

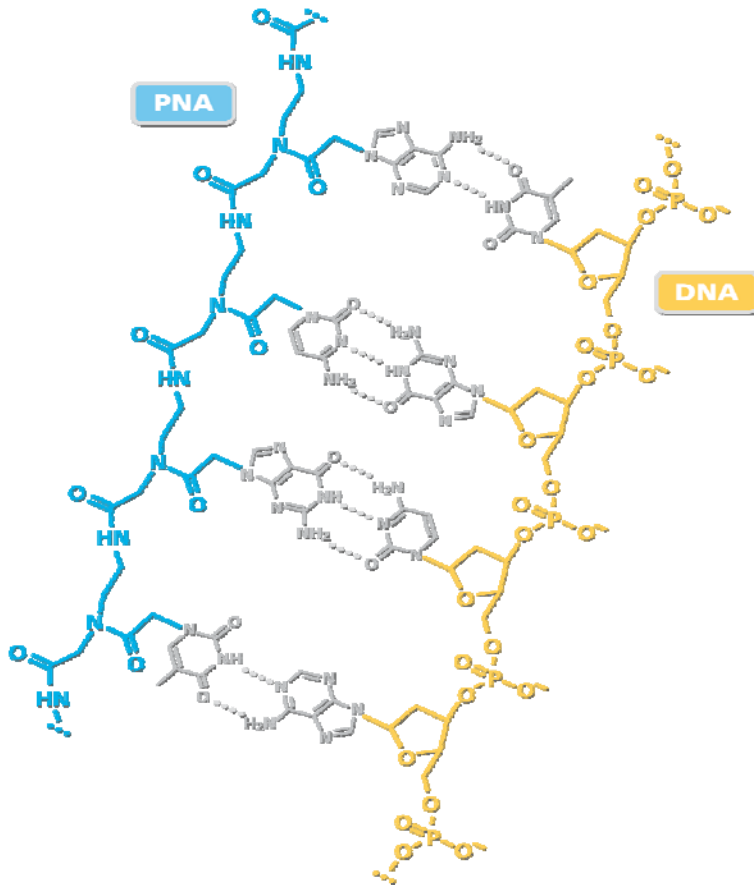
Novel Approach to the Chemical Synthesis of PNA

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Aug 29 2007

Peptide Nucleic Acid (PNA)



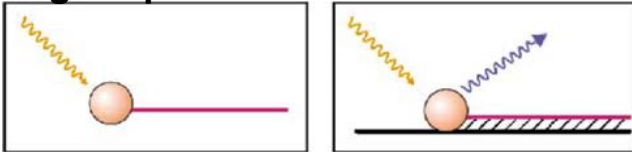
Characteristics of PNA

- Radically different from DNA
- Neutral backbone
- Higher affinity to complementary nucleic acid
- Hybridization independent of salt concentration
- Greater specificity and sensitivity of interaction
- Thermal and chemical stability
- Resistance to nucleases and proteases
- Triplex formation

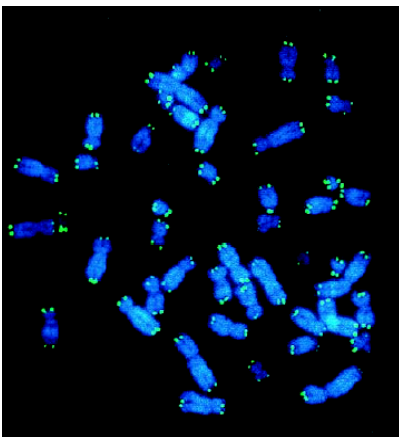
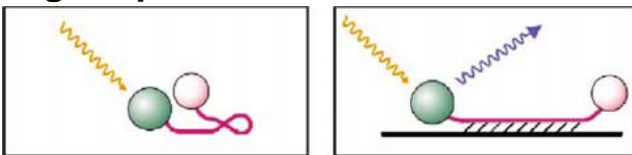
Application of PNA

Molecular Diagnostics

Light Up Probes

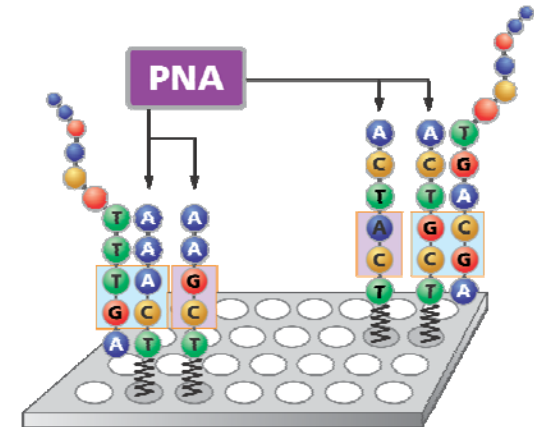


Light Speed Probes

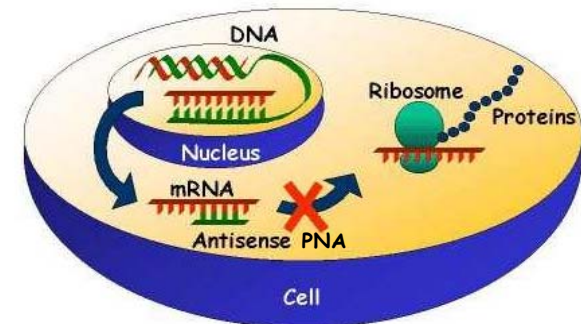


FISH
(Fluorescence
in situ Hybridization)

Microarray

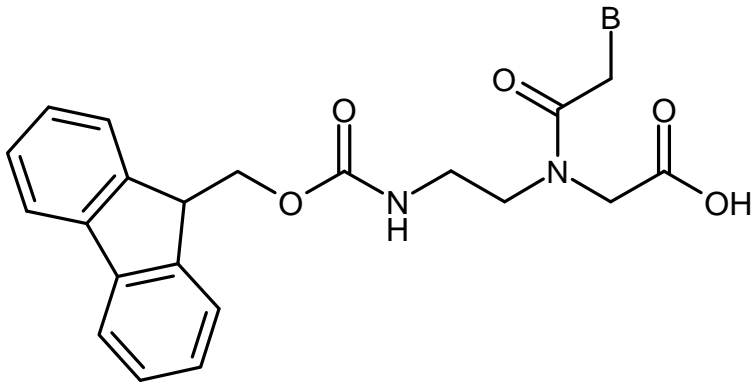


New Drug Development

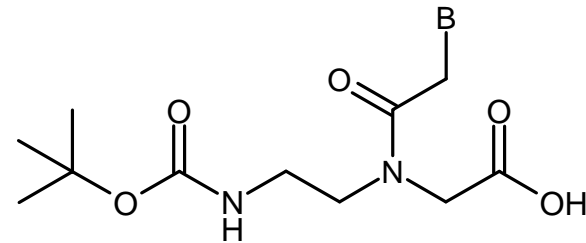


Conventional PNA Synthesis

Fmoc / Bhoc PNA Monomer



Boc / CBz PNA Monomer



Generally synthesized by well-established solid phase peptide synthesis protocols.

- Reaction depends on the coupling reagents
- Moisture sensitive (requirement of anhydrous condition)

Conventional Synthesis of PNA

Boc/Cbz Strategy:

Based on differential acid lability between two protecting groups

Cleavage and deprotection of side chains done with TFMSA / TFA

Synthesis chemistry incompatible on a DNA Synthesizer

Fmoc/Bhoc Strategy:

Mild deprotecting condition of Fmoc and compatible with various chemistries

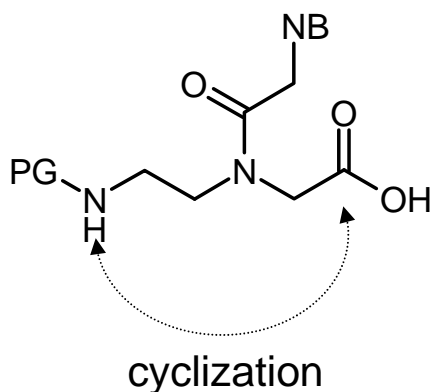
Synthesis can be carried out on a DNA Synthesizer

