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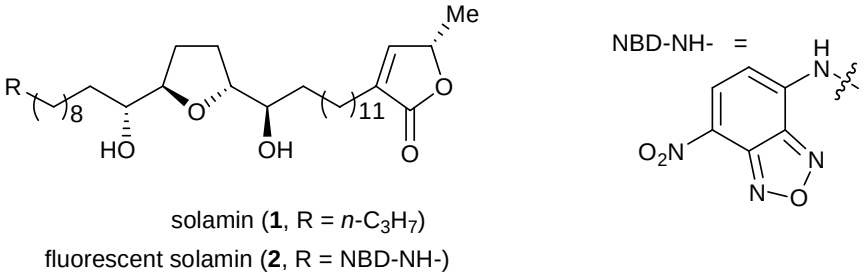
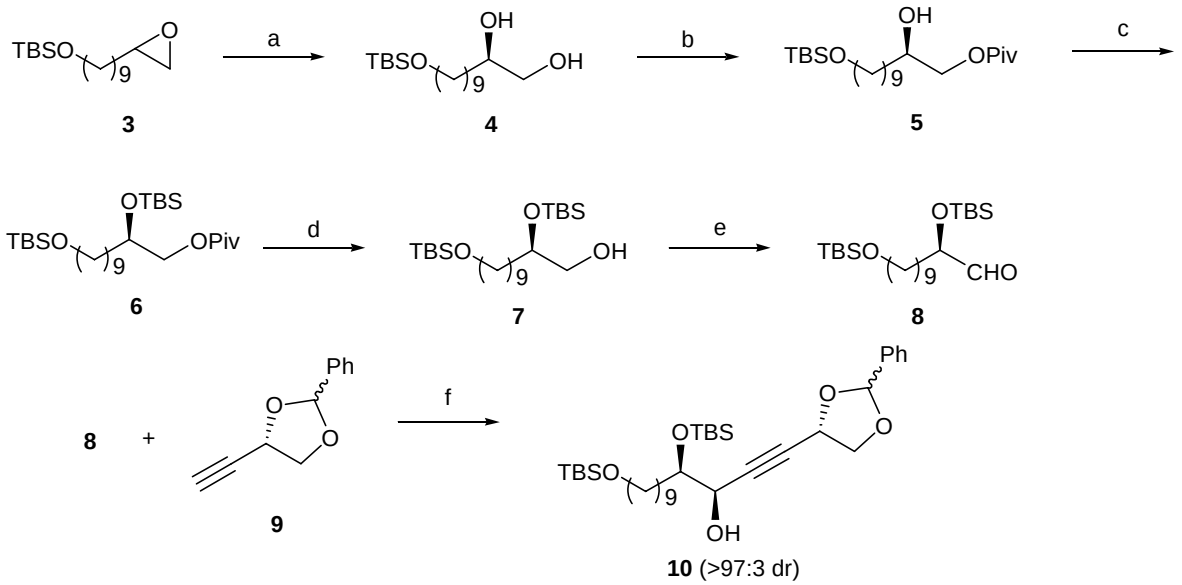
moieties seem to play an important role against biological activities in the acetogenin congeners.[8] The 7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl-amino (NBD-NH-) group was employed as a fluorescent tag due to its strong fluorescence with long wavelength, that is advantageous to observe the cell distribution.[9]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



solamin (**1**, R = *n*-C_3H_7)
fluorescent solamin (**2**, R = NBD-NH-)

