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Three-Component Reaction of 3,3-Difluorocyclopropenes, *s*-Tetrazines, and (benzo) Pyridines

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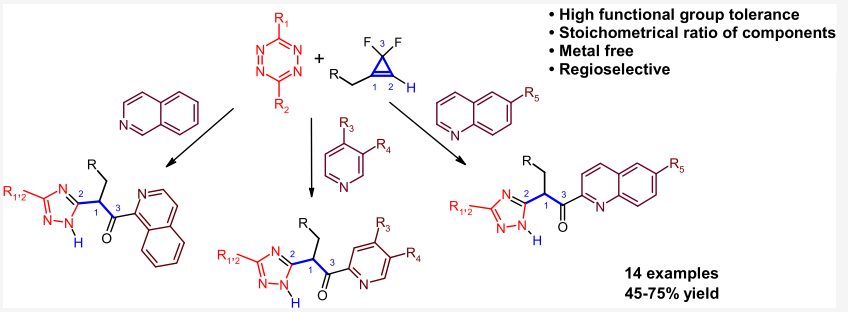

ABSTRACT: A new three-component reaction leading to 1-α-(pyridyl-2-[1,2,4]triazolyl)-2-alkyl-ethanones has been discovered while studying the reactivity of monosubstituted 3,3-difluorocyclopropenes in an inverse electronic demand Diels−Alder (IEDDA) cycloaddition−cycloreversion sequence with *s*-tetrazines. The reaction involving the above-mentioned reactants and (benzo)pyridine as a third component results in a complex transformation proceeding in mild conditions in a stoichiometric ratio of reactants and has high functional group tolerance (phenols, amides, ethers, carboxylic acids, ketones, and acrylic esters). As a result, simple pyridines are selectively functionalized at the α-position in good isolated yields. The reaction mechanism includes a rare azaphilic [4 + 2]-cycloaddition step between *s*-tetrazine and intermediate 1-hydroxyindolizine, suggested after byproduct identification and tracked with a deuterium label. To date, it is only the third known example of skewed azaphilic cycloaddition of tetrazine. The reaction is truly three-component and cannot be effectively performed stepwise.

INTRODUCTION

The inverse electronic demand Diels−Alder (IEDDA) reaction between *s*-tetrazines and simple alkenes or less reactive alkynes with nitrogen loss leading to pyridazines was first reported by Carboni and Lindsey[1−3] in 1959.

Being strained, cyclopropenes are highly reactive alkenes; in terms of the IEDDA reaction, they found application in series of works dedicated to bio-orthogonal ligation[4] due to the advantages of this type of conjugation: it proceeds fast, quantitatively, tolerates aqueous media, and produces only nitrogen as a byproduct. Another example is sequential IEDDA, involving tetrazine and two molecules of cyclopropene applied in construction of semibullvalenes[5] and homotropilidenes,[6] as was reported by Sauer et al. Gem-dimethylcyclopropene normally forms diazanorkardienes upon reaction with dimethyl *s*-tetrazine-3,6-dicarboxylate at a room temperature.[7] Reaction of cyclopropenes with 1,2,4-triazines leading to azepines is much more rarely represented.[8] To date, no examples have been found concerning the behavior of 3,3-dihalocyclopropenes in such type of [4 + 2]-cycloadditions with triazines or *s*-tetrazines.

On the other hand, *s*-tetrazines represent a class of electron-deficient aza-aromatic heterocycles, most noted as the 4-π electron component in the IEDDA reaction with electron-rich dienophiles; however, electron-poor alkenes[9] and C═N heterodienophiles such as amidines,[10] carbamates,[11] and nitriles[12] also react in particular cases. Simple *s*-tetrazines are deeply colored, weakly basic compounds that can behave as reversible oxidizers and readily accept electrons,[13] forming stable anion radicals. Being highly electron-deficient, *s*-tetrazines are prone to nucleophilic attack at the carbon atom[14] (with S, N, O nucleophiles) and more rarely at nitrogen[15] (with active C-nucleophiles). Besides normal aromatic nucleophilic substitution at carbons, the S_N(ANRORC) mechanism becomes operative, for example, in the Chichibabin hydrazination of monosubstituted *s*-tetrazines.[16] Based on the IEDDA reaction of *s*-tetrazine with four-membered dienophiles as the initial chemical act followed by subsequent small ring expansion, interesting examples of synthesized eight-membered heterocycles, such as diazocine[17]

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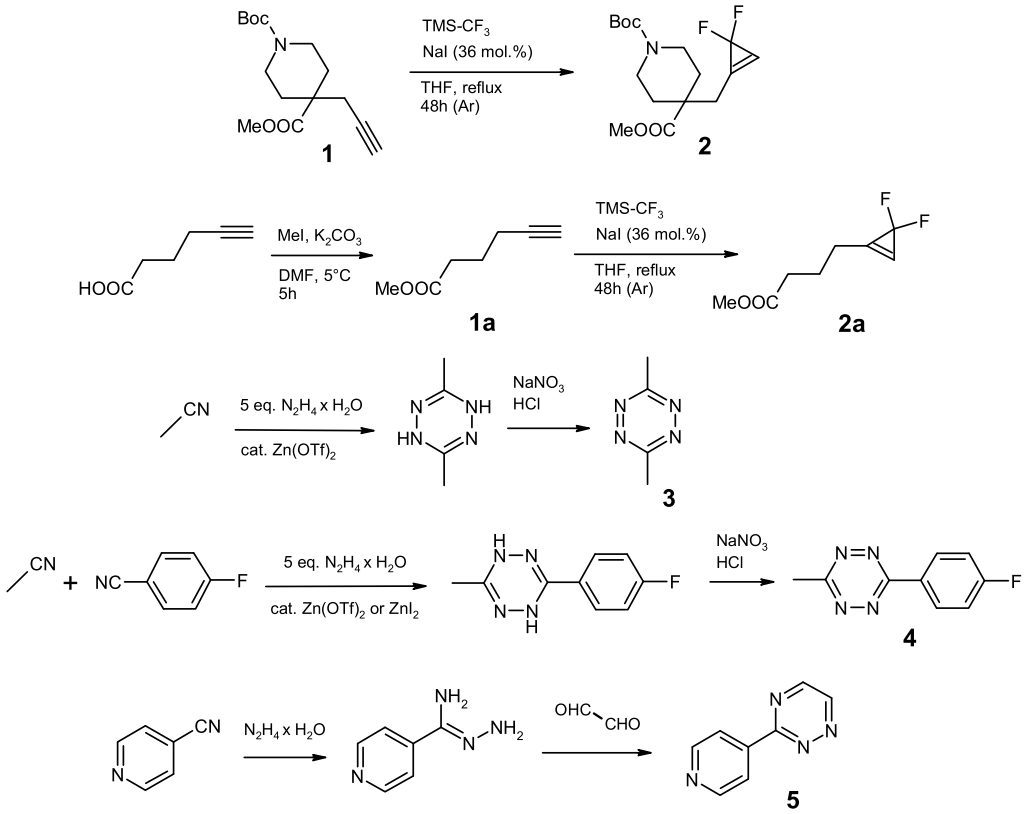
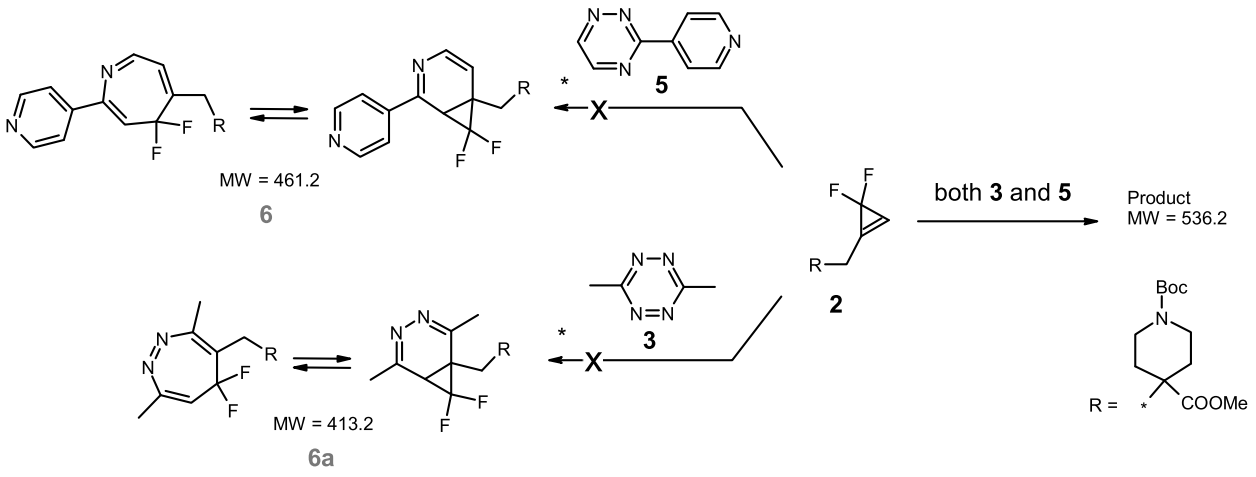
Scheme 1. Difluorocyclopropenation of Acetylenes and Synthesis of Azines



Scheme 2. Initially Planned Transformations and the Unexpected Result[a]



[a]*NO reaction took place in high-boiling-point nonpolar solvents, leaving starting materials unreacted.

and triazocine,[18] have been reported. However, four-membered thietanones reacting with *s*-tetrazines led to pyrazol-4-ols[19] instead of the expected thiadiazocine system by a nonobvious cascade of reactions, including cycloaddition, recyclization, and sulfur extrusion.

The rich variety of chemical properties for both classes—tetrazines and cyclopropenes, given as examples above—indicates that they can still conceal possible nonobvious transformations.

RESULTS AND DISCUSSION

Initial IEDDA Experiments. We planned a study of the Carboni–Lindsey reaction between 3,3-difluorocyclopropene and azines aiming to obtain unknown 4,4-difluoro-4*H*-azepine and 5,5-difluoro-5*H*-[1,2]diazepine heterocycles, interesting from the chemical point of view. Starting 4-(3,3-difluorocycloprop-1-enylmethyl)-*N*-Boc-4-methoxycarbonyl-piperidine **2** was synthesized in near-quantitative yield, by adding 3.5 equiv of the Ruppert–Parkash reagent TMS-CF_3 (as a difluorocarbene source) over 6 h to the acetylene **1** with a catalytic amount of NaI in refluxing tetrahydrofuran (THF)[20] (Scheme 1). The choice of the model acetylene **1** was made by

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J. Org. Chem. XXXX, XXX, XXX–XXX

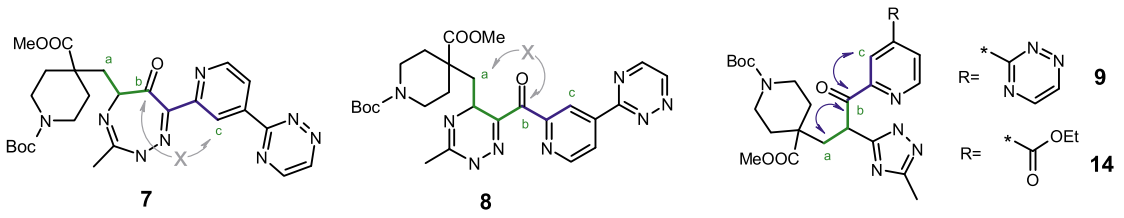

Figure 1. Structural features of the proposed products in terms of {^{1}H-^{13}C} heteronuclear multiple-bond correlation spectroscopy (HMBC) (**7** and **8** were rejected). Blue arrows, three-bond correlation (observed); gray arrows, four-bond correlation (not observed).

few criteria: it is nonvolatile, has functional groups (*N*-Boc, COOMe), and it can be easily detected by liquid chromatography−mass spectrometry (LC−MS) or specifically stained on thin layer chromatography (TLC). Analogously, hex-5-ynoic acid methyl **1a** was difluorocyclopropenated to yield **2a**. Other acetylenes with the acidic proton (e.g., OH, NH-Boc) were not compatible with the chosen Ruppert−Prakash reagent for the difluorocyclopropenation procedure.

s-Tetrazines **3**, **4** and 1,2,4-triazine **5** were prepared in two steps from appropriate nitriles according to the known literature procedures.[21,22] Upon synthesis of 2,5-dimethyltetrazine **3**, we, however, met with some difficulties such as low yield (<15%) and difficult product handling as it sublimes easily. As reduced pressure should be avoided upon manipulations with dimethyltetrazine, we applied atmospheric pressure solvent evaporation with a short Vigreux column to prepare a ∼1 M solution in dichloromethane (DCM). The concentration was quantified by ^{1}H NMR of the solution with increased relaxation delay t_1 of 10 s. A fivefold excess of hydrazine hydrate is generally used in the synthesis of the 1,4-dihydro-*s*-tetrazine intermediate; caution should be applied during the nitrous acid oxidative aromatization step, as hydrazoic acid may form.[23] Furthermore, unsubstituted *s*-tetrazine may explode upon sublimation, as known from the times of Theodor Curtius. We handled small quantities of crystalline 2,5-dimethyltetrazine and found it to be stable to friction albeit we observed its slow decomposition when exposed to light at ambient temperature.

In initial IEDDA experiments (Scheme 2), difluorocyclopropene **2** was heated with triazine **5** in high-boiling-point nonpolar aprotic solvents with no chemical interaction observed. In *p*-xylene at 130 °C, both reactants stayed intact after 18 h, and we added dimethyltetrazine **3** as a more reactive heterodiene to the reaction mixture. After heating for several hours, no changes were observed with all three reactants (**2**, **5**, and **3**) present, and the reaction vessel was cooled down to room temperature. Triazine **5** precipitated, and ethanol was added to homogenize the mixture, which was left overnight at 60 °C. After that, LC-MS indicated that a new product with a molecular weight greater and not corresponding to the expected ones (**6**, **6a**) was cleanly formed.

Structure Elucidation of the New Products. This reaction product with MW = 536.2 Da was isolated by high performance liquid chromatography (HPLC) for further structural studies. It was moderately UV-active and lower than starting triazine **5** on TLC. ^{1}H NMR preliminary analysis showed that the *N*-Boc-piperidine part was present along with the appearance of a new methyl group at 2.5 ppm as a singlet, and the triazine cycle was conserved. Surprisingly, we found that the pyridine ring of 3-pyridin-4-yl-[1,2,4]triazine **5** was substituted at the α-position, and the $-CH_2-CH-$ fragment emerged, while the characteristic 3,3′-difluorocyclopropene proton multiplet at 7.3 ppm disappeared. No fluorine atoms were detected by ^{19}F NMR, and the odd-numbered molecular weight indicated that the product must contain an odd number of nitrogen atoms. Along with the appearance of only one methyl group, we postulated formal excision of acetonitrile from dimethyltetrazine **3** in the reaction course and proposed several alternative product structures **7**−**9** (Figure 1). It is clearly seen that all three reaction components are incorporated in the proposed products.

