Special issue research article

MRC

Received: 6 June 2015 Revised: 20 October 2015 Accepted: 24 November 2015 Published online in Wiley Online Library: 13 January 2016

(wileyonlinelibrary.com) DOI 10.1002/mrc.4400

Di(ethylene glycol) methyl ether methacrylate (DEGMEMA)-derived gels align small organic molecules in methanol

Manuela E. García,[a,b] Shannon R. Woodruff,[c,d] Erich Hellemann,[a] Nicolay V. Tsarevsky[c,d] and Roberto R. Gil[a]*

Residual dipolar couplings (RDCs) constitute an important NMR parameter for structural elucidation in all areas of chemistry. In this study, di(ethylene glycol) methyl ether methacrylate (DEGMEMA)-based gels are introduced as alignment media for the measurement of RDCs of small organic molecules in polar solvents such as methanol. The low viscosity of methanol permits the execution of *J*-scaled BIRD HSQC experiments that yield very sharp lines in anisotropic conditions. The gels have excellent mechanical properties, and their compression and expansion in the swollen state can be reversed and performed multiple times. This process enables the easy loading and release of analytes. The excellent performance of these new aligning gels is demonstrated by analyzing the structure of the alkaloid retrorsine. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords: ^{1}H; ^{13}C; *J*-scaled BIRD HSQC; residual dipolar couplings; DEGMEMA gels; alignment media; retrorsine

Introduction

There is no question that NMR is one of the most powerful techniques in the area of structural analysis in organic and biological chemistry. During the past 10 years, there has been a breakthrough in the field, which is largely because of the emergence of residual dipolar coupling (RDC) analysis as a powerful tool for the constitutional, configurational, and conformational analysis of small organic molecules in solution.[1] Although RDC analysis already complements existing powerful NMR techniques, such as the measurement of NOE or ^{3}J couplings (both proton–proton[2,3] and proton-carbon[4]), RDCs also eliminate structural ambiguities that often result from these two NMR parameters.[5–9] The main advantage of RDCs over these classical NMR parameters is that they provide information of nonlocal character and allow the determination of relative orientation of internuclear vectors (e.g. CH bonds), regardless of the distance between them.

A crucial point for the measurement of anisotropic parameters is the availability of adequate alignment media to partially align the molecules of interest. While several such media for aqueous[10–12] and apolar[13–18] solvents have been found in the field of NMR spectroscopy, the situation for polar organic solvents such as DMSO (one of the most commonly used solvents in the pharmaceutical field) or methanol is different. Some interesting DMSO-compatible alignment media have been developed within the last decade. An early example was cross-linked poly(vinyl acetate) (PVAc),[19] which showed wide compatibility with most polar organic solvents. Later, cross-linked polyacrylonitrile (PAN)[20] was synthesized and employed successfully as an alignment medium in which disadvantages, such as residual signals (polymer or impurities), were overcome. However, equilibration times of the samples were long, and the synthetic procedures were rather tedious and involved the use of electron beam irradiation to achieve cross-linking. Additionally, neutral and anionically charged (containing sulfonate groups) acrylamide derivatives were copolymerized in the presence of cross-linkers, producing gels with varying fractions of ionic groups. These derivatives were found to be compatible with different solvents even though certain charged molecules could not be analyzed.[21,22] More recently, Luy and co-workers developed a versatile cross-linked poly(ethylene oxide) (PEO)[23] as an alignment medium, which covers an extensive range of solvents, from water and polar organic solvents (including DMSO, CH_3OH, and CH_3CN) to apolar organic solvents (including dioxane, THF, DCM, $CHCl_3$, and benzene). This is probably one of the most widely applicable alignment media as a result of its large solvent compatibility range. A wide range of chiral lyotropic liquid crystalline phases (LLC), compatible with organic solvents ($CDCl_3$ and DMSO), have also been used to align small organic molecules.[24] Very recently, a new type of alignment medium based on graphene oxide was reported for very polar solvents.[25]

Although it seems that there are many examples, the reality is that this area of research is in its infancy as there are many

