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Phloroglucinol sulfonic acid esters as antimalarial/anticancer agents

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The synthesis and biological testing of some phloroglucinol-derived trisulfonates and sulfonate methyl ethers has established that the tritosylate **12** is a good *in vitro* antimalarial agent and that the dinosylate methyl ether **19** is a superior agent for inhibiting human skin cancer cell lines.

Keywords: sulfonate ethers; anticancer agents; antimalarial agents

1. Introduction

The demand for antimalarials is driven by the fact that malaria is endemic in 117 countries and that 2.5 billion people are at risk, which represents 40% of the world's population (*1*). There are an estimated 300–500 million cases per year, which result in 1–2 million deaths, the majority of which are children less than 5 years of age in rural areas of sub-Saharan Africa (*2*). In this region, 10% of hospitalizations and 20–60% of physician visits are due to malaria. It is estimated that malaria has direct costs of $1.8 billion annually and results in $12 billion of lost GDP. These figures, particularly the deaths, are likely to rise steeply in the next 50 years as *Plasmodium falciparum*, the most deadly species of malaria parasite, becomes the dominant species causing malaria (*3*). While malaria is viewed as a developing-world problem it will impact other regions as travel is expected to grow dramatically in the next decade such that 50 million travelers will visit malaria-endemic areas yearly from regions where the disease is not present (*4*). Typically, these travelers have no natural immunity or experience of malaria and are thus at a high risk of contracting the disease. The widespread use of antimalarials has prompted fears that highly drug-resistant malaria will emerge and therefore there is a desperate need for novel antimalarials.

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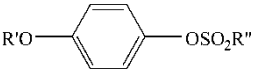
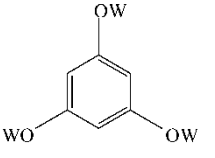
We have previously reported (*5, 6*) that hydroquinone-derived sulfonic acid esters **1** are selectively toxic to *P. falciparum* (malaria) in cell cultures.



1

Toxicity comparisons juxtaposed *Plasmodium* and Chinese hamster ovary (CHO) cells, which were included to measure the general toxicity of each compound. We have also examined (*5–8*) the effects of these compounds on C32 amelanotic melanoma cells, cancerous (MCF-7) and non-cancerous (MCF-10A) human breast epithelial cells (*8*) to determine if compounds with anti-*Plasmodium* properties are active because they target rapidly proliferating cell lines. In this report, the preparation and biological testing of a new series of phloroglucinol-derived sulfonic acid esters **2** is described.

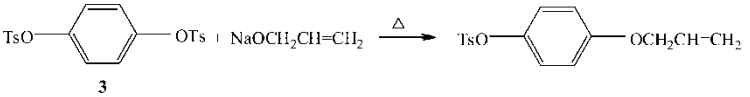


2 (W = R or RSO_2)

2. Results and discussion

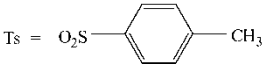
2.1. *Organic synthesis*

From the strategic standpoint, the construction of derivatives of polyphenolic structures in which there are different substituents present in the same derivative would be facilitated by using precursors in which a single phenolic hydroxyl group was available for reaction. However, our earlier work (*5, 6*) on compound **1** established that hydroquinone-derived ether sulfonates were more efficiently obtained by means of a counter-attack strategy applied to disulfonates (see Scheme 1 for an example).



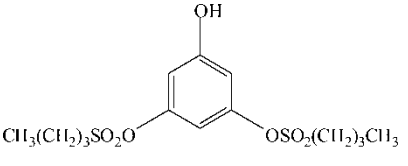
3

4 (38%)



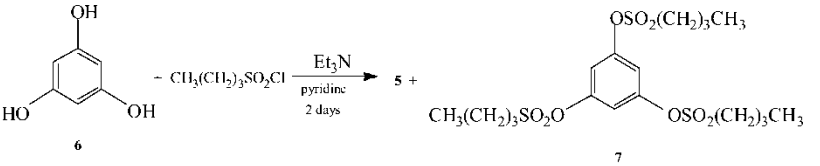
Scheme 1.

Synthetic work on phloroglucinol derivatives began with a study of conditions intended to provide the dibutylsulfonate phenol **5**.



5

Our results are summarized in Scheme 2.

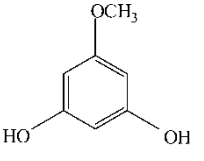


Ratio			% Yield	
6		Sulfonyl Chloride	Dibutyl Sulfonate	Tributyl Sulfonate
1	:	3.5	7	20
1	:	2.0	6	7
1	:	1.8	6	8

Scheme 2.

