

Supplementary Information

Biguanide-based synthesis of 1,3,5-triazine derivatives with anticancer activity and 1,3,5-triazine incorporated calcium citrate nanoparticles

Monnaya Chalermnon,^a Sarocha Cherdchom,^{b,c} Amornpun Sereemaspun,^d Rojrit Rojanathanes,^e and Tanatorn Khotavivattana^{*f}

- a. Faculty of Science, Department of Chemistry, Chulalongkorn University, Bangkok 10330, Thailand*
- b. Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Phayathai road, Wangmai, Patumwan, Bangkok 10330, Thailand*
- c. NanoMedicine Research Unit, Department of Anatomy, Faculty of Medicine, Chulalongkorn University, Rama 4 Road, Patumwan, Bangkok 10330, Thailand*
- d. Chula Medical Innovation Centre (CMIC), Nanomedicine Research Unit, Department of Anatomy, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand*
- e. Centre of Excellence in Materials and Bio-Interfaces Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*
- f. Centre of Excellence in Natural Products Chemistry, Department of Chemistry, Chulalongkorn University, Bangkok 10330, Thailand*

**Co-corresponding authors*

^1H NMR (400 MHz, DMSO) δ 9.75 (s, 1H), 7.41 – 7.24 (m, 8H), 7.13 – 6.97 (m, 3H).

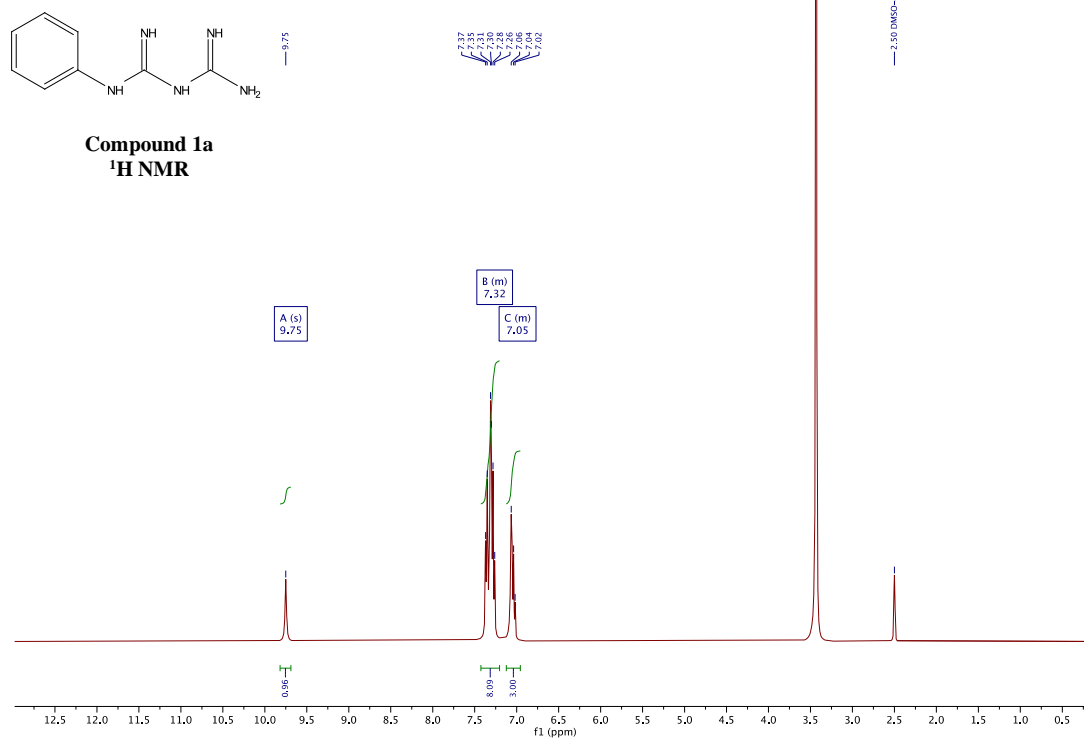


Figure 1. ^1H NMR of Phenylbiguanide hydrochloride (**1a**)

^{13}C NMR (101 MHz, DMSO) δ 161.4, 155.5, 138.8, 128.9, 123.6, 121.2.

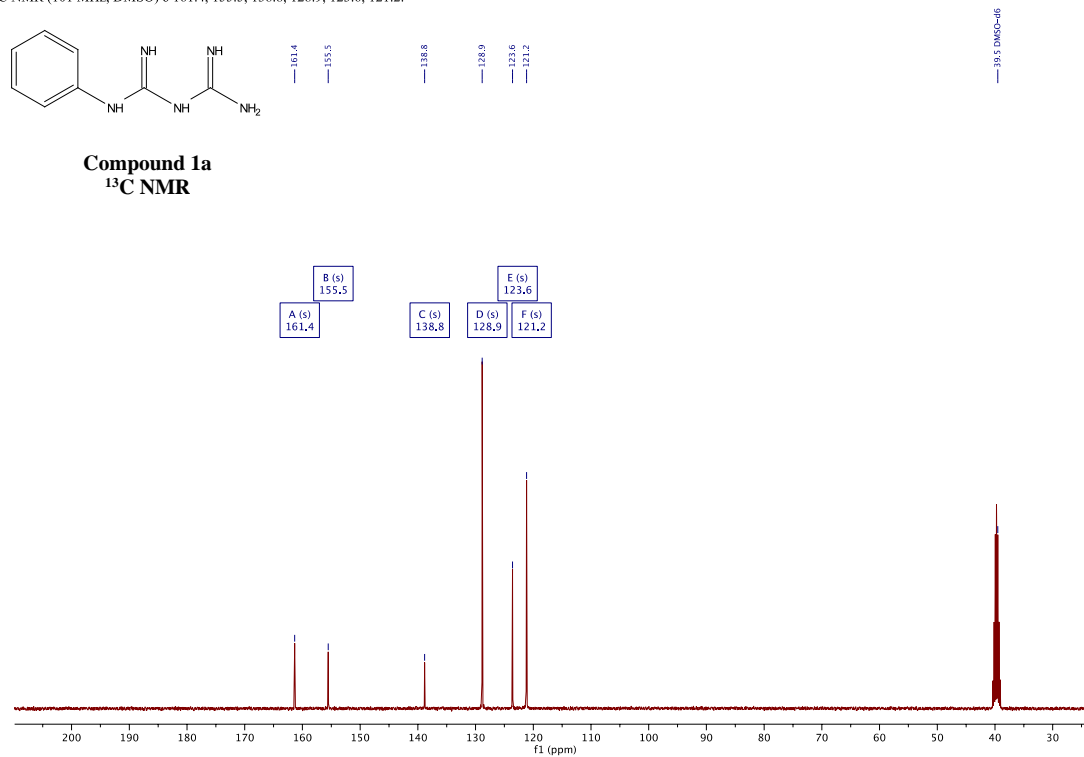


Figure 2. ^{13}C NMR of Phenylbiguanide hydrochloride (**1a**)

^1H NMR (400 MHz, DMSO) δ 7.20 (t, $J = 7.6$ Hz, 2H), 6.93 – 6.75 (m, 3H).

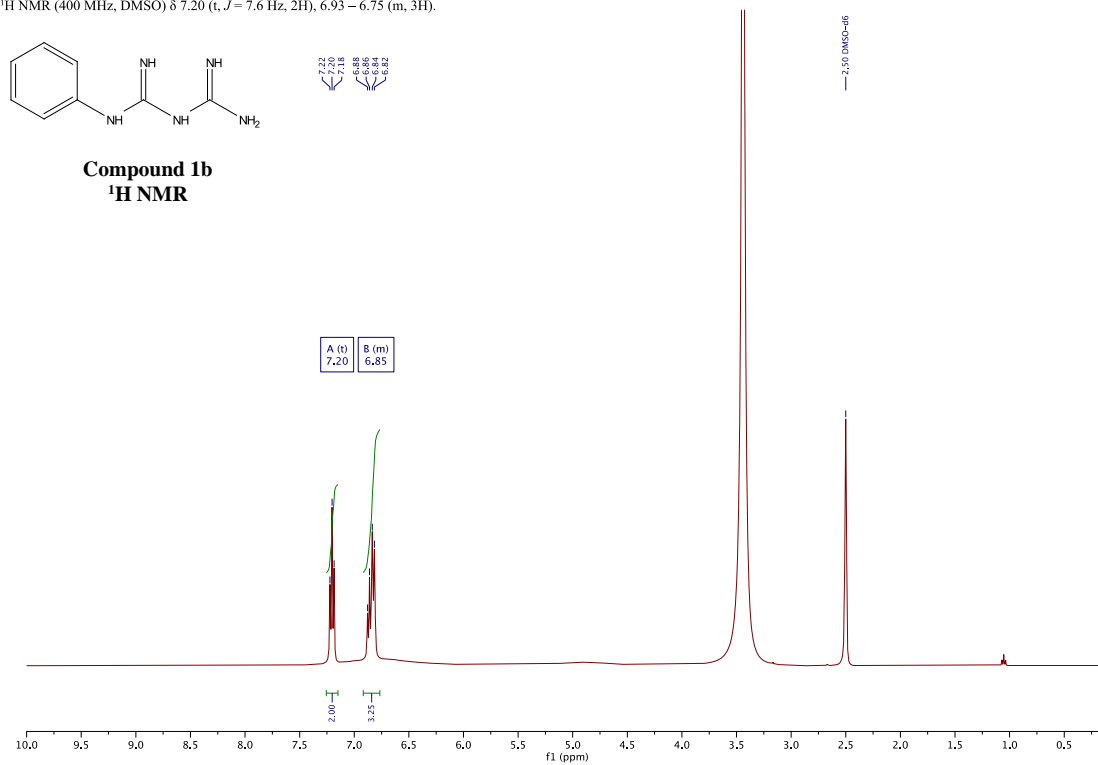


Figure 3. ^1H NMR of Phenylbiguanide (**1b**)

^{13}C NMR (101 MHz, DMSO) δ 159.7, 157.9, 150.2, 129.2, 123.0, 121.1.

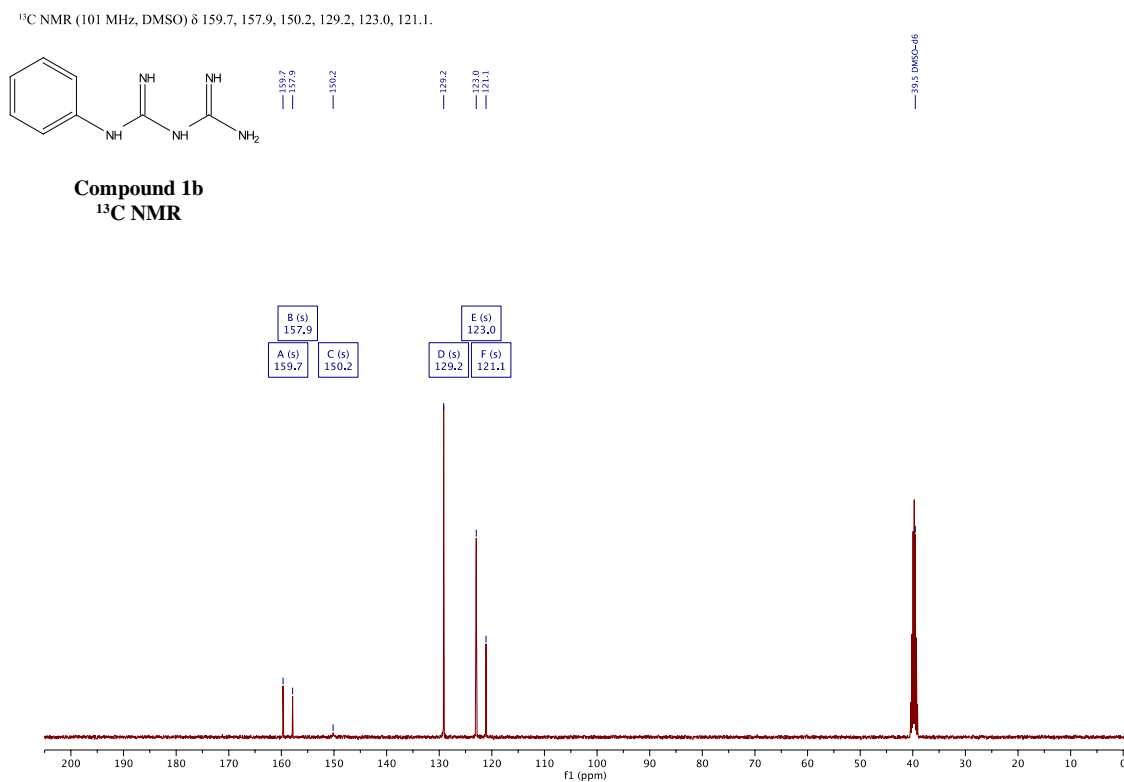


Figure 4. ^{13}C NMR of Phenylbiguanide (**1b**)

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 5.17 (brs, 2H), 3.15 (brs, 3H), 3.11 (brs, 3H), 2.49 (t, $J = 7.7$ Hz, 3H), 1.78 – 1.64 (m, 2H), 1.32 – 1.19 (m, 24H), 0.87 (t, $J = 6.7$ Hz, 3H).

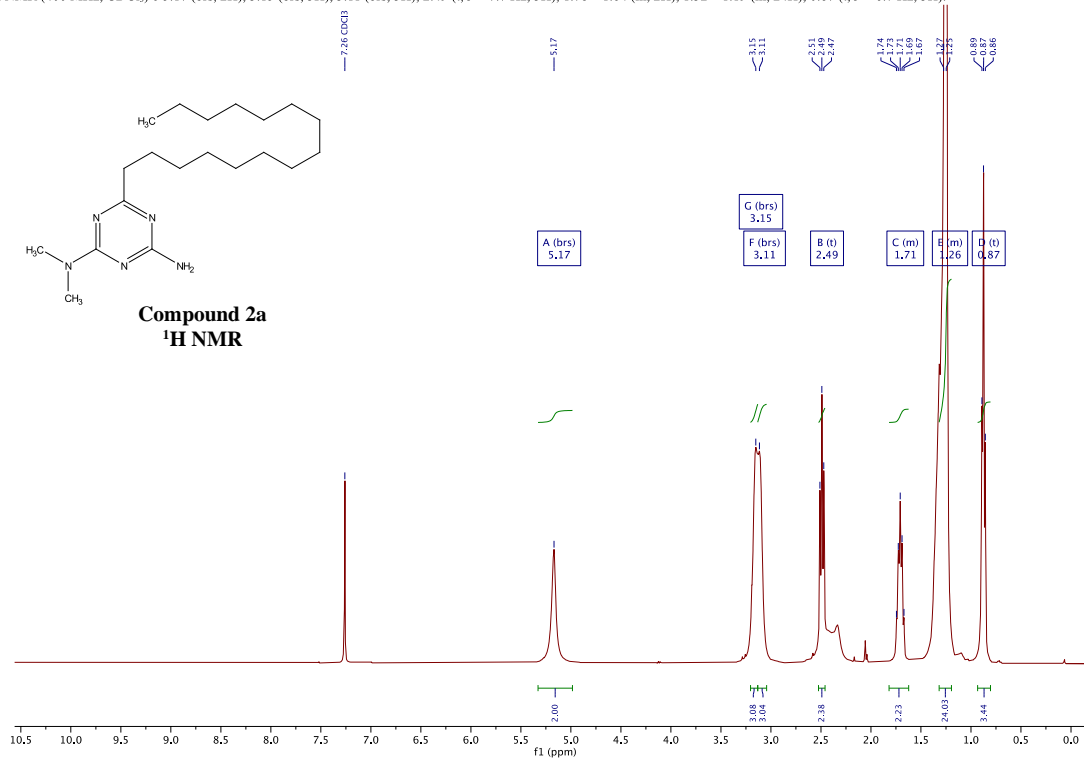


Figure 5. $^1\text{H NMR}$ of N,N' -dimethyl-6-pentadecyl-1,3,5-triazine-2,4-diamine (**2a**)

$^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 178.1, 166.3, 165.6, 38.8, 36.3, 32.1, 29.8, 27.7, 22.8, 14.2.

