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SYNTHESIS OF FLUORESCENT SOLAMIN FOR VISUALIZATION OF CELL DISTRIBUTION[†]

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Abstract - Asymmetric synthesis of fluorescent solamin was accomplished by using highly stereoselective asymmetric alkynylation and Williamson etherification as the key steps, wherein the fluorescent acetogenin with all functionality was firstly synthesized.

Annonaceous plants produce a polyketide family of natural products, so-called *annonaceous* acetogenins.¹ They have attracted considerable attention by the interesting biological activity represented by potent cytotoxicity against human cancer cell lines. The mode of action was supposed to be based on its strong inhibitory activity against a mitochondrial complex I. However, the structure–activity relationship against the complex I inhibition was not completely related to its cytotoxicity. McLaughlin suggested that the mitochondrial assay is cell-free and does not take into consideration factors such as membrane transport, intracellular transport, metabolic inactivation, *etc.*²

Poupon and co-workers reported the first synthesis of fluorescent squamocin analogue and visualization of its cell distribution.³ Their synthesis is based on degradation of a natural acetogenin. As a result, the γ -lactone moiety was lost by the modification. Recently, Yao and co-workers also reported a new fluorescent acetogenins analogue,⁴ in which the THF rings were replaced to acyclic ethers for simplification. These reports prompted us to synthesize a novel fluorescent *annonaceous* acetogenin, which retains all functionalities.

We have developed a systematic and stereoselective synthesis of *annonaceous* acetogenins.⁵ By applying this methodology, we planned to synthesize a novel fluorescent *annonaceous* acetogenin, which possesses all functional groups including the γ -lactone moiety and THF ring flanking by two hydroxy groups. Among the many acetogenin congeners, we selected solamin having a comparatively simple structure, but potent cytotoxicity against a wide range of cancer cell lines.^{6,7} We planned to introduce the fluorescent tag at the end of the unfunctionalized hydrocarbon chain, because both the γ -lactone, the THF, and hydroxy

[†] Dedicated to the great contribution to organic chemistry of the late professor Ivar Ugi.

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moieties seem to play an important role against biological activities in the acetogenin congeners.⁸ The 7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl-amino (NBD-NH-) group was employed as a fluorescent tag due to its strong fluorescence with long wavelength, that is advantageous to observe the cell distribution.⁹ Herein, we describe a highly stereoselective synthesis of the NBD-labeled mono-THF *annonaceous* acetogenin, solamin, bearing all functionalities.

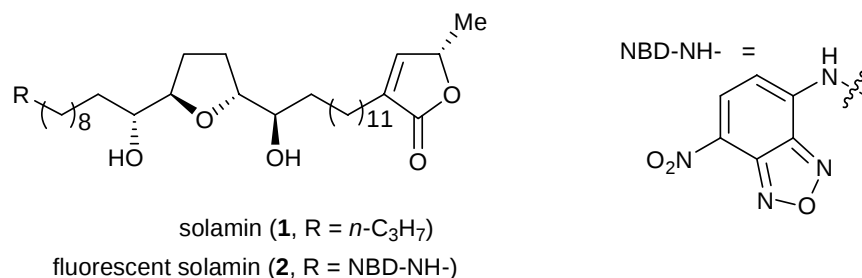
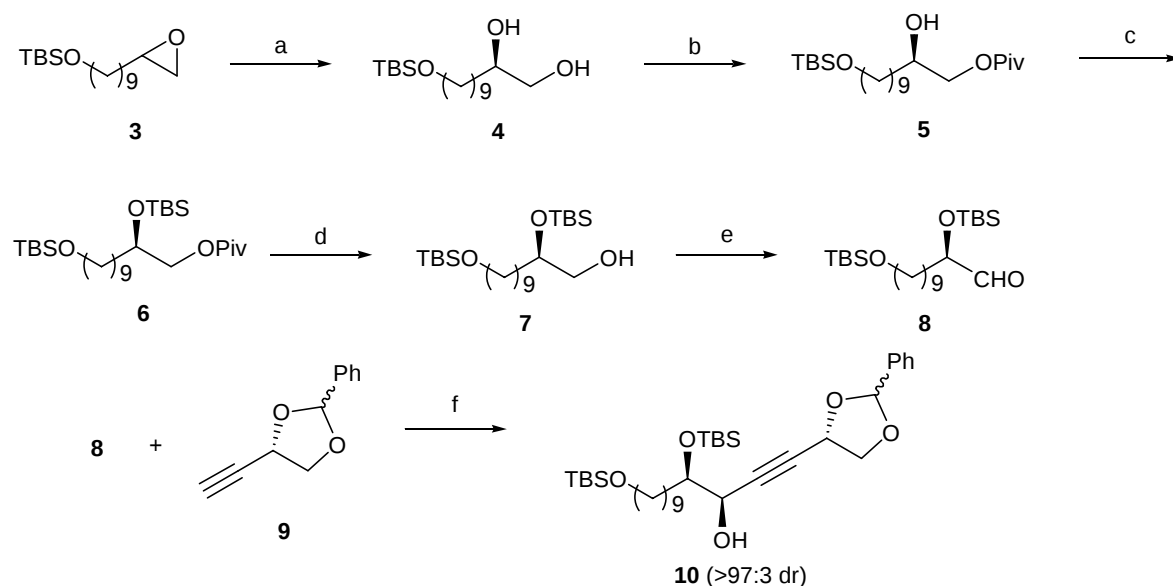


