Energizing listless pyrimidines by pre-distortion for the general synthesis of 7-
aza-indazoles from 2-hydrazonylpyrimidines via intramolecular Diels-Alder
reactions

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Abstract

Pyrimidines are almost unreactive partners in Diels-Alder cycloadditions with alkenes and alkynes, and usually only reactions under drastic conditions were historically reported. We describe how 2-hydrazonylpyrimidines, easily obtained in two steps from commercially available 2-halopyrimidines can be exceptionally activated by trifluoro acetylation. This allows a Diels-Alder cycloaddition under very mild conditions, leading to a large diversity of aza-indazoles, a ubiquitous scaffold in medicinal chemistry. This reaction is general, scalable and has an excellent functional group tolerance. Quantum mechanical calculations show how the simple N-trifluoromethylation of 2-hydrazonylpyrimidines distorts the substrate into a transition state-like geometry that readily undergoes the intramolecular Diels-Alder cycloaddition.
Cycloaddition reactions are unique tools that enable the rapid elaboration of complex scaffolds with control over regio- and stereochemistry. Applications of these venerable pericyclic reactions, and in particular the Diels-Alder cycloaddition, can be found in natural products synthesis and the preparation of pharmaceutically relevant molecules\textsuperscript{1,2}. From a strategic standpoint, the inverse electron-demand Diels-Alder cycloaddition of azines\textsuperscript{1,3-5} is of great interest as it generates nitrogen-containing heterocycles\textsuperscript{2}, a privileged scaffold in life-science research and industry\textsuperscript{6-8}. High reactivity is generally observed with an increasing number of nitrogen atoms in the azine, which reduces the aromaticity of the 6π system and also favorably influences both distortion and interaction energies required to reach the transition state of the Diels-Alder cycloaddition (Fig 1a)\textsuperscript{9}. The 1,2,4,5-tetrazines are prototypical example of highly reactive aza-diene that reacts with a diversity of dienophiles, especially electron-rich, under mild conditions\textsuperscript{5}. This rapid Diels-Alder reaction is central to numerous chemical biology studies and drug activation chemistries\textsuperscript{10-12}. Triazines can also be reactive as aza-dienes as demonstrated by elegant studies by Boger\textsuperscript{13,14}, and applications in chemical biology by Prescher\textsuperscript{15}. By contrast, near the other end of the azine spectrum, pyrimidines stand as unreactive 4π partners\textsuperscript{9,16,17}. Seminal studies by Neunhoeffer\textsuperscript{18} and van der Plas\textsuperscript{19,20} demonstrated some decades ago that the lack of reactivity of pyrimidines\textsuperscript{3} in inter- or intramolecular Diels-Alder cycloadditions has to be overcome by an exceptionally reactive dienophile (e.g. ynamines\textsuperscript{18,21-23}) or harsh reaction conditions (up to 280 °C in batch\textsuperscript{24} and 310 °C in continuous flow\textsuperscript{25}) and long reaction times (up to several days) (Fig. 1b)\textsuperscript{26}. The scope of these historical studies remained very limited, and only a handful of applications were reported\textsuperscript{16}. 
Fig. 1 Diels-Alder cycloadditions of 2-hydrazinyl-pyrimidines as an entry to relevant nitrogen-containing heterocycles. a, Successive replacements of carbon atoms by nitrogen in azines result in a reactivity increase in Diels-Alder cycloadditions with electron-rich dienophiles. Pyrimidines are among the least reactive aza-dienes. The green dashed line indicates the termini of the aza-diene forming two new C-C bonds. b, Classically, pyrimidines 3 react in Diels-Alder cycloadditions at very high temperature and for extended reaction time to yield fused pyridines 4. c, Diels-Alder cycloadditions of 2-hydrazonyl-pyrimidines 7 (prepared in 2 steps from commercial and inexpensive 5) occurs even at 20 °C. The heterocycle 8 is shared by numerous biologically active compounds, among which the marketed drug Adempas® (9, Bayer). d, Reaction design based on an activating group. Conformational equilibrium pre-organizes and distorts 7 into a transition state-like geometry. e, Pyrazoles 15 could easily be obtained from 12 and 6.
Because of their low reactivity, the potential of the Diels-Alder cycloadditions of pyrimidines remains untapped. If the reactivity challenge posed by pyrimidines could be met, this strategy would constitute a breakthrough in terms of heterocyclic chemistry, and a fertile ground for theoretical explanation. Indeed, pyrimidines are small building blocks that possess key advantages; a large collection of structurally diverse pyrimidines is accessible at low price, which stands in sharp contrast with the triazines or tetrazines (Supplementary Information).

