Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 165–168

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

ALANYL-PNA HOMODUPLEX: A-T PAIRING WITH THE *N*7-REGIOISOMER OF ADENINE

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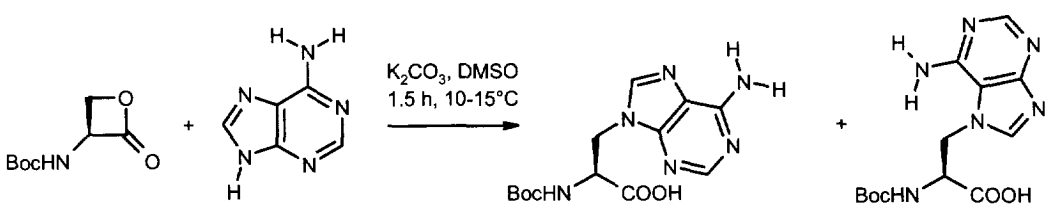
Received 17 October 1997; accepted 4 December 1997

Abstract: The *N*7-regioisomer of adeninyl alanine can be used as a building block for the synthesis of alanyl-PNA. By changing the nucleobase connectivity from *N*9 to *N*7, pairing with the Hoogsteen side is no longer possible and the order of donor/acceptor positions at the Watson-Crick side is reversed. This influences the pairing selectivity but not the stability of alanyl-PNA self-pairing complexes, as shown by UV and CD spectroscopy.

Nucleobases are purines or pyrimidines with amino or carbonyl groups in positions 2 and 6 or 2 and 4, respectively. This functionalization provides a H-bond donor/acceptor pattern which specifies recognition of the complementary base, the pairing mode and the hydrogen-bond stability of a base pair. Since nucleobases in oligomers are linked to a backbone, the constitution and conformation of the backbone limits the options for base pair formation. In order to understand base pairing not limited to primarily two base pairs and one pairing mode, as in DNA/RNA, and furthermore to ascertain the influence of small constitutional changes on the base pairing specificities, we started our investigations on alanyl-PNA[1]. The alanyl-PNA oligomer consists of a regular peptide strand composed of modified alanyl monomers, which carry the nucleo bases in the β-position as a side chain. Alternating configuration of the nucleo amino acids provides that the oligomers are repetitive in every nucleobase and are able to pair in linear double strands. Changes in the constitution of building blocks such as an additional methylene group in the nucleobase linker (homoalanyl nucleo amino acids)[2] or in the backbone (β-homoalanyl nucleo amino acids)[3] alter the pairing specificities of the corresponding PNAs and lead to interesting properties. At this point we would like to discuss the effect of using the *N*7 instead of the *N*9 regioisomer of adeninyl alanine as building block of alanyl-PNA.

Figure 1 Synthesis of the adeninyl nucleo amino acids as described by Lohse *et al.*[4]



1 **2** (22%) **3** (10%)

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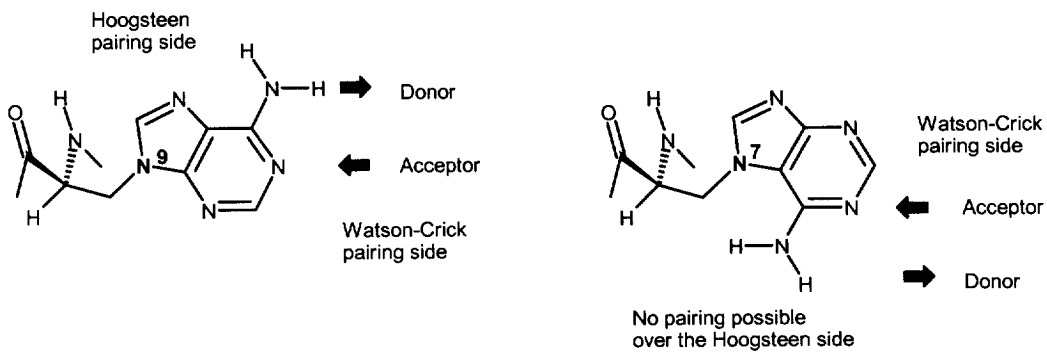
0960-894X/98/$19.00
PII: S0960-894X(97)10210-4

As described by Lohse *et al.* nucleophilic ring opening of Boc-serine lactone **1** with adenine provides Boc-*N*9-adeninyl alanine **2** and Boc-*N*7-adeninyl isomer **3** in a 2:1 ratio[4]. Both products are well characterized[4b]. Oligomerization to hexamer H-(L-Ala^{N7}A - D-AlaT)$_3$-L-Lys-NH_2 was done by solid phase peptide synthesis on a 4-methylbenzhydrylamine (MBHA)-polystyrene support loaded with L-lysine(Z)-OH[5]. The coupling reaction was activated with *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium-hexafluorophosphate (HATU) and Hünig base in DMF for Boc-D-AlaT-OH. Because of the low solubility of Boc-L-Ala^{N7}A-OH, 1-methyl-2-pyrrolidone was used instead of DMF, and the coupling reaction was extended to 8h. The coupling yield was estimated to be above 95%. Purification was done by HPLC on a RP-C18 column. Characterization of the product was done by ESI-MS, UV, CD and ^{1}H-NMR spectroscopy[6].