The difference among triazepine **7**, dihydrotriazine **8**, and triazole **9** was not obvious. Each of them has the same molecular weight corresponding to the LC-MS data, retains triazine and *N*-Boc-piperidine rings, has the $-CH_2-CH-$ fragment observed as the ABX system by ^{1}H NMR, and has eight nitrogen atoms. Dihydrotriazine **8** is expected to aromatize readily in oxidative conditions.[24] We preliminarily refuted **8** by the chemical criteria as no oxidation occurred in the experiments. To distinguish between the rest of the triazepinone **7** and triazole **9** alternative structures, and as a test of the reaction scope, we switched the pyridine component to ethylisonicotinate, and the three-component reaction (3-CR) was performed using 1.5 equiv of 3,5-dimethyltetrazine **3** and ethylisonicotinate in relation to difluorocyclopropene **2** in refluxing ethanol for 15 h. During that time, the reaction mixture gradually changed color from the distinctive red to brownish, indicating that most tetrazine was consumed. The 3-CR product was isolated in 50% yield and was used for further structure determination. The key structural difference between alternative **7**−**9** is that triazole **9** has three bonds between carbonyl $C^{(b)}$ and pyridine proton $H^{(c)}$, allowing {^{1}H-^{13}C}HMBC observation, in contrast to triazepinone **7**, where $C^{(b)}-H^{(c)}$ are separated with four bonds. The same NMR criteria were additionally used to distinguish between refuted dihydrotriazine **8** (four bonds between $C^{(b)}$ and $CH_2^{(a)}$) and triazole **9** (three bonds between $C^{(b)}$ and $CH_2^{(a)}$). Optimization of HMBC parameters for $^{3}J_{H\text{-}C} \approx 7$ Hz coupling revealed both important correlation peaks: $H^{(c)}-C^{(b)}$ and $CH_2^{(a)}-C^{(b)}$, indicating that the correct 3-CR product structure is firmly consistent with triazole **9**(**14**). Prototropic tautomerism of triazole **14** was observed by ^{1}H NMR in dimethyl sulfoxide (DMSO)-d_6; however, in $CDCl_3$, a single tautomer was seen in the temperature range 25−70 °C. In ^{13}C NMR in DMSO-d_6, both carbons of the triazole ring fully merged with the baseline but became observable in $CDCl_3$ as broadened signals. Gradual conformer stabilization (by intramolecular hydrogen-bonding between the keto-function and triazole NH, as assumed) was detected in $CDCl_3$ solution at 50 °C by slow downfield shifting (hours) of the triazole NH signal from 7.5 to 13 ppm.

Next, we conducted experiments on exclusion of one or two components of the reaction under study. At first, stability of difluorocyclopropene **2** was tested in 3-CR conditions (entry 1,

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J. Org. Chem. XXXX, XXX, XXX−XXX

Table 1. Reaction Component Exclusion Experiments

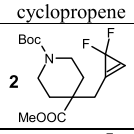
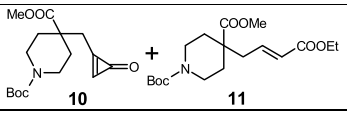
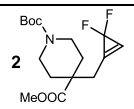
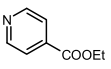
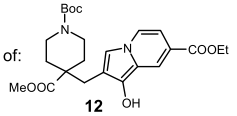
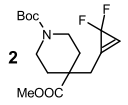
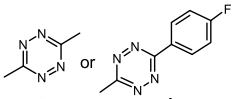
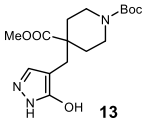
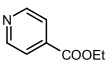
entry	difluoro-cyclopropene	tetrazine (1.2 eq.)	pyridine (1.2 eq.)	observations	conditions
1	 **2**	-	-	 **10** **11**	EtOH, 78°C, 18h
2	 **2** or **10**	-		Dimer of:  **12**	EtOH, 78°C, 18h (for **2**) or EtOH, 78°C, 2h (for **10**)
3	 **2** or **10**	 **3** **4**	-	 **13**	EtOH, 78°C, 48h (for **2**) or EtOH, 55°C, 12h (for **10**)
4	-	 **3**		no reaction	EtOH, 78°C, 20h

Table 1). On heating in ethanol at 78 °C for 18 h, clean hydrolysis of the CF_2 group gave the corresponding cyclopropenone **10** along with the product of ring-opening—ethyl acrylate **11**.[25] Further heating resulted in **11** as a sole product.

When dimethyltetrazine **3** was excluded from 3-CR (entry 2), full conversion of difluorocyclopropene after 18 h afforded a brownish product with a molecular weight of 918.4 in contrast to the expected 1-hydroxyindolizine[26] **12**. Formally, this product corresponds to 1-hydroxyindolizine **12** dimerized with a loss of H_2. It was isolated by HPLC; however, ^{1}H NMR spectra showed significantly broadened signals in the whole spectral range, obscuring the structural information. Finally, in the absence of the pyridine reaction component (entry 3), we isolated the 3-hydroxypyrazole **13** product, formed after 48 h in the reaction between tetrazine and difluorocyclopropene in refluxing ethanol. Interestingly, when unsymmetrically substituted 3-(4-fluorophenyl)-6-methyltetrazine **4** was taken instead of dimethyltetrazine **3**, the same 3-hydroxypyrazole was obtained. The closest reactions in the literature reported the interaction of 2,3-diphenylcyclopropenone with 1,2,3-triazines affording condensation products;[27] however, *s*-tetrazines reactions were not surveyed. Next, we tested the subsequent addition of ethylisonicotinate to **13** as well as dimethyltetrazine to the dimer of **12**, and after heating for 24 h in ethanol, we did not observe any 3-CR product, proving that neither **13** nor dimeric-**12** was the 3-CR intermediate. Obviously, upon exclusion of the difluorocyclopropene component (entry 4), no reaction took place between dimethyltetrazine and ethylisonicotinate. Hence, only the simultaneous presence of all three reagents furnished the 3-CR product. Summarizing, we consider that cyclopropenone **10** is the early 3-CR intermediate. It should be noted that it has been observed neither in 3-CR-s studied later nor in experiments 2 and 3 (Table 1) upon LC-MS monitoring, indicating the high conversion rate of the intermediate **10** in 3-CR.

We decided to synthesize **10** separately by hydrolyzing its precursor **2**. In wet THF under reflux conditions, no reaction took place after 18 h even with acidic additives (SiO_2 or Amberlyst-15) present, but in wet 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), the full conversion was achieved after 20 min at room temperature, giving **10** in 70% isolated yield. Cyclopropenone **10** was found to be stable enough, showing no changes after 7 day storage at room temperature and surviving heating in inert solvents (e.g., 1,2-dichloroethane (DCE), MeCN) for 18 h. We conducted component exclusion experiments (Table 1, entries 2, 3) with cyclopropenone **10** instead of difluorocyclopropene **2** and generally obtained the same results, but the formation of dimeric-**12** was much faster (<2 h). Analogously, we noticed about a four times rate increase in the reaction between cyclopropenone **10** and dimethyltetrazine: the full conversion leading to 3-hydroxypyrazole **13** was achieved only after 12 h at 55 °C in ethanol. Heating was necessary as at room temperature no **13** was obtained.

Scope of the Reaction. Next, we screened 3-CR conditions on the same model reaction among difluorocyclopropene **2**, dimethyltetrazine **3**, and ethylisonicotinate to shed light on the possible 3-CR specificities (Table 2).

Generally, heating was necessary to push the reaction forward and the presence of water was crucial (entries 5, 9, 12, 14, 16). In the absence of water, destructive side processes became prevalent (1, 2, 4) or no reaction occurred (8, 10, 11). Hence, higher temperature and anhydrous conditions had a negative effect, launching competing side reactions. The minimal temperature range was found to be 50−60 °C for the reaction completion in wet solvents (9, 12, and 16). In anhydrous acidic media MeCN/HFIP (1/1) or THF/AcOH (8/2) at room temperature, each of the three reactants remained intact. Elevating the reaction temperature to 55 °C in a more acidic THF/AcOH mixture (entry 13) gave only traces of the product at full conversion of difluorocyclopropene **2** due to the competing side reactions. When heating was employed for the reaction in EtOH/HFIP (entry 19), slight reaction acceleration was noticed, compared to EtOH alone (entry 15), due to the facilitated hydrolysis of the CF_2 group of **2** in slightly acidified media. On the other hand, DMSO media greatly suppressed the reaction (entries 6, 7), probably due to its basicity, indicating that protic catalysis was necessary. In wet HFIP at room temperature, fast conversion (∼1 h) of **2** led exclusively to the dimeric indolizine **12** product (entry 18), but at 55 °C the corresponding 3-CR product/dimeric-**12** ratio was ∼3/7 (entry 19). Thus, heating was necessary to activate the reaction with tetrazine and to suppress formation of dimeric-**12** at the same time.

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J. Org. Chem. XXXX, XXX, XXX−XXX

Table 2. Three-Component Reaction Condition Exploration

entry	solvent	T, °C	time, h	observations (LC-MS)	3-CR product
1	*i*-PrOH	80	72	full conversion,[a] complex mixture	traces
2	*t*-BuOH	70	72	full conversion, complex mixture	traces
3	*t*-BuOH/H_2O (8/2)	70	72	full conversion, side products	+
4	dioxane	100	18	decomposition	–
5	dioxane/H_2O (8/2),	70	18	full conversion, 30% yield	+
6	DMSO	70	72	only starting materials	–
7	DMSO/H_2O (8/2)	70	72	only starting materials	–
8	MeCN	55	18	only starting materials	–
9	MeCN/H_2O (8/2)	55	18	full conversion	++
10	MeCN/HFIP (1/1)	55	7	only starting materials	–
11	THF	55	18	only starting materials	–
12	THF/H_2O (8/2)	55	18	95% conversion	++
13	THF/AcOH (8/2)	55	18	full conversion, complex mixture	traces
14[b]	EtOH	70	36	full conversion	++
15	EtOH	55	18	5–10% conversion	+
16	EtOH/H_2O (8/2)	55	18	full conversion, 50% yield	+++
17	EtOH/HFIP (1/1)	55	7	30% conversion	++
18	HFIP/H_2O (20/1)	25	2	0% yield, mostly dimer **12**	–
19	HFIP/H_2O (20/1)	55	1	3-CR product/dimer **12** ~ 30/70	+

[a]Conversion is related to limiting difluorocyclopropene **2**. Dimethyltetrazine and ethylisonicotinate were used in 1.5 equiv excess. [b]EtOH used, contained ~4 vol % water.

Further, with optimized reaction conditions in hand, we focused on exploring variations, mainly in the pyridine component as the most accessible 3-CR counterpart, to understand the reaction scope and limitations. The successful combinations of reagents are presented in Table 3.

Functional groups such as ester, carbamoyl, ketone, phenol, and acrylic esters were well-tolerated. Isolated yields were on average about 50%, and in cases of isonicotinamide and 4-acetylpyridine, they were 70 and 75%, respectively (entries 3, 4). In the case of quinolines (entries 6, 7, 10), some amount of 3-hydroxypyrazole **13** emerged as a byproduct. Unsymmetrically substituted tetrazine **4** gave two 3-CR products with methyl and 4-fluorophenyl substituents at the triazole ring in a 7:3 ratio (entries 10, 11). For the cases of α-, γ-unsubstituted pyridines and quinolines (entries 6–10), only α-positions of the pyridine ring were selectively functionalized in the course of 3-CR. Unsymmetrical nicotinic acid and (*E*)-3-pyridin-3-yl-acrylic acid methyl ester (entries 8, 9) afforded two isomeric products, with the ratio dictated by the steric factor. In the particular case of the ketoacid **21** (entry 8) with possible ring-chain tautomerism, only the ring tautomer was found according to ^{1}H and ^{13}C NMR in $CDCl_3$ and DMSO-d_6.

The completion time of the reactions varied: 18 h (entries 1, 2, 10, 14, 15), 25 h (entries 3, 9, 11), and 40 h (entries 5, 7, 8). We tried to conduct 3-CR with simple unsubstituted pyridine. It differs from the common set, as it is more basic[28] ($pK_{b(H_2O)}$ = 8.83) than the other used pyridines $pK_{b(H_2O)}$ ~ 10. When the 3 equiv excess of pyridine was employed in 3-CR, no reaction occurred after 24 h, but taken stoichiometrically, ~7% conversion was observed at the same period of time. It was not completed even after 96 h (85% conversion), and this observation was in agreement with what we noticed above that bases significantly suppressed 3-CR at the first limiting step, i.e., hydrolysis of difluorocyclopropene **2** to reactive cyclopropenone **10**. We applied the trick found upon 3-CR condition exploration: HFIP as an acidic additive accelerated the reaction. In this case, 2 equiv of HFIP shortened the reaction time to adequate 48 h.

When symmetric 2,3-dimethylpyrazine was employed instead of the pyridine 3-CR component (entry 13), the known condensation[26] took place, giving the appropriate pyrrolo[1,2-*a*]pyrazinol **28** in 60% yield, whereas non-participating dimethyltetrazine **3** was recovered. Conducting an experiment between competitive 2,3-dimethylpyrazine and ethylisonicotinate with difluorocyclopropene **2**, the major product was dimeric-**12** and no appropriate pyrrolo[1,2-*a*]pyrazinol was obtained, showing the much higher reactivity of pyridine toward **2**/**10**.

2-Bromopyridine and several other aromatic heterocycles, preferentially containing the pyridine-type nitrogen, e.g., thiazole, 5-carboxy-thiazole, (1-methyl or 1*H*)-pyrazole, and 5-methoxycarbonyloxazole, were also tested as the pyridine component in 3-CR. They did not participate in the reaction, and only 3-hydroxypyrazole **13** appeared as a reaction product. Fairly basic dimethylaminopyridine showed no reaction, terminating it at the first step of CF_2 hydrolysis, and only starting materials were recovered.

