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Glyco- and Peptidomimetics from Three-Component Joullié−Ugi Coupling Show Selective Antiviral Activity

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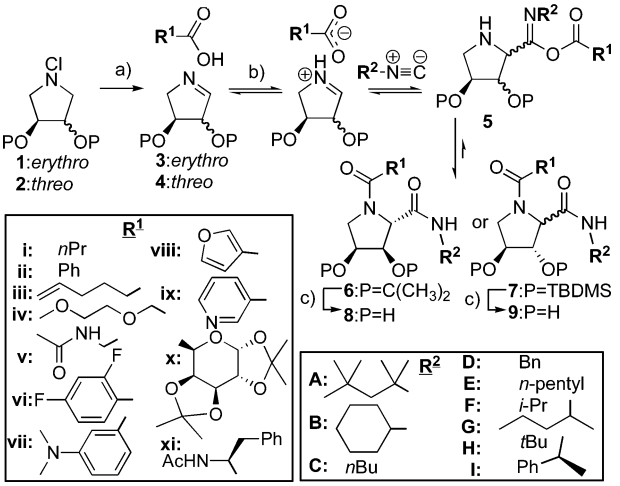
The hydroxylated pyrrolidine scaffold provides valuable sources not only of glycomimetics[1] but also of hydroxyproline derivatives.[2] With the aim of creating biodivergently targeted libraries, we have exemplified a multicomponent reaction (MCR) giving novel bisamide pyrrolidines accessed through a chlorination−elimination strategy.[3] Previous studies have shown such imines to be efficient scaffolds for reaction with organometallic reagents.[3] We demonstrate here that they are also highly effective components in MCRs that may be applied to library construction.

The mechanism proceeds via intermediates that are common to the Ugi reaction,[4] a widely used reaction in library construction.[5] However, the use of cyclic imine components in MCRs is rare: in 1989 Joullié demonstrated the role of a single cyanophenoxy dihydropyrrole.[6,7] It is all the more surprising that such a "Joullié−Ugi" process has not been applied to hydroxylated cyclic scaffolds as this would yield a ready route to compounds that could be considered as either azasugars or dihydroxyprolyl peptides. The motif thus formed would therefore potentially be effective in both carbohydrate processing (e.g., glycosidase) and/or peptide-processing (e.g., prolyl peptidase) inhibitors.[1,2,8] Several important syntheses of dihydroxyproline modules have been reported;[9] many highlight the difficulty, length,[10] relatively low yields,[11] and long reaction times[11] of prolyl amide coupling. Improved access to coupled hydroxyprolines is desirable. We hereby report that entry to the Joullié−Ugi reaction through elimination followed by facile deprotection has allowed access to one of the most wide-ranging azasugar/dihydroxyprolyl libraries,[12] which in turn has yielded potent inhibitors of two disease-associated targets, one based on inhibition of carbohydrate processing and one on peptide processing.

Erythritol **3** and threitol **4** imines, formed from treatment of *N*-chloramine precursors **1** and **2** (Scheme 1) with DBU established the unoptimized viability of reaction with *N*-acetyl glycine **v** and benzyl isocyanide **D** (Scheme 1), giving reasonable yields of elaborated bisamide (68 and 64% yield over two steps from **1** and **2**, respectively); excellent diastereoselectivity (de > 98%) was observed for erythritol **6vD**.[13] Deprotection with TFA proceeded smoothly in 90% for erythritol **8vD** and 62% for the 2,3-*trans* threitol species **9vD**. Conditions for ready parallel handling were then established: isocyanides were removed in vacuo and acids were removed by base wash, and final treatment with TFA afforded pure deprotected product without recourse to chromatography.

Scheme 1. Joullié−Ugi MCR[a]



[a] (a) DBU, THF. (b) Carboxylic acid, isocyanide, MeOH. (c) 50% TFA, THF.

