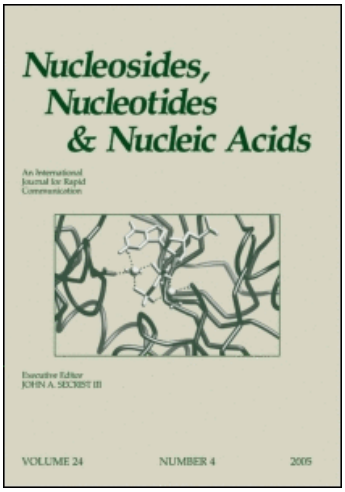

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Ceulemans, G. , Khan, K. , Van Schepdael, A. and Herdewijn, P.(1995) 'Peptide Analogues of DNA Consisting of l-α-Amino-γ-thymine Butyric Acid and l-Valine Subunits', Nucleosides, Nucleotides and Nucleic Acids, 14: 3, 813 — 816
To link to this Article: DOI: 10.1080/15257779508012478
URL: http://dx.doi.org/10.1080/15257779508012478

NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 813-816 (1995)

PEPTIDE ANALOGUES OF DNA CONSISTING OF L-α-AMINO-γ-THYMINE BUTYRIC ACID AND L-VALINE SUBUNITS

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Abstract : Reaction of *N*-Boc-L-homoserine benzylester with N^3-benzoylthymine under Mitsunobu conditions afforded *N*-Boc-L-α-amino-γ-N^3-benzoylthymine butyric acid benzylester. After removal of the *N*-benzoyl and *O*-benzyl protecting group, this compound was used in solution phase peptide synthesis.

Introduction

Molecules that bind sequence specifically to single or double stranded nucleic acids are potential candidates for therapeutics targeted at specific genes, either at the mRNA (anti-sense) or double stranded (ds) DNA (anti-gene) level. Lately there has been considerable interest in developing peptide analogues of DNA instead of modified oligonucleotides. Besides their resistance to nucleases, such peptide nucleic acids (PNA) have the advantage of being available by solid phase peptide synthesis techniques. Interest in PNA synthesis was renewed after reports published on the subject in 1991, almost simultaneously by Huang et al.[1] and Egholm et al.[2]. A molecular study dating from the same time[3] suggests that α-amino acid derived peptides of the type prepared by Buttrey et al.[4] (Fig. 1) accomodate base stacking interactions similar to those found in nucleic acids (B-form). However the oligothymine derivative prepared by the latter authors[4] failed to show any binding with poly(rA). The oligothymines synthesized by Tyaglov et al.[5] (Fig. 2) incorporate a spacer amino acid and it was reported that they do form stable complexes with complementary duplexes. Similar polymers made up of racemic amino acids and a longer nucleobase tether have been reported before[6].

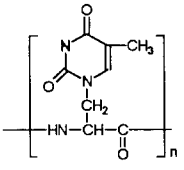

Fig. 1

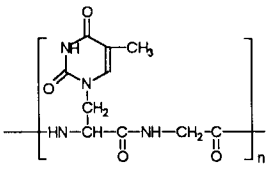

Fig. 2

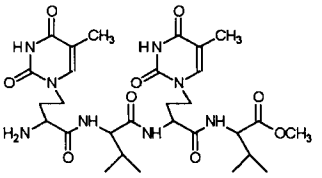

Fig. 3

Here we wish to report the synthesis of a chiral polyamide consisting of L-valine amino acids and L-α-amino-γ-thymine butyric acid (Fig. 3). The backbone of this polymer should allow every base to interact with DNA. The internucleobase distance is optimized so as to match that in DNA.

Chemistry

L-Homoserine is commercially available. Its amino and carboxylic acid function were protected by formation of an urethane and ester respectively, as has been described before[7,8]. The protected thymine nucleobase[9] was introduced at the primary hydroxyl group under Mitsunobu conditions to give *N*-Boc-α-amino-γ-N^3-benzoylthymine butyric acid benzylester[10]. Removal of the *N*-benzoyl and *O*-benzyl protecting group by treatment with 1N NaOH, after dissolving in MeOH, yielded *N*-Boc-α-amino-γ-thymine butyric acid[11,12]. This compound (Fig. 4) was used as a building block in solution phase as well as solid phase synthesis. The synthesis was carried out following the Merrifield method. L-Valine was used as spacer amino acid.

Solution phase synthesis resulted in the desired tetrapeptide (Fig. 3), containing two thymines. In the mixture released from the resin after solid phase synthesis, however, no such compound could be detected.

