**Article**

**Investigation of the Pyridinium Ylide—Alkyne Cycloaddition as a Fluorogenic Coupling Reaction**

Simon Bonte 1, Ioana Otilia Ghinea 2, Rodica Dinica 2, Isabelle Baussanne 1,* and Martine Demeunynck 1,*

1 Department of Pharmacochemistry, Université Grenoble Alpes, CNRS, DPM UMR 5063, F-38041 Grenoble, France; simontlse.12@gmail.com

2 Department of Chemistry, Physics and Environment, Faculty of Science and Environment, “Dunarea de Jos” University of Galati, 111 Domneasca Street, 800201 Galati, Romania; otilia.ghinea@gmail.com (I.O.G.); rodicad@yahoo.com (R.D.)

* Correspondence: isabelle.baussanne@ujf-grenoble.fr (I.B.); martine.demeunynck@univ-grenoble-alpes.fr (M.D.); Tel.: +33-476-635-314 (M.D.)

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**Abstract:** The cycloaddition of pyridinium ylides with alkynes was investigated under mild conditions. A series of 13 pyridinium salts was prepared by alkylation of 4-substituted pyridines. Their reactivity with propiolic ester or amide in various reaction conditions (different temperatures, solvents, added bases) was studied, and 11 indolizines, with three points of structural variation, were, thus, isolated and characterized. The highest yields were obtained when electron-withdrawing groups were present on both the pyridinium ylide, generated *in situ* from the corresponding pyridinium salt, and the alkyne (X, Z = ester, amide, CN, carbonyl, etc.). Electron-withdrawing substituents, lowering the acid dissociation constant (pKa) of the pyridinium salts, allow the cycloaddition to proceed at pH 7.5 in aqueous buffers at room temperature.

**Keywords:** indolizine; coupling reaction; ylide; dipolar cycloaddition

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**1. Introduction**

We have been interested in the indolizine chemistry for several years. Indolizine is a nitrogen-containing bicyclic heterocycle, and its derivatives display interesting biological [1–5] and optical properties [6–13] (fluorescence and circular dichroism [14,15]). Indolizines have been used as biomarkers [16] and in the fluorescent labeling of carbon nanotubes [17] and graphene [18], for instance. Among the main routes of preparation that have been designed, we can cite the metal-catalyzed cyclization of 2-alkynylpyridines [19–25] or 2-pyridine alkynyl carbinols [26–31]. The most common metal-free methodology [2,32,33] involves the 1,3-dipolar cycloaddition of pyridinium ylides with alkynes (Scheme 1) [2,34–39]. The dihydroindolizines thus formed spontaneously, aromatize under air oxidation. This chemistry has been the subject of a large number of publications, focusing, in particular, on the formation and reactivity of the ylides [34,35,40–44] and on the mechanism of the cycloaddition [33]. More recently, improvements in the reaction conditions have been reported, by using oxidant-free cycloaddition to alkenes [32], one-pot [38], microwave-activated [34,35], or biocatalyzed processes [36]. Note that most of the work reported so far in the literature involved N-benzoylmethylpyridinium-derived ylides [45,46]. The purpose of the present work was to explore the potency of the pyridinium ylide-alkyne cycloaddition as a click-type coupling reaction.
The key points to investigate were the effectiveness of this cycloaddition in mild conditions (room temperature, neutral or near neutral conditions), ease of accessibility of the reactants, and their practical pre- or post-functionalization. For all these reasons, we decided to prepare a series of ylide precursors (pyridinium salts) and to compare their reactivity in the presence of alkynes in various conditions. We first aimed to select the best partners for the application as coupling methodology. To achieve this goal, we modified the structure of the ylide precursors: reactive or easily modified substituents were introduced on the pyridinium salts (R₁ group in Scheme 1) and ester or amide groups were used to stabilize the ylide (R₂ = RO- or RNH-, in Scheme 1) in place of the benzoyl group usually reported in the literature. The structure optimization of the reactants allowed the cycloaddition to proceed at room temperature in very mild conditions including pH 7.5 aqueous buffers, yielding indolizines with three possible points of functionalization.

2. Results

2.1. Preparation and Characterization of the Pyridinium Salt

We first prepared a series of 13 pyridinium salts. To keep the symmetry of the starting molecule and to prevent formation of regioisomers after cycloaddition, substituents were only introduced at position 4 of the pyridine ring [37]. The pyridinium salts were obtained by alkylation of the pyridine derivatives with methyl 2-bromoacetate (compounds 1–7), 2-iodoacetophenone derivatives (compounds 8 and 9), or 2-bromo-N-propylacetamide (compound 10) in acetone under ultrasound activation. In the case of alkylation with diethyl 2-iodomalonate to prepare the pyridinium salts containing two carboxylic esters (compounds 11–13, R₂ = CO₂Et), very low yields were observed under these conditions. The reaction was improved by using the diethyl 2-iodomalonate in large excess. The structures, yields, pKa and ¹H-NMR data are collected in Table 1.

As previously observed [47,48], the electronic nature of the substituent present at position 4 modulates the pKa values, and the pKa variation correlates well with the Hammett constant of the R₁ group. The electron-withdrawing groups decrease the pKa values (compare 4–7 versus 1). This effect was more pronounced with mesomeric (CN or COCH₃) than inductive (CF₃) withdrawing substituents. The effect of the nature of R₁ has been studied [49] and seemed more prominent for the phenacyl analogues [47], however the measurements were made in different conditions (ylides dissolved in methanol). In another study, the deprotonation of pyridinium salts was studied by NMR in DMSO in the presence of a strong base [48]. The authors found that the effect on the deprotonation of the ring substituents was greater than the effect of the methylene substituent.

