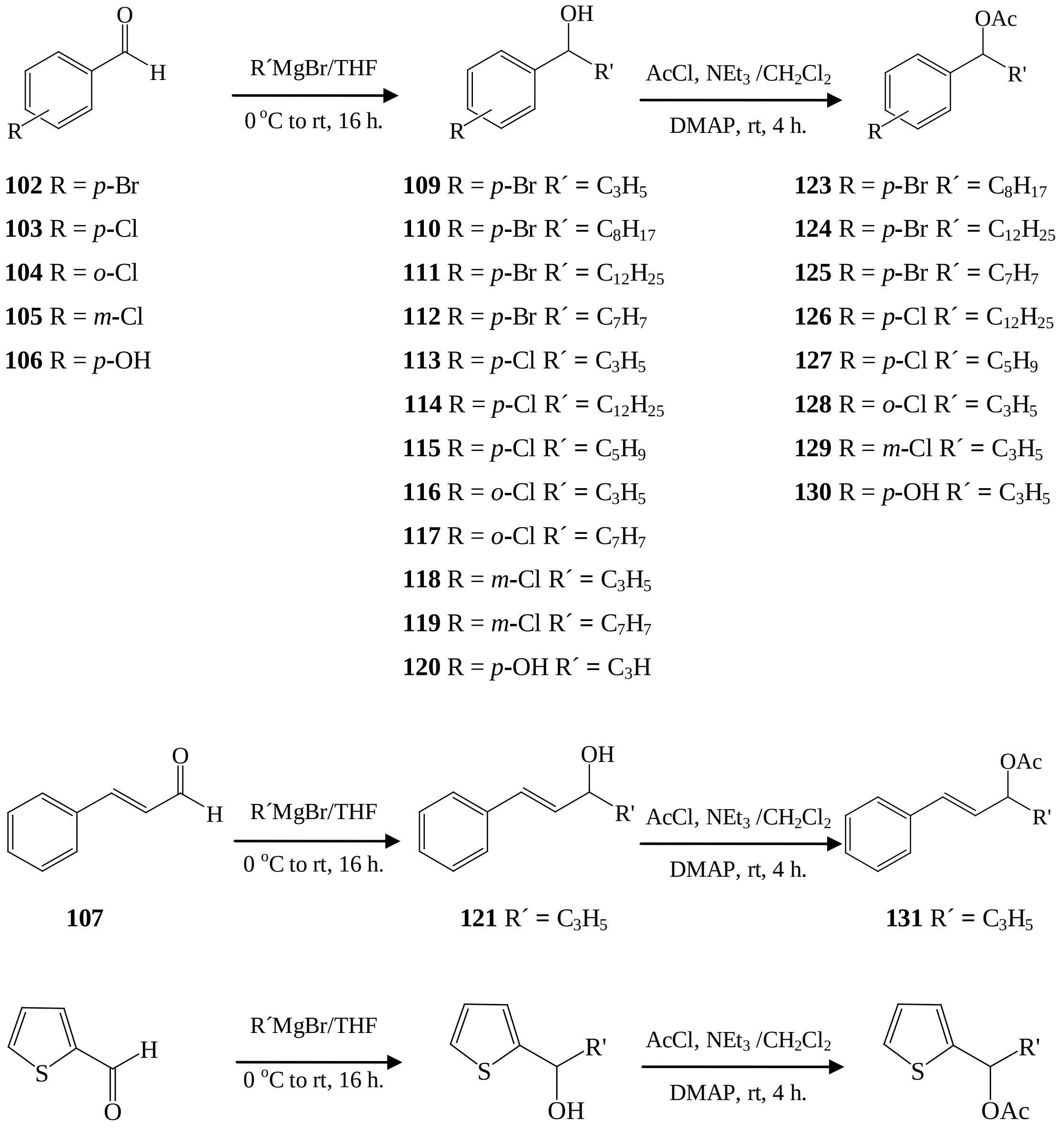
Chapter 3

Materials and method

3.1 Chemical reagents and Instrument

All anhydrous solvents and starting materials were purchased from Acros Organics and Aldrich Chemical Co. All commercial grade solvents were purchased from RCI Labscan Co, Ltd. The purity of the compounds was isolated by column chromatography on Merck precoated silica gel 60 particle sizes 63-200 µm (70-230 mesh). The reactions were monitored by thin-layer chromatography (TLC) on precoated Merck 60 F254 silica gel plates and visualized using UV light (254 nm). All ^{1}H, ^{13}C NMR spectra were recorded on a Bruker-Ultra Shield (400 and 100 MHz for ^{1}H and ^{13}C, respectively), using $CDCl_3$ as a solvent with trace of $CHCl_3$ as an internal standard. The chemical shifts (δ) are reported in ppm. Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), dt (double triplet), dd (double doublet), m (multiplet) and br s (broad singlet). IR spectra were recorded on Perkin Elmer spectrometers. Mass spectra were recorded with a Thermo Finnigan LCQ Advantage ion trap mass spectrometer.

Synthetic routes of ACA analogs are depicted in Scheme 11. Compounds **108-122** were synthesized via the Grignard reaction (procedure A) and subsequent acetylation reaction (procedure B) to give compounds **122-132**. Their structures were characterized by elemental analyses and spectral data (^{1}H, ^{13}C NMR, IR and MS)



102 R = *p*-Br
103 R = *p*-Cl
104 R = *o*-Cl
105 R = *m*-Cl
106 R = *p*-OH

109 R = *p*-Br R´ = C_3H_5
110 R = *p*-Br R´ = C_8H_{17}
111 R = *p*-Br R´ = $C_{12}H_{25}$
112 R = *p*-Br R´ = C_7H_7
113 R = *p*-Cl R´ = C_3H_5
114 R = *p*-Cl R´ = $C_{12}H_{25}$
115 R = *p*-Cl R´ = C_5H_9
116 R = *o*-Cl R´ = C_3H_5
117 R = *o*-Cl R´ = C_7H_7
118 R = *m*-Cl R´ = C_3H_5
119 R = *m*-Cl R´ = C_7H_7
120 R = *p*-OH R´ = C_3H

123 R = *p*-Br R´ = C_8H_{17}
124 R = *p*-Br R´ = $C_{12}H_{25}$
125 R = *p*-Br R´ = C_7H_7
126 R = *p*-Cl R´ = $C_{12}H_{25}$
127 R = *p*-Cl R´ = C_5H_9
128 R = *o*-Cl R´ = C_3H_5
129 R = *m*-Cl R´ = C_3H_5
130 R = *p*-OH R´ = C_3H_5

107
121 R´ = C_3H_5
131 R´ = C_3H_5

108
122 R´ = C_3H_5
132 R´ = C_3H_5

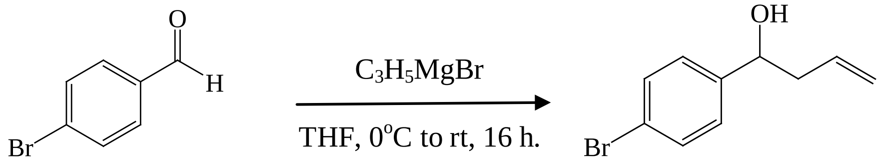
Scheme 11. Synthetic route to the ACA analogs

3.2 General procedure A to preparation of ACA analogs 109–122

General procedure was carried out according to previous researches (Azuma, et al., 2006) and (Murakami, Toyota, Ohura, Koshimizu, & Ohigashi, 2000). The aldehyde **102-108** was added to a stirring solution of anhydrous tetrahydrofuran (THF) (25 ml) at room temperature before adding Grignard reagent (1.2 eq.) which was previously dissolved in anhydrous tetrahydrofuran (THF) under N_2 atmosphere.

The mixture was allowed to stir for a further 16 hours before adding hydrochloric acid (0.5 M, 20 ml) and ethyl acetate (50 ml). The reaction mixture was extracted with ethyl acetate (3 × 20 ml), and the organic phases were combined, dried (Na_2SO_4) and evaporated to yield the crude product. Purification was carried out by column chromatography, eluting with ethyl acetate-hexane (yield 9 – 60%).

