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Soluble-Polymer Supported Synthesis of a Prostanoid Library: Identification of Antiviral Activity

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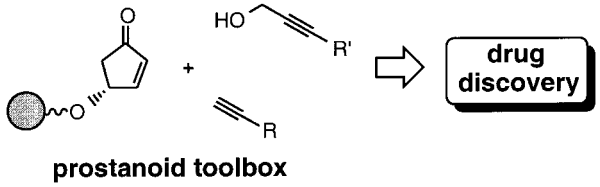
ABSTRACT



The prostaglandins are potent natural products taking part in many biological processes. The "convergent generation of diversity" from a "toolbox" of prostanoid components, augmented with additional polymer-supported transformations, can enable construction of valuable libraries. A parallel-pool strategy was used to assemble a small library of prostanoids. The inhibition of a herpes-family virus demonstrated the potential for new drug discovery.

The synthesis and screening of chemical libraries represents the current forefront in the search for compounds possessing biological activity. Of primary importance in this endeavor is the construction of complex molecules on polymer supports through adaptation of sophisticated organic reactions. A number of natural product analogues have recently highlighted the advancements in this area.[1] Our contributions have included the syntheses of the prostaglandin E_2 (PGE_2) methyl ester and the related $PGF_{2\alpha}$ on soluble polystyrene.[2]

The prostaglandin family of natural products constitutes perhaps the most physiologically potent nonprotein molecules found in mammals. These relatively low molecular weight and delicate structures play a vital role in the processes of inflammation and tissue repair and in the immune response.[3] Given their enormous potential therapeutic benefits, extensive efforts have been directed at the design and synthesis of pharmacologically useful analogues.[4] Herein, we report the development of our previous soluble-polymer technology for the preparation of a prostanoid library. Moreover, evaluation of the library for inhibition of cytomegalovirus (CMV), a herpes-family virus, revealed a prostanoid with potent antiviral activity.

The "convergent generation of diversity" from a "toolbox" of prostanoid components (enones, α-chains, *ω*-chains), augmented with additional polymer-supported transformations, can enable the assembly of arrays of valuable compounds. Central to this approach is a "parallel-pool" library strategy that incorporates the well-known techniques of split-mix and parallel combinatorial synthesis.[5] In this way, small pools of compounds (4−16 members) are manipulated during library construction through a desired number of

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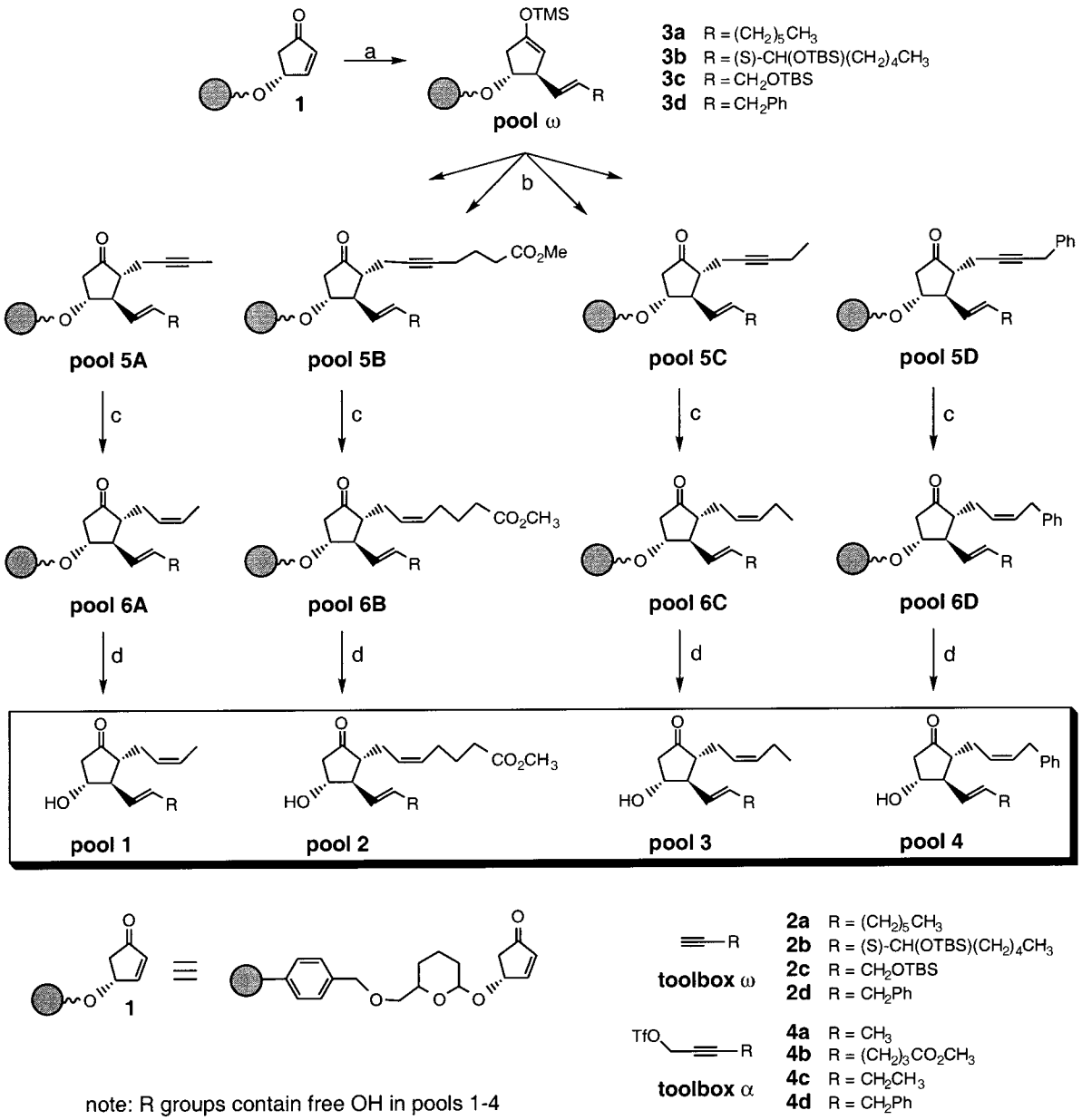