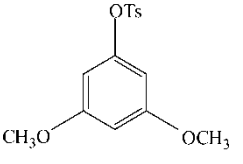
It appears that the reaction times we have found (in previous cases) to be helpful for better overall yields, lead to equilibration in reactions in which incomplete derivatization is desired. We were unable to get useful amounts of disulfonate phenol **5** in this manner. A reaction on **6** and butanesulfonyl chloride conducted with sodium hydroxide/acetone fared no better. Furthermore, an attempt to methylate **5** with methyl iodide gave no methyl ether at all. Hence, preparative work on phloroglucinol derivatives, like the hydroquinone derivatives before them, turned to polysulfonates and the counter-attack strategy.

Given the superior efficacy/selectivity of the tosylate methyl ether derived from hydroquinone in our initial report (*5*) of biological testing on malaria (*Plasmodium*), CHO and skin cancer (C32) cells, we have elected to prepare and test, *inter alia*, all of the tosyl- and methyl-substituted phloroglucinols **2**. Additionally, symmetrical trisulfonates were prepared in simple one-pot reactions on phloroglucinol **6**, however because the phloroglucinol monomethyl ether **8** was commercially available, symmetrical disulfonate methyl ethers were also readily obtained.



8

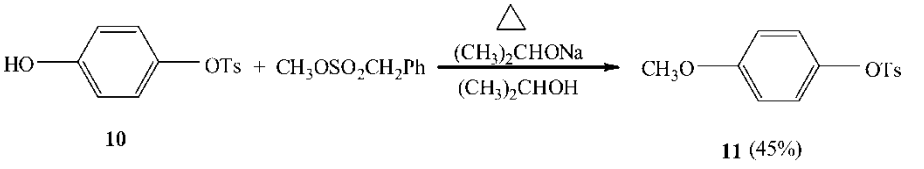
Finally, the trimethyl ether of phloroglucinol was purchased from a commercial source (Aldrich); however, the tosylate dimethyl ether **9** required synthesis.



9

Because counter-attack reactions on hydroquinone disulfonates gave significant amounts of sulfonate phenols along with sulfonate methyl ethers (*5*), exploratory work on the application

of a counter-attack strategy to phloroglucinol polysulfonates was preceded by an examination of methyl sulfonates as methylating agents for phenols. Scheme 3 presents our results using methyl benzylsulfonate as a methylating agent.

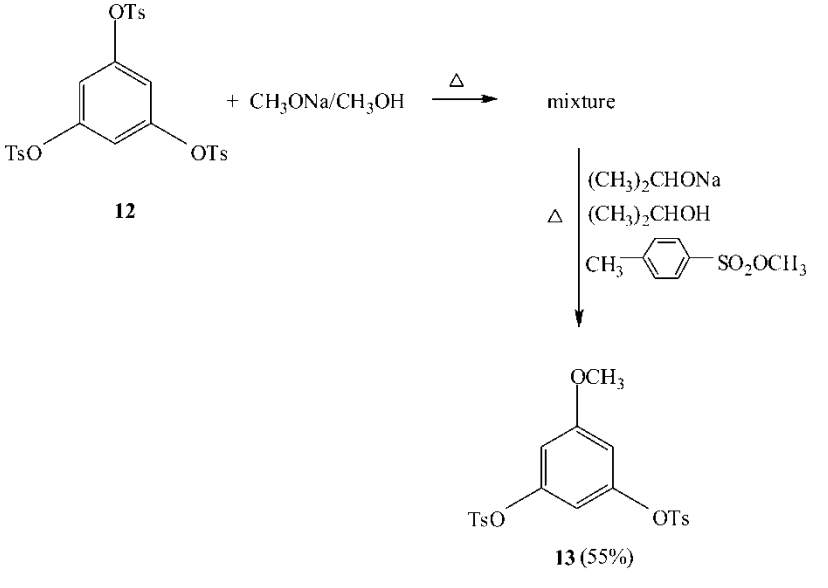


10 **11** (45%)

Scheme 3.

Very similar results were obtained when the *m*-tosyloxyphenol was methylated with methyl *p*-toluenesulfonate (*8*).

