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Synthesis of the four diastereoisomers of 3-thymine-1-(ᵗbutoxycarbonyl)aminocyclopentane-1-carboxylic acid

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Abstract—Synthetic routes to all four diastereoisomers of 3-thymine-1-(ᵗbutoxycarbonyl)aminocyclopentane-1-carboxylic acid have been developed starting from the commercially available (*S*)-dimethyl malate. The key step in the synthesis involves dialkylation of *N*-(diphenylmethylene)glycine ethyl ester with 1,4-diiodo-2(*S*)-trityloxybutane.

Selective manipulation of gene expression will make a major impact on the therapy of human disease and much research is now focused on the design and synthesis of ligands that bind to DNA in a sequence specific manner so as to inhibit transcription. Nielsen et al.[1,2] have developed polyamide nucleic acid (PNA) mimics of DNA in which the entire deoxyribose–phosphate backbone has been exchanged with a structurally homomorphous uncharged polyamide backbone composed of *N*-(2-aminoethyl)glycine units (Fig. 1). PNAs bind to both single-stranded DNA (ssDNA) and RNA with high affinity and sequence specificity and a number of oligonucleotide-dependent enzymatic functions have been inhibited on forming PNA/ssDNA or PNA/

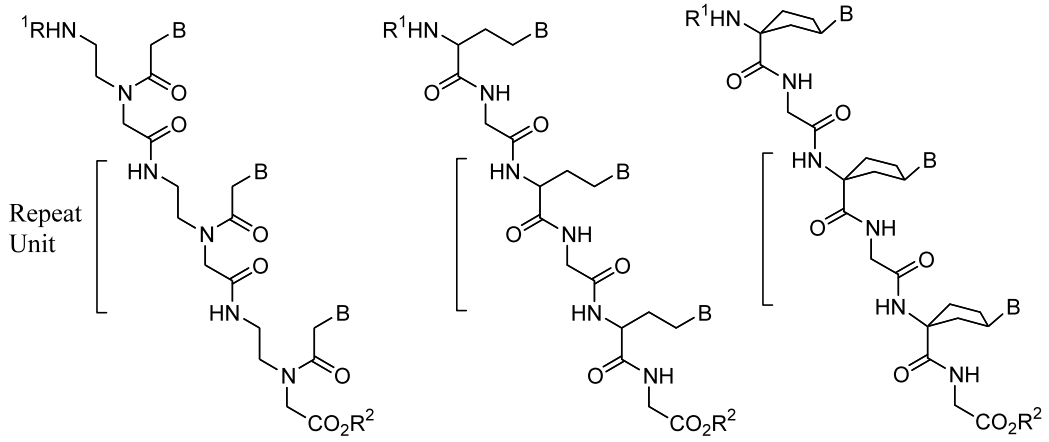

Figure 1. Comparison of PNA, α-PNA and α-cycloPNA structures. B = adenine; cytosine; guanine; thymine.

Keywords: PNA; homoserine analogues; cyclic α,α-disubstituted amino acids.