We have discovered that 2-hydrazonopyrimidines 7 can be activated towards Diels-Alder cycloaddition under mild conditions (20 or 60 °C, Fig. 1c) in sharp contrast with previous observations about pyrimidine reactivity. The corresponding cycloadducts are aza-indazoles 8, obtained in a straightforward three-step sequence from 2-halopyrimidines 5 that are commercially available, inexpensive and structurally diverse chemicals. 7-Aza-indazoles 8 are relevant nitrogen-containing heterocycles27 that can be found in the marketed drug Adempas® (9, Bayer28), Vericiguat (10, Bayer and Merck & Co.29) actually in phase III clinical trials and BMT-145027 (11, Bristol-Myers Squibb30). This conceptually new synthetic approach of 7-aza-indazoles 8 has a very wide scope, is amenable to a one-pot procedure and could be performed on a gram scale. We also report quantum mechanical calculations of this Diels-Alder reaction that shed light on the exceptional activation of the 2-hydrazonopyrimidines 7. Indeed, this phenomenon can be explained by the formation of an activated conformer s-cis,Z-7 that is distorted into a transition state-like geometry (Fig. 1d). After Diels-Alder cycloaddition, a spontaneous retro-Diels-Alder and hydrolysis of the activating group delivers the desired 7-aza-indazole 8. The nature of the activating group is thus central to prevent N-cyclisation to the corresponding pyrazole 15 (Fig. 1f)31,32, to pre-organize the system through conformational equilibria and to dramatically increase the reactivity (and thus functional group tolerance) of the overall process.
Results and discussion

Unsubstituted pyrimidines are particularly challenging substrates; the intramolecular cycloaddition of \(N\)-(but-3-yn-1-yl)pyrimidin-2-amines into 7-aza-indolines at 210 °C was reported to only lead to decomposition. The hydrazone, 7, was found to undergo very slow intramolecular Diels-Alder reactions. We screened activating groups that could be easily introduced on the hydrazone 7 to enhance reactivity. Trifluoroacetic anhydride was identified as the optimal \(N\)-acylating agent, allowing a clean cycloaddition of model substrate 16 into 7-aza-indazole 18 at room temperature in THF in the presence of 3-pentanone as a formonitrile trap (Fig. 2). This dramatic increase in reactivity of pyrimidines in Diels-Alder cycloaddition is unprecedented and opens new avenues in terms of synthetic applications. Further optimization of the reaction temperature and time with hydrazone 17 demonstrated that a complete conversion to 19 could be obtained in only 10 min at 60 °C. This latter set of conditions was selected for the exploration of the scope of this Diels-Alder/retro-Diels-Alder cycloaddition (Table 1).

![Fig. 2 Diels-Alder cycloadditions of pyrimidines under mild conditions.](image-url)

Pyrimidines 16 and 17 are efficiently converted into cycloadducts 18 and 19 at 20 or 60 °C. X-ray crystallographic structures were obtained for 17 and 19.

This new method efficiently converts unsubstituted pyrimidines into reactive aza-dienes upon treatment with TFAA, leading to 7-aza-indazoles 20 and 21 in 93% yield. 5-Bromo pyrimidines are symmetrical pyrimidines that delivered 7-aza-indazoles 22 and 23 possessing an alkyl or cycloalkyl on
the 3-position in 76-81% yield. With unsymmetrical aza-dienes such as 4-trifluoromethyl-pyrimidines, 7-aza-indazoles 24-27 were obtained in 78-90%.

