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COMMUNICATIONS

Published on Web 09/11/2008

Addressing Mechanistic Issues in the Coupling of Isonitriles and Carboxylic Acids: Potential Routes to Peptidic Constructs

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In previous papers, we have described some of the chemistry which follows from the reaction of carboxylic acids (**1**) and isonitriles (**2**) under suitable thermolytic means (Figure 1).[1] To differentiate these studies from earlier aspects of isonitrile chemistry,[2] we suggest the term "two-component coupling" (2CC) for our program. In the absence of external acyl acceptors, quite encouraging yields (70−90%) of the synthetically versatile *N*-formylamides of the type **4** are obtained. As was shown, such novel amidic (or, more precisely, imidic) structures provide new opportunities for the synthesis of modified peptide-like structures. In the presence of nucleophilic trapping agents, the rearrangement of **3**→**4** competes with the formation of interdiction products. For instance, ester-type **5** is generated from intervention by an external alcohol-type nucleophile.[1b] In the presence of amine-based acyl acceptors, simple amides can be produced (see **6**).[1a] The formation of these products can be mechanistically accommodated via the intermediacy of the formimidate carboxylate mixed anhydride (FCMA, cf. **3**). We have shown that, notwithstanding several claims to the contrary,[3,4] the isolation of such a FCMA under usual reaction conditions has not been accomplished.[5]

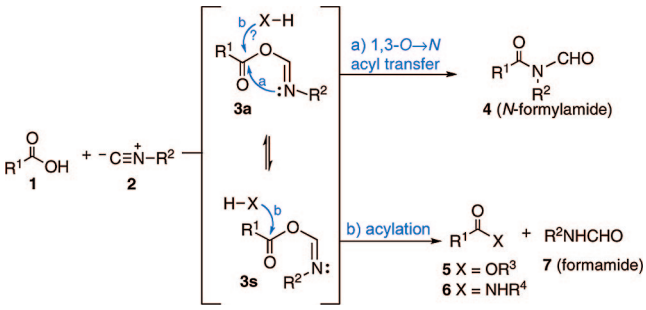

Figure 1

Two formulations present themselves to reach products **4**, **5**, and **6**. In one view, a high energy FCMA is produced, which can undergo the 1,3-O→N acyl transfer leading to **4** or nucleophilic trapping leading to **5** or **6**. Alternatively, the apparent competition between interdiction and *N*-formylamide formation may really reflect the consequence of concurrent formation of **3a** and **3s**.[6,7] One of the stereoisomers, **3a**, is perhaps noninterdictable. It is, in effect, "committed" to undergoing very rapid thermolytically induced 1,3-O→N acyl transfer leading to **4**. By contrast, production of the nonrearranging FCMA (**3s**) in the presence of a nucleophilic acceptor would lead to **5** or **6** (as well as formamide **7**). Of course, in the absence of acyl acceptor XH, the noncompetent acyl transfer FCMA form, **3s**, would revert to **3a**, which goes on to **4**. In this

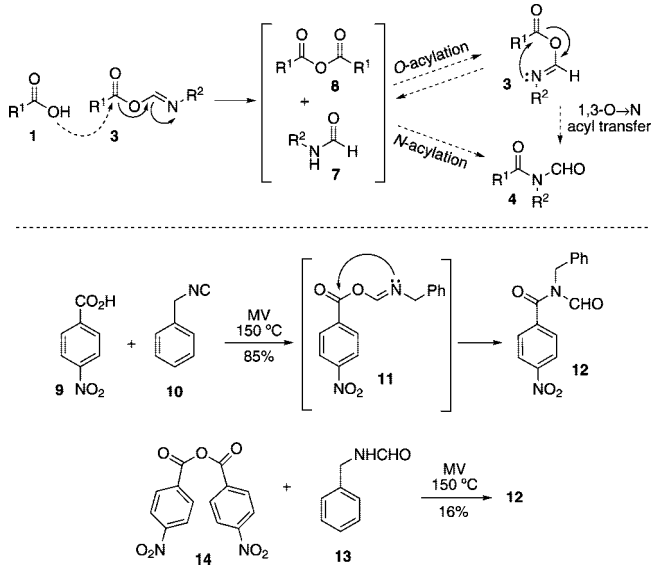

Figure 2

view, the distribution between **4** vs **5** (or **6**) really reflects, to a great extent, the distribution in the formation of FCMA stereoisomers **3a** anti (a) and **3s** syn (s). Were equilibrium between **3a** and **3s** to be more rapid than either 1,3-O→N acyl transfer or interdiction by nucleophiles, then, the two pathways would, in effect, still be competing for control of product distribution.

