ON THE SYNTHESIS, SURFACE ACTIVITY AND SUPRAMOLECULAR STRUCTURES OF PSEUDO DOUBLE-CHAINED L-Asp BASED AMPHIPHILES

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ABSTRACT

Eight new final compounds derivatives of L-Asp are synthesized. They contain L-Asp linked by an amide bond both to n-hexyl chain on its C-side and an acyl chain linked to the N-side. Four different acyl residues are condensed to the N side. The newly synthesized compounds belong to two series of structures, as four of them contain L-Asp-ß-benzyl ester, while the other four – a typical L-Asp-ß-carboxylic group. The structures of the surfactants are categorized as pseudo-double alkyl chained amphiphiles, because one of the tails has a constant length of only six carbon atoms. Some of the newly synthesized surfactants are studied as monolayers on the air/water interface. The properties of the rest of them are determined by tensiometric and conductometric methods. Nano- and micro- sized supramolecular complexes of the compounds studied are observed in monolayers using Brewster Angle Microscopy (BAM) and Scanning Electron Microscopy (SEM) for samples after solvent evaporation.

Keywords: L-Asp, amphiphile, synthesis, surfactants, Langmuir monolayer, critical aggregation concentration, supramolecular structures.

INTRODUCTION

Amphiphilic molecules having an amino acid polar head and lipophilic fatty acid chain tail, known also as lipopeptides, are intensively studied nowadays [1 - 3]. They possess remarkable biological activities but their physicochemical properties are not very well understood [4 - 7]. Like many amphiphiles, some lipopeptides when present at very low concentrations in aqueous solutions form a monolayer at the air/water interface. The study of such Langmuir monolayer at the air/water interface is possible by a special technique of compression using mechanical barriers. The compression leads to a decrease of the area per molecule related to phase transitions through several 2D phases such as gaseous, liquid expanded (LE), liquid condensed (LC), condensed, etc. [8]. These phases can be inferred from the different slopes of the surface pressure vs specific molecular area (π-A) isotherms [9]. Additional compression beyond that of the condensed phase leads to the monolayer collapse and hence 2D to 3D structural transformation takes place [10]. Two main collapses types are discussed in the literature. One of them is called a ‘constant area collapse’ when π decreases rapidly with p, while the other one is known as a ‘constant pressure collapse’ when π remains constant or increases slowly with p [11]. The type of the collapse is related to the nature of the component molecules and the subphase conditions. The structural transformations determined by π are related to formation of polymolecular self-ordered structures. 3D structures appear during the first step. Depending on the packing parameter (shape factor) they can be micelles (spherical or non-spherical) and vesicles or bilayers [12]. They are usually less than 200Å in a diameter. Their polar head groups are directed towards the water solution, while the hydrophobic fatty alkyl chains are buried in the interior. Many authors devote their efforts to the investigation of the micelle structure and morphology [13 - 22]. Almost all physical properties of a surfactant solution change sharply at the beginning of the self-aggregation (or
micellization) process. The minimum concentration of molecules required for micelles or other aggregates formation is known as a critical micelle concentration (CMC) and a critical aggregation concentration (CAC), respectively. Several methods applicable to cmc/cac determination are described in the literature [23 - 25]. The tensiometric, conductometric, colorimetric and fluorimetric procedures are the most widely used.

In fact lipopeptides amphiphiles are structurally different from the conventional surfactants because the amide bond-containing backbone is distinctly different from the hydrocarbon chain and offers inherently different physical properties. They often interact with each other so other highly organized complexes form. Such supramolecular structures built by some amphiphiles attract lately wide attention [26, 27]. The major interactions responsible for the assembly of molecules into ordered complexes refer to hydrogen bonds formation, metal coordination, as well as hydrophobic, van der Waals, π–π and electrostatic interactions [26 - 28]. The amino acid-based amphiphiles conferring absolute chirality to these molecules [29 - 36] occupy a special place in supramolecular chemistry. Many surfactants synthesized on the ground of amino acids offer interesting alternatives in respect to the conventional one. They possess desired functionality, renewability, biocompatibility, etc. New molecules are designed to be used as surfactants as well as building blocks in supramolecular chemistry [26 - 28].

