Organic LETTERS

Letter

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Merremoside D: *De Novo* Synthesis of the Purported Structure, NMR Analysis, and Comparison of Spectral Data

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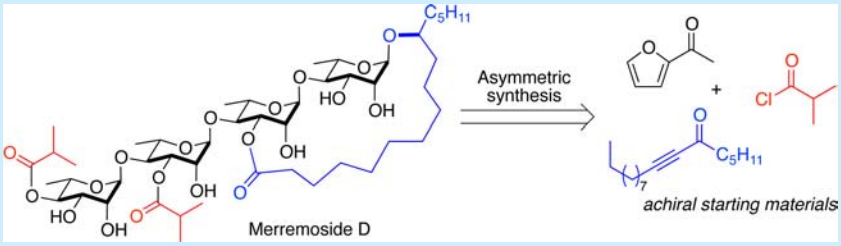
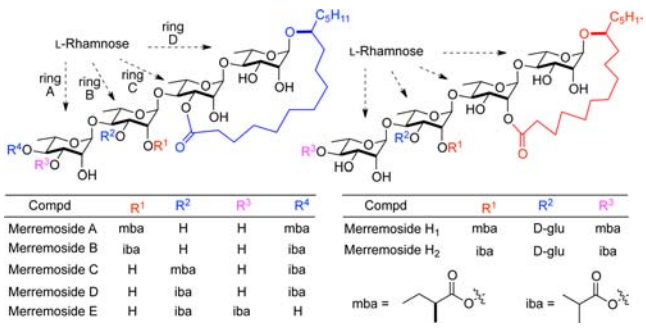
Supporting Information



ABSTRACT: The first synthesis of the purported structure of Merremoside D has been achieved in 22 longest linear steps. The *de novo* asymmetric synthesis relied on the use of asymmetric catalysis to selectively install all 21 stereocenters in the final compounds from commercially available achiral starting materials. Adiabatic gradient 2D NMR techniques (gHSQCAD, gHMBCAD, gH2BCAD, gHSQCTOXYAD, ROESYAD) were used to completely assign the structure of synthetic Merremoside D. Comparison of our assignments with the limited NMR data reported for natural Merremoside D allows for the tentative confirmation of its structure.

The merremoside family of resin glycoside type natural products was isolated from the fresh tuber of *Merremia mammosa* (Lour.) Hall. f. (convolvulaceae) by Kitagawa.[1] These structurally complex oligosaccharides possess a macrolactone (20- or 21-membered) which consisted of a bis-rhamnose disaccharide bridged at the *C*-1 and *C*-2′ or *C*-3′ by a jalapinolic acid (Figure 1). The tuber of the *Merremia mammosa*



Compd	R^1	R^2	R^3	R^4
Merremoside A	mba	H	H	mba
Merremoside B	iba	H	H	iba
Merremoside C	H	mba	H	iba
Merremoside D	H	iba	H	iba
Merremoside E	H	iba	iba	H

Compd	R^1	R^2	R^3
Merremoside H_1	mba	D-glu	mba
Merremoside H_2	iba	D-glu	iba

Figure 1. Structures of merremoside family of resin glycosides.