* *Correspondence to: Roberto R. Gil, Department of Chemistry, Carnegie Mellon University, 4400 Fifth Avenue, Pittsburgh, PA 15213, USA. E-mail: rgil@andrew.cmu.edu*

a *Department of Chemistry, Carnegie Mellon University, 4400 Fifth Avenue, Pittsburgh, PA, 15213, USA*

b *Department of Organic Chemistry, National University of Cordoba, Medina Allende y Haya de La Torre, Cordoba 5000, Argentina*

c *Department of Chemistry, Southern Methodist University, Dallas, TX, USA*

d *Center for Drug Discovery, Design, and Delivery in Dedman College, Southern Methodist University, Dallas, TX, USA*

drawbacks and challenges to be overcome. Based on the need to find new alignment media compatible with polar organic solvents, we propose the use of a new orienting gel that exhibits good alignment properties in methanol-d_4 (CD_3OD). Methanol is an appropriate solvent for important classes of molecules that are insoluble in other organic solvents, including sugars, peptides, polar natural products, and other compounds. Herein, we propose the use of compressed di(ethylene glycol) methyl ether methacrylate (DEGMEMA)-based gels for partial orientation of small molecules in methanol.

Experimental

All gels were prepared in 3-mm disposable NMR tubes, which were pre-silanized (in order to prevent strong adhesion of the gel to the glass) using the following procedure. A 10 % (v/v) solution of 1,1,1,3,3,3-hexamethyldisilazane in toluene was prepared and added to each tube. The tubes were capped with rubber septa, sealed with parafilm, and left to sit for 24 h. Afterwards, the tubes were emptied and air-dried over mild heat.

The monomer, DEGMEMA (10 ml, 5.42×10^{-2} mol, after being passed through a column filled with basic alumina in order to remove the polymerization inhibitor in the commercial reagent), was mixed with 2 ml of CH_3OH. Ethylene glycol dimethacrylate (EGDMA) was used as the cross-linker after being passed through a column filled with basic alumina, while 2,2′-azobis(2,4-dimethyl-4-methoxyvaleronitrile) (V-70) was employed as the polymerization initiator. Four vials were labeled A, B, C, and D. Each vial was charged with a different amount of V-70 and EGDMA (with V-70 being calculated at 10 mol % of EGDMA) as follows: vial A-0.6 mg of V-70 and 3.4 μl of EGDMA; vial B-0.8 mg of V-70 and 5.1 μl of EGDMA; vial C-1.1 mg of V-70 and 6.8 μl of EGDMA; and vial D-1.7 mg of V-70 and 10.2 μl of EGDMA. Then, 2.5 ml of the DEGMEMA/CH_3OH mixture were then added to each vial. Once the contents were mixed, the silanized 3-mm NMR tubes were filled with solutions A, B, C, or D. These four tubes were capped with rubber septa and submersed in an oil bath at 50 °C for 6 h. Following gelation, the gels dried in their tubes for 3 days. The tubes were then carefully broken, and the gels were removed (while taking care to not damage them) and placed in CH_3OH for 3 h to swell and wash out any remaining monomer. After washing, the gels were removed, dried, and stored in glass tubes to prevent bending during drying. The gels decreased in size significantly upon drying (2.1-mm diameter on average) and have a flexible, rubbery, and sticky consistency. For RDC measurements, 2.5-cm rods were cut and swollen in CD_3OD inside 5-mm NMR tubes. All synthesized gels were tested for their mechanical properties and ^2H quadrupolar splitting ($\Delta\nu_Q$) of the ^2H NMR signal of CD_3OD. The fraction of crosslinker relative to monomer in composition C was found to be useful for poly(methyl methacrylate)-based gels,[15] and in this study, we prepared gels with both higher and lower crosslink densities in order to identify the optimally performing one. Gel A demonstrated the best mechanical properties and was used for the following experiments.

Retrorsine, a member of the natural pyrrolizidine family of alkaloids, was used to test the alignment capabilities of Gel A. This molecule has four stereocenters with the well-established configuration, 7*R*,8*R*,12*S*,13*R* (Fig. 1).[26] For simplicity, the configuration of the stereocenters will be shown without numbers and in the same order for every diastereomer, as displayed in Fig. 2.

