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Total Synthesis of Emmyguyacins A and B, Potential Fusion Inhibitors of Influenza Virus

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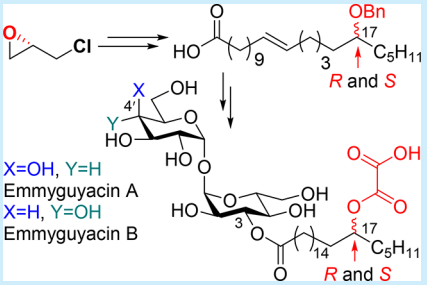
Supporting Information

ABSTRACT: Fungal glycolipids emmyguyacins A and B inhibit the pH-dependent conformational change of hemaglutinin A during replication of the Influenza virus. Herein, we report the first total synthesis and structure confirmation of emmyguyacins A and B. Our efficient route, which involves regioselective functionalization of trehalose, allows rapid access to adequate amounts of chemically pure emmyguyacin analogues including the desoxylate derivatives for SAR studies.



Although a hundred years have passed since the first major outbreak of pandemic influenza, known as "Spanish Flu", which caused tens of millions of deaths across the globe in 1918, influenza still continues to threaten human life.[1] Every few decades, a new strain of potentially deadly influenza virus appears and spreads all over the world. The 1957 outbreak which started in China and spread globally took one million lives around the world. In 1968, another outbreak caused 1 to 3 million deaths. In 2003, A(H5N1) referred to as Avian Influenza emerged, which could pass from animals to humans, and in 2009 the world witnessed the "Swine flu" A(H1N1) pandemic, which started in Mexico and spread to over 214 countries. To combat the unpredictable outbreak of flu, which involves airborne transmission and viral mutations, researchers are always looking for novel and effective therapeutics and vaccines against the flu.[2]

In 2002, Christie Boros and her group isolated two structurally related, trehalose based novel glycolipids, emmyguyacin A (**1**) and emmyguyacin B (**2**) in 3:1 proportion (Figure 1), from a potato dextrose agar fermentation of a sterile fungus species OSI 55538.[3] Structurally, these two molecules are a pair of C4′-epimers wherein compound **1** has a gluco-galacto trehalose core and **2** is a gluco−gluco diastereomer. Both glycolipids contain a novel acid chain at the 3-position, 17-oxalyloxydocosanoic acid, which was also isolated for the first time. Despite all the attempts by Boros and co-workers, the stereochemistry of the hydroxyl group of the fatty acid at C_{17} could not be determined, perhaps due to its distant position from the point of attachment to trehalose. However, they could confirm that the natural products contained both *R* and *S* enantiomers of 17-oxalyloxydocosanoic acid in undefined proportions. A mixture of compound **1** and **2** in a ratio of 95:5 was used for the biological studies and was shown to inhibit replication of influenza A virus (A/X31) in MDCK cells by inhibiting the pH dependent conformational change of the viral glycoprotein hemaglutinin A (HA), at a

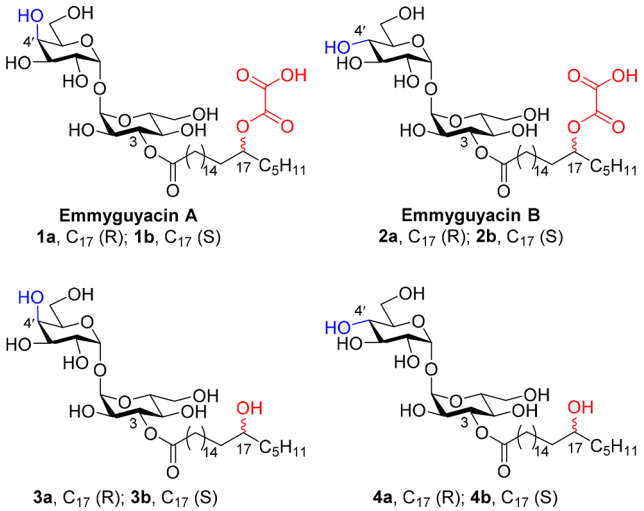


Figure 1. Structures of emmyguyacin A and emmyguyacin B.

