# The first total synthesis of aplysamine 6, an inhibitor of isoprenylcysteine carboxy methyltransferase

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## ARTICLE INFO

## ABSTRACT

The first total synthesis of aplysamine 6, an inhibitor of isoprenylcysteine carboxy methyltransferase (Icmt), was accomplished in an overall high yielding reaction sequence.

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The C-terminal isoprenylcysteine motif of the CaaX proteins targets a variety of eukaryotic proteins to a series of post-translational modifications which are important for their localization and function.[1,2] The protein farnesyltransferase or protein geranylgeranyltranferase type I catalyzes the covalent attachment of a 15-carbon farnesyl or a 20-carbon geranylgeranyl lipid to the cysteine of the CaaX motif to initiate the processing.[3] After prenylation and followed by removal of three C-terminal amino acids, the C-terminal isoprenylcysteine is methylated by isoprenylcysteine carboxy methyltransferase (Icmt).[4]

Proteins that terminate in a CaaX motif regulate a number of important pathways in oncogenesis. The best studied example is the central role of the Ras family of proteins in growth factor activation of the MAP kinase signaling cascade, and in addition, many cancers contain alterations upstream of Ras, and the resultant hyperactivation of Ras is thought to contribute to tumorigenesis in these cancers as well.[4] Recent studies using genetic disruption of Icmt have revealed that Ras proteins are significantly mislocalized and tumorigenesis is markedly impaired in cells that lack Icmt.[5]

With emerging evidence for the importance of Icmt-catalyzed CaaX protein methylation in oncogenesis, there is a growing need for specific pharmacological agents to target this process. The development of Icmt inhibitors is a new approach to find anticancer drugs.[6] In order to discover new Icmt inhibitors, bioassay-directed purification of extracts of the sponge *Pseudoceratina* sp. (Pseudoceratinidae) afforded a new bromotyrosine derivative,

aplysamine 6 (**1**).[6] Aplysamine 6 contains one bromotyrosine unit and one bromomethoxycinnamoyl unit and shows inhibition of Icmt with an $IC_{50}$ of 14 μM (assay performed in duplicate on four independent days), and is considered a new addition to the small list of inhibitors of Icmt.[6] Herein, we wish to report the first total synthesis of aplysamine 6.

Our synthetic strategy commenced with the synthesis of compound **4** and is outlined in Scheme 1. The conversion of *p*-anisaldehyde **2** to the known bromobenzaldehyde **3**[7] required an extended reaction time of 24 h. In turn, **3** was subjected to Doebner–Knoevenagel condensation[8] with malonic acid in the presence of pyridine and piperidine to afford cinnamic acid **4** in 92% yield.

The bromination of the known 4-hydroxyphenethylamine **7**,[9] prepared from **5**, was carried out in methanol with bromine at 60 °C to afford the corresponding dibromide **8** in a quantitative yield (Scheme 2). Having the desired dibromide **8** in hand, we next focused on the chemoselective acylation of dibromide **8**. The acid **4** was transformed into the corresponding acid chloride with the aid
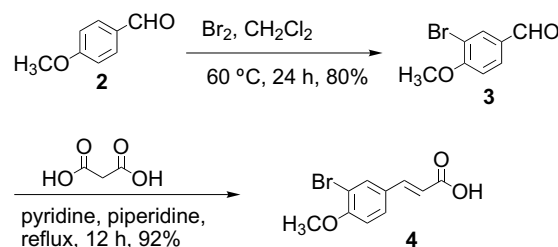