Side reaction such as trans-acylation under basic condition

Produces many more impurities compared to Boc chemistry

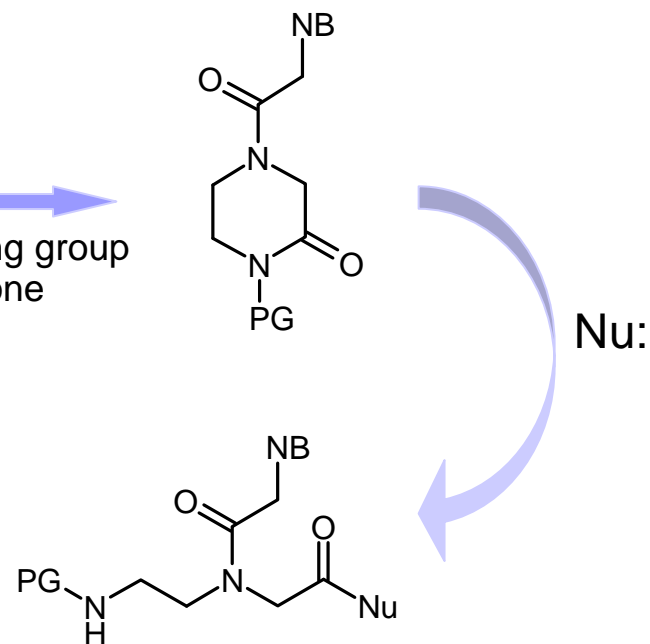
Self-activated PNA monomer

Conventional PNA Monomers



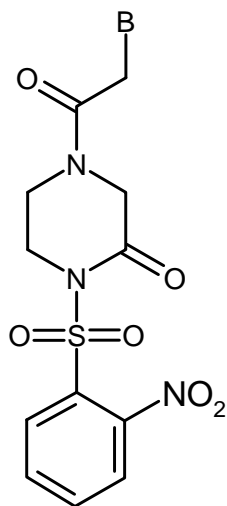
Conjunction by cyclization
Electron-withdrawing Protecting group (PG) to activate the piperazine

Self-Activated PNA Monomer



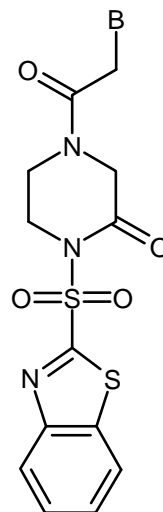
Self-activated PNA monomers

Nitrobenzenesulfonyl
PNA monomer



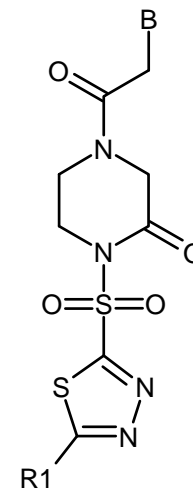
US 7,022,851 B2

Benzothiazole-2-sulfonyl
PNA monomer



US 6,969,766 B2
US 7,125,994 B2
US 7,145,006 B2
US 7,179,896 B2

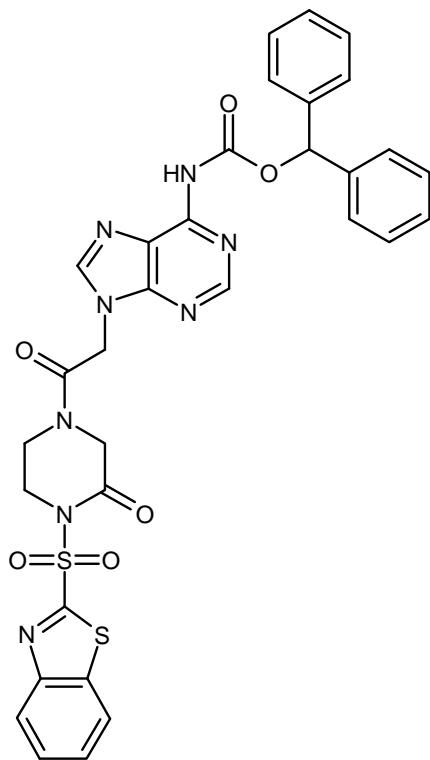
Thiadiazolesulfonyl
PNA monomer



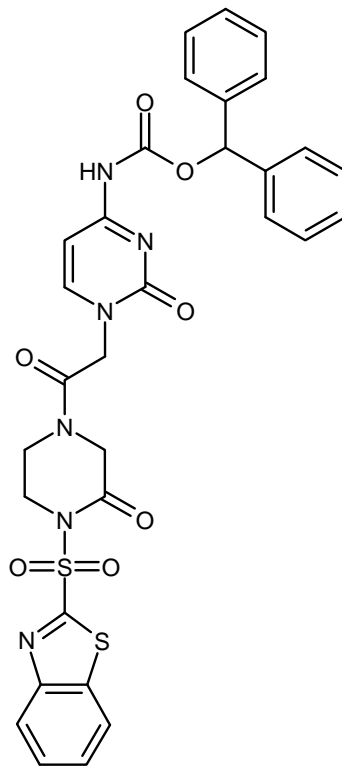
R₁ = Phenyl, Benzoyl

US 7,211,668 B2

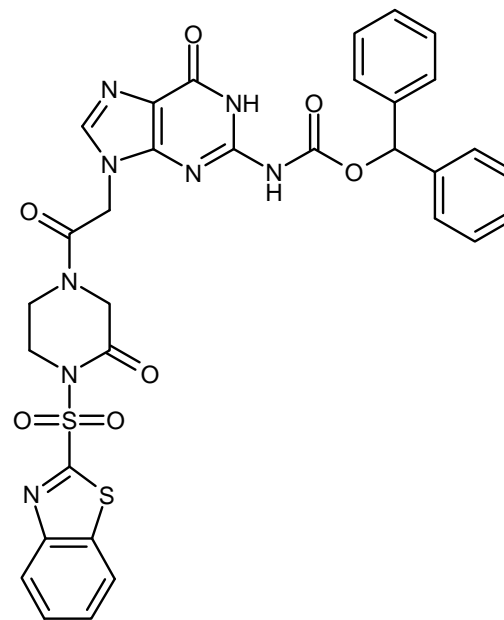
Chemical Structure of Bts Monomers



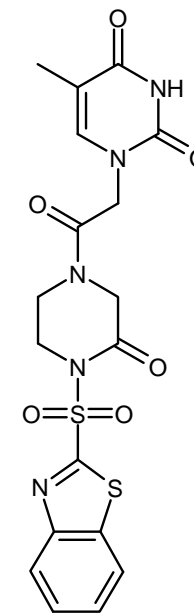
A monomer



C monomer

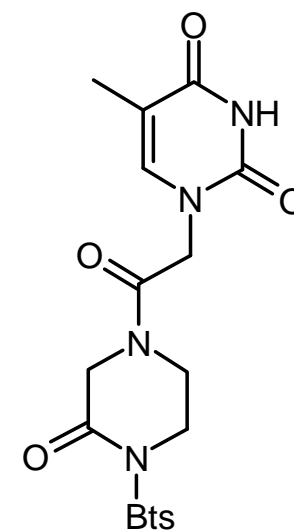
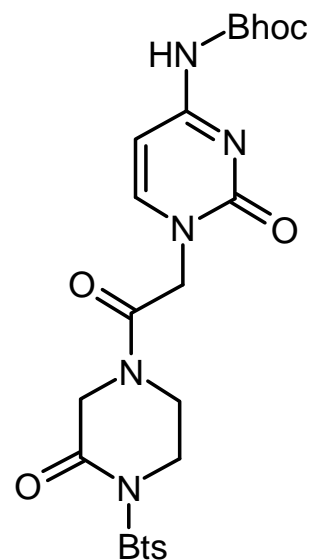
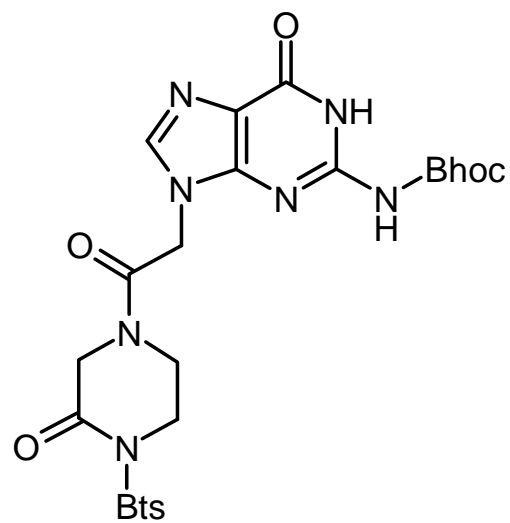
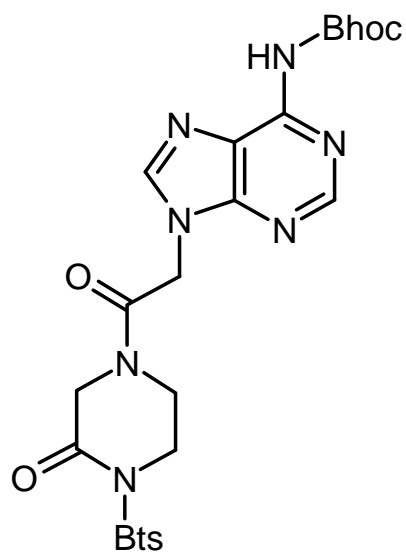