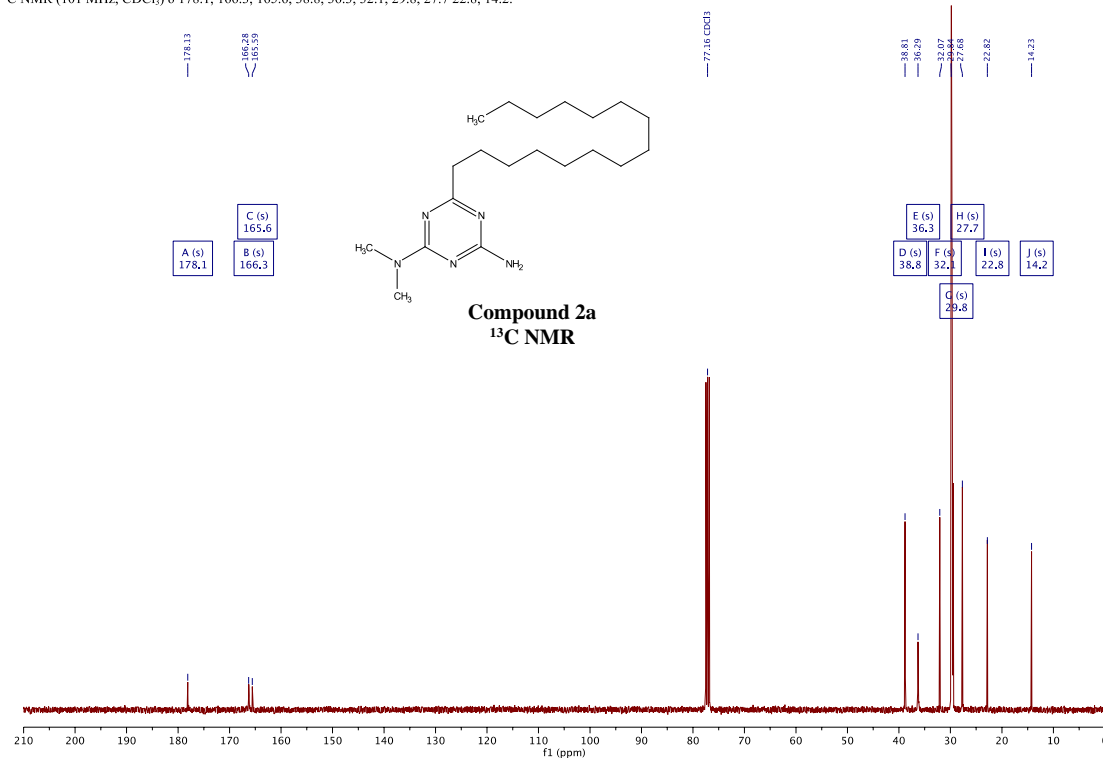


Figure 6. $^{13}\text{C NMR}$ of N,N' -dimethyl-6-pentadecyl-1,3,5-triazine-2,4-diamine (**2a**)

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 8.37 (d, $J = 7.5$ Hz, 2H), 7.53 – 7.38 (m, 3H), 5.26 (brs, 2H), 3.30 (brs, 3H), 3.17 (brs, 3H).

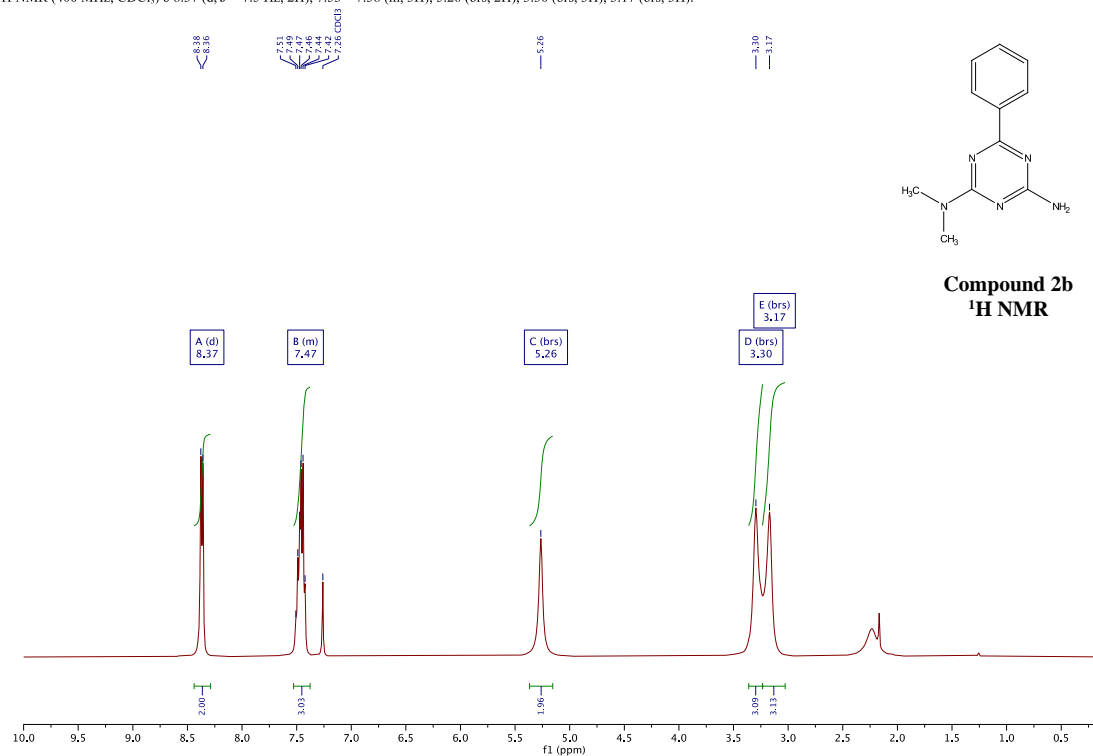


Figure 7. $^1\text{H NMR}$ of N^2,N^2 -dimethyl-6-phenyl-1,3,5-triazine-2,4-diamine (**2b**)

$^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 171.0, 167.4, 166.0, 131.4, 130.1, 128.5, 128.3, 36.4.

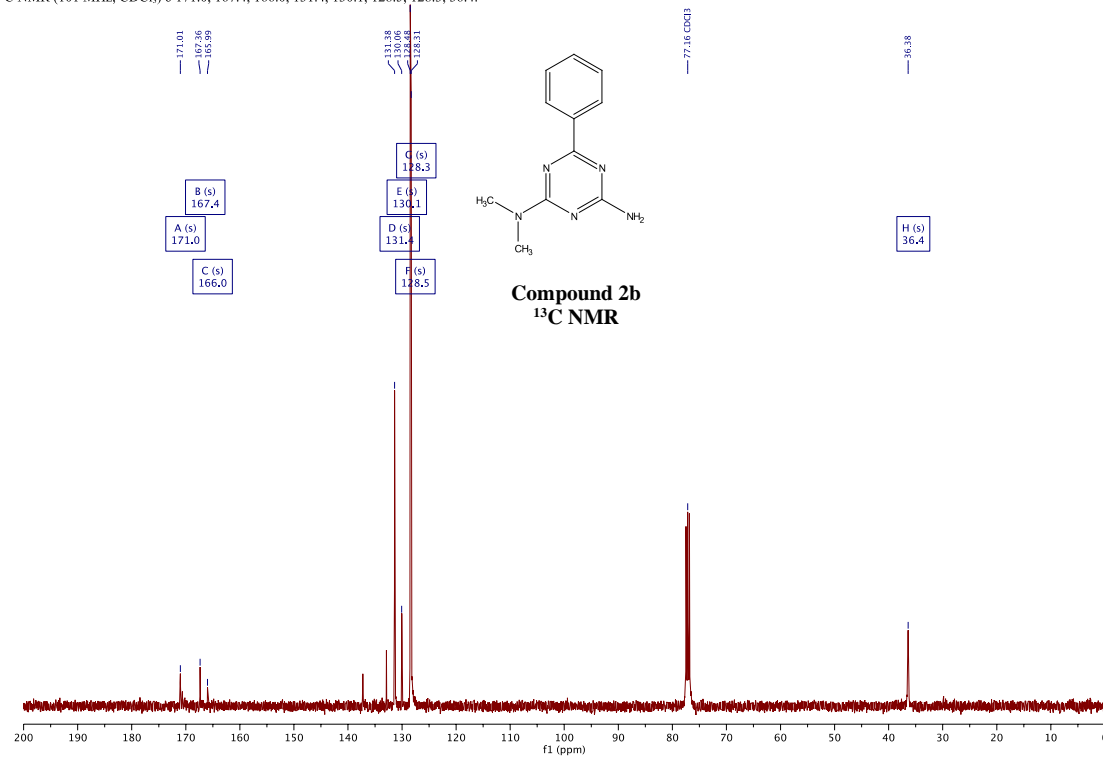


Figure 8. $^{13}\text{C NMR}$ of N^2,N^2 -dimethyl-6-phenyl-1,3,5-triazine-2,4-diamine (**2b**)

¹H NMR (400 MHz, CDCl₃) δ 8.33 (d, *J* = 7.9 Hz, 1H), 7.37 (t, *J* = 7.8 Hz, 1H), 6.95 (d, *J* = 8.3 Hz, 1H), 6.89 (t, *J* = 7.5 Hz, 1H), 5.21 (brs, 2H), 3.22 (s, 3H), 3.17 (s, 3H).

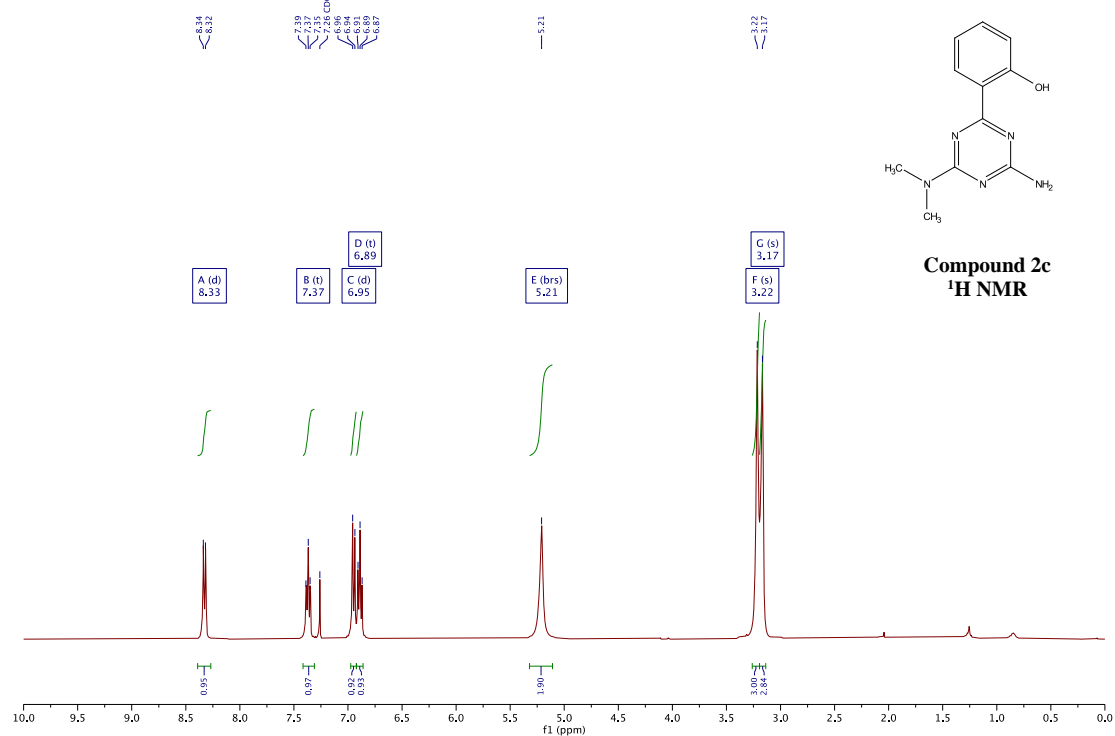


Figure 9. ¹H NMR of 2-(4-amino-6-(dimethylamino)-1,3,5-triazin-2-yl)phenol (**2c**)

¹³C NMR (101 MHz, CDCl₃) δ 171.3, 165.8, 164.4, 162.2, 134.1, 129.7, 119.1, 118.4, 118.1, 36.9, 36.7.

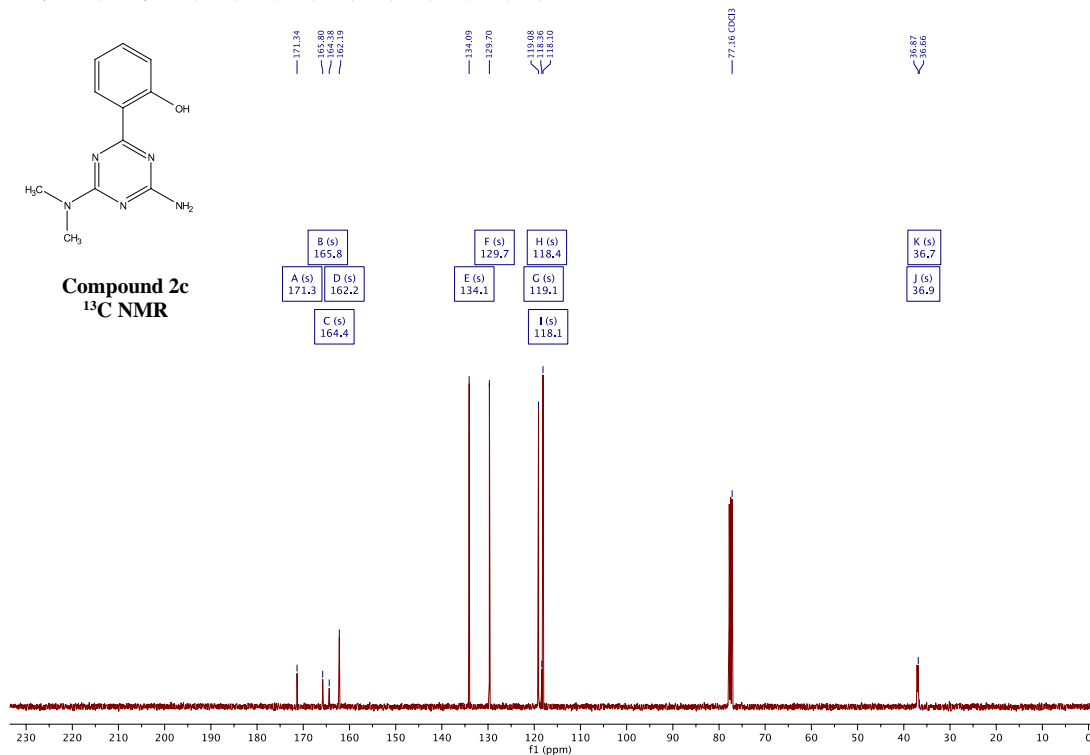


Figure 10. ¹³C NMR of 2-(4-amino-6-(dimethylamino)-1,3,5-triazin-2-yl)phenol (**2c**)

Mass Spectrum List Report

Analysis Info

Analysis Name D:\Data\Data Service\190819VA46_RC6_01_2982.d
Method nv_pos_6min_profile_wguardcol_190624.m
Sample Name A46
Comment

Acquisition Date 8/19/2019 10:23:02 PM

Operator CU.
Instrument / Ser# micrOTOF-Q II 10335

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	3.0 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	250.0 Vpp	Set Divert Valve	Waste

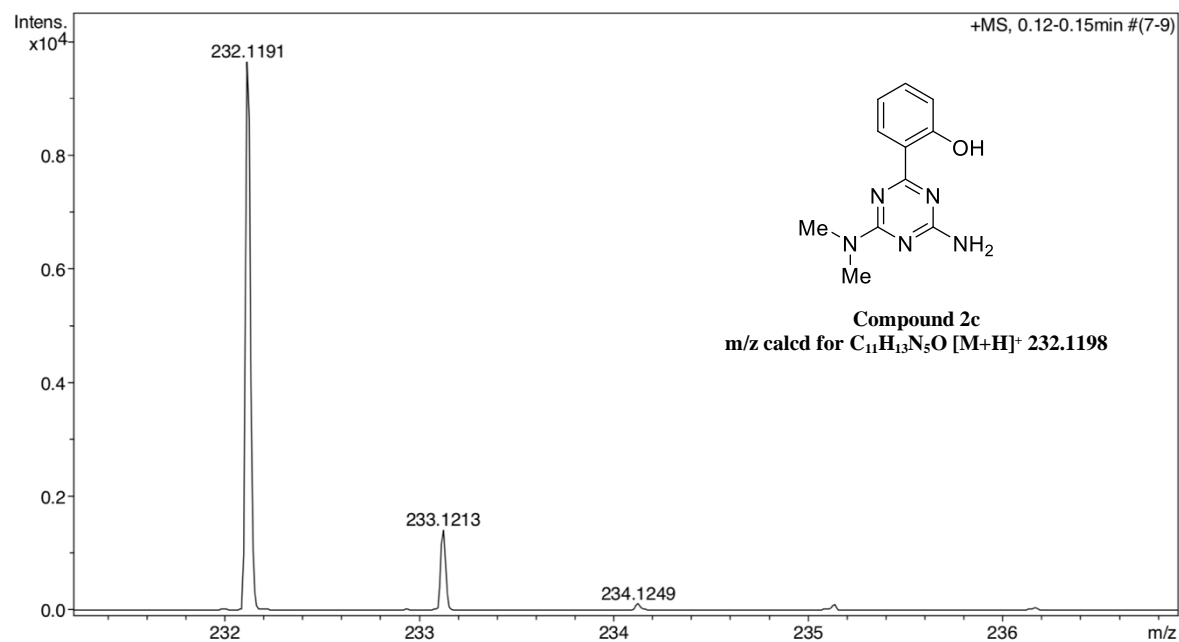


Figure 11. HRMS spectrum of 2-(4-amino-6-(dimethylamino)-1,3,5-triazin-2-yl)phenol (**2c**)

