SUPPLEMENTARY MATERIAL

Enzyme mediated-transesterification of verbascoside and evaluation of antifungal activity of synthesized analogues

Abstract: Enzymatic acylation of verbascoside, a polyhydroxylated natural product was reported in this study using five different commercial lipases and taking p-nitrophenyl alkanoates as acyl donors. Out of these enzymes, the immobilized *Candida antartica* lipase B was found as the enzyme of choice. Mono- and di-acylated products were formed, with mono as major product indicating high regio-selective nature of such transformations. A series of acyl esters of verbascoside have been synthesized by this enzymatic transesterification methodology. The lipophilicity of the synthesized analogues was also checked. The analogues were further subjected to synergistic antifungal activity with Amphotericin B (AmB) against *Candida albicians.* Four-fold reduction in minimum inhibitory concentration (MIC) of AmB was observed with few synthesized analogues such as verbascoside 4″-octanoate (**3b**), verbascoside 4″-palmitate (**3d**) and verbascoside 4″, 4′-dipalmitate (**4d**) at a concentration of 0.5 μg/ml.

Abbreviations:

DMAP: Dimethyl amino pyridine

DCC: Dicyclocarbutammide

DCM: Dichloromethane

MIC: Minimum Inhibitory concentration

Table S1

Table S1: C logP values of verbascoside and its acylated analogues

Compound	ClogP
verbascoside	-0.69
per acetylated verbascoside (**2a**)	6.24
per butyrated verbascoside (**2b**)	8.32
verbascoside 4'-butyrate (**3a**)	0.24
verbascoside 4''-octanoate (**4a**)	0.97
verbascoside 4''-4'-butyrate(**3b**)	1.41
verbascoside 4'',4'-dioctanaote (**3b)**	1.81
verbascoside 4''-myristylate (**3c**)	2.13
verbascoside 4''-palmitate (**4c**)	2.89
verbascoside 4'',4'-dimyristylate (**3d**)	2.42
verbascoside 4'',4'-dipalmitate (**4d**)	3.02

Compound Characterization S1

Per acetylated verbascoside **(2a)**

(Yield 41%); Colourless solid; mp: 158 ^{o}C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.71 (d, 1H, *J* = 16 Hz), 7.35 (d, 2H, *J* = 8.5 Hz), 7.25 (d, 2H, *J* = 7.8 Hz), 7.00 (d, 3H, *J* = 10 Hz), 6.43 (d, 1H, *J* = 15.98 Hz), 5.15 (m, 6H), 4.45 (d, 1H, *J* = 8.01 Hz), 4.14 (s, 3H), 3.92 (m, 2H), 3.73 (d, 2H, *J* = 9.23 Hz), 2.95 (m, 2H), 2.25 (t, 12H, *J* = 6.46 Hz), 2.10 (d, 7H, *J* = 2.77 Hz), 2.00 (d, 3H, *J* = 6.76 Hz), 1.86 (d, 6H, *J* = 5.5 Hz), 1.00 (d, 3H, *J* = 6.13 Hz); Maldi mass: 1025 (M + Na+).

Per butyrated verbascoside **(2b)**

(Yield 41%); Colourless solid; mp: 145 ^{o}C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.40 (d, 1H, *J* = 16 Hz), 7.00 (s, 1H), 7.15 (s, 2H), 7.25 (d, 1H, *J* = 10 Hz), 7.34 (s, 1H), 7.46 (d, 2H, *J* = 8.5 Hz), 7.70 (d, 1H, *J* = 13.5 Hz), 5.33 (t, 1H, *J* = 3.5 Hz), 5.36 (m, 3H), 5.00 (t, 1H), 4.95 (s, 1H), 4.57 (d, 1H, *J* = 8.0 Hz), 4.48 (d, 3H, *J* = 5.5 Hz), 4.10 (d, 1H, *J* = 5.6 Hz), 4.00 (t, 1H, *J* = 4.5 Hz), 3.92 (m, 1H), 3.70 (d, 2H, *J* = 8.5 Hz), 2.90 (t, 2H, *J* = 6.5 Hz), 2.65 (m, 6H), 2.42 (d, 6H, *J* = 6.5 Hz), 2.32 (m, 4H), 2.20 (m, 3H), 1.85 (m, 10H), 1.70 (d, 8H, *J* = 3.5 Hz), 1.61 (m, 5H), 1.30 (m, 2H), 0.95 (m, 19H); Maldi mass: 1224 (M+ Na+).

Compound Characterization S2

3.4.3. Verbascoside 4'-butyrate (**3a**)

Colourless solid; mp: (158 ^{o}C); $[\alpha]^{D}$ 25 -90.0 (c = 1.0, CH_3OH); IR (KBr, cm-1): 511, 561, 807, 857, 1036, 1090, 1114, 1157, 1260, 1375, 1446, 1602, 3380; ^{1}H NMR (500 MHz, DMSO): δ 7.50 (d, *J* = 15.8 Hz, 1H), 7.26 (d, *J* = 8.51 Hz, 1H), 7.19 (s, 1H), 7.0 (d, *J* = 8.19 Hz, 1H), 6.82 (d, *J* = 8.07 Hz, 1H), 6.58 (d, *J* = 7.8 Hz, 1H), 6.47 (d, *J* = 8.07 Hz, 1H) , 6.25 (d, *J* = 15.7 Hz, 1H), 5.09 (s, 1H), 4.75 (s, 1H) ,4.28 (d, *J* = 8.1 Hz, 1H), 3.97 (m ,1H), 3.81 (s, 1H), 3.71 (t, *J* = 5.54 Hz, 1H), 3.63 (dd, $J_{1,2}$ = 8.1, $J_{2,3}$= 4.5 Hz, 1H) , 3.51 (t, *J* = 10.2 Hz, 1H), 3.49 3.35 (m, 2H), 3.28 (t, *J* = 5.72 Hz, 2H), 3.20 (submerged with solvent peak, 14H), 2.8 (t, *J* = 6.32 Hz, 1H), 2.5 (q, *J* = 7.3 Hz, 1H), 2.3 (t, *J* = 2.88 Hz, 1H), 2.0 (d, *J* = 7.32 Hz, 1H), 1.7 (dd, *J* = 7.34, 3.45 Hz, 1H), 1.2 (s, 3H), 1.0 (m, 2H), 0.8 (m, 2H); ^{13}C (500 MHz, CD_3OD): 14, 19, 19.5, 30, 35, 49, 54, 61, 70, 72, 74, 75, 78, 81, 102, 104, 114, 115,117, 118, 121, 122, 124, 132, 145, 146, 165, 172; Maldi mass: (M + Na+) 717; Anal. Calcd for $C_{32}H_{42}O_{16}$: C, 57.06; H, 6.09. Found: C, 57.13; H, 6.03.

