Abstract

Determining the accurate chemical structures of synthesized compounds is essential for biomedical studies and computer-assisted drug design. The unequivocal determination of N-adamantylation or N-arylation site(s) in nitrogen-rich heterocycles, characterized by a low density of hydrogen atoms, using NMR methods at natural isotopic abundance is difficult. In these compounds, the heterocyclic moiety is covalently attached to the carbon atom of the substituent group that has no bound hydrogen atoms, and the connection between the two moieties of the compound cannot always be established via conventional $^1$H-$^1$H and $^1$H-$^{13}$C NMR correlation experiments (COSY and HMBC, respectively) or nuclear Overhauser effect spectroscopy (NOESY or ROESY). The selective incorporation of $^{15}$N-labelled atoms in different positions of the heterocyclic core allowed for the use of $^1$H-$^{15}$N ($J_{\text{HN}}$) and $^{13}$C-$^{15}$N ($J_{\text{CN}}$) coupling constants for the structure determinations of N-alkylated nitrogen-containing heterocycles in solution. This method was tested on the N-adamantylated products in a series of azolo-1,2,4-triazines and 1,2,4-triazolo[1,5-a]pyrimidine. The syntheses of adamantylated azolo-azines were based on the interactions of azolo-azines and 1-adamantanol in TFA solution. For azolo-1,2,4-triazinones, the formation of mixtures of N-adamantyl derivatives was observed. The $J_{\text{HN}}$ and $J_{\text{CN}}$ values were measured using amplitude-modulated 1D $^1$H spin-echo experiments with the selective inversion of the $^{15}$N nuclei and line-shape analysis in the 1D $^{13}$C spectra acquired with selective $^{15}$N decoupling, respectively. Additional spin–spin interactions were detected in the
An azolo-azine core with a bridgehead nitrogen atom is found in many natural products [5,6] and biologically active synthetic compounds [7,8]. The purine-like scaffold of these nitrogen-containing heterocycles is frequently used in medicinal chemistry and drug design. For example, 6-nitro-1,2,4-triazolo[5,1-c][1,2,4]triazine 4 (Figure 1A, Triazavirin®) was approved in Russia for the treatment of influenza [9]. This drug targets the viral protein haemagglutinin. The incorporation of an adamantyl moiety in azolo-azine structures could lead to the development of new multifunctional antiviral drugs.

Previously, we synthesized N-adamatylated derivatives of 1,2,4-triazolo[5,1-c][1,2,4]triazines 5 and 6 by reaction with the adamantyl cation generated from 1-adamantanol in acidic medium [10]. The azolo-azine scaffold of these compounds has several nitrogen atoms that can react with alkylation reagents [11,12] (Figure 1B). For this reason, the adamantylation of compounds 5 and 6 led to mixtures of N3- and N4-adamantylated isomers, which reisomerized into each other likely via the formation of an adamantyl cation and starting NH-heterocycle. The unambiguous determination of N-adamatylation site(s) in heterocycles 5 and 6 using well-established 1H and 13C NMR methods (such as 1D, 2D COSY, HMQC, HMBC, and INADEQUATE spectra) was difficult because the heterocyclic moiety was covalently attached to the adamantane tertiary carbon that had no bound hydrogen atoms. Nuclear Overhauser effect spectroscopy (NOESY or ROESY) also did not provide unequivocal structures of the N-adamatylated derivatives [13,14]. For example, the attachment of an adamantyl group to the N1 or N3 atom in the azole ring of compounds 5 and 6 could not be distinguished by NOE data. Similar problems with the unambiguous determination of the product structure were also found for N-arylation or N-alkylation with tert-butyl fragments in the series of 1,2,3-triazole [15,16], tetrazole [17-20], and purine [21] derivatives. Meanwhile, knowledge of the accurate chemical structures of N-substituted heterocycles is essential for biomedical studies and computer-assisted drug design, e.g., molecular docking techniques. Thus, the development of effective methods for the unambiguous determination of N-alkylation site(s) in the azolo-azine series is important.

The data that are required to solve this problem could be provided by 15N NMR spectroscopy. For monocyclic derivatives of azoles, the structures of N-alkylated regioisomers can be determined using 2D H-(-C)-N multiple bond correlation (HCNMB) experiments [22,23] using natural isotopic abun-
dance. These experiments rely on the magnetization transfer through $^{13}$C-$^{15}$N $J$-coupling constants ($J_{\text{CN}}$). However, the fusion of the azine ring to an azolo fragment increases the number of possible alkylation sites and considerably complicates the analysis of the $J_{\text{CN}}$ patterns. This issue, together with the inherently low sensitivity of natural abundance $^{15}$N NMR spectroscopy, does not always permit the unambiguous positioning of alkyl (N-adamantyl, N-tert-butyl or N-aryl) fragments in azolo-azines.

The incorporation of $^{15}$N labels in nitrogen-containing heterocycles greatly facilitates the use of NMR spectroscopy for studies of molecular structures and mechanisms of chemical transformations [10,24-29]. The labelling enhances the sensitivity of detection and permits the quantitative measurements of $J_{\text{CN}}$ and $J_{\text{IN}}$ $J$-coupling constants even in a mixture of tautomeric forms [24,25]. Additionally, a method based on amplitude-modulated spin-echo experiments was found to be the most efficient way to measure $J_{\text{IN}}$ couplings [24]. Previously, the incorporation of a single $^{15}$N label in position 1 of the 1,2,4-triazole fragment of compounds 5 and 6 and analysis of the $J_{\text{CN}}$ couplings permitted the unambiguous identification of the structures of the N3-adamantylated derivatives (Figure 1B), while the structures of the N4-adamantylated products were determined by $^{13}$C NMR spectroscopy via comparison with model compounds, N-methylated azolo-azines [10]. However, this preliminarily study did not evaluate the potential of the incorporation of several $^{15}$N-labels and simultaneous analysis of the $J_{\text{CN}}$ and $J_{\text{IN}}$ coupling constants for the determination of the N-adamantylation site(s) in heterocycles.