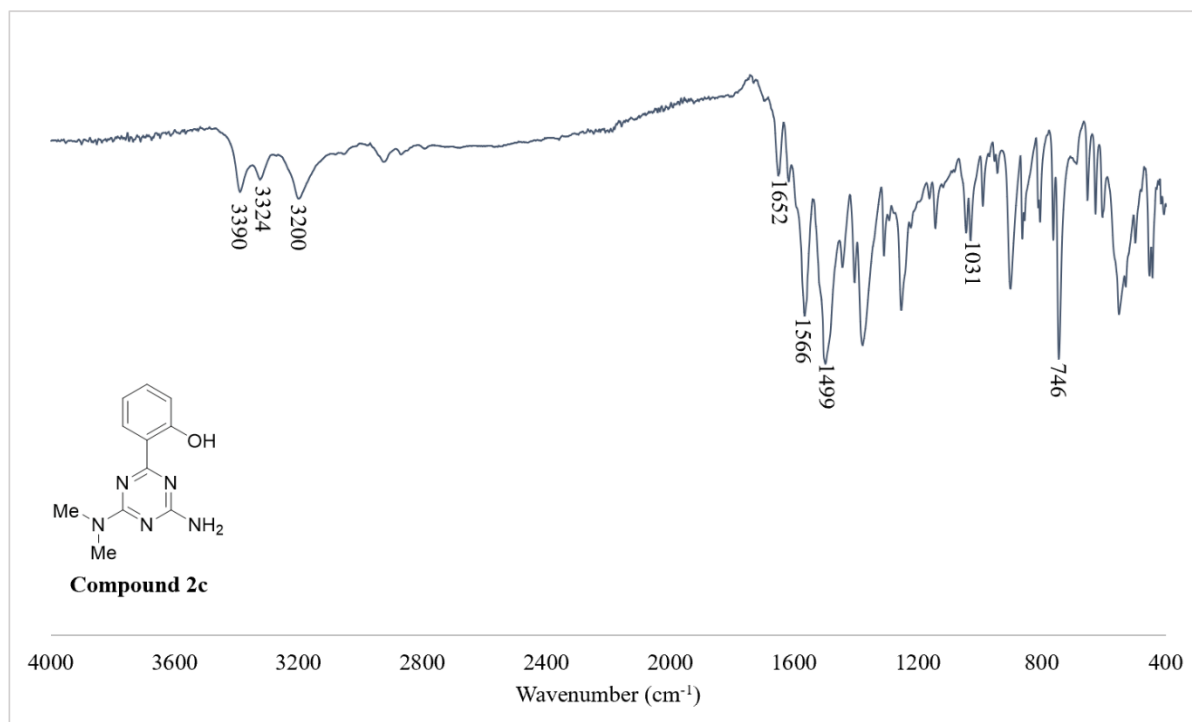


Figure 12. IR spectrum of 2-(4-amino-6-(dimethylamino)-1,3,5-triazin-2-yl)phenol (**2c**)

¹H NMR (400 MHz, CDCl₃) δ 7.95 (d, *J* = 15.9 Hz, 1H), 7.58 (d, *J* = 7.0 Hz, 2H), 7.43 – 7.30 (m, 3H), 6.83 (d, *J* = 15.9 Hz, 1H), 5.28 (brs, 2H), 3.22 (brs, 3H), 3.15 (brs, 3H).

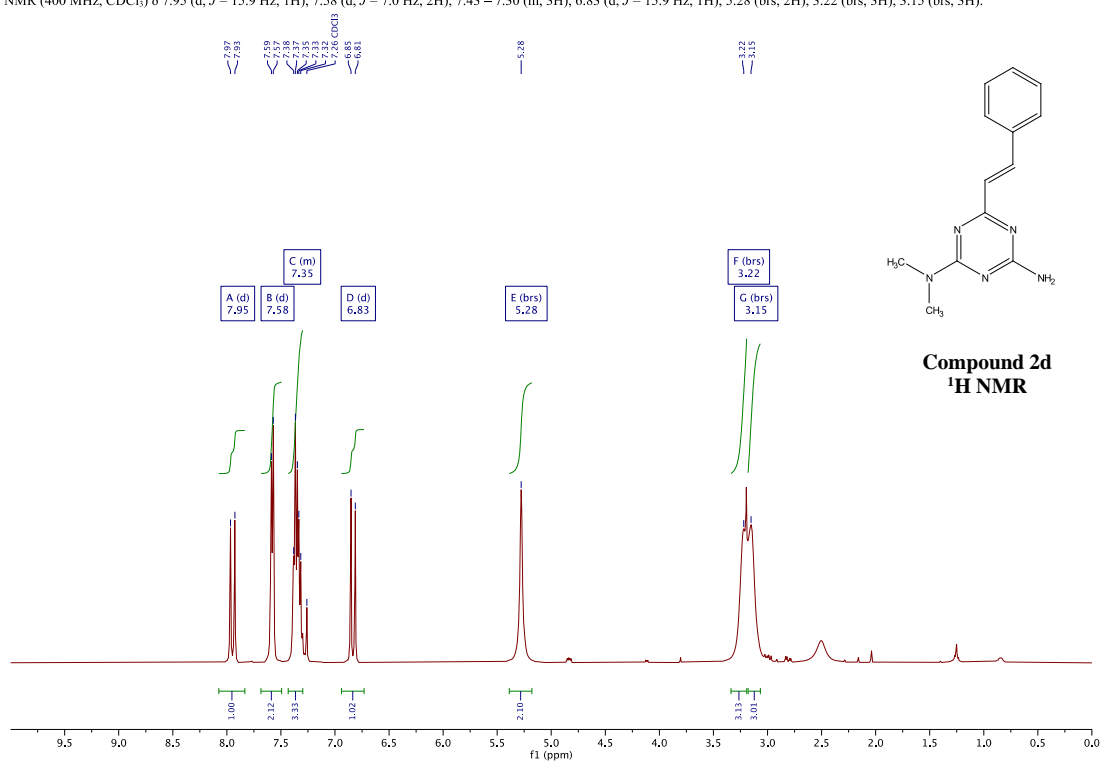


Figure 13. ¹H NMR of (*E*)-*N*²,*N*²-dimethyl-6-styryl-1,3,5-triazine-2,4-diamine (**2d**)

¹³C NMR (101 MHz, CDCl₃) δ 171.0, 167.2, 166.1, 139.7, 136.3, 129.6, 129.2, 128.3, 127.5, 36.7.

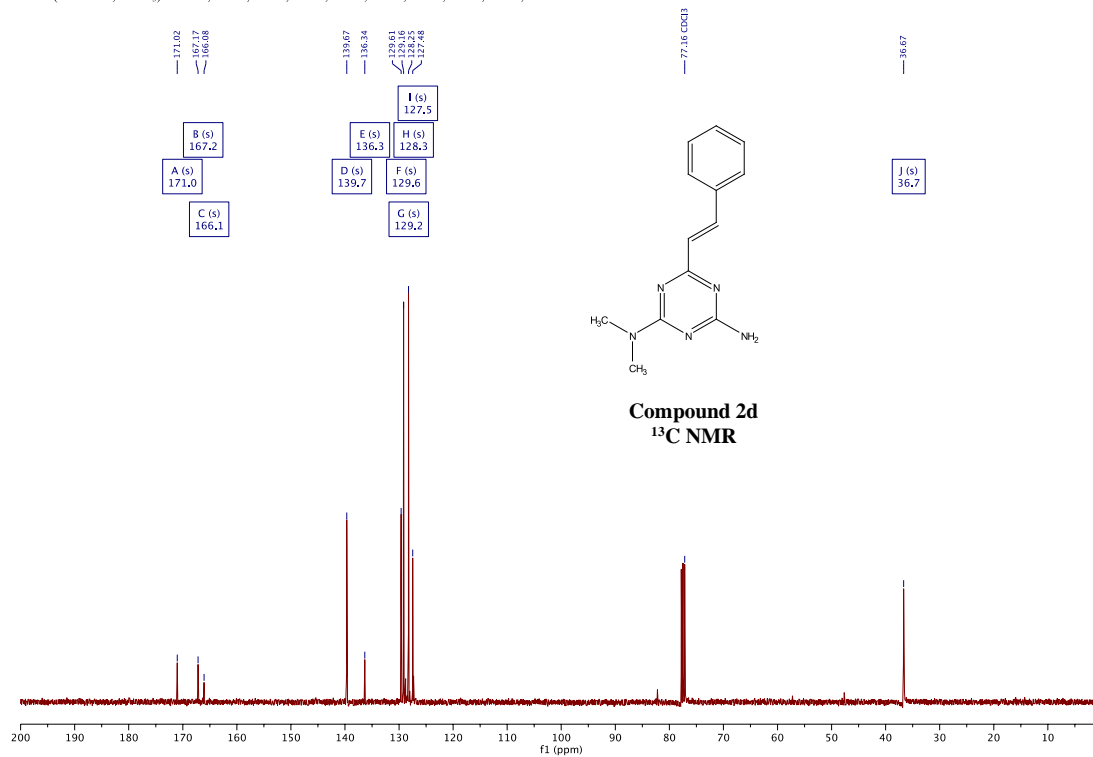


Figure 14. ¹³C NMR of (*E*)-*N*²,*N*²-dimethyl-6-styryl-1,3,5-triazine-2,4-diamine (**2d**)

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 5.68 (brs, 2H), 3.95 (s, 3H), 3.23 (s, 3H), 3.13 (s, 3H).

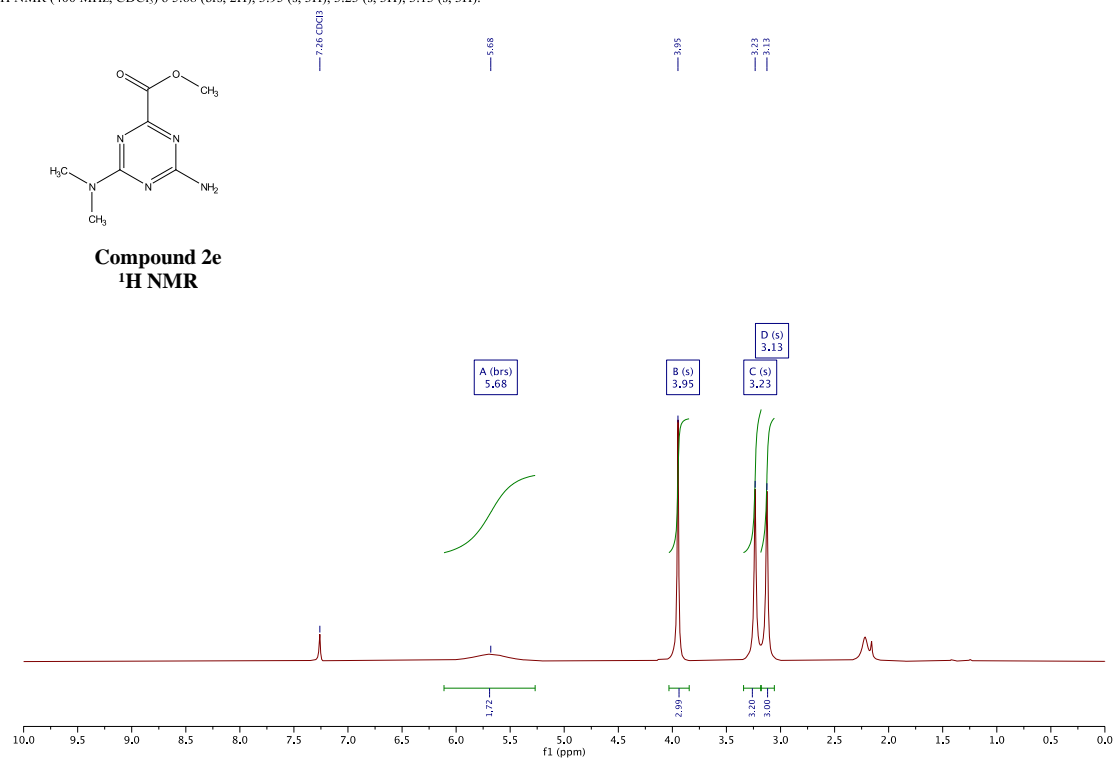


Figure 15. $^1\text{H NMR}$ of Methyl 4-amino-6-(dimethylamino)-1,3,5-triazine-2-carboxylate (**2e**)

$^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 166.5, 165.4, 164.0, 163.1, 53.1, 36.4, 36.2.

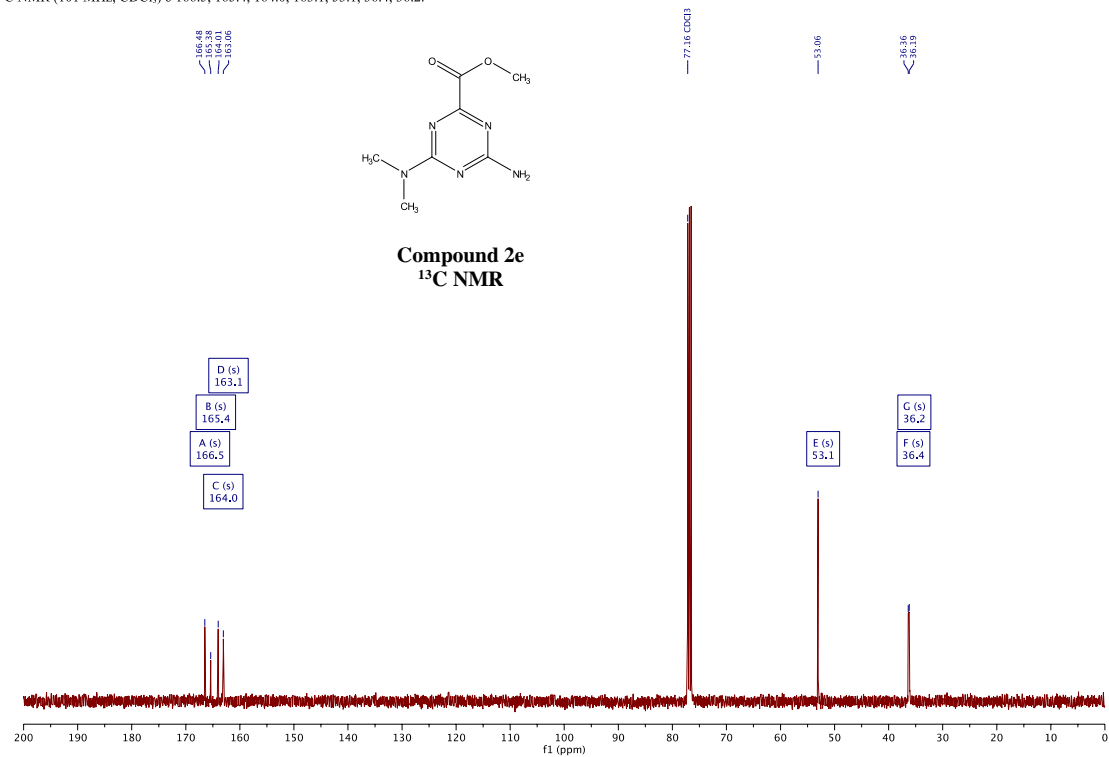


Figure 16. $^{13}\text{C NMR}$ of Methyl 4-amino-6-(dimethylamino)-1,3,5-triazine-2-carboxylate (**2e**)

Mass Spectrum List Report

Analysis Info

Analysis Name D:\Data\Data Service\190819VA52_RC7_01_2983.d
Method nv_pos_6min_profile_wguardcol_190624.m
Sample Name A52
Comment

Acquisition Date 8/19/2019 10:29:30 PM

Operator CU.
Instrument / Ser# micrOTOF-Q II 10335

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	3.0 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	250.0 Vpp	Set Divert Valve	Waste

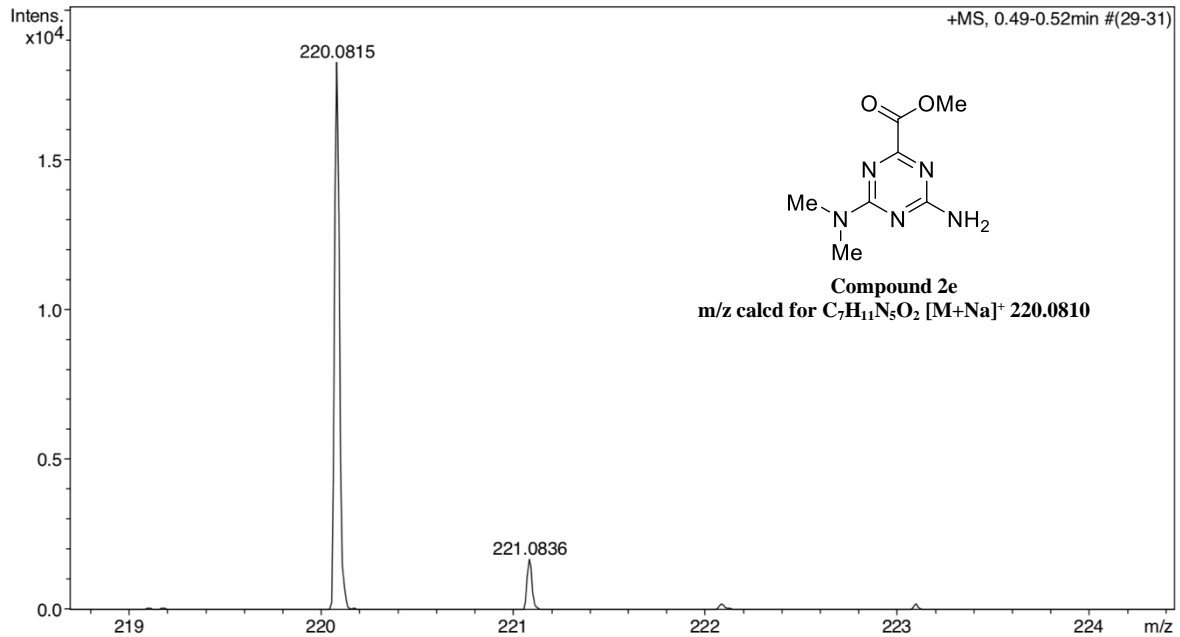


Figure 17. HRMS spectrum of Methyl 4-amino-6-(dimethylamino)-1,3,5-triazine-2-carboxylate (**2e**)

