Full Articles

Modeling of NMR spectra and signal assignment using real-time DFT/GIAO calculations*

P. A. Belaykov and V. P. Ananikov

N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 47 Leninsky prosp., 119991 Moscow, Russian Federation. Fax: +7 (499) 135 5328. E-mail: val@ioc.ac.ru

An automated algorithm for fast quantum chemical modeling of NMR spectra within the framework of the density functional theory was developed. High accuracy of calculations of NMR parameters achieved for various classes of organic compounds including heterocyclic compounds, carbohydrates, steroids, and peptides is comparable with the accuracy of experimental determination. The efficiency of computing the NMR chemical shifts using the high-performance PBE/PRIRODA method was demonstrated.

Key words: NMR spectroscopy, density functional theory, GIAO, B3LYP and PBE functionals, signal assignment, chemical shift calculations, heterocyclic compounds, carbohydrates, steroids, Cyclosporin A.

NMR spectroscopy is a key structural method in modern organic chemistry. It is widely used at present for structure elucidation, investigations of the reactivity and mechanisms of reactions involving organic molecules.[1—3] The key role of NMR spectroscopy in structure determination and in studies of the dynamics of biomolecules is commonly recognized in biochemistry, biology, and pharmacy.[4—7] The development of new instrumental methods has made possible structural studies of rather complex molecular systems including ionic liquids,[8] melts,[9] polymeric[10—12] and crystalline materials,[13,14] as well as nanoscale[15,16] and supramolecular systems.[17—19] Homo- and heteronuclear NMR spectroscopies are key methods in catalysis,[20,21] development and optimization of industrial technologies.[22,23]

A necessary step in the interpretation of results of spectroscopic measurements is the assignment of signals observed in the NMR spectra. A vast variety of multidimensional NMR techniques and a number of special approaches have been developed to solve this problem.[1—7] Recently, quantum chemical calculations of NMR chemical shifts have been widely used for signal assignment.[24—27] Calculations within the framework of the density functional theory (DFT) in the GIAO (Gauge Including Atomic Orbital) approximation ensure an optimum computational cost/accuracy ratio and are most often used.[24—27]

* Dedicated to Academician V. N. Charushin on the occasion of his 60th birthday.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 5, pp. 765—771, May, 2011.

1066-5285/11/6005-783 © 2011 Springer Science+Business Media, Inc.

In this connection, a joint analysis of experimental and calculated NMR chemical shifts is undoubtedly a convenient additional procedure for NMR studies of complex systems and for assignment of signals in the NMR spectra. Rapid increase in performance of personal computers allows NMR parameters to be determined from quantum chemical calculations that take a relatively short time comparable with the acquisition time of the 1D and 2D NMR spectra. Thus, all necessary prerequisites for making a complex experimental and theoretical analysis of NMR spectra a routine tool in chemical research are deployed.

In this work, we describe an automated algorithm for quantum chemical modeling of NMR spectra. The aim of the algorithm is to perform signal assignment and to determine the molecular structures of organic compounds. The efficiency of the algorithm proposed was tested on a number of organic molecules of different complexity.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance NMR spectrometer operating at 600 (^{1}H) and 150.9 (^{13}C) MHz in DMSO-d_6. The chemical shifts in the NMR spectra were calibrated against the residual signals of the solvent used as internal reference. The signals in the experimental NMR spectra were assigned using the 2D techniques including COSY, NOESY, HSQC, and HMBC.

Geometry optimizations were carried out with the Gaussian program[28] by the B3LYP method[29—31] in the 6-31G* basis set.[32,33] The shielding constants were calculated in the GIAO approximation[34—36] by the B3LYP method with the 6-311G(2d,p) basis set.[37,38] Density functional calculations with the PRIRODA-06 program were performed using the PBE functional in the 3z split-valence basis set (for both geometry optimization and in the GIAO approximation) with the expansion of the electron density over an auxiliary basis set.[39,40] Calculations were carried out on a personal computer with an Intel Core 2 Quad processor (clock speed 3.0 GHz). Visualization of the molecular structures was done with the MOLDEN graphic software.[41]

To compare the calculated and experimental chemical shift values, they should be transformed to a unified scale. This was done by performing calculations for the reference compound and the compound under study using the same method. The final values of the calculated chemical shifts of the compounds under investigation (δ_{calc}) were determined from the expression

$$\delta_{calc} = \delta^*_{st} - \delta^*_{sub} + \delta^0_{st},$$

where δ^*_{st} is the calculated chemical shift of the reference compound, δ^*_{sub} is the calculated chemical shift of the substance under study, and δ^0_{st} is the chemical shift assigned to the reference compound. Problems concerning the choice of optimum reference compounds for quantum chemical calculations of the NMR chemical shifts have been documented.[42,43] The accuracy of the calculations of the ^{13}C NMR chemical shifts was determined as the average of absolute values of the differences between the experimental and calculated chemical shift values. The correlation coefficient calculated using the linear regression equation $Y(X) = A + B \cdot X$ for a correlation of the experimental and calculated chemical shift values. Correctness of the application of the linear regression equation for comparing the experimental and calculated values of the NMR chemical shifts was discussed earlier.[44]

