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Supporting Information

Inhibition of Mincle signaling by chemically synthesized disaccharide-type 6-*O*-acylated steryl β-glucosides (βASGs) and their analogues derived from plants

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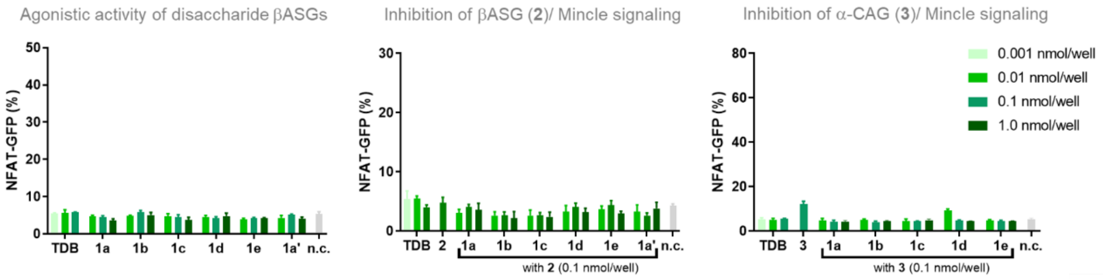


Fig. S1. Mincle-mediated signaling activity of ASGs by using 2B4 NFAT-GFP reporter cells expressing FcRγ alone. The cells were incubated on a plate with the ligands, TDB, or ^{i}PrOH as a negative control at 36 ºC for 18 h. GFP expression was evaluated by flowcytometry.

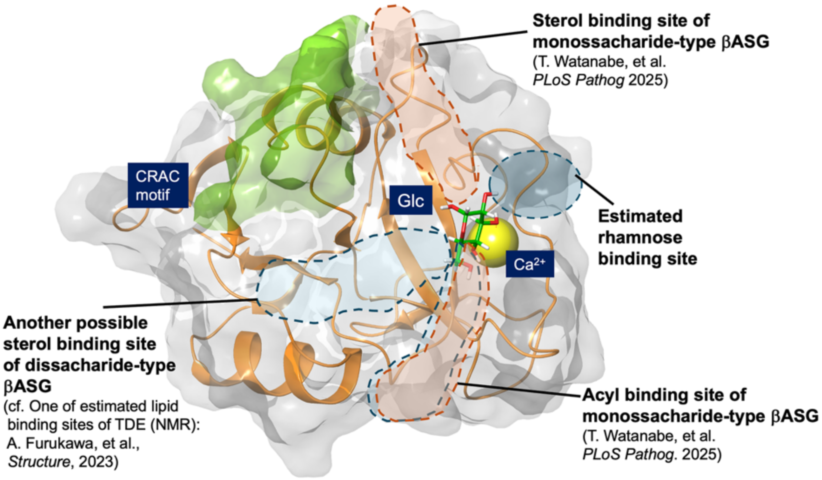
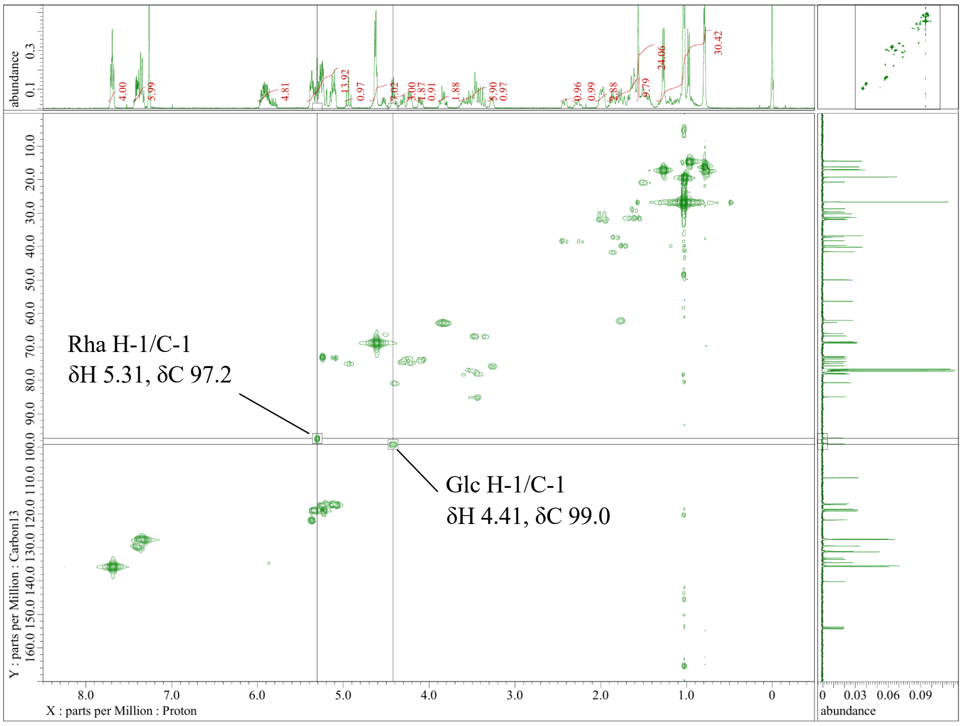
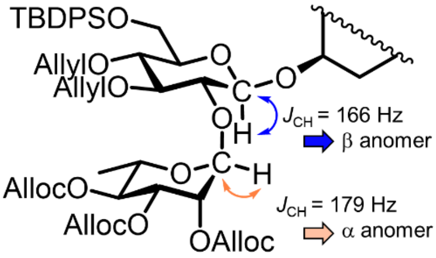


Fig. S2. Proposed model for Mincle binding sites of disaccharide βASG. Orange dotted areas were previously proposed for monosaccharide-type βASG (AEG) by Watanabe et al. (Alphafold2 model).[1] Blue dotted-areas are predicted from another model based on the docking simulation of monosaccharide-type βASG based on a Mincle model,[2] whose area is also proposed by NMR analysis of a lipid moiety of TDE.[3]

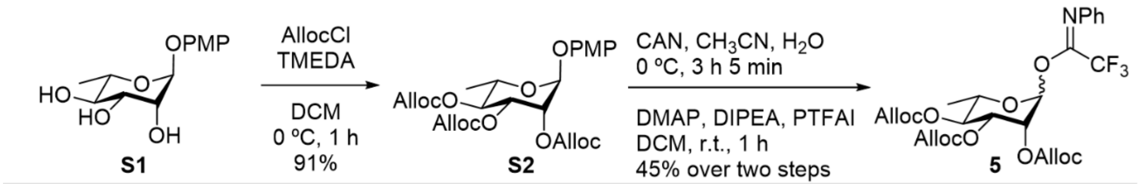
HMQC ($CDCl_3$)



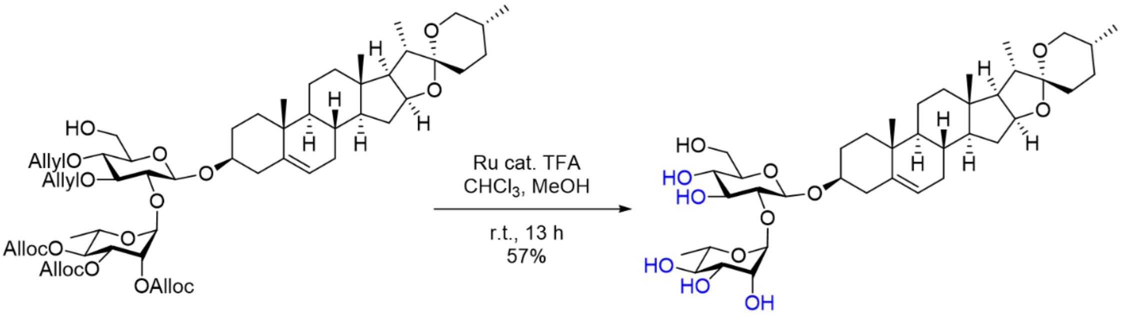


Compound 6**a**

Fig. S3. Confirmation of stereochemistry[4] of compound **6a**.



Scheme S1. Synthesis of compound **5**



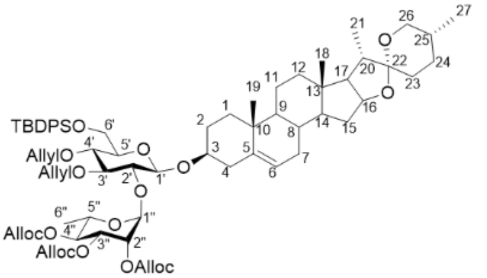
disaccharide βSG (1a')

Scheme S2. Synthesis of disaccharide βSG (**1a’**)

Experimental section

1. Synthesis

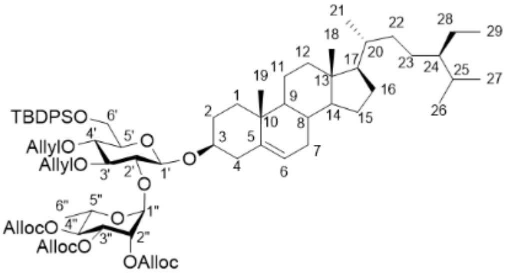
General procedures: Nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR) spectra were measured in an indicated solvent with JEOL ECX-400, JEOL ECS-400 spectrometer (^{1}H NMR: 400 MHz, ^{13}C NMR: 100 MHz). Chemical shifts are reported in parts per million (δ) relative to TMS as an internal standard. High-resolution mass spectra (HRMS) of synthetic compounds were obtained on an electronspray ionization quadrupole time of flight (ESI-QTOF) mass spectrometer (micro TOF-QII-HC; BRUKER). Analytical thin-layer chromatography (TLC) was performed on Silica gel 60 F_{254} Plates (Merck, 0.25 mm thickness). Silica gel column chromatography was performed using Silica gel 60 N (Kanto Chemical Co., 40–50 μm) using indicated solvent systems. Concentration of solutions was performed under reduced pressure using a rotary evaporator. Decahydro-2-naphthol was purchased from Tokyo Chemical Industry (Product Number: D2000) and used as received as an isomeric mixture. Ru catalyst (Ru cat.) refers to $[CpRu(CH_3CN)_3]PF_6$ in combination with quinaldic acid[5].



Diosgenyl (2”,3”,4”-*O*-trialloc-α-L-rhamnopyranosyl)- α(1→2)-3’,4’-*O*-diallyl-6’-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranoside (6a)

After **compound 4a** (163 mg, 181 μmol) and **compound 5** (227 mg, 386 μmol) were co-evaporated with toluene four times, and dried over under reduced pressure, MS4A (115 mg) was added in CH_2Cl_2 (2.5 mL). To the mixture was added TMSOTf (8.2 μL, 45 μmol) and the mixture was stirred at 0 °C for 30 min and then at r.t. for an additional 30 min. Et3N (1 drop) was added and the reaction mixture was stirred for 30 min at r.t., filtered through a PTFE membrane filter (0.5 μm, Advantec) and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/Et_2O=6/1-3/1) twice to afford **compound 6a** (221.37 mg, 95%) as colorless oil; $[\alpha]_D^{25}$ -30.8 (*c* 1.08, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 7.71–7.67 (m, 4H, Ar-H of TBDPS), 7.44–7.33 (m, 6H, Ar-H of TBDPS), 5.98–5.77 (m, 5H, -CH= of alloc, -CH= of allyl), 5.39–5.34 (m, =CH_2 of alloc), 5.33–5.23 (m, 7H, =CH_2 of alloc, =CH_2 of allyl, H-1”, H-2”) 5.20–5.15 (m, 1H, =CH_2 of allyl) 5.13– 5.09 (m, 3H, =CH_2 of allyl, H-3”), 4.94 (dd, *J* = 10.0, 10.0 Hz, 1H, H-4”), 4.63–4.61 (m, 6H, -CH_2- of alloc), 4.56–4.49 (m, 1H, H-5”), 4.43-4.38 (m, 2H, H-1’, H-16), 4.31 (dd, *J* = 12.6, 5.8 Hz, 1H, -CH_2- allyl), 4.25–4.21 (m, 2H, -CH_2-

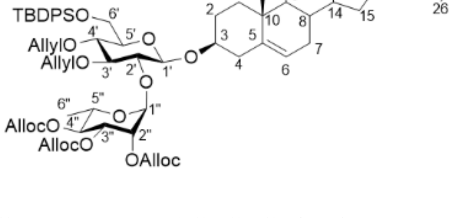
allyl), 4.10 (dd, *J* = 12.2, 5.2 Hz, 1H, -CH$_2$- allyl), 3.88–3.79 (m, 2H, H-6’), 3.64–3.35 (m, 6H, H-2’, H-3’, H-4’, H-3, H-26), 3.28–3.25 (m, 1H, H-5’), 2.44–2.41 (m, 1H, H-4), 2.29–2.23 (m, 1H, H-4), 2.00–1.97 (m, 3H, H-15, H-20), 1.89–1.73 (m, 4H, H-1, H-8, H-12, H-17), 1.69–1.43 (m, 9H, H-2, H-11, H-23, H-24, H-25), 1.33–1.26 (m, 5H, H-6’, H-7), 1.23–1.06 (m, 3H, H-1, H-12, H-14), 1.04–1.02 (m, 12H, H-19, -CH$_3$ of TBDPS), 0.98–0.88 (m, 4H, H-9, H-21), 0.79 (m, 6H, H-18, H-27); ^{13}C-NMR (CDCl$_3$) δ 154.3 (C=O of alloc), 154.1 (C=O of alloc), 153.8 (C=O of alloc), 140.2 (C-5), 135.7 (*o*-Ph of TBDPS), 135.5 (*o*-Ph of TBDPS), 134.5 (=CH$_2$ of allyl), 134.4 (=CH$_2$ of allyl), 133.5 (*ipso*-Ph of TBDPS), 133.1 (*ipso*-Ph of TBDPS), 131.3 (=CH$_2$ of alloc), 131.1 (=CH$_2$ of alloc), 129.5 (*p*-Ph of TBDPS), 129.5 (*p*-Ph of TBDPS), 127.6 (*m*-Ph of TBDPS), 127.5 (*m*-Ph of TBDPS), 121.7 (C-6), 119.1 (-CH= of alloc), 118.7 (-CH= of alloc), 118.5 (-CH= of alloc), 117.2 (-CH= of allyl), 117.0 (-CH= of allyl), 109.2 (C-22), 99.0 (C-1’ , J_{CH} = 179 Hz, α anomer), 97.2 (C-1” , J_{CH} = 166 Hz, β anomer), 85.0 (C-2’), 80.7 (C-16), 78.1 (C-4’), 77.9 (C-3’), 76.9 (C-5’), 75.7 (C-4”), 74.9 (C-3), 74.4 (-CH$_2$- of allyl), 73.6 (-CH$_2$- of allyl), 73.1 (C-3”), 72.9 (C-2”), 68.8 (-CH$_2$- of alloc), 68.7 (-CH$_2$- of alloc), 68.5 (-CH$_2$- of alloc), 66.7 (C-26), 66.0 (C-5”), 62.7 (C-6’), 62.0 (C-17), 56.4 (C-14), 50.0 (C-9), 41.5 (C-4), 40.2 (C-20), 39.7 (C-13), 38.2 (C-12), 37.2 (C-1), 36.8 (C-10), 32.0 (C-7), 31.8 (C-15), 31.3 (C-2), 31.3 (C-8), 30.2 (C-23), 29.7 (C-25), 28.7 (C-24), 26.7 (C-11), 20.7 (-CH$_3$ of TBDPS), 19.2 (C-19), 17.1 (C-6”), 17.0 (C-27), 16.2 (C-18), 14.5 (C-21); HRMS (ESI-QTOF) calcd. for $C_{73}H_{100}NaO_{18}Si$ $[M+Na]^+$ 1315.6571; found: 1315.6563.



β-sitosteryl (2”,3”,4”-*O*-trialloc-α-L-rhamnopyranosyl)- α(1→2)-3’,4’-*O*-diallyl-6’-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranoside (6b)

After **compound 4b** (59.6 mg, 66.5 μmol) and **compound 5** (59.2 mg, 101 μmol) were co-evaporated with toluene four times, and dried over under reduced pressure, MS4A (33.8 mg) was added in CH$_2$Cl$_2$ (636 μL). To the mixture was added TMSOTf (2.0 μL, 11 μmol) and the mixture was stirred at 0 °C for 30 min and then at r.t. for an additional 30 min. Et3N (1 drop) was added and the reaction mixture was stirred for 30 min at r.t., filtered through a PTFE membrane filter (0.5 μm, Advantec) and concentrated. The residue was purified by silica gel column chromatography (n-hexane/Et2O=8/1–Et$_2$O) to give a mixture, and the obtained mixture was further purified by silica gel column chromatography (n-hexane/Et$_2$O=20/1–10/1) to afford **compound 6b** (42.8 mg, 82%) as a white foam;

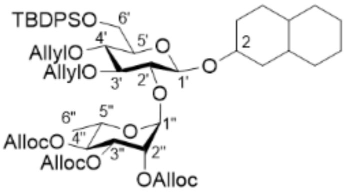
$[\alpha]_D^{25}$ -2.64 (c 1.03, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 7.70 (dd, *J* = 12.4, 2.2 Hz, 4H, Ar-H of TBDPS), 7.43–7.33 (m, 6H, Ar-H of TBDPS), 5.98–5.78 (m, 5H, -CH= of alloc, -CH= of allyl), 5.38–5.35 (m, 3H, H-6, =CH_2 of alloc), 5.32–5.24 (m, 7H, =CH_2 of allyl, =CH_2 of alloc, H-1”, H-2”), 5.20–5.16 (m, 1H, =CH_2 of allyl), 5.13–5.09 (m, 3H, =CH_2 of allyl, H-3”), 4.94 (dd, *J* = 10.4, 10.4 Hz, 1H, H-4”), 4.63–4.62 (m, 6H, -CH_2- of alloc), 4.53 (dd, *J* = 10.2, 6.6 Hz, 1H, H-5”), 4.43 (d, *J* = 7.2 Hz, 1H, H-1’ β-anomer), 4.31 (dd, *J* = 12.6, 5.4 Hz, 1H, -CH_2- of allyl), 4.25–4.21 (m, 2H, -CH_2- of allyl), 4.10 (dd, *J* = 12.6, 5.8 Hz, 1H, -CH_2- of allyl), 3.88–3.79 (m, 2H, H-6’), 3.62–3.41 (m, 4H, H-2’, H-3’, H-4’, H-3), 3.28–3.25 (m, 1H, H-5’), 2.44–2.39 (m, 1H, H-4), 2.29–2.22 (m, 1H, H-4), 2.03–1.97 (m, 3H, -CH_2- of β-sitosterol), 1.86–1.82 (m, 2H, -CH_2- of β-sitosterol), 1.71–1.42 (m, 9H, -CH and -CH_2- of β-sitosterol), 1.36–1.00 (m, 25H, H-6”, -CH_3 of TBDPS, -CH and -CH_2- of β-sitosterol), 0.93–0.81 (m, 15H, -CH_3 of β-sitosterol), 0.68 (s, 3H, H-18); ^{13}C-NMR ($CDCl_3$) δ 154.3 (C=O of alloc), 154.2 (C=O of alloc), 153.9 (C=O of alloc), 140.2 (C-5), 135.8 (*o*-Ph of TBDPS), 135.5 (*o*-Ph of TBDPS), 134.5 (=CH_2 of allyl), 133.6 (*ipso*-Ph of TBDPS), 133.2 (*ipso*-Ph of TBDPS), 131.4 (=CH_2 of alloc), 131.2 (=CH_2 of alloc), 129.6 (*p*-Ph of TBDPS), 127.6 (*m*-Ph of TBDPS), 127.5 (*m*-Ph of TBDPS), 122.1 (C-6), 119.1 (-CH= of alloc), 118.8 (-CH= of alloc), 118.6 (-CH= of alloc), 117.2 (-CH= of allyl), 117.0 (-CH= of allyl), 99.1 (C-1’ , J_{CH} = 165 Hz, β anomer), 97.3 (C-1” , J_{CH} = 175 Hz, α anomer), 85.1 (C-2’), 78.2 (C-4’), 78.0 (C-3’), 75.7 (C-5’), 74.9 (C-4”, C-3), 74.4 (-CH_2- of allyl), 73.7 (-CH_2- of allyl), 73.2 (C-3”), 73.0 (C-2”), 68.9 (-CH_2- of alloc), 68.8 (-CH_2- of alloc), 68.6 (-CH_2- of alloc), 66.1 (C-5”), 62.8 (C-6’), 56.7 (C-14), 56.0 (C-17), 50.2 (C-9), 45.8 (C-24), 42.3 (C-13), 39.7 (C-12), 38.3 (C-4), 37.3 (C-1), 36.7 (C-10), 36.1 (C-20), 33.9 (C-22), 31.9 (C-8), 31.9 (C-7), 29.8 (C-2), 29.1 (C-25), 28.2 (C-16), 26.7 (-CH_3 of TBDPS), 26.0 (C-23), 24.3 (C-15), 23.0 (C-28), 21.0 (C-11), 19.8 (C-26 or C-27), 19.3 (C-26 or C-27), 19.0 (C-21), 18.8 (C-19), 17.1 (C-6”), 12.0 (C-18), 11.8 (C-29); HRMS (ESI-QTOF) calcd. for $C_{75}H_{108}NaO_{16}Si$ $[M+Na]^+$ 1315.7299; found: 1315.7290.



Cholesteryl (2”,3”,4”-*O*-trialloc-α-L-rhamnopyranosyl)- α(1→2)-3’,4’-*O*-diallyl-6’-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranoside (6c)

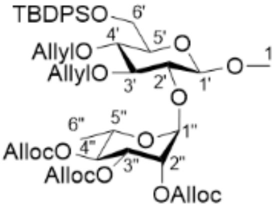
After **compound 4c** (57.8 mg, 66.7 μmol) and **compound 5** (83.2 mg, 142 μmol) were co-evaporated with toluene four times, and dried over under reduced pressure, MS4A (68 mg) was added in CH_2Cl_2

(930 μL). To the mixture was added TMSOTf (3.0 μL, 17 μmol), and the mixture was stirred at 0 ^{o} C for 30 min and then at r.t. for an additional 30 min. Et3N (1 drop) was added and the reaction mixture was stirred for 30 min at r.t., filtered through a PTFE membrane filter (0.5 μm, Advantec) and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=20/1-10/1) to give a mixture, and the obtained mixture was further purified by silica gel column chromatography (n-hexane/Et$_2$O=8/1–Et$_2$O) to afford **compound 6c** (51.9 mg, 62%) as a colorless oil; $[\alpha]_D^{25}$ -2.45 (*c* 1.01, CHCl$_3$); ^{1}H-NMR (CDCl$_3$) δ 7.71–7.68 (m, 4H, Ar-H of TBDPS), 7.43–7.33 (m, 6H, Ar-H of TBDPS), 5.97–5.78 (m, 5H, -CH= of alloc, -CH= of allyl), 5.39–5.34 (m, 3H, H-6, =CH$_2$ of alloc), 5.33–5.23 (m, 7H, =CH$_2$ of allyl, =CH$_2$ of alloc, H-1”, H-2”), 5.20–5.15 (m, 1H, =CH$_2$ of allyl), 5.13–5.09 (m, 3H, =CH$_2$ of allyl, H-3”), 4.94 (dd, J = 10.0, 10.0 Hz, 1H, H-4”), 4.63–4.61 (m, 6H, -CH$_2$- of alloc), 4.55–4.50 (m, 1H, H-5”), 4.42 (d, J = 7.6 Hz, 1H, H-1’ β-anomer), 4.31 (dd, J = 12.2, 5.8 Hz, 1H, -CH$_2$- of allyl), 4.25–4.21 (m, 2H, -CH$_2$- of allyl), 4.10 (dd, J = 12.0, 5.6 Hz, 1H, -CH$_2$- of allyl), 3.87–3.79 (m, 2H, H-6’), 3.63–3.57 (m, 1H, H-3), 3.56–3.52 (m, 1H, H-4’), 3.49–3.41 (m, 2H, H-2’, H-3’), 3.28–3.25 (m, 1H, H-5’), 2.43–2.40 (m, 1H, H-4), 2.29–2.22 (m, 1H, H-4), 2.03–1.97 (m, 3H, -CH$_2$- of cholesterol), 1.85–1.78 (m, 2H, -CH$_2$- of cholesterol), 1.64–0.98 (m, 35H, H-6”, -CH$_3$ of TBDPS, -CH and -CH$_2$- of cholesterol), 0.93–0.85 (m, 10H, -CH and -CH$_3$ of cholesterol), 0.68 (s, 3H, H-18); ^{13}C-NMR (CDCl$_3$) δ 154.3 (C=O of alloc), 154.2 (C=O of alloc), 153.9 (C=O of alloc), 140.2 (C-5), 135.8 (*o*-Ph of TBDPS), 135.5 (*o*-Ph of TBDPS), 134.5 (=CH$_2$ of allyl), 133.2 (*ipso*-Ph of TBDPS), 131.4 (=CH$_2$ of alloc), 131.2 (=CH$_2$ of alloc), 129.6 (*p*-Ph of TBDPS), 127.6 (*m*-Ph of TBDPS), 127.5 (*m*-Ph of TBDPS), 122.1 (C-6), 119.1 (-CH= of alloc), 118.8 (-CH= of alloc), 118.6 (-CH= of alloc), 117.2 (-CH= of allyl), 117.1 (-CH= of allyl), 99.1 (C-1’, J_{CH} = 178 Hz, β anomer), 97.3 (C-1” , J_{CH} = 163 Hz, α anomer), 85.1 (C-2’), 78.2 (C-4’), 78.0 (C-3’), 75.7 (C-5’), 74.9 (C-4”, C-3), 74.4 (-CH$_2$- of allyl), 73.7 (-CH$_2$- of allyl), 73.2 (C-3”), 73.0 (C-2”), 68.9 (-CH$_2$- of alloc), 68.8 (-CH$_2$- of alloc), 68.6 (-CH$_2$- of alloc), 66.1 (C-5”), 62.8 (C-6’), 56.7 (C-14), 56.1 (C-17), 50.2 (C-9), 42.3 (C-13), 39.7 (C-12), 39.5 (C-24), 38.3 (C-4), 37.3 (C-1), 36.7 (C-10), 36.2 (C-22), 35.8 (C-20), 31.9 (C-8), 31.8 (C-7), 29.8 (C-2), 28.2 (C-16), 28.0 (C-25), 26.7 (-CH$_3$ of TBDPS), 24.3 (C-15), 23.8 (C-23), 22.8 (C26 or C27), 22.6 (C26 or C27), 21.0 (C-11), 19.3 (C-21), 18.7 (C-19), 17.1 (C-6”), 11.8 (C-18); HRMS (ESI-QTOF) calcd. for C$_{73}$H$_{104}$NaO$_{16}$Si [M+Na]$^+$ 1287.6986; found: 1287.6978.



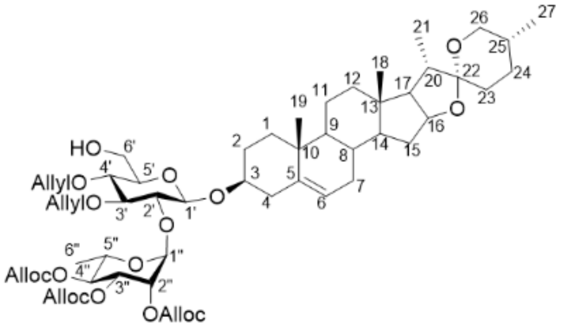
Decahydro-2-naphthol (2”,3”,4”-*O*-trialloc-α-L-rhamnopyranosyl)- α(1→2)-3’,4’-*O*-diallyl-6’-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranoside (6d)

After **compound 4d** (isomeric mixture, 73.1 mg, 115 μmol) and **compound 5** (131 mg, 223 μmol) were co-evaporated with toluene four times, and dried over under reduced pressure, MS4A (91.8 mg) was added in CH_2Cl_2 (1.6 mL). To the mixture was added TMSOTf (5.2 μL, 29 μmol), and the mixture was stirred at 0 °C for 30 min and then at r.t. for an additional 30 min. Et3N (1 drop) was added and the reaction mixture was stirred for 30 min at r.t., filtered through a PTFE membrane filter (0.5 μm, Advantec) and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=3%-28%) to give a mixture, and the obtained mixture was further purified by silica gel column chromatography (n-hexane/Et_2O=10/1–2/1) to afford **compound 6d** (90.0 mg, 76%) as colorless oil; $[\alpha]_D^{25}$ -7.01 (*c* 1.07, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 7.71–7.68 (m, 4H, Ar-H of TBDPS), 7.44–7.36 (m, 6H, Ar-H of TBDPS), 5.97–5.76 (m, 5H, -CH= of alloc, -CH= of allyl), 5.39–5.23 (m, 9H, =CH_2 of alloc, =CH_2 of allyl, H-1”), 5.18–5.08 (m, 4H, =CH_2 of allyl, H-2”, H-3”), 4.97–4.91 (m, 1H, H-4”), 4.65–4.57 (m, 6H, -CH_2- of alloc), 4.51–4.37 (m, 2H, H-5”, H-1’), 4.30 (dd, *J* = 12.4, 5.6 Hz, 1H, -CH_2- of allyl), 4.37–4.20 (m, 2H, -CH_2- of allyl), 4.09–4.03 (m, 1H, -CH_2- of allyl), 3.88–3.78 (m, 2H, H-6’), 3.57–3.37 (m, 3H, H-2, H-3’, H-4’), 3.27 (d, *J* = 9.5 Hz, 1H, H-5’), 2.00–1.83 (m, 3H, decahydro-2-naphthol), 1.70–1.20 (m, 15H, H-6”, decahydro-2-naphthol), 1.09–0.87 (m, 11H, -CH_3 of TBDPS, decahydro-2-naphthol); ^{13}C-NMR ($CDCl_3$) δ 154.5–154 (C=O of alloc), 135.7–135.5(*o*-Ph of TBDPS), 134.6–134.5 (=CH_2 of allyl), 133.5–133.2 (*ipso*-Ph of TBDPS), 131.4–131.2 (=CH_2 of alloc), 129.6 (*p*-Ph of TBDPS), 127.6–127.5 (*m*-Ph of TBDPS), 119.1–118.5 (-CH= of alloc), 117.1 (-CH= of allyl), 99.7–98.5 (C-1’), 97.4–97.2 (C-1”), 85.2–85.1 (C-2’), 78.6 (C-4’), 78.1–77.7 (C-3’), 75.9–75.8 (C-5’), 75.0 (C-4”), 74.7 (C-2), 74.4–73.5 (-CH_2- of allyl), 73.2 (C-3”), 73.1 (C-2”), 68.9–68.6 (-CH_2- of alloc), 66.0 (C-5”), 62.9–62.8 (C-6’), 42.4, 41.4, 41.3, 41.0, 39.2, 35.3, 35.2, 35.1, 34.8, 34.5, 34.1, 33.8, 33.6, 33.2, 32.1, 31.8, 31.7, 31.5, 30.4, 29.7, 28.6, 26.7, 26.5, 26.3, 25.5, 25.1, 24.7, 22.6, 20.9, 20.6, 19.3 (decahydro-2-naphthol), 17.0 (C-6”), 14.1; HRMS (ESI-QTOF) calcd. for $C_{56}H_{76}NaO_{16}Si$ $[M+Na]^+$ 1055.4795; found: 1055.4792.



