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Design and Catalyzed Activation of Mycophenolic Acid Prodrugs

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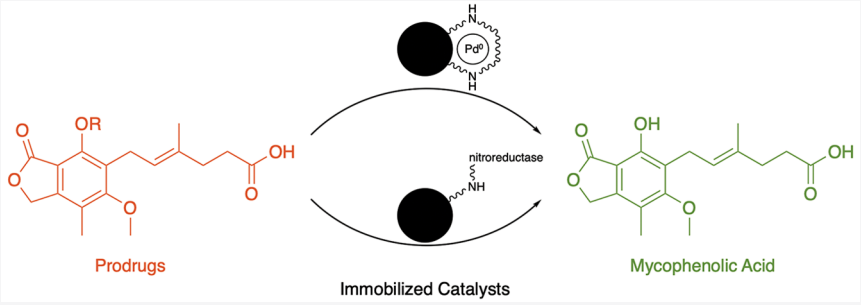

ABSTRACT: Mycophenolic acid (MPA) and its morpholino ester prodrug mycophenolate mofetil (MMF) are widely used in solid organ transplantation. These drugs prevent rejection due to their potent inhibition of inosine-5′-monophosphate dehydrogenase (IMPDH), an enzyme vital for lymphocyte proliferation. As a strategy to provide localized immunosuppression in cell transplantation, four mycophenolic acid prodrugs designed to release MPA by two distinct mechanisms were synthesized and characterized. A nitrobenzyl ether prodrug was effectively converted to MPA upon exposure to bacterial nitroreductase, while a propargyl ether was converted to the active drug by immobilized Pd^0 nanoparticles. *In vitro*, both prodrugs were inactive against IMPDH and exhibited reduced toxicity relative to the active drug, suggesting their potential for providing localized immunosuppression.

KEYWORDS: *Prodrug, Enzyme prodrug therapy, Bioorthogonal organometallic activation, Nitroreductase, Mycophenolic acid*

The transplantation of hepatocytes or pancreatic islet cell-clusters are two promising approaches to address impaired/missing liver function or Type-1 diabetes, respectively. However, these cellular transplants encounter significant challenges due to the challenging tissue isolation procedures and the complete exposure of donor cells to host tissues and blood. Our group is exploring strategies to protect these transplants by the local delivery of potent small-molecule immunomodulators. We recently demonstrated that a prodrug of the potent toll-like receptor 4 (TLR4) inhibitor TAK-242[1,2] can be covalently immobilized on islet surfaces without adverse impact on the tissue viability or function. Slow post-transplant release of the active drug from the transplant tissue can suppress innate inflammation and significantly improved outcomes in a murine model of islet transplantation.[3] More recently, we have envisioned a second strategy for localized drug delivery, wherein immobilized catalysts are cotransplanted with graft tissue in order to activate reactive prodrugs (Figure 1). In that regard, we recently reported TAK-242 prodrugs that are activated by either Pd^0 nanoparticles or nitroreductase.[4]

Subsequent to the acute innate inflammation during the early peri-transplant period, transplanted tissue is also subject to rejection by the innate and adaptive immune system.

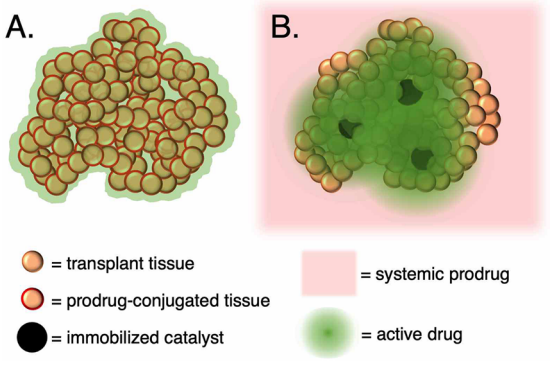

Figure 1. Two strategies for transplant-localized drug delivery. (A) Covalently modified tissue slowly releases active drug. (B) Systemically administered prodrug is activated by localized catalysts.

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Mycophenolic acid (MPA, **1**), and its morpholino ester prodrug mycophenolate mofetil (MMF, **2**, CellCept) (Figure 2) are widely used in solid organ transplantion to prevent

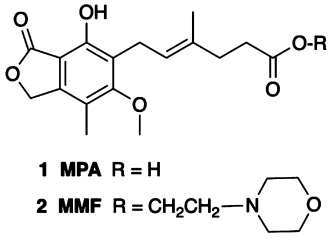


Figure 2. Structures of mycophenolic acid **1** and ester prodrug **2**.

rejection,[5,6] as they reduce lymphocyte proliferation by the inhibition of inosine-5′-monophosphate dehydrogenase (IMPDH).[7] We now report the synthesis and characterization of mycophenolic acid prodrugs for localized immunosuppression in cell transplantation.