Results and Discussion

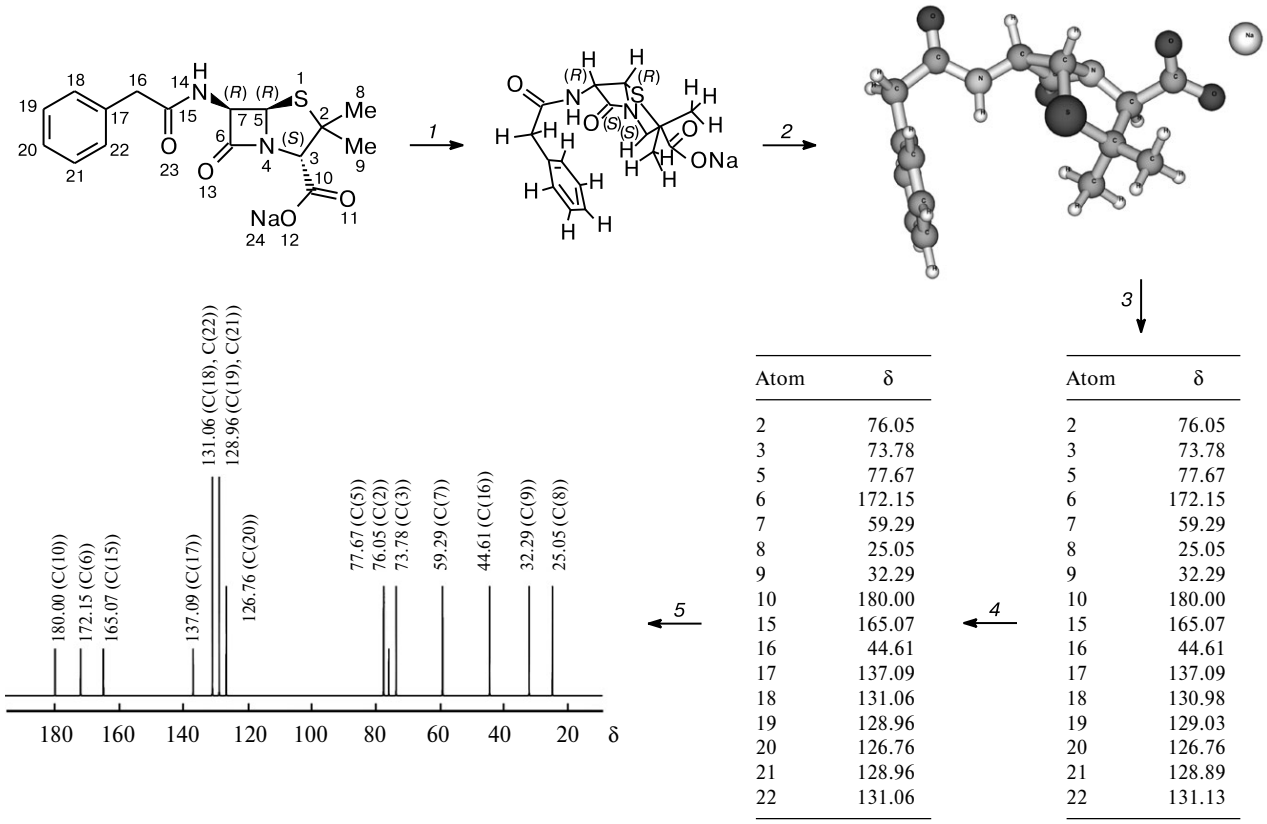
To carry out quantum chemical modeling of NMR spectra based on the structural formula, an *ad hoc* algorithm was developed (Fig. 1), which involves the following steps: *1*) transformation of the structural formula to a three-dimensional molecule; *2*) molecular geometry optimization; *3*) calculations of the NMR chemical shifts; *4*) search for magnetically equivalent nuclei and averaging of the chemical shifts; and *5*) plotting the NMR spectrum using the calculated parameters.

The structural formula was transformed (step *1*, see Fig. 1) using the algorithm of complete inclusion of possible geometric and steric factors implemented in the Conformer plugin of the Marvin software package.[45] This algorithm enables generation of molecular structures with complete analysis of the carbon skeleton, functional groups and heteroatoms, geometric isomers, and asymmetric centers. If the molecule contains an asymmetric center (Fig. 2, *a*), one enantiomer is chosen automatically and the results of calculations of the NMR spectrum are independent of the configuration of the stereocenter. If the molecule contains two and more asymmetric centers (Fig. 2, *b*), the configuration specified in explicit form is exactly used by the molecular structure generation algorithm. The first step is completed by refinement of the 3D model by the molecular mechanics method.