3.2.1 Synthesis of 1-(4-bromophenyl)but-3-en-1-ol

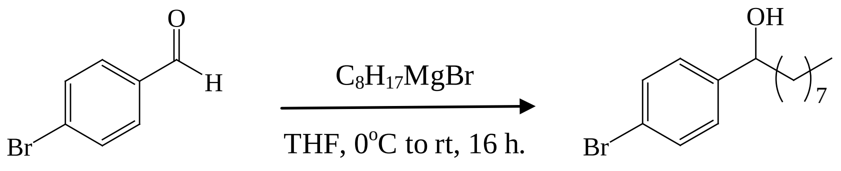


102 **109**

Scheme 12. Synthesis of 1-(4-bromophenyl)but-3-en-1-ol **109**

4-Bromobenzadehyde **102** (1 g, 5.405 mmol) was added to a stirring solution of THF at room temperature before adding 1 M allylmagnesium bromide (C_3H_5MgBr) (6.486 ml, 6.486 mmol). The reaction was prepared according to General procedure A. Purification was carried out by column chromatography, eluting from 0 to 30 % ethyl acetate in hexane to yield 1-(4-bromophenyl)but-3-en-1-ol **109** 0.6513 g, 53.1 %yield as a pale yellow oil**:** ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.02 (1H, br s, 1´-OH), 2.48 (2H, dt, J = 14, 8 Hz, H-2'), 4.71 (1H, t, J = 7.2 Hz, H-1'), 5.15 (2H, dd, J = 12, 6.8 H-4'), 5.78 (1H, dt, J = 13.6, 7 Hz, H-3'), 7.24 (2H, d, J = 7.6 Hz, H-2,6), 7.43 (2H, d, J = 7.6 Hz, H-3,5)**.** ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 43.56 (C-2'), 72.63 (C-1'), 118.50 (C-4'), 121.14 (C-4), 124.57 (C-2,6), 131.95 (C-3,5), 133.92 (C-3'), 142.82 (C-1)**.** IR (cm^{-1}): 3390 (O-H str.), 3080 (C-H aromatic str.), 1641 (C=C alkene str.), 1591.88, 1488 (C=C aromatic str.), 1067 (C-O str.). MS: *m/z* 229.3 [M $^{+\,1}$] 212, 201, 157.

3.2.2 Synthesis of 1-(4-bromophenyl)nonan-1-ol

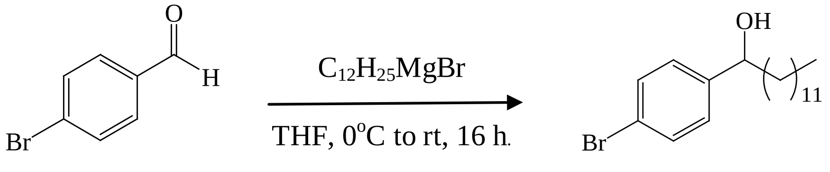


102 **110**

Scheme 13. Synthesis of 1-(4-bromophenyl)nonan-1-ol **110**

4-Bromobenzadehyde **102** (1 g, 5.405 mmol) was added to a stirring solution of THF at room temperature before adding 2 M octylmagnesium bromide ($C_8H_{17}MgBr$) (3.350 ml, 6.455 mmol). The reaction was prepared according to General procedure A. Purification was carried out by column chromatography, eluting from 0 to 20 % ethyl acetate in hexane to yield 1-(4-bromophenyl)nonan-1-ol **110** 0.8035 g, 49.7 %yield as a pale yellow oil, b.p. 175 ^{o}C: ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 0.81 (3H, t, J = 13.2 Hz, H-9'), 1.27 (12H, s, H-3'-8'), 1.61 (2H, m, H-2'), 1.88 (1H, br s, 1'-OH), 4.54 (1H, t, J = 13.2 Hz, H-1'), 7.13 (2H, d, J = 8 Hz, H-2,6), 7.40 (2H, d, J = 8.4 Hz, H-3,5). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 14.09 (C-9'), 22.65 (C-8'), 24.31 (C-3'), 25.67 (C -6'), 29.39 (C-4', 5'), 31.85 (C-7'), 38.87 (C-2'), 73.99 (C-1'), 121.14 (C-4), 127.63 (C-2, 6), 131.48 (C-3, 5), 143.93 (C-1). IR (cm^{-1}): 3353 (O-H str.), 2919, 2847 (C-H aliphatic str.), 1671, 1592 (C=C aromatic str.), 1010 (C-O str.). MS: *m/z* 299 [M $^{+1}$] 213.

3.2.3 Synthesis of 1-(4-bromophenyl)tridecan-1-ol

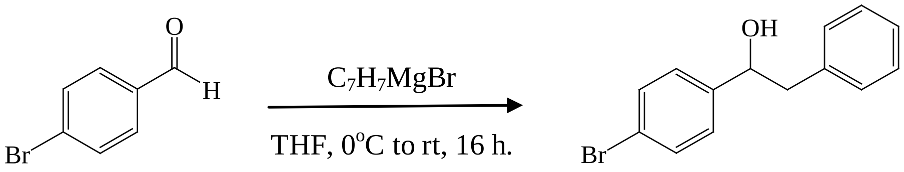


102 **111**

Scheme 14. Synthesis of 1-(4-bromophenyl)tridecan-1-ol **111**

4-Bromobenzadehyde **102** (1 g, 5.405 mmol) was added to a stirring solution of THF at room temperature before adding 1 M dodecylmagnesium bromide ($C_{12}H_{25}MgBr$) (3.243 ml, 6.486 mmol). The reaction was prepared according to General procedure A. Purification was carried out by column chromatography, eluting from 0 to 20 % ethyl acetate in hexane to yield 1-(4-bromophenyl)tridecan-1-ol **111** 0.8955 g, 50.6 %yield as a pale yellow solid: ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 0.81 (3H, t, J =12.8 Hz, H-13'), 1.27 (20H, s, H-3'-12'), 1.61 (2H, m, H-2'), 1.88 (1H, br s, 1'-OH), 4.56 (1H, t, J =13.2 Hz, H-1'), 7.15 (2H, d, J = 8.4 Hz, H-2,6), 7.39 (2H, d, J = 8.4 Hz, H-3,5). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 15.79 (C-13'), 23.38 (C-12'), 25.50 (C-3'), 29.51 (C-10'), 29.55 (C-4'), 29.60 (C-5'-9'), 33.82 (C-11'), 39.31 (C-2'), 76.73 (C-1'), 121.25 (C-4), 127.74 (C-2,6), 131.74 (C-3,5), 145.64 (C-1). IR (cm^{-1}): 3353 (O-H str.), 2919, 2847 (C-H aliphatic str.), 1487, 1465 (C=C aromatic str.), 1010 (C-O str.). Lit. m.p. 49-50 °C (Brown & Marvel, 1937).

3.2.4 Synthesis of 1-(4-bromophenyl)-2-phenylethanol

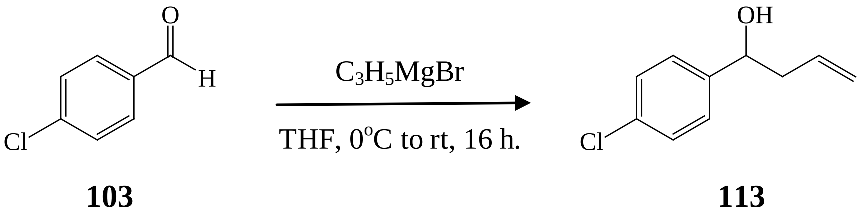


102 **112**

Scheme 15. Synthesis of 1-(4-bromophenyl)-2-phenylethanol **112**

4-Bromobenzadehyde **102** (1 g, 5.405 mmol) was added to a stirring solution of THF at room temperature before adding 2 M benzylmagnesium bromide (C_7H_7MgBr) (3.243 ml, 6.486 mmol). The reaction was prepared according to General procedure A. Purification was carried out by column chromatography, eluting from 0 to 20 % ethyl acetate in hexane to yield 1-(4-bromophenyl)-2-phenylethanol **112** 0.4997 g, 33.5 %yield as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 1.18 (1H, br s, 1'-OH), 2.89 (2H, m, H-2'), 4.79 (1H, t, J = 8 Hz, H-1'), 7.19 (7H, m, H-2,6, 4'-8'), 7.39 (2H, d, J = 8.4 Hz, H-3,5). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 44.06 (C-2'), 74.70 (C-1'), 117.39 (C-4), 126.69 (C-6'), 127.67 (C-4', 8'), 128.84 (C-5', 7'), 129.52 (C-2, 6), 130.17 (C-3, 5), 137.50 (C-3'), 142.75 (C-1). IR (cm^{-1}): 3369 (O-H str.), 3028 (C-H aromatic str.), 1591, 1487 (C=C aromatic str.), 1067 (C-O str.). MS: *m/z* 277 [M $^{+1}$] 157.

