

New Potent Human Acetylcholinesterase Inhibitors in the Tetracyclic Triterpene series with Inhibitory Potency on Amyloid β Aggregation

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Abstract

New acetylcholinesterase inhibitors in the tetracyclic triterpene series were synthesized, tested *in vitro* for the inhibition of cholinesterases (different sources of AChE and BuChE) and for the ability to prevent AChE-induced A β aggregation. Some compounds have *h*AChE IC₅₀ values in the nanomolar range and showed ability to block the AChE-induced A β aggregation. The mode of interaction between *Ee*AChE and compounds **1** and **36e** was investigated using docking and molecular dynamics simulations. These studies suggested that both compounds interact simultaneously with the catalytic and the peripheral sites of AChE, and the nature of protein-ligand interactions is mainly hydrophobic.

Keywords

Acetylcholinesterase inhibitors; N-3-isobutyrylcyclohexidine-F derivatives; Molecular modeling; Alzheimer's disease; β -amyloid aggregation; Butyrylcholinesterase; Synthesis.

1. Introduction

Alzheimer disease (AD) is a progressive neurodegenerative disorder of the central nervous system (CNS) that affects mainly aged population. AD is characterized by profound memory impairments, emotional disturbance, and also personality changes. The main pathological changes in the AD brain are extracellular amyloid plaques [1], intracellular neurofibrillary tangles containing abnormally hyperphosphorylated tau protein [2], and loss of neurons in the nucleus basalis of Meynert and the hippocampus. AD is characterized by a pronounced

alteration of the cholinergic system and other neurotransmitter systems (glutamate and serotonin). The cholinergic hypothesis postulates that memory impairments in patients with AD result from a deficit of cholinergic function in the brain [3]. Most currently prescribed AD drugs aim to increase the level of acetylcholine (ACh) in the brain by inhibiting AChE. However, clinical use of AChE inhibitors is sometimes limited mainly due to their adverse effects and modest benefits to AD patients. Therefore, novel more effective therapies, including AChE inhibitors, need to be developed. In addition to its catalytic activity, AChE exerts secondary noncholinergic functions related to its peripheral binding site on differentiation, cell adhesion, in mediating the processing and deposition of β -amyloid peptide ($A\beta$) [2]. It was postulated that AChE binds through its peripheral site to the non amyloidogenic form of β -amyloid protein acting as a chaperone protein and inducing conformational change to the amyloidogenic form with the subsequent amyloid fibril formation. Moreover, it has been shown that molecules which are able to interact with both the active and peripheral sites of AChE could prevent the aggregating activity of AChE towards $A\beta$ besides the inhibitory activity [3,4,5]. Therefore, inhibitors with dual binding to AChE represent a new therapeutic strategic option [6,7].

In continuation of our on-going research on new AChE inhibitors [8], we will present our recent studies on acetylcholinesterase inhibitor in the tetracyclic triterpene series. We previously reported that compound **1** was identified by high-throughput screening of The Institut de Chimie des Substances Naturelles' chemical library. Some hemisynthetic analogs of **1** (i.e. **2a** and **2b**) were found to be very active on *Ee*AChE and *Tc*AChE but less potent on *h*AChE (Figure 1). The aim of the present study is to develop new potent *h*AChE inhibitors in this series.

Herein we describe the synthesis, pharmacological evaluations and molecular modeling of a novel class of analogs.

These new inhibitors contain a modified heterocycle which is supposed to interact with the catalytic site and new substituents connected to the nitrogen atom N 20, which is supposed to be responsible for the binding to the peripheral site of the enzyme.

Newly synthesized compounds were tested in vitro for AChE and BuChE inhibition potency. Their selectivity for *h*AChE was also evaluated. The ability of compounds **36e**, **36f**, **36i**, and **36k** to inhibit the AChE-induced A β aggregation compared with propidium iodide and tacrine was assayed by means of a thioflavin T-based fluorometric assay [9]. A molecular modeling study (docking and molecular dynamics simulations) of **1** and **36e** was also performed on *Ee*AChE.

[Figure 1.]

2. Chemistry

Introduction of fluorine atoms on a molecule can modify its properties [10]. In addition, the presence of a trifluoromethyl group on *m*-(N,N,N,-trimethylammonio) trifluoroacetophenone (TMTFA) or zifrosilone greatly improved their acetylcholinesterase inhibition potency [11]. Replacement of the isopropyl group present in the 1' position of the tritepene tetracyclic scaffold of **1** by other substituents bearing one, three or seven fluorine atoms was first envisaged. The new fluoro analogs **9a-9c** were synthesized from cyclobuxidine-F **4** obtained by hydrolysis of *N*-3-isobutyryl-cycloxobuxidine-F **3** (Scheme 1) [12]. Preparation of amides **6a-c** was carried out by coupling trifluoroacetic anhydride or 2-fluoro-2-methylpropanoic acid or methyl heptafluoroisobutyrate with cycloxobuxidine-F **4**. The 16-hydroxyfunction was first protected as an acetate (i.e. **7a-b**) or as a benzoate (i.e. **7c**). The nitrogen atom at position 20 was protected as a salt by reaction with APTS before the transformation of the alcohol group

at position 29 to the corresponding tosylates (i.e. **8a-c**). The acetate and benzoate protective groups were then removed under basic conditions. Finally, treatment of the resulting tosylates with sodium diformylamide in DMF provided the expected fluoro oxazines **9a-c** (Scheme 1).

[Scheme 1.]

The N-substituted tetrahydropyrimidines derivatives **13a-b** were synthesized following the reactions depicted in Scheme 2 using the aldehyde **10** [8] as key intermediate, which was converted into the corresponding amines **12a-b** by aminoreduction. Subsequent heating under microwaves irradiation furnished **13a-b** (Scheme 2).

[Scheme 2.]

The new dihydro-1H-imidazole analog **20** was synthesized from N-3-isobutyrylcycloheximidine-F **3**. The 16-hydroxy function was first protected as a benzoyl (i.e. **14**). Oxidation of the alcohol group at position 29 with Jones' reagent afforded the acid (i.e. **15**). Reaction of **15** with oxalyl chloride provided the corresponding acid chloride which reacted with sodium azide to give the carbonyl azide **16**. Curtius rearrangement of **16** under microwaves irradiation furnished the isocyanate **17**. Subsequent hydrolysis with lithium hydroxide, heating in methanol, and treatment under acid conditions provided a 90/10 mixture diamine **18** (major) and the amino amide **19** (minor). Finally, the dihydro-1H-imidazole **20** was prepared by reaction of **18** and methyl orthoisobutyrate in the presence of ytterbium triflate (Scheme 3).

[Scheme 3.]

The dihydropyrimidine **25** was synthesized from cycloheximidine -F **4**. First, the primary amine was protected with tert-butyl dicarbonate and the 16-hydroxy function was transformed into a benzoate (i.e. **22**). Oxidation of the alcohol group at position 29 with Dess-Martin periodinane afforded the corresponding aldehyde **23**. Aldolisation of **23** with 3-methyl-2-

butanone led to the keto alcohol **24**. Subsequent cyclization under acid conditions followed by deprotection of the benzoate group furnished the expected dihydropyridine **25** (Scheme 4).

[Scheme 4.]

The tetrahydropyridine **27** was synthesized from the keto alcohol **24**. Dehydration of **24** was first performed using potassium carbonate to afford the α,β -unsaturated ketone **26**. The tetrahydropyridine **27** was obtained after hydrogenation of **26** over palladium hydroxide and cyclization under acid conditions in the presence of trifluoroacetic acid (Scheme 5).

[Scheme 5.]

The synthesis of compound **30** was achieved following the strategy summarized in scheme 6. Reaction of the known tosylate **28** [13] with potassium thioacetate afforded a thioacetate intermediate that was directly hydrolyzed under basic conditions to provide the thiol **29**. The 5,6-dihydro-4*H*-1,3-thiazine **30** was obtained by cyclization of **29** in the presence of trimethylaluminium (Scheme 6).

[Scheme 6.]

Synthetic routes to *N*-substituted thiazine analogs **35a-b** and carbamate **35c** were also developed. The alcohol group at position 29 of **14** was transformed into the corresponding tosylate (i.e. **31**) and then substituted with potassium thioacetate to give the corresponding thioacetate. This later was converted into its *N*-oxide by oxidation with *m*-chloroperbenzoic acid. Subsequent treatment of this *N*-oxide with a mixture of ferrous sulfate heptahydrate and iron chloride hexahydrate provided *N*-demethylated amine **32**. Hydrolysis of the thioacetate **32** provided the thiol **33** which was cyclized in the presence of trimethylaluminium (i.e. **34**). Acylation or alkylation of the amine **34** furnished the expected amides **35a-c** and the tertiary amines **36a-k** (Scheme 7).

[Scheme 7.]

3. Results and Discussion

3.1 AChE and BuChE Inhibitory Activity and AChE/BuChE Selectivity

To determine the potential interest of the newly synthesized compounds for the treatment of AD, their AChE inhibitory activity was assayed by the method of Ellman [14] using *Electrophorus electricus* and human recombinant AChE and tacrine, **1** and **3** as reference compounds. Moreover, to further study the biological profiles of the novel compounds, their butyrylcholinesterase (BuChE) inhibitory activity on human serum butyrylcholinesterase (*h*BuChE) was also determined by the same method. Recent studies have shown that in AD patients with severe pathology, BuChE increases while AChE is reduced in specific brain regions [15] this is not in agreement with a recent study which shows a decrease of BuChE activity *in vivo* [16]. The results are summarized in Table 1.

[Table 1]

The inhibitory potency depends on numerous factors. Introduction of fluoro groups at position 1' of the oxazine scaffold (i.e. **9a-9c**) decreased the inhibitory activity for AChE and BuChE.

Replacement of the oxazine ring by different heterocycle rings affected the affinity for *Ee*AChE and *h*AChE. An order of the inhibitory potency for AChE can be established among the various heterocycles : alkylated tetrahydropyrimidines (i.e. **13a-b**) \approx imidazole (i.e. **20**) < tetrahydropyrimidine (i.e. **27**) < thiazine (i.e. **30**) < dihydropyrimidine (i.e. **25**). The dihydropyrimidine **25** is the most potent inhibitor in these series (12 fold more active than **1**) and selective for *h*AChE. Compounds **13a-b** and **20** are more potent on BuChE than on AChE.

The analogs of second generation with modified nitrogen atom at position 20 were also evaluated. This series was envisaged in order to increase the binding of the inhibitors with the peripheral site of AChE and thereby increasing the inhibitory potency.

N-demethylation of the nitrogen atom of the thiazine (i.e. **34**, *hAChE* IC₅₀ = 76 nM) produced no significant change on AChE inhibition (i.e. **30**, *hAChE* IC₅₀ = 60 nM) but decreased the selectivity for AChE (i.e. **34**, 17; i.e. **30**, 89).

In the amide series (i.e. **35a-35c**) introduction of a phenyl group (i.e. **35b**) increased the inhibition activity likely as a result of a better interaction with the peripheral site of the enzyme probably *via* hydrophobic interactions. We previously reported that compound **1** was able to bind simultaneously to both catalytic and peripheral sites of AChE. Its oxazine scaffold interacted with the active site and the nitrogen atom at position 20 with the peripheral site [8].

The AChE inhibitory potency within the N-alkylated series of thiazine derivatives is related to the substituent, to the substitution of R and to the length of the linker between R and the nitrogen atom. The inhibition activity follows this order : (CH₂)₂Bn (i.e. **36c**) < (CH₂)₂Pht (i.e. **36j**) < CH₂Bn (i.e. **36b**) < (CH₂)₃Pht (i.e. **36k**) < *o*-CF₃Bn (i.e. **36g**) < *p*-MeOBn (i.e. **36f**) < Bn (i.e. **36a**) ≈ *o*-MeOBn (i.e. **36d**) < *p*-CF₃Bn (i.e. **36h**) ≈ *m*-CF₃Bn (i.e. **36h**) ≈ *m*-MeOBn (i.e. **36e**). Introduction of a CF₃ substituent on the benzyl ring increased the selectivity for *hAChE* versus *BuChE*.

3.2 Molecular Modeling Studies

In order to gain insight into the mode of interaction between this new class of inhibitors and AChE, molecular modeling studies were performed using the compound **36e**, the most potent in the series, as a representative member. The results were compared with those obtained for the apo AChE and for the complex with oxazine **1**.

Molecular docking calculations showed that compound **36e** is positioned in a conformation similar to those proposed previously for oxazine **1** [8], and that it is able to interact with both the catalytic and peripheral sites of AChE. The binding site was defined as a sphere with a radius of 20 Å around the OH group of SER200 and the protein was kept rigid during the docking, whereas the ligand was fully flexible. No hydrogen bond could be identified between oxazine **1** and the protein, and only one was present in the AChE-**36e** complex, between OH group in position 16 and OH group of TYR70. Furthermore, in order to take into account the protein flexibility and a possible induced-fit that could take place upon ligand binding, molecular dynamics simulations were carried out, using the docking results as starting conformations.

Three molecular dynamics simulations, of 10 ns each, were set up, one with the apo protein (Sim1), for comparison purposes, and the other two with oxazine **1** (Sim2) and compound **36e** (Sim3). All three structures were generally stable during the simulation, the root mean square deviation (RMSD) for protein reaching a plateau in less than 5 ns, at 1.8 Å for Sim3 and at about 2 Å for Sim1 and Sim2. In Sim2 and Sim3, the ligands attained very quickly an equilibrium state with a RMSD of 0.7 Å, which was conserved throughout the simulation (Figure S1, Supporting Information). All simulations showed three main regions with higher flexibility (around residues 73, 341 and 378), but none of them were in the binding site region (Figure S2, Supporting Information).

Generally speaking, very few protein-ligand hydrogen bonds were identified in Sim2 and Sim3, the interactions being mostly hydrophobic. In Sim2, only the interaction between the OH group in position 16 of the steroid skeleton and the OH group of TYR70 was scarcely observed during the simulation (less than 10 % of the simulation time). In Sim3, three hydrogen bonds were present all along the simulation : i) the same interaction mentioned above, for about 50 % of the simulation time; ii) a hydrogen bond between the OH group in

position 16 of compound **36e** and the carboxyl group of ASP72, present all the time; iii) an interaction between the oxygen in position 11 of steroid and the OH group of TYR121, present for about 25 % of the simulation time. Therefore, it seems that compound **36e** can establish better interactions than oxazine **1** within the binding site of AChE and these interactions may be responsible, at least in part, for the good inhibition observed for the compound **36e**.

The evolution of the protein-ligand contact surface during the simulations Sim2 and Sim3 is shown in Figure S3 (Supporting Information). Whereas during the first 5 ns the surfaces are rather comparable, during the last 5 ns the protein interaction with compound **36e** is clearly more favored than those with oxazine **1**. A possible explanation for this behavior may be that AChE better accommodates the cyclopropyl-derived scaffold of compound **36e** than the seven-membered ring-derived core of oxazine **1**. This result is also in agreement with the lower RMSD observed for protein in Sim3 (see above) and possibly could explain the good biological activity measured experimentally for compound **36e**.

In the apo simulation (Sim1), the residues forming the binding site did not show important movements, with the exception of TRP84, which underwent a 3.6 Å movement in the direction of the binding site opening, and of the TYR334-PHE330-PHE331 triplet, which underwent a concerted movement resulting in the complete closure of the gorge (Figure 2). Thus, it seems that in the absence of a ligand this closed conformation is more stable than the open conformation originally present in the X-ray structure.

[Figure 2.]

[Figure 3.]

In both Sim2 and Sim3 simulations, the ligands move about 2.8 Å towards the peripheral site (Figures 3 and 4), to reach a stable, common equilibrium position (Figure S4, Supporting Information). In the meantime, the residues surrounding the ligand move apart in order to

accommodate its presence and to maximize the hydrophobic interactions. Unexpectedly, no cation- π and π - π interactions could be evidenced between the TRP279 at the peripheral site and the positively charged nitrogen in position 20 and/or its aromatic substituent (in the case of compound **36e**).

[Figure 4.]

3.3. Inhibition of AChE-Induced A β Aggregation

Three compounds, **36e**, **36h** and **36k**, were selected to assess their abilities to inhibit A β aggregation induced by AChE using a thioflavin T-based fluorometric assay [9], compared with the reference compound propidium iodide [9], a known specific peripheral site-binding inhibitor and tacrine a known specific inhibitor of the active site [9]. The N substituted thiazines **36e**, **36h** and **36k** inhibit, at 100 μ M, the AChE-induced A β aggregation with percentages of inhibition ranging from 42% to 69% (Table 2). By observation of the data reported in Table 2, it appears that introduction of a meta CF₃ group on the N benzyl terminal group (i.e **36h**) or a phthalimide terminal group (i.e. **36k**) decrease the anti aggregating action. In contrast, introduction of a *m*-OMeBn (i.e. **36e**) favors the anti aggregating action. The best compound **36e** allows a better positioning of the methoxy aromatic ring with the peripheral site of the enzyme.

Thiazines **36e** could be classified as good inhibitor of the AChE-induced A β aggregation. The most potent described inhibitors inhibit, at 100 μ M, the AChE-induced A β aggregation with percentages of inhibition ranging from 82% to 98% [17,18,19,20,21].

[Table 2.]

4. Conclusion

We have synthesized new inhibitors in the tetracyclic triterpene series. These compounds have been synthesized from the natural product N-3-isobutyrylcyclobuxidine-F. Variations of

the heterocycle led us to identify that presence of a thiazine or a dihydropyrimidine ring enhanced the *h*AChE inhibitory potency. Alkylation of the nitrogen atom at position 20 with functionalized benzyl groups in the thiazine series also increased the *h*AChE inhibitory activity. Molecular modeling studies showed that **1** and **36e** interact simultaneously with both the catalytic and peripheral sites of *Ee*AChE, the nature of protein-ligand interactions being mainly hydrophobic. Furthermore, **36e** significantly prevented the AChE-induced $A\beta$ aggregation. Taken together, all the reported results suggested that new dual binding AChE inhibitors in the thiazine tetracyclic triterpene series are promising leads for the development of disease-modifying drugs for the future treatment of AD.

5. Experimental section

5.1. Chemistry

All commercially available reagents were used without further purification. All experiments involving water-sensitive compounds were carried out under argon and scrupulously dry conditions, using anhydrous solvents. MeOH was distilled from Mg/I₂, CH₂Cl₂ from P₂O₅, 1,4-dioxane from LiAlH₄, NEt₃ and pyridine from KOH. All separations were carried out under flash chromatographic conditions on Merck silica gel 60 (70-230 mesh) at medium pressure (200 mbar). TLC was performed on Merck silica gel plates (60F₂₅₄) with a fluorescent indicator. NMR spectra were determined on Bruker Avance-300, Bruker AC-400, Bruker AC-500 or Bruker-600 instruments and using tetramethylsilane (TMS) as reference. Chemical shifts are reported in parts per million (ppm) relative to TMS. High resolution mass spectra (HRMS) were obtained on a maldi-toff spectrometer. The assignement of the ¹H and ¹³C NMR spectra of the different compounds has been made by ¹H-¹H, ¹H-¹³C, COSY, HMBC, HMQC and by analogy with previously described compounds. The numerotation indicated on molecule is only mentioned for the NMR spectra interpretation. Infrared (IR) spectra were recorded on a Fourier Perkin-Elmer Spectrum BX

FT-IR instruments. Elemental analyses were performed by the microanalysis laboratory of the ICSN, CNRS, Gif-sur-Yvette.

5.1.1. N-[20S-(dimethylamino)-16 α -hydroxy-4 α -(hydroxymethyl)-4 β ,14 α -dimethyl-11-oxo-9,19-cyclo-5 α ,9 β -pregnan-3 β -yl]-trifluoroacetamide (6a).

To 68 mg (0.158 mmol, 1 eq.) of cycloxobuxidine-F 3 solubilised in DCM/pyridine (mixture 3/1, 4 mL) were added at 0 °C 25 μ L (0.174 mmol, 1.1 eq.) of trifluoroacetic anhydride. The mixture was stirred at 0 °C for 1 h then at RT for further 12 h. After 3 co-evaporations with 3x10 mL of 1,2-dichloroethane, the residue was dissolved in DCM, washed with 20 mL of a saturated solution of sodium hydrogen carbonate then extracted with 3x20 mL of DCM. Combined organic layers were washed with brine (20 mL), dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by silica gel column chromatography (eluent : DCM/MeOH/NH₄OH : 96/3/1) to afford the title compound as an amorphous white solid (70 mg, yield 84 %). IR (cm⁻¹) : 3276, 2944, 2872, 1698, 1698, 1555, 1454, 1157, 1187, 1209 ; ¹H NMR (300 MHz, CDCl₃): δ (ppm) 6.43 (1H, d, J_{NAH-H3} = 9.2 Hz, N_A-H), 4.16 (1H, m, H16 β), 4.12 (1H, m, H3 α), 3.42 (1H, d, J_{gem} = 12.9 Hz, H29b), 3.03 (1H, d, J_{gem} = 12.9 Hz, H29a), 2.70 (1H, dq, J_{20-17 α} = 10.8 Hz, J₂₀₋₂₁ = 6.7 Hz, H20), 2.54 (1H, d, J_{gem} = 17.2 Hz, H12 α), 2.50 (1H, ddd, J_{gem} = 13.2 Hz, J_{1 β -2} = 3.6 Hz, J_{1 β -2} = 3.4 Hz, H1 β), 2.32 (1H, d, J_{gem} = 17.2 Hz, H12 β), 2.31 (6H, sl, N_BMe₂), 2.10 (1H, dd, J_{5 α -6 β} = 12.5 Hz, J_{5 α -6 α} = 3.6 Hz, H5 α), 2.01 (3H, m, H17 α +H15 β +H8 β), 1.80 (1H, m, H2 α), 1.71 (2H, m, H2 β +H6 α), 1.57 (2H, m, H19 α +H7 β), 1.52 (1H, m, H15 α), 1.43 (1H, m, H7 α), 1.31 (1H, m, H1 α), 1.22 (3H, s, H28), 1.06 (1H, d, J_{gem} = 3.8 Hz, H19 β), 0.92 (4H, m, J₂₁₋₂₀ = 6.6 Hz, H21+H6 β), 0.86 (3H, s, H18), 0.64 (3H, s, H30) ; ¹³C NMR (75.4 MHz, CDCl₃): δ (ppm) 211.3 (C11), 158.0 (C1'), 113.9 (C2'), 78.0 (C16), 64.2 (C29), 62.4 (C20), 55.8 (C17), 52.4 (C3), 51.4 (C12), 47.1 (C14), 44.6 (C4+C13), 43.1 (C15), 41.5 (C8), 41.2 (C5), 37.2 (C10), 34.3 (C9), 30.5 (C19), 27.4 (C1), 27.2 (C2), 24.3 (C7), 20.7 (C28), 18.4 (C6), 17.9 (C18),

11.2 (C30), 10.1 (C21). MS (ESI, m/z) : 529.2 (M+H); HRMS (ESI, m/z) : calcd for C₂₈H₄₄N₂O₄F₃ : 529.3253, found : 529.3254.

5.1.2. 20S-(dimethylamino)-4 α -(hydroxymethyl)-4 β ,14 α -dimethyl-11-oxo-3 β -(trifluoroacetyl) amino]-9,19-cyclo-5 α ,9 β -pregnan-16 α -yl acetate (7a).

To 144 mg (0.27 mmol, 1 eq.) of **6a** solubilised in 5 mL of a mixture DCM/pyridine (3/1) were added at 0 °C 27 μ L (0.286 mmol, 1.05 eq.) of acetic anhydride. The mixture was stirred at 0 °C for 1 h then at RT for further 12 h. After 3 co-evaporations with 3x10 mL of 1,2-dichloroethane, the residue was dissolved in DCM, washed with 20 mL of a saturated solution of sodium hydrogen carbonate then extracted with 3x20 mL of DCM. Combined organic layers were washed with brine (20 mL), dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by neutral alumina column chromatography (eluent : DCM/MeOH : 99/1 to 97/3) to afford the title compound as an amorphous white solid (112 mg, yield 72 %). IR (cm⁻¹) : 3296, 2966, 2935, 2873, 2832, 1716, 1698, 1668, 1558, 1456, 1251, 1208, 1185, 1156 ; ¹H NMR (300 MHz, CDCl₃): δ (ppm) 6.90 (1H, d, J_{NAH-H3} = 9.0 Hz, N_A-H), 5.11 (1H, m, H16 β), 4.07 (1H, m, H3 α), 3.40 (1H, d, J_{gem} = 12.9 Hz, H29b), 3.00 (1H, d, J_{gem} = 12.9 Hz, H29a), 2.53 (1H, d, J_{gem} = 17.2 Hz, H12 α), 2.46 (2H, m, H1 β +H20), 2.36 (1H, d, J_{gem} = 17.2 Hz, H12 β), 2.21 (1H, dd, J_{17 α -20} = 11.1 Hz, J_{17 α -16 β} = 5.8 Hz, H17 α), 2.13 (6H, sl, N_BMe₂), 2.00-2.09 (3H, m, H5 α +H8 β +H15 β), 1.98 (3H, s, H32), 1.62-1.79 (3H, m, H2 α +H2 β +H6 α), 1.56 (1H, d, J_{gem} = 3.8 Hz, H19 β), 1.51 (1H, m, H7 β), 1.42 (1H, m, H15 α), 1.25-1.36 (2H, m, H7 α +H1 α), 1.14 (3H, s, H28), 1.05 (1H, d, J_{gem} = 3.8 Hz, H19 β), 0.90 (1H, m, H6 β), 0.83 (3H, s, H18), 0.80 (3H, d, J₂₁₋₂₀ = 6.3 Hz, H21), 0.62 (3H, s, H30) ; ¹³C NMR (75.4 MHz, CDCl₃): δ (ppm) 211.3 (C11), 170.6 (C31), 158.4 (q, J_{C1'-F} = 37.3 Hz, C1'), 115.8 (q, J_{C2'-F} = 287.6 Hz, C2'), 78.8 (C16), 63.9 (C29), 59.4 (C20), 54.9 (C17), 52.2 (C3), 51.8 (C12), 47.3 (C14), 44.6 (C4), 44.4 (C13), 42.9 (C15), 41.3 (C8), 41.2

(C5), 40.1 (N_BMe₂), 37.3 (C10), 33.8 (C9), 30.5 (C19), 27.4 (C1), 27.0 (C2), 24.2 (C7), 21.8 (C32), 19.5 (C28), 18.2 (C6), 17.8 (C18), 11.1 (C30), 9.77 (C21); MS (ESI, m/z) : 571.3 (M+H), 529.3; HRMS (ESI, m/z) : calcd for C₃₀H₄₆N₂O₅F₃ : 571.3359, found : 571.3385.

*5.1.3. [20S-(dimethylamino)-16 α -hydroxy-4 β ,14 α -dimethyl-11-oxo-3 β -(trifluoroacetyl)amino]-9,19-cyclo-5 α ,9 β -pregnan-4 α -yl}methyl tosylate (**8a**).*

To 110 mg (0.19 mmol, 1 eq.) of **7a** solubilised in 5 mL of anhydrous MeOH were added 37 mg (0.19 mmol, 1 eq.) of *p*-toluenesulfonic acid and a small amount of sodium sulfate. The mixture was stirred at RT for 1 h and filtered through a pad of celite. The solvent was removed and the residue was dried several minutes under vacuum. 1.5 mL of pyridine and 110 mg (0.19 mmol, 1 eq.) of tosyl chloride were added. The mixture was stirred at 90 °C for 3 h. After 3 co-evaporations with 3x10 mL of 1,2-dichloroethane, the residue was dissolved in 10 mL of a mixture MeOH/water (3/1) and a small amount of potassium carbonate was added. The mixture was stirred at RT for 3 h and the MeOH was removed under vacuum. The mixture was extracted with 3x10 mL of DCM. Combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by silica gel column chromatography (eluent : DCM/MeOH/NH₄OH : 98/1/1) to afford the title compound as an amorphous light yellow solid (70 mg, yield 50 %). IR (cm⁻¹) : 3321, 2924, 2869, 1719, 1667, 1551, 1454, 1367, 1159, 1189, 1208, 1175 ; ¹H NMR (300 MHz, CDCl₃): δ (ppm) 7.77 (2H, d, J = 8.3 Hz, H32+H36), 7.34 (d, 2H, J = 8.3 Hz, H33+H35), 5.92 (1H, d, J_{NAH-H3} = 9.8 Hz, N_A-H), 4.27-4.08 (2H, m, H3 α +H16 β), 3.86 (1H, d, J_{gem} = 9.9 Hz, H29b), 3.43 (1H, d, J_{gem} = 9.9 Hz, H29a), 2.65 (1H, m, H20), 2.53 (1H, d, J_{gem} = 17.2 Hz, H12 α), 2.45 (1H, m, H1 β), 2.43 (3H, s, H37), 2.30 (1H, d, J_{gem} = 17.2 Hz, H12 β), 2.28 (6H, sl, N_BMe₂), 2.01 (4H, m, H17 α +H5 α +H8 β +H15 β), 1.79 (1H, m, H2 α), 1.56 (1H, m, H2 β), 1.54 (1H, d, J_{gem} = 3.9 Hz, H19 β), 1.51 (1H, m, H7 β), 1.50 (2H, m, H6 α +H15 α),

1.34 (1H, m, H7 α), 1.29 (1H, m, H1 α), 1.19 (3H, s, H28), 1.01 (1H, d, $J_{\text{gem}} = 3.9$ Hz, H19 β), 0.94 (1H, m, H6 β), 0.89 (3H, d, $J_{21-20} = 6.5$ Hz, H21), 0.84 (3H, s, H18), 0.75 (3H, s, H30) ; ^{13}C NMR (75.4 MHz, CDCl_3): $\delta(\text{ppm})$ 211.1 (C11), 156.4 (q, $J_{\text{C1}'-\text{F}} = 36.8$ Hz, C1'), 145.1 (C34), 131.9 (C31), 129.8 (C33+C35), 128.1 (C32+C36), 115.7 (q, $J_{\text{C2}'-\text{F}} = 288.2$ Hz, C2'), 78.0 (C16), 70.2 (C29), 62.1 (C20), 55.8 (C17), 51.4 (C12), 51.3 (C3), 47 (C14), 44.4 (C13), 43.0 (C4), 42.9 (C15), 42.3 (C8), 41.8 (C5), 36.6 (C10), 34.1 (C9), 30.4 (C19), 27.6 (C2), 27.0 (C1), 24.3 (C7), 21.6 (C37), 20.9 (C28), 18.5 (C6), 17.9 (C18), 11.1 (C30), 10.0 (C21) ; MS (ESI, m/z) : 683.3 (M+H) ; HRMS (ESI, m/z) : calcd for $\text{C}_{35}\text{H}_{50}\text{N}_2\text{O}_6\text{SF}_3$: 683.3342, found : 683.3305.

5.1.4. 20S-(dimethylamino)-16 α -hydroxy-4 β ,14 α -dimethyl-9,19-cyclo-{2'-trifluoromethyl-5',6'-dihydro-4' α H-[1',3']oxazino[4',5':3,4]}-5 α ,9 β -pregnan-11-one (9a).