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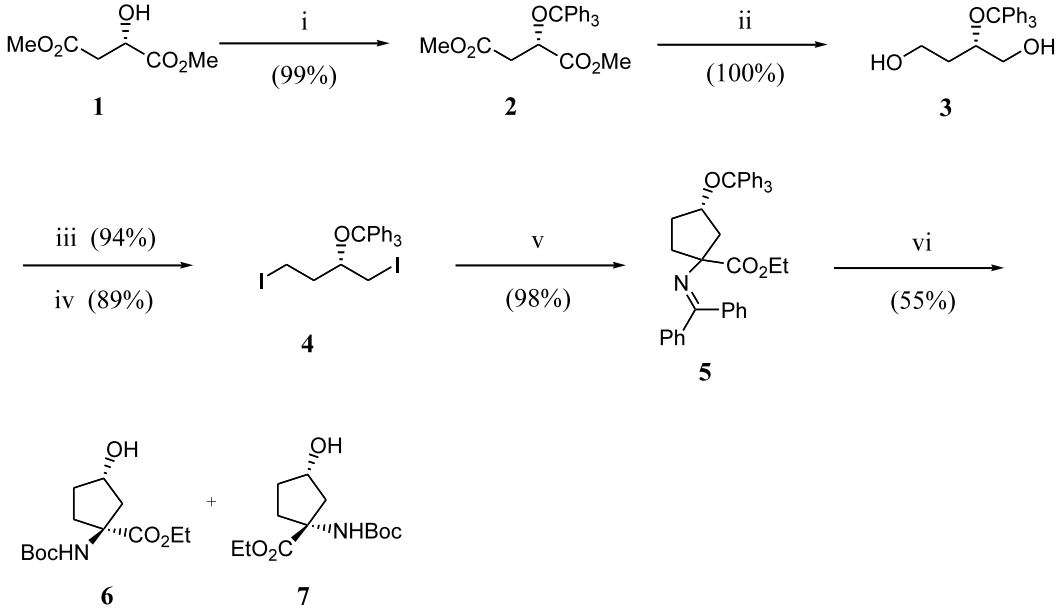
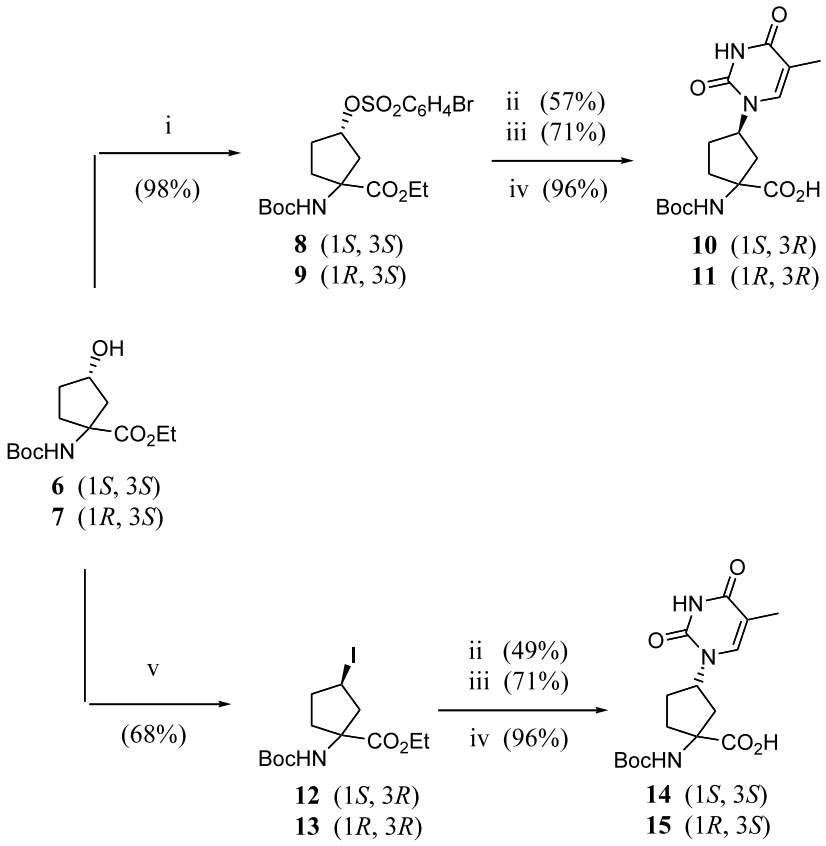
RNA complexes.[2] Homopyrimidine PNAs have been found to invade double-stranded DNA (dsDNA) by displacing the non-complementary strand to form $(PNA)_2$/DNA triplexes and a displaced strand analogous to a P-loop.[2] Homopurine PNAs also invade dsDNA but fail to form triplexes and their invasion complexes are less stable.[2] Strand invasion is of great importance because, in principle, it provides a general solution to the molecular recognition problem since duplex formation is governed by the universal Watson–Crick hydrogen bonding scheme. Unfortunately, simple mixed purine–pyrimidine PNAs do not invade dsDNA, in general, although there are a few exceptions.[3–5] Thus, there is the need to explore further PNA analogues for the purpose of expanding the strand invasion alphabet.

Recently, we have embarked on the development of true peptide mimics of DNA and have reported the design and synthesis of one such analogue, L-α-PNA (Fig. 1).[6] Unfortunately, despite molecular models indicating structural complementarity, L-α-PNA oligomers fail to hybridise to appropriate ssDNA targets.[7] We believe that the most likely explanation for this observed lack of hybridisation is side chain flexibility. That such effects may be significant is highlighted by the results of Nielsen et al. who found that reducing the side chain methylene carbonyl in PNA dramatically destabilised both PNA:DNA heteroduplexes and triplexes.[8] The issue of side chain flexibility has also been raised for acyclic DNA analogues[9,10] and for novel PNAs based on δ-amino acids.[11] Thus, we have instigated a research programme to examine side chain restricted L-α-PNAs and α-cycloPNA (Fig. 1) is one of the proposed oligomers for study.

