Spin-spin coupling constants ¹³C—¹⁵N and ¹H—¹⁵N in the investigation of azido-tetrazole tautomerism in a series of 2-azidopyrimidines*

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A new method was developed for the investigation of an azido-tetrazole equilibrium based on using a complex analysis of ${}^{13}C^{-15}N$ and ${}^{1}H^{-15}N$ spin-spin coupling constants. The use of this approach became possible due to the selective inclusion of ${}^{15}N$ isotopes into the structures of 2-azidopyrimidines and their cyclic analogs tetrazolo[1,5-*a*]pyrimidines.

Key words: azido-tetrazole tautomerism, tetrazolo[1,5-a]pyrimidine, spin-spin coupling constants, NMR spectroscopy, ¹⁵N isotope.

Practical interest in synthesis of hetaryl azides is due to their ability to participate in photochemical transformations, which allows one to use their derivatives in biological assays. For example, the 2-azidopurine derivatives are applied as photoaffine labels^{1,2} for the determination of binding sites of nucleotides with proteins and establishment of the role of these interactions.

The modification of nucleosides due to the insertion of the azido group into heterocyclic bases is known.^{3–5} This approach is promising, because the azido group is capable of transforming into the amino group, and is used in synthetic organic chemistry as well. For example, the synthesis of the hetarylamino derivatives of azines from the corresponding azidoazines was described.^{6,7}

Azaaromatic compounds with the azido group in the α -position to the nitrogen atom often demonstrate azidotetrazole tautomerism, which is widely described in the literature.^{8–12} This isomerism is characterized by the ability of the azido group in the heterocyclic derivatives to transform under the action of external factors or spontaneously and reversibly into the corresponding tetrazoles. Azido-tetrazole tautomerism makes it possible to consider tetrazoloazines as cyclic analogs of hetaryl azides.^{13,14} The studies in the area of azido-tetrazole equilibrium are necessary for controlling the reactivity of these heterocyclic systems classified as ambidentate polyfunctional compounds. So, depending on the conditions, either the tetrazole cycle, or isomeric azido group, or the azine fragment can undergo chemical transformation. It is important that the direction of the reaction often depends on the form in which the compound reacts. One of the examples of the practical successful use of the azido-tetrazole equilibrium is the hydrogenation of tetrazolo[1,5-*a*]pyridine.¹⁵ Under acidic conditions where the tetrazole form predominates, only the pyridine ring is hydrogenated, whereas the hydrogenation under basic conditions stabilizing azide affords 2-aminopyridine. One of the successful examples for the directed reduction of the tetrazole cycle is the synthesis of the modern antiepileptic drug lamotrigine and its analogs.¹⁶

It is also important to know parameters of the azidotetrazole equilibrium for the use of photoaffine labels in the field of molecular biology. This is related to the fact that the azide form is photochemically more active than the tetrazole form.¹⁷ Therefore, the maximum concentration of the azide form is necessary in experiments for the efficient application of the photoaffine label.

There is a class of hetaryl azides, whose cyclization can give two cyclic forms. For example, 2-azidopyrimidines A (X = CR³, R¹ \neq R³) containing various substituents in positions 4 and 6 of the azine ring can transform into tetrazole isomers T (X = CR³, R¹ \neq R³) and T⁷ (X = CR³, R¹ \neq R³) (Scheme 1). In the most part of cases,

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X-ray diffraction analysis is used for the unambiguous determination of the route of azido group cyclization and establishment of the structure of tetrazolo[1,5-*a*]pyrimidines $T (X = CR^3, R^1 \neq R^3)$ and $T' (X = CR^3, R^1 \neq R^3)$. However, this method is not suitable for studying the dynamics of the transition of one isomeric form to another in solutions. On the contrary, ¹H and ¹³C NMR spectroscopy allows one to directly observe azido-tetrazole rearrangements in a series of 2-azidopyrimidines, but often does not allow the structure of the formed isomers to be determined unambiguously because of the low content of hydrogen and carbon atoms in the structures of heterocycles of this class.

