Synthesis and Mechanistic Study of Steroidal Oxime Ethers

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Reaction of 5α-cholestan-6-one oxime (**1**), its 3β-acetoxy and 3β-chloro analogs, **2** and **3**, respectiveley, with $ClCH_2CH_2NH_2 \cdot HCl$ in presence of MeONa afforded 6-[(2-aminoethoxy)imino]-5α-cholestane (**4**), 3β-acetoxy-6-[(2-aminoethoxy)imino]-5α-cholestane (**5**), and 6-[(2-aminoethoxy)-imino]-3β-chloro-5α-cholestane (**6**), respectively. The structures of newly synthesized compounds have been established on the basis of physical, analytical, and spectral data. Theoretical calculations were assessed by using DFT at B3LYP/6-31G* level to describe the mechanism of the reaction. The stability and feasibility of all the generated structures studied in this report were supported by their respective fundamental frequencies and energy minima.

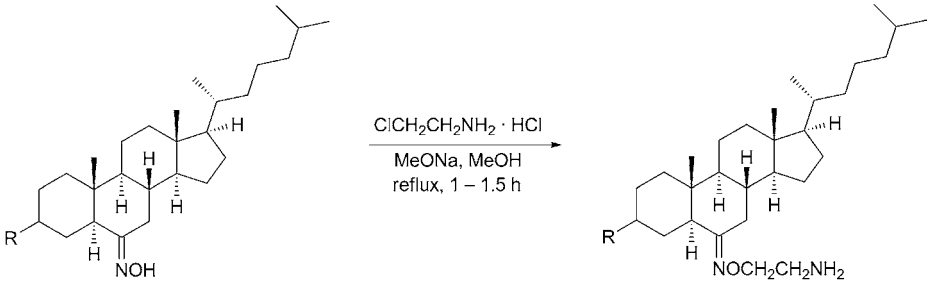
Introduction. – Oxime ethers have attracted much interest as important precursors and intermediates for the preparation of a wide variety of drugs and natural products [1]. They can be easily converted into important functional groups such as amino alcohols and hydroxy ketones [2]. Therefore, the development of methodologies for the preparation of oxime ethers is of considerable interest.

Various researchers have studied the interesting biological properties of oxime-ether derivatives such as anticonvulsant [3], anti-inflammatory [4], antineoplastic [5], anti-enteroviral [6], antimicrobial [7], anti-amoebic [8], antitumor [9], and anti-*Helicobacter pylori* activities [10].

Computational studies of organic compounds have also attracted considerable interest in recent years. Quantum chemical methods have been used to describe structure–activity mechanism of ketoxime-ether reactions [11]. *Macchia* and co-workers [12] have reported a simple molecular-modeling study of oxime ethers of oxiconazole to understand their different antifungal profiles. Literature survey revealed that the density functional theory (DFT) has high accuracy in reproducing the experimental values of quadrupole hyperfine coupling constants [13], molecular structural properties [14], frequencies and intensities [15], *etc.* However, no theoretical study has yet been reported where DFT is systematically used to explain the reaction mechanism of steroidal oxime ethers.

In the context of our research on steroid chemistry [16], here we report a convenient method of preparation of steroidal oxime ether (aminoethoxyimino) derivatives **4**–**6** (*Scheme*). With the aim of supporting the experimental data and to gain insight into the factors influencing reactivity and stability, we focus our attention on the results of the theoretical models obtained by the DFT at B3LYP/6-31G* level.

Scheme



1 R = H
2 R = AcO
3 R = Cl

4 R = H
5 R = AcO
6 R = Cl

Results and Discussion. – The 5α-cholestan-6-one oxime (**1**) and its analogs, 3β-acetoxy-5α-cholestan-6-one oxime (**2**) and 3β-chloro-5α-cholestan-6-one oxime (**3**), on treatment with $ClCH_2CH_2NH_2$ HCl in the presence of MeONa afforded the respective 6-[(2-aminoethoxy)imino]-5α-cholestane (**4**), 3β-acetoxy-6-[(2-aminoethoxy)imino]-5α-cholestane (**5**), and 6-[(2-aminoethoxy)imino]-3β-chloro-5α-cholestane (**6**). The data of **4**–**6** are given in *Tables 1* and *2*.

