The Structure of D-*xylo*-5-Hexulosonic Acid and its Gadolinium(III) Complexes in Aqueous Medium as studied by Nuclear Magnetic Resonance

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The solid-state ^{13}C n.m.r. spectrum of calcium D-*xylo*-5-hexulosonate, which exists exclusively in the β-furanose form, resembles that of the minor furanose form in solution. A ^{1}H two-dimensional nuclear Overhauser enhancement spectrum (NOESY), however, unambiguously shows that the latter component is the α-furanose form. Thus caution is required in comparing liquid- and solid-state n.m.r. spectra. Longitudinal relaxation-rate measurements show that Gd^{III} binds preferentially to the keto tautomer, in a bidentate fashion *via* a carboxylate oxygen and O(2). The first co-ordination sphere of Gd^{III} in the predominant complex is completed by furanose ligands bound in a bidentate manner *via* the two carboxylate oxygens.

D-*xylo*-5-Hexulosonic acid (5-oxo-D-gluconic acid) has found various applications, for example as a browning agent for food [1] and as a precursor of the meat flavour dihydro-4-hydroxy-5-methyl-3-furanone.[2] It is readily available by chemo- and biocatalytic oxidation of D-glucose,[3] and therefore is an attractive chiral starting compound for further synthesis. In the course of a study on metal cation-promoted reactions of D-*xylo*-5-hexulosonate, we have investigated the co-ordination of metal ions by this compound. Gadolinium(III) was used as a model ion for the group of metal ions that form complexes with bonds of a predominant electrostatic character.

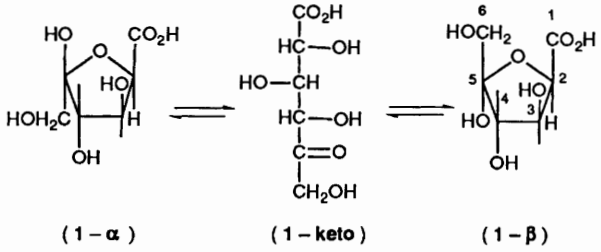

Figure 1. The mutarotation equilibrium of D-*xylo*-5-hexulosonate

Results and Discussion

Assignment of the Tautomeric Forms in Aqueous Solution.—The ^{13}C n.m.r. spectrum of sodium D-*xylo*-5-hexulosonate [Figure 2(*a*)] clearly shows the presence of the three possible mutarotation isomers (*cf.* Figure 1). The keto form (**1-keto**) is easily identified through the carbonyl signal at 214.12 p.p.m. The anomeric signals of the two furanose forms (**1-α**) and (**1-β**) are readily assigned too, appearing at 100—110 p.p.m. as a group separated from the other C(OH) signals.

It is common to compare solid- and liquid-state n.m.r. spectra in order to assign each of them. In general the chemical shifts in both states are comparable.[4] As the single isomer present in the solid calcium salt of (**1**) is known to be the β-furanose form (**1-β**),[5] we were tempted to compare its solid-state ^{13}C n.m.r. spectrum with a liquid-state spectrum of an aqueous solution of the sodium salt of (**1**). Surprisingly the chemical shift of the anomeric carbon in the cross polarisation magic angle spinning (c.p.m.a.s.) spectrum of the calcium salt [see Figure 2(*b*)] was the same as that of the minor furanose form in solution. In addition a 1:1 mixture of the sodium and calcium salts gave a similar c.p.m.a.s. spectrum, but now with an extra small peak at 104 p.p.m., which is the chemical shift for the anomeric carbon of the major tautomer in solution. It should be noted that the sample concerned was melted in its crystal water during the measurement, probably due to the high pressure as a result of the high spinning rate. In solution the ^{13}C chemical shift differences of the sodium and calcium salts are usually negligible. Therefore, these data would suggest that the major tautomer in solution is (**1-α**), which is very unlikely considering the strong arguments that have been presented by Crawford *et al.*[6] to show that the major tautomer is (**1-β**). Therefore, we have measured the ^{1}H nuclear Overhauser enhancement spectroscopy (NOESY) spectrum[7] of an aqueous solution of compound (**1**). This spectrum (see Figure 3) has a cross-peak between H(4) and H(6) of the major furanose form, whereas no cross-peak between the corresponding protons of the minor furanose form could be detected. Since the nuclear Overhauser effect is inversely proportional to the sixth power of the distance of the nuclei under consideration,[8] this spectrum shows that the distance between H(4) and H(6) in the major isomer is much shorter than that in the minor one. Therefore it can be concluded that the major isomer in solution is (**1-β**).

