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Parth B. Jariwala April 14, 2015

Synthesis of Ellagic Acid Glycoside Derivatives found in *Rubus ulmifolius*

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Abstract

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The dramatic increase in the number of antibiotic-resistant bacteria is a major problem in the field of public health. A novel approach to killing antibiotic-resistant bacteria employs compounds to shutdown bacterial defense mechanisms, thereby increasing the efficacy of current antibiotics at killing the bacteria. A previous study by Quave *et al.* found that an extract from *Rubus ulmifolius* inhibits biofilm formation, a defense mechanism, in *S. aureus*. The extract contains high levels of glycosidic derivatives of ellagic acid. The primary goal of this project is to synthesize and test glycosidic derivatives of ellagic acid for anti-biofilm and growth inhibitory activity in order to study structure activity relationships. The main synthetic approach taken is directly glycosylating ellagic acid. Several methods have been tried to glycosylate ellagic acid, and some have led to product formation, but more optimization is required to produce the glycosidic derivatives in higher yields.

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Introduction

Years of use and misuse of antibacterial agents has led to a staggering increase in the number of antibiotic-resistant bacteria.[1] Presently, for example, 40-60% of *Staphylococcus aureus* strains are methicillin-resistant (MRSA).[2] MRSA and other resistant strains have drastically increased morbidity rates, mortality rates, and healthcare costs; hence, they pose a major problem for the field of public health. A study in New York City alone found that mortality rates rose from 8% to 21% when patients were infected with MRSA versus its methicillin-susceptible counterpart, methicillin-susceptible *S. aureus* (MSSA).[3] In addition, healthcare cost increased by 22% when dealing with MRSA.[3] If this problem is not addressed immediately, these rates will continue to rise and the health community will have limited tools to combat bacterial infections.

Bacterial resistance occurs at two levels: at the individual level and the population level.[4] At the individual level, bacteria can resist antibiotics via the release of enzymes that can alter the structure of the antibiotic, efflux pumps that can actively pump the antibiotic out of the cytoplasm, and through genetic mutations (or gene transfer) that can alter the target of the antibiotic, rendering it useless.[1, 4] At the population level, bacteria can coordinate with one another through signaling mechanisms in order to increase chances of survival (e.g. quorum sensing). They can coordinate to collectively synthesize and release enzymes that target and destroy antibiotics as well as coordinate to build micro-colonies that contain extracellular barriers.[4] Such extracellular barriers can stop harmful foreign substances from physically reaching the bacterium within the colonies.

The traditional method to combat antibiotic-resistant bacteria is to simply create or find more antibiotics; generally, by finding or creating novel broad-spectrum antibiotics. Broad-

spectrum antibiotics can be viewed as economically and functionally efficient drugs, since they are effective against many species of bacteria. However, this is not the case. It now costs approximately $1 billion to take an antibacterial agent from its initial discovery to the marketplace.[5] Thus many pharmaceutical companies have found the high cost of producing antimicrobial agents unprofitable. They would rather focus their time and money developing therapeutics for extremely chronic diseases (e.g. cancer and HIV) where the lifespan of a patient can be significantly prolonged.[5] Additionally, broad-spectrum antibiotics are susceptible to resistance due to overuse. For example, vancomycin, "the drug of last resort", has become ineffective against some strains of MRSA.[6] New antibiotics such as daptomycin and linezolid have been developed to counter not only vancomycin-resistant MRSA, but also other vancomycin resistant bacteria.[1] Sadly, some vancomycin-resistant MRSA strains are already showing resistance against these new drugs (specifically, linezolid).[1]

Because of the increasing rate of resistance against broad-spectrum antibiotics and the high cost of production, alternative approaches that are more economically and functionally efficient must be explored.[4] One approach involves discovering drugs that inhibit defense mechanisms of bacteria.[4] Inhibiting such mechanisms can help give current antibiotics a better chance at killing the bacteria. Although resistance will likely develop against these drugs as well, this synergistic approach is highly economical. It allows old antibiotics to essentially be "recycled," as their efficacy will be increased.

One such defense mechanism is biofilm formation. Biofilms are composed of polymeric substances, eDNA, and proteins that are secreted by bacteria after they attach to a solid surface and form micro-colonies.[4]. A biofilm functions as a barrier that physically stops antibiotics and other harmful agents from reaching the bacteria. Furthermore, the close proximity of the bacteria

within the biofilms helps facilitate gene transfer, thereby increasing the spread of resistance.[4, 7] Medical implants are a prime location for bacteria to form biofilms in the body.[7] Infections arising from bacteria within biofilms on medical implants are hard to fight, as antibiotics cannot physically reach the bacteria. Thus, shutting down biofilm formation can facilitate the ability of antibiotics to reach and kill the bacteria.

Using bioassay-guided fractionation, Quave *et al.* found that an extract from a plant, *Rubus ulmifolius*, inhibits biofilm formation in *S. aureus*.[7] Fraction 2 (40:60 MeOH:DCM) of the butanol partition (220D) of the crude extract inhibits biofilm formation without inhibiting growth. Furthermore, the study found that this fraction (220D-F2) considerably increased the efficacy of antibiotics in killing *S. aureus*. LC-UV/MS/MS studies also revealed high levels of ellagic acid (EA) and ellagic acid glycosides (EAG) in 220D-F2. The presence of EA and EAG in 220D-F2 is quite interesting, as they are known to have many therapeutic qualities. They have been shown to have anti-tumor, anti-cancer, anti-oxidant, anti-HIV, anti-hepatitis, and anti-microbial activity.[7-8]

Since EA and EAG are present in high concentrations in 220D-F2 and they are known to have many therapeutic qualities, we are interested in determining if they are the active constituents within the extract. The primary aim of this project is to study structure activity relationships (SAR) of EAG. A library of glycosidic derivatives of ellagic acid will be tested for anti-biofilm and inhibitory growth activity in *S. aureus*. If the EAG show anti-biofilm activity, then it can be concluded that they are the active constituents of extract 220D-F2. Subsequently, the secondary aim of this project will be to elucidate the mechanism by which EAG inhibit biofilm formation in *S. aureus* via the use of biochemical techniques (e.g. protein pull-down studies). This will provide valuable insight into how biofilms form and function. If the EAG do

not show anti-biofilm activity, then it can be concluded that either they are not the active constituents of extract 220D-F2 or that the anti-biofilm activity is due to the synergistic action between EAG and other constituents in 220D-F2.

