Final Report 2011-2012 – Bande Omprakash

Synthesis of Lipid I and Lipid II monophosphate analogues of Moenomycin for inhibition of transglycosylases.

Moenomycin A is a natural product which interrupts cell wall biosynthesis by binding to the bacterial transglycosylases (TGases), causing an accumulation of cell wall intermediates and leading to lysis and cell death. It is one of the most potent transglycosylase inhibitor known to date, but its absorption upon oral administration is relatively poor due to the C25 isoprenoid chain. Removal of this unit completely abolishes its biological activity. Owing to, these limitations, intensive synthetic efforts have focused in designing and developing new moenomycin type lead compounds as novel antibiotics.[1]

In the light of this and looking at the structural features of the Lipid-II and Moenomycin, both of which interacts actively with transglycosylase, we planned to synthesize hybrid molecules by taking the structural features from both Lipid II and Moenomycin (compound **A**, **B**, and **C**, see Fig. 1).

The synthetic strategy for C-2-hydroxymethyl analogs (**A**, **B**, **C**) involves,

1) The synthesis of a donor fragment and a C_2-hydroxymethyl analog of muramic acid as acceptor fragment (see Fig. 1). The glycosylation reaction between donor and acceptor will give a disaccharide, that upon phoshphorylation will give the lipid II precursor (**B**). Lipid I analogue (**A**) can be synthesized from one of the intermediate in the synthesis of the acceptor

2) Alkylation of the phosphates with different alkyl chains or isoprenoid chains (<C20) and glycerate lipid chains (pyrophosphate mimic)

3) Incorporation of suitable peptides

A similar protocol can be used to prepare Lipid II analogue (**C**) where the C_2-hydroxymethyl group is coupled with a phosphate to form a stable cyclic phosphate.

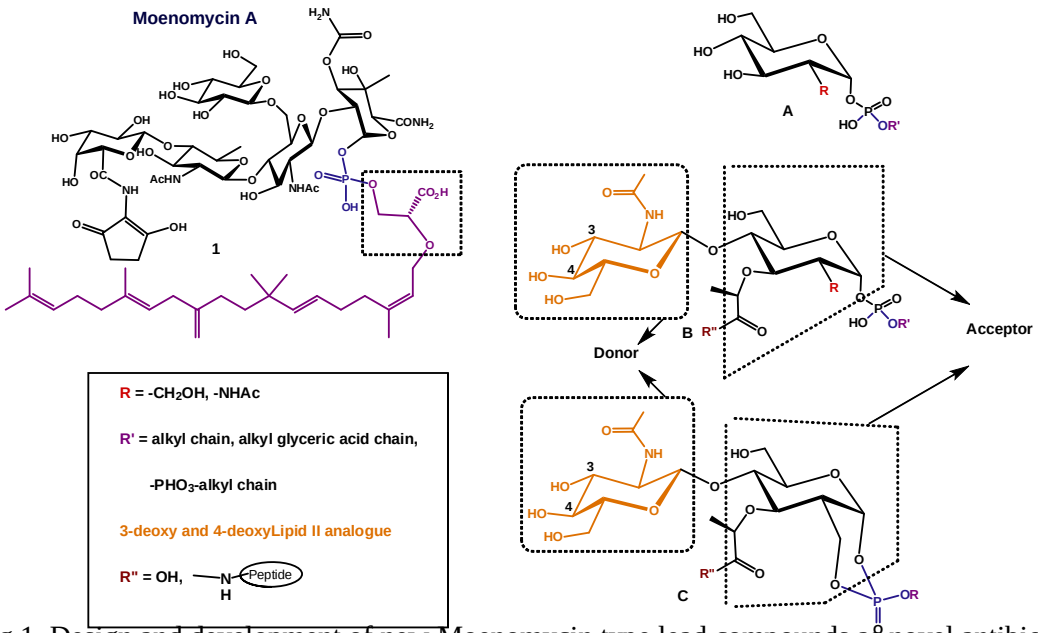
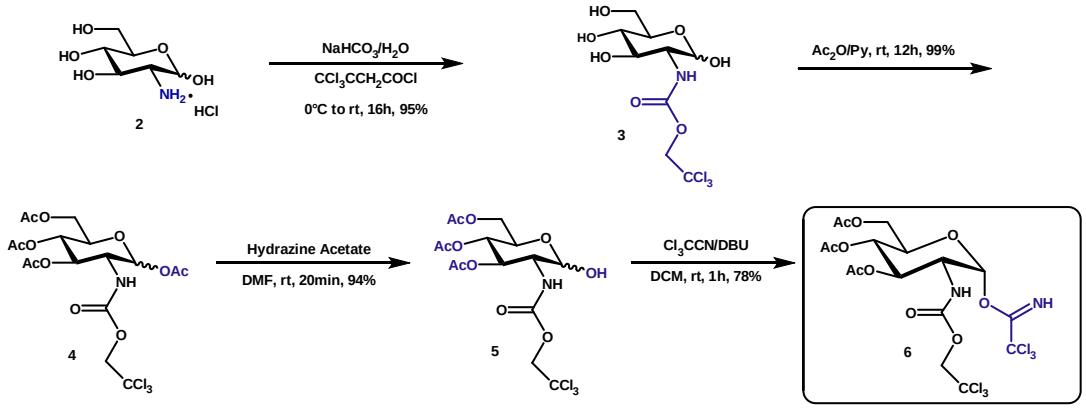


