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A Synthesis of Exiguaquinol Dessulfate

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Abstract

A concise and stereoselective synthesis of exiguaquinol dessulfate is described. Sequential application of a Diels–Alder cycloaddition, a desymmetrizing aldol addition, and a reductive Heck cyclization established most of the architecture of exiguaquinol, and a carefully choreographed introduction of the polar substituents afforded the title compound; unfortunately, naphthoquinol sulfation could not be achieved to deliver exiguaquinol. Our hypothesis regarding the configurational preference of the *N*-acyl hemiaminal, which was based upon an analysis of internal hydrogen-bonding interactions with polar functional groups, was proven correct. Unfortunately, the title compound did not demonstrate any bactericidal activity against *H. pylori* cultures.

A desymmetrization strategy led to the synthesis of a congener of exiguaquinol, an inhibitor of the *H. pylori* MurI enzyme and a potential antibiotic. The pentacyclic architecture was accessed via convergent Diels–Alder and aldol additions, followed by a reductive Heck cyclization to forge the vicinal quaternary stereogenic centers.

Graphical abstract

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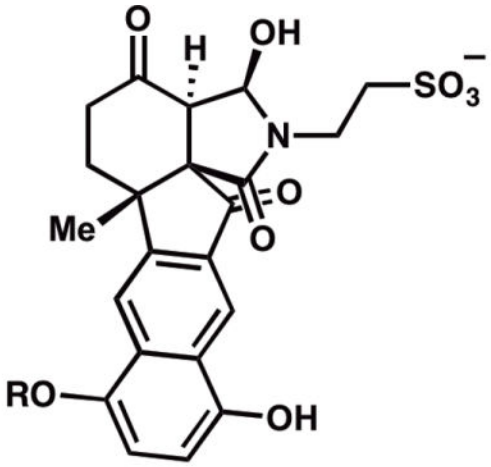

R = SO_3^-: *exiguaquinol*
R = H : *exiguaquinol dessulfate*

Keywords

antibiotic; natural product synthesis; epimers; Heck cyclization; sulfation

Over 50% of the world's population is infected with the *Helicobacter pylori* bacterium, a gram-negative pathogen that is known to cause peptic ulcers or gastritis and has been linked to an increased risk of gastric cancer.[1] Although *H. pylori* eradication is possible with "triple therapy" treatments using broad-spectrum antibiotics and proton-pump inhibitors, unwanted digestive side effects often occur from disruption of the gut microbiome.[2,3] Therefore, antibiotics with new mechanisms of action must be developed to overcome the limitations of current treatments.

In 2007, Lundqvist and coworkers at AstraZeneca described a potential pathway for the selective eradication of *H. pylori* by targeting its MurI enzyme, a glutamate racemase responsible for the interconversion of L- to D-glutamate that is essential for bacterial survival.[4] Inhibition of *H. pylori* MurI stops bacterial growth, causes drastic changes in cell morphology, and ultimately leads to cell death.[5] Based on prominent structural differences among MurI isoforms, Lundqvist and coworkers developed a class of heterocyclic MurI inhibitors selective for the *H. pylori* isoform.[4] Further studies performed by Basarab, de Jonge, and others at the same company led to the development of more potent *H. pylori* MurI inhibitors; unfortunately, pharmacokinetic issues and poor *in vivo* efficacy ultimately resulted in the abandonment of this program.[3–6]

In 2008, exiguaquinol (**1**, Figure 1), a novel pentacyclic natural product was reported by Quinn *et al.* as the first natural product inhibitor of *H. pylori* MurI.[7] While exiguaquinol exhibited only modest activity in the D-serine-*O*-sulfate assay (IC_{50} = 4.4 μM),[7,8] we considered that a modular synthesis might allow for the development of more potent unnatural analogues of **1**. This idea was supported by the docking studies of the Quinn group, which showed that **1** likely occupied the same allosteric binding site in MurI as the

AstraZeneca inhibitors, while failing to provide an appropriate group to fill a key hydrophobic pocket. Additionally, we were interested in whether or not the natural product scaffold would show efficacy against *H. pylori*. Surprisingly, the Quinn group never assayed exiguaquinol for bactericidal activity, so it's real promise as an antibiotic remained unknown. This group did, however, postulate that exiguaquinol might have its biosynthetic origins in the halenaquinol family of natural products (see **3** and **4**).[7]

