Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters 46 (2005) 8497–8501
Tetrahedron Letters

Solid-phase synthesis development of a thymidinyl and 2′-deoxyuridinyl Ugi library for anti-bacterial agent screening

Dianqing Sun and Richard E. Lee*

Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, Memphis, TN 38163, USA

Received 18 September 2005; accepted 5 October 2005
Available online 24 October 2005

Abstract—A solid-phase synthesis has been developed to make a thymidinyl and 2′-deoxyuridinyl Ugi library starting from 5′-azido-nucleosides loaded onto polystyrene butyl diethylsilane (PS-DES) resin. Library synthesis development including: Ugi reaction optimization; selection of building blocks and optimization to 96-well filter plate synthesis are reported. A 1344 member library was synthesized for anti-bacterial screening.

1. Introduction

Nucleoside motifs are commonly found in many naturally occurring antibiotics that target the early stages of bacterial cell wall biosynthesis such as tunicamycins, mureidomycins, and liposidomycins.[1] By analogy to these natural products, a thymidinyl and 2′-deoxyuridinyl nucleoside–peptide hybrid library **1** was designed and synthesized to produce potential inhibitors of bacterial cell wall biosynthesis (Fig. 1). A Ugi reaction[2] was employed to introduce four points of diversity using two nucleoside amines, aldehyde, carboxylic acid, and isocyanide inputs. The Ugi reaction is a powerful, widely used reaction for library construction and can produce attractive peptidomimetics.[3]

Parallel synthesis of small molecule drugs has become an important tool for drug discovery.[4] Solid-phase organic synthesis techniques offer simple purification of products from reactants through filtration, allowing for the rapid synthesis of arrays of compounds for drug testing.[5] Solid-phase Ugi reactions have been widely used to prepare compound libraries[3a–d,6] and herein we report the modification of these techniques for the

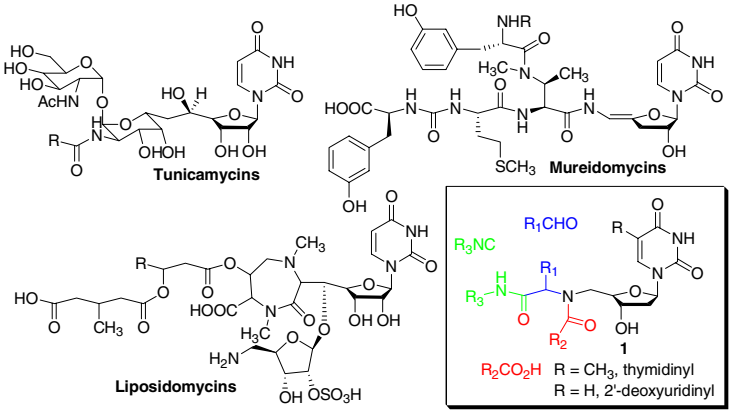


Figure 1. Naturally occurring antibiotics and our targeted library **1**.

Keywords: Thymidinyl/2′-deoxyuridinyl library; Solid-phase synthesis; Ugi reaction; 96-Well filter plate.
* Corresponding author. Tel.: +1 901 448 6018; fax: +1 901 448 6828; e-mail: relee@utmem.edu

0040-4039/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2005.10.012

preparation of a nucleoside–peptide based Ugi library by the immobilization of a nucleoside amine input.

