Bioorganic & Medicinal Chemistry Letters xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Towards new antibiotics targeting bacterial transglycosylase: Synthesis of a Lipid II analog as stable transition-state mimic inhibitor

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ARTICLE INFO

Article history:
Received 23 January 2018
Revised 12 March 2018
Accepted 13 March 2018
Available online xxxx

Keywords:
Iminosugar
Inhibitor
Lipid II
Transglycosylase
Synthesis

ABSTRACT

Described here is the asymmetric synthesis of iminosugar **2b**, a Lipid II analog, designed to mimic the transition state of transglycosylation catalyzed by the bacterial transglycosylase. The high density of functional groups, together with a rich stereochemistry, represents an extraordinary challenge for chemical synthesis. The key *2,6-anti-* stereochemistry of the iminosugar ring was established through an iridium-catalyzed asymmetric allylic amination. The developed synthetic route is suitable for the synthesis of focused libraries to enable the structure–activity relationship study and late-stage modification of iminosugar scaffold with variable lipid, peptide and sugar substituents. Compound **2b** showed 70% inhibition of transglycosylase from *Acinetobacter baumannii*, providing a basis for further improvement.

Introduction

The increasingly common occurrences of infections caused by the drug-resistant bacteria represent a major threat to public health.[1] The urgent demand for novel antibacterials has led to the search for underexploited drug targets. In the past, targeting the assembly of peptidoglycan (PG), a polymer-like structure that helps maintain the integrity of bacteria cell and protects it from lysis, has proven to be a successful strategy for the discovery of antibiotics. Our group has a long-standing interest in the enzyme transglycosylase (TG) as target, which catalyzes the polymerization of Lipid II (**1**, Fig. 1A) to generate a nascent PG before it is cross-linked by transpeptidase (Fig. 1B). First reported 50 years ago,[2a] TG is still viewed as a difficult,[2b] albeit an attractive target.[2c,2d] Located on the external surface of the cytoplasmic membrane, TG is accessible to potential inhibitors. As TG does not have any mammalian counterpart, it is possible to design new antibiotics that are specific against prokaryotic pathogens. In addition, because TG recognizes an invariant carbohydrate backbone, it may be less susceptible to the traditional mechanisms of resistance development.[3] Although, antibacterials that inhibit Lipid II polymerization by sequestering its substrate have been identified (*e.g.*, vancomycin), the direct binders of TG with potent inhibitory activities and pharmacological properties suitable for clinical use have yet to be developed. One major effort in this direction has been the optimization of moenomycin structure,[4] the only TG-specific inhibitor known to date.

Based on recent efforts towards finding the minimal required features of Lipid II/Lipid IV,[5–7] we designed structure **2** (Fig. 1A) as a potential transition-state mimic of the TG-catalyzed reaction.[5] Compound **2** consists of iminosugar ring connected to an additional ring of GlcNAc, a truncated peptide moiety with two essential methyl groups from the lactyl-alanine sequence,[5a,6a,6d] along with a phosphono-phosphate linked lipid chain,[6b] which is necessary for the proper recognition and binding. Towards structure **2**, our group has initially reported the synthesis of the truncated analog **2a** (Fig. 1A), which indeed showed inhibition of TG function.[6b] The two drawbacks of compound **2a** are of note. First, because TG is a processive enzyme,[7] it is highly unlikely that this mono-sugar derivative **2a** can reach the desired donor site of TG due to the lack of the second GlcNAc, therefore preventing enzyme to process **2a**. Hence, the observed activity could be a result of **2a** binding to the acceptor site only, and its designation as a transition-state analog inhibitor could not be fully realized. Second, the synthetic strategy developed for the assembly of **2a** is not suitable for the preparation of the highly functionalized iminosugar **2** and its derivatives,[6b] required for the detailed structure–activity relationship (SAR) study of TG inhibition. To solve the

