Multiple-Component Condensation Strategies for Combinatorial Library Synthesis

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I. Introduction

Historically, the paradigm of drug development has followed an iterative cycle of screening and synthesis, involving the manipulation of individual structures. The feedstock of molecules for this process has traditionally incorporated both natural products and proprietary and commercial compound collections. The latter usually represent a collectively monumental effort of synthesis over a period of many years. The introduction of high-throughput biological screening and the accelerated discovery of new biological targets has increased the demand on synthetic chemists to produce new compounds for testing. One response to this demand has been the development of techniques to greatly increase the speed and efficiency of compound synthesis. In the case of peptides[1−4] and oligonucleotides,[5,6] combinatorial libraries containing large numbers of individual components have afforded high-affinity ligands and potent inhibitors to a variety of targets. However, synthetic methods for these biopolymers are well-established, and it is only recently that chemists have applied some of these strategies to the currently more difficult task of generating libraries of small-molecule therapeutics.

In this Account, we will focus on multiple-component condensations (MCCs) as one subset of strategies for the generation of compound libraries. While most libraries have been generated using a linear, multistep process, MCCs provide a complementary approach to a number of structures and should find applications in library generation. Multiple-component condensations are those reactions in which three or more reactants come together in a single reaction vessel to form a new product which contains portions of all the components. These reactions may be carried out in solution or on a solid support. A catalyst or other additive which might facilitate the coupling of two other components in a reaction but which does not structurally contribute to the product is not considered a component in an MCC reaction. It is not necessary that all components condense in a mechanistically concerted fashion; however, the MCC reactions considered herein do not require extensive manipulations: they are one-pot reactions. In this and the succeeding section, it is instructive to contrast this approach with linear synthesis to highlight the differences in methods, potential library size, and output format. For example, an MCC reaction with four components provides, in a single step, a molecular scaffold characterized by a core set of atoms common to the condensation reaction and displaying aspects of the four components. In contrast, to achieve the same structure in a linear fashion, multiple steps with attendant workup cycles may be required.

It is our belief that the methods used to synthesize a particular library are dictated by (1) the need for a specific core structure and (2) the commercial availability or ease of access to inputs which give the structural variability to the core. Approaches to desired core structures vary greatly, and the chosen route may be a linear synthesis, an MCC, or a combination of the two. The term linear synthesis as used in this Account refers to a multistep process requiring the isolation of intermediates or washing of the solid support resin and re-exposure to new reagents for each step of the synthesis. While synthetic chemists may be more familiar with the distinction between "linear" and "convergent" when applied to synthetic strategies, in this Account we refer to as linear any library strategy that builds up the target molecule one step at a time. By this definition, both a solid-phase peptide synthesis and, for example, Ellman's 1,4-benzodiazepine synthesis[7] (Figure 1) constitute linear syntheses, because each constructs the target skeleton in a stepwise manner. We define "linear" in this manner as a means of distinguishing the MCC strategy, which we feel is an underutilized tool for library synthesis.[8]

This Account will focus on two related core struc-

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(1) Pinilla, C.; Appel, J.; Blondelle, S.; Dooley, C.; Dorner, B.; Eichler, J.; Ostresh, J.; Houghten, R. A. *Biopolymers* **1995**, *37*, 221−240.
(2) Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1233−1251.
(3) Pavia, M. R.; Sawyer, T. K.; Moos, W. H. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 387−396.
(4) Jung, G.; Beck-Sickinger, A. G. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 367−383.
(5) Gold, L.; Polisky, B.; Uhlenbeck, O.; Yarus, M. *Annu. Rev. Biochem.* **1995**, *64*, 763−797.
(6) Ecker, D. J.; Vickers, T. A.; Hanecak, R.; Driver, V.; Anderson, K. *Nucleic Acids Res.* **1993**, *21*, 1853−1856.
(7) Bunin, B. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1992**, *114*, 10997−10998.
(8) For a recent example, see: Wipf, P.; Cunningham, A. *Tetrahedron Lett.* **1995**, *36*, 7819−7822.

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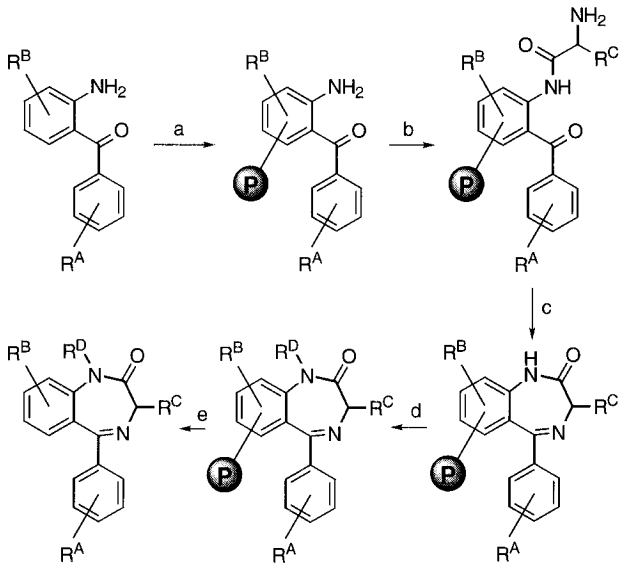


Figure 1. Ellman's 1,4-benzodiazepine synthesis. Lower-case letters represent one or more steps; the "P"-labeled sphere represents a resin bead.

tures we have synthesized in a library format using an MCC approach. We will also address some of the details of the specific protocol for the library synthesis and the output format. Use of this approach will entail certain advantages and disadvantages. For example, with respect to the synthesis of discrete (one compound/vessel) libraries, MCC reactions offer substantial efficiencies in time and effort, but do not allow for split-and-mix strategies.[24] In contrast, linear methods work well with the split-and-mix protocol.

II. Opportunities for Introduction of Diversity

The definition and measurement of diversity of a given library are important issues which are relevant to the choice of starting materials ("inputs") to be used in the synthesis of the library.[3,9] However, this topic is beyond the scope of this Account. The opportunity for introduction of diversity, that is, the ability to utilize families of different inputs, is governed by the methods employed and is independent, in theory, of the final structure. The chemistry may involve the use of a linear synthesis in which a molecule bound to a resin increases in molecular weight or undergoes functional group manipulations in a sequential fashion. Alternatively, an MCC reaction in which three or more components come together in a single-pot reaction may be utilized.[10] The best strategy for any library is dictated by the available inputs, the target structure, and the format of the library, whether pooled mixtures or discrete compounds.

