ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2021, *66*(4), 375–380
DOI: 10.33224/rrch.2021.66.4.09

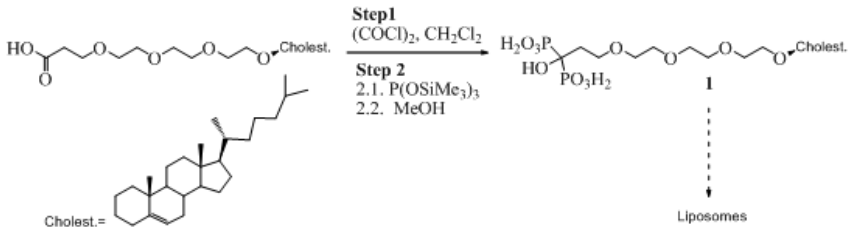
SYNTHESIS OF A HYDROXYBISPHOSPHONIC-DERIVED AMPHIPHILIC COMPOUND

Simona Cristina RIZEA,[a] Cătălina IONESCU,[b,*] Andreea PÎRVU,[a]
Véronique BARRAGAN-MONTERO[c] and Johny NEAMȚU[a]

[a] Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova, Str. Petru Rareş nr. 2-4 Craiova 200349, Dolj, Roumania
[b] University of Craiova, Faculty of Sciences, Department of Chemistry, 107i Calea Bucureşti, 200144 Craiova, Roumania
[c] LBN, University of Montpellier, Montpellier, France

Received January 19, 2021

Surface-modified liposomes with specific bone-targeting properties are promising alternatives to classical medication, thanks to their reduced systemic toxicity. In this paper, the synthesis of an amphiphilic compound containing a hydroxybisphosphonic acid (HBPA) group as hydrophilic part is reported. The compound has been prepared starting from its carboxylic acid correspondent, by conversion in its corresponding acid chloride, followed by its reaction with tris(trimethylsilyl) phosphite and then methanolysis of the silylated intermediate. A detailed description of the registered analyses that confirm product structure is also provided. Compounds with similar structure are known to enable the liposomes containing them to specifically bind to hydroxyapatite (HA) of bones.



INTRODUCTION

Systemic side-effects of classical medication may be reduced by local-targeted active principles that can be achieved, among other, by using surface-modified liposomes. Previously, liposomes decorated with hydroxybisphosphonic acid (HBPA) groups have been prepared and their bone-binding affinities have been reported.[1-3] The rationale behind these delivery systems is that HBP groups can bind to the inorganic phase of bone, hydroxyapatite (HA). Numerous research studies involving HA and its chemical properties, realized mainly in the purpose of obtaining implant applications, have been caried out.[4,5] HA is a good targeting candidate as it is present only in teeth and bones in healthy bodies. Previous studies show that a variety of functional groups can bind to HA.[6,7] The most potent among them are tetracycline and HBP group derivates.[8-10]

Liposomes are synthetic lipidic nanoparticles[11] that can entrap active principles inside them. They have received a huge amount of interest because they can be used in the passive targeting of medication to tumour sites[12] and they can be decorated with various ligands in order to actively target specific organs and cells in the body.[13] In order to achieve active targeting of liposomes to bones, synthetic amphiphilic lipids have been prepared and they have been used in liposomes

* Corresponding author. Tel/ Fax: +40 757 571 247; e-mail address:catalinagurgui@yahoo.co.uk (C. Ionescu)

preparation. These liposomes proved to possess HA affinity.

Among the previously reported amphiphilic compounds, the cholesteryl derivative possessing a HBPA group presented by Hengst *et al*.,[1] attracted our attention. Its synthesis and characterization had only briefly been presented earlier in a patent, by Greb *et al.*,[14] As part of a bigger project, we decided to prepare a compound similar to it, having the poly(oxyethylene) chain longer with a methylene group when compared with the compound reported by Greb *et al.* The structure of the target compound, namely (12-cholesteryloxy-4,7,10-trioxa)dodecan-1-hydroxy-1,1-bisphosphonic acid (Chol-TOD-HBPA) **1**, is represented in Figure 1.

