HHS Public Access

Author manuscript

Org Lett. Author manuscript; available in PMC 2019 April 03.

Published in final edited form as:

Org Lett. 2019 February 15; 21(4): 876–879. doi:10.1021/acs.orglett.8b03716.

A Semi-Synthesis of an Anticancer DPAGT1 Inhibitor from a Muraymycin Biosynthetic Intermediate

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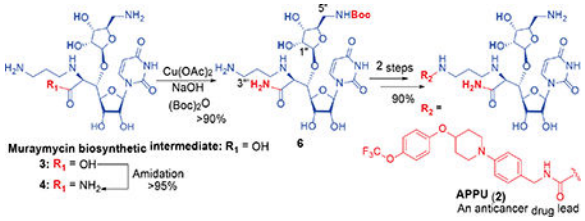
Abstract

We have explored a method to convert a muraymycin biosynthetic intermediate **3** to an anticancer drug lead **2** for *in vivo* and thorough preclinical studies. $Cu(OAc)_2$ forms a stable complex with the amide **4** and it prevents electrophilic reactions at the 2-((3-aminopropyl)amino)acetamide moiety. Under the present conditions, the desired 5”-primary amine was selectively protected with $(Boc)_2O$ to yield **6**. The intermediate **6** was converted to **2** in two steps with 90% yield.

Graphical Abstract



N-linked glycans play essential roles in many biological processes. Aberrant protein glycosylation is frequently observed in cancer cells. The integral membrane enzyme, *N*-acetylglucosaminephosphotransferase 1 (DPAGT1), plays a central role in *N*-linked glycoprotein biosynthesis, catalyzing the first step in the dolichol-linked oligosaccharide pathway. Certain cancer cells require an increase in *N*-linked glycosylation for their progression, thus, selective DPAGT1 inhibitors have therapeutic potential.[1] The only inhibitors of DPAGT1 that have been reported to date are the antibiotic tunicamycin and its derivatives. Tunicamycins have been useful experimental tools to interrogate *N*-linked glycosylation and to examine the effects on protein folding.[2] Tunicamycins inhibit growth

Supporting Information Available Experimental procedures and copies of NMRs. This is available free of charge via the Internet at http://pubs.acs.org.

across a wide range of mammalian cell lines in a non-selective fashion.[1a] In addition, physicochemical and biological properties of tunicamycins (*e.g.* water-solubility and narrow therapeutic window) are far from ideal as leads for the development of preclinical drugs. Therefore, new types of inhibitors are required. We have recently identified a strong DPAGT1 inhibitor, aminouridyl phenoxypiperidinbenzyl butanamide (APPB, **1**), by screening of an aminoribosyl-uridine library generated based on muraymycin (Figure 1).[1a,1b] APPB selectively inhibits growth of solid tumors (*e.g.* KB, LoVo, SK-OV-3, MDA-MB-432S, HCT116, Panc-1, and AsPC-1) at low μM concentrations but does not inhibit growth of healthy cells at these same concentrations.[1a] APPB displayed a remarkably selective cytotoxicity (*e.g.* IC_{50} pancreatic cancer cells/ IC_{50} healthy cells: >1/100), metabolic stability ($t_{1/2}$ >60 min.), and water solubility (~75 mg/mL for HCl salt). Thus, pharmacological studies of APPB and its related analogs using appropriate animal models are a focus of our continuing research efforts. APPB is a total synthetic product that is not feasible to access from reported natural products or muraymycin biosynthetic intermediates.[3] Based on our structure-activity relationship studies of APPB, it was realized that a urea analogue, aminouridyl phenoxypiperidinbenzyl urea (APPU, **2** in Figure 1) shows equal biological activity to APPB without reducing the favorable physicochemical properties. Importantly, APPU has the potential to be synthesized from a biosynthetic intermediate of muraymycin (**3)** whose structure corresponds to de-*N*-methyl FR-900463.[1d,4] Intermediate **3** can potentially be produced at large-scale by the disruption of the *mur30* gene in the muraymycin-producing *Streptomyces sp.* NRRL 30471 (Figure 2).[3] However, no synthetic method has been developed to convert the non-biologically active intermediate **3** to a pharmaceutically interesting product.[5]

In a semi-synthetic approach with **3**, the most challenging transformation is the selective carbamation (temporary protection) of the primary amine (C5”-position) of the aminoribose moiety that allows functionalization at the other primary amine (C3”’-position). In this article, we report a semi-synthesis of APPU (**2**) from **3** by exploring transition metals that temporarily protect the 2-((3-aminopropyl)amino)acetamide system to achieve selective carbamation at the C5”-amine (Scheme 1).

