Synthetic Methods

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[2+2+2] Cycloadditions of Siloxy Alkynes with 1,2-Diazines: From Reaction Discovery to Identification of an Antiglycolytic Chemotype**

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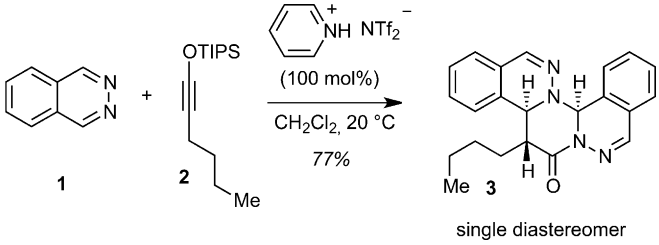
The synthesis of new nitrogen-containing heterocycles plays a pivotal role in chemical biology and medicinal chemistry, as reflected by their many applications in the development of pharmacological probes and drugs.[1] Despite notable progress, there is a significant need for the identification of new nitrogen heterocycles which target previously unexplored regions of biogenic chemical space. Among the many possible synthetic strategies to such compounds, cycloadditions involving C–N multiple bonds are particularly attractive as they generate complex cyclic products by simultaneous formation of multiple bonds starting from readily available precursors.[2] Herein, we describe the discovery and development of a formal [2+2+2] cycloaddition of siloxy alkynes with phthalazines, a process that had not been previously described for either 1,2-diazines or electron-rich alkynes.[3–7] This effort has not only afforded heterocyclic products with a unique pentacyclic ring system but has also enabled the identification of a novel chemotype that inhibits glycolytic ATP production by direct blockage of glucose uptake in CHO-K1 cells. As a result of the prevalence of the Warburg effect in many human cancers, such compounds may prove useful in the development of new therapeutics which target reprogrammed energy metabolism of rapidly proliferating cells.[8]

Our study began by examining the reaction of phthalazine (**1**) with the siloxy alkyne **2** in the presence of common Brønsted acids. While no reaction between **1** and **2** was observed in the absence of such additives, even at elevated temperatures, we found that addition of simple pyridinium salts promoted the formation of a new pentacyclic product (**3**; Scheme 1).

After examining a range of mono- and bis(pyridinium) salts in various solvents, we determined the optimum protocol to entail the use of a stoichiometric amount of pyridinium trifluoromethanesulfonimide in CH_2Cl_2 at room temperature, thus producing the lactam **3** as a single diastereomer in 77% yield. While most of the known [2+2+2] cycloadditions typically require the presence of a transition-metal catalyst,[9] the present method promotes the condensation under remarkably mild reaction conditions, using only a simple, weak Brønsted acid. The excellent diastereoselectivity of this transformation is also highly noteworthy. The atom connectivity within the reaction product was initially determined to be that in **3** and is based on extensive use of NMR spectroscopic methods. Ultimately, the structure was secured and the relative stereochemistry of the three newly created stereogenic centers was defined through X-ray crystallographic analysis (see below).



Scheme 1. [2+2+2] cycloaddition of phthalazine (**1**) with the 1-siloxy-1-hexyne **2**. Tf = trifluoromethanesulfonyl, TIPS = triisopropylsilyl.

Interestingly, while a range of substituted mono- and bis(pyridinium) trifluoromethanesulfonimides were found to be effective as reaction promoters, the use of only $HNTf_2$, in the absence of pyridine, produced **3** with lower efficiency (48% yield) and diminished diastereoselectivity (83:17). Furthermore, the use of either pyridinium chloride, pyridinium *p*-toluenesulfonate, or pyridinium triflate substantially decreased product yields or prevented the reaction. These results highlight the importance of the weakly nucleophilic trifluoromethanesulfonimide counterion,[10] an observation consistent with those made in the course of previous studies of Brønsted acid promoted transformations of siloxy alkynes.[3]

