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Author manuscript

Org Lett. Author manuscript; available in PMC 2022 June 14.

Published in final edited form as:

Org Lett. 2022 March 25; 24(11): 2170–2174. doi:10.1021/acs.orglett.2c00462.

A backbone anchoring, solid-phase synthesis strategy to access a library of peptidouridine-containing small molecules

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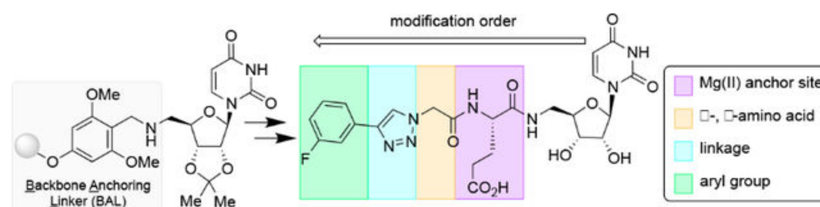
Abstract

Nucleoside diphosphate sugar (NDP-sugar) substrates provide the inspiration for nucleoside analog inhibitor scaffolds. By employing solid-phase synthesis, we provide a method to access a library of peptidouridine inhibitors with both minimal compound handling and purification steps. Specifically, this strategy is exemplified by generating uridine diphosphate sugar (UDP-sugar) mimics, which allows compound elaboration by altering the dipeptide composition, the N-terminal linkage, and a pendant aryl group. To exemplify the versatility 41 unique nucleoside analogs are presented.

Graphical Abstract



Nucleoside-containing natural products have provided a blueprint for inhibitor and antibiotic development. Many of these nucleoside-based inhibitors target glycan assembly pathways as these compounds structurally mimic the nucleoside diphosphate sugar (NDP-sugar) substrates. Specifically, uridine diphosphate (UDP)-sugar mimics (**1**) have shown inhibition towards glycosyl transferases, phosphoglycosyl transferases, and sugar-modifying enzymes (Figure 1).[1] For example, muraymycin and analogs (**2**) inhibit MraY,[2] a polytopic phosphoglycosyl transferase (PGT) responsible for transferring phospho-MurNAc-pentapeptide (Park's nucleotide) to undecaprenyl phosphate in peptidoglycan

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Experimental procedures, full spectroscopic data, and copies of ^{1}H and ^{13}C NMR, and crude HPLC traces for all new compounds (PDF)

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biosynthesis.[3] Additionally, analog libraries of sansanmycin (**3**) were found to target *Mycobacterium tuberculosis* (*Mtb*) MurX, an MraY-like enzyme in mycobacteria.[4] However, the complexity of these uridine natural products, and the analogs that are based on them, make these targets synthetically cumbersome to access. Providing a streamlined strategy to access these scaffolds, while simultaneously providing opportunities for compound diversification, will expedite the production of high-value inhibitor libraries.

Solution-phase synthesis has been predominately employed to access uridine-based small molecules;[2c, 5] however, the adaptation of a synthetic route to a solid-phase-based approach can improve yields by allowing the target to remain immobilized while excess reagents and solvents are washed away. To streamline inhibitor development, solid-phase approaches were developed by attaching uridine to the solid support via the C2' and C3' hydroxyls of the ribose,[1a, 6] the uracil nitrogen,[7] or by performing a semi-synthesis; synthesizing the peptide portion on resin, and attaching the uridine in solution.[2d] A disadvantage is that these strategies contain many manipulations to access key resin-bound intermediates to initiate inhibitor synthesis. More specifically, the previously reported solid-phase strategy that links uridine through the C2' and C3' hydroxyls contains multiple drawbacks.[1a] The amine source at the C5' position is installed on resin through a Mitsunobu reaction with tetrachlorophthalimide. Once installed, the deprotection is executed with ethylenediamine; however, primary amines are known to prematurely cleave amino acids from Wang resin which can result in compound loss and lower yields.[8] Moreover, the linker is attached to Ala-Wang resin. When treated with trifluoroacetic acid (TFA) to cleave the desired compound from the acetal linkage, simultaneous cleavage of the linker-Ala from the solid support could result in chromatography containing undesired side products. We envisioned designing a strategy that would circumvent these undesired side reactions. Furthermore, avoiding attachment at the ribose hydroxyls will have a greater impact on the nucleoside field as other nucleosides (e.g., thymidine) do not contain both C2', C3'-hydroxyls required for attachment. At this time, we realized that many analogs based on UDP-sugars scaffolds (**1**),[1a] including muraymycin (**2**),[2] and sansanmycin (**3**)[4] contain an amide linkage proximal to the uridine core (Figure 1). This linkage site can be recapitulated in solid-phase synthesis by attaching a C5'-amino-nucleoside to the solid support.