Methyl (2”,3”,4”-*O*-trialloc-α-L-rhamnopyranosyl)- α(1→2)-3’,4’-*O*-diallyl-6’-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranoside (6e)

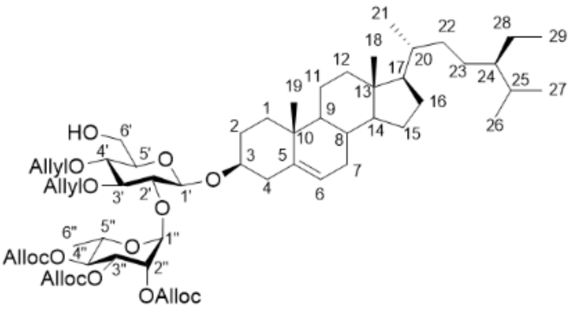
After **compound 4e** (73.0 mg, 14.2 μmol) and **compound 5** (193 mg, 329 μmol) were co-evaporated with toluene four times, and dried over under reduced pressure, MS4A (97.8 mg) was added in CH_2Cl_2 (2.0 mL). To the mixture was added TMSOTf (6.4 μL, 36 μmol), and the mixture was stirred at 0 °C for 30 min and then at r.t. for an additional 30 min. Et3N (1 drop) was added and the reaction mixture was stirred for 30 min at r.t., filtered through a PTFE membrane filter (0.5 μm, Advantec) and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=8%-66%) to give a mixture, and the obtained mixture was further purified by silica gel column chromatography (n-hexane/EtOAc = 20/1–13/1) to afford **compound 6e** (67.20 mg, 52%) as a white solid; $[\alpha]_D^{25}$ -3.01 (*c* 1.06, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 7.75–7.69 (m, 4H, Ar-H of TBDPS), 7.45–7.34 (m, 6H, Ar-H of TBDPS), 6.00–5.82 (m, 5H, -CH= of alloc, -CH= of allyl), 5.39–5.32 (m, 3H, $=CH_2$ of alloc), 5.29–5.18 (m, 7H, $=CH_2$ of allyl, $=CH_2$ of alloc, H-1”, H-2”), 5.16–5.12 (m, 3H, $=CH_2$ of allyl, H-3”), 4.95 (dd, *J* = 9.8 Hz, 1H, H-4”), 4.66–4.61 (m, 6H, $-CH_2-$ of alloc), 4.34–4.15 (m, 6H, H-5”, H-1’, $-CH_2-$ of allyl), 3.88 (d, *J* = 2.8 Hz, 2H, H-6’), 3.55–3.43 (m, 6H, H-1, H-2’, H-3’, H-4’), 3.25–3.23 (m, 1H, H-5’), 1.27 (d, *J* = 6.4 Hz, 3H, H-6”), 1.04 (s, 9H, $-CH_3$ of TBDPS); ^{13}C-NMR ($CDCl_3$) δ 154.2 (C=O of alloc), 153.9 (C=O of alloc), 135.8 (C=O of alloc), 135.5 (*o*-Ph of TBDPS), 134.6 ($=CH_2$ of allyl), 133.1 (*ipso*-Ph of TBDPS), 131.3 ($=CH_2$ of alloc), 131.3 ($=CH_2$ of alloc), 131.2 ($=CH_2$ of alloc), 129.6 (*p*-Ph of TBDPS), 127.7 (*m*-Ph of TBDPS), 127.5 (*m*-Ph of TBDPS), 119.1 (-CH= of alloc), 119.0 (-CH= of alloc), 118.8 (-CH= of alloc), 117.3 (-CH= of allyl), 117.0 (-CH= of allyl), 102.1 (C-1’, J_{CH} = 159 Hz, β anomer), 98.0 (C-1” , J_{CH} = 181 Hz, α anomer), 84.6 (C-2’), 78.7 (C-4’), 77.6 (C-3’), 75.6 (C-5’), 74.9 (C-4”), 74.6 ($-CH_2-$ of allyl), 73.7 ($-CH_2-$ of allyl), 73.2 (C-3”, C-2”), 68.9 ($-CH_2-$ of alloc), 68.8 ($-CH_2-$ of alloc), 66.3 (C-5”), 62.4 (C-6’), 56.4 (C-1), 26.7 ($-CH_3$ of TBDPS), 19.3, 16.8 (C-6”); HRMS (ESI-QTOF) calcd. for $C_{47}H_{62}NaO_{16}Si$ $[M+Na]^+$ 933.3699; found: 933.3702.



Diosgenyl (2”,3”,4”-*O*-trialloc-α-L-rhamnopyranosyl)- α(1→2)-3’,4’-*O*-diallyl-β-D-glucopyranoside (7a)

To a stirred solution of **compound 6a** (26.0 mg, 20.0 μmol) in THF (400 μL) was added 1 M TBAF in THF (90 μL, 90 μmol), and the mixture was stirred at r.t. for 1.25 h. Then 1 M TBAF in THF (40 μL, 40 μmol) was added to the mixture, which was stirred at r.t. for additional 4.25 h. Sat. aq. NH_4Cl (ca. 1 mL) was added to the reaction mixture, and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over Na_2SO_4, filtered through a cotton plug, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=5/1) to afford **compound 7a** (13.7 mg, 65%) as a colorless solid; $[\alpha]_D^{25}$ -42.6 (*c* 1.02, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 5.97–5.84 (m, 5H, -CH= of allyl, -CH= of alloc), 5.41–5.40 (m, 1H, H-5), 5.39–5.34 (m, 2H, -CH= of alloc), 5.32–5.23 (m, 8H, =CH_2 allyl, =CH_2 of alloc, H-1”, H-2”), 5.19–5.07 (m, 3H, =CH_2 of allyl, H-3”), 4.93 (dd, *J* = 10.2 Hz, 1H, H-4”), 4.66–4.57 (m, 6H, -CH_2- of alloc), 4.53–4.46 (m, 2H, H-5”, H-1’), 4.44–4.38 (m, 1H, H-16), 4.33–4.29 (dd, 12.2, 5.4 Hz, 1H, -CH_2- of allyl), 4.27–4.20 (m, 2 H, -CH_2- of allyl), 4.15–4.09 (m, 1H, -CH_2- of allyl), 3.88–3.83 (m, 1H, H-6’), 3.73–3.66 (m, 1H, H-6’), 3.60–3.47 (m, 4H, H-2’, H-3’, H-3, H-26), 3.40–3.27 (m, 3H, H-4’, H-5’, H-26), 2.45–2.41 (m, 1H, H-4), 2.29–2.24 (m, 1H, H-4), 2.02–1.93 (m, 2H, H-15), 1.90–1.80 (m, 3H, H-1, H-8, H-20), 1.77–1.69 (m, 2H, H-12, H-17), 1.66–1.38 (m, 9H, H-2, H-11, H-23, H-24, H-25), 1.30–1.21 (m, 5H, H-6”, H-7), 1.19–1.09 (m, 3H, H-1, H-12, H-14), 0.979 (s, 3H, H-19), 0.95–0.84 (m, 4H, H-9, H-21), 0.80–0.78 (m, 6H, H-18, H-27); ^{13}C-NMR ($CDCl_3$) δ 154.3 (C=O of alloc), 154.2 (C=O of of alloc), 153.8 (C=O of alloc), 139.9 (C-5), 134.4(=CH_2 of allyl), 134.3 (=CH_2 of allyl), 131.3 (=CH_2 of alloc), 131.1 (=CH_2 of alloc), 122.1 (C-6), 119.1 (-CH= of alloc), 118.8 (-CH= of alloc), 118.6 (-CH= of alloc), 117.5 (-CH= of allyl), 117.2 (-CH= of allyl), 109.2 (C-22), 99.1 (C-1’), 97.2 (C-1”), 84.7 (C-2’), 80.8 (C-16), 78.6 (C-4’), 77.7 (C-3’) 76.2 (C-5’), 75.0 (C-4”), 74.8 (C-3), 74.3 (-CH_2- of allyl), 73.7 (-CH_2- of allyl), 73.1 (C-3”), 72.8 (C-2”), 68.9 (-CH_2- of alloc), 68.8 (-CH_2- of alloc), 68.6 (-CH_2- of alloc), 66.8 (C-26), 66.1 (C-5”), 62.0 (C-6’), 61.8 (C-17), 56.4 (C-14), 50.0 (C-9), 41.6 (C-4), 40.2 (C-20), 39.7 (C-13), 38.2 (C-12), 37.1 (C-1), 36.8 (C-10), 32.0 (C-7), 31.8 (C-15), 31.3 (C-2), 30.2 (C-8, C-23), 29.6 (C-25), 28.7 (C-24), 20.8 (C-11), 19.2 (C-19), 17.1 (C-6”), 17.0

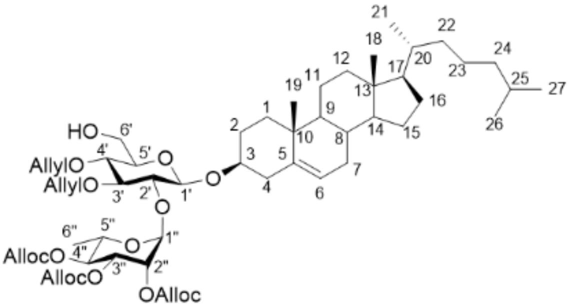
(C-27), 16.2 (C-18), 14.5 (C-21); HRMS (ESI-QTOF) calcd. for $C_{57}H_{82}NaO_{18}$ $[M+Na]^+$ 1077.5393; found: 1077.5393.



β-sitosteryl (2”,3”,4”-*O*-trialloc-α-L-rhamnopyranosyl)- α(1→2)-3’,4’-*O*-diallyl-β-D-glucopyranoside (7b)

To a stirred solution of **compound 6b** (42.8 mg, 33.1 μmol) in THF (600 μL) was added 1 M TBAF in THF (150 μL, 150 μmol), and the mixture was stirred at r.t. for 2.5 h. Then 1 M TBAF in THF (75 μL, 75 μmol) was added to the mixture, which was stirred at r.t. for additional 2 h. Sat. aq. NH_4Cl (ca. 1 mL) was added to the reaction mixture, and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over Na_2SO_4, filtered through a cotton plug, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=8%-25%) to afford **compound 7b** (27.9 mg, 80%) as a colorless solid; $[\alpha]_D^{25}$ -7.91 (*c* 1.03, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 5.97–5.85 (m, 5H, -CH= of allyl, -CH= of alloc), 5.41–5.40 (m, 1H, H-6), 5.39–5.34 (m, 2H, $=CH_2$ of alloc), 5.32–5.30 (m, 2H, $=CH_2$ of allyl or alloc, H-1”), 5.29–5.23 (m, 6H, $=CH_2$ of allyl, $=CH_2$ of alloc, H-2”), 5.19–5.16 (m, 1H, $=CH_2$ of allyl), 5.14–5.07 (m, 2H, $=CH_2$ of allyl, H-3”), 4.93 (dd, *J* = 10.4 Hz, 1H, H-4”), 4.66–4.58 (m, 6H, $-CH_2-$ of alloc), 4.54–4.47 (m, 2H, H-5”, H-1’), 4.31 (dd, *J* = 12.4, 5.8 Hz, 1H, $-CH_2-$ of allyl), 4.27–4.20 (m, 2 H, $-CH_2-$ of allyl), 4.15–4.10 (m, 1H, $-CH_2-$ of allyl), 3.87–3.83 (m, 1H, H-6’), 3.73–3.67 (m, 1H, H-6’), 3.62–3.47 (m, 3H, H-2’, H-3’, H-3), 3.37–3.27 (m, 2H, H-4’, H-5’), 2.44–2.40 (m, 1H, H-4), 2.29–2.24 (m, 1H, H-4), 2.05–1.80 (m, 6H, $-CH_2-$ of β-sitosterol), 1.71–0.99 (m, 23H, H-6”, $-CH_2-$ and -CH of β-sitosterol), 0.95–0.81 (m, 16H, -CH and $-CH_3$ of β-sitosterol) 0.68 (s, 3H, H-18) ; ^{13}C-NMR ($CDCl_3$) δ 154.3 (C=O of alloc), 154.2 (C=O of alloc), 153.9 (C=O of alloc), 139.9 (C-5), 134.5($=CH_2$ of allyl), 134.4($=CH_2$ of allyl), 131.4 ($=CH_2$ of alloc), 131.2 ($=CH_2$ of alloc), 122.4 (C-6), 119.2 (-CH= of alloc), 118.8 (-CH= of alloc), 118.6 (-CH= of alloc), 117.5 (-CH= of allyl), 117.2 (-CH= of allyl), 99.1 (C-1’), 97.2 (C-1”), 84.7 (C-2’), 78.8 (C-4’), 77.7 (C-3’), 76.3 (C-5’), 75.0 (C-4”), 74.9 (C-3), 74.4 ($-CH_2-$ of allyl), 73.7 ($-CH_2-$ of allyl), 73.1 (C-3”), 72.9 (C-2”), 68.9 ($-CH_2-$ of alloc), 68.8 ($-CH_2-$ of of alloc), 68.6 ($-CH_2-$ of alloc), 66.1 (C-5”), 61.9 (C-6’), 56.7 (C-14), 56.0 (C-17), 50.1 (C-9), 45.8 (C-24), 42.3 (C-13), 39.7 (C-12), 38.3 (C-4), 37.2 (C-1), 36.7 (C-10), 36.1 (C-20), 33.9 (C-22), 31.9 (C-

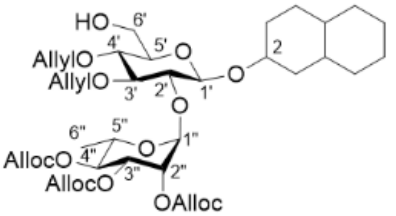
8), 31.8 (C-7), 29.7 (C-2), 29.1 (C-25), 28.2 (C-16), 26.0 (C-23), 24.3 (C-15), 23.0 (C-28), 21.0 (C-11), 19.8 (C-26 or C-27), 19.2 (C-26 or C27), 19.0 (C-21), 18.8 (C-19), 17.0 (C-6”), 12.0 (C-18), 11.8 (C-29); HRMS (ESI-QTOF) calcd. for $C_{59}H_{90}NaO_{16}$ $[M+Na]^+$ 1077.6121; found: 1077.6118.



Cholesteryl (2”,3”,4”-*O*-trialloc-α-L-rhamnopyranosyl)- α(1→2)-3’,4’-*O*-diallyl-β-D-glucopyranoside (7c)

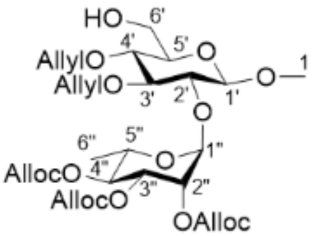
To a stirred solution of **compound 6c** (49.2 mg, 38.9 μmol) in THF (800 μL) was added 1 M TBAF in THF (175 μL, 175 μmol), and the mixture was stirred at r.t. for 2.5 h. Then 1 M TBAF in THF (90 μL, 90 μmol) was added to the mixture, which was stirred at r.t. for 2 h. Sat. aq. NH_4Cl (ca. 1 mL) was added to the reaction mixture, and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over Na_2SO_4, filtered through a cotton plug, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=8%-25%) to afford **compound 7c** (30.0 mg, 75%) as a colorless solid; $[\alpha]_D^{25}$ -7.44 (*c* 0.93, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 5.97–5.85 (m, 5H, -CH= of allyl, -CH= of alloc), 5.39–5.34 (m, 3H, =CH_2 of alloc, H-6), 5.32–5.30 (m, 2H, =CH_2 of allyl or alloc, H-1’) 5.29–5.22 (m, 6H, =CH_2 of allyl, =CH_2 of alloc, H-2”), 5.19–5.16 (m, 1H, =CH_2 of allyl), 5.14–5.07 (m, 2H, =CH_2 of allyl, H-3”), 4.93 (dd, *J* = 10.4, 10.4 Hz, 1H, H-4”), 4.66–4.58 (m, 6H, -CH_2- of alloc), 4.53–4.47 (m, 2H, H-5”, H-1’), 4.30 (dd, *J* = 12.2, 5.4 Hz, 1H, -CH_2- of allyl), 4.27–4.20 (m, 2H, -CH_2- of allyl), 4.12 (dd, *J* = 12.2, 5.8 Hz, 1H, -CH_2- of allyl), 3.88–3.83 (m, 1H, H-6’), 3.73–3.66 (m, 1H, H-6’), 3.62–3.47 (m, 3H, H-2’, H-3’, H-3), 3.37–3.27 (m, 2H, H-4’, H-5’), 2.44–2.40 (m, 1H, H-4), 2.29–2.24 (m, 1H, H-4), 2.02–1.78 (m, 6H, -CH_2- of cholesterol), 1.71–0.83 (m, 25H, H-6”, -CH_2- and -CH and -CH_3 of cholesterol), 0.95–0.85 (m, 10H, -CH and -CH_3 of cholesterol) 0.67 (s, 3H, H-18); ^{13}C-NMR ($CDCl_3$) δ 154.3 (C=O of alloc), 154.2 (C=O of alloc), 153.9 (C=O of alloc), 139.9 (C-5), 134.4 (=CH_2 of allyl), 134.3 (=CH_2 of allyl), 131.4 (=CH_2 of alloc), 131.2 (=CH_2 of alloc), 122.4 (C-6), 119.2 (-CH= of alloc), 118.8 (-CH= of alloc), 118.6 (-CH= of alloc), 117.5 (-CH= of allyl), 117.2 (-CH= of allyl), 99.1 (C-1’), 97.2 (C-1”), 84.7 (C-2’), 78.8 (C-4’), 77.7 (C-3’), 76.3 (C-5’), 75.0 (C-4”), 74.9 (C-3), 74.4 (-CH_2- of allyl), 73.7 (-CH_2- of allyl), 73.1 (C-3”), 72.9 (C-2”), 68.9 (-CH_2- of alloc), 68.8 (-CH_2- of alloc), 68.6 (-CH_2- of alloc), 66.1 (C-5”), 61.8 (C-6’), 56.7 (C-14), 56.1 (C-17), 50.1 (C-9), 42.3 (C-

13), 39.7 (C-12), 39.5 (C-24), 38.3 (C-4), 37.2 (C-1), 36.7 (C-10), 36.2 (C-22), 35.8 (C-20), 31.9 (C-8), 31.8 (C-7), 29.7 (C-2), 28.2 (C-16), 28.0 (C-25), 24.3 (C-15), 23.8 (C-23), 22.8 (C26 or C27), 22.5 (C26 or C27), 21.0 (C-11), 19.2 (C-21), 18.7 (C-19), 17.0 (C-6”), 11.8 (C-18); HRMS (ESI-QTOF) calcd. for $C_{57}H_{86}NaO_{16}$ $[M+Na]^+$ 1049.5808; found: 1049.5817.



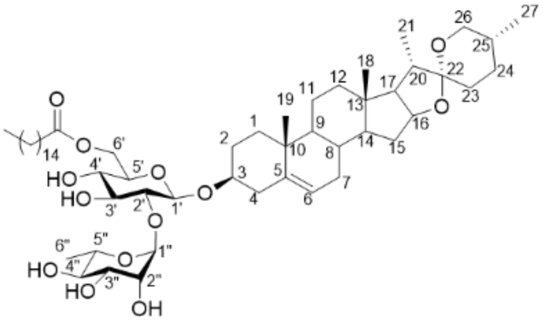
Decahydro-2-naphthol (2”,3”,4”-*O*-trialloc-α-L-rhamnopyranosyl)- α(1→2)-3’,4’-*O*-diallyl-β-D-glucopyranoside (7d)

To a stirred solution of **compound 6d** (90.0 mg, 87.1 μmol) in THF (1.7 mL) was added 1 M TBAF in THF (390 μL, 390 μmol), and the mixture was stirred at r.t. for 2.5 h. Then 1 M TBAF in THF (190 μL, 190 μmol) was added to the mixture, which was stirred at r.t. for 2 h. Sat. aq. NH_4Cl (ca. 1 mL) was added to the reaction mixture, and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over Na_2SO_4, filtered through a cotton plug, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=5%-27%) to afford **compound 7d** (53.1 mg, 77%) as a colorless solid; ^{1}H-NMR ($CDCl_3$) δ 5.97–5.85 (m, 5H, -CH= of allyl, -CH= of alloc), 5.39–5.32 (m, 4H, =CH_2 of alloc), 5.29–5.22 (m, 6H, =CH_2 of alloc, =CH_2 of allyl, H-1”, H-2”), 5.19–5.16 (m, 1H, =CH_2 of allyl) 5.15–5..08 (m, 2H, =CH_2 of allyl, H-3”), 4.97–4.89 (m, 1H, H-4”), 4.65–4.58 (m, 6H, -CH_2- of alloc), 4.52–4.42 (m, 2H, H-5”, H-1’), 4.31 (dd, J = 12.4, 6.0 Hz, 1H, -CH_2- of allyl), 4.27–4.19 (m, 2H, -CH_2- of allyl), 4.15–4.09 (m, 1H, -CH_2- of allyl), 3.88–3.84 (m, 2H, H-6’, H-2), 3.73–3.61 (m, 1H, H-6’), 3.56–3.45 (m, 2H, H-2’, H-3’), 3.37–3.28 (m, 2H, H-4’, H-5’), 2.10–1.30 (m, 14H, decahydro-2-naphthol), 1.26–1.22 (m, 5H, H-6”, decahydro-2-naphthol), 1.11–0.83 (m, 1H, decahydro-2-naphthol); ^{13}C-NMR ($CDCl_3$) δ 154.3–153.9 (C=O of alloc), 134.4–134.3 (=CH_2 of allyl), 131.3–131.1 (=CH_2 of alloc), 119.1–118.5 (-CH= of alloc), 117.5–117.1 (-CH= allyl), 99.9–99.1 (C-1’), 98.8–97.0 (C-1”), 84.8 (C-2’), 78.6 (C-4’), 78.1–77.8 (C-3’), 76.2–75.5 (C-5’), 75.0 (C-4”), 74.9 (C-3), 74.5–73.7 (-CH_2- of allyl), 73.1 (C-3”), 73.0 (C-2”), 68.9–68.6 (-CH_2- of alloc), 66.1 (C-5”), 61.9 (C-6’), 42.3, 41.2, 41.2, 41.0, 39.3, 35.2, 34.9, 34.6, 34.5, 33.9, 33.7, 33.5, 33.4, 33.1, 31.9, 31.7, 30.3, 28.5, 26.4, 26.2, 25.4, 24.9, 24.7 (decahydro-2-naphthol), 17.0 (C-6”); HRMS (ESI-QTOF) calcd. for $C_{40}H_{58}NaO_{16}$ $[M+Na]^+$ 817.3617; found: 817.3614.



Methyl (2”,3”,4”-*O*-trialloc-α-L-rhamnopyranosyl)- α(1→2)-3’,4’-*O*-diallyl-β-D-glucopyranoside (7e)

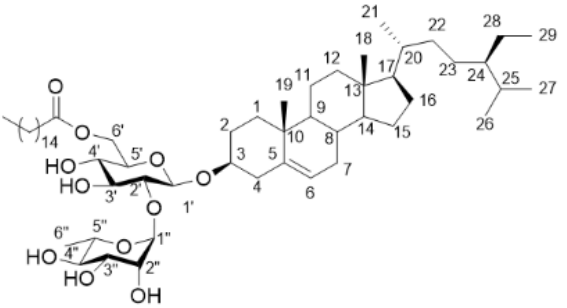
To a stirred solution of **compound 6e** (67.2 mg, 73.8 μmol) in THF (1.5 mL) was added 1 M TBAF in THF (330 μL, 330 μmol), and the mixture was stirred at r.t. for 2.5 h. Then 1 M TBAF in THF (160 μL, 160 μmol) was added to the mixture, which was stirred at r.t. for 2 h. Sat. aq. NH_4Cl (ca. 1 mL) was added to the reaction mixture, and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over Na_2SO_4, filtered filtered through a cotton plug, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=8%-35%) to afford **compound 7e** (32.7 mg, 66%) as a colorless solid; $[\alpha]_D^{25}$ -3.75 (*c* 1.30, $CHCl_3$); ^1H-NMR ($CDCl_3$) δ 5.96–5.84 (m, 5H, -CH= of allyl, -CH= of alloc), 5.37–5.30 (m, 3H, $=CH_2$ of alloc), 5.27–5.21 (m, 7H, $=CH_2$ of alloc, $=CH_2$ of allyl, H-1”, H-2”), 5.18–5.15 (m, 1H, $=CH_2$ of allyl) 5.13–5.09 (m, 3H, $=CH_2$ of allyl, H-3”), 4.92 (dd, *J* = 10.0, 10.0 Hz, 1H, H-4”), 4.67–4.56 (m, 6H, $-CH_2-$ of alloc), 4.34–4.27 (m, 1H, $-CH_2-$ of allyl), 4.24–4.17 (m, 4H, $-CH_2-$ of allyl, H-5”, H-1’), 4.16–4.10 (m, 1H, $-CH_2-$ of allyl), 3.89–3.85 (m, 1H, H-6’), 3.74–3.65 (m, 1H, H-6’), 3.45 (s, 3H, H-1), 3.47–3.43 (m, 2H, H-2’, H-3’), 3.38–3.34 (m, 1H, H-4’), 3.31–3.26 (m, 1H, H-5’), 1.24 (d, *J* = 6.4 Hz, 3H, H-6”); ^{13}C-NMR ($CDCl_3$) δ 154.2 (C=O of alloc), 154.2 (C=O of alloc), 153.9 (C=O of alloc), 134.4 ($=CH_2$ of allyl), 134.3 ($=CH_2$ of allyl), 131.3 ($=CH_2$ of alloc), 131.2 ($=CH_2$ of alloc), 131.1 ($=CH_2$ of alloc), 119.2 (-CH= of alloc), 119.0 (-CH= of alloc), 118.8 (-CH= of alloc), 117.5 (-CH= of allyl), 117.3 (-CH= of allyl), 102.4 (C-1’), 97.9 (C-1”), 84.3 (C-2’), 78.0 (C-4’), 77.4 (C-3’), 75.0 (C-5’), 74.8 (C-4”), 74.5 ($-CH_2-$ of allyl), 73.7 ($-CH_2-$ of allyl), 73.1 (C-3”, C-2”), 68.9 ($-CH_2-$ of alloc), 68.8 ($-CH_2-$ of alloc), 66.4 (C-5”), 61.6 (C-6’), 57.1 (C-1), 16.8 (C-6”); HRMS (ESI-QTOF) calcd. for $C_{31}H_{44}NaO_{16}$ $[M+Na]^+$ 695.2522; found: 695.2514.



