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Synthesis and biological evaluation of desmethylveramiline, a micromolar Hedgehog inhibitor

Guillaume Guerlet [a], Thomas Spangenberg [a], André Mann [a,*], Hélène Faure [b], Martial Ruat [b]

[a] Laboratoire d'Innovation Thérapeutique, UMR 7200 CNRS-Université de Strasbourg Faculté de Pharmacie, 74 route du Rhin, 67401 Illkirch, France
[b] Signal Transduction and Developmental Neuropharmacology group, Neurobiology and Development, CNRS UPR-3294, 1 Avenue de la Terrasse, 91198 Gif-Yvette, France

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ABSTRACT

Desmethylveramiline (**1**), an aza steroid analogue of veramiline was designed as a surrogate for cyclopamine, a reference antagonist of the Sonic Hedgehog (Shh) pathway. Desmethyveramiline (**1**) was prepared in seven steps from commercially available Fernholtz acid using the hydroformylation of a terminal olefine as the key step for the construction of the piperidine appendage. In two assays (i) the inhibition of the Shh-induced Gli-dependent luciferase activity in Shh-light2 cells, (ii) the inhibition of the SAG-induced differentiation of the mesenchymal C3H10T1/2 cells, desmethylveramiline (**1**) is an inhibitor in the μM range comparable to cyclopamine.

The Sonic Hedgehog (Shh) signaling pathway plays a pivotal role in embryogenesis and in adult tissues, including brain.[1–3] The activation of Shh signaling is initiated by the binding of the Shh ligand to the membrane receptor Patched (Ptc), which relieves the Ptc-mediated inhibition of the transmembrane protein Smoothened (Smo). Aberrant Shh signals have been reported to be involved in numerous cancers. Therefore it is commonly accepted that antagonists for Shh signaling have great potential in cancer therapy.[4–8] Cyclopamine, an alkaloid with a C-nor-D-homo steroid skeleton is the reference antagonist of the Shh signaling pathway. However cyclopamine has a poor biodisponibility and chemical stability. Furthermore, cyclopamine is readily converted to inactive veratramine in acidic media,[9] that precludes any oral administration as a drug. Interestingly, Tremblay's group have performed extensive transformations on the core structure of cyclopamine culminating in the discovery of IPI-926, a compound with improved pharmacokinetic properties.[10] In the past, other steroidal alkaloids such as solanidine and veramiline were mentioned for their teratogenic profile, but until now few data were available for their efficiency on the Shh signaling.[11] In our effort to discover new ligands interfering with Shh signaling,[12] we found of interest to prepare azasteroid **1**, a seco-analogue of cyclopamine, having a steroidal ring system and bearing at C22 a piperidine ring. Compound **1**, desmethylveramiline, was selected as target with the following rationales: (i) **1** is readily obtained from the Fernholtz acid[13] via an original approach (vide infra); (ii) **1** has a simple steroid frame in respect to the C-nor-D-homo steroid skeleton, but orients the nitrogen lone pair present in the piperidinyl ring, in similar spatial areas[14]; (iii) azasteroid of type **1** can be a surrogate for structurally more complex cyclopamine or jervine. In this Letter, we report the synthesis and the biological activity on the Shh signaling pathway of desmethylveramiline (**1**) (Fig. 1). The chemical strategy used is based on the hydroformylation of a terminal olefine.

Esterification of commercially available Fernholtz acid in methanol/H_2SO_4 provided ester **2** in 89% yield. Protection of the secondary alcohol at C3 with TBSOTf furnished **3** and subsequent LAH reduction of the ester function gave **4** in 95% (two steps). The desired aldehyde **5** was obtained as a white solid which matched reported analytical data, either by a Swern oxidation protocol (91%) or by using IBX (quant) (Scheme 1).[15] Next, **5** was subjected to a three components aza-Sakurai–Hosomi reaction under optimized conditions, a convenient route for the synthesis homallylamines from aldehydes, using a carbamate and allylsilane as partners.[16] If diastereoselectivity is a concern it has been shown that a substrate control is operating.[17] Indeed the reaction of Fernhotlz aldehyde (**5**), benzylcarbamate and allylsilane in presence of $BF_3{\cdot}Et_2O$ proceeded rapidly with an excellent diastereoselectivity at C22 (*dr* 95/5) and homoallylamine **6** was obtained with a good yield (87%). As the reaction mixture was allowed to reach room temperature, the TBS group on the secondary alcohol at C3 was cleaved in the meantime.

