstrated the ability of the Type Ia coupling to enable the construction of asparagine-linked glycosylamino acids. As shown in Scheme 2, a range of glycosyl isonitriles (**12–14**) were successfully merged with aspartate **15** to provide N-formyl glycosylamino acids (**16–18**).

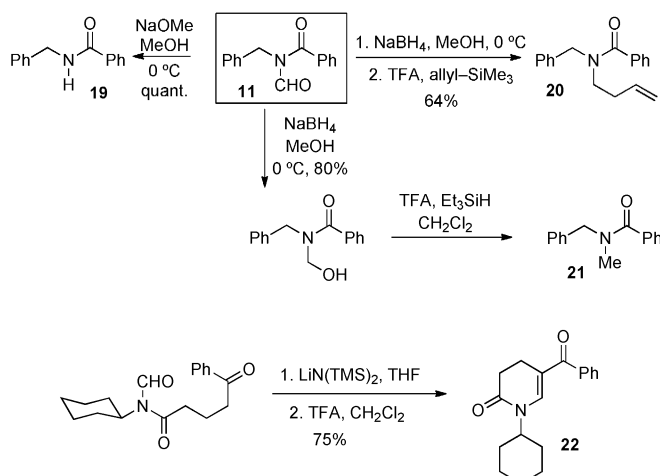
As seen above, the Type Ia coupling reaction delivers an N-formyl moiety to the nitrogen atom of the resultant amide bond. We anticipated that we would be able to achieve selective derivatization of the N-formyl bond, which was expected to be particularly vulnerable to nucleophilic cleavage due to its mixed imide-like character. Indeed, in a simple model series, we have demonstrated the selective conversion of the N-formyl group (**11**) to a variety of potentially useful functionalities, including N-H (**19**), N-homoallyl (**20**), and N-Me (**21**). In a separate system, the N-formyl amide adduct arising from a Type Ia coupling was readily cyclized to deliver the cyclic vinylogous imide, **22** (Scheme 3).

2.2. Type Ia Coupling: Mechanistic Studies

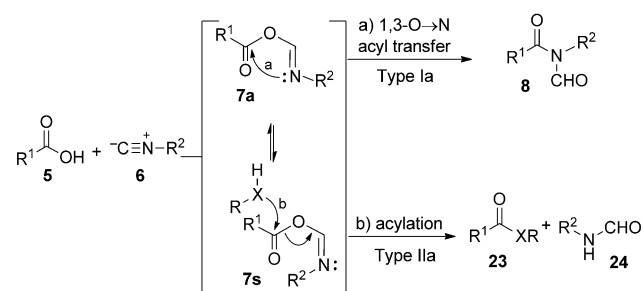
Having demonstrated the gross feasibility of our Type Ia coupling sequence, we next sought to undertake mechanistic studies into the nature of the transformation. As described above, we had postulated that the reaction would proceed through the intermediacy of a highly reactive FCMA species, which would spontaneously undergo [1,3]-O→N acyl transfer, generating N-formyl amide products (**5** + **6** → **7** → **8**, Scheme 4). We had also observed that in the presence of external nucleophilic trapping agents (RXH), there also arise products of type **23**, in which the putative FCMA intermediate has presumably been intercepted by the nucleophile prior to rearrangement. In a generic sense, we consider the formation of these interrupted coupling products to arise through what we term a “Type Ila” coupling reaction.

In light of our experiences with the high-energy FCMA species, we were skeptical of a number of reports from other groups claiming to have accomplished the isolation of such intermediates as crystalline products under standard conditions.^[12] We were particularly curious about a disclosure in which alleged FCMA species were reported to have been isolated from various benzoic acids and isonitriles in the presence of methanol at room temperature.^[12a] Based on our experiences, we predicted that even if the FCMA species were to be formed, it would have rapidly undergone nucleophilically mediated solvolysis under these conditions. We thus set out to explore these claims, and found that in each case, the observed product had been misassigned as a FCMA species.^[13] Thus, to our knowledge, no FCMA has yet been isolated under usual reaction conditions.^[14]

The formation of the observed products is consistent with the intermediacy of a highly reactive FCMA species (**7**), which rapidly undergoes either [1,3]-O→N acyl transfer to provide an N-formyl amide, **8** (Type Ia), or in the presence of an external nucleophile, acylation to generate **23** and **24** (Type Ila).^[15] Of



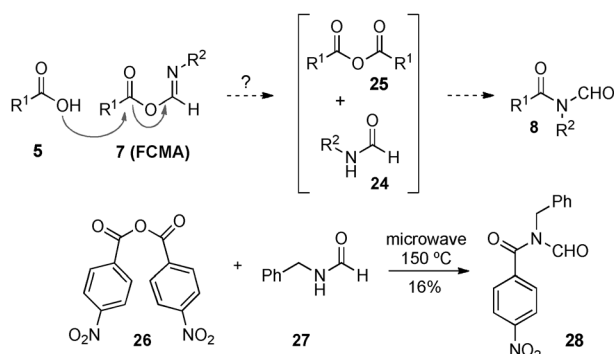
Scheme 3. Methods for the selective derivatization of the N-formyl functionality arising from Type Ia coupling.



Scheme 4. Type Ia and Type Ila coupling: Mechanistic considerations.

interest to us from a mechanistic standpoint was the product distribution observed when the FCMA is formed in the presence of an exogenous nucleophile (RXH). Under these conditions, a mixture of Type Ia (rearrangement) and Type IIa (acylation) products is obtained. It may be that a single FCMA intermediate is formed, which can either undergo rearrangement (**8**) or nucleophilic trapping (**23**). Alternatively, the observed product ratio might be a consequence of concurrent formation of two FCMA isomers—the *anti* (**7a**) and *syn* (**7s**) isomers. The former would be well positioned to undergo rapid [1,3]-O→N acyl transfer, and might be already “committed” to forming **8**. In contrast, the non-competent *syn* isomer would be susceptible to nucleophilic attack in the presence of an appropriate nucleophile. Presumably, in the absence of a suitable acyl acceptor, the non-rearranging **7s** isomer would revert to the rearranging **7a** form. We have obtained some evidence in support of this view. Under our standard coupling conditions, we observed the formation of rearranged and intercepted products in the presence of either 1 or 5 equivalents of external nucleophile (MeOH). Interestingly, a large increase in methanol did not lead to any significant increase in formation of the Type IIa methyl ester product (ca. 40%). We best interpret these results as suggesting that there are produced two geometrically isomeric FCMA intermediates: **7s**, which is highly susceptible to methanolysis, and **7a**, which is committed to undergoing intramolecular rearrangement, even in the presence of excess nucleophile.

