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Research Article

Rapid Synthesis of a Natural Product-Inspired Uridine Containing Library

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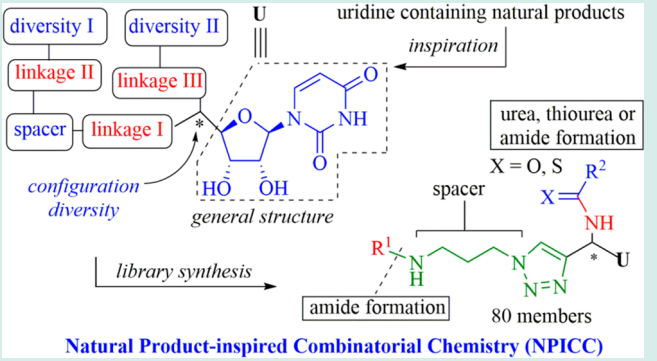
ABSTRACT: The preparation of natural product-inspired nucleoside analogs using solution-phase parallel synthesis is described. The key intermediates containing alkyne and N-protected amino moieties were developed to allow for further skeleton and substituent diversity using click chemistry and urea or amide bond formation. Rapid purification was accomplished using solid-phase extraction. The obtained library comprised 80 molecules incorporating two diversity positions and one chiral center, each of which was efficiently prepared in good purity and acceptable overall yield. A bacterial morphology study was also performed.



KEYWORDS: *combinatorial chemistry, natural product-inspired nucleosides, chemical space, semiautomated synthesis, uridine-containing library*

INTRODUCTION

Natural products are an important source of inspiration in drug discovery, medicinal chemistry, and chemical biology.[1−3] Among them, uridine-containing natural products show a wide range of bioactivities, such as anticancer, antifungal, antiviral, and antibiotic activity, and are implicated in several nucleoside metabolic pathways.[4−6] For example (Figure 1), the polyoxins, a group of antifungal nucleosides that inhibit chitin synthase, were isolated from *Streptomyces cacaoi subsp. Asoensis*;[7,8] the muraymycins were isolated from a culture broth of *Streptomyces sp.* and found to show excellent antimicrobial activity against Gram-positive bacteria;[9−12] and tunicamycin, produced by *Streptomyces lysosuperficus*, is a nucleoside antibiotic complex under investigation for its inhibition of polyisoprenyl-phosphate *N*-acetylhexosamine-1-phosphate transferases (PNPT) with view to the development of a novel anticancer drug.[13,14]

These uridine-containing natural products are all biologically relevant molecules and share a common structural feature (the 5′-substituted uridine moiety) but are otherwise structurally diverse and complex. Thus, it is difficult to clearly define their real core structures or diversity positions. Also, their structural complexity (in particular, the large number of stereogenic centers) and very low natural availability hamper their further study. To date, only straightforward one-dimensional chemical diversity of 5′-substituted uridines has been extensively reported;[15−17] to the best of our knowledge, more structurally diverse uridine-based libraries incorporating more than two diversity positions and synthesized by way of flexible, fragment-assembly strategies have not been investigated.

Our research interests include the design and synthesis of biologically interesting natural product-inspired scaffolds[18] and libraries via divergent synthesis or combinatorial chemistry.[19−22] Recently, we reported the systematic preparation of bacterial Park nucleotide-based molecules, one class of UDP-type natural products, for an enzyme substrate specificity study.[23] Inspired by naturally occurring uridine-containing molecules, nucleoside antibiotics, and uridine metabolic pathways, we realized that diverse chemical synthesis of these uridine-containing scaffolds, molecules, or libraries is needed to increase our chemical space coverage and for their further study in forward or reverse chemical genetic screening.[24−27]

Here, we report an efficient, solution-phase parallel synthetic approach to prepare a diverse uridine-containing library by assembly of multifragments or substituents within a single framework (two diversity positions, three conjugations linkages, one space, and one chiral center) under mild reaction

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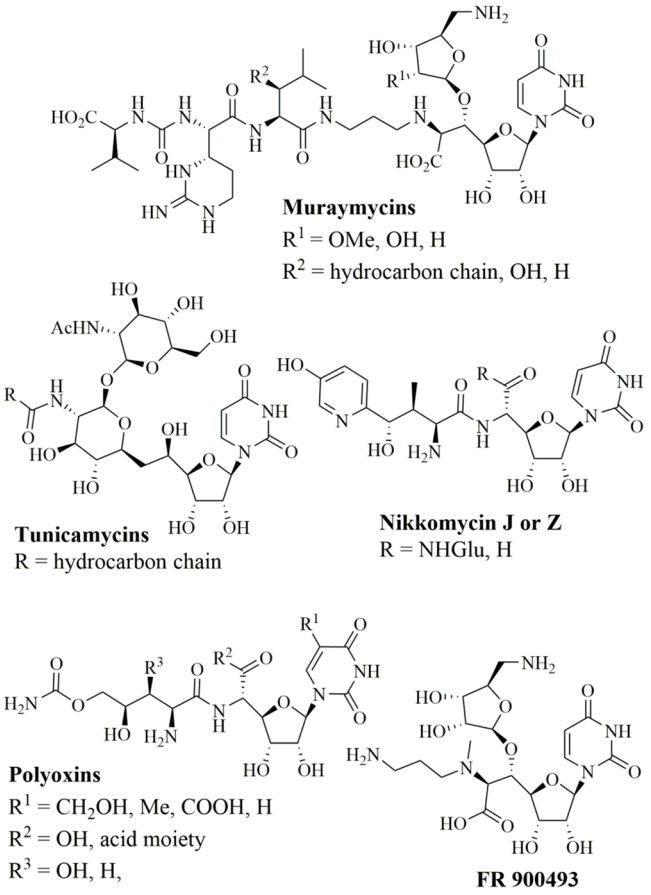
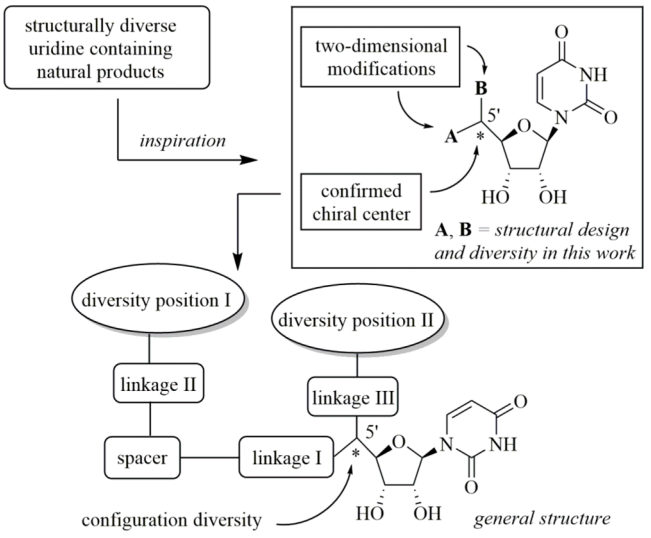


