Optimization of Three- and Four-Component Reactions for Polysubstituted Piperidines: Application to the Synthesis and Preliminary Biological Screening of a Prototype Library

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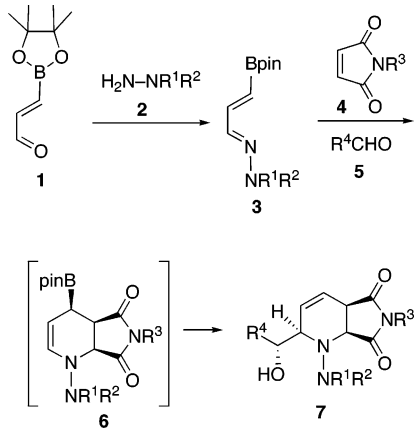
Several solid- and solution-phase strategies were evaluated for the preparation of libraries of polysubstituted piperidines of type **7** using the tandem aza[4+2]cycloaddition/allylboration multicomponent reaction between 1-aza-4-boronobutadienes, maleimides, and aldehydes. A novel four-component variant of this chemistry was developed in solution phase, and it circumvents the need for pre-forming the azabutadiene component. A parallel synthesis coupled with compound purification by HPLC with mass-based fraction collection allowed the preparation of a library of 944 polysubstituted piperidines in a high degree of purity suitable for biological screening. A representative subset of 244 compounds was screened against a panel of phosphatase enzymes, and despite the modest levels of activity obtained, this study demonstrated that piperidines of type **7** display the right physical properties (e.g., solubility) to be assayed effectively in high-throughput enzymatic tests.

1. Introduction

Multicomponent reactions (MCRs) are very attractive processes that push the limits of synthetic efficiency by using more than two reactants to create novel products with an optimal number of new bonds and functionalities.[1] MCRs are ideally suited for the construction of combinatorial libraries based on the structure of natural products and other privileged structures prone to display biological activity.[2] We have previously developed a three-component reaction based on a "one-pot" sequential aza[4+2]cycloaddition/allylboration reaction between 1-aza-4-boronobutadienes (**3**), N-substituted maleimides (**4**), and aldehydes (**5**) (Scheme 1).[3]

This process requires the pre-formation of heterodienes **3** in a separate step involving hydrazone formation between 3-boronoacrolein pinacolate (**1**) and a hydrazine (**2**). Heterodienes **3** first undergo a [4+2] cycloaddition with electronically deactivated dienophiles such as maleimides **4**, affording the bicyclic allylic boronate intermediates **6**. The latter then react with aldehydes to give the final products **7** after the hydrolytic workup. Overall, this multicomponent reaction provides an efficient preparation of polysubstituted piperidines of type **7** in a stereocontrolled fashion and with a broad substrate scope.[4] These compounds possess the right size and many of the desirable types of stable functionalities such as imide, hydroxyl, and basic amino groups. These functional groups possess hydrogen bond donor/acceptor capabilities that could confer interesting physicochemical and biological properties for applications in chemical biology and drug discovery.[5] Indeed, there are numerous examples of piperidine and imide-containing drugs and biologically active substances (Figure 1).[6,7]

Scheme 1. Three-Component Synthesis of Polysubstituted Piperidines **7** Using a Tandem Aza[4+2]cycloaddition/allylboration (pin = $OCMe_2CMe_2O$)



We were interested in undertaking the synthesis of medium-sized combinatorial libraries for biological screening applications in multiple assays. Here, we describe an investigation of both solid- and solution-phase strategies for the synthesis and purification of polysubstituted piperidines of type **7**. We report the new finding that this multicomponent reaction functions efficiently as a four-component reaction, which alleviates the need for pre-forming the heterodiene component **3**. A prototype library was assembled

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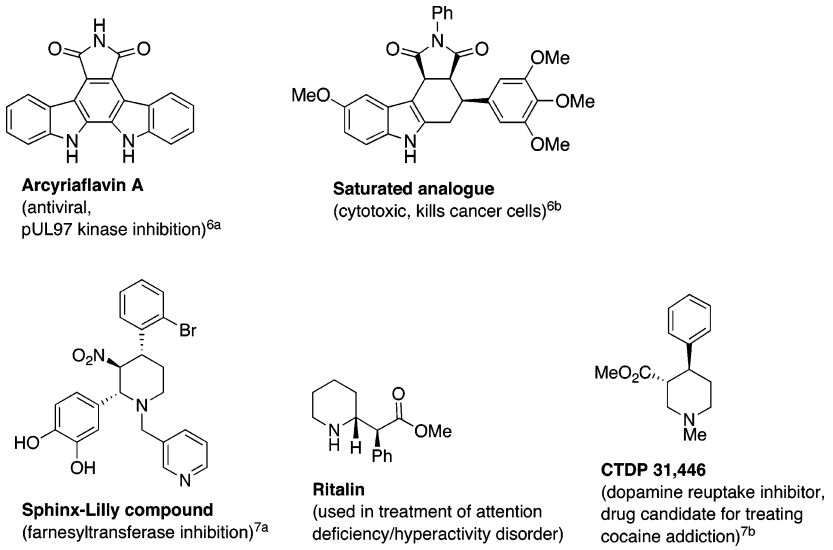
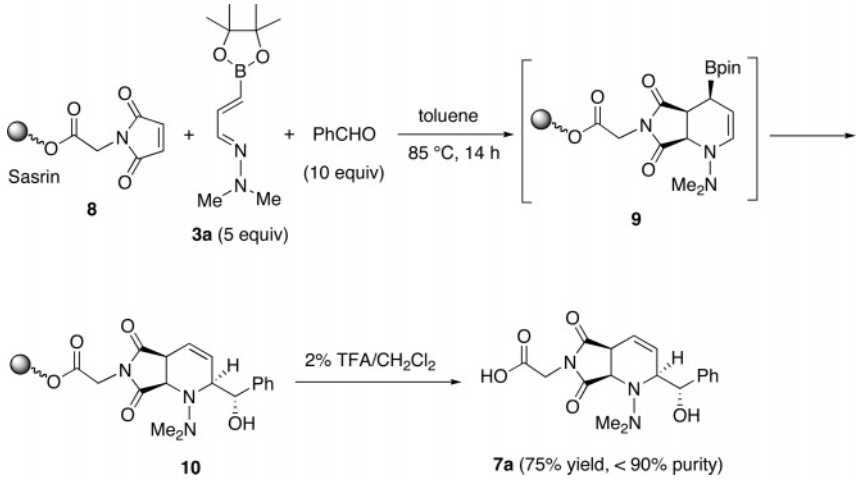
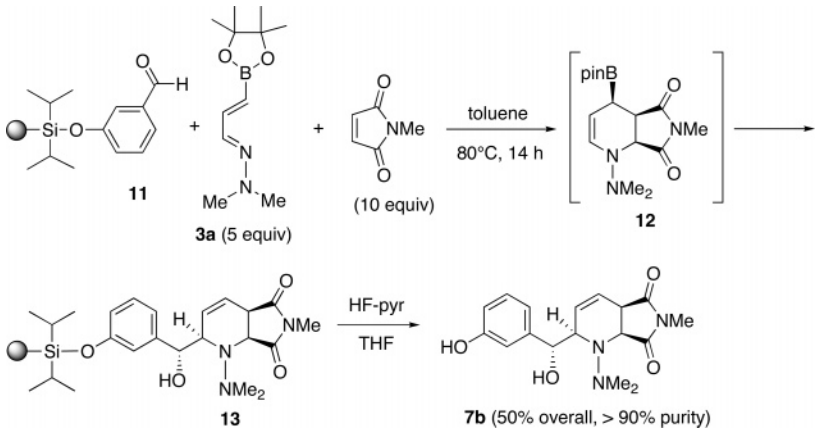


