



Total synthesis of the natural isoprenylcysteine carboxyl methyltransferase inhibitor spermatinamine

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ABSTRACT

The first total synthesis of spermatinamine, an inhibitor of isoprenylcysteine carboxyl methyltransferase (Icmt) with a bromotyrosine–spermine–bromotyrosine dimeric structure is described.

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Post-translational modifications of eukaryotic proteins are important for their cellular localization and function.¹ The covalent attachment of isoprenoid groups to proteins that contain CAAX motifs (C, cysteine; A, aliphatic amino acid; X, any amino acid) has gained considerable attention recently because it is the initial step of cell signaling pathways that control diverse biological processes.^{2,3} C₁₅-farnesyl or C₂₀-geranylgeranyl groups are the most common isoprenoids enzymatically attached to the cysteine at or near the carboxy terminus of the CAAX-containing protein⁴ through a mechanism that depends upon the sequence of amino acids of the X residue,⁵ and in general involves Zn²⁺.⁶ Three different prenyltransferases, namely farnesyltransferase (FT), geranylgeranyltransferase 1 (GGT1) and geranylgeranyltransferase 2 (GGT2) mediate transfer of isoprenoids in humans, although these enzymes have also been found in other eukaryotes including vertebrates, insects, plants, protozoa and fungi.⁴

One of the best-studied CAAX motif-containing proteins is the Ras GTPase superfamily (39 members)^{8–10} which can be prenylated at the cysteine side chain with either C₁₅ (X small amino acid: Ala, Ser) or C₂₀ (X = Leu) prenyl chains. S-prenylation of CAAX proteins is followed by additional post-translational modifications in sequence involving S-palmitoylation of neighbouring cysteine groups, specific endo proteolytic cleavage of the AAX residue (by Ras-converting enzyme Rce1, a farnesyl-S-Ras endoprotease),^{11–13} and methylation of the carboxyl cysteine residue (by isoprenylcysteine carboxyl methyltransferase, Icmt).^{11,14,15}

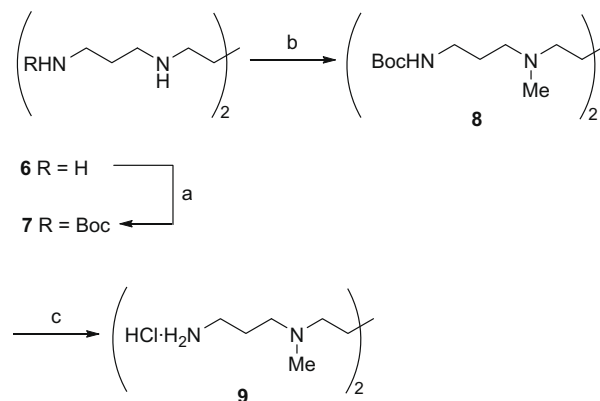
Hyperactivation of Ras is thought to contribute to carcinogenesis and tumour progression.^{3,16} Inhibition of (some of the) steps that covalently modify the Ras oncogene with a prenyl group prior to its recruitment to the plasma membrane are attractive targets for drug development.¹⁰ Given the limited success of farnesyltransferase inhibitors (TFIs), attention has been turned to other proteins on the Ras processing pathway as new anticancer drug targets.¹⁰ In fact, drugs that inhibit Icmt (methotrexate and cismethynil 1,

Fig. 1)¹⁷ have shown promising antitumour properties in tissue cultures and animal models.^{18–20}

Aplysamine 6 **2**²¹ and spermatinamine **3** (Fig. 1),²² isolated from extracts of the Verongida sponge *Pseudoceratina* sp., were the first natural products shown to inhibit Icmt. More recently, bioassay-guided purification of *Hovea parvicalyx* extracts²³ led to isolation and identification of new β-hydroxychalcone derivatives (**4** and **5**, Fig. 1) with Icmt inhibitory activity.

Spermatinamine **3** (IC₅₀ = 1.9 μM for Icmt) is representative of a group of antitumour natural products extracted from Verongidas that biosynthetically derive from the condensation of bromotyrosine and spermine.²² Due to its potential as a lead to discover more potent Icmt inhibitors with better bioavailability, we undertook the total synthesis of spermatinamine **3** and analogues. The first synthesis of aplysamine 6 **2** reported recently²⁴ prompted us to disclose our approach to spermatinamine **3**.

The synthesis of spermatinamine **3** utilizes commercially available O-methyl-L-tyrosine **10** and spermine **6** as suitable precursors



Scheme 1. Reagents and conditions: (a) BOC-ON, THF, 0 °C, 2 min, 80%; (b) 37% HCHO, NaBH₃CN, CH₃COOH, EtOH, 25 °C, 12 h, 96%; (c) 4 M HCl, 1,4-dioxane, 25 °C, 4 h, 87%.