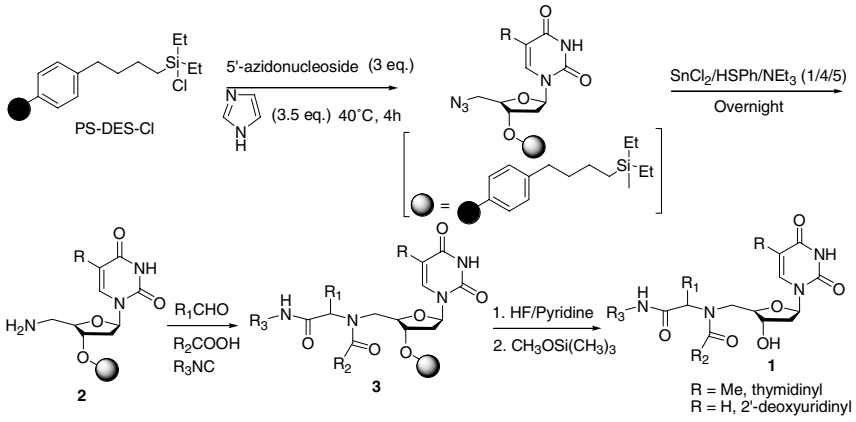
2. Solid-phase synthesis development

Key to the solid-phase synthesis of the target library was the choice of a suitable linker strategy. One that would allow the use of orthogonal chemistries to build the target library and mild cleavage conditions avoiding degradation of the nucleoside bond within the library. Therefore, a strategy was developed using polystyrene butyl diethylsilane (PS-DES) resin[7] (Scheme 1), in which the 3′-hydroxy of a nucleoside azide[8] was attached to the solid support followed by reduction of the azide using $SnCl_2$/HSPh/N(Et)$_3$ (1/4/5) in THF.[9] This yielded the target polymer-bound aminonucleoside **2** and the reaction was monitored for completion using the Kaiser test.[10] Following library synthesis, the compounds could be cleaved from the resin using either mild acid conditions or HF/pyridine, with no evidence of degradation of the products.

2.1. Ugi reaction optimization

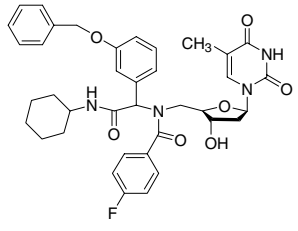
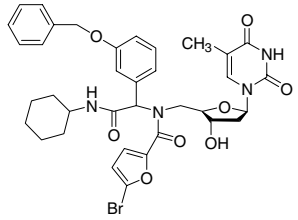
Utilization of the aminonucleoside-bound resin **2** as a support for Ugi chemistry was explored and the conditions were optimized (Scheme 1). First, two preliminary Ugi experiments, using the thymidinyl nucleoside, were performed at room temperature and 40 °C for 24 h. Following filtration, washing, cleavage, and analysis,[11] results (Table 1) showed that 40 °C gave better yields. HPLC purities of the desired Ugi products were 61% and 75% at 40 °C, compared with 43% and 61% at room temperature. Aminothymidine was the only side-product.

The second Ugi optimization experiment was designed to study the effects of solvent and molar ratios of the ingredients on reaction yields using a parallel array in Robbins 96-well Flexchem filter plates. Twelve carboxylic acids, three aldehydes, and one isocyanide (Fig. 2) were used in this study to create 12 different reactant combinations (columns 1–12) under eight different reaction conditions of solvent and molar ratios (rows A–H).



Scheme 1. Solid-phase synthesis development of library **1**.

Table 1. Solid-phase Ugi reaction at room temperature (rt) and 40 °C

Ugi product	HPLC yield (%) (rt)	HPLC yield (%) (40 °C)	rt[a] (min)	MS [MH$^+$]
	43	61	13.4	685.5
	61	75	13.4	735.5

[a] Retention time.

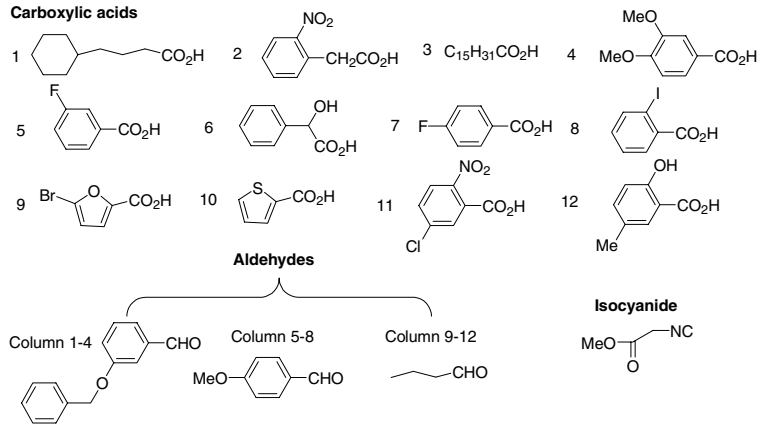


Figure 2. Building blocks used in Ugi optimization.