**Scheme 1.** Synthesis of compound **4**.

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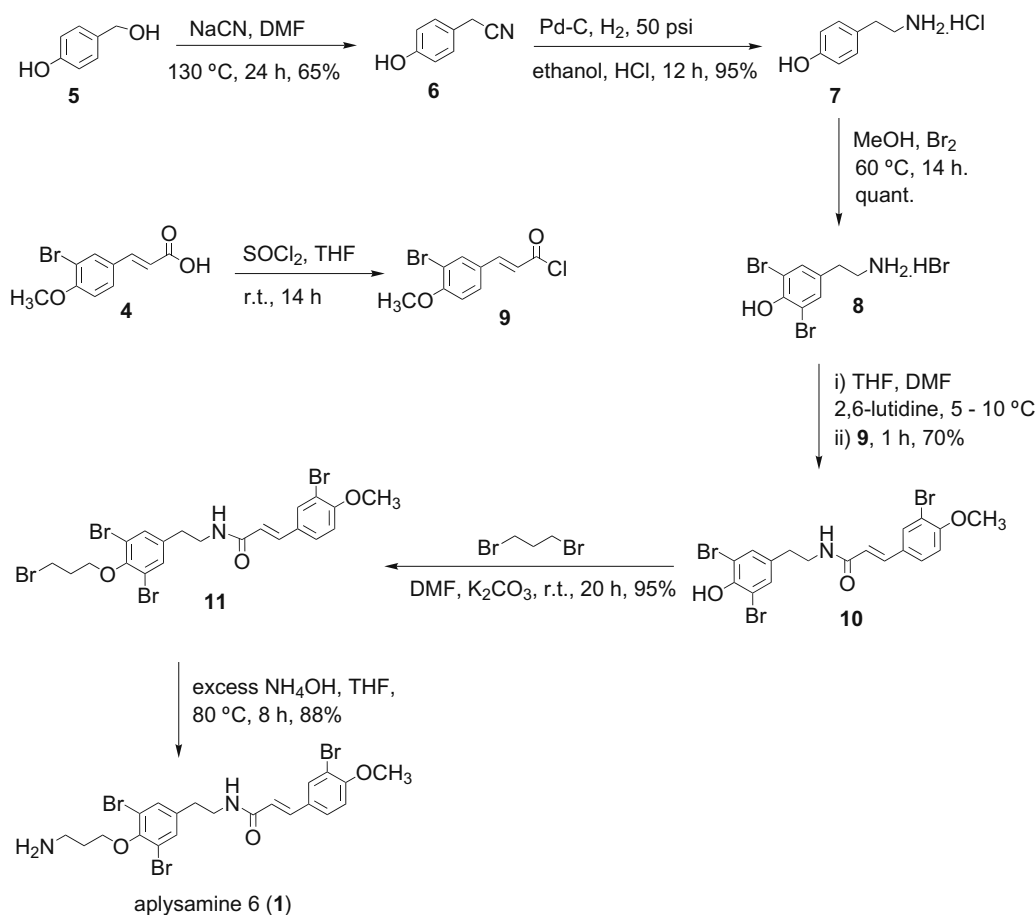
**Scheme 2.** Synthesis of aplysamine 6 (**1**) from compounds **4** and **7**.

of thionyl chloride in THF, and after evaporation of the solvents, a solution of acid chloride **9** in dry THF was added dropwise to a solution of dibromide **8** in THF at −10 °C using triethylamine as base to afford a low yield (20%) of the desired amide **10**, along with the formation of a diacylated side product which made column purification very tedious. The solubility of the dibromide **8** in THF at −10 °C was very poor and hence in a second attempt, we added the acid chloride **9** to a solution of dibromide **8** in a mixture of THF and DMF (1:1) at 5–10 °C using 2,6-lutidine as the base and this gave the desired amide **10**[10] cleanly in 70% yield after column purification. The amide **10** was alkylated by the action of excess 1,3-dibromopropane in DMF using potassium carbonate as base to give compound **11** in an excellent yield (95%), which in turn was reacted with excess ammonium hydroxide at 80 °C for 8 h in a sealed tube to produce aplysamine 6 (**1**) in an excellent yield (88%) (Scheme 2). The spectral data of our synthetic **1**[11] coincided with those of the natural material.[6]

In conclusion, we have accomplished an efficient first total synthesis of aplysamine 6 (**1**), an inhibitor of isoprenylcysteine carboxy methyltransferase, in an overall 55% yield from **7** and **4**.

## Acknowledgment

## Supplementary data

$^1$H NMR and $^{13}$C NMR spectra of aplysamine 6 (**1**) and the $^1$H NMR spectrum of compound **10** are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.10.103.

