An Affinity Probe for Isolation of Abscisic Acid-Binding Proteins

James M. Nyangulu , Marek M. Galka, Ashok Jadhav , Yuanzhu Gai,
Cindy M. Graham, Ken M. Nelson, Adrian J. Cutler, David C. Taylor,
Gary M. Banowetz, Suzanne R. Abrams*

Plant Biotechnology Institute, National Research Council of Canada,
110 Gymnasium Place, Saskatoon, Saskatchewan S7N 0W9, Canada

Supporting Information

Materials and Methods

General. Melting points are uncorrected. FTIR spectra were recorded using KBr cells on a Perking Elmer Paragon 1000. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 500 MHz Spectrometer in chloroform-d unless otherwise noted. Chemical shifts (δ) and coupling constants (J) are reported as if they are first order. High-resolution mass spectra (HRMS) were recorded in either the electron impact (EI) mode, the chemical ionization (CI) mode or in negative ion electrospray mode using capillary voltage of 2.75 KV, counter electrode 35 V, collision energy (ELAB) of 14 V and cell pressure of 1.0×10^{-3} mBar with argon. Mass spectra data are reported in mass to charge units (m/z). Optical rotations were obtained from a Perkin-Elmer 141 Polarimeter and were carried out in chloroform. Flash chromatography was performed using Merck Silica gel 60 (230-400 mesh). Merck Silica gel 60 F_{254} plates (0.2 mm) with aluminum sheet backing were used in analytical TLC. The spots were detected using UV light (254 nm), by staining with iodine, or by immersing in a developing solution and charring on a hot plate. (The developing solution was prepared by dissolving concentrated sulfuric acid (50 g), cerium (IV) sulfate (10 g) and phosphomolybdic acid hydrate (40 g) in water (1 L)). Melting points were measured on an Electrothermal 9300 melting point apparatus and are not

corrected. The solvent tetrahydrofuran (THF) was dried by distillation from sodium and benzophenone.

Gel electrophoresis (SDS-PAGE). Protein acrylamide gel electrophoresis was run at 110V using 4% and 10% stacking and running gels correspondingly. Protein samples were prepared in Laemmli buffer (BioRad) with 5% 2-mercaptoethanol. Gels were stained and developed using Silverstain Plus kit from BioRad.

Gene expression. *Study of 3-ketoacyl-CoA gene induction by biotinylated bicyclic ABA analog **17.** Embryo treatments and northern analysis. Brassica napus* cultivar Hero plants were grown in controlled environment growth chambers and microspores were isolated and cultured according to the methods described previously for cv Reston by Qi *et al*.[1] The microspore-derived embryos were enriched in the torpedo stage. Embryos in liquid culture were supplemented with 10 μM (+)-ABA or 10 μM analog (+)-**17**. An initial T_0 control was untreated. Embryos were maintained in the dark at 25^{o}C on a rotary shaker at 50 rpm. After 72 h of hormone treatment, individual plates of embryos for each treatment were harvested, rinsed with sterile distilled water and the medium was stored at –20 °C. Total RNA was isolated from harvested embryos of each treatment and fractionated on 1.2% formaldehyde arose gels.

The RNA was subsequently transferred to Hybond N+ membrane and hybridized at high stringency (65 °C) overnight with a ^{32}P-labeled *Arabidopsis thaliana* 3-ketoacyl-CoA synthase1 (*FAE1,* fatty acid elongase1) DNA probe, prepared using the Random Primers DNA labeling kit as described previously.[2]

Binding of anti-ABA monoclonal antibodies to affinity column modified with 17.

5 mL of commercially available affinity matrix (HiTrap) purchased from Amersham Biosciences was packed in to the affinity column.

The column was pre-conditioned by washing with 40 column volumes of a binding buffer (20 mM sodium phosphate, 015 M NaCl, pH 7.5). Then 10 mL of 1mM solution of (+)-**17** in binding buffer was run three times through the column at the flow rate of 0.4 mL/min. The column was stored overnight at 4 °C in order to allow complete saturation

of streptavidin sites with biotinylated probe. Then the column was washed with 20 volumes of binding buffer (1 mL/min) followed by running a 10 mL solution of anti-ABA monoclonal antibodies at 0.4 mL/min flow rate repeated three times. The column was incubated with antibodies for 2 hours and then washed three times with 20 volumes of binding buffer. The last wash fraction was collected for further analysis. Lastly the column was washed with 20 mL of 5 mM (±) ABA (in binding buffer pH 7.6) as an eluting agent and the eluent was collected for analysis. The eluted buffer wash fractions as well as ABA eluted ones were concentrated down to 100µL using Amicon Ultra-4 centrifugal Filter Devices purchased form Millipore. The concentrated fractions were then checked for the presence of antibodies using SDS-PAGE (Fig 6). A second column not modified with (+)-**17** was used as a control to check whether the antibodies remain on the column only due to the presence of the probe.

Cloning of Arabidopsis CYP707A1. The open reading frame of AtCYP707A1 was PCR amplified using Pfu polymerase (Invitrogen) from pUNI-vector clone, U10730. The gene-specific primers were designed to allow for directional cloning into pBluescript sk- as follows: CYP707A1-1F: 5' ATACGTTAGGTACC*ATG*GATATCTCCGCCTTGT 3' (*Kpn*I site is underlined, ATG is in italics), CYP707A1-1R: 5'TATGCAATGAATTCATATAGAAAAG*CTA*AAGGCAA 3' (*Eco*RI site is underlined, stop is in italics). Constructs were sequenced in both directions using gene specific primers to ensure the fidelity of the clones.