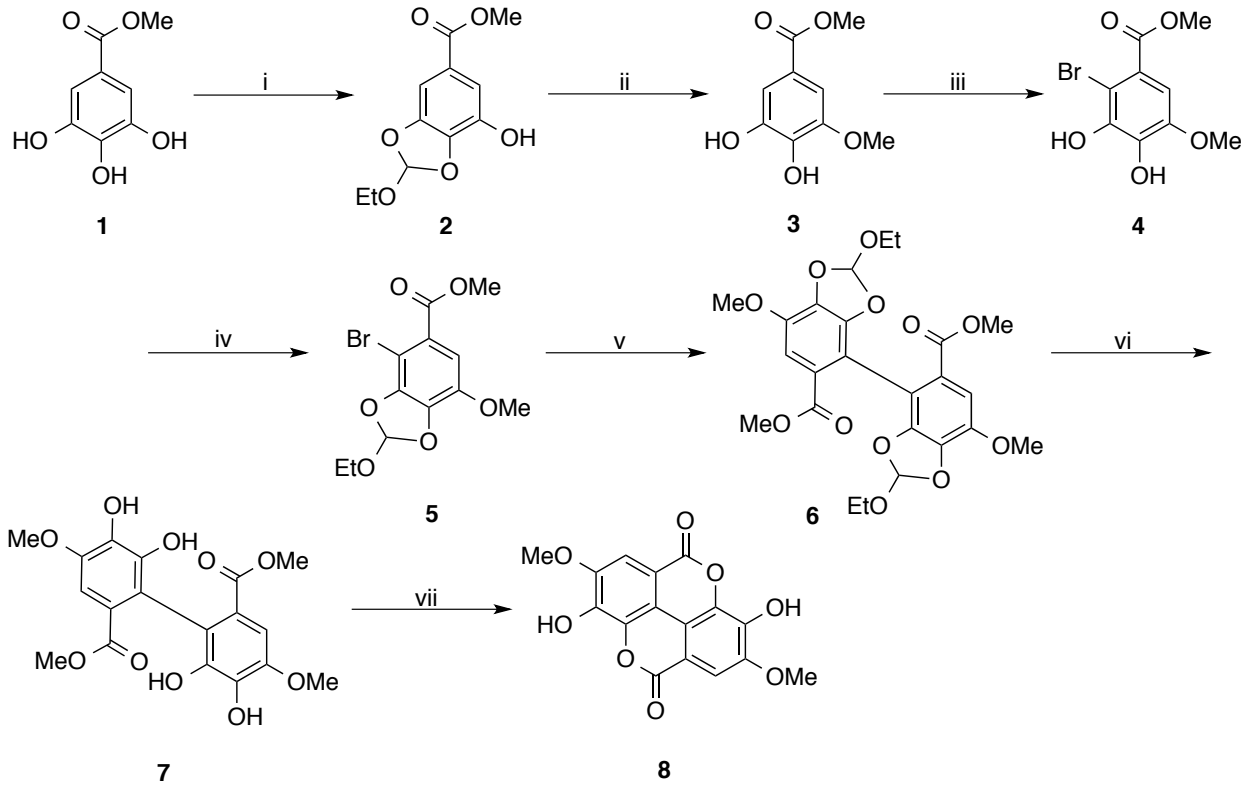
Isolating single compounds from crude extracts of plants via fractionation is a tedious process resulting in very small yields. Take for example the famed anti-cancer natural product, Taxol, which is found in the bark of the tree, *Taxus brevifolia*.[9] It took approximately 12 kg of dried bark to yield ~0.05 g Taxol (~0.004% yield).[9] Hence, this is not an ideal process to obtain reasonable yields of isolated compounds since plant material is not always readily available. Instead of isolating the EAG from *R. ulmifolius*, they will be synthesized. A synthetic approach also allows for the preparation of unnatural EAG derivatives, facilitating SAR studies.

Approaches to Synthesizing Ellagic Acid Glycosides

The two general approaches that can be taken to synthesize EAG are either total synthesis using the precursor of EA, gallic acid, or directly glycosylating EA itself.

The total synthesis approach allows for general flexibility when it comes to choosing synthesis schemes. Total synthesis of EA and its alkyl derivatives has been accomplished by the Tsuboi group.[8, 10] Scheme 1 depicts a synthetic scheme to produce the methyl derivative of EA, 4,4'-di-*O*-methylellagic acid (**8**)

Scheme 1. Preparation of 4,4'-di-*O*-methylellagic acid[8]



i. $(EtO)_3CH$, Amberlyst 15E, benzene, reflux, 18h; ii. MeI, K_2CO_3, DMF, 70°C, 12h, HCl, MeOH, rt., 2h; iii. DBDMH (0.51 Eq.), $CHCl_3$, rt., 24h; iv. $(EtO)_3CH$, Amberlyst 15E, benzene, reflux, 17h; v. Cu, DMF, 110°C, 3h then 180°C, 18h; vi. 2N HCl, MeOH, rt., 4h; vii. MeOH:H_2O (1:1), reflux, 8h, quant.

In Scheme 1, the Tsuboi group utilizes the EA precursor gallic acid (**1**) to produce 4,4'-di-*O*-methylellagic acid (**8**). The two critical steps in Scheme 1 are (ii) and (v). In step (ii), the alkyl group of interest is added to the protected gallic acid molecule (**2**). Since the methyl derivative of EA is being synthesized, methyl iodide (MeI) is added. In step (v), the brominated alkyl derivative of gallic acid (**5**) undergoes Ullmann coupling to yield a symmetrical biaryl system (**6**).[11] After deprotection and lactamization of **6** and **7**, respectively, the final product (**8**) is obtained.

