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Selective ^{15}N-Labeling and Analysis of ^{13}C−^{15}N *J* Couplings as an Effective Tool for Studying the Structure and Azide−Tetrazole Equilibrium in a Series of Tetrazolo[1,5-*b*][1,2,4]triazines and Tetrazolo[1,5-*a*]pyrimidines

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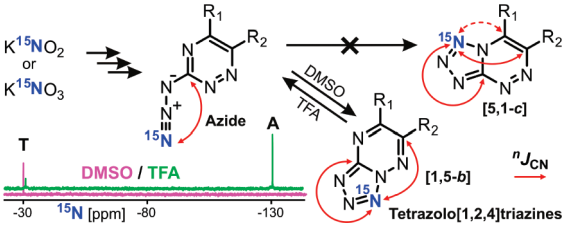

Two general methods for the selective incorporation of an ^{15}N-label in the azole ring of tetrazolo[1,5-*b*][1,2,4]triazines and tetrazolo[1,5-*a*]pyrimidines were developed. The first approach included treatment of azinylhydrazides with ^{15}N-labeled nitrous acid, and the second approach was based on fusion of the azine ring to [2-^{15}N]-5-aminotetrazole. The synthesized compounds were studied by ^{1}H, ^{13}C, and ^{15}N NMR spectroscopy in both DMSO and TFA solution, in which the azide−tetrazole equilibrium is shifted to tetrazole and azide forms, respectively. Incorporation of the ^{15}N-label led to the appearance of ^{13}C−^{15}N *J* coupling constants (J_{CN}), which can be measured easily using either 1D ^{13}C spectra with selective ^{15}N decoupling or with amplitude modulated 1D ^{13}C spin−echo experiments with selective inversion of the ^{15}N nuclei. The observed J_{CN} patterns permit unambiguous determination of the type of fusion between the azole and azine rings in tetrazolo[1,5-*b*][1,2,4]triazine derivatives. Joint analysis of J_{CN} patterns and ^{15}N chemical shifts was found to be the most efficient way to study the azido-tetrazole equilibrium.

Introduction

Derivatives of 1,2,4-triazines and pyrimidines present an important core in many natural[1−5] and synthetic[6−11] biologically active compounds. These heterocycles easily undergo different substitution reactions and ring transformations[12−14] that make them convenient precursors for the synthesis of other heteroaromatic compounds, e.g., azoloazines.[15,16] The

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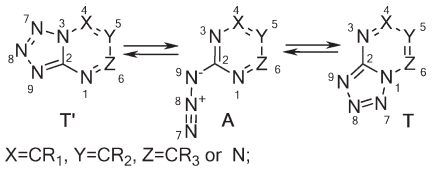
SCHEME 1. Azido−Tetrazole Tautomerism in a Series of Azido-1,2,4-triazines A (X = CR_1, Y = CR_2, Z = N) and Azidopyrimidines A (X = CR_1, Y = CR_2, Z = CR_3)



transformations of the azine ring by the Dimrorth rearrangement can easily change the type of fusion between the azole and azine rings.[17−23] Therefore, the unambiguous determination of the fusion type in azoloazines becomes an important task in organic chemistry.[17−20] A similar problem arises from the possibility of a structural rearrangement in the azole ring. In this case, the azido-tetrazole tautomerism between fused tetrazoles and their open-chain isomers (Scheme 1) should be taken into consideration. For example, the cyclization of the azido group attached to the di(tri)azine ring at the position between nitrogen atoms (structure **A**) can result in formation of two differently fused tetrazoloazines, **T** and **T′** (Scheme 1). Therefore, two structural parameters should be determined for the characterization of the fused tetrazoloazines: (1) the state of azide−tetrazole equilibrium and (2) the mode of fusion between tetrazole and azine rings.

In many previous works, topics of the azido-tetrazole transformation of azido-1,2,4-triazines similar to **A** (Z = N, X = CR_1, Y = CR_2) have been discussed, but several aspects remain unclear.[24−32] Exclusive formation of tetrazolo[1,5-*b*]-[1,2,4]triazines **T** was reported for the isomerization of 3-azido-1,2,4-triazines **A** (Scheme 1, Z = N, X = CH, Y = CAr; Z = N, X = COH, Y = CMe/CPh).[24,25] At the same time, the formation of the tetrazolo[5,1-*c*][1,2,4]triazines **T′** was described in the cyclization of benzo derivatives of 3-azido-1,2,4-triazines **A** (Scheme 1, Z = N, X = Y = benzo(C_6H_4)).[26,27]

X-ray crystallography is usually used to confirm the azoloazine structures[17−20] and for determination of the fusion mode in tetrazoloazines.[24,28,29] Unfortunately, this method only gives information about the structure of compounds in the solid state. To confirm the structure of such compounds in solution, indirect UV−vis and ^{1}H, ^{13}C NMR spectroscopic methods are usually used, based on comparison with model compounds. In this case, the spectra of tetrazoloazines are most frequently compared with those of their deaza analogues.[27,30−32] Due to the low densities of hydrogen and carbon atoms in azoloazines, the direct spectroscopic studies using well established ^{1}H and ^{13}C NMR methods (1D spectroscopy, 2D HMQC, HMBC, INADEQUATE, etc.) did not provide enough information for structural characterization of these compounds. In addition the computational methods based on calculations of relative thermodynamic stability of open-chain and cyclic forms can be used for studies of azido-tetrazole equilibrium. This approach was successfully applied for investigation of rearrangement of linear azido-1,2,4-triazines **A** (Z = N; X = Y = benzo(C_6H_4)) and azidoqunazolines **A** (X = CH; Z = Y = benzo(C_6H_4)) into their angular isomers **T′**.[33,34] To the best of our knowledge, there is no general method to study the azido-tetrazole isomerization that allows for the direct experimental determination of the equilibrium state and the structure of the tetrazole isomers in solution.

^{15}N-isotopic labeling of heterocyclic compounds increases significantly the breadth of NMR methods that can be used for determination of molecular structures and mechanisms of chemical rearrangements in solution.[35−37] In the case of tetrazoloazines, the chemical shifts of the labeled ^{15}N atoms can be an indicator for either the azide or tetrazole forms of the molecule.[38−44] Heteronuclear ^{1}H−^{15}N and ^{13}C−^{15}N *J* couplings (J_{HN} and J_{CN}) give direct information about chemical structure. Moreover, the vicinal couplings (^3J) are useful in conformational studies of molecules in solution.[45] These favorable properties of J_{HN} and J_{CN} are used widely in NMR studies of proteins and nucleic acids, in which heteronuclear *J* couplings form the basis of so-called "triple-resonance" experiments and play important role in conformational analysis.[46,47]

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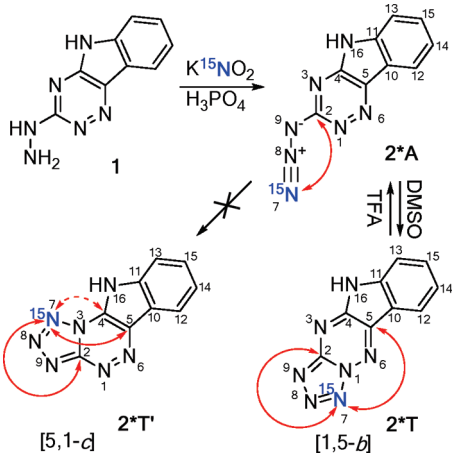
SCHEME 2. Synthesis of Selectively ^{15}N-Labeled 2* and Associated NMR J_{CN} Data Confirming Its Structure[a]



[a]The azide−tetrazole equilibrium is shifted to the tetrazole/azide form in DMSO/TFA solution. The observed J_{CN} couplings from the ^{15}N7 nucleus are shown by red arrows. The expected but unobserved coupling ($^{2}J_{CN}$) is indicated by dashed arrows. The measured J_{CN} values are presented in Table 1.

Herein we report an efficient method for the structural determination of the cyclization products of azido-1,2,4-triazines **A** (Scheme 1, X = C-R_1, Y = C-R_2, Z = N) and azidopyrimidines **A** (Scheme 1, X = Y = Z = CH) based on selective ^{15}N isotope labeling of the tetrazole ring, followed by joint analysis of ^{13}C−^{15}N *J* couplings and ^{15}N chemical shifts. This method not only allows for confirmation of the type of fusion between azole and azine rings but also permits the direct study of azide−tetrazole equilibrium in solution.

