SYNTHESIS AND DNA BINDING PROPERTIES OF NOVEL PYRROLIDINE PEPTIDE NUCLEIC ACID CARRYING 3-AMINOPYRROLIDINE-4-CARBOXYLIC ACID SPACER

NISANATH REENABTHUE

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Master of Science Degree in Chemistry
February 2012

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This thesis entitled "Synthesis and DNA binding properties of novel pyrrolidine peptide nucleic acid carrying 3-aminopyrrolidine-4-carboxylic acid spacer" submitted by Nisanath Reenabthue in partial fulfillment of the requirements for the Master of Science Degree in Chemistry is hereby approved.

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ACKNOWLEDGEMENT

I would like to express my sincere gratitude and appreciation to my advisor, Dr. Chaturong Suparpprom for his invaluable advice, guidance, kindness and encouragement throughout the experimental work and make of the thesis. I also would like to all thesis committee members, and deeply grateful for their helpful comments. Furthermore, I would like to especially thank Associate Professor Dr. Tirayut Vilaivan, Mrs. Chotima Vilaivan, Miss Chalothorn Boonlua and TV group from the Department of Chemistry, Faculty of Science, Chulalongkorn University for their important support, invaluable guidance, supervision and constant inspiration throughout this study. I would like to thank the Center of Excellence for Innovation in Chemistry (PERCH-CIC) for financial support. The author expresses her gratitude to NU staff and all officers of the Faculty of Science, Naresuan University for facility support and sacrificing their time on this program. I am grateful to all my teachers for their instructions, my friends and colleagues of Department of Chemistry, Faculty of Science, Naresuan University for their precious helps and co-operation. Lastly, I would like to thank my beloved family for their love, care, financial support and encouragement throughout my life.

Nisanath Reenabthue

Title	SYNTHESIS AND DNA BINDING PROPERTIES OF NOVEL PYRROLIDINE PEPTIDE NUCLEIC ACID CARRYING 3-AMINOPYRROLIDINE-4-CARBOXYLIC ACID SPACER
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Academic Paper	Thesis M.Sc in Chemistry, Naresuan University, 2011
Keywords	β-amino acid, PNA, APC, ACPC, pyrene

ABSTRACT

Peptide nucleic acid (PNA) is a DNA mimic in which the deoxyribose phosphate backbone was substituted with a peptide chain. In this research, novel pyrrolidinyl PNA carrying 3-aminopyrrolidine-4-carboxylic acid (APC) spacers were synthesized. These spacers were designed based on our previously reported PNA system bearing 2-amino-1-cyclopentanecarboxylic acid (ACPC) spacer, with additional nitrogen atom in the cyclopentane ring of the β-amino acid. It is expected that the new spacer will improve the solubility of the PNA and provide a handle for internal modification. The *trans*-(3*R*,4*S*)- and (3*S*,4*R*)-isomers of N^3-Fmoc/N^1-Boc-protected APC were synthesized following a known route. Diastereoselective reductive amination of the keto-ester intermediate, obtained via a Diekmann cyclization of Boc-glycine ethyl ester, with (*S*)- or (*R*)-α-methylbenzylamine followed by protecting group exchange gave the (3*R*,4*S*)- and (3*S*,4*R*)-isomers of the protected spacers in 6.5% and 4.9% overall yield, respectively. These spacers have been successfully incorporated into model PNA sequences by Fmoc solid phase peptide synthesis. Successful incorporation of the new spacers was confirmed by MALDI-TOF MS. DNA binding studies by T_m measurement of **P1**-**P10** PNA sequences, some

of which was modified with a single APC unit in the middle of the strand or full modified APC unit, suggested that the *trans*-(3*R*,4*S*)-APC spacer was compatible with the original PNA backbone carrying (1*S*,2*S*)-2-aminocyclopentanecarboxylic acid (ACPC) spacer as shown by their essentially unchanged T_m. The incorporation of fluorophores such as pyrenecarbonyl and pyrenebutyryl in the middle of the PNA strand *via* the N^1 position of the APC spacer gave modified PNA that shows a high fluorescent intensity change between single-stranded PNA and hybrid of double-stranded PNA·DNA.

