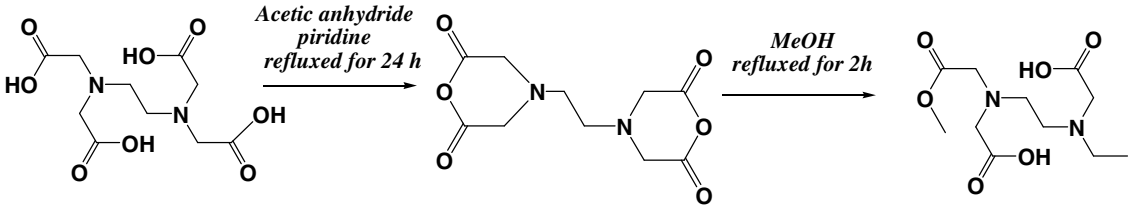
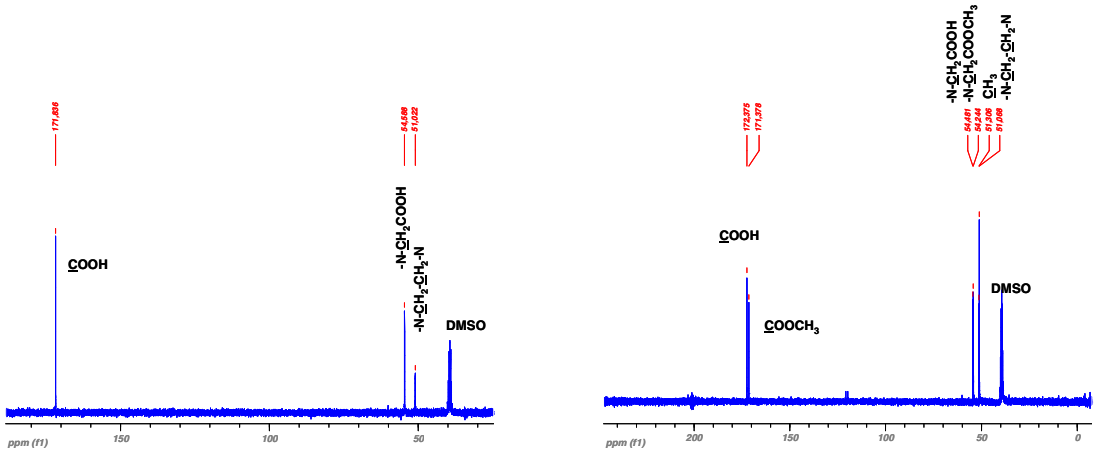
Synthesis of dimethyl ester of EDTA (Scheme 1)**:**[1] 7.28 g of EDTA (24.90 mmol) and 12 mL of pyridine (148.7 mmol) in 10 mL of acetic anhydride (106.4 mmol) were refluxed at 65 ºC for 24 hours. The solid was filtered and washed with acetic anhydride and diethyl eter, respectively. 4,68 g (18,27 mmol) of this solid were refluxed in 45 mL of methanol at 80-90ºC for 2 hours. The organic phase was partially evaporated under reduced pressure, the residue was redissolved in methanol and precipitated with ethyl acetate. Identity of the compound was confirmed by NMR (Figure 1) and purity by single point TLC in a mixture 1:1 methanol: ethyl acetate. Overall yield 76%.



Scheme 1.- Synthesis path of a dianhydride bridge derived from ethylenediaminetetraacetic.

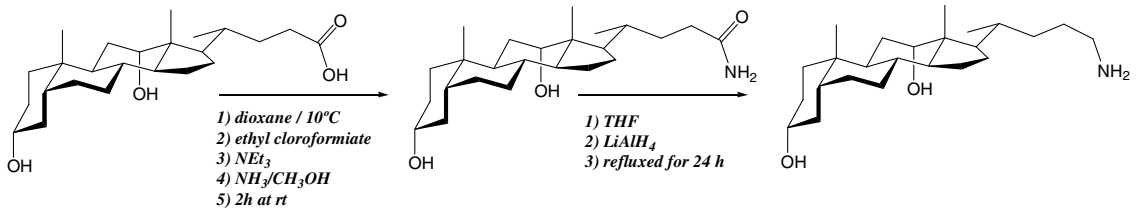


(a) **(b)**

Figure 1.- ^{13}C-NMR spectra of EDTA **(a)** and dimethyl ester of EDTA **(b)** in DMSO. 300MHz.

