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Mini Review

Discovery of Safer N-Glycosylation Inhibitors: Design Strategies and Therapeutic Potential

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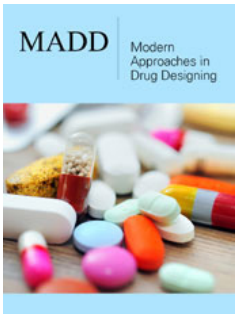
ISSN: 2576-9170



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Submission: April 21, 2026
Published: April 30, 2026

Volume 5 - Issue 2

How to cite this article: Michio Kurosu* and Bradley C Morrison. Discovery of Safer N-Glycosylation Inhibitors: Design Strategies and Therapeutic Potential. Mod Appro Drug Des. 5(2). MADD. 000606. 2026.
DOI: 10.31031/MADD.2026.05.000606

Abstract

N-Glycosylation is a fundamental post-translational modification that regulates the folding, stability, and trafficking of many oncogenic receptor proteins. The extent and structural complexity of N-glycans on these receptors are closely associated with tumor growth, progression, and metastatic potential, making this pathway an attractive therapeutic target. Although numerous glycosyltransferases participate in N-glycan biosynthesis, the selective and therapeutically effective inhibition of specific enzymes within this pathway remains a significant challenge in medicinal chemistry. The natural product tunicamycin inhibits DPAGT1, the first enzyme committed in N-glycan biosynthesis, thereby disrupting glycoprotein maturation in malignant cells. However, its promiscuous cytotoxicity, lack of selectivity, and suboptimal drug-like physicochemical properties have limited its clinical translation. In contrast, muraymycin A1 has emerged as a non-cytotoxic nucleoside antibiotic with potent and more selective DPAGT1 inhibitory activity. Importantly, muraymycin A1 and its analogues demonstrate significant antiproliferative and antimetastatic effects in DPAGT1-dependent solid tumors while exhibiting minimal toxicity toward normal cells, suggesting a therapeutically exploitable window. This short review highlights recent advances in the structural, mechanistic, and pharmacological characterization of muraymycin-based inhibitors, with an emphasis on their selectivity, improved biological profiles, and emerging translational potential. Together, these developments support targeting early steps in N-glycan biosynthesis as a promising strategy for anticancer drug discovery and future clinical applications.

Keywords: N-Glycosylation; DPAGT1 inhibition; Glycoprotein biosynthesis; Glycosyl transferase targeting; Antiproliferative agents; Antimetastatic activity; Nucleoside antibiotics; Non-cytotoxic agents

Introduction

Aberrant glycosylation is a well-established hallmark of cancer. In particular, O-glycans can provide enhanced target selectivity for therapeutic antibodies in specific biological contexts, especially in malignancies where truncated or aberrant glycoepitopes are selectively expressed [1]. A representative example is gatipotuzumab, which targets tumor-associated MUC1 bearing aberrant O-glycan structures (e.g., Tn antigen), enabling selective recognition of cancer cells while sparing normal tissues [2]. Such glycopeptide-specific epitopes confer a high degree of tumor specificity. Although N-glycans have historically been more extensively studied than O-glycans due to their conserved structures and experimental tractability, their therapeutic targeting has been limited by selectivity challenges [3,4]. N-Glycosylation is a ubiquitous and highly conserved process essential for proper protein folding, stability, and trafficking in all cells [5]. Similarly, small-molecule inhibitors targeting overexpressed enzymes in N-glycan biosynthesis have not been successfully translated into clinical applications. The first committed enzyme, DPAGT1 (dolichyl-phosphate N-acetylglucosamine-phosphotransferase 1), has been extensively studied using cytotoxic natural products such as tunicamycins, with thousands of reports describing their antitumor activity primarily *in vitro* and only rarely *in vivo*, often via intertumoral administration [6,7]. These data largely reflect

the inherent cytotoxicity of tunicamycins and have contributed to the longstanding misconception that DPAGT1 inhibition universally leads to global toxicity in both malignant and nontrans formed cells, whereas tunicamycins themselves exhibit significant membrane-disruptive effects across mammalian cell types [8-10]. Consequently, there is growing interest in leveraging safer, more selective DPAGT1 inhibitors to delineate mechanism-based toxicity and ER stress responses while enhancing therapeutic potential. This article highlights recent advances in the identification of non-cytotoxic DPAGT1 inhibitors, particularly muraymycin A1 (MA1), and discusses their rational design and optimization toward clinical applications.