Heterologous expression of CYP707A1 in yeast. The full-length cDNA of CYP707A1 in pBluescript sk- was excised with and KpnI/SstI and isolated using Qiagen's gel extraction kit. The yeast expression vector, YeDP60, was generously provided by Denis Pompon (CNRS-Centre de Génétique Moléculaire). Upon sequencing, the multiple cloning site (MCS) of YeDP60 was found to contain a start codon and 2 *Cla*I sites after the *Bam*HI site at the 5' end. The entire MCS was removed and replaced with the MCS from pUC19. The open reading frame of CYP707A1 from the pBluescript construct was directionally cloned into the modified YeDP60 vector using the enzyme pairs indicated above. The resulting constructs were used to transform the WAT11 strain of

Saccharomyces cerevisiae (Pompon D, Louerat B, Bronine A, Urban P., Meth. Enz. **1996** 272: 51-64). Transformants were grown overnight in selection media (SC-URA with 2% glucose, 60 mg/L adenine and 40mg/L tryptophan), collected by centrifugation and resuspended in YPG media (1% yeast extract, 1% bactopeptone, 2% glucose) and grown for a further 24 hours at 28 °C. The yeast was pelleted by centifugation, washed three times with inducing media (YPL - 1% yeast extract, 1% bactopeptone, 2% galactose) and used to inoculate YPL media to a final optical density of 0.5. The yeast were grown in inducing media for a further 16 hours before microsomal extraction.

Yeast microsome preparation. Yeast microsomes were prepared following the methods outlined by Urban et al. (Urban P, Werck-Reichhart D, Teutsch HG, Durst F, Regnier S, Kazmaier M, Pompon D., Eur. J. Biochem. 1994, 222(3): 843-850) with the following modifications: Yeast cells (with YeDP60 and CYP707A1) were harvested by centrifugation at 10,000 g for 10 minutes and washed in ice-cold, filter-sterilized extraction buffer (0.1% BSA, 0.33 M Sucrose, 40 mM ascorbate, 20 mM EDTA, 200 mM potassium phosphate buffer, pH 7.6). The pellet was resuspended (0.3 g/mL) in extraction buffer and PMSF was added to a final concentration of 0.5 mM. Cell walls were disrupted using an ice cooled bead beater with 0.45 - 0.5 mm glass beads. Cells were pulverized three times at one minute each allowing extract to cool between treatments. Cell extract was collected and centrifuged at 10,000 g for ten min. The supernatant was collected and microsomes precipitated on ice for 30 min after the addition of PEG3500 (10 g/mL) and NaCl (0.15 M). Extracts were centrifuged at 13,000 rpm in a Sorval SS-34 rotor for 20 min at 4 °C. Microsomal pellet was resuspended in cold 100 mM potassium phosphate buffer and 40 mM ascorbate and protein content determined using Biorad protein determination kit.
Shortly before applying the extract on the affinity column 1 mL of 1 % DHPC detergent from Avanti Polar Lipids was added in order to completely dissolve proteins.

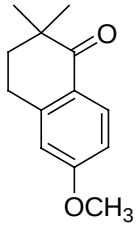
Isolation of 8'ABA-hydroxylase from yeast microsomes using affinity probe 17. Microsomes isolated from yeast suspension cultures over expressing both ABA-8'hydroxylase gene (CYP707A1) and one not over expressing this protein (YeDP60) as

control were re-dissolved in 1% CHAPS detergent in affinity binding buffer (see above). Each solution was run through a separate probe-modified affinity column (5 mL volume, pre-conditioned as described above) three times at 0.4 mL/min. Then the columns were washed three times with 20 mL of binding buffer followed by washing with 10 mM (±) ABA. The three buffer wash fractions as well as ABA wash fractions were collected and concentrated down to 100 µL using Amicon Ultra-4 centrifugal Filter Devices purchased form Millipore. The concentrated fractions were then checked for the presence of proteins by SDS-PAGE (Figure 4).

Protein identification by Mass Spectrometry. The protein bands from SDS-PAGE gels were extracted using Mass Prep Station (Robotic Protein Handling System) from The Proteome Works System™ and proteins digested by trypsin. Resulted mixture of peptides was analyzed using Micromass Q-TOF Global™ system with CAP-LC (Waters Symmetry 300 C-18 trapping column with 5µm particle diameter media and 0.35 mm x 5mm column dimensions). LC Packings™ analytical column containing PepMap™ C-18, 5 µm particle diameter media and column dimensions: 75 µm x 15 cm was used for HPLC. Peptide matching was performed by MASCOT MS/MS (matrixscience.com) ion search.

Synthesis of biotinylated probe 17.

*2,2-Dimethyl-6-methoxy-3,4-dihydro-2H-naphthalen-1-one (**4**).*[2]



4

To a suspension of NaH (13.6 g, 568 mmol) in dry THF (300 mL) in a one liter round bottomed flask was added 6-methoxy-1-tetralone **3** (20.0 g, 114 mmol) dissolved in a small amount of dry THF (40 mL). After stirring the mixture for 10 minutes at rt, methyl iodide (18.4 mL, 295 mmol) was added *via* a syringe. The mixture was then heated on an

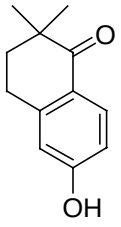
oil bath to 40 °C for 30 min, and stirring continued at rt until the starting material had disappeared. The reaction mixture was monitored by TLC with ethyl acetate: hexane (1:6) solvent mixture. The reaction was quenched by the addition of water (slowly and dropwise) to destroy excess sodium hydride. The reaction mixture was then extracted with ethyl acetate (3 x 200 mL), washed with water (2 x 200 mL) and dried over anhydrous Na_2SO_4. Evaporation of solvent *in vacuo* yielded the crude dimethyl tetralone, as a brown oil (28.7 g). The crude was carried through to the next stage without any further purification. A small sample was purified by flash chromatography (14% EtOAc in hexane) to provide the pure 6-methoxy-2, 2-dimethyl-1-tetralone **4**.

IR (ν_{max}): 2956, 2922, 1684, 1599 cm^{-1}.

^{1}H NMR δ: 1.18 (s, 3H, CH_3), 1.94 (t, 2H, *J* = 6.6 Hz, CH_2), 2.92 (t, 2H, *J* = 6.4 Hz, CH_2), 3.83 (s, 3H, CH_3), 6.65 (d, 1H, J=1.6Hz, Ar-H), 6.81 (dd, 1H, J = 8.8, 2.5 Hz, Ar-H) , 8.00 (d, 1H, *J* = 8.8 Hz, Ar-H).

HRMS: Calculated for $C_{13}H_{16}O_2$, 204.1150. Found: 204.1168.