To 34 mg (0.05 mmol, 1 eq.) of **8a** solubilised in 2 mL of anhydrous DMF were added 14 mg (0.15 mmol, 3 eq.) of sodium diformylamide. The mixture was stirred at 120 °C for 5 h, washed with 20 mL of a sodium hydrogen carbonate saturated solution and extracted with 5x30 mL of DCM. Combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by preparative TLC (eluent : DCM/MeOH/ NH_4OH : 89/10/1) to afford the title compound as an amorphous colourless solid (17 mg, yield 68 %). IR (cm^{-1}) : 3669, 2924 ($\nu_{\text{aC-H}}$), 1719, 1698, 1455, 1151, 1209 ; ^1H NMR (300 MHz, CDCl_3): $\delta(\text{ppm})$ 4.26 (1H, m, H16 β), 4.17 (1H, d, $J_{\text{gem}} = 10.3$ Hz, H29b), 3.99 (1H, d, $J_{\text{gem}} = 10.3$ Hz, H29a), 3.16 (1H, m, H3 α), 2.79 (1H, m, H20), 2.53 (1H, d, $J_{\text{gem}} = 17.1$ Hz, H12 α), 2.43 (1H, m, H1 β), 2.40 (6H, sl, $\text{N}_\text{B}\text{Me}_2$), 2.34 (1H, d, $J_{\text{gem}} = 17.1$ Hz, H12 β), 2.13 (1H, m, H5 α), 2.03 (1H, m, H15 β), 2.04 (1H, m, H17 α), 1.99 (1H, m, H2 α), 1.68 (1H, d, $J_{\text{gem}} = 4.1$ Hz, H19 β), 1.58 (1H, m, H7 β), 1.55 (1H, m, H2 β), 1.54 (1H, m, H8 β), 1.51 (1H, m, H15 α), 1.50 (1H, m, H6 α), 1.27 (1H, m, H1 α), 1.23 (1H, m, H7 α), 1.22

(3H, s, H28), 1.12 (1H, d, $J_{\text{gem}} = 4.1$ Hz, H19 β), 1.02 (1H, m, H6 β), 0.98 (3H, d, $J_{21-20} = 6.4$ Hz, H21), 0.85 (3H, s, H18), 0.87 (3H, s, H30) ; ^{13}C NMR (75.4 MHz, CDCl_3): δ (ppm) 211.1 (C11), 147.0 (C1'), 78.2 (C16), 77.4 (C29), 63.0 (C20), 59.9 (C3), 55.9 (C17), 51.4 (C12), 47.2 (C14), 44.9 (C13), 46.1 (C8), 43.2 (C15), 40.6 (C5), 36.7 (C10), 35.1 (C4), 33.9 (C9), 29.8 (C19), 28.0 (C1), 27.7 (C2), 23.7 (C7), 20.3 (C28), 18.2 (C6), 17.9 (C18), 10.9 (C30), 10.4 (C21). MS (ESI, m/z) : 511.3 (M+H) ; HRMS (ESI, m/z) : calcd for $\text{C}_{28}\text{H}_{42}\text{N}_2\text{O}_3\text{F}_3$: 511.3148, found : 511.3129.

5.1.5. *N-[20S-(dimethylamino)-16 α -hydroxy-4 α -(mercaptomethyl)-4 β ,14 α -dimethyl-11-oxo-9,19-cyclo-5 α ,9 β -pregnan-3 β -yl]-isobutyramide (29).*

To 40 mg (0.057 mmol, 1 eq.) of **28** dissolved in 0.5 mL of DMPU were added 98 mg (0.86 mmol, 15 eq.) of sodium thioacetate. The mixture was stirred at 100 °C for 5 h then diluted with 10 mL of a saturated sodium hydrogen carbonate solution and extracted with 20 mL of Et_2O . The organic layer was dried with brine, over magnesium sulfate and concentrated under vacuum. The residue was dissolved in 20 mL of a degassed mixture MeOH/water (7/3) and a small amount of potassium hydroxide was added. The mixture was stirred at RT for 3 h. The MeOH was removed under reduced pressure and the mixture was extracted with 3x20 mL of DCM. Combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by silica gel column chromatography (gradient elution : DCM/MeOH/ NH_4OH : from 99/0/1 to 90/10/1) to afford the title compound as an amorphous white solid (20 mg, yield: 67 %). IR (cm^{-1}) : 3322, 2964, 2937, 2869, 2833, 1655, 1534, 1460, 1225, 1159, 1094 ; ^1H NMR (300 MHz, CDCl_3): δ (ppm) 5.23 (1H, d, $J_{\text{NAH-3}} = 9.9$ Hz, N_A-H), 4.15 (1H, m, H3 α), 4.12 (1H, m, H16 β), 2.64 (1H, dq, $J_{20-17} = 10.8$ Hz, $J_{20-21} = 6.5$ Hz, H20), 2.53 (1H, d, $J_{\text{gem}} = 17.1$ Hz, H12 α), 2.49 (1H, m, H29b), 2.41 (1H, m, H1 β), 2.34 (1H, m, H2'), 2.29 (1H, d, $J_{\text{gem}} =$

17.1 Hz, H12 β), 2.26 (6H, bs, N_BMe), 2.22 (1H, m, H5), 2.19 (1H, m, H29a), 2.02 (2H, m, H8 β +H15 β), 1.99 (1H, m, H17 α), 1.72 (1H, m, H2 α), 1.56 (1H, d, J_{gem} = 3.4 Hz, H19 α), 1.55 (1H, m, H7 β), 1.51 (1H, m, H15 α), 1.49 (1H, m, H2 β), 1.38 (1H, m, H7 α), 1.33 (1H, m, H6 α), 1.31 (1H, m, H1 α), 1.23 (3H, s, H28), 1.17+1.14 (6H, 2d, J = 7.2 Hz, H3'+H4'), 1.03 (1H, d, J_{gem} = 3.4 Hz, H19 β), 0.92 (1H, m, H6 β), 0.89 (3H, d, J₂₁₋₂₀ = 6.5 Hz, H21), 0.85 (3H, s, H18), 0.78 (3H, s, H30); ¹³C NMR (75.4 MHz, CDCl₃): δ (ppm) 211.4 (C11), 176.7 (C1'), 78.1 (C16), 62.0 (C20), 55.7 (C17), 51.4 (C12), 49.7 (C3), 47.0 (C14), 44.4 (C13), 43.1 (C4), 42.9 (C15), 42.0 (C5), 41.9 (C8), 37.6 (C10), 35.8 (C2'), 34.2 (C9), 31.2 (C29), 30.9 (C19), 28.5 (C2), 27.4 (C1), 24.3 (C7), 20.8 (C28), 20.1+19.4 (C3'+C4'), 18.4 (C6), 17.8 (C18), 15.5 (C30), 9.9 (C21); MS (ESI, m/z) : 519.3 (M+H); HRMS (ESI, m/z) : calcd for C₃₀H₅₁N₂O₃S : 519.3620, found : 519.3611.

5.1.6. 20S-(dimethylamino)-16 α -hydroxy-4 β ,14 α -dimethyl-9,19-cyclo-{2'-isopropyl-5',6'-dihydro-4' α H-[1',3']thiazino[4',5':3,4]}-5 α ,9 β -pregnan-11-one (30). To 133 mg (0.256 mmol, 1 eq.) of **29** dissolved in 5 mL of 1,2-dichloroethane were added 640 μ L (1.28 mmol, 5 eq.) of trimethylaluminium (2M in toluene). The mixture was refluxed for 3 h then cooled to RT. The mixture was quenched with 3 mL of sodium hydroxide (1 N), diluted 10 mL of a saturated potassium hydrogen carbonate solution and extracted with 3x10 mL of DCM. Combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by silica gel column chromatography (gradient elution : DCM/MeOH/NH₄OH : from 99/0/1 to 90/10/1) to afford the title compound as an amorphous white solid (76 mg, yield: 59 %). IR (cm⁻¹) : 3330, 2962, 2933, 2866, 1661, 1633, 1462, 1260, 1124, 1157, 1088 ; ¹H NMR (300 MHz, CDCl₃): δ (ppm) 4.10 (1H, m, H16 β), 2.84 (1H, m, H3 α), 2.83 (1H, d, J_{gem} = 11.6 Hz, H29b), 2.73 (1H, d, J_{gem} = 11.6 Hz, H29a), 2.63 (1H, dq, J_{20-17 α} = 9.9 Hz, J₂₀₋₂₁ = 6.6 Hz, H20), 2.58 (1H, m, H2'),

2.51 (1H, d, $J_{\text{gem}} = 17.0$ Hz, H12 α), 2.45 (1H, dt, $J = 13.4$ Hz, $J = 3.5$ Hz, H1 β), 2.32 (1H, d, $J_{\text{gem}} = 17.0$ Hz, H12 β), 2.25 (6H, bs, N_BMe₂), 2.10 (1H, m, H8 β), 2.05 (1H, m, H2 α), 2.01 (1H, m, H15 β), 1.98 (1H, m, H17 α), 1.71 (1H, m, H2 β), 1.64 (1H, d, $J_{\text{gem}} = 3.9$ Hz, H19 α), 1.58 (1H, m, H7 β), 1.53 (1H, m, H6 α), 1.49 (1H, m, H15 α), 1.42 (1H, m, H5 α), 1.34 (1H, m, H7 α), 1.31 (1H, m, H1 α), 1.21 (3H, s, H28), 1.17 and 1.18 (6H, 2d, $J = 6.9$ Hz, H3'+H4'), 1.11 (1H, d, $J_{\text{gem}} = 3.9$ Hz, H19 β), 1.01 (1H, m, H6 β), 0.88 (3H, d, $J_{21-20} = 6.6$ Hz, H21), 0.85 (3H, s, H18), 0.76 (3H, s, H30); ¹³C NMR (75.4 MHz, CDCl₃): δ (ppm) 210.8 (C11), 165.7 (C1'), 78.3 (C16), 62.7 (C3), 62.0 (C20), 55.7 (C17), 51.4 (C12), 49.8 (C5), 47.1 (C14), 44.5 (C13), 42.8 (C15), 41.4 (C8), 40.2 (C2'), 38.7 (C29), 38.4 (C10), 34.5 (C9), 33.4 (C4), 30.6 (C2), 30.2 (C19), 28.0 (C1), 24.4 (C7), 20.8 and 21.0 (C3'+C4'), 20.7 (C28), 18.0 (C6), 17.9 (C18), 11.3 (C30), 9.9 (C21); MS (ESI, m/z) : 501.3 (M+H); HRMS (ESI, m/z) : calcd for C₃₀H₄₉N₂O₂S : 501.3515, found : 501.3537.

5.1.7. *N*-{16 α -hydroxy-4 β ,14 α -dimethyl-11-oxo-9,19-cyclo-{2'-isopropyl-5',6'-dihydro-4' α H-[1',3']thiazino[4',5':3,4]}-5 α ,9 β -pregnan-20S-yl}-*N*-methylacetamide (**35a**).

To 12 mg (0.0246 mmol, 1 eq.) of **34** dissolved in 1 mL of absolute EtOH were added 3 μ L (0.032 mmol, 1.3 eq.) of acetic anhydride. The mixture was stirred at RT for 3 h and concentrated under vacuum. 5 mL of a saturated sodium hydrogen carbonate solution were added and the mixture was extracted with 3x5 mL of DCM. Combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by silica gel column chromatography (gradient elution : DCM/MeOH/NH₄OH : from 99/0/1 to 97/2/1) to afford the title compound as an amorphous white solid (12 mg, yield: 92 %). IR (cm⁻¹) : 3306, 2962, 2930, 2868, 1662, 1612, 1454, 1258, 1114, 1082, 1016; ¹H NMR (300 MHz, CDCl₃): δ (ppm) 4.99 (1H, dq, $J_{20-17} = 11.3$ Hz, $J_{20-21} = 6.8$ Hz, H20), 4.20 (1H, m, H16 β), 2.89 (3H, s, N_BMe), 2.88 (1H, m, H3 α), 2.84 (1H, d,

$J_{\text{gem}} = 11.5 \text{ Hz}$, H29b), 2.74 (1H, d, $J_{\text{gem}} = 11.5 \text{ Hz}$, H29a), 2.58 (1H, m, H2'), 2.57 (1H, d, $J_{\text{gem}} = 17.0 \text{ Hz}$, H12 α), 2.48 (1H, dt, $J_{\text{gem}} = 13.6 \text{ Hz}$, $J_{1\beta-2} = 3.2 \text{ Hz}$, H1 β), 2.37 (1H, d, $J_{\text{gem}} = 17.0 \text{ Hz}$, H12 β), 2.11 (1H, m, H17 α), 2.08 (3H, s, H32), 2.07 (1H, m, H8 β), 2.02 (1H, m, H15 β), 1.73 (1H, m, H2 α), 1.65 (1H, d, $J_{\text{gem}} = 4.1 \text{ Hz}$, H19 α), 1.62 (1H, m, H2 β), 1.54 (2H, m, H6 α +H7 β), 1.42 (1H, m, H15 α), 1.39 (1H, m, H5 α), 1.33 (1H, m, H1 α), 1.30 (1H, m, H7 α), 1.20 (3H, s, H28), 1.20+1.18 (6H, 2d, $J = 7.0 \text{ Hz}$, H3'+H4'), 1.13 (1H, d, $J_{\text{gem}} = 4.1 \text{ Hz}$, H19 β), 1.09 (3H, d, $J_{21-20} = 6.8 \text{ Hz}$, H21), 1.03 (1H, m, H6 β), 0.96 (3H, s, H18), 0.77 (3H, s, H30); ^{13}C NMR (75.4 MHz, CDCl_3): $\delta(\text{ppm})$ 210.4 (C11), 173.7 (C31), 171.0 (C1'), 75.6 (C16), 62.6 (C3), 57.1 (C17), 51.6 (C12), 49.8 (C5), 48.2 (C20), 47.4 (C14), 46.1 (C15), 45.6 (C13), 41.4 (C8), 40.1 (C2'), 38.7 (C29), 38.4 (C10), 34.0 (C9), 33.5 (C4), 30.5 (C2+C19), 30.4 ($\text{N}_\text{B}\text{Me}$), 27.9 (C1), 24.5 (C7), 22.5 (C32), 21.1+20.8 (C3'+C4'), 19.9 (C28), 18.1 (C21), 18.0 (C18+C6), 11.3 (C30); MS (ESI, m/z) : 529.3 (M+H); HRMS (ESI, m/z) : calcd for $\text{C}_{31}\text{H}_{49}\text{N}_2\text{O}_3\text{S}$: 529.3464, found : 529.3461.

5.1.8. *20S-[benzyl(methyl)amino]-16 α -hydroxy-4 β ,14 α -dimethyl-9,19-cyclo-{2'-isopropyl-5',6'-dihydro-4' α H-[1',3']thiazino[4',5':3,4]}-5 α ,9 β -pregnan-11-one (36a).* To 13 mg (0.0267 mmol, 1 eq.) of **34** dissolved in 1 mL of acetonitrile were added 4.1 μL (0.035 mmol, 1.3 eq.) of benzyl bromide and 11 mg (0.08 mmol, 3 eq.) of potassium carbonate. The mixture was stirred at RT for 16 h, diluted with 5 mL of a saturated sodium hydrogen carbonate solution then extracted with 3x5 mL of DCM. Combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by silica gel column chromatography (gradient elution: DCM/MeOH/ NH_4OH : from 99/0/1 to 96/3/1) to afford the title compound as an amorphous white solid (14 mg, yield: 93 %). IR (cm^{-1}) : 3324, 2962, 2929, 2867, 1666, 1633, 1452, 1259, 1223, 1147, 1079, 1017 ; ^1H NMR (500 MHz, CD_3CN , 333K): $\delta(\text{ppm})$ 7.30 (5H, m, H33+H34+H35+H36+H37), 3.99 (1H,

m, H16 β), 3.72 (1H, d, $J_{\text{gem}} = 12.8$ Hz, H31), 3.59 (1H, d, $J_{\text{gem}} = 12.8$ Hz, H31), 2.86 (2H, m, H20+H29b), 2.84 (1H, m, H3 α), 2.75 (1H, d, $J_{\text{gem}} = 11.6$ Hz, H29a), 2.53 (1H, d, $J_{\text{gem}} = 17.1$ Hz, H12 α), 2.48 (1H, m, H2'), 2.35 (1H, dt, $J_{\text{gem}} = 13.6$ Hz, $J_{1\beta-2} = 3.2$ Hz, H1 β), 2.23 (1H, d, $J_{\text{gem}} = 17.1$ Hz, H12 β), 2.21 (3H, s, N_BMe), 2.12 (1H, m, H17 α), 2.08 (1H, m, H8 β), 1.97 (1H, m, H15 β), 1.95 (1H, m, H2 α), 1.63 (1H, m, H2 β), 1.53 (1H, m, H7 β), 1.51 (1H, m, H6 α), 1.50 (1H, d, $J_{\text{gem}} = 4.0$ Hz, H19 α), 1.47 (1H, m, H5 α), 1.39 (1H, m, H15 α), 1.33 (1H, m, H7 α), 1.31 (1H, m, H1 α), 1.18 (3H, s, H28), 1.13 (1H, m, H19 β), 1.12 (6H, 2d, $J = 6.7$ Hz, H3'+H4'), 1.05 (1H, m, H6 β), 0.98 (3H, d, $J_{21-20} = 6.4$ Hz, H21), 0.75 (3H, s, H18), 0.73 (3H, s, H30); ¹³C NMR (75.4 MHz, CD₃CN): δ (ppm) 210.8 (C11), 165.9 (C1'), 138.7 (C32), 129.1 (C33+C37), 128.5 (C34+C36), 127.3 (C35), 77.6 (C16), 62.7 (C3), 60.3 (C20), 59.4 (C31), 55.8 (C17), 51.4 (C12), 49.7 (C5), 47.2 (C14), 44.7 (C13), 42.8 (C15), 41.3 (C8), 40.2 (C2'), 38.7 (C29), 38.4 (C10), 35.3 (N_BMe), 34.4 (C9), 33.4 (C4), 30.5 (C2), 30.3 (C19), 28.0 (C1), 24.4 (C7), 21.0+20.8 (C3'+C4'), 20.6 (C28), 18.0 (C6), 17.7 (C18), 11.3 (C30), 10.7 (C21); MS (ESI, m/z) : 577.4 (M+H), 487.3 (M-Bn+H); HRMS (ESI, m/z) : calcd for C₃₆H₅₃N₂O₂S : 577.3828, found : 577.3835.

5.2. Biochemical Methods

5.2.1. In vitro AChE Inhibition Assay

Enzymes : *EeAChE* from *electrophorus electricus* (reference C 2888) and human recombinant *hAChE* (reference C 1682) were purchased from Sigma.

Inhibition of AChE activity was determined by the spectroscopic method of Ellman *et al.* [14], using acetylthiocholine iodide as substrate, in 96-well microtiter plates. All solutions were brought to room temperature prior to use. Aliquots of 200 μ L of a solution containing 640 μ L 10 mM DTNB in 0.1 M sodium phosphate, pH 8.0, 19.2 mL of the same buffer, and 13 μ L of a solution of AChE (100U/mL) in water, were added to each well, followed by 2 μ L

of a DMSO solution of the inhibitor. The reaction was initiated by adding 20 μL of acetylthiocholine iodide (7.5 mM) to each well, and followed by monitoring the appearance of the thiolate dianion produced by reduction of DTNB at 412 nm for 120 s at 25° C in a Molecular Devices Spectra Max 384 Plus plate reader. Each inhibitor was evaluated at several concentrations in the range of 10^{-9} - 2×10^{-5} M. Percentage inhibition was calculated relative to a control sample (DMSO), and IC_{50} values displayed represent the mean \pm standard deviation for triplicate assays.

5.2.2. *In vitro* BuChE Inhibition Assay

Enzyme : *h*BuChE from human serum (reference C 9971) was purchased from Sigma. Inhibition of BuChE activity was determined by the spectroscopic method of Ellman *et al.* [14], using butyrylthiocholine iodide as substrate, in 96-well microtiter plates. All solutions were brought to room temperature prior to use. Aliquots of 200 μL of a solution containing 640 μL 10 mM DTNB in 0.1 M sodium phosphate, pH 8.0, 19.2 mL of the same buffer, and 13 μL of a solution of BuChE (100U/mL) in water, were added to each well, followed by 2 μL of a DMSO solution of the inhibitor. The reaction was initiated by adding 20 μL of butyrylthiocholine iodide (7.5 mM) to each well, and followed by monitoring the appearance of the thiolate dianion produced by reduction of DTNB at 412 nm for 120 s at 25 °C in a Molecular Devices Spectra Max 384 Plus plate reader. Each inhibitor was evaluated at several concentrations in the range of 10^{-9} - 2×10^{-5} M. Percentage inhibition was calculated relative to a control sample, and IC_{50} values displayed represent the mean \pm standard deviation for triplicate assays.

5.2.3. *Inhibition of AChE-Induced A β_{40} Aggregation Assay*

Thioflavine T (T3516), 1,1,1,3,3,3,-hexafluoro-2-propanol (HFIP) and human recombinant

hAChE (reference C 1682) were purchased from Sigma Chemicals. β -amyloid₁₋₄₀ (*A β* 40) trifluoroacetate salt was purchased from Bachem AG (Bubendorf, Switzerland). Aliquots of 2 μ L of *A β* (1-40), lyophilized from 2 mg/mL HFIP and dissolved in DMSO at a final concentration of 230 μ M, were incubated for 24h at room temperature in 0.215 M sodium phosphate buffer (pH 8.0). For coincubation experiments, aliquots of *hAChE* (2.30 μ M, ratio 100:1) and *hAChE* in the presence of the tested compound (100 μ M) were added. Blanks containing *A β* , *hAChE*, *A β* plus the tested compound, and *hAChE* plus the tested compound in 0.215 M sodium phosphate buffer (pH 8.0) were prepared. The final volume of each vial was 20 μ L. To quantify amyloid fibril formation, the thioflavin T fluorescence method was used⁹ and monitored with a spectrofluorometer (Hitachi F-2500). Thioflavin T binds to amyloid fibrils, giving rise to an intense specific emission band at 490 nm in its fluorescent emission spectrum. Therefore, after incubation, the samples were diluted to a final volume of 2 mL with 50 mM glycine-NaOH buffer (pH 8.5) containing 1.5 μ M thioflavin T. A 300 s time scan of fluorescence intensity was carried out (λ_{exc} = 446 nm, λ_{em} = 490 nm), and values at the plateau were averaged after subtraction of the background fluorescence of the 1.5 μ M thioflavin T solution. The percent inhibition of the AChE-induced aggregation due to the presence of increasing concentrations of the inhibitor was calculated by the following expression: $100 - [(IF_i/IF_0) \times 100]$ where IF_i and IF_0 were the fluorescence intensities obtained for *A β* plus AChE in the presence and in the absence of inhibitor, respectively, after subtracting the fluorescence of respective blanks. Each assay was run in triplicate.

5.3. Computer-Aided Molecular Modeling

Molecular docking was carried out using GOLD 4.0 [22] with standard parameters. Crystal structure of *EeAChE* (pdb code 1EEA) has been chosen in order to facilitate the comparison

with the inhibition assay results, the binding site being defined as a 20 Å radius sphere around the OH group of Ser200. Although this protein has been crystallized in the apo form, the residues that form the anionic site are positioned in the open form of the gorge, thus making this structure appropriate for docking compounds presumed to interact with both the catalytic and peripheral sites of *Ee*AChE. The 3D structures of ligands were constructed with CORINA 3.44 (Molecular Networks GmbH).

Molecular dynamics simulations were carried out with GROMACS version 4.0.2 [23] using the OPLS-AA [24] force field. Each system was energy-minimized until convergence using a steepest descents algorithm. Molecular dynamics with position restraints for 200 ps was then performed followed by the production run of 10 ns. During the position restraints and production runs, the Parinello-Rahman method [25] was used for pressure coupling, and the temperature was coupled using the Nosé-Hoover method [26] at 300 K. Electrostatics were calculated with the particle mesh Ewald method [27]. The P-LINCS algorithm [28] was used to constrain bond lengths, and a time step of 2 fs was used throughout. Ligand topologies for the OPLS-AA force field were obtained using an in-house developed script. All calculations were performed using the HPC facilities at the ICSN.

Images were generated with Chimera [29] and raytraced with the POV-ray module.

Acknowledgment We thank the CNRS and ICSN for financial support. J. Rouleau was supported by fellowship from the Institut de Chimie des Substances Naturelles (ICSN-CNRS). Professor J.L.ALLEMAND is gratefully acknowledged for his interest in our work. We also thank Dr. Jordi Molgo for fruitful discussions.

Appendix. Supplementary material

Detailed experimental procedures, compound characterization data for **6b-c**, **7b-c**, **8b-c**, **9b-c**, **12a-b**, **13a-b**, **14**, **15**, **16**, **18**, **19**, **20**, **21**, **22**, **23**, **24**, **25**, **26**, **27**, **31**, **32**, **33**, **34**, **35b-c**, **36b-k**. Computer-Aided Molecular Modeling. RMSD, RMSF and protein-ligand contact surface plots from MD simulations. Equilibrium conformations for compounds **1** and **36e** in the AChE binding site can be found in the online version at

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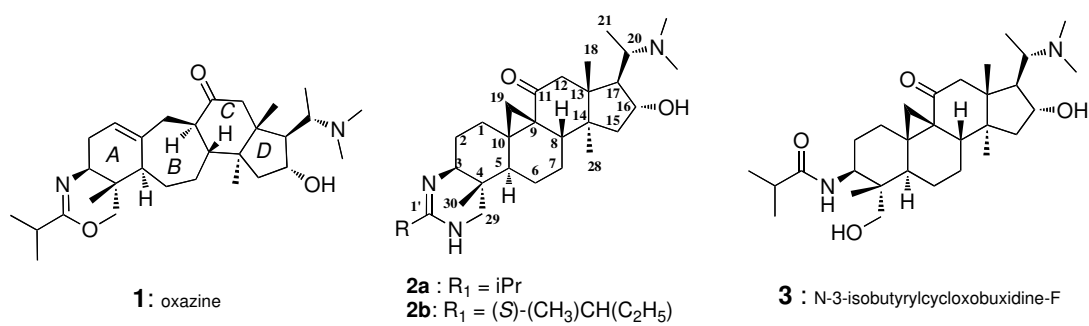
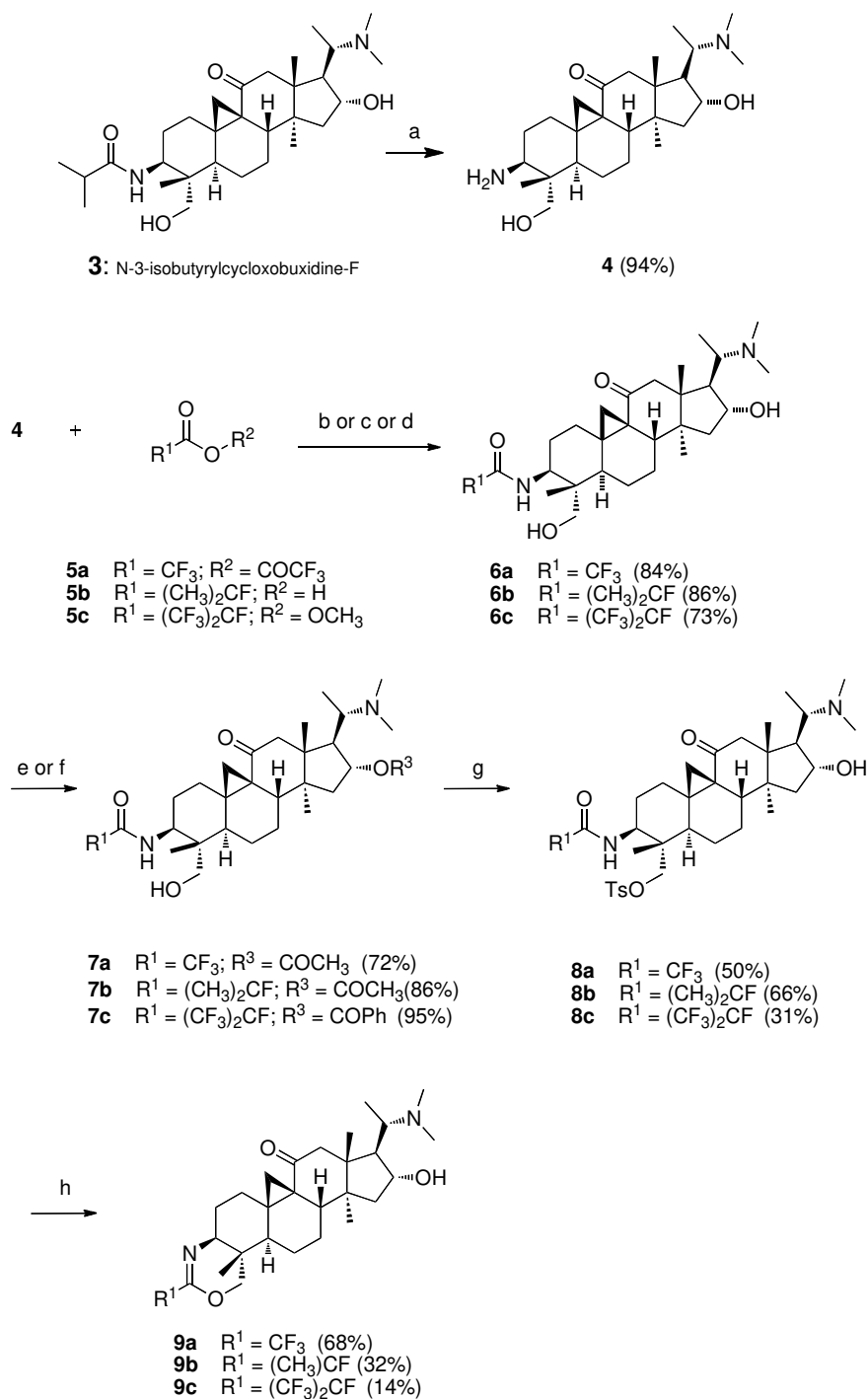
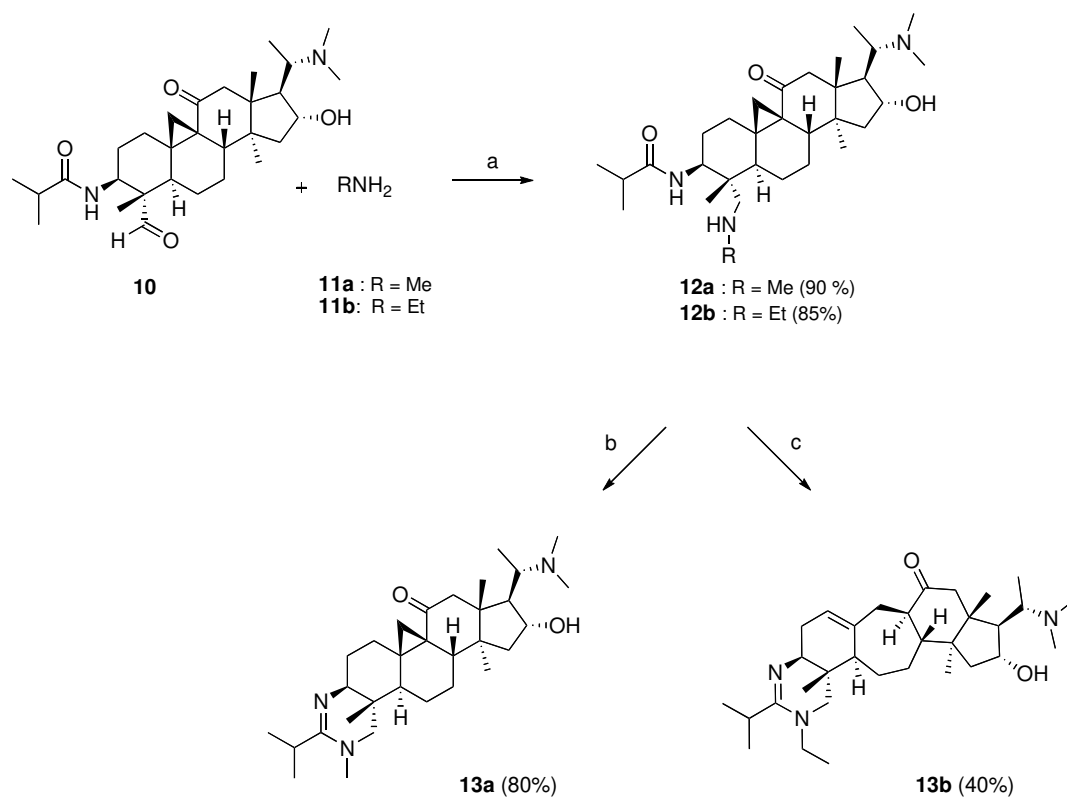


Figure 1.