Figure 1 Structure of solamin and fluorescent solamin

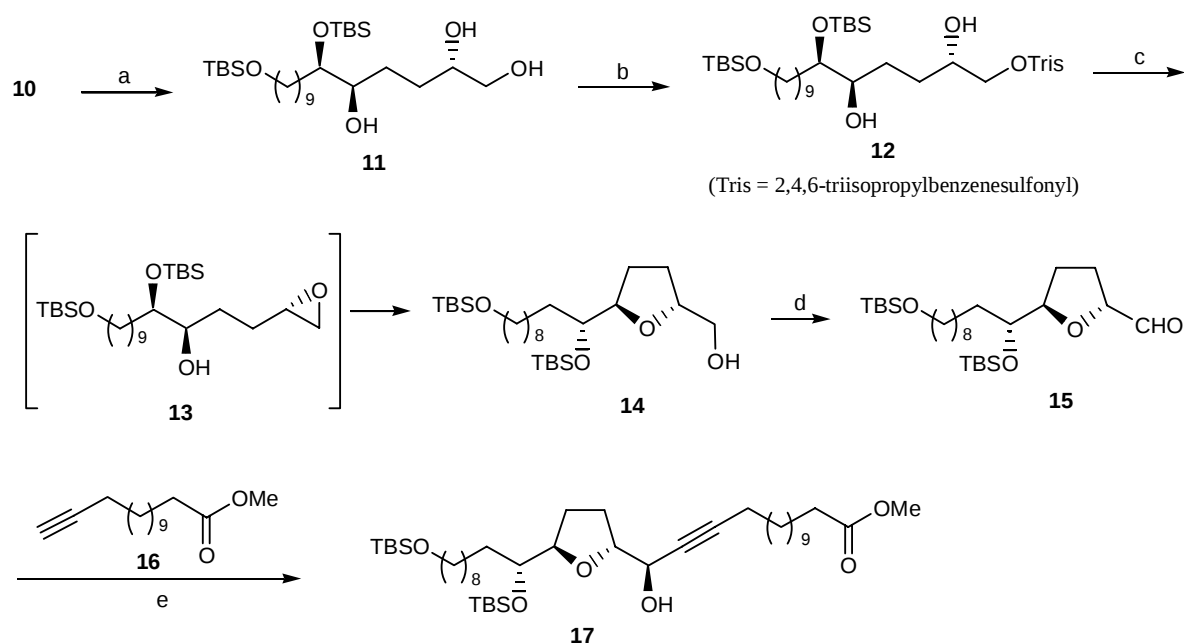
α -Oxyaldehyde (**8**) bearing a terminal oxygen was synthesized starting from kinetic optical resolution of the racemic epoxide (**3**)¹⁰ using Jacobsen's salen-cobalt complex¹¹ to give (*R*)-diol (**4**) with >98% ee (Scheme 1).^{12,13} The diol (**4**) was converted to aldehyde (**8**) in sequential reactions: 1) selective pivaloylation of the primary alcohol, 2) TBS protection of the secondary alcohol, 3) deprotection of the pivaloyl ester, and 4) oxidation with Dess–Martin periodinane.



