Supplementary Material

Comprehensive High Resolution Mass Spectrometric Analysis of DNA Phosphate Adducts Formed by the Tobacco-Specific Lung Carcinogen 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)

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Scheme S1. Synthetic route for CpopC

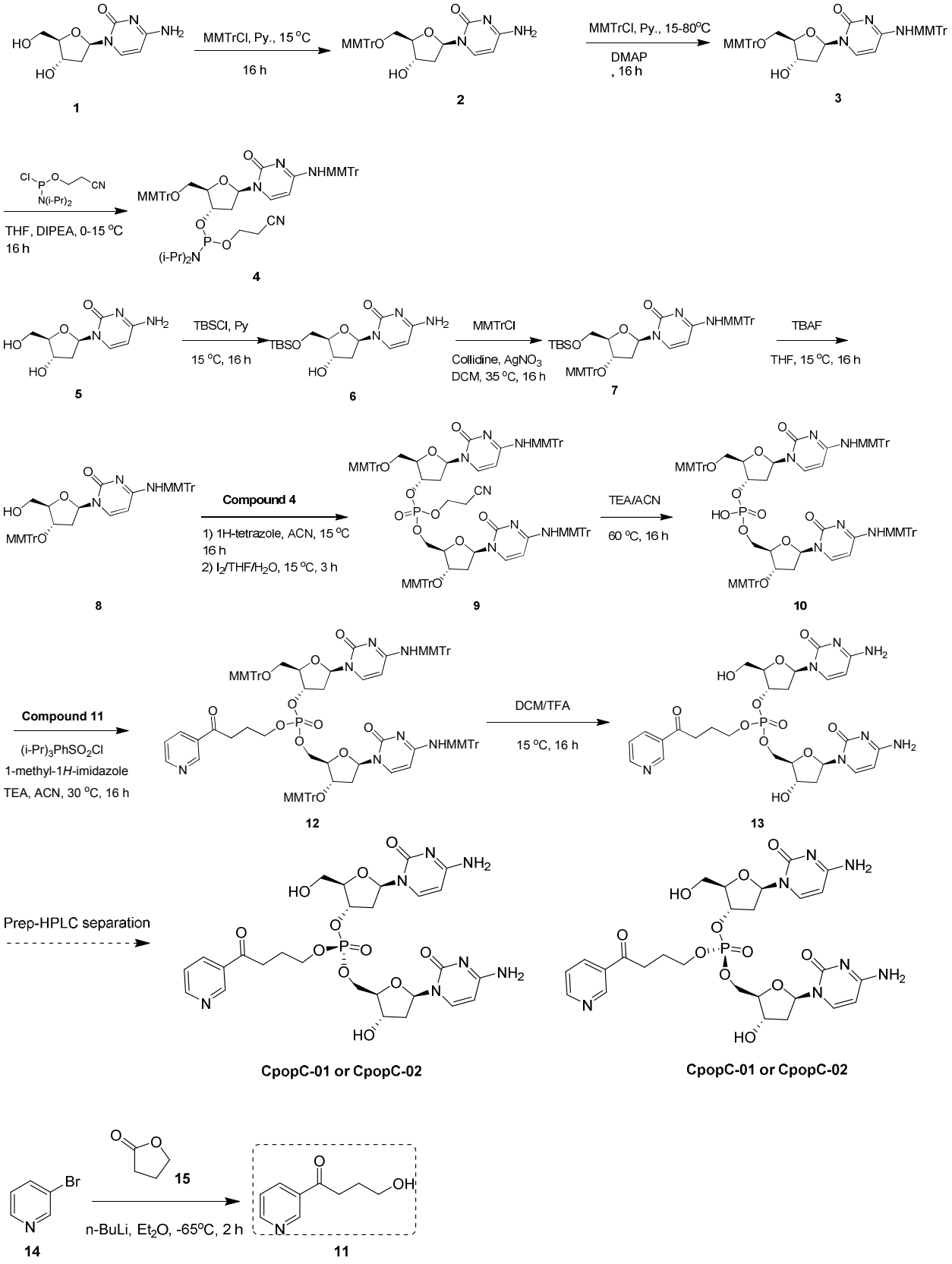
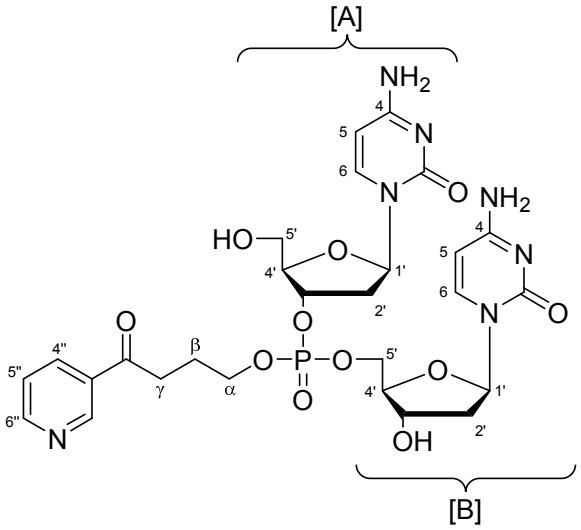


Figure S1. 1D and 2D NMR of CpopC



CpopC-1

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.97 - 2.05 (m, 3 H, Cβ-H$_2$, C2′-H[B]) 2.11 - 2.22 (m, 2 H, C2′-H′[B], C2′-H[A]) 2.39 - 2.44 (m, 1 H, C2′-H′[A]) 3.20 (t, *J* = 7.02 Hz, 2 H, Cγ-H$_2$) 3.56 - 3.61 (m, 2 H, C5′-H$_2$[A]) 3.91 - 3.95 (m, 1 H, C4′-H[B]) 4.06 - 4.09 (m, 1 H, C4′-H[A]) 4.10 - 4.17 (m, 3 H, Cα-H$_2$, C5′-H[B]) 4.17 - 4.25 (m, 2 H, C5′-H′[B], C3′-H[B]) 4.93 - 4.97 (m, 1 H, C3′-H[A]) 5.16 (t, *J* = 5.34 Hz, 1 H, C5′-OH[A]) 5.41 (d, *J* = 4.27 Hz, 1 H, C3′-OH[B]) 5.73 - 5.77 (m, 2 H, C5-H[A], C5-H[B]) 6.17 - 6.23 (m, 2 H, C1′-H[A], C1′-H[B]) 7.09 - 7.23 (br. m., 4 H, C4-NH$_2$[A], C4-NH$_2$[B]) 7.55 - 7.59 (m, 1 H, C5″-H) 7.61 (d, *J* = 7.32 Hz, 1 H, C6-H[A/B]) 7.77 (d, *J* = 7.63 Hz, 1 H, C6-H[A/B]) 8.27 - 8.32 (m, 1 H, C4″-H) 8.78 - 8.81 (m, 1 H, C6″-H) 9.12 - 9.14 (m, 1 H, C2″-H).

CpopC-1

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File Name							
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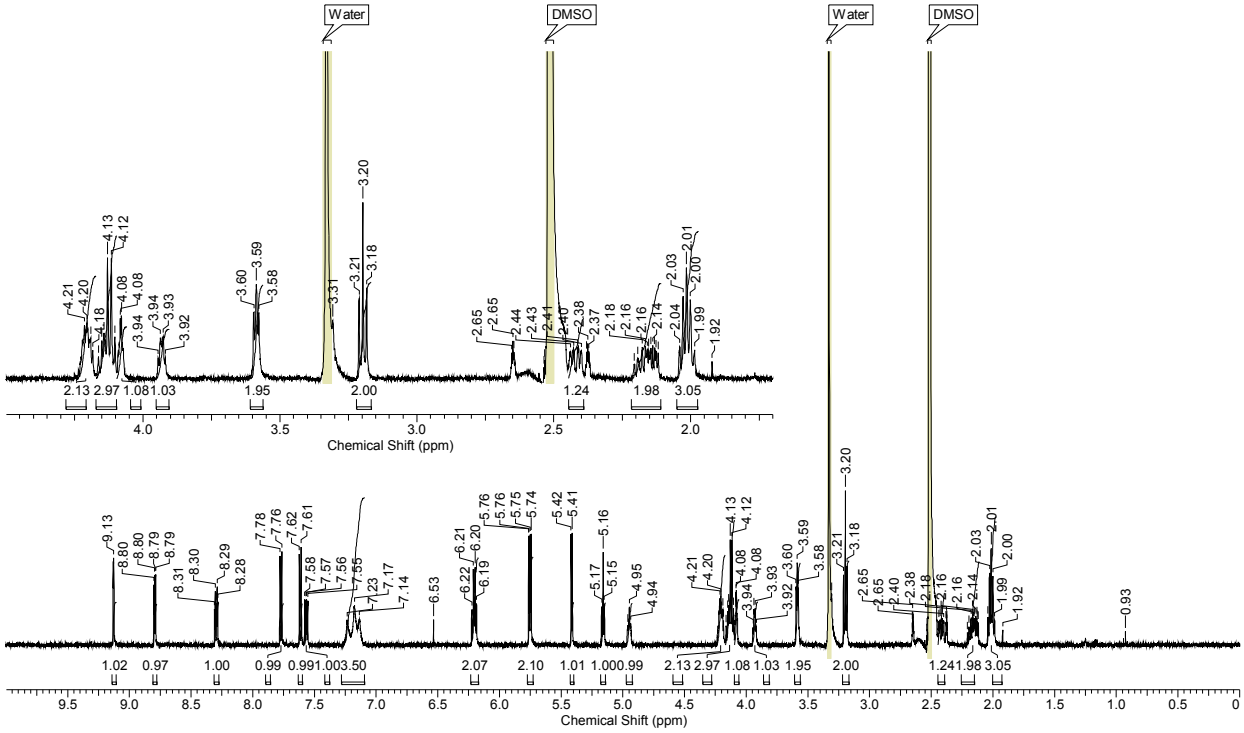
Spectrum Type	STANDARD	***Sweep Width (Hz)***	9999.70	***Temperature (degree C)***	25.128		



