Organic Process Research & Development **1999**, *3*, 177–183

Fast Scale-Up Using Solid-Phase Chemistry

Stephen P. Raillard,* Gongjun Ji, Adam D. Mann, and Ted A. Baer

Affymax Research Institute, 3410 Central Expressway, Santa Clara, California 95051

Abstract:

A novel approach using solid-phase chemistry for scale-up was developed. The method makes use of commercially available high-load Merrifield resin. Three representative solid-phase chemistries leading to a diketopiperazine, a tetramic acid, and a β-lactam were scaled-up effectively and resulted in products with good yields and high crude purities. Affording close to 1 g of product per 1 g of Merrifield resin, the high-load resins showed high-volume productivity. The advantages of the work-ups, consisting simply of resin washes, are discussed. The need for low reactant and reagent equivalents results from higher reactant concentration, and it is shown that these stoichiometries can be reduced to affordable amounts.

Introduction

The emergence of combinatorial chemistry has profoundly changed the way in which drug discovery is conducted in the pharmaceutical industry.[1] Particularly the combination of solid-phase synthesis with high-throughput screening promises to generate lead molecules in a dramatically shortened time. As a consequence, Chemical Development departments will be asked to scale-up more compounds, faster than ever, in the quest to find novel drug substances.

The attractiveness of solid-phase chemistry can be attributed to the elimination of time-consuming work-up steps and the fact that reactions can be driven to completion by using large excesses of reagents. These features make solid-phase chemistry potentially attractive also for chemical development purposes. In addition, it would be beneficial if the development work could capitalize on the initial research effort in designing and optimizing a particular solid-phase strategy. Unfortunately, due to their high costs, the popular resins generally employed in solid-phase and combinatorial chemistry are too expensive to be of interest for scale-up purposes.[2] Only Merrifield resins can be purchased for a price of approximately $1 per millimole of resin loading. The price of this resin becomes even more attractive if the resin is purchased in its high-load form.[3] The following experiments with three different solid-phase chemistries that are known from the literature investigate the applicability of truly high-load Merrifield resins for the expedient scale-up to 100 g of final product.

Results

Diketopiperazine (DKP) Synthesis (Scheme 1). An elegant entry into DKPs via solid-phase chemistry has recently been published.[4] Key features of this reaction sequence are the use of the Ugi multicomponent reaction and a cyclization-mediated cleavage to liberate the product with high purity. In their studies, the authors had employed PAM and Tentagel resins.

The synthesis of a DKP via Ugi reaction using high-load hydroxymethyl resin is shown in Scheme 1. The conversion of Merrifield resin **1** with a loading of 3.9 mmol/g to hydroxymethyl resin **3** was carried out in two steps in a modification of a literature protocol for low-load resins.[5] In the first step, the chloride was displaced with acetate in refluxing 2-methoxyethanol with 1.5 equiv of potassium acetate. This conversion was monitored by measuring the amount of liberated chloride with an ion-selective chloride electrode. The hydrolysis of **2** to the hydroxymethyl resin **3** was achieved with 2 equiv of hydroxide in water and was monitored by IR (diminishing C═O absorption) and by nanoprobe ^{1}H NMR[6] (disappearance of the acetyl methyl group). It was found that the reaction needed dioxane as a cosolvent and elevated temperatures to proceed to completion.

The coupling of fluorenylmethoxycarbonyl-valine (Fmoc-valine) with resin **3** was performed using a variation of a method developed by Sieber.[7] Using 3 equiv of Fmoc-protected valine and coupling reagents at a concentration of 0.6 M in *N*-methylpyrrolidone (NMP), **4** was generated in a 98% yield.[8] Removal of the Fmoc group under standard conditions resulted in resin **5**, to which were then added the starting materials for the Ugi reaction. This multicomponent reaction was performed according to the literature procedure. The notable difference was that the reagent equivalents employed were reduced from 5 to 3 equiv because the concentration of reagents in this reaction was 0.8 M, whereas the literature procedure had employed a concentration of 0.29 M. After two acidic and one basic cyclization-mediated cleavages with **6** and a short extractive work-up, the product **7** was isolated as a 4:3 mixture of diastereomers (HPLC at 254 nm). From 25 g of hydroxymethyl resin **3** was obtained

* To whom correspondence should be addressed. Phone: (408) 522 5858. Fax: (408) 481 9558. E-mail: Stephen_Raillard@affymax.com.

(1) Brown, D. *Mol. Div.* **1996**, *2*, 217.

(2) Prices are >$25 per millimole loading for the widely used Tentagel and Argogel resins if purchased in 100 g quantities.

(3) The price as of 11/98: Advanced Chemtech, 4.4 mmol/g resin, $0.8/mmol if bought in 1 kg quantity.

(4) Szardenings, A. K.; Burkoth, T. S.; Lu, H. H.; Tien, D. W.; Campbell, D. A. *Tetrahedron* **1997**, *53*, 6573.

(5) Bodanszky, M.; Sheehan, J. T. *Chem. Ind.* **1966**, 1597.

