Homo- and heteronuclear NMR methods for signal assignments in ^{1}H and ^{13}C specta of 1-aminoacyloxygermatran

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Introduction

Hypercoordinated group 14 element compounds have attracted considerable attention. In most germatranes, the germanium atom is pentacoordinated. However, octahedral geometry is often the characteristic of organo-germanium compounds. The secondary bonding between the donor and the germanium atoms causes the hypercoordination phenomenon, resulting in a hexacoordinated germanium atom with a distorted octahedral geometry [1, 2]. For example, octahedral germanium (IV) complexes bearing acetylacetonato ligand possess the promising anticancer activity and display high complex stability in biological media [3]. In [4] the hexa- coordinated germanium complexes catechols and biologically active pyridines (including vitamins) were obtained from GeO_2 Heptacoordinated trichlorogermyl[tris(2-methoxyphenyl)]methane of a tricapped tetrahedral structure was obtained by Y.Takeuchi with co-author [5]. S. Karlov and co-authors [6] reported that germaspirobis (ocanes) $[RN(CH_2CHR'O)_2]_2Ge$ may possess four-, five-, and six-coordinated Ge atoms. Among them, octahedrally hexacoordinated Ge atoms with the N atoms occupying cis-positions are thermodynamically the most stable.

The germatrane cycle is significantly more resistant to hydrolytic cleavage than the silatrane one, which makes it possible to use germatranes as a transport agent for biologically active fragments into living cells [7]. At the moment, a wide range of germatranes containing organic substituents at the germanium atom are known [8]. Among them, the 1-acyloxygermatranes $RC(O)OGe(OCH_2CH_2)_3N$ should be especially distinguished. The choice of a carboxylic acid as a substituent has a significant effect on the biological activity of germatranes.

The use of NMR spectroscopy for establishing the structure of germatranes (Figure **1**) has a rather long history, which is described in the review [9]. The importance of the first NMR works (in the early 80s) of the two most active research groups from the Siberian Branch of the Russian Academy of Sciences under the guidance of M.G. Voronkov and from the Institute of Organic Synthesis (The Latvian Academy of Sciences) under the guidance of É. Lukevics should be noted. It is not surprising that from these groups (predominantly synthetic chemists by training) such well-known NMR-specialists as V.A. Pestunovich, V.K. Voronov [10], S.N. Tandura, A.V. Kisin, E.E. Liepins and É.L. Kupće [11, 12] later emerged, some of them continue active NMR studies [13, 14]. The methodological and technical revolution in NMR spectroscopy at the end of the 1970s made it possible to carry out studies (exotic for that time) on almost all magnetic nuclei and so the works of these two groups on the study of germatranes on the rare nuclei ^{13}C, ^{15}N, ^{17}O, ^{73}Ge appeared [15]. However, ^{1}H NMR turned out to be the least informative because of

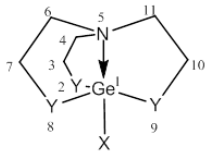

Figure 1. The structure and numbering of germatranes

high level symmetry of germatranes and the effects of strong coupling AA`XX`-type of protons in $(NCH_2CH_2O)_3$ system. The introduction of substituents at positions 3, 7, and 10 of the atrane core complicates the ^1H spectrum too much [9], the analysis of which is a separate goal.

Experiments and results

The main goal of this work is to identify signals in the ^1H and ^{13}C NMR spectra of 1-aminoacyloxygermatran (**1**) using homo- and heteronuclear correlation methods J-resolved COSY, COSY, NOESY, HSQC, and HMBC. Figure **2** shows fragments of the ^1H and J-COSY spectra, which greatly facilitate the analysis of overlapped regions of the proton spectrum and the isolation of multiplet signals and also the measurement of the scalar constants $^{2,3}J_{H\text{-}H}$.