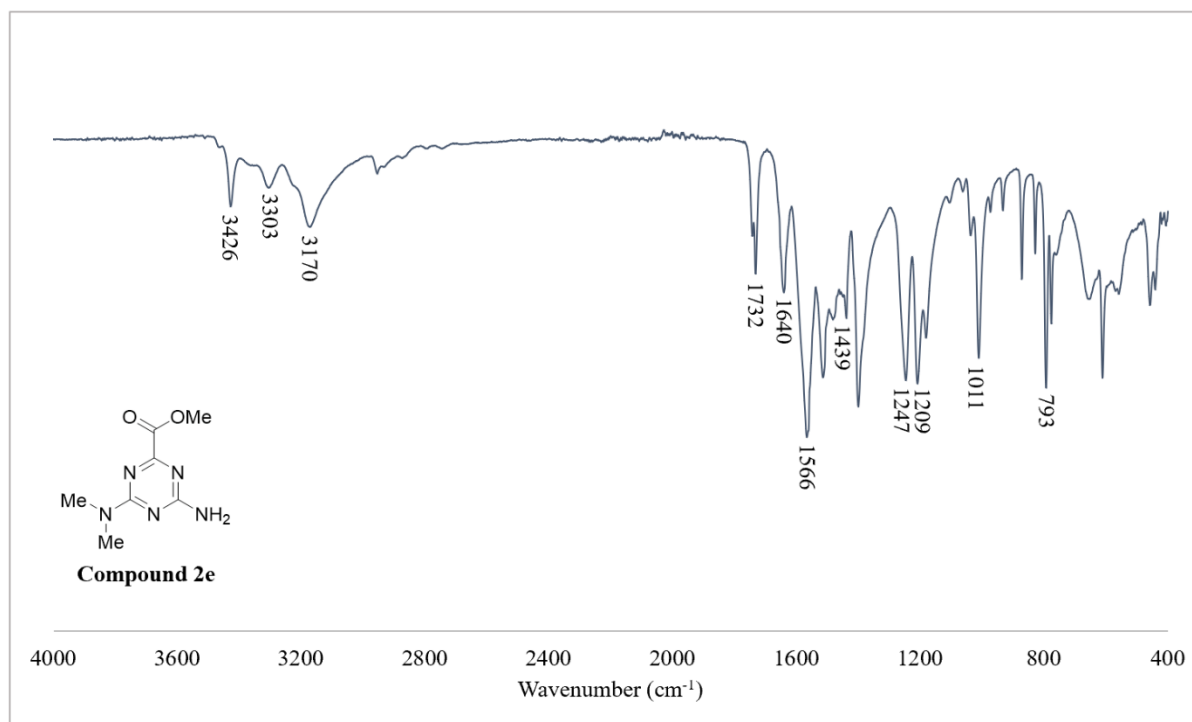


Figure 18. IR spectrum of Methyl 4-amino-6-(dimethylamino)-1,3,5-triazine-2-carboxylate (2e)

¹H NMR (400 MHz, DMSO) δ 11.08 (s, 1H), 9.01 (brs, 1H), 8.02 (brs, 1H), 3.11 (s, 3H), 3.02 (s, 3H).

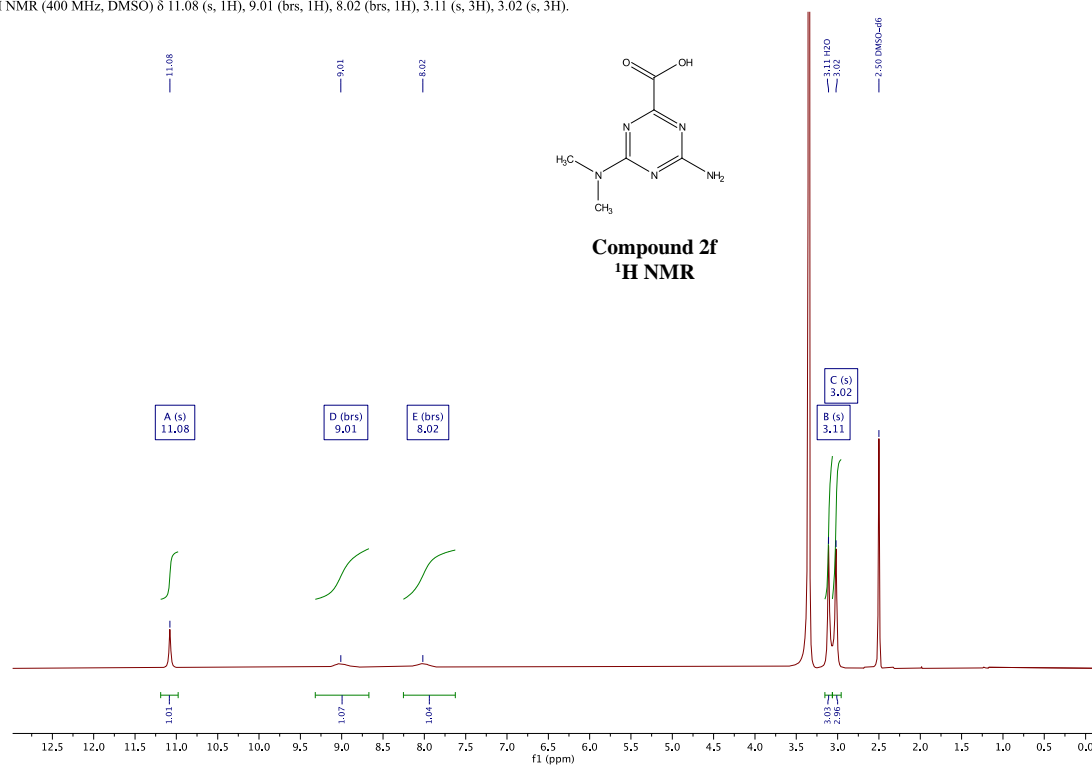


Figure 19. ¹H NMR of 4-amino-6-(dimethylamino)-1,3,5-triazine-2-carboxylic acid (**2f**)

¹³C NMR (101 MHz, DMSO) δ 171.2, 169.9, 163.8, 160.4, 37.9, 37.2.

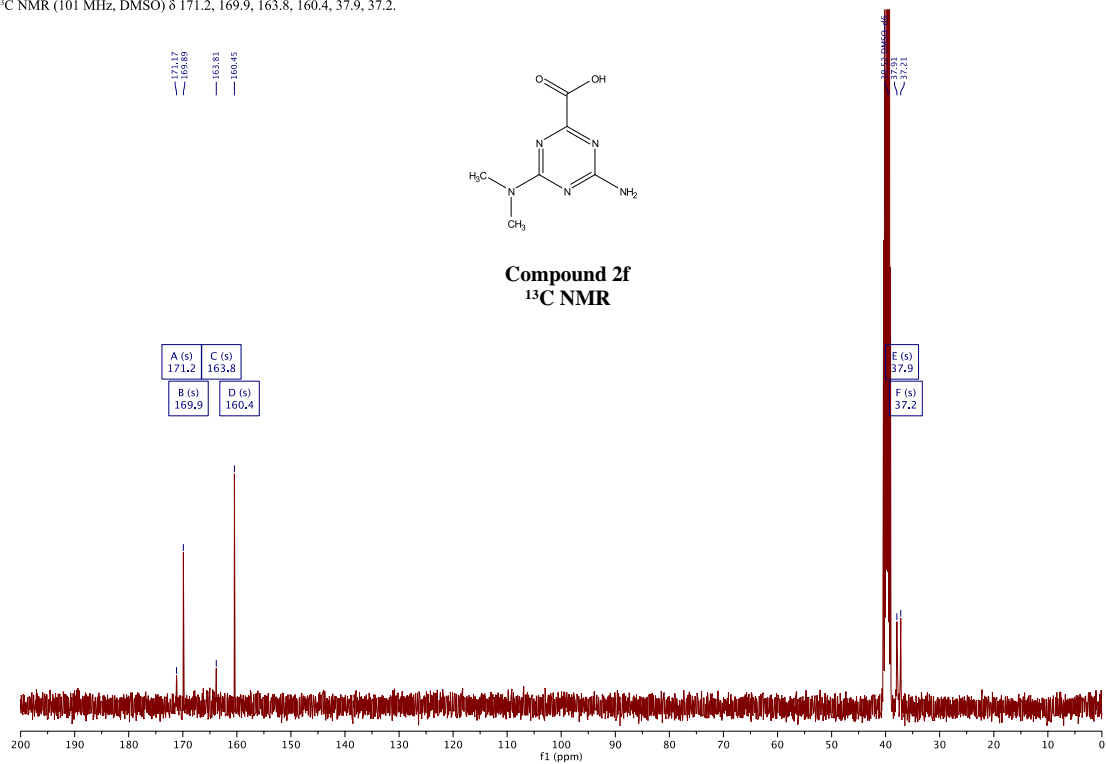


Figure 20. ¹³C NMR of 4-amino-6-(dimethylamino)-1,3,5-triazine-2-carboxylic acid (**2f**)

Mass Spectrum List Report

Analysis Info

Analysis Name D:\Data\Data Service\190819\A30_RA8_01_2948.d
Method nv_pos_6min_profile_wguardcol_190624.m
Sample Name A30
Comment

Acquisition Date 8/19/2019 5:48:44 PM

Operator CU.
Instrument / Ser# micrOTOF-Q II 10335

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	3.0 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	250.0 Vpp	Set Divert Valve	Waste

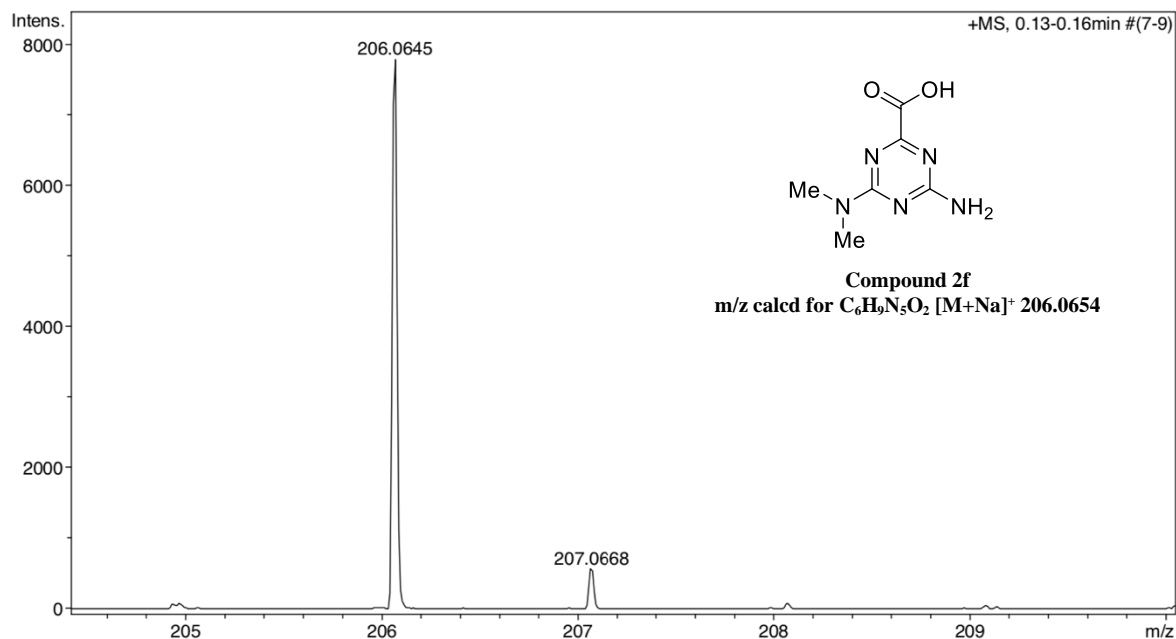


Figure 21. HRMS spectrum of 4-amino-6-(dimethylamino)-1,3,5-triazine-2-carboxylic acid (**2f**)

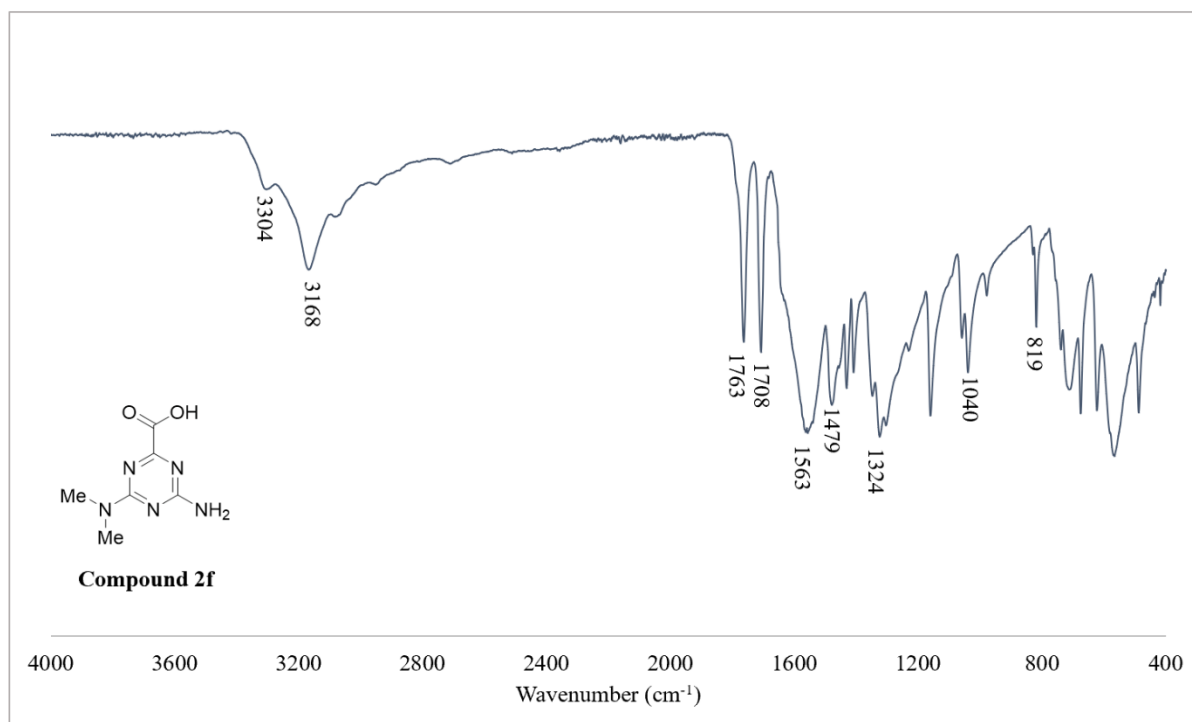


Figure 22. IR spectrum of 4-amino-6-(dimethylamino)-1,3,5-triazine-2-carboxylic acid (**2f**)

¹H NMR (400 MHz, DMSO) δ 9.38 (s, 1H), 7.77 (d, *J* = 7.9 Hz, 2H), 7.24 (t, *J* = 7.9 Hz, 2H), 6.94 (t, *J* = 7.2 Hz, 3H), 2.41 (t, *J* = 7.6 Hz, 2H), 1.73–1.60 (m, 2H), 1.29–1.20 (m, 23H), 0.84 (t, *J* = 6.7 Hz, 3H).