Mechanism Discussion. Next, having some experience in the reaction under study, we focused on understanding its mechanism. Upon addition of ethylisonicotinate to cyclopropenone **10**, immediate reaction mixture colorization (yellow) occurred with notable heat generation. LC-MS analysis immediately after mixing indicated the clean formation of monomeric 1-hydroxyindolizine **12**, which was isolated by chromatography on SiO_2. However, we found that monomeric **12** oxidatively dimerized at a rate of ~10% per hour at room temperature in air but was stable at −20 °C or in an inert atmosphere. Other oxidizers (e.g., DAIB) caused immediate dimerization of 1-hydroxyindolizine **12**. When unsubstituted pyridine was introduced instead of ethylisonicotinate, the initial monomer was not detected due to the much faster dimerization rate of the appropriate 1-hydroxyindolizine. Importantly, when the reaction between **10** and pyridine was performed in the presence of dimethyltetrazine **3**, the 3-CR product **27** was detected in LC-MS spectra, indicating that monomeric 1-hydroxyindolizine is a 3-CR intermediate.

For more detailed mechanistic investigations, we performed deuterium labeling (Scheme 3). Deuterodifluorocyclopropene **2**-D was synthesized from deuterated acetylene **1**-D. Stability of the carbon–deuterium bond was tested by heating the sample of **2**-D in wet ethanol at 65 °C for 24 h. As expected, partial hydrolysis of the CF_2 group took place, and deuterium was conserved in deuterodifluorocyclopropene **2**-D, and the appropriate deuterocyclopropenone with no deuterium exchange was noticed.

Due to poor stability of the 1-hydroxyindolizine **12** 3-CR intermediate, it was generated in situ in the NMR tube for its proper characterization.

We added a stoichiometric amount of ethylisonicotinate to cyclopropenone **10** in degassed CD_3OD and yellow 1-

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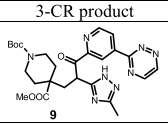
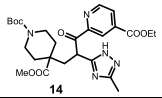
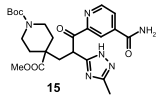
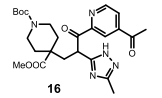
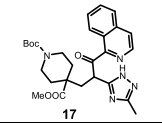
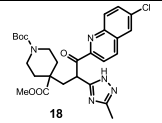
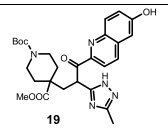
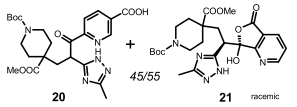
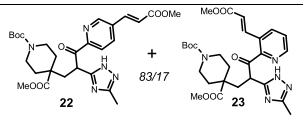
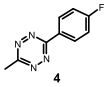
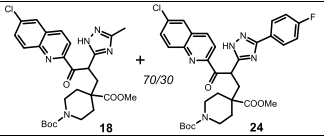
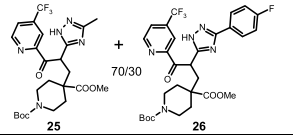
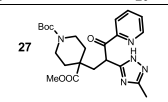
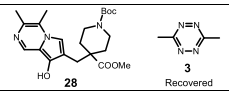
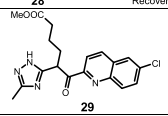
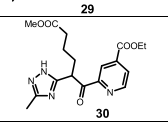
J. Org. Chem. XXXX, XXX, XXX–XXX

Table 3. Examples of a Three-Component Reaction

entry	tetrazine	pyridine	3-CR product	time, h	yield [a]
1	 **3**	 **5**	 **9**	18	50%
2	**3**		 **14**	18	50%
3	**3**		 **15**	25	70%
4	**3**		 **16**	40	75%
5	**3**		 **17**	40	55%
6	**3**		 **18**	18	50%
7	**3**		 **19**	40	50%
8	**3**		 **20** 45/55 **21** racemic	40	50[b]%
9	**3**		 **22** 83/17 **23**	25	50[b]%
10	 **4**		 **18** 70/30 **24**	18	50[b]%
11	**4**		 **25** 70/30 **26**	25	45[b]%
12	**3**		 **27**	120 48[c]	60%
13	**3**		 **28** **3** Recovered	6	60%
14	**3**		 **29**	18	45%[d]
15	**3**		 **30**	18	45%[d]

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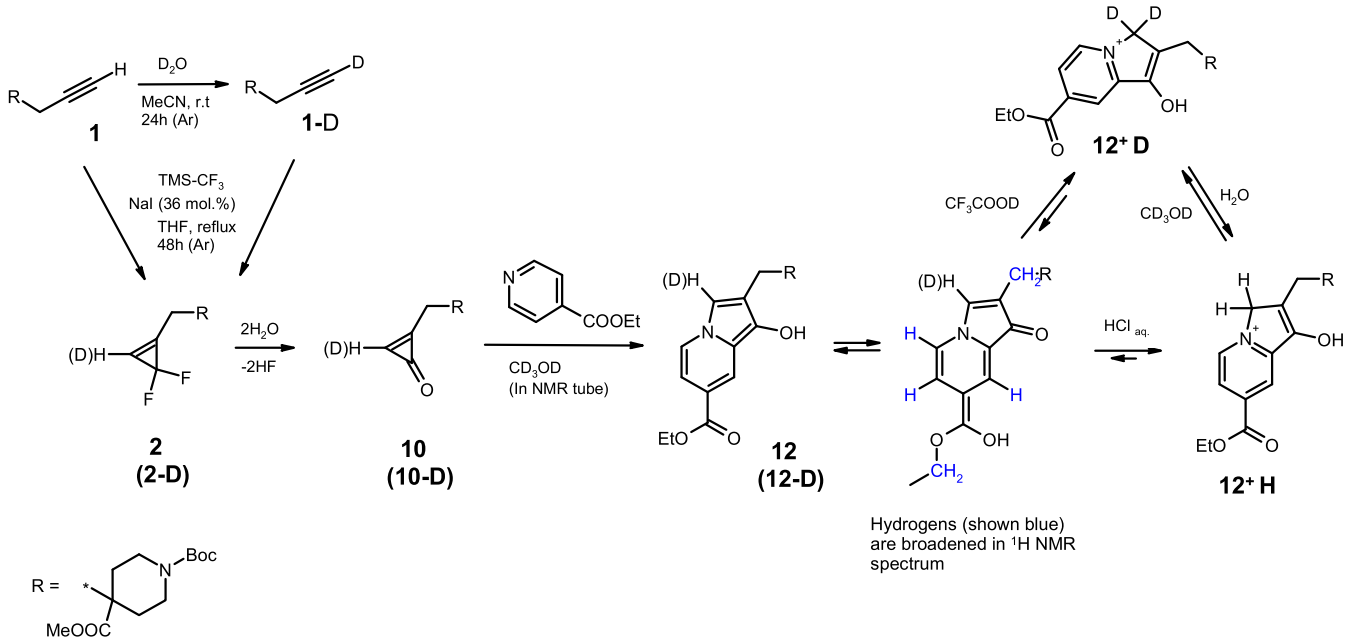
J. Org. Chem. XXXX, XXX, XXX−XXX

Table 3. continued

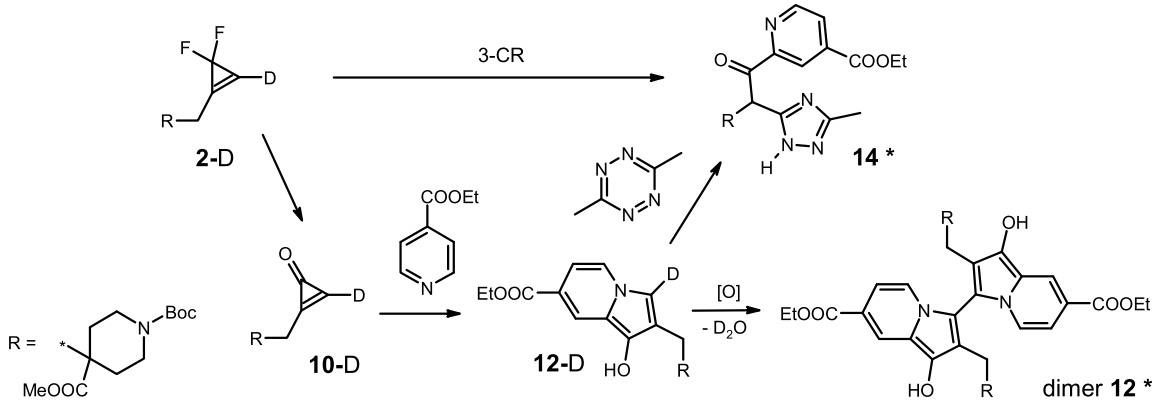
[a]Reaction conditions: 1 mmol of each component (difluorocyclopropene **2** (or **2a**), pyridine, tetrazine), EtOH (10 mL), H_2O (0.1 mL) at 65 °C. Isolated yields are given. [b]Isolated yield for both 3-CR products. [c]With 2 equiv of HFIP additive, under an argon atmosphere. [d]Difluorocyclopropene **2a** was used.

Scheme 3. Deuterium Labeling of the Difluorocyclopropene Reaction Component[a]



[a]Double-bond tautomerism and deuterium exchange in 1-hydroxyindolizine **12**.

Scheme 4. Three-Component Reaction with D-Labeled Difluorocyclopropene[a]



[a]*No deuterium was incorporated in the reaction products.

hydroxyindolizine **12** was quickly formed. As in the case of its dimer, we met significant broadening of the aromatic region signals in NMR along with the unusually broad signals of the CH_2 group in the COOEt substituent and CH_2 adjacent to the pyrrole ring. We assumed that slow double-bond tautomerism was responsible for this phenomenon, and protonation of 1-hydroxyindolizine might block the interconversions. Indeed, the observed NMR spectrum signal sharpening after hydrochloric acid addition supported the tautomerism hypothesis. More accurately, CF_3COOD acidification was enough to protonate indolizine **12** with full spectrum improvement. Pyrrole ring proton disappearance from the ^1H NMR spectrum was rationalized by deuterium exchange in acidic CD_3OD (**12**$^+$H ↔ **12**$^+$D).

Obviously, when deuterocyclopropenone **10**-D was used instead of **10** in the same NMR-tube experiment, 3-deutero-1-hydroxyindolizine **12**-D was cleanly formed, but it led to essentially the same charged tautomer **12**$^+$D after CF_3COOD acidification. Finally, we detected the CH_2 group in the pyrrole ring of charged **12**$^+$H at 5.2 ppm (proton) and 62.2 ppm (carbon) by ^1H NMR and heteronuclear single quantum coherence (HSQC), using deuterium-free solvents ($CHCl_3$ acidified with trifluoroacetyl (TFA)) where deuterium exchange is not possible.

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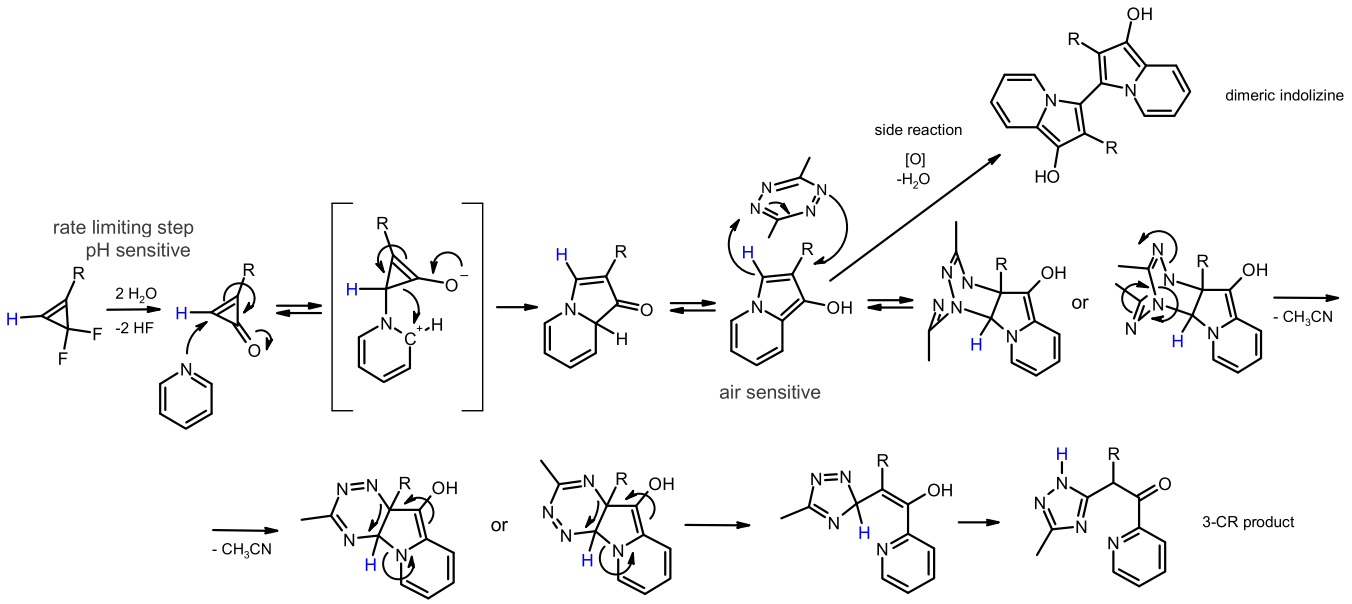
J. Org. Chem. XXXX, XXX, XXX−XXX

Scheme 5. Three-Component Reaction Mechanism



In the 3-CR where labeled deuterodifluorocyclopropene **2**-D reacted with ethylisonicotinate and dimethyltetrazine **3**, we observed deuterium loss in both the 3-CR product and dimeric-**12** (Scheme 4). As the deuterium label was conserved in monomeric 1-hydroxyindolizine **12**-D and its dimer contained no deuterium atoms, we proposed that oxidative dimerization of 1-hydroxyindolizine could occur at position 3, which is the most electron-rich and reactive position for protonation and electrophilic substitution.

Influence of air on 3-CR was qualitatively evaluated by LC-MS, and we found that a less dimeric-**12** byproduct was formed in a degassed solvent under an argon atmosphere. Hence, oxygen should be excluded from the reaction media.

With the collected experimental data in hands, we postulated the reaction mechanism with the example of dimethyltetrazine and unsubstituted pyridine (Scheme 5).

As follows from Scheme 5, proton in starting difluorocyclopropene can be tracked to the triazole 3-CR product, where it becomes exchangeable.

As the obtained 3-CR products had an amorphous glass/foam appearance, any attempts to grow a crystal were unsuccessful even when NH of the piperidine ring was deprotected and/or the methoxycarbonyl group was hydrolyzed. However, we succeeded in obtaining {^{1}H-^{15}N} HMBC optimized for 5 Hz coupling, with the correlation peaks ultimately proving the correct triazole structure (Figure 2).

