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Complete Proton Assignment in Acetylcholesterol using ge-SELINCOR-TOCSY

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Abstract: The ge-SELINCOR sandwich is used to select proton magnetization by irradiating the carbon atom directly bound to the proton. This magnetization can be transferred to coupled protons by a TOCSY mixing process (MLEV-16). A 1D experiment is obtained which gives additional information when complex H,H-COSY spectra suffer from overlapping crosspeaks. Coherence pathway selection is performed with pulsed field gradients.

Signal overlapping is one of the biggest problems in NMR spectroscopy. Sometimes the standard 1D and 2D experiments[1] do not allow the full assignment of spectral data for natural and synthetic products such as steroids[2] for example. Connectivity information from H,H-COSY-spectra cannot be fully utilized if several coupled isochrone pairs of nuclei exist in the substance. More sophisticated experiments are therefore required, and in recent times 3D experiments have been proposed which resolve signals in a third dimension.[3] Implementation of the carbon frequency domain can be beneficial, which is achieved by inserting a second evolution time into the pulse sequence. Such a H,H,C-COSY usually results in good signal separation, the main drawbacks are the long measuring times required and the high demands on computer space and power.

We present here a new carbon selective 1D method, the ge-SELINCOR-TOCSY, which utilizes the ge-SELINCOR sandwich.[4] This method is in essence the same as a 3D H,H,C-COSY, but with the added advantage that it extracts only the pertinent information. The required time for the measurement is drastically decreased. The ge-SELINCOR sandwich selects the adjacent proton(s) through a frequency selective carbon pulse, while the TOCSY component gives the connection of this proton to its homonuclear neighbours (fig. 1).

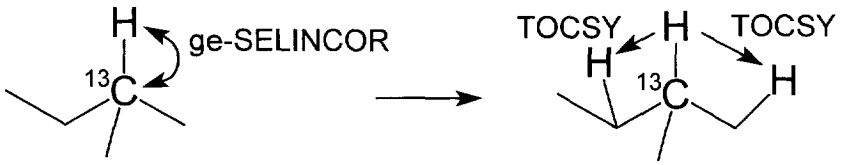

Fig. 1: Graphic representation of magnetization transfer in the ge-SELINCOR-TOCSY experiment.

RESULTS AND DISCUSSION

The H,C-COSY allows the correlation between the proton and carbon chemical shift of CH, CH_2 and CH_3 fragments, while the H,H-COSY and the long range H,C-COSY determines the connection between them. In case of overlap in the H,H-COSY a ge-SELINCOR-TOCSY can be applied to distinguish between the cross peaks. The usefulness of this new method will be demonstrated using acetylcholesterol **1** (see fig. 2).

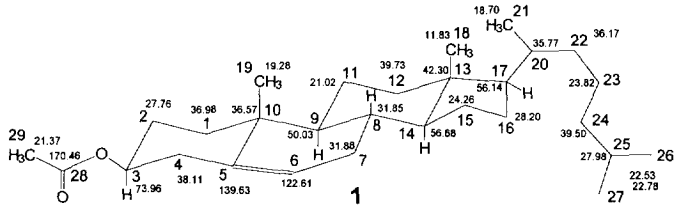

Fig. 2: Molecular formula of acetylcholesterol **1**; carbon chemical shifts are also given.

The gated decoupled ^{13}C NMR, H,H-COSY, H,H-TOCSY, ROESY, H,C-COSY and long range H,C-COSY spectra almost allow a complete assignment of all signals of **1**. In fig. 3 the expansion of the crowded ^1H NMR region of **1** is given. The predicted proton signal assignments are also included.

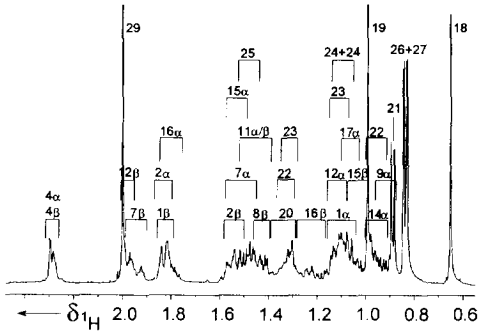

Fig. 3: Expansion of the 500 MHz ^1H NMR of **1**; β: same side as methyl group 19, α: opposite side to methyl group 19.

Table 1: ^1H NMR (500 MHz) data of 100 mg of **1** in 1 ml $CDCl_3$

#	1α	1β	2α	2β	3β	4α/β	6	7α	7β	8β	9α
δ(^{1}H)	1.128	1.850	1.855	1.567	4.600	2.314	5.370	1.543	1.970	1.450	0.952
#	11α/β	12α	12β	14α	15α	15β	16α	16β	17α	18	19
δ(^{1}H)	1.480	1.146	1.995	0.987	1.562	1.060	1.827	1.242	1.102	0.678	1.018
#	20	21	22	22	23	23	24/24	25	26	27	29
δ(^{1}H)	1.356	0.915	0.984	1.343	1.136	1.333	1.121	1.517	0.866	0.861	2.025

There are still some uncertainties however. The H,C-COSY leads to two resonances 2α and 2β corresponding to two protons bound to the same carbon. We therefore expect to see in the H,H-COSY a crosspeak correlating 2α to 2β due to a geminal coupling. It would be nice to confirm the assignment

in table 1 and prove that there are couplings between 1β-2β and 15α-16α, which are overlapped by the geminal coupling 2α-2β. It will be shown that such a task may be performed with the 1D-ge-SELINCOR-TOCSY experiment.