CpopC-1

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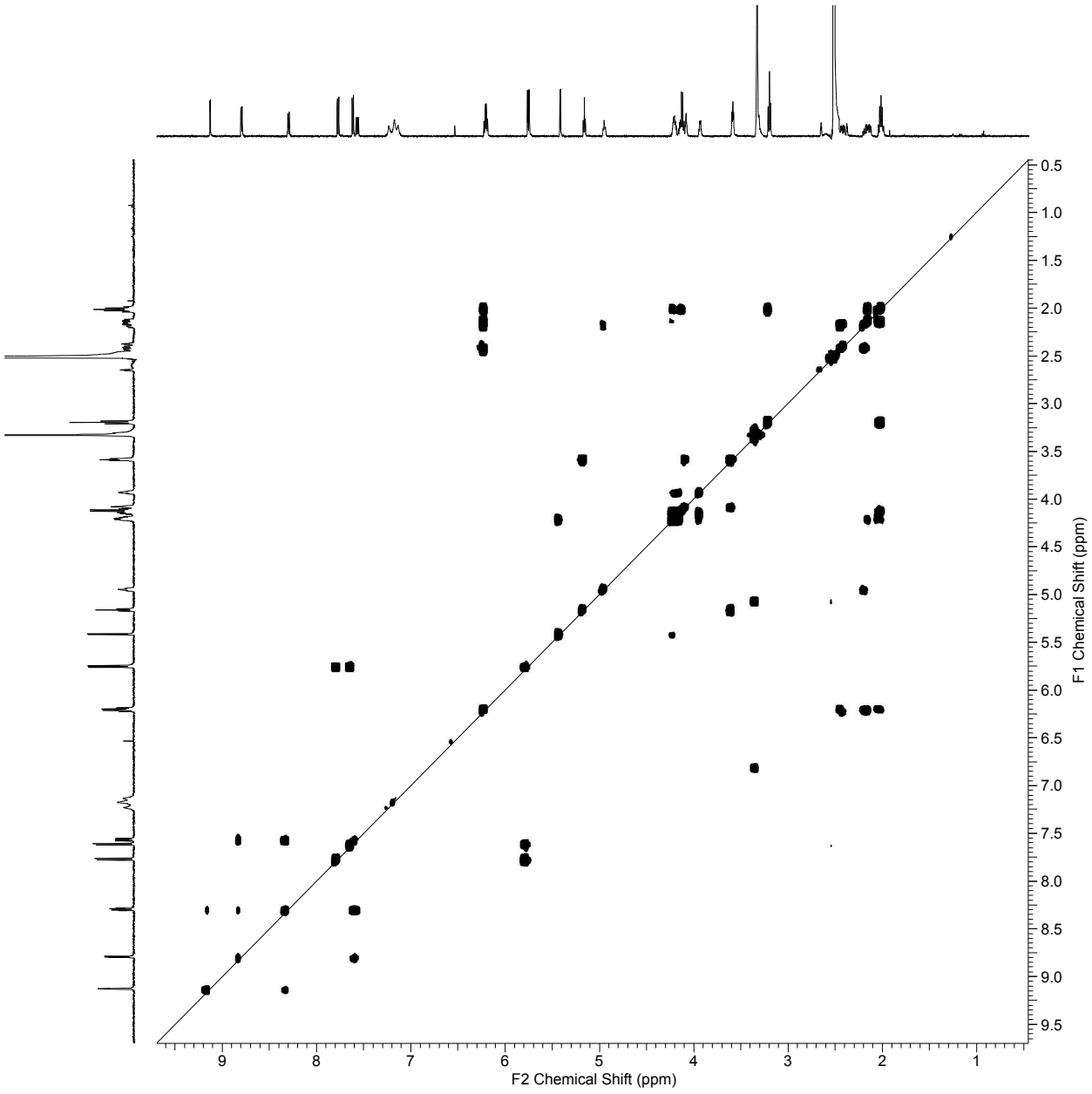
Title					



CpopC-1

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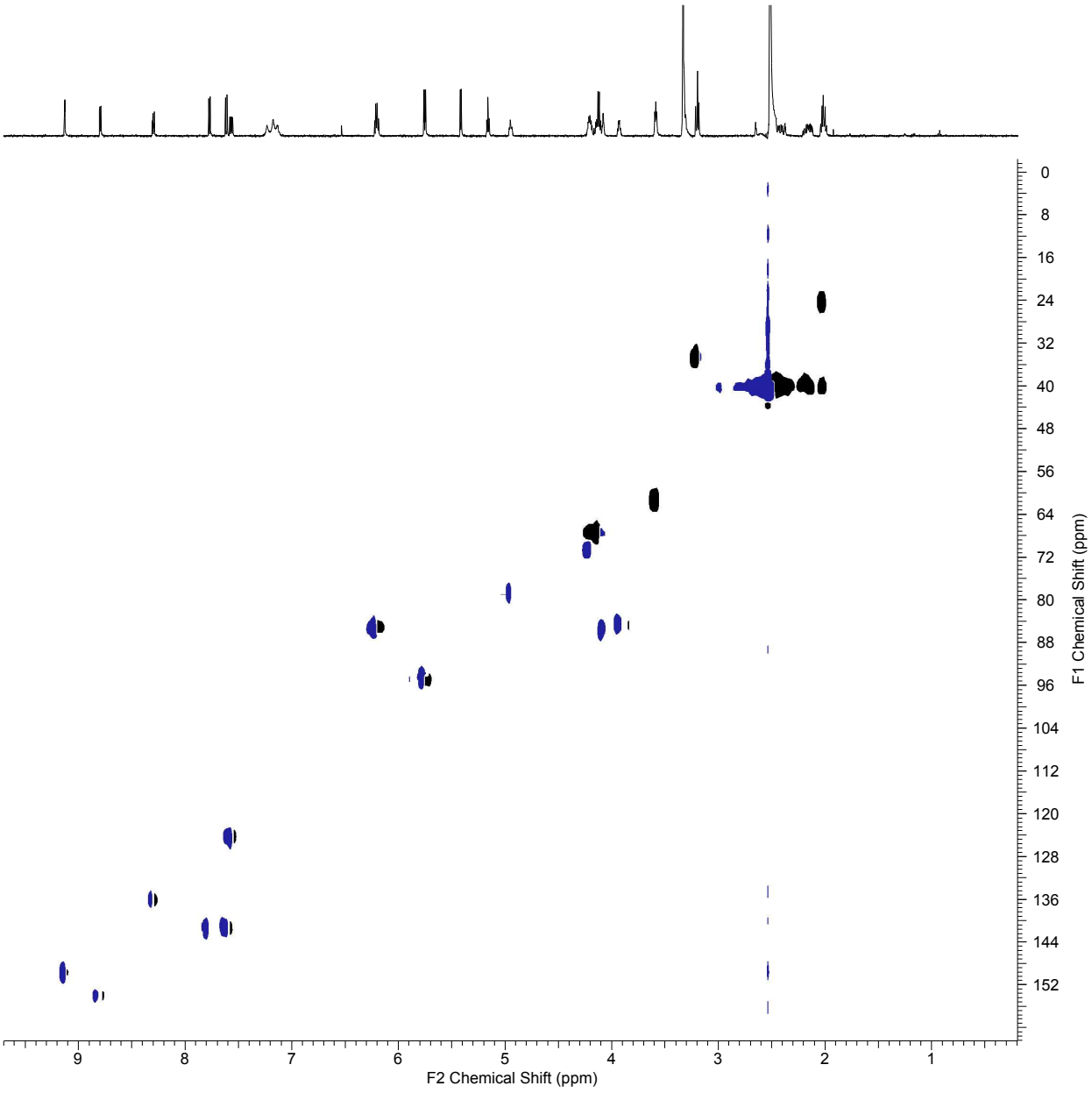
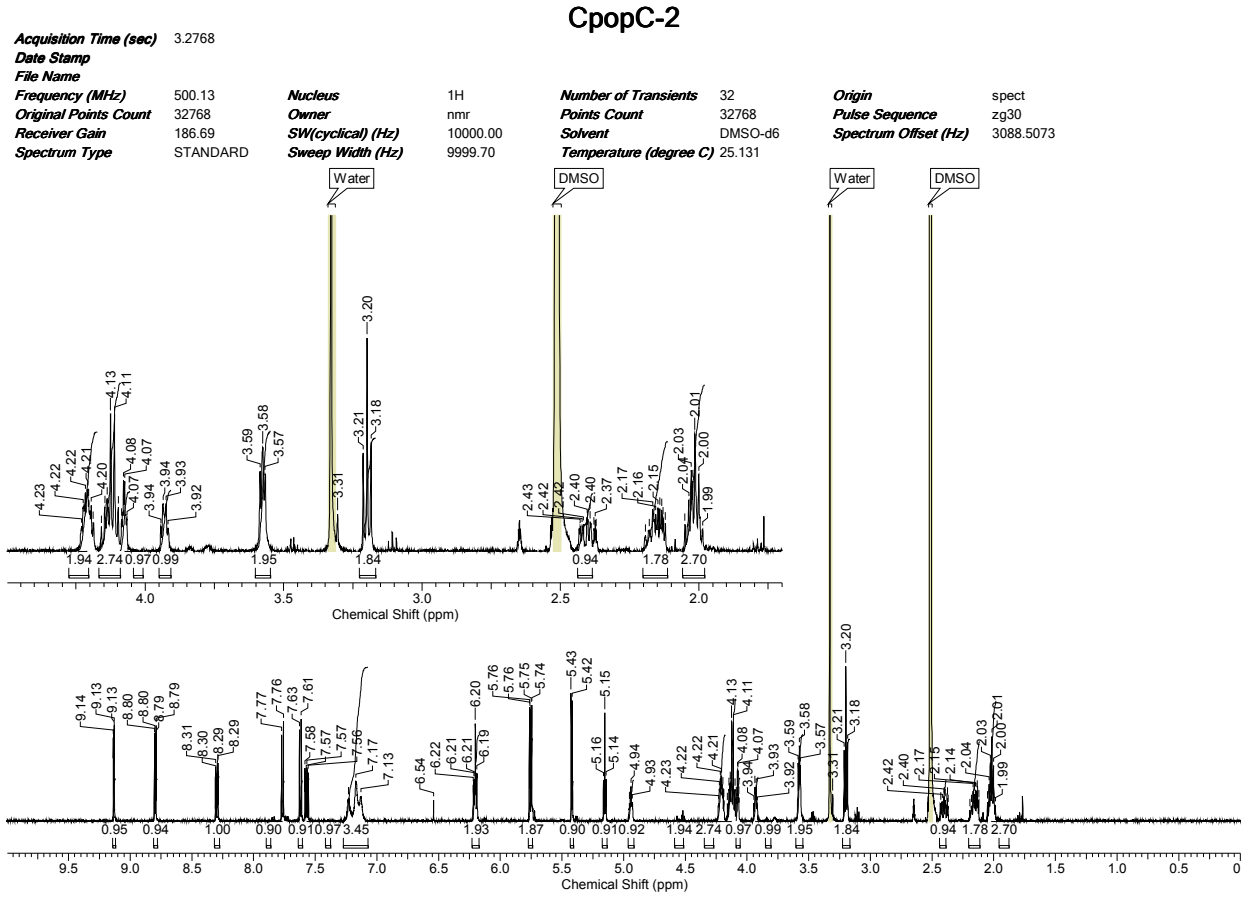
Spectrum Type	HSQC	***Sweep Width (Hz)***	(4757.25, 20726.63)	***Temperature (degree C)***	25.269
Title					



CpopC-2

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.98 - 2.06 (m, 3 H, Cβ-H_2, C2′-H[B]) 2.11 - 2.20 (m, 2 H, C2′-H′[B], C2′-H[A]) 2.39 - 2.44 (m, 1 H, C2′-H′[A]) 3.20 (t, *J* = 7.02 Hz, 2 H, Cγ-H_2) 3.55 - 3.60 (m, 2 H, C5′-H_2[A]) 3.91 - 3.95 (m, 1 H, C4′-H[B]) 4.06 - 4.09 (m, 1 H, C4′-H[A]) 4.09 - 4.17 (m, 3 H, Cα-H_2, C5′-H[B]) 4.17 - 4.25 (m, 2 H, C5′-H′[B], C3′-H[B]) 4.92 - 4.96 (m, 1 H, C3′-H[A]) 5.15 (t, *J* = 5.14 Hz, 1 H, C5′-OH[A]) 5.42 (d, *J* = 4.27 Hz, 1 H, C3′-OH[B]) 5.73 - 5.77 (m, 2 H, C5-H[A], C5-H[B]) 6.17 - 6.23 (m, 2 H, C1′-H[A], C1′-H[B]) 7.10 - 7.26 (br. m., 4 H, C4-NH_2[A], C4-NH_2[B]) 7.55 - 7.59 (m, 1 H, C5″-H) 7.62 (d, *J* = 7.32 Hz, 1 H, C6-H[A/B]) 7.77 (d, *J* = 7.63 Hz, 1 H, C6-H[A/B]) 8.28 - 8.32 (m, 1 H, C4″-H) 8.78 - 8.81 (m, 1 H, C6″-H) 9.12 - 9.15 (m, 1 H, C2″-H).



