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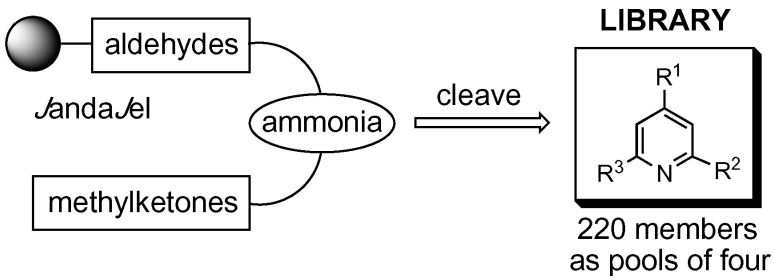

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Preparation of a Kröhnke Pyridine Combinatorial Library Suitable for Solution-Phase Biological Screening

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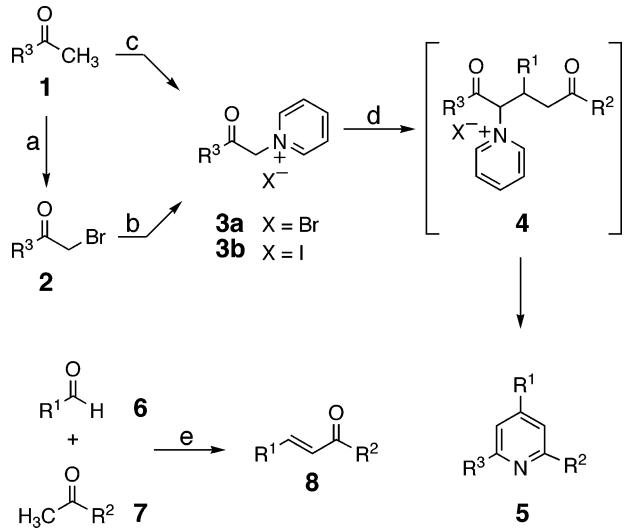
Pyridine derivatives are an important part of the repertoire in the discovery of new pharmaceuticals and agrochemicals. In this regard, combinatorial chemistry can be a powerful tool. The Kröhnke synthesis of 2,4,6-trisubstituted pyridines is ideal for combinatorial applications, since reactions generally proceed in high yields on solid phase, and three points of diversity can be independently varied. A 220-member Kröhnke pyridine library was prepared on *J*anda*J*el using a combined split-mix and parallel strategy affording 55 pools of four members each after cleavage from the resin. The library was analyzed by HPLC and LC/MS that indicated 85% of the pools were of good quality in terms of yield, purity, and members present in similar amounts. Notably, the library was prepared on a large scale (∼15 to 20 mg per member) and in a time/cost efficient manner. The pooling approach facilitated synthesis and should also prove advantageous in biological screening experiments.

Introduction

Combinatorial chemistry continues to be a focal point in academia and industry for the discovery of biologically active molecules. In this regard, the construction of heterocyclic small-molecule libraries using both solid- and solution-phase formats has been widely investigated.[1] Since pyridine derivatives are both a potential and proven source of pharmaceuticals and agrochemicals,[2] this ring system is an attractive target for combinatorial library development and screening. The Hantzsch and Bohlmann−Rahtz reactions have been explored for combinatorial pyridine synthesis, but improvements are necessary, and their two-component formats limit versatility.[3,4] Greater utility for the Hantzsch reaction has been possible in the preparation of fused-pyridine heterocycle libraries.[5] In another interesting example, a solution-phase cobalt-catalyzed cyclotrimerization of two alkynes and a nitrile afforded highly functionalized pyridines, but was hampered by a lack of regiochemical control and formation of complex mixtures.[6] To prepare a combinatorial library of pyridines for drug discovery, it is preferable to design a solid-phase strategy that is efficient, provides individual or small pools of compounds in adequate yields and purities, and in which at least three components for diversity can be independently and readily varied.

The Kröhnke pyridine synthesis represents an ideal method for combinatorial applications. In the traditional procedure, a bromoketone **2** affords pyridinium intermediate **3a** that undergoes a Michael-type addition to an α,β-unsaturated ketone **8**, followed by ring formation in the presence of ammonium acetate to give a 2,4,6-trisubstituted pyridine **5** (Scheme 1).[7] Notably, no 1,5-diketone intermediates need to be isolated, and unlike the Hantzsch reaction, no oxidation of a dihydropyridine is required, since the pyridinium species **4** is at the necessary oxidation level. Grosche et al. reported the individual solid-phase preparation of nine different pyridines in excellent yields and purities using the Kröhnke strategy.[8] A modification also on solid-phase employed a trimethylsilylenol Michael addition to form a discrete 1,5-diketone that afforded 10 pyridines in low to moderate yields and purities.[9] Other versions in solution and solid phase make use of enaminonitriles as "ammonia-enol synthons" in the addition to α,β-unsaturated ketones, although only two points of diversity can be introduced.[10] To date, the Kröhnke synthesis has not been used to create combinatorial pyridine libraries suitable for the purpose of biological screening.

Scheme 1. Generalized Kröhnke Pyridine Synthesis[a]



[a] (a) bromination (i.e., Br_2 or Bu_4NBr_3) (standard protocols); (b) pyridine, THF; (c) pyridine solvent, I_2; (d) **8**, NH_4OAc, AcOH, 20−120 °C; (e) basic conditions (i.e., NaOH or Na_2CO_3) (standard protocols).

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Approaches to small-molecule combinatorial libraries include the synthesis of all compounds in one mixture, the parallel synthesis of individual compounds, and the synthesis of "pools" of compounds. Herein, we describe the solid-phase combinatorial preparation of a diverse 220-member Kröhnke pyridine library using a pooling strategy. After cleavage of compounds from the resin, each pool contained four library members on a multimilligram scale ready for dissolution and analysis.

