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Polyamide Based Nucleic Acid Analogs - Synthesis of δ-Amino Acids with Nucleic Acid Bases Bearing Side Chains

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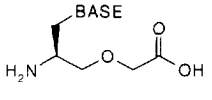
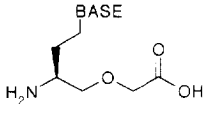
Abstract: *Nucleoamino acids of type **I** - **III** have been synthesized, which can serve as building blocks for novel polyamide based nucleic acid analogs. Key steps in the syntheses are the alkylation of serinol **1** and homoserinol **18** with tert-butyl bromoacetate or tert-butyl bromopropionate under phase transfer conditions and the introduction of thymine or uracil into the amino acid side chains by way of a Mitsunobu reaction. Cytosine derivatives were prepared through uracil → cytosine base conversion at the stage of N_δ-BOC protected amino acid tert-butyl esters.*

The emergence of antisense-based drug design strategies[1] as potentially very powerful alternatives to classical drug discovery approaches has led to widespread interest in structurally modified oligonucleotides or oligonucleotide analogs as metabolically more stable entities than their natural congeners.[2] A large variety of chemical modifications have been investigated in this context, some of which have been found to result in significant increases in RNA-binding affinity and/or nuclease resistance of the corresponding oligonucleotides.[2] While most of the resulting analogs still display many of the structural features characteristic for the deoxyribose-phosphate backbone of natural DNA (e. g. five membered ring structures and/or at least a limited number of unmodified phosphodiester linkages as part of the overall modified backbone), the discovery of peptide nucleic acids (PNA) demonstrates that even more rigorous changes in DNA structure (in the case of PNA the replacement of the entire deoxyribose-phosphate backbone by consecutive aminoethyl glycyl units) do not automatically abrogate binding to complementary RNA or DNA with high affinity and specificity.[3] As polyamide based nucleic acid analogs are intrinsically insensitive to degradation by nucleolytic enzymes[4] and in principle should be synthetically more readily accessible than many types of modified oligodeoxyribonucleotides, it is not surprising that the favorable RNA- and DNA-binding properties of PNA have led to (renewed) interest in the design of other polyamide based structures.[5]

As part of our ongoing program on the biophysical and biochemical evaluation of novel modified antisense oligonucleotides, we have also embarked on the design and synthesis of nucleoamino acids,[6] which could serve as building blocks for novel types of polyamide based nucleic acid analogs. In this communication we want to report on the synthesis of enantiomeric nucleoamino acids of type **I** and **II**[7] as well as diastereomerically homogeneous α-methylated derivatives of type **III** and their conversion into suitably protected derivatives for the solid-phase synthesis of the corresponding oligomers. In the following paper we shall then describe the synthesis of such oligonucleotide analogs and their binding to complementary RNA and DNA.



I



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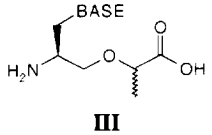

III

The synthesis of both nucleoamino acids of type **I** and **III** proceeds through differentially protected serinol **1** as an early common intermediate, which was prepared from commercially available N_α-BOC-(L)-Ser(OBn)-OH by direct reduction with $LiAlH_4$ in 65% yield.[8] To access nucleic acid base derivatives of type **I** (*Scheme 1*) compound **1** was elaborated into primary alcohol **3** *via* alkylation with *t*-butyl bromoacetate under

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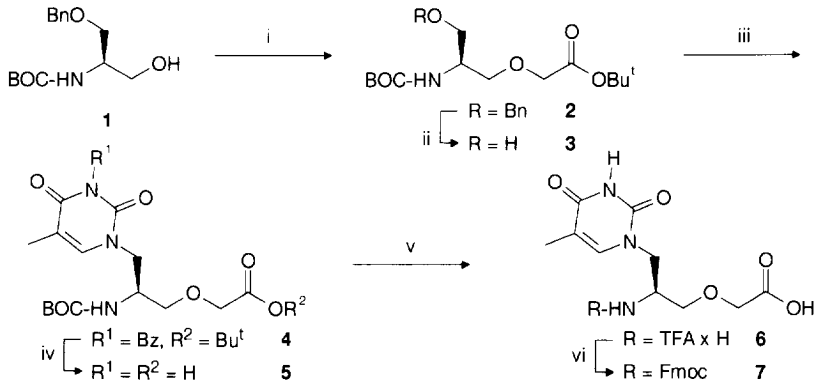