CpopC-2

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Points Count	(1024, 2048)	***Pulse Sequence***	cosygpppqf	***Solvent***	DMSO
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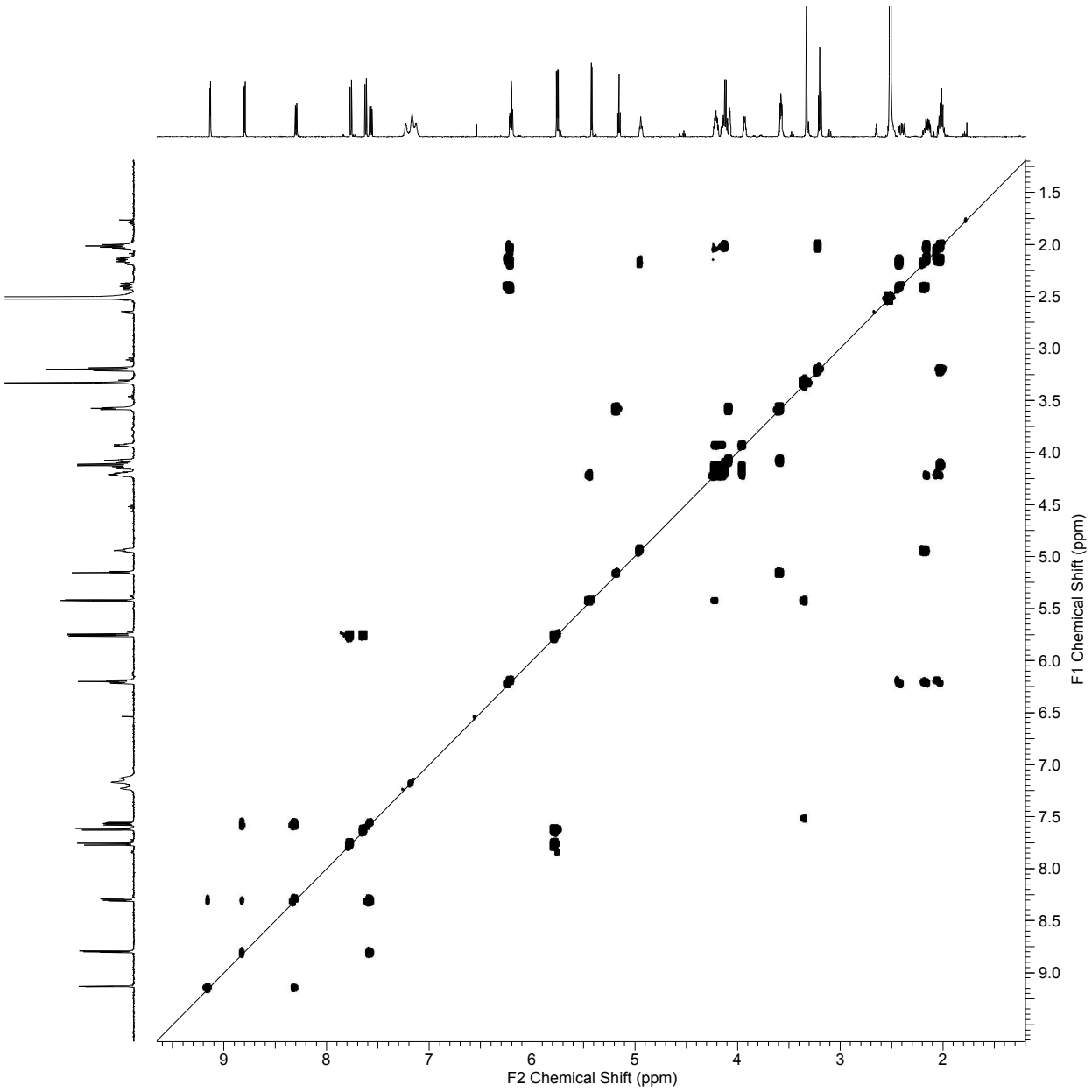
Title					



CpopC-2

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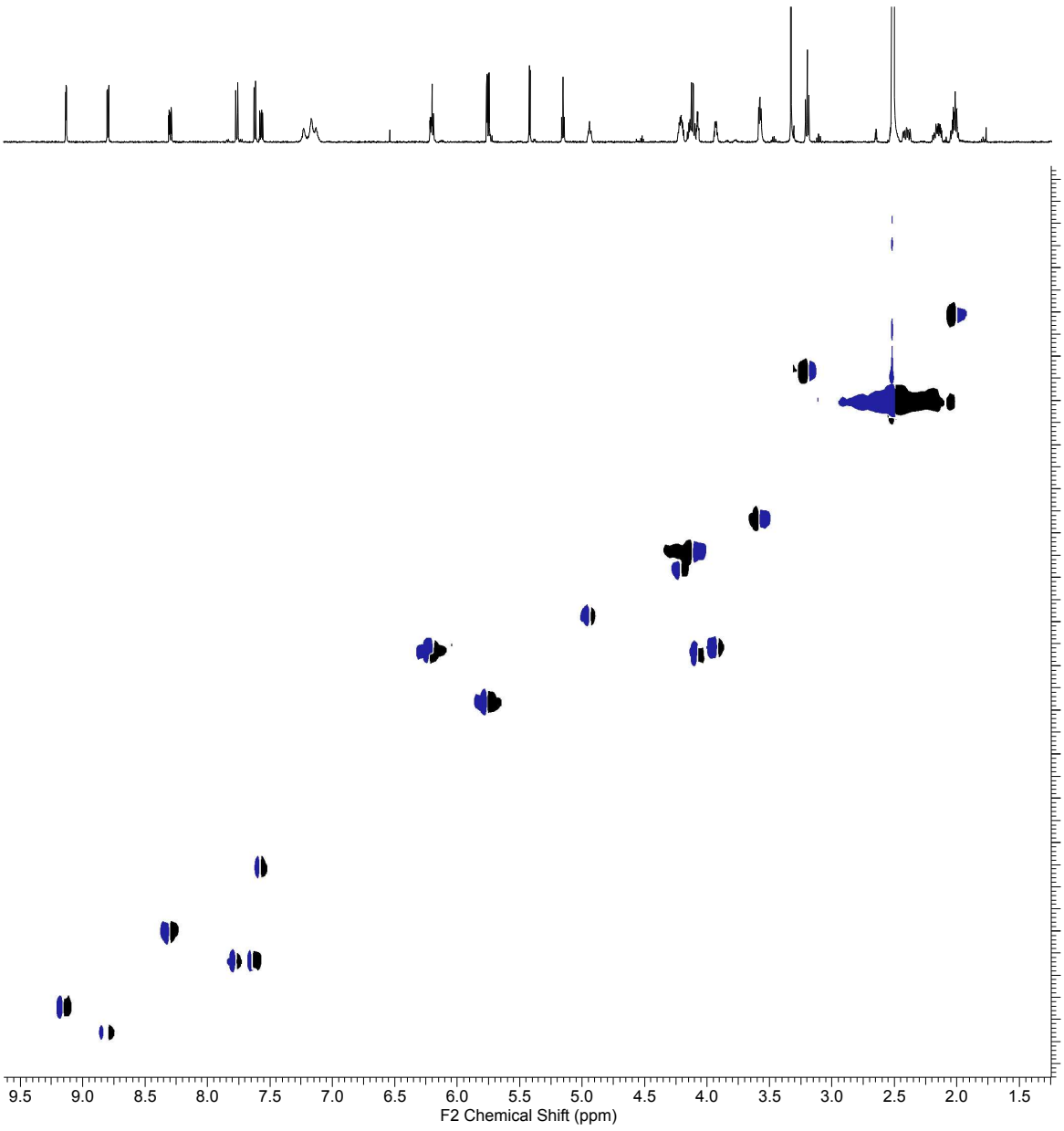
Points Count	(1024, 1024)	***Pulse Sequence***	hsqcedetgpsisp2.3	***Solvent***	DMSO
Spectrum Type	HSQC	***Sweep Width (Hz)***	(4197.58, 20726.63)	***Temperature (degree C)***	25.332
Title	CpopC - P3				



Scheme S2. Synthetic route of [$^{15}N_3$]2'-dCpdC.

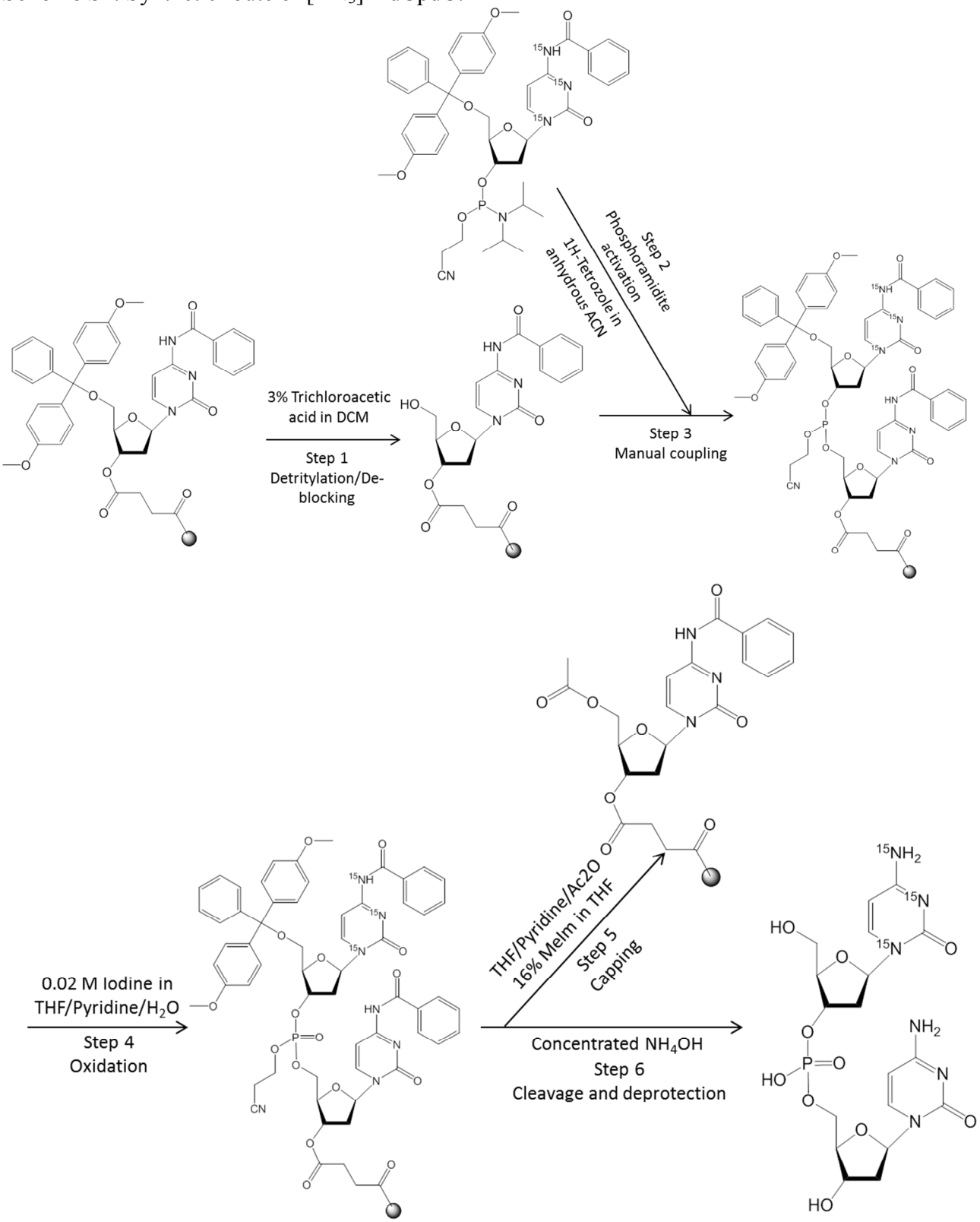


Figure S2. Extracted ion chromatograms (EIC) of CpopC and [$^{15}N_3$]CpopC obtained from full scan and product ion scan.

