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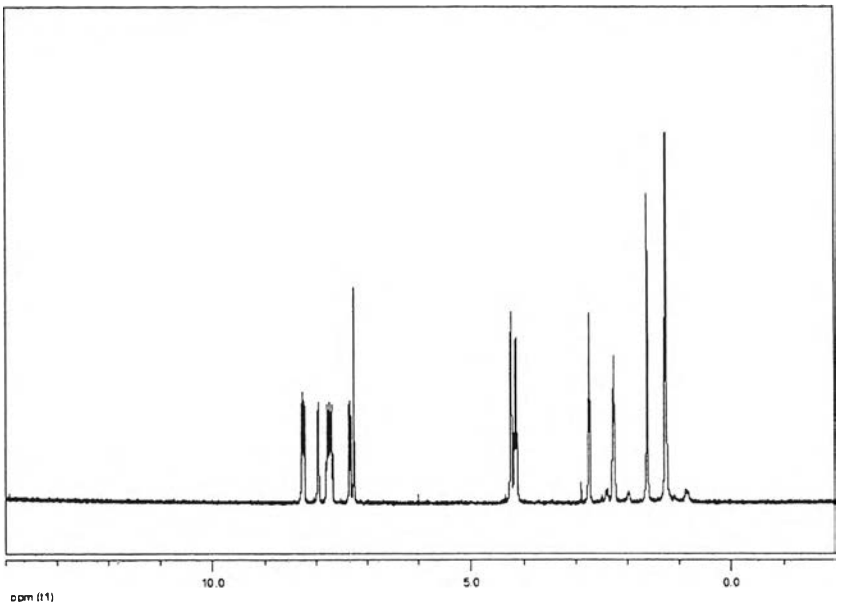
APPENDIX



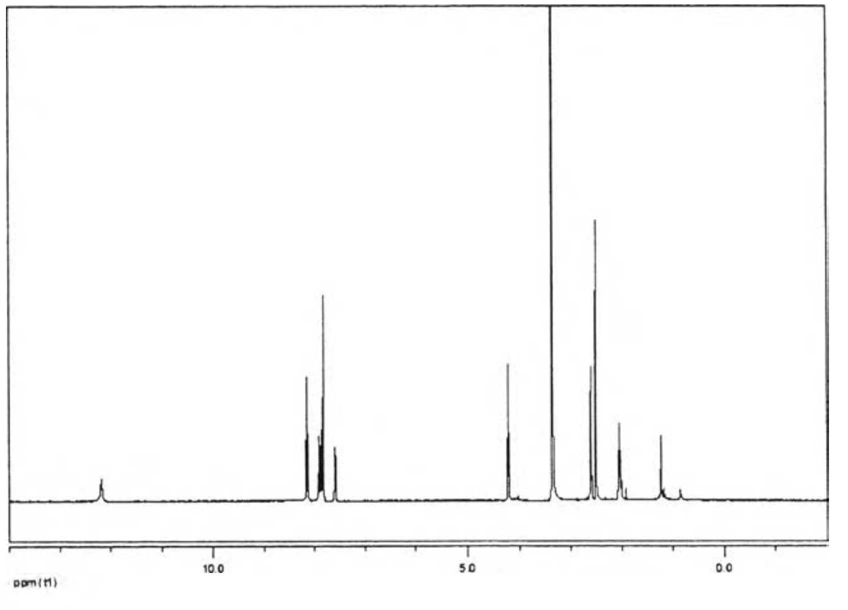


Figure A1 ^{1}H NMR spectra of ethyl 4-(anthraquinone-1-oxy)butyrate (400 MHz, $CDCl_3$) (top) and 4-(anthraquinone-1-oxy)butyric acid (400 MHz, DMSO-d_6) (bottom)

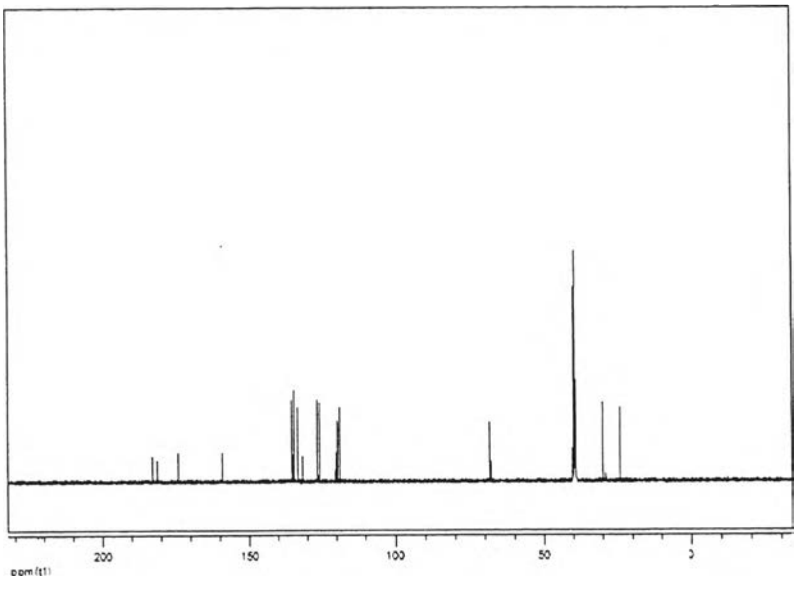


Figure A2 ^{13}C NMR spectrum of 4-(anthraquinone-1-oxy)butyric acid (100 MHz, DMSO-d_6)

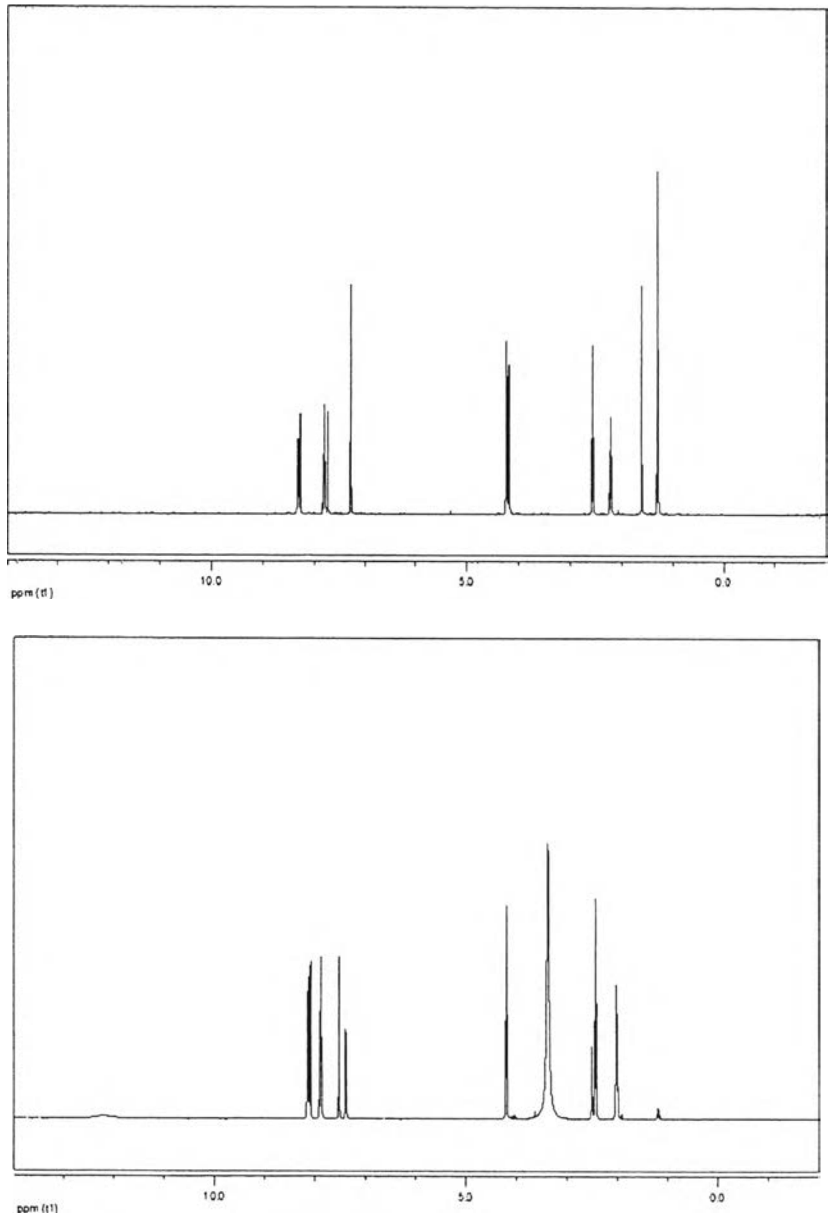


Figure A3 ^{1}H NMR spectra of ethyl 4-(anthraquinone-2-oxy)butyrate (400 MHz, $CDCl_3$) (top) and 4-(anthraquinone-2-oxy)butyric acid (400 MHz, DMSO-d_6) (bottom)

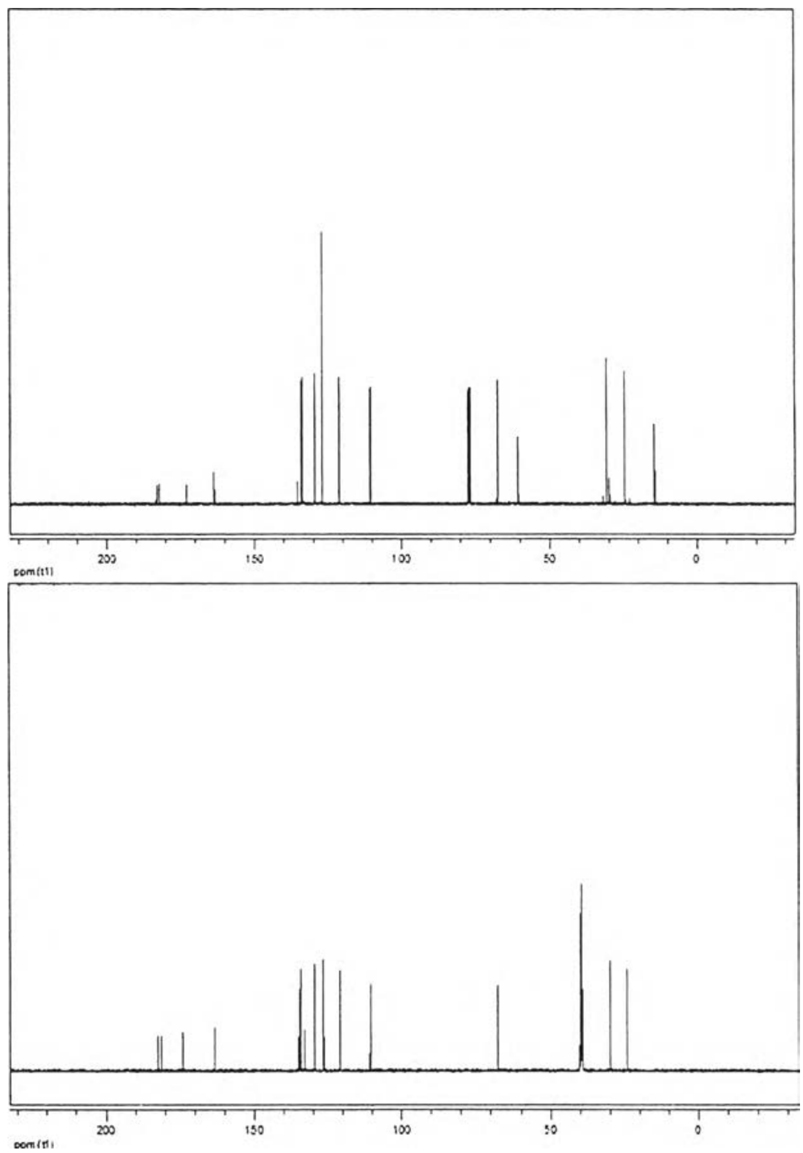


Figure A4 ^{13}C NMR spectra of ethyl 4-(anthraquinone-2-oxy)butyrate (400 MHz, $CDCl_3$) (top) and 4-(anthraquinone-2-oxy)butyric acid (400 MHz, DMSO-d_6) (bottom)

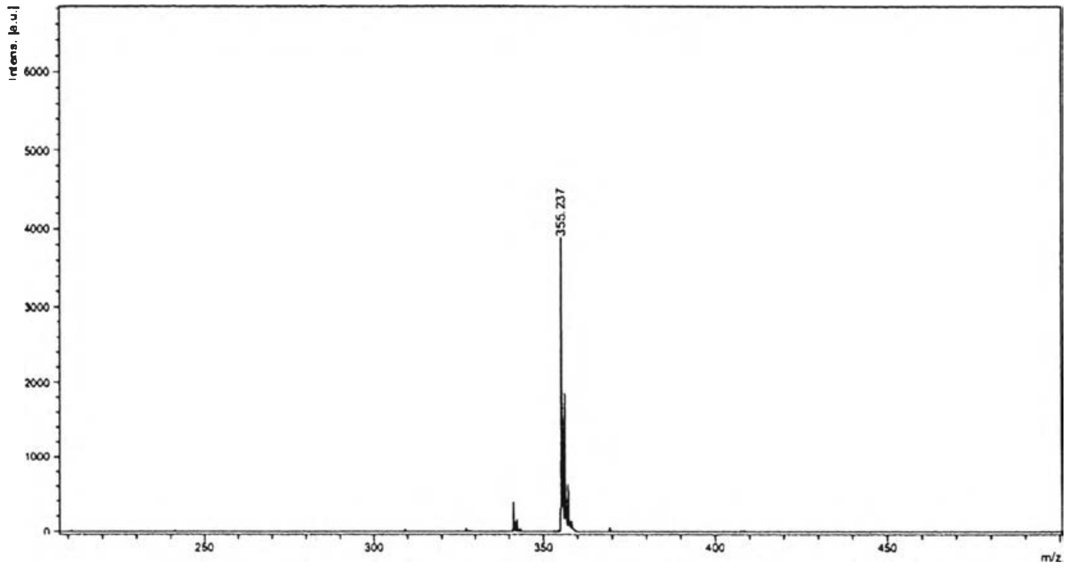


Figure A5 MALDI-TOF mass spectrum of methylene blue butyric acid (calcd for $[M+H]^+$ = 357.1)

