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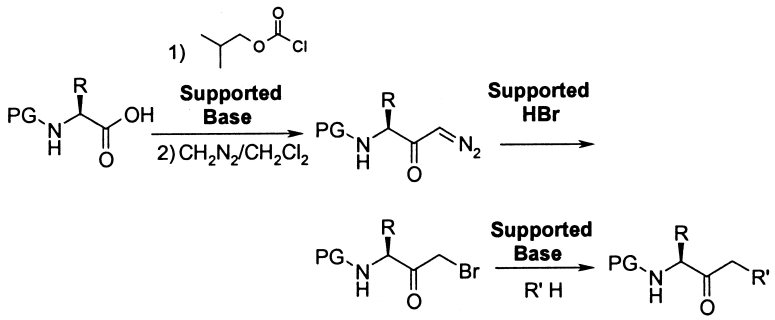

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Polymer-Supported Approach for Solution-Phase Synthesis of Cysteine Trap Protease Inhibitors: Procedure for Straightforward Optimization of the P1−P1′ Pocket

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Peptide-based reversible and irreversible cysteine proteases inhibitors are well reported in the literature. Many of these compounds have an electrophilic carbonyl group as a cysteine trap in the place of a scissile amide moiety of the natural substrate. As a common mechanism strategy, we have designed a probe library of a cysteine trap for rapid optimization of P1−P1′ pockets of different cysteine proteases. The synthesis of this library using a straightforward methodology based on polymer-supported reagents and scavengers to avoid tedious purification steps has been achieved. For the selective monobromination of diazo ketones, preparation of a new supported reagent, piperidinoaminomethylpolystyrene hydrobromide, is also described.

Introduction

Cysteine proteases are ubiquitous enzymes largely distributed in living organisms and are involved in different important metabolic processes and diseases[1] involving various therapeutic fields such as cardiovascular,[2] oncology,[3] osteoporosis,[4] and arthritis.[5] Libraries of small molecules based on a common mechanism are obviously an important step as the starting point in an optimization process for a specific therapeutic target.

Mechanism of Inhibition

Cysteine proteases possess a cysteine residue in the active site that acts as a nucleophile and attacks the carboxamide group of the cleavable peptide bond of the substrate.[6] The nucleophilicity is increased by the presence of the imidazole moiety of a histidine residue. The active form of the enzyme consists of a thiolate−imidazolium ion pair. In the first step of the enzymatic reaction a noncovalent complex of the enzyme and the substrate is formed followed by the acylation of the enzyme. The acyl−enzyme then reacts with a water molecule. According to this mechanism, different inhibitors have been designed with very potent activity, like peptidyl-aldehydes,[7] trifluoromethyl ketones,[8] diazomethyl ketones,[9] and acyloxymethyl ketones.[10] Ellman et al.[11] have recently described the solid-phase synthesis of ketone-based cysteine protease inhibitors.

Substrate specificity relies on S*n*−S*n*′ enzyme subsites matching the P*n*−P*n*′ residues of the protein to be processed.[3] Therefore, to obtain a potent and selective inhibition of a specific cysteine protease, interaction between the S subsites and the side chains of the inhibitor around a reactive group has to be achieved (Figure 1).

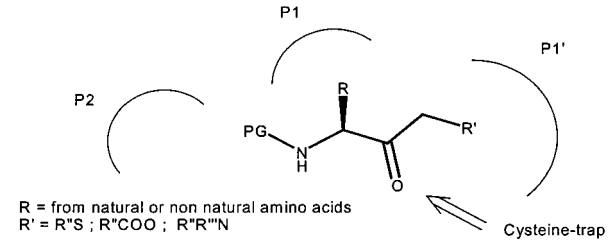

Figure 1.

Polymer-supported reagents have been in use since the 1960s and have been the subject of several review articles.[13] Synthesis using solid-supported reagents is attractive and suitable for parallel synthesis because the workup involves simple filtration and evaporation of the solvents. We have designed substituted ketones as a cysteine-trap motif to primarily optimize the P1−P1′ pockets. A chemical pathway using supported reagents at each step was introduced, making automation amenable for the production of a library.