concentrations of 9 μM. Further studies revealed that the compounds show similar biological activity in the absence of the oxalate ester.[3] Identification of compounds that would inhibit the conformational change of HA at low pH is a viable approach for the development of antiviral drugs.[4] In this regard, emmyguyacins can be looked upon as important leads. Emmyguyacin B was found to be inseparable from emmyguyacin A even upon HPLC column chromatography. So, it remains unclear as to whether the major component is responsible for the inhibition of influenza or the minor component. In order to confirm the origin of biological activity of the mixture, and to make these compounds available in pure form and ample amounts for SAR studies, it was necessary to

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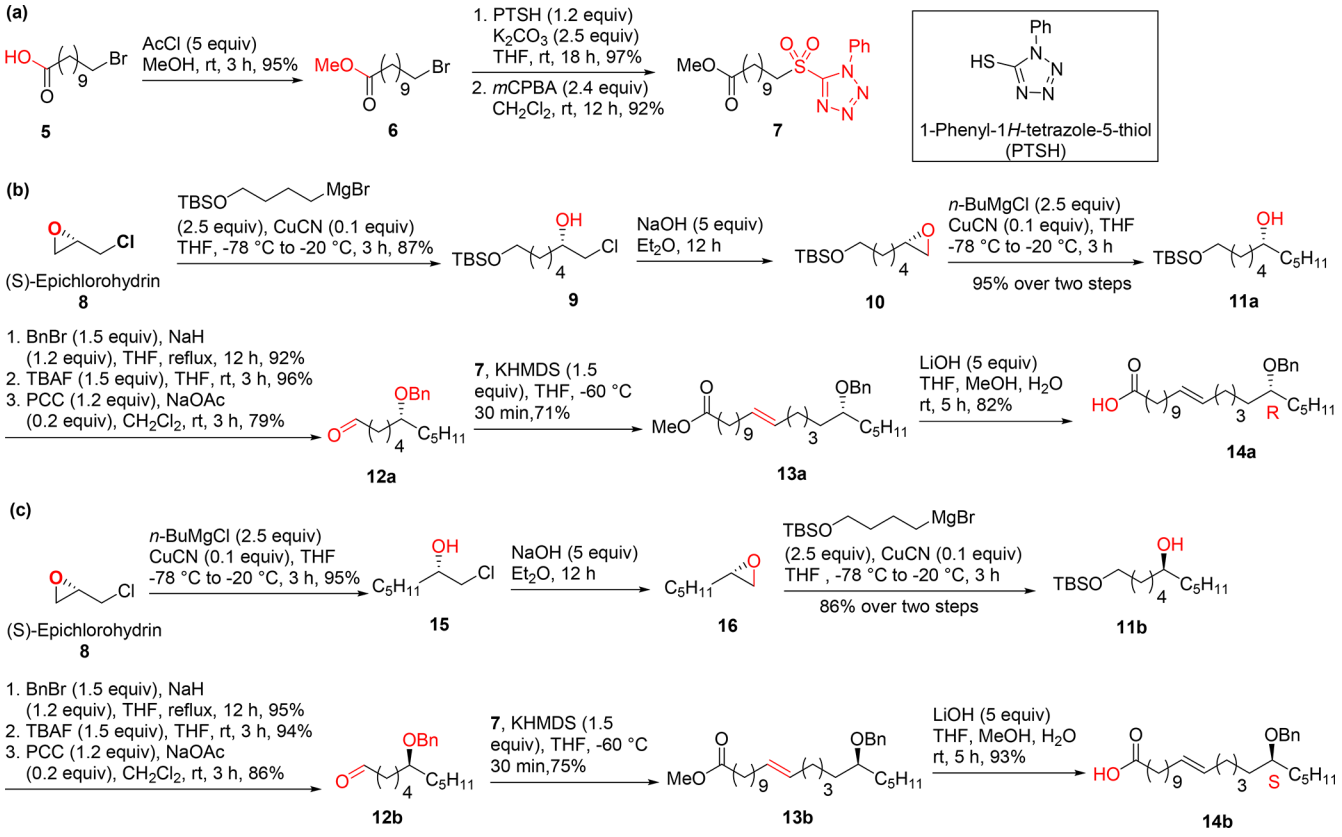
Scheme 1. Synthesis of the Side Chain of Emmyguyacins



develop a short and efficient synthetic strategy for the synthesis of these compounds. It was envisaged that a total synthesis using the optically pure *R* and *S* enantiomers of 17-oxalyloxydocosanoic acid would provide access to optically pure materials. Accordingly, we set out to synthesize *R* and *S* fatty acid containing emmyguyacins A and B as well as their corresponding four desoxylate derivatives **3a**, **3b** and **4a**, **4b**.

