

SUPPLEMENTARY MATERIAL

Exploring the Antimelanoma potential of Betulinic Acid Esters and their Liposomal Nanoformulations

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Abstract: Betulinic acid is a natural occurring pentacyclic triterpene belonging to the lupane-group, that exhibits a wide range of pharmacological activities. BA derivatives are continuously being researched due to their improved anticancer efficacy and bioavailability. The current research was conducted in order to determine the antiproliferative potential of three synthesized BA fatty esters using palmitic, stearic and butyric acids and their liposomal nanoformulations. The cytotoxic potential of BA fatty esters (Pal-BA, St-BA, But-BA) and their respective liposomal formulations (Pal-BA-Lip, St-BA-Lip, But-BA-Lip) has been assessed on HaCaT immortalized human keratinocytes and A375 human melanoma cells. Both the esters and their liposomes, acted as cy-totoxic agents against melanoma cells in a time and dose-dependent manner. The butyryl ester But-BA outperformed BA in terms of cytotoxicity (IC₅₀ 60.77 μM) while the nanoformulations St-BA-Lip, But-BA-Lip and BA-Lip also displayed IC₅₀ values (60.11, 50.71 and 59.01 μM) lower compared to BA ((IC₅₀ 65.9 μM). The morphological evaluation revealed that the A375 cells underwent morphological changes consistent with apoptosis following 48h treatment with the tested compounds, while the HaCaT cells' morphology remained unaltered. Both the esters and their liposomal formulations were able to inhibit the migration of the melanoma cells, suggesting a significant antimetastatic effect. The quantitative real-time PCR revealed that all tested samples were able to significantly increase the expression of the pro-apoptotic Bax and inhibit the anti-apoptotic Bcl-2 proteins. This effect was more potent in the case of liposomal nanoformulations versus non-encapsulated compounds, and overall, But-BA and its formulation exhibited the best results in this regard.

Keywords: betulinic acid; betulinic acid derivatives; liposomal formulation; cytotoxicity; melanoma.

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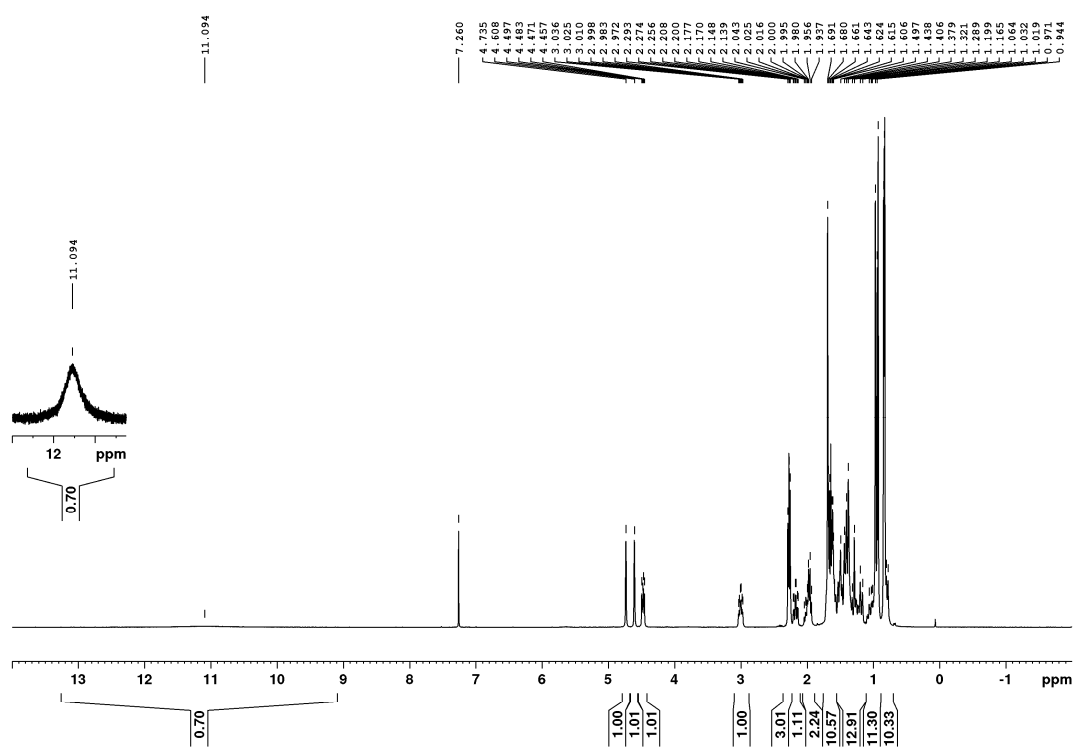


Figure S1. ^1H NMR spectrum of 3-O,28-O-diacetyl-betulin (But-BA)

