

Fast analysis of maca bioactive compounds for ecotype characterization and export quality control

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Abstract

Maca (*Lepidium meyenii* Walp.) storage hypocotyls have been used since pre-Columbian times by Andean dwellers due to their high nutritional value and energizing properties, which appear to counteract some of the negative physiological effects of high altitudes. Maca has been propelled to the status of highly successful Peruvian export through reports of its energizing/invigorating properties and its effectiveness against benign prostate hyperplasia. Although studies reporting these effects have been performed using hexane, chloroform, alcohol or aqueous extracts, none have used pure compounds and one cannot pinpoint the biologically active compounds in the plant tissue. Analytical methods are needed which can provide in a reliable, quick and simple fashion the amounts of potential bioactive compounds in export material and also, since maca is exported as the dry hypocotyls, of the quality of the postharvest treatment used to naturally dry the plant tissue. We propose two, possible methods, for the sequential evaluation of bioactive marker compounds in maca. A headspace capture system coupled to a 10 min GC-MS run for the fast analysis of benzylamines, aldehydes, alcohols, isothiocyanates and nitriles and a solid phase extraction method using ion exchange and reverse phase cartridges sequentially followed by LC-MS analysis for total glucosinolates and fatty acid benzylamides. Both methods also allow the direct comparison of maca ecotypes in order to target them in the export market for their various purported physiological effects.

Introduction

Maca (*Lepidium meyenii*, Walpers or *L. peruvianum*, Chacon), an annual herbaceous plant of the Brassicaceae family, is a native of the Andean high plateaus. It is the only reported species of the genus *Lepidium* which contains a fused hypocotyl and tap root forming a fleshy underground storage organ suited to the dry, windy and cold high-altitude environment (NRC, 1989). The plant has been cultivated and used for food and medicinal purposes since pre-Columbian times and has gained attention in recent years due to reports on medicinal properties which make it a good candidate for the nutraceuticals market (Canales et al., 2000, Dini et al., 1994). Maca production has grown from 800 to well over 2,000 metric tons in the past 7 years and the export of dried maca powder and derived products reached US\$ 5 million in 2008 (Prompex 2008).

Maca, however, presents a variety of chemical phenotypes regarding the major natural products that the plant accumulates in its tissues. These variants can be grouped into three major categories, red, yellow and black, based on hypocotyl and stem coloration. These, in turn, have a number of ecotypes which are characteristic of geographical regions where the plant is grown in Peru. Preliminary evidence indicates differences in the levels of major natural products characteristic of the species and in the reported biological effects or medical target for which these different types can be used. Gonzales and collaborators (Gonzales et al., 2005, Chung et al., 2005) report for example that black maca is useful in stimulating sperm count while red maca is useful against benign prostate hyperplasia.

Given the difficulties in obtaining phytosanitary certificates for the export of fresh tubers, the majority of the exported material is shipped in the form of powder obtained from naturally air-dried hypocotyls or from of lyophilized fresh material. The difference in composition between these two products is, as expected, fairly large. Powdered maca for export is usually obtained from roots that have been dried in the open air on site. The process takes usually one month and involves exposure to the extreme temperature cycles and strong light conditions typical at high altitudes. A significant degree of variation in the environmental conditions used for drying mean that one can expect also variations in the amounts of various hydrolytic products of the major maca storage compounds present in the tubers.

Any method aimed at targeting maca for particular medicinal or nutraceutical uses will have to deal with both chemical phenotypes and hydrolytic byproducts. In this progress report we propose a fast two-stage strategy for chemical phenotype profiling of maca and for quality control of naturally dried hypocotyls aimed for the export market.

Materials and Methods

Biological material

Fresh maca (*Lepidium meyenii*, Walpers) was bought in Lima markets. The hypocotyls were lyophilized for 3 days, pulverized in a blender and sieved (denominated the MFL fraction). Dry maca was bought in markets in the city of Huancayo dried in the traditional way (by direct exposure in the field for a month), pulverized in a ball mill and sieved (denominated the MSM fraction), both were stored at -20°C until used.

Synthetic material

Macamides were synthesized by reaction of benzylamine or 4-methoxy-benzylamine (1.5 mmol) with the respective fatty acid (caprylic, palmitic, stearic, oleic, linoleic and linolenic) (1 mmol) promoted by dicyclohexylcarbodiimide (DCC) (1.0mmol) catalyzed with pyrrolidinepyridine (PPy) (0.5mmol). The amides obtained were purified by chromatography on silica gel 60 and characterized using a Bruker UltraShield 300 MHz NMR spectrometer, a Hewlett Packard 5971A GC-MS with electron impact ionization and a Perkin Elmer 1600 Series FT-IR spectrometer. The spectral data of the synthetic amides are listed in the appendix.

Extraction procedures

1g of maca was extracted in 20ml of 70% MeOH at 70°C under nitrogen for 1h under constant agitation. The extract was vacuum-filtered through Whatman GF/A (1.6µm) glass microfiber filters and stored under nitrogen at -20°C until used.

Analytical procedures

Liquid chromatographic analyses

Macamides. Five ml of stock solution were diluted with 2ml of water and loaded onto a Merck LiChrolut RP-18 500mg SPE column preconditioned with 5ml MeOH, 5ml water and 5ml of 50% MeOH. Washed with 0.5ml 50% MeOH and eluted with 2ml of 100% MeOH.