[1] Esterhuysen, C.; Bredenkamp, M. W.; van Kralingen, L.; Adendorff, H. J.; Jacobs, E. P.; Swart, P. *Acta Crystal.* **2006**, *E62*, 355-7.

Synthesis of 24-deoxycholate amine:[2] The synthetic pathway used for the preparation of 24-deoxycholate amine involve two steps (Scheme 2).



Scheme 2.- Synthesis path of 24-deoxycholate amine.

4.17 g of deoxycholic acid (0.01 mmol) were dissolved in 50 mL of anhydrous dioxane, and freshly distilled triethylamine (1.4 mL) was added with stirring. The solution was kept cold at 10 ºC for 10 min, and then 0.01 mmol of ethyl chloroformiate (1 mL) was added to the solution at this temperature over a period of 10 min. A slight excess (17 mL) of a solution (2M) of ammonia in methanol was added and the mixture was left at 10ºC for 30 min and then at rt for 2 h with stirring. Overall yield 94%. The 24-amide was characterized by NMR (Figure 2). The NMR spectrum(in MeOD) evidence some ethyl eter, but it is not important in the next reaction. R_f deoxycholic amide (ethyl acetate:methanol 8:2)=0.27. For reference purposes: R_f deoxycholic acid (ethyl acetate: methanol 8:2)=0.37. The amide was used for the second step.

^{13}C NMR (300 MHz, MeOD): C1 (CH_2) 36.53, C2 (CH_2) 31.18, C3 (CH) 72.63, C4 (CH_2) 37.33, C5 (CH) 43.75, C6 (CH_2) 28.47, C7 (CH_2) 27.52, C8 (CH) 37.56, C9 (CH) 34.95, C10 (C) 35.38, C11 (CH_2) 29.99, C12 (CH) 71.12, C13 (C) 47.67, C14 (CH) 49.37, C15 (CH_2) 24.91, C16 (CH_2) 28.67, C17 (CH) 48.24, C18 (CH_3) 13.25, C19 (CH_3) 23.74, C20 (CH) 36.53, C21 (CH_3) 17.74, C22 (CH_2) 33.62, C23 (CH_2) 33.24, C24 (C) 178.65 ppm. **^{1}H NMR (300 MHz, MeOD):** 0.71 (s, 3H, H18); 0.93 (s, 3H, H19); 0.8 to 2.2 (m, $H_{aliphatic}$); 3.54 (bs, 1H, H3); 3.95 (bs, 1H, H12) ppm.

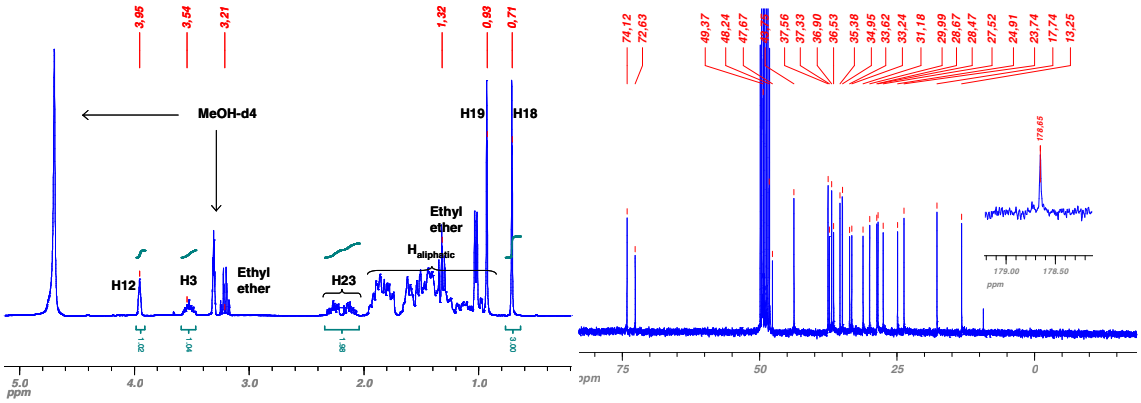


Figure 2.- ^{1}H and ^{13}C-NMR spectra of 24-amide in MeOD. 300 MHz.