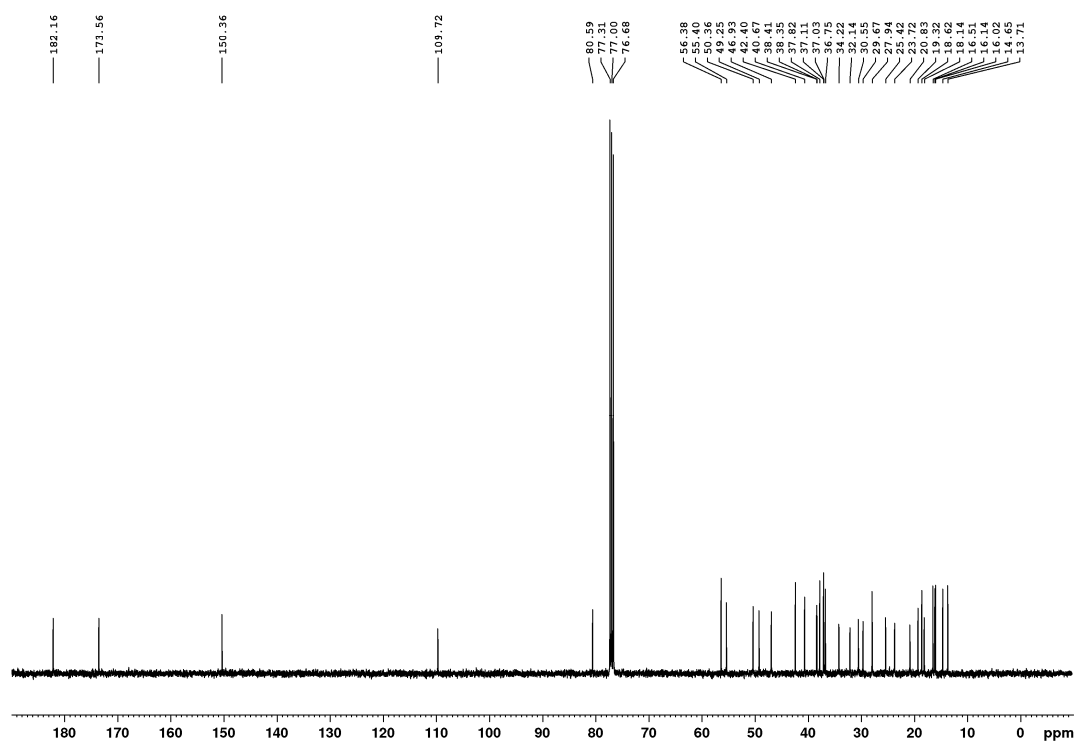


Figure S2. ^{13}C NMR spectrum of 3-O-butyryl-betulinic acid (But-BA)

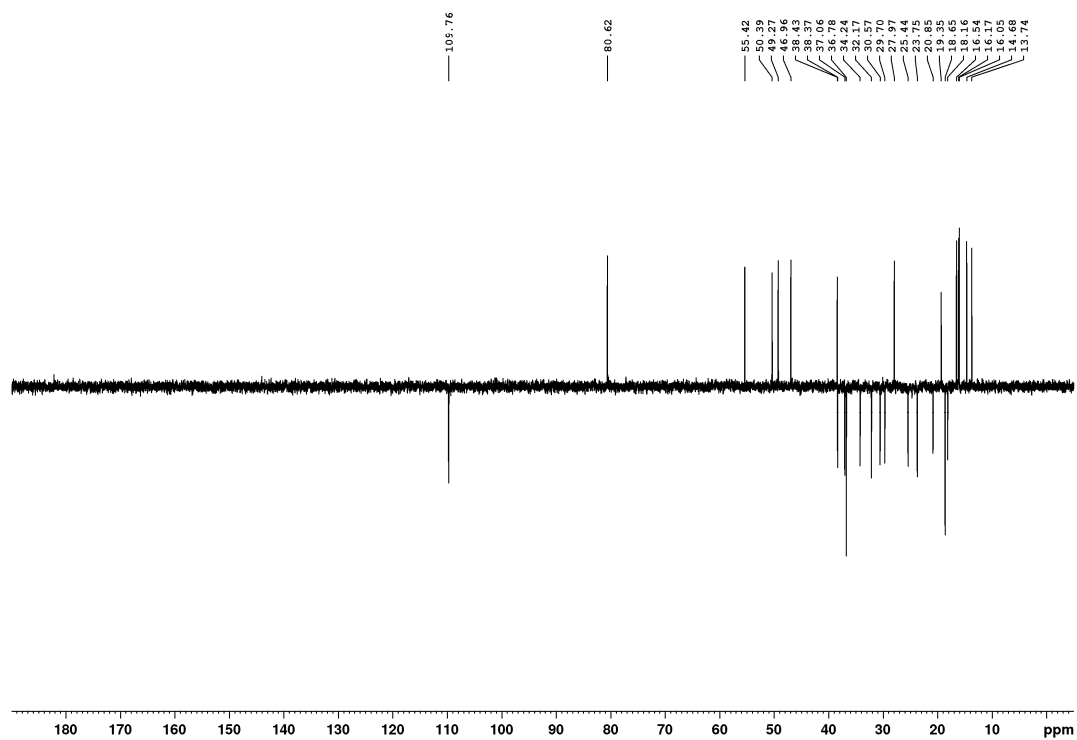


Figure S3. ^{13}C DEPT NMR spectrum of 3-O-butyryl-betulinic acid (But-BA)

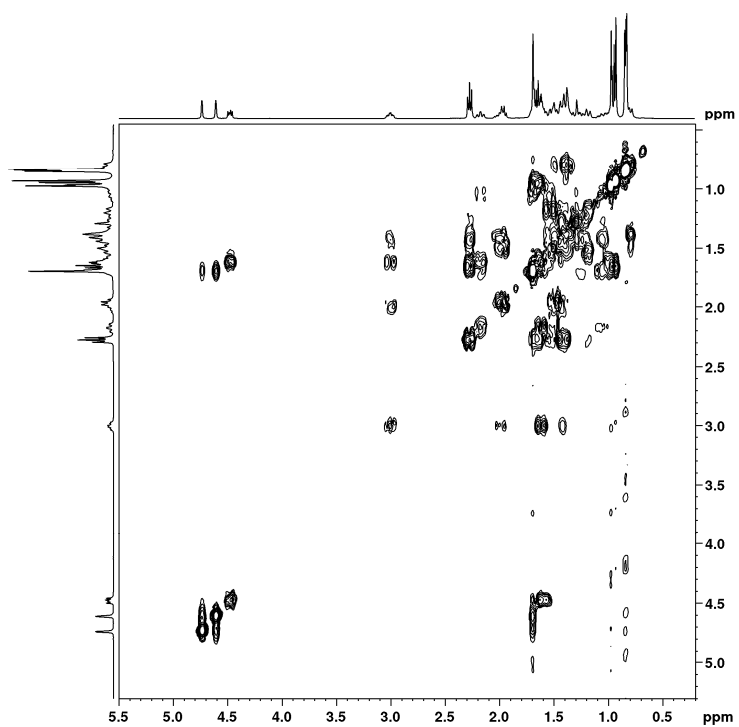


Figure S4. ^1H , ^1H -COSY NMR spectrum of 3-O-butyryl-betulinic acid (But-BA)

