Synthesis, Spectroscopic and Theoretical Studies of New Quaternary \(N,N\)-Dimethyl-3-phthalimidopropylammonium Conjugates of Sterols and Bile Acids

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Received: 12 February 2014; in revised form: 19 March 2014 / Accepted: 21 March 2014 / Published: 3 April 2014

Abstract: New quaternary 3-phthalimidopropylammonium conjugates of steroids were obtained by reaction of sterols (ergosterol, cholesterol, cholestanol) and bile acids (lithocholic, deoxycholic, cholic) with bromoacetic acid bromide to give sterol 3\(\beta\)-bromoacetates and bile acid 3\(\alpha\)-bromoacetates, respectively. These intermediates were subjected to nucleophilic substitution with \(N,N\)-dimethyl-3-phthalimidopropylamine to give the final quaternary ammonium salts. The structures of products were confirmed by spectral (\(^1\)H-NMR, \(^{13}\)C-NMR, and FT-IR) analysis, mass spectrometry (ESI-MS, MALDI) as well as PM5 semiempirical methods and B3LYP \textit{ab initio} methods. Estimation of the pharmacotherapeutic potential has been accomplished for synthesized compounds on the basis of Prediction of Activity Spectra for Substances (PASS).

Keywords: sterols; bile acids; quaternary ammonium salt; conjugates; \(N,N\)-dimethyl-3-phthalimidopropylamine; PASS; PM5 calculations; B3LYP \textit{ab initio} methods

1. Introduction

Steroids are a large class of organic compounds. They play a very important role in animals, plants and microorganisms. The best known steroid is certainly cholesterol. Cholesterol was isolated for the first time from gall stones nearly two centuries ago by Chevreul [1,2]. This sterol is an important
component of mammalian cell membranes; it is also present in significant concentrations in the brain and nervous tissue [3–6]. Cholesterol is the biosynthetic precursor of steroid hormones, bile acids, vitamin D and lipoproteins [7–9]. Like the functions of cholesterol in mammals, ergosterol is necessary to support the life of fungi. It serves two main purposes: a bulk membrane function and a sparking function. Ergosterol is a biological precursor to vitamin D$_2$ (ergocalciferol) [10–12].

All sterols are crystalline compounds with a secondary hydroxyl group in the position C(3) of the steroid skeleton, one or two double bonds and differently modified side chains. Rings A/B of the steroid skeleton may have trans geometry (the allo series) or cis (the normal series). Sterols have the hydroxy group on the C(3) position forming a number of β-sterols with respect to the average plane of the ring. By contrast bile acids have hydroxy groups on the C(3) position which prefer the α orientation [13–17].

Bile acids are major metabolites of cholesterol, being end products of its metabolism in the liver [18,19]. They are isolated from the bile of higher animals, where they are found as sodium salts of peptide conjugates with glycine and taurine. The most important are the primary bile acids, e.g., chenodeoxycholic acid and cholic acid, which are successively transformed into secondary bile acids such as ursodeoxycholic, deoxycholic and lithocholic acids [20–22]. Bile acids (e.g., lithocholic, deoxycholic and cholic) are very interesting because they display a large, rigid, and curved skeleton. Moreover, they possess chemically different polar hydroxy groups (3α, 3α,7α and 3α,7α,12α) and amphiphilic properties. Modifications of the functional groups of sterol molecules allow one to obtain systems with high pharmacological activity [23].

Quaternary alkylammonium salts play an important role in the living organisms and many functions of prokaryotic and eukaryotic cells have been shown to be alkylammonium salts dependent [24,25]. These compounds also exhibit excellent antimicrobial activity, and therefore they are used as antiseptics, bactericides and fungicides, as well as therapeutic agents. In general, quaternary alkylammonium salts with good antimicrobial activities contain one or two alkyl chains with lengths in the C$_8$–C$_{14}$ range. For the applications as softeners and hair conditioning agents hydrocarbon chain lengths between C$_{16}$ and C$_{18}$ are used [26–28]. Phthalimides, and N-substituted phthalimides are also an important class of compounds because of their biological activities as antimicrobial agents [29,30]. It has recently been shown that tetrachlorophthalimide derivatives are good α-glucosidase inhibitors [31]. The use of microbiocides of the same type for a long time may cause an increase of resistance of microorganisms to the chemicals used, which is a very serious and dangerous problem. Antimicrobial resistance of bacteria comprises a wide variety of biochemical mechanisms and processes that allow microorganisms to grow in the presence of microbiocides [32–34]. There are many ways to overcome the risk of an increasing resistance of microorganisms, however the best one is a periodically application of new microbiocides with modified structures [35,36]. Therefore, connections of sterols and bile acids with various amines or polyamines appears to be an unusually interesting potential approach to such new structures [37–40].

2. Results and Discussion

In the present work, the synthesis and physicochemical properties of some new quaternary N,N-dimethyl-3-phthalimidopropylammonium conjugates of sterols (ergosteryl 3β-bromoacetate (4),
cholesteryl 3β-bromoacetate (5), dihydrocholesteryl 3β-bromoacetate (6)) and derivatives of bile acids (methyl litocholate 3α-bromoacetate (13), methyl deoxycholate 3α-bromoacetate (14) and methyl cholate 3α-bromoacetate (15)) with N,N-dimethyl-3-phthalimidopropylamine in acetonitrile are investigated.

New quaternary 3-phthalimidopropylammonium conjugates of steroids were obtained by reaction of ergosterol (1), cholesterol (2), cholestanol (5α-cholestan-3β-ol, 3), and bile acids 10–12 with bromoacetic acid bromide to give 4–6 and 13–15. The 3β-bromoacetates of sterols and 3α-bromoacetates of bile acids, as well as N,N-dimethyl-3-phthalimidopropylamine were prepared according to the literature procedures [41,42]. The structure of products was confirmed by 1H-NMR, 13C-NMR, and FT-IR analysis, as well as ESI-MS and MALDI. The syntheses of conjugates 7–9 and 16–18 are shown in Scheme 1.

**Scheme 1.** Synthesis of quaternary phthalimidopropylammonium conjugates of sterols 7–9 (a) and bile acids 16–18 (b).

Potential pharmacological activities of the synthesized compounds have been determined on the basis of computer-aided drug discovery approach with *in silico* Prediction of Activity Spectra for Substances (PASSs) program. It is based on a robust analysis of the structure-activity relationship in a heterogeneous training set currently including about 60,000 biologically active compounds from...
different chemical series with about 4,500 types of biological activities. Since only the structural formula of the chemical compound is necessary to obtain a PASS prediction, this approach can be used at the earliest stages of investigation. There are many examples of the successful use of the PASS approach leading to new pharmacological agents [43–47]. The PASS software is useful for the study of biological activity of secondary metabolites. We have selected the types of activities that were predicted for a potential compound with the highest probability (focal activities). If predicted activity is higher than 0.7 (PA > 0.7), the substance is very likely to exhibit the activity in experiment and the chance of the substance being the analogue of a known pharmaceutical agent is also high. If predicted activity is between 0.5 and 0.7 (0.5 < PA < 0.7), the substance is unlikely to exhibit the activity in experiment and the similarity to known pharmaceutical substance is very limited.