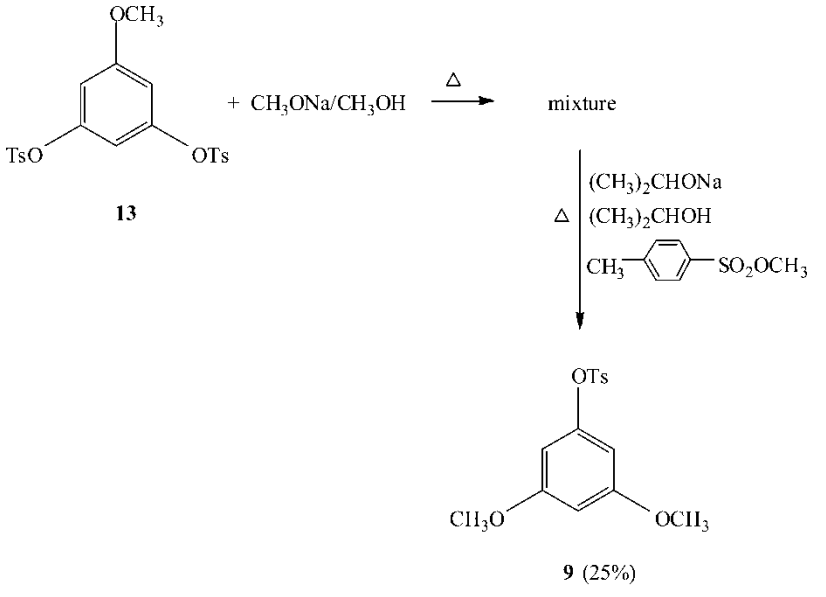
Exploration of a counter-attack strategy for the introduction of methoxy groups onto the phloroglucinol skeleton began with reactions on the tritosylate **12**. The synthesis employed reaction with methoxide ions to produce a mixture of **9** and the corresponding ditosylate phenol followed by reaction on the crude mixture, from that step, using isopropoxide/methyl *p*-toluenesulfonate as shown in Scheme 4.



12 **13** (55%)

Scheme 4.

Although the yield of **13** (Scheme 4) is much lower than the 70% obtained from reaction of **8** with *p*-toluenesulfonyl chloride, the overall result in Scheme 4 suggested that this approach would be useful for the preparation of **9**. In the event, counter-attack synthesis of **9** proceeded as outlined in Scheme 5.

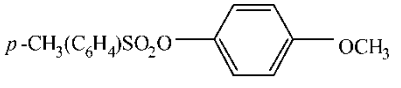
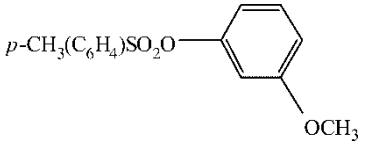
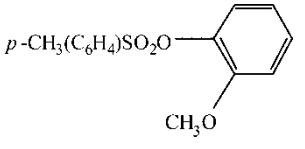


Scheme 5.

2.2. *Biological testing*

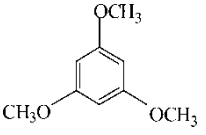
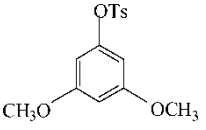
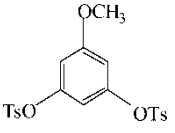
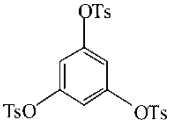
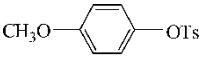
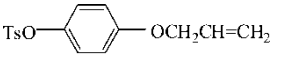
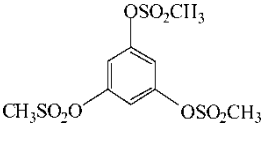
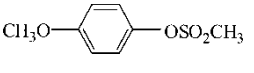
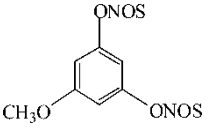
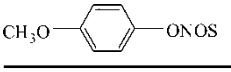
We had previously determined that selected sulfonic acid esters showed marked selectivity for disrupting the cellular processes of malaria parasites (*5, 6*). In particular, *p*-methoxyphenyl *p*-toluenesulfonate, compound **11** (see ref. (*5*) and Table 1), was shown to be selectively toxic to *Plasmodium* and melanoma (C32) cultures.

Table 1. *o*, *m*, *p*-Hydroquinone tosylate methyl ethers as antimalarial/anticancer agents. IC_{50} represents the concentration of each compound that resulted in 50% inhibition of *Plasmodium* (malaria ItG), human skin cancer (C32) cells and representative (normal) mammalian (CHO) cells. Values are representative of multiple determinations performed over several days.

