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Estrogen Receptor Ligands. 12. Synthesis of the Major Metabolites of an ERα-Selective, Dihydrobenzoxathiin Antagonist for Osteoporosis

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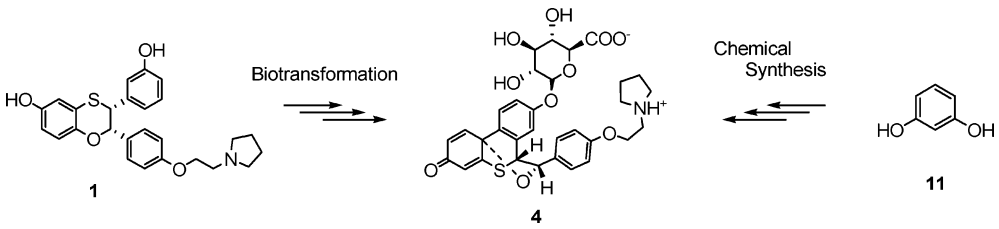
ABSTRACT



1 4 11

During the course of drug metabolism studies, a major metabolite of compound 1 was detected in rhesus monkeys and assigned structure 4. The intriguing biotransformation of 1 leading to 4 was confirmed by a 19-step total synthesis starting from resorcinol (11), the key feature of which was the construction of the oxygen bridge utilizing a phenolic oxidation and trapping sequence. In addition, the synthesis of a related metabolite (5) is described.

In conjunction with a medicinal chemistry program targeting selective estrogen receptor modulators (SERMs), dihydrobenzoxathiin **1**[1] was discovered to be a potent SERAM[2] (Selective Estrogen Receptor Alpha Modulator) that exhibited subnanomolar binding to ERα and 40-fold selectivity. Further evaluation of **1** revealed its promise for the prevention of estrogen-deficiency osteopenia in postmenopausal women, without uterotropism,[2b] and thereby **1** qualified as a developmental candidate.[3]

In preclinical studies in the rhesus monkey, the major metabolites of **1** included two *O*-glucuronides (**2** and **3**)[4] and the bridge compound **4**,[5] as depicted in Figure 1, which together accounted for approximately 70% of the metabolism. Intestinal extraction via glucuronidation, "first-pass effect", accounted for the formation of the two *O*-glucuronides. However, the intriguing biotransformation leading to **4** was seemingly unprecedented. A mechanism, which is described in Scheme 1, may be postulated to partially proceed through a quinone intermediate **7**, generated from a phenolic-radical fragmentation process embedded in the dihydrobenzoxathiin

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(1) Kim, S.; Wu, J. Y.; Chen, H. Y.; DiNinno, F. *Org. Lett.* **2003**, *5*, 685.

(2) (a) Kim, S: Wu, J. Y.; Birzin, E. T.; Frisch, K.; Chan, W.; Pai, L. Y.; Yang, Y. T.; Mosley, R. T.; Fitzgerald, P. M. D.; Sharma, N.; Dahlund, J.; Thorsell, A. G.; DiNinno, F.; Rohrer, S. P.; Schaeffer, J. M.; Hammond, M. L. *J. Med. Chem.* **2004**, *47*, 2171. (b) Kim, S.; Wu, J. Y.; Chen, H. Y.; Birzin, E. T.; Chan, W.; Yang, Y. T.; Colwell, L.; Li, S.; Dahlund, J.; DiNinno, F.; Rohrer, S. P.; Schaeffer, J. M.; Hammond, M. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2741. (c) Chen, H. Y.; Kim, S.; Wu, J. Y.; Birzin, E. T.; Chan, W.; Yang, Y. T.; Dahlund, J.; DiNinno, F.; Rohrer, S. P.; Schaeffer, J. M.; Hammond, M. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2551.

(3) Song, Z. J.; King, A. O.; Waters, M. S.; Lang, F.; Zewge, D.; Bio, M.; Leazer, J. L., Jr.; Javadi, G.; Kassim, A.; Tschaen, D. M.; Reamer, R. A.; Rosner, T.; Chilenski, J. R.; Mathre, D. J.; Volante, R. P.; Tillyer, R. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 5776.