Geometry optimization of the 3D molecular model thus constructed (step *2*, see Fig. 1) is carried out within the framework of the DFT by the B3LYP/6-31G(d) method which correctly reproduces the geometric parameters of a large number of chemical compounds at a reasonable computational cost.[46,47] Acceptable accuracy of the determination of geometric parameters can also be attained using the PBE/TZ2p method with much lower computational cost.

The NMR chemical shifts are calculated by the B3LYP/6-311G(2d,p) and PBE/TZ2p methods in the GIAO approximation (step *3*, see Fig. 1). In particular, a good accuracy (of the order of a few ppm) was attained for the NMR spectra of heteroniclei[24—27] when the input task for the chemical shift calculations in this approximation was prepared manually.

A necessary step in modeling experimental NMR spectra is the search for magnetically equivalent nuclei and the averaging of the calculated chemical shift values taking into account the conformational flexibility of molecules



Atom	δ
2	76.05
3	73.78
5	77.67
6	172.15
7	59.29
8	25.05
9	32.29
10	180.00
15	165.07
16	44.61
17	137.09
18	131.06
19	128.96
20	126.76
21	128.96
22	131.06

Atom	δ
2	76.05
3	73.78
5	77.67
6	172.15
7	59.29
8	25.05
9	32.29
10	180.00
15	165.07
16	44.61
17	137.09
18	130.98
19	129.03
20	126.76
21	128.89
22	131.13

Fig. 1. General algorithm for automated quantum chemical modeling of NMR spectra as applied to the penicillin molecule.

(step *4*, see Fig. 1). This step was done using the Topology Analysis plugin of the Marvin software,[45] and also involves a correction of the NMR signal intensities.

In the final step, the model NMR spectrum is plotted based on the calculated chemical shift values and expected signal intensities (step *5*, see Fig. 1). A convenient graphic representation of NMR spectra is provided by the xyz2jmod program.[48]

All steps described above were successfully automated within the framework of a common algorithm and the prediction accuracy of NMR spectra was tested on a number of organic molecules of different complexity. As the first example, Table 1 lists the calculated ^{13}C NMR chemical shift values obtained using the B3LYP and PBE functionals, as well as the corresponding experimental values given for comparison. The penicillin molecule containing

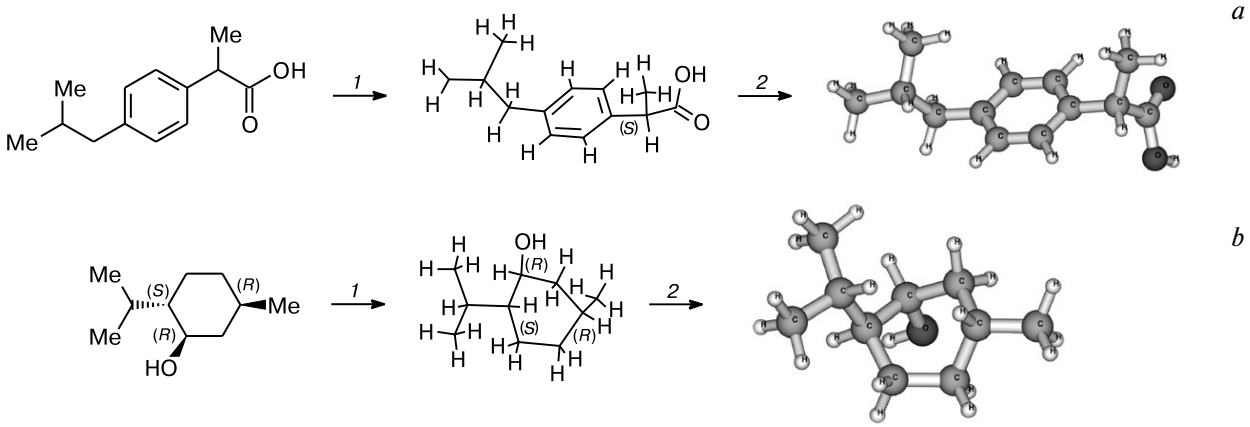


Fig. 2. Generation of the 3D structures of the molecules containing one asymmetric center (configuration not specified) and three asymmetric centers with a specified configuration taking 2-[4-(2-methylpropyl)phenyl]propane acid (*a*) and (1*R*,2*S*,5*R*)-5-methyl-2-(propan-2-yl)cyclohexan-1-ol (*b*) as the respective examples.