Results and Discussion

When considering the options for combinatorial library design and implementation, synthesis and testing of mixtures of compounds ("pools") can be often more efficient than synthesis and testing of individual compounds.[11] Similarly, pooling experiments of individual compounds have been a time- and cost-effective approach for screening libraries as part of the drug discovery process in pharmaceutical companies.[12] In the synthesis of mixtures, one disadvantage might be the significant underrepresentation of particular members in the pool(s), which during screening might mean that important leads or even the most active compound will not be identified. The specific strategies employed for library design/synthesis, pooling, and deconvolution will affect the likelihood of success. Consequently, it is critical that the synthetic chemistry is broadly applicable to a variety of structures used as building blocks, and that the pools are appropriately sized (member numbers), allowing adequate concentrations of all compounds to maximize detection of "hits".

Our intent was to manually construct a small Kröhnke pyridine library on solid phase and on a large scale (individual members at ~15 to 20 mg). Our philosophy is that libraries of 200−300 members are an ideal size by enabling synthetic efficiency, the ease of one-time preparation of large quantities for numerous screening experiments, and sufficient diversity to provide a reasonable probability for identifying a lead compound. Furthermore, weighing the options in this case, we concluded that the pooling approach would be highly applicable and time/cost-effective. Notably, the Kröhnke reaction has shown broad utility for the preparation of many different 2,4,6-trisubstituted pyridines.[7] Moreover, since other workers defined conditions that afforded excellent results on solid phase,[8] our strategy could engage both "split-mix" and "parallel" aspects. After final cleavage from the resin, compounds would be available for testing in solution.

The Kröhnke reaction permits efficient use of a given set of reactants in that a methyl ketone is used in two different steps to install groups at identical 2- and 6-positions of the pyridine nucleus (Scheme 1). Hence, only two types of building blocks, aldehydes and methyl ketones, provide three points of diversity, but in which a combinatorial approach using one "toolbox" of each of these components results in less than a theoretical maximum number of library members. We designed a library based on using four aldehydes and 10 methyl ketones (Figure 1). This would afford 220 different pyridines, and not 400, as calculated using the expression $m[(n^2 + n)/2]$, where m is the number of aldehydes and n is the number of methyl ketones. Of course, one could employ a second toolbox of methyl ketones ($4 \times 10 \times 10$ format) to yield 400 compounds, but not in a fully combinatorial fashion. In using the 4×10 format, we concluded that the preparation of 55 pools of four members each (124 total chemical reactions, including resin formation and cleavage) would be more efficient than a parallel library (484 total reactions), and far superior to strategies consisting of pools with up to 10 members. The four-member pools would be also advantageous with respect to eventual biological screening experiments.

