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LETTER

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Efficient Three-Step One-Pot Synthesis of a Novel 2,3,5-Substituted Pyrazine Library

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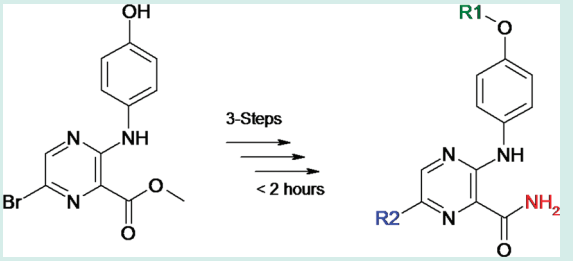
Supporting Information

ABSTRACT: The partnership between rational synthesis design and mass-triggered preparative LCMS is a powerful one, capable of furnishing very large libraries in a selective manner in a very short space of time. Herein, we communicate one example of possibly a perfect marriage between the synthetic chemistry and the subsequent purification method employed, affording a ~1000-member library supplying 50 mg on average of final compound in less than a month.

KEYWORDS: mass-triggered preparative LCMS, three-step one-pot pyrazine library, Mitsunobu, Suzuki−Miyaura



Traditional cancer chemotherapy uses cytotoxic agents targetting inhibition of DNA synthesis and function or interruption of the cell-cycle.[1] Recently, the pharmaceutical industry has been focusing on new treatments for cancer concentrating on inhibition of cell-signaling pathways, notably on kinase inhibition.[2] From this new strategy to combat this terrible disease at epidemic proportions,[3] we have already seen emerge successes on the market in the field of epidermal-growth factor receptor (EGFR) inhibition, such as gefinitib[4] and erlontib,[5] both compounds based on mimicking ATP with 4-(anilino)quinazoline scaffolds.[5] Since 2000, the industry has heavily exploited this particular structural template with many compounds currently in development;[6] therefore, the quest for new scaffolds for kinase inhibitors has become very important.[7] Alongside their core therapeutic programs, AstraZeneca initiates a certain number of nontargetted library design and synthesis projects searching for novel scaffolds, which are tested in high throughput assays in a hit-to-lead style initiative.[8] We designed our speculative library around a pyrazine-carboxamide hub offering a hinge binding capacity while leaving two vectors to explore: the selectivity pocket using Suzuki reaction[9] and the solvent region using Mitsunobu chemistry (Figure 1).[10]

The synthesis of the scaffolds started from commercially available 3-aminopyrazine-2-carboxylic acid methyl ester (**1**). Bromination followed by diazotisation/bromination (Sandemyer) of the amino group afforded only 32% yield of dibromide **2** but with no chromatography. The intermediate phenol **3** was prepared in excellent yield by S_NAr using a slight excess of 4-aminophenol, under pseudomelt conditions at 100 °C.

To meet our library goals with a 2 diversity points to explore, we wanted an efficient synthesis preferably with a single purification step at the end. We initially set our sights on a solid-phase approach attaching the scaffold to polystyrene supported Rink amide linker.[11] Saponification of **3** afforded the corresponding

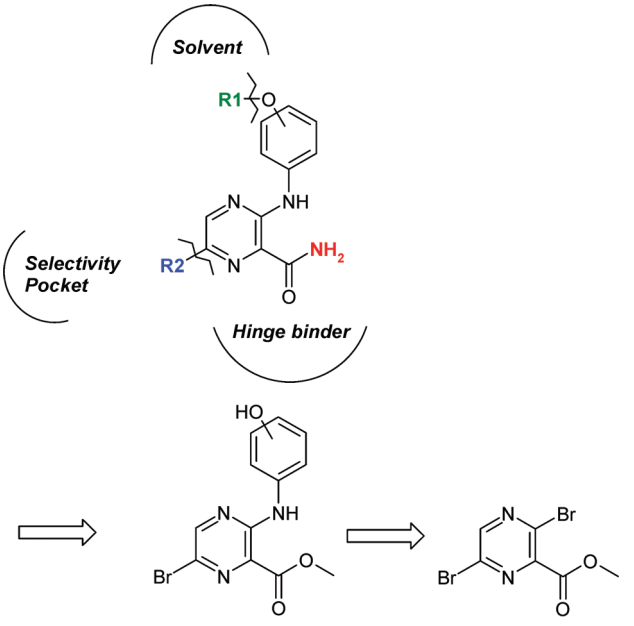


Figure 1. Proposed retrosynthetic analysis of the library scaffold.

acid which, after significant development work, was successfully bound to the Rink resin using mixed anhydride methodology (*i*-BuOCOCl, NMM, THF, 0 °C to r.t.). The subsequent Mitsunobu alkylation required a 3-fold excess of reagents to ensure a complete reaction but the following Suzuki−Miyaura

