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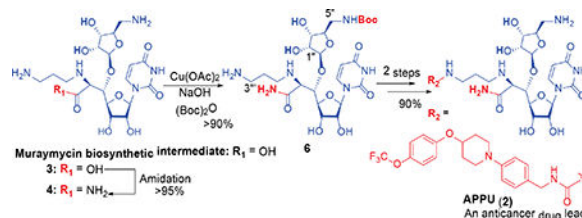
A Semi-Synthesis of an Anticancer DPAGT1 Inhibitor from a Muraymycin Biosynthetic Intermediate

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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*-acetylglucosaminylphosphotransferase 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, glyceracetone-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). CuSO₄- and CuBr₂-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 CuCl₂ 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 CuCl₂; the reaction was completed in 1 h with Cu(OAc)₂ (vs. 5 h with CuCl₂). It may be attributed to the stronger coordination ability of CuCl₂ 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 CuCl₂); **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.* D₂O, *d*₆-DMSO, *d*₆-acetone, and CD₃OD). 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 CuCl₂ resulted in less precipitate and higher-resolution NMR. ¹H-NMR of a 1:1:1 mixture of **11**, **12**, and CuCl₂ in D₂O revealed that all carbon protons of the 2-((3-aminopropyl)amino)acetamide moiety of **11** are shifted to downfield; δ values were +0.73, +0.50, +0.26, and +0.05 for C2-H, C1'-2H, C2'-2H, and C3'-2H, respectively. In ¹³C-NMR of **11**-CuCl complex, no chemical shifts were observed due to the complexation of **11** with CuCl₂. 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 CuCl₂, the C5-2H in the aminoribose derivative **12** were not shifted ($\delta = 0$), 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-CH₂Cl₂ (1/1) in the presence of Et₃N 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 ¹H- and ¹³C-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.

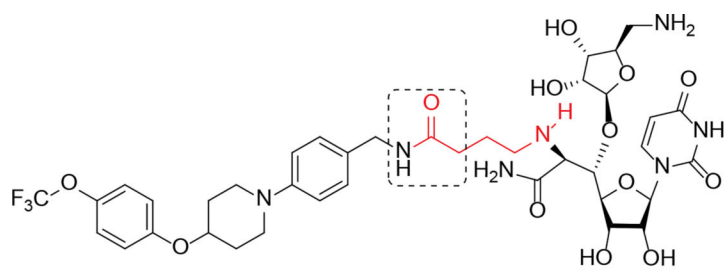
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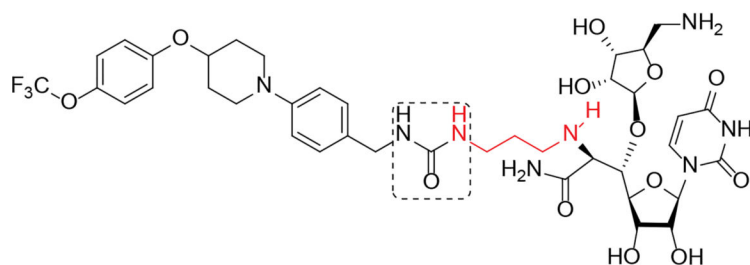
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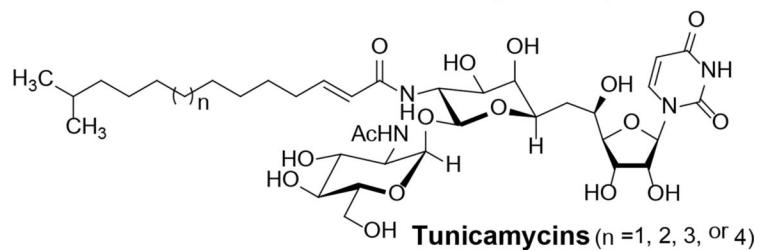
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- (7). Isolation of **3**; see Supporting Information.
- (8). The mon-Boc-protected molecule **7** and di-Boc-protected molecule **8** were synthesized via total chemical syntheses according to the procedure previously reported; see Supporting Information.
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Aminouridyl phenoxy piperidin benzyl butanamide (**APPB, 1**)



