Pergamon

Tetrahedron Letters 40 (1999) 2025–2028

TETRAHEDRON LETTERS

Adaptation of an NMR Signal Suppression Pulse Sequence for the Selective Removal of Benzylic Methylene Signals of Benzyl Ether-Protected Carbohydrates[1]

Trevor J. Rutherford, K.P. Ravindranathan Kartha, S. Kristy Readman, Peter Cura and Robert A. Field

School of Chemistry, Biomolecular Sciences Building, University of St Andrews, St Andrews, Fife KY16 9ST, U.K.

Received 10 December 1998; accepted 7 January 1999

Abstract: Application of the DPFGSE-TOCSY pulse sequence permits the selective removal of benzylic signals from the ^{1}H NMR spectra of benzyl ether-protected glycosides. Anomeric proton signals, which have a similar chemical shift, can be readily re-introduced with the aid of a TOCSY sequence. This approach is useful in cases where intense spectral overlap does not permit the straightforward identification of anomeric signals, and hence definition of anomeric configuration or determination of anomeric ratios. The representative application of this pulse sequence to analysis of iodine-promoted thioglycoside activation is reported.

Assignment of the anomeric configuration of sugar units in protected oligosaccharides can present major difficulties in carbohydrate chemistry. This problem often arises from a lack of spectral dispersion in one dimensional proton NMR spectra. In particular, the methylene signals of benzyl ether protecting groups often overlap with those of anomeric protons. Clearly one might consider the use of selectively deuterated benzyl group precursors to overcome this problem, but this represents a rather long-winded process for routine application. Herein we describe a modification of the excitation sculpting NMR pulse sequence,[2] commonly used for suppression of solvent signals, that permits the selective removal of benzyl group methylene signals followed by re-introduction of masked anomeric signals of similar chemical shift with the aid of a TOCSY sequence. The application of this combined pulse sequence in studies on thioglycoside activation is reported.

The double-pulsed field gradient spin-echo (DPFGSE) pulse sequence is routinely used for suppressing the solvent resonance line in non-deuterated aqueous solution.[2] Use of the scheme was recently demonstrated for efficient excitation sculpting in one-dimensional coherence transfer experiments.[3,4] Band-selective 1D-TOCSY experiments are particularly effective for resolving overlap in highly crowded NMR spectra, as exemplified for carbohydrates,[4,5] in which magnetisation is transferred from a selectively excited carbohydrate ring proton to other protons within the same residue. Here we adopt the alternative strategy of selective suppression of the non-carbohydrate benzylic protons.

0040-4039/99/$ - see front matter
PII: S0040-4039(99)00108-2

The DPFGSE-TOCSY scheme (Figure 1) consists of two elements in which a band-selective inversion pulse and a non-selective inversion pulse are applied between two identical pulsed field gradients. Any signal that is inverted by the selective pulse is efficiently suppressed by the filter, but can be restored by transferring magnetisation from J-coupled spins that are not suppressed. For per-benzylated saccharides the selective pulse inverts signals from a band of frequencies encompassing the benzylic methylene proton resonances. The signals from anomeric protons are restored by a TOCSY spin-lock period, providing that they are J-coupled to a proton spin that is not suppressed by the filter. The benzylic methylene protons are not J-coupled to any protons resonating outwith the suppressed region and, therefore, are not restored. The major advantage of this filtration technique over other (two-dimensional) methods of resolving overlapping ^{1}H resonances is the short total acquisition time; the time required is identical to that for an unfiltered one-dimensional experiment. The technique is therefore readily applicable to the analysis of reaction kinetics.[6]

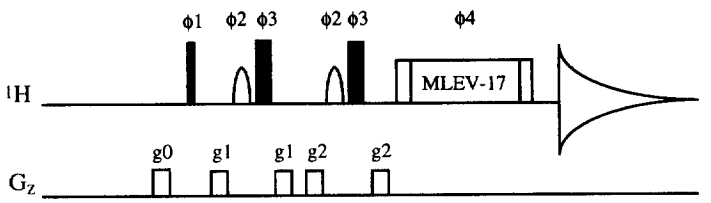

Figure 1: DPFGSE-TOCSY pulse scheme. Narrow bars represent 90° pulses and wide bars indicate 180° pulses. Shaped bars represent selective inversion pulses. The strength of all gradient pulses was 9 G/cm, with durations as follows: g0=5.0 ms, g1=0.1 ms, g2=2.0 ms. MLEV-17 spin-lock in a 10 kHz field was applied for 70 ms, with 2.5 ms trim pulses. Receiver phase=ϕ1=y,-y,-x,x; ϕ2=8x,8-x; ϕ3=8-x,8x; ϕ4=y,y,-x,-x,-y, -y,x,x.

