The synthesis of labeled azolo-1,2,4-triazines with ^{15}N isotope in the azole and azine rings

Tatiana S. Shestakova,[a] Sergey L. Deev,[b] Eugene N. Ulomskii,[a] Vladimir L. Rusinov,[a] Mikhail I. Kodess,[a] and Oleg N. Chupakhin*[a,b]

[a]Department of Organic Chemistry, Ural State Technical University, 19 Mira St., Ekaterinburg, 620002, Russian Federation
[b]Institute of Organic Synthesis, Russian Academy of Sciences
22 S. Kovalevskoi St., Ekaterinburg, 620041, Russian Federation
E-mail: chupakhin@ios.uran.ru

Dedicated to Prof. Alexander F. Pozharskii on the occasion of his 70th birthday

Abstract
Efficient methods for the incorporation of ^{15}N-isotope into 1,2,4-triazolo[5,1-*c*][1,2,4]triazines have been developed. The label can be selectively introduced into either the azolo or azine fragment of the molecule.

Keywords: 1,2,4-Triazines, azolo, azine, ^{15}N isotope, selective isotopic labeling, ^{15}N NMR

Introduction

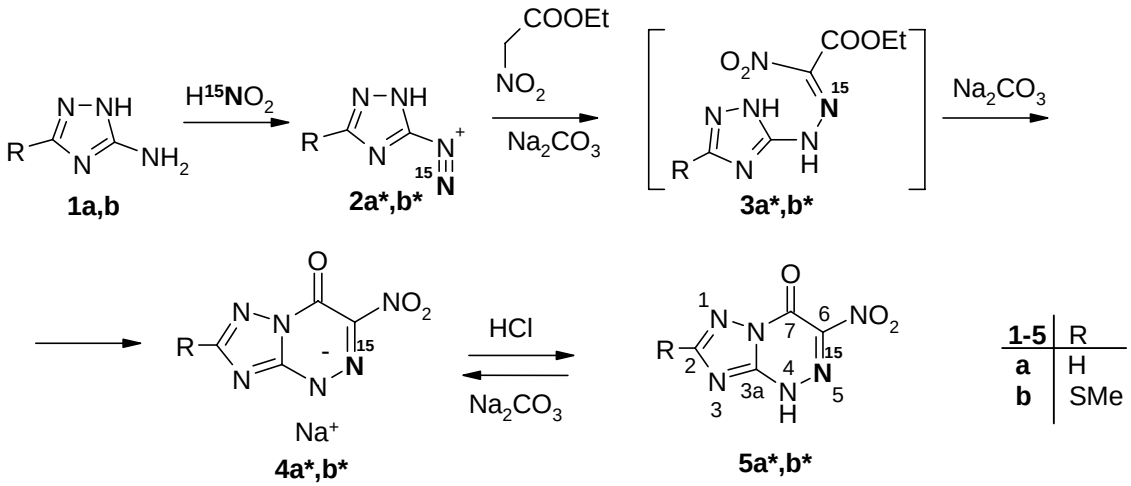
Stable isotope labeling provides valuable information on the structure of a molecule and is often used in studies of reaction mechanisms of rearrangements and transformations in heterocyclic compounds.[1-4] Incorporation of stable isotopes into the molecules of biological active compounds can be used in pharmacological studies on early phases of drug design[5-8]. It substitutes the use of radioactive isotopes and allows the investigation of drug metabolism to be carried out in humans.[5,8]

The great interest in 1,2,4-triazolo[5,1-*c*][1,2,4]triazinones and their sodium salts, along with azoloannelated 1,2,4-triazines, is due to their high activity against different kinds of viruses including influenza and bird flu (culture H5N1).[9-11] In this article, we report the synthesis and spectral characterization of ^{15}N-labeled azolo-1,2,4-triazines containing bridgehead nitrogen.

Result and Discussion

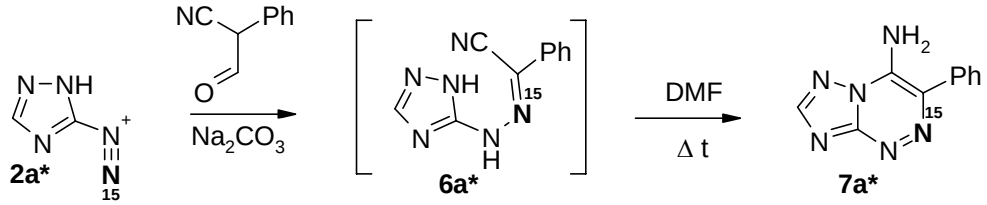
One general method for the synthesis of 1,2,4-triazolo[5,1-*c*][1,2,4]triazines and related azolo fused 1,2,4-triazines is based on the reaction of diazoazoles with CH-active methylene

