Supplemental Material for

PTX013, a potent cytotoxic agent against tumors and drug resistant cancer

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Contents of Supplementary Material S1

General Experimental Protocols

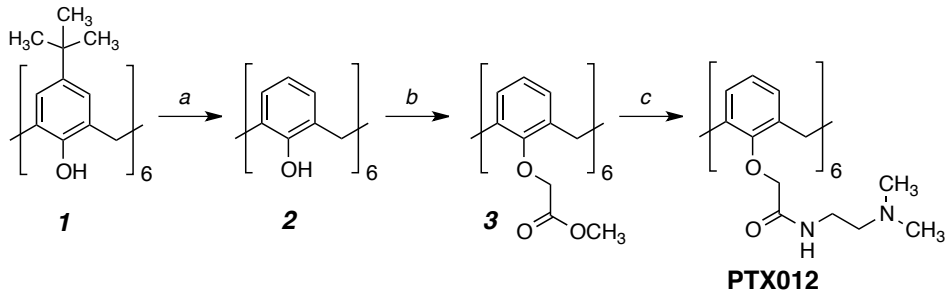
^{1}H and ^{13}C NMR spectra were recorded on a Bruker 500 (500 MHz) or Varian Inova 500 (500 MHz) instrument. ^{1}H NMR chemical shifts in $CDCl_3$ are referenced to δ 7.26 ppm ($CHCl_3$) and in methanol-d_4 to 3.31 ppm (CHD_2OD). ^{13}C NMR chemical shifts in methanol-d_4 are referenced to 49.0 ppm. The following format is used to report resonances: chemical shift in ppm [multiplicity, coupling constant(s) in Hz, integral value].

High-resolution mass spectra were recorded on a Bruker BioTOF II (ESI-TOF) instrument using PPG (MW_{ave} 725) as an internal calibrant. A solution of the analyte in methanol and then of the PPG-725 were plug-loaded into a 100 μL syringe and injected into the ESI-TOF ionization chamber. Five swaths of data (ca. 500 scans each) were analyzed, and the median value was recorded.

Synthesis Procedures and Spectroscopic Characterization Data for New Compounds.

PTX012

Scheme 1. Synthesis of **PTX012.**



a. $AlCl_3$, PhOH, toluene, rt; **b**. $BrCH_2CO_2CH_3$, Na_2CO_3, acetone, 56 °C **c**. $(CH_3)_2NCH_2CH_2NH_2$, toluene, MeOH, 90 °C.

The synthesis of known **3** was accomplished via a known 2-step sequence (dealkylation[1] to **2** followed by O-alkylation[2] to **3**) starting from the commercial available 4-*tert*-butylcalixarene (**1**).

PTX012: To a stirred solution of hexamethyl ester **3** (20 mg, 0.019 mmol, 1 equiv) dissolved in toluene (200 μL) along with several drops of methanol, was added *N,N*-dimethylethylenediamine (123 μL, 1.12 mmol, 60 equiv) under a nitrogen atmosphere. The resultant solution was heated at 90 °C for 24 h after which an additional 60 equiv of *N,N*-dimethylethylenediamine was added and the solution heated for 20.5 h. The solution was cooled and the solvents removed under reduced pressure. Diethyl ether was added to the remaining solid and the resultant gummy residue was triturated with ethanol/ethyl acetate. The supernatant was concentrated to provide **PTX012** (20 mg).

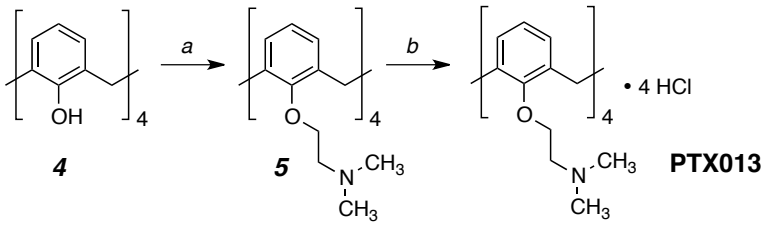
^{1}H NMR (500 MHz, methanol-d_4) δ 6.82 (d, *J* = 7.5 Hz, 12H), 6.63 (t, *J* = 7.6 Hz, 6H), 4.23 (br s, 12H), 4.04 (br s, 12H), 3.29 (mostly obscured by CD_2HOD peak, chemical shift deduced from COSY spectrum correlation to the 2.38 resonance), 2.38 (t, *J* = 6.9 Hz, 12H), and 2.23 (s, 36H).

^{13}C NMR (125 MHz, methanol-d_4) δ 170.8, 155.6, 134.9, 130.6, 126.1, 72.8, 59.1, 45.6, 37.6, and 30.7.

HRMS (ESI-TOF) *m/z* calcd for $C_{78}H_{108}N_{12}Na_2O_{12}$ $(M + 2Na)^{2+}$, 725.3997; found, 725.3994.

PTX013

Scheme 2. Synthesis of **PTX013.**



a. $ClCH_2CH_2N(CH_3)_2$•HCl, NaOH, toluene, 110 °C **b**. HCl/EtOH, rt.

Calixarene **5**: To a culture tube equipped with a stir bar was added compound **4** (150 mg, 0.354 mmol, 1 equiv) dissolved in toluene (3 mL). The solution was warmed and powdered NaOH (340 mg, 8.5 mmol, 24 equiv) was added. This suspension was refluxed for 10 min after which 2-(*N,N*-dimethylamino)ethyl chloride•HCl (410 mg, 2.85 mmol, 8 equiv) was added and the mixture was heated for 2.25 h. The mixture was cooled to rt, water was added, and the layers were separated. The organic layer was washed with 10% $HCl_{(aq)}$ (x 3) and the aqueous layers were combined and basified with $NaOH_{(aq)}$. The aqueous layer was extracted with $CHCl_3$ and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated to provide a pale yellow solid. Trituration with hexane gave **5** as an off-white solid (22 mg).

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.61 (m, 12H), 4.43 (d, *J* = 13.5, 4H), 4.04 (t, *J* = 7.5 Hz, 8H), 3.17 (d, *J* = 13.5 Hz, 4H), 2.85 (br s, 8H), and 2.31 (s, 24H).

PTX013: Acetyl chloride (71 μL) was added to ethanol (10 mL) at rt to produce a 0.1 M ethanolic HCl stock solution. In another flask calixarene **5** (10 mg, 0.014 mmol, 1 equiv) was dissolved in $CHCl_3$ (1 mL), and a portion of the stock ethanolic HCl solution (564 μL, 0.056 mmol, 4 equiv) was added. After several minutes the solvent was removed *in vacuo* to provide **PXT013** as a white solid.