2,2-Dimethyl-6-*hydroxy-3,4-dihydro-2H-naphthalen-1-one (**5**).*[3]



5

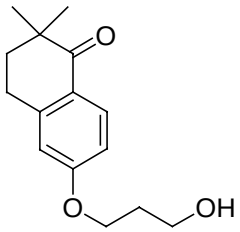
To the dimethyl tetralone **4** (28.7 g, 140.5 mmol) was added an excess amount of 48% hydrobromic acid (500 mL) and the mixture refluxed at 100 °C overnight. The reaction was quenched by pouring the reaction mixture to a large beaker (1 L) of ice/water with stirring. After stirring for 15 min, the mixture was extracted with ethyl acetate (3 x 250 mL). The organic phase was then re-extracted with 1N NaOH and allowed to stand for an hour. The aqueous phase was then acidified with 1.0 N HCl and the precipitate filtered and dried, to afford product **5** (22.7 g, 85%), as a brown solid.

Mp: 138 – 140 °C (EtOAc); lit. 141 °C.[3]

IR (ν_{max}): 3376, 2967, 2922, 1685, 1602 cm^{-1}.

^{1}H NMR δ: 1.09 (s, 3H, CH_3), 1.82 (t, 2H, *J* = 6.3 Hz, CH_2), 2.80 (t, 2H, *J* = 6.4 Hz, CH_2), 6.56 (d, 1H, *J* = 2.4 Hz, Ar-H) 6.67 (dd, 1H, *J* = 8.7, 2.4 Hz, Ar-H), 7.80 (d, 1H, *J* = 8.7 Hz, Ar-H).

*2,2-Dimethyl-6-(3'-hydroxypropoxy)-3,4-dihydro-2H-naphthalen-1-one (**6**).*



6

To a suspension of 6-hydroxy-2,2-dimethyl-1-tetralone (11.7 g, 61.6 mmol) and K_2CO_3 (25.5 g, 185.0 mmol) in anhydrous DMF (200 mL), was added 3-bromo-1-propanol (11.1 mL, 123.0 mmol). The reaction mixture was heated to 95 °C and stirred for 16 h. The reaction mixture was then poured into a 1 L beaker containing an ice/water mixture. The mixture was stirred for 30 min and then extracted with ethyl acetate (3 x 200 mL), washed with water (2 x 200 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*, leaving a brown oil. Column chromatography of the oil using silica gel with 33% EtOAc in hexane afforded the product **6** (12.8 g, 84%).

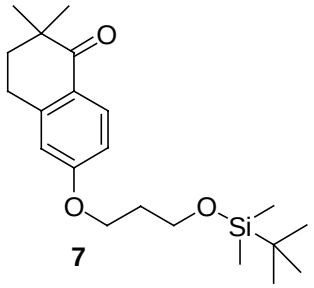
IR (ν_{max}): 3383, 3003, 2941, 1689, 1603 cm^{-1}.

^{1}H NMR δ: 1.16 (s, 3H, CH_3), 1.91 (t, 2H, *J* = 6.1 Hz, CH_2), 2.02 (t, 2H, *J* = 6.0 Hz, CH_2), 2.90 (q, 2H, *J* = 6.0 Hz, CH_2), 3.82 (t, 2H, *J* = 6.0 Hz, CH_2), 4.13 (t, 2H, *J* = 6.0 Hz, CH_2), 6.64 (d, 1H, *J* = 1.6 Hz, Ar-H), 6.78 (dd, 1H, *J* = 8.7, 2.2 Hz, Ar-H), 7.94 (d, 1H, *J* = 8.8 Hz, Ar-H).

^{13}C NMR δ: 202.0, 162.6, 145.8, 130.3, 124.9, 113.5, 112.8, 65.3, 59.7, 41.2, 36.6, 31.8, 29.6, 26.0, 24.4.

HRMS: Calculated for $C_{15}H_{20}O_3$, 248.1412. Found: 248.1487.

*6-(3'-tert-Butyldimethylsilanyloxypropoxy)-2,2-dimethyl-3,4-dihydro-2H-naphthalen-1-one (**7**).*



7

The tethered tetralone **6** (9.82 g, 40.0 mmol) was dissolved in dry dichloromethane (300 mL) and cooled to 0 °C. Imidazole (3.50 g, 52.0 mmol) was then added slowly, followed by *tert*-butyldimethylsilyl chloride (7.18g, 48.0 mmol). The reaction mixture was then allowed to stir at 0 °C for 1 h and then at rt for a further 2 h. The reaction was quenched by the addition of water (100 ml). The organic phase was then washed with water (2 x 150 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*, leaving a brown oil. Column chromatography of the oil using silica gel with 20% EtOAc in hexane yielded the desired product **7** (13.2 g, 92%).

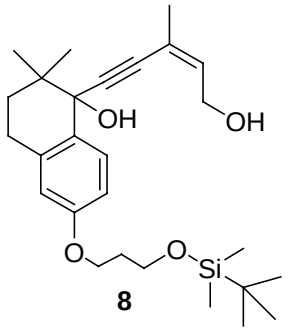
IR (ν_{max}): 2987, 2920, 1687, 1599 cm^{-1}.

^{1}H NMR δ: 0.02 (s, 6H, 2 X CH_3), 0.86 (s, 9H, 3 X CH_3), 1.17 (s, 3H, CH_3), 1.92 (m, 4H, 2 X CH_2), 2.90 (t, 2H, *J* = 6.0 Hz, CH_2), 3.76 (t, 2H, *J* = 6.1 Hz, CH_2), 4.08 (t, 2H, *J* = 6.0 Hz, CH_2), 6.63 (d, 1H, *J* = 1.6 Hz, Ar-H), 6.78 (dd, 1H, *J* = 8.8, 2.3 Hz, Ar-H), 7.96 (d, 1H, *J* = 8.7 Hz, Ar-H).

^{13}C NMR δ: 201.8, 162.8, 145.8, 130.3, 124.9, 113.6, 112.8, 64.6, 59.3, 41.3, 36.8, 32.2, 26.1, 25.9, 24.5, 18.2, -5.4.

HRMS: Calculated for $C_{21}H_{35}O_3Si$, 363.2360 (M+1). Found: 363.2375.

