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A.A. Bakibaev[1], M.Zh. Sadvakassova[2*], V.S. Malkov[1],
R.Sh. Erkasov[2], A.A. Sorvanov[1], O.A. Kotelnikov[1]

[1]National Research Tomsk State University, Tomsk, Russia;
[2]L.N. Gumilyov Eurasian National University, Nur-Sultan, Kazakhstan
(Corresponding author's e-mail: madinas-t@mail.ru)

Study of the biologically active acyclic ureas by nuclear magnetic resonance

A wide variety of acyclic ureas comprising alkyl, arylalkyl, acyl, and aryl functional groups are investigated by nuclear magnetic resonance spectroscopy. In general, spectral characteristics of more than 130 substances based on acyclic ureas dissolved in deuterated dimethyl sulfoxide at room temperature are studied. The results obtained based on the studies of ^{1}H and ^{13}C NMR spectra of urea and its N-alkyl-, N-arylalkyl-, N-aryl- and 1,3-diaryl derivatives are presented, and the effect of these functional groups on the chemical shifts in carbonyl and amide moieties in acyclic urea derivatives is discussed. An introduction of any type of substituent (electron-withdrawing or electron-donating) into urea molecule is stated to result in a strong upfield shift in ^{13}C NMR spectra relatively to unsubstituted urea. A strong sensitivity of NH protons to the presence of acyl and aryl groups in nuclear magnetic resonance spectra is pointed out. In some cases, qualitative dependencies between the chemical shifts in the NMR spectra and the structure of the studied acyclic ureas are revealed. A summary of the results on chemical shifts in the NMR spectra of the investigated substances allows determining the ranges of chemical shift variations of the key protons and carbon atoms in acyclic ureas. The literature describing the synthesis procedures are provided. The results obtained significantly expand the methods of reliable identification of biologically active acyclic ureas and their metabolites that makes it promising to use NMR spectroscopy both in biochemistry and in clinical practice.

Keywords: urea, alkylurea, arylurea, acylurea, diarylurea, urea fragment, amide group, NMR spectroscopy, chemical shift.

Introduction

Urea is the most important nitrogen metabolism product, the final amino acids exchange product. Urea is synthesized from ammonia, which is constantly formed in the body during the oxidative and non-oxidative amino acids deamination, in the hydrolysis of amides of glutamic and aspartic acids, as well as in the decomposition of purine and pyrimidine nucleotides [1]. It is well known that urea (carbamide) I is a product of nitrogen compounds metabolism in the mammals [2, 3], but, at the same time, there is literature evidences of an independent biological role of urea [4–6]. Targeted research in the field of urea chemistry made it possible to create many biologically active and medicinal products of the acyclic and heterocyclic structures that contain a urea fragment in their structure [7–11]. To better understand and explain some physical-chemical processes occurring with the participation of urea I, the latter is often represented in the form of Ia and Ib resonance structures.

It is believed that the given examples of resonance structures Ia and Ib plausibly explain the urea behavior features in spectral studies and in the urea interaction with a probable biological object.

Spectral methods to study the biologically active substances (including drugs) have been long and successfully used both for identification and establishing of the action mechanism. Spectral analysis methods are

*Corresponding author.

especially useful to reveal the structure and determine the metabolites composition. In the light of the foregoing, in recent years, the NMR spectroscopy is gaining strength for the biologically active compounds and drugs study.

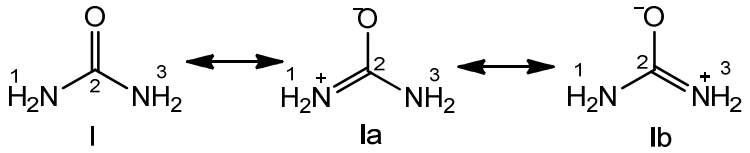


Figure 1. Urea resonance structures

The NMR studies of urea and its derivatives on ^{1}H, ^{13}C, ^{15}N nuclei are presented in literature [14–17]. Most published works address narrow specialized issues: the formation of intra- and intermolecular bonds, the establishment of rotation barriers, etc., or vice versa, NMR spectra are used only to confirm the synthesized compound structure. Thus, a targeted and systematic analysis of the ^{1}H and ^{13}C NMR spectra of acyclic urea was practically not carried out, except for the certain compounds.

In the present work, we analyzed the synthesized acyclic urea large array NMR spectral data.

Experimental

The ^{1}H and ^{13}C NMR spectra were recorded on a spectrometer "Bruker AVANCE III HD" (The Bruker Corporation, Germany), with 400 and 100 MHz operating frequencies, respectively, in DMSO and DMSO-d_6 solutions. Chemical shifts are given in the δ-scale relative to the tetramethylsilane (TMS) as an internal standard. The spectra were obtained in full decoupling mode from protons. The concentration of compounds was 0.5 % for ^{1}H NMR and 10 % for ^{13}C NMR, and the substances were synthesized and purified by known methods [18].

Results and Discussion

N-Alkyl- and N-arylalkylureas. The arylalkyl group is an independent pharmacophore fragment of many drugs and endogenous substrates [19]. On the other hand, urea is known as a well-established class of biologically active compounds [20–23]. The simultaneous presence of arylalkyl and urea groups in the compounds, as a rule, causes an increase in biological activity of the compounds studied.

To qualitatively assess the CS changes of NH protons and carbonyl carbon of N-monosubstituted urea derivatives, the ^{1}H and ^{13}C NMR spectra of compounds **1–35**, shown in Figure 2, were recorded and interpreted.

