Prolinol-Based Nucleoside Phosphonic Acids: Synthesis and Properties

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ABSTRACT

Commercially available trans-4-hydroxy-L-proline has been used as a starting material for the synthesis of prolinol-based nucleotide analogues with N-phosphonomethyl moiety attached to the nitrogen atom of prolinol ring. The synthetic methodology based on the inversion of configuration at both 1- and 4-positions led, in result, to all diastereoisomeric O-protected 4'-mesoxyprolinol-N-methylphosphonates. Alkylation of nucleobases using the synths afforded the nucleotide analogues corresponding to α- and β-nucleotides in both L- and D-series. The NMR-based conformational study of α- and β-nucleotides in aqueous solution performed at two different pH values securing either N-fully protonated or deprotonated forms revealed in both cases occurrence of the same mostly populated conformer. All final prolinol-based nucleoside phosphonic acids were tested for cytotoxic and antiviral properties, but no significant activity was found.

RESULTS AND DISCUSSION

Commercially available trans-4-hydroxy-L-proline, as well as cis-4-hydroxy-D-proline obtainable by inversion of configuration at C2 atom of the former compound, were used as key starting material for the synthesis of both L- and D-configured prolinol-based nucleoside phosphonic acids 3, resp (Scheme 1). Both 4-hydroxyprolinols were protected on nitrogen atom with benzylxy carbonyl group and then converted, in several steps into the respective unprotected diastereoisomeric 4-hydroxyprolinols.

Fig. 1 Pyrrolidine nucleoside phosphonic acids

Whereas compounds 1, isosteric with ddNMP, exerted weak antiviral properties, the individual diastereomers of compound 2 were used as monomers for solid-phase synthesis of phosphonate-based PNA.

Herein, we report the synthesis of nucleotide analogues 3 (Fig. 1) related to the α- and β-2'-deoxynucleoside 3'-phosphates, containing prolinol moiety instead of the pentofuranosyl sugar residue, in which the 4'-oxygen atom is replaced by methylene group and the pyrrolidine nitrogen atom is located in place of the 3'-sugar carbon atom. The presence of nitrogen atom at the 3'-position leads to the loss of unambiguously defined configuration at this centre and thus, the N-phosphonomethyl moiety can adopt cis or trans orientation to the nucleobase (Fig. 2).

Fig. 2 Conformational flexibility of pyrrolidine nucleoside N-methyl phosphonic acids

Scheme 1. Synthesis of pyrrolidine nucleoside N-methylphosphonic acids 3

Kabachnik-Fields reaction with the aqueous formaldehyde and diisopropyl phosphite afforded N-
-diisopropylphosphonomethyl derivatives of 4-hydroxyprolinols. Dimethoxytritylation of primary hydroxyl followed by mesylation of the secondary hydroxyl resulted in four synths suitable for the alkylation of nucleobases. The obtained protected nucleotides were transformed into free phosphonic acids 3.

The NMR spectra of α- β- L-prolinol nucleoside phosphonic acids 3 in aqueous solution showed over pH range of 3-11 only small changes of chemical shifts and the coupling constants suggesting preferred N-protonated form. Significant changes in NMR spectra were observed at pH>12 obviously due to the deprotonation at nitrogen atom.

The configurational assignment of methylene protons of pyrrolidine ring was determined from 2D-H, H-ROESY spectra using the observed NOE contacts with protons and substituents (base and the hydroxymethyl moieties) at positions 1 and 4 with known configuration. The preferred configuration at protonated nitrogen atom with NH proton cis-oriented to neighbouring CH₂OH group was derived from NOE contacts of CH₂-P(O)(OH)₂ group and supported by the theoretical calculations (Fig. 3).

Fig. 3 Schematic representation of selected calculated types of preferred conformers

CONCLUSION

We have synthesized a novel isosteric 3'-nucleotide analogues, the prolinol-based α-L- and β-L-nucleoside N-methylphosphonic acids which are distinguished for the loss of unambiguously defined configuration at the nitrogen atom in 3'-position of the prolinol ring. Conformational differences between prolinol α- and β-nucleotides determined by NMR study suggest some similarity with the natural nucleotide. Thus, the structure of β-D- and α-D-analogs could mimic the structure of natural nucleoside 3’- and 5’-phosphates, resp. Therefore, the incorporation of these β-D- and α-D-analogs into oligonucleotides could be interesting in term of hybridization properties. The direction of the synthesis on solid phase in an individual way with each analog would have to be taken into account.

For the β-D-nucleotide, the direction would be from the 3’ to 5’ end whereas for the α-D-nucleotide in reverse direction, from the 5’ to 3’ end.

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