Summary of PhD thesis

IMPROVEMENT OF NUCLEAR MAGNETIC RESONANCE (NMR) METHODS BY UTILIZING BROADBAND HOMONUCLEAR PROTON-DECOUPLING

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I. INTRODUCTION AND AIM OF THE WORK

Nuclear magnetic resonance (NMR) spectroscopy is one of the most powerful techniques for investigation of structural, dynamical properties and interactions of molecules at atomic resolution. By now, it has become a method that is widely used in most fields of chemistry and in structural biology as well. High quality NMR spectra are very much needed if one would like to gain reliable information from the measurements. Sensitivity and resolution are the two key parameters which primarily determine the quality of an NMR spectrum. In the last two decades the sensitivity of NMR measurements has increased by more than an order of magnitude, due to electronical improvements, higher magnetic fields and the introduction of cryogenically cooled probes. In contrast, the spectral resolution has been enhanced by only a factor of two if we compare the highest magnetic field spectrometers available today with the ones operated 20 years ago in terms of resolution. Therefore, there is a continuous demand for developing methods which can improve the resolution of NMR spectra.

Signal overlaps being present the most often in ^{1}H spectra originate from the limited chemical shift range (~10-15 ppm) on the one hand, and from the extensive signal splittings due to homonuclear proton-proton couplings on the other hand. In contrast, in a broadband homonuclear decoupled ^{1}H spectrum – as it is shown in Figure 1 – each multiplet has collapsed to singlet, which gives rise to several advantages. First and foremost, spectral resolution increases significantly, signal overlaps are minimized or totally absent, which is absolutely useful in the evaluation of spectra of macromolecules and mixtures (e.g. diastereomers, reaction product mixtures, metabolites). Secondly, the determination of NMR parameters (e.g. chemical shifts, heteronuclear coupling constants) becomes simpler and more precise due to the simplification of the spectra. Finally, automatic peak picking is applicable and more efficient owing to the singlet lines, thus the time needed for evaluation of complex spectra is significantly reduced.

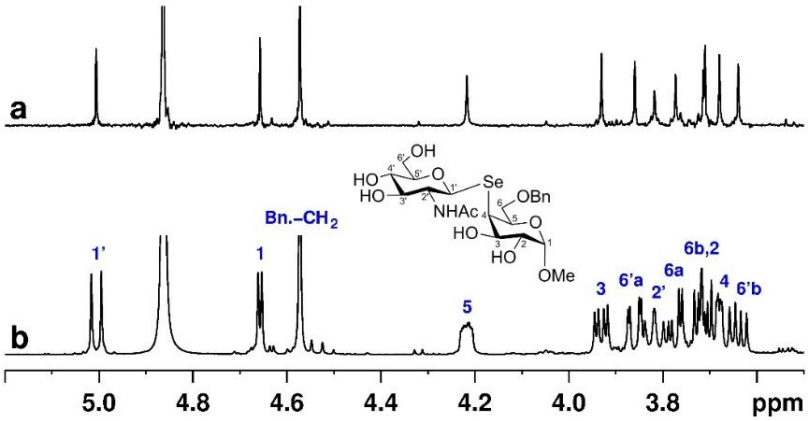

Figure 1. Comparison of broadband homonuclear decoupled ^{1}H (**a**) and normal ^{1}H (**b**) spectra

Due to the foreseeable advantages, several methods were proposed for broadband homonuclear decoupling in the decades before the turn of the millennium. However, all these techniques are applicable only in the indirect dimension of two-dimensional NMR spectra and require complicated data processing, and some of them considerably reduce sensitivity compared to the regular ones. Thus these methods have not found widespread applications. In recent years, there has been a revival in the field of broadband homonuclear decoupling (also known as pure shift NMR) owing to the novel ideas published in the literature. One of the greatest advantages of these new methods is the possibility to utilize them in multidimensional, proton-detected experiments for simplification of signals and so, for increasing resolution.

In 2012 our research group also joined to this dynamically growing NMR methodological research field with the goal of increasing the efficiency of NMR experiments by building in pulse sequence elements capable of broadband homonuclear decoupling. On the one hand, our aim was to develop broadband proton-decoupled, heteronuclear correlation experiments, which yield enhanced resolution and efficient water suppression. On the other hand, we planned the improvement of methods applicable to the determination of one- and multiple-bond heteronuclear coupling constants by broadband homonuclear decoupling. Our goal was also to demonstrate the utility of the developed methods for the measurement of various heteronuclear coupling constants in carbohydrate derivatives.

II. EXPERIMENTAL SECTION

All experiments were performed at 11.75 T (500 MHz for ^{1}H) on a Bruker Avance II NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) equipped with a BBI (broadband, inverse) or a TXI (triple-resonance, inverse) z-gradient probe. All spectra were processed, evaluated and plotted with TopSpin 2.1 or 3.0 (Bruker Biospin GmbH, Karlsruhe, Germany).