We have previously demonstrated amidation of protecting group free-amino acid derivatives with NH_4Cl (excess), EDCI, glyceroacetonide-Oxyma, and $NaHCO_3$ in water media.[1d,6] Under the same condition, **3**[7] was converted to the corresponding amide **4** in over 95% yield (Scheme 1). The amide **4** was examined in transition metal-mediated selective Boc-protection under aq NaOH in a mixture of DMF, MeOH, and H_2O. Because of the strong coordination aptitude of group 4 elements to diamines, we screened Cu, Ni, Zn, V, Co, and Fe salts for selectively synthesizing the mono-Boc-protected triamine **7** in excellent yield. The transition-metal salts tested, selectivity of mono-/di-Boc (**7**/**8**), and isolation yield for **7** are summarized in Table 1. Reactions of **4** with a majority of the metal salts in Table 1 caused precipitation in a single solvent system (*e.g.* MeOH, H_2O, or DMF); all reactions were carried out in a mixture of DMF-MeOH-H_2O (1/1/1). Without addition of a transition metal salt, Boc-protection of **4** furnished a 1:1 mixture of **7** and **8** (entry 14).[8] Boc-protection reactions in the presence of Zn salts, $CoCl_2$, or Fe species provided 1:1 ratio of (**7**/**8**) with <50% isolation yield for **7** (entries 9–13). VCl_3-mediated Boc-protection of **4** exhibited **7**/**8**

selectivity of 4:1 and **7** was isolated in 40% yield after 12 h (entry 8). All Ni species tested gave rise to the desired mono-Boc product **7** in 85% yield with >19:1 (entries 5–7). Selective Boc-protection reactions mediated via the Cu species provided the only desired product **7** in 10–95% yield (entries 1–4). $CuSO_4$- and $CuBr_2$-mediated reactions did not progress toward completion even after 12 h (entries 1 and 3). On the other hand, selective Boc-protection performed with $Cu(OAc)_2$ or $CuCl_2$ provided **7** in 90–95% yield (entries 2 and 4). Kinetically, the $Cu(OAc)_2$-mediated reaction is more favorable to the production of **7** than that with $CuCl_2$; the reaction was completed in 1 h with $Cu(OAc)_2$ (vs. 5 h with $CuCl_2$). It may be attributed to the stronger coordination ability of $CuCl_2$ to the C5”-amine, slowing down the electrophilic reaction with $(Boc)_2O$. This hypothesis was proven correct by the successful reaction in the strictly controlled condition (1.0 equiv of $CuCl_2$); **7** was synthesized in >90% in 1 h. We also examined selective Cbz-protection of **4** via the $Cu(OAc)_2$-mediated condition developed in Table 1 (Scheme 2). In both cases (CbzCl and CbzOSu), **4** could be converted to the desired mono-Cbz protected product **9** in 95% without formation of di-Cbz by-product **10**.

To obtain insight into mechanisms of the Cu-mediated selective Boc protection, we performed NMR studies for the Cu-**4** complexes. Unfortunately, all Cu salts in Table 1 showed poor solubility in the complexation with **4** in conventional deuterated solvents (*i.e.* D_2O, d_6- DMSO, d_6-acetone, and CD_3OD). Thus, we synthesized two model compounds **11** and **12**, and a mixture of **11** and **12** were applied to investigate complexation with the Cu-salts in solution. Complexation of **11** and **12** with $CuCl_2$ resulted in less precipitate and higher-resolution NMR. ^{1}H-NMR of a 1:1:1 mixture of **11**, **12**, and $CuCl_2$ in D_2O revealed that all carbon protons of the 2-((3-aminopropyl)amino)acetamide moiety of **11** are shifted to downfield; Δδ values were +0.73, +0.50, +0.26, and +0.05 for C2-H, C1’−2H, C2’−2H, and C3’−2H, respectively. In ^{13}C-NMR of **11**-CuCl complex, no chemical shifts were observed due to the complexation of **11** with $CuCl_2$. Although participation of the carbonyl oxygen in **11**-CuCl complex cannot be determined by these NMR experiments, coordination of the Cu-amide derivatives have previously been reported; based on X-ray and spectroscopic studies, the carbonyl groups of the primary amides are responsible for the formation of the stable Cu(II) complexes.[9] In our NMR analyses, in the presence of $CuCl_2$, the C5–2H in the aminoribose derivative **12** were not shifted (Δδ = 0), thus these data in solution-state may support that the C5”-amino group in **4** remains reactive in electrophilic reactions. Considering these facts, we speculate that the coordination geometries around the Cu(II) ion and 2-((3-aminopropyl)amino)acetamide moiety (highlighted in red) of **4** are as shown in **4**-CuCl complex in Figure 3.