Although two pathways could be envisaged for the retro-Diels-Alder cycloaddition\textsuperscript{14,35}, a single cycloadduct was observed in each case in the crude reaction mixture. The largest number of examples involve substituted 5-fluoro-pyrimidines, leading to aza-indazoles 29-48 in good to excellent yields.

Systematic variations of the electronic and steric natures of the $R^1$, $R^2$ and $R^3$ substituents of the cycloaddition precursor demonstrated that a broad range of motifs and functional groups are tolerated, leading to a unique collection of 7-aza-indazoles. Aromatic and heteroaromatic groups could be introduced on the 3 or 4-position of the cycloadduct, as in 29 (87%), 36 (63%), 41 (99%), and 42 (64%). Halo-substituted aromatics are also compatible with the process, as shown by 35 obtained in 88%. The latter is poised for metal-catalysed cross-coupling, leading to further chemical diversity.

Esters can be present in the 3-position of the 7-aza-indazoles (32 67%, 40 85%) as well as alkyl (19 83%, 42 64%), cycloalkyl (30 65%, 43 83%, 44 94%) or saturated $N$-heterocycles such as a piperidin-4-yl motif in 45 (70%).

To further expand the chemical space in these series, the synthesis of 7-aza-indazoles with two classes of promising medicinal chemistry substituents was studied: the saturated bioisostere of benzene, bicyclo[1.1.1]pentane as a relevant mimic of para-disubstituted aromatic group (46 86%, 47 89%)\textsuperscript{36,37}. Finally, we investigated spirocyclic substituents, as their reduced lipophilicity, their high $sp^1/sp^2$ carbon atoms ratio and their intrinsic positioning of bond vectors make them attractive rigid scaffolds for medicinal chemistry\textsuperscript{38}; the 7-aza-indazole 48 was obtained in 70% as a single compound.
Table 1 Scope of the Diels-Alder cycloadditions of pyrimidines

| Reaction conditions: 2-hydrazonopyrimidine (1 equiv.), 3-pentanone (3 equiv.), TFAA (1.5 equiv) in THF (0.2 M) at 60 °C (microwave irradiation, 150 W, ramp time: 45 s) for 10 min. Yields were determined after chromatography on silica gel. X-ray crystallographic structures were obtained for 19, 45, 46 and 47. Boc, 'Butoxycarbonyl. |
Fig. 3 Density functional theory calculations. a, Transition state structures TS1-TS6 and computed activation energies for the intramolecular Diels-Alder cycloadditions of pyrimidines 49a, 50a, and 51a for the non-activated (R = H) and 49b, 50b, and 51b for the activated (R = COCF₃) cases (Δ G‡, Δ H‡, -T Δ S‡ in red, blue, and black, respectively, in kcal/mol). b, Distortion/Interaction analysis on the intermolecular Diels-Alder cycloadditions reactions of substituted pyrimidines 55-60 with propyne, and the corresponding transition state structures TS7-TS12 are shown below each reaction. The sum of distortion energy of pyrimidine (blue arrow), distortion energy of propyne (green arrow), and interaction energy (red arrow) gives the activation energies of the process (black arrow). Energies are in kcal/mol.
Density functional theory (DFT) calculations were carried out to understand the origin of the activation of pyrimidines. Gas phase geometry optimization was carried out at the M06-2X/6-31G(d) level of theory, followed by single point energy calculations using 6-311+G(d,p) basis set with a CPCM solvation model. Studies of the pyrimidine-alkyne cycloadditions revealed that the Diels-Alder reaction is the rate-determining step, while the following retro-Diels-Alder has a low activation barrier and is significantly exergonic with the release of formonitrile (Supplementary Information). Based on the broad scope of this reaction, we simplified the system by replacing phenyl with methyl and cyclohexyl with hydrogen and focused on the substituents on pyrimidine moiety and on the impact of N-trifluoroacetylation on the activation.