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https://doi.org/10.1016/j.bmcl.2018.03.035
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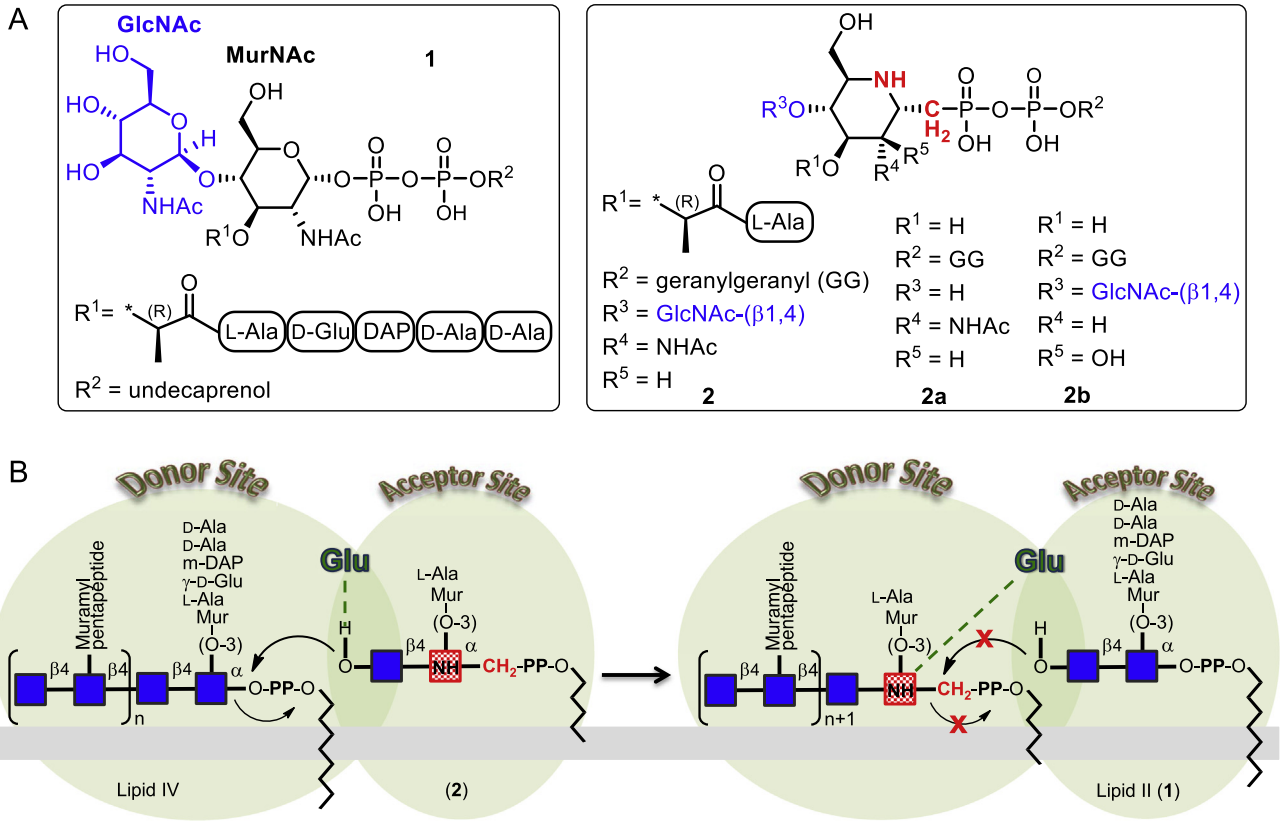
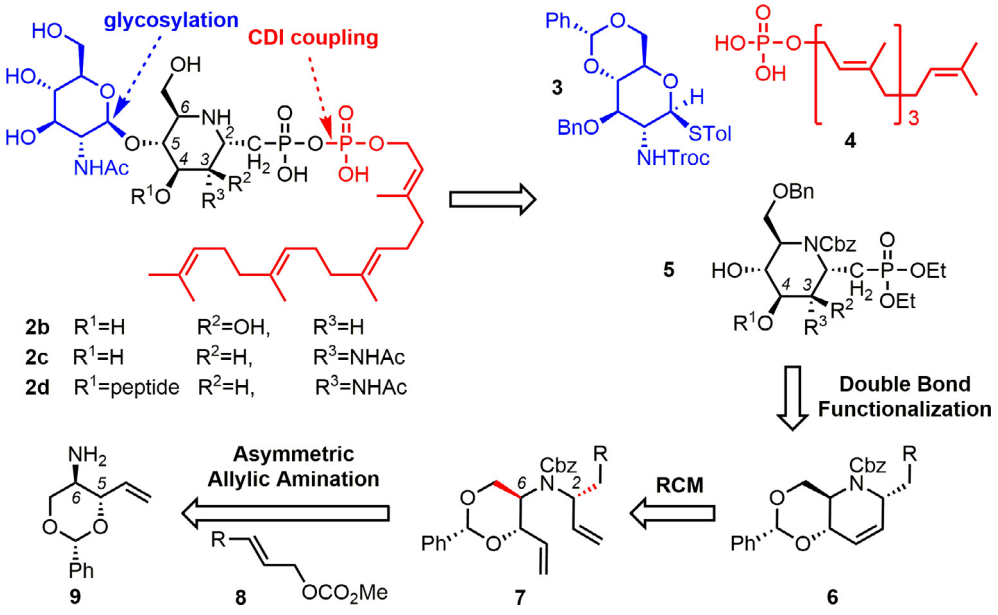