Herein, we report the selective incorporation of two $^{15}$N-labelled atoms in tetrazolo[1,5-]h][1,2,4]triazin-7-one, 1,2,4-triazolo[5,1-c][1,2,4]triazin-7-one, and 1,2,4-triazolo[1,5-a]pyrimidin-7-one and the N-adamantylation of the obtained compounds. The combined analysis of the $J_{\text{CN}}$ and $J_{\text{IN}}$ couplings permitted the straightforward determination of the adamantylation sites in these azolo-azines, even when a mixture of regioisomers is formed.

Results

Synthesis. Derivatives of 1,2,4-triazolo[1,5-a]pyrimidine [30], 1,2,4-triazolo[5,1-c][1,2,4]triazinone [31] and tetrazolo[1,5-b]-[1,2,4]triazinone [32] can be obtained by the fusion of an azine ring to an azole fragment. This method can be used for the selective incorporation of $^{15}$N atoms in different azolo-azines. Recently, we tested this approach for the syntheses of $^{15}$N-labelled tetrazolo[1,5-b][1,2,4]triazines and tetrazolo[1,5-a]pyrimidines [25] starting from $^{15}$N-labelled 5-aminotetrazole. However, due to proton tautomerism, the use of single-labelled [2-$^{15}$N]-5-aminotetrazole led to the formation of isotopomer mixtures, which complicated the subsequent NMR analysis. Meanwhile, the application of [2,3-$^{15}$N]-5-aminotetrazolo[1,5-a]pyrimidine series [33]. Thus, in the current work, [2,3-$^{15}$N]-5-aminotetrazolo[1,5-a]pyrimidine [34] was incorporated into the formed labelled $^{15}$N atoms was used to incorporate isotopic labels in the tetrazolo[1,5-b][1,2,4]triazine core (Scheme 1). The interaction of diazonium salt $8$-$^{15}$N derived from [2,3-$^{15}$N]-5-aminotetrazolo[1,5-a]pyrimidine [34] with ethyl a-formylphenylacetate (9) yielded compound $10$-$^{15}$N$_2$. It was expected that the cyclization of $10$-$^{15}$N$_2$ would give [1,2-$^{15}$N]-tetrazolo[5,1-c][1,2,4]triazine $11$-$^{15}$N$_2$. Indeed, [2,3-$^{15}$N]-tetrazolo[1,5-b][1,2,4]triazin-7-one $13$-$^{15}$N$_2$ was obtained (see below). Most likely, tetrazole $11$-$^{15}$N$_2$ underwent a ring-opening process, yielding azide $12$-$^{15}$N$_2$, and this process was followed by an alternative ring closure. This azido-tetrazole equilibrium has been previously studied in detail [25].

The coupling between compound $13$-$^{15}$N$_2$ and 1-adamantanol (14) was conducted in trifluoroacetic acid (TFA) solution under reflux. A general and convenient approach to the N-adamantylation of heterocycles involves a reaction with the adamantyl cation generated from 1-adamantanol in acidic medium [34-37]. The adamantylation of $13$-$^{15}$N$_2$ led to N2- and N1-regioisomers $15a$-$^{15}$N$_2$ and $15b$-$^{15}$N$_2$, respectively (Scheme 1). Interestingly, according to the possible resonance structures, compound $15a$-$^{15}$N$_2$ should represent a mesionic (betaine-like) structure with positive and negative charges located at the tetrazole and triazine rings, respectively. The relative concentration of regioisomers $15a$-$^{15}$N$_2$ and $15b$-$^{15}$N$_2$ was determined from the integral intensity of the corresponding signals in the 1D $^1$H and $^{15}$N NMR spectra. The regioselectivity of adamantylation depends on the reaction time. Refluxing of the $13$-$^{15}$N$_2$/14 mixture (1:1.5 mol/mol) in TFA over 5 min led to the predominant formation of N2-adamantylated derivative $15a$-$^{15}$N$_2$. The 1:2 $15a$-$^{15}$N$_2$/15b-$^{15}$N$_2$ mixture was obtained after 2 h of refluxing. This phenomenon could be explained by the reisomerization of the initially formed N2-adamantylated product ($15a$-$^{15}$N$_2$). Indeed, 2 h of refluxing of isolated $15a$-$^{15}$N$_2$ in TFA with 1.5 molar equivalents of 14 yielded a mixture of compounds $15a$-$^{15}$N$_2$ and $15b$-$^{15}$N$_2$ in the same (1:2) ratio (Scheme 1).

The use of [1-$^{15}$N]-3-amino-1,2,4-triazole $16$-$^{15}$N (98%, $^{15}$N) and labelled sodium nitrite (98%, $^{15}$N) in acidic medium allowed for the in situ production of diazonium salt $17$-$^{15}$N$_2$, which reacted with ethyl nitroacetate (18) in a sodium carbonate solution (Scheme 2). This reaction led to the formation of [1,5-$^{15}$N$_2$]-1,2,4-triazolo[5,1-c][1,2,4]triazinone $19$-$^{15}$N$_2$. Previously, the same approach was described for the incorporation of $^{15}$N atoms in azole and azine rings of compound 4 [38]. Heterocycle $20$-$^{15}$N$_2$ was obtained by the treatment of $19$-$^{15}$N$_2$ with
hydrobromic acid according to a procedure described for 6-nitro-1,2,4-triazolo[5,1-c][1,2,4]triazin-7-ones [39]. In this case, hydrogen bromide was obtained in situ by the reaction between acetyl bromide and ethanol. The adamantylation of 20-15N2 first occurred on the N3 atom of the azole ring. It was found that 5 min reflux of 20-15N2 in TFA with a 1.5 molar excess of 14 led to the structure 21a-15N2 (Scheme 2). However, prolonged (6 h) refluxing of the N3-regioisomer with 1.5 molar equivalents of 1-adamantanol (14) in a TFA solution led to complete isomerization of the compound and re-attachment of adamantane to the N4-atom of the azine ring (compound 21b-15N2).