Table 1. *Physical and Analytical Data of Compounds* **4**–**6**

Compound	Yield [%]	M.p. [°]	Molecular formula	Found (calc.) [%]		
				C	H	N
4	85	177–179	$C_{29}H_{52}N_2O$	78.39 (78.32)	11.80 (11.79)	6.35 (6.30)
5	88	182–184	$C_{31}H_{54}N_2O_3$	74.07 (74.06)	10.84 (10.83)	5.58 (5.57)
6	90	169–171	$C_{29}H_{51}ClN_2O$	72.73 (72.69)	10.76 (10.73)	5.84 (5.85)

Table 2. *Spectral Data of Compounds* **4**–**6**

Compound	IR (KBr) [cm^{-1}][a])	^{1}H-NMR ($CDCl_3$) (δ in ppm, J in Hz)[b])
4	3420 (NH_2), 1655 (C=N), 1400 (N–O), 1292 (C–O), 1225 (C–N)	5.2 (*s*, NH_2); 3.8 (*t*, J = 5.8, CH_2O); 3.18–3.22 (*m*, CH_2N); 2.50 (*t*, J = 5.7, H–C(5)); 1.34–1.45 (br. *m*, H_α–C(3))
5	3470 (NH_2), 1660 (C=N), 1735 (COOMe), 1380 (N–O), 1300 (C–C), 1220 (C–N)	5.3 (*s*, NH_2); 4.24–4.36 (br. *m*, H_α–C(3); 3.75 (*t*, J = 6.0, CH_2O); 3.08–3.12 (*m*, CH_2N); 2.6 (*t*, J = 5.6, H–C(5)); 2.1 (*s*, MeO)
6	3460 (NH_2), 1665 (C=N), 1381 (N–O), 1290 (C–O), 725 (C–Cl)	5.3 (*s*, NH_2); 3.65 (*t*, J = 6.0, CH_2O); 3.34–3.45 (br. *m*, H_α–C(3); 3.17–3.22 (*m*, CH_2N); 2.55 (*t*, J = 5.7, H–C(5))

[a]) Characteristic absorption bands. [b]) Signals of angular and side-chain Me H-atoms appeared at δ(H) 1.1–0.68.

Spectroscopic Data. Selected diagnostic bands in IR spectrum of compound **4** provided vital information for determining its structure. The IR spectrum exhibited characteristic absorption bands at 3420 (NH_2), 1655 (C=N), 1400 (N–O), 1292 (C–O), and 1225 (C–N) cm^{-1}. Besides, ^{1}H-NMR spectrum showed a *singlet* for two H-atoms at δ(H) 5.2 for NH_2, a *triplet* for two H-atoms at 3.8 for CH_2O, a *multiplet* for two H-atoms at 3.18–3.22 for the NCH_2 group, a broad *multiplet* at 1.34–1.45 for H_α–C(3). All the spectral data are in good agreement with the desired structure, namely 6-[(2-aminoethoxy)imino]-5α-cholestane (**4**). Similarly, the spectral data of compounds **5** and **6** are compatible with the structure of 3β-acetoxy-6-[(2-aminoethoxy)imino]-5α-cholestane and 3β-chloro-6-[(2-aminoethoxy)imino]-5α-cholestane, respectively.