The present communication describes eight newly synthesized L-Asp based compounds, six of which are surfactants. All new compounds contain L-Asp linked by an amide bond both to the n-hexyl chain on its C-side and another alkyl chain linked to the N-side. The carboxylic acids condensed to the N side refer to myristic acid (tetradecanoic acid, C_{14}H_{28}O_{2}), decanoic acid (C_{10}H_{20}O_{2}), hexanoic acid (C_{6}H_{12}O_{2}) and acetic acid (C_{2}H_{4}O_{2}). Four of them contain L-Asp-β-benzyl ester, while the other four contain a typical L-Asp-β-carboxylic group acting as a polar head group. These compounds could be classified as pseudo double-chained surfactants, as they contain one hydrophilic head and two hydrophobic alkyl tails one of which is of a five carbon atoms length, while the other one have chains of five, nine or thirteen carbon atoms. The six carbon alkyl chain is the longest alkyl chain that still has a reasonable solubility in water, but haxanoic and heptanoic acids possess surface activities. That is why the hydrophobic tails are considered as double-pseudo one. Some of the newly synthesized compounds are studied as monolayers on the air/water interface, while the other are subjected to cac/cmc determination by tensiometric and conductometric methods. Scanning electron microscopy (SEM) is used to observe the morphology of the nano- and micro-sized complexes obtained upon solvent evaporation.

**EXPERIMENTAL**

**Reagents:** Boc-Asp(Obzl)-OH (Alfa Aesar); Amphiphilic fatty acids, chlorides and anhydrides (Alfa Aesar); TBTU [O-(Benzotriazol-1-yl)-N,N,N',N' -tetramethyluronimium tetrafluoroborate] (Iris Biotech GmbH); DMEA [N,N-Diisopropylethylamine] (Alfa Aesar), DMF [Dimethylformamide] (Merck); Ethylacetate (Merck); Hexane (Merck); NaHCO_{3} (Merck); Trifluoroacetic acid (Alfa Aesar); Citric acid (Merck); Chloroform (Merck); Methanol (Merck); 5 % Pd/C (Sigma-Aldrich)

**Methods and apparatus**

The chemical syntheses were performed in a solution using TBTU as a condensing reagent and DMEA as a base. The products obtained were crystalline and each one was purified through recrystallization from ethylacetate. The molecular structure and the purity of each of the newly synthesized compounds were defined by 1H NMR, 13C NMR, MS spectra, their melting point and angle of optical rotation.

**Analyses**

1H and 13C spectra were recorded on Bruker Avance -II+/−600 MHz spectrometer. The 1H and 13C NMR chemical shifts are given relative to TMS. Chemical shifts are expressed in ppm, while the coupling constants - in Hz. The ESI/MS analyses were recorded on Thermo Finnigan LCQ advantage ion trap mass spectrometer. The reaction proceeding and the purity of the products were checked by TLC on precoated plates of Silica gel 60 F254 (Merck). The spots on TLC chromatograms were detected by the chlorine/o-tolidine reaction. The melting points were determined on a Kofer apparatus. Their uncorrected values are used. The specific optical rotation values were measured on Jasco P-2000 polarimeter (Tokyo, Japan) at D line of sodium lamp at 20ºC by using 1 dm quartz cell. The [α]_{D} are given in deg cm$^{-1}$ g$^{-1}$dm$^{-1}$, while the concentration - in g cm$^{-3}$. 

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Monolayer deposition

The monolayers formation was achieved by deposition of a droplet of a specified amount of the solution studied over the available area of a Teflon trough (475 cm²). The subphase was either deionized water or an acidic one. The surface pressure, π, was measured using a KSV-2200 (Finland) surface balance equipped with a platinum plate.

Critical aggregation concentration (CAC) determination

The critical aggregation concentrations of the anionic surfactants were determined applying tensiometric and conductometric approach. Solutions of a known concentration were progressively diluted and examined. The temperature was 25°C. These measurements were performed in unbuffered aqueous solutions. The CAC value was determined by plotting the surface tension against the log of the concentration of an amino acid surfactant.

Apparatus utilized for supramolecular structures study

Ramé-Hart Model 290 Automated Goniometer with DROPImage Advanced v2.4, WTW inoLab 720 with a conductivity cell Tetracon 325 (BAM - Nanofilm_ultramab (Accurion); Image sizes 720x400 micrometers) and Jeol Scanning Electron Microscope JSM-5510 (Jeol Ltd.) were used for observation of dried samples.

