EurJOC
European Journal of Organic Chemistry

Chemistry Europe
European Chemical Societies Publishing

Accepted Article

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To be cited as: *Eur. J. Org. Chem.* 10.1002/ejoc.202000051

Link to VoR: https://doi.org/10.1002/ejoc.202000051

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FULL PAPER

Gram-scale Synthesis of Tomatidine, a Steroid Alkaloid with Antibiotic Properties Against Persistent Forms of *Staphylococcus aureus*

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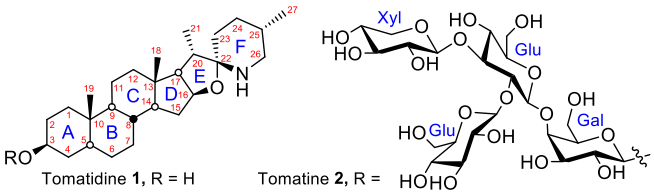
Abstract:

We herein describe the first diastereoselective synthesis of the *Solanum* alkaloid tomatidine **1**. The synthesis has been accomplished in 11 steps and 24.9 % overall yield (longest linear sequence). This methodology, which involves a convergent synthon insertion followed by a sequence of ring opening/nitrogen substitution/ring closing, allowed the generation of **1** on > 2g scale. The synthetic challenge with the diastereoselective generation of the unusual spiroaminoketal moiety was solved through a combined azide reduction/addition sequence. The first diastereoselective synthesis of the phytosteroid yamogenin is also reported. Tomatidine has shown promising antibiotic properties against persistent forms of *Staphylococcus aureus* (*S. aureus*) and methicillin-resistant *S. aureus* (MRSA). In particular, it possesses the unique ability to kill persistent forms of *S. aureus* and MRSA while simultaneously potentiating the antibiotic efficacy of aminoglycoside antibiotics against wild type strains of the bacteria.

Introduction

In 2017, the World Health Organisation (WHO) identified *S. aureus*, MRSA and other antibiotic-resistant bacterial species as "high-priority" targets for the development of new antibiotics in the fight against resistance.[1] MRSA, resistant to β-lactams and multiple other drugs, is widely found in hospital-acquired and community-acquired infections.[2] *S. aureus* and MRSA can adopt two phenotypes, the prototypical wild-type (WT) and its small-colony variants (SCV).[3] The slow-growing SCV is the result of a point mutation that occurs under pressure and affects the electron transport chain. Slow growth, intracellular persistence and high biofilm production all contribute to protect SCV from antibiotic action, forming a reservoir that leads to persistence.[2,4] SCV can revert to the virulent WT upon cessation of treatment, leading to infection relapses.[5] Our team reported that **1** possesses potent inhibitory activities against the SCV of S. *aureus* and MRSA, with minimal inhibitory concentrations (MIC) as low of 0.06 µg/mL.[6] We further demonstrated that **1** targets the ATP synthase of *S. aureus*, a protein important in energy production.[7] We previously elucidated the structure-activity relationship of **1** in order to optimize its inhibition potential against SCV,[7] and demonstrated that **1** potentiates up to 16-fold the activity of aminoglycoside antibiotics such as gentamicin or tobramycin against WT *S. aureus*.[8,9] These combined properties make tomatidine a target of interest for use in combination with aminoglycosides to treat simultaneously the WT and SCV forms of *S. aureus* and MRSA.



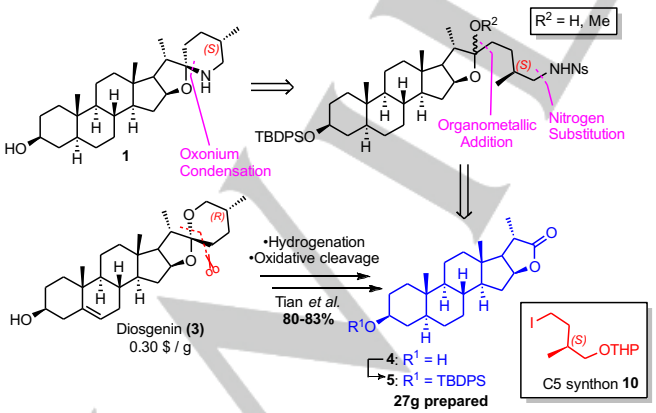
Tomatidine **1**, R = H Tomatine **2**, R =

Figure 1. Tomatidine **1** and its glycosylated form tomatine **2**

Tomatidine **1** is a steroid alkaloid found in more than 60 species of the *Solanum* plant genus.[10] It was first isolated and characterized in 1948 as its naturally occurring C3-glycosylated form tomatine **2** (Figure 1), which bears the Gal-Glu(Glu-Xyl) tetrasaccharide.[11] **1** is biosynthesized from plant cholesterol via a series of GLYCOALKALOID METABOLISM (GAME) enzymes.[12]

Isolation from plants is currently the only means to access **1** and **2**, both present in low concentrations in the leaves, roots and fruits of *Solanum* plants. Tomatine **2** needs to be subjected to acidic hydrolysis to deliver its aglycon form tomatidine **1**. Tomatidine is commercially available yet expensive, typically as a hydrochloride salt of *ca* 85% purity. Structurally, it bears six rings (A-F, Figure 1), 12 stereogenic centers and a spiroaminoketal moiety (rings E-F) also found in other biologically active products such as the herbicide hydantocidin[13], the phycotoxins azaspiracid A-E[14,15] or the immunosuppressant sanglifehrin A-D.[16] The commercial price of tomatidine (US$ 1,000-1,500/g) and its low availability limit further optimization toward novel antibiotics, hence the strategic need to develop a synthetic route. Recently, the synthesis of 25-nortomatidine was reported by Czajkowska-Szczykowska *et al.* It was obtained as a minor diastereoisomer in the synthesis of 25-norsolasodine.[17]