Theoretical Study. The reaction pathway of conversion of 5α-cholestan-6-one oxime (**1**) to 6-[(2-aminoethoxy)imino]-5α-cholestane (**4**), of 3β-acetoxy-5α-cholestan-6-one oxime (**2**) to 3β-acetoxy-6-[(2-aminoethoxy)imino]-5α-cholestane (**5**), and of 3β-chloro-5α-cholestan-6-one oxime (**3**) to 3β-chloro-6-[(2-aminoethoxy)imino]-5α-cholestane (**6**) is described by considering the optimized lower-energy structures of steroidal ketoximes **1**–**3**, oximate anions **I-1**–**I-3** as intermediates, and steroidal oxime ether derivatives **4**–**6** as products. The main focus will be the molecular properties changed during the reaction from reactant to product by considering the finally calculated DFT structures at B3LYP/6-31G* level.

Total energy, frontier molecular orbital (FMO) energies, hardness and dipole moment of all the steroid molecules calculated in this study are compiled in *Table 3*. The dipole moment (μ) first increased from reactants **1**–**3** to intermediates **I-1**–**I-3** and then decreased from intermediates **I-1**–**I-3** to products **4**–**6**. The reactants **1**–**3** have lower μ values than the corresponding products **4**–**6**, implying lower polarity of the reactants. Intermediates **I-1**–**I-3** have higher μ values than the respective reactants and products, implying higher polarity of the intermediates, and leading to the conclusion that the formation of the oximate anions modifies their charge distribution.

Table 3. *Molecular Properties of Steroidal Structures Involved in the Synthesis of 6-[(2-Aminoethoxy)-imino]-5α-cholestane* (**4**)*, 3β-Acetoxy-6-[(2-aminoethoxy)imino]-5α-cholestane* (**5**)*, and 6-[(2-Amino-ethoxy)imino]-3β-chloro-5α-cholestane* (**6**)

Molecule	Energy[a])	HOMO[b])	LUMO[b])	Hardness[b])	Dipole moment[c])
1	– 744938.654	– 6.35	0.47	3.41	0.83
2	– 887937.159	– 6.49	0.32	3.40	1.88
3	– 1033342.22	– 6.57	0.21	3.39	3.44
I-1	– 744557.001	1.13	3.65	1.26	24.35
I-2	– 887558.812	0.96	2.73	0.88	21.69
I-3	– 1032965.41	0.88	3.53	1.32	27.16
4	– 829004.130	– 6.09	0.45	3.27	1.60
5	– 972002.360	– 6.22	0.30	3.26	2.40
6	– 1117407.63	– 6.27	0.20	3.23	4.32

[a]) In kcal/mol. [b]) In eV. [c]) Calculated in Debye.

As expected, the LUMO values of all the structures calculated were higher in energy than their respective HOMO values (*Table 3*). This is reasonable, because electrons in orbitals with lower energy are more susceptible to receive the electrophilic

species [17], and the reactions occur easily. Another finding is that the hardness values decreased from reactants **1**–**3** to the respective intermediates **I-1**–**I-3**, and it increased from intermediates to the respective products **4**–**6**. Thus, it is concluded that intermediate has more softness and thus is highly likely to be converted to the product. This property supports the claim that, according to *Pearson* [18], the soft molecules are more reactive than hard molecules. In other words, the softer the material is, the more reactive it is. This is just what has been observed when starting materials **1**–**3** are converted to products **4**–**6**.

Formation of Oximate Anion Intermediates. Steroidal ketoximes **1**–**3** undergo reaction *via* formation of oximate anion intermediates **I-1**–**I-3**. The starting materials **1**–**3** have reactive oxime groups which are deprotonated in presence of a base (MeONa) to form oximate anions. From theoretical point of view, it was observed that O-atom of oxime group in structures **1**–**3** exhibited significant charges, electrostatic potentials, and highly occupied molecular orbitals (*Figs. 1* and *2*). All these properties facilitate deprotonation of oxime. Besides, there is the increase of energy of 6.31, 3, and 1.46 kcal/mol during the formation of intermediates **I-1**–**I-3**, respectively, as shown in energy curves (*Fig. 3*). This is expected because there are reported *Hartree–Fock* energies for the atoms, where it was observed that energy diminishes with increasing number of atoms increases [19].