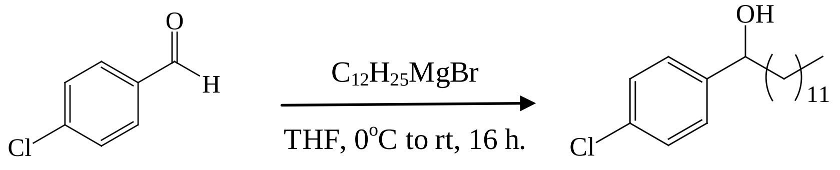
3.2.5 Synthesis of 1-(4-chlorophenyl)but-3-en-1-ol



Scheme 16. Synthesis of 1-(4-chlorophenyl)but-3-en-1-ol **113**

4-Chlorobenzadehyde **103** (5 g, 35.585 mmol) was added to a stirring solution of THF at room temperature before adding 1 M allylmagnesium bromide (C_3H_5MgBr) (42.702 ml, 42.702 mmol). The reaction was prepared according to General procedure A. Purification was carried out by column chromatography, eluting from 0 to 35 % ethyl acetate in hexane to yield 1-(4-chlorophenyl)but-3-en-1-ol **113** 3.0523 g, 47 %yield as a yellow-oil: ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.17 (1H, br s, 1´-OH), 2.49 (1H, dt, *J* = 14, 8 Hz, H-2') 4.74 (1H, t, *J* = 7.2 Hz, H-1') 5.18 (2H, dd, *J* = 12, 6.8 H-4') 5.81 (1H, dt, *J* = 13.6, 7 Hz, H-3'), 7.26 (2H, d, *J* = 7.6 Hz, H-2,6), 7.39 (2H, d, *J* = 7.6 Hz, H-3,5). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 43.51 (C-2'), 72.67 (C-1'), 118.43 (C-4'), 127.72 (C-2,6), 128.83 (C-3,5), 133.04 (C-4), 134.32 (C-3'), 142.38 (C-1). IR (cm^{-1}): 3390 (O-H str.), 3074 (C-H str. aromatic), 1644 (C=C alkene str.), 1607, 1488 (C=C aromatic str.), 1091 (C-O str.). MS: *m/z* 183.1 [M $^{+1}$] 130.

3.2.6 Synthesis of 1-(4-chlorophenyl)tridecan-1-ol

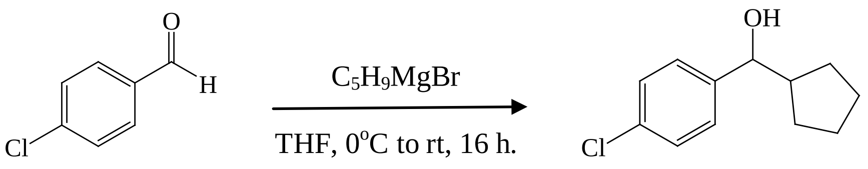


103 **114**

Scheme 17. Synthesis of 1-(4-chlorophenyl)tridecan-1-ol**114**

4-Chlorobenzadehyde **103** (2 g, 14.2278 mmol) was added to a stirring solution of THF at room temperature before adding 1 M dodecylmagnesium bromide ($C_{12}H_{25}MgBr$) (8.5367 ml, 17.0734 mmol The reaction was prepared according to General procedure A. Purification was carried out by column chromatography, eluting from 0 to 20 % ethyl acetate in hexane to yield 1-(4-chlorophenyl)tridecan-1-ol **114** 1.6399 g, 22.7 %yield as a white solid, m.p. 52 °C: ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 0.90 (3H, t, *J* =12.5 Hz, H-13'), 1.27 (20H, s, H-3'-12'), 1.75 (2H, m, H-2'), 1.77 (1H, br s, 1'-OH), 4.67 (1H, t, *J* =13.2 Hz, H-1'), 7.30 (2H, d, *J* = 8.4 Hz, H-2,6), 7.32 (2H, d, *J* = 8 Hz, H-3,5). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 14.11 (C-13'), 22.70 (C-12'), 25.70 (13'), 29.5 (C-10'), 29.52 (C-4'), 29.54 (C-5'-9'), 31.94 (C-11'), 39.99 (C-2'), 74.03 (C-1'), 121.44 (C-4), 127.07 (C-3,5), 131.78 (C-2,6), 143.41 (C-1). IR (cm^{-1}): 3390 (O-H str.), 2930, 2854 (C-H aliphatic str.), 1640, 1593 (C=C aromatic str.), 1091 (C-O str.).

3.2.7 Synthesis of (4-chlorophenyl)(cyclopentyl)methanol

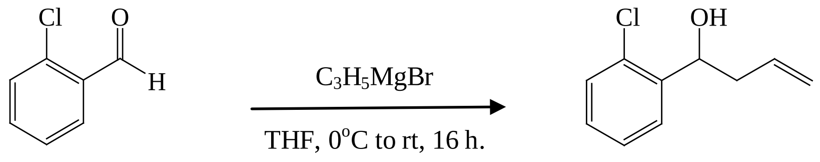


103 **115**

Scheme 18. Synthesis of (4-chlorophenyl)(cyclopentyl)methanol **115**

4-Chlorobenzadehyde **103** (1 g, 7.117 mmol) was added to a stirring solution of THF at room temperature before adding 2 M cyclopentylmagnesium bromide (C_5H_9MgBr) (4.2702 ml, 8.5404 mmol). The reaction was prepared according to General procedure A. Purification was carried out by column chromatography, eluting from 0 to 40 % ethyl acetate in hexane to yield (4-chlorophenyl)(cyclopentyl) methanol **114** 0.1974 g, 13.2 %yield as a yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 1.35 (2H, m, H-3'a,6'a), 1.45 (2H, m, H-4'a,5'a), 1.55 (2H, m, H-4'b,5'b), 1.61 (2H, m, H-3'b,6'b), 2.12 (1H, br s, 1´-OH), 2.17 (1H, sextet, *J* = 8.4 Hz, H-2'), 4.40 (1H, d, *J* = 8 Hz, H-1'), 7.20 (2H, d, *J* = 8.4 Hz, H-2,6), 7.23 (2H, d , *J* = 8.4 Hz, H-3,5). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 25.56 (C-4', 5'), 29.54 (C-3', 6'), 47.75 (C-2'), 78.36 (C-1'), 127.94 (C-3, 5), 128.38 (C-2, 6), 133.16 (C-4), 142.92 (C-1). IR (cm^{-1}): 3379 (O-H str.), 2945, 2857 (C-H aliphatic str.), 1597, 1488 (C=C *aromatic str.)*, 1089 (C-O str.).

3.2.8 Synthesis of 1-(2-chlorophenyl)but-3-en-1-ol



104

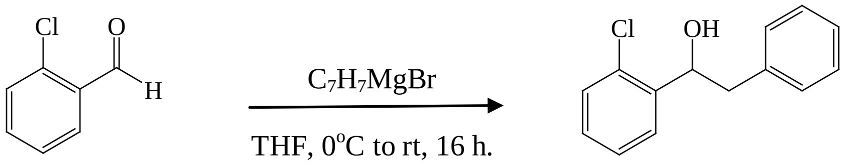
116

Scheme 19. Synthesis of 1-(2-chlorophenyl)but-3-en-1-ol **116**

2-Chlorobenzadehyde **104** (1 g, 7.117 mmol) was added to a stirring solution of THF at room temperature before adding 1 M allylmagnesium bromide (C_3H_5MgBr) (8.537 ml, 8.537 mmol). The reaction was prepared according to General procedure A. Purification was carried out by column chromatography, eluting from 0 to 30 % ethyl acetate in hexane to yield 1-(2-chlorophenyl)but-3-en-1-ol **116** 0.3449 g, 26.5 %yield as a yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 1.92 (1H, br s, 1´-OH), 2.38 (1H, dt, *J* = 14, 8 Hz, H-2'a) 2.64 (1H, dt, *J* = 8.8, 5.2 Hz, H-2'b) 4.12 (1H, dd, *J* = 14, 7.2 Hz, H-1'), 5.14 (2H, m, H-4'), 5.89 (1H, m, H-3') 7.21 (1H, t, *J* = 13.6 Hz, H-5), 7.28 (2H, m, H-4,6), 7.57 (1H, d, *J* = 7.6 Hz, H-3). ^{13}C NMR ($CDCl_3$, 100

MHz), δ (ppm): 42.00 (C-2'), 69.64 (C-1'), 118.15 (C-4'), 118.65 (C-5), 125.45 (C-6), 127.05 (C-3), 128.42 (C-4), 129.30 (C-2), 134.14 (C-3'), 141.20 (C-1)**.** IR (cm^{-1}): 3390 (O-H str.), 3074 (C-H str. aromatic), 1641 (C=C alkene str.), 1576, 1471 (C=C aromatic str.), 1033 (C-O str.). MS: *m/z* 185 [M $^{+1}$].

