ELSEVIER

Carbohydrate Research 263 (1994) 155–161

CARBOHYDRATE
RESEARCH

Note

Application of the ^{29}Si NMR 2D INEPT spin-flip *J*-resolved technique to the structural analysis of trimethylsilylated glycosides

Mouna Harket [a], Bernard De Jeso [a], Jean-Claude Lartigue [a],
Michel Petraud [b], Max Ratier [c,*]

[a] *Institut du Pin, Université Bordeaux I, 351 cours de la Libération, F-33405 Talence, France*
[b] *Centre d'Etude Structurale et d'Analyse des Molécules Organiques, Université Bordeaux I, 351 cours de la Libération, F-33405 Talence, France*
[c] *Laboratoire de Chimie Organique et Organométallique, URA 35 CNRS, Université Bordeaux I, 351 cours de la Libération, F-33405 Talence, France*

Received 2 November 1993; accepted in final form 6 May 1994

Keywords: ^{29}Si NMR; 2D INEPT spin-flip *J*-resolved technique; Trimethylsilylated glycosides

The trimethylsilylation technique has been shown to be a well-established derivatization method for hydroxyl groups in carbohydrate chemistry [1]. However, two major difficulties have been encountered in analytical applications involving ^{29}Si NMR determinations, due to the inherent low sensitivity of the ^{29}Si nucleus [2] and the need for complete and unambiguous assignment of ^{29}Si spectra.

Various empirical and experimental methods have been developed in order to improve the sensitivity and solve the assignment problems. While empirical rules have been inferred from known variations in ^{29}Si chemical shifts with substituent effects [3], experimental approaches involve specific deuteriations, selective proton decoupling experiments, shift reagents studies [4], refocused INEPT experiments [5], modified INDOR [6] or INADEQUATE [7] experiments, and two-dimensional heteronuclear shift correlations [8].

Two-dimensional methods offer many advantages, both in terms of efficiency and precision, over conventional one-dimensional approaches. Nevertheless, diffi-

---

* Corresponding author.

SSDI 0008-6215(94)00163-A

culties in performing 2D ^{29}Si–^{1}H correlated spectra result from strong cross-peaks correlating the proton and silicon nuclei within each trimethylsilyl group and small cross-peak intensities arising from other protons in the ring.

In order to circumvent this drawback, we have investigated the feasibility of the 2D INEPT spin-flip *J*-resolved technique [9], associated with computer simulations of the spectra [10]. The proposed method precludes the prerequisite tedious step of assigning ^{1}H NMR multiplets by the usual combination of ^{1}H–^{1}H homonuclear and ^{1}H–^{13}C heteronuclear correlations [11], and can yield valuable information for identification and conformational analysis in a single experiment.

We have chosen to study a series of model methyl per(trimethylsilyl)-glycosides in order to eliminate problems arising from possible anomerisation during the per(trimethylsilylation) process. Experiments were carried out on methyl 2,3,4,6-tetra-*O*-trimethylsilyl-α-D-mannopyranoside (**1**), methyl 2,3,4,6-tetra-*O*-trimethylsilyl-α-D-glucopyranoside (**2**), and methyl 2,3,4,6-tetra-*O*-trimethylsilyl-β-D-galactopyranoside (**3**). All pyranose rings have been found to occur in flattened chair conformations [12].

The decoupled ^{29}Si NMR spectra of compounds **1**–**3** are limited to the trivial determination of the number of different trimethylsilyl groups, unless the ^{29}Si chemical shifts can be assigned to each of the four trimethylsilyl groups. Such information, however, is rapidly obtainable from slices of the *J*-resolved 2D INEPT contour plot containing the four ^{29}Si–^{1}H coupled spectra (Fig. 1). Experimental spectra were assigned by comparison of observed patterns for each ^{29}Si with calculated spectra.