Figure 1. Examples of bioactive molecules containing a uridine moiety.

conditions. Scheme 1 is a schematic depiction of our synthetic strategy. Several flexible and convenient bioconjugation methods, such as Cu-catalyzed azide−alkyne cycloaddition (CuAAC),[28,29] amide bond formation,[30] and urea bond formation,[31] will be applied in this study. Notably, some natural products or bioactive compounds (see in Figure 1) possess a glycosidic linkage, but this formation condition is highly moisture sensitive and not convenient. Therefore, we decide to utilize an amide or urea moiety as our linkage III instead of the glycosidic linkage to efficiently perform the substituent diversity. In addition, the triazole formation (CuAAC) and the amide bond formation will be applied as our linkage I and II, respectively (Scheme 1). Several flexible and semiautomated equipment, including a solution-phase synthesizer, multichannel liquid handler, and centrifugal evaporator were used to accelerate the preparation process. Product purification was rapidly accomplished using solid-phase extraction.[32−34]

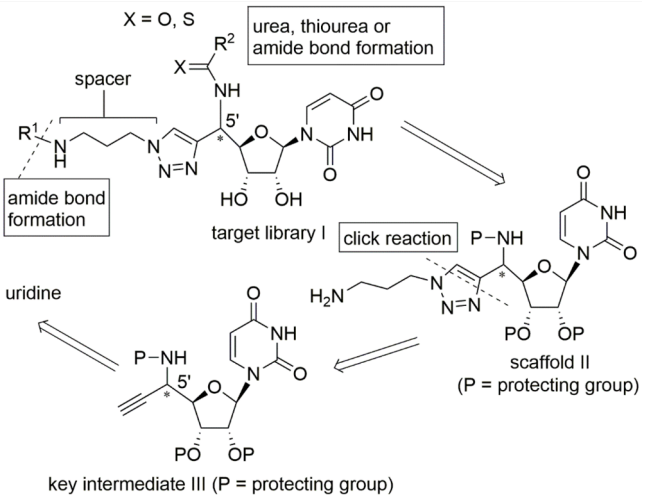
Scheme 1. Principle of Our Structural Design for Uridine-Based Chemical Space



RESULTS AND DISCUSSION

Design and Retrosynthetic Analysis. Scheme 2 depicts our retrosynthetic analysis. The general structure of target library **I** includes two structurally diverse fragments **A** and **B** and one position of configuration diversity (at the C5′ position). Fragment **A** (see in Scheme 1) incorporates several structural features including the triazole-based linkage I, the amide-based linkage II, a flexible spacer, and a lipophilic moiety. In contrast, the amino group attached at the C5′ position will be used for the attachment of fragment **B** through (thio-) urea or amide bond formation with various isocyanates or carboxylic acids. Scaffold **II** will be prepared from the key intermediate **III**, derived from uridine.

Scheme 2. Retrosynthetic Analysis of 5′-Triazole-Substituted Uridines



Synthesis of Key Intermediates 5S and 5R. As shown in Scheme 3, alkyne alcohol **1R** was prepared from uridine as previously described, with minor modifications.[35] Mesylation of **2R**, followed by S_N2 substitution, with sodium azide in DMF at 50 °C gave **4S** in 84% overall yield over two steps. Reduction of azide **4S** under Staudinger conditions (PMe_3/H_2O/THF) followed by *N*-Boc protection gave the first N-protected amino alkyne **5S** in 63% yield (two steps). N-protected amino alkyne **5R** was accessible from **1R** by either Mitsunobu reaction, mesylation, and Boc-protection or via an intramolecular Mitsunobu cyclization to give cyclized intermediate **7**, followed by ring opening with sodium azide.[36] Notably, oxidation of **1R**, followed by reduction (see Supporting Information (SI)) did not give us a better diastereoselective ratio for a separation benefit (approximately 1:1). We also found that use of PMe_3 rather than PPh_3 for the