Intermediates of solution phase synthesis have been analysed by NMR and MS. The dipeptide consisting of L-valine and L-α-amino-γ-thymine butyric acid was further investigated. The amount of diastereoisomers has been assessed to be 4.4% by capillary electrophoresis[13]. The cause of the apparent racemisation is under current investigation but most probably it resides in the alkaline deprotection conditions.

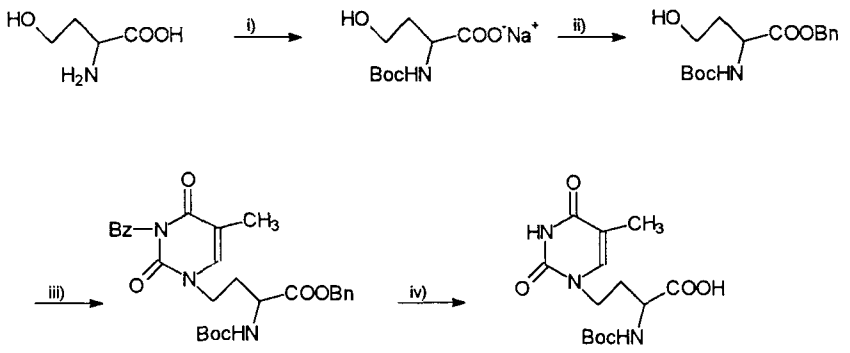

Fig. 4 - Reagents and conditions

i)Ozinskas et al.1,;i)Baldwin et al.2;iii)N3-benzoylthymine,Ph3P,DEAD,THF;iv)1N NaOH,MeOH

Conclusions

The synthesis of the monomer is straightforward and gives good yields. During the formation of the polymer in solution, yields are moderate (80%) but drop drastically upon addition of the third amino acid (50%). After four amino acid additions in solid phase synthesis on Brominated Wang Resin (purchased from Novabiochem, Switzerland), no major peak corresponding to the expected tetrapeptide could be detected on reversed phase HPLC analysis. The reason why the solid phase synthesis failed is under investigation.

Acknowledgements

This work was supported by the Nationaal Fonds voor Wetenschappelijk Onderzoek (grant n°3007692). We thank Mieke Vandekinderen for fine editorial help.

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10. The product crystallized from ethanol or ethyl acetate. UV (MeOH) : λ_{max} = 254 nm (ε=18400) ^{1}H NMR ($CDCl_3$) : δ 1.4 (9H,s,3xCH_3 Boc), 1.9 (3H,s,CH_3 thym.), 2.2(2H,m,bCH_2), 3.8 (2H,m,gCH_2), 4.4 (1H,br s,CH), 5.2 (2H,s,Bn), 5.3 (1H,d,NH), 7.1 (1H,s,6-H), 7.3-7.7 (8H,m,ar.), 7.9 (2H,d,o-Bz) ppm. ^{13}C NMR ($CDCl_3$) : δ 171.4 (COO), 163.0 (C-4), 155.0 (ureth.), 149.8 (C-2), 140.3 (C-6), 134.9 (Ci), 131.5-128.4 (ar.), 110.8 (C-5), 80.4 (C Boc), 67.9 (Bn), 51.2 (CH), 45.6 (gCH_2), 31.4 (bCH_2), 28.2 (3xCH_3 Boc), 12.3 (CH_3 thym.) ppm.

11. UV (MeOH) : λ_{max} = 272 nm (ε=7900) ^{1}H NMR (CD_3OD) : δ 1.4 (9H,s,3xCH_3 Boc), 1.9 (3H,s,CH_3 thym.), 2.2 (2H,m,βCH_2), 3.8 (2H,m, γCH_2), 4.1 (1H,br s,CH), 7.4 (1H,s,6-H) ppm. ^{13}C NMR (CD_3OD) : δ 174.3 (COO), 165.9 (C-4), 157.1 (ureth.), 152.0 (C-2), 142.3 (C-6), 110.2 (C-5), 79.8 (C Boc), 51.5 (CH), 45.6 (γCH_2), 30.5 (βCH_2), 27.8 (3xCH_3 Boc), 11.3 (CH_3 thym.) ppm. MS (+FAB) : 328 $[M+H]^+$

12. The fully deprotected compound has been described before : Nollet, A.J.H. and Pandit, U.K. *Tetrahedron* **1968**, *25*, 5989-5994

13. Analyses were done using an acid buffer containing cyclodextrin as the chiral selector.