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Perdeuterated pyridinium molten salt (ionic liquid) for direct dissolution and NMR analysis of plant cell walls

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A bi-solvent system consisting of perdeuterated pyridinium molten salt and DMSO-d_6 (dimethyl sulfoxide-d_6) has been developed for direct dissolution and nuclear magnetic resonance (NMR) analysis of plant cell walls, which will shed a new light on efficient detailed structure of the plant cell walls and benefit lignin engineering for biofuel and biomaterial research.

Increasing societal demand for sustainable development has led to considerable efforts in the search for renewable energy and materials, especially as concerns related to energy security and climate change continue to grow.[1] Substantial efforts are being made to establish processes that transform differing sources of biomass into liquid fuels and chemicals as viable alternatives to nonrenewable petroleum resources.[2] To address this challenge, research attention has been directed towards a better understanding of plant cell wall structures and their constituents which are composite materials consisting of cellulose, hemicelluloses and lignin.[3] Extensive research has been focused on lignin[2,4] due to its being a major obstacle in chemical pulping, forage digestibility, and processing of plant biomass to biofuels. Recent developments in the genetic engineering of lignin's biosynthetic pathway[5] provide new avenues to rationally designing bio-energy crops with improved processing properties by either reducing the amounts of lignin present or providing a lignin that is easier to degrade. As new plants with differing variations in lignin structure are developed, the need for rapid, high-resolution analysis of lignin becomes a pressing research issue.

Recently, Ralph and co-workers[6] have reported a 2D NMR analysis of non-derivatized ball-milled plant cell walls solubilized in a 4 : 1 mixture of DMSO-d_6 and 1-methylimidazole-d_6. This methodology provides a direct and accurate approach for the characterization of lignin and hemicellulose in plant biomass and was applied to samples of loblolly pine, quaking aspen and kenaf. This approach is notable since it precludes the need for lengthy, low-yielding lignin extraction protocols. The key to liquid NMR analysis of plant cell material is the efficient dissolution of ball-milled plant cell walls in a perdeuterated NMR solvent system.

Ionic liquids composed entirely of ions with low melting points have emerged as promising and versatile solvents for various research and industrial applications.[7] Since Rogers and co-workers' initial reports that ionic liquids can dissolve biomass, such as cellulose and wood sawdust,[8] the application of these solvents to various biomass research efforts has grown substantially. In particular, imidazolium chloride ionic liquids, such as [bmim]Cl and [Amim]Cl, and imidazolium phosphonate have been successfully used as green solvents for functionalization or pretreatment of biomass.[9,10] Our group has recently demonstrated that imidazolium type ionic liquids [mmim][OSO_3Me], [bmim][OSO_3Me], and [hmim]OTf are especially well suited to dissolve lignin for NMR analysis.[11]

Despite wide applications of imidazolium ionic liquids (see Fig. 1) as the solvents for biomass dissolution and functionalization, the tedious multi-step synthesis of perdeuterated imidazolium ionic liquids[12] has limited their application in biomass NMR characterization. To the best of our knowledge, the use of pyridinium molten salts for dissolving the biomass has not been reported. These ionic liquids are particularly interesting as unique NMR solvents due to the easy accessibility of perdeuterated pyridinium salts *via* commercially available pyridine-d_5. With the aim to develop a fast and accurate NMR characterization of plant cell walls *via* a perdeuterated ionic liquid solvent system, herein we report the first perdeuterated pyridinium ionic liquid/DMSO-d_6 bi-solvent system for direct dissolution and NMR analysis of the Wiley milled and ball-milled plant cell walls. This research will also shed a new light on the detailed structure of the plant cell walls.

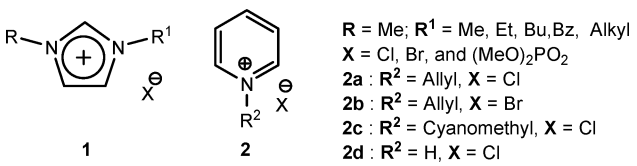

Fig. 1 Structures of imidazolium and pyridinium salts.

