Published in final edited form as:

Synthesis (Stuttg). 2016 July ; 48(13): 2065–2068. doi:10.1055/s-0035-1561608.

Improved Synthesis of MDL 73811 – a Potent AdoMetDC Inhibitor and Anti-Trypanosomal Compound

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Abstract

An improved synthesis of MDL 73811 – a potent AdoMetDC (S-adenosylmethionine decarboxylase) inhibitor and anti-trypanosomal compound with in vivo activity has been completed in four steps from commercially available 2',3'-O-isopropylideneadenosine. Utilization of Mitsunobu chemistry was crucial for the reliable and scalable introduction of the 5'-methylamine moiety, which was problematic using traditional activation/displacement chemistry as previously reported. All reactions in this synthesis were run on gram-scale resulting in a five-fold increase in yield over the original synthesis.

Abstract

Keywords

nucleosides; drug discovery

The polyamines putrescine, spermidine and spermine are biologically essential cationic amines that are synthesized from the amino acid L-ornithine and S-adenosylmethionine.¹⁻⁴ Spermidine in particular is absolutely required for eukaryotic and archaeal cell growth as it is needed for the essential covalent modification of the elongation factor eIF5A.^{5,6} Because

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Primary Data: No

Supporting Information: Yes

polyamines are essential for cell growth, inhibitors of polyamine biosynthesis have been promoted as anti-proliferative agents for a range of applications, most notably cancer^{1,3} and treatment of Human African *trypanosomiasis* (HAT) caused by the parasitic protozoan *Trypanosoma brucei*.⁷ S-Adenosylmethionine decarboxylase (AdoMetDC) has an unusual composition in *T. brucei*, and unlike the human enzyme which is a homodimer, *T. brucei* AdoMetDC is formed by heterodimerization between an active but severely impaired catalytic subunit and an inactive paralog termed prozyme.^{7,10} Several promising *in vivo* studies demonstrated that the AdoMetDC inhibitor MDL 73811 **1** (Figure 1) could cure *T. brucei* infections in mice.^{11,12} However despite the good activity of MDL 73811, rapid plasma clearance and poor brain penetration hindered its further development.¹³ A metabolically stable analog of MDL 73811 called Genz 644131, has also been reported, but while it has improved plasma half-life compared with MDL 73811, it still showed insufficient brain penetration to cure the CNS form of the disease^{12,13}. A significant liability to further development of this series for HAT has been the complex synthetic protocol, which has made it very difficult to generate analogs that may overcome the poor brain penetration of the series. Here, we describe an improved scalable synthesis of MDL 73811 (**1**) that we envision to be applicable to the eventual synthesis of analogs with improved properties.

Initial attempts to develop a robust synthetic route to **1** envisioned introduction of the *N*-(aminobut-2-enyl), *N*-methylamine group at the 5'-position using activation and displacement chemistry. Activation of commercially available 2',3'-*O*-isopropylideneadenosine **2** as either the chloride¹⁴⁻¹⁵ or tosylate^{8,15-17} followed by displacement with methylamine^{8,15,-17} to form amine **3** has been reported. In our hands however, these methods gave unsatisfactory results (especially on multi-gram scale) as yields ranged from 0-21 % (Scheme 1). We also attempted the activation of alcohol **2** to the tosylate followed by displacement with fully intact side chain amine **5**,¹⁸ but none of the desired carbamate **4** was isolated despite several attempts. We attribute the failure of this reaction due to stability issues of amine **5**.¹⁹

A switch to Mitsunobu chemistry proved crucial to the completion of a robust route to **1**. This optimized route commenced with amination of commercially available 2',3'-*O*-isopropylideneadenosine **2** with *N*-methyl-2-nitro-benzene-sulfonamide **6** under Mitsunobu conditions (Scheme 2),²⁰ a protocol that reliably yielded nosyl-protected methylamine **7** in 85% yield on >20 g scale. A simple trituration of product **7** from methanol eliminated the need for chromatographic purification. Sulfonamide **7** was then treated with thiophenol and cesium carbonate in acetonitrile²⁰ at room temperature for six hours. This method cleaved the nosyl moiety to give 5'-methylamine **3** in 81% yield. Methylamine **3** was then alkylated with *tert*-butyl (*Z*)-(4-chlorobut-2-en-1-yl)carbamate **8** in the presence of sodium iodide and triethylamine in acetonitrile for three hours at room temperature. This procedure reproducibly afforded carbamate **4** in 61% yield. Controlling both reaction time and temperature was crucial to the success of this reaction as either extended reaction times or heating the reaction mixture gave decreased yields of **4**. Carbamate **4** was then treated with 1M aqueous sulfuric acid and methanol and stirred at room temperature for 18 hours. This method resulted in deprotection of both the acetonide and Boc-carbamate moieties, and the