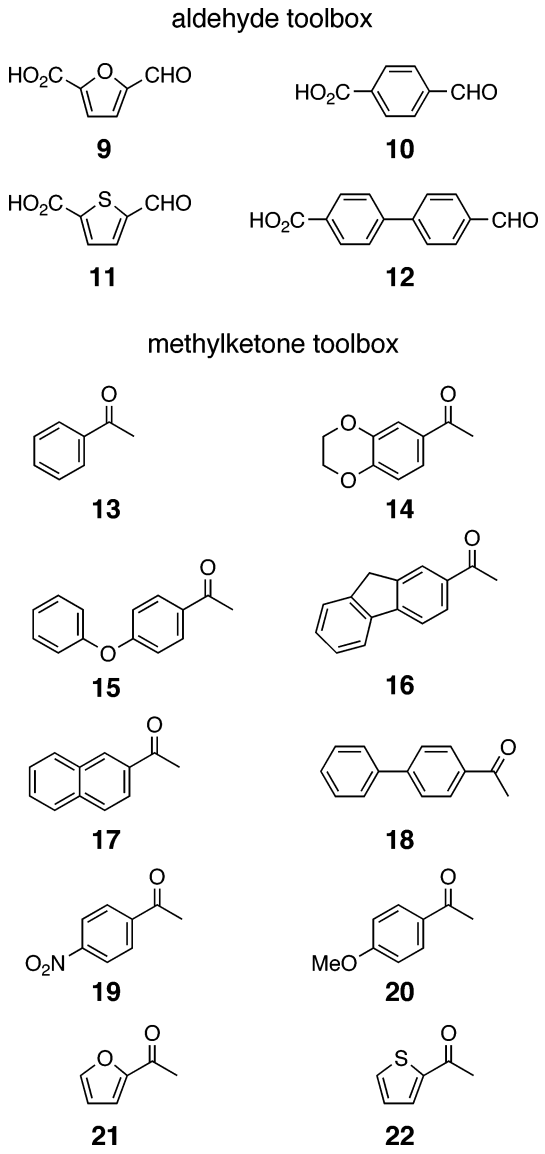
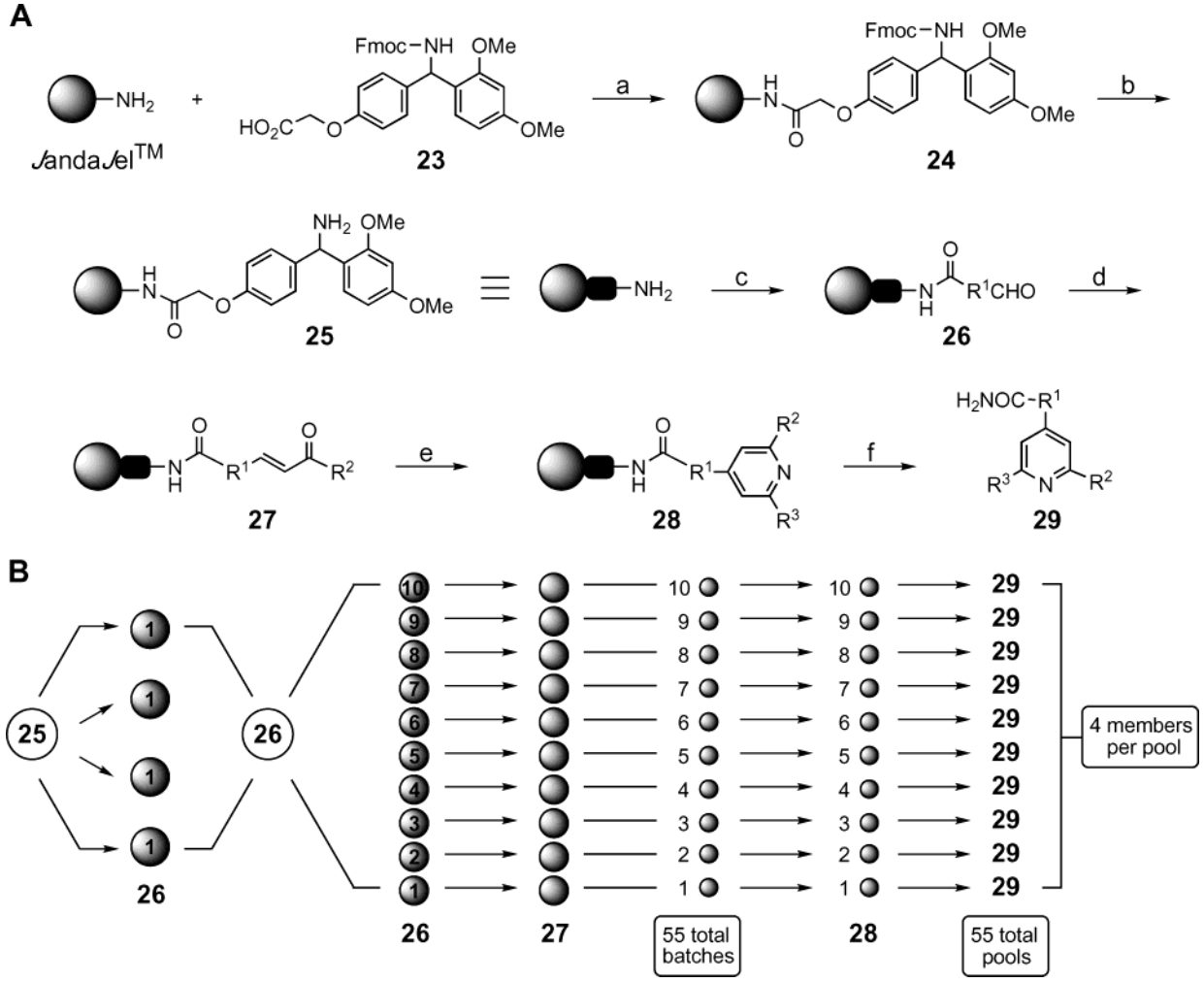


Figure 1. Building block toolboxes for the Kröhnke pyridine library. The aldehydes contribute diversity at R^1, and the methyl ketones contribute diversity at R^2 and R^3.

In light of the above considerations, we chose the aldehyde components **9**−**12** bearing a carboxylic group for attachment to *J*anda*J*el-NH_2 resin (Figure 1, Scheme 2, A). To this end, the resin was functionalized with a Rink-type linker **23** using standard amide-bond forming and Fmoc-deprotection reactions to afford **25**. The linker improves reaction yields and allows facile cleavage of products. The aldehydes **9**−**12** were then individually coupled to batches of **25** of equal weight using the diisopropylcarbodiimide (DIC) method to afford

Scheme 2. Combinatorial Solid-Phase Synthesis of a Kröhnke Pyridine Library[a]



[a] Reagents and conditions: (a) DIC, HOBt, DMF; (b) 20% piperidine/DMF; (c) DIC, HOBt, DMF, **9**−**12** (individually coupled); then, mix−split; (d) LiOH, DME, **13**−**22** (individually reacted), RT, 16 h; then, split−parallel; (e) NH_4OAc, HOAc, DMF, **13**−**22** as species **3a** (individually reacted), 90 °C, 24 h; (f) TFA, CH_2Cl_2, RT, 30 min. [b]Strategy for library preparation: Numbers **25**−**29** correspond to part A. Numbers inside shaded circles correspond to approximate weight ratios of resin. Numbers to the left of shaded circles correspond to the number of batches of resin of approximately equal weight.

four resins **26**, each of which contained a different R^1 substituent. These four resins were then mixed. To avoid eventual compound redundancy, **26** was split into a series of 10 batches of unequal weight with the largest batch containing ∼10-fold more resin than the smallest batch (Scheme 2, B).

Introduction of the R^2 substituents via a Claisen−Schmidt condensation was then carried out in parallel using a different one of the methyl ketones **13**−**22** for each batch (Scheme 2). This provided 10 unequal batches of **27**, with each containing four different α,β-unsaturated ketone resins. In a second parallel step, the R^3 substituents were then also derived from **13**−**22** via a sequential Michael-type addition and ring closure in the presence of NH_4OAc. For this step, it was first necessary to prepare the pyridinium salts **3a** or **3b** corresponding to **13**−**22**. Although the Ortoleva−King reaction directly affords the iodides **3b**,[13] we chose the bromides **3a**. Since purification of the bromoketones **2** was not required, some bromoketones were commercially available, and all **3a** readily crystallized. Hence, the batches of **27** were subdivided equally with the number of batches based on their weight ratios (10 batches from the largest, nine batches from the next, etc.) (Scheme 2B). Each of the batch sets was reacted with a corresponding set of **3a** (**3a** from **13**−**22** used for the 10 batch set, **14**−**22** for the nine batch set, etc.) to afford the entire combinatorial library of 2,4,6-trisubstituted pyridine resins **28** as 55 batches.