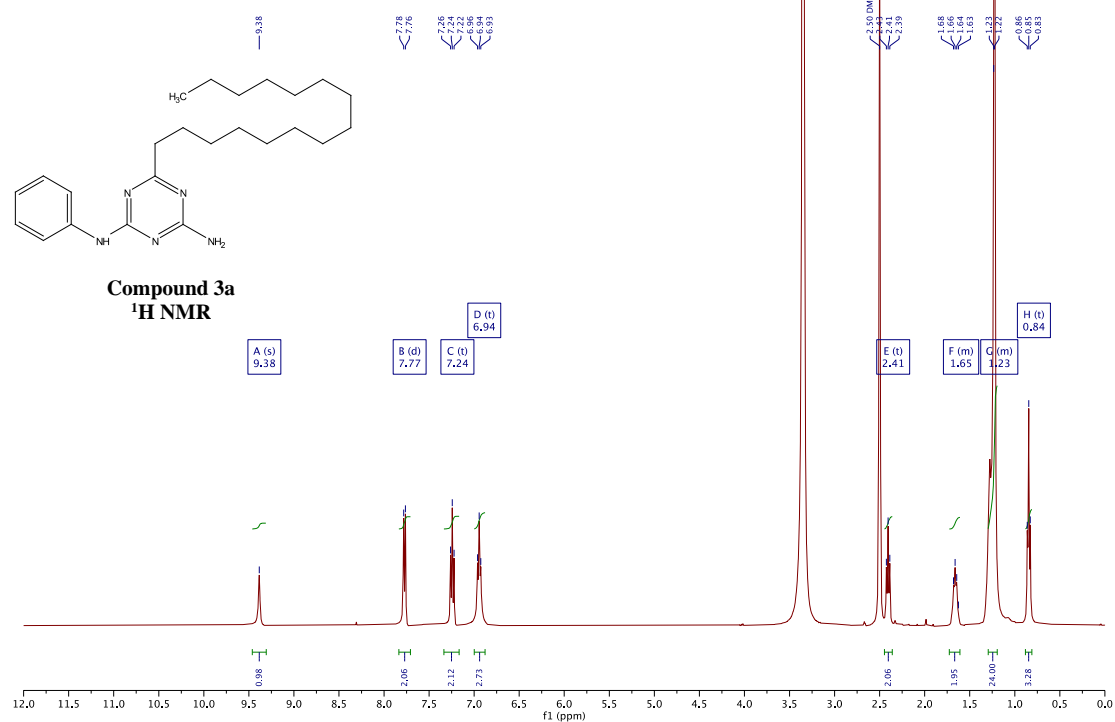


Figure 23. ¹H NMR of 6-pentadecyl-*N*²-phenyl-1,3,5-triazine-2,4-diamine (**3a**)

¹³C NMR (101 MHz, DMSO) δ 177.8, 166.6, 164.2, 140.0, 128.3, 121.7, 119.7, 37.9, 31.2, 29.0, 26.9, 22.0, 13.9.

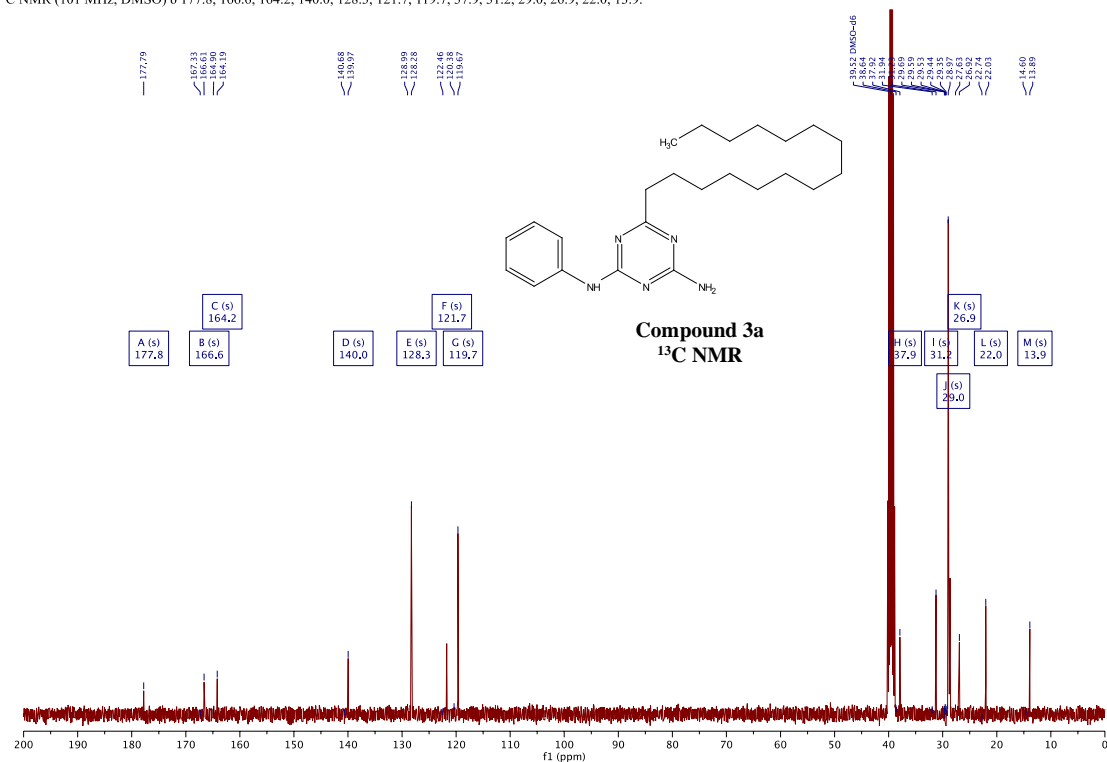


Figure 24. ¹³C NMR of 6-pentadecyl-*N*²-phenyl-1,3,5-triazine-2,4-diamine (**3a**)

Mass Spectrum List Report

Analysis Info

Analysis Name D:\Data\Data Service\190819\A40_RC5_01_2981.d
Method nv_pos_6min_profile_wguardcol_190624.m
Sample Name A40
Comment

Acquisition Date 8/19/2019 10:16:34 PM

Operator CU.
Instrument / Ser# micrOTOF-Q II 10335

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	3.0 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	250.0 Vpp	Set Divert Valve	Waste

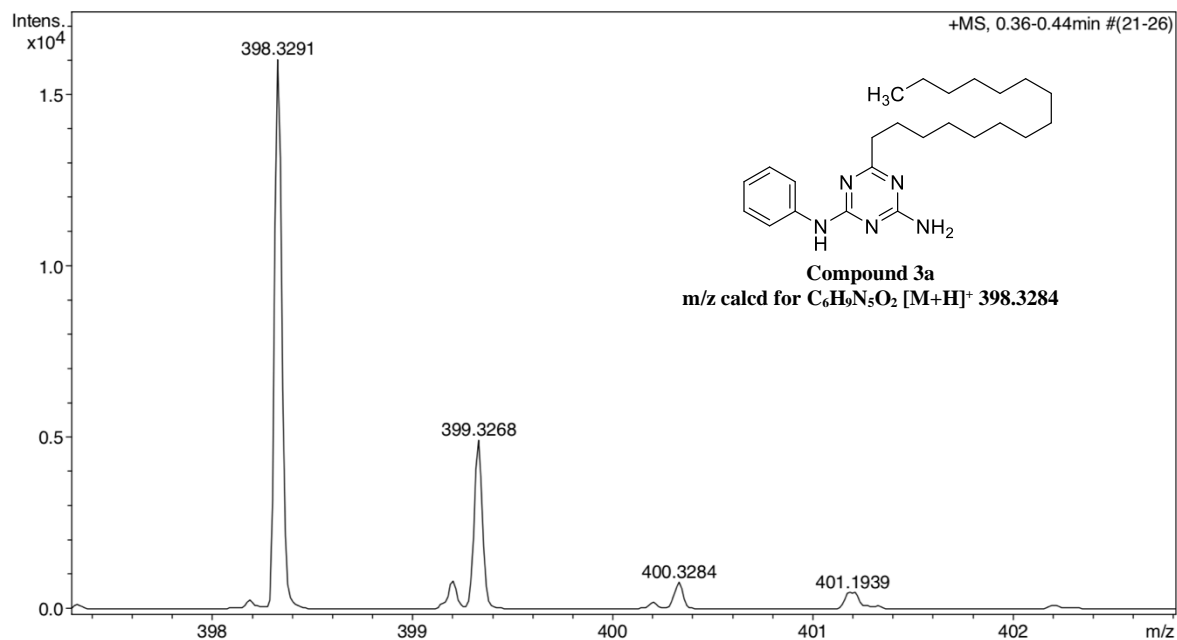


Figure 25. HRMS spectrum of 6-pentadecyl-*N*²-phenyl-1,3,5-triazine-2,4-diamine (**3a**)

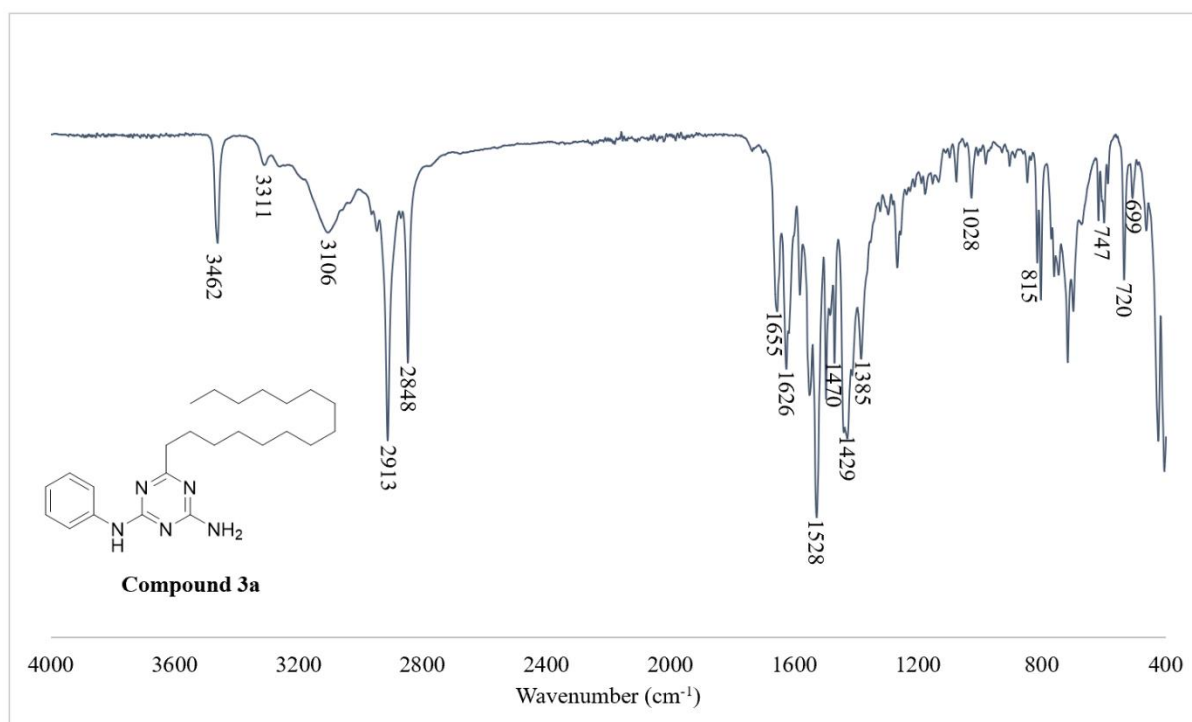


Figure 26. IR spectrum of 6-pentadecyl-*N*²-phenyl-1,3,5-triazine-2,4-diamine (**3a**)

¹H NMR (400 MHz, DMSO) δ 9.52 (s, 1H), 8.31 (d, *J* = 6.8 Hz, 2H), 7.84 (d, *J* = 8.0 Hz, 2H), 7.58 – 7.44 (m, 3H), 7.31 (t, *J* = 7.8 Hz, 2H), 7.12 (brs, 2H), 6.99 (t, *J* = 7.3 Hz, 1H).

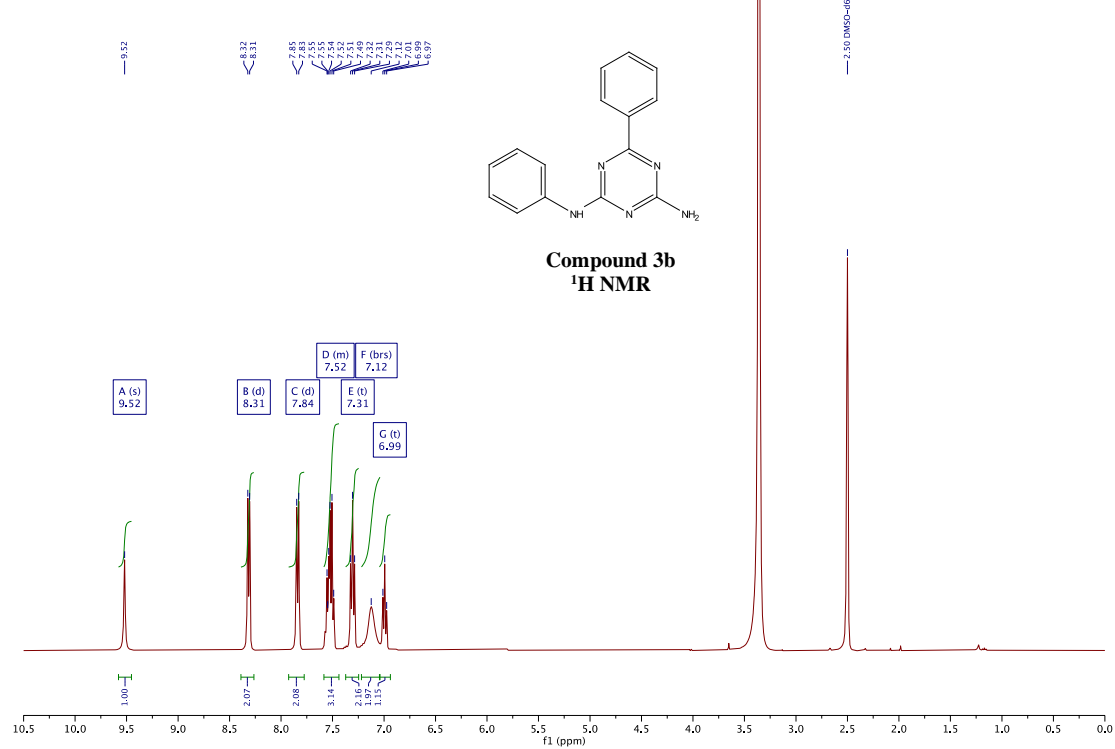


Figure 27. ¹H NMR of *N*²,6-diphenyl-1,3,5-triazine-2,4-diamine (**3b**)

¹³C NMR (101 MHz, DMSO) δ 170.7, 167.6, 165.1, 140.3, 137.2, 131.8, 128.9, 128.7, 128.2, 122.5, 120.4.

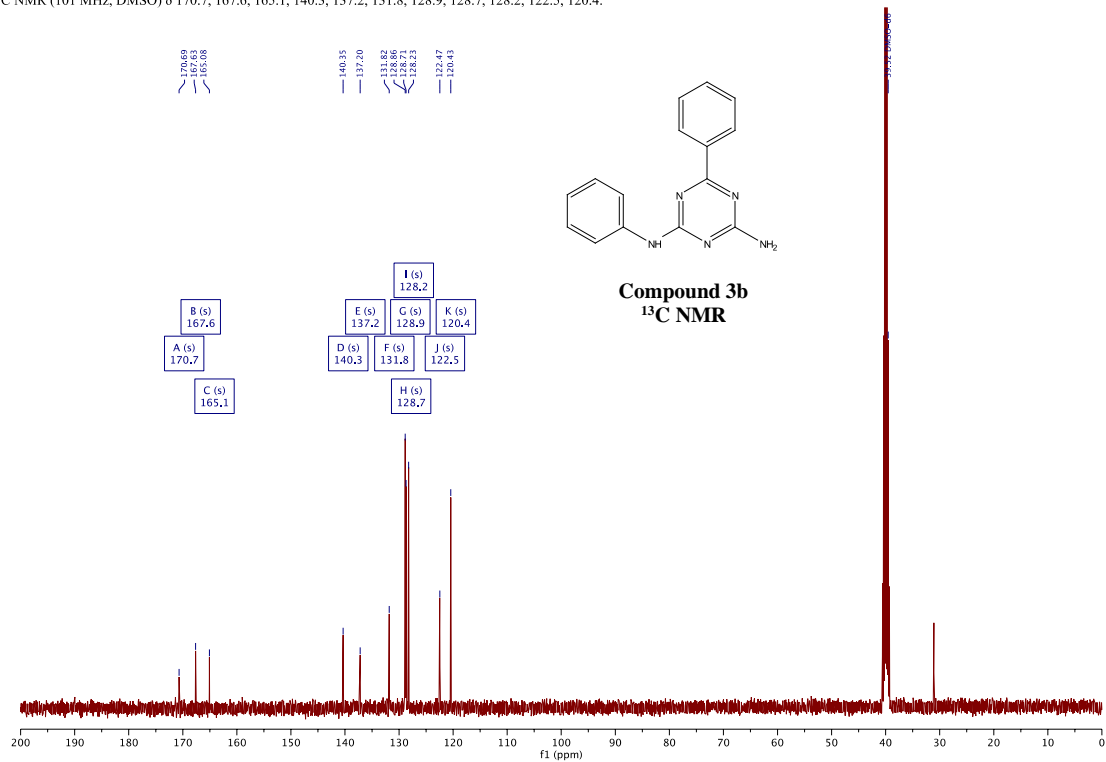


Figure 28. ¹³C NMR of *N*²,6-diphenyl-1,3,5-triazine-2,4-diamine (**3b**)

¹H NMR (400 MHz, DMSO) δ 13.49 (s, 1H), 9.77 (brs, 1H), 8.26 (d, *J* = 7.3 Hz, 1H), 7.76 (brs, 2H), 7.55 (brs, 1H), 7.44–7.35 (m, 2H), 7.33 (t, *J* = 7.2 Hz, 2H), 7.04 (t, *J* = 7.5 Hz, 1H), 6.90 (t, *J* = 8.0 Hz, 2H).

