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Poly(*N*-vinyl-2-pyrrolidone-*co*-vinyl alcohol), a Versatile Amphiphilic Polymeric Scaffold for Multivalent Probes

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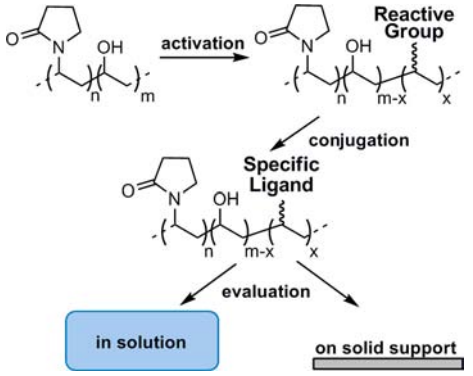
ABSTRACT



A convenient scaffold based on poly(*N*-vinyl-2-pyrrolidone-*co*-vinyl alcohol) is proposed for presenting ligands in multivalent format. This amphiphilic polymer supports synthesis of conjugates in both organic and aqueous media, permits enzymatic processing of the ligand precursor, and, finally, offers a choice of formats for evaluation of biological activity either as a soluble inhibitor or as a capture reagent after deposition on a hydrophobic surface or standard microtiter plates.

Therapeutic applications of polymer-based drugs are hindered by a limited choice of safe, biocompatible polymeric scaffolds. In order to be acceptable as a drug both the polymer and the corresponding monomers should be nontoxic, nonimmunogenic, biodegradable, or excretable via the kidneys and have no toxic metabolites.[1] A copolymer of *N*-vinyl-2-pyrrolidone (NVP) and vinyl acetate known as copovidone is a broadly used excipient.[2] We are exploring the scope of potential biochemical applications of this scaffold especially for display of diverse ligands. Here we report the synthesis and activity of heterobifunctional ligands presented on a narrow molecular weight polymeric scaffold, poly(*N*-vinyl-2-pyrrolidone-*co*-vinyl alcohol) (poly(NVP-*co*-VA), as well as other applications of this polymeric scaffold.

The unique physicochemical properties of the polymer poly(NVP-*co*-VA) render it amenable to a variety of chemical manipulations performed in either organic or aqueous media. Here, we illustrate the utility of this polymer for construction of multivalent probes that can be used either as soluble inhibitors or as a means for immobilization of ligands on a solid support.

Reversible addition−fragmentation chain transfer polymerization (RAFT) used here offers better control of polymer molecular weight and polydispersity.[3−5] Synthesis of

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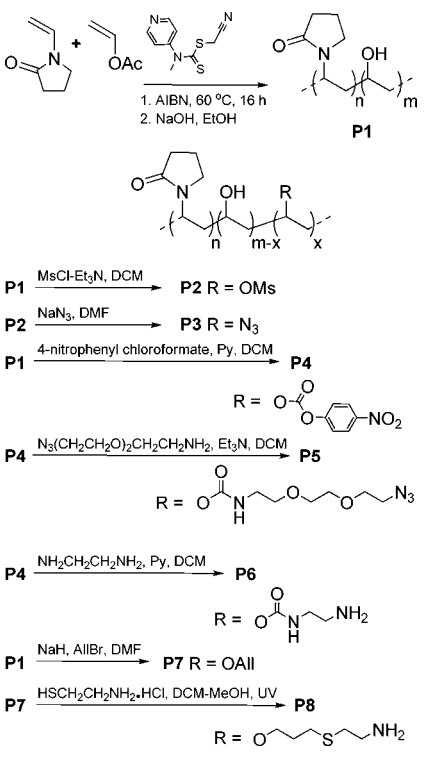
Scheme 1. Synthesis of Functionalized Polymers



poly(NVP-*co*-VA) via RAFT polymerization used the "universal" transfer reagent, cyanomethyl methyl(pyridin-4-yl)carbamodithioate.[6] The polymer was deacetylated, and the resulting polymer **P1** (M_W = 37 kDa, M_w/M_n = 1.69) was derivatized in several ways as delineated in Scheme 1 to afford side chain functional groups such as azide, amine, or activated carbonate ester that are ready for bioconjugation.