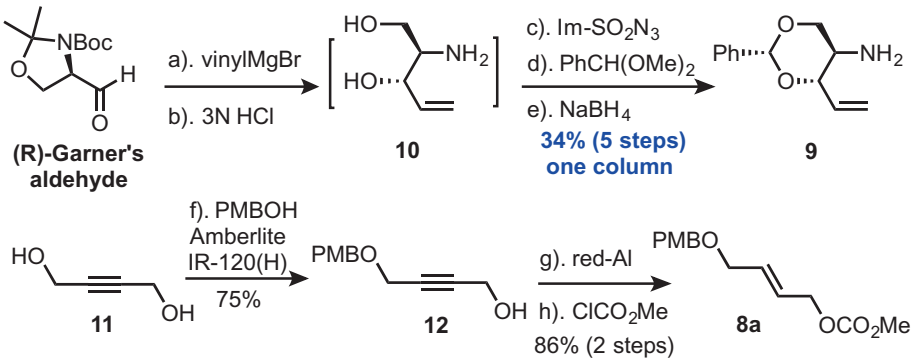
Fig. 1. (A) The structure of Lipid II (**1**) and the target iminosugar derivatives. (B) Schematic representation for the inhibition of the donor site of TG with iminosugar **2**.

drawbacks of compound **2a**, herein we report an optimized asymmetric synthesis of the iminosugar **2b** as a core inhibitor of TG. Since **2b** is a *pseudo*-disaccharide derivative of Lipid II, we envisioned that upon binding it could be processed by the enzyme and pulled into the donor site, where it can block any further transglycosylation reactions (Fig. 1B). The synthetic route developed for the assembly of **2b** can be further applied for the preparation of other analogs, *e.g.*, **2c** and **2d** (Scheme 1).

The retrosynthetic analysis of the target molecule is presented in Scheme 1. The introduction of the sugar and lipid substituents can take place at the late stage using conventional glycosylation with GlcNAc donor (**3**) and a CDI-activated lipid phosphate (**4**).

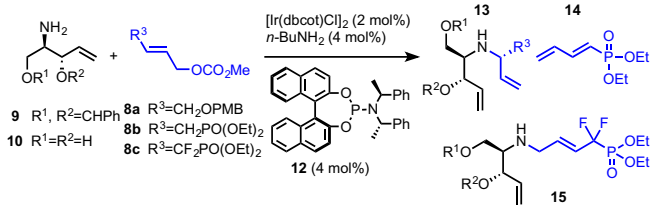


Scheme 1. Retrosynthetic analysis of target molecule **2**.



Scheme 2. Synthesis of the amine **9** and carbonate **8a**.

Table 1
Screen of substrates for the Ir-catalized allylic amination reaction[a].