Linear Synthesis. This approach is an evolution of the strategy for solid-phase synthesis of biopolymers such as polypeptides and nucleic acid oligomers. One characteristic of the linear approach to combinatorial libraries is the requirement for the initial inputs to have one of three features: they must be (a) monofunctional and bireactive, (b) bifunctional (or polyfunctional) and orthogonally reactive, or (c) monofunctional. Only the "capping" inputs (c), those which truncate the extension of a functional group, can be monoreactive/monofunctional. As shown in Figure 2, the first step of a linear synthesis must provide a functional group handle for the second coupling. Likewise, the second and third reactions must do the same up to the termination of the synthesis. A single cycle in the synthesis of a peptoid[11] involves the use of a monofunctional bireactive input (amine) followed by a bifunctional, orthogonally reactive input (amino acid). In contrast, a peptide library is built entirely from bifunctional amino acid inputs. A second example, mentioned above, is the 1,4-benzodiazepine synthesis shown in Figure 1. Two key steps involve the condensation of two bifunctional, orthogonally reactive inputs (step b) and a capping of the amide with the monofunctional alkyl halide (step d).

For a linear synthesis, the library inputs and their "diversity" are limited by the necessity to conform to requirements a, b, or c, or a combination thereof. While there are many more monofunctional (or monoreactive[12]) commercially available inputs, the diversity of available structures will be limited compared to chemistry available to bifunctional inputs. That is, monofunctional inputs can only be used to cap a functional group and not to extend the skeleton of the molecule. But the advantage of exploiting monofunctional inputs is that while the synthetic effort to generate the core scaffold (which presents orthogonally reactive functional groups) might be substantial, the ability to sequentially cap the functional groups with diverse monofunctional inputs is, in general terms, tied to their commercial availability. We believe the overall lesson is that designing linear library strategies in which the availability (synthetic or commercial) of the initial inputs is limited will result in libraries of small size and/or scope.

Multiple-Component Condensations. A second strategy for the generation of libraries involves the use of MCC reactions. This is a historically rich area of chemistry, beginning with Strecker's synthesis of amino acids in 1850, and there are many such reactions described in the literature (see Figure 3).[13−23] Most of these reactions have not been introduced on solid supports nor synthesized in a library format.

An MCC is a reaction in which three or more reactants combine in a single reaction event to yield a product that displays features of all inputs. Because each condensation is a single process, each product in a library of compounds can be synthesized in a separate reaction vessel. Ninety-six-well microtiter plates are thus ideal for this synthesis, because the product in each well is unequivocally known from the particular reactants in that well. We define library synthesis in this format as *array* synthesis, an array

(9) Simon, R. J.; Kania, R. S.; Zuckermann, R. N.; Huebner, V. D. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9367−9371.
(10) A two-component condensation is a linear one-step synthesis.
(11) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646−10647.
(12) Monoreactive compounds would be those that have more than one functional group, of which only one participates in the reaction.
(13) Strecker, A. *Justus Liebigs Ann. Chem.* **1850**, *75*, 27.
(14) Strecker, A. *Justus Liebigs Ann. Chem.* **1854**, *91*, 349.
(15) Hantzsch, A. *Ber. Dtsch. Chem. Ges.* **1890**, *23*, 1474.
(16) Biginelli, P. *Ber. Dtsch. Chem. Ges.* **1891**, *24*, 2962.
(17) Biginelli, P. *Ber. Dtsch. Chem. Ges.* **1893**, *26*, 447.
(18) Mannich, C.; Krosche, W. *Arch. Pharm. (Weinheim, Ger.)* **1912**, *250*, 647.
(19) Passerini, M. *Gazz. Chim. Ital.* **1921**, *51*, 126, 181.
(20) Bucherer, H. T. Fischbeck, H. T. *J. Prakt. Chem.* **1934**, *140*, 69.
(21) Bucherer, H. T.; Steiner, W. *J. Prakt. Chem.* **1934**, *140*, 24.
(22) Bergs, H. Ger. Patent 566 094, 1929.
(23) Khand, I. U.; Knox, G. R.; Pauson, P. L.; Watts, W. E.; Foreman, M. I. *J. Chem. Soc., Perkin Trans. 1* **1973**, *9*, 977−981.

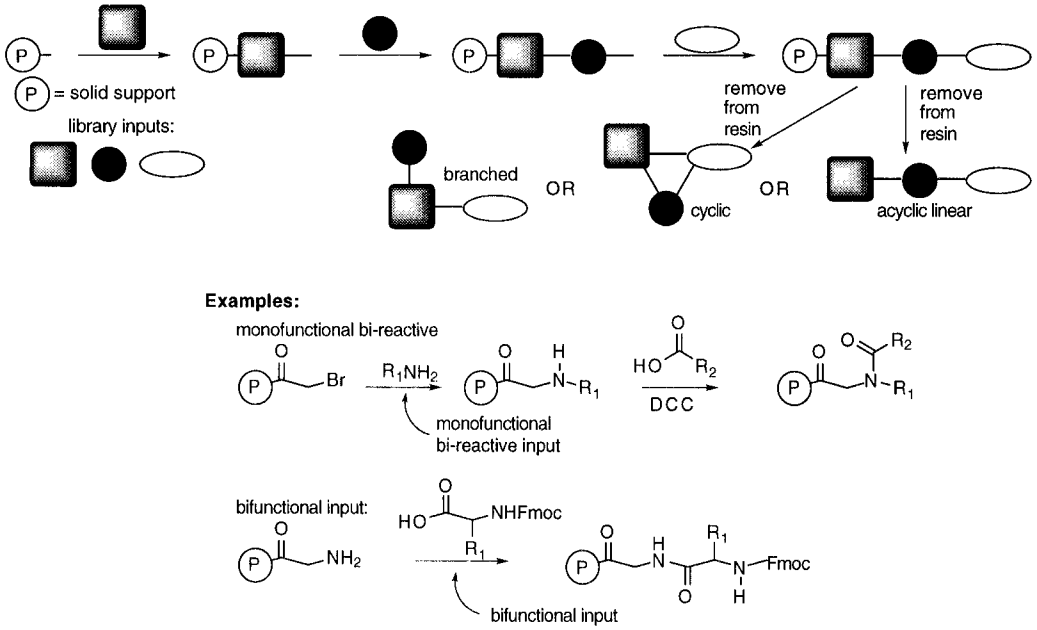


Figure 2. A linear strategy for combinatorial synthesis.