Mesylation of **P1** hydroxy groups followed by nucleophilic displacement resulted in derivative **P3** with a high payload of azide, which is directly attached to the polymer backbone. Alternatively, azide-terminated side chains in **P5** were introduced via prior activation of the hydroxyl groups as a *p*-nitrophenyl carbonate ester **P4**. Analogously, amino groups were introduced in **P6** by a condensation of intermediate **P4** with excess ethylene diamine. Amine-terminated side chains that contain no hydrolytically labile groups in **P8** were obtained by allylation of **P1** followed by photoaddition of cysteamine hydrochloride to the allylated polymer **P7**. Transformations conducted on the poly(NVP-*co*-VA) scaffold can be followed by NMR spectra after dialysis. Although the signals corresponding to poly(NVP-*co*-VA) span a wide ^{1}H NMR chemical shift range, the signals indicative of side chains modifications can often be observed and their integration permits quantification of the payload (Figure 1).

To demonstrate the utility of the functionalized polymers, several P^k di- and trisaccharide ligands containing

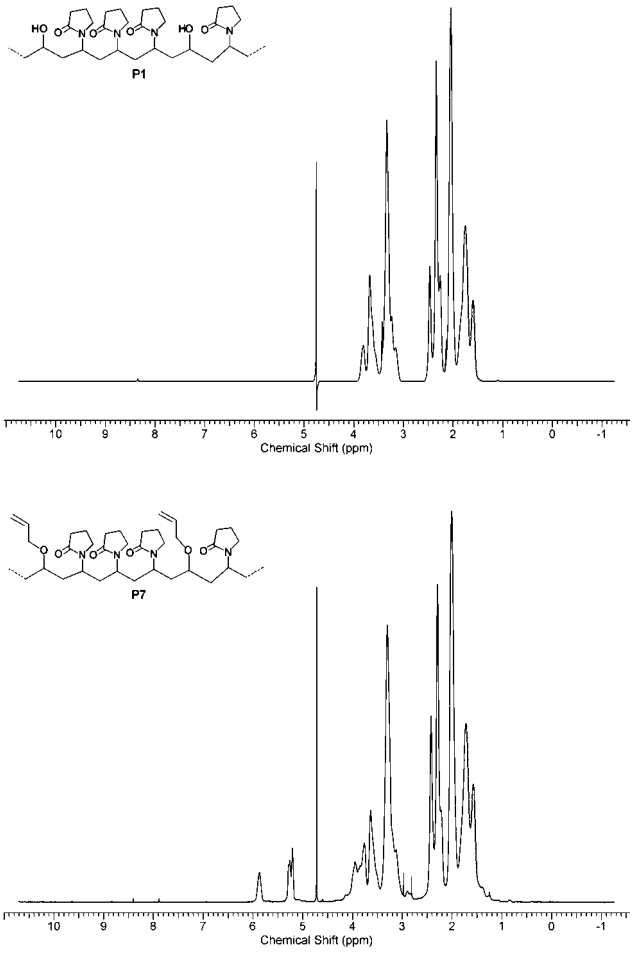


Figure 1. ^{1}H NMR spectra of polymers **P1** and **P7**.

different reactive groups were prepared and conjugated to the scaffold (Scheme 2). Thus, ligands equipped with alkyne groups were linked via the efficient Huisgen 1,3-dipolar copper(I)-catalyzed cycloaddition reaction with azide-presenting polymers **P3** and **P5**. Since polymer **P5** is equipped with a build-in spacer, the pendant ligands may contain a very short linker such as the P^k trisaccharide derivative **2**. The 11−12 atom spacer ensures that the interactions of pendant ligands with a protein target will not be hindered.

The next example demonstrates a sequence of transformations performed on a poly(NVP-*co*-VA) support that take advantage of the polymer solubility in organic solvents. The amine-presenting polymer **P6** was linked to the ligand via a carbamate bond using an activated carbonate ester of a protected disaccharide derivative **3**. After deacetylation of the product, the resulting lactose conjugate was enzymatically glycosylated to provide a P^k trisaccharide conjugate **C3**.

The final example is an efficient conjugation via an amide bond. This was achieved between polymer **P8** and a trisaccharide with a carboxylic-acid-terminated aglycon using an EDC-HOBt protocol in aqueous solution.