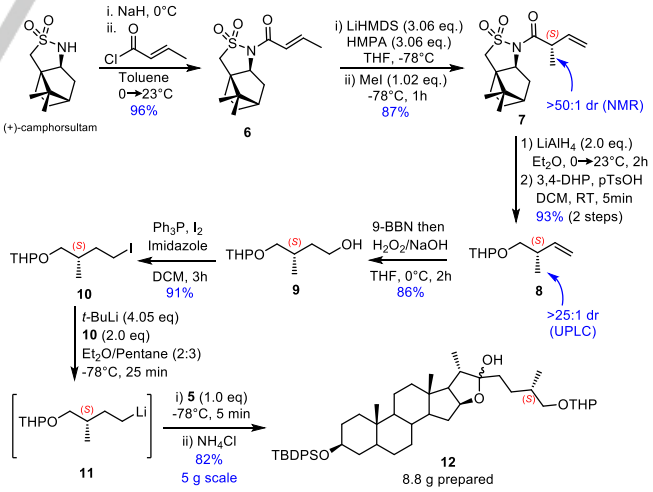
Our goal was to develop the first synthesis of **1**, aiming for a gram-scale, efficient and cost-effective process from readily available starting materials. Accordingly, the known dinorcholanic lactone **5** (Scheme 1) appeared as an attractive starting point. Indeed, **5** possesses the correct stereochemistry on rings A-D and C_{20} methyl. Following the methodology developed by Tian *et al.*, Pd-catalyzed hydrogenation, iodine-mediated Baeyer-Villiger oxidation/ lactonisation of diosgenin **3** to **4** followed by TBDPS protection of the C3 alcohol yielded dinorcholanic lactone **5** in 3 steps and high yields on 27g scale.[18] Diosgenin, a triterpene phytosteroid isolated industrially from Fenugreek,[19,20] can be purchased at low cost (US$ 0.30/g). Construction of rings E and F, on the other hand, relied on the chiral synthesis of iodide **10**.



Scheme 1. Retrosynthetic analysis of **1**

Results and Discussion

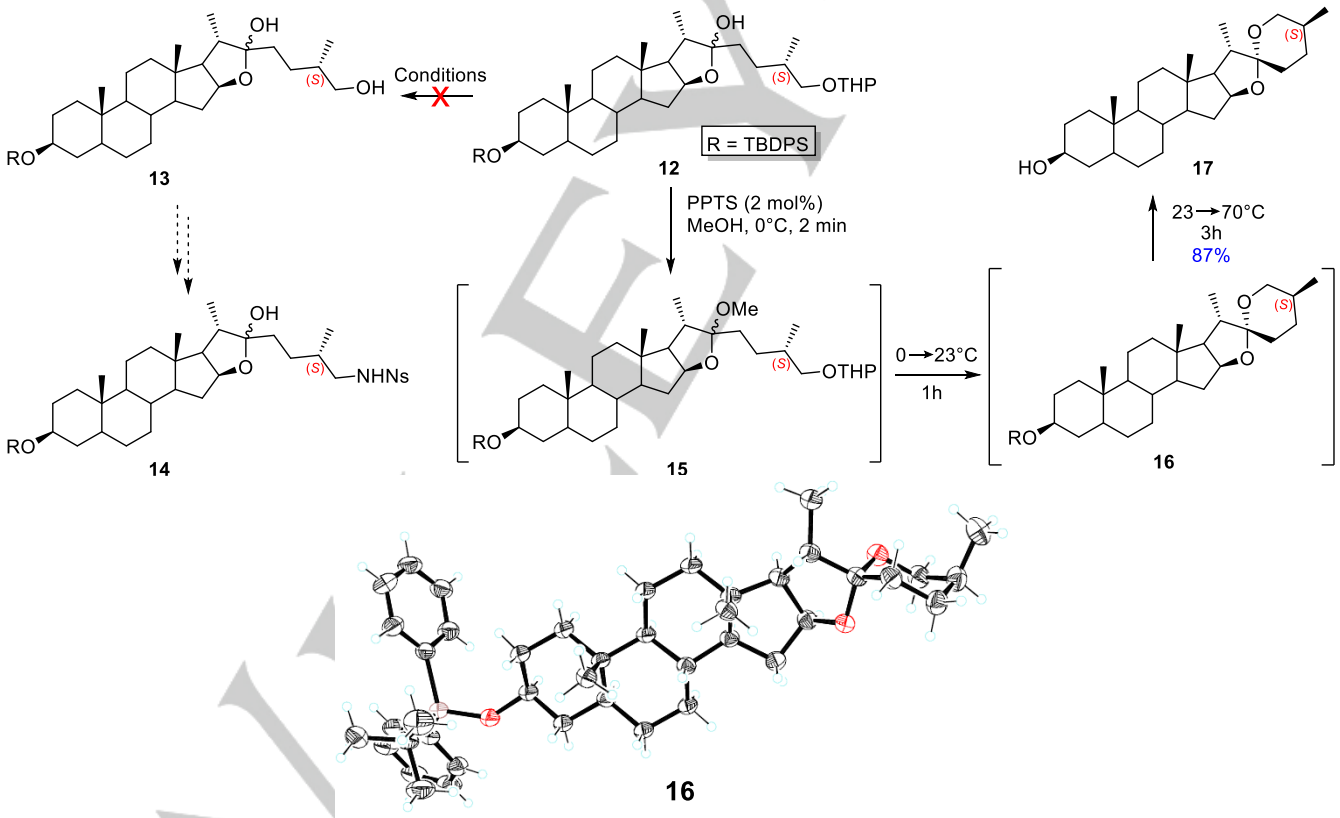
Synthesis of iodide **10** (Scheme 2) was initiated by crotonoylation of commercial (+)-camphorsultam using crotonyl chloride, providing crystalline **6** in 96% yield. Subsequent alkylation using LiHMDS and methyl iodide in THF/HMPA yielded **7** in 87% yield and >50:1 d.r. (NMR).[21,22] This methodology was preferred over Evans' oxazolidinone approach, which is associated with lower diastereoselectivity (d.r. < 3.6:1).[23] **7** was reductively cleaved using LAH, and the resulting primary alcohol protected directly as a tetrahydropyranyl (THP) ether to deliver alkene **8** in 93% yield (2 steps). The enantiomeric excess of the alcohol was determined to be >93% by chiral UPLC of the corresponding PMB ether (**8a**, see supporting information). Hydroboration of alkene **8** using 9-BBN followed by oxidative workup with sodium hydroxide and hydrogen peroxide[24] yielded primary alcohol **9** in 86% yield. Alcohol **9** was transformed into the corresponding iodide **10** using Appel conditions in 91% yield.[25] The latter underwent iodide-lithium exchange[26] using *t*-butyllithium to generate organolithium intermediate **11** *in situ*, to which was added **5** at -78°C to generate hemiketal **12** in 82% yield (5 g scale). A total of 8.8 g of **12** was prepared in this manner. To be noted, attempts to generate the Grignard reagent derived from the corresponding bromide failed to provide addition product **12** in useful yields.



