Supplementary Information

C5-Azobenzene-functionalized locked nucleic acid uridine: isomerization property, hybridization ability, and enzymatic stability

Kunihiko Morihiro,* Osamu Hasegawa, Shohei Mori, Shin-ichi Tsunoda and Satoshi Obika*

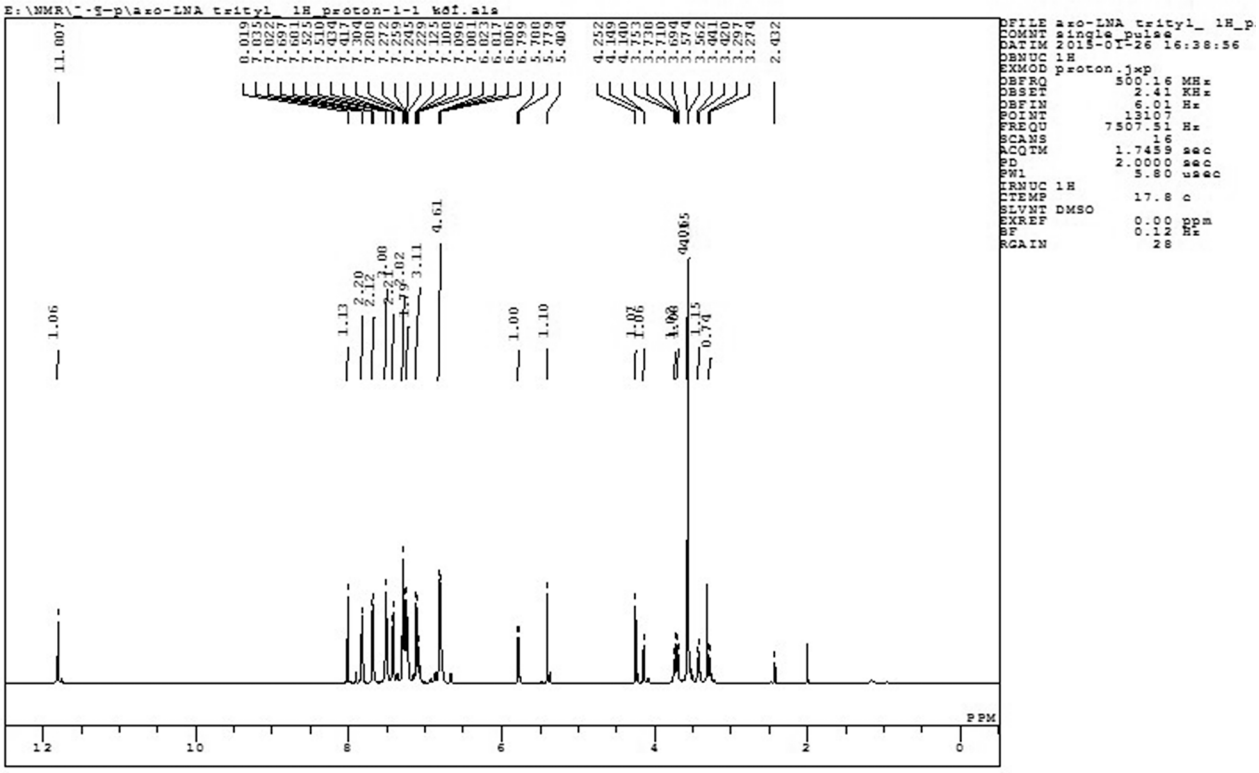
National Institute of Biomedical Innovation (NIBIO)

7-6-8 Saito-Asagi, Ibaraki, Osaka 567-0085, Japan

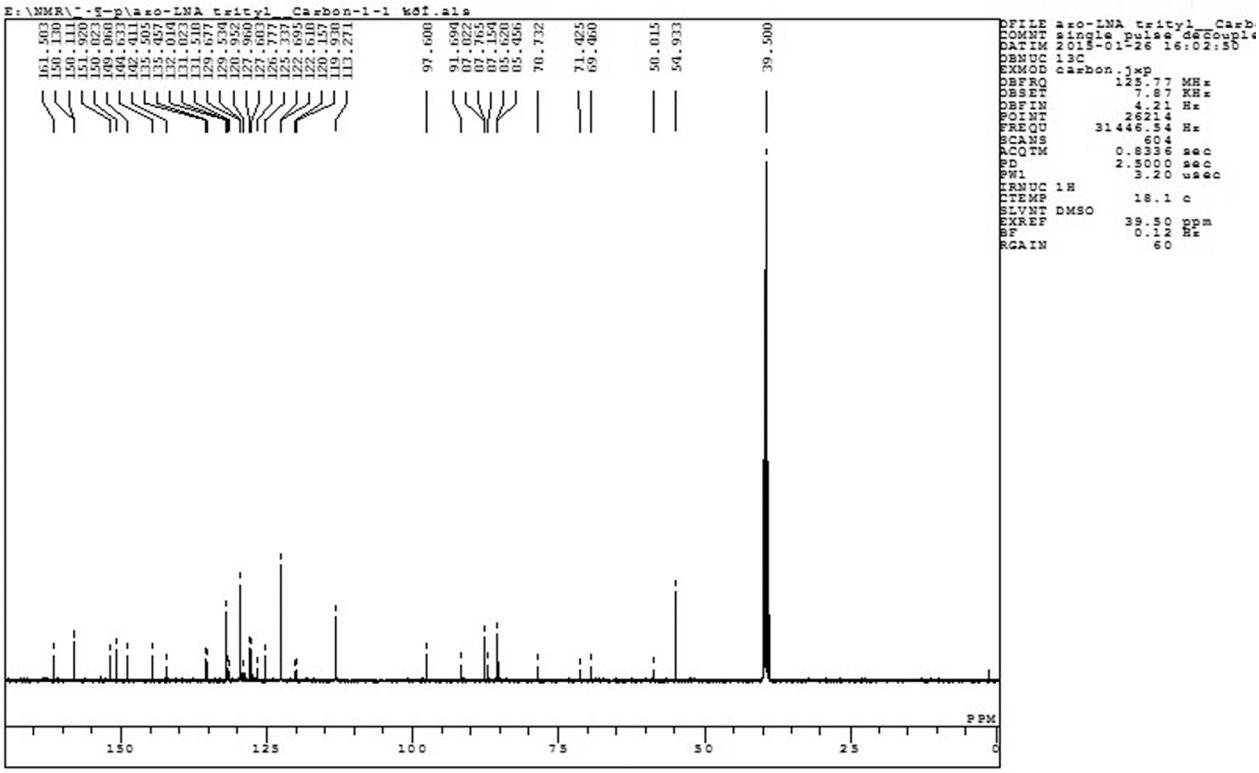
k-morihiro@nibio.go.jp; obika@phs.osaka-u.ac.jp

