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Synthesis and Biological Evaluation of Cremastrine and an Unnatural Analogue

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Abstract

In this Letter, we describe the first total synthesis of cremastrine, a pyrrolizidine alkaloid from *Cremastra appendiculata*, with anticholinergic activity as well as an unnatural analogue. The streamlined synthesis proceeds in 9 steps, 7 steps longest linear sequence, in 25.2% overall yield, and features novel methodology to construct the pyrrolizidine core. Biological evaluation of cremastrine and the unnatural analogue indicated that both are *pan*-mAChR functional antagonists.

Keywords

pyrrolizidine; cremastrine; anticholinergic; sulfinimine; Mitsunobu

Recently, Ikeda and co-workers reported on the isolation and characterization of cremastarine (**1**), a pyrrolizidine alkaloid from the Japanese tuber *Cremastra appendiculata*, a species widely used in traditional medicine in Asia for a number of therapeutic indications.[1] Preliminary biological investigation, employing a [^{3}H]-NMS binding assay, indicates that **1** is a moderately selective muscarinic acetylcholine receptor subtype 3 (M_3) ligand (M_1 K_i = 505 nM, M_2 K_i >5,000 nM M_3 K_i = 126 nM, M_4 K_i = 498 nM, M_5 K_i = 1,220 nM) an interesting pharmacological profile if the binding profile translated into functional antagonism.[1,2] As such, **1** would have the potential to treat irritable bowel syndrome, chronic obstructive pulmonary disease and asthma.[2] Based on our lab's interest in muscarinic drug discovery[3] and our recently developed methodology to rapidly construct enantiopure idolizines, pyrrolo[1,2-*a*]azepines and pyrrolo[1,2-*a*]azocines,[4] application of this methodology to the synthesis of the related pyrrolizidine core seemed warranted. In this letter, we report the first total synthesis of cremastarine (**1**) employing a new synthetic strategy, distinct from previous pyrrolizidine syntheses,[5–8] (Scheme 1), that afforded **1** in 7 total synthetic steps and an overall yield of 25.2% that enabled biological evaluation.

Efforts first focused on the synthesis of the α-hydroxy carboxylic acid **3**. Conversion of D-isoleucine **6** to α-hydroxy carboxylic acid **3** was accomplished following the Shin procedure

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wherein the α-amino acid is converted to the α-hydroxy acid with retention of stereochemistry.[9,10] Treatment of **6** with aqueous nitrous acid leads to diazotization of the α-amine stereocenter followed by hydrolysis to furnish the enantiopure α-hydroxy acid **3**.[9,10] Treatment of acid **3** with excess *tert*-butyldimethylsilyl chloride, followed by basic hydrolysis, provides coupling partner **7** in 65% yield over 3 steps, without epimerization and in agreement with literature precedent (Scheme 2).[9]

With **7** in hand, we then targeted the synthesis of the pyrrolizidine core of **1** employing our new methodology for enantiopure azacine construction employing Ellman sulfinimine technology via **4**.[4,11] Requisite aldehyde **10** is commercially available, but we also developed two complimentary routes to access the key aldehyde precursor **10** to sulfinamide **4** (Scheme 3). 4-Pentenal **8** was protected as the 1,3-dioxane, followed by oxonolysis to provide **10** in 78% yield. Alternatively, bromide **9** was converted to the nitrile congener, and reduced with DIBALH to deliver **10** in 84% yield. Condensation of commercially available (*R*)-*tert*-butanesulfinamide and aldehyde **10** gives chiral aldimine **4** in 93% yield.

From aldimine **4**, we next sought to install two stereocenters through an indium mediated allylation using an appropriately functionalized allyl bromides (Table 1).[12] Interestingly, esters and free hydroxyl moieties afforded poor conversion (30–55%), but good dr (3:1 to >6:1). Ultimately, good yield and moderate diastereoselectivity was observed when TBS-ether **5** was used providing **11** (85% conversion, >4:1 *dr*), and the stereochemistry assigned based on the established literature precedent of a six-membered ring chelation control model.[13,14] For large scale preparations, we found that stirring and sonication of the indium metal and bromide before adding the aldimine was crucial to deliver homoallylic sulfinamide **11c** with yields comparable to small-scale reactions.