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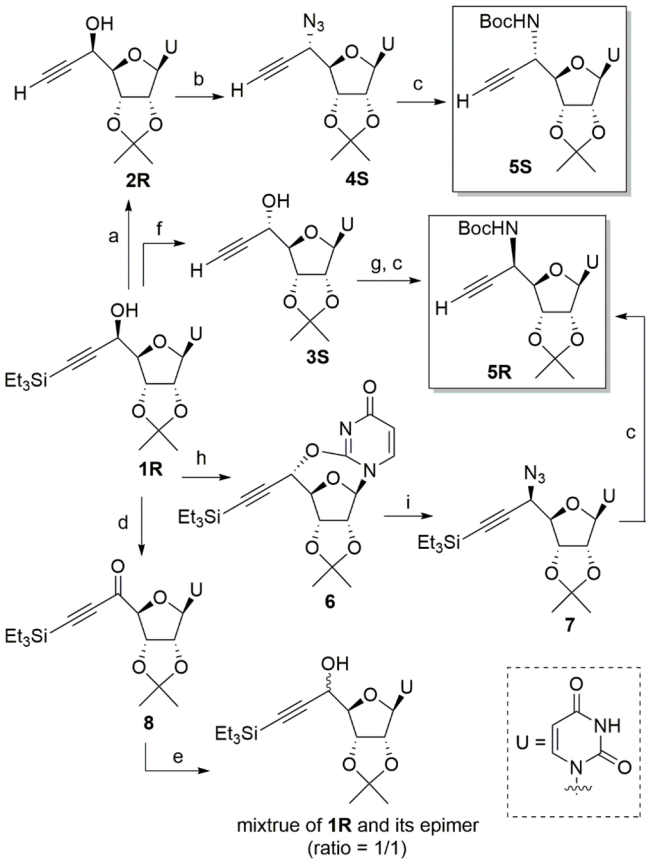
Scheme 3[a]

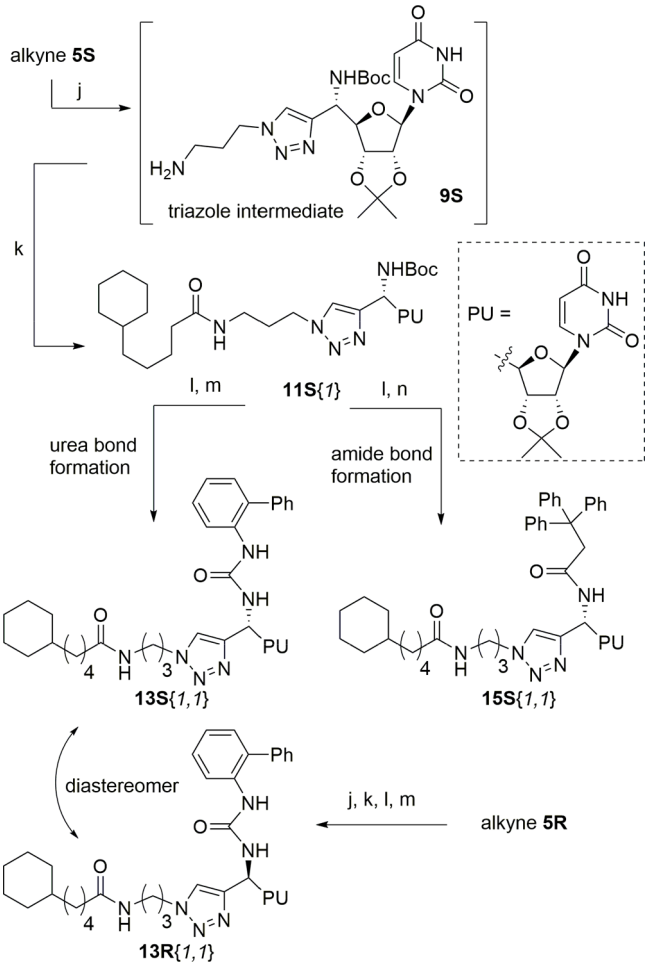


[a]Reagents and conditions: (a) TBAF, THF, rt, 0.5 h, 96%; (b) (i) MsCl, Et_3N, DCM, 0 °C, 0.5 h, (ii) NaN_3, DMF, 50 °C, 8 h, 84% over 2 steps; (c) (i) PMe_3, THF/H_2O, rt, 5 h, (ii) Boc_2O, $NaHCO_3$, rt, 2 h, 61−63% over 2 steps; (d) Dess−Martin periodinane, CH_2Cl_2, 81%; (e) $NaBH_4$, EtOH, 1:1 mixture of **1**, 99%; (f) (i) PPh_3, DIAD, THF, rt, 0.5 h, (ii) NaOH, MeOH/H_2O, rt, 5 h, 87% over 2 steps; (g) (i) MsCl, Et_3N, DCM, 0 °C, 0.5 h, (ii) NaN_3, DMF, 50 °C, 8 h, 52% over 2 steps; (h) DEAD, PPh_3, THF, 75%; (i) NaN_3, DMF, 70 °C, 1 h, 69%.

transformation of **3S** to **5R** was associated with an increase in the yield from 52% to 83%.[37,38]

Chemical Synthesis of Model Molecules. With alkynes **5S** and **5R** in hand, model studies to explore the reaction conditions necessary to accomplish the practical library synthesis could be investigated (Scheme 4). 3-Azidopropan-1-amine was conjugated to alkyne **5S** using catalytic $CuSO_4$ and sodium ascorbate via a Cu-catalyzed azide−alkyne cycloaddition (CuAAC) to give triazole intermediate **9S**, which was directly coupled with 5-cyclohexylpentanoic acid **10**{*1*} (Figure 2) to give **11S**{*1*} in 95% overall yield over two steps. The *N*-Boc deprotection of **11S**{*1*} was performed under acidic TFA conditions to give the corresponding amine intermediate, which was directly treated with 2-biphenylyl isocyanate **12**{*1*} to form a urea-based linkage. After global deprotection using 6 N HCl in MeOH, **13S**{*1,1*} was smoothly obtained after simple purification. Likewise, **13R**{*1,1*}, the C5′ epimer of **13S**{*1,1*}, was similarly prepared from alkyne **5R**. Instead of a, (thio-) urea bond formation, another conjugation method via an amide bond formation could also be established with the treatment of mixture of the amine intermediate which was prepared from *N*-Boc deprotection of **11S**{*1*}, 3,3,3-

