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Solid-phase synthesis of quinoxaline, thiazine, and oxazine analogs through a benzyne intermediate

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Abstract—A solid-phase synthetic route to quinoxaline, thiazine, and oxazine analogs is described. *N*-Alloc-3-amino-3-(2,4-difluoro-5-nitrophenyl)propanoic acid was tethered to Rink resin via its carboxylic acid group. The 4-arylfluorine was displaced with a primary amine, alcohol, or thiol to create, respectively, a resin bound aniline, phenol, or thiophenol derivative with one diversity element and one single atom (e.g., *N*, *S*, or *O*) diversity point. A fused heterocyclic system was subsequently created via a benzyne heterocyclization initiated by dehydrofluorination with strong base. Acid treatment released the desired products in high yield and moderate purity.

During the last decade, solid-phase synthetic techniques that allow for the generation of large numbers of organic compounds have tremendously aided in drug discovery.[1] Although several chemistries have proven useful for the solid-phase synthesis of bioactive compounds,[2] the number of chemical techniques available to researchers is limited due to two stringent requirements of solid-phase synthetic work: (1) each reaction must deliver the desired product in high yield and relatively high purity; and (2) chemical reagents used in the synthesis must accommodate the sensitive nature of the solid support. One solution to these problems has been to prepare the framework of the desired target in solution phase where harsh chemical conditions are more easily accommodated and to then move this 'diversity-ready' framework to solid phase for completion of the synthesis. While solution phase techniques are available in abundance, this strategy tends to require long development times. For this reason, we chose to focus on the adaptation of chemical conditions used for heterocyclic drug development based on scaffolding strategies, since this approach enables the rapid generation of numerous testing ligands.[3]

Quinoxalines are an important heterocyclic component of various biologically interesting compounds with several pharmaceutical applications. For example, quinoxalines have been used in colon cancer therapies,[4] they interact with α-aminobutyric acid A ($GABA_A$) and benzodiazepine receptors,[5] they inhibit aldose reductases,[6] and they are potent angiotensin II receptor antagonists.[7] Although both solution [5b] and solid[8] phase syntheses of 1,2,3,4-tetrahydroquinoxalines have been described, we report here the preparation of a quinoxaline containing heterocyclic that allows for the introduction of diversity elements in spatially segregated areas and, importantly, accommodates a variable heteroatom that allows for diversification to other heterocyclic analogs. Specifically, we report manipulation of the highly functionalized β-amino acid *N*-alloc-3-amino-3-(2,4-difluoro-5-nitrophenyl)propanoic acid in the creation of quinoxaline-, thiazine-, and oxazine-based libraries.[9]

We began by attempting to attach *N*-alloc-3-amino-3-(2,4-difluoro-5-nitrophenyl)propanoic acid to Rink resin through its free carboxylic acid. However, this proved problematic due to the sensitivity of the aryl-fluorine moiety; we discovered that the scaffold had conjugated to the resin via three distinct modes as illustrated in *Route 1* of Scheme 1. To resolve this problem, the synthetic route was adjusted to include pre-activation of the carboxylic acid with HOBt and DIC. Indeed,

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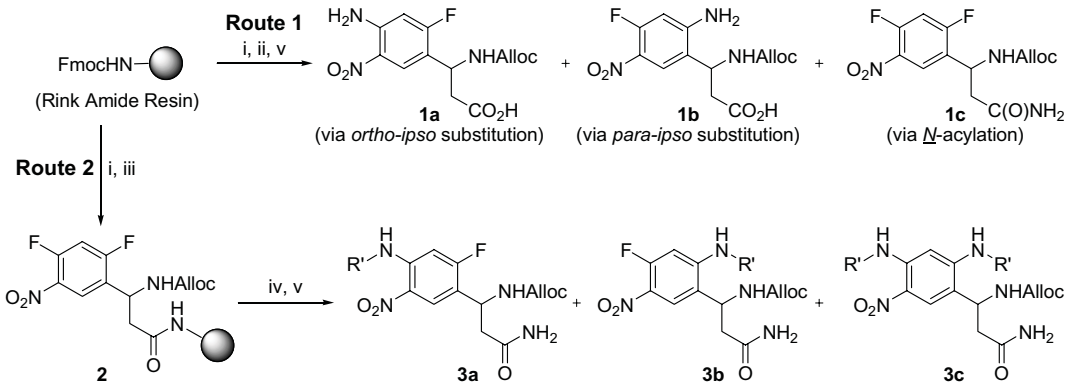


