Natural Product Synthesis

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Enantioselective Synthesis of the Complex Rocaglate (−)-Silvestrol**

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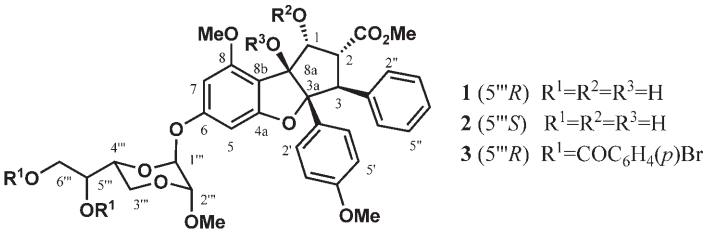
Extracts from dried roots and stems of several species of the plant genus *Aglaia* are the source of the rocaglamides, a unique group of natural products featuring a cyclopenta[*b*]tetrahydrobenzofuran skeleton.[1] The complex rocaglate silvestrol (**1**) and its epimer **2** (Figure 1) were recently isolated from the plant *Aglaia foveolata* by Kinghorn and coworkers.[2,3] The structural assignment of **1** was based on NMR spectroscopy and X-ray diffraction studies of the bis-*p*-bromobenzoate derivative **3**. Compound **1** was shown to exhibit very potent cytotoxic activity (e.g. $ED_{50} = 1.2$ nm against human lung cancer cells) which is comparable to the activity of the anticancer agent Paclitaxel (Taxol). Mechanism-of-action studies indicate that cytotoxicity induced by silvestrol in human prostate cancer (LNCaP) cells is associated with a block in the cell cycle at the G2/M checkpoint in a manner that is independent of p53.[3] In contrast to other rocaglate derivatives, silvestrol possesses a unique dioxanyloxy group at C6 of the cyclopenta[*b*]benzofuran core. Although the rocaglamides[1c,d] and the corresponding dioxanyloxy side chain[1e,f] of silvestrol have been the subject of synthetic studies, an enantioselective synthesis of silvestrol has not been reported to date. We have chosen the promising anticancer lead compound silvestrol as a synthetic target to further develop applications of our enantioselective photogeneration/cycloaddition of oxidopyryliums derived from 3-hydroxyflavones[4] and to develop chemistry pertaining to the synthesis and attachment of the structurally unique dioxanyloxy segment.



1 (5‴*R*) $R^1=R^2=R^3=H$
2 (5‴*S*) $R^1=R^2=R^3=H$
3 (5‴*R*) $R^1=COC_6H_4(p)Br$

Figure 1. (−)-Silvestrol and related derivatives.

Our retrosynthetic analysis of silvestrol (**1**) is illustrated in Figure 2. Compound **1** may be derived from C–O bond formation between 1,4-dioxan-2-ol **4** and hydroxyphenyl rocaglate derivative **5**. Compound **5** may be prepared using photocycloaddition methodology developed in our laboratory to access the methyl rocaglate core by employing protected 3-hydroxyflavone **6** as starting material.[4] The unusual dioxanyloxy fragment **4** may be obtained via 1,4-dioxan-2-one precursor **7**, which may be derived from 1,2-dibenzylthreitol derivative **8**. Intermediate **8** is readily obtained from the commercially available reagent D-dimethyl tartrate.[5]

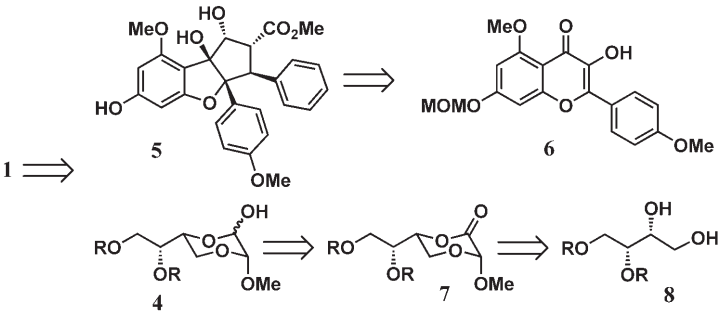


Figure 2. Retrosynthetic analysis of (−)-silvestrol (**1**).

