Supplementary Information

Qualitative and quantitative estimation of nitrogen and oxygen anions: Utilization of No-D PULCON NMR

Shrikant A Shete[a,b], Partha P Mukhopadhyay[a], Sitaram Pal[a], Prashantha Kamath[a], Sujit K Ghorai[a,b] & Bhanu N Manjunath*[a,b]

[a] Research & Technology Centre, Syngenta Biosciences Pvt. Ltd., Santa Monica Works, Corlim, Ilhas, Goa 403 110, India

[b] School of Chemical Sciences, Goa University, Taleigao, Plateau, Goa 403 206, India

E-mail: bhanu.manjunath@syngenta.com

Received 19 August 2025; accepted (revised) 7 August 2026

Qualitative and Quantitative Estimation of Nitrogen and Oxygen Anions: Utilization of No-D PULCON NMR

Contents

S1 Study of Anion generation of aniline by using LiHMDS and *n-BuLi*. 3
S2 Confirmation of anion by using electrophile addition: 5
S3 Quantitation of Anilines, indole and phenol: 12
S4 Comparative study of quantitation by using different External reference standard..... 17
S5 Experimental: 21
S6 References: 21

S1 Study of Anion generation of aniline by using LiHMDS and *n*-BuLi.

General procedure for anion generation in aniline:

To a stirred solution of Aniline (500 mg,5.26 mmol) different equivalents of 1M of LiHMDS in THF and 2M *n*-BuLi in cyclohexane were added at ambient temperature. The stirring continued at the same temperature for 10 min and reaction mixture was directly transferred into dry NMR tube and recorded No-D NMR.

Study of aniline anion generation by using LiHMDS:

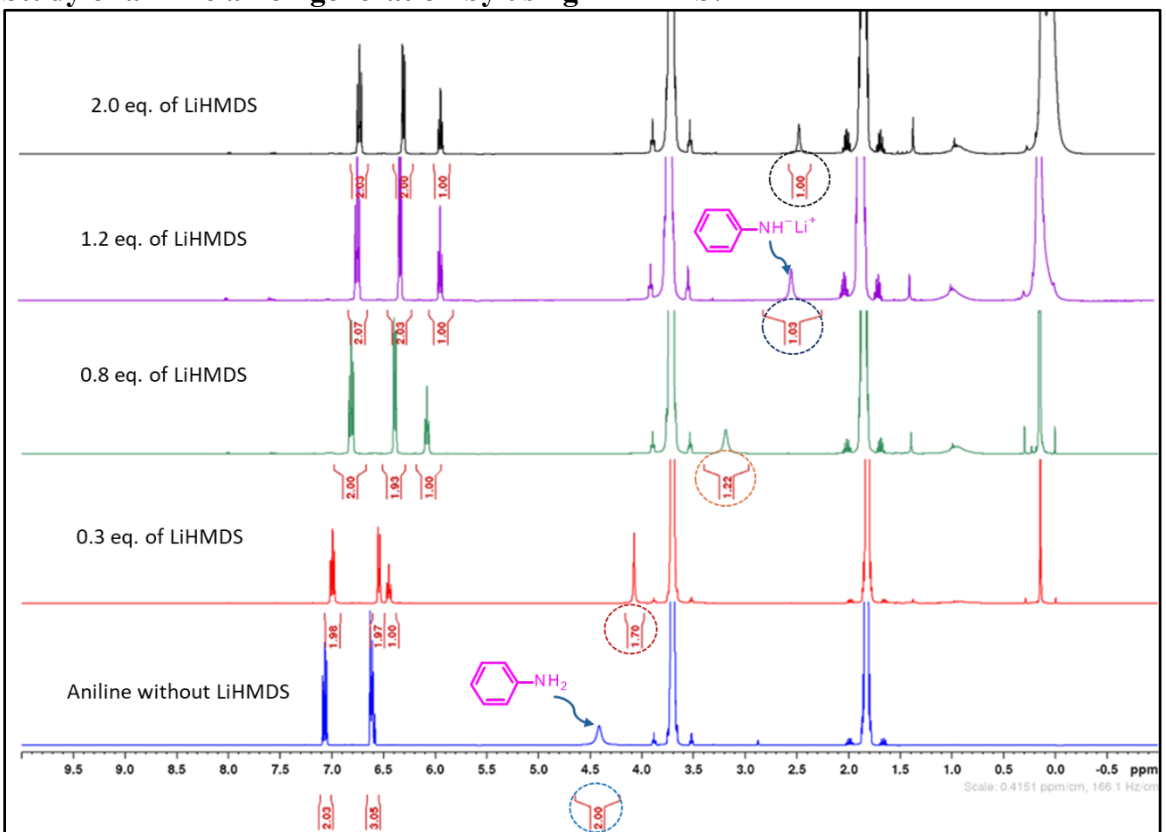


Fig. S1- No-D ^{1}H-NMR spectrum of aniline and aniline anion generated with different equivalent of LiHMDS.

Study of aniline anion generation by using *n*-BuLi:

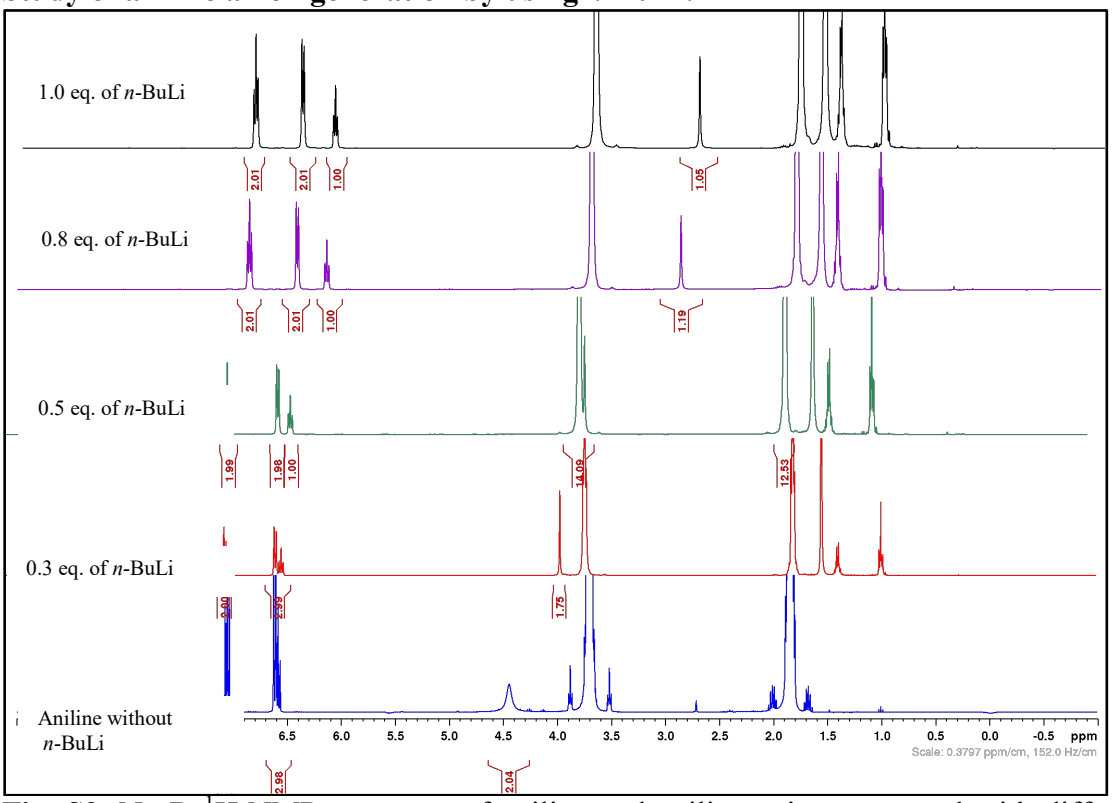


Fig. S2. No-D ^{1}H-NMR spectrum of aniline and aniline anion generated with different equivalent of *n*-BuLi.

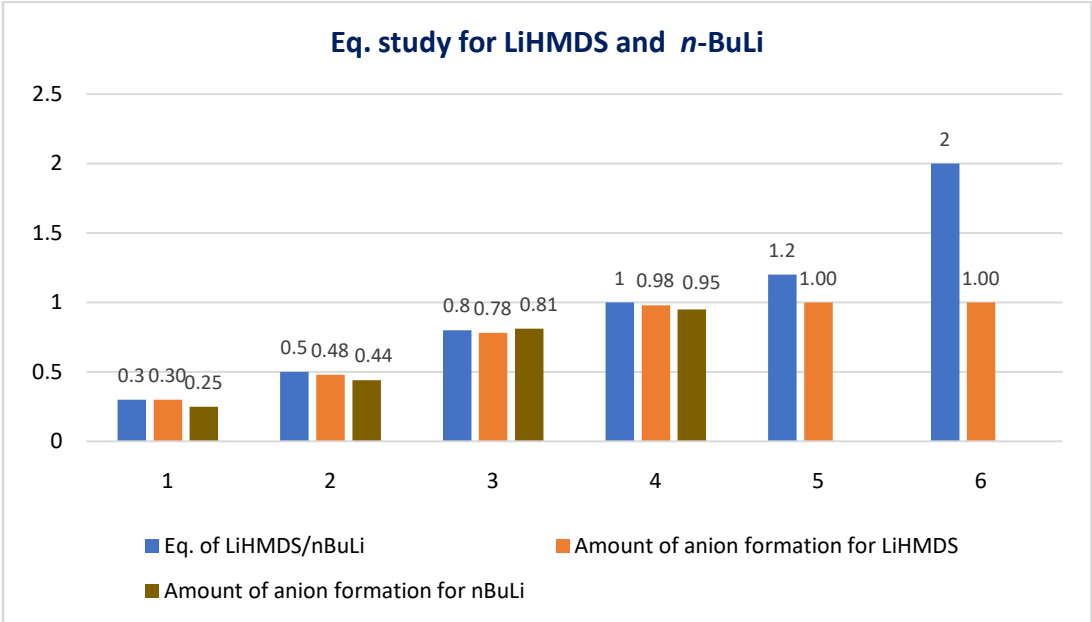
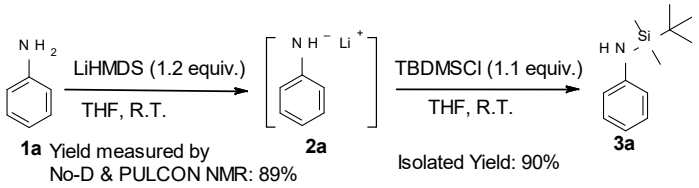


Fig. S3. Comparison of concentration of aniline anion generated with different bases LiHMDS and n-BuLi measured by using No-D NMR.