Scheme 1 can potentially be used to synthesize a doubly glycosylated EA, thereby showing that total synthesis of EAG from gallic acid is possible. The Tsuboi group created several alkyl derivatives of EA by using different alkyl groups in step (ii). For example, they used benzyl chloride (BnCl) instead of MeI to produce the benzyl derivative, 4,4'-di-*O*-benzylellagic acid. In place of BnCl or MeI, a protected and halogenated sugar can be added to produce a glycosylated gallic acid, which can then be used to form a doubly glycosylated EA using the remaining steps in Scheme 1. There are drawbacks to the total synthesis approach. In general, this approach will require much time and resources to produce a library of EAG (as can be seen by the number of steps in Scheme 1) and the final yields will be relatively low.

Since a library of EAG is desired, the direct glycosylation approach is ideal since it requires fewer steps, saving time and resources. The strength in this approach also lies in the fact that groups including Sugai *et al.*, Tatsuta *et al.*, and Morita *et al.* have synthesized glycosidic derivatives of EA by using direct glycosylation methods.[12] As with the total synthesis approach, the direct glycosylation approach has drawbacks. As will be mentioned later, many of the reagents utilized for direct glycosylation are highly hygroscopic. Not only does this impede the glycosylation process itself, but it also leads to the development of side products due to the base sensitive nature of EA. Regardless, the direct glycosylation approach was taken for this project since it requires fewer steps than the total synthesis approach.

Chemical and Physical Properties of Ellagic Acid

EA (Figure 1) is a symmetrical dilactone tetraphenol.

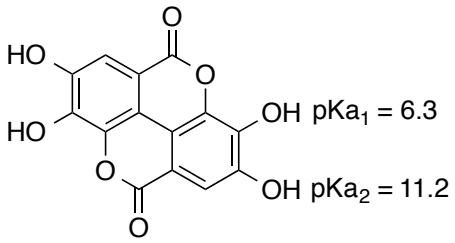
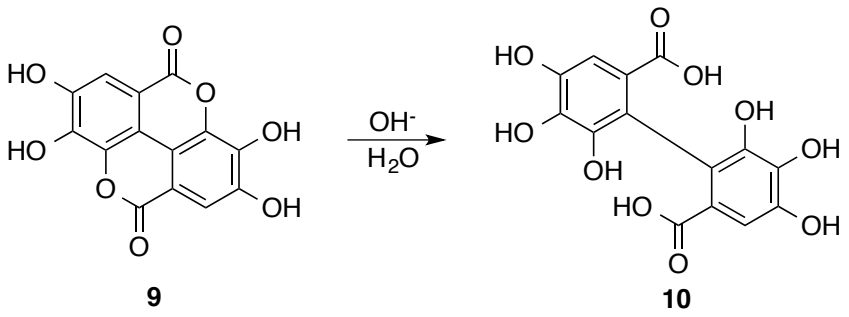


Figure 1. Ellagic acid

Since EA is symmetrical, the structure only has two chemically different hydroxyl groups. The hydroxyl groups in the para-position (*p*-OH) with respect to the ester group have a pKa value of 6.3 making them more acidic than the hydroxyl groups in the meta-position (*m*-OH), which have a pKa value of 11.2.[13] The large difference in the pKa values can be attributed to the electron withdrawing nature of the ester group.

Since EA contains two lactones, it is base sensitive.[14] Scheme 2 depicts a hypothetical situation in which EA (**9**) undergoes hydrolysis in basic conditions to yield hexahydroxydiphenic acid (**10**).

Scheme 2. Hydrolysis of ellagic acid in basic conditions



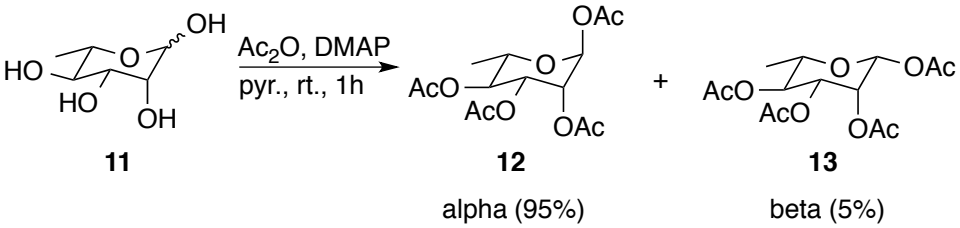
Hence, the use of basic conditions to glycosylate EA must be performed with care as the presence of water can result in lactone hydrolysis. In addition to being base sensitive, EA is highly insoluble. At room temperature, it is not soluble in nonpolar solvents such as

dichloromethane (DCM) and chloroform ($CHCl_3$). Likewise, it is minimally soluble in polar solvents. For example, 5 mg EA is soluble in approximately 25 mL ethanol (EtOH), 2.5 mL pyridine (pyr.), 0.2 mL NMP, or 2.0 mL DMSO.[15] The insolubility of EA will make the use of protecting groups vital in the synthesis of EAG.

Results and Discussion

Preparation of 1,2,3,4-tetra-*O*-acetyl-α-L-rhamnopyranose. Prior to the initial glycosylation attempt of EA, a peracetylated sugar was prepared. L-rhamnose was chosen as the sugar of interest and it was acetylated following the method developed by Donahue and Johnston (Scheme 3).[16]

Scheme 3. Preparation of 1,2,3,4-tetra-*O*-acetyl-α-L-rhamnopyranose

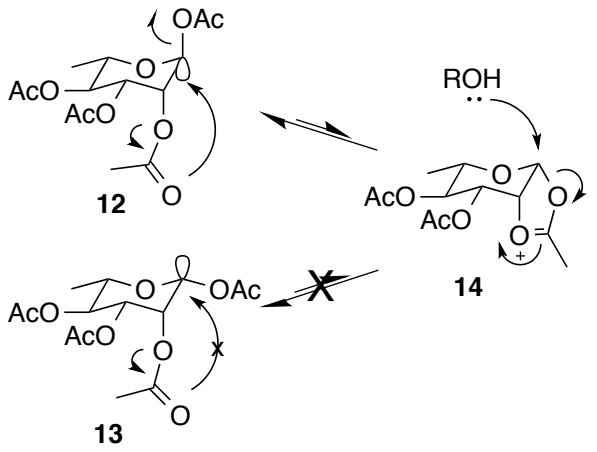


Following their method initially gave an α:β mixture of approximately 3:2 based on the integration values of the proton at the C1 position (anomeric position) in the ^1H NMR spectrum of the crude product. The α and β products (**12** and **13**, respectively) could not be separated since they have the same R_f values in virtually all solvent systems. The method was slightly modified in order to selectively produce **12**. Before the drop-wise addition of acetic anhydride (Ac_2O), L-rhamnose and 4-dimethylaminopyridine (DMAP) were stirred in pyridine at room temperature for approximately 30 min. The basic environment results in the isomerization of β-L-rhamnose to the more thermodynamically stable α-L-rhamnose. This led to an optimized α:β product mixture of approximately 20:1. The alpha configuration is more thermodynamically stable because it takes advantage of the anomeric effect. The nonbonding electrons (NBE) of the heteroatom are

aligned with the σ* orbital of the acetyl group at the anomeric position. This alignment allows for interaction between the NBE and the σ* orbital thereby stabilizing the structure and making the alpha configuration more thermodynamically favorable than the beta configuration.

