Selective Rotations Using Non-Selective Pulses and Heteronuclear Couplings

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Contents

I. Introduction

The pulse sequence kit for construction of multidimensional NMR pulse sequences contains many elements for effecting selective or seemingly selective rotations. They are employed for suppression of entire cross or diagonal peak multiplets or for blocking certain coherence transfers with the purpose of obtaining simplified multiplet patterns.

This paper will describe some known and some novel pulse sequence elements within multidimensional liquid state NMR. In general, the optimum choice of element depends on several conditions related to the exact application as for example (i) whether the element shall be part of a preparation or a mixing sequence, (ii) whether relevant spins are active or passive or both, (iii) whether there are orders of magnitude differences between the sizes of pertinent J coupling constants that can be exploited, and (iv) whether the selectivity must be obtained in a single step or a linear combination of experiments is acceptable.

II. Heteronuclear Bilinear $\pi/2$ and π Rotations in Spin Systems with a Single $^1J_{IS}$

In this section we consider selective rotations in the presence of only one large one-bond coupling,

$^1J_{IS}$, per molecule. That would apply for S $= {}^{13}$C or ^{15}N at the natural abundance level but also for S $= {}^{15}$N in fully ^{13}C, ^{15}N-labeled proteins since $^1J_{CH}$ can be suppressed. A thesis written in a local dialect (1) suggested the following two sequences for broadband selective excitation of S satellites in I $= {}^1$H spectra:

$$A : \left(\frac{\pi}{2}\right)^I_x - \frac{\tau}{2} - (\pi)^{I,S}_x - \frac{\tau}{2} - \left(\frac{\pi}{2}\right)^I_x$$

$$B : \left(\frac{\pi}{4}\right)^I_x - \tau - (\pi)^{I,S}_x - \tau - \left(\frac{3\pi}{4}\right)^I_x$$

with $\tau = (2J_{IS})^{-1}$. In addition, sequence B with $\tau = (2J_{CC})^{-1}$ and exclusively ^{13}C pulses throughout was proposed as a "poor mans" INADEQUATE for observation of ^{13}C satellites in ^{13}C spectra (1). Nowadays sequence B is known as TANGO (2) and sequence A has been referred to as a purging sandwich in ^{13}C editing experiments (3,4).

The rationale behind sequence A is to apply the equivalent of y and $-$y rotations to the satellites corresponding to spin S in α and β spin state, respectively. This is illustrated in Figure 1a, which shows that the parent signal is not excited at all since it occurs at the zero crossing of positive and negative rotations. That, of course, also follows from the propagator behind the sequence:
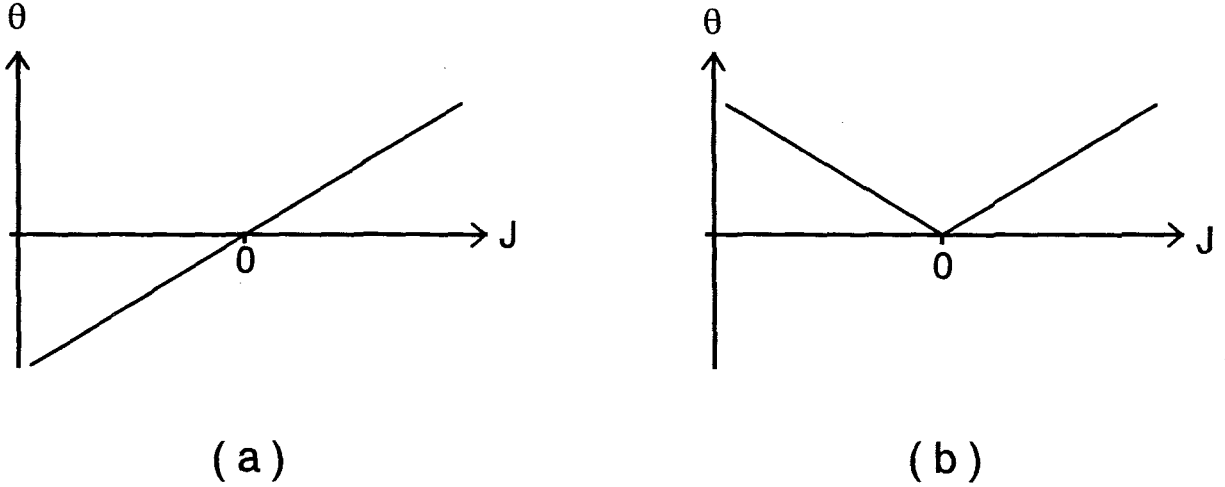
(a)   **(b)**