Scheme 1



 $X = CR^3, N; \underline{N} = {}^{14}N, {}^{15}N$ $\longrightarrow {}^{n}J_{C.N} (\underline{N} = {}^{15}N, X = N)$

Other examples for similar compounds are 3-azido-1,2,4-triazine derivatives A(X = N), which are capable of cyclizing to form two cyclic isomers of tetrazolo[5,1-c]-[1,2,4]triazines T' (X = N) and tetrazolo[1,5-b][1,2,4]triazines T (X = N) (see Scheme 1). It has previously¹⁸ been shown that the selective introduction of the ¹⁵N isotope in the γ -position of the azido group (N = ¹⁵N) of open form A(X = N) makes it possible to detect open and cyclic forms and unambiguously establish the structures of tetrazolo-1,2,4-triazines from an analysis of chemical shifts of signals in the 1D¹⁵N NMR spectra and spin-spin coupling constants (SSCCs) ${}^{13}C{-}^{15}N$ ($J_{C,N}$). For example, the formation of tetrazolo[1,5-*b*][1,2,4]triazines T ($\underline{N} = {}^{15}N, X = N$) was confirmed by two ${}^{13}C - {}^{15}N$ constants in the 1D¹³C NMR spectra. In the case of cyclization of compound $A(\underline{N} = {}^{15}N, X = N)$ to tetrazolo[5,1-c]-[1,2,4]triazines \mathbf{T}' , ${}^{n}J_{C,N}$ should be detected for all carbon atoms of the azine fragment (see Scheme 1).

In this approach, the potential of the ${}^{1}\text{H}{-}{}^{15}\text{N}$ SSCCs ($J_{\text{N,H}}$), which can be observed even in samples with the

natural abundance of the ¹⁵N isotope,¹⁹ is not observed. The selective introduction of the ¹⁵N label significantly facilitates the measurement of long-range ¹H—¹⁵N SSCCs (for example, ⁴ $J_{\rm N,H}$ through four covalent bonds), substantially decreases requirements for the amount of the substance necessary for analysis, and makes it possible to apply $J_{\rm N,H}$ for the determination of structures of more complicated organic molecules.

In this work, a complex approach based on the combined use of ${}^{13}\text{C}-{}^{15}\text{N}$ and ${}^{1}\text{H}-{}^{15}\text{N}$ SSCCs was proposed with the purpose for searching for the most efficient methods of investigation of the azido-tetrazole equilibrium for ${}^{15}\text{N}$ -labeled 2-azidopyrimidines as examples.

One of the methods for inclusion of the ¹⁵N isotope into the structure of 2-azidopyrimidines is based on the interaction of 2-hydrazinopyrimidines with labeled nitrous acid generated from K¹⁵NO₂ in an acidic medium.¹⁸ Hetaryl azide **2*A** (enrichment in ¹⁵N is 86%) containing the isotope label in the γ -position of the azido group was obtained by the same method from compound **1** (Scheme 2).





An alternative approach to the synthesis of 2-azidoazines is based on ring opening in the corresponding tetrazole derivatives. In this case, to introduce the ¹⁵N atom into the azide group/tetrazole fragment, it is necessary to use labeled 5-aminotetrazole, which builds up the azine fragment.^{16,18} The ¹⁵N isotope can be included into position 2 of 5-aminotetrazole by the treatment of aminoguanidine with nitrous acid or by the interaction of ¹⁵N-aminoguanidine **3*** with HNO₂.¹⁸ The combination of these two approaches allowed one to synthesize $[^{15}N_2]$ -5-aminotetrazole **4**** containing simultaneously two isotope labels in the heterocycle. The further condensation of compound **4**** with benzoylacetone **5** afforded tetrazolo[1,5-*a*]pyrimidine **6******T** including the ¹⁵N isotope in the β - and γ -positions of the azido group (86% enrichment in each ¹⁵N atom, Scheme 3). It should be mentioned that earlier [2-¹⁵N]-5-aminotetrazole containing one isotope label was applied to the preparation of unsubstituted ¹⁵N-tetrazolo-[1,5-*a*]pyrimidines and ¹⁵N-tetrazolo[1,5-*b*][1,2,4]triazines.^{18,20} However, in this case, a mixture of ¹⁵N isotopomers was formed at the β - and γ -positions of the azido group. The use of compound **4**** makes it possible to obtain samples of ¹⁵N-tetrazolo[1,5-*a*]pyrimidines with the homogeneous isotope composition.

Pyrimidine derivatives 2^*A and 6^{**T} were studied by the NMR method in DMSO and TFA solutions, which make it possible to shift the azido-tetrazole equilibrium towards the tetrazole isomer or azide form, respectively. In addition, the ring-chain tautomerism of sample 2^*A was additionally studied in various mixtures of DMSO and TFA. The signals from the ¹H and ¹³C nuclei were assigned using 2D ¹H—¹³C HMBC and HMQC NMR experiments.

