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POLYANION INHIBITORS OF HUMAN IMMUNODEFICIENCY VIRUS. Part IV. - POLYMERIZED ANIONIC SURFACTANTS : INFLUENCE OF THE DENSITY AND DISTRIBUTION OF ANIONIC GROUPS ON THE ANTIVIRAL ACTIVITY.

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Abstract : The synthesis of a series of new polyanions, via γ-polymerization, of ω-unsaturated multi-charged anionic surfactants, has been realized. These polyanions were evaluated for their activity against HIV-1. All tested compounds proved active without presenting toxicity to the host cells (CEM-4 or MT-4).

Various polyanions have proven inhibitory to HIV replication; their activity has been attributed to interference with virus adsorption, due to a shielding of the viral envelope glycoprotein gp120[1,2].

Recently, we have shown that polyanions obtained by γ-polymerization in micellar solutions of ω-unsaturated anionic surfactants (Figure 1a) with the polar head derived either from simple anionic groups[3], aminoacids or dipeptides[4], or saccharidic residues[5], markedly inhibit the replication of HIV-1 and HIV-2 in cell culture. All starting monomeric surfactants were monoanionic compounds.

In a recent article[2], it has been postulated that the antiviral potency and activity spectrum of polyanions could depend upon the density and the distribution of the negative charges. This hypothesis prompted us to explore the antiviral activities of polyanions obtained by γ-polymerization of ω-unsaturated multi-charged anionic surfactants, i.e. bi-, tri- or tetra-charged monomers (Figure 1b, c and d).

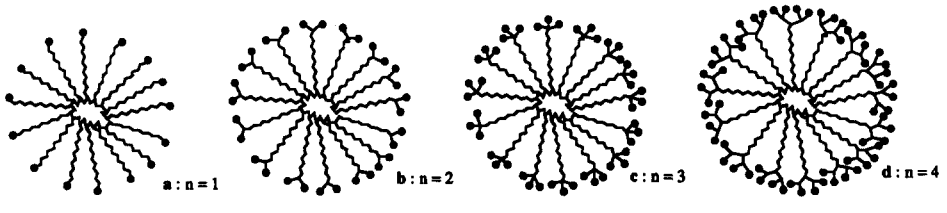


Figure 1.- Schematic structure of polyanions obtained by γ-polymerization of ω-unsaturated n-charged anionic surfactants (● : mono-anionic group).

In this work, we present the synthesis of a series of polyanions that originate from monomers of a C_{11} linear chain bearing a carbon-carbon double bond at one end, and a multi-charged anionic head at the other end. Antiviral activities of these compounds are also presented.

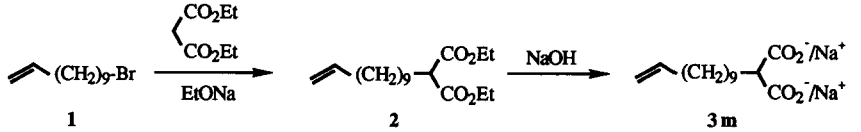
Syntheses

Except for the compound **3m** resulting from the malonic acid alkylation, all surfactants are amide derivatives obtained from natural or modified α-amino-acids, or from tris-(hydroxymethyl) aminomethane.

Dianionic compounds

1 - Compounds derived from malonic acid : the sodium 2-(undec-10-enyl)propanedioate **2** was obtained according to a classic malonic synthesis[6]. The condensation of the 11-bromoundec-10-ene **1**[3] on the diethylmalonate was realized in the presence of sodium ethoxide to give diester **2**, which was saponified by sodium hydroxyde in water/acetone solution to give the surfactant **3m** (Scheme 1) in 90% yield[7].