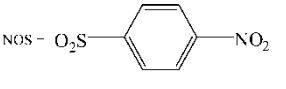
Compound tested[a]		IC_{50} malaria ItG	IC_{50} C32 cells	IC_{50} CHO cells
	**11**[b]	68.4 ± 7.2 μM	54.0 ± 3.6 μM	>1 mM
	**14**	71.9 ± 21.6 μM	39.6 ± 7.2 μM	39.7 ± 7.1 μM
	**15**	151 ± 29 μM	54.0 ± 10.8 μM	46.8 ± 14.4 μM

[a]The compounds were dissolved in dimethyl sulfoxide (DMSO) for testing. Assays containing DMSO alone were performed in parallel and when the toxic concentrations for the tested compounds in DMSO were equal to or greater than those observed for the equivalent amount of DMSO alone, the results are stated as >1 mM.
[b]Results for **11** were previously reported (*5*).

Table 2. Phloroglucinol derivatives as antimalarial/anticancer agents. IC_{50} represents the concentration of each compound that resulted in 50% inhibition of *Plasmodium* (malaria ItG), human skin cancer (C32) cells and representative (normal) mammalian (CHO) cells. Values are representative of multiple determinations performed over several days.

Compound tested[a]		IC_{50} malaria ItG	IC_{50} C32 cells	IC_{50} CHO cells
	**16**	>3 mM	547 ± 53 μM	595 ± 77 μM
	**9**	45.5 ± 3.3 μM	48.7 ± 26.0 μM	289 ± 81 μM
	**13**	69.2 ± 8.9 μM	>1 mM	>1 mM
	**12**	34.0 ± 6.8 μM	224 ± 15	>1 mM
	**11**[b]	68.4 ± 7.2 μM	53.4 ± 3.6 μM	>1 mM
	**4**[b]	9.2 ± 2.0 μM	24.0 ± 10.9 μM	>1 mM
	**17**	>1 mM	369 ± 39 μM	572 ± 50 μM
	**18**[b]	470 ± 25 μM	257 ± 40 μM	>1 mM
	**19**[c]	47.1 ± 5.9 μM	2.8 ± 0.2 μM	790 ± 55 μM
	**20**[b]	6.8 ± 2.3 μM	374 ± 105	>1 mM

[a]Compounds were dissolved in dimethyl sulfoxide (DMSO) for testing. Assays containing DMSO alone were performed in parallel and when the toxic concentrations for the tested compounds in DMSO were equal to or greater than those observed for the equivalent amount of DMSO alone, the results are stated as >1 mM.
[b]Results for these compounds were previously reported (*5, 6*).
[c]Nosylate (NOS) is:



Biological testing for the present report began with the ortho/para regioisomers of **11** (see **14** and **15** in Table 1). Interestingly, **14** and **15** failed to display the selective toxicity associated with **11** but instead were equally toxic in all cell systems assayed (see Table 1). The selective toxicity of **11** alone suggested that the compounds were toxic because they interacted with a specific target, as opposed to modifying a general environment, and that a methoxy group in the para position prevented this interaction in CHO cells. We would also expect the three compounds to cross membranes to an equal degree, and therefore we do not expect access to specific biological compartments to be an explanation for the differential toxicities we observed. To exclude the possibility that the results of the biological assays were due to breakdown products of compounds **11**, **14** or **15** we determined the effect of phloroglucinol **6** and, separately, sodium *p*-toluenesulfonate in *Plasmodium*, CHO and C32 cultures. Neither compound was active to a significant degree (*e.g.* they have IC_{50} values >1 mM) suggesting that characteristics of the intact sulfonate ethers were required for activity, although we cannot exclude the possibility that active metabolites are generated internally in some cell types.

In the hope that aryl sulfonates/ethers would prove to be an even more fruitful source of antimalarial/anticancer agents, we initiated testing on phloroglucinol derivatives which offer a larger manifold of compounds than is available from hydroquinone systems. In view of our earlier, promising results on **11** (see Table 2), the first series of phloroglucinol derivatives, **16**, **9**, **12**, **13** (see Table 2), includes all possible combinations of tosylate and methoxy groups.

What is immediately striking about the Table 2 results of these compounds is that those derivatives, **16** and **9**, in which methoxy groups outnumber tosylate groups, have unacceptable toxicity to normal (CHO) cells. The tritosylate **12** showed good activity against malaria, although it is not as efficacious as the hydroquinone derivative **4** (see Table 2).

Earlier experience (*5, 6*) with hydroquinone derivatives showed that methanesulfonates were much less selective than tosylates (cf. **11** and **18**, Table 2) and our current results (cf. **12** and **17**, Table 2) remain consistent with such a view.