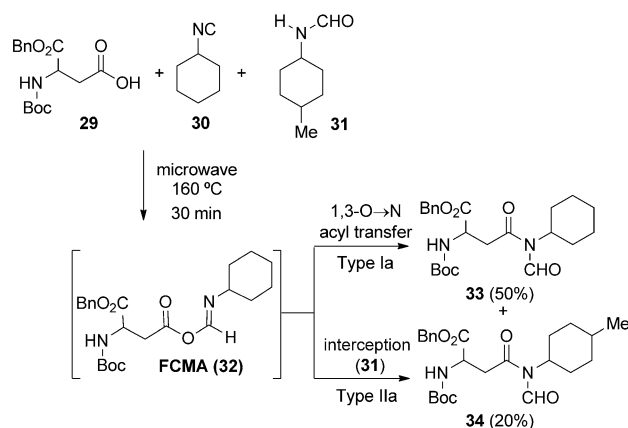
In attempting to elucidate the mechanistic intricacies of our Type Ia coupling reaction, we also took note of the possibility of an alternative FCMA-based pathway, which would not involve a [1,3]-O→N acyl rearrangement to produce N-formyl amide products. As outlined in Scheme 5, FCMA intermediate **7** would be attacked by carboxylic acid **1** to produce symmetrical anhydride **25** and formamide **24**. N-acylation of **24** with **25** would furnish the observed product (**8**). This potential pathway was examined in the context of the N-formyl amide adduct **28**, which is readily formed under our coupling conditions. Thus, if the symmetrical anhydride pathway were viable, the intermediates formed from addition of acid to FCMA (**26** and **27**) would be expected to combine to produce amide **28** under standard coupling conditions. In the event, however, only a 16% yield of **28** was obtained from



Scheme 5. Evaluation of a potential formamide-symmetrical anhydride pathway.

coupling of **26** and **27** under microwave irradiation. These results imply that the symmetrical anhydride pathway is certainly not dominant under our reaction conditions.

Finally, we sought to evaluate the reactivity of the intermediate FCMA species. In this context, we wondered whether a weakly nucleophilic formamide would be capable of nucleophilic attack on a FCMA intermediate. As described above (Scheme 5), we had previously demonstrated that an intermediate formamide would not be sufficiently nucleophilic to efficiently trap a symmetrical anhydride (**26** + **27** → **28**). As shown in Scheme 6, carboxylic acid **29**, isonitrile **30**, and formamide **31** were subjected to standard reaction



Scheme 6. Evidence for the existence of a highly reactive FCMA intermediate (**32**).

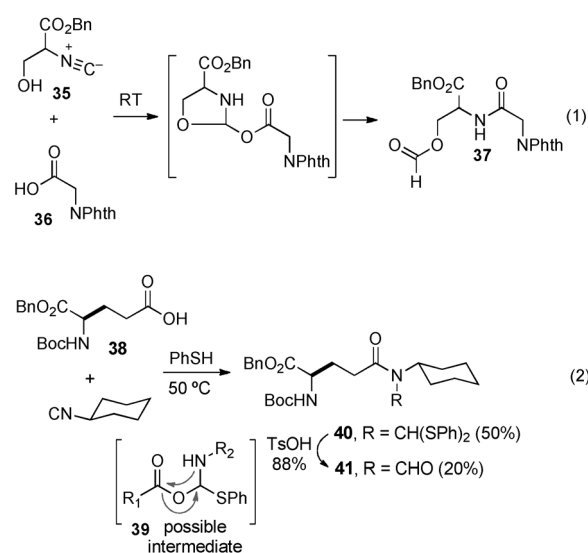
conditions. After 30 min, the N-formyl amide **33**, arising from [1,3]-O→N acyl transfer of the presumed FCMA (**32**), was isolated in 50% yield. However, we also obtained a substantial amount (20%) of cross-over product, **34**, presumably arising from interception of the intermediate species by formamide **31**. These data lend support to the supposition that a species which is a more powerful acylating agent than anhydride (presumably FCMA **32**) is produced in the course of the reaction.

2.3. Development of a Modified Type Ia Coupling Protocol

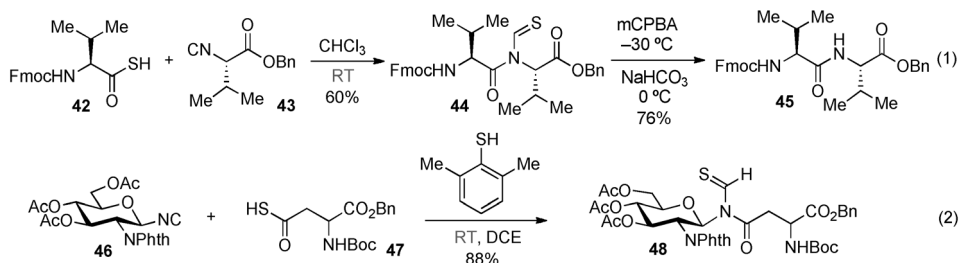
We recognized that, in order for this method to find extensive application to complex biologic targets, we would need to develop a set of conditions that would allow for reaction under ambient temperatures. The high temperatures required for coupling (150 °C) could be problematic in the context of often sensitive, but biologically important products, such as many peptides and glycans. Along these lines, we took note of an earlier finding from our laboratory, wherein Type II products (of the type **23**) could be obtained at room temperature. It seems that, although the FCMA intermediate is actually generated at ambient temperature, thermolysis is required to achieve [1,3]-O→N acyl rearrangement leading to the desired product. We had separately observed that a serine-derived isonitrile substrate, **35**, presenting a free

alcohol moiety, reacted with a carboxylic acid (**36**) at room temperature, to generate a rearrangement product, **37**, in which the formyl group had transferred from the nitrogen to the hydroxy group (Scheme 7).^[16] Interestingly, when the proximal alcohol was protected, no rearrangement product was observed. Seemingly, the resident free hydroxy group had served to promote formation or activation of the FCMA species, perhaps through an activated cyclic intermediate, as shown in Scheme 7.

In light of these observations, we sought to examine the consequences of adding an *external* nucleophile to the reaction, in the hopes of achieving a similar outcome in a generic substrate. Indeed, we were pleased to observe that, in the presence of thiophenol at 50 °C, carboxylic acid **38** underwent Type Ia coupling with cyclohexylisonitrile to afford a mixture of thioacetal (**40**) and N-formyl amide (**41**) products. The former may be converted to the latter in a single, high-yielding step (88 %). The role of the thiophenol in facilitating the rearrangement has not yet been established. In principle, at least, the transformation may well proceed through a FCMA-derived tetrahedral intermediate, **39**, which could suffer more rapid intramolecular acyl transfer than is the case with the unmodified FCMA, to generate the observed products (Scheme 7).



Scheme 7. Mild conditions for the Type Ia coupling reaction.



Scheme 8. Type Ib coupling of thioacids and isonitriles.

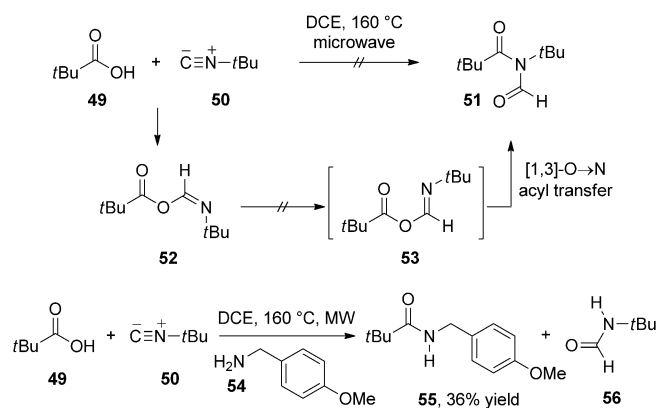
2.4. Type Ib Coupling: Isonitriles and Thioacids

Further progress toward our goal of developing a mild and general route for amide bond formation was achieved with the discovery that thioacids, as opposed to carboxylic acids, readily undergo coupling with isonitrile substrates at room temperature [Scheme 8, Eq. (1)].^[17] The merger of isonitriles and thioacids is termed a “Type Ib” coupling.^[11] A range of amino thioacids were found to react with amino acid isonitrile substrates to generate dipeptide products in good yields. Interestingly, product yields were generally observed to correlate positively with steric hindrance of the isonitrile. This trend may be attributed to suppression of thio-FCMA cleavage in hindered cases. Conceivably, these bulky thio-FCMA intermediates may also be more prone to undergo rapid *syn*→*anti* isomerization, thereby enhancing the rate of the [1,3]-S→N acyl transfer. Dethioformylation of the dipeptide products was achieved through a two-step protocol, involving treatment with mCPBA followed by sodium bicarbonate (**44**→**45**, Scheme 8).