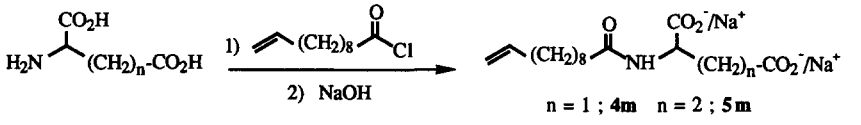
Scheme 1



1 **2** **3 m**

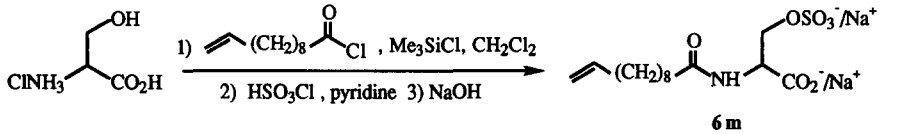
2 - Compounds derived from L-aspartic and L-glutamic acids : ω-unsaturated surfactants derived either from L-aspartic acid or L-glutamic acid were obtained according to the Schotten-Baumann procedure[8] as shown in Scheme 2. Neutralization of the intermediate diacids, under nitrogen atmosphere and at 0°C, by two equimolar amounts of sodium hydroxyde gave the corresponding di-sodium salts. The latter were recovered by lyophilization in 70% yield[9] with aspartic acid, compound **4m**; and in 90% yield[10] with glutamic acid, compound **5m**, respectively.

Scheme 2



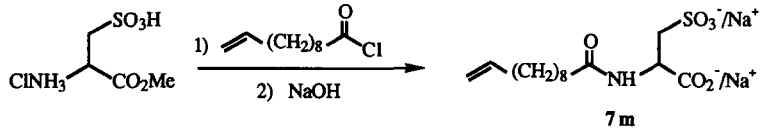
n = 1 ; **4m** n = 2 ; **5m**

3-Compounds derived from cysteinic acid and from L-serine : the compound **6m** was prepared by reacting undec-10-enoyl chloride with L-serine using a temporary protection of the carboxylic function by a trimethylsilyl ester[11]. Sulfation of the alcohol function of the amino-acid lateral chain by chlorosulfonic acid in pyridine[12,13], followed by stœchiometric neutralization with sodium hydroxyde, gave the monomer **6m** in 48% yield[14] (Scheme 3).



6 m

Scheme 3



7 m

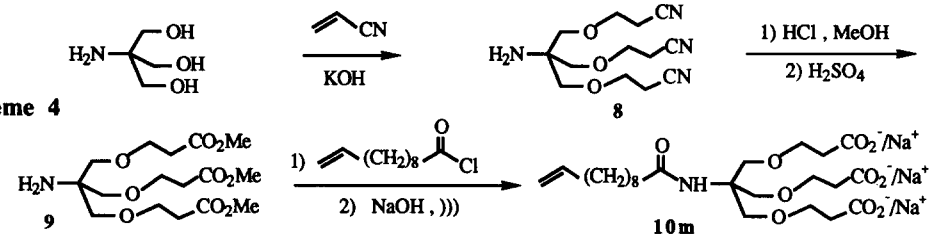
N-acylation of cysteinic methyl ester, in biphasic medium (aqueous sodium carbonate / chloroform), by undec-10-enoyl chloride, followed by a saponification reaction, gave compound **7m** in 90% yield[15] after lyophilization. Due to the fact that both reactions of the acid chloride according to the Schotten-Baumann procedure or with the temporary protection failed, the protection of the methyl ester has proven to be necessary.

Trianionic compound

The structure of this compound is shown in Scheme 4. The intermediate trinitrile **8** was obtained from the tris (hydroxymethyl) aminomethane by a Michaël addition[16,17]. This nitrile is successively treated by a methanolic chlorhydric acid solution and by 5N sulfuric acid. Initially, the nitrile function was converted to iminoester which was hydrolyzed by diluted sulfuric acid. The compound **9** was isolated as a colourless oil [17,18].

Triester **9** was reacted with undec-10-enoyl chloride in methylene chloride in the presence of triethylamine to obtain the corresponding amide which, after saponification under sonication, led to the trianionic surfactant **10m** isolated with non optimized 40% yield[19].

Scheme 4

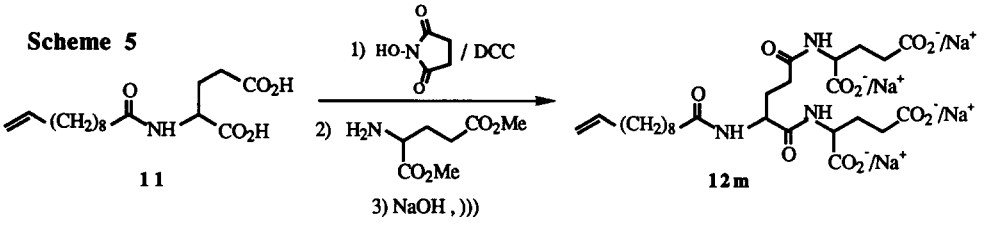


Tetra-anionic compound

A tetra-anionic surfactant was synthesized by condensing the L-glutamic acid dimethyl ester with the two carboxylic acid functions of the N-(undec-10-enoyl)-L-glutamic acid **11**[4] in the presence of the dicyclohexyl-carbodiimide / N-hydroxysuccinimide couple[20] in chloroform (Scheme 5).

