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Exploiting $^{13}C/^{14}N$ solid-state NMR distance measurements to assign dihedral angles and locate neighboring molecules†

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The RESPDOR NMR method rapidly provides multiple $^{13}C/^{14}N$ distance measurements in natural abundance solids. In this study, $^{13}C/^{14}N$ RESPDOR information is shown, for the first time, to provide accurate molecular conformation and to locate non-bonded neighboring molecules.

The development of solid-state NMR methods for accurately measuring distances between pairs of nuclei has proven to be one of the most influential developments in structural analysis of solids by NMR.[1] Such measurements were initially demonstrated for heteronuclear systems using the rotational-echo double resonance (REDOR) experiment.[2] This technique reintroduces dipolar couplings between a pair of spin = 1/2 nuclei in a rapidly rotating sample, thereby providing distances between these sites, usually denoted as I and S. A comparison of spectra in which the dipolar coupling is reintroduced *versus* spectra without the "recoupling" step provides an estimate of the coupling, even in cases of very weak couplings of tens of Hz. At present, a variety of alternatives to REDOR have been introduced[3] and, in most cases, provide a single distance between two selected (and often isotope labelled) nuclei in a sample. These techniques have now been extended to allow multiple distances to be measured within a molecule,[4] allowing for structural characterization of molecules as complex as proteins.[4d,5] One limitation to these methods is their ability to evaluate coupling between $I = 1/2$ sites and nuclei having a quadrupole moment (*i.e.* $S > 1/2$). Although nuclei with small quadrupolar couplings (C_Q) can often be accurately analysed, many quadrupolar nuclei have C_Q values of several MHz, making analysis difficult. Because NMR active

nuclei with $I = 1/2$ are relatively rare in the periodic table and most elements have at least one isotope with $S > 1/2$, this limitation can be a serious one. Fortunately, considerable effort has been made toward developing methods for analysing solids with $I = 1/2$ and $S > 1/2$ and several techniques are now available including TRAPDOR,[6] REAPDOR,[7] DEAR,[8] mod. LA_REDOR[9] and RIDER.[10]

The R-RESPDOR technique[11] also measures distances between $I = 1/2$, $S > 1/2$ sites. This technique achieves recoupling using rotary resonance on the spin 1/2 nucleus and indirectly monitors coupling by observing the I isotropic spectra. A detailed discussion of the advantages and limitations of R-RESPDOR relative to other methods is given elsewhere.[9,12] Recently, improved recoupling schemes have been incorporated into RESPDOR involving symmetry based sequences that decrease the sensitivity to the chemical shift anisotropy (CSA) of the spin 1/2 nucleus and minimize the influence of homonuclear dipolar couplings (*e.g.* $^{13}C/^{13}C$) and heteronuclear scalar couplings.[13] This approach employs the $SR4_1^2$ or SFAM recoupling schemes[14,15] and is known as S-RESPDOR. An advantage of both R- and S-RESPDOR is that distances can be obtained through a universal function describing the dephasing curves.[11,12a]

At present, distances between a wide variety of $I = 1/2$, $S > 1/2$ nuclei have been measured by R/S-RESPDOR including, for example, $^{1}H/^{51}V$, $^{13}C/^{11}B$, $^{15}N/^{51}V$, $^{13}C/^{67}Zn$.[9,12a,16] These studies demonstrate that RESPDOR can provide accurate distances even when the nuclei being measured have large CSAs, low γ-values, or involve pairs of nuclei having very similar Larmor frequencies (*e.g.* $^{13}C/^{27}Al$).[17] Some nuclei seem particularly well suited to analysis by RESPDOR. For example, natural abundance organic products containing ^{14}N have the unusual combination of numerous low abundant ^{13}C sites (1.1%) in proximity to ^{14}N which has a high natural abundance of 99.6%. This means that each $^{13}C/^{14}N$ coupled pair can be treated as an isolated spin system and multiple $^{13}C/^{14}N$ distances can potentially be measured in a single experiment. Despite the considerable success of R/S-RESPDOR and related methods, nearly all measurements report distances corresponding to directly bonded pairs of nuclei

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or sites connected through two bonds (*i.e.* valence angles). In a few cases, distances corresponding to dihedral angles (3 bonds)[11,17] and even further[11,16a] have been measured. However, in all such cases these dihedral angles have been measured in rigid moieties where no conformational flexibility is possible. At the present time, dihedral angles in flexible molecules have not been determined using dipolar recoupling methods involving $I = 1/2$ and $S > 1/2$. The ability to measure dihedral angles in flexible molecules has played a prominent role in structural analysis by NMR for over half a century[18] and the ability to obtain such measurements would greatly aid in structural analysis of molecules where single crystals cannot be obtained for X-ray diffraction or where isotope labelling with more NMR-accessible nuclei is not possible.