LIST OF CONTENTS

Chapter		Page
I	**INTRODUCTION**	1
	Rationale for the study	1
	Objectives of the study	4
	Scope of this Research	4
II	**LITERATURE REVIEW**	4
	Peptide nucleic acid (PNA)	6
	Modification of peptide nucleic acid (PNA)	10
	Application of peptide nucleic acid (PNA) as probes in nucleic acid sequence determination	16
	Synthesis of *trans*-3-aminopyrrolidine-4-carboxylic acid; alternative spacer for probe application	20
III	**RESEARCH METHODOLOGY**	22
	General Procedure	22
	Synthesis of both enantiomeric 3-aminopyrrolidine-4-carboxylic (*apc*) spacers	24
	The procedure for solid phase synthesis of PNA	33
	Solid phase peptide synthesis of *apc*PNA	40
	Biophysical studies	43
IV	**RESULTS AND DISCUSSION**	39
	Synthesis of *apc*PNA oligomers	50
	Hybridization of *apc/acpc*PNA with DNA	54
	Application of fluorescent labeled PNA as a probe for DNA sequence analysis	65

LIST OF CONTENTS (CONT.)

Chapter	Page
V CONCLUSION	72
REFERENCES	81
APPENDIX	51
BIOGRAPHY	66

LIST OF TABLES

Table		Page
1	Sequences of PNA oligomers used in this work	34
2	Gradient system for HPLC purification of PNA	40
3	Yield of PNA sequences in this study	52
4	Characterization data of PNA sequences	54
5	T_m data of PNA (P1-P6) oligomers in this study	57
6	T_m data of DNA (D1) with PNA (P2, P4) hybrid at pH 6.0-8.0	62
7	T_m data of DNA (D1) with PNA (P2, P4) hybrid at [NaCl] 0-500 mM	64
8	T_m data of PNA (P7-P10) oligomers in this study	66
9	Fluorescent intensity of the PNA (P7-P10) oligomers	71

LIST OF FIGURES

Figures		Page
1	Chemical structure of a DNA molecule and an *aeg*PNA molecule.....	1
2	Schematic representation of the antisense inhibition and antigene inhibition. An antigene oligomer (e.g. PNA) could bind to complementary sequence in the DNA and inhibit transcription of the gene. On the other hand, cells can also be treated with an antisense oligonucleotide, and hybridization to a specific mRNA sequence can inhibit the expression of a protein at the level of translation.....	2
3	Structure and fluorescence of single-fluorophore-labelled PNA is observed upon duplex formation.....	3
4	Chemical structures of pyrrolidinyl PNA molecules.....	4
5	Chemical structures of an *aeg*PNA molecule and the hybridization of *aeg*PNAs with DNAs.....	6
6	Schematic representations: (a) PNA-DNA duplex in the antiparallel mode (3'-end of the DNA facing the amino-terminal of the PNA); (b) 2PNA/DNA triplex in the preferred binding mode with antiparallel Watson-Crick strand and parallel Hoogsteen strand.....	7
7	Schematics of *aeg*PNA binding mode for double stranded DNA	8
8	Hydrogen bonding *via* Watson-Crick and Hoogsteen base pairing.....	8
9	Structure of various PNA complexes shown in side view and top view of PNA·RNA, PNA·DNA, PNA·DNA·PNA and PNA·PNA	9
10	Structure of modified PNA backbone; (a) aminoethylglycine (*aeg*) PNA, (b) 4-aminoprolyl PNA, (c) prolylglycyl PNA	11
11	Structure of modified pyrrolidine PNA	12
12	Structure of monomeric and oligomeric (a) *trans*-2-aminocyclohexane carboxylic acid (*trans*-ACHC) and (b) *trans*-2-aminocyclopentane carboxylic acid (*trans*-ACPC)	13

LIST OF FIGURES (CONT.)