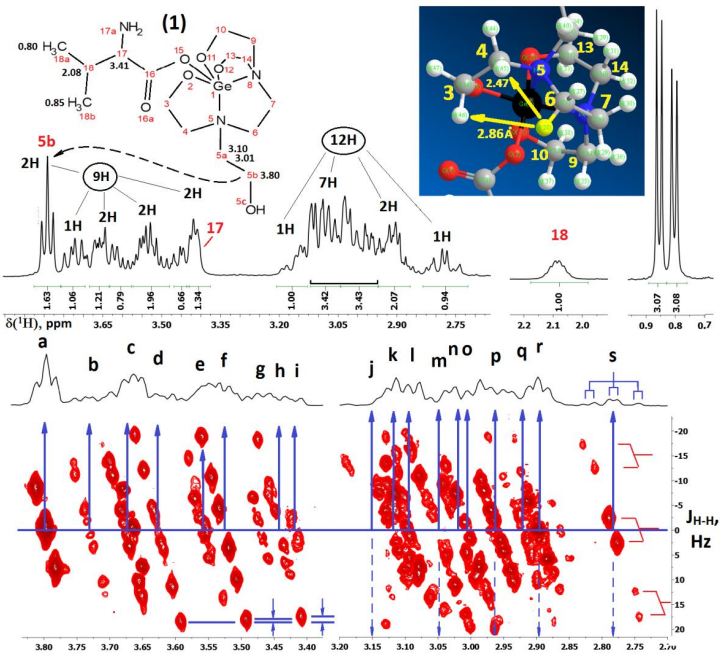

*Figure 2. ^1H (left) and J-resolved COSY (right) spectra of 1-aminoacyloxygermatran (**1**). Multiplet structure and shifts of some signals are shown by arrows. Fragment of calculated (MM2) spatial structure of compound (**1**) and atom numbering are presented in a rectangle.*

In the ^1H NMR spectrum of compound (**1**) a significant dispersion of signals is observed both in the region of 3.85 – 3.35 ppm, in which the signals of eight protons of the -O-CH_2-groups and the $C^{17}H$ proton at 3.41 ppm are located (d, J = 4 Hz, 1H) and in the region of 3.20-2.70 ppm, where the signals of all 12 protons of the N-CH_2 groups are situated. Integration of the signals (see Fig. 2, left) shows that only the region 3.12 – 2.94 ppm turns out to be too overloaded, since there are signals of 7 of the 12 methylene protons at different nitrogen atoms. The multiplet signals of other protons (-N-CH_2-CH_2-O-) belong to weakly coupled spin systems of the AB-XY type and are quite well distinguishable. The only exceptions are the signals of the methylene protons of the mobile fragment $N^5C^{5a}H_2C^{5b}H_2OH$,

one of which ($C^{5b}H_2$) has a characteristic triplet structure at 3.80 ppm, and for the second one the chemical shift difference of geminal protons is equal to 0.087 ppm. So, they form an AB-system in the region 3.15 – 2.97 ppm (see Fig. **3**). The belonging of signals to pseudoequatorial or pseudoaxial protons is easily determined on the basis of the Karplus dependence of vicinal constants on the dihedral angle [16], which leads to the following set of constants for the protons of the ethane fragment in rigid cyclic structures: the sum of the constants (i.e., the multiplet width) for the equatorial proton is always less than for axial. Therefore, the signals with a triplet structure found in the J-COSY spectrum at 3.63 (**d**), 3.53 (**f**) and 3.44 (**h**) ppm (the sum of the constants is about 30 Hz) belong to axial protons, and the signals at 3.73 (**b**), 3.67 (**c**), and 3.56 (**e**) ppm. respectively, equatorial (the sum of the constants is about 22 Hz). The existence of a geminal constant $^2J_{ax\text{-}eq} \approx -12$ Hz between axial and equatorial protons and their spatial proximity ($r_{ax\text{-}eq} = 1.78$ Å), made it easy to establish 3 pairs of $–OCH_2$-: protons: "**b-d**", "**c-f**" and "**h-e**". These pairs are especially well seen in the NOESY spectrum (Fig. **3**) as intense negative (blue) cross peaks near the diagonal with a positive (red) signal orientation.