RESULTS AND DISCUSSION

Chol-TOD-HBPA has been prepared using the carboxylic acid **2** reported earlier.[15] Two methods are reported in literature for the conversion of a carboxylic acid into its hydroxybisphosphonic analogue: (a) method A involves the usage of PCl_3 and H_3PO_4.[16] Because of the polyetheric structure of our compound, we avoided using this method; (b) method B, also used by Greb *et al*.,[14] involves two steps: conversion of the carboxylic acid in the corresponding acid chloride **3**,[17] followed by its reaction with tris(trimethylsilyl)phosphate and then methanolysis of the silylated intermediate (Scheme 1).[18]

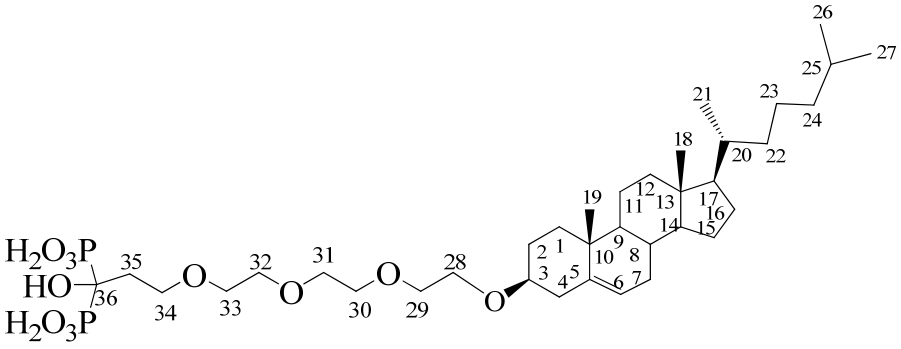
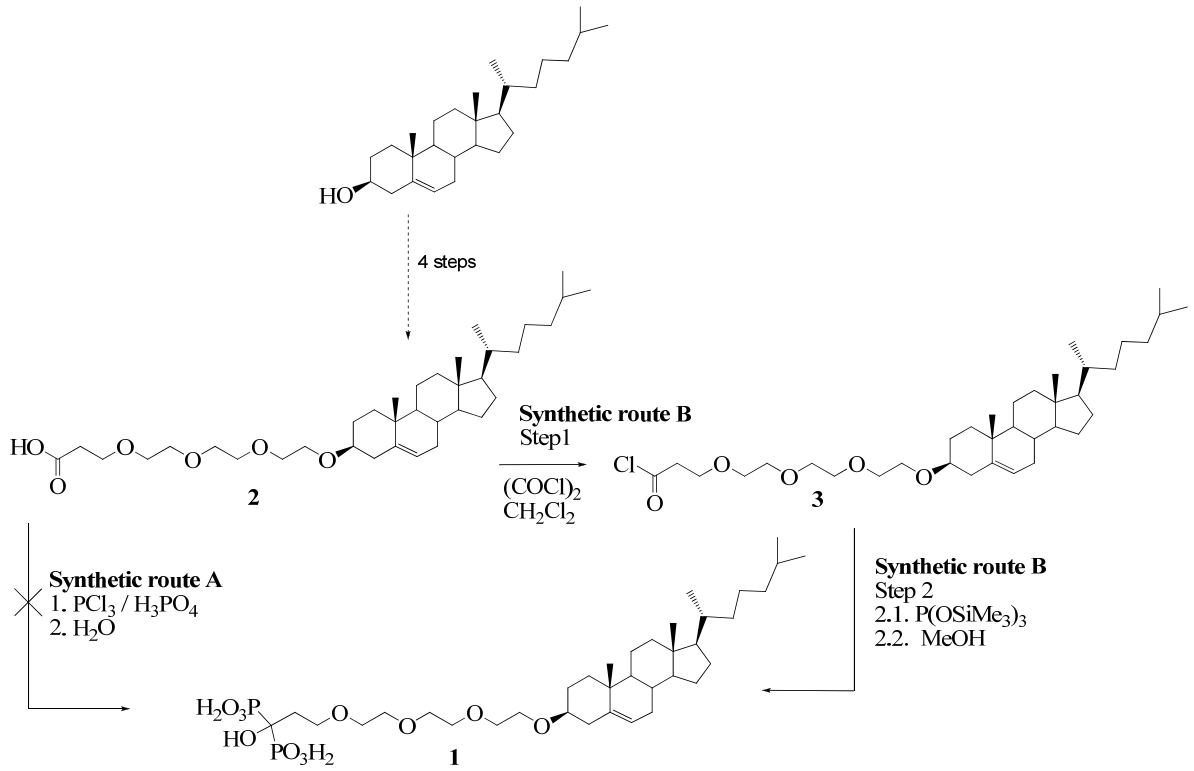