S2 Confirmation of anion by using electrophile addition:

Confirmation of aniline derivatives anion by using TBDMSCl

To a stirred solution of aniline (500 mg,5.26 mmol) in THF were added 1.2 equiv. of LiHMDS. The stirring continued for 10 min and reaction mixture was directly transferred into dry NMR tube recorded No-D NMR and measured concentration of anion by using PULCON. Side by side 1.1 equiv. of TBDMSCl in THF was added to the reaction mass and stirred for 2 hrs. at room temperature. The reaction mixture was cooled to ambient temperature, diluted with aq. NH_3Cl (30 mL) and extracted with AcOEt (3 x 50 mL) and recorded qNMR for yield determination.



Reaction scheme S1: Comparison of yield of aniline anion as lithium salt, measured by No-D PULCON NMR and isolated yield of silyl derivative from aniline anion

Yield: 90 % (992 mg); ^{1}H NMR (400 MHz, DMSO-*d6*): δ ppm 7.03 (t, *J* = 8.21 Hz, 2H), 6.72 (d, *J* = 8.85 Hz, 2H), 6.55 (t, *J* = 7.35 Hz, 1H), 5.02 (br s, 2H), 0.93 (s, 9H),0.19 (s, 6H),;^{13}C NMR{1H} (100 MHz, DMSO-d_6): δ ppm 151.2, 131.4, 119.2, 119.1, 29.0, 20.8,-1.49;

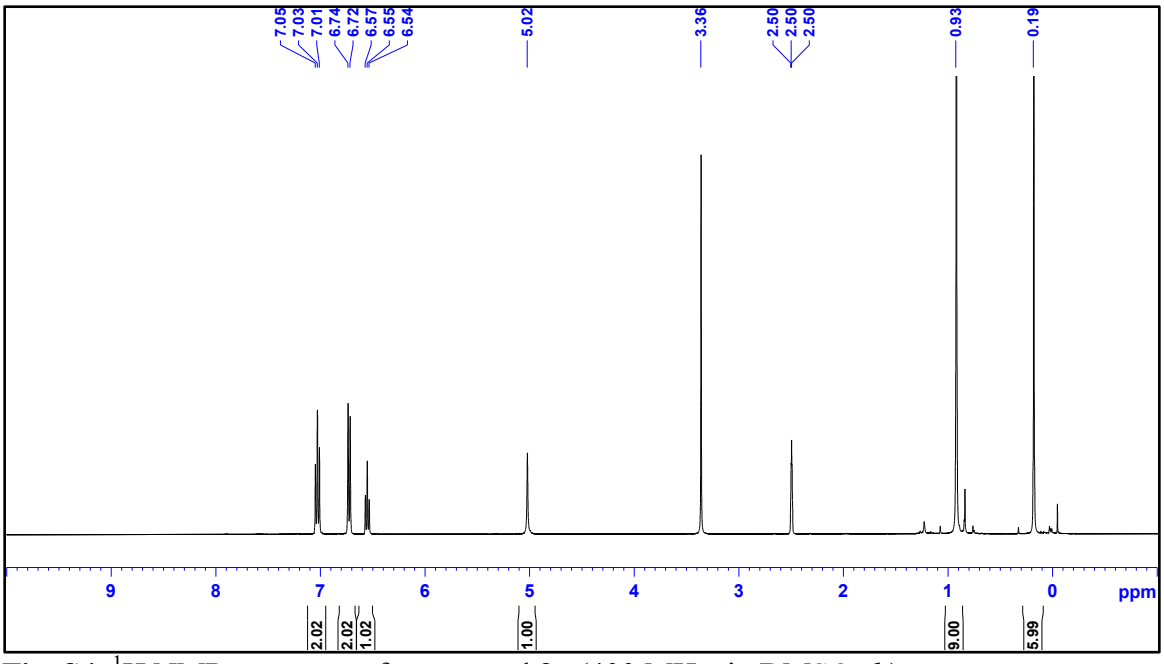


Fig. S4. ^{1}H NMR spectrum of compound **3a** (400 MHz, in DMSO-d_6)

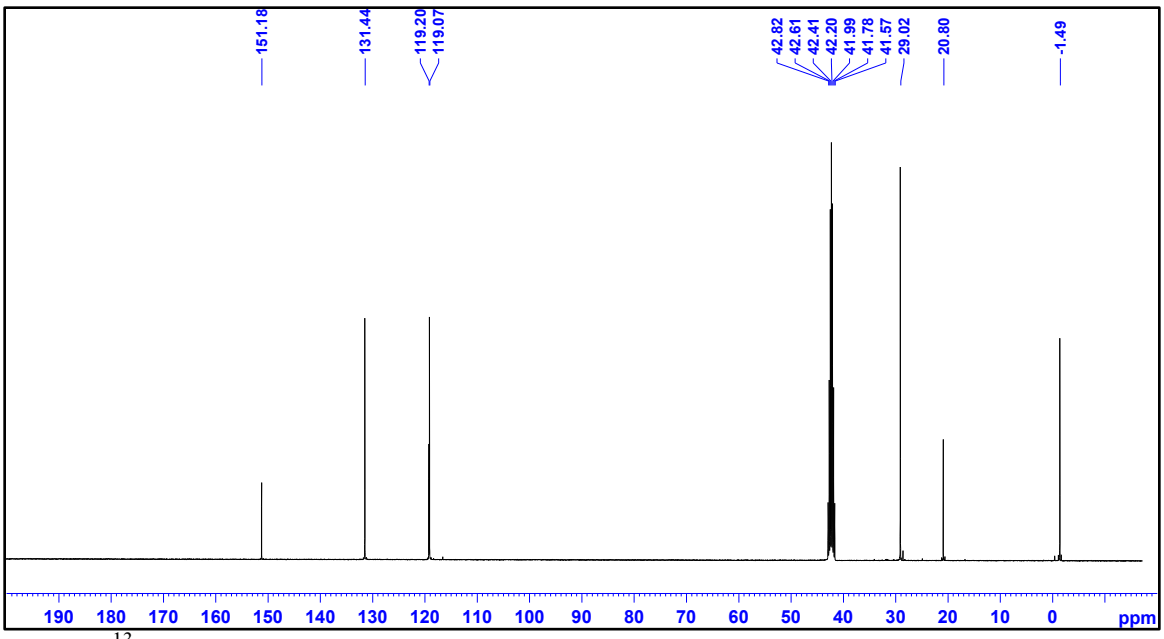


Fig. S5. ^{13}C NMR spectrum of compound **3a** (400 MHz, in DMSO-d_6)

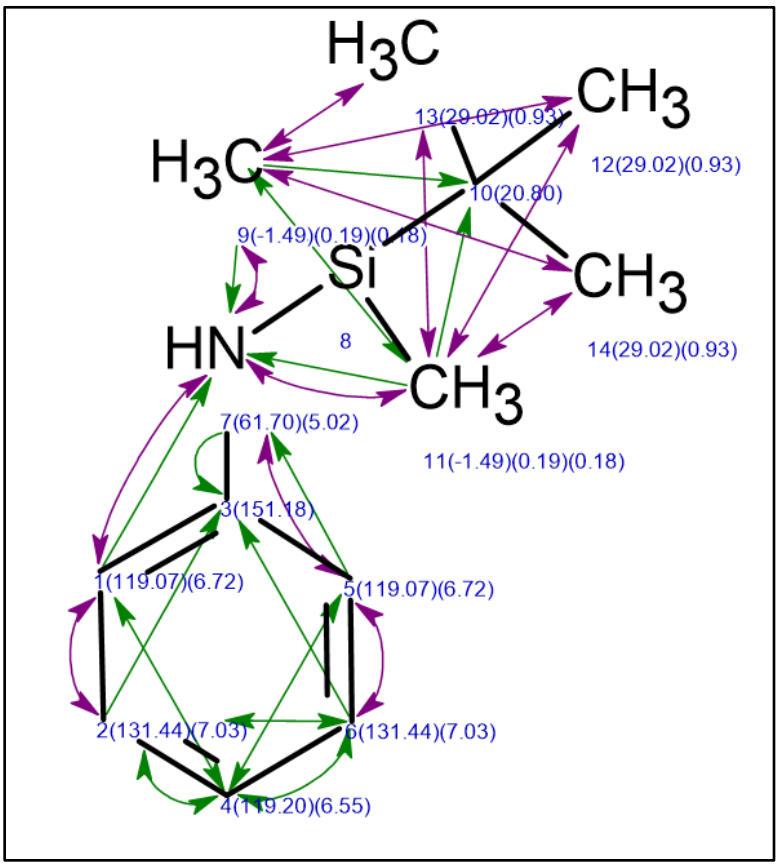
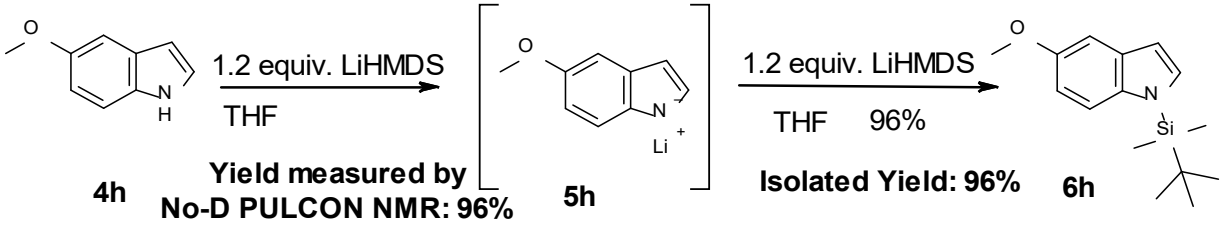


