Simplified "No-D" NMR Methods for Routine Analysis and Organometallic Reagent Concentration Determination

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Supporting Information

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1 General Information

All NMR analysis was conducted using a *Bruker Fourier Advance III 300HD* spectrometer equipped with a dual ${}^{1}H/{}^{13}C$ channel probe (*DUL 300MHz S1 with Z-gradient*). For samples run in tetrachloroethylene (TCET), the solvent ${}^{1}H$ and ${}^{13}C$ NMR spectra were referenced to tetramethylsilane (0 ppm). Data are reported as follows: chemical shift, multiplicity (br. s = broad singlet, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet). All chemicals were purchased from commercial suppliers and used as received.

2 Procedure for Analysis with Tetrachloroethylene as Solvent

A stock solution of tetramethylsilane (TMS) in TCET (0.5% v/v) was prepared and samples (~5-10 mg) made up with ~0.6 mL of this solvent in standard 5 mm NMR tubes. Within Bruker's *lcon* automation software, the *solvent/probe dependencies* are adjusted. For either the "none" solvent, or a newly defined solvent, the *associated shim routine* is set to "TOPSHIM 1h lockoff olp=-0.5 selwid=0.5" the *associated lock routine* is set to "LOCKOFF". From there, standard NMR experiments are deployed.

2.1 Table of Commonly Encountered Trace Impurities

The spectra used to construct Table 1 are presented in Section 4.