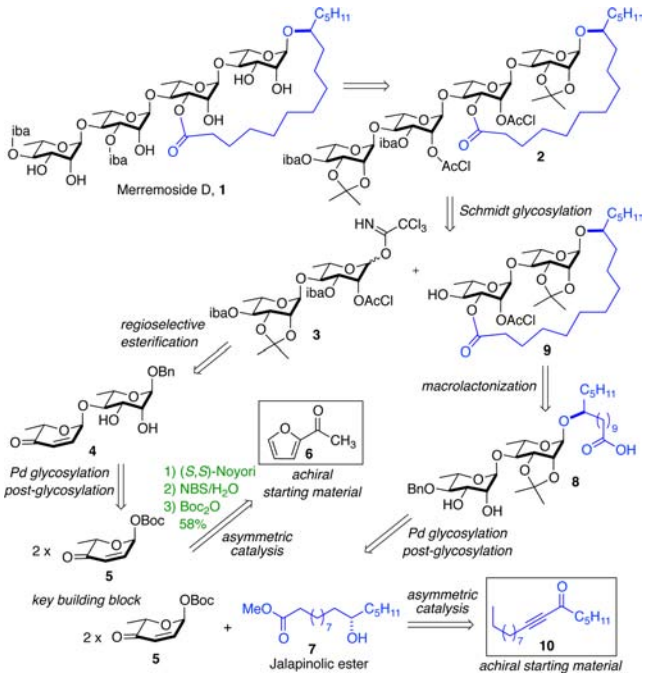
plant has been traditionally used for the treatment of illnesses associated with the throat and respiratory systems.[1a] This tradition continues to the present day, where the powder of the tuber is sold as an herbal medicine for treating patients with various maladies (*e.g.*, cancers, appendicitis, swollen veins, typhus).[2] The amphiphilic nature of the resin glycosides has been suggested to be the source of its ionophoretic activity (i.e., membrane transporter) as observed in human erythrocyte membranes.[3] While several resin glycoside natural products have been synthesized,[4] no member of the merremoside family has succumbed to total synthesis.[5] Despite their interesting biological activities, no detailed structure−activity relationship (SAR) has been carried out.

To gain a better understanding of the promising and diverse biological activities associated with this novel set of natural products, we became interested in a synthesis led SAR-study of the merremosides. In this vein, we targeted for the synthesis of merremoside D. Intrigued by the possibility that enantiomeric analogues of these target compounds would possess the ion transport properties, yet would lack the same target protein interactions, we decided to develop a *de novo* asymmetric approach to the merremosides. We have demonstrated that a *de novo* approach to carbohydrates[6] can be used for the assembly and medicinal chemistry study of oligosaccharides.[7,8] The approach combines the use of asymmetric synthesis of pyranone glycosyl donor **5**, a Pd(0)-catalyzed glycosylation and post-glycosylation transformation, which allow the enone functionality of the pyranones to serve as atom-less protecting groups for the *C*-2 to *C*-4 triol portion of the target *rhamno*-pyranose. Because the route uses asymmetric synthesis, it offers equal efficiency to access to various all D-, all L-, and mixed D/L-isomers.[9] Herein, we disclose our successful efforts in the synthesis of the purported structure of merremoside D.

Received: November 21, 2013
Published: December 19, 2013

Retrosynthetically we envisioned that construction of all the stereocenters in the merremoside D **1** could be accomplished from achiral starting materials utilizing our *de novo* approach to carbohydrates (Scheme 1). The tetrasaccharide **2** would be

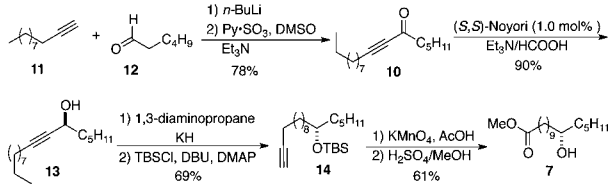
Scheme 1. Retrosynthetic Analysis: *De Novo* Approach



constructed by a convergent glycosylation between macrolactone disaccharide **9** and the imidate disaccharide **3**, with a *C*-2 chloroacetate serving as an anomeric directing group. The desired *C*-3 esterification on **3** would be obtained by tin-mediated regioselective esterification of diol **4**, which in turn would be obtained from pyranone **5**. The macrolactone disaccharide **9** would be obtained from diol-acid **8**, the bis-rhamnose unit of which would be obtained from the same building block pyranone **5**. This key building block pyranone **5** would be obtained from acetylfuran **6** in three steps.[7a,b] A highly stereoselective Pd-catalyzed glycosylation would install all the desired 1,4-α-linkage in the tetra-rhamnose backbone. The aglycon **7** (jalapinolic ester) would be obtained from alkynone **10**, using a modified asymmetric variant of a route reported by Heathcock.[10] Thus as devised, all the chiral centers in **1** would have their absolute stereochemistry resulting from the Noyori asymmetric reduction of furan **6** and ynone **10**.[11]