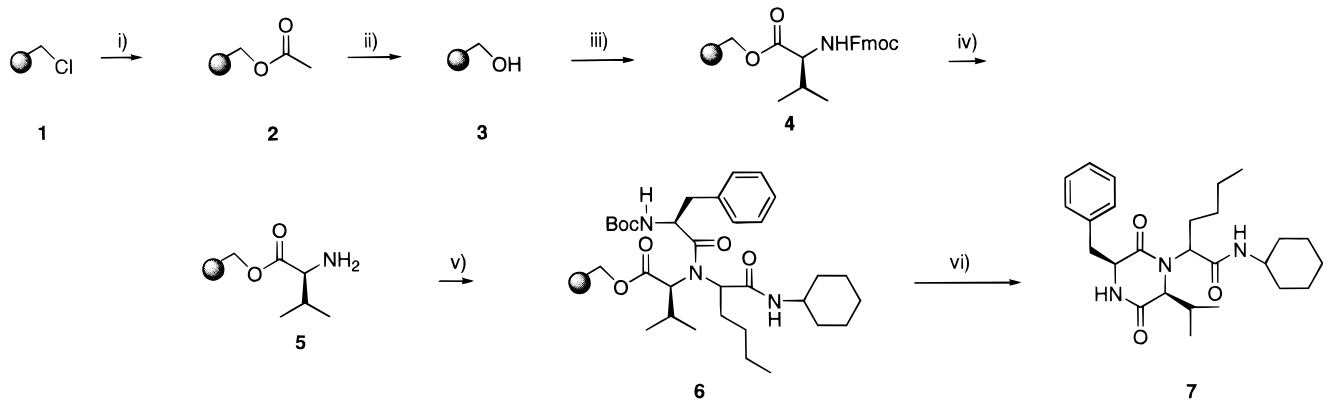
(6) Fitch, W. L.; Detre, G.; Holmes, C. P.; Shoolery, J. N.; Keifer, P. A. *J. Org. Chem.* **1994**, *59*, 7955.

(7) Sieber, P. *Tetrahedron Lett.* **1987**, *28*, 6147.

(8) This is the yield as measured by the weight gain. An alternative yield determination based on UV quantitation of the Fmoc group gave a 92% yield.

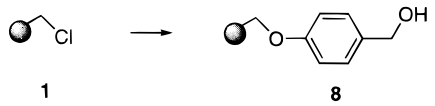
10.1021/op9800966 CCC: $18.00
Published on Web 04/02/1999

Scheme 1. **DKP synthesis**[a]



[a] Reagents and conditions: (i) KOAc, 2-methoxyethanol, reflux; (ii) NaOH, H_2O, dioxane, reflux; (iii) Fmoc-Val, 2,6-dichlorobenzoyl chloride, py, NMP; (iv) 20% piperidine/NMP; (v) C_4H_9CHO, Boc-Phe, $C_6H_{11}NC$; (vi) (1) TFA/CH_2Cl_2 (1:1), (2) 1% $HOAc/CH_3CN$, (3) 4% NEt_3/CH_3CN.

Scheme 2. **Preparation of Wang resin**[a]



[a] Reagents and conditions: 4-hydroxybenzyl alcohol, K_2CO_3, DMF, 50 °C.

34 g of DKP **7** (79% yield) with a crude purity of 93% (HPLC).

Tetramic Acid Synthesis (Scheme 3). A solid-phase synthesis on low-load polystyrene resin was recently described for 3-acyl tetramic acids.[9] The Wang resin was chosen to allow for a simplified cleavage of intermediates for reaction monitoring. Merrifield resin **1,** with a loading of 4.4 mmol/g, was allowed to react with 3 equiv of 4-hydroxybenzyl alcohol in DMF at 50 °C to yield the Wang resin **8** (Scheme 2). This resulted in a quantitative displacement of chloride and a weight gain corresponding to a complete reaction. Wang resin **8** was then mixed with 3 equiv of Fmoc-phenylalanine together with diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole (HOBt) in NMP at a concentration of 0.6 M to yield **9** (Scheme 3). This coupling step resulted in a 55% yield. It was later found that conditions employing 2,6-dichlorobenzoyl chloride resulted in improved yields of up to 80% in esterification reactions with high-load resins. The Fmoc group in **9** was removed, and a reductive alkylation with 5 equiv of anisaldehyde in trimethyl orthoformate (TMOF)/dichloromethane (1:1) was performed. This was followed by the addition of 10 equiv of solid sodium cyanoborohydride to yield **11**.[10] An extensive wash sequence with HPLC monitoring of anisaldehyde followed to eliminate anisaldehyde as a contaminant in the final product. The resulting secondary amine **11** was treated with acyl Meldrum's acid **12** in dioxane at 65 °C. Under these conditions, the Meldrum's acid was fairly unstable. The best yield of final product was generated when this acylation step was performed twice with 2.5 equiv of **12** for 2.5 h each time. After the acylation, the addition of hydroxide in a mixture of dioxane and dichloromethane was used to induce the cleavage of the product **14** into solution. Starting with 84.7 g of Wang resin **8** (which corresponds to 61 g of 4.3 mmol/g of Merrifield resin **1**), the synthesis resulted in 52.1 g of the desired tetramic acid **14** with a crude purity of 95%. This corresponds to an overall yield of 40%. This yield reflects mostly the modest coupling efficiency when using HOBT/DIC to generate **9**.

