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Regioselective synthesis of 5′-amino acid esters of some nucleosides via orthogonal protecting protocol

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ABSTRACT

Amino acid esters of nucleosides at 5′-position, as peptidomimetic prodrugs, which could be actively transported by the intestinal oligopeptide transporters 1 (PepT1), bear improved oral bioavailability. We established here a regioselective synthesis of the 5′-esters of some nucleosides via an orthogonal protecting protocol with triphenylmethyl (Tr) and allyloxycarbonyl (AOC) protecting groups. A series of 5′-esters of cytarabine and gemcitabine were selectively synthesized in over 36.0% total yields. This efficient and robust methodology will be examplified for the further study of the prodrugs of large number of antiviral and anticancer nucleosides.

1. Introduction

Nucleoside analogs have been used in clinic for many years and play very important roles in the treatment of viral infections and cancer. For example, cytarabine (1-β-D-arabino-furanosylcytosine, **1**, Fig. 1) is a therapeutic agent for the treatment of both acute and chronic myeloblastic leukemias,[1,2] while gemcitabine (2′-deoxy-2′,2′-difluorocytidine, **2**) is clinically effective for solid tumors and non-small cell lung cancer.[3,4]

Although effective, most nucleosides suffer from poor oral bioavailability and short plasma half-life due to their high polarity, low intestinal permeability and poor metabolic stability.[5,6] Thus intravenous infusion is usually utilized and the relatively complex and precise dosing schedules are necessary to maintain effective blood level. So the development of an oral alternative to intravenous administration is essential for broadening the therapeutic use of these nucleosides.

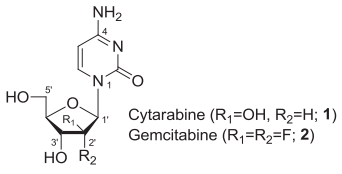

Fig. 1. The structures of cytarabine and gemcitabine.

Prodrug strategies have often been employed to increase lipophilicity by chemical modification of the parent drugs.[7] Alternatively, prodrugs targeting transporters present in the intestine have been exploited to facilitate the transport of the nucleoside analogs.[8,9] In this regard, the intestinal oligopeptide transporters 1 (PepT1) plays an important role in the oral absorption of nutrients and various drugs.[10–13] It is able to transport a wide variety of di- and tri-peptides, as well as many peptidomimetic drugs. Several PepT1-targeting prodrugs have been developed to improve the oral bioavailability of these nucleosides attribute to the enhanced affinity to the transporters.[14–20] For example, valacyclovir[14,15] and valganciclovir[16,17] are two oral-administered prodrugs of 5′-valyl ester of acyclovir and ganciclovir.

In our previous study, a series of 5′-esters of cytarabine were synthesized utilizing direct coupling of *N*-protected amino acids with *N*-Cbz-cytarabine. All the tested esters were more permeable, amongst 5′-L-Valyl ester exhibited the highest permeability of 11 times higher than the parent cytarabine.[18,19]

Nevertheless, multiple reactive functionalities in the structure of **1** made the reaction and the work-up procedure complicated (Scheme 1). The desired 5′-esters were formed together with similar amount of 3′-regioisomers, and more than 3 times careful column separation had to be carried out to purify the 5′-esters, which made it extremely tough to get the final product even in gram scale.

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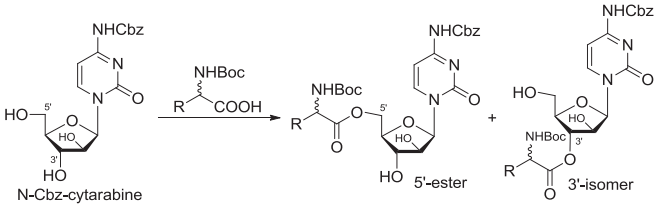
Therefore, it is necessary to develop an alternative synthetic approach with high yield and easy purification to prepare sufficient amount of the derivatives for further in-depth study. It is still prone to synthesize the 5′-esters directly starting from the parent nucleosides.

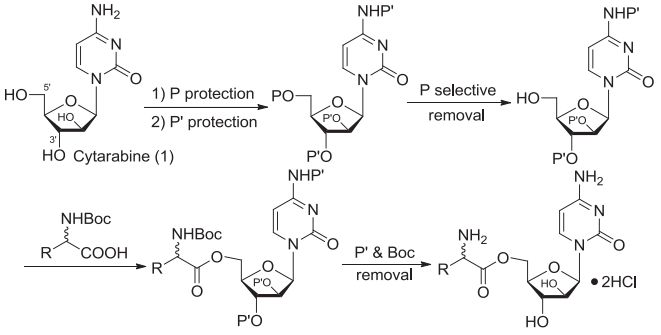


Scheme 1. The poor selectivity for the direct synthesis.

In this paper, it was developed an efficient regioselective synthesis of 5′-amino acid esters of cytarabine and gemcitabine employing a proper orthogonal protecting protocol.

2. Results and discussion

A significant challenge in the regioselective synthesis of 5′-amino acid esters of cytarabine is to select orthogonal protecting groups for 5′-hydroxyl and other active groups. As shown in Scheme 2, a bulky group (P) is supposed to selectively protect 5′-OH of cytarabine. Then all other active sites including the 2′-OH, 3′-OH, and 4-NH_2 are supposed to be protected by another group (P′), which will not be affected during the removal of P. Selective deprotection of P will provide the P′-protected cytarabine with only ONE reactive 5′-OH group, which will be further condensed with the amino acids. Finally, removal of all protecting P′ groups will afford the target products. Thus, adopting suitable protecting protocol, **NO** 3′-isomer will be produced.