3.4.4. Verbascoside 4", 4'-dibutyrate (**4a)**

As colourless solid; mp: 134 ^{o}C; $[\alpha]^{D}_{25}$ -42.0; IR (KBr, cm^{-1}): 521, 533, 758, 812, 1035, 1260, 1382, 1448, 1464, 1518, 1606, 1704, 3377; ^{1}H NMR (500 MHz, DMSO): δ 7.56 (d, *J* = 7.33 Hz, 1H), 6.82 (d, *J* = 8.4 Hz,1H), 6.65 (d, *J* = 7.9 Hz, 1H), 6.25 (d, *J* = 15.7 Hz, 1H), 5.09 (s, 1H), 4.75 (d, *J* = 9.9 Hz, 1H), 4.28 (d, *J* = 7.8 Hz, 1H), 4.01 (d, *J* = 7.4 Hz, 1H), 3.81 (s, 1H), 3.72 (m, *J* = 9.4 Hz, 1H), 3.52 (t, *J* = 6.03 Hz, 1H), 3.45 (t, *J* = 5.58 Hz, 1H), 3.41 (s, 3H), 3.31 (d, *J* = 8.4 Hz, 2H), 3.21 (m, *J* = 8.41 Hz, 7H), 2.47 (q, *J* = 3.07 Hz, 3H), 1.66 (q, *J* = 3.07 Hz, 3H), 1.19 (s, 3H), 0.97 (m, 3H); ^{13}C (500 MHz, DMSO): δ 14, 19, 19.5, 58, 62, 70, 71, 75, 76, 77, 78, 79, 82, 103, 112, 113, 115, 120, 121, 125, 133, 138, 140, 157, 168, 172 175; Maldi mass: (M + Na) 789; Anal. Calcd for $C_{37}H_{48}O_{7}$: C, 58.11; H, 6.33. Found: C, 58.08; H, 6.34.

3.4.5. Verbascoside 4"-octanoate **(3b)**

Colourless white solid; mp: 162 ^{o}C ; $[\alpha]^{D}_{25}$ -35.0 (c = 1.0, $CHCl_3$); IR (KBr, cm^{-1}): 476, 560, 854, 960, 1088, 1114, 1150, 1270, 1300, 1375, 1434, 1517, 1607, 1662, 1703, 3384; ^{1}H NMR (500 MHz, DMSO): δ 7.55 (d, *J* = 15.85 Hz, 1H), 7.47 (t, *J* = 9.24 Hz, 2H), 7.01 (d, *J* = 8.26 Hz, 1H), 6.75 (t, *J* = 7.64 Hz, 1H), 6.45 (d, *J* = 7.95 Hz, 2H), 6.39 (d, *J* = 15.84 Hz, 1H), 5.02 (s, 1H), 4.73 (s, 1H), 4.49 (t, *J* = 7.61 Hz, 1H), 3.9 (dd, *J* = 6.96, 3.23 Hz, 1H), 3.75 (m, 1H), 3.70 (dd, *J* = 15.8, 6.78 Hz, 2H), 3.31 (d, *J* = 3.62 Hz, 2H), 3.2 (dd, *J* = 8.05, 4.50 Hz,1H), 3.19 (dd, 8.4 Hz, 1H), 3.1 (dd, *J* = 9.25, 4.56 Hz, 5H), 2.70 (t, *J* = 7.26 Hz, 2H), 1.70 (t, *J* = 6.48 Hz, 2H), 1.43 (submerged with solvent peak, 7H), 0.97 (q, 6.45 Hz, 3H); ^{13}C (500 MHz, DMSO): δ 17.5, 19.5, 23, 25, 29, 29.5, 30, 32, 37, 39, 40, 61, 69, 71, 73, 75, 80, 101, 103, 115, 116, 117, 120, 124, 129, 139, 143, 145, 165, 171; Maldi Mass: (M + Na) 773; Anal. Calcd for $C_{37}H_{50}O_{16}$: C, 59.19; H, 6.71. Found: C, 59.13; H, 6.65.

3.4.6. Verbascoside *4", 4'dioctanoate* **(4b)**

As colourless solid ; mp: 176 ^{o}C; $[\alpha]^{D}_{25}$ -80.0 (1.0, $CHCl_3$); IR (KBr, cm $^{-1}$): 511, 545, 750, 807 , 857, 1036, 1078, 1125, 1145, 1200, 1350, 1145, 1530, 1656, 1710, 3380; ^{1}H NMR (500 MHz, DMSO): 7.57 (d, *J* = 15.83 Hz, 1H), 7.40 (t, *J* = 8.5 Hz, 2H) , 6.96 (d, *J* = 9.6 Hz, 1H), 6.8 (d, *J* = 7.06 Hz, 1H), 6.71 (d, *J* = 7.95 Hz, 2H), 6.39 (d, *J* = 15.84 Hz, 1H), 5.02 (s, 1H), 4.73 (t, *J* = 9.5 Hz, 1H), 4.36 (t, *J* = 7.61 Hz, 1H), 3.9 (m, *J* = 8.75 Hz, 1H), 3.66 (m, *J* = 10.85 Hz, 3H), 3.33

(dd, *J* = 6.2, 2.3 Hz, 2H), 3.29 (dd, *J* = 7.95 Hz, 1H), 3.2 (dd, *J* = 6.17 Hz, 1H), 3.11 (dd, *J* = 3.30, 1.24 Hz, 1H), 2.73 (dd, *J* = 7.26, 5.61 Hz, 3H), 2.79 (t, *J* = 6.45 Hz, 3H), 2.55 (t, *J* = 7.07 Hz, 1H), 2.51 (d, *J* =1.32 Hz, 9H), 1.62 (t, *J* = 7.06 Hz, 6H), 1.36 (s, 3H), 1.24 (submerged with solvent peak), 0.96 (q, *J* = 6.45 Hz, 3H), 0.86 (t, *J* = 6.48 Hz, 3H); ^{13}C (500 MHz, DMSO): *δ* 14, 19, 23, 25, 29, 32, 29, 30, 31, 40, 63, 70, 71, 72, 73, 75, 80, 103, 104, 117, 120, 124, 125, 128, 137, 138, 139, 145, 148, 149, 165, 170, 173; Maldi mass: (M + Na) 899; Anal. Calcd for $C_{45}H_{64}O_{17}$: C, 61.62; H, 7.36. Found: C, 61.60; H, 7.30.