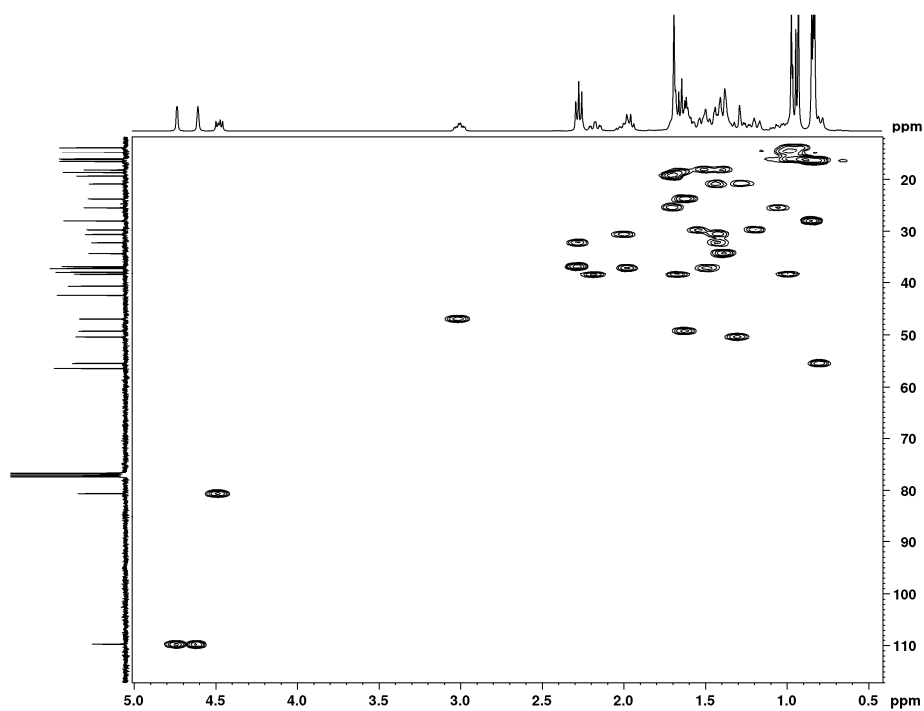


Figure S5. $^1\text{H}, ^{13}\text{C}$ -HSQC NMR spectrum of 3-O-butyryl-betulinic acid (But-BA)

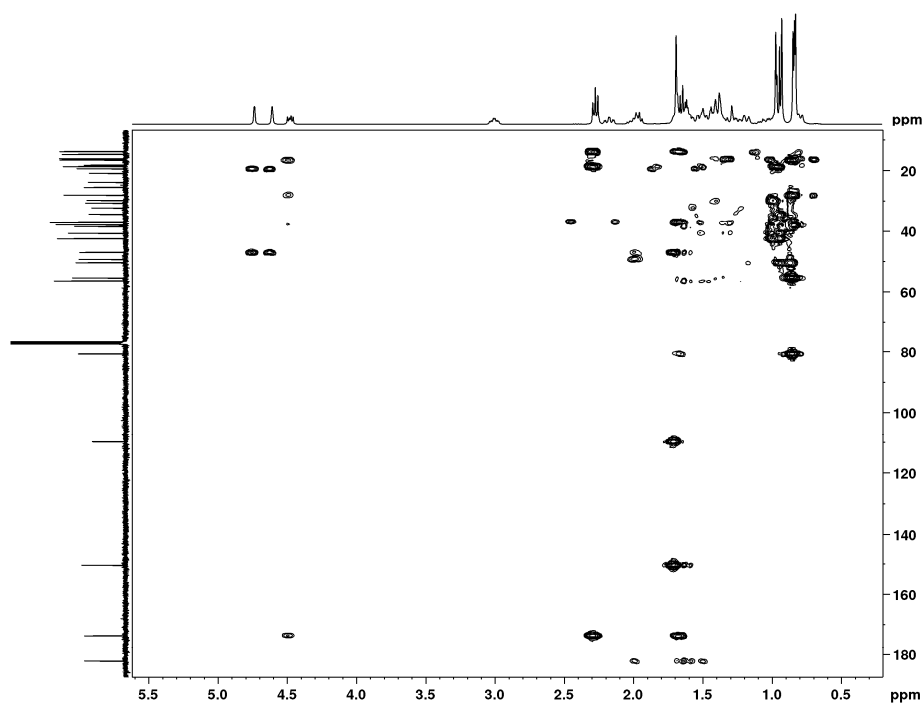
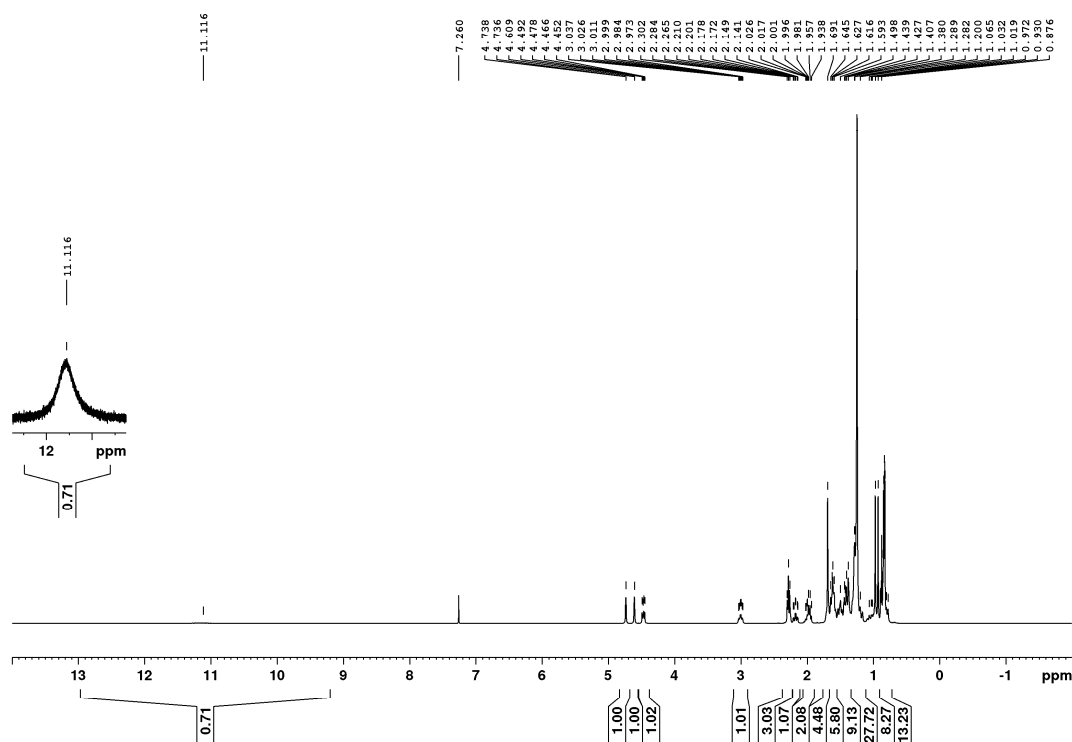