Double-labelled [1,2-15N2]-3-amino-1,2,4-triazole 16-15N2 was synthesized by the interaction of 15N2-hydrazine sulphate (98%, 15N) with S-methyl isothiourea sulphate and consecutive cyclization with formic acid (see the Supporting Information File 1). The use of 16-15N2 in a reaction with ethyl 4,4,4-trifluoroacetooacetate (22) yielded azolo-azine 23-15N2 containing two isotopic labels in the 1,2,4-triazole fragment (Scheme 3). The adamantylation of [1,8-15N2]-1,2,4-triazolo[1,5-a]pyrimidine 23-15N2 was regioselective and led to the formation of the N3-isomer 24-15N2 only. This compound did not undergo further isomerization.

Isomerization of adamantylated derivatives. The adamantyla-
tion of 1,2,4-triazolo[5,1-c][1,2,4]triazin-7-one derivatives in acidic medium is a thermodynamically controlled reaction [10], which could explain the rearrangement of N3-isomer 21a into N4-isomer 21b and the formation of the 15a/15b mixture from compound 15a. To evaluate the relative thermodynamic stabili-
ties of isomers 15a,b and 21a,b, we performed DFT calculations with the RB3LYP/6-31-G(d,p) approximation in the gas phase using the Gaussian 09 package [40]. Isomers 15b and 21b are thermodynamically more stable than counterparts 15a and 21a. The calculated relative energy differences were 8.3 kcal/mol and 6.4 kcal/mol for the 15a-15b and 21a-21b pairs, respectively (see the Supporting Information File 1).

To further study the mechanism of isomerization between com-
ounds 15a and 15b, equimolar quantities of unlabelled N2-isomer 15a and its double-labelled non-adamantylated pre-
Scheme 2: Synthesis and adamantylation of $^{15}$N-labelled $^{20-15}$N$_2$ and $J_{HN}$ and $J_{CN}$ data confirming the structures of adamantylated derivatives $^{21a,b-15}$N$_2$. The $J_{HN}$ couplings measured either in the 1D $^1$H spectra or by amplitude-modulated 1D $^1$H spin-echo experiments and detected in the 2D $^{15}$N-HMBC spectra are shown by grey, magenta, and red arrows (see the legend in the figure). The measured $J_{HN}$ values (gray and magenta) with magnitudes $J \geq 14$ Hz and $J < 0.1$ Hz are indicated by the bold solid and dashed arrows, respectively. The $^1$H-$^{15}$N cross-peaks observed in the 2D HMBC spectrum are classified into three categories: strong, medium and weak (bold solid, thin solid, and dashed red arrows, respectively). The $J_{CN}$ couplings with the $^{15}$C carbons measured in the 1D $^{13}$C spectra are classified into three categories: $J \geq 2$ Hz, $2 > J \geq 1$ Hz, and $J < 1$ Hz (bold solid, thin solid, and dashed green arrows, respectively).

Scheme 3: Synthesis and adamantylation of $^{15}$N-labelled $^{23-15}$N$_2$ and $J_{HN}$ and $J_{CN}$ data confirming the structure of adamantylated derivative $^{24-15}$N$_2$. The $J_{HN}$ couplings measured in the 1D $^1$H spectra and detected in the 2D $^{15}$N-HMBC spectra are shown by grey, cyan, and red arrows (see the legend in the figure). The measured $J_{HN}$ values (gray and cyan) with magnitudes $J \geq 3$ Hz and $J < 1$ Hz are indicated by the bold and thin solid arrows, respectively. The correlation between H$_2'$ and the unlabelled N$_3$ atom observed in the HMBC spectrum of $^{24-15}$N$_2$ is shown by a red arrow. The $J_{CN}$ couplings with the H$_1'$ adamantane carbons measured in the 1D $^{13}$C spectrum have magnitudes < 1 Hz and are shown by dashed green arrows. The $^1$J$_{NN}$ couplings observed in the 1D $^{15}$N NMR spectra of $^{23-15}$N$_2$ (13.6 Hz) and $^{24-15}$N$_2$ (13.4 Hz) are not shown.

cursor $^{13-15}$N$_2$ (isotopic enrichment 98%) were refluxed for 2 h in TFA without the addition of 1-adamantanol (14, Scheme 4). NMR analysis of the resulting mixture revealed the compounds $^{13*}$-$^{15}$N$_2$, $^{15a*}$-$^{15}$N$_2$, and $^{15b*}$-$^{15}$N$_2$ in a 5:2:3 ratio. The observed equal $^{15}$N-isotopic enrichment (~49%) in compounds $^{13*}$-$^{15}$N$_2$, $^{15a*}$-$^{15}$N$_2$, and $^{15b*}$-$^{15}$N$_2$ indicated that the equilibrium $^{15a} \rightleftharpoons ^{15b}$ was reached in the isomerization process. The obtained ratio between the adamantylated products confirmed the higher thermodynamic stability of compound $^{15b}$ relative to isomer $^{15a}$.

NMR spectroscopy and resonance assignment. The synthesized compounds were studied by NMR spectroscopy in a dimethyl sulfoxide (DMSO-d$_6$) solution using samples with concentrations with range of 30–70 mM. The obtained 1D $^{15}$N NMR spectra are shown in Figure 2, and the 1D $^1$H and
Scheme 4: Isomerization of 15a in the presence of tetrazolo[1,5-b][1,2,4]triazin-7-one 13-15N2 and isotopic enrichment of the reactants and products. The starting level of 15N-isotopic enrichment (98%) of compound 13-15N2 is shown in blue. The levels of 15N enrichment (~49%) of the obtained compounds 13*-15N2, 15a*-15N2 and 15b*-15N2 are shown in red. The levels of isotopic enrichment were determined by mass spectrometry. In addition, ~50% excess of the 15N isotopes in compounds 15a*-15N2 and 15b*-15N2 after reaction was confirmed by 13C NMR spectroscopy. In this case, the C1' signals of the labelled and unlabelled components demonstrated approximately equal integral intensities (Figure S24 in Supporting Information File 1).