A portion of resin from each batch of **28** was cleaved using TFA to afford the 55 pools of **29**, each containing four Kröhnke pyridine library members (Scheme 2). The amount of resin used provided for the preparation of ∼70 to 80 mg of each pool. At the outset, on the basis of a small-scale test of the complete reaction sequence by preparing one pyridine **29** (R^1, R^2, R^3 = Ph), and the goal of obtaining ∼15 to 20 mg of each library member, it was estimated that a minimum of ∼12 g of *J*anda*J*el-NH_2 resin would be required for library preparation. We started with ∼18 g, since we anticipated converting some of the pyridine library to a pyridinium library using the "library-from-library" concept. Unfortunately, the Kröhnke pyridines appeared to be resistant to alkylation (i.e., benzylbromide) under several conditions (unpublished results), including those of Lago et al. for 3,5-disubstituted pyridines,[14] perhaps because of steric effects. The remaining **28** can be conveniently stored for archiving and should additional quantities of **29** be required for biological assays.

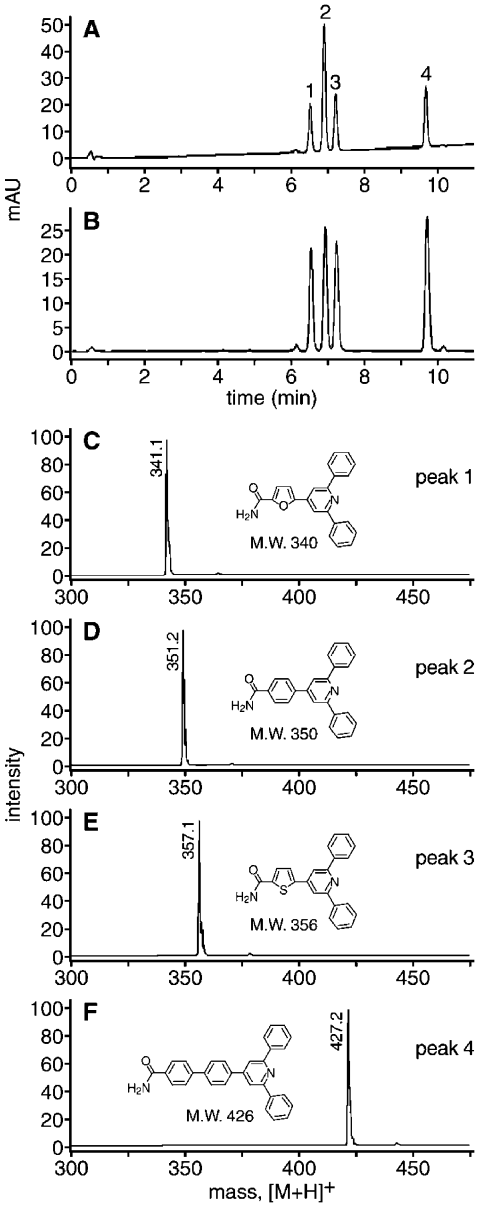


Figure 2. HPLC/LC/MS analysis of a typical pool from the Kröhnke pyridine library. The pool shown corresponds to pool 1 (see Experimental Section and Supporting Information). (A) Elution profile from analytical HPLC. (B) Elution profile from LC/MS. (C−F) Mass spectral analysis of each peak from B.

Analysis by HPLC showed that 44 of the 55 pools contained four major peaks similar to pool 1 (Figure 2A), 1 pool in which two major peaks could not be resolved, and 10 other pools in which one peak was too small and obscured to be quantitated (see Supporting Information). Interestingly, for the latter, all 10 members contained R^1 = biphenyl. In addition, as defined by the library design, three of these members were from one family (R^1 = biphenyl, R^2 = biphenyl) and in pools of good quality (yield/purity, 74−96%/83−90%), and seven members were a complete family (R^1 = biphenyl, R^2 = 9H-fluoren-2-yl), but in which the pools were of poor quality (yield/purity, 36−47%/16−31%). The reason these 10 pools were problematic, particularly the one entire family, remains unclear. We speculated that the Claisen−Schmidt reaction did not proceed adequately, but FT-IR data (1606 cm^{-1}, strong) indicated that this was not the case. Only for pools derived from **3a** (R^3 = 2-furyl) that sometimes resulted in lower purities could some conclusions be drawn, because this reagent appeared unstable and turned black under the acidic ring-forming conditions. Following from the above, on inspection by LC/MS (Figure 2B−F), peaks corresponding to 210 of the 220 expected members were identified with a correct mass.

These 210 members were also assigned relative ratios within their respective pools, with the library average calculated as 1.0/1.4/0.99/0.84 (R^1 from **9/10/11/12**, respectively). However, compound ratios were likely even closer to unity, as suggested by the HPLC response factors for **9**−**12** which showed **10** > **9** (severalfold) and **9** > **11** ∼ 12 (slightly). The presence of similar amounts of members within pools is an important determinant of good quality for a library of this type.

Finally, for all 55 pools taken together, the average yield was 85%, and the average purity was 72%. Eliminating the seven pools above of poor quality, as well as one other anomalous pool (R^2 = Ph, R^3 = 2-furyl; all peaks identified; yield/purity, 96%/28%), raises the averages to 91% and 80%, respectively. Therefore, consistent with these values, 47 of 55 (85%) of the pools could be considered of good to excellent quality. These pools are the ones that are particularly meaningful for initial biological screening purposes, although the pools of poor quality should be also tested for activity, given that significant amounts of three pyridines were detected in each case. In light of the scale of library production, hundreds or even thousands of assays would be possible at 100 μM levels of all 55 pools in 0.1−0.5 mL microtiter well or cuvette formats.