An analysis of the NMR spectra of sample 2*A in a DMSO solution (Fig. 1, *a*) showed that the azide form undergoes spontaneous cyclization to form simultaneously two cyclic isomers 2*T' and 2*T. This conclusion was drawn on the basis of chemical shifts of the ${}^{15}N_{\nu}$ nuclei (Table 1), which were detected in the range characteristic of tetrazoloazines.^{18,21,22} An analysis of the integral intensities of signals in the 1D ¹H and ¹⁵N NMR spectra made it possible to monitor the mutual transformation of two isomeric forms of compound 2^* . Immediately after the dissolution of the production of the reaction of hetaryl-hydrazine 1 with labeled nitrous acid, the ratio of isomers 2^*T and $2^*T'$ was 92 : 8 (Table 2). Further the concentration of compound $2^*T'$ increased rapidly and changed to 49 : 51 already after incubation at 30 °C within 24 h (Table 2).

The 2D ¹H-¹⁵N HMBC spectrum of a mixture of the tetrazole isomers for compounds 2*T and 2*T' contained cross-peaks between the labeled nitrogen atom $(^{15}N_{\nu})$ and protons of the azine fragment, which directly indicated the long-range ¹H-¹⁵N spin-spin interactions between the corresponding nuclei. However, a considerable broadening of the ¹H NMR signals of compound **2***T probably caused by chemical exchange processes did not allow us to analyze the value of observed $J_{\rm N,H}$ and conclude about the structure of the tetrazole isomers formed by the cyclization of azide **2*A**. Amplitude modulated 1D ¹H spin-echo experiments with selective inversion of the ¹⁵N nuclei were used for the quantitative measurement of the observed $^{1}\text{H}-^{15}\text{N}$ SSCCs. The measured values of long-range J_{N} H (see Table 1) allowed us to establish the structures of compounds 2^{T} and 2^{T} (see Scheme 2). For example, the $^{1}\text{H}-^{15}\text{N}$ spin-spin interaction at the H(5) proton and the protons of the methyl group at the C(6') atom prove that



Note. For compounds **6******T**, **6******A**, and **6******T**', ${}^{n}J_{C,N}$ and ${}^{n}J_{N,H}$ are shown for the ${}^{15}N_{\gamma}$. atom only.



Fig. 1. 1D ¹⁵N NMR spectra of compounds 2^* 24 h after dissolution in DMSO (*a*), in a DMSO–TFA (2:3) mixture (*b*), and in TFA (*c*) and of compounds 6^{**} in DMSO (*d*) and TFA (*e*).

the cyclization of compound **2*A** to tetrazole isomer **2*T** involves the N(1) atom of the pyrimidine ring. In the case of structure **2*T**['], the annelation mode for the tetrazole ring is confirmed by the observation of the single ${}^{4}J_{\rm N,H}$ between the H(5) proton and N_v nitrogen atom.

The conclusion about the structures of compounds 2*T and 2*T' is well consistent with the experimental data on the analysis of the ¹³C—¹⁵N coupling constants. Thus, in the 1D ¹³C NMR spectrum of a mixture of tetrazolo-[1,5-*a*]pyrimidines for the C(2), C(4), C(5), and C(6) signals of compound 2*T' exhibits spin-spin interactions with the ¹⁵N_{γ} nucleus (Table 3, see Scheme 2). The interaction between the ¹³C(4) and ¹⁵N_{γ} atoms (²J_{C,N} = 2.1 Hz) proves

that the N(3) atom participates in tetrazole isomer formation. In the case of isomer **2*T**, $J_{C,N}$ was observed only for the signals of two C(6) and C(6') atoms (see Table 3). The expected constants at C(2) and C(5) were not detected because of a considerable broadening of the corresponding ¹³C NMR signals, which is induced by the dynamic transformation of the cyclic forms into each other and a possible influence of prototropic tautomerism for compounds **2*T** and **2*A**. However, the presence of the spin-spin interaction between the carbon atom of the methyl group ¹³C(6') and the ¹⁵N_γ atom (³ $J_{C,N} = 0.8$ Hz) unambiguously confirms the structure of cyclic isomer **2*T** (see Scheme 2).

