Supporting Information: Hydrogen Bonding Contributes to the Selectivity of Nucleotide Incorporation Opposite an Oxidized Abasic Lesion

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Contents:

M13 Genome Construction and Replication in E. coli. The synthetic DNA insert was cloned into the M13mp7L2 vector in triplicate as previously described.[1,2] Briefly, the insert (15 pmol) was phosphorylated (12 U PNK, 37 °C, 1 h) and ligated (1200 U, 16 °C, 2 h) into 10 pmol of EcoRI-digested plasmid using complementary scaffold (15 pmol). After digestion of the scaffolds with T4 DNA polymerase (16 U, 16 °C, 1 h), the vectors were purified by phenol extraction and G-25 Sephadex filtration. The preparation of plasmid containing the ketone analogue (K) was slightly different. The oligonucleotide containing the ketone precursor[3] was phosphorylated, phenol extracted and precipitated in 70% ethanol. The pellet was resuspended in NaOAc buffer (pH 6.0, 100 mM) and treated with $NaIO_4$ (10 mM, 1 h) before being precipitated in 70% ethanol. The ligation and T4 DNA polymerase digestion were carried out in 1 × buffer 1 (20 mM bis-trispropane, 10 mM $MgCl_2$, pH 7.0) in place of the corresponding commercially-supplied reaction buffer. This is important to prevent adventitious cleavage of the oligonucleotide.

Wild-type (AB1157), polymerase II (STL1336), polymerase IV (Xs-1), polymerase V (SR1157U) and triple knockout cells (SF2108) were grown to an OD_{600} of 0.3, pelleted, and resuspended in 10 mM $MgSO_4$. The cells were irradiated at 45 J/m^2, added to 25 mL 2 × YT, and incubated at 37 °C for 45 min. The cells were pelleted, washed with cold water, and resuspended in 10% glycerol. The prepared cells (100 μL) were electroporated with 1 pmol of the vector (2.5 kV, 4.74 ms), and plated with X-Gal and IPTG.

REAP Assay to Determine Mutation Frequency. Mutation analysis was carried out using the restriction endonuclease and postlabeling (REAP) assay, which has previously been described.[2] Briefly, viral DNA was recovered from the growth medium and PCR amplified.

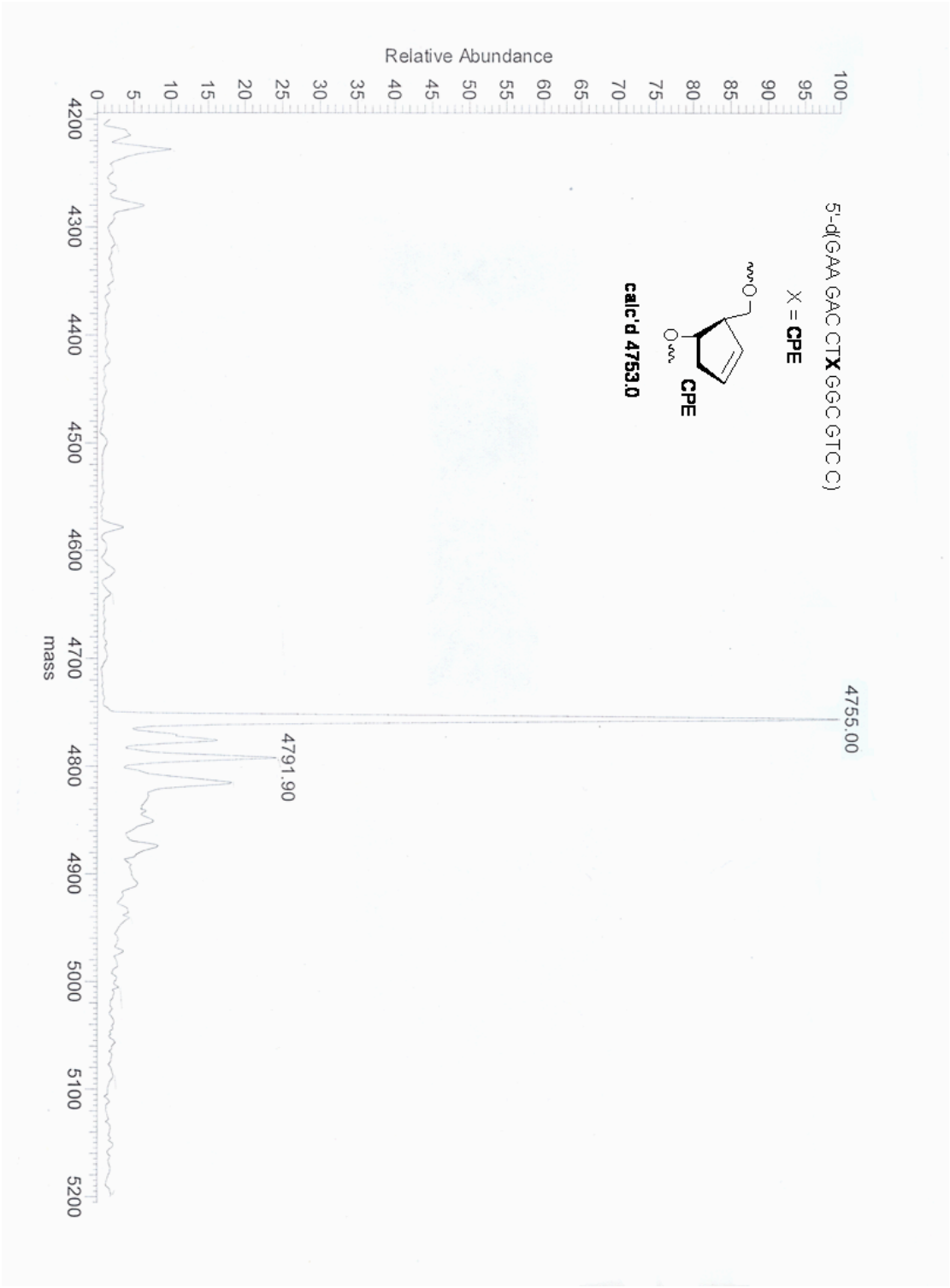
Following digestion with *Bbs*I and shrimp alkaline phosphatase, the DNA was ^{32}P-labeled and further digested with *Hae*III. The desired 18mer product was purified using 20% denaturing PAGE and desalted using a G25- Sephadex column. Finally, the samples were digested with nuclease P1 and nucleotides separated on a PEI cellulose TLC plate which was run with saturated $(NH_4)_2HPO_4$ and H_3PO_4, pH 5.8.

Molecular Modeling. The structure of MeG:L was optimized at the PM3 level in the gas phase. The distances between the N-methyl carbon of MeG, O3 and O5 of L were constrained using the corresponding distances between C1' of ddADP, O3' and O5' of T in the template strand in the Dpo4-DNA crystal structure (PDB: IJX4). The dihedral angle between N9, and the N-methyl carbon of MeG, O3 and O5 of L were also constrained using the dihedral angle in the crystal structure to prevent MeG from rotating into a conformation that was equivalent to the α-anomer of ddGDP. Failure to constrain the dihedral angle resulted in an optimized structure corresponding to the α-anomer of ddGDP. The optimized structure of MeG:L was then superimposed onto the Dpo4 crystal structure to replace ddADP:T.