3.4.7. Verbascoside 4"-myristylate **(3c)**

Colourless solid; mp: 151^{o}C; $[\alpha]^{D}_{25}$ -25.0 (c 1.0, $CHCl_3$); IR (KBr, cm^{-1}): 510, 521, 554, 786, 806, 845, 1042, 1065, 1095, 1116, 1145, 1250, 1278, 1354, 1670, 1710, 3345; ^{1}H NMR (500 MHz, DMSO): *δ* 7.65 (d, *J* = 15.83 Hz, 1H), 7.52 (t, *J* = 8.45 Hz, 2H), 7.01 (d, *J* = 8.2 Hz, 1H), 6.64 (d, *J* = 7.65 Hz, 2H), 6.50 (d, *J* = 7.95 Hz, 1H), 6.39 (d, *J* = 15.84 Hz, 1H), 5.02 (s, 1H), 4.73 (t, *J* = 9.5 Hz, 1H), 4.36 (t, *J* = 7.61 Hz, 1H), 3.9 (dd, *J* = 6.96 Hz, 1H), 3.31 (d, *J* = 3.6 Hz, 2H), 3.29 (m, 3H), 3.19 (dd, *J* = 8.4, 5.4 Hz, 2H), 3.1 (dd, *J* = 9.25, 3.45 Hz, 2H), 2.73 (m, 3H), 2.55 (t, *J* = 7.07 Hz, 3H), 2.51 (d, *J* = 1.32 Hz, 3H), 1.62 (t, *J* = 7.06 Hz, 2H), 1.24 (submerged with solvent Peak, 17H), 0.96 (q, *J* = 6.45 Hz, 3H), 0.86 (t, *J* = 6.48 Hz, 3H); ^{13}C (500 MHz, DMSO): *δ* 17.5, 19,.23, 25, 29.5, 30, 32.5, 34, 36, 37, 40, 61, 69, 71, 72, 75, 80, 102, 103, 116, 117, 120, 144, 146, 163, 165, 172; Maldi mass: 858 (M + Na^{+}); Anal. Calcd for $C_{43}H_{62}O_{16}$: C, 61.86; H, 7.48. Found: C, 61.83; H, 7.43.

*3.4.8. Verbascoside 4", 4'-dimyriatylate (***4c**)

As colourless solid; mp: 143^{o}C; [α]$^{D}_{25}$ -65.0 (c 1.0, $CHCl_3$); IR : (KBr, cm^{-1}): 501, 630, 650, 757, 820, 1039, 1419, 1642, 1719, 2871, 2936, 3399; ^{1}H NMR (500 MHz, DMSO): *δ* 7.53 (d, *J* = 15.08 Hz, 1H), 7.28 (t, *J* = 8.5 Hz, 1H), 7.20 (s, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 6.79 (d, *J* = 7.95 Hz, 1H), 6.75 (d, *J* = 7.8 Hz, 1H), 6.65 (d, *J* = 8.17, 1H), 6.25 (d, *J* = 15.84 Hz, 1H), 5.02 (s, 1H), 4.73 (s, 1H), 4.28 (t, *J* = 7.61 Hz, 1H), 3.9 (m, 1H), 3.82 (s, 1H), 3.72 (m, 1H), 3.54 (d, *J* = 4.92 Hz, 1H), 3.43 (d, *J* = 2.84 Hz, 4H), 3.21 (m, 6H), 2.78 (d, *J* = 8.56 Hz, 1H), 2.5 (m, 3H), 1.62 (t, *J* = 7.06 Hz, 3H), 1.34 (s, 3H), 1.19 (submerged with solvent peak), 0.96 (d, *J* = 6.45 Hz, 14H), 0.86 (t, *J* = 6.48 Hz, 8H); ^{13}C (500 MHz, DMSO): *δ* 14, 19, 23, 30, 31, 35, 37, 46, 58, 62, 70, 71, 74, 75, 76, 79, 80 , 82, 103, 112, 115, 117, 118, 120, 122, 130, 134, 146, 147, 148, 153, 162, 168,