These results demonstrate that great care is needed when solid- and liquid-state n.m.r. spectra are compared.

Assignment of ^{1}H *and* ^{13}C *N.M.R. Spectra.*—The ^{1}H and ^{13}C n.m.r. signals of the two furanose forms (**1-α**) and (**1-β**) were assigned by their chemical shifts and with the aid of homonuclear and heteronuclear correlation experiments (COSY and HETCOR, respectively).[9] Plots of the ^{13}C chemical shifts as a function of the pH have a sigmoidal shape with an inflection point around pH 3.5, which corresponds with the pK_a of these acids. The chemical shifts, pH effects, and the proton–proton coupling constants obtained are compiled in Table 1.

It should be noted that the differences in the magnitudes of the vicinal proton–proton coupling constants $^{3}J(H^{2}H^{3})$ and $^{3}J(H^{3}H^{4})$ between the two furanose anomers (see Table 1) points to more puckering in the relevant part of the five-membered ring of (**1-α**), which can be explained by the steric interaction of the CH_2OH group and the neighbouring OH(3). This interaction probably also hinders the rotation of the CH_2 group in that tautomer, as was witnessed by the presence of an AB system for the methylene protons, whereas only a single CH_2 signal was observed for (**1-β**).

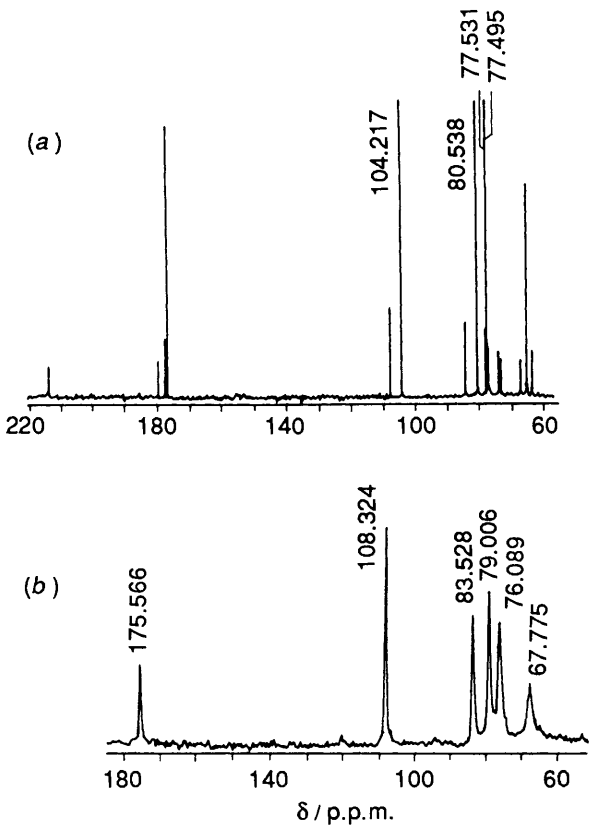

Figure 2. (*a*) Carbon-13 n.m.r. spectrum of a 2 mol dm^{-3} solution of sodium D-*xylo*-5-hexulosonate in D_2O at pD 7, 25 °C. (*b*) C.p.m.a.s. ^{13}C n.m.r. spectrum of calcium D-*xylo*-5-hexulosonate

Table 1. Chemical shifts (p.p.m.), pH effects on chemical shifts (in parentheses),[a] and proton–proton coupling constants (Hz) of the three tautomers of compound (**1**)[b]

		(1-α)	(1-β)	(1-keto)
δ(^{13}C)	C(1)	177.77	177.34	179.76
		(2.86)	(2.78)	(2.71)
	C(2)	84.57	80.72	73.74
		(2.03)	(1.59)	(1.64)
	C(3)	78.26	77.82	74.54
		(0.23)	(0.26)	(0.50)
	C(4)	81.05	77.65	77.58
		(0.50)	(0.31)	(0.77)
	C(5)	108.29	104.53	214.12
		(−0.50)	(−0.54)	(0.37)
	C(6)	63.91	65.15	67.62
		(0.10)	(0.15)	(−0.03)
δ(^{1}H)	H(2)	4.68	4.62	
	H(3)	4.38	4.43	
	H(4)	4.17	4.11	
	H(6)	3.67	3.66	
	H(6′)	3.76	3.66	
^{3}J	H(2), H(3)	4.9	5.8	
	H(3), H(4)	1.6	4.2	
$\|^{2}J\|$	H(6), H(6′)	12.0		

[a] Chemical shift difference between pD 7.2 and 1.0. [b] In a 1 mol dm^{-3} solution in D_2O at pD 7.2, 25 °C.