Figure 1. Examples of piperidine and imide-containing substances and their biochemical activity.

Scheme 2. Solid-Phase Synthesis Approach Using a Supported Maleimide



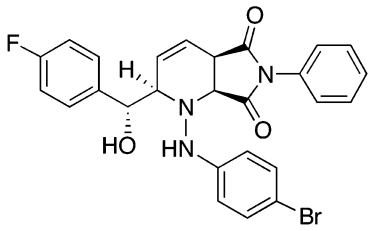
Scheme 3. Solid-Phase Synthesis Approach with a Supported Aldehyde



using solution-phase methods, and purified with ease using semipreparative HPLC with mass-based fraction collection. A representative subset of this prototype library was subjected to a preliminary evaluation of biological activity against a panel of phosphatase enzymes.

2. Results

2.1. Investigation of Solid-Phase Synthetic Strategies. Because of its ease and rapidity, as well as the possibility of using excess reagents to drive reactions to completion, we first considered solid-phase strategies for the preparation of libraries of piperidines **7**.[8] Although multicomponent reactions offer more possibilities for diversification, more issues have to be examined in the design of a solid-phase synthetic strategy. Factors such as the reaction mechanism, the cost and availability of all the components, and the site of attachment to the resin have to be considered. Indeed, one significant drawback from these approaches is the require-



7{10,7,29} MW = 522.37

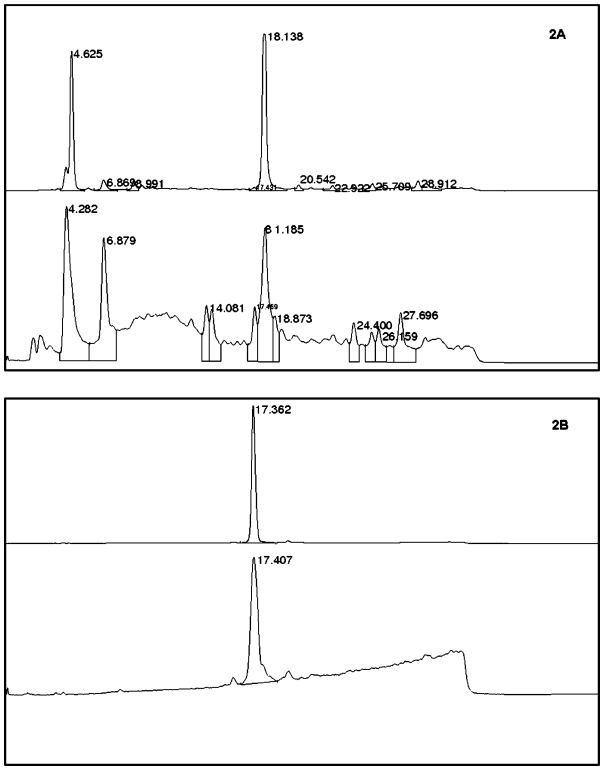
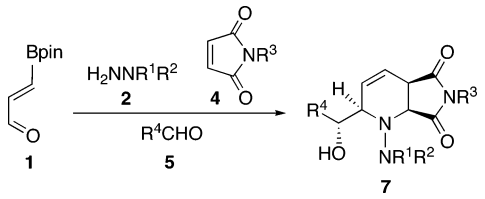


Figure 2. Typical HPLC chromatograms comparing a library compound (**7**{*10,7,29*}) in crude unpurified form (Figure 2A) and after purification (Figure 2B). For both figures, the UV (254 nm) chromatogram is on top and the ESI-MS total ion chromatogram at the bottom.

ment to "sacrifice" one component for attachment to a solid support, which usually makes it no longer possible to diversify this component. We first considered attachment through the N-substituent of the maleimide component. This attempt involved the preparation of a N-alkyl maleimide linker **8**, which then underwent the three-component reaction with heterodiene **3a** and benzaldehyde (Scheme 2). While this reaction worked reasonably well under optimized conditions using excess reagents, we were unable to optimize it to highly satisfactory levels of purity for crude product **7a** after cleavage of the resin-bound product **10**.