Figure 1: Graphical illustration of the basic ideas behind the excitation profiles for discrimination between IS and isolated I spin systems. θ is the rotation angle and the zero on the abscissa represents the refocused chemical shifts: (a) sequence A; (b) sequence B. Note that these diagrams only illustrate the ideas and that in practice J-dependent phases and amplitudes occur using sequence B and its derived sequences.

$$e^{-i\theta\{I_yS^\alpha - I_yS^\beta\}} = e^{-i\theta 2 I_y S_z} = e^{i\frac{\pi}{2}I_x}e^{-i\theta 2 I_z S_z}e^{-i\frac{\pi}{2}I_x} \tag{1}$$

where insertion of two π pulses results in sequence A. The idea of formulating the aim of a pulse sequence in terms of selective rotations and then expanding the propagator to end up with a sequence employing only non-selective pulses has been used in refs. (5,6) and exploited extensively in ref. (7).

For the rare occasions where the antiphase character of the signals resulting from sequence A is not acceptable the sequence can be extended with the spin echo

$$\frac{\tau}{2} - (\pi)^{I,S} - \frac{\tau}{2}$$

and possibly terminated by a $\pi/2$ purging pulse (8) on the S channel. The same effect can be obtained with less pulses using sequence B.

That sequence corresponds to the profile in Figure 1b. However, an expansion procedure equivalent to eqn. 1 is not immediately possible because the propagator for the selective rotations is equal to the propagator for a non-selective excitation:

$$e^{-i\theta\{I_yS^\alpha + I_yS^\beta\}} = e^{-i\theta I_y} \tag{2}$$

Hence a simple "expansion" like eqn. 2 does not have a built-in guarantee for zero excitation of the parent signals as did sequence A. It is necessary to add another idea that exploits an interaction present

only in the IS spin system and not in the isolated I spin system. Obviously this interaction can only be the J_{IS} coupling:

$$e^{-i\theta(I_xS^\alpha + I_xS^\beta)} = e^{-i(\Psi+\pi)I_x}e^{-i\pi(I_zS^\alpha - I_zS^\beta)}e^{-i(\pi-\Psi)I_x}$$
$$= e^{-i(\Psi+\pi)I_x}e^{-i\pi 2 I_z S_z}e^{-i(\pi-\Psi)I_x} \tag{3}$$

where Ψ is a parameter explained below. In analogy to sequence A the parent signal is at the zero crossing for the z rotation.

A $(\Psi + \pi)_x$ pulse followed by a π_z rotation is equivalent to a $-(\Psi + \pi)_x$ pulse. Thus for an overall $(\pi/2)_x$ rotation Ψ must be set according to

$$\theta = -(\Psi + \pi) + (\pi - \Psi) = \frac{\pi}{2} \Rightarrow \Psi = \frac{3\pi}{4} \tag{4a}$$

Sequence B in fact corresponds to a $(\pi/2)_{-x}$ rotation:

$$\theta = -(\Psi + \pi) + (\pi - \Psi) = -\frac{\pi}{2} \Rightarrow \Psi = \frac{\pi}{4} \tag{4b}$$

The π refocusing pulse is pulled out of $(\Psi + \pi)_x$ to yield

$$B' : (\Psi)_x^I - \tau - (\pi)_x^{I,S} - \tau - (\pi - \Psi)_x^I$$

So far we have implied $\pi/2$ rotations but the general sequence B' also allows an easy derivation of a π rotation according to a modified version of eqn. 4:

$$\theta = -2\Psi = \pi \Rightarrow \Psi = \pm\frac{\pi}{2} \qquad (5)$$

That leads to the sequence

$$C : \left(\frac{\pi}{2}\right)^I_x - \tau - (\pi)^{I,S}_x - \tau - \left(\frac{\pi}{2}\right)^I_x$$

which is commonly referred to as BIRD (9). Note that the only difference between sequences A and C is the interpulse delay resulting in $\pi/2$ and π rotations, respectively.

The opposite situation of rotating isolated I spin systems while not affecting or doing something different to IS systems also occurs. Sequences for that purpose can easily be derived from those above.

Sequence A corresponds to a 2π rotation for isolated I spins so, for example, a $(\pi/2)_y$ pulse can be appended for excitation. That turns the antiphase IS magnetization to the z axis; this antiphase character of the IS z magnetization is normally of no concern.

