

Title:

A novel depurination methodology to assess DNA alkylation of chloro bis-seco-cyclopropylbenzoindoles (CBIs) allowed for comparison of minor groove reactivity

Authors:

Shuai Wang, Buyun Chen, Peter Dragovich, Thomas Pillow, Leanna Staben, Jun Guo, Dian Su, Chenghong Zhang, Sudheer Bobba, Yong Ma, Jianshuang Wang, Dewakar Sangaraju, Gail Lewis Phillips, Cyrus Khojasteh, Donglu Zhang

Affiliations:

Drug Metabolism and Pharmacokinetics (SW, BC, DS, CZ, SB, YM, JW, DS, CK, DZ), Discovery Chemistry (PD, TP, LS), Discovery Biology (JG, GP), Genentech, Inc., South San Francisco, CA 94080, USA

Supplemental tables and figures

Table S1. Mass spectral characterization of CPI/CBI compounds, the related degradants and DNA adducts. *A: adenine alkylation; S: spirocyclization; OH: hydroxylation; AH: amide hydrolysis.

Compound	Reaction	R.T. (min)	[M+H] ⁺ or [M+2H] ^{2+//2}	Fragment Ions	Formula
1	1	11.42	470.1468	237.0594, 234.0756, 199.0860, 187.0860, 128.9534	C ₂₄ H ₂₅ CIN ₃ O ₅ ⁺
	1a	S	9.54	434.1703	201.1016, 234.0753, 128.9533
	1b	A	7.88	569.2250	201.1019, 234.0756, 136.0619
	1c	AH	6.17	201.1019	186.0787, 173.1070
2	2	11.08	514.1381	234.0760, 247.0268, 279.0529, 128.9534	C ₂₅ H ₂₅ CIN ₃ O ₇ ⁺
	2a	S	9.61	478.1612	234.0759, 245.0919, 213.0658, 128.9534
	2b	A	7.91	613.2124	234.0753, 245.0912, 136.0614, 213.0649
	2c	AH	6.14	245.0918	213.0655, 231.0761
3	3	12.95	563.1508	330.0891, 302.0940, 128.9533, 234.0677	C ₃₁ H ₂₉ Cl ₂ N ₂ O ₄ ⁺
	3a	mono-S	11.44	527.1722	330.0891, 302.0941, 198.0913, 294.1124
	3b	di-S	9.64	491.1969	294.1124, 198.0914, 252.1019, 266.1176, 128.9533
	3c	mono-A	10.25	662.2277	294.1119, 198.0911, 491.1973, 330.0891, 128.9534, 136.0612
	3d	A and S	8.63	626.2510	294.1120, 198.0909, 128.9533, 491.1970, 429.1646, 136.0614
	3e	A and OH	8.6	644.2621	312.1229, 198.0912, 294.1119, 136.0614
	3f	di-A	8.22	761.3052 381.1563	294.1118, 198.0911, 491.1964, 429.1674, 626.2495, 128.9534 136.0616, 294.1119, 198.0910, 128.9533, 252.1013
	3g	AH	6.15	198.0913	139.1228, 181.0647
4	4	13.73	664.1738	198.0908, 234.0674, 170.0597	C ₃₈ H ₃₂ Cl ₂ N ₃ O ₄ ⁺
	4a/4b	mono-S	12.50, 12.62	628.1980	198.0908, 234.0677, 170.0596, 128.9531
	4c	di-S	11.2	592.2218	198.0908, 128.9533, 181.0648
	4d/4e	mono-A	11.11	763.2532	198.0909, 136.0614, 128.9531, 395.1370
	4f/4g	A and S	9.77	727.2761	198.0909, 128.9533, 136.0618, 395.1375
	4h	di-A	8.92	431.6687	136.0641, 198.0908, 268.8929?
5	5	11.87	735.2139	198.0913, 395.1389, 431.1148, 128.9534	C ₄₁ H ₃₇ Cl ₂ N ₄ O ₅ ⁺
	5a/5b	mono-S	10.46, 10.63	699.2369	198.0911, 395.1383, 431.1149, 128.9536
	5c	di-S	9.09	663.2596	198.0912, 395.1394, 128.9535
	5d/5e	mono-A	9.69	834.2897	198.0910, 395.1383, 136.0616, 431.1146, 663.2580
	5f/5g	A and S	8.49	798.3143	198.0910, 395.1385, 128.9531, 136.0615
	5h/5i	A and OH	8.28	816.3246	413.1490, 198.0911, 395.1380, 136.0619
	5j	di-A	8.03	467.1884	136.0618, 198.0912, 395.1389, 663.2612

Figure S1. DNA adducts were captured in the aqueous portion, but not ethyl acetate (EA) extract portion of a CBI DNA incubation in acidic LC condition without prior depurination process.