Figures		Page
13	Structure of modified PNA carrying various *β*-amino acid spacers; (a) *N*-amino-*N*-methylglycine, (b) L-aminopyrrolidine-2-carboxylic acid (L-Apc), (c) D-aminopyrrolidine-2-carboxylic acid (D-Apc), (d) (1*R*, 2*S*)-2-aminocyclopentane carboxylic acid (L-Acpc) (e) *β*-alanine (*β*-ala)	14
14	Structure of pyrrolidinyl PNA carrying 2-aminocyclopentane carboxylic acid (ACPC) spacers	15
15	Structure of two configurations of pyrrolidinyl PNA carrying 2-aminocyclopentane carboxylic acid (ACPC) spacers	16
16	Strategy for SNP detection by the PNA/Nuclease/Dye system	17
17	Strategy for nucleic acid detection *via* light-up probe (a) chemical structure of light-up probes (b) schematic showing how mixed sequence (I) and homopyrimidine (II) light-up probes may interact with single-stranded target nucleic acid.	18
18	Structure and fluorescence of the single-fluorophore-labelled PNA is observed upon duplex formation	19
19	The *acpc*PNA was used for DNA sequence analysis by a new ion-exchange capture technique	19
20	The synthetic scheme of a protected version of 3-aminopyrrolidine-4-carboxylic acid	20
21	The new route of synthesis of 3-aminopyrrolidine-4-carboxylic acid	21
22	A diagram showing the manual technique for solid phase peptide synthesis; (a) coupling, deprotection and cleaving process; (b) washing process	34
23	The procedure for solid phase synthesis of PNA	36
24	(a) *acpc*PNA, (b) *(3R,4S)-apc*PNA and (c) *(3S,4R)-apc*PNA	46

LIST OF FIGURES (CONT.)

Figures **Page**

25 The synthetic scheme of (3*R*) -aminopyrrolidine-(4*S*)-carboxylic acid derivative 47

26 Mechanism of Diekmann cyclization of Boc-glycine ethyl ester (2) to give ethyl 1-*tert*-butoxycarbonyl-3-oxopyrrolidine-4-carboxylate (3) 49

27 TantaGel S RAM Fmoc resin 51

28 (a) HPLC chromatogram of purified PNA (P2) and (b) MALDI-TOF MS of purified PNA (P2) 53

29 The change of UV absorbance in a typical thermal denaturation experiment of DNA duplex 55

30 The first derivative plot obtained from a typical thermal denaturation experiment of DNA duplex 56

31 T_m curves of PNA (P2-6) hybrid with complementary DNA Condition: 10 mM sodium phosphate buffer pH 7.0, 100 mM NaCl, [PNA] = 1.0 μM and [DNA] = 1.0 μM 56

32 UV titration plot of DNA (D1) and T_9 PNA (P2, P3 and P4) Condition: 10 mM sodium phosphate buffer pH 7.0, 100 mM NaCl and [PNA] = 2.0 μM 59

33 CD titration spectra of DNA (D1) and T_9 PNA (P2) Condition: 10 mM sodium phosphate buffer pH 7.0, 100 mM sodium chloride, [PNA] = 2.5 μM and [DNA] = 3.0 μM 60

34 CD titration spectra of DNA (D1) and T_9 PNA (P3) Condition: 10 mM sodium phosphate buffer pH 7.0, 100 mM sodium chloride, [PNA] = 2.5 μM and [DNA] = 3.0 μM 60

35 CD titration spectra of DNA (D1) and T_9 PNA (P4) Condition: 10 mM sodium phosphate buffer pH 7.0, 100 mM sodium chloride, [PNA] = 2.5 μM and [DNA] = 3.0 μM 61

LIST OF FIGURES (CONT.)

Figures		Page
36	T_m curves of DNA (D1) with PNA (P2) hybrid at pH 6.0-8.0 Condition: 100 mM NaCl, [PNA] = 1.0 μM and [DNA] = 1.0 μM	62
37	T_m curves of DNA (D1) with PNA (P4) hybrid at pH 6.0-8.0 Condition: 100 mM NaCl, [PNA] = 1.0 μM and [DNA] = 1.0 μM	63
38	T_m curves of DNA·PNA (P2) hybrid at [NaCl] = 0, 100, 500 mM Condition: 10 mM sodium phosphate buffer pH 7.0, [PNA] = 1.0 μM and [DNA] = 1.0 μM	64
39	T_m curves of DNA·PNA (P4) hybrid at [NaCl] = 0, 100, 500 mM Condition: 10 mM sodium phosphate buffer pH 7.0, [PNA] = 1.0 μM and [DNA] = 1.0 μM	65
40	Fluorescence spectra of pyrene-labeled *apc/acpc*PNA P7 (—), P7 + complementary DNA (—), P7 + single mismatch DNA (—). Conditions: 10 mM sodium phosphate buffer, pH 7.0, [PNA] = 2.5 μM and [DNA] = 3.0 μM, excitation wavelength = 345 nm.	67
41	Fluorescence spectra of pyrene-labeled *apc/acpc*PNA P9 (—), P9 + complementary DNA (—), P9 + single mismatch DNA (—). Conditions: 10 mM sodium phosphate buffer, pH 7.0, [PNA] = 2.5 μM and [DNA] = 3.0 μM, excitation wavelength = 345 nm.	67
42	Photographs of pyrene-labeled P7 and P9 in the presence and absence of the DNA targets under UV light (365 nm). Conditions: 10 mM sodium phosphate buffer, pH 7.0, [PNA] = 2.5 μM and [DNA] = 3.0 μM	68
43	Fluorescence spectra of pyrene-labeled *apc/acpc*PNA P8 (—), P8 + complementary DNA (—), P8 + single mismatch DNA (—). Conditions: 10 mM sodium phosphate buffer, pH 7.0, [PNA] = 2.5 μM and [DNA] = 3.0 μM, excitation wavelength = 345 nm.	69