III. NEW SCIENTIFIC ACHIEVEMENTS

1. The conventional HSQC experiment was improved by broadband homonuclear decoupling based on BIRD^{d} pulse sequence element.

We inserted the BIRD^{d} (*BIlinear Rotation Decoupling*) block and a non-selective 180° proton pulse into the HSQC (*Heteronuclear Single Quantum Correlation*) pulse sequence before the proton detection period to achieve broadband homonuclear decoupling. The timing and phase program of the pulses of the experiment, which has utilized the interferogram-based acquisition strategy, was optimized by performing measurements on simple carbohydrate derivatives. The characteristic multiplet structure of correlation peaks was simplified in the spectra obtained (Figure 2).

The method developed was compared with the only, previously published proton-decoupled HSQC experiment. We found that our method was more robust, namely, it was less sensitive for the changes in the duration of INEPT (*Insensitive Nuclei Enhanced by Polarization Transfer)*/BIRD delays adjusted to the one-bond heteronuclear coupling constant.

An additional advantageous side-product of the BIRD^{d} pulse sequence element employed in the acquisition scheme is the efficient suppression of undesired long-range correlation peaks arising from strong coupling effects (Figure 2).

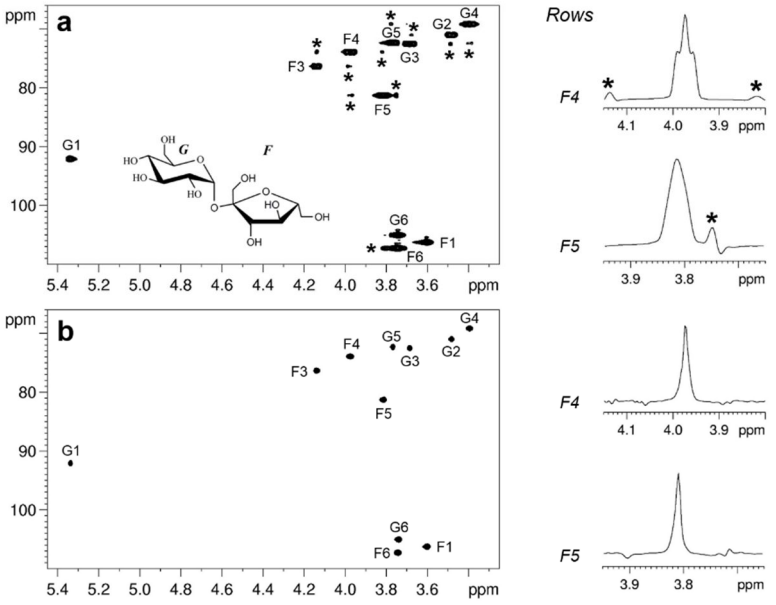

Figure 2. Comparison of ^{1}H-^{13}C HSQC spectra and selected carbon traces recorded without (**a**) and with (**b**) broadband proton decoupling for sucrose. Long-range correlation artifacts resulting from strong coupling effects in spectrum (**a**) are labeled with asterisks (*).

We incorporated BIRD decoupling into a modified perfect echo pulse sequence to create what we term a "perfectBIRD" pulse sequence element, which can remove splittings arising from geminal proton-proton couplings as well. With this the greatest disadvantage of BIRD decoupling was eliminated.

2. A real-time (instant) broadband homonuclear decoupled variant of the sensitivity-enhanced ^{1}H-^{15}N HSQC method was developed for measuring biological samples in H_2O.

After a careful optimization, efficient water suppression was achieved in the real-time broadband proton-decoupled experiment using gradient pairs of appropriate length and strength around the BIRD^{d} block and the non-selective 180° proton pulse during acquisition.

We showed that the method developed was suitable for recording ^{1}H-^{15}N HSQC spectra of small proteins dissolved in H_2O even at natural ^{15}N isotopic abundance (Figure 3).