compounds. The use of ^{15}N-labeled nitrous acid for diazotation of 2-aminoimidazole followed by coupling with Meldrum's acid results in imidazo[2,1-*c*][1,2,4]triazinone containing ^{15}N isotope in azine cycle.[1] So, diazoazoles **2a*,b*** were obtained by treatment of 2-R-amino-1,2,4-triazole **1a,b** with $K^{15}NO_2$ (86% of label) under acidic conditions. Reaction of **2a*,b*** with ethyl nitroacetate as active methylene compound in the presence of Na_2CO_3 gave salts **4a*,b*** (Scheme 1). This can results in formation of a mixture of potassium and sodium salts which is acidified to give azoloazines **5a*,b*** and then converted exclusively to sodium salt **4a*,b*** by treatment with a solution of sodium carbonate.



Scheme 1

The method described above is suitable for introduction of ^{15}N-isotope in other azolo-1,2,4-triazine derivatives. ^{15}N Labeling of atom N(5) of 7-amino-6-phenyl-1,2,4-triazolo[5,1-*c*][1,2,4]triazine **(7a*)** was achieved by reaction of 1,2,4-triazole derivative **2a*** with α-formyl-α-phenylacetonitrile (Scheme 2). It should be noted that phenylacetonitrile itself did not interact with diazoazole **2a***. The introduction of the electron-withdrawing formyl substituent increased CH-acidity of phenynacetonitrile.[12] This approach allowed the synthesis of compound **7a*** in a good yield.

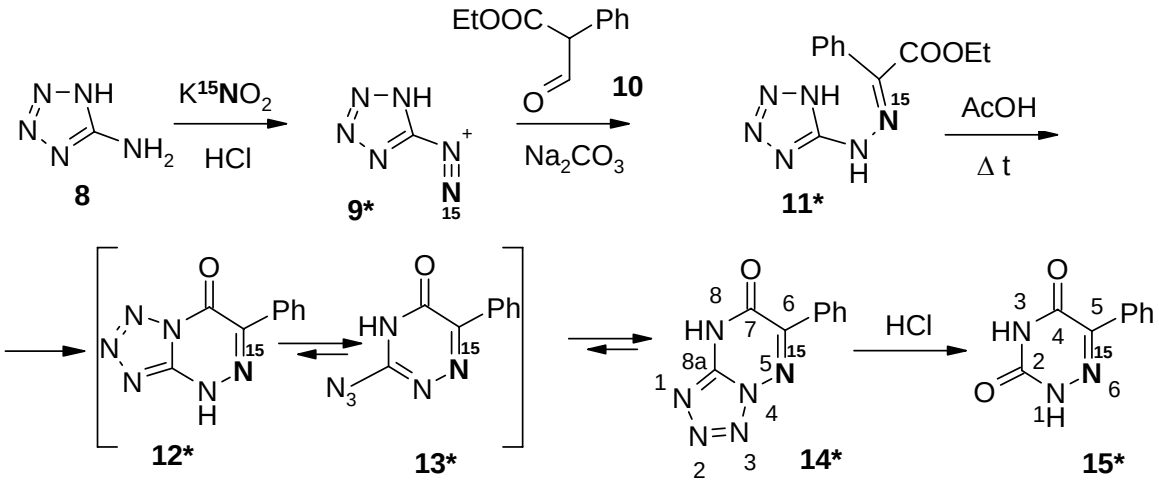


Scheme 2

The mass-spectra in combination with ^{13}C and ^{15}N NMR spectroscopic data of azoloazines **4a*,b***,**5a*,b***,**7a*** confirmed the presence of ^{15}N label (86%).

The proton-decoupled ^{13}C NMR spectra of compounds **4a*,b***,**5a*,b***,**7a*** showed ^{13}C-^{15}N coupling constants for atoms C(3a), C(6) and C(7). Moreover, the splitting of C-*ipso* (d, $^2J_{CN}$ 8.0 Hz) and C-*ortho* (d, $^3J_{CN}$ 1.2 Hz) signals of phenyl substituent was available in spectrum of hetarylamine **7a***. The signals of the carbon atom of heterocycles **4a*,b***,**5a*,b***,**7a*** was determined due to the analysis of coupling constants ^{13}C-^{15}N and multiplicity in ^1H coupled ^{13}C spectra of unlabeled derivatives **4a,b**,**5a,b**,**7a**.