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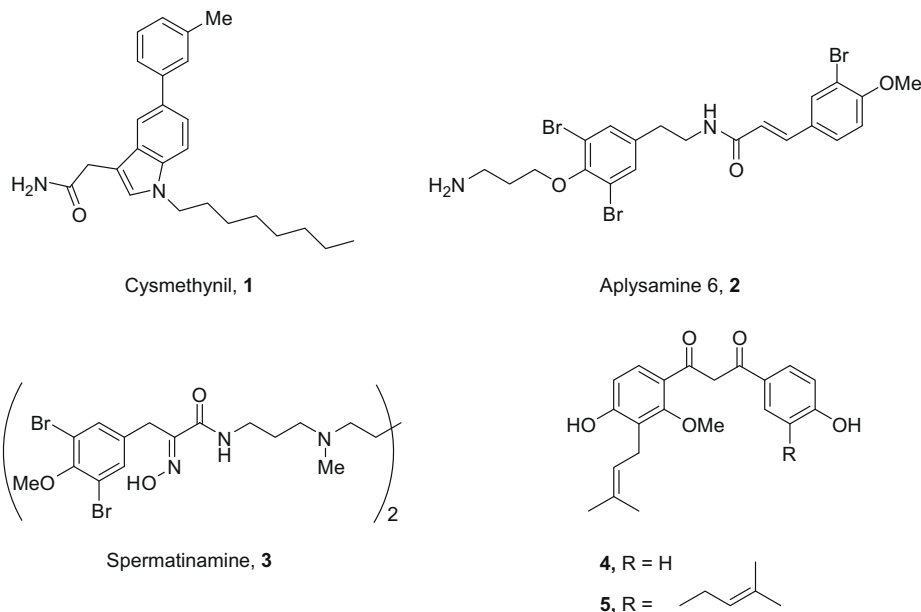
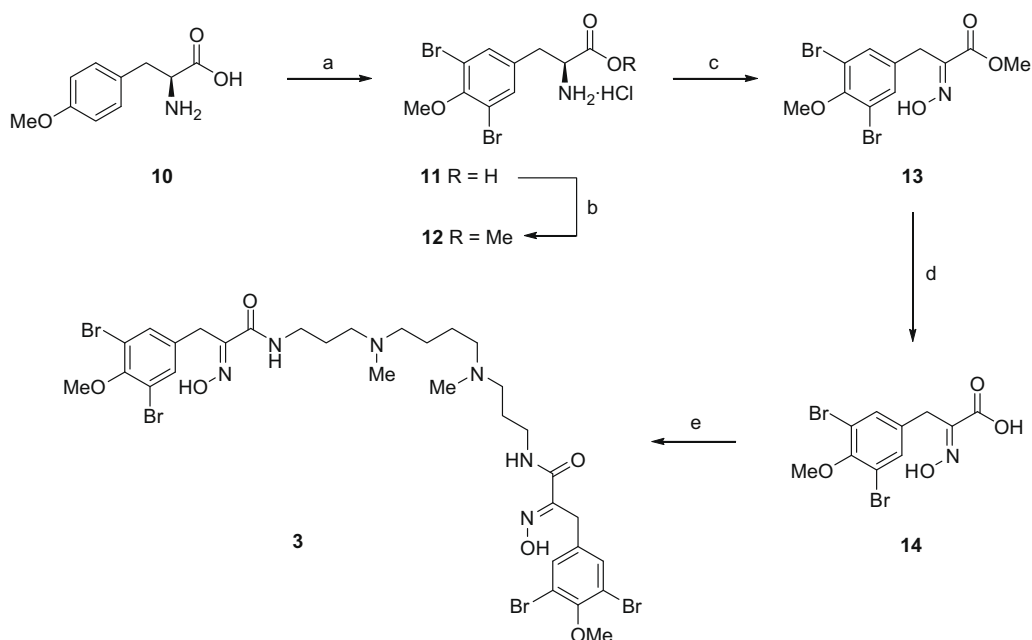


Figure 1. Chemical structure of Icmt inhibitors.

to build the bis-bromotyrosine–spermine skeleton. After the initial attempts to protect the primary amino groups of spermine **6** using Boc_2O failed, protected amine **7** could be obtained by treatment of **6** with BOC-ON^{25} in THF. Subsequently methylation of **7** by reductive amination gave **8** in good yield (Scheme 1). Final deprotection of the terminal amino group with 4 M HCl afforded hydrochloride **9** in excellent yield (Scheme 1).