Having established a general reaction protocol, we began a detailed investigation of the scope of this [2+2+2] cycloaddition. With regard to siloxy alkyne substitution, we found that both alkyl and aryl substituents are well tolerated, thus providing the expected products in good yields with high levels of diastereoselection (Table 1, entries 1–4). When the steric bulk of the substituent in direct proximity to the alkyne is increased, the yield of the reaction is lowered slightly but the diastereoselectivty remains relatively unaffected. For instance, the siloxy alkynes **6** and **8** afforded the expected products **7** (73%) and **9** (69%), respectively (Table 1, entries 2 and 3). Furthermore, the use of 1-siloxy-propyne

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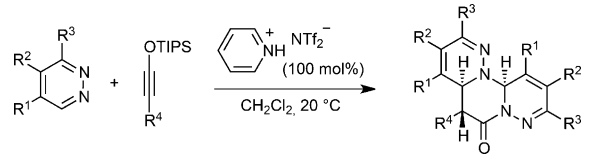
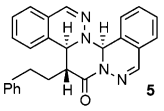
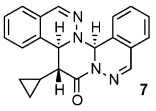
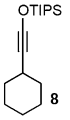
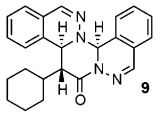
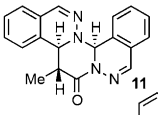
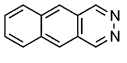
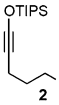
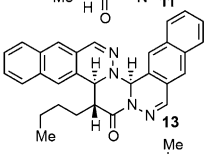
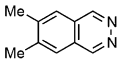
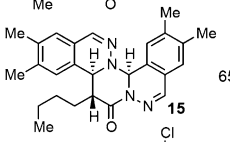
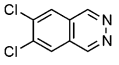
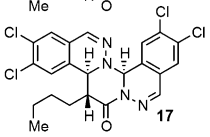
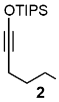
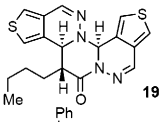
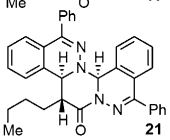
Table 1: Scope of the [2+2+2] cycloaddition of phthalazines and siloxy alkynes.



Entry	1,2-Diazine	Alkyne	Product	Yield [%][a]	d.r.[b]
1	 **1**	 **4**	 **5**	76	98:2
2	 **1**	 **6**	 **7**	73	98:2
3	 **1**	 **8**	 **9**	69	98:2
4	 **1**	 **10**	 **11**	77	>98:2
5	 **12**	 **2**	 **13**	74	>98:2
6	 **14**	 **2**	 **15**	65	91:9
7	 **16**	 **2**	 **17**	73	>98:2
8	 **18**	 **2**	 **19**	81	>98:2
9	 **20**	 **2**	 **21**	61	>98:2

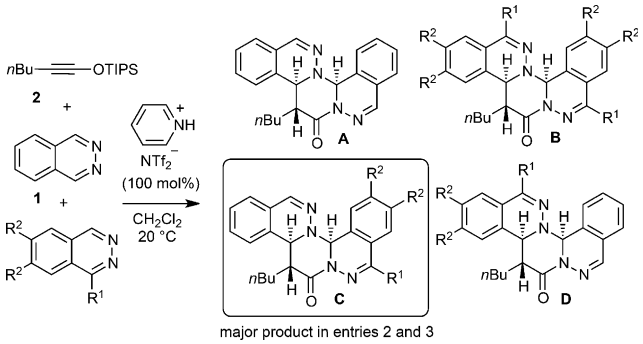
[a] Yields refer to those of the major diastereomer isolated for each reaction. [b] Diastereomeric ratios were determined by ^{1}H NMR (500 MHz) analysis of the crude reaction mixture prior to purification.

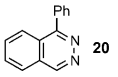
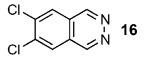
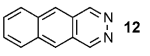
(**10**) resulted in efficient formation of the lactam **11** with high diastereoselectivity (d.r. > 98:2). Taken together, these results suggest that a wide range of siloxy alkyne substituents would be well tolerated in this reaction.