The alpha configuration is desired because it can undergo neighboring group assistance (Scheme 4), which helps to increase the rate of glycosylation and promote α-diastereoselectivity.

Scheme 4. Proposed mechanism for neighboring group assistance in per-*O*-acetyl rhamnopyranose



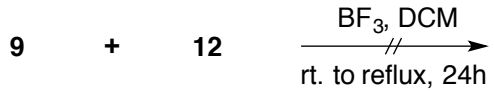
The glycosylation reaction likely proceeds through acetoxonium ion **14**, formed via nucleophilic attack of the C2 carbonyl oxygen on C1. The formation of this unstable charged intermediate helps increase the rate of glycosylation and sterically prevents nucleophilic attack of the glycosyl acceptor from the bottom face. The beta configuration (**13**) cannot undergo neighboring group assistance due to the *anti* relationship between the C2 acetyl group and the σ* orbital at the C1 position. The inability of **13** to undergo neighboring group assistance makes it functionally inert. Although it can still undergo glycosylation via an S_n2-like mechanism, the rate is likely to be very slow due to a lack of a charged intermediate.

Initial Glycosylation Attempt. Peracetylated sugars are heavily used in aromatic-*O*-glycosylation.[17] The commonly employed method when using peracetylated sugars is stirring the

desired phenol, peracetylated sugar, and boron trifluoride diethyl etherate (BF_3•$O(C_2H_5)_2$) in dry DCM or $CHCl_3$ for approximately 24h.[18] BF_3 acts as a promoter to drive the conversion of **12** to **14** in Scheme 4 by increasing the leaving ability of the acetyl group at the C1 position.

These conditions were utilized to glycosylate EA (Scheme 5).

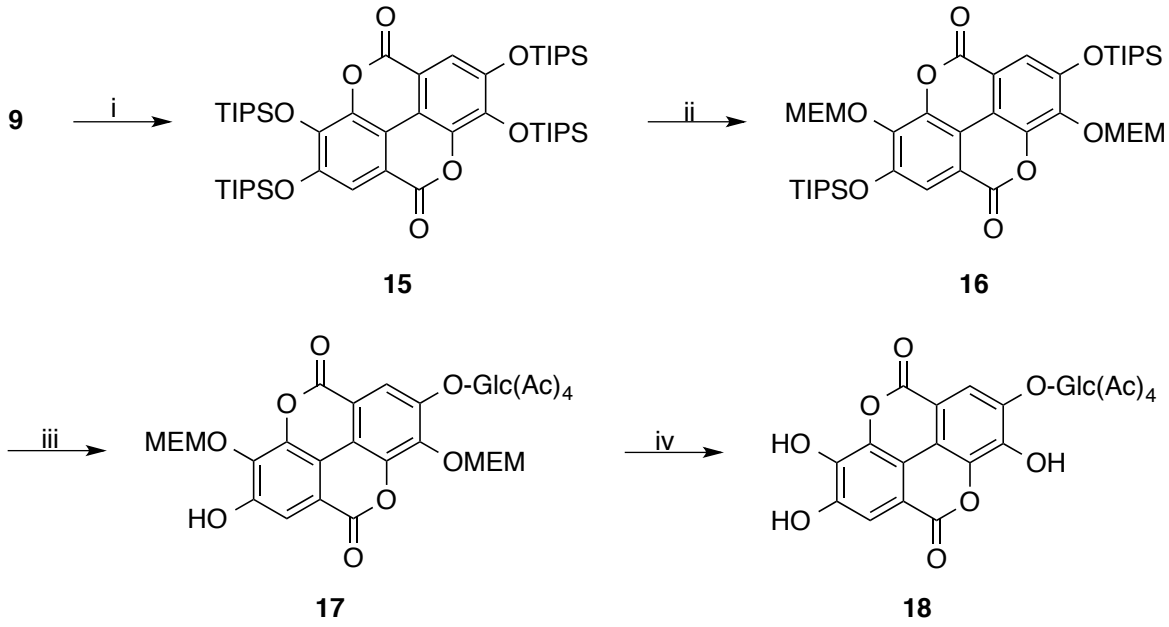
Scheme 5. Glycosylation of ellagic acid using BF_3 as a catalyst



An isomeric mixture was expected, but this was not of concern as the mixture could potentially be separated via HPLC. TLC analysis and ^{1}H NMR of the crude product indicated the presence of starting material after 24h. Hence, the reaction did not proceed at all and this was mainly due to the insolubility of EA, which was also expected. It was thought that refluxing could drive some EA into solution where glycosylation could occur. The failure of Scheme 5 necessitated protection of EA to enhance solubility prior to any glycosylation reactions.

Kobayashi *et al.* Method. The insoluble nature of EA prompts the use of protecting groups to increase the lipophilicity and hence, confer solubility in organic solvents. As stated earlier, Kobayashi *et al.* successfully synthesized a glycosidic derivative of EA, employing protecting groups to achieve solubility.[12a] Scheme 6 depicts part of the synthetic approach they take to synthesize 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl ellagic acid (**18**).