Scheme 2. Synthesis of synthon **10** and hemiketal **12**

With hemiketal **12** in hand, we set out to deprotect the THP group, with the goal to convert it to an amine such as **14** (Scheme 3).[27] However, even mild deprotection conditions (i.e. $B_{10}H_{14}$ (cat.) in MeOH[28], DDQ (cat.) in wet MeCN[29], LiCl in wet DMSO[30]) failed to yield alcohol **13** and repeatedly produced spiroketal **16** (Scheme

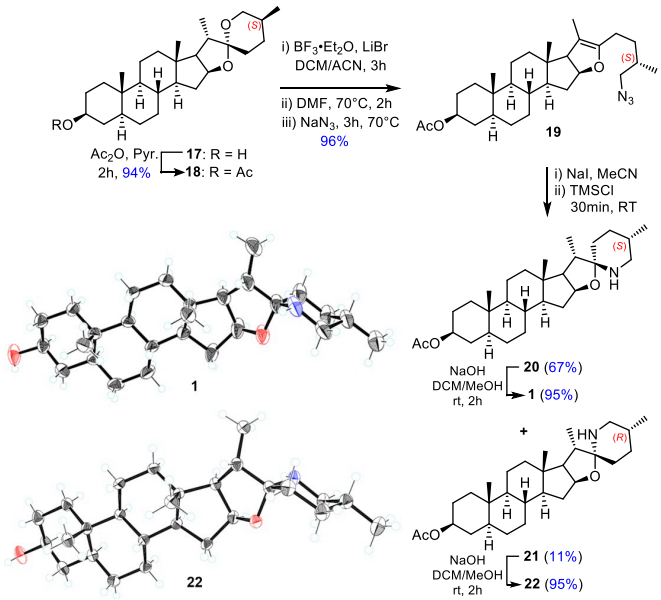
3). In more detail, when hemiketal **12** was subjected to pyridinium *p*-toluenesulfonate (cat.) in methanol, ketal **15** was obtained rapidly (< 2 min. at 0°C). Reaction was then brought to room temperature for 1 h to yield spiroketal **16** after intramolecular oxonium trapping of the newly generated alcohol. Further heating yielded desilylated spiroketal **17**. The structure of intermediate **16** (CCDC Deposition #1992541) was confirmed by single crystal X-ray crystallography (Scheme 3), revealing a silylated and reduced derivative of phytosteroid yamogenin **17**, the 25*S* diastereomer of diosgenin **3**. This is not the first report of synthetic yamogenin[31], but it is the first reported diastereoselective synthesis. Yamogenin possesses interesting anti-hyperlipidermic effects.[32] Interestingly, as opposed to tomatidine, its C25 methyl group prefers the axial orientation, likely the result of anomeric stabilization by the two spiro oxygen atoms. With spiroketal **16** in hand and the correct stereochemistry installed on C_{25}, we set out to introduce a nitrogen source at the C_{26} position, before closing ring F to generate the spiroaminoketal moiety. Various protocols have been developed to open and functionalize the F ring of spiroketal sapogenin steroids, often requiring strong oxidative and acidic conditions[33] or hazardous reagents.[34] The Lewis acid-mediated ring opening of steroidal spiroketals reported by Tian *et al* was used.[35] The TBDPS ether **16** being incompatible with these conditions, it was removed by bringing the spiroketalisation step to reflux in order to generate secondary alcohol **17** (Scheme 3) in 87% yield.[36] The C_3 alcohol was then protected as an acetate **18** in 94% yield (Scheme 4). The corresponding ω-halo enol ether was then generated using BF_3 etherate and lithium bromide, followed by heating in DMF. The intermediate bromide was substituted *in situ* by addition of sodium azide to the mixture to afford ω-azido enol ether **19** in 96% yield. With the nitrogen group installed on position C_{26}, azide reduction to the amine and subsequent cyclization onto the enol ether was achieved in one step using trimethylsilyliodide (TMSI), generated by addition of TMSCl to a solution of NaI in MeCN.[35,37]



