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Synthesis of β-lactam nucleoside chimera via Kinugasa reaction and evaluation of their antibacterial activity

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Abstract—Several β-lactam nucleoside chimeras **1** (*cis*) and **2** (*trans*) were synthesized from the corresponding *N*-propargyl nucleobases via Kinugasa reaction in moderate yields. Initial screening for antibacterial activity against ampicillin sensitive *E. coli* showed weak activity for the uracil-β-lactam chimera.

Bioconjugates or the so-called dual acting drugs have drawn significant interest in recent years. Several research groups have applied this concept by combining two different pharmacophores to successfully generate chimeric molecules. For example, a chimera of classical β-lactam antibiotics and gyrase inhibitors has given rise to extremely potent antibiotics.[1] Few years back, Ugi and co-workers[2] developed an elegant method for the synthesis of β-lactam nucleoside chimeras by using the well-known multicomponent Ugi reaction.[3] Although these molecules looked attractive targets owing to the multi-faceted activity profiles of β-lactams[4] and nucleosides,[5] the biological activity of these molecules has not been reported. In this communication, we would like to report an alternate approach to the synthesis of a structurally different β-lactam nucleic base chimeras[6] using Kinugasa reaction[7] between nitrones and various *N*-propargyl nucleic acid bases. The results of initial screening of these molecules as antibacterial agents are also reported herein.

Since the nucleotide bases have reactive functional groups, for the synthesis of the targeted β-lactams, a method was required, which has a high functional group tolerance. The Kinugasa reaction, which has drawn significant attention in recent years, satisfies such a criteria. Thus for our synthesis we needed the various *N*-propargyl bases, namely 3-propargyl adenine **3a**, and 1-propargyl uracil **3c**, 1-propargyl thymine **3d** and 3-propargyl guanine **3e**. Amongst these, propargyl uracil[8] and thymine, **3c** and **3d**, respectively, were synthesized by direct alkynylation with propargyl bromide using DMF as a solvent. For the synthesis of adenine-based alkyne, we prepared both 3-propargyl adenine and its di-*t*-Boc analogue **3b**. The latter was synthesized to enhance the solubility in organic solvents. The propargyl guanine, however, could not be prepared; in our hands, the reported acetylation followed by alkynylation ran into problems.

With the propargyl nucleobases in hand, we proceeded to do the Kinugasa reaction with the diphenyl nitrone **4** using our published procedure.[9] *N*,*N*-di-*t*-Boc propargyl adenine **3b** was reacted in the first place for better handling due to solubility. However, the reaction with the nitrone **4** in the presence of CuI and Et_3N in DMF produced only the *exo*-methylene β-lactam **8** thus showing the liability of the imidazoline ring as a nucleofugal. At the same time, the fact that we could obtain a β-lactam demonstrated that the initial cycloaddition between the nitrone and the in situ generated cuprous acetylide was occurring; it is only during the rearrangement of the intermediate isooxazoline **5** to the β-lactam **8**, concomitant elimination took place (route a).[10] One may, however, argue that the β-lactams **1b** and **2b** were first formed via the standard mechanism. These then underwent a base-induced elimination. In order to establish the actual pathway, we synthesized the di-Boc β-lactams **1b** (*cis*) and **2b** (*trans*) from the corresponding adenine β-lactams. These were subjected to the

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basic conditions employed in Kinugasa reaction. We have not noticed any formation of the *exo*-methylene β-lactam thus ruling out the latter mechanistic possibility as depicted in route b (Scheme 1).