Fig. S6. Structure confirmation data by using 2D NMR for structure **3a,** blue arrows represent the correlations derived from 2D NOESY, while green arrows highlight the ^{1}H-^{13}C/^{15}N HMBC correlations.

Confirmation of indole derivatives anion by using TBDMSCl:
Followed same procedure as like aniline.



Reaction scheme S2. Comparison of yield of indole anion as lithium salt, measured by No-D PULCON NMR and isolated yield of silyl derivative from aniline anion.

Yield: 96 % (844 mg); ^{1}H NMR (400 MHz, DMSO-*d6*): δ ppm 7.40 (d, *J* = 9.13 Hz, 1H), 7.28 (d, *J* = 3.04 Hz, 1H), 7.06 (d, *J* = 2.51 Hz, 1H), 6.74 (d, *J* = 9.11, 2.64 Hz, 1H), 6.50 (d, *J* = 3.08 Hz, 1H) 3.74 (s, 3H), 0.84 (s, 9H),0.58 (s, 6H),;^{13}C NMR{1H} (100 MHz, DMSO-d_6): δ ppm 154.0, 135.9, 132.6, 132.0, 114.7, 111.5, 104.9, 102.5, 55.6, 26.6, 19.6, -3.8; HRMS (ESI): m/z $[M + H]^+$ calc. for $C_{15}H_{24}NOSi$,262.1621; found 262.162.

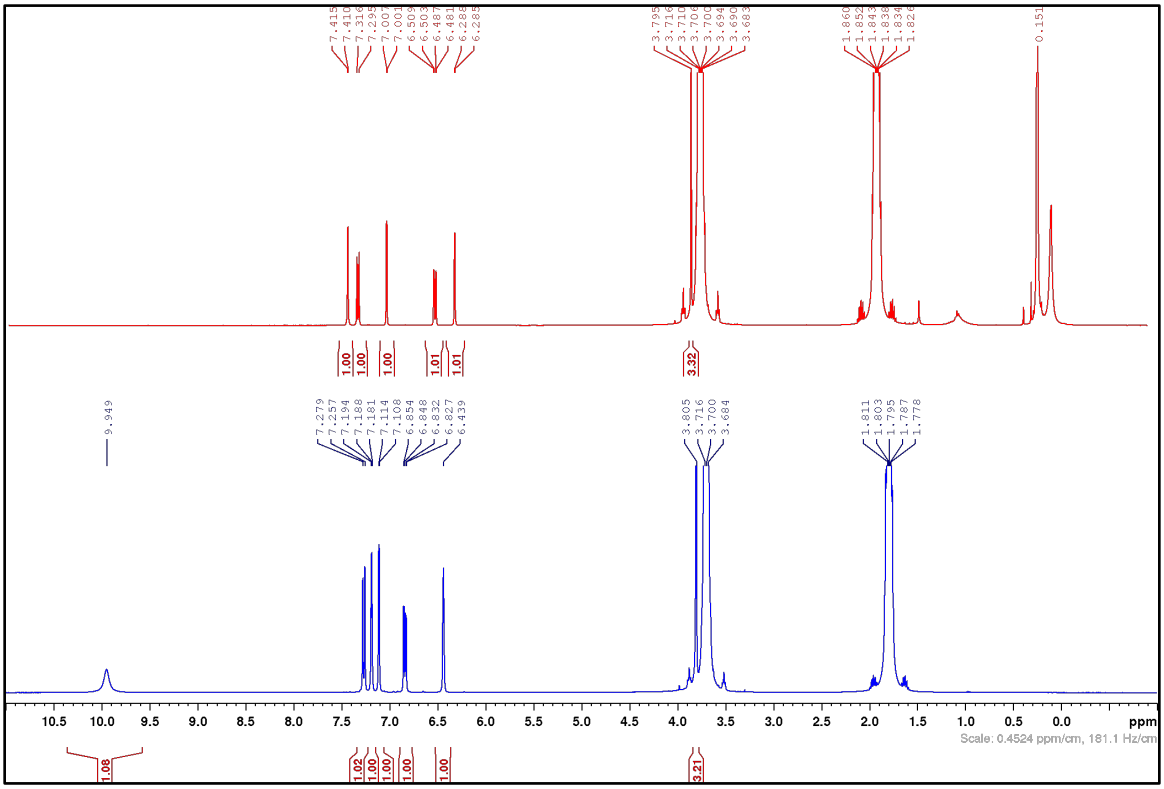


Fig. S7. Overlayed NMR spectrum of compound **4h** and **5h** in THF

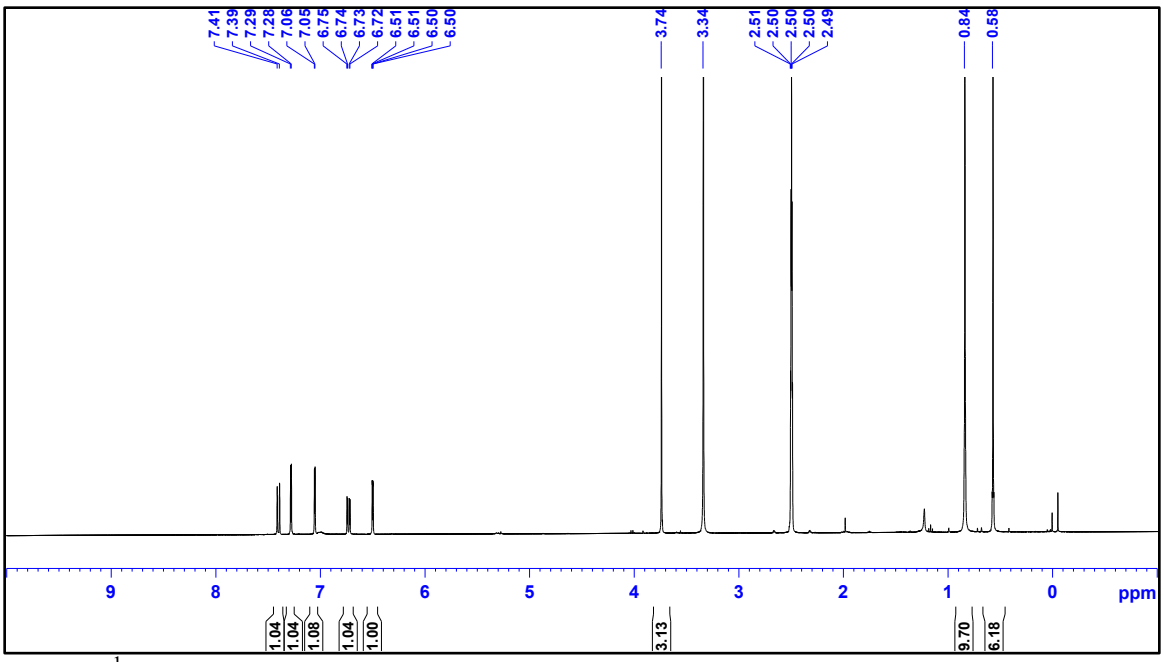


Fig. S8. ^{1}H NMR spectrum of compound **6h** (400 MHz, in DMSO-d_6)