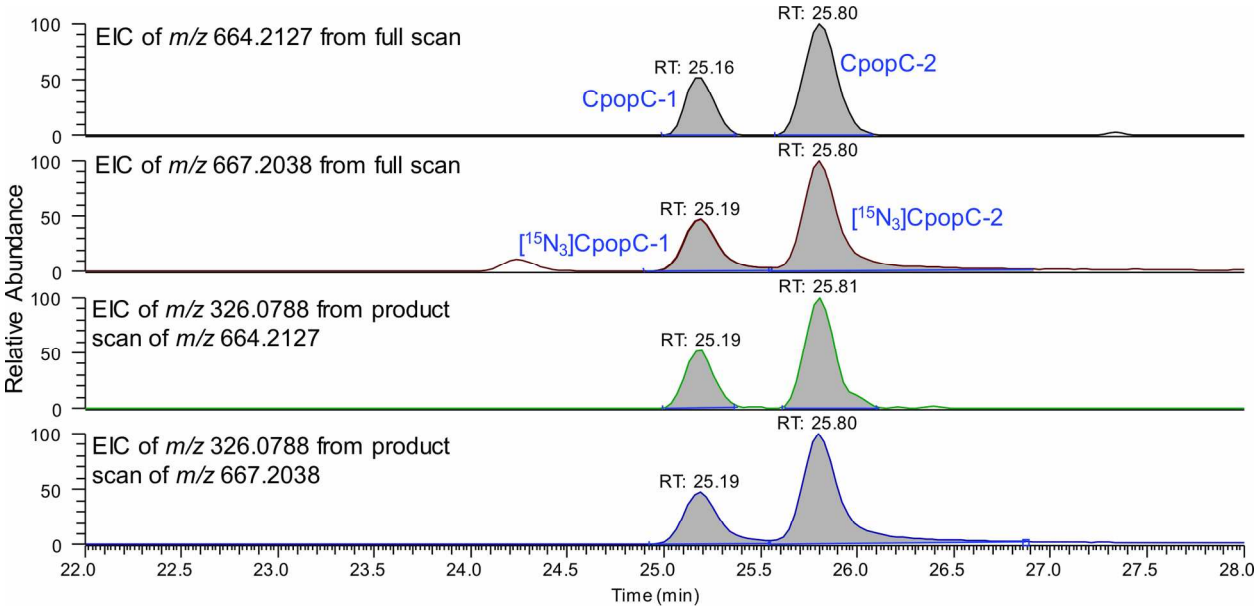


Figure S3. The product ion spectra and fragmentation pattern of four isomers of ApopC.

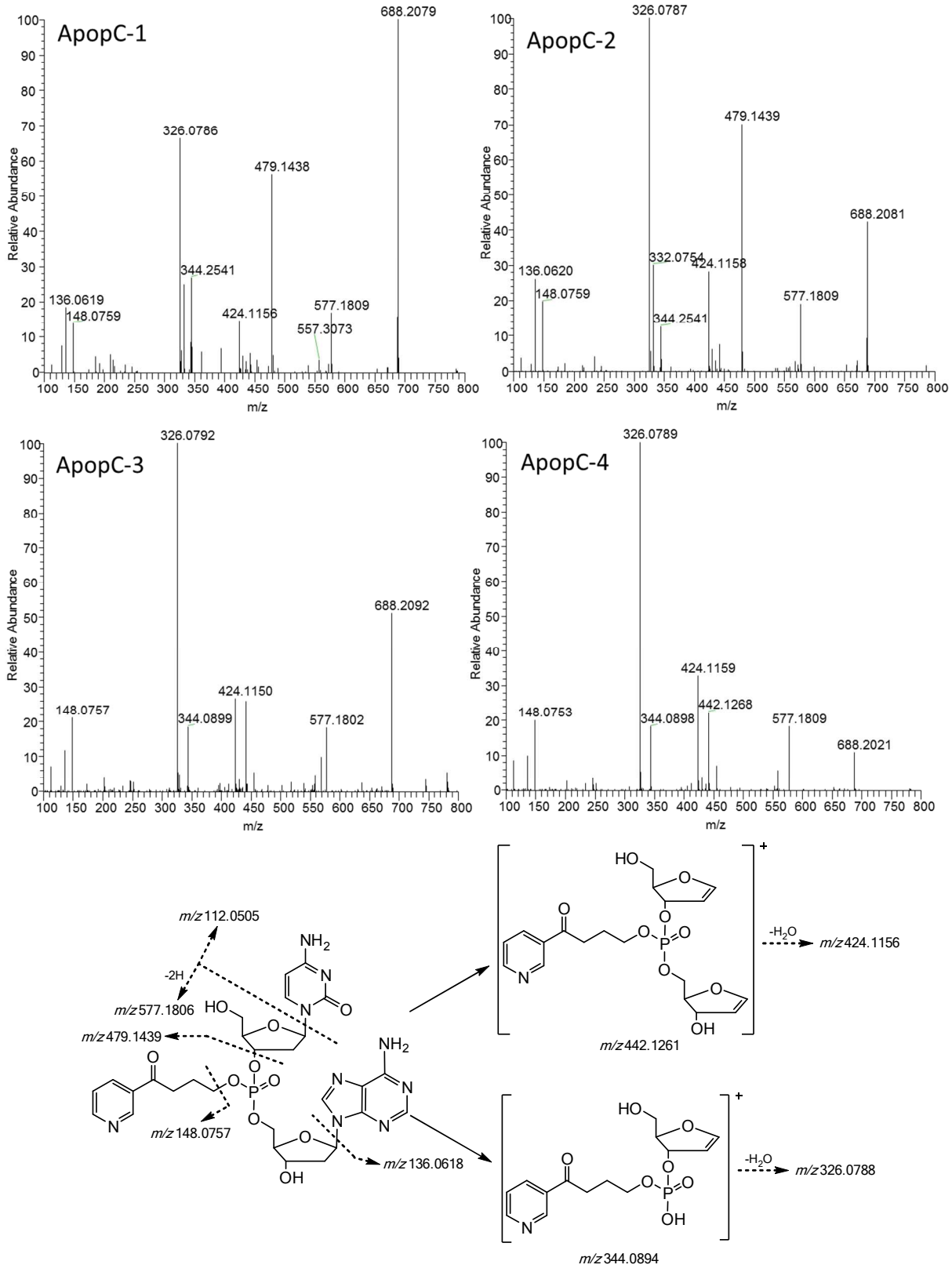


Figure S4. The extracted ion chromatograms and fragmentation patterns of B_1popB_2 PTEs in NNKOAc-treated CT-DNA.

ApopA

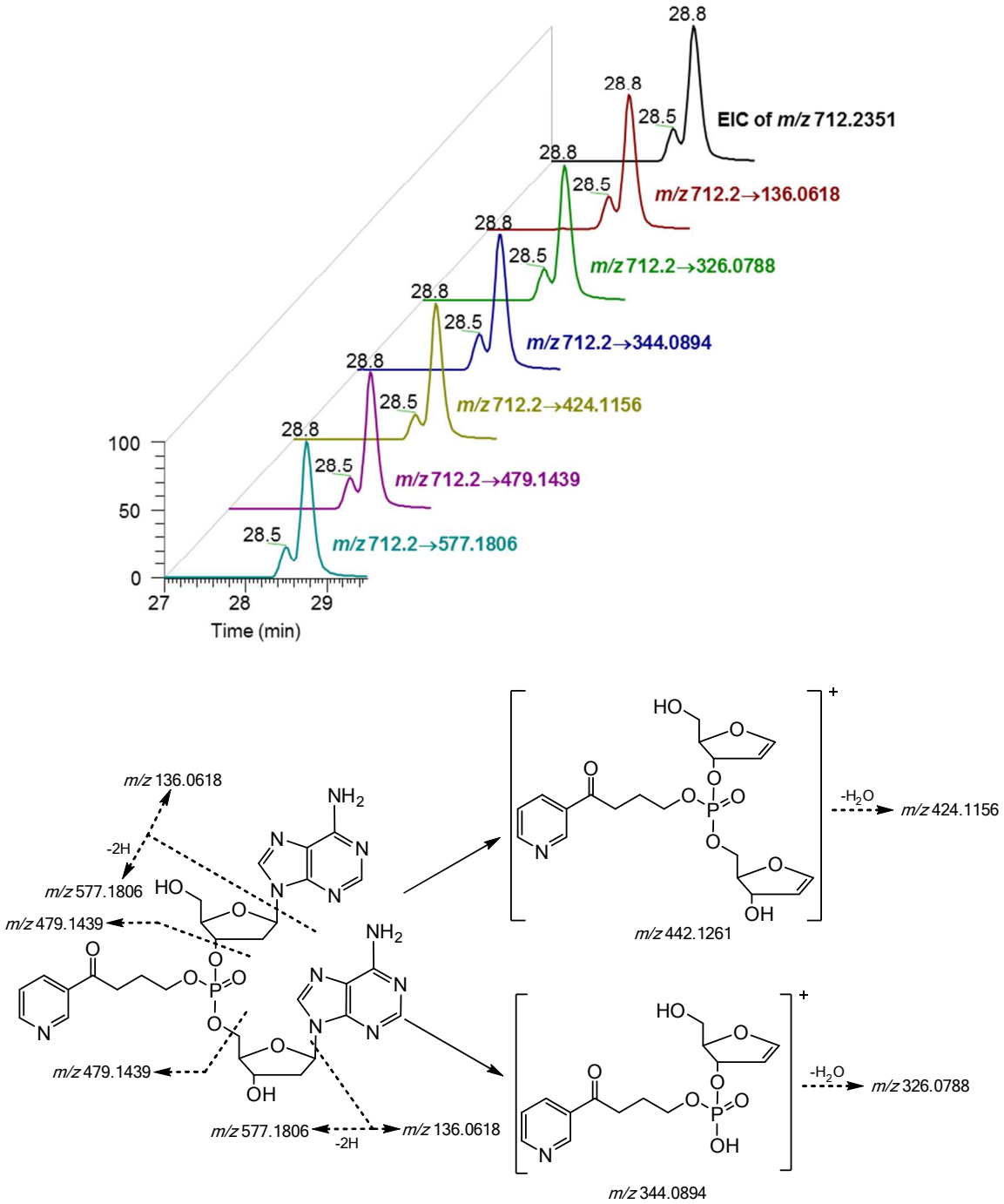


Figure S4. (Continued)

