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Synthesis of biotinylated retinoids for cross-linking and isolation of retinol binding proteins

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This article is dedicated to Professor Yoshito Kishi on his receipt of the Tetrahedron Prize

Abstract—The synthesis of (3*R*)-3-[Boc-Lys(biotinyl)-*O*]-11-*cis*-retinol bromoacetate and 3-[Boc-Lys(biotinyl)-*O*]-*all trans*-retinol chloroacetate is described. These biotinylated retinoids are instrumental in labeling the retinol binding proteins (RBPs) via a nucleophilic displacement of the haloacetate by a residue in the binding site of the protein. The covalently linked biotin will allow for a facile isolation and purification of the protein on a streptavidin column thus rendering the protein ready for a tryptic digest followed by mass spectrometric analysis. The 11-*cis* retinoid was synthesized via metal reduction of an alkyne intermediate generated from a Horner–Wadsworth–Emmons (HWE) reaction whereas the *all-trans* was synthesized via two consecutive HWE couplings.

1. Introduction

Retinoids are derivatives of vitamin A (*all trans*-retinol) which can generally be synthesized for studies directed to a better understanding of human and non-human vision. We hereby report the synthesis of (3*R*)-3-[Boc-Lys(biotinyl)-*O*]-11-*cis*-retinol bromoacetate (**1**) and 3-[Boc-Lys(biotinyl)-*O*]-*all trans*-retinol chloroacetate (**2**) (Fig. 1). These retinoids can act as ligands for proteins known as retinol binding proteins (RBPs) present in the retinal pigment epithelial (RPE) cells.[1] These RBPs are believed to be responsible for the transport of retinols in RPE cells and some are involved in catalyzing esterification, hydrolysis, and isomerization of retinols at various stages in the visual cycle. A summary of the mammalian visual cycle is presented in Fig. 2. The visual protein, rhodopsin, is comprised of the protein opsin (ca. 348 amino acids in bovine) and 11-*cis*-retinal covalently linked via a schiff base with Lys296 residue. Vision begins with the absorption of light by rhodopsin causing the isomerization of 11-*cis*-retinylidene to *all trans*-retinylidene. This occurs over several states of rhodopsin leading to a meta II state (unprotonated schiff base) which is responsible for G-protein activation ultimately leading to neural signaling.[2]

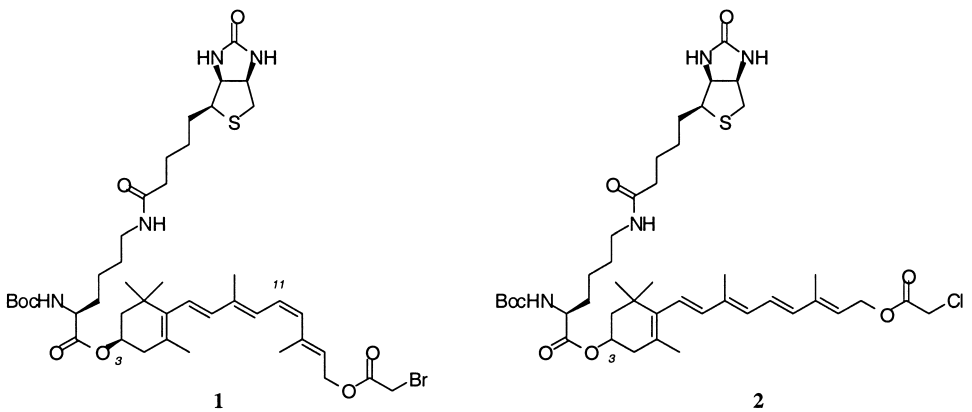

Figure 1. The target retinoid biotinylated structures.

Keywords: biotinylated retinoids; biotin; retinol binding proteins; RBP.
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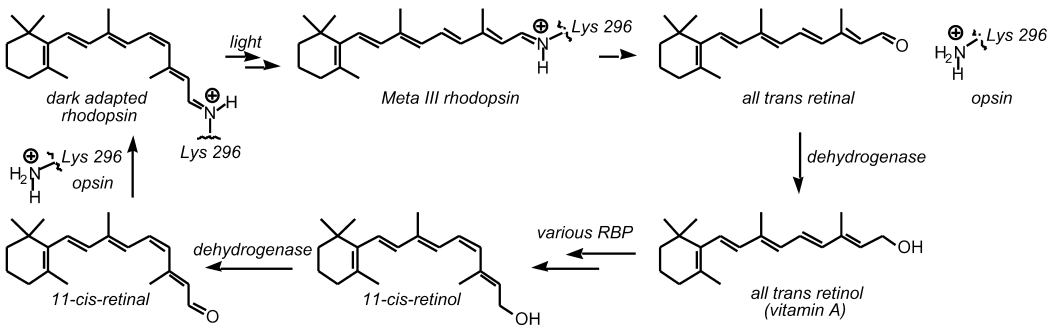


Figure 2. The mammalian visual cycle.

This isomerization is accompanied by various conformational changes of the protein which in turn trigger the binding of G-protein. These photo intermediates have been well studied through photoaffinity labeling experiments, protein digestion and mass spectrometric characterization.[3] The meta II state is then protonated generating meta III (see Fig. 2) which undergoes hydrolysis regenerating the protein opsin and *all trans*-retinal. To complete the visual cycle, the *all trans*-retinal needs to be re-isomerized back to 11-*cis*-retinal. This is achieved through a cascade of several events involving proteins such as dehydrogenase which initially reduces *all trans*-retinal to *all trans*-retinol (vitamin A). The latter is then isomerized to 11-*cis*-retinol through the action of numerous RBPs which we are interested in further investigating.[1] The actions of these proteins include the esterification of vitamin A with a palmitoyl group followed by an isomerization of the resulting palimitate through a possible hydrolytic mechanism. One such protein that has been already under investigation is known as lecithin retinol acyl transferase (LRAT).[4,5] To regenerate the visual pigment, 11-*cis*-retinol undergoes oxidation via a dehydrogenase rendering 11-*cis*-retinal ready for incorporation into opsin forming the Schiff base rhodopsin.