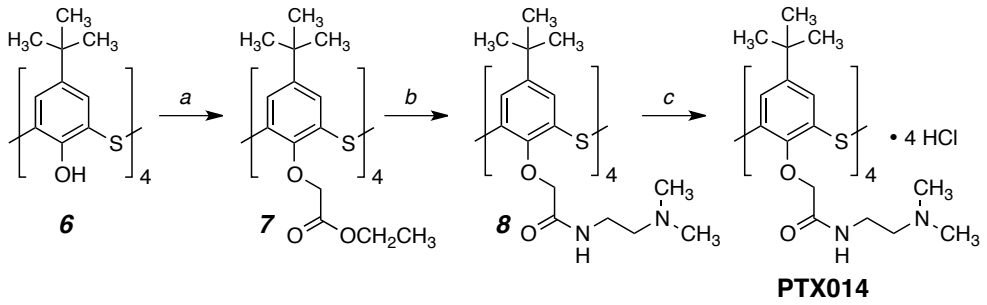
^{1}H NMR (500 MHz, methanol-d_4) δ 6.67 (br s, 12H), 4.35 (t, *J* = 7.6, 8H), 4.33 (d, *J* = 13.6 Hz, 4H), 3.64 (t, *J* = 7.2 Hz, 8H), 3.38 (d, *J* = 13.8 Hz, 4H), and 2.96 (s, 24H).

^{13}C NMR (125 MHz, methanol-d_4) δ 156.4, 135.8, 130.1, 124.6, 69.8, 57.2, 44.5, and 32.1.

HRMS (ESI-TOF): calcd for $C_{44}H_{61}N_4O_4$ $(M + H)^+$, 709.4687; found 709.4701.

PTX014

Scheme 3. Synthesis of **PTX014.**



a. $BrCH_2CO_2CH_2CH_3$, Na_2CO_3, acetone, 56 °C **b**. $(CH_3)_2NCH_2CH_2NH_2$, toluene, CH_3OH, 90 °C **c**. HCl/ CH_3OH, rt.

The synthesis of **7** was accomplished by O-alkylation[2] of known **6** as shown above.

Calixarene **8:** Tetraethyl ester **7** (26.5 mg, 0.028 mmol, 1 equiv) was dissolved in *N,N*-dimethylethylenediamine (1 mL) under a nitrogen atmosphere. The resultant solution was stirred at rt for 20 h. Water was added and the mixture was extracted with ethyl acetate. The organic layers were dried with $MgSO_4$, filtered, and concentrated to give a white solid. Crystallization from ether/ethanol provided calixarene **8** (17.5 mg).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09 (t, *J* = 5.5 Hz, 4H), 7.32 (s, 8H), 4.86 (s, 8H), 3.47 (dt, *J* = 6.0, 6.0 Hz, 8H), 2.49 (t, *J* = 6.5 Hz, 8H), 2.36 (s, 24H), and 1.1 (s, 36H).

PTX014: Acetyl chloride (71 μL) was added to methanol (10 mL) at rt to produce a 0.1 M methanolic HCl stock solution. In another flask calixarene **8** (11 mg, 0.009, 1 equiv) was dissolved in $CHCl_3$ (1 mL). A portion of the stock methanolic HCl solution (410 μL, 0.041 mmol, 4.4 equiv) was added. After several minutes the solvent was removed *in vacuo* to provide **PXT014** as a white solid.

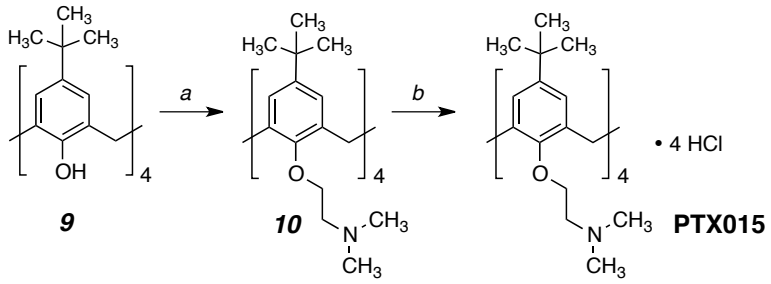
^{1}H NMR (500 MHz, methanol-d_4) δ 7.47 (s, 8H), 5.04 (s, 8H), 3.78 (t, *J* = 5.9 Hz, 8H), 3.44 (t, *J* = 5.6 Hz, 8H), 3.0 (s, 24H), and 1.14 (s, 36H).

^{13}C NMR (125 MHz, methanol-d_4) δ 172.2, 159.3, 149.2, 136.3, 129.7, 75.9, 58.3, 44.0, 35.8, 35.3, and 31.6.

HRMS (ESI-TOF) *m/z* calcd for $C_{64}H_{98}N_8O_8S_4$ $(M + 2H)^{2+}$, 617.3190; found, 617.3198.

PTX015

Scheme 4. Synthesis of **PTX015.**



a. $ClCH_2CH_2N(CH_3)_2$•HCl, NaOH, toluene, 110 ^oC; **b**. HCl/MeOH, rt.

Calixarene **10**: To a culture tube equipped with a stir bar was added compound **9** (50 mg, 0.077 mmol, 1 equiv) dissolved in toluene (1 mL). The solution was warmed and powdered NaOH (75 mg, 1.88 mmol, 24 equiv) was added. This suspension was refluxed for 10 min after which 2-(*N,N*-dimethylamino)ethyl chloride•HCl (89 mg, 0.618 mmol, 8 equiv) was added, and the mixture was heated for 2.25 h. The mixture was cooled to rt, water was added, and the layers were separated. The organic layer was washed with 10% $HCl_{(aq)}$ (x 3), and the aqueous layers were combined and basified with $NaOH_{(aq)}$. The aqueous layer was extracted with $CHCl_3$ and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated to provide a pale yellow solid. Trituration with hexane gave **10** as an off-white solid (14 mg). The ^1H NMR spectral data in $CDCl_3$ were in accord with reported literature values.[3]

PTX015: Acetyl chloride (71 μL) was added to methanol (10 mL) at rt to produce a 0.1 M methanolic HCl stock solution. In another flask was placed **10** (8.5 mg, 0.009 mmol, 1 equiv) dissolved in $CHCl_3$ (0.5 mL), and a portion of the stock methanolic HCl solution (365 μL, 0.036 mmol, 4 equiv). After several minutes the solvent was removed *in vacuo* to provide **PXT015** as a white solid.

^1H NMR (500 MHz, methanol-d_4) δ 6.92 (s, 8H), 4.38 (m, 8H), 4.29 (d, J = 12.9 Hz, 4H), 3.69 (m, 8H), 3.31 (obscured by CD_2HOD peak, chemical shift from COSY correlated with the resonance at 4.29 ppm), 2.99 (br s, 24H), and 1.1 (s, 36H).