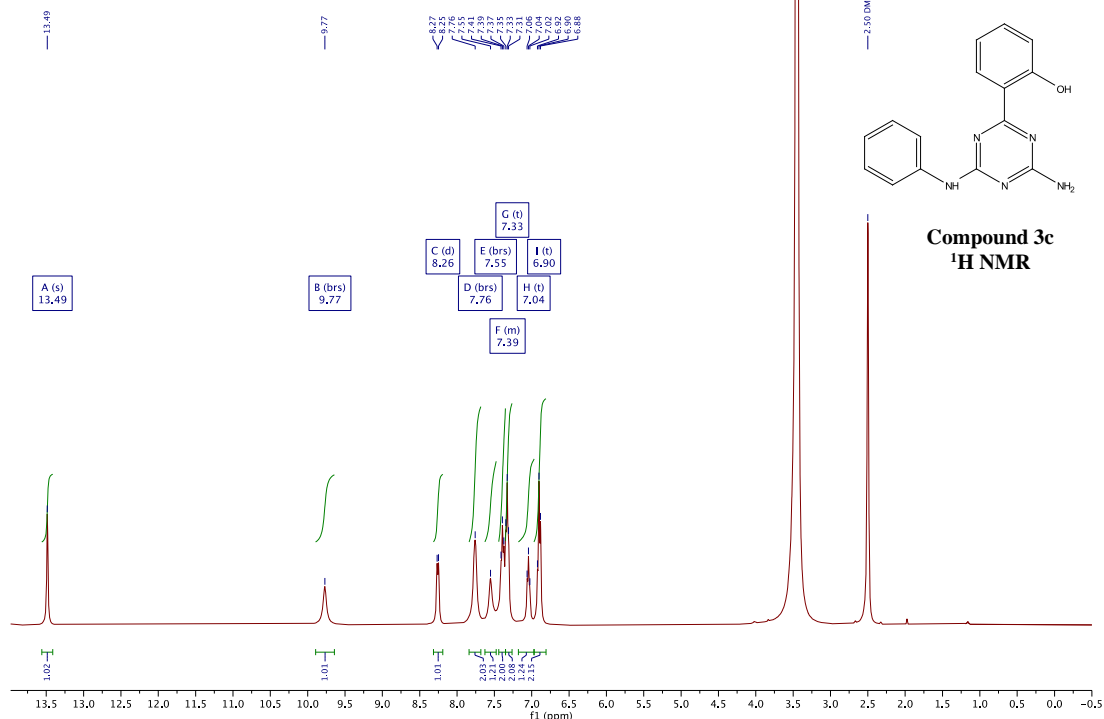


Figure 29. ¹H NMR of 2-(4-amino-6-(phenylamino)-1,3,5-triazin-2-yl)phenol (**3c**)

¹³C NMR (101 MHz, DMSO) δ 169.9, 164.7, 160.8, 138.8, 133.2, 128.3, 128.1, 122.3, 120.2, 117.9, 117.2, 117.1.

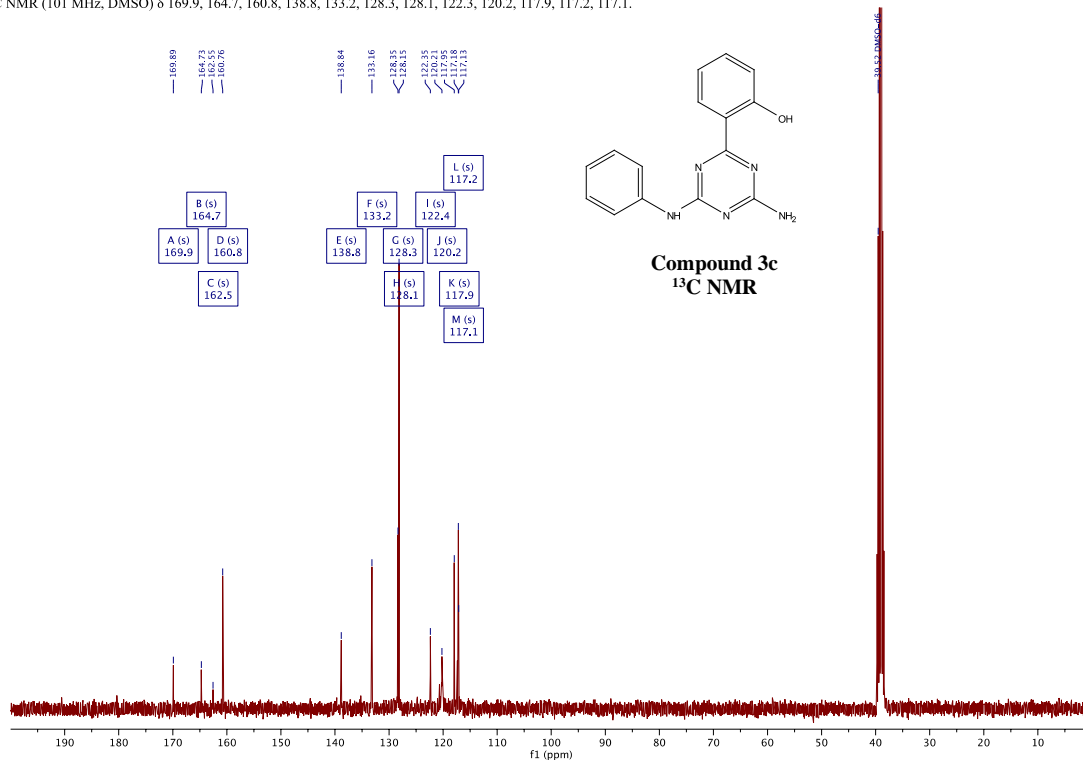


Figure 30. ¹³C NMR of 2-(4-amino-6-(phenylamino)-1,3,5-triazin-2-yl)phenol (**3c**)

Mass Spectrum List Report

Analysis Info

Analysis Name D:\Data\Data Service\190819\A38_RC4_01_2980.d
Method nv_pos_6min_profile_wguardcol_190624.m
Sample Name A38
Comment

Acquisition Date 8/19/2019 10:10:06 PM

Operator CU.
Instrument / Ser# micrOTOF-Q II 10335

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	3.0 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	250.0 Vpp	Set Divert Valve	Waste

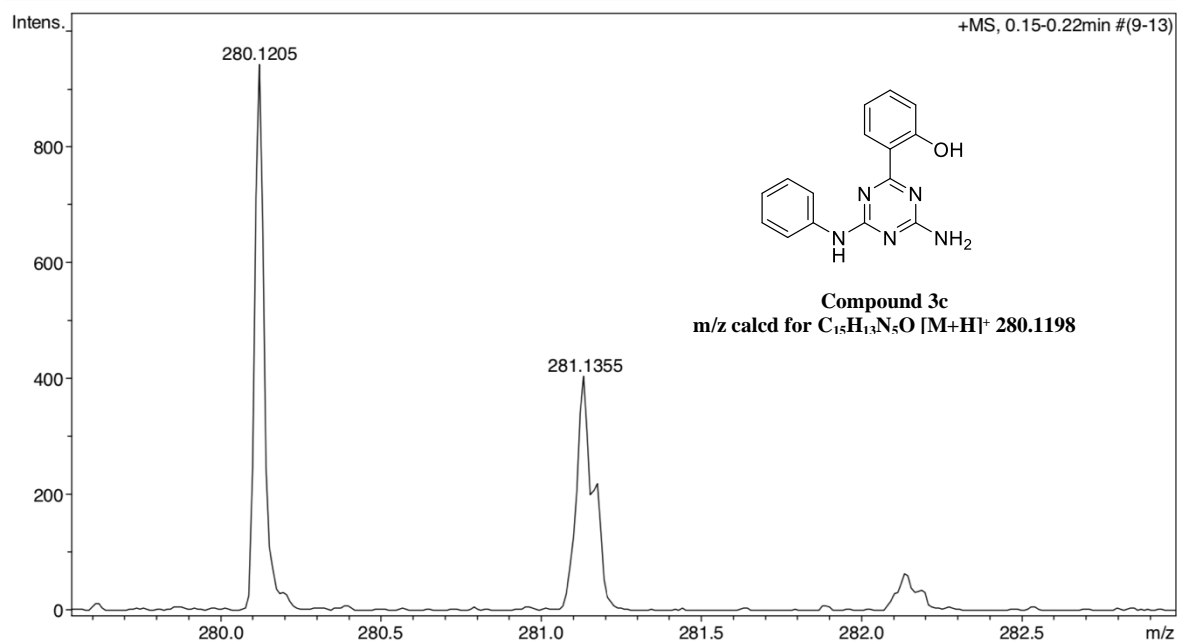


Figure 31. HRMS spectrum of 2-(4-amino-6-(phenylamino)-1,3,5-triazin-2-yl)phenol (**3c**)

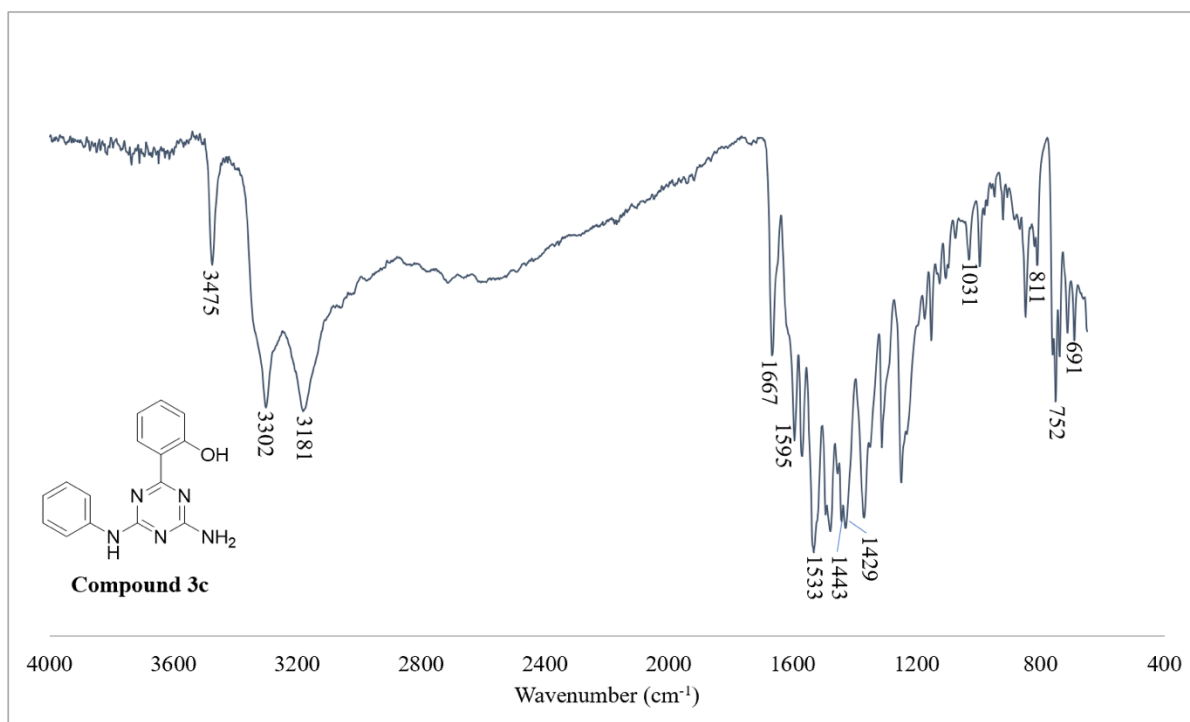


Figure 32. IR spectrum of 2-(4-amino-6-(phenylamino)-1,3,5-triazin-2-yl)phenol (**3c**)

¹H NMR (500 MHz,) δ 9.47 (s, 1H), 7.87 (d, *J* = 15.9 Hz, 1H), 7.81 (dd, *J* = 8.7, 1.2 Hz, 2H), 7.66 (d, *J* = 7.5 Hz, 2H), 7.46 – 7.36 (m, 3H), 7.28 (t, *J* = 7.6 Hz, 2H), 7.03 (brs, 2H), 6.97 (tt, *J* = 7.3, 1.2 Hz, 1H), 6.82 (d, *J* = 16.0 Hz, 1H).

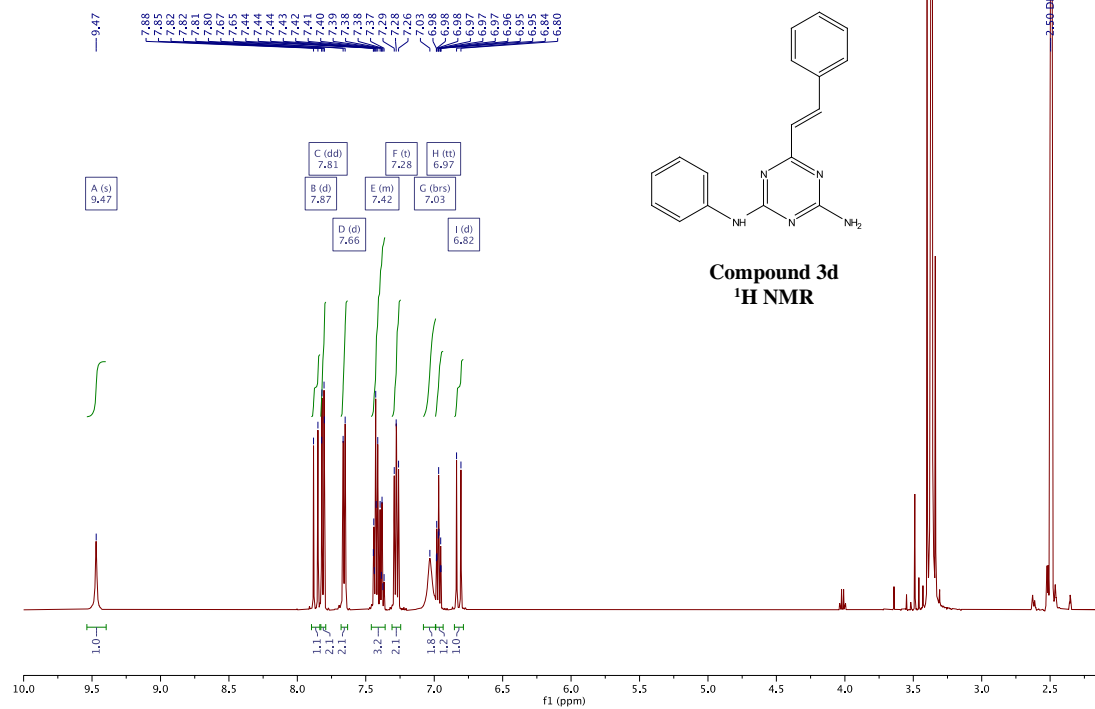


Figure 33. ¹H NMR of (*E*)-*N*²-phenyl-6-styryl-1,3,5-triazine-2,4-diamine (**3d**)

¹³C NMR (101 MHz, CDCl₃) δ 171.0, 166.6, 164.3, 141.2, 138.3, 135.5, 129.9, 129.1, 129.0, 128.2, 125.5, 124.0, 121.0.