1. ^{1}H, ^{13}C and ^{31}P spectra of new compounds

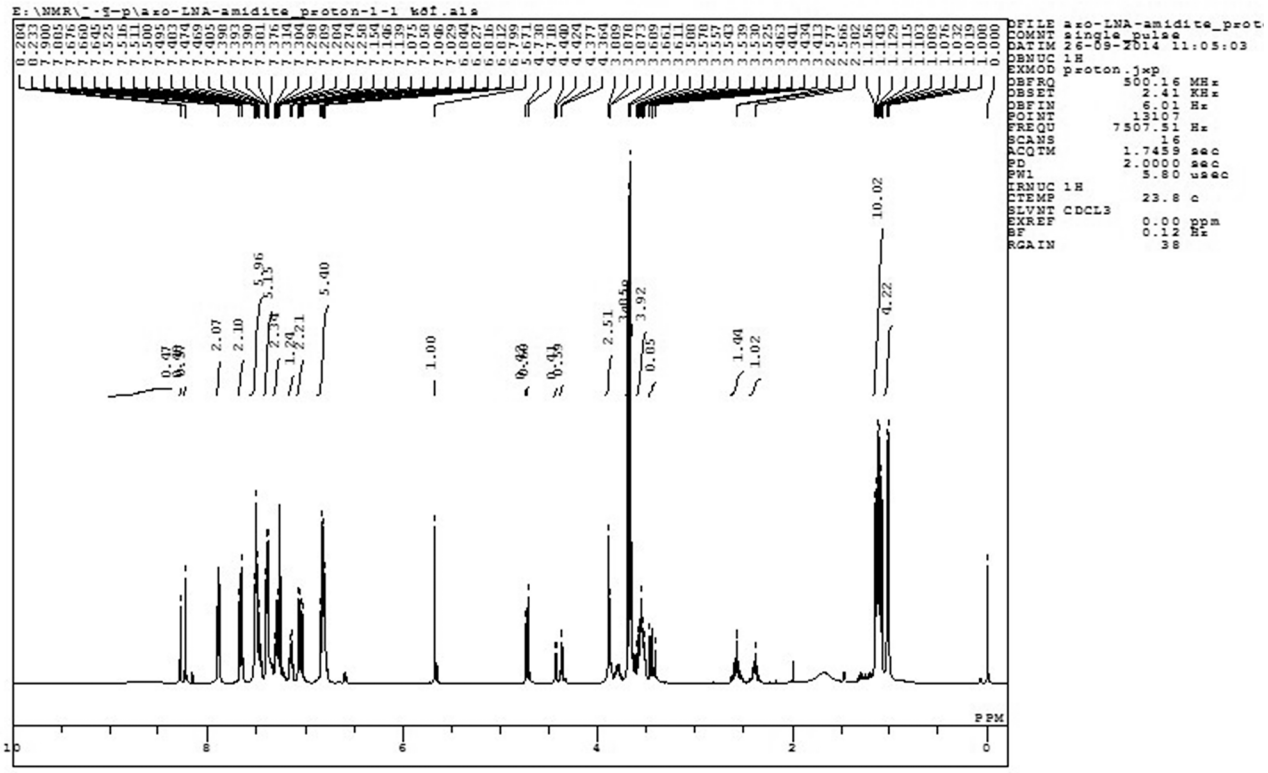
^{1}H spectrum of compound 2



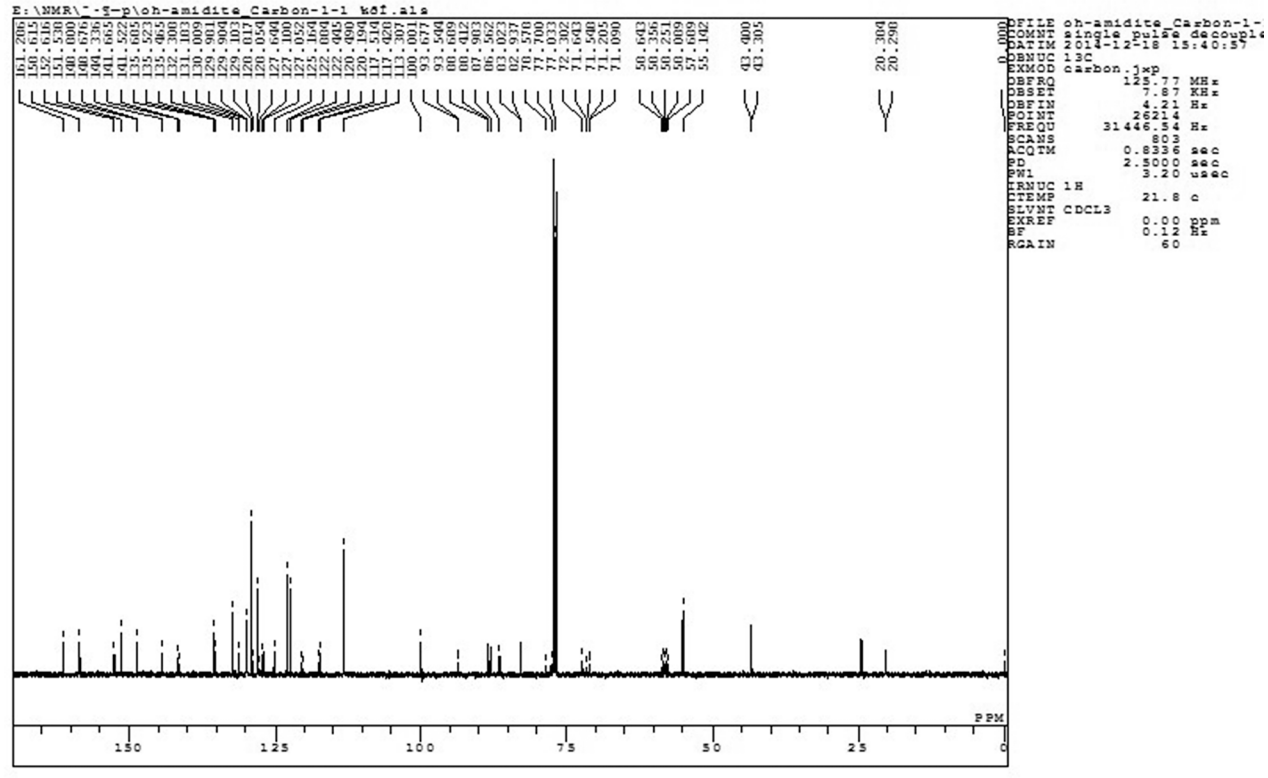
^{13}C spectrum of compound 2



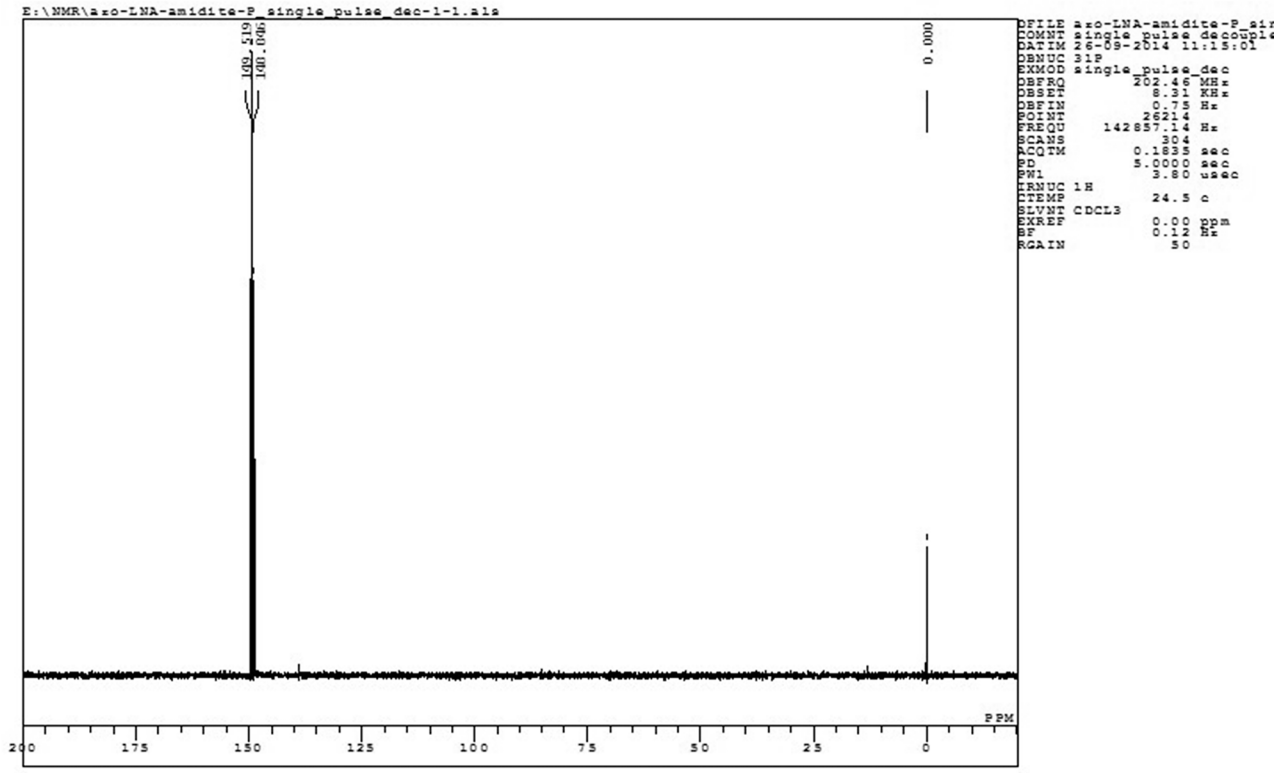
^{1}H spectrum of compound 3



^{13}C spectrum of compound 3