Macamides in the preparation were analyzed in a Merck-Hitachi LaChrom D-7000 HPLC with a Merck-Hitachi L-7450-A diode array UV detector and a LiChrospher 100 RP-18 column (250 x 4mm, 5µm particles). Temperature was set at 40°C and flow at 1ml/min. Solvent A: ACN with 0.005% TFA. Solvent B: H₂O with 5% of MeOH and 0.005% TFA. Gradient: 4min at 65%A, 65%-85%A in 2min, 85%-100% in 20min, 5min at 100%A. Injection: 20µl. Detection: 210nm.

Amines. Amines were analysed by conversion to *o*-phthaldehyde (OPA) derivatives by adding 200 µl of maca stock solution to 250 µl of borate buffer 0.4M, pH 9.5, and 50 µl of OPA reagent, mixed on a vortex and allowed to react for 3 minutes at room temperature. After derivatization 50µl samples were analysed immediately by HPLC with diode-array UV detection, using a LiChrospher 100 RP-18 column (125 x 4mm, 5µm particles). Temperature 30°C, flow: 1ml/min. UV detection at 340 nm. Solvent A: MeOH, solvent B: Na acetate buffer 20mM, pH = 6.0. Gradient: 9min 40%-90%A and then 4 min 100% A.

Glucosinolates. Glucosinolates were analyzed as desulfoglucosinolates following the procedure described by Thies (Thies1988) with some modifications. In brief, 5ml of stock with 1ml of water were loaded onto a Varian BondElut strong anion exchanger (SAX) SPE cartridge (500mg) pre-conditioned with 60% MeOH. After washing with 2ml of 60%MeOH and 3ml of water, 1 ml of MES 0.02M, pH 5.2 and 150 µl of sulfatase (E.C. 3.1.6.1, Sigma) were added and allowed to react overnight. Desulfoglucosinolates were collected the following day by sequential elution with 800 µl of EtOH 60% and 800 µl of MilliQ water and collected in the same vial. HPLC analyses were carried out by HPLC with diode array UV detection using a LiChrospher 100 RP-18 (250 mm x 4mm, 5µm particles) column. Temperature 30°C, flow 1ml/min. UV detection at 254nm. Solvent A: ACN, solvent B: water. Gradient: 6min 2%-5%A, 2min 5% - 7%, 10min 7% - 21%, 5min 21% - 29%, 2min 29% - 100% .

Gas chromatographic analyses. The analysis of isothiocyanates, nitriles, aldehydes, alcohols and amines was performed by solid phase microextraction (SPME) using PDMS coated fibers (100 μm) exposed 20 min at room temperature to powdered dried maca or to crushed fresh maca tuber cubes (0.1 g) placed within 4 ml glass vials closed with Teflon/silicone septum caps.

SPME fibers were desorbed and analysed in a Perkin Elmer AutoSystem gas chromatograph with flame ionization detection using a J&W Scientific, DB-225 (50% cyanopropylphenyl/methylpolysiloxane) column (30m x 0.32mm, 0.25 μm film thickness). Injector temperature 250 $^{\circ}\text{C}$, detector temperature 250 $^{\circ}\text{C}$, He flow 1ml/min. Temperature program: 1min at 70 $^{\circ}\text{C}$, 70 $^{\circ}\text{C}$ -220 $^{\circ}\text{C}$ in 40 $^{\circ}\text{C}/\text{min}$ and 220 $^{\circ}\text{C}$ for 5min.

Colorimetric procedures. Phenolic compounds were analyzed using the Folin reagent as modified by Kursar and Coley (2003). The unretained fraction from SPE in RP-18 of the maca extract was used for this analysis. Thirty μl of sample in 50%MeOH were added to 220 μl of MilliQ water followed by 500 μl of Folin-Ciocalteu reagent, and the mixture was allowed to react at room temperature for 6 min. 500 μl of Na_2CO_3 0.5M were then added and incubated for 2 h at 30 $^{\circ}\text{C}$ in water bath. UV absorption was read at 725nm and 760nm.

Results and discussion

General considerations

Our analytical approach focused on two different sets of analytes. The first procedure was aimed at determining the content of the most likely candidate constitutive markers for chemical phenotype in maca. These included glucosinolates, amides, total phenolics and free amines present in the extracts (Li & Ammerman, 2001, Piacente et al., 2002, Muhamad et al., 2002, Tellez et al., 2002, Zhao et al., 2005, McCollom et al., 2005). These were analysed by solid phase extraction and liquid chromatography. These compounds can be considered “standard” indicators of the putative pharmacological potency of the plant material and their abundance is a result of environmental conditions during growth and the plant genotype.

In addition to the previous method, a fast SPME-GC approach was developed to evaluate volatiles in fresh and dried plant material. Most volatiles arise as a result of hydrolysis and further metabolic events during processing of the plant material. Figure 1 shows some of the expected products of thioglucosylhydrolases, nitrile lyases and amidohydrolases among others (Winkler et al., 2006).