Carboxylic acids **i**−**ix** and isocyanides **A**−**H** (Scheme 1) were selected for a library.[12] These included hydrophobic groups since they have been shown to enhance the activity of inhibitors of glycosidases, glucosylceramide synthase, and prolyl-processing enzymes.[8,14] Test arrays probed efficiency. Reaction of **1** with *N*-acetyl glycine **v** and isocyanides **A**−**H** gave single diastereoisomers in total yields of 43−77%.[15] **1** plus acids **i**−**ix** with isocyanide **C** gave 55−99% yield also as single diastereomers. Similar studies on **2** gave similarly good to excellent yields (78−98% with **v**[15,16] plus **A**−**H** and 77−100% with **C** plus **i**−**ix** as a 1:1 mixture of diastereoisomers).[15,17] Deprotection of all adducts (TFA) proceeded quantitatively in most cases.[15] Having established a good level of generality, the library was expanded to 132 deprotected members in total yields of 42−100% from erythritol *N*-chloramine **1** and 77−100% from threitol *N*-chloramine **2**, all at >90% purity as determined by LC−MS and ^{1}H NMR.[15]

More complex homochiral components were also tested, including representative biomolecule fragments. (*S*)-*sec*-Phenethyl isocyanide **I** and *N*-Ac-L-phenylalanine **xi** gave 51 and 59% yield and >98% de[18] with **viii** and **E**, respectively. Disappointingly, proline, deprotected glucuronic, and galacturonic acids gave little product. Protection of sugar hydroxyls is typical in successful MCRs,[5,19] and gratifyingly, protected D-galacturonic acid **x**[20] gave 44% overall yield, >98% de (**1** + **x** + **E**) of azadisaccharide mimic **9xE**.

Having readily generated an array of potential glyco- and peptidomimetics, we probed their activities against 15 different

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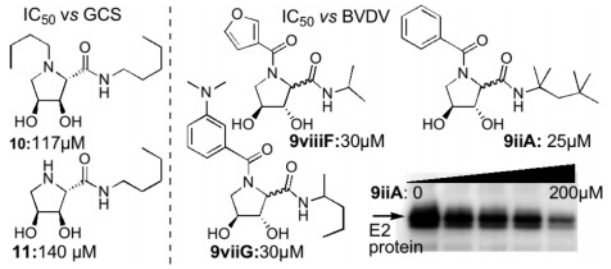

Figure 1. Identified inhibitors of glucosylceramide synthase, bovine viral diarrhoea virus, and anti-E2 Western assay of **9iiA**-treated BVDV.

sugar- and peptide-based targets. To test glycomimicry, the library was screened against five human glycosidases, five non-mammalian glycosidases, and the glycosyltransferase glucosylceramide synthase (GCS), a Gaucher's disease target.[21] The entire library showed little or no inhibition of glycosidases at 100 μM.[22] This appears to be due to requirement for a basic endocyclic nitrogen atom. Gratifyingly, treatment of **9iE** with 1.5 equiv of lithium aluminum hydride[23] allowed the rarely performed chemoselective[24] reduction of the tertiary amide in the presence of the secondary amide at C-1 and library elaboration;[25] **10** and **11** were successfully identified as GCS inhibitors with IC_{50} 117 and 140 μM, respectively (Figure 1). Inhibitors of HIF hydroxylases are of current anti-ischemic interest,[26] and elastases are implicated in, e.g., pancreatitis, rheumatoid arthritis, and emphysema. To test peptide mimicry, the library was screened against peptide-processing target enzymes that preferentially accept substrates that contain prolyl residues, FIH,[27] PHD2,[28] and porcine pancreatic elastase, but showed only low inhibition.[15]

Finally, the library was tested in whole pathogen assays against hepatitis B virus (HBV) and bovine diarrhoea virus (BVDV), which is a primary model of human HCV.[29,30] A specific pattern of potency against BVDV for aromatic R^1 and branched R^2 substituents emerged. IC_{50} values of 25 μM (**9iiA**) and 30 μM (**9viiG**, **9viiiF**, MOI = 0.5, Figure 1) compare very favorably with *N*N-DNJ (deoxynojirimycin), 10 μM, MOI = 0.1 and better than those for *N*B-DNJ (125 μM, MOI = 0.1).[31] Reduction of viral protein E2 level, lack of glycosidase, and HBV inhibition also indicated a novel, selective mechanism distinct from those of these previous imino sugars.[31] We believe this to be the first example of a BVDV inhibiting azasugar that does not affect HBV. Excitingly, no significant toxicity was observed even at highest concentration (300 μM).

In conclusion, a rarely constructed azasugar/dihydroxy prolyl array was assembled efficiently through three-component Joullié−Ugi reaction accessed using chlorination−elimination methodology and allowed the identification of inhibitors of carbohydrate- and peptide-processing targets, including disease enzyme GCS and model viral pathogen BVDV.

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Supporting Information Available: Experimental procedures and characterization data for all library members and for biological testing. This material is available free of charge via the Internet at http://pubs.acs.org.

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