Scheme 1. A solid-phase synthesis approach to 3-amino-3-(8-nitro-1,2,3,4-tetrahydroquinoxalin-6-yl)propanoic acid. Reagents and conditions: (i) $2 \times 25\%$ piperidine, 15 min; (ii) *N*-alloc-3-amino-3-(2,4-difluoro-5-nitrophenyl)propanoic acid (3 equiv), HOBt (3.5 equiv), DIC (3.5 equiv), DMF, 2 h; (iii) a solution of *N*-alloc-3-amino-3-(2,4-difluoro-5-nitrophenyl)propanoic acid (3 equiv), HOBt (3.5 equiv), DIC (3.5 equiv), and DMF was premixed for 15 min and then added to the resin, which was then stirred for, 2 h; (iv) ethylenediamine (10 equiv), DIEA (10 equiv), DMF, 14 h; (v) 95% TFA/H_2O, 2 h.

premixing this solution and then adding it to the resin resulted in selective attachment of the scaffold to the resin through carboxylic acid.

Once accomplished, we then turned to the substitution of 4-fluoro functional group with a primary amine.

It was our belief that substitution of 4-fluoro group would occur selectively in the presence of 2-fluoro group. However, we were dismayed to find that amine treatment followed by cleavage and spectroscopic evaluation of the product established that *ipso* substitution was occurring at both fluorinated positions (e.g., Scheme 1, *Route 2*). We reasoned that our use of excess amine was resulting in apparent non-selective reaction. Indeed, upon careful evaluation of the reaction, it was determined that the 2-fluoro position was more reactive and, in the presence of stoichiometric amounts of the amine, could be selectively substituted. By adjusting the amount of amine to 1.05 equiv, we were able to successfully obtain the 2-amino product.

We next turned to an investigation of intramolecular substitution at the 4-fluoro position. During several experiments targeting the synthesis of diazapines (illustrated in Fig. 1), we noted that weakly basic systems (i.e., DIEA in DMF) were not effective in driving this second substitution reaction to completion. After attempting several bases, we arrived at the strong base lithium *tert*-butoxide, which appeared to mediate the desired substitution.

The strong base requirement suggested that 4-fluoro substitution may be proceeding through a benzyne intermediate as illustrated in Figure 2. To test this hypothesis, we chose ethylenediamine for 2-fluoro substitution. Since ethylenediamine is too short to accommodate *para* substitution, the intramolecular addition to the benzyne

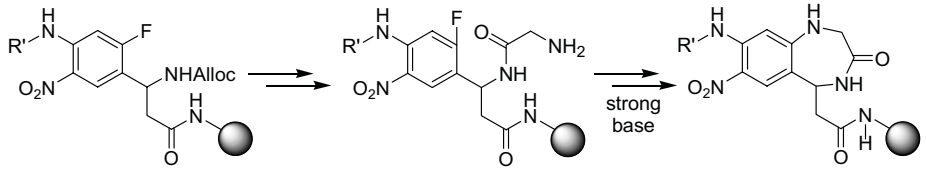


Figure 1. Planned route to benzo[*e*][1,4]diazepin-3-ones.

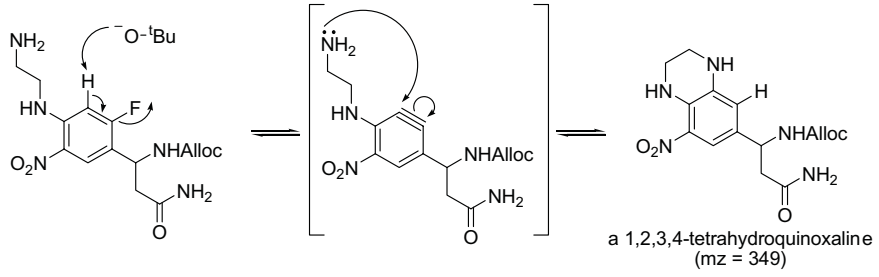
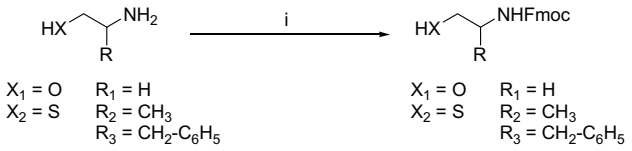


Figure 2. Benzyne as intermediate en route to 1,2,3,4-tetrahydroquinoxalines.