Thioacid substrates were also found to be competent coupling partners in reactions with glycan isonitriles.^[18] As shown in Scheme 8 [Eq. (2)], thioacid **47** and isonitrile **46** readily underwent coupling at room temperature in the presence of 2,6-dimethylthiophenol activator, to afford the desired glycosylamino acid **48** in 88 % yield.

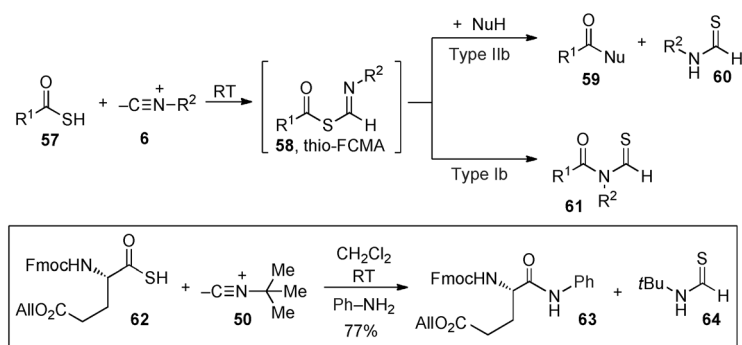
Our attempts to extend Type Ia and Type 1b couplings to highly hindered substrates were unsuccessful. In a telling

example, exposure of pivalic acid **49** and hindered isonitrile **50** to microwave irradiation under thermal conditions did not lead to formation of the rearrangement product expected on the basis of precedent (Scheme 9).^[19] It seems that either the neopentyl coupling partners could not form the FCMA species or, alternatively, a FCMA intermediate, so formed, would be unable to undergo the [1,3]-O→N migration step, presumably due to excessive steric hindrance. Interestingly, when an external nucleophile (**54**) was added to the reaction,



Scheme 9. Coupling of hindered isonitriles.

amide **55**, arising from interception of an intermediate FCMA species, was obtained. This finding would seem to establish that the neopentyl FCMA species is indeed generated under the reaction conditions, but is then unable to undergo the requisite [1,3]-O→N acyl transfer to deliver Type Ia adducts. An analogous finding was observed in Type Ib settings. The critical observation that highly hindered isonitrile substrates form FCMA intermediates that are impervious to rearrangement yet susceptible to external attack prompted us to pursue a new approach to isonitrile-based acylation.



Scheme 10. Type IIb coupling: Thio-FCMA intermediates as strong acyl donors.

2.5. Type IIb Coupling: The Use of thio-FCMA Intermediates as Particularly Strong Acyl Donors

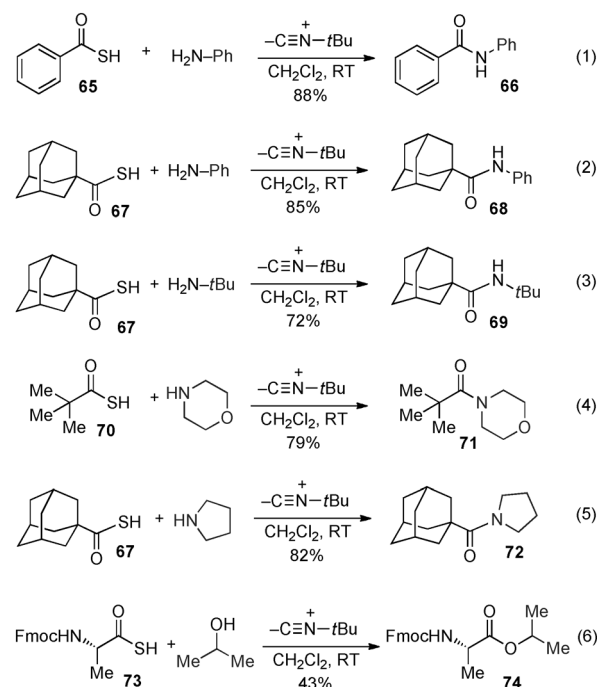
As described above, in the presence of a nucleophile, FCMA and thio-FCMA intermediates can undergo reaction by either of two competing pathways: intramolecular acyl migration (Type Ia or Ib) or bimolecular reaction with an acyl acceptor (Type IIa or IIb).^[20,11] Our initial efforts had focused mainly on promoting the acyl migration pathway, wherein the two substrates—that is, thioacid **57** and isonitrile **6**—are both represented in the final product (**61**, Scheme 10). Although we have developed methods for the efficient syntheses of a variety of value-added isonitrile substrates,^[21] we recognized that the need to pre-assemble complex isonitrile substrates could be a disincentive to the broad applicability of this approach, particularly in the context of large peptidic or glycan substrates.

Based on the knowledge that hindered isonitriles do not undergo [1,3]-acyl transfer, a promising variation presented itself. We considered the possibility of employing such hindered isonitriles to effectively suppress the Type Ib [1,3]-S→N acyl migration pathway from thio-FCMA **58**, thus favoring the bimolecular acylation (Type IIb) pathway from thio-FCMA **58**. If the failure of hindered isonitriles to undergo intramolecular acyl transfer was not contingent on the thioacid coupling partner also being hindered, it should be possible to accomplish the direct coupling of substrates such as **57** with external nucleophiles (NuH) to produce adducts of the type **59**. Under this plan, the hindered isonitrile (**6**) would serve as a simple “throwaway” (but recyclable!) reagent, and following interception by the amine nucleophile, an isonitrile-derived recyclable debris product **60** would be extruded along with the product. Needless to say, the benefit of such a plan would be that the amine substrate could be employed directly in the coupling, obviating the need to pre-assemble a high value-added isonitrile function.

We were gratified to find that, as expected from our earlier results, treatment of thioacid **62** with the bulky *tert*-butylisonitrile (**50**) and aniline readily resulted in coupling at room temperature to provide amide adduct **63** in 77% yield.

This powerful new acylation method nicely accommodates a range of substrates, including bulky thioacids and amines [Scheme 11, Eq. (2) and (3)], secondary amines [Eq. (4) and (5)] and even alcohol nucleophiles [Eq. (6)]. Of particular note is the smooth formation of products in which

both sides of the resultant amide bond are highly hindered. Needless to say, the formation of isopropyl ester **74**, under these conditions, is particularly convincing testimony to the high reactivity of the intermediate acyl donor (presumably the corresponding thio-FCMA).



Scheme 11. Scope of Type IIb coupling reaction.