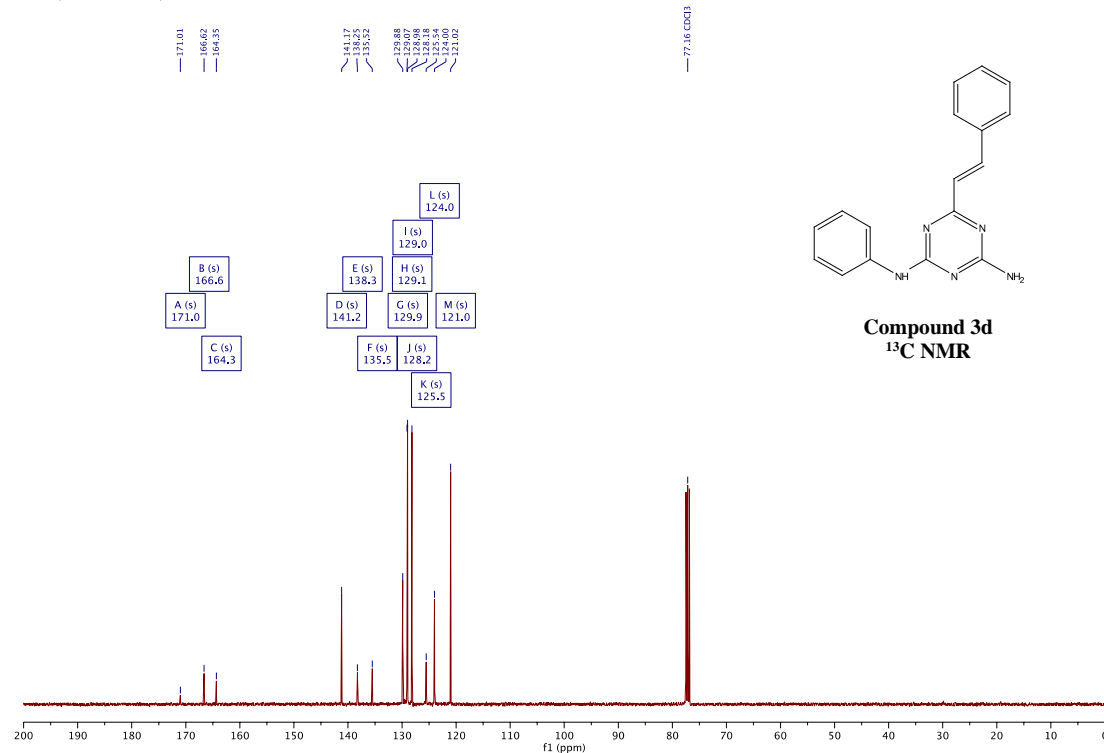


Figure 34. ¹³C NMR of (*E*)-*N*²-phenyl-6-styryl-1,3,5-triazine-2,4-diamine (**3d**)

Mass Spectrum List Report

Analysis Info

Analysis Name D:\Data\Data Service\190819\A37_RC3_01_2979.d
Method nv_pos_6min_profile_wguardcol_190624.m
Sample Name A37
Comment

Acquisition Date 8/19/2019 10:03:39 PM
Operator CU.
Instrument / Ser# micrOTOF-Q II 10335

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	3.0 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	250.0 Vpp	Set Divert Valve	Waste

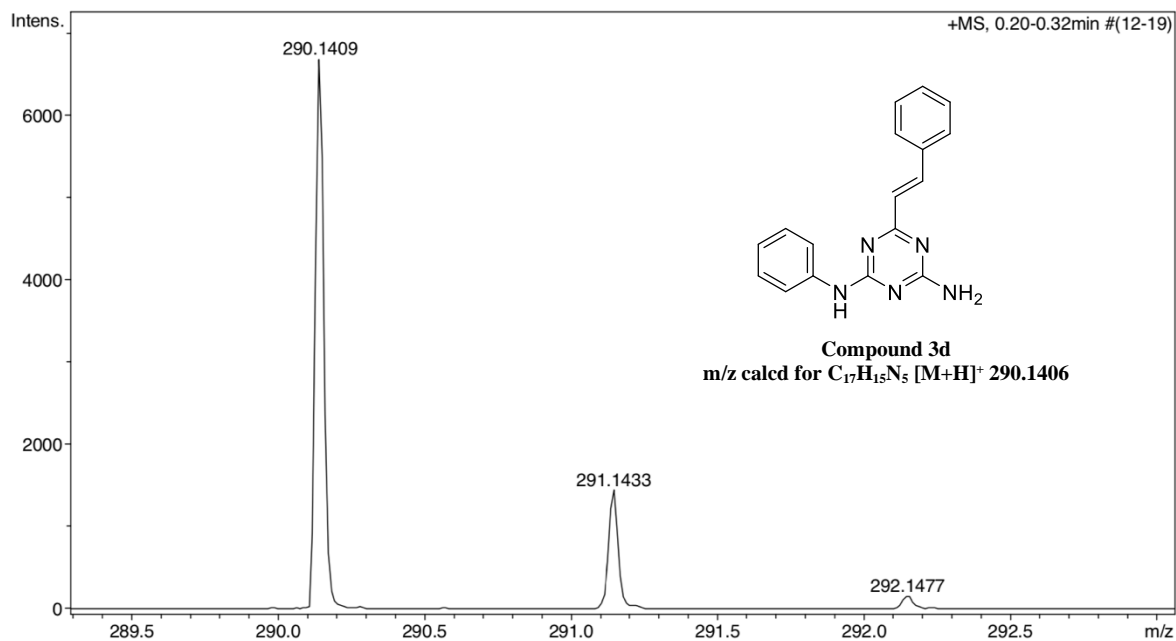


Figure 35. HRMS spectrum of (*E*)-*N*²-phenyl-6-styryl-1,3,5-triazine-2,4-diamine (**3d**)

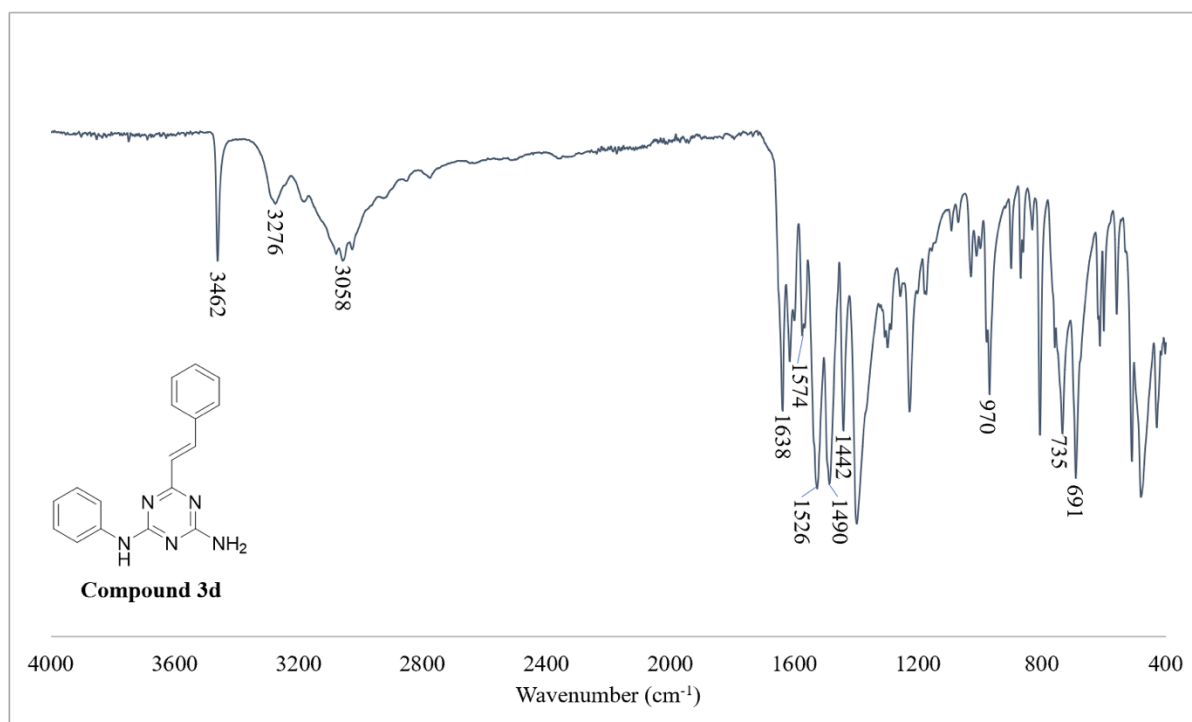


Figure 36. IR spectrum of (*E*)-*N*²-phenyl-6-styryl-1,3,5-triazine-2,4-diamine (**3d**)

¹H NMR (400 MHz, DMSO) δ 9.93 (brs, 1H), 7.78 (d, *J* = 7.9 Hz, 2H), 7.56 (brs, 1H), 7.43 (brs, 1H), 7.28 (t, *J* = 6.8 Hz, 2H), 7.01 (t, *J* = 7.9 Hz, 1H), 3.83 (s, 3H).

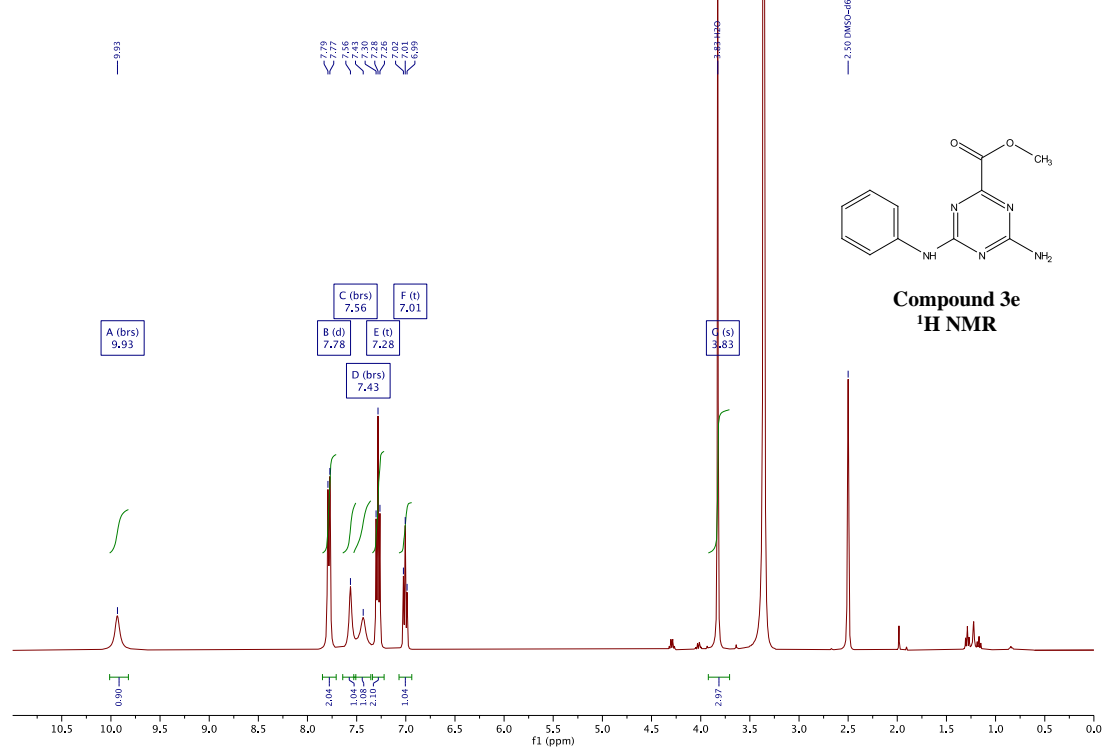


Figure 37. ¹H NMR of Methyl 4-amino-6-(phenylamino)-1,3,5-triazine-2-carboxylate (**3e**)

¹³C NMR (101 MHz, DMSO) δ 167.3, 164.5, 164.2, 164.1, 139.5, 128.7, 122.7, 120.3, 52.6.

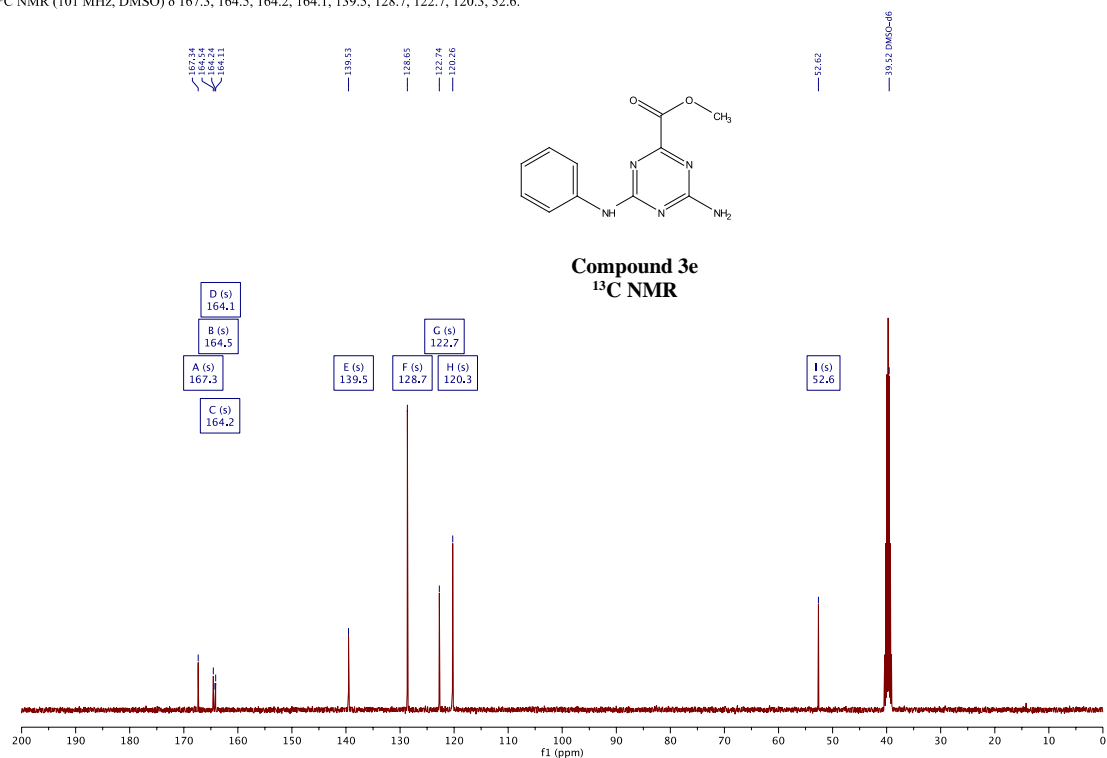


Figure 38. ¹³C NMR of Methyl 4-amino-6-(phenylamino)-1,3,5-triazine-2-carboxylate (**3e**)

¹H NMR (400 MHz, DMSO) δ 9.89 (brs, 1H), 7.78 (d, *J* = 8.1 Hz, 2H), 7.47 (brs, 2H), 7.28 (t, *J* = 7.3 Hz, 2H), 7.00 (t, *J* = 7.5, 6.7 Hz, 1H).

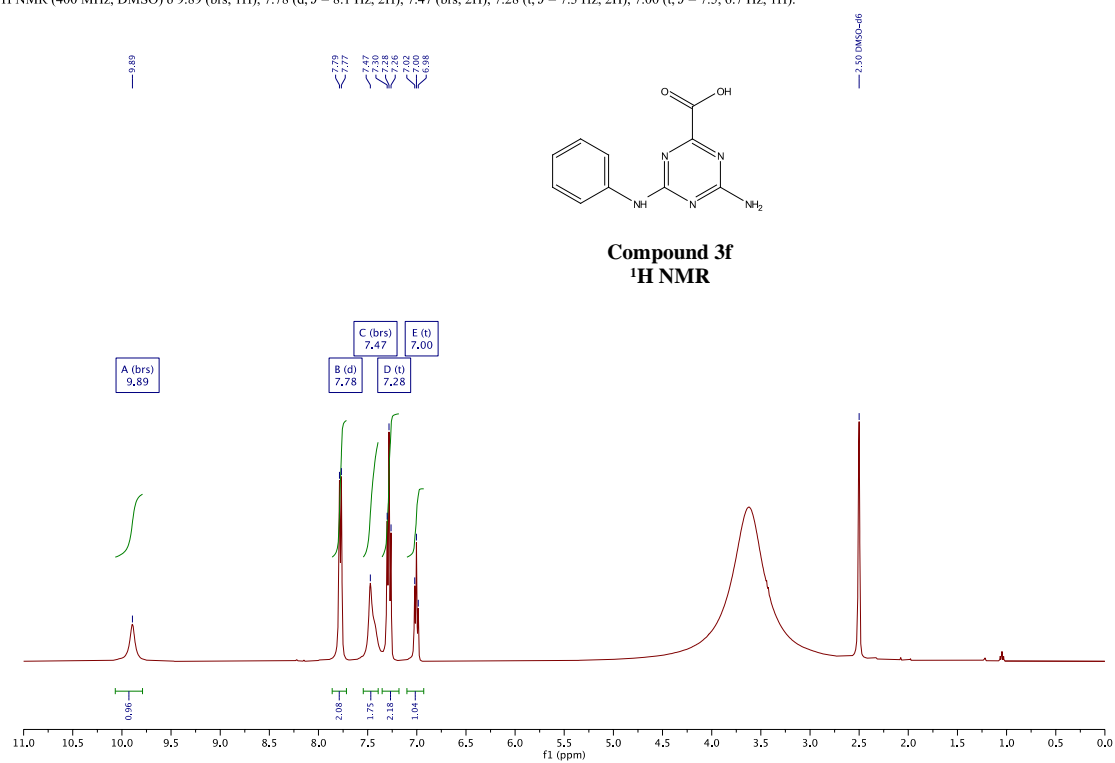


Figure 39. ¹H NMR of 4-amino-6-(phenylamino)-1,3,5-triazine-2-carboxylic acid (**3f**)

¹³C NMR (101 MHz, DMSO) δ 166.9, 165.4, 165.0, 164.3, 139.4, 128.5, 122.6, 120.2.