In the interest of isolating and investigating the RBPs involved in the transport and transformation of *all trans*-retinol to the 11-*cis*-retinol the target molecules **1** and **2** were synthesized. The *all trans* biotinylated retinoid **2** is needed as a ligand for RBPs involved in the earlier stages of

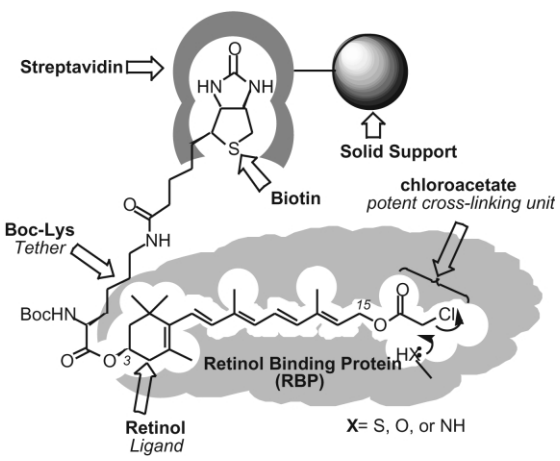


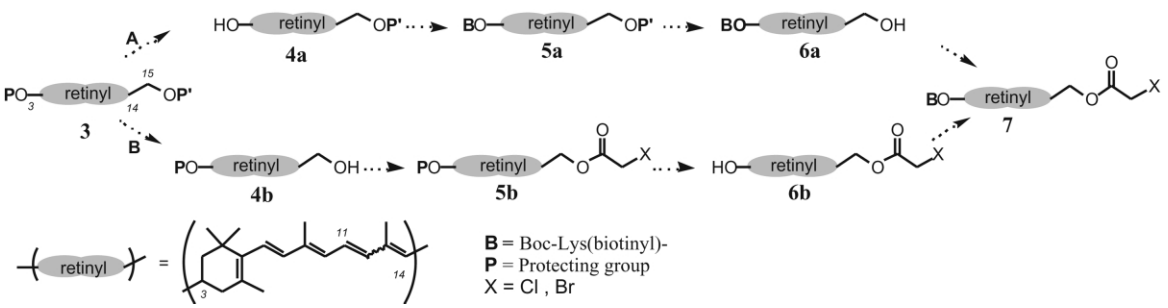
Figure 3. A schematic diagram of structure **2** in the binding site of a representative RBP featuring key elements of the structure and their role in protein labeling and isolation.

the transformation while the retinoid **1** may prove helpful for isolating RBPs that act in the later stages of pigment regeneration. The features of the target molecules are outlined in Fig. 3 as exemplified through retinoid **2**. The *all trans* retinol portion of the structures acts as a ligand for the binding site of the RBP. The chloroacetyl moiety acts as a potent electrophile for a nearby residue to carry out a nucleophilic displacement of the halogen forming a covalent bond with a residue in the active site of the protein. This nucleophilic displacement resulting in a covalent bond with the protein is referred to as the labeling step of the protein. The ultimate result is that the RBPs which inherently bind retinol (hence the name) will be specifically labeled with these structures bearing an extended tether (Boc-Lys) to a biotin. The latter will allow for the facile isolation of the protein via affinity chromatography through an avidin column. This technique proved to be successful in the isolation and identification of mammalian LRAT using biotinylated affinity labeling agents.[6] The biotin affinity labeling approach was important here because membrane bound LRAT was not susceptible to purification due to its instability when solubilized in detergent.

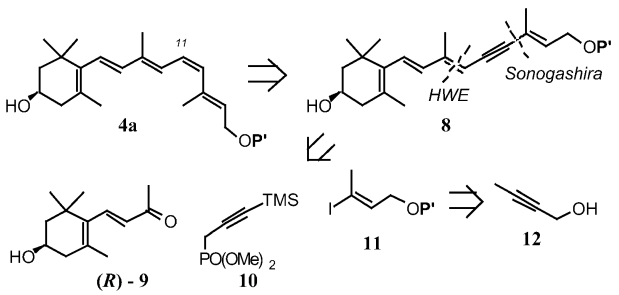
One important advantage that is featured in the target structures needs to be emphasized. The biotin allows for the successful isolation of the protein due to its remarkable binding to streptavidin with association constants often exceeding $10^{15}\,M^{-1}$.[6,7] However, this often presents a challenge for the scientist who is interested in isolating the protein via breaking this association. The above structure features a labile ester of the primary alcohol at C-15 of the retinol hydroxyl which can be easily cleaved in basic media (pH~11).

1.1. Retrosynthetic analysis of 1 and 2

Scheme 1 represents the two approaches for the installation of a biotin on one end of the retinoid and a haloester on the other end. Structure **7** represents the general formula for retinoids **1** and **2**. Structure **3** represents the retinoid backbone with some orthogonal protection on the hydroxy groups at C-3 and C-15. Route A will entail installing the Boc-Lys(biotinyl)-OH (**B**-OH) first directly after deprotection of the C-3 alcohol (unless prior steps involve releasing the 3-hydroxy) and then attachment of the haloacid via an EDC coupling reaction. Route B, however, will require the release of the primary alcohol at C-15, attachment of the haloester, and finally deprotection of the 3-hydroxy



Scheme 1. Retrosynthetic analysis using two poteintal route A and B.



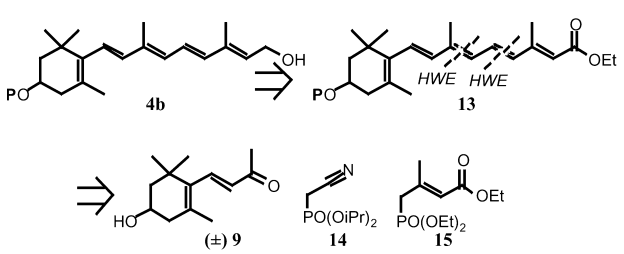
Scheme 2.

followed by esterification with **B**-OH. The first route is favored as the labile haloester which is attached last will not need to endure the steps presented in Route B. However, both routes were used as will become apparent in due course owing to the nature of the retinoid precursor **3** for each of the two target molecules, the *cis* and the *trans*.

The retrosynthesis of **4a** is outlined in Scheme 2. The 11-*cis* double bond can be generated via a zinc metal reduction of the alkyne intermediate **8**.[8] The latter can be constructed via a Horner–Wadsworth–Emmons (HWE) coupling of the 3-hydroxy-β-ionone (*R*)-**9** and phosphonate **10** followed by a Sonogashira coupling[8] with vinyl iodide **11**, which can be obtained from a reduction of **12** followed by iodination with I_2.[9]

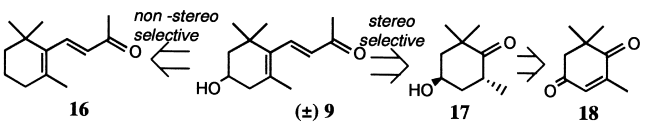
The 3-hydroxy-β-ionone can also be used in the synthesis of **4b** as shown in the retrosynthetic analysis in Scheme 3. Two consecutive HWE couplings: the first between (±)-**9** and **14** followed by DIBAH reduction to generate an aldehyde ready for the second coupling with phosphonate **15** thus generating **13**. Reduction of the latter with $LiAlH_4$ will afford the desired 3-siloxylated-*all trans*-retinol **4b**.[10,11]

Retrosynthetic Schemes 2 and 3 indicate the key role of the compound 3-hydro-β-ionone **9**. The stereochemistry at the 3-hydroxy position does not need to be defined for the



Scheme 3.