Hydroboration-oxidation of the terminal olefin of **11c** furnished primary alcohol **12** in excellent yield. Initial attempts to form the substituted pyrrolidine ring system involved mesylation of the alcohol and displacement in a 5-exo-*tet* cyclization fashion. This was unfortunately unsuccessful, but under Mitsunobu conditions, we successfully obtained substituted pyrrolidine **13**. NOESY-NMR data confirms the *syn*-stereochemical relationship of the two chiral centers on the pyrrolidine ring. From here, TBAF deprotection of the tert-butyldimethylsilyl ether gave primary alcohol **14**. Deprotection of the acetal and the sulfinyl exposed the aldehyde and amine, respectively. This was theorized to undergo an intramolecular condensation to form the imine, upon which an addition of a reducing agent would produce **2**,[11–13] the pyrrolizidine core structure. Both classical conditions,[11–13] and a variety of acids (TFA, HCl, HOAc) and reducing agents (Et_3SiH, $NaBH(OAc)_3$, $NaBH_3CN$) failed to provide the desired product **2** (Scheme 4).

Due to the failure of this key transformation, we presumed that the presence of the free hydroxyl in the protic environment led to undesired side reactions leading to very polar, complex mixtures. Therefore, we modified our approach to cyclize after formation of the ester bond. As shown in Scheme 5, an EDCI/DMAP mediated coupling between acid **7** and alcohol **14** delivered ester **15** in excellent yield. Application of the classical deprotection/reductive amination conditions (TFA:H_2O (95:5) followed by triethyl silane as the reducing agent) afforded both global deprotection of **15** as well as intramolecular condensation/reductive amination to furnish the natural product cremastrine (**1**) in 80% yield from ester **15**. Our synthetic **1** was in agreement with the data reported for natural **1**;[15] however, only tabular NMR data is available and authentic samples could not be acquired. Thus, the first total synthesis of **1** has been completed in seven steps (five steps longest linear sequence) with an overall yield of 25.2% from commercial starting materials. This expedited route also allowed for the preparation of quantities of **1** to support biological studies.

Following the route in Scheme 2, we also prepared a benzyl protected congener **16** of **7**, to explore strucutre-activity relationships (SAR) with a non-hydrolyzable protecting group in place of the free hydroxyl of **1**. As shown in Scheme 6, unnatural analog **17** was readily prepared and once again in high overall yield (26.1%).[16] Unnatural analogue **17** also provided confidence for the synthesis of **1** (since we were unable to acquire NMRs or an authentic sample), as spectral data for **17** agreed well with synthetic and natural **1**.

As cremastrine (**1**) was reported to display modest selectivity for binding to the M_3 mAChR (2- to 20-fold),[1] we evaluated both our synthetic **1** and unnatural analog **17** against all five human mAChRs (M_1–M_5) in a functional assay to determine if the modestly selective M_3 binding would translate into selective M_3 functional antagonist activity.[17] Interestingly, we found that **1** was a *pan*-mAChR functional antagonist (Fig. 1), moderately inhibiting M_1–M_5 (M_1 IC_{50} = 2.8 μM, M_2 IC_{50} > 10 μM, M_3 IC_{50} > 10 μM, M_4 IC_{50} > 10 μM, M_5 IC_{50} = 4.0 μM), with a preference for M_1 and M_5, and very weak acitivty at M_3. Thus, **1** is not a selective M_3 antagonist. Unnatural analog **17** was more potent and selective than **1** (M_1 IC_{50} = 1.9 μM, M_2 IC_{50} > 10 μM, M_3 IC_{50} = 5.0 μM, M_4 IC_{50} = 5.2 μM, M_5 IC_{50} = 2.0 μM), but still categorized as a *pan*-mAChR antagonist. These data suggest that the azabicyclo[3.3.0]octane ester chemotype is comparable to the *pan*-mAChR antagonist tropane ester chemotypes, such as atropine.[15] However, this data is still intriguing and the synthesis and evaluation of additional unnatural analogs would be of scientific value.

In summary, we have successfully extended our methodology to rapidly construct enantiopure idolizidines, pyrrolo[1,2-*a*]azepines and pyrrolo[1,2-*a*]azocines,[4] to the synthesis of pyrrolizidine alkaloids. Here, we completed the first total synthesis of the pyrrolizidine alkaolid cremastrine (**1**) in seven steps from commercial materials and in 25.2% overall yield. The methodology also allowed for the synthesis of unnatural analog such as **17**. Moreover, we demonstrated in mAChR functional assays, that both **1** and unnatural **17** are not selective M_3 antagonists, as previous binding data suggested, but rather *pan*-mAChR antagonists. This concise synthesis will allow for the synthesis of additional unnatural analogs of **1** for biological evaluation as well as for the synthesis of related pyrrolizidine-based alkaloid natural products. These efforts are in progress and will be reported in due course.