Retrorsine (3 mg) was dissolved in CD_3OD (200 μl) and placed in a 5-mm NMR tube containing the clean and fully relaxed swollen polyDEGMEMA gel stick. A Shigemi plunger was inserted into the tube and used to pump the gel several times in order to let the compound diffuse into the gel. A 1D ^1H NMR spectrum was collected; it showed that the sample was inside the gel within a few

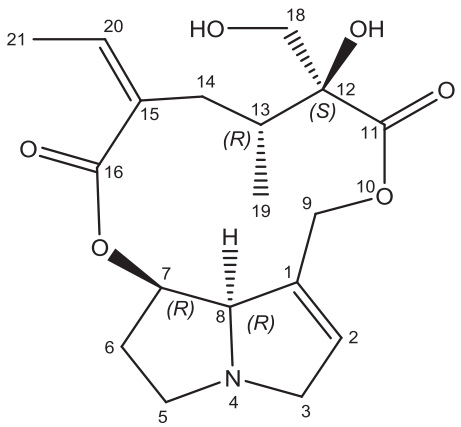

Figure 1. Retrorsine structure with its stereocenter configurations.

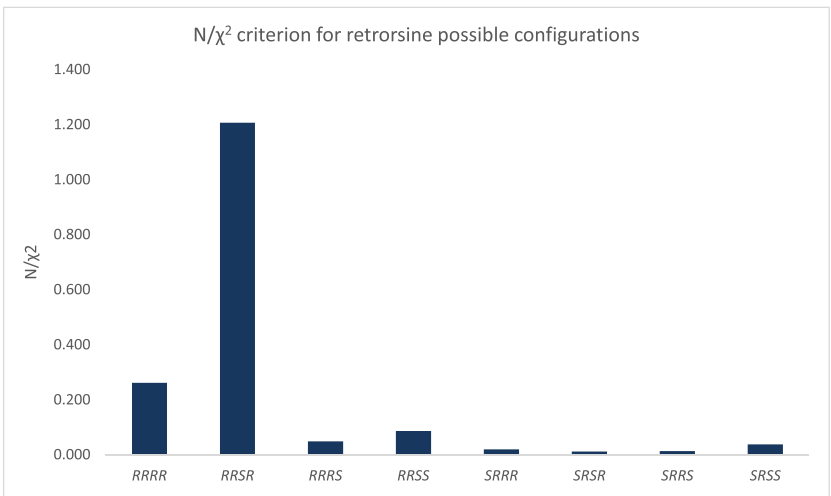

Figure 2. N/χ^2 fit for the eight possible configurations of retrorsine.

minutes time. For the purpose of measuring the $\Delta\nu_Q$ of the solvent, a ^{2}H NMR spectrum was obtained, showing a small ^{2}H quadrupolar splitting value for the –OD signal (6.14 Hz) for the fully compressed gel. Moreover, a sample (5 mg) of alkaloid was dissolved in CD_3OD for its isotropic measurements. *J*-scaled BIRD HSQC spectra with a scaling factor (κ) of 4 were acquired under both isotropic and anisotropic conditions.[27,28]

Results and Discussion

The DEGMEMA-based gels described in this work are affordable as well as easy to prepare and use. Their chemical structure, being without aromatic rings, minimizes chemical shift perturbation. In addition, they are flexible, have reversible compression, and the swelling time is short (a few hours). These gels can be stored dry, are very stable over time, and allow sample recovery. In our view, the most remarkable feature and advantage is that excellent resolution is obtained even under anisotropic conditions as a result of the low viscosity of CD_3OD.

Similar to other gels, polymer background signals are still observed and overlap with the analyte signals is probable within a limited region (3.4–3.7 ppm). Furthermore, although these gels exhibit a low degree of alignment resulting in small $^1D_{CH}$ values, these parameters can be extracted and measured with great accuracy because of the high quality of the spectra and narrow lines. It is well-known that one of the most common experimental problems to measure RDCs is the significant proton–proton dipolar broadening of the signals under anisotropic conditions. In order to overcome this disadvantage, we have been avoiding the use of F2 ^{1}H-coupled HSQC experiments to measure one-bond ^{1}H,^{13}C RDCs ($^1D_{CH}$) since 2011,[29] and instead, we have measured RDCs from the F1 ^{1}H-coupled version of HSQC experiments.[27,28] Added to this, we later found that the *J*-scaled BIRD version of this experiment generated RDCs with higher accuracy.[28,29] The resolution can be further enhanced by the use of low viscosity solvents, such as CD_3OD, as demonstrated in this work (Fig. 3).