The structures of all synthesized compounds were determined from their 1H- and 13C-NMR, FT-IR and ESI-MS spectra. Moreover, PM5 calculations and B3LYP ab initio methods were performed for all compounds. Additionally, analyses of the biological prediction activity spectra for the new esters prepared herein are good examples of in silico studies of chemical compounds. We also selected the types of activity that were predicted for a potential compound with the highest probability (focal activities) (Table 1). According to these data the most frequently predicted types of biological activity are: inhibitors glyceryl-ether monoxygenase, acylcarcinine hydrolase, alkylacetylglycerophosphatase, plasmanylethanolamine desaturase, \(N\)-(acyl)ethanolamine deacylase and protein-disulfide reductase.

### Table 1. “Probability to be Active” (PA) values for the predicted biological activity of 7–9 and 16–18.

| Focal Predicted Activity (PA > 0.80) | Compounds |
|-------------------------------------|-----------|
| Glyceryl-ether mono-oxygenase inhibitor | 0.87 0.91 0.93 0.94 0.95 |
| Acylcarcinine hydrolase inhibitor | - - 0.81 0.83 0.91 0.94 |
| Alkylacetylglycerophosphatase inhibitor | - - - 0.82 0.90 0.86 |
| Plasmanylethanolamine desaturase inhibitor | - - - - 0.71 0.78 |
| CYP3A4 substrate | - - - - 0.70 0.73 |
| \(N\)-(acyl)ethanolamine-deacylase inhibitor | - - - - 0.73 0.76 |
| Protein-disulfide reductase inhibitor | - - 0.72 - - |
| Alkenylglycerophosphocholine hydrolase inhibitor | - - - - 0.80 - |
| Oxidoreductase inhibitor | 0.87 0.77 - - - - |
| Alcohol \(O\)-acetyl-transferase inhibitor | 0.88 - - - - - |
| DELTA14-sterol reductase inhibitor | - 0.73 - - - - |
| Alkylacetylglycerophosphatase inhibitor | - - 0.84 - - - |
| Antieczematic | - - - 0.72 - - |
| Glucan endo-1,3-\(\beta\)-D-glucosidase inhibitor | - - - - - 0.73 - |
| \(\alpha\)-lactaldehyde dehydrogenase inhibitor | - - - - 0.74 - |
| \(\beta\)-lactaldehyde dehydrogenase inhibitor | - - - - - - |

The \(^1\)H-NMR spectra of compounds 7–9 show characteristic multiplets in the range 4.96–4.48 ppm assigned to the C3\(\alpha\)–H protons of the sterol skeleton. Similarly, compounds 16–18 exhibit multiplets in the range 4.92–4.55 ppm which are assigned to the C3\(\beta\)–H protons of the bile acid skeleton (Figure 1). A proton singlet at 0.83 ppm for 7 and singlets in the range of 0.68–0.64 ppm for other conjugates are
assigned to CH₃–18. The other singlets ranging from 1.04–0.79 ppm, 0.91 ppm and 0.89 ppm are assigned to CH₃–19 for 7–9, 17 and 18, respectively. In the case of compound 16 signal of the CH₃–19 group and CH₃–21 group form the multiplet. The characteristic doublets of CH₃–21, with the exception of conjugate 16, are observed at 0.98–0.90 ppm and are assigned to conjugates 7–10, 17 and 18.

**Figure 1.** ¹H-NMR spectra in the region (8.0–3.5 ppm) of the most characteristic signals of conjugates 8 (a) and 18 (b).

The characteristic doublets of the ergosterol-substituted derivatives are observed at 1.01 ppm for CH₃–28 group and 0.84 ppm for CH₃–27 and CH₃–26 groups, respectively. The ¹H-NMR spectra of conjugates 8 and 9 show doublets at 0.87 and doublet of doublets at 0.86 ppm of the C(26) and C(27)
methyl group. In the $^1$H-NMR spectra of sterol conjugates 7–9 and bile acid conjugates 16–18 a signal of protons of the COCH$_2$N$^+$ group in the range 5.35–4.55 ppm is observed. The peaks of six methyl protons of the N$^+$CH$_3$$_2$ and two methylene protons of the N$^+$CH$_2$ appear as singlets and multiplets or broad singlets in the range 3.69–3.61 ppm and 4.96–3.87 ppm, respectively. Two methylene protons of attached to the phthalimide ring–N–CH$_2$ group are seen as a broad singlets in the 3.83–3.82 ppm range.

The $^{13}$C-NMR spectra of conjugates 7–9 and 16–18 in CDCl$_3$ show characteristic signals at 15.80 ppm (7), 12.66–11.79 ppm (8, 9, 16–18), and 19.19–17.25 ppm (7, 8, 16–18), 12.18 ppm (9) which are assigned to CH$_3$–18 and CH$_3$–19, respectively. Carbon atoms of the CH$_3$–21 group give signals in the ranges 22.55–18.621 ppm. Characteristic shifts of methyl groups present in the sterol side chain (CH$_3$–26 and CH$_3$–27) are positioned in the range 22.76–19.94 ppm and 22.52–19.61 ppm, respectively. The carbon atoms of the CO$_2$CH$_3$ unit resonated in the range of 174.77–174.72 ppm and 52.49–51.45 ppm, assigned to CO$_2$ and CH$_3$, respectively.

Two important signals for C(1')=O and C(3)–O lie at 164.25–163.87 ppm and 77.97–74.87 ppm, respectively. The spectra of all conjugates show two diagnostic signals associated with CH$_2$ atoms in N$^+$–C(2')H$_2$–CO and N$^+$–C(4')H$_2$ groups. The carbon atoms of the first group are observed at 62.25–60.67 ppm and carbon atoms of the second group lie at 61.50–61.05 ppm for 8, 9 and 16–18 and 57.03 ppm for 7, respectively. The carbon atoms of N$^+$CH$_3$$_2$ group appear in the range of 53.16–51.42 ppm. The carbon atoms of (C(7')=O)$_2$N–C(6')H$_2$ group resonate in the range of 168.34–168.09 ppm and 62.25–60.67 ppm assigned to C(7')=O and C(6')H$_2$, respectively.

The proton chemical shift assignments of N,N-dimethyl-(3β-acetate-cholest-5-ene)-3-phthalimidopropylammonium bromide (8) (Table 2) are based on 2D COSY experiments, in which the proton-proton connectivity is observed through the off-diagonal peaks in the counter plot.