Chemical synthesis and purification

The newly obtained eight amino acid-based compounds belonged to two series, namely: acyl-L-Asp(OBzl)-N-hexylamide (Scheme 2, compound I) and acyl-L-Asp-N-hexylamide (Scheme 3, compound II). Those included in the first series (compound I) referred to: acetyl-L-Asp(OBzl)-N-hexylamide (1), hexanoyl-L-Asp(OBzl)-N-hexylamide (2), decanoyl-L-Asp(OBzl)-N-hexylamide (3), myristoyl-L-Asp(OBzl)-N-hexylamide (4). The compounds of the next series (compound II) referred

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**Scheme 1.** Synthesis of N-Boc- L-Asp(OBzl)–N-hexyl amide.

**Scheme 2.** Synthesis of acyl-L-Asp (OBzl) N-hexylamide (compounds I: I-I) [n=0, compound I; n=4, compound 2; n=8, compound 3; n=12, compound 4].

**Scheme 3.** Synthesis of acyl-L-Asp-N-hexylamide (Compounds II: I-II) [n=0, compound 5; n=4, compound 6; n=8, compound 7; n=12, compound 8].

**Synthesis of N-Boc- L-Asp(OBzl)-N'-hexyl amide (Boc-MA6) by the TBTU method**

1-hexylamine (1 eq) was added to the solution of Boc-Asp(OBzl)-OH (1.2 eq.) dissolved in DMF (1 g compound in 2 ml DMF) and stirred in an ice-bath till cooling to 0°C. The condensation reagent – TBTU (1.2 eq) was added to the cooled solution and finally, the base –DIEA (N-diisopropylethylamine) (1.2 eq) was introduced drop wise to the reaction mixture. The reaction was left for 2 h at 0°C and stirred overnight at a room temperature. Afterwards, 8% NaHCO₃ was added until a white precipitate was formed. This mixture was left in the refrigerator at 4°C-6°C for 1 h - 2 h and the precipitation was filtered off and washed initially with 8% NaHCO₃, then with 10% citric acid and finally with cool distilled water until the washing water attained a neutral pH value. The solid crude was recrystallized from ethyl acetate/hexane. All products were obtained after filtration as chromatographically pure white crystals.

**Compound 1 (Acetyl-L-Asp(OBzl)-N-hexylamide):** White solid (78 % yield). M.p. 90 - 91°C. ¹H NMR (600 MHz, CDCl₃): δ = 7.42 - 7.32 (m, 5H), 6.58 (d, J = 7.9 Hz, 1H), 6.58 (t, J = 5.2 Hz, 1H), 5.17 (q, J = 12.2 Hz, 2H), 4.79 (td, J = 7.5, 4.3 Hz, 1H), 3.21 (dd, J = 13.2, 7.0 Hz, 2H), 2.99 (dd, J = 17.0, 4.3 Hz, 1H), 2.69 (dd, J = 17.0, 7.2 Hz, 1H), 2.03 (s, 3H), 1.51 - 1.42 (m, 2H), 1.36 - 1.22 (m, 6H), 0.90 (t, J = 6.9 Hz, 3H) ppm.¹³C NMR (151 MHz, CDCl₃): δ = 172.05, 170.24, 170.07, 135.36, 128.65, 128.47, 128.31, 66.94, 49.28, 39.69, 35.91, 31.40, 29.30, 26.47, 22.54, 14.03 ppm.

MS (ES) (m/z): Calculated for C₁₉H₂₈N₂O₄⁺ [M + H⁺] 349.20; found 349.21.

[^] D -28±2 (c = 0.16, CHCl₃).

**Compound 2 (Hexanoyl-L-Asp(OBzl)-N-hexylamide):** White solid (60 % yield). M.p. 74°C. ¹H NMR (600 MHz, CDCl₃): δ = 7.38 - 7.34 (m, 5H), 6.81 (d, J = 8.0 Hz, 1H), 6.55 (t, J = 5.8 Hz, 1H), 5.18 - 5.12 (m, 2H), 4.81 - 4.76 (m, 1H), 3.22 - 3.17 (m, 2H), 0.88 (t, J = 6.9 Hz, 3H) ppm.¹³C NMR (151 MHz, CDCl₃): δ = 172.12, 170.49, 155.85, 135.36, 128.64, 128.43, 128.28, 80.48, 66.85, 50.58, 39.66, 36.11, 31.44, 29.36, 28.29, 26.46, 22.55, 14.04 ppm.