Figure 1. The parallel-pool synthesis of a prostanoid library: (a) (i) **2a**, **2b**, **2c**, or **2d** (5 equiv), $Cp_2Zr(H)Cl$ (5 equiv), THF, rt, 30 min, (ii) MeLi (10 equiv), −50 °C, 10 min, then CuCN (5 equiv), −50 °C, 15 min, (iii) MeLi (5 equiv), −50 °C, 15 min, (iv) **1**, THF, −50 °C, 40 min, (v) TMSCl (25 equiv), −50 °C, 40 min, then NEt_3 (50 equiv), −50 to 0 °C, 15 min; (b) (i) MeLi (4 equiv), THF, −23 °C, 40 min, (ii) **4a**, **4b**, **4c**, or **4d** (18 equiv), THF, −78 °C, 10 min, then −23 °C, 40 min; (c) H_2, 5% Pd−$BaSO_4$, quinoline, benzene/cyclohexane (1/1), 45 °C, 48 h; (d) 48% aqueous HF, THF, 45 °C, 6 h.

mixing and splitting steps, in conjunction with identical or different parallel chemical operations on the individual pools. Significantly, although a library of unbound pools is obtained at the conclusion of reaction sequences, all prior pools following convergence of the three prostanoid components can be considered as part of the total library upon release from the polymer. Each pool can then be assessed in a particular biological assay and, if positive, deconvoluted by synthesis of subpools and unique members. We describe the preparation and evaluation of a small prostanoid library that provides a model for eventual extension to larger libraries.

The soluble-polystyrene polymer **1** was functionalized with ω chains in four separate reactions that yielded **3a**−**d** and then pool ω when the polymers were mixed in equal amounts (Figure 1). The pool ω was split into four batches and each reacted with a different α-chain triflate **4a**−**d** that produced the first set of polymer-supported prostanoids. The pools 5A−D represented 16 potential members of our prostanoid library. However, our objective at this time was to evaluate prostaglandin analogues that contained the natural side-chain unsaturation. Hence, parallel reductions of the alkynyl groups were carried out that afforded pools 6A−D. A comparison

of the ^{1}H NMR spectra of individual polymer-supported pools before and after hydrogenation clearly showed the successful outcome of the reaction. The integration of all vinylic resonances was doubled after reduction relative to the distinct acetal-methine hydrogen derived from the linker and used as a standard. These data, together with ^{1}H NMR data of **3a**−**d** and pools 5A−D, indicated an overall excellent efficiency of the convergent operations.