The scope of the reaction was next evaluated with respect to a series of substituted 1,2-diazine derivatives. For instance, benzo[g]phthalazine (**12**) provided the expected product **13** in good yield with high diastereoselection (Table 1, entry 5). The reaction of the alkyne **2** with 6,7-dimethyl phthalazine (**14**) gave the lactam **15** in 65 % yield and 91:9 diastereoselectivity (Table 1, entry 6). The lower efficiency of this reaction may be attributed to the decreased electrophilicity of the phthalazine moiety resulting from the presence of the two alkyl substituents. Both the halogen-containing (**16**) and heteroaromatic (**18**) diazines served as excellent substrates in the [2+2+2] cycloaddition, thus providing the expected products **17** (73 %) and **19** (81 %), respectively (Table 1, entries 7 and 8). Finally, 1-phenyl phthalazine (**20**) was found to participate in this transformation in a regio- and diastereoselective manner, thus providing a single [2+2+2] adduct in 61 % yield (Table 1, entry 9). Simple pyridazines were unreactive under the current reaction conditions. This observation is consistent with the lower reactivity of pyridazines compared to that of phthalazines in other reactions, including [4+2] cycloadditions with electron-rich dienophiles.

We next examined a possibility of conducting a three-component reaction using two different 1,2-diazines. Reaction of **2** with **1** and **20** delivered roughly equal quantities of all four possible products (Table 2, entry 1), which was expected

Table 2: Exploring three-component [2+2+2] cycloadditions of phthalazines and siloxy alkynes.



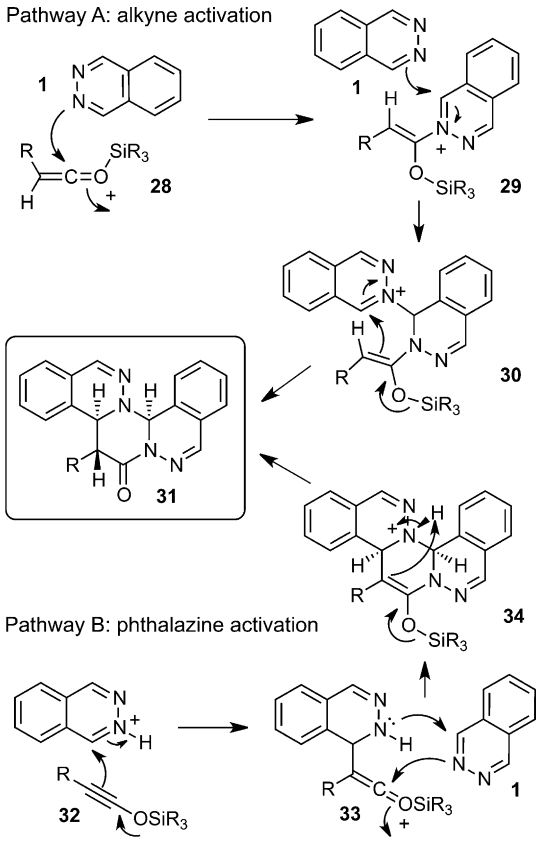
Entry	1,2-Diazine	Product ratio: **A**/**B**/**C**/**D**[a]	Yield, %[b]
1	 **20**	**3**/**21**/**22**/**23** = 29:21:23:27	56
2	 **16**	**3**/**17**/**24**/**25** = 5:19:48:28	43
3	 **12**	**3**/**13**/**26**/**27** = 0:33:67:0	68

[a] Molar ratios based on yields of individually isolated product isomers based on tentative structural assignments. [b] Combined yield (over 48 h) of isolated products for reaction performed on a 0.2 mmol scale. For all sets of products, only one diastereomer was found to be present by ^{1}H NMR (500 MHz) analysis of the crude reaction mixture.

given the small electronic perturbation provided by the phenyl substituent at C2. However, treatment of **2** with **1** and 6,7-dichlorophthalazine (**16**) with pyridinium trifluoromethane-sulfonimide afforded, predominantly, the hybrid product **24**, as well as smaller quantities of the cycloadducts **17** and **25** (Table 2, entry 2). Finally, the use of **12** in a similar three-component reaction with **1** and **2** resulted in the formation of only one (**26**) of the hybrid products as the major product along with the cycloadduct **13** (Table 2, entry 3). These results highlight the unique ability of this process to discriminate

between two similar but electronically differentiated 1,2-diazine moieties.