The proton NMR spectra of 4-substituted pyridinium salts are characterized downfield by two multiplets for H-2/H-6 and H-3/H-5, the latter being more shielded, and a singlet for the CH₂ generally found between 4.4 and 6.6 ppm. The correlation between the presence of the electron-withdrawing group R₁ and pKa (deprotonation and ylide formation) is reflected by higher δ value for the CH₂ signal. The integration of this singlet was lower than expected with compounds 4–6 and this signal may also be lacking (see 11 and 12) probably due to high H/D exchange rate in CD₃OD. We observed in the spectrum of the 4-acetylpyridinium salt 6 in CD₃OD, the presence of a second set of shielded signals, not found in DMSO-d₆, which was attributed to the formation of hemiketal or ketal derivatives.
in this solvent and that increased with time spent in solution (the simulated spectra of the different species were in agreement with the experimental data).

Table 1. Structures, yields, pKa values, and proton NMR data of the pyridinium salts.

| Salt | R1   | R2   | R3   | Yields % | R1 Hammett Cste | pKa a | 1H-NMR b δ H2/H6 | 1H-NMR b δ H3/H5 | 1H-NMR b δ CH2/CH |
|------|------|------|------|----------|-----------------|-------|-----------------|-----------------|-----------------|
| 1    | H    | OMe  | H    | 71       | 0               | 8.30  | 9.01            | 8.23            | 5.66            |
| 2    | NH2  | OMe  | H    | 83      | -0.66          | 8.84  | 8.09            | 6.92            | 5.12            |
| 3    | NHAc | OMe  | H    | 81      | 0.06           | 8.74  | 8.74            | 8.14            | 5.51            |
| 4    | COF3 | OMe  | H    | 54      | 0.54           | 8.25  | 9.39            | 8.66            | 5.83            |
| 5    | CONHPr | OMe | H    | 80      | 0.36           | 8.24  | 9.19            | 8.52            | 5.75            |
| 6    | COCH3| OMe  | H    | 64      | 0.50           | 8.04  | 9.35            | 8.63            | 5.88            |
| 7    | CN   | OMe  | H    | 60      | 0.66           | 8.16  | 9.29            | 8.64            | 5.75            |
| 8    | CN   | Ph   | H    | 70      | 8.11           | 9.30  | 8.85            | 6.61            |                 |
| 9    | CN   | Ph-NO2 | H  | 60      | 7.07           | 9.26  | 8.83            | 6.60            |                 |
| 10   | CN   | NHCH2H2 | H  | 60      | nd             | 9.24  | 8.59            | 5.60            |                 |
| 11   | H    | OEt  | CO2Et | 99     | 5.51           | 9.20  | 8.28            | Not obs.        |                 |
| 12   | COCH3| OEt  | CO2Et | 99     | 5.04           | 9.36  | 8.61            | Not obs.        |                 |
| 13   | CN   | OEt  | CO2Et | 40     | nd             | 9.44  | 8.66            | Not obs.        |                 |

a The pKa values were determined by potentiometry of 10−3 M solution of the salt in 0.1 M NaClO4. The measurements were made in triplicate; b The NMR spectra were recorded in CD3OD; c Non-reproducible data.

2.2. Reactivity Studies

The reactivity of the different salts with alkynes was compared using a reference reaction: methanol was chosen as solvent, ethyl propiolate as dipolarophile, and K2CO3 for ylide generation. The reaction mixtures were stirred at 25 °C for 18 h, and the indolizines were isolated. The ease of isolation and purification is an important point for the usefulness of the reaction. Therefore, in the following tables, we give and discuss the yields of isolated indolizines.

As known in the literature, the cycloaddition is fully regioselective, the ethyl and methyl esters being found in positions 1 and 3, respectively, in our study and easily identified.

2.2.1. Influence of the R1 Substituent

We first studied the importance of the nature of R1 on the reactivity (Table 2). Introduction of electron-withdrawing groups on the pyridine ring clearly favored the cycloaddition. Higher yields (77% and 81%) were obtained in the presence of electron-withdrawing mesomeric groups (COCH3 or CN, respectively) that efficiently stabilize the negative charge of the ylides by delocalization. In the case of the CF3 substituent (entry 6), the NMR data showed the presence of the desired indolizine 19 and of the corresponding dimethyl carboxylate, formed by trans-esterification of the ethyl ester at position 1 by methanol. To selectively prepare 19, the reaction should be performed in ethanol or in a non-nucleophilic solvent such as DMF (cf. part 2.2.2). Under these mild conditions, the 4-amino or amido-substituted pyridinium salts (entries 2 and 3), which display the lowest Hammett constant, did not react.
Influence of the Solvent and of the Nature of the Added Base

To evaluate the importance of the solvent on the cycloaddition efficiency, the reactions were performed in parallel in DMF, a polar non-nucleophilic solvent, and methanol. Three bases, \( \text{NH}_4\text{OH}, \text{NEt}_3, \) and \( \text{K}_2\text{CO}_3 \) were also tested. The data are collected in Table 3. From these experiments, compounds 6 and 7 were selected for further studies.

### Table 3. Influence of the nature of the solvent and of the base on the yields of indolizines 18 and 20 from pyridinium salts 6 and 7.

| Entry | Base (1 eq.) | Solvent | \( T \) °C | Time (h) | Yield (%) |
|-------|--------------|---------|------------|----------|-----------|
| 1     | \( \text{K}_2\text{CO}_3 \) | MeOH    | 25         | 1        | 50        |
| 2     | \( \text{K}_2\text{CO}_3 \) | MeOH    | 25         | 18       | 77        |
| 3     | \( \text{K}_2\text{CO}_3 \) | MeOH    | 25         | 1        | 45        |
| 4     | \( \text{K}_2\text{CO}_3 \) | MeOH    | 25         | 1        | 38        |
| 5     | \( \text{K}_2\text{CO}_3 \) | MeOH    | 25         | 1        | 88        |
| 6     | \( \text{NEt}_3 \) | MeOH    | 25         | 1        | 44        |
| 7     | \( \text{NEt}_3 \) | MeOH    | 25         | 1        | 45        |
| 8     | \( \text{NEt}_3 \) | MeOH    | 25         | 5        | >90       |
| 9     | \( \text{NEt}_3 \) | MeOH    | 25         | 5        | 48        |
| 10    | \( \text{NEt}_3 \) | MeOH    | 25         | 5        | 48        |
| 11    | \( \text{K}_2\text{CO}_3 \) | MeOH    | 25         | 5        | 45        |
| 12    | \( \text{K}_2\text{CO}_3 \) | MeOH    | 25         | 18       | 81        |
| 13    | \( \text{K}_2\text{CO}_3 \) | MeOH    | 25         | 5        | 70        |
| 14    | \( \text{K}_2\text{CO}_3 \) | MeOH    | 40         | 18       | 38        |
| 15    | \( \text{Phosphate buffer} \) | \( \text{H}_2\text{O} \) | 25         | 18       | 24        |
K₂CO₃ appeared as the most efficient base in both solvents (entries 1 and 5), and DMF emerged as the best choice to perform reactions with 6 (R₁ = COCH₃). The reaction proceeded quickly in this solvent with good yield obtained in 1 h (entry 5). Increasing the reaction time to 5 h (entry 8) did not significantly improve the yield. This solvent effect was also observed with the 4-cyano analog 7, for which both solvents may be alternatively used. Still, the reaction remained faster in DMF than in methanol (compare entries 11 and 13). The reaction was attempted at a higher temperature in methanol (entries 12 and 14), but leading to the formation of side-products including those resulting from trans-esterification.