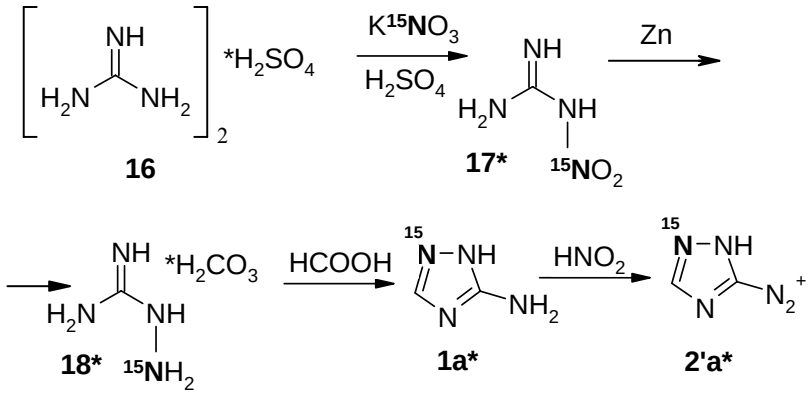
Similar synthetic approach was used for ^{15}N-labeling of the azine part of tetrazolo[1,5-*b*][1,2,4]triazines. Tetrazolyldiazonium salt **9*** reacted with ethyl phenyl(formyl)acetate **10** in the presence of sodium carbonate. The reaction resulted in formation of hydrazone **11***, which was converted into [5-^{15}N]-tetrazolo[1,5-*b*][1,2,4]triazin-7-one **(14*)** by heating in acetic acid (Scheme 3). Our earlier studies[13] have shown that the transformation of **11*** into **14*** proceeds via formation of intermediate azide **13***. The heating of tetrazolo[1,5-*b*][1,2,4]triazine **14*** under reflux in weak hydrochloric acid gave 6-azauracile **15*** in 65% yield (Scheme 3). Thus this reaction can be considered as a convenient procedure for the selective ^{15}N-labeling of azauraciles.



Scheme 3

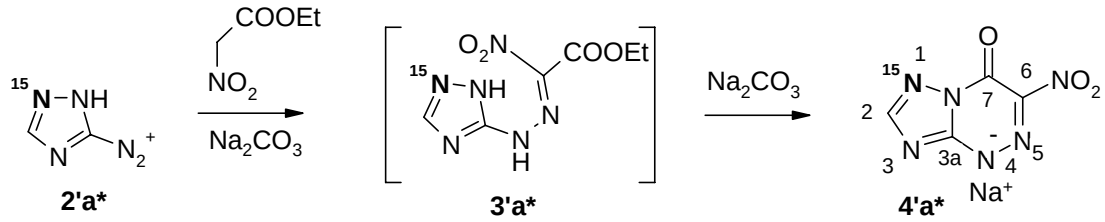
The proton-decoupled ^{13}C NMR spectra of compounds **14*** and **15*** showed the doublets for C(6) ($^1J_{CN}$ 2.8 Hz), C(8a) ($^2J_{CN}$ 2.1 Hz), C(5) ($^1J_{CN}$ 4.0 Hz), and C(2) ($^2J_{CN}$ 2.8 Hz), correspondingly. Also spectra of 1,2,4-triazine derivatives **14***, **15*** was characterized by ^{13}C-^{15}N coupling constants for signals of C-*ipso* and C-*ortho* of the phenyl substituent. The assignment of the signals of carbon atoms of heterocyclic moiety in compound **15*** was confirmed by 2D ^1H-^{13}C HMBC experiment. Additionally the presence of isotope ^{15}N in compounds **14***, **15*** has been confirmed by mass-spectra and ^{15}N NMR spectroscopy.

The other method for the preparation of ^{15}N-labeled azolo-1,2,4-triazines, which was used in this work, is based on the use of [2-^{15}N]-5-amino-1,2,4-triazole **1a***. The scope of this approach was demonstrated by the synthesis of [1-^{15}N]-6-nitro-1,2,4-triazolo[5,1-*c*][1,2,4]triazin-7-one derivative **4'a*** (Scheme 5) according to the procedures described earlier for unlabeled compounds [14-19]. We synthesized [^{15}N]-labeled 5-amino-1,2,4-triazole **1a*** starting from guanidine sulfate **16** and using enriched $K^{15}NO_2$ (86% of ^{15}N) (Scheme 4). Thus treatment guanidine **16** with $K^{15}NO_2$ in concentrated sulfuric acid gave compound **17***, which was converted to aminoguanidine **18*** by reduction with zinc, which was followed by condensation of aminoguanidine **18*** with formic acid to give 5-amino-1,2,4-triazole **1a***.