Results

Synthesis. Incorporation of an ^{15}N-isotopic label in the tetrazole part of tetrazolo[1,5-*b*][1,2,4]triazines and tetrazolo-[1,5-*a*]pyrimidines was performed by two methods using either K^{15}NO$_3$ or K^{15}NO$_2$ with 86% ^{15}N enrichment of the parent substances. The first method was based on the interaction of heteroarylhydrazides with ^{15}N-labeled nitrous acid generated from K^{15}NO$_2$. This method, used for 1,2,4-triazine derivatives, was tested on the known reaction of 2-hydrazino-1,2,4-triazines with nitrous acid.[24,25,28,29] The treatment of compound **1** with ^{15}N-labeled potassium nitrite in phosphoric acid gave tetrazolotriazine **2*T** in 56% yield through the spontaneous cyclization of azide **2*A** (Scheme 2). Despite two possible ring closures, only the linear tetracycle **2*T** was obtained rather than the angular **2*T′**.[48] (The details of the NMR and crystallographic analysis of the synthesized compounds confirming their structures are presented in a separate section of the manuscript.)

The same procedure was applied to the incorporation of an ^{15}N-isotopic label in tetrazolo[1,5-*a*]pyrimidine. The reaction of 2-hydrazinopyrimidine **3** and ^{15}N-enriched nitrous acid, however, did not proceed selectively, and a mixture of isotopomers **4*aT** and **4*bT** in 5:1 ratio was obtained in 51% total yield (Scheme 3, Method 1; please note that the identical numeration of atoms in triazines **2***, **9***, **12***, and pyrimidines **4*** instead of the IUPAC numbering was used for the convenience). The Dimroth rearrangement[49,50] is one of the most probable sources of the observed isomerization (see Scheme 3).

The second method involved the cyclization of pyrimidine or 1,2,4-triazine rings to labeled [2-^{15}N]-5-aminotetrazole **5*** and its derivatives. Synthesis of **5*** was based on the Thiele method, which included the interaction of aminoguanidine salts with nitrous acid (see Supporting Information). Because the ^{15}N-aminotetrazole **5*** exists in two tautomeric forms in solution the reaction of this compound should result in formation of a mixture of isotopomers. As expected, the condensation of tetrazole **5*** with 1,1,3,3-tetramethoxypropane led to a mixture of tetrazolopyrimidines **4*aT** and **4*bT** in a 1:1 ratio (Scheme 3, Method 2).

Cyclization of diazonium salt **6***, derived from ^{15}N-labeled aminotetrazole **5*** (see Supporting Information), with ethyl cyanoacetate **7** gave a mixture of tetrazolo[1,5-*b*][1,2,4]-triazines **9*aT** and **9*bT** in 60% yield and a 1:1 ratio (Scheme 4). Similarly, the 1:1 isotopomer mixture of tetrazolo[1,5-*b*]-[1,2,4]triazines **12*aT** and **12*bT** was obtained in the reaction of **6*** with ethyl α-formylphenylacetate **10** in 48% overall yield (Scheme 4). It should be noted that the reaction of **6*** with ethyl phenylacetate did not occur due to the low acidity of the phenylacetate. The C-formylation reaction was used previously to activate methylene-active compounds in reaction with diazonium salts.[51] In this case, the electron-withdrawing formyl group increases the acidity of the C−H bond and is eliminated from the product after azacoupling (Japp−Klingemann reaction).[52−54]

The synthesis of tetrazoloazines **9*a,bT** and **12*a,bT** provides a good illustration of how the azide−tetrazole tautomerism changes the pathway of chemical reactions. The cyclization of **8*** and **11*** (products of azacoupling of **6*** with **7** and **10**) should give tetrazolo[5,1-*c*][1,2,4]triazines **9*a,bT′** and **12*a,bT′** but likely undergoes tetrazole ring opening to give azides **9*a,bA** and **12*a,bA** followed by an alternative closure yielding tetrazolo[1,5-*b*][1,2,4]triazines **9*a,bT** and **12*a,bT** (Scheme 4).

Characterization of Fused Tetrazoloazines. NMR Spectroscopy in DMSO Solution. The synthesized compounds were studied by NMR methods in both dimethyl sulfoxide and trifluoracetic acid using samples with concentrations in the range of 90−160 mM. These solvents were chosen because in most cases the azide−tetrazole equilibrium was shifted to the tetrazole form in DMSO solution,[55−57] whereas the presence of strong acid, such as TFA, usually increases the relative concentration of

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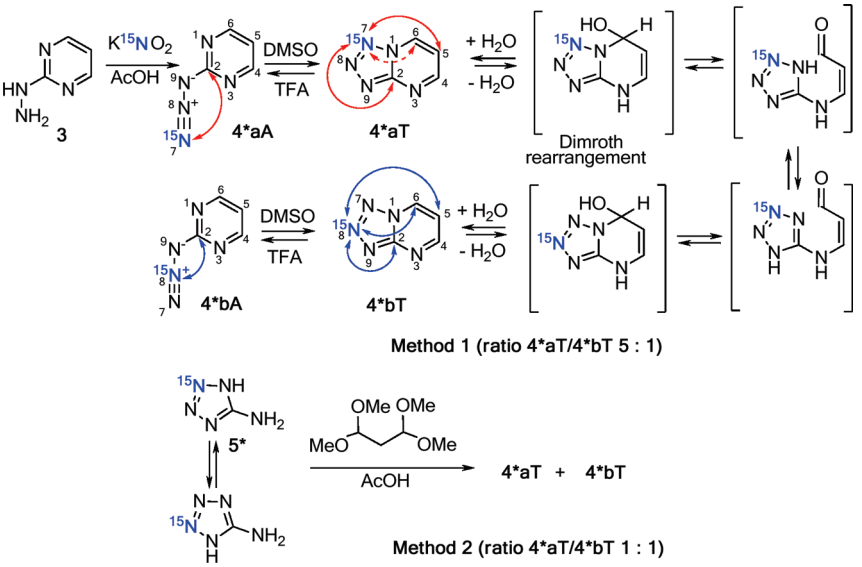
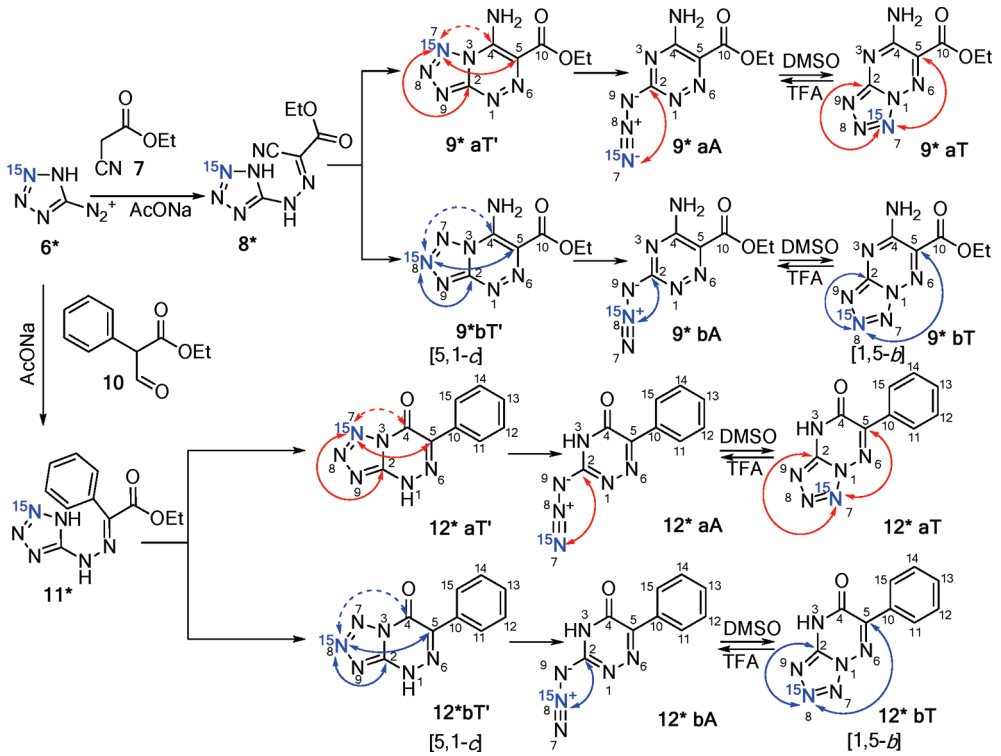
SCHEME 3. Synthesis of Selectively ^{15}N-Labeled 4* Made by Two Alternative Methods[a]



[a]The NMR J_{CN} data confirming the structures are also shown. The Dimroth rearrangement (shown on the right) is probably involved in the isomerization of **4*aT** to **4*bT** during synthesis by Method 1. The azide−tetrazole equilibrium is shifted to the tetrazole/azide form in DMSO/TFA solution. The observed J_{CN} couplings from the ^{15}N7 and ^{15}N8 nuclei are shown by red and blue arrows, respectively. The expected but unobserved coupling ($^2J_{CN}$) is indicated by dashed arrows. The measured J_{CN} values are presented in Table 1.