One-bond proton-carbon RDCs ($^1D_{CH}$) were extracted as the difference between the signal splitting observed under anisotropic conditions ($^1T_{CH} = {}^1J_{CH} + {}^1D_{CH}$) and the splitting observed under isotropic conditions ($^1J_{CH}$), as shown in Table 1. As previously reported, fitting RDC data to all possible configurations and conformations, alignment tensor determination, and calculation of Cornilescu quality (*Q*) factors[30] were performed using MSpin.[31]

Table 1. Retrorsine ^{13}C and ^{1}H NMR chemical shifts, scalar coupling constants $^1J_{CH}$, total splitting $^1T_{CH} = {}^1J_{CH} + {}^1D_{CH}$, and experimental (exp) and calculated (calc) $^1D_{CH}$ values with their corresponding experimental errors

#C	δ_C (ppm)	δ_H (ppm)[a]	$^1T_{CH}$ (Hz)	$^1J_{CH}$ (Hz)	Exp $^1D_{CH}$ (Hz)	Calc $^1D_{CH}$ (Hz)	Error (Hz)
2	135.1	6.20	165.77	168.31	−2.54	−2.54	0.35
3	62.4	a: 3.92	139.81	139.90	−0.10	−0.08	0.24
		b: 3.41	—	139.90	—	−0.08	—
5	52.6	a: 3.27	141.77	140.00	1.80	1.91	0.30
		b: 2.60	141.77	140.00	1.80	1.91	0.30
6	34.3	a: 2.43	132.24	132.53	−0.30	−0.02	0.39
		b: 2.16	132.24	132.53	−0.30	−0.02	0.39
7	75.1	5.08	151.73	159.50	−7.77	−7.66	0.10
8	77.2	4.32	140.88	144.55	−3.67	−3.74	0.17
9	59.5	a: 5.50	145.04	149.26	−4.22	−4.15	0.33
		b: 4.18	145.04	149.26	−4.22	−4.15	0.33
13	35.8	1.75	125.52	128.58	−3.07	−3.2	0.27
14	37.9	a: 2.28	129.33	130.04	−0.77	−0.66	0.47
		b: 1.80	129.33	130.04	−0.77	−0.66	0.47
18	66.7	3.66	144.26	144.21	0.05	−0.24	0.13
19	10.4	0.86	125.38	126.35	−0.97	−0.93	0.13
20	133.9	5.79	154.62	154.45	0.17	0.38	0.17
21	13.8	1.83	126.27	127.30	−1.04	−1.15	0.07

[a]Residual dipolar couplings for CH_2 groups are reported as half of the sum of the couplings of CHa and CHb. The methylene protons are designed as 'a' and 'b', according to its chemical shift order. (—) not measurable (overlapping with signals from gel).

The eight possible structures were initially energy-minimized. Conformational searches were performed using the molecular mechanics force-field in Macromodel (Schrodinger Suite); structures within a window of 2 kcal mol^{-1} were selected from the conformational search. Single tensor approximation with fit to populations was performed for the eight possible configurations and their conformations. The results are presented in the form of Cornilescu quality (*Q*) factors and as N/χ^2 (Fig. 2 and Supporting Information).[32] By using the *Q* factors, six out of the eight configurations were eliminated with certainty. The two remaining structures were the