Finally, we have rationalized 3-hydroxypyrazole **13** 3-CR byproduct occurrence as follows in Scheme 6.

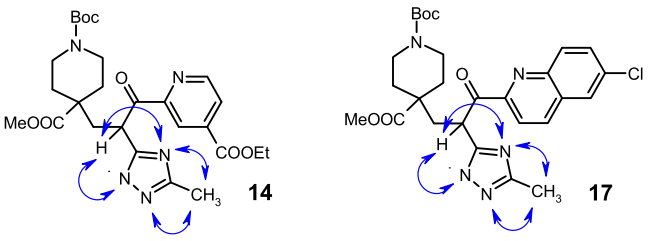


Figure 2. Observed {^{1}H-^{15}N} HMBC correlations.

This mechanism is supported by LC-MS detection of pyrazolo[1,2-*a*]tetrazin-6-one adducts with water and EtOH as the reaction intermediates when **3** reacted with **10** at room temperature.

CONCLUSIONS

The three-component reaction among monoalkyl substituted 3,3-difluorocyclopropenes, *s*-tetrazines, and (benzo)pyridines has been studied, and the 1,2,4-triazole product structure has been reliably proved. The reaction mechanism based on the studied 3-CR byproducts has been suggested and supported by deuterium labeling experiments. To our knowledge, this is the first example of azaphilic cycloaddition between tetrazine and unstable (oxygen-sensitive) 3-H-1-hydroxyindolizine. The success of this cycloaddition is the slow formation of the 1-hydroxyindolizine intermediate in the presence of tetrazine as the heterodiene at elevated temperatures. Electron-withdrawing groups (EWGs) at the pyridine component (or its benzannulation) leads to basicity decrease, facilitating the hydrolysis of the CF_2 group in difluorocyclopropene to afford reactive cyclopropenone, on the one hand, and suppressing dimerization and heterodienophilicity of EWG-deactivated 1-hydroxyindolizine, on the other hand. The kinetic balance (fine-tuning) of these competing processes is responsible for the reaction rate and the product yields, which varied from 45 to 75%. Additionally, in the course of this study, the reactivity of tetrazines as a net "hydrazine donor" for monoalkyl substituted cyclopropenones has been discovered.

EXPERIMENTAL SECTION

General Information. Unless otherwise stated, reagents and solvents were purchased from commercial suppliers (Sigma-Aldrich, Apollo Scientific, Fisher, Acros Organics, and Alfa Aesar) and used without further purification. Ethyl alcohol contained 4 vol % water. Chromatography solvents were of HPLC grade and used without further purification.

HPLC chromatography was performed on a C18 reverse-phase HPLC Column YMC-Pack Pro C18 150 × 20 mm^2, particle size 10 μm, pore size 12 nm with a gradient elution, using as a mobile phase various ratios of H_2O/MeCN with 0.1% formic acid. Flow rate was 15 mL/min, and elution time was 20 min. Analytical TLC was performed

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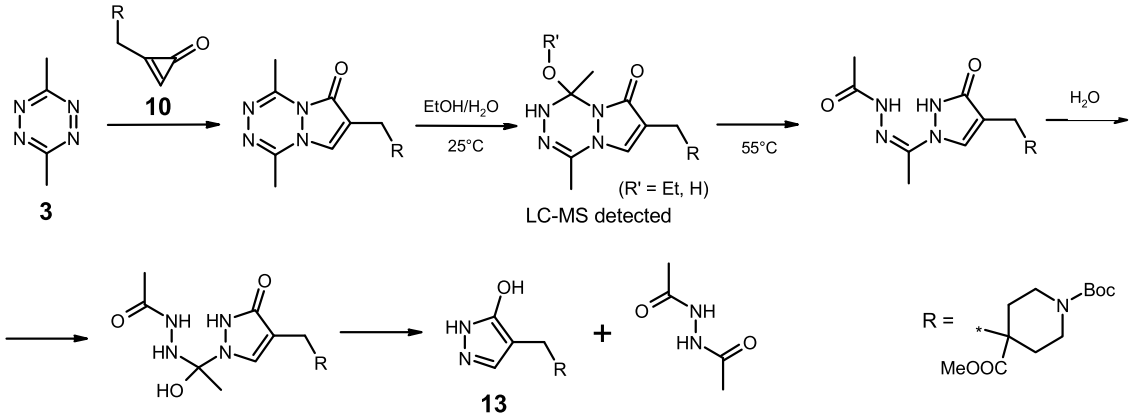
J. Org. Chem. XXXX, XXX, XXX−XXX

Scheme 6. 3-Hydroxypyrazole 13 Byproduct Formation Mechanism



using TLC silica gel 60 F_{254} plates (Merck). Column chromatography was performed on a Combiflash RF200 using Silica RediSep-Rf disposable columns.

^{1}H and ^{13}C NMR (with carbon−proton interaction decoupling) spectra were recorded on a Varian MERCURY plus 400 MHz spectrometer or a Bruker AVANCE II 300 MHz spectrometer using an internal deuterium lock at 50 °C unless otherwise stated. Chemical shifts are given in parts per million (ppm) relative to the residual solvent peak $CDCl_3$: (^{1}H NMR 7.26 ppm, ^{13}C NMR 77.0 ppm), DMSO-d_6: (^{1}H NMR 2.50 ppm, ^{13}C NMR 39.5 ppm), CD_3OD: ^{1}H NMR 3.25 ppm. In ^{13}C NMR of deuterium-containing compounds, $Cr(acac)_3$ was added as a relaxation agent. Proton and carbon shifts were additionally determined using HH and CH correlations—correlation spectroscopy (COSY), HSQC, and HMBC (over ranges of two and three bonds: $^2J_{H\text{-}C\text{-}C}$, $^3J_{H\text{-}C\text{-}C\text{-}C}$). Structural assignments were made with additional information from gCOSY, gHSQC, and gHMBC experiments.

LC-MS spectra were recorded on an Agilent 1100 Series instrument using a Phenomenex Onyx Monolithic C18, 25 × 4.6 mm^2 column detected by a photodiode array (PDA) detector at 275 ± 60 nm and by mass spectrometry (MS) APCI in positive ions. Mobile phase: A, 0.1% solution of formic acid in water; B, acetonitrile. Flow rate was 1.5 mL/min, T_{column} was 25 °C, and injection volume was 5 μL. LC-MS spectra of reaction mixtures and starting materials were recorded on Thermo Fisher Scientific Surveyor MSQ instrument using the Phenomenex Onyx Monoliythic C18 25 × 4.6 mm^2 detected by PDA at 200−800 nm, MS APCI in positive and negative ions, and an evaporative light-scattering detector (ELSD) PL-ELS 2100.

High-resolution mass spectra (HRMS) were registered on a Bruker micrOTOF-Q II hybrid quadrupole time-of-flight mass spectrometer using electrospray ionization (ESI); measurements were performed in the positive ion mode. Voltage on the capillary 4500 V; range of scanned masses m/z 50−3000; external calibration (Electrospray Calibrant Solution; Fluka, Germany); nebulizer pressure 0.4 bar; flow rate 3 μL/min; nitrogen as dry gas (6 L/min); interface temperature 180 °C. The samples were injected into the mass spectrometer chamber using a syringe injection or from the Agilent 1260 HPLC system equipped with an Agilent Poroshell 120 EC-C18 column (50 × 3.0 mm^2; particle size 2.7 μm) and an identically packed security guard using an autosampler. The samples were dissolved in acetonitrile (LC-MS grade; Panreac, Spain). The column was eluted with a gradient of acetonitrile (A) concentrations in water (MilliQ ultrapure water; Merck Millipore KGaA, Germany) with a flow rate of 400 μL/min in the following gradient parameters: 0−15% A for 6 min, 15−85% A for 1.5 min, 85−0% A for 0.1 min, and 0% A for 2.4 min.

All solvent mixtures are quoted as volumes prior to mixing (v/v).

Starting Materials: Synthetic Procedures. *4-Propargyl-N-Boc-4-methoxycarbonylpiperidine (**1**)—(CAS - 2199503-30-3).* To a stirred solution of *N*-Boc-piperidine-4-carboxylic acid methyl ester (10 mmol, 1.43 g, 1 equiv) in THF (30 mL), freshly prepared lithiumdiisopropylamide (LDA; 1 M solution in THF, 12 mL, 1.2 equiv) was added at −60 °C over 5 min. The mixture was stirred for 0.5 h, and then, propargyl bromide (1.4 g, 1.2 equiv) in THF (5 mL) was added dropwise. The reaction mixture was allowed to warm to 25 °C over 1 h. The reaction was quenched with saturated aqueous NH_4Cl (10 mL) and extracted with EtOAc (2 × 15 mL). The combined organic extracts were concentrated under reduced pressure, and the residue was purified on SiO_2, using hexane/EtOAc (8:2) as the eluent to afford **1** as a slowly crystallizing colorless liquid. Yield 90% (2.5 g).

^{1}H NMR (400 MHz, $CDCl_3$): δ 3,81 (m, ^2J ∼ 14 Hz, 2H), 3.72 (s, 3H), 3.00 (ddd, J = 13.9, 11.0, 3.0 Hz, 2H), 2.42 (d, J = 2.7 Hz, 2H), 2.10 (m, ^2J ∼ 13.6 Hz, 2H), 2.02 (t, J = 2.7 Hz, 1H), 1.55 (ddd, J = 13.6, 11.0, 4.4 Hz, 2H), 1.44 (s, 9H).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 174.7, 154.8, 79.5, 79.2, 71.4, 52.0, 45.4, 41.1 (br.), 32.3, 29.0, 28.4.

mp = 54−56 °C.

MS: Surveyor MSQ (APCI) m/z: 282.4 (M^+), 267.4 (M^+ − t-Bu + MeCN), 226.6 (M^+ − t-Bu), 182.2 (M^+ − Boc).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{15}H_{24}NO_4$ 282.1706; found: 282.1701.

*Hex-5-ynoic Acid Methyl Ester (**1a**).* Hex-5-ynoic acid (1.0 g, 9 mmol, 1 equiv) was dissolved in DMF (20 mL). Anhydrous K_2CO_3 (1.6 g, 1.3 equiv) was then added, and the suspension was cooled (ice/water bath). Iodomethane (1.9 g, 1.5 equiv) was added in one portion, and the reaction mixture was stirred at +5 °C for 5 h. TLC (hexane/EtOAc = 9:1, staining with aq $KMnO_4$) indicated full conversion, and the mixture was diluted with water (100 mL) and extracted with EtOAc (2 × 20 mL). Organic extracts were combined and carefully concentrated under reduced pressure (**1a** is volatile!), and the crude product was purified by flash chromatography on SiO_2 using EtOAc/hexane (1:15) as the eluent to afford **1a** as a light-yellow oil. Yield = 70% (0.9 g).

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.67 (s, 3H), 2.45 (t, J = 7.4 Hz, 2H), 2.26 (td, J = 6.9, 2.7 Hz, 2H), 1.95 (t, J = 2.7 Hz, 1H), 1.85 (p, J = 7.1 Hz, 2H).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 173.4, 83.2, 69.0, 51.4, 32.7, 23.7, 17.9.

MS: Surveyor MSQ (APCI), Agilent 1100 (APCI), HRMS (ESI-TOF) m/z: not observable.

*4-(1-Deuteropropargyl)-N-Boc-4-methoxycarbonylpiperidine (**1-D**).* 4-Propargyl-*N*-Boc-4-methoxycarbonylpiperidine **1** (2.8 g, 10 mmol, 1 equiv) was dissolved in 20 mL of MeCN. Anhydrous K_2CO_3 (3.45 g, 2.5 equiv) was then added, and the suspension was stirred at r.t. for 2 h. Deuterium oxide (4 mL, 20 equiv) was added thereto, and the reaction mixture was stirred for 24 h at r.t. LC-MS analysis showed deutero enrichment, and the reaction mixture was diluted with EtOAc (50 mL). The organic phase was decanted and volatiles were removed under reduced pressure to afford **1**-D with no

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J. Org. Chem. XXXX, XXX, XXX−XXX

additional purification necessary. According to NMR, 90% deuteration was achieved. Yield = 99% (2.8 g).

^{1}H NMR (400 MHz, $CDCl_3$): δ 3,82 (m, $^2J \sim 14$ Hz, 2H), 3.74 (s, 3H), 3.00 (ddd, J = 13.9, 11.0, 3.0 Hz, 2H), 2.44 (s, Hz, 2H), 2.11 (m, $^2J \sim$13.6 Hz, 2H), 1.56 (ddd, J = 13.6, 11.0, 4.4 Hz, 2H), 1.46 (s, 9H).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 174.7, 154.7, 79.4, 78.7 (t, $^2J_{C-D}$ = 7.5 Hz), 71.2 (t, $^1J_{C-D}$ = 38 Hz), 51.9, 45.3, 41.0 (br.), 32.2, 28.9, 28.3.

MS: Surveyor MSQ (APCI) m/z: 283.4 (M^+), 268.3 (M^+ − t-Bu + MeCN), 227.3 (M^+ − t-Bu), 183.2 (M^+ − Boc).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{15}H_{23}NO_4D$ 283.1768; found: 283.1751.

*4-(3,3-Difluorocycloprop-1-enylmethyl)-N-Boc-4-methoxycarbonylpiperidine (**2**).* 4-Propargyl-*N*-Boc-4-methoxycarbonylpiperidine **1** (2 g, 7.1 mmol, 1 equiv) was dissolved in dry THF (15 mL), and finely ground NaI (0.38 g, 0.36 equiv) was added at room temperature. The reaction vessel was degassed by sequential evacuation and releasing vacuum with argon (three times). Then, the reaction vessel was placed in a preheated (70 °C) oil bath and 2 M solution of TMS-CF_3 in THF (12.5 mL, 3.5 equiv) was pumped over 6 h upon stirring and left at reflux for 40 h. After reaction completion according to LC-MS and TLC (hexane/EtOAc = 7:3, R_f (**1**) ∼ 0.60, R_f (**2**) ∼ 0.55), volatiles were removed under reduced pressure and the crude product was purified by chromatography on SiO_2 using EtOAc/hexane (3:7) as the eluent to afford **2** as a light-yellow oil, which slowly crystallized on standing. Yield = 99% (2.3 g).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30 (tt, J = 1.9, 0.9 Hz, 1H), 3.82 (m, $^2J \sim$14 Hz, 2H), 3.73 (s, 3H), 3.05 (ddd, J = 13.9, 10.8, 3.0 Hz, 2H), 2.76 (td, J = 2.6, 0.8 Hz, 2H), 2.14 (m, $^2J \sim$ 13.6 Hz, 2H), 1.48 (ddd, J = 13.6, 11.0, 4.4 Hz, 2H), 1.45 (s, 9H).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 174.5, 154.8, 134.4 (t, $^2J_{C-F}$ = 11.2 Hz), 119.6 (t, $^2J_{C-F}$ = 12.1 Hz), 101.7 (t, $^1J_{C-F}$ = 270.7 Hz), 79.7, 52.2, 44.5, 40.9 (br.), 33.9, 32.9, 28.4.

mp = 65−66 °C.