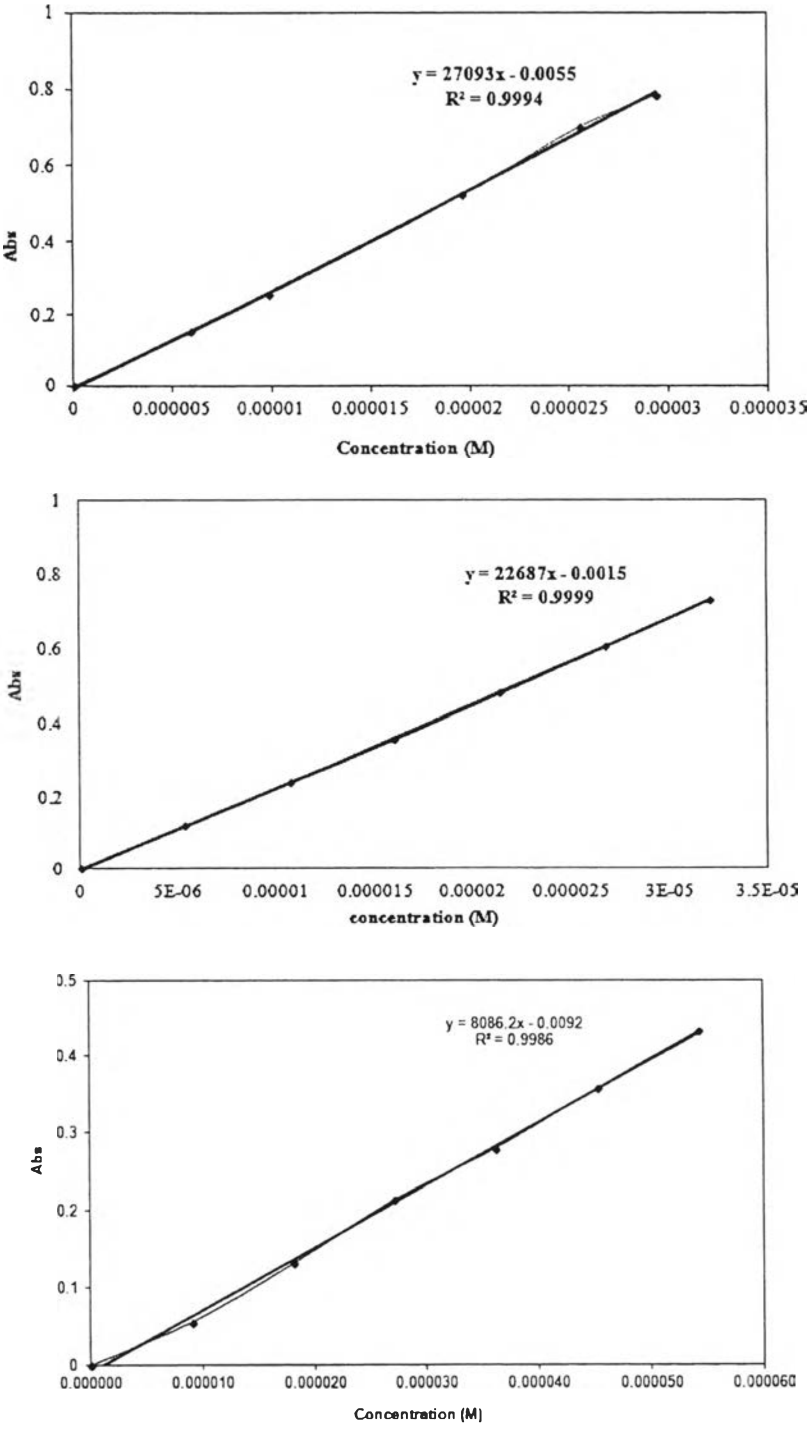
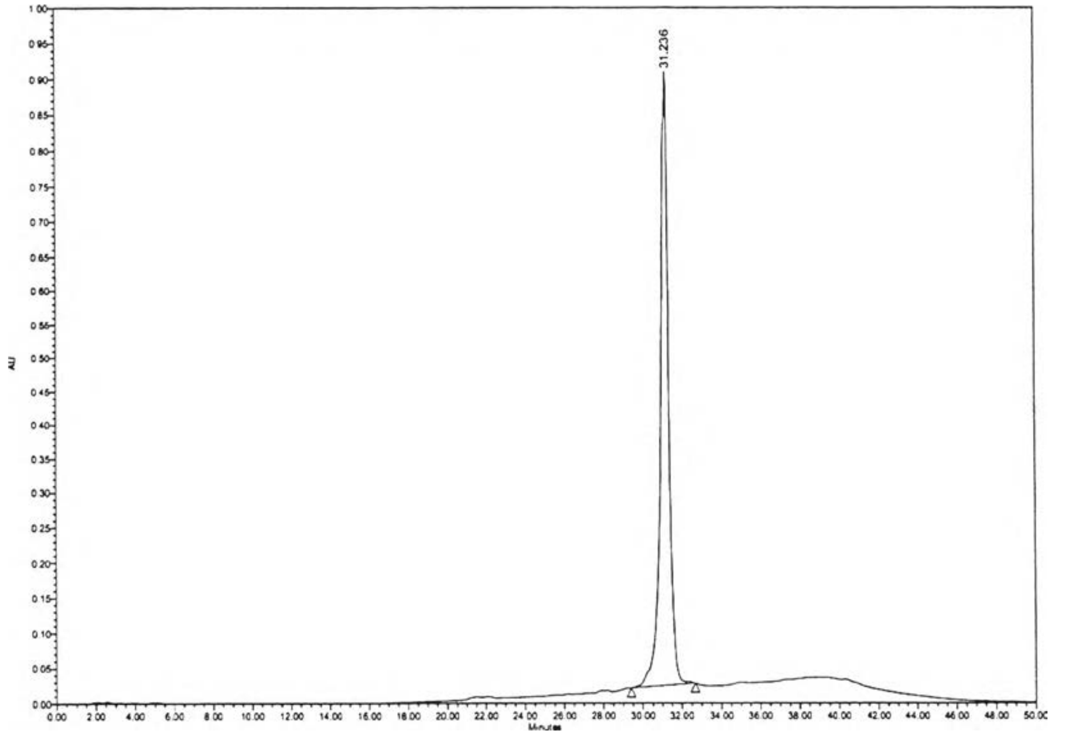


Figure A6 Determination of molar extinction coefficient at 260 nm (ε_{260}) of 4-(anthraquinone-1-oxy)butyric acid (top), 4-(anthraquinone-2-oxy)butyric acid (center) and methylene blue butyric acid (bottom) in MeOH



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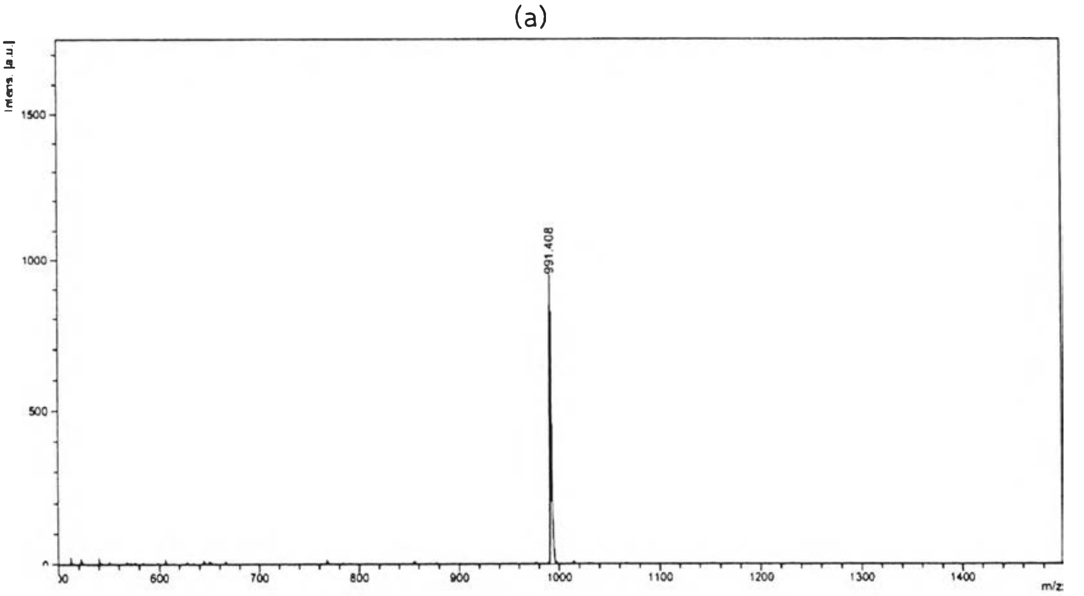
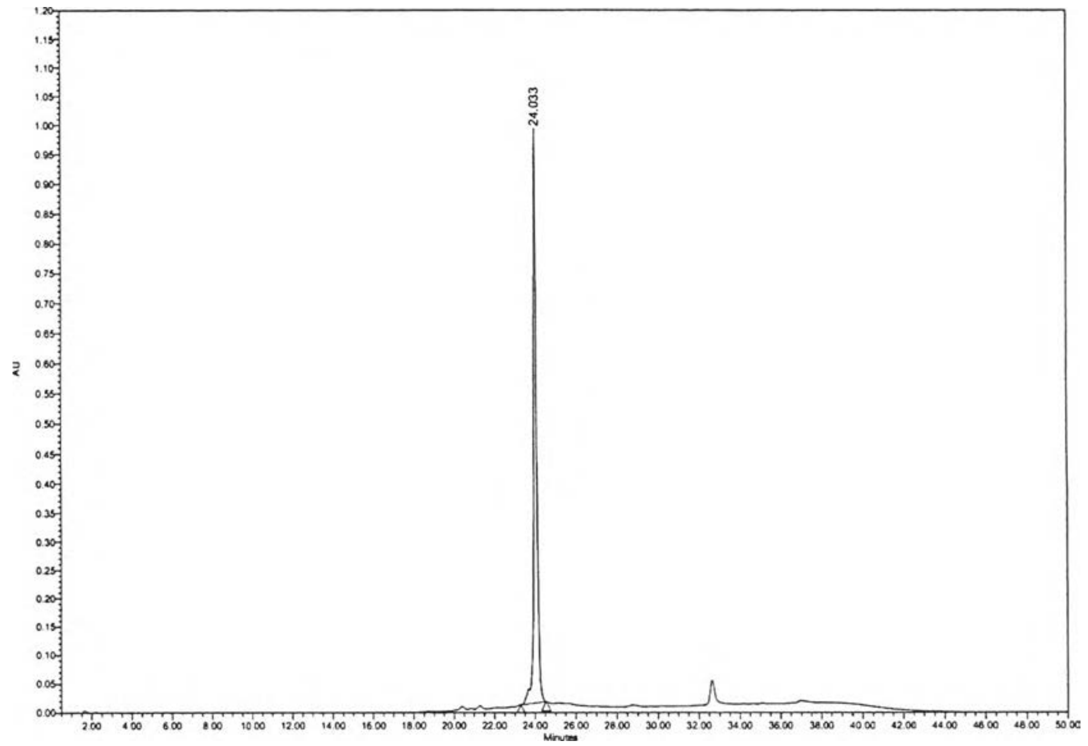


(b)

Figure A7 (a) Analytical HPLC chromatogram (ACE 5 C18-AR (150 x 4.6 mm) HPLC column, water(A)/methanol(B) (started with A:B (90:10) for 5 min followed by a linear gradient to A:B (10:90) over a period of 70 min), flow rate 0.5 mL/min) and (b) MALDI-TOF mass spectrum of PNA 1AQ-T2-Lys (calcd for $[M+H]^+$ = 991.3)



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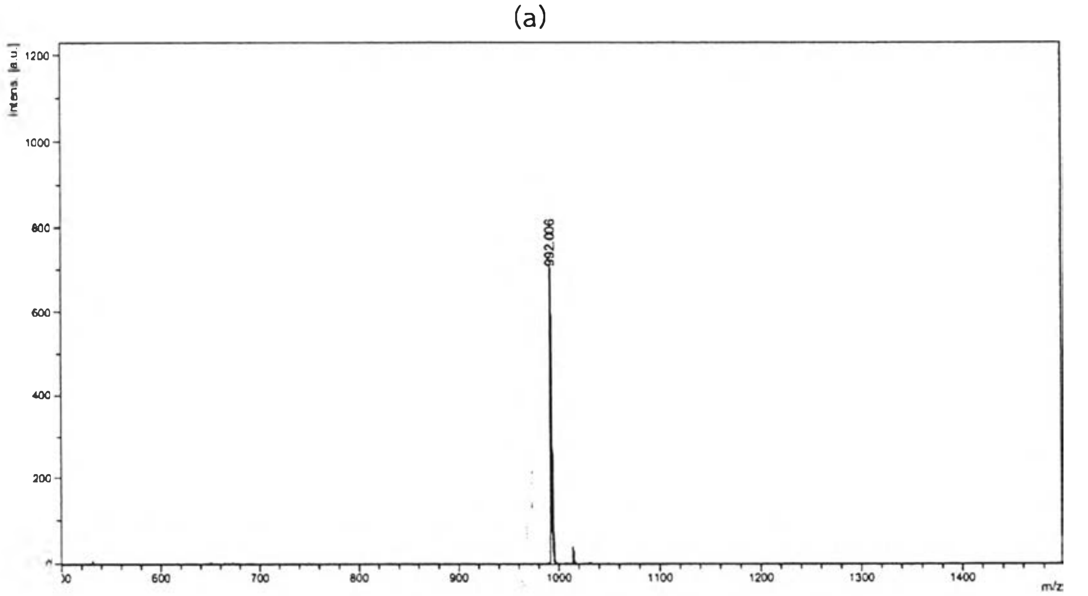
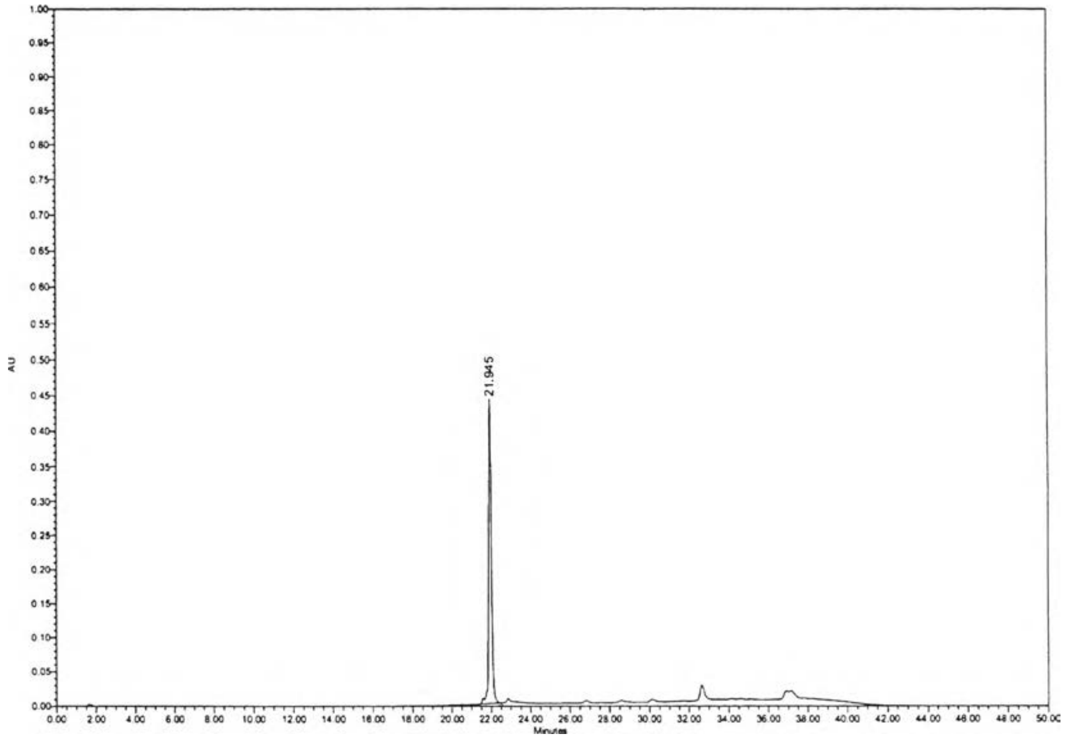


(b)

Figure A8 (a) Analytical HPLC chromatogram (ACE 5 C18-AR (150 x 4.6 mm) HPLC column, water(A)/methanol(B) (started with A:B (90:10) for 5 min followed by a linear gradient to A:B (10:90) over a period of 70 min), flow rate 0.5 mL/min) and (b) MALDI-TOF mass spectrum of PNA 2AQ-T2-Lys (calcd for $[M+H]^+$ = 992.0)