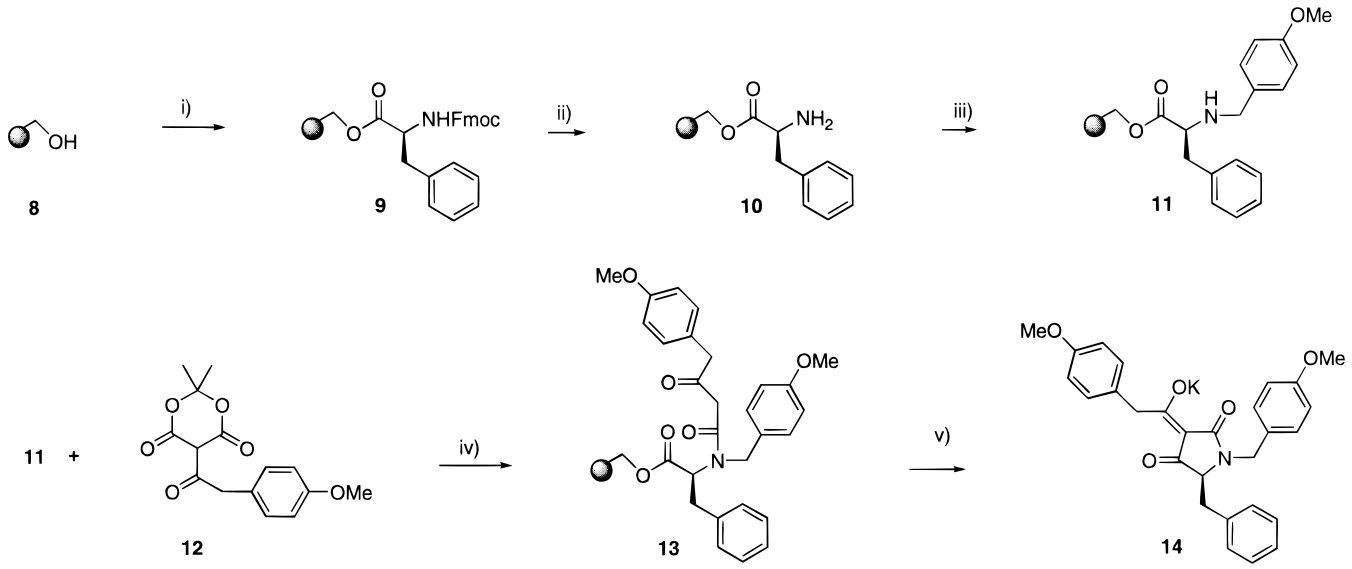
***β*-Lactam Synthesis (Scheme 5).** In this synthesis, published in 1996, Sasrin resin was used to allow for a mild cleavage of the product from the solid support.[11] The preparation of high-load Sasrin resin employed a procedure slightly modified from a recent literature protocol for low-load resin (Scheme 4).[12]

High-load 4.4 mmol/g Merrifield resin **1** was treated with 3 equiv of 2-methoxy-4-hydroxy benzaldehyde, 3 equiv of potassium carbonate, and 0.01 equiv of potassium iodide at 55 °C in DMF for 16 h to yield the resin-bound aldehyde **15**. Analysis of the liberated chloride with a chloride-selective electrode together with the weight gain of the resin indicated a complete reaction. The reduction of the aldehyde resin **15** was performed with 4 equiv of sodium borohydride in a mixture of THF, *N*-methylmorpholine (NMM), and ethanol for 16 h to yield the high-load Sasrin resin **16**. The extent of the reduction could be assessed using nanoprobe ^{1}H NMR: the aldehyde proton in **15** gave a distinct resonance at 10.3 ppm. This resonance disappeared in **16**, and the two new benzylic protons in **16** could be clearly identified and assigned at 4.6 ppm.

The Sasrin resin **16** was then used to perform a *β*-lactam synthesis (Scheme 5). The resin **16** was coupled with 3 equiv of Fmoc-valine using 2,6-dichlorobenzoyl chloride and reagent concentrations of 0.74 M.[13] This resulted in a quantitative coupling yield, as determined by the weight gain of the resin, whereas a Fmoc quantitation showed a slightly lower yield of 95%. Any unreacted hydroxy groups in **17** were then capped in a reaction with acetic anhydride and pyridine. The Fmoc group was removed to yield **18,** and the resin-bound amine was then condensed with 2.5 equiv of

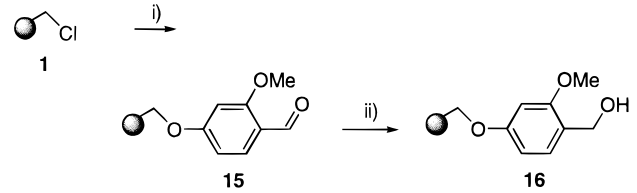
(9) Romoff, T. T.; Ma, L.; Wang, Y.; Campbell, D. A. *Synlett* **1998**, 1341.
(10) Both aldehyde and sodium borohydride equivalents were reduced by a factor of 4 and 3, respectively, compared to the literature protocol.
(11) Ruhland, R.; Bhandari, A.; Gordon, E. M.; Gallop, M. A. *J. Am. Chem. Soc.* **1996**, *118*, 253.
(12) Katritzky, A. R.; Toader, D.; Watson, K.; Kiely, J. S. *Tetrahedron Lett.* **1997**, *38*, 7849.
(13) The coupling was similarly effective when 2 equiv of Fmoc-valinyl chloride was employed together with 2 equiv of pyridine in dichloromethane.