The ability to establish distances between non-bonded sites in neighboring molecules is also of critical importance in structural analysis of solids. NMR methods have been shown to provide such constraints in a few cases where isotope enrichment was possible (*e.g.* $^{13}C/^{17}O$ and $^{13}C/^{27}Al$)[19] but such distances have not yet been measured in natural abundance $^{13}C/^{14}N$ materials. The goals of the present study are to demonstrate that $^{13}C/^{14}N$ RESPDOR analysis can provide accurate dihedral angles in flexible molecules and that intermolecular non-bonded close contacts can also be measured in natural abundance samples, allowing the proximity of neighboring molecules to be established.

As an initial example of the ability of RESPDOR to establish dihedral angles in flexible structures, an analysis was performed on the anticancer drug docetaxel (Fig. 1). The phase of docetaxel studied here differs from all known phases based upon a comparison of unit cell parameters[20] with previously reported forms.[21] The single ^{14}N present in docetaxel exhibits dipolar coupling to four ^{13}C resonances, labelled A–D in Fig. 1, and a fit to these dephasing curves provides $^{13}C/^{14}N$ distances (Table 1).

Table 1 Carbon-13 sites experiencing dipolar interactions with ^{14}N in docetaxel

Sites	^{13}C shift (ppm)	Distance (Å)	Interaction type
C1′/N	175.9	3.3	Dihedral angle
C3′/N	56.0	1.8	Bonded atoms
C5′/N	155.2	1.4	Bonded atoms
C6′/N	138.4	2.6	Valence angle

and estimated uncertainties. Criteria for deciding which ^{13}C peaks are coupled is given in ESI.† The directly bonded carbons at C3′ and C5′ can be immediately identified from distances corresponding to bond lengths. Because C5′ is a carbonyl it is assigned to the signal at 155.2 ppm while C3′ is assigned by default to the resonance at 56.0 ppm. The dipolar coupling to an aromatic resonance at 138.4 ppm provides a distance corresponding to a valence angle (*i.e.* 2.5 Å) and thus identifies this resonance as C6′. Of greatest relevance to the present study was the observation of a $^{13}C/^{14}N$ coupling to the ^{13}C peak at 175.9 ppm corresponding to a distance of 3.3 Å. The ^{13}C resonance was identified as C1′ based on a partial assignment from a refocused INADEQUATE[22] analysis (see ESI†). This $^{13}C1′/^{14}N$ distance corresponds to a C1′–C2′–C3′–N dihedral angle of ±90° as determined from DFT geometry optimizations of a series of possible dihedral angles at the B3LYP/D95* level of theory (included as ESI†). Of equal interest is the absence of a dipolar coupling between the ^{14}N and C12′. This missing coupling indicates that the *t*-butyl moiety is directed away from the ^{14}N. Here, the N–C5′–O–C12′ dihedral angle is assigned as 180° based on the missing coupling and the well-known fact that carbonyls in COOR moieties preferentially form dihedral angles in which the OR bond eclipses the C=O.[23] It is noteworthy that six of the eight crystal structures presently available for docetaxel,[21a] show similar dihedral angles at C1′–C2′–C3′–N and N–C5′–O–C12′ to the values predicted here (Table 2).

A notable omission from the measured distances is the C2′/N separation. This pair of atoms is separated by approximately 2.5 Å and thus well within the measurable range. The C2′ peak differs from peaks A–D in that the C2′ resonance is degenerate with a second signal, based on lineshape. It is presently unclear
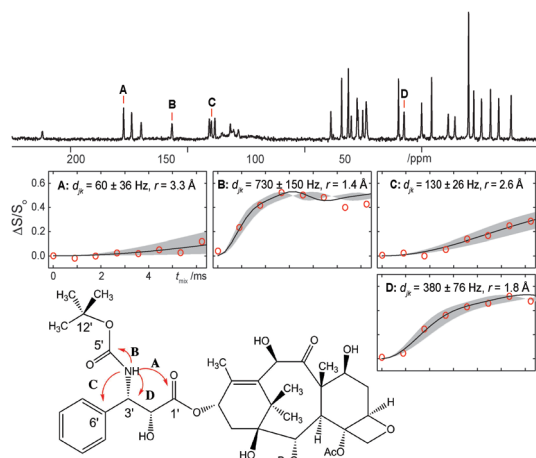