GpopG

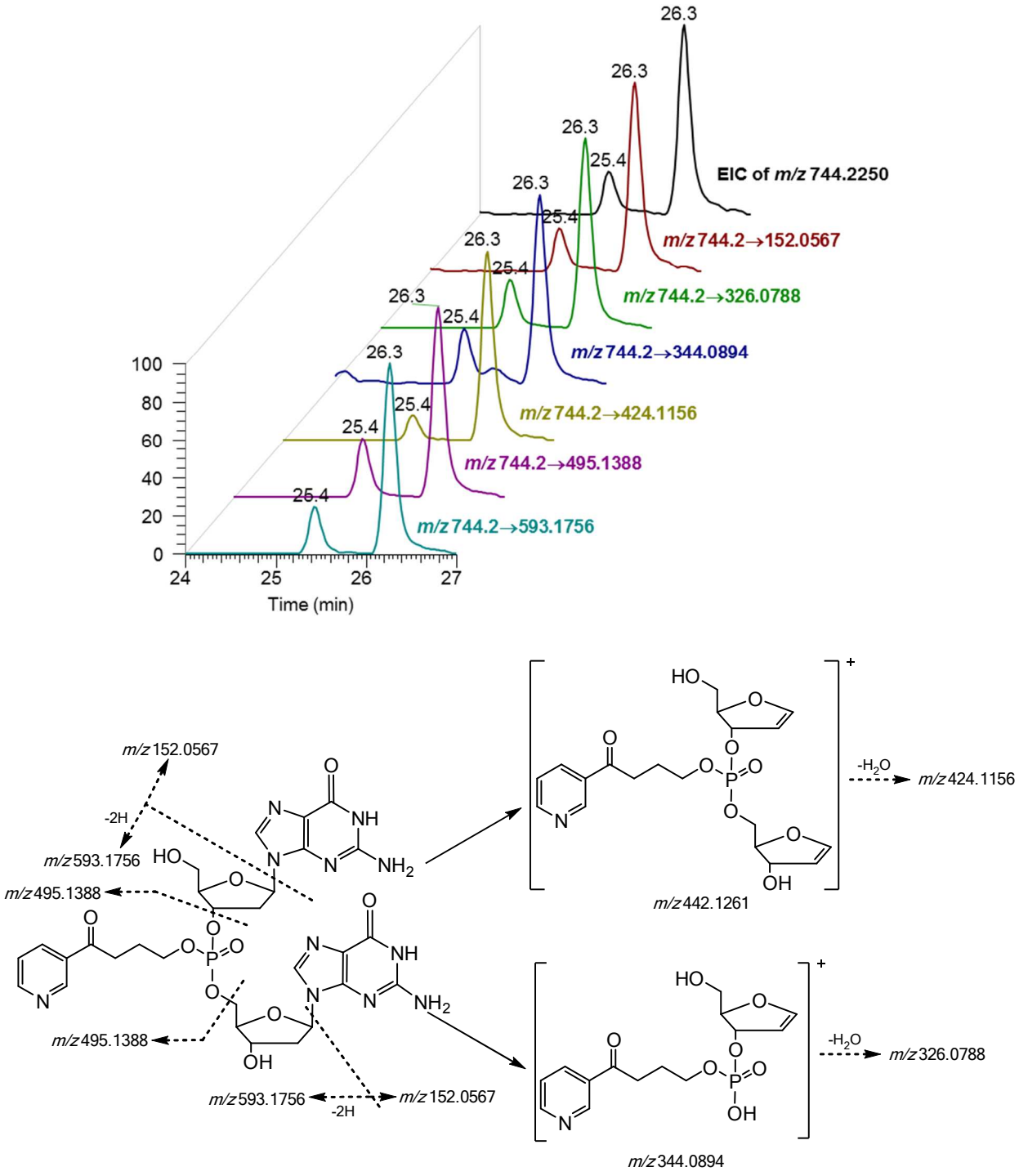
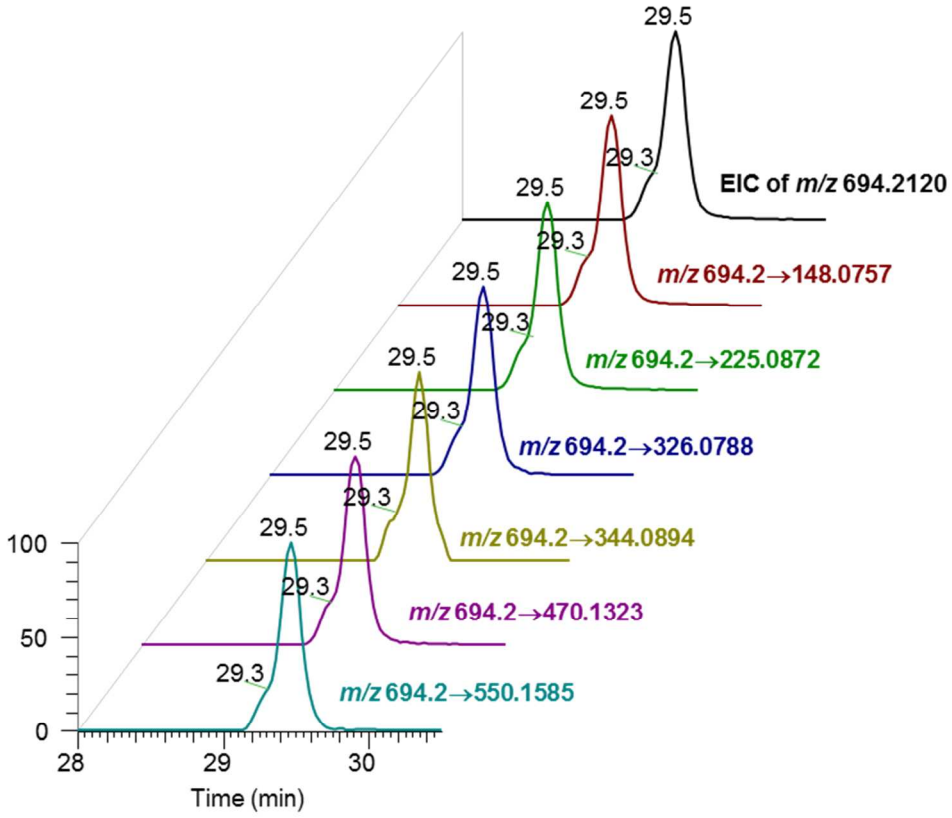


Figure S4. (Continued)

TpopT



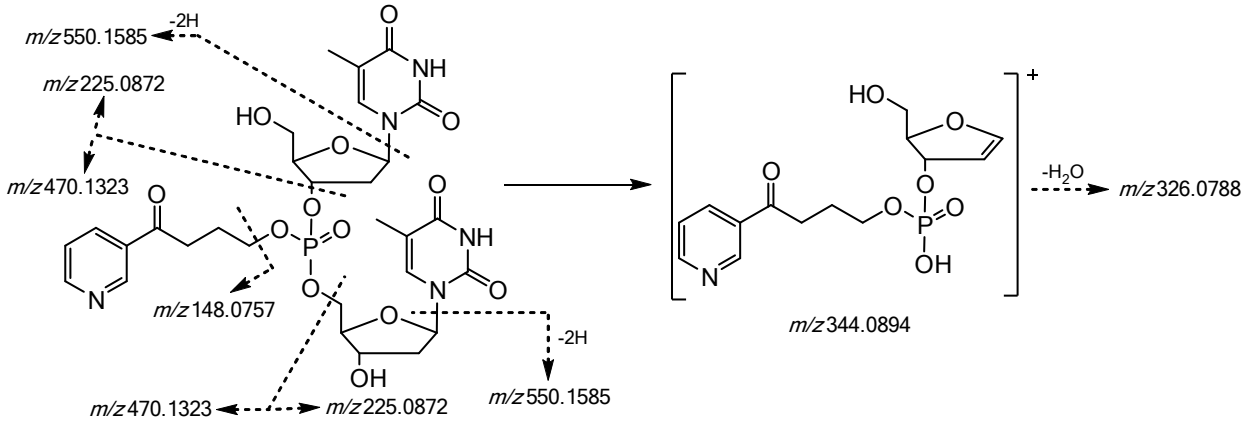
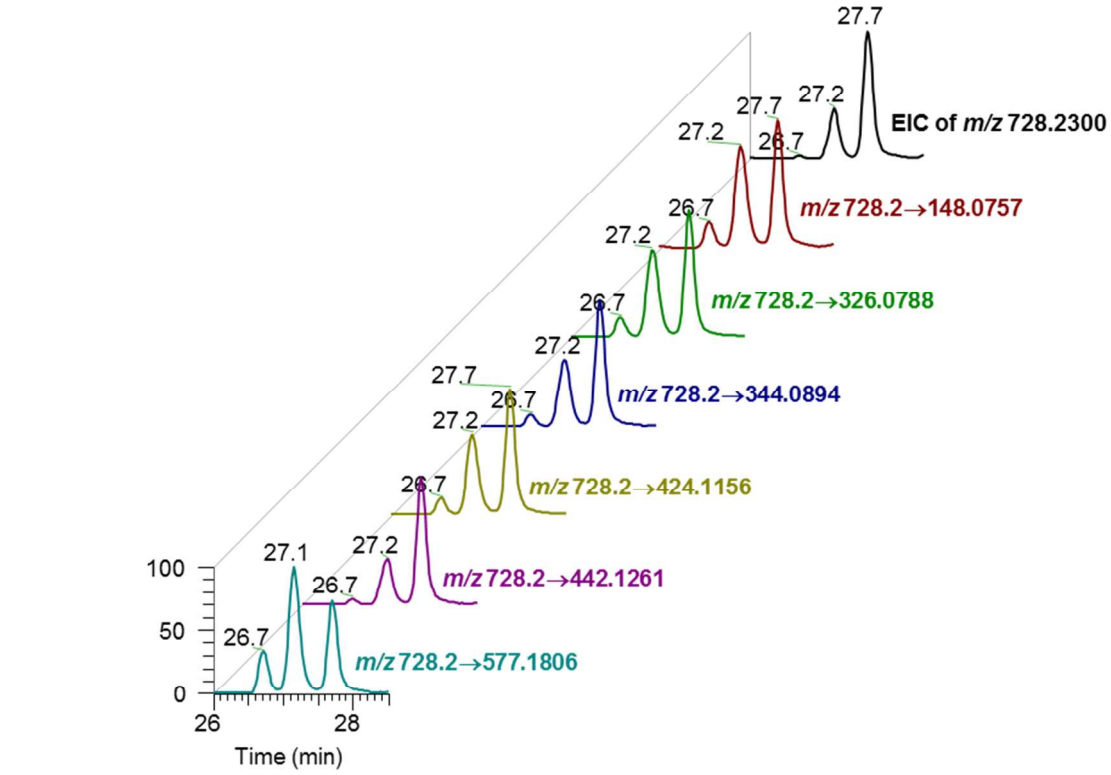


Figure S4. (Continued)