Conclusion

The Kröhnke pyridine synthesis is suited for the design and construction of combinatorial libraries. Reactions in the sequence are straightforward and provide high yields on solid phase and can incorporate three points of extensive structural diversity in the target pyridine. As demonstrated herein, a library of this type could be manually constructed in a low-tech fashion using less than $300 of commercially available *J*anda*J*el-NH_2 resin and inexpensive aldehydes and methyl ketones as building blocks. In addition, the efficiency and time/cost factor is further emphasized in that the pooling approach allowed a library of 220 members to be obtained on a large scale within a period of 1 month, including both preparation and analysis. The methodology and biological testing continue to be investigated in our laboratory.

Experimental Section

General Methods. ^1H and ^{13}C NMR spectra were measured at 600 MHz on a Brucker DRX-600 spectrometer. Chemical shifts (ppm) are reported relative to internal $CDCl_3$ (^1H, 7.26 ppm and ^{13}C, 77.0 ppm) and DMSO-d_6 (^1H, 2.50 ppm and ^{13}C, 39.5 ppm). HRMS spectra were measured using electrospray ionization (ESI) or MALDI techniques. Analytical HPLC (Hitachi) and LC/MS (Hewlett-Packard 1100 MSD) were performed using a reversed-phase Discovery HS C18 column (3 μm; 5 cm × 4.6 mm) (Supelco) with a gradient elution (30−100% solvent B; solvent A = water/0.1% formic acid, solvent B = acetonitrile/0.1% formic acid) and detection at 254 nm (see procedure for **29**). For LC/

MS, the MS (Aglient 100MSD) employed a scan range of 200−2000 *m*/*z* positive mode; fragmentor = 100. Spray chamber conditions: dry gas flow = 12 L/min, nebulizer pressure 50 psig, dry gas temp of 350 °C, and a capillary voltage of 4000. Infrared spectra were measured using neat solid samples on a Nicolet Avatar 360 FT-IR equipped with a Nicolet Smart Golden Gate (ZnSe) by the ATR technique. Glassware and solvents were dried by standard methods. Thin-layer chromatography (TLC) was performed on glass plates coated with a 0.25-mm layer of silica gel 60 F-254. The Rink linker **23** was purchased from Novabiochem. All other reagents (aldehydes, methyl ketones, bromoketones, etc.) were purchased from Aldrich, ACROS, TCI, and Halocarbon Products and were used without further purification.

4′-Formylphenyl-4-benzoic Acid 12. This compound was prepared according to standard methods.[15] To a suspension of 4-bromobenzoic acid (1.12 g, 5.60 mmol), 4-formylphenylboronic acid (1.00 g, 6.70 mmol) and $Pd(PPh_3)_4$ (320 mg, 5 mol %) in DMF (40 mL) was added 2 M Na_2CO_3 (8.2 mL, 16.4 mmol). The mixture was heated in an oil bath at 110 °C for 4.5 h. After cooling, the solid material was filtered and washed with MeOH (10 mL), and the filtrate was evaporated to dryness. To the residue was added MeOH/H_2O (50 mL/10 mL), and the solution was acidified with 1 M HCl (6 mL). The resulting precipitate was collected, followed by drying under vacuum to yield **12** (1.02 g, 80%). ^{1}H NMR (DMSO-d_6) δ: 10.08 (s, 1H), 8.07 (d, J = 8.3 Hz, 2H), 8.03 (d, J = 8.3 Hz, 2H), 7.98 (d, J = 8.3 Hz, 2H), 7.90 (d, J = 8.3 Hz, 2H). ^{13}C NMR (DMSO-d_6) δ: 192.8, 167.1, 144.7, 142.9, 135.6, 130.7, 130.2, 130.1, 127.8, 127.7, 127.3. ESI-MS $[M + Cl]^-$ 261.

Preparation of 29 (R^1, R^2, R^3 = Ph): Small-Scale Test Reaction. Using the procedures described below, reaction efficiencies were assessed by preparing a single pyridine from **24** (170 mg, 0.11 mmol capacity). Reagents were appropriately scaled, and a 5 mL disposable syringe (Norm-Ject) with a polyethylene filter (Bel-Art) was used as a reaction vessel for **24**, **25**, and **26**. After cleavage from the resin, a homogeneous compound was obtained (30 mg, 90%). ^{1}H NMR ($CDCl_3$) δ: 8.12 (d, J = 8.3 Hz, 4H), 7.98 (d, J = 8.3 Hz, 2H), 7.89 (s, 2H), 7.82 (d, J = 8.3 Hz, 2H), 7.46−7.55 (m, 6H), 6.69 (br s, 2H). ^{13}C NMR ($CDCl_3$) δ: 169.8, 157.4, 150.2, 142.1, 137.6, 133.3, 129.9, 128.4, 127.6, 127.5, 118.0. MALDI-FTMS $[M + H]^+$ 351.

***Janda*Jel Fmoc-Rink Amide 24.** To a glass bottle (100-mL Aldrich sure-seal type, but using only a screw cap) was added *J*anda*J*el-NH_2 resin (100−200 mesh; ∼1.2 mmol/g) (3.93 g, 4.71 mmol capacity), Fmoc-Rink linker **23** (3.05 g, 5.65 mmol), HOBt (0.893 g, 6.61 mmol), and DMF (90 mL). DIC (1.05 mL, 6.70 mmol) was added, and the mixture was shaken on a BigBill shaker (Barnstead Intl.) at room temperature for 12 h. A second batch was prepared simultaneously. The combined resin was poured into a 350-mL sintered glass filter and washed with DMF (3 × 200 mL), THF (3 × 200 mL), and CH_2Cl_2 (3 × 200 mL), followed by drying under vacuum to yield the *J*anda*J*el resin **24** (13.9 g, approximately quantitative). A total of 31.12 g of **24** was prepared as described. IR 1713 (linker carbamate C═O), 1676 (linker amide C═O) cm^{-1}.