Figure 2 Regioisomers of adeninyl alanine. In the *N*7 isomer the donor/acceptor order changes, and the Hoogsteen side is not available.



Linkage of adenine to the backbone over *N*7 instead of *N*9 leads to a reversed order of donor/acceptor positions at the Watson-Crick side[7]. At the same time pairing at the Hoogsteen side is no longer possible (Figure 2). The nitrogens *N*3 and *N*9 form a new bisacceptor pairing side, which has no bisdonor equivalent in adenine or thymine. Therefore, pairing between *N*7-adenine and thymine is possible only in a Watson-Crick mode. Since pairing mode, strand orientation (antiparallel/parallel) and configuration of the nucleo amino acids which form a base pair (homochiral/heterochiral) are interdependent[1], an alanyl-PNA double strand based on Watson-Crick A-T base pairs is only possible with an antiparallel strand orientation and homochiral base pairs, or parallel strands and heterochiral base pairs (Figure 3). The *N*7-adeninyl alanine can pair in two Watson-Crick modes: type 1 with a hydrogen bond between thymine-O2 and adenine-NH_2 and type 2 with a tymine-O4/adenine-NH_2 H-bond. The two pairing modes differ in the orientation of the thyminyl nucleo amino acid which can be interconverted by a 180° rotation. We examined the self-pairing of oligomer H-(L-Ala^{N7}A - D-AlaT)$_3$-L-Lys-NH_2. Only A-T base pairs that are heterochiral can form, and due to the geometrical restraints the only possibilities are parallel and Watson-Crick mode type 1, or antiparallel and Watson-Crick type 2. The latter is more likely since it allows the formation of an additional base pair and separation of the charged lysine amides[8].

Figure 3 Adenine-thymine pairing: Watson-Crick mode as one of four possible pairing modes for the *N*9 isomer. The *N*7-adenine can pair with thymine in two orientations which determine the possible geometries for self-pairing of H-(L-Ala^{N7}A - D-AlaT)$_3$-L-Lys-NH$_2$: A double strand with Watson-Crick type 1 pairing mode leads to 5 A-T pairs and parallel strand orientation, while Watson-Crick type 2 leads to 6 A-T pairs with antiparallel oriented backbones. (A = ^{N7}AlaA, T = AlaT).

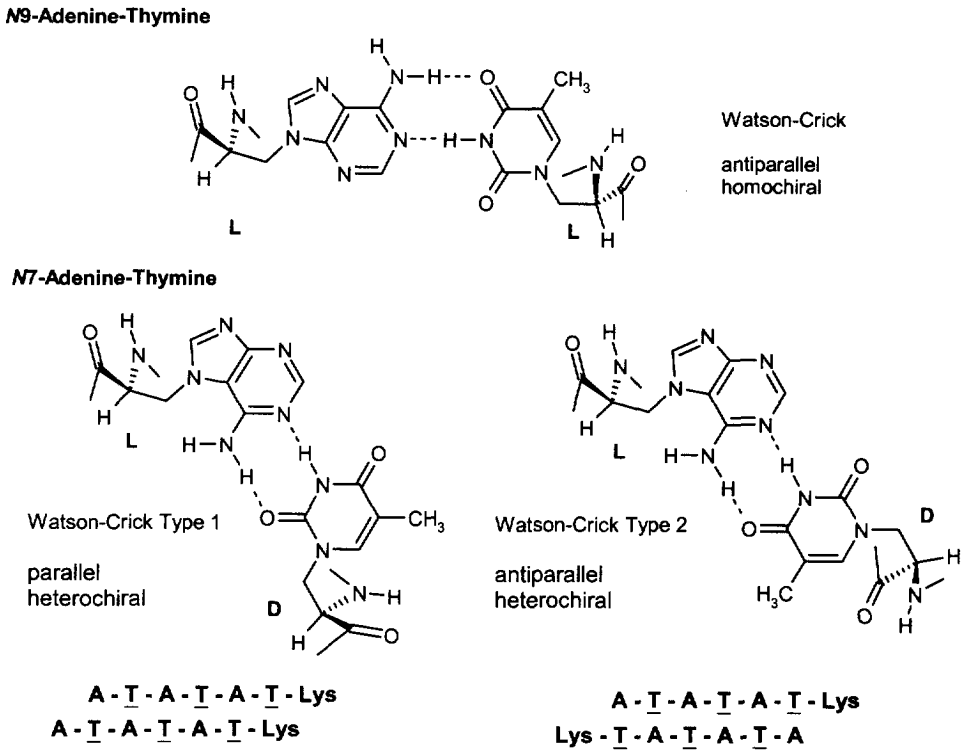
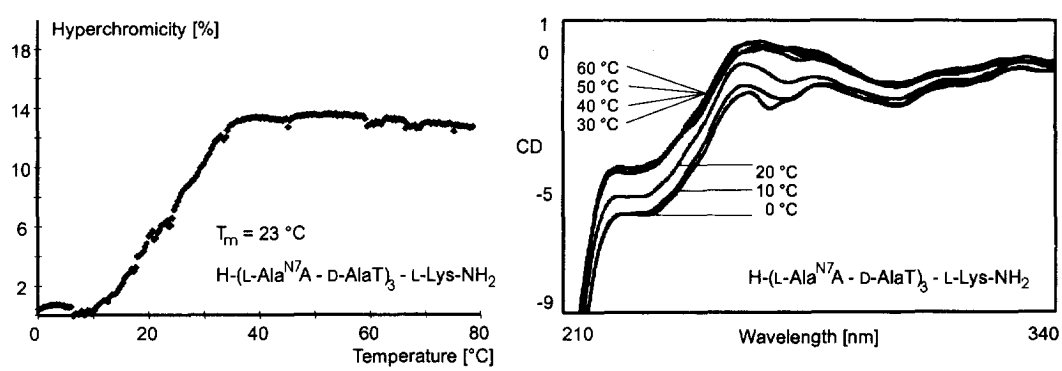