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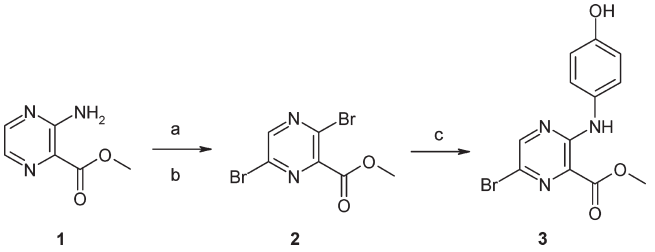
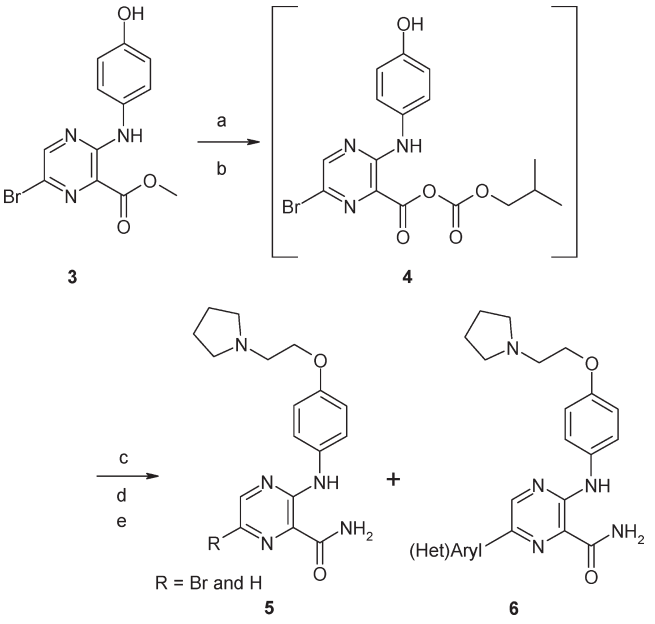
Scheme 1[a]



[a] Reagents and conditions: (a) Br_2, NaOAc, AcOH, rt, overnight, 74%; (b) $NaNO_2$, 48% HBr (aq), Br_2, AcOH, H_2O, 0−5 °C, 50 min, 43%; (c) 4-aminophenol, minimum MeOH, 100 °C, 1 h, 97%.

Scheme 2[a]

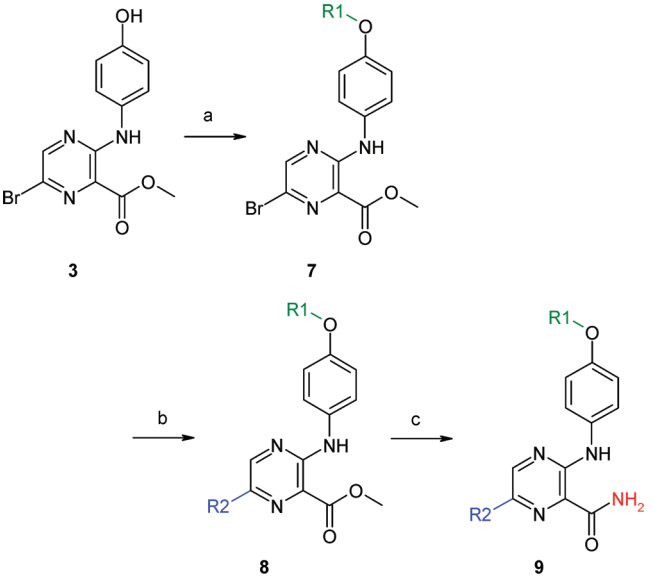


[a] Reagents and conditions: (a) LiOH, MeOH, 50 °C, 68%; (b) iBuOCOCl, NMM, THF, PS-Rink Amide, quant.; (c) 1-(2-hydroxyethyl)pyrrolidine, TPP, DTAD, DCM 0 °C to rt, quant.; (d) (Het)ArylB$(OH)_2$, Pd(dppf)Cl_2.CH_2Cl_2, 1,4-dioxane, THF−MeOH, CsF, 80 °C; (e) TFA, TES, DCM, 50%.

cross-coupling reaction could only be progressed to around 50% completion. Despite numerous attempts to improve on this conversion by changing solvent, catalytic system, we had difficulty pushing the Suzuki−Miyaura to completion on the resin so we abandonned the solid-phase approach (Scheme 2). Moreover, with the basic chain already successfully installed, the small difference in retention time between the 5-Br, the 5-H impurity and the desired compound posed certain purification issues using our standard preparative LCMS conditions.

Preferring something other than a solid-phase approach, we turned our attention to the possibility of using a straightfoward 3-step one-pot solution phase approach, cascading the 3 reaction steps together in one-pot starting with the most sensitive step and finishing with the least sensitive step. In this particular case, we postulated that the byproduct generated by the Mitsunobu reaction should be tolerated by the subsequent Suzuki−Miyaura

Scheme 3[a]



[a] Reagents and conditions: (a) 3 PS-TPP (3 mmol/g), 3 R1-OH, 3 DTAD, DCM, rt, 30 minutes, quantitative; (b) R2-B$(OH)_2$, Pd(dppf)Cl_2.CH_2Cl_2, MeOH, CsF, 120°C, microwave, 20 minutes; (c) NH_3 in MeOH (7N), 120°C, microwave, 30 minutes.

reaction leaving an ammonolysis "drown-out" of the methyl ester to afford the final primary carboxamides thus allowing for a simple one-pot process with concentrations between steps (Scheme 3).