Figure S6. $^1\text{H}, ^{13}\text{C}$ -HMBC NMR spectrum of 3-O-butyryl-betulinic acid (But-BA)



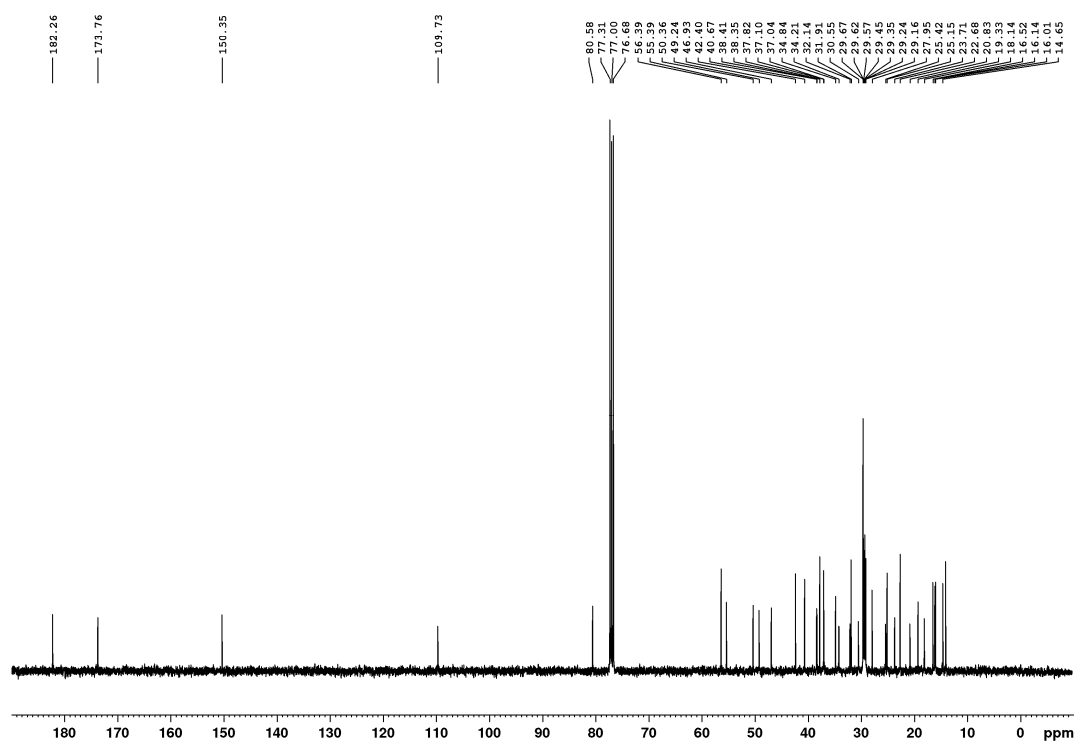


Figure S8. ^{13}C NMR spectrum of 3-O-palmitoyl-betulinic acid (Pal-BA)

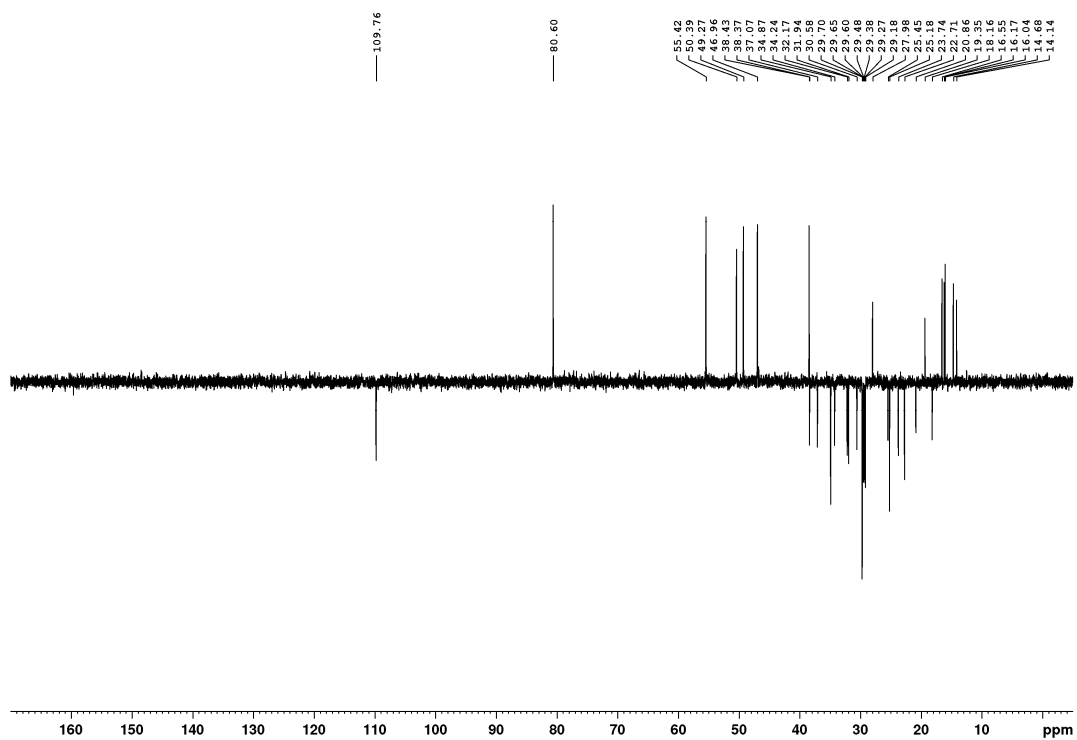


Figure S9. ^{13}C DEPT NMR spectrum of 3-O-palmitoyl-betulinic acid (Pal-BA)