The asymmetric approach to aglycon **7** began with the synthesis of alkynone **10** from undecyne **11** and hexanal **12** (Scheme 2).[10] Enantioselective (*S*,*S*)-Noyori reduction of alkynone **10** gave alkynol **13** (89% ee).[11a,b] An alkyne-zipper reaction was used to isomerize the internal alkyne **13** into the terminal alkyne,[12] which was followed by hydroxyl protection,

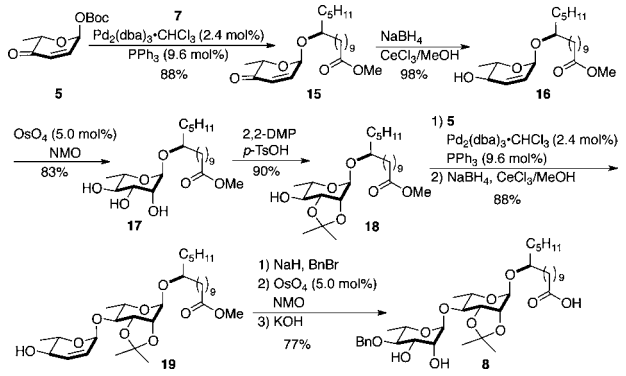
Scheme 2. Synthesis of Jalapinolic Ester



oxidative alkyne cleavage, and esterification resulting in the desired jalapinolic ester (i.e., **13** to **7**).[10]

The synthesis of the macrolactone disaccharide began with the *de novo* asymmetric synthesis of the key pyranone building block **5** (Scheme 3). The three-step synthesis of **5** involves a

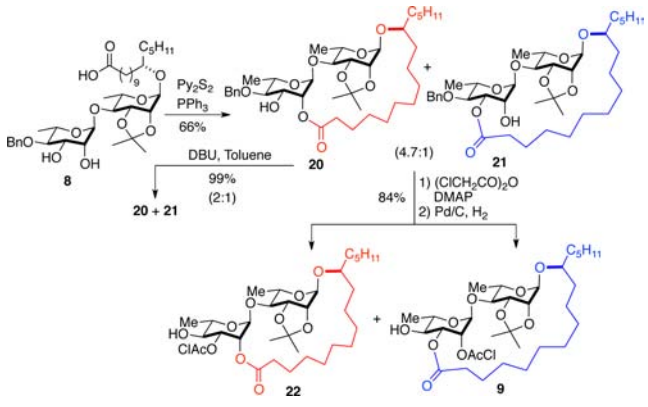
Scheme 3. Synthesis of Macrolactone Disaccharide



Noyori reduction of acylfuran **6**[11] followed by a subsequent Achmatowicz rearrangement[13] and diastereoselective *tert*-butyl carbonate (Boc)-protection of the axial anomeric alcohol. A Pd-catalyzed stereoselective glycosylation of **5** with jalapinolic ester **7** gave **15** (at this stage the minor diastereomer was removed by flash chromatography). The enone **15** was converted to glycosyl acceptor **18** via Luche reduction,[14] Upjohn dihydroxylation,[15] and *syn*-diol protection. A second Pd-catalyzed glycosylation with pyranone **5** followed by enone reduction gave allylic alcohol **19**. Benzylation of the allylic alcohol followed by alkene dihydroxylation and a subsequent methyl ester saponification resulted in the diol-acid **8**.

With the macrolactone precursor **8** in hand, we investigated the macrolactonization utilizing conditions reported by Yang et al.[5] Macrolactonization of **8** under Corey–Nicolaou conditions[16] gave a mixture of regioisomeric products **20** and **21** in a 4.7:1 ratio (Scheme 4). Extensive NMR analysis of the

Scheme 4. Macrolactonization/Synthesis of Glycosyl Acceptors



macrolactones confirmed that the major product was a *C*-2′ macrolactone **20**, and the minor product was a *C*-3′ macrolactone **21**. This observation was opposite to what was reported by Yang et al. (see Supporting Information).[5]