Influence of Temperature and pH *on the Composition.*— Compound (**1**) is stable in aqueous solution between pH 1 and 10 and up to 80 °C. Above pH 9 the (**1-keto**) form could not be observed, probably because of extensive broadening due to exchange between the keto form and its hydrate under these conditions.

As the carboxyl and the neighbouring hydroxy group are *cis* to each other in the cyclic forms, a considerable proportion of the keto form is to be expected,[10] in agreement with the observations (see Table 2). The amount of open chain increases with temperature at the expense of the β-furanose form (see Table 2), but (**1-keto**) does not become the major tautomer at

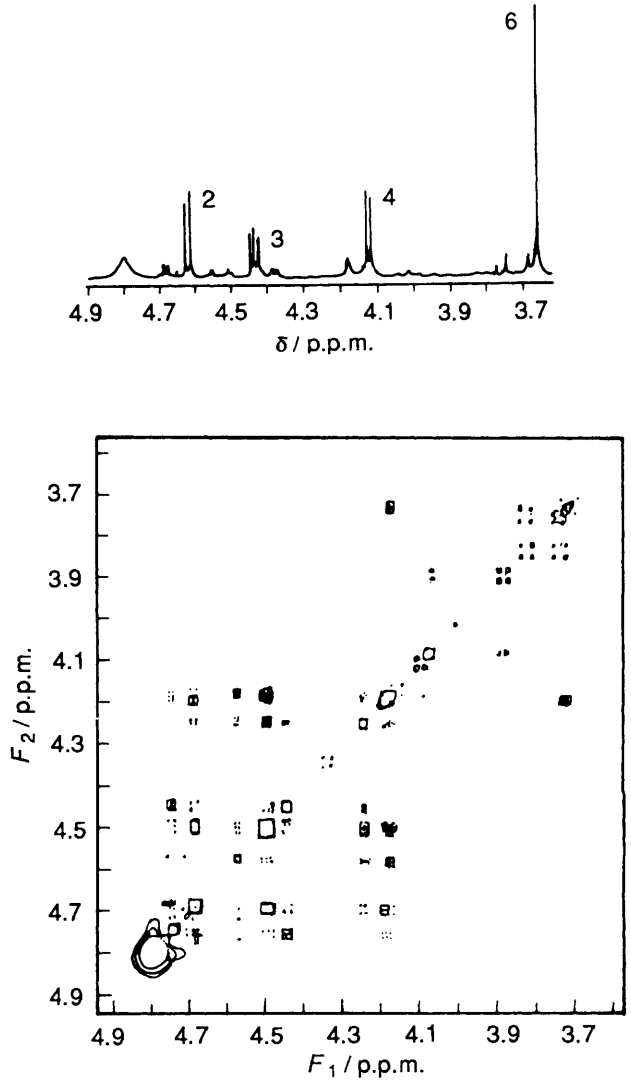

Figure 3. Proton NOESY n.m.r. spectrum of a 1 mol dm^{-3} solution of sodium D-*xylo*-5-hexulosonate in D_2O at pD 7, 25 °C, degassed

Table 2. Composition[a] (mol %) of compound (**1**) at pD 7 at different temperatures and thermodynamic parameters

T/K	(1-α)	(1-β)	(1-keto)
298	13	82	6
318	12	76	12
338	13	69	18
353	12	66	22
ΔH^{b}	−0.7	0	−5.8
ΔS^{c}	1.4	0	−14.4

[a] Determined by integration of ^{13}C n.m.r. signals of spectra measured with gated decoupling and a long acquisition delay ($5T_1$). [b] Relative to (**1-β**), in kcal mol^{-1} (cal = 4.184 J). [c] Relative to (**1-β**), in cal K^{-1} mol^{-1}.