Table 2. HPLC purity of desired products under solid-phase Ugi reaction optimization

	Solvent[a]	$R_1CHO/R_2CO_2H/R_3NC$[b]	1	2	3	4	5	6	7	8	9	10	11	12
A	DCM/MeOH(1/1), 0.6 mL	10/10/10												
B	DCM/MeOH(2/1), 0.9 mL	10/10/10												
C	DCM/MeOH(2/1), 1.5 mL	20/20/10												
D	DCM/MeOH(1/1), 1.2 mL	10/20/10												
E	DCM/MeOH(1/1), 1.2 mL	10/10/10												
F	$CHCl_3$/MeOH(3/1), 1.2 mL	10/10/10												
G	PhMe/MeCN(3/1), 1.2 mL	10/10/5												
H	THF, 0.9 mL	10/10/10												

Product yield index: Green: >80%; Blue: 50-80%; Pink: 20-50%; Red: <20%.; [a]v/v. [b]molar ratio.

Yields of the target products were determined by LC/MS analysis and are shown in Table 2. Based on these results DCM/MeOH (2/1, v/v, 0.9 mL) and 10/10/10 molar excess of reactants to resin loading (row B) was selected for use in library synthesis, as it gave optimal results in the trade off between favored Ugi reaction conditions, cost of reagents, and necessary resin swelling.

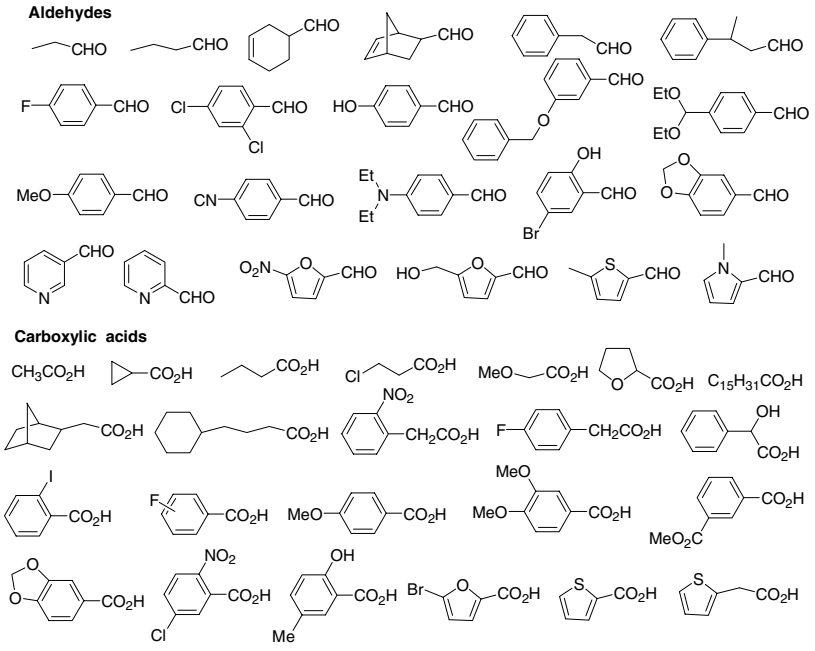


Figure 3. Building blocks evaluated on solid-phase Ugi reaction.

