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Synthesis of tetracyclic iminosugars fused benzo[*e*][1,3]thiazin-4-one and their HIV-RT inhibitory activity

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ABSTRACT

Several aza-*C*-pseudonucleosides bearing 1,3-benzothiazin-4-one (**6** and **7**) were prepared by the one-pot three-component condensation from the iminosugar aldehyde **3**, amino acid ethyl/methyl ester hydrochlorides **4**(**a**–**c**), and 2-mercaptobenzoic acid **5**. After removal of Boc and the isopropylidene groups, the target novel tetracyclic iminosugars fused benzo[*e*][1,3]thiazin-4-one **1**(**a**–**c**) and **2**(**a**–**b**) were first afforded by the intramolecular cyclo-amidation reaction. Their structures were determined by their ^{1}H, ^{13}C NMR, and HRMS (ESI) spectra and X-ray. The tetracyclic iminosugars **1**(**a**–**c**) and **2**(**a**–**b**) were examined for their HIV reverse transcriptase (RT) inhibitory activities. The result showed that all compounds could effectively inhibit RT activity. Among them, compound **2a** was the best one with the IC_{50} value of RT inhibitory activity of 0.82 μM. Structure–activity relationship analysis suggested that 1′*R* configuration in the tetracyclic azasugars was of benefit to their anti-HIV RT activity.

Iminosugars or azasugars, which exhibit effective inhibition against carbohydrate-processing enzymes, have attracted great interest for their potential clinical applications as anti-HIV, anti-diabetic, and anti-cancer agents and immunomodulators in past decades.[1] The bicyclic azasugars, including the naturally occurring compounds (**A** and **B**)[2] and the synthetic ones (**C** and **D**)[3] (Fig. 1), have also been paid much attention due to their increased possibility leading to discovering new bioactive therapeutic agents. To date, a large number of bicyclic azasugars fused nitrogen heterocycle have been synthesized and evaluated.[4] Much less efforts have been put into multicyclic analogs.[5] The tetracyclic azasugars were so far scarcely reported for their synthesis and biological activity study.

Recently, a series of novel bi- and tricyclic thiazolidin-4-one- and benzothiazin-4-one-fused azasugars (**E** and **F**, Fig. 1) have been found to exhibit strong HIV reverse transcriptase (HIV-RT) inhibitory activities.[6] It was well known that most HIV-1 nonnucleoside reverse transcriptase inhibitors (NNRTIs) generally showed butterfly-like conformation when they were binding with HIV-RT.[7] Taking account this dominant conformation as determinant for anti-HIV activity, many families of NNRTIs were initially designed, including the butterfly-like conformationally constrained NNRTIs **G** (MEN 10979) and **H** (Fig. 1).[8] Inspired by this, we conceived that the tetracyclic azasugars fused benzo[*e*][1,3]thiazin-4-one expanded from the tricyclic ones might be more beneficial for their binding with HIV-RT due to their possible butterfly-like conformation, like compounds **G** and **H**. Therefore, in this Letter, we would like to first report the design and the synthesis of the novel tetracyclic azasugars fused 1,3-benzothiazin-4-one (**1** and **2**, Fig. 2) as a continuation of our researches. Such newly synthetic azasugars were evaluated for their HIV-RT inhibitory activity in order to further investigate the structure–activity relationship (SAR).