As part of our earlier screening (*6*) of ether sulfonates, we examined the hydroquinone-derived nitrobenzenesulfonate ether **20** (see Table 2) which showed excellent antimalarial behavior but only good anticancer activity. The corresponding phloroglucinol-derived dinosylate methyl ether **19** (see Table 2) showed reciprocal biological activities, *i.e.* is they are good against malaria but excellent against human skin cancer.

When the compounds were assayed in *Plasmodium* cultures, phloroglucinol derivatives **16** and **17**, which lack tosylate groups, were both found to be inactive. This may indicate that the tosylate groups are important in the compound's concentration or compartmentalization within the parasite, in the same way that weak bases concentrate in the food vacuole. The presence of tosyl groups and their relative positions are clearly important in all cell types assayed, which is consistent with our previous conclusion that the toxicity we observe is related to the structure, and not merely the composition of the compounds. Whether this reflects the presence of specific enzymatic processes that release metabolites is difficult to discern as we would expect *Plasmodium* cultures to be the most biochemically divergent, which is consistent with the results obtained from **13**, **16** and **20.**

3. Conclusions

The synthesis of aryl sulfonate ethers is facilitated by the application of a counter-attack strategy. When the resulting compounds were tested in three cell cultures a significant number of them were selectively toxic to a single cell line, suggesting that these compounds interact with living cells in a specific manner as opposed to being toxic because they are highly reactive or merely disrupt general biochemical processes. Given the interesting results on the nosylates **19** and

20, it would appear that new *p*-nitrobenzenesulfonate ethers merit synthesis and biological testing.

4. Experimental

Infrared (IR) spectra were recorded on a Thermo Nicolet 2000 spectrometer. ^{1}H (270 MHz) and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on a JEOL JNM-GSX 270 Fourier-transform NMR system. Mass spectra were obtained on a Hewlett-Packard 5988A gas–liquid chromatography mass spectrometer (GLC/MS) system. Melting point analyses were determined on a Gallenkamp MFB-595 capillary melting point apparatus and are uncorrected.

4.1. *Parasite strains and culture*

Plasmodium falciparum cultures were grown in O blood obtained by venipuncture of volunteers. Cultures of the laboratory line ltG were maintained by the method of Trager and Jensen (*9*) using RPMI 1640 supplemented with 10% human serum (obtained from the Princess Margaret Hospital, Toronto) and 50 μM hypoxanthine (RPMI-A). *P. falciparum* susceptibility testing was performed using a lactate dehydrogenase (LDH) enzyme assay specific to the LDH enzyme found in *Plasmodium* (pLDH) (*10, 11*). Briefly, compounds to be tested were dissolved in dimethyl sulfoxide (DMSO) and were then serially diluted in 50 μL of RPMI-A in duplicate in a 96-well plate to produce a compound gradient with 2-fold dilutions. An equal volume of parasite culture (50 μL of 2% hematocrit, ∼2% parasitemia) was added to each well and the plates were then incubated at 37 °C in an atmosphere of 95% N_2, 3% CO_2, 2% O_2 for 72 h. The contents of the wells were then resuspended and a 10 μL sample was removed and added to 100 μL of pLDH enzyme assay mixture (*11*). After 1 h, the absorbance of the wells at 650 nm was determined using a microplate reader (Biorad, Mississauga, Ontario). The IC_{50} values of individual compounds were determined using a nonlinear regression analysis of the dose–response curve using the computer program SigmaPlot (Jandel Scientific).

4.2. *Mammalian strains and culture*

C32 almelanotic melanoma and CHO cells (ATCC, Manassas, VA) were grown in RPMI-1640 supplemented with 10% fetal calf serum (Sigma, St. Louis, MO), 25 mM HEPES and gentimicin. The cells were grown to 50% confluency in 96-well plates prior to the addition of either DMSO alone, or individual compounds in DMSO. After 24 h, the viability of the cells was determined by adding 10 mg/mL of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT: Sigma) in 100 μL of culture media, incubating the plates for a further hour, and then removing the media and adding 100 μL of DMSO and reading the absorbance at 650 nm (*12*). The IC_{50} values of individual compounds were determined using a nonlinear regression analysis of the dose–response curve using the computer program SigmaPlot (Jandel Scientific) and represent the mean of four independent determinations.

4.3. *Organic syntheses*

4.3.1. *Reactions of phloroglucinol with* 1*-butanesulfonyl chloride*

All of the reactions summarized in Scheme 2 were carried out in the manner described next for the 3.5:1 run.

