A Novel Concept in Combinatorial Chemistry in Solution with the Advantages of Solid-Phase Synthesis: Formation of N-Betaines by Multicomponent Domino Reactions**

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Dedicated to Professor Ivar Ugi on the occasion of his 70th birthday

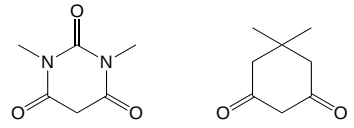
Combinatorial chemistry is an important method, both in the search for lead structures of pharmacologically active compounds and in their optimization,[1] and it also finds application in supramolecular chemistry,[2] in catalyst development,[3] and in material science.[4] Within this context combinatorial solid-phase synthesis[5] has the advantage of simple automation, since reagents can be used in excess, and impurities and by-products can be removed by simple filtration and washing procedures. Frequently, however, the transfer of reactions established in solution to the solid phase is fraught with difficulties; an additional disadvantage is that the synthesis is extended by two steps. Moreover, monitoring the course of the reaction on the solid phase is problematic using normal methods. Therefore, parallel to solid-phase synthesis new combinatorial approaches to synthesis in solution, to reactions on soluble resins,[6] and in the perfluorinated phase,[7] as well as separation of by-products by binding to polymers[8] have been developed.

Herein we describe a new, simple concept in combinatorial chemistry which combines the advantages of reactions in solution with those of solid-phase synthesis. The reagents can be used in excess, and the products can be obtained by simple precipitation in purities of up to 99%. The procedure involves a multicomponent domino Knoevenagel/hetero-Diels–Alder reaction[9] of a 1,3-dicarbonyl compound with an amino aldehyde and an enol ether, followed by a reductive amination with the formation of a betaine which can be precipitated from the solution in high purity. Organic by-products and excess reagents are separated by simple filtration. By the use of α-, β-, and γ-amino aldehydes libraries of pyrrolidine, piperidine, and azepane derivatives, respectively, may be obtained.

The required amino aldehydes **1**–**3** are accessible in high yields simply by reaction of a wide spectrum of available natural and nonnatural α-, β-, and γ-amino acids with benzyl chloroformate, followed by esterification, and reduction with diisobutylaluminum hydride (DIBAL-H) in hexane solution (Table 1).[10] The compounds are stable and storable. *N*,*N*′-dimethylbarbituric acid (**5a**), the cyclohexan-1,3-diones **5b** and **5c**, and 4-hydroxycoumarin (**5d**) were used as 1,3-dicarbonyl components. The differently substituted benzyl enol ethers **4a**–**d**[11] served as dienophiles.

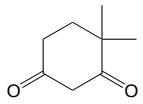
Table 1. Amino aldehydes used.

Aminoaldehyde	R^1	R^2	*n*
1a	H	H	0
1b	H	Me	0
1c	Me	H	0
1d	*i*Pr	H	0
1e	*i*Bu	H	0
1f	Bn	H	0
1g	$CH_2CH_2CH_2$		0
2	H	H	1
3	H	H	2



5a

5b



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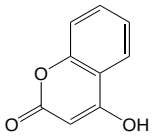

5d

The amino aldehydes **1**–**3** were treated with the 1,3-dicarbonyl components **5a**–**d** (1 equiv) and the benzyl enol ethers **4a**–**d** (4 equiv) in toluene in the presence of catalytic amounts of EDDA and trimethyl orthoformate as dehydrating agent in an ultrasonic bath at 50 °C (Scheme 1). Knoevenagel condensation of the amino aldehyde with the 1,3-dicarbonyl component occurs first with the formation of an electron-deficient, sterically fixed 1-oxo-1,3-butadiene **6** which then reacts with the benzyl enol ether in a Diels–Alder reaction with inverse-electron demand to afford a benzyl-protected acetal **7**. Subsequent hydrogenation with palladium on carbon as catalyst leads to hydrogenolysis of the benzyl acetal and the Cbz protecting group with release of an amine and an aldehyde, which under the reaction conditions lead to the desired highly substituted nitrogen heterocycles **8**–**10** of different ring size in an intramolecular reductive amination (Scheme 2, Table 2); mixtures of diastereoisomers are normally formed in the reaction.

The products thus obtained contain a 1,3-dicarbonyl unit with an C–H-acidic methylene group and an amino group; this leads to the formation of a betaine which is readily soluble in water and methanol, but can be precipitated by the addition of diethyl ether. This represents a new concept in combinatorial chemistry which should find general applicability. The scope of the procedure is considerable; for example, sterically demanding amino aldehydes and benzyl enol ethers could be used. The use of 1,3-dicarbonyl compounds with reduced reactivity did not result in a deterioration of the purity of the