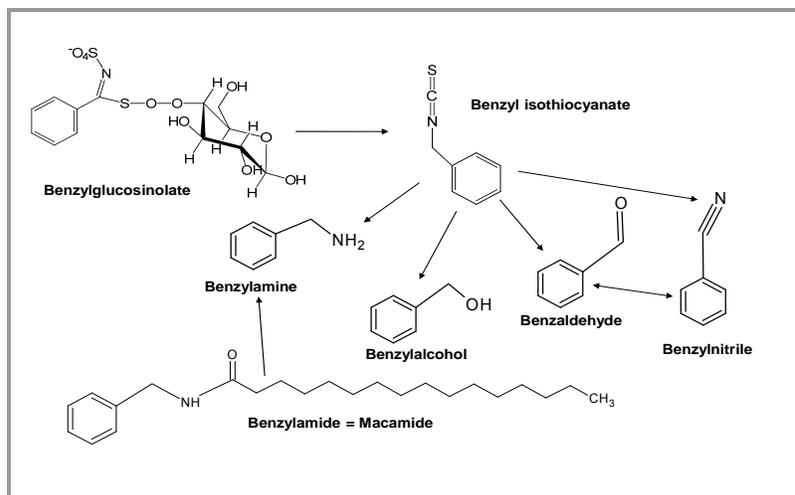


Figure 1. Volatile hydrolytic products of maca glucosinolates and amides. Putative interconversion between nitrile and aldehyde may be promoted by a nitrile lyase.

Analysis of glucosinolate, amide, amine and total phenolic content

The analysis of constitutive secondary metabolites of fresh and dried maca consisted of an extraction in 70%MeOH at 70°C for 1 h, followed by analysis of aliquots in parallel (Figure 2). Amines were analyzed directly from an aliquot of the crude extract by derivatizing it with α -phthalaldehyde reagent followed by HPLC with UV or fluorescence detection. Amides were separated from another aliquot by retention on a reverse-phase SPE cartridge at low solvent strength (50% MeOH). The unretained fraction contained most phenolic components and could be quantified colorimetrically. Amides were eluted with 100% methanol and analyzed by HPLC as described in Materials and Methods. A third aliquot of the extract was loaded onto an anion exchanger (SAX) SPE cartridge for glucosinolate analysis. Glucosinolates retained in the cartridge were treated overnight with sulfatase and the resulting desulfoglucosinolates, eluted sequentially with methanol and water and analyzed by HPLC as described in Materials and Methods.

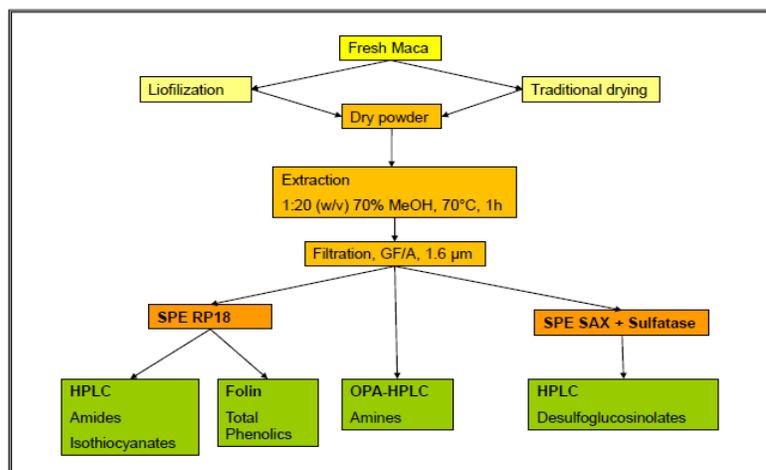


Figure 2. Flow chart describing the processing and analysis of maca constitutive components by liquid chromatography and colorimetric methods

Results using the previously mentioned procedure confirmed previous reports (Li & Ammerman, 2001) of significant loss of glucosinolates during the open air drying process. Figure 3 shows HPLC elution profiles of desulfoglucosinolates from naturally air dried maca and fresh maca dried by freeze-drying in the laboratory. Approximately 80% of the glucosinolates present in fresh maca were converted into other compounds by tissue disruption, hydrolysis and photochemical degradation due to exposure to sunlight at high altitudes and extreme climatic conditions present in the traditional drying process.

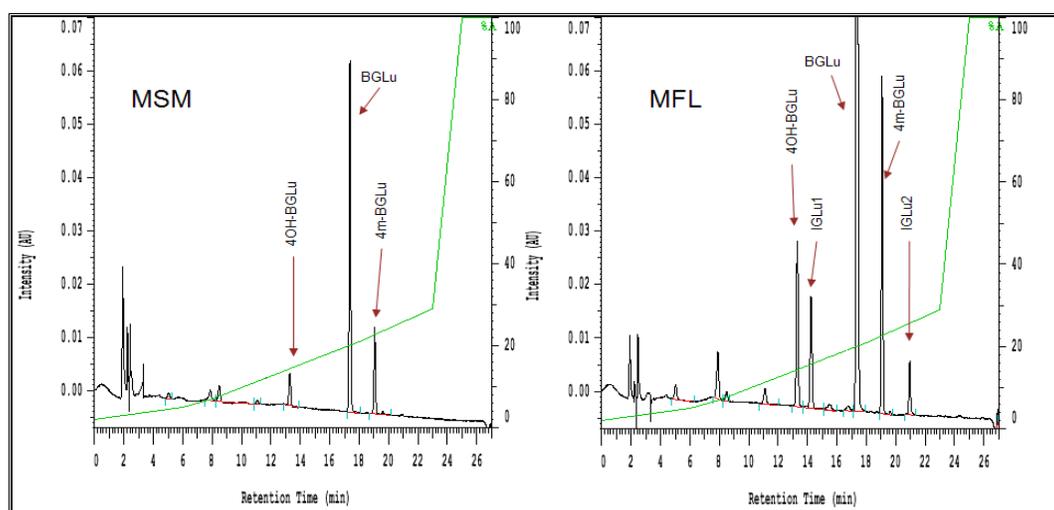


Figure 3. Glucosinolate content, determined as desulfoglucosinolates, decreases significantly in open-air dried material. The figure shows HPLC elution profiles of desulfoglucosinolates determined in naturally dried maca (MSM) and fresh lyophilized maca (MFL). BGLu, benzyl glucosinolate, 4m-BGLu, 4-methoxybenzyl glucosinolate, 4OH-BGLu, 4-hydroxybenzyl glucosinolate. IGLu 1 and 2 are indolic glucosinolates of unknown structure.