Fig. 1 – Structure and numbering system of Chol-TOD-HBPA.



Scheme 1 – Preparation of Chol-TOD-HBPA (route B - 52% yield from compound 2; for the conversion of cholesterol into the carboxylic acid 2, see ref. 15).

Chol-TOD-HBPA has been easily purified by repeated precipitations from diethyl ether. A detailed description of its ^{1}H, ^{31}P and ^{13}C NMR spectra is provided in the experimental part.

^{31}P NMR spectra prove the presence of phosphorous in the structure of our compound. In the ^{31}P CPD NMR spectrum (Figure 2), phosphorous signal was at 18.95 ppm, in agreement with literature data.[14,19] A ^{31}P-^{1}H coupling constant of 13.5Hz was be measured when the ^{31}P-^{1}H NMR spectrum was registered.

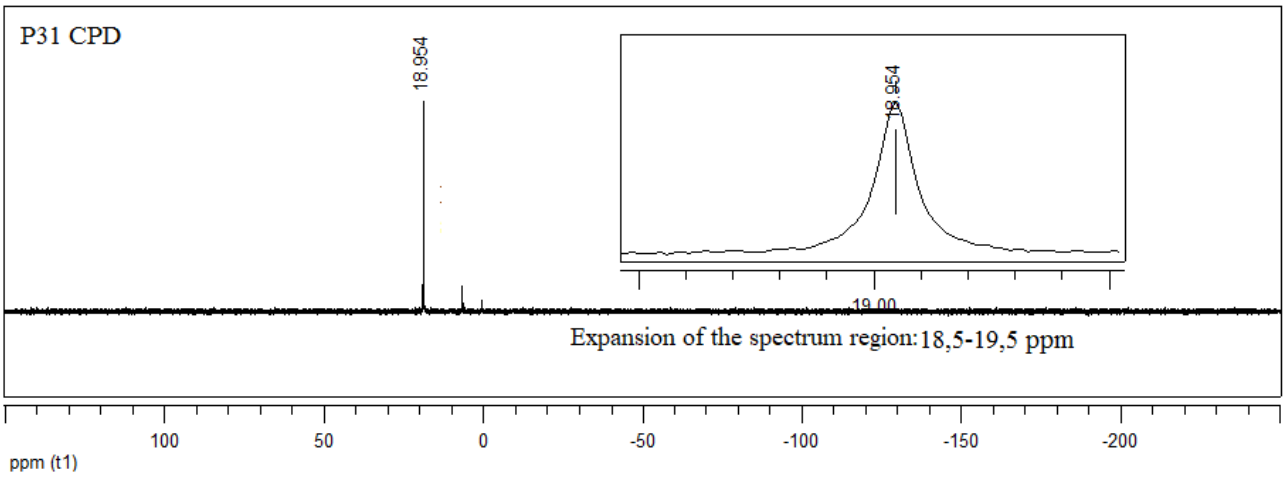


Fig. 2 – ^{31}P CPD spectrum of compound 1.