The formation of a water molecule during the amino-allylation and the presence of $BF_3{\cdot}Et_2O$ are accounting for this observed desilylation (fluoride ions are produced). The stereochemistry at C22, expected to be *S* (see below), could be explained by a modified

* Corresponding author.
E-mail address: andre.mann@unistra.fr (A. Mann).

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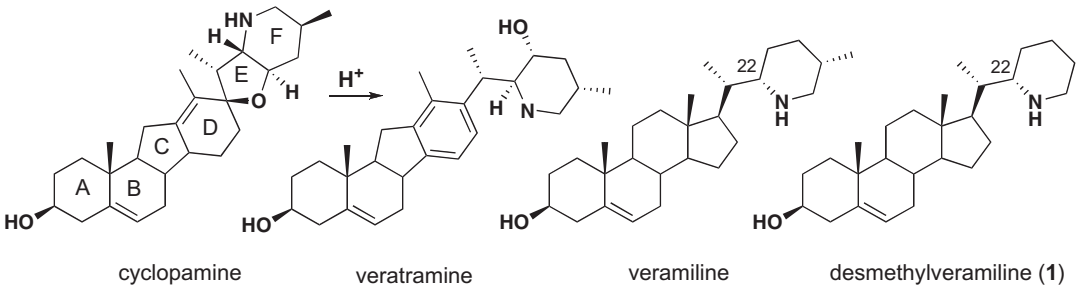
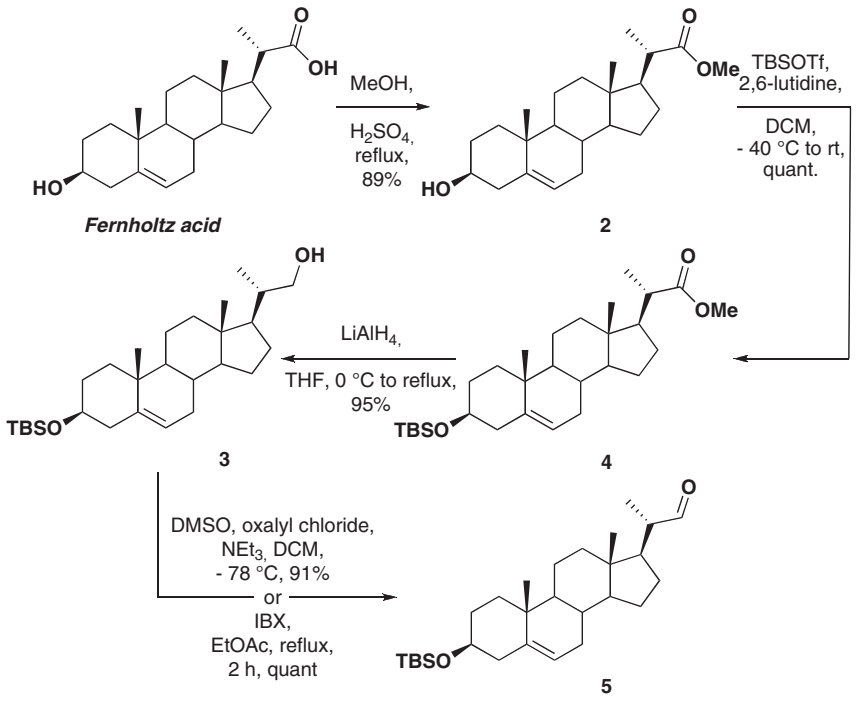
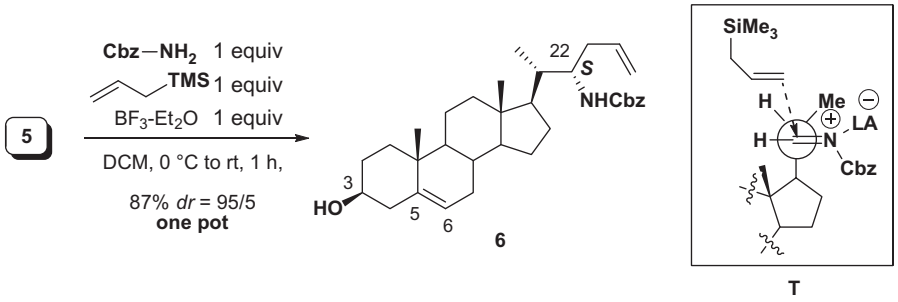