Two mechanistic pathways can explain the formation of pentacyclic lactams originating from [2+2+2] cycloadditions of phthalazines with siloxy alkynes (Scheme 2). The reaction can proceed by initial formation of the ketenium ion **28** through protonation of the siloxy alkyne by the pyridinium salt (pathway A). This electrophilic intermediate can be intercepted by **1** to give the enol silane **29**, a heterodiene with nucleophilic and electrophilic carbon atoms at opposite ends. Addition of a second phthalazine moiety to **29** would trigger cyclization of the central ring to afford the lactam **31** upon protodesilation. Alternatively, the process can begin with protonation of phthalazine by the pyridinium catalyst (pathway B). The resulting phthalazinium ion would then react with the alkyne **32** to give the ketenium ion **33**. Interception of this intermediate by another molecule of **1** would give the ammonium ion **34**, which can trigger protodesilylation of the enol silane moiety to give the observed product **31**. Both mechanistic pathways are consistent with the results of deuterium-labeling experiments performed using deuteropyridinium triflate, which resulted in exclusive deuterium incorporation at the α-carbon atom of the N-acyl hydrazone moiety.[11] Further mechanistic studies are required to establish which of the two reaction pathways affords the final cycloaddition product.

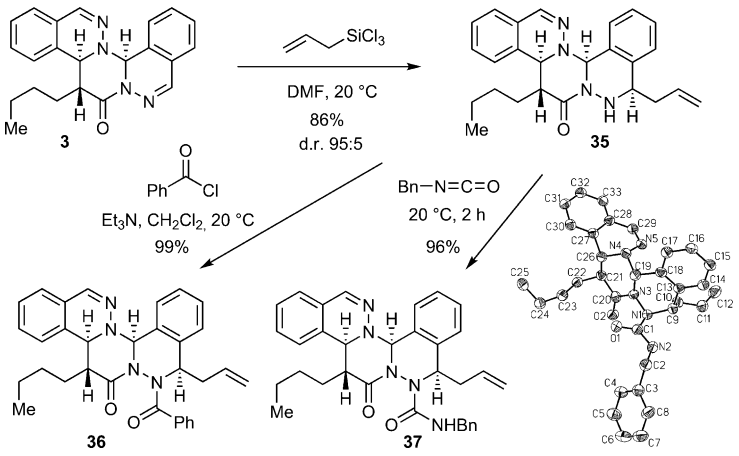


Scheme 2. Proposed reaction mechanism.

To demonstrate further the synthetic utility of the novel structural types obtained by cycloadditions of siloxy alkynes and 1,2-diazines, we have developed a route to selectively functionalize the N-acyl hydrazone moiety of the observed [2+2+2] adducts (Scheme 3). The two diazine moieties of **3** are chemically differentiated, one a hydrazone and the other an N-acyl hydrazone. While hydrazones are notoriously unreactive towards nucleophilic additions, N-acyl hydrazones are quite reactive and have proven to be valuable substrates for the synthesis of diverse classes of nitrogen-containing compounds.[12] We found that treatment of **3** with a slight excess of allyl trichlorosilane resulted in highly chemo- and diastereoselective allylation at the azomethine carbon atom of the N-acyl hydrazone moiety, thus providing the allylated product **35** in 86% yield.[13] Treatment of **35** with either benzoyl chloride or benzyl isocyanate afforded the expected acylation products, amide **36** or hydrazide **37**, respectively. The hydrazide **37** was highly crystalline and its structure was determined by X-ray crystallography, which provided unambiguous stereochemical assignment of the initial cycloadduct **3** and subsequent allylation product **35**.