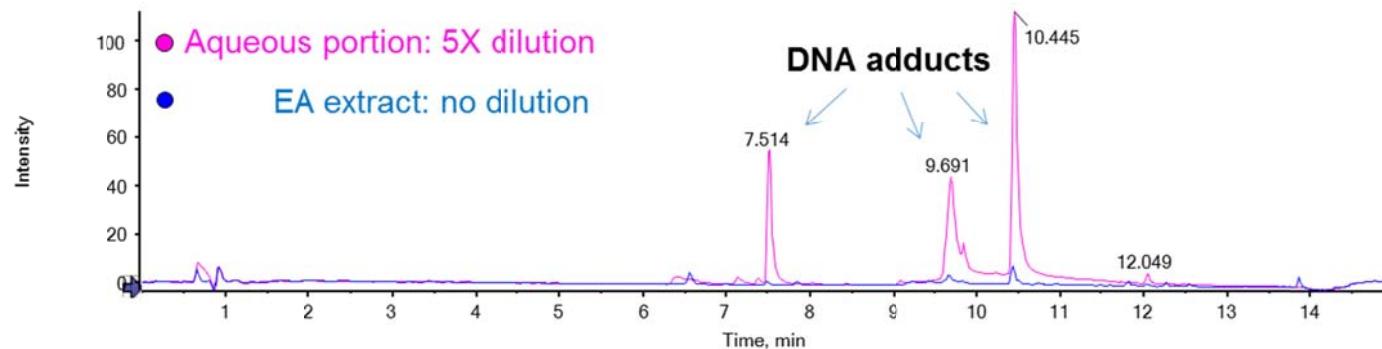


Figure S2. Comparison of acidic LC and basic LC on the analysis of CBI DNA incubation sample without depurination process.

