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# Steroidal and Nonsteroidal Sulfamates as Potent Inhibitors of Steroid Sulfatase

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Synthetic routes to potent steroidal and nonsteroidal sulfamate-based active site-directed inhibitors of the enzyme steroid sulfatase, a topical target in the treatment of postmenopausal women with hormone-dependent breast cancer, are described. Novel compounds were examined for estrone sulfatase (E1-STS) inhibition in intact MCF-7 breast cancer cells and placental microsomes. Reaction of the sodium salt of estrone with sulfamoyl chloride gave estrone 3-*O*-sulfamate (EMATE, **2**) which inhibits E1-STS activity potently (>99% at 0.1 $\mu$M in intact MCF-7 cells, $IC_{50}$ = 65 pM) in a time- and concentration-dependent manner, suggesting that EMATE is an active site-directed inhibitor. EMATE is also active in vivo orally. 5,6,7,8-Tetrahydronaphthalene 2-*O*-sulfamate (**7**) and its N-methylated derivatives (**8** and **9**) were synthesized, and **7** inhibits the E1-STS activity in intact MCF-7 cells by 79% at 10 $\mu$M. 4-Methylcoumarin 7-*O*-sulfamate (COUMATE) and its derivatives (**14**, **16**, and **18**) were prepared to extend this series of nonsteroidal inhibitors, and COUMATE reduces the E1-STS activity in placental microsomes by >90% at 10 $\mu$M. Although the orally active COUMATE is less potent than EMATE as an active site-directed inhibitor, it has the important advantage of being nonestrogenic. Analogues (**20**, **22**, **24**, **26**, **27**, **31**, **33**, **39**, and **44**) of COUMATE were synthesized to study its structure−activity relationships, and sulfamates of tetralones (**46** and **48**) and indanones (**49**, **51**, and **53**) were also prepared. While most of these compounds were found to inhibit E1-STS activity less effectively than COUMATE, one analogue, 3,4-dimethylcoumarin 3-*O*-sulfamate (**24**), was found to be some 12-fold more potent than COUMATE as an E1-STS inhibitor in intact MCF-7 cells ($IC_{50}$ = 30 nM for **24**, cf. 380 nM for COUMATE). Hence, highly potent sulfamate-based inhibitors of steroid sulfatase, such as EMATE, COUMATE, and **24**, possess therapeutic potential and will allow the importance of estrogen formation in breast tumors via the E1-STS pathway to be assessed. A pharmacophore for active site-directed sulfatase inhibition is proposed.

## Introduction

Breast cancer causes more death in women than any other neoplasm. This disease is particularly prevalent in Western countries, and a recent survey shows that the U.K. heads the incidence rate within Europe.[1] In women who develop breast cancer, about one-third to one-half will eventually die of it. Although much emphasis recently has been placed on the prevention and an earlier detection of the disease by mammography screening, there is a demand for novel therapeutic interventions in the management of breast cancer.

The close association of estrogens with the promotion of the growth and development of breast cancers has long been recognized.[2−4] In postmenopausal women, in whom breast cancers most frequently occur, approximately one-third of breast tumors are hormone-dependent.[5] There is now substantial evidence[6−9] to imply that in situ formation of estrogen from estrogen precursors is the major contribution to the estrogen content in breast tumors.

In patients with hormone-dependent breast cancer, the inhibition of the aromatization of androstenedione to estrone (E1, Figure 1) in the last step of the biosynthesis of estrogens has been a target for ablating estrogens. Many potent, well-tolerated and highly selective aromatase inhibitors are now available for clinical use. However, the response rates of patients with estrogen receptor positive (ER+) tumors to anastrozole in a recent randomized clinical trial were found to be quite low.[10,11] These findings have thus raised one particular question as to what other mechanisms may be responsible for the lack of improved response rates despite the use of highly potent aromatase inhibitor such as anastrozole.

The most likely explanation for the relative lack of clinical efficacy of aromatase inhibitors is that the estrone sulfatase (E1-STS) pathway (Figure 1) [i.e. the hydrolysis of E1S to estrone], as opposed to the aromatase pathway, is the major route of estrogen formation in breast tumors.[12,13] Evidence to support this hypothesis includes (i) a million-fold higher steroid sulfatase activity than aromatase activity in liver and normal and malignant breast tissues[14] and (ii) the origin

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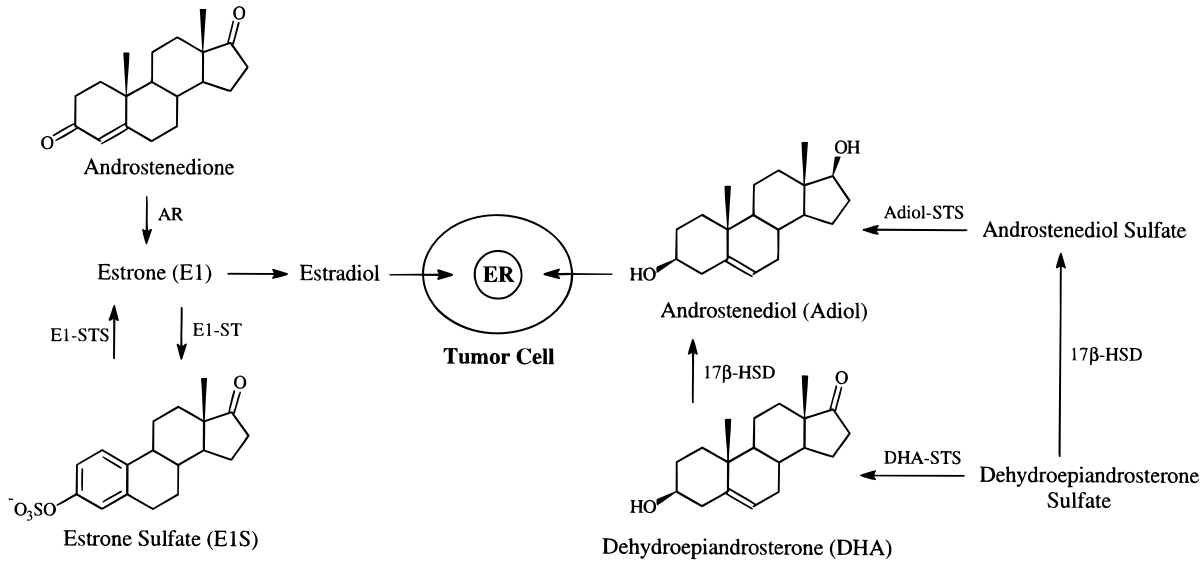


**Figure 1.** The origin of estrogenic steroids in postmenopausal women: AR, aromatase; ST, sulfotransferase; STS, sulfatase; 17$\beta$-HSD, 17$\beta$-hydroxysteroid dehydrogenase; ER, estrogen receptor.

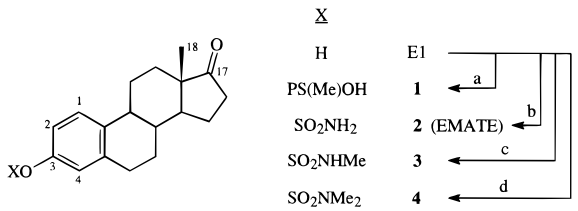


**Figure 2.** Steroidal inhibitors of steroid sulfatase: (a) MePSCl$_2$/pyridine/24 h, HOCH$_2$CH$_2$CN/24 h, NH$_4$OH/65 °C/5 h; (b) NaH/DMF, H$_2$NSO$_2$Cl; (c) NaH/DMF, MeNHSO$_2$Cl; (d) NaH/DMF, Me$_2$NSO$_2$Cl.

of E1 from E1S in breast cancer tissue is about 10 times more than that from androstenedione.[12]

In addition to the E1-STS pathway, there is a growing awareness that another potent estrogen, androstenediol (Adiol, Figure 1), may be of even greater importance in the support of growth and development of hormone-dependent breast tumors.[15−17] Nearly 90% of Adiol originates from dehydroepiandrosterone sulfate (DHA-S) once it has been hydrolyzed to DHA by DHA-sulfatase (DHA-STS)[18] (Figure 1). Although it is still unconfirmed if E1-STS and DHA-STS are isoenzymes or not, transient transfection of a placental steroid sulfatase into COS-1 cells showed that the expressed protein was able to hydrolyze both E1S and DHA-S.[19] Hence, steroid sulfatase inhibitors, when used alone or in conjunction with an aromatase inhibitor, may enhance the response of hormone-dependent tumors, such as of the breast, to this type of endocrine therapy by reducing not only the formation of E1 from E1S but also the synthesis of Adiol from DHA-S.

The emergence of the biological significance of the E1-STS pathway has prompted ourselves and several other groups[20−26] to examine a variety of compounds for the inhibition of E1-STS activity. We found that E1S analogues possessing sulfate surrogates such as 3-*O*-phosphonates and 3-*O*-thiophosphonates [e.g. estrone 3-*O*-methylthiophosphonate (E1-3-MTP, **1**, Figure 2)][27,28] and 3-*O*-sulfonates[29] were able to inhibit the hydrolysis of E1-STS efficiently in intact MCF-7 breast cancer cells and also in placental and breast tumor preparations. These findings subsequently led to our synthesis of a series of sulfamate derivatives of estrone (**2** − **4**, Figure 2), of which estrone 3-*O*-sulfamate (EMATE, **2**) is the most potent active site-directed steroidal inhibitor synthesized to date.[30,31]

Unexpectedly, a recent report has shown that EMATE and its estradiol congener possess potent estrogenic activity.[32] These findings, together with the likelihood of E1 being released during sulfatase inhibition, as suggested by its proposed mechanisms of action (Figure 3),[33,34] have rendered EMATE unsuitable for use in the treatment of hormone-dependent tumors. Recently, EMATE has also been shown to have memory-enhancing effects in rats.[35] In addition, studies in mice have suggested a role of DHA-STS activity in regulation of part of the immune response,[36,37] and such a relationship may also be found in humans. Since it is likely that some of these newly discovered potential uses of EMATE in other therapeutic areas may require drugs to be administered from an early age, it is thus anticipated that a potent orally active irreversible *nonsteroidal* E1-STS inhibitor, which is nonestrogenic and unlikely to be metabolized to compounds with hormonal activity, will have much wider clinical applications than their steroidal counterparts.

Various structurally diversified nonsteroidal E1-STS inhibitors have been reported, such as a sulfate derivative of 2-phenylindoles (e.g. **5**, Figure 4)[38] and (*p*-*O*-sulfamoyl)-*N*-alkanoyltyramines.[39,40] Our first nonsteroidal E1-STS inhibitors were 5,6,7,8-tetrahydronaphthalene 2-*O*-sulfamate (**7**, Figure 4) and its N-alkylated derivatives (**8** and **9**). The best candidate in this series was **7**, but it is a much weaker inhibitor than EMATE.[30] We then reasoned that sulfamates of alternative two-ring systems such as monohydroxylated coumarins were also likely to act as E1-STS inhibitors. After establishing that 7-sulfooxy-4-methylcoumarin (**11**, Figure 5) was a substrate for E1-STS as expected, we synthesized and examined coumarin sulfamates (**12**, **14**, **16**, and **18**), of which 4-methylcoumarin 7-*O*-sulfamate (COUMATE, **12**) was found to be the most effective inhibitor.[41]

To study the structure−activity relationships for coumarin sulfamates, we have prepared and examined

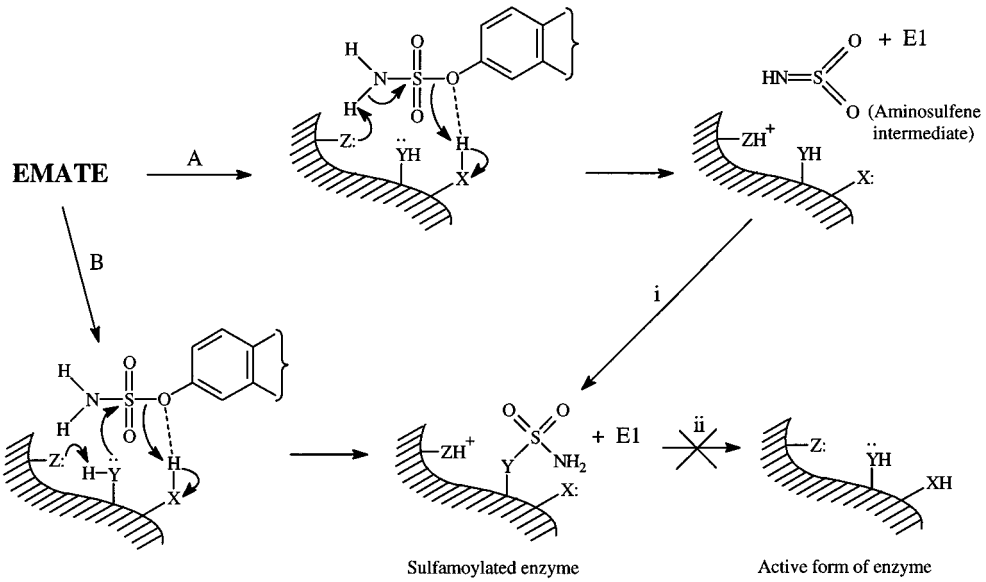


**Figure 3.** Proposed mechanisms of action of EMATE in the inhibition of estrone sulfatase (E1-STS): (A) via an aminosulfene intermediate and (B) via a nucleophilic attack by an amino acid residue in the active site. (i) Attacks by a nucleophilic amino acid residue in the active site—selective or random sulfamoylation. (ii) No hydrolysis of the sulfamoylated E1-STS by water to regenerate the active form of the enzyme. X, Y and Z: amino acid residue. (- - -) Hydrogen bonding.

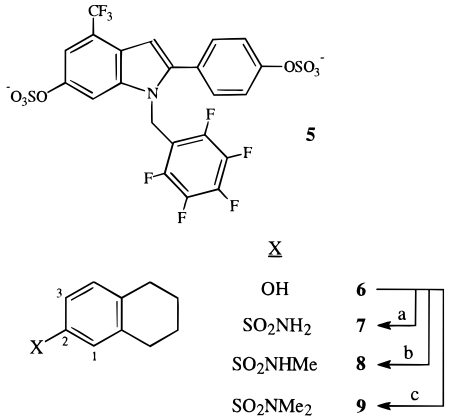


**Figure 4.** Structures of nonsteroidal inhibitors of steroid sulfatase (**5**, **7**−**9**) and 5,6,7,8-tetrahydronaphth-2-ol (**6**): (a) NaH/DMF, $H_2NSO_2Cl$; (b) NaH/DMF, $MeNHSO_2Cl$; (c) NaH/DMF, $Me_2NSO_2Cl$.

several analogues of COUMATE and its congener, **14**, in which the sulfamate group is relocated, the coumarin ring is substituted, and the conjugation of the coumarin ring system is disrupted. The mono- and bis-sulfamates of a catechol-like coumarin are also examined. In addition, we have also explored the potential of other two-ring sulfamates such as those of tetralones and indanones as effective inhibitors of E1-STS.

EMATE, COUMATE, and compounds **3**, **4**, **7**−**9**, **11**, **14**, **16**, and **18** have been reported previously in communication form in this journal.[30,41] We now describe full details of their synthesis and review their biological activities in conjunction with an SAR study on the COUMATE class of compounds and related entities.