$RNHCONH_2$
1–4, 15–16

R = H (1); R = CH_3 (2);
R = i-C_3H_7 (3); R = i-C_4H_9 (4);
R = CH_2Ph (15); R = $Ph(CH_2)_2$ (16);

$PhCH(R)NHCONH_2$
17–28

R = CH_3 (17); R = C_2H_5 (18); R = C_3H_7 (19); R = i-C_3H_7 (20); R = C_4H_9 (21); R = i-C_4H_9 (22); R = $PhCH_2$ (23); R = $PhCH_2CH_2$ (24); R = $(Ph)_2CH$-CH_2 (25); 4-C_6H_5-$C_6H_4CH(CH_3)NHCONH_2$ (26); 4-CH_3-$C_6H_4CH(CH_3)$ $NHCONH_2$ (27); $PhCH_2CH(C_2H_5)NHCONH_2$ (28);

$(R, R_1)CHNHCONH_2$
5–14, 29–35

R = R_1= CH_3 (5); R = R_1= C_2H_5 (6);
R = R_1 = C_3H_7 (7); R = R_1 = i-C_3H_7 (8);
R = R_1 = C_4H_9 (9); R = R_1 = C_6H_{13} (10);
R = CH_3, R_1 = C_4H_9 (11); R = CH_3, R_1 = C_6H_{13} (12);
R = CH_3, R_1 = $C(C_3H_9)$ (13);
R = CH_3, R_1 = i-C_3H_7 (14);
R = $PhCH_2$, R_1 = CH_3 (29); R = Ph, R_1 = i-C_4H_9 (30);
R = H, R_1 = $PhCH_2$ (31); R = H, R_1 = Ph (32);
R = R_1= Ph (33); R = R_1= $PhCH_2$ (34);
$(PhCH_2)NCONH_2$ (35);

Figure 2. N-Alkyl- and N-arylalkylureas

Based on the obtained experimental data, it can be noted that, when any R and R_1 substituent is introduced into the urea **1** molecule, the carbonyl carbon atom becomes screened regardless of the substituent nature.

Given the positive alkyl substituent inductive effect on the urea nitrogen atom, one could expect screening of the nitrogen atom and the NH proton signal, and, therefore, the displacement of its CS in the strong field region. However, as it turned out, the experimental data are not simple. Thus, if in the case of a methyl group (compound **2**), the NH proton is de-screened, then the isopropyl radical (compound **3**) shields it (Table 1). Moreover, in the spectrum for compound **4** containing the isobutyl group, the amide proton is de-screened again. Therefore, the CS of NH protons is affected not only by the substituent electronic effects. The substitution of one of the NH protons in urea **1** featuring a group with a positive inductive effect leads to an increase in the bond order in the RNH–C(O) fragment compared to the unsubstituted NH_2–C(O) fragment. This is explainable in terms of the monosubstituted urea **A** resonance structure significant contribution to the molecule general hybrid due to the stabilization by the electron-donating substituent.

However, despite the fact that the alkyl group inductive effect favors the formation of structure **A**, it is unlikely to act exactly the same for the corresponding resonance structures **B** and **C**, in which the proton is cleaved from the Alk-NH fragment (Fig. 3).

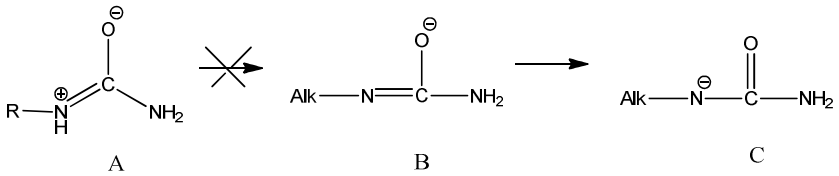


Figure 3. N-alkylurea resonance structures

Secondly, the methyl group steric effect is possible which makes the NH proton less accessible for cleavage (and substitution) with a base than the NH_2 group. Thus, the NH proton de-shielding by the methyl group can be interpreted as a result of a weak inductive effect and a small steric effect of this substituent. The isopropyl radical has a stronger inductive effect, and the branching in the form of methyl groups "shields" the amide proton from the solvent molecules. While moving from the reaction center, the electronic effect of the substituent on this center decreases. Thus, the inductive effect of the two CH_3 groups of the isobutyl radical has almost no effect on the nitrogen atom due to the large distance (through 3 bonds). This distance should also be considered by revising the steric factor. The greater distance between the methyl groups and NH protons (compared with the CH_3 groups of N-isopropylurea **3**) does not allow them to effectively "shield" the amide proton from the hydrogen bonds formation, due to which the proton signal is shifted to the low-field region.

Interestingly, the CS of NH^I proton for N-isobutyl- and N-β-phenylethylureas (compounds **4** and **31**, respectively) coincide, which once again confirms the assumption that the electronic effects are insignificant through several bonds for the compounds studied.

Table 1

Urea 1 and its N-alkyl derivatives 2–14 ^{1}H and ^{13}C chemical shifts

Compound number	The ^{13}C NMR spectra, DMSO, δ, ppm		The ^{1}H NMR spectra, DMSO-d_6, δ, ppm		
	CH	CO	CH	NH, d	NH_2, s
1	–	161.47	–	5.93	5.93
2	–	160.74	–	6.06	5.75
3	–	158.88	–	5.90	5.46
4	–	159.78	–	6.12	5.67
5	41.18	158.88	3,61	5.38	5.90
6	51.48	158.89	3.54	6.00	5.56
7	47.97	158.67	3.69	5.90	5.47
8	49.46	158.52	–	–	–
9	48.49	158.61	3.70	5.90	5.47
10	48.50	158.67	3.70	5.89	5.45
11	44.61	158.37	3.76	5.94	5.56
12	44.69	158.45	3.81	6.02	5.52
13	52.73	159.05	3.74	5.76	5.34
14	–	–	3.70	6.00	5.52

Table 2

Urea 15–35 N-arylalkyl derivatives ^{1}H and ^{13}C chemical shifts

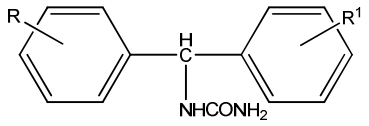
Compound number	The ^{13}C NMR spectra, DMSO, δ, ppm		The ^{1}H NMR spectra, DMSO-d_6, δ, ppm		
	CH	C=O	CH	NH	NH_2
15	43.04	159.12	4.41	6.79	5.86
16	38.13	158.97	3.19	6.36	5.87
17	48.57	158.15	4.94	6.47	5.68
18	54.62	158.37	4.72	6.66	5.65
19	52.75	158.22	4.82	6.68	5.68
20	58.58	158.45	4.67	6.71	5.71
21	53.12	158.30	4.79	6.67	5.66
22	51.33	158.32	4.89	6.69	5.70
23	54.77	158.07	5.12	6.81	5.71
24	52.83	158.37	4.88	6.82	5.72
25	51.56	158.15	4.55	6.87	5.63
26	48.04	157.62	4.99	6.74	5.69
27	48.27	158.07	4.90	6.62	5.70
28	51.70	158.52	3.84	6.00	5.55
29	46.69	158.91	3.79	5.88	5.42
30	–	158.79	–	6.54	5.55
31	–	159.50	–	6.12	5.68
32	–	159.47	–	6.56	5.72
33	56.45	157.52	6.12	7.23	5.84
34	52.39	158.35	4.12	6.08	5.58
35	–	158.89	–	–	5.83