Results and Discussion

The classical solution-phase method to prepare substituted amino ketones from protected amino acids is via mixed-anhydride transformation to aminodiazo ketones.[14] The diazo group is further replaced by bromine, using a solution of HBr in acetic acid. The resulting bromo ketone is then substituted with different nucleophiles (acids, thiols, amines, and phenols), leading to substituted ketones.[15] A reaction strategy envisaging use of supported reagents to avoid tedious workup and multiple purifications is outlined in Figure 2.

1. Synthesis of Diazo Ketones. This reaction is described in the literature using *N*-methylmorpholine (NMM). The desired diazo ketone is usually accompanied by two side products. The traces of moisture, if present, are sufficient to form the isobutyl ester during the mixed-anhydride step using isobutyl chloroformate, and the methyl ester results from the reaction of diazomethane with the partially hydrolyzed mixed

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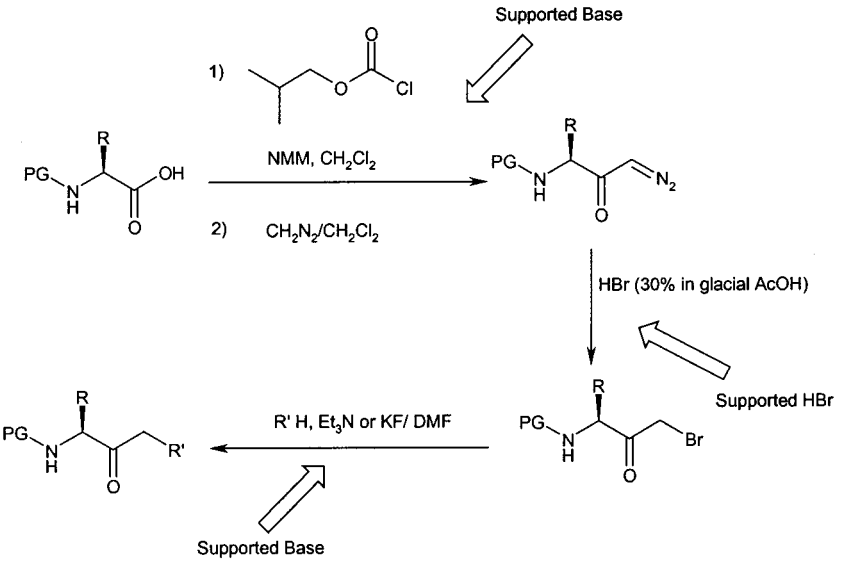


Figure 2.

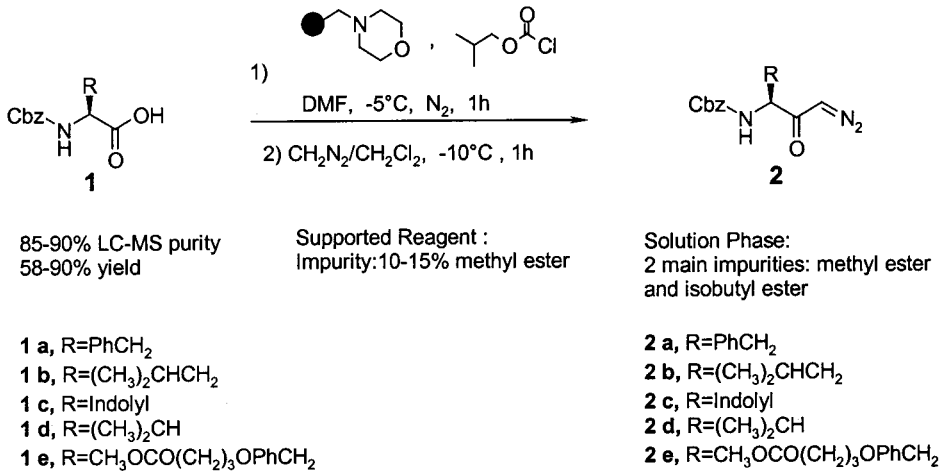


Figure 3.

anhydride. The use of polymer-supported *N*-methylmorpholine, without any particular precaution, significantly improved both the purity and the yield (Figure 3).