Scheme 6. Preparation of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl ellagic acid[12a]



i. TIPSCl, imidazole, DMAP, DMF, DCM, 50°C, 12h; ii. Step 1) TBAF, MS 4A, DCM, rt., 15 min. Step 2) MEMCl, Cs_2CO_3, DCM, -50°C, 4h; iii. Step 1) TBAF, $CHCl_3$, rt., 15 min. Step 2) acetobromoglucose, Cs_2CO_3, BTBAB, H_2O, rt., 18h; iv. TFA, DCM, rt., 1h

Kobayashi *et al.* begins the glycosylation process by protecting **9** with triisopropylsilyl (TIPS) groups to yield tetrakis-TIPS EA (**15**), which is highly soluble in DCM and $CHCl_3$. Silyl protecting groups are attractive, as they can be selectively cleaved using a soluble source of fluoride ions due to the strength of the Si-F bond.[19] Since the *p*-OH position is more acidic than the *m*-OH position, the fluoride ions will selectively cleave the silyl groups protecting the *p*-OH groups before cleaving the silyl groups protecting the *m*-OH groups. Hence, the silyl groups at the *p*-OH position can be selectively cleaved to yield bis-TIPS EA by adding 2 Eq. of a fluoride source. This selectivity makes the use of silyl protecting groups more attractive than other protecting groups. To deprotect **15**, Kobayashi *et al.* employed the common silyl-deprotecting

agent, tetrabutylammonium fluoride (TBAF). Figure 2 displays the quaternary ammonium salt formed after the addition of 2 Eq. TBAF in step (ii).

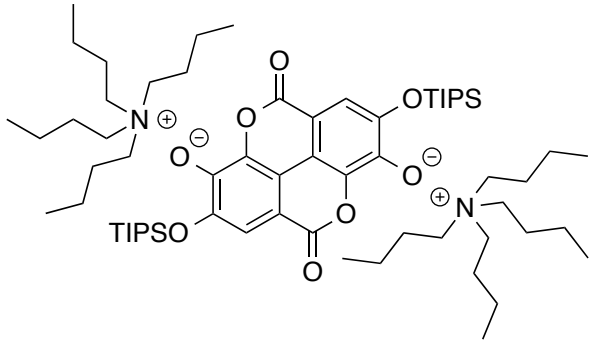


Figure 2. Formation of quaternary ammonium salt intermediate

The lipophilic nature of the tetrabutylammonium cations maintains solubility of the deprotected **15**. However, the highly hygroscopic nature of TBAF poses an issue since wet samples will contain highly basic tetrabutylammonium hydroxide (commercial samples from Sigma contain ~5% H_2O), and EA is base sensitive. In spite of this concern, TBAF was initially used for deprotection due to literature precedent in Kobayashi *et al.*

After deprotection *in situ*, the *p*-phenoxides are re-protected with 2-methoxyethoxymethyl chloride (MEMCl) in step (ii) to yield bis-TIPS, bis-MEM ellagic acid (**16**). Compound **16** permits selective *m*-OTIPS removal and subsequent two-phase glycosylation *in situ* using a glycosyl bromide to afford **17.** Finally, the MEM groups are cleaved in step (iv) to yield **18** (bis-glucosylated ellagic acid is also produced in small yields according to their report).

Tetrakis-TIPS ellagic acid was successfully synthesized following the method used by Kobayashi *et al*. Even though **15** was stored over Drierite and P_2O_5 in a vacuum desiccator, ^{1}H NMR showed the presence of water in the sample. This revealed that **15** is hygroscopic. Thus, the material was dried over magnesium sulfate ($MgSO_4$) in dry DCM and stored under an atmosphere of nitrogen.

Although **16** was successfully synthesized, a yield of greater than 5% could not be achieved. The low yield can potentially be attributed to several reasons. After the addition of 2 Eq. TBAF in step (ii), TLC analysis of the mixture shows the presence of the expected intermediate, bis-TIPS EA (Figure 2). But there is also significant baseline material present (degradation) on the TLC plate. This baseline spot continues to grow as time progresses. The basic environment that develops leads to lactone hydrolysis as depicted in Scheme 2. The other possible identity of the baseline material is over-deprotected **15**.

In addition to degradation occurring after TBAF is added, another possible reason for the low yield is the acidification of the reaction medium after the addition of MEMCl. Although TLC analysis indicates the presence of a new product after the addition of MEMCl (identified as **16** by ^{1}H NMR), it does not develop much overtime. After the reaction has proceeded for 4h, the pH of the reaction mixture is ~1-2. Since MEM protecting groups are acid-labile, protected product **16** could be decomposing during the reaction. This might explain why **16** is not being produced in high yield. Despite using rigorous anhydrous techniques, the acidic environment is likely developing from the presence of water in the reaction mixture and/or in the MEMCl reagent bottle (which was stored over 4A molecular sieves under dry nitrogen in a desiccated jar). The MEMCl will react with water to produce hydrochloric acid (HCl) and 2-methoxyethoxymethyl alcohol (MEMOH).

Many attempts were made to optimize the reaction in step (ii). Less TBAF was used to try and minimize degradation. The number of equivalences was varied from 2.0 to 1.9, but this did not help as degradation continued to occur. Another attempt to increase the yield was to drop the equivalences of MEMCl in order to decrease the acidity of the mixture. The equivalences was varied from 8.0 to 3.0, but this had no effect on the resulting pH. Both methods failed to

increase the yield of **16**. ^{1}H NMR of the dry DCM revealed no water peak. Since TBAF, MEMCl, and Cs_2CO_3 are hydroscopic, new bottles were brought and used right away, but this still failed to improve yields.

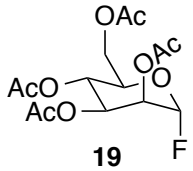
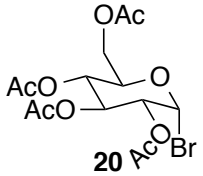
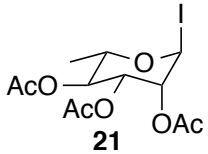
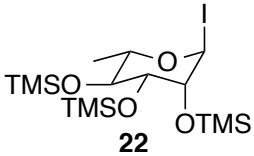
Since TBAF and MEMCl seem to be a problem, the method in Scheme 6 was slightly modified. Instead of using 2 Eq. TBAF to produce bis-TIPS ellagic acid, 1.5 Eq. TBAF was used to produce a mixture of tris-TIPS ellagic acid and bis-TIPS ellagic acid, which were separated by silica chromatography. Although tris-TIPS ellagic acid and bis-TIPS ellagic acid were successfully prepared and isolated, ^{1}H NMR revealed the presence of regioisomers. Isomers were forming due to silyl group migration during aqueous workup. Silyl group migration in basic, protic solvents is precedented.[19] To avoid regioisomerization, glycosylation of EA must be done *in situ* after silyl group removal.