The synthesis of the target tetracyclic azasugars was achieved in three steps. The key reaction for the intermediate aza-*C*-pseudonucleosides bearing 1,3-benzothiazin-4-one (**6** and **7**) was the one-pot three-component condensation from the iminosugar aldehyde **3**,[9] amino acid ethyl/methyl ester hydrochlorides **4**(**a**-**c**) (neutralized by $NaHCO_3$), and 2-mercaptobenzoic acid **5** at 40–60 °C as shown in Scheme 1. In the presence of the condensation reagent *N*,*N*′-dicyclohexyl-carbodiimide (DCC) and the promoter 4-dimethylamino-pyridine (DMAP), the one-pot synthesis afforded the diastereomeric products **6** and **7** (Table 1) in the overall yields of 12.4–59.7% following our reported procedure.[10] However, the reactions were accompanied with the generation of the byproducts **10** and **11** which were directly condensed from the aldehyde **3** and 2-mercaptobenzoic acid **5**. The consumption of the aldehyde **3** maybe caused the low yields of the three-component reactions. Moreover, steric hindrance from the methyl on L- and D-alanine might be another reason for the low yields. Especially in entry 3 (Table 1) using D-alanine methyl ester hydrochloride **4c** as amine source, **6c** was just obtained in 12.4% yield. Although only one isomer was found in this case, we could

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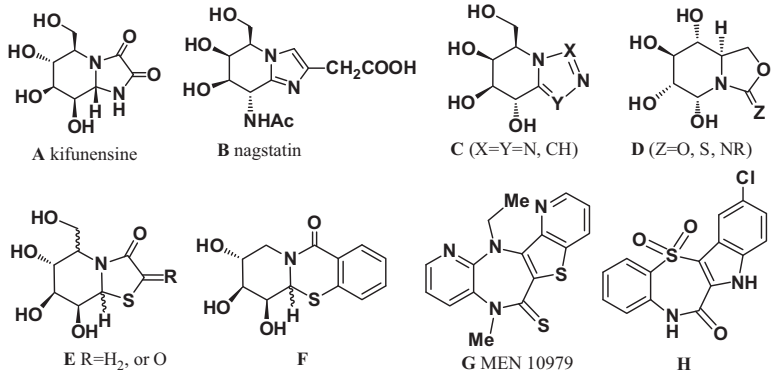


Figure 1. The structures of some bi/tricyclic azasugars **A**–**F** and conformationally constrained NNRTIs **G** and **H**.

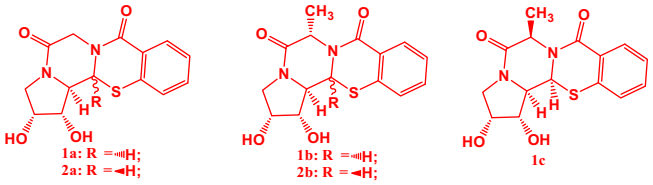
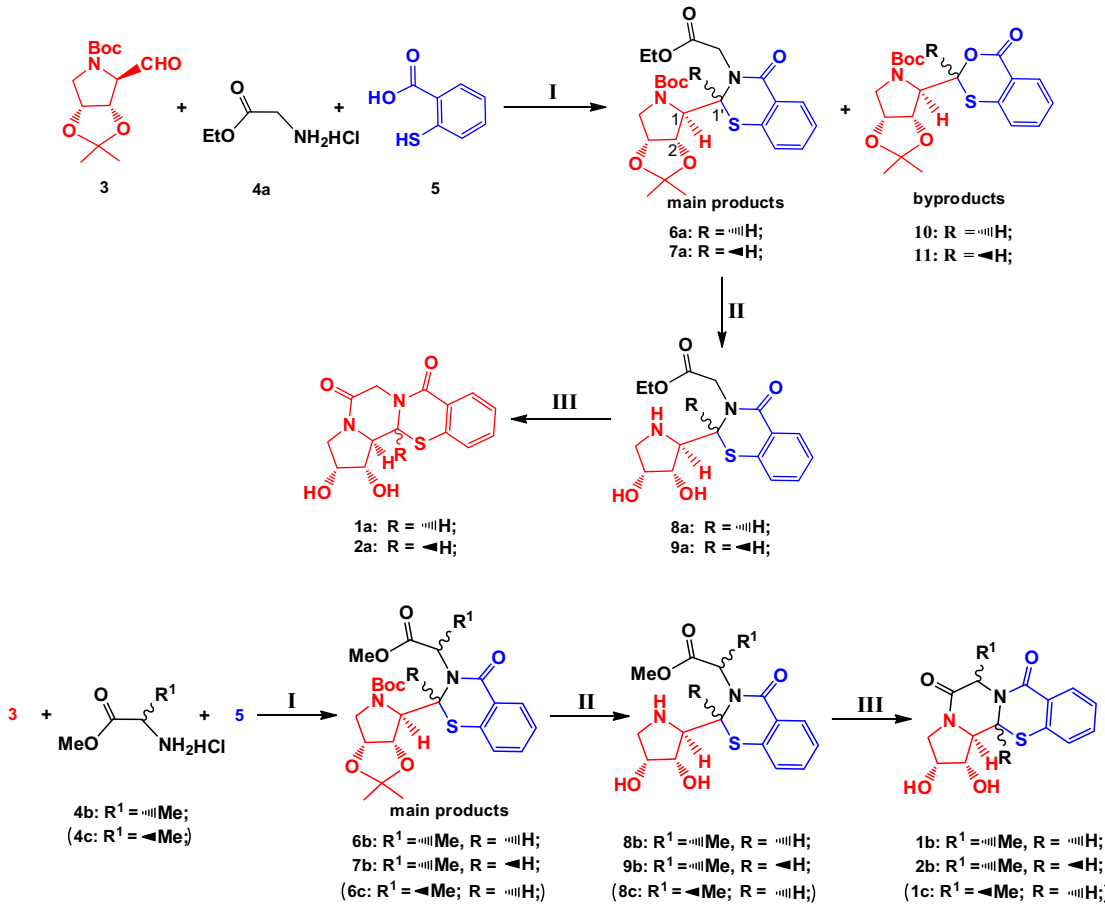