The reaction was also performed in pH 7.5 phosphate buffer (entry 15). The pyridinium salt 7 was soluble in aqueous solution, but the hydrophobic nature of the ethyl propiolate was a severe limitation. Nevertheless, we were thrilled to isolate the resulting indolizine 20 in 24% yield. This result indicated that the pKa of the salt (8.16 for 7) was compatible with the partial formation of the ylide under these conditions, thus allowing the cycloaddition reaction. It should be noted that, to our knowledge, dipolar cycloaddition involving a pyridinium ylide in neutral aqueous solution has not been reported so far.

In an effort to increase the reactivity in water, the reaction was performed with compounds 11–13 that displayed lower pKa values and would mainly exist as ylides at pH 7.5. The reactions were performed in pH 7.5 buffer solutions. For solubility reasons, the first attempts were made at 40 °C in Tris buffer. As indicated in Table 4, the cyclization was highly dependent on the nature of the substituent at position 4. There was no reaction with the unsubstituted pyridinium 11 (entry 1), and a low yield in indolizine 22 was obtained from the 4-acetyl pyridinium 12 (entry 2). The 4-cyanopyridinium 13 yielded the indolizine 23 in a reasonable 40% yield. As we had previously observed a negative effect of temperature on yields, the reaction was then performed at 25 °C under vigorous stirring and, as a result, the yield jumped to 63% (entry 4). Replacing Tris buffer with phosphate buffer (entry 5) had a negative effect on the yield that dropped to 42%. For comparison, the reactivity of 13 in organic solvents was investigated (entries 6 and 7). A very strong solvent effect was observed. While no formation of 23 occurred in methanol, it was isolated in excellent yield in DMF. The difference in reactivity between 12 and 13 in Tris buffer may be due to the unfavorable formation of the hydrate form of 12. However, these first data confirmed the feasibility of this dipolar cycloaddition in aqueous solutions starting with 4-cyanopyridinium salts 7 and 13.

Table 4. Formation of indolizine from diethyl dicarboxylate substituted pyridinium 11–13 in pH 7.5 buffer solutions.

| Entry | R₁  | Base          | Solvent          | T °C | Indolizine | Yield (%) |
|-------|-----|---------------|------------------|------|------------|-----------|
| 1     | H   | pH 7.5 Tris buffer | H₂O              | 40   | 21         | 0         |
| 2     | COCH₃ | pH 7.5 Tris buffer | H₂O              | 40   | 22         | 12        |
| 3     | CN  | pH 7.5 Tris buffer | H₂O              | 40   | 23         | 40        |
| 4     | CN  | pH 7.5 Tris buffer | H₂O              | 25   | 23         | 63        |
| 5     | CN  | pH 7.5 phosphate buffer | H₂O          | 25   | 23         | 42        |
| 6     | CN  | K₂CO₃         | MeOH             | 25   | 23         | 0         |
| 7     | CN  | K₂CO₃         | DMF              | 25   | 23         | 93        |

The mixtures of pyridinium salts (1 eq.) and ethyl propiolate (1.5 eq.) were stirred at the chosen temperatures for 18 h. Results are given as yields of isolated indolizines.
2.2.3. Effect of the R₂ Group

To extend the scope of the reaction, it was also important to compare the reactivity of pyridinium salts containing various methylene R₂ substituents. As shown in Table 5, the yield in 7-cyanoindolizine was higher starting from the methyl ester 7 than from benzoyl derivatives 8 or 9. The presence of the nitro group on the phenyl ring, lowering the pH of ylide formation, slightly increased the yield (compare entries 2 and 3).

Table 5. Effect of the nature of R₂ group on the yield of 7-cyanoquinazolines.

| Entry | Pyridinium Salt | R₂   | Indolizine | Yield (%) |
|-------|-----------------|------|------------|-----------|
| 1     | 7               | OMe  | 20         | 81        |
| 2     | 8               | Ph   | 24         | 50        |
| 3     | 9               | C₆H₄NO₂ | 25       | 67        |
| 4     | 10              | NHC₃H₇ | 26       | 62        |

The pyridinium salts and 1.5 equivalents of ethyl propiolate were stirred in methanol in the presence of K₂CO₃ (1 eq.) for 18 h at room temperature.

An interesting result was obtained with the amide containing pyridinium salt 10. The yield of indolizine 26 was moderate (62%), but this result was important as it gives an interesting alternative to the ester as a potential reactant, and this approach has not been reported so far in the literature.

2.2.4. Influence of the Dipolarophile

The last parameter to evaluate was the nature of the dipolarophile. As shown in Table 6, the reaction worked also well with propionic amide such as 27, giving the corresponding indolizine 28 in reasonable (not optimized) yield. With substituted propiolate, such as 29, a complex mixture of indolizines was obtained as shown by the presence of several fluorescent spots on TLC. No reaction was observed with less activated alkynes, as exemplified by 30 or 31.