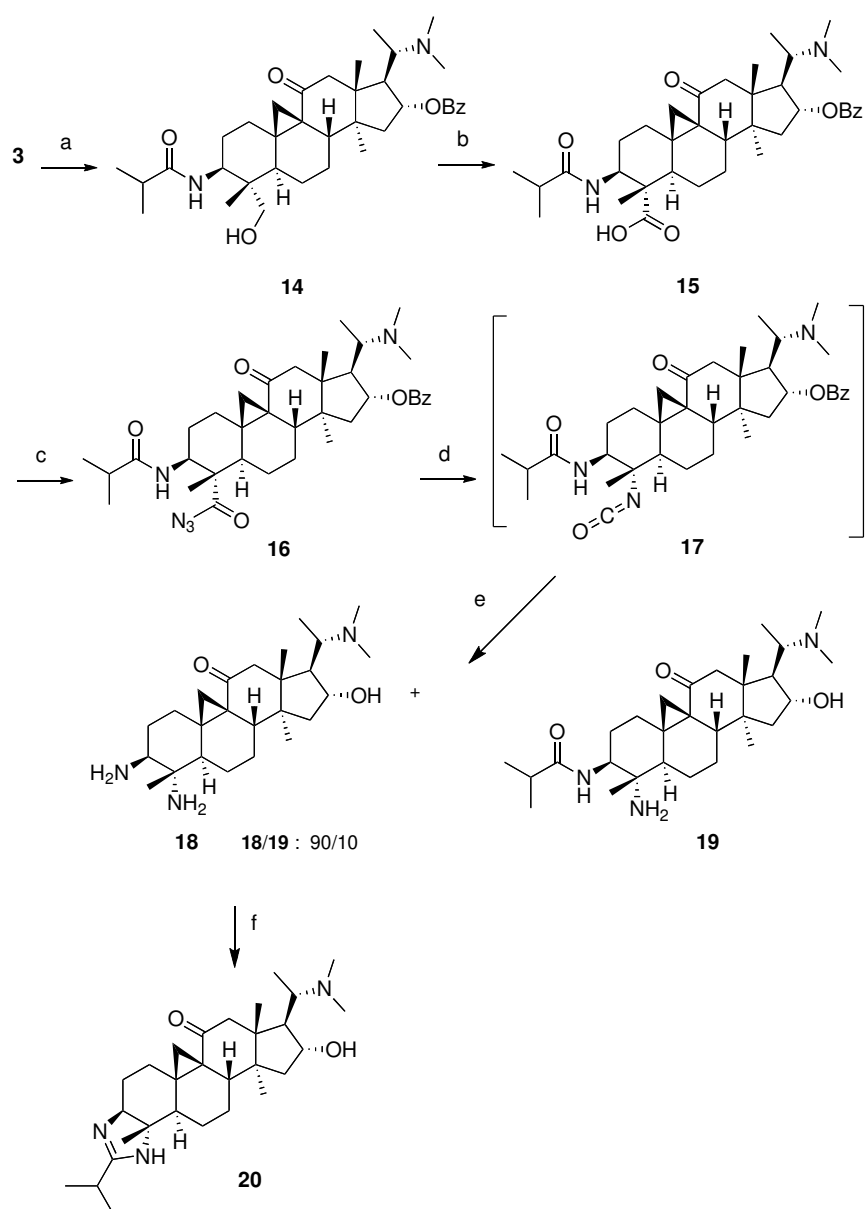
^aReagents and conditions : (a) H_2SO_4 , MeOH; (b) for **5a** : pyridine, CH_2Cl_2 ; (c) for **5b** : $(\text{ClCO})_2$, DMF then pyridine, CH_2Cl_2 ; (d) for **5c** : MeOH; (e) for **6a** and **6b** : Ac_2O , pyridine, CH_2Cl_2 ; (f) for **7c** : Bz_2O , pyridine, CH_2Cl_2 ; (g) i) APTS, Na_2SO_4 , MeOH, ii) TsCl, pyridine, iii) K_2CO_3 , MeOH, H_2O ; (h) $\text{NaN}(\text{CHO})_2$, DMF

Scheme 1.



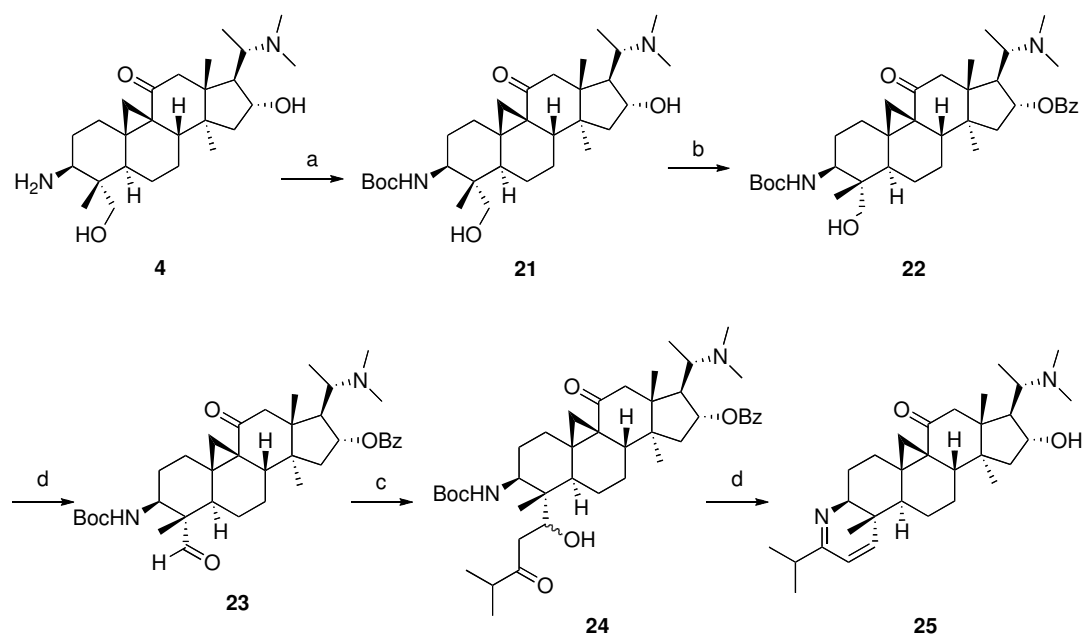
^aReagents and conditions : (a) i) RNH_2 , Na_2SO_4 , EtOH, ii) NaBH_3CN , AcOH, EtOH; (b) NEt_3 , *n*-BuOH, microwaves, 200°C, 2h; (c) NEt_3 , *n*-BuOH, microwaves, 250°C, 5h.

Scheme 2.



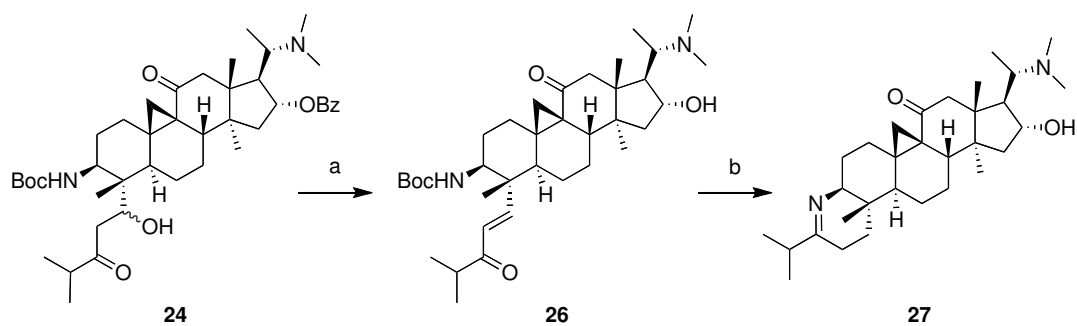
^aReagents and conditions : (a) Bz₂O, pyridine, CH₂Cl₂; (b) Jones' reagent, acetone (73%); (c) i) (ClCO)₂, NEt₃, DMF, ii) NaN₃, H₂O (74%); (d) microwaves (100%); (e) i) LiOH, THF, H₂O, ii) MeOH, iii) 1N HCl reflux (84%); (f) iPrC(OMe)₃, Yb(OTf)₃, Dioxane (85%).

Scheme 3.



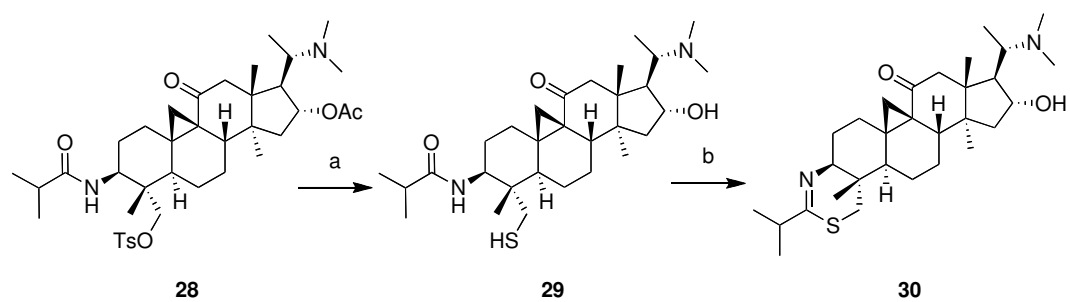
^aReagents and conditions : (a) Boc_2O , MeOH (100%); (b) Bz_2O , DMAP, CH_2Cl_2 , NEt_3 (97%); (c) Dess-Martin periodinane, CH_2Cl_2 (88%); (d) i) TFA, CH_2Cl_2 , ii) K_2CO_3 , MeOH, H_2O (63%).

Scheme 4.



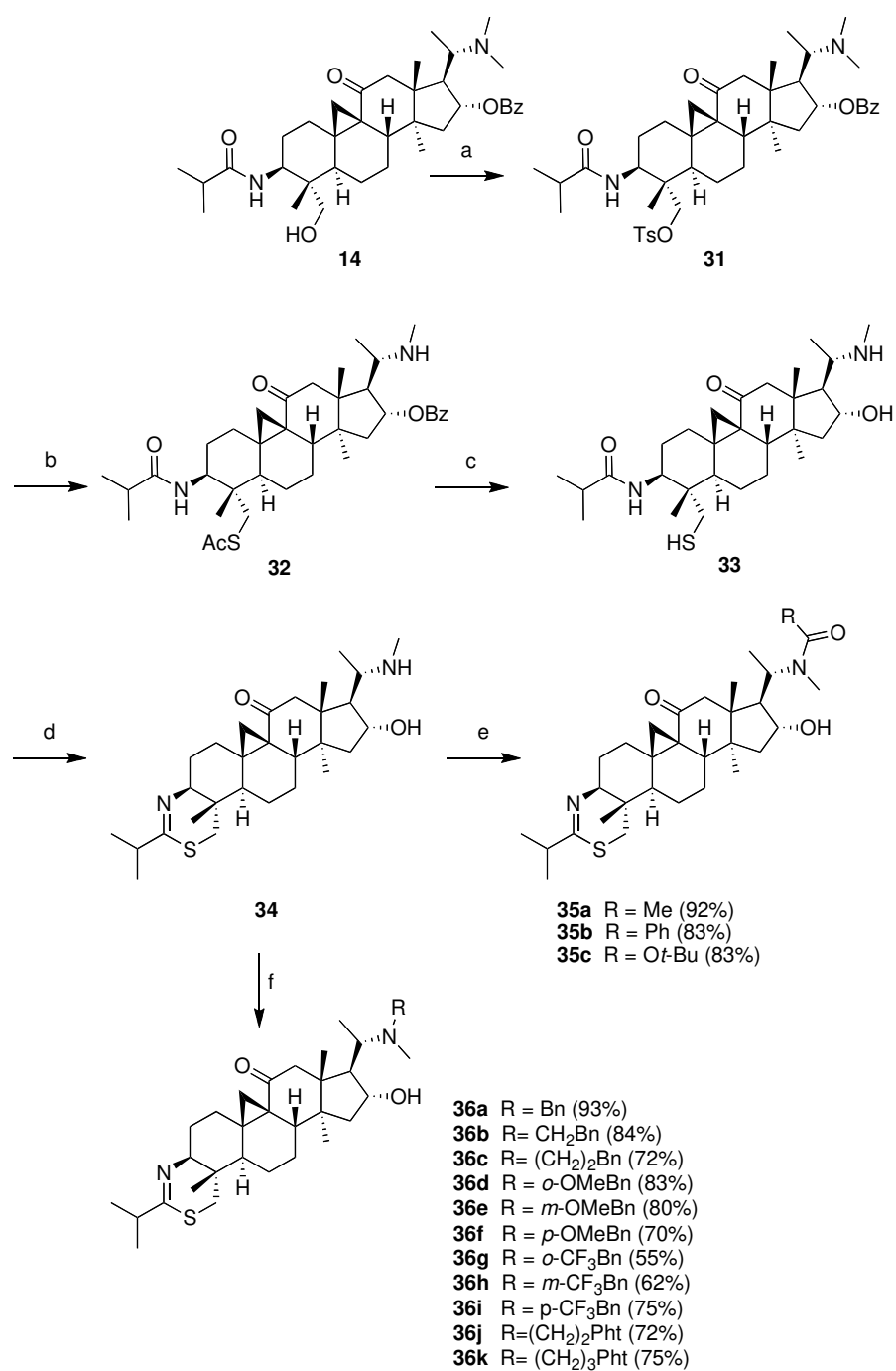
^aReagents and conditions : (a) K_2CO_3 , MeOH, H_2O (86%); (b) i) $\text{Pd}(\text{OH})_2$, H_2 , MeOH, ii) TFA, CH_2Cl_2 (68%).

Scheme 5.



^aReagents and conditions : (a) i) KSAc, DMPU, ii) KOH, MeOH, H₂O (67%). (b) AlMe₃, (ClCH₂)₂ (59%)

Scheme 6.



^aReagents and conditions : (a) i) APTS, THF; ii) TsCl, pyridine (86%); (b) i) KSAc, DMPU, ii) *m*-CPBA, CH₂Cl₂, iii) FeSO₄·7 H₂O, FeCl₃·6 H₂O, MeOH (81%); (c) KOH, MeOH, H₂O (55%); (d) AlMe₃, (ClCH₂)₂ (70%); (e) (RCO)₂O, EtOH or THF; (f) RX, K₂CO₃, CH₃CN or DMF.

Scheme 7.

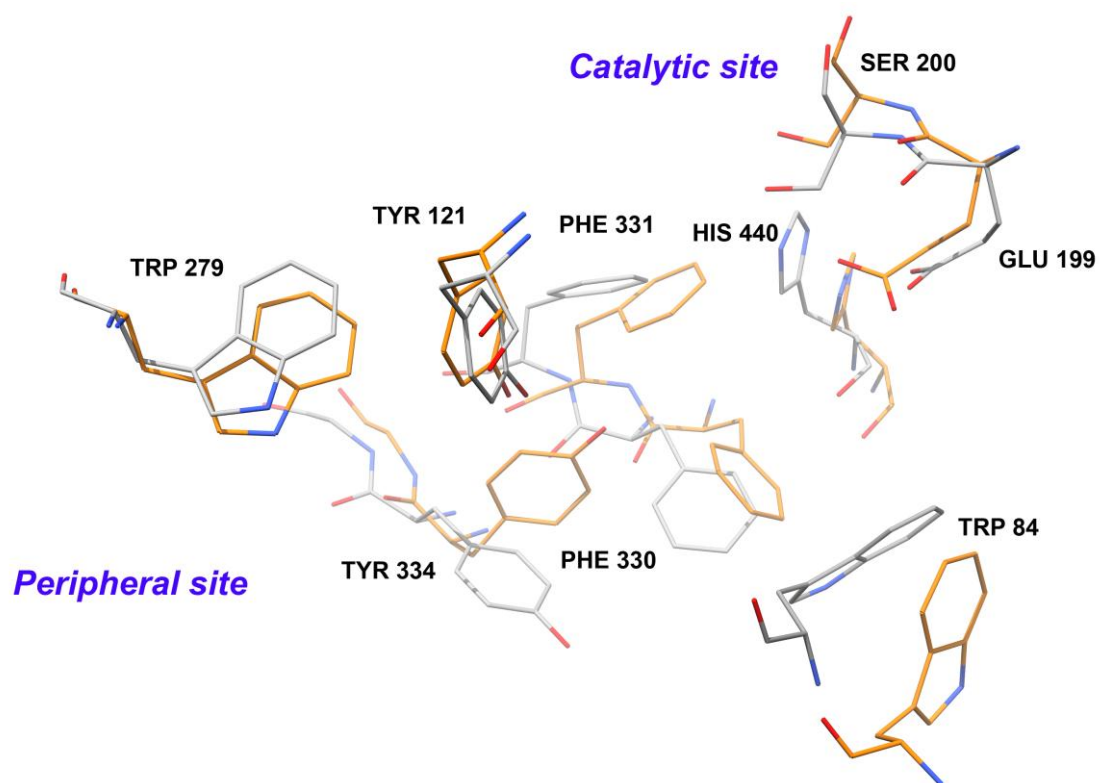


Figure 2

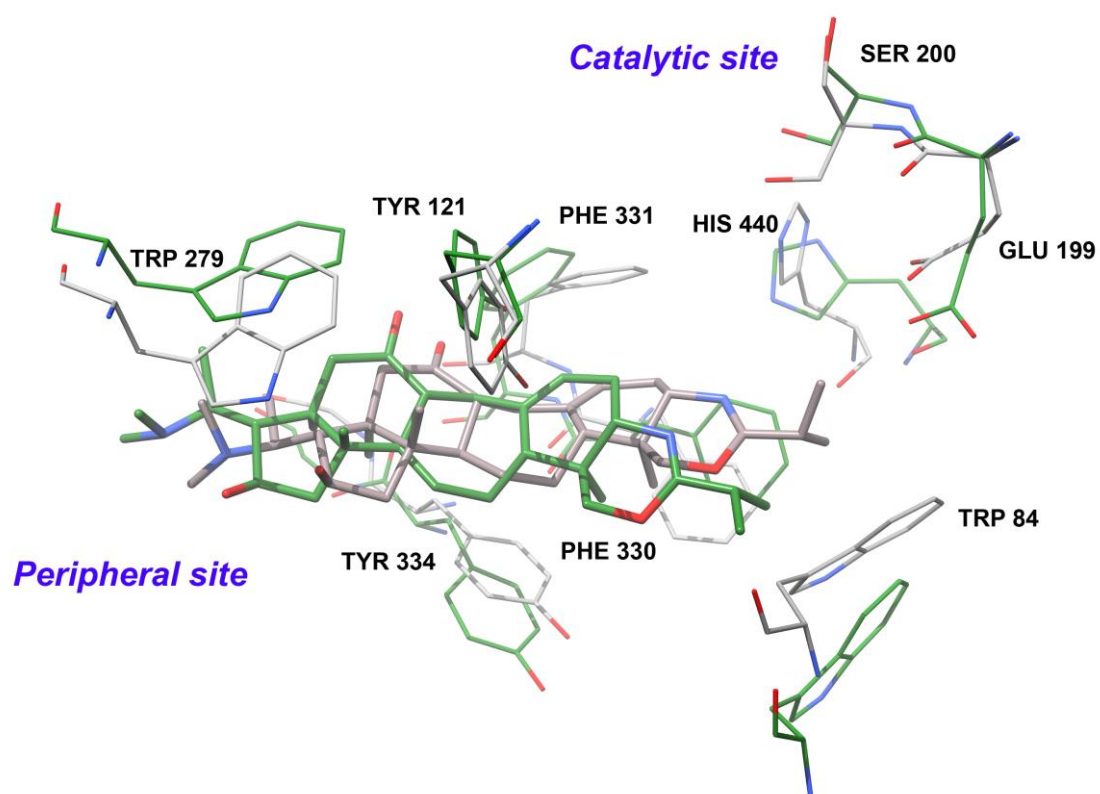


Figure 3

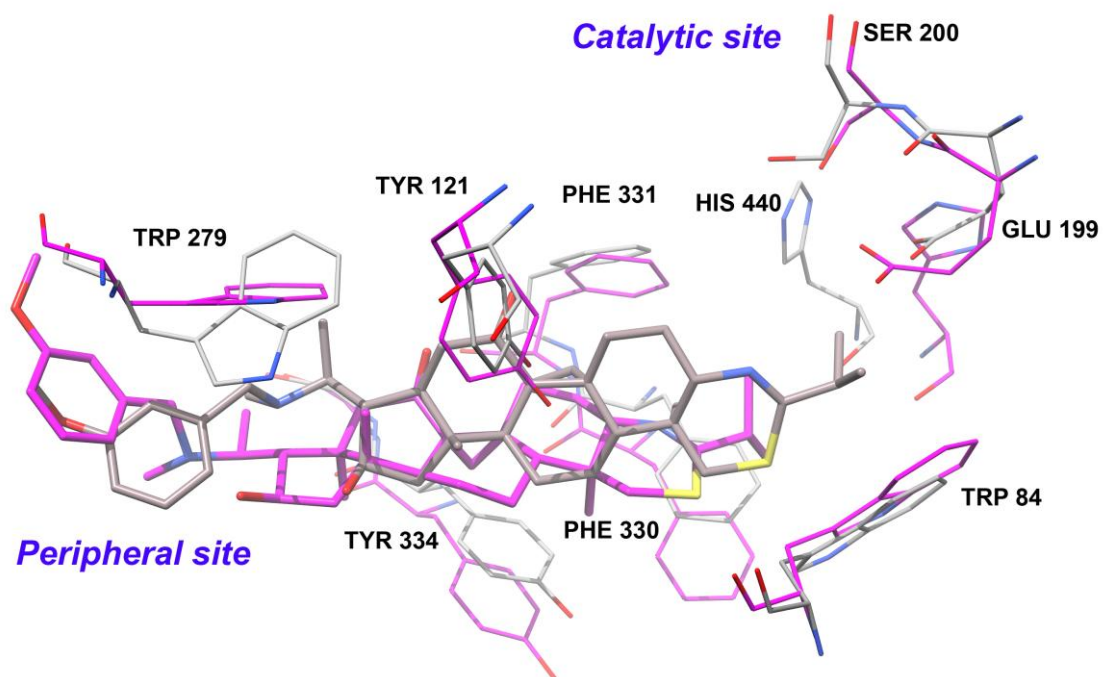


Figure 4

Figure legends

Figure 1. Structures of compounds **1-3**.

Figure 2. Selected residues of the protein binding site (apo form) at the beginning (gray) and at the end (orange) of the 10 ns molecular dynamics simulation

Figure 3. Selected residues of the protein binding site in complex with compound **1** at the beginning (gray) and at the end (green) of the 10 ns molecular dynamics simulation

Figure 4. Selected residues of the protein binding site in complex with compound **36e** at the beginning (gray) and at the end (magenta) of the 10 ns molecular dynamics simulation

Scheme legends

Scheme 1. Synthesis of oxazines **9**.

Scheme 2. Synthesis of tetrahydropyrimidines **13a-b**.

Scheme 3. Synthesis of Dihydro-1*H*-imidazole **20**.

Scheme 4. Synthesis of dihydropyridine **25**.

Scheme 5. Synthesis of tetrahydropyridine **27**.

Scheme 6. Synthesis of 5,6-dihydro-4*H*-1,3-thiazine **29**.

Scheme 7. Synthesis of compound **35a-c** and **36a-k**.

TABLES

Table 1 : AChE and BuChE Inhibitory activities of new analogs.^a

Compounds	IC ₅₀ (nM)			AChE selectivity ^b
	<i>Ee</i> AChE	<i>h</i> AChE	<i>h</i> BuChE	
1	31 ± 4 ^c	299 ^c	> 1 000 ^c	> 3
2a	> 10 000	nd	> 10 000 ^c	nd ^d
9a	> 10 000	> 10 000	> 10 000	nd
9b	336 ± 41	744 ± 103	> 10 000	> 13
9c	1701 ± 260	3003 ± 292	> 10 000	> 3
13a	> 10 000	> 10 000	2 948 ± 329	0.29
13b	> 10 000	> 10 000	3 326 ± 143	0.33
20	> 10 000	> 10 000	7 224 ± 742	0.72
25	16 ± 1.4	25 ± 1.4	679 ± 6.9	25
27	294 ± 36	618 ± 87	> 10 000	16 ^c
30	38 ± 4	60 ± 7.9	5 375 ± 430	89
34	45 ± 3.8	76 ± 14.3	1 343 ± 120	17
35a	139 ± 11.9	298 ± 19.2	> 10 000	> 33
35b	37 ± 2.9	43 ± 10.0	> 1 000	> 23
35c	186 ± 13.7	159 ± 46.7	> 10 000	> 63
36a	13 ± 0.8	7 ± 0.9	554 ± 36	79
36b	14 ± 0.9	20 ± 1.6	501 ± 40	25
36c	79 ± 7.1	76 ± 6.9	1430 ± 162	18
36d	12 ± 1.7	6 ± 1.2	333 ± 25	55
36e	6 ± 0.5	3 ± 0.2	477 ± 35	159
36f	18 ± 3.0	9 ± 0.4	217 ± 17	24
36g	24 ± 2.6	11 ± 0.9	> 10 000	> 909
36h	9 ± 1.2	3 ± 1.1	> 10 000	> 3333
36i	7 ± 0.8	3 ± 0.4	> 10 000	> 3333
36j	24 ± 1.7	70 ± 7.1	653 ± 140	9
36k	14 ± 4.5	14 ± 5.3	224 ± 19	16
tacrine	136 ± 10.8	484 ± 40	73 ± 7	0.15

^a Values are expressed as the mean ± standard error of the mean of three experiments. IC₅₀ inhibitory concentration (nM) of AChE from *Electrophorus electricus* (*Ee*AChE) or human recombinant (*h*AChE) or BuChE from human serum (*h*BuChE). ^bIC₅₀(*h*BuChE)/ IC₅₀ (*h*AChE). ^c Values from reference 8. ^d Not determined.

Table 2. A β aggregation of **36e**, **36h** and **36k**, propidium iodide and tacrine.^a

compd	AChE-induced A β_{1-40} aggregation (%)
36e	69 \pm 2
36h	56 \pm 1
36k	42 \pm 1
propidium iodide	82 \pm 2.5 ^c
tacrine	7 \pm 0.21 ^c

^a Values are expressed as mean \pm standard error of the mean from two independent measurements. ^b A 100 μ M concentration of the inhibitor was used. ^c Data from reference 9.

- > New acetylcholinesterase inhibitors in the triterpene series were synthesized. >
- > Some compounds have *human*AChE IC₅₀ values in the nanomolar range. >
- > Some analogs showed ability to block the AChE-induced A β aggregation. >
- > A docking study and molecular dynamics simulations were done. >
- > These compounds interact with both catalytic and peripheral sites of AChE. >

Supplementary material

New Potent Human Acetylcholinesterase Inhibitors in the Tetracyclic Triterpene series with Inhibitory Potency on Amyloid β Aggregation

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Table of contents

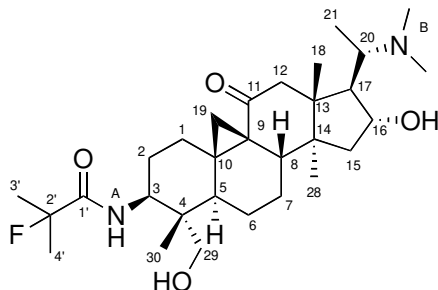
Preparation of compound 6b	S4
Preparation of compound 6c	S5
Preparation of compound 7b	S6
Preparation of compound 7c	S7
Preparation of compound 8b	S8
Preparation of compound 8c	S9
Preparation of compound 9b	S10
Preparation of compound 9c	S11
Preparation of compound 12a	S12
Preparation of compound 12b	S13
Preparation of compound 14	S14
Preparation of compound 15	S15
Preparation of compound 16	S16
Preparation of compound 18	S17
Preparation of compound 19	S18
Preparation of compound 21	S19
Preparation of compound 22	S20
Preparation of compound 23	S21
Preparation of compound 24	S22
Preparation of compound 26	S23
Preparation of compound 29	S24
Preparation of compound 31	S25
Preparation of compound 32	S26
Preparation of compound 33	S27
Preparation of compound 35b	S28
Preparation of compound 35c	S29

Preparation of compound 36a	S30
Preparation of compound 36b	S31
Preparation of compound 36c	S32
Preparation of compound 36d	S33
Preparation of compound 36f	S34
Preparation of compound 36g	S35
Preparation of compound 36h	S36
Preparation of compound 36i	S37
Preparation of compound 36j	S38
Preparation of compound 36k	S39
Purity of tested compounds	S40
Computer Aided Molecular Modeling	S41
Figure S1, RMSD plot from MD simulations	S41
Figure S2, RMSF plot from MD simulations	S42
Figure S3, Protein-ligand contact surface plot from MD simulations	S43
Figure S4, Equilibrium conformations for compounds 1 and 36e in the AChE binding site	S43

Detailed experimental procedures and compound characterization data **6b-c**, **7b-c**, **8b-c**, **9b-c**, **12a-b**, **14**, **15**, **16**, **18**, **19**, **21**, **22**, **23**, **24**, **26**, **29**, **31**, **32**, **33**, **35b-c**, **36a-d**, **36f-k**. The assignement of the ^1H and ^{13}C NMR spectra of the different compounds has been made by ^1H - ^1H , ^1H - ^{13}C , COSY, HMBC, HMQC and by analogy with previously described compounds. The numerotation indicated on molecule is only mentioned for the NMR spectra interpretation.

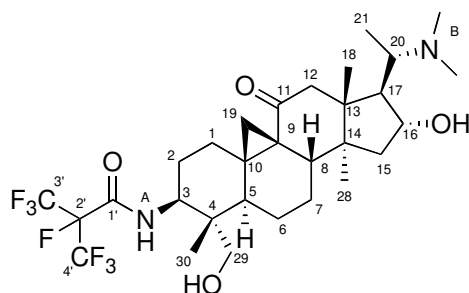
Note : The resonances of the CF_3 -group carbons are not being observed. The absence of such signals is ascribed to the slow relaxation times of these carbons and/or due to ^{13}C - ^{19}F couplings. Both of these phenomena are likely to lead to low-intensity signals for such carbons. The consequence of the free rotation of the $\text{N}_\text{B}\text{Me}_2$ group is the apparition of a broad singlet in ^1H NMR and the diminution or disappearance of the corresponding peak in ^{13}C NMR. In some cases, this chemical shift can't be determined.

***N*-[20*S*-(dimethylamino)-16 α -hydroxy-4 α -(hydroxymethyl)-4 β ,14 α -dimethyl-11-oxo-9,19-cyclo-5 α ,9 β -pregnan-3 β -yl]-2-fluoro-2-methylpropanamide (**6b**)**



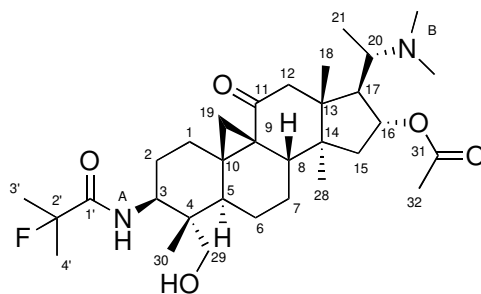
To 34 μ L (0.39 mmol, 1.5 eq.) of oxalyl chloride **solubilized** in 1 mL of DCM at 0 $^{\circ}$ C were slowly added 30 μ L (0.39 mmol, 1.5 eq.) of DMF and 30 μ L (0.34 mmol, 1.3 eq.) of 2-fluoro-2-methylpropanoic acid. The mixture was stirred at 0 $^{\circ}$ C for 10 min and slowly added to 112 mg (0.26 mmol, 1 eq.) of cyclooxobuxidine-F **3** **solubilized** in 1.5 mL of pyridine and 1 mL of DCM at -20 $^{\circ}$ C. The mixture was stirred at RT for 12 h. After three co-evaporations with 3x10 mL of 1,2-dichloroethane, the residue was dissolved in DCM, washed with a saturated solution of sodium carbonate and extracted with 3x20 mL of DCM. Combined organic layers were washed with brine (20 mL), dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by silica gel column chromatography (eluent : DCM/MeOH/NH₄OH : 96/3/1) to afford the title compound as an amorphous white solid (116 mg, yield 86 %). IR (cm⁻¹) : 3331, 2936, 2870, 1659, 1659, 1535, 1461, 1161, 1189, 1225 ; ¹H NMR (300 MHz, CDCl₃): δ (ppm) 6.31 (1H, dd, $J_{\text{NAH-H3}} = 8.9$ Hz, $J_{\text{NAH-F}} = 6.8$ Hz, N_A-H), 4.07 (2H, m, H16 β +OH29), 3.95 (1H, m, H3 α), 3.34 (1H, m, H29b), 2.92 (1H, d, $J_{\text{gem}} = 11.9$ Hz, H29a), 2.60 (1H, dq, $J_{20-17\alpha} = 10.8$ Hz, $J_{20-21} = 6.5$ Hz, H20), 2.49 (1H, d, $J_{\text{gem}} = 17.2$ Hz, H12 α), 2.42 (1H, ddd, $J_{\text{gem}} = 13.5$ Hz, $J_{1\beta-2} = 3.5$ Hz, $J_{1\beta-2} = 3.4$ Hz, H1 β), 2.27 (1H, d, $J_{\text{gem}} = 17.2$ Hz, H12 β), 2.22 (6H, bs, N_BMe₂), 1.92-2.07 (4H, m, H5 α +H17 α +H15 β +H8 β), 1.64-1.71 (3H, m, H2 α +H2 β +H6 α), 1.59 (3H, d, $J_{\text{H3'-F}} = 7.1$ Hz, H3'), 1.55 (1H, m, H19 α), 1.52 (3H, d, $J_{\text{H4'-F}} = 7.2$ Hz, H4'), 1.49 (1H, m, H7 β), 1.47 (1H, m, H15 α), 1.41 (1H, m, H7 α), 1.27 (1H, m, H1 α), 1.19 (3H, s, H28), 1.02 (1H, d, $J_{\text{gem}} = 3.7$ Hz, H19 β), 0.88 (1H, m, H6 β), 0.84 (3H, d, $J_{21-20} = 6.5$ Hz, H21), 0.81 (3H, s, H18), 0.56 (3H, s, H30); ¹³C NMR (75.4 MHz, CDCl₃): δ (ppm) 211.3 (C11), 174.4 (d, $J_{\text{C1'-F}} = 20.9$ Hz, C1'), 96.1 (d, $J_{\text{C2'-F}} = 181.1$ Hz, C2'), 78.1 (C16), 64.0 (C29), 61.9 (C20), 55.7 (C17), 51.4 (C12), 50.6 (C3), 47.0 (C14), 44.6 (C4+C13), 42.7 (C15), 41.3 (C8), 41.1 (C5), 37.5 (C10), 34.3 (C9), 30.5 (C19), 27.5 (C1), 27.2 (C2), 25.5 (d, $J_{\text{C3'-F}} = 23.6$ Hz, C3'), 24.6 (d, $J_{\text{C4'-F}} = 24.2$ Hz, C4'), 24.2 (C7), 20.7 (C28), 18.3 (C6), 17.7 (C18), 11.1 (C30), 9.8 (C21). MS (ESI, m/z) : 521.4 (M+H) ; HRMS (ESI, m/z) : calcd for C₂₈H₄₄N₂O₄F₃ : 521.3755, found : 521.3755.