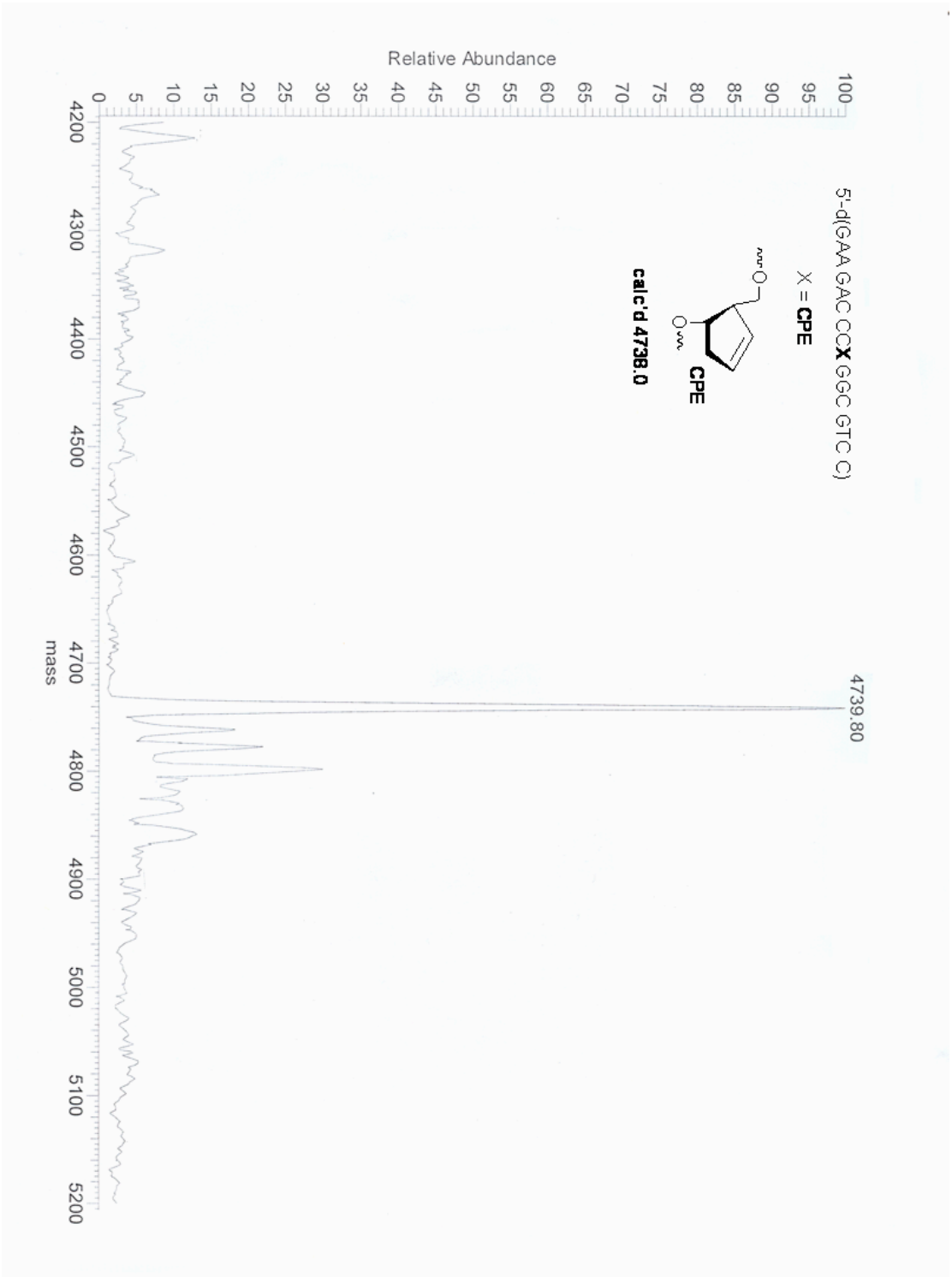
(1) In vivo Bypass Efficiencies and Mutational Signatures of the Guanine Oxidation Products 2-Aminoimidazolone and 5-Guanidino-4-nitroimidazole. Neeley, W. L.; Delaney, J. C.; Henderson, P. T.; Essigmann, J. M. *J. Biol.Chem.* **2004**, *279*, 43568-43573.

(2) Mutagenic Effects of 2-Deoxyribonolactone in Escherichia coli. An Abasic Lesion That Disobeys the A-Rule. Kroeger, K. M.; Jiang, Y. L.; Kow, Y. W.; Goodman, M. F.; Greenberg, M. M. *Biochemistry* **2004**, *43*, 6723-6733.

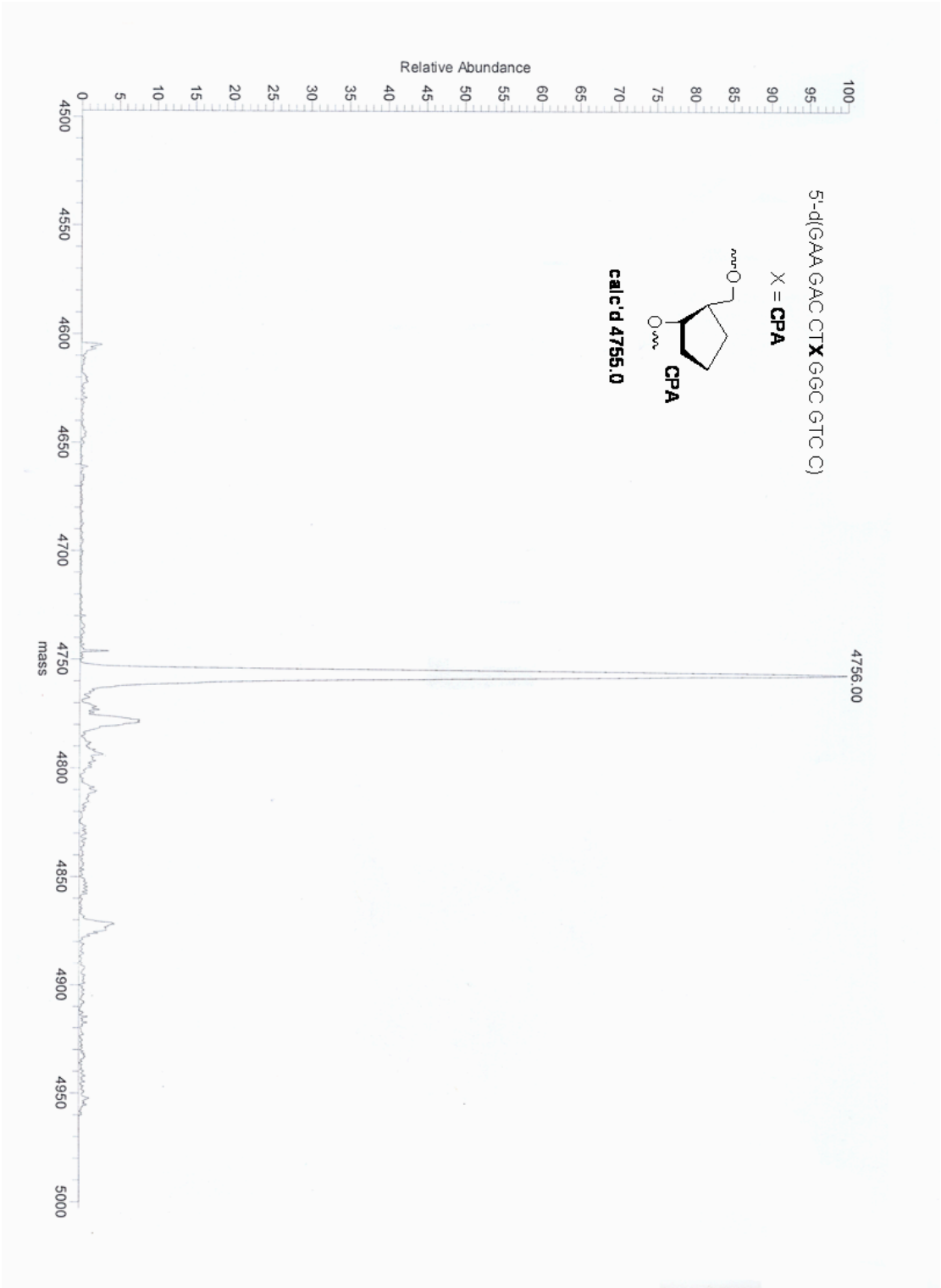
(3) Synthesis and Analysis of Oligonucleotides Containing Abasic Site Analogues. Huang, H.; Greenberg, M. M. *J. Org. Chem.* **In press**.