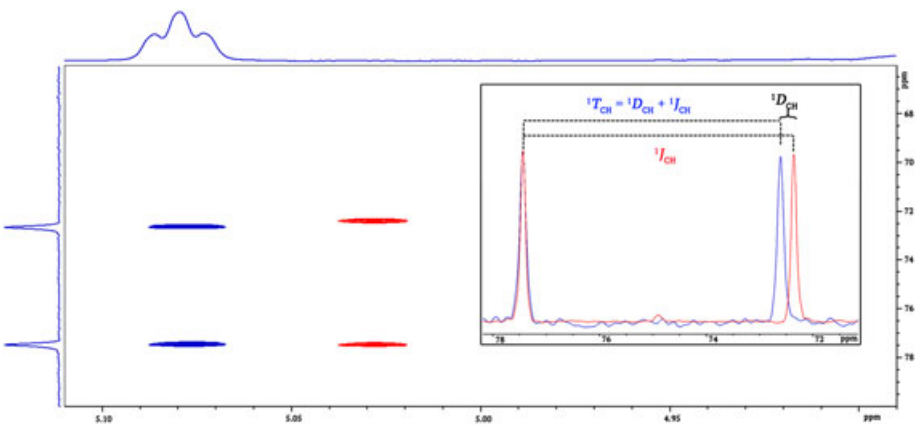

Figure 3. Extraction of CH couplings ($^1T_{CH}$ and $^1J_{CH}$) from the experimental data collected with the *J*-scaled BIRD HSQC experiment. Expanded region of C7-H7 cross-peak with isotropic spectrum shown in red and anisotropic spectrum in blue. Note that the low viscosity of methanol yields very sharp lines in anisotropic conditions.

isomers *RRRR* and *RRSR*, with *RRSR* having the lower *Q* factor (0.081 against 0.094). A better discrimination between these two structures was obtained with the N/χ^2 criterion; the incorrect structure (*RRRR*) had the lowest value and thus was eliminated by this criterion, as shown in Fig. 2. A fit of the experimental RDC data to the X-ray structure of retrorsine obtained by Pretorius and co-workers[26] was performed, resulting in a *Q* factor of 0.050 for the *RRSR* structure. The *Q* factor illustrates the good agreement between X-ray structural analysis and RDCs. The main goal of the present article is to present a new aligning gel compatible with methanol. The alkaloid retrorsine, whose configuration is well-known, was solely used to demonstrate the capabilities of this gel to produce excellent quality RDCs. As shown earlier, RDCs cannot discriminate between the isomers *RRRR* and *RRSR* without ambiguity, but both compounds should show distinct sets of NOE enhancement for the protons H-13, H-14, and H-18. NOE-derived distances should straightforwardly discriminate these two structures, but this discussion is outside the scope of this article.

The very low viscosity of methanol leads to very narrow lines for the signals of the compound, both in isotropic and anisotropic conditions. Although the range of RDC values is smaller than usual (−2.5 to +7.8), these linewidths provide highly accurate values, as reflected in the very low values of the *Q* factor for the correct structure.

In summary, the usefulness of cross-linked polyDEGMEMA for the partial alignment of molecules in polar organic solvents such as CD_3OD was demonstrated. The main advantage of these gels is the remarkable resolution obtained under anisotropic conditions as well as their excellent mechanical properties.

Acknowledgements

NMR instrumentation at CMU was partially supported by the NSF (CHE-0130903 and CHE-1039870). The authors gratefully acknowledge support from the NSF (CHE-1111684 (RRG) and CHE-1111929 (NVT)). M. E. G thanks the NSF for funding her postdoctoral stay.