Scheme 1 Reagents and Conditions: (a) Jacobsen's catalyst, Bu^tOMe, H₂O, 0 °C to room temperature, 48%; (b) PivCl, pyridine, CH₂Cl₂, 0 °C to room temperature, 95%; (c) TBSCl, imidazole, DMF, 0 °C to room temperature, 97%; (d) DIBAL-H, CH₂Cl₂, -78 °C, quantitative; (e) Dess–Martin periodinane, pyridine, CH₂Cl₂, 0 °C to room temperature, 91%; (f) Zn(OTf)₂, Et₃N, (1*R*,2*S*)-NME, toluene, room temperature, 91% (>97:3 dr).

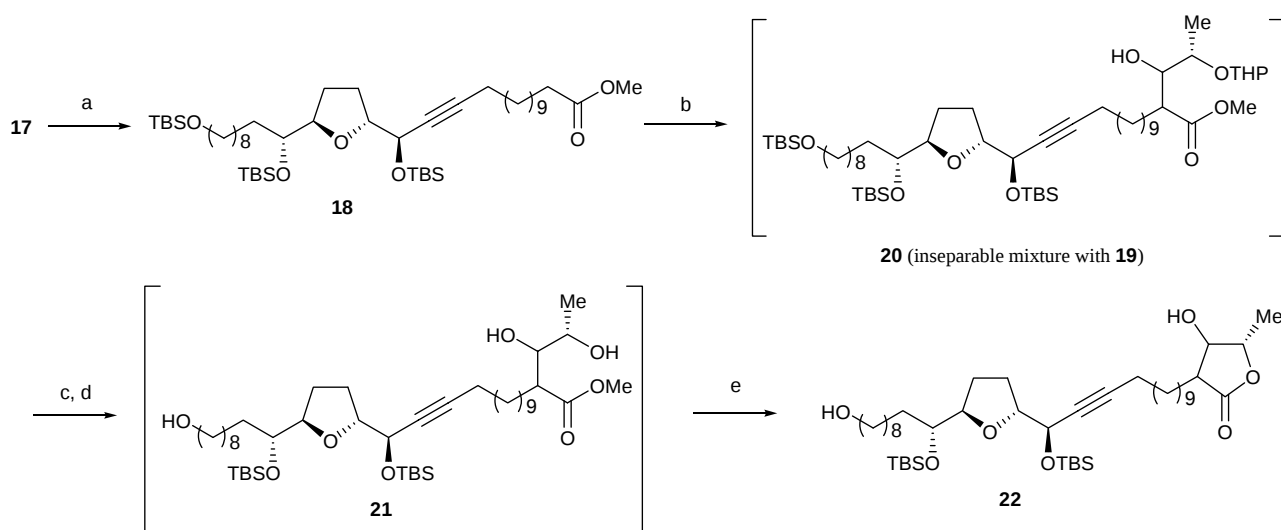
Next, asymmetric alkynylation of aldehyde (**8**) with alkyne (**9**)¹⁴ was carried out under Carreira's conditions¹⁵ with (1*R*,2*S*)-*N*-methylephedrine (NME) as a chiral ligand to give *syn*-adducts (**10**) in 91% yield with >97:3 dr. The stereochemistry was confirmed by comparison of the ¹H NMR spectral data of the corresponding *anti*-adduct synthesized with (1*S*,2*R*)-NME (85%, dr >97:3).¹⁶