Formation of Products. The products **4**–**6** were formed from **I-1**, **I-2**, and **I-3**, respectively, by the reaction of $ClCH_2CH_2NH_2$ with the corresponding oximate anions. To carry out this step of the reaction, it is necessary to have electrostatic potential, electronegative charge, and HOMO at the O-atom of the intermediates **I-1**–**I-3** as shown in *Figs. 1* and *2*. The electrostatic potential and electrostatic charges are in good agreement to follow the order of present reaction mechanism. Furthermore, the analyses of FMOs show that the intermediates have HOMOs just on their reactive parts and work in the same way as the FMO analysis based on perturbation theory predicts. The mentioned theory has been widely used to rationalize the relative reactivity pattern in the different types of reactions [20].

The relative energy curves are shown in *Fig. 3*. The reaction of **1** to **4**, **2** to **5**, and **3** to **6** proceeds *via* intermediates **I-1**–**I-3**, respectively. The energy of $ClCH_2CH_2NH_2$, $-373219.667\ kcal\,mol^{-1}$, is added to the energy of the respective reactants **1**–**3**. The energies of $ClCH_2CH_2NH_2$ and H^+ (– 375.345654 kcal/mol) are added to the energies of **I-1**–**I-3**, whereas HCl energy, – 289153.929 kcal/mol, is added to the product energy, to enable the relative-energy comparisons. It was found that intermediate **I-3** is more stable than **I-2** and **I-1** by 1.54 and 4.85 kcal/mol, respectively. Thus, it is concluded that the reaction from **3** to **6** is comparatively more favorable than those of **1** to **4** and **2** to **5**. On the other hand, product **4** is comparatively more stable than **5** and **6** by 0.28 and 0.07 kcal/mol, respectively. However, the products **4**–**6** are higher in energy than their respective starting materials **1**–**3**. Several attempts were made to calculate the transition state from reactant to intermediate and from intermediate to product, but all attempts failed. Accordingly, it is concluded that the present reaction proceeds *via* only one intermediate that is the oximate anion, while no transition state occurs during the reaction.

The structural stability and feasibility of all the calculated molecules was assessed by computing their harmonic normal modes of vibrations. The lowest-lying normal