*6-(3'-tert-Butyldimethylsilanyloxypropoxy)-2,2-dimethyl-1-[(Z)-5''-hydroxy-3''-methylpent-3''-en-1''-ynyl]- 1,2,3,4-tetrahydronaphthalen-1-ol (**8**).*



8

(*Z*)-3-Methylpent-2-en-4-yn-1-ol (side chain) (4.14 g, 43.1 mmol) in dry THF (350 mL) was cooled to –78 °C under an atmosphere of argon. *n*-Butyl lithium (37.3 mL, 2.5 M) was then added slowly, *via* syringe. The reaction mixture was allowed to stir at –78^{o}C for 45 minutes, after which, the silyl protected tethered tetralone (13.0 g, 35.9 mmol), dissolved in dry THF (75 mL) was added. The reaction mixture was stirred for a further 15 min at –78 °C and then the ice bath was removed. The reaction mixture was stirred at rt for a further 3 h, at which point, all the starting material had disappeared. The reaction was quenched by the addition of a saturated solution of NH_4Cl (150 mL). The reaction mixture was stirred for 10 min and extracted with ethyl acetate (3 x 150 mL), washed with water (2 x 150 mL) and dried over anhydrous Na_2SO_4. Evaporation of solvent yielded the desired alcohol as a brown oil. Column chromatography of the brown oil using silica gel with ethyl acetate: hexane (1:2) gave the desired clean allylic alcohol **9** (12.5 g, 76%).

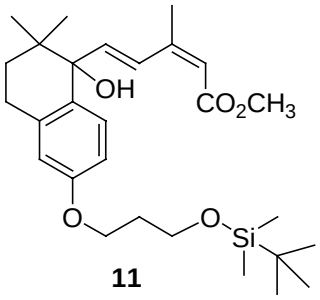
IR (ν_{max}): 3382, 3054, 2940, 2278, 1601 cm^{-1}.

^{1}H NMR (CD_3CN) δ: 0.03 (s, 6H, 2 x CH_3), 0.88 (s, 9H, 3 x CH_3), 1.03 (s, 3H, CH_3), 1.07 (s, 3H, CH_3), 1.62 (m, 1H, CH), 1.85 (s, 3H, CH_3), 1.91 (m, 3H, CH and CH_2), 2.76 (m, 2H, CH_2), 3.77 (t, 2H, *J* = 6.3 Hz, CH_2), 4.03 (t, 2H, *J*=6.3 Hz, CH_2), 4.18 (d, 2H, J=5.5 Hz, CH_2), 5.80 (t, 1H, J=5.2 Hz, CH), 6.63 (d, 1H, J = 2.6 Hz, Ar-H), 6.75 (dd, 1H, *J* = 8.7, 2.6 Hz, Ar-H) , 7.61 (d, 1H, *J* = 8.7 Hz, Ar-H).

^{13}C NMR δ: 159.2, 137.5, 137.4, 132.8, 130.6, 119.8, 114.4, 113.6, 97.9, 84.7, 74.8, 65.1, 61.3, 60.1, 38.2, 33.1, 32.0, 26.6, 26.2, 24.3, 23.8, 23.3, 18.2, -5.2.

HRMS: compound not stable.

*Methyl (2Z,4E)-5-[6'-(3''-tert-butyldimethylsilanyloxypropoxy)-2',2'-dimethyl-1'-hydroxy-1',2',3',4'- tetrahydronaphthalen-1'-yl]-3-methylpenta-2,4-dienoate (**11**).*



11

Allylic alcohol **8** (10.0 g, 21.9 mmol) in dry THF (200 mL) was cooled to –78 °C and RedAl® (13.6 mL, 43.7 mmol) added dropwise *via* syringe. The reaction mixture was stirred at –78 °C for 1 h and then allowed to warm up to 0 °C and stirred for a further 2 h. The reaction was quenched by slow addition of water (100 mL) and extracted with diethyl ether (3 x 100 mL). The organic phase was washed with water (200 mL) and dried over anhydrous Na_2SO_4. Evaporation of solvent *in vacuo*, left a crude brown oil of the allylic alcohol **9** (10.9 g), which was carried through to the next stage without any further purification. The crude allylic alcohol was dissolved in acetone (300 mL) and stirred with MnO_2 (37.8 g, 435.0 mmol) at rt, while monitoring the reaction progress by TLC (25% ethyl acetate in hexane). After stirring at rt for 3 h, all the starting material had disappeared. The reaction mixture was then filtered through a pad of Celite® and concentrated in *vacuo*. ^{1}H NMR of the crude product showed the presence of the aldehyde proton. The crude material (9.35 g) was carried through to the next stage without further purification. The crude tetralone aldehyde (9.0 g, 19.7 mmol) was dissolved in methanol (150 mL) and stirred with MnO_2 (34.2 g, 393.0 mmol), NaCN (3.38 g, 69.0 mmol) and AcOH (1.30 g, 21.7 mmol) at room temperature. After 3 h, the mixture was filtered through a pad of Celite® and concentrated *in vacuo*. The residue was brought up in distilled H_2O and extracted with ethyl acetate (3 x 150 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Column chromatography (25% EtOAc in hexane) gave the methyl ester **11** (5.80 g, 54%) over the three steps.

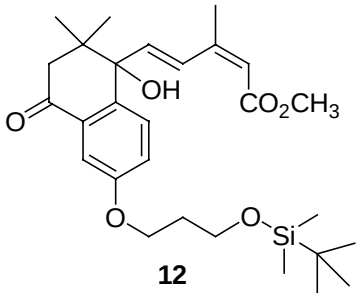
IR (ν_{max}): 3452, 3042, 2978, 1669, 1596 cm^{-1}.

^{1}H NMR δ: 0.02 (s, 6H, 2 x CH_3), 0.86 (s, 9H, 3 x CH_3), 0.94 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 1.64 (m, 1H, CH), 1.90 (m, 1H, CH), 1.92 (t, 2H, CH_2), 1.94 (s, 3H, CH_3), 2.81 (t, 2H, CH_2), 3.67 (s, 3H, CH_3), 3.76 (t, 2H, CH_2), 4.01 (t, 2H, CH_2), 5.67 (s, 1H, CH), 6.31 (d, 1H, CH), 6.61 (d, 1H, Ar-H), 6.70 (dd, 1H, CH), 7.25 (d, 1H, Ar-H), 7.80 (d, 1H, CH).