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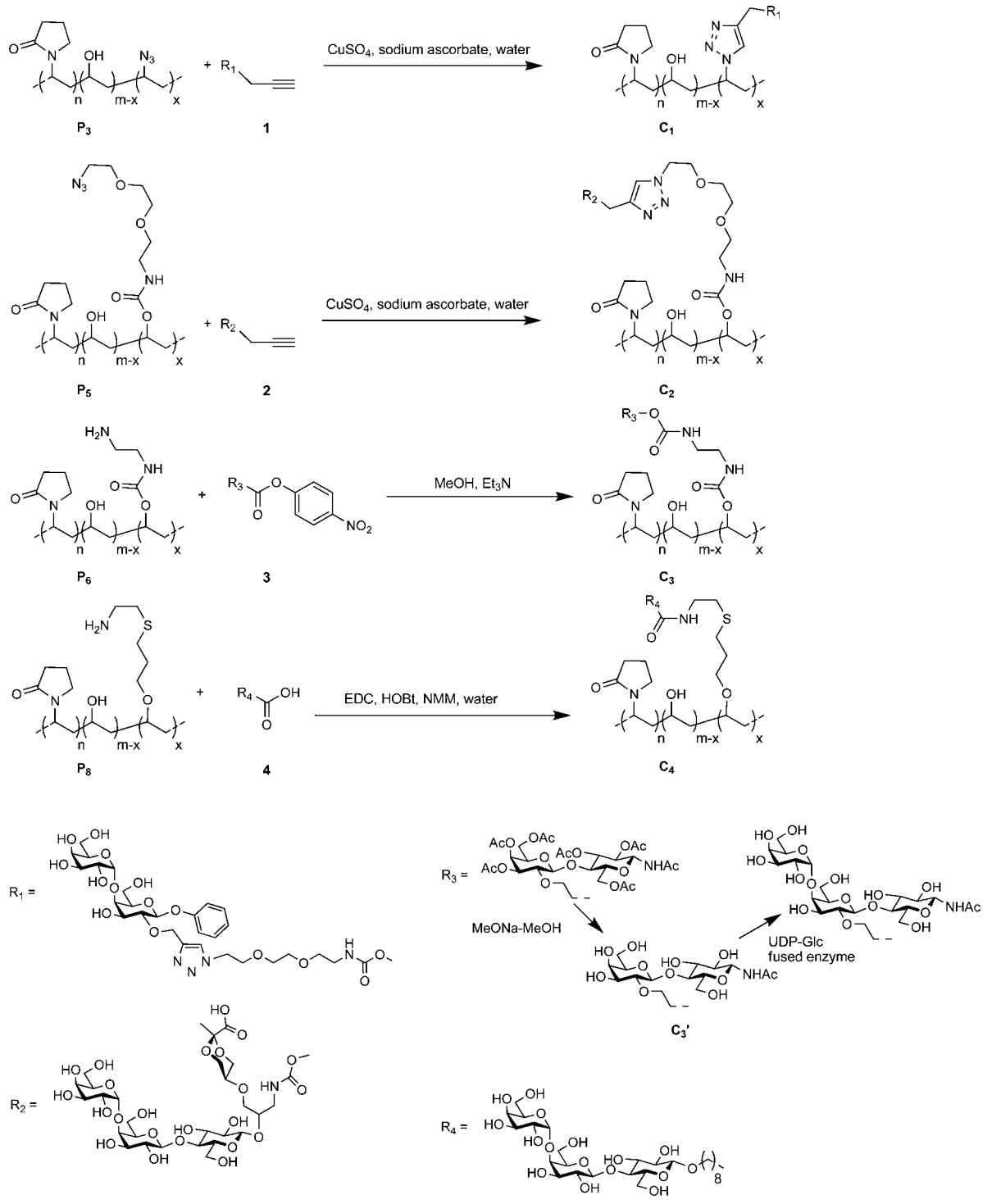
Scheme 2. Representative Examples of Conjugation with Functionalized Polymers **P3**, **P5**, **P6**, and **P8** and Chemoenzymatic Processing of Glycoconjugate **C4**



The biological activities of the prepared conjugates were evaluated by ELISA using two different formats: a binding assay and an inhibition assay. Thus, Shiga toxin type 1 (Stx1), which specifically recognizes the galabiose fragment of Gb_3 ganglioside, was shown to bind to microtiter plates coated with conjugates **C1**–**C4**, in a concentration-dependent manner (Figure 2, left panel). In this solid-phase assay, poly(NVP-*co*-VA)-based conjugates showed very low background signal (see Supporting Information, Figure S1), which is consistent with the antifouling properties for both