G monomer



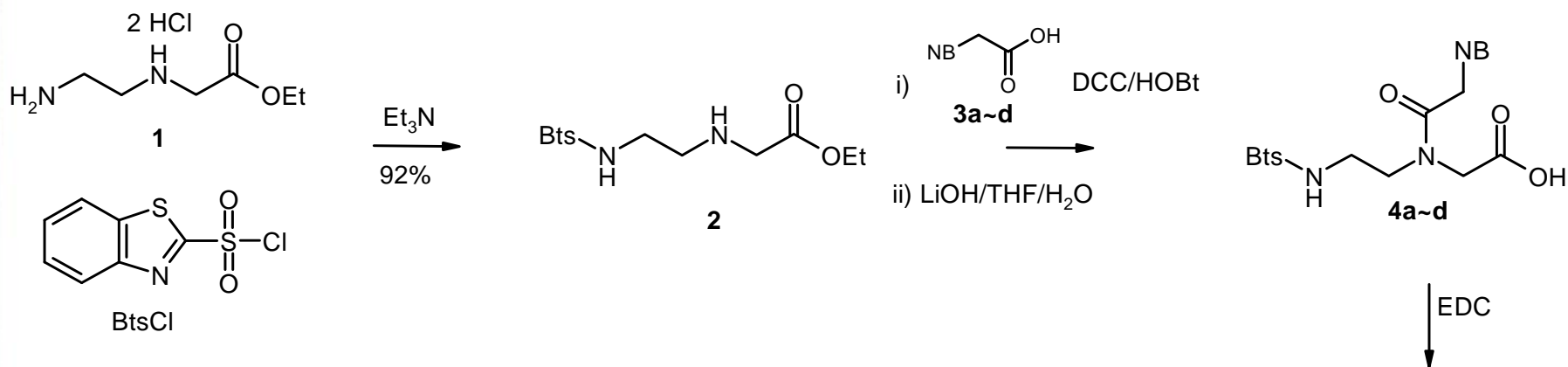
T monomer

Self-activated Bts PNA monomers



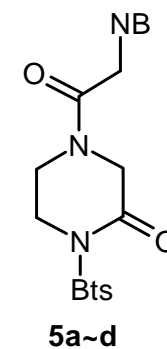
Bts : Benzothiazole-2-sulfonyl-, Bhoc : Benzhydryloxycarbonyl-

Preparation of Bts PNA Monomers

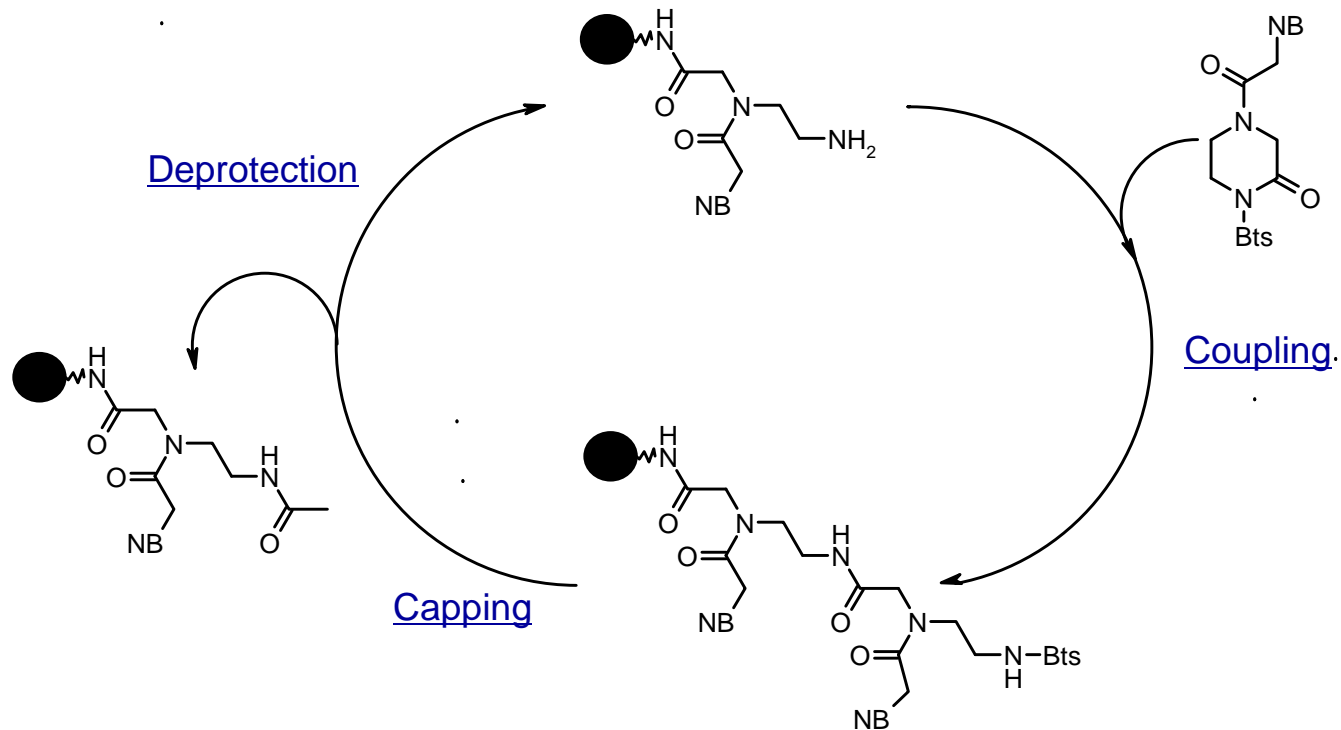


	NB	4	5
a ;	Adenine(Bhoc)	72	92
b ;	Cytosine(Bhoc)	80	95
c ;	Guanine(Bhoc)	68	90
d ;	Thymine	72	93