We next employed this chemo- and diastereoselective elaboration strategy to assemble a representative collection of 56 structurally diverse compounds,[11] which was then evaluated for their ability to inhibit aerobic glycolysis in cancer cells. To this end, we employed our recently developed screening approach, which is based on monitoring glycolytic ATP production in live cells with chemically impaired mitochondria.[14] We performed the screen by subjecting CHO-K1 cells, treated with antimycin A, to each member of the newly assembled chemical library[11] and monitored production of ATP using a well-established luciferase-based protocol. The structure of the most potent inhibitor **38** is shown in Figure 1a.

This compound elicited the expected dose-dependent ATP suppression profile shown in Figure 1b. The ATP production was inhibited much more efficiently when cells were treated with a combination of **38** and antimycin A ($IC_{50} = 11.4\ \mu M$). To further validate inhibition of aerobic



Scheme 3. Subsequent chemoselective functionalization. Bn = benzyl, DMF = *N*,*N*-dimethylformamide.

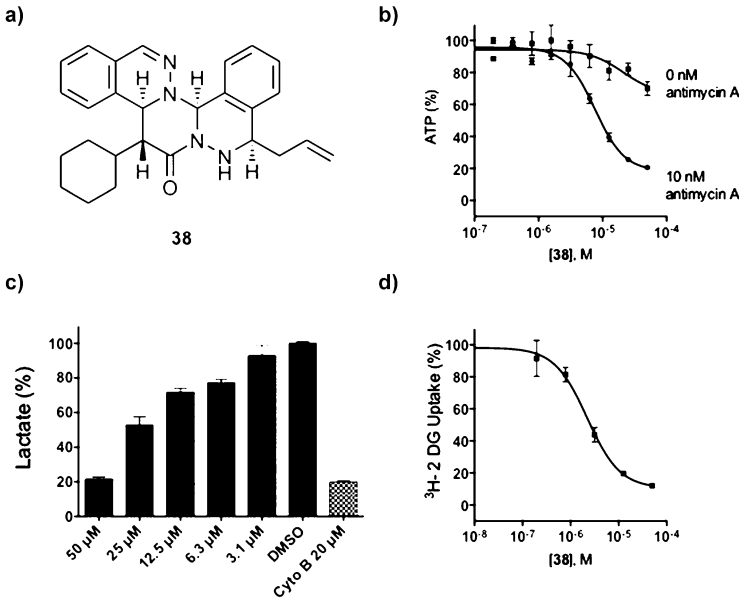


Figure 1. a) Chemical structure of **38**. b) Inhibition of cellular ATP levels in PC3 cells by **38** in the presence or absence of Complex III inhibitor, antimycin A. c) Inhibition of lactate production in A549 cells upon treatment with **38**. Cytochalasin B was used as a positive control. d) Inhibition of glucose uptake in CHO-K1 cells by **38** using ^{3}H 2-deoxyglucose scintillation experiments. All values are presented as a percentage of the vehicle treated samples. Each value is the mean SEM of duplicate values from a representative experiment.

glycolysis, we measured the effects of **38** on lactate production, which was found to decrease in a dose dependent-manner (Figure 1c). Finally, we found that **38** inhibited cellular uptake of ^{3}H-labeled 2-deoxyglucose with $IC_{50} = 2.2\ \mu M$ (Figure 1d), thus confirming the antiglycolytic effect of this compound.

In summary, we have described highly efficient and diastereoselective [2+2+2] cycloadditions of siloxy alkynes with phthalazines. This synthetic process is promoted under mild reaction conditions by simple pyridinium salts and provides rapid access to new, complex heterocyclic products, richly adorned with handles for further functionalization. Cellular screen of a representative collection of such structurally diverse compounds enabled identification of a new chemical inhibitor of aerobic glycolysis.

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