The 2CC reaction of acids and isonitriles carries with it significant potentialities for the synthesis of biologically active amidic or peptidic products, including patterns not readily accessible by current methods.[8] With new insights into the finer workings of the underlying chemistry of the 2CC method, could well come new opportunities to enlarge upon its growing menu of applications. The experiments delineated herein particularly focus on gaining better insight into the feasibility of 2CC processes in the presence of potential acyl acceptors. The findings described below chart a course for future discovery.

We first interrogated a 2CC system by evaluating an alternative pathway, which need not actually involve 1,3-O→N acyl rearrangement within **3** for producing **4**. In this proposal, interdiction (perhaps solely on the nonrearranging **3s** form) occurs by nucleophilic attack of acid **1** at the carboxyl carbonyl group of FCMA **3** (Figure 2). This step would coproduce formamide **7** and symmetric anhydride **8**.[9] *N*-acylation of **7** with **8** would afford, directly, *N*-formylamide product **4**. Of course, *O*-acylation of **7** with **8** would be an "invisible option" in that it would simply result in the reappearance of **3** and, thence, **4**.

We studied the possible formamide (cf. **7**)−symmetrical anhydride (cf. **8**) pathway, in the context of the high yielding reaction

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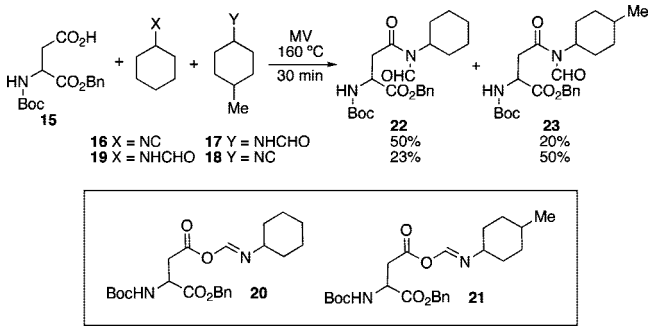


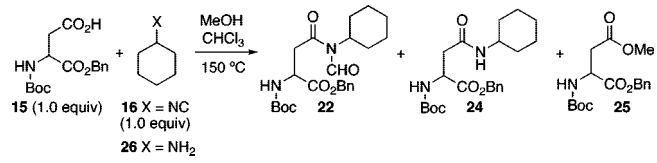
Figure 3. The distribution of **22**, **23** was determined by LC−MS of the crude sample.

of acid **9** with benzylisonitrile **10** under microwave pyrolysis. Compound **12** was obtained in 85% yield. To evaluate the viability of the alternative (symmetrical anhydride) route, we independently examined the reaction of benzylformamide **13** and separately synthesized symmetrical anhydride **14**,[10] under simulated microwave thermolysis conditions. In this experiment, we were clearly providing a far more favorable setting for such a pathway than could be available via low levels of the same intermediates generated in the course of the 2CC reaction. In the event, reaction of **13** and **14** under 2CC conditions afforded a maximum 16% yield of **12**. While we cannot assign a limiting maximal number for intervention of the symmetrical anhydride pathway in the actual 2CC processes, we conclude that the symmetrical anhydride pathway (cf. **14**) is, at best, a very minor contributor. Moreover as noted above, even in the minor pathway in which **14** may be involved, reaction with **13** may actually occur by *O*-acylation (the invisible reaction), taking one back to FCMA **11**, which advances to **12** via 1,3-O→N acyl transfer.