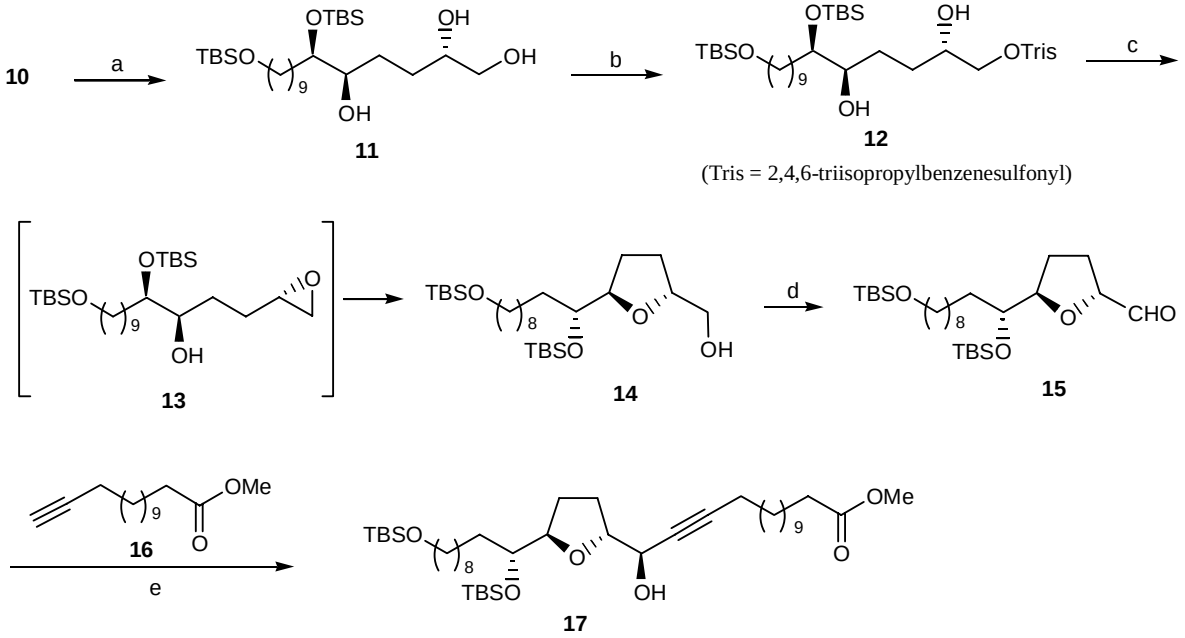
Figure 1 Structure of solamin and fluorescent solamin

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 complex[11] 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.



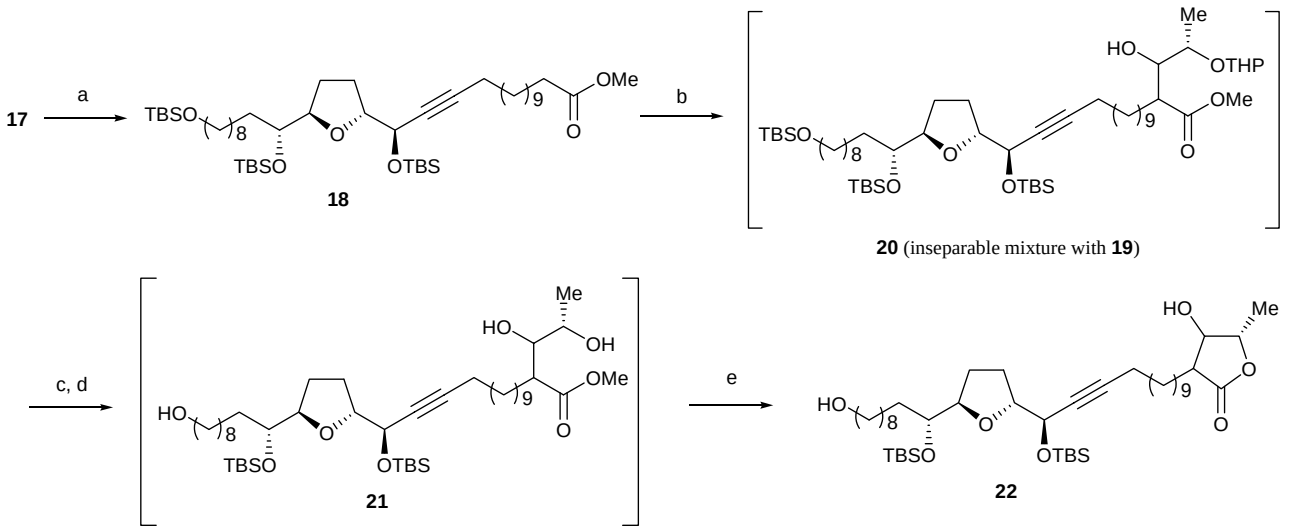
Scheme 1 *Reagents and Conditions*: (a) Jacobsen's catalyst, Bu^{t}OMe, H_2O, 0 °C to room temperature, 48%; (b) PivCl, pyridine, CH_2Cl_2, 0 °C to room temperature, 95%; (c) TBSCl, imidazole, DMF, 0 °C to room temperature, 97%; (d) DIBAL-H, CH_2Cl_2, –78 °C, quantitative; (e) Dess–Martin periodinane, pyridine, CH_2Cl_2, 0 °C to room temperature, 91%; (f) $Zn(OTf)_2$, Et_3N, (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 conditions[15] 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 ^{1}H 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** delived **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 ^{13}C NMR data of Fujimoto's model compounds after reduction of the triple bond and deprotection of the TBS group with HF.[18]