ApopG



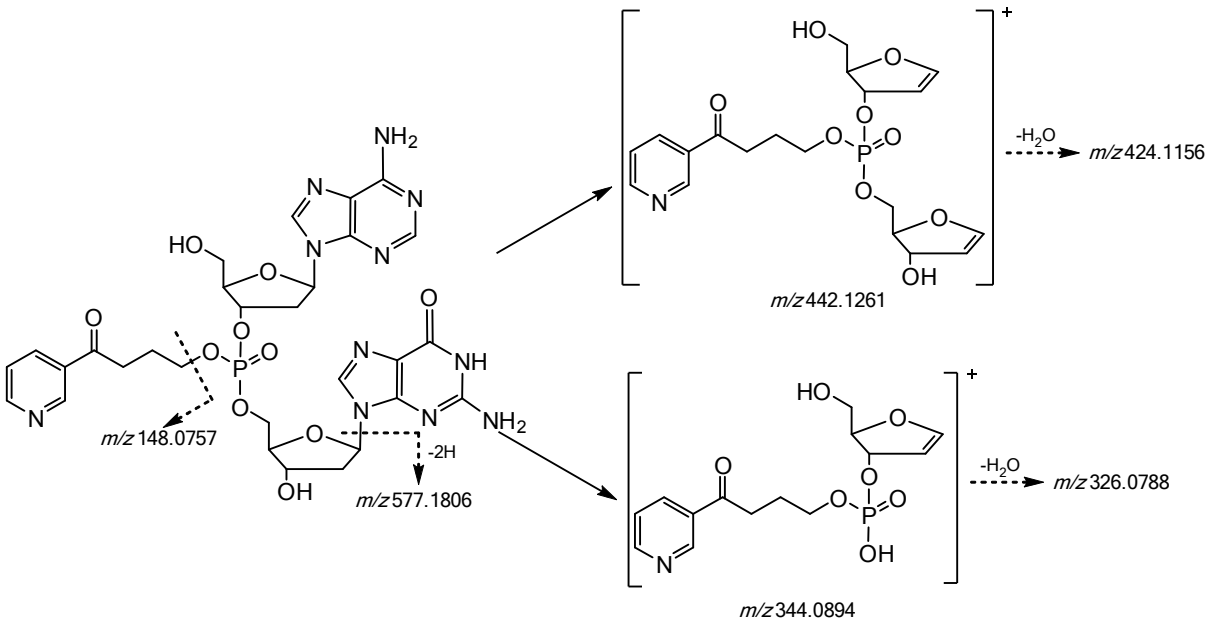


Figure S4. (Continued)

ApopT

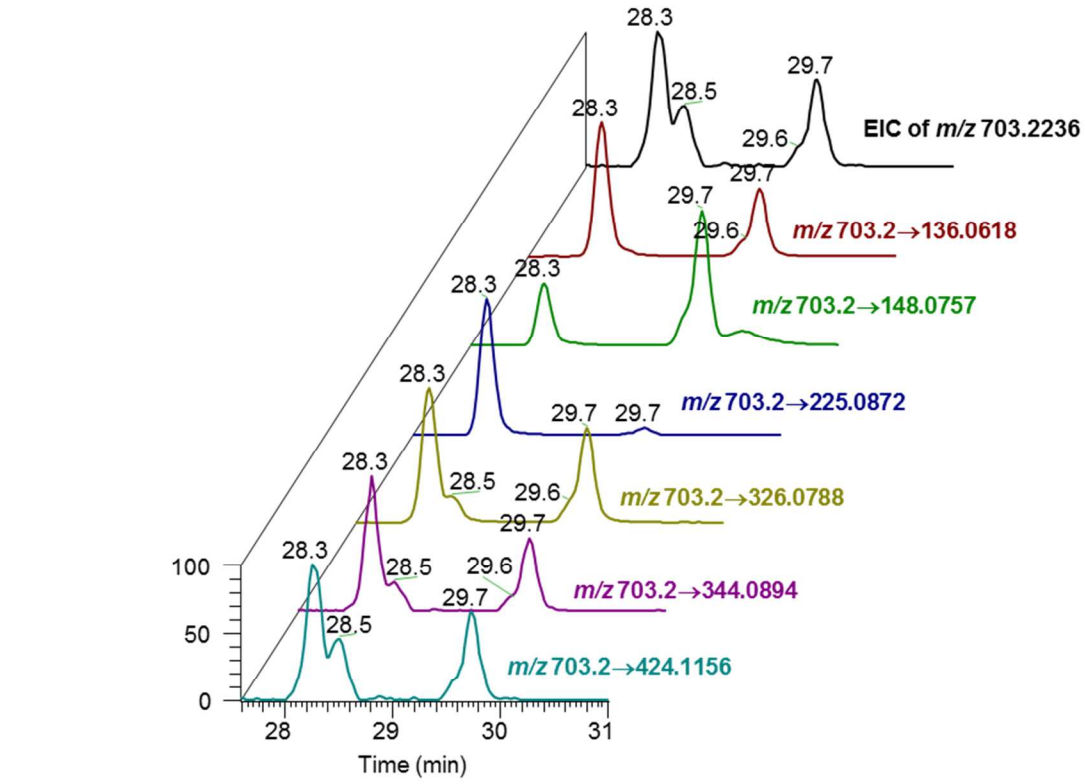


Figure S4. (Continued)

CpopG

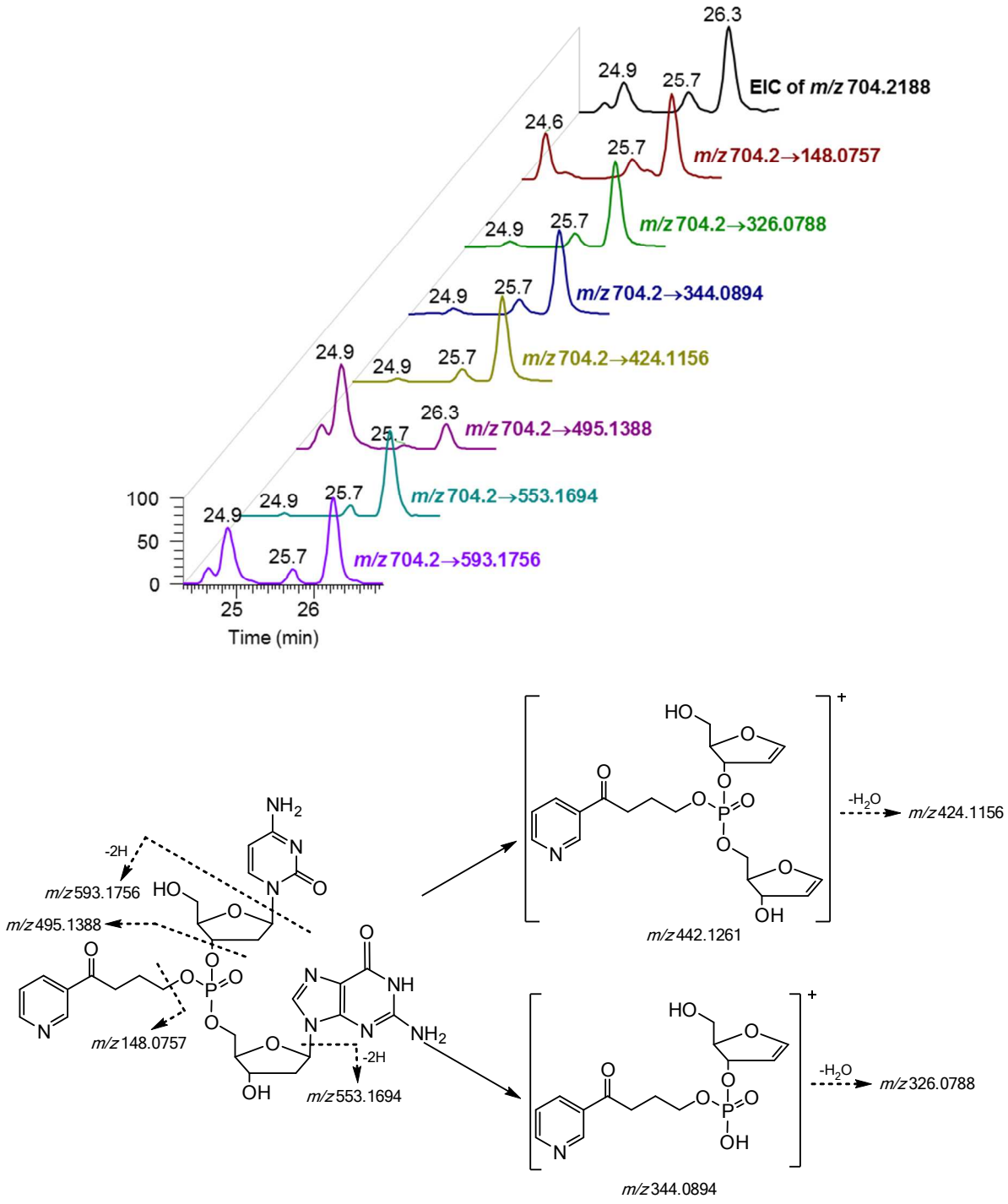


Figure S4. (Continued)

