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Original article

Unraveling the structure–activity relationship of tomatidine, a steroid alkaloid with unique antibiotic properties against persistent forms of *Staphylococcus aureus*

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ABSTRACT

Staphylococcus aureus (*S. aureus*) is responsible for difficult-to-treat and relapsing infections and constitutes one of the most problematic pathogens due to its multiple resistances to clinically available antibiotics. Additionally, the ability of *S. aureus* to develop small-colony variants is associated with a reduced susceptibility to aminoglycoside antibiotics and *in vivo* persistence. We have recently demonstrated that tomatidine, a steroid alkaloid isolated from tomato plants, possesses anti-virulence activity against normal strains of *S. aureus* as well as the ability to potentiate the effect of aminoglycoside antibiotics. In addition, tomatidine has shown antibiotic activity against small-colony variants of *S. aureus*. We herein report the first study of the structure–activity relationship of tomatidine against *S. aureus*.

1. Introduction

Antibiotic resistance is becoming an alarming health problem compounded by the lack of new therapeutic options to fight resistant pathogens [1,2]. Since the first synthetic antibiotics were introduced nearly 80 years ago, several scaffolds have become new classes of antibiotics [3,4]. As an adaptive response to this arsenal, pathogens have acquired efficient resistance mechanisms [5–7], leading eventually to multi-resistant strains [3,8]. The development of new antibacterial agents has not paralleled the development of resistance [3,4,9], and drug approvals in the antibiotic field have diminished by half in the past 20 years [10]. Additionally, there has been a lack of new chemical scaffolds among new drugs. Indeed, between 1930 and 1970, 20 different classes of antimicrobial agents were discovered, including widely used classes such as the penicillins, aminoglycosides and tetracyclines [3,5]. In the thirty years that followed, the discovery of new classes came to a standstill [4,9]. As a result, most of the recently introduced antibiotics are still based on scaffolds discovered or designed 60 years ago, which increases the likelihood of resistance development [3,9].

Among highly resistant pathogens, *Staphylococcus aureus* (*S. aureus*) has evolved methicillin-resistant (MRSA) and often multi-resistant strains. Since 2002, we have witnessed the emergence of strains resistant to vancomycin, which is generally considered the last resort antibiotic for this pathogen [7,11]. MRSA is responsible for many hospital- and community-acquired infections and is often associated with recurring and difficult-to-treat infections, which increase morbidity and mortality in both humans and animals [5,12].

Among the mechanisms that allow *S. aureus* to cause resistance and persistent infections and reduce its susceptibility to antibiotics, this pathogen often presents itself in the form of respiratory-deficient small-colony variants (SCVs) [13]. SCVs suffer from a deficient electron transport chain and a weaker proton-motive

Abbreviations: S. aureus, *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*; SCV, small-colony variant; MIC, minimal inhibitory concentration; SAR, structure–activity relationship.

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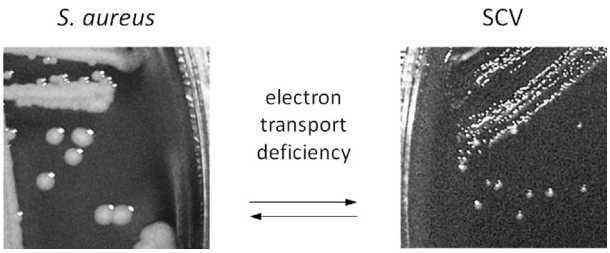


Fig. 1. Characteristics of normal and small colony variants of *S. aureus*.

force, which concertedly impede bacterial growth while increasing resistance to antibiotics such as aminoglycosides [12,14–16]. As opposed to normal strains (Fig. 1), SCVs possess characteristics that contribute to prevent acute infections, but allow *in vivo* persistence [14,15,17].

S. aureus has the ability to switch between the normal and SCV phenotypes. While normal strains exhibit virulence, fast growth and dissemination and are responsible for acute infections, SCVs are associated with colonization, biofilm production and persistent infections [16]. They possess a deficient electron transport chain, which modifies the proton-motive force (PMF) and affects susceptibility to aminoglycoside (AMG) antibiotics.

Tomatidine (**1**, Fig. 2), the aglycone version of tomatine (**2**), is an important antimicrobial defense metabolite of many solanaceous plants and possesses potential anticancer [18–21], chemosensitizer [22], anti-Leishmania [23], anti-hyperlipidemic [24], and anti-inflammatory properties [25]. Apart from its action on eukaryotic organisms, our group has recently demonstrated that tomatidine possesses anti-virulence activity against normal strains of *S. aureus* without impairing growth, while also displaying potent antibiotic activity against SCVs [12,14]. Most importantly, tomatidine has demonstrated its ability to potentiate the bactericidal activity of aminoglycoside antibiotics against the normal phenotype of *S. aureus* [12]. An example of such potentiating effect is observed when tomatidine is used in combination with gentamicin, which improves the minimal inhibitory concentration (MIC) of gentamicin by 8-fold (0.5 μg/mL to 0.06 μg/mL in the absence or presence of tomatidine, respectively). The antibacterial mechanism of action, structure–activity relationship (SAR) and target of tomatidine remain to be elucidated.

In order to better understand the antibacterial properties of tomatidine and explore its SAR, we undertook the synthesis of structural analogs. Our initial goal was to firstly understand the SAR of tomatidine, both as a potentiator of the antibiotic action of gentamicin and as a growth inhibitor of *S. aureus* SCVs. The results of these efforts are reported below.

2. Chemistry

Tomatidine **1** (Fig. 2) is a steroid alkaloid structurally characterized by 6 rings, 12 stereogenic centers, a 3β-hydroxyl group and spiro-fused E, F rings in the form of an aminoketal. It was isolated by hydrolysis of its glycosylated analog tomatine **2** [26]. The structure of tomatidine was elucidated via a combination of infrared spectroscopy, elemental analysis and X-ray crystallography [13,27]. In the absence of an identified cellular target, our initial objective was to elucidate SAR, based on the initial hypothesis that the steroidal A–D rings act as a rigid scaffold to orient pharmacophores defined by the 3β-hydroxyl group on ring A and the spiroaminoketal group on rings E, F. Accordingly, our attention focused on the two extremities of the molecule. An important question from the outset was whether the closed or the open form of the aminoketal is responsible for biological activity. Indeed, the aminoketal can be opened by hydrolysis to reveal a hydroxyl function and a tethered amino and keto functional groups on ring D [28,29].

Analogs bearing modifications on ring A were prepared as indicated in Scheme 1. The aminoketal moiety was first protected as a formamide [30] by *N*,*O*-double formylation of tomatidine hydrochloride **3** with acetic formic anhydride, followed by chemoselective deprotection of the formate ester in mild basic conditions to yield *N*-formyltomatidine **4** in quantitative yield. Compound **4** underwent a Mitsunobu inversion with acetic acid [31], followed by protective groups acidolysis to afford 3α-hydroxyl tomatidine **6** in high yields [32]. Palladium-catalyzed *O*-allylation of *N*-formyltomatidine **4** delivered *O*-allyltomatidine **8** in good yield after deformylation [33]. Direct oxidation of the C3 alcohol of compound **4** using Dess–Martin periodinane [34] delivered 3-ketotomatidine **9**, which was subsequently deprotected to yield **10** by acidic hydrolysis [32]. Finally, starting from 3-ketotomatidine intermediate **9**, amino analogs **12**, **14**, **16** and **18** were generated via reductive amination [35] followed by deprotection in low to moderate yields.

In order to better understand the SAR of the aminoketal moiety on rings E, F and ascertain whether a closed or open form of rings E and F constitute the active form of the molecule, we generated analogs modified with open rings E and/or F via two complementary approaches.

Firstly, tomatidine was used as starting materials to generate open chain derivatives (Scheme 2). Opening of ring E was performed via catalytic hydrogenation of the spiroaminoketal moiety on platinum oxide [36] to yield piperidine derivative **19**. On the other hand, dihydrofuran **22** was obtained by acetylation of the spiroaminoketal moiety of **3** to give intermediate **20**, followed by acid-catalyzed elimination then ester hydrolysis [37–39]. Subsequent reduction delivered the corresponding tetrahydrofuran **23** with no control of stereochemistry on the newly created stereogenic centers. Acetylation of tomatidine hydrochloride **3** also yielded triacetylated compound **24**, which was opened in acidic conditions to give intermediate **25** [37–39] then reduced and deprotected to afford triol **27**. Unfortunately, all attempts to hydrolyze the terminal acetamide of **27** resulted in product decomposition.

The second approach in the synthesis of analogs modified on rings E and F started from commercially available pregnenolone acetate **28**, which bears the same A–D ring system and stereochemistry as tomatidine (Scheme 3). Hydrogenation and ester hydrolysis [40] followed by ^{t}BDMS protection of the hydroxyl group

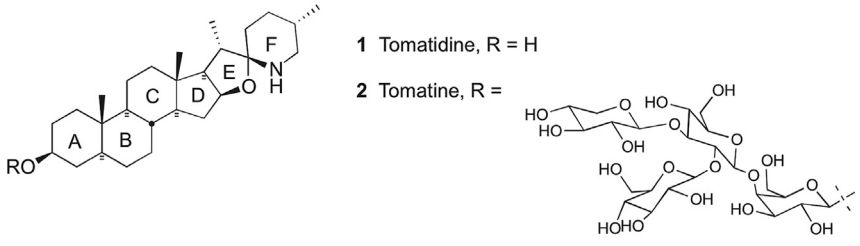
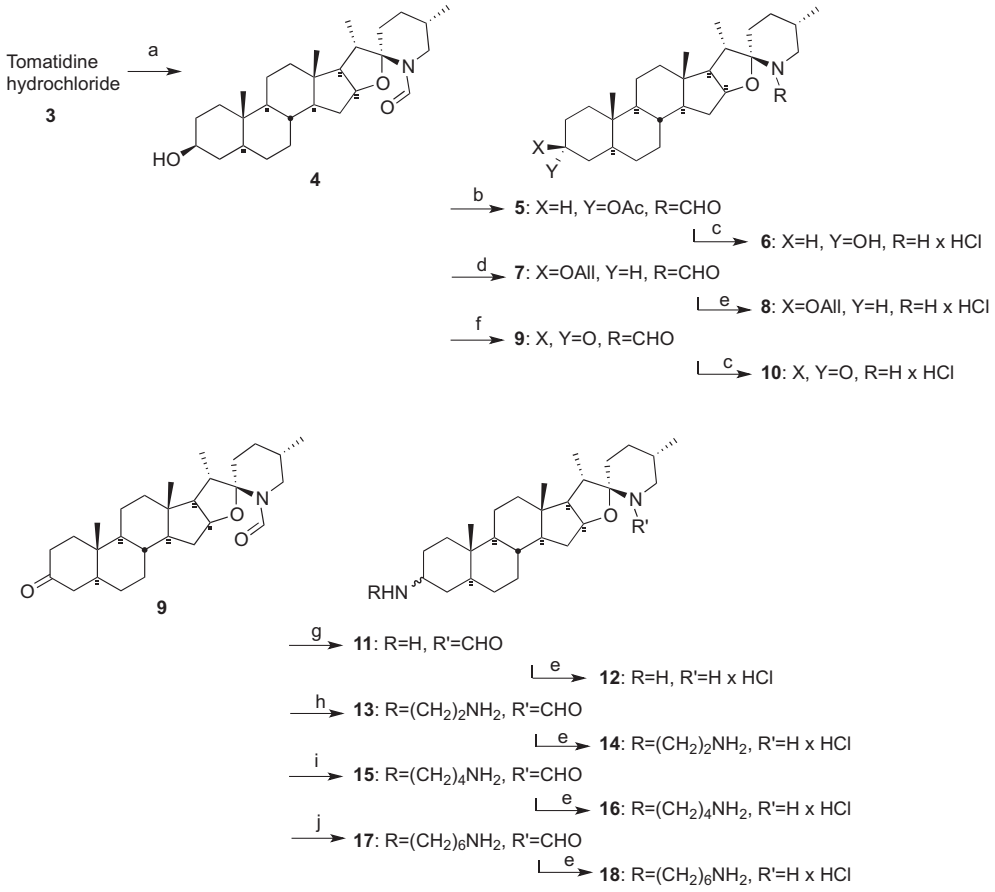
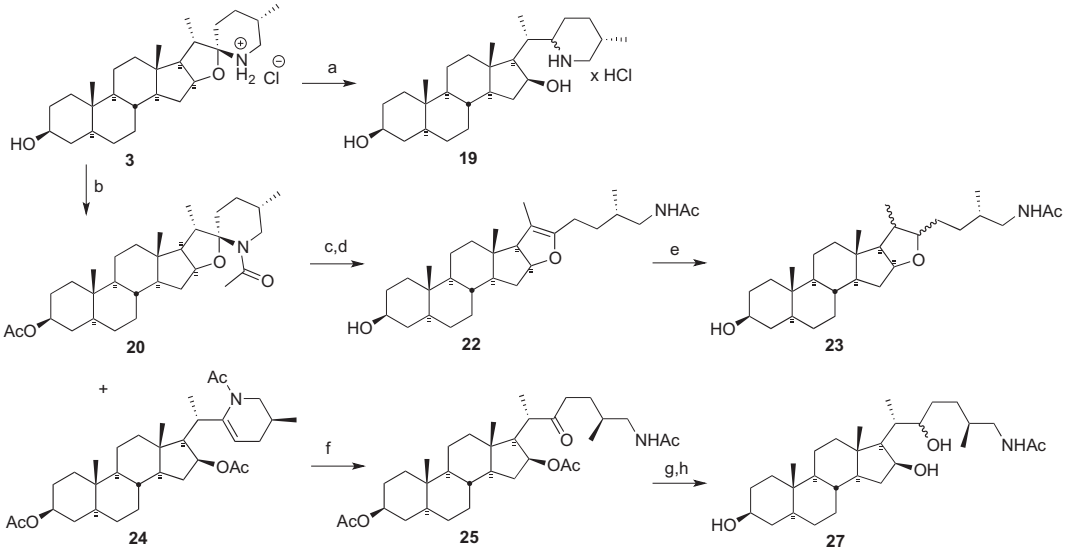


Fig. 2. The structures of tomatidine and tomatine.