Herein we present a solid-phase synthetic approach for uridine-linked small molecules using the backbone anchoring linker (BAL) resin (**4**, Scheme 1).[9] This resin has been previously employed for backbone anchoring of peptides for C-terminal modifications and macrocyclization on resin.[10] The backbone anchoring linker allows for minimal solution- and solid-phase manipulations to access the key intermediate for inhibitor elaboration. The 2',3'-*O-iso*propylidene-C5'-aminouridine (**5**) was generated in solution from uridine in three steps. To attach the protected C5'-aminouridine to resin (**4**), compound **5** was dissolved in 1% AcOH/DMF and added to the solid support to form the corresponding imine. Subsequent addition of $NaCNBH_3$ in MeOH overnight affords the resin-bound secondary amine **6**. The conversion of the reductive amination reaction was monitored by the colorimetric nitrophenylhydrazine test.[11] The secondary amine can be treated with standard solid-phase amide coupling conditions to elongate on resin. The final compound

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can be readily cleaved from the solid support with 95% TFA due to the labile nature of the linker.

We have exemplified this solid-phase synthetic strategy by developing inhibitors that may target bacterial PGTs. PGTs are responsible for catalyzing the initial membrane-committed step in the biosynthesis of N-linked glycoproteins associated with bacterial virulence.[12] Previous work has identified key features for analog development, including (1) uridine, (2) a carboxylate to substitute or coordinate a metal ion at the active site, (3) a side chain to occupy the carbohydrate-binding site, and (4) an alkyl chain to mimic a prenyl moiety.[13] These features ultimately identified the *m*-fluoro-phenyl-substituted analog (**1**) which exhibited the most encouraging inhibition of a monotopic PGT from *Campylobacter concisus* (IC_{50} of 72 ± 7 μM), a potential emerging pathogen of the human intestinal tract.[14] Herein, this compound will be referred to as the benchmark inhibitor (**1**). These results set an excellent starting point for strategic modifications of uridine analogs, thus we utilized this solid-phase strategy to provide an opportunity for structure-activity relationship (SAR) exploration. Keeping the essential uridine, we focused on exploring the Mg(II) anchor site by altering the spatial orientation (D or L) of the amino-acid side chain or the distance (Asp or Glu) of the carboxylate from the peptide backbone. Moreover, the composition of the dipeptide in the peptidouridine can be altered to explore enhanced substrate mimetic properties. To introduce pharmacologically-relevant aryl moieties, we focused on the ease of the Cu(I)-catalyzed alkyne-azide cycloaddition (CuAAC),[15] noting that an amine, amide, and urea linkage could also be installed through other elongation strategies. Lastly, we wanted to diversify the terminal aryl group as a surrogate for the carbohydrate portion of the UDP-sugar; carbohydrate binding sites are frequently lined with aromatic amino acid side chains.[16]

As the glutamic acid was previously reported to be important for binding,[1a] we first modified the length and spatial orientation of the amino-acid side chain to strategically diversify the Mg(II) anchor site. Starting from the C5’ of uridine the carboxyl-modified amino acid is the first site for variation of the benchmark inhibitor **1** (Scheme 2). The secondary amine **6** was coupled to various Fmoc-protected amino acids using standard coupling conditions, followed by Fmoc deprotection with 20% piperidine/DMF (**7**). The free N-terminus was coupled to azido acetic acid to poise **8** for a CuAAC.[15] After the cycloaddition (**9**), the resin was treated with TFA to furnish the desired purified small molecules in 12–17% yield (**10a**-**e**). In addition to carboxylate amino acids, a carboxamide (Gln, **10d**, 13% yield) and methyl ester (Glu(OMe), **10e**, 7% yield) were prepared.

The scaffold of benchmark inhibitor **1** was modified by substituting the second amino acid from the C-terminus (Scheme 3). To avoid synthesizing each amino acid with the N-terminal azide prior to coupling, the azide was installed on resin. This transformation was achieved using a diazotransfer reagent, imidazole sulfonyl azide, to conveniently convert the primary amine of the amino acid (**11**) to an azide in 2 h in the presence of Hünig’s base and DMSO.[17] After the azide is installed, the CuAAC reaction was performed to produce the penultimate molecule (**12**) in Series 1. Subsequent resin cleavage with 95% TFA furnished the deprotected, purified compounds (**13a**-**l**, Series 1) in 6–27% yield over 8 steps.