Phloroglucinol **6** (0.50 g, 3.96 mmol) was dissolved in dry pyridine (50 mL) and triethylamine (1.4 g, 13.8 mmol) was added. The reaction mixture was cooled with an ice/water bath. 1-Butanesulfonyl chloride (2.16 g, 13.8 mmol) was added dropwise over 5 min. The reaction mixture turned orange-brown. The reaction was stirred at ambient temperature for 48 h.

Chloroform (100 mL) was added and the resultant mixture washed with 2.5%V/V hydrochloric acid (100 mL aliquots) until the aqueous layer remained acidic. The organic layer was dried ($MgSO_4$), filtered and the solvent evaporated affording crude product (0.8 g). The crude product was chromatographed on silica gel (80 g) employing chlroform (80-mL fractions) for elution. Fractions 5–8 were combined and concentrated giving the tributyl sulfonate **7** (0.38 g, 0.78 mmol, 20%). The tributyl sulfonate **7** had ^{1}H NMR (270 MHz) δ 0.96 (*t*, 9H), 1.51 (sex, 6H), 1.93 (quin, 6H), 3.27 (*t*, 6H), 7.20 (*s*, 3H). Fractions 23–32 were combined and concentrated furnishing the dibutyl sulfonate **5** (0.10 g, 0.28 mmol, 7%). The dibutyl sulfonate **5** had IR 3442, 1365, 1170 cm^{-1} and ^{1}H NMR (270 MHz) δ 0.96 (*t*, 6H), 1.51 (sex, 4H), 1.93 (quin, 4H), 3.26 (*t*, 4H), 6.20 (broad *s*, 1H), 6.72 (*m*, 3H).

4.3.2. *Sulfonate ether* **11**

Sodium metal (30 mg, 1.30 mmol) was dissolved in refluxing 2-propanol (5 mL) and the solution cooled to ambient temperature. *p*-Hydroxyphenyl *p*-toluenesulfonate **10** (258 mg, 0.97 mmol) and methyl benzylsulfonate (174 mg, 0.93 mmol) were added. The reaction mixture turned yellow before it was refluxed for 2 h. Water (50 mL) and hydrochloric acid (10%, 10 mL) were added and the resultant mixture extracted with chloroform (three 50-mL aliquots). The combined organic layers were dried ($MgSO_4$), filtered and concentrated affording crude **11** (231 mg). The product was recrystallized affording clean sulfonate ether **11** (121 mg, 0.43 mmol, 45%). The product was identical to previously described material (*5*).

4.3.3. *Phloroglucinol tritosylate* **12**

Phloroglucinol **6** (1.0 g, 7.94 mmol) and triethylamine (2.45 g, 23.8 mmol) were added to dry pyridine (50 mL). The reaction mixture was cooled with an ice/water bath. Sublimed *p*-tolenesulfonyl chloride (4.52 g, 23.8 mmol) was added in small portions over 10 min. The reaction mixture was stirred at ambient temperature for 7 days.

Chloroform (250 mL) was added and the resultant mixture washed with hydrochloric acid (5%, 5–100-mL portions). The organic layer was dried ($MgSO_4$), filtered and the solvent evaporated. Crude product was chromatographed on silica gel (250 g) employing 1:1 chloroform/light petroleum (100 mL fractions) for elution Fractions 47–55 were combined and concentrated yielding tritosylate **12** which was recrystallized from methanol (50 mL). Recrystallized **12** (1.29 g, 2.20 mmol, 28%) had mp 83.1–83.5°C (found: C 54.8, H 4.2%. $C_{27}H_{24}O_9S_3$ requires C 55.1, H 4.1%). IR 1367, 1180 cm^{-1}. ^{1}H NMR (270 MHz) δ 2.45 (*s*, 9H), 6.59 (*s*, 3H), 7.34 (*d*, 6H), 7.60 (*d*, 6H). ^{13}C NMR δ 21.8, 116.0, 128.4, 130.1, 131.5, 146.3, 149.8. MS(CI): 589 ($M^+ + 1$, 10%), 155 (12%), 125 (100%).

4.3.4. *Ditosylate methyl ether* **13** *from tritosylate* **12**

Part A. Sodium metal (0.13 g) was dissolved in methanol (43.3 mL) and the solution stirred at ambient temperature for 1.5 h. A portion of this solution (40 mL), methanol (60 mL) and phloroglucinol tritosylate **12** (2.01 g, 3.40 mmol) were mixed and the reaction refluxed for 5 days. Water (150 mL) and 5% hydrochloric acid (20 mL) were added and the resultant mixture extracted with chloroform (4–100-mL aliquots). The combined organic layers were dried ($MgSO_4$), filtered and

the solvent evaporated. ^{1}H NMR established that the crude was a mixture of the ditosylate methyl ether **13** and the corresponding ditosylate phenol.