(4) Independent synthesis confirmed structures of **2** and **3**, respectively.

(5) The structure of this unusual oxygen bridge metabolite **4** and **5** was unambiguously determined by extensive 2D NMR experiments (ROESY, HMQC, HMBC) and LC/MS/MS. The absolute configuration at C3 was assumed to be (*R*) on the basis of the fact that stereocenter at C3 should remain unchanged during the biotransformation.

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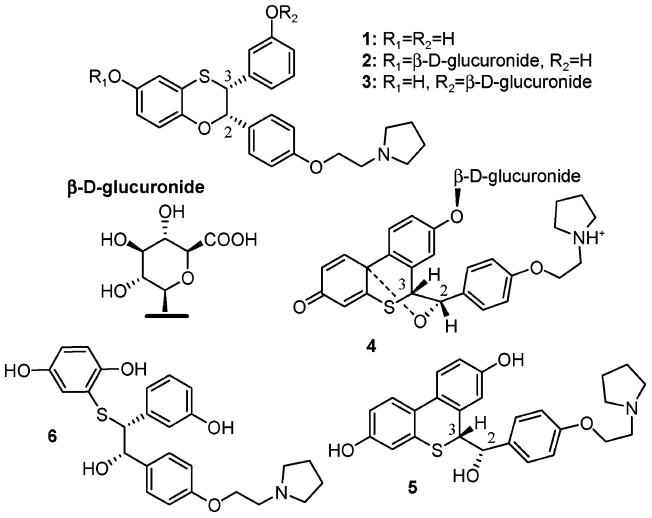
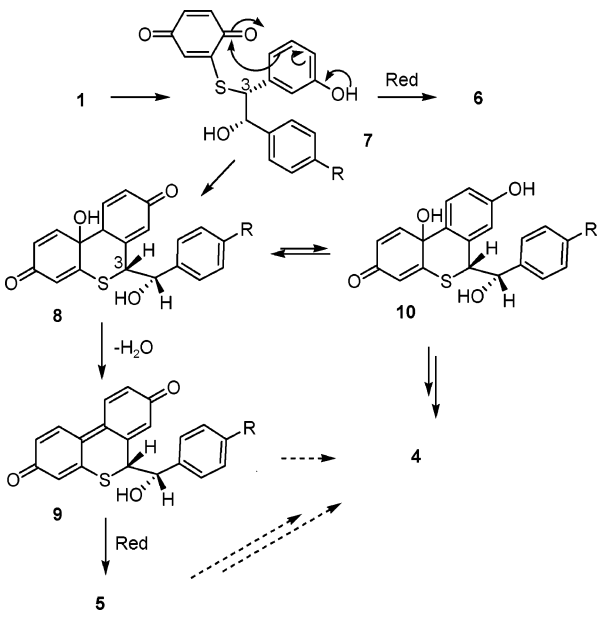


Figure 1.

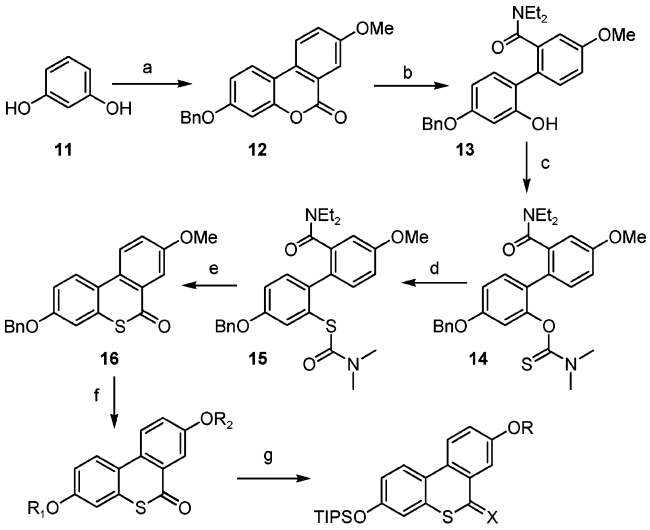
core, followed by a nucleophilic addition of a phenolic-radical in the pendant aromatic ring to the resultant benzoquinone moiety in the A-ring to provide the intermediate **8**. In turn, this initially formed intermediate would eventually yield metabolite **4** via sequential glucuronidation and bridge ether formation of the tautomeric phenol **10**. Intuitively, the isolation of **5** and **6**[6] from the in vitro incubation of **1** with monkey liver microsomes provided support for the plausibility of the above metabolic pathway. The possibility that metabolite **5** would be a biosynthetic precursor of **4**, however, could not be ruled out. Although mechanistic probing awaits further study, the unique structural features of compound **4**, combined with the fascinating biotransformaton, prompted us to attempt the total synthesis of **4** and **5**. To confirm the structure assignments, as well as to further evaluate the metabolites, we targeted the synthesis of all of the different stereoisomers and now report the details of this effort.