Table 1. Calculated and experimental ^{13}C NMR chemical shift values (ppm) for the penicillin molecule

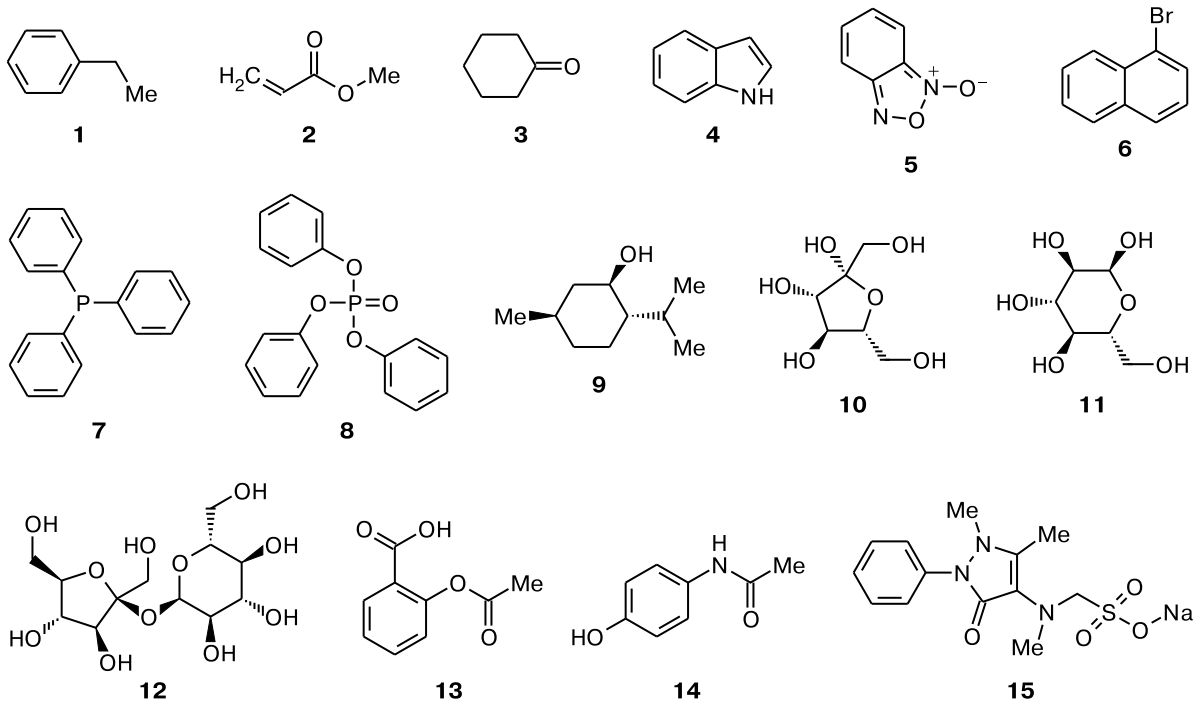
Atom number[b]	Calculations		Experiment[a]
	GIAO-B3LYP[c]	GIAO-PBE[d]	
2	73.58	76.05	64.26
3	72.33	73.78	74.04
5	76.37	77.67	66.74
6	175.51	172.15	173.32
7	57.80	59.29	57.61
8	24.53	25.05	27.51
9	31.58	32.29	31.14
10	183.18	180.00	170.00
15	166.59	165.07	170.35
16	43.23	44.61	41.41
17	137.57	137.09	136.00
18	131.26	131.06	129.03
19	129.29	128.96	128.15
20	127.56	126.76	126.37

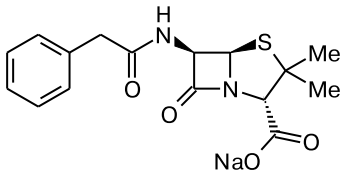
[a] Complete assignment of the signals in the spectrum was done using the 2D COSY, NOESY, HSQC, and HMBC techniques.
[b] Atom numbering scheme is given in Fig. 1.
[c] Geometry optimized by the B3LYP/6-31G(d) method, the chemical shifts calculated by the B3LYP/6-311G(2d,p) method.
[d] Geometry optimization and the chemical shift calculations were performed by the PBE/TZ2p method.

41 atoms including heteroatoms, functional groups, and three chiral centers is a relevant test compound in quantum chemical modeling of the NMR spectrum and a typical example of problems in modern synthetic organic chemistry.

A comparison of the calculated and experimental ^{13}C NMR chemical shift values shows that the algorithm developed can be successfully used to model the NMR spectra (see Table 1). The accuracy of the NMR chemical shift calculations ($\Delta\delta$ ^{13}C) was 3.67 ppm for the GIAO-B3LYP method (Gaussian program) and 3.73 ppm for the GIAO-PBE method (PRIRODA program); the correlation coefficients obtained using the linear regression equation are 0.996 and 0.995, respectively. As should be expected, the largest differences between the calculated and experimental values were obtained for the carbon atoms C(2) and C(5) bound to heteroatoms. The total computing time (steps *1*—*5*, see Fig. 1) was 6 h for the GIAO-B3LYP method and only 30 min for the GIAO-PBE method (starting approximations were similar). Thus, both methods provide a comparable accuracy of the chemical shift calculations, the GIAO-PBE method being much faster.