Scheme 3. Synthesis of intermediate **17** and ORTEP diagram of intermediate **16** (50% Thermal Probability Ellipsoids)

In these conditions, tomatidine acetate **20** was obtained in 67% yield. Interestingly, its diastereoisomer 5,6-dihydrosolasodine acetate **21** was also obtained in 11% yield. This result can be explained by imine-enamine isomerisation of the reduced amine in acidic conditions, with the neat result of *in situ* inversion of configuration of the C25 methyl group.[35] The subsequent isomerized 25*R* amine intermediate yielded **21** as a minor product, and 25*S* amine yielded tomatidine acetate **20** as the major product**.** Spiroaminoketals **20** and **21** were finally subjected to ester hydrolysis to afford **1** and **22** in 95% yield. Crystallization of

synthetic **1** (CCDC Deposition # 1992540) and **22** (CCDC Deposition # 1992542) from MeOH afforded crystals suitable for x-ray diffraction, confirming their identity (Scheme 4). This synthesis successfully delivered 2.35 g of synthetic Tomatidine.



Scheme 4. Completion of the synthesis and ORTEP diagram of Tomatidine **1** and 5,6-dihydrosolasodine **22** (50% Thermal Probability Ellipsoids)

Conclusion

In summary, we report the first synthesis of tomatidine **1** using an 11-step convergent sequence starting from cheap and commercially available diosgenin **3**. It delivered gram-scale quantities with an overall yield of 24.9 % for the longest linear sequence. The synthesis was achieved via the pivotal yamogenin intermediate **17**. This synthesis opens the way to larger amounts of materials to further study the role and structure-activity relationship of tomatidine **1** and analogs as potential antibiotic agents against persistent forms of *S. aureus* and MRSA.

Experimental Section

General Information**:** All reactions were performed in flame-dried or oven-dried glassware under an atmosphere of nitrogen using Teflon-coated stir bars. Reactions ran above room temperature (23 °C) were heated in an oil bath. Commercially available reagents were purchased from Sigma-Aldrich with the highest available purity and used without purification. Tetrahydrofuran (THF), methanol (MeOH), acetonitrile (MeCN), *N*,*N*-dimethylformamide (DMF) were purchased as DriSolv® grade, stored and used under argon atmosphere. Diethyl ether (Et_2O) and pentane were distilled over sodium under argon and kept dry using activated 3Å molecular sieves. Flash chromatography was performed on Silicycle SiliaFlash P60 silica gel (230−400 mesh, 40−63 µm particle size). Automated flash chromatography was performed using a Biotage Isolera ISO-1SV automated flash chromatography system. Thin layer chromatography (TLC) was performed on glass-backed Silicycle SiliaPlate 250 µm thickness, 60 Å porosity F-254 precoated plates from Silicycle. Compounds were visualized with UV light (254 nm) then stained with cerium ammonium molybdate followed by heating (steroids) or potassium permanganate (KMnO4) followed by heating (non-steroids). Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker Ascend 400 spectrometer. Residual chloroform ($CHCl_3$) signals were used as internal reference for ^1H (δ = 7.26 ppm) and ^{13}C (δ = 77.1 ppm) spectra. The following abbreviations were used to describe NMR multiplicities: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, sext = sextuplet, sept = septuplet and m = multiplet. Coupling constants (*J*) are given in Hertz (Hz). Infrared (IR) spectra were obtained on an ABB Bomem MB104 spectrophotometer using a diamond-attenuated total reflectance (ATR) accessory. All substances were directly applied on the ATR unit. Frequency of absorption is given in cm^{-1}. High-resolution mass spectra (HRMS) were obtained on a Nexera (Shimadzu) LC-QTOF mass spectrometer coupled to maXis (Bruker) mass spectrometer (ESI) using sodium formate as internal standard. Melting points were measured on an Electrothermal MEL-TEMP 3.0 melting point apparatus. X-Ray diffraction was performed on a KAPPA APEX-DUO (Bruker) diffractometer device using a Cu radiation source.

2-(((*S*)-2-methylbut-3-en-1-yl)oxy)tetrahydro-2*H*-pyran (8). A solution of **7** (28.3 g, 95.2 mmol, prepared as described by Cooksey, P. *et al*) in Et_2O (900 mL), was added dropwise over 2h to a suspension of lithium aluminum hydride (7.22 g, 190 mmol, 2.0 eq.) in anhydrous Et_2O (150 mL) at 0°C. The resulting grey suspension was stirred vigorously and allowed to warm to room temperature. When **7** was fully consumed as followed by TLC (1 h), the mixture was slowly quenched by successive addition of water (7.2 mL), 15% aqueous NaOH (7.2 mL) then water (21.6 mL). The suspension was dried over sodium sulfate and stirred vigorously for 30 min. The resulting suspension was then filtered through a glass-fritted funnel and the residue washed repeatedly with Et_2O (3x 100 mL) while manually stirring the slurry in the funnel before filtering again. Filtrates were combined and Et_2O was removed *via* fractional distillation until minimal Et_2O remained (ca. 50 mL). A small aliquot of the reaction crude was put aside for PMB derivatization and enantiomeric excess determination (see supporting information). To this concentrated solution was added DCM (250 mL), 3,4-Dihydro-2*H*-pyran (17.4 mL, 66.5 mmol, 2.0 eq.) and *p*-toluenesulfonic acid monohydrate (362 mg, 1.90 mmol, 0.02 eq.). This mixture was stirred until the alcohol was fully consumed as evidenced by TLC (5 min). The mixture was then diluted with DCM (200 mL) then washed with saturated aqueous $NaHCO_3$ (40 mL). The organic layer was dried with sodium sulfate then concentrated *in vacuo*. Purification by flash chromatography (step gradient 0, 2.5, 5, 7.5, 10% EtOAc:Hex) yielded tetrahydropyranyl ether 8 (15.1 g, 93%) as a light yellow oil. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 5.85-5.75 (m, 1H); 5.05 (dd, 1H, *J* = 8.8 Hz, 1.2 Hz); 4.99 (dd, 1H, *J* = 5.2 Hz, 1.2 Hz); 4.57 (t, 1H, *J* = 3.6 Hz); 3.88-3.82 (m, 1H); 3.59 (ddd, 1H, *J* = 36.4 Hz, 9.6 Hz, 6.8 Hz); 3.51-3.46 (m, 1H); 2.46 (quint, 1H, *J* = 6.8 Hz); 1.86-1.77 (m, 1H); 1.74-1.65 (m, 1H); 1.61-1.48 (m,