Our initial goal was to secure the carbon framework as shown in Scheme 2. The synthesis began with the known

Scheme 1



Scheme 2[a]



[a] Key: (a) (i) 2-bromo-5-methoxybenzoic acid, $CuSO_4$, NaOH, (ii) BnBr, Cs_2CO_3, quant; (b) Me_3Al, Et_2NHCl, 80 °C, 90%; (c) chlorodimethyl thiocarbamate, NaH, DMAP, 75%; (d) Ph_2O, 280 °C, 4 h, 75%; (e) NaOH in MeOH, then 1 N HCl, quant; (f) (i) BBr_3, 0 °C, (ii) TIPSCl, 70% for two steps, (iii) BBr_3, rt, 80%; (g) (i) LAH, 0 °C, (ii) TFA, Et_3SiH, 80% for two steps, (iii) TBSCl, Et_3N, quant yield.

compound **12**,[7] which was easily synthesized from resorcinol **11** in two steps. The resulting coumarin **12** was converted into the corresponding amide **13** in excellent yield by reaction with methylchloroaluminum amide.[8]

A low yield was observed when dimethylamine was used as the amidating reagent, due in part to concomitant reclosure to the starting material **12**. The phenol **13**[9] was immediately allowed to react with dimethylthiocarbamoyl chloride to afford the *O*-aryl dimethylthiocarbamate **14** in 75% yield, which upon pyrolysis gave the *S*-aryl dimethylthiocarbamate **15** as the product of the Newman−Kwart rearrangement.[10] A temperature of 280 °C was necessary for clean conversion;

(6) We isolated **6** as a single isomer, whose stereochemistry was believed to be (*S*), the result of a retention of the stereochemistry of C-2 in compound **1**. However, we did not confirm the stereochemistry.

(7) (a) Devlin, J. P. *Can. J. Chem.* **1975**, *53*, 343. (b) Lederer, E.; Polonsky, J. *Bull. Soc. Chim. Fr.* **1948**, 831.

(8) Levin, J. I.; Turos, E.; Weinreb, S. M. *Synth. Commun.* **1982**, *12*, 989.

(9) After overnight at room temperature, ca. 20% of the product was converted back to the starting material **12**.

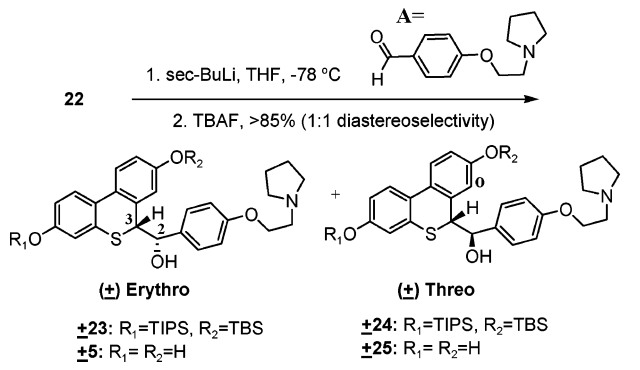
(10) Villemin, D.; Hachemi, M.; Lalaowi, M. *Synth. Commun.* **1996**, *26*, 2461.

otherwise only starting material or complex mixtures were obtained. Alkaline hydrolysis of compound **15** followed by simple addition of acid (dilute HCl) promoted smooth cyclization to the thiocoumarin **16** in high yield.