^{13}C NMR δ: 167.2, 158.9, 152.0, 143.8, 138.2, 133.8, 130.6, 126.7, 117.1, 114.4, 113.7, 78.4, 65.1, 60.2, 51.4, 37.9, 33.6, 33.2, 26.8, 26.3, 24.4, 23.5, 21.4, 17.0, -5.2.

HRMS: Calculated for $C_{28}H_{44}O_5Si$, 488.2958. Found: 488.2963.

*Methyl (2Z,4E)-5-[6'-(3''-tert-butyldimethylsilanyloxypropoxy)-2',2'-dimethyl-1'-hydroxy-4-oxo-1',2',3',4'- tetrahydronaphthalen-1'-yl]-3-methylpenta-2,4-dienoate (**12**)*



12

The methyl ester **11** (0.566 g, 1.57 mmol) in dry benzene (25 mL) was cooled to 10 °C in an ice bath. Pyridinium dichromate (2.37 g, 6.29 mmol) was then added with stirring, followed by 5.0 M – 6.0 M *tert*-butyl hydroperoxide (1.26 mL, 6.29 mmol) *via* syringe. The reaction mixture was stirred at 10 °C for 15 min and the ice bath removed. The reaction mixture was then stirred at rt for a further 2 h. The reaction mixture was diluted with diethyl ether (25 mL) and filtered over a bed of Celite®. Evaporation of solvent *in vacuo* and column chromatography of the resulting brown oil using silica gel with 20% ethyl acetate in hexane yielded the desired product **12** (0.285 g, 49%) and recovered starting material **11** (0.202 g, 36%).

IR (ν_{max}): 3458, 3068, 2962, 1684, 1648, 1598 cm^{-1}.

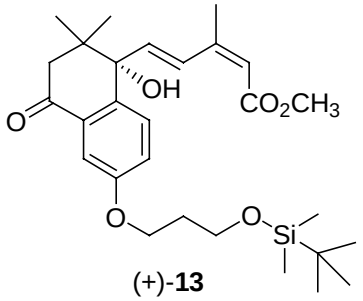
^{1}H NMR δ: 0.02 (s, 6H, 2 x CH_3), 0.86 (s, 9H, 3 x CH_3), 1.04 (s, 3H, CH_3), 1.05 (s, 3H, CH_3), 1.96 (q, 2H, J = 6.0 Hz, CH_2), 1.99 (s, 3H, CH_3), 2.53 (d, 1H, J = 17.0 Hz, ABX), 2.85 (d, 1H, J = 17.0 Hz, ABX), 3.67 (s, 3H, CH_3), 3.77 (t, 2H, J = 6.0 Hz, CH_2), 4.09 (t, 2H, J = 6.0 Hz, CH_2), 5.72 (s, 1H, CH), 6.34 (d, 1H, J = 16 Hz, CH), 7.10 (dd, 1H, J =

8.6, 2.8 Hz, Ar-H), 7.39 (d, 1H, J=8.6 Hz, Ar-H), 7.49 (d, 1H, *J* = 2.8 Hz, Ar-H), 7.86 (d, 1H, *J* = 16.0 Hz, CH).

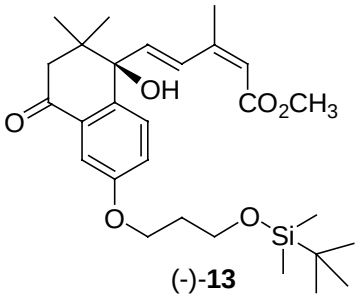
^{13}C NMR δ: 197.2, 166.5, 158.9, 146.9, 138.7, 137.9, 128.8, 127.8, 122.2, 119.9, 117.9, 110.0, 77.8, 64.8, 59.4, 51.1, 49.8, 41.1, 32.3, 29.7, 25.9, 24.4, 23.5, 21.2, 18.3, -5.4.

HRMS: Calculated for $C_{28}H_{42}O_6Si$, 502.2751. Found: 502.2726.

*Resolving silyl protected ester **12** by chiral HPLC.*



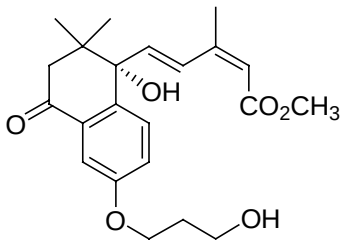
(+)-**13**



(-)-**13**

The ester **12** was resolved on a chiral HPLC column (250 mm x 21.1 mm, (*R,R*)-Whelk O-1, Kromasil, Regis Technologies Inc., USA) with 3% IPA in hexane at 7.5 mL/min and UV detection at 262 nm, to afford (+)-**13** $[\alpha]_D^{25}$ +271 (c 2.9, $CHCl_3$) and (-)-**13** $[\alpha]_D^{25}$ -274 (c 0.7 , $CHCl_3$) with retention times of 55.7 and 62.0 min respectively.

*Methyl (2Z,4E)-5-[2',2'-dimethyl-1'-hydroxy-6'-(3''-hydoxypropoxy)-4'-oxo-1',2',3',4'-tetrahydronaphthalen-1'-yl]-3-methylpenta-2,4-dienoate (**14**)*



(+)-**14**

To the silyl protected ester (+)-**13** (0.105 g, 0.21 mmol) in dry THF (10 mL) at 0 °C was added 1.0 M tetrabutylammonium fluoride (0.523 mL, 0.52 mmol) *via* syringe. The reaction mixture was allowed to warm up to rt and stirring continued for 2 h. The reaction mixture was quenched by the addition of water (5 mL) and then extracted with ethyl acetate (3 x 25 mL). The organic phase was washed with water (50 mL), dried over

anhydrous Na_2SO_4 and concentrated *in vacuo*, to yield the desired alcohol (+)-**14** as light brown oil (0.090 g). An analytical sample was purified by flash chromatography (33% EtOAc in hexane).