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Figure 2 represents the excellent compound purity after reverse-phase HPLC (RP-HPLC) purification.

The successful incorporation of amino acids that contain nucleophilic side chains (Lys, Dap) inspired us to further functionalize those sites (**14**, Scheme 3, Series 2). To perform a bi-directional synthesis on the terminal amino acid, a non-acid/base labile protecting group was required to avoid premature resin cleavage. Thus, for Series 2 compounds, an allyl-based (allyloxycarbonyl, Alloc) protecting group was used. This protecting group was removed using a Pd-based catalyst ($Pd(PPh_3)_4$) in the presence of a reducing agent, $PhSiH_3$, in CH_2Cl_2.[18] The free amine of the amino acid side chain was coupled to both short- and long-chain carboxylic acids. Treating the resin with TFA and RP-HPLC purification furnished the acyl-modified molecules (**14a-d**) in 5–10% purified yield over 10 steps. Traditionally, these lipidic modifications, in particular the long-chain analogs, are challenging to incorporate in solution due to poor solubility. By immobilizing on the solid support, these solubility issues are circumvented by avoiding work-up and chromatography after each iteration.

In addition to coupling via CuAAC to furnish a triazole, we wanted to showcase the versatility of the solid-phase strategy with three additional elongation reactions (Scheme 4). These linkages are commonly found in natural products antibiotics.[19] In this case, the aryl installation is achieved by coupling a carboxylic acid, performing a reductive amination, or reacting the terminal amine with a phenyl isocyanate, to ultimately produce amide-, amine-, or urea-linked small molecules, respectively. Specifically, the immobilized peptidouridine (**11**) was treated with a 3-F-Ph-R group with the appropriate elongation conditions.[8] For Series 3, the resin was treated with TFA to afford compounds **15a-c** in 3–18% purified yield. Series 4 included the installation of the lipidic myristoyl tail (**16a-e**), performed in a similar fashion to the triazole series from Scheme 3. First the Alloc-protecting group was removed using Pd, followed by subsequent coupling with myristic acid. The compounds were then cleaved from the resin providing the amide (**15a**, **16a**, **16d**), urea (**15b**, **16b**, **16e**), and amine (**15c**, **16c**) in 3–18% yield after RP-HPLC purification.

Lastly, the terminal aryl diversity was expanded to mimic the carbohydrate-portion of the UDP-sugar substrate by implementing the triazole linkage (Scheme 5). A significant proportion of biologically-active and, importantly, FDA-approved small molecules contain heterocycles.[20] The incorporation of these heterocyclic components aids in compound lipophilicity, polarity, solubility, and hydrogen bonding capacity.[20] For these reasons, both *N*- (**17a**, **17d-l**) and *S*-containing (**17b**) heteroaromatic groups were incorporated (4–37% yield). Additionally, heteroatom-appended aryl groups were successfully installed (**17a**, **17c**). These derivatives expanded on the uridinyl library data set previously obtained in our laboratory.[1a] Through these efforts we have established a routine to prepare ample amounts of purified materials to make 10 mM stock solutions in DMSO for plate-based inhibitor screening and distribution to other laboratories.

Although most compounds in this study were predicted to have optimal physicochemical properties,[21] compound aggregation can be a problem in small molecule libraries.[22] However, we hypothesize that the peptidyl-nucleoside structural core will prevent undesired colloid formation. As these undesired molecular features tend to be observed with flat,

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aromatic-rich molecules.[23] Even so, this inhibitor promiscuity will be investigated with our library and reported in due course.

In addition to uridine, this strategy can be applied with other natural or pseudo nucleosides,[1a, 24] including analogs with highly modified ribose moieties. Due to the structural similarities to uridine, thymidine and pseudouridine will be easy to employ. We have previously demonstrated the compatibility of pseudouridine with solid phase synthesis without the need of nucleobase protecting groups.[1a] Solution- phase synthesis to access C5'-substituted cytidine and analogs has been established,[25] suggesting this solid-phase approach is feasible. Although also conceivable, guanosine and adenosine will require nucleobase-protecting groups prior to solid-phase immobilization to prevent unwanted side reactions.

Herein, we have established a new solid-phase synthesis strategy to access peptidouridine-containing small molecules. The compounds were strategically modified to provide maximum chemical diversity in a complex inhibitor library. This approach has provided a streamlined process to access natural product-inspired, nucleoside-containing small molecules. Additionally, in our laboratory this strategy will be employed for other nucleosides, affording potential inhibitors for enzymes that accept non-uridine nucleoside diphosphate sugar substrates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT

Financial support from the National Institute of Health (GM131627 to B.I. and F32GM136023 to C.A.A.) and Massachusetts Institute of Technology Research Support Committee (MIT RSC) is gratefully acknowledged. The authors would like to thank Dr. Leah Seebald (MIT) for providing substantial edits to the manuscript. We also acknowledge the MIT DCIF for NMR analyses and Dr. Mohan Kumar (MIT) with HR-MS assistance.