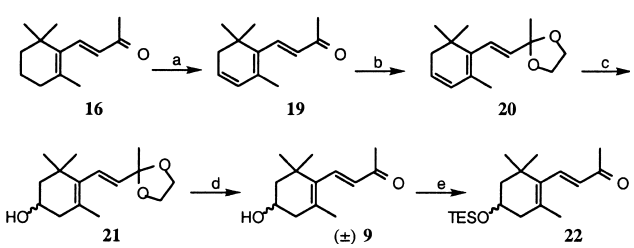
biological assays since the biotin linker is not directly involved in the binding of the protein. We have in fact generated 3-hydroxy-β-ionone **9** from two independent routes and have arbitrarily proceeded with (*R*)-**9** for the synthesis of **1** and the racemic **9** for the synthesis of **2**. The stereoselective route for the generation of (*R*)-**9** was needed for another purpose that will be published elsewhere. Scheme 4 presents that two routes for the generation of **9**. The non-stereoselective route will involve the introduction of a double at the C-3,4[12] followed by hydroboration treatment[13] to afford (±)-**9**.[14] The stereoselective route will involve the stereoselective reduction of **18** followed by a second reduction of the unhindered carbonyl resulting in **17**.[15] The latter can be transformed into (*R*)-**9** via triflation[16] followed by a Heck reaction.[17]



Scheme 4.

2. Results and discussion

The synthesis of the racemic 3-hydroxy-β-ionone (±)-**9** from β-ionone **16** was carried out according to Scheme 5.[14] Treatment of the latter with *N*-bromosuccinimide (NBS) under basic and refluxing conditions yielded a 4-bromo derivative which eliminated when heated in pyridine for 2 h affording 3,4-dehydro-β-ionone **19** in a 50% overall yield.[12] Protection of the ketone occurred smoothly with ethylene glycol in benzene catalyzed by *p*-toluenesulfonic acid in the presence of triethylorthoformate.[13] The latter acted as a water trap and its presence resulted in enhancement of the yield from 50% (with a Dean–Stark distillation) to 80%.



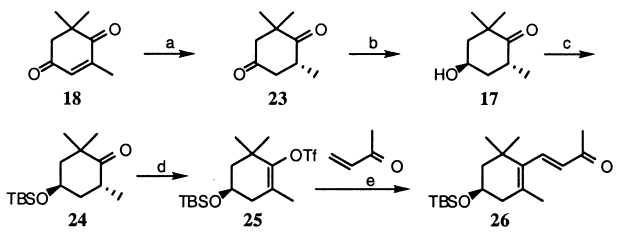
Scheme 5. *Reagents*: (a) (i) CaO, $NaHCO_3$, NBS/CCl_4 reflux, 2 h; (ii) $PhN(CH_3)_2$, 90°C, 1 h; (iii) pydrine, 90°C, 2 h; 50%; (b) $HOCH_2CH_2$-OH, TsOH, $HC(OEt)_3$, PhH, reflux, 2 h; 80%; (c) (i) 9-BBN/THF 55°C 4 h; (ii) MeOH; (iii) NaOH, H_2O_2, 0°C, 55%; (d) 5% HCl/acetone, 5 min, 99%; (e) TESCl, DMAP, Et_3N/CH_2Cl_2; 97%. TES, triethylsilyl.

Hydroboration of **20** with 9-BBN in THF followed by oxidative methanolic work up afforded a mixture of hydroxylated compound enriched in the desired **21** (racemic) with a yield of 55%.[13] Deprotection of the latter in 5% HCl in acetone for 5 min afforded (±)-**9** in quantitative yield. Silylation of 3-hydroxy-β-ionone **9** was carried out, as necessary for further transformation, using triethylsilyl as a protecting group with a yield up to 97% resulting in **22**. The overall yield of transformation from **16** to **22** was 21%.

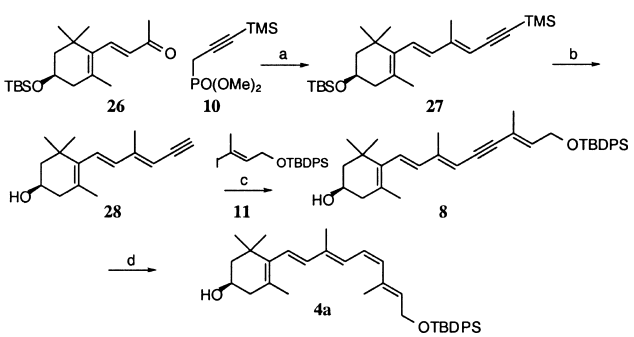
The stereoselective route for the synthesis of (3*R*)-3-hydroxy-β-ionone (*R*)-**9** shown in Scheme 6 involved an enzymatic reduction of 2,6,6-trimethyl-2-cyclohexen-1,4-dione (4-oxoisophorone) **18** with baker's yeast to generate **23** after which a Raney Nickel reduction resulted in **17**.[15] A generous amount of **17** was provided to us by Hoffmann LaRoche from which the rest of the synthesis was actually carried out. Silylation of the latter under standard conditions of DMAP and triethylamine resulted in a quantitative yield of **24**.[16] Triflation was carried out on **24** with 1.1 equiv. of LDA and Tf_2NPh to afford **25** in a modest 68% yield.[16] This was followed by a Heck reaction with methyl vinyl ketone (MVK) catalyzed by Pd(0), which forms in situ from Pd(II), to give **26** in 67% yield.[17] The overall yield of transformations from compound **17** to the desired (3*R*)-3-siloxylated-β-ionone **26** was 44%.

It was completely arbitrary and a result of synthetic timing that resulted in the usage of **26** in the synthesis of 11-*cis* retinoid **1** and the usage of the racemic precursor **22** in the synthesis of the *all trans* retinoid **2**. Scheme 7 presents the synthesis of the 11-*cis* precursor monoprotected retinoid **4a**.

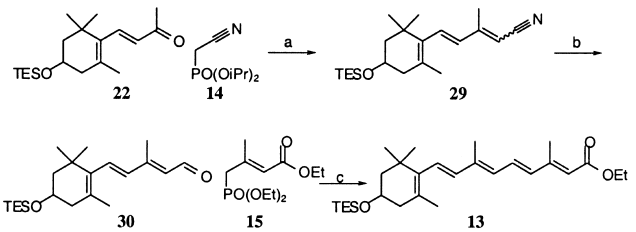
The silyloxylated β-ionone **26** was treated with phosphonate[18] **10** and BuLi to afford the protected alkyne **27** in a quantitative yield with 4:1 of the desired *E*/*Z* ratio. The isomeric mixture inseparable at that stage was subjected to a global deprotection of both silyl groups with TBAF resulting in the terminal alkyne **28** ready for a Sonogashira coupling with vinyl iodide **11** generating **8** in quantitative yield. The 9*E* and 9*Z* isomers were then easily separated at that stage due to the presence of the bulky silyl group via column chromatography. Vinyl iodide **11** was prepared via reduction of 2-butyn-1-ol **12** with titanocene dichloride[9] followed by quenching with I_2 and protection of the primary alcohol with *tert*-butyldiphenylsilyl chloride (TBDPSCl). The key step of generating the 11-*cis* from alkyne **8** utilized a copper/silver pretreated zinc reduction[8] of the latter in a 1:1 MeOH/H_2O mixture over a period of ca. 24 h resulting in a nearly quantitative yield of exclusively the 11-*cis* isomer **4a**.