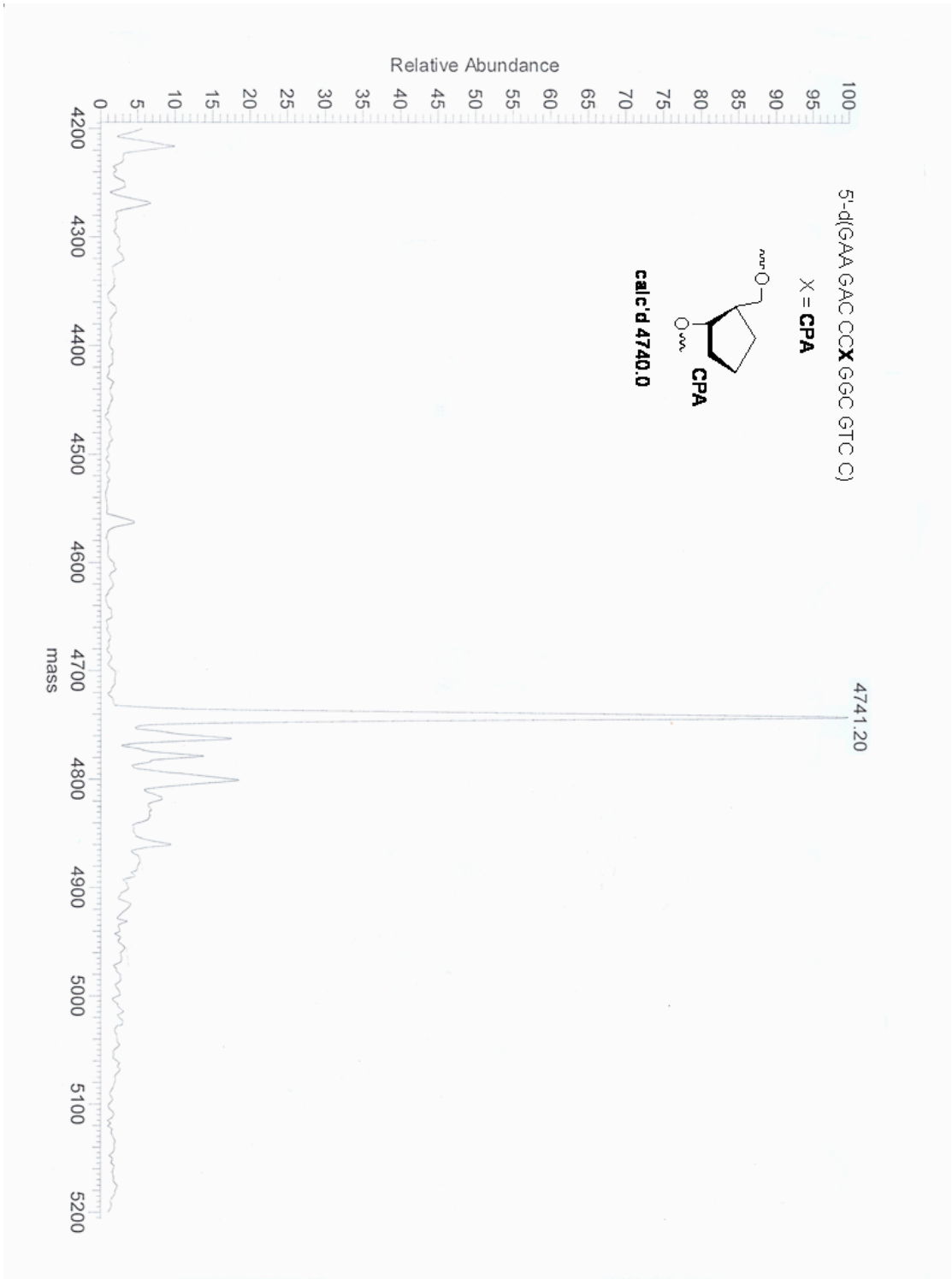
Supporting Information Figure 1. ESI-MS of **CPE** (3' to T).



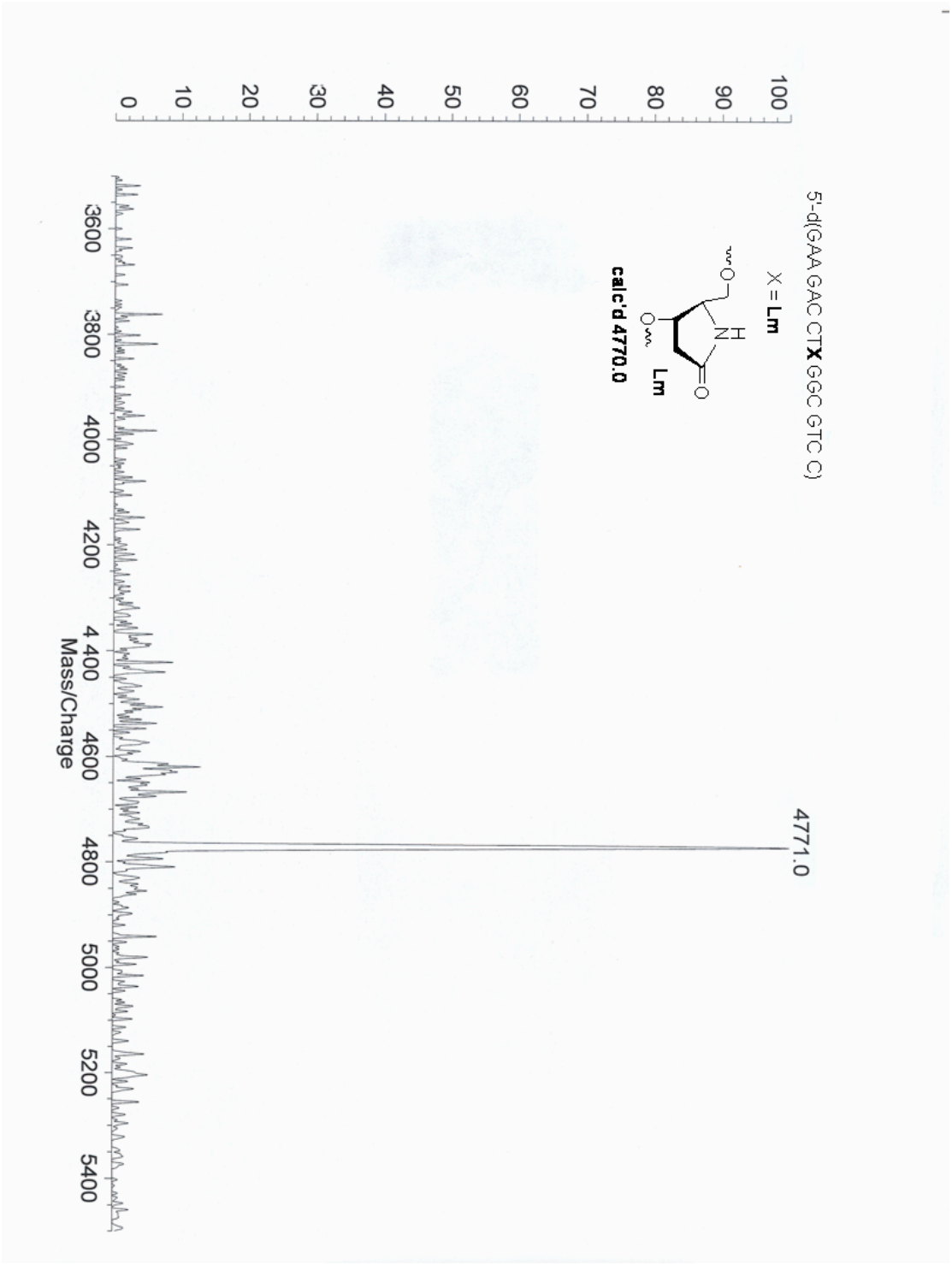
Supporting Information Figure 2. ESI-MS of **CPE** (3' to C).