Discussion

For over half a century, tunicamycins have been extensively used as chemical probes to study Endoplasmic Reticulum (ER) stress responses in transformed cells [7,8]. Despite their potent inhibition of DPAGT1, their therapeutic potential has remained largely unrealized due to substantial target-independent toxicity. This longstanding limitation has hindered efforts to define the precise role of DPAGT1 in cancer cell survival and to evaluate its suitability as a therapeutic target. Disentangling DPAGT1-dependent cytotoxicity from nonspecific toxic effects therefore represents a critical conceptual and translational advance, opening the door to the development of selective inhibitors that can exploit N-glycosylation vulnerabilities in cancer. In this context, the discovery of MA1, a structurally distinct and noncytotoxic nucleoside antibiotic, provides compelling evidence that such separation is achievable [11]. MA1 exhibits ~8.5-fold greater inhibitory potency against DPAGT1 than tunicamycin V (TM-V), yet displays a markedly improved selectivity profile. Specifically, MA1 induces antiproliferative effects across a broad spectrum of DPAGT1-dependent solid tumors, including pancreatic, gastric, prostate, breast, cervical, ovarian, melanoma, and head and neck cancers, while exhibiting minimal cytotoxicity toward low-DPAGT1-expressing cancer cells and nontrans formed cells [12]. These findings establish a clear mechanistic link between selective DPAGT1 inhibition and tumor-specific antiproliferative activity, and highlight the feasibility of developing next-generation inhibitors that retain on-target efficacy while minimizing nonspecific toxicity. MA1 has been biologically characterized across a panel of breast cancer models, including triple-negative breast cancer (TNBC), to define its antiproliferative profile and target-dependent activity [12]. MA1 is mechanistically distinguished from tunicamycins by the absence of membrane-disruptive activity that contributes to off-target cytotoxicity [9]. Accordingly, MA1 does not exhibit cytotoxicity in a panel of non-tumorigenic human cell types-including epithelial, fibroblast-like, immune, and cardiomyocyte lineages-at concentrations ≥100μM. In contrast, TM-V induces robust cytotoxicity in these cells at 0.25-0.45μM, consistent with its membrane-permeabilizing properties.

APPB was rationally designed to enable pharmacological proof-of-concept in anticancer and antimetastatic animal models. APPB exhibits high aqueous solubility (78.2mg/mL in saline), minimal hemolytic activity (ED_{50}>200μM in RBCs), and favorable metabolic stability ($t_1/_2$>60min in rat microsomes). Pharmacokinetic analysis shows AUC inf=2,900h·ng/mL. *In vivo* tolerability is significantly improved relative to TM-V, with LD_{50}>20mg/kg (i.e., mouse) compared to 1.0mg/kg for TM-V. APPB demonstrates potent antiproliferative activity against DPAGT1-overexpressing solid tumor models (IC_{50}=0.2-0.6μM) with a high selectivity index (SI>166-500).

These findings indicate that the nonselective toxicity of tunicamycins arises, at least in part, from membrane disruption, and further support MA1 as a scaffold for selectively interrogating DPAGT1-dependent biology. MA1 exhibits striking differences in cellular response compared to TM-V, including induction of G2/M cell cycle arrest rather than the G0/G1 arrest observed for TM-V [9,12]. This shift is accompanied by strong activation of apoptotic pathways and potent inhibition of cell migration. While these effects have been previously associated with tunicamycins, MA1 displays markedly enhanced activity, consistent with its significantly greater potency toward DPAGT1 (IC_{50}=0.18μM vs 1.5μM for TM-V). These findings further support a direct relationship between selective DPAGT1 inhibition and tumor-relevant cellular phenotypes. MA1 is a structurally complex nucleoside natural product, and its scalable supply for extensive *in vivo* evaluation remains a significant limitation [13,14]. While total synthesis can provide sufficient material for *in vitro* and *ex vivo* studies, structural simplification is required to enable practical pharmacological development [11]. Structure-activity relationship (SAR) analyses delineated the key functional elements necessary for DPAGT1 inhibition and antiproliferative activity. As shown in Figure 1, the hydroxy group on the ω-lipid chain does not make a significant contribution to DPAGT1 binding. Similarly, replacement of the C7′-carboxylic acid with the corresponding primary amide is well tolerated, sustaining inhibitory activity while improving synthetic tractability. In addition, removal of the cyclic guanidyl-urea moiety yields an MA1-NH_2-truncated analogue that retains DPAGT1 inhibitory activity, thereby defining a significantly simplified and synthetically accessible scaffold for further optimization [9,12]. Guided by these insights, the ω-guanidyl lipid hydroxyleucine ester moiety was replaced with a conformationally constrained, water-soluble lipid mimetic (TMPA group), affording APPB [15-17]. This modification yielded a highly water-soluble DPAGT1 inhibitor with ~7.2-fold enhanced inhibitory potency. APPB further exhibits favorable biochemical and pharmacokinetic properties, supporting its suitability for *in vivo* evaluation and pharmacological proof-of-concept studies [10]. Consistent with this profile, APPB demonstrated robust antitumor efficacy across multiple xenografts and orthotopic models, including a patient-derived breast cancer xenograft (HCI-012 PDX), triple-negative breast cancer (MDA-MB-231), and KPC-1 pancreatic cancer, following intraperitoneal administration (i.p) at 5-10mg/kg mouse. In addition to significant tumor growth inhibition, APPB induced suppression of metastatic progression under clinically relevant dosing conditions [10]. Tumors harvested from these *in vivo* studies were subjected to N-glycan profiling and immunohistochemical analyses, which confirmed that the observed tumor regression following APPB treatment is consistent with the same mechanism established *in vitro* in monolayer cultures. To