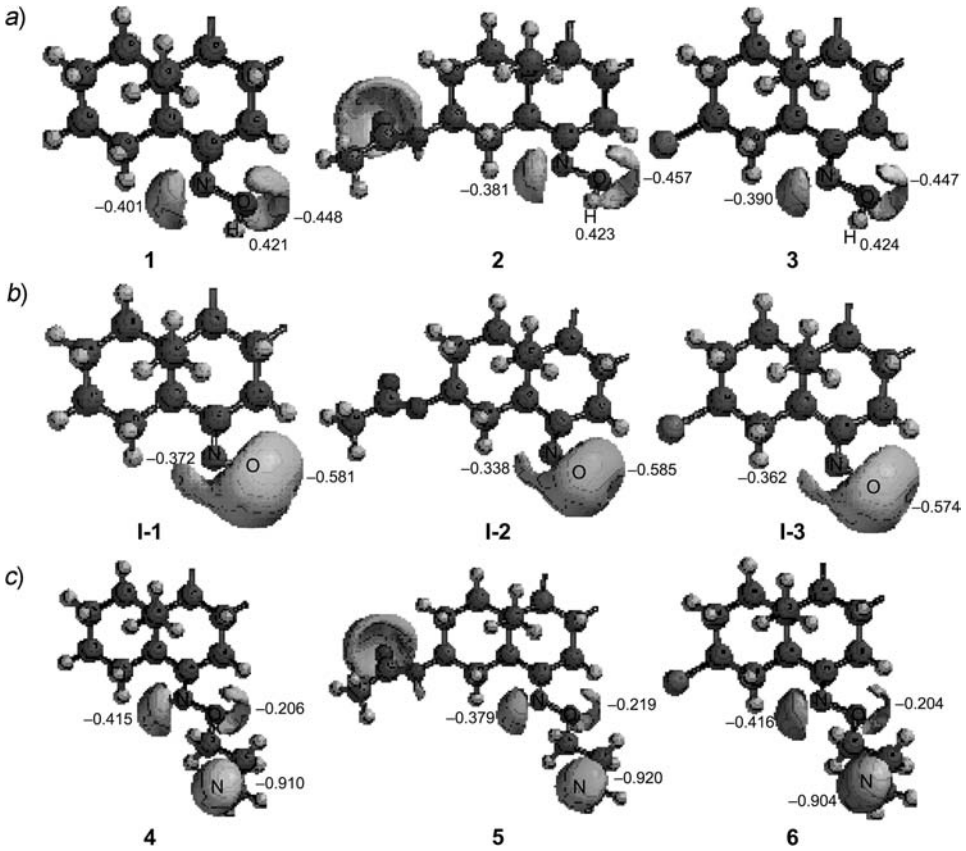


Fig. 1. a) *The encoded electrostatic potential map of 5α-cholestan-6-one oxime* (**1**)*, 3β-acetoxy-5α-cholestan-6-one oxime* (**2**)*, and 3β-chloro-5α-cholestan-6-one oxime* (**3**). b) *The electrostatic potential encoded with density of intermediates* **I-1**–**I-3**. c) *The encoded electrostatic potential map of 6-[(2-aminoethoxy)imino]-5α-cholestane* (**4**)*, 3β-acetoxy-6-[(2-aminoethoxy)imino]-5α-cholestane* (**5**)*, and 6-[(2-aminoethoxy)imino]-3β-chloro-5α-cholestane* (**6**). Some significant atomic charges are also labelled.

mode of vibration of each of the molecules **1**–**3**, **I-1**–**I-3**, and **4**–**6** is of frequency at 27.13, 22.94, 24.50, 26.50, 22.15, 24.00, 17.74, 19.70, and 20.82 cm^{-1}, respectively. The absence of imaginary frequencies indicates that the structures of all the compounds are the local minima on the corresponding potential-energy surfaces.

Based upon the experimental and theoretical evidences, the reaction mechanism is described for the formation of 6-[(2-aminoethoxy)imino]-5α-cholestane (**4**), its 3β-acetoxy and 3β-chloro analogs, **5** and **6**, respectively, from the corresponding steroidal keto-oximes **1**–**3**, respectively. An oximate anion is formed as the intermediate. The physicochemical parameters *i.e.*, the total energy, electrostatic potential, atomic charges, FMO energies, hardness, and dipole moment, were highly supportive to provide a logical explanation of the reaction mechanism. Indeed, these results could be helpful to design and synthesize pharmacologically vital compounds.

