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Synthesis and Characterization of Four Diastereomers of Mono-rhamnolipids

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ABSTRACT: Rhamnolipids are amphiphilic glycolipids biosynthesized by bacteria that, due to their low toxicity and biodegradability, are potential replacements for synthetic surfactants. The previously limited access to pure materials at the gram scale has hindered extensive characterization rhamnolipid structure-performance behavior. Here, we present an efficient and versatile synthetic methodology from which four diastereomers of the most common monorhamnolipid, α-rhamnopyranosyl-β-hydroxydecanoyl-β-hydroxydecanoate, are prepared and subsequently characterized. Exploration of their behavior at the air-water interface is reported and analyzed in terms of the absolute configuration of the lipid tail carbinols at pH 4.0 and 8.0. All diastereomers exhibit a minimum surface tension of about 28 mN/m without a significance difference between the protonated (nonionic) or deprotonated (anionic) states. At pH 4.0 (nonionic), all diastereomers have a critical micelle concentration (CMC) in the μM range. At pH 8.0 (anionic), CMC values for the (***R,R***), (***S,S***), and (***S,R***) diastereomers are approximately an order of magnitude higher than in their nonionic states whereas the (***R,S***) diastereomer exhibits a CMC about five times larger.

INTRODUCTION

Rhamnolipids are amphipathic glycolipids with outstanding surfactant properties biosynthesized by microorganisms[1-4] mainly belonging to the class of Gammaproteobacteria like *Pseudomonas aeruginosa*, although others from the Actinobacteria, Bacilli, Betaprotobacteria and Deltaproteobacteria classes have been identified as producers as well.[5] More than 50 biosynthesized congeners have been identified in mixtures, but the general structure shown in **Figure 1** is comprised of a lipid unit of (***R***,***R***)-β-hydroxyalkanoyl-β-hydroxyalkanoic acids[6] of variable chain lengths (C_6-C_{14})[7,8], *trans*-1,2-*O*-glycosylated[9] by mono- or dimeric carbohydrate L-rhamnopyranosyl units.[10]

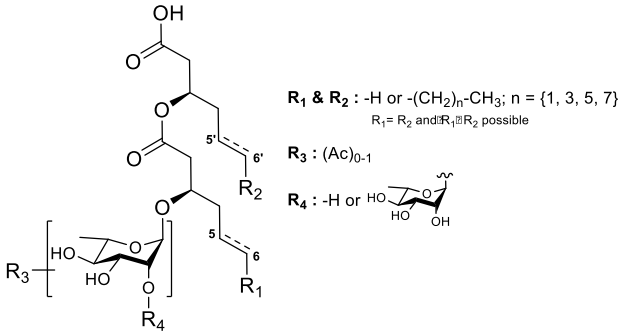


Figure 1. Structural chemical diversity of (***R***,***R***)-rhamnolipids produced by *Pseudomonas aeruginosa*. Traces of rhamnolipids with unsaturation on both alkyl chains and monoacetylated rhamnose have been harvested from cultures of *P. aeruginosa*.

Biofilm regulation,[5,11-16] antimicrobial activity,[17-21] uptake and biodegradation of poorly soluble substrates,[22-24] immune modulators and virulence factors,[25-37] and surface motility[38-42] are some of the physiological functions attributed to rhamnolipids. Applications such as complexation and flushing of heavy metals from contaminated soils,[12,43-48] hydrocarbon removal from soils,[49-51] enhancers of hydrocarbon biodegradation,[49,52-54] and use as biological control agents[55-58] have been suggested. Both, the biological roles and the developing biotechnology mentioned above rely on the powerful pH-modulated[59] surfactant properties of these materials. In their anionic form, the low critical micelle concentrations (CMCs) and high surface activity of rhamnolipids are several orders of magnitude lower than related anionic synthetic petroleum-based derivatives and current commercially available counterparts. In their nonionic form, their CMCs are comparable to nonionic synthetic petroleum-based surfactants. These characteristics make rhamnolipids competitive[60,61] green alternative biomaterials for industrial specialty surfactant sectors. However, regardless of the high interest of the industrial and scientific communities, few studies[62] have dealt with pure materials whose structure-surfactant performance can be evaluated. This occurs because until now the only practical source of rhamnolipids has been harvesting from microorganisms that produce condition-dependent multi-component mixtures of congeners. In addition, access to the interfacial and solution aggregation properties of diastereomers of these surfactants has been prohibited by virtue of the exclusive bioproduction of L-lipid units only.[4,63]

In this study we present a flexible, scalable and practical synthetic methodology for the synthesis and identification of all four diastereomers (**1**,**2**,**3**,**4**) of α-rhamnopyranosyl-β-hydroxydecanoyl-β-hydroxydecanoate (Rha-C10-C10), the most abundant biosynthesized congener, shown in **Figure 2**. Such a synthetic approach allows us to ascertain whether

stereochemistry of the lipid tails plays a pivotal role in determining solution aggregation properties or other surfactant behavior at the air-water interface.