MS (ES) (m/z): Calculated for C₁₉H₂₈N₂O₄⁺ [M + H⁺] 349.20; found 407.26.

**General Procedure for in-situ synthesis of acyl-L-Asp(OBzl)-N'-hexyl amide by deprotection of N-Boc-L-Asp(OBzl)-N'-hexyl amide**

A mixture of N-Boc-L-Asp(OBzl)-N'-hexyl amide (1 eq.) and Trifluoroacetic acid (TFA) (12 eq.) was stirred about 75 min. The excess of TFA was removed by vacuum evaporation. The solid obtained was dissolved in DMF (1g solid in 1.5 ml DMF) and DIEA was dropped in it so that pH of 7 was achieved. This solution was cooled in an ice-bath and 1.2 eq. of the corresponding fatty acid, TBTU (1.2 eq.) and DIEA (1.2 eq.) were added successively to the cooled mixture. The reaction was left for 2 h at 0°C and stirred 24 h at a room temperature. Then 8% NaHCO₃ was added to the reaction mixture, so that the product was obtained in the form white precipitate. The flask was left at a room temperature for 1 h and then moved in the refrigerator at 4°C-6°C for 1h-2 h where the precipitate consolidated. Afterwards the precipitation was filtered off and washed initially with 8% NaHCO₃, then with 10% citric acid and finally with cool distilled water until the washing water attained a neutral pH value. The solid crude was recrystallized from ethylacetate/petroleum ether and dried. All products were obtained after filtration as chromatographically pure white crystals.
H^+] 405.27; found 405.28.
\([\alpha]_D^20 = 30 \pm 1^\circ (c = 0.26, \text{CHCl}_3).\)

**Compound 3 (Decanoyl-L-Asp(OBzl)-N-hexylamide):**
White solid (92 % yield). M.p. 85 - 86°C. \(^1\)H NMR (600 MHz, CDCl3): \(\delta = 7.40 - 7.29, \text{ (m, 5H), 6.81 (d, J = 7.7 Hz, 1H), 6.54 (t, J = 5.4 Hz, 1H), 5.15 (q, J = 12.2 Hz, 2H), 4.78 (td, J = 7.3, 4.2 Hz, 1H), 3.30 - 3.09 (m, 2H), 2.99 (dd, J = 16.9, 4.2 Hz, 1H), 2.65 (dd, J = 17.0, 7.0 Hz, 1H), 2.20 (td, J = 7.3, 1.8 Hz, 2H), 1.67 - 1.55 (m, 4H), 1.44 (dd, J = 14.1, 6.9 Hz, 2H), 1.32 - 1.20 (m, 16H), 0.88 (dt, J = 7.1, 1.4 Hz, 6H) ppm. \(^1\)C NMR (151 MHz, CDCl3): \(\delta = 173.44, 172.20, 170.17, 135.33, 128.65, 128.47, 128.31, 66.94, 49.13, 39.66, 36.59, 35.69, 31.87, 31.42, 29.45, 29.34, 29.30, 29.28, 29.24, 26.49, 25.58, 22.68, 22.55, 14.13, 14.03 ppm.

MS (ES) (m/z): Calculated for C\(_{31}\)H\(_{31}\)N\(_2\)O\(_4\) \([M + H'^+]\) 461.39; found 461.40.
\([\alpha]_D^20 = 27 \pm 1^\circ (c = 0.19, \text{CHCl}_3).\)