4H); 1.03 (dd, 3H, *J* = 6.8 Hz, 4.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 141.4, 114.1, 98.9, 94.8, 72.2, 63.0, 62.2, 37.9, 30.8, 25.6, 19.9, 19.6, 16.8. FTIR-ATR (neat) ν (cm^{-1}) 3036, 2943, 1652, 1635, 1124, 1074, 1035, 965, 820, 754. HRMS-ESI (*m/z*) calcd for $C_{10}H_{18}O_2$: 193.1199 $[M+Na]^+$ found : 193.1201 $[M+Na]^+$.

(3*S*)-3-methyl-4-((tetrahydro-2*H*-pyran-2-yl)oxy)butan-1-ol (9). A 0.5M solution of 9-BBN (47.6 mL, 23.8 mmol, 2.2 eq.) was added dropwise to a cooled (0°C) solution of **8** (1.84 g, 10.8 mmol) dissolved in anhydrous THF (20 mL). The mixture was then slowly warmed to room temperature until **8** was consumed as evidenced by TLC (*ca* 1 h). The mixture was then cooled to 0°C before a premixed solution of 2M aqueous sodium hydroxide (16.2 mL, 32.4 mmol, 3 eq.) and aqueous 30% hydrogen peroxide (15.5 mL, 151 mmol, 14 eq.) was added dropwise. This mixture was then warmed to room temperature and stirred 1.5h before brine (100 mL) was added. The resulting mixture extracted with Et_2O (3x 50 mL), combined organic fractions were dried on sodium sulfate, concentrated *in vacuo* and the product purified by flash chromatography (Step gradient, 0, 10, 20, 30, 35, 40, 45, 50% EtOAc:Hex) to yield alcohol 9 (1.74 g, 86%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.59-4.56 (m, 1H); 3.86-3.80 (m, 1H); 3.70 (sept, 1H, *J* = 4.8 Hz); 3.66-3.55 (m, 2H); 3.52-3.47 (m, 1H); 3.28-3.18 (m, 1H); 2.49 (br s, 1H, O*H*); 1.90 (sept, 1H, *J* = 2.8 Hz); 1.83-1.75 (m, 1H); 1.72-1.48 (m, 7H); 0.93 (dd, 3H, *J* = 6.8 Hz, 1.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 99.2, 99.0, 73.4, 73.2, 62.4, 61.2, 38.0, 31.3, 30.6, 25.5, 19.6, 17.7. FTIR-ATR (neat) ν (cm^{-1}) 3389, 2937, 2871, 1454, 1352, 1200, 1119, 1060, 1023, 974, 902, 867, 810. HRMS-ESI (*m/z*) calcd for $C_{10}H_{20}O_3$: 211.1305 $[M+Na]^+$ found : 211.1308 $[M+Na]^+$.

2-((*S*)-4-iodo-2-methylbutoxy)tetrahydro-2*H*-pyran (10). Iodine (1.19 g, 4.70 mmol, 1.3 eq.) was added in portions to a solution of PPh_3 (1.14 g, 4.34 mmol, 1.2 eq.) and imidazole (369 mg, 5.43 mmol, 1.5 eq.) in DCM (15 mL) previously cooled to 0°C. This mixture was stirred 20 minutes at 0°C before a solution of **9** (681 mg, 3.62 mmol) in DCM (10 mL) was added dropwise over 10 minutes. The mixture was then warmed to RT and stirred vigorously for 3h. It was then diluted with DCM (25 mL), quenched with a 10% sodium thiosulfate solution (10 mL) and the resulting aqueous layer was extracted with DCM (3x 30 mL). The combined organic layers were washed with brine (15 mL), dried on sodium sulfate and concentrated *in vacuo*. Purification by flash chromatography (0-5% EtOAc:Hex) afforded **10** (1.00 g, 91%) as a colorless oil. (NOTE: product is light-sensitive and needs to be stored in a cold, dark room under inert atmosphere). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.55 (q, 1H, *J* = 3.6 Hz); 3.85-3.80 (m, 1H); 3.62-3.56 (m, 1H); 3.52-3.47 (m, 1H); 3.32-3.26 (m, 1H); 3.24-3.17 (m, 2H); 2.08-1.96 (m, 1H); 1.91-1.77 (m, 2H); 1.75-1.65 (m, 2H); 1.61-1.49 (m, 4H); 0.93 (dd, 3H, *J* = 6.8 Hz, 4.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 98.9, 72.0, 62.3, 38.2, 34.7, 30.8, 25.6, 19.6, 16.5, 5.1. FTIR-ATR (neat) ν (cm^{-1}) 2938, 2869, 1453, 1351, 1183, 1119, 1063, 1031, 973, 903, 868, 814. HRMS-ESI (*m/z*) calcd for $C_{10}H_{29}IO_2$: 321.0322 $[M+Na]^+$ found : 321.0328 $[M+Na]^+$.