For some time we have been studying the activation of thioglycosides and glycosyl halides using iodine-based reagents.[1] With a view to improving the stereoselectivity of such processes, we have undertaken a mechanistic study of the iodine-promoted activation of thioglycosides. We recently noted that the iodine-promoted activation of benzylated methyl thioglycosides can be sufficiently slow that it can be monitored effectively by NMR spectroscopy.[7] However, a major complicating factor in such studies arises from spectral overlap of signals for anomeric protons of interest and benzylic methylene signals of benzyl ether protecting groups. Although anomeric ^{1}H resonances are often resolved from the methylene signals of a per-benzylated reactant, during a reaction time-course overlap of H-1 with methylene proton signals from intermediates and products is inevitable (Figure 2a). By suppressing the signals of all benzyl methylene protons it is possible to resolve the H-1 signals of both the β-thioglycoside starting material (4.62 ppm) and the α-glycoside product (5.12 ppm)(Figure 2b). Progress of the iodine-promoted formation of per-benzylated methyl α-D-galactopyranoside could

therefore be monitored from the change in H-1 peak intensity of both starting material and product (Figure 3).[8]

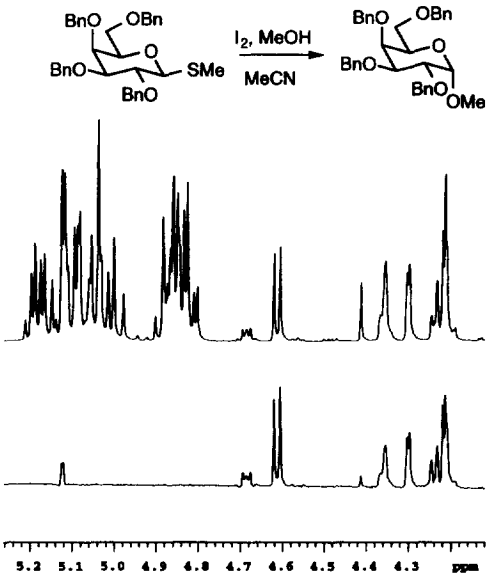

Figure 2: (a) unedited ^{1}H NMR spectrum of per-benzylated methyl thiogalactoside in $C^{2}H_3CN$, in the presence of iodine (both α-galactoside and β-thiogalactoside are present). (b) DPFGSE-TOCSY spectrum with filtration of the benzyl methylene signals, clearly resolving the anomeric proton resonances. Selective inversion was achieved using a 2.1 ms I-BURP-1 pulse.[11]

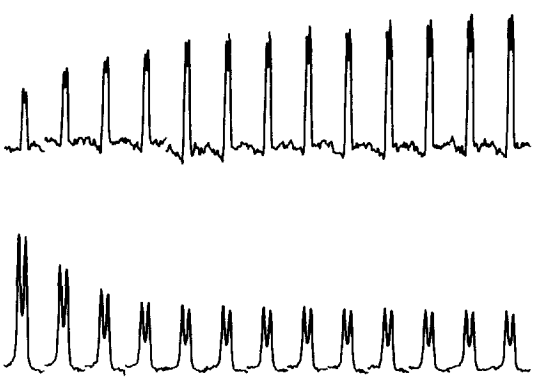

Figure 3: Representative time course (five minute intervals) demonstrating iodine-promoted methanolysis of methyl β-thiogalactoside. The signal intensities are shown for (a) the α-galactoside H-1 and (b) the β-thiogalactoside H-1.

In summary, we have used the DPFGSE-TOCSY pulse sequence for the selective filtration of benzylic signals from complex one dimensional ^{1}H NMR spectra of benzyl ether-protected glycosides. This approach is useful in cases where intense spectral overlap does not permit the straightforward identification of anomeric signals. Herein we report the application of this pulse sequence to the 'real time' analysis of iodine-promoted thioglycoside activation. Further exploitation of this sequence in studies on iodine-promoted glycosylation chemistry will be reported in due course.[7]

Acknowledgements:

We thank the Wellcome Trust (Grants 042472 and 040331), Pfizer and the University of St Andrews for financial support.

References:

1. Iodine and its' Interhalogen Compounds: Useful Reagents in Carbohydrate Chemistry X. For part IX see: Kartha, K.P.R.; Field, R.A. *Synlett,* in press.
   Further information about the NMR studies reported herein can be obtained from TJR at : School of Chemistry, The University of Birmingham, Edgbaston, BIRMINGHAM B15 2TT, e-mail: t.j.rutherford@bham.ac.uk
2. Hwang, T. L.; Shaka, A. J. *J. Magn. Reson., Ser. A,* **1995**, *112*, 275.
3. Xu, G.; Evans, J. S. *J. Magn. Reson. B,* **1996**, *111*, 183.
4. Gradwell, M. J.; Kogelberg, H.; Frenkiel, T. A. *J. Magn. Reson.,* **1997**, *124,* 267.
5. Uhrín, D.; Barlow, P. N. *J. Magn. Reson.,* **1997**, *126*, 248.
6. It should be noted that the peak integrals are dependent upon the efficiency of the spin-lock period, and are not an accurate measure of the relative concentration of separate signals. However, providing the pulse width calibration is unaffected by the changing sample conditions, any change of signal intensity is a quantitative measure of the change in concentration. The duration of a 90° pulse is a function of the magnetic susceptibility of the solvent, (which is affected by pH and ionic strength) but in this transformation the pulse width calibration before and after the experiment were identical.
7. Cura, P.; 1997, PhD thesis, University of St Andrews. Kartha, K.P.R.; Aloui, M.; Cura, P.; Readman, S.K.; Rutherford, T.J.; Field, R.A., manuscript in preparation.
8. Glycosyl iodide formation was not observed in these experiments (α-iodide H-1 expected as a doublet at approx. 7.0ppm).[9] However, thioglycoside epimerisation[10] was evident; the prevalence of this process was solvent dependent. Further details will be reported in due course.[6]
9. For the corresponding α-glucopyranosyl iodide see: Gervay, J.; Nguyen, T.N.; Hadd, M.J. *Carbohydr. Res.*, **1997**, *300*, 119.
10. Boons, G.J.; Stauch, T. *Synlett*, **1996**, 906.
11. Geen, H.; Freeman, R. *J. Magn. Reson.,* **1993**, *93*, 93.