Finally, we examined the urea-formation of the mono-Boc protected molecule **9** with the imidazole-carboxamide derivative **13** and deprotection to synthesize APPU (**2**). The coupling reaction between **9** and **13** was best performed in a mixed solvent system of DMF-CH_2Cl_2 (1/1) in the presence of Et_3N to furnish the urea **14** in 90% yield. Deprotection of **14** with 30% TFA provided APPU•TFA (**2**-TFA). Purification of **2**-TFA salt was performed by ion-exchange column (DOWEX 50Wx4, H^+) to afford **2** in quantitative yield. The synthetic APPU (**2**) in Scheme 3 was determined by ^{1}H- and ^{13}C-NMR, MS, and HPLC to be identical to a sample previously synthesized via a total synthesis (see SI)[1d].

In conclusion, we have established the semi-synthesis of an anticancer agent, APPU (**2**), from the muraymycin biosynthetic intermediate **3**. It was demonstrated that the 2-((3-aminopropyl)amino)acetamide moiety in the amide derivative **4** forms complexes with Cu species either in water or water-containing organic solvent systems. Such complexes can serve as protection of the primary amine at the C3”-position in **4**, allowing us to perform selective carbamate- formation reactions of the primary amine at C5”-position. Synthetic pathways illustrated in Scheme 1 and 3 warrant a wide range of application of the biosynthetic intermediate **3** for development of novel agents to cure human diseases (*i.e.* infectious and oncology fields). We are currently studying disruption of the mur30 gene of *Streptomyces sp*. NRRL 30471 to improve production of **3** (Figure 2).[10] Practicality of isolation and purification of **3** with the genetically modified *Streptomyces sp*. and a larger scale semi-synthesis of a DPAGT1 inhibitor with strong anticancer activity will be reported elsewhere.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

We thank the National Institutes of Health (Grant GM114611). MK also thank University of Tennessee Health Science Center for generous financial supports (UTHSC College of Pharmacy, CORNET, and UTRF awards). NMR data were obtained on instruments supported by the NIH Shared Instrumentation Grant. *Streptomyces sp*. NRRL 30471 was acquired from USDA (NRRL Culture Collection).

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(7). Isolation of **3**; see Supporting Information.

(8). The mon-Boc-protected molecule **7** and di-Boc-protected molecule **8** were synthesized via total chemical syntheses according to the procedure previously reported; see Supporting Information.

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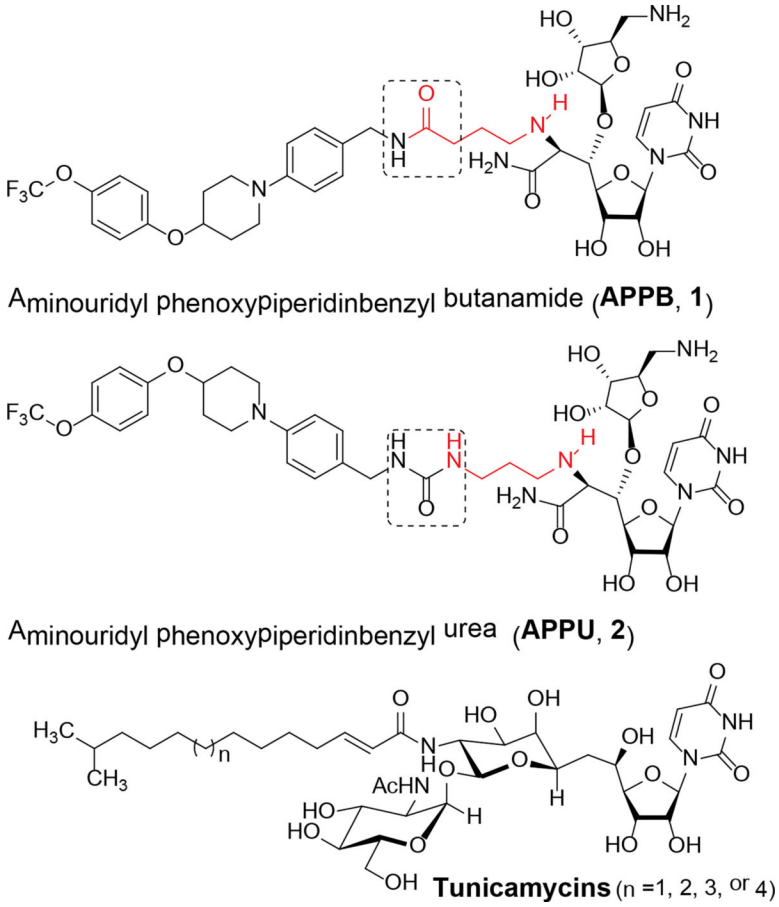


Figure 1.
Structures of Anticancer DPAGT1 Inhibitors, APPB (**1**), APPU (**2**), and tunicamycin.