Figure 1. Steroidal alkaloids with a piperidine ring.



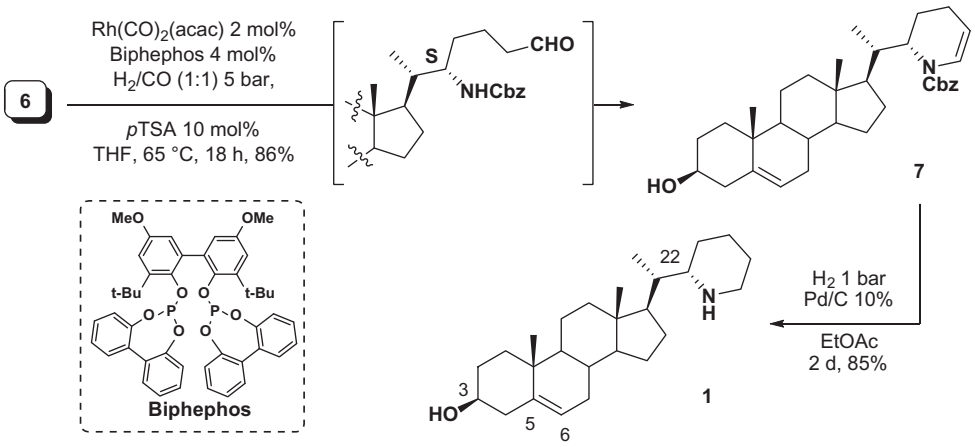
Scheme 1. Preparation of Fernholtz aldehyde **5**.



Scheme 2. 1,2-*syn*-diastereoselective aza-Sakurai–Hosomi reaction on Fernholtz aldehyde **5**.

Felkin–Anh model (the transition state **T** in the box, *Si* attack of the allylsilane, Scheme 2).[18]

Homoallylamines are attractive substrates for the construction of piperidines, indeed the missing carbon to build in the six membered heterocycle may be introduced by hydroformylation on the terminal olefine.[19,20] The control of the troublesome linear/branched ratio for the resulting aldehydes is now manageable by using Rh(I) as the metal and bulky bidendate phosphines or phosphites as the ligands.[21,22] Therefore the chemoselective hydroformylation of the terminal olefine in allylamine **6** was designed: the formation of the linear aldehyde followed by its intramolecular collapse with the resident Cbz carbamate to a piperidine ring were expected. An other hurdle may be anticipated with allylamine **6** when submitted to hydroformylation. As syngas is a mixture of H_2/CO: 1:1, the inertia of the C5–C6 double bound towards hydrogenation was questionable, but precedents show that even under



Scheme 3. Cyclohydrocarbonylation towards desmethylveramiline **1**.

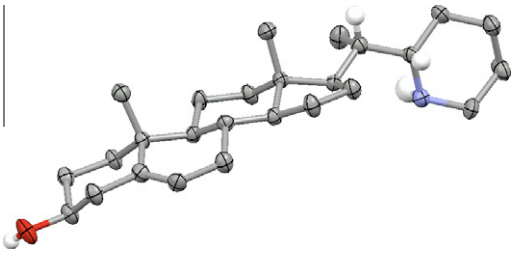
harsh conditions (120 bar, 120 °C), the internal double bound may remain intact (Scheme 3).[23] The unprotected secondary alcohol at C3 on homoallylamine **6** was also a possible chelating group for rhodium(I) present in the catalyst. In the past, we had some experience for linear hydroformylation and therefore we tested a standard catalytic mixture. Indeed when the allylamine **6** was submitted to hydroformylation in THF with a mixture of $Rh(CO)acac_2$, biphephos as ligand and 10 mol % of *p*-TSA as additive, the desired enamide **7** was obtained uneventfully as the only adduct in 86% yield. The isomeric branched aldehyde was not detected in our conditions. Indeed, in one step compound **7** is formed by the internal reaction of the homologated aldehyde with the NCbz carbamate (via a transient immonium). This sequence described as a cyclohydrocarbonylation (CHC) is now well documented. Recently our group has reported several examples using this strategy for the synthesis of natural compounds or biomolecules.[17,24–26]