To prove the efficiency of the automated algorithm proposed, we calculated the ^{13}C NMR spectra of organic compounds belonging to various classes, as well as pharmaceuticals, steroids, and a complex bioactive compound Cyclosporin A by the GIAO-PBE method. The reference compounds were benzene with $\delta^0_{st} = 128.5$ (compounds **1**—**9**, **13**—**21**, Table 2) and ethylene glycol with $\delta^0_{st} = 63.4$ (compounds **10**—**12**, see Table 2). For all compounds studied (**1**—**21** in Table 2), we present the total computing times and the durations of particular steps. The accuracy of calculations can be evaluated using the average values of the differences between the experimental and calculated NMR chemical shifts and the correlation coefficients listed in Table 2.





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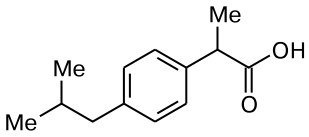

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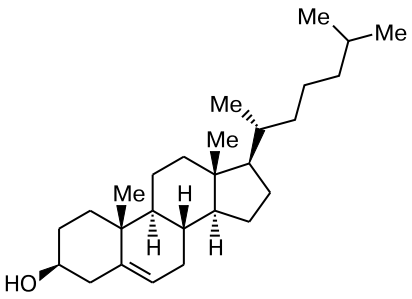

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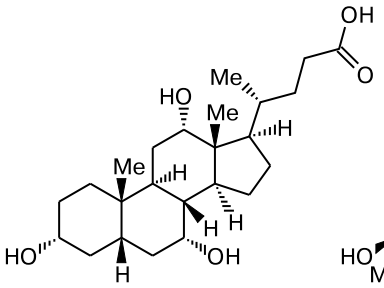

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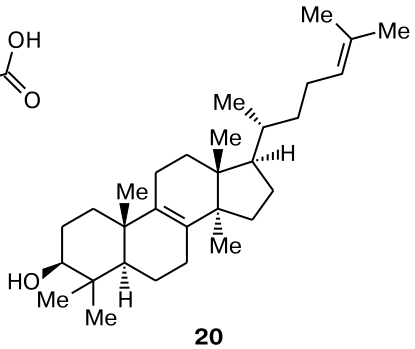

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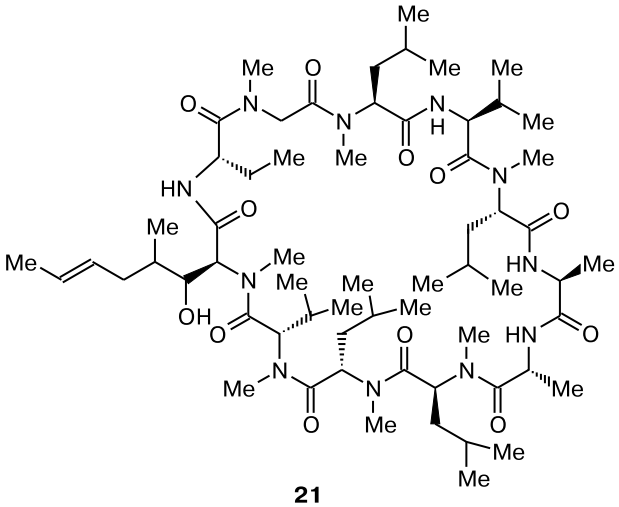

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The total computing time was less than 2 min for small organic molecules (compounds **1**—**6**, see Table 2), from a few minutes to 30 minutes for medium-size molecules (compounds **7**—**17**), and about 1 h for larger molecules (compounds **18**—**20**). Step *2* involving optimization of the molecular geometry (see Fig. 1) is the most time-consuming step. It takes 81—98% of the total computing

Table 2. Total computing time (τ) and the accuracy of the ^{13}C NMR chemical shift calculations by the PBE/TZ2p method for organic compounds **1**—**21**