***Janda*Jel Rink Amide 25.** Into each of two glass bottles (see **24**) was added the resin **24** (6.92 g, 4.68 mmol capacity) in 20% (v/v) piperidine/DMF (100 mL) and the mixture was shaken on a IKA-Vibrax-VXR (IKA-Labortechnik) at room temperature for 30 min. Completion of the reaction was confirmed by IR by observing disappearance of the C═O stretch (1713 cm^{-1}). The combined resin was poured into a 350-mL sintered glass filter and washed with DMF (3 × 200 mL), THF (3 × 200 mL), and CH_2Cl_2 (3 × 200 mL), followed by drying under vacuum to yield the *J*anda*J*el resin **25** (11.5 g, approximately quantitative). The entire lot of **24** was processed as described to give a total of 26.05 g of **25**. IR 1674 cm^{-1}.

Coupling Carboxyaldehydes 9, 10, 11, and 12; *J*anda*J*el Resins 26. The resin **25** (11.4 g, 9.28 mmol) was divided equally (2.85 g, 2.32 mmol capacity) into four glass bottles (see **24**), and DMF (50 mL) was then added to each batch. To batch 1 was coupled **9** (804 mg, 5.74 mmol), to batch 2 was coupled **10** (862 mg, 5.74 mmol), to batch 3 was coupled **11** (896 mg, 5.74 mmol), and to batch 4 was coupled **12** (1.30 g, 5.74 mmol) using HOBt (970 mg, 7.10 mmol) and DIC (1.10 mL, 7.02 mmol) for each reaction. The mixtures were shaken on a IKA-Vibrax-VXR (IKA-Labortechnik) at room temperature for 14 h. Completion of the reactions was confirmed by the Kaiser test. The resins were then mixed and poured into a 350-mL sintered glass filter and washed with DMF (3 × 200 mL), THF (3 × 200 mL), and CH_2Cl_2 (3 × 200 mL), followed by drying under vacuum to yield the resin-bound aldehydes **26** (13.4 g, approximately quantitative) containing the R^1 substituents. The entire lot of **25** was processed as described to give a total of 29.37 g of **26**. IR 1669 cm^{-1} (aldehyde and amide C═O stretching regions overlapped).

Typical Claisen−Schmidt Condensation; *J*anda*J*el Resins 27. The procedure was analogous to that described by Marzinzik et al.[10b] In a glass bottle (see **24**), a batch of resin **26** (5.00 g, 3.50 mmol capacity), acetophenone **13** (9.00 g, 75 mmol), and LiOH monohydrate (3.14 g, 75 mmol) in anhydrous DME (80 mL) was shaken on a IKA-Vibrax-VXR (IKA-Labortechnik) at room temperature for 16 h. The resin was poured into a sintered glass filter and washed with acetic acid (2 × 50 mL), DMF (3 × 50 mL), *i*-PrOH (3 × 50 mL), and CH_2Cl_2 (3 × 50 mL), followed by drying under vacuum to yield the resin-bound α,β-unsaturated ketones **27** (5.48 g, approximately quantitative) containing the four different R^1 substituents and R^2 = Ph. The remaining lot of **26** was separated into nine batches decreasing in weight by ∼0.5 g increments from the largest batch (∼5.5 g) to the smallest batch (∼0.5 g). Each batch was reacted as described with a different methyl ketone **14**−**22**. IR 1660 cm^{-1} (ketone and amide C═O stretching regions overlapped), 1602 cm^{-1} (C═C).

Preparation of Pyridinium Salts 3a. The crystalline compounds were obtained by filtration after stirring the appropriate bromoketone **2** with pyridine according to a literature procedure.[16] Methyl ketones **14** and **21** were brominated with tetrabutylammonium tribromide,[17] and **15**, **16**, and **22** were brominated with Br_2.[18] All other bromoketones were commercially available. In all cases, bromo-

ketones **2** were used without purification. Typical methods and relevant data for **3a** are as follows:

1-Phenacylpyridinium Bromide (3a, R^3 = Phenyl). ^{1}H NMR (DMSO-d_6) δ: 9.08 (d, J = 6.6 Hz, 1H), 8.76 (t, J = 7.5, 1H), 8.31 (dd, J = 6.6, 7.5 Hz, 2H), 8.09 (d, J = 8.3 Hz, 2H), 7.81 (d, J = 7.5 Hz, 2H), 7.68 (dd, J = 7.5, 8.3 Hz, 2H), 6.60 (s, 2H). ^{13}C NMR (DMSO-d_6) δ: 190.7, 146.4, 146.3, 134.7, 133.5, 129.2, 129.1, 128.3, 127.8, 66.3.

1-[(1,4-Benzodioxan-6-ylcarbonyl)methyl]pyridinium Bromide (3a, R^3 = 1,4-Benzodioxan-6-yl). ^{1}H NMR (DMSO-d_6) δ: 9.04 (d, J = 6.6 Hz, 2H), 8.74 (t, J = 7.5 Hz, 1H), 8.28 (dd, J = 6.6, 7.5 Hz, 2H), 7.60 (dd, J = 2.2, 8.3 Hz, 1H), 7.56 (d, J = 2.2 Hz, 1H), 7.12 (d, J = 8.3 Hz, 1H), 6.49 (s, 2H), 4.38−4.41 (m, 2H), 4.33−4.36 (m, 2H). ^{13}C NMR (DMSO-d_6) δ: 189.0, 149.2, 146.3, 143.5, 127.8, 126.9, 122.4, 117.6, 117.3, 65.9, 64.7, 64.0.