LIST OF FIGURES (CONT.)

Figures **Page**

44 Fluorescence spectra of pyrene-labeled *apc/acpc*PNA P10 (—), P10 + complementary DNA (—), P10 + single mismatch DNA (—). Conditions: 10 mM sodium phosphate buffer, pH 7.0, [PNA] = 2.5 μM and [DNA] = 3.0 μM, excitation wavelength = 345 nm. 69

45 Photographs of pyrene-labeled P8 and P10 in the presence and absence of the DNA targets under UV light (365 nm). Conditions: 10 mM sodium phosphate buffer, pH 7.0, [PNA] = 2.5 μM and [DNA] = 3.0 μM 70

46 ^{1}H NMR spectrum of *N*-Boc glycine ethyl ester (2) 82

47 ^{13}C NMR spectrum of *N*-Boc glycine ethyl ester (2) 82

48 ^{1}H NMR spectrum of Ethyl 1-*tert*-butoxycarbonyl-3-oxopyrrolidine-4-carboxylate (3) 83

49 ^{13}C NMR spectrum of Ethyl 1-*tert*-butoxycarbonyl-3-oxopyrrolidine-4-carboxylate (3) 83

50 ^{1}H NMR spectrum of (3*R*,4*S*)-1-(*N*-*tert*-butoxycarbonyl)-3-[(1'*S*)-phenylethylamino]-4-ethoxycarbonylpyrrolidine hydrochloride (5) 84

51 ^{13}C NMR spectrum of (3*R*,4*S*)-1-(*N*-*tert*-butoxycarbonyl)-3-[(1'*S*) - phenylethylamino]-4-ethoxycarbonylpyrrolidine hydrochloride (5) 84

52 ^{1}H NMR spectrum of (3*R*,4*S*)-1-(*N*-*tert*-Butoxycarbonyl)-3-(9*H*-fluoren-9-yl-methoxycarbonylamino)-4-carboxypyrrolidine (8) 85

53 ^{13}C NMR spectrum of (3*R*,4*S*)-1-(*N*-*tert*-Butoxycarbonyl)-3-(9*H*-fluoren-9-yl-methoxycarbonylamino)-4-carboxypyrrolidine (8) 85

54 ^{1}H NMR spectrum of (3*R*,4*S*)-1-(*N*-*tert*-Butoxycarbonyl)-3-(9*H*-fluoren-9-yl-methoxycarbonylamino)-4-(pentafluorophenoxy) carbonyl) pyrrolidine (9) 86

LIST OF FIGURES (CONT.)