Hydrogenation of the triple bond and deprotection of benzylidene acetal afforded triol (**11**) in 94% yield. Then, selective sulfonylation of the primary alcohol followed by base treatment of the resulting **12** delivered **11** to *trans*/*threo*-THF (**14**) (Scheme 2).¹⁷ After oxidation of **14** with Dess–Martin periodinane, we examined asymmetric alkynylation of methyl 13-tetradecynoate (**16**) to aldehyde (**15**). The reaction furnished *threo*-adduct (**17**) in 93% yield with high diastereoselectivity (>97:3 dr). The stereochemistry of **17** was determined by comparison with the ¹³C NMR data of Fujimoto's model compounds after reduction of the triple bond and deprotection of the TBS group with HF.¹⁸



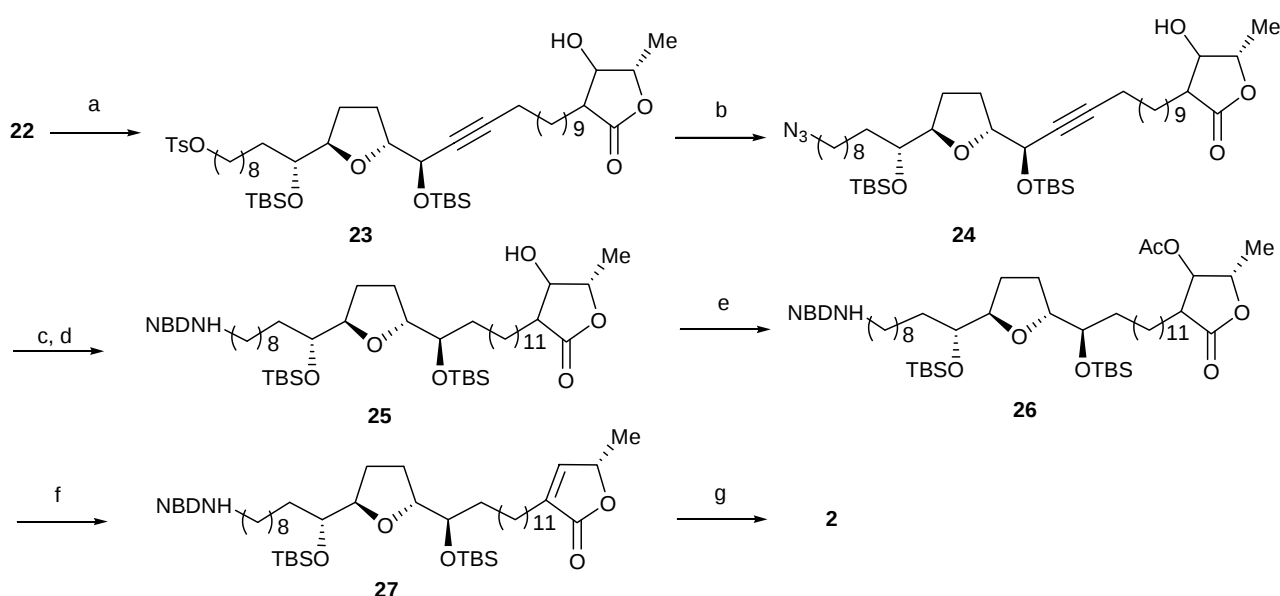
Scheme 2 Reagents and Conditions: (a) H₂, 10% Pd–C, EtOAc, room temperature, 94%; (b) TrisCl, pyridine, CH₂Cl₂, 0 °C to room temperature, 78%; (c) K₂CO₃, MeOH, 0 °C to room temperature, 67%; (d) Dess–Martin periodinane, pyridine, CH₂Cl₂, 0 °C to room temperature, 70%; (e) Zn(OTf)₂, Et₃N, (1*R*,2*S*)-NME, toluene, room temperature, 93% (>97:3 dr).