Scheme 2. Synthetic route to the creation of *N*-Fmoc derivatives used for further synthesis: (i) Fmoc-Osu, triethylamine, dioxane, N_2, 0 °C → rt.

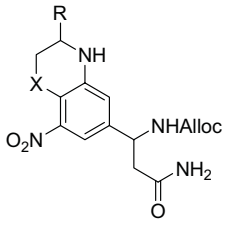
intermediate would lead to *meta* substitution. With these ideas in mind, we performed 4-fluoro substitution in the presence of lithium *tert*-butoxide and successfully obtained *N*-alloc-3-amino-3-(8-nitro-1,2,3,4-tetrahydroquinoxalin-6-yl)propanamide. Indeed, as outlined in Figure 2, mass spectral data for the reaction product established that the now intramolecular substitution reaction was leading to a 1,2,3,4-tetrahydroquinoxaline product—an observation, which establishes that the reaction is proceeding via a benzyne intermediate.

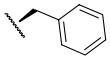
With these results in hand, we prepared a series of Fmoc-protected amino alcohols and thiols according to Scheme 2.[10] Once isolated, these compounds were taken through the reactions presented in Scheme 3. Several compounds were isolated in varying yields and purity (Table 1).

All isolated products were confirmed with spectroscopic data. The ESI-MS of each compound was determined. The presence of the nitro group on the benzene ring was confirmed by FTIR showing characteristic peaks between 1540–1600 and 1290–1575 cm^{-1}. The ^1H NMR displayed all characteristic chemical shifts and splitting patterns as well as the disappearance of all amine and amide peaks upon addition of D_2O. Finally, ^{13}C NMR showed not only the characteristic chemical shifts, but no splitting of the carbon signals were observed as would be expected had the fluorine not been displaced.

In conclusion, we report the preparation of quinoxaline, thiazine, and oxazine analogs on solid phase via a protocol that proceeds via a benzyne intermediate. The use of only two (see † in **2**, Scheme 3) of the five potential (see † and * in **2**, Scheme 3) diversification points on the scaffolding molecule lends well to future work involving library synthesis of quinoxaline, thiazine, and oxazine based libraries. Further, the establishment of a benzyne intermediate on solid phase opens unique opportunities for exploiting this reactive intermediate.

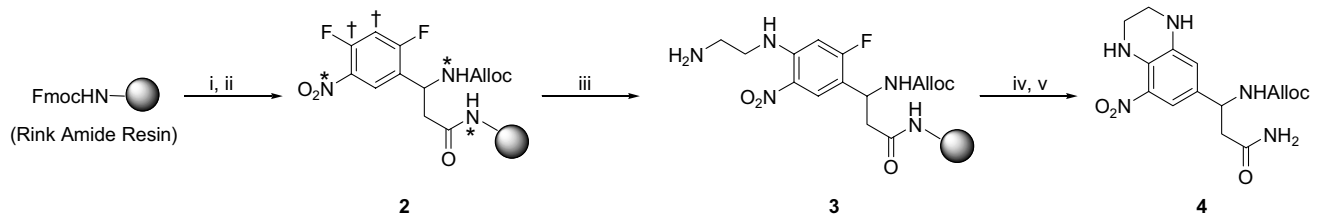
Table 1. Synthesis of quinoxaline, thiazine, and oxazine derivatives as outlined in Scheme 3



Compound	X	R	Crude yield[a] (%)	Purity[b] (%)	ES-MS[c] (M^+) Found (calcd)[c]
4	N	H	68	44	266.3 (266.1)
5a	O	H	91	74	267.6 (267.1)
5b	O		88	68	283.4 (283.1)
5c	O		82	72	281.6 (281.1)
6	S	H	88	77	357.6 (357.1)

[a] Yield of the crude product was based on Rink resin loading.
[b] Purity was obtained by measuring the crude samples using RP-HPLC at $\lambda = 254$ nm.
[c] Molecular weight was measured by ES-MS.