Alternative Approach. Step (ii) in the Kobayashi *et al.* method could not be reproduced effectively. Since degradation of tetrakis-TIPS ellagic acid seems to be occurring after the addition of TBAF and a yield of greater than 5% of **16** could not be achieved, an alternative approach was taken. Instead of adding MEMCl after deprotecting **15** with 2 Eq. TBAF, the sugar can be added instead. This will ideally lead to glycosylation at the *p*-OH position to produce both mono-glycosylated and di-glycosylated ellagic acid. This approach is ideal since it avoids the use of MEMCl to create intermediate **16**. However, orthogonal protection of *p*-OH and *m*-OH will be required to prepare *m*-*O*-glycosides.

Since this new approach still requires the use of TBAF, degradation of **15** will still occur. And since the degraded material seems to develop as time goes on, the reaction time needs to be minimized. One way to decrease the reaction time is by increasing the reactivity of the sugar. Different leaving groups at the C1 position can be used to increase reactivity. If the reactivity of

the sugar can be increased, then the reaction can theoretically proceed faster, thereby reducing degradation of **15**. Table 1 lists sugars with varying anomeric leaving groups that were attempted in the glycosylation of **15** after deprotection with 2 Eq. TBAF.

Table 1. Sugars utilized for glycosylation

Leaving Group at C1 Position	Sugar	Conditions[a]	Product Yield (%)
Acetyl Group	**12**	Cs_2CO_3, BF_3, rt. to reflux, 24h	No Rxn
Fluorine	 **19**	Cs_2CO_3, BF_3, [b] rt. to reflux, 24h	No Rxn
Bromine	 **20**	Cs_2CO_3 ,BTMAB, rt., 24h	Complex Mixture
Iodine (Per-*O*-acetyl Sugar)	 **21**	TBAI, rt. to reflux, 24h	No Rxn
Iodine (Per-*O*-TMS Sugar)	 **22**	TBAI, rt. to reflux, 24h	< 5[c]

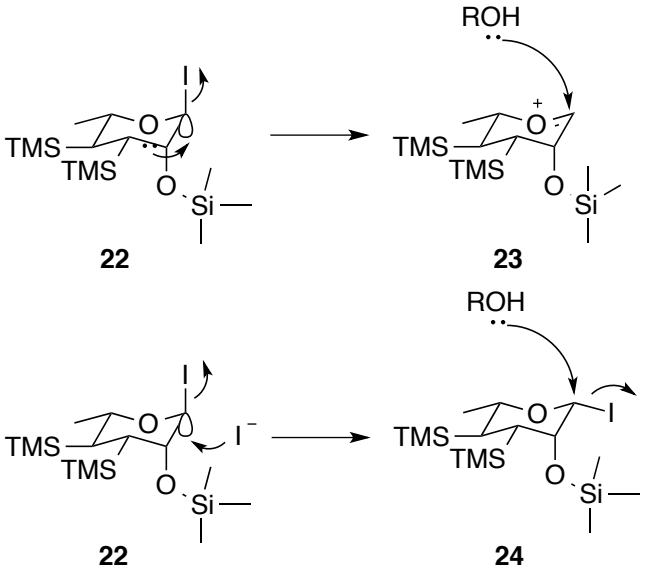
a. Prior to the addition of the sugar and reagents listed under the "Conditions" column, 2 Eq. TBAF was added to a mixture of **15** and 4A molecular sieves in dry DCM and stirred for 15

min.; b. The reaction was done without BF_3 as well; c. The product was bis-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl), bis-TIPS ellagic acid (identified by ^{1}H NMR).

TLC analysis revealed the presence of starting material after 24h for reactions involving **12** and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl fluoride (**20)**, indicating that the reactions did not proceed. Even in the presence of an activator (BF_3), the reaction did not proceed. Next, a glycosyl bromide, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**20**), was used. After 24h, TLC analysis revealed the formation of a complex mixture, which could not be separated. Proton NMR of the crude product did not show the presence of the desired product. Finally, a glycosyl iodide, 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl iodide (**21)** was used. Work done by Gervais-Hague and co-workers shows that glycosyl iodides are stable enough for use in glycosylation reactions.[20] Compound **21** was prepared from **12** in excellent yield based on ^{1}H NMR.[21] Unfortunately, TLC analysis revealed no product formation when **21** was used for glycosylation.

To further increase the reactivity of the sugar, the glycosyl iodide was protected with trimethylsilyl (TMS) groups instead of acetyl groups. L-rhamnose was protected using trimethylsilyl chloride (TMSCl) and iodinated based on previous literature to produce 2,3,4-tri-*O*-TMS-α-L-rhamnopyranosyl iodide (**22**).[21-22] Since iodine is a great leaving group, the oxonium intermediate, **23**, readily forms and is stabilized by the electron donating TMS groups (Scheme 7).

Scheme 7. Glycosylation mechanism when using TBAI



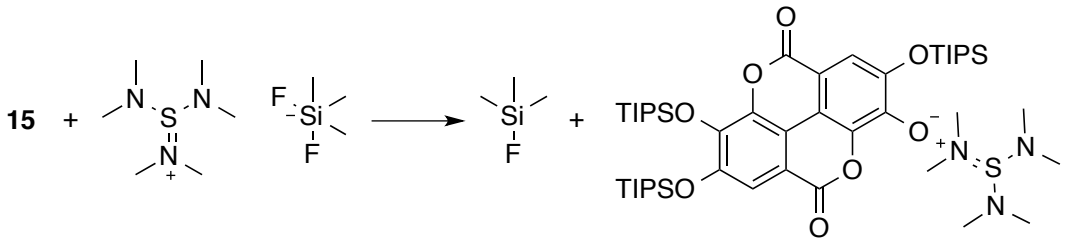
Based on studies done by the Gervay-Hague group, the use of tetrabutylammonium iodide (TBAI) is critical for reactions involving glycosyl iodides.[20] The soluble iodide anion converts **22** to its beta anomer, **24**. Based on previous studies, the glycosylation proceeds through beta-iodide **24**, which is less thermodynamically-stable than the alpha diastereomer.[20] The greater stability of the alpha configuration can be attributed to the anomeric effect. Previous work with glycosyl iodides shows that the beta configuration likely goes through an S_n2-like mechanism since only the alpha anomer of the product is produced. Glycosylation with iodide **22** produced bis-(2,3,4-tri-*O*-TMS-α-L-rhamnopyranosyl), bis-TIPS ellagic acid, which was isolated by silica chromatography in poor yield (<5%) and identified by ^{1}H NMR.