Scheme 6. *Reagents*: (a) Baker's yeast, sucrose; 24%; (b) Raney Ni; 66%; (c) TBSCl, DMAP, Et_3N/CH_2Cl_2; 97%; (d) (i) LDA (ii) Tf_2NPh; 68%; (e) $PdCl_2(PPh_3)_2$, DMF, 80°C, 30 h; 67%. TBS, *tert*-butyldimethylsilyl.



Scheme 7. *Reagents*: (a) BuLi+**10** at 0°C then 26/THF; 99%; (b) TBAF, 4 h; 99%; (c) CuI, $Pd(PPh_3)_4$, *i* $PrNH_2$; 99%; (d) Zn(Cu/Ag), MeOH/H_2O, 21 h; 99%. TMS, trimethylsilyl; TBDPS, *tert*-butyldiphenylsilyl.

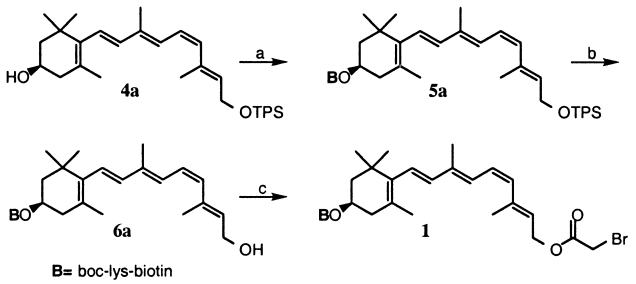


Scheme 8. *Reagents*: (a) NaH/THF; 84%; (b) DIBAH, CH_2Cl_2, −60°C; 74%; (c) BuLi, THF; 88%.

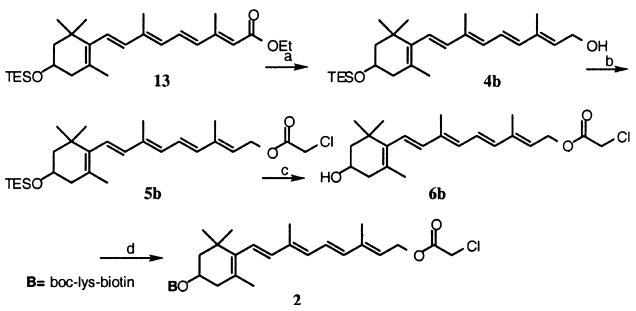
Synthesis of the *all trans* retinoid **13** involved a series of two HWE couplings outlined in Scheme 8.[10,11] The siloxylated β-ionone **22** was treated with the NaH generated anion of phosphonate **14** to afford **29** in a mixture of 3.5:1 of 9*E*/9*Z* which were separated after DIBAH reduction resulting in the desired 9*E* **30** in an overall yield of 61%. The second HWE of the latter with **15** pretreated with BuLi resulted in a 4:1 of 13*E*/13*Z* mixture separable by column chromatography to afford **13**.

Due to the nature of the structures of the retinoid precursors **4a** and **13**, both routes A and B (see Scheme 1) were utilized in installing the biotin group. For instance, in the case of **13** the only means of introducing the primary alcohol at C-15 is through an LAH reduction. Therefore the Boc-Lys-biotin (**B**) had to be introduced last (according to route B) as it would not have survived the latter reduction. Thus **13** must be first reduced with LAH to the primary alcohol, esterified with the appropiate α-haloacid, then desilylated without disturbing the labile haloester, and finally biotinylated with Boc-Lys(biotinyl)-OH. The 11-*cis* was fortunately carried through as the protected primary alcohol and a free C-3 hydroxyl allowing for the transformations depicted by route A (the more desirable of the two routes).

Scheme 9 describes the transformations required for the generation of the target biotinylated retinoid **1** according to route A. The monoprotected 3-hydroxy retinoid **4a** was subjected to EDC coupling conditions with Boc-Lys(biotinyl)-OH affording **5a** in 78% yield. This was followed by TBAF deprotection of the primary alcohol resulting in **6a** with a yield of 79%. The unstable primary alcohol was immediately treated with bromoacetic acid under EDC



Scheme 9. *Reagents*: (a) **B**-OH/DMSO, EDC, DMAP, CH_2Cl_2; 78%; (b) 2 equiv. TBAF, 0°C–rt, 2 h; 79%; (c) $BrCH_2CO_2H$, EDC, DMAP, CH_2Cl_2, −10°C, 15 min; 99%. **B**, Boc-Lys(biotinyl)-.



Scheme 10. *Reagents*: (a) $LiAlH_4$, Et_2O, −10°C, 10 min; (b) $ClCH_2CO_2H$, EDC, DMAP, CH_2Cl_2, −10°C, 5 min; (c) 5% HF/pyridine, THF, 0°C, 5 min; 27% over last three steps; (d) **B**-OH/DMSO, EDC, DMAP, CH_2Cl_2, −10°C, 5 min; 40%. **B**, Boc-Lys(biotinyl)-.

coupling conditions to generate the desired final product **1** in quantitative yield.

The transformations that brought about the final stitches in the construction of **2** in Scheme 10 were certainly a lot more cumbersome due to the undesirable route B that was pursued. Needless to say, several attempts at completing this route proved unsuccessful due to the labile nature of the chloroester (which indeed is much more unstable than its bromo analog). The latter information regarding the stability of the bromoester versus the chloroester was not available to us until after the completion of this work and hence the reason for the synthesis of the chloroester analog **2**. Moreover, preliminary unpublished biological assay results, which were not available during the time of the synthesis of the chloroester, have shown no preference toward having one halogen over the other, and therefore future targets will employ the exclusive use of α-bromoesters.

The silylated ester **13** was reduced with LAH resulting in **4b** which was subsequently used without purification in an EDC coupling reaction with chloroacetic acid affording **5b** which was purified rapidly by flash chromatography to avoid major decomposition. The latter was mildly desilylated by treatment with 5% HF/pyridine in THF for 5 min affording **6b** in a modest overall yield of 27% from the starting ester **13**. An EDC coupling of **6b** with Boc-Lys(biotinyl)-OH resulted in the formation of an apparent quantitative yield of **2** by TLC; however a maximum of 40% recovery was achieved after workup and purification of the desired compound **2**.

3. Conclusion

The synthesis of biotinylated retinoids **1** and **2** was accomplished using two different routes. Installing the biotin first, route A, was preferred due to the labile nature of α-haloesters (in particular chloroesters) which were preferably attached last. Route A was used in the synthesis of **1**, however, to avoid multiple protection and deprotection procedures, route B (attaching the chloroester first) was employed in the synthesis of **2**. The overall yield for the synthesis of **1** and **2** were 20% (from **17**) and 0.7%, respectively. The biotinylated targets are currently in use for labeling RBPs in the retinal pigment epithelial (RPE) cells allowing for facile isolation via avidin affinity chromatography, hydrolysis off the column, and final characterization by mass spectrometry.