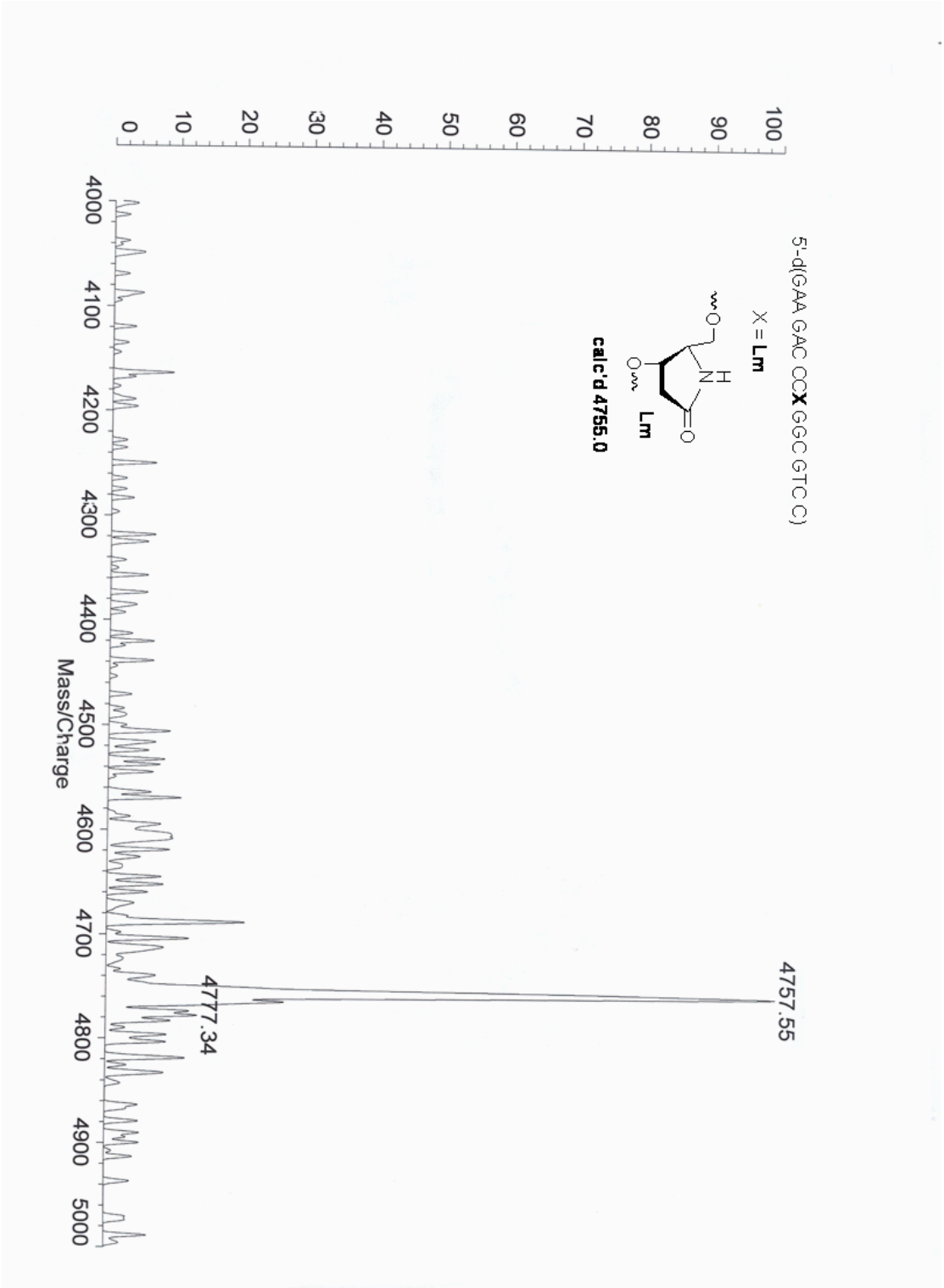
Supporting Information Figure 3. ESI-MS of **CPA** (3' to T).



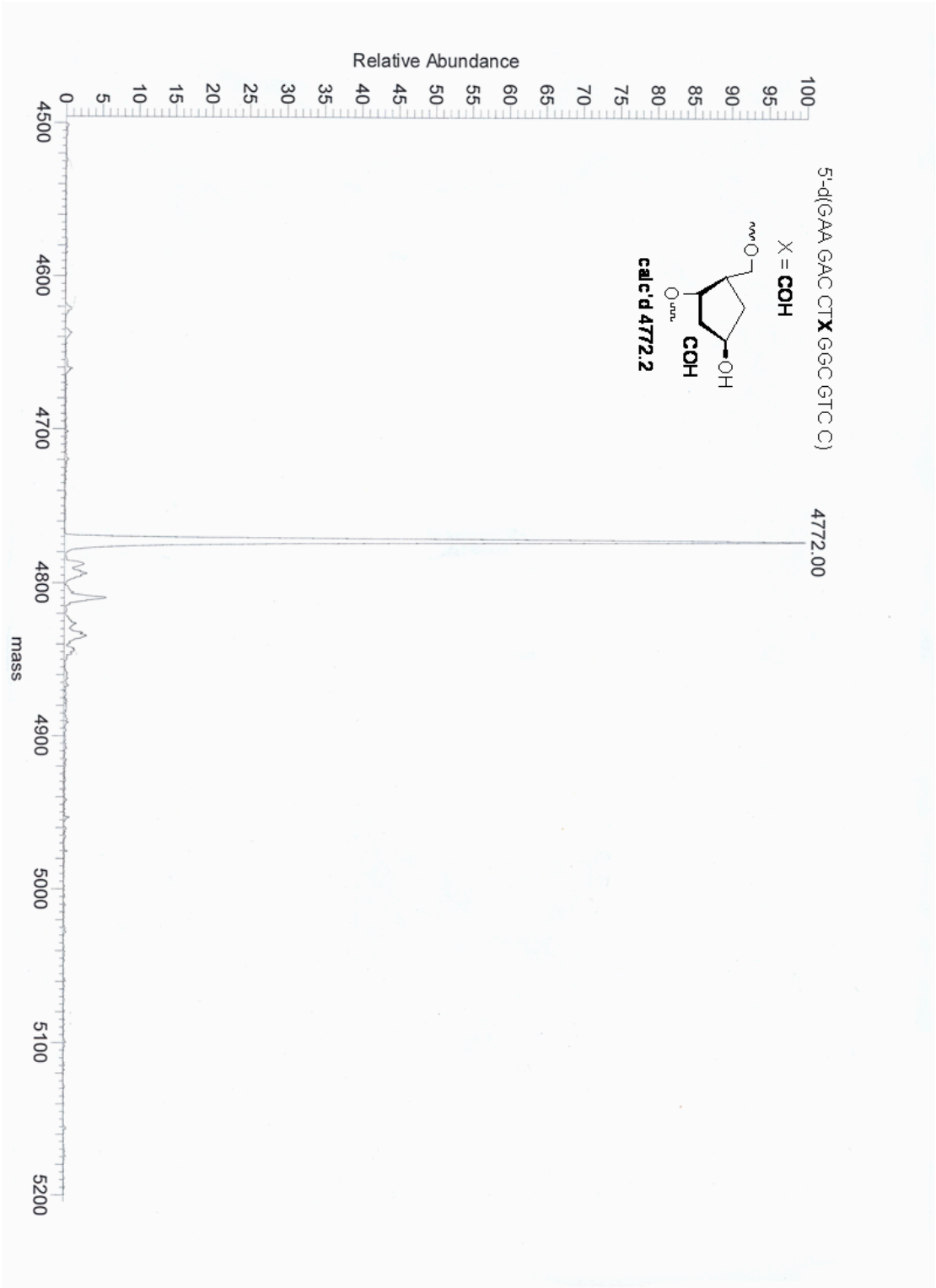
Supporting Information Figure 4. ESI-MS of **CPA** (3' to C).