The amide bond of the 24-amide was reduced by treatment with $LiAlH_4$ as follows. Reaction of 24-amide (0.41 g, 1mmol) in 80 mL of anhydrous tetrahydrofuran in the presence of the reductor agent (5 g of $LiAlH_4$) afforded the 24-amine in 65% yield. The suspension was refluxed for 24 h, and small amounts of hydride were added at 4 h intervals. The reaction mixture was treated with water, and the precipitate was extracted. Finally, the resulting solid

[2] Bellini, A. M.; Quaglio, M. P.; Guarneri, M.; Cavazzini, G. *Eur. J. Med. Chem.-Chim. Ther.* **1983**, 18 (2), 185-193.

was purified by silica gel chromatography (first with hexane: ethyl acetate 1:1, and then with methanol: triethylamine 95:5). The NMR analysis performed in MeOD demonstrated that the purified final compound was free of any included byproduct or reagent as shown by Figure 3. R_f deoxycholic amide (methanol: triethylamine 95:5)=0.80. For comparative purposes R_f deoxycholic amine (methanol: triethylamine 95:5)=0.27.

^{13}C NMR (300 MHz, MeOD): C1 (CH_2) 36.54, C2 (CH_2) 31.18, C3 (CH) 72.62, C4 (CH_2) 37.33, C5 (CH) 43.75, C6 (CH_2) 28.48, C7 (CH_2) 27.53, C8 (CH) 37.56, C9 (CH) 34.94, C10 (C) 35.38, C11 (CH_2) 30.00, C12 (CH) 71.14, C13 (C) 47.63, C14 (CH) 49.36, C15 (CH_2) 24.93, C16 (CH_2) 28.49, C17 (CH) 48.43, C18 (CH_3) 13.26, C19 (CH_3) 23.75, C20 (CH) 36.54, C21 (CH_3) 18.07, C22 (CH_2) 37.39, C23 (CH_2) 30.25, C24 (CH_2) 43.09 ppm. **^{1}H NMR (300 MHz, MeOD):** 0.72 (s, 3H, H18); 0.94 (s, 3H, H19); 0.8 to 2.2 (m, $H_{aliphatic}$); 3.53 (bs, 1H, H3); 3.96 (bs, 1H, H12); 2.62 (m, 2H, H24) ppm.

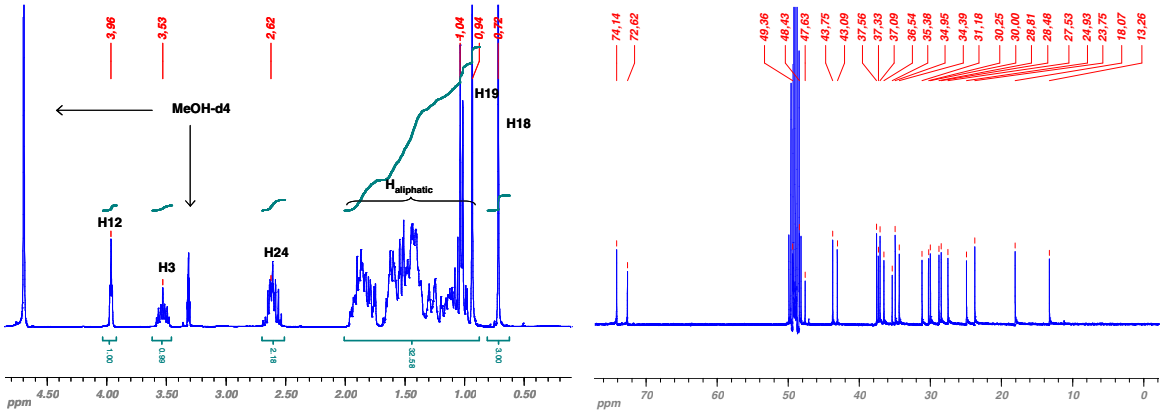
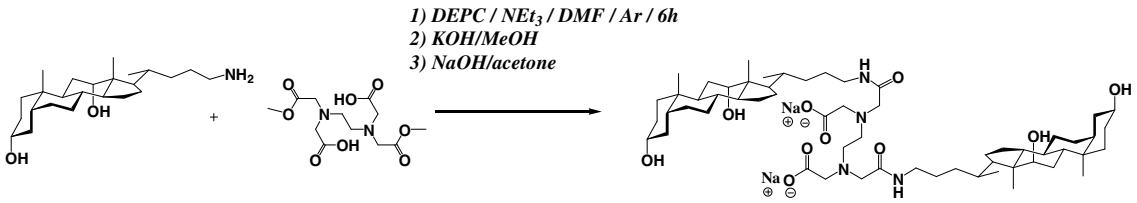


Figure 3.- ^{1}H and ^{13}C-NMR spectra of 24-amine in MeOD. 300MHz.