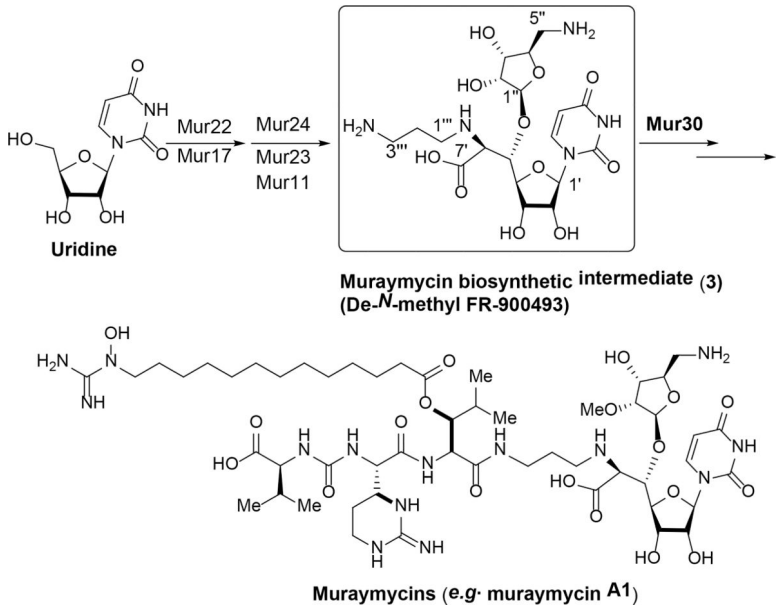


Figure 2.
Biosynthesis of Muraymycins in *Streptomyces sp.* NRRL30471.

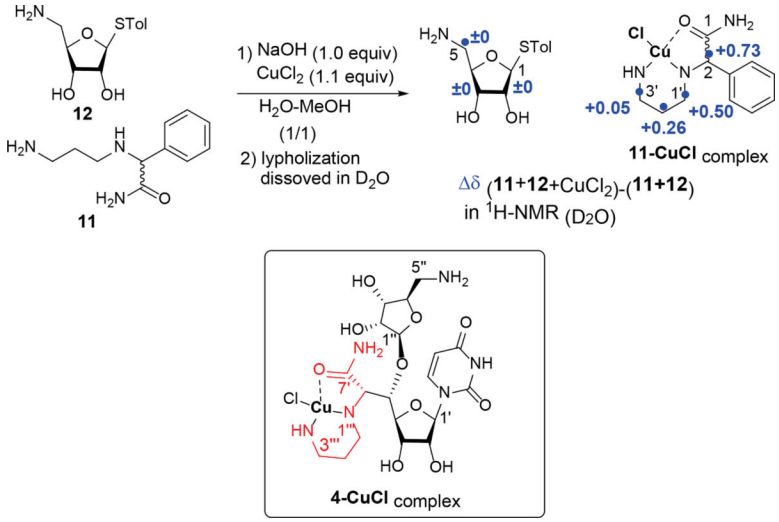
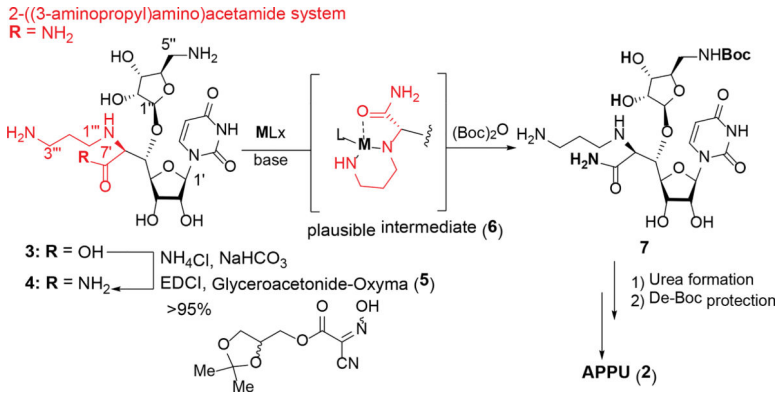
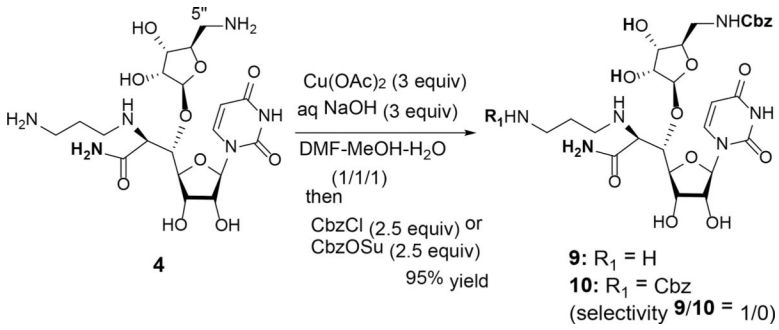