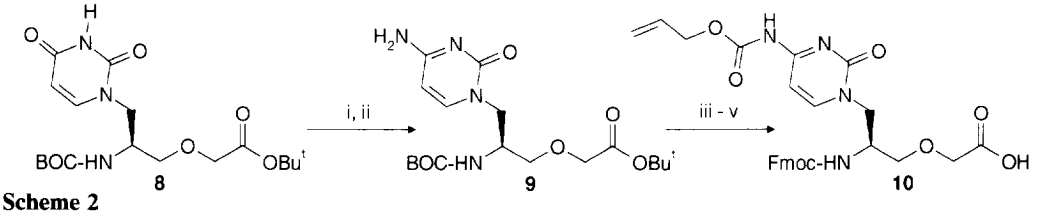
Scheme 1

i. $BrCH_2COOBu^t$ (3 equiv.), Bu_4NHSO_4 (0.25 equiv.), benzene/50% NaOH, 10°, 2.5h, 90%. ii. H_2, Pd-C, MeOH/AcOEt 1/1, RT, 1.5h, 91%. iii. DEAD (2.4 equiv.), N^3-Bz-thymine (1.9 equiv.), Ph_3P (2.4 equiv.), THF, 0° → RT, 18h, 74%. iv. 2N NaOH/MeOH 1/4 (4.0 equiv. OH^-), RT, 3.5h, 75%. v. CF_3COOH, RT, 2h. vi. Fmoc-OSu (1.15 equiv.), Na_2CO_3 (2.3 equiv.), dioxane/H_2O 2/1, RT, 18h, 84% (2 steps).

phase transfer conditions followed by removal of the benzyl protecting group by catalytic hydrogenation in 81% overall yield. Reaction of **3** with N^3-Bz-thymine under *Mitsunobu* conditions[9] provided thymine derivative **4** in 74% yield without any apparent interference from nucleophilic attack, either by the carbonyl oxygen of the BOC-group or the protected amine nitrogen, on the oxo-phosphonium intermediate formed in the *Mitsunobu* reaction.[10] Concomitant saponification of the *tert*-butyl ester and removal of the Bz-group from the base nitrogen followed by acid induced cleavage of the BOC protecting group on the δ-nitrogen led to thymine derivative **6**. For the purpose of solid-phase polyamide synthesis **6** was converted into N_δ-fluorenylmethoxycarbonyl (Fmoc-) protected amino acid derivative **7**.[11]

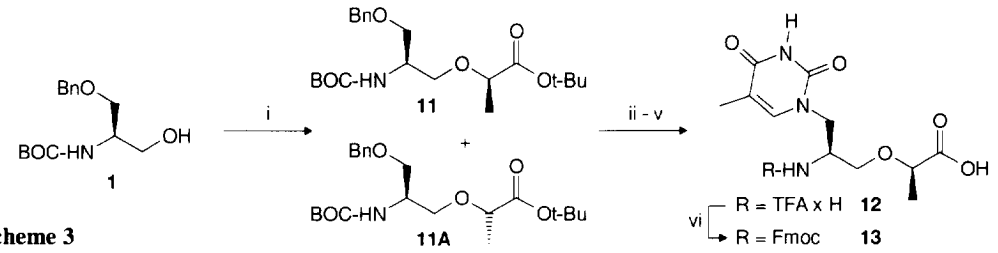
The synthesis of protected cytosine derivative **10** (*Scheme 2*) proceeded through the corresponding uracil derivative **8** (obtained from reaction of **3** and N^3-Bz-uracil under *Mitsunobu* conditions and subsequent removal of the Bz-group with conc. NH_3/dioxane) as the key intermediate. Conversion of the uracil base to cytosine *via* the N-1 substituted 4-triazolo-pyrimidine-2-one[12] followed by protection of N^4 with an allyloxycarbonyl group, concomitant cleavage of the BOC- and *tert*-butyl ester moieties under acidic conditions and finally reprotection of the δ-amino group by reaction with Fmoc-OSu[11] gave **10** in 24% overall yield for the 7-step sequence starting from **3**.

The synthesis of thymine derivatives of type **III** in the initial step involved alkylation of **1** with either (*S*)- or (*R*)-*tert*-butyl bromopropionate. As illustrated in *Scheme 3* for the synthesis of the *2R, 5S* isomer, alkylation of **1** with (*S*)-*tert*-butyl bromoproprionate under phase transfer conditions resulted in an inseparable mixture of diastereoisomers **11** and **11A** (~ 3 : 1). This mixture was elaborated into the corresponding mixture of N^3-Bz-thymine derivatives, from which the desired *2R, 5S* isomer could be isolated in diastereomerically pure form after silica gel chromatography and subsequent recrystallization (33% based on the mixture of **11** and **11A**). Subsequent protecting group manipulations gave Fmoc-protected thymine derivative **13** in 54% overall yield.