3. Cyclosporine

To further evaluate the complexity-building potential of our isonitrile-based findings under “battlefield realities”, we sought to apply them to a total synthesis of cyclosporine A.^[22] Isolated from the fungus *Tolypocladium inflatum gams*,^[23] cyclosporine A exhibits potent immunosuppressive properties and is used to facilitate organ transplantation as well as in the treatment of immune system-related disorders including psoriasis and rheumatoid arthritis.^[24] Cyclosporine A is a cyclic peptide containing seven sites of N-methylation, which are apparently essential to the observed biological activity.

Our isonitrile-based methods, which can deliver N-methyl functionality at the site of ligation, were seen to be well suited to the synthetic challenges posed by cyclosporine A.

Our plan for accomplishing the total synthesis of cyclosporine A envisioned the assembly of two peptidic fragments, **81** and **91**, through recourse to the recently discovered isonitrile-based methods (Scheme 12 and 13). In the concluding stages of the synthesis, fragments **81** and **91** were to be joined and ultimately subjected to a late-stage macrolactamization. Indeed, we also hoped to accomplish cycloamidation through application of our Type IIa acylation method.

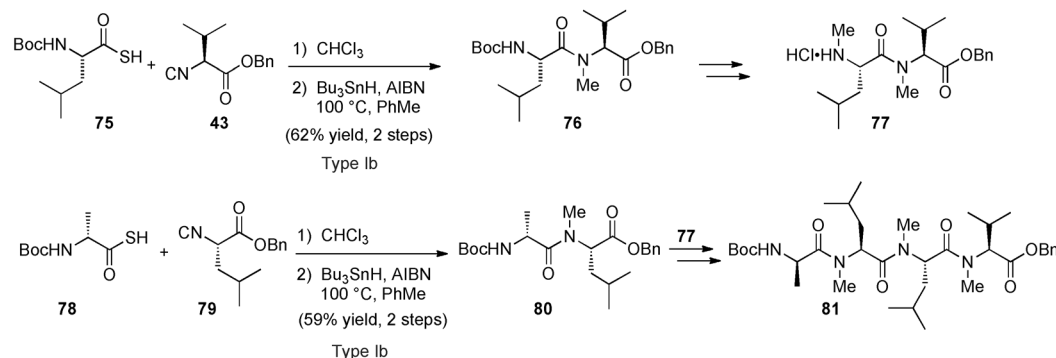
In the event, the synthesis of peptide fragment **81** was achieved through a sequence featuring two Type Ib coupling reactions between thioacid and isonitrile amino acid substrates (Scheme 12). Similarly, fragment **91**, representing the bulk of the peptidic backbone, was assembled by using Type IIB acylation between intermediates **85** and **89**, themselves prepared by using Type Ia and Type IIB methods (Scheme 13).

We were now prepared to merge fragments **81** and **91** in anticipation of the key macrolactamization step. In the event, following removal of the benzyl ester of **81**, the two peptide

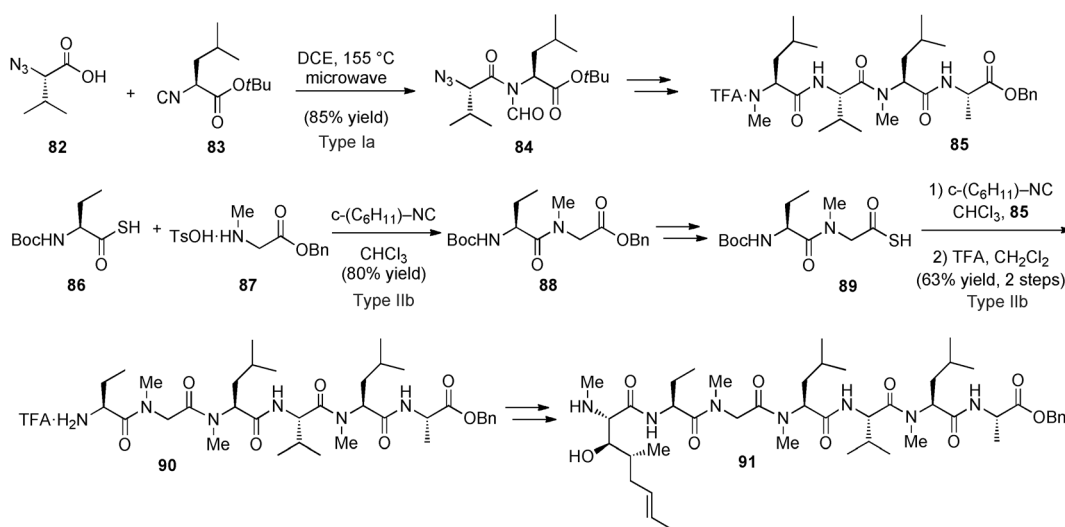
fragments combined to afford polypeptide **92**. As shown in Scheme 14, we were able to successfully extend the logic of the FCMA acylation to the macrolactamization reaction. Thus, when treated with HOBt and cyclohexylisonitrile, the deprotected carboxylic acid derived from **92** underwent macrolactamization to deliver the natural product, cyclosporine A, in 54 % yield from **92**. The success of the macrolactamization in the context of this highly complex system is presumably attributable, at least in part, to the pre-organization of the substrate through intra-strand hydrogen bonding.

In a further demonstration of the broad utility of the emerging isonitrile-based strategy toward amide bond formation, we have recently completed a second-generation route to cyclosporine A.^[25] In designing our second-generation synthesis, we took recourse to a bond-disconnection strategy not unlike that employed in our first route. As shown in Scheme 15, dipeptide **76** (see Scheme 12) was readily advanced to tetrapeptide **81** through iterative Type IIB couplings. Hydrogenolysis of **81** delivered intermediate **94** in excellent overall yield.

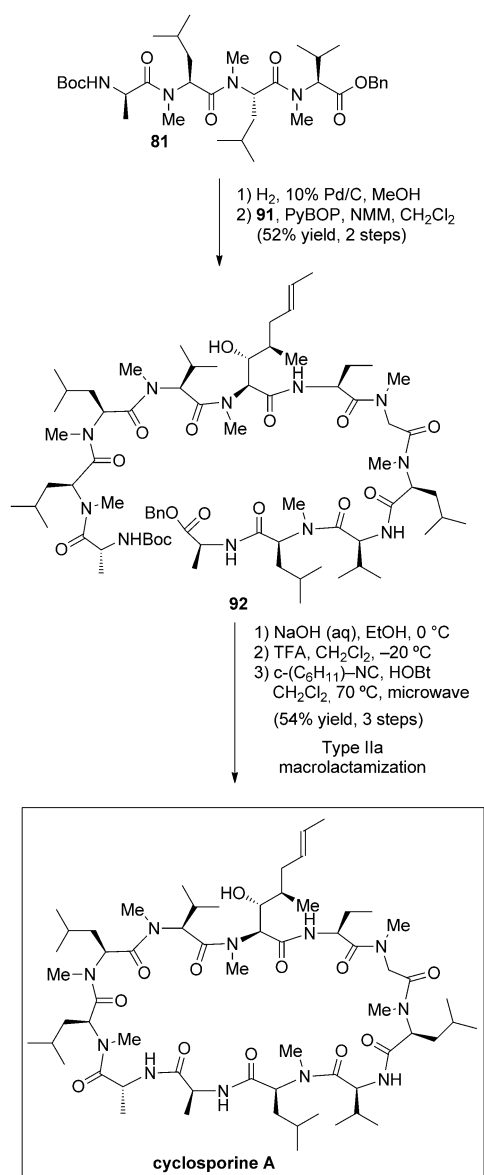
The synthesis of the heptapeptide substrate, **91**, is outlined in Scheme 16. Thus, a series of high-yielding iterative Type IIB coupling and deprotection reactions gave rise to hexapeptide **101b**. In a key transformation, peptide **101b** underwent Type IIa coupling with non-natural amino acid **102**, bearing a free alcohol functionality, to deliver heptapep-