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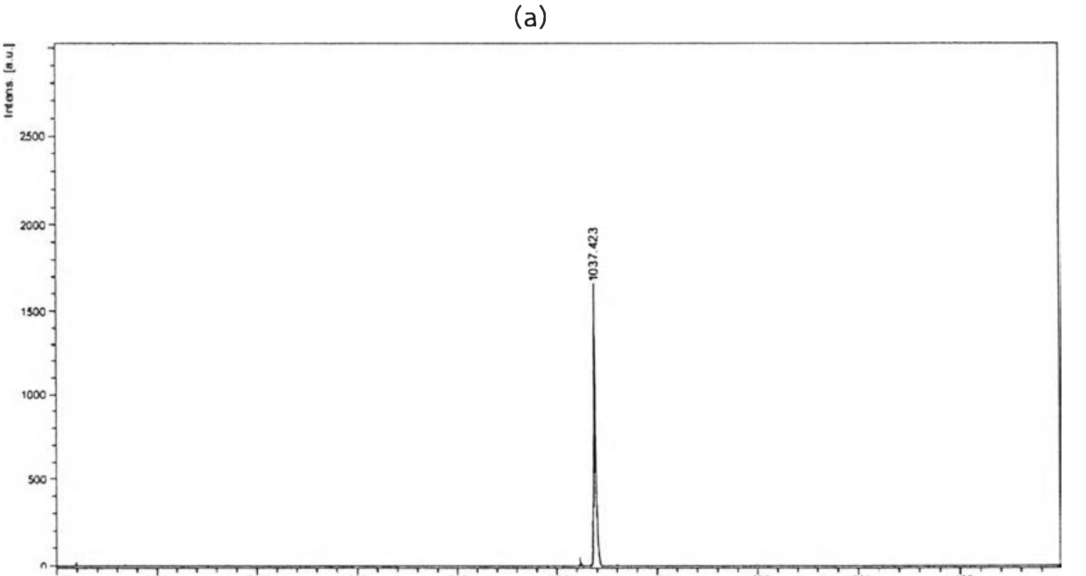
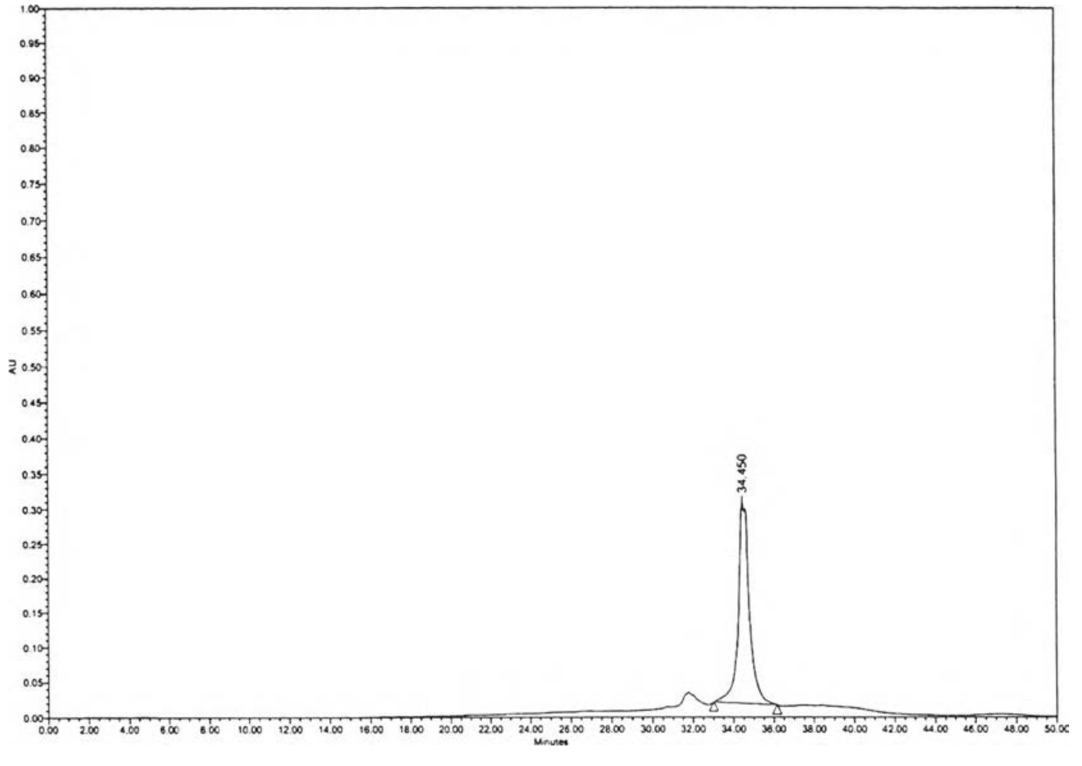
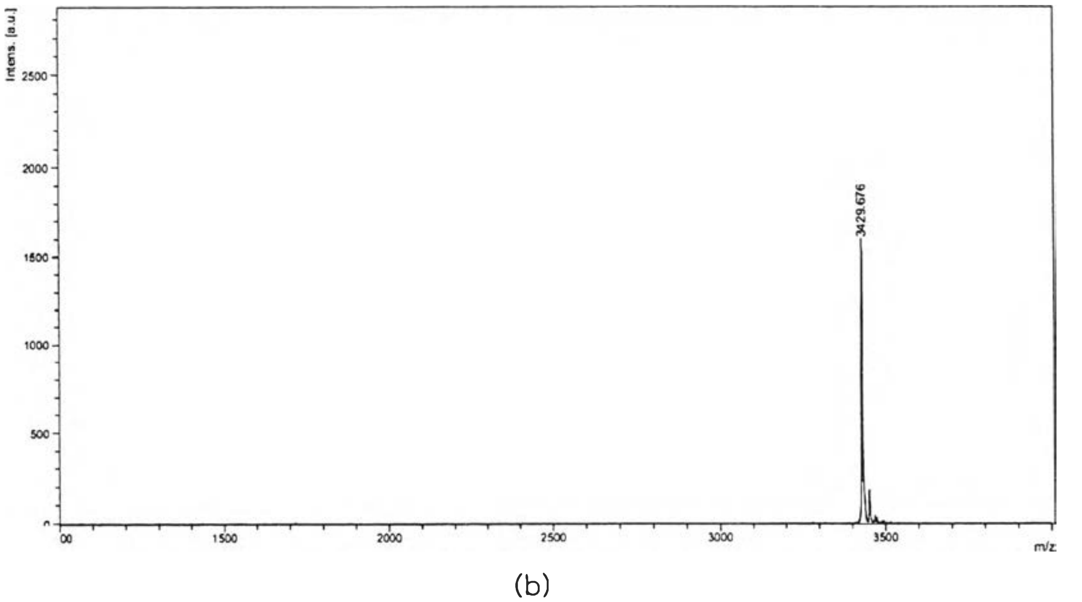


(b)

Figure A9 (a) Analytical HPLC chromatogram (ACE 5 C18-AR (150 x 4.6 mm) HPLC column, water(A)/methanol(B) (started with A:B (90:10) for 5 min followed by a linear gradient to A:B (10:90) over a period of 70 min), flow rate 0.5 mL/min) and (b) MALDI-TOF mass spectrum of PNA MB-T2-Lys (calcd for $[M+H]^+$ = 1037.4)

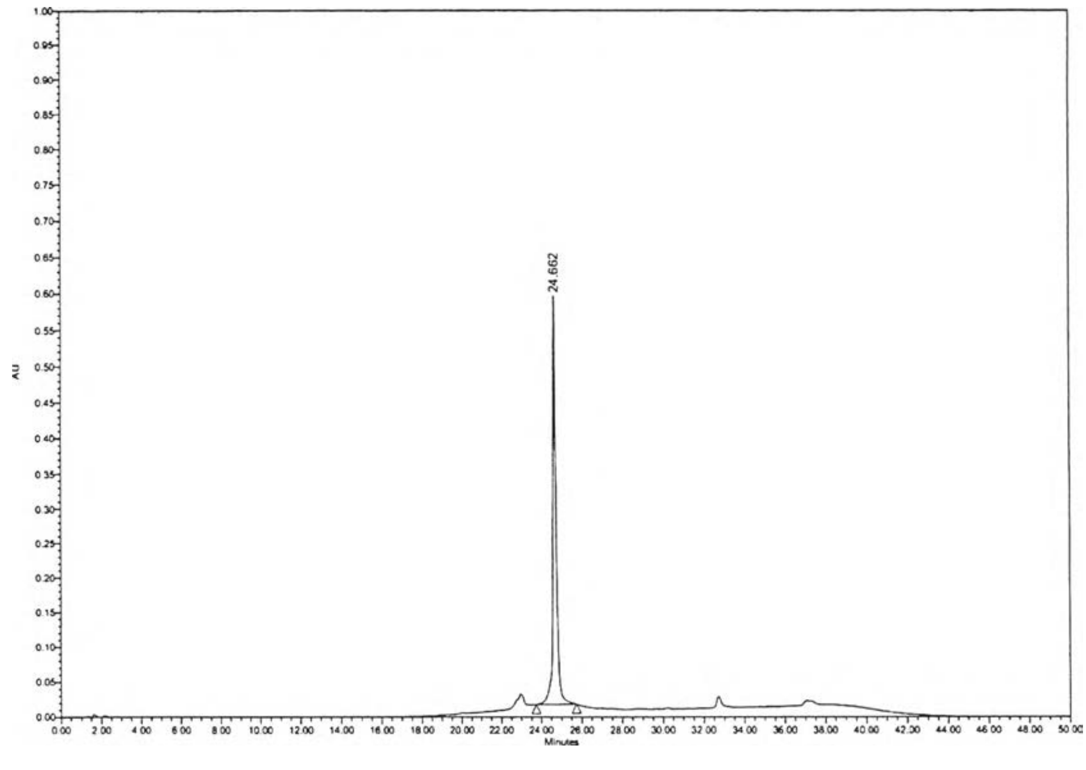


(a)

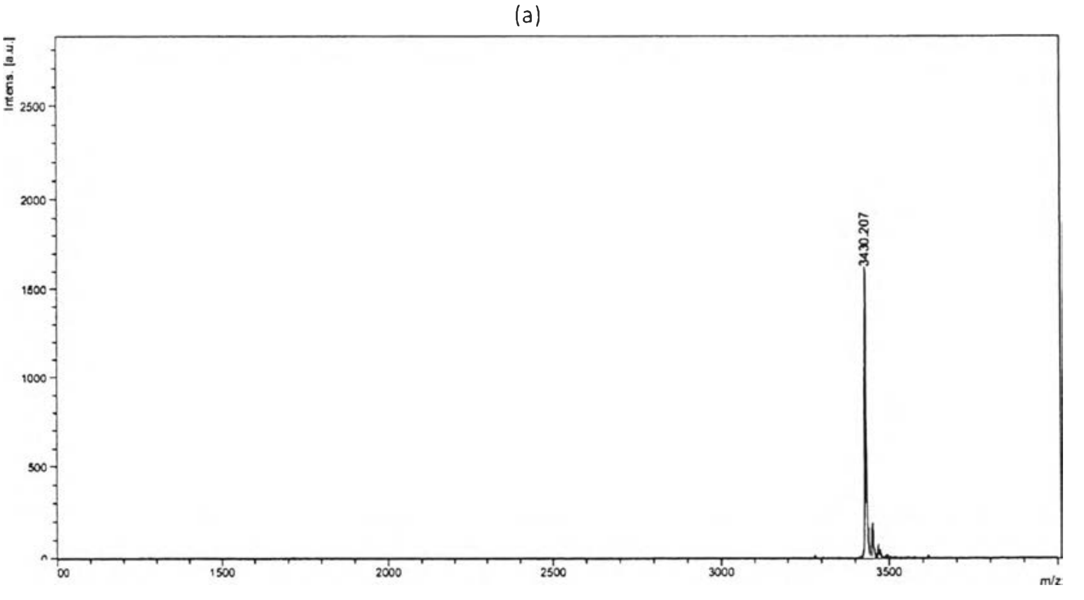


(b)

Figure A10 (a) Analytical HPLC chromatogram (ACE 5 C18-AR (150 x 4.6 mm) HPLC column, water(A)/methanol(B) (started with A:B (90:10) for 5 min followed by a linear gradient to A:B (10:90) over a period of 70 min), flow rate 0.5 mL/min) and (b) MALDI-TOF mass spectrum of PNA 1AQ-T9-Lys (calcd for $[M+H]^+$ = 3429.7)

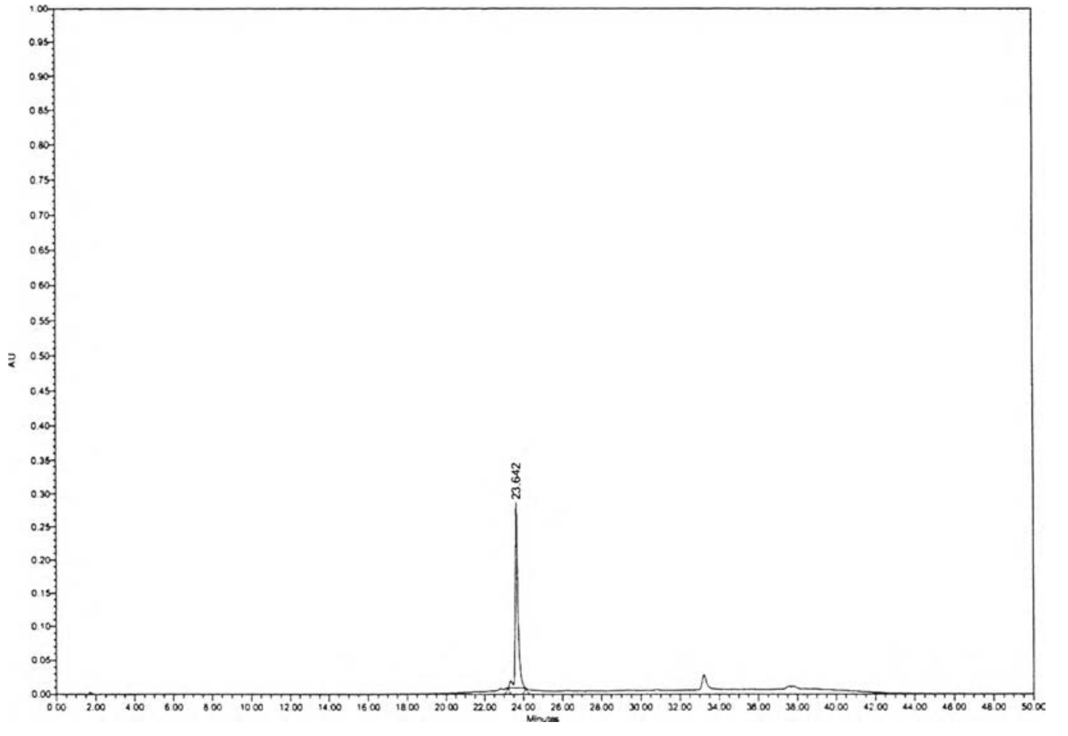


(a)