Table 6. Reaction of 4-cyanopyridinium salt 7 with various dipolarophiles.

| Entry | Dipolarophile | Indolizine (Yield) |
|-------|---------------|--------------------|
| 1     | EtO⁻CO₂Et     | 20 (81%)           |
| 2     | NH⁻Bn         | 28 (40%)           |
| 3     | Complex mixture |                   |
derivatives) allowed the reaction with propiolic ester or amide to proceed in mild conditions in a variety of conditions. The formation and stabilization of the reactive ylides that are also favored by electron-withdrawing groups.

However, one also has to keep in mind that the very first step is not the cycloaddition itself, but the formation of zwitterionic allenoate intermediate followed by the intramolecular 5-endo trig cyclization. Again, it is important to emphasize the mild conditions used in this study. Simple pyridinium ylides were shown to react at high temperatures with isolated alkynes \[39\]. However the lack of reactivity with isolated alkynes at room temperature may be useful as it allows the introduction of an isolated triple bond in the reactants for further orthogonal reactions.

3. Discussion

To summarize, the highest yields of indolizines were obtained when both partners of the reaction, the pyridinium salt (or the corresponding ylide) and the triple bond, were substituted with a strong electron-withdrawing group (acetyl, cyano, ester, or amide). The mechanism of the cycloaddition has been discussed in the literature. The reactions of pyridinium ylides with propiolates are generally described as concerted OM-controlled reactions \[33,40,43,44\]. Matsumoto \[41\] also reported the inverse electron-demand cycloaddition of cyclooctyne with pyridinium bis(methoxycarbonyl)methylides similar to 11. However, Shang and colleagues \[39\] found that, in the reaction with simple alkynes in DMF–K₂CO₃ at high temperature (120 °C), the presence of electron-donating or −withdrawing substituents on the ylides significantly lowered the yields in indolizines.

The importance of electron-withdrawing groups on both reactants is in favor of the two-step mechanism depicted in Scheme 2, involving the Michael addition of the ylide to the triple bond, with formation of zwitterionic allenoate intermediate followed by the intramolecular 5-endo trig cyclization. However, one also has to keep in mind that the very first step is not the cycloaddition itself, but the formation and stabilization of the reactive ylides that are also favored by electron-withdrawing groups.

![Scheme 2](image)

In conclusion, the reaction of pyridinium ylides with propiolic acid derivatives was explored in the perspective of its use as coupling reaction. The reaction was investigated by varying a set of parameters, i.e., the nature of the substituents, the reaction conditions, and the nature of the dipolarophile. First of all, the regioselectivity of the reaction is a positive aspect for this application. The presence of an electron-withdrawing group at position 4 of the pyridinium salt (exemplified with 4-cyano or 4-acetyl derivatives) allowed the reaction with propiolic ester or amide to proceed in mild conditions in a variety of conditions.
variety of solvents. The reaction progression was easily monitored by TLC, due to the fluorescence of the newly-formed indolizine. This point is of major interest is that, so far, most pro-fluorescent click reactions involve the use of added fluorogenic or fluorescent heterocycles [50,51].

A second key result was the reactivity in neutral aqueous solutions at room temperature. Indeed, the presence of the electron-withdrawing group at position 4 played an essential role in the formation and stabilization of the reactive ylides in these conditions. The main limitation appeared to be the water solubility of the propiolic ester.

This study allowed us to select the best partners and conditions for a highly modular pro-fluorescent click-type coupling reaction. The reactants include a pyridine containing an electron-withdrawing group (CN, COR, CO₂R, CONHR, etc.) at position 4, a 2-bromo-acetyl ester or amide, and a propiolic ester or amide. Interestingly, this methodology appears complementary to the tetrazine-alkene reaction [52,53] involving an electron-rich dipolarophile, but yielding non-fluorescent compounds.

4. Experimental Section

4.1. Material and Methods

Melting points were determined using a Reichert Thermovar apparatus (Depew, NW, USA) and are uncorrected. NMR spectra were recorded on the Bruker Avance 400 spectrometer (Bruker Corporation, Billerica, MA, USA) of the “Fédération de Recherche” ICMG (FR2607) platform, using the solvent as the internal reference; the chemical shifts are reported in parts per million (ppm) units. High-resolution mass spectra (HRMS) were performed on a Bruker maXis mass spectrometer Q-TOF (Bruker Corporation) by the “Fédération de Recherche” ICOA/CBM (FR2708) platform. Reversed-phase HPLC was performed with a µ-bondapak-C18 analytical column (Waters Corporation, Milford, MA, USA). A Waters chromatographic system was used, with two M-510 pumps and a photodiode array detector Waters 996 using Millenium 32 software. A linear gradient from 0 to 100% methanol in H₂O pH 2.5 (phosphoric acid), 2 mL/min flow rate, was used.

The reagents were purchased from Sigma Aldrich and were used without further purification. N-Benzylprop-2-yramide 27 was prepared by biocatalyzed reaction between benzylamine and ethyl propionate as reported recently by us [54]. Ethyl 3-ethynybenzoate 30 was prepared by esterification of 3-ethynylbenzoic acid following reported procedure [55].

Calculator Plugins were used for structure property prediction and calculation, Marvin 6.0.2, 2013, ChemAxon (http://www.chemaxon.com).

Copies of the NMR spectra and HPLC chromatograms of the new compounds may be found in the Supplementary Materials.

4.2. General Methods for the Synthesis of N-Heterocyclic Salts

Method A: The pyridine derivative (1 eq.) and the alkylating reagent (1.5 eq) were dissolved in dry acetone (2 mL for 1 mmol of pyridine derivative). The reaction mixture was stirred in an ultra-sound bath for 5 h to 10 h, depending on the nature of substituent present on the pyridine. The temperature of the bath was kept under 50 °C by adding ice is necessary. Then, a non-polar solvent (3 to 5 mL of Et₂O or DCM) was added, and the quaternary salt that deposited was filtered off, and washed with DCM.

Method B: The reactions between the pyridine derivatives and diethyl iodomalonate were performed in acetone in the presence of a large excess of reactant, the mixture being stirred at room temperature for two days. Then, Et₂O was added to the flask and the quaternary hygroscopic salt was filtered off and washed with DCM.