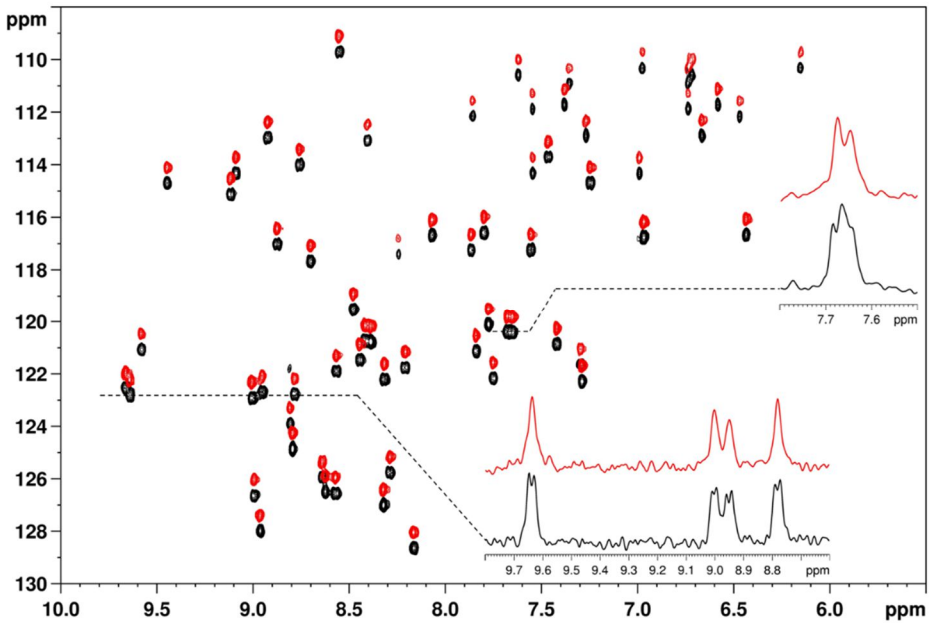

Figure 3. ^{1}H-^{15}N sensitivity-enhanced HSQC spectra of unlabeled mutant PAF^{D55N} (55 amino acids) protein in 95 % H_2O / 5 % D_2O without (black, lower) and with (red, upper) real-time homonuclear decoupling. The pure shift spectrum is shifted in the nitrogen dimension for easier comparison.

3. Interferogram-based broadband proton-decoupled CLIP/CLAP-HSQC methods were established for the determination of one-bond heteronuclear coupling constants.

Broadband homonuclear decoupling was achieved by applying an appropriate combination of BIRD^{d} pulse sequence element and a non-selective 180° proton pulse in the CLIP/CLAP-HSQC (*CLean In-Phase/CLean Anti-Phase*) experiments, being widely used for the measurement of one-bond heteronuclear couplings. With this novel method, the determination of one-bond heteronuclear coupling constants is simplified to the measurement of frequency differences between singlet maxima. As a result, accurate and reliable coupling values are provided even in the case of molecules with complex proton-proton coupling network (Figure 4).

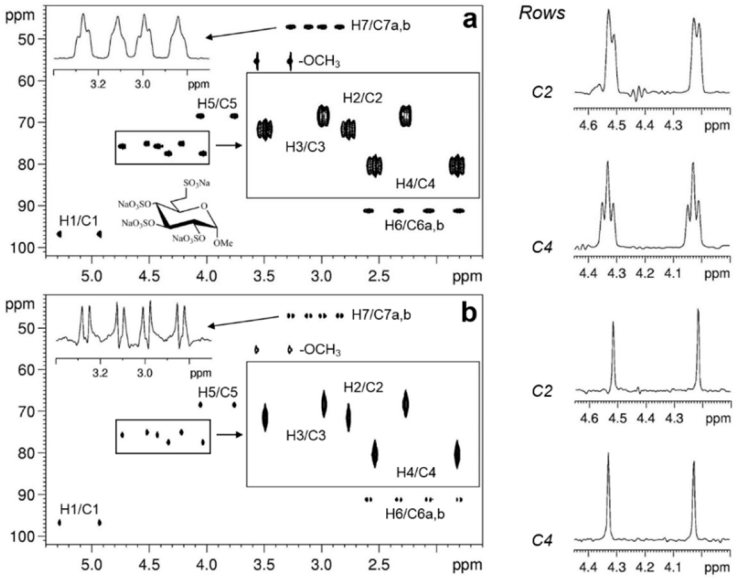

Figure 4. Comparison of ^{1}H-^{13}C CLIP-HSQC (**a**) and broadband proton-decoupled ^{1}H-^{13}C CLIP-HSQC (**b**) spectra and selected carbon traces

We investigated the robustness and tolerance of the experiments developed with regard to the mismatch of BIRD/INEPT delays. To this end, the duration of corresponding delays was adjusted to nominal values of one-bond heteronuclear couplings ranging from 100 to 180 Hz. It was found that signal intensities were significantly degraded when the delays were misset with respect to the real value of coupling constant, but the pure absorptive quality of the lineshapes remained basically unaffected. This finding predicted the potential utility of the method for anisotropic samples as well, in which the net coupling constants show large variation.

With measurements carried out in weakly-orienting medium we proved that the proton-decoupled CLIP/CLAP-HSQC method could be utilized for determination of RDCs (*Residual Dipolar Couplings*), which provide important structural and dynamical information (Figure 5).