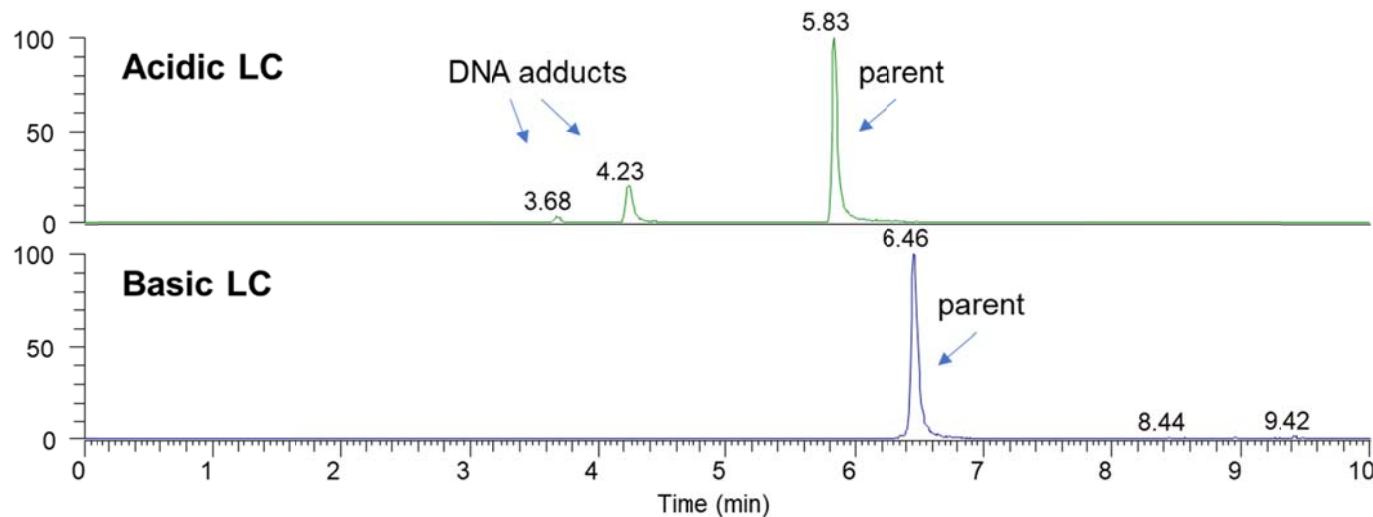
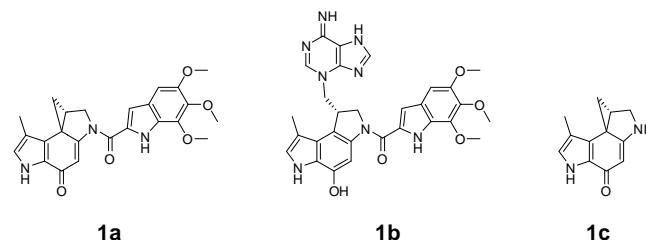
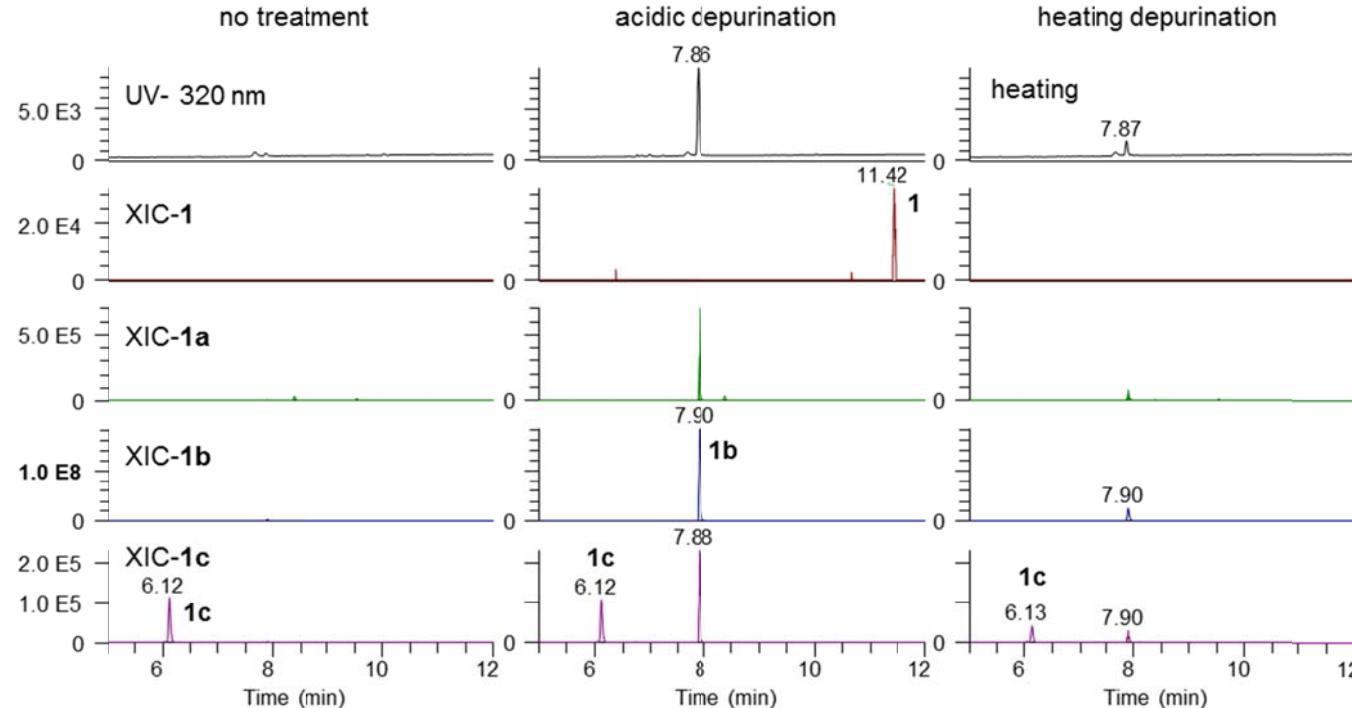