Part B. Sodium metal (0.125 g) was dissolved in hot 2-propanol (35 mL) and the solution stirred for 1.5 h at ambient temperature. A portion of the solution (28 mL), the crude product from *Part A* and methyl *p*-toluenesulfonate (0.81 g, 4.32 mmol) were admixed and the resultant solution refluxed for 2 h. Water (200 mL) and 10% hydrochloric acid (10 mL) were added and the resultant mixture washed with chloroform (4–100-mL portions). The combined organic layers were dried ($MgSO_4$), filtered and the solvent evaporated affording impure **13**. Crude ditosylate methyl ether **13** was chromatographed on silica gel (140 g) employing 3:1 chloroform/light petroleum (100-mL fractions) for elution. Fractions 13–24 were combined and concentrated affording ditosylate methyl ether **13** (1.01 g). The chromatographed material was recrystallized from methanol (6 mL) affording clean **13** (0.84 g, 1.86 mmol, 55%) which had mp 69–71 °C (found: C 56.4, H 4.6%. $C_{21}H_{20}O_7S_2$ requires C 56.2, H 4.5%). IR 1375, 1178 cm^{-1}. ^{1}H NMR (270 MHz) δ 2.44 (*s*, 6H), 3.63 (*s*, 3H), 6.19 (*t*, 1H), 6.44 (*d*, 2H), 7.31 (*d*, 4H), 7.64 (*d*, 4H). ^{13}C NMR δ 17.8, 51.9, 103.5, 105.1, 124.6, 126.0, 128.1, 141.9, 146.4, 156.7. MS(CI): 449 ($M^{+\cdot} + 1$, 61%), 229 (61%), 155 (100%).

4.3.5. *Ditosylate methyl ether* **13** *from 5-methoxyresorcinol* **8**

5-Methoxyresorcinol **8** (0.25 g, 1.80 mmol) was added to a solution of dry triethylamine (0.37 g, 3.66 mmol) in dry pyridine. The reaction mixture was cooled with an ice/water bath and sublimed *p*-toluenesulfonyl chloride (0.69 g, 3.65 mmol) was added in small portions over 5 min. The reaction mixture was stirred at ambient temperature for 7 days. Chloroform (150 mL) was added and the resultant mixture extracted with 5% V/V hydrochloric acid (three 75-mL aliqots). The organic layer was dried ($MgSO_4$), filtered and the solvent evaporated affording crude ditosylate methyl ether (0.72 g).

The crude product was chromatographed on silica gel (70 g) employing chloroform for elution (70 mL fractions). Fractions 3–5 were combined and concentrated yielding a colorless oil (0.54 g, 1.20 mmol, 70%) which crystallized on standing for several weeks. Chromatographed ditosylate methyl ether was recrystallized from methanol (4 mL) affording clean **13** (0.36 g).

4.3.6. *Tosylate dimethyl ether* **9**

Part A. Sodium metal (40 mg, 1.70 mmol) was dissolved in methanol (20 mL), the ditosylate methyl ether **13** (0.52 g, 1.16 mmol) added and the reaction mixture refluxed for 4 days. Water (100 mL) and 5% hydrochloric acid (15 mL) were added and the resultant mixture extracted with chloroform (three 100-mL aliquots). The combined organic layers were dried ($MgSO_4$), filtered and the solvent evaporated affording crude material (0.36 g). The crude product was chromatographed on silica gel (40 g) employing 3:1 chloroform/light petroleum (40-mL fractions) for elution. Fractions 7–10 were combined and concentrated furnishing the target **9** (82 mg). Fractions 18–25 were combined and concentrated yielding the tosylate methyl ether phenol (0.17 g) which had ^{1}H NMR (270 MHz) δ 2.42 (*s*, 3H), 3.64 (*s*, 3H), 5.80 (*s*, 1H), 6.07 (*t*, 1H), 6.14 (*t*, 1H), 6.27 (*t*,1H), 7.30 (*d*, 2H), 7.73 (*d*, 2H). ^{13}C NMR δ 21.7, 55.5, 100.6, 100.8, 102.5, 128.5, 129.8, 132.3, 145.6, 150.9, 157.2, 161.1.