Twelve benzylalkamides (macamides) were synthesized and characterized for use as chromatographic standards and for use in animal trials. The compounds prepared are listed in Figure 4. For the purpose of this report, only a liquid chromatographic method was used for the analysis of these compounds. An alternative GC-MS method is being evaluated. The HPLC separation of the macamides was done according to McCollom and collaborators (2005). However, modifications were introduced that improved the quality of the separation, given the large number of amides that were to be evaluated and the low levels we had previously determined in lyophilized plant tissues. The inclusion of 5% MeOH in the water solvent eliminated overlapping problems between longer chain macamides. Also, the method started at a lower acetonitrile concentration (see Materials and Methods) as we also included short-chain macamides (C8) and benzylisothiocyanate in the analytical run. These last two are good indicators of sample processing problems. The HPLC resolution of the amide standards and of potentially contaminating fatty acids is shown in Figure 5.

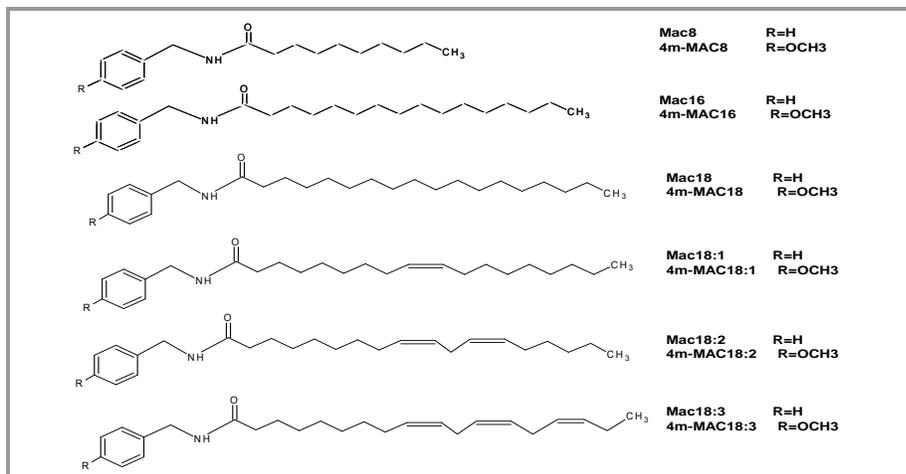


Figure 4. Amide standards synthesized for this study.

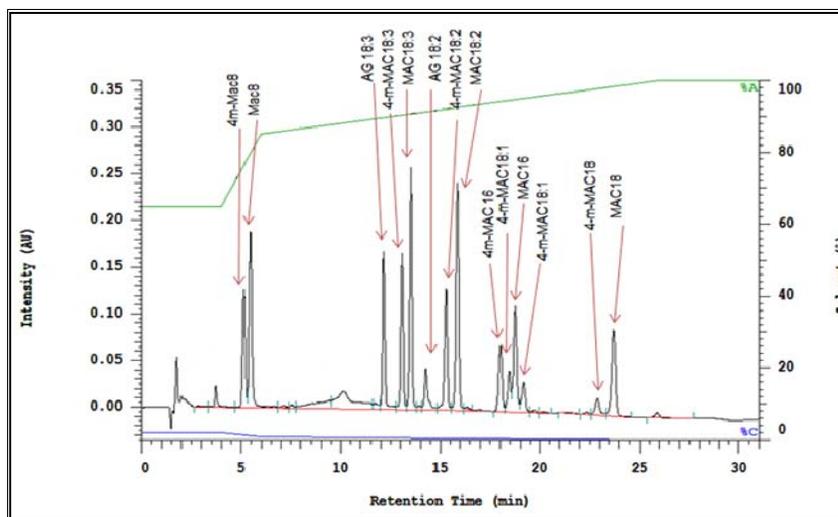


Figure 5. HPLC chromatogram of synthetic benzylalkamide (macamide) standards which can be found in maca hypocotyls. Abbreviations for the macamides are explained in Figure 4. AG 18:3 and AG 18:2 are free linolenic and linoleic acid respectively.

strategy and Figure 9 shows a GC-SPME head-space profile of maca of crushed fresh maca tubers. We expect to use this technique to further characterize the hydrolytic procedures taking place during open-air drying.

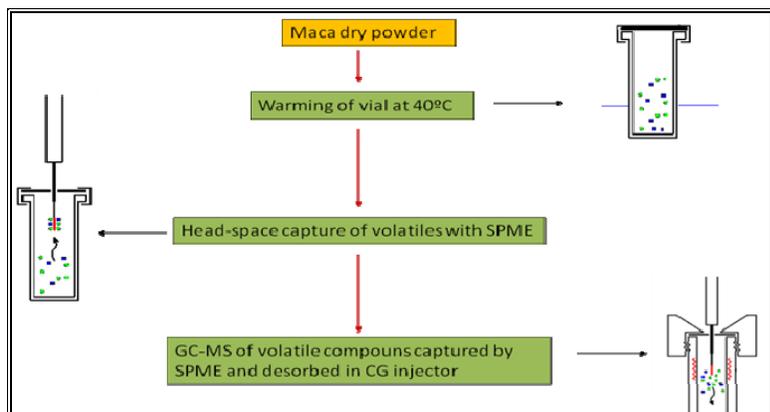


Figure 8. Analysis of volatiles in maca powders by SPME and GC-MS.