We have recently developed a computer program based on the general INEPT equation of Schenker and von Philipsborn [13], involving combinations of the different ^{1}H–X (X spin 1/2) polarization transfer contributions and intensities modulations via homonuclear couplings according to τ values. It became apparent that the general shape of each INEPT spectrum may depend on the $^{3}J_{\mathrm{H,H}}$ values of vicinal protons close to each silicon atom involved. Thus, in order to recognize the influence of J_{aa}, J_{ae}, and J_{ee} couplings on INEPT spectra, we carried out a series of simulations for various values of literature coupling constants [14]. A good agreement between computed and experimental spectra indicates the spacial arrangement around silicon atoms and the corresponding chemical shifts. A significant modification of the general shape between simulated and experimental spectra could be observed after varying ^{29}Si–^{1}H or ^{1}H–^{1}H coupling constants by ± 0.3 or ± 0.5 Hz respectively, within the 0–10 Hz range. This precision is adequate for structural determination.

The analytical potential of the method is described for **1**, for which the direct environment of C-4, the carbon atom under investigation, is highlighted and the trimethylsilyl residue under consideration italicised (Fig. 1).

As expected from the INEPT equation, modulation intensities of the Si_4 multiplet components of the Si_4–H^n correlated system are proportional to $\Sigma^p \sin[\pi\tau J(\mathrm{Si}_4, \mathrm{H}^p) \Pi^{q(q \neq p)} \cos[\pi\tau J(\mathrm{H}^p, \mathrm{H}^q)]$ where p and q run over the indices of the n proton nuclei. The enhancement factor due to the $^{3}J(^{29}\mathrm{Si}_4\mathrm{OC}^{1}\mathrm{H})$ contribution appears to be very dependent on the cosine term, providing antici-