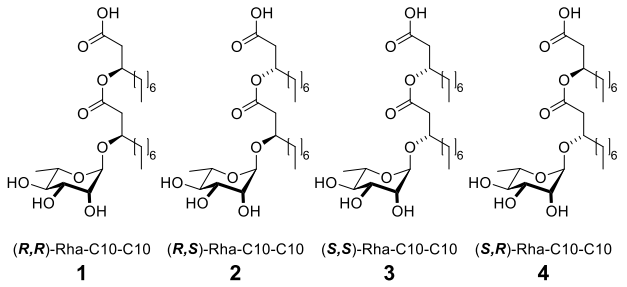


Figure 2. Diastereomers produced by chemical synthesis.

The structure of a surfactant is known to govern its properties such as CMC, surface activity and aggregation behavior.[64] Techniques including small angle neutron scattering,[65-68] fluorescence quenching,[69] and surface tensiometery[65,66,69-75] have been used to study native rhamnolipids to characterize these properties. In this work, in addition to a new synthetic methodology for production of the four diastereomers of the Rha-C10-C10 monorhamnolipids, we also report the surface activity and aggregation morphology of these synthetic forms, providing insight into possible structure-performance relationships between these variants.

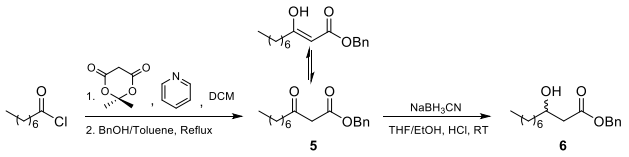
RESULTS AND DISCUSSION

Synthesis of monorhamnolipid diastereomers. To date, two approaches to the chemical synthesis of rhamnolipids have been reported.[76,77] Both approaches are versatile and can be used to produce any saturated mono- or dirhamnolipid with any desired stereochemistry. Unfortunately, these approaches are not readily amenable to production of these materials at scale, as they are labor intensive (>18 steps per diastereomer), use expensive reagents, dangerous procedures, and low yielding reactions. Here, we report a solution phase methodology in which a long shelf life, air and moisture stable peracetylated rhamnose donor can be activated in the presence of a minimally competent Lewis acid[78,79] to produce a racemic mixture of the lipid acceptor that can be resolved in a flash preparative silica column by means of enantiomer-to-diastereomer conversion[80-82] after stereocontrolled glycosylation. The pure free acid diastereomers are esterified with the second lipid unit to render a new mixture of diastereomers that is separable as free acids by conventional flash column chromatography. The rhamnose units of the pure diastereomers are deacetylated orthogonally by transesterification to yield the final products. The methodology is sufficiently flexible to allow modification of the sugar and lipid moieties to create other glycolipids as well. Other significant attributes include scalability, mild reaction conditions, high green indices, and cost-effectiveness compared to the two synthetic methods previously reported. Absolute configuration assignments are performed on the synthetic and naturally produced materials by means of Mosher's ester analysis and optical rotation measurements. Details of the synthetic methods are contained in the Supporting Information along with supporting spectral data (Figures S1-18).

Synthesis. *Preparation of the donor.* Rhamnolipid syntheses found in the literature utilize thiorhamnosides, iodorhamnosides and trichloroacetimidates. In general, carbohydrate peracetates are synthesized and then converted to more reactive donor species for glycosylation. However, the peracetate can also be an effective donor species, supported by oxonium ion stabilization via Lewis acid activation and neighboring participation of the C-2 acetate carbonyl of the peracetylated carbohydrate. Large quantities of peracetylated donor can be prepared quantitatively (e.g. 200 g) in one step at room temperature (pyridine, acetic anhydride). This donor is not water or air sensitive and is stable at room temperature, resulting in a long shelf life either as a solid or dissolved in organic solvent without special storage requirements. Peracetylations performed at room temperature yield mixtures of anomers at the same ratio as the starting sugar.