Diosgenyl α-L-rhamnopyranosyl-α(1→2)-6'-*O*-palmitoyl-β-D-glucopyranoside (1a)

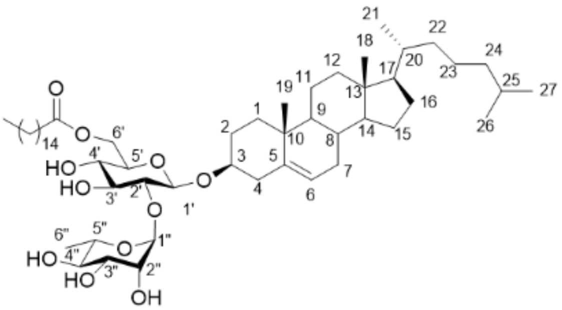
To a stirred solution of **compound 7a** (13.7 mg, 12.9 μmol) and palmitic acid (6.5 mg, 25.3 μmol) in CH_2Cl_2 (260 μL) was added EDCI· HCl (5.08 mg, 26.5 μmol), DMAP (2.0 mg, 16.3 μmol) and the mixture was stirred at 0 °C to r.t. for 3.75 h. Then, EDCI· HCl (5.08 mg, 26.5 μmol) was added to the mixture, which was stirred at r.t. for 1.5 h. H_2O (ca. 1 mL) was added to the reaction mixture, and the aqueous layer was extracted with EtOAc. The combined organic extracts was washed with 1M NaOH twice, H_2O, brine, dried over anhydrous Na_2SO_4, filtered through a cotton plug, and then concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=10/1) to give a partially purified material, which was used in the next step. A portion of the obtained material (11.2 mg) was dissolved in $CHCl_3$/MeOH=5:1 with TFA 1 % (290 μL) and Ru cat. (950 μg, 1.81 μmol) was added. The reaction mixture was stirred at r.t. for 2.5 h. Additional Ru cat. (430 μg, 0.82 μmol) was added, and the reaction mixture was stirred at r.t. for an additional 2 h. Then, Ru cat. (930 μg, 1.77 μmol) was added, and the reaction mixture was stirred for an additional 17 h. SiliaMetS® (25 mg) was added and the reaction mixture was stirred for 30 min at r.t., filtered through a PTFE membrane filter (0.5 μm, Advantec) and concentrated. The residue was purified by silica gel column chromatography (chloroform/methanol = 15/1–8/1) to give a mixture. The mixture was purified by silica gel column chromatography (chloroform/methanol = 15/1) with a pad of SiliaMetS® to afford **compound 1a** (6.63 mg, 43% over 2 steps) as a white solid; ^{1}H-NMR ($CDCl_3$/CD_3OD = 12:1, 30 °C) δ 5.35 (d, *J* = 4.8 Hz, 1H, H-6), 5.19 (s, 1H, H-1"), 4.44–4.23 (m, 4H, H-16, H-1', H-6'), 4.11–4.04 (m, 1H, H-5"), 3.94 (s, 1H, H-4'), 3.70 (dd, *J* = 9.6, 3.6 Hz, 1H, H-3'), 3.55–3.34 (m, 7H, H-3, H-26, H-2', H-5', H-3", H-4"), 3.31–3.26 (m, 1H, H-2"), 2.40–2.21 (m, 4H, α-CH_2 of palmitic acid, H-4), 2.01–1.42 (m, 18H, β-CH_2 of palmitic acid, -CH_2- and -CH- of diosgenin), 1.28–1.03 (m, 32H, H-6", -CH_2- of palmitic acid, H-1, H-7, H-12, H-14), 1.01 (s, 3H, H-19), 0.98–0.45 (m, 4H, H-9, H-21), 0.93–0.82 (t, *J* = 6.8 Hz, 3H, -CH_3 of palmitic acid), 0.80–0.79 (m, 6H, H-18, H-27); ^{13}C-NMR ($CDCl_3$/CD_3OD = 12:1, 30 °C) δ 174.4 (C=O of palmitic acid), 140.4 (C-5), 121.5 (C-6), 109.4 (C-22), 100.2 (C-1"), 99.9 (C-1'), 80.8 (C-16), 79.2 (C-2'), 77.5 (C-3), 73.4 (C-4"), 72.7 (C-3"), 71.2 (C-3'), 70.4 (C-4'), 70.3 (C-2"), 68.1 (C-5"), 66.7 (C-26), 63.6 (C-6'), 61.9 (C-17), 56.4 (C-14), 50.0 (C-9), 41.5 (C-4), 40.1 (C-20), 39.6 (C-13), 38.3 (C-12), 37.1 (C-1), 36.7 (C-10), 34.1 (α-CH_2 of palmitic acid), 31.9 (C-7), 31.8 ((ω-

2)-CH_2 of palmitic acid), 31.6 (C-2 and C-15), 31.2 (C-8), 31.2 (C-23), 30.1 (C-25), 29.6 (-CH_2 of palmitic acid), 29.4 (C-24), 29.2 (-CH_2 of palmitic acid), 29.1 (-CH_2 of palmitic acid), 28.6 (C-24), 24.8 (β-CH_2 of palmitic acid), 22.6 ((ω-1)-CH_2 of palmitic acid), 20.7 (C-11), 19.1 (C-19), 17.2 (C-6"), 16.9 (C-27), 16.1 (C-18), 14.3 (C-21), 14.0 (-CH_3 of palmitic acid); HRMS (ESI-QTOF) calcd. for $C_{55}H_{92}NaO_{13}$ $[M+Na]^+$ 983.6430; found: 983.6431.



β-sitosteryl α-L-rhamnopyranosyl-α(1→2)-6'-*O*-palmitoyl-β-D-glucopyranoside (1b)

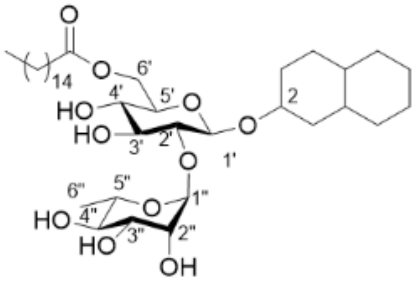
To a stirred solution of **compound 7b** (20.8 mg, 19.7 μmol) and palmitic acid (10.3 mg, 40.2 μmol) in CH_2Cl_2 (400 μL) was added EDCI· HCl (8.38 mg, 43.7 μmol), DMAP (2.50 mg, 20.4 μmol) and the mixture was stirred at r.t. for 11 h. H_2O (ca. 1 mL) was added to the reaction mixture, and the aqueous layer was extracted with EtOAc. The combined organic extracts was washed with 1M NaOH twice, H_2O, brine, dried over anhydrous Na_2SO_4, filtered through a cotton plug, and then concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=5%-40%) to give a partially purified material, which was used in the next step. A portion of the obtained material (15.8 mg) was dissolved in $CHCl_3$/MeOH=5:1 with TFA 1 % (400 μL) and Ru cat. (1.60 mg, 3.04 μmol) was added. The reaction mixture was stirred at r.t. for 12 h. SiliaMetS® (57.3 mg) was added and the reaction mixture was stirred for 30 min at r.t., filtered through a PTFE membrane filter (0.5 μm, Advantec) and concentrated. The residue was purified by silica gel column chromatography (chloroform/methanol = 3%–28%) with a pad of SiliaMetS® three times to afford **compound 1b** (7.59 mg, 73% over two steps) as a white solid; ^1H-NMR ($CDCl_3/CD_3OD$ = 12:1, 30 °C) δ 5.35 (d, *J* = 4.8 Hz, 1H, H-6), 5.19 (s, 1H, H-1"), 4.41 (d, *J* = 7.2 Hz, 1 H, H-1' β anomer), 4.35 (dd, *J* = 12.2, 2.2 Hz, 1 H, H-6'), 4.25 (dd, *J* = 12.0, 6.8 Hz, 1H, H-6'), 4.11–4.04 (m, 1H, H-5"), 3.95 (d, *J* = 1.6 Hz, 1H, H-4'), 3.70 (dd, *J* = 9.6, 3.6 Hz, 1H, H-3'), 3.56–3.38 (m, 6H, H-2', H-5', H-3", H-4", H-3), 3.30–3.26 (m, 1H, H-2"), 2.40–2.26 (m, 4H, α-CH_2 of palmitic acid, H-4), 2.03–1.80 (m, 6H, -CH_2- of β-sitosterol), 1.71–1.00 (m, 49H, H-6", -CH_2- of palmitic acid, -CH_2- and -CH of β-sitosterol), 0.97–0.81 (m, 21H, -CH_3 of palmitic acid, -CH_3 of β-sitosterol) 0.68 (s, 3H, H-18); ^{13}C-NMR ($CDCl_3/CD_3OD$ = 12:1) δ 174.3 (C=O of acyl), 140.4 (C-5), 121.8 (C-6), 100.2 (C-1"), 99.8 (C-1'), 79.3 (C-2'), 77.5 (C-3), 73.4 (C-4"), 72.7 (C-3"), 71.2 (C-3'), 70.4 (C-4'), 70.3 (C-2"), 68.1 (C-5"), 63.6 (C-6’), 56.6 (C-14), 55.9 (C-17), 50.1 (C-9) 45.7 (C-24), 42.2 (C-13), 39.6 (C-12), 38.4 (C-4), 37.2 (C-1), 36.6 (C-10), 36.0 (C-20), 34.2 (α-CH_2 of palmitic acid), 33.8 (C-22), 31.8 ((ω-2)-CH_2 of palmitic acid and C-8), 31.7 (C-7), 29.6 (-CH_2 of palmitic acid), 29.5 (-CH_2 of palmitic acid), 29.5 (C-2), 29.2 (-CH_2 of palmitic acid), 29.1 (C-25), 29.0 (-CH_2 of palmitic acid), 28.1 (C-16), 25.9 (C-23), 24.8 (β-CH_2 of palmitic acid), 24.1 (C-15), 22.9 (C-28), 22.6 ((ω-1)-CH_2 of palmitic acid), 20.9 (C-11), 19.7 (C-26 or C27), 19.1 (C-26 or C27), 18.8 (C-21), 18.6 (C-19), 17.2 (C-6”), 14.0 (-CH_3 of palmitic acid), 11.8 (C-18), 11.7 (C-29); HRMS (ESI-QTOF) calcd. for $C_{57}H_{100}NaO_{11}$ $[M+Na]^+$ 983.7158; found: 983.7158.



Cholesteryl α-L-rhamnopyranosyl-α(1→2)-6’-*O*-palmitoyl-β-D-glucopyranoside (1c)

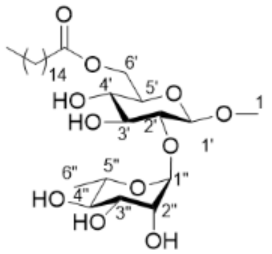
To a stirred solution of **compound 7c** (22.9 mg, 22.3 μmol) and palmitic acid (11.4 mg, 44.5 μmol) in CH_2Cl_2 (580 μL) was added EDCI· HCl (8.53 mg, 44.5 μmol), DMAP (2.70 mg, 22.1 μmol) and the mixture was stirred at r.t. for 11 h. H_2O (ca. 1 mL) was added to the reaction mixture, and the aqueous layer was extracted with EtOAc. The combined organic extracts was washed with 1M NaOH twice, H_2O, brine, dried over anhydrous Na_2SO_4, filtered through a cotton plug, and then concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=5%-40%) to give a partially purified material, which was used in the next step. A portion of the obtained material (16.1 mg) was dissolved in $CHCl_3$/MeOH=5:1 with TFA 1 % (425 μL) and Ru cat. (1.67 mg, 3.18 μmol) was added. The reaction mixture was stirred at r.t. for 12 h. SiliaMetS® (59 mg) was added and the reaction mixture was stirred for 30 min at r.t., filtered through a PTFE membrane filter (0.5 μm, Advantec) and concentrated. The residue was purified by silica gel column chromatography (chloroform/methanol = 3%–26%) with a pad of SiliaMetS® to afford **compound 1c** (6.61 mg, 56% over two steps) as a white solid; ^{1}H-NMR ($CDCl_3$/CD_3OD = 12:1, 30 °C) δ 5.35 (d, *J* = 4.8 Hz, 1H, H-6), 5.19 (s, 1H, H-1”), 4.41 (d, *J* = 7.2 Hz, 1H, H-1’ β anomer), 4.35 (dd, *J* = 12.0, 2.0 Hz, 1H, H-6’), 4.25 (dd, *J* = 12.2, 6.6 Hz, 1H, H-6’), 4.12–4.04 (m, 1H, H-5”), 3.94 (d, *J* = 2.0 Hz, 1H, H-4’), 3.69 (dd, *J* = 9.6, 3.2 Hz, 1H, H-3’), 3.56–3.37 (m, 6H, H-2’, H-5’, H-3”, H-4”, H-3), 3.31–3.26 (m, 1H, H-2”), 2.40–2.26 (m, 4H, α-CH_2 of palmitic acid, H-4), 2.03–1.78 (m, 5H, -CH_2- of cholesterol), 1.68–0.98 (m, 54H, H-6”, -CH_2- of palmitic acid, -CH_2- and -CH of cholesterol), 0.95–0.84 (m, 15H,

-CH_3 of palmitic acid, -CH_3 of cholesterol) 0.68 (s, 3H, H-18); ^{13}C-NMR ($CDCl_3$/CD_3OD = 12:1, 30 °C) δ 174.3 (C=O of palmitic acid), 140.4 (C-5), 121.8 (C-6), 100.2 (C-1”), 99.8 (C-1’), 79.3 (C-2’), 77.6 (C-3), 77.2, 73.4 (C-4”), 72.7 (C-3”), 71.2 (C-3’), 70.4 (C-4’), 70.3 (C-2”), 68.1 (C-5”), 63.6 (C-6’), 56.6 (C-14), 56.0 (C-17), 50.1 (C-9), 42.2 (C-13), 39.6 (C-12), 39.4 (C-24), 38.4 (C-4), 37.2 (C-1), 36.6 (C-10), 36.1 (C-22), 35.7 (C-20), 34.2, 31.8 (C-8), 31.7 (C-7), 29.6 (C-2), 29.5, 29.2, 29.1, 28.1 (C-16), 27.9 (C-25), 24.8, 24.1 (C-15), 23.7 (C-23), 22.6, 22.6 (C26 or C27), 22.4 (C26 or C27), 20.9 (C-11), 19.1 (C-21), 18.5 (C-19), 17.2 (C-6”), 13.9, 11.7 (C-18); HRMS (ESI-QTOF) calcd. for $C_{55}H_{96}NaO_{11}$ $[M+Na]^+$ 955.6845; found: 955.6841.



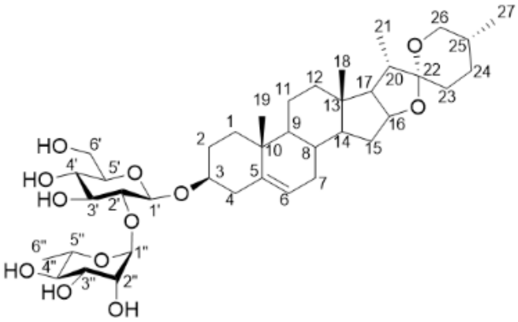
Decahydro-2-naphthol α-L-rhamnopyranosyl-α(1→2)-6’-*O*-palmitoyl-β-D-glucopyranoside (1d)
To a stirred solution of **compound 7d** (36.2 mg, 45.6 μmol) and palmitic acid (25.27 mg, 98.5 μmol) in CH_2Cl_2 (910 μL) was added EDCI· HCl (19.4 mg, 101 μmol), DMAP (5.91 mg, 48.4 μmol) and the mixture was stirred at r.t. for 11 h. H_2O (ca. 1 mL) was added to the reaction mixture, and the aqueous layer was extracted with EtOAc. The combined organic extracts was washed with 1M NaOH twice, H_2O, brine, dried over anhydrous Na_2SO_4, filtered through a cotton plug, and then concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=5%-40%) to give a partially purified material, which was used in the next step. A portion of the obtained material (18.11 mg) was dissolved in $CHCl_3$/MeOH=5:1 with TFA 1 % (600 μL) and Ru cat. (1.02 mg, 1.95 μmol) was added. The reaction mixture was stirred at r.t. for 2.5 h. Additional Ru cat. (1.06 mg, 2.02 μmol) was added, and the reaction mixture was stirred at r.t. for an additional 3 h. Then, Ru cat. (626 μg, 1.19 μmol) was added, and the reaction mixture was stirred for an additional 12 h. SiliaMetS® (60 mg) was added and the reaction mixture was stirred for 30 min at r.t., filtered through a PTFE membrane filter (0.5 μm, Advantec) and concentrated. The residue was purified by silica gel column chromatography (chloroform/methanol = 3%–28%) with a pad of SiliaMetS® to afford **compound 1d** (12.3 mg, quant. over two steps) as a white solid; ^{1}H-NMR ($CDCl_3$/CD_3OD = 12:1, 30 °C) δ 5.23-5.20 (m, 1H, H-1”), 4.43–4.34 (m, 2H, H-1’, H-6’), 4.26–4.21 (m, 1H, H-6’), 4.07–4.03 (m, 1H, H-5”),

3.93–3.92 (m, 1H, H-4’), 3.68 (dt, *J* = 9.6, 3.2 Hz, 1H, H-3’), 3.52–3.36 (m, 4H, H-2’, H-5’, H-3”, H-4”), 3.31–3.26 (m, 1H, H-2”), 2.33 (t, *J* = 7.8 Hz, 2H, α-CH_2 of palmitic acid), 2.07–1.78 (m, 3H, decahydro-2-naphthol), 1.70–1.26 (m, 37H, -CH_2 of palmitic acid, decahydro-2-naphthol), 1.15–0.93 (m, 2H, decahydro-2-naphthol), 0.90–0.88 (m, 4H, -CH_3 of palmitic acid, decahydro-2-naphthol); ^{13}C-NMR ($CDCl_3$/CD_3OD = 12:1, 30 °C) δ 174.2 (C=O of palmitic acid), 100.2 (C-1”), 99.8 (C-1’), 77.6 (C-2’), 74.9 (C-2), 73.4 (C-4”), 72.6 (C-3”), 71.1 (C-3’), 70.5 (C-4’), 70.4 (C-2”), 68.3 (C-5”), 63.7 (C-6’), 35.2 (decahydro-2-naphthol), 34.1 (α-CH_2 of palmitic acid), 33.7 ((ω-2)-CH_2 of palmitic acid), 31.8 (decahydro-2-naphthol), 29.4 (-CH_2 of palmitic acid), 29.2–29.1 (-CH_2 of palmitic acid), 24.8 (β-CH_2 of palmitic acid), 22.5 ((ω-1)-CH_2 of palmitic acid), 17.2 (C-6”), 13.9 (-CH_3 of palmitic acid); HRMS (ESI-QTOF) calcd. for $C_{38}H_{68}NaO_{11}$ $[M+Na]^+$ 723.4654; found: 723.4652.



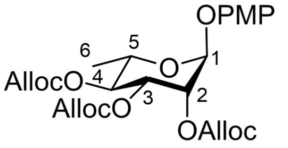
Methyl α-L-rhamnopyranosyl-α(1→2)-6’-*O*-palmitoyl-β-D-glucopyranoside (1e)

To a stirred solution of **compound 7e** (16.0 mg, 23.8 μmol) and palmitic acid (12.5 mg, 48.7 μmol) in CH_2Cl_2 (480 μL) was added EDCI· HCl (9.88 mg, 51.5 μmol), DMAP (2.97 mg, 24.3 μmol) and the mixture was stirred at r.t. for 11 h. H_2O (ca. 1 mL) was added to the reaction mixture, and the aqueous layer was extracted with EtOAc. The combined organic extracts was washed with 1M NaOH twice, H_2O, brine, dried over anhydrous Na_2SO_4, filtered through a cotton plug, and then concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=6%-50%) to give a partially purified material, which was used in the next step. A portion of the obtained material (11.4 mg) was dissolved in $CHCl_3$/MeOH=5:1 with TFA 1 % (415 μL) and Ru cat. (1.64 mg, 3.12 μmol) was added. The reaction mixture was stirred at r.t. for 12 h. SiliaMetS® (57 mg) was added and the reaction mixture was stirred for 30 min at r.t., filtered through a PTFE membrane filter (0.5 μm, Advantec) and concentrated. The residue was purified by silica gel column chromatography (chloroform/methanol = 3%–28%) with a pad of SiliaMetS® to afford **compound 1e** (5.14 mg, 73% over two steps) as a white solid; ^{1}H-NMR ($CDCl_3$/CD_3OD = 12:1) δ 5.11 (d, *J* = 1.2 Hz, 1H, H-1” α anomer), 4.39 (dd, *J* = 12.2, 2.2 Hz, 1H, H-6’), 4.26 (dd, *J* = 12.4, 6.0 Hz, 1H, H-6’), 4.21 (d, *J* = 7.6 Hz, 1H, H-1’ β anomer), 3.94–3.87 (m, 2H, H-4’, H-5”), 3.67 (dd, *J* = 9.6, 3.6 Hz, 1H, H-3’), 3.51 (s, 1H, H-1), 3.49–3.63 (m, 4H, H-2’, H-5’, H-3”, H-4”), 3.33–3.28 (m, 1H, H-2”), 2.35 (t, *J* = 7.8 Hz, 2H, α-CH_2 of palmitic acid), 1.61 (m, 2H, β-CH_2 of palmitic acid), 1.27 (m, 27H, H-6”, -CH_2- of palmitic acid), 0.88 (t, *J* = 6.8 Hz, 3H, -CH_3 of palmitic acid); ^{13}C-NMR ($CDCl_3$/CD_3OD = 12:1) δ 174.4 (C=O of palmitic acid), 102.5 (C-1’), 100.7 (C-1”), 78.1 (C-2’), 73.4 (C-4”), 72.5 (C-3”), 71.0 (C-3’), 70.3 (C-4’), 70.0 (C-2”), 68.2 (C-5”), 63.4 (C-6’), 56.6 (C-1), 34.0 (α-CH_2 of palmitic acid), 31.7 ((ω-2)-CH_2 of palmitic acid), 29.5 (-CH_2 of palmitic acid), 29.3 (-CH_2 of palmitic acid), 29.2 (-CH_2 of palmitic acid), 29.1 (-CH_2 of palmitic acid), 28.9 (-CH_2 of palmitic acid), 24.7 (β-CH_2 of palmitic acid), 22.5 ((ω-1)-CH_2 of palmitic acid), 16.8 (C-6”), 13.9 (-CH_3 of palmitic acid); HRMS (ESI-QTOF) calcd. for $C_{29}H_{54}NaO_{11}$ $[M+Na]^+$ 601.3558; found: 601.3549.



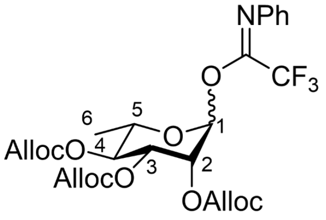
Diosgenyl α-L-rhamnopyranosyl-α(1→2)-β-D-glucopyranoside (1a’)

To a stirred solution of **compound 6a** (12.5 mg, 11.9 μmol) in $CHCl_3$/MeOH=5:1 with TFA 1 % (218 μL), was added Ru cat. (1.59 mg, 3.03 μmol). The mixture was stirred at r.t. for 11.5 h. SiliaMetS® (56 mg) was added to the mixture, which was filtered, then concentrated under reduced pressure, which was purified by silica gel column chromatography (chloroform/methanol=3%-28%) twice with a pad of SiliaMetS® to afford **compound 1a’** (4.86 mg, 57%) as a white solid;. ^{1}H-NMR ($CDCl_3$/CD_3OD = 12:1, 30 °C) δ 5.35 (d, *J* = 5.2 Hz, 1H, H-6), 5.19 (s, 1H, H-1”), 4.47–4.38 (m, 2H, H-1’, H-16), 4.11–4.04 (m, 1H, H-5”), 3.95 (s, 1H, H-4’), 3.83 (dd, *J* = 11.8, 3.0 Hz, 1H, H-3’), 3.75 (dd, *J* = 12.4, 4.4 Hz, 1H, H-6’), 3.70 (dd, *J* = 9.6, 3.2 Hz, 1H, H-6’), 3.60–3.34 (m, 7H, H-2’, H-2”, H-3”, H-4”, H-3, H-26), 3.28–3.24 (m, 1H, H-5’), 2.42–2.38 (m, 1H, H-4), 2.30–2.25 (m, 1H, H-4), 2.01–1.95 (m, 2H, H-15), 1.91–1.83 (m, 3H, H-1, H-8, H-20), 1.80–1.39 (m, 11H, -CH_2- and -CH of diosgenin), 1.33–0.83 (m, 5H, H-6”, H-7), 1.22–1.05 (m, 3H, H-1, H-12, H-14), 1.02 (s, 3H, H-19), 0.89–0.96 (d, 3H, *J* = 7.6 Hz, H-21), 0.95–0.83 (m, 1H, H-9), 0.80–0.79 (m, 6H, H-18, H-27); ^{13}C-NMR ($CDCl_3$/CD_3OD = 12:1, 30 °C) δ 140.3 (C-5), 121.7 (C-6), 109.4 (C-22), 100.3 (C-1”), 99.5 (C-1’), 80.8 (C-16), 78.7 (C-2”), 77.5 (C-3), 75.3 (C-4”), 72.8 (C-3”), 71.2 (C-3’), 70.4 (C-4’), 70.2 (C-2”), 68.1 (C-5”), 66.8 (C-26), 61.9 (C-6’), 61.8 (C-17), 56.4 (C-14), 50.0 (C-9), 41.5 (C-4), 40.2 (C-20), 39.6 (C-13), 38.3 (C-12), 37.1 (C-1), 36.8 (C-10), 31.9 (C-7), 31.6 (C-15), 31.3 (C-2), 31.2 (C-8), 30.1 (C-23), 29.6 (C-25), 29.5, 28.6 (C-24), 20.7 (C-11), 19.1 (C-19), 17.2 (C-6”), 16.9 (C-27), 16.1 (C-18), 14.3 (C-21); HRMS (ESI-QTOF) calcd. for $C_{39}H_{62}NaO_{12}$ $[M+Na]^+$ 745.4133; found: 745.4124.



p-methoxyphenyl 2,3,4-*O*-trialloc-L-rhamnopyranoside (S2)

To a stirred solution of **compound S1** (145 mg, 0.536 mmol) and *N*,*N*,*N*′,*N*′-Tetramethylethylenediamine (185 µL, 1.24 mmol) in CH_2Cl_2 (4.5 mL) was added Allyl Chloroformate (250 µL, 2.36 mmol) at 0 °C, and the mixture was stirred at 0 °C for 1 h. CH_2Cl_2 (ca. 10 mL) was added to the reaction mixture. The mixture was cooled to 0 ºC, and sat. aq. $NaHCO_3$ (ca. 10 mL) was added. The aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were washed with H_2O and brine, dried over anhydrous Na_2SO_4, filtered filtered through a cotton plug, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=8/1-4/1) to afford **compound S2** (256 mg, 91%) as colorless oil; ^{1}H-NMR ($CDCl_3$) δ 7.02–6.97 (m, 2H, Ar-H of PMP), 6.83 (m, 2H, Ar-H of PMP), 5.99–5.87 (m, 3H, -CH= of alloc), 55.45 (d, *J* = 1.6 Hz, H-1 α anomer), 5.41–5.32 (m, 5H), 5.01 (t, *J* = 9.8 Hz, 1H, H-4), 4.69–4.61 (m, 6H, -CH_2- of alloc), 4.09–4.02 (m, 1H, H-5), 3.78 (s, 3H, -OCH_3 of PMP), 1.26 (d, *J* = 6.4 Hz, 3H, H-6); ^{13}C-NMR ($CDCl_3$) δ 155.2 (C=O of alloc), 154.2 (C=O of alloc and *ipso*-Ph of PMP), 153.9 (C=O of alloc), 149.7 (*p*-Ph of PMP), 131.3 (=CH_2 of alloc), 131.2 (=CH_2 of alloc), 131.0 (=CH_2 of alloc), 119.4 (-CH= of alloc), 118.9 (-CH= of alloc), 118.9 (-CH= of alloc), 117.6 (*m*-Ph of PMP), 114.6 (*o*-Ph of PMP), 96.1 (C-1), 74.6 (C-4), 72.8 (C-2, C-3), 69.1 (-CH_2- of alloc), 68.9 (-CH_2- of alloc), 68.8 (-CH_2- of alloc), 66.9 (C-5), 55.6 (-OCH_3 of PMP), 17.2 (C-6); HRMS (ESI-QTOF) calcd. for $C_{25}H_{30}NaO_{12}$ $[M+Na]^+$ 545.1629; found: 545.1632.



2,3,4-*O*-trialloc-α-L-rhamnopyranosyl *N*-phenyltrifluoroacetimidate (5)

To a stirred solution of **compound S2** (147 mg, 282 µmol) in CH_3CN/H_2O=4/1 (14 mL) was added CAN (306 mg, 55.8 µmol), and the mixture was stirred at 0 °C. Additional portions of CAN (128 mg, 46 mg, and 65 mg) were added after 1 h, 2 h 25 min, and 3 h, respectively. After stirring for a total of 3 h 5 min, brine (ca. 10 mL) was added to the mixture, and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered filtered through a cotton plug, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=5/1-2/1) to afford a red oil. The obtained material was dissolved in CH_2Cl_2 (5.8 mL) and treated with DMAP (24.7 mg, 202 μmol), DIPEA (98 μL, 0.58 mmol), and 2,2,2-trifluoro-*N*-phenylacetimidoyl (PTFAI) chloride (50 mL, 0.32 mmol). After stirring for 1 h at r.t., the reaction mixture was concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=15/1-10/1) to afford **compound 5** (anomer mixture 74.2 mg, 45% over two steps) as white solid; $[\alpha]_D^{25}$ -13 (c 1.03, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 7.31 (t, *J* = 8.0 Hz, 2H, Ar-H of PTFAI), 7.12 (t, *J* = 7.6 Hz, 1H, Ar-H of PTFAI), 6.83 (d, *J* = 7.6 Hz, 2H, Ar-H of PTFAI), 6.29 (bs, 1H, H-1), 5.97–5.88 (m, 3H, -CH= of alloc), 5.43–5.41 (m, 1H, H-2), 5.41–5.34 (m, 3H, $=CH_2$ of alloc), 5.31–5.26 (m, 3H, $=CH_2$ of alloc), 5.20 (dd, *J* = 10.4, 3.6 Hz, 1H, H-3), 5.04 (dd, *J* = 2.4, 2.0 Hz, 1H, H-4), 4.68–4.64 (m, 6H, $-CH_2-$ of alloc), 4.08–4.02 (m, 1H, H-5), 1.34 (d, *J* = 6.4 Hz, 3H, H-6); ^{13}C-NMR ($CDCl_3$) δ 154.0 (C=O of alloc),153.9 (C=O of alloc), 153.6 (C=O of alloc), 142.8 (*ipso*-Ph of PTFAI), 131.1 ($=CH_2$ of alloc), 131.0 ($=CH_2$ of alloc), 130.8 ($=CH_2$ of alloc), 128.7 (*m*-Ph of PTFAI), 124.6 (*p*-Ph of PTFAI), 119.4 (-CH= of alloc), 119.2 (*o*-Ph of PTFAI), 118.9 (-CH= of alloc), 118.8 (-CH= of alloc), 93.2 (H-1), 73.8 (C-4), 72.4 (C-3), 71.2 (C-2), 69.2 (C-5), 68.9 ($-CH_2-$ of alloc), 68.9 ($-CH_2-$ of alloc), 68.9 ($-CH_2-$ of alloc), 17.2 (C-6); HRMS (ESI-QTOF) calcd. for $C_{26}H_{28}NaO_{11}$ $[M+Na]^+$ 610.1507; found: 610.1505.