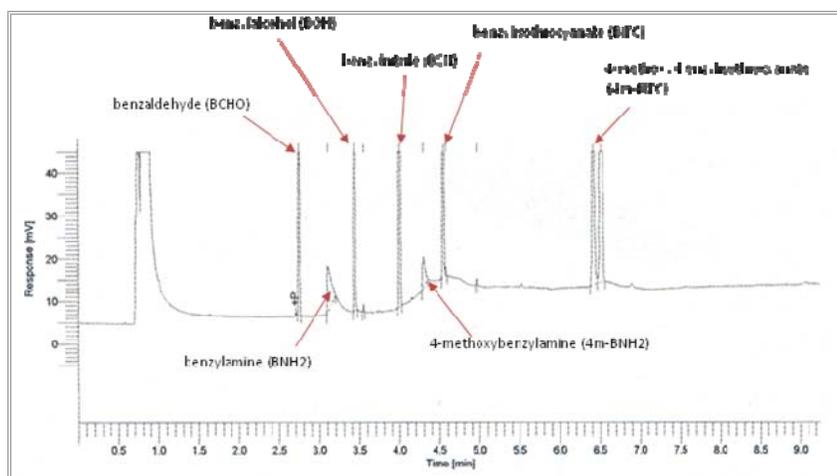


Figure 9. Hydrolysis products detected by headspace-SPME sampling after crushing 0.2 g of fresh maca tuber and sampling for 30 min at room temperature.

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Appendix

Spectroscopic characterization of synthetic amides

MAC 8: N-benzyl octanamide (N-benzylamide of caprylic acid). White crystalline solid. Molecular formula: $C_{15}H_{23}NO$ Mol. wt.: 233.35 **NMR:** $\delta = 2.202$ (t, J=7.65Hz, 2H, H=2), $\delta = 1.670$ (m, J=7.50Hz, 2H, H=3), $\delta = 1.281$ (s, br, 8H, H=4-7), $\delta = 0.872$ (t, J=6.75Hz, 3H, H=8), $\delta = 4.437$ (d, J= 5.70Hz, 2H, H=1'), $\delta = 7.306$ (m, 5H, H=3'-7'), $\delta = 5.856$ (s, 1H, N-H). **FT-IR:** 3291.67cm^{-1} N-H, 1632.36cm^{-1} C=O, 1553.99cm^{-1} N-C amide, 1452.55cm^{-1} N-C=O, 725.25cm^{-1} amide and 695.65cm^{-1} monosubstituted aromatic ring, 2953.46cm^{-1} C-H, 2928.15cm^{-1} C-H, 2915.13cm^{-1} C-H, 2850.05cm^{-1} C-H. **EI-MS:** [M+]=233, [C₉H₁₁NO+]=149, [C₇H₈N+]= 106, [C₇H₇+]= 91.

MAC 16: N-benzylhexadecanamide (N-benzylamide of palmitic acid). White crystalline solid. Molecular formula: $C_{23}H_{39}NO$. Mol. wt.: 345.56. **NMR:** $\delta = 2.213$ (t, J=7.65Hz, 2H, H=2), $\delta = 1.65$ (t, J=7.05Hz, 2H, H=3) $\delta = 1.253$ (s, br, 24H, H=4-15) $\delta = 0.88$ (t, J=6.75Hz, 3H, H=16) $\delta = 4.437$ (d, J= 7.65Hz, 2H, H=1') $\delta = 7.292$ (m, 5H, H=3'-7') $\delta = 5.852$ (s, 1H, N-H). **FT-IR:** 3291.55cm^{-1} N-H, 1631.36cm^{-1} C=O amide, 1553.54cm^{-1} N-C amide, 1452.94cm^{-1} N-C=O, 724.05cm^{-1} and 695.78cm^{-1} monosubstituted aromatic ring, 2953.33cm^{-1} C-H, 2916.15cm^{-1} C-H, 2847.57cm^{-1} C-H. **EI-MS:** [M+]=345, [C₉H₁₁NO+]=149, [C₇H₈N+]= 106, [C₇H₇+]= 91.

MAC 18: N-benzyl octadecanamide (N-benzylamide of stearic acid). White crystalline solid. Molecular formula: $C_{25}H_{43}NO$. Mol. wt.: 373.62. **NMR:** $\delta = 2.203$ (t, J= 7.5Hz, 2H, H=2), $\delta = 1.647$ (m, J= 6.90Hz, 2H, H=3) $\delta = 1.255$ (s, br 28H, H=4-17), $\delta = 0.881$ (t, J=6.60Hz, 3H, H=18), $\delta = 4.440$ (d, J=5.70Hz, 2H, H=1'), $\delta = 7.308$ (m, 5H, H=3'-7'), $\delta = 5.843$ (s, 1H, N-H). **FT-IR:** 3293.37cm^{-1} N-H amide, 1631.95cm^{-1} C=O amide, 1553.55cm^{-1} N-C amide, 1462.12cm^{-1} N-C=O, 727.82cm^{-1} and 696.81cm^{-1} monosubstituted aromatic ring, 2951.92cm^{-1} C-H, 2916.38cm^{-1} C-H, 2847.63cm^{-1} C-H. **EI-MS:** [M+]=373, [C₉H₁₁NO+]=149, [C₇H₈N+]= 106, [C₇H₇+]= 91.