Com- pound	Solvent	1 H (δ , <i>J</i> /Hz) ^{<i>a</i>}			¹⁵ N (δ , <i>J</i> /Hz) ^{<i>b</i>}	
		H(5)	Me	Other signals	N_{γ}/N_{β}	Other signals
2* T [*] c	DMSO	5.94	2.38	_	$-33.8 (N_{y})$	-259.0 (N(1));
		$({}^{4}J_{\rm NH} = 0.6)$	$({}^{4}J_{\rm H(5)\ H(6')}=0.8)$		· <i>P</i>	-136.9(N(3))
	DMSO-TFA (3:1)	5.81	2.33	_	$-33.8(N_{y})$	_
	DMSO-TFA(2:3)	5.67	2.22		$-34.5(N_y)$	_
		$({}^{4}J_{\rm NH} = 0.6)$			ν P	
2*T	DMSO	6.30 ^d	2.62	_	-34.7 (N _y)	-155.5 (N(1))
		$({}^{4}J_{\rm N} = 0.6)$	$({}^{4}J_{\rm NH} = 0.2,$, P	
			${}^{4}J_{\rm H(5)} + {}^{1}H(6) = 1.3$			
	DMSO-TFA (3:1)	6.17	2.56	_	-34.8 (N _y)	_
	DMSO-TFA(2:3)	6.05	2.47	_	$-34.2(N_{y})$	_
		$({}^{4}J_{\rm N} = 0.6)$	$({}^{4}J_{\rm NH} = 0.2)$		· //	
	TFA	6.22	2.51	_	$-32.9(N_{y})$	-154.9 (N(1))
		$({}^{4}J_{\rm N} = 0.7)$	$({}^{4}J_{\rm NH} = 0.2)$		· //	
2*A ^e	DMSO-TFA (2:3)	6.34	2.24	_	-134.4 (N _v)	_
	TFA	6.33	2.24 ^f		-131.0 (N _v)	-228.6 (N(1));
						-159.6 (N(3))
6**T	DMSO	8.15	2.99	8.34 (H(8));	-35.6 (N _y),	-140.8 (N(1));
		$({}^{4}J_{\rm N, H} = 0.82,$	$({}^{4}J_{\rm N, H} = 0.20,$	7.65 (H(9));	22.0 (N_{β})	-126.7 (N(3))
		${}^{5}J_{\rm N_{0}H} = 0.09$	${}^{5}J_{\rm N_{0}H} = 0.08,$	7.66 (H(10))	$({}^{1}J_{N_{1},N_{2}} = 16.8)$	
		· · · p,· · ·	${}^{4}J_{\mathrm{H(5),H(6^{\prime})}} = 1.0)$		т.уу. (р	
6**A	TFA	7.70	2.65 ^f	8.15 (H(8));	-131.3 (N _y),	-214.4 (N(1));
				7.48 (H(9));	-151.9 (N _B)	-136.6 (N(3))
				7.62 (H(10))	$({}^{1}J_{N_{\gamma},N_{\beta}} = 5.7)$	

Table 1. Chemical shifts of the ¹H and ¹⁵N nuclei and the $J_{H,H}$, $J_{N,H}$, and $J_{N,N}$ SSCC for compounds **2**^{*} and **6**^{**}

^{*a*} The ¹H $^{-15}$ N SSCCs ($J_{N,H}$) were measured using 1D experiments including amplitude-modulated spin-echo with ¹⁵N nuclear selective inversion. The total time delay in the spin-echo experiments (the time of magnetization evolution for $J_{N,H}$) was 0.6 and 1.2 s for compounds **2**^{*} and **6**^{**}, respectively. The experimental error in the measured values of $J_{N,H}$ does not exceed 0.05 and 0.03 Hz, respectively.

^b The chemical shifts of the ${}^{15}N_{\gamma}$ and ${}^{15}N_{\beta}$ signals were measured in the 1D ${}^{15}N$ NMR spectra. Other signals were assigned at the natural content of the ${}^{15}N$ isotope using the 2D ${}^{1}H{}^{-15}N$ HMBC spectra.

^c No signals of compound **2*T**['] in TFA were observed.

^{*d*} The signal is broadened.

^{*e*} No signals of compound 2*A in DMSO and DMSO-TFA (3 : 1) were observed.

^f The ${}^{4}J_{H(5),H(6')}$ value was not measured because of a large half-width of the corresponding signals in the spectrum.