The four diastereoisomers of 3-thymine-1-Boc-aminocyclopentane-1-carboxylic acid acid (**10**, **11**, **14** and **15** (Scheme 2)) required for construction of α-cycloPNA oligomers have been prepared as outlined in Schemes 1 and 2. Their synthesis starts from the commercially available (*S*)-dimethyl malate (**1**), which was recently employed by Ma et al. in the stereoselective synthesis of (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid.[12] The first step involved protection of the hydroxyl group of **1** with a trityl group by treatment with trityl chloride in the presence of DBU to afford **2** in a 99% yield (Scheme 1).[13] The diester **2** was then reduced using lithium borohydride in the presence of a catalytic amount of B-methoxy-9-BBN to give the diol **3** in a quantitative yield after purification (Scheme 1).[14] Mesylation of both hydroxyl groups, followed by heating the resulting dimesylate with sodium iodide in acetone, gave the more stable 1,4-diiodide product, **4**, in an overall yield of 84% (Scheme 1). Dialkylation of *N*-(diphenylmethylene)glycine ethyl ester with **4** was accomplished using 2.2 equiv. of lithium hexamethyldisilazide to yield the cyclised compound **5** as an inseparable mixture of two isomers (Scheme 1).[15] The ratio of *cis*:*trans* products was estimated to be 2:1 from the ^1H NMR of the crude material. Concomitant deprotection of the hydroxyl and amino functions under acidic conditions and reprotection of the amino moiety with a Boc group gave the two key alcohols, **6** and **7**, in 55% yield over the two steps (Scheme 1). Separation of the two isomers was achieved at this stage using flash chromatography and the stereochemistry of the minor product (19%) was assigned as (1*R*,3*S*) (i.e. **7**) from NOE experiments.



Scheme 1. *Reagents and conditions*: i. 1.2 equiv. Ph_3CCl, 1.4 equiv. DBU, DCM, rt; ii. 1.5 equiv. $LiBH_4$, 0.1 equiv. B-methoxy-9-BBN, THF, rt; iii. 2.2 equiv. MsCl, 3.3 equiv. Et_3N, DCM, rt; iv. 5 equiv. NaI, acetone, reflux; v. 1 equiv. $Ph_2CNCH_2CO_2Et$, 2.2 equiv. LiHMDS, THF, −78°C; vi. (a) 2 M (aq.) HCl, rt (b) 1.1 equiv. $(Boc)_2O$, 2 equiv. Na_2CO_3, $CHCl_3$:H_2O, reflux.



Scheme 2. *Reagents and conditions*: i. 3 equiv. $BrC_6H_4SO_2Cl$, 3 equiv. DMAP, 3 equiv. Et_3N, $CHCl_3$, rt; ii. 2.2 equiv. N^3-benzoylthymine,[16] 2 equiv. NaH, DMF, 40°C; iii. 1.5 equiv. NaOEt, EtOH, rt; iv. 2/3 M (aq.) NaOH, dioxane, rt; v. 1.2 equiv. PPh_3, 1.2 equiv. DIAD, 1.2 equiv. MeI, THF, rt.

With alcohols **6** and **7** to hand, it was now possible to make all four diastereoisomers of 3-thymine-1-Boc-aminocyclopentane-1-carboxylic acid. This was effected by firstly converting **6** or **7** into their respective brosylates **8** or **9** (Scheme 2). Preparation of the (1*S*,3*R*)- and (1*R*,3*R*)-diastereoisomers of 3-thymine-1-Boc-aminocyclopentane-1-carboxylic acid (i.e. **10** and **11**) involved alkylation of N^3-benzoylthymine[16] with either **8** or **9** in the presence of sodium hydride, removal of the benzoyl protecting group from thymine using sodium ethoxide, and alkaline ester hydrolysis (Scheme 2). Synthesis of the (1*S*,3*S*)- and (1*R*,3*S*)-diastereoisomers (i.e. **14** and **15**) first required transformation of alcohols **6** or **7** into their corresponding iodo derivatives **12** or **13** through a Mitsunobu reaction (Scheme 2).[17] Subsequent alkylation of N^3-benzoylthymine[16] with either **12** or **13**, removal of the benzoyl protecting group and alkaline ester hydrolysis afforded **14** and **15** in reasonable yields after purification.

In conclusion, we have developed synthetic routes to all four diastereoisomers of 3-thymine-1-Boc-aminocyclopentane-1-carboxylic acid starting from commercially available (*S*)-dimethyl malate. The key step in the synthesis involves dialkylation of *N*-(diphenylmethylene)glycine ethyl ester with 1,4-diiodo-2(*S*)-trityloxybutane. We are currently in the process of incorporating these monomers into α-cycloPNA oligomers.

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