**Compound 4 (Myristoyl-L-Asp(OBzl)-N-hexylamide):**
White solid (90 % yield). M.p. 72-74°C. \(^1\)H NMR (600 MHz, CDCl3): \(\delta = 7.40 - 7.30, \text{ (m, 5H), 6.85 (d, J = 7.9 Hz, 1H), 6.56 (t, J = 5.6 Hz, 1H), 5.15 (q, J = 12.2 Hz, 2H), 4.79 (td, J = 7.3, 4.2 Hz, 1H), 3.24 - 3.14 (m, 2H), 2.98 (dd, J = 16.9, 4.2 Hz, 1H), 2.66 (dd, J = 16.9, 7.1 Hz, 1H), 2.20 (td, J = 7.3, 1.9 Hz, 2H), 1.62 (dt, J = 16.3, 7.4 Hz, 4H), 1.44 (dd, J = 14.5, 7.2 Hz, 2H), 1.32 - 1.21 (m, 24H), 0.88 (t, J = 7.0 Hz, 6H) ppm. \(^1\)C NMR (151 MHz, CDCl3): \(\delta = 173.51, 172.10, 170.17, 135.33, 128.65, 128.47, 128.31, 66.94, 49.13, 39.66, 36.59, 35.69, 31.87, 31.42, 29.45, 29.34, 29.30, 29.28, 29.24, 26.49, 25.58, 22.68, 22.55, 14.13, 14.03 ppm.

MS (ES) (m/z): Calculated for C\(_{31}\)H\(_{32}\)N\(_2\)O\(_4\) \([M + H'^+]\) 461.39; found 461.40.
\([\alpha]_D^20 = 27 \pm 1^\circ (c = 0.19, \text{CHCl}_3).\)

**Compound 5 (Acetyl-L-Asp-N-hexylamide):**
White solid (90 % yield). M.p. 118 - 120°C. \(^1\)H NMR (600 MHz, [D6] DMSO): \(\delta = 8.10, \text{ (d, J = 8.1 Hz, 1H), 7.81 (t, J = 5.7 Hz, 1H), 4.49 (td, J = 7.9, 6.1 Hz, 2H), 3.06 - 2.95 (m, 2H), 2.59 (dd, J = 16.2, 6.0 Hz, 1H), 2.42 (dd, J = 16.2, 7.8 Hz, 1H), 1.82 (s, 3H), 1.43 - 1.30 (m, 2H), 1.30 - 1.12 (m, 6H), 0.85 (t, J = 7.1 Hz, 3H) ppm. \(^1\)C NMR (151 MHz, [D6] DMSO): \(\delta = 172.30, 170.83, 169.64, 49.95, 39.06, 36.9, 31.42, 29.38, 26.37, 23.06, 22.50, 14.38 ppm.

MS (ES) (m/z): Calculated for C\(_{31}\)H\(_{32}\)N\(_2\)O\(_4\) \([M + H'^+]\) 259.16; found 259.16.
\([\alpha]_D^20 = 35 \pm 2^\circ (c = 0.22, \text{CH}_3\text{OH}).\)

**Compound 6 (Hexanoyl-L-Asp-N-hexylamide):**
White solid (58 % yield). M.p. 66 - 68°C. \(^1\)H NMR (600 MHz, [D6] DMSO): \(\delta = 8.04, \text{ (d, J = 8.1 Hz, 1H), 7.73 (t, J = 5.8 Hz, 1H), 4.51 (dd, J = 14.1, 7.9 Hz, 1H), 3.12 - 2.89 (m, 2H), 2.60 (dd, J = 11.6, 4.6 Hz, 1H), 2.41 (dd, J = 16.2, 7.8 Hz, 1H), 2.08 (t, J = 7.4 Hz, 2H), 1.50 - 1.43 (m, 2H), 1.40 - 1.32 (m, 2H), 1.30 - 1.16 (m, 10H), 0.85 (td, J = 7.0, 1.3 Hz, 6H) ppm. \(^1\)C NMR (151 MHz, [D6] DMSO): \(\delta = 172.64, 172.28, 170.85, 49.81, 39.02, 36.83, 35.58, 31.44, 29.40, 29.29, 26.35, 25.31, 22.50, 22.37, 14.38, 14.32 ppm.