Isolated yield



Oligomerization of PNA



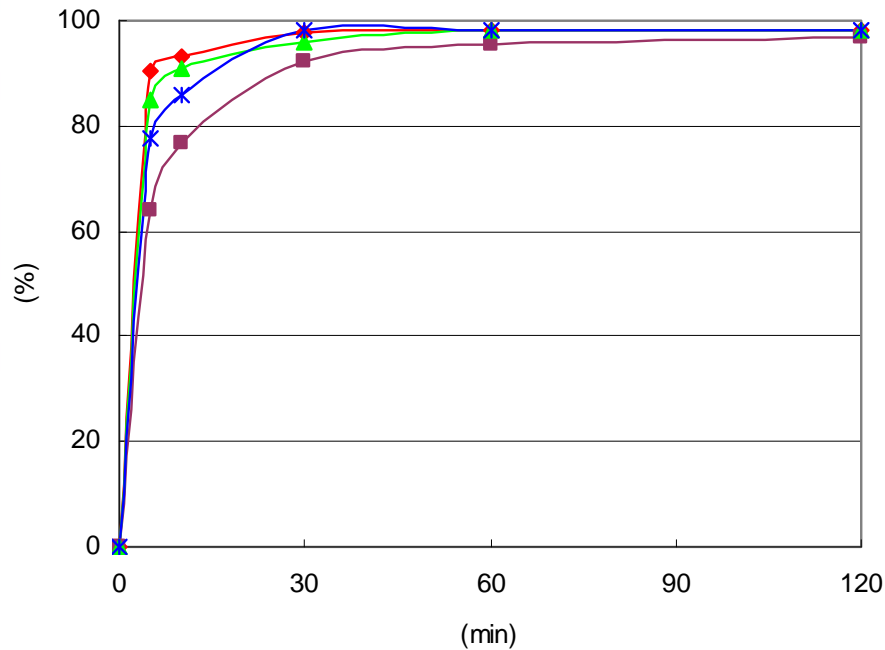
PNA Synthesis Cycle Table

Table 1. Outlines of the Bts PNA Synthesis Cycles¹²

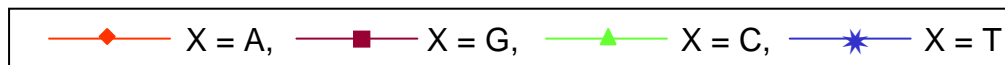
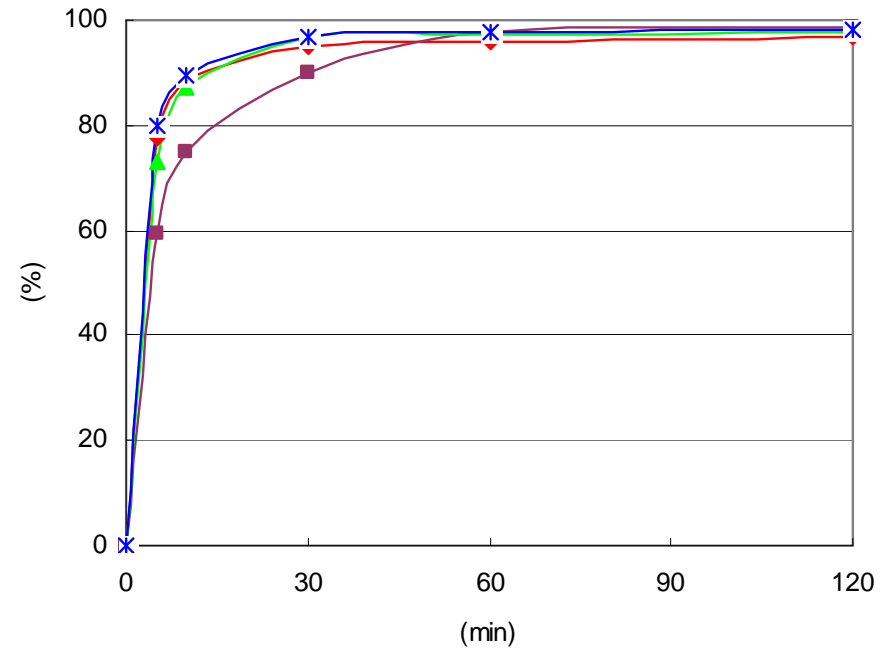
steps	reaction condition
coupling	0.3 M PNA monomer (20 equiv) and 0.2 M DIEA solution in DMF (120 min, 40 °C)
capping	(1) 5% Ac ₂ O and 6% lutidine solution in DMF (3 min, rt); (2) 10% piperidine in DMF (3 min, rt)
deprotection	0.8 M 4-methoxybenzenethiol and 0.4 M DIEA in DMF (10 min, 40 °C)

Reactivity of Bts PNA monomers

Reactivity of Bts X monomers with PNA (10-mer)



Reactivity of Bts X monomers with PNA (14-mer)





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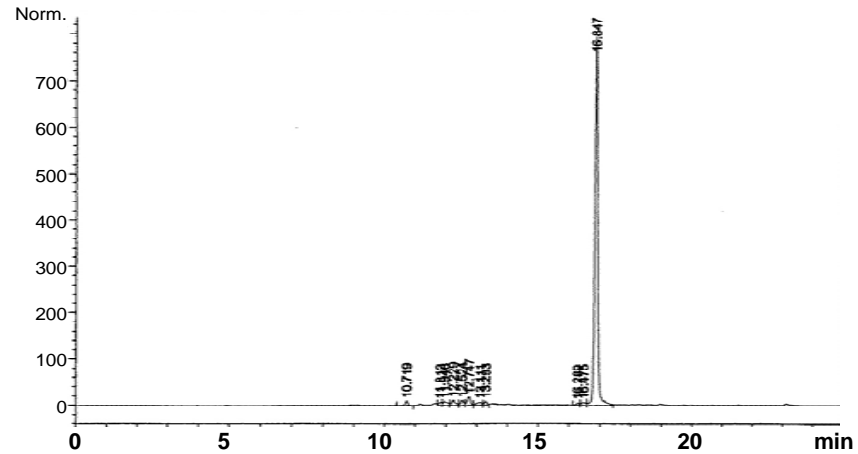
Examples of PNA by Bts Strategy



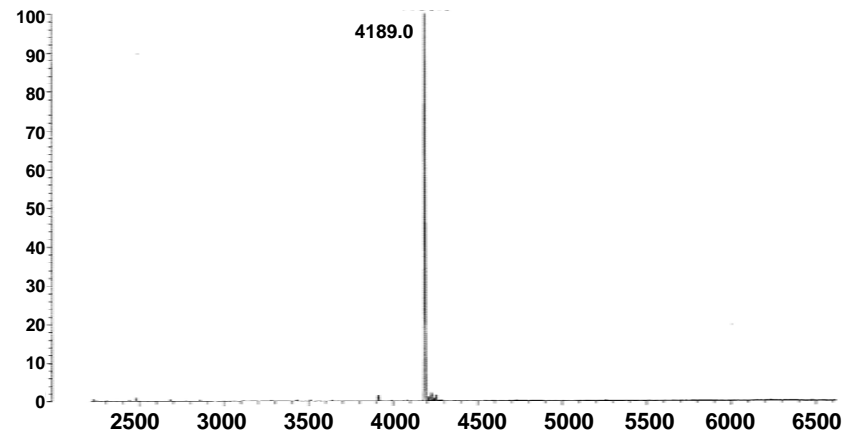
Crude PNA (15-mer) with Bts

Bts -CTC AGC ACA TCT ACA

HPLC profile : 87% ▶

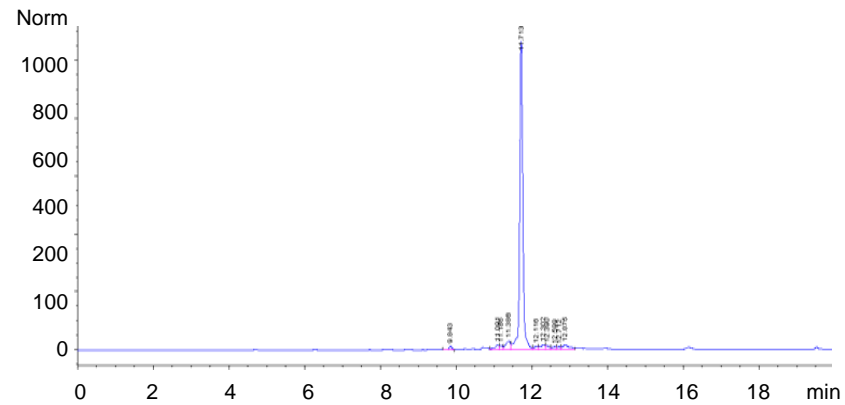


MALDI-TOF MS profile ▶



Crude PNA (15-mer) without Bts

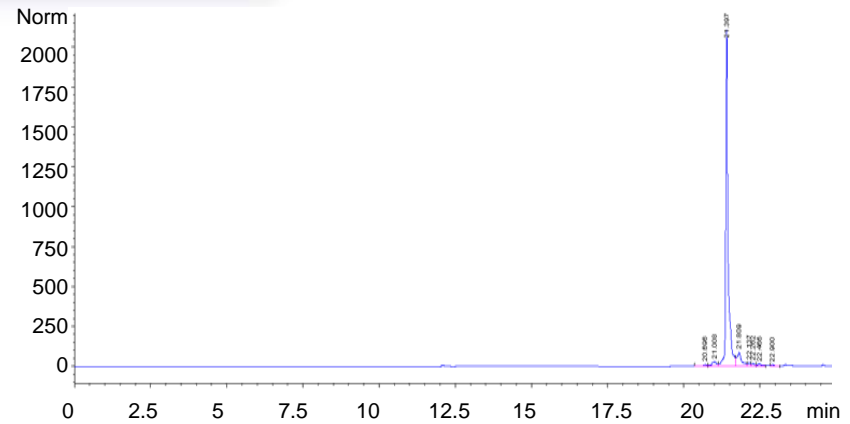
HPLC



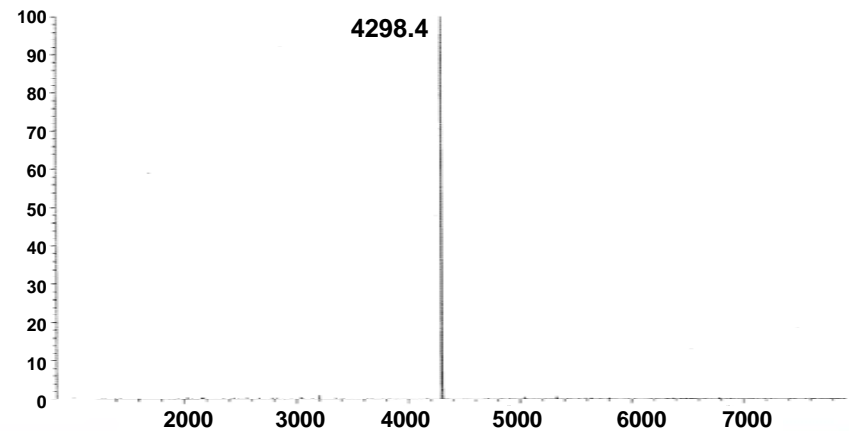
Crude PNA conjugated with peptide

PNA(10-mer)-O-peptide(10-mer)