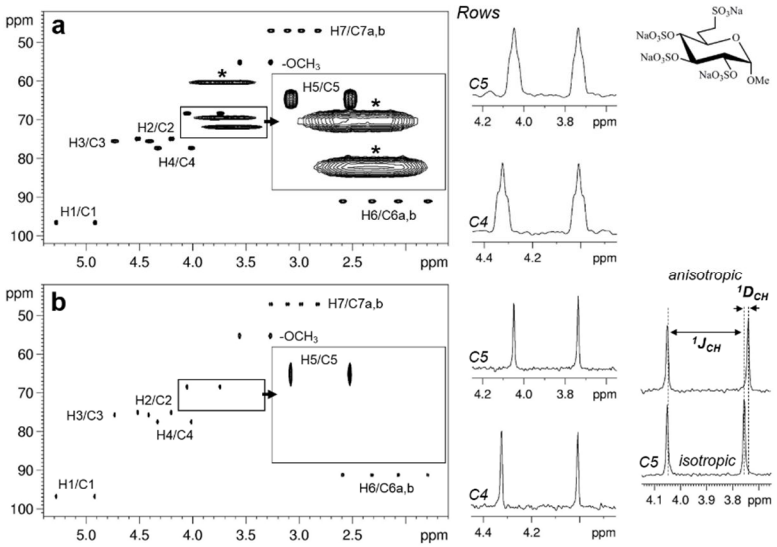

Figure 5. Comparison of ^{1}H-^{13}C CLIP-HSQC (**a**) and broadband proton-decoupled ^{1}H-^{13}C CLIP-HSQC (**b**) spectra and selected carbon traces in anisotropic medium. Correlations of the orienting medium are labeled with asterisks (*). The determination of $^{1}D_{CH}$ residual dipolar coupling (RDC) is illustrated at the bottom right inset.

By inserting the perfectBIRD pulse sequence element into the CLIP/CLAP-HSQC experiments we established an NMR method which has allowed the determination of one-bond heteronuclear couplings – even in the case of diastereotopic methylene hydrogen atoms – by simply measuring frequency differences between singlet maxima.

4. Broadband proton-decoupled CLIP/CLAP-HSQC method was improved by the real-time acquisition strategy.

The broadband homodecoupled CLIP/CLAP-HSQC experiments were considerably speeded up with the application of real-time, windowed decoupling during acquisition compared to the interferogram-based ones, so much so that the sensitivity of the original, proton-coupled method was retained.

According to our observations, real-time decoupling can bias the heteronuclear coupling constants significantly as a function of the proton offset frequency and the

duration of BIRD delay. We proved that the systematic errors could be minimized with complex phase sequencing and cycling of pulses applied during acquisition.

The dependence of heteronuclear coupling constants from proton offset frequency and duration of BIRD delay was systematically investigated for improving and optimizing the phase program. The experiment with the optimized phase program is suitable for the determination of one-bond ^{1}H-^{15}N coupling constants of small proteins even in weakly-orienting medium.

5. We developed an interferogram-based, slice-selective broadband homonuclear decoupled CPMG-HSQMBC method for the accurate determination of multiple-bond heteronuclear coupling constants.

A slice-selective, Zangger-Sterk (ZS)-type pulse sequence element was inserted into the CPMG-HSQMBC (*Carr-Purcell-Meiboom-Gill Heteronuclear Single Quantum Multiple-Bond Correlation*) experiment before the proton detection period to remove the undesired splittings caused by proton-proton couplings from the detected signals. As a result the determination of long-range heteronuclear coupling constants was simplified to measure frequency differences between singlet maxima even in the case of molecules with complex proton-proton coupling network (Figure 6).

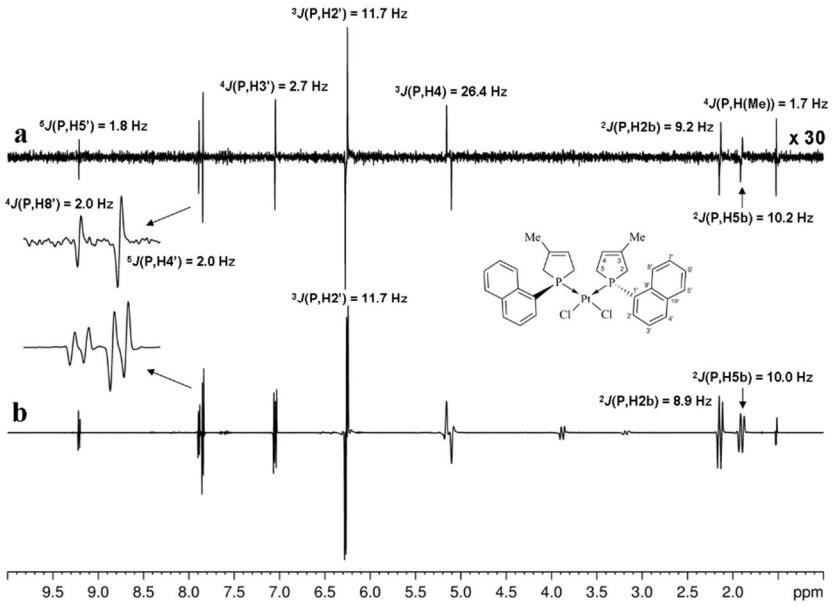

Figure 6. Comparison of Zangger-Sterk homonuclear decoupled ^{1}H-^{31}P CPMG-HSQMBC (**a**) and ^{1}H-^{31}P CPMG-HSQMBC (**b**) spectra

It was demonstrated on phosphorus containing model compounds that a broad range (~ 2-26 Hz) of multiple-bond heteronuclear coupling constants could be measured in a single experiment using our new method (Figure 6).