Table 3. Gadolinium(III)-induced longitudinal relaxation-rate enhancements (s^{-1}) of the ^{13}C nuclei of the three tautomers of compound (**1**),* at $\rho = 10^{-4}$, pD 7, and 25 °C

Nucleus	(**1**-α)	(**1**-β)	(**1**-keto)
C(1)	5.57	6.37	54.99
C(2)	1.09	2.24	35.74
C(3)	0.47	0.84	6.00
C(4)	0.59	0.67	0.48
C(5)	0.74	1.84	1.40
C(6)	0.22	1.71	1.34

* Calculated from the slopes of plots of $1/T_{obs.}$ *versus* ρ.

elevated temperature, as has been suggested by Chen *et al.*[11]

The ΔG values were calculated from the ratios of the isomers. From plots of these values *versus* $1/T$ the relative values of ΔH and ΔS for the three isomers were calculated (see Table 2). The rather large difference in entropy between the open keto-form and the two furanose forms is consistent with the assignments.

The pH appeared to have no influence on the tautomeric equilibrium (studied between pH 1 and 10).

Structure of the Gadolinium(III) *Complexes in Solution.*—Addition of a small amount of an alkaline-earth or lanthanide salt to an aqueous solution of compound (**1**) gave rise to precipitation of the metal salts. Upon addition of Gd^{III} to a 2 mol dm^{-3} solution of (**1**) the solution remained homogeneous up to a molar ratio Gd^{III}/(**1**) (ρ) of 3×10^{-4} (pH 7). Gadolinium(III) is a lanthanide cation with a relatively long electronic relaxation time,[12] that induces a large enhancement of the relaxation rates in the ligands to which it is bound. In its co-ordination behaviour Gd^{III} resembles the other lanthanide ions and Ca^{II}.[13] Its relaxation-enhancing property makes it a very suitable n.m.r. probe for these ions, particularly in the present case, where low solubilities prohibit the use of the other metal ions in co-ordination studies.

Since Gd^{III} is applied in very low concentrations with respect to the ligand ($\rho < 5 \times 10^{-4}$) in relaxation studies, information can be obtained on complexes in which Gd^{III} is co-ordinated by the maximum number of ligands possible.[14] Free compound (**1**) exists in an equilibrium involving three species, so upon addition of Gd^{III} a complex system of equilibria will be formed. The exchange between the tautomers of (**1**) is slow on the n.m.r. time-scale. This allows us to describe the system with three sets of independent equilibria, one for each tautomer. For the small range of gadolinium(III) concentrations used, it may be assumed that almost no unbound Gd^{III} is present and that the fractional distribution of Gd^{III} over the various species is constant.

Assuming that the mean residence time of a ligand in its gadolinium(III) complex is short with respect to the longitudinal relaxation time (T_1) and that the contribution of intermolecular interactions to the relaxation is negligible, the observed relaxation rate ($1/T_1 obs.$) of for example the (**1**-α) tautomer can be expressed[15,16] as in equation (1) where n is the number of the

$$1/T_1 obs. = nf_\alpha \rho / T_1(\text{Gd-}\alpha) + 1/T_1(\alpha) \quad (1)$$

(**1**-α) ligands bound in the complex, f_α is the molar fraction of Gd^{III} bound in this complex, T_1(Gd-α) is the longitudinal relaxation time of the complex, and $T_1(\alpha)$ is that of free (**1**-α). Similar expressions can be deduced for the case where more than one complex of (**1**-α) is present in solution. So, a plot of the relaxation rate of a nucleus *versus* ρ should be linear with a slope that is determined by f_α, n, and the relaxation rate of that nucleus in the gadolinium complex, or, when the ligand is involved in more than one such complex, the weighted average of the relaxation rates.

The relaxation rate of a nucleus in a gadolinium(III) complex is related to the molecular structure *via* equation (2).[17–19] Here

$$1/T_1(\text{complex}) = k/r^6 \quad (2)$$

r is the distance between Gd^{III} and the nucleus under consideration and k is a constant.

The relaxation rates of the ^{13}C nuclei of the tautomers of (**1**) were measured at four different gadolinium(III) concentrations. In all cases a linear relationship between the relaxation rate and ρ was found (correlation coefficient > 0.99). This is in accordance with equation (1). The slopes of the various lines are compiled in Table 3.