***N*-[20*S*-(dimethylamino)-16*α*-hydroxy-4*α*-(hydroxymethyl)-4*β*,14*α*-dimethyl-11-oxo-9,19-cyclo-5*α*,9*β*-pregnan-3*β*-yl]-heptafluoroisobutanamide (**6c**)**



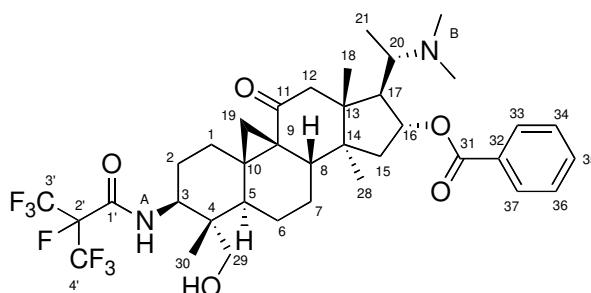
To 82 mg (0.29 mmol, 1 eq.) of cyclobuxidine-F **3** **solubilized** in 4 mL of anhydrous MeOH were added 130 mg (0.57 mmol, 3 eq.) of methyl heptafluoroisobutyrate. The mixture was stirred at RT for 24 h and the solvent was evaporated under vacuum. The residue was purified by silica gel column chromatography (eluent : DCM/MeOH/NH₄OH : 96/3/1) to afford the title compound as an amorphous white solid (86 mg, yield 73 %). IR (cm⁻¹) : 3331, 2936, 2870, 1659, 1659, 1535, 1461, 1161, 1189, 1225 ; ¹H NMR (300 MHz, CDCl₃): δ(ppm) 6.69 (1H, dm, J_{NAH-H3} = 8.7 Hz, N_A-H), 4.16 (1H, m, H3_α), 4.08 (1H, m, H16_β), 3.39 (1H, m, H29b), 2.96 (1H, d, J_{gem} = 12.4 Hz, H29a), 2.62 (1H, dq, J_{20-17α} = 10.7 Hz, J₂₀₋₂₁ = 6.6 Hz, H20), 2.53 (1H, d, J_{gem} = 17.0 Hz, H12_α), 2.48 (1H, dt, J_{gem} = 13.4 Hz, J_{1β-2} = 3.5 Hz, H1_β), 2.28 (1H, d, J_{gem} = 17.0 Hz, H12_β), 2.24 (6H, bs, N_BMe₂), 2.09 (2H, m, H5_α+H8_β), 2.00 (1H, m, H15_β), 1.98 (1H, m, H17_α), 1.74 (2H, m, H2_α+H2_β), 1.67 (1H, m, H6_α), 1.56 (1H, m, H7_β), 1.54 (1H, d, J_{gem} = 3.8 Hz, H19_α), 1.47 (1H, m, H15_α), 1.41 (1H, m, H7_α), 1.25 (1H, m, H1_α), 1.21 (3H, s, H28), 1.06 (1H, d, J_{gem} = 3.8 Hz, H19_β), 0.92 (1H, m, H6_β), 0.87 (3H, d, J₂₁₋₂₀ = 6.6 Hz, H21), 0.85 (3H, s, H18), 0.62 (3H, s, H30) ; ¹³C NMR (75.4 MHz, CDCl₃): δ(ppm) 211.0 (C11), 158.8 (d, J_{C1'-F} = 18.7 Hz, C1'), 78.0 (C16), 63.9 (C29), 61.8 (C20), 55.8 (C17), 52.1 (C3), 51.2 (C12), 46.9 (C14), 44.5 (C13), 44.3 (C4), 42.6 (C15), 41.5 (C8), 41.1 (C5), 36.8 (C10), 34.1 (C9), 30.2 (C19), 27.3 (C1), 27.1 (C2), 24.2 (C7), 20.4 (C28), 18.2 (C6), 17.5 (C18), 10.8 (C30), 9.5 (C21). MS (ESI, m/z) : 629.3 (M+H) ; HRMS (ESI, m/z) : calcd for C₃₀H₄₄N₂O₄F₇ : 629.3189, found : 629.3158.

20S-(dimethylamino)-3 β -(2-fluoro-2-methylpropanamido)-4 α -(hydroxymethyl)-4 β ,14 α -dimethyl-11-oxo-9,19-cyclo-5 α ,9 β -pregnan-16 α -yl acetate (7b**)**



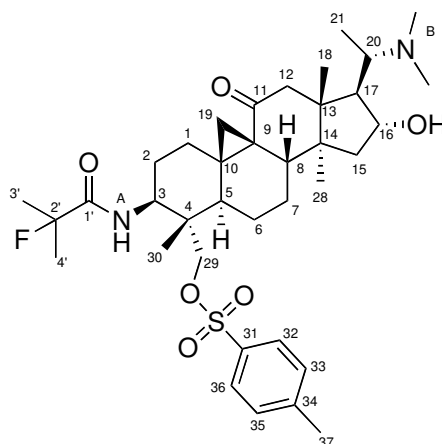
To 189 mg (0.36 mmol, 1 eq.) of **6b** solubilized in 10 mL of a mixture DCM/pyridine (3/1) were added at 0 °C 41 μ L (0.43 mmol, 1.2 eq.) of acetic anhydride. The mixture was stirred at 0 °C for 1 h then at RT for further 12 h. After 3 co-evaporations with 3x10 mL of 1,2-dichloroethane, the residue was dissolved in DCM, washed with 20 mL of a saturated solution of sodium hydrogen carbonate then extracted with 3x20 mL of DCM. Combined organic layers were washed with brine (20 mL), dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by silica gel column chromatography (eluent : DCM/MeOH/TEA : 96.5/3/0.5) to afford the title compound as an amorphous white solid (175 mg, yield 86 %). IR (cm^{-1}) : 3344, 2970, 2933, 2873, 2825, 2733, 1669, 1652, 1540, 1456, 1249, 1229, 1197 ; ^1H NMR (300 MHz, CDCl_3): δ (ppm) 6.29 (1H, dd, $J_{\text{NAH-H3}} = 8.7$ Hz, $J_{\text{NAH-F}} = 7.1$ Hz, $\text{N}_\text{A-H}$), 5.13 (1H, dd, $J_{\text{H16}\beta\text{-H15}\beta} = 8.1$ Hz, $J_{\text{H16}\beta\text{-H17}\alpha} = 5.9$ Hz, $\text{H16}\beta$), 4.12 (1H, dd, $J_{\text{OH-H29b}} = 10.5$ Hz, $J_{\text{OH-H29a}} = 5.0$ Hz, OH29), 3.99 (1H, m, $\text{H3}\alpha$), 3.38 (1H, dd, $J_{\text{gem}} = 12.9$ Hz, $J_{\text{H29b-OH}} = 10.5$ Hz, H29b), 2.95 (1H, dd, $J_{\text{gem}} = 12.9$ Hz, $J_{\text{H29a-OH}} = 5.0$ Hz, H29a), 2.55 (1H, d, $J_{\text{gem}} = 17.2$ Hz, $\text{H12}\alpha$), 2.47 (2H, m, $\text{H20+H1}\beta$), 2.39 (1H, d, $J_{\text{gem}} = 17.2$ Hz, $\text{H12}\beta$), 2.23 (1H, dd, $J_{\text{H17a-H20}} = 11.0$ Hz, $J_{\text{H17a-H16b}} = 5.9$ Hz, $\text{H17}\alpha$), 2.14 (6H, bs, N_BMe_2), 1.98-2.10 (3H, m, $\text{H5}\alpha\text{+H8}\beta\text{+H15}\beta$), 2.01 (3H, s, H32), 1.70 (3H, m, $\text{H2}\alpha\text{+H2}\beta\text{+H6}\alpha$), 1.63 (3H, d, $J_{\text{H3'-F}} = 6.9$ Hz, H3'), 1.59 (1H, d, $J_{\text{gem}} = 3.9$ Hz, $\text{H19}\alpha$), 1.56 (3H, d, $J_{\text{H4'-F}} = 6.9$ Hz, H4'), 1.47 (1H, m, $\text{H7}\beta$), 1.42 (1H, d, $J_{\text{gem}} = 14.1$ Hz, $\text{H15}\alpha$), 1.38 (1H, m, $\text{H7}\alpha$), 1.26 (1H, m, $\text{H1}\alpha$), 1.16 (3H, s, H28), 1.06 (1H, d, $J_{\text{gem}} = 3.9$ Hz, $\text{H19}\beta$), 0.85 (3H, s, H18), 0.82 (3H, d, $J_{\text{H21-H20}} = 6.5$ Hz, H21), 0.60 (3H, s, H30) ; ^{13}C NMR (75.4 MHz, CDCl_3): δ (ppm) 211.2 (C11), 174.5 (d, $J_{\text{C1'-F}} = 20.3$ Hz, C1'), 170.6 (C31), 96.1 (d, $J_{\text{C2'-F}} = 181.7$ Hz, C2'), 78.8 (C16), 64.1 (C29), 59.4 (C20), 55.1 (C17), 51.9 (C12), 50.7 (C3), 47.4 (C14), 44.6 (C4), 44.6 (C13), 43.0 (C15), 41.4 (C8), 41.2 (C5), 40.2 (N_BMe_2), 37.6 (C10), 33.9 (C9), 30.7 (C19), 27.6 (C1), 27.4 (C2), 25.6 (d, $J_{\text{C3'-F}} = 24.2$ Hz, C3'), 24.7 (d, $J_{\text{C4'-F}} = 24.2$ Hz, C4'), 24.3 (C7), 21.2 (C32), 19.5 (C28), 18.4 (C6), 17.8 (C18), 11.1 (C30), 9.8 (C21) ; MS (ESI, m/z) : 563.3 (M+H), 521.4 ; HRMS (ESI, m/z) : calcd for $\text{C}_{32}\text{H}_{52}\text{N}_2\text{O}_5\text{F}$: 563.3860, found : 563.3845.

20S-(dimethylamino)-3β-(heptafluoroisobutanamino)-4α-(hydroxymethyl)-4β,14α-dimethyl-11-oxo-9,19-cyclo-5α,9β-pregnan-16α-yl benzoate (7c)



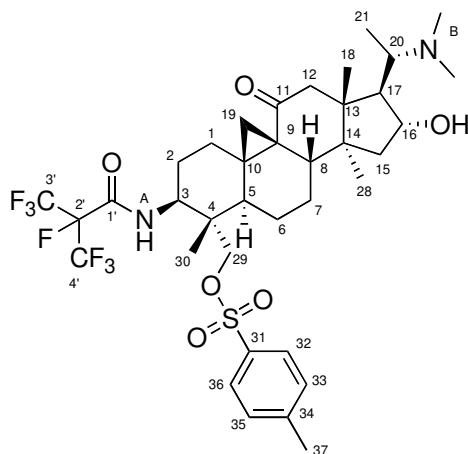
To 51 mg (0.081 mmol, 1 eq.) of **6c** solubilized in 6 mL of a mixture DCM/pyridine (3/1) were added at 0 °C 23 mg (0.092 mmol, 1.2 eq.) of benzoic anhydride. The mixture was stirred at 0 °C for 1 h then at RT for further 12 h. After 3 co-evaporations with 3x10 mL of 1,2-dichloroethane, the residue was dissolved in DCM, washed with 20 mL of a saturated solution of sodium carbonate then extracted with 3x20 mL of DCM. Combined organic layers were washed with brine (20 mL), dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by silica gel column chromatography (eluent : DCM/MeOH/NH₄OH : 98/1/1) to afford the title compound as an amorphous white solid (55 mg, yield 95 %). IR (cm⁻¹) : 3538, 3447, 2969, 2932, 2862, 2833, 1708, 1642, 1523, 1450, 1259, 1225, 1128 ; ¹H NMR (300 MHz, CDCl₃): δ(ppm) 8.04 (2H, d, J = 7.2 Hz, H33+H37), 7.55 (1H, m, H35), 7.44 (2H, m, H34+H36), 6.62 (1H, dd, J_{NAH-H3} = 8.9 Hz, J_{NAH-F} = 2.7 Hz, N_A-H), 5.42 (1H, dd, J_{H16β-H15α} = 8.1 Hz, J_{H16β-H17α} = 5.6 Hz, H16β), 4.17 (1H, m, H3α), 3.39 (1H, m, H29b), 2.96 (1H, d, J_{gem} = 12.4 Hz, H29a), 2.63 (1H, d, J_{gem} = 17.1 Hz, H12α), 2.52 (2H, m, H20+H1β), 2.46 (1H, m, H17α), 2.44 (1H, d, J_{gem} = 17.1 Hz, H12β), 1.96-2.11 (9H, m, N_BMe₂+H5α+H8β+H15β), 1.77 (2H, m, H2α+H2β), 1.67 (1H, m, H6α), 1.55-1.60 (2H, m, H19α+H15α), 1.51 (1H, m, H7β), 1.37 (1H, m, H7α), 1.30 (1H, m, H1α), 1.24 (3H, s, H28), 1.07 (1H, d, J_{gem} = 3.8 Hz, H19β), 0.95 (1H, m, H6β), 0.85 (3H, d, J₂₁₋₂₀ = 6.0 Hz, H21), 0.91 (3H, s, H18), 0.62 (3H, s, H30) ; ¹³C NMR (75.4 MHz, CDCl₃): δ(ppm) 211.2 (C11), 165.9 (C31), 158.2 (d, J_{C1'-F} = 19.2 Hz, C1'), 132.5 (C35), 130.8 (C32), 129.4 (C33+C37), 128.2 (C34+C36), 79.5 (C16), 63.8 (C29), 59.4 (C20), 55.3 (C17), 52.2 (C3), 51.8 (C12), 47.5 (C14), 44.8 (C13), 44.6 (C4), 43.2 (C15), 41.6 (C8), 41.2 (C5), 40.2 (N_BMe₂), 37.2 (C10), 33.9 (C9), 30.7 (C19), 27.4 (C1), 27.3 (C2), 24.3 (C7), 19.6 (C28), 18.4 (C6), 17.9 (C18), 11.1 (C30), 9.9 (C21) ; MS (ESI, m/z) : 733.3 (M+H) ; HRMS (ESI, m/z) : calcd for C₃₇H₄₈N₂O₅F₇ : 733.3494, found : 733.351.

{20*S*-(dimethylamino)-16*α*-hydroxy-4*β*,14*α*-dimethyl-11-oxo-3*β*-(2-fluoro-2-methylpropanamido)-9,19-cyclo-5*α*,9*β*-pregnan-4*α*-yl}methyl tosylate (8b)



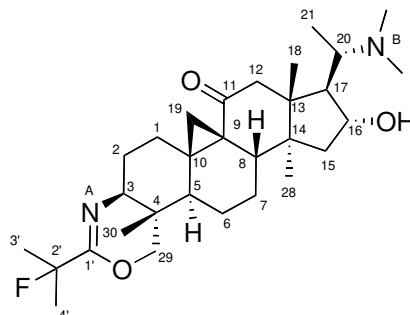
The title compound was prepared from 75 mg (0.31 mmol, 1 eq.) of **7b**, 10 mL of anhydrous MeOH, 60 mg (0.31 mmol, 1 eq.) of PTSA, 1.5 mL of pyridine and 178 mg (0.93 mmol, 3 eq.) of tosyl chloride by following the similar procedure described for the preparation of **8a**. The crude product was purified by reverse phase silica gel column chromatography (eluent : water/EtOH/HCOOH : 80/20/0.1) to afford the title compound as an amorphous white solid (149 mg, yield 66 %). IR (cm⁻¹) : 3330, 2962-2934, 2869, 1667, 1598, 1525, 1455, 1261, 1188, 1174 ; ¹H NMR (300 MHz, CDCl₃): δ(ppm) 7.81 (2H, d, J = 8.2 Hz, H32+H36), 7.33 (2H, d, J = 8.2 Hz, H33+H35), 6.07 (1H, dd, J_{NAH-H3} = 9.9 Hz, J_{NAH-F} = 7.0 Hz, N_AH), 4.71 (1H, t, J = 7.5 Hz, H16*β*), 4.11 (1H, m, H3*α*), 3.81 (1H, d, J_{gem} = 9.8 Hz, H29*b*), 3.57 (1H, d, J_{gem} = 9.8 Hz, H29*a*), 3.42 (1H, dq, J_{20-17*α*} = 11.2 Hz, J₂₀₋₂₁ = 6.6 Hz, H20), 2.96 (3H, s, H5'), 2.84 (3H, s, H6'), 2.62 (1H, d, J_{gem} = 17.0 Hz, H12*α*), 2.62 (1H, s, H37), 2.38 (1H, m, H1*β*), 2.32 (1H, d, J_{gem} = 17.0 Hz, H12*β*), 2.27 (1H, m, H17*α*), 2.25 (6H, bs, N_BMe₂), 2.09 (1H, m, H15*β*), 1.94-2.01 (2H, m, H5*α*+H8*β*), 1.75 (1H, m, H2*α*), 1.63 (1H, d, J_{gem} = 14.2 Hz, H15*α*), 1.62 (1H, m, H7*β*), 1.55 (1H, m, H19*α*), 1.51 (3H, d, J_{H4'-F} = 22.4 Hz, H4'), 1.46 (1H, m, H2*β*), 1.41 (1H, m, H6*α*), 1.39 (3H, d, J_{H3'-F} = 2.4 Hz, H3'), 1.26 (3H, d, J₂₁₋₂₀ = 6.6 Hz, H21), 1.27 (2H, m, H7*α*+H1*α*), 1.06 (1H, d, J_{gem} = 4.1 Hz, H19*β*), 0.93 (1H, m, H6*β*), 0.88 (3H, s, H18), 0.74 (3H, s, H30) ; ¹³C NMR (75.4 MHz, CDCl₃): δ(ppm) 209.6 (C11), 172.3 (d, J_{C1'-F} = 19.8 Hz, C1'), 144.7 (C34), 132.6 (C31), 129.8 (C33+C35), 128.1 (C32+C36), 96.6 (d, J_{C2'-F} = 184.3 Hz, C2'), 73.4 (C16), 70.5 (C29), 65.1 (C20), 55.4 (C17), 51.7 (C12), 49.5 (C3), 47.4 (C14), 45.9 (C13), 45.5 (C15), 44.0 (C5'), 43.1 (C4), 42.6 (C8), 41.5 (C5), 37.5 (C10), 36.1 (C6'), 33.7 (C9), 30.8 (C19), 28.0 (C1), 27.2 (C2), 25.1 (d, J_{C3'-F} = 18.4 Hz, C3'), 24.8 (d, J_{C4'-F} = 18.4 Hz, C4'), 24.3 (C7), 21.6 (C37), 20.0 (C28), 18.5 (C6), 17.9 (C18), 11.6 (C21), 10.9 (C30) ; MS (ESI, m/z) : 675.3 (M+H) ; HRMS (ESI, m/z) : calcd for C₃₇H₅₆N₂O₆FS : 675.3843, found : 675.3879.

{20*S*-(dimethylamino)-16*α*-hydroxy-4*β*,14*α*-dimethyl-11-oxo-3*β*-heptafluoroisobutanamido-9,19-cyclo-5*α*,9*β*-pregnan-4*α*-yl}methyl tosylate (8c**)**



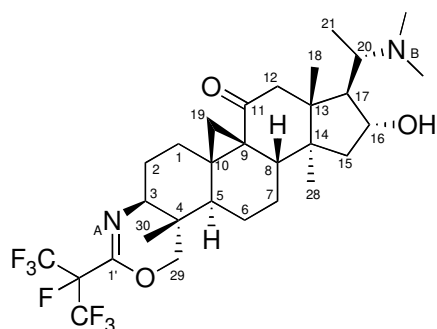
The title compound was prepared from 280 mg (0.42 mmol, 1 eq.) of **7c**, 20 mL of anhydrous MeOH, 80 mg (0.42 mmol, 1 eq.) of *p*-toluenesulfonic acid, 2.5 mL of pyridine, 398 mg (2.1 mmol, 5 eq.) of tosyl chloride by following similar procedure described for the preparation of **8a**. The basic hydrolysis of benzoyl group was proceed at 60 °C for 3 h. The crude product was purified by reverse phase silica gel column chromatography (eluent : water/CH₃CN/HCOOH : 75/25/0.1) to afford the title compound as an amorphous light yellow solid (101 mg, yield 31 %). IR (cm⁻¹) : 3342, 2932, 2873, 1716, 1670, 1539, 1457, 1236, 1176 ; ¹H NMR (300 MHz, CDCl₃): δ(ppm) 7.78 (2H, d, *J* = 8.3 Hz, H33+H35), 7.78 (2H, d, *J* = 8.3 Hz, H32+H36), 6.16 (1H, dd, *J*_{NAH-H3} = 9.8 Hz, *J*_{NAH-F} = 3.8 Hz, N_A-H), 4.21 (1H, m, H16*β*), 4.13 (1H, m, H3*α*), 3.88 (1H, d, *J*_{gem} = 10.1 Hz, H29*b*), 3.40 (1H, d, *J*_{gem} = 10.1 Hz, H29*a*), 2.65 (1H, dq, *J*_{20-17*α*} = 10.8 Hz, *J*₂₀₋₂₁ = 6.5 Hz, H20), 2.53 (1H, d, *J*_{gem} = 17.0 Hz, H12*α*), 2.48 (1H, m, H1*β*), 2.44 (3H, s, H37), 2.31 (1H, d, *J*_{gem} = 17.0 Hz, H12*β*), 1.96-2.06 (10H, m, H17*α*+N_BMe₂+H5*α*+H8*β*+H15*β*), 1.78 (1H, m, H2*α*), 1.63 (1H, m, H2*β*), 1.48-1.56 (4H, m, H6*α*+H19*α*+H15*α*+H7*β*), 1.31 (1H, m, H7*α*), 1.30 (1H, m, H1*α*), 1.20 (3H, s, H28), 1.02 (1H, d, *J*_{gem} = 4.0 Hz, H19*β*), 0.93 (1H, m, H6*β*), 0.88 (3H, d, *J*₂₁₋₂₀ = 6.5 Hz, H21), 0.84 (3H, s, H18), 0.75 (3H, s, H30) ; ¹³C NMR (75.4 MHz, CDCl₃): δ(ppm) 211.2 (C11), 156.1 (d, *J*_{C1'-F} = 18.7 Hz, C1'), 144.9 (C34), 132.3 (C31), 129.4 (C33+C35), 128.0 (C32+C36), 78.0 (C16), 70.1 (C29), 62.1 (C20), 55.7 (C17), 51.4 (C12), 51.3 (C3), 46.9 (C14), 44.4 (C13), 43.2 (C4), 42.9 (C15), 42.2 (C8), 42.0 (C5), 36.6 (C10), 34.0 (C9), 30.5 (C19), 27.8 (C1), 27.0 (C2), 24.3 (C7), 21.5 (C37), 20.9 (C28), 18.5 (C6), 17.9 (C18), 11.0 (C30), 10.0 (C21) ; MS (ESI, *m/z*) : 783.3 (M+H) ; HRMS (ESI, *m/z*) : calcd for C₃₇H₅₀N₂O₆F₇S : 783.3278, found : 783.3328.

20S-(dimethylamino)-16 α -hydroxy-4 β ,14 α -dimethyl-9,19-cyclo-{2'-(1-fluoro-1-methylethyl)-5',6'-dihydro-4' α H-[1',3']oxazino[4',5':3,4]}-5 α ,9 β -pregnan-11-one (9b)



To 32 mg (0.044 mmol, 1 eq.) of **8b** solubilized in 3 mL of anhydrous DMF were added 21 mg (0.22 mmol, 5 eq.) of sodium diformylamide. The mixture was stirred at 120 °C for 5 h, washed with 20 mL of a sodium hydrogen carbonate saturated solution and extracted with 5x10 mL of DCM. Combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by neutral alumina column chromatography (eluent : DCM/MeOH : 7/3) to afford the title compound as an amorphous colourless solid (7 mg, yield 32 %). IR (cm⁻¹) : 3329, 2934, 2869, 1669, 1456, 1157 ; ¹H NMR (500 MHz, CDCl₃): δ(ppm) 4.10 (1H, m, H16 β), 4.03 (1H, d, J_{gem} = 9.9 Hz, H29b), 3.86 (1H, d, J_{gem} = 9.9 Hz, H29a), 3.04 (1H, dm, J = 12.1 Hz, H3 α), 2.62 (1H, m, H20), 2.50 (1H, d, J_{gem} = 17.0 Hz, H12 α), 2.37 (1H, m, H2 α), 2.33 (1H, d, J_{gem} = 17.0 Hz, H12 β), 2.25 (6H, bs, N_BMe₂), 2.13 (1H, m, H5 α), 2.03 (1H, m, H15 β), 1.93-1.98 (2H, m, H17 α +H1 β), 1.67 (1H, d, J_{gem} = 4.1 Hz, H19 α), 1.50-1.59 (10H, m, H7 β +H3'+H4'+H15 α +H1 α +H8 β), 1.30 (1H, m, H2 β), 1.22 (2H, m, H6 α +H7 α), 1.10 (1H, d, J_{gem} = 4.1 Hz, H19 β), 0.92 (1H, m, H6 β), 0.88 (3H, d, J₂₁₋₂₀ = 6.1 Hz, H21), 0.84 (3H, s, H18), 0.82 (3H, s, H30) ; ¹³C NMR (125.6 MHz, CDCl₃): δ(ppm) 210.5 (C11), 158.8 (C1'), 93.7 (C2'), 78.3 (C16), 76.5 (C29), 62.1 (C20), 59.6 (C3), 55.9 (C17), 51.4 (C12), 47.2 (C14), 46.2 (C8), 44.7 (C13), 42.6 (C15), 40.7 (C5), 37.1 (C10), 34.9 (C9), 34.1 (C4), 29.7 (C19), 28.4 (C2), 28.2 (C1), 25.5 (d, J_{C3'-F} = 23.8 Hz, C3'), 25.4 (d, J_{C4'-F} = 23.8 Hz, C4'), 24.3 (C7), 20.5 (C28), 18.1 (C6), 17.9 (C18), 10.9 (C30), 10.0 (C21) ; MS (ESI, m/z) : 503.3 (M+H) ; HRMS (ESI, m/z) : calcd for C₃₀H₄₈N₂O₃F : 503.3649, found : 503.3642.

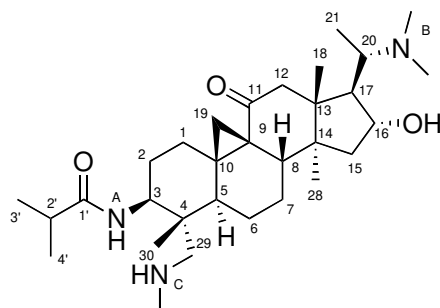
20S-(dimethylamino)-16 α -hydroxy-4 β ,14 α -dimethyl-9,19-cyclo-{2'-heptafluoroisobutyl}-5',6'-dihydro-4' α H-[1',3']oxazino[4',5':3,4]]-5 α ,9 β -pregnan-11-one (9c)



28.5 mg (0.036 mmol, 1 eq.) of **8c**, 17 mg (0.182 mmol, 5 eq.) of sodium diformylamide and 2 mL of DMF were used by following similar procedure described for the preparation of **9a**. 3 mg of **9c** were isolated as amorphous white solid (14%).

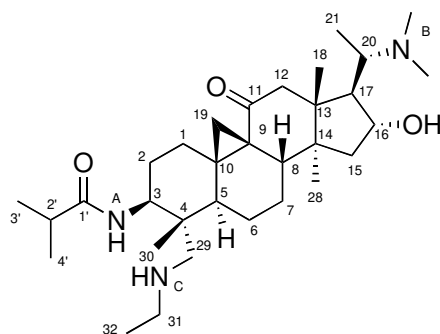
IR (cm⁻¹) : 3400, 2953, 2924, 2857, 1734, 1688, 1672, 1460, 1270, 1236 ; ¹H NMR (500 MHz, CDCl₃): δ (ppm) 4.16 (1H, d, $J_{\text{gem}} = 10.1$ Hz, H29a), 4.10 (1H, m, H16 β), 4.00 (1H, d, $J_{\text{gem}} = 10.1$ Hz, H29b), 3.20 (1H, m, H3 α), 2.63 (1H, dq, $J = 11.0$ Hz, $J = 6.4$ Hz, H20), 2.58 (1H, d, $J_{\text{gem}} = 17.1$ Hz, H12 α), 2.41 (1H, m, H1 β), 2.35 (1H, d, $J = 17.1$ Hz, H12 β), 2.42 (6H, bs, N_BMe₂), 2.17 (1H, m, H17 α), 2.12 (1H, m, H5 α), 2.06 (1H, m, H15 β), 2.01 (2H, m, H2), 1.68 (1H, d, $J_{\text{gem}} = 4.0$ Hz, H19 α), 1.58 (1H, m, H15 α), 1.56 (1H, m, H8 β), 1.14 (1H, m, H6 α), 1.34 (2H, m, H7), 1.59 (1H, m, H1 α), 1.22 (3H, s, H28), 1.12 (1H, d, $J_{\text{gem}} = 4.0$ Hz, H19 β), 0.88 (3H, d, $J = 6.4$ Hz, H21), 0.86 (3H, s, H18), 0.85 (1H, m, H6 β), 0.84 (3H, m, H30) ; ¹³C NMR (125.6 MHz, CDCl₃): δ (ppm) 209.4 (C11), 144.6 (C1'), 78.5 (C16), 77.8 (C29), 60.4 (C3), 62.1 (C20), 56.0 (C17), 51.6 (C12), 47.1 (C14), 45.8 (C8), 44.7 (C13), 44.2 (C15), 40.4 (C5), 36.4 (C10), 35.2 (C9), 34.9 (C4), 29.8 (C19), 28.1 (C1), 27.3 (C2), 22.3 (C7), 19.7 (C28), 19.3 (C6), 17.7 (C18), 11.0 (C21), 10.6 (C30) ; MS (ESI, m/z) : 611.3 (M+H) ; HRMS (ESI, m/z) : calcd for C₃₀H₄₂F₇N₂O₃ : 611.3085, found : 611.3085.