## Results and Discussion

The sulfamoyl group has been widely utilized as an activity-modifying substituent in several different classes of drugs. For example, N-substituted steroidal sulfamates have been synthesized,[42−46] including sulfamates of estradiol,[45,47−49] to block metabolic conjugation, but their ability to inhibit E1-STS was not explored. In most of our earlier works (e.g., for EMATE, **2**), the sulfamoylation of phenolic compounds was carried out directly with crude residue/crystalline sulfamoyl chloride, prepared according to the method of Appel and Berger.[50] However, the continual handling of sulfamoyl chloride in the solid state proved to be cumbersome since this reagent is extremely hygroscopic and decomposes readily even on storage at low temperature. Nevertheless, it was found subsequently that these drawbacks could largely be surmounted if the freshly prepared crude sulfamoyl chloride was stored instead as a solution in anhydrous and sulfur impurities-free toluene under nitrogen.[33]

To verify that sulfamoyl chloride is stable when stored as a solution in toluene under the conditions described above, its reactivity was examined by monitoring the yield of 2-nitrophenol *O*-sulfamate (**54**, Figure 9) in the sulfamoylation of 2-nitrophenol. 2-Nitrophenol was initially chosen for this study because of its obvious advantages of being structurally simple and also chromophoric. However, the yield of **54** was consistently poor. This was subsequently attributed to the instability of **54** in the reaction mixture. Nonetheless, some interesting results emerged during the course of this study as outlined below.

When a solution of **54** in ethyl acetate was shaken with 5% sodium bicarbonate solution, a bright orange aqueous portion resulted upon separation of layers, suggesting that the sulfamate group of **54** is cleaved in the presence of the base and sodium 2-nitrophenolate is generated. The p$K_a$s of the *N*-protons in **54** are expected to be lower than those of EMATE by virtue of the powerful electron-withdrawing 2-nitro group, and although the mechanism has not yet been fully established, it is likely that decomposition of **54** involves the removal of the N-proton(s) of the sulfamate group by the base. The resulting anion of **54** then collapses, generating the highly chromophoric 2-nitrophenolate as a consequence.

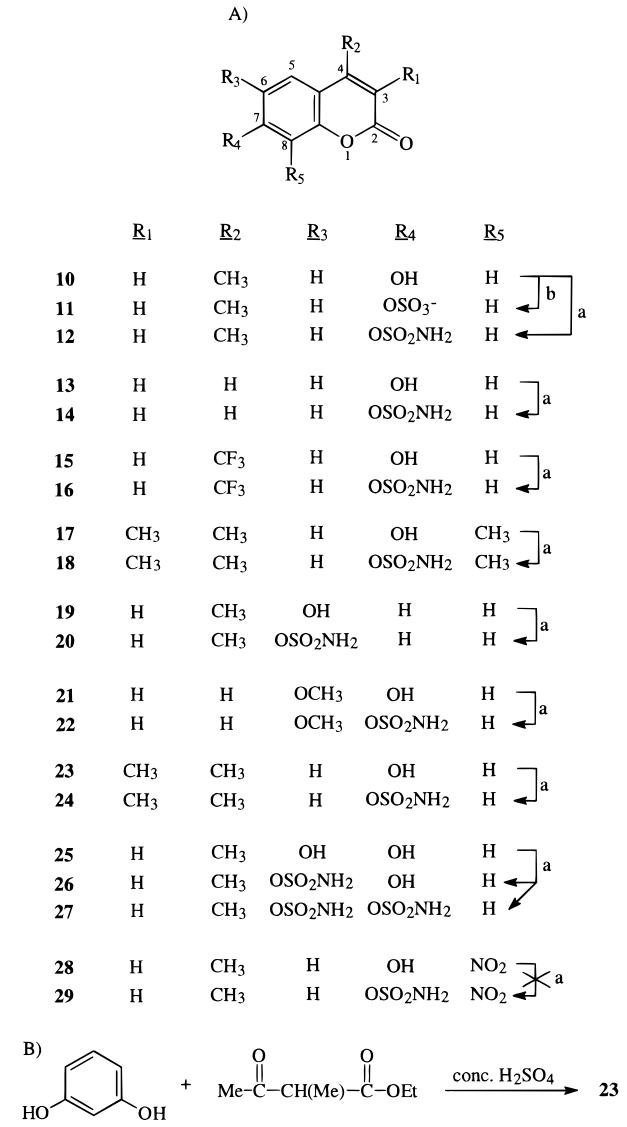


**Figure 5.** (A) Synthesis of coumarin sulfamates [**12** (COUMATE), **14, 16, 18, 20, 22, 24, 26**, and **27**] from their corresponding starting alcohols and 7-sulfooxy-4-methylcoumarin (**11**). 4-Methyl-8-nitrocoumarin 7-*O*-sulfamate (**29**) could not be prepared from **28** by method a: (a) NaH/DMF, $H_2NSO_2$-Cl; (b) pyridine−$SO_3$ complex/pyridine, NaOH in MeOH. (B) Pechmann synthesis of coumarin **23**.

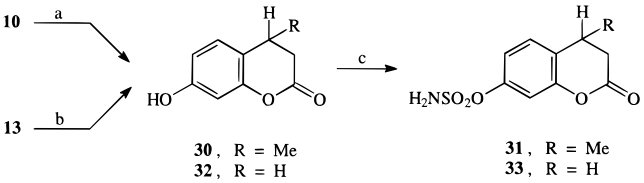


**Figure 6.** Hydrogenation of coumarins **10** and **13** to give the corresponding lactonic derivatives **30** and **32** which were sulfamoylated to **31** and **33**, respectively: (a) Pd−C (10%), $H_2$, 75 psi, 3 days; (b) Pd−C (10%), $H_2$, 40 psi, 24 h; (c) NaH/DMF, $H_2NSO_2Cl$.

Although **54** was the major product obtained from the above sulfamoylation reaction, a chromophoric fraction of higher polarity (i.e., lower $R_f$ value on silica TLC plate) was also afforded. This fraction was isolated and subsequently identified as azomethine **55** (Figure 9), which is an adduct of **54** and dimethylformamide (DMF). A retrosynthetic analysis suggests that **55** could have been formed when one of the N-protons of **54** was removed (presumably by the presence of an excess sodium hydride) and the resulting anion of **54** attacked a molecule of DMF at the carbonyl carbon followed by dehydration of the product upon acidification during the aqueous workup (Figure 9). In our usual small-scale synthesis of EMATE, such an adduct of EMATE and DMF, which is less chromophoric than **55**, was not easily detectable on TLC. However, we did isolate a fraction in our subsequent large-scale synthesis of EMATE whose $^1H$ NMR suggested the presence of the adduct (data not shown).

The dose−response curve for the inhibition of E1-STS in intact MCF-7 breast cancer cells by EMATE is shown in Figure 10A. EMATE inhibits E1-STS activity by >99% at 10, 1, and 0.1 $\mu$M with an $IC_{50}$ of 65 pM. Moreover, the $IC_{50}$ for inhibition of E1-STS activity by EMATE, measured in a placental microsome preparation (100 000 g pellet)[12] using a saturating substrate concentration (20 $\mu$M), was found to be 80 nM.[30] This demonstrates the dramatic increase in potency of EMATE, compared to E1-3-MTP, since under the same conditions in placental microsomes, the latter had an $IC_{50}$ of 43 $\mu$M.[27,28] Unlike E1-3-MTP, which is only a reversible inhibitor,[27] EMATE inhibits E1-STS irreversibly in a time- and concentration-dependent manner in placental microsome preparation (Figure 11A),[51] indicating that it acts as an active site-directed inhibitor. Subsequent studies have also shown that EMATE inhibits DHA-STS,[19,51] the enzyme which regulates the biosynthesis of the estrogenic androstenediol. Moreover, EMATE is active in vivo, inhibiting rat liver E1-STS and DHA-STS activities almost completely when given either orally or subcutaneously.[52]

The structure−activity relationships for EMATE have not been extensively studied. However, from the poorer inhibitory activities of many other E1S surrogates available to date (e.g., E1-3-MTP), it is evident that the sulfamate group of EMATE is a crucial motif for its potency as an active site-directed E1-STS inhibitor. It has also been shown that the bridging oxygen atom between the steroid nucleus and the sulfamoyl group ($H_2NSO_2O$-) is indispensable for the inhibitory activity of EMATE. Hence, analogues of EMATE, where the bridging atom is either a sulfur or nitrogen atom, are only weak non-time-dependent inactivators.[33]

In addition, Figure 10A clearly shows that a significant reduction in the inhibitory activity of EMATE resulted when the N atom of the sulfamate group is increasingly methylated. Hence, estrone 3-*O*-(*N*-methyl)sulfamate (**3**, Figure 2) and estrone 3-*O*-(*N*,*N*-dimethyl)sulfamate (**4**) inhibit E1-STS activity in intact MCF-7 breast cancer cells by only 80 and 50%, respectively, at 0.1 $\mu$M, in contrast to the almost complete inhibition by EMATE at the same concentration. Moreover, both **3** and **4** were found not to be irreversible inhibitors since E1-STS activity recovered when reassaying washed intact MCF-7 cells pretreated with these analogues.[30] It has yet to be established, however, if the reduction in potency and the apparent change in the nature of inhibition observed for **3** and **4** are due to an ineffectual operation of the proposed mechanisms of action depicted in Figure 3. Recently, we have further explored the effects of modifying the sulfamate group of EMATE on inhibition of E1-STS activity by acylating

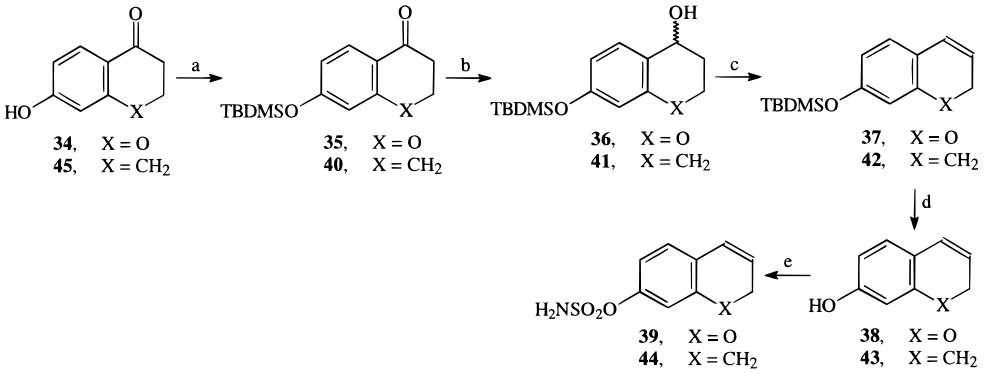


**Figure 7.** Synthesis of sulfamates **39** and **44**: (a) imidazole/DMF, TBDMSCl; (b) $LiAlH_4$/ether/−78 °C; (c) $Et_3N/CH_2Cl_2$, $MeSO_2$-Cl; (d) $(Bu)_4N^+F^-$; (e) NaH/DMF, $H_2NSO_2Cl$. TBDMS: $^t$Bu-C$(CH_3)_2$Si.

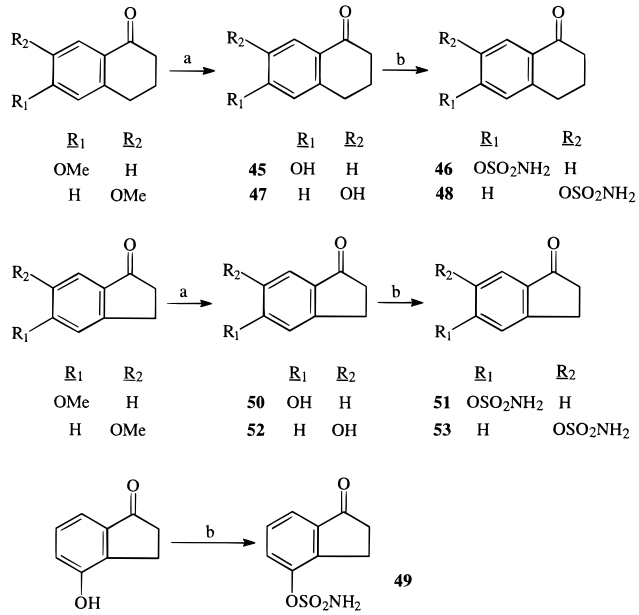


**Figure 8.** Synthesis of tetralone sulfamates (**46** and **48**) and indanone sulfamates (**49**, **51**, and **53**): (a) $AlCl_3$/toluene/reflux; (b) NaH/DMF, $H_2NSO_2Cl$.

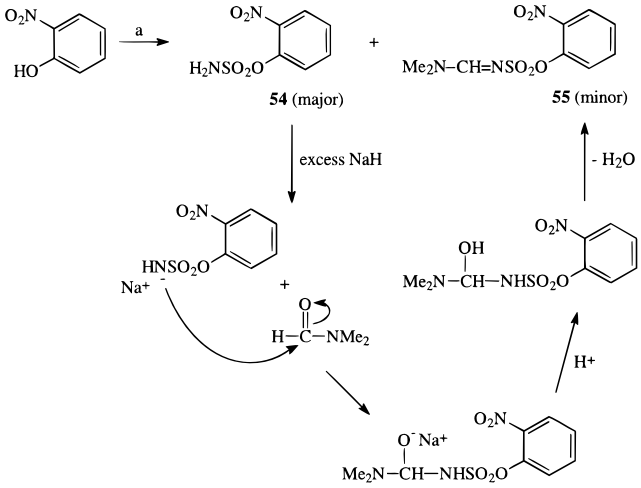


**Figure 9.** Synthesis of sulfamate **54** and azomethine **55** from 2-nitrophenol and the proposed mechanism for the formation of **55**: (a) NaH/DMF, $H_2NSO_2Cl$.

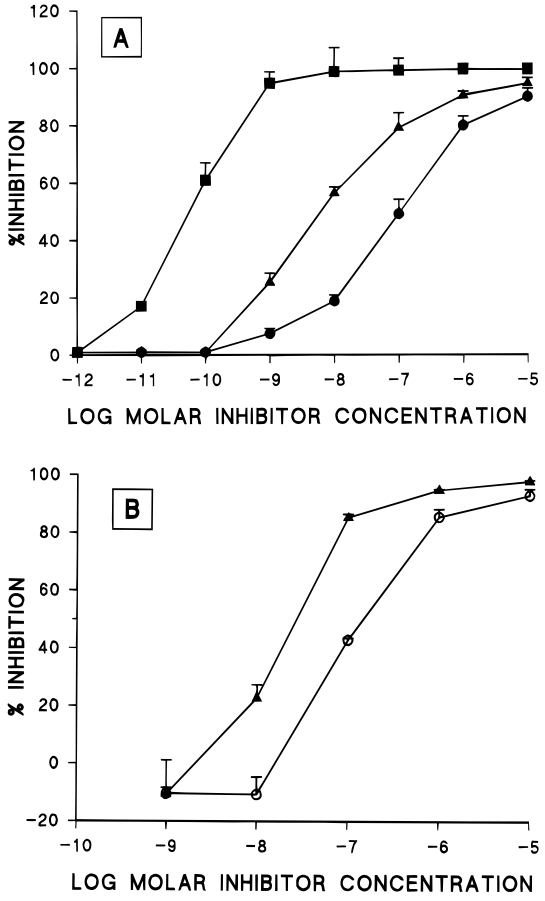


**Figure 10.** Dose−response curves for inhibition of estrone sulfatase activity in intact MCF-7 breast cancer cells by (A) estrone 3-*O*-sulfamate (EMATE, **2**, ■), estrone 3-*O*-(*N*-methyl)-sulfamate (**3**, ▲), and estrone 3-*O*-(*N*,*N*-dimethyl)sulfamate (**4**, ●); and (B) 4-methylcoumarin 7-*O*-sulfamate (COUMATE, **12**, ○) and 3,4-dimethylcoumarin 7-*O*-sulfamate (**24**, ▲). Assays were performed essentially as described previously.[29,32] Monolayers of intact MCF-7 cells in 25 $cm^3$ flasks were incubated for 20 h at 37 °C with [$^3$H]estrone sulfate (2 nM) and synthetic analogues at various concentrations. Estrone sulfatase activity was determined by measuring the total amount of $^3$H-labeled estrone and estradiol formed. Sulfatase activity in untreated cells was 100−120 fmol/20 h/$10^6$ cells. Each point represents the mean ± sd of triplicate measurements. $IC_{50}$s of EMATE, COUMATE, and **24** are 65 pM, 380 nM, and 30 nM, respectively.

the N atom and replacing its protons entirely with larger alkyl groups.[53] Hence, the N-acetylated derivative of EMATE inhibits the E1-STS activity irreversibly, but less potently than EMATE while the N-benzoylated, 3-*O*-(piperidino)sulfamate and *N*,*N*-dibenzyl derivatives of EMATE are only weak reversible inhibitors.