The effect of Gd^{III} is largest on the nuclei of the keto tautomer; at $\rho > 10^{-4}$ the signals of C(1) and C(2) of that isomer were no longer observable due to excessive broadening. This indicates that the association constants of the relevant complexes are large in comparison to those with (**1**-α) and (**1**-β). The relaxation-rate enhancement of the carboxylate carbon of (**1**-keto) is about ten times as large as that of (**1**-α) and (**1**-β). Since the carboxylate group of each of these tautomers, because of its negative charge, will be co-ordinated to Gd^{III}, it can be concluded that roughly 80% of the Gd^{III} will be bound by (**1**-keto). The relaxation rates of the carboxylate carbons of (**1**-α) and (**1**-β) are comparable, indicating that the stabilities of the gadolinium(III) complexes of the furanose forms are about the same.

The magnitude of the relaxation-rate enhancements (see Table 3) is the largest for C(1) and C(2) of (**1**-keto), suggesting that this ligand is bound to Gd^{III} in a bidentate fashion *via* one of the carboxylate oxygens and the 2-OH function. In Figure 4 the relative relaxation-rate enhancements of (**1**-keto) are compared with values calculated for a model in which the ligand is co-ordinated in this way (**3**) and a model in which Gd^{III} is bound in a tridentate fashion *via* CO_2^-, OH(2), and OH(3), (**2**). The distances required for these calculations were estimated from Dreiding models. The comparison unambiguously shows that the bidentate co-ordination is predominant. This is in agreement with previous results on the co-ordination of lanthanide cations by glycerate, which showed that that ligand is bound predominantly in a bidentate fashion.[14,20] Taga *et al.*,[21] on the other hand, have concluded on the basis of lanthanide-induced ^1H shifts that gluconate in the 1:1 lanthanide(III) complexes is bound in a tridentate fashion. A rigorous analysis of lanthanide-induced shifts is very complicated, particularly for complexes with flexible ligands.[22] The assumptions made in the interpretation of the lanthanide(III)-induced shifts of gluconate,[21] such as the neglect of the contact shifts and the position of the magnetic axis, make these conclusions rather ambiguous.

It has been shown,[5] with the use of *X*-ray diffraction, that the calcium salt of (**1**) contains the β-furanose form (**1**-β), bound *via* one of the carboxylate oxygens, O(1), and O(6). Therefore, a similar binding mode was taken into consideration for the gadolinium(III) complex of (**1**-β). A comparison of the calculated relaxation enhancements for such a model (**4**) with the observed ones shows a poor fit (see Figure 4). In particular, the relatively low observed values for C(2), C(5), and C(6) are noteworthy. The relaxation-rate enhancements of these nuclei are, however, too high for a co-ordination exclusively *via* the carboxylate group [(**5**), see Figure 4]. A good fit between experimental and calculated relaxation enhancements was obtained for a situation in which 39% of (**1**-β) is bound in a tridentate fashion and the remainder in a bidentate mode (*via* the two carboxylate oxygens) (see Table 4).

Analogously it can be shown that the α-furanose form also preferentially binds Gd^{III} *via* the carboxylate group, (**7**). Here