Scheme 4[a]



[a]Reagents and conditions: (j) 3-azidopropan-1-amine, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, *t*-BuOH, 80 °C, 3 h; (k) 5-cyclohexylpentanoic acid **10**{*1*}, EDC·HCl, Et_3N, DCM, 4 h, 95% over 2 steps from **5S**; (l) TFA, DCM, 0.5 h; (m) (i) 2-biphenylyl isocyanate **12**{*1*}, Et_3N, DCM, 16 h, (ii) 6 N HCl, MeOH, 16 h, 85% over 3 steps from **11S**{*1*}; (n) (i) 3,3,3-triphenylpropionic acid **14**{*1*}, EDC·HCl, Et_3N, DCM, 4 h, (ii) TFA, DCM, H_2O, 16 h, 83% over 3 steps from **11S**{*1*}.

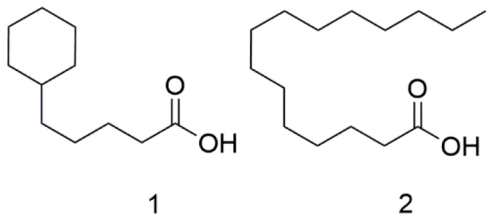


Figure 2. Set of acids **10**{*1*−*2*} for diversity.

triphenylpropionic acid **14**{*1*}, EDC, and triethylamine. After simple extraction and global deprotection, **15S**{*1,1*} was also obtained in 83% yield from **11S**{*1*}.

Library Synthesis. The successful synthesis of **13S**{*1,1*} and **15S**{*1,1*} from **5S** and **13R**{*1,1*} from **5R** encouraged us to attempt the preparation of a more diverse library. To improve efficiency, we sought to use semiautomated equipment. Requisite precursors **11S**{*1*−*2*} and **11R**{*1*−*2*} bearing a lipophilic substituent at R^1 and incorporating configuration diversity at C5′ were prepared with the assistance of a solution-

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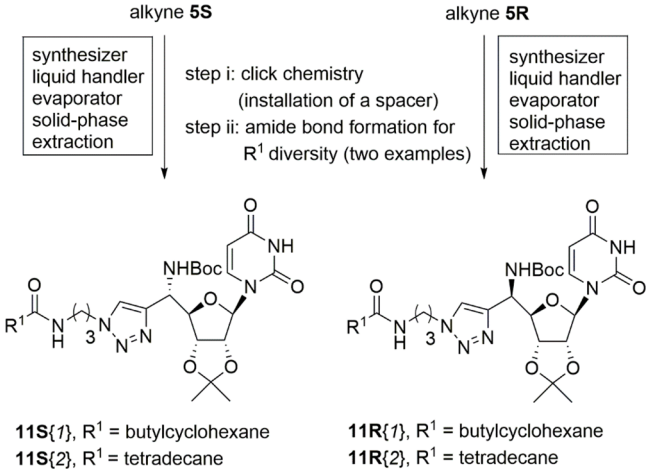
phase synthesizer, liquid handler, and evaporator as shown in Scheme 5 (also see in SI). To simplify our process, the final purification was accomplished by solid-phase extraction (SPE).

Scheme 5. General Route for the Preparation of Key Precursors 11S{*1−2*} and 11R{*1−2*} from Alkynes 5S or 5R



A total of 16 isocyanate reagents **12**{*1−16*} (Figure 3) and 16 acid reagents **14**{*1−16*} (Figure 4) were reacted with

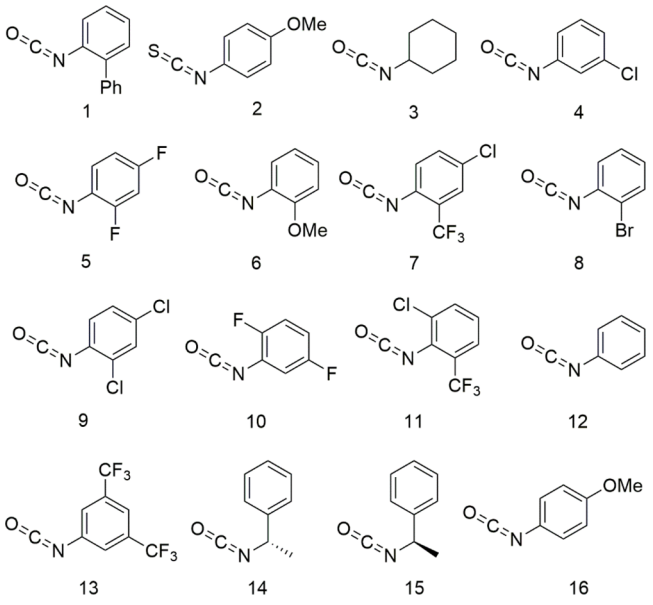


Figure 3. Set of isocyanates **12**{*1−16*} for diversity

nucleoside-based substrates **11S**{*1−2*} and **11R**{*1−2*} according to the protocols described above. Some minor changes were made to the above protocols, to accommodate the automated equipment. For example, *N*-Boc deprotection of the nucleoside-based substrate **11S**{*1*} under acidic conditions (step i) was performed, followed by direct evaporation, individual treatment with isocyanate reagents **12**{*1−16*} (step ii), and global deprotection (step iii) to obtain desired target molecules **13S**{*1−2,1−16*} and **13R**{*1−2,1−16*} after simple solid-phase extraction (Scheme 6). Likewise, the amide bond formation in step ii would also be performed to get the desired 16 compounds **15S**{*1,1−16*}. Notably, the crude products were evaporated by speed-vac and washed by