We determined several $^{n}J_{SeH}$ values in diglycosyl-(di)selenides, which was not possible with earlier methods due to signal distortions caused by proton-proton couplings.

The utility of ZS-decoupled CPMG-HSQMBC was also demonstrated for the measurement of long-range ^{1}H-^{13}C coupling constants. However, this experiment was successful only with highly concentrated (~ M range) samples owing to the significant sensitivity drop caused by slice-selection and to the unfavorable natural abundance of the ^{13}C nucleus.

6. The real-time version of Zangger-Sterk broadband proton-decoupled CPMG-HSQMBC method was established.

The ZS CPMG-HSQMBC experiment was considerably speeded up with the application of real-time, windowed decoupling during acquisition compared to the corresponding interferogram-based one. The limitations of the real-time acquisition strategy, namely, occurrence of some line broadening and artefacts reducing the quality of spectra were also evaluated.

We showed that if the spin system and assignment of the molecule studied were known in advance, the sensitivity of the experiment could be significantly enhanced by applying multiple-frequency modulated pulses. In this case each proton resonance can be detected from multiple slices of the sample if proton offsets are suitably chosen to avoid accidental recoupling effects.

According to our observations the real-time Zangger-Sterk CPMG-HSQMBC method can be utilized for the determination of multiple-bond heteronuclear coupling constants only in those molecules where the proton resonance frequency difference of coupling partners is at least 90-100 Hz. Thus, properly short (max. ~10 ms) selective proton pulse can be used in the proton-proton refocusing blocks during acquisition.

7. The CPMG-HSQMBC experiment was improved by utilizing interferogram-based PSYCHE broadband homonuclear decoupling.

The PSYCHE (*Pure Shift Yielded by CHirp Excitation*) scheme inserted into the CPMG-HSQMBC pulse sequence efficiently eliminates the unwanted proton-proton splittings from the heteronuclear multiplets, and so the desired multiple-bond heteronuclear coupling constants can be determined directly and precisely from the pure antiphase doublets (Figure 7).

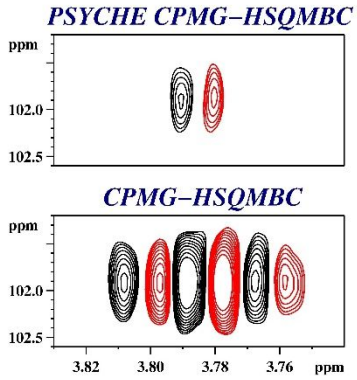

Figure 7. Comparison of representative correlation peaks of PSYCHE ^{1}H-^{13}C CPMG-HSQMBC and ^{1}H-^{13}C CPMG-HSQMBC 2D spectra

A pronounced advantage of the PSYCHE CPMG-HSQMBC experiment in contrast with the ZS-decoupled method is that the nature (characteristics) of the proton spin systems involved practically has no effect on the efficiency of homonuclear decoupling. Consequently, the PSYCHE experiment can be performed without tedious fine-tuning of experimental parameters from sample to sample.

In case of molecules with less than 50 Hz resonance frequency difference of the proton coupling partners, the PSYCHE CPMG-HSQMBC can provide nearly an order of magnitude enhancement in sensitivity compared to the interferogram-based ZS-decoupled method (Figure 8). Note that the real-time version of ZS-decoupled experiment is not applicable either in this case (see thesis statement 6, paragraph 3).

The performance of PSYCHE CPMG-HSQMBC method was illustrated by measuring $^{3}J_{CH}$ and $^{3}J_{SeH}$ values in various carbohydrates. These data are conveying valuable information on the conformations around the glycosidic linkages. It is important to note that extraction of the corresponding couplings from the conventional, proton-coupled HSQMBC multiplets may demand computer-aided fitting procedures or may even be impeded by partial signal cancellations in the mixed-phase signals.