The synthesis of the protected 3-hydroxyflavone **6** began with Friedel–Crafts acylation of phloroglucinol (**9**) using benzyloxyacetyl chloride in the presence of $AlCl_3$ following a modified procedure (Scheme 1).[6] After MOM protection and methylation, the derived aryl ketone **10** was subjected to selective MOM deprotection in the presence of a catalytic amount of iodine in methanol,[7] followed by acylation with 4-methoxybenzoyl chloride, to afford phenyl ester **11**. Baker–Venkataraman rearrangement of **11** under basic conditions (LiHMDS, THF) yielded diketone **12**.[8] Treatment of **12** with sodium acetate in acetic acid effected smooth cyclization/dehydration[9] with concomitant removal of the MOM ether. Reintroduction of the MOM protecting group and subsequent hydrogenolysis of the benzyl group provided the requisite 3-hydroxyflavone **6**.

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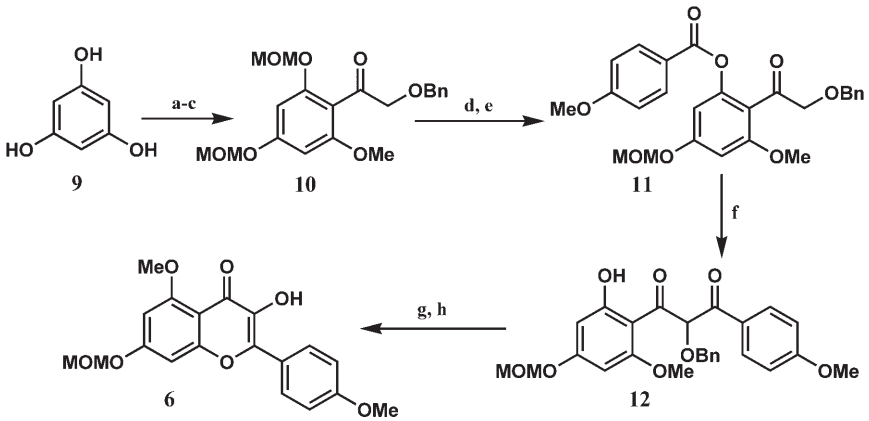
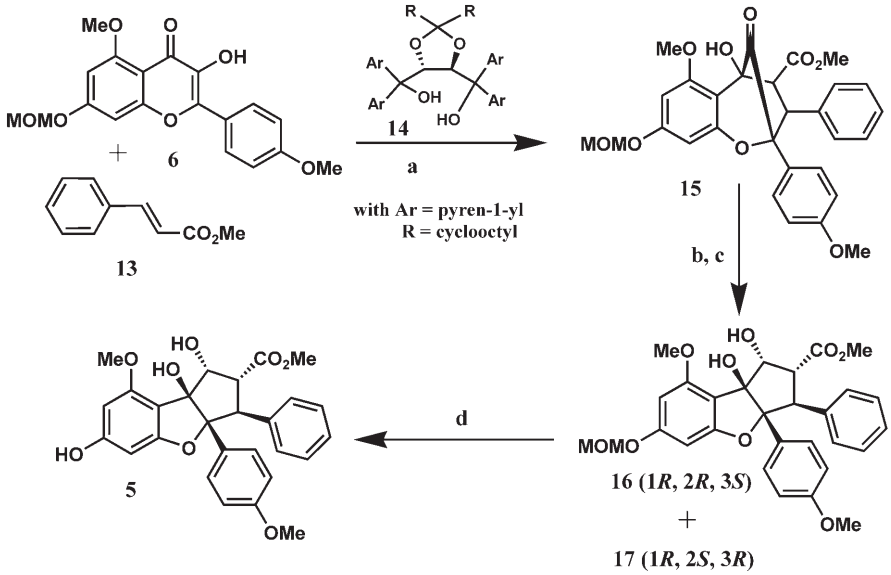


Scheme 1. a) Benzyloxyacetyl chloride, $AlCl_3$, CH_2Cl_2/Et_2O (1:1), 50 °C, 24 h, 58%; b) MOMCl, K_2CO_3, acetone, RT, 3 h, 60%; c) Me_2SO_4, K_2CO_3, acetone, 60 °C, 6 h, 93%; d) I_2, MeOH, RT, 4 h, 94%; e) 4-methoxybenzoyl chloride, Et_3N, DMAP, CH_2Cl_2, RT, 4 h, 85%; f) LiHMDS (3 equiv), THF, −20 °C, 1 h, 88%; g) AcOH, AcONa (2.5 equiv), 100 °C, 3 h; h) MOMCl, acetone, K_2CO_3, RT, 6 h, 70% over two steps; i) H_2, $Pd(OH)_2$, EtOH/THF (1:1), RT, 45 min, 92%. Bn = benzyl, MOM = methoxymethyl, DMAP = 4-dimethylaminopyridine, HMDS = hexamethyldisilazide.