Table 1.	¹ H and ¹³ C	NMR frequ	encies of c	commonly	encountered	trace im	purities with	tetrachloroeth	ylene	TCET) as solvent.	3
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Substance ^b	Proton /mult.	TCET ¹ H ^c	CDCI ₃ ¹ H ^d	Carbon	TCET ¹³ C ^c	CDCI ₃ ¹³ C ^d
residual solvent	-	-	7.26	С	120.45	77.16
water	H ₂ O /br. s	1.04	1.56	-	-	-
acetic acid	CH ₃ /s	2.02	2.10	CO	176.89	175.99
	COOH /br. s	11.83	-	CH ₃	20.09	20.81
acetone	CH ₃ /S	2.03	2.17	CU CH₂	201.5	207.07
acetonitrile	CH ₃ /s	1.89	2.10	CN	114.40	116.43
				CH₃	1.57	1.89
benzaldehyde	CHO /s	9.96	10.03	C(O)	189.50	192.67
	CH(2,0)/III CH(4)/m	7.56-7.49	7.61-7.65	CH(4)	133.53	134.64
	CH(3,5) /m	7.49-7.41	7.51-7.57	CH(2,6)	129.43	129.16
	011/	7.05	7.00	CH(3,5)	128.67	129.91
benzene	CH /s	7.25	7.36	CH	128.13	128.37
tert-butyl alconol	OH₃/s OH /br. s	1.19 1.83–1.30	1.28	C CH₃	68.20 31.36	69.15 31.25
tert-butyl methyl ether	OCH ₃ /s	3.10	3.22	С	71.85	72.87
	CH ₃ /s	1.12	1.19	OCH ₃	48.97	49.45
DUT	ArH /o	6.94	6.09	CH ₃	27.01	26.99
впі	OH /s	0.04	5.98	C(2.6)	135.16	135.87
	ArCH ₃ /s	2.21	2.27	C(4)	127.91	128.27
	ArC(CH ₃) ₃ /s	1.40	1.43	CH(3,5)	125.31	125.55
				$(CH_3)_3C$	34.06	34.25
				CH ₃) ₃ C	21.63	21.20
chloroform	CH /s	7.22	7.26	CH	77.16	77.36
cyclohexane	CH ₂ /s	1.43	1.43	CH ₂	27.17	26.94
cyclohexanone	CH ₂ /t	2.23	2.33	C(O)	207.32	212.57
	CH ₂ /m	1.86-1.75	1.84-1.86	CH ₂	42.69	41.97
	CH ₂ /m	1.73-1.62	1./1–1./3	CH ₂	28.24 26.57	27.00 24.97
1.2-dichloroethane	CH ₂ /s	3.64	3.73	CH ₂	42.69	43.50
dichloromethane	CH ₂ /s	5.23	5.30	CH ₂	53.09	53.52
diethyl ether	CH ₂ /g	3.36	3.48	CH ₂	65.73	65.19
	CH ₃ /t	1.14	1.21	CH ₃	15.45	15.20
1,2-dimethoxyethane	CH ₂ /s CH ₃ /s	3.41 3.28	3.55 3.40	CH ₂ CH ₃	72.04 58.72	71.84 59.08
dimethylformamide	CH /br. s	7.87	8.02	CO	160.59	162.62
	CH ₃ /s	2.86	2.96	CH₃	35.69	36.50
dimethylsulfoxide		2.78	2.88	CH ₃	30.99	31.45
		3.56	3.71		66.99	67 14
ethanol		3.60	3.72	CH ₂	57.7	58.28
	OH /br. s	2.45	1.32	CH ₃	18.44	18.41
ethyl acetate	CH ₂ CH ₃ /q	4.05	4.12	СО	168.77	170.36
,	CH₃CO /s	1.94	2.05	CH ₂	59.69	60.49
	CH ₂ CH ₃ /t	1.21	1.26	CH₃CO	20.60	21.04
hexamethyldisiloxane	CH ₃ /s	0.06	0.07	CH₃ CH₃	1.95	14.19
<i>n</i> -hexane	CH ₂ /m	1.37-1.21	1.27	CH ₂	31.91	31.64
	CH ₃ /t	0.89	0.88	CH ₂	22.96	22.70
		0.50	0.05	CH ₃	14.27	14.14
HMPA		2.59	2.65	CH ₃	36.90	30.87
methanor	OH ₃ /a OH /br. s	2.39	5.49 1.09		50.03	50.41
nitromethane	CH ₃ /s	4.20	4.33	CH₃	61.77	62.50
<i>n</i> -pentane	CH ₂ /m	1.38–1.19	1.27	CH ₂	34.41	34.16
	CH ₃ /t	0.90	0.88	CH ₂	22.65	22.38
2-propanol	CH /m	3 98-3 83	4 04		14.20	14.08
	OH /br. s	2.39	-	CH ₃	24.43	25.14
nvridine		1.13	1.22	CH(2.6)	140.06	140.00
pyridine	CH(2,0)/III CH(4)/m	7.56–7.48	7.68	CH(2,6) CH(4)	134.70	149.90
nyrrole	CH(3,5) /m	7.16-7.09	7.29	CH(3,5)	122.95	123.75
pyrrole	CH(2.5) /m	6.63-6.49	6.83	CH(2,5) CH(3,4)	108.38	107.98
	CH(3,4) /m	6.19-6.02	6.26			
pyrrolidine	CH ₂ (2,5) /m	2.81-2.73	2.87	$CH_2(2,5)$	47.15	46.93
	CH ₂ (3,4) /m NH /br s	1.64–1.57	1.68	$CH_{2}(3,4)$	25.73	25.56
tetrahydrofuran	OCH ₂ /m	3.67–3.58	3.76	OCH ₂	67.50	67.97
	CH ₂ /m	1.80-1.72	1.85	CH ₂	25.84	25.62
toluene	CH(3,5) /m	7.17-7.10	7.25	CH(1)	137.16	137.89
	CH ₃ /s	2.31	2.36	CH(3,5)	128.08	129.07
				CH(4)	125.26	125.33
triathulamir -	011./=	0.40	2.52	CH ₃	21.54	21.46
uletnylamine	CH ₂ /q CH ₂ /t	2.43	2.53		46.55 12.26	46.25 11 61

¹All measurements were recorded using a *Bruker Advance III* 300 MHz spectrometer and reported in ppm. ⁵Approximately 5-10 mg of each sample was used. ⁶A 0.5% v/v solution of TMS in TCET of was used, and spectra calibrated against TMS (0 ppm). ⁶The CDCl₃ data was transcribed from Fulmer *et al. Organometallics*, **2010**, *29*, 2176–2179, and Gottlieb *et al. J. Org. Chem.* **1997**, *62*, 7512–7515. ⁶The ¹³C signal experiences a 3 Hz coupling to phosphorous.

2.2 Natural Product Comparisons

A solution of cyclosporine A (14 mg) was dissolved in 0.5 mL of TCET (0.5% TMS) and $CDCI_3$, respectively (¹H; Figure 1, ¹³C; Figure 2). Calciferol (10 mg) was compared in the same way (¹H; Figure 3, ¹³C; Figure 4).



Figure 1. Comparisons between ¹H NMR spectra of cyclosporine A run in TCET (upper), and CDCI₃ (lower).