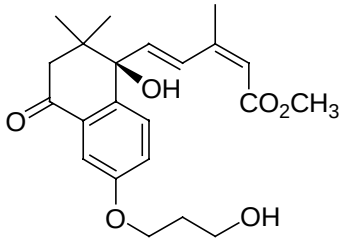
$[\alpha]_D^{25}$ +288 (c 1.0, $CHCl_3$).

IR: 3442, 2959, 1682, 1634, 1602 cm^{-1}

^1H NMR δ: 1.04 (s, 3H, CH_3), 1.05 (s, 3H, CH_3), 1.99 (s, 3H, CH_3), 2.32 (q, 2H, *J* = 6.0 Hz, CH_2), 2.54 9 (d, 1H, *J* = 17.0 Hz, CH), 3.82 (d, 1H, *J* = 17.0 Hz, CH), 3.58 (t, 2H, *J* = 6.0 Hz, CH_2), 3.67 (s, 3H, CH_3), 4.15 (t, 2H, *J* = 6.0 Hz, CH_2), 5.72 (s, 1H, CH), 6.34 (d, 1H, *J* = 16.0 Hz, CH), 7.11 (dd, 1H, *J* = 8.6, 2.7 Hz, Ar-H), 7.42 (d, 1H, *J* = 8.5 Hz, Ar-H), 7.50 (d, 1H, *J* = 2.8 Hz, Ar-H), 7.85 (d, 1H, *J* = 16 Hz, Ar-H).

^{13}C NMR δ: 197.5, 166.6, 158.5, 149.9, 139.0, 138.2, 131.9, 129.0, 127.8, 122.0, 117.6, 109.9, 77.6, 65.5, 60.4, 59.8, 51.1, 49.6, 41.1, 31.8, 24.3, 23.5, 21.2.

HRMS: Calculated for $C_{22}H_{28}O_6$, 388.1886. Found: 388.1874

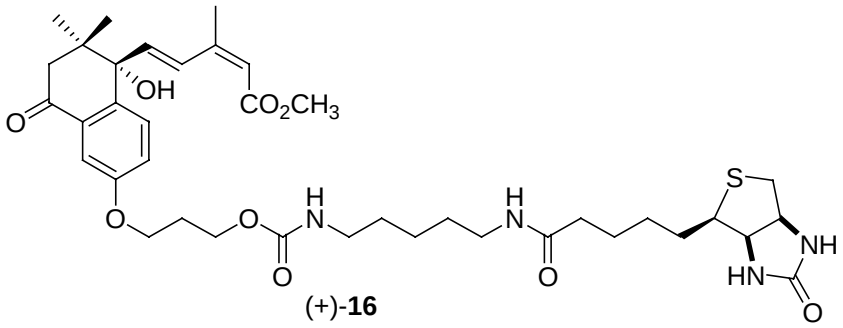


(-)-**14**

The enantiomer (-)-**14** was prepared in a similar manner from (-)-**13**.

$[\alpha]_D^{25}$ -291 (c 1.1, $CHCl_3$).

Methyl (2Z,4E)-5-((1'S)-6'-{3"-(5'"-[5''''-((3aS,6R,6aR)-hexahydro-2-oxo-1H-thieno[3,4-d]imidazol-6-yl)pentanamido]pentylcarbamoyloxy)propoxy}-2',2'-dimethyl-1'-hydroxy-4'-oxo-1',2',3',4'-tetrahydronaphthalen-1'-yl)-3-methylpenta-2,4-dienoate ***(16)***



(+)-**16**

To a solution of alcohol (+)-**14** (0.048 g, 0.124 mmol) in dry dichloromethane (3 mL) was added carbodiimide imidazole (CDI) (0.020 g, 0.124 mmol) and the reaction mixture stirred at rt for 3 h. The solvent was evaporated *in vacuo* to afford the crude product **15** (0.060 g), which was carried through to the next stage without any further purification (care should be taken in handling the CDI activated alcohol as it hydrolyses readily in presence of moisture and should therefore be used immediately after the solvent has been evaporated off). The CDI activated tetralone (0.060 g, 0.124 mmol) was dissolved in dry DMF (3 mL) in a 10 mL round bottomed flask. 5-(Biotinamido)pentylamine (0.045 g, 0.136 mmol) was then added and the reaction mixture stirred for 16 h at rt. The DMF was evaporated off *in vacuo* to leave a brown oil residue. Thin layer chromatography of the residue oil on a 20 x 20 cm silica gel plate with 10% methanol in dichloromethane yielded the desired biotinylated tetralone compound (+)-**16** (0.048 g, 53%) with an 0.4 Rf value.

$[\alpha]_D^{25}$ +124 (c 1.1, $CHCl_3$).

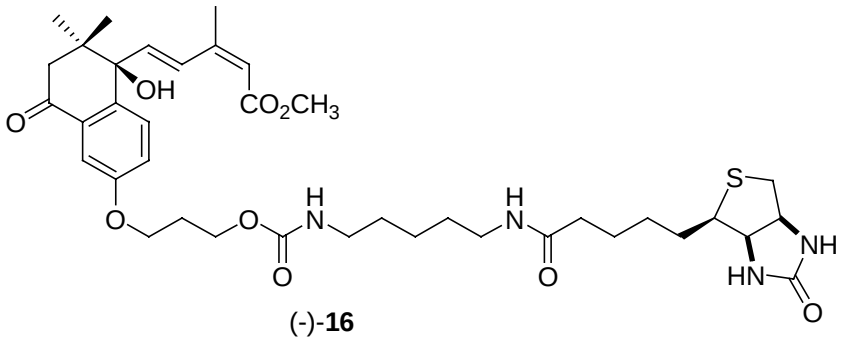
IR (ν_{max}): 3271, 2933, 1694, 1602 cm^{-1}