Synthesis Scheme of *g-2DC$_{24}$-EDTA*: The recipe is given in the text.



Scheme 3.- Synthesis path of *g-2DC$_{24}$-EDTA*.

The final di-esther product was purified by a Sephadex column (ethyl acetate: methanol, 7:3). R_f *gemini*=0.51. R_f *24-amine*=0.08.

^{13}C NMR (300 MHz, DMSO): C1 (CH_2) 35.87, C2 (CH_2) 30.95, C3 (CH) 70.67, C4 (CH_2) 37.03, C5 (CH) 42.36, C6 (CH_2) 27.71, C7 (CH_2) 26.80, C8 (CH) 36.40, C9 (CH) 33.68, C10 (C) 34.53, C11 (CH_2) 29.34, C12 (CH) 71.71, C13 (C) 46.68, C14 (CH) 48.13, C15 (CH_2) 24.19, C16 (CH_2) 27.94, C17 (CH) 47.07, C18 (CH_3) 13.13, C19 (CH_3) 23.76, C20 (CH) 35.73, C21 (CH_3) 18.00, C22 (CH_2) 33.52, C23 (CH_2) 26.64, C24 (CH_2) 39.50, -N-$\underline{C}H_2$-$\underline{C}H_2$-N- 53.01, -$\underline{C}H_2$-CONH- 58.23, -$\underline{C}H_2$-$COOCH_3$ 55.68, -O$\underline{C}H_3$ 51.85, $\underline{C}OOCH_3$ 170.56, $\underline{C}$ONH-172.20 ppm. **^{1}H NMR (300 MHz, DMSO):** 7.74 (d, 2H, amide bond); 3.78 (bs, 2H, H3); 4.08 (bs, 2H, H12); 3.44 (s, 4H, -$\underline{C}H_2$-CONH-); 3.60 (-O$\underline{C}H_3$); 3.14 (s, 4H, -$\underline{C}H_2$-$COOCH_3$); 2.67 (s, 4H, -N-$\underline{C}H_2$-$\underline{C}H_2$-N-); 0.8 to 2.2 (m, $H_{aliphatic}$); 0.58 (s, 6H, H18), 0.83 (s, 6H, H21) ppm.