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2.3 Comparison of Aromatic Samples

The utility of TCET as an NMR solvent for polycyclic aromatic and heterocyclic compounds was assessed by comparison with CDCl₃ for a small range of samples (Figure 5). 5-10 mg of 2-phenylindole (1 H; Figure 6, 13 C; Figure 7), 9-anthraenecarboxaldehyde (1 H; Figure 8, 13 C; Figure 9), and 2,4,6-triphenylbromobenzene (1 H; Figure 10, 13 C; Figure 11) were dissolved in 0.5 mL of TCET (with 0.5% TMS) and CDCl₃, respectively. In all cases signals in the 13 C spectra were well resolved.



Figure 5 Aromatic samples for comparison.







Figure 8. Comparisons between ^{1}H NMR spectra of 9-anthracenecarboxaldehyde run in TCET (upper), and CDCI₃ (lower)



Figure 9. Comparisons between ¹³C NMR spectra of 9-anthracenecarboxaldehyde run in TCET (upper), and CDCl₃ (lower)



Figure 10. Comparisons between ¹H NMR spectra of 2,4,6-triphenylbromobenzene run in TCET (upper), and CDCl₃ (lower)



Figure 11. Comparisons between ¹³C NMR spectra of 2,4,6-triphenylbromobenzene run in TCET (upper), and CDCl₃ (lower)

2.4 Resolution Tests

2.4.1 Resolution Assessed using the ¹H Lineshape Test

A set of ten heavily reused "economy" grade 5 mm NMR tubes was selected, and 0.6 mL of a 3% v/v solution of chloroform in TCET (with 0.5% v/v TMS) added to each tube. The *lineshape* test was run on each sample in automation mode. In the same set of tubes the solvent was replaced with a 3% v/v solution of chloroform in chloroform-d (99.8% D). The same *lineshape* test was then run. On average, the chloroform in chloroform-*d* solutions gave slightly better width-at-half-height values. However, the results from chloroform in TCET are comparable, and the shim quality was good in all cases (Figure 12). This comparison seeks to demonstrate shim quality under typical rather than optimal working conditions. The samples were not manually shimmed, and pristine high-resolution NMR tubes were not used.



Figure 12. Lineshape tests comparing shim quality between a 3% v/v solution of CHCl₃/CDCl₃ and CHCl₃/TCET.

2.4.2 Resolution of ¹³C NMR Signals nearby to the Solvent Resonance in TCET

To assess the resolution of ¹³C NMR spectra, and the potential for the solvent signal to obscure analyte signals we searched for compounds with signals very close to the solvent signal (120.45 ppm). The C-2 signal of nitrosobenzene was found to lie at 120.49 ppm with a signal separation

of 0.04 ppm from TCET (Figure 13). A 5 mg/mL solution using an acquisition time of 2 hours did reveal partial separation of signals. The TCET signal is sharp, and only obscures a very narrow band of the spectrum.



Figure 13. ¹³C NMR spectrum of a 5 mg/mL solution of nitrosobenzene in TCET (0.5% v/v TMS) showing partial resolution of the C-2 signal from the solvent signal at a separation of 0.04 ppm.

2 General Methods for the Construction of Sealed Double-Walled NMR Tubes

4 mm O.D. NMR tubes were purchased from Norell (S-4-HT-7, ASTM type 1 grade A glass). 5 mm O.D. NMR tubes were purchased from Norell (S-3-300-7, ASTM type 1 grade A glass). The 4 mm tubes were prepared by firstly removing the insignia lacquer with a scalpel blade, and then placing and setting ~8 mm wide pieces of PTFE "heat shrink" spacers ("TE Connectivity Clear Heat Shrink Tubing, sleeve diameter 3.2 mm", purchased from RS Components International, item: 170-6282). One spacer was placed at the bottom, and the other ~3 cm from the top of the tube. Gentle heating with a heat-gun secured the spacers. The bottom spacer was trimmed at the base of the tube, and at 4 mm above the base using a jig. The upper spacer is also trimmed to ~4 mm. To the 5 mm outer tube was added ~120 µL of the reference solution (D₂O, or cyclooctadiene in C₆D₆). Transfers were done using a long needle or pasture pipette to

prevent solvent residues near the top of the tube. The inner tube was then inserted into the outer. Air bubbles trapped around the bottom of the tube were removed by slowly separating the tubes, and then tapping them together. It is best that the solvent level of the reference solution be about one third the height of the tube. Over time, surface tension will cause fissures to run down the solvent. A high solvent level will ensure that such fissures do not reach the analysis zone of the tube.