Compound	Brutto formula	τ/min	Computing time for different steps (%)[a]	$\Delta\delta$ ^{13}C, ppm[b]	R[c]
Ethylbenzene (**1**)	C_8H_{10}	~1.6	0.35 : 87.79 : 10.80 : 0.71 : 0.35	1.3	0.999
Methacrylate (**2**)	$C_4H_6O_2$	1.8	0.31 : 94.11 : 4.96 : 0.31 : 0.31	1.7	0.999
Cyclohexanone (**3**)	$C_6H_{10}O$	~1.4	0.59 : 87.28 : 11.15 : 0.78 : 0.20	2.4	0.999
Indole (**4**)	C_8H_7N	1	0.55 : 82.64 : 15.98 : 0.55 : 0.28	1.8	0.979
Benzofuroxan (**5**)	$C_6H_4N_2O_2$	1.65	0.34 : 87.14 : 11.84 : 0.34: 0.34	2.1	0.986
Bromonaphthalene (**6**)	$C_{10}H_7Br$	~1.3	0.44 : 80.60 : 18.08 : 0.44 : 0.44	3.0	0.985
Triphenylphosphine (**7**)	$C_{18}H_{15}P$	14	0.06 : 95.74 : 4.04 : 0.14 : 0.02	1.9	0.988
Triphenyl phosphate (**8**)	$C_{18}H_{15}O_4P$	32	0.03 : 97.46 : 2.43 : 0.06 : 0.02	2.7	0.999
Menthol (**9**)	$C_{10}H_{20}O$	6	0.14 : 93.37 : 6.06 : 0.24 : 0.19	2.9	0.995
Fructose (**10**)	$C_6H_{12}O_6$	16	0.05 : 97.69 : 2.16 : 0.07 : 0.03	3.6	0.996
Glucose (**11**)	$C_6H_{12}O_6$	10	0.24 : 92.52 : 6.94 : 0.18 : 0.12	1.7	0.995
Sucrose (**12**)	$C_{12}H_{22}O_{11}$	29	0.03 : 97.97 : 1.97 : 0.02 : 0.01	3.8	0.967
Aspirin (**13**)	$C_9H_8O_4$	10	0.06 : 96.78 : 3.01 : 0.09 : 0.06	2.4	0.997
Paracetamol (**14**)	$C_8H_9NO_2$	11	0.05 : 97.64 : 2.16 : 0.10 : 0.05	3.3	0.997
Analgin (**15**) (metamizole sodium)	$C_{13}H_{16}N_3NaO_4S$	25	0.03 : 96.46 : 3.42 : 0.07 : 0.02	4.6	0.994
Penicillin (**16**)	$C_{16}H_{17}N_2NaO_4S$	30	0.04 : 96.29 : 3.59 : 0.06 : 0.02	3.7	0.995
Ibuprofen (**17**)	$C_{13}H_{18}O_2$	12	0.07 : 95.41 : 4.30 : 0.17 : 0.05	2.5	0.998
Cholesterol (**18**)	$C_{27}H_{46}O$	54	0.04 : 96.49 : 3.40 : 0.05 : 0.02	2.8	0.997
Cholic acid (**19**)	$C_{24}H_{40}O_5$	69	0.03 : 97.03 : 2.90 : 0.03 : 0.01	3.9	0.973
Lanosterol (**20**)	$C_{30}H_{50}O$	59	0.03 : 95.85 : 4.05 : 0.06 : 0.01	3.4	0.997
Cyclosporin A (**21**)	$C_{62}H_{111}N_{11}O_{12}$	780	<0.01 : 97.77 : 2.20 : 0.02 : <0.01	3.9	0.996

[a] The format is stage *1* : stage *2* : stage *3* : stage *4* : stage *5*.

[b] Average difference between the calculated and experimental chemical shift values for the corresponding signals.

[c] The coefficient of correlation between the calculated and experimental chemical shift values.

time (see Table 2). The chemical shift calculations in the GIAO approximation (step *3*, see Fig. 1) take 2—18% of the total computing time. Steps *1*, *4*, and *5* (see Fig. 1) are relatively fast and take at most 2% of the total computing time (see Table 2).

The ^{13}C NMR chemical shifts calculated for organic molecules using this automated algorithm show a good accuracy, *viz.*, the deviations from the corresponding experimental values were usually about 1—3 ppm, being less than 4 ppm even for rather large steroid systems (see Table 2). It should be noted that the measured chemical shifts can vary within 1—3 ppm depending on the detection conditions of the ^{13}C NMR spectra, on the concentration of the substance under study, the temperature, and the type of the solvent.[49,50] Thus, calculations enable automated modeling of ^{13}C NMR spectra with a nearly experimental accuracy. Good agreement between the experimental and calculated values is also confirmed by the correlation coefficients (see Table 2).

Calculations of a rather large Cyclosporin A (**21**) molecule containing 196 atoms may be treated as a rather complex test for the algorithm presented in this work. Cyclosporin A is a powerful immunosuppressant used in transplantation and has been intensively studied for some other applications.[51—53]

The total computing time for Cyclosporin A (**21**) molecule was 13 h, the average difference between the experimental and calculated ^{13}C NMR chemical shift values is less than 4 ppm (see Table 2). Good agreement between the experimental and calculated chemical shift values is illustrated in Fig. 3 (correlation coefficient is 0.996).

