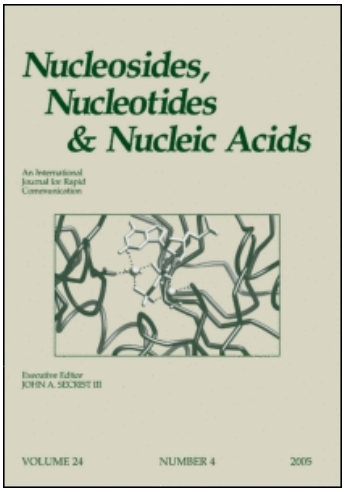

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INVESTIGATION OF NOVEL LIPID-FUNCTIONALIZED PNA MONOMERS AS POTENTIAL HIV-1 NON-NUCLEOSIDE REVERSE TRANSCRIPTASE AND/OR INTEGRASE INHIBITORS

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□ *A range of novel N-terminal lipid-functionalized peptide nucleic acid (PNA) monomers have been prepared and their abilities to inhibit HIV-1 reverse transcriptase and integrase have been examined.*

Keywords Anti-HIV agents; non-nucleoside reverse transcriptase inhibitors; integrase inhibitors; PNA analogues

INTRODUCTION

Despite the considerable success in Western countries of highly active anti-retroviral therapy (HAART), AIDS still remains one of the most urgent world health problems, being the first cause of death in Africa and the fourth leading cause of death worldwide.[1] HAART typically involves a cocktail of three drugs given in combination, which act on two different biological targets in the HIV-1 life cycle, for example, two nucleoside inhibitors of HIV-1 reverse transcriptase (NRTIs) and either a protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI).[2] Unfortunately, the efficacy of this current therapy often is hampered by the

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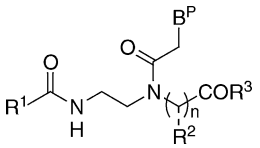


FIGURE 1 Structures of *N*-terminal lipid functionalised PNA monomers. B = Adenine, Cytosine, Thymine; P = H-, Cbz-; R^1 = CH_3-, $CH_3(CH_2)_3$-, $CH_3(CH_2)_8$-, $CH_3(CH_2)_{16}$-, C_6H_5-, $C_6H_5CH_2$-; R^2 = H-, CH_3-(L); R^3 = H-, CH_3-, C_2H_5-, $O[(CH_2)_2]_2N$-, $C_6H_5CH_2NH$-, $(CH_3)_2N$-, ClC_6H_4NH-; n = 1–2.

rapid emergence of drug resistant HIV-1 variants, the severe side-effects associated with both short- and long-term treatments and medication costs.[3] These findings highlight the continuing need for new anti-HIV drugs which are less toxic, active against the common drug resistant mutants selected by present treatments and/or inhibit novel biological targets in the viral replicative life cycle.

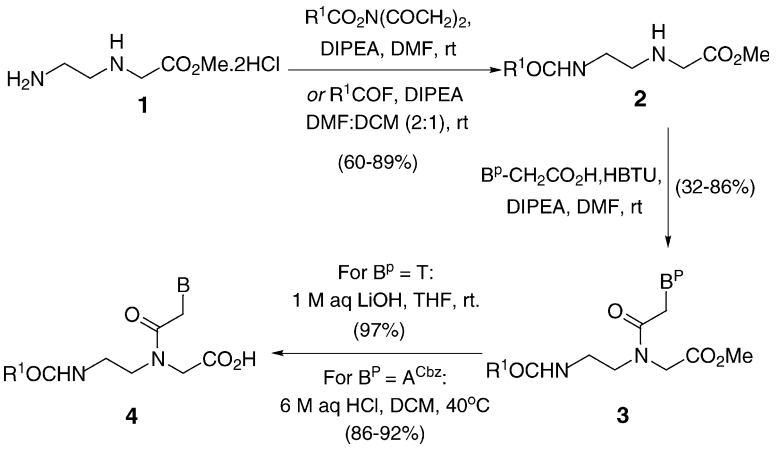
In an attempt to address some of these issues, we have recently embarked on the development of novel NNRTIs and HIV-1 integrase (IN) inhibitors. HIV-1 IN has emerged as an attractive target for future anti-viral therapies because this enzyme has been found to play a key role in stable infection and there appears to be no functional, human equivalent.[4] As part of this research, we have synthesised and evaluated a range of novel *N*-terminal lipid-functionalised peptide nucleic acid (PNA) monomers (Figure 1) and our findings are discussed here.