2. Synthesis of Bromo Ketones. To the best of our knowledge, no method has been reported for the monobromination of diazo ketone using supported reagent and no supported HBr salt is commercially available. Preliminary tests with commercially available polymer-supported pyridine tribromide resulted in an undesirable mixture of mono- and dibromide derivatives. This prompted us to prepare the supported HBr salt using different commercially available supported tertiary amines by the following methods (Figure 4): (1) resin in acetic acid−diethyl ether HBr solution; (2) bubbling of HBr gas in a diethyl ether resin suspension.

All the resins used gave the hydrobromide salt with good loading (about 3.25 mmol/g, as determined by Br microanalysis). When the hydrobromide salt was generated by method 1, undesired dibromo ketone was also formed probably because of the presence of Br_2 in the solution. HBr gas in ether circumvents this problem and gives desired monobrominated compound as the only product.

Five different hydrobromide salts were prepared and tested. Piperidinoaminomethylpolystyrene hydrobromide gave the monobromo ketone as the unique product in good yield and proved to be stable for several weeks in the refrigerator. Freshly prepared polymer-suppported pyridinium hydrobromide gave similar results but lacked stability.

3. Nucleophilic Substitution. In the literature, the nucleophilic substitution of the bromo ketone is often described using KF.[16−18] In our hands, this results in the formation of different side products. One of these was isolated and identified as the corresponding hydroxy ketone. The removal of side products involve very tedious purification and proves to be a drawback of the process.

In a model study, using 2,5-dichlorobenzoic acid as the nucleophile, we screened different polystyrene-supported bases to replace KF. Dimethylaminomethylpolystyrene turned out to be the best amine for this reaction. In addition, when the acids were used as nucleophiles, this resin also plays the role of the unused acid scavenger. (Figure 5).

4. Production of a Substituted Carboxamide Library from Amino Acids. After optimal reaction conditions were established, an 80-array library was generated using diverse nucleophiles as acids, amines, and thiols (Figure 6). Five diazo ketones and their respective bromo ketones were prepared using Quest 205. The only workup at each step

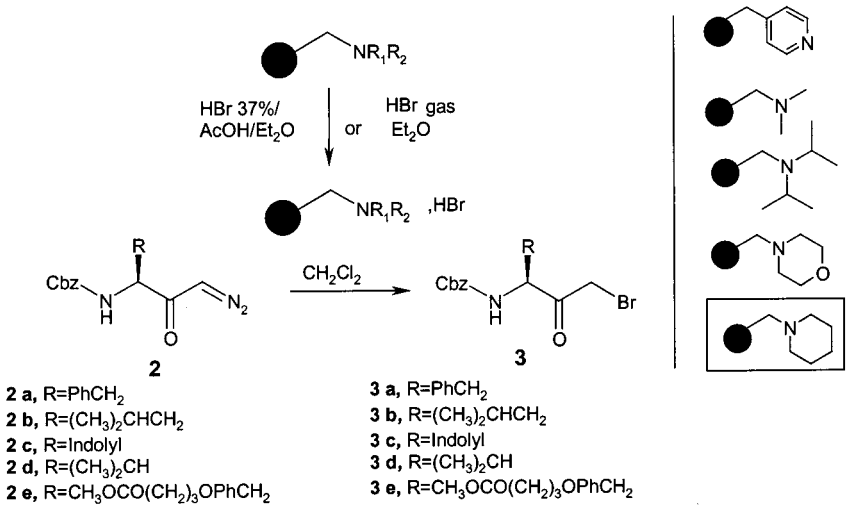


Figure 4.

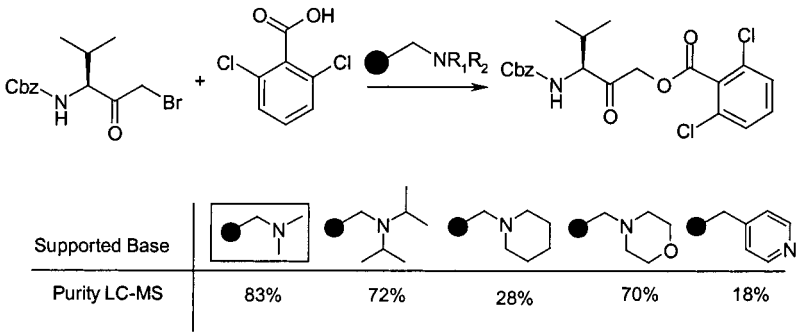


Figure 5.