Acknowledgments

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15. A solution of ester **15** (50 mg, 0.0914 mmol) in 2 mL of 95:5 trifluoroacetic acid:H2O was stirred for 1 hr at room temperature then 0.19 mL of triethyl silane was added. This solution was stirred overnight and concentrated *in vacuo* to give a crude, yellow oil. This was dissolved in 1 mL DMSO and purified by reverse phase chromatography (2% to 45 H_2O(0.1%TFA):AcCN) to give (19 mg, 80% yield) as a colorless oil. [α]D^{20} = −15.2 (*c* = 0.97 $CHCl_3$; [α]D^{20} = −27.2 (*c* = 1.0, EtOH); ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm) = 6.87 (m, 1H), 4.49 (d, *J* = 5.2, 1H), 4.33 (m, 1H), 4.20 (m, 1H), 4.18 (m, 1H), 3.55 (m, 1H), 3.49 (*t*, *J* = 7 Hz 1H), 2.88 (m, 1H), 2.8 (m, 1H), 2.65 (*dt, J* = 8.1, 1H), 1.99 (m, 1H), 1.90 – 1.80 (brm, 4H), 1.72 (m, 2H), 1.59 (m, 1H), 1.42 (m, 1H), 1.1 (*d, J* = 4 Hz, 3H), 0.99 (t, J = 7 Hz, 3H). ^{13}C NMR (100.6 MHz, pyr-d_5) δ (ppm): 174.6, 72.6, 68.1, 63.1, 55.38, 53.27, 39.9, 39.46, 26.64, 26.2, 25.93, 24.78, 15.81, 11.75; HRMS (TOF, ES+) $C_{14}H_{26}NO_3$ [M+H]+ calc'd 256.1913, found 256. 1911.
16. A solution of ester (50 mg, 0.0914 mmol) in 2 mL of 95:5 trifluoroacetic acid:H_2O was stirred for 1 hr at room temperature then 0.19 mL of triethyl silane was added. This solution was stirred overnight and concentrated *in vacuo* to give a crude, yellow oil. This was dissolved in 1 mL DMSO and purified by reverse phase chromatography (2% to 45% H_2O(0.1%TFA):AcCN) to give (19 mg, 80% yield) as a colorless oil. [α]D^{20} = (*c* = 1.12, $CHCl_3$); ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm) = 7.33 (m, 5H), 4.66 (d, *J* = 11.49, 1H), 4.38 (d, *J* = 11.50, 1H) 4.15 (dt, *J* = 