The low yield is likely due to **15** degrading in the presence of TBAF as discussed earlier. The continuous problems encountered when using TBAF prompted the search for another fluoride deprotecting reagent. The literature revealed tris(dimethylamino)sulfonium difluorotrimethylsilicate (TASF) as a milder alternative to TBAF for silyl ether cleavage of base sensitive substrates.[23] The fluoride in TASF is complexed to the mild Lewis-acid, TMSF, which

diminishes the reactivity compared to the naked fluoride of TBAF. Additionally, TASF is commercially available as an anhydrous solid, as opposed to TBAF, which is typically purchased as a solution in hygroscopic tetrahydrofuran (THF).

A small study was performed to compare TASF and TBAF in the deprotection of **15**. Treatment of **15** with 2 Eq. TBAF resulted in a complex mixture after several hours, according to TLC analysis. Encouragingly, 2 Eq. TASF produced tris-TIPS EA as the major product with **15** still present. Unlike treatment with TBAF, continuing the TASF deprotection for 24h did not result in side-reactions. The presence of unreacted **15** can be explained by the formation of the TMSF by-product, which likely competes with **15** for the fluoride ion in **25** (Scheme 8).

Scheme 8. Deprotection of tetrakis-TIPS ellagic acid using TASF

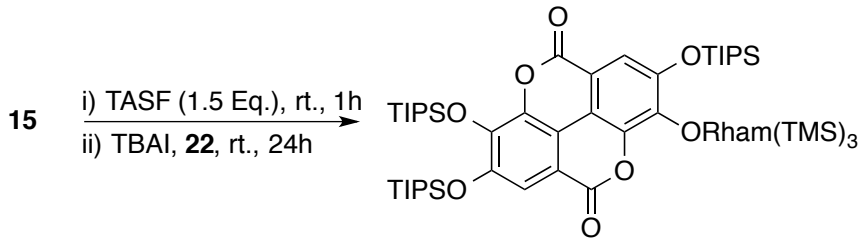


25 **26** **27**

Thus, the gaseous TMSF by-product was continuously removed by purging the reaction flask with dry nitrogen to drive the reaction forward. TLC analysis shows that purging with nitrogen promotes the conversion of **15** to **27**. Additional optimization experiments found that 1.5 Eq. TASF allows for most tetrakis-TIPS EA to be converted to tris-TIPS EA with minimal over-deprotection to bis-TIPS EA.

Scheme 9 depicts the conditions used to glycosylate **15** with **22** when using TASF instead of TBAF.

Scheme 9. Preparation of tris-TIPS, (2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl) ellagic acid



28

Initial studies reveal that **28** is produced in small yields (<5%) when using conditions in Scheme 9. Although **15** is not decomposing when using TASF, the silylated iodorhamnose (**22**) seems to be decomposing before a large quantity can react with the glycosyl acceptor. Indeed, the literature routinely uses three-fold excess of per-*O*-TMS glycosyl iodide donors to compensate for inevitable decomposition via elimination reactions.[24]

Future Directions

Since per-*O*-TMS-rhamnopyranosyl iodide (**22**) is decomposing too quickly, per-*O*-benzyl rhamnopyranosyl iodide will be used instead to glycosylate tetrakis-TIPS EA since it is more stable. If per-*O*-benzyl rhamnopyranosyl iodide fails to work, per-*O*-acetyl rhamnopyranosyl iodide (**21**) will be used again since it is more stable than per-*O*-benzyl rhamnopyranosyl iodide.

If **21** in combination with TASF fails to work, then other glycosyl donors may have to be utilized (e.g. trichloroacetimidates or thioglycosides). If these donors fail to work as well, then the project will have to move towards only glycosylating and testing analogs of EA (Figure 3).

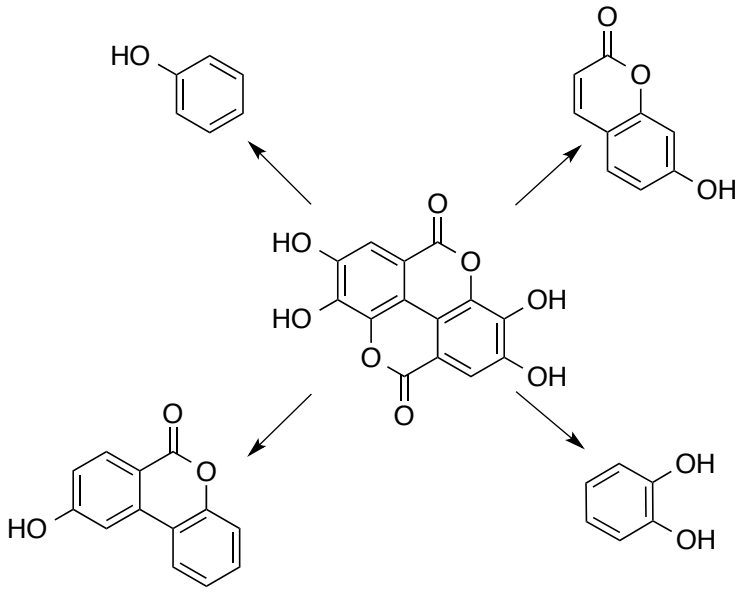


Figure 3. Analogs of ellagic acid

Many of the analogs are soluble and not highly base-sensitive. If the analogs show activity, then it can help decipher which regions in the EAG structure show anti-biofilm activity. The downside with only testing the analogs of EAG is that it cannot help decipher if the EAG are the active constituents of 220D-F2.