^{13}C NMR (125 MHz, methanol-d_4) δ 152.1, 146.1, 133.2, 125.6, 68.2, 55.3, 42.9, 33.5, 30.5, add 29.3.

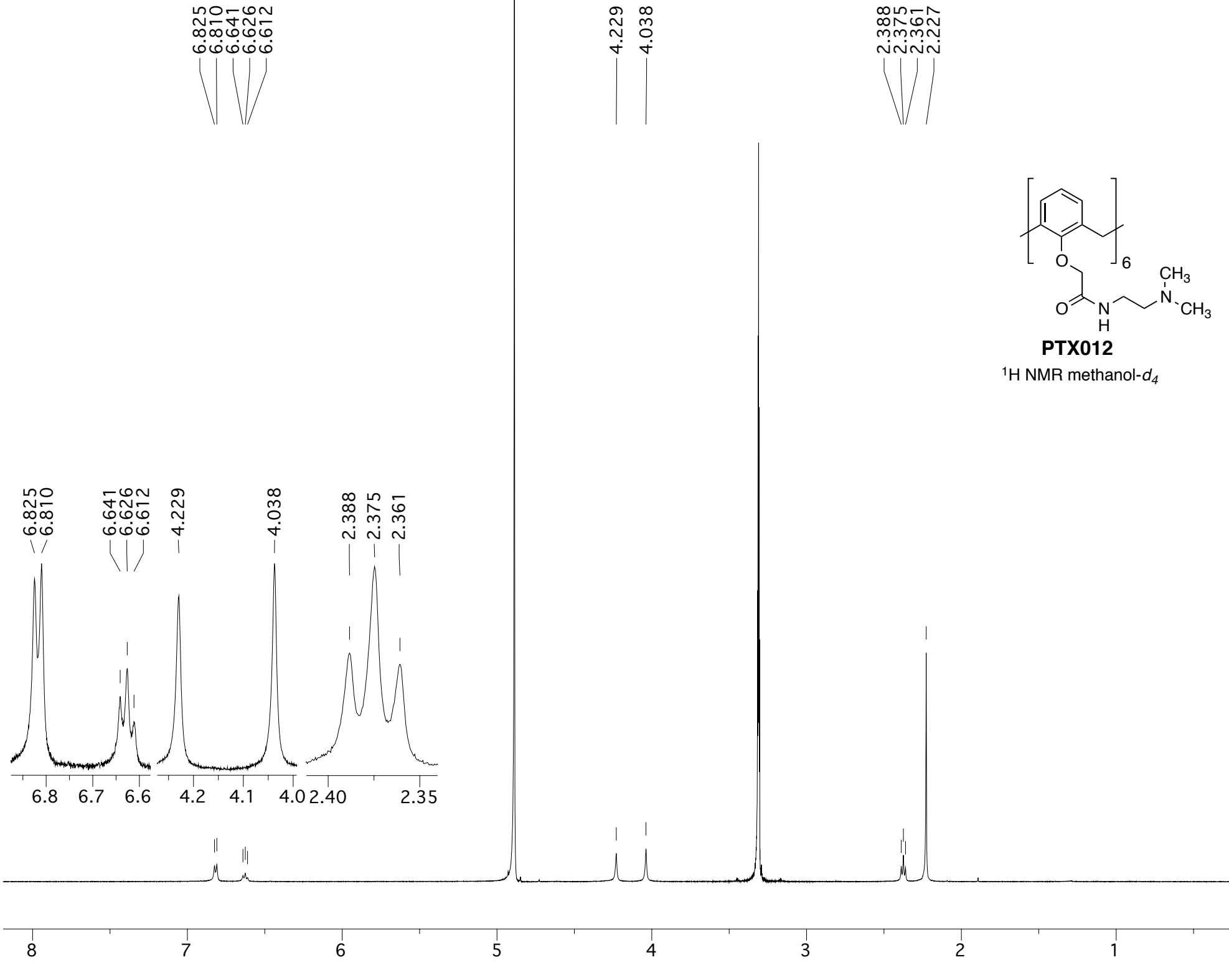
HRMS (ESI-TOF) *m/z* calcd for $C_{60}H_{93}N_4O_4$ $(M + H)^+$, 933.7191; found, 933.7207.

References

[1] V. Böhmer, D. Rathay, H. Kämmerer, The *t*-butyl group as a possible protective group in the synthesis of oligo [hydroxy-1,3-phenylene]methylenes, Org. Prep. Proc. Int. 3 (1978) 113–121.