Fig. 1 The ^{13}C NMR spectrum (top) of docetaxel with letters indicating resonances that experience $^{13}C/^{14}N$ dipolar coupling in a RESPDOR analysis. The middle plot illustrates the experimentally measured signal fractions ($\Delta S/S_0$, red circles) and corresponding simulations (black lines) with estimated uncertainties (grey regions) together with the predicted dipolar couplings and corresponding $^{13}C/^{14}N$ distances. A docetaxel structure (bottom) shows $^{13}C/^{14}N$ coupled sites.

Table 2 A comparison of dihedral angles (in degrees) in known phases of docetaxel with angles predicted from RESPDOR

Structure	C1′–C2′–C3′–N	N–C5′–O–C12′	Ref.
Anhydrate	−145.4	−132.4	27[a]
Monohydrate	58.6	−85.6	27[a]
EtOH·H$_2$O	55.5	175.4	27[a] and 21[b]
MeOH·H$_2$O	56.6	176.5	21[c]
EtOH·2H$_2$O[c]	56.8	177.3	27[a]
EtOH·2H$_2$O[c]	59.8	−171.6	27[a]
Trihydrate	56.4	−174.2	27[a] and 21[b]
Trihydrate	56.5	−174.2	27[a]
0.5 hydrate	±90.0	±180.0	Present study

Obtained from either [a] Single crystal or. [b] Powder diffraction data. [c] The two values listed are from a single phase having two molecules in the asymmetric unit.

why this poses a challenge and further work is needed. Overall, the RESPDOR analysis of docetaxel assigns four ^{13}C resonances and restricts conformations of two key dihedral angles in docetaxel.

Another important class of structural constraints is distance measurements between non-bonded nuclear sites in neighboring molecules. Recently, the ^{14}N/^{1}H connectivity to both nitrogen's in ^{14}N–^{1}H···^{14}N hydrogen bonds has been observed in solids using NMR methods that rely on dipolar coupling.[24] The feasibility of achieving similar measurements in ^{13}C/^{14}N systems has been demonstrated in a peptide.[25] This study established, from a single ^{13}C/^{14}N distance measurement, that two adjacent peptides interact with the L27 sidechain from one peptide interdigitated between the F23 and N21 sidechains of the second peptide. Unfortunately, such measurements can be complicated by multiple ^{14}N sites coupling with a given ^{13}C site. In general, peptides will suffer from this complication because all backbone ^{13}C positions are directly bonded to one ^{14}N and attached to a second ^{14}N through two bonds. In the peptide study, the risk of ^{13}C coupling to multiple ^{14}N sites was reduced by isotopically enriching all nitrogens to 96% in ^{15}N. In general, however, such isotope labelling is less feasible, and it is important to ask whether useful intermolecular ^{13}C/^{14}N couplings can also be achieved when ^{15}N isotope labelling is unattainable.

The ability of RESPDOR to provide useful intermolecular ^{13}C/^{14}N distances in natural abundance materials was evaluated using 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-1-β-D-glucopyranosyl azide (Fig. 2). A total of eight ^{13}C resonances experience dipolar couplings to a ^{14}N as summarized in Table 3. An illustration of these dipolar couplings is given in Fig. 2 with
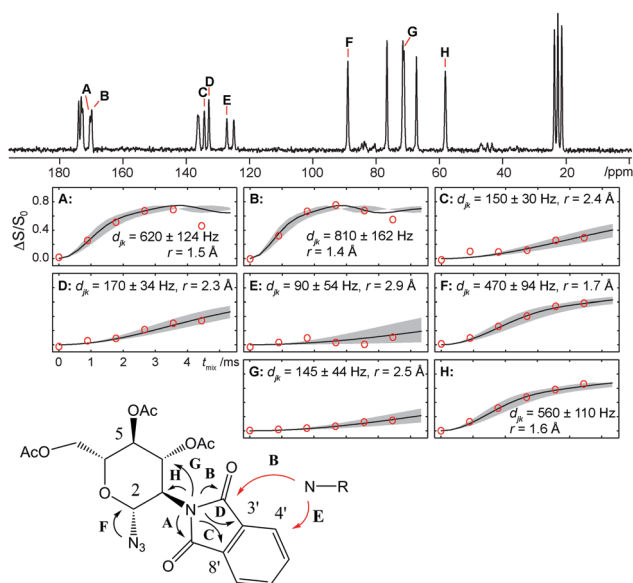