We reasoned that the solid-supported allylboronate **9**, an intermediate likely to be unstable to air and adventitious water, may be prone to generate side-products that remain bound to the support up until the final cleavage operation. To avoid this problem, immobilization of the aldehyde component would be ideal because the required allylboronate intermediate **12** would rather form in solution, and its decomposition products would be washed off from the resin-bound product in the workup steps (i.e., washings). In this event, a model reaction between diene **3a**, *N*-methyl male-

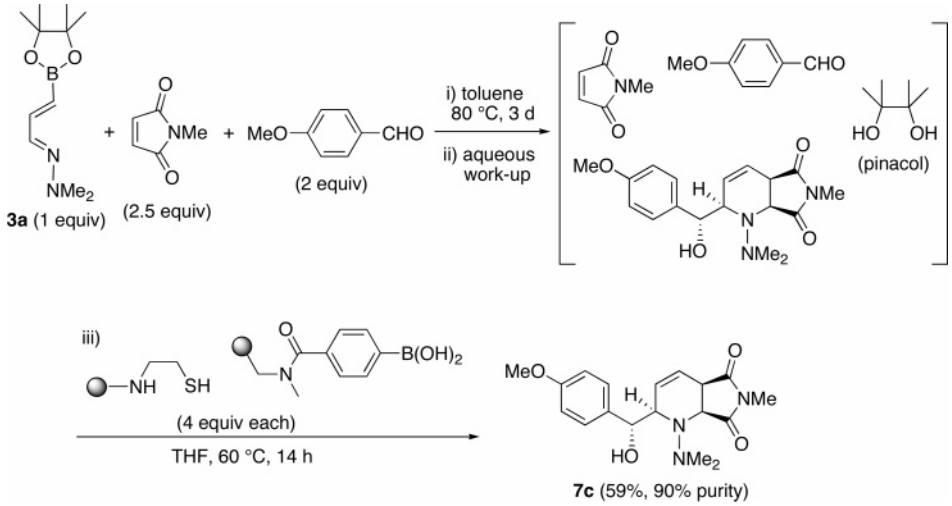
Table 1. Systematic Comparison of Three-Component (3CR) and Four-Component (4CR) Reactions[a]



						yield (%)[b]	
entry	R^1	R^2	R^3	R^4	product	3CR	4CR
1	Me	Me	Me	$C_{10}H_{21}$	**7d**	26	17
2	Me	Me	Me	Ph	**7e**	50	52
3	Me	Me	Me	2-pyr	**7f**	*c*	*c*
4	Ph	H	Me	$C_{10}H_{21}$	**7g**	37	32
5	Ph	H	Me	Ph	**7h**	53	65
6	Ph	H	Me	2-pyr	**7i**	42	55

[a] Conditions: toluene, 85 °C, 72 h. Typical scale: 1.0 mmol at 0.2 M. For 3CR: heterodiene, 1.0 equiv; maleimide, 2.0 equiv; aldehyde, 1.5 equiv. For 4CR: aldehyde **1**, 1.0 equiv; hydrazine, 1.1 equiv; maleimide, 2.0 equiv; aldehyde, 1.5 equiv. See Supporting Information for more details. [b] Isolated nonoptimized yields after flash chromatography purification. [c] No or little (<5%) product was isolated.

Scheme 4. Solution-Phase Approach Using Supported Scavengers



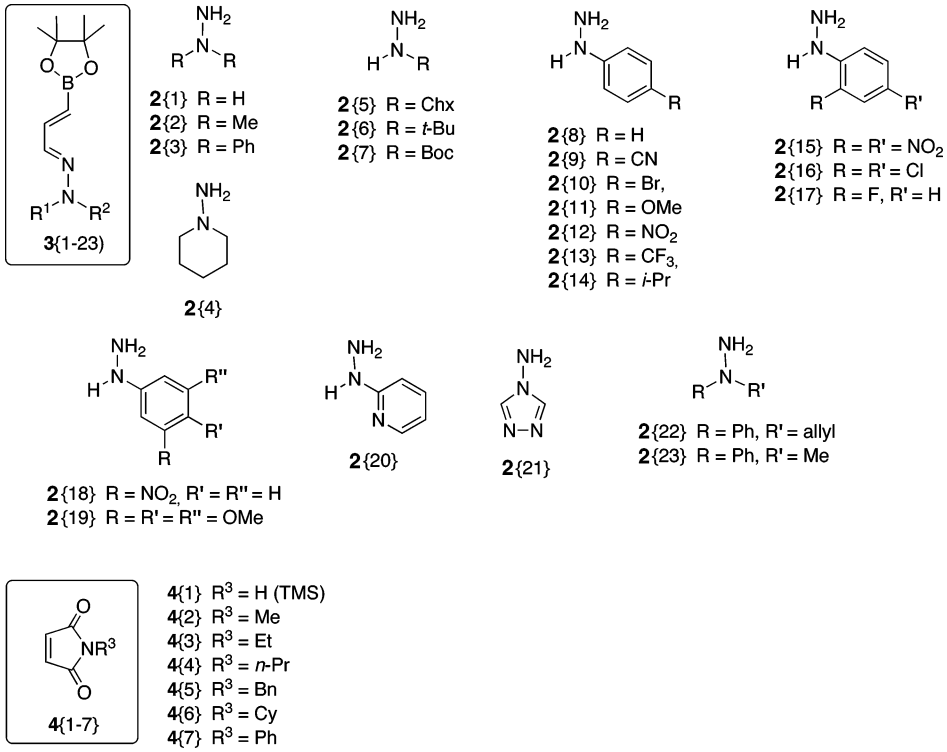


Figure 3. Hydrazine (**2**) and maleimide (**4**) building blocks used for the synthesis of a library of piperidines **7**.

imide, and siloxy resin-supported[9] aldehyde **11** (Scheme 3) did provide product **7b** in a level of homogeneity clearly superior to that of the previous approach of Scheme 2. Although this new approach would provide a rapid and straightforward access to the desired piperidine libraries, the immobilization of the aldehyde component also brings a significant disadvantage. Indeed, the aldehyde is the most easily diversifiable component of this MCR, with several hundreds available from many commercial sources. Consequently, we decided to examine other options.