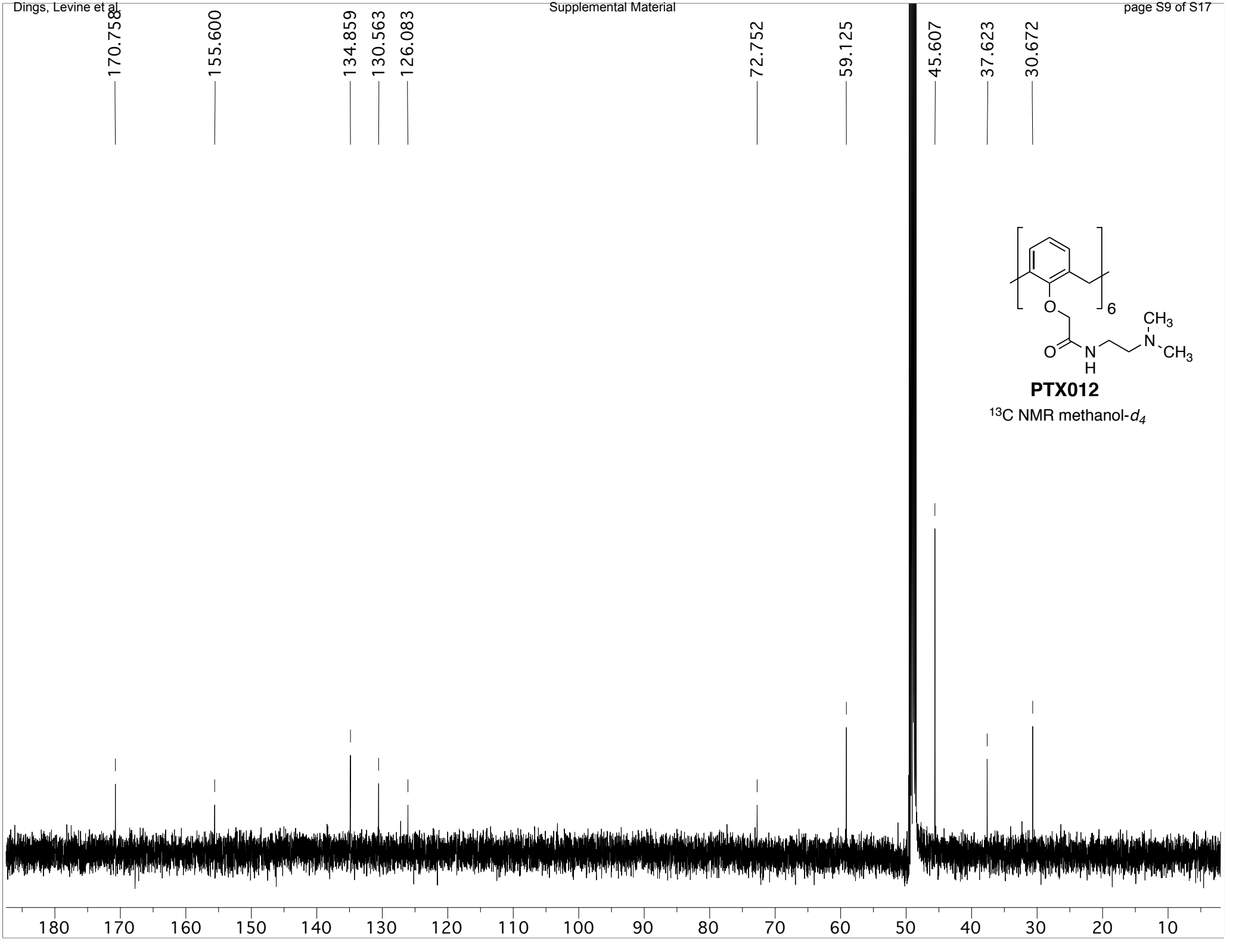
[2] F. Arnaud-Neu, E. M. Collins, M. Deasy, G. Ferguson, S. J. Harris, B. Kaitner, A. J. Lough, M. A. McKervey, E. Marques, B. L. Ruhl, M. J. Schwing-Weill, E. M. Seward, Synthesis, X-ray crystal structures, and cation-binding properties of alkyl calixaryl esters and ketones, a new family of macrocyclic molecular receptors, J. Am. Chem. Soc. 111 (1989) 8681–8691.

[3] A. F. Danil de Namor, F. J. Sueros Velarde, M. C. Cabaleiro, New lower rim calix [4]arene amine derivatives: Synthesis, characterisation and acid-base properties, J . Chem. Soc. Faraday Trans. 92 (1996) 1731–1737.