In the next step, we planned to reduce in one pot the enamide function and hydrogenolyze the Cbz N-protection in **7**. Indeed, the use Pd/C (10% on C) as catalyst in presence of a blanket of hydrogen delivered desmethylveramiline **1** (85% yield) (Scheme 3). The relative stereochemistry at carbon C22 was definitively secured by single crystal X-ray crystallography,[27] and confirmed the rationale proposed for aza-Sakurai–Hosomi reaction of allylsilane and the Fernholtz aldehyde **5** (Scheme 4). Interestingly a recent report of the Giannis' group reported the syntheses of aminosteroids bearing piperidone at C20,[28] combining chiral sulfinylimine chemistry and metathesis. In this regard the present route to **1** is complementary in considering step and atom economy.

The biological evaluation of **1** on the Shh pathway was performed using the two following assays: (i) the Gli-dependent luciferase reporter assay in Shh-light2 cells incubated for 40 h with the active aminoterminal fragment of Shh (ShhN, 5 nM),[29] (ii) the alkaline phosphatase (AP) assaythat reflects the differentiation of the mesenchymal C3H10T1/2 cells stimulated by SAG (100 nM), a synthetic Smo agonist.[30] The cell-based bioassays were performed as described.[12] Compared to Cur61414, another reference Smo

Table 1
Comparison of the inhibition induced by desmethylveramiline (**1**), Cur61414 and cyclopamine on ShhN-induced luciferase activity in Shh-light2 cells and SAG-induced differentiation of C3H10T1/2 cells

	% of inhibition				
	Shh-light2			C3H10T1/2	
Compounds	0.3 μM	3 μM	10 μM	1 μM	10 μM
1	5 ± 4	47 ± 4	87 ± 1	13 ± 4	85 ± 4
Cur61414	89 ± 7	97 ± 3	92 ± 1	78 ± 1	99 ± 2
Cyclopamine	58 ± 7	96 ± 1	98 ± 2	71 ± 3	105 ± 1



Scheme 4. Crystal structure of **1** displayed with Mercury™ (one molecule of $CDCl_3$ and most hydrogens were omitted for clarity).[27]