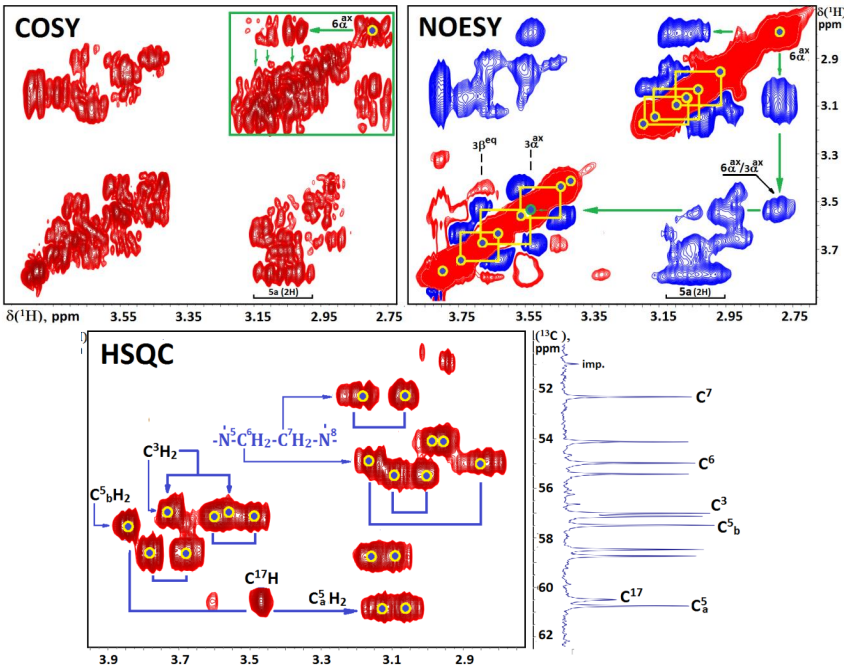

*Figure 3. Fragments of ^{13}C (right) and (left to right) COSY, NOESY and HSQC spectra of 1-aminoacyloxygermatran (**1**). Most important for ^1H and ^{13}C signal assignments scalar and through space interactions (NOEs) are shown by different color lines and arrows.*

Similar geminal pairs of protons can also be found in the region where the signals of NCH_2- groups are located. Among them, the signals of protons of the $N^5C^6H^2C^7H_2N^8$ group are the most interesting, since all their signals should be in the region of 3.20–2.70 ppm. Consequently, any of their spatial interactions with the protons of the $O–CH_2$- groups will indicate the spatial proximity of the single $N^5C^6H_2C^7H_2N^8$ fragment to the protons of the

nearest -CH_2O- fragment of the neighboring germatrane cycle. This problem was solved by comparing the cross peaks in the COSY and NOESY spectra (Fig. **3**). The first of them clearly shows that the signal at 2.78 ppm (**s**), which has the structure of a triplet of doublets (td, $J_{H\text{-}H}$ = 12.6, 12.6, 4.8 Hz, 1 H) and, therefore, it is an axial proton. It has scalar and spatial (NOE) cross peaks only within the 3.20 – 2.98 ppm. region and, consequently, this is one of the axial protons of the desired ethane fragment C^6H_2-C^7H_2. Moreover, there is one more cross-peak in the spectrum, linking this proton with the axial proton (at 3.53 ppm. **f**) of one of the -OCH_2- groups. According to calculations, only the α-proton at the C^3 atom can be such a proton. So, we can make the reasonable assignments of proton and carbon signals for two adjacent germatrane cycles: Ge-N^5-C^6H_2-C^7H_2-N^8 and Ge-N^5-C^4H_2-C^3H_2-O^2 (See ^1H and ^{13}C assignments in Fig. **3**). Additional independent HMBC data fully confirm the obtained results.

Conclusion

Using various homo- and heteronuclear NMR experiments (J-COSY, COSY, NOESY, HSQC, HMBC), the identification of signals in the ^1H and ^{13}C NMR spectra was made for a 1-aminoacyloxygermatran (**1**). The obtained values of chemical shifts and scalar constants $^{2,3}J_{H\text{-}H}$ are the basis for the further active use of NMR spectroscopy in the structural and conformational analysis of new germatrane derivatives with potential biological activity.

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