^{31}P spectrum of compound 3



2. HPLC and MALDI-TOF MS analysis of LNA-U^{Az}-modified ONs

ON 6

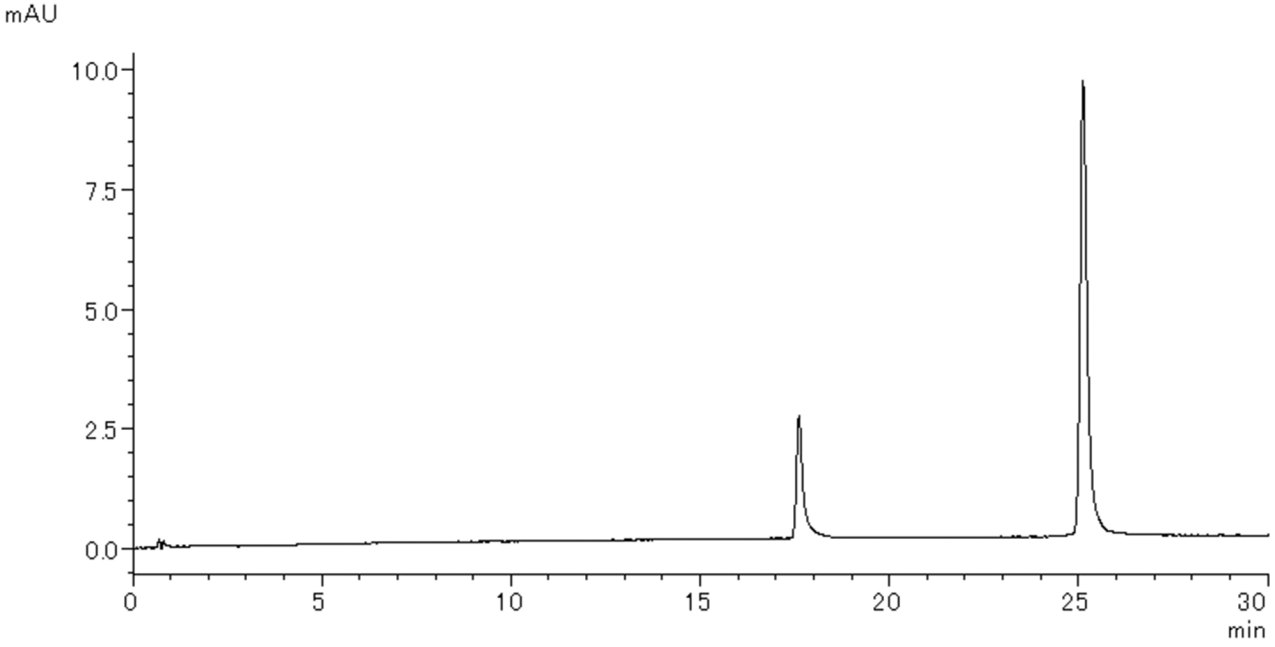
RP-HPLC

Column: Waters XBridge^{TM} OST C18 2.5 μm, 4.6 x 50 mm

Gradient: 7.5-15% MeCN (over 30 min) in triethylammonium acetate buffer (pH 7.0, 0.1 M)