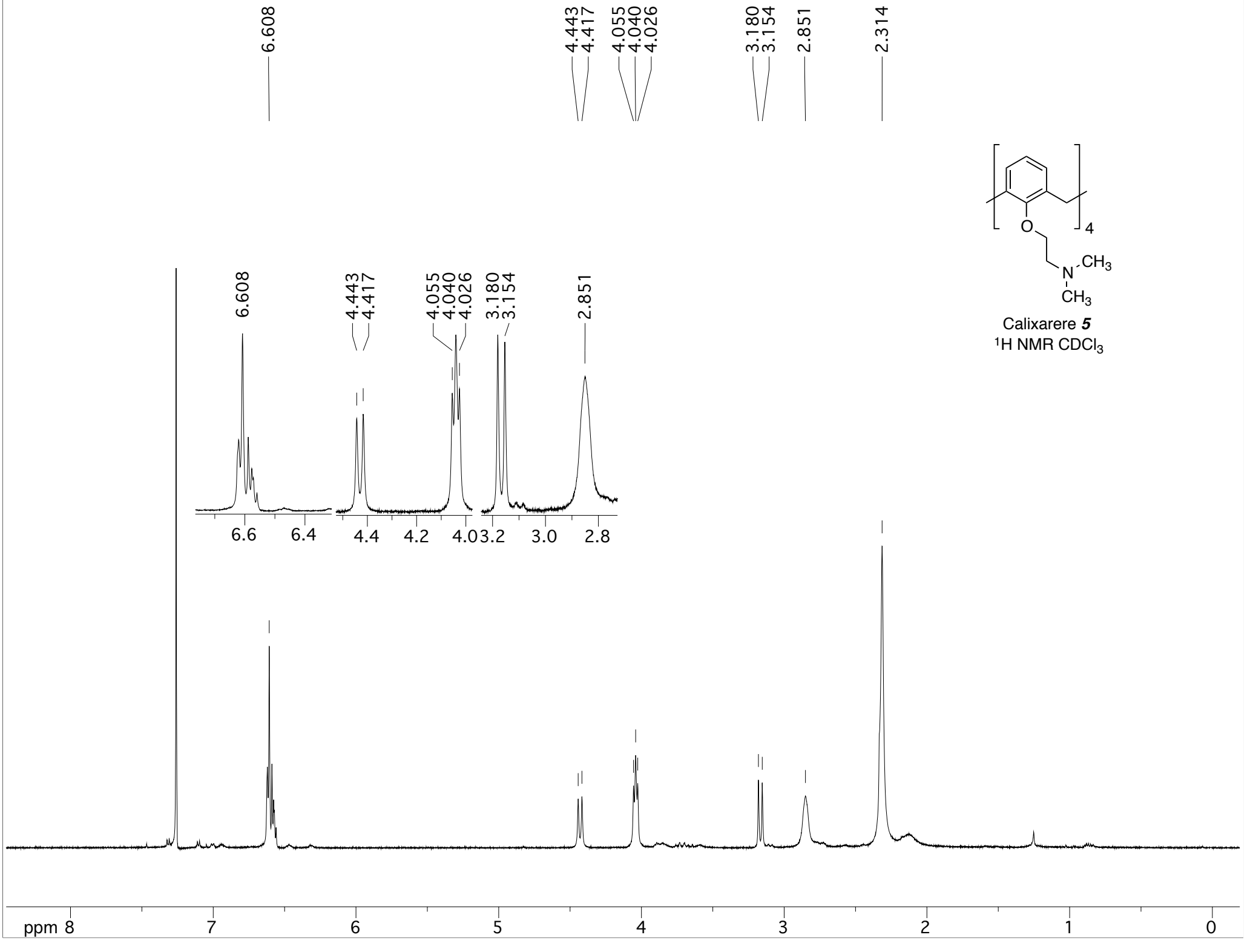
PTX012

^{1}H NMR methanol-d_4



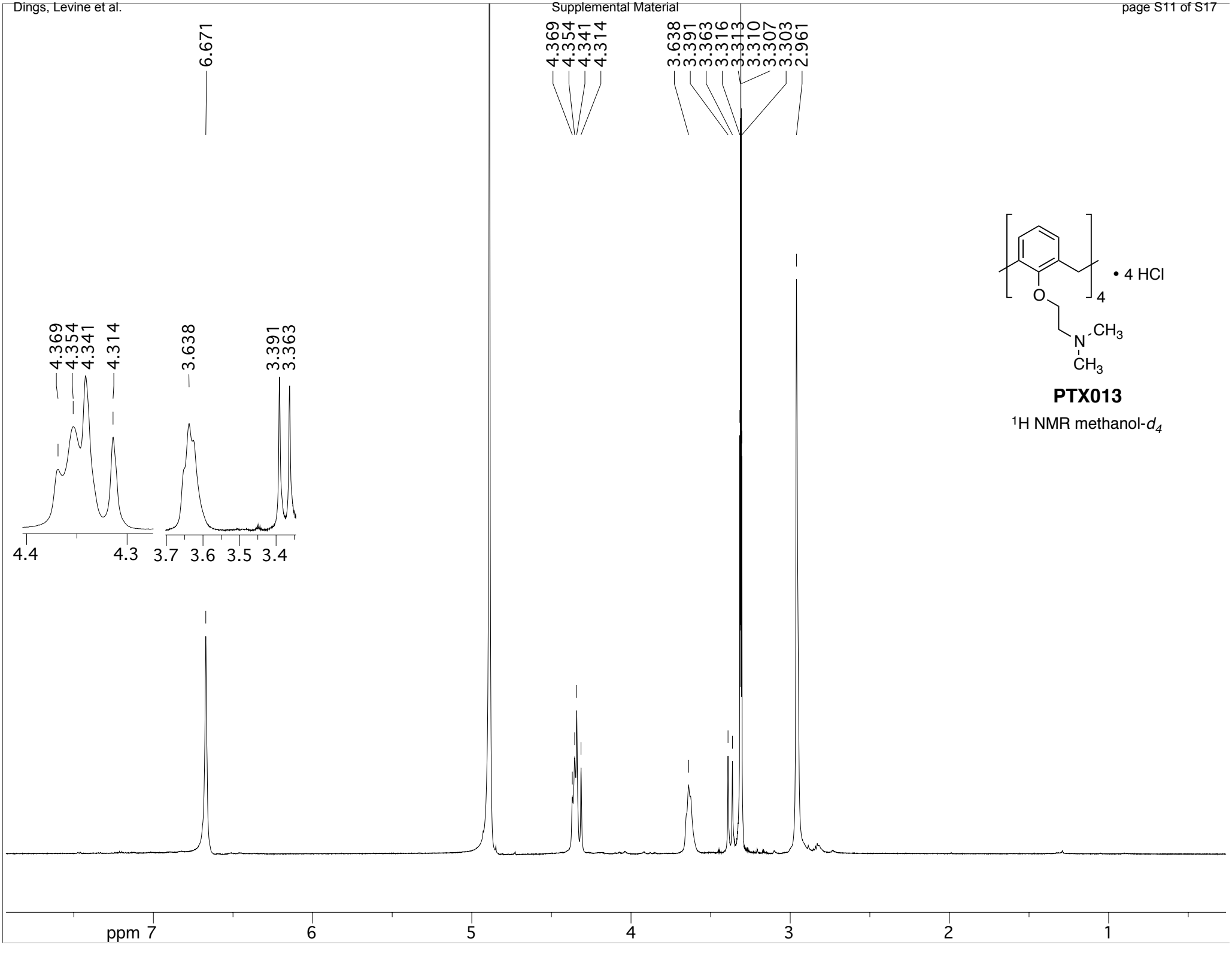
PTX012

^{13}C NMR methanol-d_4



Calixarere ***5***