Scheme 3. **Tetramic acid synthesis**[a]



[a] Reagents and conditions: (i) Fmoc-Phe, DIC, HOBt, NMP; (ii) 20% piperidine/NMP; (iii) (1) 4-OMe-C_6H_5CHO, CH_2Cl_2/TMOF (1:1), (2) $NaCNBH_3$; (iv) acyl Meldrum acid **12**, dioxane, 80 °C; (v) KOH, dioxane/CH_2Cl_2 (1:1).

Scheme 4. **Preparation of Sasrin resin**[a]



[a] Reagents and conditions: (i) 2-methoxy-4-hydroxybenzaldehyde, K_2CO_3, DMF, catalytic KI, 50 °C; (ii) $NaBH_4$, 4-methylmorpholine, THF.

benzaldehyde in a 1:1 mixture of dichloromethane and trimethyl orthoformate for 3 h.[14] The following cycloaddition reaction was performed by slowly adding 2.5 equiv of phenoxyacetyl chloride to a dichloromethane suspension of **19,** together with 2.5 equiv of triethylamine at 0 °C. The reaction mixture was allowed to warm to room temperature overnight, and the product was cleaved with dilute TFA in dichloromethane. From 169 g of Sasrin resin (which is prepared from 119 g of 4.4 mmol/g of Merrifield resin) was obtained 105 g of crude product **21** with a purity of >95%. After crystallization with toluene, 100 g of **21** with a purity of >99% was obtained as a 2:1 mixture of diastereomers. This corresponds to an overall yield from the Merrifield resin of 56%.

Discussion

Optimization of Reaction Conditions. For the scale-up of a solid-phase reaction, it is generally practical to start out by adapting the conditions optimized on the research scale; only occasionally was it necessary to deviate from published protocols for low-load resins.[15] However, to keep costs of added reagents and reactants reasonable and to make the approach attractive for scale-up, it was imperative to reduce the large amount of reagent equivalents typically employed in solid-phase synthesis. The lowest amounts of reagents necessary to reach a particular overall yield were not optimized; instead, it was shown that the large excesses commonly used in solid-phase chemistry are not a necessity for scale-up when using high-load resins.[16]

Reaction Vessels. For solid-phase reactions on multigram scales, vessels that are based on the traditional fritted peptide vessel were used.[17] To this design were added additional necks with ground glass joints as well as a jacket for heating and cooling.[18] The necks serve as convenient inlet ports for the addition of reagents, for inert gas, and for the introduction of a mechanical stirrer. Controlled mechanical stirring with a Teflon paddle on a glass rod using speeds up to 200 rpm did not generally affect the mechanical integrity of the beads.[19] For solid-phase reactions employing volumes in excess of 1 L, the vessel design included a clamped top. The easy removal of the top resulted in a simplified sampling of the resin and allowed for convenient reaction control studies. This also greatly reduced the time needed to collect the whole resin batch for the purpose of drying.

Washes. The equivalent to a traditional work-up in solution-phase chemistry is a wash sequence in solid-phase chemistry. It serves the purpose of washing away excess reagents and reaction byproducts from the resin. It was found to be advantageous to monitor the content of the washes (generally by HPLC) to reduce the amount of wash solvents. An even more important aspect of a monitored wash is the prevention of contamination of the final product.

High-Load versus Low-Load Resins. When results from reactions with low-load resins (1 mmol/g loading) were

(14) The literature procedure uses 10−15 equiv for this step.[11]

(15) Examples are (1) use of 2,6-dichlorobenzoyl chloride as a coupling agent to generate high yields in esterification reactions using high-load resins and (2) use of dioxane as a cosolvent in the preparation of the hydroxymethyl resin.

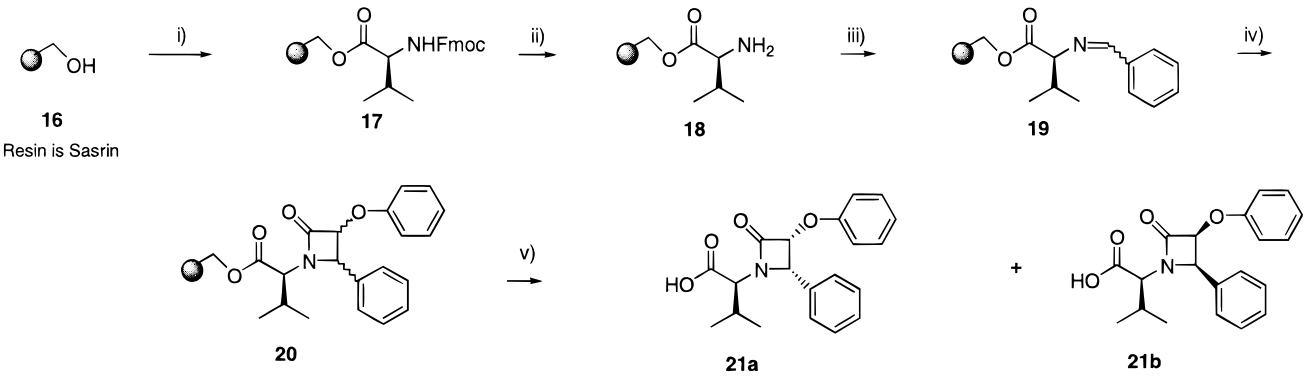
(16) For comparison, the literature protocols employed the following building block equivalents: in the DKP synthesis, 5−20 equiv; in the tetramic acid synthesis, 6−30 equiv; and in the β-lactam synthesis, 10−15 equiv.[4,9,11]

(17) These vessels are commercially available from vendors such as Chemglass, Vineland, NJ.