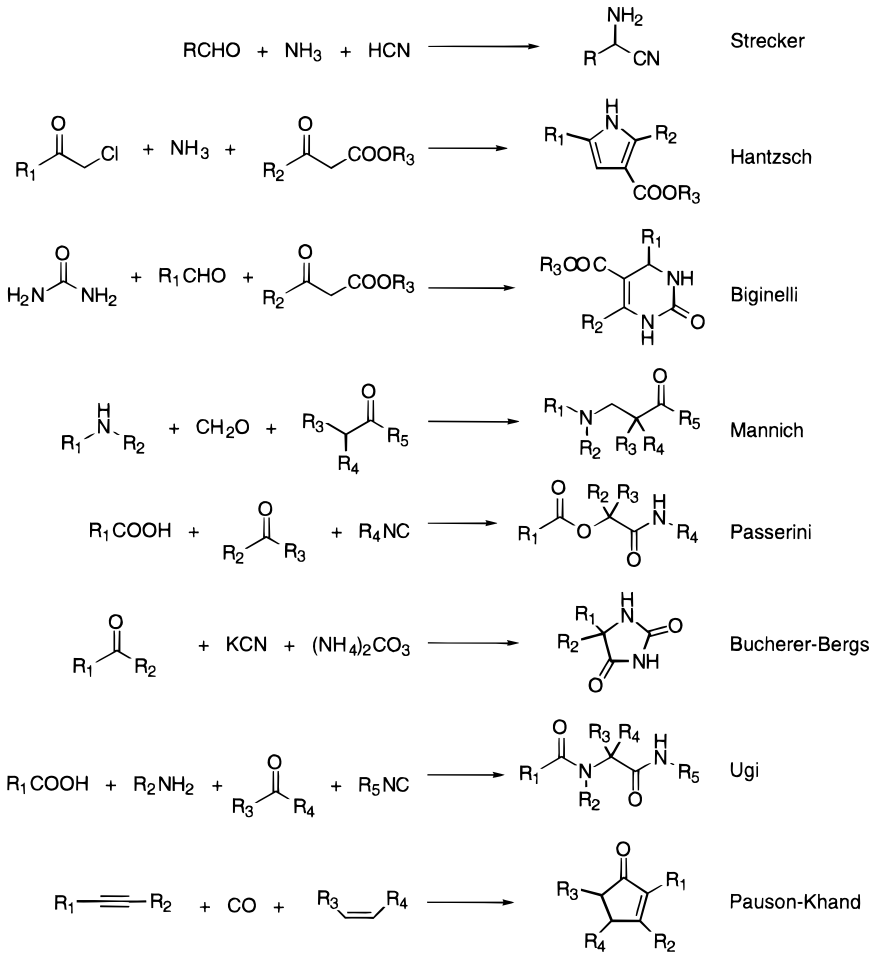


Figure 3. Some examples of multiple-component condensations.

being a group of discrete, spatially addressed compounds whose identities are defined by the inputs employed at each location in the array. The dimensionality of the array is given by the number of inputs used in each reaction. Thus, a three-component condensation yields a three-dimensional array (indeed a 3-D array can be envisioned as such, see Figure 9), subject to the stricture that no component is held constant.

The MCC can be a valuable tool for the generation of libaries based on a common core structure, because (1) the product is formed in one step, with corresponding savings in synthetic time and effort; and (2) in theory, any of the inputs to the reaction can be varied

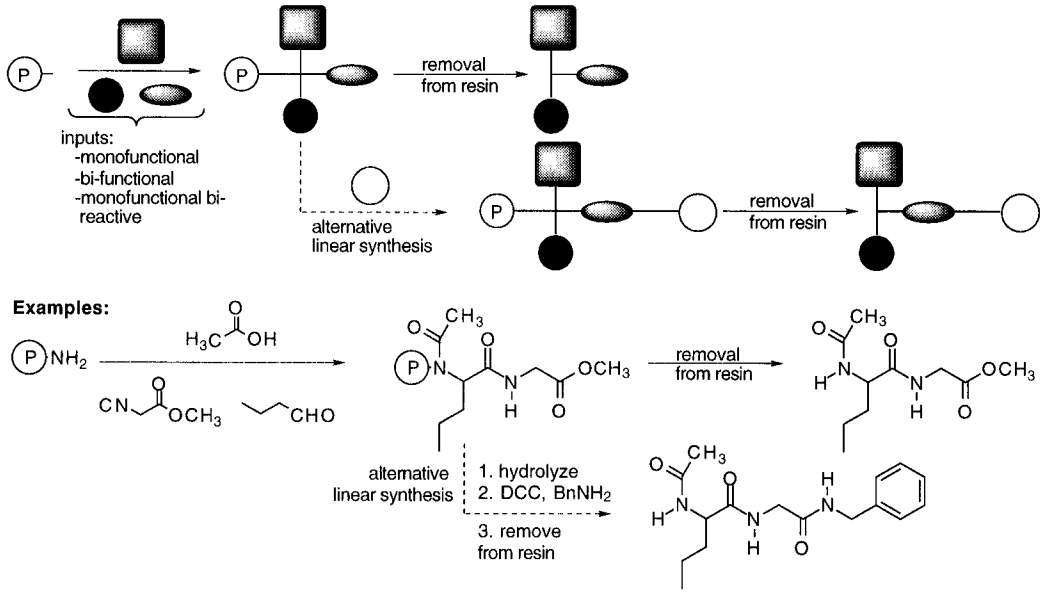


Figure 4. A convergent strategy for library synthesis using Ugi 4CC.

independently of all others, thus generating compounds whose diversity is proportional to the number and availability of the inputs. These libraries represent a substantially different approach to compound generation by not being limited to bifunctional or bireactive inputs. This strategy represents the most efficient generation of discrete (single compound/well) libraries since it involves (but is not limited to) a single reaction event on the solid support. That is, additional linear steps either pre- or postcondensation can be included. With MCC reactions, time- and labor-consuming processes such as polymer washing and reagent reintroduction are minimized. We have named this approach the multiple component condensation array synthesis (MCCAS). It is simply the application of MCC reactions to the synthesis of large arrays of compounds. One example of this approach, shown in Figure 4, involves the Ugi four-component condensation (4CC). As implemented on a solid support, this reaction brings together three monofunctional inputs (acid, aldehyde, and isocyanide) in the presence of a resin-bound amine, affording a condensation product involving all four components. It should be noted that use of a convergent strategy does not preclude additional, linear steps either before or after the key condensation. As depicted in Figure 4, post-Ugi reaction modifications can include hydrolysis of the methyl ester and standard DCC amide coupling to add an additional site of diversity.

The potential size of a library generated via a linear synthesis is a function of the number of steps and the number of individual inputs in each step. For instance, a four-step synthesis in which each step has 20 different inputs results in a library of 20^4 compounds. In contrast, a four-component condensation reaction with 20 inputs of each functional group provides the same number of compounds overall. However, in terms of number of steps, the MCC achieves the same library size by a single reaction event. Table 1 details this comparison. With a fixed number of 20 variants per input (an analogy to amino acid inputs), the advantage of an MCC over a linear process increases with the number of components. This advantage is even greater if one includes the deprotection steps often necessary in a linear synthesis (peptide synthesis, for example), which doubles the number of steps in an iterative, linear synthesis. The box in Table 1 highlights the number of components of the MCCs which will be discussed below, the Passerini 3CC and the Ugi 4CC.