desired compound MDL 73811 (**1**) was isolated in 60% yield. Our synthesis of **1** was thus completed in four steps from commercially available 2',3'-*O*-isopropylideneadenosine **2** in 25% overall yield. The seminal synthesis of **1** was completed in eight steps and 5% overall yield.⁸ Thus, our synthesis accomplished a five-fold increase in yield over four fewer steps. Our approach is reliable and scalable as yields were consistent over multiple runs on gram scale, which makes this synthesis desirable for preparation of large quantities of **1** for further biological studies. In contrast to activation/displacement chemistry, the use of Mitsunobu chemistry followed by thiophenolate cleavage of the intermediate sulfonamide provided robust yields of 5'-methylamine **3** on multi gram scale. Efforts to improve the drug-like properties of **1** through analog synthesis using this methodology and SAR are ongoing and will be reported in due course.

Unless otherwise specified, all commercially available reagents were used as received. 2'-3'-isopropylidene adenosine **2** was purchased from Chem-Impex International, Inc. All reactions using dried solvents were carried out under an atmosphere of argon in flame-dried glassware with magnetic stirring. Dry solvent was dispensed from a solvent purification system that passes solvent through two columns of dry neutral alumina. Silica gel chromatographic purifications were performed by flash chromatography with silica gel (Sigma, grade 62, 60-200 mesh) packed in glass columns; the eluting solvent was determined by thin layer chromatography (TLC). Analytical TLC was performed on glass plates coated with 0.25 mm silica gel using UV for visualization. ¹H NMR spectra were obtained on a 400 MHz NMR spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to residual solvent. Multiplicities are given as: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), m (multiplet). Proton-decoupled ¹³C NMR spectra were obtained on a 400 MHz NMR spectrometer at an operating frequency 101 MHz. IR spectra were obtained on a FT-IR spectrometer. Electrospray ionization mass spectra (ESI-MS) were recorded on a Shimadzu 2010-LCMS.

Procedures

N-Methyl-2-nitrobenzenesulfonamide (**6**)

To a cooled (0 °C) solution of methylamine hydrochloride (15.8 g, 71.3 mmol) in H₂O (43 mL) was added NaOH (17.2 g, 428 mmol). A solution of 2-nitrobenzene sulfonyl chloride in EtOAc (140 mL) was added dropwise via separatory funnel over a period of 30 minutes. The resulting biphasic mixture was stirred at 0°C for five additional minutes and was then stirred for 20 minutes at rt. The layers were then separated and the aqueous layer was extracted with EtOAc (2 × 100 mL). The combined organic layers were dried, filtered, and concentrated *in vacuo*. The residual off-white solid was crystallized from CH₂Cl₂/Hexanes to afford the desired product **6** as off-white crystals; yield: 14.6 g (95%).

IR (thin film): 3335, 3095, 1540 cm⁻¹.

¹H NMR (CDCl₃, 400 MHz): δ = 8.13 (m, 1H), 7.86 (m, 1H), 7.78–7.73 (m, 2H), 5.24 (br s, 1H), 2.78 (d, *J* = 4.8 Hz, 3H). ¹H NMR was in accordance with literature values.²¹

¹³C NMR (CDCl₃, 100 MHz): δ = 148.2, 133.7, 132.7, 132.4, 131.5 125.4, 29.8.

LC/MS (ESI): m/z [M – NH(Me)]⁺ calcd for C₆H₅NO₄S: 187.1; found: 187.1.

2'3'-O-Isopropylidene-5'-deoxy-5'-((N-methyl-2-nitrophenyl)sulfonamido)adenosine (7)

To a cooled (0 °C) suspension of 2'-3'-O-isopropylideneadenosine **2** (22.0 g, 71.8 mmol) and 2-nitro-*N*-methylbenzenesulfonamide **6** (14.6 g, 79.0 mmol) in THF (360 mL) was added PPh₃ (32.0 g, 122 mmol). DIAD (24.2 mL, 122 mmol) was then added dropwise via syringe during which time the solution turned yellow and became homogenous. The solution was then stirred for 18 h during which time the reaction was allowed to warm to rt. The now brown solution was then concentrated in *vacuo* to afford a viscous brown oil. This oil was dissolved in methanol (250 mL) and then cooled to 0 °C in an ice bath for 1.5 hours during which time a yellow precipitate formed. The precipitate was collected via suction filtration and dried under high-vacuum to afford the sulfonamide **7** as a yellow powder; yield: 30.9 g (85%); mp 240–241 °C (Lit.²⁰ 240–241 °C); [α]_D +30.0 (*c* 1, CHCl₃) (Lit.²⁰ +30.4 (*c* 1, CHCl₃)).

IR (thin film): 3324, 3175, 2988, 1710, 1645, 1544 cm⁻¹.