The assignments of the 13C and 15N signals in the synthesized compounds were obtained by analysing the 2D 13C-HMQC, 13C-HMBC and 15N-HMBC spectra and observing the 13C-15N and 1H-15N spin–spin interactions (see below). The 13C assignment procedure for 19-15N2, 20-15N2, and 21a,b-15N2 was aided by the data from a previous study of unlabelled derivatives of compound 19 [12]. The 13C-19F J-coupling constants (J_{CF}, Table 2) observed in the 1D 13C spectra facilitated the assignment of the 13C nuclei for the heterocyclic moieties of compounds 23-15N2 and 24-15N2. The observations of the 3J_{H2-C3a} coupling constants (9.2 Hz) in the 1D 13C spectra of 19-15N2 and 20-15N2 measured without proton decoupling confirmed the assignment of C3a to the signals at 160.23 ppm and 152.32 ppm, respectively. The obtained NMR assignments are collected in Table 1 (1H, 15N) and Table 2 (13C).

**13C-15N couplings for the structure determination of **N-adamantylated azoloazines. The incorporation of 15N labels into the synthesized compounds led to the appearance of 1H-15N and 13C-15N J-coupling constants (J_{CN} and J_{HN} couplings, respectively). The J_{CN} couplings became evident from the additional splitting of the corresponding signals in the 1D 13C NMR spectra and were measured by nonlinear fits of the 13C line shapes in the 1D spectra acquired with band-selective decoupling from 15N nuclei [25] (Figure 3). This method allowed for the measurement of the 13C-15N spin-spin
interactions of different magnitudes and ranges starting from the direct $J_{\text{HCN}}$ couplings (magnitudes of 1.2–12.0 Hz) to long-range $J_{\text{CN}}$ couplings (magnitudes of 0.2–0.8 Hz). The full list of measured $J_{\text{CN}}$ couplings is collected in Table 2. The couplings between adamantane carbons and the nitrogens of the heterocycles are shown in Schemes 1–3.

The $J_{\text{CN}}$ couplings observed for the C6, C7 and C8a atoms in the heterocyclic moieties of compounds 13-$^{15}$N$_2$ and 15a,b-$^{15}$N$_2$ confirmed the [1,5-$^{15}$N]-type fusion between the azole and azine rings in these structures (Table 2, Figure 3). The observation of the direct $J_{\text{C1'-N2}}$ (6.5 Hz) and other $^{13}$C-$^{15}$N interactions for the C1' ($^{3}$J$_{\text{C-N}}$ 3.8 Hz), C2' ($^{3}$J$_{\text{C-N2}}$ 0.4 Hz and $^{3}$J$_{\text{C-N3}}$

### Table 1: $^1$H and $^{15}$N chemical shifts (ppm), $^1$H-$^{15}$N and $^{15}$N-$^{15}$N J-coupling constants (Hz), and $^1$H-$^{15}$N spin–spin interactions observed in the 2D $^{15}$N-HMBC spectra of the synthesized compounds.

| Compound | $^{15}$N-labelled N2/N1 | $^{15}$N-labelled N3/N5/N8 | $^{15}$N at natural abundance | Ad | H2,H6/Ph |
|----------|-------------------------|---------------------------|-----------------------------|----|----------|
| 13-$^{15}$N$_2$ | 5.74 (N2) | -29.02 (N3) | 1.4N2-N3 16.4 | 1.4N3-N2 16.4 | 8.036 (H10) |
| 15a-$^{15}$N$_2$ | -81.81 (N2) | 46.42 (N3) | 1.4N2-N3 14.7 | 1.4N3-N2 14.7 | 2.361 (H2)$^f$ |
| 15b-$^{15}$N$_2$ | -32.69 (N2) | 42.14 (N3) | 1.4N2-N3 15.3 | 1.4N3-N2 15.3 | 2.412 (H2)$^f$ |
| 19-$^{15}$N$_2$ | -110.06 (N1) | 19.27 (N5) | 2.4H2-N1 15.9$^h$ | 2.4H2-N1 15.9$^h$ | 8.309 (H2) |
| 20-$^{15}$N$_2$ | -110.79 (N1) | 51.36 (N5) | 2.4H2-N1 16.1$^i$ | 2.4H2-N1 16.1$^i$ | 8.359 (H2) |
| 21a-$^{15}$N$_2$ | -122.55 (N1) | 2.79 (N5) | 5.4H2-N5 0.07 (w) | 5.4H2-N5 0.07 (w) | 2.390 (H2)$^f$ |
| 21b-$^{15}$N$_2$ | -110.24 (N1) | 49.26 (N5) | 2.4H2-N5 0.06 (w) | 2.4H2-N5 0.06 (w) | 2.392 (H2)$^f$ |
| 23-$^{15}$N$_2$ | -115.62 (N1) | 156.75 (N8) | 1.4H1-N8 13.6 | 1.4H1-N8 13.6 | 8.945 (H2) |
| 24-$^{15}$N$_2$ | -120.03 (N1) | 155.21 (N8) | 1.4H1-N8 13.4 | 1.4H1-N8 13.4 | 8.996 (H2) |