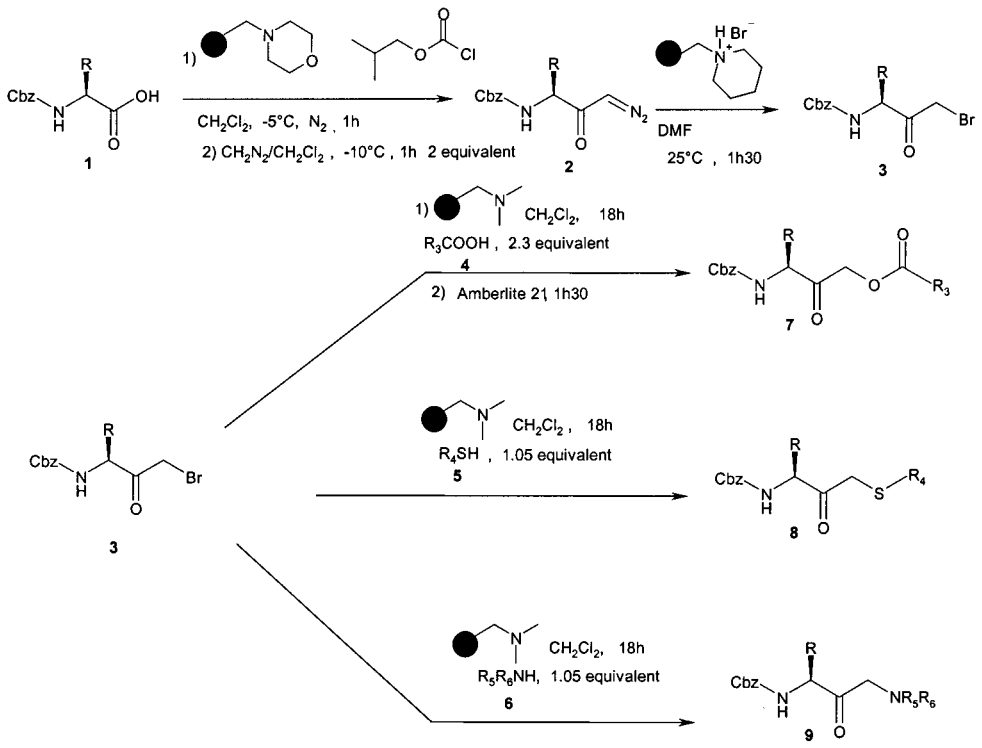
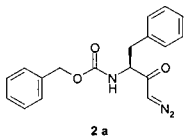
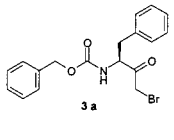
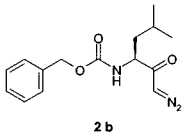
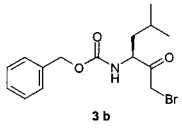
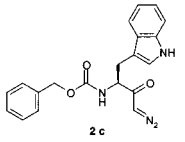
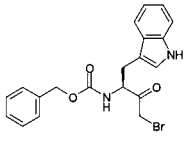
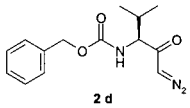
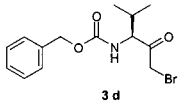
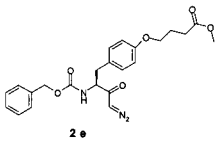
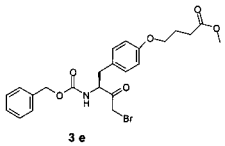


Figure 6.

was filtration followed by evaporation. The results are summarized in Table 1 and show the scope of this method. Bromo ketones are obtained in good yields and purity to perform the final diversification.