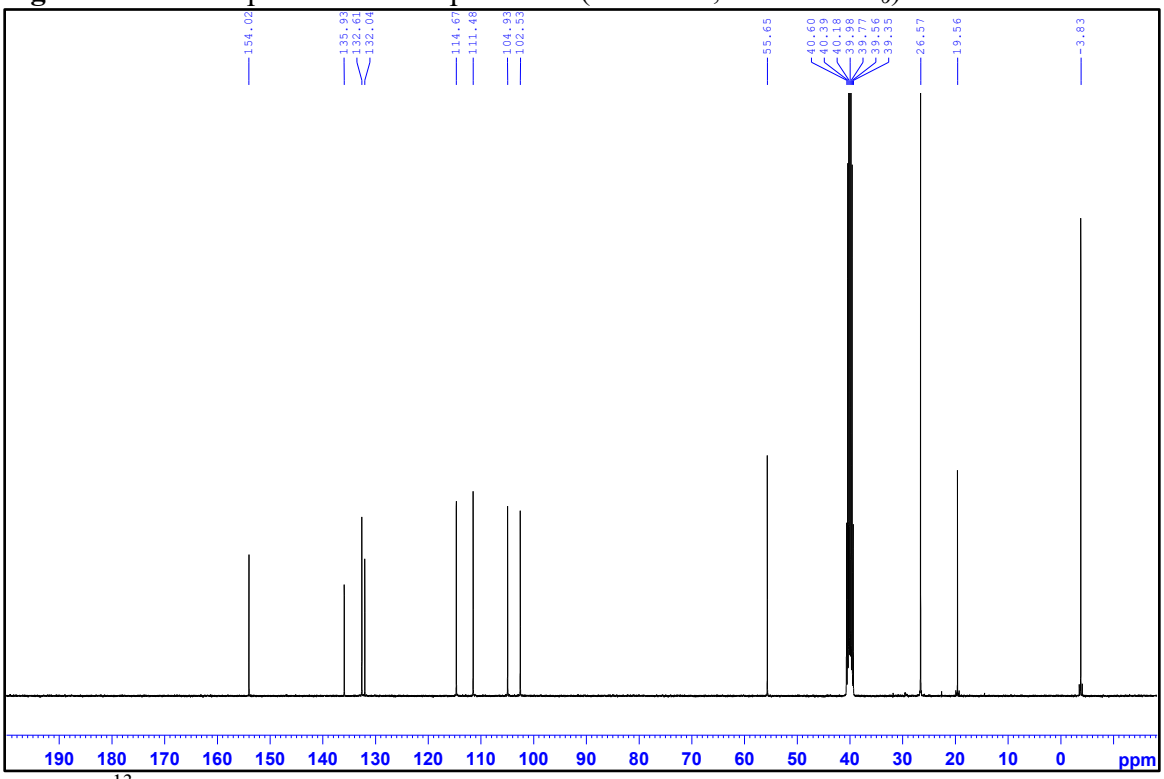


Fig. S9. ^{13}C NMR spectrum of compound **6h** (400 MHz, in DMSO-d_6)

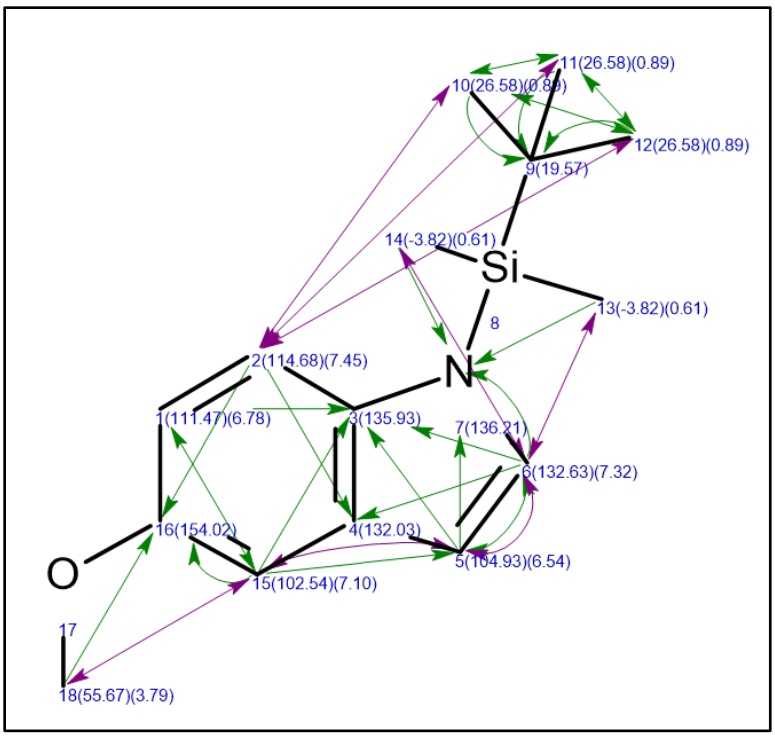
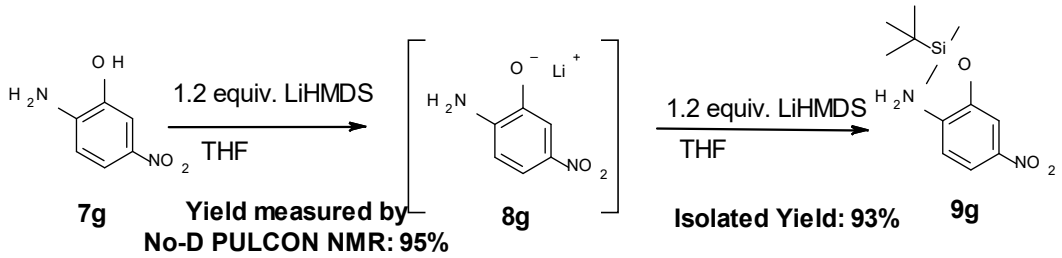


Fig. S10. Structure confirmation data by using 2D NMR for structure **6h**, blue arrows represent the correlations derived from 2D NOESY, while green arrows highlight the ^{1}H-$^{13}C/^{15}N$ HMBC correlations.

Confirmation of phenol derivatives anion by using TBDMSCl:

To a stirred solution of compound **7g** (500 mg, 3.21 mmol) in THF were added 1.0 equiv. of LiHMDS. The stirring was continued for 10 min and reaction mixture was directly transferred into dry NMR tube recorded No-D NMR and measured concentration of anion by using PULCON. Side by side 1.1 equiv. of TBDMSCl in THF was added to the reaction mass and stirred for 2 hrs. at room temperature. The reaction mixture was cooled to ambient temperature, diluted with aq. NH_3Cl (30 mL) and extracted with AcOEt (3 x 50 mL) and recorded qNMR for yield determination.

Yield: 93 % (802 mg); ^{1}H NMR (400 MHz, DMSO-*d6*): δ ppm 7.78 (dd, *J* = 8.80, 2.45 Hz, 1H), 7.52 (d, *J* = 2.45 Hz, 1H), 6.79 (d, *J* = 8.82 Hz, 1H), 6.19 (br s, 2H), 1.02 (s, 9H), 0.31 (s, 6H),;^{13}C NMR{1H} (100 MHz, DMSO-*d6*): δ ppm 184.4, 140.4, 135.9, 120.6,113.7, 112.6, 26.2, 18.6,; HRMS (ESI): m/z $[M + H]^{+}$ calc. for $C_{12}H_{20}N_2O_3Si$,269.1315; found 269.1319.



Reaction scheme S3. Comparison of yield of indole anion as lithium salt, measured by No-D PULCON NMR and isolated yield of silyl derivative from aniline anion.