After the secondary alcohol of adduct (**17**) was protected as a TBS ether, the tris-TBS compound (**18**) was coupled with THP-protected (*S*)-lactataldehyde (**19**),¹⁹ giving an inseparable mixture of the aldol product (**20**) (mixture of diastereoisomers) and the recovered aldehyde (**19**). The mixture was treated with MgBr₂ for selective deprotection of the THP group. Although the deprotection proceeded successfully, partial γ -lactonization occurred during silica gel column chromatography. Accordingly, CSA-promoted γ -lactonization of the crude (**20**) was attempted. However, deprotection of the terminal TBS group proceeded instead of the γ -lactonization to give triol (**21**). Fortunately, γ -lactonization gradually proceeded by quenching the reaction with NaHCO₃. Therefore, the reaction was allowed to stand until the lactonization was completed to give γ -lactone (**22**) in 41% yield in three steps from **18** (Scheme 3).



Scheme 3 Reagents and Conditions: (a) TBSCl, imidazole, DMF, 0 °C to room temperature, 96%; (b) 19, LDA, THF, -78 °C; (c) MgBr₂, Et₂O, room temperature; (d) CSA, MeOH-CH₂Cl₂ (1/1), 0 °C; (e) NaHCO₃, 41% from 18.

To avoid epimerization of the methyl group on the γ -lactone,²⁰ the α,β -unsaturated γ -lactone moiety was constructed at the late stage. Thus, the fluorescent tag was introduced before dehydration. Tosylation of the terminal alcohol (*p*-TsCl, pyridine) and subsequent azidation (NaN₃, DMSO) furnished 24 in 71% yield in two steps. Then, reduction of the azide to amine and reaction with NBDCl in the presence of Et₃N in MeOH gave NBD-labeled compound (25) in 97% yield in two steps. Synthesis of the fluorescent solamin 2²¹ was completed via acetylation of the secondary alcohol, DBU-promoted β -elimination, and global deprotection with HF in MeCN/THF (Scheme 4).



Scheme 4 Reagents and Conditions: (a) TsCl, pyridine, CH₂Cl₂, 0 °C to room temperature, 85%; (b) NaN₃, DMSO, room temperature, 83%; (c) H₂, 10% Pd-C, MeOH, room temperature; (d) NBDCl, Et₃N, MeOH, 0 °C to room temperature, 97% in two steps. (e) Ac₂O, pyridine, room temperature, quantitative; (f) DBU, THF, room temperature, 93%; (g) 48% aq. HF, MeCN-THF, room temperature, 92%.

In conclusion, we have accomplished a synthesis of fluorescent solamin bearing all functionalities. In addition, we synthesized the synthetic intermediate (**24**), which is a useful precursor for synthesis of solamin derivatives labeled by other fluorescent tags. Since our strategy is applicable to other *annonaceous* acetogenins with diverse stereochemistry and with a different number of THF rings, the synthetic route described here will be applicable to other fluorescent-labeled *annonaceous* acetogenins.

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- 21 Data for **2**; mp: 83.0–85.0 °C (EtOAc); $[\alpha]_D^{27} +19.8$ (c, 0.20, CHCl₃); ¹H NMR (CDCl₃, 500 MHz) δ : 1.25–1.73 (m, 39H), 1.41 (d, 3H, *J* = 6.7 Hz), 1.81 (qn, 2H, *J* = 7.3 Hz), 1.96–2.02 (m, 2H), 2.24–2.28 (m, 3H), 3.39–3.43 (m, 2H), 3.47–3.51 (m, 2H), 3.78–3.83 (m, 2H), 4.97–5.02 (m, 1H), 6.18 (d, 1H, *J* = 8.5 Hz), 6.29 (br s, 1H), 6.99–7.00 (m, 1H), 8.51 (d, 1H, *J* = 8.5 Hz); ¹³C NMR (CDCl₃, 75 MHz) δ : 19.2, 25.2, 25.5, 25.6, 26.9, 27.4, 28.5, 28.7, 29.1–29.7 (14C), 33.5, 44.0, 73.97, 74.04, 77.5, 82.5, 82.6, 98.5, 123.9, 134.3, 136.5, 143.8, 143.9, 144.2, 148.9, 174.0; IR (KBr) cm⁻¹: 3329, 2925, 1751, 1585; LRMS (FAB) *m/z* 701 (M⁺+H); HRMS (FAB) calcd for C₃₈H₆₁N₄O₈ 701.4489, found 701.4492.