3.2.9 Synthesis of 1-(2-chlorophenyl)-2-phenylethanol

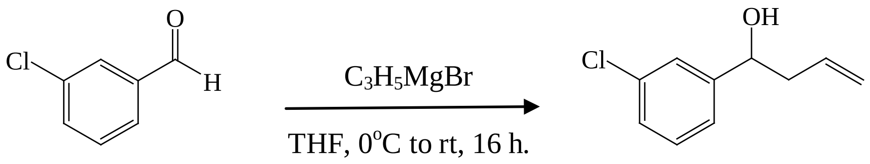


104 **117**

Scheme 20. Synthesis of 1-(2-chlorophenyl)-2-phenylethanol **117**

2-Chlorobenzadehyde **104** (1 g, 7.117 mmol) was added to a stirring solution of THF at room temperature before adding 2 M benzylmagnesium bromide (C_7H_7MgBr) (4.268 ml, 8.537 mmol). The reaction was prepared according to General procedure A. Purification was carried out by column chromatography, eluting from 0 to 30 % ethyl acetate in hexane to yield 1-(2-chlorophenyl)-2-phenylethanol **117** 0.1516 g, 9.2 %yield as a yellow solid, b.p. 64 ^{o}C: ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 1.89 (1H, br s, 1'-OH), 2.77 (1H, dd, *J* = 9.2, 4.4 Hz, H-2'a), 3.17 (1H, dd, *J* = 6, 4.4 Hz, H-2'b), 5.33 (1H, dd, *J* = 6, 9.2 Hz, H-1'), 7.06 (2H, t, *J* = 12.1 Hz, H-5, Ar'H-4), 7.12 (2H, dt, *J* = 17.1, 7.8 Hz, Ar'H-2,5), 7.13 (2H, dt, *J* = 17.1, 7.8 Hz, H-4,6), 7.15 (1H, d, *J* = 7.2 Hz, H-3), 7.18 (2H, dd, *J* = 8.4, 6 Hz, Ar'H-3,5). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 42.56 (C-2'), 72.85 (C-1'), 126.58 (Ar'C-4), 126.75 (C-5), 128.83 (Ar'C-2, 6), 128.97 (C-6, Ar'C-3, 5), 130.01 (C-3,4), 132.29 (C-2), 138.62 (C-1), 142.40 (Ar'C-1). IR (cm^{-1}): 3418 (O-H str.), 3054 (C-H aromatic str.), 1651 (C=C alkene srt.), 1604, 1496 (C=C aromatic str.), 1032 (C-O str.).

3.2.10 Synthesis of 1-(3-chlorophenyl)but-3-en-1-ol

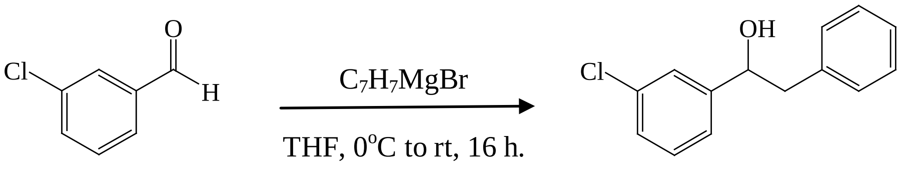


105 **118**

Scheme 21. Synthesis of 1-(3-chlorophenyl)but-3-en-1-ol **118**

3-Chlorobenzadehyde **105** (1 g, 7.117 mmol) was added to a stirring solution of THF at room temperature before adding 1 M allylmagnesium bromide (C_3H_5MgBr) (8.537 ml, 8.537 mmol). The reaction was prepared according to General procedure A. Purification was carried out by column chromatography, eluting from 0 to 30 % ethyl acetate in hexane to yield 1-(3-chlorophenyl)but-3-en-1-ol **118** 0.3433 g, 26.4 %yield as a yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 1.77 (1H, br s, 1´-OH), 2.41 (2H, m, H-2') 4.65 (1H, t, J = 8 Hz, H-1'), 5.12 (2H, dd, J = 11.2, 4.4 Hz, H-4'), 5.72 (1H, ddd, J = 17.2, 10, 6.4 Hz, H-3') 7.13 (1H, d, J = 10.2 Hz, H-2), 7.17 (1H, dd, J = 12.6, 6.4 Hz, H-6), 7.20 (2H, m, H-4,5). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 43.83 (C-2'), 72.62 (C-1'), 118.96 (C-4'), 123.98 (C-6), 126.07 (C-2), 127.54 (C-4), 129.69 (C-5), 133.91 (C-3), 134.40 (C-3'), 145.98 (C-1). IR (cm^{-1}): 3385 (O-H str.), 3074 (C-H aromatic str.), 1679 (C=C alkene str.), 1643, 1597 (C=C aromatic str.), 1054 (C-O str.). EI-MS (*m/z*, %) 182 [M $^{+1}$] 165, 141, 113, 77 (Li, Wang, Wen, & Ma, 2011).

3.2.11 Synthesis of 1-(3-chlorophenyl)-2-phenylethanol

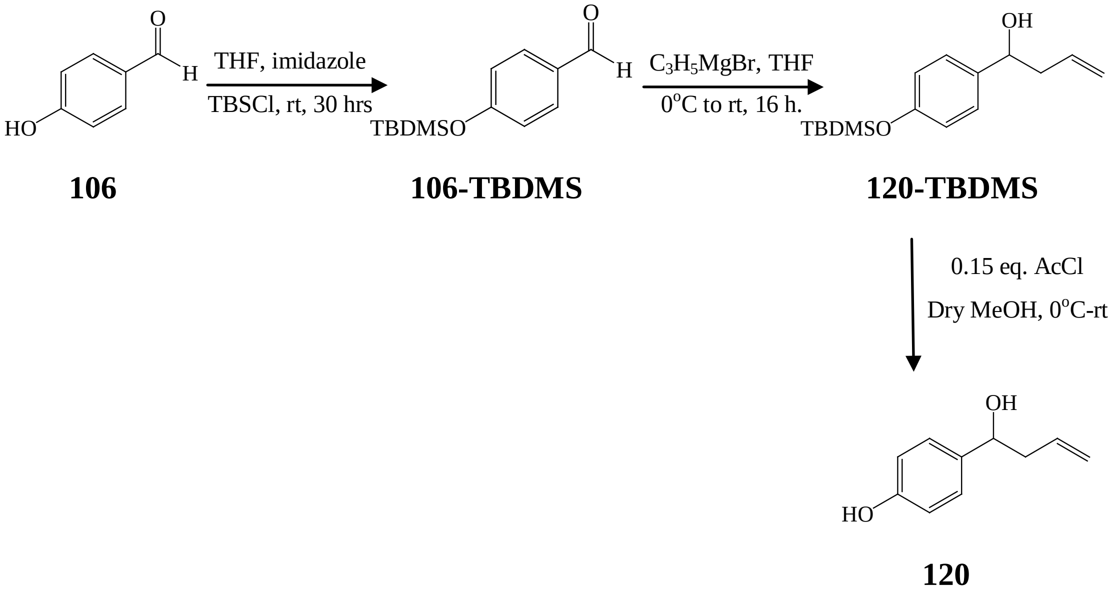


105 **119**

Scheme 22. Synthesis of 1-(3-chlorophenyl)-2-phenylethanol **119**

3-Chlorobenzadehyde **105** (1 g, 7.117 mmol) was added to a stirring solution of THF at room temperature before adding 2 M benzylmagnesium bromide (C_7H_7MgBr) (4.268 ml, 8.537 mmol). The reaction was prepared according to General procedure A. Purification was carried out by column chromatography, eluting from 0 to 30 % ethyl acetate in hexane to yield 1-(3-chlorophenyl)-2-phenylethanol **119** 0.4893 g, 29.6 %yield as a dark yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.04 (1H, br s, 1'-OH), 2.95 (1H, dd, J = 9.2, 4.4 Hz, H-2'a), 2.99 (1H, dd, J = 8.8, 4.8 Hz, H-2'b), 4.81 (1H, t, J = 8 Hz, H-1'), 7.07 (1H, d, J = 8 Hz, H-6), 7.11 (1H, t, J = 11 Hz, Ar'H-4), 7.13 (2H, d, J = 7.2 Hz, Ar'H-2,6), 7.15 (1H, t, J = 11.2 Hz, H-5), 7.19 (2H, dt, J = 17.2, 8 Hz, H-2,4), 7.23 (2H, d, J = 7.2 Hz, Ar'H-3,5). IR (cm^{-1}): 3418 (O-H str.), 3054 (C-H aromatic str.), 1597, 1574 (C=C *aromatic str.*), 1077 (C-O str.). Lit. m.p. 123 ^{o}C at 0.01 mmHg (Baciocchi, Perucci, & Rol, 1975).