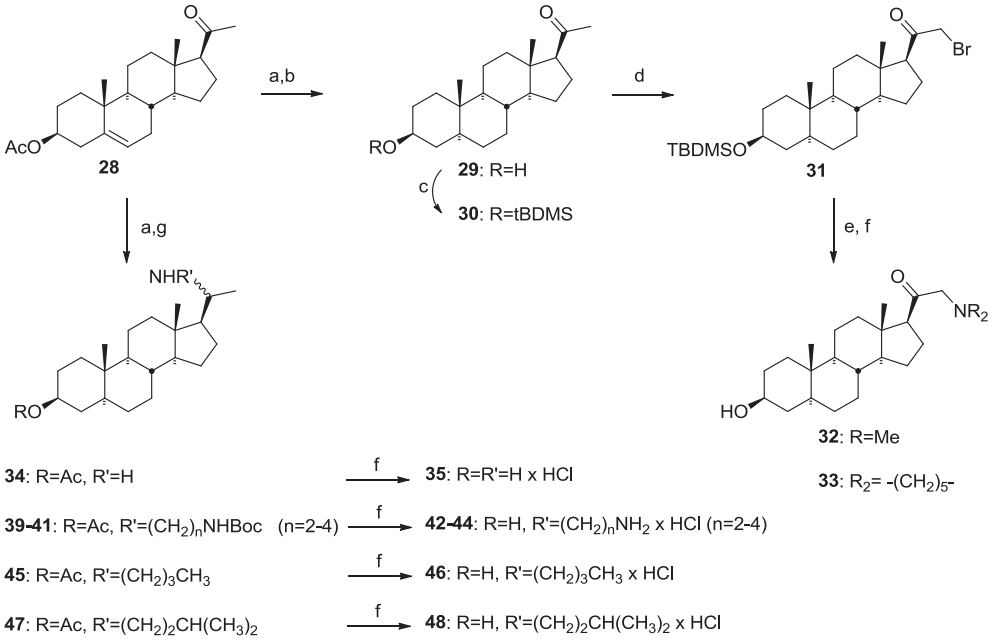


Scheme 1. Reagents and conditions: a) i) acetic formic anhydride, DIPEA, THF, rt; ii) EtOH, $NaHCO_3$ buffer pH = 9.5, rt, 79% (2 steps); b) DIAD, PPH_3, AcOH, THF, rt, 86%; c) HCl, EtOH, reflux, quant.; d) allyl methyl carbonate, $Pd_2(dba)_3$, dppp, THF, 65 °C, 76%; e) HCl, MeOH, reflux, quant.; f) Dess–Martin periodinane, DCM, rt, 68%; g) NH_4OAc, $NaBH_3CN$, MeOH, pH = 6, reflux, 69%; h) $BocNH(CH_2)_2NH_2$, $NaBH_3CN$, MeOH, pH = 6, reflux, 89%; i) $BocNH(CH_2)_4NH_2$, $NaBH_3CN$, MeOH, pH = 6, reflux, 44%; j) $BocNH(CH_2)_6NH_2$, $NaBH_3CN$, MeOH, pH = 6, reflux, 46%.

delivered keto intermediate **30** in high yields [41]. Bromination α to the keto group then yielded bromoketone **31** [42,43]. Subsequent substitution with dimethylamine or piperidine [44] followed by desilylation yielded dimethyl-substituted and pyrrolidinyl-substituted tertiary amines **32** and **33**, respectively. In parallel, reductive amination of **28** with diverse amines and monoprotected diamines followed by acidic hydrolysis produced analogs **35**, **42**–**44**, **46** and **48**.

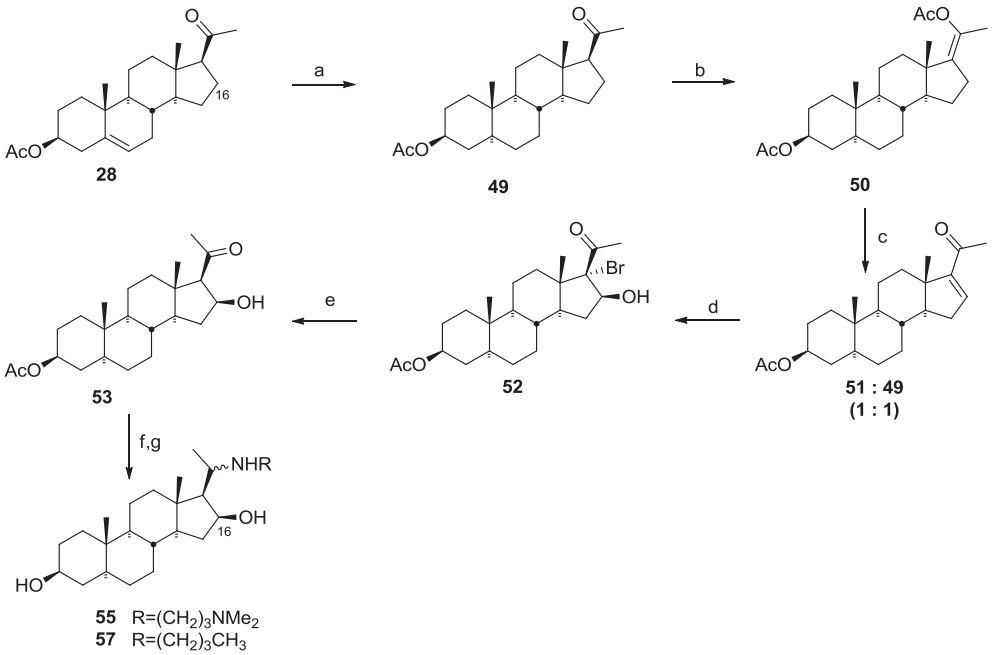


Scheme 2. Reagents and conditions: a) PtO_2, H_2 (200 PSI), AcOH rt, 54%; b) Ac_2O, pyridine, rt, 16 h, 9% **20** and 9% **24**; c) AcOH, reflux, 15 min, quant.; d) NaOMe, MeOH, 16 h, rt, 46%; e) H_2 (750 PSI), Pd/C, EtOH, HCl, 16 h, rt, 12%; f) HCl, H_2O, AcOH, rt, 1 h; g) $LiBH_4$, MeOH, THF, 16 h, rt; h) HCl, MeOH, 65 °C, 3.5 h, rt (31%, 2 steps).



Scheme 3. Reagents and conditions: a) H_2 (200 PSI), Pd/C, rt, quant.; b) NaOH, MeOH reflux, quant.; c) ^{t}BDMSCl, imidazole, DIPEA, THF, rt, 87%; d) i) KHMDS, THF, −78 °C, ii) TMSCl, rt, iii) NBS, −78 °C, 87%; e) Me_2NH (**32**) or piperidine (**33**), THF, rt, 79% f) HCl, THF, rt, 75%; g) NH_4OH, $BocNH(CH_2)_2NH_2$ (**42**), $BocNH(CH_2)_3)NH_2$ (**43**), or $BocNH(CH_2)_4NH_2$ (**44**), $NaBH_3CN$, MeOH, THF, pH 6, reflux, 40–70%.

In order to further mimic the open form of tomatidine, we synthesized analogs bearing a β-hydroxyl function in position C16, as exemplified by **55** and **57** (Scheme 4). Hydrogenation of pregnenolone acetate **28** on palladium yielded **49** in quantitative yield. Ketone **49** was converted to enol acetate **50** [45], then underwent a Wohl–Ziegler reaction to yield enone **51** along with precursor ketone **49** in a 1:1 ratio [46]. Upon reaction with *N*-bromoacetamide, the crude mixture was converted to 16-β-hydroxybromoketone **52** [47], which was then separated from **49** by flash chromatography. The bromide group of **52** was reduced with tributyltin hydride [47] to generate β-hydroxyketone **53** in 61% overall yield. Reductive amination of **53** followed by acid-mediated deprotection gave access to amines **55** and **57** with no control of the stereochemistry on the newly created stereogenic center.



Scheme 4. Reagents and conditions: a) H_2 (200PSI), Pd/C rt, quant.; b) pTSA, Ac_2O, reflux, 99%; c) NBS, CCl_4, reflux, quant.; d) *N*-bromoacetamide, H_2O, THF, 48 h, rt, 85%; e) Bu_3SnH, Et_3B, O_2, DCM, rt, 73%; f) amine, $NaBH_3CN$, MeOH, THF, pH = 6, reflux, 40–60%; g) HCl, MeOH, reflux, quant.

3. Biology

The biological evaluation of tomatidine hydrochloride **3** and newly synthesized analogs is summarized in Table 1 and expressed according to three parameters: (1) the minimal inhibitory concentration (MIC) against the normal strain of *S. aureus* ATCC29213, which represents the actual antibiotic activity of analogs against *S. aureus*; (2) the potentiation fold, which expresses the potentiating effect of analogs on the antibiotic activity of gentamicin on normal strain of *S. aureus* (gentamicin alone possesses an MIC of 0.25–0.5 μg/mL on this strain), and (3) the activity of analogs expressed as low, moderate or high (MIC ≥ 8 μg/mL, MIC = 0.5–4 μg/mL, and MIC = 0.06–0.25 μg/mL, respectively), on *S. aureus* NewbouldΔ*hemB*, a genetically derived strain which displays a stable SCV phenotype. Note that the potentiation fold (*i.e.*, the ratio of the MIC of gentamicin alone and the MIC of gentamicin in combination with tomatidine analogs) is correlated to the fractional inhibitory concentration (FIC index) [48]. To that effect, we previously showed that a potentiation fold of 4 or 8 for tomatidine is equivalent to an FIC of 0.2, which clearly demonstrates synergy with aminoglycosides [12]. We therefore interpreted a potentiation fold of 8, 4 and ≤2 as a high, moderate and low (no) synergy with gentamicin, respectively (Table 1).

4. Results and discussion

Similarly to tomatidine, most analogs possessed no antibiotic activity against the normal phenotype of *S. aureus*; as a result, these analogs alone typically possessed MIC > 64 μg/mL (Table 1). In contrast, **8**, **14** and **16** were the only analogs possessing antibiotic activity by themselves, with MIC 8–16 μg/mL.

The potentiating effect of the analogs on the inhibitory activity of gentamicin against the normal strain of *S. aureus* ATCC29213, and the antibiotic properties against the SCV, varied as a function of their structure as detailed below.

Seven compounds possessed structural variations on position 3 of ring A while keeping rings E and F of tomatidine (Scheme 1, analogs **6**–**18**). As reported in Table 1, all of these analogs kept a 4- to 8-fold synergy with gentamicin against the normal strain of *S. aureus*. The contrast between the moderate activity of tomatidine derivative **6** possessing inverted stereochemistry in C3 and the high activity of tomatidine hydrochloride **3** itself, both as aminoglycoside potentiator and SCV inhibitor, hints toward a specific interaction at this location. Whether or not this difference is steric or due to a hydrogen bond is undetermined. The strong activity of compounds **8** and **10**, both as gentamicin potentiators and against SCVs, suggests that a hydrogen bond donor effect of the 3-hydroxyl group is not important for activity. The moderate activity of compounds **12**, **14**, **16** and **18** suggests that the presence of one or two ammonium groups in position C3 reduces activity against SCVs, while being beneficial for antibiotic activity against normal strains (more specifically as in **14** and **16**). Indeed, while conserving moderate activity both against SCVs and as aminoglycoside potentiators, analogs **14** and **16** unexpectedly showed appreciable antibiotic activity on their own against the normal strain of *S. aureus*, a property that is absent in tomatidine or other analogs. Analog **8** also possesses some level of antibiotic activity by itself. These results opens the path for further optimization of tomatidine analogs as a new class of antibiotics against normal strains of *S. aureus*.

Along with analogs modified on rings E and F, we also tested dihydrosolasodine (**58**, Fig. 3), a structural isomer of tomatidine with inverted stereochemistry on the exocyclic methyl substituent on ring F and on the spiroaminoketal rings E–F junction. It is interesting to note that while tomatidine is highly active both as a potentiator and SCV inhibitor, dihydrosolasodine **58** does not possess any activity on either target. This observation reinforces the notion that specific targets are involved in the mechanism of action of tomatidine, requiring specific orientation of heteroatoms. It also suggests that the closed form of tomatidine is the active form. Indeed, the open form of rings E and F of tomatidine or solasodine would differ only by the orientation of a methyl group on a highly flexible chain, which would be unlikely to greatly influence biological activity.

Several modifications were made for the sole purpose of testing the importance of partial or full ring opening within the spiroaminoketal moiety for biological activity. While compound **19**, an analog bearing an open **E** ring, kept a moderate activity both as a potentiator on normal strains and SCVs, compounds **22** and **23**, which display open F ring, as well as fully open compound **27**, showed complete loss of both activities. Moreover, compound **4**, which bears a formyl group that both traps the aminoketal in closed configuration and masks the basic nitrogen, completely lost biological activity. Together, these observations suggest that the spiroaminoketal moiety is important in a closed configuration, and that neutralizing its H-bond donating capability is detrimental for activity. Loss of biological activity for analogs **4**, **22**, **23** and **27** may also be attributed to the loss of basicity of the amine moiety. Unfortunately, all attempts to hydrolyze the terminal acetamide in order to regenerate a basic amine at the extremity of the exocyclic chain of these analogs resulted in product decomposition. All these observations, combined with the lack of activity of dihydrosolasodine **58**, suggest that the closed form of tomatidine is the active form, and that a basic nitrogen is required for biological activity.