***N*-[20*S*-(dimethylamino)-16*α*-hydroxy-4*β*,14*α*-dimethyl-4*α*-[(methylamino)methyl]-11-oxo-9,19-cyclo-5*α*,9*β*-pregnan-3*β*-yl]-isobutyramide (12a)**



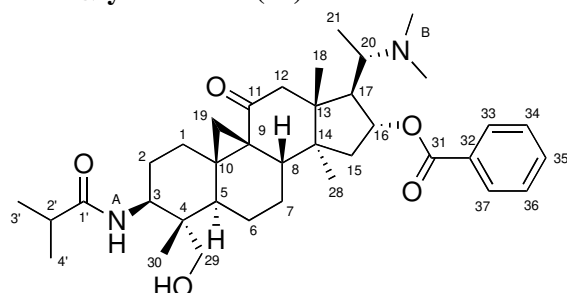
To 100 mg (0.19 mmol, 1 eq.) of aldehyde **10** **solubilized** in 1 mL of EtOH were added 100 μ L (1.4 mmol, 7 eq.) of methylamine (33 % in EtOH) and a small amount of sodium sulfate. The mixture was stirred at 60 °C for 12 h, filtered and concentrated under vacuum. The formation of imine was controlled by NMR. The residue was dissolved in 1 mL of EtOH and 34 μ L (0.6 mmol, 3 eq.) of acetic acid and 19 mg (0.3 mmol, 1.5 eq.) of sodium cyanoborohydride were added at 0 °C. The mixture was stirred at RT for 1 h, washed with 20 mL of a saturated sodium carbonate solution then extracted with 3x20 mL of DCM. Combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum to afford the title compound without further purification as an amorphous white solid (93 mg, yield 90 %). IR (cm^{-1}) : 3308, 2961, 2931, 2869, 2839, 1732, 1649, 1537, 1450, 1226, 1188, 1156, 1095 ; ^1H NMR (300 MHz, CDCl_3): δ (ppm) 5.46 (1H, d, $J_{\text{NAH-3}} = 9.7$ Hz, $\text{N}_\text{A}\text{-H}$), 4.10 (1H, m, $\text{H}_{16\beta}$), 3.97 (1H, m, $\text{H}_{3\alpha}$), 2.62 (1H, dq, $J_{20-17\alpha} = 10.7$ Hz, $J_{20-21} = 6.5$ Hz, H_{20}), 2.51 (1H, d, $J_{\text{gem}} = 17.2$ Hz, $\text{H}_{12\alpha}$), 2.44 (1H, m, $\text{H}_{1\beta}$), 2.36 (1H, m, $\text{H}_{2'}$), 2.34 (3H, s, H_{31}), 2.27 (1H, d, $J_{\text{gem}} = 17.2$ Hz, $\text{H}_{12\beta}$), 2.25 (6H, bs, $\text{N}_\text{B}\text{Me}_2$), 2.21 (2H, m, H_{29}), 2.12 (1H, m, $\text{H}_{5\alpha}$), 2.02 (1H, m, $\text{H}_{15\beta}$), 1.98 (1H, m, $\text{H}_{17\alpha}$), 1.91 (1H, m, $\text{H}_{8\beta}$), 1.68 (1H, m, $\text{H}_{2\alpha}$), 1.54 (1H, m, $\text{H}_{2\beta}$), 1.53 (1H, d, $J_{\text{gem}} = 3.5$ Hz, $\text{H}_{19\alpha}$), 1.50 (2H, m, $\text{H}_{6\alpha} + \text{H}_{15\alpha}$), 1.48 (2H, m, H_7), 1.27 (1H, m, $\text{H}_{1\alpha}$), 1.21 (3H, s, H_{28}), 1.15 and 1.18 (6H, 2d, $J = 7.3$ Hz, $\text{H}_{3'} + \text{H}_{4'}$), 1.04 (1H, d, $J_{\text{gem}} = 3.5$ Hz, $\text{H}_{19\beta}$), 0.94 (1H, m, $\text{H}_{6\beta}$), 0.84 (3H, s, H_{18}), 0.87 (3H, d, $J_{21-20} = 6.5$ Hz, H_{21}), 0.68 (3H, s, H_{30}). ^{13}C NMR (75.4 MHz, CDCl_3): δ (ppm) 211.6 (C_{11}), 176.9 ($\text{C}_{1'}$), 78.2 (C_{16}), 61.9 (C_{20}), 56.6 (C_{29}), 55.7 (C_{17}), 51.4 (C_{12}), 50.4 (C_3), 47.0 (C_{14}), 44.3 (C_{13}), 43.3 (C_4), 42.9 (C_{15}), 42.6 (C_5), 42.1 (C_8), 38.1 (C_{10}), 37.4 (C_{31}), 35.8 ($\text{C}_{2'}$), 34.3 (C_9), 31.7 (C_{19}), 28.2 (C_2), 27.6 (C_1), 24.5 (C_7), 21.0 (C_{28}), 19.4 and 20.2 ($\text{C}_{3'} + \text{C}_{4'}$), 18.6 (C_6), 17.8 (C_{18}), 13.8 (C_{30}), 9.8 (C_{21}) ; MS (ESI, m/z) : 516.4 ($\text{M} + \text{H}$) ; HRMS (ESI, m/z) : calcd for $\text{C}_{31}\text{H}_{54}\text{N}_3\text{O}_3$: 516.4165, found : 516.4159.

***N*-[20*S*-(dimethylamino)-4 α -[(ethylamino)methyl]-16 α -hydroxy-4 β ,14 α -dimethyl-11-oxo-9,19-cyclo-5 α ,9 β -pregnan-3 β -yl]-isobutyramide (**12b**)**



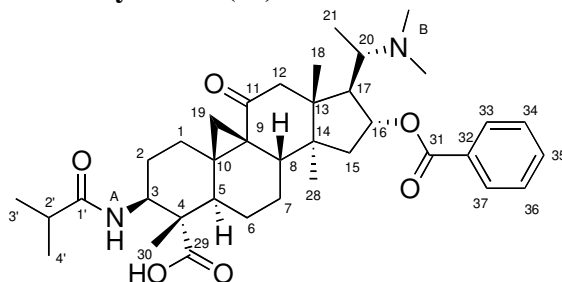
The title compound was prepared from 100 mg (0.19 mmol, 1 eq.) of aldehyde **10**, 100 μ L (1.5 mmol, 7.5 eq.) of ethylamine, 34 μ L (0.6 mmol, 3 eq.) acetic acid, 19 mg (0.3 mmol, 1.5 eq.) of sodium cyanoborohydride and 1 mL of EtOH by following similar procedure describe for the preparation of **12a**. **12b** (90 mg, yield 85 %). IR (cm^{-1}) : 3308, 2961, 2933, 2869, 2831, 1732, 1651, 1537, 1453, 1226, 1188, 1165, 1096 ; ^1H NMR (300 MHz, CDCl_3): δ (ppm) 5.46 (1H, d, $J_{\text{NAH-3}} = 9.7$ Hz, $\text{N}_\text{A}\text{-H}$), 4.10 (1H, ddd, $J = 10.0$ Hz, $J = 7.3$ Hz, $J = 2.8$ Hz, H16 β), 3.97 (1H, ddd, $J = 11.9$ Hz, $J = 9.7$ Hz, $J = 4.2$ Hz, H3 α), 2.53-2.68 (3H, m, H20+H31), 2.52 (1H, d, $J_{\text{gem}} = 17.8$ Hz, H12 α), 2.41 (1H, m, H1 β), 2.36 (1H, q, $J = 6.9$ Hz, H2'), 2.28 (1H, d, $J_{\text{gem}} = 17.8$ Hz, H12 β), 2.25 (8H, m, H29+N_BMe₂), 2.17 (1H, m, H5 α), 2.04 (1H, m, H15 β), 1.98 (2H, m, H17 α +H8 β), 1.69 (1H, m, H2 α), 1.54 (1H, d, $J_{\text{gem}} = 3.6$ Hz, H19 α), 1.53 (1H, m, H2 β), 1.50 (4H, m, H6 α +H15 α +H7), 1.28 (1H, m, H1 α), 1.22 (3H, s, H28), 1.15 and 1.17 (6H, 2d, $J = 6.9$ Hz, H3'+H4'), 1.08 (3H, t, $J_{32-31} = 7.2$ Hz, H32), 1.04 (1H, d, $J_{\text{gem}} = 3.6$ Hz, H19 β), 0.98 (1H, m, H6 β), 0.87 (3H, d, $J_{21-20} = 6.6$ Hz, H21), 0.84 (3H, s, H18), 0.69 (3H, s, H30) ; ^{13}C NMR (75.4 MHz, CDCl_3): δ (ppm) 211.6 (C11), 176.9 (C1'), 78.3 (C16), 61.9 (C20), 55.7 (C17), 53.4 (C29), 51.4 (C12), 50.4 (C3), 47.0 (C14), 44.7 (C31), 44.3 (C13), 43.1 (C4), 42.8 (C15), 42.3 (C5), 42.0 (C8), 38.1 (C10), 35.8 (C2'), 34.3 (C9), 31.5 (C19), 28.2 (C2), 27.5 (C1), 24.4 (C7), 21.0 (C28), 19.4 and 20.1 (C3'+C4'), 18.6 (C6), 17.8 (C18), 14.7 (C32), 13.8 (C30), 9.8 (C21) ; MS (ESI, m/z) : 530.5 (M+H) ; HRMS (ESI, m/z) : calcd for $\text{C}_{32}\text{H}_{56}\text{N}_3\text{O}_3$: 530.4322, found : 530.4299.

20S-(dimethylamino)-3 β -(isobutanamido)-4 α -(hydroxymethyl)-4 β ,14 α -dimethyl-11-oxo-9,19-cyclo-5 α ,9 β -pregnan-16 α -yl benzoate (14)



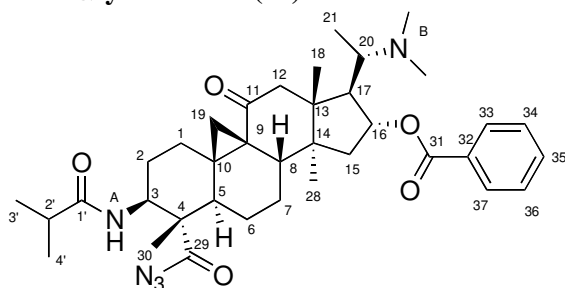
To 216 mg (0.429 mmol, 1 eq.) of *N*-3-isobutyrylcycloheximidine-F **3** solubilized in 9 mL of a mixture DCM/pyridine (3/1) were added at 0 °C 130 mg (0.515 mmol, 1.2 eq.) of benzoic anhydride. The mixture was stirred at 0 °C for 1 h and at RT for further 12 h. After 3 coevaporations with 3x10 mL of 1,2-dichloroethane, the residue was dissolved in DCM, washed with 20 mL of a saturated sodium hydrogen carbonate solution and extracted with 3x20 mL of DCM. Combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by silica gel column chromatography (eluent : DCM/MeOH/NH₄OH : 97/2/1) to afford the title compound as a white solid (260 mg, yield 100 %). IR (cm⁻¹) : 3314, 2965, 2930, 2873, 2829, 1702, 1661, 1620, 1540, 1447, 1279, 1230, 1114 ; ¹H NMR (300 MHz, CDCl₃): δ (ppm) 8.04 (2H, m, H33+H37), 7.54 (1H, m, H35), 7.43 (2H, m, H34+H36), 5.41 (2H, m, N_A-H+H16 β), 4.35 (1H, dd, J_{OH-H29b} = 10.9 Hz, J_{OH-H29a} = 4.9 Hz, OH29), 3.99 (1H, m, H3 α), 3.33 (1H, dd, J_{gem} = 12.7 Hz, J_{H29b-OH} = 10.9 Hz, H29b), 2.96 (1H, dd, J_{gem} = 12.7 Hz, J_{H29a-OH} = 4.9 Hz, H29a), 2.62 (1H, d, J_{gem} = 17.1 Hz, H12 α), 2.50 (1H, dq, J_{20-17 α} = 11.3 Hz, J₂₀₋₂₁ = 6.6 Hz, H20), 2.44 (2H, m, H1 β +H17 α), 2.43 (1H, d, J_{gem} = 17.1 Hz, H12 β), 2.40 (1H, m, H2'), 2.02-2.17 (9H, m, N_BMe₂+H5 α +H8 β +H15 β), 1.68 (2H, m, H2 α +H2 β), 1.68 (1H, m, H6 α), 1.55-1.60 (2H, m, H19 α +H15 α), 1.47 (1H, m, H7 β), 1.38 (1H, m, H7 α), 1.27 (1H, m, H1 α), 1.24 (3H, s, H28), 1.17 and 1.19 (6H, 2d, J = 6.9 Hz, H3'+H4'), 1.06 (1H, d, J_{gem} = 3.5 Hz, H19 β), 0.89 (1H, m, H6 β), 0.91 (3H, s, H18), 0.85 (3H, d, J₂₁₋₂₀ = 6.1 Hz, H21), 0.57 (3H, s, H30) ; ¹³C NMR (75.4 MHz, CDCl₃): δ (ppm) 211.2 (C11), 178.5 (C1'), 165.9 (C31), 132.5 (C35), 130.9 (C32), 129.4 (C33+C37), 128.4 (C34+C36), 79.5 (C16), 64.1 (C29), 59.5 (C20), 55.4 (C17), 52.0 (C12), 50.7 (C3), 47.6 (C14), 44.7 (C13), 44.6 (C4), 43.2 (C15), 41.5 (C8), 41.2 (C5), 40.3 (N_BMe₂), 37.8 (C10), 35.8 (C2'), 34.0 (C9), 30.8 (C19), 27.8 (C1), 27.6 (C2), 24.4 (C7), 20.1 (C28), 19.6 and 19.4 (C3'+C4'), 18.4 (C6), 17.9 (C18), 11.2 (C30), 9.9 (C21) ; MS (ESI, m/z) : 607.3 (M+H) ; HRMS (ESI, m/z) : calcd for C₃₇H₅₅N₂O₅ : 607.4070, found : 607.4111.

16 α -(benzyloxy)-20S-(dimethylamino)-3 β -(isobutanamido)-4 β ,14 α -dimethyl-11-oxo-9,19-cyclo-5 α ,9 β -pregnan-4 α -carboxylic acid (15**)**



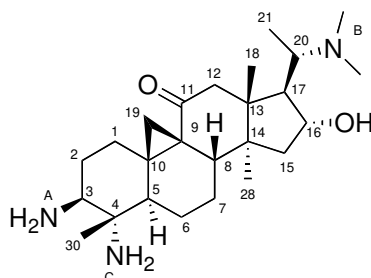
To 300 mg (0.494 mmol, 1 eq.) of **14** solubilized in 15 mL of acetone were added at 0 °C 925 μ L (2.47 mmol, 5 eq.) of Jones' reagent (2.672 M). The mixture was stirred at RT for 1 h then cooled at 0°C. 5 mL of isopropanol were slowly added to quench the excess of reagent. 5 mL of NH_4OH were added and the mixture was filtered through a pad of celite. The filtrate was concentrated under vacuum and the residue was purified by silica gel column chromatography (eluent : DCM/MeOH/ NH_4OH : 85/15/1) to afford the title compound as a white solide (224 mg, yield 73 %). IR (cm^{-1}) : 3354, 2966, 2930, 2873, 2829, 1711, 1660, 1650, 1555, 1449, 1279, 1228, 1112 ; ^1H NMR (300 MHz, CD_3OD) : δ (ppm) 8.03 (2H, m, H33+H37), 7.57 (1H, m, H35), 7.45 (2H, m, H34+H36), 5.43 (1H, m, H16 β), 4.32 (1H, m, H3 α), 2.93 (1H, dq, $J_{20-17\alpha} = 11.0$ Hz, $J_{20-21} = 6.4$ Hz, H20), 2.74 (1H, d, $J_{\text{gem}} = 18.4$ Hz, H12 α), 2.67 (1H, m, H17 α), 2.41 (1H, m, H2'), 2.38 (1H, m, H12 β), 2.34 (1H, m, H1 β), 2.23 (1H, m, H5 α), 2.31 (6H, s, $\text{N}_\text{B}\text{Me}_2$), 2.18 (1H, m, H8 β), 1.73 (2H, m, H2 α +H2 β), 1.59 (2H, m, H15 α +H15 β), 1.56 (1H, m, H19 α), 1.48 (2H, m, H7 β +H7 α), 1.33 (1H, m, H1 α), 1.29 (3H, s, H28), 1.20 (1H, m, H6 α), 1.19 (1H, m, H19 β), 1.01-1.07 (13H, m, H6 β +H21+H30+H3'+H4'), 0.96 (3H, s, H18) ; ^{13}C NMR (75.4 MHz, CD_3OD) : δ (ppm) 212.9 (C11), 181.3 (C29), 179.2 (C1'), 167.4 (C31), 134.1 (C35), 132.0 (C32), 130.4 (C33+C37), 129.6 (C34+C36), 80.9 (C16), 61.8 (C20), 55.9 (C17), 55.3 (C3), 54.5 (C4), 53.0 (C12), 48.4 (C14), 46.5 (C5), 46.4 (C13), 44.3 (C15), 42.5 (C8), 40.6 ($\text{N}_\text{B}\text{Me}_2$), 39.2 (C10), 36.1 (C2'), 35.2 (C9), 31.5 (C19), 28.9 (C1), 28.7 (C2), 25.5 (C7), 22.1 (C6), 20.1 and 20.2 (C3'+C4'), 19.9 (C28), 18.3 (C18), 11.4 (C30), 10.7 (C21) ; MS (ESI, m/z) : 621.3 (M+H) ; HRMS (ESI, m/z) : calcd for $\text{C}_{37}\text{H}_{53}\text{N}_2\text{O}_6$: 621.3904, found : 621.3896.

4 α -(azidocarbonyl)-20S-(dimethylamino)-3 β -(isobutanamido)-4 β ,14 α -dimethyl-11-oxo-9,19-cyclo-5 α ,9 β -pregnan-16 α -yl benzoate (16)



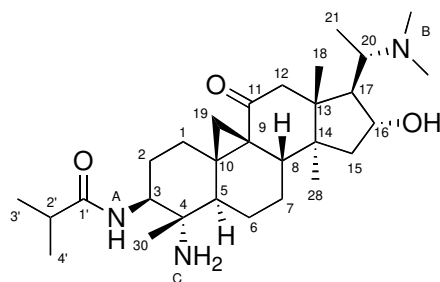
To 350 mg (0.564 mmol, 1 eq.) of **15** solubilized in 7 mL of DCM were added at -20 °C 235 μ L (1.69 mmol, 3 eq.) of TEA, 44 μ L (0.564 mmol, 1 eq.) of DMF and 74 μ L (0.845 mmol, 1.5 eq.) of oxalyl chloride. The mixture was stirred at -20 °C for 15 min then warmed to 0 °C. 183 mg (2.8 mmol, 5 eq.) of sodium azide dissolved in the minimum of water were added. The mixture was stirred at RT for further 3 h, washed with 20 mL of a saturated sodium hydrogen carbonate solution and extracted with 3x20 mL of DCM. Combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by silica gel column chromatography (eluent : ACOEt/heptane/ NH_4OH : 50/50/1) to afford the title compound as a white solid (350 mg, yield: 74 %). IR (cm^{-1}) : 3316, 2933, 2870, 2137 ($\nu_{\text{CO-N}_3}$), 1744, 1708, 1665, 1530, 1449, 1194, 1218, 1276 ; ^1H NMR (300 MHz, CDCl_3): δ (ppm) 8.05 (2H, m, H33+H37), 7.56 (1H, m, H35), 7.44 (2H, m, H34+H36), 5.41 (1H, m, H16 β), 5.36 (1H, d, $J_{\text{NAH-3}} = 9.5$ Hz, N_AH), 4.38 (1H, m, H3 α), 2.63 (1H, d, $J_{\text{gem}} = 17.0$ Hz, H12 α), 2.50 (1H, m, H20), 2.45 (2H, m, H17 α +H12 β), 2.36 (1H, m, H1 β), 2.29 (1H, m, H2'), 2.25 (1H, m, H5 α), 2.09 (6H, s, $\text{N}_\text{B}\text{Me}_2$), 2.12 (1H, m, H8 β), 1.80 (2H, m, H2 α +H2 β), 1.54-1.63 (3H, m, H15 α +H15 β +H19 α), 1.48 (2H, m, H7 β), 1.40 (2H, m, H7 α +H1 α), 1.28 (3H, s, H28), 1.05-1.11 (9H, m, H6+H19 β +H3'+H4'), 1.07 (3H, s, H30), 0.90 (3H, s, H18), 0.85 (3H, d, $J_{21-20} = 5.9$ Hz, H21) ; ^{13}C NMR (75.4 MHz, CDCl_3): δ (ppm) 210.4 (C11), 183.4 (C29), 176.2 (C1'), 165.7 (C31), 132.6 (C35), 130.7 (C32), 129.3 (C33+C37), 128.2 (C34+C36), 79.4 (C16), 59.4 (C20), 55.4 (C17), 54.7 (C4), 52.7 (C3), 51.8 (C12), 47.5 (C14), 44.6 (C13), 44.1 (C5), 42.9 (C15), 40.9 (C8), 40.2 ($\text{N}_\text{B}\text{Me}_2$), 36.2 (C10), 35.7 (C2'), 33.7 (C9), 29.4 (C19), 27.9 (C2), 27.2 (C1), 23.9 (C7), 21.1 (C6), 19.9 (C28), 19.3 and 19.4 (C3'+C4'), 17.9 (C18), 10.0 (C30), 9.9 (C21) ; MS (ESI, m/z) : 646.4 (M+H), 618.3 ; HRMS (ESI, m/z) : calcd for $\text{C}_{37}\text{H}_{52}\text{N}_5\text{O}_5$: 646.3968, found : 646.3952.

3 β ,4 α -diamino-20*S*-(dimethylamino)-16 α -hydroxy-4 β ,14 α -dimethyl-9,19-cyclo-5 α ,9 β -pregnan-11-one (18)



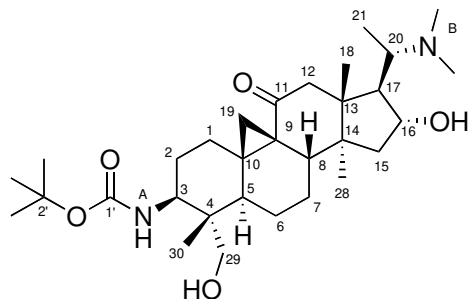
155 mg (0.24 mmol, 1 eq.) of **16** were heated without solvent in a microwave (Pmax = 200 W) at 110 °C for 10 min. 20 mL of THF, 5 mL of water and a small amount of lithium hydroxide were added and the mixture was refluxed for 12 h. 5 mL of MeOH were added and the mixture was refluxed for further 1 h. Solvents were removed under vacuum and 50 mL of HCl (2N) were added to the residue. The mixture was refluxed for 1 h and concentrated under vacuum to leave 5 mL of aqueous layer. This layer was alkalinised with NH₄OH and extracted with 5x20 mL of DCM. Combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by silica gel column chromatography (eluent : DCM/MeOH/NH₄OH : 80/20/1) to afford 76 mg of the title compound and 10 mg of **19** (respective ratio: 9/1) as amorphous white solids (overall yield: 84 %). IR (cm⁻¹) : 3210 (νN-H, νO-H), 2966-2941, 2873, 1702, 1667, 1459, 1250, 1222 ; ¹H NMR (300 MHz, CDCl₃): δ(ppm) 4.10 (1H, ddd, J = 10.1 Hz, J = 7.3 Hz, J = 2.9 Hz, H16 β), 2.62 (1H, dq, J_{20-17 α} = 10.9 Hz, J₂₀₋₂₁ = 6.5 Hz, H20), 2.50 (1H, d, J_{gem} = 17.1 Hz, H12 α), 2.43 (1H, dd, J = 10.4 Hz, J = 4.1 Hz, H3 α), 2.30 (1H, d, J_{gem} = 17.1 Hz, H12 β), 2.30 (1H, m, H1 β), 2.24 (6H, bs, N_BMe₂), 2.07 (1H, m, H8 β), 2.02 (1H, m, H15 β), 1.97 (1H, m, H17 α), 1.76 (2H, m, H2+C6 β), 1.60 (1H, m, H7 α), 1.54 (1H, d, J_{gem} = 4.0 Hz, H19 α), 1.49 (1H, m, H15 α), 1.46 (1H, m, H5 α), 1.36 (1H, m, H7 β), 1.32 (1H, m, H2 β), 1.25 (1H, m, H1 α), 1.21 (3H, s, H28), 1.02 (1H, m, H6 α), 1.05 (1H, d, J_{gem} = 4.0 Hz, H19 β), 0.87 (3H, d, J₂₁₋₂₀ = 6.6 Hz, H21), 0.85 (3H, s, H30), 0.84 (3H, s, H18) ; ¹³C NMR (75.4 MHz, CDCl₃): δ(ppm) 211.0 (C11), 78.3 (C16), 62.0 (C20), 60.2 (C3), 57.0 (C4), 55.7 (C17), 51.4 (C12), 50.7 (C5), 47.1 (C14), 44.5 (C13), 42.7 (C15), 41.2 (C8), 39.0 (C10), 35.0 (C9), 31.6 (C2), 29.7 (C19), 27.7 (C1), 24.4 (C7), 20.6 (C28), 18.4 (C6), 17.8 (C18), 15.2 (C30), 9.9 (C21) ; MS (ESI, m/z) : 418.3 (M+H) ; HRMS (ESI, m/z) : calcd for C₂₅H₄₃N₃O₂ : 418.3433, found : 418.3450.

***N*-[4 α -amino-20*S*-(dimethylamino)-16 α -hydroxy-4 β ,14 α -dimethyl-11-oxo-9,19-cyclo-5 α ,9 β -pregnan-3 β -yl]-isobutyramide (19)**



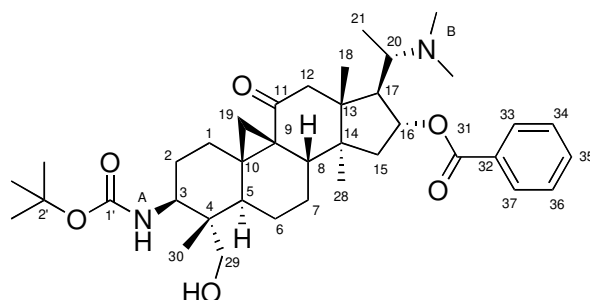
MP (°C) : 254 ; IR (cm⁻¹) : 3314, 2970, 2932, 2873, 2832, 1652, 1540, 1456, 1226, 1189, 1166 ;
¹H NMR (300 MHz, CDCl₃): δ(ppm) 5.36 (1H, d, J_{NAH-3} = 9.1 Hz, N_AH), 4.10 (1H, m, H16 β),
 3.78 (1H, m, H3 α), 2.62 (1H, dq, J_{20-17 α} = 10.9 Hz, J₂₀₋₂₁ = 6.6 Hz, H20), 2.52 (1H, d, J_{gem} =
 17.0 Hz, H12 α), 2.39 (1H, m, H2'), 2.34 (1H, m, H1 β), 2.30 (1H, m, H12 β), 2.25 (6H, s,
 N_BMe₂), 2.09 (1H, m, H8 β), 2.03 (1H, m, H15 β), 1.95 (1H, m, H17 α), 1.91 (1H, m, H6 β), 1.77
 (2H, m, H2 α +H2 β), 1.62 (2H, m, H5 α +H7 β), 1.53 (1H, d, J_{gem} = 4.0 Hz, H19 α), 1.50 (1H, m,
 H15 α), 1.38 (1H, m, H7 α), 1.33 (1H, m, H1 α), 1.22 (3H, s, H28), 1.17 and 1.18 (6H, 2d, J =
 6.9 Hz, H3'+H4'), 1.05 (1H, d, J_{gem} = 6.9 Hz, H19 β), 0.95 (1H, m, H6 α), 0.89 (3H, d, J₂₁₋₂₀ =
 6.6 Hz, H21), 0.86 (3H, s, H30), 0.84 (3H, s, H18) ; ¹³C NMR (75.4 MHz, CDCl₃): δ(ppm)
 211.1 (C11), 177.3 (C1'), 78.2 (C16), 62.0 (C20), 58.3 (C3), 56.7 (C4), 55.8 (C17), 51.4 (C12),
 50.0 (C5), 47.1 (C14), 44.5 (C13), 42.7 (C15), 41.1 (C8), 38.3 (C10), 36.0 (C2'), 35.0 (C9), 29.4
 (C19), 29.0 (C2), 27.5 (C1), 24.2 (C7), 20.6 (C28), 19.6 and 19.9 (C3'+C4'), 18.2 (C6), 17.8
 (C18), 16.2 (C30), 9.9 (C21) ; MS (ESI, m/z) : 488.4 (M+H) ; HRMS (ESI, m/z) : calcd for
 C₂₉H₅₀N₃O₃ : 488.3852, found : 488.3848.

***tert*-butyl [20*S*-(dimethylamino)-16*α*-hydroxy-4*α*-(hydroxymethyl)-4*β*,14*α*-dimethyl-11-oxo-9,19-cyclo-5*α*,9*β*-pregnan-3*β*-yl]carbamate (21)**



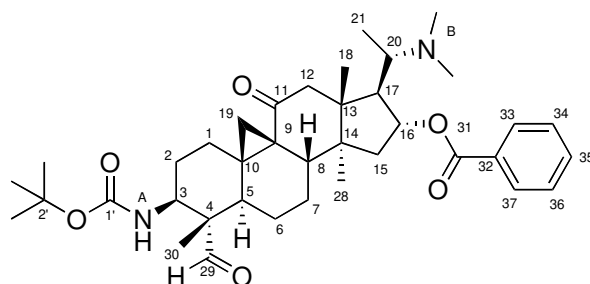
To 788 mg (1.82 mmol, 1 eq.) of cycloxbuxidine-F **4** dissolved in 25 mL of MeOH were added 429 μ L (2.0 mmol, 1.1 eq.) of di-*tert*-butyl dicarbonate. The mixture was stirred at RT for 5 h and the solvent was removed under reduced pressure. The residue was dissolved in DCM, washed with 30 mL of a saturated sodium carbonate solution and extracted with 3x30 mL of DCM. Combined organic layers were washed with brine, dried over magnesium sulfate. The solvent was removed under reduced pressure to afford the title compound as an amorphous white solid. (970 mg, yield: 100 %). IR (cm^{-1}) : 3313, 2971, 2935, 2870, 1667, 1530, 1454, 1251, 1164 ; ^1H NMR (300 MHz, CDCl_3): δ (ppm) 4.64 (1H, d, $J = 9.2$ Hz, $\text{N}_\text{A}\text{-H}$), 4.09 (2H, m, $\text{H}_{16\beta} + \text{OH}_{29}$), 3.69 (1H, ddd, $J = 12.4$ Hz, $J = 9.2$ Hz, $J = 4.1$ Hz, $\text{H}_{3\alpha}$), 3.35 (1H, dd, $J_{\text{gem}} = 12.7$ Hz, $J_{\text{H}_{29\text{b}}-\text{OH}} = 10.3$ Hz, $\text{H}_{29\text{b}}$), 3.13 (1H, dd, $J_{\text{gem}} = 12.7$ Hz, $J_{\text{H}_{29\text{a}}-\text{OH}} = 4.4$ Hz, $\text{H}_{29\text{a}}$), 2.61 (1H, dq, $J_{20-17\alpha} = 10.7$ Hz, $J_{20-21} = 6.6$ Hz, H_{20}), 2.51 (1H, d, $J_{\text{gem}} = 17.1$ Hz, $\text{H}_{12\alpha}$), 2.39 (1H, dm, $J = 13.3$ Hz, $\text{H}_{1\beta}$), 2.28 (1H, d, $J_{\text{gem}} = 17.1$ Hz, $\text{H}_{12\beta}$), 2.25 (6H, bs, $\text{N}_\text{B}\text{Me}_2$), 2.02 ($\text{H}_{5\alpha} + \text{H}_{8\beta} + \text{H}_{15\beta}$), 1.97 (1H, m, $\text{H}_{17\alpha}$), 1.72 (1H, m, $\text{H}_{2\alpha}$), 1.69 (1H, m, $\text{H}_{6\alpha}$), 1.55 (1H, d, $J = 3.9$ Hz, $\text{H}_{19\alpha}$), 1.52 (3H, m, $\text{H}_{2\beta} + \text{H}_7$), 1.48 (1H, m, $\text{H}_{15\alpha}$), 1.45 (9H, s, Me_3), 1.29 (1H, m, $\text{H}_{1\alpha}$), 1.22 (3H, s, H_{28}), 1.04 (1H, d, $J_{\text{gem}} = 3.9$ Hz, $\text{H}_{19\beta}$), 0.90 (1H, m, $\text{H}_{6\beta}$), 0.87 (3H, d, $J_{21-20} = 6.6$ Hz, H_{21}), 0.84 (3H, s, H_{18}), 0.56 (3H, s, H_{30}) ; ^{13}C NMR (75.4 MHz, CDCl_3): δ (ppm) 211.2 (C_{11}), 157.1 ($\text{C}_{1'}$), 79.9 ($\text{C}_{2'}$), 78.2 (C_{16}), 64.1 (C_{29}), 61.9 (C_{20}), 55.7 (C_{17}), 51.9 (C_3), 51.4 (C_{12}), 47.0 (C_{14}), 44.6 (C_4), 44.4 (C_{13}), 42.7 (C_{15}), 41.3+41.0 ($\text{C}_5 + \text{C}_8$), 37.7 (C_{10}), 34.0 (C_9), 30.5 (C_{19}), 28.2 (Me_3), 27.7+27.6 ($\text{C}_1 + \text{C}_2$), 24.2 (C_7), 20.7 (C_{28}), 18.3 (C_6), 17.7 (C_{18}), 10.9 (C_{30}), 9.8 (C_{21}) ; MS (ESI, m/z) : 533.3 ($\text{M} + \text{H}$) ; HRMS (ESI, m/z) : calcd for $\text{C}_{31}\text{H}_{53}\text{N}_2\text{O}_5$: 533.3954, found : 533.3965.