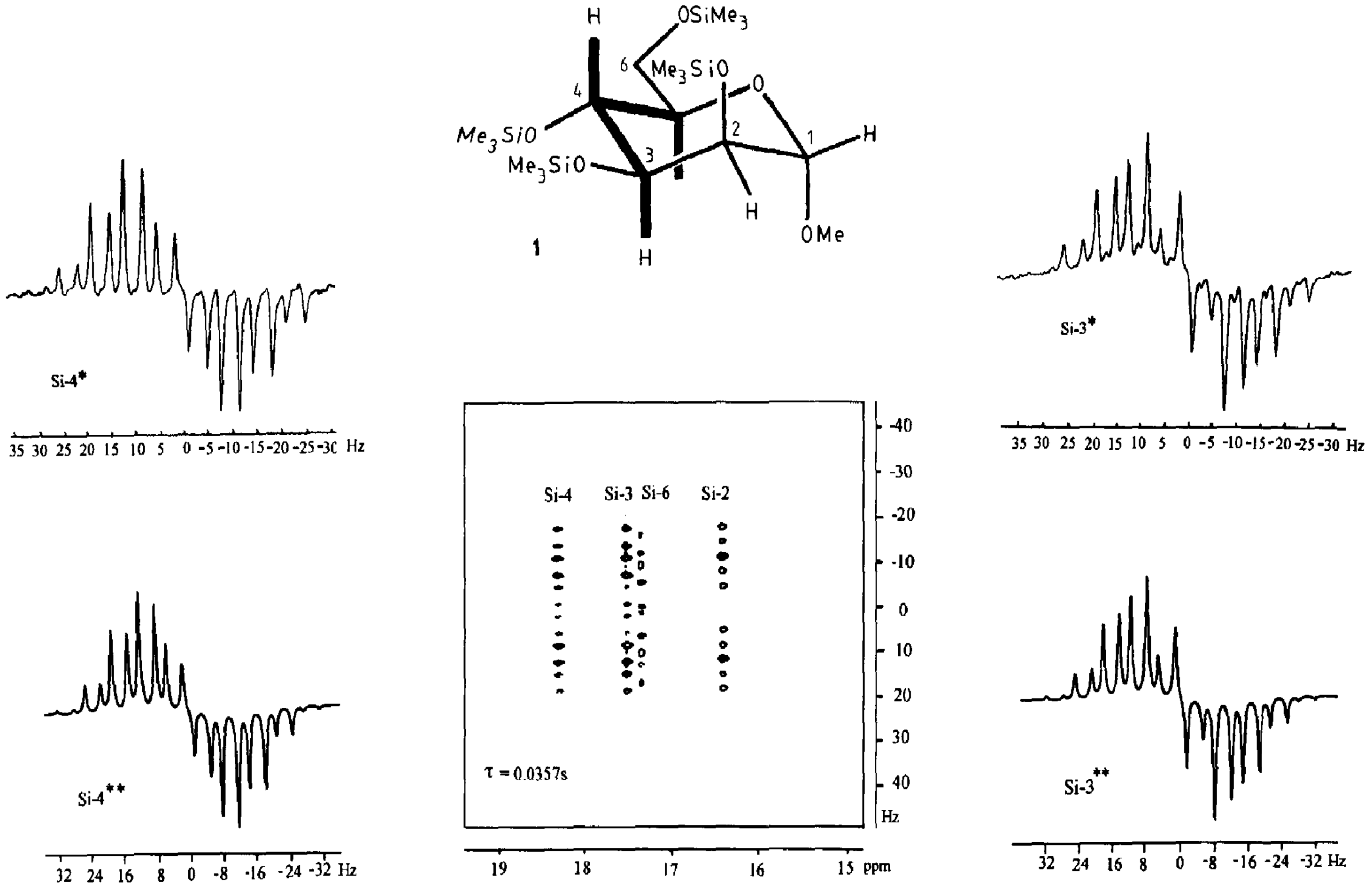

Fig. 1. ^{29}Si NMR assignment of methyl 2,3,4,6-tetra-*O*-trimethylsilyl-α-D-mannopyranoside 1 by the 2D INEPT spin-flip *J*-resolved technique: experimental * and simulated ** INEPT spectra for $\tau = 0.0357$ s.

pated modifications in the shape of the spectrum according to τ values (0.0357 and 0.06 s).

The $^{2}J(^{29}Si_{4}C^{1}H)$ coupling constant of the silicon atom, with the nine equiva-

Table 1

Configuration of the trimethylsilyl groups in methyl 2,3,4,6-tetra-*O*-trimethylsilyl-α-D-mannopyranoside **1**, methyl 2,3,4,6-tetra-*O*-trimethylsilyl-α-D-glucopyranoside **2**, methyl 2,3,4,6-tetra-*O*-trimethylsilyl-β-D-galactopyranoside **3**, and trimethylsilyl 2,3,6,2′,3′,4′,6′-hepta-*O*-trimethylsilyl-α-lactoside **4**, according to vicinal $^{1}H-^{1}H$ coupling constants values obtained by 2D INEPT spin-flip *J*-resolved experiments, for $\tau = 0.0357$ and 0.06 s.

		δ_{ppm}	$^{2}J_{Si,H}$ [a]	$^{3}J_{Si,H}$	J_{gr2-3} [b]	J_{gr2-4}	Typical H–H [c] orientations
1	Si-2	16.4	6.7	3.3	2.6	1.7	*e–a*
							e–e
	Si-3	17.5	6.6	3.9	9.1	2.6	*a–a*
							a–e
	Si-4	18.3	6.6	3.9	9.1	9.1	*a–a*
	Si-6	17.3	6.7	3.7/4.3	−11.4	1.7	
2	Si-2	16.8	6.7	3	9	3.5	*a–a*
							a–e
	Si-3	17.6	6.7	3.5	8.6	9	*a–a*
	Si-4	18	6.6	3.8	8.5	8.6	*a–a*
	Si-6	17.8	6.6	3.2/3.2	−11.5	3.5	
3	Si-2	18.7	6.6	3.4	9.2	7.4	*a–a*
	Si-3	17.7	6.6	4.2	2.7	9.2	*a–a*
							a–e
	Si-4	16.6	6.6	3	1	2.7	*e–a*
	Si-6	18.1	6.6	2.4/2.1	−9.5	5.3	
4	Si-1	17.9	6.8	4	3	–	*e–a* [d]
	Si-2	17.2	6.8	3.9	3	8.5	*a–e*
							a–a
	Si-3	16.7	6.7	3.7	8.5	8.8	*a–a*
	Si-6	17.6	6.6	2.5/2.5	−11.1	2.7	
	Si-2′	18.5	6.8	4	7.7	9	*a–a*
	Si-3′	17.8	6.8	4	9	2.6	*a–a*
							a–e
	Si-4′	15.5	6.6	3	0.7	2.6	*e–a*
							e–a
	Si-6′	17.7	6.7	2.7/2.7	−9.6	5	