In 2013, we disclosed a synthesis of a tetracyclic model system resembling exiguaquinol, but lacking much of the polar functionality, which had been designed to evaluate our C–C bond forming strategy.[9] Although the tetracycle obtained (**5**) was epimeric to exiguaquinol (**1**) at the C2 hemiaminal position, computational analysis of the ground state energies for each epimer of **1** suggested that this discrepancy is unlikely to persist once the alkyl sulfonate is installed (Figure 2).[9] Therefore, we aimed to investigate this conformational phenomenon in a synthesis of the fully functionalized natural product (**1**). Herein, we report an efficient and modular synthesis of exiguaquinol dessulfate and the evaluation of its antibacterial activity.

Our symmetry-breaking strategy, shown from a retrosynthetic perspective, began by simplification of **1** to a more manageable precursor lacking the most polar groups (**7**, Scheme 1). The cyclopentanone bearing the vicinal quaternary stereogenic centers would arise from a reductive Heck cyclization of **8**, which we planned to access using a convergent desymmetrizing aldol reaction between achiral bicyclic imide **9** and naphthaldehyde **10**. Bicycle **9** would arise from a sequence of Diels–Alder cycloaddition and imide *N*-alkylation steps starting from simple precursors.

The bicyclic imide (**16**) was synthesized by a Diels–Alder reaction between bis(phenylsulfide) diene **11** (itself made in two steps from 1,5-hexadien-3,4-diol[9]) and maleimide (**12**) (Scheme 2a). Alkylation of the succinimide nitrogen of **13** with alkyl bromide **14** and subsequent PtO_2-catalyzed alkene hydrogenation under an elevated pressure of hydrogen gas delivered imide **16**, the pronucleophile needed for convergent aldol addition.

Although strategies to access naphthaldehydes closely related to **10** have been reported, they typically require many steps and most involve the intermediacy of 1,3,6-trimethoxybenzocyclobutene or close relatives,[10] compounds we found difficult to prepare even using literature protocols.[11] Instead an alternative approach that avoided benzocyclobutene intermediates was investigated (Scheme 2b). Starting with 3,4-dibromothiophene (**17**), oxidation to the thiophene-*S*,*S*-dioxide followed by a cascade of [4+2] cycloaddition with excess benzoquinone/cheletropic extrusion of SO_2/oxidation afforded naphthoquinone **18** in a moderate yield.[12,13] Reduction using sodium dithionite and dimethylation of the resulting naphthoquinol yielded **19** with high efficiency.[14] Lastly, we were pleased to find that reductive monolithiation/formylation proceeds without aryne formation as long as temperatures are maintained below –90 °C;[15] this procedure produces naphthaldehyde **20** in nearly quantitative yield.

Access to **16** and **20** allowed us to evaluate their union in an aldol addition (Scheme 3). Standard conditions employing LDA or LiHMDS primarily led to recovery of unreacted starting material, and we attributed this poor reactivity to the electron-rich aldehyde and a sterically demanding transition structure for C–C bond formation. Fortunately, the addition of $BF_3{\cdot}OEt_2$ to the reaction mixture allowed the aldol reaction to proceed, affording **21** as a single diastereomer in high yield.[16] This reaction is rather unusual in its combined use of both a strong Lewis acid and a lithium enolate to promote an aldol addition. The aldol adduct was then protected, the sulfides were each oxidized to the corresponding sulfoxides, and subsequent thermal elimination afforded diene **22**. While we were able to obtain up to 56% yield in the thermal elimination, prolonged heating resulted in the appearance of a side product arising from skeletal rearrangement of the 1,5-diene product (see Supporting Information).[17] Imide reduction of **22** using $LiBH_4$ produced a single diastereomer of hemiaminal **23**, bearing the opposite C2 configuration as that of the natural product. Previously obtained ground state calculations of the two hemiaminal epimers suggested a thermodynamic preference for the natural configuration with the sulfonate present;[9] therefore, we aimed to epimerize this stereogenic center at a later stage in our synthesis.