Table 1. Comparison of Synthetic Steps Required for Libraries Using Either MCC or Linear Strategies

no. of components	structural variants/ input	compds generated	MCC synth steps	linear steps (with deblock)
2	20	400	1	1 (2)[a]
3	20	8 000	1	2 (4)
4	20	160 000	1	3 (6)
5	20	3 200 000	1	4 (8)
6	20	64 000 000	1	5 (10)

[a] Parentheses indicate number of linear steps if a deprotection step is required prior to each coupling.

In terms of individual manipulations to generate a library, the MCC approach is ideally suited for discrete library synthesis since it involves minimal resin washes. In this single compound/vessel format, the structure of any library component can be simply established because the library is spatially addressed: the inputs at a specific location in the array are known, thus the structure of the expected product is unequivocally and effortlessly assigned. The parallel synthesis of linear combinatorial libraries to achieve a discrete format is a larger task since numerous filtration and reagent introduction cycles are necessary. While most biopolymer automated synthesizers work in this fashion, they benefit from coupling chemistries which have been optimized over many years and from the limited number of desired inputs. A very powerful route for the synthesis of linear libraries involves the "split synthesis" method introduced by Furka.[24] This method is used heavily for the synthesis of nonpolymeric combinatorial libraries; however, some degree of deconvolution,[25,26] that is,

(24) Furka, Á.; Sebestyén, F.; Asgedom, M.; Dibó, G. *Int. J. Pept. Protein Res.* **1991**, *37*, 487−493.
(25) Janda, K. D. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 10799−10785.
(26) Erb, E.; Janda, K. D. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11422−11426.

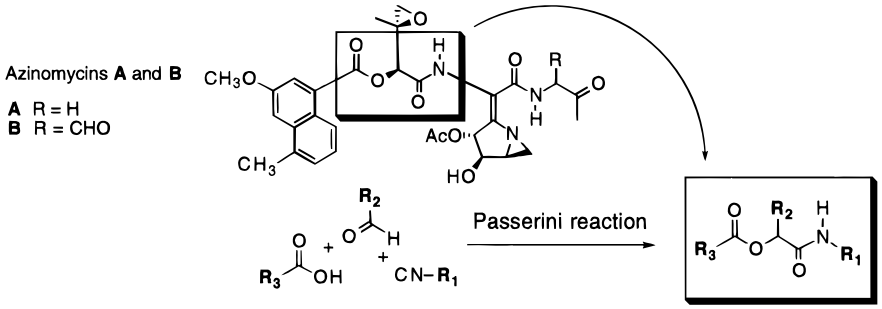
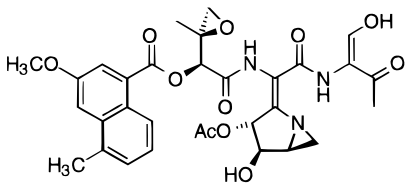


Figure 5. Synthesis of azinomycin analogs via Passerini 3CC.

identification of an active component in a library, is necessary. Deconvolution strategies are required when a library synthesis yields mixtures of compounds, instead of discrete compounds, as described above for MCC reactions. Biological testing of a set of mixtures can reveal which "pool" contains an active compound, but further effort ("deconvolution") is needed to isolate the particular compound. (No deconvolution is required for MCC arrays because of their discrete format.) In cases in which a biological assay will not accommodate compound mixtures for testing, encoding methods enable conversion of pools of compounds arising from split-and-mix strategies into discrete, identifiable compounds. Chemical encoding schemes using molecular tags[27−29] have been developed, and more recently, encoding using radiofrequency transponding microchip tags has been disclosed.[30,31,61]

III. Multiple-Component Condensations

Passerini Reaction.[19,32,33] We initiated our effort in the parallel synthesis of compound libraries as a result of our work[34,35] toward the total synthesis of the azinomycin antitumor antibiotics. Binding studies



azinomycin B

had established that the natural product cross-linked duplex DNA in the major groove.[36] Partial degradation of these adducts suggested that the aziridine and epoxide moieties were involved in the alkylation event, perhaps facilitated by the intercalation of the naphthyl ester found in the natural product. Concurrently, we had developed a highly convergent synthesis of the left-hand portion of the molecule involving the Passerini three-component condensation,[32] in which each of the components contained one of the postulated functionalities involved in binding (see Figure 5). We thus recognized that a structure−activity relationship (SAR) study of the natural product, specifically the interrelation of the key functionalities, would be accessible in this single 3CC reaction with the appropriate starting materials in place. This observation has led us to investigate the basic chemistry associated with selected MCC reactions. Specifically, a strategy was desired which would enable the rapid synthesis of azinomycin analogs. A polymer-supported strategy using the Passerini three-component condensation (3CC) was employed; however, a photocleavable polymer linker was needed to ensure survival of azinomycin analogs not stable to acidic or basic polymer-cleaving conditions.

Before conducting the Passerini reaction in a combinatorial array, we optimized the Passerini reaction conditions and the photolytic cleavage from the resin for a representative system (Scheme 1). The glycine carbamate linker **3** was constructed by reacting isocyanate **1** with the free hydroxyl of the known photolabile linker **2**.[37−41] Coupling of linker **3** to methylbenzhydrylamine (MBHA)-Gly-resin **4**[42] yielded the polymer-supported photocleavable carbamates **5**.[43] Hydrolysis of the ester **5** afforded the requisite polymer-supported acid **6**. A solution of methyl isocyanoacetate and butyraldehyde was then added to polymer **6** and the reaction allowed to stand for 2 days, affording resin-bound Passerini adduct **7**. Photolytic cleavage of the solid-supported Passerini adduct **7** and in situ acylation by acetic anhydride afforded the *N*-acyl Passerini adduct **10** as the only observable compound.[44]

These methods were then applied to the first solid-phase MCCAS. A solid support 3-(6×5×1)-MCCAS (6 isocyanides, 5 aldehydes, 1 carboxylic acid) array was generated using the MBHA-Gly-resin **6** in each reac-