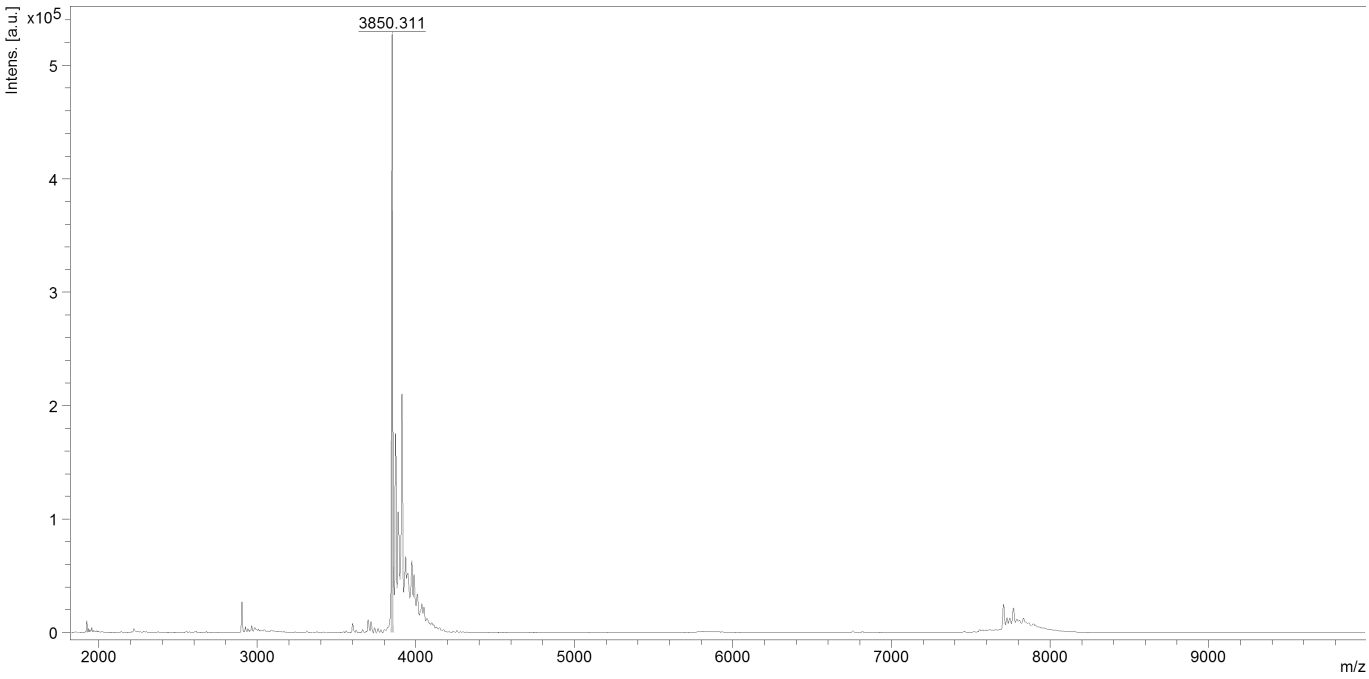
Flow rate: 1.0 mL/min

Column temperature: 37 ˚C



MALDI-TOF MS

Calcd. 3850.6 $[M-H]^-$



ON 8

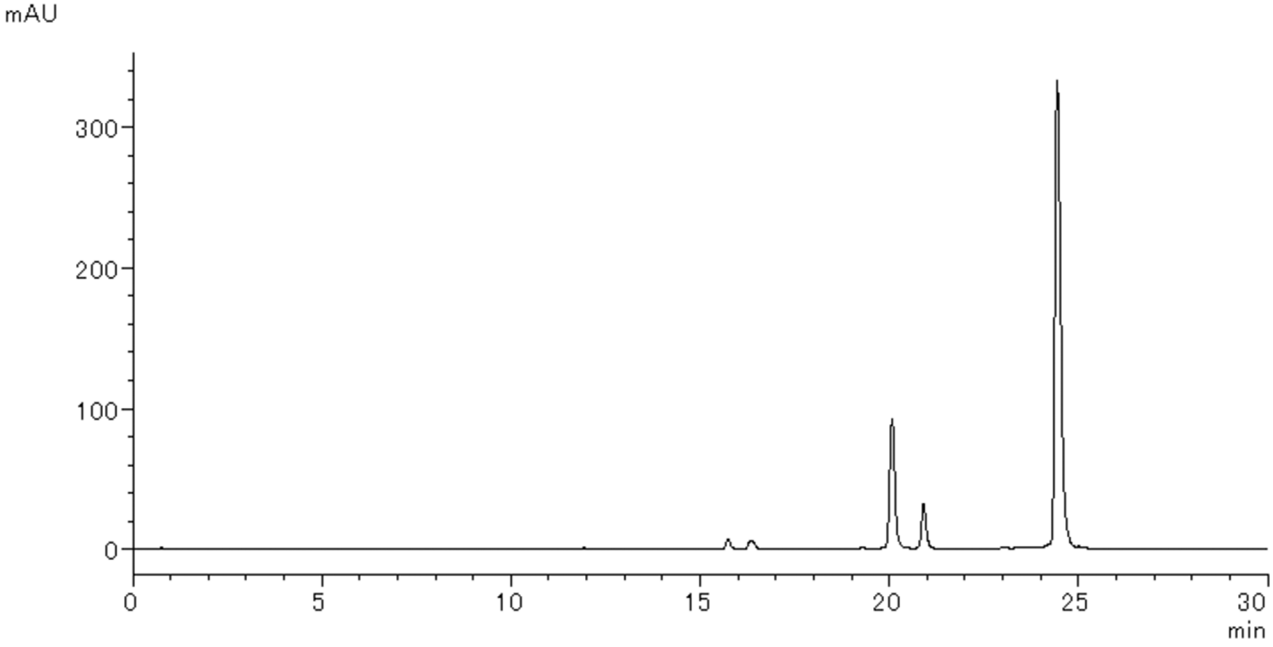
RP-HPLC

Column: Waters XBridge^{TM} OST C18 2.5 μm, 4.6 x 50 mm

Gradient: 15-40% MeCN (over 45 min) in triethylammonium acetate buffer (pH 7.0, 0.1 M)