The successful 5-*exo* reductive Heck cyclization was performed with $Pd(P\textit{t}\text{-}Bu_3)_2$ and sodium formate to assemble the fused pentacyclic skeleton of exiguaquinol (**24**). Interestingly, a boost in efficiency occurred with the addition of $[P\textit{t}\text{-}Bu_3]HBF_4$, despite reports that implicate the monoligated Pd-species as the active catalyst in related reactions.[18,19] Bis(silyl ether) **24** was deprotected with CsF and selectively oxidized to ketone **25** with DDQ. The remaining exocyclic olefin underwent dihydroxylation/diol cleavage to provide cyclohexanone **26** in good yield, thereby establishing the full pentacyclic carbon skeleton of the target.

We initially intended to install the alkyl sulfonate through displacement of a primary alkyl leaving group with sodium sulfite (Scheme 4). Treatment of **26** with PPh_3, DDQ, and Bu_4NBr resulted in clean conversion to bromide **27**;[20] however, all attempts to displace the leaving group with nucleophilic sulfite resulted in decomposition of the hemiaminal. Instead, a Mitsunobu reaction was employed to introduce a thioester in excellent yield (**29**), and subsequent oxidation with *m*CPBA delivered sulfonate **28**.[21]

On the basis of ground state calculations of the exiguaquinol epimers (Figure 2), we hypothesized that the natural configuration of the hemiaminal should be attainable at this stage. However, treatment of **28** with Cs_2CO_3 resulted in the incomplete inversion to the natural epimer along with significant amounts of the hemiaminal dehydration product (a vinylogous imide, not shown).[22] When *t*-BuOK was employed, we noticed the rapid formation of aldehyde intermediate **30**, which cyclized exclusively to **31** upon exposure to silica gel, without any deleterious dehydration. The high selectivity for the (*R*)-configured hemiaminal can be attributed to the anomeric stabilization in this orientation, an effect that is absent in the unnatural (*S*)-configured epimer (**29**).[9]

The final steps in our synthesis of exiguaquinol dessulfate relied on the demethylation of dimethoxynaphthalene precursor **31**. This task was accomplished using ceric ammonium nitrate to first access the reactive naphthoquinone (“exiguaquinone”), which was reduced on

workup with sodium dithionite to afford exiguaquinol dessulfate (**2**). Although **2** could be isolated, it was unstable and rapidly decomposed upon exposure to air—an observation consistent with Kitagawa's and Harada's findings in their seminal studies on halenaquinol.[23,24] Nevertheless, **2** was treated with a variety of sulfating reagents, including SO_3·pyridine, SO_3·DMF, DCC/H_2SO_4,[25] and $Cl_3C(CH_2)_2OSO_2Cl$;[26] unfortunately, all conditions led to decomposition of **2** and exiguaquinol (**1**) was not observed. Further efforts, including enzymatic sulfation attempts using the arylsulfotransferase from *Haliangium ochraceum*,[27] were equally unsuccessful.

We were motivated by several factors to examine the MurI inhibitory and bactericidal activity of exiguaquinol analogue **31**. We noted that in the halenaquinone/halenaquinol/halenaquinol sulfate series, the only compound with reported antibiotic activity was halenaquinone;[23,28] the fact that exiguaquinol dessulfate spontaneously oxidizes in air (to a mixture that includes "exiguaquinone") suggested that the monosulfate might simply be a protecting group of sorts, possibly in both natural product series, for either the hydroquinone or quinone forms. If that were true, sulfatase activity might be expected to unveil the more reactive forms from the corresponding monosulfate. Both of these forms, in the exiguaquinol series, were relatively unstable, so stable bis(methyl ether) **31** arose as a logical candidate with which to assess biological activity of the scaffold. The fact that the docking study of Quinn did not show an obvious role for the sulfate in binding to MurI was also a motivating factor. Furthermore, MurI inhibition had never been correlated with actual bactericidal activity of exiguaquinol, but we thought we might be able to do so with close analogue **31**. We therefore attempted to reproduce the MurI inhibition assay with the kind provision of the requisite plasmid and a published heterocyclic inhibitor from AstraZeneca; however, we were unable to recapitulate the previously published results.[4] Therefore, we simply evaluated the antimicrobial activity of **31** toward *H. pylori*; unfortunately, we did not observe any bactericidal activity whatsoever. Of course, these results are overall inconclusive with respect to the potential activity of **1**, **2**, or "exiguaquinone".