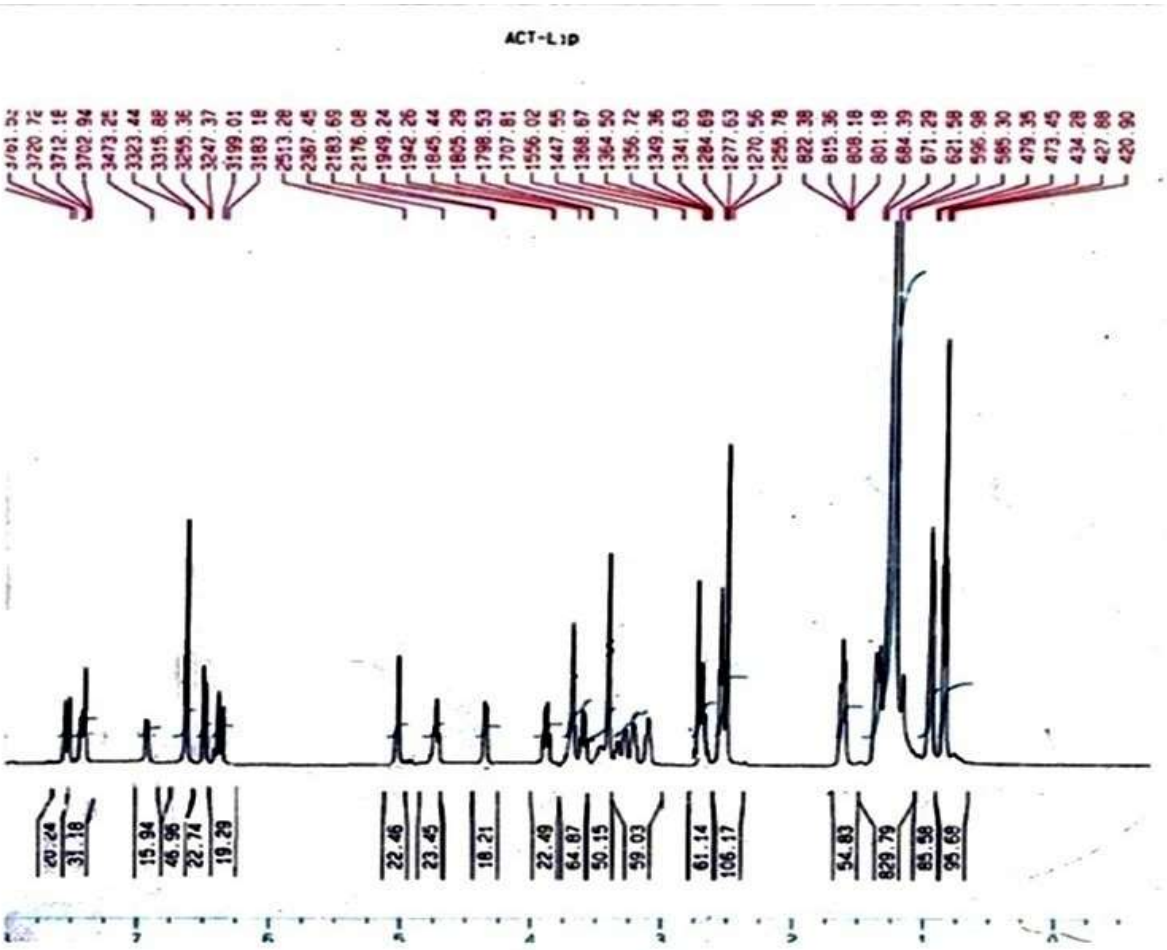
171, 173; Maldi mass: (M + Na) 1045; Anal. Calcd for $C_{58}H_{90}O_{17}$: C, 65.76; H, 8.56. Found: C, 65.73; H, 8.53.

3.4.9. Verbascoside 4''-palmitate **(3d)**

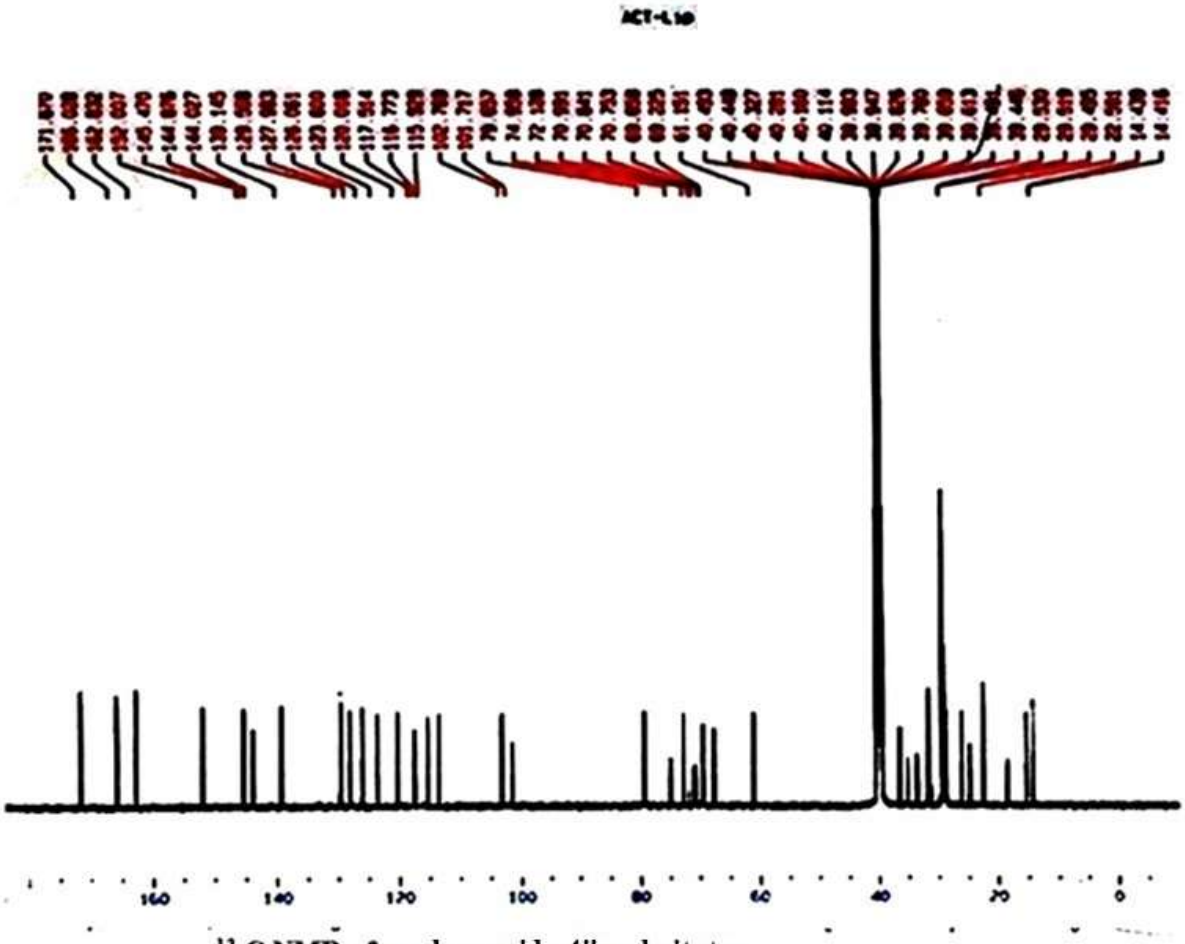
As colourless solid; mp: 183^{o} C; $[\alpha]^{D}_{25}$ -33.3 (c 1.0, $CHCl_3$); IR (KBr, cm^{-1}): 501, 523, 545, 653, 780, 790, 830, 1043, 1097, 1132, 1178, 1267, 1368, 1724, 3330; ^{1}H NMR (500 MHz, DMSO): δ 7.65 (d, *J* = 15.83 Hz, 1H), 7.47 (t, *J* = 9.24 Hz, 2H), 7.01 (d, *J* = 8.26 Hz, 1H), 6.64 (t, *J* = 7.56 Hz, 2H), 6.48 (d, *J* = 7.8 Hz, 1H), 6.25 (d, *J* = 15.79 Hz, 1H), 5.02 (s, 1H), 4.73 (t, *J* = 9.5 Hz, 1H), 4.49 (t, *J* = 7.51 Hz, 1H), 3.9 (dd, *J* = 6.96 Hz, 1H), 3.75-3.70 (m, 3H), 3.31 (d, *J* = 3.62 Hz, 2H), 3.29-3.19 (m, 5H), 2.8 (t, *J* = 7.47 Hz, 3H), 1.70 (t, *J* = 6.48 Hz, 2H), 1.43 (submerged with solvent peak, 27H), 1.03 (d, 6.45 Hz, 3H), 0.92 (t, *J* = 6.19 Hz, 3H); ^{13}C (500 MHz, DMSO): δ 15, 19, 25, 28, 32, 35, 38, 39, 50, 64, 71, 72, 73, 75, 76, 82, 104, 105, 116, 117, 119, 120, 122, 124, 125, 129, 130, 134, 142, 146, 147, 149, 165, 172; Maldi mass: (M + Na^{+}) 872; Calcd for $C_{44}H_{64}O_{16}$: C, 62.25; H, 7.60. Found: C, 62.21; H, 7.54.