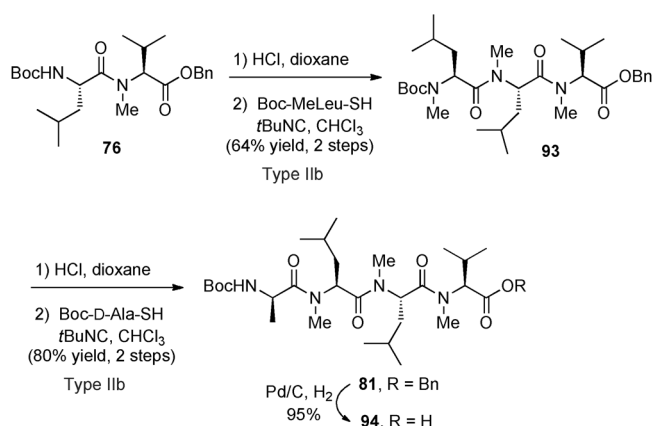
Scheme 12. Synthesis of tetrapeptide **81** en route to cyclosporine A.



Scheme 13. Synthesis of intermediate **91** en route to cyclosporine A.



Scheme 14. Completion of the total synthesis of cyclosporine A.



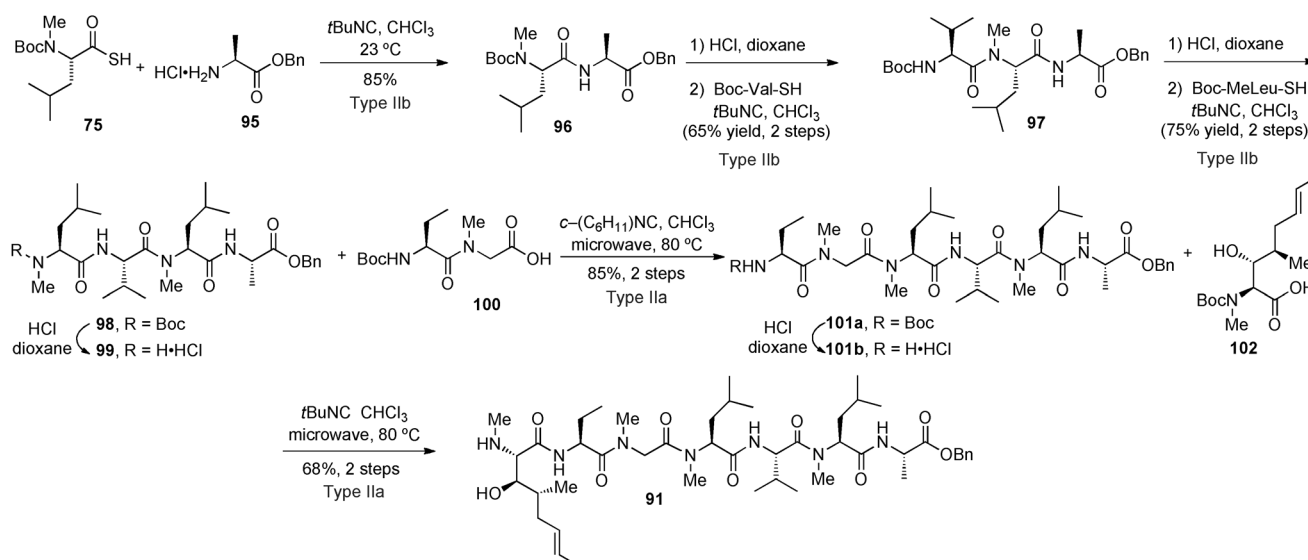
Scheme 15. Second generation route to cyclosporine A: Synthesis of tetrapeptide **94**.

tide **91** in good yield without any apparent epimerization (Scheme 16).

With the key fragments in hand, we next investigated the proposed isonitrile-based Type II coupling between **94** and **91**. Interestingly, under isonitrile-activated Type II conditions, we obtained a 71% yield of *epi*-**92**, wherein the α -carbon of MeVal (see asterisk) had suffered complete inversion (Scheme 17). In light of this finding, the requisite fragment condensation of **94** and **91** was instead accomplished, as precedented,^[22] through the use of PyBOP and NMM. With the desired undecapeptide **92** in hand, we next sought to explore the possibility of enhancing the efficiency of the Type II macrolactamization reaction by incorporating a C-terminal thioacid in lieu of the carboxylic acid at the site of cyclization. Our first-generation route had employed a Type IIa carboxylic acid-based cyclization, and the elevated temperatures required for cyclization had led to an erosion of yield, presumably due to starting material decomposition and formation of by-products. We postulated that a Type IIb thioacid-based macrolactamization would proceed under ambient temperatures, minimizing the opportunity for competing side reactions. Thus, **92** was converted to **103** in 68% overall yield. Removal of the Boc protecting group, followed by exposure to cyclohexylisocyanide in the presence of HOBT at room temperature resulted in nearly complete conversion to cyclosporine A within 10 h, with minimal formation of byproducts.

4. Synthesis of Constrained Peptides

Peptides often exhibit high affinity and selectivity for key biotargets, and thus represent, at least in principle, an important class of lead compound for drug development. However, a serious impediment to the broad success of peptides as clinical agents is their high susceptibility to protease-induced degradation.^[26] In the context of various efforts to address this kind of problem, it has been found that the introduction of conformational constraint elements to these peptidic scaffolds can serve to enhance both pharmacostability and biological activity.^[27] Along these lines, we took note that our Type I coupling protocols, which deliver an N-formyl amide at the site of ligation, could potentially be used to allow access to biologically active peptidic fragments incorporating conformational constraints. In addition to exploiting partial conformational immobilization to influence the affinities of ligands for their receptors, we envisioned that a temporary molecular constraint might serve to mitigate the aggregation tendencies often exhibited by unfolded polypeptides. Of course, we also sought to ensure that the constraint could be readily removed to restore the native peptide sequence.^[28] In this way, a temporary molecular constraint might be used in the context of a total synthesis to stabilize otherwise aggregation-prone peptides en route to the eventual more stable target. More specifically, as outlined in Scheme 18 below, we envisioned iterative Type Ia coupling reactions to generate a polypeptide of the type **108**, bearing N-formyl groups on adjacent “imide-like” nitrogens. We further anticipated conversion, by two-fold reduction, of



Scheme 16. Second generation route to cyclosporine A: Synthesis of hexapeptide **91**.

these proximal formyl groups to generate a constrained tripeptide, **109**. Elongation of the peptide in the N- and C-terminal directions would provide a target polypeptide, **110**, bearing a site-specific cyclic constraint. Finally, removal of the $\text{CH}_2\text{-O-CH}_2$ bridge would reveal the native peptide system, **111**, at a strategically propitious stage.