Experimental

General. Ellagic acid (97% purity), TIPSCl, and TMSCl were purchased from Acros Organics. Nitrogen was purchased from Nexair. 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl fluoride was purchased from Peptides International. L-rhamnose, Cs_2CO_3, boron trifluoride diethyl etherate, DMAP, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide, trimethylsilyl iodide (TMSI), TBAI, TBAF, TASF, Ac_2O, 4A molecular sieves, imidazole, MEMCl, triethylamine, and all solvents (pyridine, DCM, $CHCl_3$, toluene, and DMF) were obtained from Sigma. All solvents were dried over 4A molecular sieves for approximately three days prior to use. Proton NMR data was obtained using an INOVA 400 instrument. TLC data was obtained using silica and alumina plates purchased from Merck and Fluka Analytical, respectively. TLC data was read using both a 254 nm UVP UVGL-15 Compact UV Lamp and potassium permanganate ($KMnO_4$) stain. All reactions were carried out under nitrogen using oven dried glassware and standard syringe, cannula, and septa techniques.

1,2,3,4-tetra-*O*-acetyl-α-L-rhamnopyranose (12)

Following a previously reported method[16] (with slight modifications), a solution of L-rhamnose (0.50 g, 3.05 mmol) and DMAP (37.21 mg, 0.30 mmol) in dry pyridine (4 mL) was stirred for approximately 30 min. The stirring solution was cooled to 0°C using an ice/water bath and then acetic anhydride (3.45 mL, 36.55 mmol) was added drop-wise to the mixture. After 1h, the solvent was removed *in vacuo* and the remaining residue was diluted with toluene. The toluene solution was washed twice with 1 M HCl, twice with brine, dried over $MgSO_4$, filtered, and evaporated *in vacuo* to afford 0.81 g **12** as a colorless viscous oil (80%).

Spectral data matches reported data[16]

Tetrakis-TIPS ellagic acid (15)

Following a previously reported method[12a] (with slight modifications), TIPSCl (4.25 mL, 19.85 mmol) was added drop-wise to a stirring solution of ellagic acid (1.00 g, 3.31 mmol), imidazole (1.35 g, 19.85 mmol), and DMAP (12.00 mg, 0.10 mmol) in dry DMF (1.5 mL) and dry DCM (10 mL). The mixture was stirred at 50°C. After 24h, the reaction was quenched with saturated aqueous NH_4Cl. The mixture was extracted three times with $CHCl_3$. The combined organic layer was washed once with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting residue was washed with hot isopropanol and the precipitate was dissolved in dry DCM, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford 2.19 g **15** as a colorless powder (71%). The powder was stored under nitrogen until further use.

Spectral data matches reported data[12a]

Bis-MEM, bis-TIPS ellagic acid (16)

Following a previously reported method[12a] (with slight modifications), TBAF (0.42 mL, 0.42 mmol) was added drop-wise to a stirring solution of **15** (0.20 g, 0.22 mmol) and 4A molecular sieves in dry DCM (8 mL). After stirring for 15 min. at room temp., Cs_2CO_3 (0.28 g, 0.86 mmol) and MEMCl (0.1 mL, 0.86 mmol) were added to the mixture. After stirring for 4h at -50°C, the pH was adjusted to 5-6 by adding saturated aqueous $NaHCO_3$. The mixture was filtered and then the combined filtrate was washed three times with $CHCl_3$. The combined organic phase was washed with brine, dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The product was separated using 10:1 Hexanes:EtOAc to afford 9 mg **16** as a slightly yellow solid (5%).

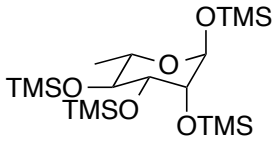
Spectral data matches reported data[12a]

2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl iodide (21)

Following a previously reported method[21], a solution of **12** (0.45 g, 1.36 mmol) in dry DCM (9 mL) was cooled to 0°C using a water/ice bath. TMSI (0.20 mL, 1.49 mmol) was added drop-wise to the stirring solution. After stirring for 2 hours at 0°C, the solvent was removed *in vacuo* and the residue was azeotroped with dry toluene several times to remove the trimethylsilyl acetate by-product to afford 0.49 g **21** as a colorless oil (90%). The oil was stored under nitrogen and in a dark environment until further use.

Spectral data matches reported data[24]

1,2,3,4-tetra-*O*-trimethylsilyl-α-L-rhamnopyranose (**29**)



29

Following a previously reported method[24] (with slight modifications), L-rhamnose (0.20 g, 1.22 mmol), triethylamine (0.90 mL, 6.33 mmol), and 4A molecular sieves were stirred in dry DMF (7 mL) for approximately 30 min. The solution was cooled to 0°C using a water/ice bath and TMSCl (0.80 mL, 6.33 mmol) was added drop-wise to the mixture. A precipitate (triethylammonium chloride) formed upon addition of TMSCl. The temperature was allowed to increase to room temperature. After 4h, the reaction mixture was diluted with pentane and crushed ice. The aqueous phase was extracted three times with pentane. The combined organic phase was washed with distilled water and brine, dried over $MgSO_4$, filtered, and evaporated *in vacuo* to afford 0.41 g **29** as a colorless oil (75%). The oil was stored under nitrogen and in a dark environment until further use.

Spectral data matches reported data[24]

2,3,4-tri-*O*-trimethylsilyl-α-L-rhamnopyranosyl iodide (22)

Following a previously reported method (with slight modifications)[24], a solution containing **29** (0.41 g, 0.91 mmol) and 4A molecular sieves in dry DCM (9 mL) was cooled to 0°C using a water/ice bath. To the stirring solution was added TMSI (0.14 mL, 1.00 mmol) drop-wise. The mixture turned light brown upon addition of TMSI. After 15 min, the solvent was evaporated *in vacuo*. Dry toluene was added to the residue under dry nitrogen and evaporated (to remove di-TMS ether) *in vacuo*. This was repeated several times. The resulting oil was stored under dry nitrogen and in a dark environment until further use – this product has to be used immediately.

Spectral data could not be obtained for **22** as it is too unstable. TLC (10:1 Hexanes:EtOAc) shows disappearance of **29** (R_f = 0.67) and formation of a spot at the baseline. This indicates that the reaction proceeded.

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