Synthesis of an 11-*cis*-locked biotinylated retinoid for sequestering 11-*cis*-retinoid binding proteins[1]

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Abstract: The synthesis of a seven-membered ring locked analogue of 11-*cis*-retinol, tethered to a cross-linking moiety on C-15 and a lysine-extended biotin on the C-3, was accomplished for its utilization as a probe to fish out retinol binding proteins that may be involved in the reisomerization of retinal from all-*trans* to 11-*cis* in the visual cycle.

Key words: retinoids, biotin, 11-*cis*-locked, cross-linking, retinol binding proteins.

Résumé : La synthèse d'un analogue de 11-*cis*-rétinol sous la forme d'un anneau verrouillé, comportant sept membres, attachés en partie, au C-15, avec un groupe ayant la possibilité de se liér aux protéines a été réalisée. En plus, l'analogue est attaché de l'autre côté sur le C-3, avec une lysine biotine étendue. Cette synthèse a été réalisée afin d'utilizer le composé comme agent détecteur des protéines qui portent des rétinols et qui pourraient être impliquées dans la réisomerization du all-*trans* retinal au 11-*cis* dans le cycle de vision.

Mots clés : rétinoides, biotine, 11-*cis*-verrouillé, liés, protéines qui portent des rétinols.

Introduction

The basic process of vision, first elucidated by Wald (1), involves the light-triggered isomerization of retinal (or vitamin A aldehyde) from its 11-*cis* conformation to the all-trans conformation. Retinal, in its 11-*cis* geometry, is bound by the apoprotein opsin, a G-protein coupled receptor, forming a protonated Schiff base with Lys296, present in Helix G of the seven-membered transmembrane protein,which generates rhodopsin, the active photoreceptor unit in the cones and rods of eyes (2, 3). Upon absorption of a photon by 11-*cis*-retinal, the retinoid undergoes an isomerization to the all-trans geometry, resulting in a conformational change in the protein that leads to the activation of the G protein, which ultimately results in vision (4). The all-*trans*-retinal is subsequently hydrolyzed off the lysine residue, freeing the protein for a fresh 11-*cis*-retinal molecule. The completion of the visual cycle is ultimately dependent on the less well-understood proteins that are capable of binding all *trans*-retinoids and converting them to the 11-*cis*-retinal geometry (5). The latter proteins are collectively termed retinol binding proteins (RBPs). Their crucial involvement in the visual cycle warrants the need for the development of tools that enable their isolation and purification for further studies of their detailed function.

We have previously synthesized (6) a biotin-linked 11-*cis*-retinol analogue (structure **1** without the trimethylene bridge linking C-10 and C-13), as well as the all-*trans*-retinol analogue, **1a**. The latter derivative, bearing a biotin moiety and a chloroacetate cross-linking unit, enabled us to successfully isolate and characterize certain retinol binding proteins (7). We attempted to use the biotin-linked 11-*cis* analogue in the isolation of RBPs involved in the isomerization of all-*trans*-retinal to 11-*cis*-retinal. Despite the synthetic accomplishment (6), the retinoid was prone to undergoing an inevitable isomerization from 11-*cis* to all-trans upon incubation in the retinal pigment epithelial (RPE) cells, thus losing its affinity toward the RBPs of interest. To circumvent this problem, we resorted to ret7 (Fig. 1), the first of the 11-*cis*-locked analogues, which is comprised of a seven-membered ring locking the double bond at the C-11 position into a *cis*-conformation (8). This retinoid was particularly useful because it retains the full chromophore properties of native 11-*cis*-retinal and readily forms, with bovine opsin, the nonbleachable pigment, rhodopsin 7 or rh7. Both the UV–vis λ_{max} of 490 nm and the CD maxima at 330 and 488 nm

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Scheme 1. Retrosynthetic analysis for the biotinylated retinoid **1**.

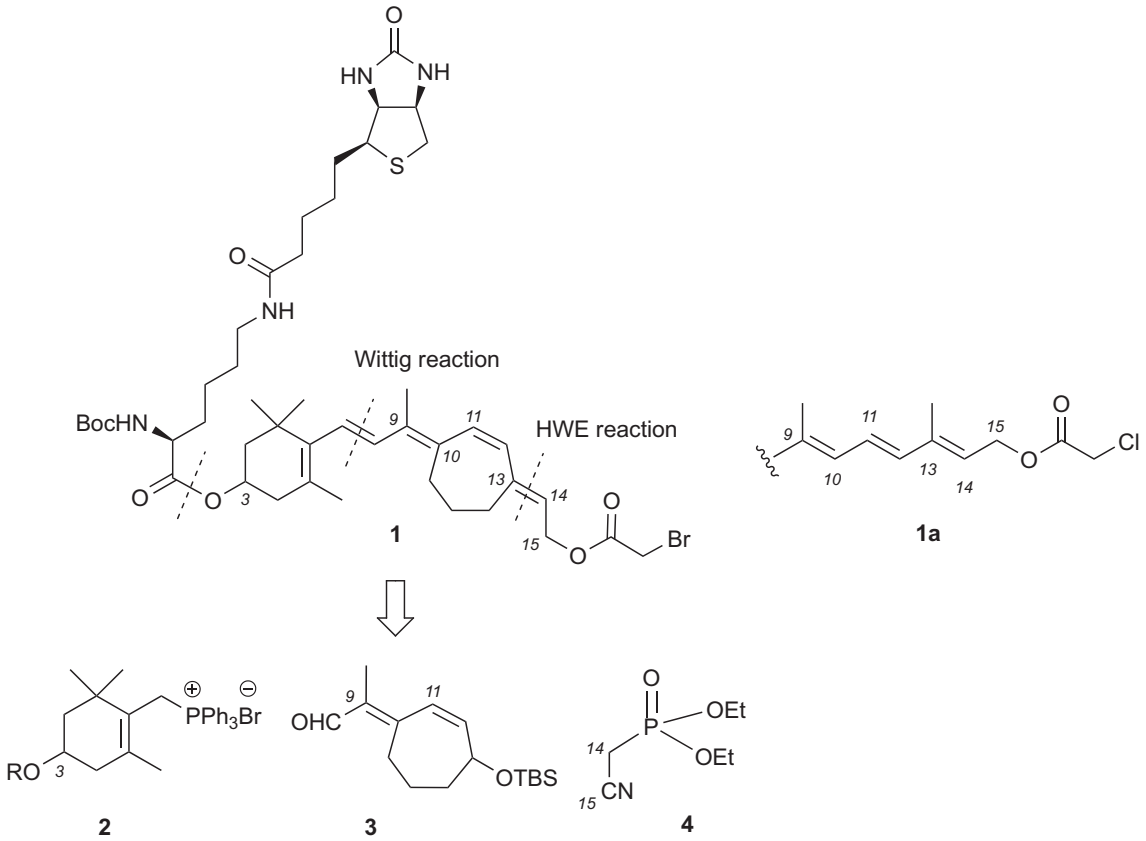
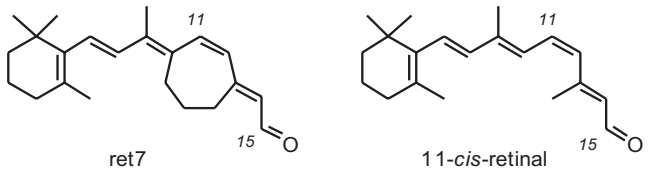


Fig. 1. The structures of ret7 and the native 11-*cis*-retinal.