Fig. 3a shows the transition state structures involved in these reactions, with the forming bond lengths labeled (in Å) and the activation energies shown below (in kcal/mol). The 4-trifluoromethyl and 5-fluoro groups on the pyrimidine scaffold lower the activation energy slightly (0-2 kcal/mol, TS3 and TS5 vs. TS1). On the other hand, the N-trifluoroacetyl has an enormous impact, decreasing the reaction barriers by 12-14 kcal/mol (TS2, TS4 and TS6 vs. TS1, TS3 and TS5 respectively). To understand the substituent effect, we studied the corresponding intermolecular reactions and analyzed the results with the distortion/interaction model (Fig. 3b). The 3-trifluoromethyl group, 5-fluoro atom, and N-trifluoroacetyl group lower the activation barriers by 2-3 kcal/mol by improving interaction energies. Analysis of the molecular orbital energies (Supplementary Information) showed that the N-trifluoroacetyl group lowers the energy of the second lowest unoccupied molecular orbital which interacts with the HOMO of alkyne, increasing the stabilizing interaction energy due to charge transfer in this inverse-electron-demand Diels-Alder reaction. However, in the intermolecular cycloaddition case, the activation from trifluoroacetyl group is small (~2 kcal/mol), far less than in the intramolecular cycloaddition case.
Fig. 4 Pre-organization and direct reaction during the intramolecular Diels-Alder cycloaddition of pyrimidine. a, Two distinct phases are proposed for the intramolecular cycloadditions of 2-hydrazonepyrimidines, pre-organization of the ground state to the reactive state followed by cycloaddition. b, Two parameters of the pre-organization phase, dihedral angle (θ) and pyramidalization of the N1 atom (ϕ). c, Ground states and reactive states of 2-hydrazonepyrimidines 51a and 51b cases (ΔG‡, ΔH‡, -TΔS‡ in red, blue, and black, respectively, in kcal/mol); dihedral angle (θ, front view) and pyramidalization of the N1 atom (ϕ, side view); calculated structures with distances in Å.
To explain the origin of this powerful effect for the intramolecular reaction, we propose a two-phase model, dividing the intramolecular reaction into: pre-organization and cycloaddition phases (Fig. 4a). The pre-organization phase refers to the process in which the reactant undergoes conformational change from the ground state to a reactive state where the aza-diene and the dienophile (pyrimidine and alkyne in this case) are close in proximity in order to react. These conformational changes can be described through dihedral angle $\theta$ and the degree of pyramidalization $\varphi$ of the N1-nitrogen atom (Fig. 4b). The cycloaddition phase refers to the actual bond forming/cleavage process.

As shown in Fig. 4c, the ground state structure of 2-hydrazonepyrimidine 51a adopts a planar geometry ($\theta = 0^\circ$). This extended geometry is supported by X-ray crystallography of alkynyl-pyrimidine 17 (Fig. 2).

Bringing the triple bond closer to pyrimidine moiety costs 7.6 kcal/mol and even in the reactive state, the triple bond of 51a orients away from the perfect perpendicular position ($\theta = 143^\circ$ and $\varphi = 144^\circ$). However, when a $N$-trifluoracetyl group is present as in 51b, due to the steric repulsion between the pyrimidine N and carbonyl O, the triple bond is naturally positioned over the pyrimidine moiety, perfectly in position for the cycloaddition reaction ($\theta = 0^\circ$ and $\varphi = 180^\circ$). In this case, the ground state is also the reactive state. In addition, in the cycloaddition phase, the $N$-trifluoroacetyl group further lowers the activation barrier by 5.8 kcal/mol due to the larger interaction energy.