our knowledge, these findings represent the first demonstration that selective DPAGT1 inhibition can achieve tumor regression and growth suppression *in vivo*. Importantly, these studies indicate a favorable safety profile, supporting the therapeutic potential of DPAGT1-targeted inhibitors. Collectively, APPB represents a compelling lead scaffold for further optimization of pharmacokinetic and pharmacodynamic properties, as well as *in vivo* therapeutic efficacy, achievable through the rational design of water-soluble lipid mimetics. Notably, the recent determination of the cryo-EM structure of the DPAGT1-APPB complex provides critical molecular insights into ligand-protein interactions [18]. These structural data will facilitate structure-guided optimization of next-generation DPAGT1 inhibitors, supporting their advancement toward comprehensive toxicological evaluation and future clinical development.

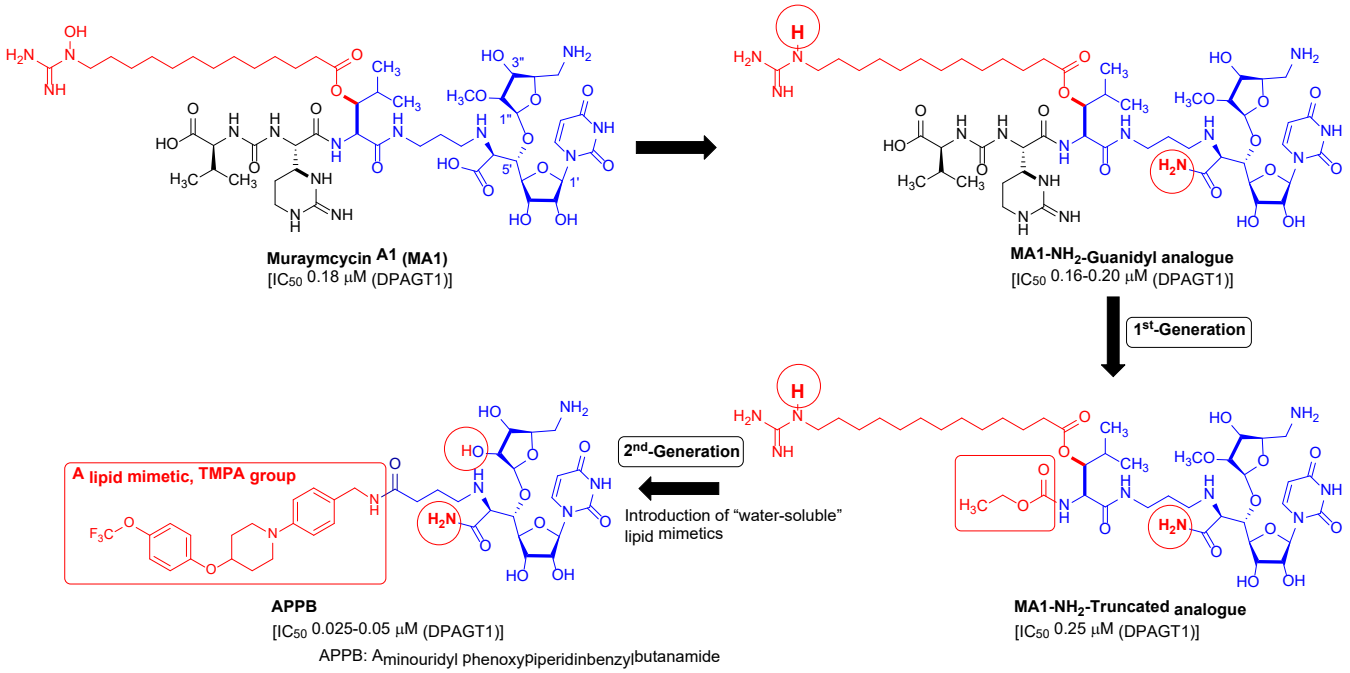

Figure 1: Discovery of a pharmacologically effective DPAGT1 inhibitor derived from muraymycin A1 (MA1).

Conclusion

A pharmacologically effective DPAGT1 inhibitor is rationally identified from the structure of noncytotoxic nucleoside antibiotic, muraymycin A1 (MA1). MA1 has previously never been investigated its DPAGT1 inhibitory activity. Cytotoxic tunicamycins were only DPAGT1 inhibitors until this discovery, and mechanistic studies using these molecules are not solely due to the DPAGT1 inhibition. MA1 does not affect growth of nontransformed cells, but shows antiproliferative activity against DPAGT1-dependednt solid cancers. Its simplified analogue design and synthesis are rationally established by Dr. Kurosu and his groups. Selective DPAGT1 inhibition operates within a partial suppression mechanism in which residual enzymatic activity sustains basal N-glycosylation in normal cells through compensatory mechanisms, including UPR-mediated transcriptional upregulation, metabolic adaptation, and reduced ER protein-folding load. In contrast, cancer cells, characterized by elevated glycoprotein flux and limited adaptive capacity, fail to compensate for reduced lipid-linked oligosaccharide biosynthesis, resulting in unresolved ER stress, impaired maturation of oncogenic glycoproteins, and apoptotic cell death. N-Glycosylation dependent solid cancers (e.g., breast cancers) are hypersensitive to DPAGT1 perturbation [12]. Nonetheless, detailed mechanistic studies to address how DPAGT1 inhibitors selectively