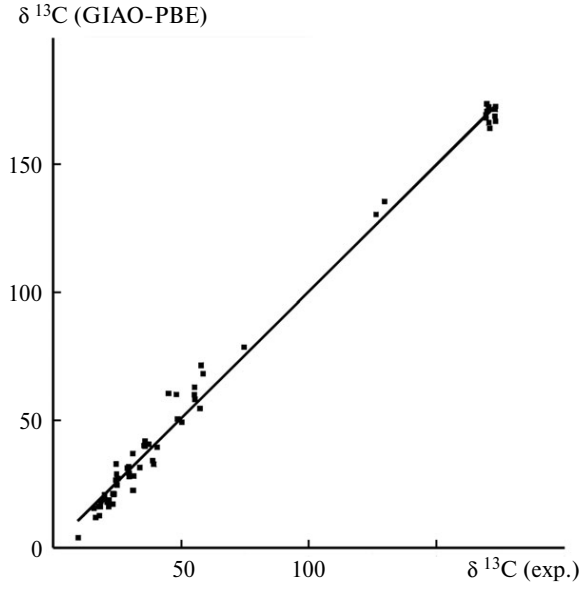

Fig. 3. Correlation between the ^{13}C NMR chemical shifts obtained from PBE/TZ2p calculations and the experimental values for Cyclosporin A (**21**) (linear regression parameters are $A = 0.336$ and $B = 1.000$; $R = 0.996$; $n = 62$).

Summing up, we have for the first time developed an automated algorithm for quantum chemical calculations of the NMR chemical shifts based on the structural formula of the compound. The correctness of reproduction of the ^{13}C NMR chemical shifts with a nearly experimental accuracy was proved by the calculations of organic molecules containing from 12 to 196 atoms. We found that theoretical modeling of NMR spectra with the B3LYP (Gaussian program) and PBE (PRIRODA program) functionals leads to the results of comparable accuracy. Calculations using the PBE functional were probed on various classes of organic molecules including heterocyclic compounds, carbohydrates, steroids, and peptides. Since NMR chemical shifts obtained from quantum chemical calculations are unambiguously assigned to particular atoms in the molecule, a comparison of calculated and experimental chemical shift values helps to assign signals in NMR spectra. The adequacy of such an approach was demonstrated in this work by comparing theoretical NMR spectra with experimental NMR spectra with the known assignments of NMR signals.

This work was financially supported by the Russian Foundation for Basic Research (Project No. 11-03-01055).

References

1. *Multidimensional NMR Methods for the Solution State*, Eds G. Morris, P. J. Emsley, John Wiley and Sons Ltd, Chichester, 2010.
2. T. Claridge, *High-Resolution NMR Techniques in Organic Chemistry*, 2nd ed., Tetrahedron Organic Chemistry, **27**, Elsevier Science, Amsterdam, 2008.
3. *Encyclopedia of Nuclear Magnetic Resonance*, Eds D. M. Grant, R. K. Harris, John Wiley and Sons, Chichester, 1996, **1—8**; 2002, **9**.
4. K. Chary, G. Govil, *NMR in Biological Systems: From Molecules to Human*, Springer, Dordrecht, 2008.
5. G. S. Rule, T. K. Hitchens, *Fundamentals of Protein NMR Spectroscopy*, Springer, Dordrecht, 2006.
6. M. Pellecchia, D. S. Sem, K. Wüthrich, *Nat. Rev. Drug Discov.*, 2002, **1**, 211.
7. D. D. Boehr, H. J. Dyson, P. E. Wright, *Chem. Rev.*, 2006, **106**, 3055.
8. V. P. Ananikov, *Chem. Rev.*, 2011, **111**, 418.
9. V. P. Ananikov, I. P. Beletskaya, *Izv. Akad. Nauk, Ser. Khim.*, 2008, 740 [*Russ. Chem. Bull., Int. Ed.*, 2008, **57**, 754].
10. T. Kitayama, K. Hatada, *NMR Spectroscopy of Polymers*, Springer, Berlin, 2010.
11. R. Kimmich, N. Fatkullin, *Adv. Polym. Sci.*, 2004, **170**, 1.
12. H. W. Spiess, *Macrom. Chem. Phys.*, 2003, **204**, 340.
13. *NMR Crystallography*, Eds R. K. Harris, R. E. Wasylishen, M. J. Duer, John Wiley and Sons Ltd, Chichester, 2009.
14. *New Techniques in Solid-State NMR*, Ed. J. Klinowski, *Topics in Current Chemistry*, Springer, Berlin, 2005, **246**.
15. V. P. Ananikov, I. P. Beletskaya, *Ros. Nanotekhnol.*, 2009, **4**, 56 [*Nanotechn. Russ.* (*Engl. Transl.*), 2010, **5**, 1].
16. C. Mayer, *Ann. Rep. NMR Spectr.*, 2005, **55**, 205.