11.26, 4.89 Hz, 2H), 3.74 (d, *J* = 5.65 Hz, 1H), 3.67 (m, 1H), 3.46 (m, 1H), 3.11 – 2.92 (m, 3H), 2.41 (sextet, *J* = 6.77 Hz, 1H), 2.01 (m, 1H), 1.88 – 1.78 (m, 3H), 1.58 (m, 2H), 1.25 (m, 3H), 0.91 (d, *J* =6.77 Hz, 3H), 0.87 (t, *J* = 7.91, 3H); ^{13}C NMR (100.6 $CDCl_3$) δ (ppm): 172.44, 137.48, 128.37, 127.86, 82.65, 72.53, 64.69, 62.72, 61.18, 44.29, 41.49, 38.00, 31.30, 29.68, 28.75, 28.05, 24.71, 15.17, 11.28; HRMS (TOF, ES+) 346.2382, found 346.2381.
16. For full details of the mAChR functional assay see: Lebois EP, Bridges TM, Dawson ES, Kennedy Jp, Xiang Z, Jadhav SB, Yin H, Meiler J, Jones CK, Conn PJ, Weaver CD, Lindsley CW. ACS Chemical Neurosci. 2010; 1:104–121.
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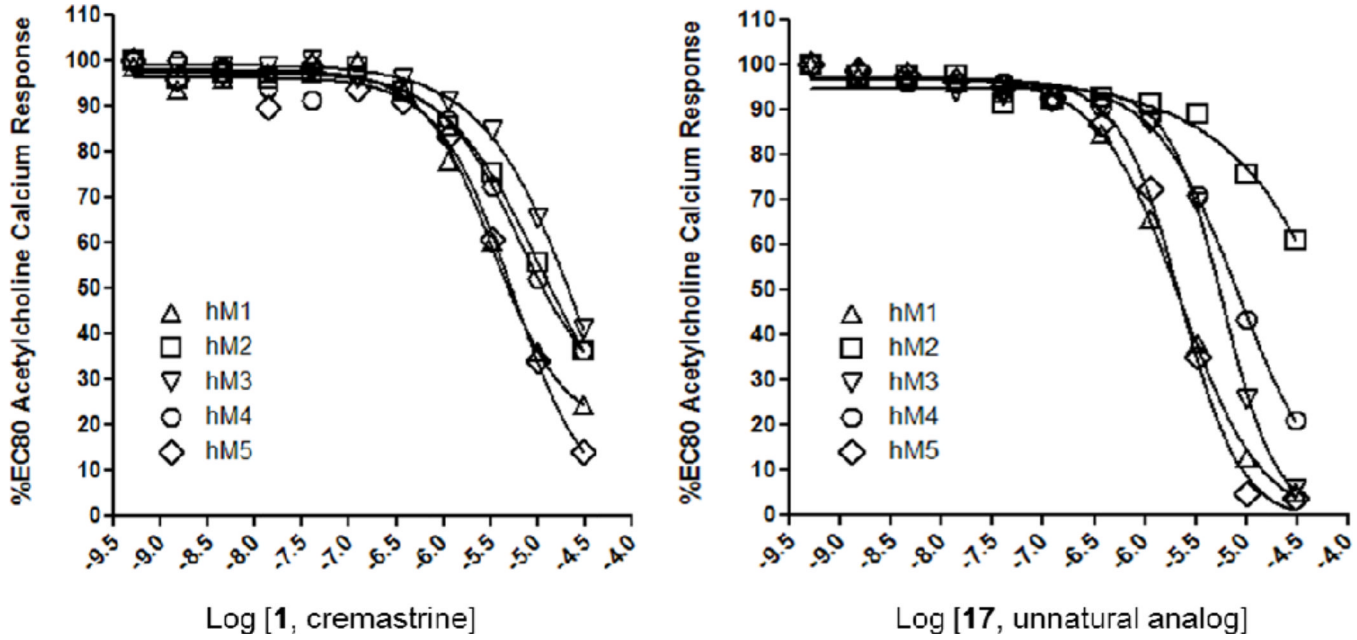
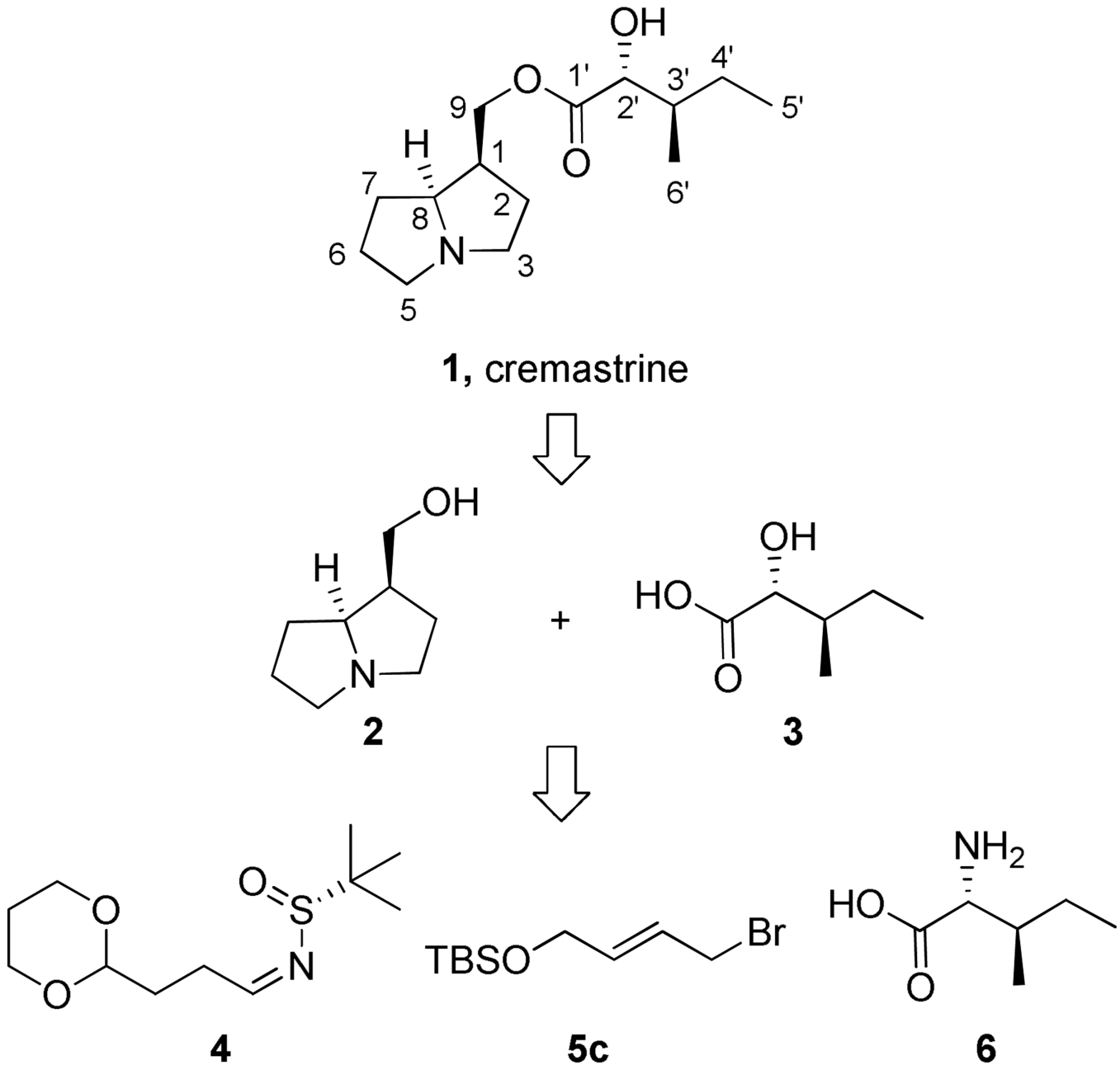
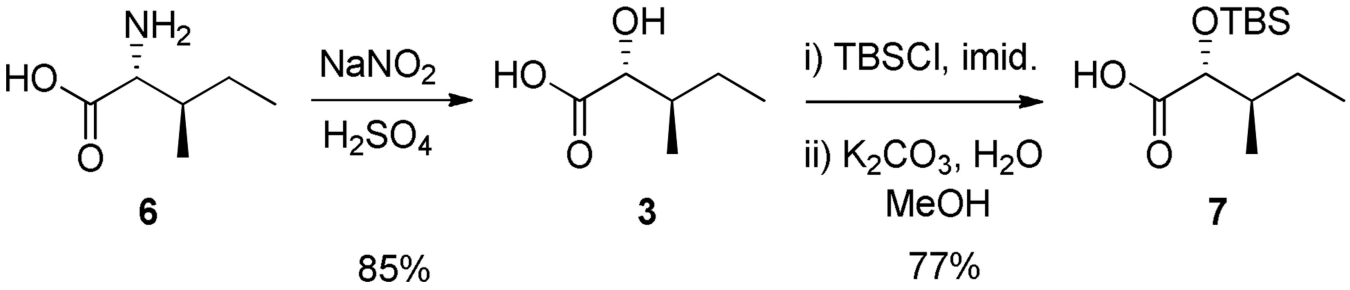