MAC 18:1: N-benzyl-(9Z)octadecenamide (N-benzylamide of oleic acid). Molecular formula: $C_{25}H_{41}NO$. Mol. wt.: 371.60. Liquid at room temperature. White crystalline solid at $-20^{\circ}C$. **NMR:** $\delta = 2,211$ (t, $J=7,65$ Hz, 2H, $H=2$), $\delta = 1,659$ (m, $J= 6,45$ Hz, 2H, $H=3$), $\delta = 1,284$ (s, br, 8H, $H=4-7$), $\delta = 2,047$ (m, $J=3,90$ Hz, 4H, $H=8,11$) $\delta = 5,342$ (m, $J=2,00$ Hz, 2H, $H=9,10$), $\delta = 1,300$ (s,br, 12H, $H=12-17$), $\delta = 0,879$ (t, $J= 6,60$ Hz, 3H, $H=18$), $\delta = 4,133$ (q, $J= 7,10$ Hz, 2H, $H=1'$), $\delta = 7,319$ (m), 5H, $H=3'-7'$) $\delta = 5,65$ (s, 1H, N-H). **FT-IR:** 3298.94 cm^{-1} N-H amide, 1639.56 cm^{-1} C=O amide, 3002.10 cm^{-1} C=C-H, 1553.61 cm^{-1} N-C amide, 1463.98 cm^{-1} N-C=O, 725.25 cm^{-1} and 695.65 cm^{-1} monosubstituted aromatic ring, 2919.45 cm^{-1} C-H, 2849.40 C-H. **EI-MS:** $[M+]=371$, $[C_9H_{11}NO+]=149$, $[C_7H_8N+]=106$, $[C_7H_7+]=91$.

MAC 18:2: N-benzyl-(9Z,12Z)octadecadienamide (acid N-benzylamide of linoleic acid). Molecular formula: $C_{25}H_{39}NO$. Mol. wt.: 369.58. Colorless oil. **NMR:** $\delta = 2,211$ (t, $J=7,65$ Hz, 2H, $H=2$), $\delta = 1,658$ (m, $J= 7,35$ Hz, 2H, $H=3$), $\delta = 1,312$ (s, br), 14H, $H=4-7$, 15-17), $\delta = 2,036$ (m, $J=6,45$ Hz, 4H, $H=8,14$) $\delta = 5,360$ (m, $J=2,70$ Hz, 4H, $H=9,10,12,13$), $\delta = 2,770$ (t, $J=5,85$ Hz, 2H, $H=11$), $\delta = 0,889$ (t, $J= 6,70$ Hz, 3H, $H=18$), $\delta = 4,457$ (d, $J=5,70$ Hz, 2H, $H=1'$), $\delta = 7,319$ (m), 5H, $H=3'-7'$), $\delta = 5,691$ (m, 1H, N-H). **FT-IR:** 3285.69 cm^{-1} N-H amide, 1645.62 cm^{-1} C=O amide, 1549.64 cm^{-1} N-C amide, 1454.07 cm^{-1} N-C=O, 725.74 cm^{-1} y 697.13 cm^{-1} monosubstituted aromatic ring, 3064.23 cm^{-1} y 3008.10 cm^{-1} C=C-H, 2953.68 cm^{-1} C-H, 2926.35 cm^{-1} C-H, 2854.34 cm^{-1} C-H. **EI-MS:** $[M+]=368$, $[C_9H_{11}NO+]=149$, $[C_7H_8N+]=106$, $[C_7H_7+]=91$.

MAC 18:3: N-benzyl-(9Z,12Z,15Z)octadecatrienamide (N- benzylamide of linolenic acid). Molecular formula: $C_{25}H_{37}NO$. Mol. wt. 367.57. Colorless oil. **NMR:** $\delta = 2,208$ (t, $J=7,65$ Hz, 2H, $H=2$), $\delta = 1,657$ (m, $J=7,20$ Hz, 2H, $H=3$) $\delta = 1,310$ (s, br, 8H, $H=4-7$) $\delta = 2,077$ (m, $J=7,05$ Hz, 4H, $H=8,17$), $\delta = 5,361$ (m, $J=3$ Hz, 6H, $H=9,10,12,13,15,16$) $\delta = 2,805$ (t, $J=5,85$ Hz, 4H, $H=11, 14$), $\delta = 0,974$ (t, $J=7,5$ Hz, 3H, $H=18$), $\delta = 4,453$ (d, $J=5,70$, 2H, $H=1'$), $\delta = 7,334$ (m, 5H, $H=3'-7'$), $\delta = 5,715$ (s, 1H, N-H). **FT-IR:** 3301.84 cm^{-1} N-H amide, 1639.13 cm^{-1} C=O amide, 1551.37 cm^{-1} N-C amide, 1453.88 cm^{-1} N-C=O, 3005.85 cm^{-1} amide, 720.74 cm^{-1} y 696.02 cm^{-1} monosubstituted aromatic ring, , C=C-H, 2962.02 cm^{-1} C-H, 2918.27 cm^{-1} C-H, 2848.44 cm^{-1} C-H. **EI-MS:** $[M+]=367$, $[C_9H_{11}NO+]=149$, $[C_7H_8N+]=106$, $[C_7H_7+]=91$.