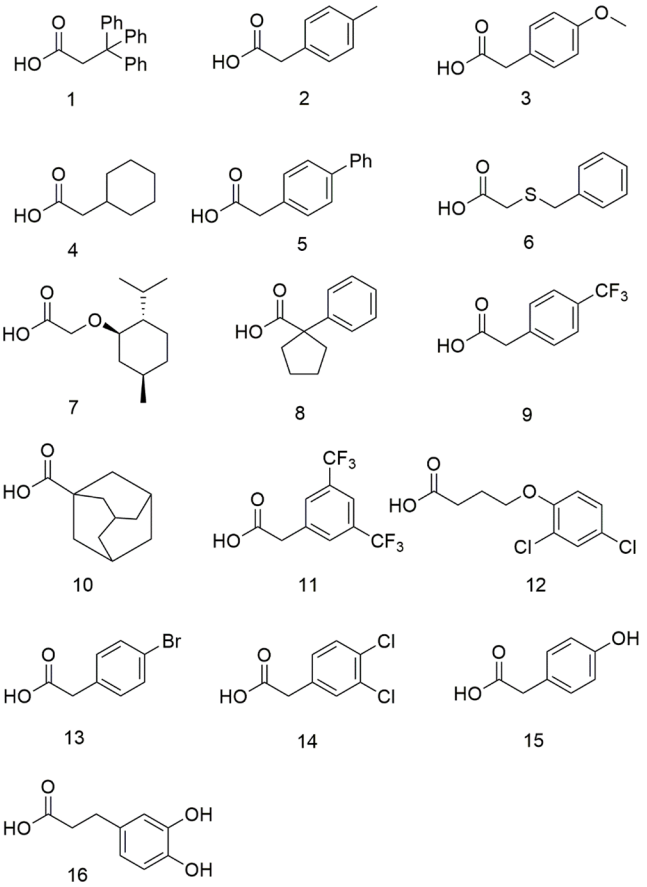
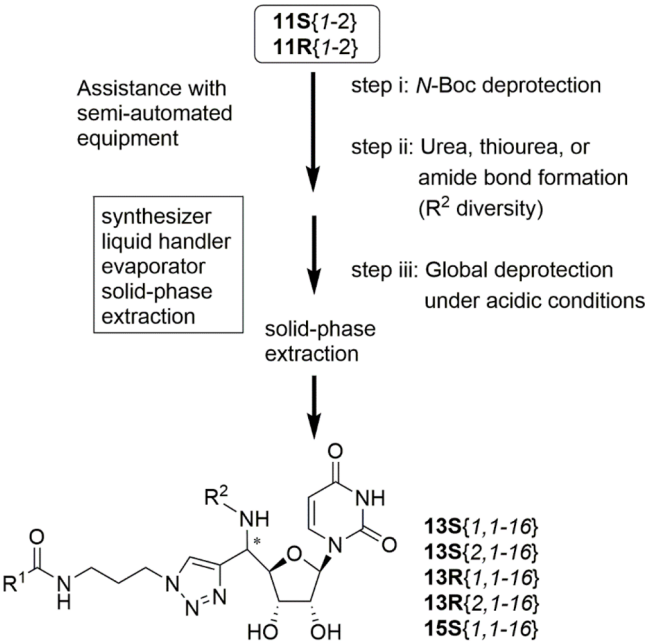


Figure 4. Set of acids **14**{*1−16*} for diversity.

Scheme 6. General Procedure for the Semiautomatic Synthesis of the Desired Library



solution-phase extraction assisted by a liquid handler. Most impurities, derived from the excess of isocyanate or acid reagents, were easily removed by further solid-phase extraction with silica gel and 1/1 mixture of ethyl acetate/hexanes as

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Table 1. Yields and Purities of the Library via a Urea or Amide Bond Formation with Nucleoside-Based Substrates and Isocyanate or Acid Reagents

product	yield (purity)[a]	product	yield (purity)[a]	product	yield (purity)[a]	product	yield (purity)[a]	product	yield (purity)[a]
13S{*1,1*}	85 (98)	**13S**{*2,1*}	56 (96)	**13R**{*1,1*}	41 (94)	**13R**{*2,1*}	28 (96)	**15S**{*1,1*}	83 (94)
13S{*1,2*}	30 (99)	**13S**{*2,2*}	57 (99)	**13R**{*1,2*}	23 (99)	**13R**{*2,2*}	36 (99)	**15S**{*1,2*}	53 (99)
13S{*1,3*}	51 (99)	**13S**{*2,3*}	56 (97)	**13R**{*1,3*}	62 (97)	**13R**{*2,3*}	52 (98)	**15S**{*1,3*}	83 (99)
13S{*1,4*}	31 (99)	**13S**{*2,4*}	32 (99)	**13R**{*1,4*}	70 (96)	**13R**{*2,4*}	50 (96)	**15S**{*1,4*}	35 (99)
13S{*1,5*}	58 (99)	**13S**{*2,5*}	67 (95)	**13R**{*1,5*}	64 (95)	**13R**{*2,5*}	54 (99)	**15S**{*1,5*}	51 (96)
13S{*1,6*}	51 (99)	**13S**{*2,6*}	49 (98)	**13R**{*1,6*}	45 (98)	**13R**{*2,6*}	53 (99)	**15S**{*1,6*}	62 (88)
13S{*1,7*}	63 (98)	**13S**{*2,7*}	51 (96)	**13R**{*1,7*}	76 (96)	**13R**{*2,7*}	39 (98)	**15S**{*1,7*}	40 (93)
13S{*1,8*}	62 (97)	**13S**{*2,8*}	36 (94)	**13R**{*1,8*}	50 (97)	**13R**{*2,8*}	45 (96)	**15S**{*1,8*}	60 (95)
13S{*1,9*}	11 (95)	**13S**{*2,9*}	57 (98)	**13R**{*1,9*}	31 (93)	**13R**{*2,9*}	25 (97)	**15S**{*1,9*}	26 (89)
13S{*1,10*}	56 (98)	**13S**{*2,10*}	21 (97)	**13R**{*1,10*}	40 (98)	**13R**{*2,10*}	41 (99)	**15S**{*1,10*}	42 (96)
13S{*1,11*}	56 (99)	**13S**{*2,11*}	50 (99)	**13R**{*1,11*}	37 (97)	**13R**{*2,11*}	56 (97)	**15S**{*1,11*}	31 (89)
13S{*1,12*}	53 (99)	**13S**{*2,12*}	53 (99)	**13R**{*1,12*}	45 (98)	**13R**{*2,12*}	24 (93)	**15S**{*1,12*}	18 (96)
13S{*1,13*}	72 (99)	**13S**{*2,13*}	60 (99)	**13R**{*1,13*}	49 (95)	**13R**{*2,13*}	46 (96)	**15S**{*1,13*}	87 (85)
13S{*1,14*}	68 (99)	**13S**{*2,14*}	53 (98)	**13R**{*1,14*}	29 (99)	**13R**{*2,14*}	51 (99)	**15S**{*1,14*}	64 (85)
13S{*1,15*}	65 (98)	**13S**{*2,15*}	39 (95)	**13R**{*1,15*}	27 (85)	**13R**{*2,15*}	35 (99)	**15S**{*1,15*}	30 (99)
13S{*1,16*}	25 (99)	**13S**{*2,16*}	8 (97)	**13R**{*1,16*}	62 (99)	**13R**{*2,16*}	41 (99)	**15S**{*1,16*}	23 (99)