This plan has indeed been reduced to practice. Thus, in a representative example, carboxylic acid **82** and isonitrile **83** underwent Type Ia coupling to afford, following cleavage of the *tert*-butyl ester, dipeptide **112** in 82% yield (Scheme 19). A second Type Ia reaction with isonitrile **43** generated tripeptide **113**, containing proximal N-formyl functionalities. Interestingly, the resident N-formyl group of **112** had played a key role in ensuring the success of this second coupling reaction, by stabilizing the intermediate FCMA against non-productive oxazolone formation. Next, treatment of **113** with LiBH_4 followed by TFA provided constrained tripeptide **114**. The N-terminal amine, which had until now been housed as an azide functionality, was exposed by reduction, and the N- and C-termini were extended to generate **115**. Finally, treatment with 0.1M HCl and 1,3-propanedithiol delivered the target linear heptapeptide **116** in 95% yield. We note that, in this study, constrained peptides (cf. **110**) were generally found to be much more solubilizable and less prone to aggregation than were their linear counterparts (cf. **111**).

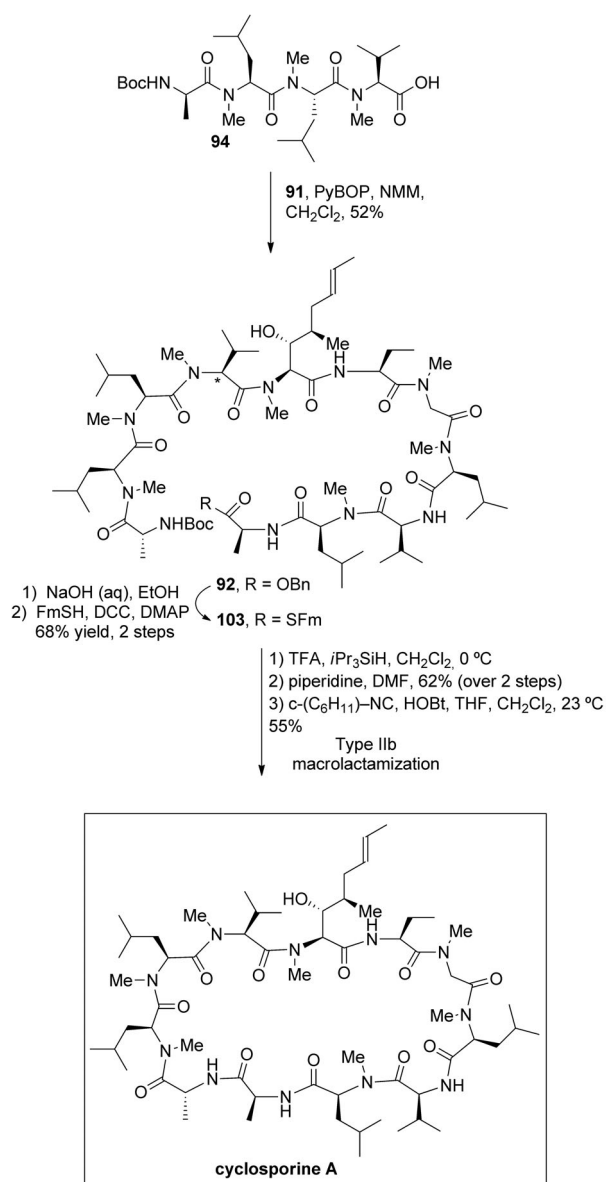
5. Beyond Isonitriles: A General Method for the Ligation of Peptides

Despite the broad utility of the isonitrile-based Type I and Type II coupling reactions described above, we were not unaware of the difficulties inherent in extending our peptide-based coupling to encompass more complex systems. In fact, the Type II coupling of even dipeptidyl thioacids is often low-yielding, apparently due to the rapid conversion of the intermediate dipeptide thio-FCMA to the non-productive

oxazolone intermediate (Scheme 20). In exploring potential solutions to this conundrum, we considered the possibility of achieving in situ conversion of the activated thio-FCMA intermediate to a still more productive acyl donor. Along these lines, we took note of the fact that peptide bonds formed through HOBt ester intermediates tend to exhibit significantly lower levels of C-terminal, oxazolone-induced epimerization.^[29] We thus envisioned a reaction setting wherein a thio-FCMA, derived from a peptidyl thioester and a “throw-away” isonitrile (i.e. cyclohexylisonitrile), would progress to become an HOBt ester. The latter would hopefully undergo ligation with the N-terminal coupling partner.

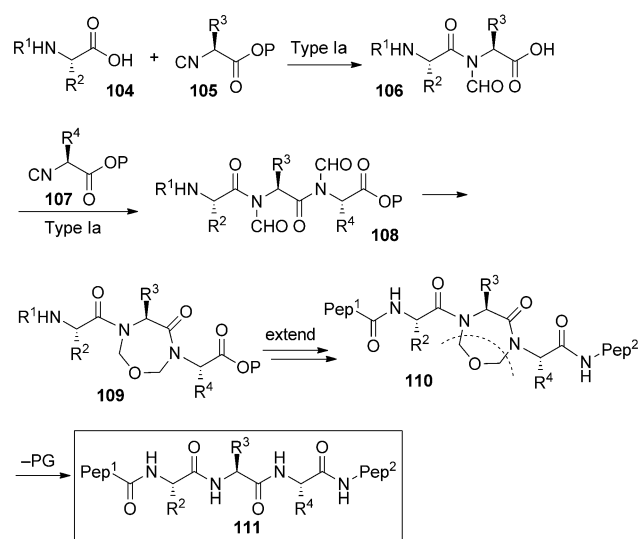
As shown in Scheme 21, this proposed solution was indeed successful.^[30] Thus, in the presence of both HOBt and cyclohexylisonitrile, thioacid peptide **117** and peptide **118** underwent ligation to deliver adduct **119**, within 20 h in 60% yield [Eq. (1)], presumably through the intermediacy of both the thio-FCMA and HOBt ester species (Scheme 20). To our surprise, however, a control experiment revealed that *in the absence of isonitrile*, the coupling of **117** and **118** in the presence of HOBt proceeded in 82% yield over only 12 h [Eq. (2)]. Moreover, these HOBt-based conditions were demonstrated to effectively promote ligation of a range of peptidyl substrates, including those bearing epimerizable C-terminal amino acid residues. In several direct comparisons, ligations performed in the presence of only HOBt actually proceeded with higher yields and faster reaction times than those executed under isonitrile/HOBt conditions. Moreover, the HOBt-promoted reaction could be extended to the ligation of glycopeptide substrates, to provide access to diglycosylated peptide adducts (Scheme 22, **120** + **121** \rightarrow **122**).