^{1}H NMR $CDCl_3$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3.363
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3.5
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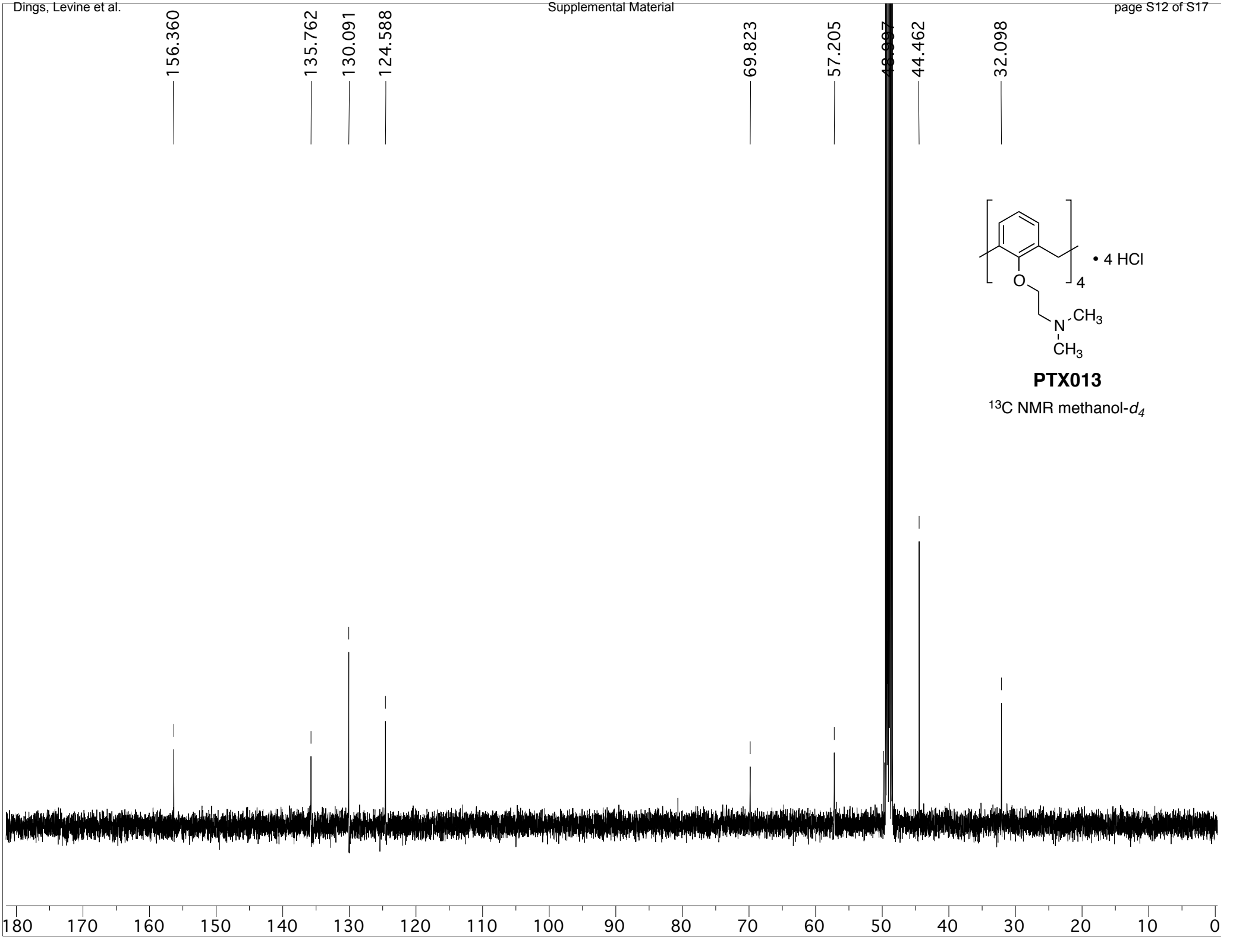