For the synthesis of glycolipids **1**−**4** (Figure 1), a short and efficient synthesis of both *R* and *S* isomers of 17-hydroxydocosanoic acid derivatives was necessary. Our synthesis of the protected unsaturated (C22:1) fatty acids is shown in Scheme 1. Both the chiral acids were synthesized from commercially available chiral synthon (*S*)-epichlorohydrin **8** via the successive Grignard addition−epoxidation−Grignard addition sequence followed by a Julia−Kocienski olefination. The key sulfone fragment **7**[5] required for olefination was prepared from 11-bromoundecanoic acid in three steps (Scheme 1a). First, methyl ester formation from acid **5** using acetyl chloride[6] and methanol gave compound **6** in 95% yield and was followed by displacement of the bromide in **6** by 1-phenyl-1*H*-tetrazole-5-thiol (PTSH) employing K_2CO_3 as a base (97%). Subsequent oxidation of the intermediate thioether with *meta*-chloroperoxybenzoic acid (92%) afforded sulfone **7**.

Scheme 1b delineates the synthesis of *R* acid **14a**. Grignard reaction of (*S*)-epichlorohydrin **8** with (4-((*tert*-butyldimethylsilyl)oxy)butyl)magnesium bromide[7] in the presence of cuprous cyanide at −78 °C furnished compound **9** in 87% yield.[8] Epoxidation using sodium hydroxide followed by a second Grignard reaction with *n*-butylmagnesium chloride furnished the *R*-alcohol **11a** in 95% yield over two steps. The free hydroxyl group in **11a** was protected as a benzyl ether, and this was followed by TBS deprotection using TBAF and oxidation of the free primary hydroxyl group using PCC to obtain aldehyde **12a** in 70% yield over three steps. Next, sulfone **7** was coupled with aldehyde **12a**, employing KHMDS[9] as a base at −60 °C to afford **13a** in 71% yield. Methyl ester hydrolysis by LiOH produced the desired side chain *R* acid derivative **14a** (82%).

The enantiomeric *S*-acid **14b** was also synthesized from **8** by simply changing the sequence of addition of the Grignard reagent (Scheme 1c). First, Grignard reaction with *n*-butylmagnesium chloride in the presence of cuprous cyanide at −78 °C furnished compound **15**[10] in 95% yield. Next, epoxide formation to arrive at **16**[11] using sodium hydroxide followed by a second Grignard reaction with (4-((*tert*-butyldimethylsilyl)oxy)butyl)magnesium bromide gave the *S*-alcohol **11b** in 86% yield over two steps. After obtaining the *S* alcohol **11b**, using the same procedure employed in Scheme 1b, the *S* acid **14b** was synthesized through the intermediacy of aldehyde **12b** (77%, 3 steps) and olefin **13b** (75%).

Keeping in mind the observed equal activity of emmyguyacins A and B in the absence of oxalate ester,[3] we proceeded to synthesize emmyguyacins A, B and their desoxylate derivatives **3** and **4** (Figure 1). Scheme 2 outlines our strategy for the synthesis of a suitably protected gluco-galacto trehalose core for the synthesis of emmyguyacins A **1** and emmyguyacin A desoxylate derivatives **3**. Following the literature reported procedure, we prepared 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl-(1 → 1′)-2′,3′,6′-tri-*O*-benzoyl-α-D-galactopyranoside **18** in three steps starting from trehalose **17**.[12] First, heptabenzoylation of trehalose **17** by controlling the temperature and stoichiometry of reagent gave 2,3,4,6- tetra-*O*-benzoyl-α-D-glucopyranosyl-(1 → 1)-2′,3′,6′- tri-*O*-benzoyl-α-D-glucopyr-