MS: Surveyor MSQ (APCI) m/z: 317.4 (M^+ − t-Bu + MeCN), 267.3 (M^+ − 65), 256.3 (M^+ − 76).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{16}H_{24}F_2NO_4$ 332.1674; found: 332.1662.

*4-(3,3-Difluorocycloprop-1-enyl)-butyric Acid Methyl Ester (**2a**).* Hex-5-ynoic acid methyl ester **1a** (0.9 g, 5 mmol, 1 equiv) was dissolved in dry THF (15 mL), and finely ground NaI (0.27 g, 0.36 equiv) was added at room temperature. The reaction vessel was degassed by sequential evacuation and releasing vacuum with argon (three times). Then, the reaction vessel was placed in a preheated (70 °C) oil bath and 2M solution of TMS-CF_3 in THF (12.5 mL, 3.5 equiv) was pumped over 6 h upon stirring and left at reflux for 18 h. After reaction completion according to TLC (hexane/EtOAc = 9:1, R_f (**1a**) ∼ 0.60, R_f (**2a**) ∼ 0.42), volatiles were removed under reduced pressure and the crude product was purified by chromatography on SiO_2 using EtOAc/hexane (2:8) as the eluent to afford **2a** as a light-yellow oil. Yield = 75% (0.65 g).

Product **2a** showed partial (∼30%) decomposition after two weeks of storage at −20 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.24 (tt, J = 1.8, 1.0 Hz, 1H), 3.66 (s, 3H), 2.56 (ttd, J = 7.3, 2.6, 0.9 Hz, 2H), 2.40 (t, J = 7.3 Hz, 2H), 1.95 (p, J = 7.5 Hz, 2H).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 172.6, 137.1 (t, $^2J_{C-F}$ = 10.1 Hz), 117.4 (t, $^2J_{C-F}$ = 11.5 Hz), 102.2 (t, $^1J_{C-F}$ = 269.7 Hz), 51.2, 32.5, 22.7, 21.4.

MS: Surveyor MSQ (APCI), Agilent 1100 (APCI) m/z: not observable.

HRMS (ESI-TOF): m/z: $[M + H]^+$ calcd for $C_8H_{11}F_2O_2$ − 2(HF) + H_2O 155.0708; found: 155.0704.

*4-(2-Deutero-3,3-difluorocycloprop-1-enylmethyl)-N-Boc-4-methoxycarbonylpiperidine (**2-D**).* The product was prepared from 4-(1-deuteropropargyl)-*N*-Boc-4-methoxycarbonylpiperidine **1**-D (1.4 g, 5 mmol.) according to the procedure described for **2** and was isolated as a light-yellow oil. Yield = 90% (1.5 g), 90% ^{2}H.

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.82 (m, $^2J \sim$ 14 Hz, 2H), 3.73 (s, 3H), 3.05 (ddd, J = 13.9, 10.8, 3.0 Hz, 2H), 2.75 (t, J = 2.6 Hz, 2H), 2.14 (m, $^2J \sim$ 13.6 Hz, 2H), 1.48 (ddd, J = 13.6, 11.0, 4.4 Hz, 2H), 1.45 (s, 9H).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 174.4, 154.7, 134.0 (t, $^2J_{C-F}$ = 11 Hz), 119.3 (tt, $^1J_{C-D}$ = 34.6 Hz, $^2J_{C-F}$ = 11.6 Hz), 101.5 (t, $^1J_{C-F}$ = 270.5 Hz), 79.6, 52.1, 40.8 (br.), 33.8, 32.8, 28.4.

MS: Surveyor MSQ (APCI) m/z: 318.5 (M^+ − t-Bu + MeCN), 267.1 (M^+ − 65), 257.4 (M^+ − 76), 233.3 (M^+ − Boc).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{16}H_{23}F_2NO_4D$ 333.1736; found: 333.1737.

*4-(3-Oxocycloprop-1-enylmethyl)-N-Boc-4-methoxycarbonylpiperidine (**10**).* 4-(3,3-Difluorocycloprop-1-enylmethyl)-*N*-Boc-4-methoxycarbonylpiperidine **2** (330 mg, 1 mmol, 1 equiv) was dissolved in hexafluoroisopropanol (HFIP) (3.5 mL), and H_2O (0.35 mL) was then added at room temperature upon stirring. After 20 min, volatiles were removed under reduced pressure and the crude product was purified by flash chromatography on SiO_2 using EtOAc as the eluent (R_f (**10**) ∼ 0.4) to afford **10** as a light-pink oil. Yield = 70% (215 mg).

^{1}H NMR (400 MHz, CD_3OD): δ 8,68 (s, 1H), 3.73 (m, $^2J \sim$ 14 Hz, 2H), 3.68 (s, 3H), 3.08 (ddd, J = 13.7, 10.3, 3.2 Hz, 2H), 2.97 (s, 2H), 2.12 (m, $^2J \sim$ 14 Hz, 2H), 1.54 (ddd, J = 14.1, 10.3, 4.2 Hz, 2H), 1.39 (s, 9H).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.44 (s, 1H), 3.81 (m, $^2J \sim$ 14 Hz, 2H), 3.74 (s, 3H), 3.08 (ddd, J = 13.7, 10.6, 3.2 Hz, 2H), 2.90 (s, 2H), 2.20 (m, $^2J \sim$ 14 Hz, 2H), 1.54 (ddd, J = 14.1, 10.3, 4.2 Hz, 1H), 1.45 (s, 9H).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 174.3, 166.9, 156.2, 154.7, 149.8, 79.8, 52.4, 44.6, 40.8 (br.), 37.3, 32.9, 28.4.

MS: Surveyor MSQ (APCI) m/z: 310.4 (M^+), 254.3 (M^+ − t-Bu), 210.2 (M^+ − Boc).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{16}H_{24}NO_5$ 310.1655; found: 310.1648.

*4-(2-Deutero-3-oxocycloprop-1-enylmethyl)-N-Boc-4-methoxycarbonylpiperidine (**10-D**).* The product was prepared from 4-(2-deutero-3,3-difluorocycloprop-1-enylmethyl)-*N*-Boc-4-methoxycarbonylpiperidine **2**-D (0.66 g, 2 mmol, 1 equiv) according to the procedure described for **10** and was isolated as a light-pink oil. Yield = 90% (0.43 g), 90% ^{2}H.

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.80 (m, $^2J \sim$ 14 Hz, 2H), 3.73 (s, 3H), 3.07 (ddd, J = 13.7, 10.6, 3.2 Hz, 2H), 2.89 (s, 2H), 2.20 (m, $^2J \sim$ 14 Hz, 2H), 1.53 (ddd, J = 13.7, 10.6, 4.3 Hz, 2H), 1.44 (s, 9H).

^{1}H NMR (400 MHz, CD_3OD): δ 3.73 (m, $^2J \sim$ 14 Hz, 2H), 3.68 (s, 3H), 3.08 (ddd, J = 13.7, 10.3, 3.2 Hz, 2H), 2.97 (s, 2H), 2.12 (dddd, J = 13.6, 5.1, 3.3, 1.5 Hz, 2H), 1.54 (ddd, J = 14.1, 10.3, 4.2 Hz, 2H), 1.40 (s, 9H).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 174.2, 166.5, 156.0, 154.6, 149.4 (t, $^1J_{C-D}$ = 32.1 Hz), 79.7, 52.3, 44.5, 40.7 (br.), 37.2, 32.8, 28.3.

MS: Surveyor MSQ (APCI) m/z: 311.4 (M^+), 255.3 (M^+ − t-Bu), 211.2 (M^+ − Boc); Agilent 1100 (APCI) m/z = 228.0, 210.0, 182.0.

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{15}H_{25}NO_4$ 311.1718; found: 311.1702.

*General Three-Component Reaction Procedure (Synthesis of Compounds **9**, **14**−**26**).* A 20 mL screw-cap clear vial was charged with 4-(3,3-difluorocycloprop-1-enylmethyl)-*N*-Boc-4-methoxycarbonylpiperidine (**2**) (330 mg, 1 mmol, 1 equiv), 3,5-dimethyltetrazine (**8**) (110 mg, 1 equiv), and appropriate pyridine or (iso)quinoline (1 mmol, 1 equiv) followed by addition of ethanol (10 mL) and water (0.1 mL). The reaction mixture was evacuated and the vacuum was released with argon (3 times); then, it was sealed and heated with stirring at 65 °C for 18−48 h. The red color of the reaction mixture gradually disappeared, indicating that tetrazine was consumed. Full conversion of **2** was ensured according to LC-MS. Volatiles were removed under reduced pressure, and the residue was redissolved in a minimal amount of MeCN (∼2 mL) for purification by HPLC with gradient elution from 25 to 55% MeCN (20 min). Alternatively, column chromatography on SiO_2 may be performed, with gradient elution from EtOAc to EtOAc/MeOH (9:1) for the compounds **9**, **14**−**19**, **22**, **23**, **25**, **29**, and **30**. Solvents from the collected fractions

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J. Org. Chem. XXXX, XXX, XXX−XXX

were evaporated to provide compounds **9** and **14**−**26** as amorphous solids.

*4-[2-(5-Methyl-4H-[1,2,4]triazol-3-yl)-3-oxo-3-(4-[1,2,4]triazin-3-yl-pyridin-2-yl)-propyl]-N-Boc-4-methoxycarbonylpiperidine **(9)**.* The product was obtained from **2** (330 mg, 1 mmol), 3,5-dimethyltetrazine **3** (110 mg, 1 equiv), and 3-pyridin-4-yl-[1,2,4]-triazine **5** (158 mg, 1 equiv) and was isolated as a gray foam. Yield = 50% (268 mg).

^{1}H NMR (300 MHz, DMSO-d_6): δ 9.49 (d, J = 2.4 Hz, 1H), 9.01 (d, J = 2.4 Hz, 1H), 8.94 (dd, J = 5.0, 0.8 Hz, 1H), 8.84 (t, J = 1.8, 0.8 Hz, 1H), 8.51 (dd, J = 5.0, 1.8 Hz, 1H), 5.60 (dd, J = 7.5, 4.9 Hz, 1H), 3.69 (m, $^2J \sim$ 14 Hz, $^3J \sim$ 4.5 Hz, 2H), 3.50 (s, 3H), 2.86 (m, 2H), 2.57 (dd, J = 14.4, 7.5 Hz, 1H), 2.28 (dd, J = 14.4, 4.9 Hz, 1H), 2.23 (s, 3H), 2.02 (m, 2H), 1.45 (m, 2H), 1.40 (s, 9H).

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.27 (d, J = 2.4 Hz, 1H), 9.05 (dd, J = 1.7, 0.8 Hz, 1H), 8.85 (dd, J = 5.0, 0.8 Hz, 1H), 8.75 (d, J = 2.4 Hz, 1H), 8.53 (dd, J = 5.0, 1.7 Hz, 1H), 5.79 (dd, J = 7.5, 5.3 Hz, 1H), 3.82 (m, 2H), 3.54 (s, 3H), 2.86 (m, 1H), 2.79 (m, 1H), 2.71 (dd, J = 14.4, 7.5 Hz, 1H), 2.37 (s, 3H), 2.33 (dd, J = 14.4, 5.3 Hz, 1H), 2.13 (m, 2H), 1.44 (m, 2H), 1.42 (s, 9H).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 196.6, 175.1, 162.0, 158.7 (br.), 156.1 (br.), 154.7, 153.4, 149.9, 148.8, 148.8, 143.5, 125.1, 121.2, 79.5, 51.8, 45.4, 41.3 (br.), 40.4, 40.0, 33.7, 33.5, 28.5, 12.8.

MS: Agilent 1100 (APCI) m/z: 537.1 (M^+), 481.1 (M^+ − t-Bu), 437.1 (M^+ − Boc).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{26}H_{33}N_8O_5$ 537.2574; found: 537.2566.

*4-[3-(4-Ethoxycarbonylpyridin-2-yl)-2-(5-methyl-4H-[1,2,4]-triazol-3-yl)-3-oxopropyl]-N-Boc-4-methoxycarbonylpiperidine **(14)**.* The product was obtained from **2** (330 mg, 1 mmol), 3,5-dimethyltetrazine **3** (110 mg, 1 equiv), and ethylisonicotinate (151 mg, 1 equiv) and was isolated as a brownish foam. Yield = 50% (264 mg).

^{1}H NMR (400 MHz, DMSO-d_6)—major tautomer (80%): δ 13.1 (s, 1H), 8.88 (d, J = 5.0 Hz, 1H), 8.27 (dd, J = 1.6, 0.8 Hz, 1H), 8.01 (dd, J = 5.0, 1.6 Hz, 1H), 5.51 (dd, J = 8.4, 3.9 Hz, 1H), 4.38 (q, J = 7.1 Hz, 2H), 3.68 (m, 2H), 3.41 (s, 3H), 2.74 (m, 2H), 2.49 (m, $^3J \sim$ 8 Hz, 1H), 2.21 (s, 3H), 2.15 (dd, J = 14.3, 3.9 Hz, 1H), 2.02 (m, 1H), 1.92 (m, 1H), 1.36 (s, 9H), 1.35 (m, 2H), 1.34 (t, J = 7.1 Hz, 3H).