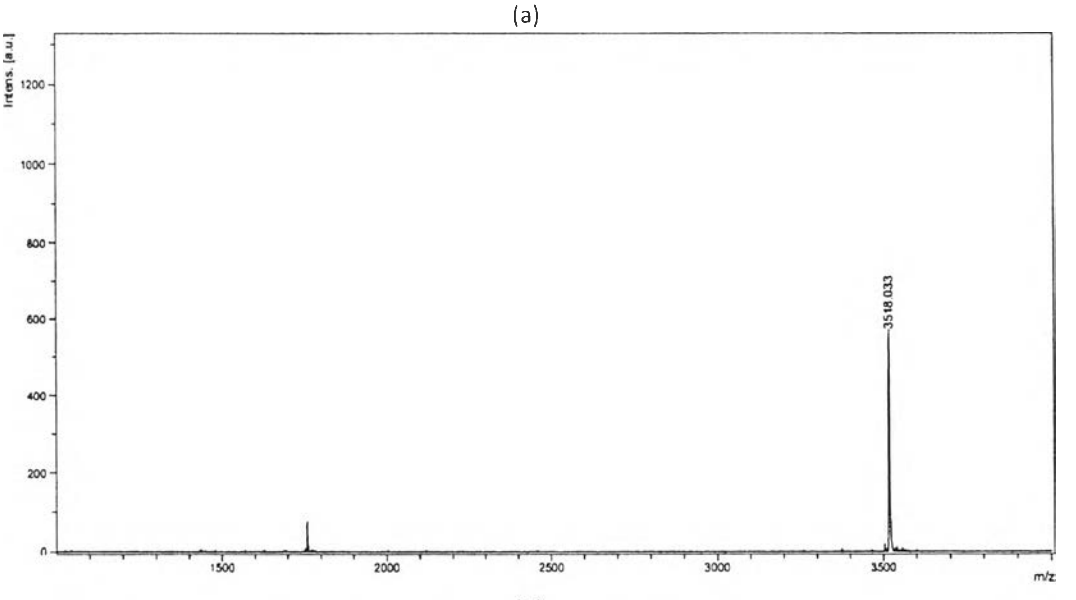


(b)

Figure A11 (a) Analytical HPLC chromatogram (ACE 5 C18-AR (150 x 4.6 mm) HPLC column, water(A)/methanol(B) (started with A:B (90:10) for 5 min followed by a linear gradient to A:B (10:90) over a period of 70 min), flow rate 0.5 mL/min) and (b) MALDI-TOF mass spectrum of PNA 2AQ-T9-Lys (calcd for $[M+H]^+$ = 3430.2)

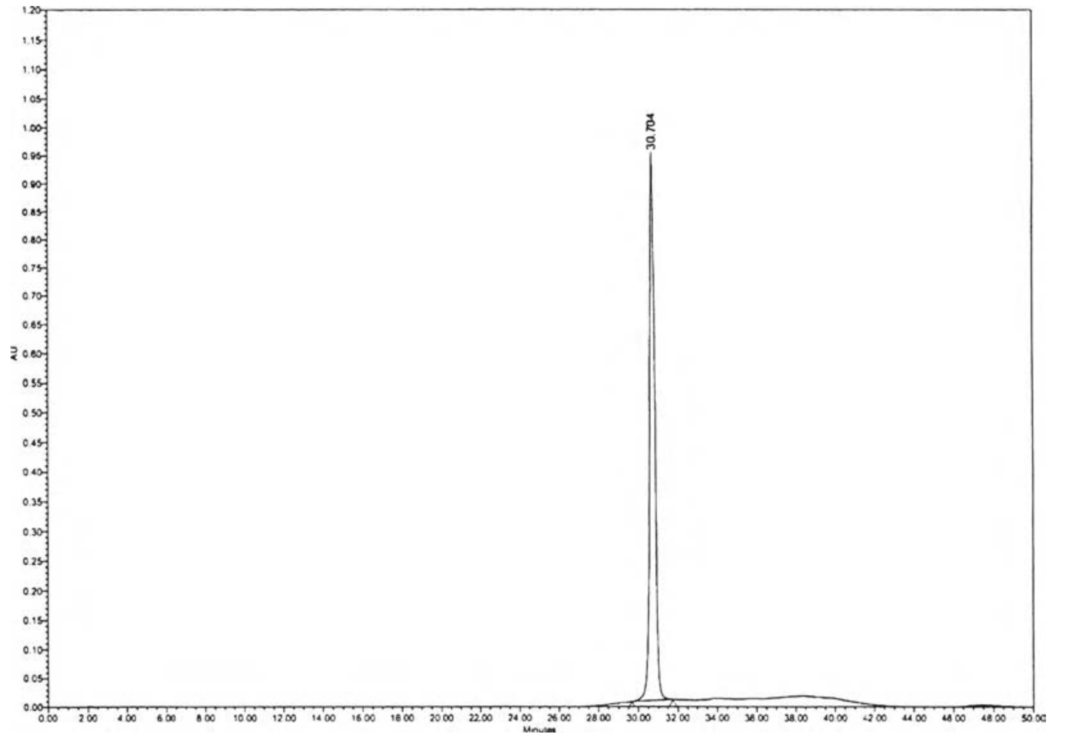


(a)

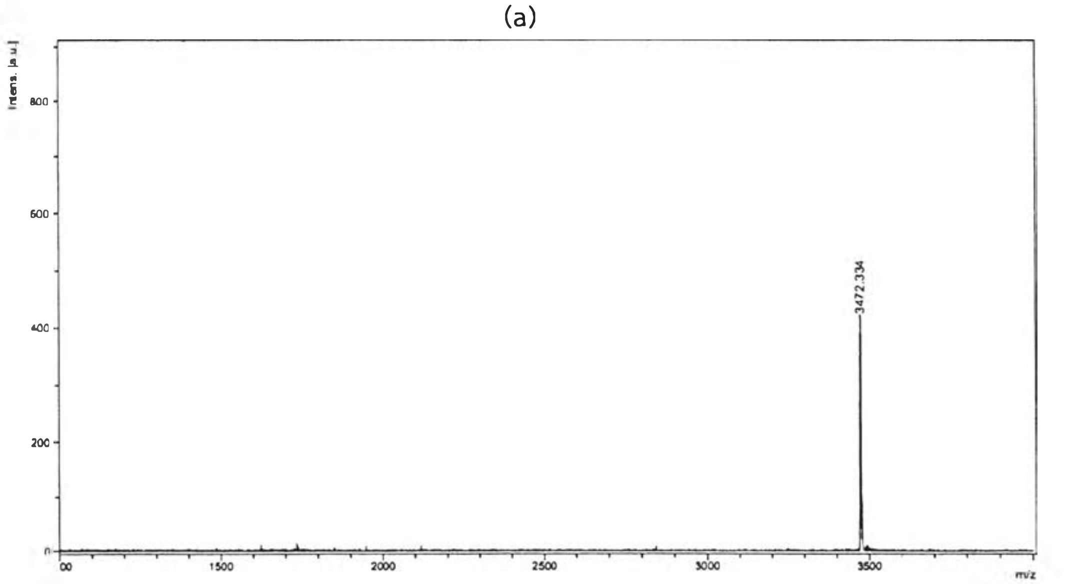


(b)

Figure A12 (a) Analytical HPLC chromatogram (ACE 5 C18-AR (150 x 4.6 mm) HPLC column, water(A)/methanol(B) (started with A:B (90:10) for 5 min followed by a linear gradient to A:B (10:90) over a period of 70 min), flow rate 0.5 mL/min) and (b) MALDI-TOF mass spectrum of PNA MB-T9-Lys (calcd for $[M+H]^+$ = 3518.0)

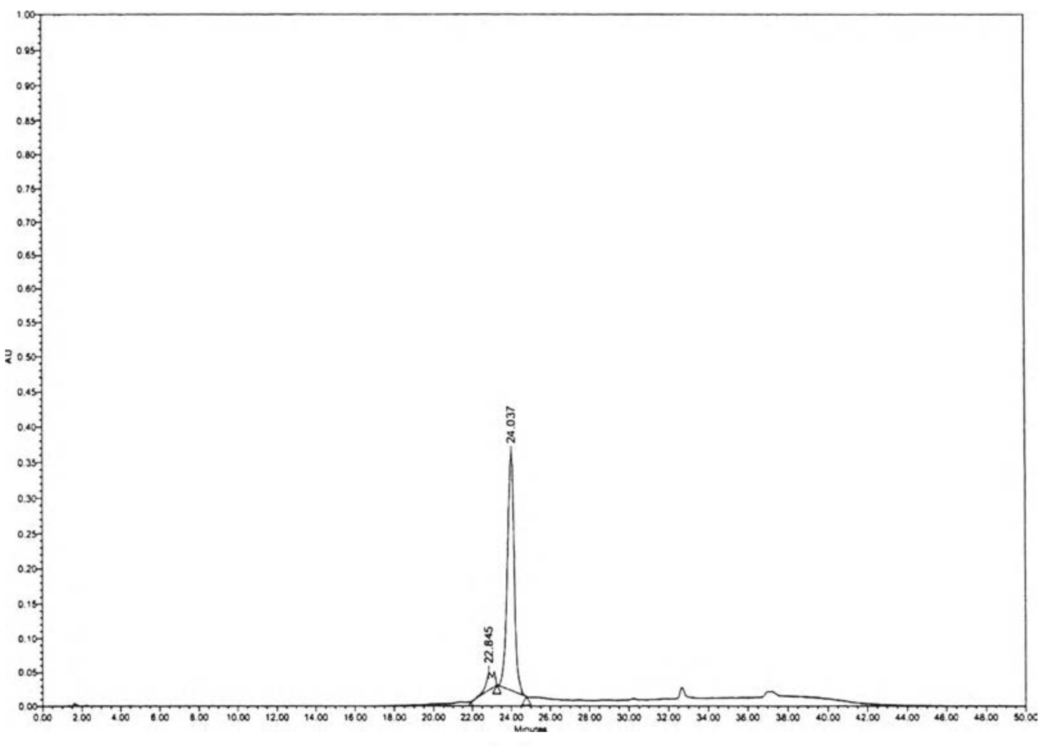


(a)

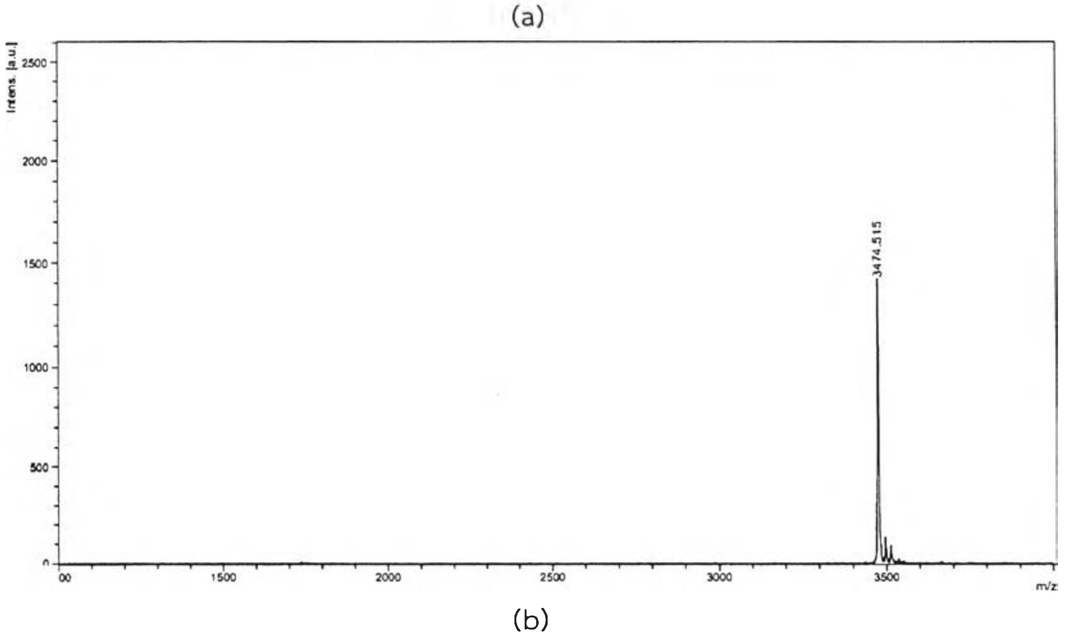


(b)

Figure A13 (a) Analytical HPLC chromatogram (ACE 5 C18-AR (150 x 4.6 mm) HPLC column, water(A)/methanol(B) (started with A:B (90:10) for 5 min followed by a linear gradient to A:B (10:90) over a period of 70 min), flow rate 0.5 mL/min) and (b) MALDI-TOF mass spectrum of PNA T5-1AQ-T4-Lys (calcd for $[M+H]^+$ = 3472.3)

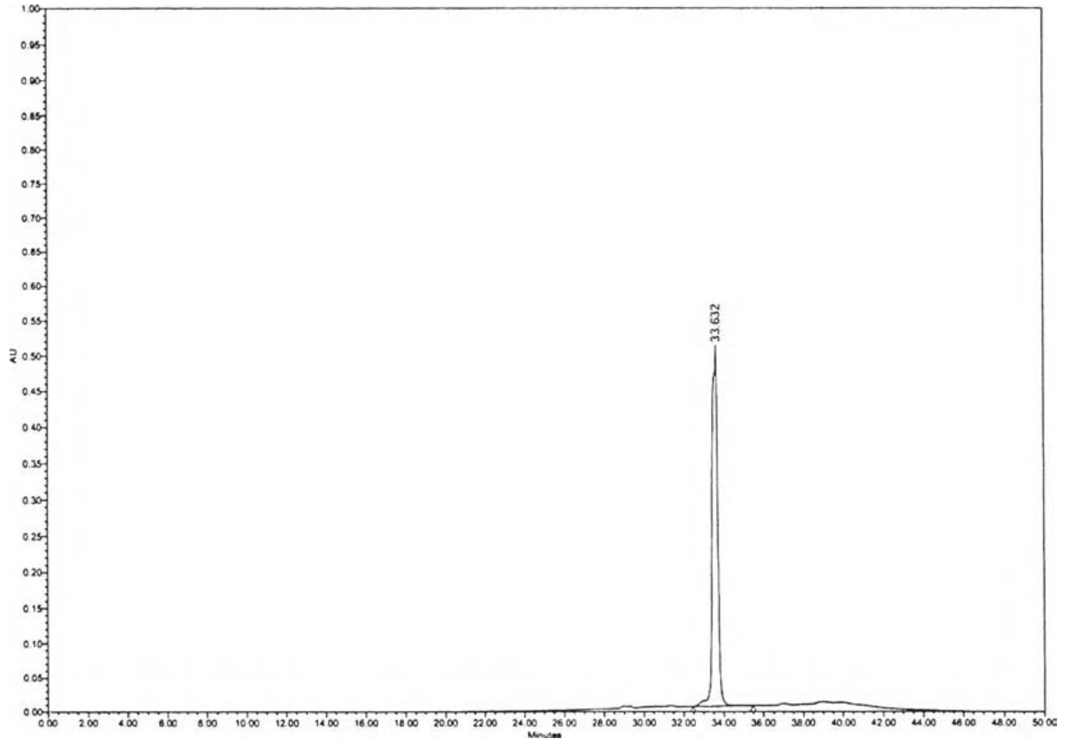


(a)

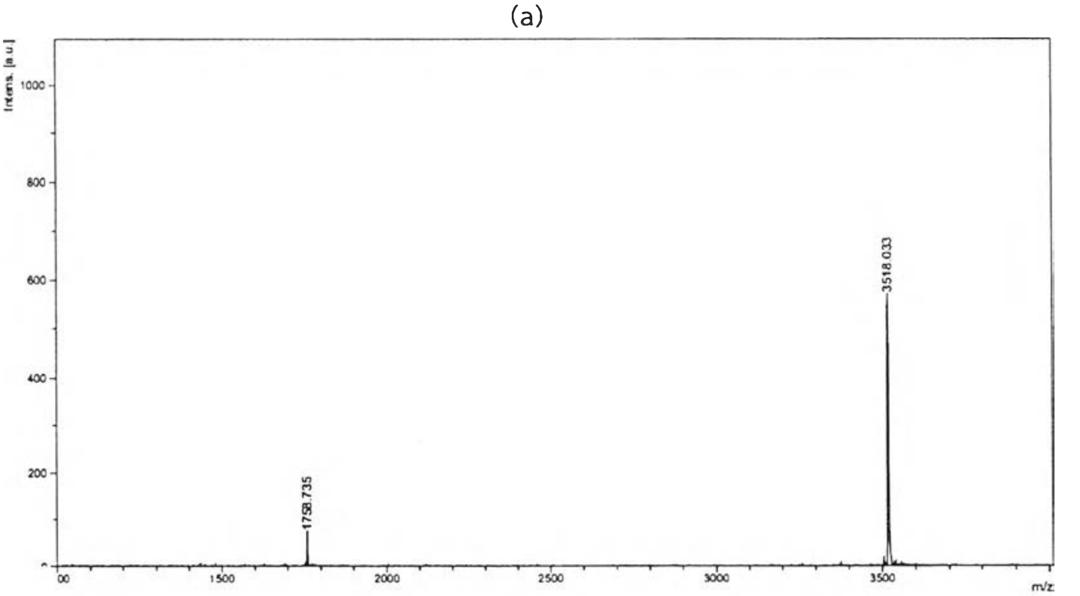


(b)