[a] $J_{Si,H} = 0.1$ Hz; $^{4}J_{Si4,H} = 0.1/0.8$ Hz (**3**); $^{4}J_{Si4',H} = 0.1/1.1$ Hz.

[b] Arbitrary numbering of the direct environment of silicon atoms in the program (gr, abbreviation for group). All vicinal coupling constants were taken as positive and geminal coupling constants $J_{6,6}$, negative [15]. $J_{H,H}$(gr1–2/1–3/1–4/3–4) = zero; $J_{H,H}$(gr3–4) = 4, 1.6 and 7.8 Hz for **1**, **2**, **3** respectively and 3 and 7.7 Hz for Si-6 and Si-6′ in **4**.

[c] Si-3/Si-4 INEPT spectra in **2**, Si-2/Si-3′ and Si-3/Si-2′ in **4** are strictly identical and cannot be assigned unequivocally (a complete assignment of the ^{29}Si spectrum of **2** has been obtained by selective deuteriation [4]).

[d] One small coupling constant value: the INEPT spectrum is identical with that obtained for two small coupling constant values.

lent methyl protons and the $^3J(^{29}Si_4OC^1H)$ long-range coupling with H-4, have been directly measured from the spectrum (^2J 6.6 Hz, ^3J 3.9 Hz). Long-range couplings with protons H-3 and H-5 cannot be measured (low J value), but a very low ^4J value ($\neq 0$) must be introduced for the computer procedure [^4J 0.1 Hz].

Computed spectra were similar to those obtained experimentally for $^3J_{H,H}$ 9 and 7 Hz for different τ values (0.0357 and 0.06 s). Hence, the Si-4 trimethylsilyl group under consideration (Fig. 1) is close to H-3–H-4 and H-4–H-5 protons in *axial–axial* orientations. The ^{29}Si chemical shift assignment is immediately inferred from the contour plot. The Si-6 experimental INEPT spectrum is not exactly superimposable to its simulation. This low distorsion could be attributed to the appearence of a second-order phenomenon. The whole molecule can be gradually assigned in this way.

The procedure allows the assignment of the configuration of the trimethylsilyl groups in the *manno, gluco*, and *galacto* derivatives **1–3** (Table 1) via J_{aa}, J_{aa} (two high $J_{H,H}$ values, 7 to 9.3 Hz), J_{ae}, J_{ae}, or J_{ae}, J_{ee} (two small $J_{H,H}$ values, 1 to 2.5 Hz), and J_{aa}, J_{ae} (high and small $J_{H,H}$ values, 1.8 to 9 Hz). A characteristic splitting in the Si-4 INEPT spectrum is observed in the galactopyranoside derivative **3**, due to a specific long-range $^4J(Si_4OCH_3)$ coupling. The origin of this coupling has been determined from the ^{29}Si–^{1}H correlated spectrum, following a method previously described for the determination of ^{119}Sn–^{1}H coupling constants [10]. Nevertheless, the method is of limited applicability when vicinal protons have identical coupling constants. Such a limitation was evident in the glucopyranoside derivative **2** for Si-3 and Si-4.

The results available so far prompted us to extend the same approach to model per(trimethylsilylated) disaccharides. Assignments of resonances of trimethylsilyl hepta-*O*-trimethylsilyl-α-lactoside (**4**) serves as a typical example.