Flame Sealing: A ~5 cm long graphite pin was fashioned from a hard graphite pencil. This was worked down with sandpaper using an electric drill as a lathe, so as to fit within the 4 mm NMR tubes. The head of the pin is made thicker so that it gently rests at the top of the tube. The pin was burned using an oxygen/LPG flame to burn out the organic binder. A photograph is shown in Figure 14.

The coaxial NMR tubes were braced at the bottom using a piece of rubber tubing, and locked into a vertically mounted electric drill. The graphite pin was placed in the top of the inner tube. The drill was maintained at a slow rotation speed (clamp on the trigger), and the rotating tube was cradled with a clamp ~4 cm below the top of the tube. The two layers of glass were then sealed together using a fine-tip oxygen/LPG flame (using a BOC 5W-A welding tip, Figure 15). The flame we used was ~3 mm long (hot part), and many welding and glassblowing accessories will achieve a suitable flame. After sealing, all tubes were pressure tested by heating in an oven at ~10 °C above the boiling point of the reference solvent.



Figure 14. Photographs and illustration of preparation of the 4 mm inner tube, the graphite pin, and a double-walled NMR tube after sealing. Expansions show the placement of the PTFE spacers.



Figure 15. Left, welding apparatus used. Right, picture of flame sealing, showing the red-hot graphite pin.

2.5 Resolution Tests

The lineshape test was recorded with a 3% v/v solution of chloroform in tetrachloroethylene. The "humpcal" resolution test gave "width-at-half-height" values of typically less than 0.5 Hz (e.g. Figure 16).



Figure 16. Lineshape test on D_2O encapsulated double-walled NMR tubes using a 3% v/v of chloroform in TCET.

2.6 Example Spectra of Neat Solvents

Double-walled tubes with D_2O as the encapsulated reference solution were used to record spectra of neat, protonated solvents. Examples of ethyl acetate (Figure 17), tetrahydrofuran (Figure 18), and toluene (Figure 19) are shown below.



Figure 17. ¹H and ¹³C NMR spectra of neat ethyl acetate using a D₂O encapsulated double-walled NMR tube.



Figure 18. ¹H and ¹³C NMR spectra of neat tetrahydrofuran using a D₂O encapsulated double-walled NMR tube.

96.95



210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm Figure 19. 1 H and 13 C NMR spectra of neat toluene using a D₂O encapsulated double-walled NMR tube.

3 Construction and Calibration of Organometallic Concentration Determination Tubes

A stock solution of 1,5-cyclooctadiene (368 μ L, 3.0 mmol) in benzene-d₆ was made up to 1.5 mL, giving a solution of ~2.0 M. To a set of six 5 mm NMR tubes was added 120 μ L of this solution. The prepared inner tubes were added, and flame-sealed in the normal way. A 1.00 M stock solution of 1,2,4,5-tetramethylbenzene (13.42 g, 100.0 mmol) was made up to 100 mL in A-grade volumetric glassware. Aliquots of ~0.5 mL of this solution was added to each tube, and the *apparent* concentration of 1,5-cyclooctadiene in the inner chamber determined by quantitative ¹H NMR comparison between 1,2,4,5-tetramethylbenzene and cyclooctadiene (Figure 20). The apparent concentration of the calibrant is sensitive to the exact dimensions of both the inner and outer chambers, such that each tube is unique. (Our set of tubes gave calibration values of: 0.390 M, 0.372 M, 0.373 M, 0.342 M, 0.382 M, and 0.382 M). It is worth noting that using any significantly higher concentration of cyclooctadiene in the outer tube reduces the ²H signal to a level where automated Locking becomes unreliable (on our instrument).



Figure 20. Example of cyclooctadiene/benzene-d₆ encapsulated double-walled NMR tube calibration against a standard solution of durene.

3.1 General Procedure for Concentration Determination of Organometallic Reagents

A pre-calibrated set of cyclooctadiene NMR tubes was dried under high-vacuum in a Schlenk tube equipped with a rubber septum, and then maintained under argon. A solution of the analyte was then transferred *via* syringe to each of the tubes. The rubber septum was removed, and under a blanket of argon, small silicone stoppers (Norrell item: TS-4-SR) were used to stopper each tube. The tubes were then taken out, and the stoppers secured with PTFE tape.