Figures		Page
55	^{13}C NMR spectrum of (3*R*,4*S*)-1-(*N*-*tert*-Butoxycarbonyl)-3-(9*H*-fluoren-9-yl-methoxycarbonylamino)-4-(pentafluorophenoxy) carbonyl) pyrrolidine (9)	86
56	Mass spectrum of (3*R*,4*S*)-1-(*N*-*tert*-Butoxycarbonyl)-3-(9*H*-fluoren-9-yl-methoxycarbonylamino)-4-(pentafluorophenoxy)carbonyl) pyrrolidine (9)	87
57	^{1}H NMR spectrum of (3*R*,4*S*)-1-(2,2,2-trifluoroacetyl)-3-(9*H*-fluoren-9-yl-methoxycarbonylamino)-4-((pentafluorophenoxy)carbonyl) pyrrolidine (10)	88
58	^{13}C NMR spectrum of (3*R*,4*S*)-1-(2,2,2-trifluoroacetyl)-3-(9*H*-fluoren-9-yl-methoxycarbonylamino)-4-((pentafluorophenoxy)carbonyl) pyrrolidine (10)	88
59	Mass spectrum of (3*R*,4*S*)-1-(2,2,2-trifluoroacetyl)-3-(9*H*-fluoren-9-yl-methoxycarbonylamino)-4-((pentafluorophenoxy)carbonyl) pyrrolidine (10)	89
60	^{1}H NMR spectrum of (3*S*,4*R*)-1-(*N*-*tert*-butoxycarbonyl)-3-[(1'*S*)-phenylethylamino]-4-ethoxycarbonylpyrrolidine hydrochloride (12)	89
61	^{13}C NMR spectrum of (3*S*,4*R*)-1-(*N*-*tert*-butoxycarbonyl)-3-[(1'*S*)-phenylethylamino]-4-ethoxycarbonylpyrrolidine hydrochloride (12)	90
62	^{1}H NMR spectrum of (3*S*,4*R*)-1-(*N*-*tert*-Butoxycarbonyl)-3-(9*H*-fluoren-9-yl-methoxycarbonylamino)-4-carboxypyrrolidine (15)	90
63	^{13}C NMR spectrum of (3*S*,4*R*)-1-(*N*-*tert*-Butoxycarbonyl)-3-(9*H*-fluoren-9-yl-methoxycarbonyl amino)-4-carboxypyrrolidine (15)	91
64	^{1}H NMR spectrum of (3*S*,4*R*)-1-(*N*-*tert*-Butoxycarbonyl)-3-(9*H*-fluoren-9-yl-methoxycarbonylamino)-4-((pentafluorophenoxy) carbonyl) pyrrolidine (17)	91

LIST OF FIGURES (CONT.)

Figures **Page**

65 ^{13}C NMR spectrum of (3*S*,4*R*)-1-(*N*-*tert*-Butoxycarbonyl)-3-(9*H*-fluoren-9-yl-methoxycarbonylamino)-4-((pentafluorophenoxy) carbonyl) pyrrolidine (17) 92

66 Mass spectrum of (3*S*,4*R*)-1-(*N*-*tert*-Butoxycarbonyl)-3-(9*H*-fluoren-9-yl-methoxycarbonylamino)-4-((pentafluorophenoxy)carbonyl) pyrrolidine (17) 92

67 HPLC chromatogram of Bz-TTTTTTTTT-Lys-NH_2 (P2) 93

68 HPLC chromatogram of Bz-TTTTTTTTT -Lys-NH_2 (P3) 93

69 HPLC chromatogram of Bz-TTTTTTTTT-Lys-NH_2 (P4) 94

70 HPLC chromatogram of Bz-TTTTTTTTT-Lys-NH_2 (P5) 94

71 HPLC chromatogram of Bz-GTAGATCAC T-Lys-NH_2 (P6) 95

72 HPLC chromatogram of Ac-TTTT$^{(Py)}$TTTTT-Lys-NH_2 (P7) 95

73 HPLC chromatogram of Ac-TTTT$^{(PyBu)}$TTTTT-Lys-NH_2 (P8) 95

74 HPLC chromatogram of Ac-GTAGA$^{(Py)}$TCAC T-Lys-NH_2 (P9) 96

75 HPLC chromatogram of Bz-GTAGA$^{(PyBu)}$TCAC T-Lys-NH_2 (P10)..... 97

76 MALDI-TOF mass spectrum of Bz-TTTTTTTTT-Lys-NH_2 (P2) 97

77 MALDI-TOF mass spectrum of Bz-TTTTTTTTT -Lys-NH_2 (P3) 98

78 MALDI-TOF mass spectrum of Bz-TTTTTTTTT-Lys-NH_2 (P4) 98

79 MALDI-TOF mass spectrum of Bz-TTTTTTTTT-Lys-NH_2 (P5) 99

80 MALDI-TOF mass spectrum of Bz-GTAGATCAC T-Lys-NH_2 (P6).....

81 MALDI-TOF mass spectrum of Ac-TTTT$^{(Py)}$TTTTT-Lys-NH_2 (P7) 100

82 MALDI-TOF mass spectrum of Ac-TTTT$^{(PyBu)}$TTTTT-Lys-NH_2 (P8)..... 100

LIST OF FIGURES (CONT.)