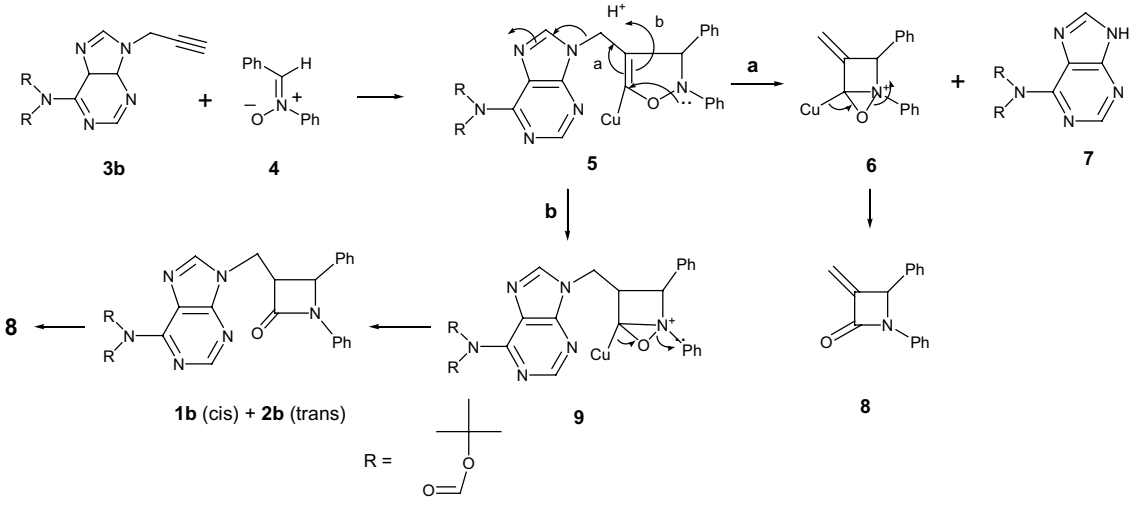
Realizing that the two *t*-butyloxy carbonyl groups at the 6-amino group enhance the electron withdrawing character of the imidazole ring in adenine, we decided to carry out the reaction with propargyl adenine itself for which the imidazole ring is not activated further by the electron withdrawal from the adjacently fused pyrimidine ring. On the contrary the presence of free amine should reduce the electron withdrawing character. Gratifyingly, the reaction was successful and we obtained moderate yields of *cis* and *trans* β-lactams **1a** and **2a**, respectively (Scheme 2). The reaction of propargyl uracil and thymine with the nitrone were also successful, both producing a mixture of *cis* (**1b**,**c**) and *trans* β-lactam (**2b**,**c**) in respectable yields (Table 1). NMR and mass spectroscopy characterized the structures of the various nucleoside-β-lactams chimeras. For example, for the adenine-based β-lactam **1a**, the characteristic signals for the ring hydrogens H-3 and H-4 appeared at δ 4.39 and 5.50 for the *cis* isomer with the H-4 appearing as a doublet with a characteristic[11] coupling constant of 6.0 Hz. For the corresponding *trans* isomer, H-4 appeared as a doublet at δ 5.01 with a characteristic[11] J value of 2.5 Hz. The H-3, shielded by the phenyl, appeared as a multiplet at δ 3.55. The other β-lactams showed NMR spectra compatible with their structures.

Table 1. Results of Kinugasa reaction of propargyl bases with the phenyl nitrone

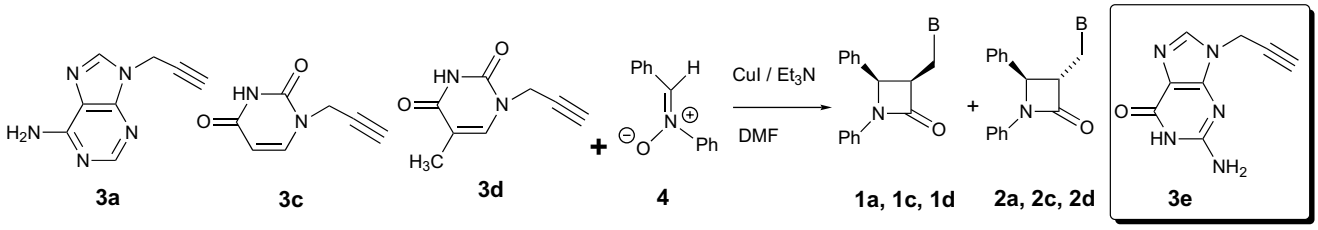
Nitrone	Propargyl base	Time (h)	Combined yield (%)	Ratio of *cis* and *trans*
Phenyl (**4**)	Adenine (**3a**)	36	64	1:6
Phenyl (**4**)	Uracil (**3c**)	36	60	2.5:1
Phenyl (**4**)	Thymine (**3d**)	36	60	2:1

With the chimeras of β-lactam and nucleobases in hand, we turned our attention to check their antibacterial activities, if any. However, poor solubility of the materials in many commonly used water miscible organic solvents proved to be a problem. Finally, the activity could be checked in DMF solution using the 'holed-plate' assay[12] against penicillin sensitive *E. coli* strain. The uracil-β-lactam (initially given as a mixture of *cis* and *trans*) was found to be active against the strain with the activity potency of about 20% of that of ampicillin. Similar activity was found when individual *cis* and *trans* isomers **1c** and **2c** were used for antibacterial screening. No firm conclusion could be drawn for the adenine and thymine based β-lactams as these immediately precipitates out upon contact with water present in the agar medium.

In conclusion, we have demonstrated the utility of Kinugasa reaction in the synthesis of β-lactam nucleobase chimeras. The uracil-based chimera has been shown to possess some activity, which may possibly be improved



Scheme 1.



Scheme 2.

by synthesizing water-soluble entities. Current efforts in our laboratory are aimed towards that end.

General procedure for preparation of β-lactam

To a solution of *N*-propargyl bases **3a**, **3c** or **3d** (1.1 mmol) in DMF, triethylamine (1.1 mmol) was added under argon. The mixture was stirred for 30 min at 0 °C. CuI (1 mmol) was added and stirring was continued for 5 min at room temperature. Solution of phenyl nitrone **4** (1 mmol) in DMF was added drop wise for about 15 min. The reaction mixture was stirred for 36 h at rt, after which it was poured into water and filtered through Celite. The Celite bed was thoroughly washed with EtOAc. The organic layer was washed with saturated solution of NH_4Cl, water and brine and dried over Na_2SO_4. Filtration followed by removal of solvent gave solid residue from which product was isolated by column chromatography over Si-gel using hexane–EtOAc or DCM–methanol (for adenine β-lactams) as eluent.