The urea **1–35** NMR spectra analysis shows that significant changes in the structures of the substituted N-alkyl-2–14 and N-arylalkylureas **15–35** are reflected to a greater extent in the δ (CO) CS in the ^{13}C spectra of N-alkyl substituted ureas than N-arylalkylureas. The total range of changes in δ (CO) CS in these compounds is 3.22 ppm, and when compared with urea **1** itself, a noticeable strong field shift up to 4 ppm is observed. The carbonyl atom greatest screening can be seen in arylalkylurea **33**, which is apparently due to the action of spatial factors. Despite this, the difference between the most and the least shielded carbon atoms in N-alkyl substituted ureas is δ (CO) 2.37 ppm, in arylalkyl-substituted ureas this signal is Δδ (CO) 1.98 ppm.

The analysis of CS values for methine carbon atom (CH) in the ^{13}C spectra of ureas **2–35** indicates its higher sensitivity to structural variations compared to the carbonyl carbon atom. As can be seen from the data given in tables 1 and 2, the difference between the most and the least shielded CH- carbon signals for alkyl ureas **5–13** is Δδ = 11.55 ppm, and for arylalkylureas **15–35** the value is Δδ = 20.45 ppm. In the series of compounds **5–13**, there is a tendency towards a strong-field shift of δ (CH) groups as the substituent volume R(R_1) increases, with the exception of compound **13** featuring the most unscreened signal. Among the N-arylalkyl-substituted compounds **15–35**, the CH carbon is screened in the highest extent in the case of compound **16**, which is most likely due to the spatial influence of a more branched alkyl radical and phenyl core on the methine carbon atom.

The comparison of CSs δ (NH_2) in the ^{1}H NMR spectra of urea **1** and its derivatives **2–35** shows that the signal of these protons in alkyl derivatives **2–14** is screened to a greater extent (up to 0.56 ppm) than in compounds **15–35** (up to 0.45 ppm). The analysis of CS values of NH- and CH-protons of compounds **2–35** shows that for alkylureas **2–14**, the CS change of these protons is less pronounced (Δδ (NH) = 0.74 ppm, Δδ (CH) = 0.27 ppm), than for arylalkylureas **15–35** (Δδ (NH) = 1.35 ppm, Δδ (CH) = 2.93 ppm). At the same time, it can be observed that for N-arylalkyl compounds **15–35**, the CS protons of CH and NH are shifted to weak fields by ca. 1 ppm as compared with the CS protons in CH and NH groups of N-alkylurea. This phenomenon can be explained by the anisotropic effect of the nearby phenyl ring.

N,N'-Benzhydrylureas. Benzhydrylureas are low toxic substances and are characterized by a wide physiological activity range, e.g., anticonvulsant [20, 21], antihypoxic [22], enzyme-inducing effects on cytochrome-P-450, a dependent liver monooxygenase system [21]. In this part of the article, we present the ^{1}H and ^{13}C NMR spectra of studied N-benzhydrylureas **36–70** showed in Figure 4. Table 3 shows the chemical shifts of N-benzhydrylureas **36–70** in the ^{1}H and ^{13}C NMR spectra.



36–70

R = R_1 = H (36); R = H, R_1 =4-F (37);
R = H, R_1 = 4-Cl (38); R = H, R_1 = 4-Br (39);
R = H, R_1 = 4-NO_2 (40); R = H, R_1 = 4-CH_3O (41);
R = H, R_1 = 3-F (42); R = H, R_1 = 3-Cl (43);
R = H, R_1 = 3-Br (44); R = H, R_1 = 3-I (45);
R = H, R_1 = 2-F (46); R = H, R_1 = 2-Cl (47);
R=H, R_1=2-Br (48); R = H, R_1 = 2-I (49);
R = H, R_1 = 2-CH_3 (50); R = 4-CH_3, R_1 = 3-F (51);
R = 4-CH_3, R_1 = 3-CH_3 (52);
R = 4-CH_3, R_1 = 2-CH_3 (53);
R = 4-Cl, R_1 = 3-Cl (54); R = 4-Cl, R_1 = 2-Cl (55);
R = R_1 = 3,8-Cl (56); R = R_1 = 2,8-Cl (57);
R = R_1 = 3-Br (58); R = H, R_1 = 3-CH_3 (59);
R = H, R_1 = 4-CH_3 (60); R = H, R_1 = 2,5-Cl (61);
R = H, R_1 = 4-OH (62); R = H, R_1 = 3-F, 4-CH_3 (63);
R = H, R_1 = 3,4-CH_3 (64); R = H, R_1 = 3-NO_2 (65);
R = H, R_1 = 4-N (66); R = H, R_1 = α-нафтилметил (67);
R = 4-Cl, R_1 = бензофурил (68);
R = 4-F, R_1 = бензофурил (69);
R = 4-Cl, R_1 = 4-Cl (70).