3.4.10. Verbascoside 4'', 4'-dipalmitate **(4d)**

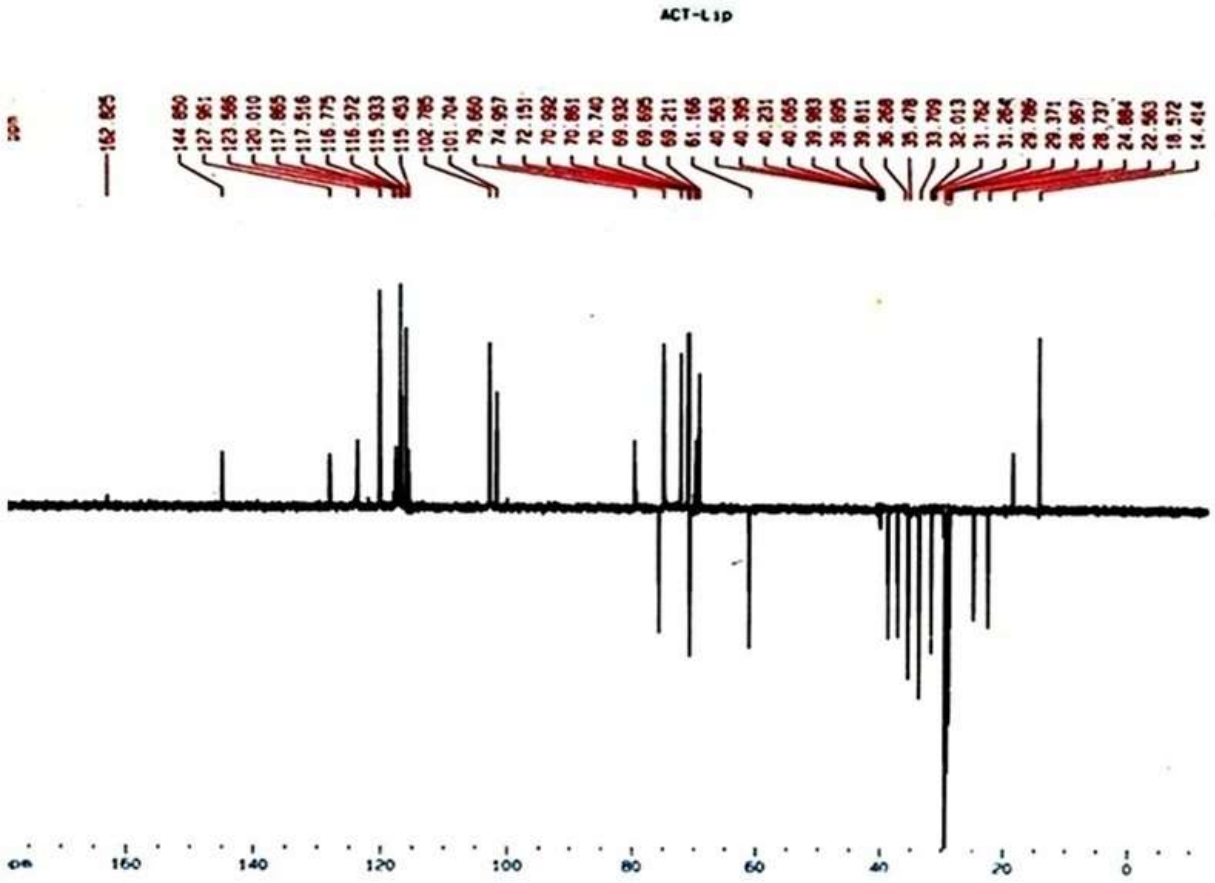
As colourless solid; mp: 188 ^{o}C; $[\alpha]^{D}_{25}$ -54.0 (c 1.0, $CDCl_3$); IR (KBr, cm^{-1}): 521, 590, 630, 675, 750, 845, 1035, 1420, 1645, 1739, 2834, 3345; ^{1}H NMR (500 MHz, DMSO); δ 7.53 (d, *J* = 15.08 Hz, 1H), 7.28 (t, *J* = 8.5 Hz, 1H), 7.2 (s, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 6.79 (d, *J* = 7.95 Hz, 1H), 6.75 (d, *J* = 7.8 Hz, 1H), 6.65 (d, *J* = 8.17 Hz, 1H), 6.25 (d, *J* =15.84 Hz, 1H), 5.02 (s, 1H), 4.73 (s, 1H), 4.28 (t, *J* = 7.61 Hz, 1H), 3.9 (m, *J* = 8.75 Hz, 1H), 3.82 (s, 1H), 3.72 (m, 1H), 3.54 (d, *J* = 4.92 Hz, 2H), 3.43 (d, *J* = 2.84 Hz, 4H), 3.21 (m, 6H), 2.78 (d, *J* = 8.56 Hz, 1H), 2.5 (m, 3H), 1.62 (t, *J* = 7.06 Hz, 3H), 1.34 (s, 3H), 1.19 (submerged with solvent peak), 0.96 (d, *J* = 6.45 Hz, 14H), 0.86 (t, *J* = 6.48 Hz, 8H); ^{13}C (500 MHz, DMSO): δ 14, 19, 23, 30, 31, 35, 37, 46, 58, 62, 70, 71, 74, 75, 76, 79, 80, 82, 103, 112, 115, 117, 118, 120, 122, 130, 139, 146, 147, 148, 153, 165, 170, 173; Maldi mass: (M + Na) 1069; Anal. Calcd for $C_{60}H_{94}O_{17}$: C, 66.76; H, 8.71. Found: C, 66.73; H, 8.53.