Fig 1. Design and development of new Moenomycin type lead compounds as novel antibiotic

The convergent approach for the synthesis of C_2-hydroxymethyl analogs (**C**) involves the synthesis of the donor fragment (as shown in scheme 1) and the synthesis of a C_2-hydroxymethyl acceptor part(as shown in scheme 2).

Synthesis of donor[2] (Scheme 1)



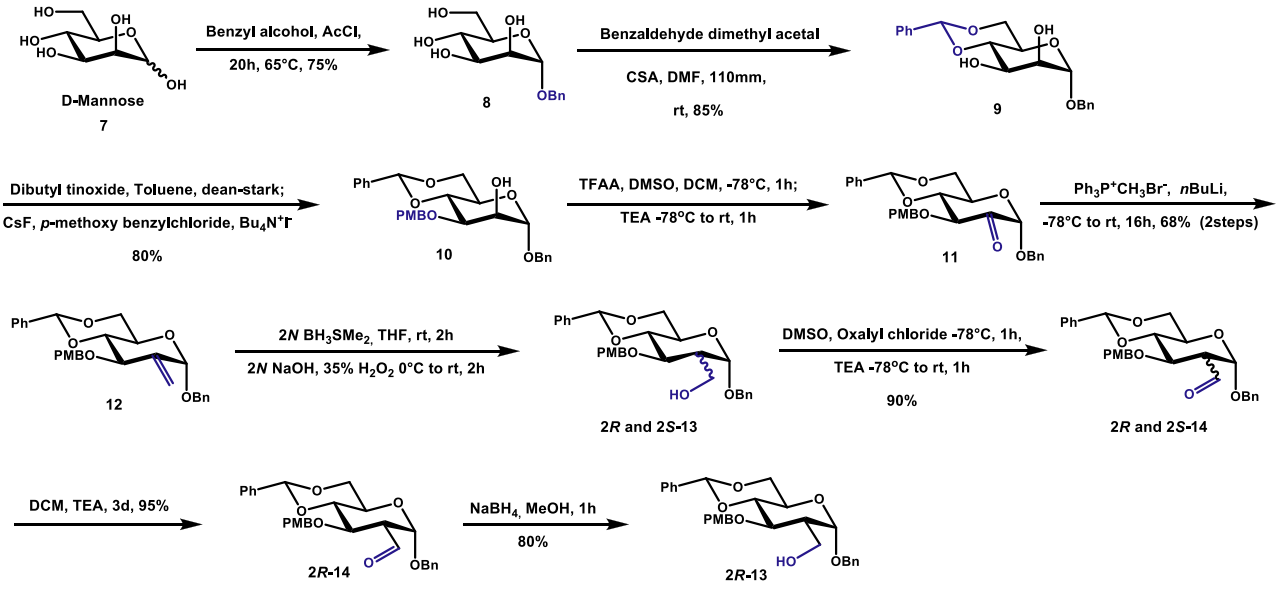
Scheme 1

The donor fragment was synthesized from D-glucosamine hydrochloride.[2] The amine functionality was protected with -Troc to obtain **3**. Compound **3** was exhaustively acetylated to obtain tetraacetate **4**.

Treatment of compound **4** with hydrazine acetate resulted in regioselective hydrolysis of the anomeric *O*-acetyl group to obtain compound **5**. The anomeric position in compound **5** was activated for nucleophilic attack, by treating **5** with trichloroacetonitrile to obtain the donor part (**6**).

