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Synthesis of Enantiomerically Pure Backbone Alkyl Substituted Peptide Nucleic Acids Utilizing the Et-DuPHOS-Rh$^+$ Hydrogenation of Enamido Esters

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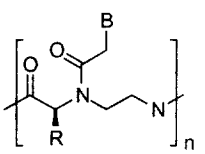
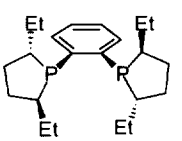
Abstract: A series of enantiomerically pure alkyl substituted peptide nucleic acid (PNA) monomers were synthesized by incorporating novel α-amino acids produced by the Et-DuPHOS-Rh$^+$ catalytic asymmetric hydrogenation of enamido esters.

Peptide Nucleic Acids (PNAs) are oligonucleotide mimics in which the ribose phosphate backbone is replaced with an achiral 2-aminoethylglycine peptidomimetic backbone (Fig. 1, B=nucleobase). PNAs have shown excellent sequence selective binding properties to both DNA and RNA.[1] As well as being useful diagnostic tools,[2] PNAs have also shown promise as antisense and antigene agents.[3] However, as therapeutic compounds, the parent PNAs (Fig. 1, R=H) appear to have limited utility due to inherently low solubility and inability to transverse cell membranes.[1]

Figure 1.



The physical properties of PNA oligomers such as binding affinity, hydrophobicity and solubility could potentially be tuned to suit a specific purpose by variation of the R substituent. Since naturally occurring oligonucleotides are chiral, the importance of the stereochemistry of the substituents on the PNA backbone must be addressed because of the potential effect on the complexation to DNA or RNA. Examples of PNAs containing backbone substituents have appeared in the literature.[4] The published strategies rely on naturally occurring α-amino acids for the backbone substituents which provides for only a limited range of functionalities. Our goal was to use the Et-DuPHOS-Rh$^+$ (**1**-Rh$^+$) catalyzed asymmetric hydrogenation of enamido esters[5] to produce novel amino acids that could be incorporated into the backbone of PNA monomers.



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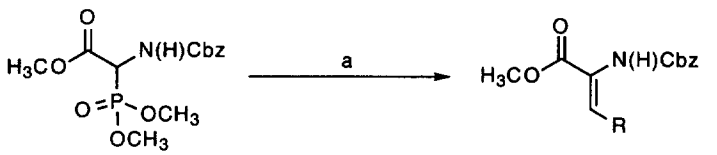
PII: S0040-4039(99)00518-3

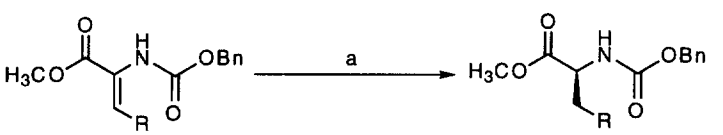
The enamido ester substrates **3** were prepared as shown in Scheme 1. A Horner-Emmons type olefination was performed between methyl-2-(*N*-Cbz-amino)-2-dimethylphosponylacetate **2**[6,7] and butyrylaldehyde, hexanal, and cyclohexane carboxaldehyde using tetramethylguanidine (TMG) as base in THF. The *Z*-enamido esters **3a-c** were formed selectively (>20:1 Z/E) in yields of 79%, 73% and 72%, respectively.



2 **3**

Scheme 1. Synthesis of *N*-Cbz enamido esters **3**; **3a**, R=propyl; **3b**, R=pentyl; **3c**, R=cyclohexyl. (a) RCHO, TMG, THF, 0 °C, 3 h.

The *N*-Cbz enamido esters **3** were then hydrogenated in the presence of **1**-Rh^+ as shown in Scheme 2.[5a] Table 1 lists the stereochemistry of the ligand used and substrate to catalyst ratios employed, as well as enantiomeric excesses and absolute configurations for **4**. The enantioselectivities for the hydrogenations of **3** were all above 98% as determined by chiral GC. The absolute stereochemistries of **4** were assigned based upon analogy with known compounds.[5]