We then asked whether a formamide would be sufficiently nucleophilic to intervene if it were present from the beginning as an added component in the 2CC reaction. In the light of the experiment described above, for a formamide such as **13** to interdict in the context of the 2CC reaction, there would have to be generated a much stronger acyl donor than is a symmetrical anhydride such as **14**. To approach this question, we studied the 2CC reaction of aspartate derivative **15** with cyclohexylisonitrile **16** in the presence of 4-methylcyclohexylformamide **17**.[11] As shown in Figure 3, there are produced substantial amounts of crossover products (see **22** and **23**). Essentially, the same level of crossover occurred in the reaction of 4-methylisonitrile **18** in the presence of cyclohexylformamide **19** (see products **22** and **23**). We take these experiments to teach that during the course of the 2CC reactions, there must be produced highly reactive acylating agents (presumably FCMA structures **20** and **21**) capable of aspartylating even a relatively weak nucleophile such as a formamide (cf. **17** or **19**). Such a step accounts for the substantial crossover observed. The simplest interpretation of the events envisions the FCMA (**20** or **21**) reacting with the NH group of cyclohexylformamide **17** or **19** to give directly, **22** or **23**.[12] Of course, an alternative "invisible" possibility envisions O-acylation of the 4-methylcyclohexyl formamide **17** leading to FCMA **21** which goes on to **23** by the postulated 1,3-O→N acyl transfer.

We next probed the question of intervention in the 2CC chemistry by methanol (presumably via its reaction with FCMA **20**) to produce methyl ester **25**. This reaction had been demonstrated earlier,[1b] but was now interrogated at a more subtle level. We asked whether trapping by methanol and formation of 1,3-O→N acyl transfer product are truly competitive within the same molecular species, or whether the apparent competition really stems from the formation of two molecular entities **3a** and **3s** each of which is, in effect,

Table 1[a]



entry	MeOH	22[a]	24	25
1	1.0 equiv	30%	4%	38%
2	5.0 equiv	14%	11%	41%

[a] The distribution of **22**, **24**, **25** was determined by ^{1}H NMR of the crude sample.

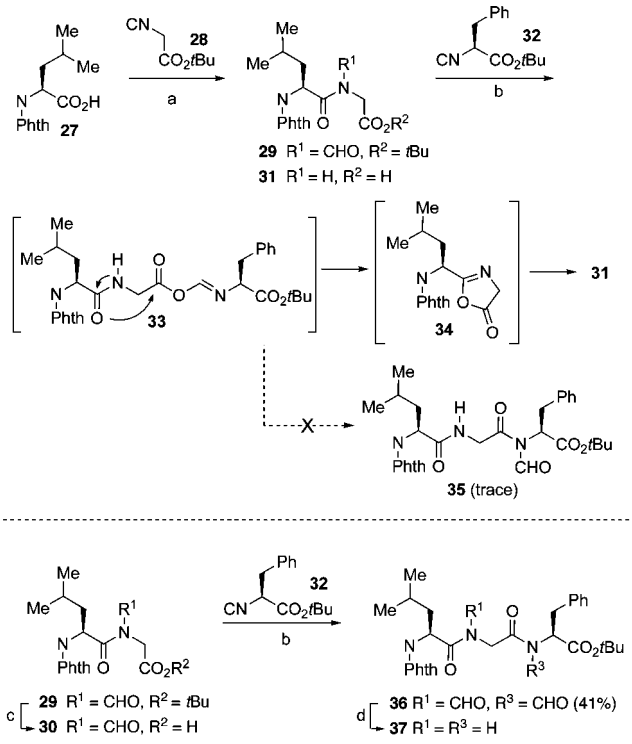
committed to a particular program (vide supra, see pathways in Figure 1). To get at this problem, we studied the consequences of administering 5 equiv of methanol (rather than 1) in the 2CC reaction of acid **15** and isonitrile **16**. Remarkably, the yield of methyl ester did not increase significantly, even following a sharp upgrade in the concentration of the trapping agent (methanol). We take this experiment to suggest that already at the level of a single equivalent of methanol, there exists an intermediate whose thermally induced methanolysis reaction is sufficiently fast as to provide for highly efficient trapping. Thus, administration of additional equivalents of methanol does not materially change the amount of methyl ester which is generated. What does change (see Table 1) is the ratio of *N*-formyl compound **22** to amide **24**. This result reflects the role of excess methanol in increasing the level of methanolytic transformation of **16** to the corresponding amine **26**. The latter goes on to produce amide **24**, as previously shown. However, the important message is that a large increase of the trapping agent has not affected to any significant extent the level of interdiction.