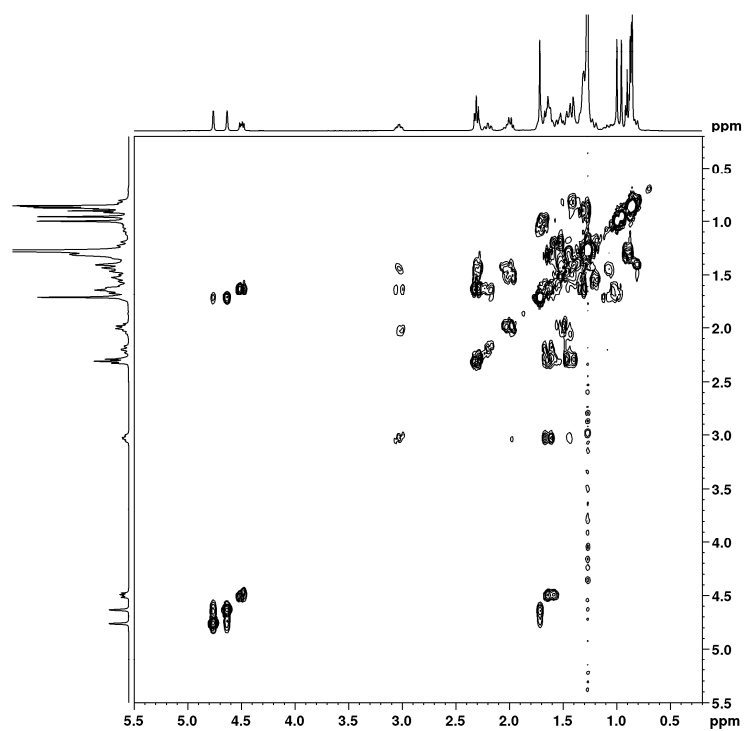


Figure S10. H,H-COSY NMR spectrum of 3-O-palmitoyl-betulinic acid (Pal-BA)

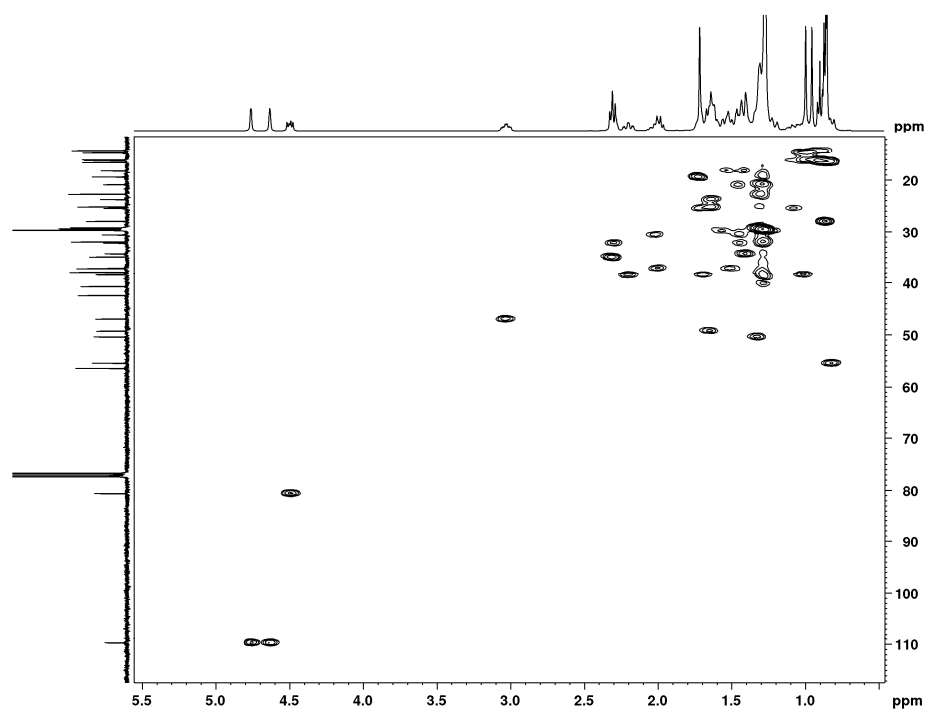


Figure S11. H,C-HSQC NMR spectrum of 3-O-palmitoyl-betulinic acid (Pal-BA)

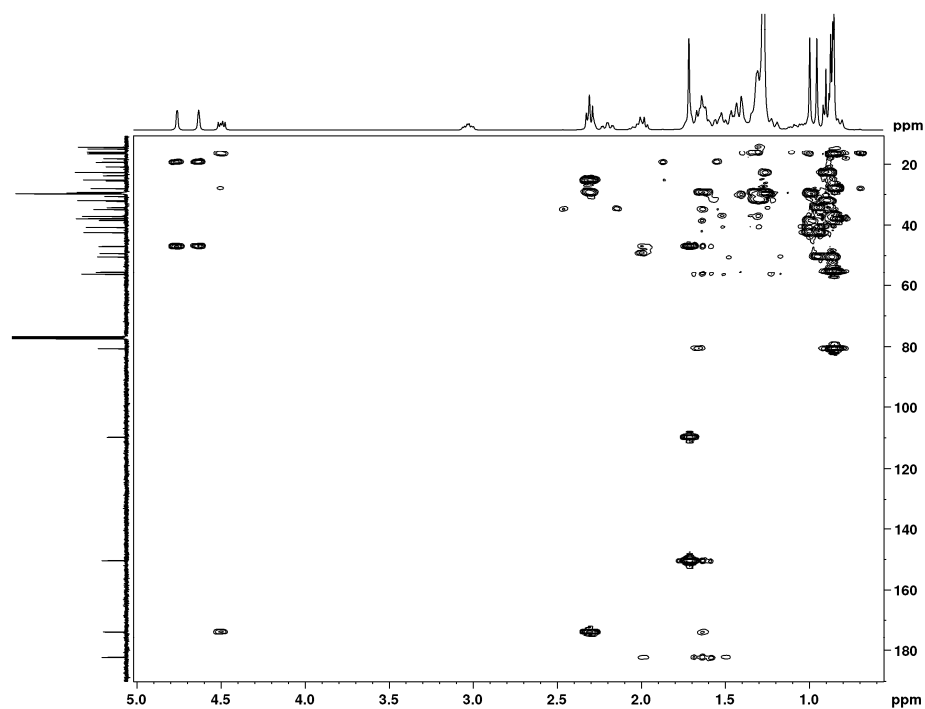


Figure S12. H,C-HMBC NMR spectrum of 3-O-palmitoyl-betulinic acid (Pal-BA)