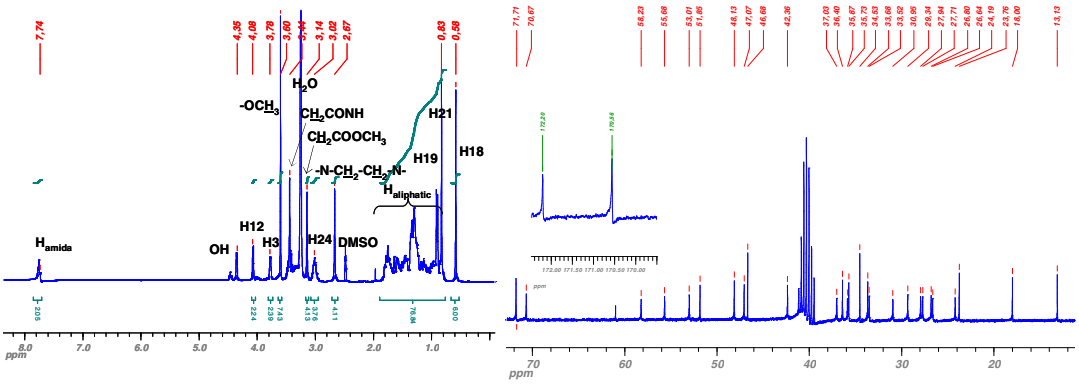
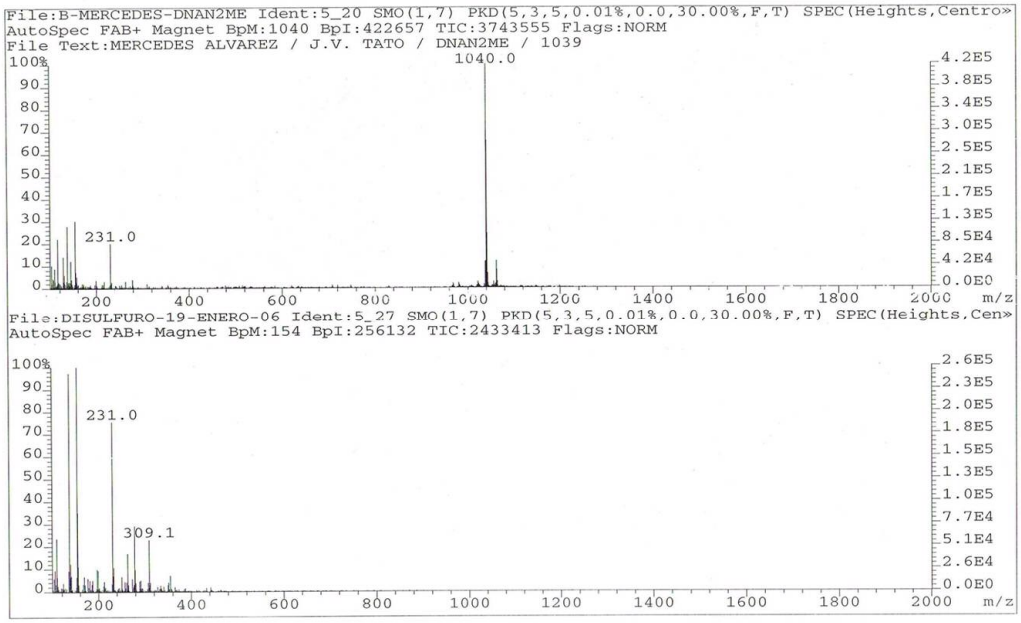


Figure 4.- ^{1}H and ^{13}C-NMR spectra of *g-2DC$_{24}$-EDTA* (di-ester) in DMSO. 300MHz.

FAB-MS: m/z 1039.47 calc for $C_{60}H_{102}N_4O_{10}$, found: 1039.77.



Synthesis of the diacid form of *g-2DC$_{24}$-EDTA*

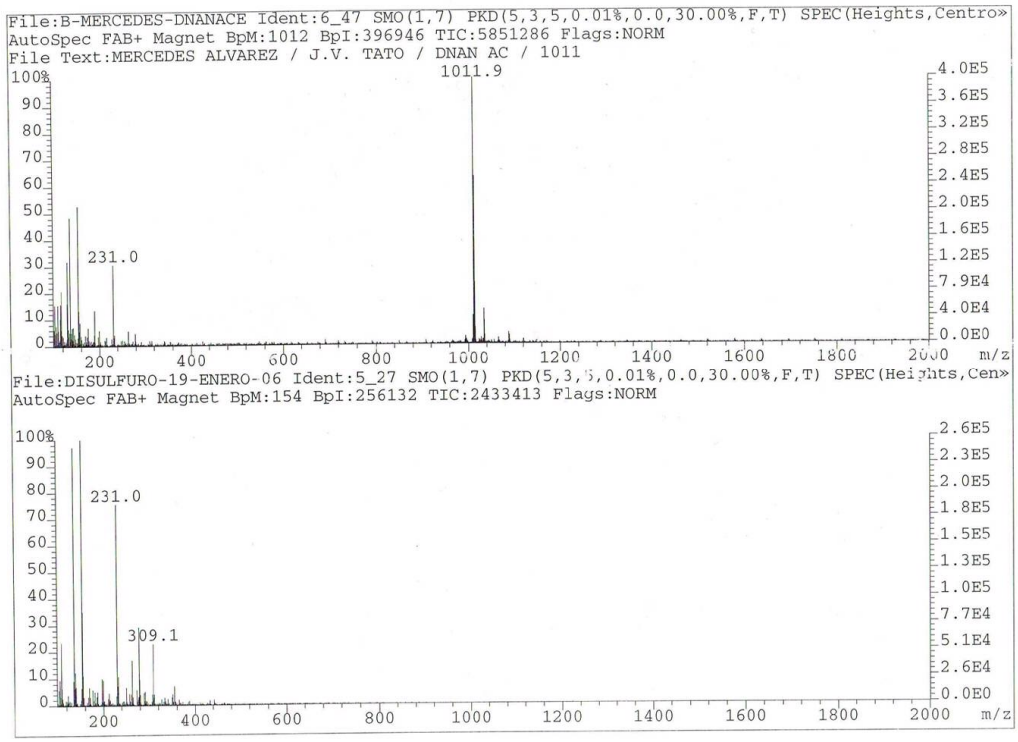
To remove the ester methyl groups, the compound was refluxed with KOH 1 M in methanol for one hour at 80ºC. The solvent was evaporated and the solid redissolved in water (200mL) and acidified with HCl (pH~1). When the solution is cooled, the compound precipitates it is diacid form. The precipitate was filtered and dried in vaccum oven. R_f (ethyl acetate: methanol, 1:9) =0.58. Overall yield 45%