Figure 2. The synthetic tetracyclic azasugars fused benzo[*e*][1,3]thiazin-4-one.

not absolutely rule out small amounts of the other product **7c** from the reaction, its presence was not evident from examination of the crude reaction mixtures. After Boc and the isopropylidene groups in **6** and **7** were removed in 90% CF_3COOH to achieve the corresponding products **8** and **9**, then the treatment of **8** or **9** with triethylamine at 50 °C gave the targeted tetracyclic azasugars fused benzo[*e*][1,3]thiazin-4-one **1** and **2** in good yields by intramolecular cyclo-amidation from secondary amine and ester. However, it should be mentioned that, under the same condition, the final cyclization could not be effectively performed using β-alanine as amine source which would form seven-membered ring.

The structures of all the newly synthesized tetracyclic iminosugars were determined by their ^1H, ^{13}C NMR, and HRMS (ESI) spectra. Both analytical and spectral data of compounds are in agreement with the proposed structures. The typical coupling



Scheme 1. The synthesis of the tetracyclic azasugars **1**(**a**–**c**) and **2**(**a**–**b**) using iminosugar aldehyde **3** as starting material. Reagents and conditions: (I) toluene, $NaHCO_3$, DCC, DMAP, 40–60 °C; (II) 90% CF_3COOH–H_2O, rt; (III) MeOH, NEt_3, 50 °C.

Table 1
The synthesis of aza-C-pseudonucleosides bearing 1,3-benzothiazin-4-one (**6** and **7**) by the one-pot three-component condensation

Entry	Amino acid ester hydrochloride	Products		Total yields[a] (%)	Ratio of **6**/**7**[b]
		6-1′*S*	**7**-1′*R*		
1	Glycine	30.2	29.5	59.7	1:1
2	L-Alanine	22.1	14.7	36.8	1.5:1
3	D-Alanine	12.4	—[c]	12.4	1:0

[a] Isolated yield.
[b] Determined by ^{1}H NMR.
[c] Not found.