Compounds **32**, **33**, **35**, **42**–**44** (Scheme 3) were inactive against both the normal strain and SCVs. Analogs **46** and **48** retained noticeable potentiation activity against *S. aureus* compared to tomatidine, while they lost all or partial activity against SCVs. These results suggest that two distinct mechanisms of action might be at play against normal strains of *S. aureus* and SCVs since compound **46** possesses a 64-fold decreased activity against SCVs while retaining full gentamicin potentiation against normal strain.

Similarly to analogs **46** and **48**, compound **19**, which possesses both a 16β-hydroxyl functional group, a charged amino group in close proximity to the steroidal skeleton as well as an alkyl substituent, lost some activity against SCVs. Compounds **46** and **48** both exhibit charged an amino group in close proximity to the steroidal skeleton and alkyl substitution, but not the 16β-hydroxyl moiety. They kept moderate to high potentiation activity against normal strains of tomatidine, yet lost considerable activity on the SCV phenotype (≥64 fold). In order to test whether the 16β-hydroxyl group rescues bacteriostatic activity, we synthesized analogs **55** and **57**. However the presence of the hydroxyl group in analog **55** abrogated both potentiating and anti-SCV activity.

As a summary of SAR, we found that a hydrogen bond acceptor in position 3 plays a role in biological activity against SCVs, and β-configuration is preferred (*e.g.*, **3**, **8** vs **6**). Alkylation of this substituent it tolerated (*e.g.*, **8**). On the other hand, potentiation of gentamicin activity on normal strains seems to possess a broader SAR (*e.g.*, **14**, **16**, **18**). Second, the stereochemistry of the spiroaminoketal functional group in rings E and F appears critical for antibiotic activity against SCVs (*e.g.*, tomatidine **1** vs dihydrosolasodine **58**). The aminoglycoside potentiator activity, however, seems more affected by the presence of an amino group proximal to the steroidal skeleton than by ring opening. Alkylation of this tethered amino group seems to be preferred. Third, open rings E, F analogs and analogs bearing a distant tethered amino group remained inactive, as well as analogs bearing a 16β-hydroxyl moiety. Since chemical modifications on ring A were tolerated, but not modifications on rings E and F, the spiroaminoketal moiety

Table 1
Biological activities of tomatidine analogs against *S. aureus* of the normal and SCV phenotypes.

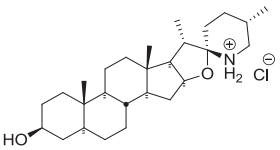
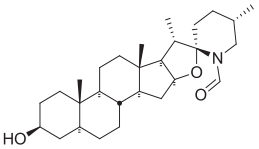
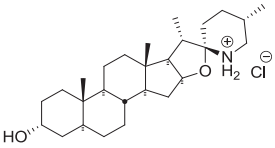
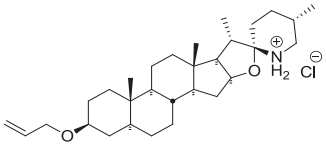
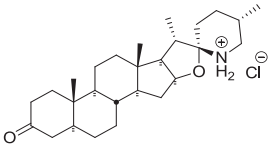
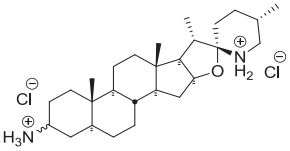
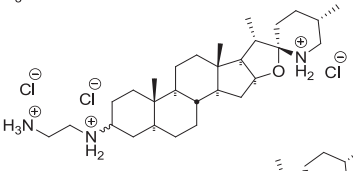
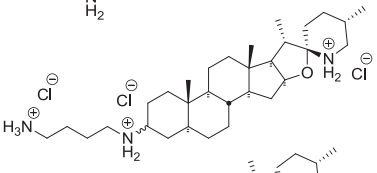
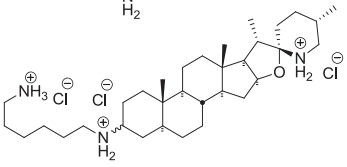
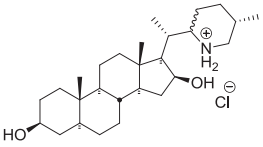
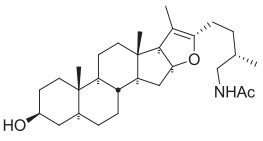
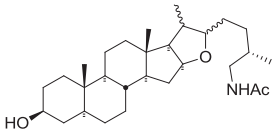
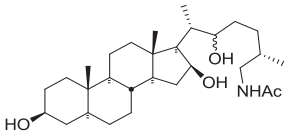
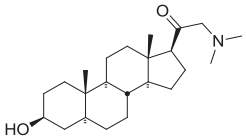
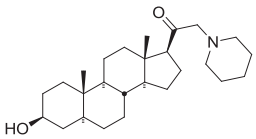
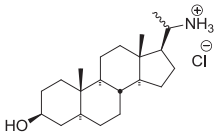
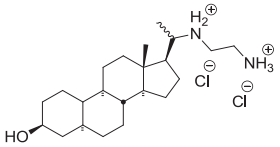
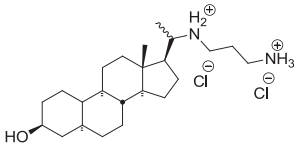
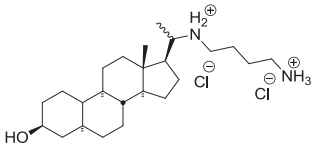
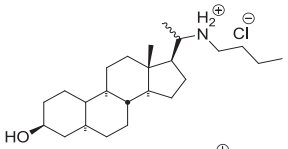
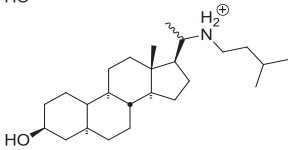
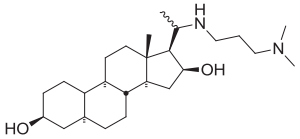
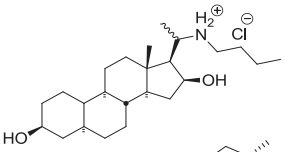
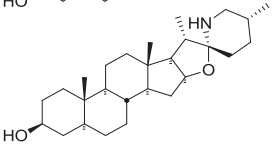
Compound		MIC vs ATCC29213 (μg/mL)	Combination MIC vs ATCC29213 (potentiation fold with gentamicin)[a]	MIC vs SCV[b] (μg/mL)
3		>64	0.06 (8×) High	0.06 High
4		>64	0.25 (2×) Low	>8 Low
6		>64	0.12 (4×) Moderate	4 Moderate
8		16	0.06 (8×) High	0.25 High
10		>64	0.06 (8×) High	0.25 High
12		32	0.06 (8×) High	1 Moderate
14		8–16 Active	0.06–0.12 (4–8×) Moderate	0.5–1 Moderate
16		16 Active	0.06–0.12 (4–8×) Moderate	0.5 Moderate
18		32	0.12–0.25 (2–4×) Moderate	1 Moderate
19		32	0.12 (4×) Moderate	4 Moderate
22		>64	0.25 (1×) Low	>8 Low

Table 1 (*continued*)

Compound		MIC vs ATCC29213 (μg/mL)	Combination MIC vs ATCC29213 (potentiation fold with gentamicin)[a]	MIC vs SCV[b] (μg/mL)
23		>64	0.25 (1×) Low	>8 Low
27		>64	0.25 (1×) Low	>8 Low
32		>64	0.25 (2×) Low	>8 Low
33		>64	0.25 (2×) Low	>8 Low
35		64	0.25 (2×) Low	>8 Low
42		32	0.25 (2×) Low	>8 Low
43		32	0.25 (2×) Low	>8 Low
44		32	0.25 (2×) Low	>8 Low
46		64	0.06 (8×) High	4–8 Moderate
48		>64	0.12 (4×) Moderate	>8 Low
55		>64	0.25 (2×) Low	>8 Low

(*continued on next page*)

Table 1 (*continued*)

Compound	MIC vs ATCC29213 (μg/mL)	Combination MIC vs ATCC29213 (potentiation fold with gentamicin)[a]	MIC vs SCV[b] (μg/mL)
57 	>64	0.25 (2×) Low	>8 Low
58 	>64	0.5 (1×) Low	>8 Low

[a] Potentiation fold of analogs is defined as the ratio of the MIC of gentamicin alone and the MIC of gentamicin in combination with the analog used at 8 μg/mL (except for compounds **14** and **16** that were used at 4 μg/mL).

[b] Activity of the analogs against the SCV strain NewbouldΔ*hemB* (MIC = ≥8 μg/mL: low activity, MIC = 0.5–4 μg/mL: moderate activity, MIC = 0.06–0.25 μg/mL: high activity).

seems to play a critical role in this activity. Finally, analogs bearing diamines in position 3 of ring A possess antibiotic activities of their own against normal strains of *S. aureus*, although whether this new activity is due to molecular interactions with a cellular target, increased cellular uptake or increased solubility remains unknown.

5. Conclusion

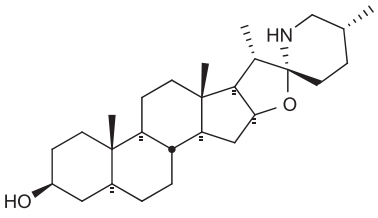
In conclusion, we report here the first SAR study of tomatidine on *S. aureus* and SCVs, both as a synergistic agent with gentamicin, and as an antibiotic on its own. Although no cellular target has been identified yet, this SAR suggests a target-based rather than a nonspecific mode of action. The segregation of biological activities (potentiation of aminoglycoside activity and SCV growth inhibition) in some analogs also suggests the existence of two mechanisms of action in *S. aureus* against the normal and SCV phenotypes. Finally, these works support the hypothesis that the closed rather than the open spiroaminoketal moiety of tomatidine is important for activity, and that the 3-hydroxyl group can be further modified to both refine activity and to confer to this series antibiotic activity against normal strains of *S. aureus*.

6. Experimental section

6.1. Biological tests

6.1.1. Bacterial strains and growth conditions

S. aureus strain ATCC29213 was used as the normal growth phenotype strain. NewbouldΔ*hemB*, in which the gene *hemB* has been disrupted by insertion of the *ermA* cassette by homologous recombination to generate a respiratory deficient strain [49], was used as a stable small colony variant (SCV) phenotype. Both strains were maintained on triptic soy agar (BD, Mississauga, ON, Canada).



Dihydrosolasodine **60**

Fig. 3. The structure of dihydrosolasodine.

6.1.2. Antibiotic susceptibility testing

MICs were determined by a broth microdilution technique in cation-adjusted Mueller-Hinton broth (BD, Mississauga, ON, Canada), following the recommendations of the Clinical and Laboratory Standards Institute (CLSI) [29], except that the incubation period was extended to 48 h (instead of 24 h) and the medium used was brain-heart infusion (BD, Mississauga, ON, Canada) to allow adequate growth of the SCV strain. Tomatidine hydrochloride **3** (Sigma, Oakville, ON, Canada), gentamicin sulfate (Sigma, Oakville, ON, Canada) and the combination of gentamicin and tomatidine were always included as comparators in all MIC tests performed for the new chemical entities. Tomatidine and all new analogs were solubilized at 2 mg/mL in DMSO. The gentamicin MIC for *S. aureus* ATCC29213 is 0.5–1 μg/mL when gentamicin is used alone and 0.03–0.12 μg/mL when gentamicin is used in combination with tomatidine at 8 μg/mL. This concentration of tomatidine is well above the minimal tomatidine concentration showing a potentiating effect with aminoglycosides in synergy studies [12]. The MIC of tomatidine used alone on *S. aureus* NewbouldΔ*hemB* is 0.03–0.12 μg/mL, whereas tomatidine has no significant activity against *S. aureus* strains of the normal growth phenotype (MIC of >32 g/L against ATCC29213) [12].

6.2. Chemistry

All moisture-sensitive reactions were performed in an inert, dry atmosphere of argon in flame-dried glassware. Air-sensitive liquids were transferred via syringe or cannula through rubber septa. Reagent grade solvents were used for extractions and flash chromatography. THF was distilled from sodium/benzophenone under argon; methanol was distilled from magnesium under argon. All other solvents purchased from commercial sources were used without further purification. Tomatidine hydrochloride was purchased from Molekula. All other reagents were generally purchased from Aldrich and used with no further purification.

Reactions were monitored by analytical thin-layer chromatography (Canadian Life Science, TLC, GLASS plates SIL 60 G-25 UV 254). The plates were visualized first with UV illumination followed by heating with ceric ammonium molybdate (CAM) [1% (w/v) ammonium cerium sulfate, 2.5% (w/v) molybdenum trioxide, 1:9H_2SO_4/H_2O]. Flash column chromatography was performed manually on SiliaFlash® P60 silica. The solvent compositions

reported for all chromatographic separations are on a volume/volume (v/v) basis.

High-pressure liquid chromatography (HPLC) was performed on an Agilent 1100 apparatus using an ACE C18 column, 250 × 21.2 mm, with 5 μm silica and 15.5% carbon load.

NMR spectra were recorded at room temperature on a Bruker Spectrospin 300 spectrometer at 300 MHz (^{1}H) and 75 MHz (^{13}C), or on a Varian Unity Inova at 600 MHz (^{1}H) and 150 MHz (^{13}C). ^{1}H NMR spectra are reported in parts per million (ppm) on the δ scale relative to the residual signals of chloroform (δ 7.26 ppm) or methanol (δ 3.31 ppm) as an internal standard with the following multiplicity – s: singlet; d: doublet; t: triplet; q: quartet; quint: quintet; m: multiplet. ^{13}C NMR spectra are reported in ppm on the δ scale relative to $CDCl_3$ (δ 77.0 ppm) or CD_3OD (δ 49.0 ppm). High-resolution mass spectrometry (HRMS) was performed on a Maxis (Bruker) Q-TOF.

*6.2.1. General procedure for synthesis of Boc-diaminoalkanes (**A**)*

Boc-alkanediamines **36**–**38** were synthesized as reported by Jensen et al. [50].