Prodrug derivatives of MPA have been previously reported. The ester prodrug mycophenolate mofetil (**2**) is rapidly converted to MPA (**1**) by serum and liver esterases, and while it provides improved oral bioavailability it does not afford opportunity for localized delivery.[8] More recently, a clickable, acid labile MPA prodrug was reported for the modification of hydrogels, which could be implanted to provide a localized release of the active drug upon exposure to a lower pH at a site of inflammation.[9] In contrast, our strategy for localized delivery involves the systemic administration of prodrug and its localized activation at a target site. We chose to evaluate two complementary approaches for prodrug activation - bioorthogonal organometallic (BOOM) chemistry,[10−13] where metal catalysts reveal the active drug, and directed enzyme prodrug therapy (DEPT),[14−20] which utilizes exogenous enzymes for prodrug activation.

For the preparation of BOOM-reactive prodrugs we chose to explore propargyl derivatives of MPA, which were anticipated to release the parent drug (and hydroxyacetone) upon exposure to Pd nanoparticles embedded in TentaGel resin.[21−24] Accordingly, MPA was reacted with propargyl bromide and DBU, readily affording the bis-propargyl prodrug **3** (Scheme 1). Aqueous hydrolysis of ester **3** then gave the monopropargyl derivative **4** in good yield. We also prepared *p*-nitrobenzyl (pNB) protected prodrugs, which could be converted to the active compound by nitroreductase, a bacterial enzyme commonly used in DEPT.[25,26] For these derivatives, enzymatic reduction of the prodrug nitro group is followed by a 1,6-elimination, releasing the active compound.[27,28] Reaction of MPA with *p*-nitrobenzyl chloride and triethyl amine provided the bis-pNB prodrug **5** (Scheme 1), which was readily hydrolyzed, yielding mono-pNB derivative **6** in good yield.

With MPA derivatives **3**−**6** in hand, their suitability as prodrugs was first examined. First, these compounds were evaluated for residual IMPDH2 activity (MPA inhibits both isoforms of IMPDH) using a standard *in vitro* assay. In this assay, while 10 μM MPA completely inhibits the enzyme, none of the derivatives showed significant activity at this concentration (Figure 3).

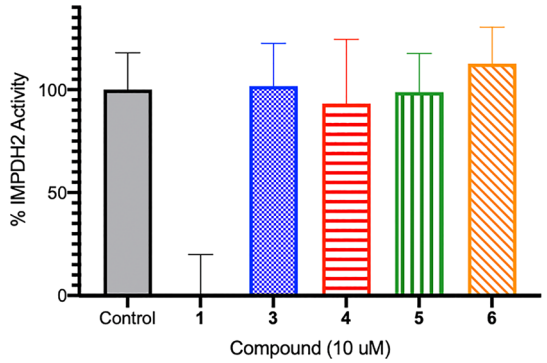
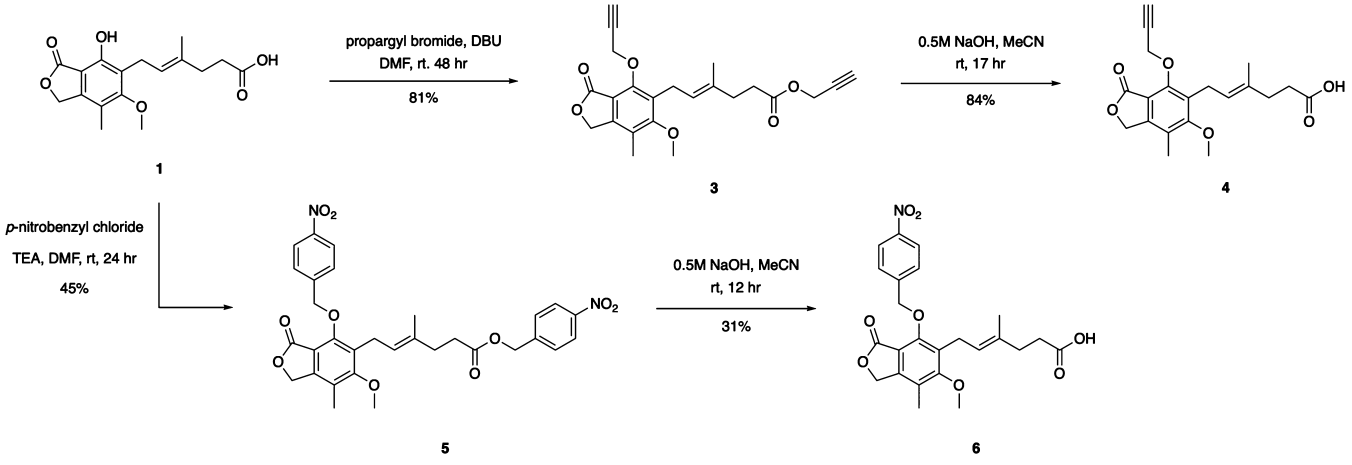