From a mechanistic perspective, these results are quite interesting, albeit not yet explicated. Of course, the finding that the isonitrile is not required for ligation certainly does not preclude the possibility that the mechanism proposed above (Scheme 20) is, in fact, operational when the isonitrile is present. However, the efficient formation of product in the

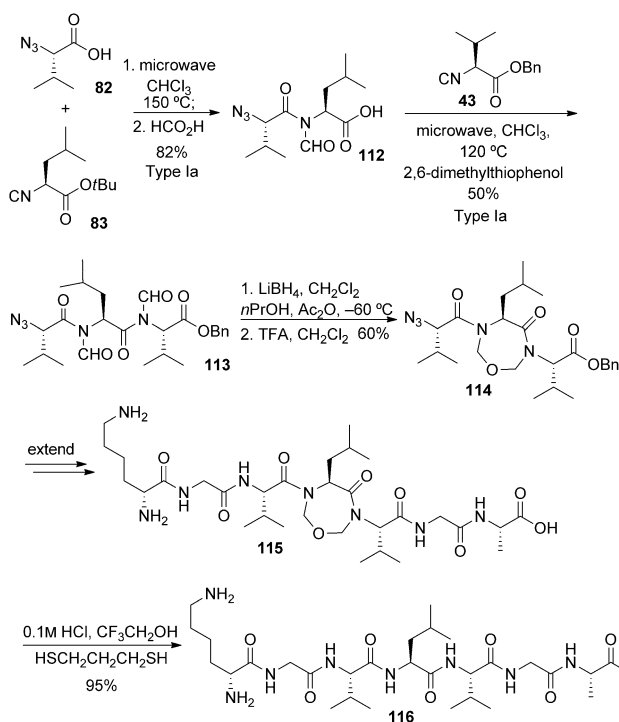


Scheme 17. Second-generation total synthesis of cyclosporine A.

absence of isonitrile does establish the existence of a pathway to ligation from thioacid and amine starting materials, *which is not dependent on the intermediacy of a FCMA species*. It is, in principle, possible that the thioacid peptide is inherently capable of participating in direct ligation with an amine coupling partner; in other words, that SH is a sufficiently powerful leaving group to allow for acylation. However, consistent with the findings of Sheehan and Johnson^[31] and, more recently, those of Orgel and Liu,^[32] it is perhaps more likely that these HOBT-mediated ligations are proceeding through some, as yet unspecified, oxidative mechanism. A series of mechanistic studies have provided support for this theory. Thus, when attempts were made to minimize opportunities for fortuitous oxidation (i.e., by performing the reaction under an argon atmosphere) the yield of amide was notably reduced. Conversely, the ligations proceeded more

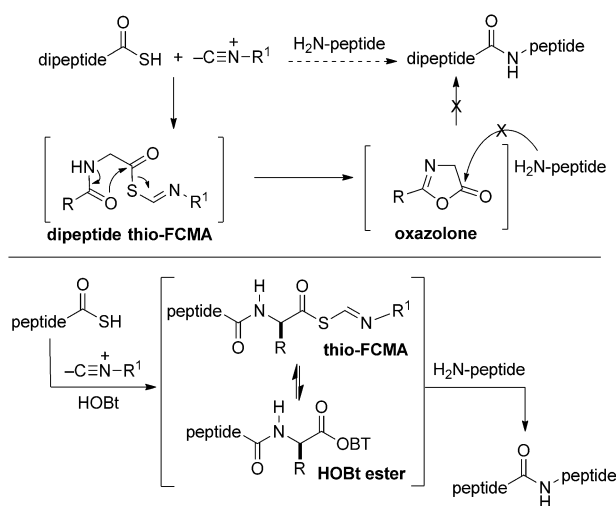


Scheme 18. Synthesis of constrained peptides through isonitrile coupling.

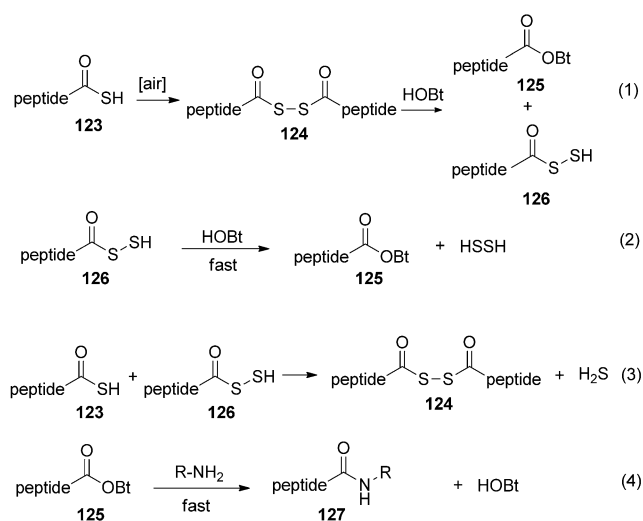


Scheme 19. Application of Type Ia coupling to the synthesis of a temporary peptide constraint.

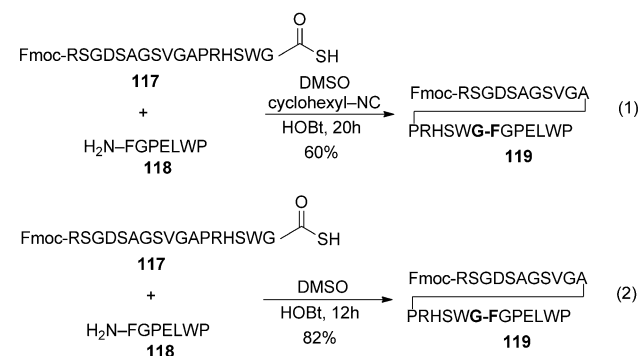
rapidly in the presence of iodine, an established oxidizing agent, than in its absence. Although the exact nature of the transformation has not yet been defined, one can envision a reaction pathway, initiated by low levels of thioacid oxidation, with turnover to restore the C-terminal acyl donor. Any number of plausible pathways could well serve to sustain a high-turnover oxidation-initiated route. One such potential model, involving trace formation of a diacyl disulfide



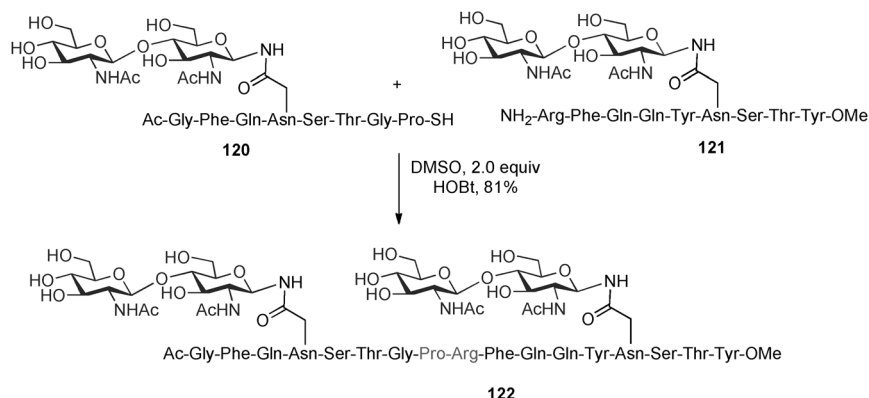
Scheme 20. Thio-FCMA ligation: The challenge.



Scheme 23. Proposed mechanistic pathway for oxidative peptide ligation.



Scheme 21. Type Ib (1) and oxidative (2) ligations of peptides.



Scheme 22. Oxidative ligation of glycopeptides.

intermediate (**124**), is outlined in Scheme 23. However, we have certainly not ruled out the possibility that the oxidative component of the pathway in fact operates through “stoichiometric” rather than catalytic levels of oxidizing agent with high turnover.