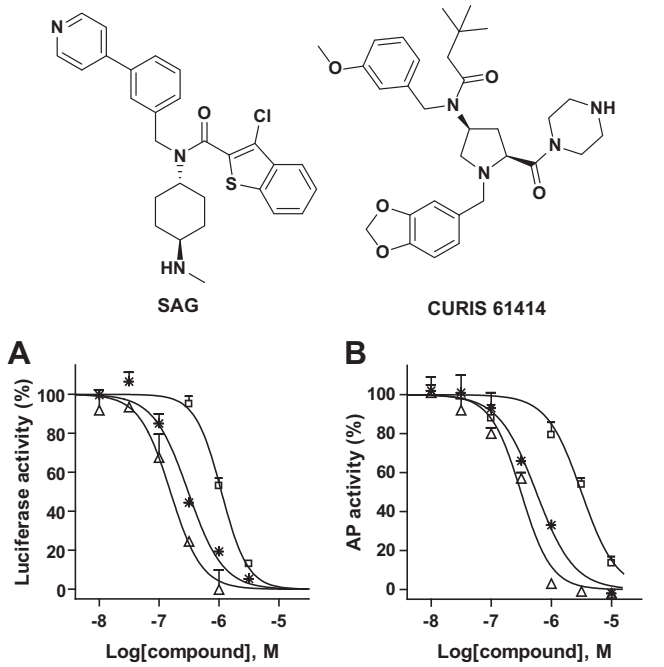


Figure 2. Dose dependent activity of **1** (open squares), Cur61414 (open triangle) and cyclopamine (stars): (**A**) ShhN-induced luciferase activity in Shh-light2 cells; Cur61414: IC_{50} = 0.1 μM; cyclopamine: IC_{50} = 0.3 μM; desmethylveramiline (**1**): IC_{50} = 1.1 μM. (**B**) SAG-induced differentiation of C3H10T1/2 cells; Cur61414: IC_{50} = 0.3 μM; cyclopamine: IC_{50} = 0.6 μM; desmethylveramiline (**1**): IC_{50} = 3.2 μM. The data shown are representative of independent experiments and are the means ± SEM of triplicates. The values are expressed as % of the maximal response.

antagonist[31] and cyclopamine, desmethylveramiline (**1**) is a micromolar inhibitor of the Shh signaling pathway as measured in the luciferase-based and the AP assays (Table 1). Interestingly aza-steroid (**1**) reveals an inhibitory activity towards Shh signaling in the same range than cyclopamine. In the Shh-light2 assay, desmethylveramiline (**1**) (IC_{50} = 1.1 μM) is three to four times less potent than cyclopamine itself (IC_{50} = 0.3 μM) (Fig. 2). These results demonstrate that aza-steroid **1**, missing some structural elements in respect to cyclopamine such as the C-nor-D-Homo substructure and the E-furane ring, can operate as a micromolar inhibitor of Shh signaling in the two distinct assays. Those results are in line with recent results reported by Winckler's group for a steroidal analogue of cyclopamine (Table 1).[14]

In conclusion we have shown that the combination of an aza-Sakurai-Hosomi with a hydroformylation reaction is a powerful tactic for the preparation of 25-desmethyl-veramiline (**1**), a steroidal alkaloid, in seven steps from Fernholtz acid (57% overall yield); from a biological point of view that **1** is a micromolar inhibitor on Shh signaling. It comforts the assumption that a well designed aza-steroid may match the activity of cyclopamine. Further studies on this topic are currently undertaken in our laboratory.[32]

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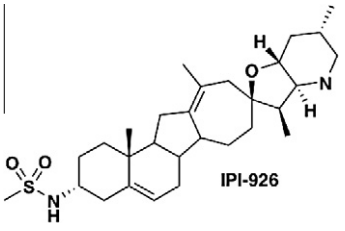
References and notes

1. Scales, S. J.; de Sauvage, F. J. *Trends Pharmacol. Sci.* **2009**, *30*, 303.
2. Traiffort, E.; Angot, E.; Ruat, M. *J. Neurochem.* **2010**, *113*, 576.
3. (a) Rudin, C. M.; Hann, C. L.; Laterra, J.; Yauch, R. L.; Callahan, C. A.; Fu, L.; Holcomb, T.; Stinson, J.; Gould, S. E.; Coleman, B.; LoRusso, P. M.; Von Hoff, D. D.; de Sauvage, F. J.; Low, J. A. N. *Engl. J. Med.* **2009**, *361*, 1173; (b) Martinez, M. C.; Larbret, F.; Zobairi, F.; Coulombe, J.; Debili, N.; Vainchenker, W.; Ruat, M.; Freyssinet, J.-M. *Blood* **2006**, *108*, 3012.
4. Beachy, P. A.; Karhadkar, S. S.; Berman, D. M. *Nature* **2004**, *432*, 324.
5. Lum, L.; Beachy, P. A. *Science* **2004**, *304*, 1755.
6. (a) Ruiz i Altaba, A.; Sanchez, P.; Dahmane, N. *Nat. Rev. Cancer* **2002**, *2*, 361; (b) Couvé-Privat, S.; Le Bret, M.; Traiffort, E.; Queille, S.; Coulombe, J.; Bouadjar, B.; Avril, M. F.; Ruat, M.; Sarasin, A.; Daya-Grosjean, L. *Cancer Research* **2004**, *64*, 3559.
7. (a) Borzillo, G. V.; Lippa, B. *Curr. Top. Med. Chem.* **2005**, *5*, 147; (b) Mahindroo, N.; Punchihewa, C.; Fujii, N. *J. Med. Chem.* **2009**, *52*, 3829; (c) Peukert, S.; Miller-Moslin, K. *ChemMedChem* **2010**, *5*, 500; (d) Herestsch, P.; Tzagkaroulaki, L.; Giannis, A. *Angew. Chem., Int. Ed.* **2010**, *49*, 3418; (e) Heretsch, P.; Tzagkaroulaki, L.; Athanassios Giannis, A. *Bioorg. Med. Chem.* **2010**, *18*, 6613.
8. Cooper, M. K.; Porter, J. A.; Young, K. E.; Beachy, P. A. *Science* **1998**, *280*, 1603.
9. Keeler, R. F. *Teratology* **1970**, *3*, 169.
10. Tremblay, M. R.; Lescarbeau, A.; Grogan, M. J.; Tan, E.; Lin, G.; Austad, B. C.; Yu, L.-C.; Behnke, M. L.; Nair, S. J.; Hagel, M.; White, K.; Conley, J.; Manna, L. D.; Alvarez-Diez, T. M.; Hoyt, J.; Woodward, C. N.; Sydor, J. R.; Pink, M.; MacDougall, J.; Campbell, M. J.; Cushing, J.; Ferguson, J.; Curtis, M. S.; McGovern, K.; Read, M. A.; Palombella, V. J.; Adams, J.; Castro, A. C. *J. Med. Chem* **2009**, *52*, 4400.