2.2. Investigation of Solution-Phase Strategies. Development of a Four-Component Variant. Next we considered generating libraries via the three-component solution-phase reaction variant and by using solid-supported scavengers to provide a rapid and simple purification of the piperidine products. To this end, a model reaction between components **3a**, *N*-methyl maleimide, and *p*-methoxybenzaldehyde was optimized (Scheme 4). It was necessary to use an excess of maleimide and aldehyde to obtain full consumption of the heterodiene. At the end of the reaction, a basic aqueous workup was performed to hydrolyze the resulting pinacol borate product. Then, the crude material was treated with an excess of an aminothiol scavenger, which removed both excess maleimide and excess aldehyde. A supported boronic acid resin was used concomitantly to scavenge the pinacol byproduct. Although product **7c** was obtained in a good degree of purity, this procedure failed to remove all of the residual pinacol.

Solution-phase strategies coupled with HPLC purification were explored next. This approach was deemed valuable in a multicomponent reaction where products can be prepared in only one or two synthetic operations. Moreover, at this stage, another possible improvement of the chemistry of Scheme 1 was considered. We contemplated the idea that it may not be necessary to pre-form the heterodienes **3**. It was thought that the precursors, 3-boronoacrolein (**1**), and the hydrazines **2** may react preferentially even in the presence of the second aldehyde, thus providing a four-component reaction. Such a four-component reaction would lead to significant savings of time and effort in the preparation of libraries of piperidines **7** by avoiding the preparation of the heterodienes as a separate step. To this end, a systematic comparison between the three-component reaction (with preformed heterodiene **3**) and the four-component variant (using **1** and **2**) was planned. The results are shown in Table 1.

From the results of a representative matrix of six examples, including two heterodienes made from two electronically different hydrazines, it is clear that both protocols are quite comparable for all types of aldehydes examined. Although the yields are lower than desired, they are certainly acceptable considering that the reaction involves four components. Aromatic aldehydes provide cleaner reactions in both cases, which is reassuring. Indeed, because of the widespread commercial availability of aromatic aldehydes, they constitute the bulk of the possible and most interesting examples towards library generation. The use of enolizable aliphatic aldehydes was less effective in both the three- and the four-component reactions. A probable reason for this is the possibility for enamine formation and ensuing condensations between the aldehyde components. Nonetheless, the possibility of generating libraries of polysubstituted piperidines with a four-component reaction significantly simplifies the practical aspects of this multicomponent chemistry. Moreover, the recent advent of powerful HPLC technologies based on mass-based fraction collection[10] led us to consider this method for the purification of libraries of piperidines **7**. These basic piperidines are cationic in water and were thus expected to be ideal analytes for an instrument based on electrospray

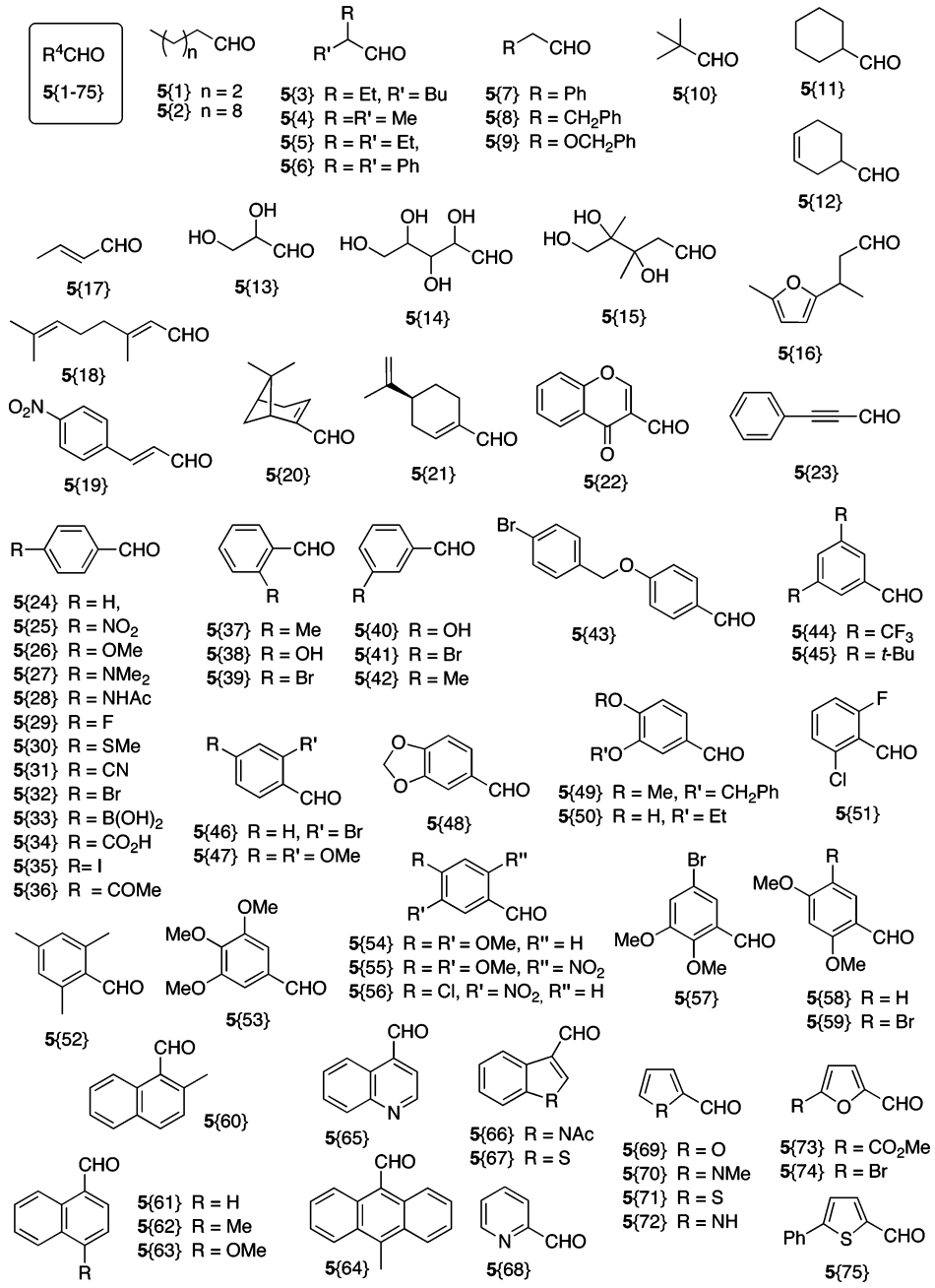


Figure 4. Aldehyde (**5**) building blocks used for the synthesis of a library of piperidines **7**.