Treatment of pools 6A−D, in parallel, with fluoride liberated the final 16-member library as pools 1−4 (26−38% yields based on average molecular weights) in which each contained four compounds. As an example, pool 1 contained prostanoids **7aa**, **7ab**, **7ac**, and **7ad** denoted with two letters that indicated the origin from toolbox α and toolbox ω chains, respectively (Figure 2). The ^{1}H NMR and

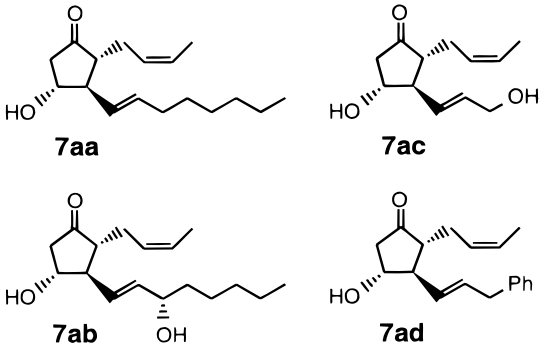

Figure 2. The prostanoids contained in pool 1 of the library.

HPLC analyses of pools 1−4 showed characteristic proton signals and four well-separated chromatographic peaks. In light of subsequent biological data (vide infra), each member of pool 1 was independently synthesized, which confirmed structure and stereochemistry. Interestingly, unlike other prostaglandin analogues, **7ad** could not be synthesized in solution and was therefore prepared via our polymer-supported synthesis followed by an additional purification step that yielded analytical material. While the reasons for success only on the polymer were not clear, control experiments ruled out a linker effect and one possible solvent-like microenvironment effect (e.g., the solution reaction failed not only in the standard THF solvent but also in toluene). Thus, soluble-polymer synthesis might provide access to some valuable prostanoids not otherwise obtainable by conventional methods. For all pools only minor amounts of other compounds, such as des-α-chain prostanoids from incomplete alkylation of pool ω, were evident. After analysis, the final pools were entered into a screening assay for inhibition of a productive CMV infection.

CMV is prevalent in most healthy adults in a latent state, although it rarely reactivates and causes disease in immunocompetent individuals.[6] However, in cases of immunosuppression, as in AIDS patients and transplant recipients, there can be dire consequences of morbidity and mortality.[7] Since prostaglandins are immunomodulators, we investigated the effect of the library prostanoids on the replication of murine CMV in cultured cells. After synthesis, each final pool was tested and pool 1 effected a significant inhibition of viral titer (Table 1). Members **7aa**−**7ad** of this pool were

Table 1. Inhibition of MCMV Growth in NIH 3T3 Cells by Prostanoids[a]

prostanoid(s)	viral titer (PFU/mL)[b]	activity (%)[c]
PGE_2	1.32×10^5	47
PGE_2 Me ester	3.60×10^5	>100
pool 1	1.70×10^4	6
pool 2	4.02×10^5	>100
pool 3	1.17×10^5	42
pool 4	1.02×10^5	36
7aa	6.80×10^3	2
7ab	1.10×10^5	39
7ac	1.30×10^5	46
7ad	1.60×10^5	57

[a] MCMV = murine CMV; see Supporting Information for assay procedure. [b] PFU = plaque forming units. [c] The viral titer that remained as a percentage compared to the control titer (without exogenous prostanoid) after addition of 20 μM prostanoid(s).

individually examined for antiviral activity. The results of these experiments showed that **7aa** was clearly the most potent.

Work by others on human CMV and ganciclovir, the most commonly used antiviral agent, suggested that our compound compared favorably, having approximately an order of magnitude less potency.[8] The remainder of the library, the alkynyl prostanoids obtained after cleavage of pools 5A−D, will be studied in future experiments. Although previous studies have established a link between prostaglandins and CMV replication, the exact nature of this connection is not well understood. Interestingly, such studies have generally revealed that blocking prostaglandin synthesis inhibits viral growth,[9] an effect contrary to that observed here. Yet, some prostaglandins up-regulate and others down-regulate immune function. Several of the prostanoids present in our library, such as the previously unknown **7aa**, might indeed boost cell-mediated factors that inhibit the virus. Our results are significant, since there are few antiviral agents or drug therapies clinically available for CMV infection and these often lack desirable efficacy.[10]

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We demonstrated a parallel-pool strategy as one approach to the construction of prostanoid libraries. It is anticipated that perhaps a 4-fold increase in overall diversity (i.e., up to 128 members) will be feasible with additional split-mix and parallel operations. In this regard, mass spectrometry should prove useful for examining the composition of larger libraries.[11] The advantages of the parallel-pool approach versus individual compound syntheses will be fully appreciated in the preparation of hundreds of compounds via several libraries. Finally, from a lead compound as discovered herein, a focused, second-generation library could provide even more effective prostanoids as well as aid in the delineation of the mechanism of action of these novel antiviral agents. Hence, high-throughput screening of many new prostaglandin analogues provides an excellent opportunity for drug discovery.

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Supporting Information Available: Experimental procedures, spectral data, and biological assay methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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