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11. Gaffield, W.; Keeler, R. F. *Pure Appl. Chem.* **1994**, *66*, 2407.
12. (a) Manetti, F.; Faure, H.; Roudaut, H.; Gorojankina, T.; Traiffort, E.; Schoenfelder, A.; Mann, A.; Solinas, A.; Taddei, M.; Ruat, M. *Mol. Pharmacol.* **2010**, *78*, 658; (b) Roudaut, H.; Traiffort, E.; Gorojankina, T.; Vincent, L.; Faure, H.; Schoenfelder, A.; Mann, A.; Manetti, F.; Solinas, A.; Taddei, M.; Ruat, M. *Mol. Pharmacol.* **2011**, *79*, 453.
13. Fernholtz acid was purchased from Steraloids Inc. (USA).
14. Winkler, J. D.; Isaacs, A.; Laura Holderbaum, L.; Tatard, V.; Nadia Dahmane, N. *Org. Lett.* **2009**, *11*, 2824.
15. Atot, M.; Schmidt, S. J.; Adams, J. L.; Dolle, R. E.; Kruse, L. I.; Frey, C. L.; Barone, J. M. *J. Med. Chem.* **1992**, *35*, 100.
16. Veenstra, S. J.; Schmid, P. *Tetrahedron Lett.* **1997**, *38*, 997.
17. Spangenberg, T.; Airiau, E.; Thuong, M. B. T.; Donnard, M.; Billet, M.; Mann, A. *Synlett* **2008**, 2859.
18. Loh, T.-P.; Xu, J.; Hu, Q.-Y.; Vittal, J. J. *Tetrahedron: Asymmetry* **1565**, *2000*, 11.
19. Ojima, I.; Tsai, C.-Y.; Tzamarioudaki, M.; Bonafoux, D. *Org. React.* **2000**, *56*, 1.
20. Breit, B.; Seiche, W. *Synthesis* **2001**, 1.
21. Billig, E.; Abatjoglou, A. G.; Bryant, D.R. US patent 4,668,651 **1987**; *Chem. Abstr.* **1987**, *107*, 7392.
22. Cuny, G. D.; Buchwald, S. L. *J. Am. Chem. Soc.* **1993**, *115*, 2066.
23. Toros, S.; Gemes-Pesci, I.; Heil, B.; Maho, S.; Tuba, Z. *J. Chem. Soc., Chem. Commun.* **1992**, *11*, 858.
24. Chiou, W.-H.; Schoenfelder, A.; Sun, L.; Mann, A.; Ojima, I. *J. Org. Chem.* **2007**, *72*, 9418.
25. Airiau, E.; Chemin, C.; Girard, N.; Lonzi, G.; Mann, A.; Petricci, E.; Salvadori, J.; Taddei, M. *Synthesis* **2010**, *17*, 2901.
26. Airiau, E.; Spangenberg, T.; Girard, N.; Schoenfelder, A.; Salvadori, J.; Taddei, M.; Mann, A. *Chem. Eur. J.* **2009**, *14*, 10938.
27. *Crystallographic data for* **1**: formula: $C_{26}H_{43}NO$, $CHCl_3$; space group: P 2_1 2_1 2_1; cell lengths: a: 7.3450(2); b: 14.1260(4); c: 26.1680(8); cell angles: α: 90.00; β: 90.00; γ: 90.00; cell volume: 2715.0 Z: 4 Z′: 0; R-factor (%): 6.0. The data have been registered at the Cambridge Crystallographic Data Bank with the following number CCDC 801379.
28. Fousteris, M. A.; Schubert, U.; Roell, D.; Roediger, J.; Bailis, N.; Nikolaropoulos, S. S.; Baniahmad, A.; Giannis, A. *Bioorg. Med. Chem.* **2010**, *18*, 6960.
29. Pascual, O.; Traiffort, E.; Baker, D. P.; Galdes, A.; Ruat, M.; Champagnat, J. *Eur. J. Neurosci.* **2005**, *22*, 389.
30. Chen, J. K.; Taipale, J.; Young, K. E.; Maiti, T.; Beachy, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 14071.
31. Williams, J. A.; Guicherit, O. M.; Zaharian, B. I.; Xu, Y.; Chai, L.; Wichterle, H.; Kon, C.; Gatchalian, C.; Porter, J. A.; Rubin, L. L.; Frank, Y.; Wang, F. Y. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 4616.
32. *Experimental for selected compounds*