(4S,6aS,8aS,9S,10R,11aS)-4-((tert-butyldiphenylsilyl)oxy)-6a,8a,9-trimethyl-10-((3S)-3-methyl-4-((tetrahydro-2H-pyran-2-yl)oxy)butyl)octadecahydro-1H-naphtho[2',1':4,5]indeno[2,1-b]furan-10-ol (12). Freshly titrated *t*-BuLi[38] (1.63 M in Pentane, 21.3 mL, 34.7 mmol, 4.05 eq.) was added dropwise to a mixture of **10** (5.12 g, 17.2 mmol, 2.0 eq.) previously dissolved in 13 mL anhydrous Et_2O and 19 mL anhydrous pentane at -78°C. This mixture was stirred for 25 min at -78°C until a solution of **5** (5.02 g, 8.58 mmol) in pentane (25 mL) / anhydrous THF (20 mL) was transferred dropwise *via canula*. The mixture was stirred vigorously at -78°C for 5 min, quenched with sat. aqueous NH_4Cl (50 mL), then warmed to room temperature. Water (75 mL) was added and the mixture was transferred to a separatory funnel. The aqueous layer was extracted with Et_2O (3x 75 mL) and the combined organic phases were dried over sodium sulfate and concentrated *in vacuo*. Purification by flash chromatography (0-30% EtOAc:Hex) afforded 12 (5.35 g, 82%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.68-7.66 (m, 4H); 7.43-7.34 (m, 6H); 4.58-4.53 (m, 2H); 3.87-3.83 (m, 1H); 3.61-3.48 (m, 3H); 3.21 (ddd, 1H, *J* = 25.6 Hz, 9.6 Hz, 6.0 Hz); 1.05 (s, 9H); 1.01 (d, 3H, *J* = 6.8 Hz); 0.94 (dd, 3H, *J* = 6.8 Hz, 4.8 Hz); 0.80 (s, 3H) 0.75 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 135.9, 135.1, 129.5, 127.5, 110.6, 98.9, 81.5, 77.4, 72.9, 62.9, 62.3, 56.4, 54.4, 44.9, 41.0, 40.0, 38.5, 37.1, 36.5, 35.6, 35.2, 33.6, 32.4, 31.8, 30.8, 28.7, 27.7, 27.1, 25.6, 21.1, 19.7, 19.3, 17.3, 16.5, 15.7, 12.5. FTIR-ATR (neat) ν (cm^{-1}) 3421, 2929, 2855, 1451, 1380, 1110, 1062, 1028, 903, 863, 818, 740, 701, 612. HRMS-ESI (*m/z*) calcd for $C_{48}H_{72}O_5Si$: 779.5041 $[M+Na]^+$ found : 779.5046 $[M+Na]^+$.

(4S,5'S,6aS,8aS,9S,10R,11aS)-5',6a,8a,9-tetramethyldocosahydrospiro[naphtho[2',1':4,5]indeno[2,1-b]furan-10,2'-pyran]-4-ol (17). *p*-Toluenesulfonic acid monohydrate (44.1 mg, 232 µmol, 0.02 eq) was added to a stirring mixture of **12** (8.77 g, 11.6 mmol) in anhydrous MeOH (350 mL) at 0°C. After 2 min, hemiketal **12** (R_f 0.5, 25% EtOAc:Hex) was fully transformed into **15** (R_f 0.85) as evidenced by TLC. The mixture was then warmed to room temperature. After 1 h, ketal **15** was fully transformed into spiroketal **16** (R_f 0.90) by TLC. A crystal of **16** suitable for X-ray analysis could obtained by *in vacuo* concentration of an aliquot at this stage followed by purification by flash chromatography then slow crystallization from MeOH. The mixture was then heated to 70°C. After 3h, TLC showed complete conversion of intermediate **16** to **17** (Rf 0.40). The mixture was then concentrated *in vacuo* and purified by flash chromatography (0-50% EtOAc:Hex) to yield **17** (4.19 g, 87%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.39 (q, 1H, *J* = 7.2 Hz); 3.94 (dd, 1H, *J* = 10.8 Hz, 2.4 Hz); 3.58 (sept, 1H, *J* = 4.8 Hz); 3.29 (d, 1H, *J* = 11.2 Hz); 1.07 (d, 3H, *J* = 7.2 Hz); 0.98 (d, 3H, *J* = 6.8 Hz); 0.81 (s, 3H); 0.75 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 109.7, 81.1, 71.4, 65.3, 62.2, 56.4, 54.5, 44.9, 42.3, 40.7, 40.2, 38.3, 37.1, 35.7, 35.3, 32.4, 31.7, 31.6, 28.7, 27.2, 26.1, 25.9, 21.2, 16.6, 16.2, 14.5, 12.5. FTIR-ATR (neat) ν (cm^{-1}) 3255, 2928, 2847, 1448, 1368, 1221, 1174, 1056, 983, 953, 920, 849, 774. HRMS-ESI (*m/z*) calcd for $C_{27}H_{44}O_3$: 439.3183 $[M+Na]^+$ found : 439.3178 $[M+Na]^+$.