17. S. V. Kharlamov, Sh. K. Latypov, *Usp. Khim.*, 2010, **79**, 699 [*Russ. Chem. Rev.* (*Engl. Transl.*), 2010, **79**, 635].
18. A. Pastor, E. Martínez-Viviente, *Coord. Chem. Rev.*, 2008, **252**, 2314.
19. M. Pons, O. Millet, *Prog. Nucl. Magn. Res. Spectrosc.*, 2001, **38**, 267.
20. *In situ NMR Methods in Catalysis*, Eds J. Bargon, L. T. Kuhn, Topics in Current Chemistry, Springer, Berlin, 2007, **276**.
21. T. Blasco, *Chem. Soc. Rev.*, 2010, **39**, 4685.
22. D. A. W. Wendisch, *Appl. Spectrosc. Rev.*, 1993, **28**, 165.
23. S. Ghosh, *Nuclear Magnetic Resonance for Process Industries*, CRC Press, Boca Raton, FL, 2010.
24. *Calculation of NMR and EPR Parameters. Theory and Applications*, Eds M. Kaupp, M. Bühl, V. C. Malkin, Wiley-VCH Verlag GmbH and Co., Weinheim, Germany, 2004.
25. G. Bifulco, P. Dambruoso, L. Gomez-Paloma, R. Riccio, *Chem. Rev.*, 2007, **107**, 3744.
26. J. Vaara, *Phys. Chem. Chem. Phys.*, 2007, **9**, 5399.
27. J. C. Facelli, *Modeling NMR Chemical Shifts*, in *Modern Magnetic Resonance*, Ed. G. A. Webb, Springer, Dordrecht, 2006, 53.
28. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, Jr., J. A. Montgomery, T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, J. A. Pople, *GAUSSIAN 03*, Gaussian, Inc., Pittsburgh (PA), 2003.
29. A. D. Becke, *Phys. Rev. A*, 1988, **38**, 3098.
30. C. Lee, W. Yang, R. G. Parr, *Phys. Rev. B*, 1988, **37**, 785.
31. A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648.
32. R. Ditchfield, W. J. Hehre, J. A. Pople, *J. Chem. Phys.*, 1971, **54**, 724.
33. W. J. Hehre, R. Ditchfield, J. A. Pople, *J. Chem. Phys.*, 1972, **56**, 2257.
34. K. Wolinski, J. F.Hilton, P. Pulay, *J. Am. Chem. Soc.*, 1990, **112**, 8251.
35. J. R. Cheeseman, G. W. Trucks, T. A. Keith, M. J. Frisch, *J. Chem. Phys.*, 1996, **104**, 5497.
36. G. Rauhut, S. Puyear, K. Wolinski, P. Pulay, *J. Phys. Chem.*, 1996, **100**, 6310.
37. R. Krishnan, J. S. Binkley, R. Seeger, J. A. Pople, *J. Chem. Phys.*, 1980, **72**, 650.
38. A. D. McLean, G. S. Chandler, *J. Chem. Phys.*, 1980, **72**, 5639.
39. D. N. Laikov, *Chem. Phys. Lett.*, 1997, **281**, 151.
40. D. N. Laikov, *Chem. Phys. Lett.*, 2005, **416**, 116.
41. G. Schaftenaar, J. H. Noordik, *J. Comput.-Aided Mol. Des.*, 2000, **14**, 123.
42. V. P. Ananikov, *Centr. Eur. J. Chem. (CEJC)*, 2004, **2**, 196.
43. V. P. Ananikov, I. P. Beletskaya, *Izv. Akad. Nauk, Ser. Khim.*, 2003, 771 [*Russ. Chem. Bul.*, *Int. Ed.*, 2003, **52**, 811].
44. V. P. Ananikov, *Bruker Spin Report*, 2005, N156/157, 41.
45. *Marvin 5.4.1.1*, ChemAxon, Calculator Plugins, 2010, http://www.chemaxon.com/.
46. W. Koch, M. C. Holthausen, *A Chemists´s Guide to Density Fuctional Theory*, Wiley-VCH, Weinheim, 2001.
47. D. S. Sholl, J. A. Steckel, *Density Functional Theory: A Practical Introduction*, John Wiley and Sons, Hoboken, New Jersey, 2009.
48. A. M. Genaev, *xyz2jmod*, N. N. Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Branch of the Russian Academy of Sciences, http://limor1.nioch.nsc.ru/quant/program/xyz2jmod.
49. H.-O. Kalinowski, S. Berger, S. Braun, *Carbon-13 NMR Spectroscopy*, John Wiley and Sons, Chichester, 1988.
50. *Carbon-13 NMR Chemical Shifts in Structural and Stereochemical Analysis*, Eds K. Pihlaja, E. Kleinpeter, VCH Publishers, Deerfield Beach, FL, 1994.
51. R. Y. Calne, D. J. G. White, S. Thiru, D. B. Evans, P. McMaster, D. C. Dunn, G. N. Craddock, D. B. Pentlow, K. Rolles, *Lancet*, 1978, **2**, 1323.
52. S. Britton, R. Palacios, *Immun. Rev.*, 1982, **65**, 5.
53. J. Lee, S. S. Kim, *J. Int. Med. Res.*, 2010, **38**, 1561.

Received February 25, 2011;
in revised form April 20, 2011