Fig. 4B and C illustrate a general application of the ge-SELINCOR-TOCSY experiment. In fig. 4B the ge-SELINCOR experiment (irradiating carbon C-20) allows to selectively detect proton H-20 of **1**. The ge-SELINCOR-TOCSY experiment as shown in fig. 4C spreads the magnetization from proton H-20 to all its neighbours, thus enhancing protons H-17, H-21 and H-22. In conclusion a carbon atom is selectively excited and the resultant proton magnetization is used to examine all its proton-coupled partners. Note that a short spin lock is used.

The uncertainties concerning the overlap due to the geminal coupling H2α/β in the H,H-COSY of **1** can be examined using ge-SELINCOR with a C-1 selective pulse, resulting in an enhancement of protons H1α and H1β (fig. 4D). The ge-SELINCOR-TOCSY (fig. 4e) would be expected to increase signals H2α/β. H2α is superimposed on H1β, however the expected signal H2β is clearly visible, thus proving that H1α and/or H1β is coupled to H2β. The connectivity between H15α and H16α can be similarly proved. Fig. 4F gives the ge-SELINCOR spectrum obtained on selecting carbon C-16, while fig. 4G demonstrates the coupling of H16α and/or H16β to H15α/β and H-17.

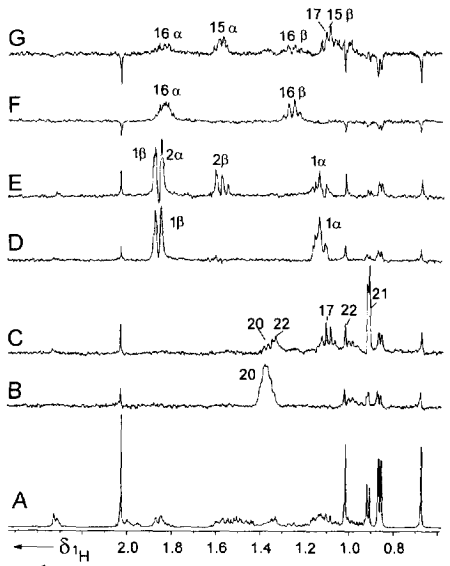

Fig. 4: ^{1}H NMR-spectra of **1**: A: ^{1}H NMR; B, D, F: ge-SELINCOR; C, E, G: ge-SELINCOR-TOCSY (selection of C-20, C- 1, C-16).

EXPERIMENTAL

Fig. 5 shows the ge-SELINCOR-TOCSY pulse sequence. Pulsed field gradients for coherence pathway selection[5] ensure that signals of protons bound to ^{12}C are suffiently suppressed. A MLEV-16[6] spin lock for the TOCSY[7] mixing time is used to transfer magnetization after the ge-SELINCOR

sandwich. Due to the low sensitivity of this carbon selective experiment an in phase magnetization spinlock transfer is more beneficial than a COSY analogue one, because in complex spin systems a COSY like transfer would lead to unobservable multiple quantum coherences, resulting in a loss of magnetization. The MLEV-16 spinlock was used because it doesn't change the coherence order.[8] A very short spinlock of 20 ms was applied, therefore only the directly coupled protons are reached.

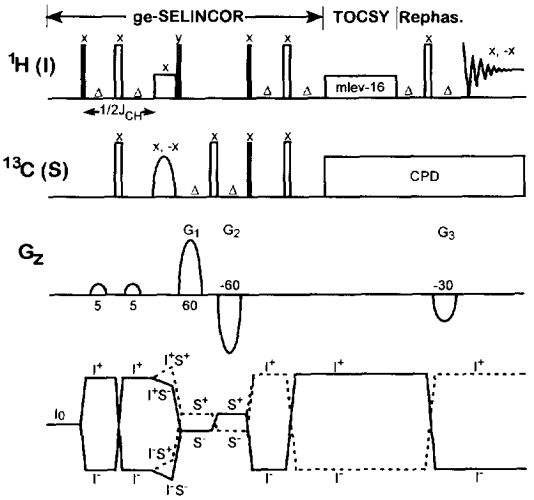

Fig. 5: The ge-SELINCOR-TOCSY experiment: All measurements were performed at 300 K on a Bruker AMX-500 spectrometer, equipped with a multinuclear inverse probehead with self-shielded coils. A Bruker z-gradient accessory delivered sinusoidal gradients up to 23 G/cm. 90° Gauss shaped selective carbon pulses (40- 80 ms) were used. Each ge-SELINCOR measurement took 45 min and each ge-SELINCOR-TOCSY 3 h. As a sample 100 mg of **1** in 1 ml $CDCl_3$ was used. Δ was adjusted accordingly to a $^1J_{CH}$-coupling. The chosen coherence pathway is shown below.

It has thus been shown that carbon selection using ge-SELINCOR can be expanded to a TOCSY experiment, which yields useful information in assignments of proton spectra. This experiment may be performed without the need for isotopic labelling and therefore it is of general interest.

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