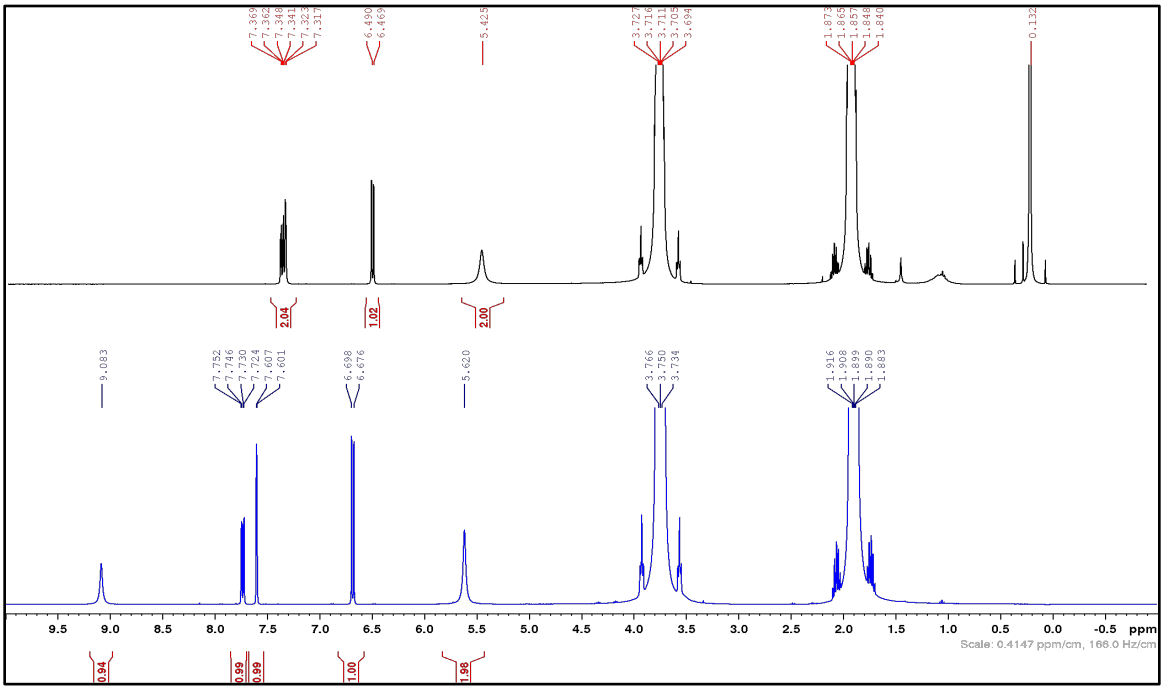


Fig. S11. Overlayed NMR spectrum of compound **7g** and **8g** in THF

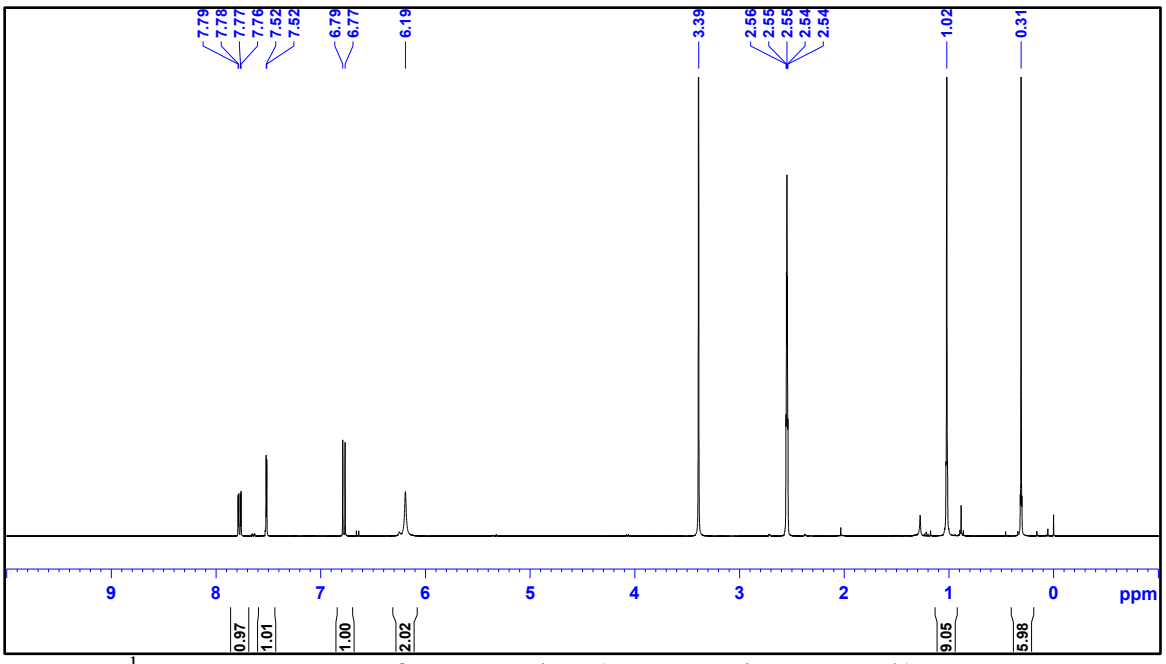


Fig. S12. ^{1}H NMR spectrum of compound **9g** (400 MHz, in DMSO-d_6)

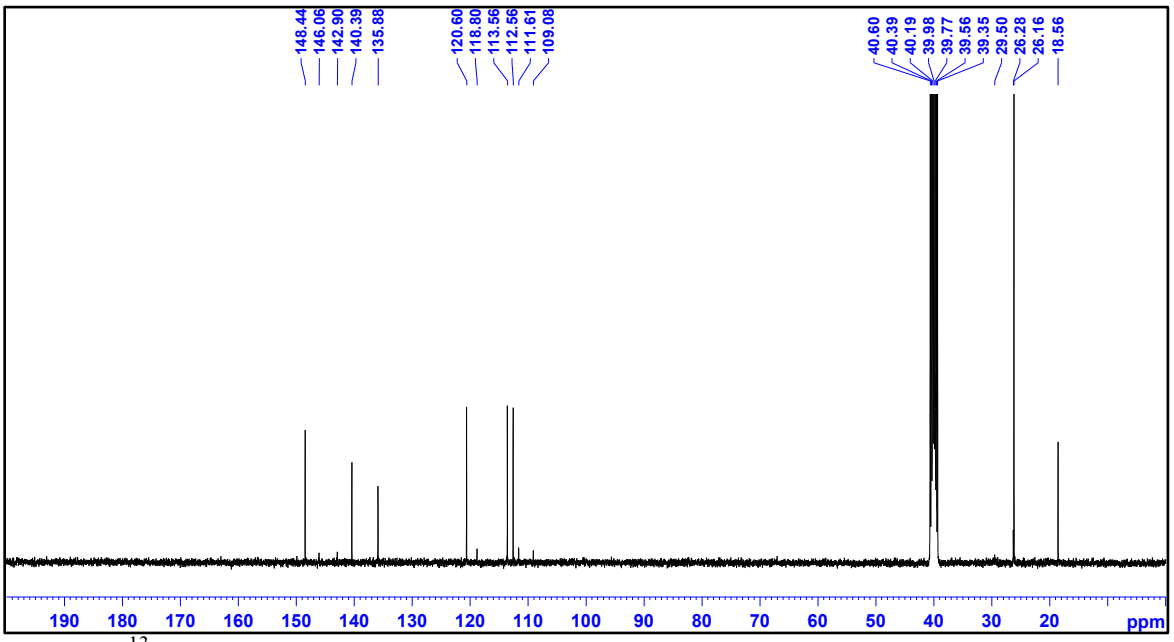


Fig. S13. ^{13}C NMR spectrum of compound **9g** (400 MHz, in DMSO-d_6)

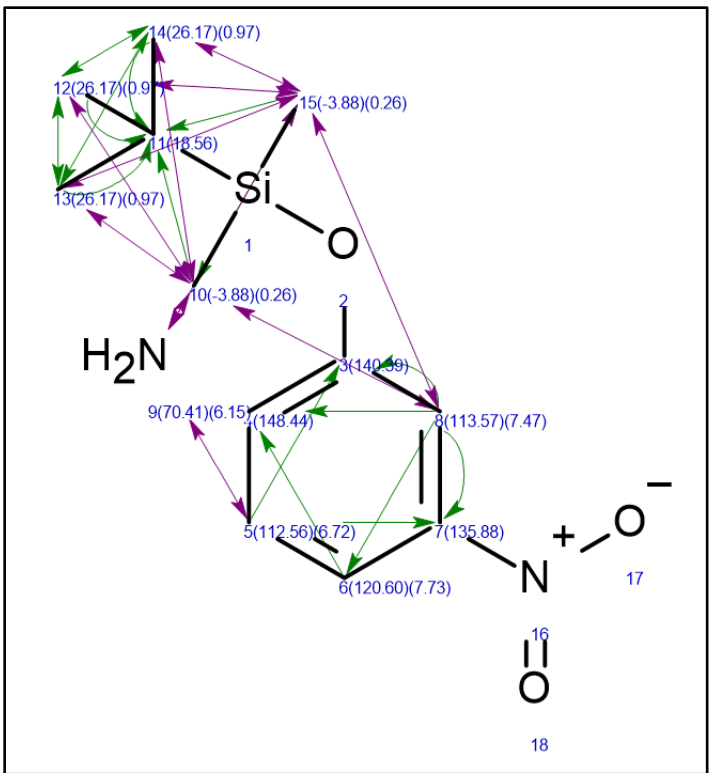


Fig. S14. Structure confirmation data by using 2D NMR for structure **9g**, blue arrows represent the correlations derived from 2D NOESY, while green arrows highlight the ^{1}H-^{13}C HMBC correlations.

S3 Quantitation of Anilines, indole and phenol:

Quantitation of Anilines

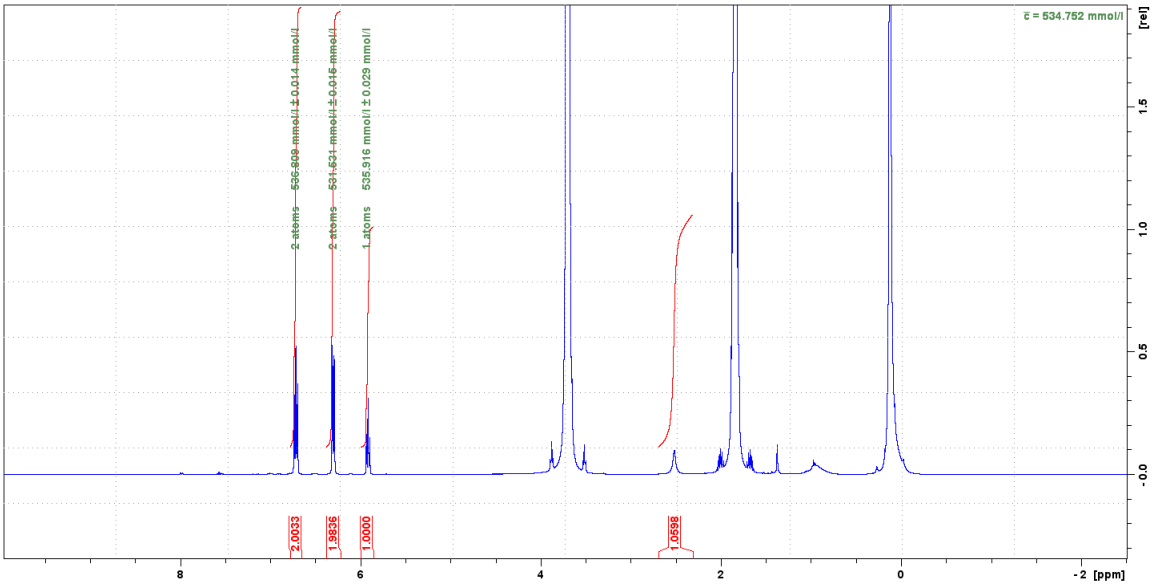
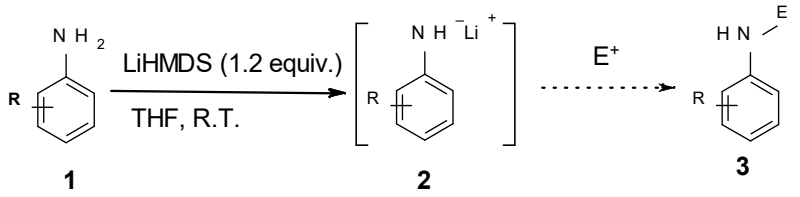
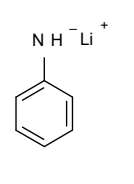


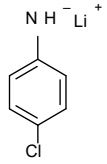
Fig. S15. Quantitation of aniline anion generated by using 1.2 equiv. LiHMDS in THF and quantified by using PULCON.