impair cancer-dependent N-glycosylation without globally poisoning cells should be performed. The experiments must demonstrate target engagement, differential pathway dependence, compensatory capacity, and downstream functional selectivity between cancer and normal cells. Selective DPAGT1 inhibition induces compensatory regulatory responses in which proteins antagonistic to metastasis are upregulated. Suppression of the EMT driver Snail (a zinc-finger transcriptional repressor) is accompanied by increased E-cadherin (a calcium-dependent cell-cell adhesion protein) expression, consistent with Mesenchymal-to-Epithelial Transition (MET) and providing a mechanistic basis for the observed antimetastatic activity [10,12]. During APPB treatment, several seemingly "paradoxical" protein upregulations were observed despite attenuation of oncogenic signaling. Integration of RNA-seq and glycoproteomics data indicates that these responses arise from compensatory transcriptional and proteostatic reprogramming induced by DPAGT1 inhibition. Reduced N-glycosylation destabilizes key glycoproteins involved in proliferative and metastatic pathways, while activating stress-responsive programs that promote epithelial differentiation. Accordingly, the observed upregulation reflects adaptive network rewiring rather than direct pathway activation, contributing to the antimetastatic phenotype.

In summary, the cytotoxic effects of tunicamycins have historically been attributed to inhibition of DPAGT1. However, converging mechanistic, cellular, and structural evidence now demonstrates that this interpretation is fundamentally flawed. Instead, tunicamycin-induced cytotoxicity arises predominantly from DPAGT1-independent liabilities, including membrane-disruptive effects, rather than selective target engagement. These findings overturn the longstanding assumption that DPAGT1 inhibition is intrinsically toxic and unsuitable for therapeutic development. The emergence of selective DPAGT1 inhibitors establishes a new paradigm, enabling precise interrogation of DPAGT1-dependent biology and redefining the therapeutic potential of targeting N-glycosylation.

Acknowledgements

This work was supported by the National Institutes of Health (R01GM114611). We gratefully acknowledge Dr. Katsuhiko Mitachi (University of Tennessee Health Science Center) and Dr. William M. Clemons (California Institute of Technology) for their valuable discussions and collaborative contributions.