Figure 1.

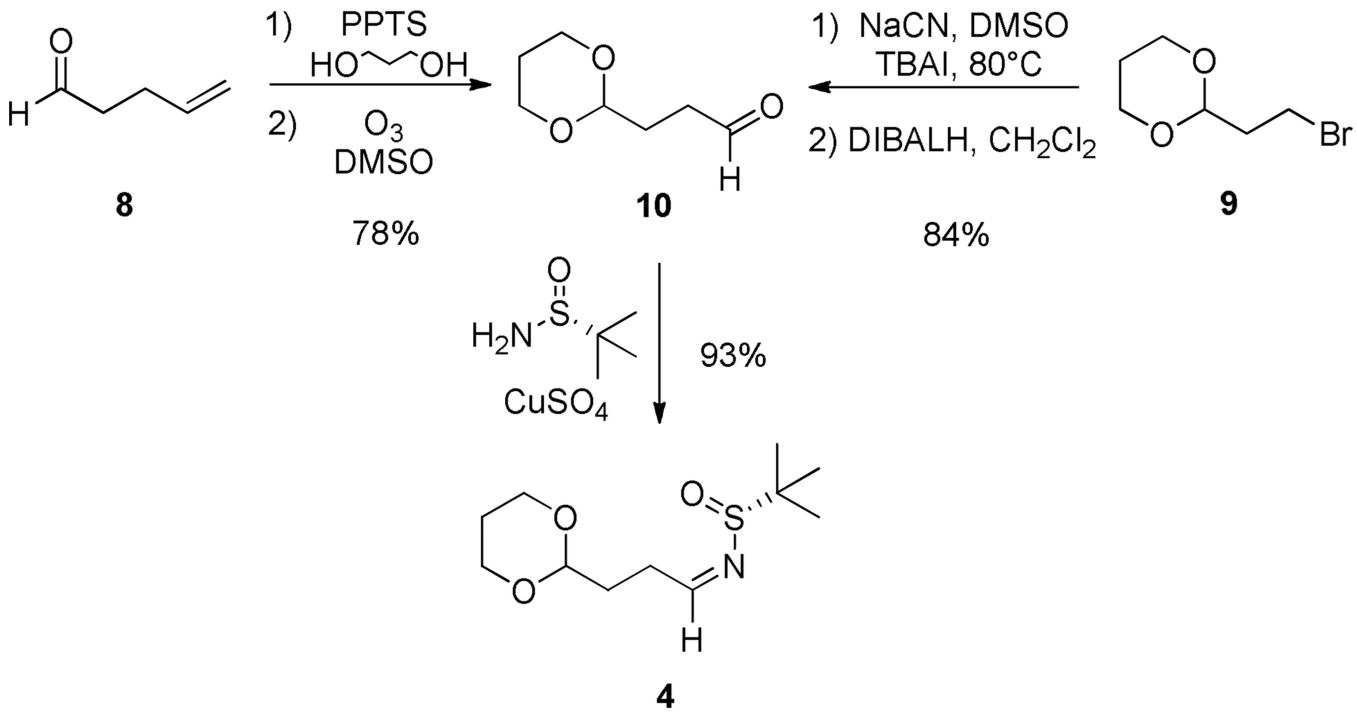
Human mAChR (M_1–M_5) functional assay with cremastrine **1** and unnatural analog **17**, indicating that both are functional antagonists of all five mAChRs.



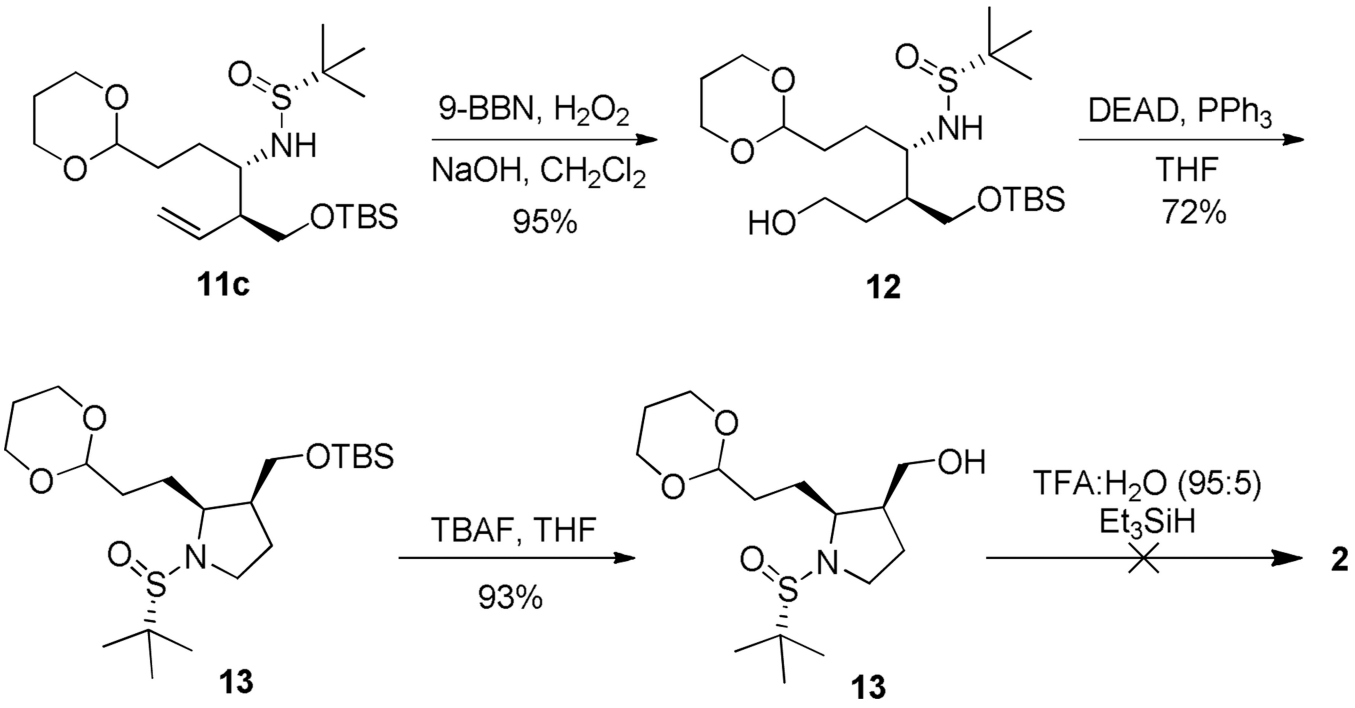
Scheme 1.
Retrosynthetic analysis of cremastrine (**1**).