Synthesis of C_2 hydoxymethylene analog of acceptor (Scheme 2)

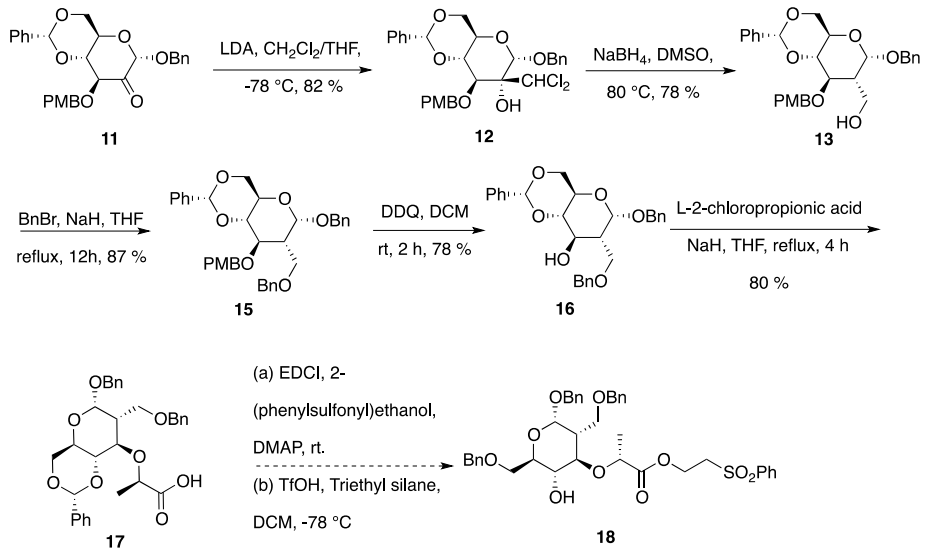
The synthesis of C_2 hydoxymethylene analog of acceptor was envisioned starting from D-Mannose. The anomeric hydroxyl group of D-mannose was protected with a benzyl group to obtain benzyl glycoside **8**. Compound **8** was treated with benzaldehyde dimethyl acetal for the protection of the 1,3-dihydroxy functionality to obtain compound **9**. The C-3 hydroxy group in compound **9** was regioselectively protected by reaction with tributyl tin oxide followed by treatment with *p*-methoxy benzyl chloride to obtain **10** in good yield. Compound **30** was subjected to Swern oxidation giving the unstable ketone **11** which was immediately subjected to Wittig reaction to obtain exocyclic olefin **12**. This olefin was subjected to hydroboration to obtain C-2 hydroxy methylene compound **13** as a 1:1 diastereomeric mixture. Since we only needed compound with an equatorial hydroxymethyl group (2*R*-**13**), this mixture was subjected to Swern oxidation followed by epimerization of 2*S* to 2*R* in presence of triethyl amine to afford 2*R*-**13**.



Scheme 2

Our initial synthetic efforts for the synthesis of C-2 hydroxymethylene analogue of acceptor, involved multistep transformations of compound **10** to compound (2*R*-**13**) with an equatorial hydroxymethyl group[3] (Scheme 2).

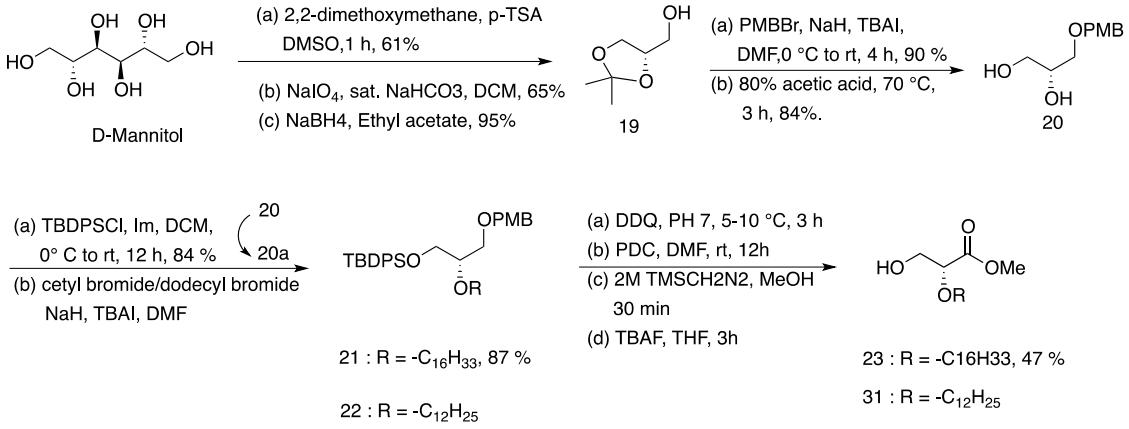
However, this strategy was cumbersome, five steps from **11** to **2*R*-13,** thus we envisaged an alternative pathway to achieve the target compound **13** from compound **11**, is shown in Scheme 3. One carbon homologation at C-2 position of compound **11** was achieved with lithium chlorocarbenoid[4,5] generated from dichlormethane and LDA (lithium diisopropylamide), *via* steroselcective introduction of dichloromethyl function to compound **11** to obtain benzyl 4,6-*O*-benzylidene-2-*C*-dichloromethyl-α-D-glucopyranoside (**12**). Hydride reduction of **12** with $NaBH_4$ (sodium borohydride) in DMSO at 80 °C gave benxyl 4,6-*O*-benzylidene-2-*C*-hydroxymethyl-α-D-glucopyranoside (**13**) in 65 % yield over two steps.