^{1}H NMR (400 MHz, $CDCl_3$): δ 10.88 (br. s, 1H), 8.81 (dd, J = 4.9, 0.8 Hz, 1H), 8.54 (t, J = 1.7, 0.8 Hz, 1H), 8.00 (dd, J = 4.9, 1.7 Hz, 1H), 5.73 (dd, J = 7.5, 5.2 Hz, 1H), 4.43 (q, J = 7.1 Hz, 2H), 4.43 (q, J = 7.1 Hz, 2H), 3.83 (m, 2H), 3.55 (s, 3H), 2.87 (m, 1H), 2.80 (m, 1H), 2.70 (dd, J = 14.4, 7.5 Hz, 1H), 2.37 (s, 3H), 2.32 (dd, J = 14.4, 5.2 Hz, 1H), 2.13 (m, 2H), 1.45 (m, 2H), 1.44 (s, 9H), 1.41 (t, J = 7.1 Hz, 3H).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 196.8, 175.3, 164.3, 159.2 (br.), 155.9 (br.), 154.8, 153.3, 149.8, 139.3, 126.3, 122.1, 79.5, 62.1, 51.7, 45.3, 41.2 (br.), 40.4, 39.9, 33.6, 33.4, 28.4, 14.1, 12.5.

^{15}N NMR (30 MHz, $CDCl_3$) δ 323.5, 251.7, 246.0, 231.3, 86.1.

MS: Agilent 1100 (APCI) m/z: 530.0 (M^+), 473.9 (M^+ − t-Bu), 429.9 (M^+ − Boc).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{26}H_{36}N_5O_7$ 530.2615; found: 530.2605.

*4-[3-(4-Carbamoylpyridin-2-yl)-2-(5-methyl-4H-[1,2,4]triazol-3-yl)-3-oxopropyl]-N-Boc-4-methoxycarbonylpiperidine **(15)**.* The product was obtained from **2** (330 mg, 1 mmol), 3,5-dimethyltetrazine **3** (110 mg, 1 equiv), and isonicotinamide (122 mg, 1 equiv) and was isolated as a white foam. Yield = 70% (350 mg).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.82 (dd, J = 5.0, 0.8 Hz, 1H), 8.33 (dd, J = 1.7, 0.8 Hz, 1H), 8.33 (br. s, 1H), 7.98 (dd, J = 5.0, 1.7 Hz, 1H), 7.69 (br. s, 1H), 5.57 (dd, J = 8.0, 4.4 Hz, 1H), 3.70 (m, 2H), 3.43 (s, 3H), 2.78 (m, 1H), 2.73 (m, 1H), 2.47 (m, $^2J \sim$ 14 Hz, $^3J \sim$ 8 Hz, 1H), 2.21 (s, 3H), 2.16 (dd, J = 14.3, 4.5 Hz, 1H), 2.02 (m, 1H), 1.95 (m, 1H), 1.38 (s, 9H), 1.35 (m, 2H).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.75 (d, J = 4.9 Hz, 1H), 8.31 (br. d, J = 1.6 Hz, 1H), 7.90 (dd, J = 4.9, 1.6 Hz, 1H), 5.74 (dd, J = 7.2, 5.5 Hz, 1H), 7.83 (m, 2H), 3.54 (s, 3H), 2.86 (m, 1H), 2.79 (m, 1H), 2.63 (dd, J = 14.4, 7.2 Hz, 1H), 2.39 (s, 3H), 2.29 (dd, J = 14.4, 5.3 Hz, 1H), 2.11 (m, 2H), 1.44 (s, 9H), 1.43 (m, 2H).

^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$, 25 °C): δ = 197.0, 175.3, 167.1, 158.7 (br.), 155.2, 154.9, 152.5, 150.1, 141.9, 125.7, 120.2, 79.8, 52.0, 45.2, 41.4 (br.), 40.9 (br.), 40.1, 39.9, 33.4 (br.), 28.4, 12.0.

MS: Agilent 1100 (APCI) m/z: 501.1 (M^+), 445.1 (M^+ − t-Bu), 401.1 (M^+ − Boc).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{24}H_{33}N_6O_6$ 501.2462; found: 501.2457.

*4-[3-(4-Acetyl-pyridin-2-yl)-2-(5-methyl-2H-[1,2,4]triazol-3-yl)-3-oxopropyl]-N-Boc-4-methoxycarbonylpiperidine **(16)**.* The product was obtained from **2** (330 mg, 1 mmol), 3,5-dimethyltetrazine **3** (110 mg, 1 equiv), and 4-acetylpyridine (121 mg, 1 equiv) and was isolated as a brownish foam. Yield = 75% (375 mg).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.86 (dd, J = 5.0, 0.8 Hz, 1H), 8.43 (dd, J = 1.7, 0.8 Hz, 1H), 7.90 (dd, J = 5.0, 1.7 Hz, 1H), 5.74 (dd, J = 7.5, 5.3 Hz, 1H), 3.84 (m, 2H), 3.57 (s, 3H), 2.88 (m, 1H), 2.81 (m, 1H), 2.70 (dd, J = 14.4, 7.5 Hz, 1H), 2.66 (s, 3H), 2.39 (s, 3H), 2.32 (dd, J = 14.4, 5.3 Hz, 1H), 2.13 (m, 2H), 1.44 (s, 9H), 1.43 (m, 2H).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 197.0, 196.4, 175.3, 159.3 (br.), 155.9 (br.), 154.8, 153.7, 150.2, 144.2, 124.4, 120.6, 79.5, 51.7, 45.3, 41.2 (br.), 40.4, 39.8, 33.6, 33.4, 28.4, 26.6, 12.5.

MS: Agilent 1100 (APCI) m/z: 500.0 (M^+), 443.9 (M^+ − t-Bu), 400.0 (M^+ − Boc).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{25}H_{34}N_5O_6$ 500.2509; found: 500.2503.

*4-[3-Isoquinolin-1-yl-2-(5-methyl-4H-[1,2,4]triazol-3-yl)-3-oxopropyl]-N-Boc-4-methoxycarbonylpiperidine **(17)**.* The product was obtained from **2** (330 mg, 1 mmol), 3,5-dimethyltetrazine **3** (110 mg, 1 equiv), and isoquinoline (129 mg, 1 equiv) and was isolated as a greenish foam. Yield = 55% (280 mg).

^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.58 (m, 2H), 8.01 (m, 2H), 7.79 (ddd, J = 8.2, 6.9, 1.4 Hz, 1H), 7.72 (ddd, J = 8.2, 6.9, 1.5 Hz, 1H), 5.61 (dd, J = 6.7, 5.4 Hz, 1H), 3.69 (m, 2H), 3.50 (s, 3H), 2.86 (m, 2H), 2.59 (dd, J = 14.4, 6.8 Hz, 1H), 2.27 (dd, J = 14.4, 5.4 Hz, 1H), 2.17 (s, 3H), 2.03 (m, $J^2 \sim$ 14 Hz, 2H), 1.42 (m, 2H), 1.40 (s, 9H).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.89 (d, J = 8.4 Hz, 1H), 8.53 (d, J = 5.5 Hz, 1H), 7.80 (dd, J = 8.0, 1.4 Hz, 1H), 7.75 (d, J = 5.5 Hz, 1H), 7.66 (ddd, J = 8.2, 6.7, 1.4 Hz, 1H), 7.61 (ddd, J = 8.2, 6.8, 1.5 Hz, 1H), 5.87 (t, J = 6.3 Hz, 1H), 3.80 (m, 2H), 3.50 (s, 3H), 2.84 (m, 2H), 2.70 (dd, J = 14.4, 6.7 Hz, 1H), 2.38 (dd, J = 14.4, 5.9 Hz, 1H), 2.31 (s, 3H), 2.13 (m, 2H), 1.44 (m, 2H), 1.42 (s, 9H).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 199.1, 175.4, 158.9, 156.5, 154.8, 151.4, 141.0, 137.1, 130.4, 129.2, 126.9, 126.8, 126.6, 124.9, 79.5, 51.7, 45.4, 42.4, 41.2, 39.6, 33.6, 33.4, 28.4, 12.7.

MS: Agilent 1100 (APCI) m/z: 508.1 (M^+), 452.1 (M^+ − t-Bu).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{27}H_{34}N_5O_5$ 508.2560; found: 508.2559.

*4-[3-(6-Chloroquinolin-2-yl)-2-(5-methyl-4H-[1,2,4]triazol-3-yl)-3-oxopropyl]-N-Boc-4-methoxycarbonylpiperidine **(18)**.* The product was obtained from **2** (330 mg, 1 mmol), 3,5-dimethyltetrazine **3** (110 mg, 1 equiv), and 6-chloroquinoline (164 mg, 1 equiv) and was isolated as a brownish foam. Yield = 50% (270 mg).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17 (d, J = 8.6 Hz, 1H), 8.14 (d, J = 9.0 Hz, 1H), 8.12 (d, J = 8.6 Hz, 1H), 7.84 (d, J = 2.3 Hz, 1H), 7.71 (dd, J = 9.0, 2.3 Hz, 1H), 5.97 (dd, J = 7.2, 5.7 Hz, 1H), 3.84 (m, 2H), 3.53 (s, 3H), 2.90 (m, 1H), 2.82 (m, 1H), 2.70 (dd, J = 14.4, 7.2 Hz, 1H), 2.38 (dd, J = 14.4, 5.7 Hz, 1H), 2.38 (s, 3H), 2.16 (m, 2H), 1.50 (m, 2H), 1.44 (s, 9H).

^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$, 70 °C): δ 197.5, 175.3, 158.9, 156.0, 154.9, 152.1, 145.5, 136.2, 135.0, 132.2, 131.2, 130.2, 126.3, 119.8, 79.5, 51.6, 45.4, 41.2 (br.), 40.2, 40.0, 33.6, 33.5, 28.4, 12.4.

^{15}N NMR (30 MHz, $CDCl_3$): δ 311.6, 240.4, 258.4, 225.6, 85.9.

MS: Agilent 1100 (APCI) m/z: 542.1 (M^+), 486.0 (M^+ − t-Bu), 442.0 (M^+ − Boc).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{27}H_{33}{}^{35}ClN_5O_5$ 542.2170; found: 542.2165.

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J. Org. Chem. XXXX, XXX, XXX−XXX

4-[3-(6-Hydroxyquinolin-2-yl)-2-(5-methyl-4H-[1,2,4]triazol-3-yl)-3-oxopropyl]-N-Boc-4-methoxycarbonylpiperidine ***(19)***. The product was obtained from **2** (330 mg, 1 mmol), 3,5-dimethyltetrazine **3** (110 mg, 1 equiv), and 6-hydroxy-quinoline (145 mg, 1 equiv) and was isolated as a pinkish foam. Yield = 50% (260 mg).

^{1}H NMR (300 MHz, $CDCl_3$, 70 °C): δ 10.86 (br, s, 1H), 7.83 (d, J = 8.6 Hz, 1H), 7.71 (d, J = 8.6 Hz, 1H), 7.64 (d, J = 9.2 Hz, 1H), 7.13 (dd, J = 9.2, 2.6 Hz, 1H), 6.87 (d, J = 2.6 Hz, 1H), 6.05 (t, J = 6.4 Hz, 1H), 3.80 (m, 2H), 3.51 (s, 3H), 2.91 (m, 2H), 2.64 (dd, J = 14.3, 6.4 Hz, 1H), 2.43 (dd, $^3J \sim$ 6.5 Hz, 1H), 2.41 (s, 3H), 2.15 (m, 2H), 1.45 (m, 2H), 1.44 (s, 9H).

^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$, 70 °C): δ 197.3, 175.4, 158.1 (br.), 158.0, 156.4 (br.), 155.1, 148.6, 142.3, 135.1, 132.1, 131.5, 123.1, 119.0, 108.6, 79.9, 51.8, 45.5, 41.3 (br.), 40.0, 39.7, 33.8, 33.3, 28.5, 12.3.

MS: Agilent 1100 (APCI) m/z: 524.2 (M^+), 468.1 ($M^+ - t$-Bu), 424.0 (M^+ − Boc).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{27}H_{34}N_5O_6$ 524.2509; found: 524.2502.

4-[3-(5-Carboxypyridin-2-yl)-2-(5-methyl-4H-[1,2,4]triazol-3-yl)-3-oxo-propyl]-N-Boc-4-methoxycarbonylpiperidine ***(20)***. The product was obtained from **2** (330 mg, 1 mmol), 3,5-dimethyltetrazine **3** (110 mg, 1 equiv), and nicotinic acid (123 mg, 1.1 mmol) and was isolated as a brownish foam. The major isomer had a greater retention time on LC-MS (45/**55**). Yield = 137 mg, 50% (for both isomers).

^{1}H NMR (300 MHz, $CDCl_3$): δ 12.00 (br. s, 2H), 9.22 (d, J = 2.0 Hz, 1H), 8.36 (dd, J = 8.1, 2.0 Hz, 1H), 8.04 (d, J = 8.1 Hz, 1H), 5.83 (dd, J = 7.1, 5.5 Hz, 1H), 3.85 (m, 2H), 3.55 (s, 3H), 2.87 (m, 2H), 2.71 (dd, J = 14.5, 7.1 Hz, 1H), 2.41 (s, 3H), 2.38 (dd, ^3J = 5.5 Hz, 1H), 1.45 (m, 2H), 1.44 (s, 9H).

^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): δ 196.4, 175.3, 166.5, 158.3 (br.), 156.1 (br.), 155.1, 154.6, 150.7, 138.5, 129.6, 122.5, 79.9, 51.8, 45.4, 41.3 (br.), 40.3, 39.4, 33.7, 33.5, 28.5, 12.3.

MS: Agilent 1100 (APCI) m/z: 502.1 (M^+), 446.1 ($M^+ - t$-Bu), 402.1 (M^+ − Boc).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{24}H_{32}N_5O_7$ 502.2295; found: 502.2296.

4-[(R)-2-((R)-1-Hydroxy-3-oxo-1,3-dihydroisobenzofuran-1-yl)-2-(5-methyl-1H-[1,2,4]triazol-3-yl)-ethyl]-N-Boc-4-methoxycarbonyl-piperidine ***(21)***. The product was obtained from **2** (330 mg, 1 mmol), 3,5-dimethyltetrazine **3** (110 mg, 1 equiv), and nicotinic acid (123 mg, 1 equiv) and was isolated as a white foam. The minor isomer had a smaller retention time on LC-MS (**45**/55). Yield = 110 mg, 50% (for both isomers).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.73 (dd, J = 4.8, 1.5 Hz, 1H), 8.52 (br. s, 2H), 8.11 (dd, J = 7.8, 1.5 Hz, 1H), 7.47 (dd, J = 7.8, 4.8 Hz, 1H), 4.72 (br. s, 1H), 3.75 (m, 2H), 3.47 (s, 3H), 2.87 (m, 1H), 2.80 (m, 1H), 2.51 (br. d, $^2J \sim$ 14.5 Hz, 1H), 2.39 (br. d, $^2J \sim$ 14.5 Hz, 1H) 2.38 (s, 3H), 2.10 (m, $^2J \sim$ 13.5 Hz, 1H), 2.00 (m, $^2J \sim$ 13.5 Hz, 1H), 1.42 (s, 9H), 1.35 (m, 2H).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 175.2, 167.6, 159.2 (br.), 158.6, 155.5, 155.0, 152.3, 136.5, 126.0 (br.), 125.4, 108.9, 79.8, 51.8, 45.2, 42.5, 41.1 (br.), 38.3, 33.5, 33.0, 28.4, 12.0.