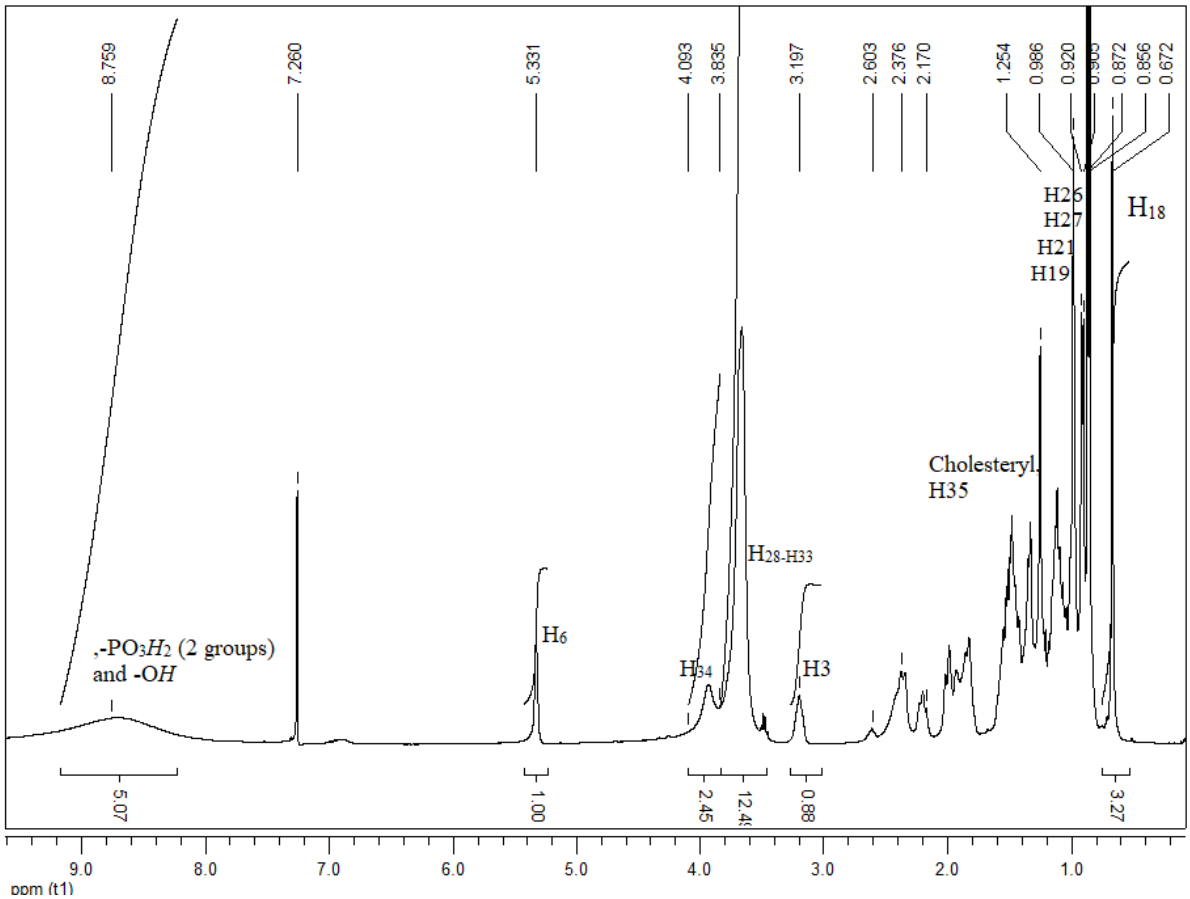


Fig. 3 – ^{1}H NMR spectrum of compound 1 (in CDCl3 as solvent).