Typical procedure: Synthesis of N-Fmoc-2-aminoethanethiol. *N*-Fmoc-2-aminoethanethiol was prepared according to the literature procedures[10] with the following modifications. 9-Fluorenylmethyl succinimidyl carbonate (Fmoc-Osu) (3.0 g, 8.9 mmol) was dissolved in dioxane (125 mL) and the resulting solution was cooled to 4 °C with stirring under nitrogen as 2-aminoethanethiol (1.2 g, 10.7 mmol) was added via syringe (20 min). Triethylamine (8.7 g, 85.5 mmol, 11.9 mL) was added and this final solution was stirred for 12 h during which time the ice bath expired. The mixture was washed with 1 N HCl and the organic layer was collected and dried over $MgSO_4$. The solvent was removed under reduced pressure, and the resulting solid was recrystallized from hexanes to yield the desired compound (10.9 g, 94% yield). FT-IR (selected peaks, cm^{-1}): 3320, 2560, 1735, 1540, 1349. ES-MS (M^+): 299.7. ^1H NMR (400 MHz, DMSO-d_6, w/TMS as internal standard) δ 7.27–7.86 (m, 8 H), 5.47 (s, 1H, erasable with D_2O), 4.52 (d, 2H), 4.24 (t, 1H), 3.39 (m, 2H), 2.76 (m, 2H),



Scheme 3. Solid-phase synthesis approach to 3-amino-3-(8-nitro-1,2,3,4-tetrahydroquinoxalin-6-yl)propanamides. Reagents and conditions: (i) 2 × 25% piperidine, 15 min; (ii) a solution of *N*-alloc-3-amino-3-(2,4-difluoro-5-nitrophenyl)propanoic acid (3 equiv), HOBt (3.5 equiv), DIC (3.5 equiv), and DMF was premixed for 15 min and then added to the resin, which was then stirred for, 2 h; (iii) ethylenediamine (1.05 equiv), DIEA (1.1 equiv), DMF, 30 h; (iv) LiO^{t}Bu (1 M in THF) (2 equiv), DMF, 24 h; (v) 95% TFA/H_2O, 2 h.

1.47 (s, 1H, exchangeable with D_2O). ^{13}C NMR (400 MHz, DMSO-d_6, w/TMS as internal standard) δ 159.4, 145.2, 140.7, 128.4, 127.9, 127.4, 125.3, 69.0, 57.3, 45.6, 29.1.

Typical procedure: Synthesis of N-alloc-3-amino-3-(8-nitro-1,2,3,4-tetrahydroquinoxalin-6-yl)propanamide (**4**). Rink amide–MBHA resin (0.25 g, 0.5 mmol/g) was swollen in DMF (8 mL) for 2 h at which time Fmoc-deprotection was carried out twice with 25% piperidine in DMF (15 min). The resin was washed with an appropriate volume of DMF (2×), DCM (3×), DMF (3×), and combined with a premixed solution of *N*-alloc-3-amino-3-(2,4-difluoro-5-nitrophenyl)propanoic acid (3 equiv), HOBt (3.5 equiv), and DIC (3.5 equiv) in DMF. The beads were rotated in this solution for 2 h at which time a Kaiser test showed the absence of a primary amine. The resin beads were then incubated with ethylenediamine (1.0 equiv, 7.51 mg, 8.35 μL) and DIEA (1.1 equiv) in DMF for 36 h. The presence of a primary amine was detected by the Kaiser test and the beads were washed thoroughly. The resulting beads were then incubated with lithium *tert*-butoxide (1 M in THF, 2 equiv) in a small amount of DMF for 24 h at which time a primary amine was no longer detected by the Kaiser test. After thorough washing with DMF (2×), MeOH (5×), H_2O (5×), and DCM (10×), the resin was subject to cleavage conditions (95% TFA/H_2O) for 2 h. The cleavage solution was collected by filtration and dried by evaporation under a steady stream of nitrogen. Ether was added to precipitate the crude product (yield: 68%; purity: 44%), which was then purified by HPLC to a purity of >99%. Lyophilization delivered the desired product as a yellow powder. ES-MS (M+): 266.3. FT-IR (selected signals, cm^{-1}): 3376, 1845, 1742, 1735, 1543, 1350. ^1H NMR (400 MHz, DMSO-d_6, w/TMS as internal standard) δ 8.41 (s, 1H, exchangeable with D_2O), 7.11 (s, 1H), 6.80 (s, 2H, erasable by D_2O), 6.52 (s, 1H), 5.60 (m, 1H), 5.25 (m, 2H), 5.03 (m, 1H), 4.68 (m, 2H), 3.86 (s, 1H, exchangeable with D_2O), 3.82 (s, 1H, exchangeable with D_2O) 3.31 (t, 2H), 3.29 (t, 2H), 2.75 (m, 2H). ^{13}C NMR (400 MHz, DMSO-d_6, w/TMS as internal standard) δ 172.3, 158.4, 135.1, 133.8, 133.6, 132.2, 120.0, 118.2, 115.7, 111.9, 65.4, 51.2, 44.5, 40.6, 39.4.

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