Our synthesis of (±)-exiguaquinol dessulfate, which takes advantage of non-obvious symmetry elements, was completed in 19 steps (longest linear sequence) from commercially available starting materials. Initial experiments employing chiral bases in the desymmetrizing aldol addition showed promising enantioselectivity (37% ee, see Supporting Information) in the formation of **21**, demonstrating proof-of-principle for an enantioselective synthesis of **1** in the event that the sulfation problem can be solved.[29] In the course of this endeavour, we demonstrated that our computation-based hypothesis about the configurational preferences of the C2 hemiaminal were correct. Finally, given the presumed biosynthetic relationship with the halenaquinol family of natural products, we extend the possibility that the compound that we have made, exiguaquinol dessulfate, could be an as-yet-uncovered natural product.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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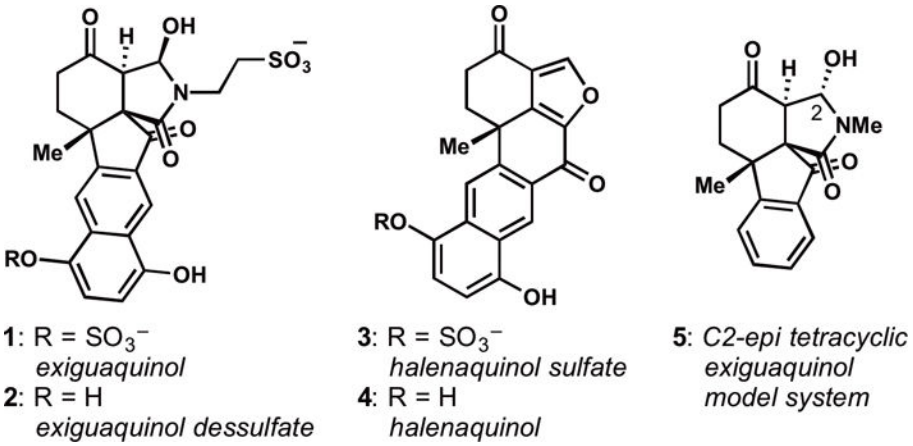


Figure 1.

Structures of exiguaquinol and its unsulfated congener, the previously synthesized tetracyclic “core” of exiguaquinol, and the biogenetically related natural products halenaquinol and halenaquinol sulfate.