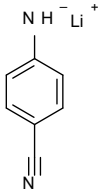
1 2 3



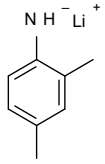
2a (89%)



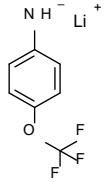
2b (98%)



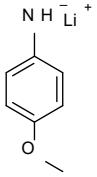
2c (92%)



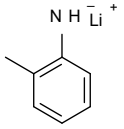
2d (93%)



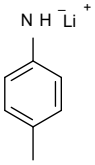
2e (92%)



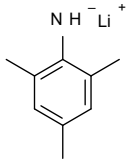
2f (92%)



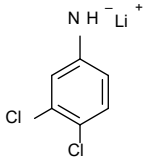
2g (96%)



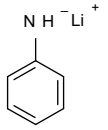
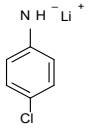
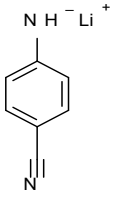
2h (95%)

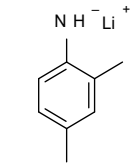
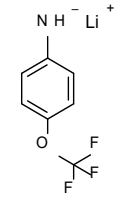
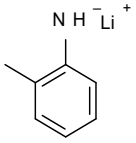
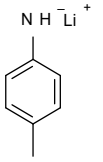
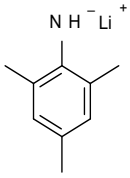
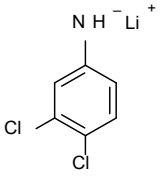


2i (89%)

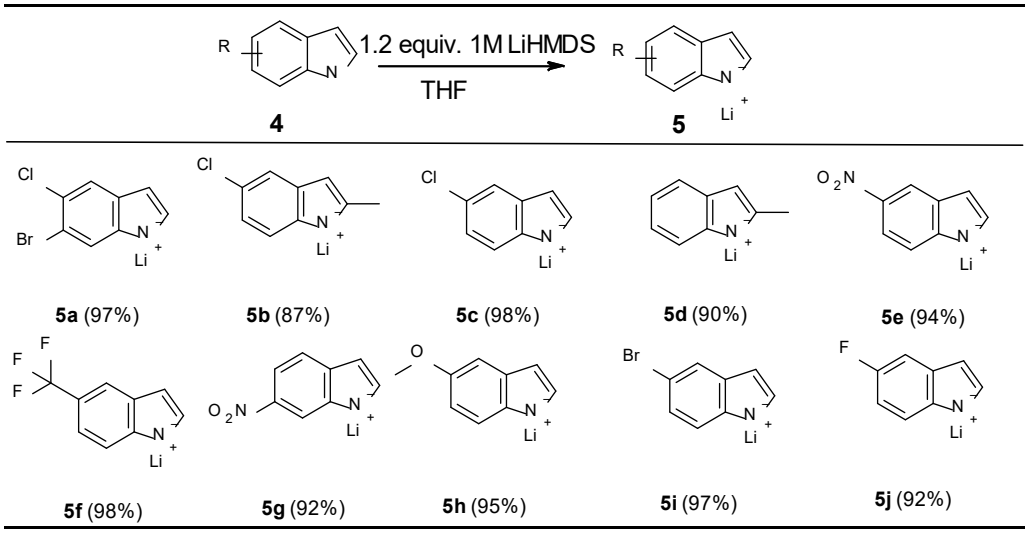


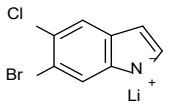
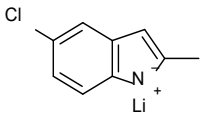
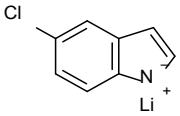
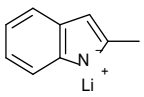
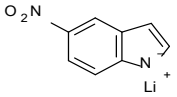
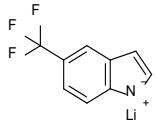
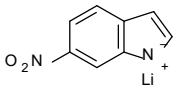
2j (99%)

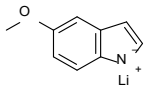
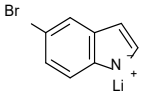
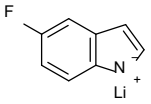
Sr. No.	Substrate	Theoretical	Reading 1		Reading 2		Reading 3		Average	
			mmol/l	%	mmol/l	%	mmol/l	%	mmol/l	%
2a		0.5986	0.5365	89.63	0.5381	89.89	0.5377	89.83	0.5374	89.78
2b		0.5047	0.4948	98.03	0.4997	99.01	0.4886	96.81	0.4943	97.95
2c		0.5199	0.4927	94.77	0.4808	92.48	0.4638	89.21	0.4791	92.15

2d		0.5149	0.4778	92.79	0.4716	91.59	0.4645	90.21	0.4713	91.53
2e		0.4377	0.3906	89.24	0.4119	94.11	0.4144	94.68	0.4056	92.67
2g		0.5387	0.5262	97.68	0.5342	99.16	0.5313	98.63	0.5306	98.49
2h		0.5387	0.5145	95.51	0.5148	95.56	0.5178	96.12	0.5157	95.73
2i		0.4931	0.4520	91.66	0.4602	93.33	0.4562	92.52	0.4561	92.50
2j		0.4562	0.4533	99.36	0.4557	99.89	0.4405	96.56	0.4498	98.60

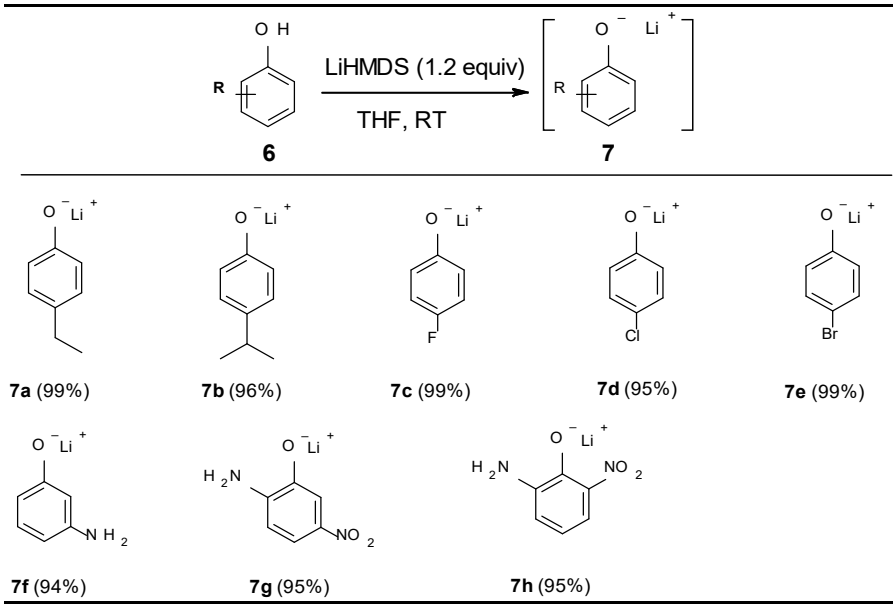
Quantitation of indole

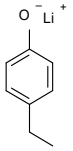
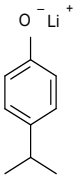


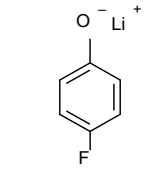
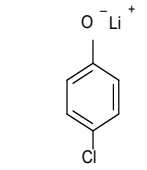
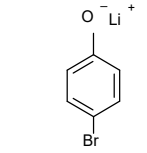
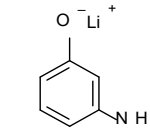
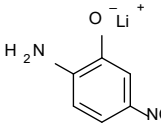
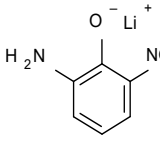
Sr. No.	Substrate	Theoretical	Reading 1		Reading 2		Reading 3		Average	
			mmol/l	%	mmol/l	%	mmol/l	%	mmol/l	%
5a		0.3829	0.3684	96.21	0.3742	97.73	0.3701	96.66	0.3709	96.87
5b		0.4516	0.3924	86.89	0.3902	86.40	0.3902	86.40	0.3909	86.57
5c		0.4699	0.4574	97.34	0.4660	99.17	0.4623	98.38	0.4619	98.30
5d		0.4980	0.4336	87.07	0.4573	91.83	0.4490	90.16	0.4466	89.69
5e		0.4560	0.4328	94.91	0.4348	95.35	0.4226	92.68	0.4300	94.31
5f		0.4285	0.4170	97.32	0.4234	98.81	0.4199	97.99	0.4201	98.04
5g		0.4560	0.4174	91.53	0.4208	92.27	0.4252	93.25	0.4211	92.35

5h		0.4759	0.4438	93.25	0.4531	95.21	0.4554	95.69	0.4508	94.72
5i		0.4082	0.3868	94.75	0.3952	96.81	0.4034	98.82	0.3951	96.79
5j		0.4932	0.4497	91.18	0.4552	92.30	0.4547	92.19	0.4532	91.89