Scheme 2

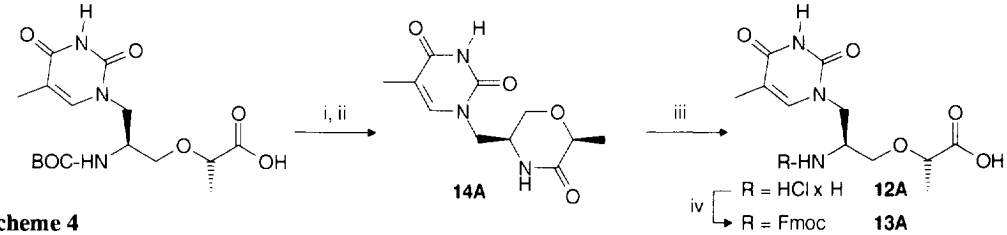
i. $POCl_3$ (2.5 equiv.), 1.2.4-triazole (22.5 equiv.), Et_3N (23 equiv.), CH_3CN, RT, 2h, 69% from **3**. ii. Conc. NH_3/dioxane 1/3, RT, 9h, 80%. iii. CH_2=$CHCH_2OC(O)Cl$ (2.3 equiv.), pyridine, Et_3N, $DMAP_{cat.}$, 79%. iv. CF_3COOH, RT, 30 min, 75%. v. Fmoc-OSu (1.15 equiv.), Na_2CO_3 (2.3 equiv.), H_2O/dioxane 1/1, RT, 20h, 73%.



Scheme 3

i. (*S*)-$BrCH(CH_3)COOBu^t$ (2.0 equiv.), Bu_4NHSO_4 (0.25 equiv.), benzene/50% NaOH 1/1, 10°, 1.5h, 85%. ii. H_2, 10% Pd-C, AcOEt/MeOH 1/1, quant.. iii. DEAD (2.5 equiv.), Ph_3P (2.5 equiv.), N^3-Bz thymine (2 equiv.), THF, 0°, 1h, RT, 16h, 33% (de > 99%). iv. 2N NaOH/MeOH 1/4 (4.0 equiv. OH^-), RT, 14h, 82%. v. CF_3COOH, RT, 30 min. vi. Fmoc-OSu (1.15 equiv.), Na_2CO_3 (2.3 equiv.), dioxane/H_2O 1/1, RT, 16h, 72% (2 steps).

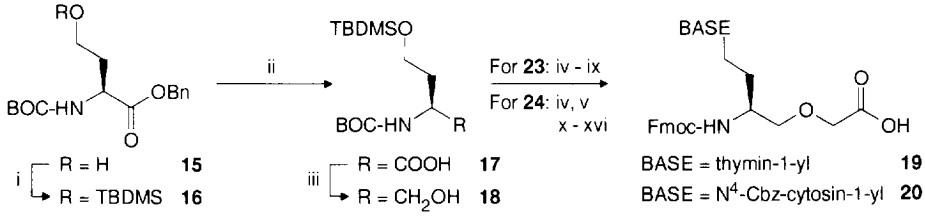
Starting from **1** and (*R*)-*tert*-butyl bromopropionate the synthesis of the Fmoc-protected *2S, 5S*-isomer **13A** proceeded through lactam **14A** as key intermediate (*Scheme 4*);[13] in contrast to its various linear precursors this compound could be obtained in diastereomerically pure form after purification by flash chromatography and was subsequently reconverted to the free amino amino acid **12A** (as the hydrochloric acid salt) by treatment with 6N HCl. Reaction of **12A** with Fmoc-OSu provided **13A** in 12 % overall yield based on **1**.



Scheme 4

i. CF_3COOH, RT, 30 min. ii. THF, refl., 4h, 36% (> 99% de). iii. 6N HCl, 80°, 2h. iv. Fmoc-OSu (1.15 equiv.), Na_2CO_3 (2.3 equiv.), dioxane/H_2O 1/1, RT, 16h, 72% (2 steps).