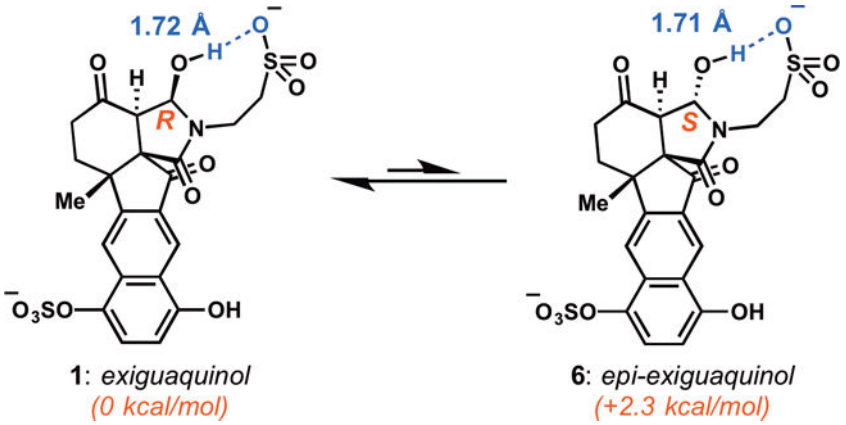
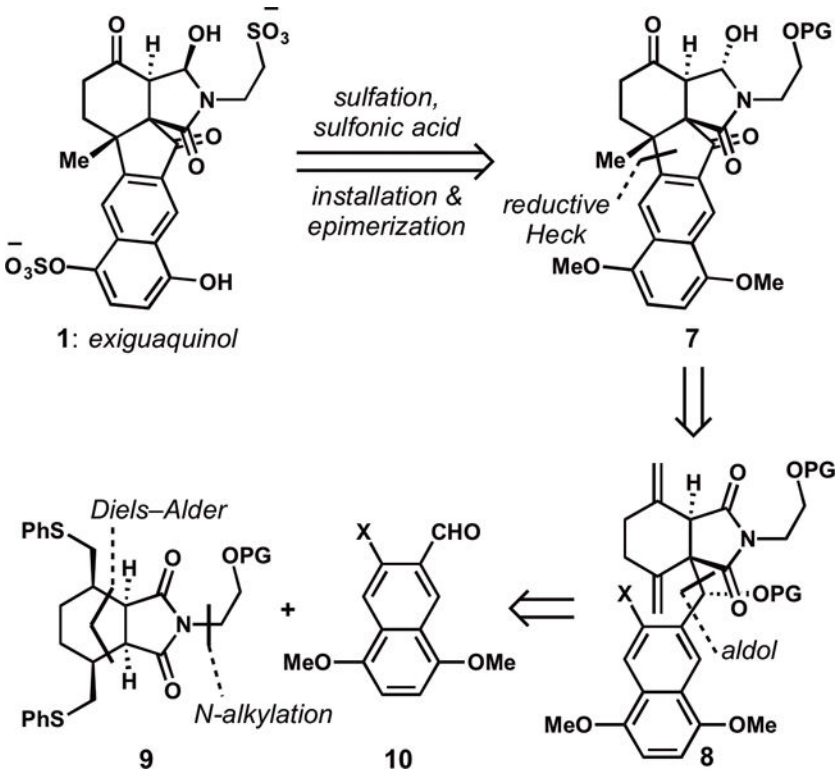
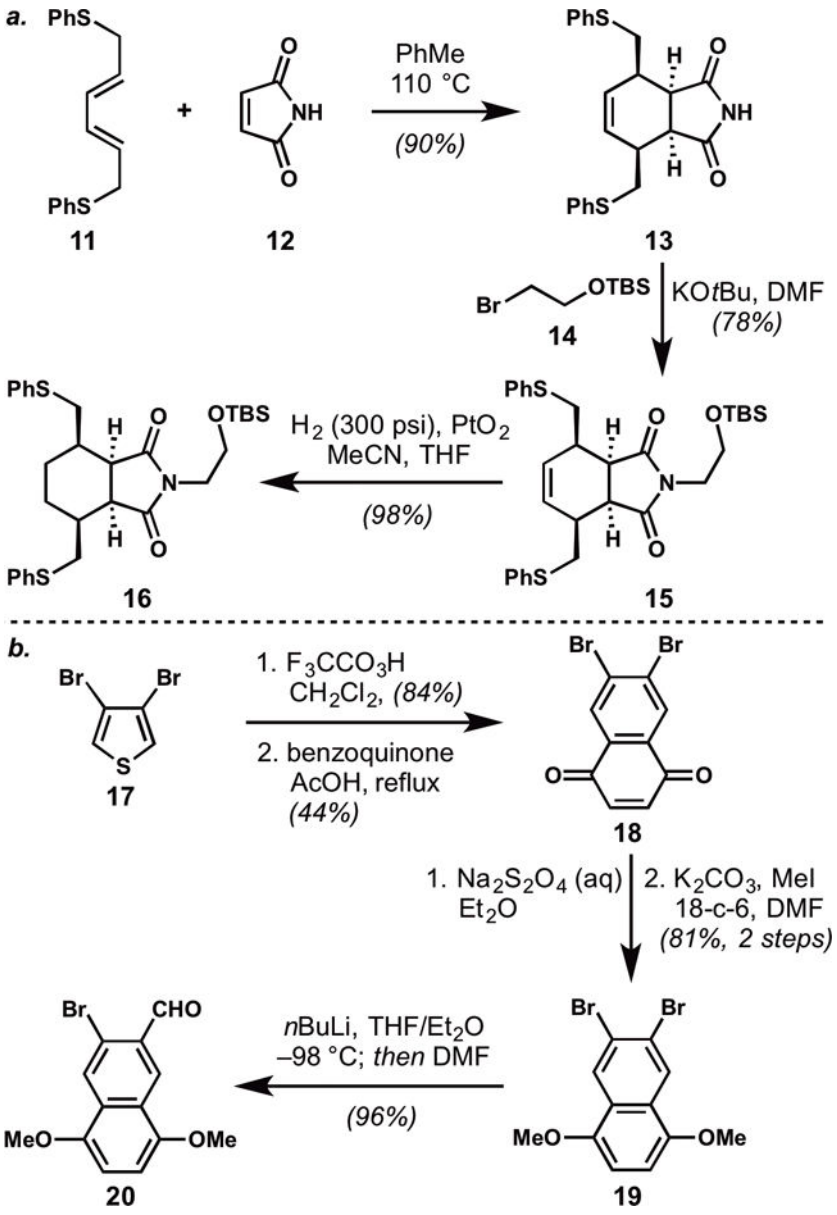