SCHEME 4. Synthesis of Selectively ^{15}N-Labeled 9* and 12* and NMR J_{CN} Data Confirming Their Structures[a]



[a]The azide−tetrazole equilibrium is shifted to the tetrazole/azide form in DMSO/TFA solution. The observed J_{CN} couplings from ^{15}N7 and ^{15}N8 nuclei are shown by red and blue arrows, respectively. The expected but unobserved couplings ($^2J_{CN}$ and $^3J_{CN}$) are indicated by dashed arrows. The measured J_{CN} values are presented in Table 1.

the azide form.[40,43,57] The collected ^{13}C and ^{15}N NMR data are presented in Figures 1−5 and Tables 1 and 2. The ^{1}H NMR data, as well as representative 2D ^{13}C-HMQC and ^{13}C/^{15}N-HMBC spectra, are shown in Supporting Information.

Selective incorporation of an ^{15}N label into tetrazolo[1,5-*b*]-[1,2,4]triazines **2*T**, **9*a,bT**, and **12*a,bT** and tetrazolo[1,5-*a*]-pyrimidines **4*a,bT** allowed for the detection of ^{13}C−^{15}N *J*-couplings, which became apparent as additional splittings of

TABLE 1. ^{13}C Chemical Shifts (ppm) and $^{13}C-^{15}N$ J-Coupling Constants (Hz) of the Studied Compounds[a]

compound	solvent	C2	C4/C6	C5	other signals
2*T	DMSO	148.92 $^2J_{C-N7}$ 3.1	150.57	141.92 $^3J_{C-N7}$ 1.4	116.06(C10) 146.77(C11) 124.22(C12) 113.49(C13) 123.31(C14) 134.89(C15)
	TFA	143.17 $^2J_{C-N7} < 0.3$[b]	149.64[c,d]	143.69 $^3J_{C-N7}$ 1.7	114.54(C10)[d] 144.96(C11) 124.56(C12)[c] 113.76(C13)[c,d] 136.65(C15)[c] 125.56(C14)
2*A	TFA	155.94 $^3J_{C-N7}$ 0.5	149.07	140.31	116.02(C10)[d] 142.58(C11) 123.43(C12)[c] 113.76(C13)[c,d] 134.97(C15)[c] 126.12(C14)
4*a,bT[e]	DMSO	154.91 $^2J_{C-N7}$ 2.3 $^2J_{C-N8}$ 2.5	160.25(C4) 135.51(C6) $^2J_{C6-N7}$ 3.8[f] $^3J_{C6-N8} < 0.3$[g]	113.74 $^3J_{C-N7}$ 1.0 $^4J_{C-N8}$ 0.5	
4*a,bA[e]	DMSO	161.80 $^3J_{C-N7}$ 0.6[h] $^2J_{C-N8}$ ND[h]	160.10(C4/6)	118.30	
	TFA	157.35 $^3J_{C-N7}$ 0.5 $^2J_{C-N8}$ 0.7	158.40(C4/6)[c]	116.98	
9*a,bT	DMSO	150.07 $^2J_{C-N7}$ 3.0 $^2J_{C-N8}$ 2.2	154.67	132.87 $^3J_{C-N7}$ 1.1 $^4J_{C-N8}$ 1.0	162.05(C10) 14.27(Me) 63.65(OCH_2)
	TFA	144.63 $J_{C-N7/8}$ ~0.5[i]	155.68	132.72 $J_{C-N7/8}$ ~1.0[i]	160.73(C10) 11.65(Me) 65.32(OCH_2)
9*a,bA	DMSO	156.58[j] $J_{C-N7/8}$ ND[j]	156.33[j]	133.35[j]	164.56(C10) 14.46(Me) 62.10(OCH_2)
	TFA	157.87 $^3J_{C-N7}$ 0.5 $^2J_{C-N8}$ 0.8	156.29	129.77	161.23(C10)[d] 11.64(Me) 64.93(OCH_2)
12*a,bT	DMSO	146.01 $^2J_{C-N7}$ 3.3 $^2J_{C-N8}$ 2.1	154.35	152.14 $^3J_{C-N7}$ 1.6 $^4J_{C-N8}$ 0.9	131.34(C10) 132.00(C13) 128.75(C11/15) 129.98(C12/14)
	TFA	143.53[c] $J_{C-N7/8}$ ND[k]	153.04	152.99 $^3J_{C-N7}$ 1.6 $^4J_{C-N8}$ 0.7	126.49(C10) 133.07(C13) 129.14(C11/15)[c] 128.05(C12/14)
12*a,bA	TFA	156.85 $^3J_{C-N7}$ 0.5 $^2J_{C-N8}$ 0.8	161.31[d]	149.96[d]	128.24(C10) 133.28(C13) 128.66(C11/15)[c] 128.63(C12/14)

[a]Unless otherwise stated, the listed J_{CN} values represent the average between two independent measurements using ^{13}C line-shape analysis and amplitude modulated spin−echo experiments (see Supporting Information). Both types of measurements gave identical values of J_{CN} within experimental error (±0.15 Hz). ^{13}C chemical shifts were referenced indirectly relative to proton signal of tetramethylsilane (TMS). [b]The J_{CN} coupling constant was not detected, probably due to broadening of the corresponding ^{13}C resonance. [c]The signal demonstrated additional broadening or splitting, which was not connected with J_{CN} or J_{CH} couplings. [d]The signal partially overlapped with the ^{13}C signals of TFA-*d* or with the ^{13}C signals of fluorine-containing impurities. [e]The mixture of **4*a/4*b** isotopomers (5:1) synthesized by Method 1 of Scheme 3 were used for J_{CN} measurements. [f]The J_{CN} value was measured only by ^{13}C line-shape analysis. Measurement using spin−echo was impossible due to fast transverse relaxation of the corresponding ^{13}C nucleus. [g]The J_{CN} coupling constant was not detected, probably due to low abundance of the **4*b** isotopomer. [h]The $^3J_{C-N7}$ value was measured by only ^{13}C line-shape analysis. Measurement of the $^2J_{C-N8}$ coupling was impossible due to low abundance of the **4*b** isotopomer. The measurements using spin−echo were impossible due to the low concentration of the azide form of **4*a,bA** in DMSO solution. [i]The J_{CN} values were estimated from ^{13}C line-shape analysis. The precise measurements were impossible due to fast conversion of tetrazoles **9*a,bT** to azides **9*a,bA** in TFA solution. [j]The assignment of ^{13}C resonances in the azide fragment and measurement of J_{CN} were impossible due to the low concentration of the azide form **9*a,bA** in DMSO solution. The presented tentative assignment is obtained by comparison with spectra of **9*a,bA** in TFA solution. [k]Measurements of J_{CN} couplings were impossible due to significant broadening of the corresponding ^{13}C resonance.

the corresponding signals in the ^{13}C NMR spectra. It should be noted that in some cases the J_{CN} splittings were unresolved, thus requiring double-resonance ($^{13}C/^{15}N$) spectroscopy for their detection. To detect and quantitatively characterize the $^{13}C-^{15}N$ interactions, two alternative methods were employed (see Supporting Information). In the first approach, the values of J_{CN} were extracted from nonlinear fits of the ^{13}C line shapes in the 1D spectra acquired with or without band-selective decoupling from ^{15}N nuclei. The second method was based on the amplitude modulated spin−echo technique.[47] In this case, the value of J_{CN} was extracted quantitatively from the intensities of multiplets in 1D ^{13}C spin−echo spectra measured with and without selective inversion of the ^{15}N nucleus of interest in the middle of echo period. Both methods gave J_{CN} values with comparable accuracy (see Table 1 and Supporting Information). The analysis of the J_{CN} patterns permitted the straightforward identification of the mode of fusion between azole and azine fragments in tetrazolo-1,2,4-triazine derivatives and significantly aided assignment of ^{13}C signals in azine moieties of the compounds, where standard 2D protocols based on $^1H-^{13}C$ *J*-couplings (^{13}C-HMQC and ^{13}C-HMBC) were ineffective due to the absence of protons. Moreover, the line shape analysis of ^{13}C multiplets confirmed the 86% excess of the ^{15}N isotope, expected from the degree of ^{15}N enrichment in the parent substances.