(3β,20*S*)-pregn-5-ene-22*S*-benzylbut-24-enylcarbamate (**6**). In a flame dried flask under argon was introduced **21** (300 mg, 0.675 mmol) and anhydrous DCM (2.25 mL). Benzylcarbamate (102 mg, 0.675 mmol) was added and the mixture was cooled to 0 °C. Trimethylallylsilane (107 μL, 0.675 mmol) was added followed by the dropwise addition of freshly distilled trifluoroboron etherate (87 μL, 0.675 mmol). The mixture turns pink after 1 h at 0 °C then blue after allowing the temperature to reach room temperature for 1.5 h. Saturated aqueous $NaHCO_3$ was then added and the mixture was extracted with DCM (three times). The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (heptane/EtOAc 7:3) to yield a white solid (296 mg, 87%). R_f = 0.20 (heptane/EtOAc 7:3). Mp: 151–152° C. $[\alpha]_D^{20}$ −39.2 (*c* 1, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.33 (m, 5H), 5.72 (m, 1H), 5.33 (m, 1H), 5.07 (m, 2H), 4.97 (m, 1H), 4.47 (m, 1H), 3.86 (m, 1H), 3.50 (m, 1H), 0.98 (s, 3H), 0.86 (d, *J* = 6,8 Hz, 3H), 0.66 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.0 (CO), 140.9 (Cquat), 136.7 (Cquat), 135.3 (CH), 128.6 (CHAr), 128.1 (CHAr), 121.6 (CH), 117.1 (CH_2), 71.6 (CH), 66.9 (CH), 66.7 (CH_2), 56.7 (CH), 53.0 (CH), 50.2 (CH), 42.3 (CH_2–CH), 39.8 (CH_2), 39.0 (CH_2), 38.7 (CH), 37.4 (CH_2), 36.5 (Cquat), 32.0 (CH), 31.9 (CH_2), 31.6 (CH_2), 28.3 (CH_2), 24.3 (CH_2), 21.2 (CH_2), 19.5 (CH_3), 12.5 (CH_3), 11.8 (CH_3). HR-MS (ESI positive): calculated (M+1) 506.3629, found (M+1) 506.3615.
(3β,20*S*)-pregn-5-ene-22*S*-benzyl-25,26-dehydropiperidincarboxylate (**7**). In a dry autoclave under argon was introduced *para*-toluenesulfonic acid monohydrate (17 mg, 0.089 mmol). In a flame dried schlenk under argon was introduced $Rh(CO)_2$(acac) (4 mg, 0.019 mmol), anhydrous and degassed THF followed by biphephos (23 mg, 0.036 mmol). *CO evolution was observed*. Compound **22** (100 mg, 0.196 mmol) was then added and the mixture was transferred in the autoclave and the schlenk was rinsed three times with THF (V_{tot} = 11 mL). After three H_2/CO (1:1) flushing cycles, the pressure was set at 5 bar and the autoclave was heated at 65 °C (internal temperature) for 18 h. After cooling, the mixture was transferred into a flask and the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (heptane/EtOAc 7:3) to yield the desired enamide **23** as a white solid (296 mg, 87%). Mp: 128–129 °C. $[\alpha]_D^{20}$ −56.1 (*c* 1, $CHCl_3$). ^{1}H NMR (200 MHz $CDCl_3$): δ 7.29 (m, 5H), 6.88 (m, 1H), 5.33 (m, 1H), 5.17 (m, 2H), 4.88 (m, 1H), 4.19 (m, 1H), 3.52 (m, 1H), 1.25 (d, *J* = 6.8 Hz, 3H), 1.24 (s, 3H), 0.97 (s, 3H).^{13}C NMR (50 MHz $CDCl_3$): δ 154.1 (CO), 141.0 (Cquat), 136.6 (Cquat), 129.2 (C_{36}), 128.7 (CHAr), 126.0 (CHAr), 121.8 (CH), 106.6 (CH), 71.9 (CH), 67.7 (CH_2), 56.3 (CH), 55.7 (CH), 53.7 (CH), 50.3 (CH), 42.5 (CH_2), 39.8 (CH_2), 37.5 (CH_2), 36.7 (Cquat), 32.0 (CH CH_2), 31.8 (CH_2), 29.9 (CH_2), 28.0 (CH_2), 24.5 (CH_2), 21.2 (CH_2), 19.6 (CH_3), 16.3 (CH_3), 11.8 (CH_3).
25-desmethylveramiline (**1**)
In a flame dried flask under argon was introduced **23** (77 mg, 0.149 mmol) in anhydrous EtOAc (3 mL) and Pd/C 10% (15 mg). A hydrogen balloon was bubbled in the mixture for 1 min and the mixture was stirred for 2 days