$^a$The $^{15}$N chemical shifts were referenced indirectly relative to MeNO$_3$. The $^{15}$N-signals of the labelled atoms were observed in the 1D $^{15}$N NMR spectra, and the $^{15}$N-signals at natural isotopic abundance were observed in the 2D $^{15}$N-HMBC spectra. $^b$The $J_{\text{HN}}$ coupling constants were measured in the 1D $^{15}$N NMR spectra. The estimated error in the $J_{\text{HN}}$ values is ±0.1 Hz. $^c$Unless otherwise stated, the $J_{\text{HN}}$ values were measured using amplitude-modulated 1H spin-echo experiments with delays for the evolution of JHN up to 1 s. The estimated error in the $J_{\text{HN}}$ values is 0.02 Hz, and the lower limit of reliable $J_{\text{HN}}$ measurements is 0.04 Hz. $^d$The cross-peaks in the 2D $^{15}$N-HMBC spectra were classified into three categories (weak – w; medium – m; strong – s). Weak peaks approximately correspond to $J_{\text{HN}} < 0.5$ Hz; strong peaks approximately correspond to $J_{\text{HN}} > 2$ Hz and medium peaks correspond to the other values. The degree of isotopic enrichment was accounted for. It was assumed that the intensity of the HMBC cross-peak is proportional to the sin$^2$(2$\pi$JHN/Δ), where Δ is the delay used for the magnetization transfer (62–125 ms). $^e$Indicates unobserved HMBC cross-peaks. $^f$The $^1$H chemical shifts were referenced relative to the residual signal of DMSO-d$_6$ at 2.50 ppm. $^g$The signal demonstrated additional splitting, which is likely related to the slow exchange between the rotamers of adamantane substituents (see text for details). $^h$The measurement of the $J_{\text{HN}}$ values was impossible due to the fast transverse relaxation of the corresponding $^1$H nuclei. $^i$The $J_{\text{HN}}$ coupling constants were measured in the 1D $^{1}$H NMR spectra. The estimated error is 0.1 Hz.

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The C5 and C3a signals overlap. The signal demonstrated additional broadening, which was not related to the couplings. Precise measurements of JCN couplings were impossible due to low intensity of the corresponding 13C resonance. The signal is overlapped with the N2 atom of the tetrazole ring (Scheme 1). However, the detection of geminal (JC1,N2 2.7 Hz), vicinal (JC2,N2 1.1 Hz and JJC1,N3 0.3 Hz) and long-range (JJC3,N2 0.3 Hz) couplings in the 1D 13C NMR spectra of 15b-152N2 revealed the attachment of the adamantyl fragment to the N1 atom of the tetrazole ring.

The structures of compounds 19-152N2, 20-152N2, 21a,b-152N2, 23-152N2 and 24-152N2 were also confirmed by the measured 13JCN patterns (Table 2). The presence of characteristic vicinal 3JC6,N1 coupling (magnitudes of 1.1-1.3 Hz) and other cou-
The detection of a single \( ^3J_{\text{CH}} \) coupling (0.4 Hz) with the adamantane carbons in compound 21a-^15N2 indicated that the substituent group is attached to the N3 atom of the 1,2,4-triazole ring (Figure 2, Scheme 2). Similarly, the N3-adamantyla-
tion in compound 24-^15N2 was characterized by two weak \( ^3J_{\text{CH}} \) couplings (0.4/0.6 Hz) detected for the C1' atom (Figure 2, Scheme 3). In contrast, the attachment of the adamantane fragment to the N4 atom of the triazine ring in compound 21b-^15N2 led to a large set of observable \( J_{\text{CN}} \) couplings, including geminal \( (^2J_{\text{CN}} \text{ at } 5.0 \text{ Hz}) \), vicinal \( (^3J_{\text{CN}} \text{ at } 1.7 \text{ Hz}) \) and long-range \( (^4J_{\text{CN}} \text{ at } 0.2 \text{ Hz}) \) and \( ^5J_{\text{CN}} \) (0.4 Hz) couplings (Figure 2, Scheme 2).

\(^1\text{H}-^1\text{H}\) couplings for the characterization of N-adamantyl-
tation sites in fused azolo-azines. The signal splittings due to the \( J_{\text{HN}} \) couplings were observed in the \(^1\text{H}\) spectra only in a limited number of cases (compounds 20, 21a,b, 23, 24, see Scheme 2 and Scheme 3). In the other cases, the \( J_{\text{HN}} \) couplings were measured by amplitude-modu-
ated 1D \(^1\text{H}\) spin-echo experiments with selective inversion of the \(^1\text{H}\) nuclei \(^\text{[24]}\) (Figure 4A, Table 1) or detected using the conventional 2D \(^15\text{N-HMBC}\) spectra (Figure 4B and Figures S19–S23 in Supporting Information File 1). These methods allowed for the straightforward detection and measurements of the geminal \( ^2J_{\text{HN}} \) (values of 13.8–16.1 Hz), vicinal \( ^3J_{\text{HN}} \) (values of 0.83–6.6 Hz) and long-range \( ^4/5J_{\text{HN}} \) (values of 0.04–0.9 Hz) couplings for the isotopically enriched nitrogen atoms. The \(^1\text{H}-^1\text{N}\) spin–spin interactions with the unlabelled \(^1\text{N}\) nuclei (at natural abundance) were also detected in the \(^15\text{N-HMBC}\) experiments (Table 1). The intensities of the HMBC cross-peaks for the \(^15\text{N}\)-labelled nuclei demonstrated an approximate correlation with the measured \( J_{\text{HN}} \) values (see Table 1). This provides a way to qualitatively estimate the \( J_{\text{HN}} \) magnitudes for unla-

### Figure 3: Signals of the C1' and C6 atoms in the proton-decoupled 1D \(^13\text{C}\) NMR spectra of 25–42 mM \(^{15}\text{a,b-15N}_2\), 21a,b-\(^15\text{N}_2\) and 24-\(^15\text{N}_2\) in DMSO-\(d_6\) (45 °C). The spectra were measured without (black traces) and with band-selective decoupling from the \(^{15}\text{N}_1/^{15}\text{N}_2\) or \(^{15}\text{N}_3/^{15}\text{N}_5/^{15}\text{N}_8\) nuclei (blue or red traces, respectively). The values of \( J_{\text{CN}} \) obtained by line-shape analysis are listed. The additional split-
ings of the C1' signals are due to the presence of two structural forms of adamantane substituents (see text for details).