*6.2.2. General procedure for reductive amination of 3β-acetoxy-5-alpha-pregnan-20-one (**B**)*

Reductive amination with pregnanolone acetate derivatives were synthesized as reported by Xie et al. [51].

*6.2.3. Representative procedure for substitution of bromine by amino compounds (**C**)*

In a 20 mL vial, **53** was solubilized in THF (0.1 M). 2.0 eq of the corresponding amine was added, and the reaction was stirred for 1 h at room temperature. THF was removed *in vacuo*. The resulting solid was suspended in water then extracted three times with EtOAc. The combined organic layers were washed with brine, dried on anhydrous magnesium sulfate, then the solvent was removed *in vacuo*. The crude compound was purified by flash chromatography.

The compound was solubilized in a mixture of THF and 1 M HCl (4:1 solution) and stirred for 2 h while monitoring by TLC. Upon completion, a saturated solution of aqueous sodium bicarbonate was added until alkaline pH was achieved, and THF was removed *in vacuo*. The remaining aqueous layer was extracted with EtOAc (3×), and the combined organic layers were washed with brine, dried on anhydrous magnesium sulfate and concentrated under reduced pressure. The crude compound was purified by flash chromatography.

*6.2.4. General method for acidic deprotections (**D**)*

In a 25 mL round bottom flash equipped with a condenser, the starting material was solubilized in 5 mL anhydrous MeOH. To this solution was added a solution of 0.9 mL acetyl chloride in 6 mL anhydrous MeOH. The reaction heated to reflux for 1 h, monitored by TLC. The reaction was allowed to cool at room temperature, then solvent was removed *in vacuo* to yield the desired compound.

*6.2.5. N-Formyltomatidine (**4**)*

In a 250 mL round flask, tomatidine hydrochloride (200 mg, 0.44 mmol, 1.0 eq) was suspended in dry THF (20 mL) then acetic formic anhydride (380 mg, 4.42 mmol, 10.0 eq) was added, followed by DIPEA (390 μL, 2.20 mmol, 5.0 eq). The reaction was stirred for 15 min, then monitored by TLC (50% EtOAc/Hexanes, CAM stain, R_f = 0.23 for mono-formylated compound, 0.49 for diformylated compound). The volatiles were removed by evaporation under reduced pressure. The compound was then diluted in a mixture of 125 mL EtOH and 50 mL aqueous $NaHCO_3$ buffer (pH = 9.5) and stirred for one week, monitored by TLC until the complete disappearance of the diformylated compound. EtOH was then evaporated, and the resulting aqueous phase was extracted with EtOAc (3 × 25 mL). The combined organic phases were dried on anhydrous magnesium sulfate then evaporated under reduced pressure.

The crude product was purified by flash chromatography (25% EtOAc/Hexanes) to give 155 mg (79%) of the desired compound (**4**) as a white solid (m.p. 184–187 °C).

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 8.41 (s, 1H), 4.29 (d, 1H, J = 11.5 Hz), 4.13 (dd., 1H, J_1 = 7.3 Hz, J_2 = 15.5 Hz), 3.58 (quint, 1H, J = 4.7 Hz), 2.65 (t, 1H, J = 11.5), 2.54 (quint, 1H, J = 7.1 Hz), 1.98 (quint, 1H, J = 5.28 Hz) 1.87 (d, 1H, J = 13.7 Hz), 1.82–1.72 (m, 3H), 1.72–1.63 (m, 3H), 1.61–1.45 (m, 7H), 1.40 (d, 1H, J = 13.0 Hz), 1.38–1.22 (m, 8H), 1.15 (dt, 1H, J_1 = 12.3 Hz, J_2 = 3.9 Hz), 1.12–1.06 (m, 2H), 1.05 (d, 3H, J = 6.8 Hz), 0.95 (dt, 1H, J_1 = 13.7 Hz, J_2 = 3.6 Hz), 0.91 (d, 3H, J = 5.9 Hz), 0.89–0.84 (m, 1H), 0.82 (s, 6H), 0.64 (dt, 1H, J_1 = 11.39, J_2 = 3.6 Hz).

^{13}C NMR (75.5 MHz, $CDCl_3$) δ (ppm) 158.9, 97.8, 78.5, 72.1, 62.3, 55.8, 54.3, 44.8, 40.2, 38.1, 36.9, 36.8, 35.5, 35.0, 32.2, 31.4, 30.7, 29.7, 29.2, 28.5, 27.5, 21.0, 18.9, 17.0, 15.3, 12.3.

HRMS calculated for $C_{28}H_{45}O_3NNa^+$: 466.3292, found: 466.3308 (MNa^+).

*6.2.6. 3α-Acetoxy-N-formyl-tomatidine (**5**)*

In a 10 mL round bottom flask, *N*-formyltomatidine (**4**) (60 mg, 0.135 mmol, 1.0 eq) was dissolved in 3 mL of anhydrous THF, along with triphenylphosphine (71 mg, 0.27 mmol, 2.0 eq) and acetic acid (22 μL, 0.38 mmol, 2.8 eq). Diisopropylazodicarboxylate (40 μL, 0.202 mmol, 1.5 eq) was added, and the reaction was stirred at room temperature. After 4 h, an additional 20 μL diisopropylazodicarboxylate, 30 mg triphenylphosphine and 20 μL of acetic acid were added, and the reaction was stirred overnight, monitored by TLC (50% AcOEt/Hexanes, R_f: 0.5). The reaction was then concentrated under reduced pressure, suspended in water and extracted with EtOAc (3×). The combined organic fractions were washed with brine, dried on anhydrous magnesium sulfate and evaporated under reduced pressure. The crude compound was purified by flash chromatography (10% EtOAc/hexanes to 15% EtOAc/hexanes) to yield 56 mg (86%) of compound **5**. The compound was contaminated with residual diisopropylazodicarboxylate as evidenced by ^{1}H NMR, yet was used as such for the next reaction.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.43 (s, 1H), 5.00 (m, 3H, DIAD) 4.31 (d, 1H, J = 11.8 Hz), 4.99 (m, 1H), 4.16 (quad, 1H, J = 7.1 Hz), 2.68 (t, 1H, J = 12.1 Hz), 2.56 (s, 4H), 2.07 (s, 3H), 2.06–1.98 (m, 2H), 1.90 (d, 1H, J = 12.4 Hz), 1.84–1.74 (m, 3H), 1.74–1.51 (m, 5H), 1.48 (s, 4H), 1.42–1.20 (m, 25H, DIAD), 1.14 (d, 2H, J = 4.7 Hz), 1.08 (d, 3H, J = 7.1 Hz), 0.93 (d, 4H, J = 6.0 Hz), 0.85 (s, 3H), 0.83 (s, 3H), 0.81–0.73 (dt, 1H, J_1 = 11.5 Hz, J_2 = 3.3 Hz).

*6.2.7. 3α-Hydroxytomatidine hydrochloride (**6**)*

In a 25 mL round flask, 28 mg **5** (0.058 mmol) was refluxed for 3 h in 6 mL EtOH and 3 mL aqueous HCl 2.5 N (TLC: 10% MeOH/AcOET with 0.5% NEt_3, R_f: 0.5). Upon completion, the volatiles were removed under reduced pressure and the remaining water was removed by lyophilization.

^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 4.40 (quad, 1H, J = 8.8 Hz), 3.98 (s large, 1H), 3.68 (m, 2H), 3.14 (m, 1H), 2.94 (t, 1H, J = 11.0 Hz), 2.23 (m, 1H), 2.13–1.99 (m, 3H), 1.91 (m, 3H), 1.80–1.45 (m, 10H), 1.40–1.15 (m, 17H), 1.12 (d, 3H, J = 6.6 Hz), 1.01 (d, 3H, J = 6.0 Hz), 0.93 (s, 3H), 0.86 (s, 4H).

^{13}C NMR (75.5 MHz, CD_3OD) δ (ppm) 96.2, 81.1, 65.7, 61.7, 55.6, 54.3, 40.8, 40.7, 39.6, 38.8, 35.8, 35.2, 34.9, 32.0, 31.9, 31.4, 28.2, 28.1, 25.8, 25.3, 20.8, 20.3, 17.3, 16.0, 13.5, 10.2.

HRMS calculated for $C_{27}H_{46}O_2N^+$: 416.3523, found: 416.3534 (MH^+), 438.3350 (MNa^+).

*6.2.8. 3β-Allyloxy-N-formyltomatidine (**7**)*

In a 3 mL round bottom flask equipped with a condenser and placed under argon atmosphere, compound **4** (30 mg, 0.068 mmol) was dissolved in 1 mL THF. Tris(dibenzylideneacetone) dipalladium(0) (3 mg, 0.003 mmol, 0.05 eq), 1,3-bis(diphenylphosphino) propane (5 mg, 0.012 mmol, 0.18 eq) and allyl methyl carbonate (0.2 mL, 1.76 mol, 26 eq) were then added successively. The reaction was brought to 65 °C for 6 h, and monitored by TLC (50% EtOAc/hexanes, UV/CAM, R_f: 0.20 (starting material), 0.50 (desired compound)). Upon completion, the reaction was allowed to cool down to room temperature, then the solvent was removed *in vacuo*. The crude compound was purified by flash chromatography (20% EtOAc/Hexanes) to yield 25 mg (76%) of the desired compound.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.41 (s, 1H), 5.93 (ddt, 1H, J_1 = 17.3 Hz, J_2 = 10.5 Hz, J_3 = 5.7 Hz), 5.34 (dq, 1H, J_1 = 17.1 Hz, J_2 = 1.4 Hz), 1.25 (dq, 1H, J_1 = 10.3 Hz, J_2 = 1.3 Hz), 4.60 (dt, 2H, J_1 = 5.8 Hz, J_2 = 1.4 Hz), 4.53 (quint, 1H, J = 5.5 Hz), 4.29 (d, 1H, J = 11.9 Hz), 4.16–4.08 (m, 1H), 2.65 (t, 1H, J = 11.3 Hz), 2.54 (t, 1H, J = 7.0 Hz), 2.01–1.94 (m, 1H), 1.94–1.84 (m, 2H), 1.81–1.68 (m, 4H), 1.65 (s, 2H), 1.62–1.47 (m, 6H), 1.46–1.35 (m, 2H) 1.35–1.28 (m, 41H), 1.22–1.08 (m, 2H), 1.05 (d, 3H, J = 7.0 Hz), 1.02–0.92 (m, 1H), 0.90 (d, 4H, J = 5.8 Hz), 0.83 (s, 3H), 0.82 (s, 1H), 0.65 (dt, 1H, J_1 = 10.7 Hz, J_2 = 4.3 Hz).

*6.2.9. 3β-Allyloxytomatidine hydrochloride (**8**)*

Following procedure **D**, **7** (16 mg, 0.033 mmol), was converted into 16.2 mg (quantitative yield) of the desired compound (R_f: 0.51 in 50/50 AcOEt/Hexanes).

^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 5.98–5.83 (m, 1H), 5.28 (d, 1H, J = 17.1 Hz), 5.19 (d, 1H, J = 10.5 Hz), 4.53 (d, 2H, J = 5.5 Hz), 4.48 (m, 1H), 4.36 (m, 1H), 3.68 (m, 1H), 3.15–3.04 (m, 1H), 2.88 (t, 1H, J = 11.87 Hz), 2.32–2.11 (m, 1H), 2.07–1.92 (m, 2H), 1.90–1.41 (m, 10H), 1.40–1.12 (m, 11H), 1.08 (d, 4H, J = 6.2 Hz), 0.95 (d, 4H, J = 6.5 Hz) 0.87 (s, 3H), 0.86–0.79 (m, 4H), 0.75–0.57 (m, 2H).

^{13}C NMR (75.5 MHz, CD_3OD) δ (ppm) 154.6, 117.1, 96.1, 81.1, 77.3, 67.6, 61.7, 61.7, 55.4, 54.0, 44.4, 40.8, 40.7, 39.6, 36.3, 35.2, 34.9, 33.6, 31.9, 31.4, 28.2, 28.1, 27.0, 25.7, 25.3, 20.7, 17.2, 15.9, 13.2, 11.1.

HRMS calculated for $C_{30}H_{50}O_2N^+ \cdot H_2O$: 474.3942, found: 474.3590 ($MH^+ + H_2O$).

*6.2.10. N-Formyl-3-oxotomatidine (**9**)*

In a 10 mL round bottom flask, *N*-formyltomatidine **4** (50 mg, 0.113 mmol, 1.0 eq) and Dess–Martin periodinane (95 mg, 0.225 mmol, 2.0 eq) were stirred in 6.5 mL DCM. The reaction was monitored by TLC (50% AcOEt/Hexanes, R_f: 0.24). Upon completion, the reaction was quenched for 30 min with $Na_2S_2O_3$ (0.2 M), then extracted 3× with EtOAc. The combined organic phases were washed with brine, dried on anhydrous magnesium sulfate then evaporated under reduced pressure. The crude compound was purified by flash chromatography (50% EtOAc/Hexanes) to yield 34 mg (68%) of the desired compound.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.46 ppm (s, 1H), 4.32 (d, 1H, J = 12.8 Hz), 4.17 (quad, 1H, J = 8.9 Hz), 2.69 (t, 1H, J = 12.6 Hz), 2.58 (quint, 1H, J = 6.6 Hz), 2.52–2.24 (m, 3H), 2.16–2.11 (m, 1H), 2.11–1.98 (m, 3H), 1.91 (d, 1H, J = 14.0 Hz), 1.86–1.68 (m, 4H), 1.69–1.42 (m, 7H), 1.40–1.20 (m, 7H), 1.19–1.12 (m, 2H), 1.09 (d, 3H. J = 7.1 Hz), 1.05 (s, 2H), 0.94 (d, 4H, J = 5.5 Hz), 0.88 (s, 3H), 0.77 (dt, 1H, J_1 = 12.6 Hz, J_2 = 4.8 Hz).