[a]Yields after overall synthesis. Purities shown in parentheses.

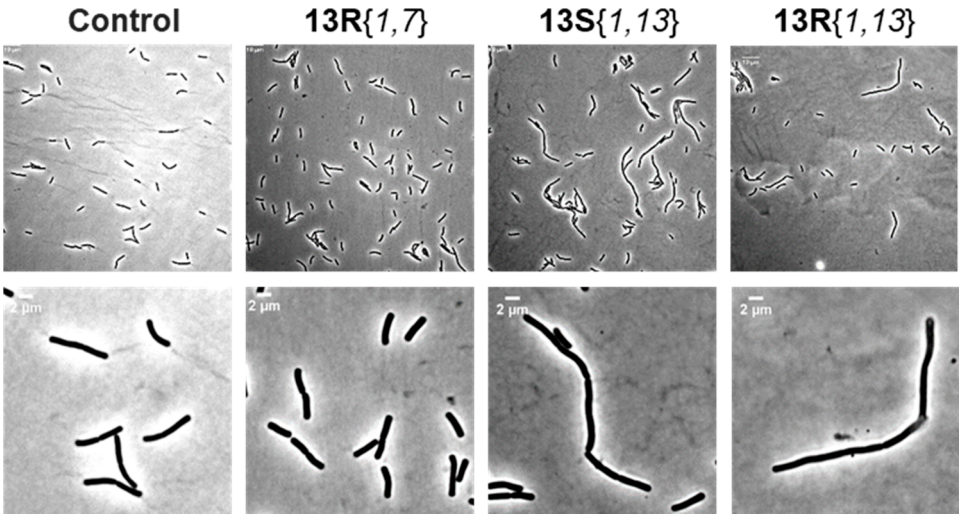

Figure 5. Morphological study of the compounds toward *Bacillus subtilis*. The bacterial cells (OD_{600nm} = 0.1−0.2) were incubated with 100 μm compounds. The images show the effect of the compounds indicated by their code number after 3 h on *B. subtilis* 168 cells.

eluent, and desired products were subsequently collected by using pure EtOAc as the eluent.

Library members (80 members) were characterized by NMR, HRMS, HPLC, and optical rotation. Purities ranged from 85% to 99%, with an average purity of 97% (Table 1). Yields ranged from 8% to 87% in three steps. The low yields of some products might be due to the solid-phase extraction in the unoptimized condition; whereas, their purity was qualified for further biological study.

Biological Evaluation of Selected Compounds. With the uridine-based library in hand, biological studies could commence. Due to their accessibility, we choose to undertake antibacterial and bacterial morphological studies.[39,40] None of the compounds synthesized were found to exhibit antibacterial activity against Gram-negative bacterial strains, such as *Acinetobacter baumannii*, at a concentration of 100 μm, presumably because they could not penetrate the bacterial membranes (data not shown). However, in the morphological study, several compounds were found to perturb the growth of *Bacillus subtilis* (Figure 5). For example, the phenotype of bacterial cells treated with **13S**{*1,13*} or **13R**{*1,13*} consisted of long filament and undivided cells compared with the control group. Most of the affected bacteria attached to each other via their poles. These observations indicate that bacterial cell division is perturbed and cell separation is obstructed by these compounds. In contrast, compounds **13R**{*1,7*} had no effect on bacterial shape, despite their structures differing only subtly from compounds **13S**{*1,13*} and **13R**{*1,13*}. The underlying mechanisms, conditions, and other factors underlying both these contrasting results and the changes observed remain to be explored.

CONCLUSION

A structurally diverse, natural product-inspired uridine-containing library (80 members) incorporating two substituent diversity positions and one configuration diversity position was efficiently synthesized by elaboration of the key intermediate **5S** and **5R** (bearing an alkyne group and Boc-*N*-protected amino group). Semiautomated equipment including a solution-phase synthesizer, multichannel liquid handler, and centrifugal

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vacuum concentrator were used to speed up the synthesis of this library, and the rapid purification of its members was accomplished using solid-phase extraction. The conditions developed in this work are anticipated to enable the generation of even more structurally diverse, uridine moiety-containing libraries in the future. A preliminary bacterial morphology study showed that subtle structural differences of uridine compounds lead different morphology and shape; a more extensive bioevaluation of this library (forward chemical genetic screen) is underway, and the results will be published in due course.