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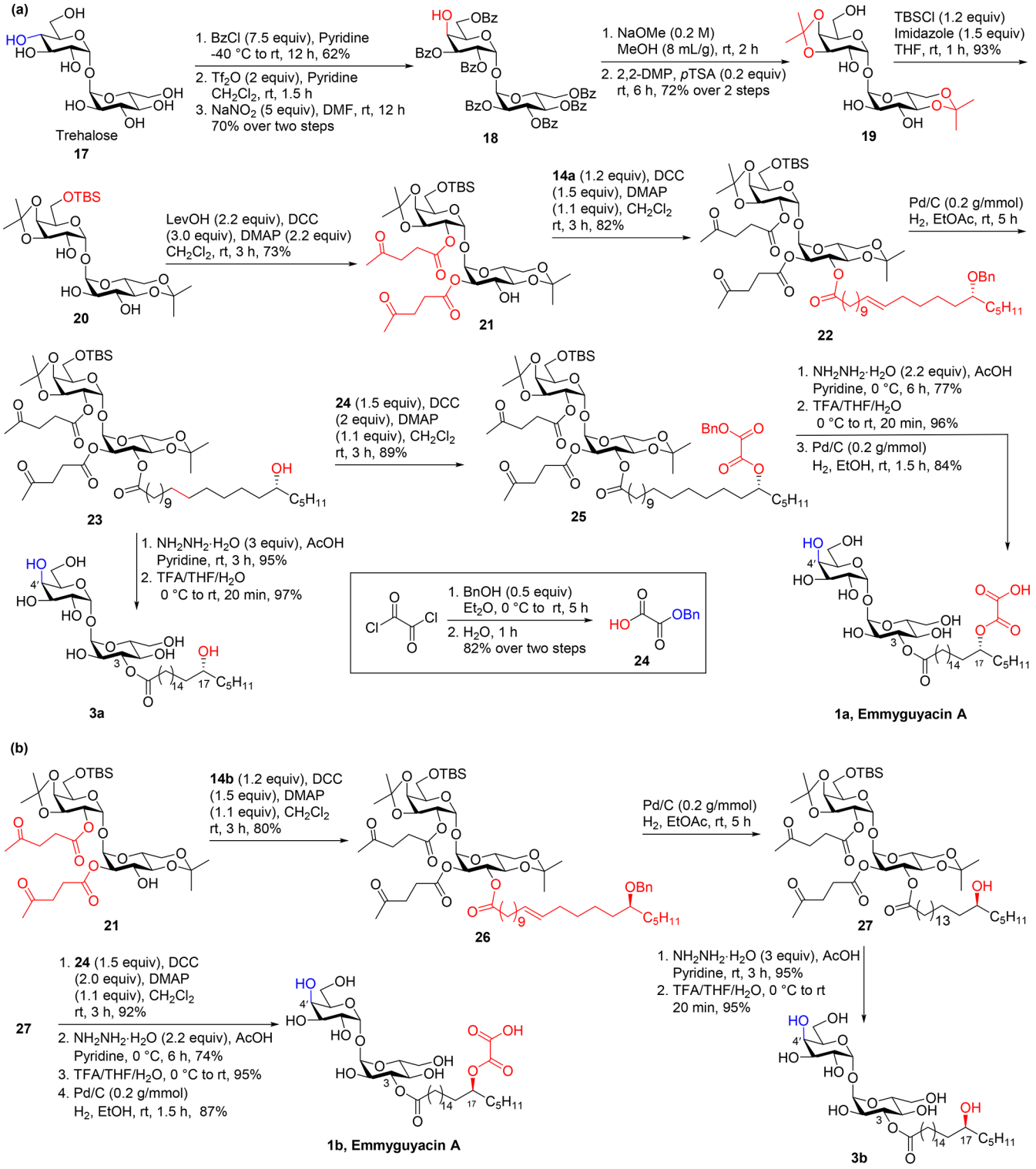
Scheme 2. Synthesis of Emmyguyacin A (1) and Derivative 3



anoside (62%). Earlier, Richardson and co-workers have reported a hepta-pivaloylation of trehalose under similar conditions and yield.[13] Next, the free C4-hydroxyl group of the corresponding hepta-benzoate was converted into triflate using triflic anhydride in pyridine and subsequently displaced with $NaNO_2$[14] in an S_N2 manner[15] to obtain compound **18** with inversion of stereochemistry at the C-4 position in 70% yield over 2 steps. Debenzoylation of **18** followed by isopropylidene protection using 2,2-dimethoxypropane and *p*TSA afforded the 4,6,3′,4′-di-*O*-isopropylidene derivative **19** (72%, 2 steps). The primary hydroxyl group of **19** was protected as a TBS ether to obtain triol **20** (93%). Next, the C2 and C2′ hydroxyl groups of triol **20** were to be selectively masked with a suitable orthogonal protecting group which could be removed in the presence of C3 ester at a later stage of synthesis. For this purpose, we opted to use levulinic esters which can be selectively cleaved by using hydrazine hydrate.[16] By controlling the equivalents of levulinic acid, we could obtain