Aminouridyl phenoxy piperidin benzyl urea (**APPU, 2**)



Tunicamycins (n = 1, 2, 3, or 4)

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

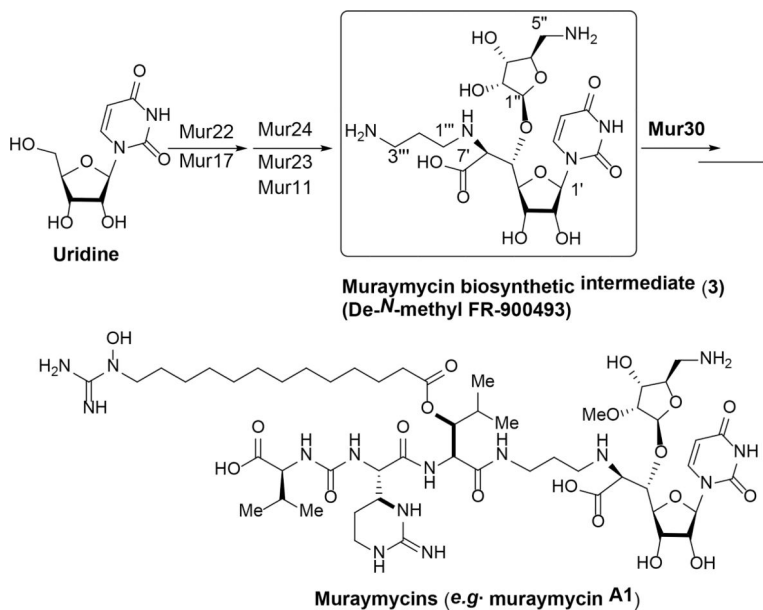


Figure 2.
Biosynthesis of Muraymycins in *Streptomyces* sp. NRRL30471.

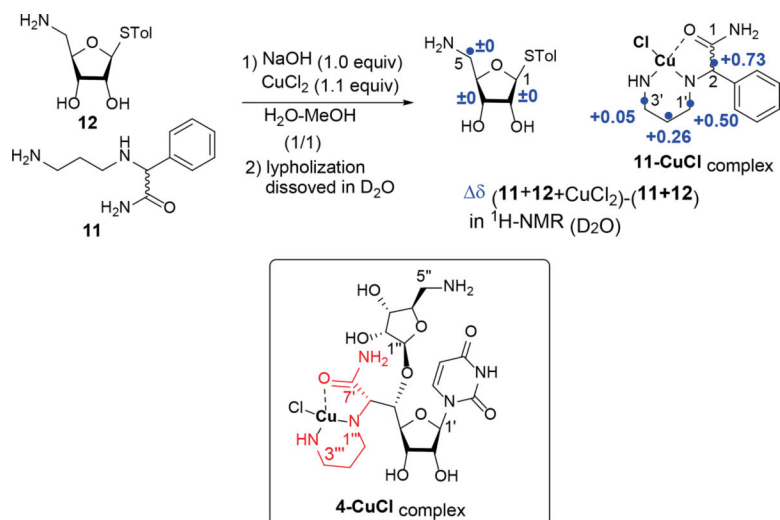
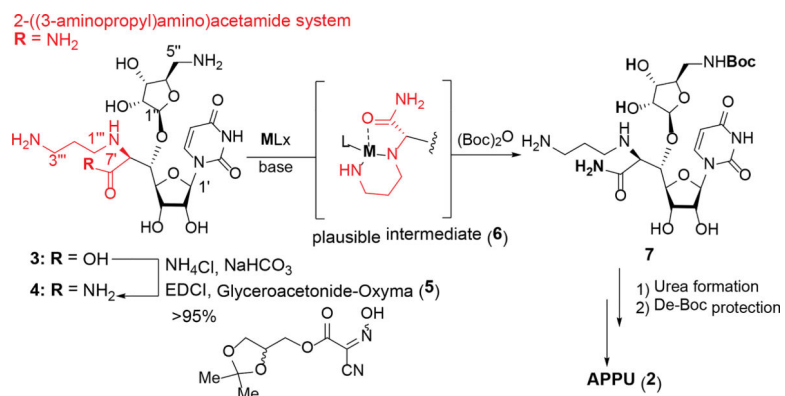
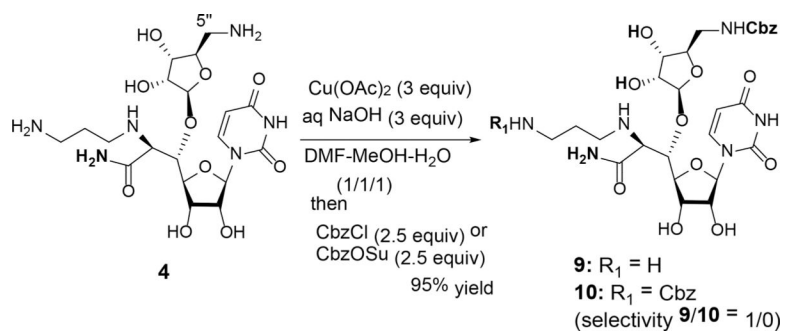