Di-acid form NMR assigments

^{13}C NMR (300 MHz, DMSO): C1 (CH_2) 35.47, C2 (CH_2) 31.05, C3 (CH) 70.77, C4 (CH_2) 36.43, C5 (CH) 42.36, C6 (CH_2) 27.71, C7 (CH_2) 26.81, C8 (CH) 37.00, C9 (CH) 33.68, C10 (C) 34.53, C11 (CH_2) 29.33, C12 (CH) 71.82, C13 (C) 46.58, C14 (CH) 48.14, C15 (CH_2) 24.22, C16 (CH_2) 27.95, C17 (CH) 47.07, C18 (CH_3) 13.14, C19 (CH_3) 23.78, C20 (CH) 35.84, C21 (CH_3) 18.06, C22 (CH_2) 33.64, C23 (CH_2) 26.24,

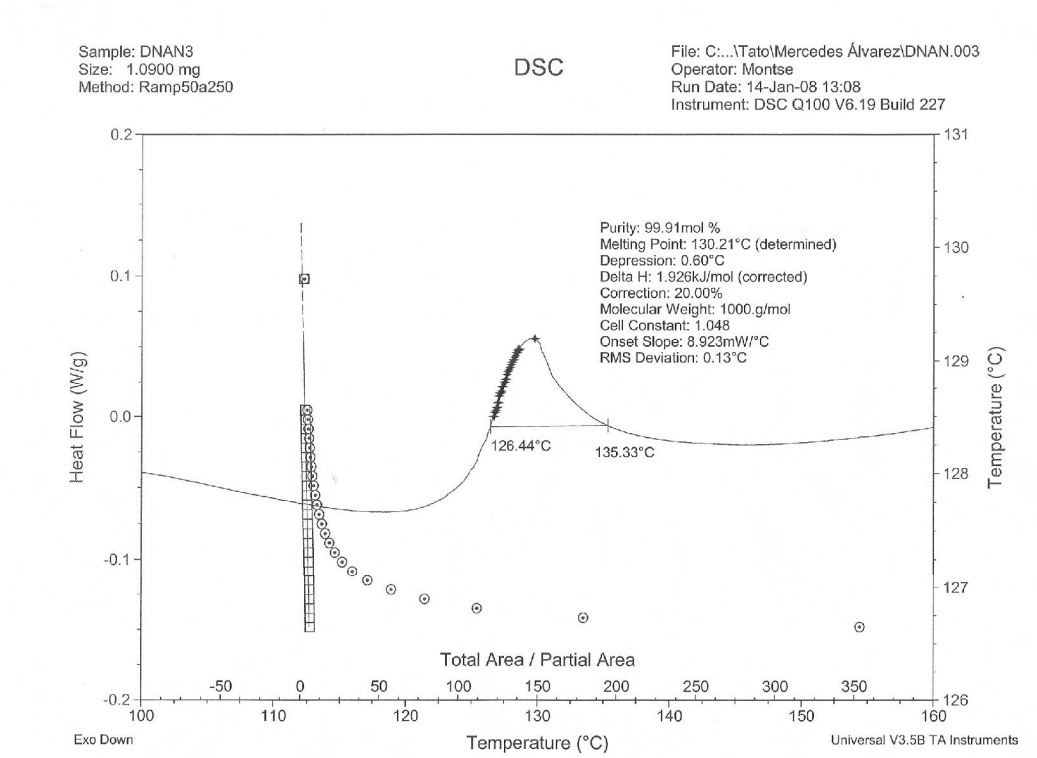
C24 (CH_2) 40.51, -N-$\underline{C}H_2$-$\underline{C}H_2$-N- 53.14, -$\underline{C}H_2$-CONH- 58.40, -$\underline{C}H_2$-COOH 56.20, -O$\underline{C}H_3$ 51.85, $\underline{C}OOCH_3$ 170.56, $\underline{C}$ONH-172.20 ppm. **^{1}H NMR (300 MHz, DMSO):** 7.84 (d, 2H, amide bond); 4.16 (bs, 2H, H12); 3.79 (bs, 2H, H3); 3.44 (s, 4H, -C$\underline{H}_2$-CONH-); 3.19 (s, 4H, -C$\underline{H}_2$-COOH); 3.04 (s, 4H,); 2.70 (s, 4H, -N-C$\underline{H}_2$-C$\underline{H}_2$-N-); 0.8 to 2.2 (m, $H_{aliphatic}$); 0.59 (s, 6H, H18), 0.83 (s, 6H, H21) ppm.