Figure A14 (a) Analytical HPLC chromatogram (ACE 5 C18-AR (150 x 4.6 mm) HPLC column, water(A)/methanol(B) (started with A:B (90:10) for 5 min followed by a linear gradient to A:B (10:90) over a period of 70 min), flow rate 0.5 mL/min) and (b) MALDI-TOF mass spectrum of PNA T5-2AQ-T4-Lys (calcd for $[M+H]^+$ = 3473.9)

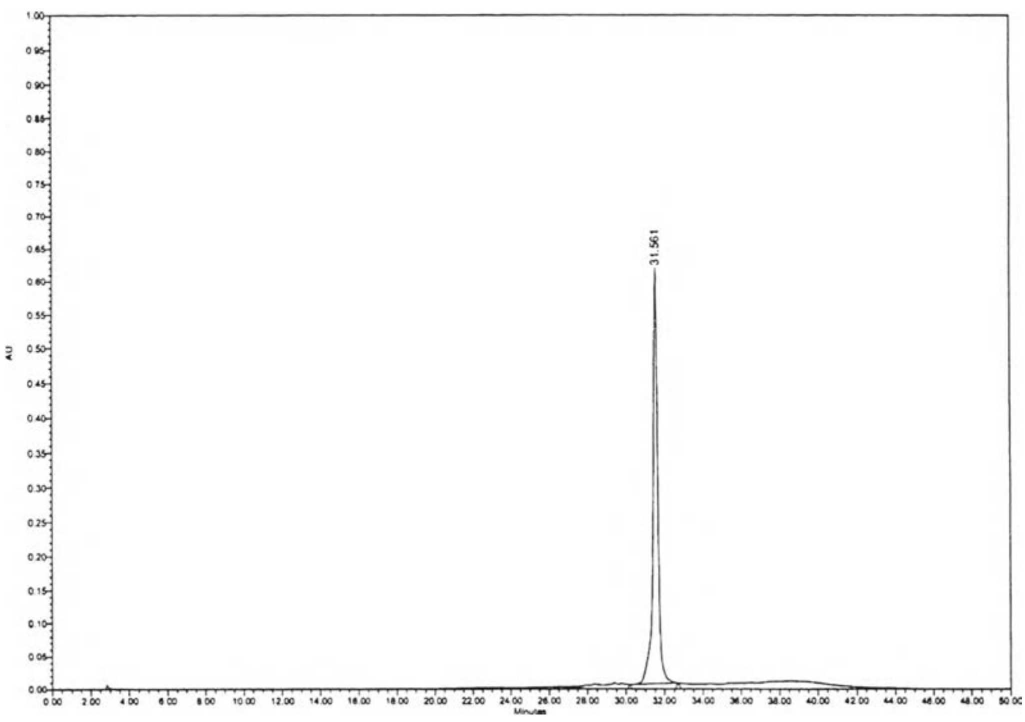


(a)

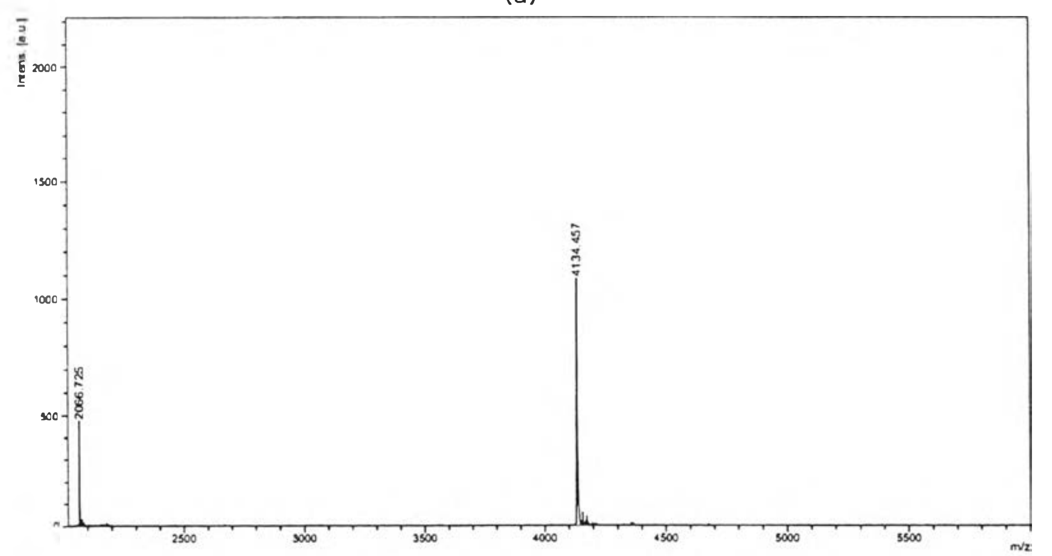


(b)

Figure A15 (a) Analytical HPLC chromatogram (ACE 5 C18-AR (150 x 4.6 mm) HPLC column, water(A)/methanol(B) (started with A:B (90:10) for 5 min followed by a linear gradient to A:B (10:90) over a period of 70 min), flow rate 0.5 mL/min) and (b) MALDI-TOF mass spectrum of PNA T5-MB-T4-Lys (calcd for $[M+H]^+$ = 3518.0)

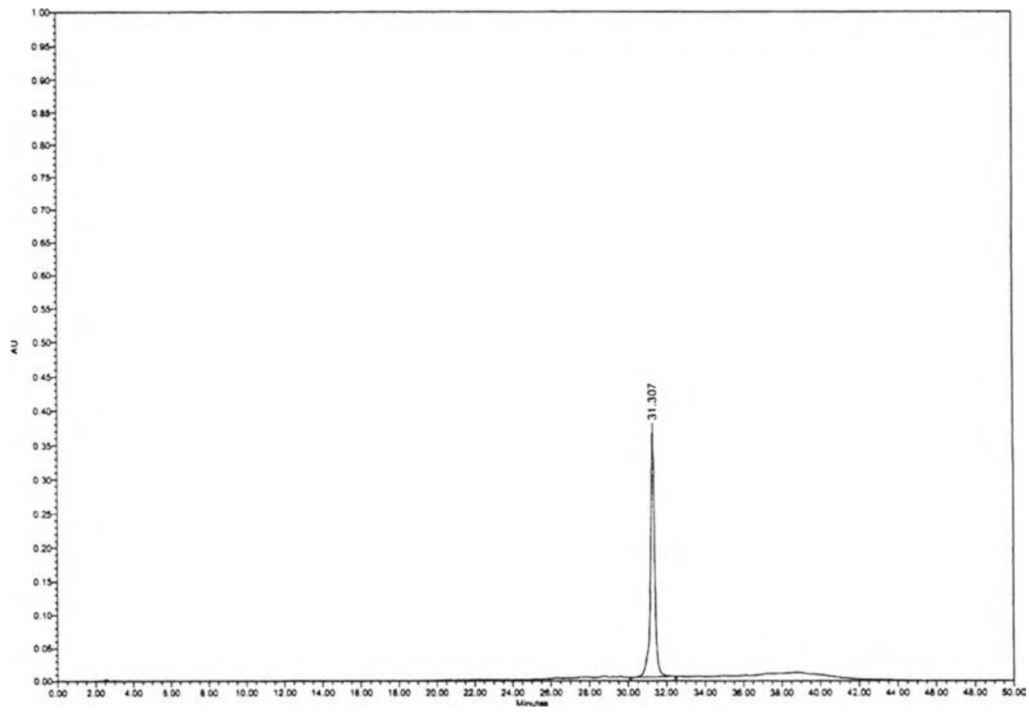


(a)

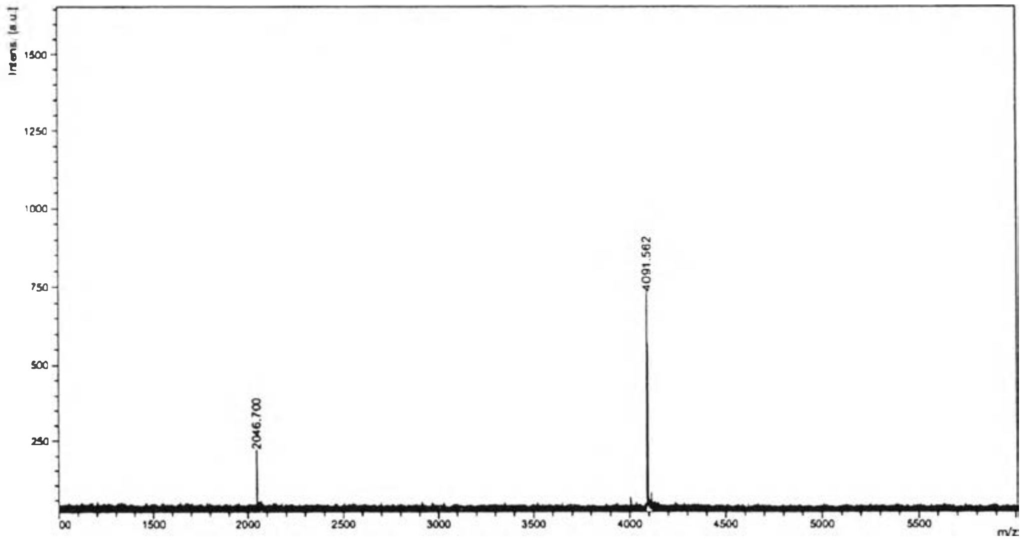


(b)

Figure A16 (a) Analytical HPLC chromatogram (ACE 5 C18-AR (150 x 4.6 mm) HPLC column, water(A)/methanol(B) (started with A:B (90:10) for 5 min followed by a linear gradient to A:B (10:90) over a period of 70 min), flow rate 0.5 mL/min) and (b) MALDI-TOF mass spectrum of PNA 2AQ-WSSV-Lys (calcd for $[M+H]^+$ = 4136.7)

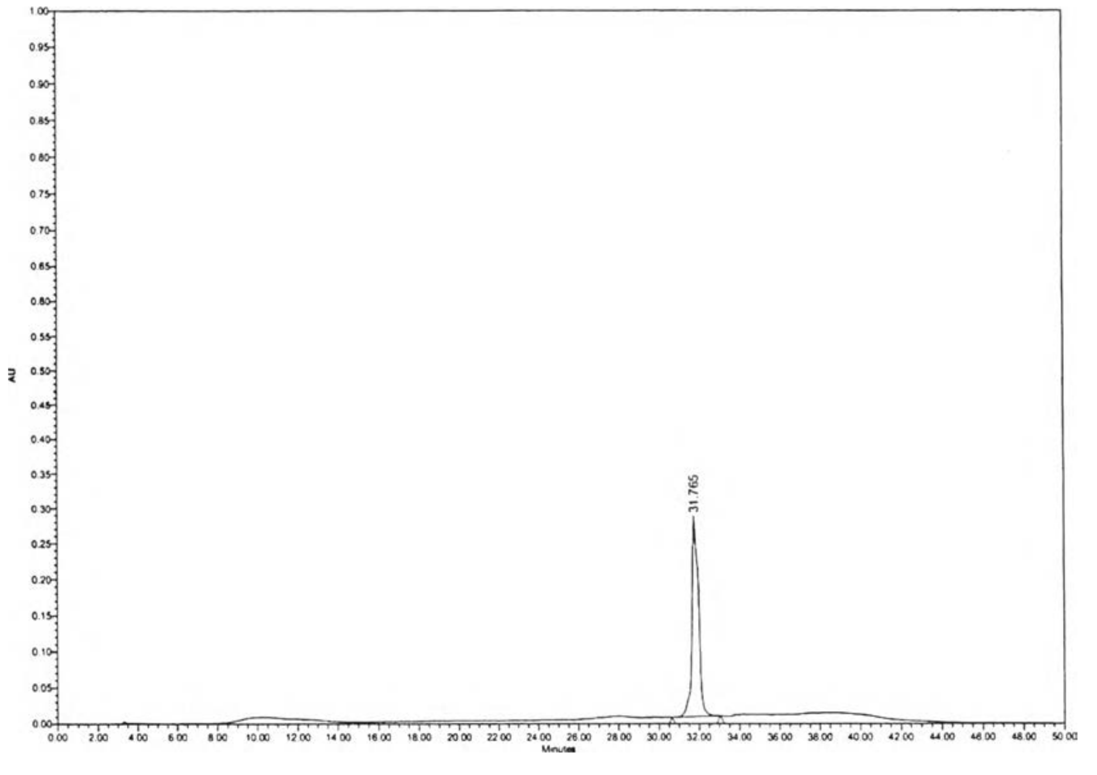


(a)

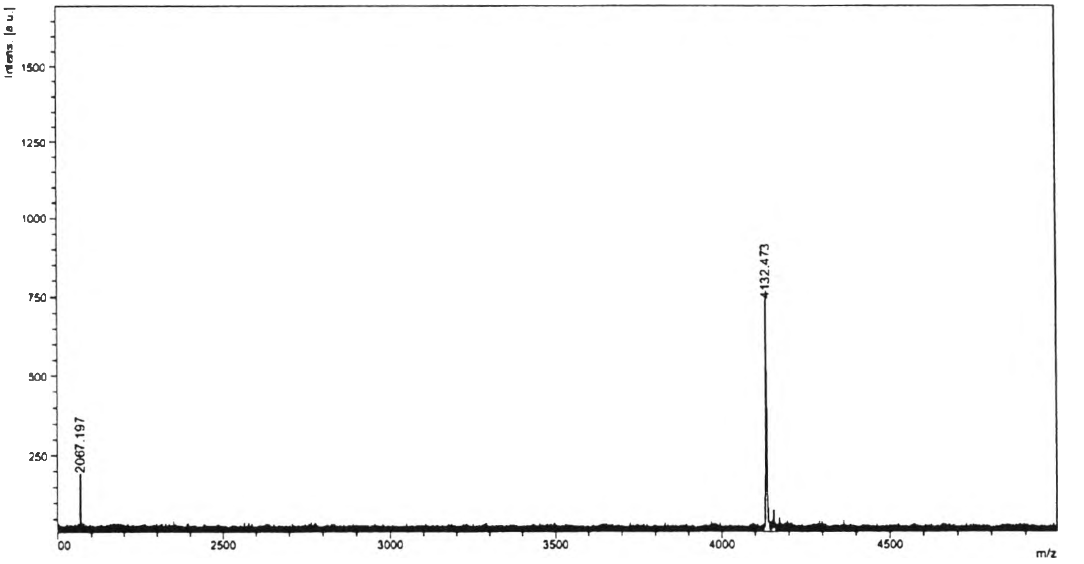


(b)

Figure A17 (a) Analytical HPLC chromatogram (ACE 5 C18-AR (150 x 4.6 mm) HPLC column, water(A)/methanol(B) (started with A:B (90:10) for 5 min followed by a linear gradient to A:B (10:90) over a period of 70 min), flow rate 0.5 mL/min) and (b) MALDI-TOF mass spectrum of PNA 2AQ-WSSV-Ser (calcd for $[M+H]^+$ = 4091.6)