Acquisition settings: ¹H NMR were recorded with the samples spinning at 20 Hz. The acquisition time was set at 30 sec and the recycle delay at 30 sec. For all other parameters, the default settings were used. The number of transients is not critical. Either 2 scans or 16 scans were recorded (2 scans is sufficient). These acquisition settings ensure quantitative spectra, and generally reflect those used by Hoye (Hoye, T. R.; Eklov, B. M.; Voloshin, M. *Org. Lett.* **2004**, *6*, 2567). Hoye's method carefully adjusted the pulse power and duration so as to avoid spectral artefacts. On our spectrometer we find this is unnecessary, likely due to the good performance of the automatic receiver gain calibration. We've used an acquisition time and recycle delay of 30 sec rather than 20 sec as a (probably redundant) precaution.

Commercial supplies of *n*-butyllithium (2.0 M in cyclohexane), methyllithium (3.1 M in dimethoxymethane), vinylmagnesium bromide (1.0 M in THF), allylmagnesium bromide (1.0 M in Et₂O) lithium diisopropylamide (1.0 M in THF/hexanes) and ethylaluminium dichloride (25% wt.% in toluene) where obtained from Sigma Aldrich and used as received. Isopropylmagnesium chloride lithium/ chloride complex was prepared according to the following:

IsopropyImagneisum chloride/ lithium chloride complex solution in THF: Magnesium turnings (1.94 g, 80.0 mmol) were cut into small pieces, ground using a mortar and pestle, and transferred to a round-bottomed flask equipped with a condenser and addition funnel. Dry lithium chloride was added (2.76 g, 65.0 mmol), and the addition funnel charged with dry THF (100 mL). A few millilitres of the solvent were added, followed by several drops of 1,2-dibromoethane. The mixture was stirred and gently heated with a heat gun until a vigorous reaction with the magnesium was observed. The mixture was then heated at reflux using an oil bath. The addition funnel was then charged with 2-chloropropane (5.9 mL, 65 mmol), and this solution added drop-wise over 30 min, and then stirred for a further 3 hours at reflux. The solution was cooled, and then transferred to a Schlenk vessel *via* cannula.

Generally we observed that NMR concentration determination gives poor and inconsistent results for "old" stocks of organometallic reagents. This is likely due to decomposition products interfering with calibrant/analyte signals.



Figure 21. Upper: Concentration determination of n-butyllithium. Lower: Titration of Methyllithium



Figure 22. *Upper:* Concentration determination of isopropylmagnesium chloride/ lithium chloride complex *Lower:* Titration of vinylmagnesium bromide



Figure 23. Upper: Concentration determination of allyImagnesium bromide. Lower: Titration of lithium disopropylamide.



Figure 24. Concentration determination of ethylaluminium dichloride.

4 NMR Analysis of Trace Impurities

This section presents the spectra used to construct **Table 1**. All materials (roughly 5-10 mg) were dissolved in TCET (0.5% v/v TMS) ~0.6 mL and recorded individually. ¹H and ¹³C NMR spectra were recorded and assignments were assisted by HSQC experiments where needed.



Figure 25. ¹H NMR spectrum in TCET: water. Water is very sparingly soluble in TCET and the identification of its signal was aided by the subsequent addition of one drop of methanol-*d4*.









Figure 29. ¹H and ¹³C NMR spectra in TCET: benzaldehyde.



Figure 30. ¹H and ¹³C NMR spectra in TCET: benzene.



210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm Figure 31. ¹H and ¹³C NMR spectra in TCET: *tert*-butylalcohol.

-1.19





Figure 33. ¹H and ¹³C NMR spectra in TCET: 2,6-di-*tert*-butyl-4-methylphenol.







Figure 36. ¹H and ¹³C NMR spectra in TCET: cyclohexanone.



Figure 37. ¹H and ¹³C NMR spectra in TCET: 1,2-dichloroethane.





Figure 39. ¹H and ¹³C NMR spectra in TCET: diethyl ether.



Figure 40. ¹H and ¹³C NMR spectra in TCET: dimethylformamide.



---2.45

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

Figure 41. ¹H and ¹³C NMR spectra in TCET: dimethylsulfoxide.



Figure 42. ¹H and ¹³C NMR spectra in TCET: 1,4-dioxane.









210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm Figure 45. ¹H and ¹³C NMR spectra in TCET: hexamethyldisiloxane.

3

2

1

0

ppm





Figure 47. ¹H and ¹³C NMR spectra in TCET: hexamethylphosphoramide.









Figure 50. ¹H and ¹³C NMR spectra in TCET: *n*-pentane.









210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm Figure 54. ¹H and ¹³C NMR spectra in TCET: pyrrolidine.





210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm Figure 56. ¹H and ¹³C NMR spectra in TCET: toluene.



Figure 57. ¹H and ¹³C NMR spectra in TCET: triethylamine.