*6.2.11. 3-Oxotomatidine hydrochloride (**10**)*

In a 25 mL round flask, 34 mg **9** (0.077 mmol) was refluxed for 2 h in 10 mL EtOH and 5 mL aqueous HCl 2.5 N (TLC: 10% MeOH/AcOEt with 0.5% NEt_3, R_f: 0.54). Upon completion, the ethanol and HCl were removed under reduced pressure and the remaining water was removed by lyophilisation to give a white solid (m.p. 160–163 °C).

^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 4.60 (m, 2H), 4.37 (q, 1H, J = 9.0 Hz), 2.80 (m, 1H), 2.70 (t, 1H, J = 12.0 Hz), 2.55–2.30 (m, 1H), 2.24–2.14 (m, 1H), 2.08–1.94 (m, 3H), 1.91–1.68 (m, 5H), 1.63–1.50 (m, 6H), 1.49–1.12 (m, 12H), 1.10 (s, 3H), 1.06 (d, 3H, J = 7.1 Hz), 0.96 (d, 3H, J = 5.5 Hz), 0.89 (s, 2H), 0.82 (s, 1H), 0.76–0.66 (m, 2H).

^{13}C NMR (75.5 MHz, CD_3OD) δ (ppm) 213.5, 100.2, 98.0, 79.2, 62.0, 55.3, 53.7, 44.0, 42.2, 41.8, 40.7, 39.7, 38.3, 35.5, 34.8, 31.9, 31.7, 28.5, 28.0, 27.4, 20.9, 18.1, 16.0, 14.3, 10.3.

HRMS calculated for $C_{27}H_{44}O_2N^+$: 414.3372, found: 414.3376 (MH^+), 436.3192 (MNa^+).

*6.2.12. N-Formyl-3-aminotomatidine (**11**)*

In a 25 mL round flask, 44 mg **4** (0.1 mmol) was dissolved in methanol (6 mL) along with ammonium acetate (77 mg, 1.0 mmol, 10 eq). The pH was adjusted to 6 with acetic acid, sodium cyanoborohydride (6.9 mg, 0.11 mmol, 1.1 eq) was added and the reaction was refluxed overnight until complete as monitored by TLC (10% MeOH/AcOEt with 0.5% NEt_3, R_f: 0). Solvents were removed under reduced pressure, and the solid was suspended in water. The pH was adjusted to 8 with saturated aqueous $NaHCO_3$. The mixture was extracted with 3× EtOAc, and the combined organic fractions were washed with brine, dried on anhydrous magnesium sulfate and evaporated under reduced pressure. The crude compound was purified by flash chromatography (10% MeOH/89%EtOAc/1% NEt_3) to yield 21 mg (48%) of the desired compound.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.40 (s, 1H), 5.91 (broad s, 2H), 4.28 (d, 1H, J = 11.5 Hz), 4.12 (m., 1H), 2.86 (quint, 1H, J = 7.1 Hz), 2.65 (t, 1H, J = 11.5), 2.53 (quint, 1H, J = 7.1 Hz), 2.04–1.92 (m, 3H) 1.90–1.82 (m, 1H), 1.80–1.42 (m, 11H), 1.38–1.18 (m, 9H) 1.17–1.06 (m, 2H), 1.04 (d, 3H, J = 7.1 Hz), 0.90 (d, 4H, J = 6.2 Hz), 0.81 (s, 6H), 0.69–0.57 (m, 1H).

*6.2.13. 3-Aminotomatidine hydrochloride (**12**)*

Following procedure **D**, 21 mg (0.047 mmol) of compound **11** were converted to 23 mg (100%) of compound **12** (R_f: 0 in 10% MeOH/AcOEt with 0.5% NEt_3).

^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 4.37 (q, 1H, J = 7.1 Hz), 3.16–3.00 (m, 2H), 2.88 (t, 1H, J = 11.1 Hz), 2.20 (t, 1H, J = 7.1 Hz), 2.09–1.94 (m, 2H), 1.91–1.63 (m, 10H), 1.62–1.46 (m, 6H), 1.44–1.15 (m, 12H), 1.09 (d, 3H, J = 7.2 Hz), 1.06–0.98 (m, 3H), 0.95 (d, 3H, J = 6.8 Hz), 0.90 (s, 3H), 0.87 (s, 5H), 0.80–0.60 (m, 3H).

^{13}C NMR (75.5 MHz, CD_3OD) δ (ppm) 96.2, 81.1, 61.7, 55.4, 53.8, 50.3, 44.6, 40.8, 39.5, 36.2, 35.2, 34.8, 32.5, 31.7, 31.4, 29.4, 28.2, 28.0, 26.1, 25.8, 25.3, 20.6, 17.3, 15.9, 13.3, 11.1.

HRMS calculated for $C_{27}H_{47}ON_2^+$: 415.3683, found: 415.3695.

*6.2.14. N-Formyl-3-(N-Boc-aminoethyl)aminotomatidine (**13**)*

Following the procedure used for synthesis of **11**, 110 mg **6** (0.25 mmol), **36** (200 mg, 1.25 mmol, 5 eq) and sodium cyanoborohydride (17 mg, 0.27 mmol, 1.1 eq) were used to yield 130 mg (85%) of compound **13** (R_f: 0.05 in 10% MeOH/AcOEt with 0.5% NEt_3). ^{1}H NMR shows the presence of residual starting diamine, which was removed in the next step.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.39 (s, 1H), 5.02 (s large, 1H), 4.28 (d, 1H, J = 11.5 Hz), 4.12 (m, 1H), 3.24–3.15 (m, 2H), 2.77–2.69 (m, 2H), 2.64 (m, 1H) 2.54 (m, 1H), 2.44 (m, 1H), 2.04–1.46 (m, 18H) 1.43 (s, 12H), 1.33–1.15 (m, 14H), 1.04 (d, 3H, J = 6.9 Hz), 0.90 (d, 3H, J = 5.7 Hz), 0.87–0.82 (m, 2H), 0.81 (s, 3H), 0.78 (s, 3H), 0.69–0.57 (m, 1H).

*6.2.15. 3-(N-Aminoethyl)-aminotomatidine hydrochloride (**14**)*

Following procedure **D**, 130 mg of compound **13** were deprotected in quantitative yield (R_f: 0 in 10% MeOH/AcOEt with 0.5%

NEt_3). The crude compound was purified preparative HPLC to yield 48 mg (38%) of desired compound. The HPLC elution parameters were as follow (percentage of acetonitrile in water): 0 min, 20%; 2 min, 20%; 27 min, 95%; 32 min, 95%.

^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 4.36 (m, 1H), 3.18–3.03 (m, 2H), 2.89 (t, 1H, J = 10.7 Hz) 2.20 (m, 1H), 2.08–1.90 (m, 3H), 1.80–1.50 (m, 18H), 1.45–1.14 (m, 12H) 1.09 (d, 3H, J = 7.1 Hz), 0.69 (d, 3H, J = 6.7 Hz), 0.89 (s, 3H), 0.85 (s, 4H), 0.83–0.66 (m, 2H).

^{13}C NMR (75.5 MHz, CD_3OD) δ (ppm) 96.1, 81.1, 61.6, 57.6, 56.3, 55.4, 53.7, 44.5, 41.1, 40.8, 40.7, 39.5, 38.6, 35.7, 35.3, 34.8, 31.7, 31.4, 30.7, 28.9, 28.0, 25.8, 25.3, 24.4, 20.6, 17.2, 15.9, 11.1, 10.4.

HRMS calculated for $C_{29}H_{52}ON_3^+$: 458.4105, found: 458.4105 (MH^+).

6.2.16. N-Formyl-3-(N-Boc-aminobutyl)aminotomatidine (**15**)

Following the procedure used for synthesis of **11**, 27 mg **6** (0.060 mmol), **38** (57 mg, 0.30 mmol, 5 eq) and sodium cyanoborohydride (4.1 mg, 0.066 mmol, 1.1 eq) were used to yield 16 mg (44%) of compound **15** (R_f: 0.07 in 10% MeOH/AcOEt with 0.5% NEt_3).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.40 (s, 1H), 5.02 (broad d, 1H), 4.28 (d, 1H, J = 11.5 Hz), 4.12 (m, 1H), 3.14–3.04 (s large, 3H), 2.80–2.50 (m, 5H), 2.42 (m, 1H), 2.04–1.93 (m, 2H), 1.86 (d, 1H, J = 12.8 Hz), 1.79–1.62 (m, 5H), 1.60–1.46 (m, 12H), 1.42 (s, 12H), 1.35–1.10 (m, 10H), 1.04 (d, 5H, J = 6.9 Hz), 0.90 (d, 4H, J = 5.8 Hz), 0.81 (s, 3H), 0.78 (s, 3H), 0.69–0.57 (m, 1H).

6.2.17. 3-(N-Aminobutyl)-aminotomatidine hydrochloride (**16**)

Following procedure **D**, 16 mg of compound **15** were deprotected in quantitative yield (R_f: 0 in 10% MeOH/AcOEt with traces if NEt_3). The crude compound was purified by preparative HPLC to yield 15 mg (100%) of desired compound. The HPLC elution parameters were as follow (percentage of acetonitrile in water): 0 min, 5%; 2 min, 5%; 27 min, 95%; 32 min, 95%.

^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 4.37 (q, 1H, J = 4.8 Hz), 3.38 (broad s, 1H), 3.18–3.15–2.87 (m, 7H), 2.20 (t, 1H, J = 6.5 Hz), 2.08–1.82 (m, 6H) 1.82–1.63 (m, 11H), 1.61–1.50 (m, 5H), 1.44–1.14 (m, 12H), 1.08 (d, 4H, J = 7.4 Hz), 0.96 (d, 3H, J = 6.5 Hz), 0.89 (s, 3H), 0.87 (s, 4H), 0.79–0.69 (m, 1H).

^{13}C NMR (75.5 MHz, CD_3OD) δ (ppm) 96.5, 80.7, 61.8, 57.1, 55.4, 54.9, 53.8, 45.2, 44.6, 43.6, 41.0, 39.6, 38.6, 36.2, 35.3, 34.8, 31.7, 31.5, 30.8, 29.1, 28.5, 28.0, 26.1, 25.4, 24.5, 24.3, 22.7, 22.3, 20.6, 17.4, 15.9, 13.4, 11.0.

HRMS calculated for $C_{31}H_{57}ON_3^{2+}$: 243.7245, found: 243.7253.

6.2.18. N-Formyl-3-(aminohexyl)aminotomatidine (**17**)

Following the procedure used for the synthesis of **11**, 20 mg **6** (0.045 mmol), hexamethylenediamine (30 μL, 0.225 mmol, 5 eq) and sodium cyanoborohydride (3.2 mg, 0.050 mmol, 1.1 eq) were used to yield 11 mg (46%) of compound **17** (R_f: 0 in 10% MeOH/AcOEt with 0.5% NEt_3).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 4.32–4.25 (m, 1H), 4.18–4.08 (m, 1H), 3.08 (broad s, 3H), 2.74–2.49 (m, 5H), 2.03–1.93 (m, 2H) 1.91–1.81 (m, 1H), 1.80–1.70 (m, 2H), 1.70–1.41 (m, 11H), 1.39–1.09 (m, 14H), 1.05 (d, 3H, J = 7.0 Hz), 0.90 (d, 3H, J = 5.4 Hz), 0.87–0.82 (m, 2H), 0.82 (s, 3H), 0.80 (s, 3H), 0.69–0.59 (m, 1H).

6.2.19. 3-(N-Aminohexyl)-aminotomatidine hydrochloride (**18**)

Following procedure **D**, 11 mg of compound **17** were deprotected in quantitative yield (R_f: 0 in 10% MeOH/AcOEt with 0.5% NEt_3). The crude compound was purified by preparative HPLC to yield 12.5 mg (100%) of compound **18**. The HPLC elution parameters were as follow (percentage of acetonitrile in water): 0 min, 20%; 2 min, 20%; 27 min, 95%; 32 min, 95%.

^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 4.37 (m, 1H), 3.39 (m, 1H), 3.16–3.05 (m, 1H) 3.04–2.82 (m, 6H), 2.20 (t, 1H, J = 6.7 Hz), 2.08–1.95 (m, 3H), 1.90–1.82 (m, 3H), 1.80–1.52 (m, 15H), 1.50–1.16 (m, 18H), 1.09 (d, 3H, J = 6.4 Hz), 1.06–0.99 (m, 3H), 0.96 (d, 3H, J = 6.8 Hz), 0.90 (s, 3H), 0.88 (s, 4H), 0.75–0.60 (m, 1H).

^{13}C NMR (75.5 MHz, CD_3OD) δ (ppm) 96.1, 81.0, 61.6, 57.0, 55.5, 53.8, 53.5, 44.5, 44.2, 40.8, 39.5, 38.8, 35.6, 34.7, 31.5, 31.3, 31.2, 30.8, 29.0, 28.2, 27.6, 26.9, 25.9, 25.5, 25.4, 22.2, 20.2, 17.2, 15.8, 13.1, 10.3.

HRMS calculated for $C_{33}H_{61}ON_3^{++}$: 257.7402, found: 257.7406.

6.2.20. (25S)-26-Amino-3β,16β-dihydroxy-5a-cholestano-22,26-piperidine (**19**)

Tomatidine hydrochloride (50 mg, 0.11 mmol) was dissolved in 20 mL glacial acetic acid. Platinum oxide (50 mg) was added and the reaction was stirred under hydrogen atmosphere at 200 PSI overnight. Reaction mixture was then filtered on Celite® and concentrated *in vacuo* (R_f: 0.67 in 10% MeOH/AcOEt with 0.5% NEt_3). The crude compound was purified by flash chromatography (50% EtOAc/Hexanes, with 25% AcOEt step increase to 100% AcOEt, then 5% MeOH step increase to 90% EtOAc/MeOH) to yield 27 mg (54%) of compound **19** as a white solid (m.p. 188 °C with decomposition).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 4.22–4.14 (m, 1H), 3.52–3.42 (m, 1H), 3.38 (d, 1H, J = 12.8 Hz), 3.14 (m, 1H), 2.65 (t, 1H, J = 12.8 Hz), 2.34–2.11 (m, 3H), 2.00–1.87 (m, 7H), 1.75–1.61 (m, 5H), 1.56–1.41 (m, 5H) 1.40–1.05 (m, 16H), 1.01 (d, 3H, J = 7.2 Hz), 0.96 (d, 4H, J = 6.7 Hz), 0.90 (broad s, 5H) 0.82 (s, 3H), 0.70–0.59 (m, 1H).