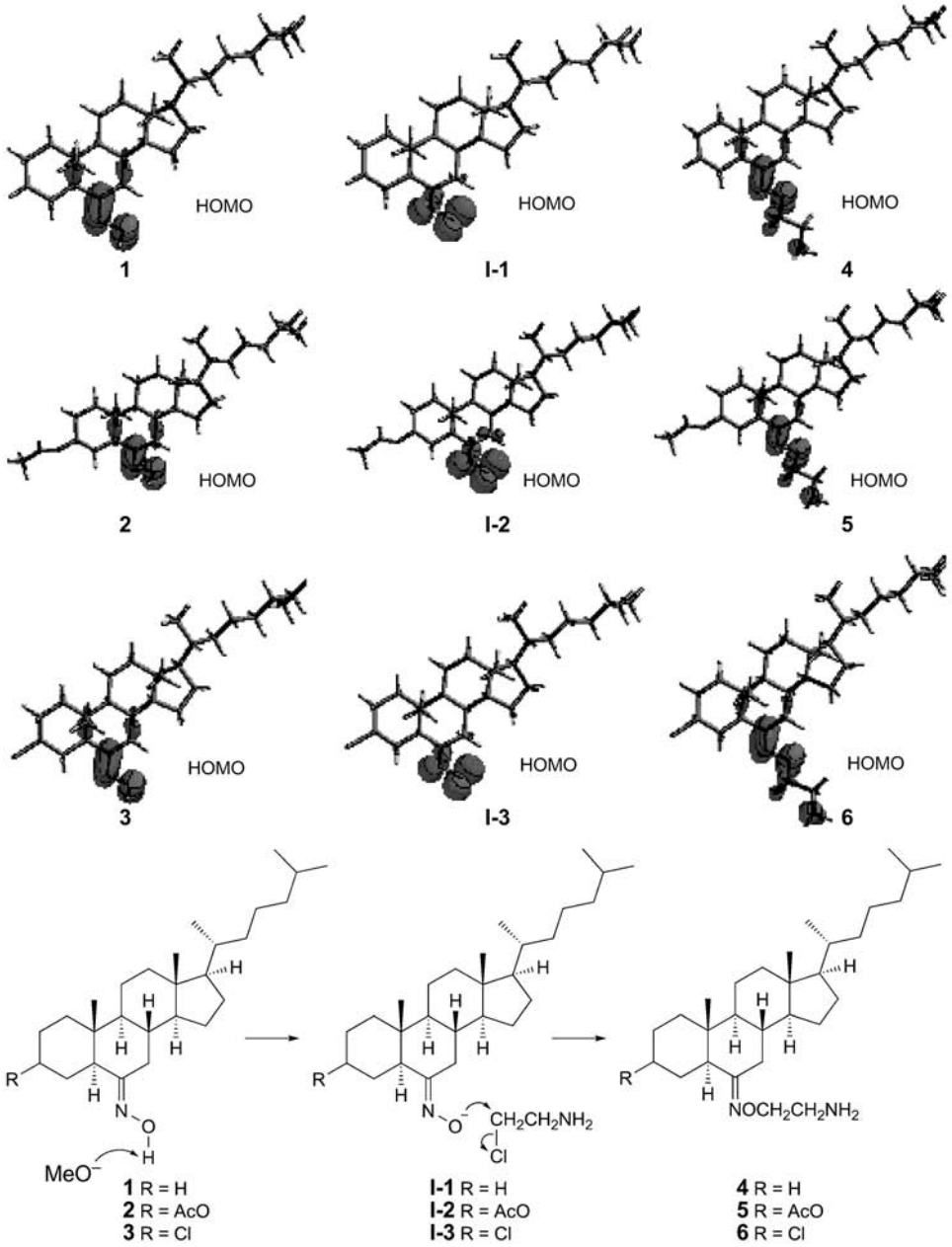


Fig. 2. *The highest occupied molecular orbital* (HOMO) *during the reaction mechanism of formation of 6-[(2-aminoethoxy)imino]-5α-cholestane* (**4**)*, and its analogs **5** and **6** from corresponding steroidal ketoximes* **1**–**3** *are shown.* **I-1**–**I-3** are the intermediates of the reactions.