One set of signals was observed in the ^{13}C, ^{15}N, and ^1H NMR spectra of **2*T** in DMSO solution (Figures 1a and 2a; Figure S1a in Supporting Information) that indicated the presence of only one tautomeric form. The 1D ^{13}C NMR spectra of **2*T** showed J_{CN} splittings for signals from the C2 and C5 nuclei (Figure 1a, Table 1). These coupling constants proved the formation of the cyclic structure with [1,5-*b*] type of fusion between tetrazole and 1,2,4-triazine rings. If the alternative structure **2*T′** with the [5,1-*c*] type of fusion were present, then one should observe J_{CN} splittings for all ^{13}C nuclei (C2, C4, and C5) of the triazine fragment (Scheme 2). Similarly, the spectra of azide **2*A** should be characterized

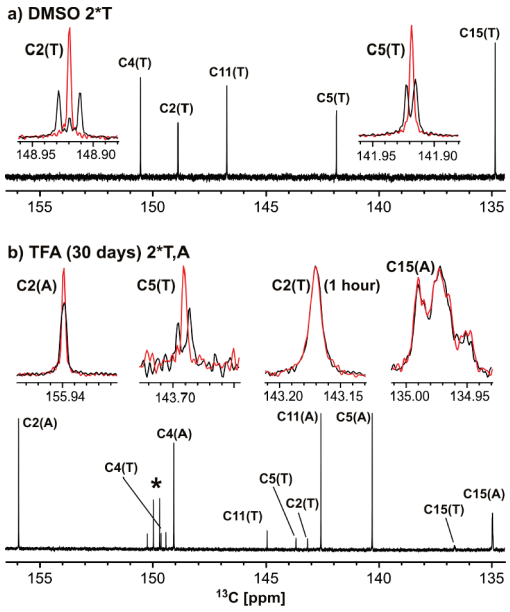


FIGURE 1. Fragments of proton decoupled 1D ^{13}C NMR spectra of 90 mM **2*T** in DMSO-d_6 (a) and TFA-*d* (b). The spectrum in panel (b) was measured 30 days after dissolving. The ^{13}C multiplets demonstrating J_{CN} couplings are enlarged and are shown above the spectra. The fragments of the spectra acquired with band-selective decoupling from the ^{15}N7 nucleus are shown in red. The signal of a fluorine-containing impurity in the TFA is marked by an asterisk.

by the presence of J_{CN} splittings for only the C2 nucleus. (Here we assume that all geminal ($^2J_{CN}$) and vicinal ($^3J_{CN}$) couplings have detectable amplitude > 0.3 Hz.) The signal of the ^{15}N7 nucleus was observed in the 1D ^{15}N NMR spectrum at -30.5 ppm (Figure 2a, Table 2), within the spectral range reported previously for fused tetrazoloazines (from -33 to -24 ppm).[40,43]

Two signals corresponding to ^{15}N-labeled atoms were detected in the 1D ^{15}N NMR spectrum of a mixture of isotopomers **4*aT** and **4*bT** of tetrazolo[1,5-*a*]pyrimidine in DMSO solution (Figure 2a, Table 2). These signals indicated ^{15}N-label incorporation at the N7 (δ -31.5 ppm) and N8 (δ 22.6 ppm) positions, respectively. The assignment of the ^{15}N7/8 resonances was confirmed by a 2D ^{15}N-HMBC spectrum (Figure S6 in Supporting Information). The integral intensity of these signals in the 1D ^{15}N NMR spectrum provided the ratio of the **4*a,bT** isotopomers formed during the preparation according to Methods 1 and 2 (Scheme 3). The detection of J_{CN} couplings for the C2, C5, and C6 nuclei (Figure 3a, Table 1) proved the formation of fused tetrazole (Scheme 3). Interestingly, the small amount of azide form **4*a,bA** ($\sim$ 5%) was detected in both the ^{13}C and ^{1}H NMR spectra (Figure 3a, Figure S2a in Supporting Information). The low concentration of this form did not allow observation of the corresponding nitrogen resonances in the 1D ^{15}N spectra. Similarly, the J_{CN} splitting, which was only observed for the C2 nucleus, confirmed the azide structure of **4*a,bA** (Figure 3a).

According to the obtained data, the ring opening associated with the tetrazole to azide rearrangement of **4*a,bT** in DMSO is characterized by a relatively large downfield shift of the C2 ($\Delta\delta$ 6.89 ppm), C5 ($\Delta\delta$ 4.56 ppm), and C6 ($\Delta\delta$ 24.59 ppm) resonances. It should be noted that partial rearrangement of the tetrazolo[1,5-*a*]pyrimidine to the corresponding azide in DMSO was observed previously by Denisov et al.[56]

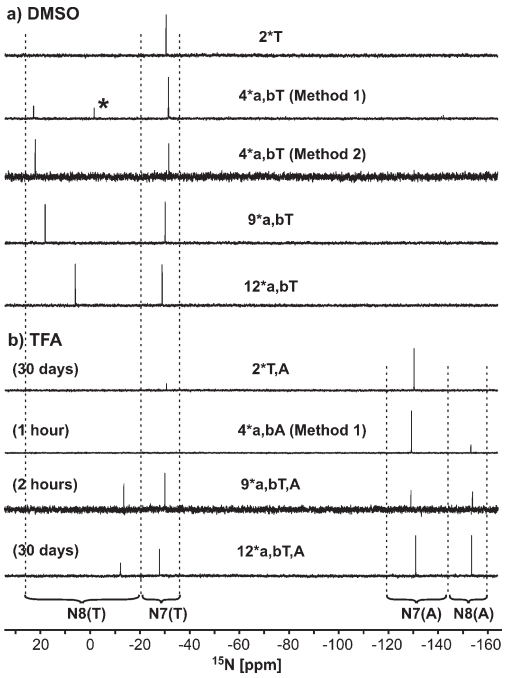


FIGURE 2. 1D ^{15}N NMR spectra of 90−160 mM **2*T**, **4*a,bT**, **9*a,bT**, and **12*a,bT** in DMSO-d_6 (a) and TFA-*d* (b). The time passed after dissolution of compounds with TFA and method used for synthesis of **4*a,bT** are given above the spectra. The spectral ranges for signals of ^{15}N7 and ^{15}N8 nuclei in the tetrazole and azide forms of the compounds are denoted by vertical dashed lines. The signal of a $K^{15}NO_3$ impurity is marked by an asterisk.

TABLE 2. ^{15}N Chemical Shifts (ppm) of the Studied Compounds[a]

compound	solvent	δ^{15}N7	δ^{15}N8	δ^{15}N of other signals[b]
2*T	DMSO	−30.5		
	TFA	−30.7		
2*A	TFA	−130.4		
4*a,bT	DMSO	−31.5	22.6	−42.7 N9 −104.0 N3 −142.0 N1
4*a,bA	TFA	−129.3	−153.3	
9a,bT	DMSO	−30.1	18.0	
	TFA	−29.5	−13.6	
9a*,b*A	TFA	−129.2	−153.9	
12*a,bT	DMSO	−28.9	5.8	
	TFA	−27.9	−12.2	
12*a,bA	TFA	−131.0	−153.6	

[a] ^{15}N chemical shifts were referenced indirectly relative to nitromethane ($MeNO_2$). [b] The assignment of signals was obtained at natural ^{15}N abundance using a 2D ^{1}H,^{15}N-HMBC spectrum.

Similarly, two signals corresponding to ^{15}N-labeled atoms (δ^{15}N7 -30.1 ppm and δ^{15}N8 18.0 ppm) were observed in the 1D ^{15}N NMR spectrum of **9*a,bT** in DMSO solution (Figure 2a, Table 2) indicating the formation of a mixture of ^{15}N7/8 isotopomers with 1:1 ratio. (Here and in the case of **12*a,bT** assignment of N7 and N8 signals was obtained by comparison with ^{15}N spectra of compounds **4*a,bT** and **2*T**.) The detection of J_{CN} couplings between the labeled nitrogen atoms and C2 and C5 nuclei (Figure 4a, Table 1) proved the formation of the fused tetrazole with a [1,5-*b*] type of fusion between tetrazole and 1,2,4-triazine rings. If the alternative structure **9*a,bT′** with a [5,1-*c*] type of fusion were present, the J_{CN} coupling should be detected for all ^{13}C nuclei (C2, C4, and C5) of the triazine fragment (Scheme 4).