2
1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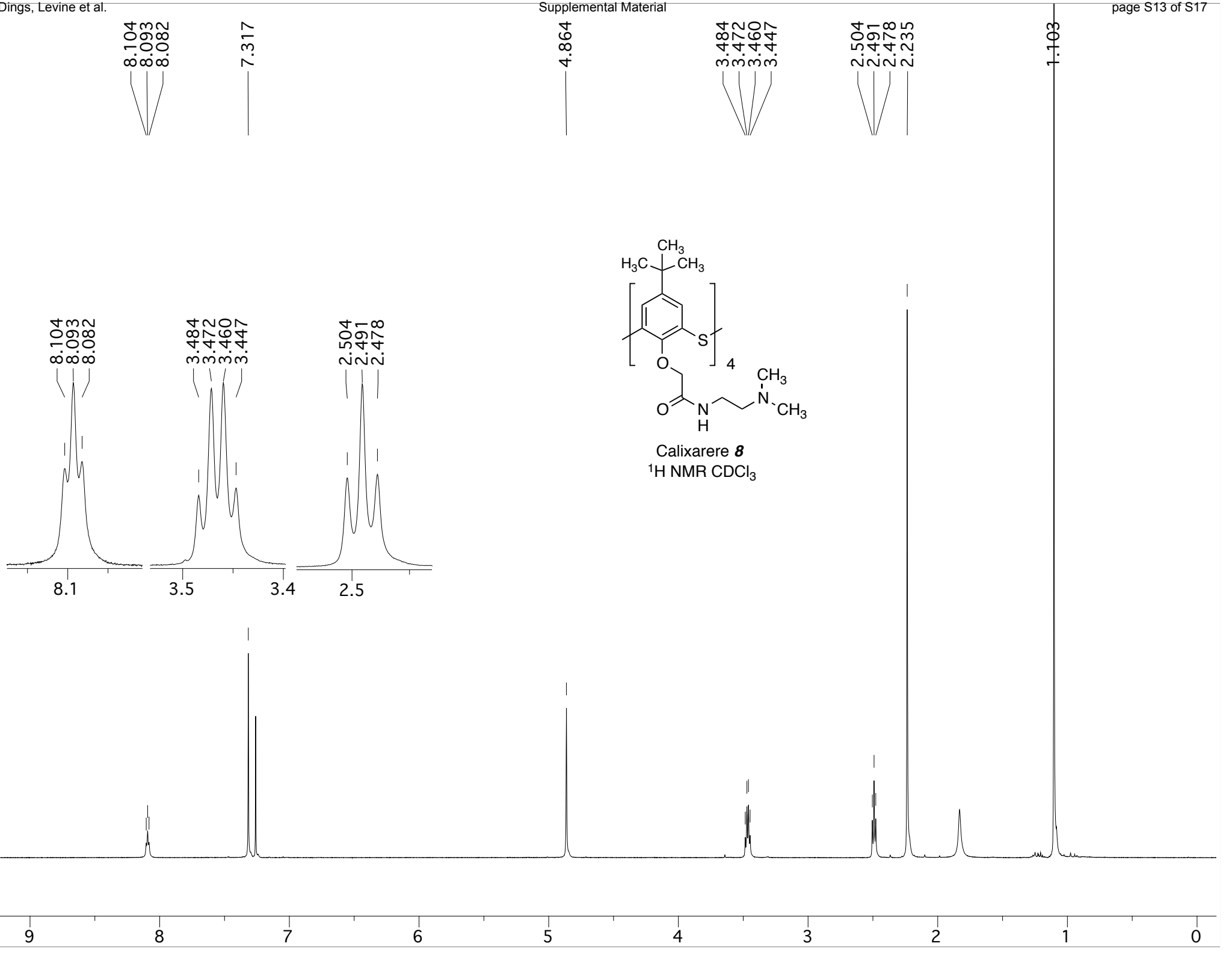
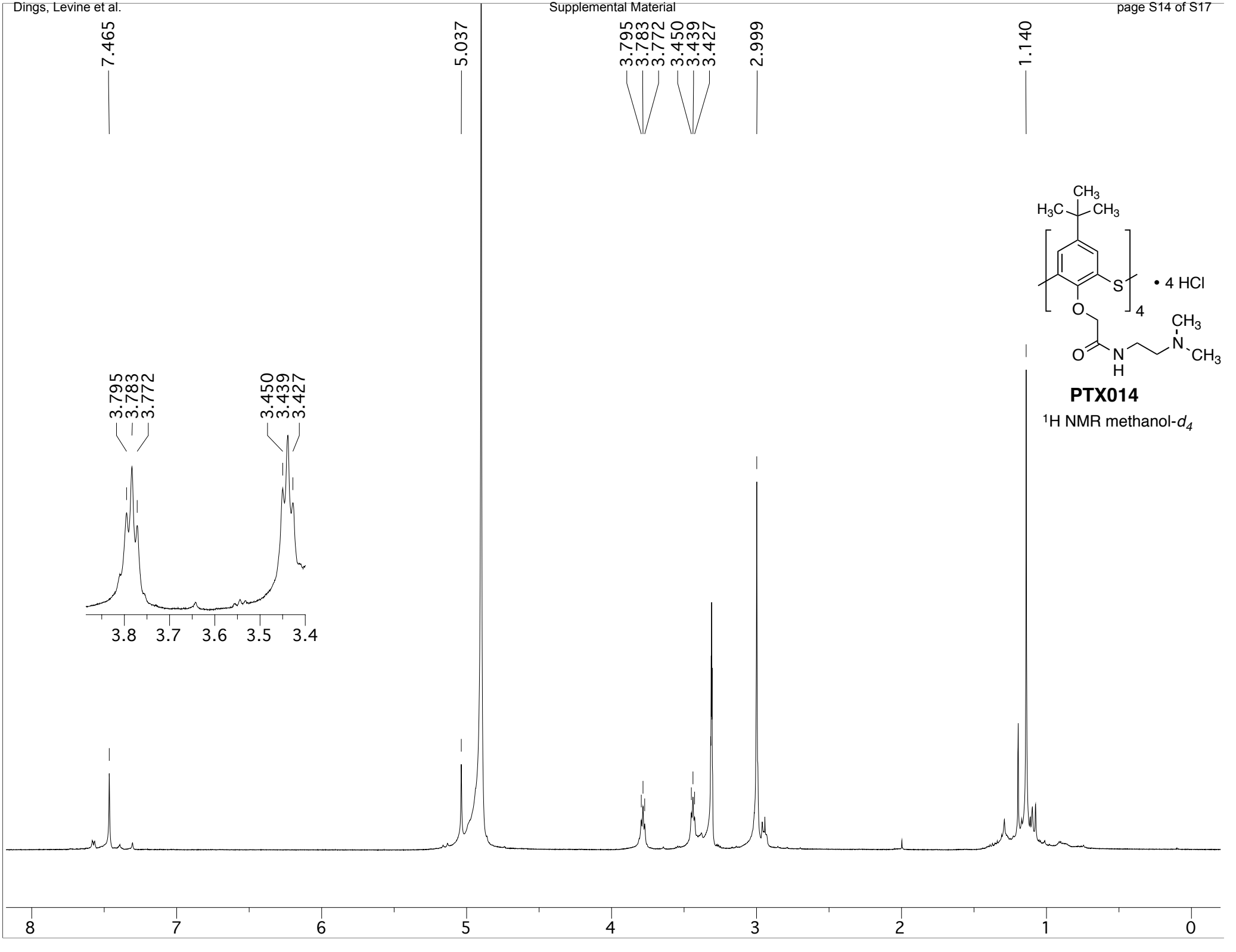
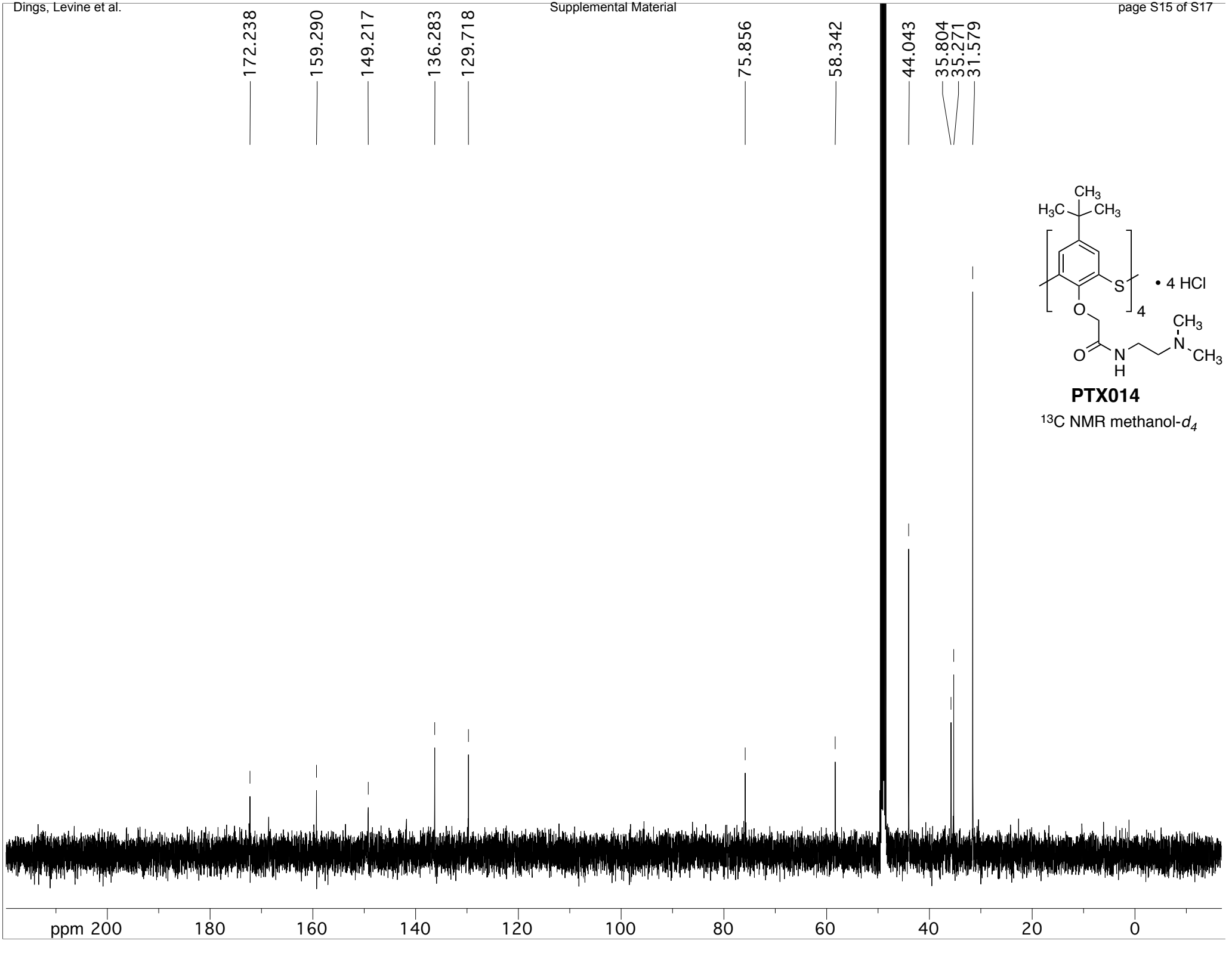