**Table 2.** Chemical shifts (δ, ppm) in D$_2$O and calculating GIAO nuclear magnetic shielding tensors (σ$_{\text{calc}}$) for N,N-dimethyl-(3β-acetate-cholest-5-ene)-3-phthalimidopropylammonium bromide (8). The predicted GIAO chemical shifts were computed from the linear equation $\delta_{\text{exp}} = a + b \sigma_{\text{calc}}$ with a and b determined from the fit the experimental data.

|        | $\delta_{\text{exp}}$ | $\delta_{\text{calc}}$ | σ$_{\text{calc}}$ | $\delta_{\text{exp}}$ | $\delta_{\text{calc}}$ | σ$_{\text{calc}}$ |
|--------|------------------------|-----------------------|-----------------|------------------------|-----------------------|-----------------|
| C1'    | 163.90                 | 160.96                | 98.55           | H2'                    | 4.80                  | 5.94            | 26.538         |
| C2'    | 62.05                  | 66.51                 | 168.03          | H3'                    | 3.66                  | 3.58            | 28.992         |
| C3'    | 52.38                  | 44.94                 | 183.90          | H4'                    | 3.95                  | 4.94            | 27.566         |
| C4'    | 61.05                  | 58.56                 | 173.88          | H5'                    | -                     | -               | 30.748         |
| C5'    | 23.76                  | 20.94                 | 201.56          | H6'                    | 3.83                  | 4.16            | 28.389         |
| C6'    | 37.63                  | 44.15                 | 184.48          | H9'                    | 7.83                  | 7.08            | 25.346         |
| C7'    | 168.12                 | 154.39                | 105.71          | H10'                   | 7.77                  | 7.00            | 25.430         |
| C8'    | 131.72                 | 134.68                | 117.88          | H3                     | 3.66                  | 3.58            | 28.987         |
| C9'    | 123.49                 | 128.37                | 122.52          | H6                     | 5.35                  | 5.04            | 27.472         |
| C10'   | 134.22                 | 137.31                | 115.95          | H18                    | 0.68                  | 0.80            | 32.113         |
| C3     | 76.57                  | 78.28                 | 159.37          | H19                    | 0.99                  | 0.82            | 31.924         |
| C5     | 138.76                 | 138.26                | 115.25          | H21                    | 0.92                  | 0.58            | 32.112         |
| C6     | 123.33                 | 127.64                | 123.06          | H26,27                 | 0.87                  | 0.76            | 31.924         |
| C18    | 11.79                  | 9.93                  | 209.66          | -                      | -                     | -               | -              |
| C19    | 19.19                  | 35.13                 | 191.12          | -                      | -                     | -               | -              |
The relations between the experimental $^1$H- and $^{13}$C-NMR chemical shifts ($\delta_{\text{exp}}$) and the Gauge-Independent Atomic Orbitals (GIAO) isotropic magnetic shielding tensors ($\sigma_{\text{calc}}$) for 8 are shown in Figure 2. Both correlations are linear, described by the equation: $\delta_{\text{exp}} = a + b\sigma_{\text{calc}}$. The $a$ and $b$ parameters are given in Table 2. The very good correlation coefficients ($r^2 = 0.9379$) for $^1$H and ($r^2 = 0.9984$) for $^{13}$C correlations of $N,N$-dimethyl-(3β-acetate-cholest-5-ene)-3-phthalimidopropyl-ammonium bromide confirm the optimized geometry of 8.

**Figure. 2.** Experimental chemical shifts ($\delta_{\text{exp}}$) for $N,N$-dimethyl-(3β-acetate-cholest-5-ene)-3-phthalimidopropylammonium bromide (8) vs. isotropic magnetic shielding constants ($\sigma_{\text{calc}}$) from the GIAO/B3LYP/6-31G(d,p) calculations; (a) carbons-13 and (b) protons.

|        | $\delta_{\text{exp}}$ | $\delta_{\text{calc}}$ | $\sigma_{\text{calc}}$ | $\delta_{\text{exp}}$ | $\delta_{\text{calc}}$ | $\sigma_{\text{calc}}$ |
|--------|------------------------|-------------------------|-------------------------|------------------------|-------------------------|-------------------------|
| C21    | 18.65                  | 25.79                   | 197.99                  | -                      | -                      | -                       |
| C26    | 22.76                  | 14.29                   | 206.45                  | -                      | -                      | -                       |
| C27    | 22.50                  | 11.67                   | 208.38                  | -                      | -                      | -                       |
| a      | -                      | -                       | 294.9163                | -                      | -                      | 31.4274                |
| b      | -                      | -                       | -1.3593                 | -                      | -                      | -0.9606                |
| $r^2$  | -                      | -                       | 0.9831                  | -                      | -                      | 0.9443                 |

*a* intercept; *b* slope; *c* correlation coefficient.

The correlation between the experimental chemical shifts and calculated isotropic screening constants are better for carbon atoms than for protons. The protons are located on the periphery of the molecule and thus they are exposed to stronger interactions with solvent than carbon atoms, which are more hidden inside of structure. The differences between the exact values of the calculated and experimental shifts for protons are probably due to the fact that the shifts are calculated for single molecules in gas phase, whereas experimental values are due to the condensed phase. For this reason the agreement between the experimental and the calculated data for protons are worse than for carbons.

PM5 semiempirical calculations were performed using the WinMopac 2003 program and B3LYP calculation are performed using the GAUSSIAN 03 program package with the 6-31G(d,p) basis set. The final heat of formation (HOF) and energies for the sterols 1–3, bile acids 10–12 as well as their
conjugates 7–9 and 16–18 is presented in Table 3. Representative conjugates of sterol 7 and bile acid 18 are shown in Figure 3.

**Table 3.** Heat of formation (HOF) [kcal/mol] and energy [a.u.] of sterols (1–3), bile acids (10–12) and their conjugates (7–9, 16–18).

| Compound | Heat of Formation [kcal/mol] | ΔHOF [kcal/mol] | Energy [a.u.] | ΔEnergy [a.u.] |
|----------|-----------------------------|----------------|--------------|----------------|
| 1        | −97.1208                    | −1113.175477   | −1118.205536 | −1119.450757   |
| 2        | −140.1058                   | −1111.48056    | −1112.057617 | −1113.205757   |
| 3        | −162.7945                   | −1119.450757   | −1121.057617 | −1122.205757   |
| 7        | −177.1668                   | −80.0460       | −4569.562486 | −3456.387009   |
| 8        | −220.6382                   | −80.5324       | −4570.807880 | −3452.602344   |
| 9        | −243.3231                   | −80.5286       | −4605.917678 | −3486.46921    |
| 10       | −236.1585                   | −1204.997432   | −1206.507617 | −1208.015757   |
| 11       | −318.1685                   | −1337.190617   | −1340.707617 | −1342.215757   |
| 12       | −306.6215                   | −70.4630       | −4640.248926 | −3435.251494   |
| 16       | −388.9986                   | −70.5370       | −4714.391510 | −3451.351494   |
| 17       | −306.6215                   | −70.4630       | −4640.248926 | −3435.251494   |
| 18       | −388.9986                   | −70.5370       | −4714.391510 | −3451.351494   |

ΔHOF = HOF$_{conjugates}$ (7–9) − HOF$_{sterols}$ (1–3); ΔHOF = HOF$_{conjugates}$ (16–18) − HOF$_{bile acids}$ (10–12); ΔEnergy = Energy$_{conjugates}$ (7–9) − Energy$_{sterols}$ (1–3); ΔEnergy = Energy$_{conjugates}$ (16–18) − Energy$_{bile acids}$ (10–12).