In spite of the fact that isomerization of tetrazoles 2*T and $2^{*}T'$ to each other proceeds through structure $2^{*}A$. no azido form was observed in a DMSO solution. Thus, it can be concluded that, under these conditions, the concentration of isomer 2*A does not exceed 0.1%. For the stabilization of azide 2*A, deuterated trifluoroacetic acid was added to a solution of a mixture of compounds 2*T and 2*T'. The DMSO: TFA volume ratio was varied from 9:1 to 2:3. The highest concentration of open form 2*A (29%) was observed for the highest content of TFA in a solution (see Table 2). The change in the parameters of chemical exchange between diverse isomeric forms of compound 2* induced by acid addition resulted in a significant broadening of the half-width of the ${}^{13}C(2)$ and ${}^{13}C(5)$ signals of compound 2^{T} and made it possible to measure J_{CN} for these nuclei (see Table 3). It is noteworthy that the chemical shifts of the ¹³C NMR signals of compounds $2^{*}T$ and $2^{*}T'$ changed insignificantly upon the addition of TFA to DMSO, which facilitated the assignment of the signals of these compounds at different TFA concentrations.

An analysis of the NMR spectra of sample 2*A in a TFA solution revealed that, under these conditions, azide form 2*A is predominant and only 2*T, whose concentration is 4%, of the tetrazole isomers exists in the solution (see Table 2). The structure of compound 2*A was confirmed by the observation of the spin-spin interaction between the C(2) carbon and N_γ nitrogen nuclei (${}^{3}J_{C,N} = 0.6$ Hz). In addition, the signal from the ${}^{15}N_{\gamma}$ atom (see Table 1) was observed in the region characteristic of azido-azines.^{18,21,22} It was impossible to detect and measure the ${}^{13}C{}^{-15}N$ coupling constants for compound 2*T in TFA because of the low concentration of this isomeric form. However, the amplitude-modulated spin-echo experi-

Table 2. Azido-tetrazole equilibrium of compounds 2* and 6**

Com- pound	Solvent	Ratio of isomers $\mathbf{T} : \mathbf{T}^{\prime} : \mathbf{A}$		
2*	DMSO ^a	$92:8:0^{b}$		
	DMSO ^c	$49:51:0^{d}$		
	DMSO-TFA (9:1)	50:50:0		
	DMSO-TFA (3:1)	49:51:0		
	DMSO-TFA (3:2)	49:51:0		
	DMSO-TFA (1:1)	51:47:2		
	DMSO-TFA (2:3)	47:24:29		
	TFA	$4:0:96^{e}$		
6**	DMSO	100:0:0		
	TFA	0:0:100		

^{*a*} The ratio of isomers was determined immediately after dissolution.

^b Only one isomer **2*T** has been observed earlier²³ immediately after the dissolution of the compound in DMSO.

^{*c*} The ratio of isomers 1 day after dissolution.

^{*d*} The ratio of isomers $2^{*}T : 2^{*}T'$ equal to 52 : 48 has earlier²³ been observed after incubation at 100 °C in a DMSO solution within 5 h.

^e Isomer 2*T was not earlier detected in TFA.²³

ments revealed the spin-spin interaction with the ${}^{15}N_{\gamma}$ nucleus at the H(5) proton and the protons of the methyl group at the C(6') atom, which proved the structure of tetrazolo[1,5-*a*]pyrimidine **2*T**. It should be emphasized that the amplitudes of the long-range ${}^{1}H^{-15}N$ SSCCs measured for compound **2*T** in DMSO and TFA solutions coincide within the experimental error (see Table 1). It is also important that the results on studying the azidotetrazole equilibrium of compound **2A** using an analysis of the ${}^{1}H^{-15}N$ and ${}^{13}C^{-15}N$ SSCCs supplement the earlier published data²³ (see Table 2), and the experiments in DMSO-TFA mixtures made it possible to reliably detect in solutions already three tautomeric forms: **2T**['], **2T**, and **2A**.

The obtained sample of compound **6******T** was studied by the NMR method in DMSO and TFA solutions. The 1D ¹⁵N NMR spectrum of compound **6******T** recorded in DMSO exhibits two doublets with the SSCC $J_{N,N} \approx 16.8$ Hz, which was confirmed by the introduction of two isotope ¹⁵N labels in the N_γ and N_β positions of the tetrazole fragment (see Fig. 1, *d*, Table 1). In this case, the signals from the ¹⁵N_γ (δ –35.6) and ¹⁵N_β (δ 22.0) nuclei were detected in the region characteristic of condensed tetrazaloazines.^{18,21,22} In addition, the 2D ¹H—¹⁵N HMBC spectrum of compound **6******T** contained cross-peaks between the labeled nitrogen atoms and the protons of the azine fragment caused by the long-range ¹H—¹⁵N coupling constants, which directly indicated the cyclic structure of the compound.