Entry	Amine	8	Additive	Result[b]
1	**10**·HCl	**8a**	NaH_2PO_4	**13** (trace)
2	**10**	**8a**	–	**13** (trace)
3	**9**	**8a**	–	**13** (>95%)[c]
4	**9**	**8b**	–	**14**
5	**9**	**8b**	NaH_2PO_4	**14**
6	**9**	**8c**	–	**15**

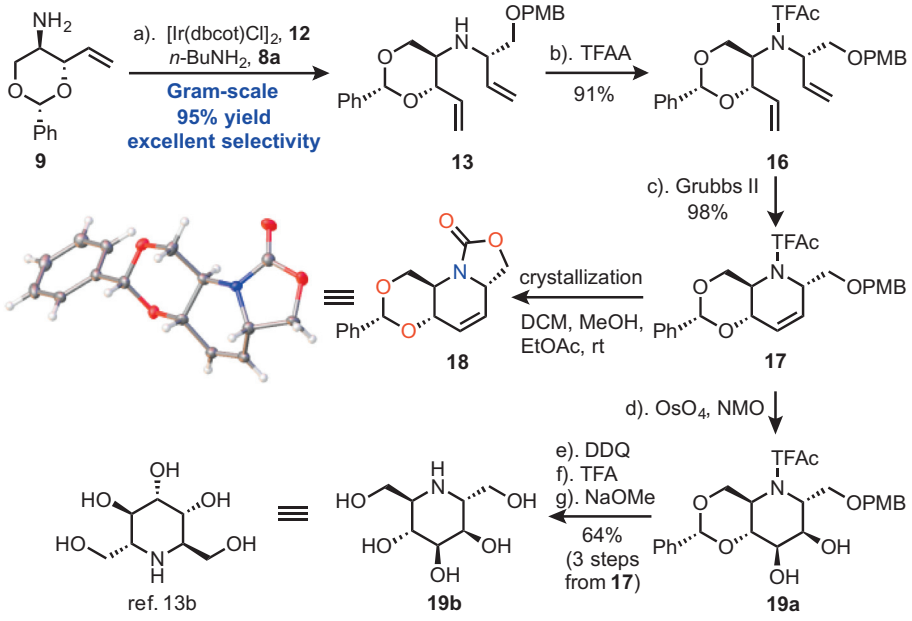
[a] Standard reaction conditions: **8** (1.1 equiv.), **9** or **10** (1.0 equiv.), $[Ir(dbcot)Cl]_2$ (2 mol%), **12** (4 mol%), *n*-$BuNH_2$ (4 mol%), DMSO, 50 °C, 12 h.
[b] Determined by LCMS.
[c] Isolated yield.

Functional groups at positions C-3 and C-4 can be installed via double bond functionalization of **6**. Although the proposed synthesis leads to a mannosyl-like iminosugar, the hydroxyl group R^2 is well positioned for the inversion with azide to provide the NHAc substituent R^3 if required. The most challenging part is the 2,6-*anti* configuration of the key iminosugar core, which could be accessed through a ligand-controlled asymmetric allylic amination.[8] The stereo centers at positions C-5 and C-6 of amine **9** can be obtained from (*R*)-Garner's aldehyde. The dense array of functional groups, high polarity and rich stereochemistry of the target molecule signify a substantial synthetic challenge. The most difficult steps of our synthesis, which we successfully solved, include installation of the 2,6-*anti* configuration of iminosugar, the C-P bond formation and pyrophosphate coupling of the complex *pseudo*-disaccharide to a long hydrophobic lipid chain.