References

[1] R. R. Gil, C. Griesinger, A. Navarro-Vázquez, H. Sun, *In Structure Elucidation in Organic Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, **2015**, 279–324.
[2] M. Karplus. *J. Chem. Phys.* **1959**, *30*, 11–15.
[3] C. A. G. Haasnoot, F. Deleeuw, C. Altona. *Tetrahedron* **1980**, *36*, 2783–2792.
[4] N. Matsumori, D. Kaneno, M. Murata, H. Nakamura, K. Tachibana. *J. Org. Chem.* **1999**, *64*, 866–876.
[5] C. M. Thiele. *Eur. J. Org. Chem.* **2008**, *34*, 5673–5685.
[6] C. M. Thiele. *Concepts Magn. Reson. Part A* **2007**, *30A*, 65–80.
[7] G. Kummerlöwe, B. Luy. *TrAC. Trends Anal. Chem.* **2009**, *28*, 483–493.
[8] G. Kummerlöwe, B. Luy, A. W. Graham. *Annu. Rep. NMR Spectros* **2009**, *68*, 193–232.
[9] R. R. Gil. *Angew. Chem., Int. Ed.* **2011**, *50*, 7222–7224.
[10] A. Bax. *Protein Sci.* **2003**, *12*, 1–16.
[11] A. Bax, A. Grishaev. *Curr. Opin. Struct. Biol.* **2005**, *15*, 563–570.
[12] J. H. Prestegard, C. M. Bougault, A. I. Kishore. *Chem. Rev.* **2004**, *104*, 3519–3540.
[13] J. C. Freudenberger, P. Spiteller, R. Bauer, H. Kessler, B. Luy. *J. Am. Chem. Soc.* **2004**, *126*, 14690–14691.
[14] B. Luy, K. Kobzar, H. Kessler. *Angew. Chem. Int. Ed.* **2004**, *43*, 1092–1094.
[15] R. R. Gil, C. Gayathri, N. V. Tsarevsky, K. Matyjaszewski. *J. Org. Chem.* **2008**, *73*, 840–848.
[16] G. Kummerlöwe, S. Knoer, A. O. Frank, T. Paululat, H. Kessler, B. Luy. *Chem. Commun.* **2008**, *44*, 5722–5724.
[17] C. M. Thiele. *J. Org. Chem.* **2004**, *69*, 7403–7413.
[18] N.-C. Meyer, A. Krupp, V. Schmidts, C. M. Thiele, M. Reggelin. *Angew. Chem. Int. Ed.* **2012**, *51*, 8334–8338 S8334/8331-S8334/8358.
[19] J. C. Freudenberger, S. Knör, K. Kobzar, D. Heckmann, T. Paululat, H. Kessler, B. Luy. *Angew. Chem. Int. Ed.* **2005**, *44*, 423–426.
[20] G. Kummerlöwe, J. Auernheimer, A. Lendlein, B. Luy. *J. Am. Chem. Soc.* **2007**, *129*, 6080–6081.
[21] P. Haberz, J. Farjon, C. Griesinger. *Angew. Chem. Int. Ed.* **2005**, *44*, 427–429.
[22] M. Schmidt, H. Sun, P. Rogne, G. K. E. Scriba, C. Griesinger, L. T. Kuhn, U. M. Reinscheid. *J. Am. Chem. Soc.* **2012**, *134*, 3080–3083.
[23] C. Merle, G. Kummerloewe, J. C. Freudenberger, F. Halbach, W. Stoewer, C. L. von Gostomski, J. Hoepfner, T. Beskers, M. Wilhelm, B. Luy. *Angew. Chem. Int. Ed.* **2013**, *52*, 10309–10312.
[24] B. Luy. *J. Indian Inst. Sci.* **2010**, *90*, 119–132.
[25] X. Lei, Z. Xu, H. Sun, S. Wang, C. Griesinger, L. Peng, C. Gao, R. X. Tan. *J. Am. Chem. Soc.* **2014**, *136*, 11280–11283.
[26] P. C. Coleman, E. D. Coucourakis, J. A. Pretorius. *S. Afr. J. Chem.* **1980**, *33*, 116–119.
[27] J. Furrer, M. John, H. Kessler, B. Luy. *J. Biomol. NMR* **2007**, *37*, 231–243.
[28] C. M. Thiele, W. Bermel. *J. Magn. Reson.* **2012**, *216*, 134–143.
[29] P. Trigo-Mouriño, A. Navarro-Vázquez, J. Ying, R. R. Gil, A. Bax. *Angew. Chem., Int. Ed.* **2011**, *50*, 7576–7580 S7576/7571-S7576/7531.
[30] G. Cornilescu, J. L. Marquardt, M. Ottiger, A. Bax. *J. Am. Chem. Soc.* **1998**, *120*, 6836–6837.
[31] A. Navarro-Vazquez. *Magn. Reson. Chem.* **2012**, *50*, S73–S79.
[32] G. Kummerlowe, B. Crone, M. Kretschmer, S. F. Kirsch, B. Luy. *Angew. Chem.* **2011**, *50*, 2643–2645.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's website.