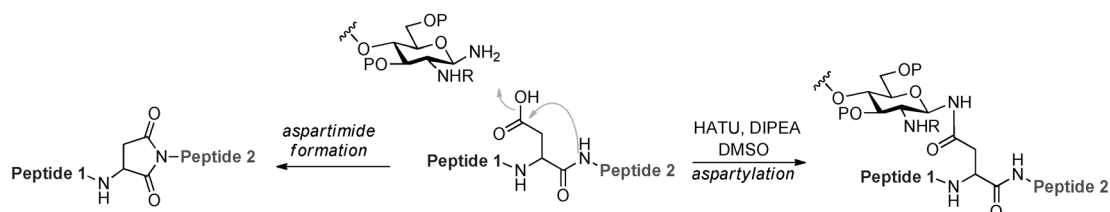
6. A Powerful Route for the Formation of N-Glycosyl Asparagine Glycopeptides

We have been pursuing the development of improved methods for the merger of oligosaccharide and peptide domains. Currently, the standard approach to glycopeptide assembly involves appendage of an anomeric β -glycosylamine, through Lansbury aspartylation, to a properly protected aspartate acyl donor, which is housed within the target peptide fragment.^[33] Several factors tend to erode the efficiency of the Lansbury aspartylation, starting with the weak nucleophilicity of the glycan amine at the reducing end of the glycan. Further complicating aspartylation is a competing

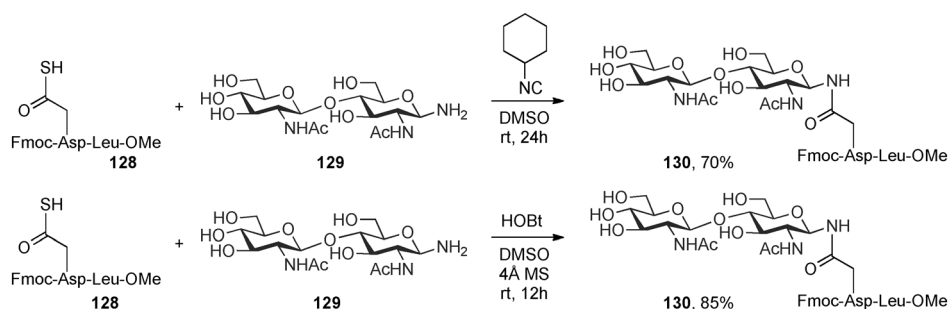
base-induced decomposition pathway, wherein the putative aspartyl donor undergoes cyclization with the NH of the adjacent amino acid to generate a non-productive aspartimide side product (Scheme 24). In certain peptide substrates, particularly those bearing glycine, serine, or alanine residues at the C-terminus of the aspartate, the tendency toward aspartimide formation can become prohibitive.^[34]

We recognized that the mild, base-free acylation methods recently discovered in our laboratory, as described above, could well be suited to the formidable challenge of glycan aspartylation. As shown in Scheme 25, we were indeed able to accomplish convergent

aspartylation under Type IIb coupling conditions. Thus, in the presence of the “sacrificial” cyclohexylisocyanide, peptide thioacid **128** and glycosylamine **129** were joined to afford glycopeptide **130** in 70% yield, accompanied by low levels of peptide carboxylic acid, aspartimide, and products presumably arising from Type Ib intramolecular S→N rearrangement of the thio-FCMA. In light of our discovery that HOBt



Scheme 24. Merger of glycan and peptide domains through aspartylation; undesired base-induced aspartimide formation.



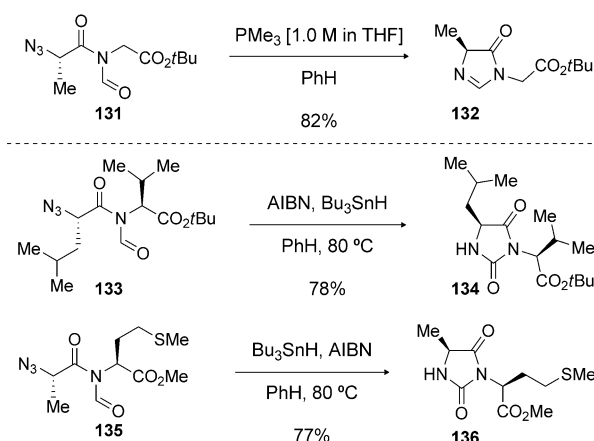
Scheme 25. Isonitrile-based and oxidative aspartylation methods.

can promote thioacid coupling, presumably through the oxidative mechanism described above, we next examined the reaction of **128** and **129** in the presence of HOBt and in the absence of isonitrile mediation. We were indeed pleased to observe that aspartylation occurred more rapidly, to yield glycopeptide **130**, in 85 % yield, along with quite low levels of carboxylic acid and aspartimide side products. It is possible that this reaction also proceeds through an oxidative mechanism, analogous to those proposed above (see Scheme 23, R = glycan).^[35]

7. Generation of Chiral Heterocycles

The biological and pharmaceutical importance of heterocyclic compounds in medicinal chemistry needs little elaboration. We have recently begun the pursuit of methods for the generation of pharma type heterocycles with pendant enantiodefined chirality, from the N-formyl amide products of our Type Ia coupling reactions. This work arose from a fundamental interest in exploring the reactivity of the previously little known N-formyl amide functional group, which is now available through the Type I reaction. We have found that the azide moiety in these products offers an excellent handle for the selective functionalization of the formyl carbonyl group (Scheme 26). In considering the particularly reactive character of the imide-like N-formyl group, the possibility of a Staudinger reaction of a resident azide group followed by aza-Wittig reaction presented itself. In the event, we found that treatment of **131** (obtained by coupling of the azido acid with glycine isonitriles) with trimethylphosphine in benzene resulted in formation of imidazolinones such as **132** in good yield. We were anticipating an alternative method for activation of the azide for reaction, which would be initiated by a presumed radical reduction of the azido group. Indeed,

reaction of **133** with *n*-SnBu₃H gave rise to hydantoin **134** in good yield, seemingly as a single stereoisomer. A similar conversion of **135** to **136** demonstrated applicability of the reaction to a methionine type side chain. We are currently studying the scope of this reaction, not to mention its non-obvious mechanism. These results suggest that a broad study of peptidyl N-formylamides could well be productive.



Scheme 26. Application of Type Ia coupling to the synthesis of heterocycles.

8. Conclusions

In summary, we have described herein recent advances from our laboratory in the field of acylation chemistry, arising from revisiting a classical functional group in organic chemistry with the benefit of contemporary mechanistic insights. We have thus come upon new isonitrile-based coupling methods and applied them to the syntheses of complex “biologic” targets of potential therapeutic import. Finally, these investigations have led to the somewhat fortuitous discovery of a widely general oxidative coupling pathway between thioacid and amine substrates, which even encompasses aspartylation of highly complex “reducing end” glycosylamines. We anticipate that continuing research fol-

lowing these new directions could lead to further discoveries of value to chemical synthesis.

Abbreviations

AIBN	azobisisobutyronitrile
All	allyl
Bn	benzyl
Boc	<i>tert</i> -butoxycarbonyl
DCC	dicyclohexylcarbodiimide
DCE	1,2-dichloroethane
DIPEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMSO	dimethylsulfoxide
Fm	9-fluorenylmethyl
Fmoc	9-fluorenylmethoxycarbonyl
HATU	<i>O</i> -(7-azabenzotriazol-1-yl)-tetramethyluronium hexafluorophosphate
HOBt	1-hydroxybenzotriazole
mCPBA	<i>m</i> -chloroperbenzoic acid
NMM	<i>N</i> -methylmorpholine
PG	protecting group
Phth	phthaloyl
PyBOP	benzotriazolyl-1-oxy-tripyrrolidinophosphonium hexafluorophosphate
TFA	trifluoroacetic acid
Ts	<i>p</i> -toluenesulfonyl

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