2.Biology

Mincle GFP reporter assay[6]

The 2B4 NFAT-GFP reporter cells were established in the author's laboratory (S. Yamasaki, Osaka University) as previously described.[6] Briefly, the host 2B4 T-cell hybridoma, which carries an NFAT-GFP reporter, was transfected with a pMX-IRES-hCD8 vector encoding either human or mouse Mincle, along with an expression plasmid for the FcRγ chain.

For the ligand stimulation assay, 2B4 NFAT-GFP reporter cells were cultured in RPMI 1640 (Nacalai Tesque) supplemented with 10% fetal bovine serum (FBS; Biowest). The ligands were dissolved in CH_2Cl_2/MeOH (2:1) at 1 mM, diluted in isopropanol, and added to 96-well plates at the indicated concentrations. After evaporation of the solvent, the 2B4-NFAT-GFP reporter cells (6×10^4 cells/well) expressing human Mincle, mouse Mincle, or FcR_g only were seeded and incubated at 37°C for 18 h. The activation of the cells was monitored by measuring GFP expression using flow cytometry.
All assays were performed in triplicate, except for one condition in Fig. 2C (mMincle, compound 1e at 1.0 nmol/well, n = 2). Data were analyzed and visualized using GraphPad Prism software. Results are presented as the mean ± s.d. of representative data from at least two or three independent experiments.

Mincle Binding Assay[7]

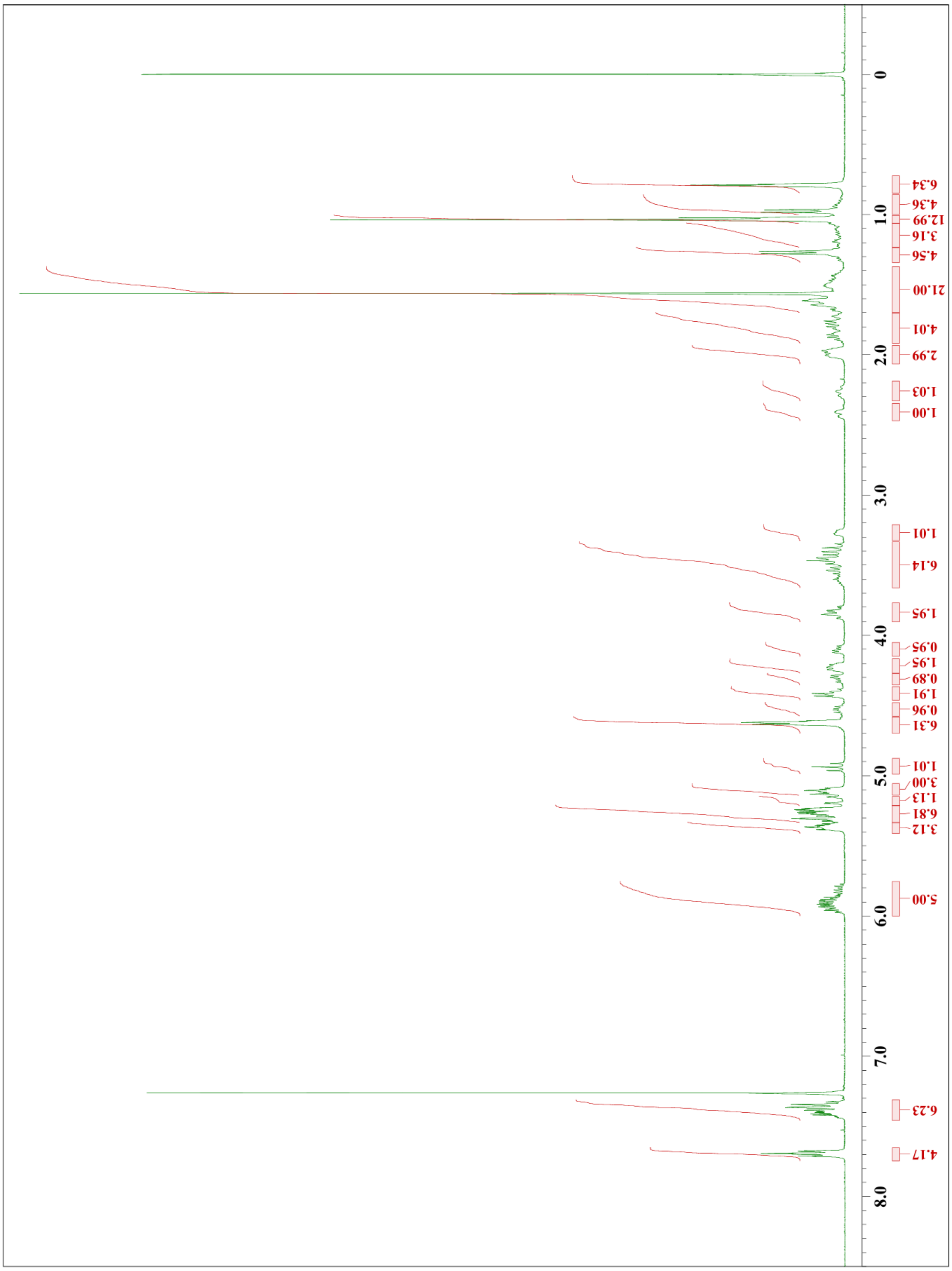
The ligands solution (1 mM in CH_2Cl_2/MeOH (2:1)) were diluted in isopropanol and added to 96-well plates with the indicated concentrations, followed by evaporation of the solvent. After blocking with 5% BSA in Tris sodium magnesium (TMS) buffer, the plates were incubated with human Mincle-Fc (hMincle-Fc) or human IgG-Fc (hIgG-Fc) as a negative control at r.t. for 4 h. The bound fusion proteins were detected using a direct ELISA format. The plates were washed five times with TMS buffer followed by incubation with the HRP-conjugated anti-human IgG antibody (1:4000 dilution) at r.t. for 1 h. After final washing, the binding was visualized using substrate solution (at r.t. for 30 min in dark) and the reaction was quenched with stop solution. The absorbance at 450 nm was measured using a microplate reader.

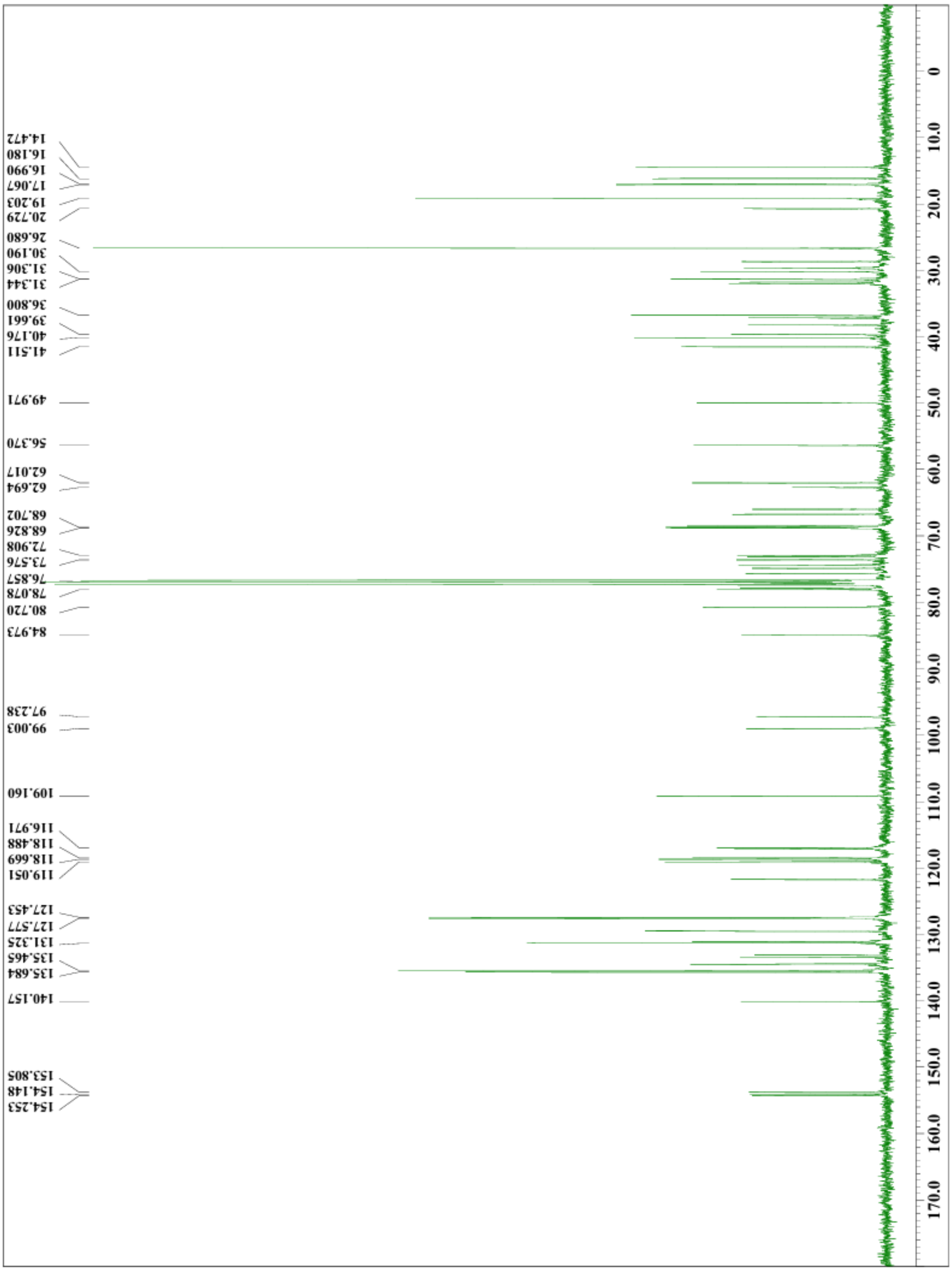
Reference

1. T. Watanabe, M. Nagai, Y. Ishibashi, M. Iwasaki, M. Mizoguchi, M. Nagata, T. Imai, K. Takato, A. Imamura, Y. Kakuta, T. Teramoto, M. Tani, J. Matsuda, H. Ishida, S. Yamasaki, N. Okino and M. Ito, *PLoS Pathog.*, 2025, **21**, e1013089.
2. a) T. Matsumaru, K. Sueyoshi, K. Okubo, S. Fujii, K. Sakuratani, R. Saito, K. Ueki, S. Yamasaki and Y. Fujimoto, *Bioorg. Med. Chem.,* 2022, **75**, 117045.
   b) T. Matsumaru, R. Ikeno, Y. Shuchi, T. Iwamatsu, T. Tadokoro, S. Yamasaki, Y. Fujimoto, A. Furukawa, K. Maenaka, *Chem. Commun.,* 2019, **55**, 711–714.
3. A. Furukawa, Y. Shuchi, J. Wang, P. Guillen-Poza, S. Ishizuka, M. Kagoshima, R. Ikeno, H. Kumeta, S. Yamasaki, T. Matsumaru and T. Saitoh, *Structure*, 2023, **31**, 1077–1085.e5.
4. C. Fontana, G. Widmalm, *Chem. Rev.,* 2023, **123**, 1040–1120.
5. S. Tanaka, H. Saburi, Y. Ishibashi and M. Kitamura, *Org. Lett.* 2004, **6**, 1873–1875.
6. S. Yamasaki, E. Ishikawa, M. Sakuma, H. Hara, K. Ogata and T. Saito, *Nat Immunol*, 2008, **9**, 1179–1188.
7. M. Watanabe, Z. Omahdi and S. Yamasaki, in *Lectin Purification and Analysis: Methods and Protocols*, J. Hirabayashi, Springer, New York, 2020, **vol. 2132**, ch. 12, pp. 119–128.

NMR spectra

diosgenyl (2,3,4-*O*-trialloc-α-L-rhamnopyranosyl)-(1→2)-3,4-*O*-diallyl-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranoside (6a)

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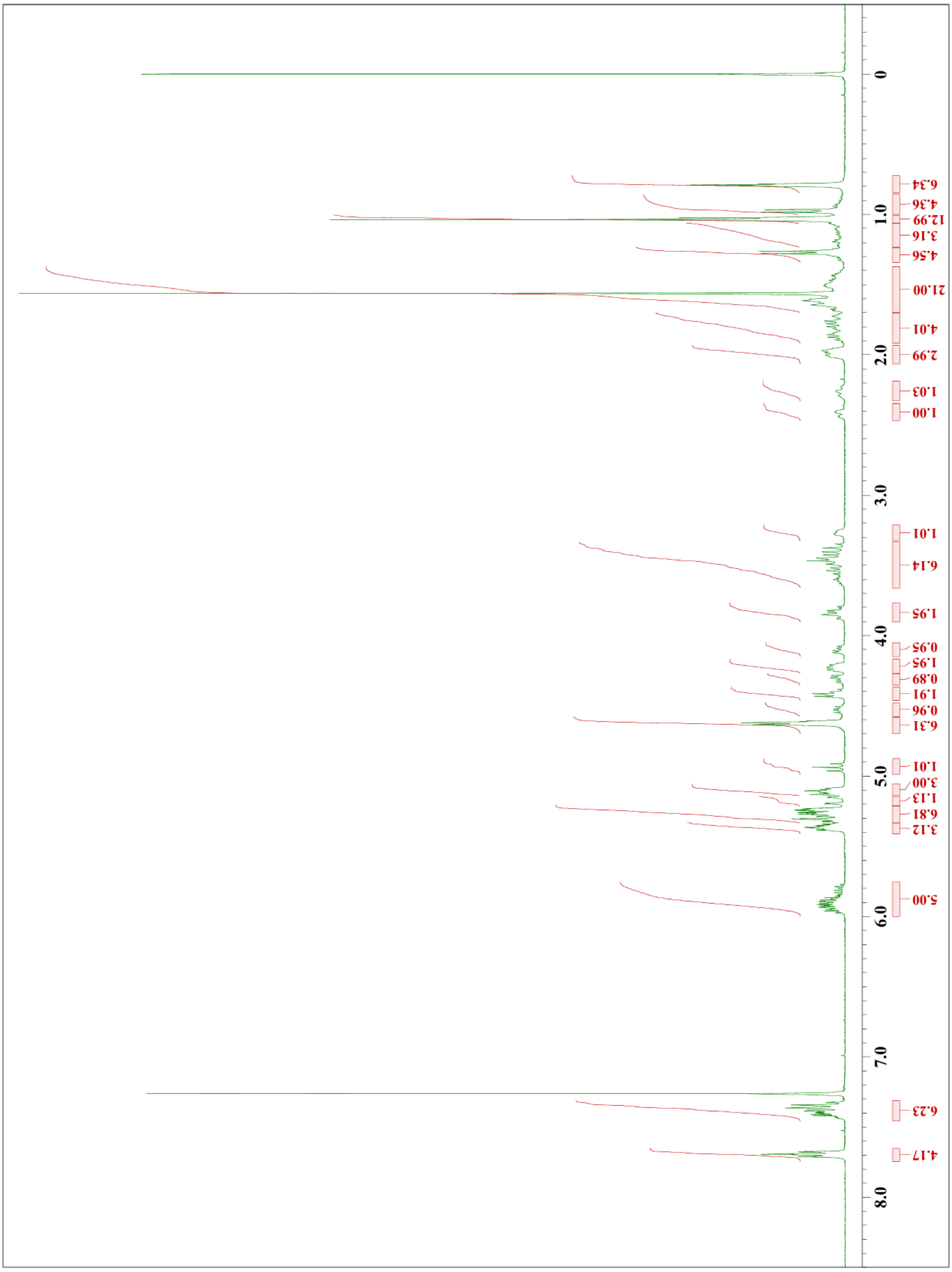
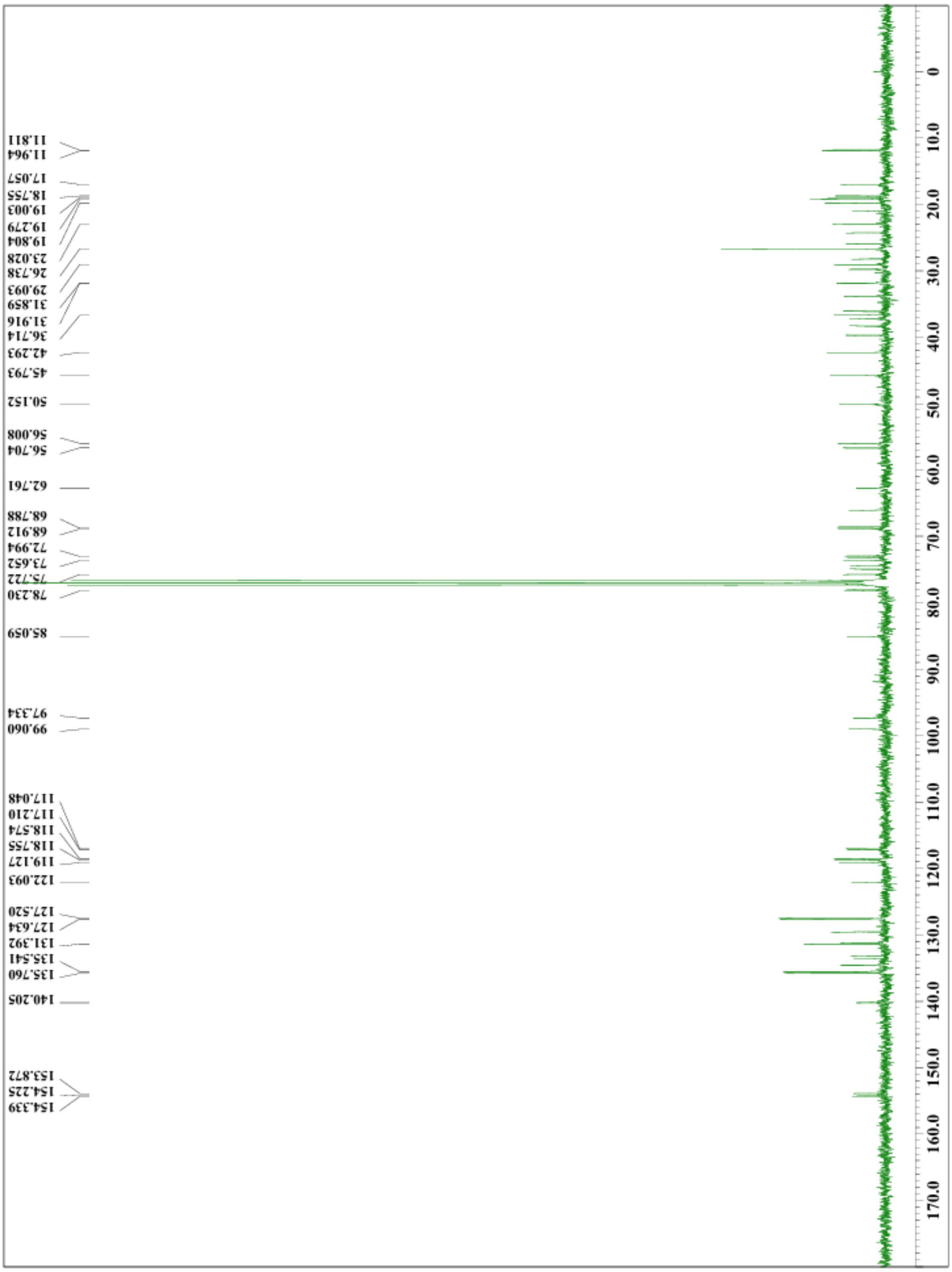
β-sitosteryl (2,3,4-*O*-trialloc-α-L-rhamnopyranosyl)-(1→2)-3,4-*O*-diallyl-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranoside (6b)

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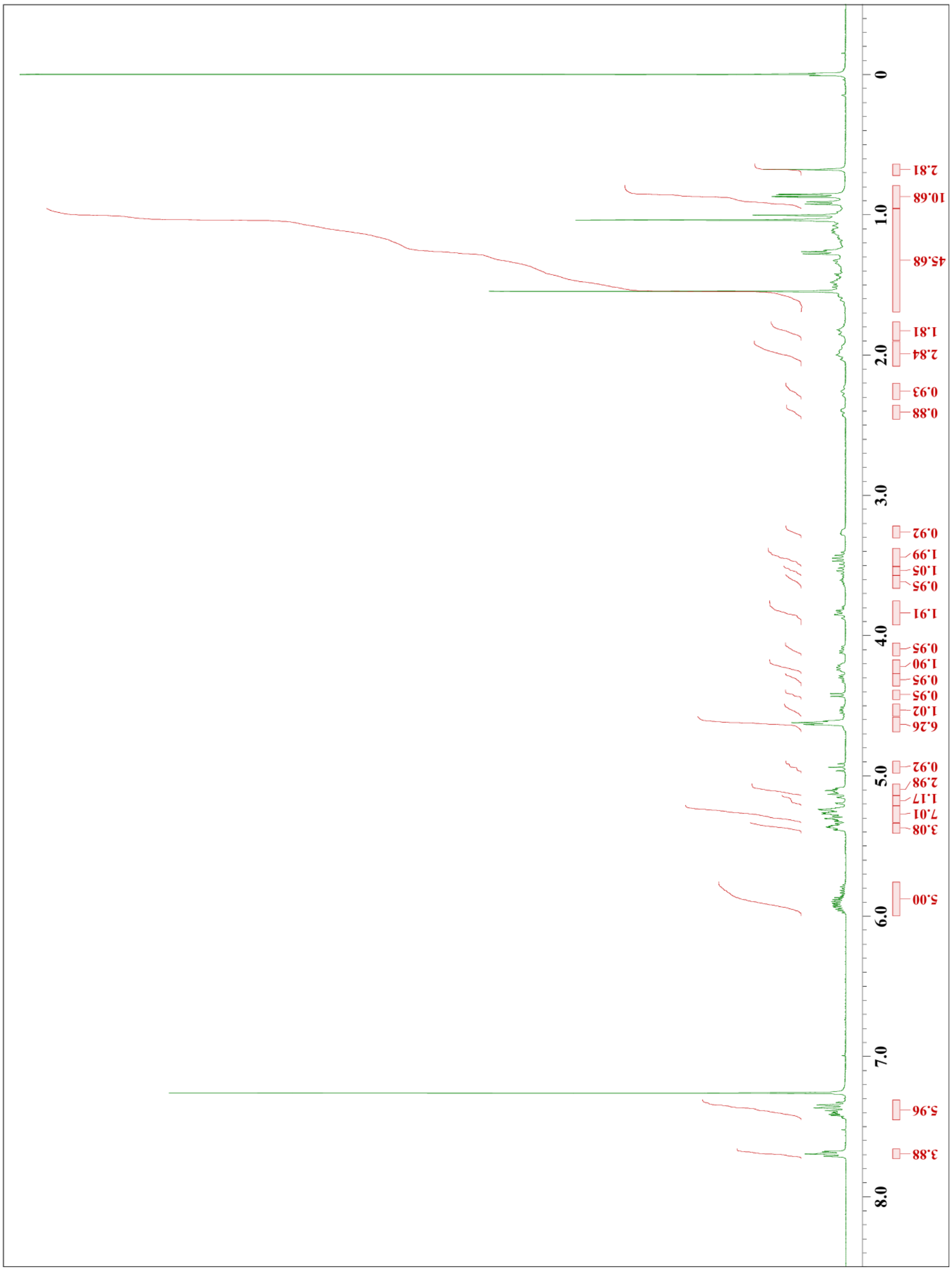
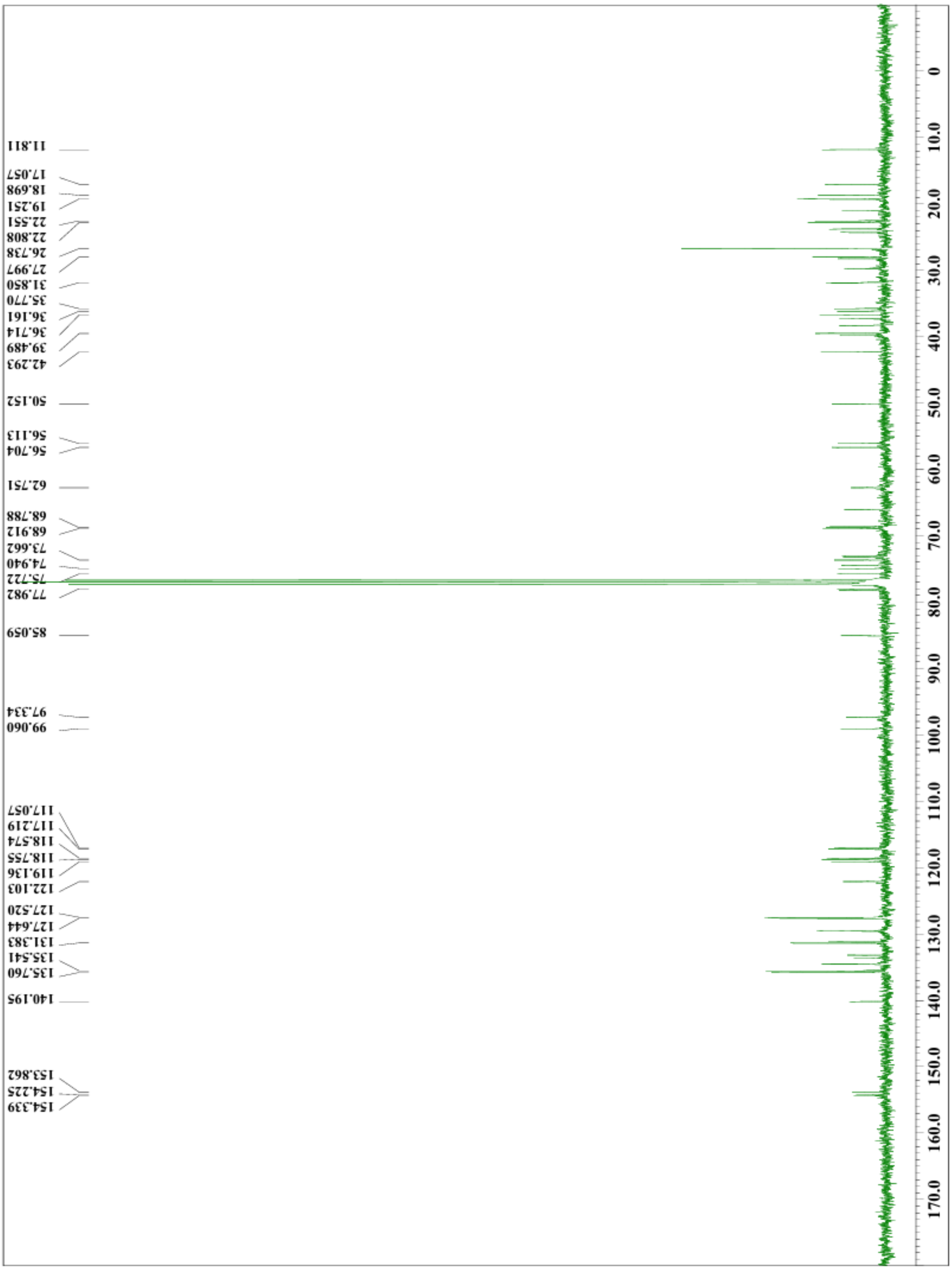
cholesteryl (2,3,4-*O*-trialloc-α-L-rhamnopyranosyl)-(1→2)-3,4-*O*-diallyl-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranoside (6c)