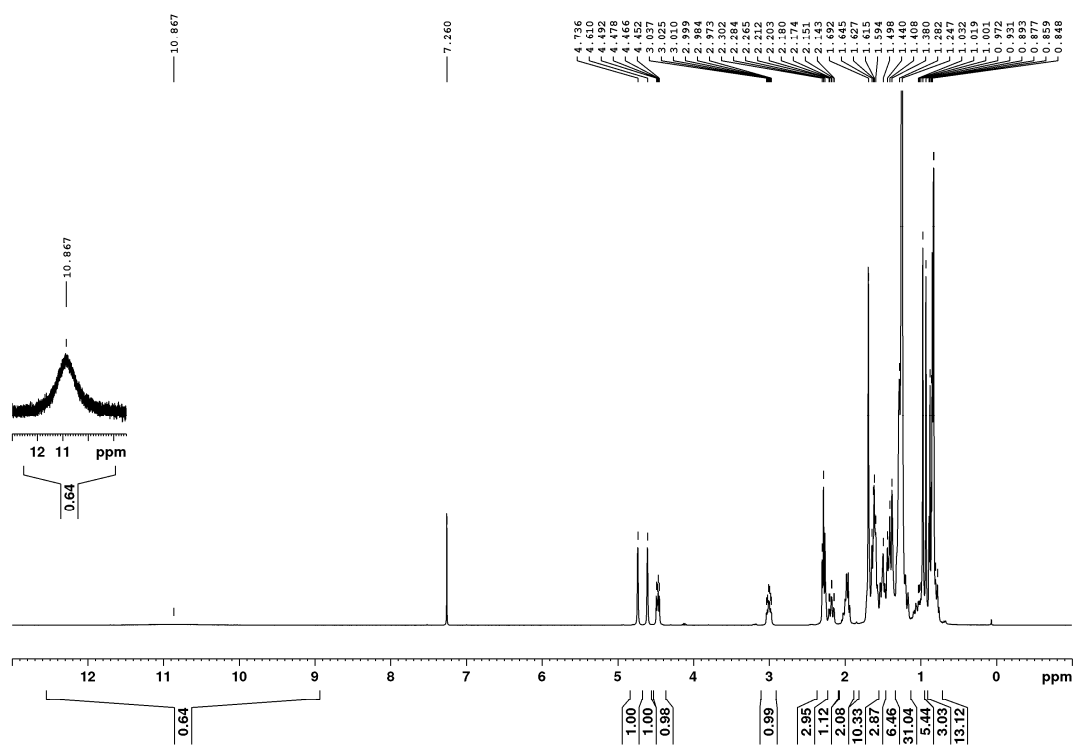


Figure S13. ^1H NMR spectrum of 3-O-stearoyl-betulinic acid (St-BA)

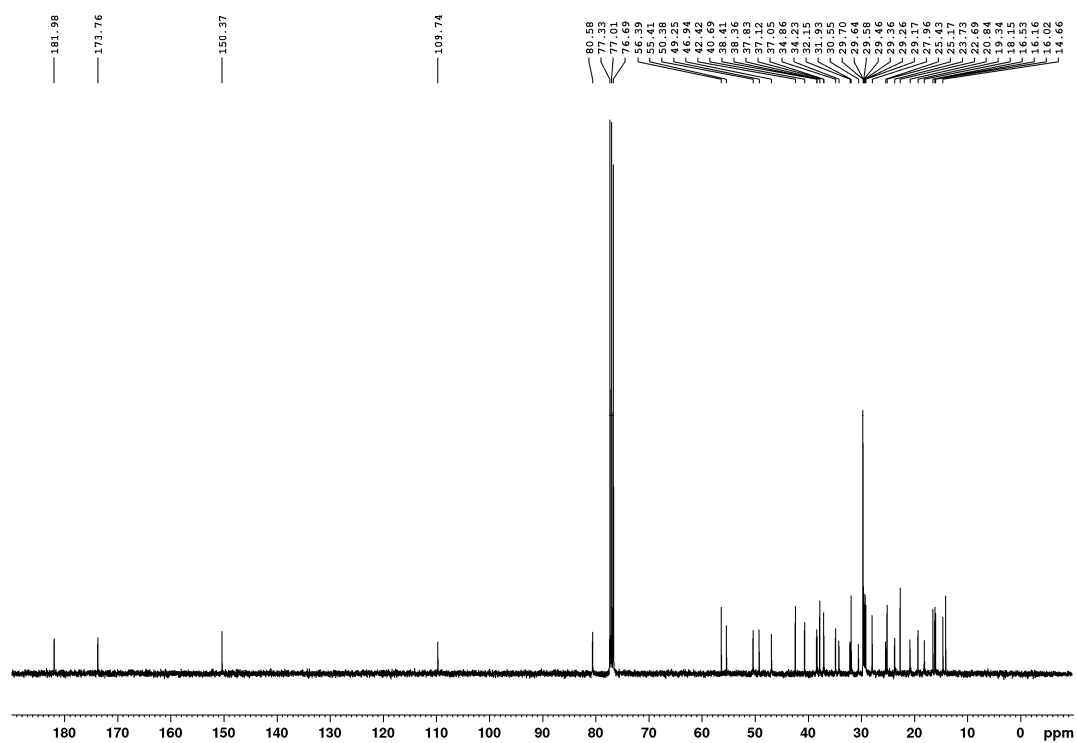


Figure S14. ¹³C NMR spectrum of 3-O-stearoyl-betulinic acid (St-BA)