(4S,5'S,6aS,8aS,9S,10R,11aS)-5',6a,8a,9tetramethyldocosahydrospiro[naphtho[2',1':4,5]indeno[2,1-b]furan-10,2'-pyran]-4-yl acetate (18). Acetic anhydride (7.60 mL, 80.5 mmol, 8.0 eq.) was added to a stirring mixture of **17** (4.19 g, 10.1 mmol) previously dissolved in pyridine (40 mL) at 50°C. This mixture was stirred at 50°C until **17** was fully consumed as shown by TLC (*ca* 2h), cooled to room temperature then poured into stirred ice-cold water (150 mL). The resulting suspension was stirred vigorously for 15 min while cooled in an ice bath then vacuum filtered using a Buchner funnel. The resulting solid

was washed with ice-cold water and further dried by vacuum filtration 15 min before it was dried *in vacuo* to yield **18** (4.35 g, 94%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.67 (sept, 1H, *J* = 4.8 Hz); 4.39 (q, 1H, *J* = 7.2 Hz); 3.94 (dd, 1H, *J* = 10.8 Hz, 2.8 Hz); 3.29 (d, 1H, *J* = 11.2 Hz); 2.01 (s, 3H); 1.07 (d, 3H, *J* = 7.2 Hz); 0.98 (d, 3H, *J* = 6.8 Hz); 0.83 (s, 3H); 0.75 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.8, 109.8, 81.0, 73.8, 65.2, 62.1, 56.3, 54.3, 44.7, 42.2, 40.6, 40.1, 36.8, 35.7, 35.2, 34.1, 32.2, 31.8, 28.6, 27.6, 27.2, 26.0, 25.9, 21.6, 21.1, 16.6, 16.2, 14.4, 12.4. FTIR-ATR (neat) ν (cm^{-1}) 2923, 2898, 2847, 1724, 1447, 1379, 1240, 1174, 1132, 1049, 1028, 987, 920, 854, 611. HRMS-ESI (*m/z*) calcd for $C_{29}H_{46}O_4$: 481.3288 $[M+Na]^+$ found : 481.3277 $[M+Na]^+$

(4S,6aS,8aS,11aS)-10-((S)-4-azido-3-methylbutyl)-6a,8a,9-trimethyl-2,2a,3,4,5,6,6a,6b,7,8,8a,8b,11a,12,12a,12b-hexadecahydro-1H-naphtho[2',1':4,5]indeno[2,1-b]furan-4-yl acetate (19). Dry LiBr (8.01 g, 92.2 mmol, 10.0 eq.) and **18** (4.23 g, 9.22 mmol) were loaded into a flame-dried 500ml round bottom flask. DCM (80 mL) and anhydrous MeCN (40 mL) were then added and the mixture stirred vigorously before BF_3•Et_2O (11.4 mL, 92.2 mmol, 10.0 eq.) was added dropwise. The reaction was stirred vigorously at RT for 3h until two slightly more polar spots (R_f 0.4 (major) and 0.35 (minor)) appeared**.** The mixture was slowly quenched with sat. aqueous $NaHCO_3$ (CAUTION : Vigorous gas generation) until no more bubbling occurred, diluted with water then extracted with DCM (3x 75 mL). The combined organic layers were washed with brine, dried on sodium sulfate then concentrated *in vacuo*. The crude mixture was then dissolved in DMF (40 mL) and stirred at 70°C for 2h before addition of sodium azide (1.80 g, 27.7 mmol, 3.0 eq.). The mixture was stirred an additional 2h at this temperature then cooled to room temperature, diluted with water and extracted with DCM (3x 75 mL). The combined organic phases were washed with brine (40 mL), dried on sodium sulfate then concentrated *in vacuo*. Purification by flash chromatography (0-15% EtOAc:Hex) yielded **19** (4.28 g, 96%) as a white waxy solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.74-4.64 (m, 2H); 3.23 (dd, 1H, *J* = 12.0 Hz, 5.6 Hz); 3.09 (dd, 1H, *J* = 12.0 Hz, 7.2 Hz); 2.45 (d, 1H, *J* = 10.0 Hz); 2.18-2.07 (m, 3H); 2.01 (s, 3H); 1.57 (s, 3H); 0.96 (d, 3H, *J* = 6.8 Hz); 0.83 (s, 3H); 0.65 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.5, 151.2, 103.9, 84.3, 73.5, 64.3, 57.6, 54.7, 54.2, 44.6, 43.5, 39.6, 36.7, 35.5, 34.9, 34.0, 33.0, 32.3, 31.4, 28.5, 27.4, 23.1, 21.4, 21.1, 17.4, 14.2, 12.2, 11.6. FTIR-ATR (neat) ν (cm^{-1}) 2915, 2845, 2095, 1731, 1449, 1367, 1237, 1029, 959, 896, 860, 664, 608. HRMS-ESI (*m/z*) calcd for $C_{29}H_{45}N_3O_3$: 506.3353 $[M+Na]^+$ found : 506.3340 $[M+Na]^+$.