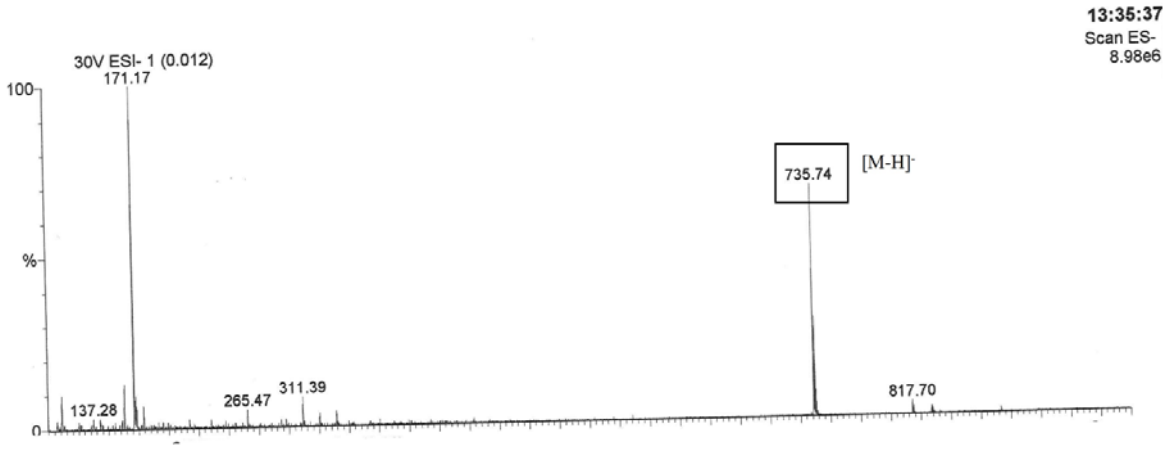


Fig. 4 – Mass Spectrometry analysis of compound 1.

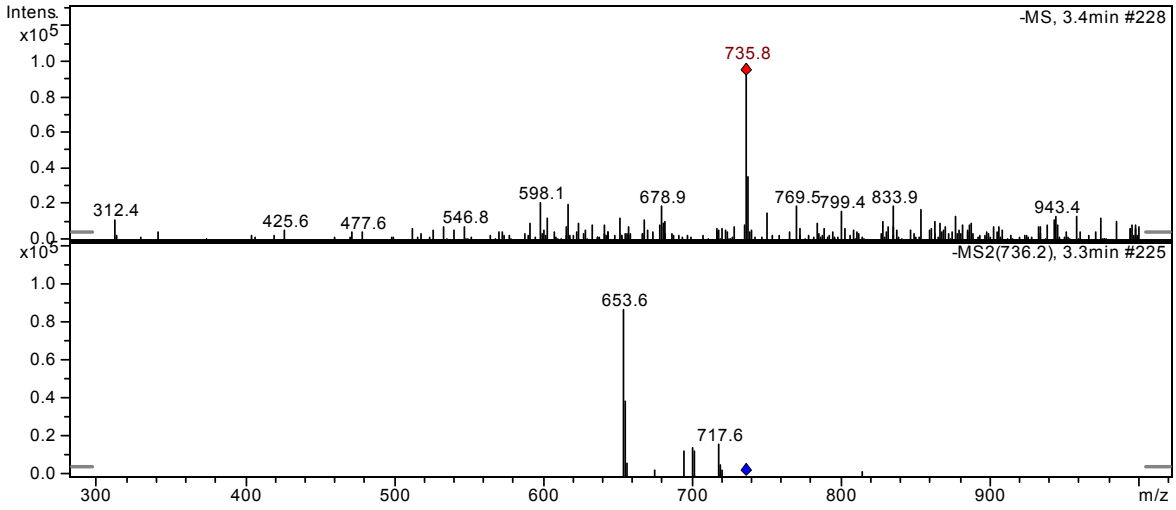


Fig. 5 – HPLC/MS analysis of compound 1.