The intermediate tetra-ester, isolated after chromatography on silica gel, was saponified by 4N sodium hydroxyde under sonication, neutralized and finally lyophilized to lead to **12m** in 80% yield[21].

Scheme 5



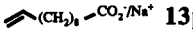
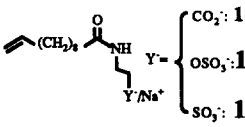
Polymerization reaction

Monomeric surfactants **3m** to **12m** were polymerized by γ-irradiation of a 0.1M aqueous micellar solution according to a previously described procedure[3] to lead to polymerized surfactants **3p** to **12p**. Polyanions thus prepared were purified by gel permeation chromatography (Sephadex-G50) to remove traces of the monomers. The polyanions were recovered by lyophilization. ^1H NMR spectroscopy in D_2O enable us to verify the disappearance of the vinylic protons and the absence of degradation during γ-irradiation.

Biological activity

The different compounds (**3p** to **12p**) were evaluated for their antiviral activity according to well-established procedures[3]. The compounds were evaluated against HIV in the T4-lymphocyte cell lines CEM-4 and MT-4. (Table 1). In addition, cytotoxicity of the different compounds was determined for the cell lines used. First of all, it is noteworthy that the polyanions were nontoxic for CEM-4 cells as well as for MT-4 cells at concentrations up

Table 1.- Anti-HIV-1 (strain HTLV-$III_{B/LAI}$) activity of polyanions in CEM-4 and MT-4 cells [a]

polyanions	CC_{50} [b] µg/mL	RT		MTT (CEM-4)		MTT (MT-4)	
		IC_{50} [c] µg/mL	SI [d]	IC_{50} [c] µg/mL	SI [d]	IC_{50} [c] µg/mL	SI [d]
3p	>100	1.7	>58	n.d. [f]	n.d.	4.9	>20
4p	>100	8	>12	n.d.	n.d.	0.8	>125
5p	>100	0.2	>500	0.8	>125	n.d.	n.d.
6p	>100	8	>12	5.9	>17	n.d.	n.d.
7p	>100	8	>12	7.6	>13	n.d.	n.d.
10p	>100	3	>32	3	>32	n.d.	n.d.
12p	>100	30	>3	24	>4	1.1	>90
 **13p** [e]	100	3.5	>28	n.d.	n.d.	3.6	>28
 CO_2^-: **14p** [e]	770	0.4	1925	0.1	7700	0.2	3850
Y⁻= OSO_3^-: **15p** [e]	>100	8	>12	11	>9	90	>1
SO_3^-: **16p** [e]	>100	2	>50	0.8	>125	0.2	>500

a) All data represent the average value of at least two separate experiments. b) 50% Cytotoxic Concentration, or coumpound concentration required to reduce the viability of uninfected cells by 50% at 5 days of incubation in the presence of the compound. c) 50% Inhibitory Concentration, or compound concentration required to reduce by 50% HIV-1-induced cytopathicity, based on the Reverse Transcriptase (RT) activity or on the MTT assay. d) 50% Selectivity Index or Ratio of CC_{50} to IC_{50}. e) See reference 3. f) not determined.

to 100 µg/mL. As shown in Table 1, all the polyanions proved inhibitory to HIV-1 in CEM-4 and MT-4 cells. The 50% inhibitory concentration (IC_{50}) based on either the MTT assay[3] or reverse transcriptase (RT) activity, fell in the range of 3-24 µg/mL (MTT) and 0.2-30 µg/mL (RT), respectively.

The activities of the polyanions **3p**, **4p**, **6p** and **7p**, obtained from dianionic surfactants can be compared to those of previously studied polyanions **13p**,**14p**,**15p** and **16p**, derived from analogous monoanionic surfactants in which one carboxylate group is missing (Table 1).

It clearly appears that the introduction of a second anionic group, - i.e. a carboxylate group -, at the end of each hydrocarbon chain, does not significantly change the anti-HIV-1 activity. Likewise, increasing the charge number to three (compound **10p**) or four (compound **12p**) on each hydrocarbon chain, does not markedly affect the anti-HIV-1 response .