4. Experimental

4.1. General

All chemicals were obtained from Aldrich except for Boc-Lys(biotinyl)-OH which was purchased from BAChem. The solvents THF, Et_2O, and CH_2Cl_2 were obtained dry from the solvent purification system via passage through an activated alumina cartridge. Column chromatography was performed using ICN silica gel (32–63 mesh). ^1H NMR spectra were obtained on Bruker DMX 400 or 500 MHz spectrometers and are reported in parts per million (ppm) relative to TMS (δ), with coupling constants (J) in Hertz (Hz). The residual protic sovlents (in $CDCl_3$ or C_6D_6) were used as an internal reference. Low and high resolution FAB mass spectra were measured on a JEOL JMS-DX303 HF mass spectrometers using a glycerol matrix and Xe ionizing gas. ESI and APCI spectra were measured on a JEOL LC mate mass spectrometer. The *all trans* retinoids were handled under dim light conditions especially when containing more than 3 conjugated double bonds. The 11-*cis* compounds were handled strictly in the darkroom under dim red lights. Retinoids are best stored in benzene solutions frozen at −78°C, unless they are not soluble.

4.2. Synthesis of biotinylated retinoid 1

4.2.1. (*R*)-5-(*tert*-Butyldimethylsiloxy)-1,3,3-trimethyl-2-[3-methyl-6-(trimethylsilyl)-hexa-1,3-dien-5-ynyl]-cyclohexene (27). Dimethyl (3-trimethylsilyl-2-propynyl)phosphonate **10** (0.550 g, 2.5 mmol) in 10 mL of THF at 0°C was treated with *n* BuLi (2.5 mmol) to give a red solution. The reaction mixture was then stirred at room temperature for 15 min, after which time **26** (0.400 g, 1.24 mmol) in 1 mL of THF was added. The mixture was stirred for an additional 2 h, and was then quenched with aqueous NH_4Cl, extracted with Et_2O, washed with brine, and dried over Na_2SO_4. The product was purified by column chromatography using 2% Et_2O in hexanes as an eluent to afford **27** as an oil (0.511 g, 99% yield; 4:1 9*E*/9*Z*). ^1H NMR (500 MHz, $CDCl_3$): δ=6.90 (d, 1H of 9*Z*, J=16.4 Hz), 6.31 (d, 1H of 9*Z*, J=16.4 Hz), 6.21 (d, 1H of 9*E*, J=16.4 Hz), 6.07 (d, 1H of 9*E*, J=16.4 Hz), 5.44 (s, 1H of 9*E*), 5.37 (s, 1H of 9*Z*), 3.93 (m, 1H), 2.22 (m, 1H), 2.07 (s, 3H of 9*E*), 2.1–2.0 (m, 1H), 1.92 (s, 3H of 9*Z*), 1.78 (s, 3H of 9*Z*), 1.68 (s, 3H of 9*E*),

1.49 (t, 1H, J=12.2 Hz), 1.11 and 1.08 (s, 2×3H), 0.91 (s, 6H), 0.20 (s, 9H). [$C_{25}H_{44}OSi_2+H^+$]: calcd 417.30; found 417.2 (APCI+).

4.2.2. (*R*)-5-Hydroxy-1,3,3-trimethyl-2-[3-methyl-6-hexa-1,3-dien-5-ynyl]-cyclohexene (28). Acetylene **27** (0.511, 1.23 mmol) in 5 mL THF at 0°C was treated with TBAF (5 mL, 1 M in THF, 5 mmol) and the stirred at room temperature for 2 h. The mixture was then quenched with aqueous NH_4Cl, extracted with Et_2O, washed with brine, and dried over Na_2SO_4. The product was purified by column chromatography using 30% EtOAc in hexanes as an eluent to afford **28** as an oil (0.282 g, 99% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.83 (d, 1H of 9*Z*, J=16.4 Hz), 6.31 (d, 1H of 9*Z*, J=16.4 Hz), 6.26 (d, 1H of 9*E*, J=16.4 Hz), 6.11 (d, 1H of 9*E*, J=16.4 Hz), 5.44 (s, 1H of 9*E*), 5.37 (s, 1H of 9*Z*), 4.02 (m, 1H), 3.32 (s, 1H of 9*E*), 3.20 (s, 1H of 9*Z*), 2.4 (dd, 1H, J=17.2, 5.3 Hz), 2.10 (s, 3H), 2.15–2.00 (m, 1H), 1.96 (s, 3H of 9*Z*), 1.8 (m, 1H) ovrlp with 1.8 (s, 3H of 9*Z*), 1.74 (s, 3H of 9*E*), 1.51 (t, 1H, J=11.9 Hz), 1.1–1.08 (2s, 2×3H). [$C_{25}H_{22}O+H^+$]: calcd 231.17; found 231.1 (APCI+).

4.2.3. (*R*)-3-Hydroxy-retin-11-yne-O-*tert*-butyldiphenylsilyl (8). Vinyl iodide **11** (872 mg, 1.6 mmol) was dissolved in *i*PrNH$_2$ (4 mL), and tetrakis(triphenylphosphine)palladium (13.8 mg, 0.0124 mmol) was then added. The solution was stirred at room temperature for 5 min prior to the addition of CuI (2.4 mg, 0.0124 mmol). After 5 min of stirring, aceylene **28** (282 mg, 1.23 mmol) was introduced, and the mixture was stirred at room temperature for 2 h. The reaction was then quenched by the removal of solvent followed by the addition of Et_2O and then NH_4Cl. The resultant mixture was extracted with Et_2O, washed with brine, and dried over Na_2SO_4. The product was purified by column chromatography using 12–20% EtOAc in hexanes as an eluent to afford **8** as an oil (432 mg of 9*E*, and 660 mg total weight, 99% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.69 (m, 4H), 7.42 (m, 6H), 6.19 (d, 1H, J=16.0 Hz), 6.10 (d, 1H, J=16.0 Hz), 6.02 (t, 1H, J=5.1 Hz), 5.54 (s, 1H), 4.29 (d, 2H, J=6.28 Hz), 4.00 (m, 1H), 2.38 (dd, 1H, J=16.9, 5.2 Hz), 2.1–2.0 (m, 1H) ovrlp with 2.06 (s, 3H), 1.77 (ddd, 1H, J=12.1, 3.3, 2.1 Hz), 1.72 (s, 3H), 1.67 (s, 3H), 1.47 (t, 1H, J=11.9 Hz), 1.35 (d, 1H, J=4.9 Hz), 1.06 and 1.05 (2s, 2×3H) ovrlp with 1.05 (s, 9H). [$C_{36}H_{46}O_2Si+H^+$]: calcd 539.33; found 539.3 (APCI+).