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10. *Typical procedure for the synthesis of amide* **10**: To a solution of compound **4** (0.3 g, 1.17 mmol) in dry THF (10 mL) at 0 °C was added thionyl chloride (0.42 g, 3.51 mmol) dropwise and the reaction was stirred overnight at room temperature. The solvent was evaporated and the acid chloride **9** was kept under high vacuum for 1 h. In another flask, a solution of compound **8** (0.45 g, 1.29 mmol) in a mixture of dry THF (15 mL) and DMF (15 mL) was cooled to between 5 and 10 °C, followed by the dropwise addition of 2,6-lutidine (0.55 g, 5.16 mmol) and after being stirred for 0.5 h, a solution of **9** in dry THF (6 mL) was added dropwise to the reaction mixture over the course of 15 min. The reaction mixture was stirred for 1 h at the same temperature, then diluted with ethyl acetate and washed successively with 2 N HCl, brine, sat. NaHCO$_3$, and brine and dried over sodium sulfate. Column chromatography of the dark orange oily material, eluted with ethyl acetate:hexanes (4:6), afforded amide **10** as a white crystalline solid (0.43 g, 70%); mp: 172–173 °C. Anal. Calcd for C$_{18}$H$_{16}$Br$_3$NO$_3$ requires C, 40.48; H, 3.02; N, 2.62. Found: C, 40.42; H, 3.06; N, 2.59. IR (KBr) ($\nu_{max}$/cm$^{-1}$) 3415, 3250, 1675, 1530, 1485, 1245, 1150. $^1$H NMR

(DMSO-$d_6$; 500 MHz): $\delta$ 2.66 (t; 2H; $J$ = 7 Hz); 3.36 (C$H_2$NHCO-; obscured by the DMSO signal); 3.86 (s; 3H); 6.50 (d; 1H; $J$ = 15.8 Hz); 7.13 (d; 1H, $J$ = 8.5 Hz); 7.30 (d; 1H; $J$ = 15.8 Hz); 7.39 (s; 2H); 7.54 (dd; 1H; $J$ = 8.5, 2.1 Hz); 7.77 (d; 1H; $J$ = 2.1 Hz); 8.02 (t; 1H). $^{13}$C NMR (DMSO-$d_6$; 125.7 MHz): $\delta$ 33.3 (CH$_2$); 39.6 (CH$_2$; obscured by DMSO peak); 56.3 (OCH$_3$); 111.0 (C); 112.8 (CH); 119.2 (2C); 121.2 (CH); 128.4 (CH); 129.1 (C); 131.6 (CH); 131.5 (C); 132.2 (2 CH); 136.7 (CH); 149.0 (C); 156.1 (C); 164.9 (C).

11. *Data for* **1**: Yield: (0.055 g, 88%); colorless gum. Anal. Calcd for C$_{21}$H$_{23}$Br$_3$N$_2$O$_3$ requires C, 42.67; H, 3.92; N, 4.74. Found: C, 42.59; H, 3.98; N, 4.66. IR (KBr)

($\nu_{max}$/cm$^{-1}$) 3410, 3273, 3061, 1682, 1540, 1497, 1458, 1260, 1201, 1138, 1050. $^1$H NMR (DMSO-$d_6$; 500 MHz): $\delta$ 1.92 (m; 2H); 2.60 (t; 2H; $J$ = 6.4 Hz); 2.72 (m; 2H; $J$ = 6.4 Hz); 3.39 (m; 2H; $J$ = 6.4 Hz); 3.85 (s; 3H); 3.96 (t, 2H; $J$ = 6.5 Hz); 6.50 (d; 1H; $J$ = 15.6 Hz); 7.12 (d; 1H; $J$ = 8.5 Hz); 7.32 (d; 1H; $J$ = 15.6 Hz); 7.49 (s; 2H); 7.53 (dd; 1H; $J$ = 8.5, 1.8 Hz); 7.76 (d; 1H; $J$ = 1.8 Hz); 8.05 (t; 1H). $^{13}$C NMR (DMSO-$d_6$; 125.7 MHz): $\delta$ 28.9 (CH$_2$); 33.5 (CH$_2$); 39.6 (CH$_2$; obscured by the DMSO signal); 45.5 (CH$_2$); 56.4 (OCH$_3$); 71.4 (CH$_2$); 111.1 (C); 112.9 (CH); 117.3 (2C); 121.1 (CH); 128.5 (CH); 129.0 (C); 131.6 (CH); 132.9 (2 CH); 136.9 (CH); 139.0 (C); 150.7 (C); 156.1 (C); 165.0 (C).