1-(2-Methoxy-2-oxoethyl)pyridinium bromide (1) was prepared according to the general method A from pyridine (1 mmol) and methyl 2-bromoacetate. The resulting pyridinium 1 was obtained in 71% yield.
(165 mg) as a white powder. mp 167–168 °C [lit. [56] 174–175 °C]; 1H-NMR (400 MHz, CD3OD) δ 9.01 (dd, 2H, J = 6.8, 1.2 Hz), 8.74 (m, 1H), 8.23 (t, 2H, J = 7.6, 6.8 Hz), 5.66 (s, 2H), 3.90 (s, 3H).

4-Amino-1-(2-methoxy-2-oxoethyl)pyridinium bromide (2) was prepared according to the general method A from 4-aminopyridine (1 mmol) and methyl 2-bromoacetate. The resulting pyridinium salt 2 was obtained in 83% yield (205 mg) as a white powder. mp 132–134 °C [lit. [57] 175–176 °C]; 1H-NMR (400 MHz, CD3OD) δ 8.09 (dd, J = 6 Hz, 2H), 6.92 (dd, J = 6 Hz, 2H), 5.12 (s, 2H), 3.86 (s, 3H).

4-Acetamido-1-(2-methoxy-2-oxoethyl)pyridinium bromide (3) was prepared according to the general method A from 4-acetamidopyridine (1 mmol) and methyl 2-bromoacetate. The resulting pyridinium salt 3 was obtained in 81% yield (234 mg) as a white powder. mp 104–106 °C; 1H-NMR (400 MHz, CD3OD) δ 11.61 (s, 1H), 8.74 (d, 2H, J = 7.6 Hz), 8.14 (d, 2H, J = 7.2 Hz), 5.51 (s, 2H), 3.78 (s, 3H), 2.26 (s, 3H); 13C-NMR (100 MHz, CD3OD) δ 171.1, 167.3, 152.5, 146.6, 114.3, 58.6, 53.0, 24.7; HRMS (ESI) m/z calcd for C10H13N2O5 209.0926, obsd 209.0919.

4-Trifluoromethyl-1-(2-methoxy-2-oxoethyl)pyridinium bromide (4) was prepared according to the general method A from commercial 4-trifluoromethylpyridine (2 mmol) and methyl 2-bromoacetate. The resulting quaternary salt 4 was obtained in 54% yield (162 mg) as a yellow powder. mp 127–130 °C; 1H-NMR (400 MHz, CD3OD) δ 9.39 (d, 2H, J = 6.8 Hz), 8.66 (d, 2H, J = 6.8 Hz), 5.83 (s, 2H), 3.94 (s, 3H); 13C-NMR (100 MHz, CD3OD) δ 165.9, 148.6 (2C), 145.9, 145.5, 124.6 (2C), 122.5, 119.8, 61.0, 52.8; HRMS (ESI) m/z calcd for C10H17F3N2O5 237.1234, obsd 237.1232.

4-(N-Propylcarbamoyl)-1-(2-methoxy-2-oxoethyl)pyridinium bromide (5) was prepared according to the general method A from 4-N-propylcarbamoylpyridine (0.9 mmol) and methyl 2-bromoacetate. The resulting quaternary salt 5 was obtained in 64% yield (716 mg) as a red powder. mp 149–150 °C; 1H-NMR (400 MHz, CD3OD) δ 8.63 (d, 2H, J = 6.8 Hz); 5.88 (s, 2H, CH2); 3.80 (s, 3H, OCH3); 2.78 (s, 3H, CH3); 13C-NMR (100 MHz, CD3OD) δ 195.5, 166.6, 149.2, 147.8 (2C), 125.7 (2C), 60.4, 53.3, 27.5; HRMS (ESI) m/z calcd for C12H17N2O5 220.0577, obsd 220.0580.

4-Acetyl-1-(2-methoxy-2-oxoethyl)pyridinium bromide (6) was prepared according to the general method A from 4-acetylpyridine (4.1 mmol) and methyl 2-bromoacetate. The resulting quaternary salt 6 was obtained in 64% yield (716 mg) as a red powder. mp 149–150 °C; 1H-NMR (400 MHz, CD3OD) δ 9.35 (dd, 2H, J = 6.8 Hz); 8.63 (dd, 2H, J = 6.8 Hz); 5.88 (s, 2H, CH2); 3.80 (s, 3H, OCH3); 2.78 (s, 3H, CH3); 13C-NMR (100 MHz, CD3OD) δ 195.5, 166.6, 149.2, 147.8 (2C), 125.7 (2C), 60.4, 53.3, 27.5; HRMS (ESI) m/z calcd for C10H12N2O5 194.0812, obsd 194.0810.

4-Cyano-1-(2-methoxy-2-oxoethyl)pyridinium iodide (7) was prepared according to the general method A from 4-cyanopyridine (1 mmol) and methyl 2-bromoacetate. The resulting pyridinium salt 7 was obtained in 60% yield (154 mg) as a white powder. mp 208–209 °C [lit. [58] 181–183 °C]; 1H-NMR (400 MHz, CD3OD) δ 9.29 (dd, 2H, J = 5.4 Hz), 8.64 (dd, 2H, J = 5.4 Hz), 5.75 (s, 2H), 3.92 (s, 3H).

4-Cyano-1-(2-oxo-2-phenylethyl)pyridinium iodide (8) was prepared according to the general method B from commercial 4-cyanopyridine (2 mmol) and 2-iodo-acetonaphene. The resulting pyridinium salt 8 was obtained in 70% yield (493 mg) as a red powder. mp 132–134 °C [lit. [47] 114–118 °C]; 1H-NMR (400 MHz, DMSO-d6) δ 9.30 (d, 2H, J = 6.9 Hz), 8.84 (d, 2H, J = 6.9 Hz), 8.09 (dd, 2H, J = 8.5, 7.1 Hz), 7.80–7.85 (m, 1H), 7.67–7.72 (m, 2H), 6.61 (s, 2H).