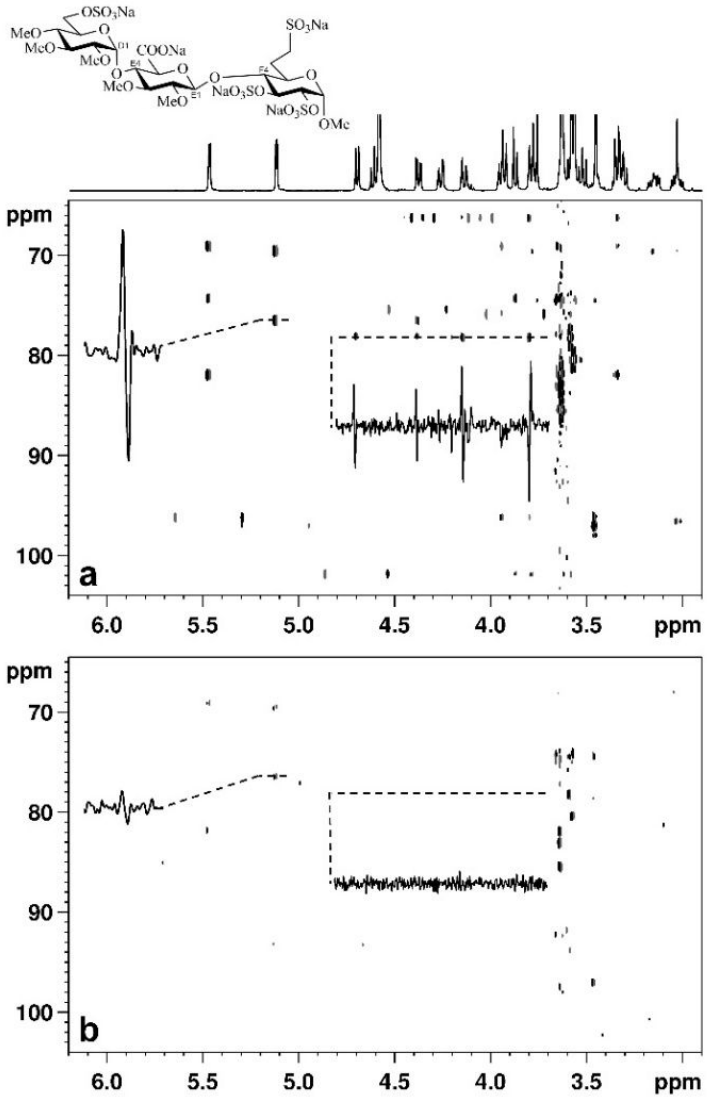

Figure 8. Comparison of PSYCHE ^{1}H-^{13}C CPMG-HSQMBC (**a**) and ZS ^{1}H-^{13}C CPMG-HSQMBC (**b**) 2D spectra and selected carbon traces of a heparin-analog trisaccharide

IV. POSSIBLE APPLICATIONS OF THE RESULTS

During our research we have joined to that dynamically growing area of NMR spectroscopy which has been focusing on the improvement of NMR methods' performance by building in various pulse sequence elements capable of broadband homonuclear decoupling. In line with this, we have developed such broadband proton-decoupled, heteronuclear correlation experiments that yield enhanced spectral resolution and allow automatic peak picking during spectral analysis. As a result, these novel experiments can considerably help the assignment of NMR spectra of complex molecules and multicomponent systems (e.g. mixtures of diastereomers or reaction products, metabolites), and so the structure elucidation or verification of these compounds. On the other hand, broadband homonuclear decoupled NMR methods providing spectra devoid of undesired proton-proton splittings have been developed for precise and direct determination of one- and multiple-bond heteronuclear coupling constants, which are invaluable and widely applied tools for structure elucidation and conformational analysis of organic compounds.

In conclusion, as our improved NMR methods provide more precise and reliable data to disclose structure-activity relationships than the earlier ones, thus they may contribute to the rational design and development of drug substances with improved biological profile.

V. PUBLICATIONS

Publications related to the dissertation

English articles in international journals:

1. István Timári, Lukas Kaltschnee, Andreas Kolmer, Ralph W. Adams, Mathias Nilsson, Christina M. Thiele, Gareth A. Morris, Katalin E. Kövér: **Accurate determination of one-bond heteronuclear coupling constants with "pure shift" broadband proton-decoupled CLIP/CLAP-HSQC experiments**
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2. Lukas Kaltschnee, Andreas Kolmer, István Timári, Volker Schmidts, Ralph W. Adams, Mathias Nilsson, Katalin E. Kövér, Gareth A. Morris, Christina M. Thiele: **"Perfecting" pure shift HSQC: full homodecoupling for accurate and precise determination of heteronuclear couplings**
Chemical Communications, **2014**, 50, 15702-15705. (IF.: 6,834)

3. István Timári, Tünde Z. Illyés, Ralph W. Adams, Mathias Nilsson, László Szilágyi, Gareth A. Morris, Katalin E. Kövér: **Precise measurement of long-range heteronuclear coupling constants by a novel broadband proton-proton - decoupled CPMG-HSQMBC method**
Chemistry - A European Journal, **2015**, 21, 3472-3479. (IF. (2014.): 5,731)