Final nucleophilic substitution was performed in the multiblock from Bohdan. The 96-array multiblock equipped with frits allowed direct filtration of the desired compounds in preweighed vials for archiving after evaporation. Five

Table 1. Purity and Yield for Compounds **2a**−**e** and **3a**−**e**

Diazoketone	Purity % [a]	Yield %	Bromoketone	Purity % [b]	Yield %
 **2a**	90	89	 **3a**	85	92
 **2b**	90	90	 **3b**	85	85
 **2c**	90	78	 **3c**	90	87
 **2d**	>95	71	 **3d**	>95	89
 **2e**	85	58	 **3e**	80	85

[a] Purity determined by ^{1}H NMR. [b] Yield determined by weight.

acids, five thiols, four secondary amines, and two primary amines were used as nucleophiles.

As shown in Table 2, except for the two primary amines, all nucleophiles gave the expected compounds with acceptable purity and yield. Primary amines probably resulted in dialkylation and hence were unable to give the desired compounds.

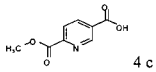
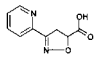
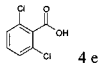
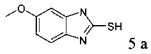
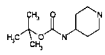
In summary, we have designed a synthetic procedure using supported reagents and scavengers for the preparation of useful acyl ketones, thio ketones, and amino ketones from amino acids as potential cysteine protease inhibitors for the straightforward optimization of the P1−P1′ pockets of the targeted enzymes. An 80-member library has been generated using this pathway. The process obviates tedious workup and purification and can be adapted for the generation of a large library on a robot. In addition, for the selective monobromination of diazo ketones, the preparation a new supported reagent, piperidinoaminomethylpolystyrene hydrobromide, has also been described.

Experimental Section

General. *N*-methylmorpholinepolystyrene was purchased from Argonaut (1.75 mmol/g). Dimethylaminomethylpolystyrene was purchased from Fluka (loading 3−4 mmol/g). *N*-Cbz-capped amino acids **1a**−**d** were purchased from Aldrich, and **1e** was prepared according to a published procedure.[19] Nucleophiles **4b**, **4e**, **5b**−**e**, and **6b** (Table 2) were purchased from Aldrich, and **5a** was purchased from Maybridge, **6a** from Astatec, and **6c** from Finorga. **4a**, **4c**−**d**, and **6d**−**f** were prepared according to the literature.[20−24] Analytical grade solvents were used for reactions and resin washing. ^{1}H NMR analyses were recorded on a Bruker DPX 300 spectrometer. HPLC−mass spectrometry analyses were performed using a Micromass platform coupled to a 1100 HP HPLC equipped with a YMC ODS-AQ (3 μm, 50 mm × 4 mm) column and in +ve electrospray ionization mode. The mobile phase was acetonitrile/water 65/35 (elution time 6 min). HPLC for amino ketones was performed on a Shimadzu 10 AVP with Alltech Platinum EPS C18 (3 μm, 33 mm × 7 mm) column using methanol/water 60/40 and 1% TFA and then directly introduced for MS detection. Flash chromatography was carried out on a Biotage Quad 3 using a flash 12 M (12 mm × 16 cm silica KP-sil 32−63 μm, 60 Å) column.

General Procedure for the Preparation of Diazo Ketones. In five Quest 205 reactors, cooled to −10 °C, under a nitrogen atmosphere, *N*-methylmorpholinepolystyrene is suspended in dichloromethane (29 mL) and allowed to swell for 15 min. A precooled solution (−10 °C, 3 mmol) of *N*-Cbz amino acid **1a**−**e** (see Table 1) in dichloromethane (13.2 mL) is slowly added (one to each reactor) followed by dropwise addition, through a septum, of isobutyl chloroformate (1.03 equiv). Reaction mixtures are stirred for 1 h at −5 °C and filtered, and the resin is washed with dichlo-

Table 2. Purity and Yield of Generated Library[c]