While the alkylation of **3** can be adequately achieved using a 3-fold excess of Mitsunobu reagents using free triphenylphosphine, we used polymer-supported triphenylphosphine in order to reduce the mass of crude product to purify at the end of the sequence and avoid contamination of final compounds with triphenylphosphine oxide.[12] The presence of large quantities of triphenylphosphine oxide (Ph_3PO) also led to difficulty in collecting the final compounds due to the strong mass response (ES+) of Ph_3PO which tended to drag over a wide area of the spectrum. These problems were eliminated using polymer-supported triphenylphosphine. Therefore, after the alkylation step was complete, the resulting suspension was filtered to remove the polymer and the filtrate concentrated, dissolved in MeOH and the resulting solution of **7** was exposed to standard Suzuki reaction conditions under microwave conditions. The subsequent solutions of **8** were concentrated and treated with methanolic ammonia under microwave conditions to afford the final compounds **9** (Scheme 3).

An important aspect of this approach was the quality of the crude reaction mixtures after 3 quick operations with excess reagents. A typical crude LCMS analysis is shown in the Supporting Information. One can quickly deduce from the spectra, that after each stage, the crude profile remains almost 100% pure by u.v. allowing for easy final stage purification. In addition, this class of compounds had a strong mass response allowing for collection by mass-triggered LCMS.

Library Synthesis. Encouraged by the excellent LCMS crude profile after each operation during the validation work, we embarked on a simple type of 10-tube multiparallel experiment.[13] The reaction mixture obtained after the Mitsunobu alkylation was evaporated to dryness taken up in methanol and the solution was divided into 10 separate microwave pressure tubes for the

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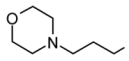
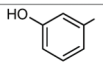
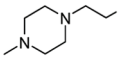
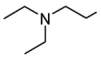
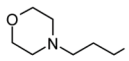
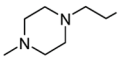
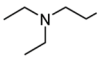
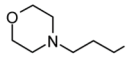
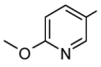
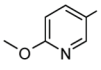
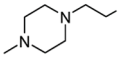
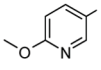
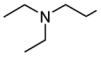
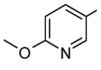
Table 1. Selected Results from This "One-Pot" 3-Step Pyrazine Library Preparation

Compound	R1	R2	% Yield	% Purity at 254 nm – (^{1}H-NMR)
9a			45	100 (100)
9b			55	97 (98)
9c			43	98 (97)
9d			66	98 (96)
9e			53	100 (100)
9f			66	100 (100)
9g			80	100 (100)
9h			52	100 (100)
9i			47	100 (99)
9j			54	100 (99)
9k			67	92 (99)
9l			56	100 (99)

subsequent Suzuki−Miyaura cross-coupling. After 30 min in the microwave, the reaction mixtures were concentrated to dryness in their pressure tubes, recapped and dissolved in a fresh solution of methanolic ammonia (7 N) and reacted under microwave irradiation to effect the transesterification to the primary carboxamide. The resulting crude reaction mixtures were concentrated to dryness and dissolved in DMF, filtered and purified directly without any workup prior to injection. The DMF solutions were purified using a Waters X-Terra reverse-phase column (C-18, 5 μm silica, 19 mm diameter, 100 mm length, flow rate of 40 mL/minute) and decreasingly polar mixtures of water (containing 1% acetic acid) and acetonitrile as eluent. The fractions containing the desired compound were evaporated to dryness to afford the final compounds (**9**), generally as solids with average purities of >95% as judged qualitatively by U.V. (254 nm) and ^{1}H NMR. Yield and purity data for selected compounds can be seen in Table 1.

To summarize, we have developed an efficient 3-step one-pot reaction by rational reaction design, relying on cascading reactions types of increasing tolerance with simple concentrations between steps. In this particular case, we started the sequence with the most sensitive reaction (Mitsunobu alkylation) and finished with the least sensitive reaction (ammonolysis "drown out") and proved that, with a well designed route and a rapid preparative mass-triggered LCMS purification method, one can access large libraries in a matter of weeks on pharmacologically interesting and diverse skeletons. We also hope that this paper does illustrate nicely that, chemistry permitting of course, one can achieve acceptable to excellent final purity profiles and yields without the constraints of developing a solid-phase supported synthesis.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Experimental procedures, ^{1}H NMR and LCMS data of a selection of compounds highlighted in the manuscript. This material is available free of charge via the Internet at http://pubs.acs.org.

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