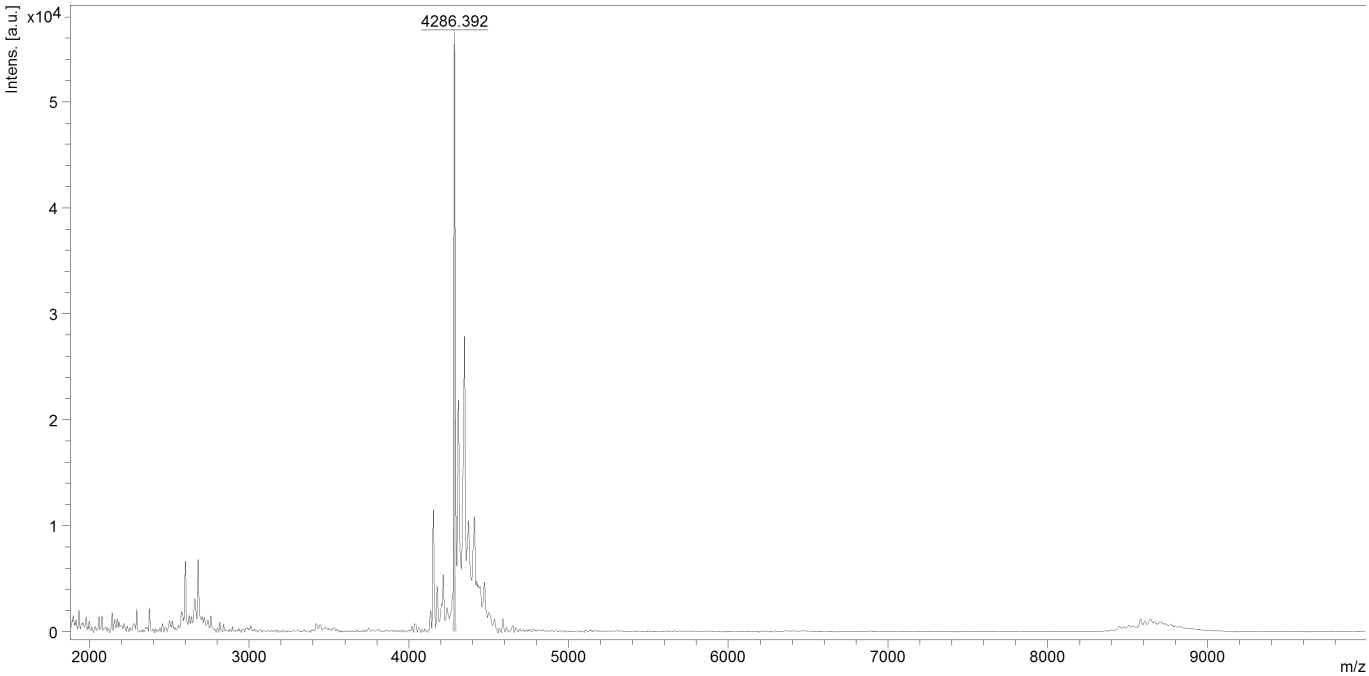
Flow rate: 1.0 mL/min

Column temperature: 50 ˚C



MALDI-TOF MS

Calcd. 4287.0 $[M-H]^-$



ON11

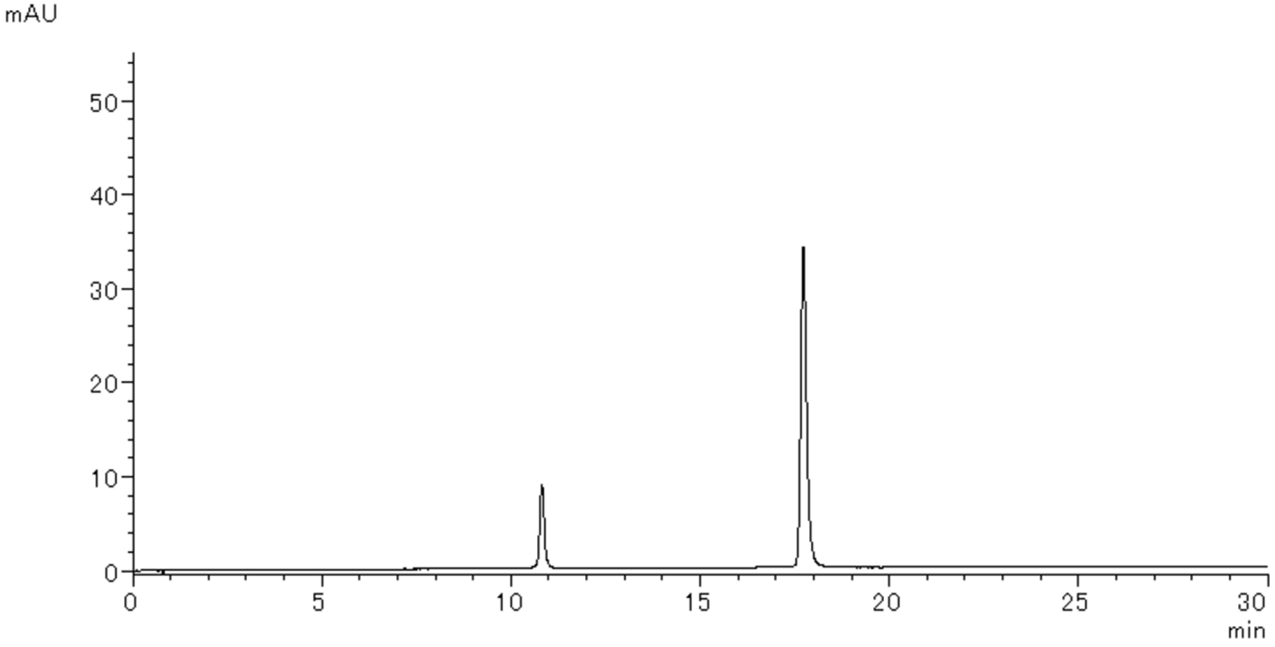
RP-HPLC

Column: Waters XBridge^{TM} OST C18 2.5 μm, 4.6 x 50 mm

Gradient: 5-20% MeCN (over 15 min) in triethylammonium acetate buffer (pH 7.0, 0.1 M)