^1H NMR (CD_3OD) δ: 1.04 (s, 3H, CH_3), 1.08 (s, 3H, CH_3), 1.33 (m, 2H), 1.43 (m, 2H), 1.50 (m, 4H), 1.63 (m, 3H), 1.72 (m, 1H), 2.04 (s, 3H, CH_3), 2.10 (m, 2H,), 2.19 (t, 2H, *J* = 7.5 Hz), 2.60 (d, 1H, *J* = 16.5 Hz, CH_2), 2.69 (d, 1H, *J* = 13 Hz), 2.70 (d, 1H, *J* = 16.5 Hz, CH_2), 2.92 (dd, 1H, *J* = 5.0, 12.5 Hz), 3.08 (t, 2H, *J* = 7.0 Hz), 3.15 (t, 2H, *J* = 7.0 Hz), 3.20 (dt, 1H, *J* = 9.0, 5.5 Hz), 3.63 (s, 3H, CH_3), 4.14 (t, 2H, *J* = 6.0 Hz), 4.21 (t, 2H, *J* = 6.0 Hz), 4.29 (dd, 1H, *J* = 4.5, 8.0 Hz), 4.48 (dd, 1H, *J* = 4.5, 8.0 Hz), 5.72 (s,

1H, CH), 6.50 (d, 1H, *J* = 16 Hz, CH), 7.21 (dd, 1H, *J* = 2.5, 8.5 Hz, Ar-H), 7.47 (d, 1H, *J* = 2.5 Hz, Ar-H), 7.50 (d, 1H, *J* = 8.5 Hz, Ar-H), 7.62 (br d, 1H, *J* = 16 Hz, CH).

^{13}C NMR ($CDCl_3$) δ: 197.9, 173.5, 166.7, 163.9, 158.4, 156.9, 149.9, 139.3, 138.5, 132.0, 129.5, 128.0, 122.3, 117.6, 109.5, 77.5, 70.1, 64.6, 63.7, 61.8, 61.3, 60.2, 55.5, 51.1, 49.8, 41.3, 40.5, 39.2, 35.7, 29.4, 28.8 (2), 27.9 (2), 25.6, 24.4, 23.7, 23.6, 21.3.

HRMS: Calculated for $C_{38}H_{55}N_4O_9S$, 743.3690 (M+1). Found: 743.3688.



(-)-**16**

The diastereomer (-)-**16** was prepared in a similar manner from (-)-**14**.

$[\alpha]_D^{25}$ -95 (c 1.2, $CHCl_3$).

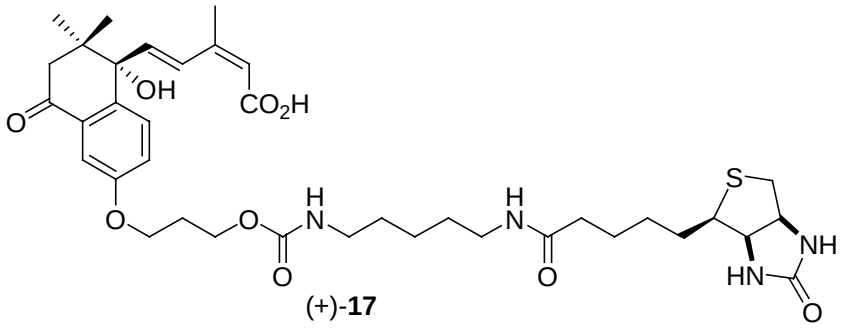
IR (ν_{max}): 3296, 2927, 1689, 1602 cm^{-1}

^{1}H NMR δ: ^{1}H NMR (CD_3OD) δ: 1.04 (s, 3H, CH_3), 1.08 (s, 3H, CH_3), 1.34 (m, 2H), 1.43 (m, 2H), 1.50 (m, 4H), 1.63 (m, 3H), 1.72 (m, 1H), 2.04 (s, 3H, CH_3), 2.10 (m, 2H,), 2.19 (t, 2H, *J* = 7.0 Hz), 2.60 (d, 1H, *J* = 17 Hz, CH_2), 2.70 (d, 1H, *J* = 13 Hz), 2.70 (d, 1H, *J* = 17 Hz, CH_2), 2.92 (dd, 1H, *J* = 5.0, 13 Hz), 3.09 (t, 2H, *J* = 7.0 Hz), 3.15 (t, 2H, *J* = 7.0 Hz), 3.20 (dt, 1H, *J* = 8.0, 6.0 Hz), 3.63 (s, 3H, CH_3), 4.14 (t, 2H, *J* = 6.0 Hz), 4.21 (t, 2H, *J* = 6.0 Hz), 4.29 (dd, 1H, *J* = 4.5, 7.5 Hz), 4.48 (dd, 1H, *J* = 4.5, 8.0 Hz), 5.72 (s, 1H, CH), 6.50 (d, 1H, *J* = 16 Hz, CH), 7.21 (dd, 1H, *J* = 2.5, 8.5 Hz, Ar-H), 7.47 (d, 1H, *J* = 2.5 Hz, Ar-H), 7.50 (d, 1H, *J* = 8.5 Hz, Ar-H), 7.62 (br d, 1H, *J* = 16 Hz, CH).

^{13}C NMR ($CDCl_3$) δ: 198.0, 173.6, 166.5, 164.1, 158.2, 156.8, 149.9, 139.5, 138.6, 131.9, 129.5, 127.9, 122.1, 117.4, 109.3, 77.4, 70.1, 64.5, 63.5, 61.7, 61.2, 60.1, 55.5, 49.7, 41.2, 40.5, 39.1, 35.6, 29.3, 28.8, 28.7, 28.0, 27.9, 25.5, 24.3, 23.7, 23.6, 21.2.

HRMS: Calculated for $C_{38}H_{55}N_4O_9S$, 743.3690 (M+1). Found: 743.3628.

*(2Z,4E)-5-((1'S)-6'-{3"-(5'"-[5''''-((3aS,6R,6aR)-hexahydro-2-oxo-1H-thieno[3,4-d]imidazol-6-yl)pentanamido]pentylcarbamoyloxy)propoxy}-2',2'-dimethyl-1'-hydroxy-4'-oxo-1',2',3',4'-tetrahydronaphthalen-1'-yl)-3-methylpenta-2,4-dienoic acid (**17**).*



(+)-**17**

The biotinylated ester (+)-**16** (40 mg, 5.39 x 10^{-2} mmol) was dissolved in 10 mL methanol and few drops of 1M KOH were added to the solution to pH 11. The reaction mixture was refluxed at 40 °C overnight. The pH of the reaction mixture was adjusted to 7 using 1M HCl and solvent evaporated. The crude material was purified using preparative TLC in 10% MeOH/CH_2Cl_2 + 0.1 % AcOH. 30 mg (4.12 x 10^{-2} mmol) of pure product with 75% yield was obtained.