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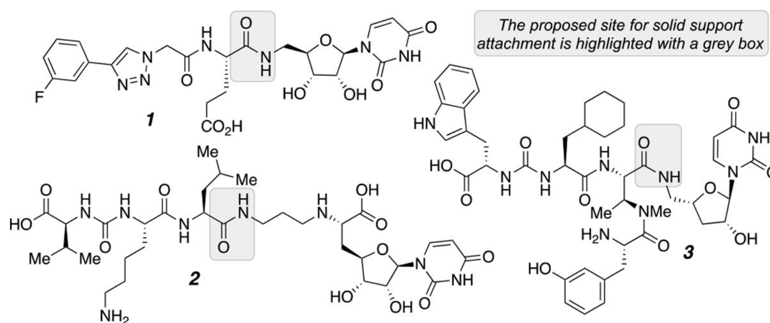

Figure 1.
Select small molecule analogs derived from uridine natural products.

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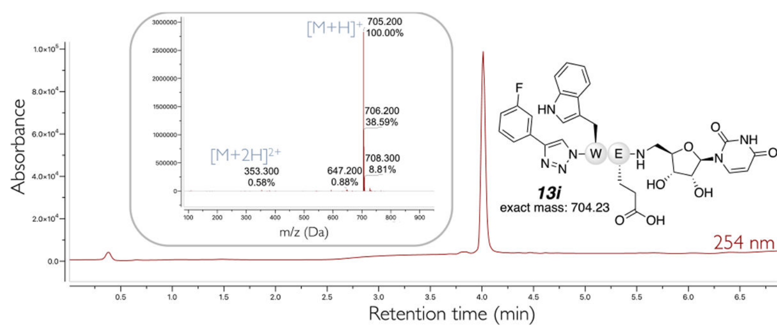

Figure 2.

LCMS trace at 254 nm and ESI-MS spectrum of purified compound **13i** from Scheme 3.

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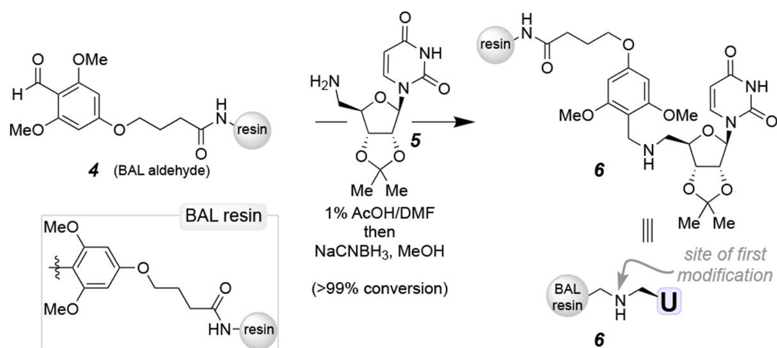

Scheme 1.

Attachment of C5’-aminouridine to the aldehyde-functionalized solid support.

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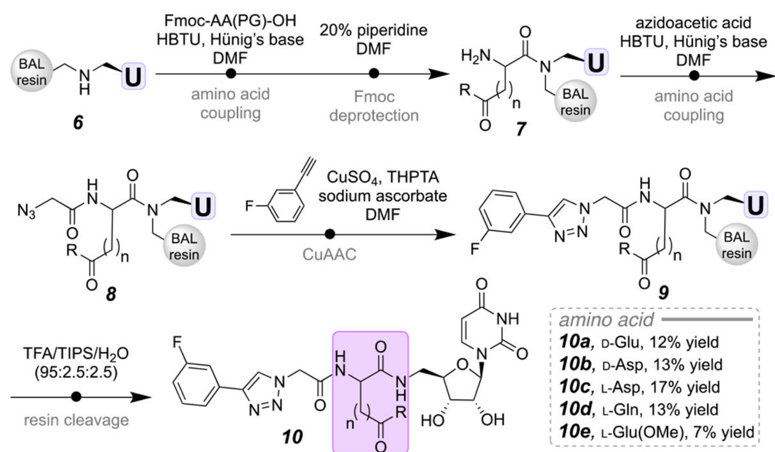

Scheme 2.