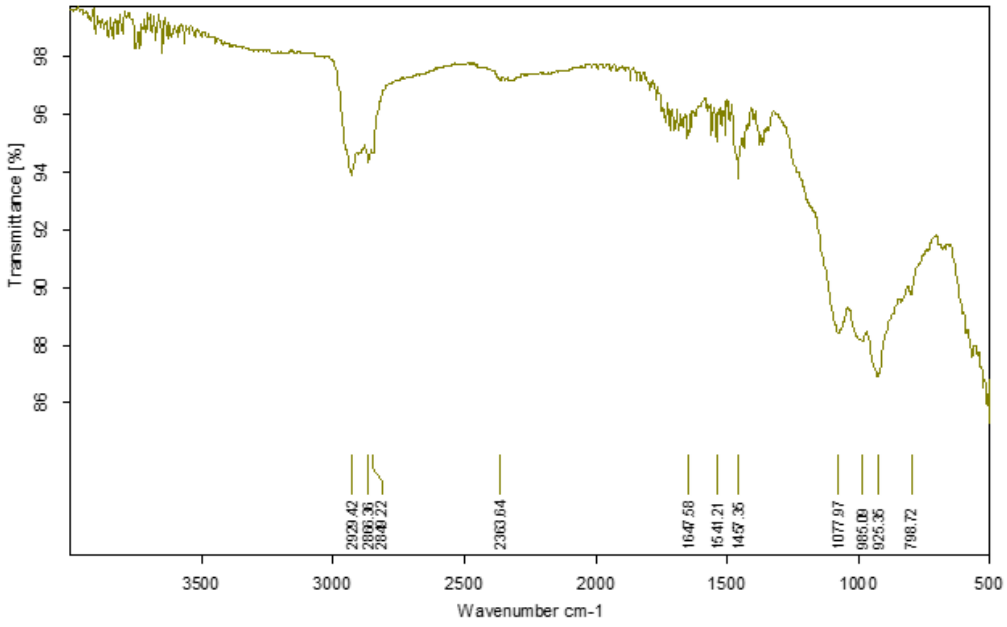


Fig. 6 – FT-IR Spectrum of compound 1.

In the ^{1}H NMR spectrum, all the peaks characteristic for cholesterol and the ethoxylated spacer are present, with specific NMR signals as previously detailed in similar compounds.[15,20] When compared with its precursor, compound 2, the acidic proton that was present at 5.3 ppm (overlapped with H_6) disappeared. The presence of acid protons can be seen in compound 1 at 8.76 ppm as a broad singlet (Figure 3). ^{13}C NMR spectrum also confirms the structure of our product.

The hydroxybisphosphonic acid was also analysed by mass spectrometry, either by direct MS analysis (Figure 4) or coupled with High Performance Liquid Chromatography (HPLC/MS – Figure 5). Due to its acidic structure, the compound ionizes in negative mode, being observed either as the molecular ion at 735.8 $[M-H]^-$(Figure 4, Figure 5), either as a fragment obtained by the loss of a phosphate group, at 653.6 $[M-H_3O_3P]^-$ (Figure 5).

The IR spectrum (Figure 6) contains the main bands characteristic for the compound. In the region 564-1652 cm^{-1}, there are found bands characteristic for hydrocarbons (cholesterol and -CH_2- groups from spacer). These bands are due to: C-H in- and out-of-plane blending; C-C stretching; CH_2 deformation, bending and waging; CH_2 and CH_3 bending, deformation vibrations and symmetric stretching, according to literature data describing FT-IR spectroscopic studies of cholesterol.[21] The intense bands observed between 925-1077 cm^{-1} are due to the two phosphonic acid groups: (a) υ (P-OH) at 925 cm^{-1};[22] (c) P=O group at 1077 cm^{-1}.[23] This band (1077 cm^{-1}) may also be assigned to aliphatic ethers (C-O). Cholesterol double bond is also visible in this region, more precisely at 985 cm^{-1}, due to =C-H bending;[21] The three intense bands near 2900 cm^{-1} (at 2849 cm^{-1}; 2866 cm^{-1}; 2929 cm^{-1}) are typical for cholesterol and are due to CH_2 symmetric stretching; CH_2 and CH_3 symmetric and asymmetric stretching.[21] An important observation is that the broad band typical for OH groups between 3000-3500 cm^{-1} isn't visible at its habitual position in this spectrum (for example, the O-H stretching appears at 3398 cm^{-1} in cholesterol). This fact is verified in the case of similar compounds, (for example in case of analogous 1-hydroxyethane-1,1'-diphosphonic acid, δOH of the alcoholic group has been assigned at 1350 cm^{-1}).[23] This can be explained by hydrogen bonding, as it is known that the -OH group in such compounds is involved in intra- and intermolecular hydrogen bonding, leading to cyclic dimmers.[24]