ionization detection. Indeed, optimization of a general reverse-phase separation method on a semipreparative C8 column provided an efficient means of purifying the piperidines even in the presence of a large excess of reagents and possible byproducts. The comparison of typical chromatograms for a library member in the crude form (Figure 2A) and the corresponding, purified product (Figure 2B) clearly demonstrates the power of this approach for the purification of piperidines of type **7**.

2.3. Library Design and Preparation. To design the libraries, we arbitrarily selected sets of commercially available components based on the optimization of structural and functional diversity (Figures 3 and 4). Thus, 23 hydrazines (**2**), 7 N-substituted maleimides (**4**), and 75 aldehydes (**5**) were employed for a possibility of 12 075 combinations of resulting piperidines **7** (Figure 5). Of that large number, however, much fewer were attempted because of restricted time and resources. Nonetheless, as a proof-of principle, all building blocks were employed at least once. The compounds were synthesized in a 0.1 mmol scale in sets of 48 using a parallel semiautomated synthesizer with both the three-component and four-component protocols. To this end, the reactants were mixed and heated at 85 °C for 72 h. The vessels were cooled to room temperature, and a saturated aqueous solution of $NaHCO_3$ was added to each vessel. The organic phases were transferred to vials that were transferred onto a rapid evaporator. Over 80% of all combinations attempted provided the desired piperidine products, for a total of 944 piperidines (the full list is available in the Supporting Information). A few of the components, such as hydrazine **2**{*21*}, gave very low yields of products. It should also be noted that the use of microwave heating failed to provide any advantage with this multicomponent reaction. To focus on product purity rather that recovered yields, a very

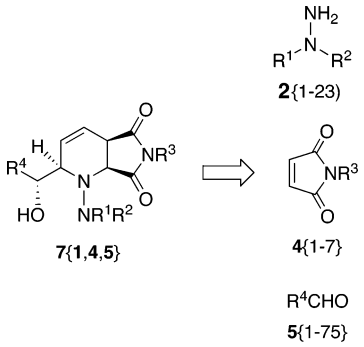


Figure 5. Generic library member with numbering system.

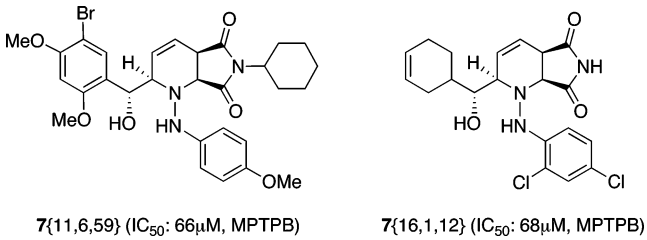


Figure 6. Two members of a library subset evaluated in preliminary screening and identified as weak inhibitors of the protein tyrosine phosphatase enzyme MPTPB.

conservative fraction collection threshold was employed. Thus, while the conventional thermal conditions provided piperidine products only with typically 10−30% recovered yields, most products were obtained in 3−10 mg quantity and in 80−100% homogeneity according to a random analysis (vide infra). This careful purification protocol explains the lower yields compared to the individual examples involving flash-chromatographic purification on normal silica gel. As shown in the Supporting Information, 51 randomly selected compounds (ca. 5% of the library) were analyzed and characterized using HPLC-ESMS and ^{1}H NMR spectroscopy. Of this sampling, 43 of the 51 (84%) were isolated in a purity greater than 80% according to UV detection, and 30 of these (59%) had a purity of over 90%.

2.4. Preliminary Screening of a Subset of the Piperidine Library. Our HPLC-purified piperidine libraries were evaporated and stored as solids or films in small glass vials. A representative subset of 244 members of the compound collection was made into 10 millimolar concentration stock solutions in DMSO. These compounds were evaluated in high-throughput assays for their ability to inhibit a panel of protein phosphatase enzymes. Protein phosphatases are a broad and therapeutically relevant class of enzymes, which, in concert with their congeners protein kinases, control phosphorylation processes crucial to the cell cycle and numerous other biological processes.[11] Thus far, there are only a relatively small number of known classes of inhibitors for these important enzyme targets.[12] Here, the piperidine library subset was evaluated for inhibition of the protein tyrosine phosphatases PTP1B, MPTPA, MPTPB, VEPTP, and PP1 and the dual-specificity phosphatases Cdc25A and VHR. All the assays were based on the monitoring, by UV absorption at 405 nm, of the dephosphorylation of 4-nitrophenyl phosphate in aqueous buffered solutions containing 1% DMSO in the presence of a detergent to avoid nonspecific binding and aggregation phenomena.[13] The evaluation of compounds displaying 50% or more inhibitory activity at 100 μM concentration were followed up with measurements of half-inhibitory concentrations (IC_{50}). These values were obtained from quadriplicate measurements, and the integrity and purity of all hits was verified by HPLC-ESMS analysis. Although only two of the piperidines **7** (Figure 6) were demonstrated to be weak inhibitors of one phosphatase enzyme, performing these assays allowed us to confirm that the piperidines of type **7** are generally soluble and stable in assay solutions and are thus suitable for undergoing general high-throughput assays against many different targets. Occasional reanalysis of selected compounds, stored as films or solids, revealed none or negligible decomposition over the course of several months. As only a small subset of these compounds were screened against only one family of enzymes, many opportunities remain to be considered for these piperidine libraries as we have engaged into several collaborations for high-throughput screening.