**Figure 3.** Molecular models of representative compounds 7 and 18 calculated by the PM5 method.

The lowest values of HOF for sterols are observed for cholestanol 3 and its conjugate 9, where there are no double bonds which stabilize the molecule and hinder its reactivity, in contrast to conjugates of ergosterol 7 and cholesterol 8 where the double bonds increase the reactivity of the molecule, thereby increasing values of HOF. The HOF relationship for methyl esters of bile acids 10–12 and their corresponding conjugates 16–18 can be explained in a similar manner. In this case, the number of hydroxyl groups in the steroid skeleton lowers the value of the determinant of HOF. This spatial arrangement of bile acids can facilitate the formation of stable host-guest complexes. These complexes may be stabilized by hydrogen bonding or electrostatic interactions that arise from the number of hydroxyl groups in the bile acid molecule. Similar correlations have been observed using the B3LYP method.

The spatial arrangement and interaction of the conjugates 7 and 18 are shown in Figure 4. The final heat of formation is −1249.429 kcal/mol for 7 and −1358.893 kcal/mol for 18 and the distances between the quaternary nitrogen and the anion bromide are 4.34 Å and 4.33 Å, respectively.
Compensation charges occurs only through intermolecular electrostatic interaction. This is a very good confirmation of the conclusion that interactions reduce HOF. The dipole moments and selected geometry parameters were calculated at the PM5 and B3LYP/6-31G(d,p) level of theory are presented in Table 4.

**Figure 4.** Molecular models of conjugates 7 and 18 calculated by the PM5 method.

### Table 4. Calculated structural parameters (B3LYP/6-31G(d,p), **PM5**) for sterol conjugates 7–9 as well as bile acid conjugates 16–18.

| Parameters       | 7     | 8     | 9     | 16    | 17    | 18    |
|------------------|-------|-------|-------|-------|-------|-------|
| Dipole moments (Debye) | 9.0817 | 8.9725 | 8.8533 | 8.9578 | 8.4897 | 7.7646 |
| Bond lengths [Å]  |       |       |       |       |       |       |
| N(1)-C(7')       | 1.451 | 1.451 | 1.451 | 1.459 | 1.459 | 1.459 |
|                  | 1.428 | 1.427 | 1.428 | 1.431 | 1.432 | 1.430 |
| N(1)-C(3')       | 1.495 | 1.494 | 1.494 | 1.492 | 1.492 | 1.491 |
|                  | 1.471 | 1.471 | 1.471 | 1.469 | 1.469 | 1.469 |
| C(1')-O(3)       | 1.261 | 1.260 | 1.260 | 1.262 | 1.263 | 1.262 |
|                  | 1.219 | 1.220 | 1.219 | 1.220 | 1.220 | 1.220 |
| N(2)-C(4')       | 1.573 | 1.570 | 1.573 | 1.572 | 1.572 | 1.571 |
|                  | 1.546 | 1.546 | 1.546 | 1.543 | 1.544 | 1.543 |
| N(2)-C(2')       | 1.570 | 1.574 | 1.570 | 1.571 | 1.572 | 1.571 |
|                  | 1.527 | 1.527 | 1.527 | 1.525 | 1.525 | 1.525 |
| C(7')-O(1)       | 1.210 | 1.210 | 1.210 | 1.208 | 1.208 | 1.208 |
Table 4. Cont.

| Parameters                        | 7     | 8     | 9     | 16    | 17    | 18    |
|-----------------------------------|-------|-------|-------|-------|-------|-------|
| Bond angles [°]                   |       |       |       |       |       |       |
| C(6')-C(5')-C(4')                | 111.5 | 112.0 | 112.2 | 108.8 | 109.1 | 108.8 |
| N(1)-C(6')-C(5')                 | 110.8 | 110.7 | 110.9 | 108.0 | 108.1 | 107.7 |
| C(4')-N(2)-C(2')                 | 110.4 | 110.5 | 110.4 | 110.4 | 110.5 | 110.8 |
| N(2)-C(2')-C(1')                 | 105.3 | 105.4 | 105.5 | 105.6 | 105.8 | 105.6 |
| C(2')-C(1')-O(3)                 | 105.6 | 105.5 | 105.6 | 105.6 | 105.6 | 105.4 |
| Torsion angles [°]                |       |       |       |       |       |       |
| C(7')-N(1)-C(6')-C(5')           | −58.1 | −60.0 | −60.8 | 58.3  | 57.3  | 58.5  |
| C(8')-C(7')-N(1)-C(6')           | −82.2 | −80.3 | −79.4 | 86.8  | 85.5  | 77.6  |
| C(5')-C(4')-N(2)-C(2')           | −177.9| −178.5| −178.8| 178.2 | 178.9 | 178.1 |
| C(3')-N(2)-C(2')-C(1')           | −170.0| 171.4 | 172.1 | 178.3 | 178.0 | 178.2 |
| C(6')-C(5')-C(4')-N(2)           | 53.6  | 56.3  | 56.1  | 56.3  | 56.4  | 56.5  |
| Hydrogen bonds and short contacts lengths distances [Å] |       |       |       |       |       |       |
| C(4')-H···Br                      | 3.149 | 3.147 | 3.147 | 3.157 | 3.159 | 3.159 |
| C(2')-H···Br                      | 3.283 | 3.288 | 3.288 | 3.434 | 3.291 | 3.303 |
| N(2)···Br                         | 3.077 | 3.076 | 3.075 | 3.067 | 3.064 | 3.068 |
| C(4')-H···Br                      | 3.075 | 3.080 | 3.075 | 3.051 | 3.045 | 3.303 |
| C(2')-H···Br                      | 3.370 | 3.372 | 3.373 | 3.369 | 3.368 | 3.370 |
| Angles [deg]                      |       |       |       |       |       |       |
| C(4')-H···Br                      | 3.459 | 3.464 | 3.463 | 3.458 | 3.463 | 3.459 |
| C(2')-H···Br                      | 153.0 | 153.6 | 153.9 | 153.5 | 153.3 | 153.4 |
| Hydrogen bonds and short contacts lengths distances [Å] |       |       |       |       |       |       |
| C(4')-H···Br                      | 144.1 | 143.8 | 143.9 | 139.1 | 138.2 | 139.4 |
| C(2')-H···Br                      | 157.7 | 158.0 | 158.0 | 158.4 | 158.5 | 158.4 |
| C(6')-C(5')-C(4')-N(2)           | 149.6 | 150.5 | 150.1 | 152.0 | 153.1 | 152.5 |

The calculated bond lengths and bond angles for 7–9 and 16–18 optimized by the PM5 and B3LYP methods are quite similar, however the bond lengths N(2)-C(2') and N(2)-C(4') are different. Also the torsion angles calculated by the PM5 and B3LYP methods are slightly different, especially the C(5')-C(4')-N(2)-C(2') angle for fatty acid conjugates 16–18. This shows a crucial role of electrostatic interaction between oppositely charged groups in the structure of the investigated compounds (Table 4). Hydrogen bonds and short contact lengths distances are shorter for compounds calculated by B3LYP method. These data prove that in the gas phase the type of quantum chemical methods used play an
important role in the molecular structure of ionic compounds. The solid-state IR spectra of sterols and bile acids conjugates are shown in Figures 5 and 6, respectively.