3.2.12 Synthesis of 4-(1-hydroxybut-3-enyl)phenol



Scheme 23. Synthesis of 4-(1-hydroxybut-3-enyl)phenol **120**

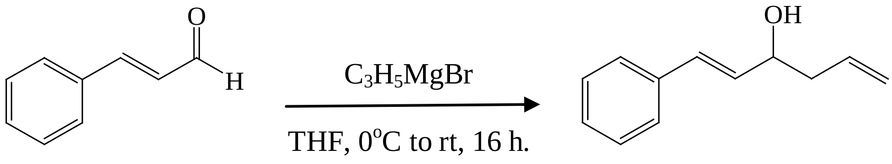
We performed protection method according to previous research (Yoo, Kim, Oelgemoller, & Shim, 2004). To a stirred solution of *p*-hydroxybenzaldehye **106** (5 g, 45.9433 mmol) in anhydrous tetrahydrofuran (100 ml) was added imidazole (3.31 g, 48.6202 mmol). After a clear solution was obtained, tert-butyldimethylsillyl chloride (7.33g, 48.6206 mmol) was added to give a cloudy white solution. The mixture was allowed to stired 30 hours at room temperature, then quenched with water (100 ml) and diluted with CH_2Cl_2 (50 ml). The organic layer was separated and washed with brine (100 ml). The organic layer was then dried and the solution was evaporated to give the crude product. Purification was carried out by column chromatography, eluting from 0 to 30 % ethyl acetate in hexane to yield 4-(*tert*-butyldimethylsilyl) benzaldehyde **106-TBDMS** 4.2054 g, 43.5 %yield as a brownish yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 0.19 (6H, s, 1'-2CH_3), 0.949 (9H, s, 2'-3CH_3), 6.95 (2H, d, *J* = 8.8 Hz, H-3,5), 7.79 (2H, d, *J* = 8.4 Hz, H-2,6), 9.88 (1H, s, CHO)

4-(*Tert*-butyldimethylsilyl)benzaldehyde **106-TBDMS** (1.5124 g, 6.3982 mmol) was added to a stirring solution of THF at room temperature before adding 1.7 M allylmagnesium bromide (C_3H_5MgBr) (4.5164 ml, 4.5164 mmol). The reaction was prepared according to General procedure A. Purification was carried out by column chromatography, eluting from 0 to 30 % ethyl acetate in hexane to yield 1-(4-(tert-butyldimethylsilyl)phenyl)but-3-en-1-ol **120-TBDMS** 1.2329 g, 86.4 %yield as a yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 0.12 (6H, s, 1'-2CH_3), 1.03 (9H, s, 2'-3CH_3), 2.17 (1H, br s, 1´-OH), 2.53 (1H, t, *J* = 7.6, H-2'), 4.72 (1H, t, *J* = 7.2 Hz, H-1'), 5.16 (2H, dd, *J* = 12, 6.9 H-4'), 5.82 (1H, m, H-3'), 6.78 (2H, d, *J* = 7.7 Hz, H-2,6), 7.13 (2H, d, *J* = 7.6 Hz, H-3,5)

According to previous research (Tewaria, Nizara, Manea, Georgea, & Prasad, 2006), A mixture of 1-(4-(tert-butyldimethylsilyl)phenyl)but-3-en-1-ol **120-TBDMS** (1.2329 g, 4.4276 mmol), acetylchloride (0.0521 ml, 0.6641 mmol) and methanol (10 ml) was allowed to stired 24 hours at room temperature. After completion of the reaction, the mixture was added to CH_2Cl_2 and water (20 ml each). An aqueous $NaHCO_3$ solution was added until the reaction attained pH 7.0. The organic layer was washed with water (25 ml) and evaporated to afford the crude product**.** Purification was carried out by column chromatography, eluting from 0 to 40 % ethyl acetate in hexane to 4-(1-hydroxybut-3-enyl)phenol **120** yield 0.4360 g, 60 %yield as a yellow oil, b.p. 299 ^oC: ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.06 (1H, br s, 1'-OH), 2.41 (2H, m, H-2'a), 2.57 (2H, m, H-2'b), 4.12 (1H, t, *J* = 7.4 Hz, H-1'), 5.02 (2H, dd, *J* = 16, 8.1 Hz, H-4'), 5.71 (1H, m, H-3'), 6.82 (2H, d, *J* = 8 Hz, H-3,5) 7.15 (1H, d, *J* =

7.2 Hz, H-2,6)**.** ^{13}C NMR ($CDCl_3$, 100 MHz), *δ* (ppm): 42.18 (C-2'), 83.39 (C-1'), 115.64 (C-3,5), 117.48 (C-4'), 128.48 (C-2,6), 134.48 (C-1), 134.84 (C-3'), 155.62 (C-4)**.**

3.2.13 Synthesis of (*E*)-1-phenylhexa-1,5-dien-3-ol

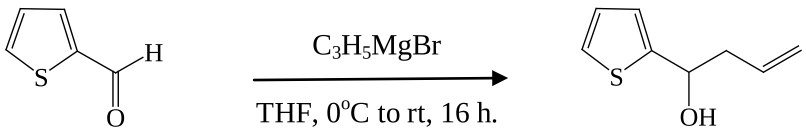


107 **121**

Scheme 24. Synthesis of (*E*)-1-phenylhexa-1,5-dien-3-ol **121**

Cinnamaldehyde **107** (1 g, 7.567 mmol) was added to a stirring solution of THF at room temperature before adding 1 M allylmagnesium bromide (C_3H_5MgBr) (9.0799 ml, 9.0799 mmol). The reaction was prepared according to General procedure A. Purification was carried out by column chromatography, eluting from 0 to 30 % ethyl acetate in hexane to yield (*E*)-1-phenylhexa-1,5-dien-3-ol **121** 0.1601 g, 12.1 %yield as a yellow color oil: ^{1}H NMR ($CDCl_3$, 400 MHz), *δ* (ppm): 2.02 (1H, br s, 3'-OH), 2.44 (2H, dd, *J* =10.4, 7.2 Hz, H-4'), 5.04 (1H, dt, *J* = 16.4, 10 Hz, H-3'), 5.44 (2H, dd, *J* = 13.2, 6.4 Hz, H-6'), 5.72 (1H, m, H-5'), 6.08 (1H, dd, *J* = 16, 8.8 Hz, H-2'), 6.56 (1H, d, *J* = 12.8 Hz, H-1'), 7.17 (1H, t, *J* = 14.4 Hz, H-4), 7.24 (2H, t, *J* = 14 H-3,5), 7.30 (2H, d, *J* = 7.2 Hz, H-2,6)**.** ^{13}C NMR ($CDCl_3$, 100 MHz), *δ* (ppm): 42.62 (C-4'), 72.86 (C-3'), 118.51 (C-6'), 124.79 (C-2'), 125.41 (C-2, 6), 127.82 (C-4), 128.07 (C-3, 5), 130.58 (C-1'), 131.42 (C-5'), 133.27 (C-1). IR (cm^{-1}): 3080 (C-H aromatic str.), 3028 (C=C alkene str.), 2919 (C-H str.), 1762 (C=O str.), 1111 (C-O str.). Lit m.p. 278 ^{o}C and EIMS *m/z* (%). 174 (m^{+} 25), 147 (18), 133 (45), 103 (100), 91 (10), 77 (58), 51 (35) (Kannekanti, Molli, & Batthula, 2011).

3.2.14 Synthesis of 1-(thiophen-2-yl)but-3-en-1-ol



108 **122**

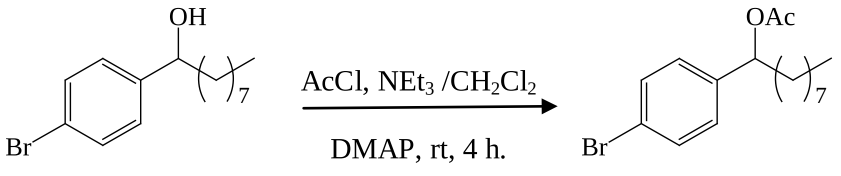
Scheme 25. Synthesis of 1-(thiophen-2-yl)but-3-en-1-ol **122**

Thiophene-2-carbaldehyde **108** (1 ml, 8.929 mmol) was added to a stirring solution of THF at room temperature before adding 1 M allylmagnesium bromide (C_3H_5MgBr) (10.714 ml, 10.714 mmol). The reaction was prepared according to General procedure A. Purification was carried out by column chromatography, eluting from 0 to 60 % ethyl acetate in hexane to yield 1-(thiophen-2-yl)but-3-en-1-ol **122** 0.8113 g, 59 %yield as a orangish yellow color oil: ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.1 (1H, br s, 1'-OH), 2.54 (2H, m, H-2'), 4.92 (1H, t, J = 12.8 Hz, H-1'), 5.08 (2H, dd, J = 16, 9.2 Hz, H-4'), 5.76 (1H, m, H-3'), 6.89 (2H, d, J = 8 Hz, H-4,5) 7.18 (1H, d, J = 6 Hz, H-3). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 43.57 (C-2'), 69.42 (C-1'), 119.55 (C-4'), 124.48 (C-4), 124.55 (C-5), 126.01 (C-3), 134.56 (C-3'), 138.33 (C-1). IR (cm^{-1}): 3385 (O-H str.), 3080 (=C-H str.), 1635 (C=C alkene str.) 1036 (C-O str.). MS: *m/z* 155 [M^{+1}]. Lit. b.p. 89-90.5 °C at 1 mmHg (Schuetz & Houff, 1955).