HPLC profile : 87% ▶



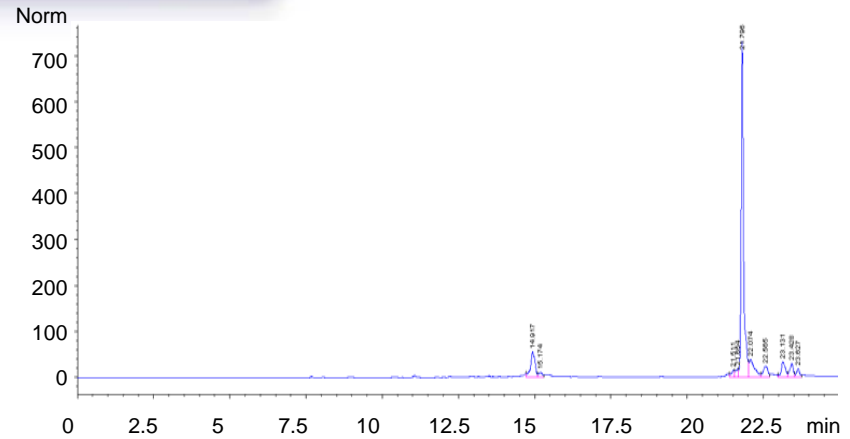
MALDI-TOF MS profile ▶



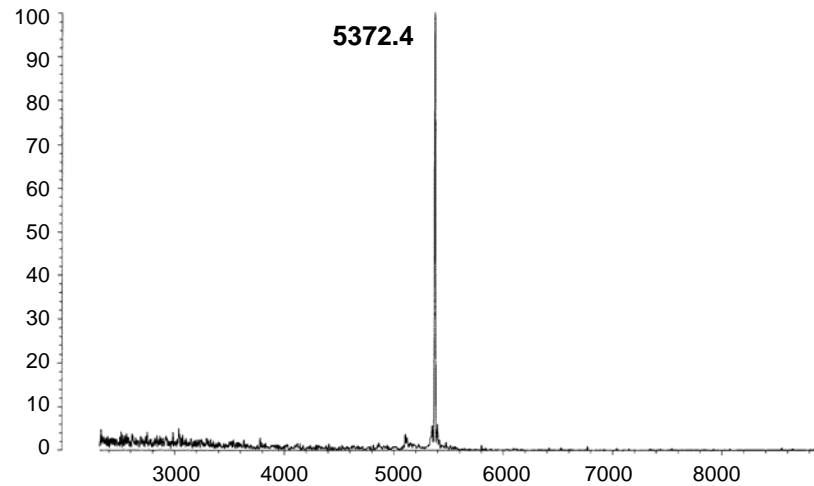
Crude Bis-PNA

Fmoc-OO-PNA(10-mer)-OOO-
-PNA(10-mer)-K

HPLC profile : 85% ▶



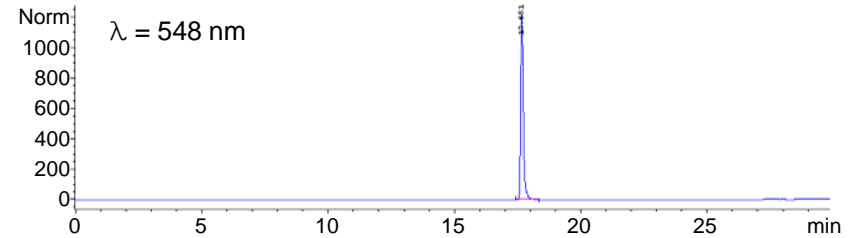
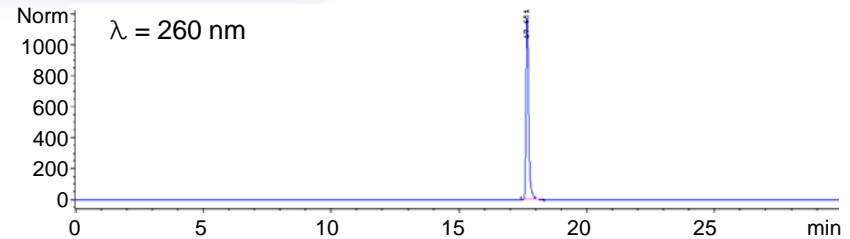
MALDI-TOF MS profile ▶



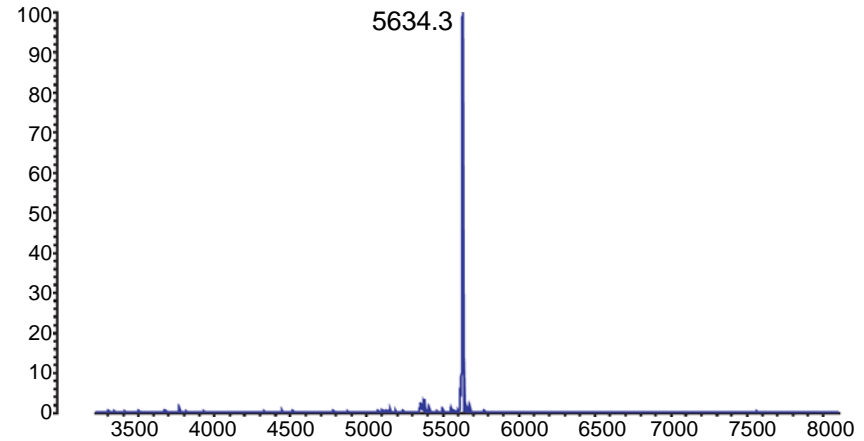
Fluorescently labeled PNA (purified)