3. Conclusion

In summary, we have examined several solid- and solution-phase strategies for the preparation of libraries of polysubstituted piperidines of type **7** using the tandem aza[4+2]-cycloaddition/allylboration multicomponent reaction. Following the optimization of a novel four-component variant of this chemistry that circumvents the need for pre-forming the azabutadiene component, we have chosen a solution-phase parallel synthesis coupled with compound purification by HPLC with mass-based fraction collection. This procedure delivered a library of 944 polysubstituted piperidines in a high degree of purity suitable for biological screening. A representative subset of 244 compounds was screened against a panel of phosphatase enzymes, and despite the modest levels of activity obtained, this preliminary study demonstrated that piperidines of type **7** display the right properties (e.g., solubility and stability) to be assayed effectively in high-throughput enzymatic tests. Larger libraries of polysubstituted piperidines are currently been synthesized and evaluated in several other high-throughput biological assays.

4. Experimental Section

4.1. General Methods. Synthesis. Toluene was distilled prior to use and dried by standard methods. HPLC-grade acetonitrile was obtained from commercial suppliers and used without further purification. HPLC-grade deionized water was employed. LCMS analyses were performed on a Hewlett-Packard/Agilent 1100 MSD using an electron spray ionization detector (scan range: 100 to 1000 M/Z). The piperidine library was synthesized using a Trident Synthesizer External-Thermal agitation unit (Biotage). All resins used in this study were purchased from NovaBiochem. ^{1}H and ^{13}CNMR spectra were obtained in $CDCl_3$ solutions at 400 MHz or 300 MHz, unless otherwise indicated. Chemical shifts (δ) were reported in parts per million (ppm) relative to the singlet at 7.26 ppm of $CDCl_3$ for ^{1}H and to the centerline of a triplet at 77.0 ppm of $CDCl_3$ for ^{13}C. The glycinyl maleimide was attached to Sasrin resin according to a literature procedure.[14] *m*-Hydroxybenzaldehyde was

attached to the silyl resin according to the reported procedure.[9] Azadienes were prepared according to the previously reported procedure.[3b]

Biochemical Screening.[13] All enzyme assays were performed by means of automated systems consisting either of a Zymark SciClone ALH 500 in conjunction with a Twister II or a Tecan EvoWare robot. In both cases, they were connected to a Genios Pro MTP reader. The reaction volume was 10 μL. For phosphatases, the reaction was started by the addition of 5 μL *p*-nitrophenyl phosphate to 5 μL of a solution containing the respective enzymes which had been preincubated for 10 min with inhibitors. Reaction velocity was determined from the slope of the absorbance change at 405 nm and related to control values in absence of the inhibitor. For IC_{50} determinations, a dilution series by the factor 2 was performed starting with 100 μM as the highest concentration. From this, absolute IC_{50} values were calculated from linear extrapolations of reaction velocity as a function of the logarithmic of concentration. This non-biased approach did not allow for the determination of IC_{50} values larger than 100 μM. IC_{50} values obtained with different charges of DMSO solutions might vary by a factor 2. PTPN2 active phosphatase was obtained from Stratgene.com. The phosphatases CDC25A, PTP1b, MPTPA, MPTPB, and SHP2 were obtained from Prof. Schwalbe, Frankfurt. CIN was obtained from Prof. Gola, Düsseldorf. PP1, VHR, and CDC25A were obtained from Kirill Alexandrov, MPI Dortmund. VE-PTP was obtained from Prof. Vestweber, MPI Münster.

All reaction mixtures contained 1 mM DTE (1,4-dithio-D,L-threitol) added on the day of the experiment from 100 mM stock) and 0.0125% (v/v) of the detergent NP-40 (Calbiochem 492015). The buffers consisted of 50 mM Tris, 50 mM NaCl, 0.1 mM EDTA, pH 8.0 in the case of CDC25A, or 25 mM HEPES, 50 mM NaCl, 2.5 mM EDTA, pH7.2 in the case of PTP1b, PTPN2, SHP2, CIN, MPTPA, and MPTPB, 50 mM Tris, 50 mM NaCl, 0.1 mM EDTA, pH 8.0, 40 mM Tris, 30 mM $MgCl_2$, 20 mM KCl, pH 8.1, or 25 mM MOPS, 5 mM EDTA, pH 6.5 in the case of VHR. The twofold dilution series were obtained from 10 μL of a buffered enzyme solution containing 200 μM of inhibitor. Of this, 5 μL was removed and mixed with 5 μL buffered enzyme solution resulting in a 2-fold dilution. This step was repeated 9 times. Five microliters of the final dilution was removed, so that each well consisted of 5 μL buffered enzyme inhibitor mix. After addition of 5 μL of *p*-nitrophenyl phosphate to 50 mM in the case of CDC25A, to 5 mM in the case of CIN, PP1, and VHR, or to 1 mM for all other phosphatases, the reactions were observed at 405 nm in a spectrophotometer kept at 37 °C. The inhibitor concentrations were 100 μM for the initial screens and 100, 50, 25, 12.5, 6.25 μM, respectively, for the dilution series. For all enzymes, their concentration was adjusted to an initial absorbance change of 1−2 OD_{405}/h. All IC_{50} determinations were performed in quadriplicate.