EXPERIMENTAL

General methods

Thin layer chromatography was carried out with pre-coated silica gel plates (E. Merck, Silica gel 60 F254) and the spots were detected by immersing the plates in 10% phosphomolybdic acid solution in ethanol followed by heating. The NMR spectra were recorded in $CDCl_3$ at 20°C on a Bruker DRX-400 spectrometer working at 400.13 MHz for ^{1}H, at 100.62 MHz for ^{13}C and at 161.97MHz for ^{31}P. The chemical shifts (δ) are reported in ppm and the coupling constants (*J*) are reported in Hz. NMR spectra were recorded with the standard BRUKER sequences. The numbering system used in the assignments of the NMR spectra is presented in Figure 1. The mass spectrum was registered on a Waters Q/Tof mass spectrometer and the HPLC/MS analysis was realized using an Agilent 1100 HPLC system coupled with an Agilent 1100 Ion Trap SL mass spectrometer. FT-IR spectrum was recorded on a Bruker ATR ZnSe spectrophotometer, within the range of 4000-550 cm^{-1}, at room temperature with a spectral resolution of 2 cm^{-1}.

Experimental procedure

Synthesis of (12-cholesteryloxy-4,7,10-trioxa)dodecan-1-hydroxy-1,1-bisphosphonic acid **1.** Oxalyl chloride (0.163 mL, 1.87 mmol) has been added dropwise under nitrogen to a solution of **2** (0.275g, 0.467 mmol) in 4 mL of distilled dichloromethane at 0°C. The mixture was allowed to warm at r.t. and was stirred for 3 hours. The volatile fractions have been removed under vacuum and the obtained acid chloride has been used directly in the next step. Tris(trimethylsilyl)phosphite (0.07 mL, 0.2 mol) has been added under nitrogen to the acid chloride previously prepared and the obtained mixture has been stirred at room temperature for 1 hour. The volatile fractions have been removed under vacuum. Methanol (0.5 mL) has been added to the residue and the obtained solution has been stirred for 1h30min. After solvent removal under vacuum, H_3PO_4 residues have been co-evaporated with Et_2O, yielding a light-brown gum. The product has been washed several times with Et_2O. ^{1}H NMR (400.13 MHz, CDCl3): 0.67 (s, 3H, H_{18}); 0.86 (d, 3H, $^{3}J_{H26-H25}$=6.6 Hz, H_{26}); 0.87 (d, 3H, $^{3}J_{H27-H25}$=6.6 Hz, H_{27}); 0.91 (d, 3H, $^{3}J_{H21-H20}$=6.3 Hz, H_{21}); 0.99 (s, 3H, H_{19}); 1.04-2.71(m, cholesteryl group protons and H_{35}); 3.20 (m, 1H, H_3); 3.54-3.78 (m, 12H, H_{28-H33}); 3.84-4.01 (m, 2H, H_{34}); 5.29-5.38 (m, 1H, H_6); 8.64 (broad s, 5H,-PO_3H_2 and -O*H*); ^{13}C NMR (100.62 MHz, CDCl3) δ(ppm): 11.9 (C18); 18,8 (C21); 19.4 (C19); 21.1 (C11); 22.6 and 22.8 (C26 and C27); 24.0(C23); 24.3 (C15); 28.0 (C25); 28.1 and 28.2 (C2 and C16); 29.7 (C35); 31.9 and 32.0 (C8 and C7); 35.7 (C20); 36.2 (C22); 36.8 (C10); 37.2 (C1); 38.8 (C4); 39.5 (C24); 39.8 (C12); 42.3 (C13); 50.1 (C9); 56.1 (C17); 56.8 (C14); 67.2, 70.1, 70.2, 70.3, 70.5, 73.0 (C28-C34); 79.8 (C3); 121.7 (C6);140.7 (C5); ^{31}P NMR (161.97MHz, CDCl3) δ(ppm): 18.96 ppm (t, J=13.5Hz); SM: (ESI$^-$/MeOH), m/z: 735.7 $[M-H]^-$; HPLC/MS (ESI$^-$), m/z: 735.8 $[M-H]^-$ and 653.6 $[M-H_3O_3P]^-$.