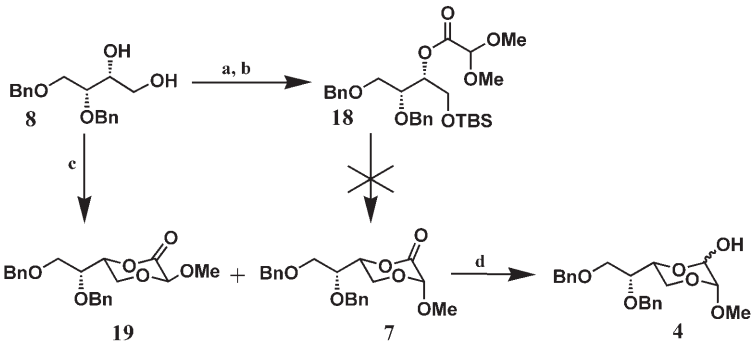
With compound **6** in hand, we proceeded to evaluate the asymmetric synthesis of methyl rocaglate fragment **5** by employing enantioselective [3+2] photocycloaddition mediated by functionalized TADDOL derivatives (TADDOL = α,α,α′,α′-tetraaryl-2,2-dimethyl-1,3-dioxolan-4,5-dimethanol).[4b] During our previous investigations, we found that both the nature of the aryl substituent and ketal side chain of the TADDOL framework, as well as low-temperature reaction conditions, were crucial factors for high enantioselectivity. Photocycloaddition ($h\nu > 350$ nm) of 3-hydroxyflavone **6** and methyl cinnamate (**13**) in the presence of chiral additive **14**, bearing a 1-pyrenyl substituent and cyclooctyl ketal, at −70 °C using $PhCH_3/CH_2Cl_2$ (2:1) as solvent led to the formation of cycloadduct **15** as well as its ketol-shift isomer[4a] after purification on SiO_2 (Scheme 2). After an α-ketol rearrangement/hydroxy-directed reduction sequence,[4a] *endo*-rocaglate derivative **16** was isolated in 57% yield and 71% *ee* along with the corresponding *exo* stereoisomer **17**. Compound **16** was then subjected to MOM deprotection using TMSBr in CH_2Cl_2 to afford hydroxyphenyl rocaglate derivative **5**. Fortunately, we were able to increase the enantiomeric excess of **5** through recrystallization to afford centrosymmetric racemate crystals[4b] and **5** with 87% *ee* (75% recovery) in the mother liquor.



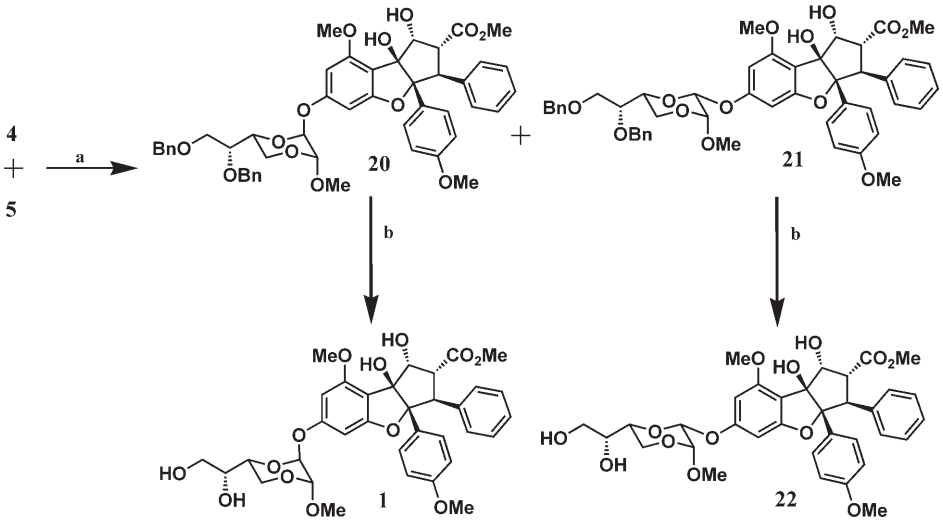
Scheme 2. a) $h\nu > 350$ nm, CH_2Cl_2/toluene, −70 °C, 10 h, 66%; b) MeONa (2.5 equiv), MeOH, 60 °C, 30 min, 89%; c) $Me_4NBH(OAc)_3$, MeCN, AcOH, 57% (**16**), 13% (**17**); d) TMSBr, CH_2Cl_2, 3 h, −78 °C, 84% yield, 87% *ee* after recrystallization. TMS = trimethylsilyl.