(18) Custom-made vessels were from Stanford Glass, Palo Alto, CA.

(19) The bead shape and size were monitored using a microscope.

***Scheme 5.* β-Lactam synthesis[a]**



[a] Reagents and conditions: (i) Fmoc-Val, 2,6-dichlorobenzoyl chloride, py, DMF; (ii) (1) Ac_2O, py, (2) 20% piperidine/NMP; (iii) C_6H_5CHO, TMOF/CH_2Cl_2 (1:1); (iv) C_6H_5-O-CH_2-COCl, NEt_3, CH_2Cl_2, 0 °C; (v) TFA/CH_2Cl_2 (1:9).

***Table 1.* Chemistries performed using high-load Merrifield resin**

chemistry	Merrifield equivalent (g)[a]	crude yield in g	crude yield in %[b]	lit. yield (%)[b]	crude purity (%)
DKP	28	34	79	98[4]	93[d]
tetramic acid	61	52	65	na[c]	95[e]
β-lactam	115	105	62	80[11]	95[d]

[a] Calculated amount of Merrifield resin that corresponds to the amounts of hydroxymethyl, Wang or Sasrin resin that were employed in the synthesis. [b] The yield is reported based on the basis of the loading of the Fmoc-amino acid-loaded resin. [c] Not applicable because tetramic acid **14** is a bis-methoxy analogue of a compound prepared in the referred work. Yields of comparable analogues were reported to be in the range of 66−92%.[9] [d] By HPLC. No values for crude purities were reported in the corresponding publications.[4,9,11] [e] Purities of comparable analogues were reported to be >95%.[9]

compared to the results achieved with the high-load resins, the following observations were made:

(i) Significantly more product per unit measure of beads could be produced with high-load resins. This is a consequence of the fact that higher concentrations of reagents and reactants can be achieved with high-load resins.

(ii) At least 50% less wash solvents were needed per unit measure of product when using high-load resin.

(iii) Smaller vessel sizes could be employed to generate a given amount of product when using high-load resins.

These findings, together with the lower cost per millimole of loading, indicate that the high-load Merrifield resin is a superior and cost-effective resin for scale-up.

Conclusion

Table 1 summarizes the results in the scale-up of solid-phase reactions using commercially available high-load Merrifield resins.

All reactions that were investigated were amenable to fast scale-up, and each of the three syntheses was performed in 2 days or less.[20] The following cost-saving features make scale-up on solid phase particularly attractive:

(i) The possibility to use established literature or research procedures developed for different supports with only minor modifications can be expected to result in a fast scale-up protocol in chemical development. Slightly lower yields with high-load resins (see Table 1) are more than offset by reduced resin costs and higher volume productivity: more product and less waste per unit measure of resin are produced with high-load resins.

(ii) Greatly simplified work-up procedures (washes) allow reducing synthesis time dramatically.

(iii) Multistep reactions can be expected to be completed in only a few days and have been shown to result generally in crude products with purities exceeding 90%.

(iv) The cost for high-load Merrifield resin to produce a product on a 100 g scale via solid-phase reactions can be expected to be on the order of a few hundred dollars.[21]

(v) High concentrations of reactants and reagents can be employed. This allows lowering the necessary stoichiometries without significantly compromising the final product yield and purity.

The results indicate that high-load Merrifield resins are attractive supports for the fast scale-up of chemistries developed for solid-phase chemistry.

Experimental Section

General. All reagents and solvents were obtained from commercial suppliers and used without further purification. The following Merrifield resins were used for the scale-up synthesis: Advanced Chemtech, 4.4 mmol/g, 100−200 mesh; Polymer Laboratories, 3.9 mmol/g, 50−100 mesh. Both suppliers produce their resins by copolymerisation of styrene with chloromethylstyrene that includes 1% divinylbenzene as cross-linker. ^{1}H NMR spectra were recorded on a Varian Gemini 400-MHz instrument. ^{1}H NMR nanoprobe experiments were performed using a Varian Inova 400-MHz NMR machine with a Varian magic angle spinning nanoprobe. Chloride determinations were done with an Orion 710 pH/mV meter using an Orion Ion plus chloride electrode. Elemental analyses were carried out at UC Berkeley Chemistry Laboratories, Berkeley, CA. Synthesis sequences were performed in modified peptide vessels except where otherwise noted. These vessels contained a jacket for heating/cooling, a coarse frit, and multiple necks; stirring occurred

(20) This assumes that the linker has been coupled to the resin. Otherwise, this step adds an additional day for Wang resin and 2 days for the Sasrin and hydroxymethyl resins.

(21) The cost is $400 if the β-lactam is used as a model compound for this calculation (based on the per-kilogram price of $3600 for the 4.4 mmol/g Advanced Chemtech Merrifield resin).

via a mechanical stirrer with a Teflon paddle on a glass rod. All reactions were stirred at speeds of <200 rpm. A single wash consisted of adding 10−100% excess of solvent over the minimal amount to swell the resin, stirring for 5 min, and filtering under vacuum or with inert gas pressure. Wash volumes are not optimized.