Calixarere ***8***
^{1}H NMR $CDCl_3$



PTX014

^{1}H NMR methanol-d_4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S
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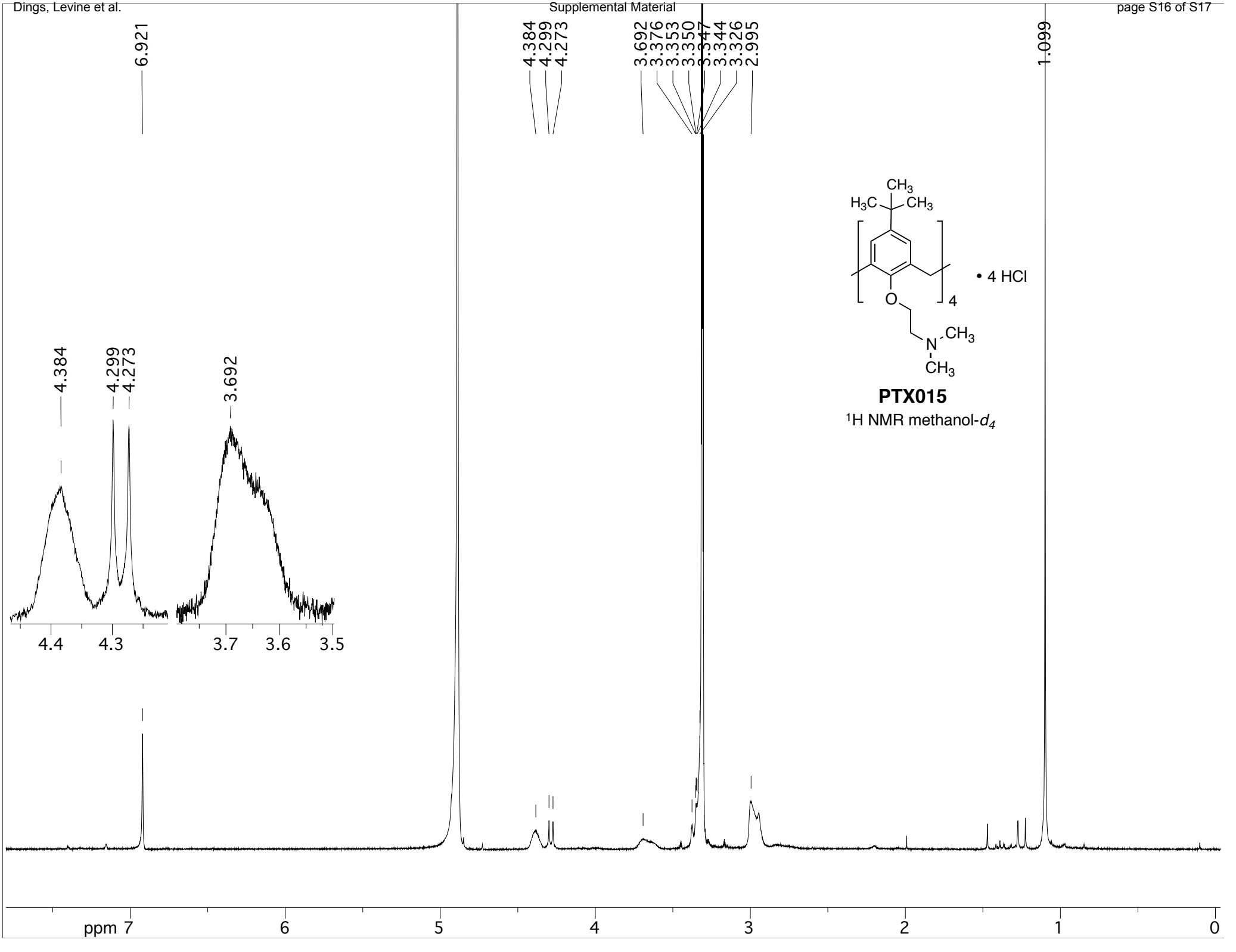
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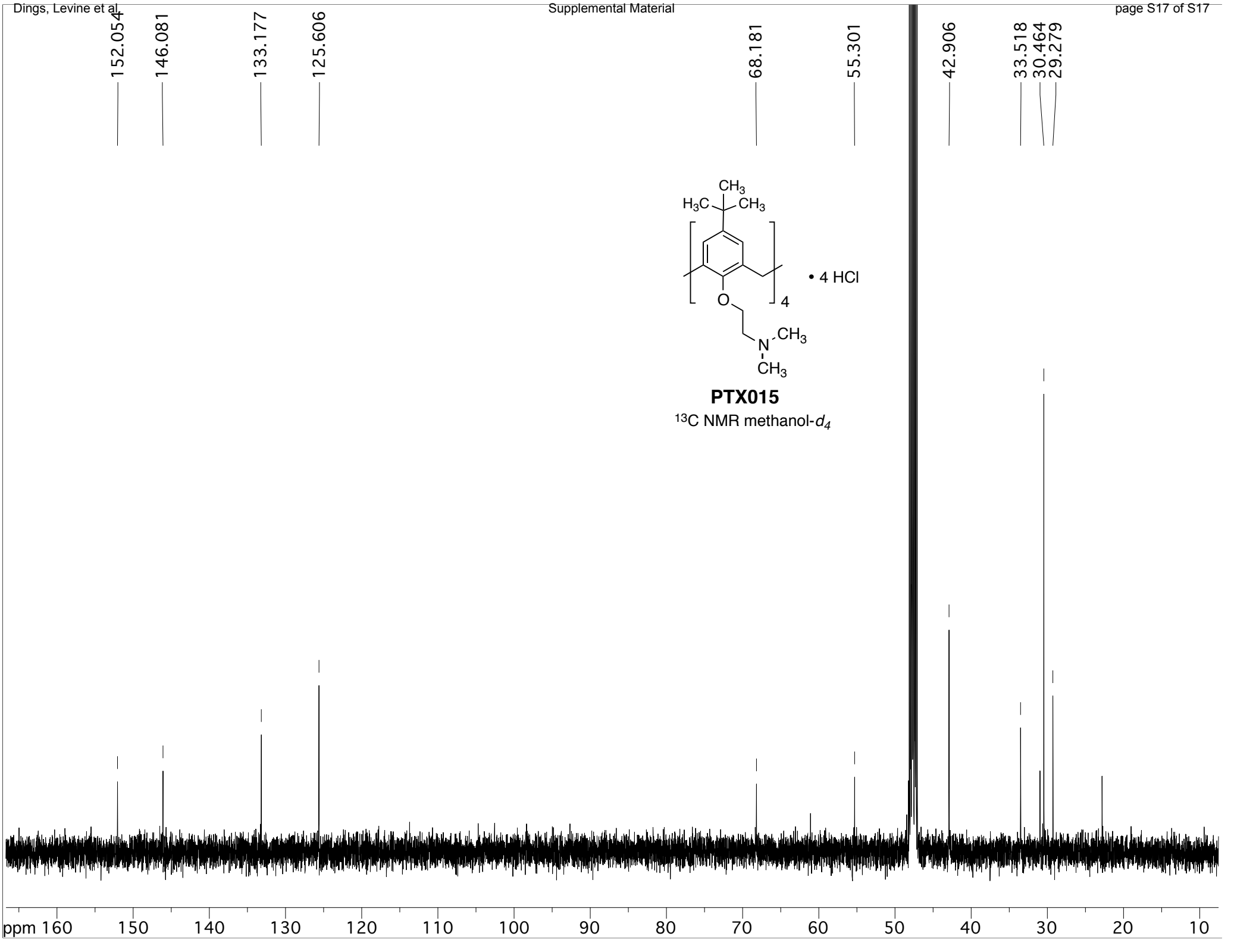
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