Figures **Page**

83 MALDI-TOF mass spectrum of Ac-GTAGA$^{(Py)}$TCAC T-Lys-NH_2 (P9)..... 101

84 MALDI-TOF mass spectrum of Bz-GTAGA$^{(PyBu)}$TCAC T-Lys-NH_2 (P10)... 101

85 The melting curves of PNA P1 hybrid with DNA D1. The T_m measured at a ratio of PNA:DNA =1:1, [PNA] = 1.0 μM and [DNA] = 1.0 μM, 10 mM sodium phosphate buffer pH 7.0, 100 mM NaCl. 102

86 The melting curves of PNA P1 hybrid with DNA D1. The T_m measured at a ratio of PNA:DNA =1:1, [PNA] = 1.0 μM and [DNA] = 1.0 μM, 10 mM sodium phosphate buffer pH 6.0-8.0, 100 mM NaCl..... 102

87 The melting curves of PNA P2 hybrid with DNA D1 and D2. The T_m measured at a ratio of PNA:DNA =1:1, [PNA] = 1.0 μM and [DNA] = 1.0 μM, 10 mM sodium phosphate buffer pH 7.0, 100 mM NaCl..... 103

88 The melting curves of PNA P2 hybrid with DNA D1. The T_m measured at a ratio of PNA:DNA =1:1, [PNA] = 1.0 μM and [DNA] = 1.0 μM, 10 mM sodium phosphate buffer pH 6.0-8.0, 100 mM NaCl..... 103

89 The melting curves of PNA P2 hybrid with DNA D1. The T_m measured at a ratio of PNA:DNA =1:1, [PNA] = 1.0 μM and [DNA] = 1.0 μM, 10 mM sodium phosphate buffer pH 7.0, 0, 100,500 mM NaCl. 104

90 The melting curves of PNA P3 hybrid with DNA D1. The T_m measured at a ratio of PNA:DNA =1:1, [PNA] = 1.0 μM and [DNA] = 1.0 μM, 10 mM sodium phosphate buffer pH 7.0, 100 mM NaCl. 104

91 The melting curves of PNA P4 hybrid with DNA D1 and D2. The T_m measured at a ratio of PNA:DNA =1:1, [PNA] = 1.0 μM and [DNA] = 1.0 μM, 10 mM sodium phosphate buffer pH 7.0, 100 mM NaCl..... 105

LIST OF FIGURES (CONT.)

Figures **Page**

92 The melting curves of PNA P4 hybrid with DNA D1. The T_m measured at a ratio of PNA:DNA =1:1, [PNA] = 1.0 μM and [DNA] = 1.0 μM, 10 mM sodium phosphate buffer pH 6.0-8.0, 100 mM NaCl. 105

93 The melting curves of PNA P4 hybrid with DNA D1. The T_m measured at a ratio of PNA:DNA =1:1, [PNA] = 1.0 μM and [DNA] = 1.0 μM, 10 mM sodium phosphate buffer pH 7.0, 0, 100, 500 mM NaCl. 106

94 The melting curves of PNA P5 hybrid with DNA D1. The T_m measured at a ratio of PNA:DNA =1:1, [PNA] = 1.0 μM and [DNA] = 1.0 μM, 10 mM sodium phosphate buffer pH 7.0, 100 mM NaCl. 106

95 The melting curves of PNA P6 hybrid with DNA D3 and D4. The T_m measured at a ratio of PNA:DNA =1:1, [PNA] = 1.0 μM and [DNA] = 1.0 μM, 10 mM sodium phosphate buffer pH 7.0, 100 mM NaCl..... 107

96 The melting curves of PNA P7 hybrid with DNA D3 and D4. The T_m measured at a ratio of PNA:DNA =1:1, [PNA] = 1.0 μM and [DNA] = 1.0 μM, 10 mM sodium phosphate buffer pH 7.0, 100 mM NaCl..... 107

97 The melting curves of PNA P8 hybrid with DNA D3 and D4. The T_m measured at a ratio of PNA:DNA =1:1, [PNA] = 1.0 μM and [DNA] = 1.0 μM, 10 mM sodium phosphate buffer pH 7.0, 100 mM NaCl..... 108

98 The melting curves of PNA P9 hybrid with DNA D3 and D4. The T_m measured at a ratio of PNA:DNA =1:1, [PNA] = 1.0 μM and [DNA] = 1.0 μM, 10 mM sodium phosphate buffer pH 7.0, 100 mM NaCl..... 108

LIST OF FIGURES (CONT.)