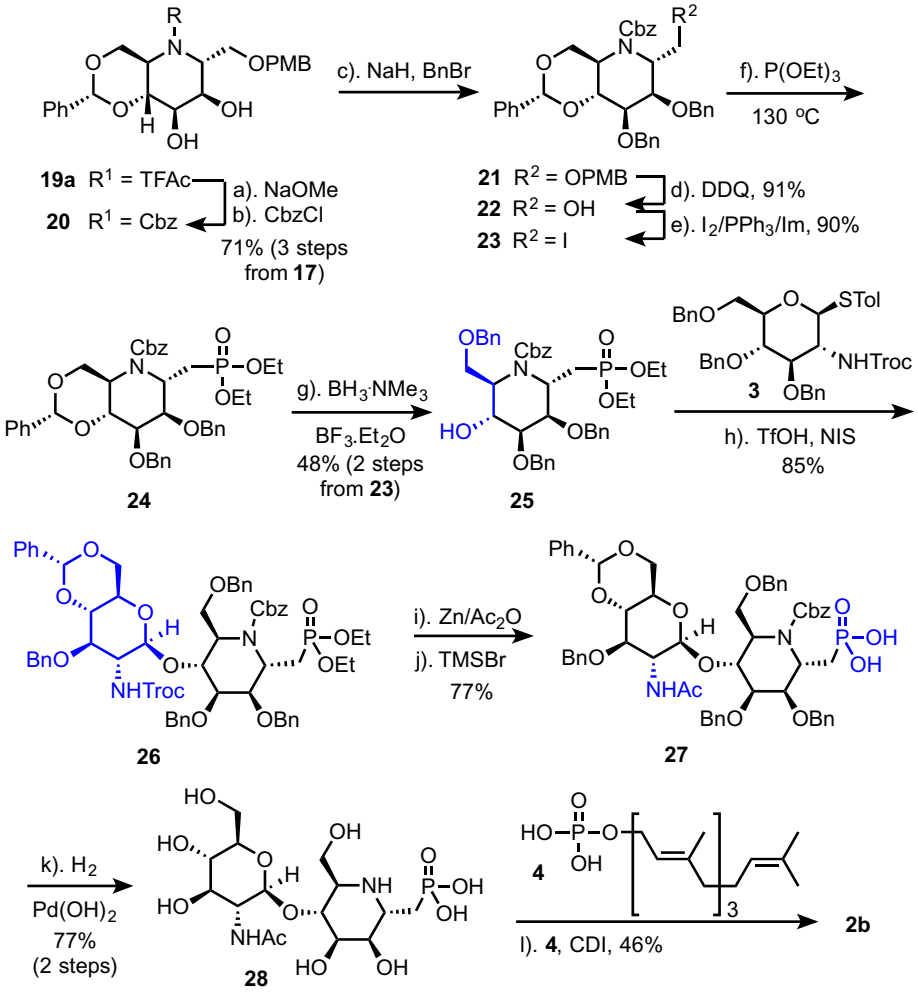
The starting amine **9** was prepared on a gram-scale through a 5-step sequence from the (*R*)-Garner's aldehyde requiring only one purification step (Scheme 2). Carbonate **8a** was obtained in a high yield via a 3-step sequence. Compounds **8b** and **8c** were synthesized in a similar manner according to the known procedures.[9]

Using chiral amines **9** and **10**, and different carbonates **8a-c**, we tested the conditions for the asymmetric allylic amination reaction. The results are summarized in Table 1. During the initial screen using **10** (or its HCl salt) and carbonate **8a** (entries 1–2, Table 1),[8d] we observed a trace amount of the desired product **13** together with unreacted starting material. Because the free hydroxyl groups might influence reactivity, we then switched to the protected amine **9**, which provided **13** in an excellent yield and selectivity. Since it is very difficult to introduce the phosphonate group into the highly functionalized iminosugar at the late stage,[10] we decided to test esters **8b-c** under the optimized reaction conditions. Unfortunately, using **8b** we could only observe the product of elimination (**14**). The reaction with **8c**, where the acidic protons are replaced with fluorine atoms,[11] gave only a linear diene (**15**). Nevertheless, the availability of intermediate **13** allowed us to pursue the synthesis of our target molecule.

Synthesis of amine **13** was scaled up without any impact on the stereoselectivity or yield, and afforded gram-quantities of material. Upon switching to inert atmosphere, the loading of iridium catalyst can be lowered to 1 mol% (Scheme 3). With the key intermediate **13**, we aimed to close the ring and to introduce substituents at positions C-3 and C-4. For the optimization of ring-closing metathesis conditions, we screened different salts of amine **13**, which however failed to provide the cyclized product. Trifluoroacetamide protection of amine (**16**),[12] however, allowed a high-yield preparation of ene-**17** using the second-generation Grubbs catalyst. Crystallization of the intermediate **17** resulted in compound **18** (Scheme 3),[13a] a crystal structure of which confirmed the 2,6-*anti* selectivity of the asymmetric allylic amination step. Next, we performed an osmium-catalyzed dihydroxylation of ene-**17**, which gave diol **19a**. To verify the stereochemistry of the last step, we carried out a global deprotection to obtain **19b**,



Scheme 3. Synthesis of the iminosugar core and determination of the absolute stereochemistry.