At this juncture, the synthetic plan required a suitable protecting group strategy for the installation of the glucuronide in the B ring, and phenolic oxidation in the A-ring that would build the ether bridge framework. The benzyl group was selectively removed with 1.5 equiv of boron tribromide at 0 °C to the corresponding phenol **17**, which without purification was transformed to the TIPS-protected compound **18**. Then selective deprotection of methyl group was realized with an excess of boron tribromide at room temperature to afford the desired compound **19** in quantitative yield. This operation was dictated by our inability to cleave the methoxy group at a latter part of the synthesis due to the acid lability[11] of the final compound **4**. Partial reduction of compound **19** with LAH in THF at 0 °C gave the 10-hydroxy thiopyran **20**, which was further reduced to compound **21** with TFA/Et_3SiH. It should be noted that this reduction initially gave the dimeric ether (not shown)[12] upon treatment with 10−15 equiv of TFA; however, an excess amount of acid was required to drive the reduction to completion. The protection of the phenol with TBSCl was uneventful, which ultimately set the stage for the completion of target compounds **4** and **5**.

Having secured a viable sequence to the core **22**, we then focused on the installation of the pyrrolidine side chain, which should then directly provide metabolite **5**. Treatment of thiopyran **22** with *n*-BuLi at −78 °C resulted in a deep-reddish-colored lithiated complex. This α-thio anion[13] seemed stable at −78 °C but slowly decomposed at higher temperatures (> −40 °C), and reaction with the aldehyde **A** provided a mixture of products (±)-erythro **23** and (±)-threo **24**, in high yield, with 1:1 diasteroselectivity, as determined by ^{1}H NMR (Scheme 3). No further attempt was made to

Scheme 3



improve the stereoselectivity, since both diastereomers were needed for structural elucidation of both metabolites **4** and **5**. To our delight, chromatographic separation of both diastereomers, as well as the enantiomers, was realized by HPLC using a chiracel OD column. Separately, all four isomers underwent cleavage of the silicon protecting groups with TBAF at room temperature to afford the respective stereoisomers of **5**: (+)-**5**, (−)-**5**, (+)-**25**, and (−)-**25**. Among them, compound (−)-**5** was found to be identical in all respects to an authentic sample of the metabolite (^{1}H NMR, ^{13}C NMR, MS, HPLC,[14] $[\alpha]_D = -148°$ in MeOH, $c = 0.466$).

The configurations of the newly created stereogenic centers at $C_{2,3}$ were assigned as follows. For **23** and **24**, the coupling constant of $H_{2,3}$, from the observed AX pattern, were large at $J = 9.2$ and 9.6 Hz, respectively, suggesting that the two protons adopted an antiperiplanar conformation. In this way, both isomers **23** and **24** would have minimal gauche interactions. However, most striking was the chemical shift of H_o in the B ring of threo **24**, which resonated at δ 5.98 ppm. In contrast, the same proton of the erythro isomer **23** was found at δ 6.86 ppm. The unusual, higher field chemical shift of the threo isomer was attributed to the anisotropic effect of circulating π-electrons of the phenyl group of the side chain. Accordingly, the configuration at $C_{2,3}$ in **5** was designated erythro, **23**.[15]

With the enantiomer (−)-**5** in hand, we continued to pursue the synthesis of **4** starting with enantiomer (−)-**23**, whose stereochemistry at $C_{2,3}$ was believed to be related to the metabolite, based primarily on the mechanism by which metabolite **4** could be derived from **5**. Toward this end, the benzylic alcohol (−)-**23** was converted to the TES ether **26** in 90% yield after treatment with TESOTf. Selective deprotection of the phenolic TBS ether **26** was effected by exposure to mild basic medium[16] to afford the monophenol **27** in 75% yield. More basic conditions, such as TBAF or Cs_2CO_3, tended to give a mixture of phenols in a nonselective manner. Subsequent glycosylation was achieved in 70% yield under Schmidt conditions, wherein the phenol **27** in CH_2Cl_2 was treated with chloroimidate **B** and catalytic BF_3-etherate, at −10 °C.[17] This coupling provided a single product **28** with the desired β-stereochemistry at the anomeric center,[18] as present in the metabolite **4**. Exhaustive desilylation with TBAF in the presence of HOAc delivered the penultimate intermediate **29** in 80% yield (Scheme 4).[19]

(11) An independent stability study of **4** showed that only 60% of the parent compound remained after 3 days at room temperature, in pH 7 buffer solution. However, **4** was relatively stable at −10 °C. The instability of the bridge ether may be due to the inherent strain in the ring system.