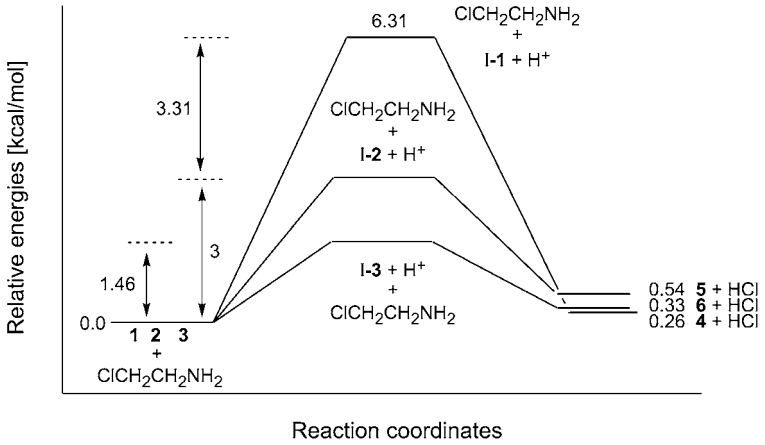


Fig. 3. *Energy curve of formation of oxime ethers.* Relative energies of reactants **1**–**3**, intermediates **I-1**–**I-3**, and products **4**–**6** are given. The energy of $ClCH_2CH_2NH_2$ is added to the reactant, energies of $ClCH_2CH_2NH_2$ and proton are added to the intermediate, and the energy of HCl molecule is added to the product, for the energy comparison.

Conclusions. – In summary, we have developed a simple and convenient method of preparation of 6-[(2-aminoethoxy)imino]-5α-cholestane (**4**), 3β-acetoxy-6-[(2-aminoethoxy)imino]-5α-cholestane (**5**), and 3β-chloro-6-[(2-aminoethoxy)imino]-5α-cholestane (**6**) by the reaction of respective steroidal ketoxime and $ClCH_2CH_2NH_2 \cdot HCl$.

Mechanism of the reaction is described successfully by using DFT calculations with the detailed analysis of local properties such as atomic charges and frontier orbitals. It was concluded that the reaction proceeds *via* formation of an oximate anion intermediate.

Experimental Part

General. All chemicals were of anal. grade and used without further purification. M.p.: *Kofler* apparatus; uncorrected. IR Spectra: *Perkin–Elmer 782* infrared spectrophotometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^{1}H-NMR Spectra: *Bruker BZH-300* instrument; $CDCl_3$ soln.; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz.

Preparation of Steroidal Oxime Ethers ((Aminoethoxy)imino Derivatives). To a soln. of 5α-cholestan-6-one oximes **1**–**3** [21] (1.092 mmol) in MeOH (10 ml) was added $ClCH_2CH_2NH_2 \cdot HCl$ (0.155 g, 1.335 mmol) at r.t., followed by a freshly prepared soln. of MeONa (2.76 mmol) dropwise. The mixture was refluxed for 1 to 1.5 h. The completion of the reaction was monitored by TLC. The mixture was cooled to r.t., and the precipitate was collected by filtration, washed with H_2O, and air-dried. The crude product obtained was crystallized from MeOH to give compounds **4**–**6**.

Computational Method. The molecules studied in this article are *5α-cholestan-6-one oxime* (**1**), *3β-acetoxy-5α-cholestan-6-one oxime* (**2**), *3β-chloro-5α-cholestan-6-one oxime* (**3**), oximate anions **I-1**–**I-3**, *6-[(2-aminoethoxy)imino]-5α-cholestane* (**4**), *3β-acetoxy-6-[(2-aminoethoxy)imino]-5α-cholestane* (**5**), and *6-[(2-aminoethoxy)imino]-3β-chloro-5α-cholestane* (**6**).

The following parameters were calculated: total energy, electrostatic charges, dipole moment (μ), electrostatic potential, and energy of FMOs; HOMO, LUMO to determine the hardness (η) [22] as an index of molecular reactivity.

All electronic structures were obtained by *ab initio* calculations. The computational calculations started with the fully optimized, semiempirical PM3 level of theory, followed by the *Hartree–Fock* (*HF*)

model using minimal STO-3G basis set. The resulting energy, wavefunction, *Hessian* matrix, and the geometry of molecules obtained were used to perform the next level of calculation with split-valence basis set 3-21G(*) and then further calculated with HF/6-31G* basis set. Finally, DFT calculations with the B3LYP functional were performed using 6-31G* basis set. The described reaction took place in protic solvent MeOH, and that solvation of the involved species was not taken into account for the performed calculations.

The fundamental frequencies of all the optimized structures were also calculated and assigned as minima (all real frequencies). The '*d*' polarized functions were added for heavy atoms. The molecules studied were built with Spartan'04 for *Windows* [23] graphical software for quantum-chemical calculations.

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