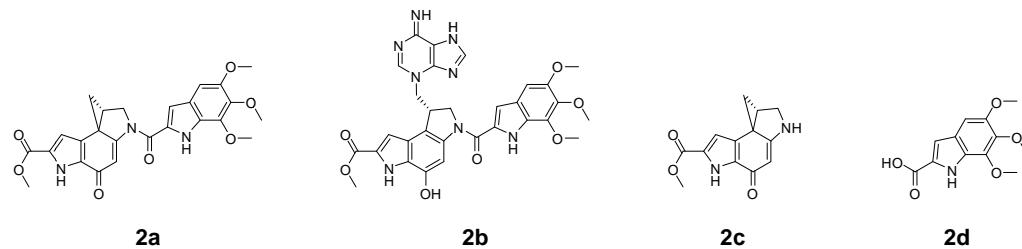
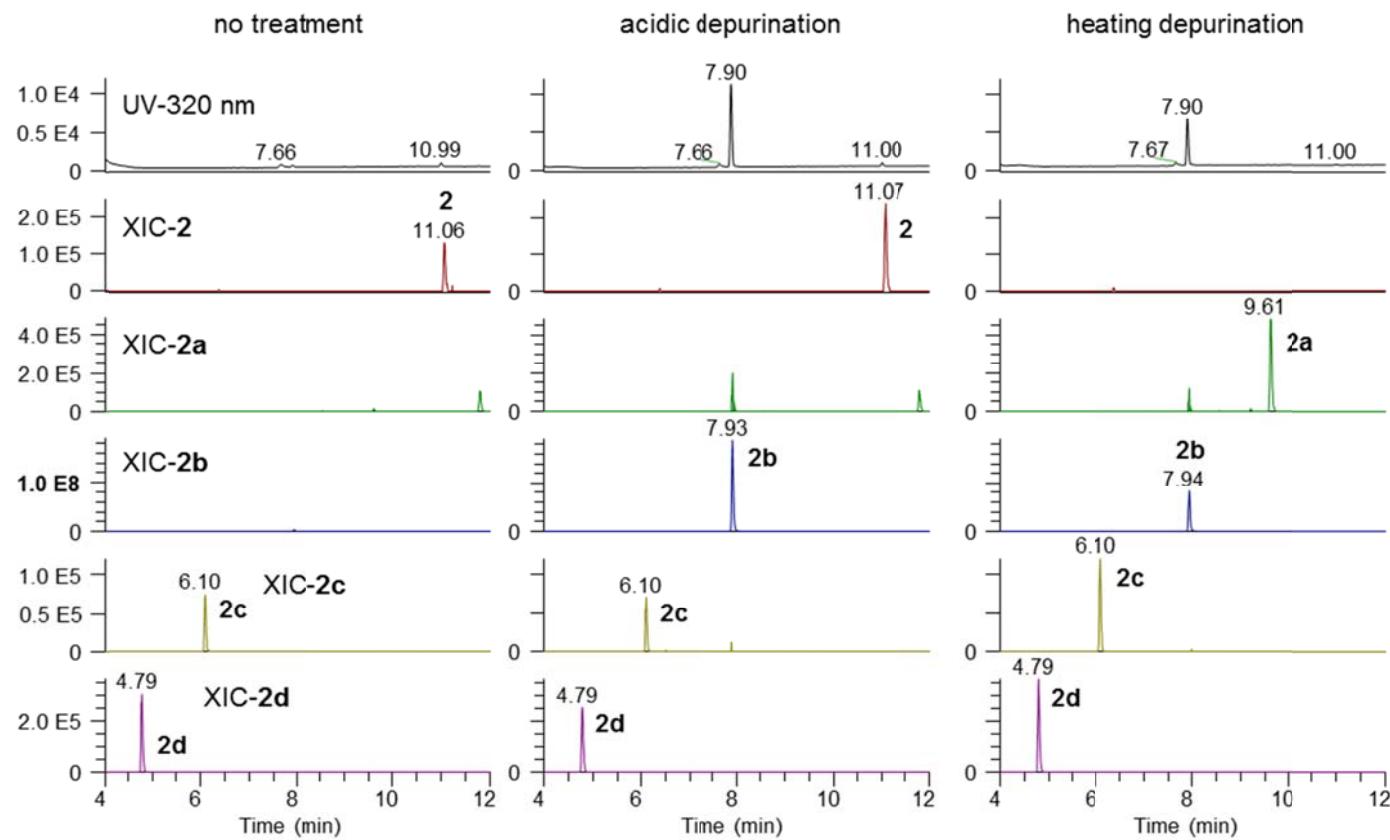