(digitonin–phosphate buffer, pH 7) are similar to those of the native chromophore (UV–vis λ_{max} at 500 nm and CD maxima at 340 and 490 nm), indicating that the binding sites are the same. These aspects have made this pigment a popular substrate for various spectroscopic measurements (9–11).

We hereby report the synthesis of 11-*cis*-locked retinoid **1** bearing an appropriate functionality for isolation through cross-linking and which possesses the additional advantage of resisting isomerization under light conditions as well as in the media of RPE cells.

The target structure **1** has significant features that merit highlighting. It has the basic 11-*cis*-retinoid structure that can bind selectively to certain RBPs involved in the reisomerization of all-*trans*- to 11-*cis*-retinal. It also harbors an active cross-linking unit, the bromoacetate functionality, which can form a stable covalent bond with the RBPs and it also has the (Boc)Lys-biotinyl linker anchored at C-3, which will facilitate isolation of the covalently linked protein via an avidin affinity chromatography. Moreover, the ret7 moiety in the structure ensures its stability against light and (or) the RPE cell medium. Based on our earlier studies, the presence of the seven-membered ring would not prevent the retinoid from binding into the protein (8, 12, 13).

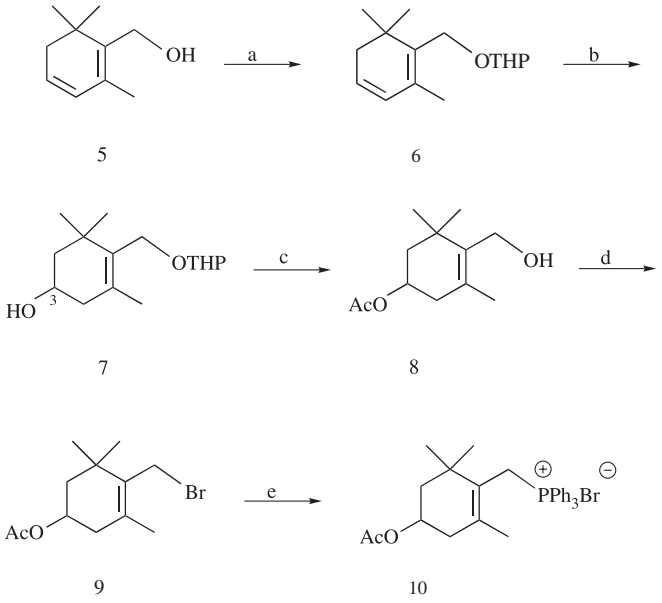
Results and discussion

The retrosynthetic logic for **1** is shown in Scheme 1. It stems from our earlier work conducted on the seven-membered ring unit **3**, synthesized from 2-cyclohepten-1-one in five steps (14). The hydroxylated β-ionone ring structure can be introduced by employing a Wittig reaction between aldehyde **3** and synthon **2**, the latter being prepared from dehydro-β-cyclocitral (also known as safranal) in seven steps as outlined in Scheme 2. The Horner–Wadsworth–Emmons (HWE) reaction can be used to install a nitrile group at the C-14 position with reagent **4**, followed by its reduction to the corresponding aldehyde, which would be subjected to further reduction to the corresponding primary alcohol onto which a bromoester can be esterified in the final stages.

Synthon **3** has been prepared from the commercially available 2-cyclohepten-1-one, according to our earlier report (14). The synthesis featured an allylic oxidation of 2-cyclohepten-1-one, a three-carbon homologation by HWE reaction with the reagent bearing the nitrile functionality, and lastly, a partial reduction of the nitrile to the corresponding aldehyde.

The synthesis of **10** (the synthon **2** unit) is outlined in Scheme 2. Dehydro-β-cyclocitrol (**5**), prepared from dehydro-β-cyclocitral (15), was protected as the tetrahydropyranyl (THP) ether (→**6**, 81%) and subsequently treated

Scheme 2. Reagents and conditions: (*a*) DHP, *p*-TsOH, CH_2Cl_2, 0 °C (81%); (*b*) 9-BBN, THF, Δ, then MeOH, NaOH, H_2O_2, 0 °C → 50 °C (55%); (*c*) (*i*) Ac_2O, pyridine, CH_2Cl_2, rt, (*ii*) *p*-TsOH, MeOH, rt (60%, two steps); (*d*) PPh_3, CBr_4, THF (94%); (*e*) PPh_3, benzene, Δ (quant.).]



with 9-borabicyclo[3.3.1]nonane (9-BBN) to undergo a hydroboration reaction affording the 3-hydroxy **7** in 55% yield. As in the case of the biotinylated all-trans analogue **1a** (6, 7), no attempts were made to resolve the C-3 enantiomers. The 3-hydroxy group was protected as the acetate by treatment with acetic anhydride (Ac_2O) – pyridine. The allylic hydroxy group was then liberated by acidic cleavage of the THP group to give alcohol **8**, which was transformed to the corresponding bromide **9** using triphenylphosphine (PPh_3) and carbon tetrabromide (94% yield). The phosphonium salt **10** was synthesized from **9** by treatment with PPh_3 under reflux conditions.

Scheme 3 shows the critical steps that assemble the retinoid from synthons **3** and **10**. A Wittig reaction between aldehyde **3** and phosphonium salt **10** afforded the desired tetraene **11a** (33%), along with a considerable amount of **11b** (40%). The trans geometry of the olefin thus formed was confirmed from the coupling constant (J = 16 Hz) observed for the H-7 and H-8 protons. The TBS group of **11a** was removed using tetrabutylammonium fluoride (TBAF) to give the free allylic alcohol, which, upon treatment with MnO_2, underwent smooth oxidation to afford the conjugated ketone **12** in 84% yield. A two-carbon extension through HWE reaction was carried out on ketone **12** with cyanomethylphoshonate (**4**) to afford the (*E*)-isomer **13** in 27% yield (the (*Z*)-isomer, 53%). The *E*/*Z* geometry was determined by NOE experiments for the H-12 and H-14 protons. Nitrile **13** was subjected to partial reduction with diisobutylaluminum hydride (DIBAH) to the corresponding aldehyde accompanied by an inevitable reductive cleavage of the acetate group at the C-3 position. After reprotection of the free alcohol with an acetyl group, resulting in **14**, the aldehyde was further reduced to the corresponding primary alcohol by treatment with sodium borohydride and was then protected as a TBS ether to afford **15**. Basic cleavage of the 3-acetyl group gave the corresponding 3-OH followed by esterification with biotinyl-(Boc)Lys-OH to give **16** (78% yield, two steps). The TBS group was deprotected, releasing the primary alcohol, which was eventually subjected to esterification with bromoacetic acid to afford the target structure **1** in 66% yield.