A good fit was found between simulated and experimental spectra for computed data listed in Table 1. Identification of the silicon signals is based on the general and characteristic shapes of the INEPT spectra described for typical H-H orientations. Thus, reference to Table 1 leads to four types of different silicon sites [Si-1 (*ae*), Si-2 and Si-3′ (*aa/ae*), Si-3 and Si-2′ (*aa/aa*), Si-4′ (*ea/ea*) with $^4J(Si_{4'}–H_{3'})$ long-range coupling]. Si-6 and Si-6′ INEPT spectra are easily assigned from the distinct and characteristic J_{HH} literature values [15] affecting the corresponding INEPT shapes.

1. Experimental

Trimethylsilylations were carried out using a standard procedure [16]. All the experiments were performed on a Bruker AC 200 spectrometer (^{1}H, 200.132 and ^{29}Si, 39.761 MHz.), fitted with an Aspect 3000 data system using a 10-mm broad-band probe (^{29}Si 90° pulse width, 15 μs, ^{1}H 90° pulse width through the decoupler coil, 29 μs). ^{29}Si spectra were recorded at room temperature as a 30% (w/v) solution in C_6D_6 for compounds **1–3** and acetone-d_6 for **4**.

The coupling constants were measured by the heteronuclear ^{1}H–^{29}Si J-resolved

2D INEPT technique [9], in the phase sensitive mode, using the sequence $(90°_x\text{H})-\tau-(180°_x\text{H})(180°_x\text{Si})-\tau-(90°_y\text{H})(90°_x\text{Si})-t_1/2-(180°_x\text{H})(180°_x\text{Si})-t_1/2-$(acquisition in TPPI mode, with proton decoupling).

The spectrum was recorded with spectral widths of 355 + / − 50 Hz in the F_2 and F_1 dimension respectively. The initial $S(t_1 t_2)$ data matrix of each experiment consisted of 2048 × 512 W. The interferograms in the t_1 dimension were zero-filled to 1024 W, before Fourier transformation with respect to t_1. The t_2 data were transformed after being exponentially weighted using a line broadening factor of 2 Hz over 2K points. The t_1 interferograms were multiplied with a $\pi/4$ shifted sine bell squared function and Fourier transformed over 1024 W points. A total of 16 transients was accumulated for the 512 increments of t_1. The relaxation delay was 1 s, $\tau = 1/4J = 0.0357$ s.

Non-refocused INEPT spectra were simulated on an AT type computer using the program previously described for ^{119}Sn [10].

Acknowledgments

We are indebted to the CNRS and the "Conseil Régional d'Aquitaine" for financial support.