EXPERIMENTAL SECTION

General Information and Instrumentation. All chemicals were obtained from commercial suppliers and used without further purification. Flash column chromatography was performed on silica gel of 40−63 μm particle size. NMR spectra were recorded on dilute solutions in D_2O, $CDCl_3$ and MeOD on Bruker AVANCE 600 spectrometers at ambient temperature. High-resolution ESI mass spectra were recorded on a Bruker Daltonics spectrometer. Parallel synthesis was performed on Mini-Block synthesizer and the reaction vessel (115 × 12) mm. Multiple-functional liquid handler (Freedom EVO, TECAN) was utilized for extraction and separation. Solvent evaporation was performed on Thermo Scientific Savat Explorer SpeedVac Concentrator Explorer-220.

Preparation of the Key Intermediate 5S. A mixture of **4S** (144 mg, 0.356 mmol) and NaN_3 in DMF (0.2 mL, 0.21 mmol) was stirred at 70 °C for 1.0 h. The reaction residue was extracted with EtOAc. The organic layers were dried over $MgSO_4$ and concentrated. The reaction mixture was purified by column chromatography EtOAc/hexane = 1/1) to give **5S** (110 mg, 0.246 mmol, 69%). R_f = 0.3 (EtOAc/hexane = 1/1). ^{1}H NMR (600 MHz, CD_3OD) δ 9.78 (brs, 1H), 7.49 (d, 1H, J = 8.1 Hz), 5.90 (d, 1H, J = 3.3 Hz), 5.76 (d, 1H, J = 8.1 Hz), 4.82 (dd, 1H, J = 6.4, 3.2 Hz), 4.76 (dd, 1H, J = 6.4, 3.2 Hz), 4.50 (d, 1H, J = 5.2 Hz), 4.23 (dd, 1H, J = 5.1, 3.2 Hz), 1.57 (s, 3H) 1.33 (s, 3H), 0.98 (t, 9H, J = 7.9 Hz), 0.62 (m, 6H, J = 7.9 Hz). ^{13}C NMR (150 MHz, CD_3OD) δ 163.4, 150.1, 140.9, 114.8, 102.9, 97.3, 92.9, 92.3, 86.2, 83.7, 80.9, 54.6, 27.0, 25.1, 7.2 (×3), 4.0 (×3). HRMS calcd for $[C_{20}H_{29}NO_5Si+H]^+$ 448.2011, found 448.2013.

Preparation of the Key Intermediate 5R. A mixture of **3S** (23.0 mg, 0.07 mmol) and 1 M trimethylphosphine in THF (0.2 mL, 0.21 mmol) was added in THF/H_2O (1/1) and stirred for 5 h. The reaction mixture was added di-*tert*-butyl dicarbonate (0.03 mL, 0.14 mmol) and sodium bicarbonate (11.6 mg, 0.14 mmol). The reaction mixture was stirred for 2 h. The reaction residue was extracted with EtOAc. The organic layers were dried over $MgSO_4$ and concentrated. The reaction mixture was added TBAF (1.0 M in THF, 2.4 mL, 2.40 mmol) and stirred for 0.5 h, then concentrated, purified by column chromatography (EtOAc/hexane = 2/1) to give **5R** (13.5 mg, 0.044 mmol, 63% over 3 steps). R_f = 0.7 (EtOAc/hexane = 2/1). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.4 (brs, 1H), 7.67 (d, 1H, J = 8.0 Hz), 7.30 (d, 1H, J = 9.1 Hz), 5.73 (d, 1H, J = 1.4 Hz), 5.57 (d, 1H, J = 8.0 Hz), 5.15 (d, 1H, J = 6.1 Hz), 4.87 (dd, 1H, J = 6.3, 3.0 Hz), 4.58 (m, 1H, J = 9.1, 7.7 Hz), 4.02 (dd, 1H, J = 9.1, 3.0 Hz), 3.30 (s, 3H), 1.46 (s, 3H), 1.32 (s, 9 H), 1.29 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 163.2, 155.0, 150.2, 142.8, 113.2, 101.8, 92.5, 86.7, 83.2, 81.6, 81.4, 78.7, 74.5, 43.9, 28.1 (×3), 26.8, 25.0. HRMS calculated for $[C_{19}H_{25}N_3O_7 + H]^+$ 430.1585, found 430.1565.

General Procedure of Model Molecules 11S{*1*−*2*} and 11R{*1*−*2*}. To a solution of alkyne **5S** or **5R** (1 equiv) and 3-azidopropan-1-amine (1.5 equiv) in *tert*-butanol was successively added $CuSO_4$ (0.1 equiv) and sodium ascorbate (0.3 equiv). The mixture was stirred at 80 °C for 3 h and diluted with EtOAc and water. The aqueous phase was extracted with EA, and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated to get amine. A solution of amine(1 equiv) in DCM was added with acid(1.5 equiv), EDC·HCl (1.5 equiv) and triethylamine (3 equiv). After 4 h, the reaction was quenched and the organic layer was extracted with 1 N HCl. The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum. The residue was purified by column chromatography (EtOAc) to get the corresponding product.