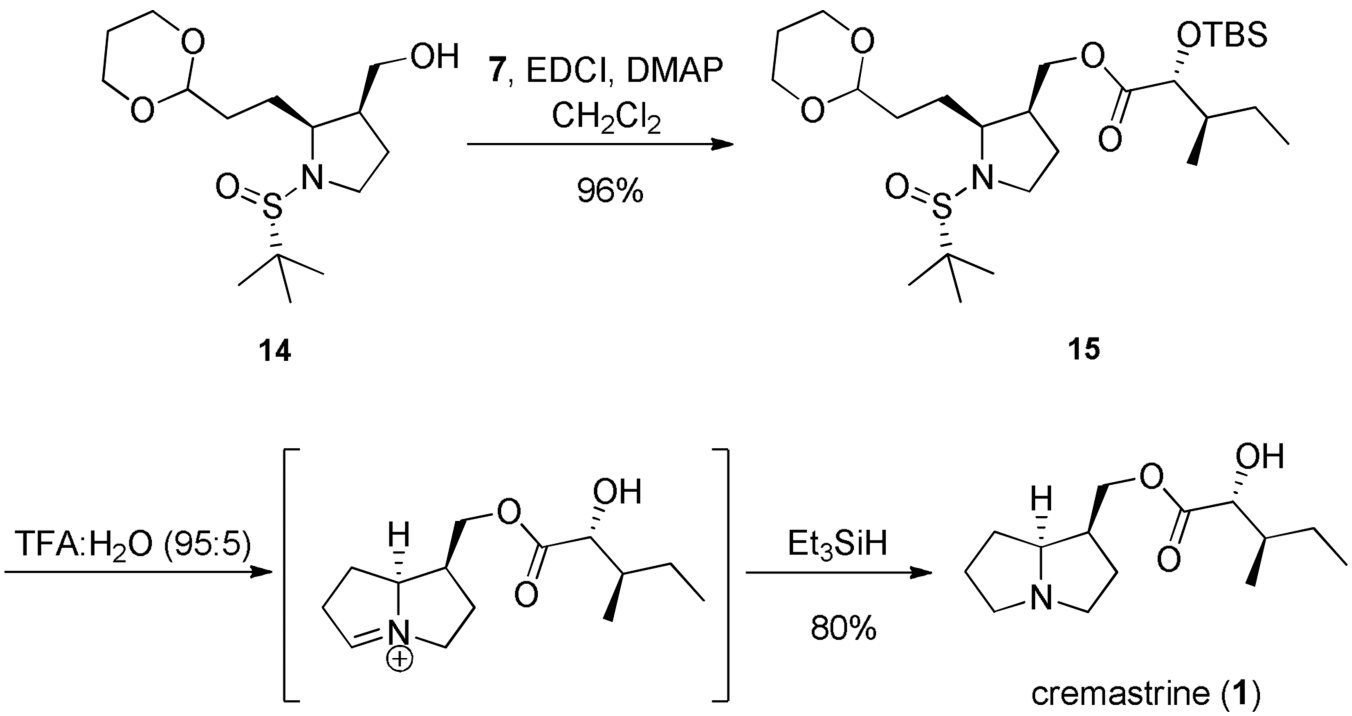
Scheme 2.
Synthesis of α-hydroxy acid **3** and protected congener **7**.