It seems that for this type of polyanions, the anti-HIV-1 activity is not influenced by the local charge density or charge distribution.

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7. Compound **3m**: Yield = 94%. mp >250°C. **IR** : KBr, ν cm-1: 2928-2856 (C-H); 1590 (C=O, CO_2^-). **1H NMR** : (250 MHz, D_2O), δ ppm: 1.3 (m, 14H, $H_{2'}$ to $H_{8'}$); 1.7 (q, 2H, $H_{1'}$, $J_{2\text{-}1'}$=7.5Hz, $J_{2'\text{-}1'}$=7.5Hz); 2.1 (m, 2H, H9'); 3.1 (t, 1H, H_2, $J_{2\text{-}1'}$=7.5Hz) ; 5.0 (m, 2H,$H_{11'}$, $J_{11b'\text{-}10'}$ = 18Hz, $J_{11a'\text{-}10'}$=10.5Hz); 5.8 (m, 1H, $H_{10'}$, $J_{10'\text{-}11b'}$=18Hz, $J_{10'\text{-}11a'}$=10.5Hz, $J_{10'\text{-}9'}$ =7Hz). **MS** : (FAB^+, G), m/z: 279 $(M\text{-}Na+2H)^+$; 301 $(M+H)^+$; 323 $(M+Na)^+$; 623 $(2M+Na)^+$; 923 $(3M+Na)^+$. **Anal** : $C_{14}H_{22}O_4Na_2$.

8. a) Schotten, C. *Ber.* **1884**, *17*, 2544. b) Baumann, E. *Ber.* **1886**, *19*, 3218.

9. Compound **4m**: Yield = 70%. mp (acide) = 83-85°C. R_f : 0.6 (20% aqueous NH_3/H_2O/EtOH, 16/12/100, v/v/v). $[\alpha]_D^{25}$ (salt) = +0.8° (H_2O). **IR** : KBr, ν cm^{-1}: 3397 (N-H); 2928-2855 (C-H); 1580-1406 (C=O, CO_2^-). **1H NMR** : (250 MHz, $CDCl_3$), δ ppm (acide): 1.1 (m, 10H, $H_{4'}$ to $H_{8'}$); 1.4 (m, 2H, $H_{3'}$); 1.8 (q, 2H, $H_{9'}$, $J_{9'\text{-}8'}$=7Hz, $J_{9'\text{-}10'}$=6Hz); 2.0 (t, 2H, $H_{2'}$, $J_{2'\text{-}3'}$=7Hz); 2.3 (m, 2H, H_3); 4.4 (q, 1H, H_2, $J_{2\text{-}3}$=7Hz, $J_{2\text{-}NH}$=7.5Hz); 4.8 (m, 2H, $H_{11'}$, $J_{11b'\text{-}10'}$=17.1Hz, $J_{11a'\text{-}10'}$=10Hz); 5.8 (m, 1H, $H_{10'}$, $J_{10'\text{-}11b'}$=17.1Hz, $J_{10'\text{-}11a'}$=10Hz); 6.8 (m, 1H, NH); 10.25 (m, 2H, $(CO_2H)_2$). **13C NMR** : (50.32 MHz, $CDCl_3$), δ ppm (acid): 25,56 to 36,19 ($C_{2'}$ to $C_{9'}$ and C_3); 48.59 (C_2); 114.34 ($C_{11'}$); 139.08 ($C_{10'}$); 174.47 ($C_{1'}$); 175.04 and 175.27 (C_1 and C_4). **MS** : (FAB^+, NBA), acid, m/z: 322 $(M+Na)^+$; 300 $(M+H)^+$. **Anal** : $C_{15}H_{23}NO_5Na_2$.