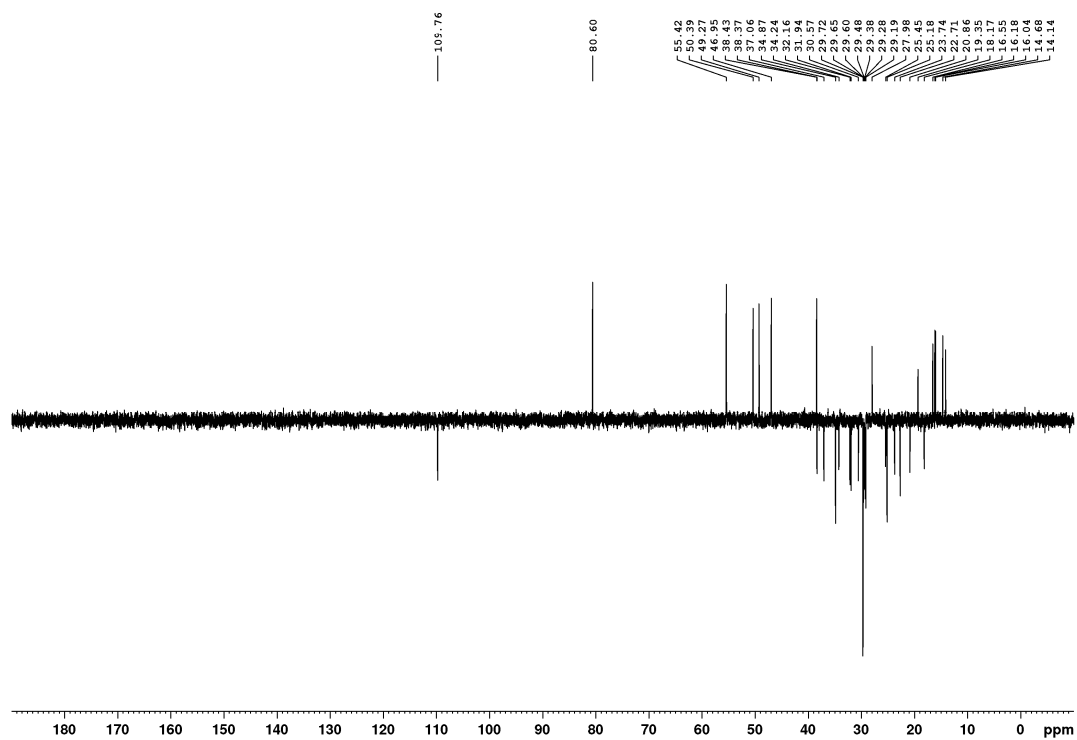


Figure S15. ¹³C DEPT NMR spectrum of 3-O-stearoyl-betulinic acid (St-BA)

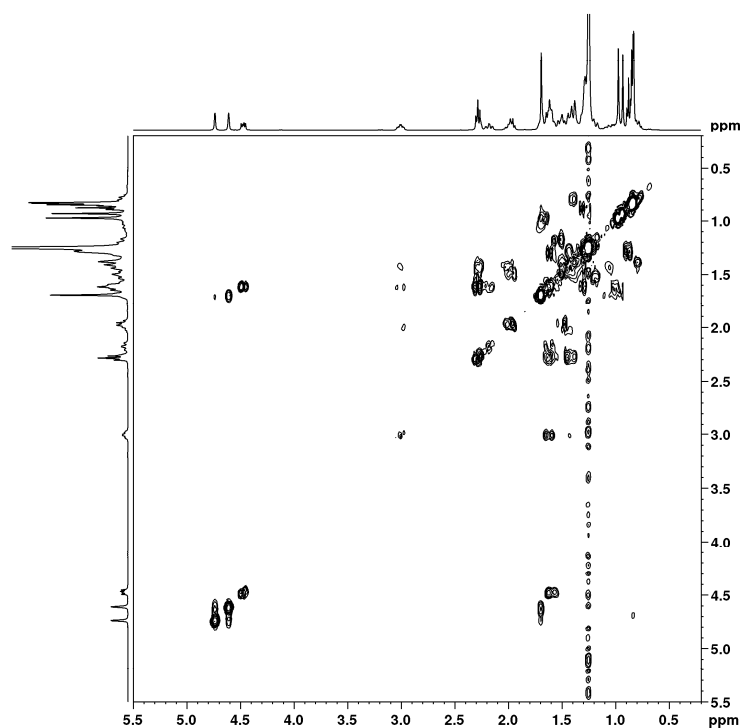


Figure S16. H,H-COSY NMR spectrum of 3-O-stearoyl-betulinic acid (St-BA)

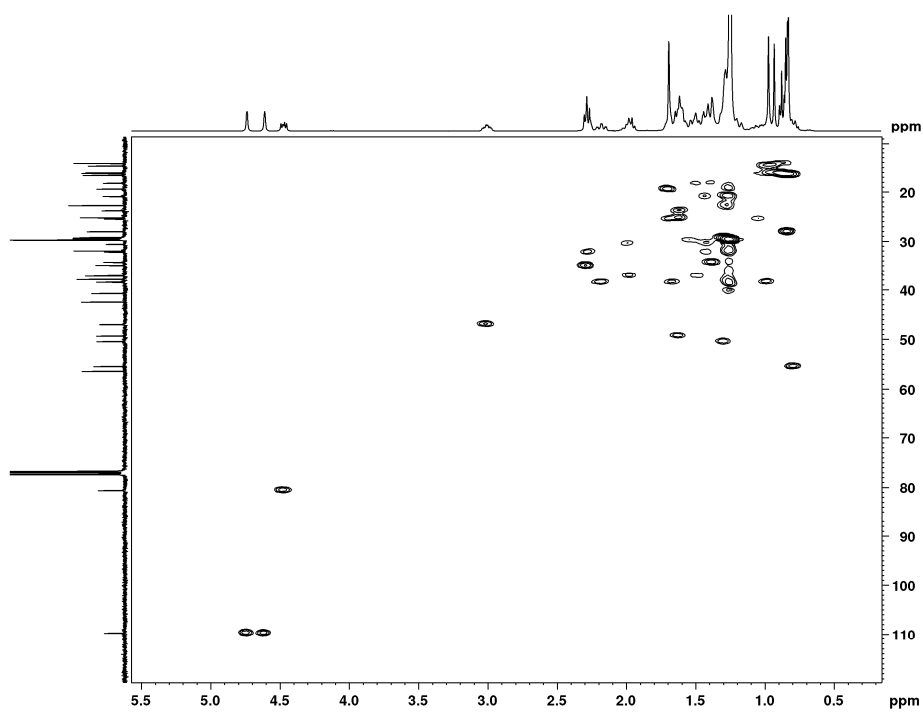


Figure S17. H,C-HSQC NMR spectrum of 3-O-stearoyl-betulinic acid (St-BA)