(27) Baldwin, J. J.; Burbaum, J. J.; Henderson, I.; Ohlmeyer, M. H. J. *J. Am. Chem. Soc.* **1995**, *117*, 5588−5589.
(28) Nestler, H. P.; Bartlett, P. A.; Still, W. C. *J. Org. Chem.* **1994**, *59*, 4723−4724.
(29) Ohlmeyer, M. J. P.; Swanson, R. N.; Dillard, L. W.; Reader, J. C.; Asouline, G.; Kobayashi, R.; Wigler, M.; Still, W. C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10922−10926.
(30) Moran, E. J.; Sarshar, S.; Cargill, J. F.; Shahbaz, M. M.; Lio, A.; Mjalli, A. M. M.; Armstrong, R. W. *J. Am. Chem. Soc.* **1995**, *117*, 10787−10788.
(31) Nicolaou, K. C.; Xiao, X.-Y.; Parandoosh, Z.; Senyei, A.; Nova, M. P. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2289−2291.
(32) Combs, A. P. Ph.D. Thesis, University of California, Los Angeles, 1993.
(33) Ugi, I.; Lohberger, S.; Karl, R. In *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I., Eds.; Pergamon: New York, 1991; Vol. 2, pp 1083−1109.
(34) Moran, E. J.; Tellew, J. E.; Zhao, Z.; Armstrong, R. W. *J. Org. Chem.* **1993**, *58*, 7848.
(35) Armstrong, R. W.; Tellew, J. E.; Moran, E. J. *J. Org. Chem.* **1992**, *57*, 2208.
(36) Armstrong, R. W.; Salvati, M. E.; Nguyen, M. *J. Am. Chem. Soc.* **1992**, *114*, 3145.
(37) Pillai, V. N. R. *Synthesis* **1980**, 1−26.
(38) Williams, P. L.; Gairi, M.; Albericio, F.; Giralt, E. *Tetrahedron* **1991**, *47*, 9867−9880.
(39) Rich, D. H.; Gurwara, S. K. *J. Am. Chem. Soc.* **1975**, *97*, 1575.
(40) Rich, D. H.; Gurwara, S. K. *Tetrahedron Lett.* **1975**, 301−304.
(41) Atherton, E.; Sheppard, R. C. *Solid Phase Peptide Synthesis*; *A Practical Approach*; IRL: Oxford, 1989; pp 63−74.
(42) MBHA-resin (1.12 mmol/g) was obtained from Novabiochem. Standard peptide coupling procedures gave MBHA-Gly-resin **4**.
(43) Photolysis of MHBA-resin **5** in CH_3CN with excess acetic anhydride afforded the *N*-acylglycinate ester as the only observed product by TLC and ^1H NMR. After hydrolysis, this product was not observed under identical photolysis conditions.

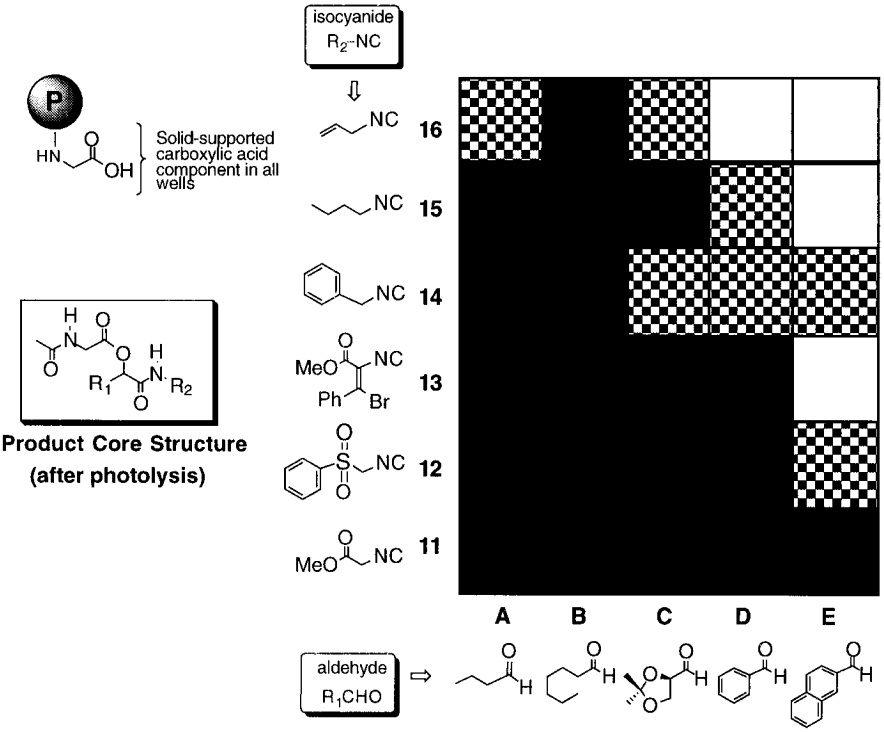
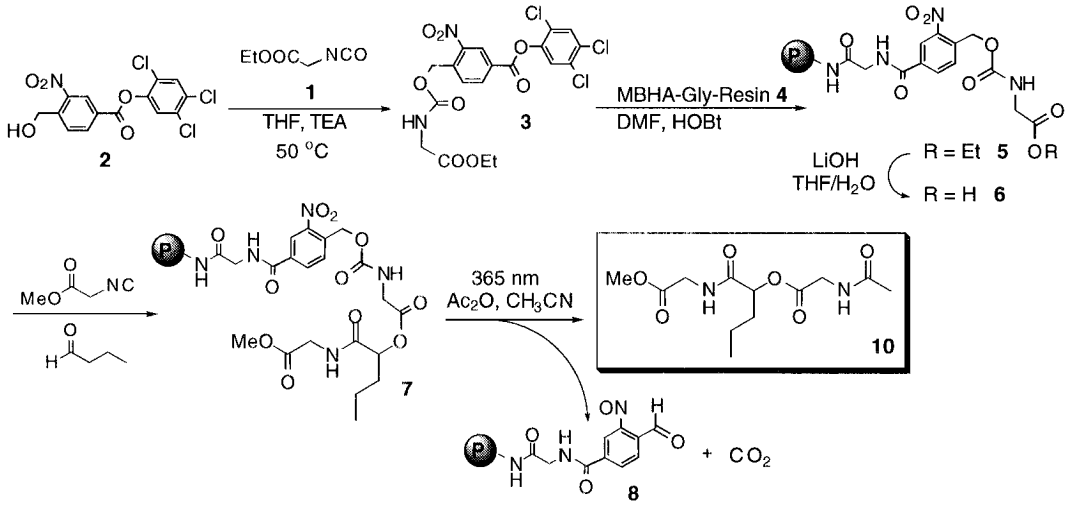


Figure 6. MCCAS of a Passerini library. Dark squares: >70% isolated yield. Checkered squares: 30−70% isolated yield. White squares: no product detected.

Scheme 1. Conditions for Passerini MCCAS of Azinomycin Analogs



tion well, followed by photolysis in the presence of acetic anhydride (Figure 6). The isocyanides (**11**−**16**) and aldehydes (**A**-**E**) were chosen in such a manner as to test the generality of the solid-phase Passerini reaction and maximize the structural diversity of products formed. The isolated products were each analyzed by thin layer chromatography (TLC) and low-resolution mass spectra (LRMS). LRMS of compounds in all wells provided proof of the existence of desired products, while TLC and ^{1}H NMR demonstrated excellent product purity (only a single product was observed in each). TLC was also particularly diagnostic for determining reaction success, since the relative polarities (R_f's) of the desired products were consistent between rows. Analysis of the reactivity profile of individual reagents revealed the same general trend observed in the MCCAS solution studies. The alkyl aldehydes (**A**−**C**) afforded the desired Passerini products in nearly all wells, while the aromatic aldehydes (**D**, **E**) displayed significantly lower reactivity toward selected isocyanides.