The exact structure of compound 6^{**T} in a DMSO solution was determined by an analysis of the ${}^{1}H{-}{}^{15}N$ SSCCs. The use of the spin-echo experiments with ${}^{15}N$

nuclear selective inversion made it possible to observe the long-range $J_{\rm N,H}$ between the protons of the C(6')H₃ methyl group and two labeled nitrogen atoms (${}^{4}J_{\rm N_{\gamma},H} \approx 0.20$ Hz, ${}^{5}J_{\rm N_{\beta},H} \approx 0.08$ Hz, see Table 1). The obtained results show that the condensation of ${}^{15}\rm{N_2}$ -aminotetrazole **4**** with benzoylacetone **5** gives tetrazolopyrimidine **6******T**, since spin—spin interactions ${}^{6}J_{\rm N_{\gamma},H(6')}$ and ${}^{6}J_{\rm N_{\beta},H(6')}$ should be absent in the case of formation of alternative structure **6******T**' (see Scheme 3). This result also indicates the efficiency of an analysis of ${}^{4}J_{\rm N,H}$ and ${}^{5}J_{\rm N,H}$ as a method of determination of azole cycle annelation to the azine fragment in tetrazolo[1,5-*a*]pyrimidines.

The quantitative measurement of the ${}^{13}C{-}^{15}N$ SSCCs in a DMSO solution for compound **6******T** confirmed the conclusions about the structure of this substance obtained by an analysis of the ${}^{1}H{-}^{15}N$ constants. Thus, ${}^{13}C{-}^{15}N$ spin—spin interaction was observed for the C(2), C(5), C(6), and C(6') atoms (see Scheme 3, Table 3). In the case of formation of alternative structure **6******T**', the ${}^{13}C{-}^{15}N$ coupling constants should be detected for the C(4) and C(7) signals, and the ${}^{13}C{-}^{15}N$ constants at the C(6') atom should be absent.

The chemical shifts of the ${}^{15}N_{\gamma}$ and ${}^{15}N_{\beta}$ signals (δ -131.3 and -151.9, respectively) in a TFA solution indicates the complete rearrangement of tetrazole isomer **6**T** to open form **6**A** (see Fig. 1, *e*, Table 1). Signal assignment in the 1D ${}^{15}N$ NMR spectrum of compound **6**A** was performed on the basis of the earlier published data. 18,21,22 The absence of the ${}^{1}H-{}^{15}N$ SSCCs in the 1D ${}^{1}H$ NMR spectrum and the presence of the single ${}^{13}C-{}^{15}N$ constant in the ${}^{13}C$ NMR spectrum unambiguously confirms the structure of azide **6**A**.

Thus, we showed the efficiency of the complex analysis of the ¹³C-¹⁵N and ¹H-¹⁵N coupling constants in studying the azido-tetrazole tautomerism in selectively ¹⁵N-labeled azidoazines for examples of pyrimidines 2 and 6. It was demonstrated that the measurement and analysis of $J_{\rm C N}$ makes it possible to unambiguously establish the structure of the azide and tetrazole forms in solution, although requires significant concentrations of the studied substances. At the same time, the use of $J_{\rm N,H}$ provides information on the structure of weakly populated (minor) isomeric forms. For instance, an analysis of the ¹H—¹⁵N spin-spin interaction confirmed the structure of isomer 2^{T} , whose population in a TFA solution is ~4%. The high sensitivity of ¹H NMR spectroscopy compared to that of ¹³C NMR spectroscopy at the natural abundance makes it possible to reliably measure the heteronuclear ¹H-¹⁵N SSCCs with a significantly lower amplitude (see Tables 1 and 3) and within a considerably shorter time that it is required for measuring the ${}^{13}C{-}^{15}N$ SSCCs (minutes and hours, respectively). Summating the aforesaid, we may conclude that the combined application of the ¹³C-¹⁵N and ¹H-¹⁵N SSCCs in investigation of isomerization of azidoazines to tetrazolo-