Preliminary studies directed towards isolation and characterization of RBPs employing **1** performed by Rando's group (Harvard Medical School, Boston, Massachusetts) have shown that some RBPs have been sequestered and identified. The results of the isolation and characterization of the RBPs using **1** will be reported in a subsequent manuscript.

Conclusion

The synthesis of biotinylated retinoid **1** was accomplished via a series of Wittig reactions, esterification of a (Boc)Lys-biotin on the 3-hydroxy position of the β-ionone ring, and the final attachment of the active bromoester cross-linking unit. The significance of the seven-membered ring lock is evident as the preliminary biological studies using this molecule have already proven the robust nature of the 11-*cis*-olefin unit. The target structure will be able to cross-link to RBPs that are involved in the crucial isomerization process that regenerates 11-*cis*-retinal. Avidin affinity chromatography will enable facile purification of the sequestered protein, and basic hydrolysis will release the isolated protein from the affinity column by cleaving the ester at the C-15 position. The purified protein can be further studied and characterized for the elucidation of its detailed function.

Experimental section

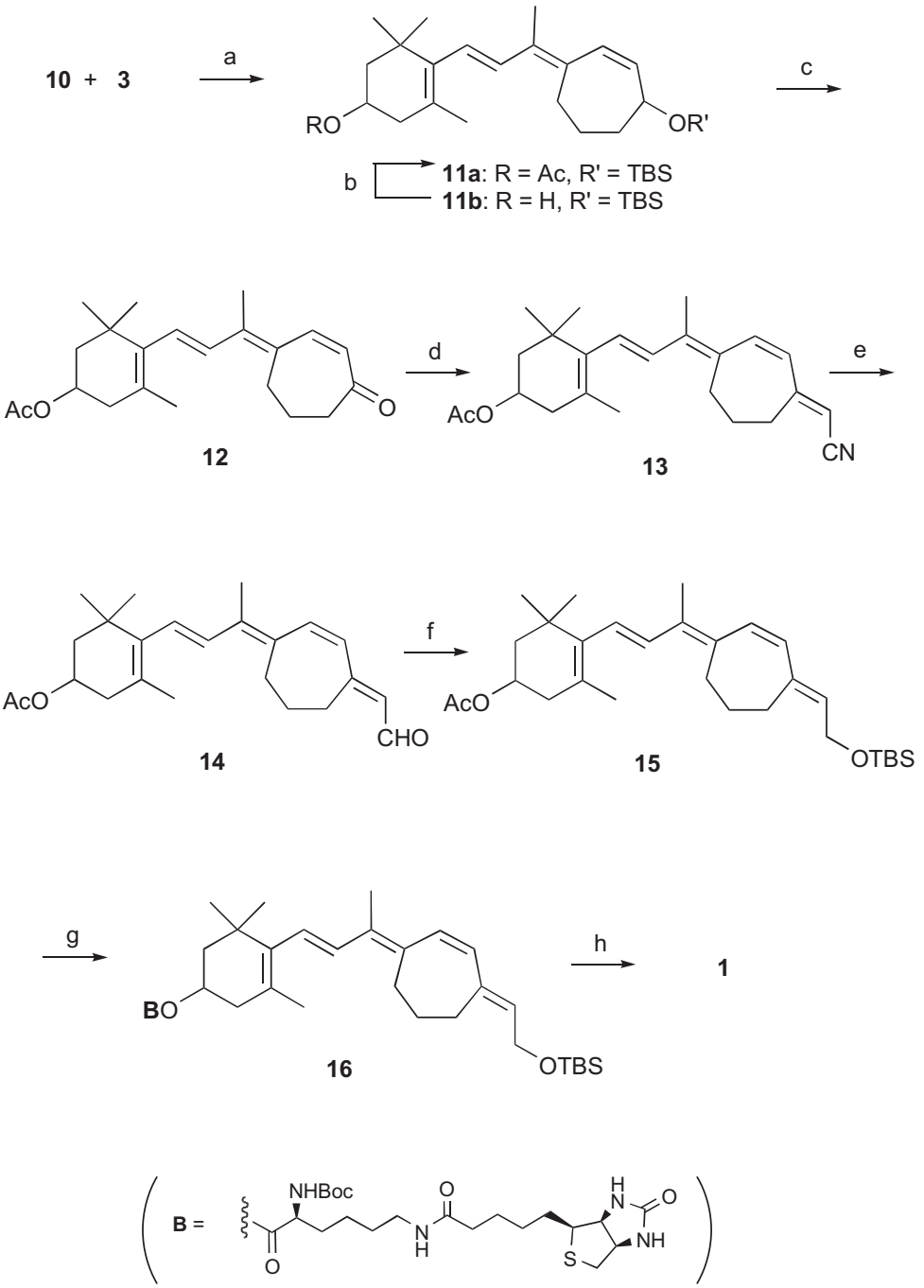
General considerations

^{1}H NMR spectra were measured on a Bruker DMX 400 or Bruker DPX 300 spectrometer (400 or 300 MHz). The chemical shifts are expressed in ppm downfield from the signal of tetramethylsilane, used as an internal standard in the solvent $CDCl_3$. Splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), qn (quintet), m (multiplet), and br (broad). High resolution mass spectrometry (HRMS) were measured on a JEOL (Tokyo) JMS-HX110/110 tandem mass spectrometer equipped with a Xe beam FAB gun (6 kV), using 3-nitrobenzyl alcohol as a matrix. Analytical and preparative thin layer chromatography (TLC) was carried out using precoated silica gel plates (Merck silica gel $60F_{254}$). The silica gel used for column chromatography was 230–400 mesh (Merck). All reactions were carried out under an Ar atmosphere using predried solvents. Anhydrous CH_2Cl_2, Et_2O, and THF were obtained from the solvent purification system via passage through an activated alumina cartridge.

Tetrahydropyranyl safranol (6)

To a solution of safranol **5** (7.67 g, 50.4 mmol) in CH_2Cl_2 (100 mL) was added 3,4-dihydro-2*H*-pyran (DHP, 8.48 g, 101 mmol). The mixture was stirred and cooled to –10 °C and *p*-toluenesulfonic acid monohydrate (*p*-TsOH, 190 mg, 1.00 mmol) was then added. After 10 min of stirring, triethylamine (1.0 mL) was added to quench the reaction.