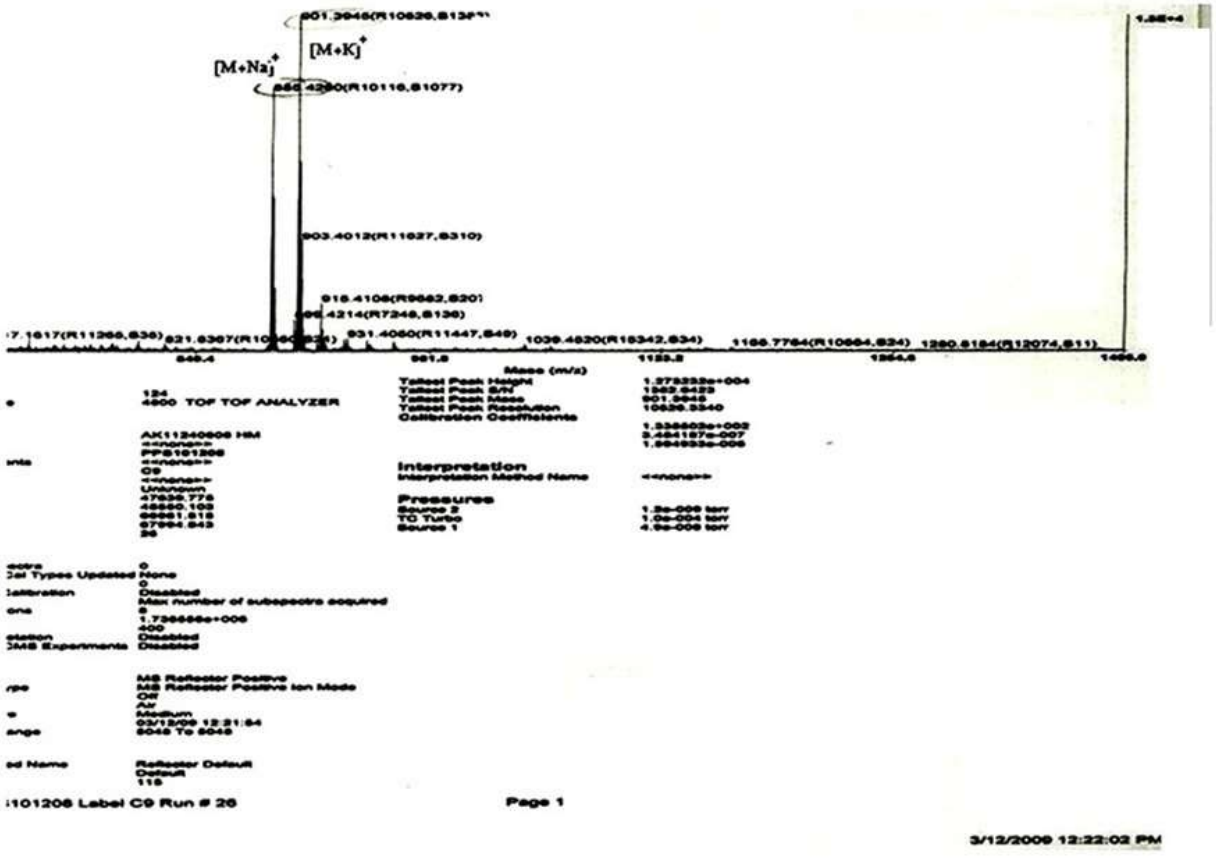
^{1}H NMR of Verbascoside 4''-palmitate (3d)



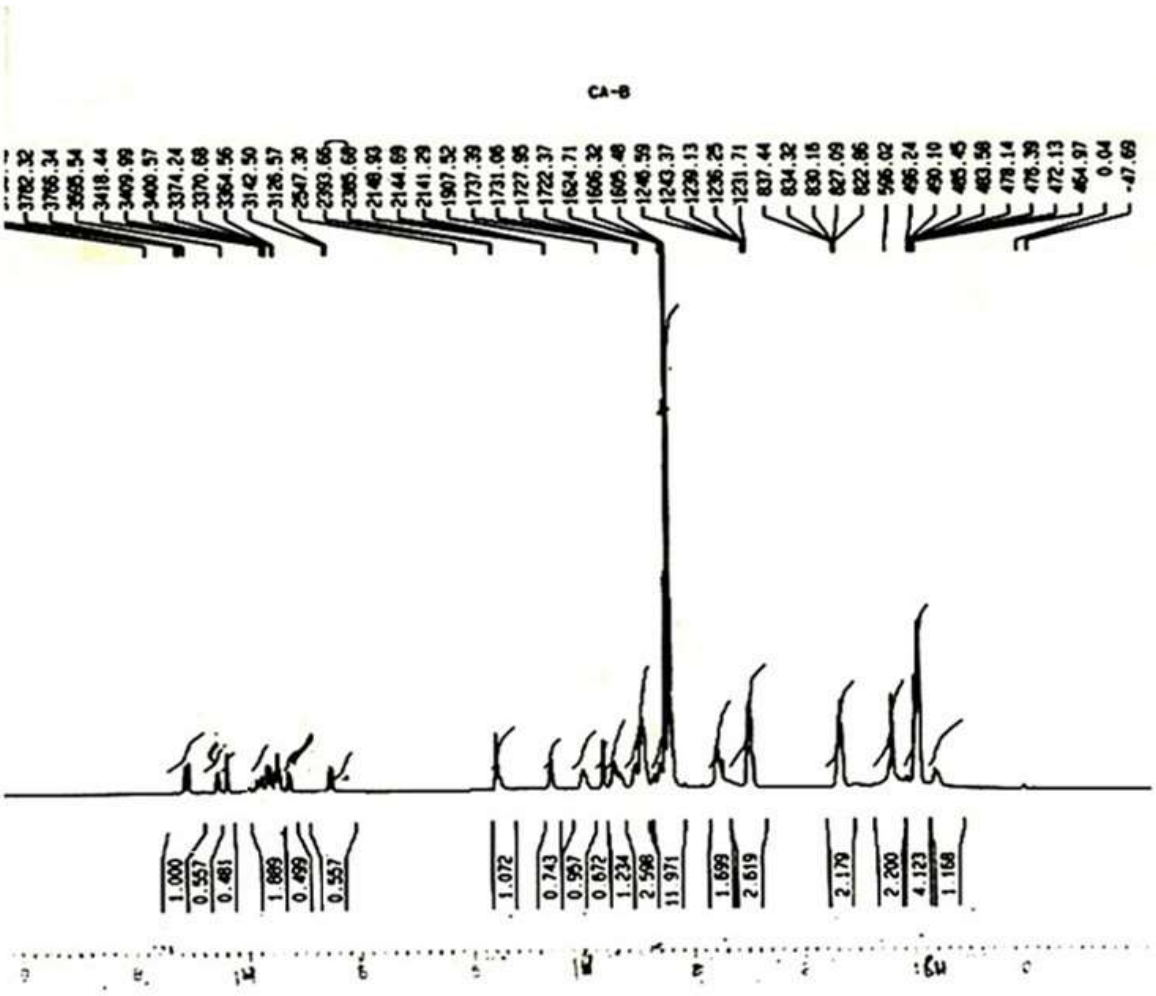
^{13} C NMR of verbascoside 4''-palmitate