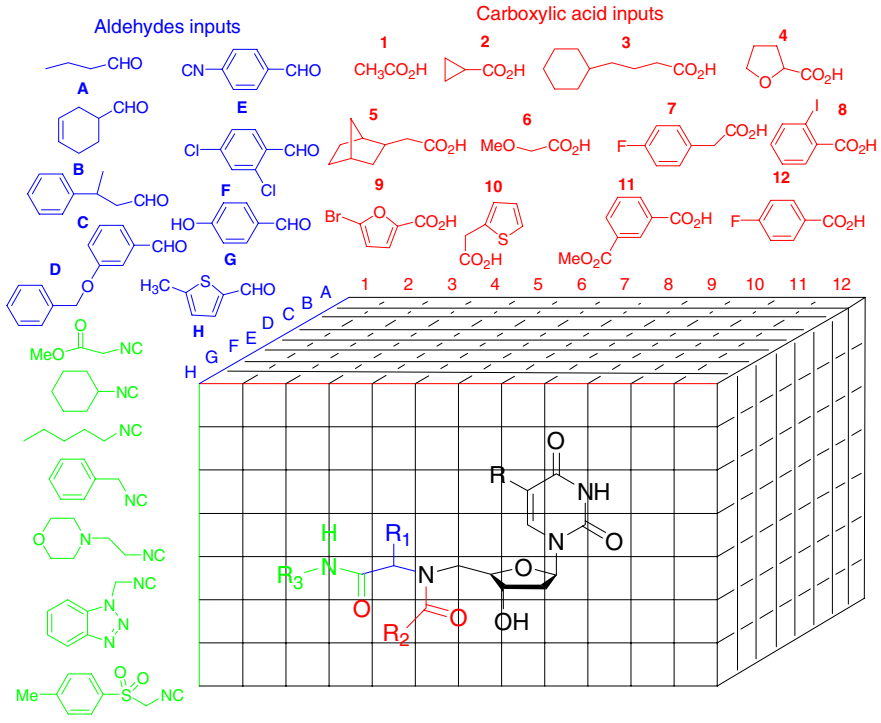


Figure 4. Ugi reaction library diagram.

2.2. Selection of building blocks

In order to maximize the structural diversity of the building blocks in the library, 24 carboxylic acids and 22 aldehydes with aliphatic, aromatic, and heterocyclic motifs were investigated for their compatibility with the Ugi conditions in the 96-well filter plates (Fig. 3). Generally speaking, the reactivity order of aldehydes was as follows: aliphatic > aromatic > heterocyclic. The purity of desired products was more sensitive to aldehyde structure than the carboxylic acid inputs. These findings are consistent with the previous reports.[3] Using this data, 12 carboxylic acids (**1**–**12**) and eight aldehydes (**A**–**H**) (Fig. 4) were selected for library synthesis based on solubility, chemistry feasibility, and library structural diversity.

3. Synthesis of Ugi library 1

Library synthesis was performed in 14 Robbins 96-well Flexchem filter plates. Nucleoside amine **2** resin was reacted with 8 aldehydes (each allocated to discrete rows **A**–**H**), 12 carboxylic acids (each allocated to discrete columns **1**–**12**), and 1 isocyanide per plate to afford solid-supported Ugi product **3** in an $8 \times 12 \times 7$ array for each of the two nucleosides (Scheme 1 and Fig. 4). Final cleavage was performed in HF/pyridine in THF. The excess HF was quenched by adding methoxytrimethylsilane.[12] This allowed simple evaporation to generate Ugi products suitable for biological screening and removal of byproducts. Based on the protocol,[13] a 1344 member library was synthesized. As anticipated, based on HPLC analysis and ^{1}H NMR diastereomeric Ugi products (ca. 1–1 ratio) were obtained.[14]

In summary, a solid-phase synthesis was developed to make a thymidinyl and 2′-deoxyuridinyl nucleoside–peptide library on PS-DES resin. Library synthesis development is reported including: Ugi reaction optimization; selection of building blocks and optimization to 96-well filter plate format. Based on our protocol, a 1344 member library has been synthesized. The compounds produced were in acceptable purity for primary antibacterial screening. Active compounds could be readily resynthesized and tested to obtain accurate minimum inhibitory concentration (MIC) values. Members of this library are showing promising biological activity, which will be reported in due course.