MS: Agilent 1100 (APCI) m/z: 501.9 (M^+).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{24}H_{32}N_5O_7$ 502.2302; found: 502.2296.

4-[3-[5-((E)-2-Methoxycarbonylvinyl)-pyridin-2-yl]-2-(5-methyl-2H-[1,2,4]triazol-3-yl)-3-oxopropyl]-N-Boc-4-methoxycarbonylpiperidine ***(22)***. The product was obtained from **2** (330 mg, 1 mmol), 3,5-dimethyltetrazine **3** (110 mg, 1 equiv), and (*E*)-3-pyridin-3-yl acrylic acid methyl ester (163 mg, 1 equiv) along with the minor product (**23**) and was isolated as a brown foam. Major isomer (**83**/17). Yield = 110 mg, 50% (for both isomers).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.98 (d, J = 2.2 Hz, 1H), 8.31 (dd, J = 8.2, 2.2 Hz, 1H), 7.97 (d, J = 8.2 Hz, 1H), 7.75 (d, J = 16.2 Hz, 1H), 6.88 (d, J = 16.2 Hz, 1H), 5.54 (dd, J = 8.1, 4.3 Hz, 1H), 3.76 (s, 3H), 3.70 (m, 2H), 3.44 (s, 3H), 2.75 (m, 2H), 2.49 (m, $^2J \sim$ 14 Hz, $^3J \sim$ 8 Hz, 1H), 2.21 (s, 3H), 2.15 (dd, J = 14.3, 4.3 Hz, 1H), 2.05 (m, 1H), 1.95 (m, 1H), 1.38 (s, 9H), 1.34 (m, 2H).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.77 (d, J = 2.0 Hz, 1H), 8.05 (d, J = 8.1 Hz, 1H), 7.92 (dd, J = 8.1, 2.0 Hz, 1H), 7.68 (d, J = 16.1 Hz, 1H), 6.57 (d, J = 16.1 Hz, 1H), 5.71 (dd, J = 7.5, 5.1 Hz, 1H), 3.83 (s, 3H), 3.83 (m, 2H), 3.54 (s, 3H), 2.86 (m, 1H), 2.80 (m, 1H), 2.68 (dd, J = 14.4, 7.5 Hz, 1H), 2.36 (s, 3H), 2.30 (dd, J = 14.4, 5.1 Hz, 1H), 2.12 (m, 2H), 1.43 (m, 2H), 1.43 (s, 9H).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 196.6, 175.3, 166.3, 158.9 (br.), 156.4 (br.), 154.8, 152.9, 148.8, 139.8, 135.3, 133.7, 123.0, 122.4, 79.5, 52.0, 51.7, 45.3, 41.2 (br.), 40.2, 39.9, 33.6, 33.4, 28.4, 12.7.

MS: Agilent 1100 (APCI) m/z: 542.2 (M^+), 486.1 ($M^+ - t$-Bu), 432.0 (M^+ − Boc).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{27}H_{36}N_5O_7$ 542.2615; found: 542.2608.

4-[3-[3-((E)-2-Methoxycarbonylvinyl)-pyridin-2-yl]-2-(5-methyl-4H-[1,2,4]triazol-3-yl)-3-oxopropyl]-N-Boc-4-methoxycarbonylpiperidine ***(23)***. The minor isomer (83/**17**) was not isolated as a pure compound, and nonoverlapping signals of the ^{1}H NMR aromatic region (6−9 ppm) are given.

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.66 (dd, J = 4.7, 1.5 Hz, 1H), 8.22 (dd, J = 8.0, 1.6 Hz, 1H), 7.98 (d, J = 16.0 Hz, 1H), 7.61 (dd, J = 8.0, 4.6 Hz, 1H), 6.56 (d, J = 16.0 Hz, 1H), 5.44 (dd, J = 7.4, 4.7 Hz, 1H).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.60 (d, J = 4.4 Hz, 1H), 8.23 (d, J = 16.0 Hz, 1H), 7.83 (d, J = 7.8 Hz, 1H), 7.41 (dd, J = 7.8, 4.4 Hz, 1H), 6.23 (d, J = 16.0 Hz, 1H).

MS (Mixture with regioisomer **22**): Agilent 1100 (APCI) m/z: 542.2 (M^+), 486.1 ($M^+ - t$-Bu), 432.0 (M^+ − Boc).

HRMS (Mixture with regioisomer **22**) (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{27}H_{36}N_5O_7$ 542.2615; found: 542.2598.

4-{3-(6-Chloroquinolin-2-yl)-2-[5-(4-fluorophenyl)-2H-[1,2,4]-triazol-3-yl]-3-oxopropyl}-N-Boc-4-methoxycarbonylpiperidine ***(24)***. The product was obtained from **2** (330 mg, 1 mmol), 3-(4-fluorophenyl)-6-methyltetrazine **4** (190 mg, 1 equiv), and 6-chloroquinoline (164 mg, 1 equiv) along with the major product (**18**) and was isolated as a brown foam. Minor product (70/**30**). Yield = 93 mg, 50% (for both products).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19 (d, J = 8.6 Hz, 1H), 8.13 (d, J = 8.6 Hz, 1H), 8.12 (d, J = 9.0 Hz, 1H), 8.00 (m, 2H), 7.84 (d, J = 2.3 Hz, 1H), 7.70 (dd, J = 9.0, 2.3 Hz, 1H), 7.06 (m, 2H), 6.01 (t, J = 6.5 Hz, 1H), 3.84 (m, 2H), 3.48 (s, 3H), 2.97 (m, 1H), 2.85 (m, 1H), 2.72 (dd, J = 14.5, 6.5 Hz, 1H), 2.52 (dd, J = 14.5, 6.5 Hz, 1H), 2.25 (m, $^2J \sim$ 14 Hz, 1H), 2.15 (m, $^2J \sim$ 14 Hz, 1H), 1.57 (m, 1H), 1.47 (m, 1H), 1.44 (s, 9H).

^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$, 60 °C): δ 196.7, 175.4, 163.8 (d, $^1J_{C-F}$ = 249.3 Hz), 159.8 (br.), 156.4, 154.9, 151.7, 145.4, 136.6, 135.4, 132.0, 131.6, 130.4, 128.6 (d, $^3J_{C-F}$ = 8.4 Hz), 126.5, 126.1 (br.), 119.9, 115.6 (d, $^2J_{C-F}$ = 21.7 Hz), 79.7, 51.9, 45.3, 41.2 (br.), 40.0, 39.9, 33.9, 33.3, 28.5.

MS: Agilent 1100 (APCI) m/z: 622.1 (M^+), 566.1 ($M^+ - t$-Bu), 522.1 (M^+ − Boc).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{32}H_{34}{}^{35}ClFN_5O_5$ 622.2233; found: 622.2224.

4-[2-(5-Methyl-2H-[1,2,4]triazol-3-yl)-3-oxo-3-(4-trifluoromethyl-pyridin-2-yl)-propyl]-N-Boc-4-methoxycarbonylpiperidine ***(25)***. The product was obtained from **2** (330 mg, 1 mmol), 3-(4-fluorophenyl)-6-methyltetrazine **4** (190 mg, 1 equiv), and 6-chloroquinoline (164 mg, 1 equiv) along with the minor product (**26**) and was isolated as a brownish foam. Major product (**70**/30). Yield = 165 mg, 45% (for both products).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.84 (d, J = 5.0 Hz, 1H), 8.23 (m, 1H), 7.65 (dd, J = 5.0, 1.7 Hz, 1H), 5.72 (dd, J = 7.6, 5.1 Hz, 1H), 3.83 (m, 2H), 3.54 (s, 3H), 2.86 (m, 1H), 2.79 (m, 1H), 2.67 (dd, J = 14.4, 7.6 Hz, 1H), 2.37 (s, 3H), 2.30 (dd, J = 14.4, 5.1 Hz, 1H), 2.12 (m, 2H), 1.44 (m, 2H), 1.42 (s, 9H).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 196.4, 175.3, 159.5 (br.), 155.6 (br.), 154.9, 153.6, 150.1, 139.8 (q, $^2J_{C-F}$ = 34.8 Hz), 122.6 (q, $^3J_{C-F}$ = 3.4 Hz), 122.5 (q, $^1J_{C-F}$ = 273.4 Hz), 118.6 (q, $^3J_{C-F}$ = 3.6 Hz), 79.6, 51.8, 45.3, 41.2 (br.), 40.4, 39.9, 33.6, 33.4, 28.4, 12.4.

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J. Org. Chem. XXXX, XXX, XXX−XXX

MS: Agilent 1100 (APCI) m/z: 526.0 (M^+), 469.9 ($M^+ - t$-Bu), 425.9 (M^+ − Boc).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{24}H_{31}F_3N_5O_5$ 526.2278; found: 526.2268.

4-[2-[5-(4-Fluorophenyl)-2H-[1,2,4]triazol-3-yl]-3-oxo-3-(4-trifluoromethyl-pyridin-2-yl)-propyl]-N-Boc-4-methoxycarbonylpiperidine ***(26)***. The product was obtained from **2** (330 mg, 1 mmol), 3-(4-fluorophenyl)-6-methyltetrazine **4** (190 mg, 1 equiv), and 6-chloroquinoline (164 mg, 1 equiv) along with the major product (**25**) and was isolated as a brown foam. Minor product (70/**30**). Yield = 81 mg, 45% (for both products).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.86 (d, J = 5.0 Hz, 1H), 8.27 (m, 1H), 7.97 (m, 2H), 7.69 (dd, J = 5.0, 1.7 Hz, 1H), 7.07 (m, 2H), 5.78 (t, J = 6.4 Hz, 1H), 3.85 (m, 2H), 3.52 (s, 3H), 2.94 (m, 1H), 2.83 (m, 1H), 2.70 (dd, J = 14.5, 6.7 Hz, 1H), 2.44 (dd, J = 14.5, 6.2 Hz, 1H), 2.20 (m, $^2J \sim$ 14 Hz, 1H), 2.14 (m, $^2J \sim$ 14 Hz, 1H), 1.49 (m, 2H), 1.44 (s, 9H).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 195.5, 175.4, 163.8 (d, $^1J_{C-F}$ = 249.5 Hz), 159.7, 156.7, 154.9, 153.3, 150.1, 140.1 (q, $^2J_{C-F}$ = 34.9 Hz), 128.5 (d, $^3J_{C-F}$ = 8.3 Hz), 126.0 (d, $^4J_{C-F}$ = 3.2 Hz), 123.0 (q, $^3J_{C-F}$ = 3.1 Hz), 122.4 (q, $^1J_{C-F}$ = 273.5 Hz), 118.9 (q, $^3J_{C-F}$ = 3.5 Hz), 115.7 (d, $^2J_{C-F}$ = 21.9 Hz), 79.7, 51.9, 45.2, 41.2 (br.), 40.3, 39.7, 33.7, 33.3, 28.5.

MS: Agilent 1100 (APCI) m/z: 606.2 (M^+), 550.1 ($M^+ - t$-Bu), 506.1 (M^+ − Boc).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{29}H_{32}F_4N_5O_5$ 606.2340; found: 606.2339.

4-[2-(5-Methyl-2H-[1,2,4]triazol-3-yl)-3-oxo-3-pyridin-2-yl-propyl]-N-Boc-4-methoxycarbonylpiperidine ***(27)***. A 20 mL screw-cap clear vial was charged with 4-(3,3-difluoro-cycloprop-1-enylmethyl)-*N*-Boc-4-methoxycarbonylpiperidine **2** (330 mg, 1 mmol, 1 equiv), 3,5-dimethyltetrazine **8** (110 mg, 1 equiv), and pyridine (79 mg, 1 equiv) followed by addition of ethanol (10 mL), water (0.1 mL), and HFIP (340 mg, 2 equiv). The reaction mixture was evacuated and the vacuum was released with argon (three times); then, it was sealed and heated with stirring at 70 °C for 48 h. The red color of the reaction mixture gradually disappeared, indicating that tetrazine was consumed. Full conversion of **2** was ensured according to LC-MS. Volatiles were removed under reduced pressure, and the residue was redissolved in a minimal amount of MeCN (∼2 mL) for purification by HPLC with gradient elution from 20 to 50% MeCN (20 min). Solvents from the collected fractions were evaporated to provide **27** as a light-brown foam. Yield = 60% (274 mg).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.63 (dt, J = 4.7, 1.4 Hz, 1H), 8.00 (dt, J = 7.9, 1.0 Hz, 1H), 7.78 (td, J = 7.7, 1.7 Hz, 1H), 7.42 (ddd, J = 7.6, 4.7, 1.2 Hz, 1H), 5.74 (dd, J = 7.3, 5.5 Hz, 1H), 3.80 (m, 2H), 3.50 (s, 3H), 2.84 (m, 1H), 2.77 (m, 1H), 2.63 (dd, J = 14.4, 7.3 Hz, 1H), 2.35 (s, 3H), 2.29 (dd, J = 14.4, 5.5 Hz, 1H), 2.09 (m, $^2J \sim$ 14 Hz, 2H), 1.41 (s, 9H), 1.40 (m, 2H).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 197.2, 175.2, 158.6, 156.1, 154.8, 152.1, 149.0, 137.0, 127.3, 122.9, 79.6, 51.7, 45.2, 41.1, 40.0, 39.8, 33.5, 33.4, 28.4, 12.4.

MS: Agilent 1100 (APCI) m/z: 458.0 (M^+), 402.0 ($M^+ - t$-Bu), 358.0 (M^+ − Boc).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{23}H_{32}N_5O_5$ 458.2404; found: 458.2405.