¹H NMR (DMSO-*d*₆, 400 MHz): δ = 8.29 (s, 1H), 8.14 (s, 1H), 7.90 (d, *J* = 9.6 Hz, 1H), 7.83–7.78 (m, 2H), 7.70 (m, 1H), 7.33 (br s, 2H), 6.18 (d, *J* = 2.0 Hz, 1H), 5.42 (dd, *J* = 6.4, 2.4 Hz, 1H), 5.05 (dd, *J* = 6.0, 3.2 Hz, 1H), 4.29 (m, 1H), 3.62–3.56 (m, 2H), 2.74 (s, 3H), 1.51 (s, 3H), 1.30 (s, 3H). ¹H NMR was in accordance with literature values.²⁰

¹³C NMR (DMSO-*d*₆, 100 MHz): δ = 156.5, 153.2, 149.0, 148.1, 140.6, 134.9, 132.7, 130.7, 130.1, 124.7, 119.6, 114.1, 89.3, 84.7, 83.6, 82.2, 51.7, 36.1, 27.4, 25.6.

LC/MS (ESI): m/z [M + H]⁺ calcd for C₂₀H₂₄N₇O₇S: 506.1; found: 506.1

2'3'-O-Isopropylidene-5'-deoxy-5'-methylaminoadenosine (3)

To a stirred solution of nosyl-protected amine **7** (4.50 g, 8.90 mmol) in CH₃CN (50 mL) was added Cs₂CO₃ (5.79 g, 17.8 mmol) followed by the dropwise addition of thiophenol (1.80 mL, 17.8 mmol). The resulting bright yellow solution was stirred at rt for 6 h before being filtered through celite and concentrated in *vacuo* to afford a yellow oil. The oil was purified by flash chromatography (silica gel, 0–20% 1%NH₄OH in MeOH–100–80% CH₂Cl₂) to afford the product amine **3** as a yellow foam; yield: 2.30 g (81%); [α]_D –20.1 (*c* 1, MeOH) (Lit.²⁰ –19.6 (*c* 1, MeOH)).

IR (thin film): 3326, 3180, 2987, 2938, 1648, 1599 cm⁻¹.

¹H NMR (CDCl₃, 400 MHz): δ = 8.32 (s, 1H), 7.89 (s, 1H), 6.17 (br s, 2H), 5.99 (d, *J* = 3.2 Hz, 1H), 5.45 (dd, *J* = 6.4, 3.2 Hz, 1H), 5.00 (dd, *J* = 6.4, 3.2 Hz, 1H), 4.35 (m, 1H), 2.90–2.79 (m, 2H), 2.41 (s, 3H), 1.59 (s, 3H), 1.36 (s, 3H). ¹H NMR was in accordance with literature values.²⁰

¹³C NMR (CDCl₃, 100 MHz): δ = 155.7, 153.1, 149.4, 139.8, 120.4, 114.6, 90.8, 85.2, 83.3, 82.3, 53.6, 36.5, 27.3, 25.4.

LC/MS (ESI): m/z [M + H]⁺ calcd for C₁₄H₂₁N₆O₃: 312.2; found 312.2

***Tert*-butyl (*Z*)-(4-chlorobut-2-en-1-yl)carbamate (**8**)**

To a stirred solution of the commercially available hydrochloride salt of (*Z*)-4-chlorobut-2-en-1-amine (3.00 g, 21.1 mmol) in THF (100 mL) was added Boc₂O (4.85 mL, 21.1 mmol) followed by the dropwise addition of ⁴Pr₂NEt (7.35 mL, 42.2 mmol). The resulting dark brown solution was stirred at rt for 6 h before being poured into water (100 mL). The solution was then extracted with Et₂O (3 × 100 mL). The combined organic layers were washed with 10% aqueous HCl (100 mL) followed by saturated aqueous NaHCO₃ (100 mL). The organic layer was dried (Na₂SO₄), filtered, and concentrated in *vacuo* to afford a brown solid. The resulting solid was crystallized from CH₂Cl₂/Hexanes to afford the desired product carbamate **8** as a white crystalline solid; yield: 4.23 g (97%); mp 65-66 °C.

¹H NMR (CDCl₃, 400 MHz): δ = 5.74 (m, 1H), 5.62 (m, 1H), 4.65 (br s, 1H), 4.11 (d, *J* = 7.6 Hz, 2H), 3.81 (d, *J* = 6.7 Hz, 2H), 1.43 (s, 9H). ¹H NMR was in accordance with literature values.²²

¹³C NMR (CDCl₃, 100 MHz): δ = 155.7, 131.1, 127.8, 79.6, 38.7, 37.1, 28.4.