Figure 3. IMPDH2 activity assay. IMPDH2 was treated with MPA (1) and derivatives **3**−**6** and stimulated with inosine-5′-monophosphate and nicotinamide adenine dinucleotide (NAD+). Conversion of NAD+ to NADH was monitored at 340 nm.

The acute toxicity of the new compounds was evaluated *in vitro* in a pancreatic β-cell model (MIN6 cell line[29]) and in primary human hepatocytes (Figure 4). Briefly, cells were cultured in the presence of the various new compounds or MPA for 48 h and then dehydrogenase activity was determined with a colorimetric assay (CCK8). In the β-cell model MPA was relatively toxic (EC50 = 0.6 μM), while prodrug **6** was the lone prodrug exhibiting toxicity, albeit at a 40-fold higher concentration (EC50 = 24.0 μM). In human hepatocytes, the

Scheme 1. Synthesis of MPA Prodrugs 3−6



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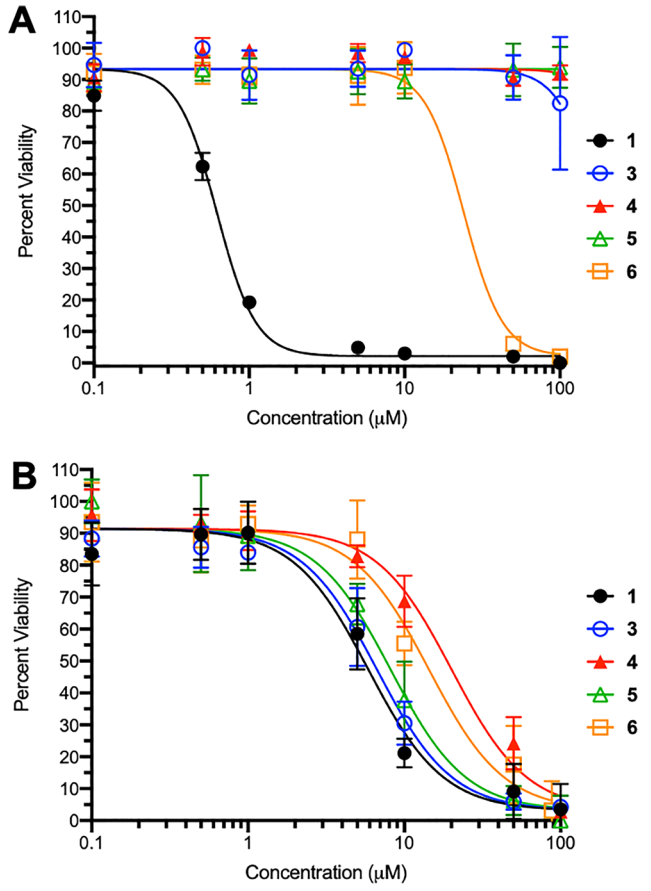

Figure 4. Toxicity was assessed for the five compounds by using CCK-8 assay on (a) MIN6 cells and (b) human hepatocytes.

two acid prodrugs (**4** and **6**) were the least toxic (EC50 values of 20.1 and 14.4 μM, respectively). The toxicity of the two ester prodrugs **3** and **5** (EC50 values of 6.7 and 8.3 μM, respectively) were more similar to the parent drug (EC50 = 5.9 μM).