10. Compound **5m**: Yield = 90%. mp (acid) = 77-79°C. R_f : 0.5 (20% aqueous NH_3/H_2O/EtOH, 16/12/100, v/v/v).$[\alpha]_D^{25}$ (salt) = +0.2° (H_2O). **IR** : KBr, ν cm^{-1}: 3392 (N-H); 2928-2853 (C-H); 1580-1409 (C=O, CO_2^-). **1H NMR** : (250 MHz, $CDCl_3$), δ ppm, (acid): 1.2 (m, 12H, $H_{4'}$ to $H_{8'}$ and H_3); 1.4 (m, 2H, $H_{3'}$); 1.7 (q, 2H, $H_{9'}$, $J_{9'\text{-}8'}$=7Hz, $J_{9'\text{-}10'}$=6Hz); 2.1 (t, 2H, $H_{2'}$, $J_{2'\text{-}3'}$=7Hz); 2.3 (m, 2H, H_4); 4.4 (q, 1H, H_2, $J_{2\text{-}3}$=7Hz, $J_{2\text{-}NH}$=7,5Hz); 4.8 (m, 2H, $H_{11'}$, $J_{11b'\text{-}10'}$=17.1Hz, $J_{11a'\text{-}10'}$=10Hz); 5.8 (m, 1H, $H_{10'}$, $J_{10'\text{-}11b'}$=17.1Hz, $J_{10'\text{-}11a'}$=10Hz); 6.8 (m, 1H, NH); 10.3 (m, 2H, $(CO_2H)_2$). **13C NMR** : (50.32, $CDCl_3$), δ ppm (acid): 25,64 to 36,29 ($C_{2'}$ to $C_{9'}$ and C_3, C_4); 51.77 (C_2); 114.21 ($C_{11'}$); 139.11 ($C_{10'}$); 175.04 and 175.19 (C_1 and C_5); 177.41 ($C_{1'}$). **MS** : (FAB^+, NBA), (acid), m/z: 322 $(M+Na)^+$; 300 $(M+H)^+$. **Anal** : $C_{16}H_{25}NO_5Na_2$.

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14. Compound **6m**: Yield = 48%. mp > 250°C. R*f* : 0.43 (AcOEt/MeOH, 5/5,v/v). $[\alpha]_D^{25}$ = +15.3° (c = 0.011, H_2O). **IR** : KBr, ν cm^{-1}: 3335 (N-H); 2921-2855 (C-H, CH_2); 1637 (C=O, amide); 1616 (C=O, CO_2^-); 1140 (S=O, sulfate). **^{1}H NMR** : (250 MHz, D_2O), δ ppm: 1.37 (m, 10H, $H_{4'}$ to $H_{8'}$); 1.54 (m, 2H, $H_{9'}$); 2.08 (m, 2H, $H_{3'}$); 2.23 (t, 2H, $H_{2'}$, $J_{2'-3'}$=7.6Hz); 3.22 (m, 2H, H_3); 4.45 (m, 1H, H_2); 4.93 (m, 2H, $H_{11'}$, $J_{11b'-10'}$=17.4Hz, $J_{11a'-10'}$=10.4Hz); 5.87 (m, 1H, $H_{10'}$, $J_{10'-11b'}$=17.4Hz, $J_{10'-11a'}$=10.4Hz).
^{13}C NMR : (50.32, D_2O), δ ppm: 26.0 ($C_{3'}$); 28.94 to 29.19 ($C_{4'}$ to $C_{8'}$); 33.90 ($C_{9'}$); 36.63 ($C_{2'}$); 55.12 (C_2); 69.55 (C_3); 114.77 ($C_{11'}$) ; 141.34 ($C_{10'}$) ; 175.76 ($C_{1'}$); 177.59 (C_1). **MS** : (FAB$^-$, TG), m/z : 350 (A^{2-}+H)$^-$; 372 (A^{2-}+Na)$^-$; 767 (2A-2H+3Na)$^-$. **Anal** : $C_{14}H_{23}NO_7SNa_2$.