The synthesis of the dioxanyloxy fragment **4** was initiated with (2*S*,3*S*)-1,2-di-*O*-benzylidenethreitol (**8**), which was readily obtained in four steps from commercially available D-dimethyl tartrate.[5] Our first strategy to obtain 1,4-dioxan-2-one **7** was based on a three-step synthesis starting from **8** involving protection of the primary alcohol, followed by esterification with dimethoxyacetic acid, and final deprotection of the primary alcohol and attempted transacetalization of **18** under acid conditions (e.g. *p*-TsOH, CSA, K-10 clay) (Scheme 3). However, using this pathway we could not isolate the desired dioxane derivative **7**. The major product isolated was characterized as the primary ester derivative obtained from acyl transfer during the deprotection step. A similar approach led us to investigate a one-pot process for functionalization of the 1,2-diol **8** through regioselective alkylation followed by lactonization. We thus envisioned the use of a tin acetal as a reactive intermediate for regioselective alkylation. Formation of the *O*-stannylene acetal[10] derived from **8**, followed by addition of freshly prepared methyl 2-bromo-2-methoxy acetate,[11] afforded **7** (45%) and its stereoisomer **19** (33%). The overall process **8**→**7** represents a tandem alkylation/lactonization sequence.[12] Attempted epimerization of dioxanyl derivative **19** was unsuccessful and led mostly to decomposition. Finally, DIBAL reduction of **7** produced 1,4-dioxan-2-ol **4** as a mixture of diastereoisomers.[13]

With the two fragments **4** and **5** in hand, we evaluated a series of conditions for their coupling (Scheme 4).[14] Utilization of glucosidation methods using fluoride or trichloroacetamidate reagents derived from **4** led to unsatisfactory yields of coupling products and significant decomposition. After



Scheme 3. a) TBSCl, imidazole, Et_3N, DMF, RT, 6 h; b) dimethoxyacetic acid, DCC, DMAP, THF, RT, 82% over two steps; c) nBu_2SnO, benzene, reflux 9 h, $CH_3OCHBrCO_2Me$, TBAI, benzene, 70°C, 2 h 45% (**7**), 33% (**18**); d) DIBAL-H, toluene, −78°C, 1 h, 83%. TBS = *tert*-butyldimethylsilyl, DCC = dicyclohexyl carbodiimide, TBAI = tetra-*n*-butylammonium iodide, DIBAL-H = diisobutylaluminum hydride.



Scheme 4. a) DIAD, F-PPh_3, toluene, 4-Å MS, RT, 42% (**20**), 20% (**21**); b) H_2, $Pd(OH)_2$, EtOH, 87%. DIAD = diisopropylazodicarboxylate, F-PPh_3 = diphenyl-[4-(1H,1H,2H,2H-perfluorodecyl)phenyl]phosphine.

considerable experimentation, we identified the Mitsunobu reaction[1f,15] as a suitable transformation for fragment coupling of **4** and **5**. However, in this particular instance, separation of the triphenylphosphine oxide by-product from the desired products was found to be problematic. Fortunately, use of a fluorous-tagged triphenylphosphine reagent (F-PPh_3)[16] enabled facile product purification after filtration through fluorous silica gel. The desired coupling product was isolated as a separable mixture of diastereoisomers **20** (42%) and **21** (20%). Finally, hydrogenation of **20** and **21** using Pearlman's catalyst afforded silvestrol (**1**) and its 1‴ stereoisomer **22**. Data for synthetic **1** were confirmed to be identical with those reported for natural (−)-silvestrol[2] including ^1H and ^{13}C NMR, mass, and IR spectra, $[\alpha]_D$ values, HPLC, and TLC R_f values.[17]