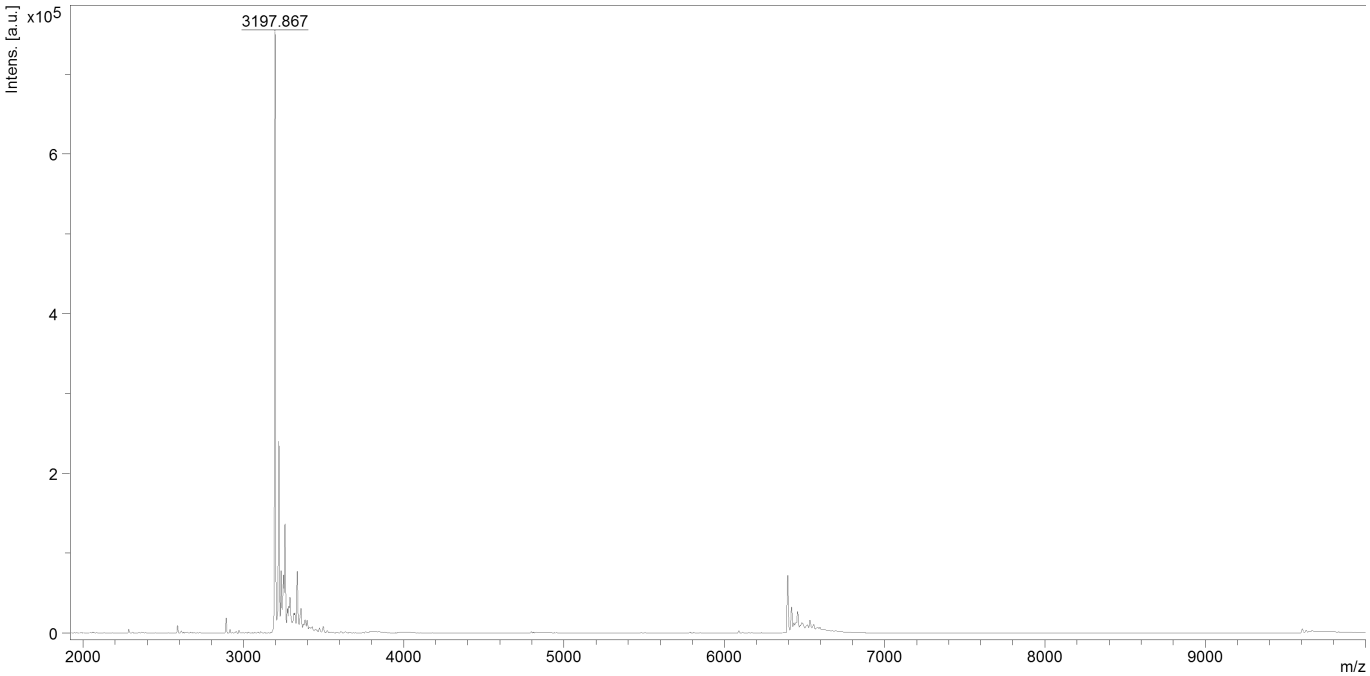
Flow rate: 1.0 mL/min

Column temperature: 50 ˚C



MALDI-TOF MS

Calcd. 3197.2 $[M-H]^-$



ON13

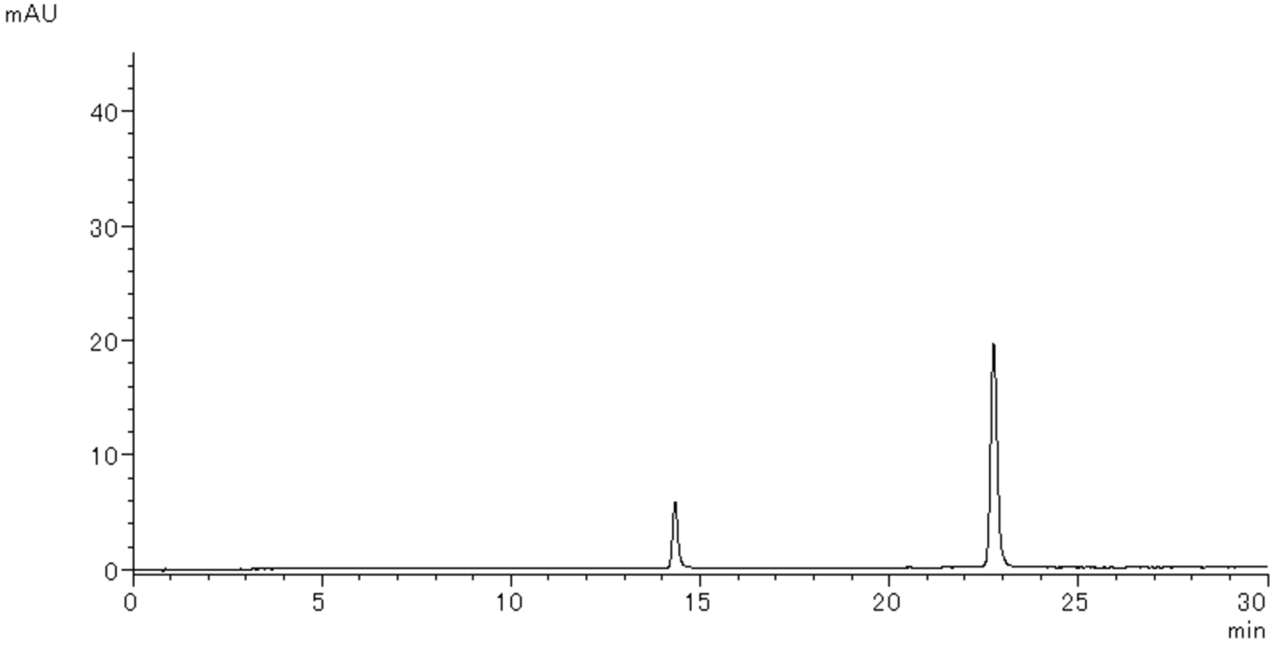
RP-HPLC

Column: Waters XBridge^{TM} OST C18 2.5 μm, 4.6 x 50 mm

Gradient: 5-20% MeCN (over 15 min) in triethylammonium acetate buffer (pH 7.0, 0.1 M)