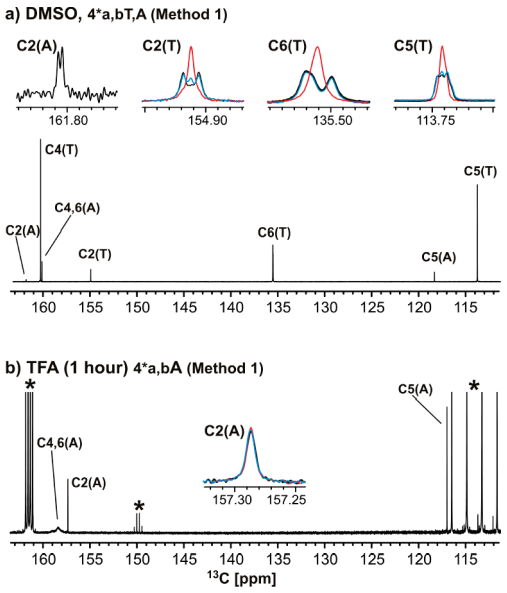


FIGURE 3. Proton decoupled 1D ^{13}C NMR spectra of 160 mM **4*a,bT** in DMSO-d_6 (a) and TFA-*d* (b). The spectrum in panel (b) was measured 1 h after dissolving. The ^{13}C multiplets demonstrating J_{CN} couplings are enlarged and shown above the spectra. The fragments of spectra acquired with band-selective decoupling from ^{15}N7 and ^{15}N8 nuclei are shown in red and blue, respectively. The signals of TFA and a fluorine-containing impurity are marked by asterisks.

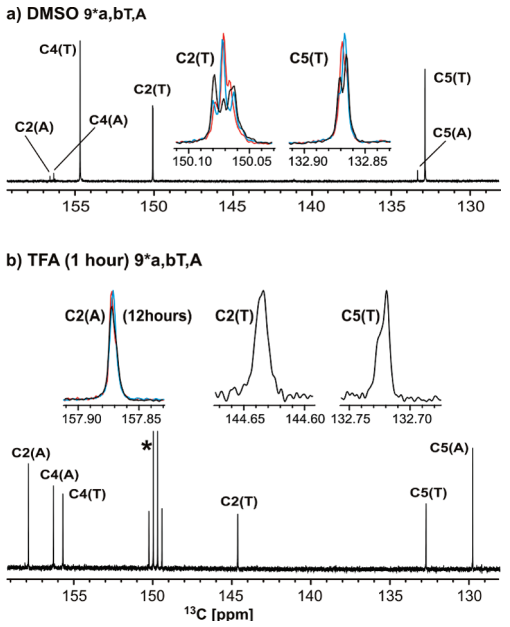


FIGURE 4. Fragments of proton decoupled 1D ^{13}C NMR spectra of 130 mM **9*a,bT** in DMSO-d_6 (a) and TFA-*d* (b). The spectrum in panel (b) was measured 1 h after dissolving. The ^{13}C multiplets demonstrating J_{CN} couplings are enlarged and shown above the spectra. The fragments of spectra acquired with band-selective decoupling from the ^{15}N7 and ^{15}N8 nuclei are shown in red and blue, respectively. The signal of a fluorine-containing impurity in the TFA is marked by an asterisk. Please note that the shown tentative assignment of **9*a,bA** in DMSO was obtained by comparison with spectra of **9*a,bA** in TFA.

Analogously to compounds **4*a,bT**, a small amount of the azide form **9*a,bA** (~4%) was detected in the ^{13}C and ^{1}H NMR spectra (Figure 4a, Figure S3a in Supporting Information). This form was also characterized by a relatively large downfield shift of the C2 ($\Delta\delta$ 6.51 ppm) resonance. The low concentration of the azide form did not allow for detection or measurement of the corresponding J_{CN} couplings.

In contrast to **4*a,bT** and **9*a,bT**, but similar to **2*T**, a single set of resonances was observed in the 1D ^{13}C and ^{1}H NMR spectra of **12*a,bT** in DMSO solution (Figure 5a, Figure S4a in Supporting Information). In this case, two nitrogen signals with identical intensities and chemical shifts (δ^{15}N7 −28.9 and δ^{15}N8 5.8 ppm), typical for fused tetratrazoloazines, were detected in the 1D ^{15}N NMR spectrum (Figure 2a, Table 2). The above observations indicated that compounds **12*a,bT** are only in the tetrazole form in DMSO solution and represent a 1:1 mixture of ^{15}N7/8 isotopomers (Scheme 4). The observation of the ^{13}C−^{15}N splitting for the C2 and C5 carbon signals in the 1D ^{13}C NMR spectrum of **12*a,bT** unambiguously confirmed the [1,5-*b*] type of fusion between the tetrazole and 1,2,4-triazine rings (Figure 5a, Table 1).

The NMR-derived structures of compounds **2*T**, **4*a,bT** and **12*a,bT** in DMSO-d_6 solution are in good agreement with X-ray diffraction data. Suitable crystals for X-ray structure determination were prepared in the case of unlabeled analogues of **2*T**, **4*a,bT** and **12*a,bT** (Figure 6).

The results showed that all compounds existed as the tetrazole isomers in the crystalline form. The X-ray data also confirmed the [1,5-*b*] type of fusion between the tetrazole and azine rings in the 1,2,4-triazine derivatives.

Tetrazole to Azide Rearrangement. NMR Spectroscopy in TFA Solution. According to the NMR data, the tetrazolo-[1,5-*b*]pyrimidines **4*a,bT** undergo rapid rearrangement to

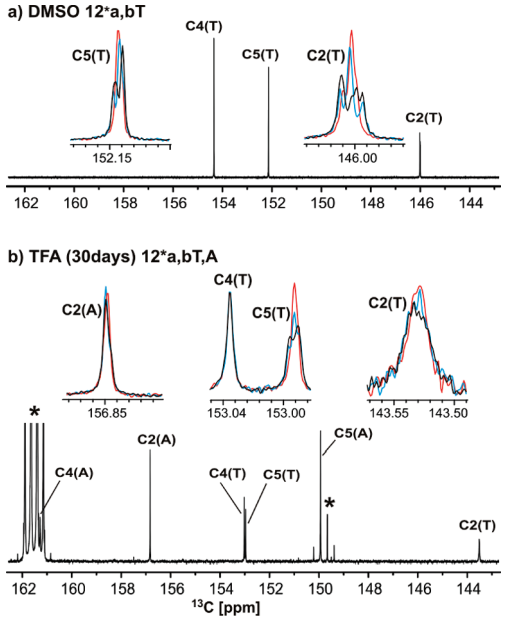


FIGURE 5. Fragments of proton decoupled 1D ^{13}C NMR spectra of 110 mM **12*a,bT** in DMSO-d_6 (a) and TFA-*d* (b). The spectrum in panel (b) was measured 30 days after dissolving. The ^{13}C multiplets demonstrating J_{CN} couplings are enlarged and shown above the spectra. The fragments of spectra acquired with band-selective decoupling from ^{15}N7 and ^{15}N8 nuclei are shown in red and blue, respectively. The signals of TFA and a fluorine-containing impurity are marked by asterisks.

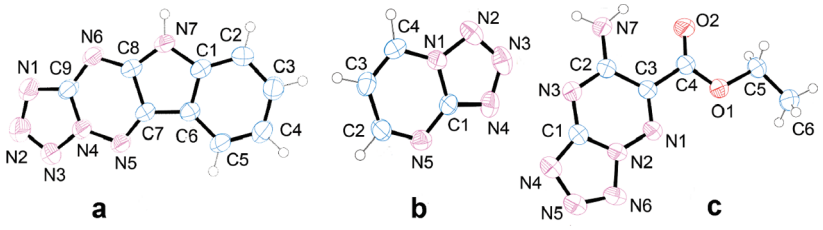


FIGURE 6. ORTEP diagrams of the X-ray structure of compounds **2T** (a), **4T** (b), and **12T** (c).

the **4*a,bA** azide forms in TFA solution (Scheme 3). As expected for the azide form, the presence of $^{13}C-^{15}N$ *J*-couplings was detected only for the C2 nucleus (Figure 3b, Table 1). All of the carbon resonances of **4*a,bA** in TFA solution were shifted upfield relative to the positions observed for **4*a,bA** in DMSO. Nevertheless, these shifts had moderate amplitude (see Figure 3b), and the C2 and C5 resonances of **4*a,bA** in TFA still demonstrate downfield shifts when compared to the corresponding resonances of **4*a,bT** in DMSO. The signal of the C4/C6 nuclei of the azide form in TFA demonstrated significant line broadening (Figure 3b), which can be ascribed to protonation of the N1/3 nitrogen atoms of the pyrimidine fragment. The resonances of the ^{15}N7 (δ −129.3 ppm) and ^{15}N8 (δ −153.3 ppm) nuclei were observed in a characteristic region for azidoazines (from −154 to −129 ppm).[40,43] The investigation of the **4*a,bT** mixture synthesized according to Method 1 of Scheme 2 and having a 5:1 ratio of ^{15}N7/8 isotopomers allowed assignment of the N7 and N8 resonances using their integral intensities in the 1D ^{15}N NMR spectrum (Figure 2b, Table 2). All obtained data confirm the full rearrangement of tetrazolo[1,5-*b*]pyrimidine to the azide form under acidic conditions and are in a good agreement with the data reported by Temple et al.[57]