Cyclopenta[*b*]benzofurans are known protein synthesis inhibitors.[18,19] Accordingly, we compared the relative potencies of silvestrol (**1**) and its 1‴ diastereoisomer **22** in an in vitro translation system utilizing a rabbit reticulocyte lysate programmed with firefly luciferase (FLuc) mRNA.[20] Titration of silvestrol (**1**) revealed an IC_{50} value of approximately 0.4 μM, whereas **22** showed an IC_{50} value of about 2 μM (Figure 3a), indicating that **22** is fivefold less active than **1**. The relative potency of **1** and **22** was also assessed in vivo by exposing cells to the two compounds, followed by monitoring the incorporation of ^{35}S-methionine into proteins. The results indicate a 10-fold difference in IC_{50} values between silvestrol (**1**) and **22**, with silvestrol being more potent for inhibition of protein synthesis in HeLa cells (Figure 3b).

In conclusion, we have accomplished the enantioselective synthesis of the rocaglate natural product and antitumor agent (−)-silvestrol. The key strategy involves an enantioselective dipolar cycloaddition of oxidopyrylium ylides derived from excited-state intramolecular proton transfer (ESIPT) of 3-hydroxyflavones using specifically functionalized TADDOL derivatives as chiral Brønsted acids. The unusual 1,4-dioxanyl unit was generated from readily available starting materials using a tandem alkylation/lactonization sequence. Initial biological studies indicate that silvestrol has approximately a 5–10-fold greater activity as an inhibitor of protein synthesis in vivo and in vitro in HeLa cells than its 1‴ diastereomer, illustrating the influence of the stereochemistry of the dioxanyl moiety on biological activity.[21] Further studies toward the synthesis of related rocaglamide derivatives and biological evaluation of silvestrol and related molecules will be reported in due course.

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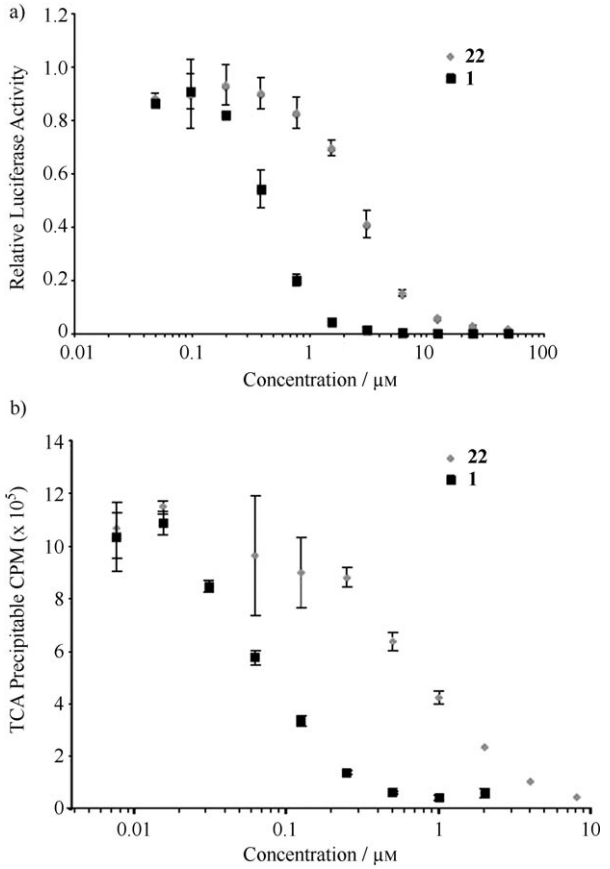

Figure 3. a) Dose-dependent inhibition of translation in vitro by **1** and **22** in rabbit reticulocyte lysates. Firefly luciferase activity of FF/HCV/Ren mRNA in the presence of compound **1** or **22** was normalized to the value obtained in the presence of vehicle (DMSO). b) Dose-dependent inhibition of protein synthesis in vivo in HeLa cells by **1** and **22**. TCA: separation of radiolabeled protein from unincorporated 35S-methionine by precipitation. The material was quantified by liquid scintillation counting.

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