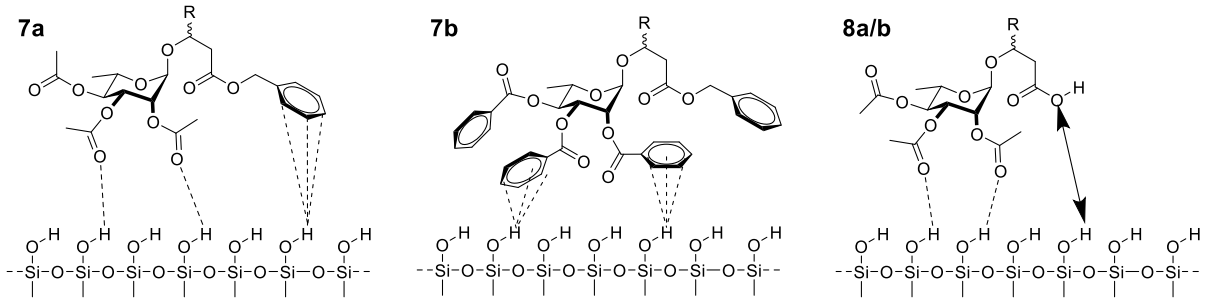
Glycosylation. Our glycosylation approach, shown in **Scheme 1**, exploits the minimally competent Lewis acid concept developed in our laboratories.[78,79] Three soft metal Lewis acid salts were tested as catalysts: $InBr_3$, $Bi(OTf)_3$ and $Sc(OTf)_3$. Perbenzoylated and peracetylated rhamnose were tested as donors. Catalytic amounts of the less hygroscopic bismuth (III) triflate promoted the glycosylation of primary and racemic secondary alcohols in high yields.[79] The Bi(III) salts are less toxic[83,84] and less hygroscopic than the In(III) and Sc(III) salts,[85,86] and proved superior in this case. Only alpha anomers were observed due to the anomeric effect coupled to the trans 'participating group' at C2 of the L-rhamnose acetates. Further explanation for this stereospecific coupling may include weak neighboring group participation and/or solvent interactions.

Synthesis of the racemic lipid units and the resolution of the racemic mixture. Several synthetic methodology precedents for the preparation of β-hydroxyalkanoic acids have been reported in the literature due to the ubiquity of this lipid moiety in biomolecules found in Gram negative bacterial cell walls.[87] Access to optically pure β-hydroxyalkanoic acids is afforded by stereoselective reduction of the β-ketoalkanoic esters using Noyori's catalytic hydrogenation, resolving agents of racemic mixtures, enzymatic reduction,[88,89] and recently, the exploitation of Mitsunobu chemistry.[90] In our approach, the lipid units are prepared as racemic mixtures of benzyl β-hydroxyalkanoic esters in two steps: formation of the benzyl β-keto ester using Meldrum's acid carbonyl chemistry[91-93] and reduction of the keto group with $NaBH_3CN$ under acidic conditions[94] as shown in Scheme 1.



Scheme 1

The racemic mixture was resolved by normal phase liquid chromatography (NP-LC) with silica (flash gel) by means of enantiomer-to-diastereomer conversion after glycosylation with a fully protected rhamnose donor. Attempts to resolve the racemic mixture by flash column chromatography relied on inducing differential intramolecular and intermolecular interactions with stationary/mobile phase systems that shift the partition coefficients of the members of the racemate. This principle was tested with the glycosides **7a**, **7b** and **8a/b** as shown in **Scheme 2**.



Scheme 2

Although separation of the **7a** diastereomeric mixture was achieved by analytical thin layer chromatography (TLC) and reversed phase high performance liquid chromatography (RPLC), it proved impossible by preparative NP-LC. On the other hand, up to 7 g of the free acid (**8a/b**) of the diastereomeric mixture **7a** have been resolved efficiently in a 500 g silica column with 1:1 EtOAc/hexanes + 1%v/v AcOH as the mobile phase. It was fortunate that such separation was achieved with the peracetylated rhamnoside, retaining the orthogonality of our protecting group scheme. Apparently, the van der Waals intermolecular interactions of the electron rich benzyl and acetyl groups are not sufficiently different among diastereomers to facilitate their separation. Alternately, it may be that hydrogen bonding of the carboxylic acid with silanols is the interaction that governs separation of the diastereomeric mixture.

Addition of the second lipid tail. The diastereomeric free acids **8a** and **8b** were coupled by Steglich[95] esterification with a second benzyl β-hydroxydecanoate ester (**6**). After reductive debenzylation, the diastereomeric mixture of peracetylated rhamnolipid free acids is separable by NP-LC with similar conditions, column loading and resolution.

The four diastereomers are deacetylated via Zemplèn[96] conditions (sodium methoxide in methanol) to produce the four Rha-C10-C10 monorhamnolipid congeners (**1**, **2**, **3** & **4**).