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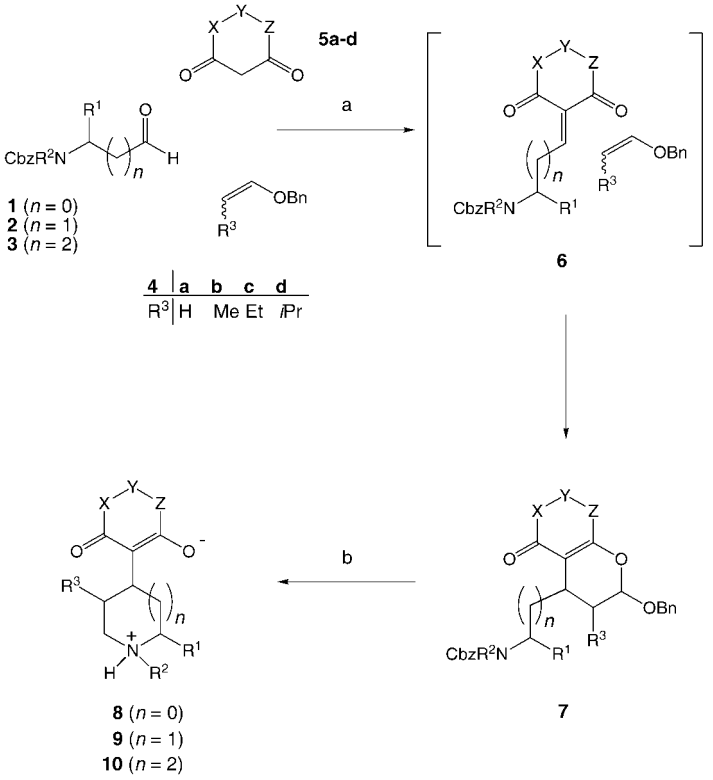
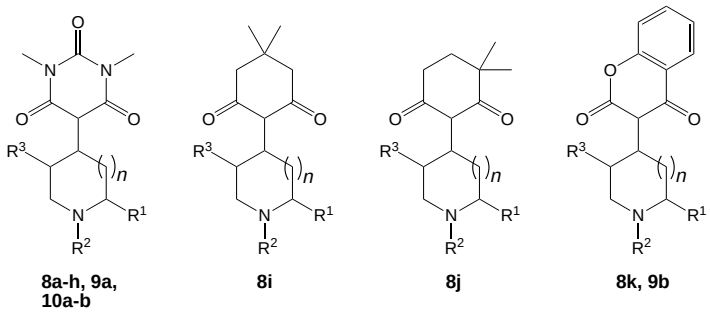


Scheme 1. Domino sequence comprising Knoevenagel, hetero-Diels–Alder reaction, and hydrogenation starting from amino aldehydes, 1,3-dicarbonyl compounds, and enol ethers. a) toluene, EDDA, ultrasound, 50 °C, 15 h; b) Pd/C, H_2, 1 bar, RT, 24 h. EDDA = ethylenediammonium diacetate, Cbz = benzyloxycarbonyl, Bn = Benzyl.



Scheme 2. Products from the domino Knoevenagel/hetero-Diels–Alder/hydrogenation sequence.

products, in spite of the required extended reaction times. Only reaction with the disubstituted *N*-Cbz-2-amino-2-methylpropionaldehyde and benzyl-2-methylprop-1-enyl ether did not lead to the desired products, presumably because of steric overload.

Unlike solid-phase combinatorial chemistry the reactions may be monitored at all times by thin-layer chromatography, and where required the intermediates investigated without difficulty by normal spectroscopic methods. Finally, one further advantage is the lack of requirement for the use of expensive resins as well as the development and optimization of suitable coupling and decoupling reactions. The domino sequence of Knoevenagel, hetero-Diels–Alder reaction, and hydrogenation allows rapid access to a number of N-heterocycles of different ring sizes and substitution patterns with a betaine structure which can be obtained in high purity by precipitation in a process which is also suitable for automation.

Table 2. Products of the domino sequence: pyrrolidine, piperidine, and azepane derivatives **8**, **9**, and **10**, respectively.

Product	R^1	R^2	R^3	*n*	Purity [%][a]	Yield [%]
8a	H	H	Et	0	97	53
8b	H	Me	*i*Pr	0	> 90[b]	47
8c	Me	H	Me	0	97	45
8d	*i*Pr	H	H	0	95	55
8e	*i*Bu	H	H	0	99	59
8f	*i*Bu	H	Et	0	> 95	41
8g	$CH_2CH_2CH_2$		H	0	> 90	51
8h	Bn	H	H	0	97	65
8i	Me	H	H	0	> 95	71
8j	Me	H	H	0	> 95	59
8k	Me	H	H	0	95	61
9a	H	H	H	1	> 95	60
9b	H	H	H	1	> 95	69
10a	H	H	H	2	98	58
10b	H	H	Me	2	> 95	55

[a] Determined by HPLC unless otherwise indicated. [b] Purity determined by ^{1}H NMR spectroscopy.