Quantitation of phenols



Sr. No.	Substrate	Theoretical	Reading 1		Reading 2		Reading 3		Average	
			mmol/l	%	mmol/l	%	mmol/l	%	mmol/l	%
7a		0.5133	0.5091	99.18	0.5086	99.08	0.5042	98.23	0.5073	98.83
7b		0.4916	0.4775	97.13	0.4671	95.02	0.4642	94.43	0.4696	95.52

7c		0.5301	0.5259	99.21	0.5254	99.11	0.5207	98.23	0.5240	98.85
7d		0.5032	0.4778	94.95	0.4855	96.48	0.4816	95.71	0.4816	95.46
7e		0.4426	0.4447	100.47	0.4319	97.528	0.4427	100.02	0.4397	99.36
7f		0.4494	0.4187	93.17	0.4257	94.73	0.4187	93.17	0.4210	93.69
7g		0.3663	0.3420	93.37	0.3491	95.30	0.3481	95.03	0.3464	94.57
7h		0.3663	0.3419	93.34	0.3475	94.87	0.3495	95.41	0.3463	94.54

S4 Comparative study of quantitation by using different External reference standard

External reference standard preparation procedure:

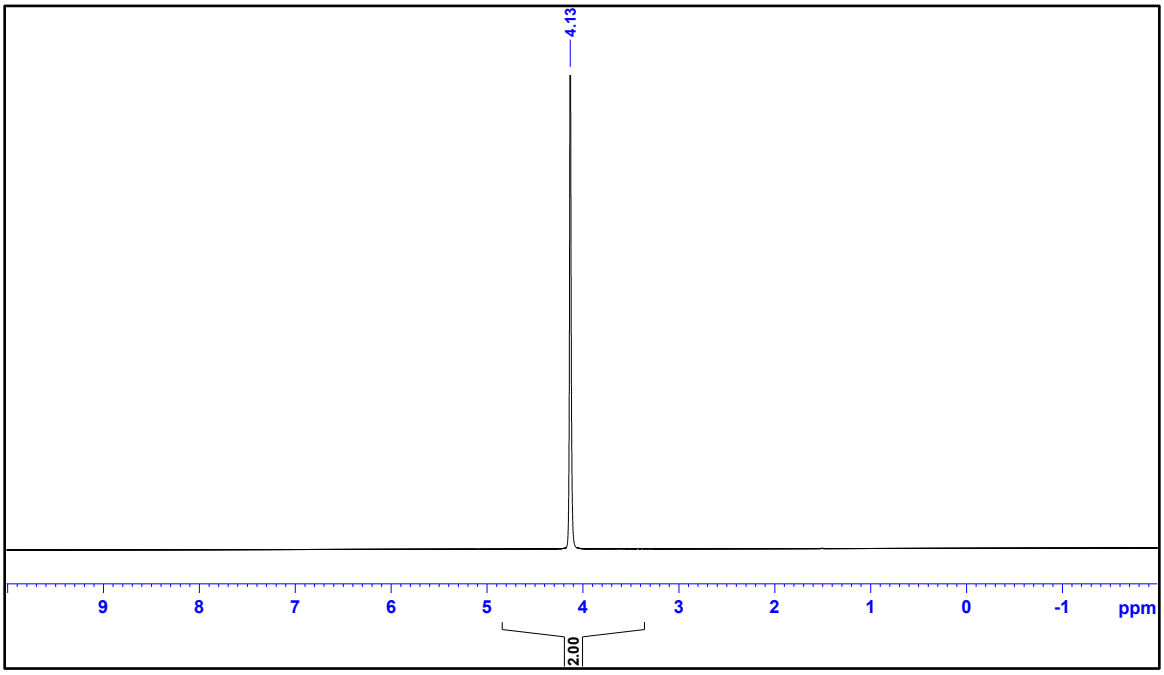
The specific details of the external standards used are as follows:

1. The external standards were individually prepared in 10.0ml volumetric flasks, dissolved in THF.
2. The quantities used for each standard were:
   - 1.2553g of 1,2,4,5-tetramethylbenzene
   - 1.1702g of 1,3,5-trimethoxybenzene
   - 1.4952g of 1,5-cyclooctadiene
3. The concentration of the external reference standards was determined to be:
   - 935.25 mmol/l for 1,2,4,5-tetramethylbenzene
   - 695.76 mmol/l for 1,3,5-trimethoxybenzene

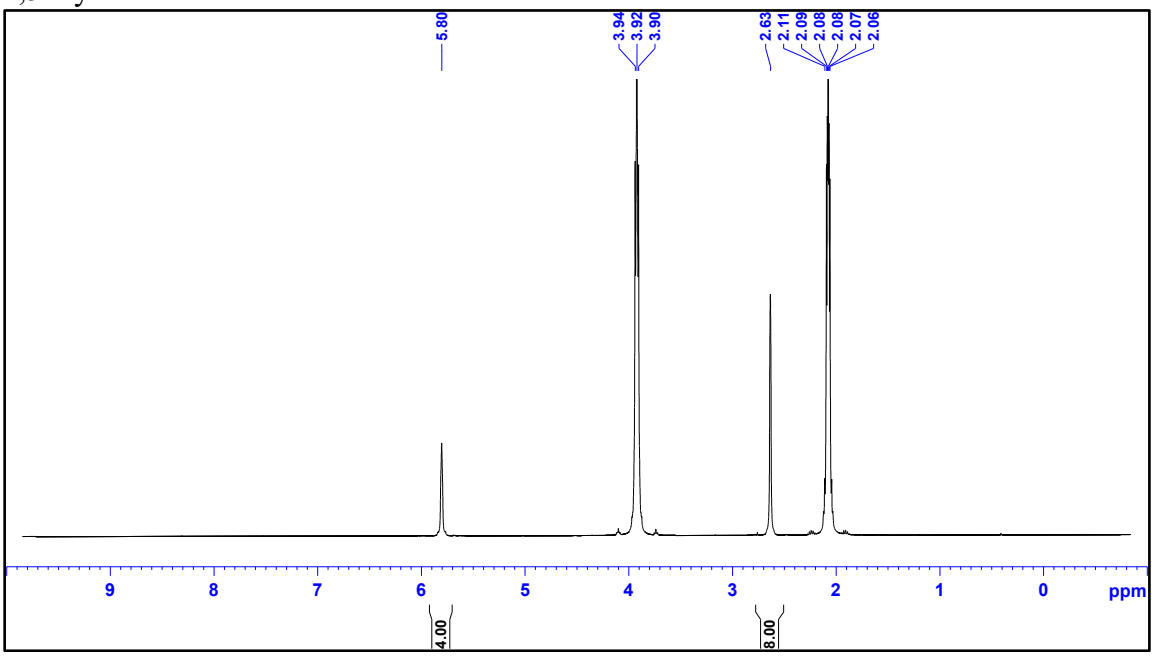
- 1382.14 mmol/l for 1,5-cyclooctadiene

NMR spectrum of External reference standard in THF:

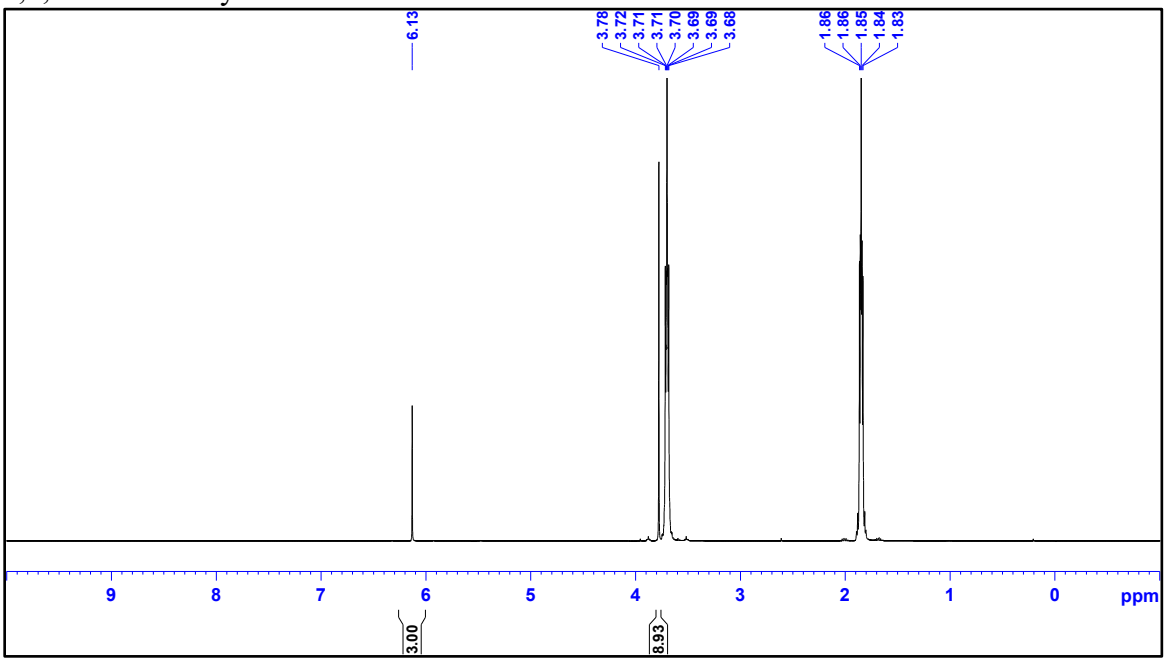
Water



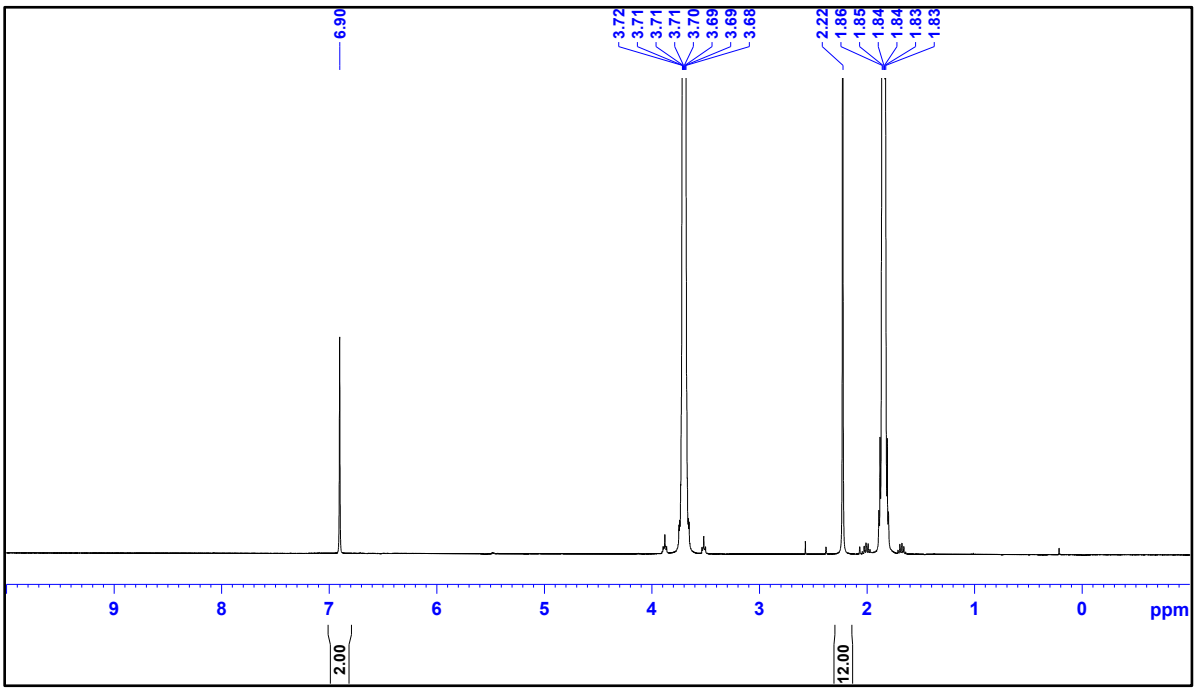
1,5-Cyclooctadiene



1,3,5 -Trimethoxy benzene



1,2,4,5-Tetramethylbenzene



Comparison of anions results with different external standards:

Table S1: Anion concentrations result determined by using four different external standards Water, 1,5 COD, 1,3,5 TMB, and 1,2,4,5 TB.

Name of Compound	Measured concentration of anions (%) by using external standards				Avg	Std. Dev	RSD
	Water	1,3,5 TMB	1,2,4,5 TB	1,5 COD			
2,4-Dimethylaniline	91.53	92.19	91.4	91.75	91.72	0.35	0.38
o-Toluidine	98.49	99.23	98.35	98.73	98.70	0.39	0.39
2,4,6-Trimethylaniline	92.5	93.19	92.38	92.73	92.70	0.36	0.39
3,4-Dichloroaniline	98.6	99.33	98.28	98.85	98.77	0.44	0.45
6-Bromo-5-chloroindole	96.87	97.57	96.74	97.1	97.07	0.37	0.38
5-Chloroindole	98.3	98.96	98.15	98.52	98.48	0.35	0.36
5-Trifluromethylindole	98.04	98.75	97.91	98.28	98.25	0.37	0.38
5-Methoxyindole	94.72	95.41	93.77	94.96	94.72	0.69	0.73
4-Flurophenol	98.85	99.57	98.75	99.09	99.07	0.37	0.37
4-Chlorophenol	95.46	96.41	95.18	95.95	95.75	0.54	0.57

S5 Experimental:

Materials used for study:

All starting material and solvent were purchased from Aldrich Chemical Co., Thermo Fisher Sci., Chempure, Tokyo Chemical Industry, Alfa Aesar Chemicals, Avra Synthesis Private Limited and used as supplied.

NMR instrumentation: Bruker 400MHz, console: Avance III HD with TOPSPIN 3.6.3 software.

NMR tube: Wilmad OD=5mm length= 7”

Long Needle: BD Micro Lance (0.9*40mm)

Syringe: Dispovan 1ml.

Parameters used for data acquisition:

Table S2: Acquisition parameters used for sample acquisition in BRUKER TOPSPIN software.

Sr. No.	Name of Parameter	Parameters
1	PULPROG	zg
2	TD	64102
3	SWH [Hz, ppm]	8012.82, 20.0204
4	AQ [sec]	3.9999647
5	RG	1.78
6	DW [µsec]	62.400
7	D1 [sec]	30.000000000
8	DS	2
9	NS	8
10	Channel	f1
11	SFO1 [MHz]	400.2318010
12	O1 [Hz, ppm]	1801.04, 4.500
13	NUC1 1H	1H
14	P1 [µsec]	14.0

S6 References:

1. Chew W & Sharratt P, *Anal. Methods.* 2 (2010) 1412. DOI: https://doi.org/10.1039/C0AY00257G.

2. Rathore A S, Bhambure R & Ghare V, *Anal. Bioanal. Chem*. 398 (2010) 137. DOI: 10.1007/s00216-010-3781-x

3. Malig T C, Koenig J D B, Situ H, Chehal N K, Hultin P G & Hein J E, *React. Chem. Eng.* 2 (2017) 309. DOI: https://doi.org/10.1039/C7RE00026J.

4. Hart R J, Pedge N I, Steven A R & Sutcliffe K, *Org. Process Res. Dev.* 19 (2015) 196. DOI: https://doi.org/10.1021/op500027w

5. Hoye T R, Eklov B M & Voloshin M, *Org. Lett*. 6 (2004) 953. DOI: https://doi.org/10.1021/ol049145r

6. Hoye T R, Aspaas A V, Eklov B M & Ryba, T D, *Org. Lett*. 7 (2005) 2205. DOI: https://doi.org/10.1021/ol0506011

7. Fallon T & Meek T, *Org. Lett.* 19 (2017) 5752. DOI: https://doi.org/10.1021/acs.orglett.7b02665

8. Creary X, *J. Org. Chem*. 88 (2023) 11545. DOI: https://doi.org/10.1021/acs.joc.3c00807

9. Gama L A, Merlo B B, Lacerda Jr V, Romão W & Neto A C, *Microchem Journal.* 118 (2015) 12. https://doi.org/10.1016/j.microc.2014.07.014.

10. Burton I W, Quilliam M A & Walter J A, *Anal. Chem.* 77 (2005) 3123. DOI: https://doi.org/10.1021/ac048385h

11. Benedito L E C, Maldaner A O & Oliveira A L, *Anal. Methods.* 10 (2018) 489. DOI: https://doi.org/10.1039/C7AY02343J.

12. Lagerquist L, Rahkila J & Eklund P, *ACS Sustainable Chem. Eng*. 7 (2019) 9002. DOI: https://doi.org/10.1021/acssuschemeng.9b01269.

13. Frank O, J K Kreissl, Daschner A & Hofmann T, *J. Agric. Food Chem.*62 (2014) 2506. DOI: https://doi.org/10.1021/jf405529b

14. Okaru A O, Scharinger A, Rezende T R, Teipel J, Kuballa T, Walch SG & Lachenmeier D W, *Foods* 9 (2020) 47 DOI: https://doi.org/10.3390/foods9010047.

15. Teipel J C, Hausler T, Sommerfeld K, Scharinger A, Walch S G, Lachenmeier D W & Kuballa T, *Foods.* 9 (2020)1355. DOI: https://doi.org/10.3390/foods9101355

16. Wider G & Dreier L, *J. Am. Chem. Soc*. 128 (2006) 2571. DOI: https://doi.org/10.1021/ja055336t

17. Hoult D I & Richards R E, *J. Magn. Reson. 24* (1976) 71. DOI: https://doi.org/10.1016/0022-2364(76)90233-X.

18. Hoult D I, *Concepts Magn. Reson.* 12 (2000) 173. DOI: https://doi.org/10.1002/1099-0534(2000)12:4<173::AID-CMR1>3.0.CO;2-Q

19. Hoult D I, *J. Magn. Reson.* 213 (2011) 344, DOI: https://doi.org/10.1016/j.jmr.2011.08.005.

20. Watanabe R , Sugai C , Yamazaki T , Matsushima R , Uchida H , Matsumiya M, Takatsu A & Suzuki T, *Toxins*. 8 (2016) 294 DOI: https://doi.org/10.3390/toxins8100294

21. Jakhar V K, Johnson E C, Kavuturu A, Heller J K, Veige A S & Ghiviriga I, *Org. Lett.* 23 (2021), 4945. DOI: https://doi.org/10.1021/acs.orglett.1c01006

22. Clayden J, Greeves N & Warre S, Organic Chemistry, 2nd edition, *Oxford*, (2012).

23. Tyburn J & Coutant J, User manual for electronic to access in-vivo concentration, Bruker, (2016).

24. Brand J C, Cook M D & Roberts B P, *J. Chem. Soc., Perkin Trans.* 2 (1984) 1187 DOI: 10.1039/P29840001187.

25. Gerasimov M , Lewicka D M , Orbaugh D M K , Qandil A M & Nichols D E, *J Med Chem*. 42 (1999) 4257. DOI: https://doi.org/10.1021/jm990325u