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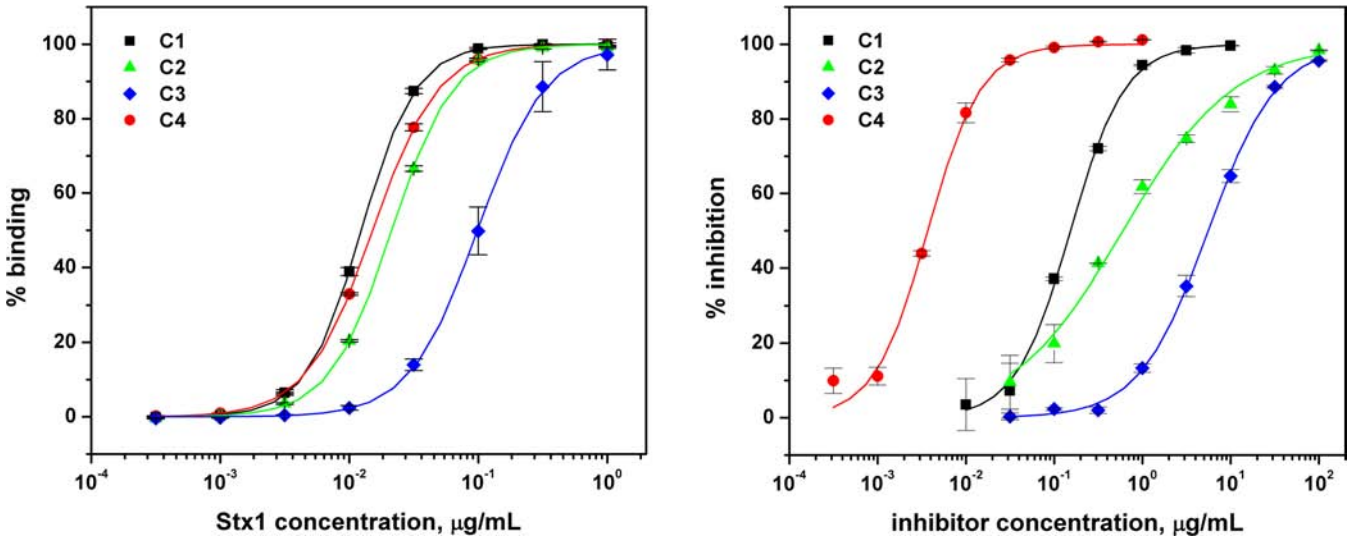

Figure 2. Solid-phase assays performed using prepared glycoconjugates. Left panel: binding of Shiga toxin type 1 to microtiter plates coated with **C1**−**C4**; (black square) **C1**, EC_{50} = 12 ng/mL; (green triangle) **C2**, EC_{50} = 21 ng/mL; (blue diamond) **C3**, EC_{50} = 98 ng/mL; (red circle) **C4**, EC_{50} = 15 ng/mL. Right panel: inhibition of binding of Shiga toxin type 1 to microtiter plates coated with a P^k trisaccharide analogue; (black square) **C1**, IC_{50} = 150 ng/mL; (green triangle) **C2**, IC_{50} = 700 ng/mL; (blue diamond) **C3**, IC_{50} = 5.6 μg/mL; (red circle) **C4**, IC_{50} = 4 ng/mL. Error bars represent standard deviations for triplicates.

polyNVP and polyVA due to their low nonspecific binding to proteins.[7,8] The same conjugates **C1**−**C4** can also act as soluble inhibitors of Stx1 binding to P^k trisaccharide analogue coated plates (Figure 2, right panel).

It is noteworthy that the coating of poly(NVP-*co*-VA)-based glycoconjugates to microtiter plates is sufficiently robust to permit multiple sequential assays on the same plate without apparent fading of the signal (Figure S1). Color development with enzyme conjugates that employ HRP is quenched at the end of the binding assay by addition of 1 M phosphoric acid, conditions that also disrupt ligand−receptor interactions and allow for washing the receptor off the plate while the poly(NVP-*co*-VA)-based conjugates remain bound and ready for the next round of assay. The ease of physical coating with the desired ligand and its persistence, comparable to covalent attachment, may find application in microarray manufacturing.

In conclusion, we have demonstrated the utility of a polymer, poly(*N*-vinyl-2-pyrrolidone-*co*-vinyl alcohol), as a support for both synthesis and biological evaluation of multivalent ligands. Solubility in organic media expands the scope of synthetic manipulation conducted with the pendant ligand prior to testing, while purification can be effected by size exclusion methods. In addition to testing the multivalent probes for interactions with target receptors in solution, the polymeric probes can be readily immobilized on hydrophobic surfaces.

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Supporting Information Available. Experimental procedures, spectroscopic and analytical data of new compounds, and details on solid-phase assays. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.