3.2 General procedure B to preparation of ACA analogs 123-132

ACA analogs **109–122** in CH_2Cl_2 was added NEt_3 (5.35 eq.), AcCl (5.35 eq.) and DMAP and then stir for 4 hours at room temperature. The reaction was then added water (10 ml), extracted with CH_2Cl_2 (3 × 20 ml), and the organic phases were combined, dried (Na_2SO_4) and evaporated to yield the crude product. Purification was carried out by column chromatography, eluting with ethyl acetate-hexane (yield 13 - 90%).

3.2.15 Synthesis of 1-(4-bromophenyl)nonyl acetate

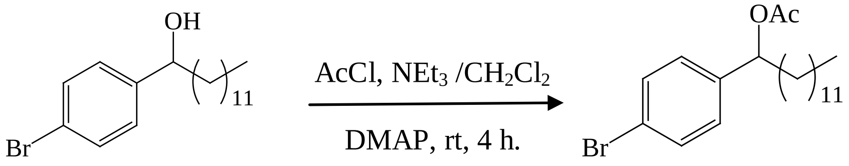


110 **123**

Scheme 26. Synthesis of 1-(4-bromophenyl)nonyl acetate **123**

1-(4-Bromophenyl)nonan-1-ol **110** (0.1002 g, 0.3342 mmol) in CH_2Cl_2 was added NEt_3 (0.1403 ml, 1.7880 mmol), AcCl (0.2384 ml, 1.7880 mmol) and DMAP. The reaction was prepared according to General procedure B. Purification was carried out by column chromatography, eluting from 0 to 30 % ethyl acetate in hexane to yield 1-(4-bromophenyl)nonyl acetate **123** 0.0358 g, 35.29 %yield as a pale yellow color oil: ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 0.82 (3H, t, J = 12 Hz, H-9'), 1.29 (10H, s, H-3'-7'), 1.68 (2H, s, H-8'), 1.94 (2H, m, H-2'), 2.19 (3H, t, J = 7 Hz, 1'-OAc), 5.73 (1H, t, J = 11.4 Hz, H-1'), 7.18 (2H, d, J = 8,2 Hz, H-2,6), 7.46 (2H, d, J = 8 Hz, H-3,5). IR (cm^{-1}): 2918, 2853 (C-H aliphatic str.), 1760 (C=O str.), 1235 (C-O str.).

3.2.16 Synthesis of 1-(4-bromophenyl)tridecyl acetate

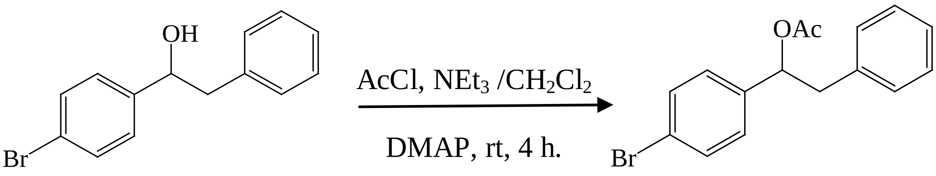


111 **124**

Scheme 27. Synthesis of 1-(4-bromophenyl)tridecyl acetate **124**

1-(4-Bromophenyl)tridecan-1-ol **111** (0.2282 g, 1.2221 mmol) in CH_2Cl_2 was added NEt_3 (0.6603 ml, 6.5382 mmol), AcCl (0.5132 ml, 6.5382 mmol) and DMAP. The reaction was prepared according to General procedure B. Purification was carried out by column chromatography, eluting from 0 to 20 % ethyl acetate in hexane to yield 1-(4-bromophenyl)tridecyl acetate **124** 0.2212 g, 48.8 %yield as a yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 0.88 (3H, t, *J* = 12.8 Hz, H-13'), 1.64 (20H, s, H-3'-12'), 1.86 (2H, m, H-2'), 2.26 (3H, s, 1'-OAc), 5.70 (1H, t, *J* = 14 Hz, H-1'), 7.22 (2H, d, *J* = 8 Hz, H-2,6), 7.47 (2H, d, *J* = 8.4 Hz, H-3,5). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 14.09 (C-13'), 23.35 (OCH_3), 25.65 (C-12'), 29.21 (C-3'), 29.35 (C-4'-9'), 31.89 (C-10'), 36.11 (C-11'), 50.26 (C-2'), 76.72 (C-1'), 121.91 (C-4), 128.46 (C-2, 6), 131.57 (C-3, 5), 139.24 (C-1), 139.95 (COO). IR (cm^{-1}): 2914, 2842 (C-H aliphatic str.), 1746 (C=O str.), 1234 (C-O str.).

3.2.17 Synthesis of 1-(4-bromophenyl)-2-phenylethyl acetate

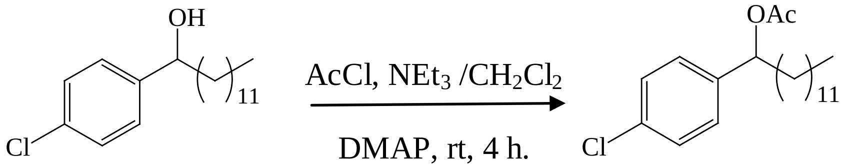


112 **125**

Scheme 28. Synthesis of 1-(4-bromophenyl)-2-phenylethyl acetate **125**

1-(4-Bromophenyl)-2-phenylethanol **112** (0.0898 g, 0.3240 mmol) in CH_2Cl_2 was added NEt_3 (0.1750 ml, 1.7334 mmol), AcCl (0.1360 ml, 1.7334 mmol) and DMAP. The reaction was prepared according to General procedure B. Purification was carried out by column chromatography, eluting from 0 to 25 % ethyl acetate in hexane to yield 1-(4-bromophenyl)-2-phenylethyl acetate **125** 0.0224 g, 23.2 %yield as a yellow oil, b.p. 108 ^{o}C: ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.12 (3H, s, 1'-OAc), 3.04 (1H, dd, *J* = 4.8, 6.4, H-2'a), 3.13 (1H, dd, *J* = 2.8, 7.6, H-2'b), 5.89 (1H, t, *J* = 8 Hz, H-1'), 7.06 (3H, d, *J* = 7.2 Hz, H-2,6,6'), 7.11 (2H, d, *J* = 8 Hz, H-4',8'), 7.23 (2H, dd, *J* = 4.2, 8.4 Hz, H-5',7'), 7.48 (2H, d, J = 8.4 Hz, H-3,5).

3.2.18 Synthesis of 1-(4-chlorophenyl)tridecyl acetate

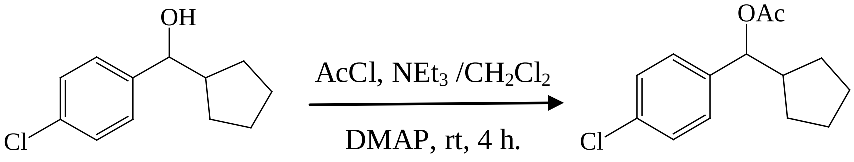


114 **126**

Scheme 29. Synthesis of 1-(4-chlorophenyl)tridecyl acetate **126**

1-(4-Chlorophenyl)tridecan-1-ol **114** (0.2539 g, 1.0448 mmol) in CH_2Cl_2 was added NEt_3 (0.5646 ml, 5.5897 mmol), AcCl (0.4388 ml, 5.5897 mmol) and DMAP. The reaction was prepared according to General procedure B. Purification was carried out by column chromatography, eluting from 0 to 20 % ethyl acetate in hexane to yield 1-(4-chlorophenyl)tridecyl acetate **126** 0.2098 g, 47.2 %yield as a pale yellow oil: ^1H NMR ($CDCl_3$, 400 MHz), δ (ppm): 0.88 (3H, t, J = 12 Hz, H-13'), 1.24 (16H, s, H-3'-12'), 1.73 (1H, m, H-2'a), 1.86 (1H, m, H-2'b), 2.26 (3H, t, J = 7 Hz, 1'-OAc), 4.56 (1H, t, J =12.8 Hz, H-1'), 7.25 (2H, d, J = 8.4 Hz, H-2,6), 7.30 (2H, d, J = 8 Hz, H-3,5). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 14.11 (C-13'), 18.20 (OCH_3), 21.16 (C-12'), 22.70 (C-3'), 25.43 (C-10'), 29.44 (C-4'-9'), 31.93 (C-11'), 36.24 (C-2'), 75.45 (C-1'), 127.95 (C-3,5), 128.63 (C-2,6), 133.57 (C-4), 143.93 (C-1), 165.57 (COO).