MS (ES) (m/z): Calculated for C\(_{31}\)H\(_{32}\)N\(_2\)O\(_4\) \([M + H'^+]\) 315.22; found 315.23.
\([\alpha]_D^20 = 39 \pm 2^\circ (c = 0.21, \text{CH}_3\text{OH}).\)

**Compound 7 (Decanoyl-L-Asp-N-hexylamide):**
White solid (94 % yield). M.p. 102 - 103°C. \(^1\)H NMR (600 MHz, [D6] DMSO): \(\delta = 8.04, \text{ (d, J = 8.1 Hz, 1H), 7.72 (t, J = 5.7 Hz, 1H), 4.50 (dd, J = 14.3, 7.8 Hz, 1H), 3.20 - 2.90 (m, 2H), 2.60 (dd, J = 16.2, 6.3 Hz, 1H), 2.41 (dd, J = 16.2, 7.7 Hz, 1H), 2.07 (t, J = 7.3 Hz, 2H), 1.54 - 1.41 (m, 2H), 1.39 - 1.30 (m, 2H), 1.25 (dt, J = 20.1, 6.9 Hz, 18H), 0.85 (td, J = 7.0, 1.2 Hz, 6H) ppm.

A general procedure of hydrogenolysis of the benzyl group of acyl-L-Asp(OBzl)-N-hexylamide (compounds \(I\)) and obtaining Acyl-L-Asp-N-hexylamide (compounds \(II\))
\(^{13}\)C NMR (151 MHz, [D6] DMSO): \(\delta = 172.64, 172.30, 170.87, 49.81, 39.02, 35.63, 31.77, 31.46, 29.41, 29.39, 29.33, 29.18, 29.04, 26.38, 25.64, 22.58, 22.52, 14.44, 14.38 \text{ppm.}\)

MS (ES) (m/z): Calculated for \(\text{C}_{20}\text{H}_{38}\text{N}_{2}\text{O}_{4}^+ \) [M + H\(^+\)] 371.28; found 371.29.

\([\alpha]_D^{20} = -33 \pm 2 \text{ (c = 0.26, CH}_3\text{OH).}\)

**Compound 8 (Myristoyl-\text{L}-\text{Asp-N-hexylamide):**
White solid (70 % yield). M.p. 81 - 82°С, \(^1\)H NMR (600 MHz, [D6] DMSO) \(\delta = 8.04\) (d, \(J = 8.1\) Hz, 1H), 7.76 (s, 1H), 4.49 (dd, \(J = 14.4, 7.8\) Hz, 2H), 3.11 - 2.90 (m, 2H), 2.58 (dd, \(J = 16.2, 7.6\) Hz, 1H), 2.12 (dt, \(J = 61.4, 7.0\) Hz, 2H), 1.45 (s, 2H), 1.34 (d, \(J = 7.1\) Hz, 2H), 1.22 (d, \(J = 11.9\) Hz, 26H), 0.85 (t, \(J = 7.0\) Hz, 6H)ppm. \(^{13}\)C NMR (151 MHz, [D6] DMSO): \(\delta = 172.59, 172.36, 170.94, 49.86, 39.01, 36.90, 35.63, 34.04, 31.76, 31.46, 29.53, 29.52, 29.49, 29.43, 29.33, 29.18, 29.04, 26.38, 25.65, 24.94, 22.57, 22.52, 14.43, 14.38 \text{ppm.}\)

MS (ES) (m/z): Calculated for \(\text{C}_{24}\text{H}_{46}\text{N}_{2}\text{O}_{4}^+ \) [M + H\(^+\)] 426.35; found 427.35.

\([\alpha]_D^{20} = -25 \text{ (c = 0.25, CH}_3\text{OH).}\)

**A general procedure of obtaining Acyl-L-Asp-N-hexylamide sodium salts:**
Compound II (1g) was mixed with 2 ml of distilled water and stirred on a magnetic stirrer for 2 h at a room temperature. 1 M NaOH was added to the mixture in an amount equivalent to the acid used (compound II). The acid was dissolved in parallel to its sodium salt formation. The solution pH changed from alkaline to neutral. Then the water was evaporated and the corresponding salt was obtained. The yields were quantitative - 98% \(\pm 100\%\). Obviously compounds I and 8 did not possess surface activities. They were synthesized and studied in order to juxtapose their behavior to that of the other members of the corresponding series. The three new nonionic and three new anionic surfactants were studied on the ground of their compression isotherm or CAC data.