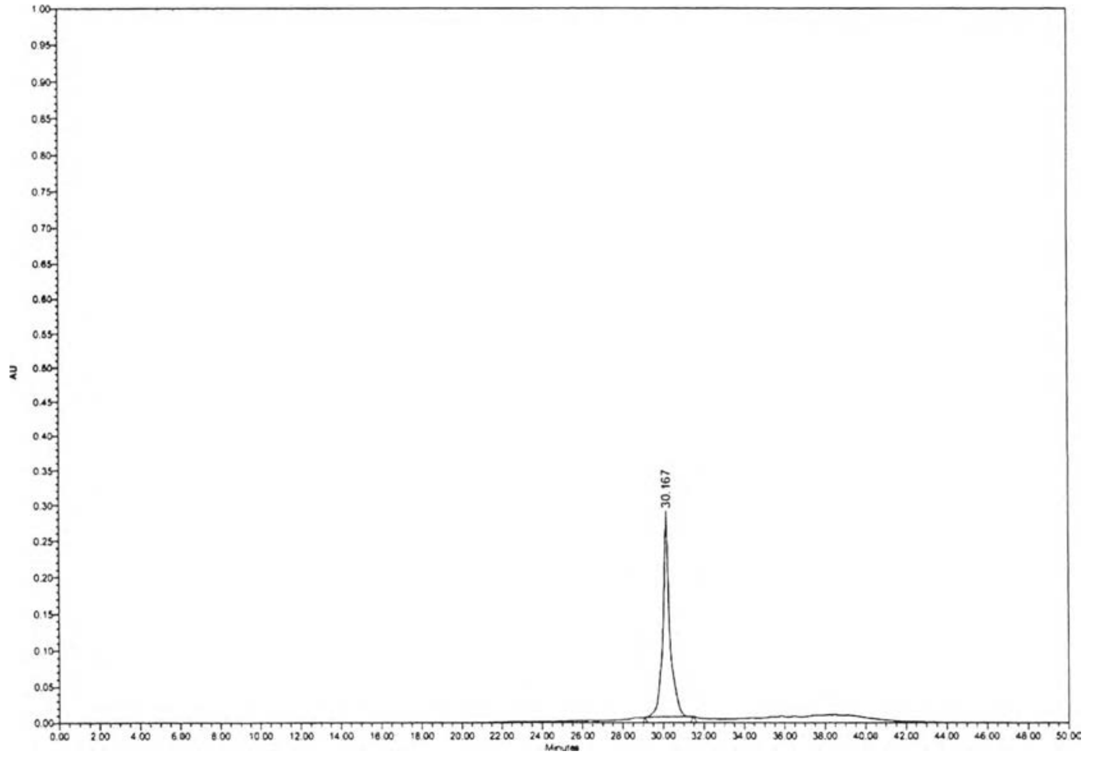


(a)

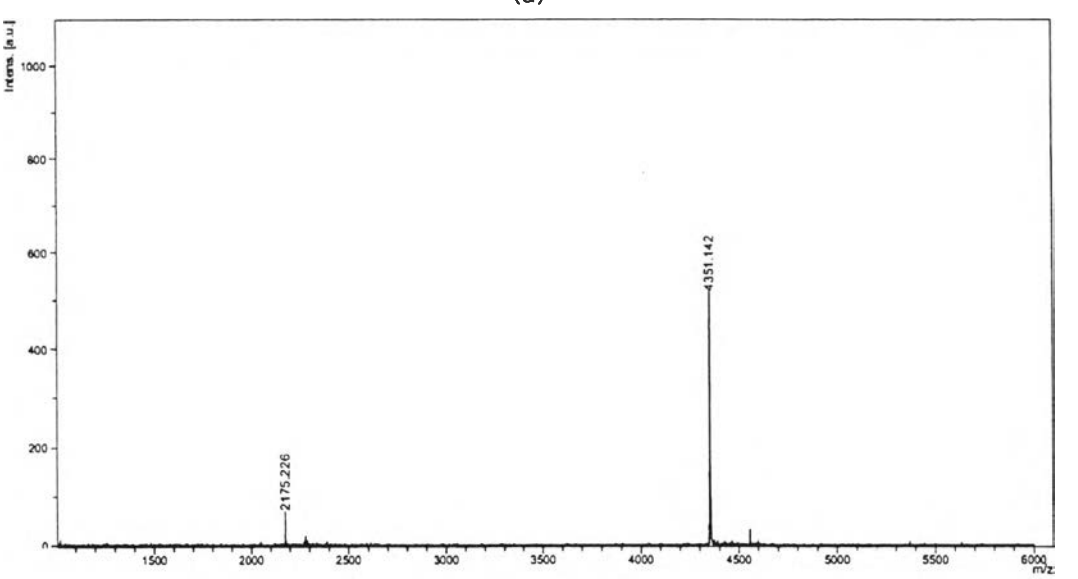


(b)

Figure A18 (a) Analytical HPLC chromatogram (ACE 5 C18-AR (150 x 4.6 mm) HPLC column, water(A)/methanol(B) (started with A:B (90:10) for 5 min followed by a linear gradient to A:B (10:90) over a period of 70 min), flow rate 0.5 mL/min) and (b) MALDI-TOF mass spectrum of PNA 2AQ-WSSV-Glu (calcd for $[M+H]^{+}$ = 4132.5)

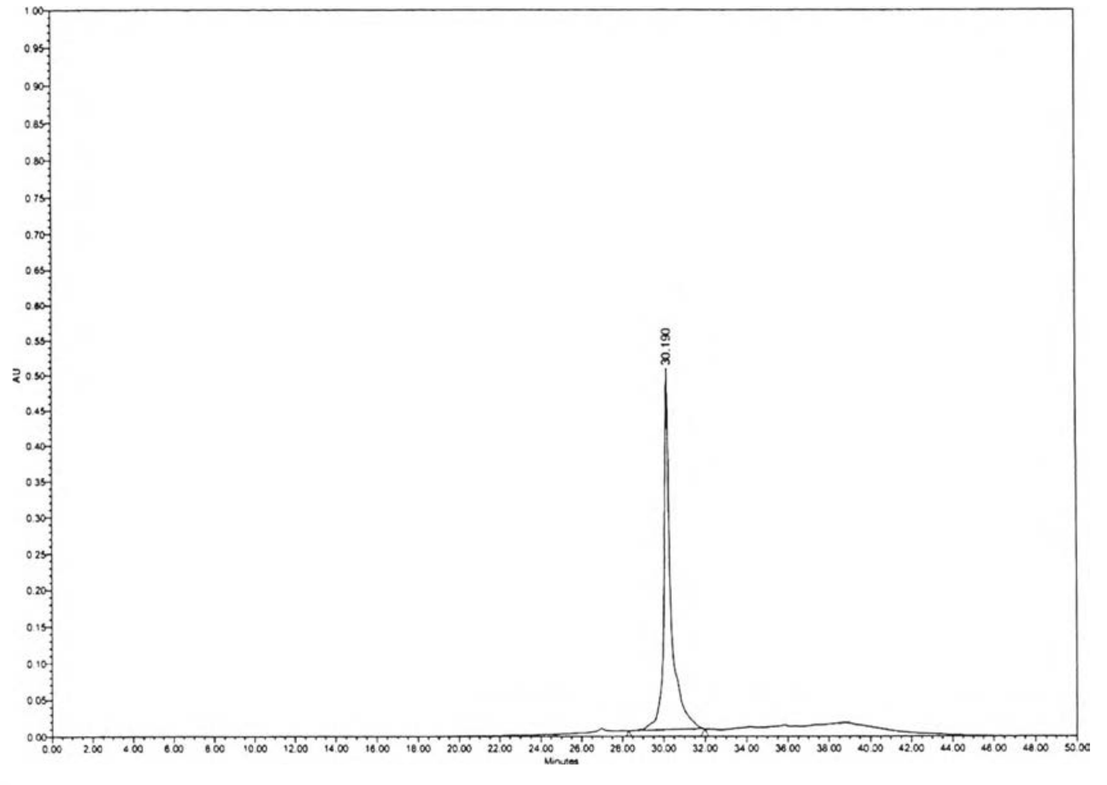


(a)

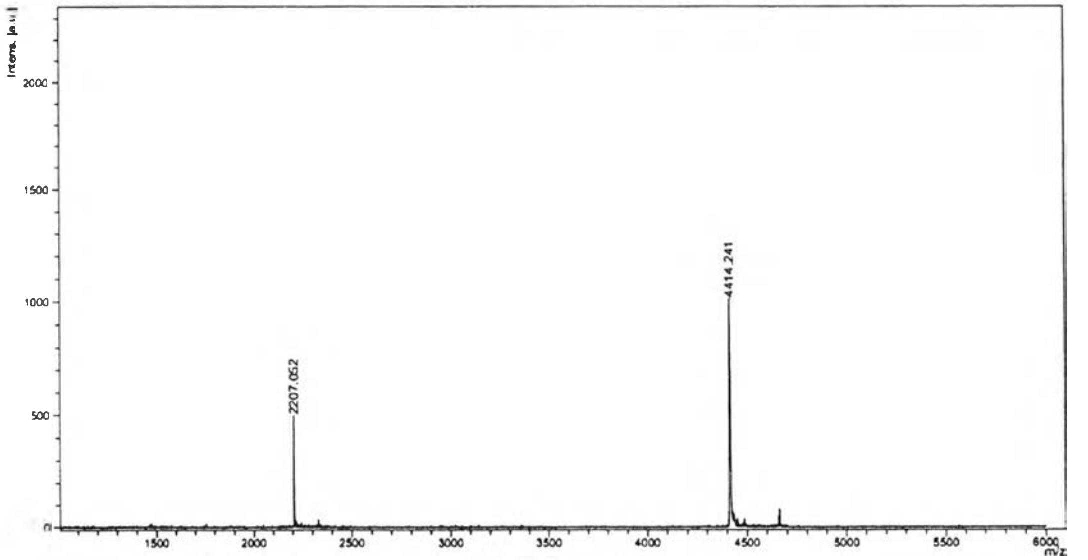


(b)

Figure A19 (a) Analytical HPLC chromatogram (ACE 5 C18-AR (150 x 4.6 mm) HPLC column, water(A)/methanol(B) (started with A:B (90:10) for 5 min followed by a linear gradient to A:B (10:90) over a period of 70 min), flow rate 0.5 mL/min) and (b) MALDI-TOF mass spectrum of PNA 2AQ-B1502-Lys (calcd for $[M+H]^+$ = 4351.1)

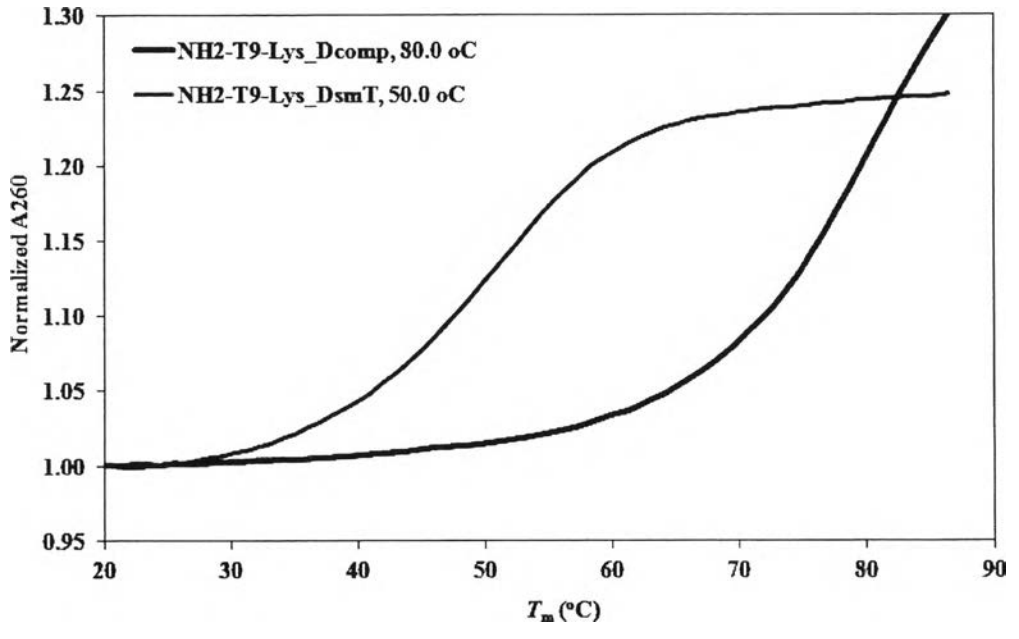


(a)



(b)

Figure A20 (a) Analytical HPLC chromatogram (ACE 5 C18-AR (150 x 4.6 mm) HPLC column, water(A)/methanol(B) (started with A:B (90:10) for 5 min followed by a linear gradient to A:B (10:90) over a period of 70 min), flow rate 0.5 mL/min) and (b) MALDI-TOF mass spectrum of PNA 2AQ-B1513-Lys (calcd for $[M+H]^+$ = 4414.4)



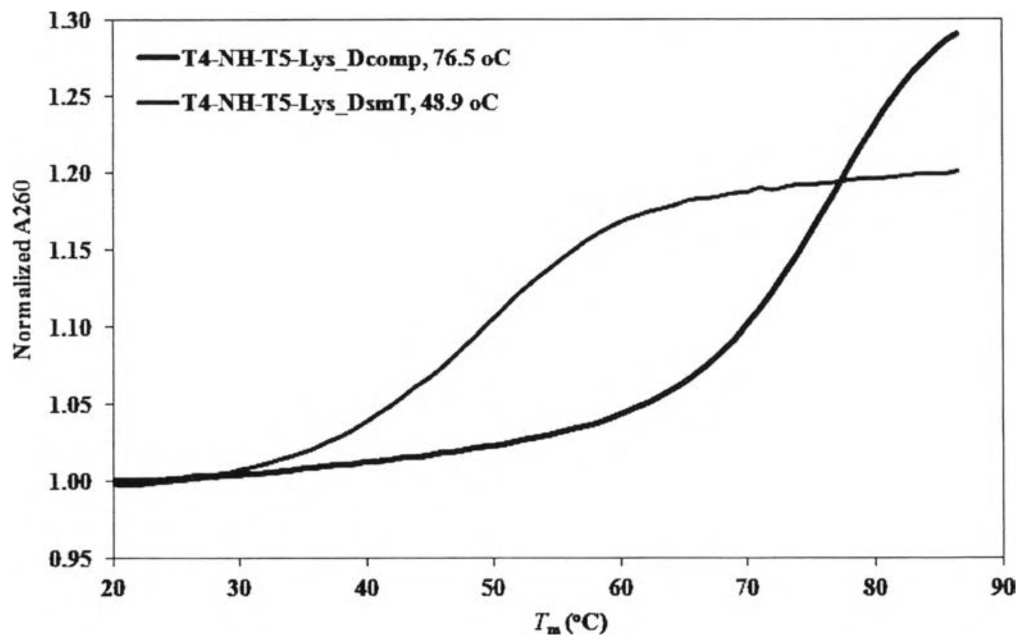
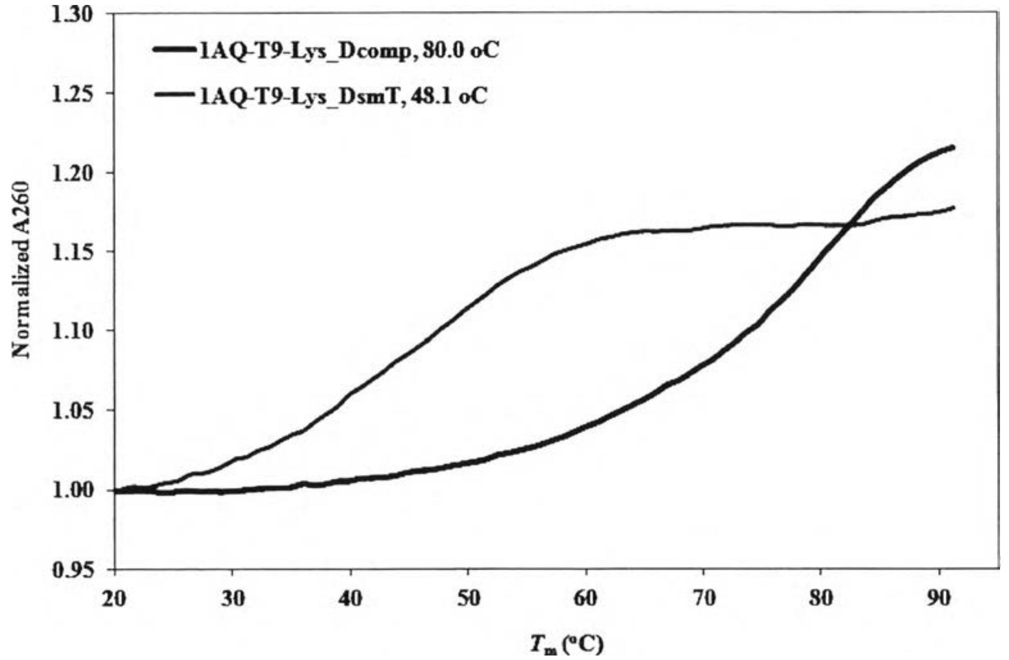