As shown in Scheme 2, the synthesis of 3,5-dibromo-*O*-methyl-*L*-tyrosine **11** was carried out by direct bromination of commercial *O*-methyl-*L*-tyrosine **10** with bromine in a water/37% hydrochloric acid mixture at 0 °C.²⁶ Treatment of acid **11** with thionyl chloride in MeOH afforded amino ester **12** which was then converted to

oxime ester **13** using sodium tungstate and 30% aqueous hydrogen peroxide.²⁷ Only freshly opened bottles of hydrogen peroxide afforded good to excellent yields in the oxidation of amino ester **12**. *E*-Oxime ester **13** was obtained as the only geometric isomer and its configuration was confirmed by comparison with that of spermatinamine **3**.²² After saponification, oxime acid **14** was condensed with methylated spermine **9** following the procedure described by Hoshino²⁸ which uses *N*-hydroxyphthalimide and dicyclohexylcarbodiimide (DCC) as activating agents to couple oxime acids with amines, to give spermatinamine **3** in 50% overall yield (Scheme 2).²⁹ The spectroscopic data of synthetic **3** matched those of the natural specimen.²² Other coupling agents (HOBT,



Scheme 2. Reagents and conditions: (a) Br_2 , 37% HCl, H_2O , 0 °C, 15 min, 56%; (b) SOCl_2 , MeOH, 80 °C, 4 h, 85%; (c) $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$, EtOH, H_2O_2 , H_2O , 25 °C, 4 h, 68%; (d) LiOH, (1:1) THF/ H_2O , 25 °C, 14 h, 95%; (e) **9**, *N*-hydroxyphthalimide, DCC, 1,4-dioxane, 25 °C, 8 h, 50%.

N-hydroxysuccinimide, EDC) failed to afford amide **3** with the unprotected oxime group, thus lengthening the sequence to reach the target.^{30,31}

Although as indicated²² spermatinamine **3** does not exhibit the required drug-like pharmacokinetic properties, the synthetic approach described herein should pave the way for the preparation and biological evaluation of novel analogs that might expand the number of marine natural product-inspired anticancer drugs³² in particular as inhibitors of relatively unexplored targets, such as lcmt.¹⁷

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.06.087.

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- N-Hydroxyphthalimide (0.07 g, 0.41 mmol) and DCC (0.08 g, 0.41 mmol) were added to a solution of acid **14** (0.15 g, 0.41 mmol) in dioxane (1 mL). After the mixture had been stirred for 8 h at room temperature, a solution of amine **9** (0.06 g, 0.20 mmol), Et₃N (0.11 mL, 0.82 mmol) and MeOH (1 mL) was added. The reaction mixture was stirred for 12 h, the solvent was removed in vacuo and the residue was purified by column chromatography (SiO₂, 95:5 CH₃OH/NH₄OH) to afford 0.09 g (50%) of **3** as a white solid, mp: 172–173 dec. (MeOH/Et₂O). ¹H NMR (DMSO-*d*₆, 400.13 MHz) (data for monomer): δ 8.18 (t, *J* = 5.4 Hz, 1H, NH), 7.45 (s, 2H, 2H^{2'}), 3.76 (s, 2H, 2H³), 3.74 (s, 3H, OCH₃), 3.17 (q, *J* = 5.6 Hz, 2H, 2H^{1''}), 2.4–2.3 (m, 4H, 2 × CH₂), 2.16 (s, 3H, NCH₃), 1.60 (quintet, *J* = 6.5 Hz, 2H, 2H^{2''}), 1.4–1.3 (m, 2H, CH₂) ppm. ¹³C NMR (CD₃OD, 100.62 MHz) (data for monomer): δ 165.5 (s), 153.9 (s), 152.1 (s), 137.5 (s), 134.6 (d, 2×), 118.7 (s, 2×), 61.1 (q), 58.0 (t), 56.1 (t), 41.6 (q), 38.7 (t), 28.9 (t), 26.7 (t), 24.8 (t) ppm. IR: ν 3500–3100 (br, NH and OH), 2944 (m, C–H), 2864 (w, C–H), 1662 (s, C=O and C=N), 1524 (s), 1470 (s), 1421 (m), 1259 (m), 1000 (s), 752 (m), 740 (m) cm^{−1}. MS (ESI⁺): *m/z* (%) 931 ([M+1]⁺ [⁸¹Br₃] [⁷⁹Br], 37), 929 ([M+1]⁺ [⁸¹Br₂] [⁷⁹Br₂], 54), 927 ([M+1]⁺ [⁸¹Br] [⁷⁹Br₃], 39), 492 (100), 406 (99). HRMS (ESI⁺) calcd for C₃₂H₄₅⁸¹Br₃⁷⁹BrN₆O₆, C₃₂H₄₅⁸¹Br₂⁷⁹Br₂N₆O₆ and C₃₂H₄₅⁸¹Br⁷⁹Br₃N₆O₆ [M+1]⁺ 931.0075, 929.0092 and 927.0109; found: 931.0051, 929.0082 and 927.0094.
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