Preparation of High-Load Resins. *Hydroxymethyl Resin **3***. A mixture of Merrifield resin **1** (80 g, 0.31 mol)[22] and potassium acetate (46 g, 0.47 mol) was heated to reflux in 2-methoxyethanol (1 L) for 16 h. The mixture was cooled to room temperature, and the resin was filtered and washed with water (3 × 500 mL), NMP (3 × 500 mL), dichloromethane (3 × 500 mL), and dioxane (3 × 500 mL).[23] To the resin **2** was added dioxane (610 mL), followed by water (610 mL) and sodium hydroxide (24 g, 0.63 mol). The mixture was heated to reflux for 16 h, after which the product resin was filtered and washed with water (3 × 500 mL), NMP (3 × 500 mL), methanol (3 × 500 mL), and dichloromethane (3 × 500 mL). Drying in a vacuum oven at 50 °C and 50 mmHg resulted in 72 g of white resin **3** (98% yield). The IR showed complete absence of the C═O from the intermediate acetyl resin **2**.

*Wang Resin **8***. A mixture of Merrifield resin **1** (150 g, 0.66 mol),[24] 4-hydroxybenzyl alcohol (240 g, 1.9 mol), potassium carbonate (267 g, 1.9 mol), and potassium iodide (1 g, 0.006 mol**)** in DMF (1.78 L) was stirred at 55 °C for 16 h. The resin was filtered and washed with DMF (3 × 500 mL), water (3 × 1000 mL), NMP (2 × 500 mL), methanol (2 × 500 mL), and dichloromethane (2 × 600 mL). Drying in a vacuum oven at 50 °C and 50 mmHg resulted in 202 g of off-white dry resin **8** (90% yield).

*Sasrin Resin **16**. Step i*. A mixture of Merrifield resin **1** (125 g, 0.55 mol),[24] 2-methoxy-4-hydroxybenzaldehyde (250 g, 1.6 mol), potassium carbonate (230 g, 1.6 mol), and potassium iodide (1 g, 0.006 mol**)** in DMF (1.78 L) was stirred at 55 °C for 16 h. The resin was filtered and washed with DMF (3 × 500 mL), methanol (3 × 500 mL), and dichloromethane (3 × 500 mL). Drying in a vacuum oven at 50 °C and 50 mmHg resulted in 183 g of off-white resin **15** (96% yield).

Step ii. To a mixture of aldehyde resin **15** (150 g, 0.435 mol)[25] in THF (1.5 L) were added sodium borohydride (69 g, 1.82 mol), NMM (1 L**),** and ethanol (1 L). The mixture was stirred at room temperature for 16 h. Then water (500 mL) was slowly added. The resin was then filtered and washed with water (500 mL), THF (3 × 500 mL), NMP (3 × 500 mL), methanol (3 × 500 mL), and dichloromethane (3 × 500 mL). Drying in a vacuum oven at 50 °C and 50 mmHg resulted in 145 g of off-white resin **16** (97% yield).

DKP Synthesis: 1*N*-Cyclohexyl-2-[3-benzyl-6-isopropyl-2,5-dioxo-(3*S*,6*S*)-perhydro-1-piperazinyl] Hexanamide 7. *Coupling of Fmoc-Amino Acid.* A mixture of hydroxymethyl resin **3** (25 g, 97.5 mmol) and Fmoc-valine (100 g, 0.3 mol) in NMP (500 mL) was stirred for 15 min. To the mixture were added pyridine (24 mL, 0.31 mol) and 2,6-dichlorobenzoyl chloride (45 mL, 0.31 mol), and stirring was continued for 24 h. The resin **4** was filtered and then washed with NMP (5 × 300 mL), methanol (3 × 300 mL), and dichloromethane (5 × 300 mL). It was then dried in a vacuum oven at 50 °C and 50 mmHg for 1 day, after which it weighed 56.5 g (100% yield).

Deprotection of Fmoc. The Fmoc deprotection was carried out by adding 500 mL of a 20% piperidine in NMP solution to the resin **4** (56.5 g, 97.5 mmol) and stirring the mixture for 30 min. The resin was then filtered and washed with NMP (5 × 500 mL), methanol (3 × 500 mL), and dichloromethane (5 × 500 mL). It was then dried in a vacuum oven at 50 °C and 50 mmHg for 1 day, after which it weighed 56.5 g (100% yield).

Ugi Reaction. To the washed resin **5** were added dichloromethane (170 mL) and valeraldehyde (29 mL, 0.27 mol). After 30 min of stirring, a solution of Boc-phenylalanine (70 g, 0.27 mol) in 170 mL of methanol was added as well as cyclohexyl isocyanide (33 mL, 0.27 mol), and stirring was continued for 15 h. The resin was then filtered and washed with methanol (3 × 500 mL), NMP (3 × 500 mL), methanol (3 × 500 mL), and then dichloromethane (5 × 500 mL).

Boc Deprotection. To the resin **6** was added 500 mL of dichloromethane/TFA (1:1), and the mixture was stirred for 1 h. The resin was then drained and washed quickly with dichloromethane (9 × 500 mL) before a final wash with acetonitrile (250 mL).