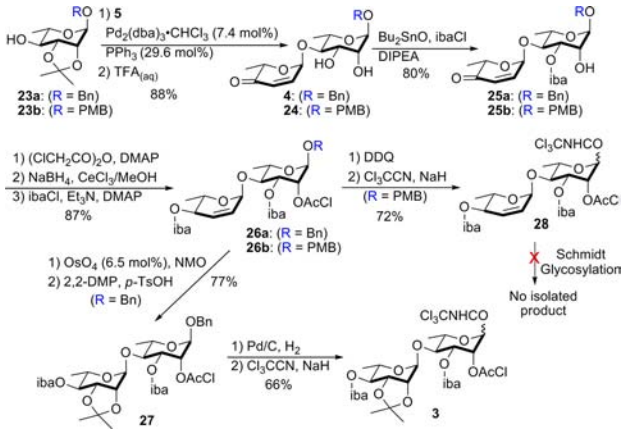
To our delight, we found that the major *C*-2′ regioisomeric macrolactone **20** could be isomerized to the *C*-3′ macrolactone

dx.doi.org/10.1021/ol403369h | Org. Lett. 2014, 16, 492–495

21. Under the isomerization conditions (1 equiv of DBU/toluene), the two macrolactones **20** and **21** reached a thermodynamic equilibrium (2:1) in 12 h. The remaining hydroxyl group on the macrolactones (**20** and **21**) was protected as chloroacetic ester, and the benzyl ether was removed to form the glycosyl acceptors (**20** to **22** and **21** to **9**) respectively.

The donor disaccharide portion was also prepared using our *de novo* asymmetric strategy. At the outset, we hoped to be able to carry the *C*-2/*C*-3 alkene functionality throughout the synthesis, thus serving as an atom-less protecting group (Scheme 5).[7e] The pyranone **5** was converted to protected

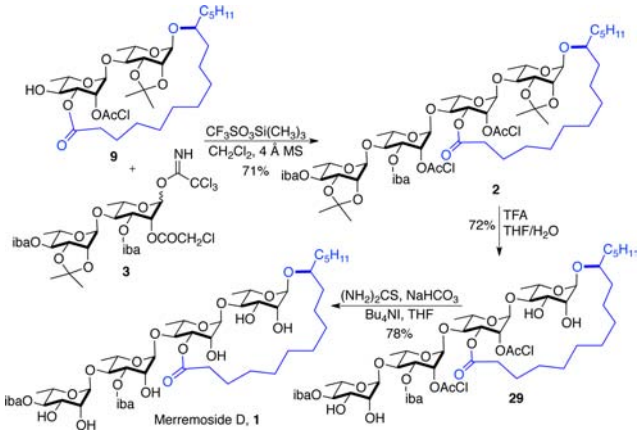
Scheme 5. Glycosyl Donor Synthesis/Attempted Glycosylation



rhamno-pyranosides **23a**/**b** in four steps (Pd-catalyzed glycosylation, Luche reduction, Upjohn dihydroxylation, and acetonation).[17,18] A subsequent Pd-catalyzed glycosylation with pyranone **5** followed by acetonide removal gave *syn*-diol **4**/**24**. This set the platform for the regioselective introduction of the required *C*-3 iba-ester, which was accomplished via a tin-mediated esterification with isobutyryl chloride (ibaCl).[19] The *C*-2 hydroxyl of **25a**/**b** was then protected as chloroacetic ester. Subsequent Luche reduction/esterification gave allylic esters (**26a**/**b**). At this stage disaccharide **26b** was converted into our desired disaccharide donor **28** with the key *C*-2/*C*-3 double bond (DDQ; Cl_3CCN/NaH). Unfortunately we were not able to find a promoter for glycosylation that did not cleave the allylic glycosidic bond in **28**. Thus suggesting a limit to the use of *C*-2/3-alkenes in traditional glycosylations.