15. Compound **7m**: Yield = 90%. mp > 250°C. R*f* : 0.43 (AcOEt/MeOH, 5/5, v/v). $[\alpha]_D^{25}$ = -9.07° (c = 0.105, H_2O).**IR** : KBr, ν cm^{-1}: 3346 (N-H); 2921-2844 (C-H, CH_2); 1643 (C=O, amide); 1599.7 (C=O, CO_2^-); 1044 (S=O, sulfonate). **^{1}H NMR** : (250 MHz, D_2O), δ ppm: 1.25 (m, 10H, $H_{4'}$ to $H_{8'}$); 1.67 (t, 2H,$H_{9'}$); 2.11 (m, 2H, $H_{3'}$); 2.38 (t, 2H, $H_{2'}$, $J_{2'-3'}$=7Hz); 4.35 (m, 2H, H_3) ; 4.55 (m,1H, H_2); 5,05 (m, 2H, $H_{11'}$, $J_{11b'-10'}$=17.4Hz, $J_{11a'-10'}$=10.4Hz); 6.0 (m, 1H, $H_{10'}$, $J_{10'-11b'}$ =17.4Hz, $J_{10'-11a'}$=10.4Hz).**^{13}C NMR** : (50,32, D_2O), δ ppm: 25.85 ($C_{3'}$); 28.92 to 29.18 ($C_{4'}$ to $C_{8'}$); 33.89 ($C_{9'}$); 36.69 ($C_{2'}$); 52.66 (C_3); 52.82 (C_2); 114.76 ($C_{11'}$); 141.37 ($C_{10'}$); 177.2 ($C_{1'}$); 177.31 (C_1). **MS** : (FAB$^-$, TG), m/z : 350 (A^{2-}+H)$^-$; 372 (A^{2-}+Na)$^-$; 767 (2A-2H+3Na)$^-$. **Anal** : $C_{14}H_{23}NO_6SNa_2$.

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19. Compound **10m**: Yield = 40%. mp =182-184°C.**IR** : KBr, ν cm^{-1}: 3474-3415 (N-H); 2927-2854 (C-H); 1639 (C=O amide); 1573 (C=O, CO_2^-). **^{1}H NMR** : (250 MHz, D_2O), δ ppm: 1.2 (m, 10H, $H_{4'}$ to $H_{8'}$); 1.5 (m, 2H, $H_{3'}$); 2.0 (q, 2H, $H_{9'}$, $J_{9'-8'}$=6.8Hz, $J_{9'-10'}$=6.8Hz); 2.1 (t, 2H, $H_{2'}$, $J_{2'-3'}$=8Hz); 2.4 (t, 6H, H_2, $J_{2'-3'}$= 6,8Hz); 3.7 (t, 12H, H_3, H_4, J_{2-3}=6.8Hz); 4.9 (m, 2H, $H_{11'}$); 5.8 (m, 1H, $H_{10'}$). **MS** : (FAB+, NOBA), m/z: 592 (M+Na)$^+$; 570 (M+H)$^+$; 548 (M-Na+2H)$^+$; 526 (M-2Na+3H)$^+$. **Anal** : $C_{24}H_{38}NO_{10}Na_3$.

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21. Compound **12m**: Yield = 80%. mp >260°C.$[\alpha]_D^{25}$ = -0.16° (H_2O). **IR** : KBr, ν cm^{-1}: 3396 (N-H); 2928-2855 (C-H); 1580 (C=O, CO_2^-). **^{1}H NMR** : (250 MHz, D_2O), δ ppm: 1.2 (m, 12H, $H_{4'}$ to $H_{8'}$ and H_3); 1.5 (m, 2H, $H_{3'}$); 2.0 (m, 6H, $H_{9'}$, H_{13}, H_8); 2.2 (m, 6H, $H_{2'}$, $H_{3'}$, H_9); 2.4 (m, 4H, H_4, H_{14}); 4.3 (t, 1H, H_7, J_{7-8}=7Hz); 4.4 (m, 2H, H_2, H_{12}); 4.8 (m, 2H, $H_{11'}$, $J_{11b'-10'}$=17Hz, $J_{11a'-10'}$ =10Hz); 6.0 (m, 1H, $H_{10'}$, $J_{10'-11b'}$=17Hz, $J_{10'-11a'}$=10Hz, $J_{10'-9'}$=6.5Hz).**^{13}C NMR** : (50.32, D_2O), δ ppm: 26.2 ($C_{3'}$); 29.1 to 32.8 ($C_{3'}$ to $C_{8'}$ and C_3, C_{13}); 34.0 ($C_{9'}$); 35.2 (C_4, C_{14}); 36.6 ($C_{2'}$); 54.0 (C_7); 56.3 (C_2, C_{12}); 115.0 ($C_{11'}$); 141.6 ($C_{10'}$); 173.7 to 178.2 ($C_{1'}$, C_6 and C_{10}); 179.5 to 183.0 (C_1, C_5, C_{11} and C_{15}). **MS** : (FAB$^-$, Glycerol), m/z : 636 (M-Na)$^-$; 658 (M-H)$^-$; 614 (M-2Na+H)$^-$. **Anal** : $C_{26}H_{37}N_3O_{11}Na_4$.

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