Figure S3. DNA adducts identified from compound and DNA incubation after acidic or heating depurination. CBI/CPI compounds (10 μ M) were incubated with calf thymus DNA (0.5 mg/ml) in 10 mM Tris-HCl buffer (pH 7.4) at 37 °C for 24 hr. After the incubation, samples were processed under three different procedures as follows: diluted 4 times with water; diluted 4 times with 0.1 % formic acid in water; diluted 4 times with water and heated at 90 °C for 10 min. The processed samples were injected to LC-UV-MS/MS for further analysis.

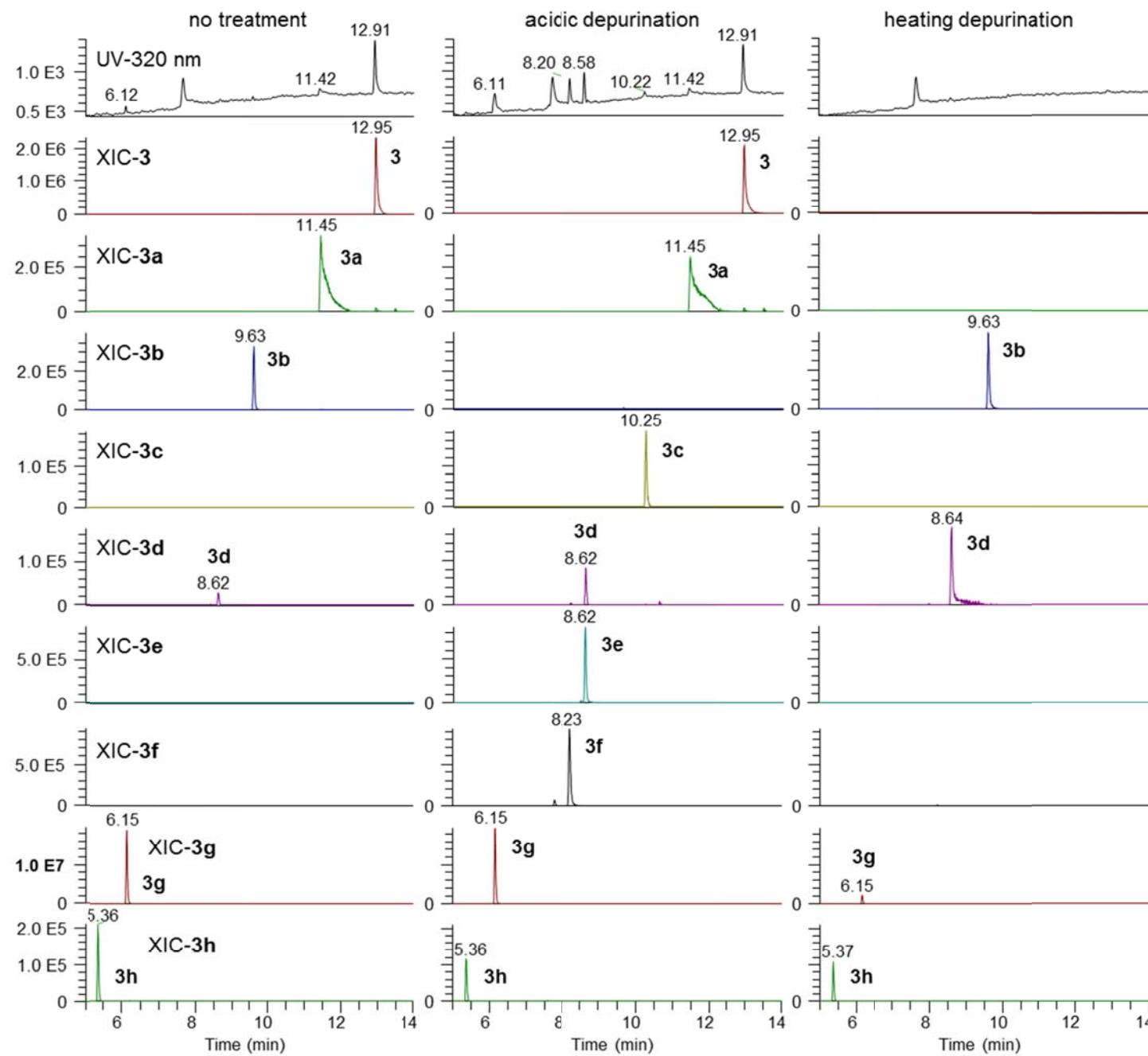
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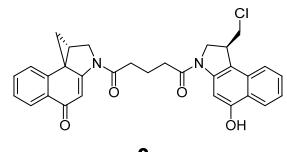


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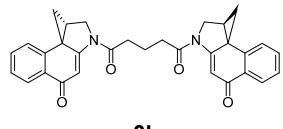


c)

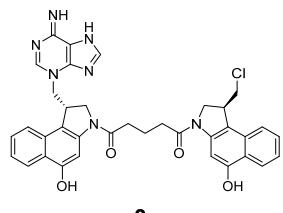




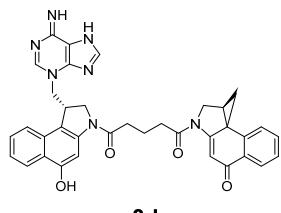
3a



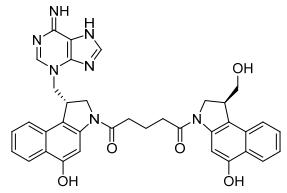
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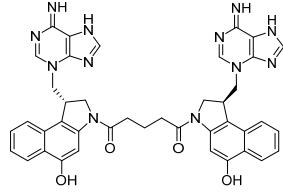
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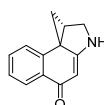
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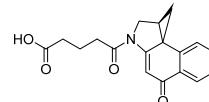
3e