### Figure 4: Detection and quantification of the \(^1\text{H}-^1\text{N}\) spin–spin interactions in compound 15a-\(^15\text{N}_2\) (DMSO-\(d_6\), 45 °C). (A) Fragment of the 1D \(^1\text{H}\) amplitude-modulated spin-echo spectrum measured without (black trace) or with selective inversion of the \(^1\text{N}_2\) (blue trace) or \(^1\text{N}_3\) (red trace) nuclei. The spectrum was measured using a spin-
echo delay (delay for the evolution of \( J_{\text{HN}} \)) of 1 s. The measured \( J_{\text{HN}} \) values are listed. The signals of \(^{13}\text{C}\)-DMSO at natural isotope abundance and the signals of impurities are marked by # and *, re-
spectively. The concentration of these impurities relative to the con-
centration of 15a-^15N2 does not exceed 2%. The up-field region of the spectrum is drawn with increased scaling. (B) Fragment of the 2D \(^15\text{N-HMBC}\) spectrum of 15a-^15N2. The \(^1\text{H}-^1\text{N}\) cross-peaks be-
tween the adamantane protons and \(^15\text{N}\)-labelled atoms are shown.
belled and $^{15}$N-labelled nuclei using the relative intensities of the HMBC cross-peaks, corrected for the degree of the isotope enrichment. The measured $J_{1HN}$ couplings and HMBC $^1H-^{15}N$ spin–spin interactions are shown in Schemes 1–3.

Compound 15a-$^{15}N_2$ was characterized by a set of $J_{1HN}$ couplings detected for the H2' $^{4}J_{H2'-N2,N3}$, H3' $^{4}J_{H3'-N2,N3}$ and H4' $^{4}J_{H4'-N2,N3}$ atoms of the adamantane group (Figure 4A, Table 1, Scheme 1). These spin–spin interactions, with the exception of 5$J_{HN}$, were also observed in the 2D $^{15}N$-HMBC spectrum (Figure 4B). The observed $J_{1HN}$ pattern indicated that the adamantane substituent is attached to the $^{15}$N-labelled atom (N2) of the tetrazole ring. Similarly, the observation of the 5$J_{HN}$ coupling constant ($\approx$ 0.04 Hz) and the medium intensity H2'-N4 HMBC cross-peak at natural $^{15}$N abundance revealed that compound 15b-$^{15}N_2$ contains an N-adamantane moiety attached to the unlabelled N1 atom (Scheme 1). Note that the weak cross-peak corresponding to the long-range 5$J_{HN}$ coupling was observed in the HMBC spectrum of 15b-$^{15}N_2$ (Figure S20 in Supporting Information File 1), but the magnitude of this $J$ coupling was under the limit of reliable $J_{1HN}$ measurements (0.04 Hz). Thus, if the $J_{1HN}$ couplings are too small to be measured quantitatively, the $^{15}$N-HMBC experiment could provide useful information about the position of the adamantane substituent. However, the assignment of the $^{15}$N-labelled atoms in compounds 15a,b-$^{15}N_2$ (differentiation between N2 and N3 resonances) could not be achieved using the $J_{1HN}$ and $^{15}$N-HMBC data alone. The absence of protons in the tetr Lizolo[1,5-b][1,2,4]triazine core of these compounds dictates the necessity of $J_{1HN}$ analysis for the unambiguous assignment of $^{15}$N-labelled nuclei.

In contrast to the situation observed for compounds 15a,b-$^{15}N_2$, the $J_{1HN}$ interactions with the H2 proton in the 1,2,4-triazolo[5,1-c][1,2,4]triazines 19-$^{15}N_2$, 20-$^{15}N_2$, and 21a,b-$^{15}N_2$ and the H2 and H6 protons in the 1,2,4-triazolo[1,5-a]pyrimidines 23-$^{15}N_2$ and 24-$^{15}N_2$ permitted the straightforward assignments of the labelled $^{15}$N atoms (see Scheme 2 and Scheme 3). The attachment of an adamantyl substituent to the N4 atom in compound 21b-$^{15}N_2$ was confirmed by the measured long-range 4$J_{HN}$ coupling constant (0.06 Hz) and the medium intensity H2'-N4 HMBC cross-peak observed at natural $^{15}$N abundance (Table 1, Scheme 2). Notably, the weak cross-peak corresponding to the 4$J_{HN}$ coupling was also detected in the $^{15}$N-HMBC spectrum (Figure S22D in Supporting Information File 1).

For the adamantylated heterocycles 21a-$^{15}N_2$ and 24-$^{15}N_2$, the $J_{1HN}$ interactions between the adamantane protons and the labelled N1, N5 or N8 atoms were not detected by amplitude-modulated $^1H$ spin-echo or $^{15}$N-HMBC experiments. Meanwhile, the interactions between the H2' proton of the adamantane and the unlabelled N3 atom of the heterocyclic moieties of the compounds were observed in the $^{15}$N-HMBC spectra (Scheme 2 and Scheme 3). These results confirmed the coupling of the adamantane bridgehead C1' carbon with the N3 nitrogen of the azole ring in 21a-$^{15}N_2$ and 24-$^{15}N_2$.

The identification of adamantylation sites based on $^{15}$N-HMBC data requires the preliminary assignment of the nitrogen atoms at natural isotopic abundance. For compounds 21a,b-$^{15}N_2$ and 24-$^{15}N_2$, the required $^{15}$N assignment could be obtained by observing the $^{15}$N-HMBC correlations from the H2 and H6 protons. However, the detection of the corresponding cross-peaks was hindered by the presence of large (>3 Hz) $J_{1HN}$ couplings with the isotopically enriched $^{15}$N-nuclei. The suppression of the magnetization transfer through the geminal 2$J_{HN}$ couplings by setting a delay in the $^{15}$N-HMBC experiment to 1$J_{1HN}$ (62.5–71.4 ms) permitted the observation of the correlations between H2 and the unlabelled N3 and N8 atoms in compounds 21a,b-$^{15}N_2$ (Figures S21 and S22 in Supporting Information File 1). Meanwhile, the presence of additional large vicinal couplings ($^{3}J_{HN}$ and $^{3}J_{HN,N}$) made this strategy not applicable for compound 24-$^{15}N_2$. In this case, the supposed assignment of the N3 resonance was indirectly confirmed by the similarity of its chemical shifts in compounds 21a-$^{15}N_2$ and 24-$^{15}N_2$.