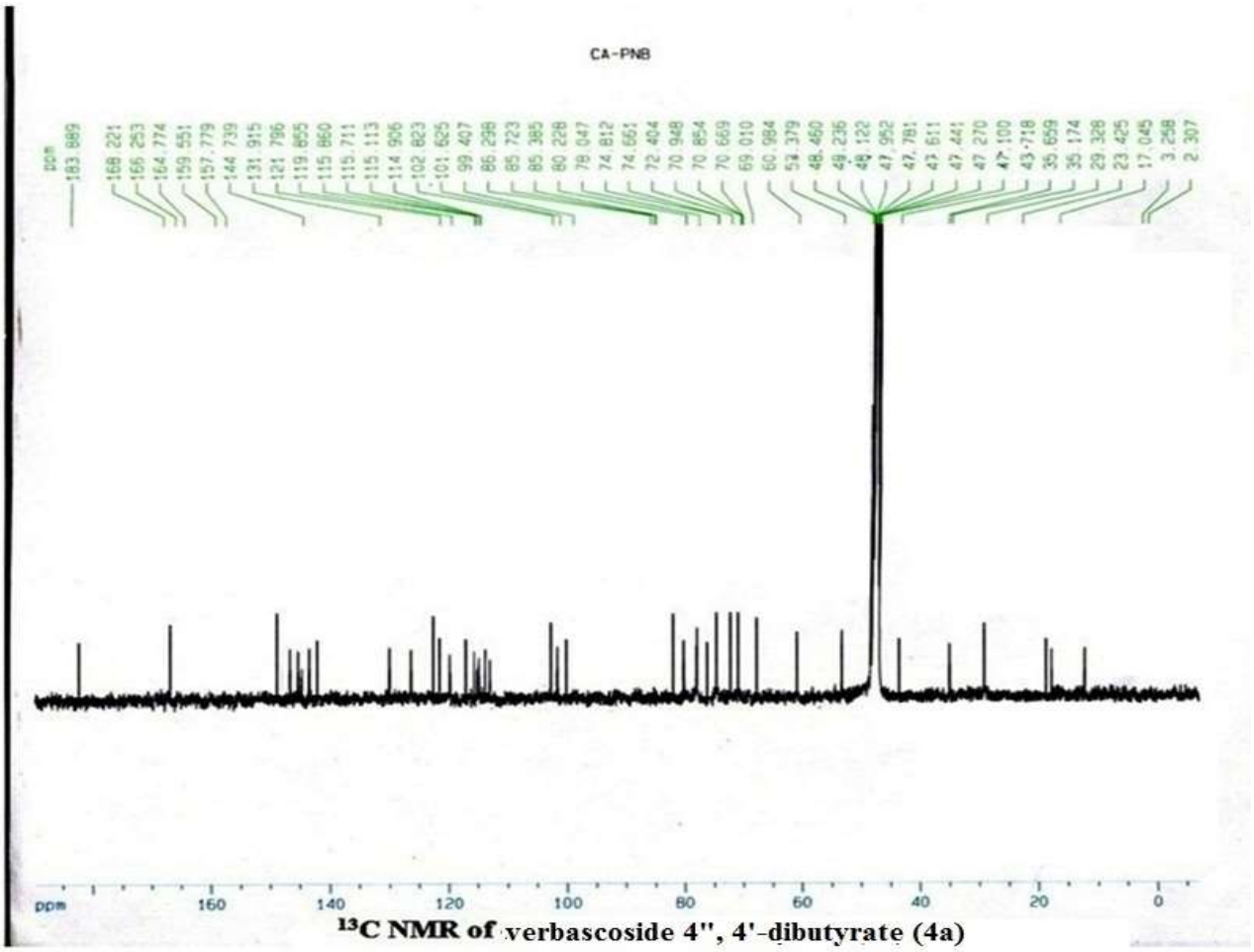
DEPTT of verbascoside 4''-palmitate (3d)