3.2.19 Synthesis of (4-chlorophenyl)(cyclopentyl)methyl acetate

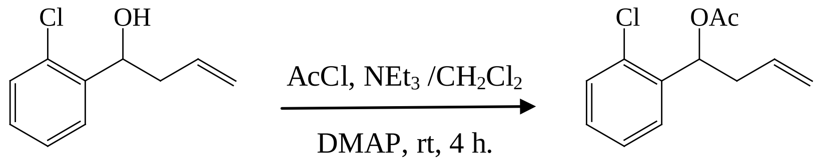


115 **127**

Scheme 30. Synthesis of (4-chlorophenyl)(cyclopentyl)methyl acetate **127**

(4-Chlorophenyl)(cyclopentyl)methanol **115** (0.5000 g, 2.3730 mmol*)* in CH_2Cl_2 was added NEt_3 (1.2823 ml, 12.6956 mmol*)*, AcCl (0.9966 ml, 12.6956 mmol*)* and DMAP. The reaction was prepared according to General procedure B. Purification was carried out by column chromatography, eluting from 0 to 40 % ethyl acetate in hexane to yield (4-chlorophenyl)(cyclopentyl)methyl acetate **127** 0.1974 g, 13.2 %yield as a yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 1.35 (2H, m, H-3'a,6'a), 1.45 (2H, m, H-4'a,5'a), 1.55 (2H, m, H-4'b,5'b), 1.61 (2H, m, H-3'b,6'b), 2.14 (3H, s, 1'-OAc) 2.16 (1H, sextet, *J* = 8.4 Hz, H-2'), 5.44 (1H, d, *J* = 8 Hz, H-1'), 7.20 (2H, d, *J* = 8.4 H-2,6), 7.24 (2H, d , *J* = 8.4 Hz, H-3,5). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 25.20 (OCH_3), 29.30 (C-4', 5'), 30.19 (C-3', 6'), 45.31 (C-2'), 80.61 (C-1'), 128.59 (C-3, 5), 128.71 (C-2, 6), 133.83 (C-4), 139.23 (C-1), 166.34 (COO).

3.2.20 Synthesis of 1-(2-chlorophenyl)but-3-enyl acetate

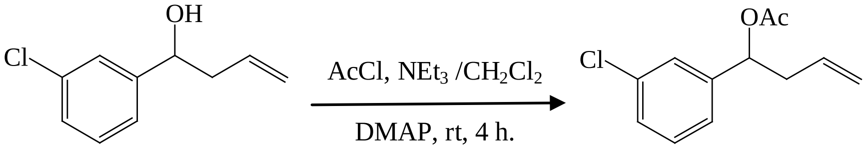


116 **128**

Scheme 31. Synthesis of 1-(2-chlorophenyl)but-3-enyl acetate **128**

1-(2-Chlorophenyl)but-3-en-1-ol **116** (0.15 g, 0.8212 mmol*)* in CH_2Cl_2 was added NEt_3 (0.3860 ml, 4.3936 mmol*)*, AcCl (0.3449 ml, 4.3936 mmol*)* and DMAP. The reaction was prepared according to General procedure B. Purification was carried out by column chromatography, eluting from 0 to 25 % ethyl acetate in hexane to yield 1-(2-chlorophenyl)but-3-enyl acetate **128** 0.1009 g, 54.5 %yield as a dark yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.19 (3H, t, *J* = 10 Hz, 1´-OAc), 2.55 (2H, t, *J* = 12 Hz, H-2'), 5.15 (1H, t, *J* = 8, Hz, H-4'a), 5.17 (1H, t, *J* = 8 Hz, H-4'b) 5.88 (1H, m, H-3'), 6.22 (1H, t, *J* = 12 Hz, H-1'), 7.21 (1H, dd, *J* = 16, 8 Hz, H-5), 7.37 (1H, d, *J* = 8 Hz, H-6), 7.38 (1H, t, *J* = 8 Hz, H-4). 7.58 (1H, d, *J* = 8 Hz, H-3).^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 20.07 (OCH_3), 39.51 (C-2'), 72.49 (C-1'), 109.59 (C-4'), 118.32 (C-5), 125.44 (C-6), 126.90 (C-4), 126.90 (C-3), 129.89 (C-2), 130.03 (C-3'), 132.72 (C-1), 168.00 (COO). IR (cm^{-1}): 3076 (C-H aromatic str.), 2971, 2919 (C-H str.), 1762 (C=O str.), 1435 (C=C alkene str.), 1110 (C-O str.).

3.2.21 Synthesis of 1-(3-chlorophenyl)but-3-enyl acetate

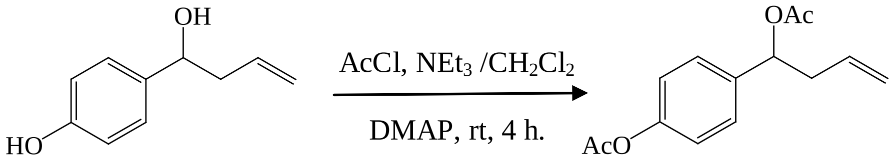


118 **129**

Scheme 32. Synthesis of 1-(3-chlorophenyl)but-3-enyl acetate **129**

1-(3-Chlorophenyl)but-3-en-1-ol **118** (0.2 g, 1.0950 mmol*)* in CH_2Cl_2 was added NEt_3 (0.5917 ml, 5.8583 mmol*)*, AcCl (0.4599 ml, 5.8583 mmol*)* and DMAP. The reaction was prepared according to General procedure B. Purification was carried out by column chromatography, eluting from 0 to 30 % ethyl acetate in hexane to yield 1-(3-chlorophenyl)but-3-enyl acetate **129** 0.0386 g, 31.1 %yield as a yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 1.77 (3H, s, 1´-OAc), 2.47 (1H, dt, *J* = 13.6, 7.2 Hz, H-2'a), 2.49 (1H, dt, *J* = 14, 6.8 Hz, H-2'b) 5.01 (2H, dd, *J* = 11.2, 4.4 Hz, H-4'), 5.56 (1H, t, *J* = 7.6, Hz, H-1'), 5.73 (1H, sextet, *J* = 9.7 Hz, H-3') 7.15 (1H, d, *J* = 6.4 Hz, H-6), 7.18 (1H, dd, *J* = 13.1, 7.2 Hz, H-5), 7.25 (2H, d, *J* = 8 Hz, H-2,4). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 21.24 (OCH_3), 40.53 (C-2'), 75.60 (C-1'), 109.69(C-4'), 118.79 (C-6), 118.93 (C-2), 123.99 (C-4), 125.26 (C-5), 128.07 (C-3'), 128.13 (C-3), 141.80 (C-1), 166.19 (COO). IR (cm^{-1}): 3078 (C-H aromatic str.), 2935 (C-H str.), 1744 (C=O str.), 1434 (C=C alkene str.), 1176 (C-O str.).

3.2.22 Synthesis of 1-(4-acetoxyphenyl)but-3-enyl acetate

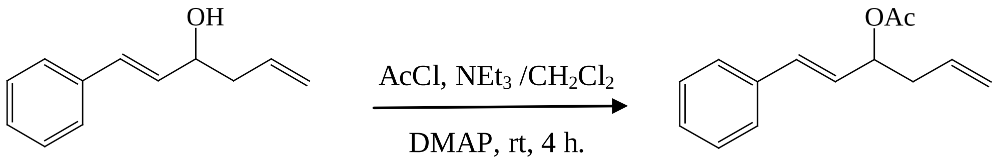


120 **130**

Scheme 33. Synthesis of 1-(4-acetoxyphenyl)but-3-enyl acetate **130**

4-(1-Hydroxybut-3-enyl)phenol **120** (0.1736 g, 1.0572 mmol) in CH_2Cl_2 added NEt_3 (0.5713 ml, 5.6562 mmol), AcCl (0.444 ml, 5.6562 mmol) and DMAP. The reaction was prepared according to General procedure B. Purification was carried out by column chromatography, eluting from 0 to 30 % ethyl acetate in hexane to yield 1-(4-acetoxyphenyl)but-3-enyl acetate **130** 0.1961 g, 89.9 %yield as a colorless oil: ^1H NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.38 (3H, s, 1´-OAc), 2.52 (1H, m, H-2'a), 2.55 (1H, m, H-2'b), 3.22 (3H, s, 4-OAc), 4.17 (1H, t, J = 7.3, Hz, H-1'), 5.03 (1H, dd, J = 12.2, 6.3 Hz, H-4'a), 5.06 (1H, dd, J = 13, 6.4 Hz, H-4'b), 5.75 (1H, m, J = 8 Hz, H-3'), 7.08 (2H, d, J = 7.2 Hz, H-3,5), 7.28 (2H, d, J = 8 Hz, H-2,6). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 21.12 (OCH_3-4), 42.50 (OCH_3-1'), 56.72 (C-2'), 83.11 (C-1'), 117.01 (C-4'), 121.45 (C-3, 5), 127.39 (C-2, 6), 134.72 (C-3'), 139.25 (C-1), 150.06 (C-4), 169.40 (2 × COO). Matsuda and co-workers reported EI-MS: *m/z* (%): 248 (M^+, 1), 146 (100) (Matsuda, Ando, Morikawa, Kataoka, & Yoshikawa, 2005).