Scheme 3. Reagents and conditions: (*a*) *n*-BuLi, THF, –78 °C → rt, (for **11a**, 33%; for **11b**, 40%); (*b*) Ac_2O, pyridine, DMAP, rt for **11b** (63%); (*c*) (*i*) TBAF, THF, 0 °C → rt (99%), (*ii*) MnO_2, CH_2Cl_2, 0 °C → rt (84%); (*d*) **4**, NaH, THF, 0 °C → rt (*E*:*Z*, 27%:53%); (*e*) (*i*) DIBAH, Et_2O, –78 °C → 0 °C (82%), (*ii*) Ac_2O, pyridine, CH_2Cl_2, 0 °C → rt (50%); (*f*) (*i*) $NaBH_4$, MeOH–$CHCl_3$ (1:10), 0 °C, (*ii*) TBSCl, imidazole, CH_2Cl_2, 0 °C (70%, two steps); (*g*) (*i*) K_2CO_3, MeOH, 0 °C → rt, (*ii*) biotinyl (Boc)lys-OH (**B**-OH), EDC, DMAP, CH_2Cl_2, 0 °C → rt (78%, two steps); (*h*) (*i*) TBAF, THF, 0 °C → rt (60%), (*ii*) $BrCH_2COOH$, EDC, DMAP, 0 °C → rt (66%).



The mixture was poured into water (150 mL) and extracted with Et_2O (3 × 150 mL). The organic layers were washed with brine, combined, dried over $MgSO_4$, and concentrated under reduced pressure. Purification of the residue by silica gel column chromatography (EtOAc–hexanes, 10:90) afforded the THP ether **6** (9.60 g, 40.8 mmol, 81%) as a colorless oil. ^{1}H NMR ($CDCl_3$, ppm) δ: 5.75 (2H), 4.61 (1H, brs), 4.31 (1H, d, *J* = 11.2 Hz), 4.07 (1H, d, *J* = 11.2 Hz), 3.91 (1H, m), 3.52 (1H, m), 2.05 (2H, s), 1.81 (3H, s), 1.81–1.69 (6H), 1.04, 1.01 (each 3H, s). ^{13}C NMR ($CDCl_3$, ppm) δ: 134, 129.9, 129.1, 126, 97.7, 63.4, 62.4, 40.3, 33.5, 31.0, 26.7 (2C), 25.9, 19.9, 18.4. FAB-HRMS calcd. for $C_{15}H_{24}O_2$ *m*/*z*: 236.1776 [M^+]; found: 236.1769.

Tetrahydropyranyl 3-hydroxysafranol (7)

To a solution of THP ether **6** (6.66 g, 28.2 mmol) in THF (40.0 mL), 9-BBN (0.5 mol/L solution in THF, 113 mL, 56.4 mmol) was added slowly over 20 min. The reaction

mixture was heated up to 60 °C and was stirred for 4 h. After the reaction mixture was cooled to room temperature, methanol (68.0 mL) and an aq. NaOH solution (3 mol/L, 9.4 mL) were added to it. Subsequently, H_2O_2 (30% solution, 8.0 mL) was added slowly to the stirring reaction mixture using an ice bath. The reaction mixture was heated for 2 h at 55 °C. The mixture was poured into water (100 mL) and extracted with EtOAc (3 × 100 mL). The organic layers were washed with brine, combined, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by silica gel column chromatography (EtOAc–hexanes, 30:70) afforded a diastereomeric mixture of **7** (3.94 g, 15.5 mmol, 55%). The ^{1}H NMR spectra indicated that the sample consisted of a mixture of diastereomers (50:50). ^{1}H NMR ($CDCl_3$, ppm) δ: 4.55 (2 × 0.5H, dt, *J* = 3.2, 8.5 Hz), 4.26, 4.17, 3.80, 3.74 (each 0.5H, d, *J* = 10.7 Hz), 3.93–3.81 (0.5H × 2 × 2), 3.50 (0.5H × 2, m), 2.37 (0.5H × 2, brs), 2.27 (0.5H × 2, brdt), 2.01 (0.5H × 2, brdd), 1.78 (0.5H × 2, m), 1.69, 1.68 (each 1.5H, s), 1.70–1.62 (0.5H × 2 × 2), 1.56–1.40 (0.5H × 2 × 5), 1.06, 1.02, 1.00, 0.993 (each 1.5H, s). ^{13}C NMR ($CDCl_3$, ppm) δ: 134, 133, 131.5, 131.4, 98.9, 98.6, 64.9 (0.5C × 2), 63.5, 63.4, 62.4, 62.3, 48.2 (0.5C × 2), 42.3 (0.5C × 2), 36.8 (0.5C × 2), 30.7 (0.5C × 2), 29.4, 29.2, 28.7, 28.6, 25.5 (0.5C × 2), 19.7 (0.5C × 2), 19.69, 19.63. FAB-HRMS calcd. for $C_{15}H_{26}O_3$ *m*/*z*: 254.1882 [M^+]; found: 254.1891.

3-*O*-Acetoxysafranol (8)

To a solution of hydroxy **7** (1.00 g, 3.93 mmol) in pyridine (7.0 mL), acetic anhydride (3.5 mL) was added at 0 °C. The resultant mixture was stirred at room temperature. After 12 h of stirring, the mixture was azeotropically concentrated with toluene (×3) in vacuo. The crude sample was subjected to the next step without purification. The acetate was treated with a catalytic amount of *p*-TsOH (10.0 mg) in methanol (60.0 mL). The resultant mixture was allowed to stir for 12 h at room temperature. The mixture was neutralized with Et_3N (1.0 mL) and concentrated under reduced pressure. Silica gel purification of the residue (EtOAc–hexanes, 25:75) gave the 3-*O*-acetyl safranol **8** (576 mg, 2.72 mmol, 70%, in two steps). ^{1}H NMR ($CDCl_3$, ppm) δ: 4.97 (1H, m), 4.13, 4.07 (each 1H, d, *J* = 11.5 Hz), 2.35 (1H, dd, *J* = 5.7, 16.9 Hz), 2.04 (1H, dd, *J* = 9.4, 16.9 Hz), 2.00 (3H, s), 1.71 (1H, m) overlapping with 1.72 (3H, s), 1.52 (1H, t, *J* = 11.9 Hz), 1.45 (1H, br, CH_2OH), 1.09, 1.05 (each 3H, s). ^{13}C NMR ($CDCl_3$, ppm) δ: 170, 137, 127, 68.2, 58.2, 43.8, 38.2, 36.4, 29.2, 28.5, 21.5, 19.5.

3-*O*-Acetoxysafranyl bromide (9)

To a solution of alcohol **8** (576 mg, 2.72 mmol) in THF (20.0 mL) at 0 °C, PPh_3 (1.07 g, 4.08 mmol) and CBr_4 (1.35 g, 4.08 mmol) were added. The resultant mixture was stirred at room temperature for 1 h. The mixture was poured into water (50 mL) and extracted with Et_2O (3 × 50 mL). The organic layers were washed with brine, combined, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified with silica gel chromatography (EtOAc–hexanes, 2:98) to afford the allyl bromide **9** (710 mg, 2.55 mmol, 94%). ^{1}H NMR ($CDCl_3$, ppm) δ: 4.99 (1H, m), 4.07, 3.96 (each 1H, d, *J* = 8.2 Hz), 2.43 (1H, dd, *J* = 4.5, 13.8 Hz), 2.11 (1H, dd, *J* = 7.4, 13.8 Hz), 2.02 (3H, s), 1.72 (1H, m) overlapping with 1.75 (3H, s), 1.58 (1H, t, *J* = 9.5 Hz), 1.20, 1.12 (each 3H, s). ^{13}C NMR ($CDCl_3$, ppm) δ: 169, 133.4, 144.2, 67.2, 43.5, 38.4, 36.7, 29.0, 28.6, 28.3, 21.1, 19.6. FAB-HRMS calcd. for $C_{12}H_1{}^{8}{}^{79}BrO_2$ *m*/*z*: 273.0490 [M – H]$^+$; found: 273.0497.