Figure 4. N-Benzhydrylurea

Table 3

^{1}H and ^{13}C chemical shifts for N-benzhydrylureas 36–70

Compound number	The ^{13}C NMR spectra, DMSO, δ, ppm		The ^{1}H NMR spectra, DMSO-d_6, δ, ppm		
	CH	**C=O**	**CH, m**	**NH, d**	**NH_2, s**
36	56.45	157.52	6.12	7.23	5.84
37	55.86	157.44	6.15	7.26	5.85
38	55.78	157.52	6.15	7.27	5.83
39	56.17	158.21	6.13	7.30	5.86
40	56.01	157.83	6.07	7.12	5.78
41	55.85	157.59	6.06	7.06	5.80
42	56.26	156.39	5.99	7.10	5.74
43	56.57	158.01	6.17	7.30	5.85
44	55.86	157.61	6.00	7.05	5.76
45	56.23	157.77	6.41	7.27	5.92
46	51.08	159.83	6.46	7.29	5.88
47	53.91	157.44	6.46	7.25	5.85
48	56.01	157.29	6.40	7.25	5.83
49	59.74	157.22	6.29	7.25	5.84
50	53.24	157.52	6.26	7.06	5.75
51	56.01	157.57	6.04	7.01	5.80
52	55.71	157.59	6.05	7.26	5.80
53	55.93	157.59	6.11	7.22	5.87
54	56.30	157.67	6.05	7.16	5.80
55	53.02	157.52	6.25	7.02	5.77
56	55.63	157.44	6.01	7.28	5.76
57	53.24	157.87	6.43	7.41	5.85
58	55.71	157.44	6.18	7.38	5.89
59	56.97	158.34	5.86	7.04	5.63
60	57.06	158.66	5.99	7.14	5.82
61	54.62	157.96	6.13	7.08	5.67
62	56.63	162.80	5.77	6.86	5.56
63	56.63	158.44	5.93	7.03	5.73
64	57.05	158.56	5.92	7.03	5.75
65	56.76	158.27	6.05	7.22	5.70
66	56.64	158.35	5.91	7.14	5.72
67	53.79	158.14	6.65	7.02	5.62
68	51.29	158.53	6.15	7.20	5.79
69	51.20	158.91	6.12	7.20	5.76
70	56.49	158.42	5.95	7.15	5.76

At first, we note the difference between the most and least shielded δCH and δCO signals in N-benzhydrylureas **36–70**: ΔCH=8.66 ppm, ΔCO=6.41 ppm. In this part of the work, we established the methine carbon atom screening effect relatively to the unsubstituted benzhydrylurea **36** by the substituent in the ortho-position (benzhydrylurea **46–50, 55, 57**). Noteworthy is the weakening of the CH groups screening effect with increasing substituent volume in the ortho position of benzhydrylureas, and for the most "bulky" substituent, i.e., iodine (compound **49**), the δCH signal is even shifted to the weak fields area. We emphasize that the chlorine atom and the methyl group characterized by the same steric constants located in the ortho position cause the same CH-carbon CS.

In the ^{1}H NMR spectra of the N-benzhydrylureas **36–70** (Table 3), the CS of methine protons are in the range of 5.77–6.65 ppm, and it can be seen that for ortho-halogen derivatives **46–50, 57** (but not for ortho-methyl derivatives) and compound **67**, these protons are the most unscreened. The difference between the signals of most and the least shielded NH protons (ΔNH = 0.52 ppm) and the NH_2 protons CS (ΔNH_2 = 0.36 ppm) are less pronounced, despite the type of mono- or disubstitution in the aryl fragment of N-benzhydrylureas **36–70**.

Acylureas. N-Arylalkyl-N-acylureas are known as biologically active compounds of various actions [20–22], and some representatives of these compounds have found application in clinical practice [19]. It is also important that the arylalkylureid fragment is a key component of the heterocycles of the barbituric, hydantoin, and quinazolinedione series, which are effective physiologically active substances of the most diverse actions [1–6, 24].

In this part of the work, we studied the effect on CS in the ^{1}H and ^{13}C NMR spectra of substituted N-alkyl(arylalkyl)-N'-acylureas **71–99** on a changes in structural parameters in the urea, amide, alkyl, and molecules acyl fragments presented in Figure 5:

$RNH^{I}CONH^{II}COR_1$
71–82

R = H, R_1 = CH_3 (71); R = i-C_5H_{11}, R_1 = i-C_4H_9 (72);
R = i-C_5H_{11}, R_1 = C_2H_5 (73);
R = i-C_3H_7, R_1 = CH_2-C_6H_5 (74);
R = i-C_5H_{11}, R_1 = C_6H_5 (75);
R = i-C_5H_{11}, R_1 = $CH(C_6H_5)_2$ (76);
R = i-C_3H_7, R_1 = C_6H_5 (77);
R = i-C_5H_{11}, R_1 = CH_2-C_6H_5 (78);
R = i-C_5H_{11}, R_1 = C_3H_7 (79);
R = i-C_5H_{11}, R_1 = CH_3 (80);
R = i-C_5H_{11}, R_1 = н-C_4H_9 (81);
R = i-C_3H_7, R_1 = C_2H_5 (82);

$PhCH(R)NH^{I}CONH^{II}COR_1$
83–95

R = H, R_1 = i-C_3H_7 (83); R = R^1 = CH_3 (84);
R = CH_3, R_1 = C_2H_5 (85); R = CH_3, R_1 = C_3H_7 (86);
R = CH_3, R_1 = C_4H_9 (87); R = CH_3, R_1 = Ph (88);
R = CH_3, R_1 = $PhCH_2$ (89); R = Ph, R_1 = CH_3 (90);
R = Ph, R_1 = CF_3 (91); R = 3-Cl-C_6H_4, R_1 = CF_3 (92);
R = C_3H_7, R_1 = 3-Cl-Ph (93); R = C_4H_9, R_1 = $PhCH_2$ (94);
R = CH_3, R_1 = 4-F-Ph (95);

$PhCH_2CH(R)NH^{I}CONH^{II}COR_1$
96–99

R = CH_3, R_1 = C_2H_5 (96); R=CH_3, R_1=i-C_4H_9 (97);
R = CH_3, R_1 = i-C_3H_7 (98); R=$PhCH_2$, R_1=C_2H_5 (99).

Figure 5. N-Alkyl (arylalkyl)-N'-acyl substituted urea

Chemical shifts in the ^{1}H and ^{13}C NMR spectra of N,N'-acylurea **71–99** are presented in Tables 4 and 5.