4.2. Syntheses. Solid-Phase Synthesis of Piperidine 7a. A solution of azadiene **3a** (111 mg, 0.53 mmol) in dry toluene (5.0 mL) was added to a round-bottom flask containing Sasrin resin-supported maleimide **8** (200 mg, 0.102 mmol) swelled in dry toluene (2.0 mL). Next, benzaldehyde (0.105 mL, 1.02 mmol) was added and the reaction mixture was heated at 85 °C for 14 h under N_2. The reaction mixture was cooled to room temperature, and the resin was separated by filtration. The resin was washed with dry toluene (3 × 5 mL), THF (3 × 5 mL), and THF/MeOH (3 × 5 mL, 1:1 v:v). Next, the resin was vortexed with MeOH (5 mL) to cleave the borate intermediate. Finally, the resin was washed with DMF (3 × 5 mL), DCM (5 × 5 mL), and Et_2O (5 × 5 mL) and dried to a constant weight on high vacuum for 12 h. A solution of TFA in DCM (2%, 3 mL) was added, and the resin was vortexed for 1 h. The resin was separated by filtration and washed with a solution of TFA (2%, 3 × 3 mL). The filtrates were combined and concentrated to give the crude product **7a** (27 mg, 75% yield). ^{1}H NMR (300 MHz, acetone-*d*): δ 7.60−7.20 (m, 14H), 6.05−5.95 (m, 2H), 5.63−5.50 (m, 2H), 5.05 (d, J = 8.4 Hz, 1H), 4.33−4.17 (m, 11H), 4.02−3.85 (m, 3H), 2.98 (s, 10H). MS (ESI) Calcd for $C_{13}H_{24}N_3O_5$: 374.17. Found: 342.10 [(M + H) − H_2O].

Solid-Phase Synthesis of Piperidine 7b. The Diels−Alder/allylboration reaction was performed according to the procedure described for compound **7a**. Cleavage and workup conditions were according to the literature procedure.[9] Crude compound **7b** was obtained in a 50% yield. Crude ^{1}H NMR (300 MHz, methanol-*d*): δ 7.50−7.42 (m, 3H), 7.18−7.05 (m, 2H), 6.80−6.75 (m, 3H), 5.95−5.85 (m, 1H), 5.70−5.60 (m, 1H), 4.55 (d, J = 9.0 Hz, 1H), 3.74 (d, J = 8,7 Hz, 1H), 4.25−3.57 (m, 1H), 3.00 (s, 3H), 2.43 (s, 6H). MS (ESI) Calcd for $C_{17}H_{21}N_3O_3$: 331.15. Found: 332.10 ([M + H]).

Solution-Phase Synthesis of Piperidine 7c Using Supported Scavengers. Crude compound **7c** was prepared on a 0.1 mmol scale according to the previously described procedure.[3b] After the aqueous workup, crude compound **7c** was dissolved in THF (5 mL), and the aminothiol and boronic acid scavenger (0.4 mmol) were added. The reaction mixture was refluxed for 14 h under N_2. The reaction mixture was allowed to cool down to room temperature, and the resins were separated by filtration and washed with THF (5 × 5 mL). The filtrates were combined and evaporated to give the final product **7c** in a 59% yield. ^{1}H NMR (300 Hz, $CDCl_3$): δ 7.28−7.20 (m, 3H), 6.90−6.82 (m, 2H), 5.92 (ddd, J = 1.5, 3.9, 11.1 Hz, 1H), 5.67 (ddd, J = 1.8, 4.2, 10.2 Hz, 1H), 4.41 (d, J = 9.3 Hz, 1H), 4.25 (s, 1H), 3.80 (s, 3H), 3.70 (d, J = 9.9 Hz, 1H), 3.48−3.40 (m, 2H), 3.05 (s, 3H), 2.47 (s, 6H). ^{13}C NMR (100.58 MHz, $CDCl_3$): δ 176.8, 174.9, 159.4, 132.2, 130.3, 128.2, 121.1, 113.8, 75.1, 61.4, 57.3, 55.4, 43.8, 38.8, 25.4. MS (ESI) Calcd for $C_{18}H_{23}N_3O_4$ ([M + H]): 346.17. Found: 346.10.

General Procedure for Library Synthesis of Piperidines 7 Using the Three-Component Reaction. A solution of an aza-diene (0.1 mmol, 1.0 equiv) in dry toluene (0.5 mL) was transferred into a 4 mL vial of a 48-vial cassette of Biotage Trident External Thermal-Agitation Unit, followed by a solution of a maleimide (0.2 mmol, 2.0 equiv) in dry toluene (0.5 mL) and an aldehyde (0.15 mmol, 1.5 equiv). The vial was capped, and the cassette was agitated for 72 h at 85 °C. The cassette was cooled to room temperature, and the 48 vials were opened. A saturated aqueous solution of $NaHCO_3$

(0.1 mL) was added to each vial. The vials were capped again, and the cassette was agitated for 0.5 h at 50 °C. The cassette was allowed to cool to room temperature; then it was taken out of the reactor, and the vials were opened. The organic layer of each of the reactions was passed through a homemade cartridge (a cotton plug was put into 3 mL syringe, followed by sand, silica gel, and anhydrous $MgSO_4$) into a 4 mL vial. The aqueous layer was extracted with AcOEt (2 × 1 mL) and was passed through the cartridge into the same vial. The vials were placed into a Genevac rack, and solvents were removed under vacuum. Next, the crude compounds were analyzed by HPLC using the analytical method. The analysis and purification methods are described below.

General Procedure for Library Synthesis of Piperidines 7 Using the Four-Component Reaction. A solution of a boronoacrolein (0.1 mmol, 1.0 equiv) in dry toluene (0.25 mL) was transferred into a 4 mL vial of 48-vial cassette of Biotage Trident external thermal agitation unit, followed by a solution of a hydrazine (0.11 mmol, 1.1 equiv) in dry toluene (0.25 mL), maleimide (0.25 mmol, 2.5 equiv) in dry toluene (0.5 mL), and an aldehyde (0.15 mmol, 1.5 equiv). The vial was capped, and the cassette was agitated for 72 h at 85 °C. The cassette was allowed to cool down to room temperature, and the 48 vials were opened. A saturated aqueous solution of $NaHCO_3$ (0.1 mL) was added to each vial. The rest of the workup was the same as described for the three-component reaction.