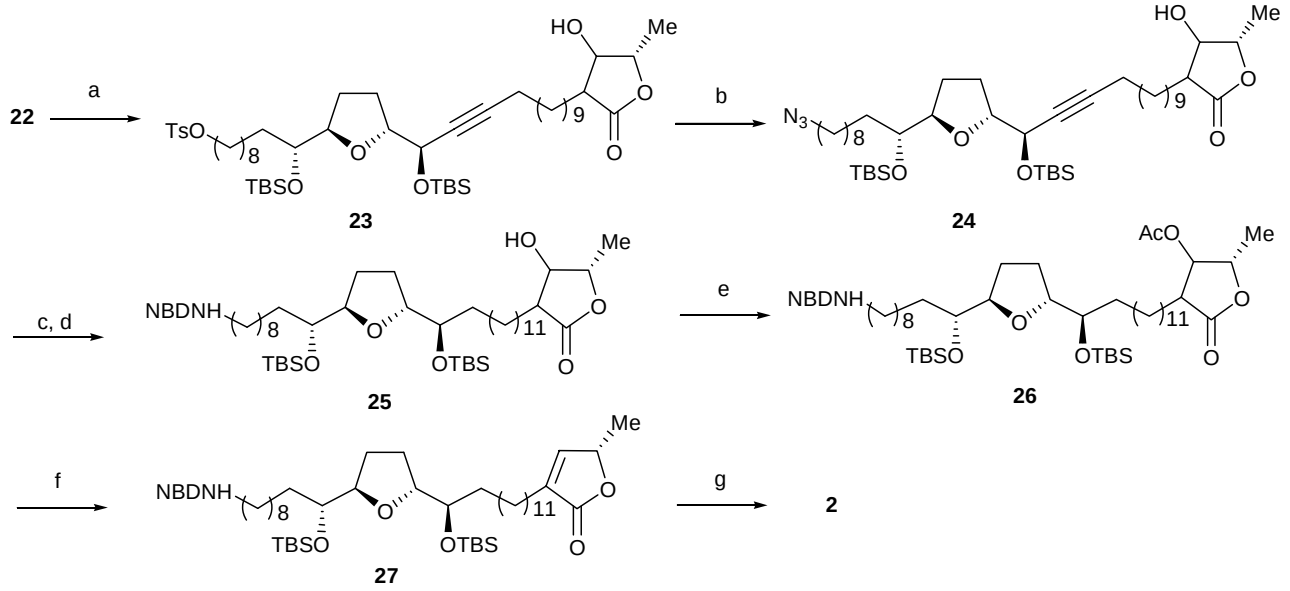
Scheme 2 *Reagents and Conditions*: (a) H_2, 10% Pd–C, EtOAc, room temperature, 94%; (b) TrisCl, pyridine, CH_2Cl_2, 0 °C to room temperature, 78%; (c) K_2CO_3, MeOH, 0 °C to room temperature, 67%; (d) Dess–Martin periodinane, pyridine, CH_2Cl_2, 0 °C to room temperature, 70%; (e) $Zn(OTf)_2$, Et_3N, (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 $MgBr_2$ 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 $NaHCO_3$. 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) $MgBr_2$, Et_2O, room temperature; (d) CSA, MeOH–CH_2Cl_2 (1/1), 0 °C; (e) $NaHCO_3$, 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 (NaN_3, DMSO) furnished **24** in 71% yield in two steps. Then, reduction of the azide to amine and reaction with NBDCl in the presence of Et_3N in MeOH gave NBD-labeled compound (**25**) in 97% yield in two steps. Synthesis of the fluorescent solamin **2**[21] 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, CH_2Cl_2, 0 °C to room temperature, 85%; (b) NaN_3, DMSO, room temperature, 83%; (c) H_2, 10% Pd–C, MeOH, room temperature; (d) NBDCl, Et_3N, MeOH, 0 °C to room temperature, 97% in two steps. (e) Ac_2O, 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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16 In comparison of related compounds synthesized in our laboratory, the hydroxy proton of the *anti*-adduct appears at higher field than that of the *syn*-adduct. On the other hand, the signals of the methin protons at both C2 and C3 positions were shifted more down-field in the *anti*-adduct than in the *syn*-adduct.

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21 Data for **2**; mp: 83.0–85.0 °C (EtOAc); $[\alpha]_D^{27}$ +19.8 (*c*, 0.20, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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 $C_{38}H_{61}N_4O_8$ 701.4489, found 701.4492.