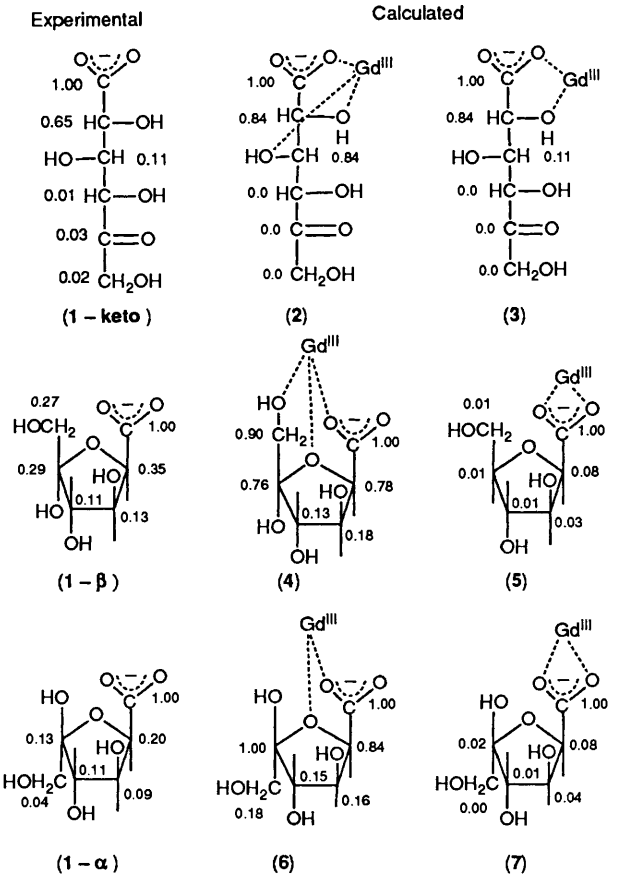

Figure 4. Comparison of experimental gadolinium(III)-induced longitudinal relaxation rates (^{13}C) with those calculated for various models. The structures are depicted schematically

Table 4. Comparison of experimental and calculated[a] relative gadolinium(III)-induced longitudinal relaxation-rate enhancements for the ^{13}C nuclei of the three tautomers of compound (**1**)

	(1-α)		(1-β)		(1-keto)	
Nucleus	exptl.	calc.[a]	exptl.	calc.[b]	exptl.	calc.[c]
C(1)	1.00	1.00	1.00	1.00	1.00	1.00
C(2)	0.20	0.23	0.35	0.35	0.65	0.84
C(3)	0.09	0.06	0.13	0.09	0.11	0.11
C(4)	0.11	0.04	0.11	0.06	0.01	0.0
C(5)	0.13	0.21	0.29	0.30	0.03	0.0
C(6)	0.04	0.03	0.27	0.36	0.02	0.0

[a] Taking into account fractional populations of models shown in Figure 4. [b] 19% (**6**), 81% (**7**). [c] 39% (**4**), 61% (**5**). [d] 100% (**3**).

only about 20% is bound in a bidentate fashion *via* one of the carboxylate oxygens and O(1), (**6**).

It can be concluded that Gd^{III} has a high preference for bidentate co-ordination of the keto form, (**1-keto**), in aqueous solution. This can be attributed to steric effects. The furanose forms, (**1-α**) and (**1-β**), are predominantly bound to Gd^{III} *via* the carboxylate group. Probably, these forms are used to complete the first co-ordination sphere of the complex with (**1-keto**). It should be noted that the predominant gadolinium complex in solution does not correspond with the structure of the solid calcium complex of (**1**). The lanthanide cations are chemically very similar to Ca^{II} and often the structures of solid calcium complexes and the corresponding lanthanide(III) complexes in solution are alike. The present case is one of the rare exceptions.

Experimental

D-*xylo*-5-Hexulosonic acid was supplied as the calcium salt by AKZO Research B.V. (Arnhem, The Netherlands). The sodium salt was obtained by passing the calcium salt through a strongly acidic Dowex column, followed by careful neutralization with aqueous NaOH. Freeze-drying of the solution obtained afforded the sodium salt.

The ^1H and ^{13}C n.m.r. spectra were recorded on a Varian VXR-400S spectrometer at 399.95 and 100.58 MHz, respectively. Sodium 2,2-dimethyl-2-silapentane-5-sulphonate and the methyl signal of t-butyl alcohol (31.2 p.p.m.) were used as internal reference for ^1H and ^{13}C chemical shifts, respectively. The NOESY spectrum was measured with a degassed sample, using a mixing time of 3 s.

The longitudinal relaxation rates (^{13}C) were measured on a sample containing 1 cm^3 of a 2 mol dm^{-3} solution of compound (**1**) in D_2O at pD 7 in the presence of different amounts of Gd^{III}. The ρ value was varied from 0 to 3×10^{-4} by addition of a 6.5 mmol dm^{-3} solution of $Gd(NO_3)_3 \cdot 6H_2O$ in water *via* a microsyringe. The relaxation times (T_1) were measured with the use of the 180°–τ–90° inversion recovery pulse sequence.

The ^{13}C c.p.m.a.s. spectra were measured with a broadband Doty probe. The contact time was 0.6 ms and the spinning rate 6 kHz.

Proton decoupling was used in both solid- and solution-state ^{13}C n.m.r. spectroscopy.

Acknowledgements

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