3-*O*-Acetoxysafranyl triphenylphosphonium bromide (10)

To a solution of allyl bromide **9** (710 mg, 2.55 mmol) in benzene (20.0 mL) at room temperature, PPh_3 (670 mg, 2.55 mmol) was added. The resultant mixture was heated up to the reflux temperature (~85 °C bath) for 12 h. After the mixture was cooled to room temperature, the volatiles were evaporated in vacuo. The residue was washed with benzene (×3) and dried under the reduced pressure for 1 day to give **10** (1.37 g, 2.55 mmol, quantitative yield). ^{1}H NMR ($CDCl_3$, ppm) δ: 7.9–7.6 (15H), 4.97 (1H, m), 4.58, 4.42 (each 1H, t, *J* = 14.0 Hz), 2.26 (1H, m), 2.04 (3H, s), 1.95 (1H, m), 1.71 (1H, dd, *J* = 2.0, 12.0 Hz), 1.51 (1H, t, *J* = 12.0 Hz), 1.07 (3H, d, *J* = 4.0 Hz), 0.88, 0.84 (each 3H, s). FAB-HRMS calcd. for $C_{30}H_{34}O_2P$ *m*/*z*: 457.2291 [M – Br]$^+$; found: 457.2285.

3-*O*-Acetyl-13-*O*-TBS (11a)

To a solution of the triphenylphosphonium bromide **10** (657 mg, 1.22 mmol) in THF (7.0 mL) at –78 °C, *n*-BuLi (2.5 mol/L solution in hexanes, 1.4 mL, 3.50 mmol) was added, and the resultant mixture was allowed to warm to room temperature and was stirred at this temperature for 1 h to generate the corresponding ylide. To a solution of aldehyde **3** (460 mg, 1.64 mmol) in THF (10.0 mL), the ylide solution was added. After 14 h of stirring at the same temperature, the mixture was poured into water and extracted with Et_2O (3 × 30 mL). The organic layers were washed with brine, combined, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by silica gel column chromatography (EtOAc–hexanes, 5:95 and 20:80) afforded the acetyl **11a** (245 mg, 534 μmol, 33%) and alcohol **11b** (276 mg, 663 μmol, 40%) as a colorless oil. The ratio of diastereomers was not determined and this sample was used as a mixture.

To a solution of 3-hydroxy **11b** (294 mg, 706 μmol) in pyridine (3.0 mL) at room temperature, Ac_2O (1.5 mL) and *N*,*N*-dimethylaminopyridine (DMAP, 5.0 mg) were added, and the resultant mixture was stirred at the same temperature for 12 h. The mixture was poured into a satd. aq. solution of $NaHCO_3$ (30 mL) and extracted with Et_2O (3 × 30 mL). The organic layers were washed with brine, combined, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by silica gel column chromatography (EtOAc–hexanes, 2:98) provided the acetyl **11a** (205 mg, 446 μmol, 63%) as a colorless oil. The ratio of diastereomers was not determined, and this sample was used as a diastereomeric mixture. ^{1}H NMR for **11a** ($CDCl_3$, ppm, major signals are only described.) δ: 6.49, 6.09 (each 1H, dd, *J* = 1.8, 16.0 Hz), 6.45, 5.69 (each 1H, dd, *J* = 2.2, 11.8 Hz), 5.06 (1H, m), 4.46 (1H, m), 2.67 (1H, dt, *J* = 5.0, 13.7 Hz), 2.44 (1H, dd, *J* = 5.8, 17.0 Hz), 2.23 (1H, m), 2.10 (1H, dd, *J* = 9.4, 16.6 Hz), 2.04 (3H, s), 1.87 (3H, s), 1.83–1.75 (2H), 1.72 (3H, s), 1.8–1.7 (2H), 1.58 (2H, t, *J* = 12.0 Hz), 1.10, 1.07 (each 3H, s), 0.906 (3H × 3, s), 0.090, 0.081 (each 3H, s). ^{13}C NMR

($CDCl_3$, ppm) δ: 170, 138, 137, 134, 132, 130, 129, 125.8, 125.0, 71.0, 68.4, 43.9, 38.3, 36.6, 35.6, 30.0, 29.8. 28.4, 25.8 (3C), 23.8, 21.4 (2C), 18.2, 14.3, –4.6 (2C). FAB-HRMS calcd for $C_{28}H_{46}O_3Si$ *m/z*: 458.3216 [M^+]; found: 458.3196. ^1H NMR for **11b** ($CDCl_3$, ppm, major signals are only described.) δ: 6.11, 6.49 (each 1H, d, *J* = 16.0 Hz), 5.69, 6.46 (each 1H, d, *J* = 11.9 Hz), 4.46 (1H, m), 4.01 (1H, m), 2.68 (1H, dt, *J* = 5.4, 8.8 Hz), 2.38 (1H, dd, *J* = 5.4, 16.6 Hz), 2.24 (1H, m), 2.04 (1H, m), 1.86 (3H, s), 1.71–1.85 (4H) overlapping with 1.73 (3H, s), 1.65 (1H, brs), 1.48 (1H, t, *J* = 11.9 Hz), 1.07 (3H × 2, s), 0.907 (3H × 3, s), 0.0815, 0.0914 (each 3H, s). ^{13}C NMR ($CDCl_3$, ppm) δ: 138, 136, 134, 132, 130, 130, 125.67, 125.64, 71.0, 64.9, 48.2, 42.4, 37.0, 35.6, 30.2, 29.8, 28.6, 25.8 (3C), 23.8, 21.5, 18.2, 14.4, –4.6 (2C). FAB-MS *m/z* (%, rel. int.): 416 (52, M^+), 398 (25, [M – H_2O]$^+$). FAB-HRMS calcd. for $C_{26}H_{44}O_2Si$ *m/z*: 416.3111 [M^+]; found: 416.3106.