Figure 2.
Computed ground state energies for exiguaquinol and its C2 epimer.



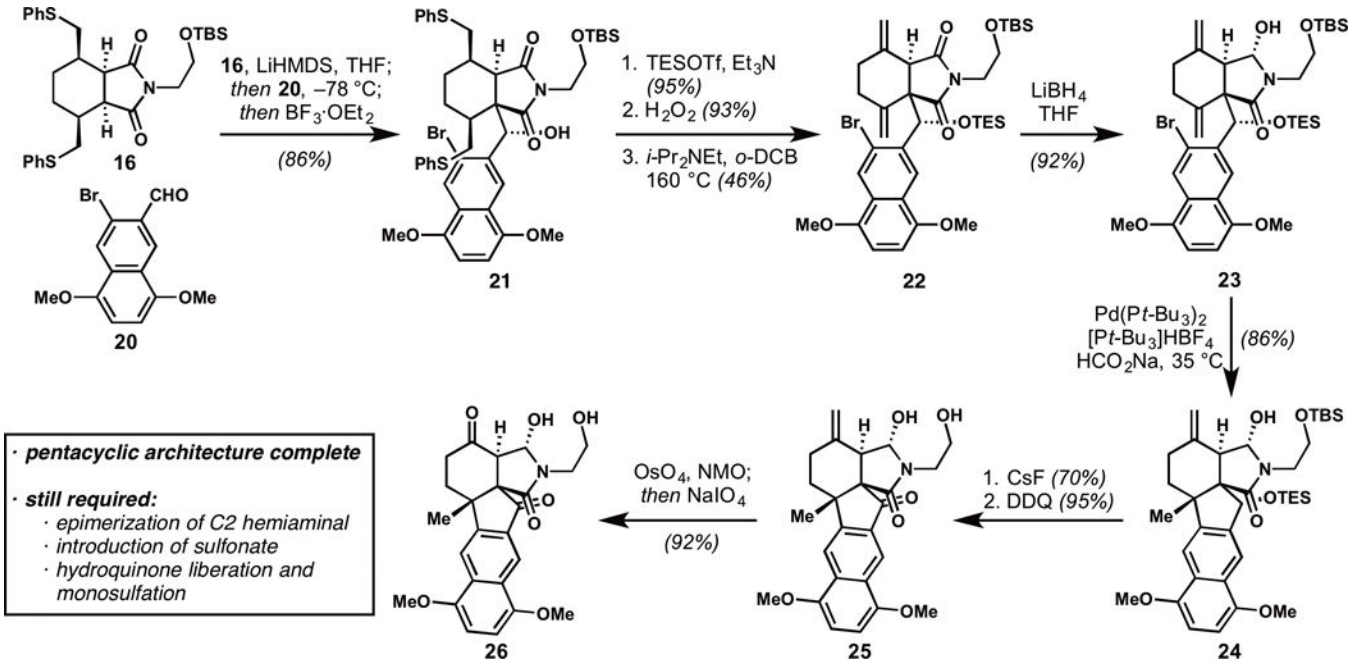
Scheme 1.