3f

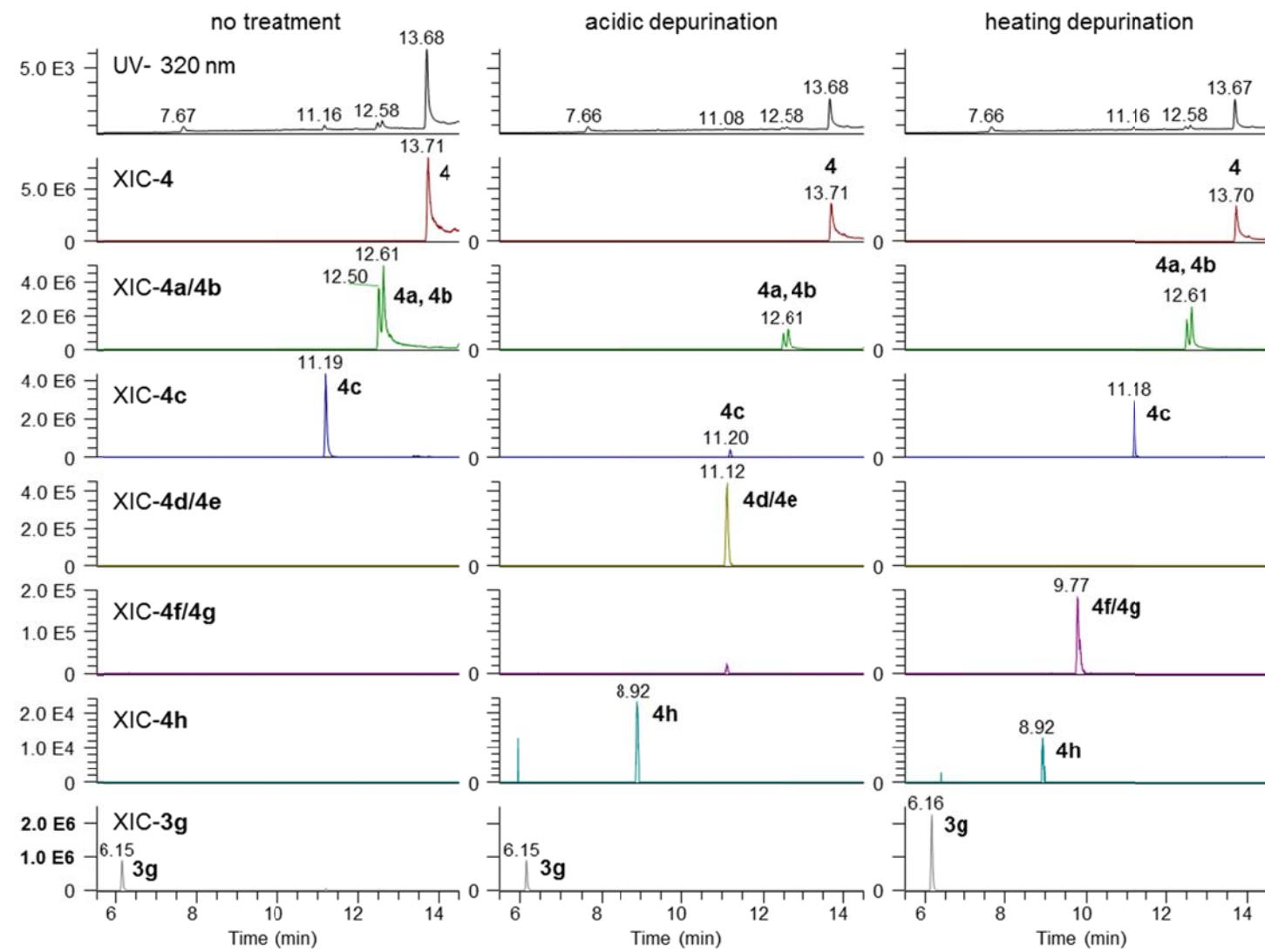


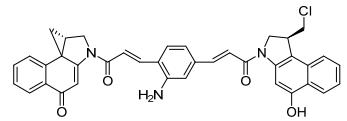
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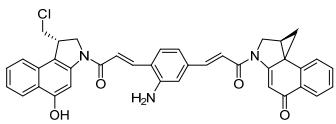
3h

d)

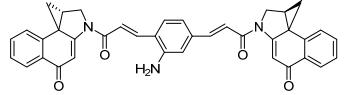




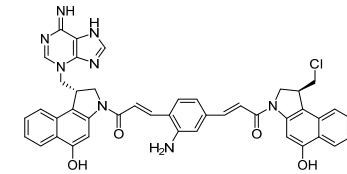
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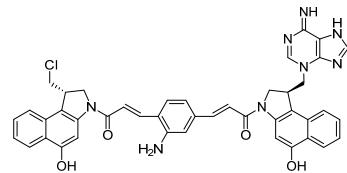
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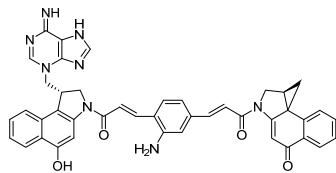
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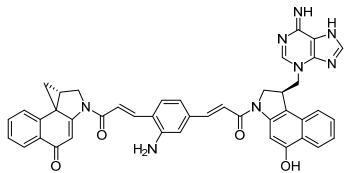
4d