In any form of endocrine therapy, it is often desirable to treat patients with nonsteroidal agents since the

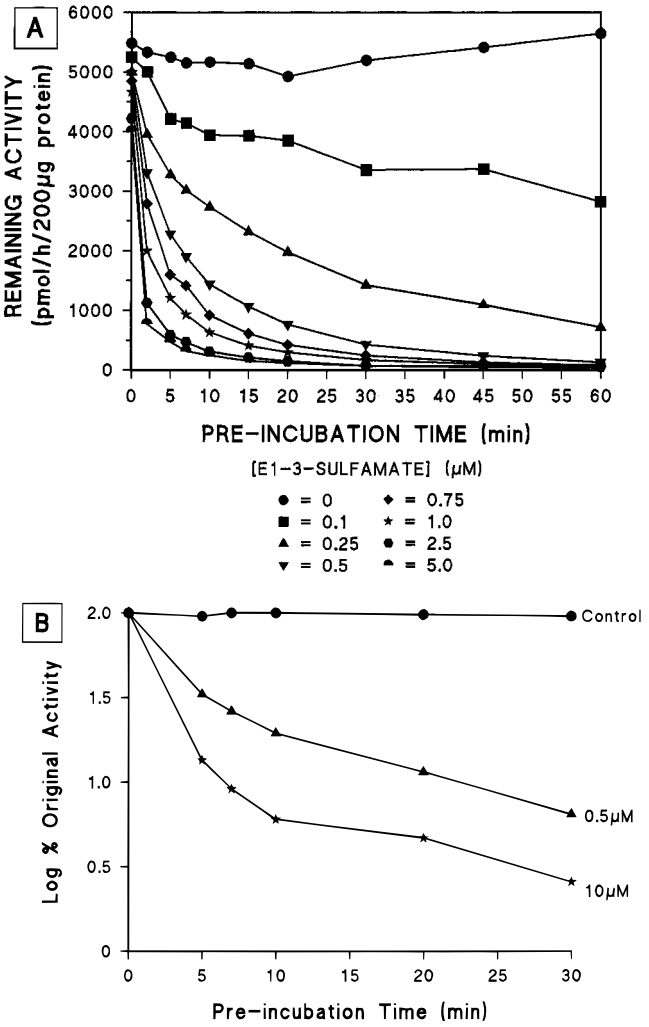


**Figure 11.** Time- and concentration-dependent inactivation of estrone sulfatase by (A) estrone 3-*O*-sulfamate (EMATE, **2**)[51] and (B) 4-methylcoumarin-7-*O*-sulfamate (COUMATE, **12**). Placental microsomes (200 $\mu$g of protein) were preincubated with **2** at various concentrations for 0−60 min and **12** at 0.5 and 10 $\mu$M for 0−30 min at 37 °C followed by incubation with dextran−charcoal for 10 min at 4 °C. Dextran−charcoal was sedimented by centrifugation, and portions of the supernatants were then incubated with [$^3$H]estrone sulfate (20 $\mu$M) for 1 h at 37 °C to assess remaining sulfatase activity. Duplicate experiments were run at each concentration, but assays for residual activity were taken at different times in each experiment.

steroidal counterparts may themselves, or through their metabolites, exert unwanted endocrinological effects. Indeed, despite its high potency as an E1-STS inhibitor, the use of EMATE in treating hormone-dependent breast cancers is restricted since it was found to be five times more estrogenic than ethinylestradiol orally in the rat.[32] Our first series of nonsteroidal inhibitors was based upon tetrahydronaphth-2-ol (THN, **6**, Figure 4) derivatives **7** − **9**, which are presumably A/B ring mimics of EMATE. The best candidate in this series was **7**, albeit it was much less potent than EMATE.[30] Its N-methylated derivatives, **8** and **9**, are even weaker inhibitors,[30] and thus it again appears that, like their steroidal counterparts **3** and **4** (Figure 2), the extent of inhibition decreases with increasing methylation of the sulfamate group. We have also established that **7** acts only as a weak time- and concentration-dependent inhibitor (data not shown). Hence, preincubation of placental microsomes with **7** at 10 $\mu$M for 60 min resulted in less than 10% inhibition of E1-STS activity.

With the limited success of achieving inhibition of E1-STS with **7**, we chose the monohydroxylated coumarin structure as an alternative template whose ring system is ubiquitous in Nature and commonly incorporated in many pharmaceuticals. The core coumarin, 7-hydroxycoumarin (**13**, Figure 5), differs structurally from THN by possessing a cyclic $\alpha,\beta$-unsaturated lactone in place of a saturated cyclic hydrocarbon. It is important, however, to first demonstrate that coumarin sulfates themselves are substrates for E1-STS, and hence we synthesized 7-(sulfooxy)-4-methylcoumarin (**11**, Figure 5) to examine if this sulfate is hydrolyzed by the enzyme. When **11** was incubated with placental microsomes in the absence of EMATE, only the free coumarin **10** was detected. However, inclusion of EMATE in the reaction mixture completely abolished the hydrolysis of **11** by E1-STS. These results therefore indicate that the coumarin sulfate **11** is a substrate for this enzyme.

It was then reasoned that sulfamates of coumarin derivatives are likely to act as steroid sulfatase inhibitors. 4-Methylcoumarin 7-*O*-sulfamate (COUMATE, **12**, Figure 5), coumarin 7-*O*-sulfamate (**14**), 4-(trifluoromethyl)coumarin 7-*O*-sulfamate (**16**) and 3,4,8-trimethylcoumarin 7-*O*-sulfamate (**18**) were hence synthesized and evaluated for E1-STS inhibition using intact MCF-7 breast cancer cells or placental microsomes as previously described.[27,30,51] The two enzyme systems adopted for the in vitro screening of potential inhibitors are complementary. The intact MCF-7 breast cancer cell system essentially shows the ability of a potential inhibitor to inhibit E1-STS in a live cell. However, to establish that a potential inhibitor does compete strongly with the natural substrate for the enzyme active site, we also screen a given compound for inhibition of E1-STS in placental microsomes at concentrations between 10 and 100 $\mu$M in the presence of a much higher concentration of E1S (20 $\mu$M) [cf. in the intact MCF-7 cell system, the E1S present is only at the physiological concentration (2 nM) whereas the concentrations of the synthetic agent used are in the range 0.1−10 $\mu$M]. Our experience has demonstrated that a prospective E1-STS inhibitor warranting further development will in general show good inhibitory activities in both enzyme systems.

The free parent coumarins (**10**, **13**, **15**, and **17**, Figure 5) were all found to be devoid of E1-STS inhibitory activity in intact MCF-7 breast cancer cells when tested at concentrations of up to 10 $\mu$M. In contrast, the corresponding coumarin sulfamates (**12**, **14**, **16**, and **18**, Figure 5) inhibit E1-STS activity in the same preparation in a dose-dependent manner (Table 1). The inhibition at 10 $\mu$M ranges from 71.5% for **16** to 93.1% for COUMATE. However, when screened in placental microsomes, only COUMATE achieves >90% inhibition at all three of the concentrations tested whereas the inhibition of E1-STS by **14** and **16** is in the region of 78% at 10 $\mu$M (Table 1). Although sulfamate **18** shows inhibitory activity comparable to that of **16** in intact MCF-7 cells, it inhibits the enzyme very poorly in placental microsomes with an inhibition of less than 20% at all three concentrations. The reason for these differences in inhibitory activities of **18** is still unclear.

**Table 1.** Inhibition of Estrone Sulfatase Activity in Intact MCF-7 Breast Cancer Cells by Sulfamates **7, 12, 14, 16, 18, 20, 22, 24, 26, 27, 31, 33, 39, 44, 46, 48, 49, 51**, and **53** at Various Concentrations[a]

| | % inhibition ± SD of E1-STS activity in intact MCF−7 cells at various concentrations | | |
|---|---|---|---|
| compd | 0.1 $\mu$M | 1 $\mu$M | 10 $\mu$M |
| **7** | 51.7 ± 2.5 | 56.7 ± 0.4 | 78.8 ± 1.9 |
| **12*** | 42.9 ± 0.7 | 85.6 ± 2.7 | 93.1 ± 2.2 |
| **14** | 23.9 ± 4.1 | 66.0 ± 7.2 | 90.4 ± 2.3 |
| **16** | 9.9 ± 3.9 | 28.3 ± 5.3 | 71.5 ± 3.6 |
| **18** | 9.3 ± 4.2 | 46.4 ± 5.0 | 75.1 ± 5.5 |
| **20** | <10 | 17.4 ± 3.4 | 39.1 ± 5.3 |
| **22** | 33.8 ± 5.3 | 34.1 ± 1.8 | 72.1 ± 1.3 |
| **24**† | 85.4 ± 1.1 | 94.9 ± 0.3 | 98 ± 0.4 |
| **26** | <10 | <10 | <10 |
| **27** | <10 | <10 | 31.2 ± 2.6 |
| **31** | <10 | <10 | 32.3 ± 7.4 |
| **33** | 12.0 ± 8.8 | 30.2 ± 3.9 | 28.5 ± 5.2 |
| **39** | 11.4 ± 3.1 | 68.2 ± 2.4 | 99.3 ± 1.1 |
| **44** | 49.0 ± 4.8 | 60.6 ± 2.4 | 76.2 ± 10.2 |
| **46** | <10 | 11.7 ± 8.2 | 78.1 ± 2.9 |
| **48** | 10.4 ± 3 | 47.8 ± 1.7 | 93.5 ± 0.8 |
| **49** | <10 | 15.5 ± 9.9 | 74.1 ± 2.8 |
| **51** | 13.5 ± 3 | 22.6 ± 5.8 | 71.3 ± 1 |
| **53** | 33 ± 2 | 91 ± 0.6 | 99 ± 2 |

[a] For details of the assay, refer to the legend of Figure 10. *<10% at 1 and 10 nM. †<10% at 1 nM and 22.7% ± 4.7 at 10 nM.

It is evident that the most effective inhibitor in this series is COUMATE. Its $IC_{50}$ for inhibition of E1-STS, measured using intact MCF-7 cells, was found to be 380 nM (Figure 10B). As with EMATE, COUMATE inhibits placental microsomes E1-STS activity in a time- and concentration-dependent manner and in a biphasic fashion (Figure 11B), indicating a similar mechanism of action to that proposed for EMATE, as depicted in Figure 3. At 10 $\mu$M, COUMATE reduces the original E1-STS activity by 95% after preincubation of the enzyme with the inhibitor for 20 min (Figure 11B). Also, at the same concentration, COUMATE inhibits placental microsomal DHA-STS activity by 94%.[54] In contrast to EMATE, however, COUMATE is devoid of estrogenic activity since it did not stimulate the growth of intact MCF-7 breast cancer cells or the uteri of ovariectomized rats.[54] It has also been shown that COUMATE is able to block completely the ability of E1S to stimulate uterine growth in ovariectomized rats.[54] Moreover, COUMATE is orally active in vivo and, after multiple dosing (10 mg/kg/day for 7 days), inhibits E1-STS activity in rat liver by 85%.[54] However, the duration of this inhibition is limited since liver E1-STS activity is almost fully restored 7 days after single or multiple dosing with COUMATE.[54] When EMATE was examined in vivo in a similar manner, the rat liver E1-STS activity remained inhibited (>95%) for over a week following the cessation of dosing.[52]

The significant increase in the inhibition of E1-STS activity by coumarin sulfamates (**12**, **14**, **16**, and **18**) in comparison to **7** can presumably be attributed to their different ring systems. The initial E1-STS/coumarin sulfamate complex may form more readily than E1-STS/**7** since there may be more favorable interactions between the amino acid residues in the active site and the $\alpha,\beta$-unsaturated lactone motif of the coumarin derivative. Subsequent inactivation of the enzyme may thus be accomplished more efficiently with the coumarin sulfamates. Alternatively, the enhanced inhibitory activity exhibited by the coumarin sulfamates may be due, in part, to a facilitated sulfamoylation of the enzyme. It is likely that the breaking of the S−O bond in the sulfamate group of **12**, **14**, **16**, and **18** during sulfamoylation of E1-STS is assisted by the extended conjugation present in the coumarin motif which improves the leaving group ability of coumarins (**10**, **13**, **15**, and **17**, Figure 5).

In light of the encouraging inhibitory activities of COUMATE, it is important to have a better understanding of its structure−activity relationships since this inhibitor represents a good lead template for the design of more potent nonsteroidal E1-STS inhibitors. Hence, we have synthesized several analogues of COUMATE and its congener, **14**, in which (i) the sulfamate group has been relocated (**20**, Figure 5), ii) the aromatic ring has been substituted (**22**, Figure 5), (iii) both the 3- and 4-positions have been substituted with methyl groups (**24**, Figure 5), and (iv) the coumarin ring system has been disrupted (**31** and **33**, Figure 6; **39** and **44**, Figure 7). The mono- (**26**) and bis-sulfamates (**27**) (Figure 5) of the catechol-like coumarin, **25**, have also been prepared.

4-Methylcoumarin 6-*O*-sulfamate (**20**, Figure 5) and 6-methoxycoumarin 7-*O*-sulfamate (**22**) were prepared by direct sulfamoylation of their corresponding starting coumarins (**19** and **21**). 3,4-Dimethyl-7-hydroxycoumarin (**23**, Figure 5), required for the synthesis of sulfamate **24**, was prepared in 25% yield by a Pechmann reaction involving the condensation of resorcinol with ethyl 2-methylacetoacetate in the presence of concentrated sulfuric acid. When 6,7-dihydroxy-4-methylcoumarin, **25**, was treated with 2 equiv of sodium hydride followed by an excess of sulfamoyl chloride (5 equiv), a mixture of the catechol-like coumarin sulfamates, **26** and **27**, was formed. The separation of the monosulfamate, **26** from the bis-sulfamate, **27**, was achieved by preparative TLC. An attempt to sulfamoylate 7-hydroxy-4-methyl-8-nitrocoumarin (**28**, Figure 5) in DMF using sulfamoyl chloride failed to give the desired 4-methyl-8-nitrocoumarin 7-*O*-sulfamate (**29**, Figure 5).

The conjugation in 7-hydroxycoumarin (**13**, Figure 5) was disrupted by hydrogenation in the presence of 10% palladium−charcoal. While the disubstituted double bond of **13** was completely reduced in 24 h at 40 psi, the sterically more hindered trisubstituted double bond in 4-methyl-7-hydroxycoumarin (**10**, Figure 5) remained unaffected under this hydrogenation condition. (±)-3,4-Dihydro-7-hydroxy-4-methylcoumarin (**30**, Figure 6) was subsequently obtained in 71% yield when **10** was hydrogenated at 75 psi for 3 days. Direct hydrogenation of COUMATE and **14** (Figure 5) to **31** and **33**, respectively, has not yet been attempted. However, from our experience with other sulfamates, it is likely that the sulfamate groups of COUMATE and **14** will be stable to hydrogenation conditions used in the above experiments, and hence this may be a more direct route to **31** and **33**.