4-Cyano-1-(2-oxo-2-(para-nitrophenyl)ethyl)pyridinium iodide (9) was prepared according to the general method A from commercial 4-cyanopyridine (1 mmol) and 2-iodo-4-nitroacetophenone. The resulting quaternary salt 9 was obtained in 60% yield (239 mg) as a yellow powder. mp 182–184 °C [lit. [47] 187–189 °C]; 13C-NMR (100 MHz, DMSO-d6) δ 189.2, 150.7, 147.6 (2C), 138.0, 130.8 (2C), 129.8 (2C), 128.1, 124.2 (2C), 114.8, 67.3.
4-Cyano-1-[(N-propylcarbamoyl)methyl]pyridinium bromide (10) was prepared according to the general method A from 4-cyanopyridine (1 mmol) and 2-bromo-N-propylacetamide. The resulting pyridinium-salt 10 was obtained in 60% yield (160 mg) as a yellow powder. mp 185–186 °C; 1H-NMR (400 MHz, CD3OD) δ 9.24 (d, 2H, J = 6.8 Hz), 8.59 (d, 2H, J = 6.8 Hz), 5.60 (s, 2H), 3.26 (t, 2H, J = 7.2 Hz), 1.58–1.64 (m, 2H), 0.99 (t, 3H, J = 7.2 Hz); 13C-NMR (100 MHz, CD3OD) δ 165.0, 149.1 (2C), 131.7 (2C), 130.4, 115.3, 63.9, 42.9, 23.5, 11.8; HRMS (ESI) m/z: [M + H]+ calcd for C11H14N3O 204.1131, obsd 204.1132.

1-(1,3-Diethoxy-1,3-dioxopropan-2-yl)pyridinium iodide (11) was prepared according to the general method B from pyridine (5.5 mmol) and ethyl 2-iodomalonate. The resulting quaternary salt 11 was obtained in 89% yield (1.80 g) as a white powder. mp 151–152 °C [lit. [59] 154–155 °C]; 1H-NMR (400 MHz, CD3OD) δ 9.20 (t, 2H, J = 7.2 Hz), 8.81–8.85 (m, 1H), 8.28 (dd, 2H, J = 7.2 Hz, 8 Hz), 4.37–4.52 (m, 4H), 1.40 (t, 6H, J = 7.2 Hz).

4-Acetyl-1-(1,3-diethoxy-1,3-dioxopropan-2-yl)pyridinium iodide (12) was prepared according to the general method B from 4-acetylpyridine (4 mmol) and ethyl 2-iodomalonate. The resulting pyridinium-salt 12 was obtained in 99% yield (1.66 g) as a yellow powder. mp 130–131 °C [lit. [37]: 196–198 °C]; 1H-NMR (400 MHz, CD3OD) δ 9.36 (d, 2H, J = 7.0 Hz), 8.61 (d, 2H, J = 7.0 Hz), 4.39–4.51 (m, 4H), 2.84 (s, 3H), 1.41 (t, 6H, J = 7.1 Hz).

4-Cyano-1-(1,3-diethoxy-1,3-dioxopropan-2-yl)pyridinium iodide (13) [37] was prepared according to the general method B from 4-cyanopyridine (4.8 mmol) and ethyl 2-iodomalonate. The resulting pyridinium-salt 13 was obtained in 40% yield (750 mg) as a white powder. mp 120–122 °C [lit. [37] 240 °C decomposed]; 13C-NMR (100 MHz, CD3OD) δ 163.3 (2C), 151.6, 149.7 (2C), 132.0, 131.4 (2C), 115.2, 65.8 (2C), 14.2 (2C).

4.3. General Methods for the Ylide-Alkyne Cycloaddition

Method C: The cycloaddition was performed with 1 eq. of the quaternary salt, 1.1 eq. of the alkyne derivative, and 1 eq. of K2CO3 in methanol or DMF. The pH of the solution was close to 9. The reaction mixture was stirred at room temperature under air atmosphere for 18 h. Then, water was added and the corresponding indolizine was precipitated, filtered off and washed with water.

Method D: The cycloaddition was performed in Tris-buffer pH 7.5 (Tris-buffered saline tablets from sigma, one tablet dissolved in 15 mL of deionized water produces 50 mM Tris-HCl, 150 mM sodium chloride) with 1 eq. of the quaternary salt and 1.1 eq of the alkyne derivative. The reaction mixture was stirred at 40 °C under air atmosphere for 18 h. Then, the reaction mixture cooled in an ice bath, and the precipitate that formed was filtered off and washed with water.

1-Ethyl 3-methyl indolizine-1,3-dicarboxylate (14). The reaction was performed with 1 (100 mg) and ethyl propiolate in methanol, following method C. The indolizine 14 was obtained in 59% yield (63 mg) as a white powder. mp 97–99 °C [lit. [60] 92–93 °C]; 1H-NMR (400 MHz, CDCl3) δ 9.56 (dd, 1H, J = 7.0, 1.2 Hz), 8.34 (m, 1H), 8.04 (s, 1H), 7.37 (m, 1H), 7.04 (td, 1H, J = 6.8, 1.2 Hz), 4.43 (q, 2H, J = 7.2 Hz), 3.97 (s, 3H), 1.47 (t, 3H, J = 7.2 Hz).