Cleavage Step. A solution of 1% acetic acid in acetonitrile (500 mL) was added to the resin, and the mixture was stirred for 3 h. The resin was drained through the coarse filter of the vessel, and the cleavage was repeated with an additional 1% acetic acid in acetonitrile solution (500 mL). The resin was drained and washed with acetonitrile (3 × 250 mL). The wash solutions were combined with the filtrates from the cleavages. Meanwhile, a solution of 4% triethylamine in acetonitrile (500 mL) was added to the resin and stirred for 5 h. The resin was drained and washed with acetonitrile (3 × 250 mL). The wash solutions and the filtrates of the base-induced cleavages were combined with the ones from the acid-induced cleavage and evaporated. The resulting oil was dissolved in a mixture of ethyl acetate (250 mL) and water (100 mL). The water phase was separated, and the organic phase was extracted twice with 1 N hydrochloric acid (100 mL) and then washed with brine (200 mL) and water (100 mL). Evaporation of the solvent resulted in 34 g of product **7** as a colorless oil with 93% purity (79% yield from hydroxymethyl resin **3**). By HPLC (detection at 254 nm), the compound consisted of two diastereomers in a 4:3 ratio.[26]

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86−1.0 (m, 6H), 1.16−1.45 (m, 11H), 1.55−1.75 (m, 4H), 1.77−1.93 (m, 2H), 1.99−2.11 (m, 2H), 2.36−2.4 (m, 1H), 2.8−2.88 (m, 1H),

(22) Resin from Polymer Laboratories, Amherst, MA.
(23) Chloride analysis using a chloride-sensitive electrode indicated a quantitative release of chloride.
(24) Resin from Advanced Chemtech, Louisville, KY.
(25) This results from a loading of 2.89 mmol/g for the aldehyde resin, which is calculated based on a 100% conversion from the Merrifield resin.
(26) The literature reports a 3:2 ratio of diastereomers, but no HPLC detection wavelength was specified. The ^{1}H NMR spectrum was found to be identical to the reported one.[4]

3.4−3.61 (m, 2H), 3.99 (d, 1H, J = 3 Hz), 4.2−4.27 (m, 1H), 4.82 (t, 1H, J = 7 Hz), 5.8−6.0 (broad, 2H), 7.2−7.4 (m, 5H).

Tetramic Acid Synthesis: 5(*S*)-5-Benzyl-3-[1-hydroxy-2-(4-methoxyphenyl)ethylidene]-1-(4-methoxybenzyl)-2,4-azolane Dione 14. *Coupling of Fmoc-Amino Acid.* To a mixture of Wang resin **8** (84.7 g, 0.26 mol)[27] and Fmoc-phenylalanine (252 g, 0.75 mol) in NMP (1.25 L) were added anhydrous 1-hydroxybenzotriazole (101 g, 0.75 mol) and diisopropylcarbodiimide (118 mL, 0.75 mol). The mixture was stirred for 20 h in a 5-L three-neck flask, after which the resin was filtered and washed with NMP (3 × 500 mL), methanol (3 × 500 mL), and dichloromethane (3 × 500 mL). The resin **9** was dried in a vacuum oven for 16 h at 50 mmHg and 50 °C, after which it weighed 137 g (55% yield).

Deprotection Reaction. The Fmoc deprotection was carried out by adding a 20% piperidine in DMF solution (625 mL) to the resin **9** (137 g, 155 mmol)[28] and stirring the mixture for 30 min. The resin was then filtered and washed with DMF (7 × 625 mL).

Reductive Alkylation. To the resin **10** was added anhydrous trimethyl orthoformate (625 mL), and the mixture was stirred for 10 min. The resin was then drained under vacuum, and anisaldehyde (66.4 mL, 0.56 mol) was added directly onto the resin, followed by trimethyl orthoformate (300 mL), dichloromethane (300 mL), and acetic acid (6.2 mL). The resin was stirred, sodium cyanoborohydride (67 g, 1.08 mol)-was added over a period of 30 min, and stirring was continued for 15 h. The resin was then filtered under vacuum and washed with methanol (2 × 500 mL), DMF (3 × 500 mL), and dichloromethane (5 × 500 mL).

Acylation Step. A solution of the acyl Meldrum's acid **12** (113 g, 0.38 mol)[29] in dioxane (630 mL) was added to the resin **11,** and the mixture was stirred at 65 °C for 2.5 h. The resin was then filtered and washed with dioxane. The above reaction was repeated with a fresh batch of **12** under the same conditions. Then the resin was filtered and washed with NMP (3 × 500 mL), dioxane (6 × 500 mL), and a 1:1 mixture of methanol and dioxane (2 × 600 mL).

Cyclization. The resin **13** was transferred into a round-bottom flask, and a mixture of 0.1 N KOH in methanol (1550 mL, 0.155 mol) and dioxane (1550 mL) was added. After 1 h of stirring, the resin was filtered. The resin was washed with a 1:1 mixture of methanol and dioxane (1 L) and filtered. Both filtrates were combined and evaporated to dryness to yield 52.1 g of the product **14** (yield = 65% from **9**) with a purity of 95.4% (HPLC).[30]

(27) The Wang resin was prepared according to the protocol described above. The original Merrifield resin had a loading of 4.3 mmol/g and could be converted quantitatively (as judged by the weight gain of the resin) to the Wang resin. This weight gain leads to a calculated loading of 3.12 mmol/g for the Wang resin.

(28) The loading of the resin was determined to be 1.13 mmol/g by UV quantitation of the Fmoc group. This number corresponds to a 60% yield in the Fmoc coupling step (0.155/0.26 × 100).