4.2.4. (*R*)-3-Hydroxy-11-*cis*-retin-*O*-*tert*-butyldiphenylsilyl (4a). Argon was bubbled through a suspension of Zn dust (10 g) in distilled H_2O (60 mL) for 15 min after which $Cu(OAc)_2$ (1 g) was added and the flask sealed immediately. The mixture was stirred vigorously at room temperature for 15 min prior to the addition of $AgNO_3$ (1 g) which resulted in an exothermic reaction. The mixture was stirred for 30 min. The resultant activated Zn was then filtered and washed successively with H_2O, MeOH, acetone, and Et_2O. The moist activated Zn was then transferred to a flask to which 20 mL of H_2O and 20 mL of MeOH were added. Acetylene **8** (194 mg, 0.36 mmol) dissolved in 2 mL MeOH was then added to the activated Zn and the reaction mixture was stirred at room temperature in the dark for 24 h. The Zn dust was then filtered through Celite, washed with Et_2O and H_2O. The aqueous layer was extracted further with EtOAc and the combined organic layers were washed with brine and dried over Na_2SO_4. The solvent was removed and the residue was suspended in 50% EtOAc/hexanes and filtered through a silica plug to afford purified **4a** as an oil with an exclusive 11-*cis* geometry (194 mg, 99% yield). ^{1}H NMR (500 MHz, C_6D_6): δ=7.81 (m, 4H), 7.71 (m, 6H), 6.86 (d, 1H, J=12.0 Hz), 6.36 (t, 1H, J=11.8 Hz), 6.31 (d, 1H, J=16.1 Hz), 6.17 (d, 1H, J=16.1 Hz), 5.99 (t, 1H, J=6.2 Hz), 5.88 (d, 1H, J=11.6 Hz), 4.37 (d, 2H, J=6.3 Hz), 3.79 (m, 1H), 2.21 (dd, 1H, J=16.8, 5.3 Hz), 1.95 (dd, 1H, J=16.8, 9.4 Hz), 1.82 (s, 3H), 1.68 (s, 3H), ovrlp with 1.6–1.5 (ddd, 1H, J=12.1, 3.3, 2.1 Hz), 1.53 (s, 3H), 1.42 (t, 1H, J=12.0 Hz), 1.17 (s, 9H), 1.05 and 1.04 (2s, 2×3H). [$C_{36}H_{48}O_2Si+H^+$]: calcd 541.35; found 541.3 (APCI+).

4.2.5. 3-[Boc-Lys(biotinyl)-*O*]-11-*cis*-retin-*O*-*tert*-butyldiphenylsilyl (5a). To a 1 mL solution of retinoid **4a** (16 mg, 0.03 mmol) in CH_2Cl_2 was added a mixture of Boc-Lys(biotinyl)-OH (28 mg, 0.06 mmol) in 100 μL DMSO and EDC (18 mg, 0.094 mmol) in 1 mL of CH_2Cl_2. After 5 min of stirring, DMAP (1 mg, 0.008 mmol) was added and the reaction mixture was stirred for 20 h after which it was washed with $NaHCO_3$, NH_4Cl, extracted with CH_2Cl_2 and chromatographed with 5–12% MeOH/CH_2Cl_2 gradient affording **5a** as an oil (23 mg, 78% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.7 (m, 4H), 7.4 (m, 6H), 6.56 (d, 1H, J=12.0 Hz), 6.32 (t, 1H, 11.9 Hz), 6.09 ('s', 2H), 5.95 (br t, 1H), 5.87 (d, 1H, J=11.7 Hz), 5.7 (t, 1H) ovrlp with (br, 1H), 5.16 (d, 1H, J=8.4 Hz), 5.10 (m, 1H), 5.00 (br, 1H), 4.52 (dd, 1H, J=7.2, 5.3 Hz), 4.33 (d, 1H, J=6.6 Hz) ovrlp with (t, 2H), 4.23 (br t, 1H), 3.24 (dd, 2H, J=6, 5 Hz), 3.17 (dd, 1H, J=7.0, 4.6 Hz), 2.93 (dd, 1H, J=12.8, 4.9 Hz), 2.74 (d, 1H, J=12.8 Hz), 2.41 (dd, 1H, J=17.5, 5.3 Hz), 2.20 (m, 2H), 2.11 (dd, 1H, J=17.2, 9.6 Hz), 1.93 (s, 3H), 1.9–1.5 (m, 8H), 1.67 (s, 3H), 1.64 (s, 3H), 1.5–1.4 (m, 6H), 1.45 (s, 9H), 1.10 and 1.07 (2s, 2×3H), 1.05 (s, 9H). [$C_{57}H_{82}N_4O_7SSi+H^+$]: calcd 995.5752; found 995.5745 (HRMS).

4.2.6. 3-[Boc-Lys(biotinyl)-*O*]-11-*cis*-retinol (6a). To a 500 μL solution of **5a** (10 mg, 0.01 mmol) in THF (at 0°C) and two beads of activated molecular sieves 4 Å was added TBAF (18 μL of 1 M/THF, 0.018 mmol) and the reaction mixture was stirred to rt for 1 h. The mixture was then chromatographed and eluted with 5–12% MeOH/CH_2Cl_2 gradient affording **6a** as a film coating the vial (6 mg, 79% yield). Due to the extreme instability of this compound, TLC was the sole means of characterization and the product was used directly in following step.

4.2.7. 3-[Boc-Lys(biotinyl)-*O*]-11-*cis*-retinol bromoacetate (1). To a 1 mL solution of **6a** (6 mg, 0.008 mmol) in CH_2Cl_2 at −10°C, and two beads of activated molecular sieves 4 Å, was added EDC (13 mg, 0.068 mmol) and bromoacetic acid (4 mg, 0.029 mmol). The mixture was stirred for 2 min prior to the addition of DMAP (1 mg, 0.008 mmol) after which it was stirred for an additional 15 min. The reaction mixture was then washed with $NaHCO_3$ and NH_4Cl, dried over Na_2SO_4 and then chromatographed using 5–8% MeOH/CH_2Cl_2 affording **1** as a film coating the inside of a vial (6.9 mg, 99% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ=6.50 (d, 1H, J=12.0 Hz), 6.39

(t, 1H, *J*=11.9 Hz), 6.11 (s, 2H), 5.99 (br t, 1H), 5.89 (d, 1H, *J*=11.6 Hz), 5.68 (br, 1H), 5.64 (t, 1H, *J*=7.0 Hz), 5.19 (d, 1H, *J*=8.3 Hz), 5.10 (m, 1H), 5.02 (br, 1H), 4.81 (d, 2H, *J*=7.2 Hz), 4.52 (dd, 1H, *J*=7.5, 5.1 Hz), 4.33 (dd, 1H, *J*=7.7, 4.8 Hz), 4.24 (br t, 1H), 3.85 (s, 2H), 3.24 (dd, 2H, *J*=6, 5 Hz), 3.17 (dd, 1H, *J*=12.0, 7.3 Hz), 2.93 (dd, 1H, *J*=12.8, 4.9 Hz), 2.74 (d, 1H, *J*=12.8 Hz), 2.42 (dd, 1H, *J*=17.5, 5.4 Hz), 2.20 (dd, 2H, *J*=12.1, 7.0 Hz), 2.12 (dd, 1H, *J*=17.2, 9.6 Hz), 1.91 (2s-ovrlp, 2×3H), 1.8–1.5 (m, 8H), 1.71 (s, 3H), 1.5–1.1.4 (m, 6H), 1.45 (s, 9H), 1.11 and 1.08 (2s, 2×3H). [$C_{43}H_{65}BrN_4O_8S+H^+$]: calcd 877.3785; found 877.3791 and 879.2012 (HRMS) due to Br isotope effect.