In contrast to the **4*a,bT** heterocycles, **2*T**, **9*a,bT**, and **12*a,bT** did not undergo fast conversion to the azide form in TFA solution. This difference allowed the simultaneous NMR detection of both isomeric forms. The position of the tetrazole−azide equilibrium has been characterized quantitatively by the integral intensities of signals of both isomers in the 1D ^{1}H and ^{15}N NMR spectra (Table 3). In the case of compounds **9*a,bT**, the almost complete rearrangement of the tetrazole isomers to azides was observed after 12 h. Compounds **12*a,bT** and **2*T** were not transformed completely to the corresponding azides even after 1 month in TFA solution. The similar slow isomerization of tetrazole to azide was reported previously for tetrazolo[1,5-*a*]pyridines.[40]

The NMR spectral parameters of compound **2*T** were determined in TFA solution immediately after dissolving the tetrazole form, after 16 days, and again after 30 days. During this period the relative population of the azide form **2*A** increased gradually from 0% to 87% (Table 3). The tetrazole form of **2*T** in TFA solution was characterized by the ^{15}N7 resonance at −30.7 ppm (Figure 2b) and by the presence of a $^{3}J_{C5-N7}$ coupling constant with magnitude 1.7 Hz (Figure 1b, Table 1). These values were in good agreement with data observed for **2*T** in DMSO (δ^{15}N7 −30.5 ppm and $^{3}J_{C5-N7}$ 1.4 Hz), thus proving the cyclic structure of **2*T** in TFA with the [1,5-*b*] type of fusion between the azole and azine rings. The J_{CN} interaction between the C2 and N7 nuclei was not detected (possibly due to broadening of C2 resonance), indicating that the associated coupling constant has significantly smaller amplitude in TFA (< 0.3 Hz) than in DMSO solution (3.1 Hz). In addition to the line broadening, the C2 resonance of **2*T** demonstrated a relatively large upfield shift ($\Delta\delta$ −5.75 ppm) upon transition of the compound from DMSO to TFA solution (Figure 1, Table 1). Similar changes in ^{13}C chemical shifts of common carbon atoms in azole and azine rings were reported previously for tetrazolo[1,5-*a*]pyridines.[43]

TABLE 3. Tetrazole−Azide Isomerization of the Studied Compounds in Different Solvents

azide:tetrazoloazine[a]	solvent DMSO	solvent TFA	time after dissolution with TFA
2*A:2*T	0:100	0:100	1 h
2*A:2*T		67:33	16 days
2*A:2*T		87:13	30 days
4*a,bA:4*a,bT	5:95[b]	100:0	1 h
9*a,bA:9*a,bT	4:96[b]	18:82[b]	1 h
9*a,bA:9*a,bT		40:60	2 h
9*a,bA:9*a,bT		97:3[c]	12 h
12*a,bA:12*a,bT	0:100	0:100	1 h
12*a,bA:12*a,bT		60:40	30 days

[a]The relative concentrations of the azide and tetrazole forms were determined from integral intensities of the corresponding signals in the 1D ^{1}H and ^{15}N NMR spectra. [b]Signals of the azide form were not detected in the ^{15}N spectra. [c]Signals of the tetrazole form were not detected in the ^{15}N spectra.

Compound **2*A** in TFA was characterized by the ^{15}N7 resonance at −130.4 ppm (Figure 2b) and by the $^{3}J_{C2-N7}$ coupling constant with a magnitude of 0.5 Hz (Figure 1b, Table 1). The other J_{CN} interactions were not detected in the spectra, thus confirming the azide structure of **2*A**. The comparison of chemical shift values between **2*T** and **2*A** in TFA indicated that upon ring opening during the tetrazole to azide rearrangement the C2 nucleus demonstrates a significant downfield shift ($\Delta\delta$ 12.77 ppm), although the C4 and C5 resonances exhibit moderate upfield shifts (Figure 1b, Table 1). Additional splittings were observed also for some signals of the benzene fragment of both **2*T** and **2*A** (Table 1). These effects are possibly connected with proton/deuterium exchange at the N16 atom.

The rearrangement of **9*a,bT** to **9*a,bA** in TFA solution was relatively fast when compared to compound **2*T**. The ^{1}H, ^{15}N, and ^{13}C NMR spectra measured at 1 and 12 h after dissolving **9*a,bT** in TFA demonstrated that the tetrazole and azide isomers of the compounds (Table 3) were the predominant forms. The assignment of the N7 and N8 signals in both isomers was obtained by comparison with ^{15}N spectra of compounds **4*a,bA** and **2*T,A** (Figure 2b, Table 2). The J_{CN} interactions for the C2 and C5 carbon atoms of tetrazole **9*a,bT** were observed in the ^{13}C NMR spectra (Figure 4b, Table 1), thus proving that the tetrazole structure

was retained and that the [1,5-*b*] type of fusion for **9*a,bT** occurred. The precise measurements of J_{CN} values for **9*a,bT** was impossible due to fast conversion of tetrazole to azide. Simplified line-shape analysis, however, revealed that the $^{2}J_{C2-N7}$ and $^{2}J_{C2-N8}$ interactions in **9*a,bT** have significantly lower values in TFA (≤0.5 Hz) than in DMSO solution (3.3/2.1 Hz). Similarly to the observation for compound **2*T**, the C2 resonance demonstrates relatively large upfield shift ($\Delta\delta$ −5.44 ppm) upon transition of tetrazole **9*a,bT** from DMSO to TFA solution (Figure 4). In addition, a significant upfield shift of the N8 resonance ($\Delta\delta$ −31.6 ppm) was observed.

As expected, the azide isoforms **9*a,bA** in TFA solution were characterized by the presence of $^{13}C-^{15}N$ *J*-coupling constants for only the C2 carbon (Figure 4b, Table 1). Interestingly, comparison of the NMR spectra of **9*a,bA** measured in DMSO and TFA solutions does not reveal large changes in the ^{13}C chemical shifts (Figure 4). The largest differences (up to 3.6 ppm) were observed for the C5 atom of the triazine fragment and the atoms of the ethoxycarbonyl group, which is covalently linked to C5 (Table 1). The comparison of the ^{13}C chemical shift values between **9*a,bT** and **9*a,bA** in TFA solution indicated that the resonance of the C2 atom demonstrates a large downfield shift ($\Delta\delta$ 13.24 ppm) upon opening of the tetrazole ring. In contrast, the other resonances of the 1,2,4-triazine fragment shift moderately upfield (Figure 4b).

The NMR spectral parameters of compounds **12*a,bT** were determined in TFA immediately after dissolving the tetrazole form and 30 days after preparation of the solution. During this period of time the relative population of the azide forms of **12*a,bA** increased from 0% to 60% (Table 3). The assignment of the N7 and N8 signals in both isomers was made by comparison with ^{15}N spectra of other compounds (Figure 2b, Table 2). The significant broadening of the C2 signal observed in the ^{13}C spectra of **12*a,bT** in TFA did not allow for identification of J_{CN} couplings from this carbon atom (Figure 5b). The $^{3}J_{C5-N7}$ and $^{4}J_{C5-N8}$ values for the C5 nucleus were measured easily. The obtained values of 1.6 and 0.7 Hz, respectively, correspond nicely to the *J*-couplings observed for the C5 carbon of **12*a,bT** in DMSO solution (1.6 and 0.9 Hz, respectively, Table 1). This similarity confirms the retention of the bicyclic structure with a [1,5-*b*] type of fusion between the azole and azine fragments for **12*a,bT** in TFA (Scheme 4). Similar to other compounds, the transition of tetrazole **12*a,bT** from DMSO to TFA solution was associated with upfield shifts of the $^{15}N8$ and $^{13}C2$ resonances ($\Delta\delta$ −18.0 and −2.49 ppm, respectively).