**Figure 5.** FT-IR spectra of sterol conjugates 7 (red) and 8 (blue) in the 3,100–400 cm$^{-1}$ region.

![Figure 5](image1.png)

**Figure 6.** FT-IR spectra of bile acid conjugates 16 (blue), 17 (red), 18 (black) in the 3100–400 cm$^{-1}$ region

![Figure 6](image2.png)

The FT-IR spectra of conjugates show characteristic bands at 1,775–1,772 cm$^{-1}$ which are due to the asymmetric carbonyl group $\nu_{as}C=O$ stretching vibrations in a phthalimide moiety (Figures 5–8) [48,49]. The symmetric $\nu_{s}C=O$ stretching vibration appears in the FT-IR spectrum as an intense and broad nonsymmetrical band at 1,716–1,699 cm$^{-1}$ suggesting the small nonequivalence of carbonyl groups in the phthalimide moiety. Moreover strong characteristic bands in the region 1,251–1,240 cm$^{-1}$ are present, which are assigned to the $\nu(C-O)$. 
**Figure 7.** FT-IR spectra of bile acid conjugates in the carbonyl group region 16 (blue), 17 (red), 18 (black).

**Figure 8.** Spectrum of \(N,N\)-dimethyl-(3β-acetate-5β-cholestan)-3-phthalimidopropyl-ammonium bromide (9); (a) FT-IR (b) calculated scaled FT-IR spectrum.
The FT-IR spectra of bile acid conjugates (Figure 7, blue line) shows characteristic vibration bands of $\nu_{\text{as}}\text{C}=\text{O}$ and $\nu_{\text{s}}\text{C}=\text{O}$ in a phthalimide moiety at 1,773 cm$^{-1}$ and at 1,712 and 1,699 cm$^{-1}$, respectively. In this case the $\nu_{\text{as}}\text{C}=\text{O}$ and $\nu_{\text{s}}\text{C}=\text{O}$ bands are more nonsymmetrical in comparison to the carbonyl bands of 17 and 18. The nonequivalence of $\nu_{\text{as}}\text{C}=\text{O}$ in the FT-IR spectrum is observed for $N,N$-bis-(phthalimidopropyl)-$N$-propylamine [50]. In contrast to the above examples, in $N,N$-dimethyl-3-phthalimidopropylammonium hydrochloride monohydrate and $N$-$n$-butyltetrachlorophthalimide no split of the carbonyl bands in FT-IR spectra were observed, in spite of the different interactions of each carbonyl group in the supramolecular structure [51,52]. The $\nu$(COO) stretching vibrations of carboxy groups are observed at 1,747–1,736 cm$^{-1}$ (Figure 7).

The room-temperature solid-state FT-IR and the calculated spectrum of 9 are shown in Figure 8. The band frequencies, relative intensities and their assignments in the 4,000–400 cm$^{-1}$ range are listed in Table 5. For convenience of comparison, the band intensities for the calculated spectrum are scaled.

**Table 5.** Observed and calculated B3LYP/6-31G(d,p) vibrational frequencies and infrared intensities for $N,N$-dimethyl-(3$\beta$-acetate-5$\beta$-cholestan)-3-phthalimidopropylammonium bromide (9).