Cy3-OO-TAACCTAACCTAACCC

HPLC profile of purified PNA ►

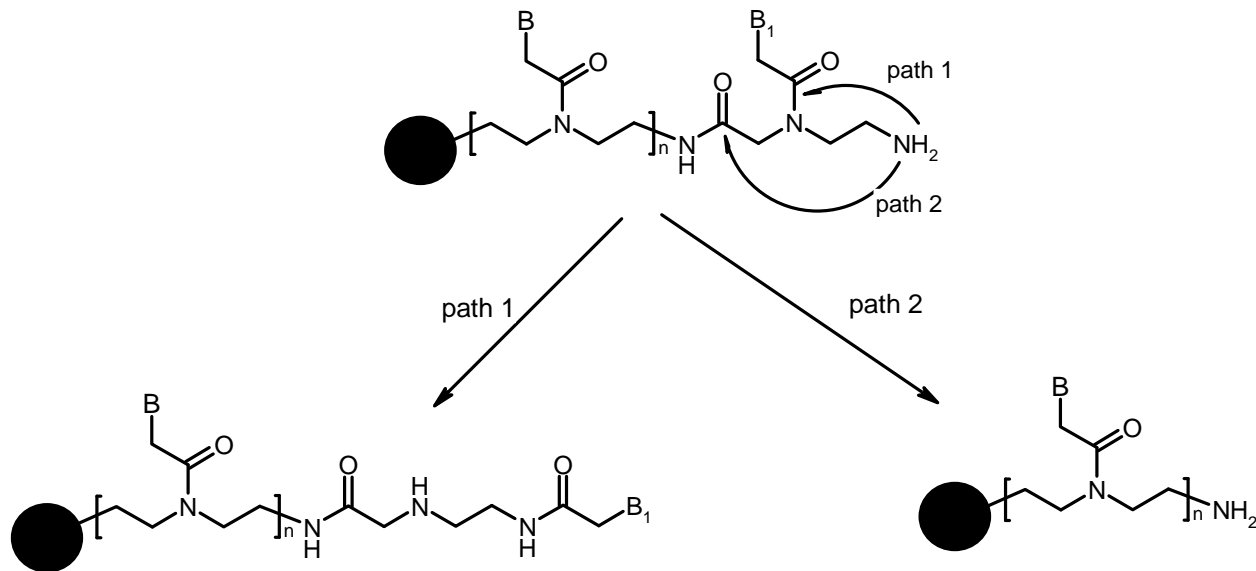


MALDI-TOF MS profile ►



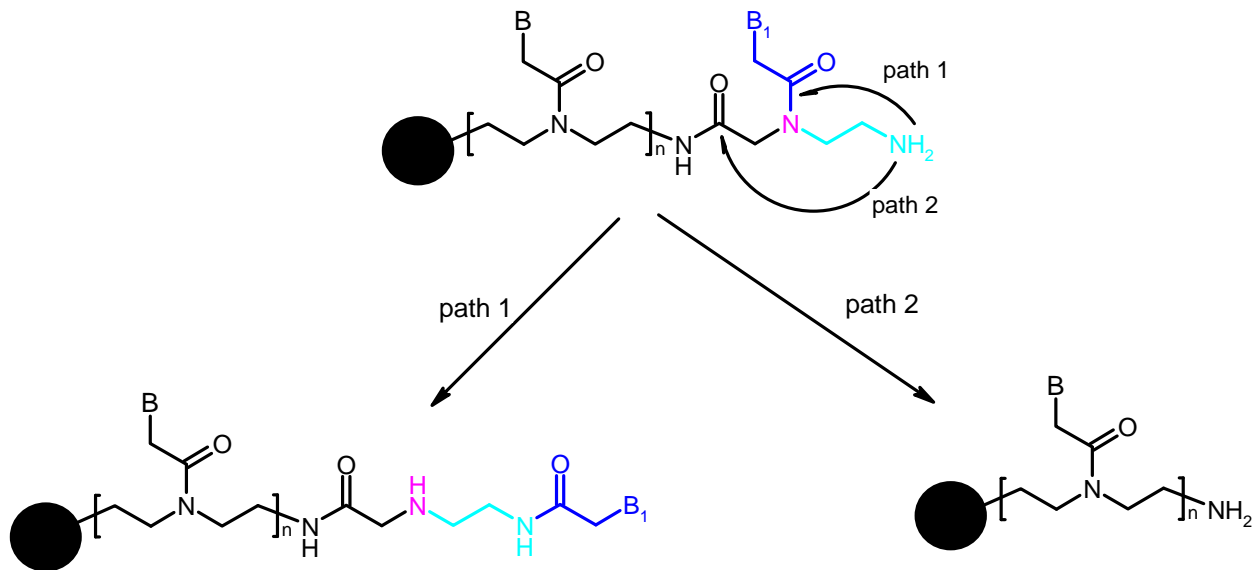
Trans-acylation – side reaction

mechanism



Trans-acylation – side reaction

mechanism



Trans-acylation during deprotection

PG-XTT-Resin

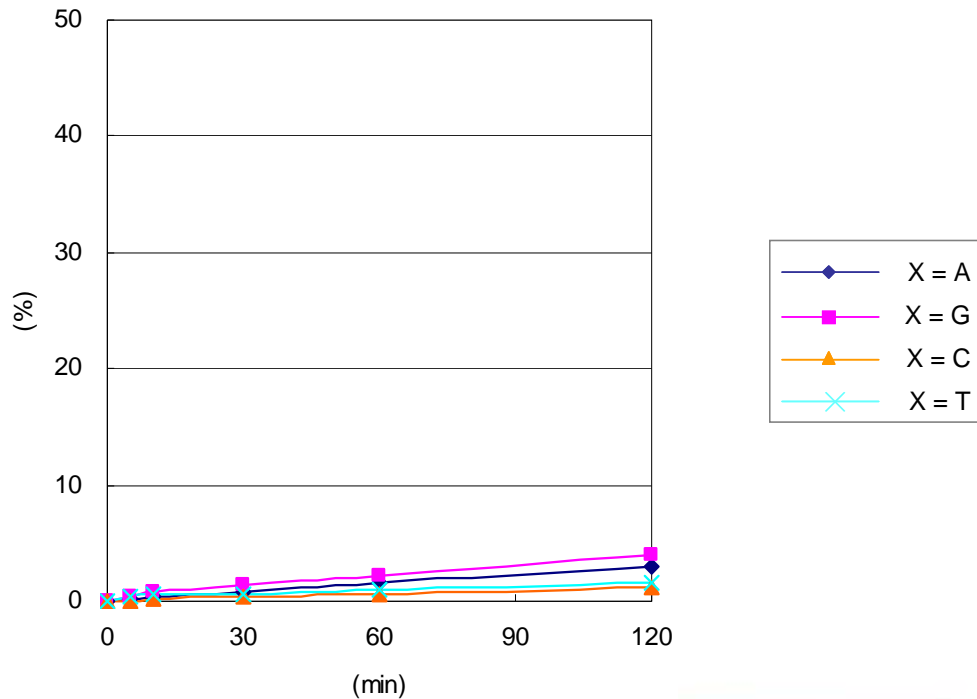
Deprotecting condition

XTT-Resin +

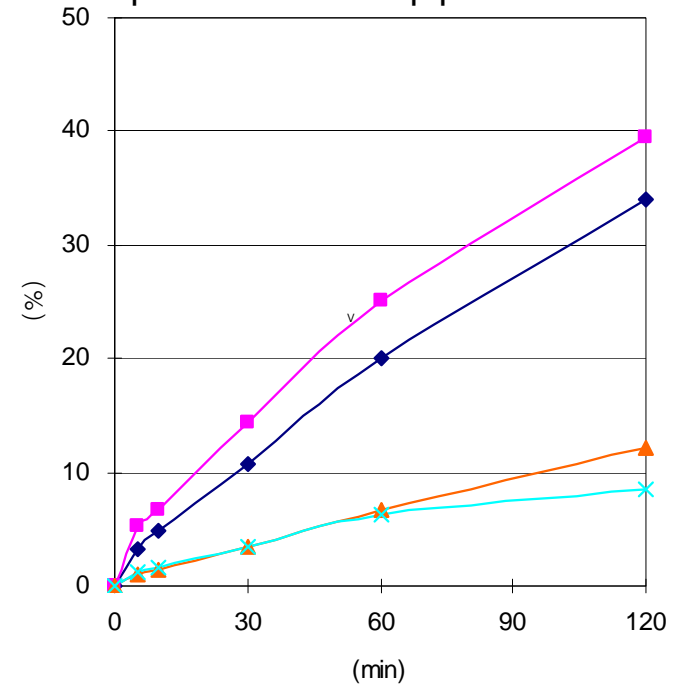
X^{ta}TT-Resin

PG : protecting group

Trans-acylation of Bts PNA

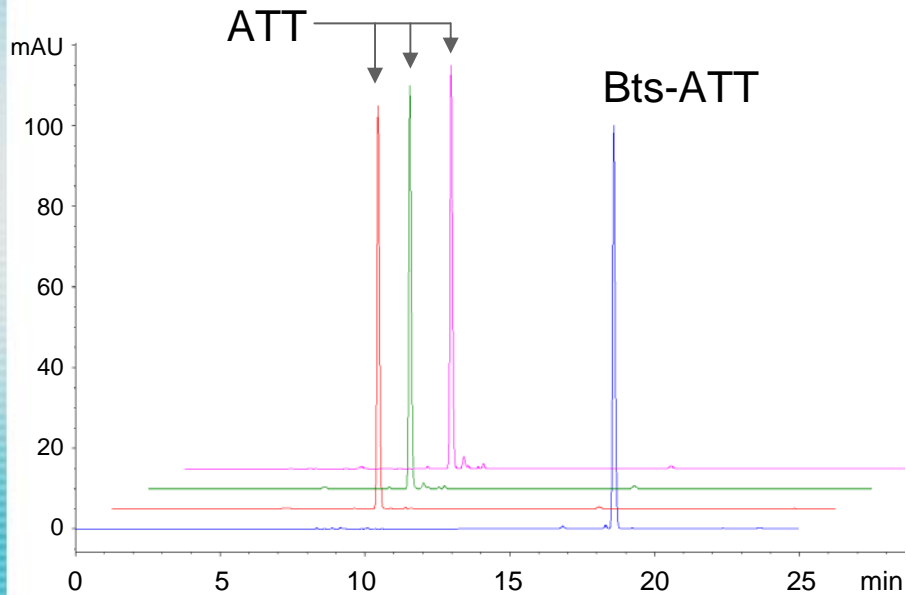


Trans-acylation of Fmoc PNA
Fmoc Deprotection : 20% piperidine in DMF

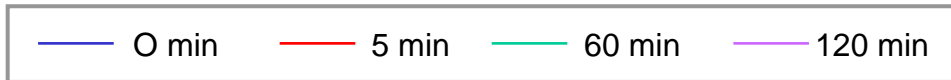
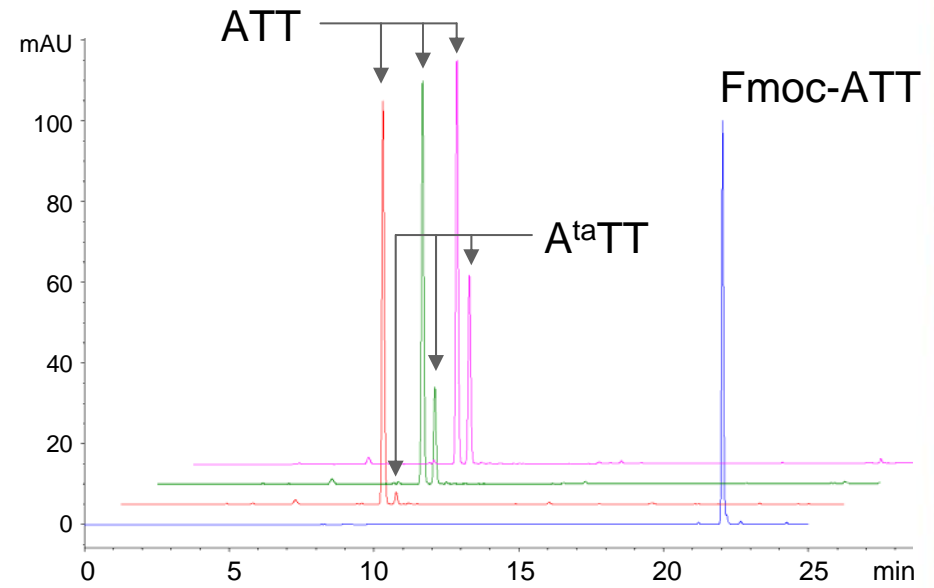