Table 4

^{1}H and ^{13}C chemical shifts for N-alkyl-N'-acylureas 71–82

Compound number	The ^{13}C NMR spectra, DMSO, δ, ppm		The ^{1}H NMR spectra, DMSO-d_6, δ, ppm	
	COR_1	C=O	NH^I	NH^{II}
1	2	3	4	5
71	172.62	154.43	7.19 7.74	10.15
72	174.81	153.75	8.38	10.25
73	176.16	153.76	8.34	10.24
74	173.50	152.88	8.19	10.56
75	168.70	153.97	8.68	10.70
76	173.93	153.56	8.30	10.77
77	168.83	153.22	8.61	10.70

Continuation of Table 4

1	2	3	4	5
78	173.33	153.73	8.30	10.59
79	175.38	153.74	8.34	10.24
80	172.70	153.67	8.31	10.28
81	175.53	153.75	8.34	10.24
82	176.14	153.00	8.26	10.19

Table 5

Chemical shifts in the ^{1}H and ^{13}C NMR spectra for N-arylalkyl-N'-acylureas 83–29

Compound number	The ^{13}C NMR spectra, DMSO, δ, ppm			The ^{1}H NMR spectra, DMSO-d_6, δ, ppm		
	CONH	CH	COR	NH^{I}, d	NH^{II}, s	CH, t
83	153.59	–	174.58	9.04	10.53	-
84	152.54	48.64	172.79	8.94	10.54	5.10
85	152.62	48.67	176.14	9,00	10.51	5.11
86	152.54	48.64	175.48	9,01	10.51	5.10
87	152.50	48.62	175.49	8.43	10.28	3.99–4.06
88	153.21	49.17	169.44	9.29	10.81	5.19
89	152.52	48.87	173.46	8.90	10.82	5.09
90	153.23	57.04	173.65	9.50	10.77	6.23, 6.31
91	151.58	58.16	–	8.73	11.73	6.10, 6.16
92	149.94	55.74	–	8.75	10.80	6.05, 6.12
93	153.21	55.36	167.75	9.06	10.92	4.79 k
94	153.46	53.39	173.56	8.12	10.52	3.72–3.64
95	153.11	49.38	167.93	9.06	10.83	4.98
96	153.13	46.87	176.25	8.36	10.25	3.97–4.04
97	153.10	46.90	174.97	8.41	10.25	3.96–4.03
98	153.34	46.94	179.41	8.42	10.28	3.99–4.04
99	153.36	52.53	176.20	8.42	10.19	4.27 m

We found that the introduction of additional phenyl core to the one of the reaction centers (to CH-carbon, compound **91**) compared with other compounds leads to some screening of the amide carbonyl group but at the same time causes a significant weak field shift (by 11.29 ppm) of the methine carbon atom signal (compound **90, 91**). In our previous works [25, 26], we reported that the introduction of substituents into the ortho position of diphenylmethyl system causes progressive de-screening of CH-carbon with an increase in the substituents volume at the nitrogen atom in the diphenylmethyl fragment of the studied compounds. As can be seen from the table 5, the diphenylmethyl system formation (compounds **90–92**) leads to a sharp weak-field shift of the CH-carbon relative to CH CS of compounds **84–89, 93–99**. A comparative analysis of the CS CH carbon atom of N-diphenylmethylamides (according to [19]), N-diphenylmethylureas (according to [25]), and N-diphenylmethylureides **90–92** established that the CH-carbon signals of compounds **90–92** are more unscreened than those of the precursors. The established fact of the highest CH-carbon of N-diphenylmethylureids **90–92** de-screening in the compared series of compounds is obviously related mainly to the spatial factor, i.e., with an increase in the substituent size in the molecules of diphenylmethyl group (the ureide group is significantly larger than the amide [19] or the urea [25] ones).

In addition, it can be noted that due to its powerful electron-withdrawing effect the N'-trifluoroacetyl group (compounds **91, 92**) has a greater diamagnetic effect on the amide carbonyl carbon atom than the alkyl acyl and alkylaryl acyl R_1 radicals. Summing up the influence of acyl groups, it can be noted that the acyl radicals cause a strong field displacement of the amide carbonyl group of the starting N-arylalkylureas [26, 27] and N-alkyl-N'-acylureas **71–82**. The difference between the most and the least carbonyl atom screened signals in the ^{13}C spectra of acylureas **71–99** is δ = 4.49 ppm. The most shielded CS of the atom in compounds **71–99** can be observed in N-diphenylmethylurea **92**.

In the ^{1}H NMR spectra of acylureas **71–99**, the acyl groups, being electron acceptors, cause regular weak-field shifts of the amide proton signals for compounds **71–99** and the methine proton for compounds **83–99** compared to the CS of similar protons in the initial N-aryalkylureas [26]. The difference between the most and the least shielded CH-carbon signals for compounds **83–99** is δ = 2.59 ppm, and for NH^{I} and NH^{II}

groups (compounds **71–99**), 2.03 ppm and 1.58 ppm, respectively. The CH proton chemical shifts in the spectra of ^{1}H acylureas **83–99** are more screened than the signals of these protons in N-diphenylmethylureids **20–22**. We noticed that the most de-shielded NH^{II} protons group signals belong to these compounds **90–92**, which is connected, as mentioned before, with the diphenylmethyl system formation on the one hand and the trifluoroacetyl group effect on the other.

Urea aryl derivatives. It is well known that the electron-withdrawing substituent decreases the bond length in the RC-N fragment, and the substituent that is able to pair with the carbonyl group π-system increases it due to the resonance effects.

The N-phenylurea resonance structures are as follows: the carbonyl group to a large extent gathers the electron density from the unsubstituted nitrogen atom, since the lone electron pair of the N^{I} atom is delocalized in the benzene ring π system. This is also evidenced by the large de-screening of the NH_2 group. However, the anisotropic and steric influence of the aromatic fragment mainly contributes to the carbonyl carbon screening.

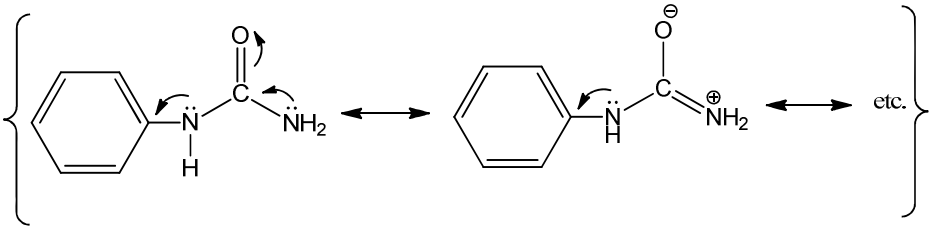


Figure 6. Electron density distribution in N-Phenylureas

The way how the direct conjugation affects the system CS can be judged by comparing the CSs in N-phenylurea with the data obtained for the remaining compounds in which the alkyl and alkylacyl radicals are present as substituents. Below we present a qualitative analysis of ^{1}H and ^{13}C NMR spectra of aryl derivatives of urea **100–107** (Fig. 7).