Conflict of Interest

Bradley Morrison is the Founder & CEO of Anviron, a license partner of University of Tennessee and sponsor of anti-cancer research using glycobiology approaches including APPB. Michio Kurosu serves as Interim Vice President of Research and as a member of Anviron's Board of Advisors.

References

1. Rømer TB, Aasted MKM, Dabelsteen S, Groen A, Schnabel J, et al. (2021) Mapping of truncated O-glycans in cancers of epithelial and non-epithelial origin. Br J Cancer 125: 1239-1250.
2. Rajesh C, Radhakrishnan P (2023) The (Sialyl) Tn antigen: Contributions to immunosuppression in gastrointestinal cancers. Front Oncol 12: 1093496.
3. Lin Y, Lubman DM (2024) The role of N-glycosylation in cancer. Acta Pharmaceutica Sinica B 14: 1098-1110.
4. Pinho SS, Reis CA (2015) Glycosylation in cancer mechanisms and clinical implications. Nature Rev Cancer 15: 540-555.
5. Moharir A, Peck SH, Budden T, Lee SY (2013) The role of N-glycosylation in folding, trafficking, and functionality of lysosomal protein CLN5. PLoS One 8: e74299.
6. Kurosu M (2019) Structure-based drug discovery by targeting N-glycan biosynthesis, dolichyl-phosphate N-acetylglucosamine phosphotransferase. Future Med Chem 11: 927-933.
7. Mitachi K, Mingle D, Effah W, Sánchez-Ruiz A, Hevener KE, et al. (2022) Concise synthesis of tunicamycin V and discovery of a cytostatic DPAGT1 inhibitor. Angew Chem Int Ed Engl 61: e202203225.
8. Kurosu M, Mitachi K (2025) DPAGT1-perspective as an anticancer drug target. Molecules 30: 4049.
9. Mitachi K, Abdelsattar AS, Skorupinska-Tudek K, Swiezewska E, Drake RR, et al. (2026) ω-Lipid chain–engineered tunicamycin analogues as safer antiproliferative and antimetastatic biochemicals. Bioorg Chem 175: 109835.
10. Mitachi K, Kurosu M (2023) Development of novel DPAGT1 inhibitors based on tunicamycin V and its homologous structures. J Synth Org Chem 81: 220-234.
11. Mitachi K, Cheng-Sánchez I, Sánchez-Ruiz A, Sarabia F, Kurosu M (2025) Total synthesis of muraymycin A1: A protecting group strategy for nucleoside antibiotic synthesis. Org Lett 27: 9448-9453.
12. Mitachi K, Daria D, Kirsh JM, Effah W, Narayanan R, et al. (2026) New insights of muraymycin A1 and its analogs as DPAGT1 inhibitors. Bioorg Chem 169: 109402.
13. Mitachi K, Aleiwi BA, Schneider CM, Siricilla S, Kurosu M (2016) Stereocontrolled total synthesis of muraymycin D1 having a dual mode of action against mycobacterium tuberculosis. J Am Chem Soc 138: 12975-12980.
14. McDonald LA, Barbieri LR, Carter GT, Lenoy E, Lotvin J, et al. (2002) Structures of the muraymycins, novel peptidoglycan biosynthesis inhibitors. J Am Chem Soc 124: 10260-10261.
15. Mitachi K, Kansal R, Hevener K, Gillman C, Yun HG, et al. (2020) DPAGT1 Inhibitors of capuramycin analogues and their antimigratory activities of solid tumors. J Med Chem 63: 10855-10878.
16. Mitachi K, Kurosu SM, Eslamimehr S, Lemieux MR, Ishizaki Y, et al. (2019) Semi-synthesis of an anticancer DPAGT1 Inhibitor from a muraymycin biosynthetic intermediate. Org Lett 21: 876-879.
17. Mitachi K, Yun HG, Kurosu SM, Eslamimehr S, Lemieux MR, et al. (2018) Novel FR-900493 analogs that inhibit outgrowth of clostridium difficile spores. ACS Omega 3: 1726-1739.
18. Kaudeer BY, Kirsh JM, Mitachi M, Ochoa JM, Soroush-Pejrimovsky MT, et al. (2026) Structures of bacterial and human phosphoglycosyltransferases bound to a common inhibitor inform selective therapeutics. bioRxiv.