Figure 4 UV melting curve and CD spectra of H-(Ala^{N7}A-AlaT)$_3$-Lys-NH$_2$ (12 μM, 0.1 M NaCl, 0.01 M Na_2HPO_4/H_3PO_4, pH 7, 260 nm).



The pairing stability of the oligomer H-(L-Ala^{N7}A - D-AlaT)$_3$-L-Lys-NH_2 was examined by temperature dependent UV and CD spectroscopy (Figure 4). The sigmoidal shape of the UV melting curve indicates a stability for dissociation of a double strand of T_m = 23°C[9]. This stability agrees well with the results of UV melting curves measured for A-T pairing hexamers in the alanyl-PNA series (T_m = 25°C, reverse Watson-Crick or reverse Hoogsteen mode)[1] and a mixed alanyl/homoalanyl-PNA (T_m = 19°C, Watson-Crick or Hoogsteen mode)[2]. The CD-spectra measured at various temperatures also indicate a dissociation of the double strand between 10 and 30°C.

Similar to the *N*9-adeninyl alanine, the *N*7-adeninyl regioisomer can be incorporated into alanyl-PNA oligomers and forms A-T base pairs with comparable stability but reversed selectivity. Because of a blocked Hoogsteen side and the pairing specificities, the pairing mode of self-pairing H-(L-Ala^{N7}A - D-AlaT)$_3$-L-Lys-NH_2 most likely is Watson-Crick type 2.

Acknowledgments

This work was supported by Deutsche Forschungsgemeinschaft, Fonds der Chemischen Industrie and Leonhard-Lorenz-Stiftung. We are grateful to Professor H. Kessler for generous support.

References and Notes

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4. a) Lohse, P.; Oberhauser, B.; Oberhauser-Hofbauer, B.; Baschang, G.; Eschenmoser, A.; *Croatica Chim. Acta* **1996**, *69*, 535-562. b) Lohse, P.; 'Synthese und Eigenschaften von oligomeren Nukleodipeptamidinium-Salzen', Dissertation, ETH 9937, Zürich, **1992**. The synthesis of Boc-thyminyl alanine follows a similar procedure and is also described in ref. 4a,b.
5. Abbreviations used: L-Ala^{N7}A (β-*N*7-adeninyl-L-alanine), D-AlaT (β-*N*1-thyminyl-D-alanine), Lys-NH_2 (lysine amide introduced for solubility).
6. ^{1}H-NMR (250 MHz, D_2O): δ (ppm) 8.36 (s, 2H, adenine), 8.29 (s, 1H, adenine), 8.19 (s, 2H, adenine), 8.16 (s, 1H, adenine), 7.33 (m, 2H, thymine), 7.27 (s, 1H, thymine), 5.05-4.40 (m, 12H, Hβ Ala), 4.10 (m, 1H, Hα Lys), 4.25-3.75 (m, 6H, Hα Ala), 2.98 (t, 2H, J = 8 Hz, Hε Lys), 1.83 (s, 3H, thymine), 1.77 (m, 6H, thymine), 1.75-1.60 (m, 4H Lys), 1.45-1.25 (m, 2H, Lys). ESI-MS of H-(Ala^{N7}A-AlaT)$_3$-Lys-NH_2 *m/z* = 1343.6 $(MH)^+$, 672.2 $(MH_2)^{2+}$.
7. Rotation of the Cα-Cβ-bond by 180° also provides a reversed donor/acceptor pattern but this conformer is disfavored by eclipsed bonds and a 1,5-repulsion. For further discussion compare ref. 2.
8. Antiparallel strand orientation in general seems to be favored in the alanyl-PNA series. Alanyl-PNA sequences with alternating *N*9-adenine and thymine most likely favor a Hoogsteen A-A and a reverse Watson-Crick T-T pairing[1]. Since we can exclude Hoogsteen pairing for *N*7-adenine A-A, T-T pairing is also very unlikely. The backbone distance in case of Watson-Crick A-A is much larger than T-T.
9. The stability of the self-pairing complex is concentration dependent (T_m = 19°C at 5 μM, 23°C at 12 μM, 26°C at 24 μM concentration) which excludes an intramolecular process.