$PhNH^{I}CON^{II}(R, R_1)$
100–107

R=R_1=H (100); R=H, R_1=C_2H_5 (101); R=R^1=CH_3 (102); R=R^1=C_2H_5 (103); R=H, R_1=$COCH_3$ (104); R=H, R_1=$COCH_2Cl$ (105); R=H, R_1=$COCF_3$ (106); $CH_3CH_2OC_6H_4NHCONH_2$ (107)

Figure 7. Urea aryl derivatives

Table 6 presents the chemical shifts in the ^{1}H and ^{13}C NMR spectra of compounds **100–107**.

Table 6

Arylureas 1–8 chemical shifts in the ^{1}H and ^{13}C NMR

Compound number	The ^{13}C NMR spectra, DMSO, δ, ppm	The ^{1}H NMR spectra, DMSO-d_6, δ, ppm	
	C=O	NH^{I}	NH^{II} (NH_2)
100	156.35	8.77	6.15
101	153.59	9.81	8.87
102	153.22	9.75	–
103	153.74	9.79	–
104	150.90	10.77	10.89
105	150.38	10.42	11.15
106	148.66	10.04	12.07
107	156.85	8.34	5.79

As can be seen from the table 6, under the influence of all substituents (except for compound **8**), the carbonyl atom CS in the ^{13}C spectrum is shifted to a strong field relative to phenylurea **100** itself. You can also notice that under the influence of the electron-donating properties of alkyl substituents, the signal is shifted to 3 ppm (101–103), and with an acetyl substituent, even more shields up to 6 ppm. But the N-tri-

fluoroacetyl group (compound **106**) has the greatest effect on the shift of the C=O group signal, the difference between the most and the least shielded atoms is 8.19 ppm compared with the unsubstituted phenylurea **100**. Probably, this is due to the fact that this substituent has a greater diamagnetic effect on the amide carbonyl carbon atom than the alkyl and alkylacyl R (R_1)-radicals.

The CS analysis of compounds **100–107** (except for the NH^{II} group of compound **107**) relatively to phenylurea **1** shows that in the ^1H spectrum, the amide protons of both groups undergo significant descreening, moreover, the NH^{II} group protons proved to be more sensitive to the electronic influence of the substituents δNH^{II} = 6.28 ppm than the protons of NH^{I} (δNH^{II} = 2.43 ppm).

Urea diaryl derivatives. In a number of spectral methods to identify and establish the action mechanisms of the biologically active compounds, the NMR spectroscopy is becoming more widespread [27]. In this part of the work, we present a qualitative analysis of N,N'-diarylureas that are characterized by diverse activity [28, 29].

Previously, the effect of various substituents on the characteristics of NMR spectra in a para-substituted phenylureas series was studied [30]. Based on the analysis of ^1H and ^{13}C NMR spectra, the electronic effect of the urea fragments of N-phenyl-N'-alkyl(acyl)urea on the benzene ring was estimated before.

In connection with the foregoing, it was interesting to evaluate the substituent effects in the aryl core on the CS of carbon and hydrogen atoms in urea fragment of diarylureas **108–122** (Fig. 8).

$C_6H_4(R)NH^{I}CONH^{II}Ph$

108–122

R = R_1 = H (108); R = 4-CH_3 (109); R = 4-OH (110); R = 4-CH_3O (111); R = 4-NH_2 (112); R = 2-CH_3 (113); R = 4,4'-O-CH_3 (114); R = 4-NO_2 (115); R = 4-COOH (116); R = 4-CH_3CO (117); R = 4-I (118); R = 2-NO_2 (119); R = 4,4'-CH_3 (120); R = 4,4'-I (121); Ph-NHCONH-C_6F_5 (122)

Figure 8. Urea diaryl derivatives

Table 7 represents the CS values in the ^1H and ^{13}C NMR spectra of diarylureas **108–122**.

Table 7

N,N'-diarylurea 108–122 ^1H and ^{13}C chemical shifts

Compound number	The ^{13}C NMR spectra, DMSO, δ, ppm	The ^1H NMR spectra, DMSO-d_6, δ, ppm	
	C=O	NH^{I}	NH^{II}
108	152.69	8.89	8.89
109	152.62	8.84	8.78
110	152.55	8.77	8.56
111	152.69	8.83	8.71
112	152.55	8.85	8.77
113	153.15	8.15	9.25
114	153.24	8.50	8.50
115	151.91	9.10	9.62
116	152.20	9.04	9.30
117	152.05	9.03	9.33
118	151.98	9.80	9.91
119	151.93	9.84	10.08
120	153.05	8.62	8.62
121	153.26	9.91	9.91
122	151.98	8.71	9.30

Judging by the carbonyl carbon atom (δCO) CS values, first of all, we note that in diphenylureas **108–122**, the CS of this carbon atom is noticeably shielded to 4.5 ppm compared to phenylurea **100** itself. Probably, this is due to the fact that the N'-arylation of phenylureas (compounds **108–122**) leads to an increase in the amide conjugation in N(I)-CO or N(II)-CO fragments due to the influence of the spatial factors, for example, due to the urea fragment effect of steric compression of molecules with N'-aryl radicals. The range of changes of δCO CS in diphenylurea **108–122** is small: ΔCO = 1.35 ppm. Since the changes in the

δCO CS are not clearly connected with the electronic characteristics of R substituents, the δCO CS vibrations are apparently also caused by the urea fragment geometry deformation by aryl radicals.

The changes in the CS of urea protons (ΔNH(I) = 1.76 ppm, ΔNH(II) = 1.52 ppm) indicate their insignificant sensitivity to the electronic influence of variable R substituents. Thus, the electron-donating substituents (compounds **109–114, 120**) cause NH(I) and NH(II) protons strong-field shifts, and electron-withdrawing substituents (compounds **115–119, 121, 122**) are a shift to weak fields, and NH(II) protons are more susceptible to the influence of the R substituents.