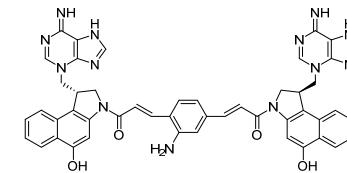
4e



4f

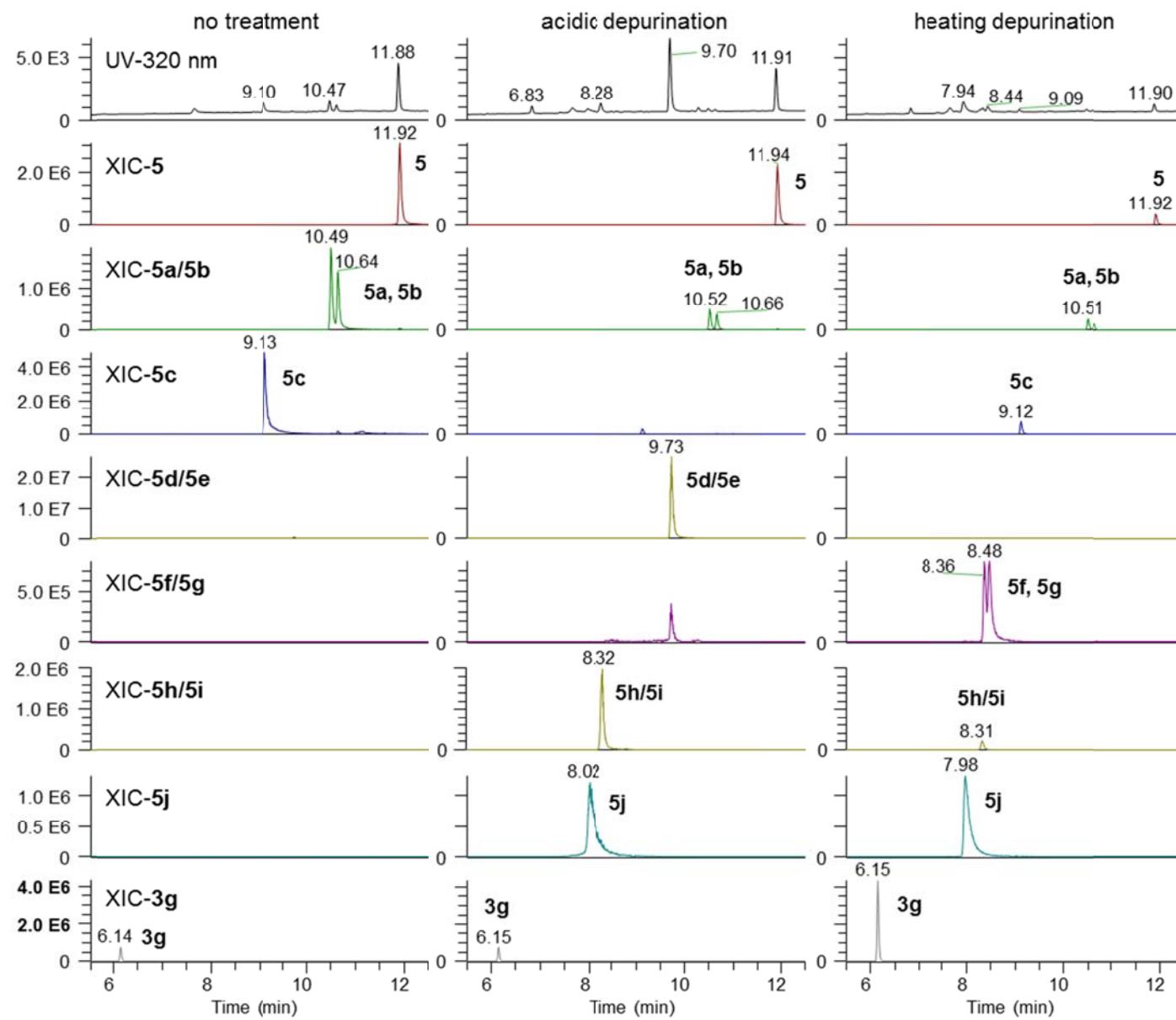


4g



4h

e)