**\(\pi\)-\text{A isotherm analysis:**}
Fig. 1 depicts \(\pi\)-\text{A isotherms of the insoluble monolayers of compounds} 2, 3 and 4 at the air–water inter-

**Critical aggregation concentration (CAC) determination:**
Compounds 5, 6, 7 and 8 have the general structure presented in scheme 3, compound II. With the exception of 5 they all have good solubility in water of neutral and acidic pH. On the other hand, 5 is not a surfactant. The sodium salts of 6, 7 and 8 were readily water soluble and all experiments referring to 6, 7 and 8 CAC determination were performed with their sodium salts.

**RESULTS AND DISCUSSION:**
Nine new compounds were synthesized. One of them is a precursor, while the other eight belong to two different homologous series. Six of these compounds are pseudo double-chained surfactants: three - nonionactive and three - anionactive. Two of the final compounds do not possess surface activities and their synthesis is carried out aiming a juxtaposition of their properties to those of the other members of the two series. The surface activity properties of the nonionactive surfactants (2, 3 and 4) are studied by applying the technology of the Langmuir monolayers on an air/water interface.
face. The results presented in this figure show that 3 and 4 are very close in their behavior. Both have monolayers isotherms typical for fatty acids as reported in ref. [37]. The section prior to the collapse can be divided in two main linear segments of different slopes. The changes in the surface pressure ($\pi$) are as follows. At the beginning $\pi$ is very low, close to zero. The initial compression leads to its slow increase. At point of 4 mN m$^{-1}$ (for 4) $\pi$ increases sharply to a collapse pressure ($\pi_c$) of ca. 16-18 mN m$^{-1}$ with a slight decrease of the molecular surface area. It is well known that the surface pressure of the liquid-expanded (LE) state in decreases rather slowly with the decrease of the molecular surface area. Obviously, both compounds are in LE state at the beginning and some rearrangements take place at 105 Å$^2$ and 90 Å$^2$ leading to the formation of a new liquid-condensed phase (LC). The liquid monolayer is destructed at $\pi_c$ point. The collapse point ($\pi_c$) of both compounds is about 18 mN m$^{-1}$. The behavior of 3 and 4 differs after the collapse point ($\pi_c$). A constant area collapse is observed for 3, while 4 shows a constant pressure collapse. The differences between the two collapsed states are assumed to be due to head group - subphase interactions [11]. As both compounds have in our case identical head groups and subphases, interactions of an equal type are expected. There are many investigations of the monolayers morphology [37], the growth [38] and the structure [39] during the collapse, but it is still not clear when and why a monolayer collapses at a constant area and nearly at constant pressure [40]. The compression of the monolayer of 2 and the $\pi$ - A isotherm recorded show no collapsing point till 20 Å. It is easily seen in the figure that the compression leads to rearrangement of the molecules at the air/water interface. Most probably the liquid-expanded phase starts to transform into a liquid-condensed phase at pressure of 2 mN m$^{-1}$-3 mN m$^{-1}$. Further pressure increase leads to parallel condensation and dissolution of the compound.

**CAC determination**

The aggregation process of pseudo double-chained amino acid based surfactants is studied tensiometrically by measuring solutions of concentrations above and below CAC. The surface tension vs. log C plots for two of the studied surfactants (for the sodium salts of 3 and 4) are presented in Fig. 2. Data referring to CAC of both compounds obtained conductometrically are close to these found tensiometrically. The sodium salt of compound 3 has CAC = 4.0 mM estimated conductometrically (compare to 7.0 mM found tensiometrically); the sodium salt of compound 4 has CAC = 0.5 mM estimated conductometrically (compare to 0.25 mM determined tensiometrically). It is obvious that the data obtained conductometrically are in an accord with those obtained tensiometrically. The latter values are chosen for subsequent consideration. The CAC and surface tension values at CAC determined tensiometrically are summarized in Table 1. As expected, CMC depends strongly on the length of the hydrophobic chain attached to the N side of the L-Asp. CMC decreases with an increase of C atoms number in the alkyl chain. The relationship of log CAC vs. carbon atoms in the hydrophobic chains is presented in Fig. 3. It shows a dependence close to a linear one.

As evident from Table 1, the surface tension ($\sigma$) which is an important parameter determining the interfacial efficiency has very close values for 7 and 8. That could due to the self-assembling properties of these compounds forming supramolecular complexes at another scale (see Figs. 4 - 6).