Scheme 4

Triazole **1a*** was then used in diazotization followed by aza-coupling reaction of **2'a*** with nitroacetate in the presence of Na_2CO_3 to yield the sodium salt of 6-nitro-1,2,4-triazolo[5,1-*c*][1,2,4]triazin-7-one **4'a*** (Scheme 5).



Scheme 5

The presence of 86% of isotope ^{15}N in the structure **4'a*** was determined by mass-spectrometry and ^{15}N NMR spectroscopy. The ^{13}C-^{15}N coupling constants for C(2) (d, $^1J_{CN}$ 3.7 Hz), C(6) (br.d, $^3J_{CN} \approx 2$ Hz) and C(7) (d, $^2J_{CN}$ 3.7 Hz) were characteristics of proton-decoupling in ^{13}C NMR spectrum and confirmed the position of labeled atom N(1).

In the ^{1}H NMR spectrum of **4'a*** the doublet of H(2) (δ 8.35 ppm, $^{2}J_{HN}$ 16.0 Hz), and the remaining singlet of the unlabeled 6-nitro-1,2,4-triazolo[5,1-*c*][1,2,4]triazin-7-one were observed.

In summary, the traditional methods for the synthesis of azolo-1,2,4-triazine derivatives with the bridgehead nitrogen atom can be effectively used for ^{15}N-labeling both.

Experimental Section

General Procedures. The NMR spectra were measured on «Bruker DRX-400» spectrometer in DMSO-d_6. ^{1}H and ^{13}C spectra were recorded at 400 and 100 MHz, respectively, by using TMS as reference. ^{15}N NMR spectra were recorded at 40.5 MHz by using liquid ammonia as external standard. Chemical shifts are given in ppm and *J* values are in Hz.

The mass spectra of compounds **4a*,b*, 4a,b, 4'a*, 5a*,b*, 5a,b, 7a*, 7a, 14*, 14, 15*, 15** and **18*** were obtained using a quadrupole Shimadzu LCMS-2010 system with a Supelco LC-18 column (4.6 × 250 mm), where temperature of 60 °C was maintained. The mobile phase was methanol (100 %). Negative chemical APCI ionization in the selective ion monitoring (SIM) mode was used. For compound **18*** the measurement was carried under positive ESI (probe voltage 1.5 kV, mobile phase CH_3CN/H_2O (1:1 v/v)). The mass spectra of compounds **17*, 1a*** were obtained on a Varian MAT-311A instrument; samples were introduced by a direct inlet system; the ionizing electron energy was 70 eV; the ionization chamber temperature was 100-300 °C. Microanalyses were performed on Perkin Elmer PE 2400 series II CHNS/O analyzer. The IR spectra were recorded in KBr pellets on a Specord 75 IR spectrometer. $K^{15}NO_3$ (86 % of label) was purchased from «Isotope» Corporation (Russian Federation). $K^{15}NO_2$ was prepared by reduction of $K^{15}NO_3$ according to the procedure described earlier.[20]

Sodium salt of [5-^{15}N]-6-nitro-1,2,4-triazolo[5,1-*c*][1,2,4]triazin-7-one (4a*). 70% HNO_3 (1 ml) was added to a solution of 5-amino-1,2,4-triazole **1a** (0.3g, 3.57 mmol) in water (3 ml). The reaction mixture was cooled to 0 °C, a solution of $K^{15}NO_2$ (0.43 g, 5 mmol) in water (2 ml) was added dropwise at vigorous stirring, and reaction mixture was kept at 0 ^{o}C for 30 min. The resulting mixture containing diazonium salt was added to a mixture of ethyl nitroacetate (0.47 g, 3.57 mmol) and sodium carbonate (1.4 g) in water (7 ml). The reaction mixture was stirred at room temperature for 2 h. The precipitate was filtered off, dried and treated with 5N hydrochloric acid (1 ml). The precipitate was filtered off, dried, suspended in a 17% sodium carbonate solution and filtered off. The product was purified by recrystallization from 50% aqueous acetic acid to give the sodium salt of **4a***. Yield 0.29 g (31%); mp > 300 °C; MS (APCI, m/z (rel. %)) 182 (100%) $[M\text{-}Na]^{-}$; IR: CO 1690, NO_2 1350, 1520; ^{1}H NMR : δ 8.39 (s, 1H, H(2)); ^{15}N NMR: δ 400.1 (N(5)); ^{13}C NMR: δ 144.1 (d, C(7), $^{2}J_{CN}$ 0.9 Hz), 144.2 (br.d, C(6), $^{1}J_{CN}$ ≈ 2 Hz), 154.7 (s, C(2)), 159.7 (d, C(3a), $^{2}J_{CN}$ 2.1 Hz); Anal. Calcl. for