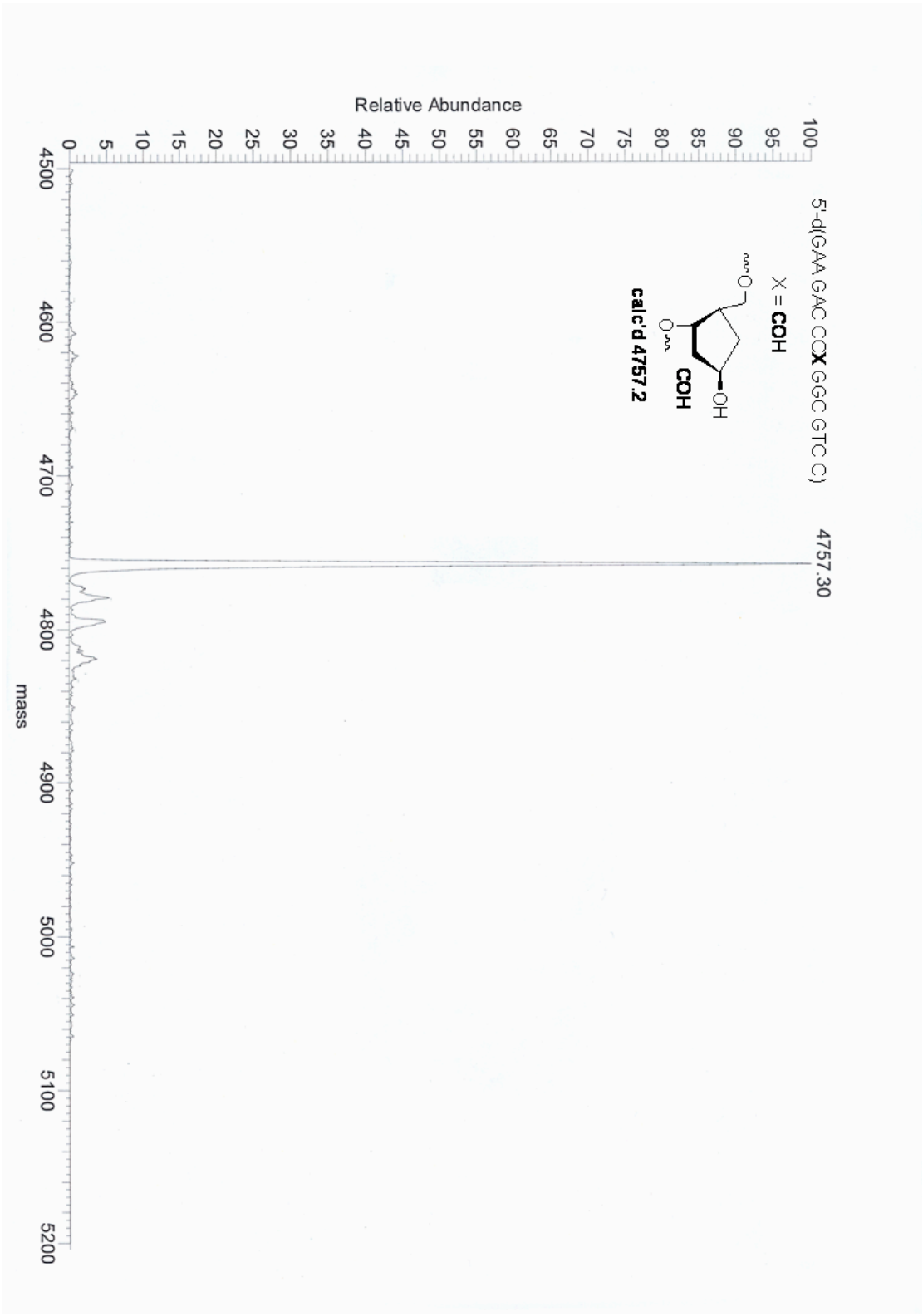
Supporting Information Figure 5. MALDI-TOF-MS of **Lm** (3' to T).