| IR   | IR$_{\text{calc.}}$ | IR$_{\text{calc,scaled}}$ | INT  | Proposed Assignment |
|------|---------------------|--------------------------|------|---------------------|
| 3,462w | 3,477               | 3,344                    | 3.34 | $\nu\text{CH}$      |
| 3,459w | 3,467               | 3,333                    | 17.8 | $\nu\text{CH}$      |
| 3,380w | 3,464               | 3,330                    | 5.26 | $\nu\text{CH}$      |
| 3,304w | 3,419               | 3,286                    | 18.2 | $\nu\text{CH}_2$    |
| 3,243w | 3,391               | 3,259                    | 381  | $\nu\text{CH}_2$    |
| 3,060w | 3,252               | 3,125                    | 10.9 | $\nu\text{CH}_2$    |
| 3,048s | 3,225               | 3,098                    | 6.74 | $\nu\text{CH}_2$    |
| 2,952s | 3,189               | 3,063                    | 8.81 | $\nu\text{CH}_3$    |
| 2,933s | 3,167               | 3,042                    | 16.7 | $\nu\text{CH}_3$    |
| 2,930s | 3,111               | 2,988                    | 485  | $\nu\text{CH}_3$    |
| 2,867s | 2,988               | 2,869                    | 515  | $\nu\text{CH}_2$    |
| 2,851s | 2,966               | 2,847                    | 994  | $\nu\text{CH}_2$    |
| 2,719w | -                   | -                        | -    | $\nu\text{CH}--\text{Br}$ |
| 2,649w | 2,531               | 2,486                    | 1982 | $\nu\text{CH}--\text{Br}$ |
| 2,534w | -                   | -                        | -    | $\nu\text{CH}--\text{Br}$ |
| 1,770m | 1,845               | 1,761                    | 16.9 | $\nu_{\text{as}}\text{CO}$ |
| 1,742s | 1,811               | 1,728                    | 55.6 | $\nu\text{COO}$     |
| 1,712s | 1,800               | 1,718                    | 190  | $\nu\text{CO}$      |
| 1,635vw | 1,728              | 1,650                    | 2.38 | $\nu\text{CC}$     |
| 1,614w | 1,706               | 1,627                    | 2.13 | $\nu\text{CC}$     |
| -     | 1,679               | 1,600                    | 4.59 | $\nu\text{CC}$     |
| -     | 1,671               | 1,593                    | 29.7 | $\nu\text{CC}$     |
| -     | 1,636               | 1,558                    | 17.7 | $\nu\text{CC}$     |
| 1,467s | 1,608               | 1,532                    | 14.2 | $\nu\text{CC}$     |
| 1,454m | 1,581               | 1,505                    | 2.76 | $\nu\text{CC}$, $\beta\text{CH}_2$ |
| 1,445 m | 1,577              | 1,502                    | 14.1 | $\beta_{\text{as}}\text{CH}_3$ |
| 1,437m | 1,529               | 1,455                    | 17.2 | $\beta\text{CH}_2$  |
| 1,396s | 1,432               | 1,361                    | 175  | $\beta\text{OH}$   |
| IR  | IR<sub>calc.</sub> | IR<sub>calcscaled</sub> | INT  | Proposed Assignment |
|-----|-------------------|-------------------|------|---------------------|
| 1,375 m | 1,430 | 1,359 | 15.7 | $\beta$CH<sub>3</sub> |
| 1,364 m | 1,424 | 1,353 | 178 | $\delta$CO |
| 1,335 w | 1,406 | 1,336 | 3.82 | $\nu$CC |
| 1,313 w | 1,379 | 1,310 | 18.5 | $\nu$CN |
| 1,285 w | 1,361 | 1,292 | 10.4 | $\nu$CC |
| 1,272 w | 1,343 | 1,275 | 34.6 | $\nu$CC, $\beta$CH<sub>2</sub> |
| 1,251 m | 1,331 | 1,263 | 82.7 | $\nu$CO |
| 1,228 m | 1,290 | 1,224 | 17.7 | $\beta$CH<sub>2</sub> |
| 1,209 s | 1,268 | 1,202 | 232 | $\nu$CC |
| 1,188 m | 1,251 | 1,186 | 10.8 | $\nu$CC |
| 1,176 m | 1,236 | 1,172 | 4.70 | $\nu$CC |
| 1,150 w | 1,225 | 1,161 | 11.5 | $\nu$CN |
| 1,142 w | 1,217 | 1,153 | 135 | $\nu$CN |
| 1,132 w | 1,214 | 1,150 | 141 | $\beta$CH |
| 1,108 w | 1,202 | 1,138 | 25.0 | $\nu$CN |
| 1,089 w | 1,192 | 1,128 | 169 | $\nu$CN |
| 1,074 w | 1,179 | - | 10.6 | $\gamma$CH<sub>2</sub> |
| 1,044 w | 1,141 | 1,116 | 14.3 | $\gamma$CH<sub>2</sub> |
| 1,037 w | 1,124 | 1,079 | 3.42 | $\gamma$CH<sub>2</sub> |
| 1,026 w | 1,106 | 1,063 | 3.28 | $\delta$CH<sub>2</sub> |
| 1,017 m | 1,098 | 1,045 | 5.41 | $\beta$CH<sub>2</sub> |
| 1,001 m | 1,074 | 1,037 | 32.5 | $\gamma$CH<sub>2</sub> |
| 0.798 w | 1,047 | 1,014 | 13.0 | $\beta$CCC |
| 0.964 w | 1,025 | 988 | 3.87 | $\beta$CO |
| 0.950 w | 1,016 | 967 | 13.6 | $\gamma$CH<sub>2</sub> |
| 0.935 w | 0.998 | 958 | 3.96 | $\beta$CH<sub>2</sub> |
| 0.927 w | 0.966 | 941 | 43.5 | $\gamma$CH<sub>2</sub> |
| 0.904 w | 0.963 | 909 | 25.0 | $\gamma$CH<sub>2</sub> |
| 0.897 w | 0.920 | 907 | 6.08 | $\beta$CCC |
| 0.887 w | 0.901 | 907 | 2.12 | $\beta$CCC |
| 0.860 vw | 0.895 | 865 | 2.04 | $\tau$ring |
| 0.842 vw | 0.879 | 847 | 5.89 | $\nu$CC |
| 0.802 vw | 0.867 | 825 | 3.62 | $\beta$CCC |
| 0.787 vw | 0.813 | 814 | 13.5 | $\beta$CCC |
| 0.720 m | 0.764 | 761 | 11.0 | $\beta$CCC |
| 0.710 vw | 0.751 | 714 | 1.86 | $\beta$CNC |
| 0.692 vw | 0.746 | 701 | 8.70 | $\beta$ring |
| 0.663 vw | 0.698 | 694 | 3.00 | $\gamma$CH |
| 0.636 vw | 0.659 | 612 | 0.13 | $\gamma$CH |
| 0.625 vw | 0.657 | 610 | 28.5 | $\gamma$CH |
| 0.605 vw | 0.650 | 603 | 3.71 | $\beta$ring |
| 0.600 vw | 0.625 | 579 | 6.76 | $\beta$NCC |
| 0.545 vw | 0.610 | 565 | 15.1 | $\gamma$CC |
| 0.531 w | 0.566 | 522 | 7.31 | $\beta$CCC |
| 0.512 vw | 0.551 | 508 | 14.9 | $\tau$ring |
Table 5. Cont.

| IR     | IR_{calc.} | IR_{calc.\,scaled} | INT | Proposed assignment |
|--------|------------|---------------------|-----|---------------------|
| 498 vw | 540        | 497                 | 12.4| τring               |
| 482 vw | 515        | 472                 | 10.3| γCCC                |
| 438 vw | 469        | 428                 | 1.23| Lattice mode        |
| 419 vw | 434        | 394                 | 11.4| Lattice mode        |
| 409 vw | 409        | 370                 | 9.39| Lattice mode        |
| -      | 386        | 348                 | 5.04| Lattice mode        |
| -      | 345        | 308                 | 22.0| Lattice mode        |
| -      | 329        | 292                 | 57.4| Lattice mode        |
| -      | 282        | 247                 | 3.62| Lattice mode        |
| -      | 257        | 223                 | 10.3| Lattice mode        |
| -      | 205        | 172                 | 5.27| Lattice mode        |
| -      | 177        | 145                 | 1.08| Lattice mode        |
| -      | 102        | 72                  | 1.63| Lattice mode        |
| -      | 56         | 28                  | 2.59| Lattice mode        |

The abbreviations are: s: strong; m: medium; w: weak; vw: very weak; ν: stretching; β: in plane bending; δ: deformation; ω: wagging; γ: out of plane bending and τ: twisting.

The DFT harmonic vibrational wavenumbers are usually higher than the experimental values. However, in this case the overall agreement between the experimental and calculated frequencies for (9) is very good (Figure 9).

**Figure 9.** Correlation between the experimental and calculated wavenumbers (cm$^{-1}$) for (9); $\nu_{\text{scaled}} = -26.3525 + 0.9689\nu_{\text{calc.}}$, $r^2 = 0.9973$.

Any discrepancy noted between the observed and calculated frequency may be due to the fact that the calculations have been done for a single molecule in the gaseous state contrary to the experimental spectrum recorded in the presence of intermolecular interactions. The scaling procedure, as recommended by Palafox was used [53,54]. The scaled IR spectrum is shown in Figure 8b and the
predicted frequencies are listed in Table 3 as \( \nu_{\text{scaleq}} \). Scaling of the harmonic vibrational frequencies reproduce the experimental solid-state FT-IR frequencies with the r.m.s. error of 38.1 cm\(^{-1}\). The vibrational band assignments of 9 were made using Gauss-View molecular visualization program [55].