It is interesting to note that our studies[1] have covered a varied range of acyl acceptors such as formamide **17** (or **19**), methanol, and amines. Yet, globally, there are produced roughly comparable levels of nucleophilic trapping (ca. 30−50%), relative to 1,3-O→N acyl migration. These admittedly preliminary findings tend to suggest the idea that under the microwave thermolytic conditions, the stereochemistry of the FCMA which is produced (**3a** vs **3s**) determines to a great extent whether it undergoes 1,3-O→N acyl transfer or interdiction.

Finally, we report on some early yet encouraging results pointing to the applicability of the 2CC reaction to the synthesis of novel peptides and peptidic constructs. We presumed that central to the 2CC method is the intermediacy of a FCMA of the general type **3a**. As shown above, products can arise from either trapping by an external acyl acceptor or 1,3-O→N acyl transfer. In contemplating application to peptide and modified peptide synthesis, we noted that the potential acyl acceptor is intramolecular, that is, the amide bond flanking the site of proposed elongation of the C-terminus through the 2CC reaction.

Our feasibility−exploring foray started with the phthalimidoyl leucine derivative (**27**, Scheme 1). The free acid was coupled to glycine isonitrile ester (**28**). Obviously, the selection of glycine served to bypass issues of racemization at the isonitrile terminus. Happily, reaction of **27** with **28** gave rise to **29** in 70% yield. We next attempted to couple compound **31** with phenylalanine isonitrile ester **32**. This compound was prepared in optically pure form by a known method.[13] Given the strong acyl donating character of the presumed FCMA (derived from the reaction of the carboxyl group of **31** with the isonitrile function of **32**), we were in effect asking whether it would be intercepted by the neighboring amide bond of **31**. In the event, reaction of **31** and **32** gave at best traces of tripeptide **35**. Presumably, reaction of **31** and **32** produced FCMA

Scheme 1[a]



[a] Key: (a) MV, 160 °C, 30 min, $ClCH_2CH_2Cl$, 70%; (b) MV, 150 °C, 30 min, $CHCl_3$; (c) HCO_2H, 4 h, room temp; (d) $NaHCO_3$, MeOH, room temp, 2 h, 60%.

33. In its anti form (cf. **3a** in Figure 1) **33** should be a candidate for rearrangement to **35** via the usual 1,3-O→N acyl migration to complete the 2CC reaction. We postulated that the failure of the reaction to occur perhaps reflects formation of the intermediate oxazalone **34**,[14] wherein the neighboring peptidic leucine amidic bond interdicts the FCMA function in **33**. Apparently, **34** is not a sufficiently competent acyl donor to go on to **35**. Rather, **34** suffers eventual hydrolysis back to **31**.

Given this interpretation, a fascinating possibility presented itself. The thought was that the intramolecular acyl acceptor ability of an *N*-formylamide system **30** (which is in fact derived from the primary product of the 2CC reaction between **27** and **28**) would be much reduced relative to the amide system within **31**. Accordingly, the 2CC reaction between compounds **30** and **32** was studied. Happily, the projected reaction occurred to produce the bis formylamide **36**. While the yield of this coupling step producing the novel diformyl compound **36** is presently rather modest, we see this as a most encouraging direction for the buildup of polypeptides through the 2CC method. In the case at hand, cleavage of both carbonyl groups provided a 60% yield of tripeptide **37**.

In summary then, the message at this stage of the inquiry is that, at least in the presence of *N*-formylamide, the intermediate peptidyl FCMA is sustainable and can participate in the next 2CC reaction. Future results describing applications of the 2CC reaction to novel peptides and peptidic constructs will be forthcoming.

Acknowledgment. Support for this research was provided by the National Institutes of Health (CA28824 to S.J.D.). We thank Rebecca Wilson for editorial consultation and Dana Ryan for assistance with the preparation of the manuscript. We also thank Dr. George Sukenick, Ms. Hui Fang, and Ms. Sylvi Rusli, (NMR Core Facility, Sloan-Kettering Institute) for mass spectral and NMR spectroscopic analysis.

Supporting Information Available: Detailed experimental procedures, copies of all spectral data, and compound characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA804709S