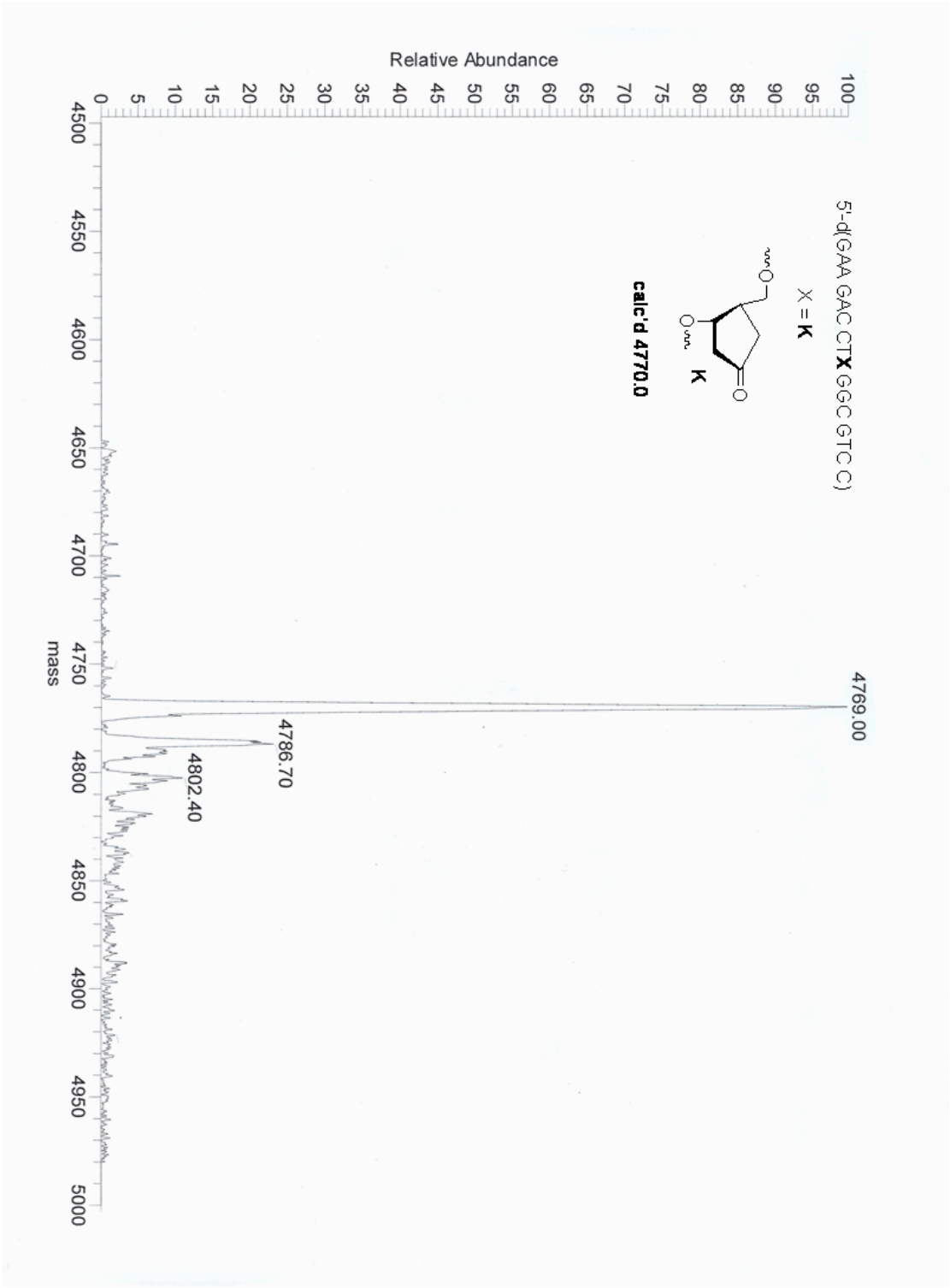
Supporting Information Figure 6. MALDI-TOF-MS of **Lm** (3' to C).



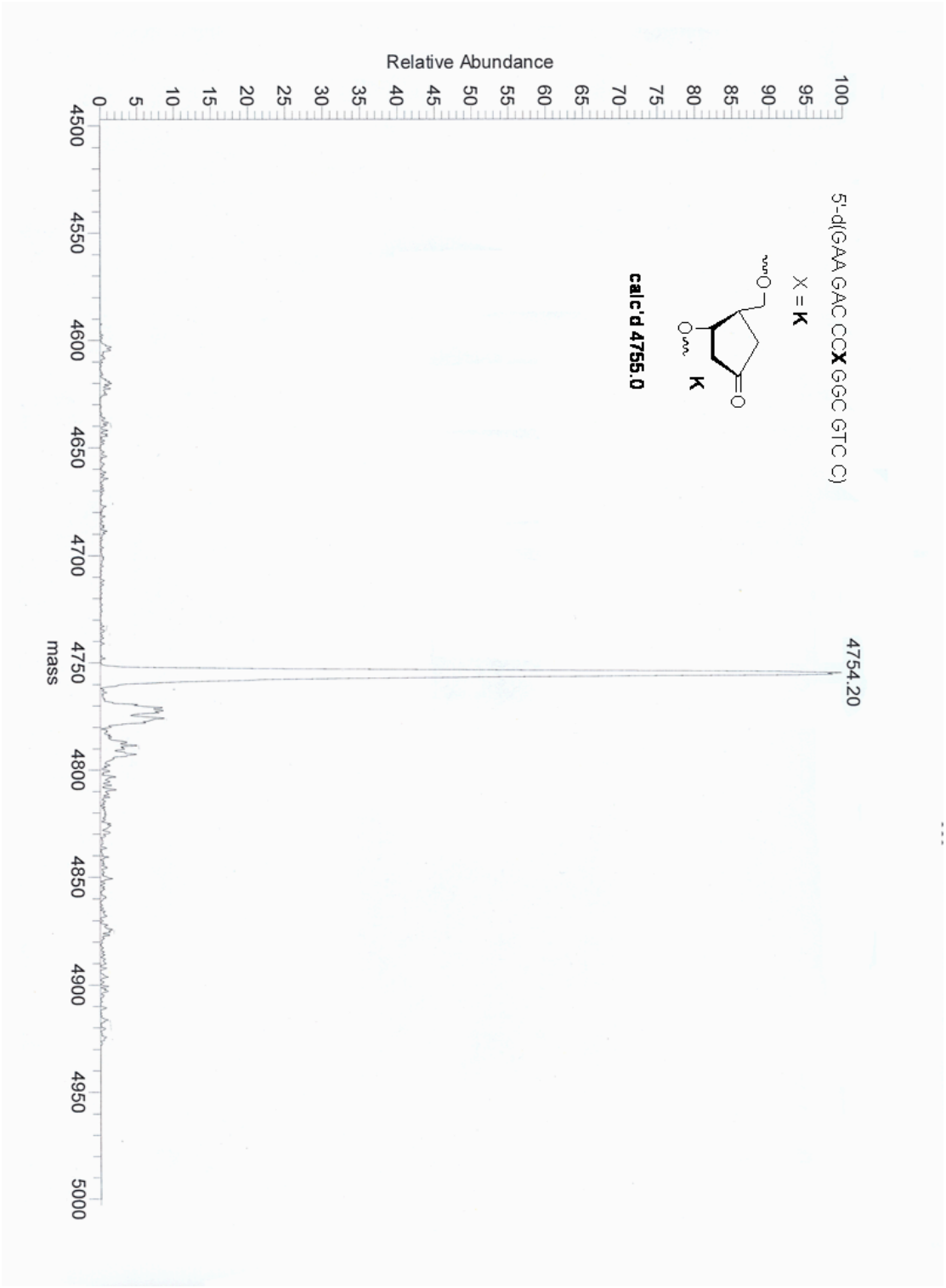
Supporting Information Figure 7. ESI-MS of **COH** (3' to T).