The entire synthesis is represented in **Figure 3**. In summary, a synthetic methodology to render the four diastereomers in five chemically distinct steps using solution base chemistry of a battery of well-described chemical transformations has been achieved. The diastereomeric enrichment allows avoidance of tedious procedures like enantiomer recrystallizations, and dangerous and sometimes not accessible enantioselective conversions like Noyori's asymmetric hydrogenation.

Absolute configuration assignment of the carbinol groups of the lipid tails. Assignment of the absolute configuration of the carbinol groups at the lipid tails of each of the diastereomers in terms of their relative R_f values was achieved using a combination of ^{1}H-NMR Mosher's ester analysis[97-99] and optical rotation measurements. In order to determine the absolute configuration of the carbinols of the inner lipid tails, the glycosidic bond of a sample of deacetylated **8a** and **8b** was exo-cleaved[100-104] by methanolysis.[29] The resulting methyl β-hydroxydecanoate esters were esterified with (***R***)- and (***S***)-MTPA-Cl to render the (***S***)- and (***R***)-Mosher's esters, **Figure 4a**. The four Mosher's esters obtained were characterized by ^{1}H-NMR and the chemical shifts were compared according to $\Delta\delta^{SR} = (\delta_S - \delta_R)$. As a control, the absolute configuration of the carbinols of the methyl ester lipid products of the acidic methanolysis of the monorhamnolipids produced by *Pseudomonas aeruginosa* ATCC 9027 was also determined by Mosher's ester analysis. Absolute configuration assignment of the secondary lipid tails was performed in similar fashion. Samples of the four diastereomeric, double lipid tail rhamnolipids (Rha-C10-C10) were hydrolyzed with 2 M NaOH to obtain the 3- hydroxydecanoic acid, and esterified with BF_3-MeOH,[105-107] **Figure 4b**. After isolation by flash column chromatography, optical rotation measurements

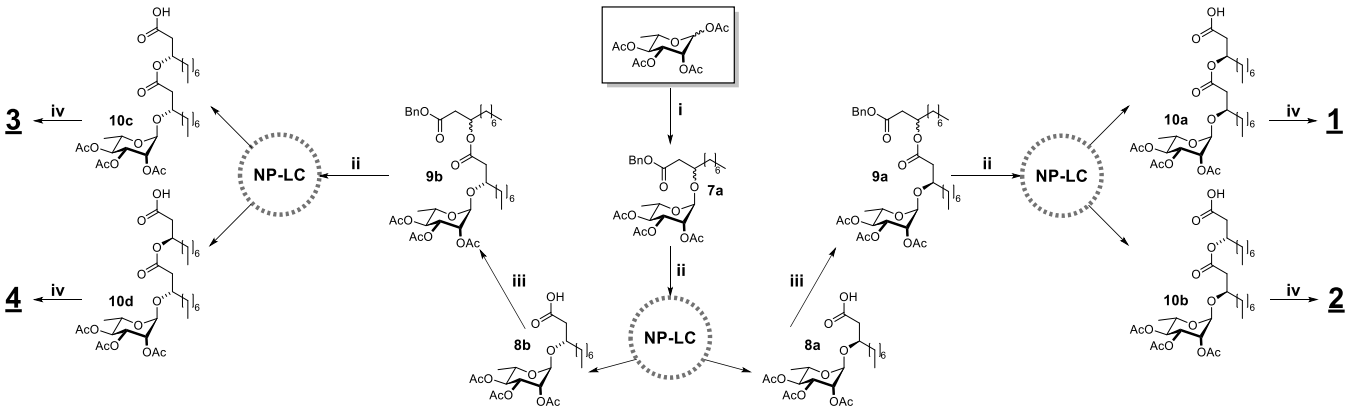


Figure 3. Synthetic methodology for manufacturing the diastereomers (***R,R***), (***R,S***), (***S,S***) and (***S,R***) of Rha-C10-C10. The stages where the diastereomers are enriched by preparative normal phase liquid chromatography (NP-LC) are marked by dashed circles. The steps involved are **i**) glycosylation of **6** in presence of $Bi(OTf)_3$ and dry MeCN refluxed for 2.5 h; **ii**) debenzoylation with Pd/C, 1 atm H_2 and dry THF at RT overnight; **iii**) Steglich esterification of **6** in presence of EDC and DMAP in dry CH_2Cl_2 at RT; and **iv**) deacetylation under Zemplèn conditions with MeONa/MeOH.