Acknowledgements

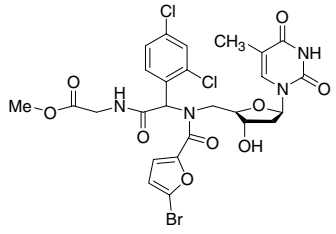
We thank National Institutes of Health grant P01AI057836 for financial support and Robin Lee for help with the preparation of this document.

References and notes

1. Kimura, K.; Bugg, T. D. H. *Nat. Prod. Rep.* **2003**, *20*, 252–273.
2. For reviews, see: (a) Domling, A.; Ugi, I. *Angew. Chem., Int. Ed.* **2000**, *39*, 3168–3210; (b) Ugi, I.; Heck, S. *Comb. Chem. High Throughput Screening* **2001**, *4*, 1–34; (c) Ugi, I. *Pure Appl. Chem.* **2001**, *73*, 187–191; (d) Nair, V.; Rajesh, C.; Vinod, A. U.; Bindu, S.; Sreekanth, A. R.; Mathen, J. S.; Balagopal, L. *Acc. Chem. Res.* **2003**, *36*, 899–907; (e) Zhu, J. *Eur. J. Org. Chem.* **2003**, 1133–1144.
3. (a) Tempest, P. A.; Brown, S. D.; Armstrong, R. W. *Angew. Chem., Int. Ed.* **1996**, *35*, 640–642; (b) Kim, S. W.; Shin, Y. S.; Ro, S. *Bioorg. Med. Chem. Lett.* **1998**, *8*,

1665–1668; (c) Hoel, A. M. L.; Nielsen, J. *Tetrahedron Lett.* **1999**, *40*, 3941–3944; (d) Golebiowski, A.; Jozwik, J.; Klopfenstein, S. R.; Colson, A.-O.; Grieb, A. L.; Russell, A. F.; Rastogi, V. L.; Diven, C. F.; Portlock, D. E.; Chen, J. J. *J. Comb. Chem.* **2002**, *4*, 584–590; (e) Gedey, S.; Van der Eycken, J.; Fülöp, F. *Org. Lett.* **2002**, *4*, 1967–1969; (f) Liu, L.; Li, C. P.; Cochran, S.; Ferro, V. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2221–2226; (g) Chapman, T. M.; Davies, I. G.; Gu, B.; Block, T. M.; Scopes, D. I. C.; Hay, P. A.; Courtney, S. M.; McNeill, L. A.; Schofield, C. J.; Davis, B. G. *J. Am. Chem. Soc.* **2005**, *127*, 506–507.