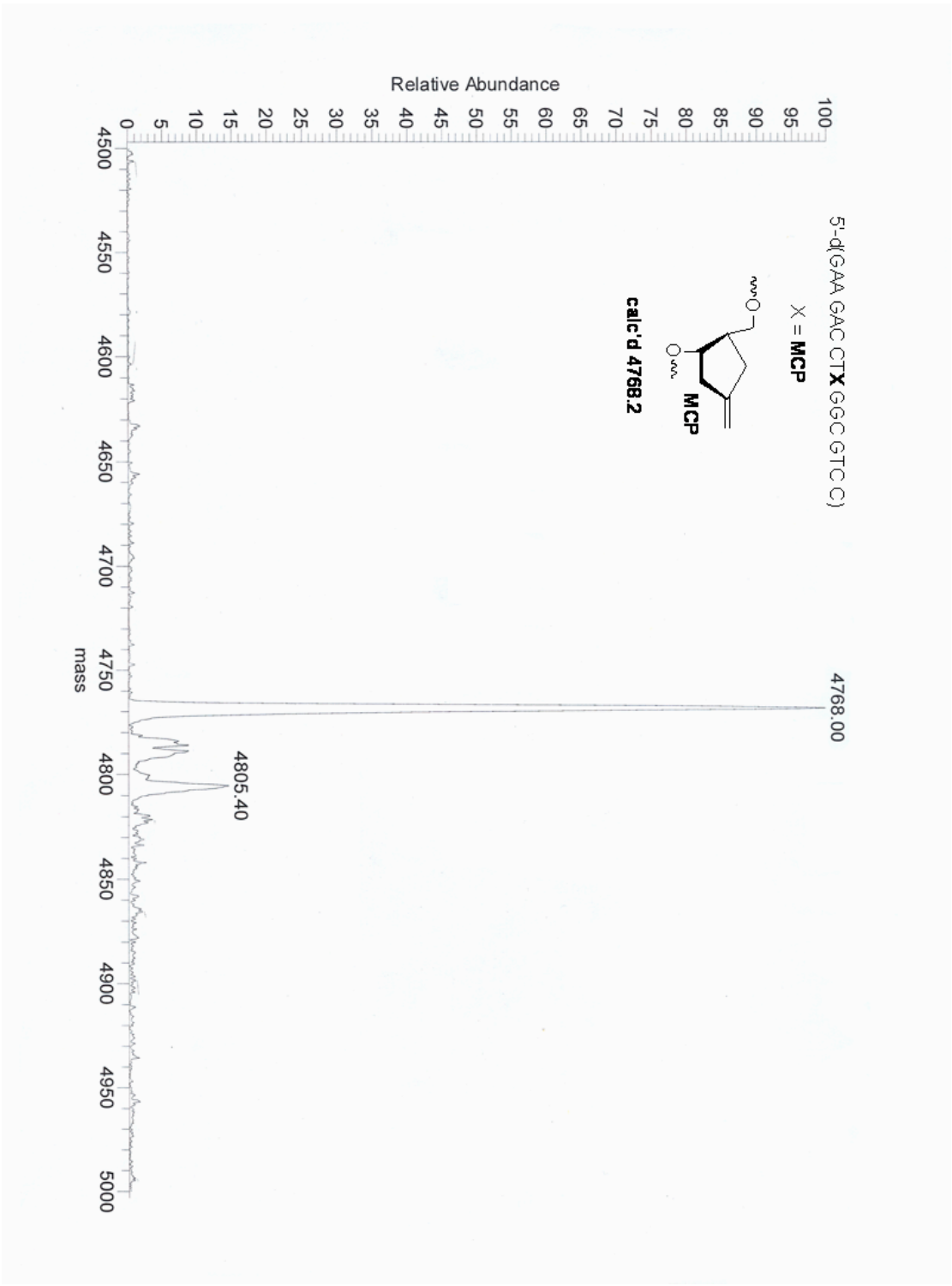
Supporting Information Figure 8. ESI-MS of **COH** (3' to C).



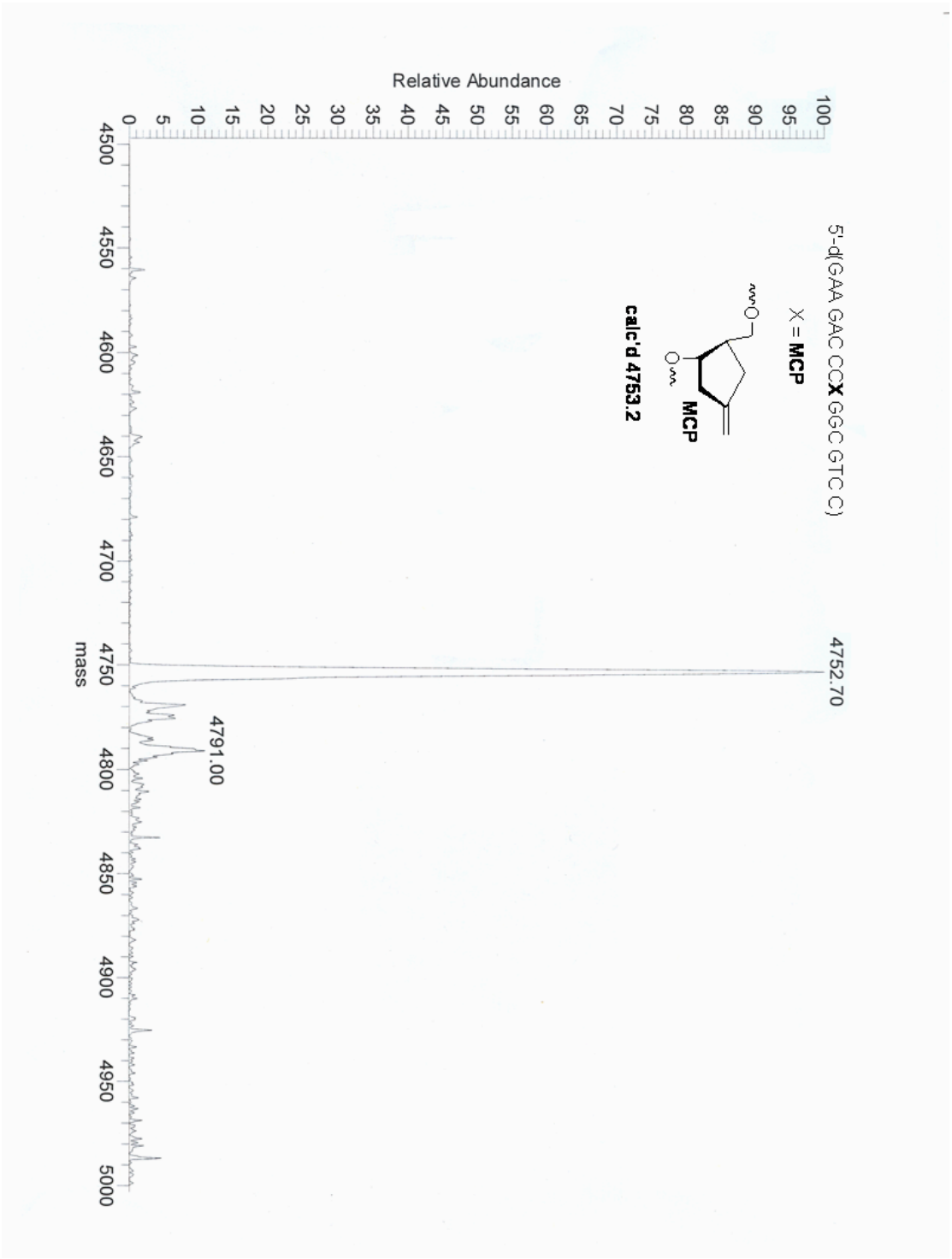
Supporting Information Figure 9. ESI-MS of **K** (3' to T).



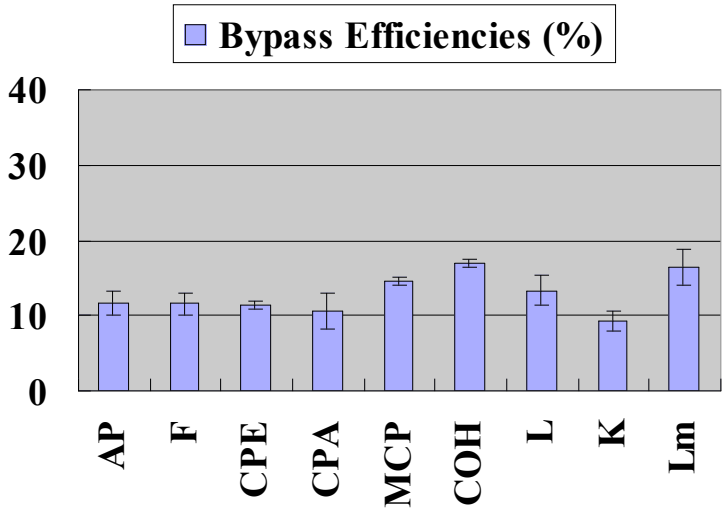
Supporting Information Figure 10. ESI-MS of **K** (3' to C).