**NMR and X-ray diffraction data revealing several rotameric configurations of adamantane substituents.** The $^{13}$C signals of the N-adamantyl substituents in compounds 15a,b-$^{15}N_2$ and 21a,b-$^{15}N_2$ measured at 45 °C demonstrated additional splitting, which was not connected to the $^1H$-$^{13}$C and $^{13}$C-$^{15}$N J-couplings. The C1' and C2' signals of adamantane (and C3' for 21a-$^{15}N_2$) were split into two components with a relative intensity ratio of $\approx$10:7 and a frequency difference of 0.5–1.2 Hz (Figure 3, Table 2). This revealed the presence of the two structural forms of the N-adamantylated heterocycles in solution with a slow (characteristic time $\geq$ 1 s) exchange between them. The rotation of the N-adamantyl substituents around the N–C1' bond in the bulky bicyclic heterocycles is likely hindered, and the observed conformational heterogeneity corresponds to the different rotameric configurations of the substituent. To test this hypothesis, additional NMR measurements at elevated temperature were carried out for compound 21a-$^{15}N_2$. The $^{13}$C 1D NMR spectrum measured at 70 °C with $^1H$ and $^{15}$N decoupling did not demonstrate additional splitting (Figure S25 in Supporting Information File 1). This confirmed that the studied NMR samples contained unique and chemically pure compounds, while the heterogeneity observed at 45 °C was connected to the presence of different rotameric states.
To confirm the determined positions of the N-adamantane substitutions, compounds 15a and 15b were studied by X-ray crystallography. Suitable crystals of 15a and 15b were obtained by slow evaporation from ethyl acetate solutions. The solved X-ray structures were in full agreement with the results of the $J_{CN}$ and $J_{IN}$ analysis and confirmed the N2-substituted mesionic form for compound 15a as well as the attachment of adamantane to the N1 atom in compound 15b. In accordance with expectations, the adamantane groups in the crystals of 15a and 15b were found disordered between two conformations with different rotameric configurations around the N–Cl' bond (Figure 5 and Supporting Information Files 2 and 3). These forms differ by the rotation around the N–Cl' bond by 40–60°; thus, in each of them, one of the C2' atoms of the adamantane substituent is located approximately in plane with the heterocyclic moiety of the compound. The populations of the two conformational forms in the single crystals of 15a and 15b (4:1 and 17:3, respectively) differ from the populations of the conformers observed by NMR spectroscopy in DMSO solution (10:7). Interestingly, for 15a, the major conformer corresponds to a rotameric state with a screened N1 atom, but in the major conformer of 15b, the N2 atom of the heterocycle is screened. Notably, similar structural disorder was previously observed in the crystals of adamantylated tetrazolopyrazole derivatives [37,41].

**Discussion**

Comparison of different NMR approaches for the determination of N-alkylation sites in fused heterocycles. The obtained data permit a comparison of the abilities of different NMR parameters ($^{13}$C and $^{15}$N chemical shifts, $J_{IN}$ and $J_{CN}$) to provide structural information about the N-adamantylation sites in bicyclic heterocycles. The previous studies of azolol[5,1-c][1,2,4]triazin-7-ones, 1,2,4-triazolo[1,5-a]pyrimidin-7-ones and tetrazo-lo-azines revealed that the $^{13}$C chemical shifts of the nearest carbon atoms to N-alkyl fragments could be used as indicators for the formation of N-alkylated azolo-azines [12,42]. For the presently studied compounds, we can expect considerable changes in the chemical shifts of the bridgehead C3a and C8a atoms. The shifts of the other carbon atoms from the heterocyclic parts moieties of the compounds (C2, C5, C6, and C7) may also provide useful structural information.

In the studied tetrazo-lo-triazines and tetrazo-lo-pyrimidines (compounds 13–$^{15}$N2 and 15a,b–$^{15}$N2 and compounds from work [25]), the resonances of the bridgehead C8a atom were observed over a relatively narrow spectral range (144–155 ppm). In the triazo-lo-triazines and triazo-lo-pyrimidines 19,$^{15}$N2, 20,$^{15}$N2, 21a,b,$^{15}$N2, 23,$^{15}$N2, and 24,$^{15}$N2, the similar bridgehead C3a atoms are shifted slightly downfield (149–160 ppm). Here, we observed that N-adamantylation of the nitrogen atom directly attached to the C3a, C8a or C2 atoms induced up-field shifts of the corresponding $^{13}$C signal. The majority of these shifts had relatively small magnitudes (Δ$^{13}$C ≈0.9 to −2.8 ppm), and a large shift was only observed for the C2 resonance of 21a,$^{15}$N2 (Δ$^{13}$C ≈11.3 ppm (Table 2)). However, these up-field shifts could not be used to determine the N-adamantylation site. The attachment of the adamantane moiety to other nitrogen atoms could lead to similar $^{13}$C shifts. For example, similar Δ$^{13}$C values (−0.9 and −1.1 ppm) were observed for the C2 resonances in compounds 21b–$^{15}$N2 and 24–$^{15}$N2, where the adamantane fragments are attached to N3 and N4, respectively. Note that the C2 and N4 atoms are separated by three covalent bonds.