4-m-MAC 8: 4'-methoxy-N-benzyl-octanamide (4'-methoxy-N-benzylamide of caprylic acid). Molecular formula: $C_{16}H_{25}NO_2$. Mol. wt.: 263.38. White crystalline solid. **NMR:** $\delta = 2.182$ (t, $J=7.65$ Hz, 2H, $H=2$), $\delta = 1.634$ (m, $J=7.50$ Hz, 2H, $H=3$), $\delta = 1.274$ (s,br, 8H, $H=4-7$), $\delta = 0.868$ (t, $J= 6.75$ Hz, 3H, $H=8$), $\delta = 4.366$ (d, $J= 6.60$ Hz, 2H, $H=1'$) $\delta = 6.86$ (d, $J=6.90$ Hz, 1H, $H=3'$) $\delta = 7.209$, (d), $J= 8.70$ Hz, 1H, $4', 6'$), $\delta = 6.86$ (d), $J=6.90$ Hz, 1H, $H=7'$) $\delta = 3.789$ (s, 3H, O-Me), $\delta = 5.755$ (s, 1H, N-H). **FT-IR:** 3289.51 cm^{-1} N-H amide, 1631.67 cm^{-1} C=O amide, 1553.93 cm^{-1} N-C amide, 1463.84 cm^{-1} N-C=O amide, 1514.79 cm^{-1} C=C-O methoxy group, 1254.11 cm^{-1} y 1031.86 cm^{-1} C-O methoxy group, $815.80 - 810.71$ cm^{-1} disubstituted aromatic ring in *para* position, 2916.06 cm^{-1} C-H, 2953.75 cm^{-1} C-H, 2928.95 cm^{-1} C-H, 2850.19 cm^{-1} C-H. **EI-MS:** $[M+]=263$, $[C_{10}H_{13}NO_2+]=179$, $[C_8H_{10}NO+]=136$, $[C_8H_9O+]=121$

4'-m-MAC 16: 4'-methoxy-N-benzylhexadecanamide (4'-methoxy-N-benzylamide of palmitic acid). Molecular formula: $C_{24}H_{41}NO_2$. Mol. wt.: 375.59. White crystalline solid. **NMR:** $\delta = 2.187$ (t, $J=7.50$, 2H, $H=2$), $\delta = 1.680$ (m , 2H, $H=3$), $\delta = 1.246$ (s, br, 24H, $H=4-15$) $\delta = 0.872$ (t, $J=6.45$ Hz, 3H, $H=16$), $\delta = 4.367$ (d, $J=5.10$ Hz, 2H, $H=1'$), $\delta = 6.866$ (d), $J=8.40$ Hz, 1H, $H=3', 7'$) $\delta = 7.207$ (d), $J=8.40$ Hz 1H, $H=6', 7'$) $\delta = 3.787$ (s, 3H, OMe), $\delta = 5.789$ (s, 1H, N-H). **FT-IR:** 3299.90 cm^{-1} N-H amide, 1639.54 cm^{-1} C=O amide, 1551.58 cm^{-1} N-C amide, 1462.63 cm^{-1} N-C=O amide, 808.23 cm^{-1} disubstituted aromatic ring in *para* position, 2954.05 cm^{-1} C-H, 2917.06 cm^{-1} C-H, 2848.74 cm^{-1} C-H, 1515.43 cm^{-1} C=C-O methoxy group, 1260.74 cm^{-1} y 1032.26 cm^{-1} C-O methoxy group. **EI-MS:** $[M+]=375$, $[C_{10}H_{13}NO_2+]=179$, $[C_8H_{10}NO+]=136$, $[C_8H_9O+]=121$

4'-m-MAC 18: 4'-methoxy-N-benzyl-octadecanamide (4'-methoxy-N-benzylamide of stearic acid). Molecular formula: $C_{26}H_{45}NO_2$. Mol. wt.: 403.64. White crystalline solid. **NMR:** $\delta = 2.192$ (t, $J=7.65$ Hz, 2H, $H=2$), $\delta = 1.637$ (m, $J=7.05$ Hz, 2H, $H=3$), $\delta = 1.250$ (s,br, 28H, $H=4-17$), $\delta = 0.876$ (s, $J=6.60$ Hz, 3H, $H=18$) $\delta = 4.378$ (d), $J=5.40$ Hz, 2H, $H=1'$), $\delta = 6.875$ (d), $J=8.70$ Hz, 1H, $3', 7'$), $\delta = 7.216$ (d), $J=8.40$ Hz, 1H, $H=4', 6'$), $\delta = 3.796$ (s, 3H, OMe), $\delta = 5.697$ (s), 1H, N-H). **FT-IR:** 3291.52 cm^{-1} N-H amide, 1630.05 cm^{-1} C=O amide, 1554.01 cm^{-1} N-C amide, 1462.30 cm^{-1} N-C=O amide, $815.80 - 807.68$ cm^{-1} disubstituted aromatic ring in *para* position, 1514.73 cm^{-1} C=C-O methoxy group, 1254.39 cm^{-1} y 1029.54 cm^{-1} C-O methoxy group, 2917.05 cm^{-1} C-H, 2847.86 cm^{-1} C-H. **EI-MS:** $[M+]=402$, $[C_{10}H_{13}NO_2+]=179$, $[C_8H_{10}NO+]=136$, $[C_8H_9O+]=121$