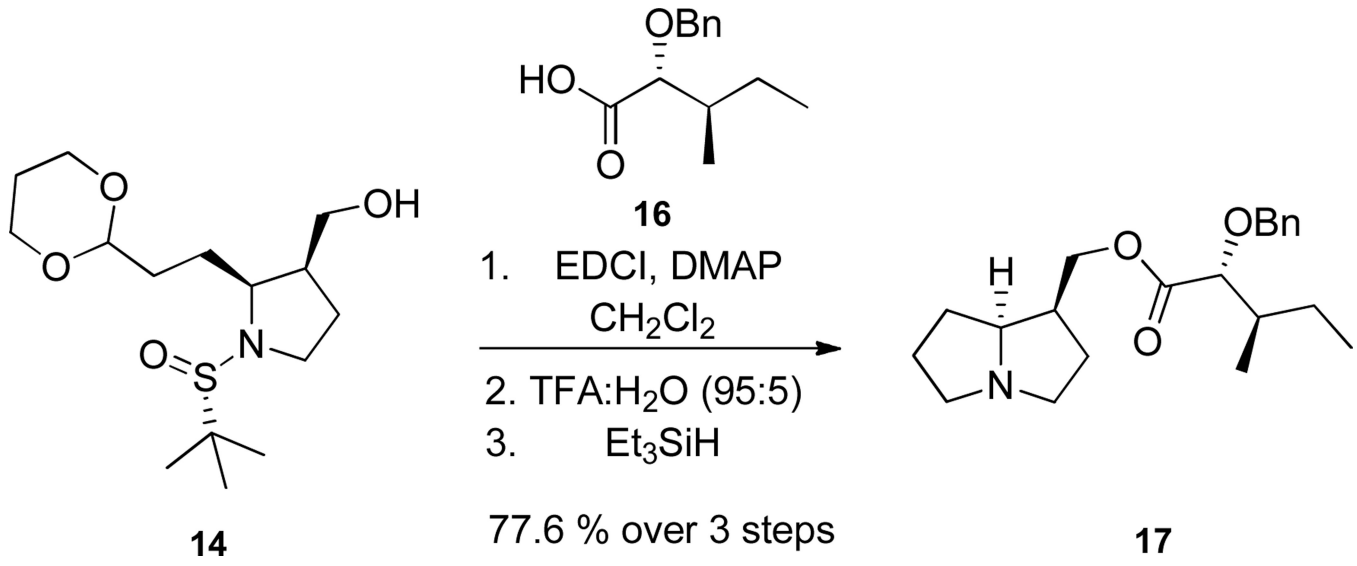
Scheme 3.
Two approaches for the synthesis of sulfinamide **4**.



Scheme 4.
Attempted synthesis of pyrrolidine core **2**.



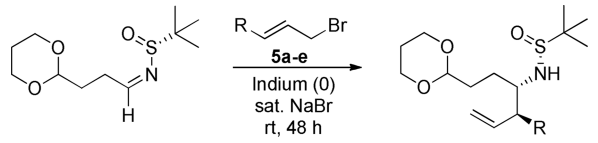
Scheme 5.
Completion of the synthesis of cremastrine (**1**).



Scheme 6.
Synthesis of unnatural analog **17**.

Table 1

In(0) allylations of sulfinamide (**4**) with diverse allyl bromides.



4 **11a-e**

entry	R	bromide	sulfinamide	yield (%)[a]	*dr*[b]
1	CO_2Me	**5a**	**11a**	55	>6:1
2	CO_2Et	**5b**	**11b**	50	>6:1
3	CH_2OTBS	**5c**	**11c**	85	4:1
4	CH_2OPMB	**5d**	**11d**	0	-
5	CH_2OH	**5e**	**11e**	31	3:1