*Compound **11S**{1}*. Yield: 95% over 2 steps. R_f = 0.2 (EA). ^{1}H NMR (600 MHz, MeOD) δ 7.91 (s, 1H), 7.60 (d, 1H, J = 8.0 Hz), 5.70 (br, 1H), 5.66 (d, 1H, J = 8.0 Hz), 5.17 (d, 1H, J = 5.0 Hz), 5.11 (d, 1H, J = 5.0 Hz), 4.41 (t, 2H, J = 7.0 Hz), 4.36 (br, 1H), 3.19 (t, 2H, J = 7.0 Hz), 2.18 (t, 2H, J = 7.5 Hz), 2.11−2.05 (m, 2H), 1.73−1.63 (m, 5H), 1.60−1.55 (m, 2H), 1.52 (s, 3H), 1.42 (s, 9H), 1.35−1.30 (m, 5H), 1.25−1.15 (m, 6H), 0.92−0.85 (m, 2H). ^{13}C NMR (150 MHz, MeOD) δ 176.7, 166.4, 157.9, 152.3, 147.5, 145.4, 124.7, 115.7, 103.0, 95.6, 89.1, 85.2, 82.5, 80.7, 50.4, 49.7, 39.0, 38.5, 37.5, 37.3, 34.7 (× 2), 31.3, 28.9 (×3), 27.9, 27.7, 26.6 (×3), 26.4, 25.7. HRMS calculated for $[C_{33}H_{51}N_7O_8 + H]^+$ 674.3872, found 674.3895.

*Compound **11S**{2}*. Yield: 97% over 2 steps. R_f = 0.5 (EA). ^{1}H NMR (600 MHz, MeOD) δ 7.91 (s, 1H), 7.60 (d, 1H, J = 8.0 Hz), 5.70 (br, 1H), 5.66 (d, 1H, J = 8.0 Hz), 5.17 (br, 1H), 5.11 (d, 1H, J = 4.8 Hz), 4.41 (t, 2H, J = 6.9 Hz), 4.36 (br, 1H), 3.19 (t, 2H, J = 6.6 Hz), 2.18 (t, 2H, J = 7.5 Hz), 2.11−2.05 (m, 2H), 1.62−1.57 (m, 2H), 1.52 (s, 3H), 1.42 (s, 9H), 1.34−1.26 (m, 25H), 0.92−0.87 (m, 2H). ^{13}C NMR (150 MHz, MeOD) δ 176.7, 166.4, 157.9, 152.3, 147.5, 145.4, 124.7, 115.7, 103.0, 95.6, 89.1, 85.2, 82.5, 80.7, 50.4, 49.7, 37.5, 37.3, 33.2, 31.3, 30.9 (× 5), 30.8, 30.6 (× 2), 30.5, 28.9 (× 3), 27.6, 27.2, 25.7, 23.9, 14.6. HRMS calculated for $[C_{37}H_{61}N_7O_8 + H]^+$ 732.4654, found 732.4677.

*Compound **11R**{1}*. Yield: 91% over 2 steps. R_f = 0.2 (EA). ^{1}H NMR (600 MHz, MeOD) δ 7.89 (s, 1H), 7.52 (d, 1H, J = 7.9 Hz), 5.74 (br, 1H), 5.64 (d, 1H, 7.9 Hz), 5.16 (d, 1H, 6.7 Hz), 5.02 (br, 1H), 4.96 (br, 1H), 4.40 (t, 2H J = 6.8 Hz), 4.32 (br, 1H), 3.18 (t, 2H J = 6.5 Hz), 2.18 (t, 2H, J = 7.4 Hz), 2.10−2.04 (m, 2H), 1.75−1.63 (m, 5H), 1.60−1.51 (m, 2H), 1.53 (s, 3H), 1.45 (s, 9H), 1.35−1.30 (m, 5H), 1.26−1.14 (m, 6H), 0.92−0.84 (m, 2H). ^{13}C NMR (150 MHz, MeOD) δ 176.7, 166.3, 157.8, 152.0, 147.2, 144.7, 124.7, 115.8, 103.0, 94.8, 88.9, 85.2, 82.9, 80.9, 50.2 (×2), 39.0, 38.5, 37.5, 37.3, 34.7 (×2), 31.3, 28.8 (×3), 28.0, 27.7, 27.6 (×3), 27.4, 25.7. HRMS calculated for $[C_{33}H_{51}N_7O_8 + H]^+$ 674.3872, found 674.3909.

*Compound **11R**{2}*. Yield: 73% over 2 steps. R_f = 0.5 (EA). ^{1}H NMR (600 MHz, MeOD) δ 7.91 (s, 1H), 7.60 (d, 1H, J = 8.0 Hz), 5.70 (br, 1H), 5.66 (d, 1H, J = 8.0 Hz), 5.17 (br, 1H), 5.11 (d, 1H, J = 4.8 Hz), 4.41 (t, 2H, J = 6.9 Hz), 4.36 (br, 1H), 3.19 (t, 2H, J = 6.6 Hz), 2.18 (t, 2H, J = 7.5 Hz), 2.11−2.05 (m, 2H), 1.62−1.57 (m, 2H), 1.52 (s, 3H), 1.42 (s, 9H), 1.34−1.26 (m, 25H), 0.92−0.87 (m, 2H). ^{13}C NMR (150 MHz, MeOD) δ 176.7, 166.4, 157.9, 152.3, 147.5, 145.4, 124.7, 115.7, 103.0, 95.6, 89.1, 85.2, 82.5, 80.7, 50.4, 49.7, 37.5,

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37.3, 33.2, 31.3, 30.9 (×5), 30.8, 30.6 (×2), 30.5, 28.9 (×3), 27.6, 27.2, 25.7, 23.9, 14.6. HRMS calculated for $[C_{37}H_{61}N_7O_8 + H]^+$ 732.4654, found 732.4677.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acscombsci.0c00011.

Full experimental details including characterization and NMR spectra of new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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