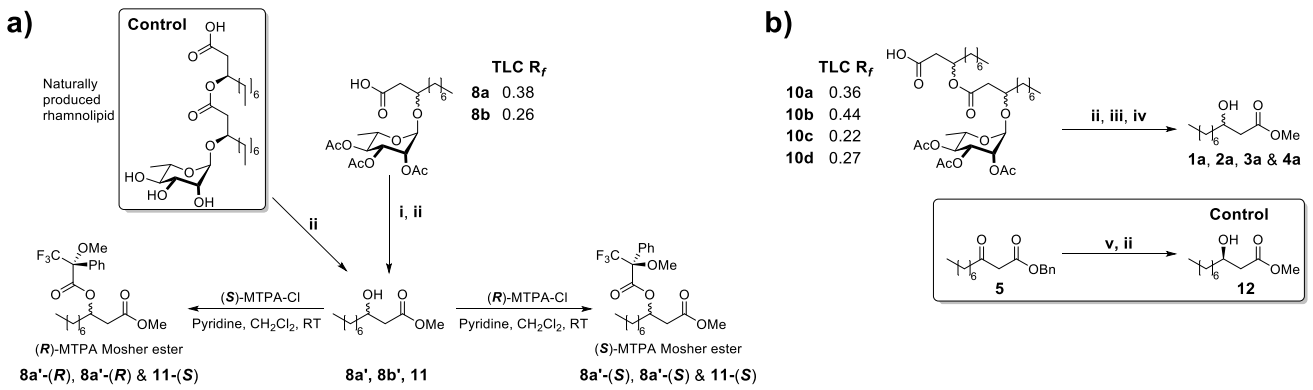


Figure 4. Approaches followed to determine the absolute configuration of a) the inner lipid tail carbinols by Mosher's ester analysis and b) the outer lipid tail carbinols by optical rotation measurements. For the inner lipid tail, the Mosher's esters of the naturally produced rhamnolipid were used as controls. Methyl (**R**)-3-hydroxydecanaote was obtained by Noyori's enantioselective hydrogenation and used as control for the optical rotation measurements. Derivatization reactions: **i**) MeONa, MeOH, RT; **ii**) MeOH, HCl 0.625 M, 70 °C, 12 h; **iii**) 2M NaOH(aq), RT; **iv**) 1.3 M BF_3-MeOH, 1.1 eq DMP 50 °C, 15 min; **v**) (**R**)-BINAP, EtOH, H_2 45 PSI, 115 °C, 72 h.

Table 1. Absolute configuration assignments for the carbinols of lipid tails.

Compound	R_f	Absolute configuration of carbinol(s) at lipid tail(s)[a]
8a	0.38	(***R***)
8b	0.26	(***S***)
10a	0.36	(***R***,***R***)
10b	0.44	(***R***,***S***)
10c	0.22	(***S***,***S***)
10d	0.27	(***S***,***R***)

[a]Assignments determined from Mosher's ester analysis and optical rotation measurements. See Supporting Information for additional details. R_f values obtained with analytical TLC with 1:1 hexanes/ethyl ether +1% v/v AcOH as mobile phase.

of the four chloroform solutions were performed and compared with the literature values of D-3-hydroxy esters and acids of similar or longer chain length.[6,10,108-111] As a control for the correlation of absolute configuration with the levorotary and dextrorotary attributes of the individual methyl (±)-3-hydroxydecanoate esters, we measured the optical rotation values of methyl (***R***)-3-hydroxydecanoate (**12**) prepared in nominally high %ee (>99%) by a well described Noyori's catalytic hydrogenation method,[88,112-114] and methyl 3-(***S***)-hydroxydecanoate ester (**8b'**) with confirmed absolute configuration by Mosher's ester analysis. All data pertaining to this analysis can be found in the Supporting Information. **Table 1** summarizes the assignments in terms of TLC R_f values of the acetylated glycolipids.

Surface tensiometry at pH 4.0 and 8.0. Surface tension measurements were made on each of the Rha-C10-C10 diastereomers using the du Noüy ring method. Due to the carboxylic acid moiety (pKa 5.5 for rhamnolipid aggregates[59]), solution conditions were adjusted to pH 4.0 or 8.0 to create solutions of the protonated nonionic or deprotonated anionic monorhamnolipids, respectively. Plots of surface tension (γ) as a function of Rha-C10-C10 concentration at pH 4.0 and 8.0 are shown in **Figures 5a** and **b**, respectively. CMCs at each pH are determined as the intersection between the two best straight lines through the data and are displayed in **Table 2** for each diastereomer.