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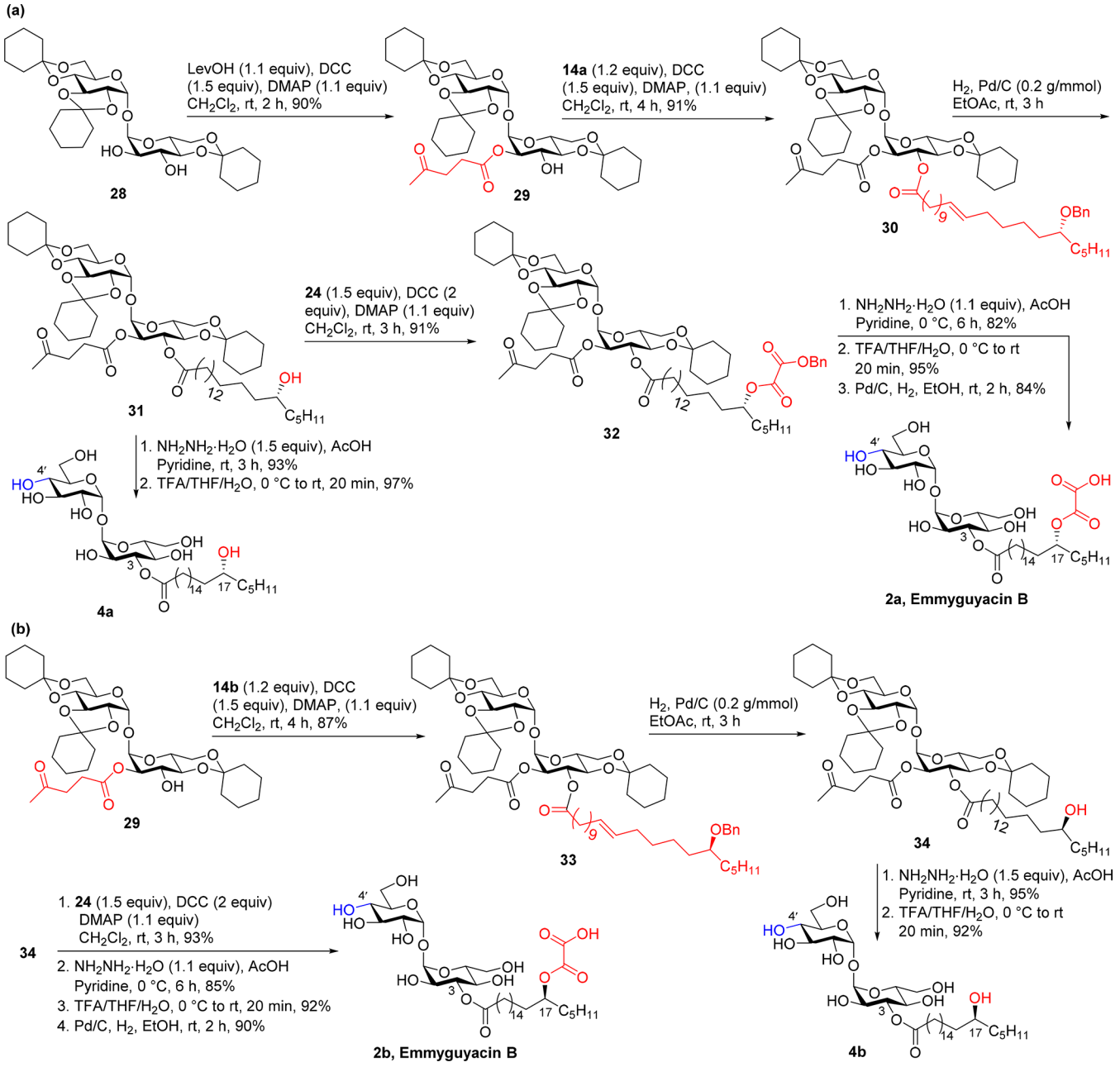
Scheme 3. Synthesis of Emmyguyacin B (2) and Derivative 4