A similar situation was observed for the carbon atoms that are separated from the N-adamantylation site by two covalent bonds (Table 2). The attachment of adamantane to the N2 atom in compound 15a–$^{15}$N2 induced a large down-field shift (Δ$^{13}$C ≈+8.6 ppm) of the C8a resonance, while modification of the N4 atom in compound 21b–$^{15}$N2 induced an up-field shift (Δ$^{13}$C ≈−3.0 ppm) of the C6 resonance. Thus, the obtained data did not reveal an easily interpreted correlation between the $^{13}$C chemical shifts and the position of N-adamantane substitu-

![Figure 5: ORTEP diagrams of the X-ray structures of compounds 15a–$^{15}$N2 (a) and 15b–$^{15}$N2 (b). For clarity, the H atoms are omitted. The observed disorder of the adamantane fragment is shown by black ellipsoids and dashed bonds (the carbon atoms are unlabelled).]
analysed the H-13N and adamantylation sites. For this purpose, we measured and data are required for the unambiguous determination of the for the C1’ atom had similar magnitudes indicated that addition- JCN and CN as well as the CN and couplings that the 1CN2 were relatively large (6.5–2.7 Hz), while the geminal and long- CN varied in magnitude. The direct and vicinal couplings (Schemes 1–3, Figure 2). The observed values greatly synthesized compounds, the bridgehead C1’ atom of the 15N chemical shifts could not reliably determine the adamanty- lation of the neighbouring N1 atom in the tetrazole fragment. In contrast, the coupling of the adamantyl fragment to the N4 atom in compound 21b.15N2 did not considerable change the chemical shift of the neighbouring 15N5 nucleus (Δδ ≈ +2.1 ppm). For clarity, we should mention that the information that could be obtained from the 15N chemical shifts is restricted by the pattern of the 15N-label incorporation. In some cases, the isotopic labels were located far from the position of the attached adamantane group. This fact could partially explain the lack of correlation between chemical shifts and structure.

The obtained data indicated that changes in the 13C and 15N chemical shifts could not reliably determine the adamantyla- tion sites in azolo-azines. Therefore, we focused our study on the analysis of 1H-15N and 13C-15N spin–spin interactions. Despite the relatively ‘sparse’ placement of 15N labels, in all the synthesized compounds, the bridgehead C1’ atom of the adamantyl fragment demonstrated detectable JCN couplings (Schemes 1–3, Figure 2). The observed JCN values greatly varied in magnitude. The direct and vicinal couplings (11JCN) were relatively large (6.5–2.7 Hz), while the geminal and long- range interactions (3JCN) were small (0.6–0.2 Hz). The fact that the 1JCN and 2JCN as well as the 3JCN and 4JCN couplings for the C1’ atom had similar magnitudes indicated that additional data are required for the unambiguous determination of the adamantylation sites. For this purpose, we measured and analysed the 13C-15N and 1H-15N spin–spin interactions for the other atoms of the adamantane groups (Schemes 1–3). These additional sets of 24JCN and 25JHN data reliably identified the N-adamantylation sites in the all studied compounds. The proposed structures of 15a,b.15N2 were independently confirmed by X-ray diffraction data.

One of the advantages of JCN and JHN data compared with chemical shift data is the usefulness of ‘negative’ information. In the majority of the cases, the absence of a detectable 13C-15N or 1H-15N spin–spin interaction indicates the remote localization of the adamantane substituent and labelled nitrogen of the heterocycle. The obtained results showed that the structural information provided by the 1H-15N spin–spin interactions (measured by 1D 1H spin-echo experiments or detected in 2D 15N-HMBC experiments) is similar to the information obtained from the JCN couplings. However, these approaches are not equivalent. On one hand, the acquisition of JHN data requires less measurement time and less sophisticated equip- ment compared with that of JCN data (conventional broadband probe and two-channel NMR spectrometer versus triple-reso- nance probe and three-channel spectrometer, respectively). On the other hand, the structural characterization of the N-adamantylation site(s) in heterocycles based on the JHN data requires the preliminary assignment of the 15N resonances. Therefore, the combination of these approaches based on the analysis of JCN and JHN couplings represents the most effective NMR tool for the determination of adamantylation sites in azolo-azines.

Possible mechanisms of the isomerization of N-adamantylated derivatives 15a and 15b. The isomerization of unla- belled 15a in the presence of 13a-15N2 (Scheme 4) elucidated the possible mechanism of the isomerization of 15a-15N2 into 15b-15N2. This experiment confirmed that this rearrangement occurs via the formation of adamantyl cation 25 and hetero- cyclic base 13.15N3 (Scheme 5). Moreover, the equilibration of the isotope composition over the reaction products (15a*.15N2, 15b*.15N2 and 13*.15N2) indicated that the transformation of 15a into 15b is reversible. Note that the protonation of compound 13 and its adamantylated derivatives probably plays an important role in the 15a=15b conversion in TFA solution.
The precise positions of the attached protons are unknown, and this determination requires additional investigation, but the analysis of calculated Mulliken charges in compounds 15a and 15b (see Supporting Information File 1, Scheme S2) suggests that the most negatively charged atom N8 undergoes the initial protonation. Similar mechanisms can be proposed to describe the isomerization of compounds 21a and 21b.

Conclusion
We reported the selective incorporation of two 15N atoms at different positions of 1,2,4-triazolo[1,5-a]pyrimidine, azolo-1,2,4-triazines and their N-adamantylated derivatives. The selective incorporation of the 15N-labels also permitted the study of the mechanism of isomerization of N-adamantylated tetrazolo[1,5-b]N-labels into the azolo-azine rings of heterocyclic compounds with a high abundance of nitrogen nuclei, where 13C chemical shifts and 1H-1H NOE data cannot provide reliable structural information. The incorporation of the 15N-labels also permitted the study of the mechanism of isomerization of N-adamantylated tetradozo[1,5-b][1,2,4]triazine-7-one in TFA solution. The formation of an adamantyl cation and NH-tetrazolo-triazine during the isomerization reaction was confirmed.

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Supporting Information
1. Supporting Information File 1
Detailed experimental procedures, the synthesis of labelled compounds, crystallographic information for 15a-15N2 and 15b-15N2, computational data, and 1D 1H, 13C and 2D 15N-HMBC spectra of the synthesized compounds. [http://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-13-250-S1.pdf]

2. Supporting Information File 2
Crystallographic data for 15a-15N2. [http://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-13-250-S2.cif]

3. Supporting Information File 3
Crystallographic data for 15b-15N2. [http://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-13-250-S3.cif]