The conjugation of the coumarin motif can also be disrupted by replacing the carbonyl group in the lactone with a methylene group. An attempt to convert a model coumarin to the corresponding $\alpha,\beta$-unsaturated ether by the method of Kraus et al.[55] failed. Since there is no apparent working direct synthetic route to com-

**Table 2.** Inhibition of Estrone Sulfatase Activity in Placental Microsomes by Sulfamates **7, 12, 14, 16, 18, 20, 22, 24, 26, 27, 31, 33, 39, 44, 46, 48, 49, 51**, and **53** at Various Concentrations[a]

| | % inhibition ± SD of E1-STS activity in placental microsomes at various concentrations | | |
|---|---|---|---|
| compd | 10 $\mu$M | 50 $\mu$M | 100 $\mu$M |
| **7** | <10 | <10 | 12.5 ± 3.0 |
| **12*** | 93.0 ± 0.8 | 97.8 ± 0.1 | 99 ± 0.4 |
| **14** | 78.2 ± 0.1 | 91.9 ± 0.8 | 94.4 ± 0.1 |
| **16** | 79.4 ± 0.1 | 94.3 ± 0.3 | 96.3 ± 0.3 |
| **18** | 17.4 ± 3.1 | 11.5 ± 1.8 | 12.5 ± 1.6 |
| **20** | <10 | <10 | <10 |
| **22** | <10 | 42.9 ± 1.9 | 69.9 ± 1.3 |
| **24**[†] | 96.9 ± 4.1 | 98.8 ± 1.3 | 99.2 ± 1.6 |
| **26** | <10 | <10 | <10 |
| **27** | <10 | <10 | <10 |
| **31** | 53.4 ± 1.6 | 70.6 ± 2.8 | 82.1 ± 1.0 |
| **33** | <10 | 15.0 ± 0.9 | 34.4 ± 0.3 |
| **39** | 19.9 ± 2.1 | 36.9 ± 1.8 | 47.2 ± 2.3 |
| **44** | <10 | 33.2 ± 1.5 | 34.5 ± 6.0 |
| **46** | 16.1 ± 5.8 | 54.3 ± 4.0 | 56.1 ± 4.6 |
| **48** | 12.3 ± 1.0 | 16.5 ± 2.1 | 27.0 ± 1.1 |
| **49** | <10 | <10 | <10 |
| **51** | 19.6 ± 3.1 | 37.7 ± 0.9 | 50.3 ± 1.2 |
| **53** | <10 | <10 | <10 |

[a] [$^3$H]Estrone sulfate ($4 \times 10^5$ dpm), adjusted to 20 $\mu$M with unlabeled substrate, with or without the inhibitor at various concentrations, was incubated with placental microsomes (125 $\mu$g of protein/mL) for 30 min. The product formed was isolated by extraction into toluene with [4-$^{14}$C]estrone ($7 \times 10^3$ dpm) being used to monitor procedural losses. Each value represents the mean ± s.d. of triplicate measurements. *<10% at 0.1 $\mu$M and 62.7% ± 0.5 at 1 $\mu$M. [†]34.7% ± 0.1 at 0.1 $\mu$M and 88.2% ± 1.9 at 1 $\mu$M.

pounds of this type, a multistep approach is required for the synthesis of 2*H*-1-benzopyran-7-ol (**38**, Figure 7), the starting material for the corresponding sulfamate (**39**, Figure 7). Hence, 2,3-dihydro-7-hydroxy-4*H*-1-benzopyran-4-one (**34,** Figure 7) was synthesized from resorcinol and acrylnitrile according to the method of Breytenbach and Rall[56] and then silylated. The resulting protected pyranone (**35**, Figure 7) was reduced and the resulting mixture of the enantiomeric alcohols (**36**, Figure 7) dehydrated to give the silylated pyranol (**37**, Figure 7). Finally, deprotection of **37** gave crude 2*H*-1-benzopyran-7-ol, **38**, which was then sulfamoylated immediately, without further purification, because of noticeable decomposition. The sulfamate **39** obtained was also unstable and had to be purified by preparative TLC before analysis and testing. It appears that this class of compounds is intrinsically unstable as the structurally related analogue, 2,7,8-trimethyl-2-(4,8,12-trimethyltrideca-3-(*E*),7(*E*),11-trienyl)-2-*H*-benzopyran-6-ol, has also been reported as showing noticeable oxidative decomposition upon storage.[57]

The above multistep approach to **38** was also applied to the synthesis of 7,8-dihydronaphth-2-ol (**43**, Figure 7). Although alcohol **43** was found to be unstable in a manner similar to its analogue **38**, 7,8-dihydronaphthalene 2-*O*-sulfamate (**44**, Figure 7), in contrast to the benzopyran sulfamate **39**, is a stable crystalline product.

Upon examination of the abilities of these COUMATE analogues (**20**, **22**, **24**, **26**, **27**, **31**, **33**, **39**, and **44**) to inhibit E1-STS activity (Tables 1 and 2), almost all of them were found to be less effective inhibitors of E1-STS than COUMATE with the exception of 3,4-dimethylcoumarin 7-*O*-sulfamate (**24**), which inhibits the enzyme to a greater extent. These results have also shown a general pattern that analogues which inhibit E1-STS activity poorly in intact MCF-7 breast cancer cells (Table 1) also perform poorly when examined in placental microsomes (Table 2). The only exception is sulfamate **31** where the situation is apparently reversed.

The dose−response curves for the inhibition of E1-STS in intact MCF-7 breast cancer cells by COUMATE and **24** are compared in Figure 10B and the $IC_{50}$ of **24** is found to be 30 nM, some 12-fold lower than that of COUMATE. It is likely that the higher potency observed for **24** is the result of its extra methyl group at the 3-position which might have rendered a tighter binding of the inhibitor to the enzyme active site through hydrophobic interactions with neighboring amino acid residues. Although no time- and concentration-dependent inactivation studies have been performed, it is reasonable to expect that **24**, like COUMATE, is also an active site-directed inhibitor.

An introduction of a substituent at either the C-6 (**22** cf. **14**, and **27** cf. COUMATE, Tables 1 and 2) or the C-8 position (**18** cf. **24**, Tables 1 and 2) of the coumarin ring significantly reduces the inhibitory activity of the sulfamate. Since coumarin sulfamates are anticipated to be A/B ring mimics of EMATE, our results have therefore highlighted the limited tolerance of the enzyme to substituents at these positions. It is possible that the methoxy group in **22**, the C-8 methyl group in **18**, and the second sulfamate group in **27** may have caused the sulfamate group at C-7 to be shifted in the binding site from the usual position occupied by the sulfamate group of EMATE/COUMATE, and hence it could not be activated effectively for the sulfamoylation of the enzyme. These substituents may also conceivably shield the sulfamate group in such a manner that the putative proton abstraction, the first vital step proposed for the mechanism of E1-STS inhibition by EMATE, is prevented from occurring.

Relocation of the sulfamate group from the usual C-7 to the C-6 position virtually abolishes the inhibitory activities of the coumarin sulfamates in both of the biological systems (**20** and **26**, cf. COUMATE, Tables 1 and 2). It is likely that on binding to the enzyme active site, the sulfamate groups in **20** and **26**, which are at the C-6 position of the coumarin ring, as opposed to the C-7 position in COUMATE, are not in close proximity to the essential amino acid residues responsible for their subsequent activation. The poor inhibition shown by sulfamates **20** and **26** could also be a consequence of the relatively poor leaving group abilities of their respective starting coumarins (**19** and **25**, Figure 5). It is reasonable to expect that the p$K_a$s of **19** and **25** will be similar to that of a simple phenol (ca. 10) since their hydroxy group at the C-6 position is not conjugated to the $\alpha,\beta$-unsaturated lactone moiety of the coumarin ring.

The significance of the coumarin moiety to the inhibitory activities of COUMATE and **14** is further exemplified by the poor inhibition observed for their analogues where the conjugation of the ring system is disrupted. When the $\alpha,\beta$-olefinic double bond was saturated (**31** and **33**, Figure 6) or the lactone disrupted, either by replacement of the carbonyl group with a methylene group (**39**, Figure 7) or by removal of the entire moiety (**44**, Figure 7), the analogues so afforded were found to be less effective inhibitors of E1-STS than COUMATE

(Tables 1 and 2). For sulfamates **39** and **44**, their poor inhibitory activities observed might be attributed to a weaker binding to the enzyme active site since a site of potential hydrogen bonding with neighboring amino acid residues has been deleted. However, the differences in the inhibitory activities of **31** and **33** when compared to those of their α,β-unsaturated lactone counterparts are unlikely to be explained simply by the structural modification to COUMATE and **14**. It is anticipated that these sulfamates (COUMATE, **14**, **31**, and **33**) will all bind in a similar manner and tightness to the enzyme active site. We therefore attribute the superior inhibition shown by COUMATE and **14** to the better leaving group abilities of their starting coumarins, **10** and **13**, respectively, as a result of the conjugation in their coumarin rings. It is now clear that a study on the p$K_a$ values of **6**, **10**, **13**, **15**, **17**, **19**, **21**, **23**, **25**, **30**, **32**, **38**, and **43** is warranted in order to establish if a correlation between the leaving group abilities of these phenols and the inhibitory activities of their corresponding sulfamates exists.

Finally, to explore the potential of other two-ring sulfamates as effective inhibitors of E1-STS, we have synthesized and examined tetralone 6-*O*-sulfamate (**46**, Figure 8), indanone 5-*O*-sulfamate (**51**, Figure 8), and the sulfamates of their analogues (**48**, **49**, and **53**, Figure 8). Although some of these sulfamates show encouraging inhibition in intact MCF-7 breast cancer cells (**48** and **53**, Table 1), all of the sulfamates inhibit E1-STS activity in placental microsomes to a lesser extent than COUMATE (Table 2). For sulfamates **46** and **51**, preliminary results from our studies in progress have indicated that the p$K_a$ values of the starting alcohols, 6-hydroxy-1-tetralone (**45**, Figure 8), and 5-hydroxy-1-indanone (**50**, Figure 8) respectively, are in the same range as that of 7-hydroxy-4-methylcoumarin (**10**, Figure 5) [ca. 8.1 (unpublished results)]. It is therefore interesting to note that the inhibitory activity of COUMATE cannot be reproduced by simply replacing the coumarin ring with other two-ring systems having leaving group abilities similar to that of **10**. The relatively disappointing inhibitory activities observed for this series of tetralone and indanone sulfamates could well be the result of less effective binding of these compounds to the enzyme active site.

In conclusion, EMATE (**2**, Figure 2) is the most potent active site-directed steroidal E1-STS inhibitor synthesized to date. However, a significant reduction in the inhibitory activity of EMATE with an apparent change in the nature of inhibition results when the N atom of its sulfamate group is increasingly methylated. An attempt to design and synthesize nonsteroidal E1-STS inhibitors, in the light of the potent estrogenicity of EMATE, has led to the development of COUMATE (**12**, Figure 5). This is a significant improvement over our first lead nonsteroidal candidate, 5,6,7,8-tetrahydronaphthalene 7-*O*-sulfamate (**7**, Figure 4). COUMATE is an orally active and nonestrogenic active site-directed steroid sulfatase inhibitor, albeit less potent than EMATE. Modifications made to the structure of COUMATE by (i) the relocation of its C-7 sulfamate group to the C-6 position, (ii) substitutions of the aromatic ring at the C-6 or C-8 positions, and (iii) the disruption of the conjugated coumarin ring all proved to be detrimental to its biological activity. Replacement of the entire coumarin ring of COUMATE with other simple two-ring systems having similar leaving group potentials have also resulted in analogues which are less effective inhibitors of E1-STS. However, when the 3-position of COUMATE is substituted with a methyl group, the analogue 3,4-dimethylcoumarin 3-*O*-sulfamate, **24**, is some 12-fold more potent than COUMATE as an E1-STS inhibitor in intact MCF-7 breast cancer cells. These structure−activity relationship studies conducted on COUMATE clearly show that its coumarin ring is pivotal for inhibition of E1-STS and that highly potent, irreversible, and nonestrogenic E1-STS inhibitors, which are structurally related to COUMATE, may subsequently be designed and developed for therapeutic use in the treatment of hormone-dependent breast cancers.

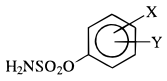


**Figure 12.** Proposed pharmacophore for an EMATE-like steroid sulfatase inhibitor. X = H or a substituent; Y = additional functionalities (including fused or adjacent/remote ring structures).

All of the studies reported here, and by ourselves and others since the publication of our initial report,[30] have demonstrated the absolute requirement for at least a phenol sulfamate ester for activity. It seems clear that this must be the active pharmacophore required for steroid sulfatase inhibition, but that other groups, not necessarily a ring structure (see refs 39 and 40), presumably provide the extra motifs required for effective binding to the active site and/or activation of the sulfamoyl group and can modulate activity and estrogenicity.[61] We therefore propose the pharmacophore illustrated in Figure 12 as the definitive molecular motif required in an EMATE-like steroid sulfatase inhibitor. No compound has yet been synthesized, acting like EMATE, that does not possess this functionality which we believe will be a crucial factor in the design of further active site-directed inhibitors of steroid sulfatase.

## Experimental Section

All chemicals were either purchased from Aldrich Chemical Co. (Gillingham, Dorset, U.K.) or Lancaster Synthesis (Morecambe, Lancashire, U.K.). Crystalline starting alcohols were dried in vacuo at 60 °C for 3 h prior to use. All organic solvents, of A.R. grade, were supplied by Fison plc (Loughborough, U.K.) and stored over 4 Å molecular sieves. Anhydrous dimethylformamide (DMF), used for all the sulfamoylation reactions, was purchased from Aldrich and was stored under a positive pressure of $N_2$ after use. Sulfamoyl chloride was prepared by an adaptation of the method of Appel and Berger[50] and was stored as a solution in toluene as described by Woo et al.[33] No titration was attempted on this sulfamoyl chloride solution whose molarity was estimated according to the weight of the original crude sulfamoyl chloride obtained after workup. An appropriate volume of this solution was freshly concentrated in vacuo immediately before use. For the synthesis of EMATE, the crude sulfamoyl chloride solid was used instead. *N*-Methylsulfamoyl chloride was prepared from *N*-methylsulfamic acid by treatment with phosphorus pentachloride;[58] *N*,*N*-dimethylsulfamoyl chloride was purchased from Aldrich. Pyridine was dried by refluxing over sodium hydroxide pellets followed by distillation and stored over 4 Å molecular sieves.

E1S and E1 were purchased from Sigma Chemical Co. (Poole, U.K.). [6,7-$^3$H]E1S (specific activity, 50 Ci/mmol) and

[4-$^{14}$C]E1 (specific activity, 52 mCi/mmol) were purchased from New England Nuclear (Boston, MA). [6,7-$^{3}$H]E1 (specific activity, 97 Ci/mmol) was obtained from the Amersham International Radiochemical Centre (Amersham, U.K.).

Thin layer chromatography (TLC) was performed on precoated plates (Merck TLC aluminum sheets silica gel 60 $F_{254}$, Art. No. 5554). Product(s) and starting material (SM) were detected by either viewing under UV light or treating with a methanolic solution of phosphomolybdic acid followed by heating. Preparative TLC was performed on precoated plates (Merck TLC silica gel 60 $F_{254}$, 20 × 20 cm, layer thickness 2 mm, art. no. 5717) and bands were visualized under UV light. Flash column chromatography was performed on silica gel (Sorbsil C60). IR spectra were determined by a Perkin-Elmer 782 infrared spectrophotometer, and peak positions are expressed in cm$^{-1}$. $^{1}$H and DEPT-edited $^{13}$C NMR spectra were recorded with a JEOL JMN-GX270 and JMN-GX400 NMR spectrometers, and chemical shifts are reported in parts per million (ppm, $\delta$) relative to tetramethylsilane (TMS) as an internal standard. For compounds **35**, **37**, **40**, and **42**, where no TMS was included, the chemical shifts are reported relative to $CDCl_3$ at 7.26 ppm. Mass spectra were recorded at the Mass Spectrometry Service Centre, University of Bath, and the EPSRC National Mass Spectrometry Service Centre, Swansea. FAB-mass spectra were carried out using *m*-nitrobenzyl alcohol (NBA) as the matrix unless stated otherwise. Elemental analyses were performed by the Microanalysis Service, University of Bath, and are within ± 0.4% of theory unless otherwise stated. Melting points were determined using a Reichert-Jung Thermo Galen Kofler block and are uncorrected.