Nucleophile \ R					
 4 a	**86**[a]	**96**	**82**	**97**	**83**
	44[b]	46,2	40,0	50,8	44,4
 4 b	**81**[a]	**84**	**80**	**89**	**68**
	50,1[b]	44,0	30,9	41,7	54,7
 4 c	**91**[a]	**93**	**81**	**97**	**80**
	47,9[b]	44,7	33,4	49,5	49,6
 4 d	**53**[a]	**79**	**63**	**86**	**79**
	31,6[b]	32,6	25,1	38,7	35,1
 4 e	**88**[a]	**94**	**88**	**98**	**80**
	67,4[b]	65,0	60,1	65,7	68,0
 5 a	**89**[a]	**84**	**88**	**89**	**73**
	95[b]	88,8	90,2	87,9	87,9
 5 b	**92**[a]	**91**	**96**	**97**	**86**
	89,9[b]	85,1	85,1	82,7	83,4
 5 c	**82**[a]	**89**	**88**	**90**	**78**
	90,8[b]	87,7	88,5	84,9	88,5
 5 d	**73**[a]	**70**	**74**	**64**	**67**
	90,0[b]	87,4	85,8	79,9	86,8
 5 e	**87**[a]	**68**	**90**	**96**	**77**
	88,0[b]	87,5	92,0	84,8	89,9
 6 a	**77**[a]	**67**	**82**	**84**	**73**
	86,8[b]	85,4	88,6	96,1	89,2
 6 b	**68**[a]	**64**	**80**	**57**	**58**
	88,4[b]	86,1	85,5	80,5	91,0
 6 c	**69**[a]	**54**	**74**	**76**	**55**
	76,7[b]	75,4	77,1	76,0	81,8
 6 d	**54**[a]	**59**	**65**	**74**	**51**
	88,8[b]	87,0	90,7	87,0	85,7
 6 e	nd	nd	nd	nd	nd
 6 f	nd	nd	nd	nd	nd

[a] Purity determined by LC−MS. [b] Yield determined by weight. [c] nd ≡ not detected.

romethane (20 mL). The obtained filtrate for each *N*-Cbz-capped amino acid is again cooled to −10 °C under a nitrogen atmosphere, and 2 equiv of diazomethane (0.3 M solution in dichloromethane prepared by standard procedure) is added dropwise. The reaction mixture was stirred for 1 h and then gradually brought to room temperature. After elimination of excess diazomethane, under a flux of nitrogen, the organic phase is concentrated. The diazo ketones are obtained with 5−10% impurity of the methyl ester (Table 1).

3-(*N*-Benzyloxycarbonyl)amino-4-phenyl-1-diazobutane-2-one (2a): ^{1}H NMR ($CDCl_3$) δ 3.04 (d, 2H), 4.49 (m, 1H), 5.09 (s, 2H), 5.20 (s, 1H), 5.36 (d, 1H), 7.02−7.43 (m, 10H); MS (ES) 324 (MH^+).

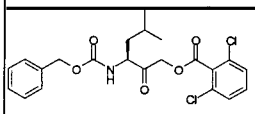
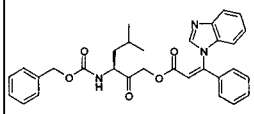
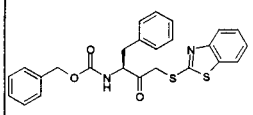
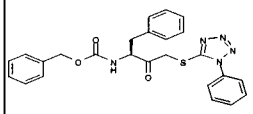
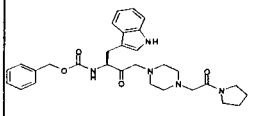
3-(*N*-Benzyloxycarbonyl)amino-5-methyl-1-diazohexane-2-one (2b): ^{1}H NMR ($CDCl_3$) δ 0.93 (m, 6H), 1.38 and 1.45 (2 m, 2H), 1.71(m, 1H), 4.27 (m, 1H), 5.12 (s, 2H), 5.20 (d, 1H), 5.42 (s, 1H) 7.35(m, 5H); MS (ES) 290 (MH^+).

3-(*N*-Benzyloxycarbonyl)amino-4-(3-indolyl)-1-diazobutane-2-one (2c): ^{1}H NMR ($CDCl_3$) δ 3.22 (m, 2H), 4.58 (m, 1H), 5.10 (s, 2H), 5.17 (s, 1H), 5.44 (d, 1H), 7.00 (d, 1H), 7.10−7.40 (m, 8H), 7.62 (d, 1H), 8.07 (s, 1H); MS (ES) 363 (MH^+).