CpopT

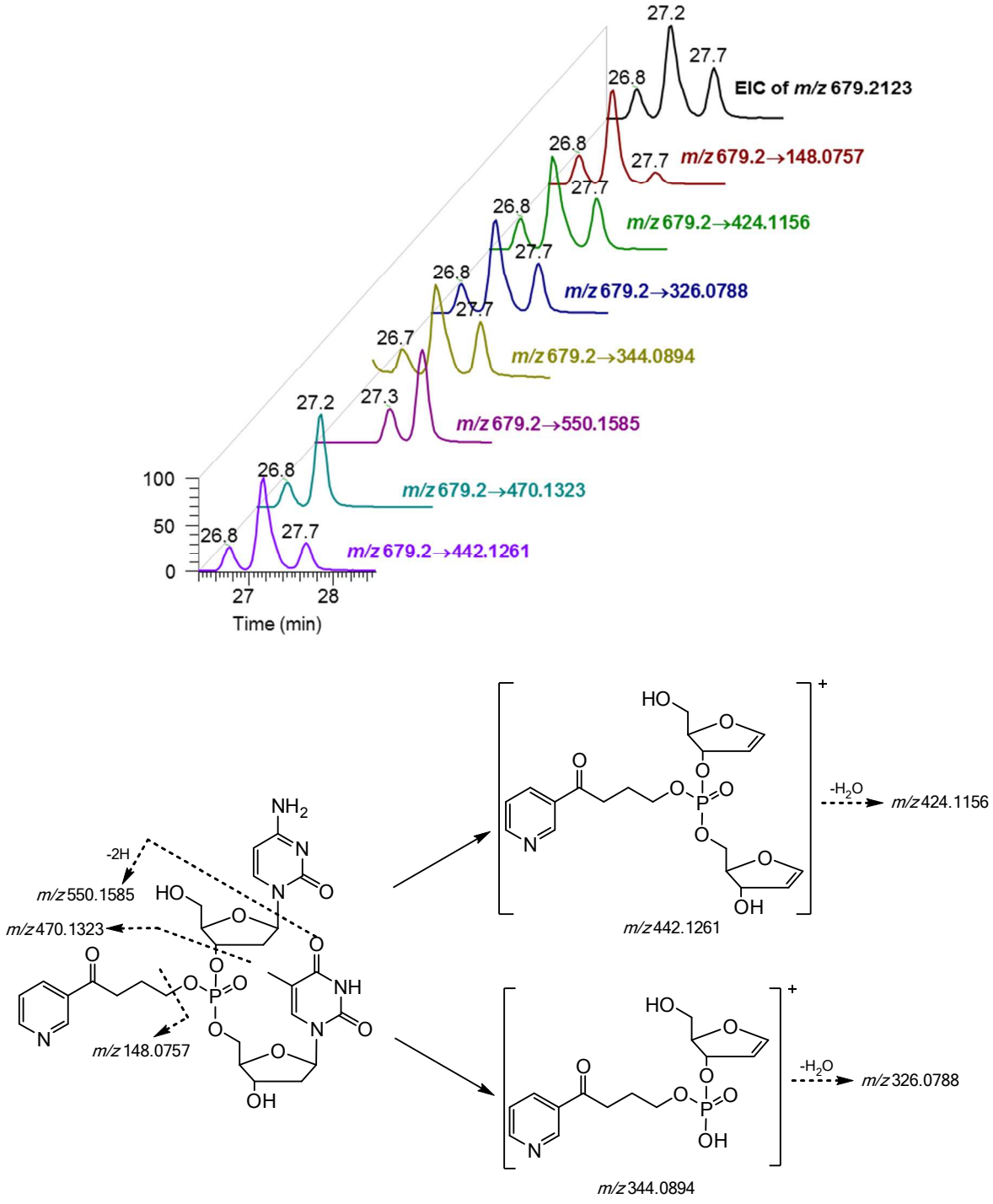
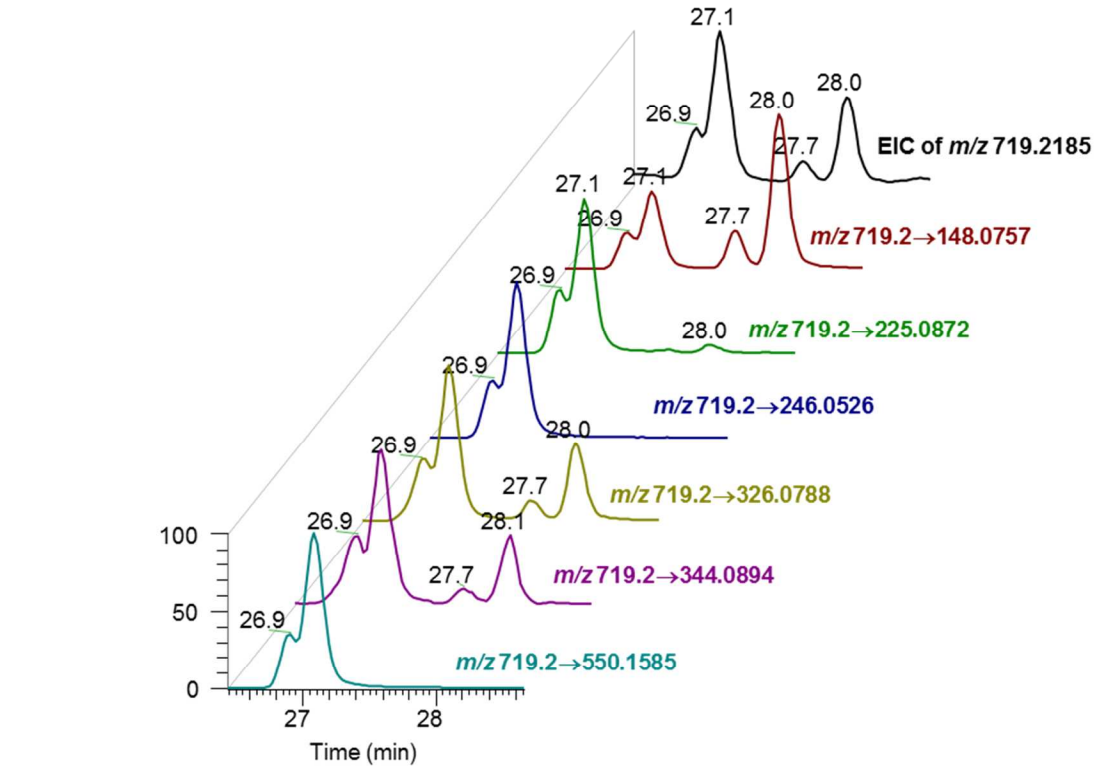
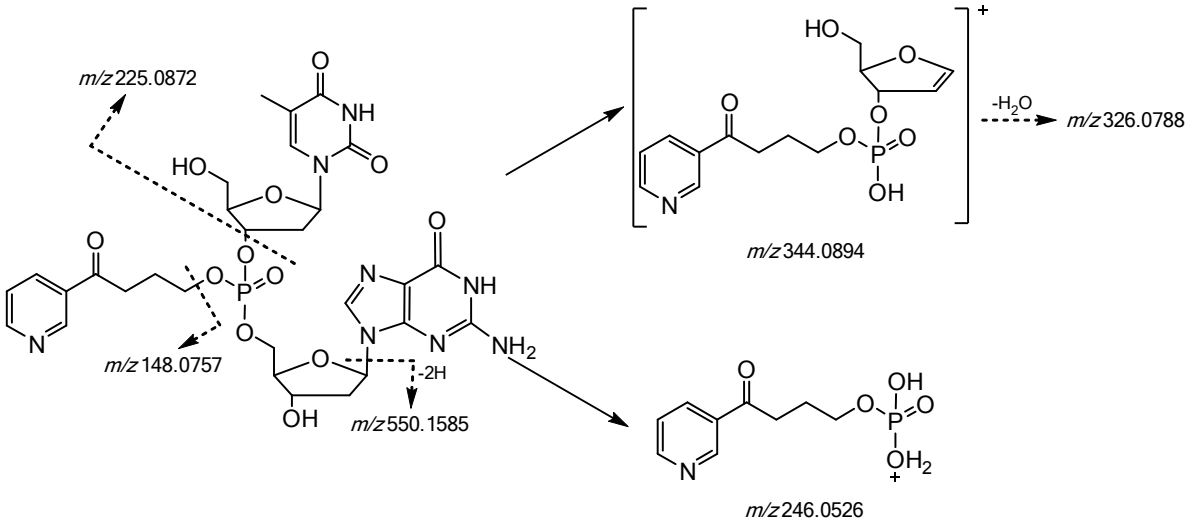


Figure S4. (Continued)

GpopT





Scheme S3. Structure of B_1p[2-(3-pyridyl)-2,3,4,5-tetrahydrofuranyl]B_2.

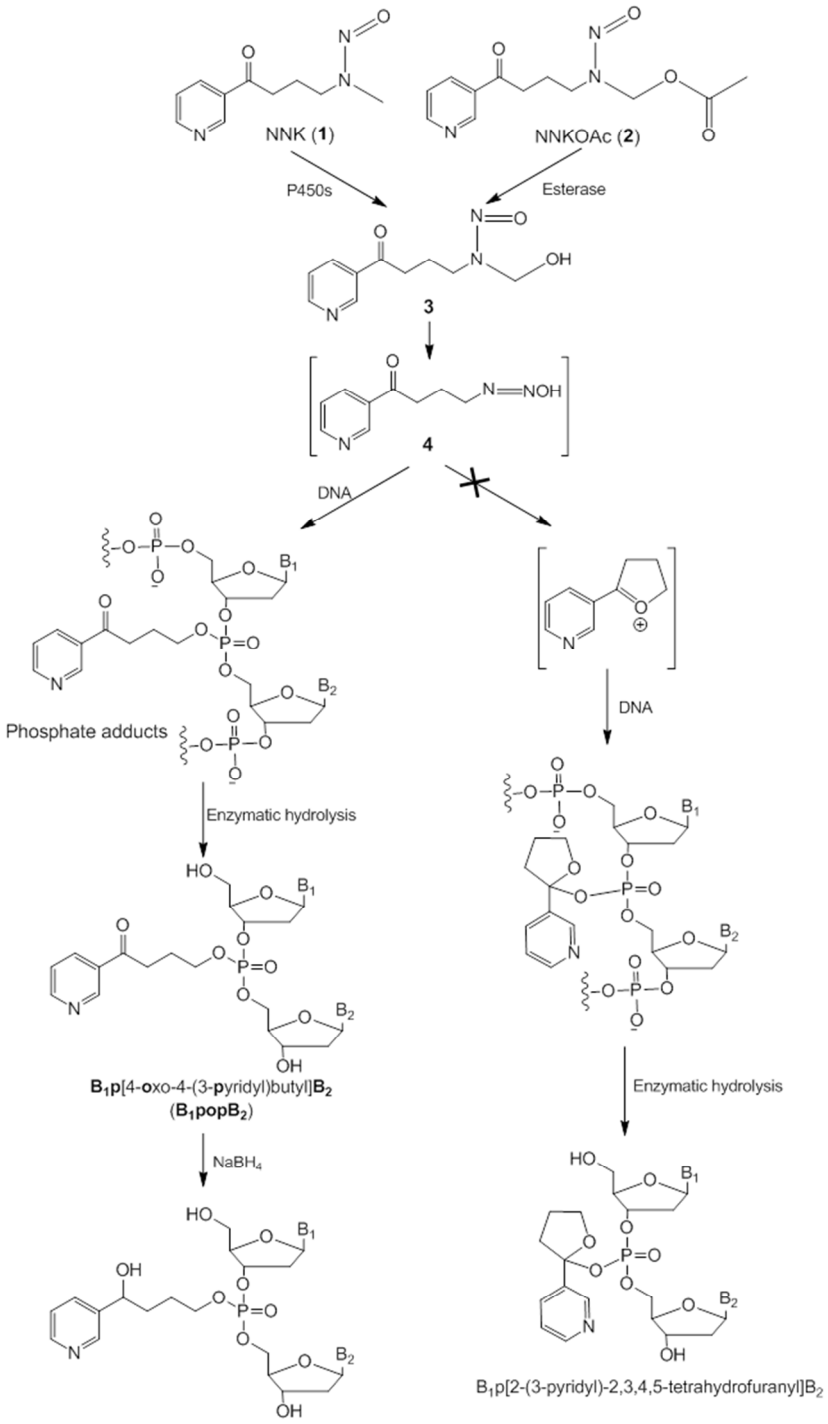


Table S1. The B_1popB$_2$ PTEs detected in rats acutely and chronically treated with NNK.

PTE	Isomer number			
	Possibility	Detected		
		Acute treatment	Chronic treatment	
		Liver	Liver	Lung
ApopA	2	2	2	2
CpopC	2	2	2	2
GpopG	2	2	1	2
TpopT	2	2	2	2
ApopC	4	4	3	3
ApopG	4	3	3	3
ApopT	4	2	3	3
CpopG	4	2	2	2
CpopT	4	3	3	2
GpopT	4	3	2	2
Total	32	25	23	23

Figure S5. Levels of DNA phosphate adducts in liver (A) and lung (B) DNA of NNK-treated rats in the chronic exposure group (5 ppm in drinking water for 30, 50 and 70 weeks)