This DFT study shows that $N$-trifluoroacetyl group pre-organizes the triple bond, not only changing the s-trans hydrazone conformation to s-cis, but also rotating the Ar—N bond so that the hydrazone is perpendicular to the diazine, placing the alkyne in perfect position for cycloaddition. The activation by the $N$-trifluoroacetyl group is thus due to the electronic substituent effect, preorganization, and more favorable entropy.
Fig. 5 NMR insights, gram scale one-pot reaction and synthetic applications. a, Diels-Alder cycloaddition of 16 at 20 °C in THF was followed by $^{19}$F NMR over 24 h. Intermediates 61 and 63 were observed. Reaction half time is 8 h at 20 °C, which corresponds to $E_{\text{Act}} = 23.3$ kcal/mol. b, Gram scale experiment starting from 5-bromo-2-chloropyrimidine 64 (CAS #32779-36-5), hydrazine monohydrate (CAS # 7803-57-8) and 4-phenylbut-3-yn-2-one 66 (CAS #1817-57-8), all commercial and inexpensive chemicals. c, Synthesis of the key intermediate 70 of the Bayer synthesis of Vericiguat from commercially available 67 (CAS #104408-28-8) and 68 (CAS #379224-67-6). ppm, parts per million; THF, tetrahydrofuran; TFA, trifluoroacetic acid; TFAA, trifluoroacetic anhydride; Bu, butyl; Bn, benzyl, Ph, phenyl.
To gain further insights into the reaction mechanism, the cycloaddition reaction of 16 was followed by $^{19}$F NMR in THF at 20 °C for 24 h (Fig. 5a). A clean transformation of 16 into its trifluoroacetylated analog 61 was observed almost instantaneously, followed by the slow formation of 63 and its hydrolysed counterpart 18 due to traces of water. Tricyclic intermediate 62 was not observed, nor protonated 63 or 18. The measured half-life is about 8 hours, which corresponds to an activation free energy of 23.3 kcal/mol, according to the Eyring equation and first order rate law. The computed activation free energy from DFT calculations is 24.2 kcal/mol, close to experimental data. From a practical point of view, this Diels-Alder cycloaddition of 2-hydrazone pyrimidines is amenable to gram scale in a one-pot process, as demonstrated with the synthesis of 22 in 74% yield from commercially available compounds (Fig. 5b). 5-Bromo-2-chloropyrimidine 64 can be transformed into the corresponding 5-bromo-2-hydrazinopyrimidine 65 in quantitative yield using hydrazine monohydrate in ethanol at 60 °C for 40 min. Crude 65 could then be reacted with the commercial ynone 66 using a sub-stoichiometric quantity of trifluoroacetic acid (20 mol%) in THF at 60 °C for 20 min. When the hydrazone formation is complete, trifluoroacetic anhydride and 3-pentanone are added. After 1 h at 60 °C, the 7-aza-indazole 22 is obtained as the only product in the crude reaction mixture; after purification by silica gel chromatography, 1.1 g of analytically pure 22 could be obtained. The relevance of this extraordinary reactivity of 2-hydrazone pyrimidines in Diels-Alder reaction under mild conditions was further explored with the synthesis of an intermediate to Vericiguat (BAY 1021189 from Bayer), a soluble guanylate cyclase (sGC) stimulator for the chronic heart failure in Phase III clinical trials (Fig 5c). Vericiguat possesses a 7-aza-indazole scaffold substituted by a 2-fluorobenzyl on N1, a pyrimidine motif on C3 and a fluorine atom on C5; this compound could be obtained in 6 steps according to the Bayer medicinal chemistry route. In contrast, the latter was obtained in this work in only 4 steps (including a one-pot reaction) from commercially available compounds 67 and 68.
The one-pot condensation/domino Diels-Alder reactions proceeds smoothly at 60 °C and yields a single compound that is treated with tetrabutylammonium fluoride in THF at 0 °C. The disubstituted 7-aza-indazole 69 is finally converted to 70 using 2-fluorobenzyl bromide and cesium carbonate in DMF at room temperature in 67% (N1/N2 benzylation ratio = 60:40). This synthesis of 72 requires only a single chromatography at the very last step.