13-Ketone (12)

To a solution of **11a** (205 mg, 446 mmol) in THF (10.0 mL) at 0 °C, tetrabutylammonium fluoride (TBAF, 1.0 mol/L solution in THF, 892 μL, 892 μmol) was added, and the resultant mixture was allowed to warm to room temperature and was continuously stirred at this temperature. After 3 h of stirring, the mixture was poured into water (30 mL) and extracted with Et_2O (3 × 30 mL). The organic layers were washed with brine, combined, dried over $MgSO_4$, and concentrated under reduced pressure. Purification of the residue by silica gel column chromatography (EtOAc–hexanes, 15:85) afforded the 13-hydroxy compound (145 mg, 425 μmol, 99%) as a colorless oil. ^1H NMR ($CDCl_3$, ppm) δ: 6.51, 5.73 (each 1H, d, *J* = 11.8 Hz), 6.47, 6.10 (each 1H, d, *J* = 15.8 Hz), 5.05 (1H, m), 4.47 (1H, m), 2.70 (1H, m), 2.44 (1H, dd, *J* = 5.4, 6.8 Hz), 2.23 (1H, m), 2.12 (1H, m), 2.05 (3H, s), 2.00–1.89 (2H), 1.88 (3H, s), 1.81–1.68 (2H, m), 1.71 (3H, s), 1.58 (2H, t, *J* = 11.9 Hz), 1.10, 1.07 (each 3H, s).

To a solution of the 13-hydroxy (209 mg, 607 μmol) in CH_2Cl_2 (15.0 mL) at 0 °C, MnO_2 (1.60 g, 18.2 mmol) was added, and the resultant mixture was stirred at this temperature. After 40 min of stirring at the same temperature, the mixture was filtered and the volatiles were removed under reduced pressure. Purification of the residue by silica gel column chromatography (EtOAc–hexanes, 20:80) afforded ketone **12** (174 mg, 507 μmol, 84%) as a colorless oil. ^1H NMR ($CDCl_3$, ppm) δ: 7.32, 5.92 (each 1H, d, *J* = 12.0 Hz), 6.55, 6.32 (each 1H, d, *J* = 18.0 Hz), 5.03 (1H, m), 2.63 (2H, t, *J* = 6.6 Hz), 2.57 (2H, t, *J* = 6.6 Hz), 2.45 (1H, dd, *J* = 5.4, 16.9 Hz), 2.11 (1H, dd, *J* = 9.6, 16.9 Hz), 2.04, 2.02 (each 3H, s), 1.87 (2H, qn, *J* = 6.6 Hz), 1.79 (1H, brt), 1.72 (3H, s), 1.58 (1H, t, *J* = 12.0 Hz), 1.10, 1.07 (each 3H, s). ^{13}C NMR ($CDCl_3$, ppm) δ: 203, 170, 143, 137.9, 137.0, 134, 132, 130, 129, 126, 68.1, 43.8, 42.9, 38.3, 36.5, 31.2, 29.9, 28.4, 23.1, 21.4, 21.3, 15.0. FAB–HRMS calcd. for $C_{22}H_{31}O_3^+$ *m/z*: 343.2273 [M + H]$^+$; found: 343.2266.

13-Nitrilemethylene (13)

To a solution of NaH (freshly prepared by washing with hexanes and drying under reduced pressure, 140 mg, 5.84 mmol) in THF (15.0 mL) at 0 °C, $(EtO)_2P(O)CH_2CN$ (**4**, 944 μL, 5.84 mmol) was added, and the resultant mixture was allowed to warm to room temperature and was stirred at this temperature for 1 h to generate the corresponding ylide. To a solution of ketone **12** (174 mg, 507 μmol) in THF (25.0 mL), the generated ylide was added. After 12 h of stirring at the same temperature, the mixture was poured into water (30 mL) and extracted with Et_2O (3 × 30 mL). The organic layers were washed with brine, combined, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by silica gel column chromatography (EtOAc–hexanes, 7:93) afforded the (*E*)-nitrile **13** (47.1 mg, 135 μmol, 27%) and the (*Z*)-nitrile (99.3 mg, 265 μmol, 53%) as yellowish oils, respectively. ^1H NMR for (*E*)-isomer **13** ($CDCl_3$, ppm) δ: 6.82, 6.13 (each 1H, d, *J* = 11.3 Hz), 6.52, 6.25 (each 1H, d, *J* = 15.9 Hz), 5.15 (1H, s), 5.04 (1H, m), 2.67 (2H, t, *J* = 6.7 Hz), 2.52 (2H, t, *J* = 6.7 Hz), 2.46 (1H, dd, *J* = 5.9, 17.2 Hz), 2.12 (1H, dd, *J* = 9.5, 17.2 Hz), 2.05, 1.96 (each 3H, s), 1.87 (2H, qn, *J* = 6.7 Hz), 1.79 (1H, m), 1.73 (3H, s), 1.59 (1H, t, *J* = 12.2 Hz), 1.11, 1.08 (each 3H, s). ^{13}C NMR ($CDCl_3$, ppm) δ: 170, 162, 139, 138, 134 (2C), 132, 129, 128, 126, 117, 96.3, 68.2, 43.9, 38.4, 36.6, 33.0, 31.3, 30.0, 28.5, 26.9, 21.5, 21.4, 14.9. FAB–HRMS calcd. for $C_{24}H_{31}NO_2$ *m/z*: 365.2355 [M^+]; found: 365.2354. ^1H NMR for the (*Z*)-isomer ($CDCl_3$, ppm) δ: 6.94, 6.57 (each 1H, d, *J* = 11.4 Hz), 6.50, 6.25 (each 1H, d, *J* = 16.0 Hz), 5.07 (1H, s), 5.04 (1H, m), 2.51 (2H, t, *J* = 6.7 Hz), 2.46 (1H, m) 2.42 (2H, t, *J* = 6.7 Hz), 2.11 (1H, dd, *J* = 9.5, 17.0 Hz), 2.04, 1.97 (each 3H, s), 1.83 (2H, qn, *J* = 6.7 Hz), 1.72 (3H, s), 1.59 (2H, t, *J* = 11.9 Hz), 1.11, 1.08 (each 3H, s).

14-Aldehyde (14)

To a solution of the (*E*)-nitrile **13** (35.7 mg, 97.7 μmol) in Et_2O (3.0 mL) at –78 °C, DIBAH (1.0 mol/L solution in toluene, 900 μL, 900 μmol) was added, and the resultant mixture was stirred at 0 °C. After 40 min of stirring, methanol (0.63 mL) was carefully added, followed by a 30% aqueous solution of Rochelle salt (potassium sodium tartrate) (1.08 mL), the resultant mixture was then stirred for 40 min. The organic layer was washed with a 30% Rochelle salt aqueous solution (2 × 0.45 mL and 1 × 0.23 mL). The combined aqueous layer was back-extracted with Et_2O (3 × 30 mL). The organic layers were washed with brine, combined, dried over $MgSO_4$, and concentrated under reduced pressure. Purification of the residue by silica gel column chromatography (Et_2O–hexanes, 50:50) afforded the aldehyde without an acetyl group at C-3 (26.1 mg, 80.1 μmol, 82%) as an oil. ^1H NMR ($CDCl_3$, ppm) δ: 10.0 (1H, d, *J* = 8.2 Hz), 6.90, 6.24 (each 1H, d, *J* = 11.3 Hz), 6.53, 6.27 (each 1H, d, *J* = 15.9 Hz), 5.93 (1H, d, *J* = 8.2 Hz), 3.97 (1H, m), 2.85 (2H, t, *J* = 6.7 Hz), 2.56 (2H, t, *J* = 6.7 Hz), 2.40 (1H, dd, *J* = 6.2, 17.0 Hz), 2.05 (1H, dd, *J* = 10.0, 17.0 Hz), 1.98 (3H, s), 1.89 (2H, qn, *J* = 6.7 Hz), 1.83–1.74 (1H, m), 1.74 (3H, s), 1.49 (1H, t, *J* = 11.8 Hz), 1.08 (3H × 2, s).