A second, analogous array synthesis was performed in solution, resulting in compounds shown in Figure 7. These analogs were then assayed for cytotoxicity toward the HCT116 human colon carcinoma cell line as well as two drug-resistant sublines: HCT116/VM46 expressing the MDR phenotype and HCT116/VP35 resistant to verapamyl and topoisomerase II-active drugs; data is tabulated in Table 2. As can be seen, compounds **17**, **18**, and **20** exhibit potencies only 5-fold less that that of the natural azinomycin B.

(44) Photolytic cleavage of Woodward's analogous *N*-(nitrobenzyl)-carbamoyl-amino acids were achieved in good yields only when additives such as hydroxylamine were included in the photolytic solvent to competitively inhibit Schiff base formation of the product amino acid with the *o*-nitrosobenzaldehyde photolysis product **8**. In fact, no Passerini product was obtained upon photolysis of resin **7** without the addition of acetic anhydride.

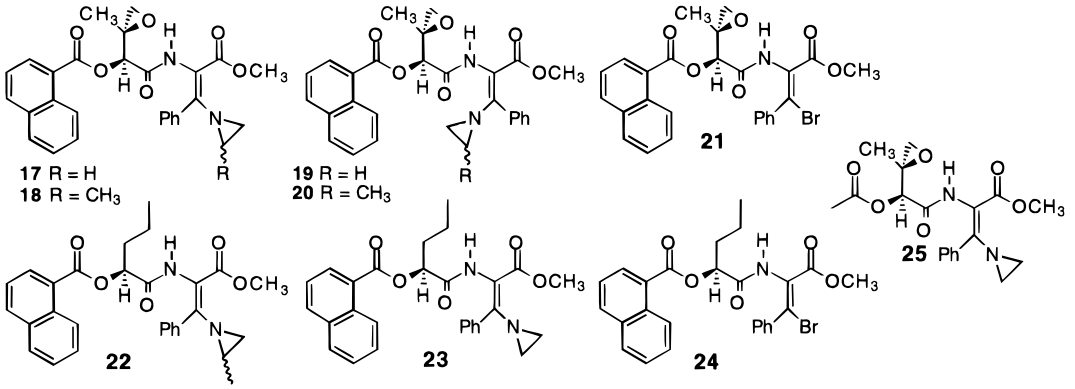


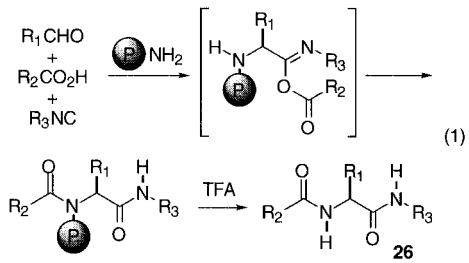
Figure 7. Selected members of MCCAS of azinomycin analogs.

Table 2. In Vitro Cytotoxicities of Azinomycin Analogs in HCT Human Colon Carcinoma Cell Lines[a]

compd no.	HCT116	HCT116/VM46	HCT116/VP35
18	4.39	5.56	5.27
18	5.4	1.6	2.6
19	12.4	13.2	11.0
20	6.76	7.7	6.4
21	>30	>30	>30
22E	>30	>30	>30
22Z	25.3	27.2	25.5
24E/Z	>30	>30	>30
23E	>30	>30	>30
23Z	28.6	38.4	27.3
25Z	>30	>30	>30
25E	>30	>30	>30
azinomycin B	0.838		

[a] IC_{50} (μM), cytotoxicity assessed by XTT assay after 72 h continuous drug exposure.

Ugi Reaction.[33,45] The Ugi 4CC is ideally suited for the construction of chemical libraries based on the α-acylamino amide core structure **26** (eq 1).[46] These libraries represent the solid support synthesis of structurally diverse mono- and disubstituted α-amino acids. Furthermore, these structures provide an entry into the synthesis of heteroaromatic derivatives that rely on a pool of α-amino acid starting materials.[47] While any of the four functional group inputs can be tethered covalently to a solid support, we chose the amino group due to its availability on a variety of matrices. A secondary amide results upon cleavage from the solid support.



(1)

A library was generated consisting of 96 members (12 acids × 8 aldehydes × 1 amine × 1 isocyanide) distributed as one product per well in a 96-well microtiter plate (Figure 8).[48] The inputs for this library were chosen to represent a wide range of donor and acceptor substituents since some components in the solution Ugi synthesis are known to have low reactivity. Thus, a substituent effect analysis of the 4CC reaction on a solid support can be obtained in a single plate (96 individual reactions) synthesis.

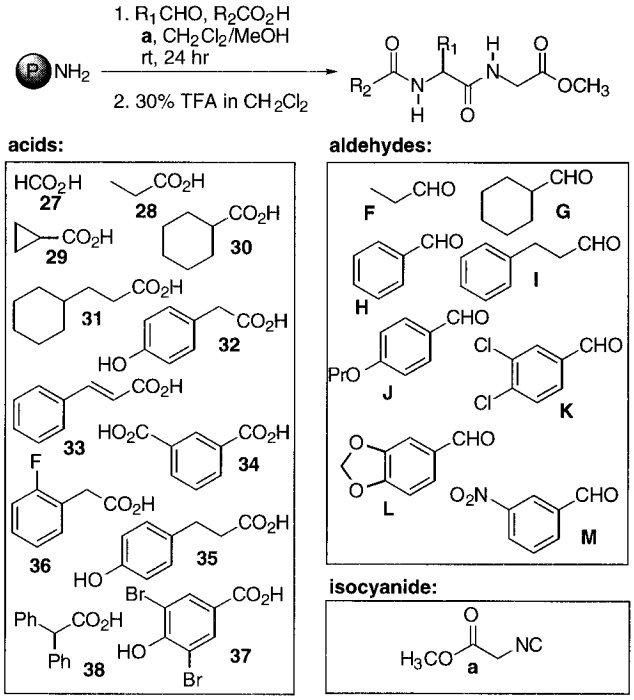


Figure 8. Inputs for solid-supported Ugi MCCAS.