4. Péter Király, Ralph W. Adams, Liladhar Paudel, Mohammadali Foroozandeh, Juan A. Aguilar, István Timári, Matthew J. Cliff, Mathias Nilsson, Péter Sándor, Gyula Batta, Jonathan P. Waltho, Katalin E. Kövér, Gareth A. Morris: **Real-time pure shift ^{15}N HSQC of proteins: a real improvement in resolution and sensitivity**
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5. István Timári, László Szilágyi, Katalin E. Kövér: **PSYCHE CPMG–HSQMBC: An NMR spectroscopic method for precise and simple measurement of long-range heteronuclear coupling constants**
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RSC Advances, bírálat alatt/under review

Publications not related to the dissertation

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7. Mihály Herczeg, László Lázár, Zsuzsanna Bereczky, Katalin E. Kövér, István Timári, János Kappelmayer, András Lipták, Sándor Antus, Anikó Borbás: **Synthesis and anticoagulant activity of bioisosteric sulfonic acid analogues of the antithrombin-binding pentasaccharide domain of heparin**
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8. Attila Borics, Jayapal Reddy Mallareddy, István Timári, Katalin E. Kövér, Attila Keresztes, Géza Tóth: **The effect of Pro^2 modifications on the structural and pharmacological properties of endomorphin-2**
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9. Magdolna Csávás, Tamás Demeter, Mihály Herczegh, István Timári, Katalin E. Kövér, Pál Herczegh, Anikó Borbás: **Rapid synthesis of self-assembling 1,2-thiomannobioside glycoconjugates as potential multivalent ligands of mannose-binding lectins**
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12. Péter L. Parajdi-Losonczi, Attila C. Bényei, Éva Kováts, István Timári, Tereza R. Muchova, Jana Kasparkova, Péter Buglyó: **$[(\eta^6\text{-}p\text{-cym})Ru(H_2O)_3]^{2+}$ binding capability of aminohydroxamates – a solution and solid state study**
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13. Krisztina Fehér, István Timári, Kinga Rákosi, János Szolomájer, Tünde Z. Illyés, Ádám Bartók, Zoltán Varga, György Panyi, Gábor K. Tóth, Katalin E. Kövér: **Probing pattern and dynamics of disulfide bridges using synthesis and NMR of an ion channel blocker peptide toxin with multiple diselenide bonds**
Chemical Science, **2016**, 7, 2666-2673. (IF. (2014.): 9,211)

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14. Timári István, Komáromi István, Fehér Krisztina, E. Kövér Katalin: **Heparin-analóg pentaszacharidok szerkezete és antitrombin III fehérjével való kölcsönhatása: NMR vizsgálatok és elméleti számítások**
Pro Scientia Aranyérmesek XI. Konferenciája, Konferenciakötet, Budapest, **2013**, 119-123.

15. Gróf Pál, Knapp Krisztina, Schlosser Gitta, Nagy Tamás Milán, Timári István, Borics Attila, Kövér Katalin, Csík Gabriella, Majer Zsuzsa: **Diszulfidhidat tartalmazó ciklikus peptidek UV-besugárzásának hatására keletkező szabadgyökök és szulfhidril-csoportok detektálása**
Magyar Tudomány, **2016**, 177, 50-54.

Lectures related to the dissertation

1. Timári István, Illyés Tünde Zita, Szilágyi László, E. Kövér Katalin: **Információnyerés információvesztéssel avagy a szélessávú proton-lecsatolás alkalmazásai**
Meeting of the NMR Working Group of the Hungarian Academy of Sciences, 9-10 May 2013, Pécs, Hungary

2. István Timári, Lukas Kaltschnee, Andreas Kolmer, Ralph W. Adams, Mathias Nilsson, Tünde Zita Illyés, László Szilágyi, Christina M. Thiele, Gareth A. Morris, Katalin E. Kövér: **Losing some sensitivity to gain more information or the novel applications of broadband proton-decoupling**
15th Austrian Chemistry Days, 23-26 September 2013, Graz, Austria

3. Timári István: **Mágneses magrezonancia (NMR) módszerek fejlesztése szélessávú proton-lecsatolás beépítésével**
Spring Conference of University of Debrecen Hatvani István College for Advanced Studies, 2-3 May 2014, Debrecen, Hungary

4. Timári István, Batta Gyula, E. Kövér Katalin: **Valós idejű szélessávú proton-lecsatolt, heteronukleáris NMR módszerek fejlesztése fehérjék vizsgálatára**
Meeting of the Peptide Chemistry Working Group of the Hungarian Academy of Sciences, 28-30 May 2014, Balatonszemes, Hungary

5. Timári István, E. Kövér Katalin: **Különféle szélessávú protonlecsatolt NMR módszerek összehasonlítása: előnyök és hátrányok**
Meeting of the NMR Working Group of the Hungarian Academy of Sciences, 2-3 October 2014, Balatonszemes, Hungary