Fig. 2 The ^{13}C NMR spectrum (top) of 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-1-β-D-glucopyranosyl azide with A–H indicating resonances that experience ^{13}C/^{14}N dipolar coupling in RESPDOR. The middle plot illustrates experimentally measured signal fractions ($\Delta S/S_0$, red circles), corresponding simulations (black lines) with uncertainties (grey areas), predicted dipolar couplings and ^{13}C/^{14}N distances. The bottom structure shows coupled ^{13}C/^{14}N sites.

Table 3   Carbon-13 sites experiencing dipolar interactions with ^{14}N in the glycosyl azide

Sites	^{13}C shift (ppm)	r (Å)	Interaction type
C2/N-azide	87.3	1.7	Bonded atoms
C3/N-amide	56.3	1.6	Bonded atoms
C4/N-amide	70.1	2.5	Valence angle
C2′/N-amide	167.7	1.4	Bonded atoms plus intermolecular coupling
C3′/N-amide	131.4	2.3	Valence angle
C4′/N-amide	125.8	2.9	Intermolecular coupling
C8′/N-amide	132.9	2.4	Valence angle
C9′/N-amide	168.1	1.5	Bonded atoms

estimated uncertainties illustrated as grey bounding curves. Based on distances observed, four peaks (A, B, F and H) can be classified as ^{13}C/^{14}N bonds, three as valence angles (C, D and G) and one (E) as an intermolecular contact or a dihedral angle. For directly bonded sites, a prior solution state NMR analysis of the glycosyl azide[26] taken together with empirical shift prediction rules, provide approximate ^{13}C assignments and these are sufficient to assign C2 and C3 as resonances F and H, with chemical shifts of 87.3 and 56.3 ppm, respectively. Similarly, an expectation of where the phthalimide carbonyls (A and B) resonate, taken together with the longer-range coupling to C2′ (described below), serve to assign C2′ and C9′ as the resonances at 167.5 and 168.1 ppm, respectively. Signals C and D occur in the aromatic region allowing the lines at 132.9 and 131.6 ppm to be assigned to C3′and C8′, respectively. The C3′ and C8′ signals cannot be unambiguously assigned and should be considered interchangeable. By default, resonance G is assigned to C4 with a shift of 70.1 ppm.

The ^{13}C/^{14}N coupling shown as peak E (Fig. 2) is consistent with an intermolecular interaction between C4′ and a ^{14}N from a neighboring molecule. The coupled carbon was designated C4′ rather than C5′ based on ^{13}C shift assignments described in ESI.† It is considered unfeasible that an intramolecular interaction between N-amide and C4′ is responsible for the observed coupling because the symmetry of the phthalimide moiety would require that a second coupling be observed between N-amide and C7′, yet such coupling is not found. Further evidence for an intermolecular rather than intramolecular ^{13}C4′/^{14}N-amide interaction is found in the magnitudes of the couplings at C2′ and C9′ where nearly identical couplings are anticipated due to symmetry. However, the coupling of C2′/N-amide is nearly 200 Hz larger than that between C9′/N-amide, indicating that at least one neighboring ^{14}N is coupling to C2′. Taken together, these data indicate the presence of a second molecule positioned in such a way that a ^{14}N interacts closely with both C2′ and C4′. While it is beyond the scope of the current study to exhaustively evaluate all structural possibilities, the requirement that one or more nitrogen atoms from a neighboring molecule must lie within 3 Å of C4′ and also interact with C2′ represents a significant constraint upon any proposed crystal structure. At the present time, a crystal structure of this glycosyl azide has not been reported.

A possible complication to this prediction of intermolecular dipolar coupling at C4′ is the possibility that an intramolecular

coupling between the ^{14}N-azide and C4′ could also explain this result. However, the rigidity of both of these functional groups causes them to become increasingly separated from one another as the distance from the pyran ring increases as illustrated in ESI.† Moreover, the stereochemistry directs the azide and phthalimide groups in opposite directions. These considerations make it unlikely that the observed ^{13}C/^{14}N coupling originates from an intramolecular interaction with the N-azide.