Biological studies were performed essentially as described previously.[27,30,51] For more details, see legends of individual figures or tables. To examine whether the sodium salt of 7-(sulfooxy)-4-methylcoumarin (**11**, Figure 5) could act as a substrate for E1-STS, 100 $\mu$g of the compound was incubated for 1 h with placental microsomes in the absence or presence of EMATE (10 $\mu$M). The product formed at the end of the incubation was extracted into diethyl ether. After evaporation of solvent, the residue obtained was examined by TLC using ethyl acetate/methanol (4:1) as eluent. In this mobile phase, the coumarin sulfate, **11**, and the parent 7-hydroxy-4-methylcoumarin (**10**, Figure 5) have $R_f$ values of 0.79 and 0.95, respectively.

**Estrone 3-*O*-Sulfamate (2).** Sodium hydride (60% dispersion, 70 mg, 1.73 mmol) was added to a stirred solution of estrone (230 mg, 850 $\mu$mol) in anhydrous DMF (5 mL) at 0 °C. Subsequently, sulfamoyl chloride (200 mg, 1.73 mmol) was added carefully, and the resulting mixture was allowed to warm to room temperature whereupon stirring was continued for a further 96 h. The reaction mixture was then poured onto a cold, saturated solution of sodium bicarbonate (20 mL). The aqueous solution was extracted with dichloromethane (5 × 15 mL), and the combined organic extracts were dried over anhydrous $MgSO_4$. Removal of the solvent in vacuo followed by repeated coevaporation with toluene (3 × 30 mL) to remove the final traces of DMF gave a viscous pale brown oil which solidified on standing. Purification was effected using flash chromatography (chloroform/methanol, 96:4). The final product was further purified by precipitation from chloroform by addition of pentane, and **2** was afforded as a white crystalline solid (230 mg, 659 $\mu$mol, 76%): mp 195−197 °C; TLC (chloroform/methanol, 96:4) $R_f$ 0.36; $^{1}$H NMR (270 MHz, methanol-$d_4$) $\delta$ 0.91 (3H, s, $CH_3$), 1.46−1.67 (6H, m), 1.89−2.54 (7H, series of m), 2.92 (2H, m), 7.04 (2H, br d, $J$ = 10.44 Hz, C2-H and C4-H), and 7.33 (1H, br d, $J$ = 8.42 Hz, C1-H); $^{13}$C NMR (100.4 MHz, methanol-$d_4$) $\delta$ 14.53 (q, C18), 22.80 (t), 27.24 (t), 27.73 (t), 30.68 (t), 33.05 (t), 37.01 (t), 39.76 (d), 45.73 (d), 50.27 (s, C13), 51.86 (d), 120.76 (d), 123.54 (d), 127.89 (d), 139.83 (s), 150.27 (s), 223.87 (s, CO); MS (EI, 70 eV) *m*/*z* (rel intensity) 349 (9, $M^+$), 270 [100, $(M - HNSO_2)^+$]. Anal. ($C_{18}H_{23}NO_4S$) C, H, N.

**Estrone 3-*O*-(*N*-Methyl)sulfamate (3).** This was prepared in a manner similar to the preparation of **2**. Estrone (290 mg, 1.07 mmol), sodium hydride (60% dispersion, 90 mg, 2.25 mmol), *N*-methylsulfamoyl chloride (280 mg, 2.14 mmol), and anhydrous DMF (5 mL) were used. Purification was effected using flash chromatography (chloroform/methanol, 98:2) to afford **3** as a cream-colored powder (90 mg, 0.247 mmol, 24%): $^{1}$H NMR (270 MHz, $CDCl_3$) $\delta$ 0.91 (3H, s, $CH_3$), 1.40−1.68 (6H, m) 1.97−2.57 (7H, series of m), 2.91 (2H, m), 2.94 (3H, d, $J$ = 5.13 Hz, C$H_3$NH), 4.70 (1H, br q, exchanged with $D_2O$, $J$ = 5.13 Hz, MeNH), 7.04 (2H, m, C2-H and C4-H), and 7.30 (1H, d, $J$ = 8.06 Hz, C1-H); $^{13}$C NMR (100.4 MHz, $CDCl_3$) $\delta$ 13.78 (q, C18) 21.54 (t), 25.72 (t), 26.21 (t), 29.39 (t), 30.23 (q, $CH_3$NH), 31.46 (t), 35.81 (t), 37.88 (d), 44.09 (d), 47.87 (s, C13), 50.34 (d), 118.84 (d),121.79 (d), 126.72 (d), 138.60 (s), 138.66 (s), 147.97 (s), 220.82 (s, CO); MS (CI, isobutane) *m*/*z* (rel intensity) 364 [13, $(M + H)^+$], 271 [70, $(M + H - MeNSO_2)^+$], 69 (100). Anal. ($C_{19}H_{25}NO_4S \cdot {}^1/_4H_2O$) C, H, N.

**Estrone 3-*O*-(*N*,*N*-Dimethyl)sulfamate (4).** Sodium hydride (60% dispersion, 74 mg, 1.85 mmol) was added to a stirred solution of estrone (500 mg, 1.85 mmol) in anhydrous DMF (5 mL) at 0 °C. After the evolution of hydrogen had ceased, *N*,*N*-dimethylsulfamoyl chloride (531 mg, 3.70 mmol) was added and the resulting mixture was left to stir overnight at room temperature. The reaction mixture was then quenched with brine (100 mL) and the aqueous portion extracted with ethyl acetate (100 mL). The organic fraction that separated was further washed with brine (5 × 50 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo to give a white residue which was fractionated by flash chromatography (chloroform/ethyl acetate, 15:1 to 9:1, gradient). The crude product obtained was further purified by recrystallization from ethyl acetate/hexane (1:1) to afford **4** as white crystals (170 mg, 450 $\mu$mol, 24%): mp 176−177 °C; $^{1}$H NMR (270 MHz, $CDCl_3$) $\delta$ 0.92 (3H, s, $CH_3$), 1.4−2.6 (13H, m), 2.92 (2H, m, C6-$H_2$), 2.99 [6H, s, $N(CH_3)_2$], 7.03 (2H, m, C2-H and C4-H), and 7.29 (1H, d, $J_{C2-H,C1-H} \approx 8$ Hz, C1-H); $^{13}$C NMR (100.4 MHz, $CDCl_3$) $\delta$ 13.75 (q, C18), 21.50 (d), 25.66 (t), 26.17 (t), 29.32 (t), 31.43 (t), 35.78 (t), 37.82 (d), 38.08 (q, $CH_3N$), 38.73 (q, $CH_3N$), 44.05 (d), 47.84 (s, C13), 50.31 (d), 118.71 (d) 121.63 (d), 126.59 (d), 138.30 (s), 138.43 (s), 148.00 (s), 220.69 (s, CO); MS (EI, 70 eV) *m*/*z* (rel intensity) 377 (100, $M^+$), 269 [20, $(M - Me_2NSO_2)^+$]. Anal. ($C_{20}H_{27}NO_4S$) C, H, N.

**5,6,7,8-Tetrahydronaphthalene 2-*O*-Sulfamate (7).** This was prepared in a manner similar to the preparation of **4**. 5,6,7,8-Tetrahydronaphth-2-ol (500 mg, 3.306 mmol), sodium hydride (60% dispersion, 132 mg, 3.306 mmol), sulfamoyl chloride (2 equiv), and DMF (5 mL) were used. Purification of the syrupy light brown crude was effected using flash chromatography (ethyl acetate/hexane, 1:4 to 1:2, gradient) to give **7** as a clear pale yellow syrup which solidified to a cream-colored waxy solid when kept under a stream of nitrogen over the weekend (624 mg, 2.745 mmol, 83%): TLC (ethyl acetate/hexane, 1:1) $R_f$ 0.58, cf. $R_f$ 0.66 (SM); IR (KBr) 3440, 3260, 3100, 2920, 2840, 1610, 1500, 1370, 1180; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.79 (4H, m, C6-$H_2$ and C7-$H_2$), 2.75 (4H, m, C5-$H_2$ and C8-$H_2$), 4.90 (2H, br s, exchanged with $D_2O$, $OSO_2NH_2$), 7.03 (2H, m, C1-H and C3-H) and 7.08 (1H, d, $J$ = 9.2 Hz, C4-H); $^{13}$C NMR (100.4 MHz, $CDCl_3$) $\delta$ 22.64 (t), 22.87 (t), 28.83 (t), 29.35 (t), 118.94 (d), 122.09 (d), 130.23 (d), 136.32 (s), 138.98 (s), 147.61 (s); MS (FAB+) *m*/*z* (rel intensity) 381.0 [14, $(M + H + NBA)^+$], 227.1 (100, $M^+$), 147.1 [45, $(M - H_2NSO_2)^+$]; MS (FAB−) *m*/*z* (rel intensity) 380.0 [26, $(M + NBA)^-$], 226.0 [100, $(M - H)^-$]; HRMS (FAB+) *m*/*z* 227.061 74 ($M^+$), calcd for $C_{10}H_{13}NO_3S$ 227.061 62. Anal. ($C_{10}H_{13}NO_3S$) C, H, N.

**5,6,7,8-Tetrahydronaphthalene 2-*O*-(*N*-Methyl)sulfamate (8).** This was prepared according to the procedure used for the preparation of **2**. 5,6,7,8-Tetrahydronaphth-2-ol (150 mg, 1.01 mmol), sodium hydride (60% dispersion, 80 mg, 2.02 mmol), *N*-methylsulfamoyl chloride (260 mg, 2.02 mmol), and DMF (5 mL) were used. Purification was effected using preparative TLC (chloroform/methanol, 96:4) to give **8** as a yellow/green viscous oil (90 mg, 0.373 mmol, 37%): $^{1}$H NMR (270 MHz, $CDCl_3$) $\delta$ 1.77 (4H, quintet, $J$ = 3.25 Hz, C6-$H_2$ and C7-$H_2$), 2.74 (4H, br d, $J$ = 4.22 Hz, C5-$H_2$ and C8-$H_2$), 2.87 (3H, d, $J$ = 5.13 Hz, C$H_3$NH), 4.85 (1H, br q, exchanged with $D_2O$, $J$ = 4.94 Hz, $CH_3NH$), 6.95−7.25 (3H, m, C1-H, C3-H,

and C4-H); $^{13}$C NMR (100.4 MHz, $CDCl_3$) $\delta$ 22.61 (t), 22.80 (t), 28.74 (t), 29.29 (t), 30.10 (q, $CH_3NH$), 118.61 (d), 121.76 (d), 130.16 (d), 135.97 (s), 138.89 (s), 147.51 (s); MS (CI, isobutane) *m/z* (rel intensity) 242 [100, $(M + H)^+$].

**5,6,7,8-Tetrahydronaphthalene 2-*O*-(*N*,*N*-dimethyl)-sulfamate (9).** This was prepared in a manner similar to the preparation of **2**. 5,6,7,8-Tetrahydronaphth-2-ol (300 mg, 2.02 mmol), sodium hydride (60% dispersion, 160 mg, 4.05 mmol), *N*,*N*-dimethylsulfamoyl chloride (580 mg, 4.05 mmol), and DMF (5 mL) were used. Purification of the crude material was effected using flash chromatography (chloroform) to yield **9** as a colorless oil (370 mg, 1.45 mmol, 72%): $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 1.78 (4H, quintet, $J$ = 3.25 Hz, C6-$H_2$ and C7-$H_2$), 2.75 (4H, br d, $J$ = 6.04 Hz, C5-$H_2$ and C8-$H_2$), 2.958 (3H, s, $CH_3N$), 2.960 (3H, s, $CH_3N$), 6.98−7.07 (3H, m, C1-H, C3-H, and C4-H); $^{13}$C NMR (100.4 MHz, $CDCl_3$) $\delta$ 22.70 (t), 22.93 (t), 28.80 (t), 29.39 (t), 38.66 (q, $CH_3N$), 38.73 (q, $CH_3N$), 118.61 (d), 121.76 (d), 130.10 (d), 135.68 (s), 138.79 (s), 147.71 (s); MS (EI, 70 eV) *m/z* (rel intensity) 255 (59, $M^+$), 147 [100, $(M - Me_2NSO_2)^+$].

**7-(Sulfooxy)-4-methylcoumarin, Sodium Salt (11).** To a stirred solution of 7-hydroxy-4-methylcoumarin (1.0 g, 5.676 mmol) in anhydrous pyridine (20 mL), under an atmosphere of $N_2$, was added sulfur trioxide−pyridine complex (1.8 g, 11.35 mmol), and the reaction mixture was stirred overnight. After removal of pyridine, methanol (20 mL) was added to the creamy syrup obtained, and the resulting light yellow solution was basified (pH ∼ 8) by dropwise addition of sodium hydroxide in methanol (1 M). The bright yellow precipitate formed was collected by filtration and washed with fresh methanol. The combined methanolic filtrates were then concentrated to 30−40 mL, and diethyl ether (ca. 120 mL in total) was added in portions until complete precipitation had occurred. The light beige precipitate was collected by filtration and recrystallized from methanol/diethyl ether (1:1) to give **11** as light creamy yellow crystals (409 mg, 1.604 mmol, 28%): mp 172−175 °C dec; TLC (methanol/diethyl ether, 1:2) $R_f$ 0.68, cf. $R_f$ 0.83 (SM); $^1$H NMR (270 MHz, DMSO-$d_6$) $\delta$ 2.41 (3H, s, $CH_3$), 6.27 (1H, s, C3-H), 7.20 (2H, m, C6-H and C8-H), and 7.70 (1H, d, $J$ = 8.8 Hz, C5-H); MS (FAB−) *m/z* (rel intensity) 431.0 [15, $(M + NBA)^-$], 408.0 [8, $M - Na + NBA)^-$], 255.0 [100, $(M - Na)^-$], 175.1 [14, $(M - Na - SO_3)^-$]; HRMS (FAB−, glycerol) *m/z* 254.998 23 [$(M - Na)^-$], calcd for $C_{10}H_7O_6S$ 254.996 33. Anal. ($C_{10}H_7O_6NaS\cdot H_2O$) C, H, N. HPLC [Spherisorb ODS5 column (25 × 4.6 mm); mobile phase, MeOH/$H_2O$ (70:30); flow rate, 1 mL/min; $\lambda_{max}$, 316 nm; $t_R$ = 1.5 min (cf. 7-hydroxy-4-methylcoumarin = 3.6 min).

**4-Methylcoumarin 7-*O*-Sulfamate (12).** A stirred solution of 4-methyl-7-hydroxycoumarin (500 mg, 2.753 mmol) in anhydrous DMF (5 mL) was treated with sodium hydride (60% dispersion, 110 mg, 2.753 mmol) at 0 °C under an atmosphere of $N_2$. After evolution of hydrogen had ceased, sulfamoyl chloride (2 equiv) was added. The reaction mixture was stirred at room temperature overnight and then poured into water (150 mL). The resulting mixture was extracted with ethyl acetate (150 mL) and the organic portion that separated was washed with brine (5 × 100 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo at 40 °C. Purification by flash chromatography (chloroform/acetone, 8:1 to 2:1, gradient) gave the crude product which was further purified by recrystallization from acetone/chloroform (3:5) to give **12** as colorless rhombic crystals (281 mg, 1.101 mmol, 40%): mp 165−167 °C; TLC (chloroform/acetone, 4:1) $R_f$ 0.26, cf. $R_f$ 0.44 (SM); IR (KBr) 3320, 3180, 3080, 1700, 1620, 1560, 1380, 1195, 1125; $^1$H NMR (400 MHz, acetone-$d_6$) $\delta$ 2.50 (3H, d, $^4J_{C3-H,Me}$ = 1.22 Hz, $CH_3$), 6.33 (1H, d, $^4J_{Me,C3-H}$ = 1.22 Hz, C3-H), 7.30 (1H, d, $J_{C6-H,C8-H}$ = 2.14 Hz, C8-H), 7.31 (1H, dd, $J_{C8-H,C6-H}$ = 2.44 Hz and $J_{C5-H,C6-H}$ = 8.54 Hz, C6-H), 7.40 (2H, br s, $D_2O$ exchanged, $OSO_2NH_2$), and 7.86 (1H, d, $J_{C6-H,C5-H}$ = 8.24 Hz, C5-H); $^{13}$C NMR (100.4 MHz, acetone-$d_6$) $\delta$ 18.53 (q, $CH_3$), 110.99 (d), 115.17 (d, C3), 119.12 (d), 119.25 (s), 127.27 (d, C5), 153.19 (s), 153.54 (s), 154.96 (s), 160.14 (s). (These assignments were confirmed by $^{13}$C−$^1$H correlation experiments); MS (FAB+) *m/z* (rel intensity) 256.1 [100, $(M + H)^+$], 177.1 [20, $(M + H - SO_2NH)^+$]; MS (FAB−) *m/z* (rel intensity) 407.1 [15, $(M - H + NBA)^-$], 254 [100, $(M - H)^-$], 175.1 [32, $(M - SO_2NH_2)^-$]. Anal. ($C_{10}H_9NO_5S$) C, H, N.