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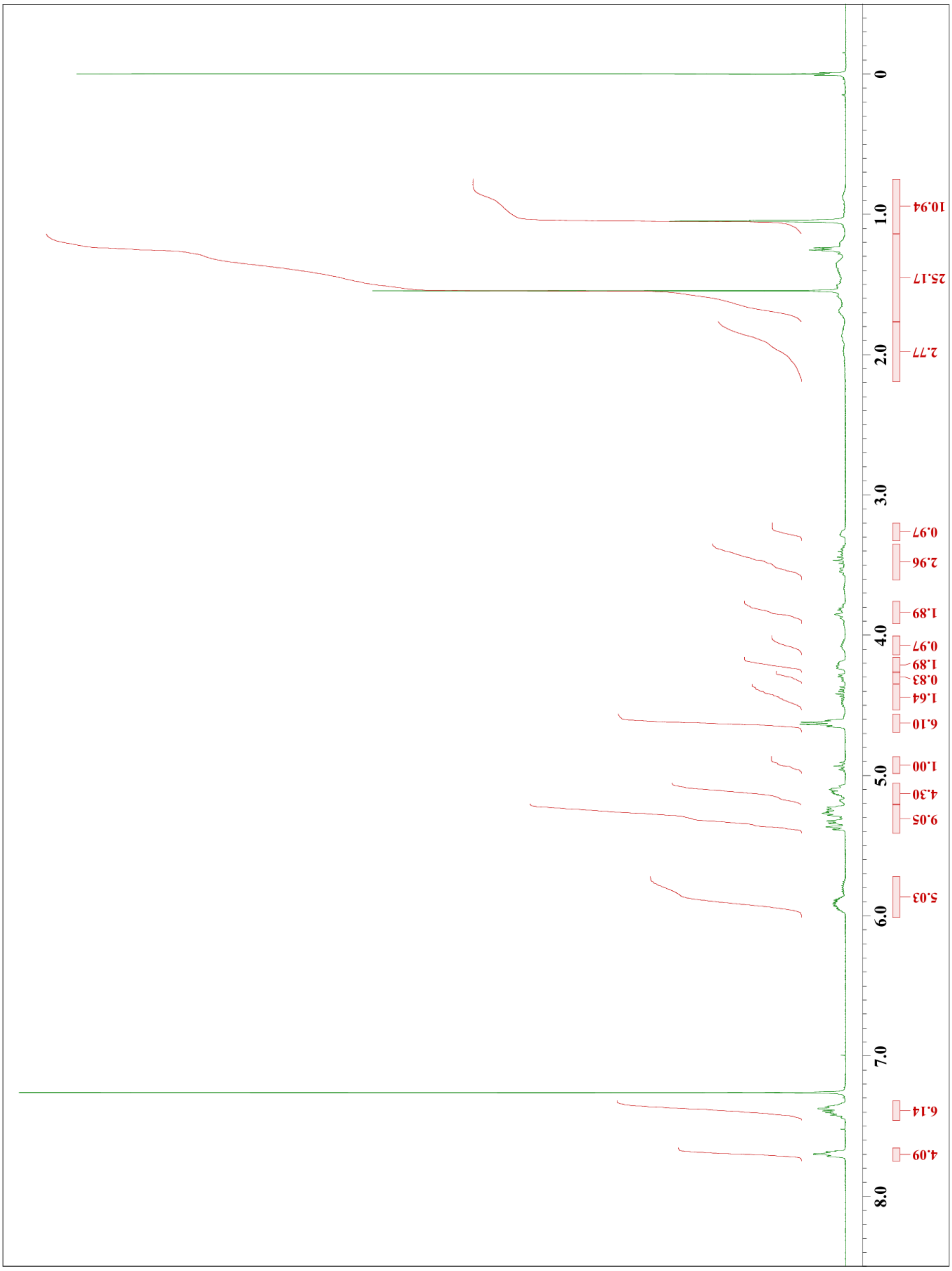

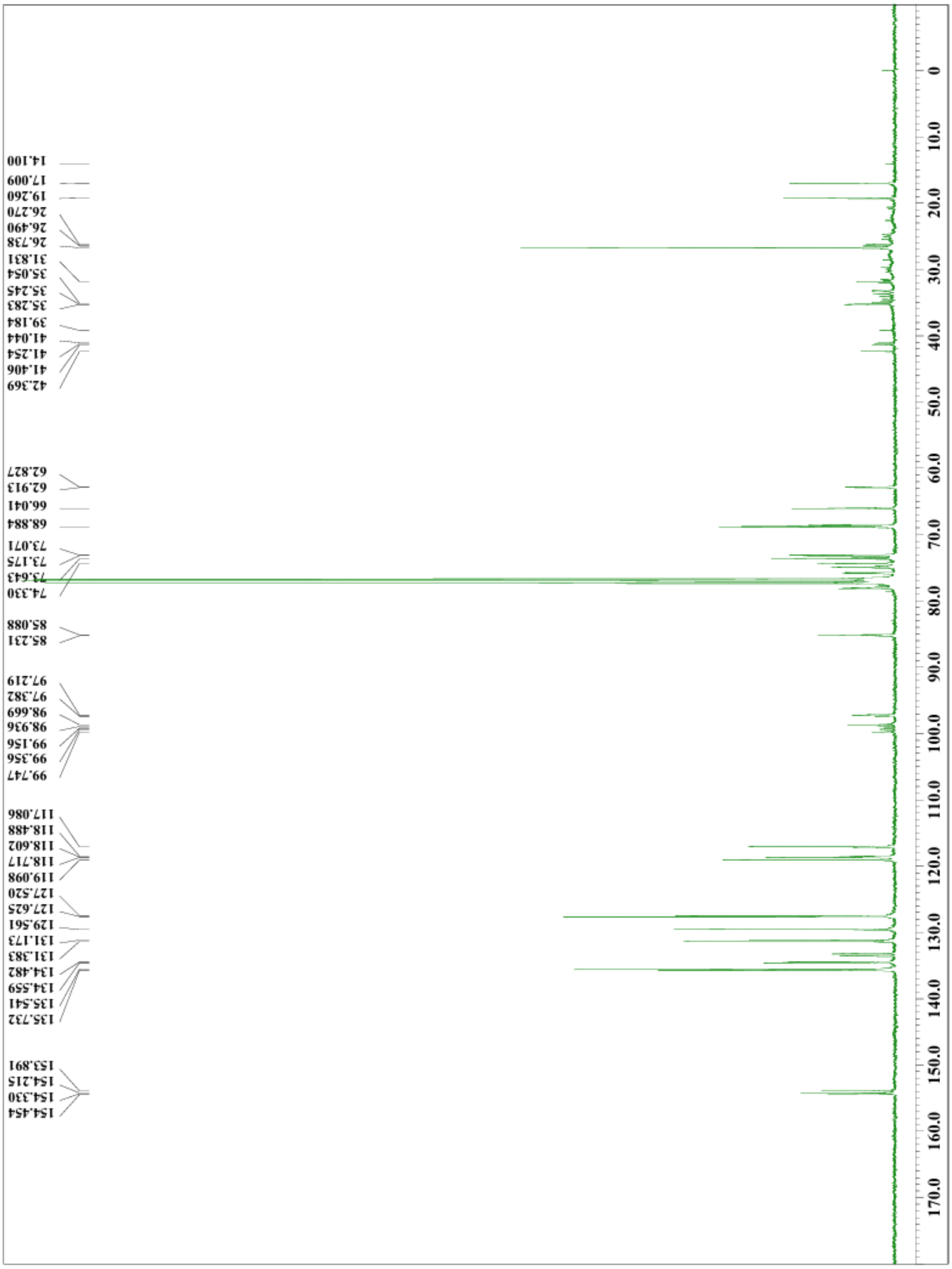
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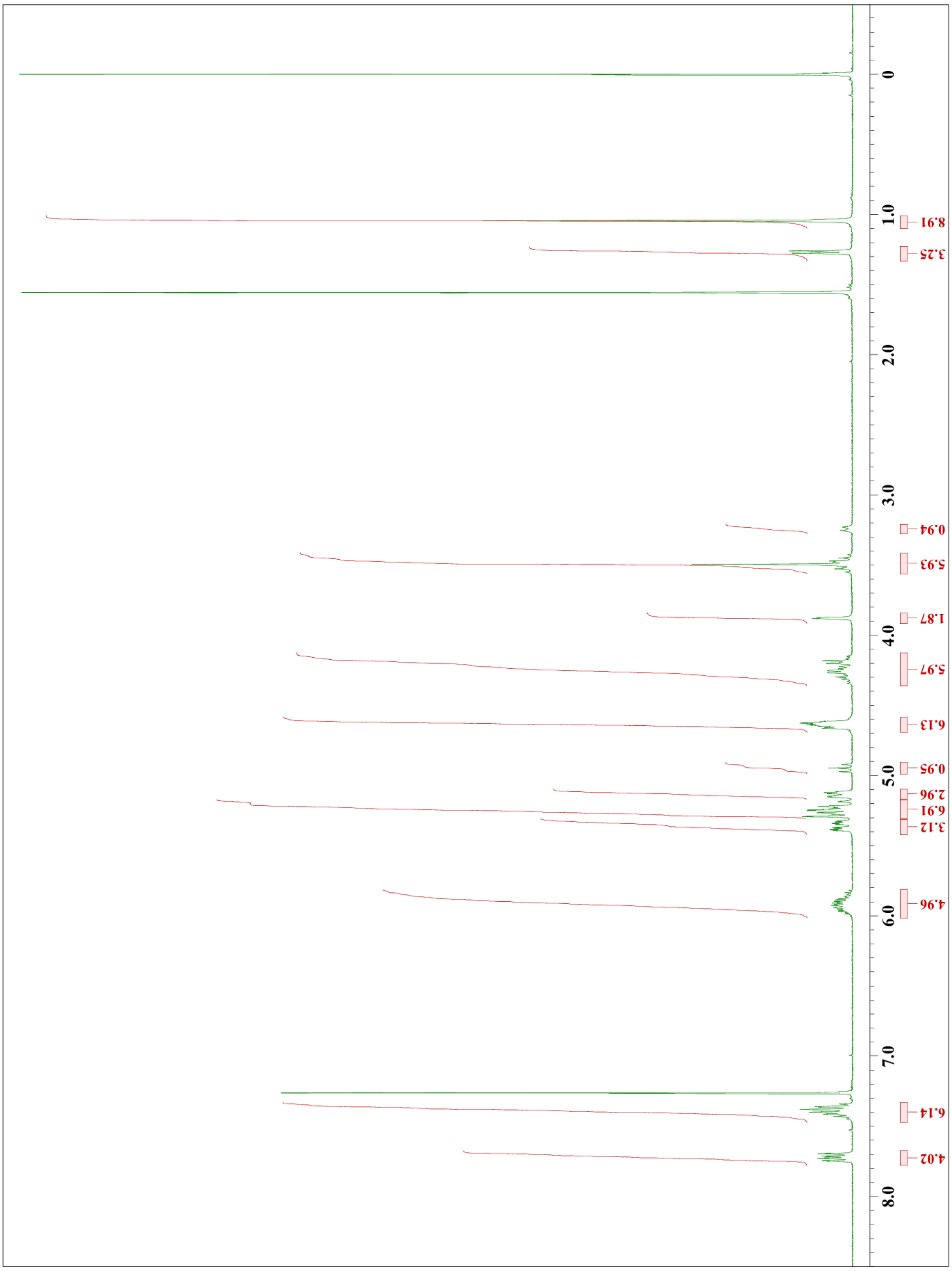
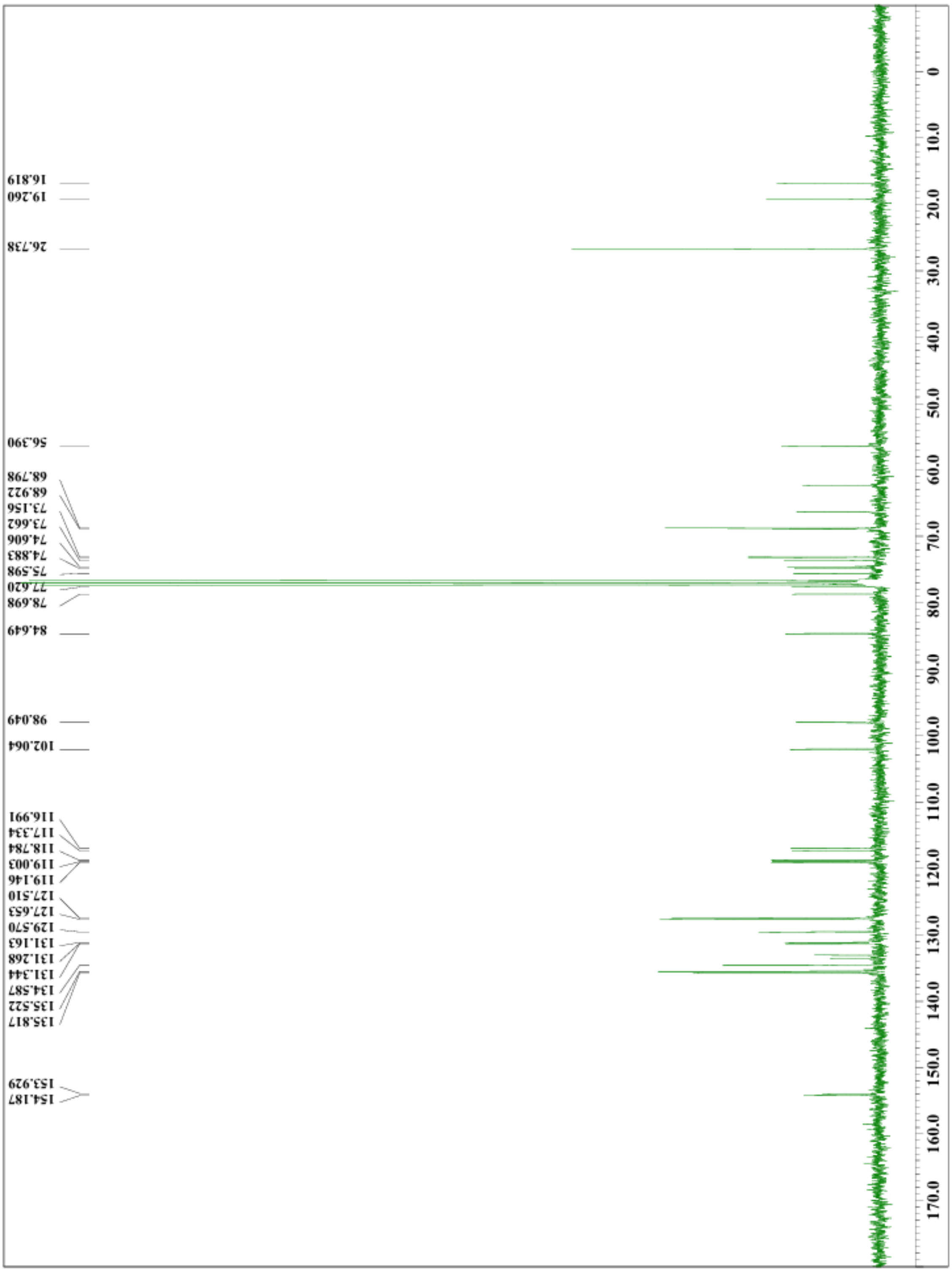
methyl (2,3,4-*O*-trialloc-α-L-rhamnopyranosyl)-(1→2)-3,4-*O*-diallyl-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranoside (6e)

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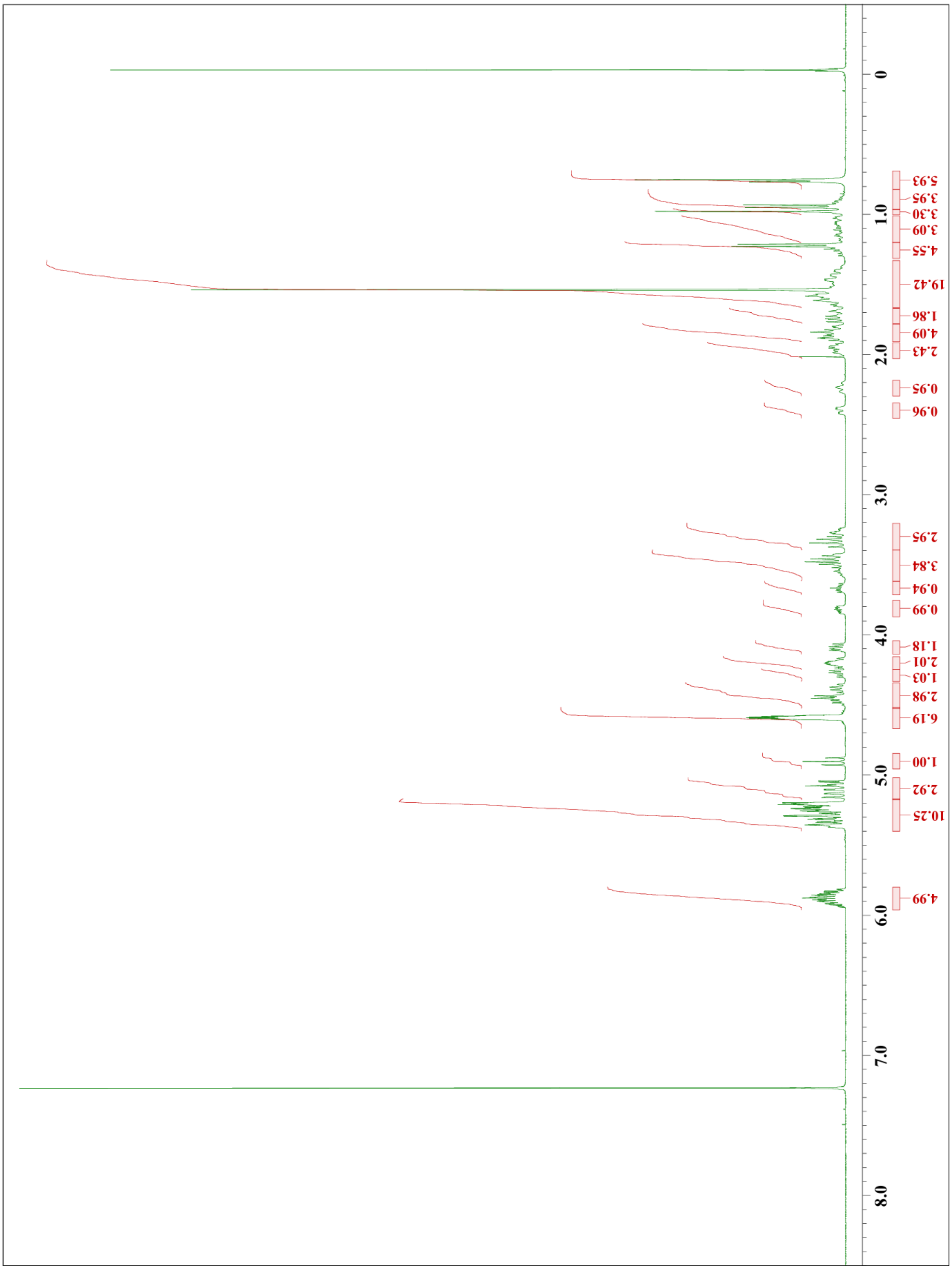

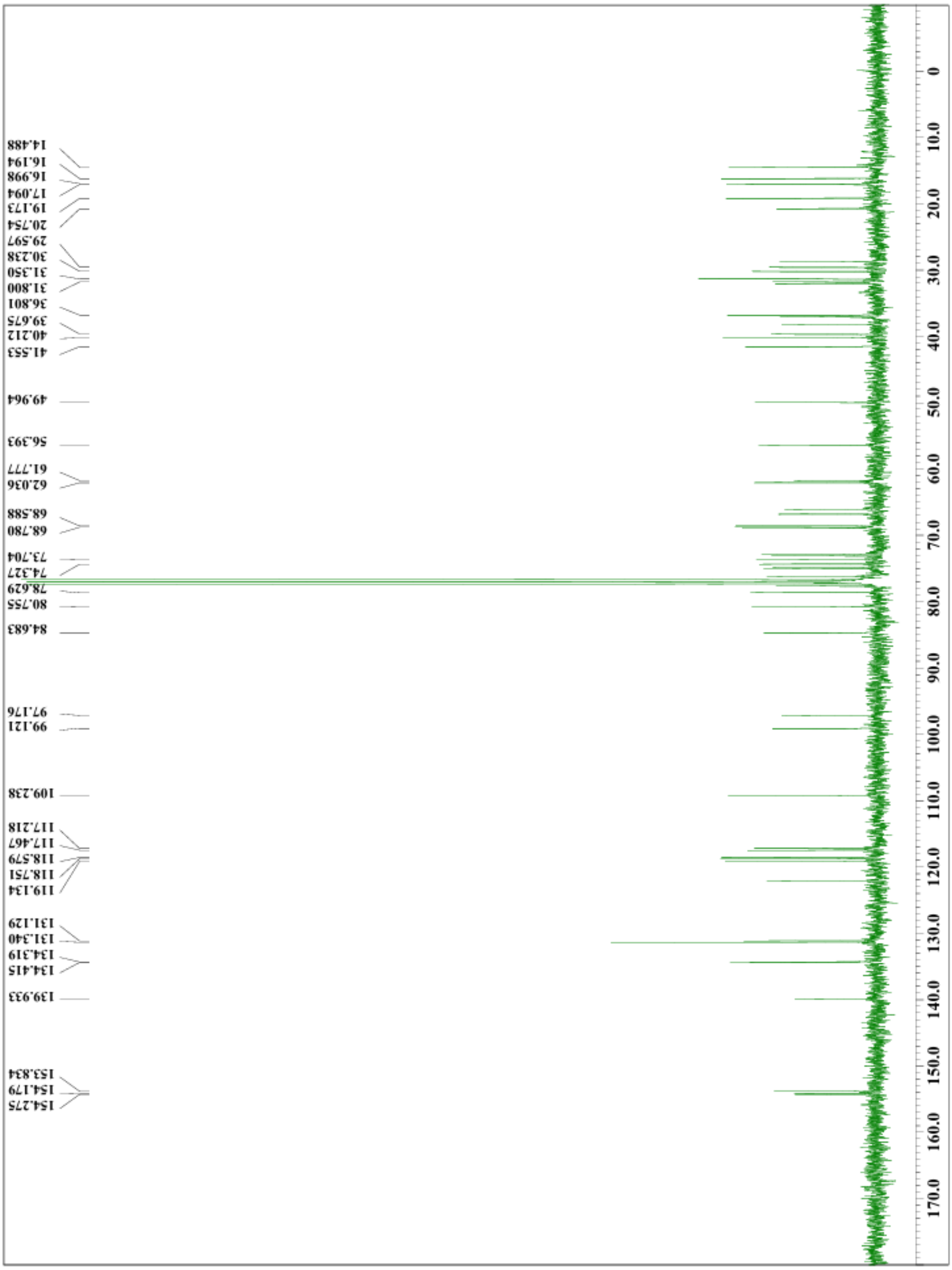
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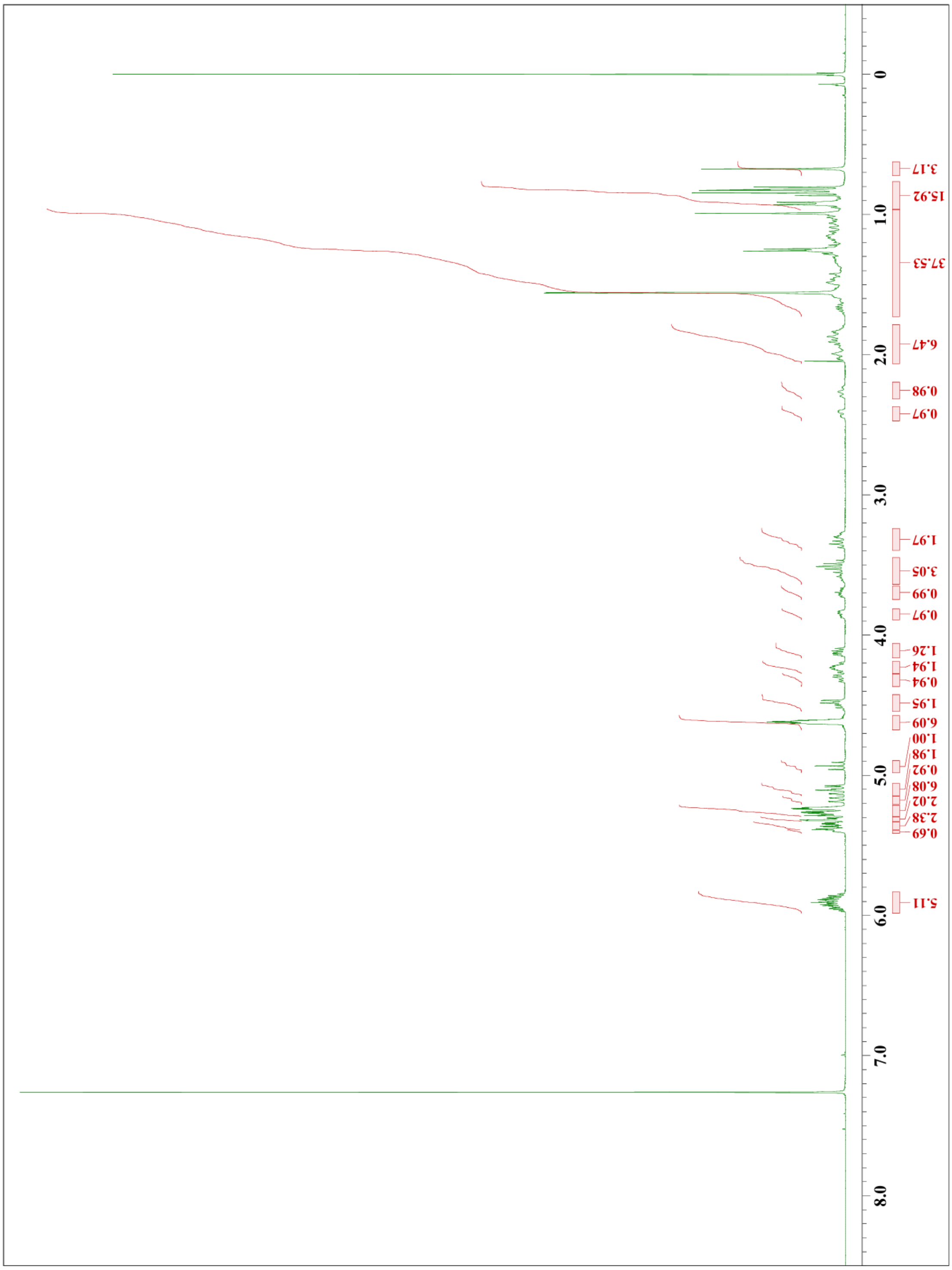
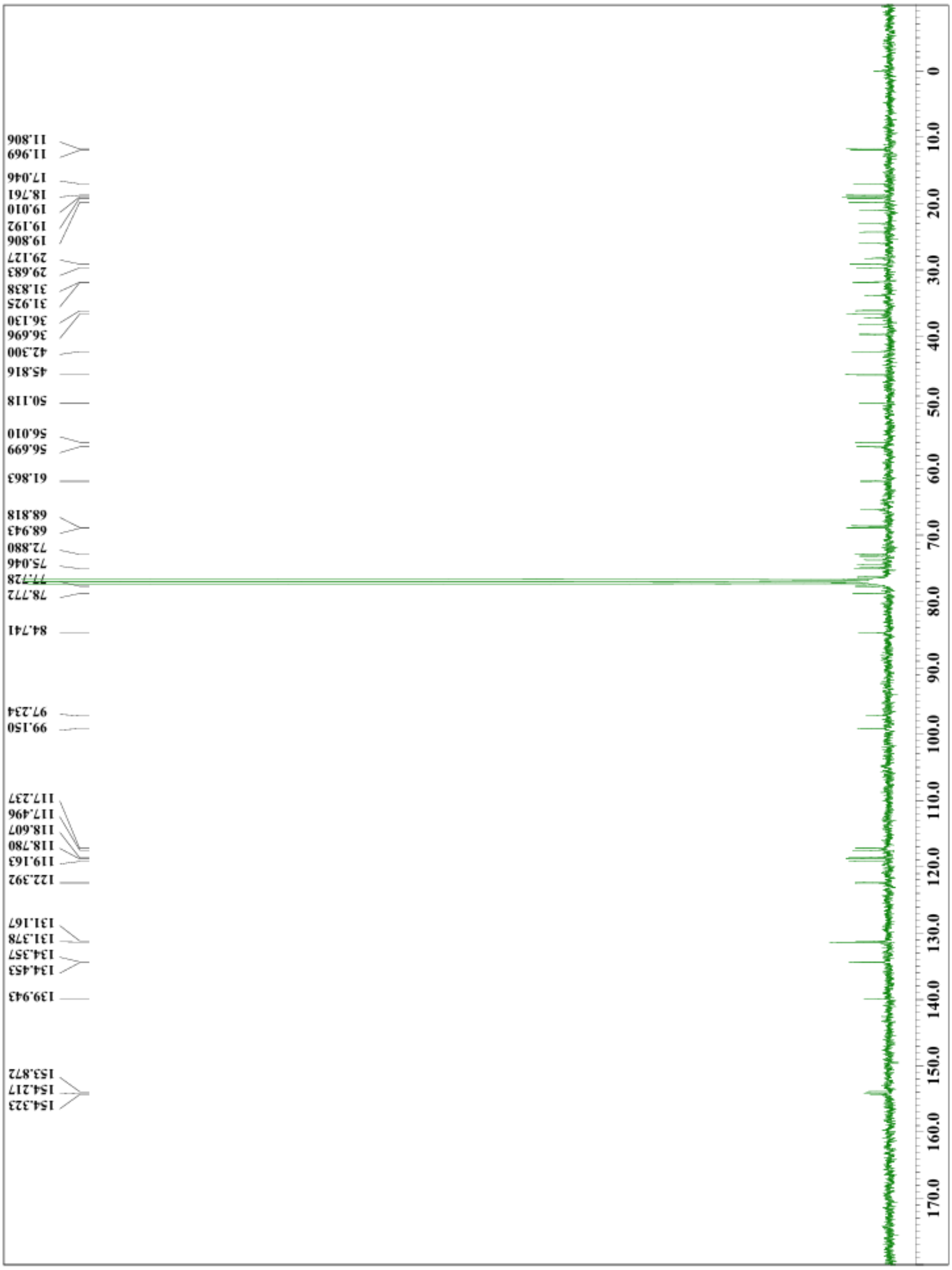
β-sitosteryl (2,3,4-*O*-trialloc-α-L-rhamnopyranosyl)-(1→2)-3,4-*O*-diallyl-β-D-glucopyranoside (7b)