The analysis of the ^{13}C multiplets of **12*a,bA** in the 1D NMR spectra revealed the presence of J_{CN} interactions for only the C2 nucleus, which confirmed the azide structure of this compound (Figure 5b). The comparison of the ^{13}C chemical shift values between **12*a,bT** and **12*a,bA** in TFA solution indicated that the ring opening associated with the tetrazole to azide rearrangement is characterized by relatively large downfield shifts of the C2 ($\Delta\delta$ 13.32 ppm) and C4 ($\Delta\delta$ 8.27 ppm) signals and a moderate upfield shift of the C5 ($\Delta\delta$ −3.03 ppm) resonance (Figure 5b). It should also be noted that in doubly $^{1}H,^{15}N$-decoupled 1D ^{13}C NMR spectrum of a **12*a,bT** and **12*a,bA** mixture the C11/C15 resonances of phenyl substitutes of both the tetrazole and azide forms demonstrated additional splitting (data not shown). These effects can be ascribed to the possible protonation of different nitrogen atoms of the 1,2,4-triazine fragment under acidic conditions.[58]

Discussion

$^{13}C/^{15}N$ NMR Markers Provide a Way To Characterize Fused Tetrazoles and Their Azides in Solution. Selective incorporation of the ^{15}N isotope in the azole ring of tetrazolo-[1,5-*a*]pyrimidine and tetrazolo[1,5-*b*][1,2,4]triazine derivatives allows the structure of these compounds to be characterized and provides a way to track the state of the azide−tetrazole equilibrium by NMR spectroscopy. Three types of NMR markers were found to be the most useful for characterizing fused tetrazoles and their open-chain isomers. These markers are (1) the ^{15}N chemical shifts of the nuclei from azole fragment, (2) the ^{13}C chemical shift of the bridge-head carbon C2, which belongs to both the azole and azine rings (Scheme 1), and (3) the J_{CN} coupling constants between the nitrogen nuclei of azole and the carbon nuclei of azine ring.

In all studied compounds, the resonances of the $^{15}N7$ and $^{15}N8$ nuclei were found in specific spectral ranges (Table 2, Figure 2). In the tetrazole form, these signals were observed at δ −31.5 to −27.9 ppm and at δ −13.6 to 22.6 ppm. Upon ring opening and formation of azides, however, the signals moved to δ −131.0 to −129.2 ppm and δ −153.9 to −153.3 ppm, respectively. Despite the relatively large solvent-dependent shifts observed for the $^{15}N8$ resonance in tetrazoles, the spectral ranges listed above do not overlap. This lack of overlap makes it possible to use the chemical shifts and integral intensities of the $^{15}N8$ and $^{15}N7$ resonances as characteristic criteria for the identification of different tautomeric forms and measurement of their relative populations in solution. The obtained data correspond well with results reported previously for tetrazolo[1,5-*a*]pyridine.[40,43] In the present work, we were able to individually assign the resonances of the $^{15}N7$ and $^{15}N8$ nuclei and to track changes of their chemical shifts upon the tetrazole to azide rearrangement.

1D ^{15}N NMR spectroscopy did not permit detection of the small amount of the azide forms of **4*a,bT** and **9*a,bT** in DMSO solution. In this case, the chemical shift of the bridge-head atom C2 could be used as an additional criterion for the identification of the different tautomeric forms. The relatively large downfield shifts of the $^{13}C2$ resonance ($\Delta\delta$ 6.5−13.3 ppm) upon tetrazole to azide rearrangement were observed in both DMSO and TFA (Table 1). As a result, the resonance of this nucleus did not overlap with the spectral regions corresponding to tetrazoles (δ 143.1−154.9 ppm) or azides (δ 155.9−161.8 ppm) in the 1D ^{13}C NMR spectrum (Table 1).

Neither the ^{15}N nor ^{13}C chemical shifts allow for determination of the type of fusion between the azole and azine rings in tetrazolo[1,5-*b*][1,2,4]triazine derivatives **2*T**, **9*a,bT**, and **12*a,bT**. Nevertheless, analysis of $^{13}C-^{15}N$ *J*-coupling patterns provides a straightforward way to solve this problem. The detection of J_{CN} coupling constants for the C2 and C5 atoms in the ^{13}C NMR spectra of these compounds in DMSO confirmed unambiguously the [1,5-*b*] type of fusion (Table 1). The presence of alternative structures **2*T′**, **9*a,bT′**,

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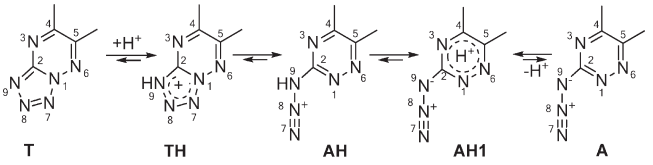
and **12*a,bT′** with the [5,1-*c*] type of fusion was excluded by the absence of J_{CN} splitting for the signal of C4 carbon (see Schemes 2, 3, and 4). Despite the absence of detectable J_{CN} interactions for the C2 atom of **2*T**, **9*a,bT**, and **12*a, bT** in TFA solution (see below), these compounds probably preserve the [1,5-*b*] structure. This assumption is supported by the good correspondence between values of the J_{CN} coupling constants observed for the C5 carbon in DMSO and TFA and by the absence of J_{CN} couplings for C4 (Table 1). The presented results indicate that the alternative isomeric structures **T′** with a [5,1-*c*] type of fusion either do not form or have very low populations even under acidic conditions in which the dynamic opening of the azole ring takes place. At the same time, the formation of a structure with [5,1-*c*] type of fusion was observed for azolo-1,2,4-triazines, which were obtained by cyclization of the azine ring to diazotized 5-amino-1,2,4-triazoles[35,59,60] or 5-hydrazino-1,2,4-triazoles.[61] In this case 1,2,4-triazolo[5,1-*c*][1,2,4]-triazines are thermodynamically more stable than alternative bicyclical isomers.[62] Interestingly, the formation of a [5,1-*c*] type of fusion in some of these compounds was confirmed by qualitative NMR analysis of J_{CN} couplings using selective ^{15}N labeling.[60] It is worth noting that structures with the [5,1-*c*] type of fusion between azole and azine rings should be formed transiently during the synthesis of compounds **9*T** and **12*T** (Scheme 4).

As expected, the azide forms were characterized by the presence of J_{CN} interactions with only the C2 carbon. The example of **4*a,bT** in DMSO (Figure 3a) indicates that diagnostic J_{CN} couplings can be detected even for small amounts of the azide form, which otherwise preclude detection of the corresponding resonances in 1D ^{15}N NMR spectra. Therefore, the ^{13}C−^{15}N couplings can be used to determine the state of the azide−tetrazole equilibrium in a series of tetrazolo-[1,5-*b*][1,2,4]triazines and tetrazolo[1,5-*a*]pyrimidines in both neutral and acidic media.

Protonation of a Specific Nitrogen Atom in the Azole Ring Is a Possible First Step in the Tetrazole to Azide Rearrangement under Acidic Conditions. The relatively large upfield shifts of the ^{15}N8 signals of tetrazoles **9*bT** ($\Delta\delta$ −31.6 ppm) and **12*bT** ($\Delta\delta$ −18.0 ppm) were observed upon changing solvents from DMSO to TFA (Table 2). According to the data reported for tetrazolo[1,5-*a*]pyridines[43] the shielding of the N8 nucleus in acidic conditions can be explained by protonation of a neighboring nitrogen atom in the tetrazole fragment. Because the N7 nucleus did not exhibit significant changes in chemical shift upon transition of **2*T**, **9*aT**, and **12*aT** from DMSO to TFA, we can conclude that nitrogen N9 is the most probable site of tetrazoles protonation in strong acidic TFA (Scheme 5). This assumption is further supported by the observation of an upfield solvent-dependent shift ($\Delta\delta$ −2.5 to −5.7 ppm) for the resonance of the neighboring C2 carbon (Table 1). The same changes in chemical shifts were reported for bridge-head carbon atom in tetrazolo[1,5-*a*]pyridines.[43]

SCHEME 5. Protonation of Tetrazolo[1,5-*b*][1,2,4]triazines Is the Possible First Step in the Tetrazole to Azide Rearrangement under Acidic Conditions



The observation of a single but broadened resonance for the ^{13}C2 nuclei of tetrazolazines **2*T**, **9*T** and **12*T** in TFA (line-width, $\Delta\nu$ ∼2−4 Hz in TFA and ∼0.6−0.8 Hz in DMSO) indicates that the process of protonation is occurring in the fast to intermediate exchange limit on the NMR time scale. Assuming that the chemical shift of the ^{13}C2 nuclei in the deprotonated tetrazole is equal to the chemical shift observed in DMSO, we can conclude that the protonation/deprotonation process is proceeding with a characteristic time of about or slightly less than 1 ms. This exchange process (proceeding quickly on the time scale of ^{13}C−^{15}N *J*-couplings) is most likely responsible for the observed suppression of J_{CN} interactions for the tetrazole ^{13}C2 nuclei in TFA solution. It worth noting that attachment of deuterium (originated from TFA-*d*) to the N9 atom should lead to the appearance of additional ^{2}H−^{13}C *J*-couplings, which could also be manifested as visual broadening of the ^{13}C2 resonance. No changes in the line shape of the ^{13}C2 resonance were detected in the 1D ^{13}C spectra measured with broadband decoupling from deuterium, however, thus ruling out ^{2}H−^{13}C *J*-couplings as a possible source of ^{13}C2 line-broadening.