**Coumarin 7-*O*-Sulfamate (14).** This was prepared from 7-hydroxycoumarin (500 mg, 3.082 mmol) in a manner similar to the preparation of **12**. Purification by flash chromatography (chloroform/acetone, 8:1 to 2:1, gradient) gave the crude product which was further purified by recrystallization from ethyl acetate/hexane (1:1) to give **14** as dull white crystals (239 mg, 991.0 $\mu$mol, 32%): mp 170.0−171.5 °C; $^1$H NMR (270 MHz, DMSO-$d_6$/$CDCl_3$, ca. 1:25) $\delta$ 6.42 (1H, d, $J$ = 9.7 Hz, C3-H), 7.29 (1H, dd, $J$ = 2.3 and 8.5 Hz, C6-H), 7.38 (1H, d, $J$ = 2.2 Hz, C8-H), 7.51 (2H, br s, exchanged with $D_2O$, $OSO_2NH_2$), 7.54 (1H, d, $J$ = 8.4 Hz, C5-H), and 7.76 (1H, d, $J$ = 9.7 Hz, C4-H); MS (EI, 70 eV) *m/z* (rel intensity) 241.0 (10, $M^+$), 162.0 [97, $(M - SO_2NH)^+$], 134.0 (100); HRMS (EI, 70 eV) *m/z* 241.006 84 ($M^+$), calcd for $C_9H_7NO_5S$ 241.004 49. Anal. ($C_9H_7NO_5S$) C, H, N.

**4-(Trifluoromethyl)coumarin 7-*O*-Sulfamate (16).** This was prepared from 7-hydroxy-4-(trifluoromethyl)coumarin (900 mg, 3.832 mmol) in a manner similar to the preparation of **12**. Purification by flash chromatography (diethyl ether/chloroform, 1:4) gave the crude product which was further purified by recrystallization from ethyl acetate/hexane (1:3) to give **16** as white needle-shaped crystals (160 mg, 517.4 $\mu$mol, 14%): mp 165−168 °C; $^1$H NMR (270 MHz, acetone-$d_6$) $\delta$ 6.99 (1H, d, $^4J_{F,H} \approx$ 1 Hz, C3-H), 7.46 (1H, dd, $J$ = 2.4 and 8.1 Hz, C6-H), 7.48 (1H, d, $J$ = 2.4 Hz, C8-H), 7.53 (2H, br s, exchanged with $D_2O$, $OSO_2NH_2$), and 7.89 (1H, m, C5-H) [$^1$H NMR spectrum of **16** in DMSO-$d_6$/$CDCl_3$ (ca. 1:15) showed partial decomposition to the starting coumarin]; MS (EI, 70 eV) *m/z* (rel intensity) 309.0 (2, $M^+$), 230.0 [77, $(M - SO_2NH)^+$], 202.0 (100); HRMS (EI, 70 eV) *m/z* 308.987 43 ($M^+$), calcd for $C_{10}H_6F_3NO_5S$ 308.991 88. Anal. ($C_{10}H_6F_3NO_5S$) C, H, N.

**3,4,8-Trimethylcoumarin 7-*O*-Sulfamate (18).** This was prepared from 7-hydroxy-3,4,8-trimethylcoumarin (1.0 g, 4.896 mmol) in a manner similar to the preparation of **12**. The crude material was recrystallized from hot ethyl acetate whereupon 238 mg of unreacted starting material was recovered. The residue, obtained from the evaporation of the mother liquor, was fractionated by flash chromatography (diethyl ether) to give the crude product which was further purified by recrystallization from acetone/hexane (1:2) to give **18** as pale yellow crystals (312 mg, 1.101 mmol, 22%): mp 197−202 °C; $^1$H NMR (270 MHz, acetone-$d_6$) $\delta$ 2.18 (3H, s, $CH_3$), 2.38 (3H, s, $CH_3$), 2.46 (3H, s, $CH_3$), 7.37 (1H, d, $J$ = 8.8 Hz, C6-H), 7.39 (2H, br s, exchanged with $D_2O$, $OSO_2NH_2$) and 7.68 (1H, d, $J$ = 8.8 Hz, C5-H); MS (EI, 70 eV) *m/z* (rel intensity) 283.1 (10, $M^+$), 204.1 [45, $(M - SO_2NH)^+$], 43.1 (100); HRMS (EI, 70 eV) *m/z* 283.049 67 ($M^+$), calcd for $C_{12}H_{13}NO_5S$ 283.051 44. Anal. ($C_{12}H_{13}NO_5S$) C, H, N.

**4-Methylcoumarin 6-*O*-Sulfamate (20).** This was prepared from 6-hydroxy-4-methylcoumarin (500 mg, 2.810 mmol) in a manner similar to the preparation of **12**. The crude material was recrystallized from warm acetone/hexane (2:1) to yield **20** as soft creamy rod-shaped crystals (285 mg, 1.118 mmol). Further recrystallization of the residue, recovered from the evaporation of the mother liquor, from acetone/hexane (2:1) gave a second crop of **20** (114 mg, 447 $\mu$mol, 56% overall): mp 214−223 °C; $^1$H NMR (270 MHz, acetone-$d_6$) $\delta$ 2.50 (3H, s, $CH_3$), 6.41 (1H, s, C3-H), 7.24 (2H, br s, exchanged with $D_2O$, $OSO_2NH_2$), 7.41 (1H, d, $J$ = 9.0 Hz, C8-H), 7.57 (1H, dd, $J$ = 2.0 and 9.4 Hz, C7-H) and 7.71 (1H, d, $J$ = 2.0 Hz, C5-H); MS (FAB+) *m/z* (rel intensity) 256.1 [100, $(M + H)^+$]; MS (FAB−) *m/z* (rel intensity) 407.9 [35, $(M - H + NBA)^-$], 253.9 [100, $(M - H)^-$]; HRMS (FAB+) *m/z* 256.026 32 [$(M + H)^+$], calcd for $C_{10}H_{10}NO_5S$ 256.027 97. Anal. ($C_{10}H_9NO_5S$) C, H, N.

**6-Methoxycoumarin 7-*O*-Sulfamate (22).** This was prepared from 7-hydroxy-6-methoxycoumarin (70 mg, 346.0 $\mu$mol) in a manner similar to the preparation of **12**. Purification by flash chromatography (chloroform/acetone, 4:1) gave the crude product which was further purified by recrystallization from acetone/hexane (1:2) to give **22** as beige crystals (59 mg, 217.5 $\mu$mol, 63%): mp 174−176 °C; $^1$H NMR (270 MHz, acetone-$d_6$)

$\delta$ 3.92 (3H, s, $OCH_3$), 6.43 (1H, d, $J$ = 9.7 Hz, C3-H), 7.31 (2H, br s, exchanged with $D_2O$, $OSO_2NH_2$), 7.36 (1H, s, C8-H), 7.44 (1H, s, C5-H), and 7.98 (1H, d, $J$ = 9.7 Hz, C4-H); MS (FAB+) $m/z$ (rel intensity) 271.9 [100, $(M + H)^+$], 191.9 [20, $(M - HNSO_2)^+$]; MS (FAB−) $m/z$ (rel intensity) 269.8 [100, $(M - H)^-$], 190.9 [40, $(M - H_2NSO_2)^-$]; HRMS (FAB+) $m/z$ 272.022 46 [$(M + H)^+$], calcd for $C_{10}H_{10}NO_6S$ 272.022 88. Anal. ($C_{10}H_9NO_6S$) C, H, N.

**7-Hydroxy-3,4-dimethylcoumarin (23).** Resorcinol (1.21 g, 11.0 mmol) was dissolved in hot ethyl 2-methylacetoacetate (1.6 g, 10.0 mmol). The mixture was then cooled to 0 °C, and concentrated sulfuric acid (98%, 15 mL) was added dropwise with stirring. The resulting yellow/brown mixture was stirred overnight at room temperature and then poured onto a mixture of ice/water. The bright yellow precipitate was collected by suction filtration and washed exhaustively with water. After the precipitate was redissolved in acetone, the solution was evaporated and drying of the mass obtained was effected azeotropically with isopropyl alcohol. Recrystallization was performed by first redissolving the crude product in hot isopropyl alcohol (30 mL) followed by the dropwise addition of hexane (30 mL). Upon cooling, **23** (369 mg, 1.942 mmol) was separated as pale beige crystals. The residue, recovered from the evaporation of the mother liquor, was recrystallized in a similar manner to give a second crop of **23** (105 mg, 552.6 $\mu$mol, 25% overall); mp 245−265 °C; $^1$H NMR (270 MHz, DMSO-$d_6$) $\delta$ 2.05* (3H, s, C3-$CH_3$), 2.33* (3H, s, C4-$CH_3$), 6.68 (1H, d, $J$ = 2.6 Hz, C8-H), 6.78 (1H, dd, $J$ = 2.6 and 8.8 Hz, C6-H), 7.60 (1H, d, $J$ = 8.8 Hz, C5-H), and 10.36 (1H, s, exchanged with $D_2O$, OH); MS (FAB+) $m/z$ (rel intensity) 191.1 [100, $(M + H)^+$]; MS (FAB−) $m/z$ (rel intensity) 343.3 [46, $(M + NBA)^-$], 189.2 [100, $(M - H)^-$]. Anal. ($C_{11}H_{10}O_3$) C, H. *These assignments were aided by a $^1$H NMR spectrum of **10** in DMSO-$d_6$.

**3,4-Dimethylcoumarin 7-*O*-Sulfamate (24).** This was prepared in a manner similar to the preparation of **12** from **23** (400 mg, 2.103 mmol), sodium hydride (1 equiv), but using more sulfamoyl chloride (5 equiv). Purification by flash chromatography (chloroform/acetone, 15:1 to 8:1, gradient) gave the crude product which was further purified by recrystallization from acetone/hexane (8:3) to give **24** as white crystals (75 mg, 278.5 $\mu$mol). Upon recrystallization of the residue, recovered from the evaporation of the mother liquor, from acetone/hexane (2:5) gave a second crop of **24** (70 mg, 259.9 $\mu$mol, 25% overall); mp 194−196 °C; TLC (ethyl acetate/chloroform, 1:1) $R_f$ 0.46, cf. $R_f$ 0.55 (**23**); IR (KBr) 3380, 3200, 3090, 1680, 1610, 1560, 1390, 1200; $^1$H NMR (270 MHz, acetone-$d_6$) $\delta$ 2.17 (3H, s, C3-$CH_3$), 2.47 (3H, s, C4-$CH_3$), 7.35 (4H, m, reduced to 2H on exchange with $D_2O$, C6-H, C8-H, and $OSO_2NH_2$), and 7.85 (1H, d, $J$ = 8.1 Hz, C5-H); MS (FAB+) $m/z$ (rel intensity) 270.1 [100, $(M + H)^+$], 190.1 [10, $(M - HNSO_2)^+$]; MS (FAB−) $m/z$ (rel intensity) 268.2 [100, $(M - H)^-$], 189.2 [28, $(M - H_2NSO_2)^-$]; HRMS (FAB+) $m/z$ 270.044 82 [$(M + H)^+$], calcd for $C_{11}H_{12}NO_5S$ 270.043 62. Found: C, 48.4; H, 4.05; N, 5.34. $C_{11}H_{11}NO_5S$ requires C, 49.06; H, 4.12; N, 5.20.

**7-Hydroxy-4-methylcoumarin 6-*O*-Sulfamate (26) and 4-Methylcoumarin 6,7-*O*,*O*-Disulfamates (27).** These were obtained when 6,7-dihydroxy-4-methylcoumarin (500 mg, 2.550 mmol) was sulfamoylated in a manner similar to the preparation of **12** using sodium hydride (2 equiv) and sulfamoyl chloride (5 equiv). A 120 mg sample of the crude material (330 mg) was purified by fractionating on preparative TLC (ethyl acetate/acetone/hexane, 2:1:1).

The band at $R_f$ 0.74, cf. SM ($R_f$ 0.46), gave a beige residue which was further purified by recrystallization from ethyl acetate/hexane (1:2) to give **27** as white crystals (24 mg, 68.53 $\mu$mol, 7%): mp 169−172 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.44 (3H, s, $CH_3$), 6.50 (1H, s, C3-H), 7.55 (1H, s, C8-H), 7.81 (1H, s, C5-H), 8.21 (2H, br s, exchanged with $D_2O$, $OSO_2NH_2$), and 8.40 (2H, br s, exchanged with $D_2O$, $OSO_2NH_2$); MS (FAB+) $m/z$ (rel intensity) 350.9 [100, $(M + H)^+$], 272.0 [30, $(M + H - SO_2NH)^+$]; MS (FAB−) $m/z$ (rel intensity) 348.9 [100, $(M - H)^-$], 269.9 [40, $(M - SO_2NH_2)^-$], 191.0 [30, $(M - H - 2SO_2NH)^-$]; HRMS (FAB+) $m/z$ 350.996 01 [$(M + H)^+$], calcd for $C_{10}H_{11}N_2O_8S_2$ 350.995 68. Anal. ($C_{10}H_{10}N_2O_8S_2$) C, H, N.

The band at $R_f$ 0.62 gave a white residue which was further purified by recrystallization from acetone/hexane (1:2) to **26** as white crystals (60 mg, 221.4 $\mu$mol, 24%): mp 203−205 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.37 (3H, s, $CH_3$), 6.22 (1H, s, C3-H), 6.89 (1H, s, C8-H), 7.59 (1H, s, C5-H), ∼8 (2H, br s, exchanged with $D_2O$, $OSO_2NH_2$) and ∼11 (1H, very br s, OH); MS (FAB+) $m/z$ (rel intensity) 272.1 [100, $(M + H)^+$], 255.2[65, $(M + H - NH_3)^+$], 193.2[35, $(M + H - SO_2NH)^+$]; MS (FAB−) $m/z$ (rel intensity) 270.0 [100, $(M - H)^-$], 191.1[30, $(M - SO_2NH_2)^-$]; HRMS (FAB+) $m/z$ 272.023 27 [$(M + H)^+$], calcd for $C_{10}H_{10}NO_6S$ 272.022 88. Found: C, 43.8; H, 3.37; N, 5.11. $C_{10}H_9NO_6S$ requires C, 44.28; H, 3.34; N, 5.16.