Com-	Solvent	$^{13}\mathrm{C}~(\delta, J/\mathrm{Hz})^a$					
pound		C(2)	C(4)	C(5)	C(6)	Other signals	
2*T ^{~b}	DMSO	151.53	154.38	98.66	154.96	19.27 (Me)	
	DMSO–TFA (3 : 1)	$({}^{2}J_{C,N} = 2.4)$ 151.31 $({}^{2}L_{-} = 2.4)$	$({}^{2}J_{C,N} = 2.1)$ 154.24 $({}^{2}L_{-} = 2.1)$	$({}^{3}J_{C,N} = 0.8)$ 98.39 $({}^{3}I_{n} = 0.8)$	$({}^{4}J_{C,N} = 0.4)$ 154.54 ^c $({}^{4}I_{c} = 0.4)$	18.65 (Me)	
	DMSO-TFA (2:3)	$(J_{C,N} - 2.4)$ 150.59	$(J_{C,N} - 2.1)$ 154.47	$(3_{C,N} - 0.8)$ 98.32	$(J_{C,N} = 0.4)$ 154.70 ^c	17.90 (Me)	
2*T	DMSO	150.98 ^{c,d}	161.27 ^{c,d}	109.10 ^{c,d}	145.31 ^c	16.33 (Me)	
	DMSO–TFA (3 : 1)	150.78	160.99 ^c	108.91	$({}^{2}J_{C,N} = 3.4)$ 145.06 $({}^{2}I_{L} = 3.4)$	$({}^{3}J_{C,N} = 0.8)$ 15.77 (Me) $({}^{3}I_{L} = 0.8)$	
	DMSO—TFA (2 : 3) TFA	$({}^{\bullet}C, N {}^{\bullet}2.7)$ 149.64 $-e^{e}$	161.58 e	108.15 108.34	146.15 148.31	14.99 (Me) 14.35 (Me)	
2*A ^f	DMSO—TFA (2 : 3) TFA	157.26 157.61 $({}^{3}J_{C N} = 0.6)^{g}$	173.55 173.10	103.81 102.86	160.73 160.84	17.54 (Me) 16.91 (Me)	
6**T	DMSO	155.50 ${}^{2}J_{C,N_{\gamma}} = 2.6,$ ${}^{2}J_{C,N_{\beta}} = 2.5)$	164.38	$110.02 ({}^{3}J_{C,N_{\gamma}} = 0.6, {}^{4}J_{C,N_{\beta}} = 0.5) $	147.16 $({}^{2}J_{C,N\gamma} = 3.1)$	17.24 (Me) ${}^{(3)}J_{C,N_{\gamma}} = 0.7,$ ${}^{4)}J_{C,N_{\beta}} = 0.2)$ 135.80 (C(7)); 128.59 (C(8)); 129.74 (C9)); 132.68 (C(10))	
6**A	TFA	156.42 $(J_{CN\gamma} = 0.6,^g J_{CN\beta} = 0.7)^g$	172.64	111.89	162.46	18.09 (Me); 131.36 (C(7)); 128.53 (C(8)); 129.23 (C(9)); 135.44 (C(10))	

Table 3. Chemical shifts of the 13 C nuclei and the $J_{C,N}$ SSCC of compounds 2* and 6**

^b No signals of compound **2*T**['] in TFA were observed.

^c The signal is broadened.

^d The signal was assigned upon the addition of TFA.

^e The signal was not observed because of the low concentration of the corresponding isomer.

^f No signals of compound **2*A** were observed in DMSO and DMSO–TFA (3 : 1).

^g The $J_{C,N}$ values were measured using 1D ¹³C NMR experiments including amplitude-modulated spin-echo with ¹⁵N nuclear selective inversion. The total time delay in spin-echo experiments (the time of magnetization evolution for $J_{C,N}$) was 0.4 s. The experimental error in the measured $J_{C,N}$ values does not exceed 0.15 Hz.

azines makes it possible to study these processes more exactly and efficiently.

Experimental

¹H, ¹³C, and ¹⁵N NMR spectra were recorded on an Avance 700 spectrometer (Bruker) equipped with a triple resonance probe (¹H, ¹³C, ¹⁵N) using DMSO-d₆ and DMSO-d₆—TFA-d mixtures as solvents and Me₄Si (¹³C, ¹H) and MeNO₂ (¹⁵N) as internal and external standard, respectively. Chemical shifts in the ¹H, ¹³C, and ¹⁵N NMR spectra of solutions in TFA-d were obtained relative to the signals of the residual protons of the COOH group (δ 11.50), Me₄Si, and MeNO₂, respectively. The NMR spectra of compound **2*** in DMSO-d₆ and DMSO-d₆—TFA-d mixtures were measured at 30 °C, the NMR spectra of compound 6^{**} in DMSO-d₆ were recorded at 42 °C, and the NMR spectra of compounds 2^* and 6^{**} in TFA-d were detected at 27 °C.