References

[1] K.M. Brobst, *Methods Carbohydr. Chem.*, 6 (1972) 3–8;
C.C. Sweeley, R. Bentley, M. Makita, and W.W. Wells, *J. Am. Chem. Soc.*, 85 (1963) 2497–2507.
[2] C. Brevard and P. Granger, *Handbook of High Resolution Multinuclear NMR*, Wiley, New York, 1982, p 100;
P.C. Lauterbur, in F.C. Nachod and W.D. Phillips (Eds.), *Determination of Organic Structure by Physical Methods*, Academic Press, New york, Vol. II, 1982, p 465.
[3] J. Schraml, J. Pola, H. Jancke, G. Engelhardt, M. Černý, and V. Chvalovský, *Collect. Czech. Chem. Commun.*, 41 (1976) 360–367;
J. Schraml, P. Koehler, K. Licht, and G. Engelhardt, *J. Organomet. Chem.*, 121 (1976) C1–C3;
D.J. Gale, A.H. Haines, and R.K. Harris, *Org. Magn. Reson.*, 7 (1975) 635–636;
J. Schraml, E. Petráková, O. Pihar, J. Hirsch, and V. Chvalovský, *ibid.* 21 (1983) 666–669;
J. Schraml, J. Včelák, M. Černý, and V. Chvalovský, *Collect. Czech. Chem. Commun.*, 48 (1983) 2503–2508;
J. Schraml, S. Kučar, J. Zelený, and V. Chvalovský, *ibid.*, 50 (1985) 1176–1183;
J. Schraml, M.F. Larin, and V.A. Pestunovich, *ibid.*, 50 (1985) 343–347;
D.J. Gale and N.A. Evans, Org. Magn. Reson., 21 (1983) 567–569.
[4] A.H. Haines, R.K. Harris, and R.C. Rao, *Org. Magn. Reson.*, 9 (1977) 432–437.
[5] J. Schraml, J. Magn. Reson., 59 (1984) 515–517; J. Schraml, *Collect. Czech. Chem. Commun.*, 48 (1983) 3402–3406.
[6] J. Schraml, M.F. Larin, and V.A. Pestunovich, *Collect. Czech. Chem. Commun.*, 50 (1985) 343–347.
[7] J. Past, J. Puskar, M. Alla, E. Lippmaa, and J. Schraml, *Mag. Reson. Chem.*, 23 (1985) 1076–1079.
[8] E. Liepinsh, I. Sekaciş and E. Lukevics, *Magn. Reson. Chem.*, 23 (1985) 10–11;
J. Schraml, E. Petráková, J. Pelnař, M. Kvíčalová, and V. Chvalovský, *J. Carbohydr. Chem.*, 4 (1985) 393–403;

R. Nardin and M. Vincendon, *J. Magn. Reson.*, 61 (1985) 338–342;
J. Schraml, E. Petráková, and J. Hirsch, *Magn. Reson. Chem.*, 25 (1987) 75–79;
J. Schraml, E. Petráková, J. Hirsch, J. Čermák, V. Chvalovský, R. Teeäär, and E. Lippmaa, *Collect. Czech. Chem. Commun.*, 52 (1987) 2460–2473.

[9] D.M. Thomas, M.R. Bendall, D.T. Pegg, D.M. Doddrell, and J. Field, *J. Magn. Reson.*, 42 (1981) 298–306;
W.E. Hull, in W.R. Croasmun and R.M.K. Calson (Eds.), *Two Dimensional NMR Spectroscopy Applications for Chemists and Biochemists*, VCH, 1987, pp 169–178.

[10] V. Gouron, B. Jousseaume, M. Ratier, J.C. Lartigue, and M. Petraud, *Magn. Reson. Chem.*, 28 (1990) 755–758;
M. Ratier, B. Jousseaume, N. Noiret, N. Petit, J.C. Lartigue, and M. Petraud, *Magn. Reson. Chem.*, 31 (1993) 176–181.

[11] J. Schraml, J. Hirsch, E. Petráková, E. Krahé, and C. Bliefert, *Collect. Czech. Chem. Commun.*, 49 (1984) 1605–1616.

[12] D.G. Streefkerk, M.J.A. de Bie, and J.F.G. Vliegenthart, *Tetrahedron*, 29 (1973) 833–844;
J.C.P. Schwarz, *Chem. Commun.*, (1973) 505–508.

[13] K.V. Schenker and W. von Philipsborn, *J. Magn. Reson.*, 61 (1985) 294–305.

[14] G. Kotowycz and R.U. Lemieux, *Chem. Rev.*, 73 (1973) 669–698;
D.G. Streefkerk, M.J.A. de Bie, and J.F.G. Vliegenthart, *Tetrahedron*, 29 (1973) 833–844.

[15] L.D. Hall and J.F. Manville, *Carbohydr. Res.*, 4 (1967) 271–273;
D.G. Streefkerk, M.J.A. de Bie, and J.F.G. Vliegenthart, *Tetrahedron*, 29 (1973) 833–844.

[16] M.F. Chaplin, in M.F. Chaplin and J.F. Kennedy (Eds.), *Carbohydrate Analysis, a Practical Approach*, IRL Press, Oxford, 1987, pp 1–36.