We believed that these compounds were worthy of investigation because the PNA monomers themselves bore many of the essential structural features required for potential NNRTIs[5a] and IN[5b] inhibitors. In addition, we envisaged that the lipid tails would either facilitate further hydrophobic interactions with appropriate amino acids at the binding sites of the enzymes or assist in stabilizing the interactions made by the PNA monomer. Indeed, the attachment of terminal lipophilic groups to anti-HIV oligonucleotides has been reported to enhance their antiviral effect.[5c]

RESULTS AND DISCUSSION

Synthesis of *N*-Terminal Lipid Functionalized PNA Monomers

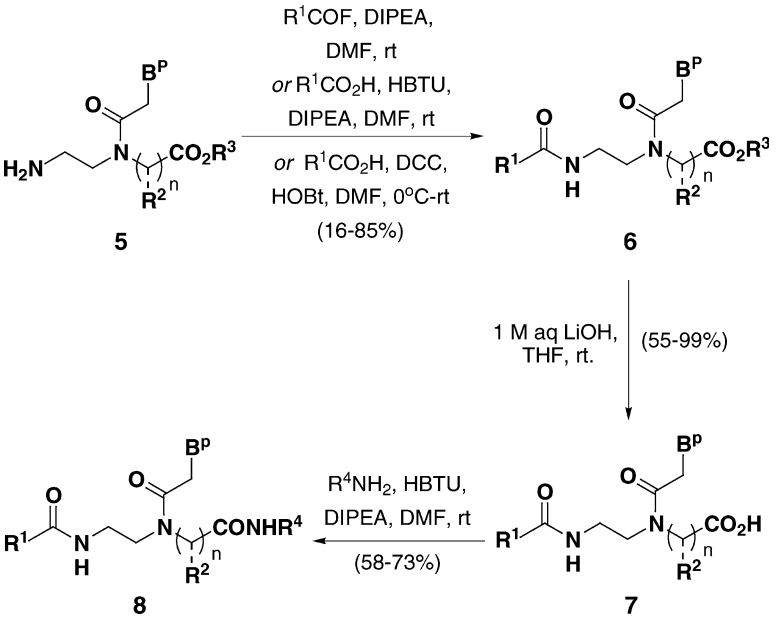
The *N*-terminal lipid functionalized PNA monomers were prepared by either coupling the appropriate lipid moiety to *N*-(2-aminoethyl)glycine methyl ester hydrochloride **1** prior to attaching the required nucleobase acetic acid derivative (Scheme 1)[6] or coupling the lipid group to the free amino terminus of the appropriate PNA monomer alkyl ester **5** (Scheme 2).[7]



SCHEME 1 $B^p = N^6$-Benzyloxycarbonyladenin-9-yl (A^{Cbz}), thymin-1-yl (T); B = adenin-9-yl (A), thymin-1-yl (T); $R^1 = CH_3(CH_2)_3$-, $CH_3(CH_2)_8$-, $CH_3(CH_2)_{16}$-.

HIV-1 RT and IN Inhibition Studies

The anti-viral activities of the *N*-terminal lipid functionalized PNA monomers have been evaluated and one compound, **4** (Scheme 1; B = T and $R^1 = CH_3(CH_2)_{16}$-), has been identified as a reasonable



SCHEME 2 $B^p = N^6$-Benzyloxycarbonyladenin-9-yl (A^{Cbz}), N^4-benzyloxycarbonylcytosin-1-yl (C^{Cbz}), thymin-1-yl (T); $R^1 = CH_3$-, $CH_3(CH_2)_3$-, $CH_3(CH_2)_8$-, $CH_3(CH_2)_{16}$-, C_6H_5-, $C_6H_5CH_2$-; R^2 = H-, CH_3-(L); R^3 = Me, Et; $R^4 = O[(CH_2)_2]_2N$-, $C_6H_5CH_2NH$-, $(CH_3)_2N$-, ClC_6H_4NH-; n = 1–2.

inhibitor of both HIV-1 RT and IN with ID_{50} = 65 μM and ED_{50} = 22 μM respectively. Time dependent inhibition studies of wild-type HIV-1 RT by **4** (B = T and R^1 = $CH_3(CH_2)_{16}$-) have revealed that, although it has a very slow association rate ($k_{on} = 31 \pm 1\ M^{-1}s^{-1}$ cf. $1.8 \pm 0.2 \times 10^3\ M^{-1}s^{-1}$ for efavirenz[8]), once formed the enzyme-inhibitor complex is extremely stable, as indicated by the very low dissociation rate ($k_{off} = 16.3 \pm 0.3 \times 10^{-5}\ s^{-1}$ cf. $1.2 \pm 0.5 \times 10^{-4}\ s^{-1}$ for efavirenz[8]). In addition, mechanism of action studies have shown this compound to be a non-competitive inhibitor of HIV-1 RT. A gel analysis of the anti-IN activity of **4** (B = T and $R^1 = CH_3(CH_2)_{16}$-) has been performed. This revealed that this compound affected both processes catalysed by IN (i.e. 3′ processing and strand transfer) equally (IC_{50} = 22 μM in each case). Further studies on this compound and related analogues are in progress.

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