2'3'-*O*-Isopropylidene-5'-deoxy-5'-(((*Z*)-4-(*tert*-butoxycarbonyl)amino)but-2-en-1-yl)(methyl)amino)adenosine (4**)**

To a stirred solution of methylamine **3** (2.00 g, 6.24 mmol) in CH₃CN (18 mL) was added *tert*-butyl (*Z*)-(4-chlorobut-2-en-1-yl)carbamate **8** (1.41 g, 6.87 mmol), NaI (1.03 g, 6.87 mmol), and Et₃N (0.88 mL, 6.87 mmol). The mixture was stirred at rt for 3 h, filtered through a pad of Celite, and rinsed with EtOAc and concentrated in *vacuo*. The residual oil was dissolved in EtOAc (25 mL) and washed with saturated aqueous NaHCO₃ (25 mL). The layers were separated and the organic layer was dried (Na₂SO₄), filtered, and concentrated in *vacuo* to afford a clear oil. The oil was purified by flash chromatography (silica gel, 0-10% 1% NH₄OH in MeOH-100-90% CH₂Cl₂) to afford the product carbamate **4** as a white foam; yield: 1.87 g (61%); [α]_D +5.0 (*c* 0.7, CHCl₃).

IR (thin film): 3325, 3176, 2927, 1699, 1646 cm⁻¹.

¹H NMR (CDCl₃, 400 MHz): δ = 8.34 (s, 1H), 8.01 (br s, 1H), 6.08 (br s, 1H), 5.83 (br s, 2H), 5.54-5.44 (m, 3H), 5.06 (br s, 1H), 4.92 (br s, 1H), 4.41 (br s, 1H), 3.76-3.61 (m, 2H), 3.14-2.86 (m, 2H), 2.59-2.49 (m, 2H), 2.27 (s, 3H), 1.61 (s, 3H), 1.44 (s, 9H), 1.39 (s, 3H).

¹³C NMR (CDCl₃, 100 MHz): δ = 155.8, 155.5, 153.1, 149.3, 140.1, 129.5, 128.8, 120.3, 114.3, 91.1, 85.1, 84.1, 83.3, 79.3, 58.8, 54.5, 42.6, 37.6, 28.4, 27.0, 25.3.

LC/MS (ESI): *m/z* [M + H]⁺ calcd for C₂₃H₃₆N₇O₅: 490.3; found 490.3

5'-(((*Z*)-4-Amino-2-butenyl)methylamino)-5'-deoxyadenosine (MDL 73811) (1**)**

To a stirred solution of carbamate **4** (1.25 g, 2.55 mmol) in methanol (15 mL) was added 1M aqueous H₂SO₄ (15 mL). The resulting solution was stirred at rt for 18 h before being concentrated in *vacuo*. The solution was basified to pH >9 with 20% aqueous NaOH and the solution was concentrated in *vacuo*. To the residual solid was added ⁴BuOH (15 mL) and the solution was dried (Na₂SO₄), filtered, and concentrated in *vacuo* to afford a clear oil. The oil

was purified by flash chromatography (silica gel, 0-40% 1% NH₄OH in MeOH–100-60% CH₂Cl₂) to afford the product amine **1** as a white solid; yield: 0.53 g (60 %); mp 260 °C (decomposes) (Lit.²³ 260 °C (decomposes)).

IR (thin film): 3281, 2939, 1624, 1578 cm⁻¹.

¹H NMR (CH₃OD, 400 MHz): δ = 8.26 (s, 1H), 8.19 (s, 1H), 5.98 (d, *J* = 4.4 Hz, 1H), 5.64–5.42 (m, 2H), 4.71 (m, 1H), 4.31–4.11 (m, 2H), 3.72–3.64 (m, 2H), 3.17 (d, *J* = 6.7 Hz, 2H), 2.81 (d, *J* = 5.7 Hz, 2H), 2.31 (s, 3H). ¹H NMR was in accordance with literature values.^{8,10}

¹³C NMR (CDCl₃, 100 MHz): δ = 157.3, 153.9, 150.5, 141.5, 131.6, 128.6, 120.6, 90.8, 83.3, 74.7, 73.7, 60.4, 55.3, 43.1, 38.4.

LC/MS (ESI): *m/z* [M + H]⁺ calcd for C₁₅H₂₄N₇O₃: 350.2; found 350.2.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors acknowledge Jack Hunt for synthetic contributions to this project. This work was supported by funds from the National Institutes of Health grant R01AI090599, (to MAP and JKDB). MAP and JKDB acknowledge the support of the Welch Foundation (grants I-1257 and I-1422, respectively). MAP holds the Beatrice and Miguel Elias Distinguished Chair in Biomedical Science and the Carolyn R. Bacon Professorship in Medical Science and Education. JKDB holds the Julie and Louis Beecherl, Jr., Chair in Medical Science.

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Figure 1. Structure of MDL 73811 (1)

Scheme 1. Attempted 5'-amination reactions

Scheme 2. Improved Synthesis of MDL 738