Experimental Section

Synthesis of **8h**: **1f** (35.4 mg, 125 μmol), *N*,*N*′-dimethylbarbituric acid (**5a**) (19.5 mg, 125 μmol) and benzylvinyl ether (**4a**) (134 mg, 1.00 mmol) were allowed to react in toluene (0.5 mL) and trimethyl orthoformate (0.1 mL) with a few crystals of EDDA in an argon atmosphere in a 10-mL pressure flask in an ultrasonic bath for 15 h at 50–60 °C. After removal of the solvent in vacuo, the residue was dissolved in methanol (4 mL) and stirred in a hydrogen atmosphere in the presence of palladium on carbon (10%, 12.5 mg) for 24 h. Subsequently, the catalyst was removed by filtration through a little celite, washed with methanol, and the solvent removed in vacuo. The residue was dissolved in a little methanol and the product was precipitated by the addition off diethyl ether. The white, amorphous precipitate was filtered off and washed with diethyl ether. Compound **8h** was 97% pure as determined by HPLC. Decomposition > 250 °C; ^{1}H NMR (300 MHz, CD_3OD): $\delta = 2.05-2.45$ (2m, 2H; 4-H), 2.90–2.95 (m, 2H; PhCH_2), 3.20 (s, 6H; $2 \times NCH_3$), 3.30–3.55 (m, 3H; 2-H, 5-H), 4.22–4.32 (m, 1H; 3-H), 7.15–7.30 (m, 5H; Ph-H); ^{13}C NMR (75 MHz, [D_6]DMSO): $\delta = 26.17$ ($2 \times NCH_3$), 27.21 (C-4), 39.87 (C-3), 35.28, 43.49 (C-1″, C-5), 61.83 (C-2), 81.15 (C-5′), 125.5, 128.3, 128.7 (C-3″, C-4″, C-5″, C-6″) 131.0 (C-2″), 152.6 (C-2′), 162.0 (C-4′, C-6′); MS (70 eV): *m*/*z* (%): 315.2 (1) [M^+], 224.1 (100) [$M^+ - Bn$], 91.0 (10) [$C_7H_7^+$]; HR-MS: calcd for $C_{17}H_{21}N_3O_3$: 315.1582, found: 315.1582.

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Spatially Resolved Detection of Neurotransmitter Secretion from Individual Cells by Means of Scanning Electrochemical Microscopy

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Microelectrochemical methods, especially constant-potential amperometry and fast-scan cyclic voltammetry,[1] provide possibilities to investigate biological systems with cellular or subcellular spatial resolution determined mainly by the size of the available microelectrodes.[2] Among the biological phenomena studied so far at single cells or substructures of single cells are individual exocytosis events,[3] oxygen consumption,[4] photosynthetic activity,[5] and ion channel distribution.[6] In these experiments, the electrochemical sensor—usually a microdisk electrode with a tip diameter of a few micrometers—has to be positioned in close proximity to the object. The approach of the microelectrode to the biological object is, in general, performed under control of an optical microscope using manual- or piezo-actuated micromanipulators for positioning of the sensor tip. The electrode is slowly moved towards the cell until it slightly touches the cell membrane, which is visualized by its bending. The tip is then retracted for a defined distance allowing, in principal, adjustment of the membrane-to-electrode distance within a range of several hundred nanometers to two micrometers. Although this approach has been successfully used in recent years,[3] there are significant drawbacks, such as a) possible contamination of the electrode surface due to its contact with the cell membrane, b) insufficient reproducibility of the tip-to-cell distance, which becomes even more difficult using smaller microelectrodes, that are hardly visible in the optical microscope, c) eventual deterioration of the cell at the contact point or mechanical depolarization of the cell, and d) the impossibility to investigate sequentially different spots on the same cell.

After initial investigations of biological samples by means of scanning electrochemical microscopy (SECM),[7] attempts have been recently undertaken to visualize the metabolism and the redox activity of individual cells.[8] However, one of the major problems in conventional SECM experiments is the constant z-height of the microelectrode, which does not allow differentiation between variations in the tip-to-sample distance and changes of the local electrochemical activity. In order to overcome these limitations, we have introduced a shear-force based constant-distance control into the SECM.[9] The benefits and limitations had been described in detail recently.[10] In short, the microelectrode vibrates at its resonance frequency with typical amplitudes of only a few nanometers with use of a piezo-pusher. Simultaneously, a laser beam is focused onto the very end of the vibrating electrode and the resulting Fresnel diffraction pattern is projected onto a split photodiode. Amplitude and phase information about the vibrating tip is obtained by the amplification of the difference signal from the split photodiode with respect to the agitation signal using a lock-in amplifier. With decreasing tip-to-sample distance, increasing shear forces between tip and sample surface lead to a damping of the vibration amplitude and to a phase shift, which can be used to continuously keep a predefined damping value related to a constant distance of about 50 – 100 nm by means of a software-controlled feedback loop.

Thus, adaptation of the shear-force based constant-distance control of the tip-to-sample gap to biological preparations at the single-cell level should, on the one hand, allow the problems occurring with manual microelectrode positioning to be overcome and, on the other hand, enable detectable variations of chemical species at different sites of a biological preparation.

Using platinum microelectrodes sealed in glass capillaries, which can be easily positioned over hard sample surfaces using the shear-force positioning mode, no satisfying results could be obtained. In preliminary experiments using adher-

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