3 β -[(*tert*-butoxycarbonyl)amino]-20*S*-(dimethylamino)-4 α -(hydroxymethyl)-4 β ,14 α -dimethyl-11-oxo-9,19-cyclo-5 α ,9 β -pregnan-16 α -yl benzoate (22)



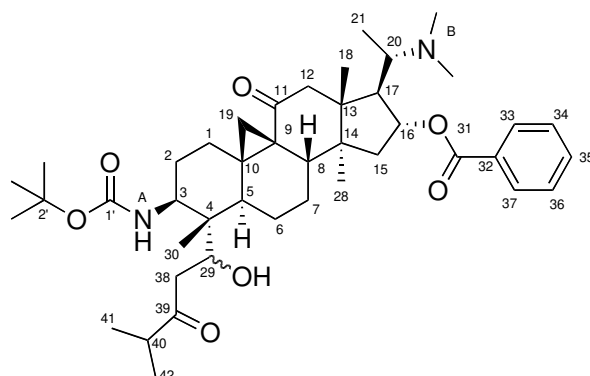
To 970 mg (1.82 mmol, 1 eq.) of **21** dissolved in 25 mL of DCM were added 481 mg (1.9 mmol, 1.05 eq.) of benzoic anhydride, 384 μ L (2.7 mmol, 1.5 eq.) of trimethylamine and a catalytic amount of DMAP. The mixture was stirred at RT for 12 h then washed with 30 mL of a saturated sodium carbonate solution. The aqueous layer was extracted with 2x30 mL of DCM. Combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by silica gel column chromatography (eluent : DCM/MeOH/NH₄OH : 97/2/1) to afford the title compound as an amorphous white solid (1.125 g, yield: 97 %). IR (cm⁻¹) : 3364, 2969, 2932, 2871, 2823, 1711, 1672, 1524, 1276, 1164 ; ¹H NMR (300 MHz, CDCl₃): δ (ppm) 8.04 (2H, m, H33+H37), 7.55 (1H, m, H35), 7.43 (2H, m, H34+H36), 5.42 (1H, dd, J = 8.0 Hz, J = 5.7 Hz, H16 β), 4.47 (1H, d, J = 9.1 Hz, N_A-H), 3.70 (1H, ddd, J = 12.6 Hz, J = 9.1 Hz, J = 4.0 Hz, H3 α), 3.34 (1H, d, J_{gem} = 12.6 Hz, H29b), 3.13 (1H, d, J_{gem} = 12.6 Hz, H29a), 2.62 (1H, d, J_{gem} = 17.0 Hz, H12 α), 2.56 (1H, m, H20), 2.44 (1H, m, H17 α), 2.42 (1H, d, J_{gem} = 17.0 Hz, H12 β), 2.41 (1H, m, H1 β), 2.17 (1H, m, H15 β), 2.11 (6H, bs, N_BMe₂), 2.08 (2H, m, H5 α +H8 β), 1.71 (1H, m, H2 α), 1.69 (1H, m, H6 α), 1.57 (2H, m, H15 α +H19 α), 1.53 (3H, m, H2 β +H7), 1.45 (9H, s, Me₃), 1.26 (1H, m, H1 α), 1.24 (3H, s, H28), 1.06 (1H, d, J_{gem} = 3.6 Hz, H19 β), 0.91 (3H, s, H18), 0.90 (1H, m, H6 β), 0.87 (3H, d, J₂₁₋₂₀ = 6.3 Hz, H21), 0.56 (3H, s, H30) ; ¹³C NMR (75.4 MHz, CDCl₃): δ (ppm) 211.2 (C11), 165.9 (C31), 157.2 (C1'), 132.5 (C35), 130.8 (C32), 129.4 (C33+C37), 128.2 (C34+C36), 80.1 (C2'), 79.5 (C16), 64.2 (C29), 59.5 (C20), 55.3 (C17), 52.0 (C3), 52.0 (C12), 47.5 (C14), 44.7 (C4+C13), 43.2 (C15), 41.4+41.1 (C5+C8), 40.2 (N_BMe₂), 37.9 (C10), 34.0 (C9), 30.8 (C19), 28.3 (Me₃), 27.9+27.8 (C1+C2), 24.4 (C7), 19.6 (C28), 18.5 (C6), 17.9 (C18), 11.0 (C30), 10.0 (C21) ; MS (ESI, m/z) : 637.3 (M+H) ; HRMS (ESI, m/z) : calcd for C₃₈H₅₇N₂O₆ : 637.4216, found : 637.4191.

3 β -[(*tert*-butoxycarbonyl)amino]-20*S*-(dimethylamino)-4 α -formyl-4 β ,14 α -dimethyl-11-oxo-9,19-cyclo-5 α ,9 β -pregnan-16 α -yl benzoate (23**)**



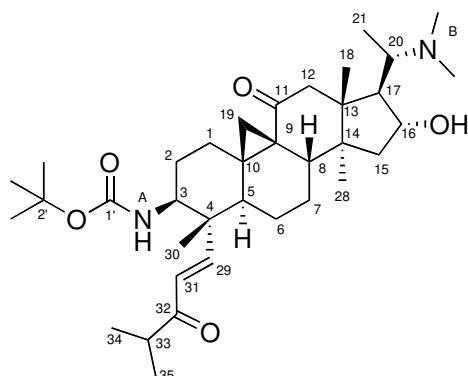
To 1.125 g (1.77 mmol, 1 eq.) of **22** dissolved in 12 mL of moist DCM were added 974 mg (2.296 mmol, 1.3 eq.) of Dess-Martin periodinane. The mixture was stirred at RT for 3 h, diluted with 50 mL of DCM and washed with 50 mL of water. The aqueous layer was extracted with 2x30 mL of DCM. Combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum to afford the title compound as an amorphous white solid (981 mg, yield: 88 %). IR (cm⁻¹) : 3374, 2974, 2933, 2872, 2823, 1704, 1667, 1516, 1278, 1157 ; ¹H NMR (300 MHz, CDCl₃): δ (ppm) 9.42 (1H, s, H₂₉), 8.04 (2H, m, H₃₃+H₃₇), 7.55 (1H, m, H₃₅), 7.44 (2H, m, H₃₄+H₃₆), 5.41 (1H, dd, J = 8.0 Hz, J = 5.5 Hz, H_{16 β}), 4.44 (1H, d, J = 9.3 Hz, N_A-H), 4.00 (1H, m, H_{3 α}), 2.64 (1H, d, J_{gem} = 17.2 Hz, H_{12 α}), 2.52 (1H, dq, J = 11.4 Hz, J = 6.1 Hz, H₂₀), 2.46 (1H, d, J_{gem} = 17.2 Hz, H_{12 β}), 2.43 (2H, m, H_{1 β} +H_{17 α}), 2.15 (1H, m, H_{15 β}), 2.10 (8H, m, N_BMe₂+H_{5 α} +H_{8 β}), 1.86 (1H, m, H_{2 α}), 1.61 (1H, d, J = 4.0 Hz, H_{19 α}), 1.56 (1H, d, J = 14.6 Hz, H_{15 α}), 1.52 (1H, m, H_{2 β}), 1.49 (2H, m, H₇), 1.41 (1H, m, H_{1 α}), 1.39 (9H, s, Me₃), 1.27 (3H, s, H₂₈), 1.07 (1H, d, J_{gem} = 4.0 Hz, H_{19 β}), 1.02 (2H, m, H₆), 0.93 (3H, s, H₃₀), 0.91 (3H, s, H₁₈), 0.86 (3H, d, J₂₁₋₂₀ = 6.1 Hz, H₂₁) ; ¹³C NMR (75.4 MHz, CDCl₃): δ (ppm) 210.4 (C₁₁), 203.8 (C₂₉), 165.8 (C₃₁), 154.9 (C_{1'}), 132.5 (C₃₅), 130.7 (C₃₂), 129.4 (C₃₃+C₃₇), 128.2 (C₃₄+C₃₆), 79.7 (C_{2'}), 79.4 (C₁₆), 59.5 (C₂₀), 56.7 (C₄), 55.4 (C₁₇), 51.9 (C₃), 51.8 (C₁₂), 47.4 (C₁₄), 44.6 (C₁₃), 43.0 (C₁₅), 41.8 (C₅), 41.1 (C₈), 40.2 (N_BMe₂), 35.8 (C₁₀), 33.5 (C₉), 29.4 (C₁₉), 28.2 (Me₃), 27.7+27.5 (C₁+C₂), 24.0 (C₇), 20.9 (C₆), 19.3 (C₂₈), 17.9 (C₁₈), 9.9 (C₂₁), 7.6 (C₃₀) ; MS (ESI, m/z) : 635.3 (M+H) ; HRMS (ESI, m/z) : calcd for C₃₈H₅₅N₂O₆ : 635.4060, found : 635.4053.

3 β -[(*tert*-butoxycarbonyl)amino]-20*S*-(dimethylamino)-4 α -(1-hydroxy-4-methyl-3-oxopentyl)-4 β ,14 α -dimethyl-11-oxo-9,19-cyclo-5 α ,9 β -pregnan-16 α -yl benzoate (24**)**



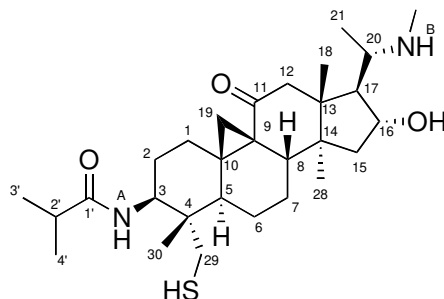
To 126 μ L (1.17 mmol, 10 eq.) of 3-methyl-2-butanone dissolved in 1 mL of THF were added at -78°C 587 μ L (1.17 mmol, 10 eq.) of LDA (2M in THF/heptane/ethylbenzene). The mixture was stirred for 15 min then slowly added to 75 mg (0.117 mmol, 1 eq.) of **23** dissolved in 0.5 mL of THF at -78°C . The mixture was stirred at -10°C for 3 h, diluted with 10 mL of a saturated sodium hydrogen carbonate solution and extracted with 3x10 mL of DCM. Combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum., over magnesium sulfate and concentrated under vacuum. The crude product was purified by silica gel column chromatography (eluent : DCM/MeOH/ NH_4OH : 96/3/1) to afford the title compound as an amorphous light yellow solid (67 mg, yield: 79 %). IR (cm^{-1}) : 3364, 2969, 2932, 2872, 2823, 1708, 1670, 1518, 1278, 1172 ; ^1H NMR (300 MHz, CDCl_3): δ (ppm) 8.04 (2H, m, H33+H37), 7.55 (1H, m, H35), 7.44 (2H, m, H34+H36), 5.41 (1H, dd, $J = 7.8$ Hz, $J = 5.6$ Hz, H16 β), 4.42 (1H, d, $J = 10.0$ Hz, N_A -H), 4.04 (1H, d, $J = 9.3$ Hz, H29), 3.61 (1H, m, H3 α), 3.37 (1H, bs, OH), 3.08 (1H, d, $J = 16.8$ Hz, H38a), 2.65 (1H, sept, $J = 6.9$ Hz, H40), 2.61 (1H, d, $J_{\text{gem}} = 17.0$ Hz, H12 α), 2.51 (1H, m, H20), 2.45 (1H, m, H17 α), 2.44 (1H, d, $J_{\text{gem}} = 17.0$ Hz, H12 β), 2.42 (1H, m, H38b), 2.36 (1H, m, H1 β), 2.15 (2H, m, H15 β +H8 β), 2.09 (6H, bs, $\text{N}_\text{B}\text{Me}_2$), 1.97 (1H, m, H6 α), 1.80 (1H, m, H5 α), 1.72 (1H, m, H2 α), 1.60 (1H, d, $J = 3.6$ Hz, H19 α), 1.57 (1H, m, H15 α), 1.53 (1H, m, H2 β), 1.50 (2H, m, H7), 1.40 (9H, s, Me_3), 1.28 (3H, s, H28), 1.24 (1H, m, H1 α), 1.13 (1H, m, H19 β), 1.10+1.09 (6H, 2d, $J = 6.9$ Hz, H41+H42), 1.00 (1H, m, H6 β), 0.91 (3H, s, H18), 0.88 (3H, s, H30), 0.85 (3H, d, $J_{21-20} = 6.0$ Hz, H21) ; ^{13}C NMR (75.4 MHz, CDCl_3): δ (ppm) 216.3 (C39), 211.1 (C11), 165.8 (C31), 155.7 (C1'), 132.5 (C35), 130.9 (C32), 129.4 (C33+C37), 128.2 (C34+C36), 79.6 (C2'), 79.5 (C16), 71.5 (C29), 59.4 (C20), 55.4 (C17), 52.7 (C3), 52.0 (C12), 47.5 (C14), 46.1 (C4), 44.6 (C13), 43.9 (C5), 43.1 (C15), 42.0 (C38), 41.3 (C8), 41.0 (C40), 40.2 ($\text{N}_\text{B}\text{Me}_2$), 38.0 (C10), 34.1 (C9), 30.6 (C19), 29.2 (C2), 28.3 (Me_3), 27.8 (C1), 24.4 (C7), 19.9 (C6), 19.5 (C28), 18.1+18.0 (C41+C42), 17.9 (C18), 11.5 (C30), 9.9 (C21) ; MS (ESI, m/z) : 721.4 ($\text{M}+\text{H}$) ; HRMS (ESI, m/z) : calcd for $\text{C}_{43}\text{H}_{65}\text{N}_2\text{O}_7$: 721.4792, found : 721.4763.

***tert*-butyl [20*S*-(dimethylamino)-16*α*-hydroxy-4*β*,14*α*-dimethyl-4*α*]-[(1*E*)-4-methyl-3-oxopent-1-en-1-yl]-11-oxo-9,19-cyclo-5*α*,9*β*-pregnan-3*β*-yl]carbamate (**26**)**



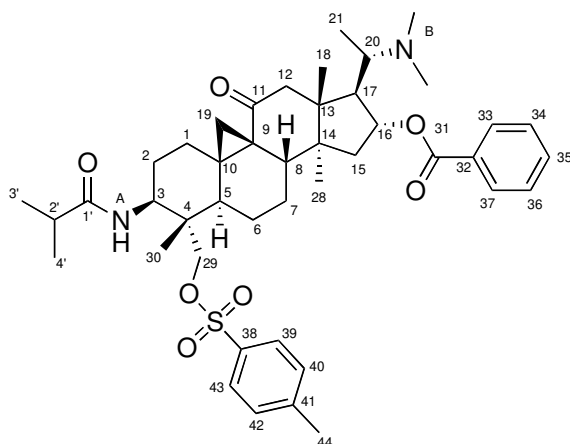
To 89 mg (0.123 mmol, 1 eq.) of **24** dissolved in 20 mL of a mixture MeOH/water (7/3) was added a small amount of potassium carbonate. The mixture was refluxed for 12 h then the MeOH was removed under vacuum. The mixture was diluted with 10 mL of a saturated sodium carbonate solution and extracted with 3x20 mL of DCM. Combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by silica gel column chromatography (eluent : DCM/MeOH/NH₄OH : 96/3/1) to afford the title compound as an amorphous colourless solid (64 mg, yield: 86 %). IR (cm⁻¹) : 3325, 2970, 2933, 2870, 2836, 1698, 1665, 1520, 1250, 1164 ; ¹H NMR (500 MHz, CDCl₃): δ(ppm) 6.72 (1H, d, J = 16.5 Hz, H29), 5.92 (1H, d, J = 16.5 Hz, H31), 4.39 (1H, d, J = 9.5 Hz, N_A-H), 4.10 (1H, m, H16*β*), 3.69 (1H, m, H3*α*), 3.07 (1H, m, H33), 2.62 (1H, dq, J = 10.7 Hz, J = 6.4 Hz, H20), 2.52 (1H, d, J_{gem} = 17.1 Hz, H12*α*), 2.38 (1H, dm, J = 14 Hz, H1*β*), 2.32 (1H, d, J_{gem} = 17.1 Hz, H12*β*), 2.25 (6H, bs, N_BMe₂), 2.07 (1H, dd, J = 10.4 Hz, J = 7.6 Hz, H8*β*), 2.00 (1H, m, H15*β*), 1.98 (1H, m, H17*α*), 1.80 (1H, m, H2*α*), 1.77 (1H, m, H5*α*), 1.60 (1H, d, J = 3.7 Hz, H19*α*), 1.50 (3H, m, H2*β*+H7), 1.48 (1H, dd, J = 14.3 Hz, J = 2.4 Hz, H15*α*), 1.35 (1H, m, H1*α*), 1.34 (9H, s, Me₃), 1.25 (1H, m, H6*α*), 1.23 (3H, s, H28), 1.10+1.09 (6H, 2d, J = 6.7 Hz, H34+H35), 1.05 (1H, d, J = 3.7 Hz, H19*β*), 0.97 (1H, m, H6*β*), 0.95 (3H, s, H30), 0.87 (3H, d, J₂₁₋₂₀ = 6.4 Hz, H21), 0.83 (3H, s, H18) ; ¹³C NMR (75.4 MHz, CDCl₃): δ(ppm) 210.8 (C11), 204.9 (C32), 155.0 (C29), 154.2 (C1'), 128.7 (C31), 79.1 (C2'), 78.2 (C16), 62.0 (C20), 55.8 (C3+C17), 51.4 (C12), 47.8 (C14), 47.1 (C4), 46.8 (C5), 44.5 (C13), 42.6 (C15), 41.2 (C8), 36.6 (C10), 36.4 (C33), 34.4 (C9), 29.7 (C19), 28.2 (Me₃), 27.7 (C1+C2), 24.2 (C7), 20.7+20.6 (C34+C35), 17.8 (C28), 18.6 (C6), 17.8 (C18), 10.8 (C30), 9.9 (C21) ; MS (ESI, m/z) : 599.4 (M+H), HRMS (ESI, m/z) : calcd for C₃₆H₅₉N₂O₅ : 599.4424, found : 599.4454.

N-[20*S*-(dimethylamino)-16 α -hydroxy-4 α -(mercaptomethyl)-4 β ,14 α -dimethyl-11-oxo-9,19-cyclo-5 α ,9 β -pregnan-3 β -yl]-isobutyramide (**29**).



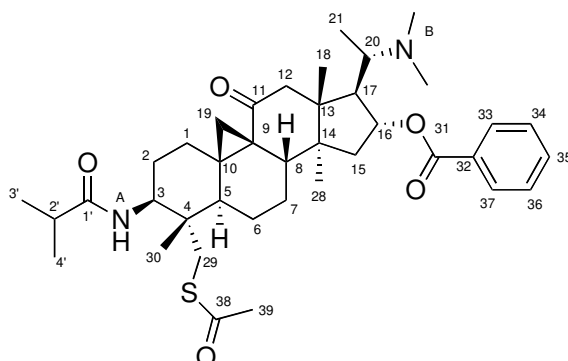
To 40 mg (0.057 mmol, 1 eq.) of **28** dissolved in 0.5 mL of DMPU were added 98 mg (0.86 mmol, 15 eq.) of potassium thioacetate. The mixture was stirred at 100 °C for 5 h then diluted with 10 mL of a saturated sodium hydrogen carbonate solution and extracted with 20 mL of Et₂O. The organic layer was dried with brine, over magnesium sulfate and concentrated under vacuum. The residue was dissolved in 20 mL of a degassed mixture MeOH/water (7/3) and a small amount of potassium hydroxide was added. The mixture was stirred at RT for 3 h. The MeOH was removed under reduced pressure and the mixture was extracted with 3x20 mL of DCM. Combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by silica gel column chromatography (gradient elution : DCM/MeOH/NH₄OH : from 99/0/1 to 90/10/1) to afford the title compound as an amorphous white solid (20 mg, yield: 67 %). IR (cm⁻¹) : 3322, 2964, 2937, 2869, 2833, 1655, 1534, 1460, 1225, 1159, 1094 ; ¹H NMR (300 MHz, CDCl₃): δ(ppm) 5.23 (1H, d, J_{NAH-3} = 9.9 Hz, N_A-H), 4.15 (1H, m, H3 α), 4.12 (1H, m, H16 β), 2.64 (1H, dq, J₂₀₋₁₇ = 10.8 Hz, J₂₀₋₂₁ = 6.5 Hz, H20), 2.53 (1H, d, J_{gem} = 17.1 Hz, H12 α), 2.49 (1H, m, H29b), 2.41 (1H, m, H1 β), 2.34 (1H, m, H2'), 2.29 (1H, d, J_{gem} = 17.1 Hz, H12 β), 2.26 (6H, bs, N_BMe), 2.22 (1H, m, H5), 2.19 (1H, m, H29a), 2.02 (2H, m, H8 β +H15 β), 1.99 (1H, m, H17 α), 1.72 (1H, m, H2 α), 1.56 (1H, d, J_{gem} = 3.4 Hz, H19 α), 1.55 (1H, m, H7 β), 1.51 (1H, m, H15 α), 1.49 (1H, m, H2 β), 1.38 (1H, m, H7 α), 1.33 (1H, m, H6 α), 1.31 (1H, m, H1 α), 1.23 (3H, s, H28), 1.17+1.14 (6H, 2d, J = 7.2 Hz, H3'+H4'), 1.03 (1H, d, J_{gem} = 3.4 Hz, H19 β), 0.92 (1H, m, H6 β), 0.89 (3H, d, J₂₁₋₂₀ = 6.5 Hz, H21), 0.85 (3H, s, H18), 0.78 (3H, s, H30) ; ¹³C NMR (75.4 MHz, CDCl₃): δ(ppm) 211.4 (C11), 176.7 (C1'), 78.1 (C16), 62.0 (C20), 55.7 (C17), 51.4 (C12), 49.7 (C3), 47.0 (C14), 44.4 (C13), 43.1 (C4), 42.9 (C15), 42.0 (C5), 41.9 (C8), 37.6 (C10), 35.8 (C2'), 34.2 (C9), 31.2 (C29), 30.9 (C19), 28.5 (C2), 27.4 (C1), 24.3 (C7), 20.8 (C28), 20.1+19.4 (C3'+C4'), 18.4 (C6), 17.8 (C18), 15.5 (C30), 9.9 (C21) ; MS (ESI, m/z) : 519.3 (M+H) ; HRMS (ESI, m/z) : calcd for C₃₀H₅₁N₂O₃S : 519.3620, found : 519.3611.

20S-(dimethylamino)-3 β -isobutyrylamino-4 β ,14 α -dimethyl-4 α -{[(*p*-methylbenzenesulfonyl)oxy]methyl}-11-oxo-9,19-cyclo-5 α ,9 β -pregnan-16 α -yl benzoate (31)



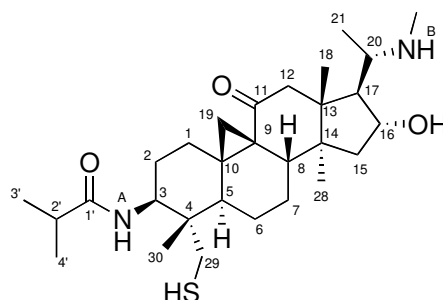
To 747 mg (1.2 mmol, 1 eq.) of **14** dissolved in 30 mL of THF were added 234 mg (1.2 mmol, 1 eq.) of *p*-toluene sulfonic acid. The mixture was stirred at RT for 30 min then concentrated under reduced pressure. The residue was dissolved in 7 mL of pyridine and 1.173 g (6.1 mmol, 5 eq.) of tosyl chloride were added. The mixture was stirred at 90 °C for 3 h. After 3 co-evaporations with 3x20 mL of 1,2-dichloroethane the residue was dissolved in DCM, washed with 30 mL of a saturated sodium hydrogen carbonate solution and extracted with 3x30 mL of DCM. Combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by silica gel column chromatography (eluent : DCM/MeOH/NH₄OH : 97/2/1) to afford the title compound as an amorphous light yellow solid (805 mg, yield: 86 %). IR : 3322, 2964, 2929, 2869, 2823, 1711, 1666, 1529, 1600, 1450, 1359, 1278, 1228, 1188, 1175 ; ¹H NMR (300 MHz, CDCl₃): δ(ppm) 8.05 (2H, m, H33+H37), 7.78 (2H, d, J = 8.3 Hz, H39+H43), 7.56 (1H, m, H35), 7.45 (2H, m, H34+H36), 7.30 (2H, d, J = 8.3 Hz, H40+H42), 5.42 (1H, dd, J = 8.1 Hz, J = 5.7 Hz, H16 β), 5.30 (1H, d, J_{NAH-H3} = 10.0 Hz, N_A-H), 4.09 (1H, m, H3 α), 3.80 (1H, d, J_{gem} = 9.6 Hz, H29b), 3.55 (1H, d, J_{gem} = 9.6 Hz, H29a), 2.62 (1H, d, J_{gem} = 17.3 Hz, H12 α), 2.54 (1H, dq, J_{20-17 α} = 11.3 Hz, J₂₀₋₂₁ = 6.0 Hz, H20), 2.45 (1H, dd, J_{17 α -20} = 11.3 Hz, J_{17 α -16 β} = 5.7 Hz, H17 α), 2.43 (1H, d, J_{gem} = 17.3 Hz, H12 β), 2.39 (3H, s, H37), 2.36 (1H, m, H1 β), 2.23 (1H, m, H2'), 2.12 (1H, m, H5 α), 2.10 (6H, bs, N_BMe₂), 2.03 (1H, m, H8 β), 2.01 (1H, d, J = 12.2 Hz, H15 β), 1.71 (1H, m, H2 α), 1.58 (1H, d, J = 12.2 Hz, H15 α), 1.56 (1H, d, J_{gem} = 3.4 Hz, H19 β), 1.46 (3H, m, H2 β +H6 α +H7 β), 1.29 (1H, m, H1 α), 1.26 (1H, m, H7 α), 1.23 (3H, s, H28), 1.04 (3H, d, J_{4'-3'} = 7.0 Hz, H4'), 1.03 (1H, d, J_{gem} = 3.4 Hz, H19 α), 0.98 (3H, d, J_{3'-4'} = 7.0 Hz, H3'), 0.91 (3H, s, H18), 0.85 (3H, d, J₂₁₋₂₀ = 6.0 Hz, H21), 0.84 (1H, m, H6 β), 0.73 (3H, s, H30); ¹³C NMR (75.4 MHz, CDCl₃): δ(ppm) 210.8 (C11), 175.9 (C1'), 165.8 (C31), 144.4 (C41), 132.5 (C35), 132.4 (C38), 130.6 (C32), 129.6 (C40+C42), 129.2 (C33+C37), 128.1 (C34+C36), 128.0 (C39+C43), 79.3 (C16), 71.2 (C29), 59.3 (C20), 55.1 (C17), 51.8 (C12), 49.4 (C3), 47.3 (C14), 44.5 (C13), 43.0 (C15), 42.9 (C4), 42.7 (C5), 41.3 (C8), 40.1 (N_BMe₂), 37.1 (C10), 35.5 (C2'), 33.6 (C9), 30.1 (C19), 28.0 (C2), 27.0 (C1), 24.1 (C7), 21.4 (C37), 19.6 (C3'), 19.4 (C28), 19.2 (C4'), 18.5 (C6), 17.7 (C18), 10.8 (C30), 9.8 (C21); MS (ESI): 761.3 (M+H); HRMS (ESI, m/z): calcd for C₄₄H₆₁N₂O₇S : 761.4199, found : 761.4197.

4 α -(acetylthiomethyl)-20S-(dimethylamino)-3 β -(isobutyrylamino)-4 β ,14 α -dimethyl-11-oxo-9,19-cyclo-5 α ,9 β -pregnan-16 α -yl benzoate (32**)**



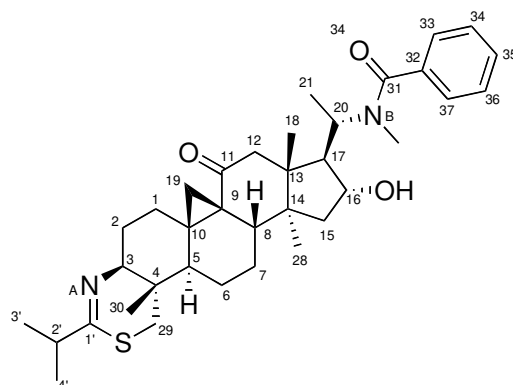
To 805 mg (1.06 mmol, 1 eq.) of **31** dissolved in 7.5 mL of DMPU were added 1.811 mg (15.8 mmol, 15 eq.) of potassium thioacetate. The mixture was stirred at 100 °C for 5 h, diluted with 70 mL of a saturated sodium hydrogen carbonate solution and extracted with 70 mL of Et₂O. The organic layer was dried with brine, over magnesium sulfate and concentrated under reduced pressure. The residue was dissolved in 40 mL of DCM and 261 mg (1.06 mmol, 1 eq.) of *m*-CPBA were added at 0 °C. The mixture was stirred at 0 °C for 1 h and 10 mL of MeOH, 285 mg (1.06 mmol, 1 eq.) of iron chloride and 588 mg (2.1 mmol, 2 eq.) of iron sulfate were added. The mixture was stirred at 10 °C for further 3 h and 3 mL of NH₄OH were added. The mixture was filtered through a pad of celite and the solvents were removed under vacuum. The crude product was purified by silica gel column chromatography (eluent : DCM/MeOH/NH₄OH : 96/3/1) to afford 555 mg of **32** (yield: 81 %) as an amorphous light yellow solid. IR (cm⁻¹) : 3317, 2967, 2932, 2869, 2824, 1704, 1660, 1626, 1526, 1450, 1278, 1228, 1113 ; ¹H NMR (300 MHz, CDCl₃): δ (ppm) 8.05 (2H, m, H33+H37), 7.55 (1H, m, H35), 7.44 (2H, m, H34+H36), 5.42 (1H, d, $J_{\text{NAH-H3}} = 9.3$ Hz, N_A-H), 5.41 (1H, m, H16 β), 3.95 (1H, ddd, $J = 12.2$ Hz, $J = 10.0$ Hz, $J = 4.3$ Hz, H3 α), 3.03 (1H, d, $J_{\text{gem}} = 13.8$ Hz, H29b), 2.85 (1H, d, $J_{\text{gem}} = 13.8$ Hz, H29a), 2.62 (1H, d, $J_{\text{gem}} = 17.0$ Hz, H12 α), 2.50 (1H, m, H20), 2.43 (2H, m, H12 β +H17 α), 2.35 (1H, m, H2'), 2.33 (1H, m, H1 β), 2.30 (3H, s H39), 2.15 (1H, m, H15 β), 2.10 (7H, m, N_BMe, H8 β), 1.75 (1H, m, H5 α), 1.72 (1H, m, H2 α), 1.60 (1H, d, $J_{\text{gem}} = 4.1$ Hz, H19 β), 1.57 (1H, m, H15 α), 1.49 (1H, m, H7 β), 1.47 (1H, m, H2 β), 1.25 (3H, s, H28), 1.24 (3H, m, H1 α +H6 α +H7 α), 1.16+1.14 (6H, 2d, $J_{3'-4'} = 6.9$ Hz, H3'+H4'), 1.07 (1H, d, $J_{\text{gem}} = 4.1$ Hz, H19 β), 0.98 (1H, m, H6 β), 0.90 (3H, s, H18), 0.89 (3H, s, H30), 0.86 (3H, d, $J_{21-20} = 6.0$ Hz, H21); ¹³C NMR (75.4 MHz, CDCl₃): δ (ppm) 210.7 (C11), 195.6 (C38), 176.3 (C1'), 165.7 (C31), 132.5 (C35), 130.7 (C32), 129.3 (C33+C37), 128.2 (C34+C36), 79.4 (C16), 56.4 (C20), 55.3 (C17), 52.0 (C3), 51.8 (C12), 47.4 (C14), 44.6 (C13), 44.5 (C5), 42.9 (C15), 42.8 (C4), 40.9 (C8), 40.2 (N_BMe), 37.6 (C10), 35.7 (C2'), 35.4 (C29), 33.9 (C9), 30.6 (C39), 29.7 (C19), 28.4 (C2), 27.2 (C1), 24.2 (C7), 19.7 (C28), 19.4 (C3'+C4'), 18.8 (C6), 17.8 (C18), 15.0 (C30), 9.8 (C21); MS (ESI, m/z) : 665.3 (M+H); HRMS (ESI, m/z) : calcd for C₃₉H₅₇N₂O₅S : 665.3988, found : 665.3980.