4. For review, see: Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555–600.
5. *Solid Phase Organic Syntheses*; Czarnik, A. W., Ed.; John Wiley & Sons: New York, 2001.
6. Examples of solid-phase Ugi reactions, see: (a) Lee, D.; Sello, J. K.; Schreiber, S. L. *Org. Lett.* **2000**, *2*, 709–712; (b) Cheng, J.-F.; Chen, M.; Arrhenius, T.; Nadzan, A. *Tetrahedron Lett.* **2002**, *43*, 6293–6295; (c) Campian, E.; Lou, B.; Saneii, H. *Tetrahedron Lett.* **2002**, *43*, 8467–8470; (d) Portlock, D. E.; Naskar, D.; West, L.; Ostaszewski, R.; Chen, J. J. *Tetrahedron Lett.* **2003**, *44*, 5121–5124; (e) Cristau, P.; Vors, J.-P.; Zhu, J. *Tetrahedron Lett.* **2003**, *44*, 5575–5578; (f) Henkel, B.; Sax, M.; Dömling, A. *Tetrahedron Lett.* **2003**, *44*, 7015–7018; (g) Hanyu, M.; Murashima, T.; Miyazawa, T.; Yamada, T. *Tetrahedron Lett.* **2004**, *45*, 8871–8874; (h) Gedey, S.; Van der Eycken, J. V.; Fülöp, F. *Lett. Org. Chem.* **2004**, *1*, 215–220.
7. PS-DES resin (100–200 mesh, loading: 0.74–1.58 mmol/g) is commercially available from Argonaut Technologies. For resin activation and loading of alcohols, see: (a) Hu, Y.; Porco, J. A.; Labadie, J. W.; Gooding, O. W.; Trost, B. M. *J. Org. Chem.* **1998**, *63*, 4518–4521; (b) Dragoli, D. R.; Thompson, L. A.; O'Brien, J.; Ellman, J. A. *J. Comb. Chem.* **1999**, *1*, 534–539.
8. Bannwarth, W. *Helv. Chim. Acta* **1988**, *71*, 1517–1527.
9. (a) Bartra, M.; Romea, P.; Urpí, F.; Vilarrasa, J. *Tetrahedron* **1990**, *46*, 587–594; (b) Kick, E. K.; Ellman, J. A. *J. Med. Chem.* **1995**, *38*, 1427–1430; (c) Maltais, R.; Tremblay, M. R.; Poirier, D. *J. Comb. Chem.* **2000**, *2*, 604–614.
10. Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595–598.
11. Analytical RP-HPLC was conducted on an Agilent 1100 chemstation, using an Alltech platinum EPS C18 column (100 Å, 5 μm, 4.6 × 150 mm) with precolumn 4.6 × 10 mm, flow rate 1.0 mL/min, and a gradient of solvent A (water with 1% acetic acid) and solvent B (acetonitrile): 0–2.00 min 100% A; 2.00–17.00 min 0–100% B; 17.00–19.00 min 100%. UV detection at 254 nm. Mass spectra were recorded on a Bruker Esquire LC–MS using ESI.
12. Hu, Y.; Porco, J. A. *Tetrahedron Lett.* **1998**, *39*, 2711–2714.
13. Protocol for Ugi library synthesis in 96-well filter plate: A neutral buoyancy suspension of solid supported amine **2** (1.4 g, 1.8 mmol) in 140 mL DCM/THF (2/1, v/v) was evenly distributed into a 96-well filter plate (0.019 mmol per well) and the solvent was removed using a filtration manifold. Then a solution of aldehyde A–H in anhydrous DCM (0.3 mL per well, 0.19 mmol, 10 equiv, 0.63 M) was added to their corresponding row in the block, except for aldehyde G, which was added in (0.4 mL, 0.19 mmol, 10 equiv, 0.47 M in anhydrous DCM/MeOH, 3/1, v/v), the reaction plate was sealed, and agitated at room temperature for 0.5 h, followed by the addition of a solution of the appropriate carboxylic acid in anhydrous MeOH to the appropriate column (0.3 mL per well, 0.19 mmol, 10 equiv, 0.63 M) except for column 11 (0.6 mL per well, 0.19 mmol, 10 equiv, 0.32 M in anhydrous DCM/MeOH, 1/1, v/v). The reaction was agitated at room temperature for another 0.5 h, then a solution of isocyanide (19.7 mmol) in anhydrous DCM (30 mL) was distributed into all 96 wells (0.3 mL per well, 0.19 mmol, 10 equiv, 0.65 M). The reaction plate was sealed and agitated at 35–40 °C for 2 days. After cooling to room temperature, the resins were filtered, washed with DMF (3 × 1 mL), MeOH (3 × 1 mL), DCM (3 × 1 mL) THF (3 × 1 mL), and dried in vacuo. For cleavage, 0.3 mL of HF/pyridine in THF (0.4 M) was added to each well of the 96-well plate, the reaction mixture was agitated at room temperature for 2.5 h, followed by the addition of methoxytrimethylsilane (50 μl, 0.36 mmol, 3 equiv relative to HF) to each well. The plate was sealed and continuously agitated at room temperature for another 3.5 h. The solution was filtered and collected into the collection plate, the resins were washed with MeOH (2 × 1 mL), and the combined filtrate was evaporated to yield crude products. Primary hits from biological screening were resynthesized on a Quest 210 synthesizer and purified by preparative RP-HPLC, structure was characterized by MS and ^{1}H NMR, purity was determined by analytical HPLC.
Representative examples of Ugi product characterization. Crude yield: 76 mg (46%).