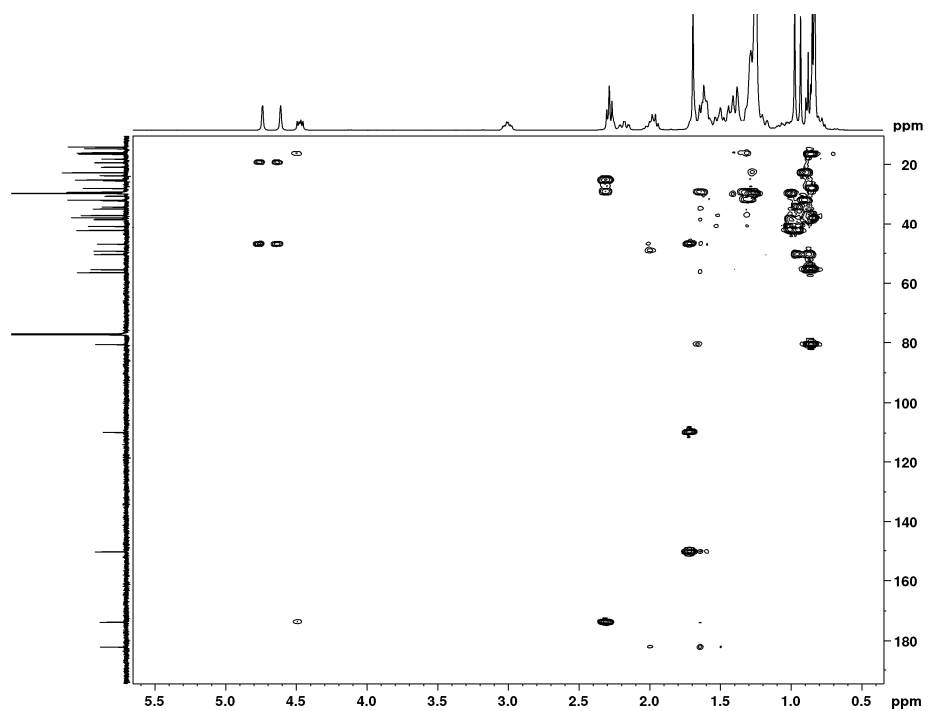


Figure S18. $^1\text{H}, ^{13}\text{C}$ -HMBC NMR spectrum of 3-O-stearoyl-betulinic acid (St-BA)

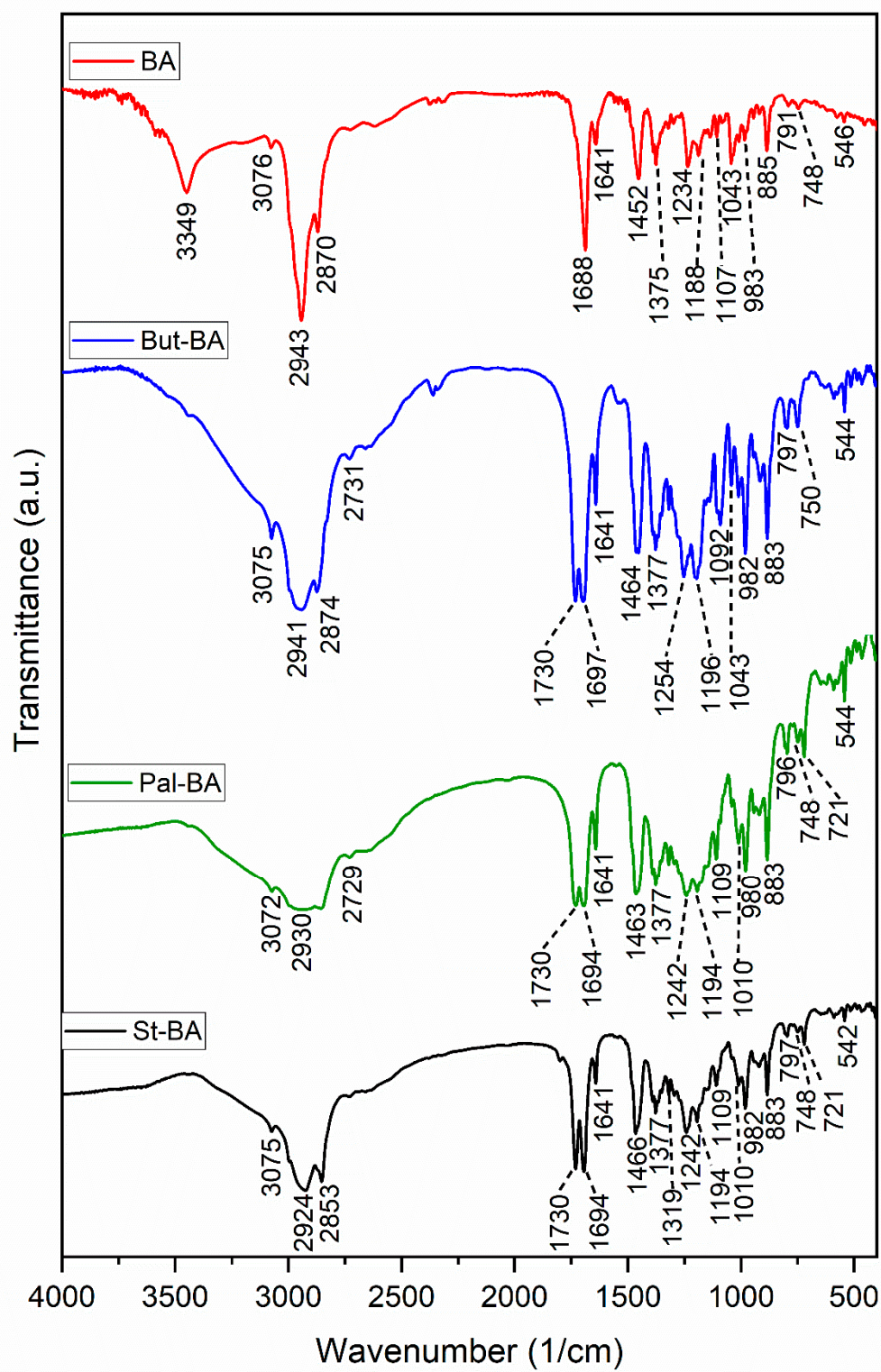


Figure S19. FTIR spectra of BA, But-BA, Pal-BA and St-BA