To facilitate the search of a suitable perdeuterated pyridinium salt for direct biomass dissolution and NMR analysis, four pyridinium ionic liquids (**2a–2d**, see Fig. 1) were investigated for biomass dissolution with DMSO-d_6 as co-solvent and ball-milled poplar as the model sample. Due to the pyridinium salt's high melting point, the co-solvent DMSO-d_6 was used to render the sample able to be dissolved at a lower temperature and facilitate NMR analysis by reducing viscosity of the resulting mixtures. A mixture of 1 : 2 [Apyr]Cl (1-allylpyridinium chloride, **2a**) and DMSO-d_6 (1.00 g) was found to dissolve 35 mg ball-milled poplar at 60 °C in 1 h as the suspended poplar particles disappeared and a homogeneous solution resulted, with increased viscosity and coloration of the solution. Furthermore,

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Table 1 Solubility of biomass in ionic liquid/DMSO-d_6 bi-solvent system[a]

Ionic Liquid (IL)	IL : DMSO-d_6	Biomass	Time/h	Solubility/ mg g^{-1}
[Apyr]Cl **2a**	1 : 2	Poplar[b]	1	35
[Apyr]Br **2b**	1 : 2	Poplar[b]	6	<5
[Cmpyr]Cl **2c**	1 : 2	Poplar[b]	1	70
[Hpyr]Cl **2d**	1 : 2	Poplar[c]	1	80
None	—	Poplar[b,c]	6	—
[Cmpyr]Cl **2c**	1 : 2	Poplar[d]	6	70
[Hpyr]Cl **2d**	1 : 2	Poplar[d]	6	80
[Cmpyr]Cl-d_7 **2c′**	1 : 2	Poplar[b]	1	70
[Hpyr]Cl-d_6 **2d′**	1 : 2	Poplar[b]	1	80
[Hpyr]Cl-d_6 **2d′**	1 : 2	Poplar[d]	6	80
[Hpyr]Cl-d_6 **2d′**	1 : 2	Switchgrass[d]	6	100

[a] In all experiments dry biomass dispersed in 1.0 g bi-solvent system consisting of pyridinium salt and DMSO-d_6 was stirred at 60 °C under nitrogen for specific time except where indicated. [b] Sample was the ball-milled biomass. [c] The mixture was heated at 80 °C. [d] Sample was non-ball-milled biomass (20 mesh).

direct ^{1}H and ^{13}C NMR analysis of the dissolved poplar solution supported this claim, as the spectral data contained signals readily attributed to lignin and polysaccharides. In contrast, the use of [Apyr]Br (1-allylpyridinium bromide, **2b**) to replace [Apyr]Cl led to significantly reduced solubility of poplar under identical conditions (<5 mg g^{-1}).

It is therefore clear that chloride as the ionic liquid anion is crucial for our present system, which agrees with Rogers' speculation that chloride is highly effective in interrupting the extensive hydrogen-bonding cellulose network presented in plant cell walls. In view of these promising results, we investigated additional pyridinium ionic liquids [Cmpyr]Cl (cyanomethylpyridinium chloride, **2c**) and [Hpyr]Cl (pyridinium chloride, **2d**) for the bi-solvent system. Compared with [Apyr]Cl as the ionic liquid, both [Cmpyr]Cl and [Hpyr]Cl were found to significantly increase poplar's solubility, as shown in Table 1. Further control experiments showed that in the absence of pyridinium chloride [Cmpyr]Cl or [Hpyr]Cl, no dissolution of poplar can be detected by direct NMR analysis even under elevated temperature and prolonged heating. Thus, the presence of the pyridinium chloride salt is crucial for the dissolution of the ball-milled sample.[13]