3-(*N*-Benzyloxycarbonyl)amino-4-methyl-1-diazopentane-2-one (2d): ^{1}H NMR ($CDCl_3$) δ 0.94 (d, 6H) 2.10 (m, 1H), 4.13 (s, 1H), 5.10 (s, 2H), 5.39 (m, 2H), 7.35 (m, 5H); MS (ES) 276 (MH^+).

3-(*N*-Benzyloxycarbonyl)amino-4-(4-methoxycarbonyl-butyl-1-oxy)phenyl-1-diazobutane-2-one (2e): ^{1}H NMR ($CDCl_3$) δ 1.97 and 2.10 (2 m, 2H), 2.41 and 2.52 (2 t, 2H), 2.97 and 3.04 (2 m, 2H), 3.68 (s, 3H) 3.97 and 4.10 (2 t, 2H), 4.42 and 4.62 (2 m, 1H), 5.08 (s, 2H), 5.21 (s, 1H)

Table 3.

Examples	LC/MS ES/MH$^+$	1H NMR, δ, ppm in CDCl3 (300MHz)
	451	0.94 (br d, 6H), 1.46 and 1.69 (2 m, 2H), 1.72 (m, 1H), 4.61 (m, 1H), 5.04 and 5.13 (AB, 2H), 5.11 (br s, 2H), 5.23 (br s, 1H), 7.28-7.38 (m, 8H).
	526	0.93 (m, 6H), 1.47 and 1.55 (2m, 2H), 1.68 (m, 1H), 4.39 (m, 1H), 4.84 (AB, 2H), 5.10 (AB, 2H), 5.26 (d, 1H), 6.72 (s, 1H), 6.94-8.03 (m, 14H), 8.59 (s, 1H).
	463	3.12 and 3.24 (2dd, 2H), 4.05 and 4.39(2D, 2H), 4.94 (m, 1H), 5.09 (AB, 2H), 5.71 (d 1H), 7.05-7.40 (m, 12H), 7.75 (m, 2H).
	474	3.06 and 3.24 (2dd, 2H), 4.27 and 4.44 (2d, 2H), 4.73 (m, 1H), 5.09 (s, 2H), 5.37 (d, 1H), 7.05-7.40(m, 10H), 7.58(m, 5H).
	532	1.70-2.05 (m, 10H), 2.20-2.60 (m, 4H), 3.05 (AB,2H), 3.08 (AB, 2H), 3.32 (2AB, 4H), 4.79 (m, 1H), 4.98 (s, 2H), 5.46 (d, 1H), 6.85 (s, 1H), 6.90-7.22 (m, 8H), 7.53 (d, 1H), 8.00 (s, 1H).

5.37 (d, 1H), 6.63 and 6.93 (AA′BB′, 4H), 7.33 (m, 5H); MS (ES) 440 (MH^+).

Preparation of *N*-Piperidinomethylpolystyrene Hydrobromide for Monobromination. In a three-necked flask equipped with an inlet for HBr gas and an outlet for HBr trap, 1 g of dried *N*-piperidinomethylpolystyrene (loading 3.5 mmol/g) is cooled to 0 °C in dry diethyl ether (35 mL). HBr gas is slowly bubbled in the gently stirred suspension for an hour. The colored suspension is filtered under a nitrogen atmosphere, and the resin is washed several times with dry diethyl ether, dried in a vacuum, and stored in a refrigerator. The bromine loading is determined by microanalysis to be 3.25 mmol/g. This resin was found to be stable for several weeks in the refrigerator.

General Procedure for the Preparation of Bromo Ketones. In a parallel reaction, a solution of diazo ketone (0.055 mmol) in DMF (0.6 mL) (Table 1) is added dropwise to the *N*-piperidinomethylpolystyrene hydrobromide (11.8 equiv), and the obtained suspension is placed on a Ping-Pong shaker. After filtration and several washings of the resin (DMF), the filtrate is evaporated (Table 2).