It is notable that three potentially measurable ^{13}C/^{14}N dipolar couplings are not observed in the glycosyl azide. Specifically, dihedral angles for N–C3–C4–C5, N–C2′–C3′–C4′, and N–C9′–C8′–C7′ would all be expected to have measurable ^{13}C/^{14}N separations unless these dihedral angles are approximately 180° where the separation is approximately 3.8 Å. In the case of N–C2′–C3′–C4′ and N–C9′–C8′–C7′, the dihedral angles are indeed forced to be roughly 180° by the rigid ring system. Similarly, the N–C3–C4–C5 has a 180° dihedral angle when the pyran ring adopts a chair orientation; hence, a chair conformation of the ring is predicted. Notably, solution NMR analysis of the glycosyl azide[26] predicts a chair conformation for the ring, supporting the conformation proposed.

An independent estimation of uncertainties in ^{13}C/^{14}N distances from RESPDOR was obtained by analysing tyramine HCl, a molecule with a known crystal structure (see ESI†). An uncertainty of ±0.1 Å was observed (N = 3) for intramolecular couplings. This result is consistent with the decision to report all RESPDOR distances to one decimal point.

It has long been anticipated that ^{13}C/^{14}N NMR distance measurements will become an important tool in structural analysis of solids.[13] Usually structurally useful information is assumed to involve distances longer than 3.0 Å, as these lengths correspond to dihedral angles and intermolecular contacts. Nevertheless, ^{13}C/^{14}N separations > 3.0 Å have seldom been reported and, to the authors' knowledge, never used to established structure. This study demonstrates that such structural information can now be obtained and that it provides both predictions of dihedral angles and proximity of neighboring molecules. It is notable that in the present study, no distances beyond 3.8 Å were observed. In fact, four ^{13}C/^{14}N pairs analysed herein have separations near 3.8 Å, yet dipolar couplings were not observed. While the absence of detected couplings was used here to assign one dihedral angle in docetaxel and another three in the glycosyl azide, this outcome indicates that the range of ^{13}C/^{14}N distances measurable by RESPDOR is likely limited to separations of less that 4 Å. The original RESPDOR study as well as the tyramine HCl analysis reported here (see ESI†), did, in fact, detect ^{13}C/^{14}N distances near 3.8 Å,[11] indicating that such couplings can be measured in favourable cases. This curious discrepancy in outcomes when measuring long ^{13}C/^{14}N distances is due, perhaps, to differences in molecular dynamics which can alter ^{13}C/^{14}N couplings.[27] We anticipate that experiments that provide higher signal-to-noise ratios such as dynamic nuclear polarization enhanced measurements will allow a more thorough study of the role of dynamics and provide more routine measurements of longer distances.

Conflicts of interest

There are no conflicts to declare.