Tomatidine acetate (20). Sodium iodide (2.93 g, 19.6 mmol, 2.2 eq) and **19** (4.30 g, 8.89 mmol) were dissolved in anhydrous MeCN (120 mL). This mixture was stirred for 30 min before a solution of freshly distilled trimethylsilyl chloride (2.60 mL, 20.4 mmol, 2.3 eq) in MeCN (20 mL) was added dropwise *via canula*. The resulting mixture was stirred for 30 min at room temperature, quenched with saturated aqueous sodium thiosulfate (20 mL), pH ajusted to 9-10 using 5% NaOH (8 mL) then concentrated under reduced pressure. The remaining suspension was extracted with chloroform (3x 75 mL). The combined organic layers were washed with brine, dried with sodium sulfate, then concentrated *in vacuo*. Purification by flash chromatography (0-40% EtOAc:Hex, 1% Et_3N) afforded **20** (2.73 g, 67%) and **21** (440 mg, 11%) as white solids. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.67 (sept, 1H, *J* = 4.8 Hz); 2.76-2.68 (m, 2H); 2.01 (s, 3H); 0.95 (d, 3H, *J* = 6.8 Hz); 0.85 (d, 3H, *J* = 6.4 Hz); 0.83 (s, 3H); 0.81 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.8, 99.2, 78.6, 73.8, 62.1, 55.8, 54.4, 50.4, 44.8, 43.2, 41.0, 40.3, 36.8, 35.7, 35.2, 34.1, 32.8, 32.3, 31.2, 28.7, 28.6, 27.6, 26.8, 21.6, 21.2, 19.5, 17.1, 16.0, 12.4. FTIR-ATR (neat) ν (cm^{-1}) 2919, 2847, 1727, 1451, 1365, 1237, 1132, 1030, 974, 888, 743, 660. HRMS-ESI (*m/z*) calcd for $C_{29}H_{47}NO_3$: 458.3629 $[M+H]^+$ found : 458.3630 $[M+H]^+$.

Tomatidine (1) A 3M aqueous solution of NaOH (9.94 mL, 29.8 mmol, 5.0 eq.) was added to a solution of **20** (2.73 g, 5.96 mmol) previously dissolved in DCM (25 mL) and MeOH (75 mL). The mixture was then stirred at room temperature until **20** was fully consumed as shown by TLC (2h). pH was then adjusted to 7-8 using 1N HCl (*ca* 12 mL) and the mixture was concentrated *in vacuo*, suspended in water (50 mL) then extracted with chloroform (3x 75 mL). The combined organic layers were dried on sodium sulfate and concentrated *in vacuo*. Purification by flash chromatography (10-60% EtOAc:Hex, 1% Et_3N) afforded Tomatidine **1** (2.35 g, 95%) as a white solid. A crystal suitable for X-ray analysis was obtained by slow crystallization from MeOH. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.12 (q, 1H, 8.4 Hz); 3.57 (sept, 1H, J = 4.8 Hz); 2.79-2.76 (m, 1H); 2.72 (t, 1H, *J* = 10.8 Hz); 2.01-1.95 (m, 1H); 0.95 (d, 3H, *J* = 7.2 Hz); 0.85 (d, 3H, *J* = 6.4 Hz); 0.81 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 99.2, 78.7, 71.4, 62.1, 55.9, 54.6, 50.4, 44.9, 43.2, 41.0, 40.4, 38.4, 37.1, 35.7, 35.2, 32.8, 32.5, 31.7, 31.2, 28.8, 28.7, 28.4, 26.8, 21.2, 19.5, 17.1, 16.0, 12.5. FTIR-ATR (neat) ν (cm^{-1}) 3330, 2916, 2852, 1445, 1382, 1137, 1050, 975, 900, 874, 787, 657, 628. HRMS-ESI (*m/z*) calcd for $C_{27}H_{45}NO_2$: 416.3523 $[M+H]^+$ found : 416.3520 $[M+H]^+$.

5,6-Dihydrosolasodine (22)

A 3M aqueous solution of NaOH (401 µL, 1.20 mmol, 5.0 eq.) was added to a solution of **21** (110 mg, 240 µmol) previously dissolved in DCM (1 mL) and MeOH (3 mL). The mixture was then stirred at room temperature until **21** was fully consumed as shown by TLC (2h). pH was then adjusted to 7-8 using 1N HCl (*ca* 1 mL) and the mixture was concentrated *in vacuo*, suspended in water (5 mL) then extracted with chloroform (3x 10 mL). The combined organic layers were dried on sodium sulfate and concentrated *in vacuo*. Purification by flash chromatography (10-60% EtOAc:Hex, 1% Et_3N) afforded 5,6-Dihydrosolasodine **22** (99 mg, 95%) as a white solid. A crystal suitable for X-ray analysis was obtained by slow crystallization from MeOH. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.28 (q, 1H, *J* = 7.6 Hz) 3.56 (sept, 1H, *J* = 4.8 Hz) 2.68-2.65 (m, 1H) 2.59 (t, 1H, *J* = 11.2 Hz) 2.02-1.95 (m, 1H) 1.88 (t, 1H, *J* = 7.2 Hz) 1.80-1.77 (m, 1H) 0.94 (d, 3H, *J* = 6.8 Hz) 0.83 (d, 3H, *J* = 6.0 Hz) 0.81 (s, 3H) 0.77 (s, 3H) ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 98.3, 79.2, 71.3, 62.9, 56.4, 54.5, 47.6, 44.9, 41.4, 40.9, 40.3, 38.3, 37.1, 35.7, 35.2, 34.0, 32.4, 32.2, 31.6, 31.2, 30.2, 28.7, 21.2, 19.4, 16.7, 15.4, 12.5 FTIR-ATR (neat) ν (cm^{-1}). 3358, 2930, 2846, 1673, 1447, 1347, 1129, 1065, 1047, 974, 957, 884, 774, 680. 670. HRMS-ESI (*m/z*) calcd for $C_{27}H_{45}NO_2$: 416.3523 $[M+H]^+$ found : 416.3527 $[M+H]^+$.

Acknowledgements

This work is dedicated to the memory of Alexandre Poirier. Financial support from PROTEO, the Cystic Fibrosis Foundation of Canada and Aligo Innovation is gratefully acknowledged. EM is a member of the FRQNT-funded PROTEO Network as well as the FRQS-funded Quebec Network for Drug Discovery.

Keywords: Tomatidine • Alkaloid • Staphylococcus Aureus • Spiroaminoketal • Natural Product

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