1,3-disubstituted ureas. By completing the assessment of qualitative changes in the CS of NH protons, carbonyl carbon, and methine atoms in the monosubstituted and disubstituted urea derivatives, we move to the final group of N,N and N,N' disubstituted ureas, amide protons and carbonyl carbon chemical shifts (Table 8), and the compounds themselves (Fig. 9).

The overall picture continues the trends observed for the N-monoprotected urea derivatives. The introduction of groups possessing donor properties shields the amide protons, and the groups capable of conjugation de-screen them. Carbonyl carbon, as expected, is shielded more strongly than in the case of monosubstitution.

It is noteworthy that in compounds **124** and **127**, the substituent enters into conjugation not only with the nitrogen atom directly bonded to it, but also with the other via the carbonyl group π system. This conclusion can be done if we consider the change in the proton of the amide linkage bound chemical shift to the tert-butyl group during the transition from compound **125** to **126**. The conjugation violation noticeably affects the signal of this NH proton, i.e., the signal is shifted towards a strong field (Δ = 0.62).

$RNHCONHR_1$
123–128

R = R_1 = CH_3 (123); R = t-C_4H_9, R_1 = COC_2H_5 (124);
R = t-C_4H_9, R_1 = Ph (125); R = t-C_4H_9, R_1 = $PhCH_2$ (126);
R = $PhCH_2$, R_1 = Ph (127); R = Ph_2CH, R_1 = Ph_2CH (128);

$(R,R_1)CHNHCONHCH(R,R_1)$
129–130

R = Ph, R_1 = 3-Cl-C_6H_4 (129);
R = 4-CH_3Ph, R_1 = 3-CH_3Ph (130)

Figure 9. N,N'-Disubstituted ureas

Table 8

^{1}H and ^{13}C chemical shifts for N,N'-disubstituted ureas 123–130

Compound number	The ^{13}C NMR spectra, DMSO, δ, ppm		The ^{1}H NMR spectra, DMSO-d_6, δ, ppm		
	CH	C=O	CH, m	NH, d	NH, d
123	–	160.0	–	5.90	5.90
124	–	153.59	–	8.38	10.12
125	–	156.11	–	5.96	6.68
126	–	157.51	–	5.73	6.06
127	–	156.0	–	6.26	8.50
128	56.88	156.22	5.92	6.90	6.90
129	56.57	156.57	6.01	7.01	7.01
130	57.07	156.76	6.85	5.78	5.78

From the data given in Table 8 it can be seen that in contrast to N-methylation, the N,N- and N,N'-arylalkylation of urea causes the carbonyl carbon atom screening. For comparison, it can be noted that the N,N-dimethylation of urea de-shields the carbonyl carbon atom by 6.1 ppm, whereas N,N-dibenzylation (compound **34**) shields 2.5 ppm this urea carbon atom. Given the fact that the arylalkyl groups in compounds **123–130** in relation to the urea carbonyl group exhibit at least lower electron-donating properties than the methyl group, it can be concluded that the δCO screening effect in compounds **123–130** upon urea N-arylalkylation is primarily due to the spatial factors. Since the steric characteristics of arylalkyl groups are significantly greater relative to methyl and due to their steric "bulkiness", it is possible that the urea arylalkyl groups **123–126** cause spatial stresses in the urea fragment of molecules simultaneously enhancing the conjugation in the amide fragment with a corresponding increase in the studied compounds amide bond order, and, as a consequence, the δCO screening in urea **123–126** relative to urea or its N-methyl derivatives. The strong field displacements of the largest carbonyl carbon atoms are observed in compound **124**, which is

caused by the presence of the most steric "bulky" radical, on the one hand, and the acyl radical effect on the other.

The CS comparison of methine carbon atoms in the ^{1}H spectrum of compounds **128–130** shows that this carbon atom in these compounds is significantly unscreened (relative to α-phenylethylurea **16**). As the substituent volume in the urea grows, the symbatic changes in the CS δCH occur, in other words, the larger are the steric characteristics of the substituents, the more likely the weak-field signal shift is observed.

The introduction of any substituent type (electron-donating or electron-withdrawing) causes a noticeable strong-field shift of the CS of C=O group in all the compounds studied in comparison with the pristine urea. This is especially pronounced for N-arylureas **100–107** and N-acylureas **71–99**. In the NMR ^{1}H spectra of the studied urea **130**, the N-arylureas **100–107** and N-acylureas **71–99** NH protons are the most sensitive to structural variations.

Conclusions

Numerous experimental data analysis for **130** N-substituted ureas cited before made it possible to determine the main intervals of chemical shifts to identify the atomic resonating groups in the NMR ^{1}H and ^{13}C spectra. The summarized data presented in table 9 are useful to identify urea in various studies and can be used by both chemists and biochemists or clinicians.

Table 9

Urea chemical shift intervals

No.	The chemical compound or fragment thereof	The ^{1}H NMR spectra, ppm	The ^{13}C NMR spectra, ppm
			C=O
1	R-NH_2	5.7–6.2	156.4–158.2
2	AlkNHCO	5.8–6.06	158.5–158.9
3	ArNHCO	8.6–10.1	151.9–156.9
4	ArAlkCHNHCO	7.0–7.4	156.6–159.9
5	Ar(Alk)NHCONHCOR_1	8.7–9.46	148.7–153.6
6	ArNHCONHCOR_1	8.6–12.5	148.7–153.6

To conclude, not only do the results of ^{1}H and ^{13}C NMR studies of ureas facilitate the opportunities to determine the qualitative and quantitative changes of the spectral characteristics from the structural parameters in specific series of the synthesized substances, but also provide a good evidence for their structure. The chemical shifts of protons and carbon atoms in the NMR spectra of the substituted ureas can be used as initial data to correlate with the biological activity and other properties. The obtained results can be used as spectral database for chemists, biochemists and pharmacologists using similar substances in practice.