The relatively low CMC values highlight strong interactions between the hydrophobic tails, consistent with the behavior of similar glycolipid surfactants.[115-120] At pH 8.0, the (***R***,***R***)-Rha-C10-C10 diastereomer has the highest CMC and (***R***,***S***)-Rha-C10-C10 has the lowest, whereas CMC values for (***S***,***S***)-Rha-C10-C10 and (***S***,***R***)-Rha-C10-C10 are comparable. These values suggest that the (***R***,***S***)-Rha-C10-C10 diastereomer has a molecular structure best suited to form aggregates in solution (see space filling structures in Figure S19 of the Supporting Information.) Specifically, the lipid tails are oriented in a way that makes it more energetically favorable for the molecules to aggregate than remain as free monomers when compared to the other diastereomers.

At pH 4.0, the (***R***,***S***)-Rha-C10-C10 diastereomer has the highest CMC and the others have comparable values. At pH 4.0, all diastereomers are nonionic, eliminating Coulombic repulsion between the headgroups and lowering the CMC. However, (***R***,***S***)-Rha-C10-C10 must assume a structure for which aggregation is less energetically favorable compared to the other diastereomers in their similar nonionic forms.

Chen *et al.* have reported CMC values for native monorhamnolipid mixtures (comprised primarily of the (***R***,***R***)-Rha-C10-C10 congener) of 180 and 360 μM at pH 7 and 9, respectively.[66] These values fall above and below that for the chemically synthesized (***R***,***R***)-Rha-C10-C10 diastereomer at pH 8.0 reported in **Table 2**, consistent with the known trend that CMC decreases with decreasing pH.[73] These values suggest that a mixture of monorhamnolipid congeners not of the form (***R***,***R***)-Rha-C10-C10 has only a minimal effect on the aggregation properties when compared to the chemically pure (***R***,***R***)-Rha-C10-C10 diastereomer produced by chemical synthesis.

The CMC values reported in **Table 2** are also similar to the values measured for the native monorhamnolipid mixture produced by *Pseudomonas aeruginosa* ATCC 9027, an exclusive producer of monorhamnolipids.[69] At pH 8, this native mixture exhibits a CMC of 200 μM while at pH 4, this mixture exhibits a CMC of 21 μM. Given that the composition of the mixture ranges from ~70-85% of the (***R***,***R***)-Rha-C10-C10, the similarity in values between the chemically synthesized diastereomers and the native mixture is not surprising.

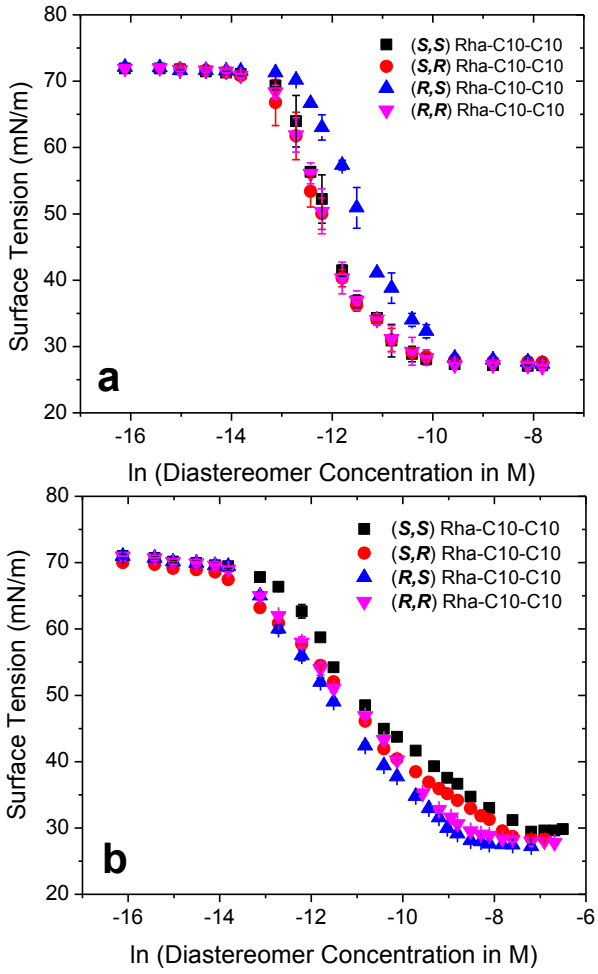


Figure 5. Surface tension as a function of Rha-C10-C10 concentration. a) Solutions adjusted to pH 4.0 with a minimal amount of HCl, and b) pH 8.0 with a minimal amount of NaOH.

The minimum surface tension values achieved with these synthetic diastereomers is also on the same order as previously reported values for native monorhamnolipid mixtures, indicating similar air-water interface adsorption of the diastereomers and the native congener mixture of monorhamnolipids.[66,69] Minimum surface tension values are in the range of 27-29 mN/m at both pH values. This would indicate that protonation of the carboxylic acid moiety and lipid tail stereochemistry have little effect on the adsorption of these surfactants at the air-water interface.