1-Ethyl 3-methyl 7-(propylcarbamoyl)indolizine-1,3-dicarboxylate (17). The reaction was performed with 5 (40 mg) and ethyl propiolate in methanol, following method C. The indolizine 17 was obtained in 66% yield (27 mg) as a white powder. mp 159–161 °C; 1H-NMR (400 MHz, CDCl3) δ 9.52 (m, 1H), 8.64 (dd, 1H, J = 1.2 Hz, 2.0 Hz), 8.00 (s, 1H), 7.47 (dd, 1H, J = 2.0 Hz, 5.2 Hz), 6.38 (s br, 1H), 4.39 (q, 2H, J = 7.2 Hz), 3.94 (s, 3H), 3.46 (m, 2H), 1.68 (q, 2H, J = 7.2 Hz), 1.43 (t, 3H, J = 7.2 Hz), 1.01 (t, 3H, J = 7.2 Hz); 13C-NMR (100 MHz, CDCl3) δ 165.3, 164.1, 161.3, 137.7, 131.4, 127.9, 124.7, 117.1, 115.6, 113.1, 107.3, 60.3, 51.7, 42.0, 22.9, 14.5, 11.5; HRMS (ESI) m/z: [M + H]+ calcd for C17H21N2O5 333.1443, obsd 333.1445.
1-Ethyl 3-methyl 7-acetylindolizine-1,3-dicarboxylate (18). The reaction was performed with 6 (100 mg) and ethyl propiolate in methanol, following method C. The indolizine 18 was obtained in 77% yield (40 mg) as a red powder. mp 151–152 °C; 1H-NMR (400 MHz, CDCl3) δ 9.55 (m, 1H), 8.98 (m, 1H), 8.07 (s, 1H), 7.57 (dd, 1H, J = 7.4 Hz, 1.9 Hz), 5.34 (s, 2H), 4.46 (q, 2H, J = 7.2 Hz), 4.00 (s, 3H), 2.75 (s, 3H), 1.49 (t, 3H, J = 7.2 Hz); 13C-NMR (100 MHz, CDCl3) δ 196.0, 163.8, 161.3, 137.5, 132.8, 127.6, 124.8, 121.4, 116.4, 111.8, 109.6, 60.4, 51.8, 26.1, 14.5; HRMS (ESI) m/z: [M + H]+ calc for C15H16NO3 290.1023, obsd. 290.1022. [M + Na]+ calc for C15H15NaNO3 312.0842, obsd. 312.0840.

1-Ethyl 3-methyl 7-((trifluoromethyl)indolizine-1,3-dicarboxylate (19a) and 1-methyl 3-methyl 7-((trifluoromethyl) indolizine-1,3-dicarboxylate (19b). The reaction was performed with 4 (50 mg) and ethyl propiolate in methanol, following method C. The mixture of indolizines 19a and 19b (30/70 ratio) was obtained in 55% yield (27 mg) as a yellow powder. 1H-NMR (400 MHz, CDCl3) δ 9.60 (d, 1H, J = 7.2 Hz), 8.64 (s, 1H), 8.04 (s, 1H), 7.12 (dd, 1H, J = 7.2, 2.0 Hz), 4.40 (q, 2H, J = 7.2 Hz, CH2 of 19a), 3.95 and 3.94 (2s, 2 × 3H, OMe of 19a and 19b), 1.42 (t, 3H, J = 7.2 Hz, CH3 of 19a); 13C-NMR (100 MHz, CDCl3) δ 164.0, 163.5, 161.3, 136.8, 128.4, 127.16, 125.7 (q, 225 Hz, CF3), 124.9, 124.6, 121.9, 117.5, 117.4, 116.1, 110.1, 107.9, 60.5, 51.8, 51.6, 14.5; HRMS (ESI) 19a: m/z calc for C14H13F3O4 316.0791, obsd 316.0796, 19b: m/z calc for C13H12F3O4 302.0635, obsd 302.0638.

1-Ethyl 3-methyl 7-cyanoundolizine-1,3-dicarboxylate (20). The reaction was performed with 7 (50 mg) and ethyl propiolate in methanol, following method C. The indolizine 20 was obtained in 81% yield (37 mg) as a white powder. mp 114–115 °C; 1H-NMR (400 MHz, CDCl3) δ 9.58 (dd, 1H, J = 7.2, 0.8 Hz), 8.74–8.75 (m, 1H), 8.06 (s, 1H), 7.07 (dd, 1H, J = 7.2, 2.0 Hz), 4.42 (q, 1H, J = 7.0 Hz), 3.98 (s, 3H), 1.44 (t, 3H, J = 7.2 Hz); 13C-NMR (100 MHz, CDCl3) δ 163.2, 161.1, 136.0, 128.2, 125.9, 125.1, 117.5, 114.1, 109.0, 108.0, 60.7, 52.0, 14.5; HRMS (ESI) m/z: [M + H]+ calc for C14H13F3O4 316.0791, obsd 316.0796, 13-Diethyl 7-acetylindolizine-1,3-dicarboxylate (22) [61]. The reaction was performed with 12 (100 mg) and ethyl propiolate, following method D. The indolizine 22 was obtained in 12% yield (9 mg) as a white powder. mp 125–127 °C. 1H-NMR (400 MHz, DMSO-d6) δ 9.47 (dd, 1H, J = 0.8, 7.4 Hz), 8.81 (d, 1H, J = 0.8 Hz), 7.90 (s, 1H), 7.62 (dd, 1H, J = 1.9, 7.4 Hz), 4.35–4.40 (m, 4H), 2.69 (s, 3H), 1.35–1.41 (m, 6H).

1,3-Diethyl 7-cyanoundolizine-1,3-dicarboxylate (23). The reaction was performed with 13 (15 mg) and ethyl propiolate, following method D. The indolizine 23 was obtained in 40% yield (4.9 mg) as a white powder. mp 103–104 °C; 1H-NMR (400 MHz, CDCl3) δ 9.64 (dd, 1H, J = 0.8, 7.2 Hz), 8.78 (s, 1H), 8.11 (s, 1H), 7.11 (dd, 1H, J = 1.6, 7.2 Hz), 4.45–4.50 (m, 4H), 1.46–1.51 (m, 6H); 13C-NMR (100 MHz, CDCl3) δ 163.2, 161.1, 136.1, 128.2, 125.8, 125.0, 117.5, 116.9, 114.1, 109.0, 108.0, 60.4, 60.7, 52.0, 14.5 (identical to the commercial compound).