^{13}C NMR (75.5 MHz, $CDCl_3$) δ (ppm) 70.3, 70.1, 59.6, 57.0, 54.2, 53.9, 51.0, 44.7, 42.4, 39.9, 37.4, 36.7, 35.1, 32.7, 31.7, 31.1, 30.6, 28.9, 28.4, 22.3, 20.6, 17.3, 17.3, 12.0, 11.6, 11.3.

HRMS calculated for $C_{27}H_{48}O_2N^+$: 418.3680, found: 418.3677 (MH^+).

6.2.21. 3α-Acetoxy-N-acetyltomatidine (**20**)

Compound **20** was synthesized as reported by to Sato et al. [37]. The compound was purified by flash chromatography (25% AcOEt/Hexanes) (R_f: 0.6 in 50% AcOEt/Hexanes).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 4.67 (m, 1H), 4.15 (m, 1H), 4.45–3.26 (m, 2H), 3.14–3.04 (m, 1H), 2.09 (s, 3H), 2.01 (s, 3H) 2.00–1.93 (m, 1H) 1.84–1.55 (m, 8H), 1.52–1.40 (m, 4H), 1.37–1.23 (m, 5H), 1.19 (d, 3H, J = 6.7 Hz), 1.10–0.94 (m, 4H), 0.86 (d, 3H, J = 6.0 Hz), 0.82 (s, 3H), 0.81 (s, 3H), 0.68–0.57 (m, 1H).

6.2.22. (25S)-3β-Acetoxy-27-acetylamino-5a-furost-20(22)-ene (**21**)

Compound **21** was synthesized as reported by to Sato et al. [37] (R_f: 0.45 in 10% MeOH/AcOEt).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 5.58 (broad s, 1H), 4.75–4.60 (m, 2H), 3.12 (t., 2H, J = 6.4 Hz), 2.45 (d, 1H, J = 10 Hz), 2.19–2.05 (m, 3H), 2.01 (s, 4H), 1.97 (s, 4H), 1.86–1.60 (m, 8H) 1.56 (s, 3H), 1.55–1.44 (m, 5H), 1.42–1.11 (m, 12H), 1.07–0.94 (m, 4H), 0.90 (d, 3H, J = 6.8 Hz), 0.83 (s, 4H), 0.71–0.66 (m, 1H), 0.65 (s, 3H).

6.2.23. (25S)-26-Acetylamino-3β-hydroxy-5a-furost-20(22)-ene (**22**)

In a 10 mL round flask, compound **21** (10 mg, 0.02 mmol, 1.0 eq) was dissolved in 3 mL anhydrous methanol along with sodium methoxide (1.4 mg, 0.024 mmol, 1.2 eq). The reaction was stirred at room temperature overnight until completion as judged by TLC (R_f: 0.28 in 10% MeOH/AcOEt). Solvents were removed *in vacuo*, and the crude compound was purified by flash chromatography (50% EtOAc/Hexanes with triethylamine with 25% step increase of AcOEt to 100% EtOAc with 0.5% triethylamine) to yield 4.3 mg (46%) of compound **22**.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 5.56 (broad s, 1H), 4.76–4.67 (m, 1H), 3.59 (sept, 1H, $J = 4.8$ Hz), 3.12–3.09 (t, 2H, $J = 5.9$ Hz), 2.45 (d, 1H, $J = 10.8$ Hz), 2.20–2.06 (m, 3H), 1.98 (s, 3H), 1.84–1.63 (m, 4H) 1.56 (s, 5H), 1.54–1.43 (m, 4H), 1.42–1.33 (m, 3H), 1.32–1.21 (m, 5H) 1.15–0.94 (m, 4H), 0.90 (d, 3H, $J = 6.8$ Hz), 0.82 (s, 4H), 0.65 (s, 4H).

^{13}C NMR (75.5 MHz, $CDCl_3$) δ (ppm) 170.0, 151.3, 104.1, 84.4, 71.3, 64.3, 54.8, 54.3, 45.3, 44.8, 43.6, 39.7, 38.2, 37.0, 35.6, 34.9, 34.0, 32.8, 32.4, 31.6, 31.5, 28.6, 23.4, 23.1, 21.2, 17.5, 14.2, 12.4, 11.6.

HRMS calculated for $C_{29}H_{47}O_3NNa^+$: 480.3448, found: 480.3457.

*6.2.24. (25S)-26-Acetylamino-3β-hydroxy-5a-furostane (**23**)*

Compound **22** (40 mg, 0.089 mmol, 1.0 eq) was dissolved in 25 mL ethanol (95%) along with 0.5 mL HCl and 10 mg palladium on activated charcoal (10% w/w). The reaction was stirred under hydrogen atmosphere (750 PSI) at room temperature overnight. TLC showed the desired compound in small proportions (R_f: 0.43 in 10% MeOH/AcOEt). The reaction mixture was filtered on Celite®, concentrated *in vacuo*, and the crude compound was purified by flash chromatography (100% EtOAc) to yield 5 mg (12%) of compound **23**.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 5.59 (broad s, 1H), 4.21–4.14 (m, 1H), 3.88 (dt, 1H, $J_1 = 10.0$ Hz, $J_2 = 3.8$ Hz), 3.58 (sept, 1H, $J = 5.5$ Hz), 3.23–3.03 (m, 2H), 2.75–2.61 (m, 1H), 2.04–1.99 (m, 1H), 1.97 (s, 3H) 1.95–1.75 (m, 3H), 1.74–1.62 (m, 3H), 1.56–1.32 (m, 7H), 1.31–1.22 (m, 6H), 1.16–1.11 (m, 2H) 1.08 (d, 3H, $J = 7.8$ Hz), 0.98 (s, 3H), 0.91 (d, 3H, $J = 6.4$ Hz), 0.81 (s, 3H), 0.65–0.54 (m, 1H).

^{13}C NMR (75.5 MHz, $CDCl_3$) δ (ppm) 83.3, 83.1, 71.3, 61.2, 58.0, 54.3, 45.4, 44.9, 40.2, 38.2, 37.5, 37.0, 34.9, 33.4, 32.3, 32.1, 32.0, 31.5, 28.6, 28.2, 20.7, 17.8, 16.9, 12.4, 11.3.

HRMS calculated for $C_{29}H_{50}O_3N^+$: 460.3785, found: 460.3801.

*6.2.25. (25S)-26-Amino-3β,16β-acetoxy-N-acetyl-5a-cholest-22-eno-22,26-piperidine (**24**)*

Compound **24** was synthesized as reported by Sato et al. [37]. The compound was purified by flash chromatography (25% AcOEt/Hexanes) (R_f: 0.45 in 50% AcOEt/Hexanes).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 5.19 (t, 1H, $J = 3.6$ Hz), 4.94 (s large, 1H), 4.66 (sept, 1H, $J_1 = 4.8$ Hz), 2.36–2.17 (m, 2H), 2.10 (s, 3H), 2.01 (s, 3H), 1.96 (s, 3H) 1.84–1.32 (m, 12H), 1.27 (d, 3H, $J = 7.2$ Hz), 1.24–1.12 (m, 5H), 1.05–0.94 (m, 3H), 0.92 (d, 3H, $J = 6.8$ Hz), 0.82 (s, 3H), 0.80 (s, 3H), 0.64 (dt, 1H, $J_1 = 10.5$ Hz, $J_2 = 3.8$ Hz).

*6.2.26. (25S)-26-Acetylamino-3β,16β-diacetoxy-5a-cholest-22-one (**25**)*

Compound **25** was synthesized as reported by Sato et al. [37] (R_f: 0.5 in 10% MeOH/AcOEt).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 5.91 (t, 1H, $J = 5.4$ Hz), 4.97 (dt, 1H, $J_1 = 8.2$ Hz, $J_2 = 3.9$ Hz), 4.67 (sept, 1H, $J = 5.3$ Hz), 3.15–3.01 (m, 2H), 2.96–2.86 (m, 1H), 2.75–2.63 (m, 1H), 2.40–2.22 (m, 2H), 2.01 (s, 3H), 1.99 (s, 3H), 1.94 (s, 3H), 1.82–1.76 (m, 3H), 1.70 (dt, 1H, $J_1 = 13.4$ Hz, $J_2 = 4.2$ Hz), 1.65–1.48 (m, 6H), 1.47–1.31 (m, 4H), 1.29–1.17 (m, 5H), 1.12 (d, 1H, $J = 7.0$ Hz), 1.07–0.95 (m, 4H), 0.88 (d, 3H, $J = 6.5$ Hz), 0.83 (s, 3H), 0.83 (s, 3H), 0.73–0.62 (m, 1H).

*6.2.27. (25S)-26-Acetylamino-3β,16β-diacetoxy-22-hydroxy-5a-cholestane (**26**)*

In a 2 mL vial, **25** (16 mg, 0.028 mmol, 1.0 eq) was dissolved in 0.3 mL of a solution of THF and MeOH (30 mL THF and 0.125 mL MeOH, for 1.1 eq of MeOH in reaction). The reaction was cooled down to 0 °C then $LiBH_4$ (0.7 mg, 0.031 mmol, 1.1 eq) was added. After 1 h, 1 mg of $LiBH_4$ was added and the reaction was allowed to stir overnight at room temperature (R_f: 0.32 in 10% MeOH/AcOEt). The reaction was then quenched with acetone for 30 min, then with water for 15 min. Solvents were removed *in vacuo* and the crude product was suspended in aqueous $NaHCO_3$ then extracted 3× with EtOAc. The organic phases were dried on anhydrous magnesium sulfate, concentrated *in vacuo*, and the crude product was purified on flash chromatography (100% EtOAc) to yield 5 mg (31%) of compound **26**.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 5.60 (s large, 1H), 5.09–5.01 (m, 1H), 4.68 (sept, 1H, $J = 5.4$ Hz), 3.4 (d, 1H, $J_1 = 8.5$ Hz), 3.28–3.05 (m, 2H), 2.41–2.30 (m, 1H), 2.23–2.06 (m, 2H), 2.03 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.97–1.32 (m, 18H) 1.30–0.99 (m, 14H), 0.95 (d, 4H, $J = 6.5$ Hz), 0.92 (d, 5H, $J = 6.6$ Hz), 0.87 (s, 3H), 0.82 (s, 4H), 0.71–0.61 (m, 1H).

*6.2.28. (25S)-26-Acetylamino-3β,16β,20-trihydroxy-5a-cholestane (**27**)*

Following procedure **D**, **26** (5 mg, 0.008 mmol), was converted into 4.2 mg (quantitative yield) of compound **27** (R_f: 0.45 in 10% MeOH/AcOEt).

^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 4.49 (q, 1H, $J = 6.4$ Hz), 4.05–4.00 (m, 1H), 3.53–3.47 (m, 1H), 3.13–2.96 (m, 2H), 2.31–2.13 (m, 3H), 2.02–1.96 (m, 2H), 1.93 (s, 2H), 1.90 (s, 1H), 1.80–1.48 (m, 11H), 1.44–1.36 (m, 6H), 1.34–1.20 (m, 16H), 1.17–1.10 (m, 5H), 1.02–0.97 (m, 2H), 0.94–0.88 (m, 8H), 0.85 (s, 3H), 0.84 (m, 3H), 0.72–0.65 (m, 1H).

^{13}C NMR δ (ppm) 85.3, 82.3, 71.2, 65.2, 56.0, 55.2, 45.9, 45.6, 40.2, 38.3, 37.4, 35.4, 33.8, 33.6, 32.8, 32.5, 31.7, 31.5, 31.2, 30.1, 29.8, 29.5, 27.8, 26.7, 21.9, 21.5, 20.3, 17.3, 15.8, 14.7, 12.1.

HRMS calculated for $C_{29}H_{52}O_4NNa^+$: 500.3716, found: 500.3714.

*6.2.29. 3β-Hydroxy-5α-pregnan-20-one (**29**)*

In a 500 mL round bottom flask, 3β-acetoxy-5α-pregnan-20-one (**49**) (5 g, 13.9 mmol) was dissolved in 400 mL of MeOH, 150 mL H_2O and 30 mL NaOH 1 M. The mixture was refluxed for 3 h (R_f: 0.27 in 50% AcOEt/hexanes). The organic solvents were removed under reduced pressure and a suspension could be observed in the remaining aqueous phase. The solid was isolated by filtration, rinsed with cold water and dried overnight at room temperature to yield 4.34 g (98%) of desired compound.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 3.60 (m, 1H, $J = 4.5$ Hz), 2.52 (t, 1H, $J = 8.8$ Hz), 2.22–2.12 (m, 1H), 2.11 (s, 1H), 2.04–1.96 (m, 1H), 1.86–1.76 (m, 1H), 1.75–1.52 (m, 7H), 1.49–1.06 (m, 12H), 1.04–0.84 (m, 3H), 0.81 (s, 3H), 0.68 (dt, 1H, $J_1 = 10.4$ Hz, $J_2 = 3.8$ Hz), 0.60 (s, 3H).

^{13}C NMR (75.5 MHz, $CDCl_3$) δ (ppm) 209.7, 71.2, 63.8, 56.7, 54.2, 44.8, 44.3, 39.1, 38.2, 37.0, 35.5, 32.0, 31.4, 31.0, 28.6, 24.4, 22.8, 21.3, 13.5, 12.3.