Subsequent studies demonstrated that the non-ball-milled poplar sample (an average particle size of 20 mesh by Wiley mill) exhibited paralleled solubility in 1 : 2 [Cmpyr]Cl/DMSO-d_6 or [Hpyr]Cl/DMSO-d_6 system, though a prolonged time of 6 h is needed to dissolve the sample. It is noteworthy to stress that the dissolution of Wiley milled biomass is of exceptional interest, since ball-milling has been reported to induce some chemical changes in the structure of lignin.[14,15] At this stage, perdeuterated [Hpyr]Cl-d_6 **2d′** was prepared from pyridine-d_5 (see Fig. 2), and the perdeuterated bi-solvent system consisting of 1 : 2 [Hpyr]Cl-d_6/DMSO-d_6 was tested for direct dissolution and NMR analysis of plant cell walls.[16]

The ^{1}H NMR spectra of both ball-milled and Wiley milled poplar samples dissolved in the [Hpyr]Cl-d_6/DMSO-d_6 system, as shown in Fig. 3a and 3b, serve to illustrate signals from both lignin and polysaccharides.[17] Furthermore, both ball-milled and Wiley milled poplar samples showed parallel ^{13}C spectra, as

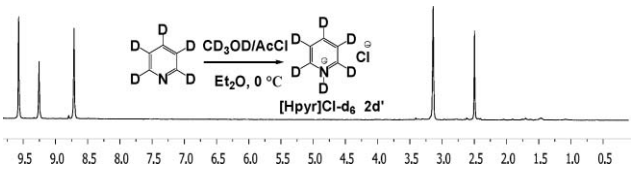

Fig. 2 Preparation and ^{1}H NMR spectra of [Hpyr]Cl-d_6 (in DMSO-d_6 with 99.9 atom% D).

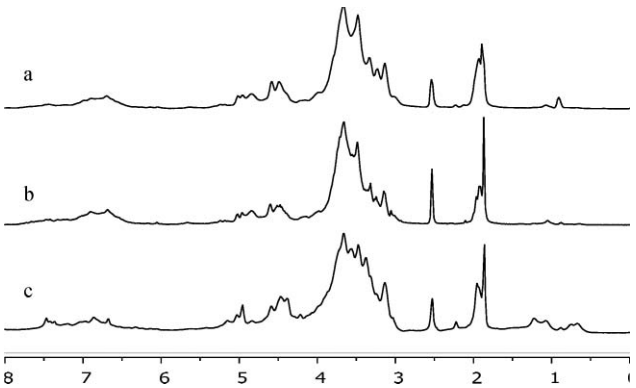

Fig. 3 ^{1}H NMR spectra of biomass solution after dissolving poplar in 1 : 2 pyridinium molten salt/DMSO-d_6 (1.0 g) at 60 °C. (a) Ball-milled poplar (80 mg) in 1 : 2 [Hpyr]Cl-d_6/DMSO-d_6; (b) Wiley milled poplar (80 mg) in 1 : 2 [Hpyr]Cl-d_6/DMSO-d_6; (c) Wiley milled switchgrass sample (100 mg) in 1 : 2 [Hpyr]Cl-d_6/DMSO-d_6.

presented in Fig. 4a and 4b.[18] The signals at δ 61.5, 74.1, 75.8, 76.9, 80.1, and 103.0 ppm were in part attributed to cellulose. Whereas, the lignin methoxyl group corresponded to the signal at δ 57 ppm and δ 58–88 ppm can be attributed, in part, to C_β in β-O-4, C_γ/C_α in β-O-4, β-5, and β-β. The signal at δ 106 ppm was attributed to C2/6 resonance of syringyl-like lignin structures, and between 110 and 120 ppm to C2, C5 and C6 resonance of guaiacyl-like lignin structures (see Fig. 5 for structure of cellulose, β-O-4, β-β, syringyl and guaiacyl-like lignin structures). In addition, ^{1}H and ^{13}C NMR analysis of Wiley milled switchgrass revealed similar spectral data, as shown in Fig. 3c and Fig. 4c.