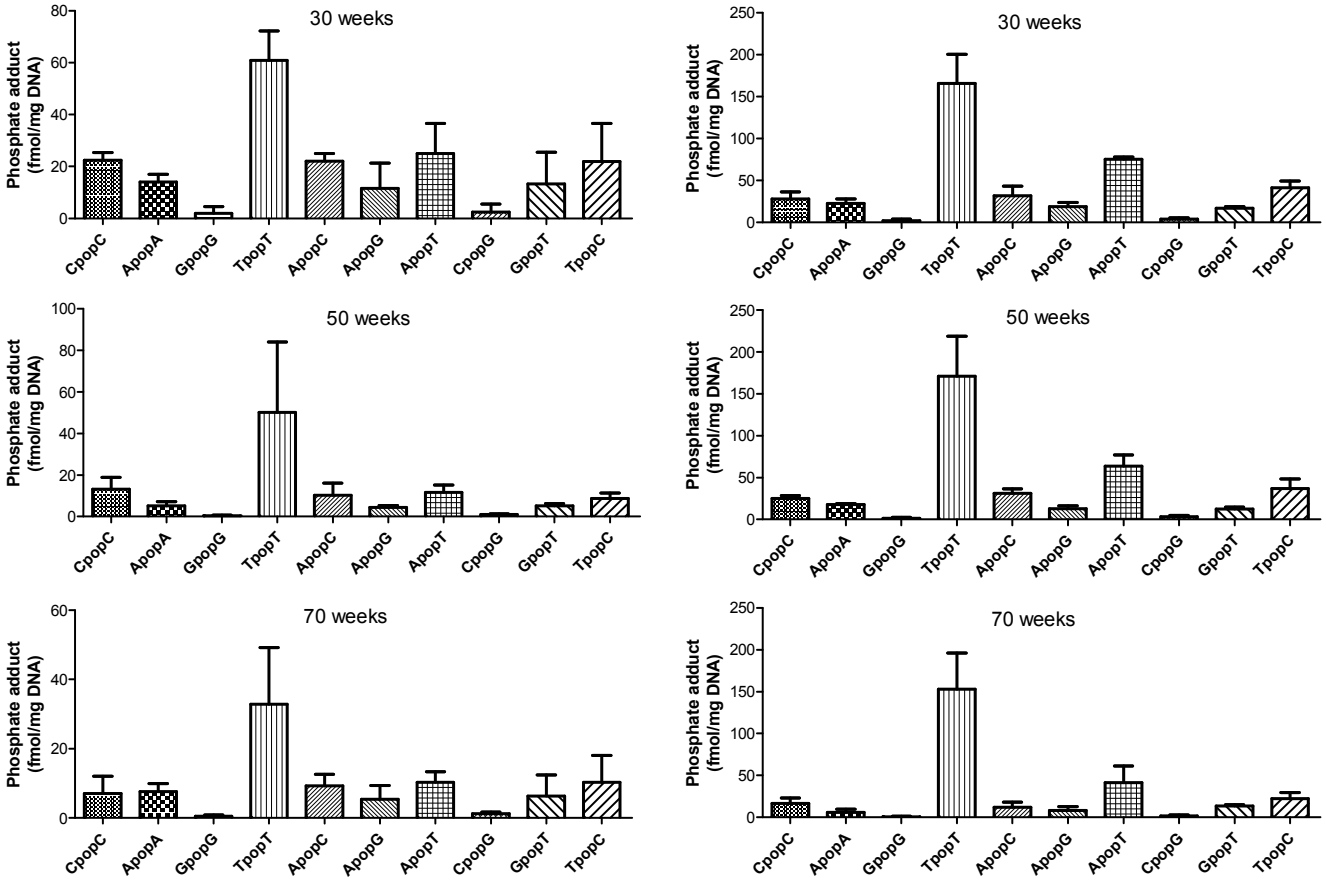
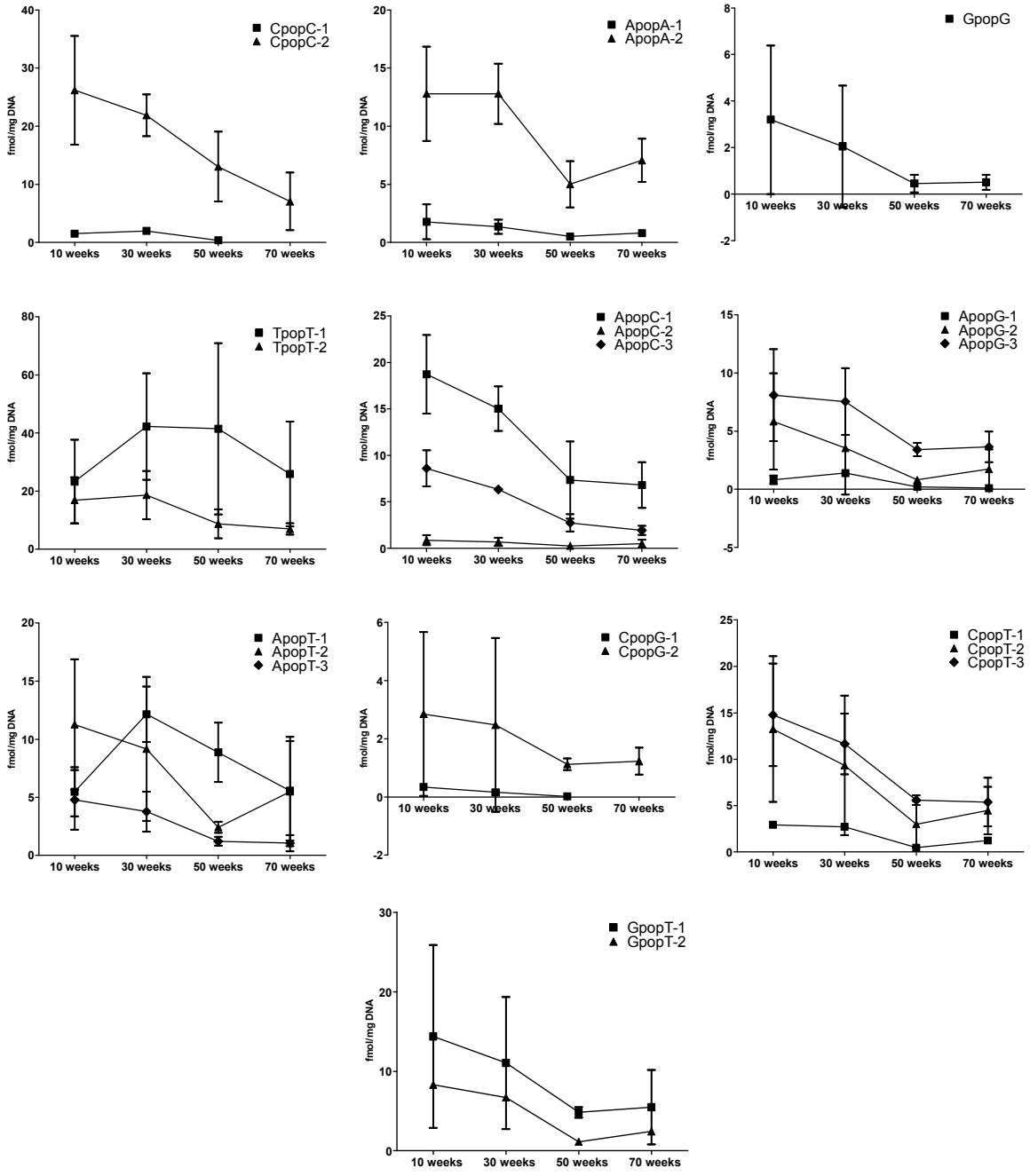
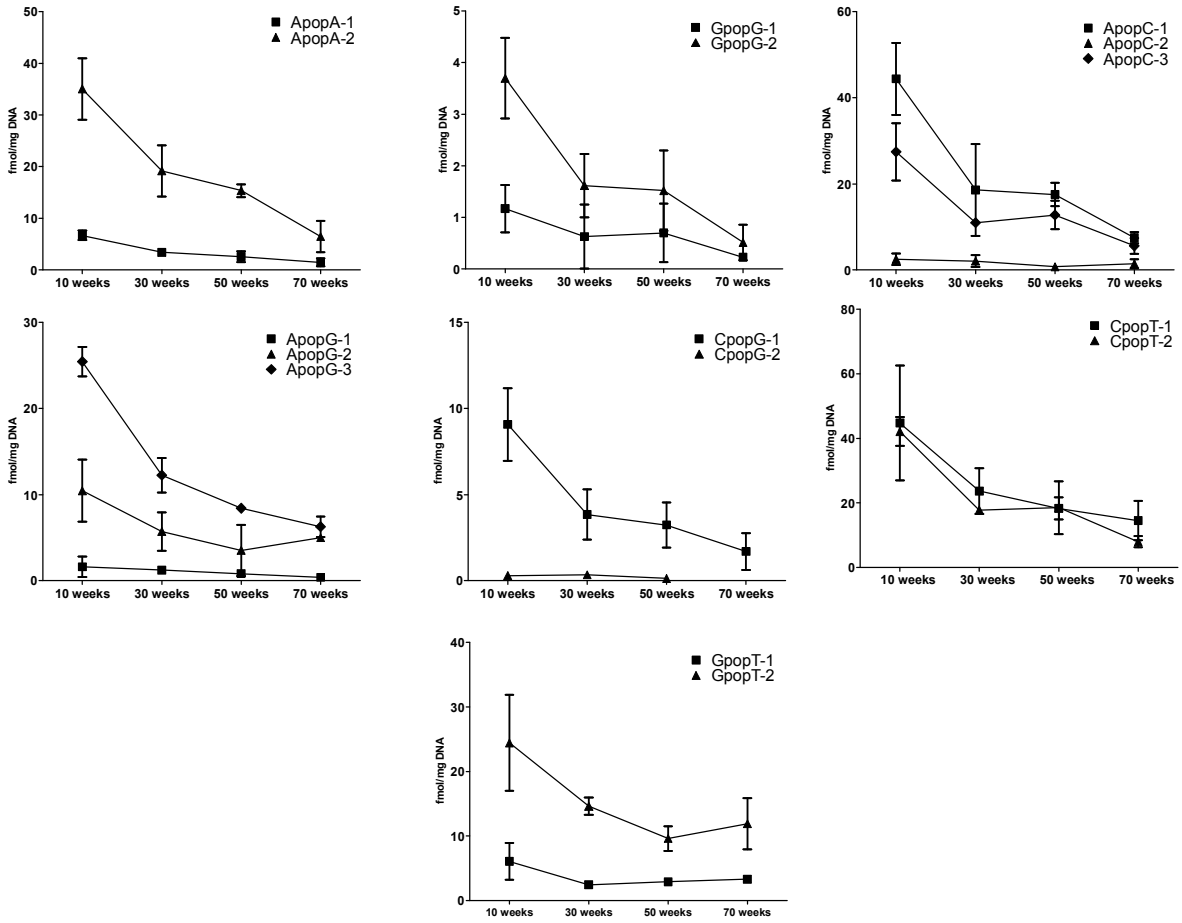


Figure S6. Levels of DNA phosphate adducts in liver (A) and lung (B) DNA from NNK-treated rats in the chronic exposure group (5 ppm in drinking water for 10, 30, 50 and 70 weeks)



A

Figure S6. (Continued)



B

Table S2. Levels of three major POB adducts and total DNA phosphate adducts in NNKOAc-treated CT-DNA, in liver DNA from NNK-treated rats in the acute exposure group (0.1 mmol/kg per day for 4 days), liver and lung DNA of NNK-treated rats in the chronic exposure group (5 ppm in drinking water for 10 weeks)

Group	POB adduct (fmol/mg DNA)			Total phosphate adduct (fmol/mg DNA)
	O^6-POB-dG	7-POB-G	O^2-POB-dT	
NNKOAc-treated DNA	808607	1178653	1080774	170420
NNK (0.1mmol/kg)-treated rat liver DNA	2048 ± 254	26222 ± 1174	19301 ± 2503	20018 ± 5626
NNK (5ppm)-treated rat liver DNA				
10 weeks	<LOQ	501 ± 53	1808 ± 840	190 ± 49
30 weeks	<LOQ	331 ± 36	2144 ± 26	134 ± 64
50 weeks	<LOQ	257 ± 32	1531 ± 161	120 ± 36
70 weeks	<LOQ	230 ± 90	910 ± 6	89 ± 4
NNK (5ppm)-treated rat lung DNA[a]				
10 weeks	34 ± 21	970 ± 148	3591 ± 414	475 ± 95
30 weeks	9 ± 9	751 ± 29	4809 ± 193	417 ± 43
50 weeks	9 ± 2	688 ± 65	4409 ± 320	346 ± 41
70 weeks	4 ± 2	315 ± 75	1983 ± 467	218 ± 15

[a] The data of POB adduct levels are from Balbo, S., et al. *Carcinogenesis* **2014**, *35*, 2798.