学位論文の要約

題目　Sequence-Specific Alkylation By Pyrrole-Imidazole Polyamide *Seco*-CBI Conjugates To Target Cancer-Associated Mutations
（変異がん遺伝子を標的としたピロール・イミダゾールポリアミド *seco*-CBI コンジュゲートによる配列特異的アルキル化）

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序論
General Introduction

The DNA minor groove is of therapeutic interest as it acts as the binding site for many small molecules. *N*- Methylpyrrole (Py) and *N*-methylimidazole (Im) polyamides are a class of such small molecules that can be tailored to bind to predetermined sequences of DNA. Extensive studies have demonstrated Py-Im polyamides ability to regulate gene expression, with good bioavailability and limited toxicity. They can be conjugated with histone deacetylase (HDAC) inhibitors and transcription activating domains to activate gene expression, or alkylating agents to inhibit gene expression. Several alkylating Py-Im polyamide conjugates have demonstrated selective tumour suppression, and we have successfully designed alkylating Py-Im polyamide *seco*-CBI Conjugates to target KRAS mutations at codons 12 and 13. Py-Im polyamides could provide a customizable therapeutic approach for treatments for various diseases including cancer.

Chapter 1: Selective Targeting Of KRAS Codon 12 Mutation Sequence By Pyrrole–Imidazole Polyamide Seco-CBI Conjugates

Mutation of KRAS is a key step in many cancers, with mutations most frequently occurring at codon 12. Targeting KRAS is notoriously difficult. Here we present a novel approach to target KRAS by targeting the genetic information. Four alkylating hairpin *N*-methylpyrrole-*N*-methylimidazole polyamide *seco*-CBI conjugates were designed to target the KRAS codon 12 mutation sequence. Conjugate 4 displayed higher affinity towards the G12D mutation sequence than the G12V sequence, and a computer-minimized model suggests that conjugate 4 can bind more efficiently to the G12D match sequence than 1 base pair mismatch sequence. Conjugate 4 was modified for Next Generation Sequencing and Bind-n-Seq analysis

supported the evidence that it can target the G12D mutation sequence exceptionally high affinity and also the G12V mutation sequence with much higher affinity than the wild type sequence.

Chapter 2: Sequence-Specific DNA Alkylation Targeting for KRAS Codon 13 Mutation by Pyrrole–Imidazole Polyamide seco-CBI Conjugates

Hairpin *N*-methylpyrrole-*N*-methylimidazole polyamide *seco*-CBI conjugates 2-6 were designed for synthesis by Fmoc solid-phase synthesis, and their DNA-alkylating activities against the KRAS codon 13 mutation were compared by high-resolution denaturing gel electrophoresis with 225 base pair (bp) DNA fragments. Conjugate 5 had high reactivity towards the KRAS codon 13 mutation site, with alkylation occurring at the A of the sequence 5'-ACGTCACCA-3' (site 2), including minor 1 bp-mismatch alkylation against wild type 5'-ACGCCACCA-3' (site 3). Conjugate 6, which differs from conjugate 5 by exchanging one Py unit with a β unit, showed high selectivity but only weakly alkylated the A of 5'-ACGTCACCA-3' (site 2). The hairpin polyamide *seco*-CBI conjugate 5 thus alkylates according to Dervan's pairing rule with the pairing recognition which β/β pair targets T-A and A-T pairs. SPR and a computer-minimized model suggest that 5 binds to the target sequence with high affinity in a hairpin conformation, allowing for efficient DNA alkylation.

Chapter 3: Sequence-specific DNA alkylation by tandem Py-Im polyamide conjugates

Tandem *N*-methylpyrrole–*N*-methylimidazole (Py–Im) polyamides were designed, and synthesized with good sequence-specific DNA alkylating activities. Three alkylating tandem Py–Im polyamides with different linkers, which have the same 10 bp DNA sequence recognition moiety, were evaluated for their reactivity and selectivity on DNA alkylation using high-resolution denaturing gel electrophoresis. All three conjugates displayed high reactivity for the target sequence. Especially, conjugate **1** with a β-alanine linker displayed the most selective sequence-specific alkylation toward the target 10-bp DNA sequences. The tandem Py–Im polyamide conjugates displayed greater sequence specific DNA alkylation than conventional hairpin Py–Im polyamide conjugates (**4** and **5**). For further research, the design of tandem Py-Im polyamides conjugates would play an important role in targeting specific gene sequences.