Scheme 4. Diversification of iminosugar scaffold and synthesis of target molecule **2b**.

which matched the NMR data reported for the iminosugar scaffold previously described in the literature (Scheme 3).[13b]

The remaining sequence of steps is presented in Scheme 4. The TFAc protection of amine **19a** was replaced by the base-compatible Cbz-protection giving the diol-**20** in a high overall yield. Next, protection of the diol with the benzyl group (**21**), removal of PMB and iodination of **22** afforded intermediate **23**. One of the most challenging steps in our synthesis was the installation of C-P bond. After screening a variety of different substrates and reagents, the reaction of iodo-derivative **23** with triethyl phosphite was identified as the only effective method, which provided phosphonate **24** in moderate yield. Selective opening of the benzylidene ring afforded acceptor **25**,[14] which was glycosylated with donor **3** to give the desired *pseudo*-disaccharide **26**.[15] Subsequent transformation of NHTroc to NHAc, hydrolysis of phosphonate ester[16] and global removal of protecting groups yielded the key intermediate **28**. Coupling of **28** with CDI-activated geranylgeranyl phosphate **4** afforded target molecule **2b**.

The inhibition of TG by **2b** was determined using HPLC-based assay.[17] At 50 μM, **2b** showed 70% inhibition of TG from Gram-negative *A. baumannii*. The observed activity of **2b** is comparable with di- and monosaccharide mimics of moenomycin that bind to the donor site of TG.[4a] This result validates our strategy for the design of TG inhibitors and suggests that the presence of a peptide moiety may be required to improve potency of **2b**. The related analogs are being investigated in our laboratory, and the results will be reported in a due course.

In conclusion, we have developed an efficient route towards the Lipid II analog **2b** from the commercially available (*R*)-Garner's aldehyde. The key step, installation of the 2,6-*anti*-stereochemistry of iminosugar was achieved using the iridium-catalyzed asymmetric allylic amination procedure, which was optimized to the gram-scale process. The developed route could be used to access other Lipid II mimics, particularly **2c** and **2d**, which are expected to have better binding affinities towards TG, than **2b**. These structures will serve as a template for further SAR and structural studies, hence accelerating the development of new antibiotics.

Acknowledgments

We thank Dr. Gembicky (UCSD crystallography facility) for the X-ray diffraction analysis of **18**. Professor Timor Baasov is acknowledged for the help with preparation of this communication. This work was supported by the National Institute of Health (AI072155), Academia Sinica (Taiwan) and the Kwang Hua Foundation.

A. Supplementary data

Supplementary data (procedures and characterization of compounds) associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2018.03.03.

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11. CF2 group is expected to be a better bioisotere of oxygen, than CH_2. Meanwell NA. J Med Chem. 2011;54:2529.
12. Protection of 13 with Cbz group was found to be problematic (screen of reaction conditions resulted in no reaction or decomposition of 13).
13. (a) We believe that transformation of 17 to 18 took place via intramolecular attack of the OPMB ether to the carbonyl of trifluoroacetyl group to form cyclized intermediate, which broke down with migration of CF3 to give 18 and 1-methoxy-4-(2,2,2-trifluoroethyl)benzene.;
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14. Other known methods (e.g., TFA/TES, or TfOH/TES) for the selective opening of benzylidene ring afforded only fully deprotected diol in our hands.
15. The presence of Cbz-protecting group leads to broadening of signals in ^{1}H NMR making it unsuitable for the assignment of the stereochemistry at the anomeric center. Nevertheless, ^{1}H NMR of the fully deprotected intermediate 28 confirms formation of the glycosidic β-linkage.
16. The TMSBr/pyridine/acetonitrile procedure was found to be the only effective method for the hydrolysis of phosphonate ester. Use of DCM instead of acetonitrile or switching to triethylamine base has led to decomposition of starting material, whereas bulky base, such as 2,6-lutidine, was not efficient at promoting the desired transformation.
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