CONCLUSIONS

In this paper, the synthesis and the detailed characterization of an amphiphilic compound with a complex structure has been realized. The

compound 1 has been obtained in one step from his carboxylic acid analogue 2 with 52% yield. Taking into account the synthesis of compound 2, the hydroxybisphosphonic acid 1 can be obtained from cholesterol in 5 steps, with 15% yield. It can be used in the preparation of liposomes able to target bones, in order to achieve selective delivery of medication to bones.

REFERENCES

1. V. Hengst, C. Oussoren, T. Kissel and G. Storm, *Int. J. Pharm.*, **2007,** *331*, 224–227.
2. T. Anada, Y. Takeda, Y. Honda, K. Sakurai and O. Suzuki, *Bioorg. Med. Chem. Lett.* **2009,** *19,* 4148–4150.
3. B. W. Geho and J. R. Lau, Therapy By Vesicle Delivery To The Hydroxyapatite Of Bone, *US Patent 4942036,* **1990.**
4. G. Ciobanu, M. Harja and L. Rusu, *Rev. Roum. Chim.*, **2017**, *62*, 449–454.
5. M. N. Stefanuț, A. Căta, D. Roşu, M. Milea and I. M. C. Ienaşcu, *Rev. Roum. Chim*., **2017,** *62*, 481–487.
6. For a review, see: D. Wang, S. C. Miller, P. Kopecková and J. Kopecek, *Adv. Drug Deliv. Rev.,* **2005***, 57,* 1049–1076 and references therein.
7. For a review, see: T.Luhmann, O. Germershaus, J. Groll and L. Meinel, *J. Control. Release*, **2012,** *161*, 198–213.
8. H. Fleisch, *Endocr. Rev.,* **1988,** *19*, 80–100.
9. R. G. Russell and M. J. Rogers, *Bone,* **1999***, 25,* 97–106.
10. C. T. Leu, E. Luegmayr, L. P. Freedman, G. A. Rodan and A. A. Reszka, *Bone,* **2006,** *38,* 628–636.
11. A. D. Bangham and R. W. Horne, *J. Mol. Biol.,* **1964,** *8,* 660-668.
12. M. Yokoyama, *J. Artif. Organs*, **2005,** *8,* 77–84.
13. L. Juillerat-Jeanneret and F. Schmitt, *Med. Res. Rev.*, **2007**, *27,* 574–590.
14. W. Greb, O. Shyhskov, G. V. Roschenthaler and V. Hengst, *WO/2005/070952*, **2005**.
15. C. Ionescu, C. S. Rizea, V. Barragan-Montero and J. Neamțu, *Farmacia,* **2013**, *61*, 725–735.
16. M. Lecouvey and Y. Leroux, *Heteroatom Chem.,* **2000**, *11,* 556–561.
17. F. M. Menger and K. D. Gabrielson, *Angew. Chem. Int. Ed. Engl.,* **1995***, 34,* 2091–2106.
18. G. Erwann, E. Degache, J. Liquier and M. Lecouvey, *Eur. J. Org. Chem.,* **2004,** *14,* 2983–2987.
19. E. Guenin, E. Degache, J. Liquier and M. Lecouvey, *Eur. J. Org. Chem.* **2004**, *35,* 2983–2987.
20. S. C. Rizea, C. Ionescu, V. Barragan-Montero and J. Neamțu, *Curr. Health Sci. J.,* **2012,** *38,* 183–187.
21. U. Gupta, V. K. Singh, V. Kumar and Y. Khajuria, *Mater. Focus,* **2014**, *3*, 2011–2017.
22. L. C. Thomas and R. A. Chittenden, *Spectrochim. Acta*, **1964**, *20*, 489–502.
23. M. C. Zenob, C. V. Luengo, M. J. Avena and E. H. Rueda, *Spectrochim. Acta,* **2008**, Part A 70, 270–276.
24. D. Albouy, A. Brun, A. Munoz and G. Etemad-Moghadam, *J. Org. Chem.,* **1998**, *63*, 7223–7230.