HPLC profile of trans-acylation

Bts PNA

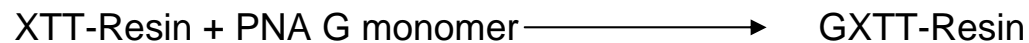


Fmoc PNA



A^{ta} : trans-acylated A

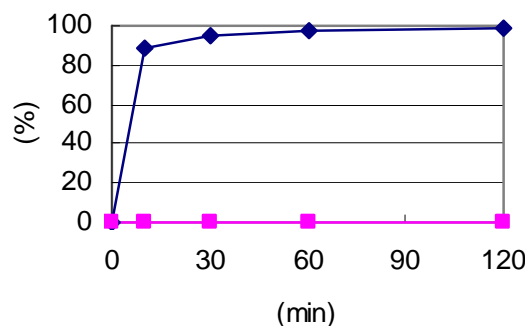
Reactivity of PNA monomer with trans-acylated PNA



Bts strategy

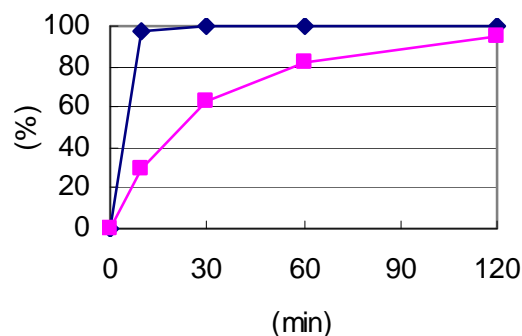
Coupling condition

0.3M Bts G(Bhoc) monomer & 0.2M DIEA

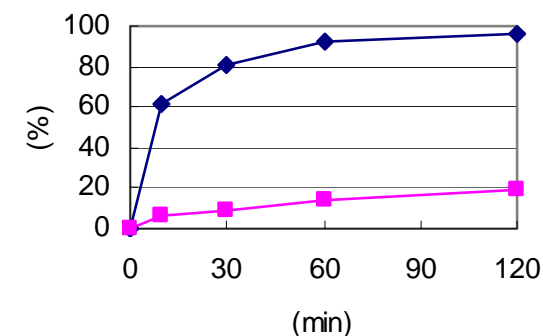


Fmoc strategy

Fmoc G(Bhoc) monomer /DIC/HOBt (1/2/1), rt



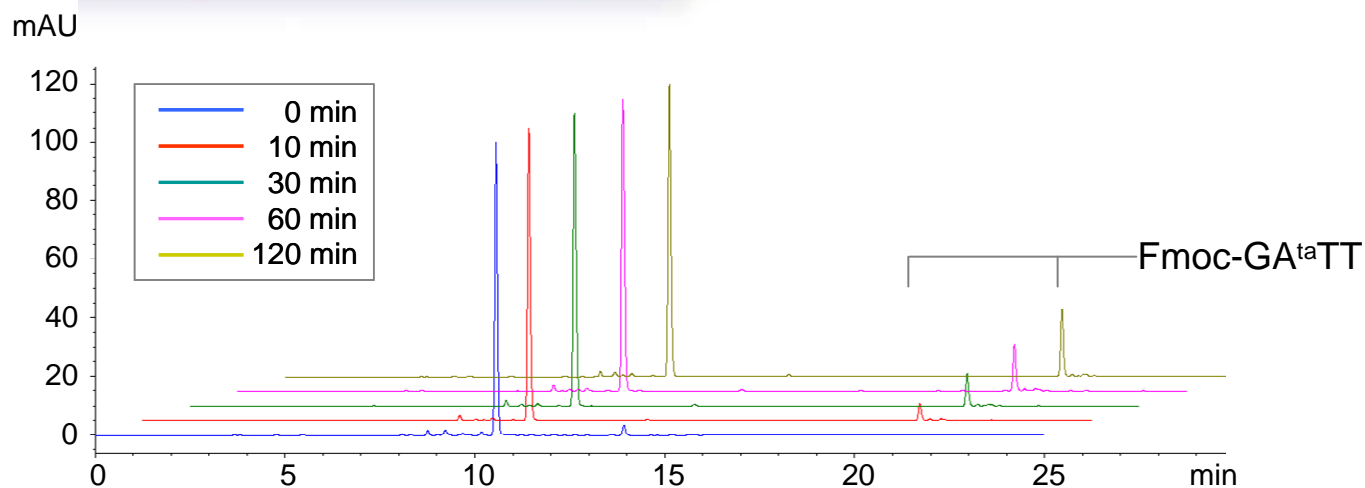
Fmoc G(Bhoc) monomer / PyBOP/Lutidine/DIEA (2/1/0.5/0.5)