HPLC Method for Analysis and Semipreparative Purification. After the reaction workup, solvents were removed using a Genevac rapid evaporator. The crude library members were dissolved in HPLC grade CH_3CN (200 μL per 30 mg of final product); the solutions were centrifuged to precipitate particular solids, and the supernatants were transferred into HPLC conical vial inserts (300 μL). Next, the compounds were injected into the LCMS instrument equipped with an analytical Zorbax C18 column (4.6 × 150 mm, 5 μm). The solvent system was CH_3CN/H_2O (45% B at 0 min, 70% B in 30 min) containing HCO_2H (both, 0.05%); the flow rate was 1 mL min^{-1}, and the column temperature was 40 °C. The compounds were monitored by UV at 254 nm and by ESI-MS (scan range = 100 to 1000 *m*/*z*). When the reaction was successful the product was separated from impurities by semipreparative LCMS using mass-based fraction collection. The system was equipped with a semipreparative Zorbax RxC8 column (9.4 × 250 mm, 5 μm) with a guard column. The solvent system was the same as in the analytical method, with a flow rate of 3 mL min^{-1}, and the column temperature was 40 °C. The percentage of the compounds obtained with a purity of higher than 80% was estimated to be 84%, on the basis of the analysis of 51 randomly picked library members (see Supporting Information).

Synthesis of Piperidines 7d−7i (Table 1). These compounds were prepared as described above for the library synthesis, however, at a scale of 1.0 mmol (5.0 mL of dry toluene) in a high-pressure vessel. The workup was performed without a cartridge (with anhydrous $MgSO_4$ added to the ethyl acetate extracts, then filtered, and concentrated). Purification was effected by flash-chromatography on silica gel.

7d. ^{1}H NMR (300 Hz, $CDCl_3$): δ 6.11 (ddd, J = 2.1, 4.5, 10.5 Hz, 1H), 5.97 (ddd, J = 1.5, 3.9, 10.8 Hz, 1H), 4.34 (d, J = 8.7 Hz, 1H), 3.71 (s, 1H), 3.46−3.38 (m, 1H), 3.26−3.18 (m, 1H), 3.0 (s, 3H), 2.80−2.72 (m, 1H), 2.45 (s, 6H), 1.64−1.40 (m, 4H), 1.37−1.20 (m, 20H), 0.92−0.84 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 177.3, 175.1, 130.7, 121.3, 72.3, 59.3, 57.3, 43.7, 38.5, 33.3, 31.9, 29.9, 29.7, 29.6, 29.4, 25.7, 25.2, 22.7, 14.2. MS (ESI) Calcd for $C_{21}H_{37}N_3O_3$ ([M + H]): 380.28. Found 380.15.

7e. Compound **7e** was previously characterized.[3b]

7f. Compound **7f** was not obtained.

7g. ^{1}H NMR (300 Hz, $CDCl_3$): δ 7.3−7.2 (m, 6H), 7.03−6.98 (m, 2H), 6.90−6.84 (m, 1H), 6.25 (dd, J = 4.2, 10.2 Hz, 1H), 6.02 (ddd, J = 1.8, 4.2, 10.2 Hz, 1H), 4.36 (d, J = 8.4 Hz, 1H), 3.57 (bs, 1H), 3.37 (dd, J = 3.6, 8.99 Hz, 1H), 3.34−3.25 (m, 1H), 3.04 (s, 3H), 1.7−1.38 (m, 6H), 1.37−1.13 (m, 37H), 0.92−0.87 (m, 7H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.6, 146.4, 129.5, 120.8, 114.8, 114.1, 66.5, 37.4, 33.2, 31.9, 29.7, 29.6, 29.5, 29.2, 22.7, 14.1. MS (ESI) Calcd for $C_{25}H_{37}N_3O_3$ ([M + H]): 428.58. Found: 428.15

7h. ^{1}H NMR (300 Hz, $CDCl_3$): δ 7.21−7.33 (m, 9H), 7.03−7.09 (m, 2H), 6.88−6.94 (m, 1H), 6.12 (dd, J = 3.3, 10.5 Hz, 1H), 5.6 (bs, 1H), 5.56 (ddd, J = 2.1, 4.5, 10.5 Hz, 1H), 4.45 (d, J = 8.0 Hz, 1H), 4.07 (d, J = 9.9 Hz, 1H), 3.98 (bs, 1H), 3.7−3.6 (m, 1H), 3.59 (bs, 1H), 3.08 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.6, 146.3, 139.4, 137.7, 129.6, 128.5, 128.4, 127.1, 124.9, 121.0, 114.1, 113, 8, 67.5, 61.5, 37.6, 25.3. MS (ESI) Calcd for $C_{21}H_{21}N_3O_3$ ([M + H]): 364.16. Found: 364.41

7i. ^{1}H NMR (300 Hz, $CDCl_3$): δ 8.56−8.52 (m, 1H), 7.68−7.61 (m, 1H), 7.37−7.33 (m, 1H), 7.24−7.15 (m, 3H), 6.86−6.78 (m, 1H), 6.14 (ddd, J = 1.8, 3.9, 10.5 Hz, 1H), 5.87 (ddd, J = 2.4, 4.2, 10.5 Hz, 1H), 4.47 (d, J = 6.9 Hz, 1H), 4.34 (d, J = 8.4 Hz, 1H), 3.98−3.91 (m, 1H), 3.63−3.55 (m, 1H), 3.45 (s, 3H). ^{13}C NMR (100.58 MHz, $CDCl_3$): δ 174.7, 159.1, 148.4, 146.4, 136.8, 129.4, 128.6, 122.9, 121.6, 120.4, 113.5, 75.3, 65.2, 60.7, 39.0, 25.0. MS (ESI) Calcd for $C_{20}H_{20}N_4O_3$ ([M + H]): 365.15. Found: 365.00.

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Supporting Information Available. Reproductions of ^{1}H and ^{13}C NMR spectra for compounds **7a−7i**, full list of library members, reproductions of HPLC-UV-ESMS chromatograms, and ^{1}H NMR spectra for 51 randomly selected library members. This material is available free of charge via the Internet at http://pubs.acs.org.

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