$C_4HN_5{}^{15}NO_3Na\times3H_2O$ (259.08) : C, 18.53; H, 2.72; N, 32.82. Found: C, 18.78; H, 2.71; N, 32.73.

Sodium salt of 6-nitro-1,2,4-triazolo[5,1-*c*][1,2,4]triazin-7-one (4a) was prepared according to the procedure described earlier [21]. MS (APCI, m/z (rel. %)) 181 (100%) $[M-Na]^-$; ^{13}C NMR: δ 144.2 (s, C(7)), 144.3 (br.s, C(6)), 154.8 (d, C(2), $^1J_{CH}$ 207.1 Hz), 159.7 (d, C(3a), $^3J_{CH}$ 9.2 Hz).

Sodium salt of [5-^{15}N]-6-nitro-2-methylthio-1,2,4-triazolo[5,1-*c*][1,2,4]triazin-7-one (4b*). Concentrated HCl (1 ml) was added to a solution of 5-amino-2-methylthio-1,2,4-triazole **1b** (0.46 g, 3.57 mmol) in water (4 ml) and the mixture was cooled to 0 °C. A solution of $K^{15}NO_2$ (0.43 g, 5 mmol) in water (2 ml) was added dropwise at vigorous stirring and the reaction mixture was stirred at 0 °C for 30 min. The resulting yellow solution of the diazonium salt was added to a mixture of ethyl nitroacetate (0.47 g, 3.57 mmol) and sodium carbonate (1.4 g) in water (7 ml). The reaction mixture was stirred at room temperature for 2 h. The precipitate was filtered off, dried then treated with 5N hydrochloric acid (2 ml) and again filtered off and dried. The product was suspended in a 17% aqueous sodium carbonate solution, the precipitate was filtered off and recrystallized from 50% acetic acid to give 0.40 g (39%) of sodium salt **4b***; mp > 300 °C; MS (APCI, m/z (rel. %)) 228 (100%) $[M-Na]^-$, 229 (9.1%) $[M+1-Na]^-$, 230 (5.28%) $[M+2-Na]^-$; IR: CO 1695, NO_2 1350, 1525; ^1H NMR: δ 2.64 (s, 3H, SMe); ^{15}N NMR: δ 397.2 (N(5)); ^{13}C NMR: δ 13.4 (s, SMe), 143.0 (d, C(7), $^2J_{CN}$ 1.2 Hz), 144.6 (br.d, C(6), $^1J_{CN} \approx 2$ Hz), 160.2 (d, C(3a), $^2J_{CN}$ 2.1 Hz), 165.9 (s, C(2)); Anal. Calcl. for $C_5H_3N_5{}^{15}NO_3SNa\times2H_2O$ (287.14): C, 20.90; H, 2.46; N, 29.61. Found: C, 21.01; H, 2.32; N, 29.23.

[5-^{15}N]-6-Nitro-1,2,4-triazolo[5,1-*c*][1,2,4]triazin-7-ones (5a*,b*). Sodium salt **4a*** (0.1 g, 0.39 mmol) was added to 5N HCl (2 ml) and the reaction mixture was stirred at room temperature for 15 min. The formed precipitate was filtered off to give compound **5a* (R=H)**. Yield 0.05 g (71%); mp > 300 °C; MS (APCI, m/z (rel. %)) 182 (100%) $[M-H]^-$; IR: CO 1690, NO_2 1355, 1515; ^1H NMR: δ 8.57 (s, 1H, H(2)), 9.18 (br.s, 1H, NH); ^{15}N NMR: δ 352.6 (s, N(5)); ^{13}C-NMR: δ 143.2 (br.s, C(6), $^1J_{CN} \approx 4$ Hz), 143.6 (d, C(7), $^2J_{CN}$ 0.9 Hz), 153.7 (s, C(2)), 153.8 (d, C(3a) $^2J_{CN}$ 2.1 Hz); Anal. Calcl. for $C_4H_2N_5{}^{15}NO_3$ (183.01): C, 26.23; H, 1.10; N, 46.45. Found: C, 26.29; H, 1.04; N, 46.45.