Figure A21 Melting curves of unmodified PNAs T9-Lys (top) and T4-(apc)-T5-Lys (bottom) (1 µM) after hybridized with complementary DNA (dA_9, 1 µM, blue) compared with single base mismatched DNA ($dA_5\underline{T}A_4$, 1 µM, red) in 10 mM phosphate buffer pH 7.0



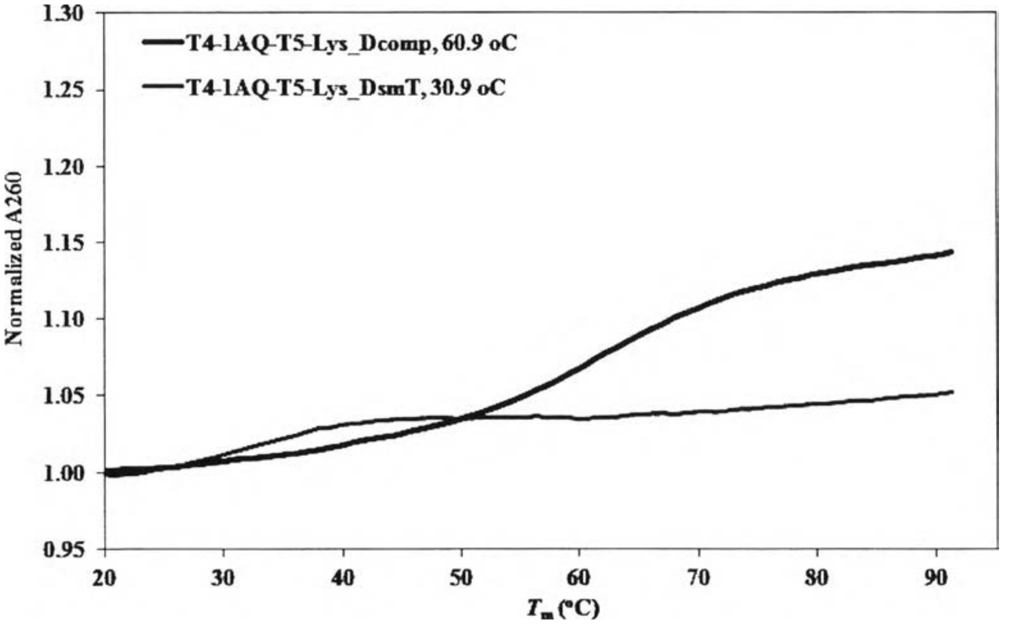
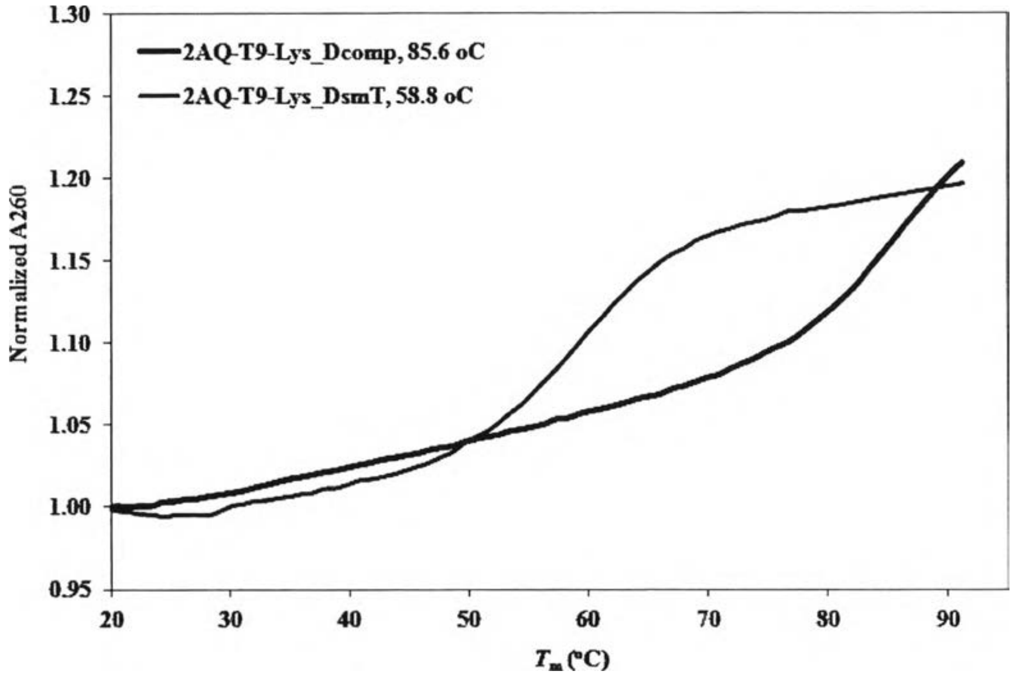


Figure A22 Melting curves of PNAs 1AQ-T9-Lys (top) and T4-1AQ-T5 –Lys (bottom) (1 μM) after hybridized with complementary DNA (dA_9, 1 μM, blue) compared with single base mismatched DNA ($dA_5\underline{T}A_4$, 1 μM, red) in 10 mM phosphate buffer pH 7.0



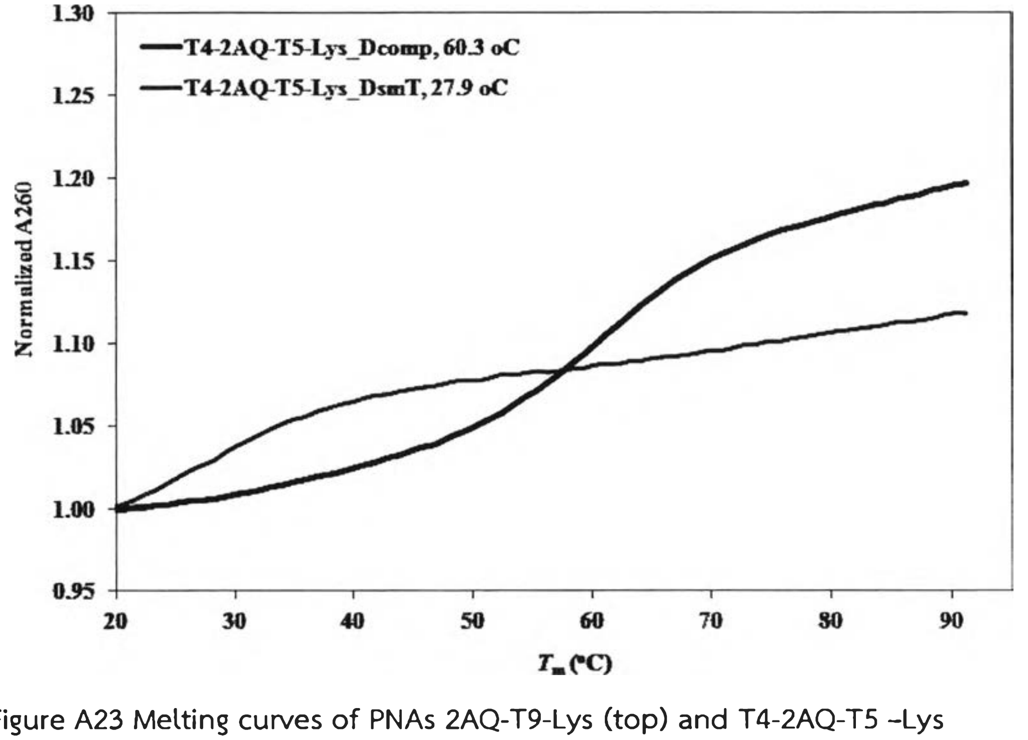
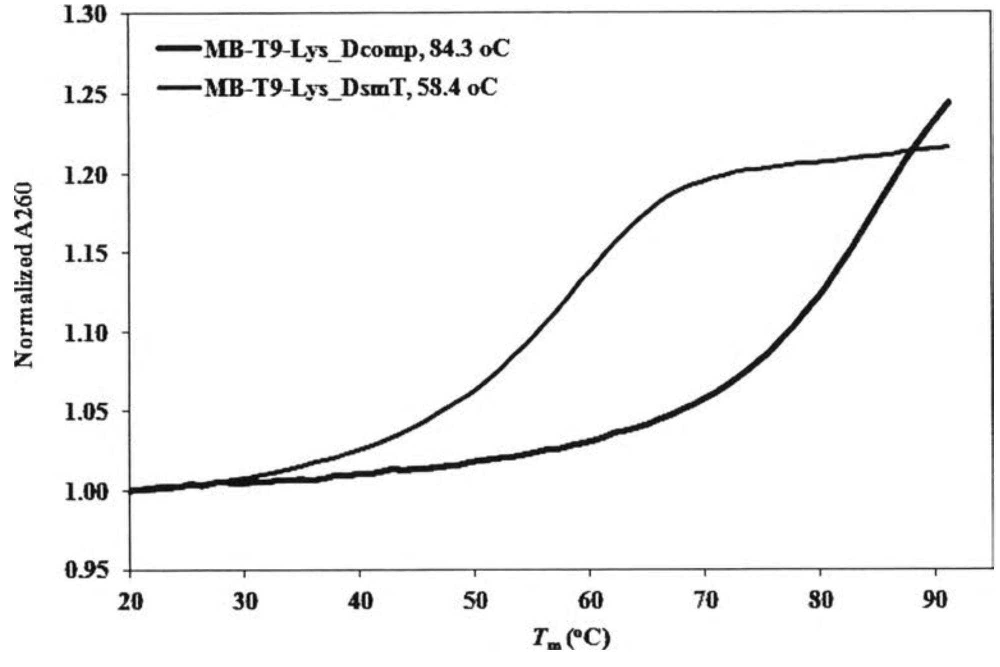


Figure A23 Melting curves of PNAs 2AQ-T9-Lys (top) and T4-2AQ-T5 -Lys (bottom) 1 µM after hybridized with complementary DNA (dA_9, 1 µM, blue) compared with single base mismatched DNA ($dA_5\underline{T}A_4$, 1 µM, red) in 10 mM phosphate buffer pH 7.0



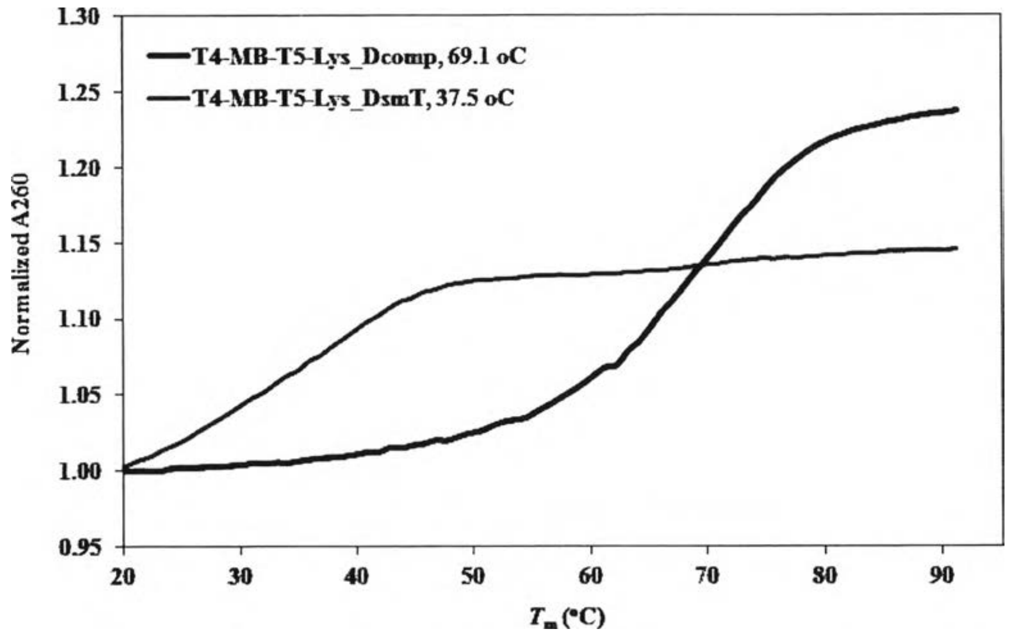


Figure A24 Melting curves of PNAs MB-T9-Lys (top) T4-MB-T5 -Lys (bottom) 1 μM after hybridized with complementary DNA (dA_9, 1 μM, blue) compared with single base mismatch DNA ($dA_5\underline{T}A_4$, 1 μM, red) in 10 mM phosphate buffer pH 7.0