(29) The acyl Meldrum's acid **12** was prepared according to a procedure mentioned in a recent publication.[9]

(30) The tetramic acid **14** is a bis-methoxy analogue of a compound that was described in the original publication by Romoff et al.[9] Its crude purity of 95.4% by HPLC is comparable to the reported purities of >95%.[9]

^{1}H NMR (400 MHz, DMSO): δ 2.95 (dd, 1H, J_1 = 4.9 Hz, J_2 = 14 Hz), 3.11 (dd, 1H, J_1 = 4.9 Hz, J_2 = 14 Hz), 3.38 (d, 1H, J = 9.3 Hz), 3.46 (d, J = 9.3 Hz), 3.75 (s, 6H), 3.94 (d, 1H, J = 12.5 Hz), 4.13 (d, 1H, J = 12.5 Hz), 5.04 (d, J = 14 Hz), 6.8−7.3 (m, 15H). Anal. Calcd for $C_{28}H_{26}KNO_5 \cdot H_2O$: C, 65.48; H, 5.69; N, 2.72. Found: C, 65.55; H, 5.60; N, 2.51.

***β*-Lactam Synthesis: *cis*-1-[(*S*)-2-(3-Methyl)butanoic acid]-3-phenoxy-4-phenyl-azetidine-2-one 21.** *Coupling of Fmoc-Amino Acid*. To a mixture of Sasrin resin **16** (169 g, 0.49 mol) and Fmoc-valine (500 g, 1.48 mol) in NMP (2 L) were added 2,6-dichlorobenzoyl chloride (211 mL, 1.48 mol) and pyridine (120 mL, 1.48 mol). The mixture was stirred in a round-bottom flask for 18 h, after which it was filtered. The resin was washed with NMP (3 × 750 mL), methanol (3 × 600 mL), and dichloromethane (3 × 600 mL) and dried in a vacuum oven for 16 h at 50 mmHg and 50 °C, which resulted in 326 g of white resin **17** (yield = 100%).

Capping Reaction. To the resin **17** were added acetic anhydride (800 mL) and pyridine (8 mL, 0.1 mol**)**, and the mixture was stirred for 3 h at 45 °C. Then the resin was filtered and washed with NMP (3 × 750 mL), methanol (3 × 750 mL), and dichloromethane (3 × 600 mL).

Deprotection Reaction. The Fmoc deprotection was carried out by adding a 20% piperidine in DMF solution (1000 mL) to the resin **17** and stirring the mixture for 60 min. The resin was then filtered and washed with NMP (3 × 750 mL), methanol (3 × 750 mL), and dichloromethane (3 × 750 mL).

Imine Formation. To the resin **18** were added dichloromethane (650 mL), anhydrous trimethyl orthoformate (650 mL), and benzaldehyde (122 mL, 1.2 mol), and the mixture was stirred at room temperature for 3 h. It was then filtered and washed with NMP (3 × 750 mL), methanol (3 × 750 mL), and dichloromethane (3 × 600 mL).

Cycloaddition. To the resin **19** was added dichloromethane (650 mL), and the mixture was cooled to 0 °C. To the mixture was added triethylamine (163 mL, 1.18 mol). A solution of phenoxyacetyl chloride (163 mL, 1.18 mol) in dichloromethane (200 mL) was then added over a period of 30 min with the temperature at 0 °C. After the addition of the chloride, the temperature was raised to room temperature over a period of 2 h, and the mixture was stirred for an additional 12 h. The resin **20** was then filtered and washed with NMP (3 × 750 mL), methanol (3 × 750 mL), and dichloromethane (3 × 600 mL).

Cleavage Reaction. A 9:1 mixture of dichloromethane and trifluoroacetic acid (1000 mL) was added to the resin **20**, and the mixture was stirred for 1 h. The resin was then filtered and washed with dichloromethane (1000 mL). This wash eluent was combined with the cleavage filtrate, and the solution was evaporated to dryness. This resulted in an oil (105 g) with a purity of 95% (HPLC). The addition of toluene (1000 mL) caused the product to solidify, and triturating with hexane yielded **21** as a white solid with a purity of >99% by HPLC (100 g, yield = 60% from Sasrin resin **16**).

^{1}H NMR (400 MHz, $CDCl_3$) for 2.5:1 mixture of 2 cis diastereomers, major isomer: δ 0.91 (d, 3H, J = 6.9 Hz),

0.95 (d, 3H, $J = 6.9$ Hz), 2.15−2.12 (m, 1H), 4.11 (d, 1H, $J = 6.95$ Hz), 5.22 (d, 1H, $J = 4.7$ Hz), 5.54 (d, 1H, $J = 4.7$ Hz), 7.13−6.72 (m, 5H), 7.42−7.25 (m, 5H). Minor isomer: δ 1.02 (d, 3H, $J = 6.6$ Hz), 1.18 (d, 3H, $J = 6.6$ Hz), 2.72−2.63 (m, 1H), 3.68 (d, 1H, $J = 8.15$ Hz), 4.96 (d, 1H, $J = 4.8$ Hz), 5.48 (d, 1H, $J = 4.7$ Hz), 7.13−6.72 (m, 5H), 7.42−7.25 (m, 5H). Anal. Calcd for $C_{20}H_{21}NO_4$: C, 70.78; H, 6.24; N, 4.13. Found: C, 70.68; H, 6.30; N, 3.75.

Received for review December 14, 1998.

OP9800966