Scheme 2

The compound **13** was subjected to benzyl protection followed by deprotection of the PMP group in presence of DDQ to afforded **16** in 67 % yield over two steps. *O*-alkylation of **16** with L-2-chloropropionic acid afforded compound **17** in 80% yield. Further the carboxylic acid moiety of **17** will be protected with 2-(phenylsulfonyl)ethyl ether subsequent treatment with trifilc acid in presence of triethylsilane will afford the C-2 hydoxymethylene acceptor **18**.

For the synthesis of phosphoglycerates,[6,7] we have developed a protocol[8] as show in Scheme 4. This protocol was used to scale up C16 phosphoglycerate chains. The synthesis begins from D-mannitol, that serves as a chiral pool for the glycerate moiety and was used to obtain acetonide protected alcohol **19** on multigram scale.[9] The (*S*)-acetonide-protected glycerol **19** was subjected to *p*-methoxybenzyl (PMB) protection as it can be selectively removed under neutral conditions with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ). Acetonide deprotection and regioselective protection of the primary hydroxyl group with TBDPS gave the key intermediate **20a**. This key intermediate **20a** was cetylated by treatment with sodium hydride and cetyl bromide to afford **21** in 87 % yield. The PMB group was carefully removed with DDQ to afford required product in 84% yield. This PMB deprotected compound was subsequently oxidized, methylated and TBDPS deprotection afforded the desired glycerate cetyl chain (**23**) in 47 % yield over four steps. The glycerate dodecyl compound (**31**) will be prepared in the same way.



Scheme 4

Conclusions:

- Synthesis of C-2 methylene analoguge of accepotor is near completion with the shorter synthetic pathway (Scheme 3)
- Synthesized C16 phosphoglycerate chain for the phosphorylation of C-2 methylene analogue of acceptor.

References

1. Ostash, B. & Walker, S. Moenomycin family antibiotics: chemical synthesis, biosynthesis, and biological activity. *Nat Prod Rep* **27**, 1594-1617 (2010).
2. Lioux, T., *et al.* Synthesis of peptidoglycan units with UDP at the anomeric position. *Collect Czech Chem C* **70**, 1615-1641 (2005).
3. Horváth, A., Ruttens, B. & Herdewijn, P. Stereoselective synthesis of (−)-ara-cyclohexenyl-adenine. *Tetrahedron Letters* **48**, 3621-3623 (2007).
4. Sato, K., Bokura, M., Moriyama, H. & Igarashi, T. Total Synthesis of a Novel Beta-Glucosidase Inhibitor, Cyclophellitol Starting from D-Glucose. *Chem Lett*, 37-40 (1994).
5. Sato, K., Suzuki, K., Ueda, M., Katayama, M. & Kajihara, Y. A Novel Reagent for the Synthesis of Branched-Chain Functionalized Sugars - Dichloromethyllithium. *Chem Lett*, 1469-1472 (1991).
6. Hecker, S.J., Minich, M.L. & Lackey, K. Synthesis of compounds designed to inhibit bacterial cell wall transglycosylation. *J. Org. Chem.* **55**, 4904-4911 (1990).
7. Kahne, D.E., *et al.* Degradation and reconstruction of moenomycin A and derivatives: Dissecting the function of the isoprenoid chain. *J. Am. Chem. Soc.* **128**, 14012-14013 (2006).
8. Dumbre, S., *et al.* Synthesis of Modified Peptidoglycan Precursor Analogues for the Inhibition of Glycosyltransferase. *J. Am. Chem. Soc.* (2012) DOI: 10.1021/ja302099u.
9. Thomas, B.N., *et al.* Phosphonate lipid tubules II. *J. Am. Chem. Soc.* **124**, 1227-1233 (2002).