3 **4**

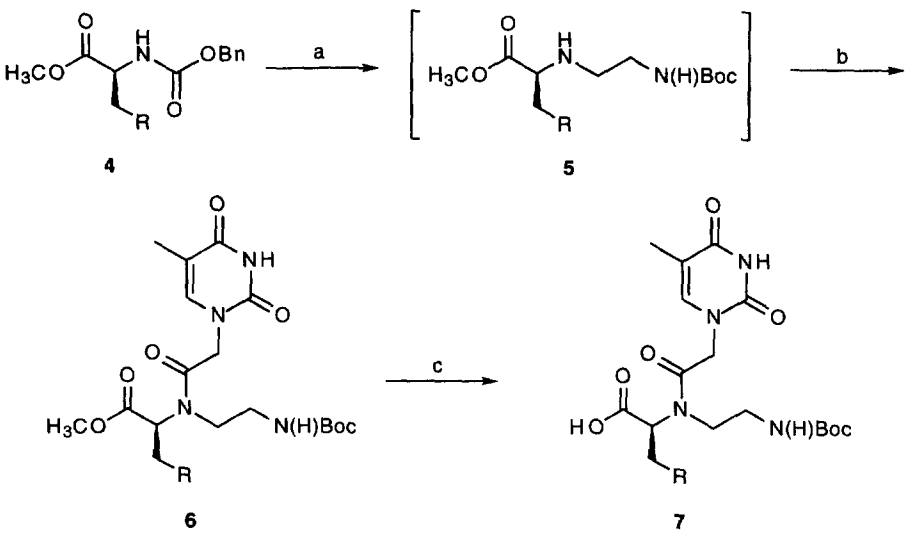
Scheme 2. (a) [(*S,S*) or (*R,R*) **1** Rh COD]$^+$ $^-$OTf, MeOH, 60 psi H_2, 20.5 to 40 h.

Table 1. Et-DuPHOS-Rh^+ catalyzed asymmetric hydrogenation of enamido esters **3a-c**.

Substr.	Ligand	Product	S/C	Conv. (%)[a]	e.e. (%)[b]	Config.
3a (R=Propyl)	(*S,S*)-**1**	**4a**	2600	100	98.9	*S*-(+)
3b (R=Pentyl)	(*S,S*)-**1**	**4b**	2370	100	99.6	*S*-(+)
3c(R=Cyhex)	(*R,R*)-**1**	**4c**	1140	100	98.7	*R*-(+)

[a] Conversion determined by ^{1}H NMR. [b] Enantiomeric excess determined by chiral GC with a Chirasil-*L*-Val column.

The chiral *N*-Cbz α-amino esters **4** were then elaborated into PNA monomers as shown in Scheme 3. The synthesis of the monomers is similar to that employed by Nielsen *et al.*[8] The PNA monomer backbones **5** were assembled by the *in situ* hydrogenolysis of the Cbz group followed by the reductive amination between the resulting free amine and *N*-Boc glycine aldehyde.[9] The products **5** were not isolated or purified and were used directly in step b.



Scheme 3. (a) N-Boc glycine aldehyde, 10% Pd/C, MeOH, 60 psi H_2, 4h; (b) DCC, 2-(thymin-1-yl) acetic acid, 1:1 DMF/CH_2Cl_2, 0 °C, 3h; (c) 1N NaOH, DME, 0.25 h.

2-(Thymin-1-yl)acetic acid was prepared according to literature procedures[10] and was coupled to the secondary amine of the PNA backbones **5** using dicyclohexylcarbodiimide (DCC). The yields of **6** were moderate at 38%, 48% and 63% respectively after purification. Only the thymine monomers were produced in this study but other nucleobase substituted acetic acids could be incorporated as per literature procedures.[8] The methyl esters **6** were then saponified with 1*N* NaOH in DME at 0 °C to give the free acid monomers **7**.[11] No racemization was observed under these conditions.

N-Cbz enamido esters have not only proven to be excellent substrates for Et-DuPHOS-Rh^+ catalytic asymmetric hydrogenation reaction but the resulting *N*-Cbz amino esters are also useful precursors for the expedient synthesis of chiral backbone substituted PNA monomers. The demonstrated synthetic sequence describes the potential to access either enantiomer of a variety chiral PNA monomers.