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А.А. Бакибаев, М.Ж. Садвакасова, В.С. Мальков,
Р.Ш. Еркасов, А.А. Сорванов, О.А. Котельников

Ядролық магниттік резонанс әдісімен биологиялық белсенді ациклдік мочевиналарды зерттеу

Мочевина және оның N-ациклдік туындылары адам қызметінің түрлі салаларында әр түрлі қолдануды табатын құрамында азоты бар органикалық қосылыстардың маңызды класы болып табылады. Сонымен, олар көптеген биохимиялық процестерде маңызды рөл атқарып, ауыл шаруашылығында, косметологияда, тамақ өнеркәсібінде және т.б. салаларда кеңінен қолданылып, органикалық синтезде азоттандыру реагенті ретінде әрекет етеді. N-орынбасқан ациклдік мочевинаны сәйкестендіру және талдау әдістерінің кең қатарында ЯМР-спектроскопия жетекші рөлге ие емес, ал бұл саладағы белгілі мәліметтер байланыссыз және жүйесіз сипатқа ие. Бұл жағдай алкил-, арилалкил-, ацил- және арилорынбасқан туындыларды қамтитын ациклдік мочевиналардың кең шеңберіне ядролық-магнитті резонанс әдісімен зерттеу жүргізуге түрткі болды. Жұмыста ^{1}H және ^{13}C ЯМР мочевина спектрлерінің және оның N-алкил-, N-арилалкил-, N-арил — және 1,3-диарил туындылары спектрлерінің негізінде алынған нәтижелері ұсынылған және осы функционалдық топтардың мочевина атомдарының карбонильді және амидті фрагменттерінің химиялық ығысуына әсері талқыланған. Жекелеген жағдайларда ЯМР спектріндегі химиялық ығысулар мен зерттелген қосылыстардың құрылымы арасында кейбір сапалық тәуелділік белгіленген. Зерттелген қосылыстардың химиялық ығысуларын өлшеу нәтижелерін қорыту ациклдік мочевиналарда протондар мен көміртегі атомының химиялық ығысуларының өзгеру аралықтарын анықтауға мүмкіндік берді. Алынған мәліметтер биологиялық белсенді ациклдік мочевиналар мен олардың метаболиттерін сенімді сәйкестендіру әдістерін едәуір кеңейтеді, ал бұл ЯМР-спектроскопияны биохимия және клиникалық практика үшін тартымды етеді.

Кілт сөздер: мочевина, N-алкилмочевина, N-арилмочевина, N-ацилмочевина, N,N-диарилмочевина, карбамид фрагменті, амидтік топ, ЯМР-спектроскопия, химиялық ығысу.

А.А. Бакибаев, М.Ж. Садвакасова, В.С. Мальков,
Р.Ш. Еркасов, А.А. Сорванов, О.А. Котельников

Исследование биологически активных ациклических мочевин методом ядерного магнитного резонанса

Мочевина и ее N-ациклические производные представляют собой важный класс азотсодержащих органических соединений, который находит разнообразное применение в различных отраслях человеческой деятельности. Так, они играют важную роль во многих биохимических процессах, широко используются в сельском хозяйстве, косметологии, пищевой промышленности и т.д., выступают в качестве азотирующего реагента в органическом синтезе. Среди широкого арсенала методов идентификации и анализа N-замещенных ациклических мочевин ЯМР-спектроскопия занимает далеко не лидирующую роль, а имеющиеся сведения в этой области знания носят отрывочный и несистемный характер. Данное обстоятельство послужило побудительным мотивом для проведения исследований методом ядерно-магнитного резонанса широкого круга ациклических мочевин, охватывающих их алкил-, арилалкил-, ацил- и арилзамещенных производных. В настоящей работе представлены результаты, полученные на основании изучения ^{1}H и ^{13}C ЯМР-спектров мочевины и ее N-алкил-, N-арилалкил-, N-арил- и 1,3-диарилпроизводных, и определено влияние этих функциональных групп на химические сдвиги карбонильных и амидных фрагментов атомов мочевины. В отдельных случаях установлены некоторые качественные зависимости между химическими сдвигами в спектрах ЯМР и структурой исследованных соединений. Обобщение результатов измерения химических сдвигов изученных соединений позволило авторам определить интервалы изменения химических сдвигов протонов и атома углерода в ациклических мочевинах. Полученные данные существенно расширяют методы надежной идентификации биологически активных ациклических мочевин и их метаболитов, что делает привлекательным использование ЯМР-спектроскопии для биохимии и в клинической практике.

Ключевые слова: мочевина, N-алкилмочевины, N-арилмочевины, N-ацилмочевины, N,N-диарилмочевины, карбамидный фрагмент, амидная группа, ЯМР-спектроскопия, химический сдвиг.

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Information about authors

Bakibaev, Abdigali Abdimanapovich — Doctor of Chemical sciences, Engineer, Institute for Problems of Chemical and Energetic Technologies SB RAS, Socialisticheskaya street, 1, 659322, Biysk, Russia; e-mail: bakibaev@mail.ru; https://orcid.org/0000–0002–3335–3166.

Sadvakassova, Madina Zhumabaykyzy — 2-year PhD student of specialty chemistry, L.N. Gumilyov Eurasian National University, Nur-Sultan, K. Munaitpasov Street, 13, 010008, Kazakhstan; e-mail: madinas-t@mail.ru.

Malkov, Victor Sergeevich — Candidate of Chemical sciences, Head of laboratory of organic synthesis, National Research Tomsk State University, Lenin avenue, 36, 634050, Tomsk, Russia; e-mail: malkov.vics@gmail.com; https://orcid.org/0000–0003–4532–2882

Erkasov, Rakhmetulla Sharapidenovich — Doctor of Chemical Sciences, Professor of the Department of Chemistry, L.N. Gumilyov Eurasian National University, Nur-Sultan, K. Munaitpasov Street, 13, 010008, Kazakhstan; e-mail: erkass@mail.ru.

Sorvanov, Alexander Alexandrovich — 1-year postgraduate of specialty organic chemistry, National Research Tomsk State University, Tomsk, Lenin avenue, 36, 634050, Russia; e-mail: wellitson@gmail.com.

Kotelnikov, Oleg Alexeevich — Junior researcher, National Research Tomsk State University, Lenin avenue, 36, 634050, Tomsk, Russia; e-mail: kot_o_a@mail.ru; https://orcid.org/0000–0002–1241–1312.