**(±)-3,4-Dihydro-7-hydroxy-4-methylcoumarin (30).** A solution of 7-hydroxy-4-methylcoumarin (1.0 g, 5.676 mmol) in ethanol (100 mL) was shaken with Pd−C (10%, 3.0 g) under hydrogen at 75 psi for 3 days. After removal of the supported catalyst by suction filtration, the filtrate was evaporated, and the residue obtained was fractionated by flash chromatography (diethyl ether). The yellow/green syrupy crude product was further purified by recrystallization from chloroform/hexane (5:3) to give **30** as white crystals (719 mg, 4.035 mmol, 71%): mp 105−111 °C; $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 1.31 (3H, d, $J$ = 7.0 Hz, $CH_3$), 2.55 (1H, dd, $J_{C4-H,C3-H_A}$ = 7.5 Hz and $J_{C3-H_B,C3-H_A}$ = 15.8 Hz, C3-$H_A$), 2.83 (1H, dd, $J_{C4-H,C3-H_B}$ = 5.5 Hz and $J_{C3-H_A,C3-H_B}$ = 15.8 Hz, C3-$H_B$), 3.11 (1H, m, C4-H), 5.23 (1H, s, exchanged with $D_2O$, OH), 6.62 (2H, m, C6-H and C8-H), and 7.08 (1H, d, $J$ = 7.9 Hz, C5-H). Anal. ($C_{10}H_{10}O_3$) C, H.

**(±)-3,4-Dihydro-4-methylcoumarin 7-*O*-Sulfamate (31).** This was prepared from **30** (500 mg, 2.806 mmol) in a manner similar to the preparation of **12**. Purification by flash chromatography (chloroform/acetone, 20:1 to 4:1, gradient) gave the crude product which was further purified by recrystallization from ethyl acetate/hexane (1:1) to give **31** as white crystals (357 mg, 1.387 mmol, 49%): mp 152−155 °C; $^1$H NMR (400 MHz, acetone-$d_6$) $\delta$ 1.32 (3H, d, $J$ = 7.0 Hz, $CH_3$), 2.63 (1H, dd, $J_{C4-H,C3-H_A}$ = 7.0 Hz and $J_{C3-H_B,C3-H_A}$ = 15.9 Hz, C3-$H_A$), 2.93 (1H, dd, $J_{C4-H,C3-H_B}$ = 5.6 Hz and $J_{C3-H_A,C3-H_B}$ = 16.0 Hz, C3-$H_B$), 3.30 (1H, m, C4-H), 7.00 (1H, d, $J$ = 2.4 Hz, C8-H), 7.11 (1H, dd, $J$ = 2.3 and 8.4 Hz, C6-H), 7.22 (2H, br s, exchanged with $D_2O$, $OSO_2NH_2$), and 7.44 (1H, d, $J$ = 8.2 Hz, C5-H); MS (FAB+) $m/z$ (rel intensity) 411.0 [24, $(M + H + NBA)^+$], 258.1 [100, $(M + H)^+$]; MS (FAB−) $m/z$ (rel intensity) 410.0 [22, $(M + NBA)^-$], 256.0 [100, $(M - H)^-$]. Anal. ($C_{10}H_{11}NO_5S$) C, H, N.

**3,4-Dihydro-7-hydroxycoumarin (32).** A solution of 7-hydroxycoumarin (2.0 g, 12.34 mmol) in ethanol (120 mL) was shaken with Pd−C (10%, 2.0 g) under hydrogen at 40 psi for 24 h. After removal of the supported catalyst by suction filtration, the filtrate was evaporated and the residue obtained was fractionated by flash chromatography (diethyl ether). The light brown crude product was further purified by recrystallization from toluene to give **32** as white crystals (1.25 g, 7.623 mmol, 61%): mp 134−137 °C (lit.[59] mp 132−133 °C); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.78 (2H, m, C3-$H_2$), 2.93 (2H, t, $J \approx$ 7.0 Hz, C4-$H_2$), 5.42 (1H, s, exchanged with $D_2O$, OH), 6.60 (2H, m, C6-H and C8-H), and 7.04 (1H, d, $J$ = 8.8 Hz, C5-H); MS (FAB+) $m/z$ (rel intensity) 165.1 [100, $(M + H)^+$]; HRMS (FAB+) $m/z$ 165.056 23 [$(M + H)^+$], calcd for $C_9H_9O_3$ 165.055 17. Anal. ($C_9H_8O_3$) C, H.

**3,4-Dihydrocoumarin 7-*O*-Sulfamate (33).** This was prepared from **32** (500 mg, 3.049 mmol) in a manner similar to the preparation of **12**. Purification by flash chromatography (chloroform/acetone, 20:1 to 4:1, gradient) gave the crude product which was further purified by recrystallization from ethyl acetate/hexane (2:1) to give **33** as white crystals (378 mg, 1.554 mmol, 51%): mp 169.5−171.5 °C; $^1$H NMR (400 MHz, DMSO-$d_6$/$CDCl_3$, ca. 1:15) $\delta$ 2.80 (2H, t, $J \approx$ 7.3 Hz, C3-$H_2$), 3.02 (2H, t, $J \approx$ 7.3 Hz, C4-$H_2$), 7.10 (2H, m, C6-H and C8-H), 7.16 (2H, br s, exchanged with $D_2O$, $OSO_2NH_2$), and 7.22 (1H, d, $J$ = 7.6 Hz, C5-H); MS (FAB+) $m/z$ (rel intensity) 397.0 [30, $(M + H + NBA)^+$], 244.1 [100, $(M + H)^+$], 164.1 [26, $(M - SO_2$-

NH)$^+$]; MS (FAB−) *m/z* (rel intensity) 396.0 [22, (M + NBA)$^-$], 242.0 [100, (M − H)$^-$]; HRMS (FAB+) *m/z* 244.027 44 [(M + H)$^+$], calcd for $C_9H_{10}NO_5S$ 244.027 97. Anal. ($C_9H_9NO_5S$) C, H, N.

**2,3-Dihydro-7-hydroxy-4*H*-1-benzopyran-4-one (34).** This compound was synthesized from resorcinol and acrylnitrile as described by Breytenbach and Rall.[56]

**2,3-Dihydro-7-[dimethyl(1,1-dimethylethyl)silyl]-4*H*-1-benzopyran-4-one (35).** To a stirred mixture of **34** (800 mg, 4.873 mmol) and imidazole (1.35 g, 19.63 mmol) in DMF (5 mL) under nitrogen at room temperature was added dimethyl-(1,1-dimethylethyl)silyl chloride (1.03 g, 6.628 mmol). After being left to stir overnight, the reaction mixture was diluted with diethyl ether (100 mL). The resulting mixture was then washed with 1 M HCl (100 mL) and then brine (4 × 50 mL), dried ($MgSO_4$), filtered, and evaporated. The residue obtained was fractionated by flash chromatography (ethyl acetate/hexane, 1:8 to 1:2, gradient) to give **35** as a pale green/yellow liquid (1.33 g, 4.777 mmol, 98%): $^1$H NMR (400 MHz, $CDCl_3$, no TMS) $\delta$ 0.24 [6H, s, $Si(CH_3)_2$], 0.98 [9H, s, $C(CH_3)_3$], 2.75 (2H, t, $J$ = 6.4 Hz, C3-$H_2$), 4.50 (2H, t, $J$ = 6.4 Hz, C2-$H_2$), 6.37 (1H, d, $J$ = 2.1 Hz, C8-H), 6.50 (1H, dd, $J$ = 2.3 and 8.7 Hz, C6-H), and 7.80 (1H, d, $J$ = 8.6 Hz, C5-H); MS (EI, 70 eV) *m/z* (rel intensity) 278.2 (24, M$^+$), 221.1 (100, [M − $C(CH_3)_3$]$^+$), 179.1 (6, [M + H − $Si(CH_3)C(CH_3)_3$]$^+$), 85.1 (8, [$SiC(CH_3)_3$]$^+$), 57.1 (23, [$C(CH_3)_3$]$^+$).

**2*H*-1-Benzopyran-7-ol (38).** Lithium aluminum hydride (5.1 mL, 1.0 M in THF) was added to a stirred solution of **35** (1.28 g, 4.598 mmol) in diethyl ether (10 mL) at −78 °C under nitrogen. The reaction mixture was allowed to warm to room temperature over 2 h and then poured cautiously onto ice. After acidification with hydrochloric acid (1 M, 20 mL), the resulting mixture was extracted with diethyl ether (100 mL). The organic layer that separated was further washed with brine exhaustively, dried ($MgSO_4$), filtered, and evaporated to give crude **36** as a light brown syrup (1.14 g): TLC (ethyl acetate/hexane, 1:1) $R_f$ 0.51, cf. $R_f$ 0.67 (**35**). This crude product was used immediately in the next reaction without further purification.

A mixture of the crude enantiomeric alcohols **36** (1.14 g), triethylamine (2.5 mL, 17.76 mmol), and methanesulfonyl chloride (350 $\mu$L, 4.432 mmol) in dichloromethane (20 mL) was stirred at room temperature for 24 h. After the reaction mixture was concentrated in vacuo, the residue afforded was redissolved in diethyl ether (100 mL) and washed with water (5 × 50 mL). The combined aqueous washes were then back-extracted with chloroform (4 × 50 mL). The organic extracts were combined, dried ($MgSO_4$), filtered, and evaporated. The crude residue was fractionated by flash chromatography (ethyl acetate/hexane, 1:16 to 1:8, gradient) to give the silylated 2*H*-1-benzopyran-7-ol (**37**) as a pale green/yellow oil (840 mg, 3.201 mmol, 70%): $^1$H NMR (270 MHz, $CDCl_3$, no TMS) $\delta$ 0.19 [6H, s, $Si(CH_3)_2$], 0.97 [9H, s, $C(CH_3)_3$], 4.77 (2H, m, C2-$H_2$), 5.62 (1H, dt, $J_{C2-H_2,C3-H} \approx$ 3.5 Hz and $J_{C4-H,C3-H}$ = 9.7 Hz, C3-H), 6.29 (1H, d, $J$ = 2.9 Hz, C8-H), 6.35 (1H, dd, $J$ = 2.4 and 8.1 Hz, C6-H), 6.37 (1H, d, $J$ = 9.7 Hz, C4-H), and 6.81 (1H, d, $J$ = 8.1 Hz, C5-H); MS (EI, 70 eV) *m/z* (rel intensity) 262.2 (55, M$^+$), 205.1 (100, [M − $C(CH_3)_3$]$^+$), 163.0 (13, [M + H − $Si(CH_3)C(CH_3)_3$]$^+$).

A mixture of tetra-*n*-butylammonium fluoride (2.0 mL, 1 M in THF), **37** (500 mg, 1.905 mmol), and diethyl ether (25 mL) was stirred at room temperature for 10 min. The reaction mixture was then diluted with diethyl ether (100 mL) and the resulting mixture washed with brine (5 × 50 mL), dried ($MgSO_4$), filtered, and evaporated to give crude 2*H*-1-benzopyran-7-ol (**38**) as an unstable light brick red liquid (380 mg); TLC (ethyl acetate/hexane, 1:2) $R_f$ 0.53, cf. $R_f$ 0.68 (**37**). This crude **38** was sulfamoylated immediately.

**2*H*-1-Benzopyran 7-*O*-Sulfamate (39).** This was prepared from crude **38** (380 mg) in a manner similar to the preparation of **12**. Purification of the crude material obtained immediately by flash chromatography (ethyl acetate/hexane, 1:4 to 1:1, gradient) gave **39** as a greenish yellow syrup (240 mg, 1.056 mmol, 55%). This syrup exhibited noticeable decomposition after 24 h at room temperature but appeared to be stable if stored under nitrogen at −20 °C. Sulfamate **39** was further purified by preparative TLC (ethyl acetate/hexane, 1:1) before analysis and bioassay: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 4.84 (2H, m, C2-$H_2$), 4.98 (2H, br s, exchanged with $D_2O$, $OSO_2NH_2$), 5.78 (1H, dt, $J_{C2-H_2,C3-H} \approx$ 3.5 Hz and $J_{C4-H,C3-H}$ = 10.1 Hz, C3-H), 6.40 (1H, dt, $^4J \approx$ 1.6 Hz and $J_{C3-H,C4-H}$ = 7.9 Hz, C4-H), 6.74 (1H, d, $J$ = 2.4 Hz, C8-H), 6.82 (1H, dd, $J$ = 2.4 and 8.2 Hz, C6-H), and 6.95 (1H, d, $J$ = 8.2 Hz, C5-H); MS (FAB+) *m/z* (rel intensity) 226 [47, (M − H)$^+$], 147 [100, (M − $SO_2NH_2$)$^+$]; HRMS (FAB+) *m/z* 226.017 34 [(M − H)$^+$], calcd for $C_9H_8NO_4S$ 226.017 41.

**6-[Dimethyl(1,1-dimethylethyl)silyl]-1-tetralone (40).** The silylation reaction was performed in a manner similar to **35**. 6-Hydroxy-1-tetralone (**45**) (1.8 g, 11.10 mmol), DMF (10 mL), imidazole (1.9 g, 27.74 mmol), and dimethyl(1,1-dimethylethyl)silyl chloride (2.1 g, 13.51 mmol) were used. The residue obtained from the workup was fractionated by flash chromatography (ethyl acetate/hexane, 1:8) to give **40** as a pale yellow liquid (2.93 g, 10.60 mmol, 96%); $^1$H NMR (270 MHz, $CDCl_3$, no TMS) $\delta$ 0.23 [6H, s, $Si(CH_3)_2$], 0.98 [9H, s, $C(CH_3)_3$], 2.13 (2H, quintet, $J \approx$ 6 Hz, C3-$H_2$), 2.60 (2H, t, $J \approx$ 6.5 Hz, C4-$H_2$), 2.89 (2H, t, $J \approx$ 6.0 Hz, C2-$H_2$), 6.65 (1H, d, $J$ = 2.7 Hz, C5-H), 6.74 (1H, dd, $J$ = 2.3 and 8.7 Hz, C7-H) and 7.95 (1H, d, $J$ = 8.6 Hz, C8-H); MS (EI, 70 eV) *m/z* (rel intensity) 276.2 (35, M$^+$), 261.3 [7, (M − $CH_3$)$^+$], 219.1 (100, [M − $C(CH_3)_3$]$^+$), 192.1 (8, [M + H − $SiC(CH_3)_3$]$^+$), 163.1 (8, [M + 2H − $Si(CH_3)_2C(CH_3)_3$]$^+$), 115.1 (12, [$Si(CH_3)_2C(CH_3)_3$]$^+$), 57.1 (15, [$C(CH_3)_3$]$^+$).

**7,8-Dihydronaphth-2-ol (43).** The multistep synthesis to **38** from **35** was used to prepare this compound from **40**. **40** (2.49 g, 9.009 mmol), diethyl ether (30 mL), and lithium aluminum hydride (9.9 mL, 1.0 M in THF) were used, and crude **41** was afforded as a pale yellow syrup (2.10 g).

Crude enantiomeric alcohols **41** (2.10 g), triethylamine (4.5 mL, 31.96 mmol), methanesulfonyl chloride (700 $\mu$L, 8.862 mmol), and dichloromethane (50 mL) were used. Fractionation of the crude residue by flash chromatography (ethyl acetate/hexane, 1:8) gave the silylated 7,8-dihydronaphth-2-ol (**42**) as a pale yellow oil (1.40 g, 5.376 mmol, 60%): $^1$H NMR (270 MHz, $CDCl_3$, no TMS) $\delta$ 0.19 [6H, s, $Si(CH_3)_2$], 0.98 [9H, s, $C(CH_3)_3$], 2.28 (2H, m, C7-$H_2$), 2.73 (2H, t, $J$ = 8.2 Hz, C8-$H_2$), 5.89 (1H, dt, $J_{C7-H_2,C6-H} \approx$ 4.4 Hz and $J_{C5-H,C6-H}$ = 9.5 Hz, C6-H), 6.40 (1H, d, $J$ = 9.7 Hz, C5-H), 6.62 (2H, m, C1-H and C3-H), and 6.87 (1H, d, $J$ = 8.6 Hz, C4-H); MS (EI, 70 eV) *m/z* (rel intensity) 260.2 (38, M$^+$), 203.1 (100, [M − $C(CH_3)_3$]$^+$).