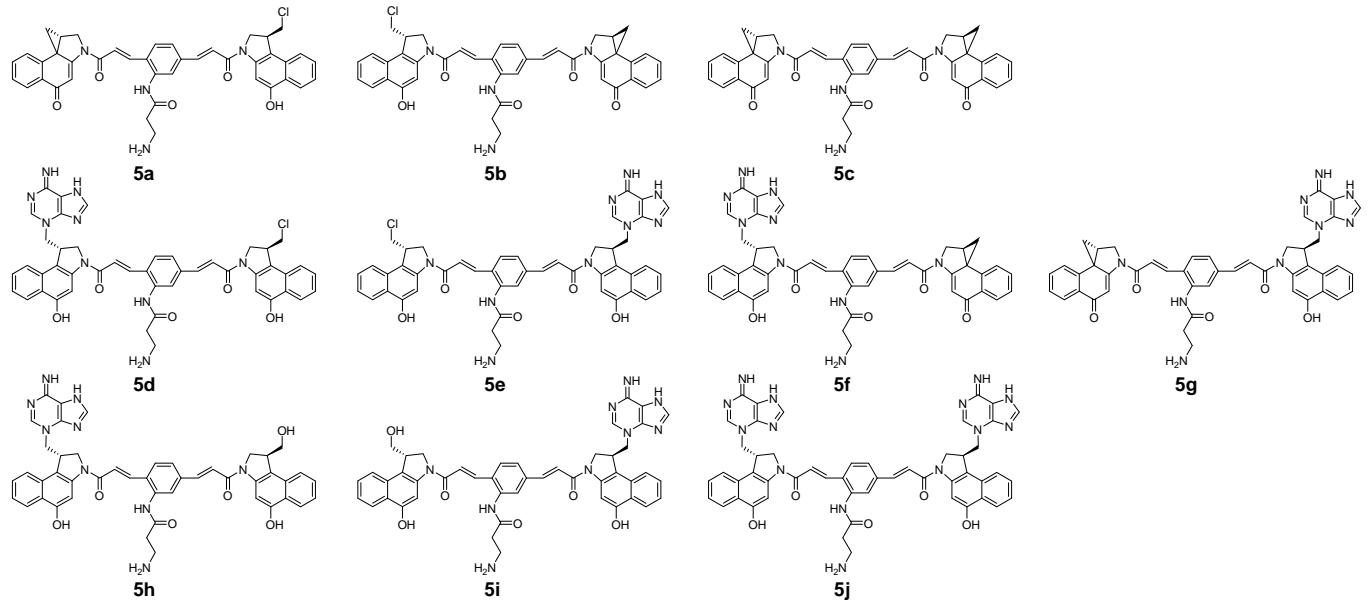


Figure S4. Time and temperature dependent degradation of compound **5** in Tris-HCl buffer (pH 7.4).

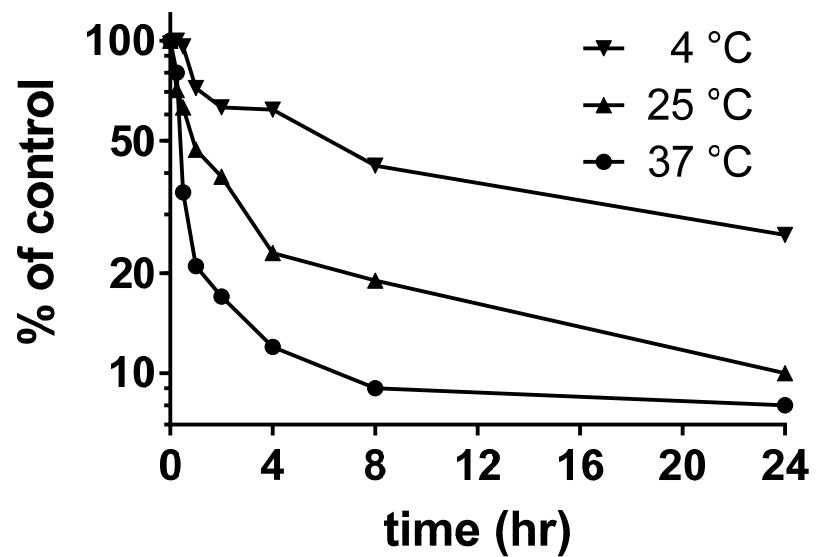


Figure S5. Chloro mono-adenine adduct **5d/5e** and di-adenine adduct **5j** changes from neutral thermal hydrolysis depurination at different temperatures.

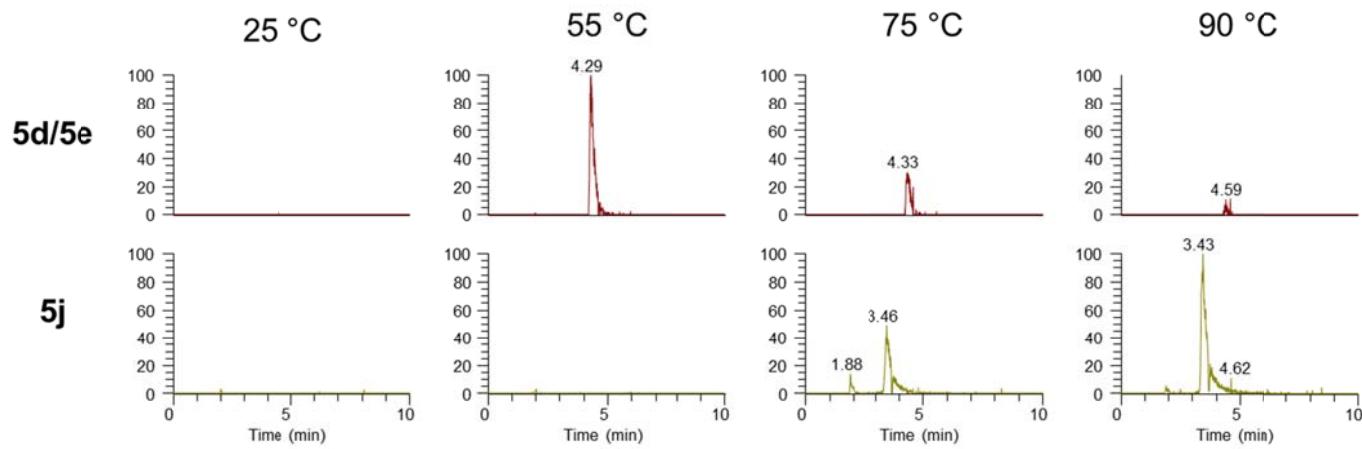


Figure S6. Time-dependent release of chloro mono-adenine adduct **5d/5e** from formic acidic depurination of compound **5** and DNA incubation sample.