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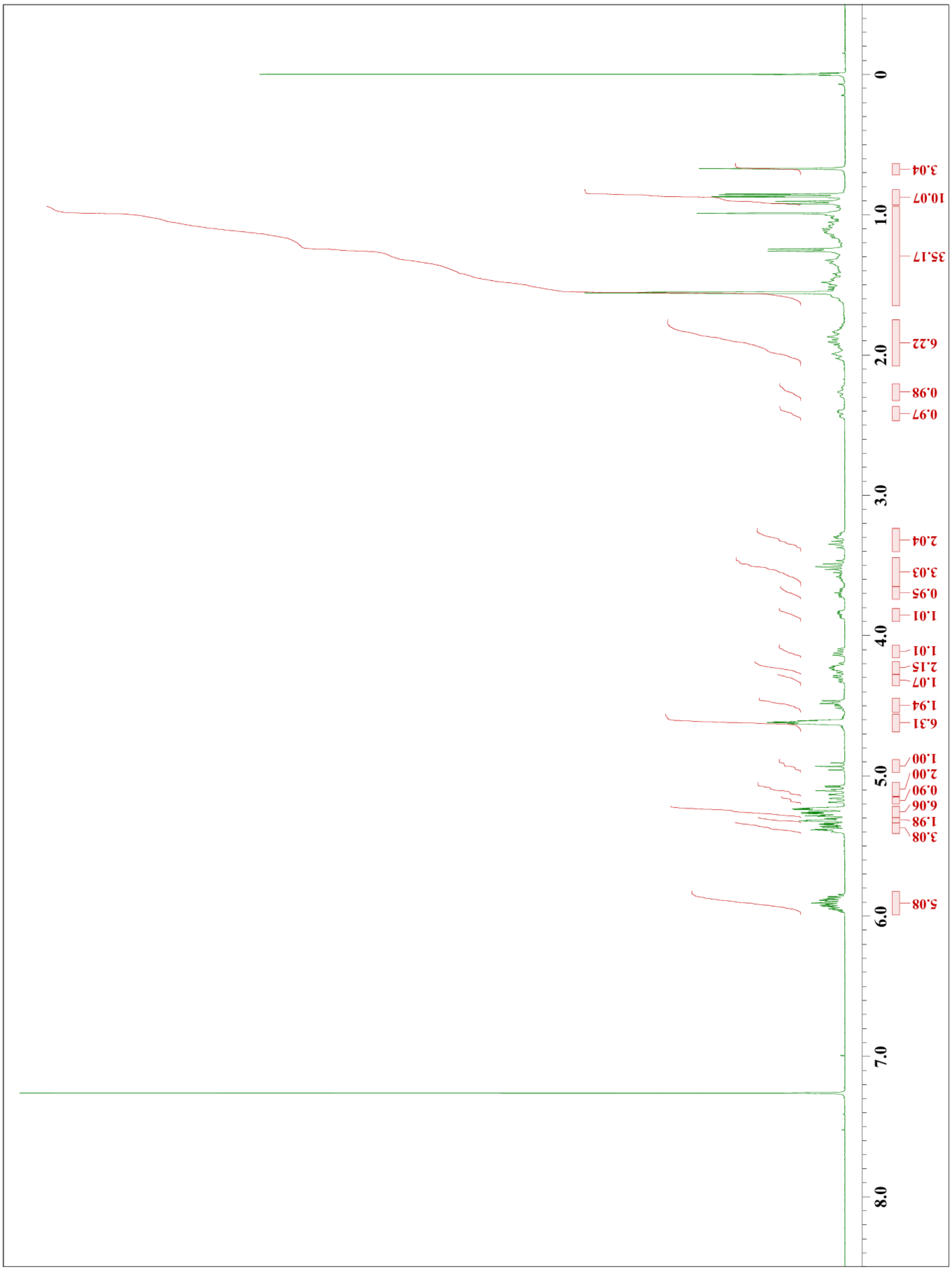
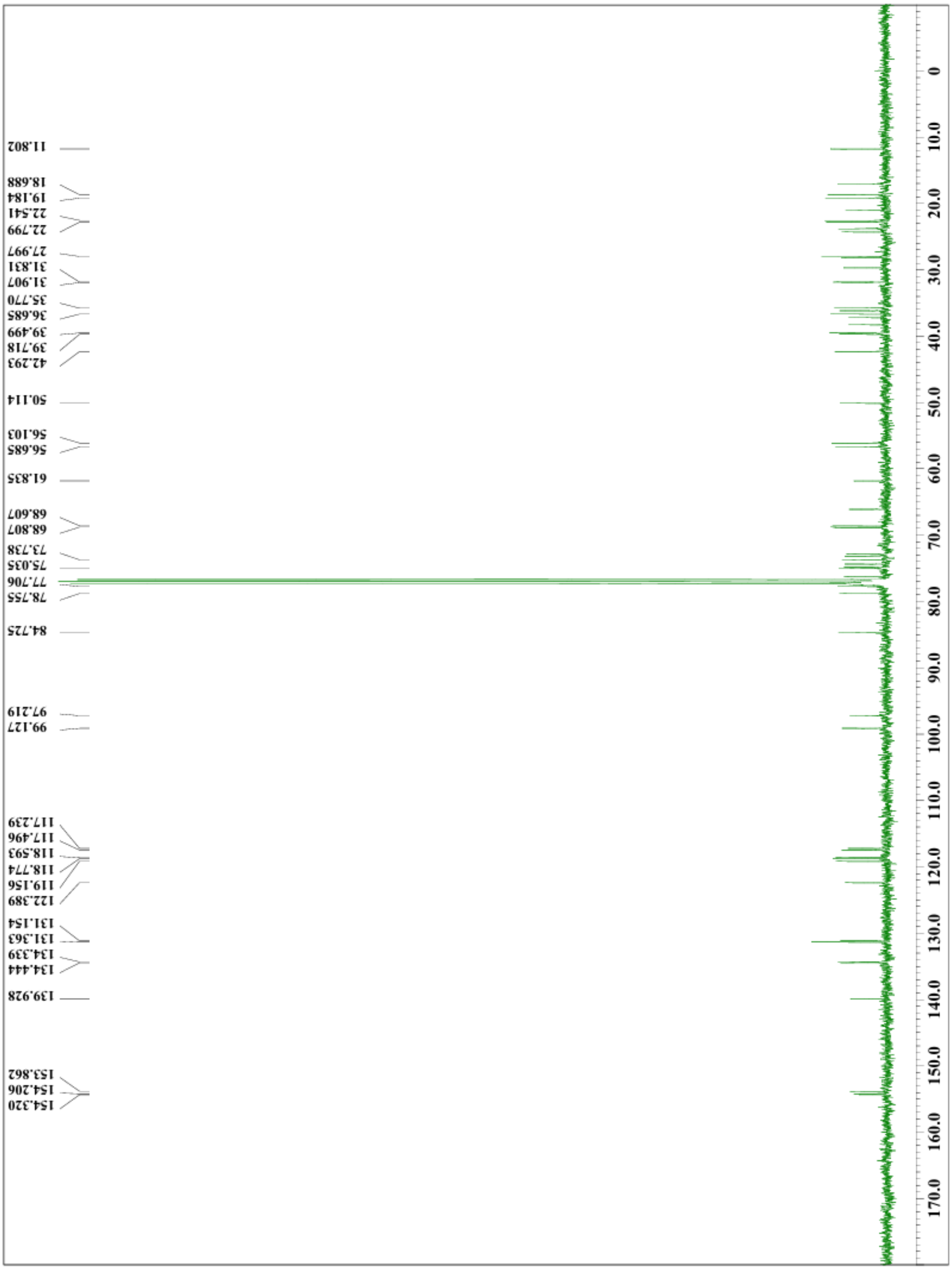
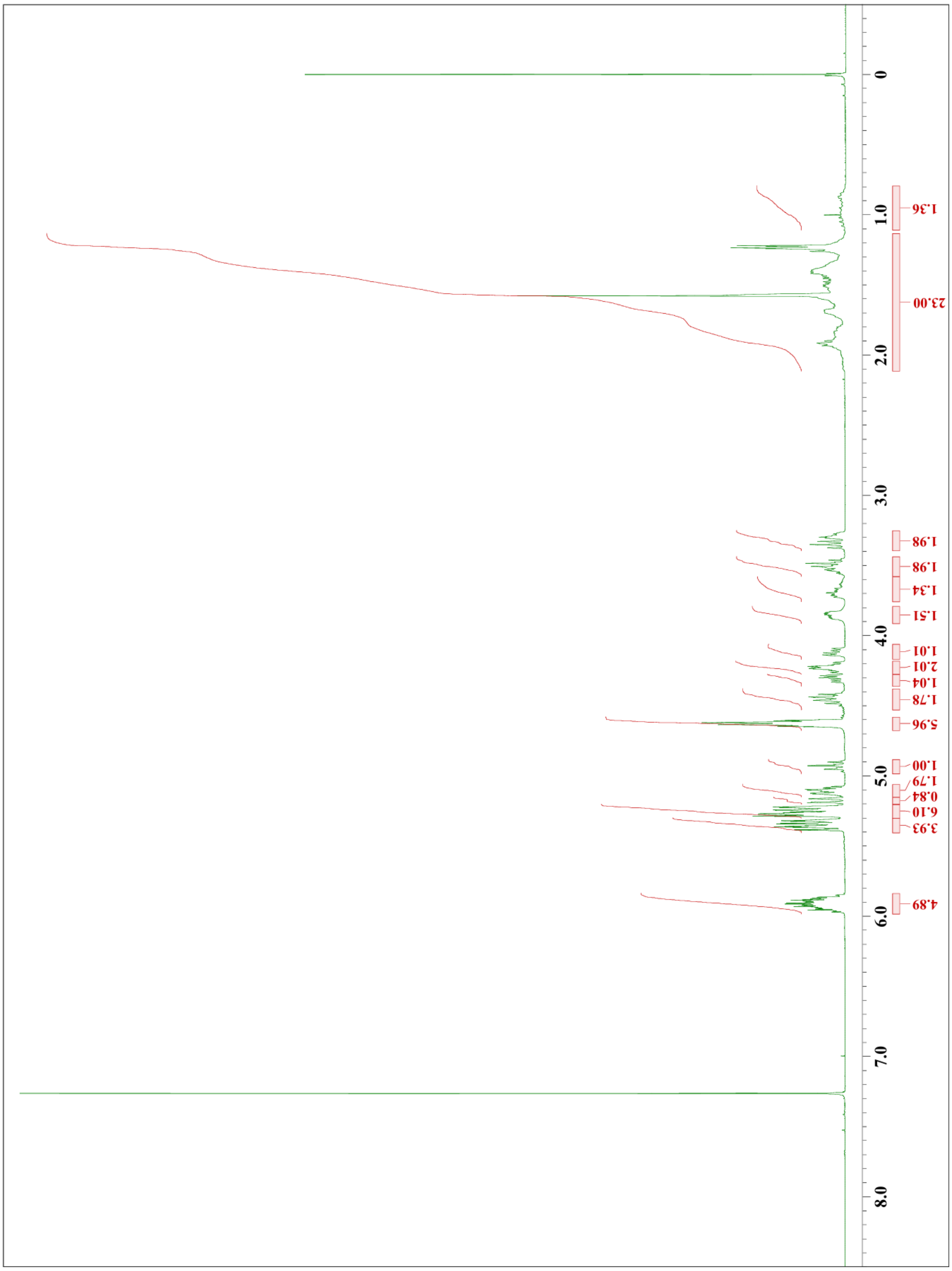
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
170.0

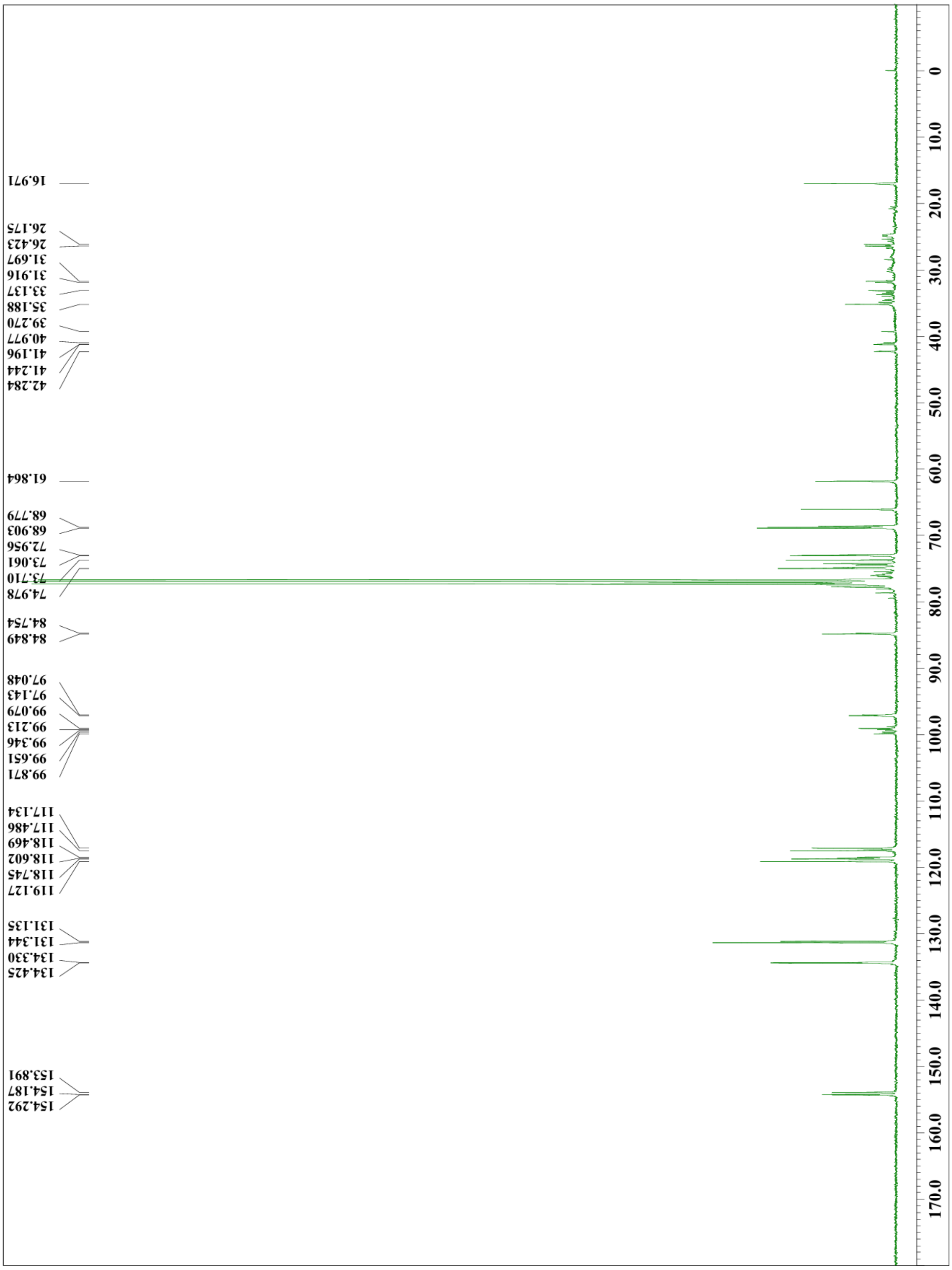
cholesteryl (2,3,4-*O*-trialloc-α-L-rhamnopyranosyl)-(1→2)-3,4-*O*-diallyl-β-D-glucopyranoside (7c)



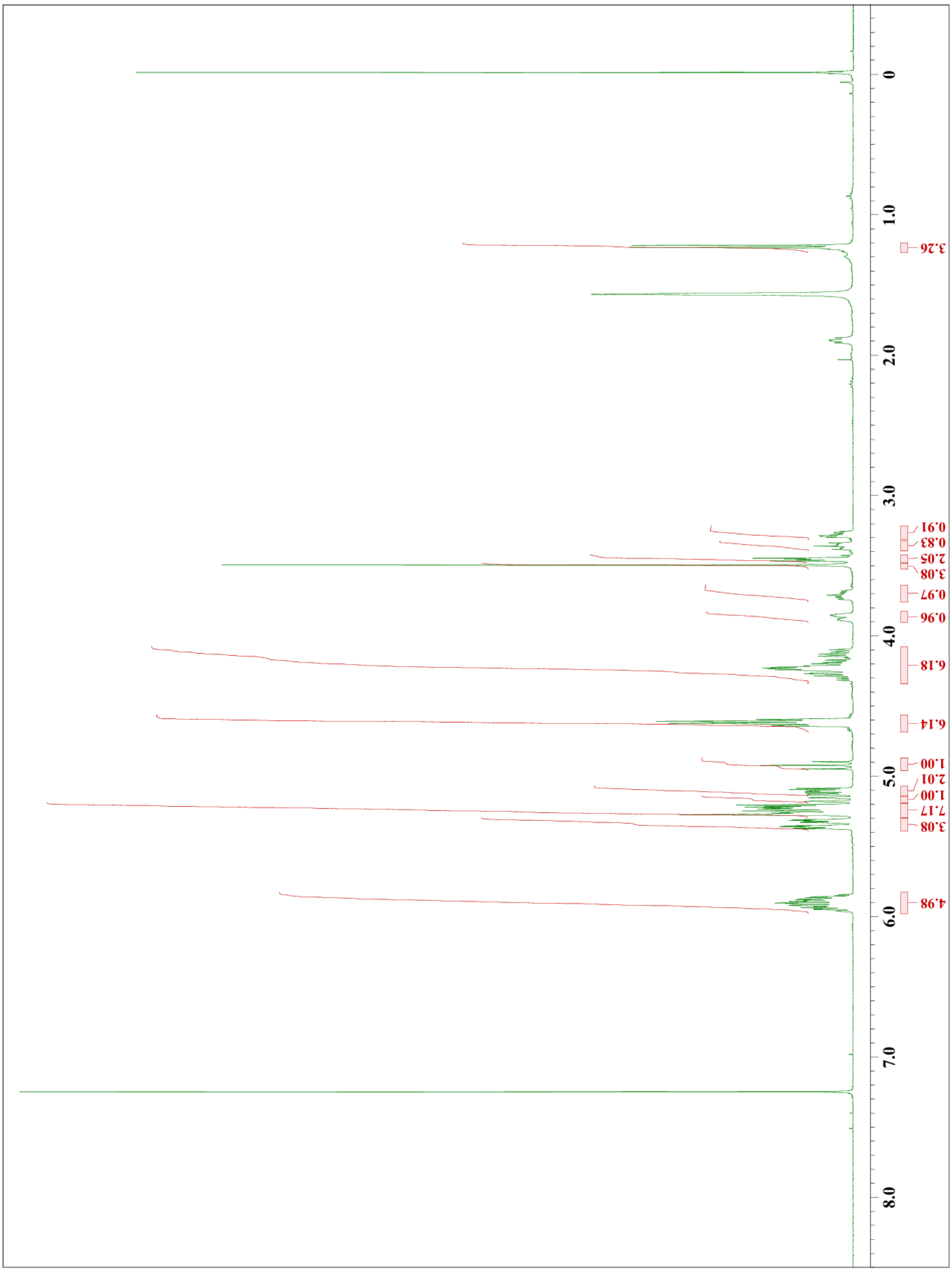

11.802
18.688
19.184
22.541
22.799
27.997
31.831
31.907
35.770
36.685
39.499
39.718
42.293
50.114
56.103
56.685
61.835
68.607
68.807
73.738
75.035
77.706
78.755
84.725
97.219
99.127
117.239
117.496
118.593
118.774
119.156
122.389
131.154
131.363
134.339
134.444
139.928
153.862
154.206
154.320
0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
170.0

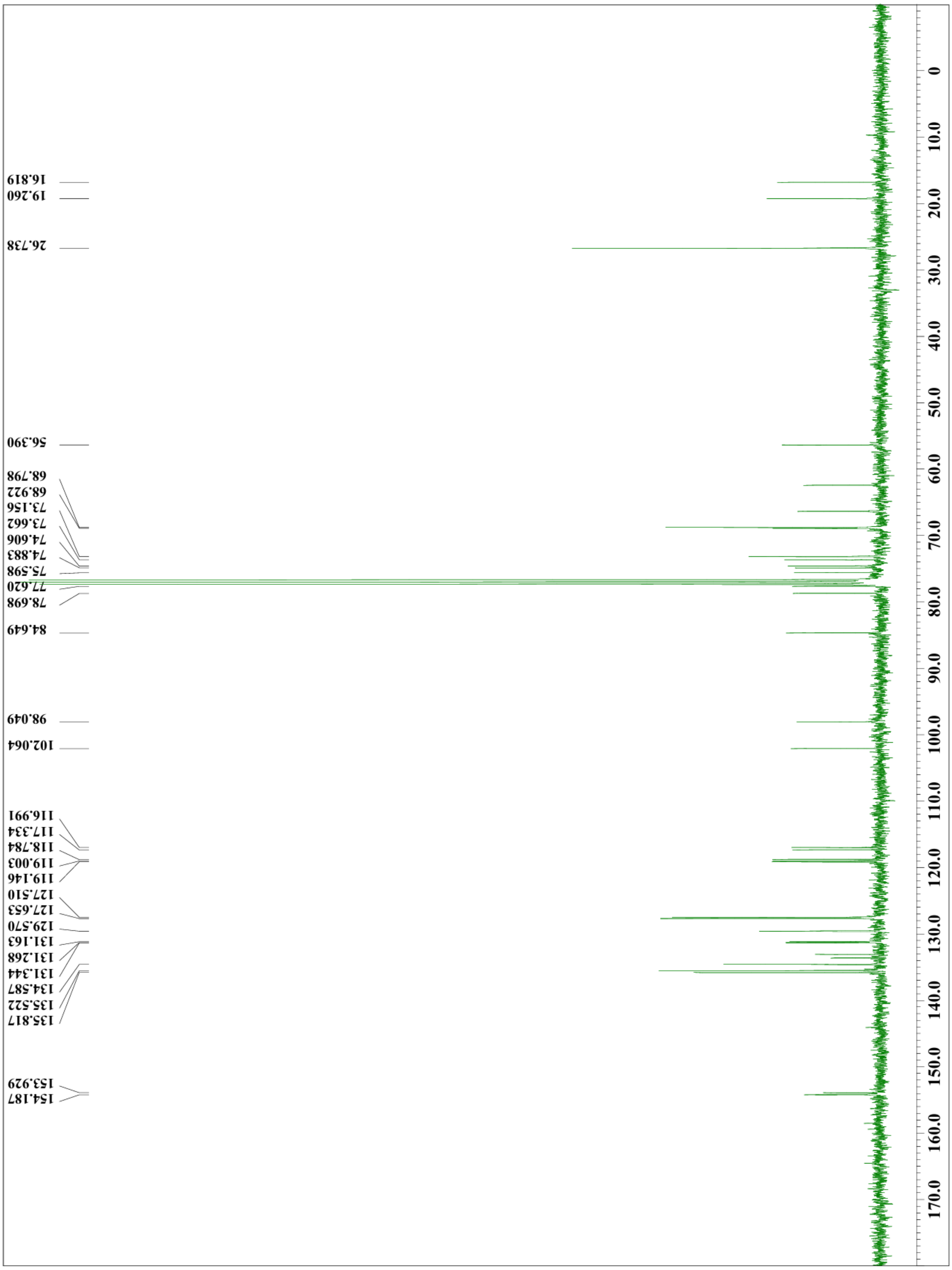
decahydro-2-naphthol (2,3,4-*O*-trialloc-α-L-rhamnopyranosyl)-(1→2)-3,4-*O*-diallyl-β-D-glucopyranoside (7d)



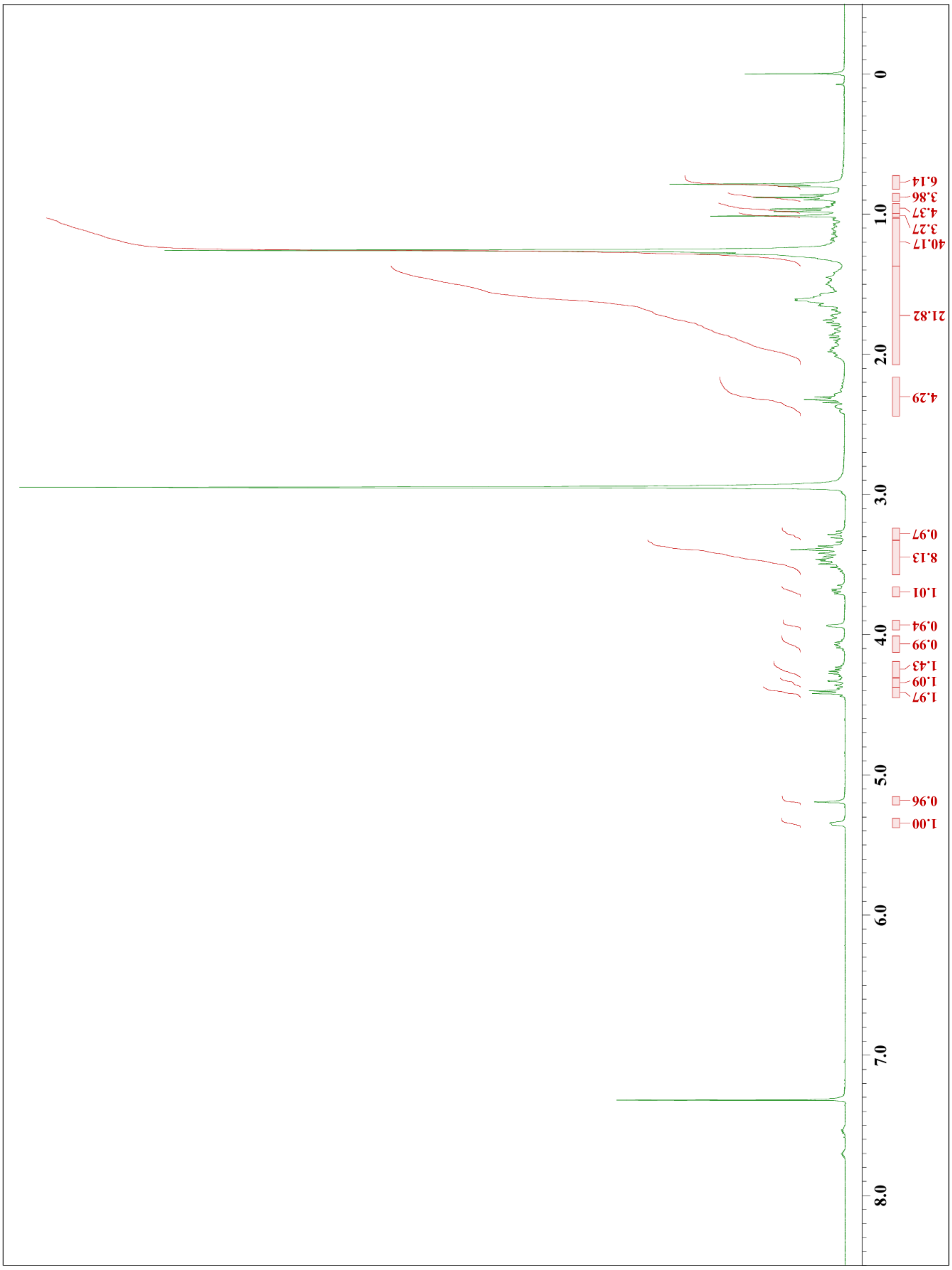

16.971
26.175
26.423
31.697
31.916
33.137
35.188
39.270
40.977
41.196
41.244
42.284
61.864
68.779
68.903
72.956
73.061
73.710
74.978
84.754
84.849
97.048
97.143
99.079
99.213
99.346
99.651
99.871
117.134
117.486
118.469
118.602
118.745
119.127
131.135
131.344
134.330
134.425
153.891
154.187
154.292
0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
170.0