<table>
<thead>
<tr>
<th>Compound (sodium salts of)</th>
<th>CAC (mM)</th>
<th>CAC (μg/ml)</th>
<th>$\sigma$-CAC (mN/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>15</td>
<td>5.04x10$^3$</td>
<td>43.5</td>
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<tr>
<td>7</td>
<td>7</td>
<td>2.74x10$^3$</td>
<td>28.8</td>
</tr>
<tr>
<td>8</td>
<td>0.25</td>
<td>112.08</td>
<td>31.1</td>
</tr>
</tbody>
</table>

Table 1. Values for CACs and corresponding surface tensions for the studied surfactants (the sodium salts of 6, 7 and 8).

![Fig. 2. Surface tension vs. logarithm of concentration curves for the sodium salts of 6, 7 and 8. All measurements are at 22°C.](image-url)
Supramolecular structures

Microscopic techniques are used to observe supramolecular formations in the monolayers and the compounds samples after the solvent evaporation.

The BAM images of the monolayer formed by compound 3 show the formation of aggregates of a length of about 50 μm - 100 μm, while the picture of the monolayer of compound 4 displays twisted and entangled fibers of a width of 1μm - 2 μm and a length of several hundreds of micrometers (see Fig. 4). The dried material from the both compounds is studied by SEM. The results obtained shown in Fig. 5 confirm the presence of monolayers. Obviously compound 4 tends to aggregate in well formed fibers (Fig. 5(b)), while compound 3 also self arranges in fibers but they are much shorter and sticked to each other (Fig. 5(a)).

Fig. 6 (a) presents the SEM image of the sodium salt of compound 6, Fig. 6 (b) – that of compound 7.
while Fig. 6 (c) - that of compound 8. A tendency of delamination and shaping of closely situated separate micro-dimensional structures of clear boundaries is easily seen. Slides of a thickness of nanometers are arranged at a distance of several tens to hundreds of nanometers in case of compound 6 (Fig. 6(a)). Compound 7 contains a mixture of rods of micro-dimensions and a blurry unshaped mass. The image of 8 (Fig. 6(c)) reveals well shaped rods, distinctly apart from each other, with a diameter of about 1 μm and a length of several to several tens of micrometers. These rods are very similar in shape to those of other alpha-amino acid derivatives of an analogous chemical structure, although prepared along different routes [41 - 43].

The observation of the supramolecular complexes formed by the studied compounds after the solvent evaporation shows that the best shaped as separated structures are the rods obtained from the solution of compound 4A. The results referring to the structures observed lead to the conclusion that the compounds from the series containing carboxylic or carboxylate group have better expressed self-assembly properties. The series containing a benzyl ester fragment displays also self-assembling properties although only compound 4 self-organizes in separate structures (see Fig. 5).

CONCLUSIONS

Eight new final compounds, derivatives of the alpha amino acid L-Asp, are synthesized. They all contain two alkyl tails. Six of these compounds possess a surface activity. They are called in this communication “pseudo-double-chained surfactants” since one of the alkyl tails has a length of only six carbon atoms, while the rest contain five, nine or thirteen alkyl carbons and one carbonyl carbon. Three compounds belong to a nonionic series of structures, while another three have anionic structures. They all tend to self-organize in supramolecular arrangements of nano- and micromedimensions, but the anionic surfactants form significantly better shaped structures. Two of the non-ionic surfactants (compounds 3 and 4) give stable monolayers (2D structures) of similar characteristics and collapsing pressure values (about 18 mN m⁻¹). The third nonionic surfactant has a higher solubility in water and its π-A isotherm does not show a collapsing point. All anionic analogs do not form monolayers because of their significantly higher solubility in water.

Fig. 6. SEM images of sodium salts of a) 6, b) 7 and c) 8. The bar represents 1 micron.
The presence of supramolecular complexes, bigger than micelles, provides to define the determined critical concentrations as critical aggregate concentrations (CAC). The CAC of the anionic surfactants is in the concentration range typical of many surfactants. The phenomena observed are still not well studied, but they are in the focus of different research teams. The appearance of filamentous micro-dimensional objects in the Langmuir monolayer is not so far reported.

The application of the newly synthesized compounds as surfactants and building units in nano- and micro-structural chemistry is a topic of future investigation of the compounds described here as well as of their analogues.

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