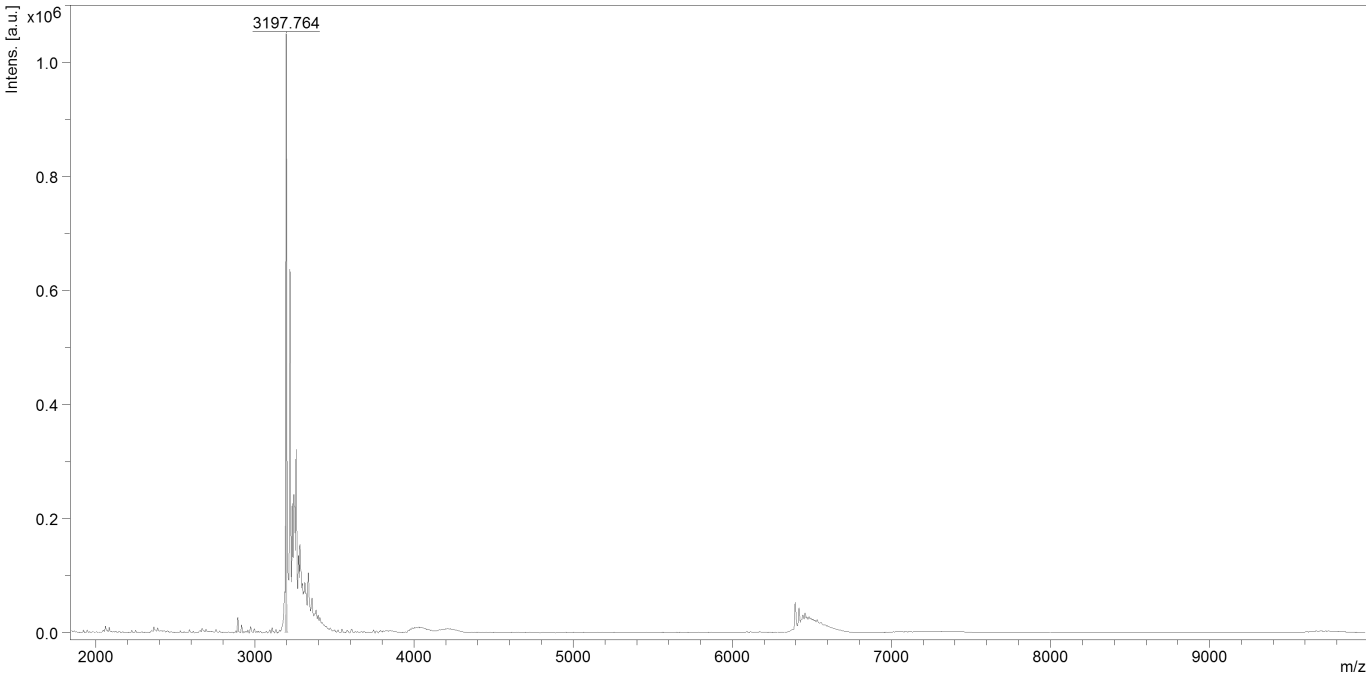
Flow rate: 1.0 mL/min

Column temperature: 50 ˚C



MALDI-TOF MS

Calcd. 3197.2 $[M-H]^-$



3. Single-mismatch discrimination ability of LNA-U^{Az}-modified ON

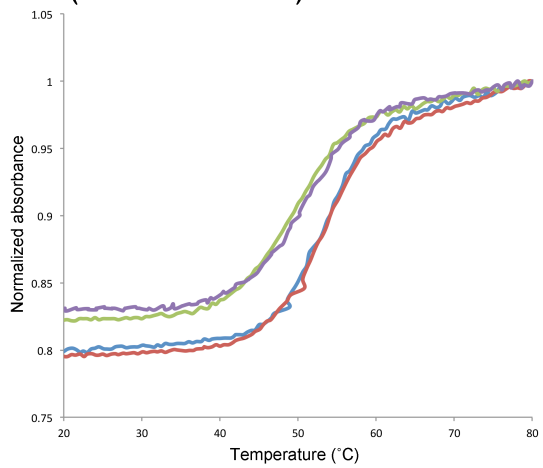
Table S1. Single-mismatch discrimination ability of **LNA-U^{Az}**-modified ON against RNA strands[a]

duplex	T_m (˚C)[b]	ΔT_m (˚C)[c]
5′-d(GCGTT**X**TTTGCT)-3′ 3′-r(CGCAAAAAACGA)-5′	48	-
5′-d(GCGTT**X**TTTGCT)-3′ 3′-r(CGCAAGAAACGA)-5′	38	-10
5′-d(GCGTT**X**TTTGCT)-3′ 3′-r(CGCAACAAACGA)-5′	32	-16
5′-d(GCGTT**X**TTTGCT)-3′ 3′-r(CGCAAUAAACGA)-5′	33	-15

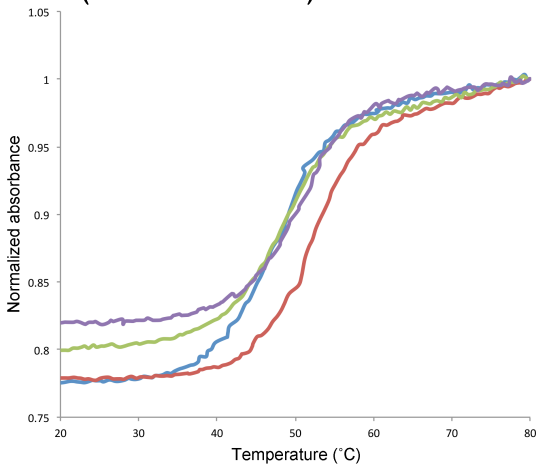
[a]UV melting profiles were measured in 10 mM sodium phosphate buffer (pH 7.2) containing 4 μM ON and 100 mM NaCl at a scan rate of 0.5˚ C/min at 260 nm. **X** = **LNA-U^{Az}**. [b]The T_m value given is the average of three independent measurements. [c]ΔT_m values are calculated relative to the T_m values of full matched duplex.

4. UV melting curves

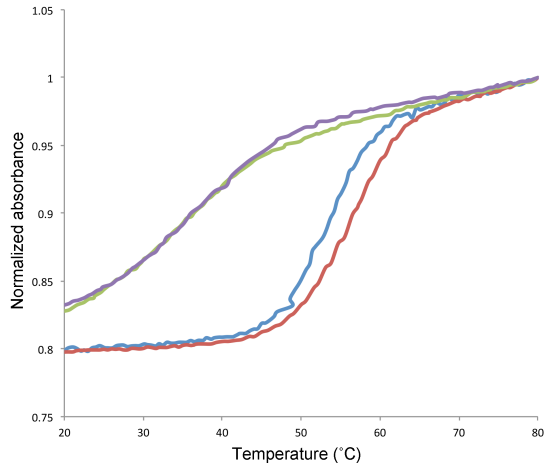
5′-d(GCGTT**X**TTTGCT)-3′
3′-d(CGCAAAAAACGA)-5′



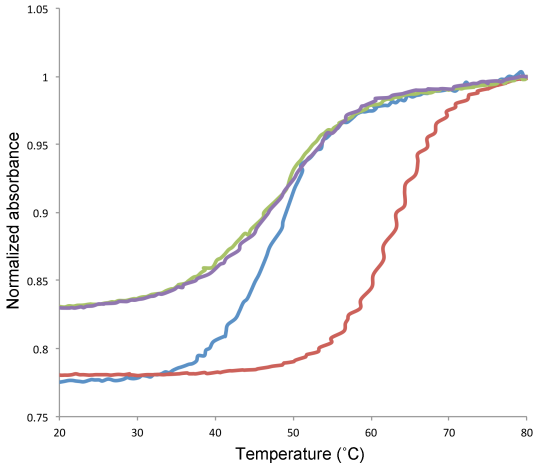
5′-d(GCGTT**X**TTTGCT)-3′
3′-r(CGCAAAAAACGA)-5′



