Nucleosides with 1,4-dioxane as sugar moiety

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ABSTRACT

A synthetic route towards novel nucleosides with 1,4-dioxane as the sugar moiety has been developed. The dioxane moiety features a second anomic center, which has been phosphitylated giving a diastereomeric mixture of the corresponding phosphoramidites.

INTRODUCTION

Over the years a wide variety of six-membered nucleosides and nucleotides have been reported in literature. Well known examples include homo-DNA¹ and hexitol nucleic acid (HNA).² Furthermore, simplified nucleoside analogs with 1,4-heteroatom-cyclohexanes as the sugar part, have been reported but have not been incorporated into oligonucleotides.³,⁴ Formal 1,4-dioxane derivatives formed by hydration of 2',3'-dialdehyde-2',3'-seco-nucleosides have also been reported as a stable form of such dialdehydes.⁵,⁶

Inspired by the interesting properties of the previous examples, we designed a route pursuing the synthesis of phosphoramidite derivatives suitable for automated incorporation of monomers X and Y into oligonucleotides.

RESULTS AND DISCUSSION

Starting from known uridine derivative 1,⁷ one-pot oxidative cleavage using sodium periodide followed by sodium borohydride reduction afforded seco-uridine derivative 2 in 89% yield (Scheme 1).

The electron-withdrawing effect of the nucleobase is expected to render the 2'-hydroxy group more acidic than the 3'-hydroxy group, as has been observed for the ring-closed analogues. As a consequence, preferential benzylation of the 2'-hydroxy group could be envisioned. Initial attempts indicated that low temperature was advantageous, leading to preferential 2'-O-benzylation. Thus, reaction at −70 °C using pyridine as base afforded benzylesters 3, 4 and 5 in 67%, 5% and 11% yield, respectively. Changing the base to imidazole, DBU, NaHMDS or K₂CO₃ did not improve formation of desired 2'-O-benzyol derivative 3 (Scheme 1).

![Scheme 1](https://academic.oup.com/nass/article-abstract/52/1/269/1107458)

In the subsequent oxidation, the need to retain the 5'-O-dimethoxytrityl group of alcohol 3 renders acidic oxidation methods unsuitable, as cleavage leads to two primary hydroxy groups (3'-OH and 5'-OH) which are only distinguishable by the prochirality of the β-position (i.e., C4'). As a result, methods such as chromium based oxidations or Swern oxidation are unsuitable. Other DMSO-based oxidations as Pfitzner-Moffatt and Parikh-Döering oxidation proved unsuccessful, as did the otherwise widespread Dess-Martin periodinane oxidation. However, oxidation of the 3'-hydroxy group of alcohol 3 was readily effected by IBX⁸ in EtOAc at near-reflux with pyridine as excess base. Debenzylation of aldehyde 6 was carried out by sodium hydroxide in methanol at room temperature affording hemiacetal 7R/S as an anomeric mixture (Scheme 2).
A few examples of phosphitylation of carbohydrates at the anomeric position have appeared in literature demonstrating the applicability of this method to form glycosidic phosphates. Gratifyingly, the anomeric mixture of hemiacetal 7R/S reacted readily with 2-cyanoeethyl N,N-disopropylphosphoramidochlorodite in dichloromethane affording the four diastereoisomers of phosphoramidite 8A-D in 73% combined yield (Scheme 2). Changing the solvent to acetonitrile did not change the ratio between the four diastereomers formed.

![Scheme 2](image)

Separation of phosphoramidites 8A-D was pursued, as direct incorporation of the diastereomeric mixture into oligonucleotides would result in an undesirable mixture of diastereomeric ONs (incorporation of both monomer X and Y). The mixture could be separated into two portions by silica gel column chromatography, each containing two phosphoramidites. The relative C3'-sterochemistry of the four diastereoisomers was established by coupling the separated binary mixtures with 2-cyanoeethyl. As the phosphorous atom of the formed phosphate triesters is achiral, the triesters are C3'-epimers allowing relative assignment of stereochemistry at this position of the four phosphoramidites. Both binary mixtures give rise to spectra having two peaks – an intensive peak at 139.8 ppm and a less intensive peak at 140.8 ppm, corresponding to a preference formation of one C3'-epimer during the phosphitylation reaction on hemiacetal 7R/S. This also proves that the binary mixtures each consist of two compounds having different C3' stereochemistry. Direct incorporation of the two binary mixtures into oligonucleotides would therefore also yield an undesirable mixture of diastereomeric ONs.

Since standard silica gel chromatography did not afford separated C3'-epimers, the possibility of separation by RP-HPLC is currently being evaluated.

**CONCLUSION**

A viable and efficient route has been established, allowing synthesis of hemiacetal 7R/S in five steps from known uridine derivative 1. Notably, regioselective 2'-O-benzylation of 2',3'-secu-uridine derivative 2 have been achieved in good yield. So far, full separation and determination of complete configuration of diastereomeric phosphoramidites 8A-D have not been realized, and thus, incorporation into oligonucleotides has not been attempted. Based on the results obtained so far, separation by RP-HPLC seems reasonable, which would allow biophysical evaluation of 1,4-dioxane as sugar moiety of modified oligonucleotides.

**REFERENCES**


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