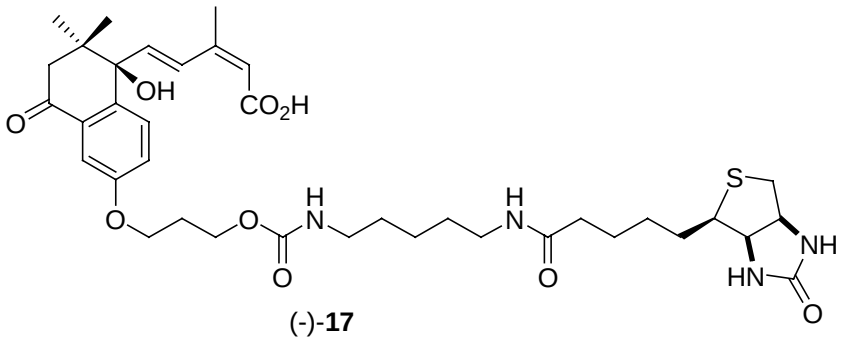
$[\alpha]_D^{25}$ +98 (c 1.1, MeOH).

IR (ν_{max}): 3500-2500, 3292, 3082(w), 2932, 1684, 1603 cm^{-1}.

^{1}H NMR (CD_3OD) δ: 1.03 (s, 3H, CH_3), 1.07 (s, 3H, CH_3), 1.33 (m, 2H), 1.42 (m, 2H), 1.49 (m, 4H), 1.63 (m, 3H), 1.72 (m, 1H), 2.02 (s, 3H, CH_3), 2.09 (m, 2H), 2.18 (t, 2H, *J* = 7.5 Hz), 2.59 (d, 1H, *J* = 16 Hz, CH_2), 2.69 (d, 1H, *J* = 13 Hz), 2.71 (d, 1H, *J* = 16 Hz, CH_2), 2.91 (dd, 1H, *J* = 5.0, 12.5 Hz), 3.08 (m, 2H), 3.15 (m, 2H), 3.19 (dt, 1H, *J* = 14, 6.0 Hz), 4.12 (t, 2H, *J* = 6.0 Hz), 4.20 (t, 2H, *J* = 6.0 Hz), 4.29 (dd, 1H, *J* = 4.5, 8.0 Hz), 4.48 (dd, 1H, *J* = 4.5, 7.5 Hz), 5.71 (s, 1H, CH), 6.45 (d, 1H, *J* = 16 Hz, CH), 7.19 (dd, 1H, *J* = 2.5 , 8.5 Hz, Ar-H), 7.44 (d, 1H, *J* = 2.5 Hz, Ar-H), 7.49 (d, 1H, *J* = 8.5 Hz, Ar-H), 7.62 (br d, 1H, *J* = 16 Hz, CH).

^{13}C NMR ($CDCl_3$ + 6 drops CD_3OD) δ: 198.4, 173.8, 168.6, 164.2, 158.3, 157.0, 150.1, 139.0, 138.7, 131.8, 129.3, 128.1, 122.3, 118.2, 109.3, 77.4, 64.5, 61.9, 61.2, 60.1, 55.5, 49.7, 41.3, 40.4, 39.2, 39.1, 35.7, 35.6, 28.7, 28.6, 28.2, 27.9, 25.4, 24.2, 23.6, 23.4, 21.2.

HRMS (ES^-): Calculated for $C_{37}H_{51}N_4O_9S$, 727.3377 (M-1). Found: 727.3362.



(-)-**17**

The diastereomer (-)-**17** was prepared in a similar manner from (-)-**16**.

$[\alpha]_D^{25}$ -51 (c 1.3, MeOH).

IR (ν_{max}): 3500-2500, 3316, 2928, 1693 cm^{-1}.

^{1}H NMR (CD_3OD) δ: 1.03 (s, 3H, CH_3), 1.07 (s, 3H, CH_3), 1.33 (m, 2H), 1.42 (m, 2H), 1.49 (m, 4H), 1.62 (m, 3H), 1.71 (m, 1H), 2.03 (s, 3H, CH_3), 2.09 (m, 2H), 2.18 (m, 2H), 2.59 (d, 1H, *J* = 16 Hz, CH_2), 2.70 (d, 1H, *J* = 12.5 Hz), 2.70 (d, 1H, *J* = 16 Hz, CH_2), 2.91 (dd, 1H, *J* = 4.5, 12.5 Hz), 3.08 (m, 2H), 3.14 (m, 2H), 3.19 (m, 1H), 4.12 (m, 2H), 4.20 (m, 2H), 4.28 (dd, 1H, *J* = 4.5, 7.5 Hz), 4.47 (dd, 1H, *J* = 5.5, 7.0 Hz), 5.71 (s, 1H, CH), 6.47 (d, 1H, *J* = 16 Hz, CH), 7.19 (dd, 1H, *J* = 2.0, 8.5 Hz, Ar-H), 7.44 (s, 1H, Ar-H), 7.49 (d, 1H, *J* = 8.5 Hz, Ar-H), 7.64 (br d, 1H, *J* = 16 Hz, CH).

^{13}C NMR ($CDCl_3$ + 6 drops CD_3OD) δ: 198.4, 173.9, 168.4, 164.1, 158.2, 157.0, 149.9, 138.9, 138.6, 131.7, 129.2, 127.9, 122.2, 118.1, 109.2, 77.4, 64.4, 61.7, 61.2, 60.0, 55.4, 49.6, 41.3, 41.2, 40.3, 39.0, 35.7, 35.5, 29.1, 28.6, 28.1, 27.8, 25.3, 24.1, 23.6, 23.3, 21.0.

HRMS: Calculated for $C_{37}H_{51}N_4O_9S$, 727.3377 (M-1). Found: 727.3356.

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(2) Hattersley, P. J.; Lockhart, I. M.; Wright, M. *Journal of the Chemical Society [Section] C: Organic* **1969**, 217-22.

(3) Almansa, C.; Carceller, E.; Bartroli, J.; Forn, J. *Synthetic Communications* **1993**, *23*, 2965-71.