Taking in mind the relatively large magnitude of solvent-dependent shifts of ^{15}N8 signal, we can propose that species protonated at the N9 atom (**TH**, Scheme 5) represent the major population of the **9*bT** and **12*bT** tetrazoles in TFA solution. According to the data reported by Cmoch et al.,[41] the azides (**A**, Scheme 5) in TFA solution also should experience protonation at some of the nitrogen atoms (N1, N3, or N6) of the azine ring (**AH1**, Scheme 5), but the favored site of protonation for 1,2,4-triazine derivatives was not determined unambiguously.[63] The broadening of the ^{13}C4/C6 signal observed for azidopyrimidine **4*A** in TFA solution confirms the presence of dynamic protonation of N1 and N3 nitrogen atoms. Interestingly the chemical equivalence of these nitrogens leads to a relatively narrow line ($\Delta\nu$ ∼1.0 Hz) for the ^{13}C2 resonance. At the same time the observation of narrow lines ($\Delta\nu$ ∼0.6−1.0 Hz) for the ^{13}C2, ^{13}C4 and ^{13}C5 resonances in azides **2*A**, **9*A**, and **12*A** could indicate that the process of protonation is occurring in the fast exchange limit on the NMR time scale and/or the deprotonated state (**A**) has vanishing population in TFA solution.

Protonation of the N9 atom of tetrazole should weaken the N1−N7 bond,[40] and as a result this shifts the equilibrium toward the open-chain azide form. At the same time the protonation of the N1 atom in azide should protect the open-chain form of the compound from recyclization. Thus we can envisage the tetrazole to azide conversion in TFA solution as a multistep process (Scheme 5), in which the initial fast

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protonation of N9 atom is followed by slower opening of the azole ring. In this case ring opening should give N9 protonated azide (**AH**), which is certainly unstable and undergoes a quick prototropy so that the proton would appear to one of the nitrogen atoms of the azine ring. The partial protonation of the N1 atom should stabilize the opened form, which additionally shifts the equilibrium toward azide. In this case the different stability of tetrazoles **2*T**, **4*T**, **9*T**, and **12*T** in TFA solution (Table 3) could be ascribed to different degree of N1 protonation in corresponding azides. The observation of small amounts of azides of **4*A** and **9*A** in DMSO solution and very fast ring opening of corresponding tetrazoles in TFA permit speculation that mechanisms of tetrazole−azide interconversion in both solvents are similar. In this case either protonation of terazole or protonation of azide or both of these reactions could be responsible for shift of the azide−tetrazole equilibrium to the opened form in TFA solution.

Conclusion

We reported, by the example of tetrazolo[1,5-*b*][1,2,4]-triazines and tetrazolo[1,5-*a*]pyrimidines, a method describing the synthesis of selectively ^{15}N-labeled heterocyclic compounds using simple isotopically enriched parent substances. The selective incorporation of the ^{15}N-label into the azolo core of tetrazoloazines leads to the appearance of ^{13}C−^{15}N *J*-couplings, which can be measured easily using conventional NMR instruments equipped with triple-resonance (^{1}H−^{13}C−^{15}N) probes. The obtained data indicate that J_{CN} couplings, together with ^{15}N chemical shifts, provide a valuable source of additional structural information that can be used during structure verification and for studies of mechanisms of chemical transformations (e.g., the tetrazole to azide rearrangement under acidic conditions). The selective ^{15}N-labeling extends the applicability of direct NMR methods for studies of heterocyclic compounds with a high abundance of nitrogen nuclei, in which conventional NMR techniques based on ^{1}H−^{13}C and ^{13}C−^{13}C *J*-couplings (HMQC, HMBC, INADEQUATE, etc.) are ineffective due to low proton and carbon densities.

Experimental Section

Compounds **1** and **3** were prepared as previously described.[48,64]

[1-^{15}N]-Tetrazolo[1′,5′:2,3][1,2,4]triazino[5,6-*b*]indole (2*T). A solution of 3-hydrazino-1,2,4-triazino[5,6-*b*]indole (**1**) (0.100 g, 0.50 mmol) in 4 mL of polyphosphoric acid was cooled to 0−5 °C, and aqueous potassium [^{15}N]-nitrite (0.090 g, 1.05 mmol in 2 mL of water) was added dropwise so that the temperature of the reaction mixture was kept below 5 °C. After stirring for 30 min at room temperature, 5 mL of water was added. The precipitate was filtered off, washed with cool water, and dried to afford compound **2*T** (0.060 g, 56%): mp 290 °C; MS (EI) *m*/*z* 212 [M$^+$]; HMRS (ESI) *m*/*z* calcd for $C_9H_5N_6{}^{15}N$ [M + H]$^+$ 213.0655, found 213.0651.

Mixture of [^{15}N]-Tetrazolo[1,5-*a*]pyrimidines (4*a,bT). Method 1. A solution of 2-hydrazinopyrimidine (**3**) (0.100 g, 0.91 mmol) in an HOAc/H_2O mixture (3:1, 5 mL) was cooled to 0−5 °C, and aqueous potassium [^{15}N]-nitrite (0.157 g, 1.82 mmol in 2 mL of water) was added dropwise so that the temperature of the mixture was kept below 5 °C. After stirring for 2 h at 5 °C, 10 mL of water was added. The resulting solution was extracted with chloroform (3 × 10 mL). Evaporation of the solvent afforded a mixture of compounds **4*a** and **4*bT** (6:1, correspondingly; 0.057 g, 51%).

Method 2. Malonaldehyde bis(dimethyl acetal) (0.031 mL, 0.24 mmol) was added to a solution of [2-^{15}N]-5-aminotetrazole monohydrate (**5***) (0.025 g, 0.24 mmol) in 1 mL of acetic acid. The reaction mixture was refluxed for 15 min. After cooling to room temperature, the precipitate was filtered off and dried to afford a mixture compounds **4*a** and **4*bT** (1:1, 0.012 g, 41%): mp 120 °C; MS (EI) *m*/*z* 122 [M$^+$]. Anal. Calcd (%) for $C_4H_3N_4{}^{15}N$: C 39,33, H 2.48, N 58,19. Found: C 39.26, H 2,44, N 57.98.

Mixture of [^{15}N]-Tetrazolo[1,5-*b*][1,2,4]triazines (9*a,bT). A mixture of ethyl cyanoacetate (**7**) (0.21 mL, 1.93 mmol) and NaOAc (1.5 g, 18.30 mmol) in water (5 mL) was added to the resulting solution of diazonium salt **6***. The reaction mixture was stirred at room temperature for 64 h. The precipitate formed was filtered off and dried to give a mixture of compounds **9*aT** and **9*bT** (0.24 g, 60%): mp 171 °C; MS (EI) *m*/*z* 210 [M$^+$]; HRMS (ESI) *m*/*z* calcd for $C_6H_8O_2N_6{}^{15}N$ [M + H]$^+$ 211.0709, found 211.0704.

Mixture of [^{15}N]-Tetrazolo[1,5-*b*][1,2,4]triazinones (12*a,bT). A mixture of ethyl phenyl(formyl)acetate **10** (0.40 g, 2.10 mmol) and sodium carbonate (0.6 g) in water (3 mL) and ethanol (2 mL) was added to the resulting solution of diazonium salt **6***. The resulting solution was stirred at room temperature for 2 h, and concentrated HCl (1 mL) was added. The reaction mixture was filtered off from the precipitate formed, which was then dissolved in 2 mL of acetic acid. The solution was refluxed for 2 h and cooled. The precipitate formed was filtered off and dried to give a mixture of compounds **12*aT** and **12*bT** (0.2 g, 48%): mp 225 °C; MS (EI) *m*/*z* 215 [M$^+$]; HRMS (ESI) calcd for $C_9H_7ON_5{}^{15}N$ [M + H]$^+$ 216.0651, found 216.0647.

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Supporting Information Available: Detailed experimental procedures, synthesis of compounds **5*** and **6***, crystallographic information and X-ray data for **2**, **4**, and **9** in CIF format, Figures S1−S6, measurement of J_{CN} coupling constants, table containing ^{1}H chemical shift data, 1D ^{1}H, and representative 2D ^{13}C-HMQC, 2D ^{13}C-HMBC and 2D ^{15}N-HMBC spectra of synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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