The resulting sequence may be simplified according to

$$e^{-i\frac{\pi}{2}I_x}e^{-i\frac{\pi}{2}I_y}e^{i\frac{\pi}{2}I_z} = e^{-i\frac{\pi}{2}I_y} \qquad (6)$$

where the z rotation was added for simplification purposes. Combined with sequence A we obtain:

$$D : \left(\frac{\pi}{2}\right)^I_x - \frac{\tau}{2} - (\pi)^{I,S}_x - \frac{\tau}{2} - \left(\frac{\pi}{2}\right)^I_y$$

A π rotation of the isolated I spin is obtained by adding a $(\pi)_{-x}$ I pulse to sequence A:

$$E : \left(\frac{\pi}{2}\right)^I_x - \frac{\tau}{2} - (\pi)^{I,S}_x - \frac{\tau}{2} - \left(\frac{\pi}{2}\right)^I_{-x}$$

This sequence results in transverse antiphase IS magnetization (as does sequence A) which might or might not represent a problem depending on the application.

The IS spin systems can be left invariant if the opposite sequences are based on sequence B and C instead of sequence A. Then $\pi/2$ and π rotations are

$$F : \left(\frac{\pi}{4}\right)^I_x - \tau - (\pi)^{I,S}_x - \tau - \left(\frac{3\pi}{4}\right)^I_{-x}$$

and

$$G : \left(\frac{\pi}{2}\right)^I_x - \tau - (\pi)^{I,S}_x - \tau - \left(\frac{\pi}{2}\right)^I_{-x}$$

respectively. Another sequence related to F acts as a $(\pi)^I$ pulse in IS systems:

$$F' : \left(\frac{\pi}{4}\right)^I_x - \tau - (\pi)^{I,S}_x - \tau - \left(\frac{\pi}{4}\right)^I_x$$

Sequence G is used as part of the preparation sequence in multidimensional NMR experiments where a subsequent delay ensures that the magnetization of non-hetero-labeled molecules largely vanishes at the starting point of the actual experiment (10). It has also been used as a refocusing pulse in evolution periods (11, 12).

For selective excitation of isolated I spin systems as part of a preparation period sequence D offers itself. However, a better approach is based on low-pass J filtering (13) because the spins of interest experience less pulses. Simple first order low-pass J filters are

$$G : \left(\frac{\pi}{2}\right)^I - \tau - \left(\frac{\pi}{2}\right)^S_{\pm x}$$

$$G' : \left(\frac{\pi}{2}\right)^I - \Delta - (\pi)^S$$

where the two experiments indicated for G are co-added while G' requires coaddition of two experiments with $\Delta = \tau = (2J_{IS})^{-1}$ and $\Delta = 0$, respectively. Alternatively, pulsed field gradients can be combined with sequence G in order to effect the filter in a single step.

In connection with mixing processes in multidimensional experiments the most useful sequence is A. It can, for example, be employed for coherence transfer from ^{15}N to the directly bonded protons while leaving the α protons unperturbed (14), a crucial feature in experiments for measurement of J coupling constants via E.COSY type multiplet patterns (15-17).

We return to the sequences of this section in Section III covering small-angle bilinear rotations.

III. Selective Rotations where None are Possible

This self-contradictory title covers the situations where no J couplings can be exploited to differenti-

ate between spins that consequently all experience the same perturbation. Nevertheless it is still possible to obtain an apparent selectivity by employing a small-angle rotation or by suppressing the spectral traces of lack of selectivity. These tricks are only of interest in connection with mixing processes and are completely irrelevant for preparation sequences.

The concept of small-angle rotations is intimately connected with the notion of active and passive spins. For given coherences, active spins are those that are transverse before or after (including before *and* after) the mixing process whereas passive spins are longitudinal in both periods surrounding the pertinent mixing sequence.

As far as active spins are concerned, variation in the rotation angle fundamentally causes an amplitude modulation. On the other hand, passive spins either stay in the spin state they are in or get inverted which is expressed by the intensity distribution within multidimensional multiplets. Small rotation angles leave passive spins largely unperturbed (18,19) thus emphasizing the multiplet components corresponding to preserved spin states of passive spins. However, perfect preservation of spin states only occurs in the limit of a vanishing perturbation where also the amplitude factor from the active spins vanishes except for the trivial case of diagonal peaks.