Compound **5b* (R=SMe)** was obtained by the same procedure. Yield 0.13 g (85%); mp > 300 °C; MS (APCI, m/z (rel. %)) 228 (100%) $[M-H]^-$, 229 (8.28%) $[M+1-H]^-$, 230 (5.91%) $[M+2-H]^-$; IR: CO 1690, NO_2 1345, 1522; ^1H NMR: δ 2.65 (s, 3H, SMe), 9.8 (br.s, 1H, NH); ^{15}N NMR: δ 350.8 (N(5)); ^{13}C NMR: δ 13.5 (s, SMe), 142.5 (s, C(7), $^2J_{CN}$ 0.9 Hz), 143.1 (br.s, C(6), $^1J_{CN}$ 5.2 Hz), 154.9 (d, C(3a), $^2J_{CN}$ 2.4 Hz), 166.6 (s, C(2)); Anal. Calcl. for $C_5H_4N_5{}^{15}NO_3S$ (229.11): C, 26.19; H, 1.76; N, 37.10. Found: C, 26.13; H, 1.76; N, 36.57.

6-Nitro-1,2,4-triazolo[5,1-*c*][1,2,4]triazin-7-one (5a) was prepared according to the procedure described earlier[21]. MS (APCI, m/z (rel. %)) 181 (100%) $[M-H]^-$; ^{13}C NMR: δ 143.3 (br.s, C(6)), 143.7 (s, C(7)), 154.6 (d, C(3a), $^3J_{CH}$ 8.9 Hz), 153.9 (d, C(2) $^1J_{CH}$ 211.4 Hz).

[5-^{15}N]-7-Amino-6-phenyl-1,2,4-triazolo[5,1-*c*][1,2,4]triazine (7a*). Concentrated 70% HNO_3 (1 ml) was added to a solution of 5-amino-1,2,4-triazole **1a** (0.3 g, 3.57 mmol) in water (3 ml). The reaction mixture was cooled to 0 °C, a solution of $K^{15}NO_2$ (0.43 g, 5 mmol) in water (2 ml)

was added dropwise at vigorous stirring, and reaction mixture was kept at 0 °C for 30 min. Then yellow solution of resulting diazonium salt was added at 0 °C to a mixture of α-phenyl-α-formylacetonitrile (0.51 g, 3.57 mmol) and 17% sodium carbonate solution (5 ml). The reaction mixture was stirred at 0 °C for 5 h. The formed precipitate was filtered off and dried. The product was dissolved in DMF (4 ml), and the solution was heated under reflux for 3 h. The solid precipitated upon cooling to room temperature was filtered off and dried to afford 0.45 g (60%) of compound **7***; mp 285 °C; MS (APCI, m/z (rel. %)) 212 (100%) $[M-H]^-$; IR: NH_2 3290, 3230; ^{1}H NMR: δ 7.49 – 7.58 (m, 3H, H*m*, H*p*); 7.73 (d, 2H, H*o*); 8.66 (br.s, 2H, NH_2); 8.71 (s, 1H, H(2)); ^{15}N NMR: δ 410.6 (N(5)); ^{13}C NMR: δ 128.7 (s, C*m*+C*p*), 129.0 (d, C*o*, $^3J_{CN}$ 1.2 Hz), 133.7 (d, C*i*, $^2J_{CN}$ 8.0 Hz), 133.2 (br.d, C(6) $^1J_{CN}$ 5.2 Hz), 139.6 (s, C(7)), 155.47 (d, C(3a), $^2J_{CN}$ 1.8 Hz), 156.2 (s, C(2)); Anal. Calcl. for $C_{10}H_8N_5{}^{15}N$ (213.21): C, 56.34; H, 3.78; N 39.88. Found: C, 55.99; H, 3.58; 40.23.

7-Amino-6-phenyl-1,2,4-triazolo[5,1-*c*][1,2,4]triazine (7a) was prepared according to the procedure described earlier[12]. MS (APCI, m/z (rel. %)) 211 (100%) $[M-H]^-$; ^{13}C MNR: δ 128.7 (C*m*+C*p*), 129.0 (C*o*), 133.3 (br.s, C(6)), 133.7 (C*i*), 139.6 (s, C(7)), 155.4 (d, C(3a), $^3J_{CH}$ 8.0 Hz), 156.2 (d, C(2), $^1J_{CH}$ 207.7 Hz).