***N*-[16 α -hydroxy-4 α -(mercaptomethyl)-4 β ,14 α -dimethyl-20*S*-(methylamino)-11-oxo-9,19-cyclo-5 α ,9 β -pregnan-3 β -yl]-isobutyramide (**33**)**



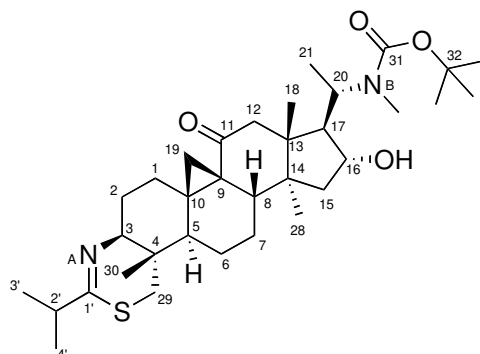
To 240 mg (0.24 mmol, 1 eq.) of **254** dissolved in 50 mL of a degassed mixture of MeOH/H₂O (4/1) was added a small amount of potassium hydroxide. The mixture was stirred at RT for 5 h then MeOH was removed under vacuum. The aqueous layer was extracted with 3x30 mL of DCM. Combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by silica gel column chromatography (gradient elution : DCM/MeOH/NH₄OH : from 99/0/1 to 90/10/1) to afford the title compound as an amorphous white solid (102 mg, yield: 55 %). IR (cm⁻¹) : 3315, 2964, 2937, 2869, 1660, 1650, 1537, 1454, 1227, 1156, 1090 ; ¹H NMR (300 MHz, CDCl₃): δ(ppm) 5.17 (1H, d, J_{NAH-3} = 10.1 Hz, N_A-H), 4.15 (1H, m, H3 α), 4.14 (1H, m, H16 β), 2.54 (1H, d, J_{gem} = 17.1 Hz, H12 α), 2.21 (1H, m, H29b), 2.47 (1H, m, H20), 2.45 (3H, s, N_BMe), 2.41 (1H, dt, J = 13.7 Hz, J = 3.5 Hz, H1 β), 2.36 (1H, d, J_{gem} = 17.1 Hz, H12 β), 2.34 (1H, m, H2'), 2.23 (2H, m, H5+H29a), 2.01 (2H, m, H8 β +H15 β), 1.73 (2H, m, H2 α +H17 α), 1.56 (1H, d, J_{gem} = 3.7 Hz, H19 α), 1.53 (1H, m, H7 β), 1.52 (1H, m, H15 α), 1.48 (1H, m, H2 β), 1.39 (1H, m, H7 α), 1.33 (2H, m, H1 α +H6 α), 1.21 (3H, s, H28), 1.17+1.15 (6H, 2d, J = 7.0 Hz, H3'+H4'), 1.09 (3H, d, J₂₁₋₂₀ = 6.1 Hz, H21), 1.03 (1H, d, J_{gem} = 3.7 Hz, H19 β), 0.89 (1H, m, H6 β), 0.85 (3H, s, H18), 0.78 (3H, s, H30); ¹³C NMR (75.4 MHz, CDCl₃): δ(ppm) 211.4 (C11), 176.7 (C1'), 77.9 (C16), 60.6 (C17), 58.5 (C20), 51.5 (C12), 49.7 (C3), 47.1 (C14), 44.6 (C13), 43.1 (C4), 42.9 (C15), 42.0 (C5), 41.9 (C8), 37.6 (C10), 35.9 (C2'), 34.2 (C9), 33.8 (N_BMe), 31.2 (C29), 30.9 (C19), 28.6 (C2), 27.5 (C1), 24.4 (C7), 20.7 (C28), 20.1 (C3'), 19.4 (C4'), 18.6 (C21), 18.5 (C6), 18.0 (C18), 15.5 (C30); MS (ESI, m/z) : 505.3 (M+H); HRMS (ESI, m/z) : calcd for C₂₉H₄₉N₂O₃S : 505.3464, found : 505.3458.

***N*-{16 α -hydroxy-4 β ,14 α -dimethyl-11-oxo-9,19-cyclo-[2'-isopropyl-5',6'-dihydro-4' α H-[1',3']thiazino[4',5':3,4]}-5 α ,9 β -pregnan-20*S*-yl}-*N*-methylbenzamide (**35b**)**



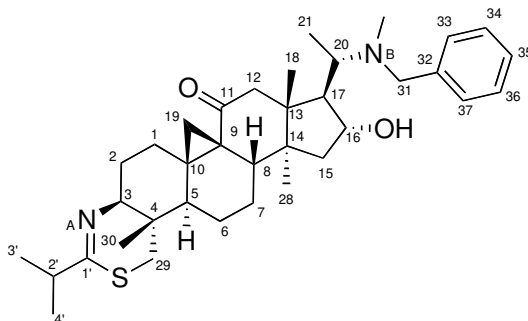
13 mg (0.0267 mmol, 1 eq.) of **34**, 9 mg (0.0347 mmol, 1.3 eq.) of benzoic anhydride and 1 mL of EtOH were used by following similar procedure described for the preparation of **35a** to give the title compound as an amorphous white solid (12.5 mg, yield: 83 %). IR (cm⁻¹) : 3333, 2962, 2931, 2868, 1666, 1613, 1598, 1574, 1447, 1257, 1115, 1084, 1025 ; ¹H NMR (300 MHz, CDCl₃): δ(ppm) 7.39 (5H, m, H33+H34+H35+H36+H37), 5.14 (1H, m, H20), 4.41 (1H, m, H16 β), 2.88 (1H, m, H3 α), 2.86 (3H, s, N_BMe), 2.84 (1H, d, J_{gem} = 11.2 Hz, H29b), 2.75 (1H, d, J_{gem} = 11.2 Hz, H29a), 2.68 (1H, m, H2'), 2.60 (1H, d, J_{gem} = 17.0 Hz, H12 α), 2.47 (1H, m, H1 β), 2.42 (1H, d, J_{gem} = 17.0 Hz, H12 β), 2.26 (1H, m, H17 α), 2.09 (2H, m, H8 β +H15 β), 1.73 (1H, m, H2 α), 1.67 (1H, d, J_{gem} = 4.0 Hz, H19 α), 1.59 (1H, m, H2 β), 1.57 (2H, m, H7), 1.54 (1H, m, H6 α), 1.43 (2H, m, H5 α +H15 α), 1.30 (1H, m, H1 α), 1.24 (3H, s, H28), 1.22 (3H, m, H21), 1.20+1.18 (6H, 2d, J = 6.8 Hz, H3'+H4'), 1.14 (1H, m, H19 β), 1.02 (3H, s, H18), 0.95 (1H, m, H6 β), 0.78 (3H, s, H30); ¹³C NMR (75.4 MHz, CDCl₃): δ(ppm) 210.7 (C11), 172.6 (C31), 172.0 (C1'), 137.6 (C32), 129.3 (C35), 128.3 (C34+C36), 126.8 (C33+C37), 75.9 (C16), 62.6 (C3), 56.9 (C17), 51.6 (C12), 49.9 (C5), 48.5 (C20), 47.4 (C14), 45.9 (C15+C13), 41.5 (C8), 40.0 (C2'), 38.7 (C29), 38.5 (C10), 34.0 (C9), 33.5 (C4), 31.4 (N_BMe), 30.5 (C2+C19), 27.9 (C1), 24.6 (C7), 21.1+20.8 (C3'+C4'), 19.9 (C28), 18.1 (C18+C21), 17.7 (C6), 11.3 (C30); MS (ESI, m/z) : 591.3 (M+H); HRMS (ESI, m/z) : calcd for C₃₁H₄₉N₂O₃S : 591.6320, found : 591.3627.

***tert*-butyl {16 α -hydroxy-4 β ,14 α -dimethyl-11-oxo-9,19-cyclo-[2'-isopropyl-5',6'-dihydro-4' α H-[1',3']thiazino[4',5':3,4]}-5 α ,9 β -pregnan-20S-yl} methylcarbamate (**35c**)**



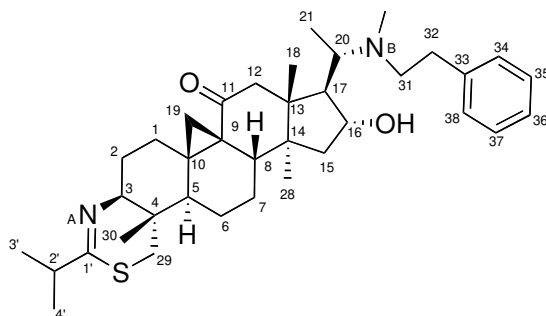
17 mg (0.035 mmol, 1 eq.) of **34**, 22 μ L (0.105 mmol, 3 eq.) of di-*tert*-butyl dicarbonate and 1 mL of THF were used by following similar procedure described for the preparation of **35a** to give the title compound as an amorphous white solid (15 mg, yield: 83 %). IR (cm^{-1}) : 3457, 2965, 2931, 2869, 1668, 1633, 1455, 1260, 1140 . ^1H NMR (500 MHz, CDCl_3): δ (ppm) 4.46 (1H, m, H20), 4.26 (1H, m, H16 β), 2.88 (1H, dd, J = 12.8 Hz, J = 3.5 Hz, H3 α), 2.84 (1H, d, J_{gem} = 11.6 Hz, H29b), 2.76 (3H, s, N_BMe), 2.74 (1H, d, J_{gem} = 11.6 Hz, H29a), 2.68 (1H, m, H2'), 2.55 (1H, d, J_{gem} = 17.1 Hz, H12 α), 2.47 (1H, m, H1 β), 2.36 (1H, d, J_{gem} = 17.1 Hz, H12 β), 2.13 (1H, m, H17 α), 2.10 (1H, m, H2 α), 2.07 (1H, m, H8 β), 2.03 (1H, m, H15 β), 1.72 (1H, m, H2 β), 1.65 (1H, d, J_{gem} = 3.7 Hz, H19 α), 1.57 (2H, m, H7), 1.55 (1H, m, H6 α), 1.43 (2H, m, H5 α +H15 α), 1.32 (10H, m, H1 α +Boc), 1.21 (3H, s, H28), 1.19+1.18 (6H, 2d, J = 6.7 Hz, H3'+H4'), 1.12 (1H, m, H19 β), 1.10 (3H, m, H21), 1.02 (1H, m, H6 β), 0.93 (3H, s, H18), 0.77 (3H, s, H30) ; ^{13}C NMR (75.4 MHz, CDCl_3): δ (ppm) 210.5 (C11), 171.6 (C1'), 156.7 (C31), 79.7 (C32), 76.0 (C16), 62.6 (C3), 57.8 (C17), 51.6 (C12), 50.8 (C20), 49.8 (C5), 47.3 (C14), 45.5 (C13), 45.2 (C15), 41.5 (C8), 40.1 (C2'), 38.7 (C29), 38.5 (C10), 34.1 (C9), 33.5 (C4), 30.5 (C19), 30.2 (C2), 28.5 (N_BMe+N_BBoc), 27.9 (C1), 24.5 (C7), 21.1 (C28), 20.8+19.9 (C3'+C4'), 18.2 (C21), 18.1 (C6), 17.9 (C18), 11.3 (C30); MS (ESI, m/z) : 587.4 (M+H) ; HRMS (ESI, m/z) : calcd for $\text{C}_{34}\text{H}_{55}\text{N}_2\text{O}_4\text{S}$: 587.3883, found : 587.3884.

20S-[benzyl(methyl)amino]-16 α -hydroxy-4 β ,14 α -dimethyl-9,19-cyclo-{2'-isopropyl-5',6'-dihydro-4' α H-[1',3']thiazino[4',5':3,4]}-5 α ,9 β -pregnan-11-one (36a).



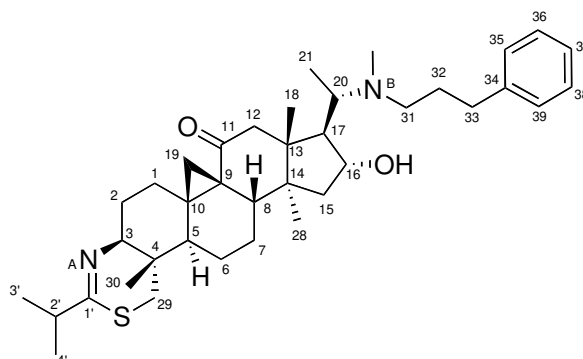
To 13 mg (0.0267 mmol, 1 eq.) of **34** dissolved in 1 mL of acetonitrile were added 4.1 μ L (0.035 mmol, 1.3 eq.) of benzyl bromide and 11 mg (0.08 mmol, 3 eq.) of potassium carbonate. The mixture was stirred at RT for 16 h, diluted with 5 mL of a saturated sodium hydrogen carbonate solution then extracted with 3x5 mL of DCM. Combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by silica gel column chromatography (gradient elution: DCM/MeOH/NH₄OH : from 99/0/1 to 96/3/1) to afford the title compound as an amorphous white solid (14 mg, yield: 93 %). IR (cm⁻¹) : 3324, 2962, 2929, 2867, 1666, 1633, 1452, 1259, 1223, 1147, 1079, 1017 ; ¹H NMR (500 MHz, CD₃CN, 333K): δ (ppm) 7.30 (5H, m, H33+H34+H35+H36+H37), 3.99 (1H, m, H16 β), 3.72 (1H, d, $J_{\text{gem}} = 12.8$ Hz, H31), 3.59 (1H, d, $J_{\text{gem}} = 12.8$ Hz, H31), 2.86 (2H, m, H20+H29b), 2.84 (1H, m, H3 α), 2.75 (1H, d, $J_{\text{gem}} = 11.6$ Hz, H29a), 2.53 (1H, d, $J_{\text{gem}} = 17.1$ Hz, H12 α), 2.48 (1H, m, H2'), 2.35 (1H, dt, $J_{\text{gem}} = 13.6$ Hz, $J_{1\beta-2} = 3.2$ Hz, H1 β), 2.23 (1H, d, $J_{\text{gem}} = 17.1$ Hz, H12 β), 2.21 (3H, s, N_BMe), 2.12 (1H, m, H17 α), 2.08 (1H, m, H8 β), 1.97 (1H, m, H15 β), 1.95 (1H, m, H2 α), 1.63 (1H, m, H2 β), 1.53 (1H, m, H7 β), 1.51 (1H, m, H6 α), 1.50 (1H, d, $J_{\text{gem}} = 4.0$ Hz, H19 α), 1.47 (1H, m, H5 α), 1.39 (1H, m, H15 α), 1.33 (1H, m, H7 α), 1.31 (1H, m, H1 α), 1.18 (3H, s, H28), 1.13 (1H, m, H19 β), 1.12 (6H, 2d, $J = 6.7$ Hz, H3'+H4'), 1.05 (1H, m, H6 β), 0.98 (3H, d, $J_{21-20} = 6.4$ Hz, H21), 0.75 (3H, s, H18), 0.73 (3H, s, H30) ; ¹³C NMR (75.4 MHz, CD₃CN): δ (ppm) 210.8 (C11), 165.9 (C1'), 138.7 (C32), 129.1 (C33+C37), 128.5 (C34+C36), 127.3 (C35), 77.6 (C16), 62.7 (C3), 60.3 (C20), 59.4 (C31), 55.8 (C17), 51.4 (C12), 49.7 (C5), 47.2 (C14), 44.7 (C13), 42.8 (C15), 41.3 (C8), 40.2 (C2'), 38.7 (C29), 38.4 (C10), 35.3 (N_BMe), 34.4 (C9), 33.4 (C4), 30.5 (C2), 30.3 (C19), 28.0 (C1), 24.4 (C7), 21.0+20.8 (C3'+C4'), 20.6 (C28), 18.0 (C6), 17.7 (C18), 11.3 (C30), 10.7 (C21) ; MS (ESI, m/z) : 577.4 (M+H), 487.3 (M-Bn+H); HRMS (ESI, m/z) : calcd for C₃₆H₅₃N₂O₂S : 577.3828, found : 577.3835.

20S-[methyl(2-phenylethyl)amino]-16 α -hydroxy-4 β ,14 α -dimethyl-9,19-cyclo-{2'-isopropyl-5',6'-dihydro-4' α H-[1',3']thiazino[4',5':3,4]}-5 α ,9 β -pregnan-11-one (36b)



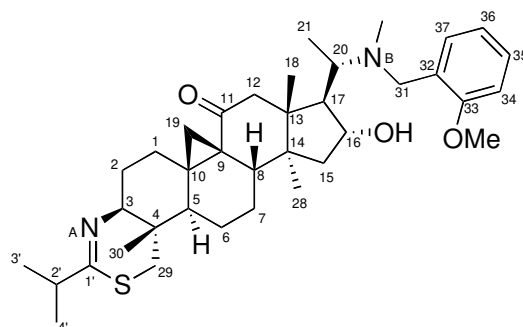
18.5 mg (0.038 mmol, 1 eq.) of **34**, 7 μ L (0.05 mmol, 1.3 eq.) of (2-bromoethyl)benzene, 16 mg (0.114 mmol, 3 eq.) of potassium carbonate and 1 mL of acetonitrile were used by following similar procedure described for the preparation of **36a** to give the title compound as an amorphous white solid (19 mg, yield: 84 %). For this reaction, the mixture was refluxed for 12 h to give the desired product. IR (cm^{-1}) : 3381, 3025 (H), 2961, 2930, 2865, 1666, 1632, 1496, 1454, 1260, 1224, 1154, 1117, 1016 ; ^1H NMR (500 MHz, CD_3CN , 333K): δ (ppm) 7.27 (5H, m, H34+H35+H36+H37+H38), 4.01 (1H, m, H16 β), 2.91 (1H, d, $J_{\text{gem}} = 11.7$ Hz, H29b), 2.86 (1H, m, H20), 2.82 (4H, m, H31+H32), 2.79 (2H, m, H3 α +H29a), 2.56 (1H, d, $J_{\text{gem}} = 17.0$ Hz, H12 α), 2.50 (1H, m, H2'), 2.39 (1H, dt, $J_{\text{gem}} = 13.2$ Hz, $J_{1\beta-2} = 3.4$ Hz, H1 β), 2.29 (3H, s, N_BMe), 2.25 (1H, d, $J_{\text{gem}} = 17.0$ Hz, H12 β), 2.13 (1H, m, H8 β), 2.05 (1H, m, H17 α), 2.01 (1H, m, H15 β), 1.97 (1H, m, H2 α), 1.68 (1H, m, H2 β), 1.57 (1H, m, H7 β), 1.54 (1H, d, $J_{\text{gem}} = 4.0$ Hz, H19 α), 1.52 (1H, m, H6 α), 1.50 (1H, m, H5 α), 1.37 (1H, m, H15 α), 1.36 (2H, m, H1 α +H7 α), 1.21 (3H, s, H28), 1.18 (1H, m, H19 β), 1.17+1.16 (6H, 2d, $J = 6.8$ Hz, H3'+H4'), 1.09 (1H, m, H6 β), 0.95 (3H, d, $J_{21-20} = 6.6$ Hz, H21), 0.88 (3H, s, H18), 0.79 (3H, s, H30) ; ^{13}C NMR (75.4 MHz, CD_3CN): δ (ppm) 211.3 (C11), 165.3 (C1'), 141.8 (C33), 129.8 (C34+C38), 129.5 (C35+C37), 127.1 (C36), 78.9 (C16), 64.1 (C20), 62.5 (C3), 57.6 (C17), 57.3 (C31), 52.7 (C12), 50.6 (C5), 48.4 (C14), 46.1 (C13), 44.2 (C15), 42.6 (C8), 40.7 (C2'), 40.0 (C29), 38.9 (C10), 35.9 (C32), 35.6 (N_BMe), 34.4 (C9), 33.4 (C4), 31.8 (C2), 30.6 (C19), 29.3 (C1), 25.4 (C7), 21.5+21.4 (C3'+C4'), 21.0 (C28), 19.1 (C6), 18.5 (C18), 11.9 (C30), 11.6 (C21); MS (ESI, m/z) : 591.4 (M+H); HRMS (ESI, m/z) : calcd for $\text{C}_{37}\text{H}_{55}\text{N}_2\text{O}_2\text{S}$: 591.3984, found : 591.3972.

20S-[methyl(3-phenylpropyl)amino]-16 α -hydroxy-4 β ,14 α -dimethyl-9,19-cyclo-{2'-isopropyl-5',6'-dihydro-4' α H-thiazine[4',5':3,4]}-5 α ,9 β -pregnan-11-one (36c)



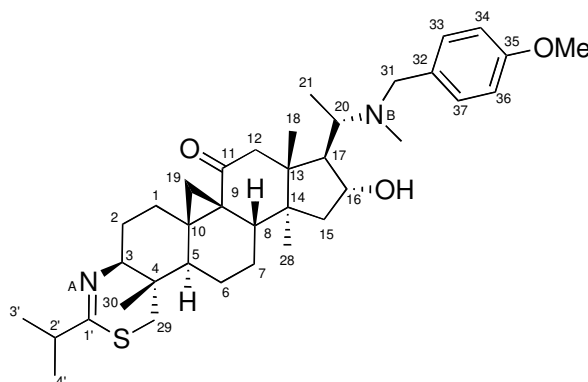
15 mg (0.031 mmol, 1 eq.) of **34**, 13 mg (0.092 mmol, 3 eq.) of potassium carbonate, 6 μ L (0.043 mmol, 1.3 eq.) of 1-bromo-3-phenylpropane and 0.5 mL of DMF were used by following similar procedure described for the preparation of **36a** to give the title compound as an amorphous white solid (14 mg, yield: 72 %). IR (cm^{-1}) : 3381, 3025, 2962, 2930, 2863, 1666, 1632, 1454, 1259, 1224, 1156, 1018 ; ^1H NMR (500 MHz, CD_3CN): δ (ppm) 7.21 (2H, m, H36+H38), 7.11 (3H, m, H35+H37+H39), 4.04 (1H, m, H16 β), 2.78 (1H, dd, J = 12.8 Hz, J = 4.0 Hz, H3 α), 2.76 (1H, d, J = 11.9, H29b), 2.66 (1H, d, J_{gem} = 11.9 Hz, H29a), 2.65 (1H, m, H20), 2.56 (2H, t, J = 8.1 Hz, H33), 2.51 (1H, septuplet, J = 7.0 Hz, H2'), 2.44 (2H, m, H31), 2.43 (1H, d, J_{gem} = 17.1 Hz, H12 α), 2.38 (1H, dt, J_{gem} = 13.4 Hz, J = 4.0 Hz, H1 β), 2.24 (1H, d, J_{gem} = 17.1 Hz, H12 β), 2.09 (3H, s, $\text{N}_\text{B}\text{Me}$), 2.01 (2H, m, H2 α +H8 β), 1.96 (1H, m, H15 β +H17 α), 1.75 (2H, m, H32), 1.62 (1H, m, H2 β), 1.57 (1H, d, J_{gem} = 4.0 Hz, H19 α), 1.53 (1H, m, H7 β), 1.49 (1H, m, H6 α), 1.42 (1H, d, J = 14.0 Hz, J = 2.7 Hz, H15 α), 1.35 (1H, d, J = 12.5 Hz, J = 3.7 Hz, H5 α), 1.24 (2H, m, H1 α +H7 α), 1.14 (3H, s, H28), 1.11+1.10 (6H, 2d, J = 7.0 Hz, H3'+H4'), 1.04 (1H, d, J = 4.0 Hz, H19 β), 0.98 (1H, m, H6 β), 0.82 (3H, d, J = 6.7 Hz, H21), 0.78 (3H, s, H18), 0.69 (3H, s, H30); ^{13}C NMR (75.4 MHz, CD_3CN): δ (ppm) 211.7 (C11), 165.8 (C1'), 141.9 (C34), 128.4 (C35+C36+C38+C39), 125.9 (C37), 78.2 (C16), 62.7 (C3), 60.4 (C20), 56.2 (C31), 55.7 (C17), 51.4 (C12), 49.8 (C5), 47.2 (C14), 44.6 (C13), 42.8 (C15), 41.4 (C8), 40.3 (C2'), 38.8 (C29), 38.5 (C10), 34.5 ($\text{N}_\text{B}\text{Me}$ +C9), 33.7 (C33), 33.4 (C4), 30.6 (C2), 30.3 (C19), 30.2 (C32), 28.0 (C1), 24.5 (C7), 21.0+20.8 (C3'+C4'), 20.7 (C28), 18.1 (C6), 17.9 (C18), 11.3 (C30), 10.9 (C21); MS (ESI, m/z) : 605.3 (M+H); HRMS (ESI, m/z) : calcd for $\text{C}_{38}\text{H}_{57}\text{N}_2\text{O}_2\text{S}$: 605.4141, found : 605.4152.

20S-[(2-methoxybenzyl)(methyl)amino]-16 α -hydroxy-4 β ,14 α -dimethyl-9,19-cyclo-{2'-isopropyl-5',6'-dihydro-4' α H-[1',3']thiazino[4',5':3,4]}-5 α ,9 β -pregnan-11-one (36d)



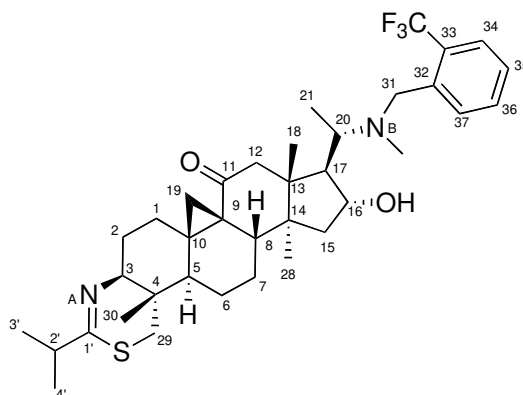
15 mg (0.031 mmol, 1 eq.) of **34**, 6 μ L (0.037 mmol, 1.2 eq.) of 2-methoxybenzyl chloride, 6.5 mg (0.046 mmol, 1.5 eq.) of potassium carbonate and 1 mL of DMF were used by following similar procedure described for the preparation of **36a** to give the title compound as an amorphous white solid (13 mg, yield: 69 %). For this reaction, the mixture was stirred at 130 °C for 30 min to give the desired product. IR (cm⁻¹) : 3324, 2962, 2929, 2867, 1666, 1633, 1452, 1259, 1223, 1147, 1079, 1017 ; ¹H NMR (500 MHz, CD₃CN): δ (ppm) 7.27 (1H, m, H37), 7.19 (1H, m, H35), 6.92 (1H, m, H36), 6.89 (1H, m, H34), 3.85 (3H, s, OMe), 3.73 (1H, m, H16 β), 3.63 (2H, bs, H31), 2.86 (1H, m, H3 α), 2.84 (1H, d, J = 11.3, H29a), 2.80 (1H, m, H20), 2.74 (1H, d, J_{gem} = 11.3 Hz, H29b), 2.60 (1H, sept, J = 6.8 Hz, H2'), 2.51 (1H, d, J_{gem} = 16.4 Hz, H12 α), 2.45 (1H, dm, J_{gem} = 13.8 Hz, H1 β), 2.32 (1H, d, J_{gem} = 16.4 Hz, H12 β), 2.18 (3H, s, N_BMe), 2.05 (2H, m, H2 α +H8 β), 1.99 (1H, m, H17 α), 1.93 (1H, m, H15 β), 1.69 (1H, m, H2 β), 1.64 (1H, d, J_{gem} = 3.8 Hz, H19 α), 1.53 (2H, m, H6 α +H7 β), 1.45 (2H, m, H5 α +H15 α), 1.34 (1H, m, H1 α), 1.31 (1H, m, H7 α), 1.20+1.18 (6H, 2d, J = 6.8 Hz, H3'+H4'), 1.18 (3H, s, H28), 1.10 (1H, d, J = 3.8 Hz, H19 β), 0.97 (1H, m, H6 β), 0.96 (3H, m, H21), 0.77 (3H, s, H30), 0.75 (3H, s, H18); ¹³C NMR (75.4 MHz, CD₃CN): δ (ppm) 211.0 (C11), 165.8 (C1'), 158.2 (C33), 131.2 (C35), 128.7 (C33), 126.8 (C32), 120.1 (C36), 110.3 (C34), 77.9 (C16), 62.7 (C3), 58.7 (C20), 55.6 (C31+C17), 55.1 (OMe), 51.4 (C12), 49.8 (C5), 47.1 (C14), 44.5 (C13), 42.8 (C15), 41.3 (C8), 40.3 (C2'), 38.8 (C29), 38.4 (C10), 34.5 (N_BMe+C9), 33.4 (C4), 30.6 (C2), 30.2 (C19), 28.0 (C1), 24.4 (C7), 21.0+20.8 (C3'+C4'), 20.6 (C28), 18.0 (C6), 17.7 (C18), 11.4 (C30), 10.8 (C21); MS (ESI, m/z) : 607.3 (M+H); HRMS (ESI, m/z) : calcd for C₃₇H₅₅N₂O₃S : 606.3933, found : 607.3923.

20S-[(4-methoxybenzyl)(methyl)amino]-16 α -hydroxy-4 β ,14 α -dimethyl-9,19-cyclo-{2'-isopropyl-5',6'-dihydro-4' α H-[1',3']thiazino[4',5':3,4]}-5 α ,9 β -pregnan-11-one (36f)



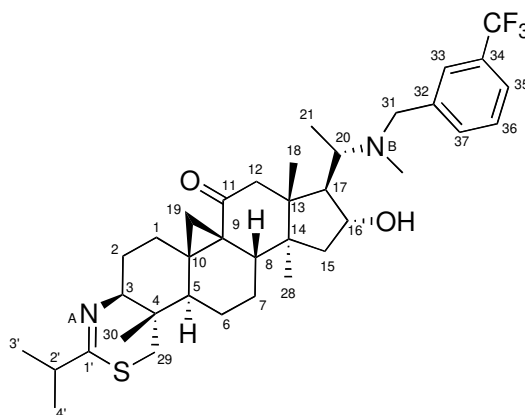
16 mg (0.033 mmol, 1 eq.) of **34**, 14 mg (0.099 mmol, 3 eq.) of potassium carbonate, 6 μ L (0.043 mmol, 1.3 eq.) of 4-methoxybenzyl chloride and 0.5 mL of DMF were used by following similar procedure described for the preparation of **36a** to give the title compound as an amorphous white solid (14 mg, yield: 70 %). IR (cm^{-1}) : 3343, 2964, 2932, 2867, 1665, 1611, 1512, 1462, 1246, 1173, 1173, 1035 ; ^1H NMR (500 MHz, CD_3CN): δ (ppm) 7.21 (2H, d, J = 8.5 Hz, H33+H37), 6.86 (2H, d, J = 8.5 Hz, H34+H36), 3.96 (1H, bs, H16 β), 3.80 (3H, s, OMe), 3.58 (2H, bs, H31), 2.85 (1H, dd, J = 12.8 Hz, J = 4.0 Hz, H3 α), 2.82 (1H, d, J = 11.3, H29b), 2.81 (1H, m, H20), 2.73 (1H, d, J_{gem} = 11.3 Hz, H29a), 2.59 (1H, septuplet, J = 7.0 Hz, H2'), 2.51 (1H, d, J_{gem} = 17.0 Hz, H12 α), 2.44 (1H, dt, J_{gem} = 13.4 Hz, J = 4.0 Hz, H1 β), 2.31 (1H, d, J_{gem} = 17.0 Hz, H12 β), 2.17 (3H, s, N_BMe), 2.05 (3H, m, H2 α +H8 β +H17 α), 1.95 (1H, m, H15 β), 1.69 (1H, m, H2 β), 1.63 (1H, d, J_{gem} = 4.0 Hz, H19 α), 1.57 (1H, m, H7 β), 1.52 (1H, m, H6 α), 1.46 (1H, m, H15 α), 1.41 (1H, m, H5 α), 1.30 (2H, m, H1 α +H7 α), 1.20 (3H, s, H28), 1.18+1.17 (6H, 2d, J = 7.0 Hz, H3'+H4'), 1.09 (1H, d, J = 4.0 Hz, H19 β), 0.98 (1H, m, H6 β), 0.95 (3H, m, H21), 0.77 (3H, s, H30), 0.75 (3H, s, H18); ^{13}C NMR (75.4 MHz, CD_3CN): δ (ppm) 210.8 (C11), 166.1 (C1'), 158.9 (C35), 130.4 (C33+C37), 113.9 (C34+C36), 77.8 (C16), 62.7 (C3), 60.4 (C31), 58.9 (C20), 55.7 (C17), 55.2 (OMe), 51.5 (C12), 49.8 (C5), 47.2 (C14), 44.7 (C13), 42.7 (C15), 41.4 (C8), 40.2 (C2'), 38.7 (C29), 38.4 (C10), 34.5 (N_BMe), 34.4 (C9), 33.4 (C4), 30.5 (C2), 30.3 (C19), 28.0 (C1), 24.4 (C7), 21.1+20.8 (C3'+C4'), 20.6 (C28), 18.0 (C6), 17.8 (C18), 11.3 (C30), 10.7 (C21); MS (ESI, m/z) : 745.4 (M+K), 607.3 (M+H); HRMS (ESI, m/z) : calcd for $\text{C}_{37}\text{H}_{55}\text{N}_2\text{O}_3\text{S}$: 607.3933, found : 607.3925.