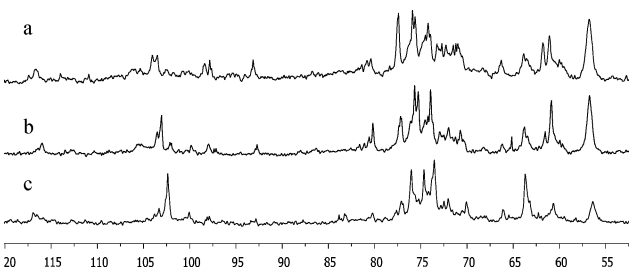

Fig. 4 ^{13}C NMR spectra of sample solution after dissolving biomass sample in 1 : 2 pyridinium molten salt/DMSO-d_6 (1.0 g) at 60 °C. (a) Ball-milled poplar (80 mg) in 1 : 2 [Hpyr]Cl-d_6/DMSO-d_6; (b) Wiley milled poplar (80 mg) in 1 : 2 [Hpyr]Cl-d_6/DMSO-d_6; (c) Wiley milled switchgrass (100 mg) in 1 : 2 [Hpyr]Cl-d_6/DMSO-d_6.

Switchgrass lignin contains a typical HGS lignin that can be readily analyzed by HSQC NMR. The HSQC spectra of the plant cell wall are summarized in Fig. 6 to illustrate the aliphatic side chain and the aromatic ring ^{13}C-^{1}H correlations of the lignin component, by comparing with the literature data.[19] The main

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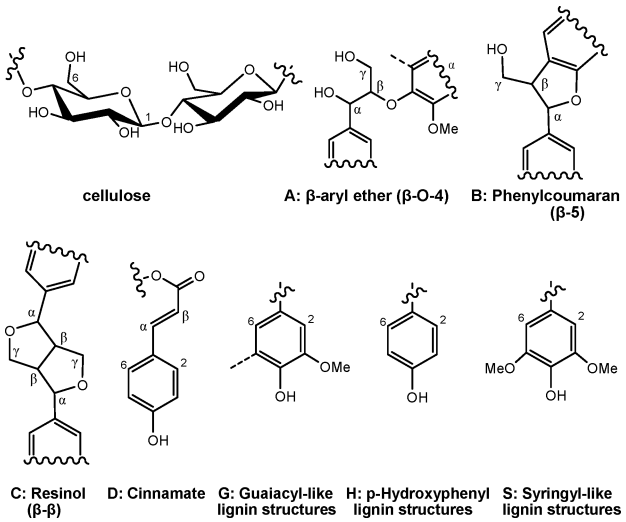

Fig. 5 Structures of cellulose and sub-lignin units.

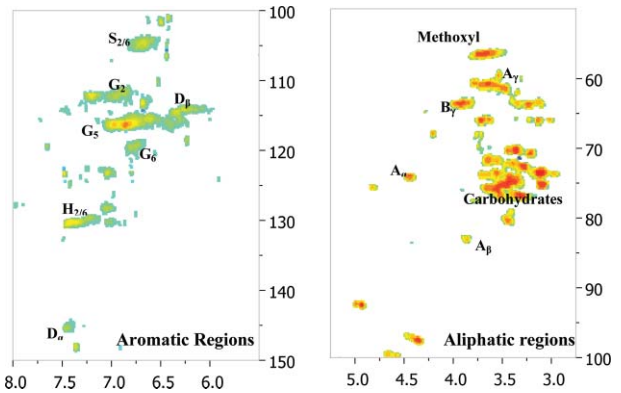

Fig. 6 2D HSQC spectra of switchgrass solution after dissolving 100 mg Wiley milled sample in 1 : 2 [Hpyr]Cl-d_6/DMSO-d_6 (1.0 g) at 60 °C.