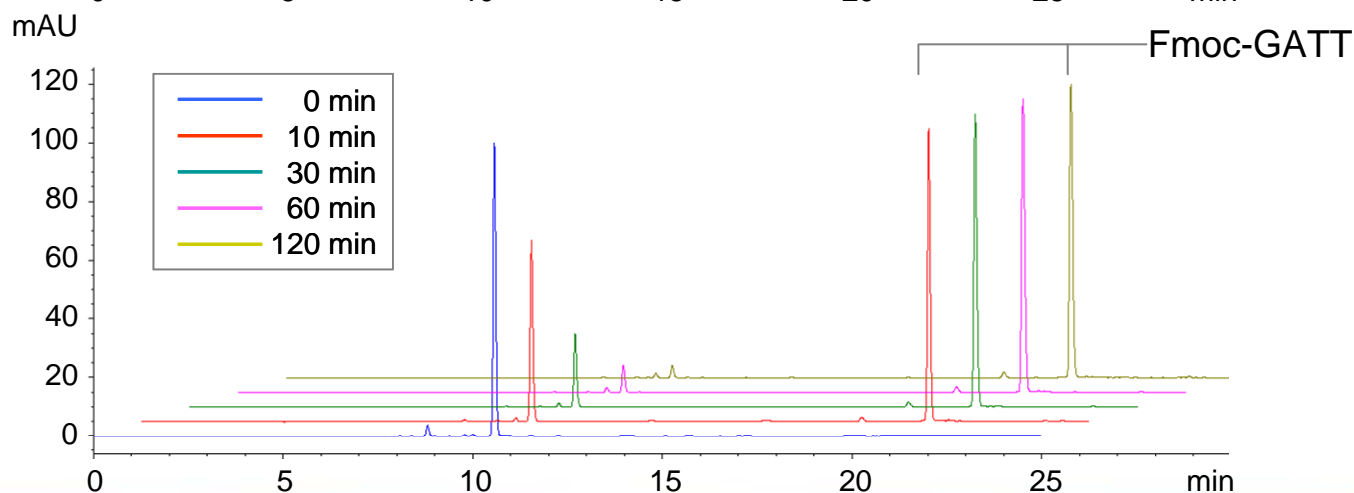
X = A  X = A^{ta} 

Coupling of PNA with Fmoc PNA monomer

A^{ta}TT-resin
+
Fmoc G monomer

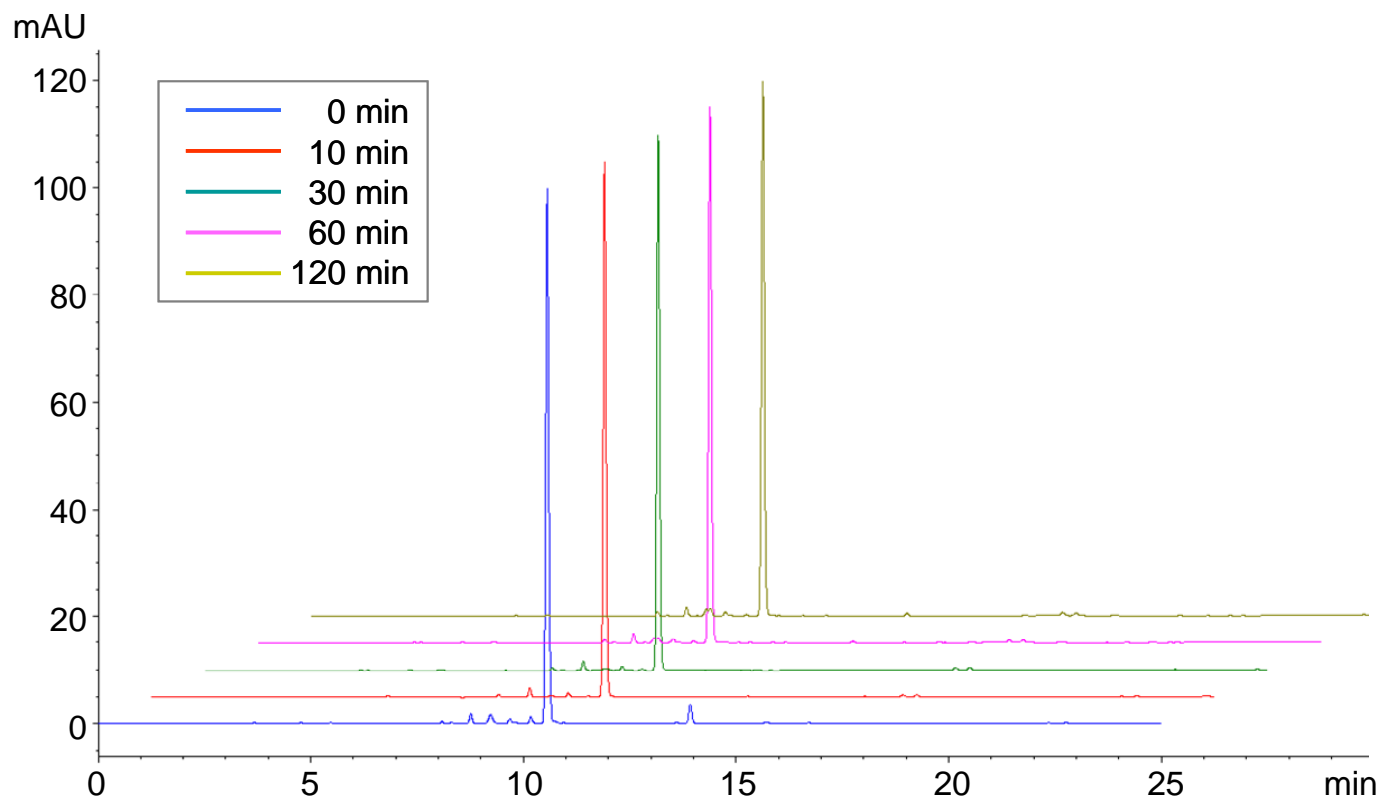


ATT-resin
+
Fmoc G monomer



Coupling of transacylated PNA with Bts PNA monomer

Reaction of A^{ta}-TT-resin with Bts G monomer

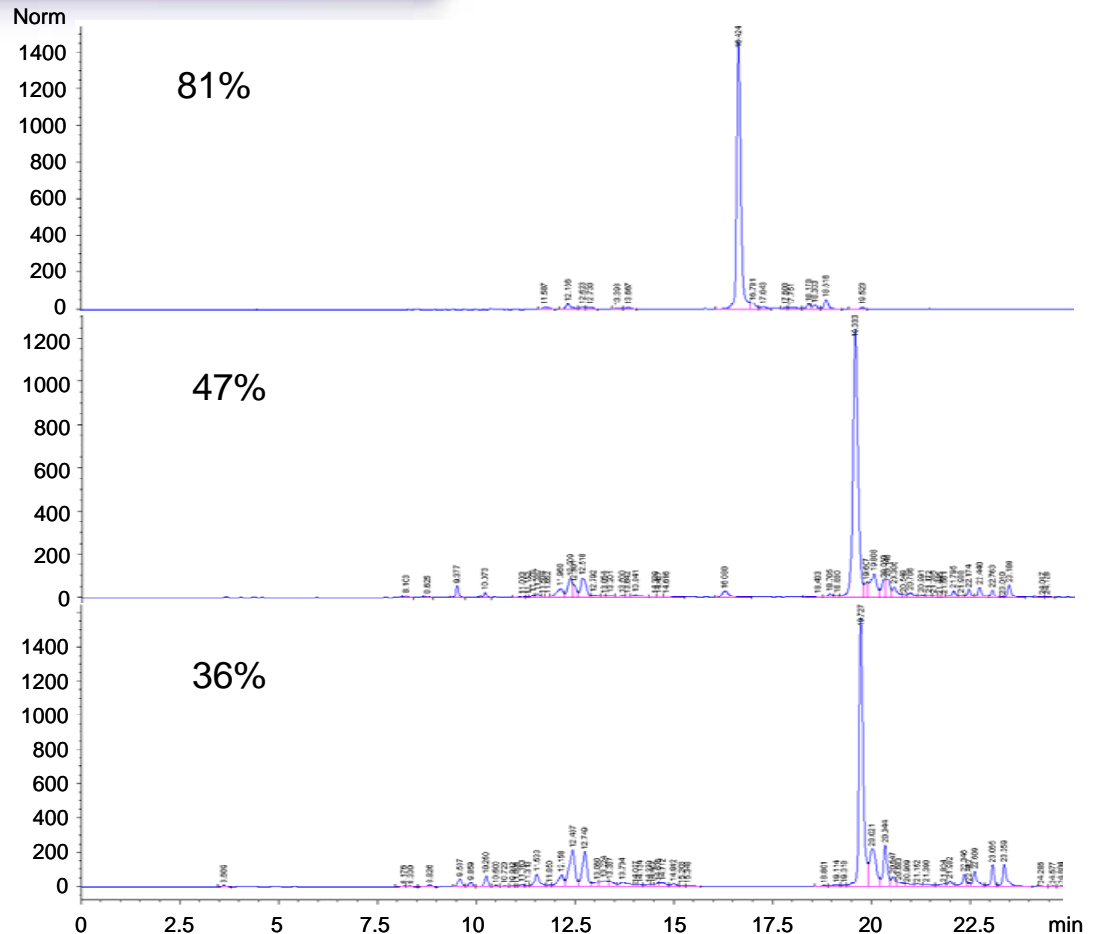


Comparison of Fmoc and Bts Chemistry

Crude PNA prepared
by Bts Chemistry
(Bts-TCATCCATATAACTG)

Crude PNA prepared
by Fmoc Chemistry using
DIC as a coupling reagent.
(Fmoc-TCATCCATATAACTG)

Crude PNA prepared
by Fmoc Chemistry using
PyBOP as a coupling reagent.
(Fmoc-TCATCCATATAACTG)



Bts Chemistry & Fmoc Chemistry

Monomers	Cyclic Bts PNA monomer	Fmoc PNA monomer
Monomer recovery after coupling	70 ~ 80%	-
Large scale synthesis of monomers	Good	-
Solubility of monomers	Good	Moderate
Cost of oligomer synthesis	Low	High
Solvent for oligomer synthesis (Anhydrous requirement)	DMF (Not necessary)	NMP (Necessary)
Coupling reagent	None	required
Trans-acylation during deprotection	+	+++
Reactivity of monomer with trans-acylated product	Not Detected	high

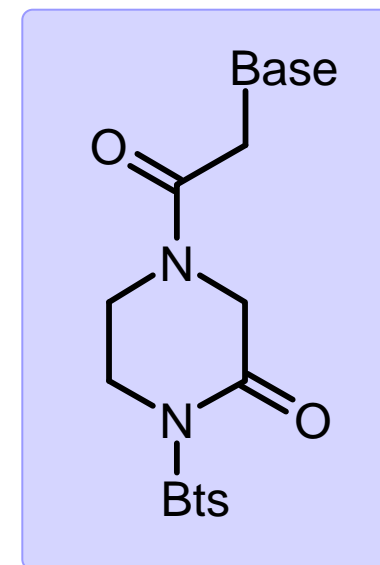
Rules Loosened with BTS



	Fmoc Chemistry	Bts Chemistry
Probe Length	< 18	< 24
Purine Content	< 60 %	< 80% No restriction for upto 10mers
Purine Stretch	\leq 4 bases	\leq 5 bases No restriction for upto 10mers

Conclusion

- ◆ We have developed novel Bts monomers and chemistry to synthesize PNA.
 - ◆ Monomers don't need Chromatographic purification
 - ◆ Excess monomers can be recycled and re-used if necessary
 - ◆ Monomers have excellent stability and solubility
- ◆ Bts monomers during Oligomerization
 - Excellent coupling yield,
 - Coupling reagent & anhydrous solvent not required
 - Deprotection under mild condition
 - Less trans-acylation during deprotection and
 - No reactivity to trans-acylated PNA





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Thank you.