In parallel with solution reactions, the product yields were most sensitive to the structure of the aldehyde inputs. Aliphatic aldehydes and those containing electron-donating groups exhibit overall good yields,[49] whereas aldehydes with electron-withdrawing groups (**K** and **M**) afforded only limited yields (20−30%) even in the presence of a large excess of reagents or repeated exposure to fresh reagents. For acid inputs, the yields involving phenolic derivatives **32**, **35**, and **37** were generally low, in part due to their precipitation from solution over the 48 h reaction period. For the purposes of quantification, four reactions were carried out on a larger scale (0.12 mM) affording, after removal from resin, the following yields:[50] **33Ga**, 30 mg, 69%; **34Ja**, 40 mg, 80%; **36Fa**,

(45) Tempest, P. A.; Brown, S. D.; Armstrong, R. W. *Angew. Chem., Int. Ed. Engl.*, in press.

(46) The ability to generate large numbers of structural analogs via this reaction was addressed by Prof. Ugi: *Isonitrile Chemistry*; Ugi, I., Ed.; Academic: New York, 1971. For a solution library strategy, see: Ugi, I.; Dömling, A.; Hörl, W. *Endeavour* **1994**, *18*, 115−123.

(47) Coppola, B. P.; Noe, M. C.; Schwartz, D. J.; Il Abdon, R. L.; Trost, B. M. *Tetrahedron* **1994**, *50*, 93−116.

(48) The Ugi reaction generates a new stereocenter when an aldehyde is used as the carbonyl input. Each well in the microtiter plate consists of a single racemic product.

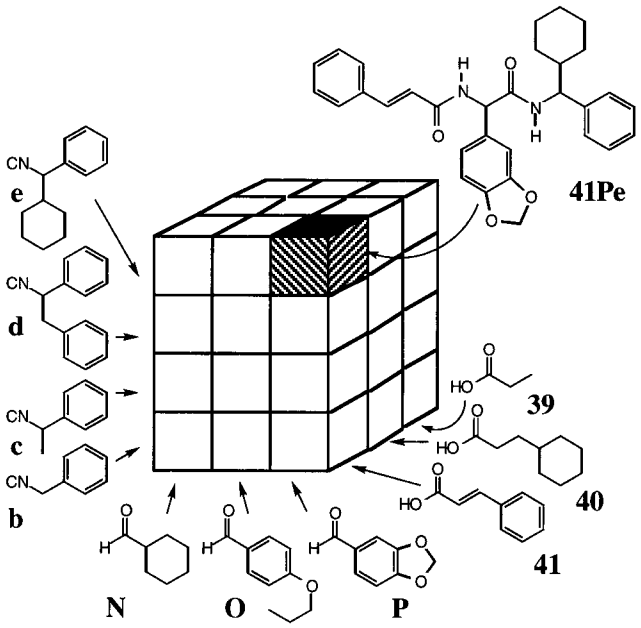


Figure 9. Chemical inputs and positional decoding of product structures for a three-dimensional four-component array.

26 mg, 68%; and **38Ia**, 41 mg, 77%. Similar yields are observed for the solution reactions. The entire library was assayed by low-resolution mass spectrometry and analyzed by ^{13}C and ^{1}H NMR, which revealed >90% purity.[51]

One of the limitations of the Ugi reaction as a tool for library generation is lack of available isocyanides: while there are literally thousands of acids, amines, and aldehydes/ketones, there are perhaps fewer than two dozen commercially available isocyanides, a severe impediment. We sought to address this problem via the pregeneration of a library of isocyanides as inputs to an Ugi-based library. In general, this strategy involves the solution condensation of two subunits prior to solid support synthesis.

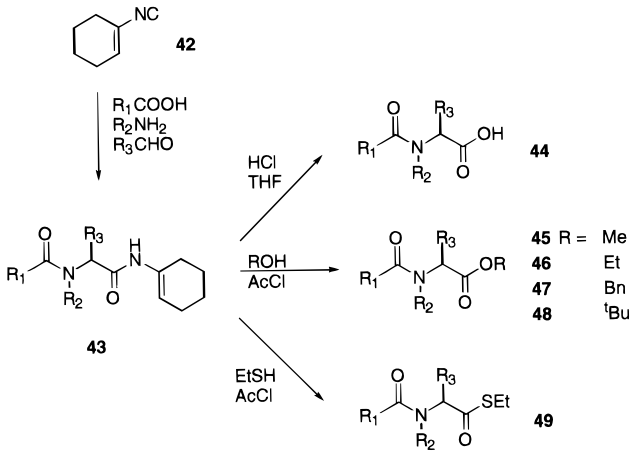
Figure 9 details our efforts toward such a library. Isocyanides **c**−**e** were synthesized from benzyl isocyanide (**b**) via α-lithiation with butyllithium followed by an alkyl halide quench. All new isocyanides were carried on as crude reaction mixtures after an aqueous wash into the array with aldehydes **N**, **O**, and **P**, acids **39**, **40**, and **41**, and Rink amine polymer. A representative product of this five-component condensation, **41Pe**, is shown in Figure 9. Yields after TFA cleavage from the solid support were variable (11−61%),[52] but all expected products were observed by mass spectrometry, and ^{1}H NMR indicated >90% purity. The

(49) Yields (%) are based on equivalents of amine on resin and are reported for the three-step process (a) removal of FMOC from resin, (b) four-component condensation, and (c) TFA cleavage: **27Fa**, 59; **28Fa**, 95; **29Fa**, 95; **30Fa**, 79; **31Fa**, 95; **32Fa**, 45; **33Fa**, 95; **34Fa**, 95; **35Fa**, 85; **36Fa**, 17; **37Fa**, 8; **38Fa**, 51; **27Ga**, 67; **28Ga**, 14; **29Ga**, 70; **30Ga**, 59; **31Ga**, 52; **32Ga**, 41; **33Ga**, 64; **34Ga**, 71; **35Ga**, 42; **36Ga**, 2; **37Ga**, 9; **38Ga**, 37; **27Ha**, 81; **28Ha**, 65; **29Ha**, 77; **30Ha**, 57; **31Ha**, 93; **32Ha**, 23; **33Ha**, **71**; **34Ha**, 68; **35Ha**, 21; **36Ha**, 2; **37Ha**, 4, **38Ha**, 36; **27Ia**, 58; **28Ia**, 65; **29Ia**, 95; **30Ia**, 79; **31Ia**, 95; **32Ia**, 98; **33Ia**, 96; **34Ia**, 90; **35Ia**, 93; **36Ia**, 91; **37Ia**, 64; **38Ia**, 50; **27Ja**, 42; **28Ja**, 86; **29Ja**, 37; **30Ja**, 64; **31Ja**, 61; **32Ja**, 70; **33Ja**, 80; **34Ja**, 76; **35Ja**, 25; **36Ja**, 4; **37Ja**, 78; **38Ja**, 54; **27Ka**, 46; **28Ka**, 30; **29Ka**, 14; **30Ka**, 11; **31Ka**, 30; **32Ka**, 0; **33Ka**, 48; **34Ka**, 31; **35Ka**, 0; **36Ka**, 35; **37Ka**, 28; **38Ka**, 26; **27La**, 55; **28La**, 94; **29La**, 46; **30La**, 52; **31La**, 64; **32La**, 33; **33La**, 66; **34La**, 70; **35La**, 0; **36La**, 5; **37La**, 47; **38La**, 33; **27Ma**, 5; **28Ma**, 0; **29Ma**, 0; **30Ma**, 24; **31Ma**, 45; **32Ma**, 0; **33Ma**, 0; **34Ma**, 30; **35Ma**, 5; **36Ma**, 0; **37Ma**, 21, **38Ma**, 18.