cross signals in the aromatic region correspond to syringyl-like (S) and guaiacyl-like (G) units which appeared separately. The S unit cross peaks for the C2,6/H2,6 ($S_{2/6}$) correlation appear at δ_C/δ_H 104.7/6.8 ppm. The G units showed different correlations at δ_C/δ_H 112.1/6.9, 115.8/6.7, 119.4/6.9 ppm for C2/H2 (G_2), C5/H5 (G_5) and C6/H6 (G_6), respectively, as shown in Fig. 6. Strong correlation signals at δ_C/δ_H 130.5/7.4 and 128.9/7.1 ppm were observed to reveal the presence of *p*-hydroxyphenyl (H in Fig. 5) unit. In addition, the HSQC spectra clearly revealed correlation signals at δ_C/δ_H 114.0/6.4 ppm (D_β) and 145.9/7.4 ppm (D_α) from *p*-coumarate and ferulate (Fig. 5), which agree with the report that the grass lignin contains *p*-coumarate and ferulate units.[20] In the aliphatic (side chain) region of lignin, the cross signal for methoxyl group was the most prominent at δ_C/δ_H 57.3/3.7 ppm with a significant amount of β-O-4 substructures (A in Fig. 5) as observed for α-, β- and γ-C positions at δ_C/δ_H 74.5/4.5, 84.8/3.8, and 60.0/3.6 ppm, respectively. The presence of phenyl coumaran substructures (B in Fig. 5) was confirmed by C–H correlations for δ_C/δ_H 63.8/3.9 ppm. Similarly, the Wiley milled poplar sample also affords satisfactory HSQC spectra, as shown in Fig. 7.

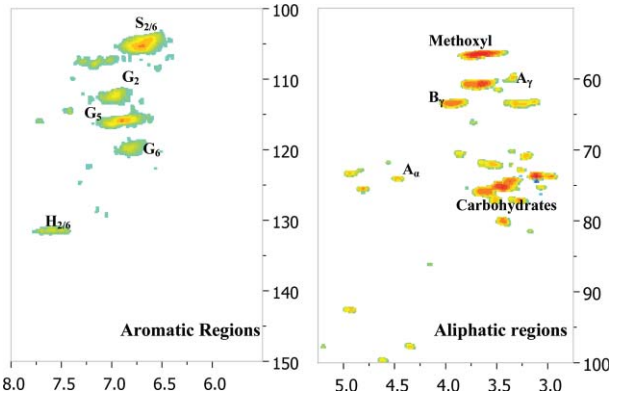

Fig. 7 2D HSQC spectra of poplar solution after dissolving 80 mg Wiley milled sample in 1 : 2 [Hpyr]Cl-d_6/DMSO-d_6 (1.0 g) at 60 °C.

The unique properties and easy preparation of perdeuterated pyridium molten salt [Hpyr]Cl-d_6 offer significant benefits over imidazolium molten salts for NMR analysis of plant cell walls. Most importantly, the use of non-ball-milled samples in our present method can provide a more efficient and accurate characterization of lignin in the plant cell walls. Future applications of this novel ionic liquid based solvent system include the determination of lignin content and HSG ratio of lignin *via* direct dissolution and NMR analysis of the plant cell walls.

Experimental

Materials

Poplar and switchgrass were procured from the National Renewable Energy Laboratory (NREL), which were Wiley milled to pass a 20-mesh screen. The samples were air dried overnight and then Soxhlet extracted with a benzene–ethanol (2 : 1, v/v) mixture for 24 h followed by an additional 24 h ethanol extraction. The extracted residue was air-dried overnight and then dried to constant weight under vacuum before use. Ball milled samples are prepared by rotary ball-milling in a porcelain jar with ceramic balls for 7 days. DMSO-d_6 (99.9 atom% D), pyridine-d_5 (99.5 atom% D), methanol-d_4 (99 atom% D) and all other solvents and reagents were obtained from Sigma–Aldrich.

Plant cell walls dissolution

Dry cell wall sample (ball-milled or Wiley milled), 1.00 g mixture of 1 : 2 IL/DMSO-d_6 (DMSO-d_6, 99.9 atom% D) and spin bar (10 mm × 3 mm) were added to 10 mL vial, and the mixture was flushed with nitrogen and then capped. After being vigorously stirred in a 60 °C (or 80 °C) oil bath for the specific time, the solution was transferred to a 5 mm NMR tube, flushed with nitrogen, and then capped before NMR analysis.