To overcome this problem, glycosyl donor **3** was synthesized from **26a** by functionalizing the double bond and anomeric activation (OsO_4/NMO; 2,2-DMP/H^+; Pd/C, H_2; Cl_3CCN/NaH). As expected, the anomeric disaccharide bond in **3** was stable under Schmidt's glycosylation conditions.[20] Thus exposing a 2:1 mixture of disaccharide glycosyl donor **3** and disaccharide glycosyl acceptor **9** to 12% TMSOTf ($CF_3SO_3Si(CH_3)_3$) afforded tetrasaccharide **2** in 71% yield with complete α-selectivity via the anchimeric assistance of the *C*-2 chloroacetate (Scheme 6). Deprotection of the tetrasaccharide **2** began with the removal of both acetonide groups to furnish tetraol **29**. Finally the two chloroacetates in **29** were removed to provide merremoside D (**1**) with thiourea without any deleterious effects to the macrolactone and the ester substituents. While the physio-chemical properties (specific rotation, melting point, and HRMS) of the synthetic **1** match those reported for the natural material, unfortunately the

Scheme 6. Total Synthesis of Merremoside D



comparison of the spectral data was more complicated (see SI). The structural identity of synthetic **1** was confirmed by detailed 1D and 2D NMR analysis (see SI). The ^1H and ^{13}C NMR spectra of the synthetic merremoside D (**1**) were acquired in $CDCl_3$, pyridine-d_5, and pyridine-d_5/D_2O (5:1) and used for comparison with the *limited NMR data reported for the isolated merremoside D*. For instance, only 21 signals were reported for the ^1H NMR (pyridine-d_5/D_2O) and 7 signals for the ^{13}C NMR data (pyridine-d_5).

While it is likely that these two materials are the same, the ill-defined ratio of the pyridine-d_5/D_2O mixture used to record the ^1H NMR spectra of the natural material rendered it difficult to match the chemical shifts between the two samples with absolute certainty (*e.g.*, depending on D_2O/H_2O concentration the D_{ppm} for various signals varied from $\leq$0.78 ppm to 0.55 ppm in pyridine-d_5). For instance, seven of the 21 signals had D_{max} greater than 0.1 ppm with three of those being greater than 0.2 ppm. Similar spectral inconsistencies due to concentration/solvent effects have been observed in related oligosaccharides.[21] Our efforts to find the identical solvent ratio used in the isolation were further confounded by the observation of ester migration/hydrolysis in synthetic **1** upon standing in pyridine-d_5/D_2O (see SI). In contrast to the chemical shifts, there was excellent agreement between the ^1H–^1H coupling constants for the 14 multiplets reported for natural merremoside D and the multiplets for synthetic **1**, regardless of the ratio of the pyridine-d_5/D_2O mixture. Similarly, there was significantly better agreement between the limited ^{13}C NMR data (seven signals) reported for natural **1** in pyridine-d_5 and those found for synthetic **1** (see SI). For instance, five of the seven signals were within 0.4 ppm and two are within 0.7 ppm, which is consistent with the known effects associated with small amounts of D_2O on carbohydrates.[1a,3,21]

In conclusion, the first total synthesis of the purported structure of merremoside D was achieved in 22 longest linear steps with a 3% overall yield. The route demonstrates the power of a *de novo* asymmetric approach to a stereochemically complex (21 stereocenters) oligosaccharide natural product. The approach provided a sufficient quantity of material (29 mg) for both structural and biological evaluation, enabling the screening against an array of organisms. In addition, the approach exposes some practical limitations of the use of atom-less protecting groups (i.e., *C*-2/3 alkene) with traditional glycosylation technology, in contrast to the Pd-catalyzed glycosylation. Detailed NMR analysis was used to confirm the

dx.doi.org/10.1021/ol403369h | *Org. Lett.* 2014, 16, 492–495

structural identity of the synthetic material, which was consistent with the data reported for the natural **1**. However, the lack of complete and reliable ^{1}H and ^{13}C NMR data precludes a conclusive confirmation of the structural assignment for merremoside D. Further efforts to elucidate the full biological structure−activity relationships of the merremoside family of natural products are ongoing.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and spectral data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

‖E.U.S. and H.L.W. contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to the NIH (GM088839) and NSF (CHE-0749451) for their generous support of our research program.

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(2) *Merremia mammosa* is an herbal cancer medicine; for its use, see: http:// www.khazanahherbal.com/product.php?category=21&product_id=111.

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