Scheme 2. Our protocol for the synthesis of 5′-amino acid esters of cytarabine.

Bulky Triphenylmethyl (Tr) group has been proved to be a suitable P protecting group for this protocol.[21,22] 5′-OH of cytarabine was easily blocked by Tr with TrCl in pyridine in over 90% yield. The removal of Tr was performed smoothly under acidic condition (Scheme 3).[23]

The tuning of the P′ protecting group of all other reactive sites in the structure was the key consideration. First, benzyl group (Bn), which was widely used for the protection of hydroxyl and amino groups, was selected. 5′-Tr-cytarabine was reacted with BnBr and NaH in THF to provide N,N,2′,3′-tetraBn-5′-Tr-cytarabine. After removal of Tr, condensation of resulting alcohol with Boc-L-Val was carried out with DCC and DMAP as catalyst to provide the desired 5′-ester. Unfortunately, deprotection could partially occur under atmospheric or high pressure hydrogenation catalyzed by Pd/C or Raney Ni. Two of the four benzyls still remained in the structure.

Next, we turned to use allyl as the P′ protecting group, which could be removed by *trans*-allylation reaction catalyzed by some homogeneous palladium catalysts.[24] Similarly, treating 5′-Tr-cytarabine with allyl bromide in the presence of NaOH in DMF, N,N,2′,3′-tetraallyl-5′-Tr-cytarabine was easily afforded. Then model reaction was carried out using the Tr removed N,N,2′,3′-tetraallyl-cytarabine to explore the efficiency of the removal of the allyl groups. It was treated by 'Pd' catalyst such as $PdCl_2$, $Pd(OAc)_2$ or $Pd(PPh_3)_4$[25–27] with or without phosphine ligand, and dimethylbarbituric acid (NDMBA) or $Et_2NH_2{\cdot}HCOOH$ was used as allyl scavenger. Disappointingly, no desired product was obtained in all cases, and trans-allylation only partially occurred detected by ESI-MS analysis.

It was pondered that the partial transallyaltion might be caused due to the different reactivity between O-allyl and N-allyl. This drove us to tune an allyl donor out for the synthesis, and the allyloxycarbonyl (AOC) appeared to be a suitable one. After the protection of the OH and NH_2, all the allyls existed as the O-allyl in the carbonate or carbamate functionality. Hence, the transallylation might be smoothly occurred. In this regard, AOC protecting strategy for the protection of hydroxyl and amino groups of nucleosides[28–32] attracted our attention. Fortunately, it worked well and showed satisfactory results in this study.

As shown in Scheme 3, AOC was employed to protect the 2′-OH & 3′-OH and 4-NH_2 of 5′-Tr-cytarabine by treating with allyloxycarbonyl chloride (AOC-Cl). After the Tr-deprotection of **Icyt** with hydrochloric EtOAc, the afforded **IIcyt** was then coupled with Boc-L-Val to provide **IIIcyt**. Complete removal of AOC of **IIIcyt** was extensively screened using $Pd(PPh_3)_4$ as the catalyst and $^{n}BuNH_2$/HCOOH, dimedone, Et_2NH/HCOOH, NDMBA,[27–29] or $TolSO_2Na$ as the allyl scavenger. It was found that the combination of $Pd(PPh_3)_4$ and $^{n}BuNH_2$/HCOOH gave the best result to afford **IVcyt** (Table 1).

In order to double confirm the regioselectivity, a single crystal of **IVcyt** suitable for X-ray analysis was crystallized from acetone, which proved to be the desired regioisomer (Fig. 2).

Finally, the target 5′-L-Val-cytarabine (**Vcyt-1**, 5′-L-Val-ester) was obtained as hydrochloric salt after treating with HCl in EtOAc for the removal of Boc. Scaling up the process to 20-g scale under optimal condition, **Vcyt-1** was obtained without any column separation for 6 steps from cytarabine in 39.2% overall yield.

Similar derivatives of cytarabine (**Vcyt-2**–**Vcyt-7**) with aliphatic L-Val, D-Val, L-Ala, L-Leu or L-Ile, and aromatic L-Phe or D-Phe amino acids, were also synthesized utilizing the above strategy in more than 36.0% overall yields (Table 2).

In order to study the scope and limitation of this methodology, gemcitabine was also selected as a parent drug, and some of its 5′-amino acid esters (**Vgem-1**–**Vgem-7**) with the aliphatic or aromatic amino acids were regioselectively obtained in over 36.0% total yields (Scheme 3, Table 2).

3. Conclusion

A series of 5′-amino acid esters of cytarabine and gemcitabine were synthesized in over 36.0% total yields in this study. A regioselective synthesis was established via orthogonal protecting strategy employing Tr and AOC as protecting groups. The bulky Tr was used to selectively protect 5′-OH of the nucleosides, while 2′-OH, 3′-OH, and 4-NH_2 were protected by AOC group. Selective deprotection of Tr had no effect on AOC and only 5′-esters were exclusively observed in the following condensation with *N*-protected amino acids. This efficient and robust methodology will be examplified for the further study of the prodrugs of large number of antiviral and anticancer nucleosides.

4. Experimental section

All solvents were dried and distilled before use, and all reagents were procured from commercial sources and used without further