under 1 atm of H_2. A filtration over a celite pad was performed and MeOH was used to rinse the solid waste. Solvents were removed under reduced pressure and the residue was purified by silica gel chromatography (DCM/MeOH 95:5 then 8:2 with 5% NEt_3) to yield the desired product as a white solid (44 mg, 85%). Mp: decomposition (>200 °C). $[\alpha]_D^{20}$ −48.6 (*c* 1, $CHCl_3$). ^1H NMR (400 MHz $CDCl_3$): δ 5.32 (br d, *J* = 5.3 Hz, 1H), 3.51 (m, 1H), 3.15 (br d, *J* = 12.2 Hz, 1H), 2.57 (m, 2H), 2.26 (m, 2H), 1.97 (m, 2H), 1.83 (m, 4H), 1.61–1.25 (m, 16H), 1.18 (td, *J* = 12.2, 4.5 Hz, 1H), 1.07 (m, 3H), 0.99 (s, 3H), 0.93 (d, *J* = 6.1 Hz, 3H), 0.67 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 141.2 (Cquat), 121,6 (CH), 71.7 (CH), 59.8 (CH), 56.9 (CH), 52.7 (CH), 50.4 (CH), 47.6 (CH_2), 42.4 (CH_2), 40.9 (CH), 39.9 (CH_2), 37.5 (CH_2), 36.6 (CH), 32.0 ($CH–CH_2$), 31.9 (CH_2), 31.0 (CH_2), 28.0 (CH_2), 26.5 (CH_2), 25.3 (CH_2), 24.3 (CH_2), 21.3 (CH_2), 19.6 (CH_3), 13.5 (CH_3), 11.8 (CH_3). HR-MS (ESI positive): calculated (M+1) 386.3417, found (M+1) 386.3425.