4'-m-MAC 18:1: 4'-methoxy-N-benzyl-(9Z)octadecenamide (4'- methoxy -N-benzylamide of oleic acid). Molecular formula: $C_{26}H_{43}NO_2$. Mol. wt.: 401.63. Liquid at room temperature. White crystalline solid at -20°C. **NMR:** $\delta = 2,189$ (t, J=5,85, 2HH=2), $\delta = 1,645$ (m, J= 7,00Hz, 2H, H=3), $\delta = 1,293$ (s, br, 12H, H=4-7, 12-17)) $\delta = 1,996$ (m, J= 3,90Hz, 4H, H=8, 11) $\delta = 5,342$ (m, J=2,0Hz, 2H, H=9,10) $\delta = 0,879$ (t, J=6,75Hz, 3H, H=18), $\delta = 4,384$ (d, J= 5,70 Hz, 2H, H=1'), $\delta = 6,88$ (d, J= 6,6Hz, 2H, H=3',7') $\delta = 7,214$ (d), J=4,50Hz, 2H, 4', 6'), $\delta = 3,800$ (s, 3H, OMe), $\delta = 5,608$ (s, 1H, N-H). **FT-IR:** 3298.94 cm^{-1} N-H amide, 1639.56 cm^{-1} C=O amide, 1553.61 cm^{-1} N-C amide, 1463.98 cm^{-1} N-C=O amide, 810.11 cm^{-1} disubstituted aromatic ring in *para* position, 1514.79 cm^{-1} C=C-O methoxy group, 1255.11 cm^{-1} y 1031.97 cm^{-1} C-O methoxy group, 3002.10 cm^{-1} C=C-H, 2919.45 cm^{-1} C-H, 2849.40 C-H. **EI-MS:** [M+]= 400, [$C_{10}H_{13}NO_2+$]= 179, [$C_8H_{10}NO+$]=136, [C_8H_9O+] = 121

4'-m-MAC 18:2: 4'-methoxy-N-benzyl-(9Z,12Z)octadecadienamide (4'-methoxy-N-benzylamide of linoleic acid). Molecular formula: $C_{26}H_{41}NO_2$. Mol. wt.: 399.61. Colorless oil. **NMR:** $\delta = 2,19$ (t, J= 7,65 Hz, 2H, H=2), $\delta = 1,629$ (m, 2H, H=3), $\delta = 1,307$ (s,br, 14H, H=4-7, 15-17), $\delta = 2,035$ (m, J= 6,45Hz, 4H, H=8,14), $\delta = 5,349$ (m), J=2,70 Hz, 4H, H=9,10,12,13), $\delta = 2,768$ (t, J=5,70 Hz, 2H, H=11), $\delta = 0,888$ (t, J= 6,75 Hz, 3H, H=18), $\delta = 4,384$ (d, J=5,70 , 2H, H= 1'), $\delta = 6,873$ (d), J=8,70 Hz, 2H, H=3',7') $\delta = 7,221$ (d), J= 8,70, 2H, 4', 6'), $\delta = 3,800$ (s, 3H, H=OMe) $\delta = 5,615$ (m, 1H, N-H). **FT-IR:** 3289.51 cm^{-1} N-H amide, 1553.93 cm^{-1} N-C amide, 1631.67 cm^{-1} C=O amide, 1463.84 cm^{-1} N-C=O amide, 815.80 - 810.71 cm^{-1} disubstituted aromatic ring in *para* position, 1514.79 cm^{-1} C=C-O methoxy group, 1254.11 cm^{-1} y 1031.86 cm^{-1} C-O methoxy group, 3006.43 cm^{-1} C=C-H, 2916.06 cm^{-1} C-H ,2953.75 cm^{-1} C-H, 2928.95 cm^{-1} C-H, 2850.19 cm^{-1} C-H. **EI-MS:** [M+]= 398, [$C_{10}H_{13}NO_2+$]= 179, [$C_8H_{10}NO+$]=136, [C_8H_9O+] = 121

4'-m-MAC 18:3: 4'-methoxy-N-benzyl-(9Z,12Z,15Z)octadecatrienamide (4'- methoxy -N-benzylamide of linolenic acid). Molecular formula: $C_{26}H_{39}NO_2$. Mol. wt.: 397.59. Colorless oil. **NMR:** $\delta = 2,187$ (t, J= 7,50Hz, 2H, H=2) $\delta = 1,644$ (m), J=7,20Hz ,2H, H=3), $\delta = 1,306$ (s,br, 8H, H=4-7), $\delta = 2,054$ (m, J=6,15Hz, 4H, H=8,17), $\delta = 5,369$ (m, J=2,70Hz , 6H, H=9,10,12,13,15,16), $\delta = 2,804$ (t, J=5,70 Hz, 4H, H=11,14), $\delta = 0,973$ (t, J=7,50 Hz, 3H, H=18), $\delta = 4,381$ (d, J=5,70 Hz, 2H, H=1'), $\delta = 6,871$ (d), J=6,6 Hz, 2H, H=3',7'), $\delta = 7,219$ (d), J= 8,40Hz, 2H, 4', 6'), $\delta = 3,798$ (s, 3H, OMe), $\delta = 5,627$ (s, N-H). **FT-IR:** 3300.51 cm^{-1} N-H amide, 1639.31 cm^{-1} C=O amide, 1552.42 cm^{-1} N-C amide, 1460.04 cm^{-1} N-C=O amide, 810.09 cm^{-1} disubstituted aromatic ring in *para* position, 1513.82 cm^{-1} C=C-O methoxy group, 1254.69 cm^{-1} y 1031.81 cm^{-1} C-O methoxy group, 3005.83 cm^{-1} C=C-H, 2916.51 cm^{-1} C-H , 2848.08 cm^{-1} C-H. **EI-MS:** [M+]= 396, [$C_{10}H_{13}NO_2+$]= 179, [$C_8H_{10}NO+$]=136, [C_8H_9O+] = 121