20S-[*o*-(trifluoromethyl)benzylamino]-16 α -hydroxy-4 β ,14 α -dimethyl-9,19-cyclo-[2'-isopropyl-5',6'-dihydro-4' α H-[1',3']thiazino[4',5':3,4]]-5 α ,9 β -pregnan-11-one (36g)



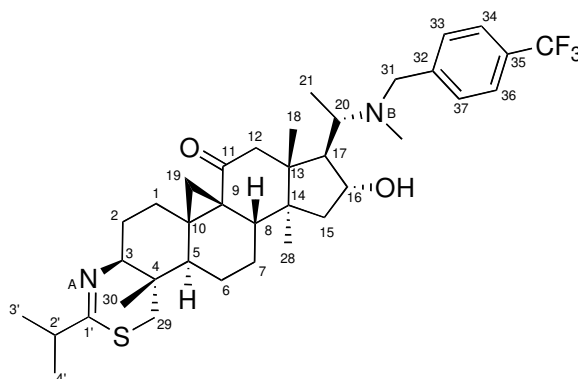
To 15 mg (0.031 mmol, 1 eq.) of **34** dissolved in 0.5 mL of DMF were added 13 mg (0.093 mmol, 3 eq.) of potassium carbonate and 10 mg (0.040 mmol, 1.3 eq.) of 2-trifluoromethylbenzyl bromide. The mixture was stirred at 80 °C for 12 h, diluted with 10 mL of a saturated sodium hydrogen carbonate solution and extracted with 3x10 mL of Et₂O. Combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by silica gel column chromatography (gradient elution : Hept/Acetone/NH₄OH : from 60/40/1 to 40/60/1) to give the title compound as an amorphous white solid (11 mg, yield: 55 %). IR (cm⁻¹) : 3455, 2960, 2930, 2867, 1666, 1633, 1454, 1311, 1257, 1160, 1120, 1037 ; ¹H NMR (500 MHz, CD₃CN, 300K): δ (ppm) 7.57 (2H, m, Har), 7.47 (1H, t, J = 7.6 Hz, Har), 7.29 (1H, t, J = 7.6 Hz, Har), 3.91 (1H, bs, H16 β), 3.91 (2H, bs, H31), 2.76 (1H, dd, J = 12.8 Hz, J = 4.3 Hz, H3 α), 2.75 (1H, d, J_{gem} = 11.6 Hz, H29b), 2.71 (1H, dq ; J = 11.3 Hz, J = 6.4 Hz, H20), 2.65 (1H, d, J_{gem} = 11.6 Hz, H29a), 2.51 (1H, septuplet, J = 7.0 Hz, H2'), 2.45 (1H, d, J_{gem} = 17.4 Hz, H12 α), 2.37 (1H, dt, J_{gem} = 13.7 Hz, J_{1 β -2} = 3.7 Hz, H1 β), 2.26 (1H, d, J_{gem} = 17.4 Hz, H12 β), 2.13 (3H, s, N_BMe), 2.00 (1H, m, H17 α), 1.98 (2H, m, H2 α +H8 β), 1.90 (1H, dd, J = 14.0 Hz, J = 10.1 Hz, H15 β), 1.62 (1H, qd, J = 13.4 Hz, J = 4.0 Hz, H2 β), 1.56 (1H, d, J_{gem} = 4.3 Hz, H19 α), 1.49 (1H, m, H7 β), 1.46 (1H, m, H6 α), 1.39 (1H, dd, J = 14.0 Hz, J = 2.7 Hz, H15 α), 1.34 (1H, dd, J = 12.5 Hz, J = 3.7 Hz, H5 α), 1.27 (1H, m, H7 α), 1.25 (1H, m, H1 α), 1.14 (3H, s, H28), 1.11+1.10 (6H, 2d, J = 7.0 Hz, H3'+H4'), 1.03 (1H, d, J_{gem} = 4.3 Hz, H19 β), 0.95 (1H, m, H6 β), 0.93 (3H, d, J₂₁₋₂₀ = 6.7 Hz, H21), 0.69 (3H, s, H18), 0.69 (3H, s, H30); ¹³C NMR (75.4 MHz, CD₃CN): δ (ppm) 211.4 (C11), 165.8 (C1'), 137.7 (C32), 131.9 (Car), 127.2 (Car), 77.8 (C16), 62.7 (C3), 59.7 (C20), 56.1 (C31+C17), 51.5 (C12), 49.8 (C5), 47.2 (C14), 44.9 (C13), 42.8 (C15), 41.4 (C8), 40.3 (C2'), 38.8 (C29), 38.5 (C10), 34.4 (N_BMe+C9), 33.4 (C4), 30.6 (C2), 30.3 (C19), 28.0 (C1), 24.4 (C7), 21.0+20.8 (C3'+C4'), 20.5 (C28), 18.0 (C6), 17.8 (C18), 11.3 (C30), 10.9 (C21), MS (ESI, m/z) : 645.4 (M+H); HRMS (ESI, m/z) : calcd for C₃₇H₅₂N₂O₂SF₃ : 645.3702, found : 645.3704.

20S-[m-(trifluoromethyl)benzylamino]-16 α -hydroxy-4 β ,14 α -dimethyl-9,19-cyclo-{2'-isopropyl-5',6'-dihydro-4' α H-[1',3']thiazino[4',5':3,4]}-5 α ,9 β -pregnan-11-one (36h)



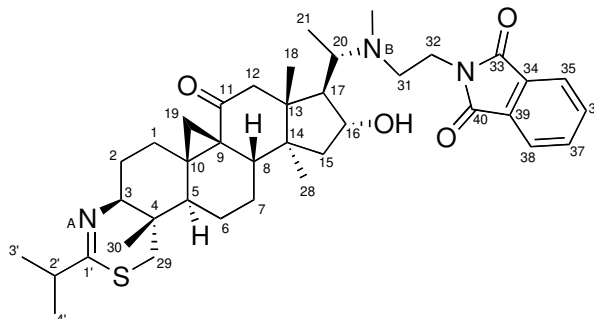
16 mg (0.031 mmol, 1 eq.) of **34**, 13 mg (0.093 mmol, 3 eq.) of potassium carbonate, 10 mg (0.040 mmol, 1.3 eq.) of 3-trifluoromethylbenzyl bromide and 0.5 mL of DMF were used by following similar procedure described for the preparation of **36e** to give the title compound as an amorphous white solid (13 mg, yield: 62 %). IR (cm⁻¹) : 3358, 2955, 2928, 2854, 1666, 1632, 1450, 1327, 1163, 1224, 1072 ; ¹H NMR (500 MHz, CD₃CN, 333K): δ(ppm) 7.52 (3H, m, H33+H35+H37), 7.46 (1H, t, J = 7.9 Hz, H36), 4.01 (1H, bs, H16 β), 3.67 (1H, bs, H31), 2.84 (1H, dd, J = 12.8 Hz, J = 4.3 Hz, H3 α), 2.83 (1H, d, J_{gem} = 11.3 Hz, H29b), 2.79 (1H, dq, J = 11.0 Hz, J = 6.7 Hz, H20), 2.73 (1H, d, J_{gem} = 11.3 Hz, H29a), 2.58 (1H, septuplet, J = 7.0 Hz, H2'), 2.5 (1H, d, J_{gem} = 17.1 Hz, H12 α), 2.44 (1H, dt, J_{gem} = 13.4 Hz, J_{1 β -2} = 3.4 Hz, H1 β), 2.32 (1H, d, J_{gem} = 17.1 Hz, H12 β), 2.18 (3H, s, N_BMe), 2.08 (1H, m, H17 α), 2.05 (2H, m, H2 α +H8 β), 1.99 (1H, m, H15 β), 1.70 (1H, m, H2 β), 1.63 (1H, d, J_{gem} = 4.0 Hz, H19 α), 1.56 (1H, m, H7 β), 1.53 (1H, m, H6 α), 1.49 (1H, m, H15 α), 1.41 (1H, m, H5 α), 1.32 (2H, m, H1 α +H7 α), 1.21 (3H, s, H28), 1.18+1.16 (6H, 2d, J = 7.0 Hz, H3'+H4'), 1.13 (1H, J_{gem} = 4.0 Hz, H19 β), 1.02 (1H, m, H6 β), 0.99 (3H, d, J₂₁₋₂₀ = 6.7 Hz, H21), 0.77 (3H, s, H18), 0.76 (3H, s, H30); ¹³C NMR (75.4 MHz, CD₃CN): δ(ppm) 210.7 (C11), 165.9 (C1'), 139.7 (C32), 132.3 (C37), 129.1 (C36), 125.8 (C33), 124.2 (C35), 78.0 (C16), 62.7 (C3), 60.7 (C31), 59.7 (C20), 55.9 (C17), 51.4 (C12), 49.8 (C5), 47.2 (C14), 44.8 (C13), 42.8 (C15), 41.4 (C8), 40.3 (C2'), 38.7 (C29), 38.5 (C10), 34.4 (N_BMe+C9), 33.4 (C4), 30.6 (C2), 30.4 (C19), 28.0 (C1), 24.4 (C7), 21.0+20.8 (C3'+C4'), 20.6 (C28), 18.0 (C6), 17.7 (C18), 11.3 (C30), 10.8 (C21); MS (ESI, m/z) : 645.4 (M+H); HRMS (ESI, m/z) : calcd for C₃₇H₅₂N₂O₂SF₃ : 645.3702, found : 645.3708.

20S-[*p*-(trifluoromethyl)benzylamino]-16 α -hydroxy-4 β ,14 α -dimethyl-9,19-cyclo-[2'-isopropyl-5',6'-dihydro-4' α H-[1',3']thiazino[4',5':3,4]]-5 α ,9 β -pregnan-11-one (36i)



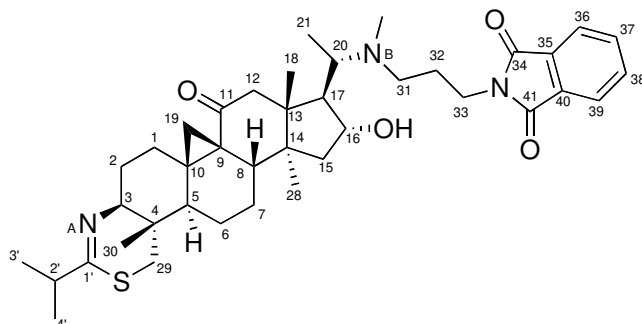
16 mg (0.031 mmol, 1 eq.) of **34**, 13 mg (0.093 mmol, 3 eq.) of potassium carbonate, 10 mg (0.040 mmol, 1.3 eq.) of 4-trifluoromethylbenzyl bromide and 0.5 mL of DMF were used by following similar procedure described for the preparation of **36e** to give the title compound as an amorphous white solid (12 mg, yield: 57 %). IR (cm⁻¹) : 3361, 2968, 2929, 2866, 1666, 1636, 1617, 1456, 1323, 1161, 1122, 1065, 1017 . ¹H NMR (500 MHz, CD₃CN, 333K): δ (ppm) 7.61 (2H, d, *J* = 8.2 Hz, H34+H36), 7.44 (2H, d, *J* = 8.2 Hz, H33+H37), 4.04 (1H, bs, H16 β), 3.69 (2H, bs, H31), 2.86 (1H, dd, *J* = 12.8 Hz, *J* = 4.0 Hz, H3 α), 2.85 (1H, d, *J*_{gem} = 11.6 Hz, H29b), 2.81 (1H, m, H20), 2.75 (1H, d, *J*_{gem} = 11.6 Hz, H29a), 2.61 (1H, septuplet, *J* = 7.0 Hz, H2'), 2.54 (1H, d, *J*_{gem} = 17.1 Hz, H12 α), 2.46 (1H, dt, *J*_{gem} = 13.7 Hz, *J*_{1 β -2} = 3.5 Hz, H1 β), 2.33 (1H, d, *J*_{gem} = 17.1 Hz, H12 β), 2.19 (3H, s, N_BMe), 2.11 (2H, m, H8 β +H17 α), 2.07 (1H, m, H2 α), 2.01 (1H, m, H15 β), 1.71 (1H, m, H2 β), 1.65 (1H, d, *J*_{gem} = 4.0 Hz, H19 α), 1.59 (1H, m, H7 β), 1.55 (1H, m, H6 α), 1.51 (1H, m, H15 α), 1.44 (1H, m, H5 α), 1.32 (2H, m, H1 α +H7 α), 1.23 (3H, s, H28), 1.20+1.18 (6H, 2d, *J* = 7.0 Hz, H3'+H4'), 1.13 (1H, d, *J* = 4.0 Hz, H19 β), 1.00 (3H, d, *J*₂₁₋₂₀ = 6.7 Hz, H21), 0.88 (1H, m, H6 β), 0.79 (3H, s, H18), 0.77 (3H, s, H30); ¹³C NMR (75.4 MHz, CD₃CN): δ (ppm) 210.7 (C11), 165.8 (C1'), 142.8 (C32), 129.3 (C33+C37), 125.5 (C34+C36), 78.0 (C16), 62.6 (C3), 60.8 (C31), 60.0 (C20), 55.9 (C17), 51.4 (C12), 49.8 (C5), 47.2 (C14), 44.8 (C13), 42.7 (C15), 41.3 (C8), 40.3 (C2'), 38.7 (C29), 38.5 (C10), 34.4 (N_BMe+C9), 33.4 (C4), 30.5 (C2), 30.4 (C19), 28.0 (C1), 24.4 (C7), 21.1+20.8 (C3'+C4'), 20.6 (C28), 18.0 (C6), 17.8 (C18), 11.3 (C30), 10.8 (C21); MS (ESI, *m/z*) : 645.4 (M+H); HRMS (ESI, *m/z*) : calcd for C₃₇H₅₂N₂O₂SF₃ : 645.3702, found : 645.3728.

2-{2-[[16 α -hydroxy-4 β ,14 α -dimethyl-11-oxo-9,19-cyclo-{2'-isopropyl-5',6'-dihydro-4' α H-[1',3']thiazino[4',5':3,4]]-5 α ,9 β -pregnan-20S-yl](methyl)amino]ethyl}-1H-isindole-1,3(2H)-dione (36j)



15.7 mg (0.032 mmol, 1 eq.) of **34**, 24 mg (0.097 mmol, 3 eq.) of 2-(2-bromoethyl)isindole-1,3-dione, 13 mg (0.097 mmol, 3 eq.) of potassium carbonate and 1 mL of acetonitrile were used by following similar procedure described for the preparation of **36a** to give the title compound as an amorphous white solid (15.4 mg, yield: 72 %). For this reaction, the mixture was refluxed for 12 h to give the desired product. IR (cm⁻¹) : 3464, 2962, 2930, 2873, 1772, 1711, 1668, 1633, 1394; ¹H NMR (500 MHz, CD₃CN, 333K): δ (ppm) 7.80 (2H, m, H35+H38), 7.76 (2H, m, H36+H37), 3.87 (1H, m, H16 β), 3.80 (2H, m, H32), 3.70 (2H, m, H31), 2.85 (1H, d, J_{gem} = 11.9 Hz, H29b), 2.82 (1H, dd, J = 12.8 Hz, J = 4.0 Hz, H3 α), 2.75 (1H, d, J_{gem} = 11.9 Hz, H29a), 2.70 (1H, m, H20), 2.51 (1H, d, J_{gem} = 17.1 Hz, H12 α), 2.46 (1H, m, H2'), 2.31 (1H, m, H1 β), 2.30 (3H, s, N_BMe), 2.19 (1H, d, J_{gem} = 17.1 Hz, H12 β), 2.04 (1H, dd, J = 10.4 Hz, J = 7.0 Hz, H8 β), 1.98 (1H, dd, J = 10.7 Hz, J = 7.0 Hz, H17 α), 1.93 (1H, m, H2 α), 1.84 (1H, dd, J = 14.0 Hz, J = 10.4 Hz, H15 β), 1.60 (1H, m, H2 β), 1.48 (3H, m, H6 α +H7 β +H19 α), 1.44 (1H, m, H5 α), 1.29 (1H, m, H1 α), 1.27 (1H, m, H7 α), 1.20 (1H, m, H15 α), 1.11 (10H, m, H3'+H4'+H19 β +H28), 1.03 (1H, m, H6 β), 0.91 (3H, d, J_{21-20} = 6.4 Hz, H21), 0.78 (3H, s, H18), 0.72 (3H, s, H30). ¹³C NMR (75.4 MHz, CD₃CN): δ (ppm) 211.3 (C11), 169.6 (C33+C40), 135.1 (C36+C37), 133.2 (C34+C39), 123.8 (C35+C38), 78.4 (C16), 63.5 (C3+C20), 56.9 (C17), 52.2 (C12), 50.2 (C5), 47.9 (C14), 45.7 (C13), 43.9 (C15), 42.0 (C8), 40.5 (C2'), 39.4 (C29), 38.8 (C10), 37.0 (C32), 35.1 (C9), 34.0 (C4), 31.4 (C2), 30.5 (C19), 28.7 (C1), 25.1 (C7), 21.3+21.2 (C3'+C4'), 20.7 (C28), 18.7 (C6), 18.1 (C18), 11.5 (C21+C30); MS (ESI, m/z) : 660.3 (M+H) ; HRMS (ESI, m/z) : calcd for C₃₉H₅₄N₃O₄S : 660.3835, found : 660.3817.

2-{3-[[16 α -hydroxy-4 β ,14 α -dimethyl-11-oxo-9,19-cyclo-{2'-isopropyl-5',6'-dihydro-4' α H-[1',3']thiazino[4',5':3,4]}-5 α ,9 β -pregnan-20S-yl](methyl)amino]propyl]-1H-isoindole-1,3(2H)-dione (36k)



11.3 mg (0.023 mmol, 1 eq.) of **34**, 9.3 mg (0.035 mmol, 1.5 eq.) of 2-(3-bromopropyl)isoindole-1,3-dione, 10 mg (0.070 mmol, 3 eq.) of potassium carbonate and 1 mL of acetonitrile were used by following similar procedure described for the preparation of **36a** (75%) to give the title compound as an amorphous white solid (11.7 mg, yield: 75%). For this reaction, the mixture was refluxed for 30 h to give the desired product. IR (cm⁻¹) : 2960, 2928, 2866, 1770, 1711, 1669, 1635, 1395; ¹H NMR (500 MHz, CD₃CN, 333K): δ (ppm) 7.81 (2H, m, H36+H39), 7.76 (2H, m, H37+H38), 4.20 (1H, m, H16 β), 3.69 (2H, m, H33), 2.86 (1H, d, J_{gem} = 11.6 Hz, H29b), 2.82 (2H, m, H3 α +H20), 2.75 (1H, d, J_{gem} = 11.6 Hz, H29a), 2.61 (2H, m, H31), 2.53 (1H, d, J_{gem} = 17.1 Hz, H12 α), 2.48 (1H, sept, J = 6.7 Hz, H2'), 2.35 (1H, dm, J = 13.4 Hz, H1 β), 2.28 (3H, s, N_BMe), 2.23 (1H, d, J_{gem} = 17.1 Hz, H12 β), 2.09 (2H, m, H8 β +H17 α), 2.02 (1H, m, H15 β), 1.95 (1H, m, H2 α), 1.89 (1H, m, H32), 1.64 (1H, m, H2 β), 1.52 (3H, m, H6 α +H7 β +H19 α), 1.46 (1H, dd, J = 12.2 Hz, J = 3.4 Hz, H5 α), 1.40 (1H, dd, J = 14.0 Hz, J = 2.4 Hz, H15 α), 1.30 (2H, m, H1 α +H7 α), 1.17 (3H, s, H28), 1.13 (7H, m, H3'+H4'+H19 β), 1.06 (1H, m, H6 β), 0.93 (3H, d, J_{21-20} = 6.4 Hz, H21), 0.86 (3H, s, H18), 0.74 (3H, s, H30); ¹³C NMR (75.4 MHz, CD₃CN): δ (ppm) 211.1 (C11), 169.1 (C34+C41), 134.8 (C37+C38), 132.9 (C35+C40), 123.6 (C36+C39), 77.4 (C16), 63.3 (C3+C20), 56.6 (C17), 52.1 (C12), 50.1 (C5), 47.8 (C14), 45.6 (C13), 43.9 (C15), 41.9 (C8), 40.4 (C2'), 39.3 (C29), 38.7 (C10), 36.4 (C33), 35.0 (C9), 33.8 (C4), 31.2 (C2), 30.2 (C19), 28.5 (C1), 27.2 (C32), 25.0 (C7), 21.3+21.2 (C3'+C4'), 20.7 (C28), 18.5 (C6), 18.2 (C18), 11.5 (C30), 11.2 (C21); MS (ESI, m/z) : 674.4 (M+H); HRMS (ESI, m/z) : calcd for C₄₀H₅₆N₃O₄S : 674.3992, found : 674.3975.

Purity of tested compounds

The purity of tested compounds were measured using reversed-phase UHPLC (HSS C-18, 2.1x50 mm s-1.8 μ M). All compounds for which biological data is reported are >95 % purity. Compounds were eluted with 95/5 A/B for 0.3 min then with a gradient of 5-100% B/A for 4.2 min followed by 0/100 A/B isocratic for 0.5 min at flow rate of 0.6 ml/min, where solvent A was 0.1 % formic acid in H₂O and solvent B was 0.1 % formic acid in MeCN. Purity was determined from the absorbance at 230 nm.

**TABLE LISTING THE DEGREE OF PURITY FOR TESTED COMPOUNDS
(percent and retention time)**

Compound	purity
9a	>98% (2.16 min)
9b	>96% (2.21 min)
9c	>95% (2.18 min)
13a	>95% (1.73 min)
13b	>95% (1.44 min)
20	>99% (1.70 min)
25	>95% (2.86 min)
27	>95% (2.96 min)
30	>97% (1.35 min)
34	>95% (1.39 min)
35a	>98% (1.12 min)
35b	>95% (2.75 min)
35c	>95% (3.08 min)
36a	>95% (1.42 min)
36b	>95% (1.74 min)
36c	>95% (1.78 min)
36d	>95% (1.75 min)
36e	>95% (1.75 min)
36f	>98% (1.71 min)
36g	>95% (1.81 min)
36h	>95% (1.87 min)
36i	>95% (1.87 min)
36j	>95% (1.68 min)
36k	>96% (1.73 min)

Computer-Aided Molecular Modeling

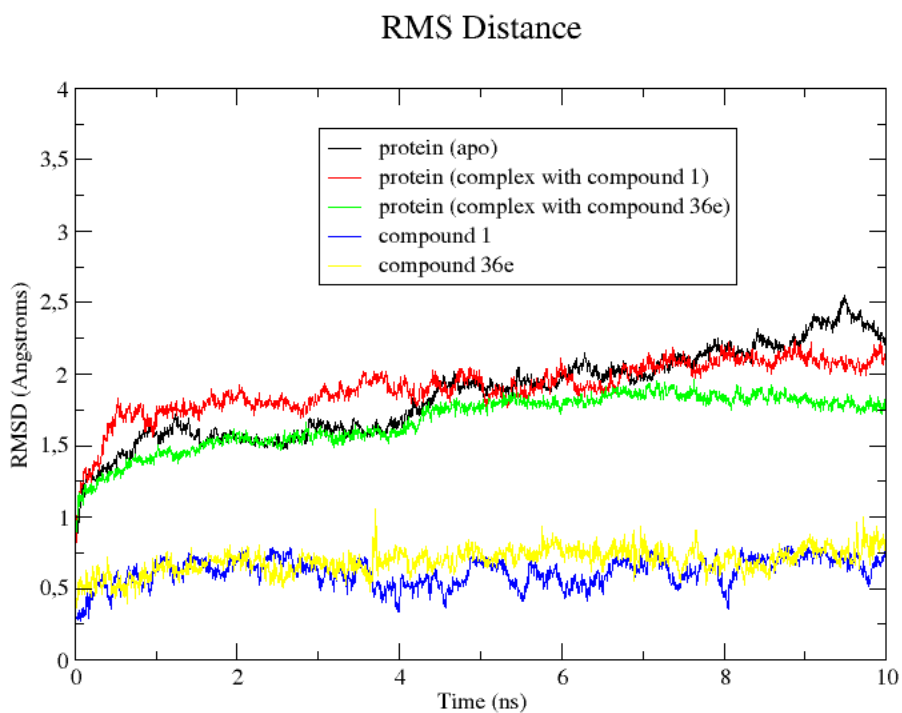


Figure S1. Root mean square deviation (RMSD) for compounds **1** (blue) and **36e** (yellow), and for protein in apo form (black) and in complex with compounds **1** (red) and **36e** (green), during the 10 ns molecular dynamics simulations

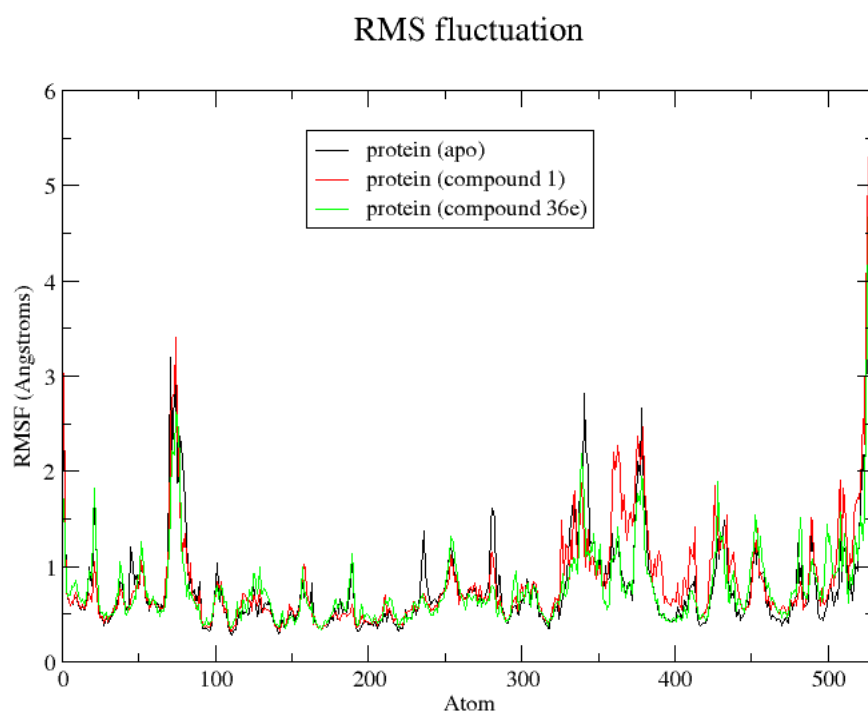


Figure S2. Root mean square fluctuation (RMSF) of CA atoms for protein in apo form (black) and in complex with compounds **1** (red) and **36e** (green), during the 10 ns molecular dynamics simulations

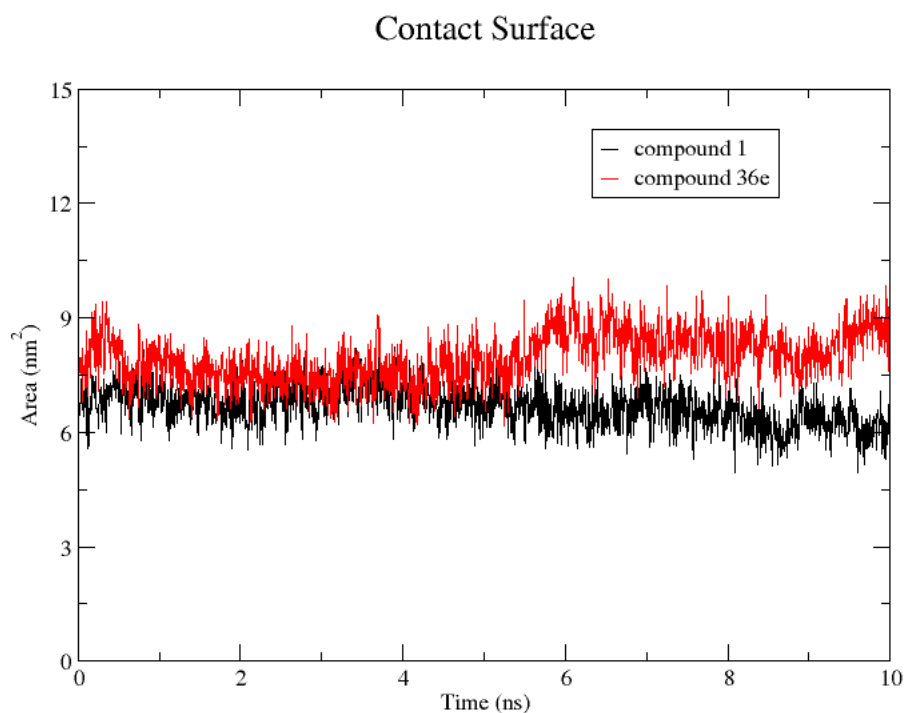


Figure S3. Contact surface between protein and compounds **1** (black) and **36e** (red), during the 10 ns molecular dynamics simulations

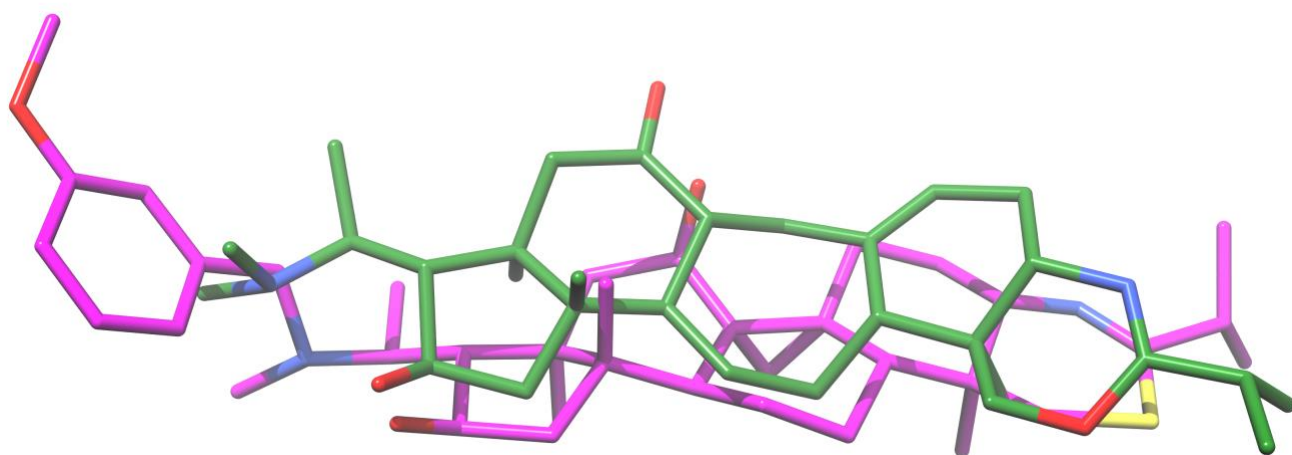


Figure S4. Superposition of equilibrium conformations in the AChE binding site for compounds **1** (green) and **36e** (magenta), after 10 ns molecular dynamics simulations