Two earlier developed methods¹⁸ were used to measure the ${}^{13}\text{C}{-}^{15}\text{N}$ coupling constants: the method of nonlinear approximation of line shapes in the 1D ${}^{13}\text{C}$ NMR spectra detected with ${}^{15}\text{N}$ nuclear selective decoupling and without it and the method of quantitative SSCCs measurement in amplitude-modulated spin-echo 1D ${}^{13}\text{C}$ experiments accumulated with selective inversion of ${}^{15}\text{N}$ nuclear magnetization in the spin-echo sequence and without it. 18 The ${}^{1}\text{H}{-}^{15}\text{N}$ coupling constant values were quantitatively measured using amplitude-modulated spin-echo 1D ${}^{1}\text{H}$ experiments. Delays (2× Δ) in the range from 0.4 to 1.2 s were used in the spin-echo sequence (the total time of magnetization evolution for $J_{\text{C,N}}$ and $J_{\text{N,H}}$). Adiabatic impulses

^{*a*} The ¹³C $^{-15}$ N(7) SSCCs ($J_{C,N}$) were measured using the nonlinear approximation of the line shapes in the 1D ¹³C NMR spectra recorded with ¹⁵N selective decoupling and without it. The experimental error in measured $J_{C,N}$ values does not exceed 0.15 Hz.

(WURST-20) with a length of 10-20 ms and a width of the inversion range of ~1 kHz (14 ppm for ^{15}N) were used for selective decouping and ^{15}N nuclear inversion.

High-resolution mass spectra were measured with a Finnigan LTQ FT mass spectrometer (Germany) equipped with a superconducting magnet with a field intensity of 7 T and an Ion Max electrosprayer.

Potassium 15 N-nitrite 18 (with 86% 15 N enrichment) and 15 N-aminoguanidine 24 3** (with 86% 15 N enrichment) were synthesized according to earlier described procedures. Benzoylacetone (5) was purchased from Aldrich.

2-Hydrazino-6-methylpyrimidin-4-one (1) was synthesized by an earlier described method.²⁵

[¹⁵N]-2-Azido-6-methylpyrimidin-4-one (2*A). A solution of K¹⁵NO₂ (0.08 g, 0.93 mmol in 1 mL of H₂O) was added to a solution of compound 1 (0.1 g, 0.71 mmol in 3.00 mL of AcOH) cooled to 5 °C. The reaction mixture was stirred for 2 h at 5 °C, H₂O (10 mL) was added, and the mixture was treated with AcOEt (3×7 mL). The organic layers were separated, dried with anhydrous Na₂SO₄, and evaporated. Compound **2***A was obtained in a yield of 0.054 g (50%), m.p. 240–242 °C. Found: m/z 153.05399 [M + H]⁺. C₅H₅N₄¹⁵NO. Calculated: [M + H]⁺ = 153.0542.

[¹⁵N₂]-5-Aminotetrazole monohydrate (4**). A 5 *M* solution of K¹⁵NO₂ (1 mL) was added to a suspension of [¹⁵N]-aminoguanidine carbonate **3*** (0.75 g, 5.5 mmol) in 1 mL of 5 *M* nitric acid at the temperature \leq 40 °C. Sodium acetate (1.5 g) was added to the obtained yellow solution, and the mixture was refluxed for 5 min. The solution was cooled, and the precipitate formed was filtered off and dried. Compound **4**** was obtained in a yield of 0.38 g (66%), m.p. 200 °C. Found: *m/z* 88.04034 [M + H]⁺. CH₃N₃¹⁵N₂. Calculated: [M + H]⁺ = 88.04074.

[¹⁵N₂]-7-Methyl-5-phenyltetrazolo[1,5-*a*]pyrimidine (6**T). Benzoylacetone (5) (0.39 g, 2.41 mmol) was added to a solution of compound 4** (0.25 g, 2.38 mmol) in AcOH (2 mL). The reaction mixture was refluxed for 1.5 h. After cooling, the precipitate formed was filtered off and dried. Compound 6**T was obtained in a yield of 0.25 g (49%), m.p. 179–183 °C. Found: m/z 214.08730 [M + H]⁺. C₁₁H₉N₃¹⁵N₂. Calculated: [M + H]⁺ = = 214.08768.

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