HRMS calculated for $C_{21}H_{34}O_2$: 318.2559, found: 318.2552.

*6.2.30. 3β-t-Butyldimehylsilyloxy-5α-pregnan-20-one (**30**)*

In a 250 mL round bottom flask, *3β-hydroxy-5α-pregnan-20-one* **29** (4.34 g, 13.6 mmol) was dissolved in 120 mL THF. Imidazole (2.3 g, 34 mmol, 2.5 eq), *t*-butyldimethylsilyl chloride (2.56 g, 17 mmol, 1.25 eq) and diisopropylethylamine (4.7 mL, 27.2 mmol, 2.0 eq) were successively added then the reaction was stirred overnight at room temperature (R_f: 0.39 in 50% AcOEt/hexanes). The brown mixture was concentrated under reduced pressure then diluted in EtOAc. The mixture was washed with water, 2× sat. sodium bicarbonate, 2× brine, and dried over anhydrous magnesium sulfate. The organic solvents were removed *in vacuo*. The crude product was purified by flash chromatography (25% EtOAc/Hexanes) to yield 5.26 g (89%) of the desired compound as a white solid (120–123 °C).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 3.54 (sept, 1H, $J = 5.8$ Hz), 2.51 (t, 1H, $J = 9.4$ Hz), 2.21–2.11 (m, 1H), 2.10 (s, 3H), 2.05–1.96 (m, 1H), 2.72–1.54 (m, 8H), 1.49–1.01 (m, 12H), 1.00–0.90 (m, 2H), 0.88

(s, 10H), 0.79 (s, 3H), 0.66 (dt, 1H, $J_1 = 11.6$ Hz, $J_2 = 4.8$ Hz), 0.59 (s, 3H), 0.05 (s, 6H).

6.2.31. 3β-t-Butyldimethylsilyloxy-21-bromo-5α-pregnan-20-one (**31**)

In a 50 mL round bottom flask under argon atmosphere, compound **30** (500 mg, 1.15 mmol) was cooled to −78 °C in anhydrous THF. Potassium bis(trimethylsilyl)amide (1 M in THF; 1.27 mL, 1.27 mmol, 1.1 eq) was added and the mixture was stirred for 15 min. Trimethylsilyl chloride (150 μL, 1.15 mmol, 1.0 eq) was added and the mixture was stirred for 1 h at room temperature while monitoring by TLC (50% EtOAc/Hexanes, UV/CAM, R_f: 0.35: starting material, 0.77: silylated compound). The reaction was cooled to −78 °C before addition of *N*-bromosuccinimide (204 mg, 1.15 mmol, 1.0 eq). After 1 h of stirring at −78 °C, monitored by TLC (same conditions as above, $R_f = 0.52$ for brominated compound), the reaction was quenched with saturated aqueous sodium bicarbonate and THF was evaporated under reduced pressure. The crude compound was suspended in water and the solution was extracted with 3× EtOAc. The combined organic layers are washed with brine, dried on anhydrous magnesium sulfate and concentrated *in vacuo*. The compound was purified by flash chromatography (2% EtOAc/Hexanes to 6% EtOAc/Hexanes) to yield 510 mg (87%) of desired compound.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 3.87 (d, 2H, $J = 2.7$ Hz), 3.51 (sept, 1H, $J = 5.3$ Hz), 2.78 (t, 1H, $J = 8.9$ Hz), 2.14 (q, 1H, $J = 9.2$ Hz), 1.87 (dt, 1H, $J_1 = 11.7$ Hz, $J_2 = 2.7$ Hz), 1.73–1.52 (m, 6H), 1.46–1.11 (m, 11H), 1.08–0.88 (m, 2H), 0.85 (s, 10H), 0.76 (s, 3H), 0.63 (dt, 1H, $J_1 = 12.1$ Hz, $J_2 = 2.9$ Hz), 0.59 (s, 3H), 0.01 (s, 6H).

6.2.32. 3β-Hydroxy-21-(N,N-dimethyl) amino-5α-pregnan-20-one (**32**)

Following procedure **C**, 125 mg of **31** (0.24 mmol) were used to give 68 mg of silylated intermediate. Upon deprotection, 20 mg of compound **32** (38% overall yield) were obtained as a white solid (m.p. 105–107 °C) (R_f: 0.13 in 100% AcOEt with 0.5% NEt_3).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 3.58 (sept, 1H, $J = 5.2$ Hz), 3.13 (dd, 2H, $J_1 = 21.4$ Hz, $J_2 = 21.7$ Hz), 2.55 (t, 1H, $J = 8.8$ Hz), 2.28 (s, 6H), 2.16 (d, 1H, $J = 9.3$ Hz), 1.91–1.75 (m, 4H), 1.74–1.52 (m, 7H), 1.43–1.20 (m, 8H), 1.20–1.04 (m, 3H), 1.03–0.83 (m, 3H), 0.79 (s, 1H), 0.72–0.61 (m, 1H), 0.60 (s, 3H).

^{13}C NMR (75.5 MHz, CD_3OD) δ (ppm) 206.5, 70.3, 60.3, 56.6, 55.9, 54.1, 44.7, 43.8, 38.7, 38.2, 37.5, 36.8, 35.4, 31.8, 30.6, 29.5, 28.5, 24.1, 23.4, 22.4, 20.9, 12.4, 11.3.

HRMS calculated for $C_{23}H_{39}O_2N$: 362.3059, found: 362.3059.

6.2.33. 3β-Hydroxy-21-piperidino-5α-pregnan-20-one (**33**)

Following procedure **C**, 100 mg of **31** (0.195 mmol) were used to give 80 mg of silylated intermediate. Upon deprotection, 46 mg of compound **33** (74% overall yield) were obtained (R_f: 0.16 in 100% AcOEt with 0.5% NEt_3).

^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 3.56 (sept, 1H, $J = 4.8$ Hz), 3.08 (s, 2H), 2.58 (t, 1H, $J = 9.8$ Hz), 2.37 (s large, 4H), 2.13 (d, 1H, $J = 10.3$ Hz), 1.86 (dt, 2H, $J_1 = 11.1$ Hz, $J_2 = 3.9$ Hz), 1.78 (d, 1H, $J = 11.1$ Hz), 1.72–1.48 (m, 10H), 1.45–1.19 (m, 10H), 1.18–1.03 (m, 3H), 1.01–0.83 (m, 3H), 0.77 (s, 3H), 0.70–0.62 (m, 1H), 0.58 (s, 3H).

^{13}C NMR (75.5 MHz, CD_3OD) δ (ppm) 71.3, 60.2, 56.8, 54.7, 54.2, 44.8, 39.0, 38.2, 37.0, 35.5, 34.5, 32.1, 31.5, 28.6, 25.7, 24.6, 23.9, 23.0, 21.3, 13.7, 12.4.

HRMS calculated for $C_{26}H_{43}O_2N$: 402.3372, found: 402.3380.

6.2.34. N-Boc-1,2-diaminoethane (**36**)

Following procedure **A**, 770 μL diaminoethane was used to yield 76 mg (41%) of desired product as a colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 5.28–5.11 (broad s, 1H), 3.13 (q, 2H, $J = 5.7$ Hz), 2.76 (t, 2H, $J = 5.7$ Hz), 2.47–2.20 (broad s, 2H), 1.38 (s, 9H).

6.2.35. N-Boc-1,3-diaminopropane (**37**)

Following procedure **A**, 970 μL diaminopropane was used to yield 100 mg (50%) of desired product as a colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 5.16 (broad s, 1H), 3.60 (broad s, 2H), 3.20 (q, 2H, $J = 6.1$ Hz), 2.83 (t, 2H, $J = 6.1$ Hz), 1.69 (quint, 2H, $J = 6.1$ Hz), 1.42 (s, 9H).

6.2.36. N-Boc-1,4-diaminobutane (**38**)

Following procedure **A**, 1.16 mL diaminobutane was used to yield 171 mg (79%) of desired product as a colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 4.94 (broad s, 1H), 3.04 (broad s, 4H), 2.69 (broad s, 2H), 1.45 (broad s, 4H), 1.36 (s, 9H).

6.2.37. 3β-Acetoxy-20-(Boc-aminoethyl)amino-5α-pregnane (**39**)

Following procedure **B**, 100 mg **36** (0.62 mmol, 1.17 eq) was used to yield 100 mg (41%) of desired product (R_f: 0 in 25% AcOEt/hexanes).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 5.02 (broad s, 1H), 3.55 (m, 1H, $J = 4.9$ Hz), 3.27–3.05 (m, 2H), 2.85–2.68 (m, 1H), 2.65–2.45 (m, 2H), 2.03–1.92 (m, 1H), 1.88–1.51 (m, 10H), 1.47–1.35 (broad s, 11H), 1.33–1.02 (m, 16H), 0.98 (d, 4H, $J = 9.2$ Hz), 0.94–0.82 (m, 3H), 0.80 (s, 3H), 0.69 (s, 3H), 0.65–0.55 (m, 1H).

6.2.38. 3β-Acetoxy-20-(Boc-aminopropyl)amino-5α-pregnane (**40**)

Following procedure **B**, 230 mg of **37** (0.605 mmol, 2.4 eq) was used to yield 132 mg (50%) of desired product (R_f: 0 in 75% AcOEt/hexanes).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 5.60 (broad s, 0.2H) 5.07 (broad s, 0.6H), 4.65 (sept, 1H, $J = 5.1$ Hz), 3.16 (sept, 1H, $J = 3.6$ Hz), 1.99 (s, 3H), 1.98–1.84 (m, 2H), 1.84–1.51 (m, 7H), 1.41 (s, 9H), 1.36–1.08 (m, 9H), 1.06 (d, 1H, $J = 6.1$ Hz), 1.03–0.97 (m, 2H), 0.95 (d, 2H, $J = 6.1$ Hz), 0.79 (s, 3H), 0.66 (s, 2H), 0.63 (s, 1H), 0.62–0.57 (m, 1H).

6.2.39. 3β-Acetoxy-20-(Boc-aminobutyl)amino-5α-pregnane (**41**)

Following procedure **B**, 315 mg of **38** (1.67 mmol, 3.0 eq) was used to yield 198 mg (73%) of desired product (R_f: 0 in 50% AcOEt/hexanes).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 4.92 (broad d), 4.65 (sept, 1H, $J = 6.2$ Hz), 3.09 (broad s, 2H), 2.78–2.52 (m, 2H), 2.52–2.38 (m, 1H), 1.99 (s, 3H), 1.95–1.84 (m, 1H), 1.82–1.45 (m, 12H), 1.41 (s, 10H), 1.35–1.10 (m 10H), 1.10–1.05 (m, 4H) 0.97 (d, 3H, $J = 5.7$ Hz), 0.91–0.81 (m, 1H), 0.76 (s, 3H), 0.66 (s, 2H), 0.63 (s, 1H), 0.61–0.56 (m, 1H).

6.2.40. 3β-Hydroxy-20(aminoethyl)amino-5α-pregnane hydrochloride (**42**)

Following procedure **D**. 100 mg **39** (0.216 mmol) was used to yield 85 mg (96%) of desired compound as a white solid (m.p. 240 °C with decomposition) (R_f: 0 in 10% MeOH/AcOEt with 0.5% NEt_3).

^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 3.48 (m, 1H), 3.43–3.35 (m, 3H), 2.00–1.45 (m, 10H), 1.40–1.20 (m, 10H), 1.18–0.87 (m, 5H), 0.82 (s, 3H), 0.76 (s, 4H).

^{13}C NMR (75.5 MHz, CD_3OD) δ (ppm) 70.3, 58.6, 55.9, 55.4, 55.0, 54.4, 54.0, 53.0, 52.0, 44.8, 44.7, 42.8, 42.4, 40.9, 39.1, 37.4, 36.8, 35.5, 35.2, 31.7, 30.7, 28.4, 23.5, 22.8, 20.7, 20.5, 14.9, 14.6, 11.7, 11.3.

HRMS calculated for $C_{23}H_{42}ON_2^+$: 362.3370, found: 363.3377 (MH^+).

*6.2.41. 3β-Hydroxy-20(aminopropyl)amino-5α-pregrane hydrochloride (**43**)*

Following procedure **D**, 132 mg **40** (0.277 mmol) was used to yield 125 mg (100%) of desired compound as a white solid (m.p. 205 °C with decomposition) (R_f: 0 in 10% MeOH/AcOEt with 0.5% NEt_3).

^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 3.45–3.34 (m, 2H), 3.13 (t, 2H, J = 6.9 Hz), 3.05 (t, 2H, J = 7.9 Hz) 2.09 (m, 2H), 1.97–1.79 (m, 1H), 1.79–1.62 (m, 5H), 1.56–1.45 (m, 2H), 1.43 (m, 4H), 1.40–1.33 (m, 3H), 1.33–1.21 (m, 7H), 1.17–1.04 (m, 3H), 1.03–1.86 (m, 2H), 0.82 (s, 3H), 0.73 (d, 3H, J = 6.1 Hz), 0.71–0.60 (m, 1H).

^{13}C NMR (75.5 MHz, CD_3OD) δ (ppm) 70.3, 57.8, 55.9, 55.4, 54.4, 54.0, 52.7, 51.8, 44.8, 42.7, 42.3, 40.9, 40.1, 39.1, 37.4, 36.8, 35.5, 35.2, 31.7, 30.7, 28.4, 25.9, 23.9, 23.5, 23.0, 20.7, 20.5, 15.1, 14.2, 11.7, 11.3.

HRMS calculated for $C_{24}H_{45}ON_2^+$: 377.3526, found: 376.3535 (MH^+).

*6.2.42. 3β-Hydroxy-20(aminobutyl)amino-5α-pregrane hydrochloride (**44**)*

Following procedure **D**, 198 mg **41** (0.40 mmol) was used to yield 186 mg (98%) of desired compound as a white solid (m.p. 255 °C with decomposition) (R_f: 0 in 10% MeOH/AcOEt with 0.5% NEt_3).