1-[2-Oxo-(4-phenyloxyphenyl)ethyl]pyridinium Bromide (3a, R^3 = 4-Phenyloxyphenyl). ^{1}H NMR (DMSO-d_6) δ: 9.03 (d, J = 6.6 Hz, 2H), 8.74 (t, J = 7.9 Hz, 1H), 8.28 (dd, J = 6.6, 7.9 Hz, 2H), 8.10 (d, J = 7.5 Hz, 2H), 7.50 (d, J = 7.5 Hz, 2H), 7.30 (t, J = 7.5 Hz, 1H), 7.15−7.21 (m, 4H), 6.50 (s, 2H). ^{13}C NMR (DMSO-d_6) δ: 189.2, 162.5, 154.7, 146.4, 146.3, 131.1, 130.6, 127.9, 125.2, 120.2, 117.5, 66.1.

1-[2-(9H-Fluoren-2-yl)-2-oxoethyl]pyridinium Bromide (3a, R^3 = 9H-Fluoren-2-yl). ^{1}H NMR (DMSO-d_6) δ: 9.08 (d, J = 6.6 Hz, 2H), 8.81 (t, J = 7.7 Hz, 1H), 8.32−8.37 (m, 3H), 8.25 (d, J = 8.1 Hz, 1H), 8.19 (d, J = 8.1 Hz, 1H), 8.14 (d, J = 6.6 Hz, 1H), 7.76 (d, J = 6.6 Hz, 1H), 7.50−7.58 (m, 4H), 6.59 (s, 2H), 4.16 (s, 2H). ^{13}C NMR (DMSO-d_6) δ: 190.3, 147.2, 146.4, 146.3, 144.7, 143.6, 139.6, 131.8, 128.6, 127.9, 127.7, 127.2, 125.5, 125.0, 121.5, 120.5, 66.3, 36.5.

1-[2-(2-Naphthyl)-2-oxoethyl]pyridinium Bromide (3a, R^3 = 2-Naphthyl). ^{1}H NMR (DMSO-d_6) δ: 9.10 (d, J = 6.6 Hz, 2H), 8.87 (s, 1H), 8.78 (t, J = 7.9 Hz, 1H), 8.32 (dd, J = 6.6, 7.9 Hz, 2H), 8.24 (d, J = 8.1 Hz, 2H), 8.17 (d, J = 8.6 Hz, 2H), 8.10 (d, J = 8.1 Hz, 2H), 8.05 (d, J = 8.6 Hz, 2H), 7.77 (t, J = 8.1 Hz, 1H), 7.72 (t, J = 8.1 Hz, 1H), 6.69 (s, 2H). ^{13}C NMR (DMSO-d_6) δ: 190.6, 146.4, 135.6, 130.8, 130.7, 129.7, 129.5, 128.9, 127.9, 127.5, 123.2, 66.3.

4-Phenylphenacylpyridinium Bromide (3a, R^3 = 4-Biphenyl). ^{1}H NMR (DMSO-d_6) δ: 9.10 (d, J = 6.6 Hz, 2H), 8.77 (t, J = 7.9 Hz, 1H), 8.32 (dd, J = 6.6, 7.9 Hz, 2H), 8.16 (d, J = 8.8 Hz, 2H), 7.99 (d, J = 8.8 Hz, 2H), 7.83 (d, J = 7.5 Hz, 2H), 7.55 (t, J = 7.5 Hz, 2H), 7.48 (t, J = 7.5 Hz, 1H), 6.63 (s, 2H). ^{13}C NMR (DMSO-d_6) δ: 190.3, 162.7, 146.4, 146.3, 145.9, 138.5, 129.2, 129.0, 128.8, 127.9, 127.2, 127.1, 66.3.

1-[2-(4-Nitrophenyl)-2-oxoethyl]pyridinium Bromide (3a, R^3 = 4-nitrophenyl). ^{1}H NMR (DMSO-d_6) δ: 9.09 (d, J = 6.1 Hz, 1H), 8.78 (t, J = 7.9 Hz, 1H), 8.48 (d, J = 8.8 Hz, 2H), 8.31−8.35 (m, 4H), 6.66 (s, 2H). ^{13}C NMR (DMSO-d_6) δ: 190.1, 150.6, 146.6, 146.3, 138.2, 129.8, 127.9, 124.2, 66.5.

1-[2-(4-Methoxyphenyl)-2-oxoethyl]pyridinium Bromide (3a, R^3 = 4-methoxyphenyl). ^{1}H NMR (DMSO-d_6) δ: 9.06 (d, J = 6.6 Hz, 1H), 8.75 (t, J = 7.9 Hz, 1H), 8.29 (dd, J = 6.6, 7.9 Hz, 2H), 8.06 (d, J = 8.8 Hz, 2H), 7.19 (d, J = 8.8 Hz, 2H), 6.53 (s, 2H), 3.91 (s, 3H). ^{13}C NMR (DMSO-d_6) δ: 189.0, 164.3, 146.3, 130.7, 127.8, 126.3, 114.4, 65.9, 55.8.