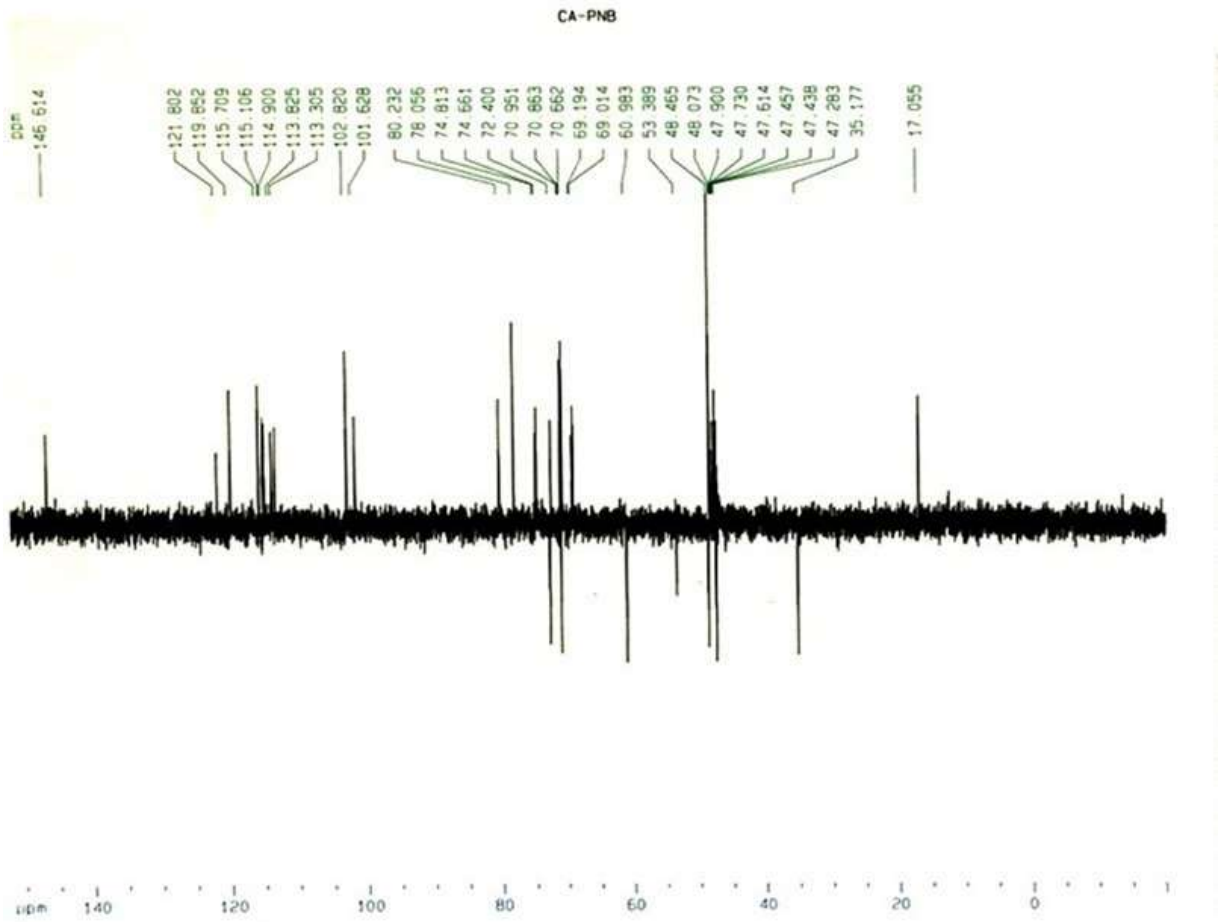
MALDI-MS of verbascoside 4''-palmitate(3d)



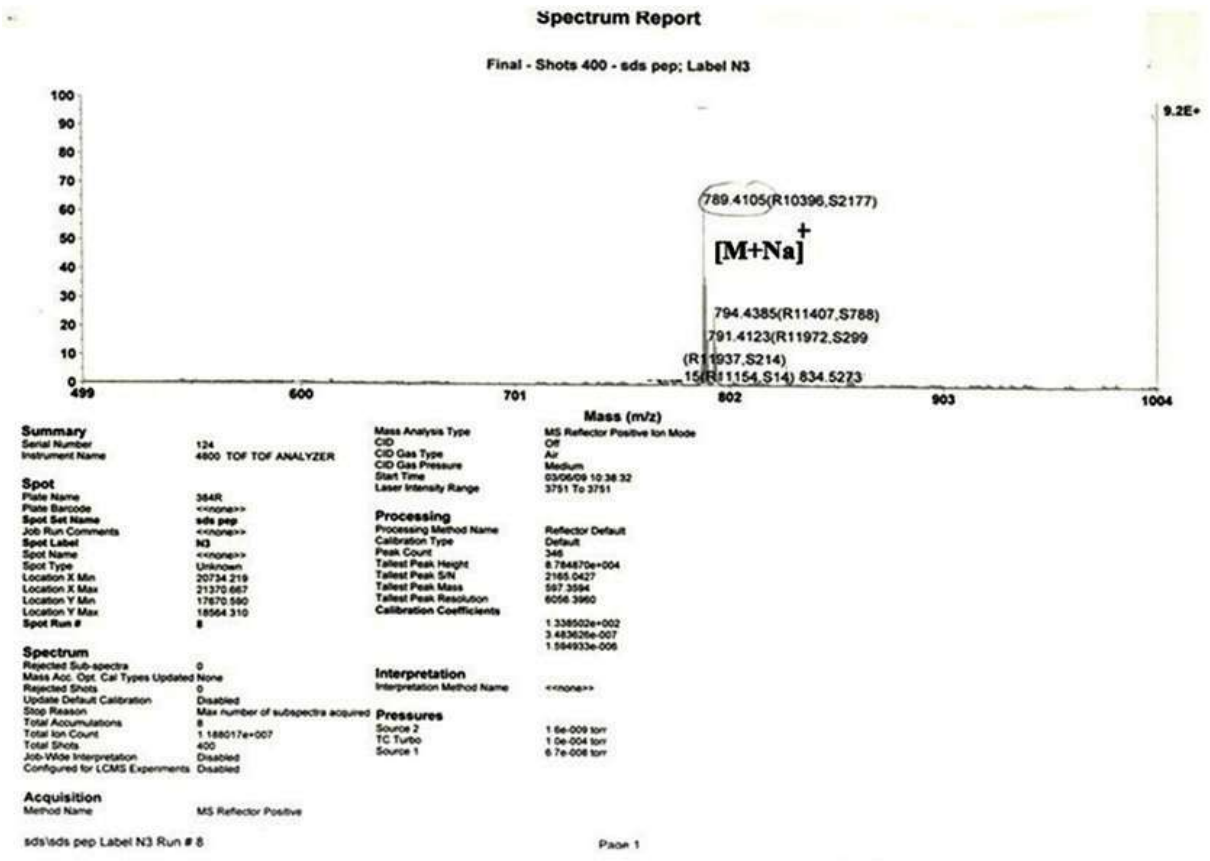
1H NMR of verbascoside 4'', 4'- dibutyrate (4a)



^{13}C NMR of verbascoside 4'', 4'-dibutyrate (4a)



Deppt of verbascoside *4'',4'-dibutyrate* (**4a**)



Maldi MS of verbascoside *4'',4'-dibutyrate* (**4a**)