Part B. Sodium metal (13 mg, 0.56 mmol) was dissolved in 2-propanol (2.5 mL). The tosylate methyl ether phenol (0.17 g from Part A) and methyl *p*-toluenesulfonate (0.11 g, 0.55 mmol) were added and the reaction mixture refluxed for 2 h. Water (50 mL) and 10% hydrochloric acid

(3 mL) were added and the resultant mixture extracted with chloroform (3–50-mL aliquots). The combined organic layers were dried ($MgSO_4$), filtered, and the solvent evaporated affording crude product (0.18 g). The crude product was chromatographed on silica gel (18 g) employing 3:1 chloroform/light petroleum (18-mL fractions). Fractions 4–9 were combined and concentrated furnishing the target dimethyl tosylate **9** and a small amount of methyl *p*-tolenesulfonate (0.13 g).

Chromatographed **9** from Parts A and B (0.21 g) was recrystallized from methanol affording clean tosylate dimethyl ether **9** (91 mg, 0.29 mmol, 25%) which had mp 63.0–63.7°C (found: C 58.2, H 5.3%. $C_{15}H_{16}O_5S$ requires C 58.3, H 5.2%). IR 1378, 1175 cm^{-1}. ^1H NMR (270 MHz) δ 2.43 (*s*, 3H), 3.67 (*s*, 6H), 6.12 (*d*, 2H), 6.30 (*t*, 1H), 7.30 (*d*, 2H), 7.72 (*d*, 2H). ^{13}C NMR δ 21.7, 55.5, 99.4, 100.7, 128.6, 129.7, 132.6, 145.3, 151.1, 161.0. MS: 308 ($M^{+\cdot}$, 52%), 244 (100%), 155 (37%), 91 (94%).

4.3.7. *Trimesylate* **17**

Phloroglucinol **6** (1.10 g, 6.81 mmol) and triethylamine (2.1 g, 20.4 mmol) were added to dry pyridine (55 mL). The reaction mixture was cooled with an ice/water bath and methanesulfonyl chloride (2.33 g, 20.4 mmol) was added dropwise. The reaction mixture was stirred at ambient temperature for 5 days. Methylene chloride (200 mL) was added and the resultant mixture washed with 5% hydrochloric acid (5–100-mL aliquots). The organic layer was dried ($MgSO_4$), filtered and the solvent evaporated. The product was recrystallized from methanol (110 mL) yielding trimesylate **17** (0.90 g, 2.5 mmol, 37%) which had mp 150.0–152.0°C (found: C 30.2, H 3.3%. $C_9H_{12}O_9S_3$ requires C 30.0, H 3.4%). IR 1369, 1180 cm^{-1}. ^1H NMR (270 MHz, CD_3COCD_3) δ 3.41 (*s*, 9H), 7.44 (*s*, 3H). ^{13}C NMR δ (CD_3COCD_3) 31.0, 117.1, 151.5.

4.3.8. *Dinosylate methyl ether* **19**

5-Methoxyresorcinol **8** (1.6 g, 11.3 mmol) was dissolved in a solution of dry triethylamine (0.7 g, 6.95 mmol) in dry pyridine (50 mL) and the reaction mixture cooled with an ice/water bath. *p*-Nitrobenzenesulfonyl chloride (1.6 g, 7.14 mmol) was added in small portions over 20 min. The reddish-purple reaction mixture was stirred at ambient temperature for 2 weeks. Chloroform (200 mL) was added and the resultant mixture extracted with 5% hydrochloric acid (100-mL aliquots) until the aqueous pH remained acidic. The organic layer was dried ($MgSO_4$), filtered and the solvent evaporated. The residue was dissolved in hot methanol (450 mL) and crystals grown at room temperature. Filtration provided clean dinitrobenzenesulfonate methyl ether **19** (1.1 g). A second crop (0.12 g) was obtained after methanol (225 mL) was distilled off. Total recrystallized **19** (1.26 g, 2.47 mmol, 22%) had mp 139.8–140.5°C (found: C 44.5, H 2.9%. $C_{19}H_{14}N_2O_{11}S_2$ requires C 44.7, H 2.8%). IR 1530, 1386, 1348, 1191 cm^{-1}. ^1H NMR (270 MHz) δ 3.71 (*s*, 3H), 6.27 (*t*, 1H), 6.52 (*d*, 2H), 8.03 (*d*, 4H), 8.41 (*d*, 4H). ^{13}C NMR δ 56.1, 107.8, 108.6, 124.6, 129.9, 140.5, 149.5, 151.3, 161.2.

4.3.9. *Tosylate methyl ethers* **14**, **15**

Tosylate methyl ethers **14** and **15** were prepared as described previously (*8*).

4.3.10. *Trimethyl ether* **16**

Trimethyl ether **16** was purchased from Aldrich Chemicals.

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