1-(2-Furylmethyl)pyridinium Bromide (3a, R^3 = 2-Furyl). To a solution of 2-acetylfuran (3.50 g, 32 mmol) in CH_2Cl_2/MeOH (5/2) (140 mL) was added tetrabutylammonium tribromide (16.8 g, 35 mmol) at room temperature. The mixture was stirred for 2 h until a decoloration of the orange solution took place. The mixture was evaporated, water (50 mL) was added, the mixture was extracted with diethyl ether (3 × 50 mL), and the combined organic layers were dried over $MgSO_4$. Evaporation of the solvent afforded the bromoketone (**2**, R^3 = 2-furyl) (5.96 g). To a solution of the bromoketone in anhydrous THF (10 mL) was added pyridine (1.60 mL, 20 mmol), and the mixture was stirred for 14 h at room temperature. The resulting precipitate was collected, followed by drying under vacuum to yield 1-(2-furylmethyl)-pyridinium bromide (2.22 g, 49%). ^{1}H NMR (DMSO-d_6) δ: 9.02 (d, J = 6.7 Hz, 2H), 8.73 (t, J = 7.9 Hz, 1H), 8.27 (dd, J = 6.7, 7.9 Hz, 2H), 8.22 (d, J = 1.5 Hz, 1H), 7.73 (d, J = 3.5 Hz, 1H), 6.90 (dd, J = 1.5, 3.5 Hz, 1H), 6.28 (s, 2H). ^{13}C NMR (DMSO-d_6) δ: 179.0, 149.2, 146.5, 146.3, 127.8, 127.1, 120.4, 113.3, 65.0.

1-(2-Thienylmethyl)pyridinium Bromide (3a, R^3 = 2-Thienyl). To a solution of 2-acetylthiophene (3.96 g, 31 mmol) and $AlCl_3$ (64 mg) in anhydrous diethyl ether (20 mL) was added bromine (1.60 mL, 31 mmol) dropwise with stirring at room temperature. After 30 min, the reaction mixture was washed with 1 M Na_2CO_3 (20 mL), and the organic layer was dried over $MgSO_4$. Evaporation of the solvent afforded the bromoketone (**2**, R^3 = 2-thienyl) (6.19 g). To a solution of the bromoketone in anhydrous THF (10 mL) was added pyridine (1.30 mL, 16 mmol), and the mixture was stirred for 14 h at room temperature. The resulting precipitate was collected, followed by drying under vacuum to yield 1-(2-thienylmethyl)pyridinium bromide (3.78 g, 43%). ^{1}H NMR(DMSO-d_6) δ: 9.09 (d, J = 7.0 Hz, 2H), 8.75 (t, J = 7.9 Hz, 1H), 8.29 (dd, J = 7.0, 7.9 Hz, 2H), 8.25 (dd, J = 1.3, 4.8 Hz, 1H), 8.24 (dd, J = 1.3, 4.0 Hz, 1H), 7.42 (dd, J = 4.0, 4.8 Hz, 1H), 6.50 (s, 2H). ^{13}C NMR (DMSO-d_6) δ: 183.6, 146.5, 146.3, 139.2, 136.9, 135.1, 129.3, 127.8, 65.6.

Typical Michael-Addition/Ring Closure; *J*anda*J*el Resins 28. The procedure was similar to that described by Grosche et al.[8] In a disposable culture tube (25 × 150 mm), a batch of resin **27** (R^2 = Ph) (500 mg, 0.320 mmol capacity), 1-(2-phenyl-2-oxoethyl)pyridinium bromide (**3a**, R^3 = Ph) (550 mg, 2.0 mmol), and NH_4OAc (700 mg, 91 mmol) in DMF/AcOH (5/3) (6 mL) were magnetically stirred at 90 °C for 24 h. During heating, the tube was kept open to the atmosphere. The resin was washed with DMF (3 × 15 mL), THF/MeOH (1:1) (3 × 15 mL), MeOH (3 × 15 mL), and CH_2Cl_2 (3 × 15 mL), followed by drying under vacuum to afford the resin-bound pyridines **28** (525 mg). Similar reactions were conducted on the remaining 54 batches of **27** in a combinatorial fashion.

Typical Cleavage from Resin; 2,4,6-Trisubstituted Pyridines 29. Cleavage of **28** (R^2, R^3 = Ph) (350 mg) was performed in a screw-cap vial (20 mL) with TFA/CH_2Cl_2

(1/3) (5 mL) at room temperature for 30 min. The solution was concentrated to dryness to provide the crude pyridines, which were then lyophilized twice from *t*-BuOH/H_2O (1/1) (workup for TFA removal) to yield **29** (74 mg, 86%) (denoted as pool 1; see also Supporting Information). Each of the pyridines in pool 1 contained one of the four different R^1 subtitutents, R^2, R^3 = Ph, and showed four peaks on HPLC (ratio, uncorrected: 1.0/1.8/1.4/1.4, R^1 from **9/10/11/12**) and the expected four peaks of correct mass on LC/MS. MS $[M + H]^+$ 341, 351, 357, 427. From the 10 different batches of resin **27**, a total of 55 pools of **29** were prepared. The chromatography conditions were as described in General Methods with the following gradient programs: pools 1, 3−10, 20−24, 26−55; HPLC: $t = 0$ min (A/B = 70:30), $t = 30$ min (A/B = 0:100), stop $t = 35$ min; LC/MS $t = 0$ min (A/B = 70:30), $t = 15$ min (A/B = 0:100), stop $t = 19$ min; pools 2, 11−19, 25; HPLC and LC/MS: $t = 0$ min (A/B = 70:30), $t = 5$−10 min (A/B = 50:50), $t = 15$−20 min (A/B = 40:60), $t = 25$−30 min (A/B = 30:70), $t = 33$ min (A/B = 0:100), stop $t = 37$ min. The relative quantitation of products by HPLC/LC/MS was accurate to ±10%.

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Supporting Information Available. Tables of additional pyridine library data. This material is available free of charge via the Internet at http://pubs.acs.org.

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