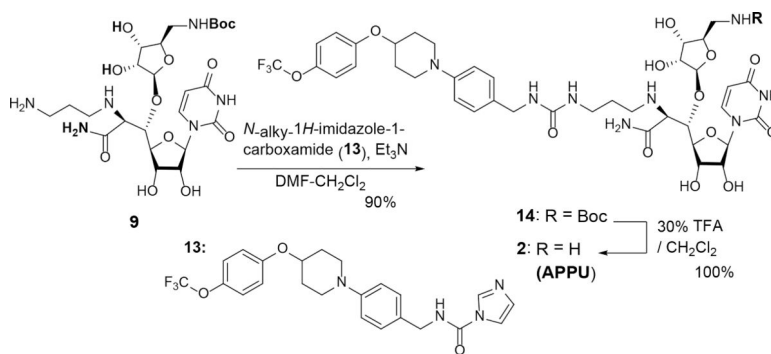
Figure 3.
Plausible 4-CuCl Complex Proposed Based on NMR Analyses.



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

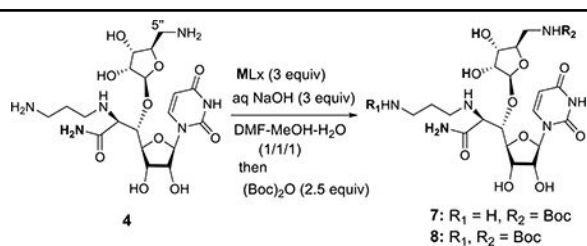


Scheme 2.
Selective Cbz-protection of **4**.



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	CuCl₂	12(5) ^{b,e}	1 : 0	90
5	NiCl ₂ •6H ₂ O	12(5) ^b	>19 : 1	85
6	Ni(OAc) ₂ •nH ₂ O	12(5) ^b	>19 : 1	85
7	Ni(NO ₃) ₂ •6H ₂ O	12(5) ^b	>19 : 1	85
8	VCl ₃	12	4 : 1	40
9	Zn(NO ₃) ₂ •6H ₂ O	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 ₂ O	12(3) ^b	1 : 1	40
13	FeCl ₃ •6H ₂ O	12(3) ^b	1 : 1	40
14	-	12(1) ^b	1 : 1	35

^aReaction condition: **3** (1.0 equiv), MLx (3.0 equiv), and NaOH (1N, 4.0 equiv) in DMF-MeOH-H₂O (1/1/1, 0.05M), after 30 min. (Boc)₂O (2.5 equiv)

^btime in the parenthesis: time required for the reaction to be completed

^cSelectivity was determined via HPLC analysis for the reaction mixtures after 12h

^dIsolated yield

^ethe same reaction with CuCl₂ (1.0 equiv) provided **6** in >90 yield in 1 h.