5′-d(GCGT**XXX**TTGCT)-3′
3′-d(CGCAAAAAACGA)-5′



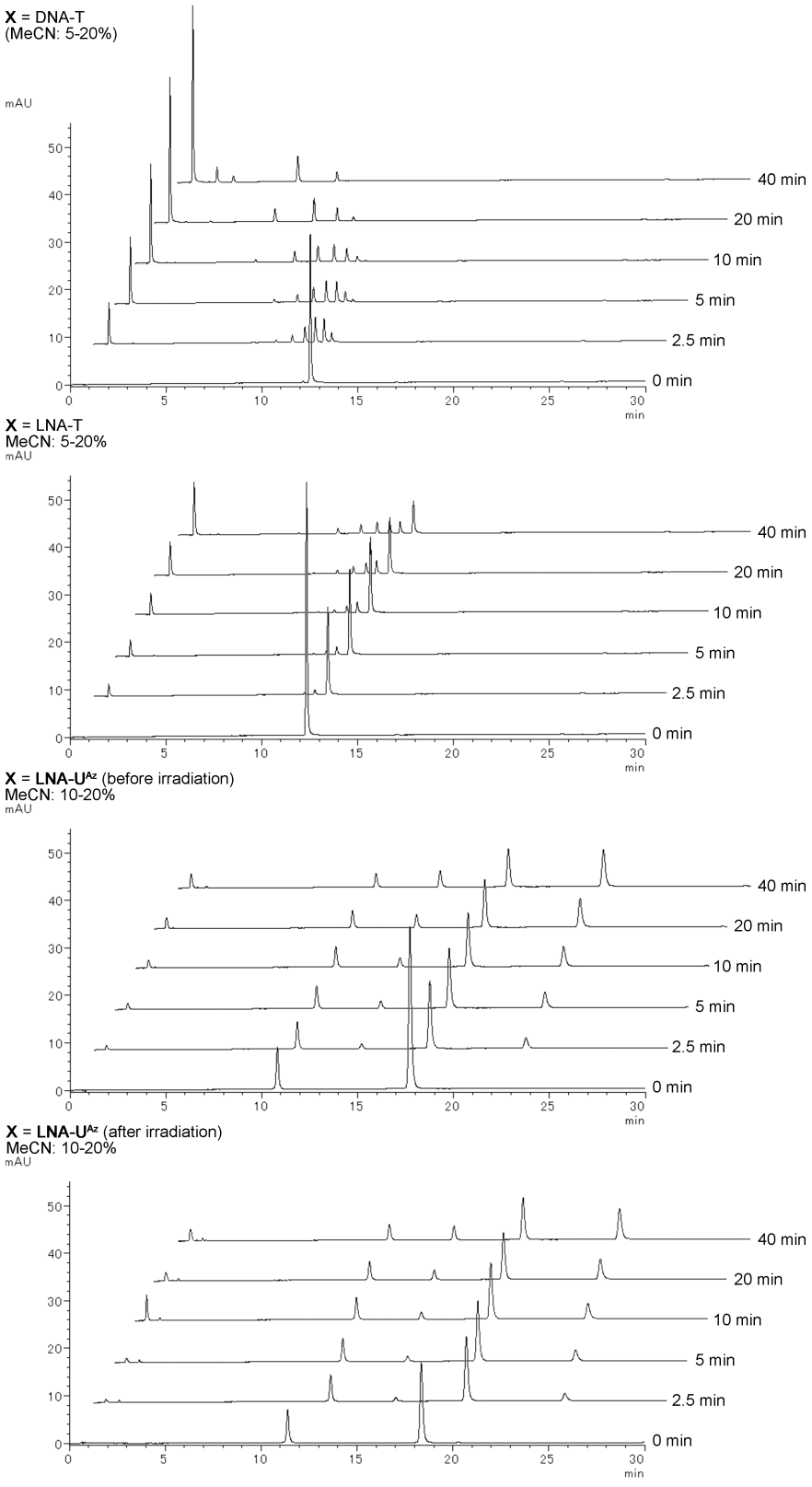
5′-d(GCGT**XXX**TTGCT)-3′
3′-r(CGCAAAAAACGA)-5′



X = DNA-T (blue), LNA-T (red), **LNA-U^{Az}** (before irradiation; green), **LNA-U^{Az}** (after irradiation; purple)

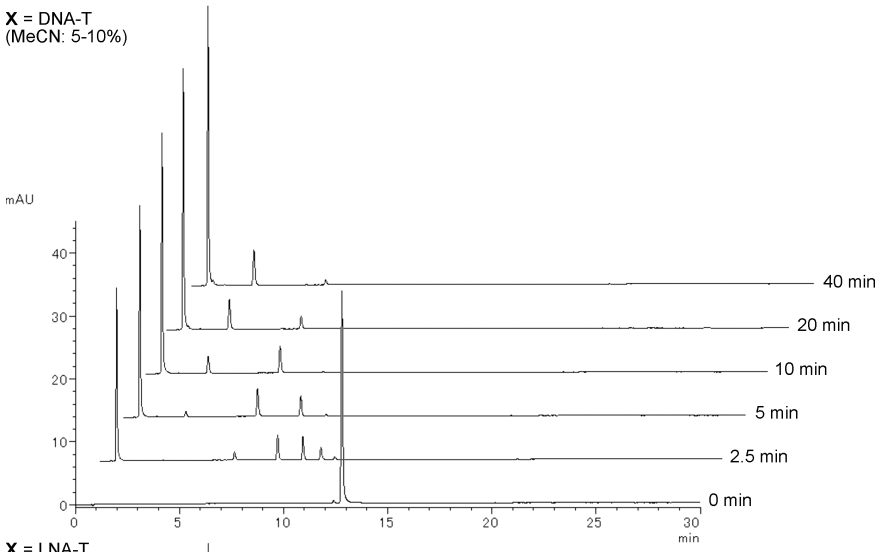
5. HPLC profiles of enzymatic degradation experiments

5’-d(TTTTTTTT**X**T)-3’

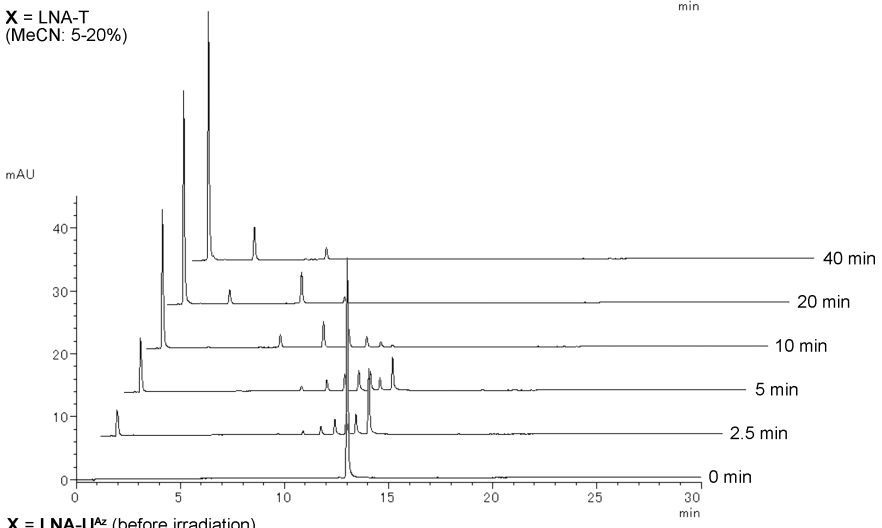


5’-d(TTTTTTTTT**X**)-3’

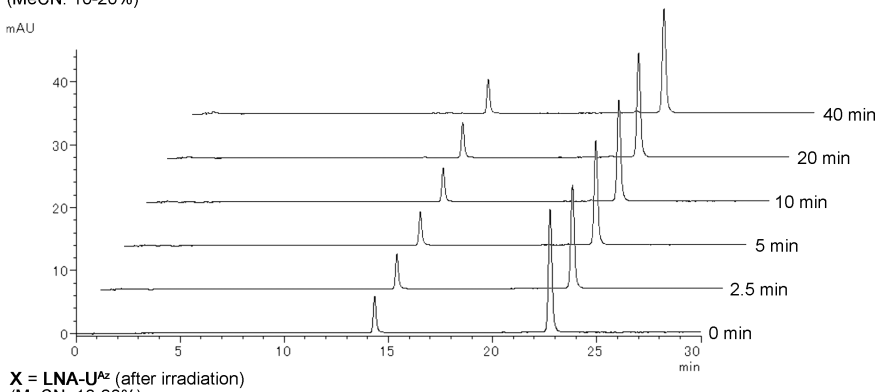
X = DNA-T
(MeCN: 5-10%)



X = LNA-T
(MeCN: 5-20%)



X = **LNA-U**^{Az} (before irradiation)
(MeCN: 10-20%)



X = **LNA-U**^{Az} (after irradiation)
(MeCN: 10-20%)