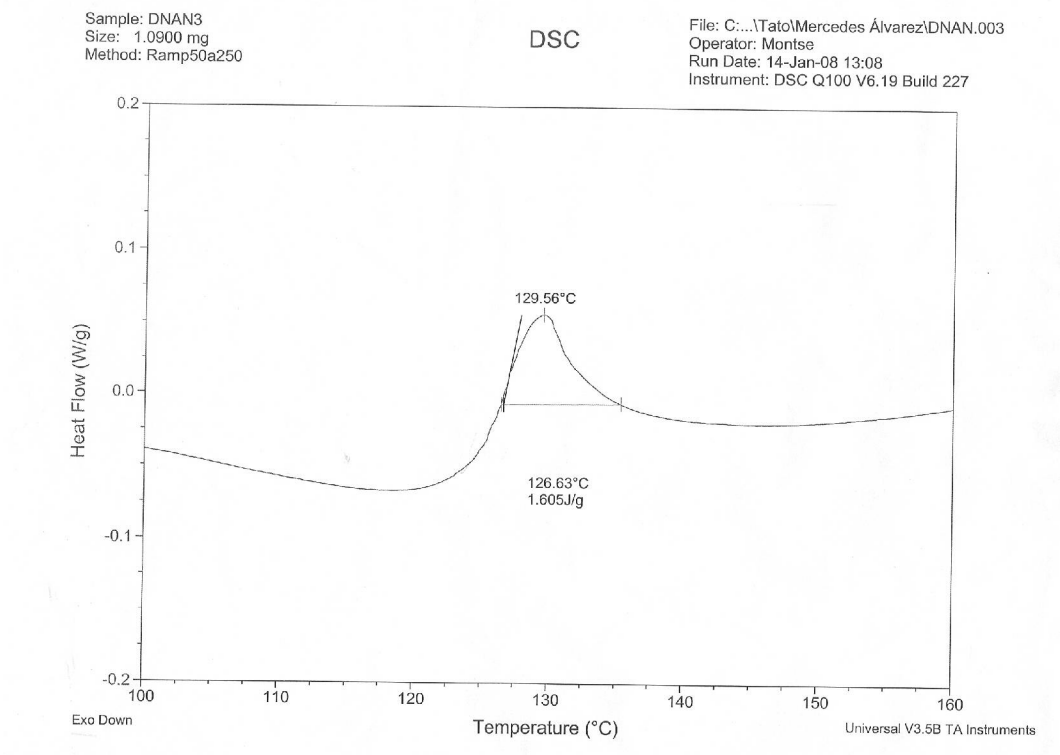
FAB-MS: m/z 1011.42 calc for $C_{58}H_{98}N_4O_{10}$, found: 1011.73.



Melting point.

Melting point measurements were carried out in a differential scanning calorimeter Q100 of TA Instruments. The obtained value is 129.56 ºC.





HPLC analysis

Purity of the compound was also checked by HPLC analysis.A Waters model 441 absorbance detector at λ=254 nm was used. Chromatographic analysis was performed using a reverse phase C-18 column (3.9×150 mm.) from Waters. An isocratic mobile phase containing 20%methanol/80% water was used. Mobile phase flow rate was set to 0.4 mL/min with a run time of 30 min.