6. Timári István, E. Kövér Katalin: **Mágneses magrezonancia (NMR) módszerek fejlesztése, avagy hogyan készül az „NMR-szimfónia"**
12th Conference of Pro Scientia Gold Medalists, 6-8 November 2014, Eger, Hungary

7. Katalin E. Kövér, István Timári, Lukas Kaltschnee, Andreas Kolmer, Ralph W. Adams, Mathias Nilsson, Tünde Z. Illyés, László Szilágyi, Christina M. Thiele, Gareth A. Morris: **Precise measurement of heteronuclear coupling constants: novel applications of broadband proton-proton decoupling**
Magnetic Moments in Central Europe 2015, 25 February – 1 March 2015, Krynica-Zdrój, Poland

Posters related to the dissertation

1. István Timári, Lukas Kaltschnee, Andreas Kolmer, Ralph W. Adams, Mathias Nilsson, Christina M. Thiele, Gareth A. Morris, Katalin E. Kövér: **Measuring one-bond heteronuclear coupling constants with improved resolution. Utilizing the potential of novel broadband proton-decoupled HSQC-based methods**
EUROMAR 2013 (European Magnetic Resonance Conference), 30 June – 5 July 2013, Hersonissos, Crete, Greece

2. István Timári, Zita Tünde Illyés, Ralph W. Adams, Mathias Nilsson, László Szilágyi, Gareth A. Morris, Katalin E. Kövér: **Highly accurate measurement of long-range heteronuclear coupling constants with a novel broadband proton-decoupled CPMG-HSQMBC method**
EUROMAR 2013 (European Magnetic Resonance Conference), 30 June – 5 July 2013, Hersonissos, Crete, Greece

3. Lukas Kaltschnee, Andreas Kolmer, István Timári, Ralph W. Adams, Mathias Nilsson, Katalin E. Kövér, Gareth A. Morris, Christina M. Thiele: **Pure Shift HSQC measurements with perfectBIRD decoupling – a method to decouple diastereotopic protons**
EUROMAR 2013 (European Magnetic Resonance Conference), 30 June – 5 July 2013, Hersonissos, Crete, Greece

4. Lukas Kaltschnee, Andreas Kolmer, István Timári, Ralph W. Adams, Mathias Nilsson, Katalin E. Kövér, Gareth A. Morris, Christina M. Thiele: **Pure Shift HSQC Measurements with perfectBIRD Decoupling – a Method to Decouple Diastereotopic Protons**
35th FGMR Discussion Meeting and Joint Conference of the German, Italian and Slovenian Magnetic Resonance Societies, 9-12 September 2013, Frauenchiemsee, Germany

5. Lukas Kaltschnee, Andreas Kolmer, István Timári, Ralph W. Adams, Mathias Nilsson, Katalin E. Kövér, Gareth A. Morris, Christina M. Thiele: **Pure Shift HSQC measurements with perfectBIRD decoupling – a method to decouple diastereotopic protons**
SMASH 2013 (Small Molecule NMR Conference), 22-25 September 2013, Santiago de Compostela, Spain

6. István Timári, Gyula Batta, Katalin E. Kövér: **Real-time broadband proton-decoupled (pure shift) methods with efficient water suppression: sensitivity enhanced ^{1}H-^{15}N HSQC and TROSY experiments of labelled and unlabelled proteins**
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7. Lukas Kaltschnee, Andreas Kolmer, István Timári, Volker Schmidts, Ralph W. Adams, Mathias Nilsson, Katalin E. Kövér, Gareth A. Morris, Christina M. Thiele: **Applications of pure shift HSQC experiments with "perfectBIRD" decoupling**
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8. István Timári, Ralph W. Adams, Mathias Nilsson, Tünde Z. Illyés, László Szilágyi, Gareth A. Morris, Katalin E. Kövér: **Simple and precise measurement of heteronuclear coupling constants by novel broadband homonuclear decoupled NMR methods**
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List of publications related to the dissertation

Foreign language scientific article(s) in international journal(s) (5)

1. **Timári, I.**, Szilágyi, L., Kövér, K.E.: PSYCHE CPMG-HSQMBC: An NMR Spectroscopic Method for Precise and Simple Measurement of Long-Range Heteronuclear Coupling Constants.
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14. **Timári I.**, Komáromi I., Fehér K., E. Kövér K.: Heparin-analóg pentaszacharidok szerkezete és antitrombin III fehérjével való kölcsönhatása: NMR vizsgálatok és elméleti számítások. In: Pro Scientia Aranyérmesek XI. Konferenciája : Szeged, 2012. november 8-10. : előadások. Szerk.: Szőllősi László, Pro Scientia Aranyérmesek Társ., Budapest, 119-123, 2013. ISBN: 9789638828927

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