Modification of the metal-binding site amino acid in the PGT benchmark inhibitor.

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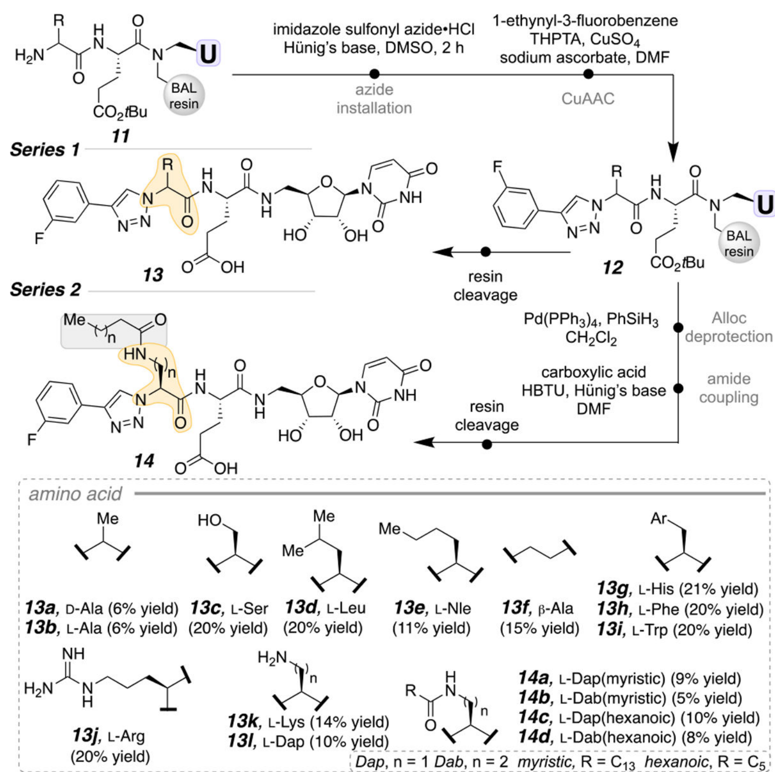

Scheme 3.

Glutamic acid-containing inhibitors with various modifications to the dipeptide scaffold.

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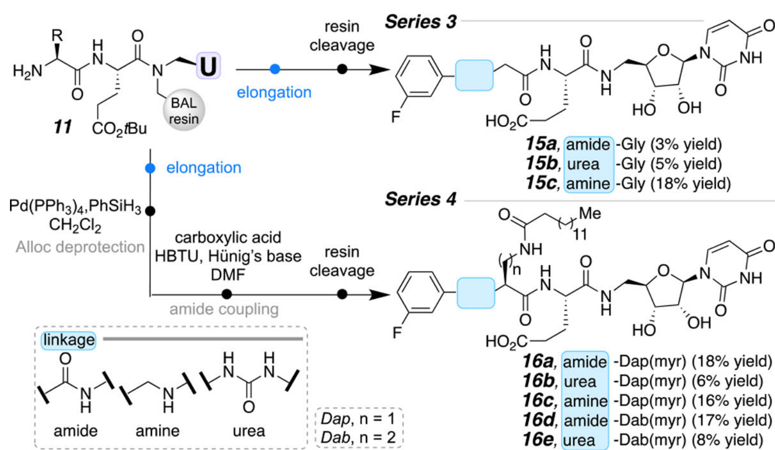

Scheme 4.

Modifying the aryl-connective moiety for both Gly and lipidic-containing dipeptides.

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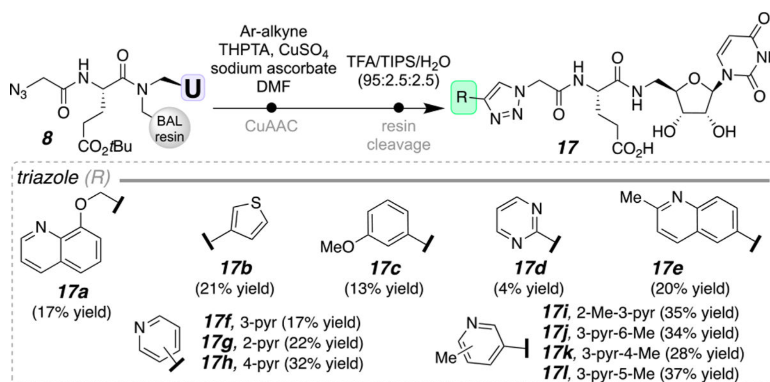

Scheme 5.

Terminal aryl modifications using the triazole connective moiety.