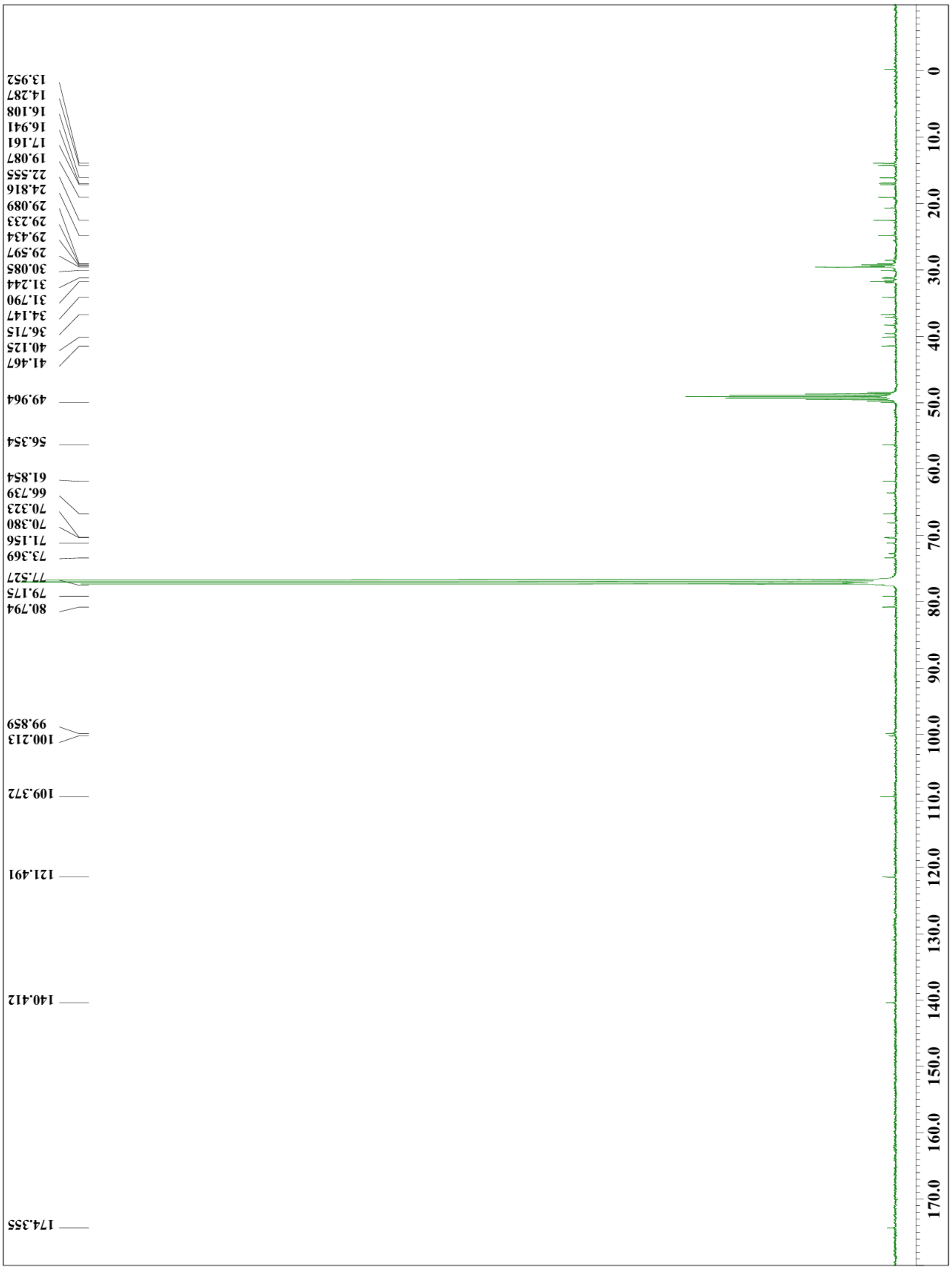
methyl (2,3,4-*O*-trialloc-α-L-rhamnopyranosyl)-(1→2)-3,4-*O*-diallyl-β-D-glucopyranoside (7e)



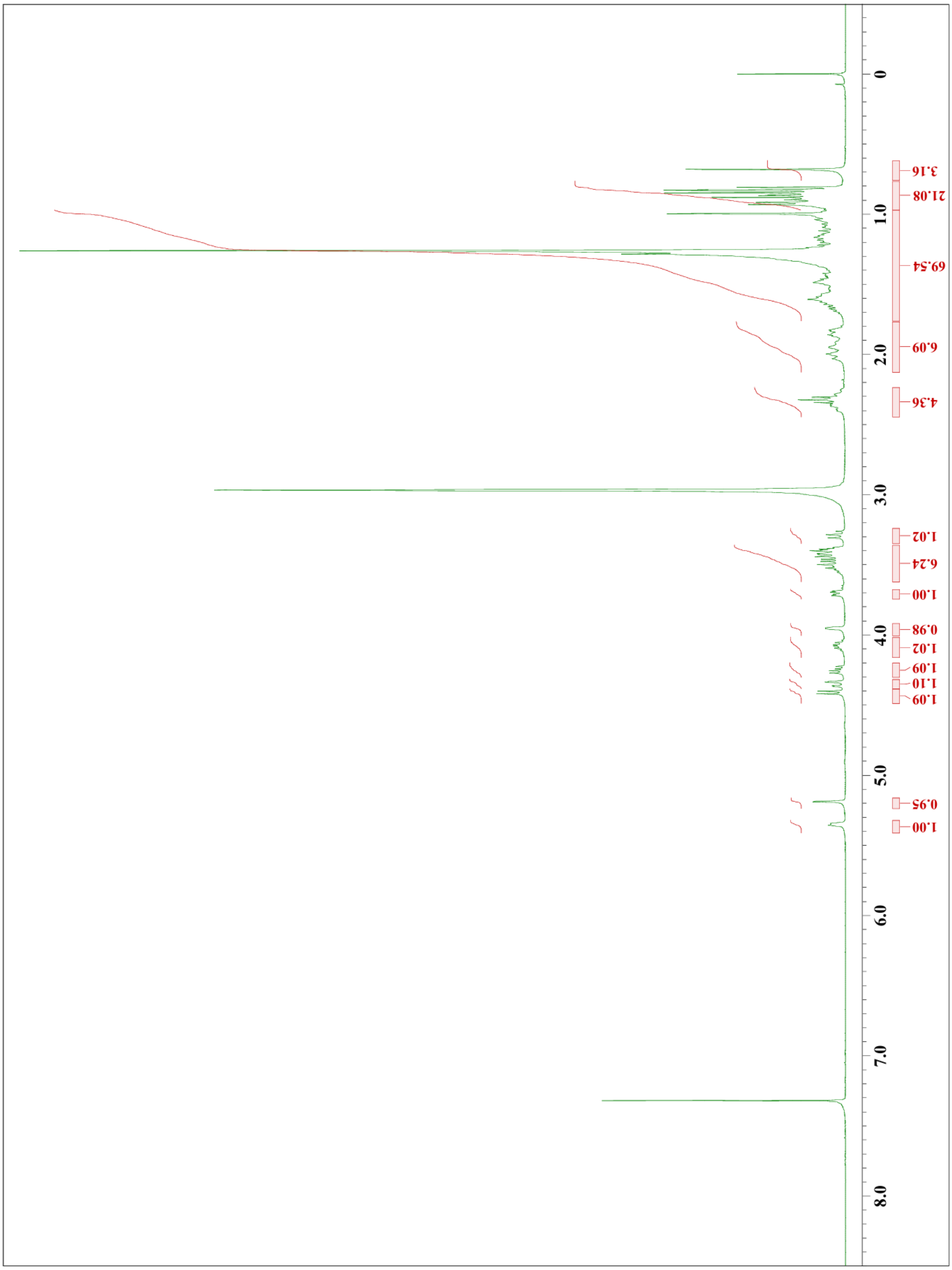

16.819
19.260
26.738
56.390
68.798
68.922
73.156
73.662
74.606
74.883
75.598
77.620
78.698
84.649
98.049
102.064
116.991
117.334
118.784
119.003
119.146
127.510
127.653
129.570
131.163
131.268
131.344
134.587
135.522
135.817
153.929
154.187
0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
170.0

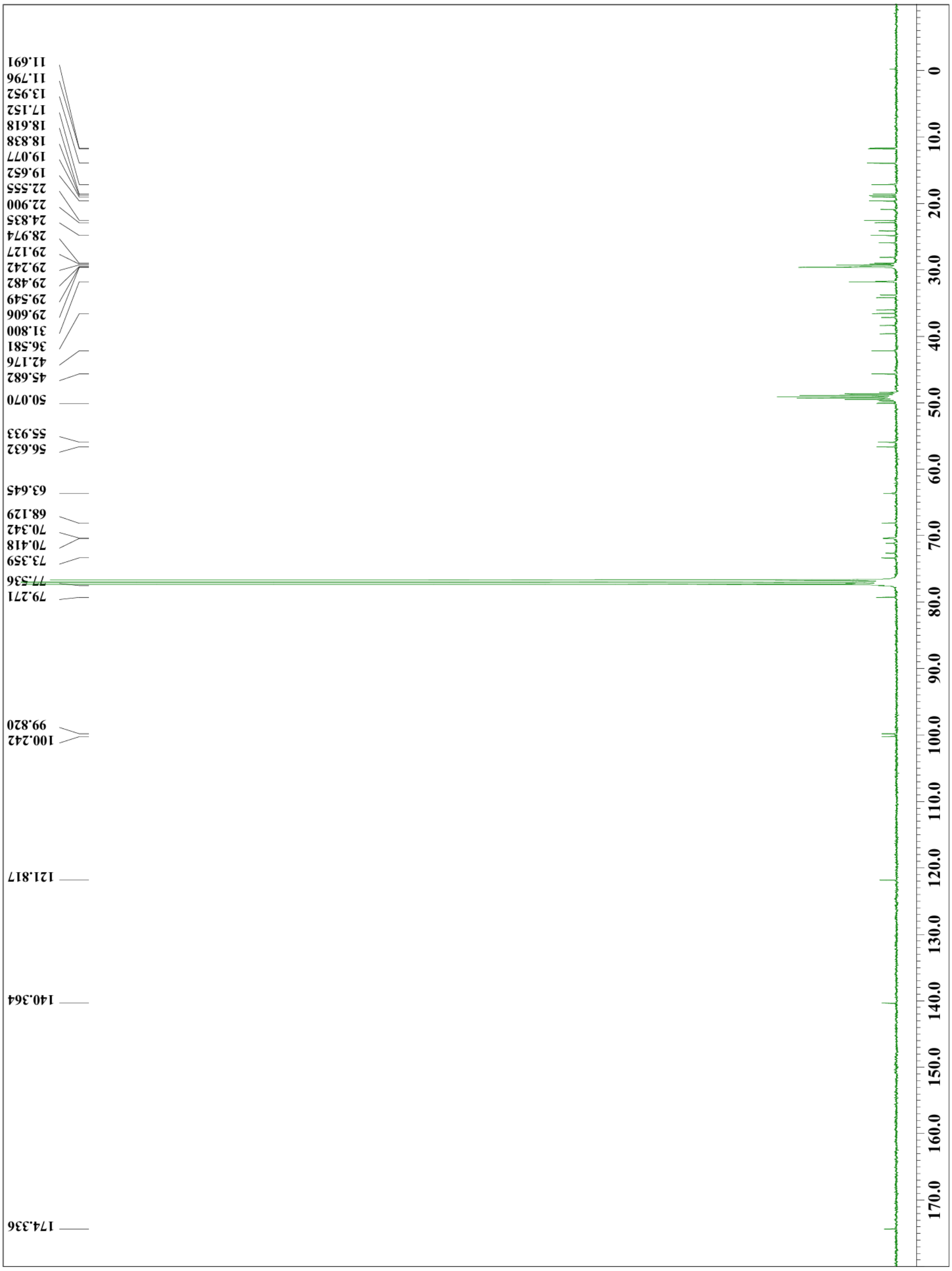
diosgenyl α-L-rhamnopyranosyl-(1→2)-6-*O*-palmitoyl-β-D-glucopyranoside (1a)



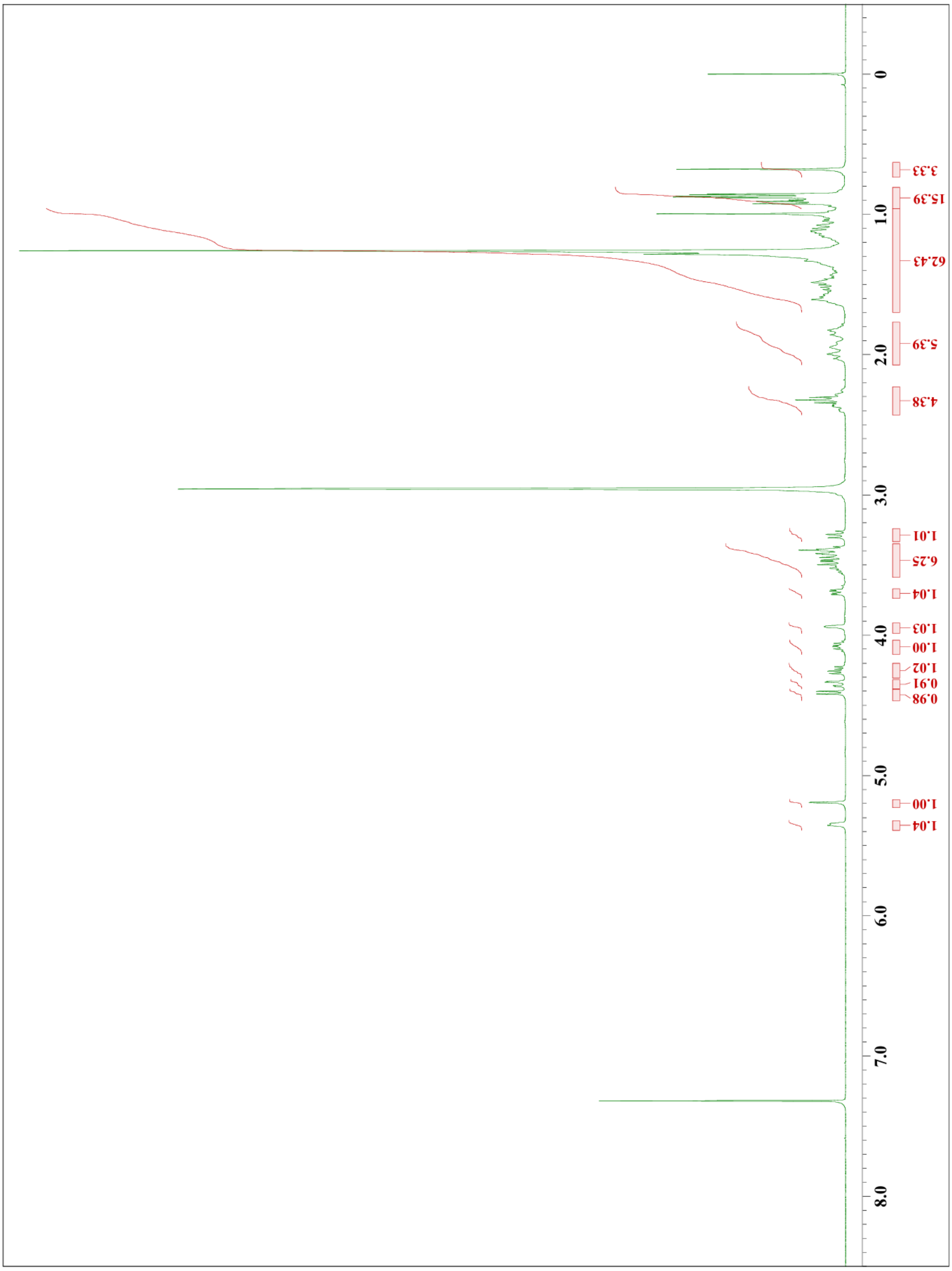

13.952
14.287
16.108
16.941
17.161
19.087
22.555
24.816
29.089
29.233
29.434
29.597
30.085
31.244
31.790
34.147
36.715
40.125
41.467
49.964
56.354
61.854
66.739
70.323
70.380
71.156
73.369
77.527
79.175
80.794
99.859
100.213
109.372
121.491
140.412
174.355
0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
170.0

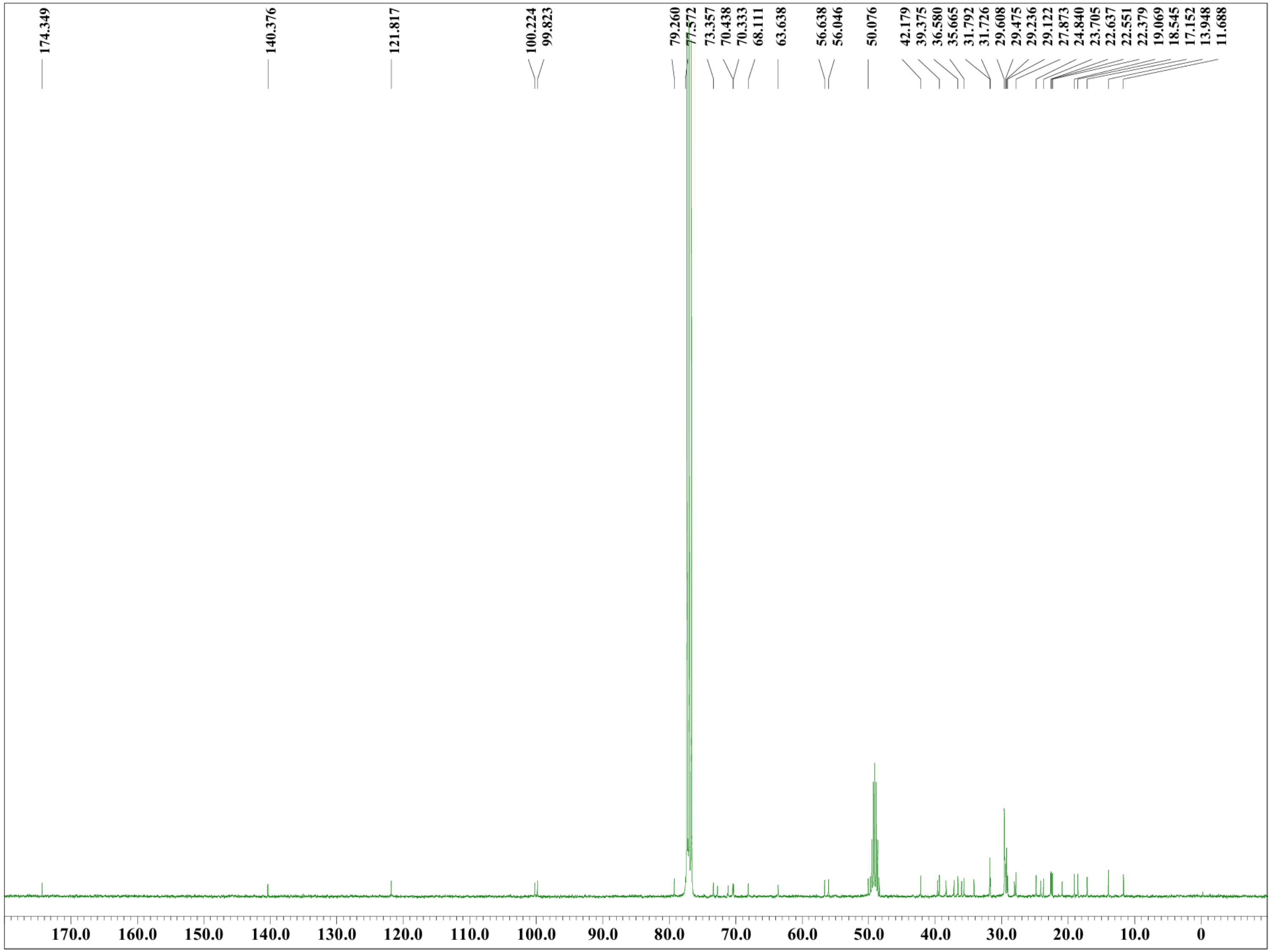
β-sitosteryl α-L-rhamnopyranosyl-(1→2)-6-*O*-palmitoyl-β-D-glucopyranoside (1b)



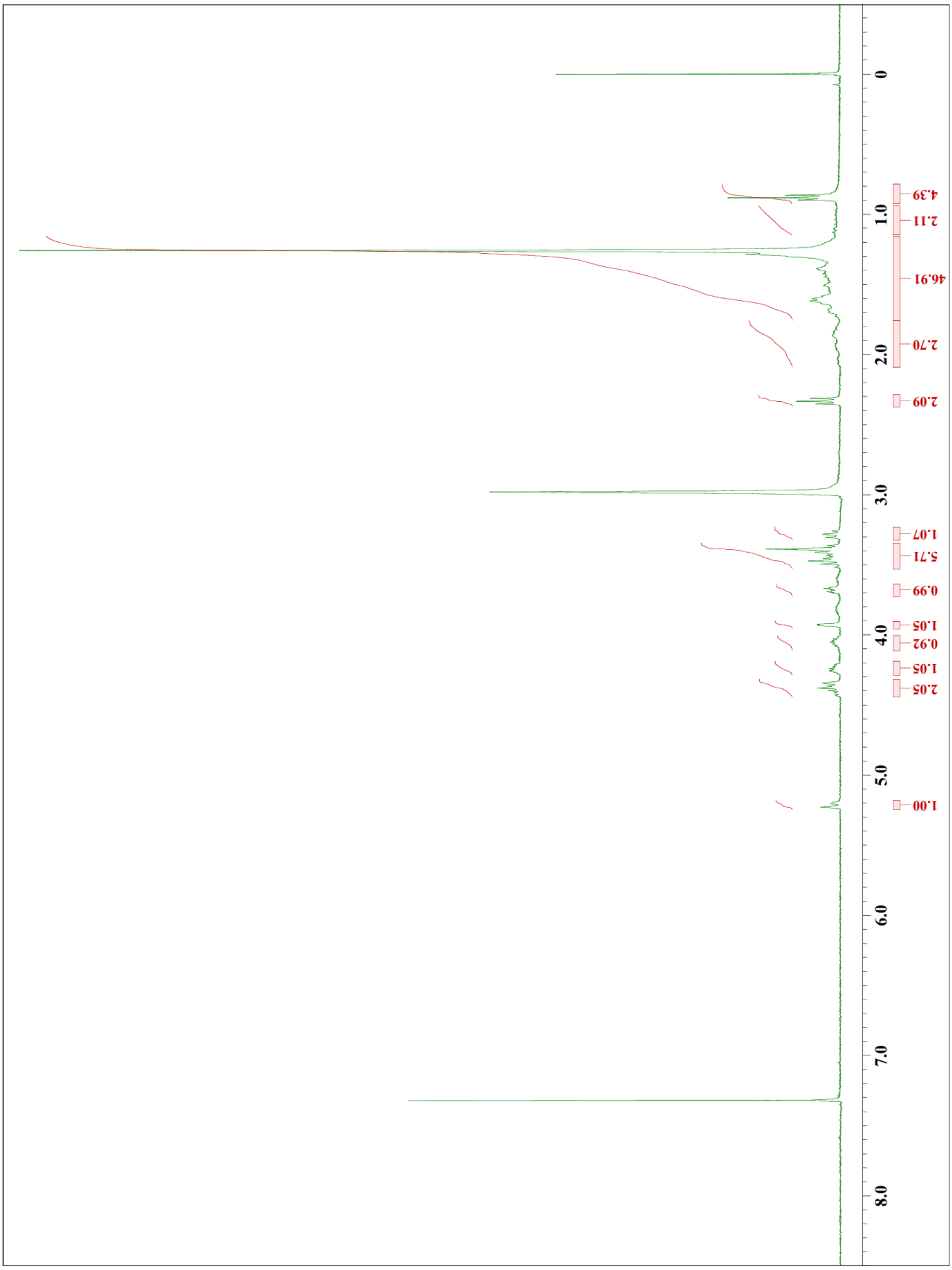

11.691
11.796
13.952
17.152
18.618
18.838
19.077
19.652
22.555
22.900
24.835
28.974
29.127
29.242
29.482
29.549
29.606
31.800
36.581
42.176
45.682
50.070
55.933
56.632
63.645
68.129
70.342
70.418
73.359
77.536
79.271
99.820
100.242
121.817
140.364
174.336
0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
170.0

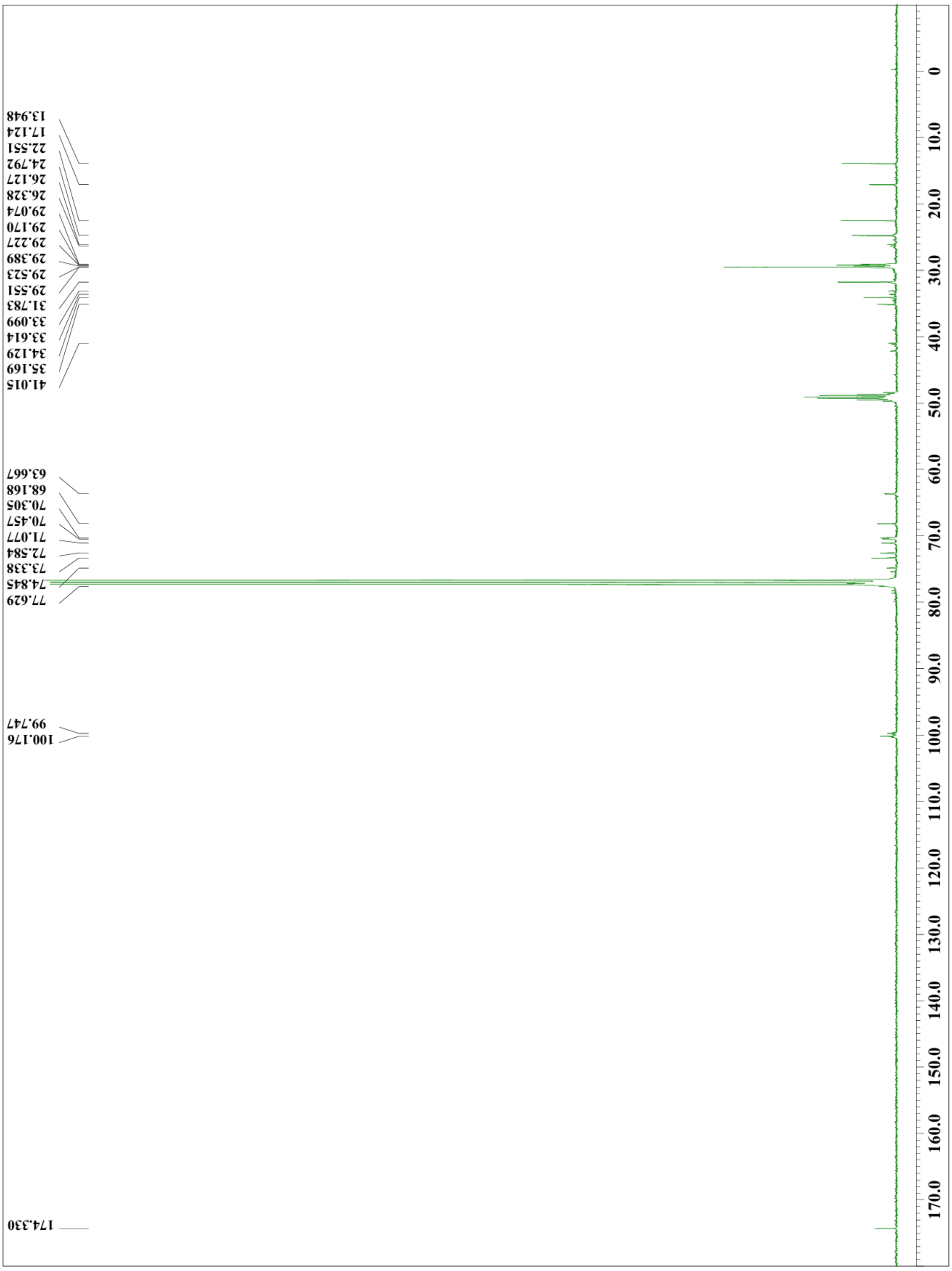
cholesteryl α-L-rhamnopyranosyl-(1→2)-6-*O*-palmitoyl-β-D-glucopyranoside (1c)



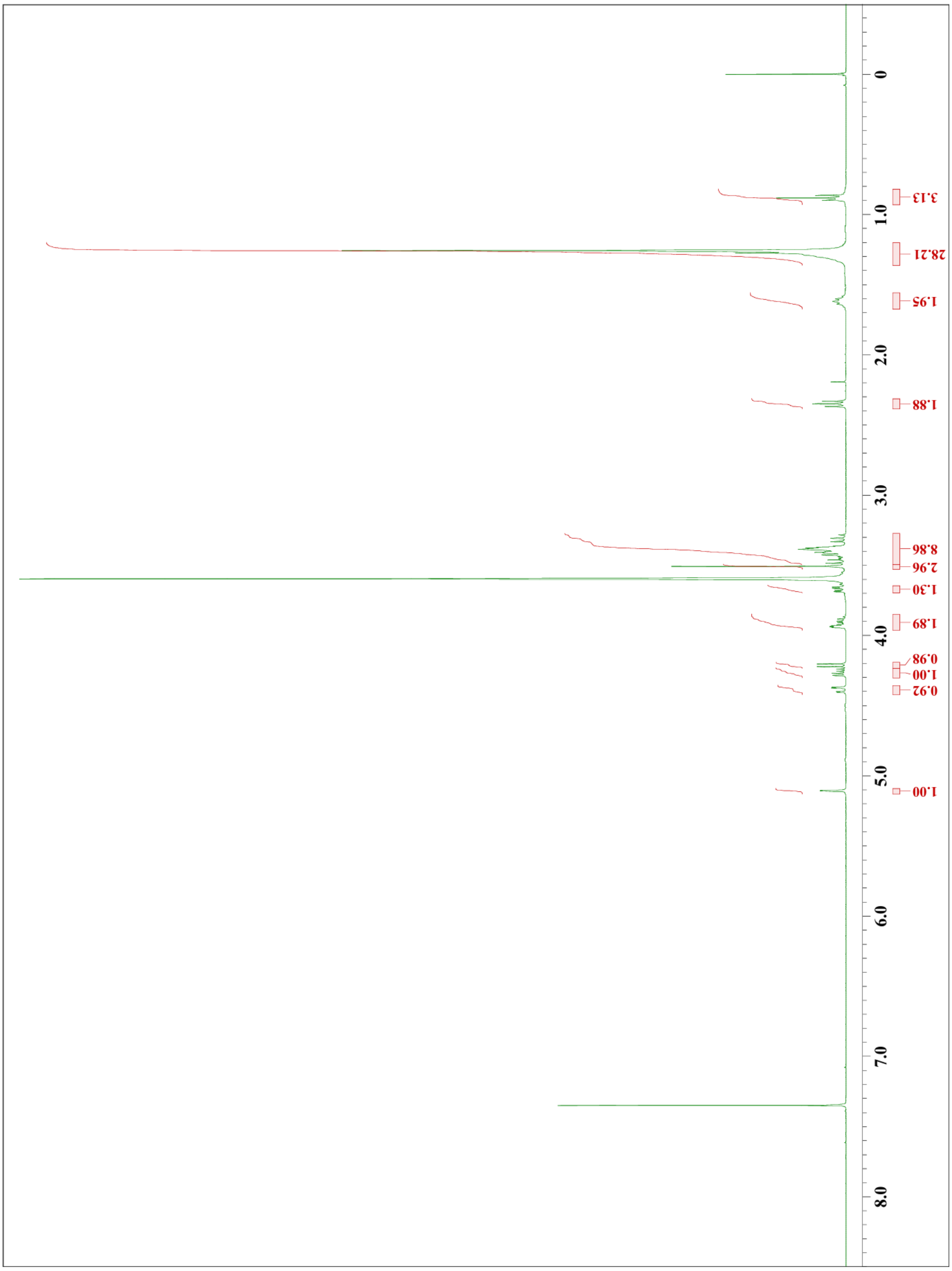

174.349
140.376
121.817
100.224
99.823
79.260
77.572
73.357
70.438
70.333
68.111
63.638
56.638
56.046
50.076
42.179
39.375
36.580
35.665
31.792
31.726
29.608
29.475
29.236
29.122
27.873
24.840
23.705
22.637
22.551
22.379
19.069
18.545
17.152
13.948
11.688
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0