Key strategic elements of our approach to exiguaquinol presented as a retrosynthetic analysis



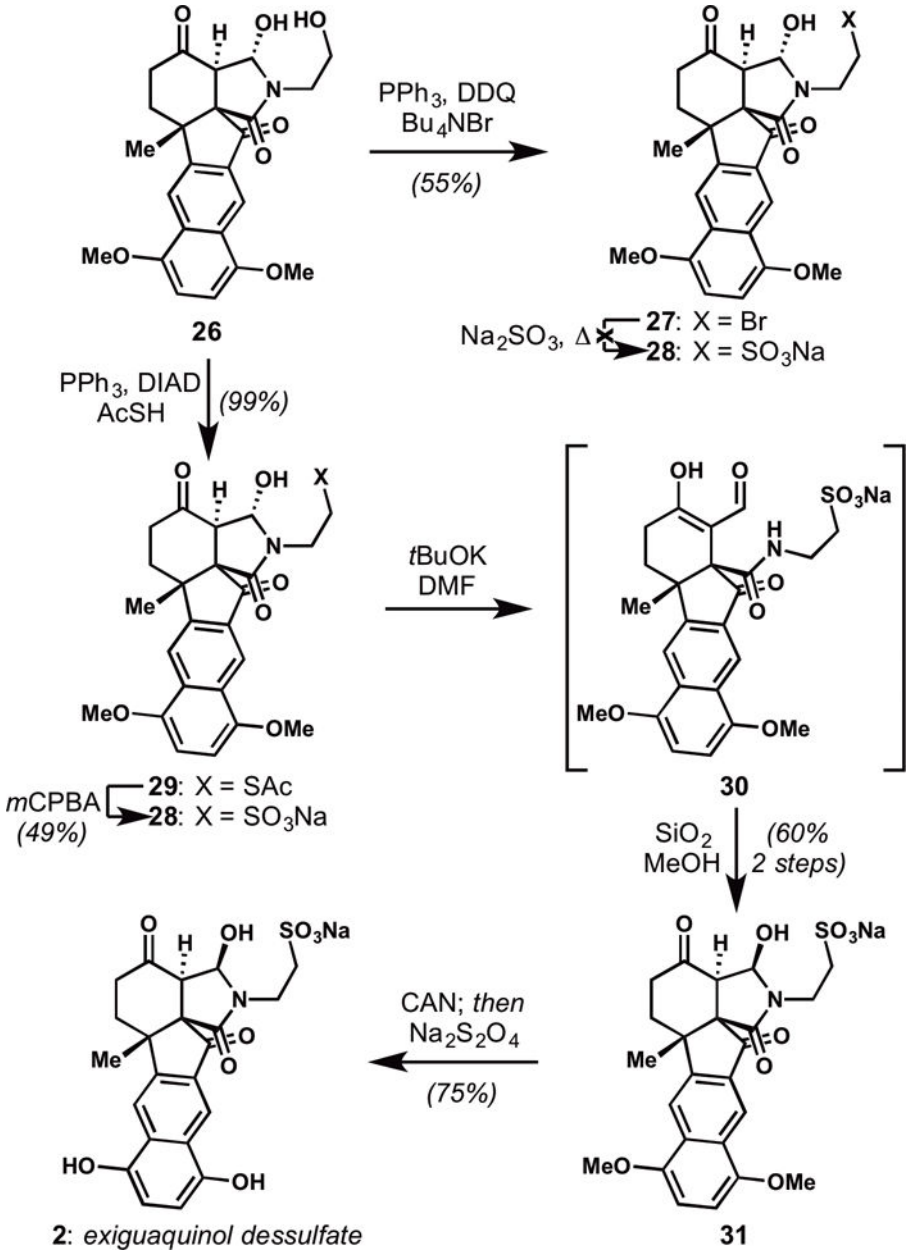
Scheme 2.

Synthesis of substrates for convergent aldol addition reaction



Scheme 3.

Completion of the pentacyclic architecture of exiguaquinol by convergent aldol addition and reductive Heck cyclization.



Scheme 4.

Completion of the synthesis of the exiguaquinol dessulfate.