The E.COSY and TR (20) techniques are the outcome of the challenge to obtain multiplets corresponding to 100% preservation of passive spin states while maintaining significant amplitude factors for the active ones. However, in particular in connection with heteronuclear NMR satisfactory performance is obtained by just applying a small-angle pulse.

Small-angle bilinear rotations have been employed in homonuclear proton NMR (21,22) but so far only one application (23) of small-angle heteronuclear bilinear rotations based on sequences of the type described in the preceding section has appeared. They are relevant in connection with molecules containing more than one heteronuclear spin isotope of the same type, like for example ^{13}C-labeled proteins.

A small-angle version of sequence A is

$$
A_\beta : \left(\frac{\pi}{2}\right)^I_x - \frac{\tau_\beta}{2} - (\pi)^{I,S}_x - \frac{\tau_\beta}{2} - \left(\frac{\pi}{2}\right)^I_x
$$

where the small angle comes from a short delay τ_β.

Sequence A_β does not have an opposite equivalent because the major part of IS spin systems behave as isolated I spin systems for short τ_β delays. In contrast, both versions are possible by modifications of sequence B because here the interpulse delay must be constant and the small angles β are obtained by adjustment of pulse angles.

Analytical derivations are possible based on the equivalents of eqns. 3 and 4:

$$
\theta = -2\Psi = \beta \Rightarrow \Psi = -\frac{\beta}{2} \qquad (7)
$$

That results in the sequence

$$
H : \left(\frac{\beta}{2}\right)^I_x - \tau - (\pi)^{I,S}_x - \tau - \left(\pi - \frac{\beta}{2}\right)^I_x
$$

which effects a β pulse in the IS spin systems while leaving the isolated I spins invariant. Another sequence that rotates by $(\pi - \beta)$ in IS spin systems but inverts isolated I spins follows immediately from sequence H:

$$
H' : \left(\frac{\beta}{2}\right)^I_x - \tau - (\pi)^{I,S}_x - \tau - \left(\frac{\beta}{2}\right)^I_{-x}
$$

The opposite sequences where the isolated I spins experience a β pulse can in analogy to eqn. 3 and sequence B' be written on the general form

$$
I : (\Psi)^I_x - \tau - (\pi)^{I,S}_x - \tau - (\pi - \Psi + \beta)^I_x
$$

Because the flip angle for the isolated I spins is invariant to Ψ the flip angle in the IS spin systems can be selected independently in analogy to eqn. 4:

$$
\theta = \beta - 2\Psi \qquad (8)
$$

For $\theta = 0$ and π, respectively, we obtain the sequences

$$
J : \left(\frac{\beta}{2}\right)^I_x - \tau - (\pi)^{I,S}_x - \tau - \left(\pi - \frac{\beta}{2}\right)^I_{-x}
$$

and

$$
J' : \left(\frac{\pi - \beta}{2}\right)^I_x - \tau - (\pi)^{I,S}_x - \tau - \left(\frac{\pi - \beta}{2}\right)^I_x
$$

All sequences with a total interpulse delay $2\tau = (J_{IS})^{-1}$ described so far in the paper are special cases of sequence I and could have been derived from that one using eqn. 8. It should also be mentioned that phase shifts have been ignored because they normally are immaterial; for example sequence J is not an invariant operation in IS spin systems but rather a $(\pi)_z$ rotation. Finally, we suggest that the small angle or β-TANGO sequences be referred to as BANGO.

IV.   Discussion and Conclusions

The paper has described pulse sequences for selective rotations where dominant one-bond heteronuclear coupling constants are responsible for the selectivity. It has been shown how such sequences are derived; originally the simple vector model with rotations of vectors in three-dimensional space was employed and this level of sophistication is still completely adequate for all sequences above.

A novel general class of pulse sequences were introduced:

$$\left(\frac{\beta^{I} - \beta^{IS}}{2}\right)^{I}_{x} - \tau - (\pi)^{I,S}_{x} - \tau - \left(\pi + \frac{\beta^{I} + \beta^{IS}}{2}\right)^{I}_{x}$$

β^{I} and β^{IS} are the desired flip angles for isolated I spin systems and IS spin systems, respectively, and τ is $(2J_{IS})^{-1}$.

Selective pulse sequences of the type described in this paper have been used mainly as part of preparation periods in multidimensional experiments but it seems that their potential in mixing sequences has not been fully exploited in particular in connection with experiments generating E.COSY type multiplet patterns.

V.   References

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