(50) Reactions were performed as described for the 96-well experiment. Yields are based on equivalents of FMOC amine on Rink resin.

(51) Similar purity was observed in the 96-well experiment.

Scheme 2. Use of 1-Isocyanocyclohexene for the Generation of Diverse 4CC Products



pre-4CC solution condensation of the isocyanide input effectively produces a 5CC library on the solid support. This strategy has been extended to an overall 6CC reaction.[53] In such a scheme, 10 structural variants of each chemical input in a 6CC reaction generate a 10^6 compound library.

Universal Isocyanide. We have also recently devised another strategy designed to overcome the lack of commercially available isocyanides.[54] By using 1-isocyanocyclohexene (**42**) in the Ugi 4CC and then treating the product **43** with acid and one of a variety of nucleophiles, the cyclohexene moiety is switched to a new functionality (Scheme 2). This methodology is not limited to purified products; after the four components of the Ugi are allowed to condense, addition of acid to the methanolic solution of crude **43** yields the methyl ester derivative as a single product in high yield. This represents a one-pot procedure in which the isocyanide component contributes only a single carbon atom to the final product.

We have found that a wide variety of inputs are tolerated in this 4CC and subsequent transformations. Aliphatic and aromatic acids, aldehydes, and ketones all undergo the 4CC with aliphatic amines and 1-isocyanocyclohexene. 1-Isocyanocyclohexene itself is somewhat susceptible to air oxidation and to heat-initiated polymerization,[55−57] but we have found it to be an indefinitely stable reagent when stored at −30 °C under an inert atmosphere.

Subsequent transformations of the 4CC product of **42** also support the generality of this reaction: almost all 4CCs thus far submitted to the conditions in Scheme 2 undergo the indicated transformations. The one exception is R^1 = H; when formic acid is used as

(52) Yields (%) are based on equivalents of amine on resin and are reported for the three-step process (a) removal of FMOC from resin, (b) four-component condensation, and (c) TFA cleavage: **39Nb**, 55; **40Nb**, 11; **41Nb**, 10; **39Ob**, 55; **40Ob**, 50; **41Ob**, 31; **39Pb**, 51; **40Pb**, 30; **41Pb**, 19; **39Nc**, 31; **40Nc**, 40; **41Nc**, 50; **39Oc**, 42; **40Oc**, 52; **41Oc**, 50; **39Pc**, 38; **40Pc**, 31; **41Pc**, 30; **39Nd**, 38; **40Nd**, 40; **41Nd**, 31; **39Od**, 50; **40Od**, 40; **41Od**, 55; **39Pd**, 32; **40Pd**, 40; **41Pd**, 28; **39Ne**, 40; **40Ne**, 30; **41Ne**, 30; **39Oe**, 18; **40Oe**, 30; **41Oe**, 23; **39Pe**, 10; **40Pe**, 30; **41Pe**, 20.

(53) Condensation of α-lithiobenzyl isocyanide with benzaldehyde followed by addition of acetic anhydride affords a three-input isocyanide. This crude reaction mixture undergoes a 4CC reaction on a solid support.

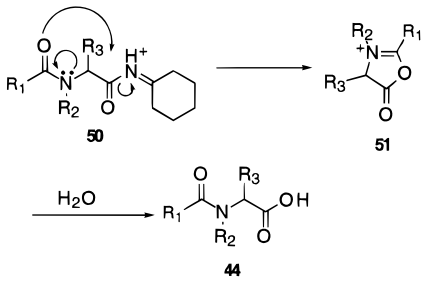
(54) Keating, T. A.; Armstrong, R. W. *J. Am. Chem. Soc.* **1995**, *117*, 7842−7843.

(55) Barton, D. H. R.; Bowles, T.; Husinec, S.; Forbes, J. E.; Llobera, A.; Porter, A. E. A.; Zard, S. Z. *Tetrahedron Lett.* **1988**, *29*, 3343−3346.

(56) Baldwin, J. E.; Yamaguchi, Y. *Tetrahedron Lett.* **1989**, *30*, 3335−3338.

(57) Keating, T. A.; Armstrong, R. W. Unpublished results.

Scheme 3



the acid input, the 4CC proceeds smoothly, but only primary amide results from hydrolysis instead of carboxylic acid **44**. In our initial communication,[54] we postulated a münchnone[58,59] intermediate (Scheme 3) to explain the unusual reactivity of the *N*-cyclohexenyl amide **43**. Protonation of **43** yields **50**, which can cyclize to azomethine ylide **51**. Attack by water (or other nucleophile) results in product **44**. We noted that extremely rapid cleavage of the *N*-formyl amide under acidic conditions would render an intermediate of type **51** impossible. This is borne out by our results.

(58) Huisgen, R.; Gotthardt, H.; Bayer, H. O.; Schaefer, F. C. *Angew. Chem., Int. Ed. Engl.* **1964**, *3*, 136−137.
(59) Padwa, A.; Burgess, E. M.; Gingrich, H. L.; Roush, D. M. *J. Org. Chem.* **1982**, *47*, 786−791.
(60) Keating, T. A.; Armstrong, R. W. *J. Am. Chem. Soc.*, in press.
(61) Tempest, P. A.; Armstrong, R. W. *J. Org. Chem.*, submitted for publication.

In more recent work,[60] we have focused on confirming the intermediacy of **51**.

Using these methods, carboxylic acids **44**, esters **45**−**48**, and thioesters **49** can be made. This remarkably powerful strategy transforms the Ugi reaction into a condensation of carboxylic acids, amines, aldehydes/ketones, and alcohols or thiols, all abundantly available reagents. In addition, the use of 1-isocyanocyclohexene obviates the need to synthesize, handle, and store a large number of isocyanides.

IV. Conclusion

In this Account, we have attempted not only to present our recent work in the area of combinatorial synthesis but also to illustrate what we believe to be excellent strategies for future investigation. If the major goals of combinatorial synthesis are the generation of not only large numbers of compounds but also diverse compounds that enjoy relative ease of synthesis, then multiple-component condensations as a class of reactions possess many rich opportunities. We believe that the core structures of many classes of important pharmacophores are accessible from such reactions. Our goals for the future include the further pursuit of the chemistry described herein, as well as the adaptation of other multiple-component condensations to solid support and array synthesis.

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