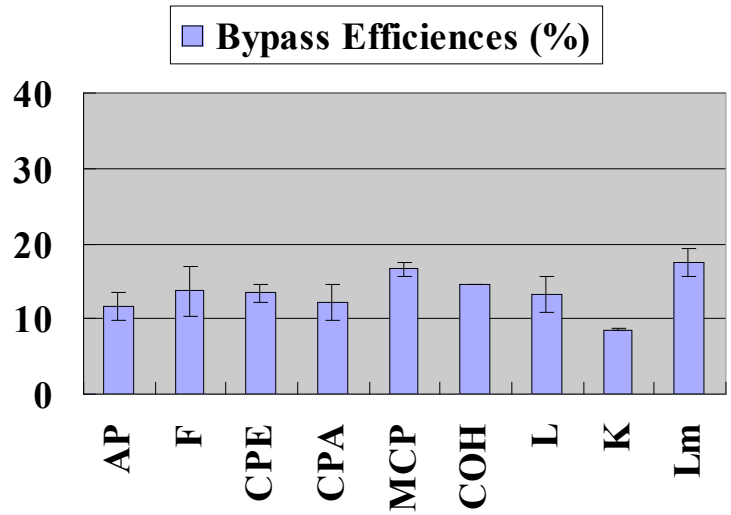
Supporting Information Figure 11. ESI-MS of **MCP** (3' to T).



Supporting Information Figure 12. ESI-MS of **MCP** (3' to C).



Supporting Information Figure 13. Bypass efficiency in wild type *E. coli.* local sequence: 5'-TXG-3'.



Supporting Information Figure 14. Bypass efficiency in wild type *E. coli.* local sequence: 5'-CXG-3'.

Supporting Information Table 1. Bypass efficiency in various polymerase-deficient *E. coli*.

Lesion and Sequence	Pol II$^-$	Pol IV$^-$	Pol V$^-$	Triple-knockout
TFG (%)	8.1 ± 0.1	6.4 ± 1.8	8.8 ± 0.2	0
CFG (%)	6.9 ± 0	4.9 ± 0.8	9.9 ± 1.4	0
TLG (%)	9.4 ± 0.1	7.9 ± 0.7	7.7 ± 0.3	0
CLG (%)	7.8 ± 1.0	6.1 ± 0.6	9.3 ± 1.0	0
TKG (%)	7.6 ± 2.8	4.1 ± 0.8	0.9 ± 0.1	0
CKG (%)	5.6 ± 1.2	3.3 ± 0.5	0.8 ± 0.2	0
TLmG (%)	13.9 ± 1.9	10.4 ± 1.4	9.9 ± 1.4	0
CLmG (%)	16.2 ± 3.1	9.2 ± 0	12.2 ± 0.6	0

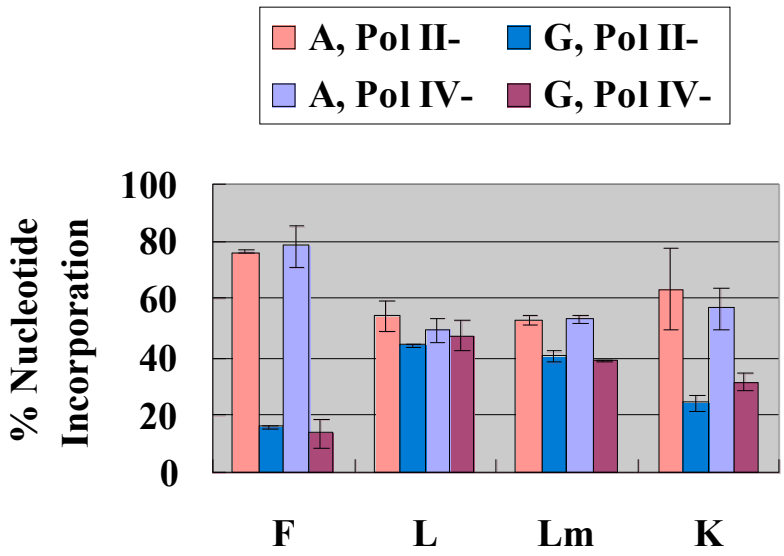
Supporting Information Table 2. Percent single-nucleotide-deletion product in wild type *E. coli*.

X	TXG	CXG
AP (%)	1.0 ± 1.0	9.3 ± 0.8
F (%)	22.6 ± 6.0	72.3 ± 9.6
CPE (%)	47.2 ± 12.3	50.8 ± 11.9
CPA (%)	72.2 ± 11.0	68.9 ± 11.1
COH (%)	3.3 ± 1.3	11.4 ± 6.4
MCP (%)	7.9 ± 3.1	12.9 ± 2.5
L (%)	11.2 ± 8.4	23.4 ± 11.9
K (%)	1.6 ± 1.5	6.1 ± 4.7
Lm (%)	7.0 ± 1.8	37.3 ± 9.3

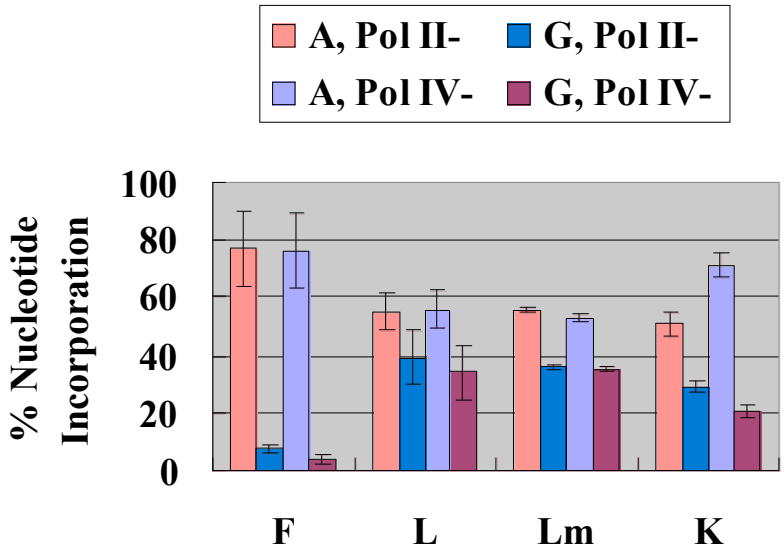
Supporting Information Table 3. Percent single-nucleotide-deletion product in various polymerase-deficient *E. coli*.

Lesion and Sequence	Pol II$^-$	Pol IV$^-$	Pol V$^-$
TFG (%)	19.9 ± 3.6	11.4 ± 2.0	100
CFG (%)	54.6 ± 4.8	80.0 ± 3.5	100
TLG (%)	14.7 ± 4.3	10.5 ± 0.3	100
CLG (%)	15.7 ± 2.7	15.6 ± 0.6	100
TKG (%)	0	0	- *
CKG (%)	3.2 ± 0.4	1.3 ± 1.3	- *
TLmG (%)	5.6 ± 2.0	4.2 ± 0.1	100
CLmG (%)	36.6 ± 10.6	32.7 ± 1.3	100

* Bypass efficiency < 1%



Supporting Information Figure 15. Percent nucleotide incorporation opposite the lesions in Pol II and Pol IV deficient *E. coli.* local sequence: 5'-TXG-3'.



Supporting Information Figure 16. Percent nucleotide incorporation opposite the lesions in Pol II and Pol IV deficient *E. coli.* local sequence: 5'-CXG-3'.