(12) (a) Hori, M.; Kataoka, T.; Shimizu, H.; Komatsu, O.; Hamada, K. *J. Org. Chem.* **1987**, *52*, 3668. (b) Ridley, D. D.; Smal, M. A. *Aust. J. Chem.* **1983**, *36*, 795.

(13) The α-thio anion seemed to be quite sensitive to oxygen, and therefore THF was rigorously degassed by N_2 prior to use. Unterhalt, B.; Bruening, S. *Sci. Pharm.* **1997**, *65*, 1.

(14) The four isomers of **5** were well separated on a Chiracel OD-RH column (4.6 mm × 150 mm, 3 μm).

(15) For the isothiochromane system, see: (a) Tomooka, K.; Wang, L. F.; Okazaki, F.; Nakai, T. *Tetrahedron Lett.* **2000**, 6121. (b) Bohme, H.; Sutoyo, P. N. *Phosphorous Sulfur Silicon Relat. Elem.* **1982**, *13*, 235.

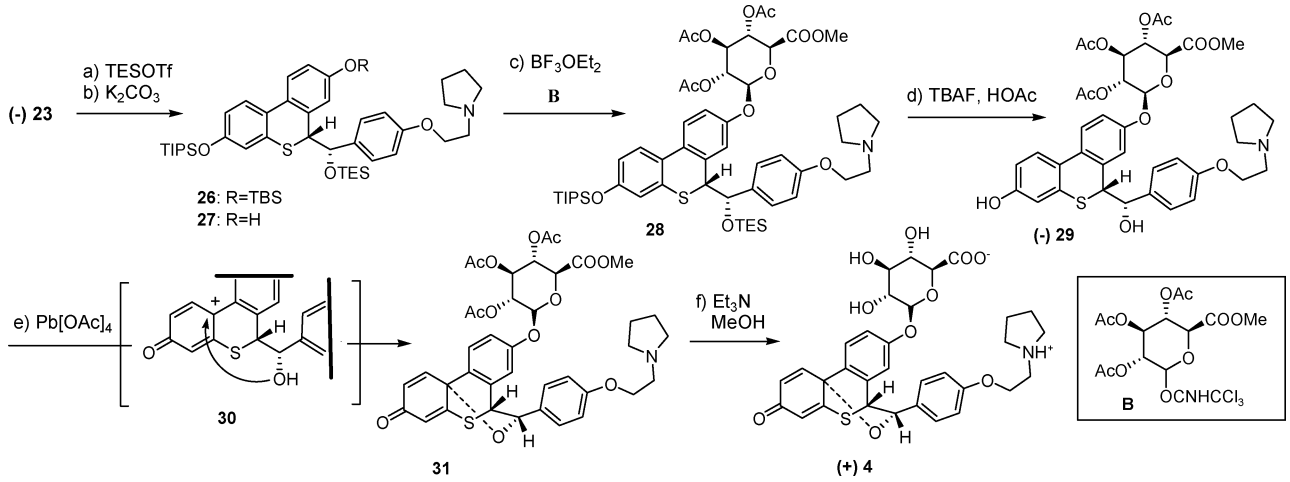
(16) Wilson, N. S.; Keay, B. A. *Tetrahedron Lett.* **1997**, *38*, 187.

(17) (a) Schmidt, R. R. *Pure Appl. Chem.* **1989**, *61*, 1257. Chloro imidate B was prepared in 3 steps from the commercially available methyl-1,2,3,4-tetra-*O*-acetyl-D-glucopyranuronate.