Figures **Page**

99 The melting curves of PNA P10 hybrid with DNA D3 and D4. The T_m measured at a ratio of PNA:DNA =1:1, [PNA] = 1.0 μM and [DNA] = 1.0 μM, 10 mM sodium phosphate buffer pH 7.0, 100 mM NaCl..... 109

LIST OF ABBREVIATIONS

δ	:	Chemical shift
μL	:	microliter
μmol	:	micromole
$[\alpha]_D$	:	specific rotation
A	:	adenine
A^{Bz}	:	N^6-benzoyladenine
Ac	:	acetyl
Ac_2O	:	acetic anhydride
AcOH	:	acetic acid
aq	:	aqueous
Boc	:	*tert*-butoxycarbonyl
Bz	:	benzoyl
c	:	concentration
C	:	cytosine
calcd	:	calculated
C^{Bz}	:	N^4-benzoylcytosine
CCA	:	α-cyano-4-hydroxy cinnamic acid
$CDCl_3$	:	deuterated chloroform
d	:	doublet
D_2O	:	deuterium oxide
DCM	:	dichloromethane
DIAD	:	diisopropylazodicarboxylate
DIEA	:	diisopropylethylamine
DMF	:	*N,N'*-dimethylformamide
DMSO-d_6	:	deuterated dimethylsulfoxide
DNA	:	deoxyribonucleic acid
Dpm	:	diphenylmethyl
ds	:	double strand
equiv	:	equivalent (s)
Fmoc	:	9-fluorenylmethoxycarbonyl

LIST OF ABBREVIATIONS (CONT.)

FmocOSu	:	9-fluorenylmethyl succinimidyl carbonate
Fmol	:	femtomole
FRET	:	fluorescence resonance energy transfer
g	:	gram
G	:	guanine
G^{Ibu}	:	N^2-isobutyrylguanine
h	:	hour
HATU	:	*O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate
HOAt	:	1-hydroxy-7-azabenzotriazole
HPLC	:	high performance liquid chromatography
Hz	:	hertz
Ibu	:	isobutyryl
IL-10	:	interleukin-10
J	:	coupling constant
Lys	:	lysine
m	:	multiplet
MALDI-TOF	:	matrix-assisted laser desorption/ionization-time of flight
MeCN	:	acetonitrile
MeOH	:	methanol
MeOTs	:	methyl tosylate
mg	:	milligram
MHz	:	megahertz
Min	:	minute
mL	:	milliliter
mM	:	millimolar
mmol	:	millimol
mRNA	:	messenger ribonucleic acid
MS	:	mass spectrometry
m/z	:	mass to charge ratio

LIST OF ABBREVIATIONS (CONT.)

nm	:	nanometer
NMR	:	nuclear magnetic resonance
°C	:	degree Celsius
OD_{xxx}	:	optical density at xxx nm (= A_{xxx})
PCR	:	polymerase chain reaction
Pfp	:	pentafluorophenyl
PfpOTfa	:	pentafluorophenyl trifluoroacetate
PG	:	an unspecified protecting group
Ph	:	phenyl
PNA	:	peptide nucleic acid or polyamide nucleic acid
ppm	:	part per million
pmol	:	picomole
R_f	:	retention factor
RNA	:	ribonucleic acid
s	:	singlet
Ser	:	serine
S/N	:	signal to noise ratio
ss	:	single strand
t	:	triplet
T	:	thymine
T^{Bz}	:	N^3-benzoylthymine
TEA	:	triethylamine
TFA	:	trifluoroacetic acid
THF	:	tetrahydrofuran
TLC	:	thin layer chromatography
T_m	:	melting temperature
t_R	:	retention time
UV	:	ultraviolet