Conclusions
Pyrimidines are intrinsically unreactive aza-dienes in Diels-Alder cycloadditions, and this lack of reactivity under mild conditions has hampered the access to a diversity of original nitrogen-containing heterocycles from these simple and inexpensive building blocks. We show that 2-hydrazonopyrimidines can be profoundly activated using a simple trifluoroacetyl group, leading to a domino Diels-Alder/retro-Diels-Alder cycloaddition even at room temperature. This reaction is general, presents an excellent functional group tolerance and can be scaled up on a gram-scale in a one-pot process. A straightforward synthesis of a key intermediate of Bayer’s Vericiguat, a soluble guanylate cyclase (sGC) stimulator for the chronic heart failure in Phase III clinical trials, illustrates the potential of this cycloaddition strategy. Central to this method is the impressive lowering of activation energy of the Diels-Alder reaction, that was analyzed by density functional theory calculations including an application of the distortion/interaction-activation strain model to intramolecular reactions. The trifluoroacetyl activating group preorganizes the cycloaddition precursor, electronically activates the aza-diene and confers a favorable entropy on the transition state of the Diels-Alder cycloaddition.
Methods

Procedure for the synthesis of 22 on a gram-scale. To a solution of 5-bromo-2-chloropyrimidine 64 (2 g, 10.34 mmol, 1.00 eq.) in EtOH (6.90 mL, 1.50 M) under nitrogen was added hydrazine monohydrate (1.01 mL, 2.00 eq.). The solution was stirred at 60 °C for 40 minutes then cooled down to room temperature. The precipitate was filtered, successively washed with EtOH and Et₂O, and dried under vacuum. 65 was obtained as a white powder (1.95 g, 10.36 mmol, quantitative). To a solution of 5-bromo-2-hydrazinylpyrimidine 65 (1 g, 5.29 mmol, 1.10 eq.) in THF (dried over sodium/benzophenone, 9.60 mL, 0.55 M) under nitrogen was added 4-phenylbut-3-yn-2-one 66 (693.40 mg, 4.81 mmol, 1.00 eq.) and trifluoroacetic acid (0.07 mL, 0.96 mmol, 0.20 eq.). After 20 minutes at 60 °C, TLC analysis indicated complete consumption of the starting materials. 3-Pentanone (1.52 mL, 14.43 mmol, 3.00 eq.) and trifluoroacetic anhydride (2.01 mL, 14.43 mmol, 3.00 eq.) were added and stirring was continued for 1 h at 60 °C. The reaction mixture was cooled to room temperature, diluted with EtOAc and washed twice with an aqueous saturated Na₂CO₃ solution. The organic layer was dried over MgSO₄, filtered and evaporated under vacuum. After silica gel chromatography (petroleum ether/ethyl acetate 80:20), 22 was obtained as a yellow powder (1.02 g, 3.54 mmol, 74%).

Data availability

Crystallographic data for the structures reported in this Article have been deposited at the Cambridge Crystallographic Data Centre under deposition numbers CCDC 1903453 (17), CCDC 1903455 (19), CCDC 1911671 (45), CCDC 1911386 (46) and CCDC 1911387 (47). Copies of the data can be obtained free of charge via https://www.ccdc.cam.ac.uk/structures/. All other data supporting this research can be obtained can be found in the Supplementary Information.


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Author Contribution

VLF, VB and NB designed, performed and analyzed experiments. NB conceived the study. CS and TF provided the building blocks leading to compounds 45-48. YC, VG, FL and KNH performed the DFT calculations. NB, FL and KNH wrote the manuscript with contributions from all authors.

Competing interests

VLF, YC, VG, VB, FL, KNH and NB declare no competing interests. CS and TF are Team Leader and CEO (respectively) of Spirochem, a Swiss CRO company selling the building blocks leading to compounds 45-48.

Additional information

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