(18) For **28**, a 9.2 Hz trans diaxial coupling constant was observed between 1′-H and 2′-H in the glycoside. For comparison, it was 7.6 Hz in metabolite **4**.

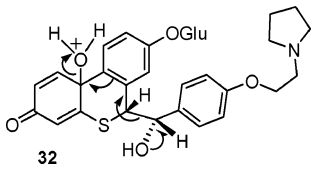
(19) Addition of HOAc was required because of the basic lability of the acetate group in the glycosidic moiety.

Scheme 4[a]



[a] Key: (a) TESOTf, Et_3N, CH_2Cl_2, 90%; (b) K_2CO_3, EtOH, H_2O, 75%; (c) Schmidt reagent **B**, CH_2Cl_2, BF_3OEt_2, 70%; (d) TBAF, HOAc, rt, 80%; (e) $Pb[OAc]_4$, CH_2Cl_2, $NaHCO_3$, 5 min, rt; (f) $Et_3N/MeOH/H_2O$ = 1:1:4, THF, 40% for two steps.

At this point in the synthetic plan, it was envisioned that nucleophilic attack by the hydroxyl group to the arene-oxonium ion[20] in intermediate **30**, generated from a two-electron oxidation of **29**, would lead to the desired six-membered bridge ether **31**. To this end, a number of oxidants[21] were employed (DDQ, PIDA, and ferric isocyanate), but these attempts produced only a small amount of the desired product **31**, along with complex mixtures. Interestingly, a careful examination of the crude material identified the presence of aldehyde **A** (see Scheme 3), which may have resulted from a retro-Aldol fragmentation of **32**,[11]



an intermediate that could rapidly degrade from the bridge ether **31** in the acidic medium. The putative companion intermediate (not shown) was not isolable. After extensive experimentation, clean oxidation was accomplished by brief exposure of **29** to $Pb[OAc]_4$ buffered with either $NaHCO_3$ or pyridine. This protocol consistently gave rise to the desired product **31**, which without further purification underwent hydrolysis under basic conditions.[22] Purification of the crude product by reverse-phase HPLC provided the desired zwitterionic compound (+)-**4**, in 40% yield for the two steps.[23] To our delight, the synthetic material was found to be identical in all respects to the metabolite (^1H NMR, ^{13}C NMR, MS, coelution on HPLC, $[\alpha]_D = +87.5°$ in 1:2 H_2O/AcCN, $c = 0.8$). The other stereoisomers, (+)-**23**, (+)-**24**, and (−)-**24**, were also converted into the analogous derivatives by application of the same procedures. Upon HPLC comparison,[24] it was found that they were all distinct from the metabolite.

In summary, the confirmation of the structural assignments of the metabolites **4** and **5** of the dihydrobenzoxathiin derivative **1** was accomplished by an independent, 19-step total synthesis.

Acknowledgment. We gratefully acknowledge Professor Barry M. Trost for helpful discussions during his scientific consultations.

Supporting Information Available: Characterization (^1H NMR, 2D NMR, ^{13}C NMR, and HPLC) of synthetic and authentic **4** and **5** and spectroscopic data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) (a) Quideau, S.; Lebon, M.; Lamidey, A. *Org. Lett.* **2002**, *4*, 3975. (b) Quideau, S.; Pouysegu, L.; Oxoby, M.; Looney, M. A. *Tetrahedron* **2001**, *57*, 319.

(21) (a) Venkateswarlu, R.; Kamakshi, C.; Moinuddin, S. G. A.; Subhash, P. V.; Ward, R. S.; Pelter, A.; Coles, S. J.; Hursthouse, M. B.; Light, M. E. *Tetrahedron* **2001**, *57*, 273 (b) Wipf, P.; Km, Y. *J. Org. Chem.* **1993**, *58*, 1649.

(22) Vaccaro, W.; Davis, H. R., Jr. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 313.

(23) Metachem Basic C8 4.6 mm × 30 mm, 5 μm, H_2O/CH_3CN.

(24) HPLC conditions: Phenomenex-Luna 5 μM Phenyl-Hexyl, 4.6 mm × 250 mm, H_2O/CH_3CN.