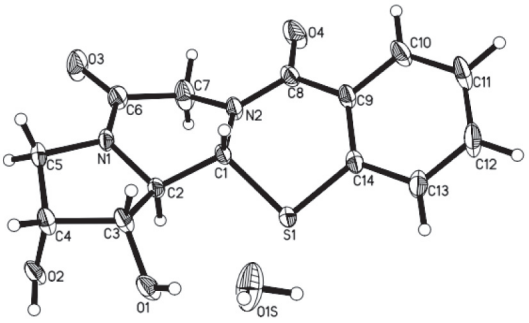
constants of 1-H and 1′-H (Table 2) indicated that compounds **1**(**a**–**c**) with small $J_{1,1'}$ values should have *cis*-relationship between 1-H and 1′-H, while their corresponding diastereomers are of *trans* forms. Thus, the absolute configuration of compounds **1**(**a**–**c**) could be determined to be of (1′*S*), the others were of (1′*R*). The absolute configuration of compound **2a** was determined to be of (1′*R*) by its X-ray crystallographic data (Fig. 3),[11] being consistent with the above NMR results.

HIV-1 reverse transcriptase (RT) inhibitory activity was preliminarily evaluated with the tetracyclic azasugars **1** and **2** (Fig. 2) by determining their percentage inhibition of HIV-RT activity in HIV-1-RT kit by comparison with AZT.[12] The results are shown in Table 3. It could be seen that all the newly synthesized tetracyclic azasugars **1** and **2** showed significant HIV-RT inhibitory activity, better than that of positive control AZT. Especially, compounds **2a** showed a more significant HIV-RT inhibitory activity with the IC_{50} value of 0.82 μM which indicated that **2a** might be better accommodated into the HIV-1 RT binding site. The inhibitory activities of the tetracyclic azasugars **2**(**a**–**b**) with 1′*R* configuration are much higher than those of their stereoisomers **1**(**a**–**b**) with 1′*S* configuration, respectively, which indicated that the stereochemistry of C-1′ in *R* configuration would be favorable to the HIV-RT inhibitory activities of the compounds.

In conclusion, novel tetracyclic azasugars fused benzo[*e*][1,3]thiazin-4-one **1** and **2** were first synthesized by the intramolecular cyclo-amidation reaction of the unprotected aza-*C*-pseudonucleosides, which were afforded by the one-pot three-component condensation from the iminosugar aldehyde, amino acid ester hydrochlorides, and 2-mercaptobenzoic acid. Their structures were determined by NMR, HRMS (ESI) spectra and X-ray crystallographic data. All the newly synthesized tetracyclic azasugars showed significant HIV-RT inhibitory activity. Among them, compound **2a** was the best one with the IC_{50} value of RT inhibitory activity of 0.82 μM. The structure activity relationship (SAR) analysis indicated that the stereochemistry of C-1′ in *R* configuration would be favorable to their anti-HIV-RT inhibitory activity.

Table 2
The coupling constants (Hz) of 1-H and 1′-H in **1** and **2**

cis (1′*S*)	$J_{1,1'}$/Hz	*trans* (1′R)	$J_{1,1'}$/Hz
1a	4.8	**2a**	7.2
1b	3.6	**2b**	10.2
1c	3.6		



2a

Figure 3. X-ray crystallographic structure of **2a**.

Table 3
In vitro HIV-1-RT kit assay for the tetracyclic azasugars

Compds	IC_{50} (μM) (HIV-RT kit assay)	Compds	IC_{50} (μM) (HIV-RT kit assay)
1a	8.21 ± 1.24	**2a**	0.82 ± 0.27
1b	9.65 ± 2.36	**2b**	4.21 ± 1.03
1c	2.01 ± 0.68	AZT	20.14 ± 1.32

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Supplementary data

Supplementary data (experimental procedures and characterization data for compounds **1**(**a**–**c**) and **2**(**a**–**b**)) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.02.049.

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11. CCDC-1433230 for **2a** contain the supplementary crystallographic data for this Letter. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_ request/cif.
12. Reverse Transcriptase Assay, Colorimetric kit, Roche Diagnostics GmbH, Roche Applied Science, Sandhofer Strasse 116, D-68305 Mannheim, Germany.