3. Experimental

3.1. General

The NMR spectra were measured with a Varian Mercury 300 MHz NMR spectrometer (Oxford, UK), operating at 300.07 and 75.4614 for \(^1\)H and \(^{13}\)C, respectively. Typical conditions for the proton spectra were: pulse width 32°, acquisition time 5 s, FT size 32 K and digital resolution 0.3 Hz per point, and for the carbon spectra pulse width 60°, FT size 60 K and digital resolution 0.6 Hz per point, the number of scans varied from 1200 to 10,000 per spectrum. The \(^{13}\)C and \(^1\)H chemical shifts were measured in CDCl\(_3\) relative to an internal standard of TMS. Infrared spectra were recorded in the KBr pellets using a FT-IR Bruker IFS 66 spectrometer (Karlsruhe, Germany). The ESI (electron spray ionization) mass spectra were recorded on a Waters/Micromass (Manchester, UK) QZ mass spectrometer equipped with a Harvard Apparatus (Saint Laurent, QC, Canada), syringe pump. The sample solutions were prepared in methanol at the concentration of approximately \(10^{-5}\) M. The standard ESI-MS mass spectra were recorded at the cone voltage 110 V. The MALDI (matrix-assisted laser desorption/ionization) mass spectra were recorded on a Waters Maldi Q-Tof Premiere. The sample solutions were prepared in methanol at the concentration of approximately \(10^{-5}\) M. The matrix was 2,5-dihydroxybenzoic acid (gentisic acid) and the standard was \(\beta\)-cyclohextrin (\(m/z\) 1157.3218).

PM5 semiempirical calculations were performed using the WinMopac 2003 program [56–58]. The calculations were performed using the GAUSSIAN 03 program package [59] at the B3LYP [60–62] levels of theory with the 6-31G(d,p) basis set [63]. The NMR isotropic shielding constants were calculated using the standard Gauge-Independent Atomic Orbital (GIAO) approach of Gaussian 03 [64,65].

3.2. Synthesis

The appropriate 3\(\beta\)-bromoacetate of sterols or 3\(\alpha\)-bromoacetate of bile acids (0.20 mmol) were dissolved in CH\(_3\)CN (6 mL) under reflux. Then \(N,N\)-dimethyl-3-phthalimidopropylamine (0.24 mmol) was added and the mixture heated under reflux for 3 h. The precipitate was filtered and crystallized from CH\(_3\)CN-EtOH (90:1), to give white solids.

\(N,N\)-Dimethyl-(3\(\beta\)-acetate-ergosta-5,7,22-triene)-3-phthalimidopropylammonium bromide (7): white solid (76%), m.p. 185–187 °C. \(^1\)H-NMR: \(\delta_H\) 7.85 (bs, 2H, Ar–H), 7.75 (bs, 2H, Ar–H), 5.35 (s, 2H, COCH\(_2\)N\(^+\)), 5.31–5.12 (m, 4H, 6, 7, 22, 23–H), 4.96–4.58 (m, 3H, 3\(\alpha\)–H, N +CH\(_2\)), 3.82 (bs, 2H, CH\(_2\)–N–phthalimide ring), 3.69 (s, 6H, N\(^+(\text{CH}_3)\)), 1.04 (s, 3H, CH\(_3\)–19), 1.01 (d, \(J = 6.0\) Hz, 3H, CH\(_3\)–28), 0.93 (d, \(J = 6.7\) Hz, 3H, CH\(_3\)–21), 0.84 (d, \(J = 6.7\) Hz, 6H, CH\(_3\)–26 and CH\(_3\)–27), 0.83 (s, 3H, CH\(_3\)–18). \(^{13}\)C-NMR: \(\delta_C\) 168.17 (C-7'), 163.98 (C-1'), 140.11 (C-8), 139.96 (C-5), 135.43 (C-22), 134.32, 132.08 (C-23), 131.81, 123.57, 118.11 (C-6), 118.03 (C-7), 74.87 (C-3), 62.40, 61.67, 60.67 (C-2'), 57.03 (C-4'), 53.16 (C-3'), 44.76, 42.79, 40.80, 40.72, 38.86, 36.82, 36.65, 36.44, 36.40, 34.93, 34.03, 33.81, 33.05, 27.58, 27.45, 26.43, 25.10, 25.04, 22.81, 21.77 (C-21), 20.96 (C-19), 19.94, 19.61, 18.28, 18.20, 17.63, 15.80 (C-18). FT-IR (KBr) \(\nu_{\text{max}}\): 2957, 2871, 1773, 1740, 1713, 1466, 1437, 1396,
N,N-Dimethyl-(3β-acetate-cholest-5-ene)-3-phthalimidopropylammonium bromide (8): white solid (82%), m.p. 199–200 °C. 1H-NMR: δH 7.85–7.80 (m, 2H, Ar–H), 7.76–7.70 (m, 2H, Ar–H), 5.35 (d, J = 4.9 Hz, 1H, 6–H), 4.80 (bs, 2H, COCH2N+), 4.69–4.48 (m, 1H, 3α–H), 4.00–3.89 (m, 2H, N+(CH3)2), 3.83 (bs, 2H, CH2–N–phthalimide ring), 3.66 (s, 6H, N+(CH3)2), 0.99 (s, 3H, CH3–19), 0.92 (d, J = 6.4 Hz, 3H, CH3–21), 0.87 (dd, J = 6.6, 1.4 Hz, 6H, CH3–26 and CH3–27), 0.68 (s, 1H, CH3–18). 13C-NMR: δC 168.12 (C-7'), 163.90 (C-1'), 138.76 (C-5'), 134.22, 131.72, 123.49, 123.33 (C-6), 76.57 (C-3), 62.05 (C-2'), 61.05 (C-4'), 56.60, 56.06, 52.38 (C-3'), 49.93, 42.24, 39.62, 39.44, 37.63, 36.75, 36.44, 36.11, 35.71, 34.63, 31.84, 31.72, 29.64, 28.15, 27.95, 27.42, 24.21, 23.76, 22.76, 22.50, 20.95, 19.19 (C-19), 18.65 (C-21), 11.79 (C-18). FT-IR (KBr) νmax: 2943, 2868, 1774, 1737, 1713, 1637, 1395, 1364, 1252, 1210, 1089, 925. ESI-MS (m/z): 670 (100%) [C43H61N2O4]+. MALDI-MS (m/z): 669.9. HRMS: caleld 669.5678 for C43H61N2O4; found 669.5630.