To a solution of the aldehyde (26.1 mg, 80.0 μmol) in a mixture of CH_2Cl_2 (1.0 mL) and pyridine (0.2 mL) at 0 °C, Ac_2O (0.1 mL) and DMAP (2.0 mg) were added, and the resultant mixture was allowed to warm to room temperature and was stirred at this temperature. After 1 h of stirring, the volatiles were removed in vacuo. Purification of the residue by silica gel column chromatography (Et_2O–hexanes, 15:85) afforded the alcohol (14.5 mg, 39.3 μmol, 50%) as an oil.

^{1}H NMR ($CDCl_3$, ppm) δ: 10.0 (1H, d, *J* = 8.2 Hz), 6.90, 6.24 (each 1H, d, *J* = 11.3 Hz), 6.53, 6.25 (each 1H, d, *J* = 15.9 Hz), 5.93 (1H, d, *J* = 8.2 Hz), 5.05 (1H, m), 2.85 (2H, t, *J* = 6.7 Hz), 2.56 (2H, t, *J* = 6.7 Hz), 2.45 (1H, dd, *J* = 5.5, 16.4 Hz), 2.12 (1H, dd, *J* = 9.4, 16.4 Hz), 2.05 (3H, s), 1.98 (3H, s), 1.90 (2H, qn, *J* = 6.7 Hz), 1.79 (1H, m), 1.74 (3H, s), 1.59 (1H, t, *J* = 12.0 Hz), 1.11, 1.08 (each 3H, s). ^{13}C NMR ($CDCl_3$, ppm) δ: 190, 170, 160, 139, 138, 135, 134, 132.4, 132.2, 128.8, 128.5, 126, 68.2, 43.8, 38.4, 36.6, 31.2, 29.9, 29.0, 28.4 (2C), 21.4 (2C), 14.9. FAB–HRMS calcd. for $C_{24}H_{31}O_3$: 367.2273 $[M - H]^+$; found: 367.2271.

15-*O*-TBS (15)

To a solution of the aldehyde **14** (14.5 mg, 39.3 μmol) in a mixture of $CHCl_3$ (0.2 mL) and methanol (1.0 mL) at –10 °C, $NaBH_4$ (8.9 mg, 236 μmol) was added. After 2 min of stirring, a satd. aq. solution of NH_4Cl (20 mL) was added and the resultant mixture was extracted with EtOAc (3 × 20 mL). The organic layers were washed with brine, combined, and dried over $MgSO_4$. ^{1}H NMR ($CDCl_3$, ppm) δ: 6.50, 6.10 (each 1H, d, *J* = 15.9 Hz), 6.47, 6.12 (each 1H, d, *J* = 11.3 Hz), 5.66 (1H, t, *J* = 7.0 Hz), 5.04 (1H, m), 4.25 (2H, d, *J* = 7.0 Hz), 2.52 (2H, t, *J* = 6.7 Hz), 2.44 (1H, dd, *J* = 6.0, 17.6 Hz), 2.36 (2H, t, *J* = 6.7 Hz), 2.11 (1H, dd, *J* = 8.9, 17.6 Hz), 2.05 (3H, s), 1.92 (3H, s), 1.83–1.70 (3H), 1.72 (3H, s), 1.58 (1H, t, *J* = 11.9 Hz), 1.10, 1.07 (each 3H, s).

To a solution of the crude alcohol in CH_2Cl_2 (1.0 mL) at room temperature, imidazole (16.0 mg, 197 μmol) was added. The mixture was cooled to 0 °C, and TBSCl (11.8 mg, 78.6 μmol) was added at this temperature. After 2 h of stirring, the mixture was poured into water (20 mL) and the resultant mixture was extracted with Et_2O (3 × 20 mL). The organic layers were washed with brine, combined, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by silica gel column chromatography (EtOAc–hexanes, 7:93) afforded **15** (13.4 mg, 27.6 μmol, 70%, in two steps) as a colorless oil. ^{1}H NMR ($CDCl_3$, ppm) δ: 6.51, 6.08 (each 1H, d, *J* = 16.0 Hz), 6.42, 6.11 (each 1H, d, *J* = 11.4 Hz), 5.57 (1H, t, *J* = 6.4 Hz), 5.05 (1H, m), 4.28 (2H, d, *J* = 6.4 Hz), 2.52 (2H, t, *J* = 6.7 Hz), 2.44 (1H, dd, *J* = 5.5, 17.0 Hz), 2.31 (2H, t, *J* = 6.7 Hz), 2.10 (1H, dd, *J* = 9.3, 17.0 Hz), 2.05, 1.91 (each 3H, s), 1.82–1.73 (3H), 1.72 (3H, s), 1.58 (1H, t, *J* = 12.0 Hz), 1.10, 1.07 (each 3H, s), 0.906 (3H × 3, s), 0.0813 (3H × 2, s). ^{13}C NMR ($CDCl_3$, ppm) δ: 170, 139, 138, 135, 134, 132, 131, 130.4, 130.3, 125.7, 125.0, 68.4, 59.9, 44.0, 38.5, 36.7, 31.3, 30.4, 30.1, 28.9, 28.6, 27.9, 26.1 (3C), 21.6, 18.5, 14.7, –4.80 (2C). FAB-HRMS calcd. for $C_{30}H_{48}O_3Si$ *m*/*z*: 484.3373 $[M^+]$; found: 484.3366.

3-*O*-Biotinyl (Boc)Lys-15-*O*-TBS (16)

To a solution of **15** (5.4 mg, 11.1 μmol) in methanol (1.0 mL) at 0 °C, K_2CO_3 (2.0 mg, 14.5 μmol) was added. The mixture was stirred and allowed to warm to room temperature. After 12 h of stirring, the mixture was poured into a satd. aq. solution of NH_4Cl (20 mL) and the resultant mixture was extracted with EtOAc (3 × 20 mL). The organic layers were washed with brine, combined, dried over $MgSO_4$, and concentrated under reduced pressure. ^{1}H NMR ($CDCl_3$, ppm) δ: 6.51, 6.11 (each 1H, d, *J* = 15.9 Hz), 6.43, 6.11 (each 1H, d, *J* = 11.4 Hz), 5.57 (1H, t, *J* = 6.3 Hz), 4.28 (2H, d, *J* = 6.3 Hz), 4.00 (1H, m), 2.52 (2H, t, *J* = 6.6 Hz), 2.38 (1H, dd, *J* = 5.0, 16.7 Hz), 2.31 (2H, t, *J* = 6.6 Hz), 2.04 (1H, dd, *J* = 9.7, 16.7 Hz), 1.91 (3H, s), 1.77 (1H, m), 1.73 (2H, qn, *J* = 6.7 Hz), 1.73 (3H, s), 1.47 (2H, t, *J* = 11.9 Hz), 1.07 (3H × 2, s), 0.906 (3H × 3, s), 0.0807 (3H × 2, s).