4.3. Synthesis of biotinylated retinoid 2

4.3.1. 3-Triethylsiloxy-β-ionone (22). Triethylchlorosilane (1.5 mL, 9.0 mmol) was added to a solution of 3-hydroxy-β-ionone (±)-**9** (1.55 g, 7.4 mmol), Et_3N (1.2 mL, 16.2 mmol), and DMAP (1.8 g, 14.8 mmol) in 15 mL of CH_2Cl_2 at 0°C. The reaction mixture was stirred for 1 h at room temperature. The mixture was then quenched with cold H_2O and extracted with CH_2Cl_2, washed with cold 1% HCl, brine, and then dried over Na_2SO_4. The product was purified by column chromatography using 15–20% EtOAc in hexanes as an eluent to afford **22** as an oil (2.33 g, 97% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.23 (d, 1H, *J*=16.4 Hz), 6.13 (d, 1H, *J*=16.4 Hz), 3.97 (m, 1H), 2.33 (m, 1H) ovrlp with 2.32 (s, 3H), 2.17 (dd, 1H, *J*=16.8, 9.4 Hz), 1.79 (s, 3H), 1.71 (ddd, 1H, *J*=12.1, 3.3, 2.1 Hz), 1.53 (t, 1H, *J*=11.9 Hz), 1.13 and 1.11 (2s, 2×3H), 1.00 (t, 9H, *J*=8.0 Hz), 0.64 (q, 6H, *J*=8.0 Hz). [$C_{19}H_{34}O_2Si+H^+$]: calcd 323.24; found 323.3 (ESI+).

4.3.2. 5-(4-Hydroxy-2,6,6-trimethyl-cyclohex-1-enyl)-3-methyl-penta-2,4-dienenitrile (29). In a 25 mL rb flask, NaH (124 mg, 60% dispersion in mineral oil, 3.1 mmol) was placed and washed with hexanes prior to suspension in dry THF (5 mL) at 0°C. Then phosphonate **14** (636 mg, 3.1 mmol) was added neat to the NaH and the reaction allowed to stir for 30 min at room temperature. The reaction mixture was cooled again to 0°C prior to the addition of ketone **22** (500 mg, 1.55 mmol) as a 1 mL solution in THF. The reaction mixture was stirred for 2 h at room temperature, then quenched with cold NH_4Cl, and extracted with Et_2O. The combined organic extracts were washed with brine and dried over Na_2SO_4 and product was purified by column chromatography using 20% Et_2O in hexanes affording **29** as an oil (447 mg, 84% yield, 3.5:1 9*E*/9*Z*). This compound was characterized after the DIBAH reduction described in Section 4.3.3.

4.3.3. 5-(4-Hydroxy-2,6,6-trimethyl-cyclohex-1-enyl)-3-methyl-penta-2,4-dienal (30). To a solution of **29** (447 mg, 1.3 mmol) in CH_2Cl_2 (5 mL) at −60°C was added DIBAH (2 mL of 1 M, 2.0 mmol) and the reaction stirred for 1 h prior to quenching with cold wet SiO_2/H_2O. The residue was then suspended in 10% Et_2O/hexanes and loaded on a column for purification to afford **30** as an oil (263 mg of 9*E*, total mass of products: 335 mg, 74% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ=10.13 (d, 1H, *J*=8.2 Hz), 6.68 (d, 1H, *J*=16.2 Hz), 6.21 (d, 1H, *J*=16.2 Hz), 5.94 (d, 1H, *J*=8.2 Hz), 3.94 (m, 1H), 2.29 (dd, 1H, *J*=16.8, 5.4 Hz) ovrlp with 2.31 (s, 3H), 2.11 (dd, 1H, *J*=16.8, 8.9 Hz), 1.71 (m, 1H) ovrlp with 1.73 (s, 3H), 1.52 (t, 1H, *J*=12.1 Hz), 1.10 (2s, 2×3H), 0.98 (t, 9H, *J*=8.0 Hz), 0.62 (q, 6H, *J*=8.0 Hz). [$C_{21}H_{36}O_2Si+H^+$]: calcd 349.26; found 349.3 (APCI+).

4.3.4. 3-Triethylsiloxy-*all trans*-retinoic acid, ethyl ester (13). Phosphonate **15** (459 mg, 422 μL, 1.74 mmol) was dissolved in 5 mL of THF and mixture stirred at 0°C for 10 min prior to the dropwise addition of *n*-BuLi (1 mL of 1 M in THF, 1.5 mmol). The reaction was stirred for 30 min and then aldehyde **30** (263 mg, 0.754 mmol) in 5 mL THF was added to the reaction mixture at 0°C (under dim light conditions). The mixture was stirred at room temperature for 2 h and then quenched with NH_4Cl and extracted with Et_2O, washed with brine, and dried over Na_2SO_4. The product was purified by column chromatography using 2% Et_2O in hexanes affording a 4:1 mixture of 13*E*/13*Z* of **13** (230 mg of 13*E*, total mass of products: 305 mg, 88% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ=6.98 (dd, 1H, *J*=15.0, 11.5 Hz), 6.29 (d, 1H, *J*=15.1 Hz), 6.22 (d, 1H, *J*=16.2 Hz). 6.1 (m, 2H), 5.78 (s, 1H), 4.17 (q, 2H, *J*=7.1 Hz), 3.95 (m, 1H), 2.48 (s, 3H), 2.26 (dd, 1H, *J*=16.8, 5.4 Hz), 2.10 (dd, 1H, *J*=16.8, 9.4 Hz), 1.99 (s, 3H), 1.71 (s, 3H), 1.67 (ddd, 1H, *J*=12.1, 3.3, 2.1 Hz), 1.51 (t, 1H, *J*=11.9 Hz), 1.28 (t, 1H, *J*=7.1 Hz), 1.08 and 1.07 (2s, 2×3H), 0.98 (t, 9H, *J*=8.0 Hz), 0.62 (q, 6H, *J*=8.0 Hz). [$C_{28}H_{46}O_3Si+H^+$]: calcd 459.33; found 459.3 (APCI+).