N,N-Dimethyl-(3β-acetate-β-cholestan)-3-phthalimidopropylammonium bromide (9): white solid (83%), m.p. 191–192 °C. 1H-NMR: δH 7.83 (d, J = 3.90 Hz, 2H, Ar–H), 7.74 (m, 2H, Ar–H), 4.88–4.55 (m, 3H, COCH2N+, 3α–H), 3.92 (bs, 2H, CO2CH3 and N+(CH3)2), 3.83 (bs, 2H, CH2–N–phthalimide ring), 3.61 (s, 6H, N+(CH3)2), 0.90 (d, J = 6.5 Hz, 3H, CH3–21), 0.86 (dd, J = 6.7, 1.9 Hz, 6H, CH3–26 and CH3–27), 0.79 (s, 3H, CH3–19), 0.65 (s, 1H, CH3–18). 13C-NMR: δC 168.18 (C-7'), 163.89 (C-1'), 134.26, 131.74, 123.51, 76.68 (C-3), 62.25 (C-2'), 61.27 (C-4'), 56.34, 55.79, 54.10, 52.51 (C-3'), 44.62, 42.52, 39.90, 39.45, 36.56, 36.11, 35.74, 35.34, 34.65, 33.55, 31.90, 29.65, 28.44, 28.18, 27.96, 27.13, 24.14, 23.79, 22.78, 22.56, 22.52, 21.15, 18.62 (C-21), 12.18 (C-19), 12.02 (C-18). FT-IR (KBr) νmax: 2952, 2867, 1770, 1742, 1712, 1467, 1467, 1395, 1364, 1252, 1210, 1089, 927. ESI-MS (m/z): 661 (100%) [C42H65N2O4]+. MALDI-MS (m/z): 659.9. HRMS: caleld 659.5812 for C42H65N2O4; found 659.5802.

N,N-Dimethyl-(methyl litocholate)-3-phthalimidopropylammonium bromide (16): white solid (96%), m.p. 104–105 °C. 1H-NMR: δH 7.86–7.82 (m, 2H, Ar–H), 7.77–7.73 (m, 2H, Ar–H), 4.84–4.67 (m, 3H, CO2CH3 and N+(CH3)2), 3.92 (bs, 2H, CO2CH3 and N+(CH3)2), 3.83 (bs, 2H, CH2–N–phthalimide ring), 3.66 (s, 9H, CO2CH3 and N+(CH3)2), 0.91 (m, 6H, CH3–19 and CH3–21), 0.64 (s, 3H, CH3–18). 13C-NMR: δC 174.72 (C-24), 168.09 (C-7'), 163.87 (C-1'), 134.24, 131.73, 123.45, 77.97 (C-3), 62.11 (C-2'), 61.14 (C-4'), 56.34, 56.21, 54.10, 52.51 (C-3'), 44.62, 42.52, 39.90, 39.45, 36.56, 36.11, 35.74, 35.34, 34.65, 33.55, 31.90, 29.65, 28.44, 28.18, 27.96, 27.13, 24.14, 23.79, 22.78, 22.56, 22.52, 21.15, 18.62 (C-21), 12.18 (C-19), 12.02 (C-18). FT-IR (KBr) νmax: 2955, 2870,
1774, 1736, 1716, 1485, 1466, 1405, 1393, 1201, 1091, 967. ESI-MS (m/z): 680 (100%) [C_{40}H_{59}N_{2}O_{7}]^{+}. MALDI-MS (m/z): 679.5. HRMS: calcd 679.5358 for C_{40}H_{59}N_{2}O_{7}; found 679.5341.

\[ N,N\text{-Dimethyl-(methyl cholate)-3-phthalimidopropylammonium bromide} \text{ (18): white solid (85%), m.p. 204–205 °C.} \]
\[ \text{\textsuperscript{1}H-NMR:} \delta_H 7.86–7.83 \text{ (m, 2H, Ar–H), 7.76–7.69 \text{ (m, 2H, Ar–H), 4.95 \text{ (s, 2H, COCH}_2\text{N}^+)}} \]
\[ 4.66–4.55 \text{ (m, 1H, 3β–H), 3.97 \text{ (bs, 1H, 12β–H), 3.92–3.89 \text{ (m, 2H, CH}_2\text{–N–phthalimide ring), 3.80 \text{ (bs, 1H, 7β–H), 3.63 \text{ (s, 3H, CO}_2\text{CH}_3,})}} \]
\[ 3.62 \text{ (s, 6H, N}(^+)\text{(CH}_3\text{)₂}, 0.97 \text{ (d, } J = 5.8 \text{ Hz, 3H, CH}_3\text{–21), 0.89 \text{ (s, 3H, CH}_3\text{–19), 0.66 \text{ (s, 3H, CH}_3\text{–18).} \text{\textsuperscript{13}C-NMR:} \delta_C 174.77 \text{ (C-24), 168.34 \text{ (C-7'), 164.25 \text{ (C-1'), 134.19, 131.78, 123.49, 76.57 \text{ (C-3), 72.62 \text{ (C-12), 67.75 \text{ (C-7), 61.86 \text{ (C-2'), 61.50 \text{ (C-4'), 52.50 \text{ (C-3'), 51.41 \text{ (C-25), 46.95, 46.35, 41.89, 41.22, 39.54, 35.26, 34.86, 34.66, 34.37, 31.13, 30.91, 28.37, 27.45, 26.68, 26.30, 23.13, 22.55, 22.32 \text{ (C-21), 17.25 \text{ (C-19), 12.47 \text{ (C-18).}}}}}} \]
\[ \text{FT-IR (KBr)} \text{ } \nu_{\text{max}}: 2938, 2870, 1772, 1739, 1711, 1468, 1437, 1399, 1370, 1208, 1073, 953. \text{ESI-MS (m/z):} 696 (100%) [C_{40}H_{59}N_{2}O_{8}]^{+}. \text{MALDI-MS (m/z):} 695.5. \text{HRMS: calcd 695.5192 for C}_{40}\text{H}_{59}\text{N}_{2}\text{O}_{8}; \text{found 695.5242.} \]

4. Conclusions

In conclusion, six new quaternary N,N-dimethyl-3-phthalimidopropylammonium conjugates of sterols (compounds 7–9) and bile acids (compounds 16–18) were prepared by the reactions of ergosteryl 3β-bromoacetate, cholesteryl 3β-bromoacetate, dihydrocholesteryl 3β-bromoacetate as well as methyl litocholate 3α-bromoacetate, methyl deoxycholate 3α-bromoacetate and methyl cholate 3α-bromoacetate, with N,N-dimethyl-3-phthalimidopropylamine in acetonitrile. These new compounds were characterized by spectroscopic and molecular structure methods. The obtained conjugates may find applications in molecular recognition and in pharmacology, especially as compounds with a high antimicrobial activity.

Acknowledgments

This work was supported by the funds from Adam Mickiewicz University, Faculty of Chemistry.

Author Contributions

The listed authors contributed to this work as described in the following. Bogumił Brycki gave the concepts of work, interpreted the results and prepared the manuscript. Hanna Koenig carried out of the synthetic work, interpreted the results and cooperated in the preparation of the manuscript. Tomasz Pospieszny performed semiempirical calculations and Prediction of Activity Spectra for Substances (PASS). Iwona Kowalczyc performed quantum chemical calculations. All authors contributed with valuable discussions and scientific input and approved the final version.

Conflicts of Interest

The authors declare no conflict of interest.
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**Sample Availability**: Samples of the compounds 4–9 and 13–18 are available from the authors.

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