The above "phase-transfer catalysis/*Mitsunobu*" strategy has also been employed in the syntheses of side chain homologated thymine and cytosine derivatives of type **II**, which involved differentially protected (*S*)-homoserinol **18** as the central intermediate and substrate in the phase transfer alkylation step (*Scheme 5*). Compound **18** was synthesized in 3 steps and 70% overall yield from known alcohol **15**[14] *via* silylation with



Scheme 5

i. TBDMS-Cl (1.1 equiv.), Et_3N (1.2 equiv.), $DMAP_{cat.}$, CH_2Cl_2, RT, 21h, 81%. ii. H_2, 10% Pd-C, quant.. iii. a. *iso*-BuOC(O)Cl (1.2 equiv.), N-methylmorpholine (1.2 equiv.), DME; b. $NaBH_4$ (1.8 equiv.), H_2O, 87%. iv. $BrCH_2COOBu^t$ (2.0 equiv.), Bu_4NHSO_4 (0.25 equiv.), benzene/50% NaOH 3/1, 10°, 2h, 89%. v. TBAF (1.0 equiv.), THF, RT, 30 min, 89%. vi. DEAD (2.4 equiv.), Ph_3P (2.4 equiv.), N^3-Bz-thymine (1.9 equiv.), THF, 0° → RT, 2h, 75%. vii. 2N NaOH/MeOH/DMF 1/2.5/3.5 (4.0 equiv. OH^-), RT, 4h, 94%. viii. CF_3COOH, RT, 30 min. ix. Fmoc-OSu (1.15 equiv.), Na_2CO_3 (2.3 equiv.), dioxane/H_2O 5/4, RT, 18h, 61% (2 steps). x. DEAD (2.4 equiv.), Ph_3P (2.4 equiv.), N^3-Bz-uracil (1.9 equiv.), THF, 0° → RT, 2h, 76%. xi. Conc. NH_3/dioxane 5/1, RT, 3h, 86%. xii. $POCl_3$ (2.5 equiv.), 1.2.4-triazole (22.5 equiv.), Et_3N (23 equiv.), CH_3CN, RT, 3h. xiii. Conc. NH_3/dioxane 1/3, RT, 5h, 80% (2 steps). xiv. BnOC(O)Cl (2 equiv.), CH_2Cl_2, Et_3N (2.5 equiv.), $DMAP_{cat.}$, 65%. xv. CF_3COOH, RT, 30 min. xvi. Fmoc-OSu (1.0 equiv.), Na_2CO_3 (2.0 equiv.), H_2O/dioxane 1/1, RT, 18h, 92% (2 steps).

TBDMS-Cl, hydrogenolytic cleavage of the benzyl ester moiety, and reduction of the carboxylic acid with *iso*-butylchloroformate/$NaBH_4$.[14] After alkylation of **18** with *tert*-butyl bromoacetate and subsequent removal of the TBDMS-group the resulting primary alcohol was elaborated into Fmoc-protected thymine derivative **19** by the same sequence of reactions as depicted in *Scheme 1* for the transformation of **3** into **7**. In complete analogy with the preparation of **10** (*Scheme 2*), N_8-Fmoc-protected cytosine derivative **20** was obtained from **18** via the homologated uridine derivative corresponding to **8**, except that the exocyclic amino group of the cytosine base in **20** is protected by a benzyloxycarbonyl (Cbz-) group rather than as allyl carbamate as in **10**.

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8. Breton, P.; Monsigny, M.; Mayer, R. *Int. J. Peptide Protein Res.* **1990**, *35*, 346 - 351. The optical purity of **1** was determined by chiral analytical HPLC. Using (*S*)-2-(benzyloxymethyl)-2-aminoethanol as a reference (prepared from Nα-BOC-D-Ser(OBn)-OH in analogy to **1**), the ee of **1** was shown to be >99%. As none of the subsequent steps in the synthesis of **7**, **10**, **13**, and **13A** is conceivably accompanied by epimerization of the chiral center α to the amino group, the optical purity of **1** will also be reflected in these final products.
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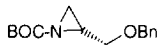
10. The synthesis of aziridine **21** from **1** under *Mitsunobu* conditions has been described (Ho, M.; Chung, J. K. K.; Tang, N. *Tetrahedron Lett.* **1993**, *34*, 6513 - 6516). We have also observed that reaction of the tosylate derived from **1** with NaN_3 in DMF at 80° predominantly provides oxazolidinone **22**.



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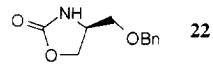

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Although we cannot formally rule out the possibility that the formation of **4** proceeds through **21** as an intermediate, the reactivity profile that we have observed for the latter compound in reactions with other N-nucleophiles ($H_2NCH_2COOBu^t$, CH_3-$HNCH_2COOBu^t$, N_3^-) makes this a rather unlikely possibility.
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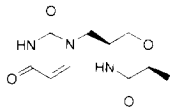

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14. a. Rodriguez, M.; Llinares, M.; Doulut, S.; Heitz, A.; Martinez, J. *Tetrahedron Lett.* **1991**, *32*, 923 - 926. b. G. Kokotos, Synthesis **1990**, 299 - 301. It has been shown that this method proceeds with retention of optical activity (ref. 14b).With regard to the optical purity of **19** and **20** *cf.* ref. 8.

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