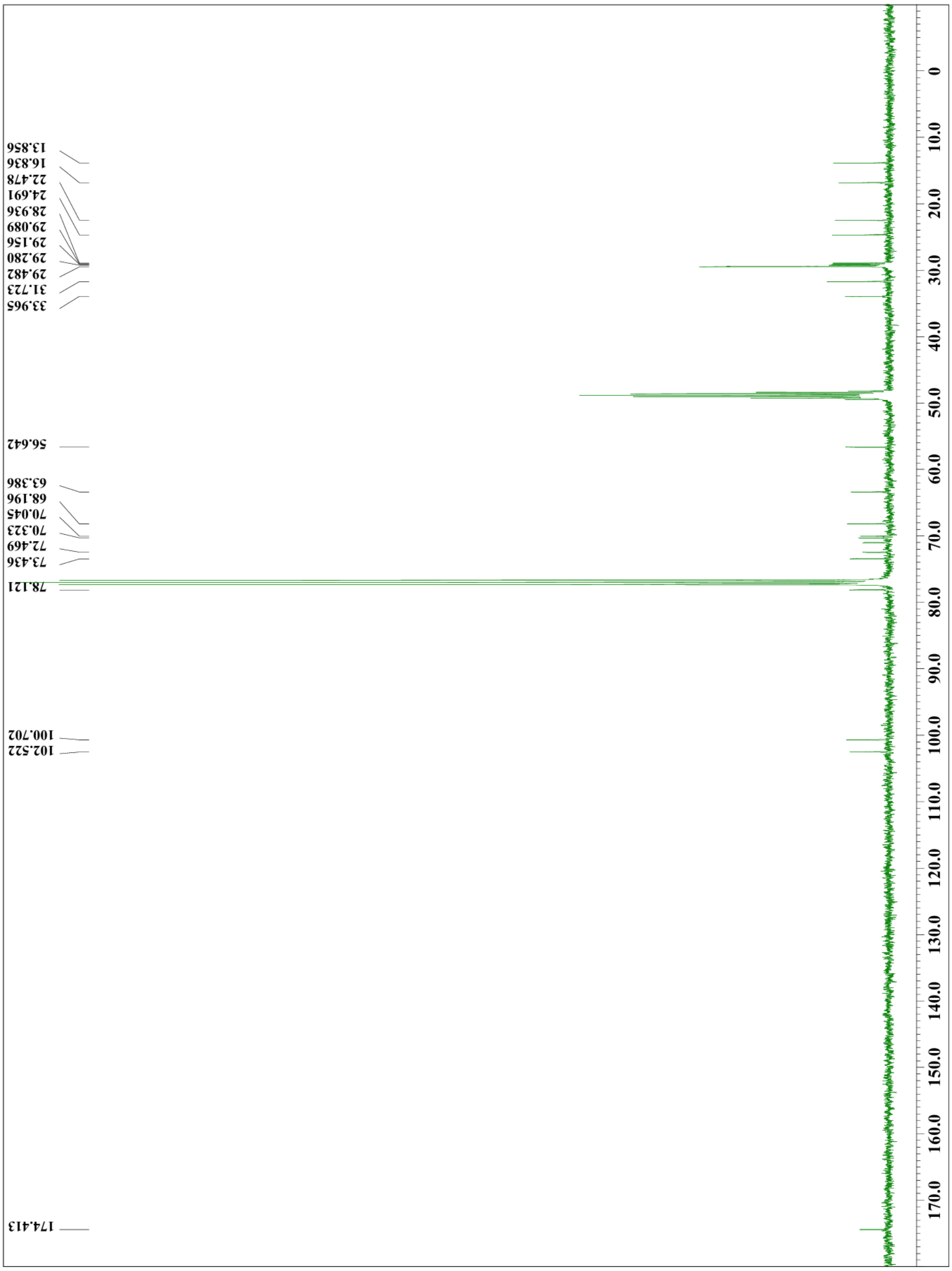
decahydro-2-naphthol α-L-rhamnopyranosyl-(1→2)-6-*O*-palmitoyl-β-D-glucopyranoside (1d)



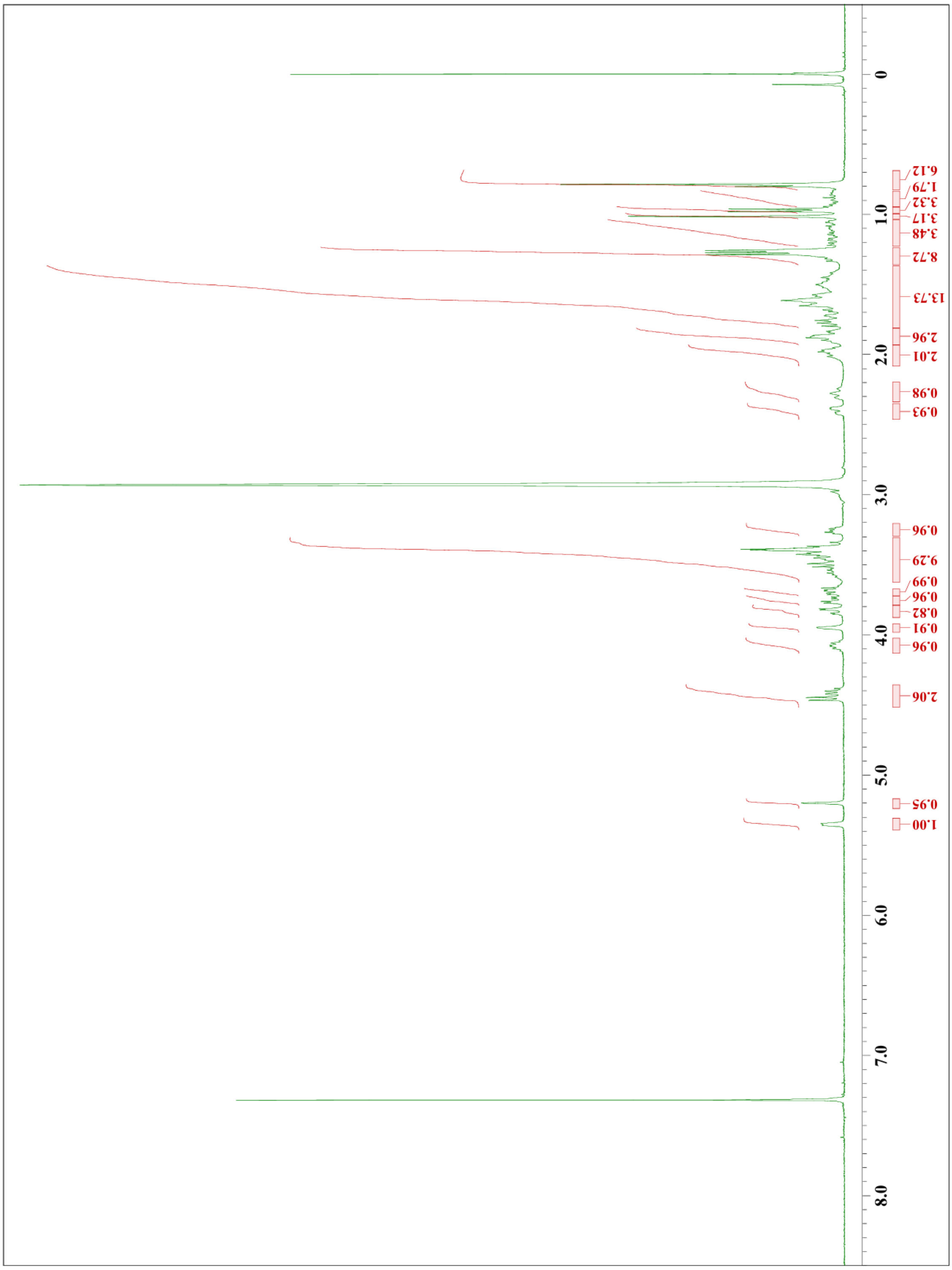

13.948
17.124
22.551
24.792
26.127
26.328
29.074
29.170
29.227
29.389
29.523
29.551
31.783
33.099
33.614
34.129
35.169
41.015
63.667
68.168
70.305
70.457
71.077
72.584
73.338
74.845
77.629
99.747
100.176
174.330
0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
170.0

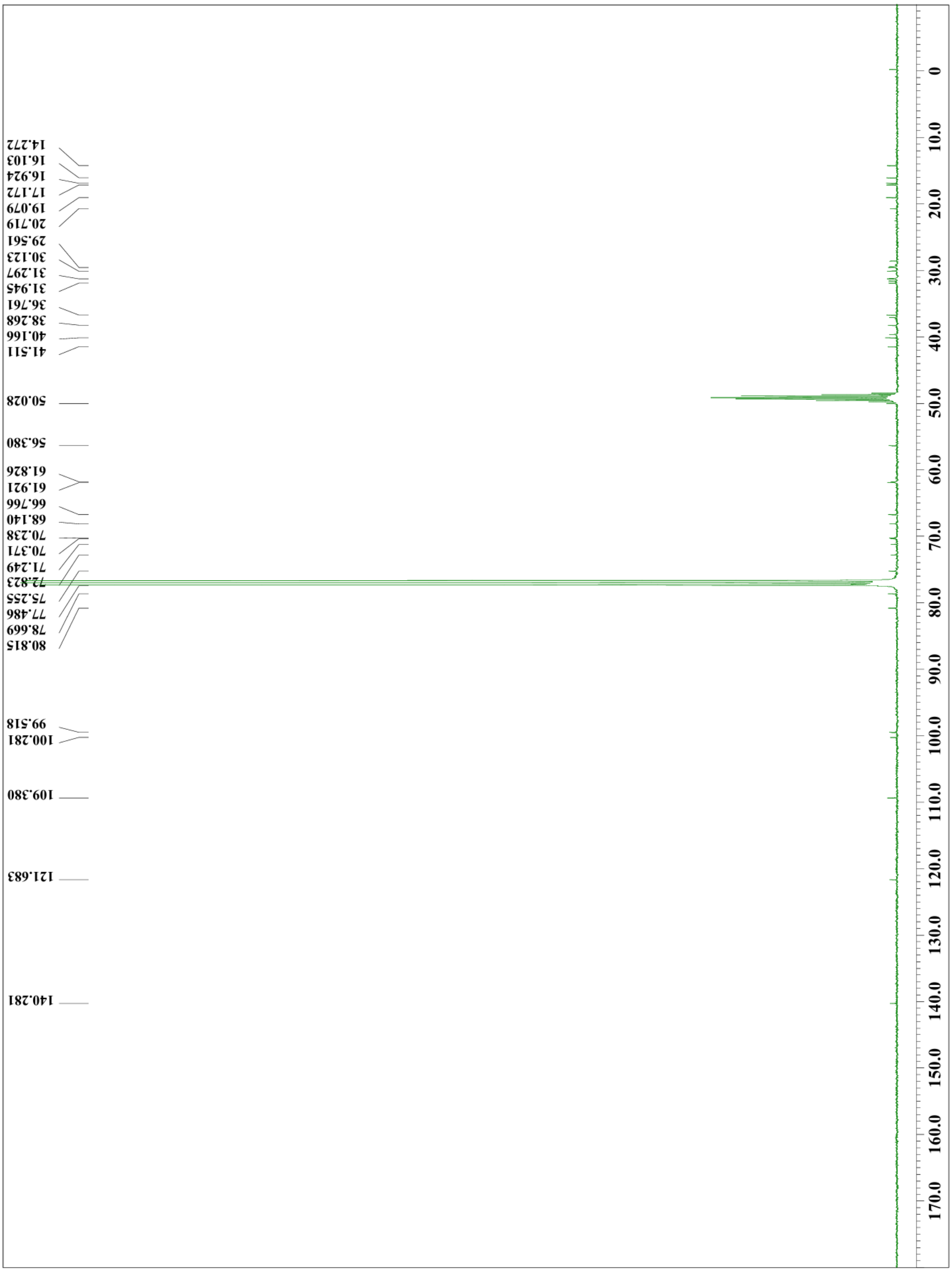
methyl α-L-rhamnopyranosyl-(1→2)-6-*O*-palmitoyl-β-D-glucopyranoside (1e)



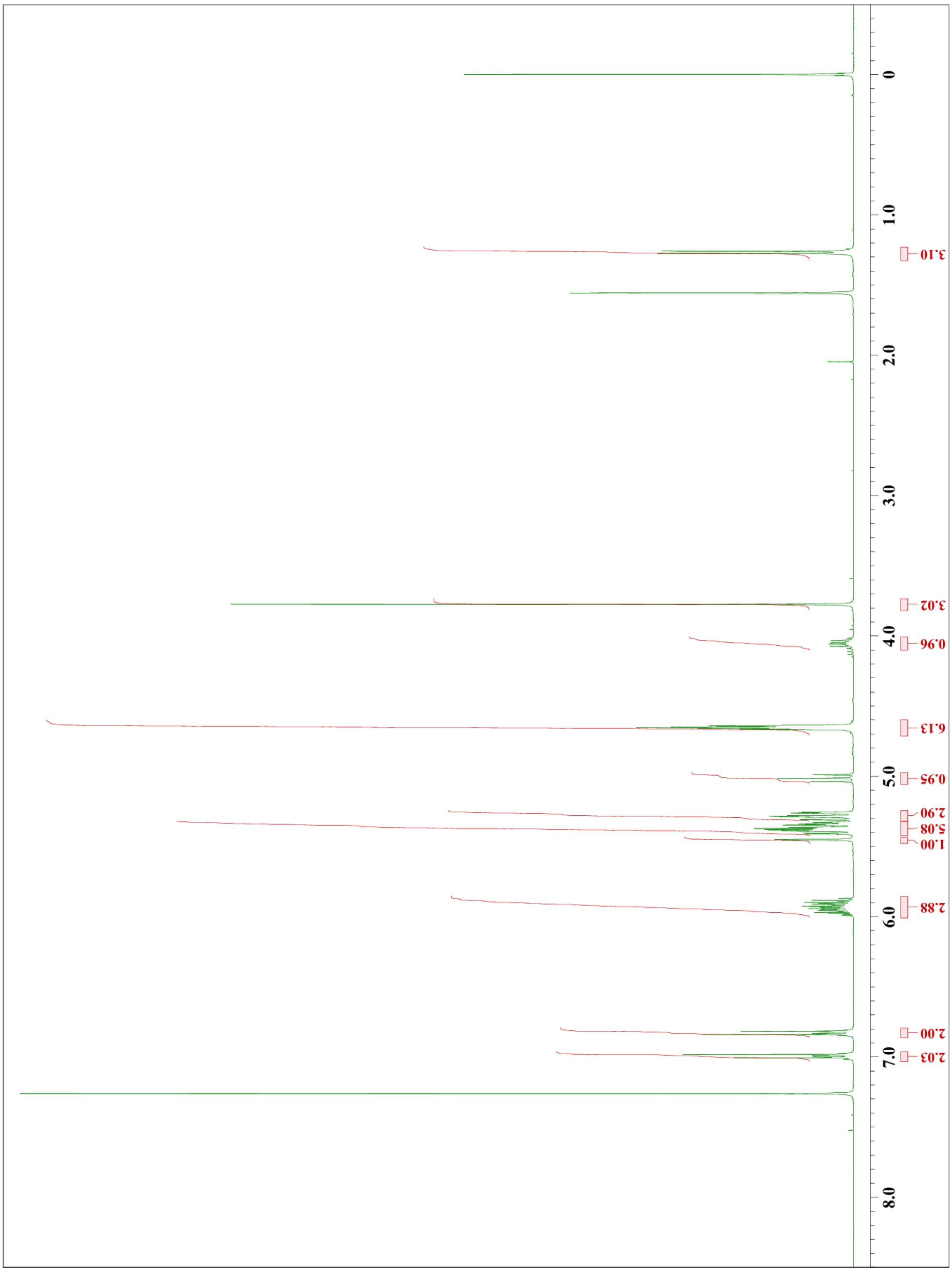

13.856
16.836
22.478
24.691
28.936
29.089
29.156
29.280
29.482
31.723
33.965
56.642
63.386
68.196
70.045
70.323
72.469
73.436
78.121
100.702
102.522
174.413
0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
170.0

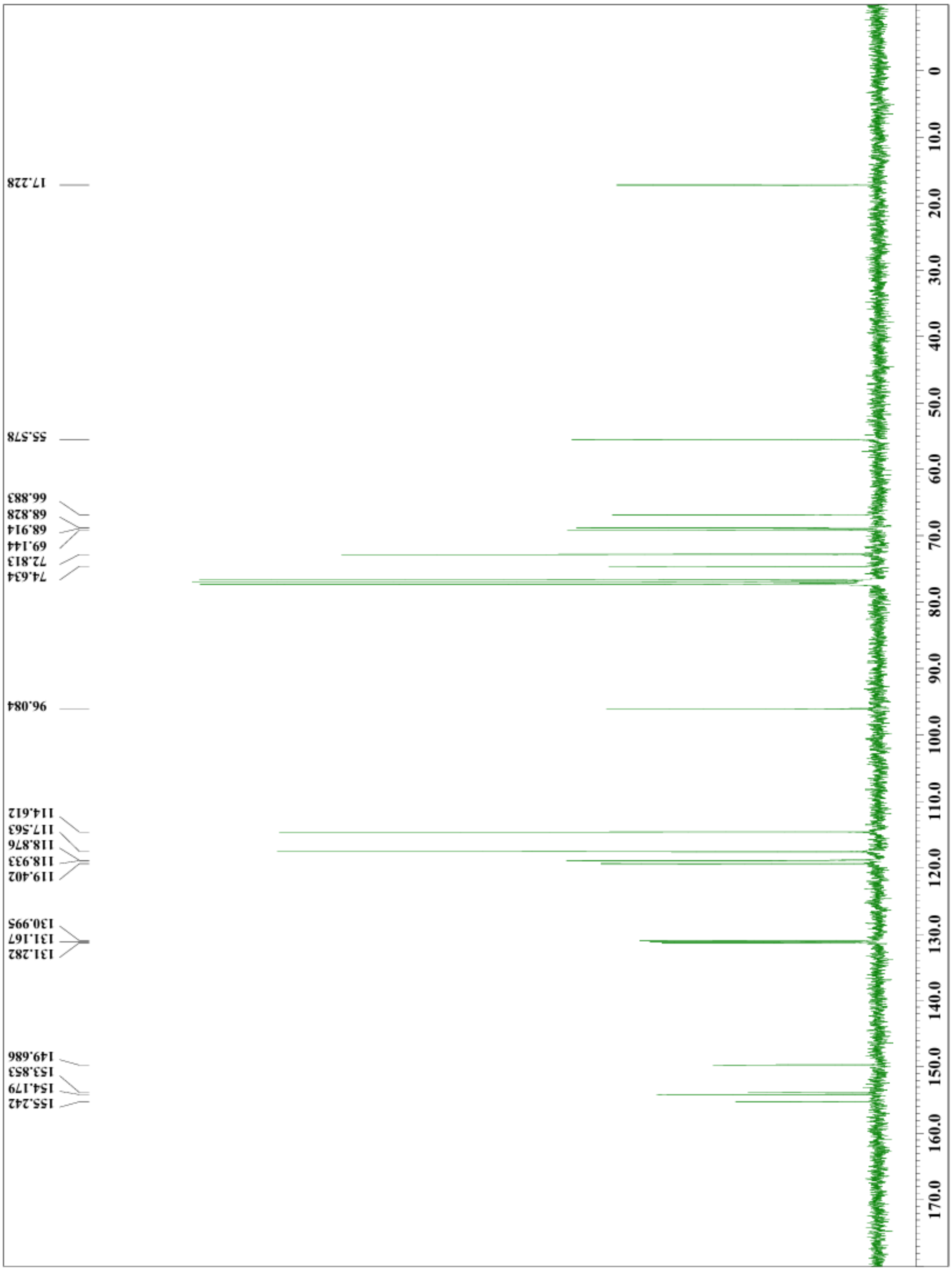
diosgenyl α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (1a')



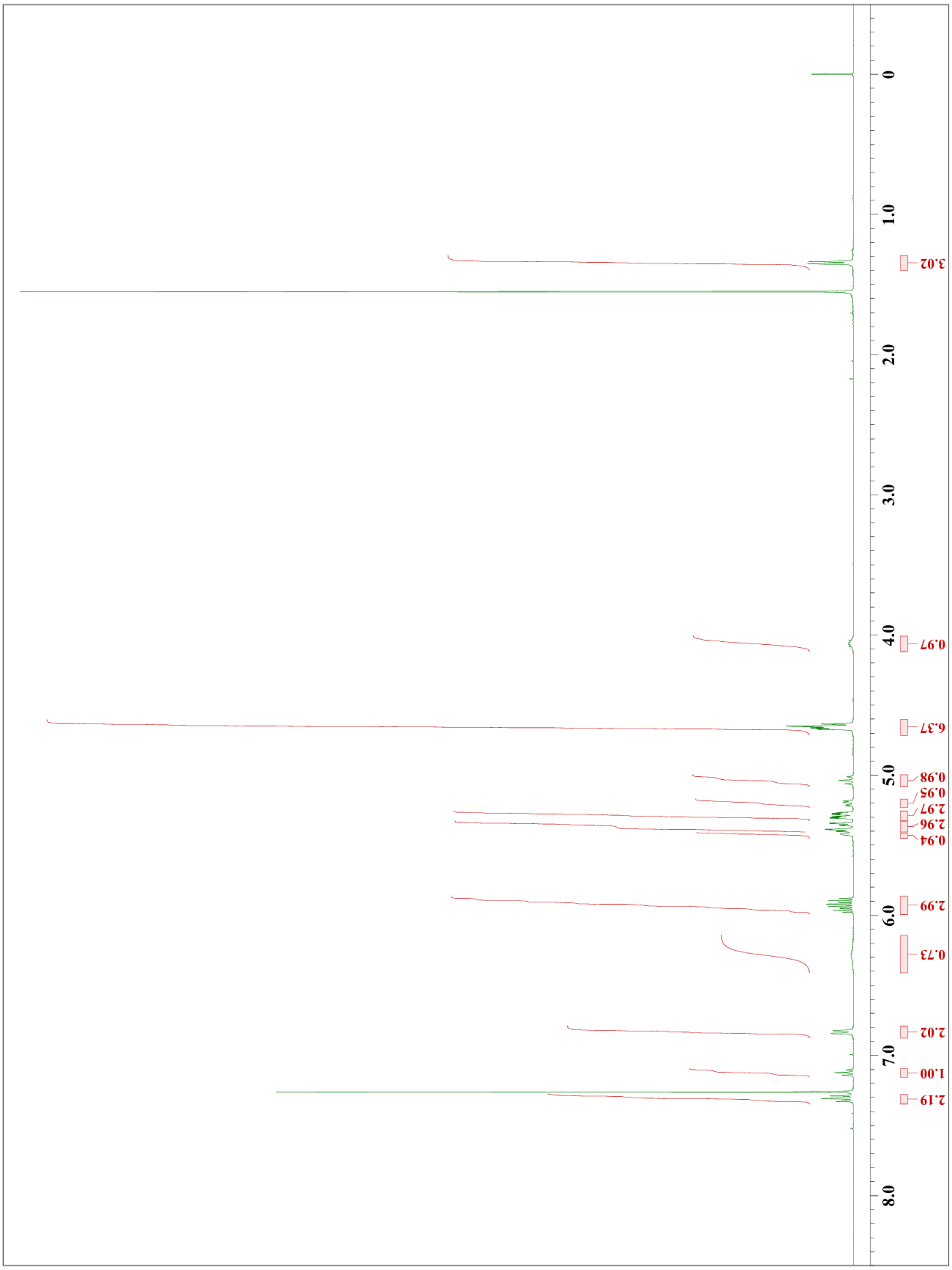

14.272
16.103
16.924
17.172
19.079
20.719
29.561
30.123
31.297
31.945
36.761
38.268
40.166
41.511
50.028
56.380
61.826
61.921
66.766
68.140
70.238
70.371
71.249
72.823
75.255
77.486
78.669
80.815
99.518
100.281
109.380
121.683
140.281
0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
170.0

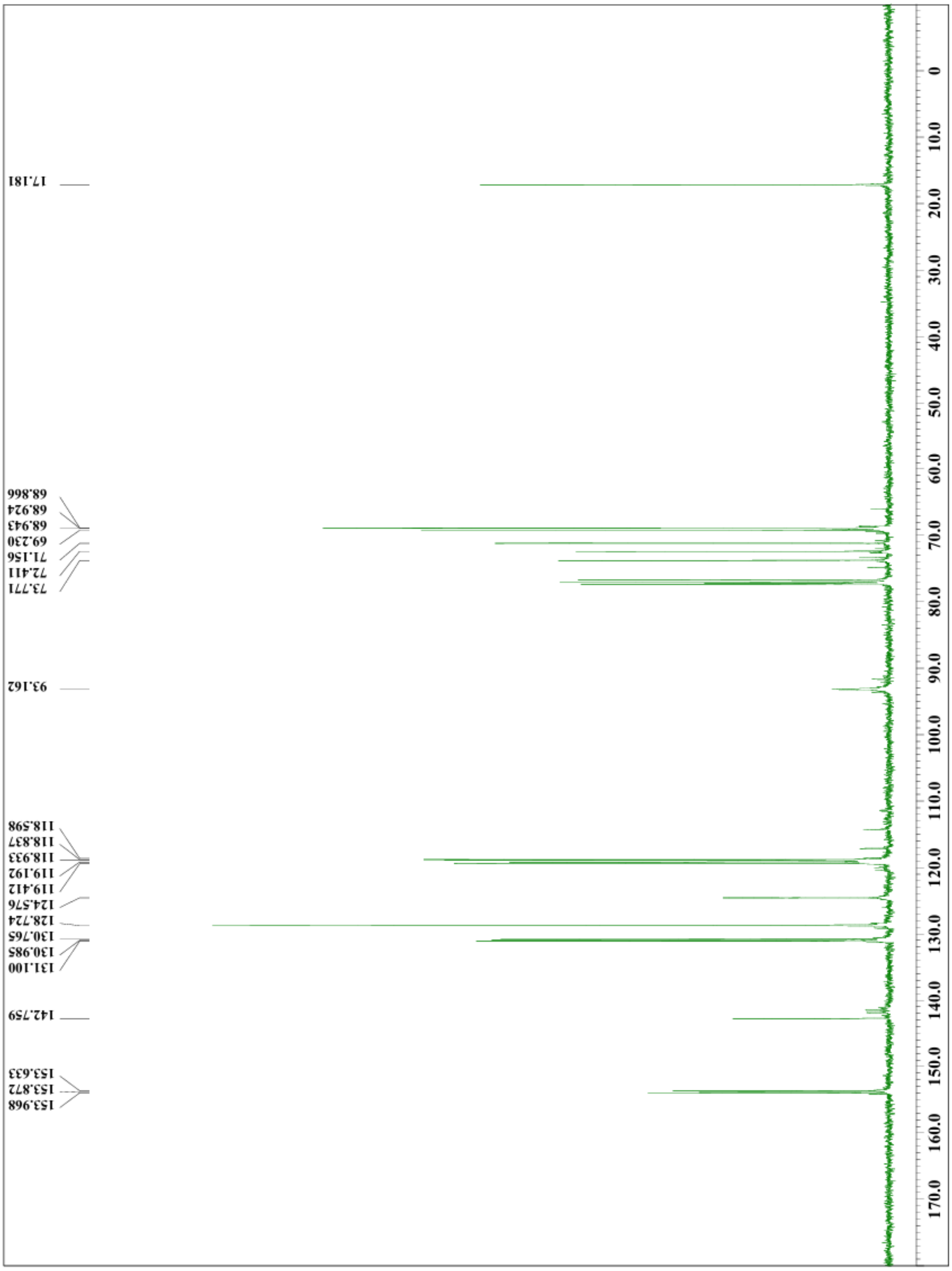
_p_-methoxyphenyl 2,3,4-_O_-trialloc-L-rhamnopyranoside (S2)




17.228
55.578
66.883
68.828
68.914
69.144
72.813
74.634
96.084
114.612
117.563
118.876
118.933
119.402
130.995
131.167
131.282
149.686
153.853
154.179
155.242
0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
170.0

2,3,4-*O*-trialloc-α-L-rhamnopyranosyl *N*-phenyltrifluoroacetimidate (5)




17.181
68.866
68.924
68.943
69.230
71.156
72.411
73.771
93.162
118.598
118.837
118.933
119.192
119.412
124.576
128.724
130.765
130.985
131.100
142.759
153.633
153.872
153.968
0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
170.0