The molecular cross-sectional areas at the air-water interface are obtained from the surface tension data using the Gibbs adsorption isotherm:

$$\Gamma = -\frac{1}{\mathrm{m}RT}\left(\frac{\partial\gamma}{\partial lnC}\right)$$

where Γ is the surface excess, R is the gas constant, T is temperature, γ is the surface tension, C is the surfactant concentration and m is the Gibbs prefactor (m = 1 for nonionic monorhamnolipids at pH 4, m = 2 for anionic monorhamnolipids at pH 8). Molecular areas are calculated from the experimentally determined surface excess values using the slopes of the surface tension plots and are reported in **Table 2**.

Several aspects of these data are noteworthy. First, in previous literature studies of molecular cross-sectional areas for native

Table 2. Surface tension results for the monorhamnolipid diastereomers at pH 4.0 and 8.0.

Rha-C10-C10	pH[a]	CMC (μM)[b]	γ_{CMC} (mN/m)	Area (Å^2/molec)
(***R***,***R***)	4.0	16 ± 4	27.5 ± 0.1	21± 4
	8.0	270 ± 77	28.1 ± 0.2	117 ± 12
(***R***,***S***)	4.0	25 ± 1	28.8 ± 0.1	23 ± 1
	8.0	79 ± 3	27.4 ± 0.2	80± 1
(***S***,***S***)	4.0	18 ± 3	27.5 ± 0.1	21 ± 2
	8.0	201 ± 51	29.5 ± 0.2	93 ± 7
(***S***,***R***)	4.0	15 ± 1	28.2 ± 0.1	21 ± 1
	8.0	180± 24	28.5 ± 0.2	103 ± 4

[a]pH 4.0 values calculated from data in Figure 5a; pH 8.0 values calculated from data in Figure 5b. [b]Standard deviations determined from measurements on three independently prepared samples.

monorhamnolipid mixtures, values of 66 and 77 Å^2/molecule at pH 7 (0.063 M K_2PO_4 and 0.037 M NaOH) and 9 (0.023 borax and 0.008 M HCl), respectively,[66] and 86 Å^2/molecule at pH 8[69] were reported. These values are slightly lower than but similar to that for the pure (***R***,***R***)-Rha-C10-C10 diastereomer at pH 8.0 reported in **Table 2**; these slight differences may result from the different buffers used for these studies. At pH 8.0, the (***R***,***S***)-Rha-C10-C10 diastereomer has the lowest cross-sectional area, with the values increasing by about 10 Å^2/molecule in the order (***S***,***S***), (***S***,***R***), and (***R***,***R***). These values suggest differences in lipid tail orientation of the surfactants adsorbed at the air-water interface leading to differences in packing. Based on these values, the (***R***,***S***)-Rha-C10-C10 is more tightly packed compared to (***R***,***R***)-Rha-C10-C10.

The second noteworthy aspect of the molecular areas in **Table 2** are the unusually low values of ~21 Å^2/molecule observed at pH 4. This is similar to the value of ~23 Å^2/molecule observed for the native monorhamnolipid mixture from *P. aeruginosa* ATCC 9027 at pH 4. Given that the cross-sectional area of a single alkyl chain is ~21 Å^2/molecule, we hypothesize that the very tight packing of these double chain monorhamnolipids at pH 4 induces formation of small lamellar packets of bilayer monorhamnolipid, contiguous with the monorhamnolipid monolayer at the water surface, that protrude into the aqueous subphase. These features result in an underestimated surface area for the monorhamnolipid layer at the water surface, thereby resulting in artificially low values of molecular cross-sectional area.

Pyrene fluorescence for probing aggregation state. In the deprotonated anionic state, the native monorhamnolipids form globular micellar structures, growing into larger lamellar structures at higher concentrations.[66,69] To gain further insight into the solution aggregation behavior of these chemically synthesized diastereomers, the fluorescence behavior of a polarity sensitive probe, pyrene, was used. Pyrene, which is known to have high affinity for non-polar environments,[121] is useful for monitoring solution aggregation[122] through changes in the intensity ratio of the III/I vibronic bands of the fluorescence spectrum. As the local polarity of the pyrene becomes increasingly nonpolar, this ratio systematically increases. This ratio was used here to confirm solution aggregate formation by the four Rha-C10-C10 diastereomers by introducing pyrene to pH 8.0 solutions of the diastereomers at varying concentrations. As shown by the results in **Figure 6**, this ratio increases with Rha-C10-C10 concentration for all four diastereomers,