3.2.23 Synthesis of (*E*)-1-phenylhexa-1,5-dien-3-yl acetate

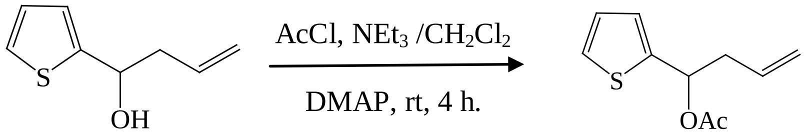


121 **131**

Scheme 34. Synthesis of (*E*)-1-phenylhexa-1,5-dien-3-yl acetate

(*E*)-1-phenylhexa-1,5-dien-3-ol **121** (0.3080 g, 1.7677 mmol) in CH_2Cl_2 was added NEt_3 (0.9552 ml, 9.4572 mmol), AcCl (0.7424 ml, 9.4572 mmol) and DMAP. The reaction was prepared according to General procedure B. Purification was carried out by column chromatography, eluting from 0 to 30 % ethyl acetate in hexane to yield (*E*)-1-phenylhexa-1,5-dien-3-yl acetate **131** 0.1322 g, 26.3 %yield as a yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.10 (3H, s, 3´-OAc), 2.44 (2H, m, H-4'), 5.03 (1H, dd, *J* = 14.8, 7 Hz, H-6'a), 5.07 (1H, dd, *J* = 15, 8.2 Hz, H-6'b), 5.65 (1H, m, H-3'), 5.72 (1H, m, H-5'), 6.08 (1H, dd, *J* = 16, 8 Hz, H-2'), 6.54 (1H, d, *J* = 12.8 Hz, H-1'), 7.17 (1H, dd, *J* = 14.2, 6 Hz, H-4), 7.23 (2H, t, *J* = 13 Hz, H-3,5), 7.30 (1H, d, *J* = 7.2 Hz, H-2,6). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 21.24 (OCH_3), 37.18 (C-4'), 73.78 (C-3'), 110.15 (C-6'), 118.17 (C-2'), 127.15 (C-2, 6), 127.98 (C-4), 128.94 (C-3, 5), 128.58 (C-1'), 132.70 (C-5'), 133.14 (C-1), 165.20 (COO). IR (cm^{-1}): 3080 (C-H aromatic str.), 3028.27 (C=C alkene str.), 2919 (C-H str.), 1762 (C=O str.), 1111 (C-O str.). MS: *m/z* 216 [M $^{+\ 1}$]; Anal. Calcd. For $C_{14}H_{16}O_2$: C 77.75, H 7.45; found C 77.25, H 7.38 (Chandrasekhar, Mohanty, & Raza, 1999).

3.2.24 Synthesis of 1-(thiophen-2-yl)but-3-enyl acetate



122 **132**

Scheme 35. Synthesis of 1-(thiophen-2-yl)but-3-enyl acetate **132**

1-(Thiophen-2-yl)but-3-en-1-ol ol **122** (0.1000 g, 0.6487 mmol) in CH_2Cl_2 was added NEt_3 (0.2949 ml, 5.8583 mmol), AcCl (0.3503 ml, 3.4689 mmol) and DMAP. The reaction was prepared according to General procedure B. Purification was carried out by column chromatography, eluting from 0 to 40 % ethyl acetate in hexane to yield 1-(thiophen-2-yl)but-3-enyl acetate **132** 0.0640 g, 50.1 %yield as a yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.09 (3H, s, 1´-OAc), 2.49 (2H, 2.77, H-2'), 5.08 (1H, dd, J = 16, 8 Hz, H-4'a), 5.14 (1H, dd, J = 16, 8 Hz, H-4'b), 5.69 (1H, m, H-3'), 6.98 (1H, t, J = 17 Hz, H-1'), 6.97 (1H, d, J = 4 Hz, H-5), 7.06 (1H, d, J = 4 Hz, H-4), 7.29 (1H, d, J = 4.1 Hz, H-3). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 18.29 (OCH_3), 40.71 (C-2'), 70.48 (C-1'), 118.46 (C-4'), 125.65 (C-3), 125.87 (C-5), 125.95 (C-4), 132.90 (C-3'), 142.92 (C-1), 170.11 (COO). IR (cm-1): 3080 (C-H aromatic str.), 2935 (C-H str.), 1738 (C=O str.), 1436 (C=C alkene str.), 1219 (C-O str.).

3.3 Assay of Anti-*Mycobacterium tuberculosis* H37Ra

All synthesized compounds were tested for their activity against *M. tuberculosis* H37Ra at National Science and Technology Development Agency (NSTDA), using Green fluorescent Protein (GFP)-based fluorescent detection method. The maximum concentration of test sample was 50 μg/ml. Rifampicin, streptomycin, isoniazid and ofloxacin were used as positive controls and 0.5% DMSO was used as negative control. Green fluorescent protein (GFP) expressing *Mycobacterium tuberculosis* strain H37Ra was established by Changsen and co-workers (2003). H37Ra *gfp* is cultivated on 7H10 agar containing 30 ug/ml kanamycin at 37 ^{o}C for 4 weeks or until growth is observed. Starter cultures are prepared by fully looping 2-3 single colony into 7H9 broth supplemented with 0.2% v/v glycerol, 0.1 % w/v of casitone, 0.05% v/v Tween 80, 10% v/v Middlebrook OADC enrichment solution (BD Biosciences) and 30 µg/ml of kanamycin. The mixture was then incubated at 37^{o}C in 200 rpm shaker incubator until the optical density (OD) at 550 nm is between 0.5 and 1. For batch cultivation, the starter cultures were transferred at the rate of 1/10 volume to the 7H9 broth and incubated at 37 ^{o}C in 200 rpm shaker incubator until the OD550 nm is approximately 0.5 to 1. The cells were pelleted, washed and suspended in PBS buffer, and then sonicated 8 times for 15 seconds each. The sonicated samples were then aliquoted and frozen at -80°C for up to 2 to 3 months prior to use. Titer stocks were determined by colony forming unit (cfu) assay and the seeding density for anti-TB assay was optimized by serial dilutions. The dilution that grows at logarithmic phase on day 7 will be used as an optimal bacterial seeding density. For assay in 384-well format, the seeding is approximately 2×10^4 to 1×10^5 cfu/ml/well. The assay was performed in duplicate; each well containing 5 µl of test samples serially diluted in 5% dimethyl sulfoxide, followed by 45 µl of cell suspension prepared as described above. Plates were incubated at 37 °C for 7 days and the fluorescence signals are measured using SpectraMax M5 microplate reader (Molecular Devices, USA) in the bottom-reading mode at the excitation and emission wavelengths of 485 nm and 535 nm. Fluorescence signals on day zero are used as background, which is used to subtract the signals on day 7. The Percentage of growth inhibition is calculated from the mean of fluorescence unit of cells treated with sample (FUT) and untreated cells (FUC), as the following equation:

$$\% \text{ Inhibition} = [1- (\text{FUT} / \text{FUC})] \times 100$$

The lowest drug concentration that inhibits cell growth by 90% was reported as the Minimum Inhibitory Concentration (MIC). Rifampicin, streptomycin, isoniazid and ofloxacin were used as positive controls, and 0.5% DMSO was used as a negative control (Changsen, Franzblau, & Palittapongarnpim, 2003).

3.4 Assay of Cytotoxicity

Some ACA analaogs were tested for their cytotoxicity at National Science and Technology Development Agency (NSTDA), using primate cell line (*Vero*) assay. Green fluorescent protein (GFP) detection method was used at maximum test concentration of 50 μg/ml. The assay is carried out by adding 45 μl of cell suspension at 3.3×10^4 cells/ml to each well of 384-well plates containing 5 μl of test compounds previously diluted in 0.5% DMSO, and then incubating for 4 days in 37 ^{o}C incubator with 5% CO_2. Fluorescence signals were measured by using SpectraMax M5 microplate reader (Molescular Devices, USA) in the bottom reading mode with excitation and emission wavelengths of 485 and 535 nm. Fluorescence signal at day 4 was subtracted with background fluorescence at day 0. The percentage of cytotoxicity is calculated by the following equation, where FU_T and FU_c represent the fluorescence units of cells treated with test compound and untreated cells, respectively.

$$\% \text{ cytotoxicity} = [1-(FU_T/FU_C)] \times 100$$

IC_{50} values were derived from dose response curves, using 6 concentrations of 2-fold serially diluted samples, by the SOFTMax Pro software (Molecular device). Ellipticine and 0.5 %DMSO were used as a positive and a negative control, respectively (Hunt, Jordan, De Jesus, & Wurm, 1999).