Silylated 7,8-dihydronaphth-2-ol (**42**) (500 mg, 1.920 mmol), diethyl ether (25 mL), and tetra-*n*-butylammonium fluoride (2.0 mL, 1.0 M in THF) were used and gave 7,8-dihydronaphth-2-ol (**43**) as an unstable light yellow/brown syrup (390 mg). This crude **43** was sulfamoylated immediately.

**7,8-Dihydronaphthalene 2-*O*-Sulfamate (44).** This was prepared from crude **43** (390 mg) in a manner similar to the preparation of **12**. Purification by flash chromatography (ethyl acetate/hexane, 1:8 to 1:4, gradient) gave the crude product which was further purified by recrystallization from ethyl acetate/hexane (1:10) to afford **44** as fine white crystals (210 mg, 822.7 $\mu$mol, 43%): mp 100−102 °C; $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 2.32 (2H, m, C7-$H_2$), 2.81 (2H, t, $J$ = 8.2 Hz, C8-$H_2$), 4.93 (2H, br s, exchanged with $D_2O$, $OSO_2NH_2$), 6.07 (1H, dt, $J_{C7-H_2,C6-H} \approx$ 4.6 Hz and $J_{C5-H,C6-H}$ = 9.5 Hz, C6-H), 6.45 (1H, d, $J$ = 9.7 Hz, C5-H), and 7.0−7.15 (3H, m, Ar-H); MS (FAB+) *m/z* (rel intensity) 225.1 (100, M$^+$), 145.1 [30, (M − $SO_2NH_2$)$^+$]; MS (FAB−) *m/z* (rel intensity) 224.0 [100, (M − H)$^-$]. Anal. ($C_{10}H_{11}NO_3S$) C, H, N.

**6-Hydroxy-1-tetralone (45).** 6-Methoxy-1-tetralone (6.0 g, 34.04 mmol) was added to a stirred suspension of aluminum chloride (11.3 g, 85.1 mmol) in anhydrous toluene (150 mL) at room temperture, under nitrogen. The reaction mixture was refluxed for 30 min, cooled, and cautiously quenched with water. The resulting mixture was then extracted with ethyl acetate (150 mL). The organic portion that separated was washed with water exhaustively, dried ($MgSO_4$), filtered, and evaporated in vacuo. Recrystallization of the orange brown

residue from hot toluene (three times) gave **45** as fine beige crystals (5.09 g, 31.38 mmol, 92%): mp 151−155 °C (lit.[60] mp 148−152 °C); $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 2.11 (2H, quintet, $J \approx 6$ Hz, C3-$H_2$), 2.63 (2H, t, $J \approx 6.0$ Hz, C4-$H_2$), 2.91 (2H, t, $J \approx 6.0$ Hz, C2-$H_2$), 6.54 (1H, br s, exchanged with $D_2O$, OH), 6.70 (1H, d, $J = 2.4$ Hz, C5-H), 6.78 (1H, dd, $J = 2.6$ and 8.6 Hz, C7-H), and 7.98 (1H, d, $J = 8.6$ Hz, C8-H); MS (EI, 70 eV) *m*/*z* (rel intensity) 162.1 (60, $M^+$), 134.0 (100). Anal. ($C_{10}H_{10}O_2$) C, H.

**1-Tetralone 6-*O*-Sulfamate (46).** This was prepared from **45** (1.0 g, 6.165 mmol) in a manner similar to the preparation of **12**. Purification by flash chromatography (chloroform/acetone, 8:1 to 2:1, gradient) gave the crude product which was further purified by recrystallization from acetone/hexane (7:4) to afford **46** as dull white rod-shaped crystals (447 mg, 1.853 mmol, 30%): mp 174−175 °C; $^1$H NMR (270 MHz, acetone-$d_6$) $\delta$ 2.13 (2H, quintet, $J \approx 6$ Hz, C3-$H_2$), 2.62 (2H, t, $J \approx 6.6$ Hz, C4-$H_2$), 3.02 (2H, t, $J \approx 5.8$ Hz, C2-$H_2$), 7.28 (4H, m, reduced to 2H upon exchange with $D_2O$, C5-H, C7-H, and $OSO_2NH_2$), and 7.99 (1H, d, $J = 8.8$ Hz, C8-H); MS (FAB+) *m*/*z* (rel intensity) 242.1 [100, $(M + H)^+$]; MS (FAB−) *m*/*z* (rel intensity) 240.0 [100, $(M - H)^-$], 161.0 [10, $(M - SO_2NH_2)^-$]. Anal. ($C_{10}H_{11}NO_4S$) C, H, N.

**7-Hydroxy-1-tetralone (47).** This was prepared from 7-methoxy-1-tetralone (4.0 g, 22.69 mmol) in a manner similar to the preparation of **45**. Recrystallization of the orange brown residue from acetone/hexane (1:5) afforded **47** as fine white crystals (3.49 g, 21.52 mmol, 95%): mp 165−167 °C; $^1$H NMR (270 MHz, acetone-$d_6$) $\delta$ 2.07 (2H, m, C3-$H_2$), 2.55 (2H, t, $J \approx 6.5$ Hz, C4-$H_2$), 2.87 (2H, t, $J = 5.9$ Hz, C2-$H_2$), 7.02 (1H, dd, $J = 2.9$ and 8.2 Hz, C6-H), 7.17 (1H, d, $J = 8.1$ Hz, C5-H), 7.39 (1H, d, $J = 2.6$ Hz, C8-H), and 8.55 (1H, br s, exchanged with $D_2O$, OH); MS (FAB+) *m*/*z* (rel intensity) 163.1 [100, $(M + H)^+$]; MS (FAB−) *m*/*z* (rel intensity) 314.3 [65, $(M - H + NBA)^-$], 161.1 [100, $(M - H)^-$]. Anal. ($C_{10}H_{10}O_2$) C, H.

**1-Tetralone 7-*O*-Sulfamate (48).** This was prepared from **47** (1.0 g, 6.164 mmol) in a manner similar to the preparation of **12**. Purification by flash chromatography (chloroform/acetone, 4:1) gave the crude product which was further purified by recrystallization from acetone/hexane (1:1) to give **48** as beige crystals (433 mg, 1.797 mmol, 29%): mp 150−152 °C; $^1$H NMR (270 MHz, acetone-$d_6$) $\delta$ 2.16 (2H, m, C3-$H_2$), 2.63 (2H, t, $J \approx 6.5$ Hz, C4-$H_2$), 3.02 (2H, t, $J \approx 6$ Hz, C2-$H_2$), 7.21 (2H, br s, exchanged with $D_2O$, $OSO_2NH_2$), 7.47 (2H, m, C5-H and C6-H), and 7.84 (1H, d, $J \approx 1$ Hz, C8-H); MS (FAB+) *m*/*z* (rel intensity) 242.2 [100, $(M + H)^+$]; MS (FAB−) *m*/*z* (rel intensity) 394.1 [43, $(M + NBA)^-$], 240.1 [100, $(M - H)^-$]. Anal. ($C_{10}H_{11}NO_4S$) C, H, N.

**1-Indanone 4-*O*-Sulfamate (49).** This was prepared from 4-hydroxy-1-indanone (500 mg, 3.375 mmol) in a manner similar to the preparation of **12**. Purification by flash chromatography (chloroform/acetone, 8:1) gave the crude product which was further purified by recrystallization from ethyl acetate/hexane (1:3) to give **49** as brown/yellow crystals (80 mg, 352.4 $\mu$mol, 11%): mp 193−196 °C; $^1$H NMR (270 MHz, acetone-$d_6$) $\delta$ 2.67 (2H, m, C3-$H_2$), 3.25 (2H, t, $J \approx 5.9$ Hz, C2-$H_2$), 7.39 (2H, br s, exchanged with $D_2O$, $OSO_2NH_2$), 7.52 (1H, t, $J = 7.7$ Hz, C6-H), 7.64 (2H, m, C5-H and C7-H); MS (FAB+) *m*/*z* (rel intensity) 228.1 [100, $(M + H)^+$]; MS (FAB−) *m*/*z* (rel intensity) 226.0 [100, $(M - H)^-$]; HRMS (FAB+) *m*/*z* 228.031 21 [$(M + H)^+$], calcd for $C_9H_{10}NO_4S$ 228.033 05. Anal. Found: C, 46.0; H, 3.94; N, 5.83. $C_9H_9NO_4S$ requires C, 47.57; H, 3.99; N, 6.16.

**5-Hydroxy-1-indanone (50).** This was prepared from 5-methoxy-1-indanone (1.0 g, 6.166 mmol) in a manner similar to the preparation of **45**. Recrystallization of the orange brown residue from acetone/hexane (1:5) gave **50** as fine yellow/brown crystals (787 mg, 5.312 mmol, 86%): mp 182−185 °C; $^1$H NMR (270 MHz, acetone-$d_6$) $\delta$ 2.55 (2H, m, C3-$H_2$), 3.04 (2H, t, $J \approx 6$ Hz, C2-$H_2$), 6.9 (2H, m, C4-H and C6-H), 7.53 (1H, d, $J = 8.1$ Hz, C7-H), and 9.37 (1H, br s, exchanged with $D_2O$, OH); MS (FAB+) *m*/*z* (rel intensity) 149.1 [100, $(M + H)^+$]; MS (FAB−) *m*/*z* (rel intensity) 147.1 [100, $(M - H)^-$]; HRMS (FAB+) *m*/*z* 149.058 79 [$(M + H)^+$], calcd for $C_9H_9O_2$ 149.060 25. Found: C, 72.40; H, 5.46. $C_9H_8O_2$ requires C, 72.96; H, 5.44.

**1-Indanone 5-*O*-Sulfamate (51).** This was prepared from **50** (250 mg, 1.687 mmol) in a manner similar to the preparation of **12**. Purification by flash chromatography (chloroform/acetone, 4:1) gave the crude product which was further purified by recrystallization from acetone/hexane (1:2) to afford **51** as beige crystals (121 mg, 532.9 $\mu$mol, 31%): mp 174−176 °C; $^1$H NMR (270 MHz, acetone-$d_6$) $\delta$ 2.68 (2H, t, $J \approx 6$ Hz, C3-$H_2$), 3.19 (2H, t, $J \approx 5.9$ Hz, C2-$H_2$), 7.36 (3H, m, 2H exchanged with $D_2O$, $OSO_2NH_2$ and C6-H), 7.51 (1H, s, C4-H) and 7.71 (1H, d, $J = 8.5$ Hz, C7-H); MS (FAB+) *m*/*z* (rel intensity) 228.1 [100, $(M + H)^+$], 147.1 [10, $(M - SO_2NH_2)^+$]; MS (FAB−) *m*/*z* (rel intensity) 226.1 [100, $(M - H)^-$], 147.1 [20, $(M - SO_2NH_2)^-$]; HRMS (FAB+) *m*/*z* 228.032 69 [$(M + H)^+$], calcd for $C_9H_{10}NO_4S$ 228.033 05. Anal. ($C_9H_9NO_4S$) C, H, N.

**6-Hydroxy-1-indanone (52).** This was prepared from 6-methoxy-1-indanone (2.5 g, 14.95 mmol) in a manner similar to the preparation **45**. Recrystallization of the yellow/brown residue from hot toluene afforded **52** as light yellow/brown crystals (2.0 g, 13.50 mmol, 90%): mp 156−158 °C; $^1$H NMR (270 MHz, DMSO-$d_6$/$CDCl_3$, ca. 1:20) $\delta$ 2.66 (2H, m, C3-$H_2$), 3.04 (2H, t, $J \approx 5.7$ Hz, C2-$H_2$), 7.13 (2H, m, C5-H and C7-H), 7.31 (1H, d, $J = 8.9$ Hz, C4-H), and 9.23 (1H, br s, exchanged with $D_2O$, OH). Anal. ($C_9H_8O_2$) C, H.

**1-Indanone 6-*O*-Sulfamate (53).** This was prepared from **52** (545 mg, 3.683 mmol) in a manner similar to the preparation of **12**. Purification by flash chromatography (chloroform/acetone, 4:1) gave the crude product which was further purified by recrystallization from acetone/hexane (1:2) to give **53** as yellow crystals (315 mg, 1.388 mmol, 37%): mp 155−157 °C; $^1$H NMR (270 MHz, acetone-$d_6$) $\delta$ 2.71 (2H, m, C3-$H_2$), 3.19 (2H, t, $J \approx 5.9$ Hz, C2-$H_2$), 7.26 (2H, br s, exchanged with $D_2O$, $OSO_2NH_2$), 7.57 (2H, m, C5-H and C7-H) and 7.67 (1H, d, $J = 9.2$ Hz, C4-H); MS (FAB+) *m*/*z* (rel intensity) 228.0 [100, $(M + H)^+$], 148.0 [15, $(M - SO_2NH)^+$]; MS (FAB−) *m*/*z* (rel intensity) 225.9 [100, $(M - H)^-$], 146.9 [8, $(M - SO_2NH_2)^-$]; HRMS (FAB+) *m*/*z* 228.033 56 [$(M + H)^+$], calcd for $C_9H_{10}NO_4S$ 228.033 05. Anal. ($C_9H_9NO_4S$) C, H, N.

**2-Nitrophenol *O*-Sulfamate (54) and Azomethine (55).** 2-Nitrophenol (1.391 g, 10.0 mmol) was sulfamoylated in a manner similar to the preparation of **12**. Purification by flash chromatography (ethyl acetate/hexane, 1:1) gave the crude 2-nitrophenol *O*-sulfamate which was further purified by recrystallization from hot chloroform to afford **54** as white crystals (333 mg, 745.8 $\mu$mol). The residue recovered, from the evaporation of the mother liquor, was recrystallized from chloroform/hexane to give further crops of **54** (a total of 252 mg, 564.4 $\mu$mol, 26% overall); mp 102−103 °C; $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 5.29 (2H, br s, exchanged with $D_2O$, $OSO_2NH_2$), 7.48 (1H, m, C4-H or C5-H), 7.66 (1H, dd, $J = 1.5$ and 8.3 Hz, C3-H or C6-H), 7.71 (1H, m, C4-H or C5-H), and 8.05 (1H, dd, $J = 1.5$ and 8.3 Hz, C3-H or C6-H); MS (EI, 70 eV) *m*/*z* (rel intensity) 218 (2, $M^+$), 139 [100, $(M - SO_2NH)^+$]; MS (CI, isobutane) *m*/*z* (rel intensity) 219 [27, $(M + H)^+$], 202 [10, $(M + H - NH_3)^+$], 140 [100, $(M + H - SO_2NH)^+$], 122 [15, $(M - OSO_2NH_2)^+$]. Anal. ($C_6H_6N_2O_5S$) C, H, N.

A fraction of higher polarity was also collected from the flash column which upon recrystallization from hot isopropyl alcohol afforded **55** as white crystals (163 mg, 597.1 $\mu$mol, 6%): mp 122−123 °C; $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 3.03 (3H, s, N−$CH_3$), 3.22 (3H, s, N−$CH_3$), 7.39 (1H, m, Ar-H), 7.65 (2H, m, Ar-H), 7.90 (1H, dd, $J = 1.3$ and 8.2 Hz, Ar-H), and 8.09 (1H, s, N=CH); $^{13}$C NMR (100.4 MHz, $CDCl_3$) $\delta$ 35.79 (q), 41.99 (q), 125.33 (d), 125.93 (d), 126.85 (d), 134.23 (d), 142.94 (s), 143.21 (s), 161.36 (d); MS (EI, 70 eV) *m*/*z* (rel intensity) 273 (4, $M^+$), 135 [100, $(Me_2NCH{=}NSO_2)^+$]; MS (CI, isobutane) *m*/*z* (rel intensity) 274 [28, $(M + H)^+$], 135 [100, $(Me_2NCH{=}NSO_2)^+$]. Anal. ($C_9H_{11}N_3O_5S$) C, H, N.

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