[5-^{15}N]-6-Phenyltetrazolo[1,5-*b*][1,2,4]triazin-7-one (14*). Concentrated HCl (1 ml) was added to a solution 5-aminotetrazole **8** in water (10 ml). The reaction mixture was cooled to -2 °C, a solution of $K^{15}NO_2$ (0.26 g, 2 mmol) in water (2 ml) was added dropwise at vigorous stirring, and the reaction mixture was kept at -2 °C for 30 min. A mixture of ethyl phenyl(formyl)acetate **10** and sodium carbonate (0.6 g) in water (3 ml) and ethanol (2 ml) was added to the resulting diazonium salt. The mixture was stirred at room temperature for 2 h, and then treated with concentrated HCl (1 ml). The precipitate that formed was filtered off, dissolved in acetic acid (3 ml) and the solution was heated under reflux for 2 h. The solid precipitated upon cooling to room temperature was filtered off and dried to afford 0.21 g (50%) of compound **14***; mp 225 °C; MS (APCI, m/z (rel. %)) 214 (100%) $[M-H]^-$; IR: CO 1710; ^{1}H NMR: δ 7.56 (m, 2H, H*m*); 7.63 (tt, 1H, H*p*, *J* 7.3, 1.5 Hz); 8.02 (dd, 2H, H*o*, *J* 8.6, 1.5 Hz); ^{15}N NMR: δ 306.2 (N(5)); ^{13}C NMR: δ 128.3 (s, C*m*), 129.5 (d, C*o*, $^3J_{CN}$ 2.8 Hz), 130.8 (d, C*i*, $^2J_{CN}$ 8.6 Hz), 131.5 (s, C*p*), 145.5 (d, C(8a), $^2J_{CN}$ 2.1 Hz), 151.7 (d, C(6), $^1J_{CN}$ 2.8 Hz), 153.9 (s, C(7)); Anal. Calcl. for $C_9H_6N_5{}^{15}NO$ (215.17): C, 50.24; H, 2.81; N, 39.51. Found: C, 50.00; H, 2.71; N, 39.47.

6-Phenyltetrazolo[1,5-*b*][1,2,4]triazin-7-one (14) was prepared according to the procedure described earlier[13]. MS (APCI, m/z (rel. %)) 213 (100%) $[M-H]^-$; ^{13}C NMR: δ 128.3 (C*m*), 129.5 (C*o*), 130.9 (C*i*), 131.5 (C*p*), 145.6 (s, C(8a)), 151.7 (t, C(6), $^3J_{CH}$ 3.7 Hz), 154.0 (s, C(7)).

[6-^{15}N]-5-Phenyl-2,4-dioxo-6-azauracil (15*). Tetrazolotriazine **14*** (0.21 g, 1 mmol) was added to 5N HCl (3 ml). The resulting suspension was heated under reflux for 1 h. The solid precipitated upon cooling to room temperature was filtered off and dried to afford 0.13g (65%) of compound **15***; mp 220 °C; MS (APCI, m/z (rel. %)) 189 (100%) $[M-H]^-$; IR: CO 1690, 1710, 1520; ^{1}H NMR: δ 7.44 (m, 3H, H*m*, H*p*), 7.87 (m, 2H, H*o*), 12.08 (s, 1H, N(3)H), 12.50 (d, 1H, N(1)H, $^2J_{NH}$ 8.4 Hz); ^{15}N NMR: δ 334.2 (N(6)); ^{13}C NMR: δ 127.9 (d, C*o*, $^3J_{CN}$=2.8), 128.0 (s, C*m*), 129.3 (s, C*p*), 132.4 (d, C*i*, $^2J_{CN}$ 8.6 Hz), 141.1 (d, C(5), $^1J_{CN}$ 4.0 Hz), 149.2 (d, C(2), $^2J_{CN}$

2.8 Hz), 156.8 (s, C(4)); Anal. Calcl. for $C_9H_7N_2{}^{15}NO_2$ (190,16): C, 56.84; H, 3.71; N, 22,62. Found: C, 56.72; H, 3.70; N, 22.73.