Before characterizing the conversion of the prodrugs to MPA we evaluated the aqueous stability of these compounds in the absence of the activating catalysts. Not surprisingly, the disubstituted esters **3** and **5** slowly hydrolyzed in neutral phosphate buffered saline (PBS), while the monosubstituted ethers **4** and **6** were stable under these conditions (Figure 5). Due to the instability of the disubstituted compounds, coupled

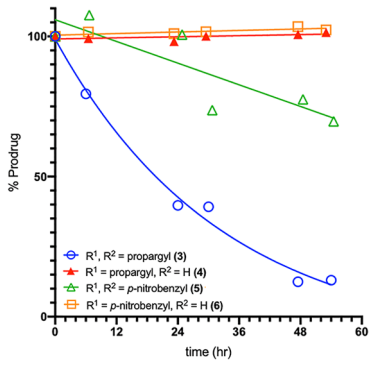

Figure 5. Aqueous stability of prodrugs **3**–**6** assessed via incubation in PBS (pH 7.4, 5% DMSO) at 37 °C in the absence of catalyst.

with their poor aqueous solubility, ethers **4** and **6** were selected for further evaluation as catalyst activated prodrugs.

We then evaluated the release of active MPA from prodrugs **4** and **6** using catalysts immobilized on functionalized 30 μm polystyrene beads. For the activation of propargyl ether **4** we utilized the same Pd^0-modifiedTentaGel resins[13] as in our previous studies on propargyl-substituted TAK-242 prodrugs.[3] For activating the nitrobenzyl ether **6** we conjugated nitroreductase to amino terminated polystyrene beads via a simple reductive amination procedure,[30] Using Pd^0-modified beads (1 mg/mL, PBS w/5% DMSO, 37 °C), propargyl prodrug **4** underwent clean conversion to the parent drug MPA (**1**) over ~2 days (Figure 6). Likewise, enzymatic

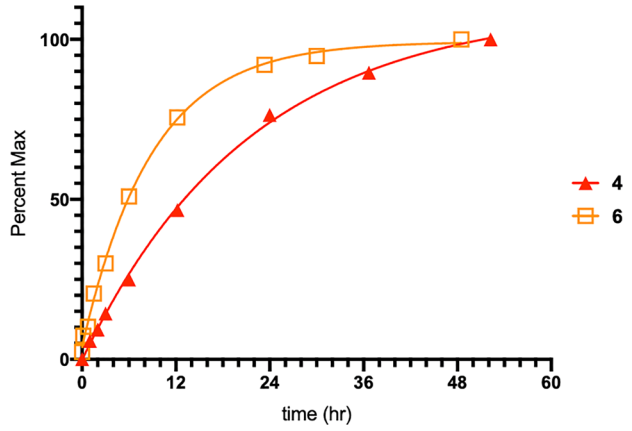

Figure 6. Catalyzed release of mycophenolic acid (**1**) was determined by incubating **4** and **6** in PBS (pH 7.4, 5% DMSO) with the appropriate solid supported catalyst: (a) prodrug **4** with Pd^0; (b) prodrug **6** with nitroreductase and NADH.

activation of nitrobenzyl prodrug **6** with nitroreductase beads (2 mg/mL, PBS, 5% DMSO, 37 °C) in the presence of the reducing cofactor β-nicotinamide adenine dinuclotide (NADH, 1 mg/mL) led to the efficient production of free MPA (**1**). The reduced amine intermediate was not observed, suggesting that the 1,6-elimination was rapid. We found that the resins could be reused, and quantified the MPA release from **4**, showing that the conversion was clean and quantitative (see Supporting Information). Nitrobenzyl prodrug **6** was not unmasked by treatment with Pd^0-modified beads, demonstrating the specificity of the activation chemistry.

In summary, we have synthesized four mycophenolic acid derivatives and characterized the two most promising compounds as prodrugs for localized immunosuppression. The prodrugs do not exhibit IMPDH2 activity characteristic of the parent drug, show less *in vitro* toxicity than the parent drug in two relevant cell lines, and are stable in buffer but rapidly converted to MPA by treatment with the appropriate solid-supported catalyst. We are presently evaluating the use of these prodrugs for the protection of islet and hepatocyte[31] grafts.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications Web site. The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.1c00079.

Full experimental details for the synthesis of prodrugs **3**–**6**, HPLC measurement of prodrug hydrolysis,

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catalytic activation and release of parent drug, nitroreductase immobilization, IMPDH2 activity assay, and cell toxicity assays (PDF)

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Author Contributions

M.A.P. synthesized and characterized the prodrugs, performed the kinetic analyses, and produced the manuscript. J.M.Q. performed the toxicity assay on human hepatocytes and contributed to the manuscript preparation. C.M.D. performed the toxicity assay on MIN6 cells and contributed to the manuscript preparation. M.C.L., B.N., and R.R.K. guided the experimentation and edited the manuscript. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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