Figure 3.

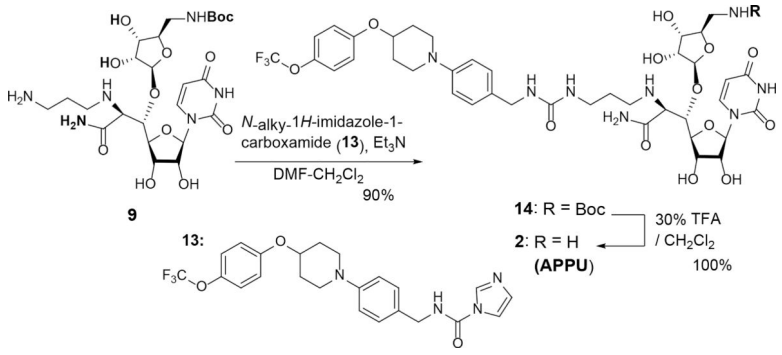
Plausible **4-CuCl** Complex Proposed Based on NMR Analyses.



Scheme 1.
Semi-synthetic Strategy of **1** from **2**.



Scheme 2.
Selective Cbz-protection of **4**.

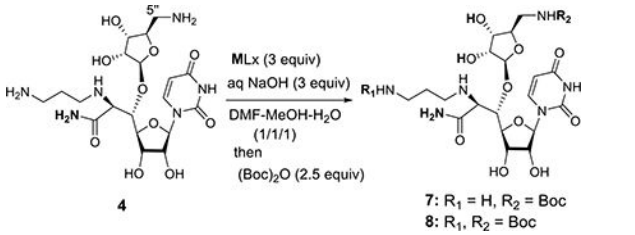


Scheme 3.

Synthesis of APPU (**1**) from **9**.

Table 1.

Selective Boc-protection of **4**.[a]



Entry	MLx	Time (h)	Selectivity (7 : 8)[c]	Yield for 7 (%)[d]
1	$CuSO_4$	12	1 : 0	20
2	**$Cu(OAc)_2$**	12(**1**)[b]	**1 : 0**	**95**
3	$CuBr_2$	12	1 : 0	10
4	**$CuCl_2$**	12(**5**)[b,e]	**1 : 0**	**90**
5	$NiCl_2•6H_2O$	12(5)[b]	>19 : 1	85
6	$Ni(OAc)_2•nH_2O$	12(5)[b]	>19 : 1	85
7	$Ni(NO_3)_2•6H_2O$	12(5)[b]	>19 : 1	85
8	VCl_3	12	4 : 1	40
9	$Zn(NO_3)_2•6H_2O$	12(3)[b]	1 : 1	45
10	$ZnCl_2$	12(3)[b]	1 : 1	40
11	$CoCl_2$	12(3)[b]	1 : 1	45
12	$FeSO_4•H_2O$	12(3)[b]	1 : 1	40
13	$FeCl_3•6H_2O$	12(3)[b]	1 : 1	40
14	-	12(1)[b]	1 : 1	35

[a] Reaction condition: **3** (1.0 equiv), **M**Lx (3.0 equiv), and NaOH (1N, 4.0 equiv) in DMF-MeOH-H_2O (1/1/1, 0.05M), after 30 min. $(Boc)_2O$ (2.5 equiv)

[b] time in the parenthesis: time required for the reaction to be completed

[c] Selectivity was determined via HPLC analysis for the reaction mixtures after 12h

[d] Isolated yield

[e] the same reaction with $CuCl_2$ (1.0 equiv) provided **6** in >90 yield in 1 h.