3-(*N*-Benzyloxycarbonyl)amino-4-phenyl-1-bromobutane-2-one (3a): ^{1}H NMR ($CDCl_3$) δ 3.08 (m, 2H), 3.81 and 3.92 (AB, 2H), 4.83 (m, 1H), 5.09 (s, 2H), 5.30 (s, 1H), 7.06−7.44 (m, 10H); MS (ES) 377 (MH^+).

3-(*N*-Benzyloxycarbonyl)amino-5-methyl-1-bromohexane-2-one (3b): ^{1}H NMR ($CDCl_3$) δ 0.97 (m, 6H), 1.45 and 1.65 (2 m, 2H), 1.74 (m, 1H), 4.04 and 4.10 (AB, 2H), 4.65 (m, 1H), 5.11 (s, 2H), 5.18 (s, 1H), 7.35 (m, 5H); MS (ES) 343 (MH^+).

3-(*N*-Benzyloxycarbonyl)amino-4-(3-indolyl)-1-bromobutane-2-one (3c): ^{1}H NMR ($CDCl_3$) δ 3.25 and 3.30 (2 dd, 2H), 3.79 and 3.91 (AB, 2H), 4.93 (m, 1H), 5.05 and 5.12 (AB, 2H), 5.41 (m, 1H), 6.99 (d, 1H), 7.09−7.4 (m, 8H), 7.62 (m, 1H), 8.09 (br s, 1H); MS (ES) 416 (MH^+).

3-(*N*-Benzyloxycarbonyl)amino-4methyl-1-bromopentane-2-one (3d): ^{1}H NMR ($CDCl_3$) δ 0.94 (d, 6H), 2.23 (m, 1H), 4.03 and 4.08 (AB, 2H), 4.61 (dd, 1H), 5.11 (s, 2H), 5.31 (s, 1H), 7.36 (m, 5H); MS (ES) 329 (MH^+).

3-(*N*-Benzyloxycarbonyl)amino-4-(4-methoxycarbonylbutyl-1-oxy)phenyl-1-bromobutane-2-one (3e): ^{1}H NMR ($CDCl_3$) δ 2.10 (q, 2H), 2.52 (t, 2H), 3.01 (m, 2H), 3.69 (s, 3H), 3.97 (t, 2H), 4.63 and 4.78 (2 m, 1H), 5.08 (s, 2H), 5.24 (d, 1H), 6.88 and 6.90 (AA′BB′, 4H), 7.34 (m, 5H); MS (ES) 479 (MH^+).

General Procedure for Nucleophilic Substitution. Stock solutions of bromo ketones (0.05 mmol) are prepared in dimethylformamide (0.6 mL).

A total of 28.6 mg of dimethylaminomethylpolystyrene (∼1.7 equiv) is introduced in each well of two miniblocks (Bohdan, 48 wells on each block), and the blocks are closed carefully with a provided seal to prevent mixing.

Selected on chemical diversity with the help of the software Reagent Selector from MDL, 16 nucleophiles are dissolved in DMF to obtain a 0.35 M solution of each nucleophile. These solutions were introduced into the miniblock as the following, for each bromo ketone: (1) five selected acids, 0.33 mL (0.115 mmol, 2.3 equiv); (2) five selected thiols, 0.15 mL (0.053 mmol, 1.05 equiv); (3) four selected secondary amines, 0.15 mL (0.053 mmol, 1.05 equiv); (4) two selected primary amines, 0.15 mL (0.053 mmol, 1.05 equiv). A total of 0.05 mmol of solution of each bromo ketone is manually introduced into each well in a serial manner. These reaction solutions on miniblocks are stirred on a Bohdan orbital shaker for 18 h and filtered, and the resin was rinsed 3−5 times with dichloromethane. The obtained thio ketones and amino ketones are evaporated in

pretarred vials, and acyl ketones are sequestered with Amberlite 21 (~100 mg) for 90 min to eliminate excess acids, if any still left, with gentle orbital stirring, filtered, rinsed, and evaporated in pretarred vials. Each compound of the generated 80-member library is analyzed by LC−MS. Primary amines failed to give desired product under the described conditions probably because of dialkylation (Table 2).

By random sampling, the products listed in Table 3 are also characterized by ^{1}H NMR for the confirmation of the structures of the generated library.

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