1-Ethyl 3-benzoyl-7-cyanoundolizine-1,3-dicarboxylate (24). The reaction was performed with 8 (50 mg) and ethyl propiolate in methanol, following method C. The indolizine 24 was obtained in 50% yield (22.4 mg) as an orange powder. mp 136–137 °C; 1H-NMR (400 MHz, CDCl3) δ 9.99 (d, 1H, J = 6.8 Hz), 8.83 (s, 1H), 7.95 (s, 1H), 7.87 (d, 2H, J = 6.8 Hz), 7.67 (d, 1H, J = 6.4 Hz), 7.60 (d, 2H, J = 7.2 Hz), 7.20 (d, 1H, J = 6.8 Hz), 4.47 (q, 2H, J = 7.2 Hz), 1.47 (t, 3H, J = 7.2 Hz); 13C-NMR (100 MHz, CDCl3) δ 186.1, 163.2, 138.9, 136.9, 132.3, 129.3, 129.1, 129.0, 128.7, 128.4, 125.6, 124.2, 117.3, 114.8, 109.7, 109.4, 60.8, 14.53; HRMS (ESI) m/z calc for C19H15N2O5 319.1077, obsd 319.1081.

1-Ethyl 3-(4-nitrobenzoyl)-7-cyanoundolizine-1,3-dicarboxylate (25). The reaction was performed with 9 (50 mg) and ethyl propiolate in methanol, following method C. The indolizine 25 was obtained in 67% yield (29.6 mg) as a yellow powder. mp 143–144 °C; 1H-NMR (400 MHz, CDCl3) δ 9.92 (d, 1H, J = 7.2 Hz), 8.75 (s, 1H), 8.34 (d, 2H, J = 8.5 Hz), 7.90 (d, 2H, J = 8.4 Hz), 7.76 (s, 1H), 7.17 (m, 1H), 4.35 (q, 2H, J = 7.1 Hz), 1.35 (t, 3H, J = 7.1 Hz); 13C-NMR (100 MHz, CDCl3) δ 183.9, 163.0, 150.0, 144.3, 137.7, 130.1, 129.9, 129.6, 129.3, 125.9, 124.1, 123.9, 123.6, 117.2, 115.7, 110.9, 110.2, 61.2, 14.7; HRMS (ESI) m/z: [M + H]+ calc for C19H14N3O5 364.0927, obsd 364.0928.
I-Ethyl 7-cyano-3-(N-propylcarbamoyl)indolizine-1-carboxylate (26). The reaction was performed with 10 (20 mg) and ethyl propiolate in methanol, following method C. The indolizine 26 was obtained in 62% yield (15.6 mg) as a white powder. mp 170–171 °C; $^1$H-NMR (400 MHz, CDCl$_3$) δ 9.83 (dd, 1H, $J$ = 7.4, 1.0 Hz), 7.80 (s, 1H), 7.08 (dd, 1H, $J$ = 7.4, 1.8 Hz), 6.17 (br s, 1H), 4.51 (q, 2H, $J$ = 7.2 Hz), 3.40–3.55 (m, 2H), 1.72–1.79 (m, 2H), 1.53 (t, 3H, $J$ = 7.2 Hz), 1.01 (t, 3H, $J$ = 7.2 Hz); $^{13}$C-NMR (100 MHz, CDCl$_3$) δ 163.4, 160.7, 135.1, 128.7, 125.8, 119.9, 119.2, 117.7, 113.4, 108.2, 107.3, 60.7, 41.3, 23.0, 14.6, 11.5; HRMS (ESI) m/z: [M + H]$^+$ calcd for C$_{16}$H$_{18}$N$_3$O$_3$ 300.1342, obsd 300.1348.

Methyl 1-(N-benzylcarbamoyl)-7-cyanoindolizine-3-carboxylate (28). The reaction was performed with propiolamide 27 and pyridinium salt 7 (50 mg) in methanol, following method C. The indolizine 28 was obtained in 40% yield (26 mg) as a white powder. mp 196–199 °C; $^1$H-NMR (400 MHz, CDCl$_3$) δ 9.56 (dd, 1H, $J$ = 7.4, 1.2 Hz), 9.07 (dd, 1H, $J$ = 1.8, 1.2 Hz), 7.77 (s, 1H), 7.42–7.44 (m, 4H), 7.36–7.39 (m, 1H), 7.10 (dd, 1H, $J$ = 7.4, 1.8 Hz), 6.29 (br s, 1H), 4.72 (d, 2H, $J$ = 5.6 Hz), 3.99 (s, 3H); $^{13}$C-NMR (100 MHz, CDCl$_3$) δ 162.9, 160.9, 138.1, 136.0, 128.9 (2C), 128.0 (2C), 127.8, 127.7, 126.8, 120.1, 117.5, 116.3, 114.2, 111.5, 107.5, 51.9, 43.7; HRMS (ESI) m/z: [M + H]$^+$ calcd for C$_{19}$H$_{16}$N$_3$O$_3$ 334.1186, obsd 334.1183, [M + Na]$^+$ calcd for C$_{19}$H$_{15}$N$_3$O$_3$ 356.1006, obsd. 356.1007.

4.4. pKa Determination

Potentiometric measurements were performed in a jacketed cell thermostated at 25.0 °C, kept under an inert atmosphere of purified argon, using an automatic titrator (Metrohm, DMS Titrino 716, Herisau, Switzerland) connected to a microcomputer. The free hydrogen concentrations were measured with a glass-Ag/AgCl combined electrode (Metrohm) filled with 0.1 M NaCl. The electrode was calibrated with three standard buffers at pH 4, 7, and 10. NaCl was employed as supporting electrolyte to maintain the ionic strength at 0.10 M.

Samples of 0.2 mmol of pyridinium salts were dissolved in 20 mL of freshly prepared 0.1 M NaClO$_4$. Aliquot of 10 mL were titrated with 0.02 M NaOH. A minimum of three sets of data was used in each case. Equilibrium constants and species distribution diagrams were calculated by using the program HYPERQUAD 2003.

Supplementary Materials: Supplementary materials can be accessed at: http://www.mdpi.com/ 1420-3049/ 21/3/332/s1.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

| Abbreviation | Definition |
|--------------|------------|
| DMF          | N,N-dimethylformamide |
| DMSO-D$_6$   | deuterated dimethylsulfoxide |
| TLC          | thin-layer chromatography |
| TRIS         | Tris(hydroxymethyl)aminomethane |

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**Sample Availability:** Samples of the compounds are not available from the authors.

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