NMR characterization

All one-dimensional qualitative ^{1}H and ^{13}C NMR spectra were acquired using a Bruker Avance/DMX 400 MHz spectrometer. ^{1}H (400 MHz, 256 scans) and ^{13}C NMR (100.59 MHz, 12288 scans) was operated at 323 K for each sample. Two-dimensional HSQC NMR correlation spectra were recorded in a Bruker

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DRX 500 spectrometer. The HSQC analysis was performed using a standard Bruker pulse sequence with a 90° pulse, 0.11 s acquisition time, a 1.5 s pulse delay, a $^{1}J_{C\text{-}H}$ of 145 Hz and acquisition of 256 data points.

Synthesis and characterization of ionic liquids

The general preparation procedure of pyridinium salts **2a–c**, and [Cmpyr]Cl-d_7 **2c**-d_7 is as follows: to a dry toluene (10 mL) solution of chloroacetonitrile-d_2 (1.5 g, 20 mmol), pyridine-d_5 (1.68 g, 20 mmol) was added dropwise under nitrogen atmosphere at room temperature. The reaction mixture was stirred with reflux at 110 °C for 12 h. After removal of toluene under reduced pressure, the resulting solid was washed with an excess amount of anhydrous diethyl ether repeatedly. The residual solid was dissolved in dichloromethane, and the resulting solution was passed through a column filled with neutral activated alumina. After removal of dichloromethane, residual solid was dried *in vacuo* at 60 °C for 24 h to give 2.70 g [Cmpyr]Cl-d_7 **2c′** as a grey solid in 85% yield.

The preparation precedure of **2d′** [Hpyr]Cl-d_6 is described as follows: a mixture of methanol-d_4 (1.44 g, 40 mmol) and pyridine-d_5 (3.36 g, 40 mmol) in 20 mL anhydrous diethyl ether was added into 50 mL round bottom flask and stirred in ice water for 2 min. Then, a solution of acetyl chloride (3.14 g, 40 mmol) in 5 mL anhydrous diethyl ether was added into the reaction mixture in 5 min. The reaction mixture was stirred for 30 min and filtered to get the white solid, which was further purified *in vacuo* to remove the trace amount of diethyl ether to afford 4.37 g pyridinium chloride-d_6 in 90% yield.