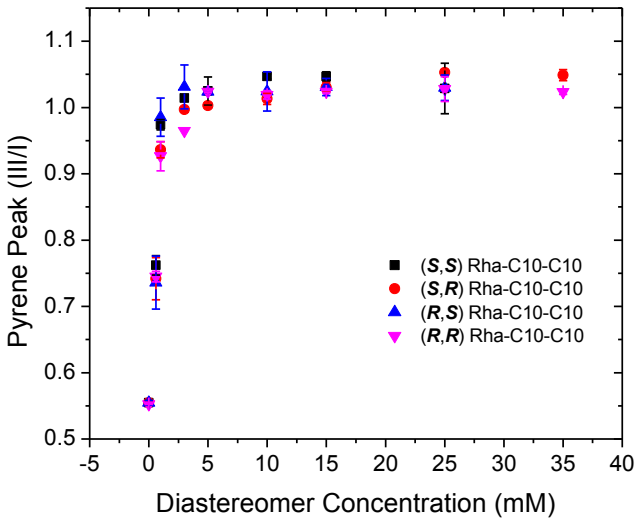


Figure 6. Pyrene III/I peak intensity ratios for all diastereomers as a function of surfactant concentration using 250 nM pyrene at pH 8.0 in 10 mM phosphate buffer.

confirming aggregate formation. This ratio does not level off until concentrations >5 mM are reached for all diastereomers, suggesting that full incorporation of pyrene does not occur until concentrations well above the CMC are reached. This behavior is consistent with size and structural changes in the early stags of solution aggregation, with the formation of pre-micellar aggregates at concentrations just above the apparent CMC likely.[69] Structural changes and growth of monorhamnolipid aggregates as a function of concentration are consistent with previous reports from this[69] and other[66,73] laboratories that aggregates on native monorhamnolipid mixtures. To further elucidate the detailed aggregation behavior of these different diastereomers, more in-depth studies are needed. These studies are underway in these laboratories and will be reported at a later date.

CONCLUSIONS

An efficient synthetic methodology for the production of four diastereomers of the monorhamnolipid Rha-C10-C10 is described. This process has considerably fewer steps than employed in the current methodologies available. Our methods are solution based, and make use of conventional and scalable well-described chemical transformations. The use of peracetylated donors and minimally competent Lewis acids improves the practicality and scalability of the method. Assignments of the absolute configuration of the carbinols at the lipid tails were performed by means of Mosher's ester ^{1}H-NMR analysis and optical rotation measurements. In addition to production of these rhamnolipid diastereomers, the method has proven to be flexible enough to test structure-performance relationships in other areas of the molecule including lipid tail symmetry and lipid tail length. The results of these studies will be reported at a later date.

Each Rha-C10-C10 diastereomer shows similar surface activity, CMC, and γ_{CMC} at pH 4 and 8, suggesting little difference in air-water interface chemistry among the four diastereomers. The values of molecular area for the diastereomers are similar although not identical, suggesting slightly different molecular orientations at the air-water interface. The molecular areas for all four diastereomers are significantly lower at pH 4 than at pH 8 due to fewer repulsive interactions between the nonionic compared to the anionic forms of these monorhamnolipds. The CMC values for the diastereomers at pH 4 are generally similar with the exception of (***R***,***S***)-Rha-C10-C10 that likely has poorer packing compared to the others. Thus, these stereochemical differences in diastereomer structure confer slight differences in surfactant performance among these molecules.

In total, these studies support chemical synthesis as a viable alternative path to biosynthesis for the production of monorhamnolipids. Given the reasonable yields of the synthetic procedure, the ability to tailor surfactant performance through choice of alkyl chain length, and the considerably greater ease at compound purification, chemical synthesis may be the more attractive pathway to fabrication of these materials for many applications.

ASSOCIATED CONTENT

Supporting Information

Materials and methods for the synthesis of monorhamnolipid diastereomers; instrumentation details and sample preparation for the surface tension measurements and the pyrene fluorescence experiments for aggregation; detailed synthetic procedures for the manufacture and isolation of rhamnolipid diastereomers; protocols for absolute configuration assignments of the carbinols on the lipid tails; relevant NMR and MS spectroscopic data. (PDF) This information is available free of charge on the ACS Publications website at DOI: XX.XXXX/jacs.

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Notes

Two authors of this work (JEP, RMM) have equity ownership in GlycoSurf, LLC that is developing products related to the research being reported. The terms of this arrangement have been reviewed and approved by the University of Arizona in accordance with its policy on objectivity in research.

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