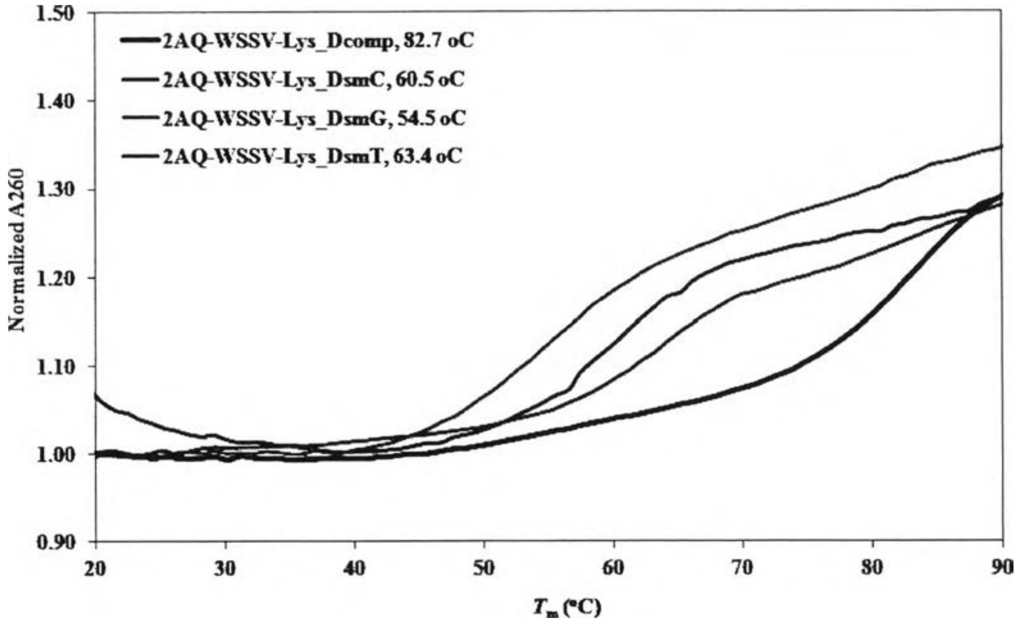
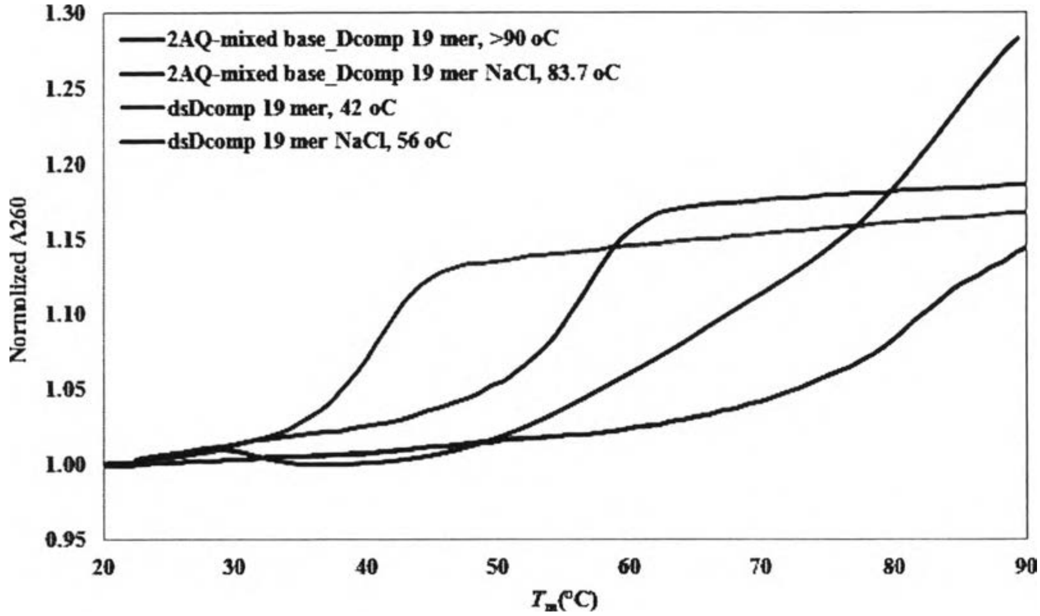


Figure A25 Melting curves of PNA 2AQ-WSSV-Lys (1 μM) after hybridized with complementary DNA (1 μM, blue) compared with three other single base mismatched DNA (1 μM, red, green and purple) in 10 mM phosphate buffer pH 7.0



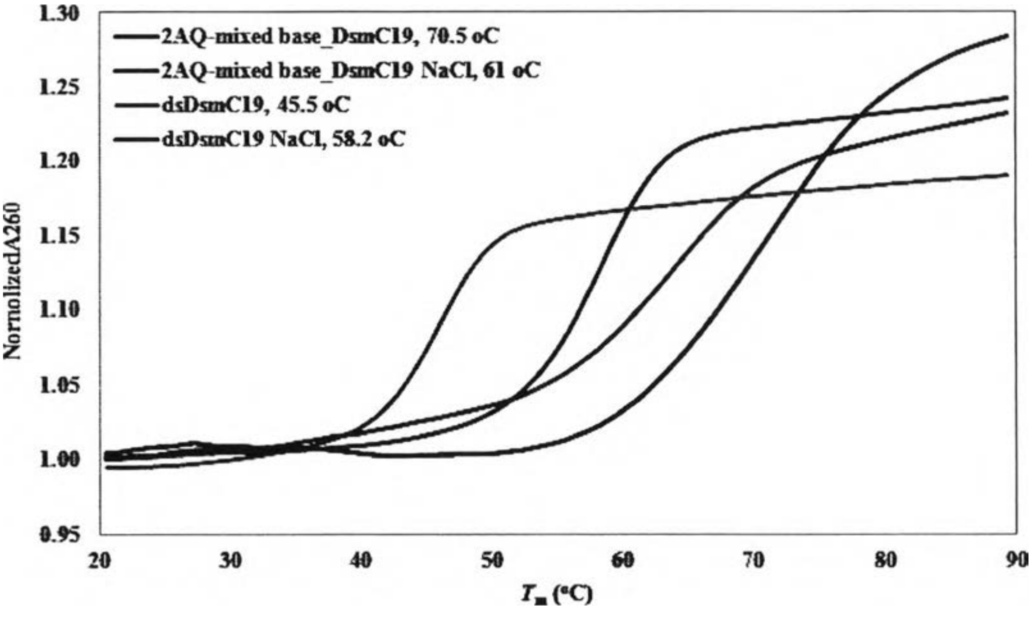
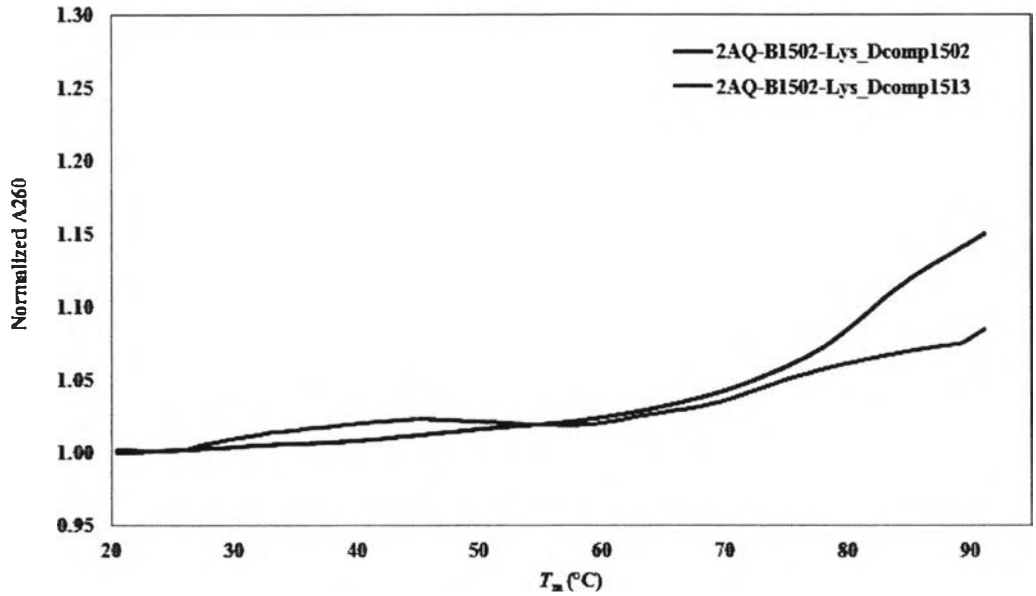


Figure A26 Melting curves of PNA 2AQ-WSSV-Lys (1 µM) after hybridized with 19bp complementary (1 µM) (top) and single base mismatched DNAs (1 µM) (bottom) (blue: without NaCl, red: with 100 mM NaCl). The corresponding melting curves for DNA duplexes (dsDcomp19mer and dsDsmC19mer) (1 µM) are shown in green (without NaCl) and purple (with 100 mM NaCl). All experiments were conducted in 10 mM phosphate buffer pH 7.0.



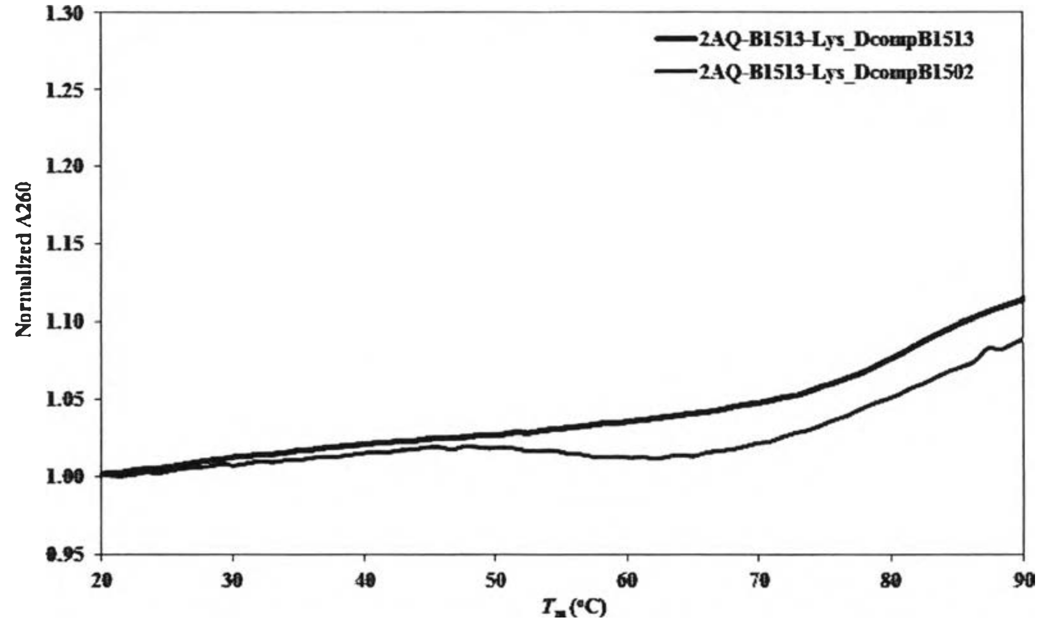
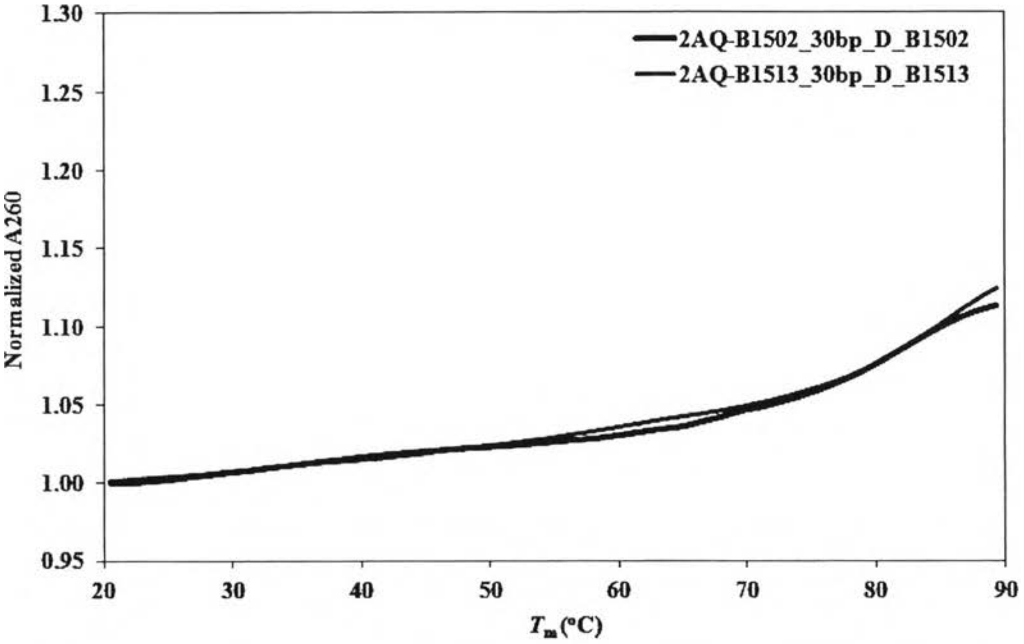


Figure A27 Melting curves of PNAs 2AQ-B1502-Lys (top) and 2AQ-B1513-Lys (bottom) (1 μM) after hybridized with complementary DNA (1 μM, blue) and single base mismatched DNA (1 μM, red) in 10 mM phosphate buffer pH 7.0



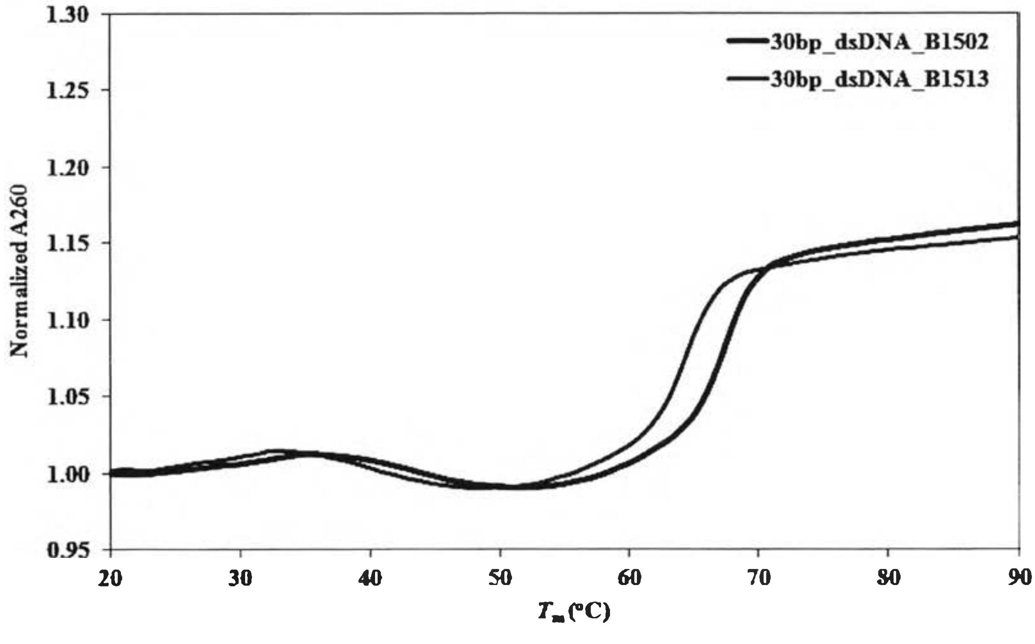


Figure A28 Melting curves of PNAs 2AQ-B1502-Lys (blue) and 2AQ-B1513-Lys (red) (top) (1 μM each) after hybridized with 30bp complementary DNA targets (1 μM) (top) and the melting curves for 30bp DNA duplexes corresponding to B1502 (1 μM, blue) and B1513 (1 μM, red) (bottom). All experiments were conducted in 10 mM phosphate buffer pH 7.0.

VITA

Miss. Jutatip Kongpeth was bom on April 18th, 1989 in Nakhon Srithammarat, Thailand. She obtained her Bachelor's Degree of Science, major in Chemistry from Faculty of Science, Chulalongkorn University in 2010. She began studying her Master's degree at the Department of Chemistry, Faculty of Science, Chulalongkorn University in 2011 and graduated in 2014.

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Experiences

2012

- Teaching assistant at Department of Chemistry, Faculty of Science, Chulalongkorn University (taking care of Varian Mercury 400+ and Bruker Avance 400 NMR instruments) (June 2012 to present)

2013

- Poster presentation "Electrochemically active peptide nucleic acid probes" in Thailand Research Fund's Senior Research Scholar annual meeting (RTA 5280002) at Prince of Songkla University (11 Jan 2013)
- Poster presentation "Synthesis of methylene blue-labeled pyrrolidinyl peptide nucleic acid and applications as a probe for electrochemical detection of DNA sequences" in the 39th Congress on Science and Technology of Thailand (STT39) at BITEC, Bangkok (21-23 Oct 2013).

2014

- Oral presentation "Immobilization-free electrochemical dna sensors based on peptide nucleic acid probes " in Pure and Applied Chemistry International Conference 2014 at Khon Kaen University (8-10 Jan 2014) (Best oral presentation award in Analytical Chemistry session)