Acknowledgements

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Notes and references

1 I. T. Horvath and P. T. Anastas, *Chem. Rev.*, 2007, **107**, 2169–2173.
2 (*a*) A. J. Ragauskas, C. K. Williams, B. H. Davison, G. Britovsek, J. Cairney, C. A. Eckert, W. J. Frederick, J. P. Hallett, D. J. Leak, C. L. Liotta, J. R. Mielenz, R. Murphy, R. Templer and T. Tschaplinski, *Science*, 2006, **311**, 484–489; (*b*) L. Petrus and M. A. Noordermeer, *Green Chem.*, 2006, **8**(10), 861–867; (*c*) J. H. Clark, V. Budarin, F. E. I. Deswarte, J. J. E. Hardy, F. M. Kerton, A. J. Hunt, R. Luque, D. J. Macquarrie, K. Milkowski, A. Rodriguez, O. Samuel, S. J. Tavener, R. J. White and A. J. Wilson, *Green Chem.*, 2006, **8**, 853–860; (*d*) A. Corma, S. Iborra and A. Venlty, *Chem. Rev.*, 2007, **107**, 2411–2502; (*e*) G. W. Huber and A. Corma, *Angew. Chem., Int. Ed.*, 2007, **46**, 7184–7201; (*f*) Y. Pu, D. Zhang, P. M. Singh and A. J. Ragauskas, *Biofuels, Bioprod. Biorefin.*, 2008, **2**, 58.
3 (*a*) *Plant Cell Wall Polymers. Biogenesis and Biodegradation*, ed. N. G. Lewis, and M. G. Paice, vol. 399, Am. Chem. Soc., Washington, DC, 1989, pp. 1–676; (*b*) *Forage Cell Wall Structure and Digestibility*, ed. H. G. Jung, D. R. Buxton, R. D. Hatfield, and J. Ralph, ASA-CSSA-SSSA, Madison, WI, 1993, pp. 1–974.
4 L. B. Davin and N. G. Lewis, *Curr. Opin. Biotechnol.*, 2005, **16**(4), 407–415.
5 (*a*) A. R. Gaspar, J. A. Gamelas, A. F. Jose, D. V. Evtuguin and N. C. Pascoal, *Green Chem.*, 2007, **9**(7), 717–730; (*b*) M. Sticklen, *Curr. Opin. Biotechnol.*, 2006, **17**(3), 315–319; (*c*) L. Pena and A. Seguin, *Trends Biotechnol.*, 2001, **19**(12), 500–506; (*d*) M. Baucher, M. Petit-Conil and W. Boerjan, *Crit. Rev. Biochem. Mol. Biol.*, 2003, **38**, 305–350.
6 D. J. Yelle, J. Ralph and C. R. Frihart, *Magn. Reson. Chem.*, 2008, **46**, 508–517.
7 (*a*) T. Welton, *Chem. Rev.*, 1999, **99**, 2071; (*b*) P. Wasserchеid and W. Keim, *Angew. Chem., Int. Ed.*, 2000, **39**, 3772; (*c*) R. A. Sheldon, *Chem. Commun.*, 2001, 2399; (*d*) S. Zhu, Y. Wu, Q. Chen, Z. Yu, Ziniu, C. Wang, S. Jin, Y. Ding and G. Wu, *Green Chem.*, 2006, **8**(4), 325–327.
8 (*a*) D. A. Fort, R. C. Remsing, R. P. Swatloski, P. Moyna, G. Moyna and R. D. Rogers, *Green Chem.*, 2007, **9**(1), 63–69; (*b*) R. P. Swatloski, S. K. Spear, J. D. Holbrey and R. D. Rogers, *J. Am. Chem. Soc.*, 2002, **124**, 4974.
9 (*a*) Y. Fukaya, K. Hayashi, M. Wada and H. Ohno, *Green Chem.*, 2008, **10**(1), 44–46; (*b*) J. Vitz, T. Erdmenger, C. Haensch and U. S. Schubert, *Green Chem.*, 2009, **11**, 417–424.
10 (*a*) C. Tsioptsias, A. Stefopoulos, I. Kokkinomalis, L. Papadopoulou and C. Panayiotou, *Green Chem.*, 2008, **10**(9), 965–971; (*b*) H. Zhao, G. A. Baker, Z. Song, O. Olubajo, T. Crittle and D. Peters, *Green Chem.*, 2008, **10**(6), 696–705; (*c*) C. Li, Q. Wang and Z. K. Zhao, *Green Chem.*, 2008, **10**(2), 177–182; (*d*) H. Xie, A. King, I. Kilpelainen, M. Granstrom and D. S. Argyropoulos, *Biomacromolecules*, 2007, **8**(12), 3740–3748; (*e*) I. Kilpelaeinen, H. Xie, A. King, M. Granstrom, S. Heikkinen and D. S. Argyropoulos, *J. Agric. Food Chem.*, 2007, **55**(22), 9142–9148; (*f*) C.-F. Liu, R.-C. Sun, A.-P. Zhang, M.-H. Qin, J.-L. Ren and X. A. Wang, *J. Agric. Food Chem.*, 2007, **55**(6), 2399–2406; (*g*) A. P. Abbott, T. J. Bell, S. Handa and B. Stoddart, *Green Chem.*, 2006, **8**(9), 784–786; (*h*) S. Barthel and T. Heinze, *Green Chem.*, 2006, **8**(3), 301–306; (*i*) J. Wu, J. Zhang, H. Zhang, J. He, Q. Ren and M. Guo Meili, *Biomacromolecules*, 2004, **5**(2), 266–268.