4-(8-Hydroxy-3,4-dimethyl-pyrrolo[1,2-a]pyrazin-7-ylmethyl)-N-Boc-4-methoxycarbonylpiperidine ***(28)***. A 20 mL screw-cap clear vial was charged with 4-(3,3-difluoro-cycloprop-1-enylmethyl)-*N*-Boc-4-methoxycarbonylpiperidine **2** (330 mg, 1 mmol, 1 equiv) and 2,3-dimethylpyrazine (119 mg, 1.1 equiv) followed by addition of ethanol (10 mL) and water (0.1 mL). The reaction mixture was heated with stirring at 65 °C for 7 h. Full conversion of **2** was ensured according to LC-MS. Volatiles were removed under reduced pressure, and the residue was purified by column chromatography on SiO_2 (R_f (**28**) ∼ 0.45 in EtOAc, yellow spot (vis.)) with gradient elution from hexane/EtOAc (1/1) to pure EtOAc. Solvent from the collected fractions was evaporated to provide **28** as a dark-yellow foam. Yield = 60% (250 mg).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.53 (s, 1H), 6.91 (s, 1H), 3.71 (dt, J = 13.5, 4.3 Hz, 2H), 3.62 (s, 3H), 2.83 (s, 3H), 2.29 (s, 6H), 1.92 (m, $^2J \sim$ 13.7 Hz, 2H), 1.42 (ddd, J = 14.3, 10.9, 4.2 Hz, 2H), 1.36 (s, 9H).

^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6): δ 174.8, 153.8, 138.5, 136.8, 129.0, 119.7, 115.3, 111.3, 109.2, 78.4, 51.4, 46.5, 40.7 (br.), 32.5, 32.1, 27.9, 19.0, 12.8.

MS: Agilent 1100 (APCI) m/z: 418.1 (M^+).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{22}H_{32}N_3O_5$ 418.2342; found: 418.2337.

6-(6-Chloroquinolin-2-yl)-5-(5-methyl-2H-[1,2,4]triazol-3-yl)-6-oxohexanoic Acid Methyl Ester ***(29)***. The product was obtained from **2a** (176 mg, 1 mmol), 3,5-dimethyltetrazine **3** (110 mg, 1 equiv), and ethylisonicotinate (151 mg, 1 equiv) according to the general 3-CR procedure and was isolated as a dark-yellow foam. Yield = 45% (165 mg).

^{1}H NMR (400 MHz, $CDCl_3$): δ 10.00 (br. s, 1H) 8.04 (m, 3H), 7.75 (d, J = 2.3 Hz, 1H), 7.60 (dd, J = 9.0, 2.3 Hz, 1H), 5.81 (dd, J = 7.8, 6.5 Hz, 1H), 3.57 (s, 3H), 2.37 (s, 3H), 2.36 (t, J = 7.5 Hz, 2H), 2.16 (m, 2H), 1.71 (p, J = 7.5 Hz, 2H).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 198.6, 173.7, 158.1, 156.1, 151.9, 145.3, 136.2, 134.9, 132.1, 131.1, 130.1, 126.2, 119.5, 51.4, 43.8, 33.5, 30.9, 22.7, 12.3.

MS: Agilent 1100 (APCI) m/z: 386.9 (M^+).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{19}H_{20}{}^{35}ClN_4O_3$ 387.1224; found: 387.1218.

2-[5-Methoxycarbonyl-2-(5-methyl-2H-[1,2,4]triazol-3-yl)-pentanoyl]-isonicotinic Acid Ethyl Ester ***(30)***. The product was obtained from **2a** (176 mg, 1 mmol), 3,5-dimethyltetrazine **3** (110 mg, 1 equiv), and 6-chloroquinoline (164 mg, 1 equiv) according to the general 3-CR procedure and was isolated as a dark-yellow foam. Yield = 45% (173 mg).

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.18 (br. s, 1H), 8.75 (dd, J = 4.9, 0.8 Hz, 1H), 8.51 (dd, J = 1.7, 0.8 Hz, 1H), 7.96 (dd, J = 4.9, 1.6 Hz, 1H), 5.56 (dd, J = 7.8, 6.6 Hz, 1H), 4.40 (q, J = 7.1 Hz, 2H), 3.59 (s, 3H), 2.36 (s, 3H), 2.33 (t, J = 7.4 Hz, 2H), 2.14 (m, 2H), 1.68 (p, J = 7.6 Hz, 2H), 1.38 (t, J = 7.1 Hz, 3H).

^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 198.1, 173.7, 164.3, 158.3, 156.4, 153.4, 149.8, 139.2, 126.3, 122.0, 62.1, 51.4, 44.5, 33.6, 30.5, 22.7, 14.1, 12.5.

MS: Agilent 1100 (APCI) m/z: 375.0 (M^+).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{18}H_{23}N_4O_5$ 375.1669; found: 375.1662.

Three-Component Reaction Byproducts and Intermediates. *4-(7-Ethoxycarbonyl-1-hydroxyindolizin-2-ylmethyl)-N-Boc-4-methoxycarbonylpiperidine* ***(12)***, ***(12$^+$)***—*NMR-Tube Experiment.* 4-(3-Oxocycloprop-1-enylmethyl)-*N*-Boc-4-methoxycarbonylpiperidine **10** (16 mg, 0.05 mmol, 1 equiv) was dissolved in CD_3OD, and ^{1}H NMR was recorded. Then, ethylisonicotinate (9 mg, 1.2 equiv) was added in the NMR tube and mixed. Immediate yellow colorization appeared, and ^{1}H NMR indicated the disappearance of starting materials and formation of **12** in slow equilibrium with its double-bond tautomer. Conc. HCl_{aq} (10 μL) or, alternatively, 10 μL of CF_3COOD was then added in the reaction mixture NMR tube, and line-sharpening of the signals was observed along with ∼1.4 ppm downfield shift of the pyridine ring proton signals (in the experiment with HCl_{aq}) and disappearance of the proton (7.0 ppm), adjacent to the pyrrole cycle of the indolizine core due to ^{1}H−D exchange. Upon attempted isolation of **12** by SiO_2 chromatography, slow oxidative dimerization progressed and the color changed from yellow to brown-green, affording finally dimeric-**12**. R_f (for **12** and dimeric-**12**) (hexane/EtOAc 7/3) ∼ 0.5, yellow spot (vis.).

^{1}H NMR (400 MHz, neutral CD_3OD): δ 8.08 (br. s, 1H), 7.67 (br. s, 1H), 7.03 (br. s, 1H), 6.67 (br. s, 1H), 4.25 (br. s, 2H), 3.78 (dt, J = 13.8, 4.2 Hz, 2H), 3.64 (s, 3H), 2.88 (m, $^2J \sim$ 11 Hz, 2H), 2.83 (br. s, 2H), 2.01 (m, $^2J \sim$ 14 Hz, 2H), 1.46 (ddd, J = 13.9, 11.1, 4.3 Hz, 2H), 1.37 (s, 9H), 1.30 (t, J = 7.1 Hz, 3H).

^{1}H NMR (400 MHz, acidic ($HCl_{(aq)}/CD_3OD \sim 1/55$)): δ 9.11 (dd, J = 6.3, 0.9 Hz, 1H), 8.42 (t, J = 1.7, 0.9 Hz, 1H), 8.15 (dd, J = 6.4, 1.7 Hz, 1H), 4.46 (q, J = 7.1 Hz, 2H), 3.74 (s, 3H), 2.97 (m, 2H),

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J. Org. Chem. XXXX, XXX, XXX−XXX

2.88 (s, 2H), 2.10 (m, ^2J ~ 14 Hz, 2H), 1.52 (ddd, J = 14.3, 10.8, 4.2 Hz, 2H), 1.40 (t, J = 7.1 Hz, 3H), 1.39 (s, 9H).

^{1}H NMR (400 MHz, acidic (CF_3COOD/CD_3OD ~ 1/55)): δ 8.06 (dd, J = 1.9, 0.9 Hz, 1H), 7.65 (dd, J = 7.4, 0.9 Hz, 1H), 6.66 (dd, J = 7.4, 1.8 Hz, 1H), 4.24 (q, J = 7.1 Hz, 2H), 3.77 (dt, J = 13.9, 4.3 Hz, 2H), 3.64 (s, 3H), 2.88 (ddd, J = 13.9, 11.1, 3.0 Hz, 2H), 2.83 (s, 2H), 2.00 (dt, J = 13.3, 3.0 Hz, 2H), 1.46 (ddd, J = 13.7, 11.0, 4.2 Hz, 2H), 1.37 (s, 9H), 1.30 (t, J = 7.1 Hz, 3H).

^{13}C{^{1}H} NMR (75 MHz, acidic ($CF_3COOD/CDCl_3$ ~ 1/55)): δ 178.1, 161.9, 156.2, 154.2, 146.8, 145.5, 141.4, 122.2, 121.8, 119.1, 82.9, 64.2, 62.2, 53.4, 46.4, 40.7, 32.9, 32.7, 28.1, 13.6.

MS (for **12**): Agilent 1100 (APCI) m/z: 461.0 (M^+), 404.9 (M^+ − t-Bu), 361.0 (M^+ − Boc).

HRMS (for **12**) (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{24}H_{33}N_2O_7$ 461.2288; found: 461.2278.

MS (for dimeric-**12**): Agilent 1100 (APCI) m/z: 919.0 (M^+), 863.0 (M^+ − t-Bu), 819.0 (M^+ − Boc), 762.9 (M^+ − Boc − t-Bu).

HRMS (for dimeric-**12**) (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{48}H_{63}N_4O_{14}$ 919.4318; found: 919.4336.

Boc-Deprotected Dimeric-(12)—Attempted NMR Characterization. As ^{1}H NMR of dimeric-**12** was even more obscured by the signal broadening in the full spectral range than in 1-hydroxyindolizine **12**, we removed two Boc groups by the following procedure: dimeric-**12** (40 mg) was dissolved in 2 mL of 4M HCl in dioxane and left stirring for 18 h. After that, LC-MS indicated that both Boc groups were removed, the solvent was evaporated under reduced pressure, and the residue was purified by HPLC with gradient elution from 5 to 15% MeCN (0.1% HCOOH) for 20 min. Solvents from the collected fractions were evaporated to provide Boc-deprotected dimeric-**12** as a dark-yellow solid. Yield = 80% (35 mg). ^{1}H NMR (CD_3OD) of the obtained product was still broadened in the full spectral range and noninterpretable. Addition of conc. HCl_{aq} (10 μL) to the NMR sample revealed the signals of the piperidine substituent.

^{1}H NMR (400 MHz, acidic ($HCl_{(aq)}$ CD_3OD)): δ 4.32 (br. s, 2H), 3.29 (br. s, 3H), 3.10 (br. d, ^2J ~ 13 Hz, 1H), 2.90 (br. d, ^2J ~ 13 Hz, 1H), 2.70 (br. t, ^2J ~ 12 Hz, 1H), 2.63 (br. t, ^2J ~ 12 Hz, 1H), 1.90 (br. d, ^2J ~ 12 Hz, 2H), 1.70 (br. t, ^2J ~ 13 Hz, 1H), 1.30 (t, ^3J ~ 7 Hz, 3H), 1.30 (m, 1H).

MS: Agilent 1100 (APCI) m/z: 718.9 (M^+).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{38}H_{47}N_4O_{10}$ 719.3293; found: 719.3288.

4-(7-Ethoxycarbonyl-1-hydroxy-3-deuteroindolizin-2-ylmethyl)-N-Boc-4-methoxycarbonylpiperidine (12-D)—NMR Tube Experiment. The experiment was conducted analogously as for **12** using 4-(2-deutero-3-oxocycloprop-1-enylmethyl)-*N*-Boc-4-methoxycarbonylpiperidine **10**-D. CF_3COOD (10 μL) acidification of the sample led to (**12**$^+$). Again, slow oxidative dimerization progressed and the color changed from yellow to brown-green. LC-MS spectra of the dimer were identical to dimeric-**12** fragmentation, indicating that deuterium atoms are lost during oxidative dimerization.

^{1}H NMR (400 MHz, neutral CD_3OD): δ 8.06 (br. s, 1H), 7.65 (br. s, 1H), 6.66 (br. s, 1H), 4.25 (br. s, 2H), 3.78 (dt, J = 13.8, 4.3 Hz, 2H), 3.64 (s, 3H), 2.88 (m, ^2J ~ 12 Hz, 2H), 2.83 (br. s, 2H), 2.01 (m, ^2J ~ 14 Hz, 2H), 1.46 (ddd, J = 14.6, 11.1, 4.3 Hz, 2H), 1.37 (s, 9H), 1.30 (t, J = 7.1 Hz, 3H).

MS (for **12**-D): Agilent 1100 (APCI) m/z: 462.0 (M^+), 405.9 (M^+ − t-Bu), 362.0 (M^+ − Boc).

HRMS (for **12**-D) (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{24}H_{32}N_2O_7D$ 462.2338; found: 462.2347.

4-(5-Hydroxy-1H-pyrazol-4-ylmethyl)-N-Boc-4-methoxycarbonylpiperidine (13). A 20 mL screw-cap clear vial was charged with 4-(3,3-difluorocycloprop-1-enylmethyl)-*N*-Boc-4-methoxycarbonylpiperidine **2** (330 mg, 1 mmol, 1 equiv) and 3,5-dimethyltetrazine **8** (133 mg, 1.2 equiv) followed by addition of ethanol (10 mL) and water (0.1 mL). The reaction mixture was heated with stirring at 78 °C for 48 h. The red color of the reaction mixture gradually faded, indicating that tetrazine was consumed. Full conversion of **2** was ensured according to LC-MS. Volatiles were removed under reduced pressure, and the residue was redissolved in minimal amount of MeCN (~2 mL) for purification by HPLC with gradient elution from 20 to 40% MeCN (20 min). Solvents from the collected fractions were evaporated to provide **13** as a white solid. (R_f (**13**) ~ 0.3 in EtOAc). Yield = 25% (85 mg).

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.30 (br, s, 2H), 7.05 (s, 1H), 3.70 (m, ^2J ~ 14 Hz, 2H), 3.61 (s, 3H), 2.84 (m, ^2J ~ 14 Hz, 2H), 1.38 (s, 9H), 1.38 (s, 9H), 1.35 (m, ^2J ~ 14, ^2J ~ 10.6, ^2J ~ 4.3 Hz, 2H).

^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6): δ 174.9, 159.5 (br.), 153.8, 128.8 (br.), 97.7, 78.4, 51.3, 46.3, 40.7 (br.), 31.9, 31.3, 27.9.

mp = 208−210 °C.

MS: Agilent 1100 (APCI) m/z: 340.0 (M^+), 284.0 (M^+ − t-Bu), 240.0 (M^+ − Boc).

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{16}H_{26}N_3O_5$ 340.1873; found: 340.1861.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.0c02292.

Copies of LC-MS, ^{1}H, and ^{13}C{^{1}H} NMR and HRMS spectral data (PDF)

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Notes

The authors declare no competing financial interest.

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