5-Phenyl-2,4-dioxo-6-azauracil (15). Tetrazolotriazine **14** (0.21 g, 1 mmol) was added to 5N HCl (3 ml). The resulting suspension was heated under reflux for 1 h. The solid precipitated upon cooling to room temperature was filtered off and dried to afford 0.13g (65%) of compound **15**; mp 220 °C; MS (APCI, m/z (rel. %)) 188 (100%) $[M-H]^-$; ^{13}C NMR: δ 128.0 (C*o*), 128.1 (C*m*), 129.4 (C*p*), 132.4 (C*i*), 141.2 (dq, C(5), $^3J_{CH}$ 7.1, 3.6 Hz), 149.3 (d, C(2), $^2J_{CH}$ 6.7 Hz), 156.8 (d, C(4), $^2J_{CH}$ 0.9 Hz); Anal. Calcl. for $C_9H_7N_3O_2$ (189.16): C, 57.14; H, 3.73; N, 22.21. Found: C, 56.93; H, 3.45; N, 22.35.

[^{15}N]-Nitroguanidine (17*). The mixture of guanidine sulfate (7g, 0.064 mol) and $K^{15}NO_3$ (6g, 0.054 mol) was added to concentrated H_2SO_4 at 0 °C. The reaction mixture was stirred at room temperature for 12 h and then added dropwise to water (100 ml) under vigorous stirring. The formed precipitate was filtered off and dried to give nitroguanidine **17***, which was used without further purification. Yield 4 g (71%); mp 225-230 °C, MS: m/z 105 (M^+).

[^{15}N]-Aminoguanidine hydrogen carbonate (18*). The mixture of [^{15}N]-nitroguanidine **17* (**4 g, 0.038 mol), zinc powder (14 g) and water (20 ml) was added to 50% aqueous acetic acid (5 ml) at 5 ^{o}C. The resulting mixture was heated at 50 ^{o}C for 5 min and filtered. The presipitate was washed with hot water (2×20 ml). The combined filtrates were treated with ammonium chloride (5 g) and sodium bicarbonate (5 g). The precipitate was filtered off to give aminoguanidine hydrogen carbonate **18***, which was used without further purification. Yield 2.8 g (54%); mp 172 °C, MS: (ESI, m/z (rel. %)) 76 (72%) $[M-H_2CO_3+H]^+$.

[2-^{15}N]-5-Amino-1,2,4-triazole (1a*). [^{15}N]-Aminoguanidine hydrogen carbonate **18*** (2.8 g, 0.02 mol) was added to 20% H_2SO_4 (5 ml). The solution was heated at 100 ^{o}C for 20 min. Then the solvent was evaporated in vacuum. The residue was dissolved in formic acid (3 ml) and a drop of concentrated nitric acid was added. The reaction mixture was heated at 100 ^{o}C for 12 h. After addition of sodium carbonate (1.2 g) the suspension was evaporated to dryness. Residue was treated with hot ethanol (50 ml). The solvent was evaporated in vacuum to give colorless crystals, which were recrystallized from ethyl acetate to give compound **1a***, which was used without further purification. Yield 1.1 g (65%); mp 138-141 °C; MS: m/z 85 (M^+).

Sodium salt of [1-^{15}N]-6-nitro-1,2,4-triazolo[5,1-*c*][1,2,4]triazin-7-one (4'a*). Concentrated 70% HNO_3 was added to a solution of 5-amino-1,2,4-triazole **1a*** (0.34 g, 4 mmol) in water (3 ml). The reaction mixture was cooled to 0 °C, and a solution of $NaNO_2$ (0.28 g, 4 mmol) in water (2 ml) was added dropwise at vigorous stirring. The reaction mixture was kept at 0 ^{o}C for 30 min and then added to mixture of ethyl nitroacetate (0.52 g, 4 mmol) and 17 % water solution of Na_2CO_3 (8 ml) at 0 °C. The reaction mixture was stirred at room temperature for 2 h. The precipitate was filtered off and recrystallized from 50 % acetic acid to give the titled compound. Yield 0.3 g (30 %); mp > 300 °C; MS (APCI, m/z (rel. %)) 182 (100%) $[M-Na]^-$; IR: CO 1690, NO_2 1350, 1520; ^1H NMR: δ 8.35 (d, 1H, H(2), $^2J_{NH}$ 16.0 Hz); ^{15}N NMR: δ 272.3 (N(5)); ^{13}C NMR: 144.0 (d, C(7), $^2J_{CN}$ 3.7 Hz), 144.2 (br.d, C(6) $^3J_{CN} \approx 2$ Hz), 154.6 (d, C(2),$^1J_{CN}$ 3.7 Hz),

159.6 (s, C(3a)); Anal. Calcl. for $C_4HN_5{}^{15}NO_3Na\times3H_2O$ (259.08) : C, 18.53; H, 2.72; N, 32.82. Found: C, 18.65; H, 2.61; N, 32.83.

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