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11. Representative data for intermediates **3a**, **4a**, **6a** and final monomer **7a**;
Methyl-2-(benzyloxycarbonylamino)-2-hexenoate (3a). ^{1}H NMR ($CDCl_3$, 300 MHz) d 7.37 (m, 5H), 6.65 (t, 1H, J=7.4 Hz), 6.21 (b, 1H), 5.15 (s, 2H), 3.75 (s, 3H), 2.19 (dt, 2H, J= 7.4 Hz, 7.4 Hz), 1.48 (tq, 2H, J=7.4 Hz, 7.4H), 0.93 (t, 3H, J=7.4 Hz). ^{13}C NMR ($CDCl_3$, 75.6 MHz) d 165.1, 154.0, 138.3, 135.9, 128.5, 128.2, 128.1, 125.1, 67.3, 52.3, 30.4, 21.5, 13.9. HRMS (FAB) *m/z* 278.1388; $(M+H)^+$ cald. for $C_{15}H_{20}O_4N$, 278.1392. **Methyl-2-(*S*)-2-(benzyloxycarbonylamino)-2-hexanoate (4a).** ^{1}H NMR ($CDCl_3$, 300 MHz) d 7.36 (m, 5H), 5.26 (d, 1H, J=8.0 Hz), 5.11 (s, 2H), 4.37 (dt, 1H, J=5.4 Hz, 5.4 Hz), 3.74 (s, 3H), 1.80 (m, 1H), 1.64 (m, 1H), 1.31 (m, 4H), 0.89 (t, 3H, J=6.8 Hz). ^{13}C NMR ($CDCl_3$, 75.6 MHz) d 173.1, 155.8, 136.2, 128.5, 128.1, 66.9, 53.8, 52.3, 32.3, 27.2, 22.2, 13.8. HRMS (FAB) *m/z* 280.1538; $(M+H)^+$ cald. for $C_{15}H_{22}O_4N$, 280.1549. $[\alpha]^{25}_D$=+7.42 (c=0.0122, $CHCl_3$). Enantiomeric excess determination; for analysis by chiral GC, **4a** was converted to the *N*-Boc derivative using hydrogenolysis in the presence of 10% Pd/C and Boc anhydride; Chirasil-*L*-Val column, inj. press. 10 psi, T=130 °C , t_R=6.97 (*R*), 7.32 (*S*) min. **Methyl-2-(*S*)-2-[*N*-2(*tert*-butoxycarbonylamino)ethyl-*N*-((thymin-1-yl)acetyl)amino]hexanoate (6a).** ^{1}H NMR ($CDCl_3$, 300 MHz) d 9.98 (b, 1H), 7.06 (s, 0.33H), 6.99 (s, 0.67H), 5.72 (t, 0.8H, J=5.8 Hz), 5.21 (m, 0.2H), 4.72-4.42 (m, 2H), 4.23 (dd, 1H, J=9.5 Hz, 5.6 Hz), 3.79 (s, 0.6H), 3.47 (s, 2.4H), 3.69-3.55 (m, 1H), 3.49-3.20 (m, 3H), 2.12-1.75 (m, 2H), 1.91 (s, 3H), 1.44 (s, 9H), 1.43-1.21 (m, 4H), 0.90 (t, 3H, J=6.9 Hz). ^{13}C NMR ($CDCl_3$, 75.6 MHz) d 171.9, 167.3, 164.5, 155.9, 151.12, 151.07, 141.0, 110.4, 79.6, 60.2, 60.0, 52.8, 52.4, 48.1, 46.7, 39.2, 28.5, 28.22, 28.16, 22.1, 13.7, 12.2. HRMS (FAB) *m/z* 455.2508, $(M+H)^+$ cald. for $C_{21}H_{35}N_4O_7$, 455.2506. $[\alpha]^{25}_D$=-12.94 (c=0.0034, $CHCl_3$). **Methyl-2-(*S*)-2-[N-2(*tert*-butoxycarbonylamino)ethyl-N-((thymin-1-yl)acetyl)amino]hexanoic acid (7a).** ^{1}H NMR ($CDCl_3$, 400 MHz) d 10.01 (b, 0.4H), 9.79 (b, 0.1H), 9.50 (b, 0.5H), 7.49 (b, 0.4H), 7.15-6.98 (m, 1H), 5.59 (b, 0.5H), 5.18 (b, 0.1H), 4.72-4.05 (m, 2H), 4.02-3.60 (m, 2H), 3.50-3.20 (m, 3H), 2.16-1.94 (m, 1H), 1.89 (s, 2H), 1.77 (s, 1H), 1.49 (s, 3H), 1.41 (s, 5H), 1.38-1.20 (m, 5H), 0.94-0.82 (m, 3H). ^{13}C NMR ($CDCl_3$, 100.6 MHz) d 173.9, 167.1, 164.4, 157.7, 156.2, 151.9, 141.2, 111.0, 82.1, 79.8, 60.8, 49.1, 47.5, 40.3, 39.1, 31.6, 29.7, 28.9, 28.4, 22.6, 22.4, 14.1, 13.9, 12.3, 11.8. HRMS (FAB) *m/z* 441.2359; $(M+H)^+$ cald. for $C_{20}H_{33}O_7N_4$, 441.2349. $[\alpha]^{25}_D$=-11.11 (c=0.0183, $CHCl_3$).