11 Y. Pu, N. Jiang and A. J. Ragauskas, *J. Wood Chem. Technol.*, 2007, **27**(1), 23–33.
12 (*a*) C. Hardacre, J. D. Holbrey and S. E. J. McMath, *Chem. Commun.*, 2001, 367–368; (*b*) R. Giernoth and D. Bankmann, *Eur. J. Org. Chem.*, 2008, 2881–2886.
13 It is important to stress that under identical conditions, the 1 : 2 [bmim]Cl/DMSO-d_6 bi-solvent system showed significantly lower solubility (45 mg g^{-1} for the ball-milled poplar sample) than the [Hpyr]Cl/DMSO-d_6 system, confirming the advantage of the pyridinium salt for biomass dissolution.
14 (*a*) H. M. Chang, E. B. Cowling, W. Brown, E. Adler and E. G. Miksche EG, *Holzforschung*, 1975, **29**, 153–159; (*b*) T. Kondo, T. Watanabe, T. Ohshita and T. Kyuma, *J. Sci. Food Agric.*, 1995, **68**(3), 383–388; (*c*) T. Ikeda, K. Holtman and J. F. Kadla, *J. Agric. Food Chem.*, 2002, **50**(1), 129–135.
15 It has to be mentioned that the dissolution of Wiley milled biomass also allows significantly increased efficiency for a fast NMR characterization of the plant cell walls, due to the prolonged ball-milling procedure (up to 7 days), which is essential for the biomass dissolution in the 1-methylimidazole-d_6/DMSO-d_6 system.
16 It has to be pointed out that the [Cmpyr]Cl/DMSO-d_6 system not only resulted in higher viscosity of the biomass solution, but perdeuterated [Cmpyr]Cl-d_7, prepared from pyridine-d_5, in combination with DMSO-d_6 for the biomass dissolution showed a significant signal at 6.10 ppm, which comes from D/H exchange due to the acidity of the N+CD_2CN moiety in [Cmpry]Cl-d_7 and active protons in dissolved biomass sample. Thus, it is obvious that [Hpyr]Cl-d_6/DMSO-d_6 can serve as the better system for NMR of plant cell wells, especially for 2D NMR analysis.
17 It is worthy of note that signals between 6.0 ppm and 8.0 ppm in ^{1}H NMR spectra are exclusively attributed to lignin, and this allows us to use a linear extrapolation method to determine lignin content, and a manuscript is currently in preparation.
18 Despite of similarity of the qualitative ^{13}C NMR spectra of ball-milled and Wiley milled poplar sample, quantitative ^{13}C NMR analyses of Wiley-milled and ball-milled plant cell wall are under investigation to confirm and extend these results, and reveal HSG lignin ratio *via* integration of various lignin carbons.
19 (*a*) S. A. Ralph, J. Ralph and L. L. Landucci, *NMR Database of Lignin and Cell Wall Model Compounds*, 2004. Available

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at http://ars.usda.gov/Services/docs.htm?docid=10491; (*b*) J. Rencoret, G. Marques, A. Gutierrez, D. Ibarra, J. Li, G. Gellerstedt, J. I. Santos, J. J. Barbero, A. T. Martinez and J. C. del Rio, *Holzforschung*, 2008, **62**(5), 514–526; (*c*) J. C. Rio, J. Rencoret, G. Marques, A. Gutierrez, D. Ibarra, J. I. Santos, J.-J. Barbero, L. Zhang and A. T. Martinez, *J. Agric. Food Chem.*, 2008, **56**, 9525–9534.

20 J. Ralph, J. M. Marita, S. A. Ralph, R. D. Hatfield, F. Lu. R. M. Ede, J. Peng, S. Quideau, R. F. Helm, J. H. Grabber, H. Kim, G. Jimenez-Monteon, Y. Zhang, H.-J. G. Jung, L. L. Landucci, J. J. Mackay, R. R. Sederoff, C. Chapple, and A. M. Boudet *Advances in Lignocellulosics Characterization*, ed. D. S. Argyropoulos, Tappi Press, Atlanta, USA, 1999, pp. 55–108.

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