the 2,2′-di-*O*-acylated compound **21** in 73% yield. The remaining C3-OH group was acylated with the acyl chain **14a** to obtain compound **22** in 82% yield. Simultaneous removal of the benzyl protecting group and double bond reduction in **22** under palladium catalyzed hydrogenation conditions gave compound **23**, which is the key intermediate for the synthesis of emmyguyacin A **1a** and emmyguyacin A derivative **3a**. Selective removal of both the levulinic ester protecting groups in **23** using hydrazine hydrate (95%) followed by hydrolysis of the intermediate under acidic conditions afforded the C_{17} *R*-acid containing the desoxylate derivative of emmyguyacin **3a** in 97% yield. Next, for the synthesis of emmyguyacin A, compound **23** was acylated with oxalic acid monobenzyl ester **24**, which could be synthesized from oxalyl chloride.[17] Finally, removal of both the levulinic groups using hydrazine acetate (77%), followed by aqueous TFA hydrolysis (96%), removed the TBS ether as well as the isopropylidene acetal, and debenzylation of the intermediate under catalytic hydrogenation conditions (84%) afforded emmyguyacin A (**1a**). Scheme 2b delineates the total synthesis of emmyguyacin A **1b** and desoxylate derivative **3b** using *S* acid **14b**, starting from intermediate **21** and following the same procedure employed in Scheme 1a, via O3 esterification, hydrogenolysis, oxalate ester formation (only for **1b**), and global deprotection in similar yields. The ^{1}H and ^{13}C NMR of emmyguyacin A matched perfectly well with the data of isolated emmyguyacin A reported by Boros and co-workers, thus confirming its proposed structure (see Supporting Information).[3] Both emmyguyacins **1a** and **1b** show identical ^{1}H and ^{13}C NMR spectral data.

For the synthesis of emmyguyacin B (**2**) (Scheme 3) we started with the known trehalose tricyclohexylidene acetal

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derivative **28**,[18] which can be conveniently prepared from trehalose in a single step in 69% yield.[19] Regioselective 2-*O*-acylation of 2,3-diol **28** with levulinic acid via a DCC-mediated coupling furnished the monoacylated compound **29** in 90% yield. The remaining C3-OH was acylated with acid **14a** in the presence of DCC and DMAP to obtain compound **30** in 91% yield. Palladium catalyzed hydrogenation of compound **30** gave saturated alcohol **31**. Deprotection of levulinic ester group in compound **31** with hydrazine hydrate followed by treatment of the intermediate with aqueous TFA furnished the desired desoxylate emmyguacin B derivative (**4a**) in 90% yields over two steps. Alternatively, compound **31** could be acylated with oxalic acid monobenzyl ester **24** to obtain compound **32** in 91% yield. Finally, removal of the levulinic group was carried out by using hydrazine acetate (82%) followed by aqueous TFA hydrolysis (95%) and debenzylation of the intermediate under catalytic hydrogenation conditions to afford emmyguyacin B (**2a**) in 84% yield.

Scheme 3b delineates the total synthesis of emmyguyacin B **2b** and derivative **4b** using *S* acid **14b** along similar lines starting from intermediate **29** and following the same procedure employed in Scheme 2a, via O3 esterification, hydrogenolysis, oxalate ester formation (only for **2b**), and global deprotection in equally good yields.

In conclusion, we have accomplished the first total synthesis of emmyguyacins A and B containing *R* and *S* forms of 17-oxalyloxydocosanoic acid in an efficient manner in overall yields of 2.7% (**1a**), 3.7% (**1b**), 12.2% (**2a**), and 13.1% (**2b**). The synthetic route involves a total of 24 steps for emmyguyacin A and 18 steps for emmyguyacin B with a longest linear sequence of 15 steps each. En route we could also synthesize the corresponding four desoxylate derivatives of the emmyguyacins. The synthesized glycolipids which are available in pure enantiomeric forms for the first time can now be tested for influenza virus inhibition studies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b03073.

^{1}H and ^{13}C NMR spectra for all compounds and ^{1}H−^{1}H COSY spectra for all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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