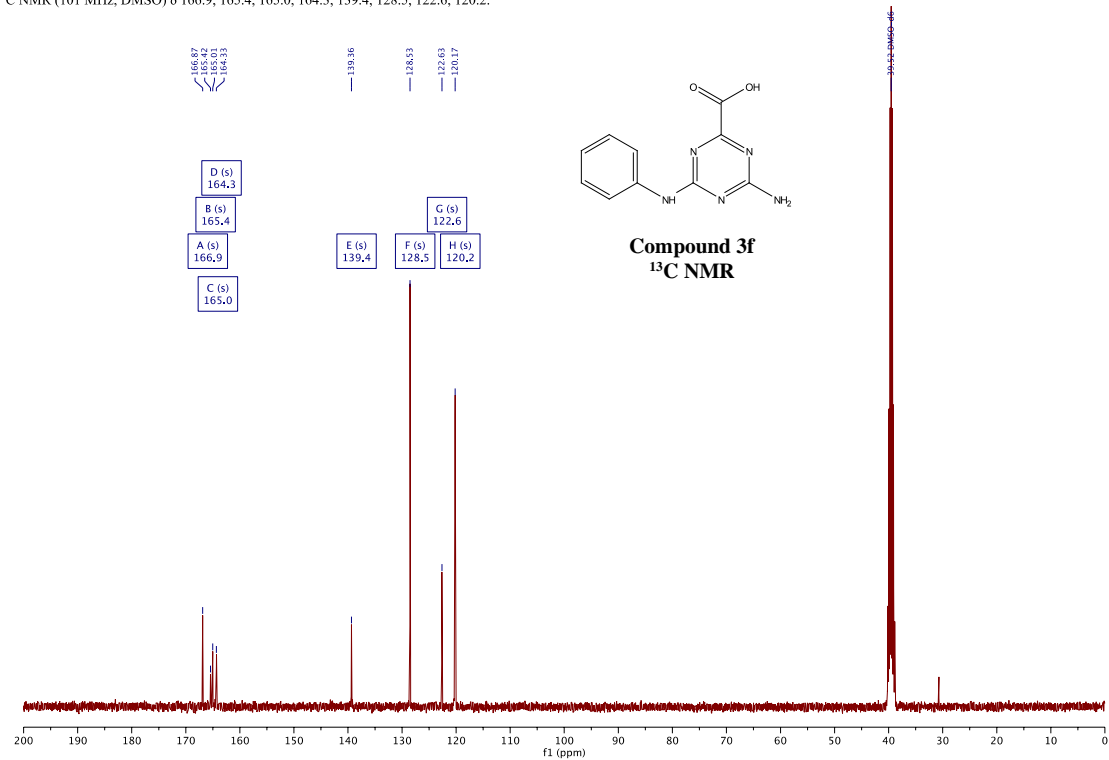


Figure 40. ¹³C NMR of 4-amino-6-(phenylamino)-1,3,5-triazine-2-carboxylic acid (**3f**)

Mass Spectrum List Report

Analysis Info

Analysis Name D:\Data\Data Service\190827\A53_RB1_01_3017.d
Method nv_pos_6min_profile_wguardcol_190624.m
Sample Name A53
Comment

Acquisition Date 8/27/2019 9:33:05 PM

Operator CU.
Instrument / Ser# micrOTOF-Q II 10335

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	3.0 Bar
Focus	Not active	Set Capillary	4000 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	250.0 Vpp	Set Divert Valve	Waste

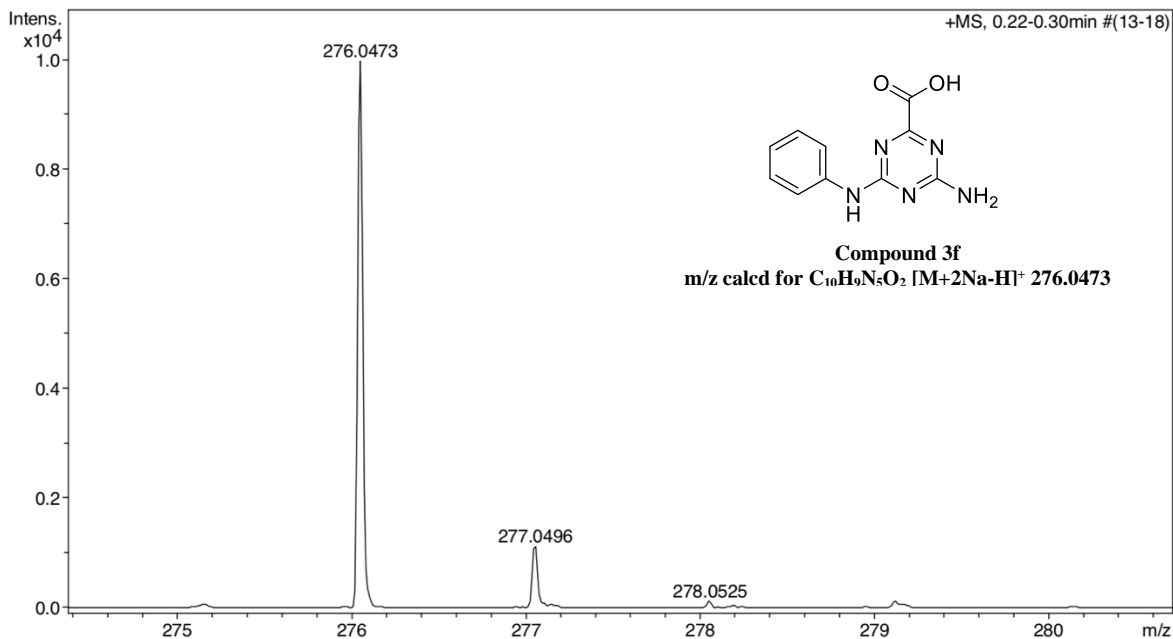


Figure 41. HRMS spectrum of 4-amino-6-(phenylamino)-1,3,5-triazine-2-carboxylic acid (**3f**)

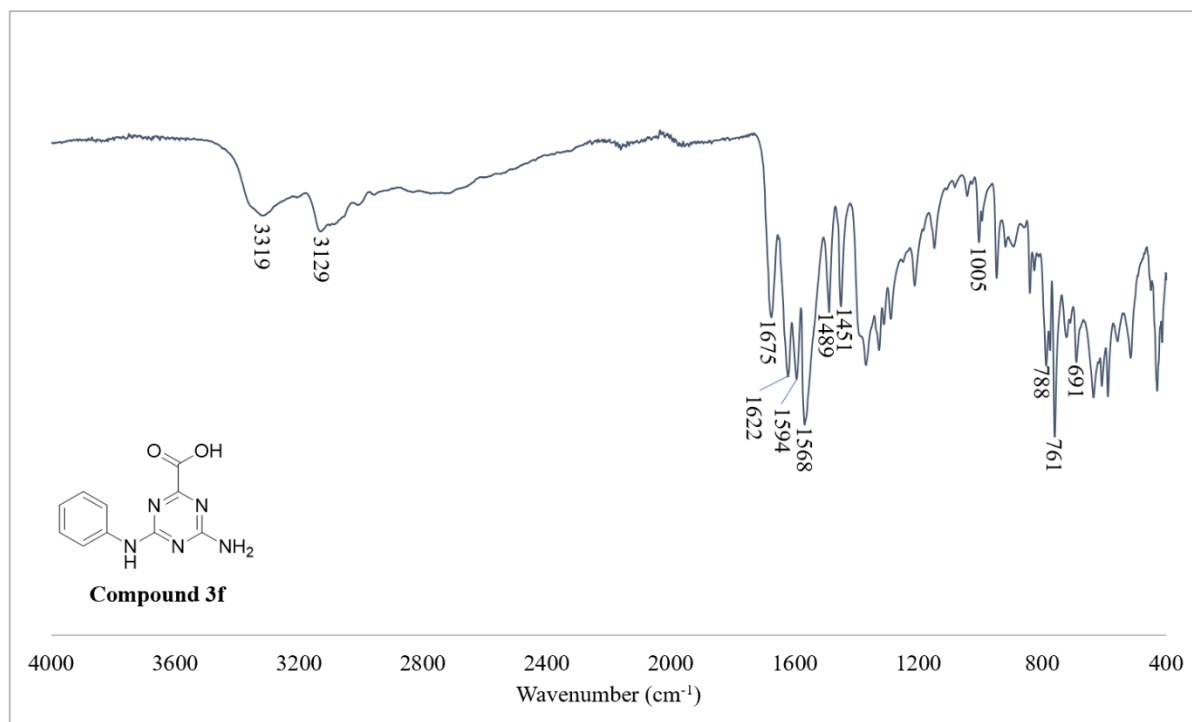


Figure 42. IR spectrum of 4-amino-6-(phenylamino)-1,3,5-triazine-2-carboxylic acid (**3f**)

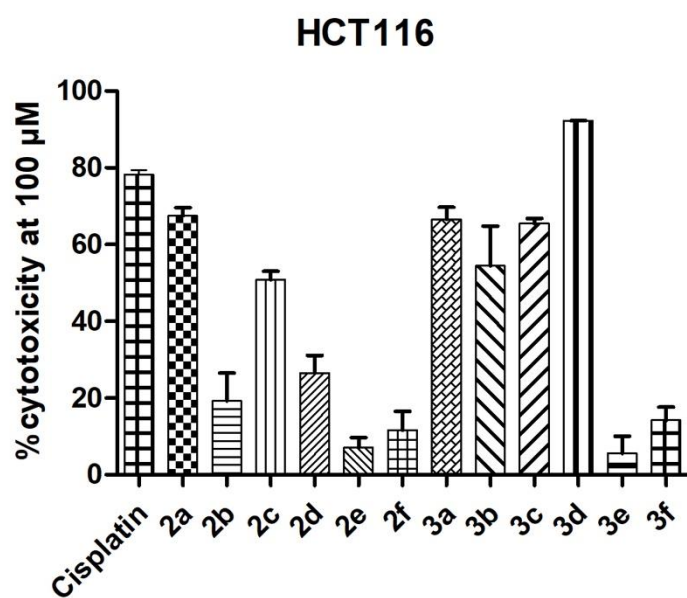
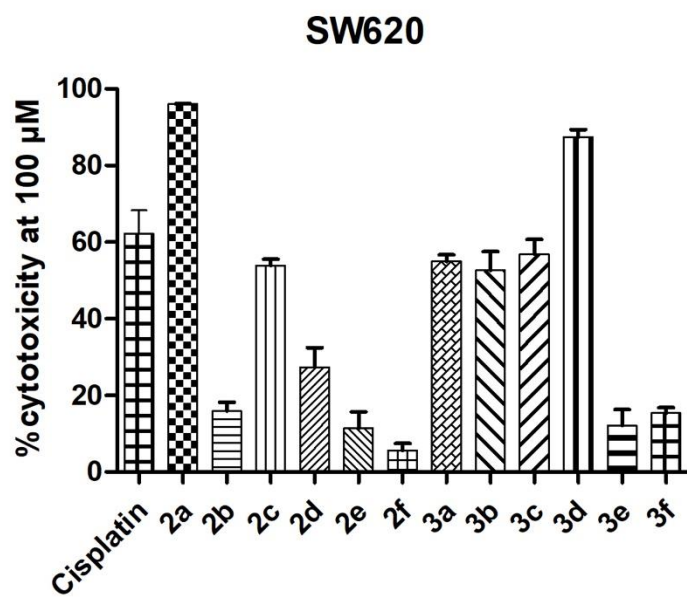


Figure 43. The cytotoxicity of twelve 1,3,5-triazine derivatives at 100 μM .

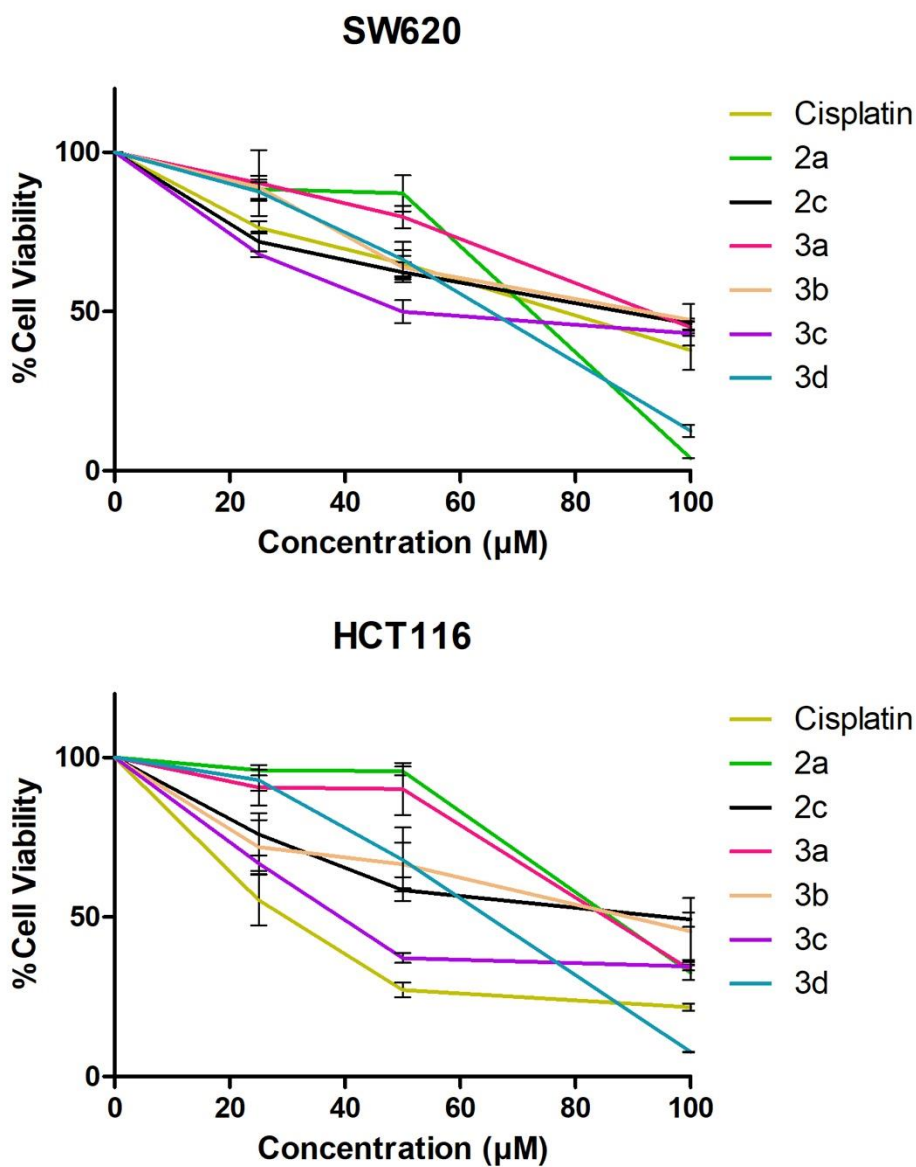


Figure 44. The concentration-response curves of the six 1,3,5-triazine derivatives at different concentrations (25, 50 and 100 µM) against SW620 and HCT116 cell lines for the calculation of IC₅₀ values.

	Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 369.3	Peak 1: 319.6	94.9	113.4
PdI: 0.464	Peak 2: 4908	5.1	701.4
Intercept: 1.01	Peak 3: 0.000	0.0	0.000
Result quality: Refer to quality report			

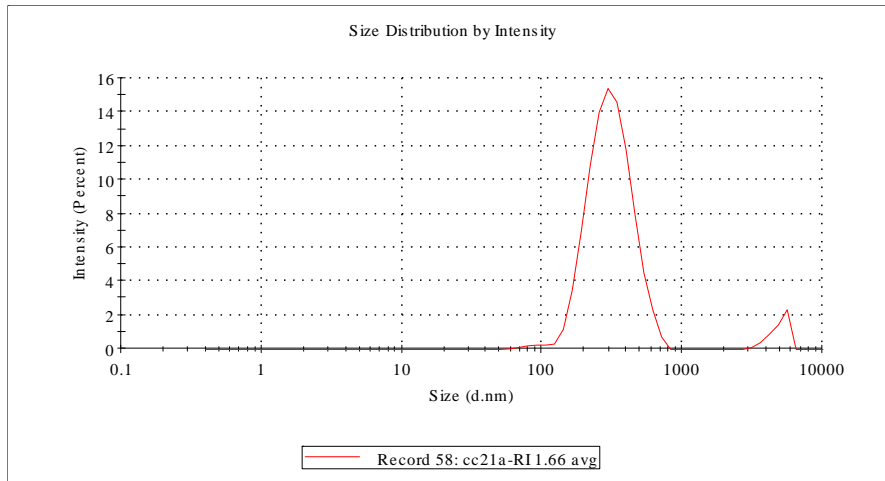


Figure 45. Particle size distribution determined by DLS – Condition A

	Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 300.3	Peak 1: 326.3	94.7	140.4
PdI: 0.322	Peak 2: 4915	5.3	693.6
Intercept: 0.947	Peak 3: 0.000	0.0	0.000
Result quality: Good			

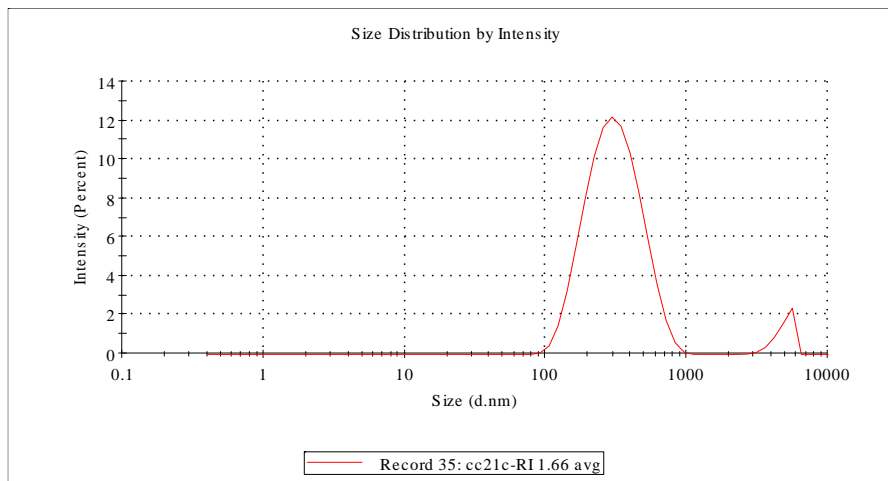


Figure 46. Particle size distribution determined by DLS – Condition C