First diastereomeric isomer after preparative HPLC: 8 mg (5%). $R_f = 0.48$ (methanol/chloroform = 1/10) (v/v). ^{1}H NMR (500 MHz, CD_3OD): δ 7.59 (d, $J = 8.3$ Hz, 1H), 7.49 (d, $J = 2.2$ Hz, 1H, 6-*H*), 7.31 (dd, $J = 2.2$ and 8.5 Hz, 1H), 7.21 (d, $J = 3.4$ Hz, 1H), 7.15 (s, 1H), 6.65 (d, $J = 3.7$ Hz, 1H), 6.31 (s, 1H), 5.96 (t, $J = 6.8$ and 6.6 Hz, 1H, *H*-1′), 4.22 (q, 1H, *H*-4′), 4.14 (d, $J = 17.1$ Hz, 1H, C*H*^{a}H^b), 3.99 (d, $J = 17.1$ Hz, 1H, CH^a*H*^{b}), 3.89 (br s, 1H, *H*-3′), 3.77 (s, 3H, CH_3OCO), 3.65 (br s, 2H, CH_2-5′), 2.24 (br s, 1H, C*H*^{b}-2′), 2.14 (m, 1H, C*H*^{a}-2′), 1.92 (d, $J = 0.7$ Hz, 3H, 5-CH_3). MS (ESI): $m/z = 689.0$ $[MH]^+$, 711.0 $[MNa]^+$. HPLC purity: 95%, $t_R = 11.4$ min.
Second diastereomeric isomer after preparative HPLC: 6 mg (4%). $R_f = 0.53$ (methanol/chloroform = 1/10) (v/v). ^{1}H NMR (500 MHz, CD_3OD): δ 7.69 (d, $J = 8.5$ Hz, 1H), 7.61 (d, $J = 2.2$ Hz, 1H, 6-*H*), 7.43 (d, $J = 8.1$ Hz, 1H), 7.40 (br s, 1H), 7.21 (d, $J = 3.4$ Hz, 1H), 6.66 (d, $J = 3.4$ Hz, 1H), 6.16 (s, 1H), 6.05 (t, $J = 6.6$ and 6.4 Hz, 1H, *H*-1′), 4.16 (sextet, 1H, *H*-4′), 4.03 (d, $J = 4.4$ Hz, 2H, CH_2), 3.99 (br s, 1H, *H*-3′), 3.94 (br s, 2H, CH_2-5′), 3.75 (s, 3H, CH_3OCO), 2.36 (m, 1H, C*H*^{b}-2′), 2.21 (m, 1H, C*H*^{a}-2′), 1.92 (s, 3H, 5-CH_3). MS (ESI): $m/z = 688.9$ $[MH]^+$, 711.0 $[MNa]^+$. HPLC purity: 100%, $t_R = 11.8$ min.

14. Quality assessment of the prepared Ugi library was performed by LC/MS using one randomly selected column (eight samples) per plate. Our library gave acceptable purities after cleavage from resin: 47% of analyzed samples had HPLC purity greater than 80%, 28% of samples gave the expected product with purity ranging from 50% to 80%, 10% of samples had purity ranging from 20% to 50% and 15% had purity less than 20%. 5′-Aminonucleoside was the major side-product of our developed library.