^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 3.48 (m, 1H), 3.09–2.91 (m, 4H), 2.01–1.63 (m, 11H), 1.61–1.46 (m, 3H), 1.36 (d, 3H, J = 6.2 Hz), 1.32–1.21 (m, 6H), 1.20–1.03 (m, 3H), 1.03–0.86 (m, 2H), 0.82 (d, 3H, J = 3.4 Hz), 0.73 (s, 4H).

^{13}C NMR (75.5 MHz, CD_3OD) δ (ppm) 70.3, 57.6, 55.9, 55.5, 54.4, 54.2, 52.8, 51.9, 44.8, 43.2, 42.3, 39.1, 38.6, 37.4, 36.8, 35.2, 31.7, 30.7, 28.4, 25.9, 24.3, 23.5, 23.3, 22.8, 20.7, 20.5, 15.1, 14.2, 11.7, 11.3.

HRMS calculated for $C_{25}H_{47}ON_2^+$: 391.3683, found: 391.3689 (MH^+).

*6.2.43. 3β-Acetoxy-20-butylamino-5α-pregnane (**45**)*

Following procedure **B**, 50 mg of 3β-acetoxy-5α-pregnan-20-one **49** (0.14 mmol), 137 μL (1.39 mmol, 10 eq) of butylamine and 10 mg of sodium cyanoborohydride (0.15 mmol, 1.1 eq) were converted to 24.2 mg (41%) of desired product (R_f: 0.33 in 10% MeOH/AcOEt with 0.5% NEt_3).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 4.67 (sept, 1H, J = 5.9 Hz), 2.75–2.65 (m, 1H), 2.60–2.52 (m, 1H), 2.50–2.41 (m, 1H), 2.01 (s, 3H), 2.00–1.84 (m, 5H), 1.82–1.44 (m, 11H), 1.40–1.10 (m 12H), 1.09 (d, 5H, J = 6.5 Hz), 1.07–0.95 (m, 3H), 0.91 (t, 3H, J = 7.4 Hz), 0.81 (s, 3H), 0.65 (s, 4H).

*6.2.44. 3β-Hydroxy-20-butylamino-5α-pregnane hydrochloride (**46**)*

Following procedure **D**, 15 mg of **45** (0.036 mmol), were converted to 13.5 mg (100%) of desired product (R_f: 0.05 in 10% MeOH/AcOEt with 0.5% NEt_3).

^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 3.48 (m, 1H), 3.20 (m, 1H), 2.97 (m, 2H), 1.97–1.84 (m, 2H), 1.79–1.07 (m, 23H), 1.01–0.85 (m, 5H), 0.81 (s, 3H), 0.72 (s, 3H), 0.69–0.61 (m, 1H).

^{13}C NMR (75.5 MHz, CD_3OD) δ (ppm) 70.4, 57.4, 56.0, 54.1, 52.7, 44.7, 42.7, 42.6, 39.1, 37.4, 36.8, 35.2, 35.2, 31.7, 30.7, 28.4, 27.9, 25.8, 23.8, 20.8, 19.5, 15.1, 12.5, 11.3, 10.9.

HRMS calculated for $C_{25}H_{46}ON^+$: 376.3574, found: 376.3578 (MH^+).

*6.2.45. 3β-Acetoxy-20-isopentylamino-5α-pregnane (**47**)*

Following procedure **B**, 100 mg of 3β-acetoxy-5α-pregnan-20-one **49** (0.28 mmol), 200 μL (1.72 mmol, 6.2 eq) of isopentylamine and 18 mg of sodium cyanoborohydride (0.29 mmol, 1.05 eq) were converted to 46 mg (41%) of desired product (R_f: 0.36 in 10% MeOH/AcOEt with 0.5% NEt_3).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 5.62 (s large, 2H), 4.65 (sept, 1H, J = 6.2 Hz), 3.26–2.57 (m, 2H), 1.99 (s, 5H), 1.85–1.37 (m, 11H), 1.35–1.15 (m, 8H), 1.12 (d, 2H, J = 6.3 Hz), 1.09–0.96 (m, 3H), 0.92 (d, 1H, J = 6.3 Hz), 0.89 (d, 6H, J = 6.4 Hz), 0.790 (s 3H), 0.69–0.55 (m, 4H).

*6.2.46. 3β-Hydroxy-20-isopentylamino-5α-pregnane hydrochloride (**48**)*

Following procedure **D**, 46 mg of **47** (0.11 mmol), were converted to 48 mg (100%) of desired product (R_f: 0.07 in 10% MeOH/AcOEt with 0.5% NEt_3).

^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 3.49 (m, 1H), 2.98 (m, 1H), 1.89–1.45 (m, 12H), 1.45–1.00 (m, 17H), 0.96 (d, 6H, J = 6.4 Hz), 0.91–0.86 (m, 2H), 0.82 (s, 3H), 0.72 (s, 3H), 0.70–0.63 (m, 2H).

^{13}C NMR (75.5 MHz, CD_3OD) δ (ppm) 70.4, 55.6, 54.4, 54.0, 52.0, 44.8, 42.3, 37.5, 37.4, 36.8, 35.2, 34.4, 31.7, 30.7, 28.4, 25.9, 23.5, 23.4, 21.4, 21.2, 20.6, 14.3, 11.5, 11.3.

HRMS calculated for $C_{26}H_{48}ON^+$: 390.3730, found: 390.3733 (MH^+).

*6.2.47. 3β,21-Diacetoxy-5α-pregn-17,20-diene (**50**)*

In a 250 mL round bottom flask 3β-acetoxypregnan-20-one **49** (4 g, 11 mmol) was dissolved in acetic anhydride (150 mL) along with 4 g (21 mmol, 1.9 eq) *p*-toluenesulfonic acid. The mixture was brought to 150 °C for 4 h (R_f: 0.53 in 50% AcOEt/hexanes). Afterward, the reaction was concentrated *in vacuo* then dissolved in DCM. The resulting mixture was neutralized with 1 M NaOH, then washed subsequently with water and brine. The solution was dried on anhydrous magnesium sulfate and solvent was removed *in vacuo* to yield 4.42 g (100%) of crude compound as a brown oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 4.73–4.41 (m, 1H), 2.37–2.16 (m, 2H), 2.09 (s, 3H), 2.02 (s, 3H), 1.87 (t, 1H, J = 1.8 Hz), 1.77 (s, 3H), 1.75–1.59 (m, 4H), 1.57 (s, 3H, H_2O), 1.54–1.09 (m, 11H), 1.07–0.86 (m 3H), 0.82 (s, 3H), 0.81 (s, 3H), 0.74–0.58 (m, 1H).

*6.2.48. 3β-Acetoxy-5α-pregn-16-en-20-one (**51**)*

Compound **51** was synthesized as reported by Djerassi et al. [46].

The compound was obtained as a mixture of desired enone **51** (R_f: 0.69 in 100% AcOEt) and 3β-acetoxy-5α-pregnan-20-one **49** in a 1:1 ratio (determined by NMR). The mixture was used as such for the subsequent reactions.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 6.68–6.64 (m, 0.5H), 4.65 (sept, 1H, J = 6.2 Hz), 2.35–2.24 (m, 1H), 2.22 (s, 3H), 2.17–2.07 (m, 2H), 1.98 (s, 3H), 1.95–1.58 (m, 6H), 1.52–1.28 (m, 5H), 1.20–1.10 (m, 1H), 1.07–0.93 (m, 2H), 0.87 (s, 3H), 0.85–0.81 (m, 4H), 0.78–0.58 (m, 1H).

*6.2.49. 17.3β-Acetoxy-16β-hydroxy-17α-bromo-5α-pregn-16-ene (**52**)*

Compound **52** was synthesized as reported by Ravindar et al. [47] (R_f: 0.63 in 100% AcOEt).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 4.88–4.82 (m, 1H), 4.61 (sept, 1H, J = 6.2 Hz), 2.34 (s, 3H), 2.32–2.03 (m, 1H), 1.94 (s, 3H), 1.86–1.48 (m, 10H), 1.47–1.34 (m, 3H), 1.33–1.10 (m 10H), 1.08 (s, 3H), 1.03–0.80 (m, 4H), 0.78 (s, 3H), 0.73–0.62 (m, 1H).

*6.2.50. 3β-Acetoxy-16β-hydroxy-5α-pregn-16-ene (**53**)*

Compound **53** was synthesized as reported by Ravindar et al. [47] (R_f: 0.69 in 100% AcOEt).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 4.68 (sept, 1H, J = 6.2 Hz), 4.58–4.51 (m, 1H), 2.32–2.22 (m, 2H), 2.20 (s, 3H), 2.02 (s, 3H), 2.00–1.96 (m, 1H), 1.85–0.50 (m, 9H), 1.50–1.10 (m, 8H), 1.08–0.95 (m, 3H), 0.94 (s, 3H), 0.84 (s, 3H), 0.76–0.54 (m, 1H).

*6.2.51. 3β-Acetoxy-16β-hydroxy-20 (N,N-dimethylaminopropyl) amino-5α-pregnane (**54**)*

Following procedure **B**, 20 mg of 16-hydroxypregnanolone acetate **53** (0.053 mmol), 40 μL (0.32 mmol, 6.0 eq) of dimethylaminopropylamine and 3.6 mg of sodium cyanoborohydride (0.058 mmol, 1.1 eq) were converted to 10.3 mg (42%) of desired product (R_f: 0.49 in 10% MeOH/AcOEt with 0.5% NEt_3).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 4.66 (sept, 1H, J = 4.7 Hz), 4.37 (m, 1H), 3.12 (d quint, 1H, J_1 = 47.7 Hz, J_2 = 6.8 Hz), 2.58–2.45 (m, 1H), 2.38 (t, 1H, J = 6.3 Hz), 2.21 (s, 6H), 2.00 (s, 3H), 1.97–1.34 (m, 12H), 1.33–1.02 (m, 12H), 0.97 (s, 3H), 0.96–0.85 (m, 3H), 0.82 (s, 0.69–0.57 (m, 2H)).

*6.2.52. 3β,16β-Dihydroxy-20 (N,N-dimethylaminopropyl)amino-5α-pregnane (**55**)*

Following procedure **D**, 10.3 mg of **54** (0.022 mmol), were converted to 9.4 mg (100%) of desired product as a white solid (m.p. 225 °C with decomposition) (R_f: 0 in 10% MeOH/AcOEt with 0.5% NEt_3).

^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 4.38 (m, 1H), 3.73 (m, 1H), 3.47 (m, 3H), 3.11 (m, 1H), 2.90 (s, 6H), 2.28–2.08 (m, 2H), 1.98–1.90 (m, 1H), 1.77–1.53 (m, 6H), 1.48 (d, 4H, J = 6.56 Hz) 1.45–1.15 (m, 11H), 1.06 (s, 3H), 1.04–0.85 (m, 3H), 0.82 (d, 3H, J = 6.8 Hz), 0.75–0.63 (m, 1H).

^{13}C NMR (75.5 MHz, CD_3OD) δ (ppm) 72.2, 70.3, 56.8, 54.7, 54.3, 54.1, 53.9, 44.7, 42.2, 42.1, 42.0, 41.5, 38.8, 37.4, 36.7, 36.4, 35.2, 34.7, 31.6, 30.7, 28.4, 21.1, 20.5, 14.8, 13.1, 11.3.

HRMS calculated for $C_{26}H_{46}O_2N_2^+$: 421.3789, found: 421.3793.

*6.2.53. 3β-Acetoxy-16β-hydroxy-20-butylkamino-5α-pregnane (**56**)*

Following procedure **B**, 20 mg of 16-hydroxypregnanolone acetate **55** (0.053 mmol), 31 μL (0.32 mmol, 6.0 eq) of butylamine and 3.6 mg of sodium cyanoborohydride (0.058 mmol, 1.1 eq) were converted to 15.6 mg (68%) of desired product (R_f: 0.55 in 10% MeOH/AcOEt with 0.5% NEt_3).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 4.68 (sept, 1H, J = 4.7 Hz), 4.35–4.15 (m, 1H), 2.32–2.19 (m 1H), 2.10–2.03 (m, 1H), 2.01 (s, 3H), 1.85–1.59 (m, 4H), 1.56 (H_2O), 1.53–1.39 (m, 5H), 1.33 (d, 3H, J = 6.3 Hz), 1.32–1.08 (m, 8H), 1.01 (s, 3H), 0.0.98–0.86 (m, 3H), 0.84 (s, 3H), 0.71–0.59 (m, 1H).

*6.2.54. 3β,16β-Dihydroxy-20-butylamino-5α-pregnane hydrochloride (**57**)*

Following procedure **D**, 15.6 mg of **56** (0.035 mmol), were converted to 7.6 mg (49%) of desired compound as a white solid (m.p. 212 °C with decomposition) (R_f: 0.04 in 10% MeOH/AcOEt with 0.5% NEt_3).

^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 4.23–4.00 (dm, 1H), 3.55–3.45 (m, 1H), 3.02–2.88 (m, 1H), 2.22–2.08 (m, 2H), 1.77–1.64 (m, 4H), 1.56–1.40 (m, 6H), 1.35–1.25 (m, 8H), 1.22–1.05 (m, 4H), 0.97 (s, 3H), 0.92–0.86 (m, 4H), 0.85 (S, 3H), 0.71–0.60 (m, 1H).

^{13}C NMR (75.5 MHz, CD_3OD) δ (ppm) 72.2, 70.3, 56.7, 54.5, 54.4, 54.1, 53.9, 44.7, 44.4, 41.9, 38.8, 37.4, 36.7, 35.2, 34.7, 31.6, 30.6, 28.4, 27.7, 20.4, 19.5, 14.9, 12.5, 11.3.

HRMS calculated for $C_{25}H_{46}O_2N^+$: 392.3523, found: 392.3523 (MH^+).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.11.019.

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