Notes and references

1 H. Eckert, *Solid State Nucl. Magn. Reson.*, 2015, **72**, 1.
2 T. Gullion and J. Schaefer, *J. Magn. Reson.*, 1989, **81**, 196.
3 C. P. Jaroniec in *NMR Crystallography*, ed. R. K. Harris, R. E. Wasylishen and M. J. Duer, Wiley, Chichester, 2009, p. 137.
4 (*a*) C. P. Jaroniec, C. Filip and R. G. Griffin, *J. Am. Chem. Soc.*, 2002, **124**, 10728; (*b*) C. A. Michal and L. W. Jelinski, *J. Am. Chem. Soc.*, 1997, **119**, 9059; (*c*) C. P. Jaroniec, B. A. Tounge, J. Herzfeld and R. G. Griffin, *J. Am. Chem. Soc.*, 2001, **123**, 3507; (*d*) J. J. Helmus, P. S. Nadaud, N. Hofer and C. P. Jaroniec, *J. Chem. Phys.*, 2008, **128**, 052314.
5 J. J. Helmus, K. Surewicz, M. I. Apostol, W. K. Surewicz and C. P. Jaroniec, *J. Am. Chem. Soc.*, 2011, **133**, 13934.
6 C. P. Grey and W. S. Veeman, *Chem. Phys. Lett.*, 1992, **192**, 379.
7 T. Gullion, *Chem. Phys. Lett.*, 1995, **246**, 325.
8 J. R. Sachleben, V. Frydman and L. Frydman, *J. Am. Chem. Soc.*, 1996, **188**, 9786.
9 E. Nimerovsky, R. Gupta, J. Yehl, M. Li, T. Polenova and A. Goldbourt, *J. Magn. Reson.*, 2014, **244**, 107.
10 K. Saalwächter and K. Schmidt-Rohr, *J. Magn. Reson.*, 2000, **145**, 161.
11 Z. Gan, *Chem. Commun.*, 2006, 12.
12 (*a*) X. Lu, O. Lafon, J. Trébosc and J.-P. Amoureux, *J. Magn. Reson.*, 2012, **215**, 34; (*b*) F. Pourpoint, Y. Yehl, M. Li, R. Gupta, J. Trebosc, O. Lafon, J.-P. Amoureux and T. Polenova, *ChemPhysChem*, 2015, **16**, 1619.
13 L. Chen, Q. Wang, B. Hu, O. Lafon, J. Trébosc, F. Deng and J.-P. Amoureux, *Phys. Chem. Chem. Phys.*, 2010, **12**, 9395.
14 A. Brinkmann and A. P. M. Kentgens, *J. Am. Chem. Soc.*, 2006, **128**, 14758.
15 R. Fu, S. A. Smith and G. Bodenhausen, *Chem. Phys. Lett.*, 1997, **272**, 361.
16 (*a*) F. Pourpoint, J. Trébosc, C. Bonhomme, O. Durupthy, N. Steunou, O. Lafon and J.-P. Amoureux, *J. Phys. Chem. C*, 2014, **118**, 18580; (*b*) E. Nimerovsky and A. Goldbourt, *J. Magn. Reson.*, 2012, **225**, 130.
17 F. Pourpoint, J. Trébosc, R. M. Gauvin, Q. Wang, O. Lafon, F. Deng and J.-P. Amoureux, *ChemPhysChem*, 2012, **13**, 3605.
18 M. J. Minch, *Concepts Magn. Reson.*, 1994, **6**, 41.
19 (*a*) L. Chopin, S. Vega and T. Gullion, *J. Am. Chem. Soc.*, 1998, **120**, 4406; (*b*) S. Li, F. Pourpoint, J. Trébosc, L. Zhou, O. Lafon, M. Shen, A. Zheng, Q. Wang, J.-P. Amoureux and F. Deng, *J. Phys. Chem. Lett.*, 2014, **5**, 3068.
20 The phase studied here crystallized in the space group $P2_1$ with a = 8.46 Å, b = 13.39 Å, c = 19.408 Å, β = 102.4°.
21 (*a*) L. Vella-Zarb, R. E. Dinnebier and U. Baisch, *Cryst. Growth Des.*, 2013, **13**, 440; (*b*) F. Gueritte-Voegelein, D. Guenard, L. Mangatal, P. Potier, J. Guilhem, M. Cesario and C. Pascard, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, 1990, **46**, 781; (*c*) L. Zaske, M. A. Perrin, C. Daiguebonne and O. Guillou, *Mater. Sci. Forum*, 2004, **443–444**, 411.
22 S. Cadars, J. Sein, L. Duma, A. Lesage, T. N. Pham, J. H. Baltisberger, S. P. Brown and L. Emsley, *J. Magn. Reson.*, 2007, **188**, 24.
23 G. I. L. Jones and N. L. Owen, *J. Mol. Struct.*, 1973, **18**, 1.
24 (*a*) A. S. Tatton, T. N. Pham, F. G. Vogt, D. Iuga, A. J. Edwards and S. P. Brown, *CrystEngComm*, 2012, **14**, 2654; (*b*) A. S. Tatton, T. N. Pham, F. G. Vogt, D. Iuga, A. J. Edwards and S. P. Brown, *Mol. Pharmaceutics*, 2013, **10**, 999; (*c*) G. N. Manjunatha Reddy, D. S. Cook, D. Iuga, R. I. Walton, A. Marsh and S. P. Brown, *Solid State Nucl. Magn. Reson.*, 2015, **65**, 41.
25 D. A. Middleton, *Magn. Reson. Chem.*, 2011, **49**, 65.
26 L. Szilágyi and Z. Gyorgydeák, *Carbohydr. Res.*, 1985, **143**, 21.
27 M. Dračínsky and P. Hodgkinson, *CrystEngComm*, 2013, **15**, 8705.