4.3.5. 3-Triethylsiloxy-*all trans*-retinol (4b). To a 5 mL suspension of $LiAlH_4$ (~10 mg, ~0.3 mmol) in Et_2O at 0°C a solution of **13** (74 mg, 0.16 mmol) in 2 mL of Et_2O was added. The reaction mixture was stirred for 10 min and then quenched with NH_4Cl, extracted with EtOAc, and then dried over Na_2SO_4. The resultant crude oil **4b** (70 mg) was not further purified and was directly used in the following step. ^{1}H NMR (500 MHz, C_6D_6): δ=6.65 (dd, 1H, *J*=15.0, 11.2 Hz), 6.30 (2d, 2×1H, *J*=15.1, 5.9 Hz), 6.21 (2d, 2×1H), 5.59 (t, 1H, *J*=6.6 Hz), 4.13 (m, 1H), 4.00 (t, 2H, *J*=5.6 Hz), 2.40 (dd, 1H, *J*=17.2, 5.3 Hz), 2.31 (dd, 1H, 17.2, 9.6 Hz), 1.9–1.7 (2dd, 2×1H), 1.88 (s, 3H), 1.73 (s, 3H), 1.62 (s, 3H), 1.17 and 1.14 (2s, 2×3H), 1.06 (t, 9H, *J*=7.9 Hz), 0.68 (q, 6H, *J*=7.9 Hz), 0.55 (t, 1H, *J*=5.6 Hz). [$C_{26}H_{44}O_2Si+H^+$]: calcd 417.3189; found 417.3193 (HRMS).

4.3.6. 3-Triethylsiloxy-*all trans*-retinol chloroacetate (5b). To a 2 mL solution of the crude **4b** (70 mg, ~0.16 mmol) in CH_2Cl_2 at −10°C, and two beads of activated molecular sieves 4 Å, was added EDC (86 mg, 0.45 mmol) and chloroacetic acid (18 mg, 0.19 mmol). The mixture was stirred for 5 min prior to the addition of DMAP (7.8 mg, 0.07 mmol) after which it was stirred for an additional 5 min. The reaction mixture was immediately washed with $NaHCO_3$ and NH_4Cl, dried over Na_2SO_4 and then chromatographed rapidly using 10% EtOAc/hexanes affording **5b** as an oil. The material was not weighed and used directly in the following step. ^{1}H NMR (500 MHz, C_6D_6): δ=6.65 (dd, 1H, *J*=15.1, 11.3 Hz), 6.3–6.1 (m, 4H), 5.49 (t, 1H, *J*=7.3 Hz), 4.55 (d, 2H, 7.3 Hz), 4.12 (m, 1H), 3.40 (s, 2H), 2.39 (dd, 1H, *J*=16.8, 5.6 Hz), 2.30 (dd, 1H, *J*=16.8, 9.4 Hz), 1.87 (ddd, 1H, *J*=13.5, 1.8, 1.7 Hz), ovrlp with 1.84 (s, 3H), 1.76 (s, 3H), ovrlp with 1.74 (t, 1H,

J=11.9 Hz), 1.62 (s, 3H), 1.16 and 1.14 (2s, 2×3H), 1.08 (t, 9H, J=7.9 Hz), 0.68 (q, 6H, J=7.9 Hz).

4.3.7. 3-Hydroxy-*all trans*-retinol chloroacetate (6b). To a 2 mL solution of **5b** (the entire sample from the above procedure) in THF at 0°C, and two beads of activated molecular sieves 4 Å, was added 100 μL of HF/pyridine. The mixture was stirred for 5 min prior to concentration and direct loading on a rapid pre-washed column eluted with 20–35% EtOAc/hexanes affording **6b** as a coating on the inside of a flask (16 mg, 27% yield over last three steps). ^{1}H NMR (500 MHz, C_6D_6): δ=6.64 (dd, 1H, J=15.1, 11.3 Hz), 6.3–6.1 (m, 4H), 5.49 (t, 1H, 7.3 Hz), 4.55 (d, 2H, 7.3 Hz), 3.82 (m, 1H), 3.41 (s, 2H), 2.23 (dd, 1H, J=16.8, 5.5 Hz), 1.99 (dd, 1H, J=16.8, 9.4 Hz), 1.82 (s, 3H), 1.71 (s, 3H), 1.64 (ddd, 1H, J=13.5, 1.8, 1.7 Hz), ovrlp with 1.62 (s, 3H), 1.44 (t, 1H, J=11.9 Hz), 1.07 and 1.06 (2s, 2×3H). [$C_{22}H_{31}ClO_3$+H^+]: calcd 379.2040; found 379.2038 (HRMS).

4.3.8. 3-[Boc-Lys(biotinyl)-*O*]-*all trans*-retinol chloroacetate (2). To a 2 mL solution of **6b** (8 mg, 0.02 mmol) in CH_2Cl_2 at −10°C, and five beads of activated molecular sieves 4 Å, was added a mixture of Boc-Lys(biotinyl)-OH (20 mg, 0.04 mmol) in 200 μL DMSO and EDC (12 mg, 0.063 mmol) in 1 mL of CH_2Cl_2. After 5 min of stirring, DMAP (1 mg, 0.008 mmol) was added and the reaction mixture was stirred for 4 h which was sufficient for a quantitative amount of product by TLC. The reaction mixture was washed with $NaHCO_3$, NH_4Cl, extracted with CH_2Cl_2, dried over Na_2SO_4 and chromatographed rapidly with 5% MeOH/CH_2Cl_2 affording **2** as a film coating the inside of a vial (6.8 mg, 40% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ=6.66 (dd, 1H, J=15.1, 11.3 Hz), 6.23 (d, 1H, J=15.1 Hz), 6.11 (d, 1H, J=11.3 Hz), ovrlp with 6.10 (s, 2H), 6.03 (br t, 1H), 5.89 (br, 1H), 5.62 (t, 1H, J=7.3 Hz), 5.17 (br, 2H), 5.09 (m, 1H), 4.85 (d, 2H, J=7.3 Hz), 4.53 (dd, 1H, J=7.2, 4.9 Hz), 4.34 (dd, 1H, J=7.7, 4.8 Hz), 4.23 (br, 1H), 4.07 (s, 2H), 3.24 (br, 2H), 3.17 (dd, 1H, J=11.9, 7.3 Hz), 2.93 (dd, 1H, J=12.8, 4.9 Hz), 2.75 (d, 1H, J=12.8 Hz), 2.43 (dt, 1H, J=16.4, 5 Hz), 2.20 (br t, 2H), 2.12 (dd, 1H, J=17.2, 9.6 Hz), 1.96 (s, 3H), 1.91 (s, 3H), 1.9–1.7 (m, 8H), ovrlp with 1.71 (s, 3H), 1.7–1.2 (m, 6H), ovrlap with 1.45 (s, 9H), 1.10 and 1.07 (2s, 2×3H). [$C_{43}H_{65}ClN_4O_8S$+H^+]: calcd 833.4290; found 833.4281 and 835.3402 (HRMS) due to Cl isotope effect.

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