To a mixture of the crude 3-OH compound, biotinyl (Boc)Lys-OH (7.9 mg, 16.7 μmol),1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 6.4 mg, 33.3 μmol) at 0 °C, and CH_2Cl_2 (0.7 mL) were added. DMAP (2.0 mg) was also added at this temperature. The mixture was warmed to room temperature and stirred. After 3 h of stirring, the mixture was poured into a satd. aq. solution of $NaHCO_3$ (20 mL) and the resultant mixture was extracted with $CHCl_3$ (20 mL). The organic layer was washed with a satd. aq. NH_4Cl solution, water, and brine, followed by drying over $MgSO_4$. Purification of the residue by silica gel column chromatography (methanol–$CHCl_3$, 7:93) afforded **16** (7.8 mg, 8.69 μmol, 78%, in two steps) as a colorless oil. ^{1}H NMR ($CDCl_3$, ppm) δ: 6.52, 6.11 (each 1H, d, *J* = 16.1 Hz), 6.44, 6.13 (each 1H, d, *J* = 11.3 Hz), 6.24 (1H, brs), 6.18 (1H, brs), 5.59 (1H, t, *J* = 6.5 Hz), 5.45–5.38 (1H, m), 5.37 (1H,brs), 5.20 (1H, d, *J* = 6.5 Hz), 5.10 (1H, m), 4.53 (1H, dd, *J* = 4.8, 7.5 Hz), 4.37–4.28 (1H, m), 4.29 (2H, d, *J* = 6.5), 4.23 (1H, m), 3.23 (2H, qn, *J* = 5.9 Hz), 3.16 (1H, m), 2.92 (1H, dd, *J* = 4.8, 12.6 Hz), 2.75 (1H, d, *J* = 12.6 Hz), 2.52 (2H, t, *J* = 6.4 Hz), 2.44 (1H, m), 2.32 (2H, t, *J* = 7.4 Hz), 2.20 (2H, t, *J* = 7.4 Hz), 2.13 (1H, m), 1.91 (3H, s), 1.85–1.50 (15H), 1.73 (3H, s), 1.45 (3H × 3, s), 1.10, 1.08 (each 3H, s), 0.908 (3H × 3, s), 0.0813 (3H × 2, s). FAB-HRMS calcd. for $C_{49}H_{81}N_4O_7SSi^+$ *m*/*z*: 897.5590 $[M + H]^+$; found: 897.5574. FAB-HRMS calcd. for $C_{49}H_{81}N_4NaO_7SSi$: 920.5493 $[M + H + Na]^+$; found: 920.5489.

3-*O*-Biotinyl (Boc)Lys 15-*O*-bromoacetate (1)

To a solution of **16** (4.1 mg, 4.57 μmol) in THF (1.0 mL) at 0 °C, TBAF (1.0 mol/L solution in THF, 9.2 μL, 9.2 μmol) was added. The mixture was warmed to room temperature and stirred. After 2 h of stirring, the mixture was poured into water (20 mL) and the resultant mixture was extracted with $CHCl_3$ (3 × 20 mL). The organic layer was washed with brine, combined, dried over $MgSO_4$, and concentrated under reduced pressure. Purification of the residue by silica gel column chromatography (methanol–$CHCl_3$, 10:90) afforded the biotinylated alcohol (2.1 mg, 2.68 μmol, 60%) as a colorless oil. ^{1}H NMR ($CDCl_3$, ppm) δ: 6.52, 6.13 (each 1H, d, *J* = 15.3 Hz), 6.49, 6.15 (each 1H, d, *J* = 11.1 Hz), 5.90 (1H, brs), 5.68 (1H, t, *J* = 6.8 Hz), 5.56 (1H, brs), 5.17 (1H, d, *J* = 7.9 Hz), 5.10 (1H, m), 4.90 (1H, brs), 4.52 (1H, m), 4.33 (1H, m), 4.26 (2H, d, *J* = 6.8 Hz), 4.25 (1H, brs), 3.23 (2H, brs), 3.17 (1H, q, *J* = 4.4 Hz), 2.93 (1H, dd, *J* = 4.8, 12.9 Hz), 2.74 (1H, d, *J* = 12.9 Hz), 2.53 (2H), 2.44 (1H, m), 2.37 (2H, t, *J* = 6.6 Hz), 2.28–2.18 (2H), 2.18–2.10 (1H, m), 1.92 (3H, s), 1.85–1.48 (16H), 1.73 (3H, s), 1.45 (3H × 3, s), 1.10, 1.08 (each 3H, s).

To a solution of the biotinylated alcohol (2.1 mg, 2.68 μmol) in CH_2Cl_2 (0.7 mL) at room temperature, $BrCH_2COOH$ (2.0 mg, 14.4 μmol) was added. The mixture was cooled to –10 °C and EDC (3.1 mg, 16.1 μmol) and DMAP (1.0 mg) were added. After 15 min of stirring at the

same temperature, the mixture was poured into a satd aq. solution of $NaHCO_3$ (20 mL) and the resultant mixture was extracted with $CHCl_3$ (20 mL). The organic layer was washed with a satd aq. NH_4Cl solution, water and brine, followed by drying over $MgSO_4$. After concentration in vacuo, purification of the residue by silica gel column chromatography (methanol–$CHCl_3$, 7:93) afforded the biotinylated bromoacetate **1** (1.6 mg, 1.77 μmol, 66%) as a colorless oil. ^{1}H NMR ($CDCl_3$, ppm) δ: 6.53, 6.13 (each 1H, d, J = 11.4 Hz), 6.50, 6.12 (each 1H, d, J = 15.7 Hz), 5.86 (1H, t, J = 5.0 Hz), 5.58 (1H, t, J = 7.3 Hz), 5.51 (1H, brs), 5.16 (1H, d, J = 8.6 Hz), 5.10 (1H, m), 4.87 (1H, brs), 4.77 (2H, d, J = 7.3 Hz), 4.51 (1H, dd, J = 4.7, 7.8 Hz), 4.32 (1H, dd, J = 4.7, 7.6 Hz), 4.23 (1H, m), 3.83 (2H, s), 3.23 (2H, qn, J = 6.1 Hz), 3.16 (1H, m), 2.92 (1H, dd, J = 4.7, 12.6 Hz), 2.73 (1H, d, J = 12.6 Hz), 2.51 (2H, t, J = 7.0 Hz), 2.39 (2H, t, J = 6.5 Hz), 2.10 (1H, m), 2.14 (2H, t, J = 6.5 Hz), 2.10 (1H, m), 1.92 (3H, s), 1.88–1.30 (16H), 1.72 (3H, s), 1.45 (3H × 3, s), 1.10, 1.08 (each 3H, s).

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