Spectral data

^1H NMR spectra were recorded at 200 MHz in $CDCl_3$ unless otherwise mentioned while ^{13}C NMR spectra were recorded at 50 MHz in d_6-DMSO.

cis β-Lactam with adenine (1a)

ν_{max} 3304, 3136, 1744, 1664, 1597 cm^{-1}; δ_H (d_6-DMSO): 3.97, 4.20 (2H, 2 × dd, J = 5.7, 14.0, 7.9 Hz), 4.39 (1H, m), 5.50 (1H, d, J = 6.0 Hz), 7.37–7.03 (10H, m), 8.08 (1H, s); δ_C 41.04, 56.27, 58.17, 116.78, 123.87, 125.91, 127.09, 128.37, 128.93, 129.17, 137.03, 137.17, 140.78, 149.49, 152.57, 155.95, 164.08; Mass (ES) 371 (MH^+), 393 (MNa^+); HRMS: calcd for $C_{21}H_{19}N_6O + H^+$ 371.1623 found 371.1622.

trans β-Lactam with adenine (2a)

ν_{max} 3313, 3117, 1735, 1679, 1637, 1600 cm^{-1}; δ_H 3.55 (1H, m), 4.73 (2H, app d, J = 6.0 Hz), 5.01 (1H, d, J = 2.5 Hz, H-4), 5.82 (2H, br s), 7.43–7.00 (10H, m), 7.97 (1H, s), 8.44 (1H, s); δ_C 41.80, 58.34, 59.64, 116.78, 118.54, 123.87, 125.30, 128.28, 128.62, 128.75, 133.92, 136.06, 136.49, 140.38, 152.10, 155.08 and 163.55; Mass (ES) 371 (MH^+), 393 (MNa^+); HRMS: calcd for $C_{21}H_{19}N_6O + H^+$ 371.1623 found 371.1625.

cis β-Lactam with uracil (1c)

ν_{max} 3390, 3041, 1744, 1673 cm^{-1}; δ_H 3.65 (2H, d, J = 7.3 Hz), 4.13 (1H, m), 5.30 (1H, d, J = 6.0 Hz), 5.48 (1H, dd, J = 1.8, 6.0 Hz), 6.50 (1H, d, J = 7.9 Hz), 7.52–7.06 (10H, m), 8.42 (1H, br s); δ_C 51.32, 56.13, 57.86, 100.52, 116.77, 125.99, 127.21, 129.00, 129.10, 129.21, 134.23, 137.12, 145.44, 150.70, 163.61 and 164.96; Mass (ES) 348 (MH^+), 370 (MNa^+); HRMS: calcd for $C_{20}H_{17}N_3O_3 + H^+$ 348.1349 found 348.1339.

trans β-Lactam with uracil (2c)

δ_H 3.39 (1H, m), 4.27, 4.24 (2H, 2 × dd, J = 6.4, 7.4, 9.3 Hz), 5.00 (1H, d, J = 2.5 Hz), 5.71 (1H, dd, J = 2, 5.8 Hz), 7.48–7.04 (10H, m), 8.56 (1H, br s); δ_C 44.20, 45.83, 58.08, 102.81, 117.05, 124.38, 125.63, 128.75, 129.21, 129.38, 134.23, 136.77, 144.36, 150.93, 162.91 and 163.88; Mass (ES) 348 (MH^+), 370 (MNa^+); HRMS: calcd for $C_{20}H_{17}N_3O_3 + H^+$ 348.1349 found 348.1342.

cis β-Lactam with thymine (1d)

ν_{max} 3432, 3154, 3028, 1750, 1701, 1676 cm^{-1}; δ_H 1.80 (3H, s), 3.72, 3.61 (2H, 2 × dd, J = 7.4, 8.0, 14.9 Hz), 4.21 (1H, m), 5.29 (1H, d, J = 5.7 Hz), 6.08 (1H, s), 7.43–7.09 (10H, m), 8.20 (1H, br s); δ_C 12.02, 51.74, 54.95, 56.30, 107.81, 116.84, 123.89, 127.42, 128.61, 129.07, 129.27, 134.34, 137.14, 141.35, 150.87, 164.21, 164.88; Mass (ES) 362 (MH^+), 384 (MNa^+); HRMS: calcd for $C_{21}H_{19}N_3O_3 + H^+$ 362.1506 found 362.1492.

trans β-Lactam with thymine (2d)

ν_{max} 3435, 3025, 1741, 1704, 1673 cm^{-1}; δ_H 1.90 (3H, s), 3.40 (1H, m), 4.20, 4.35 (2H, 2 × dd, J = 5.2, 14.7, 6.6 Hz), 5.06 (1H, d, J = 2.4 Hz), 7.44–7.04 (10H, m), 9.05 (1H, br s); δ_C 12.01, 43.83, 44.26, 51.74, 108.36, 116.84, 125.89, 127.41, 128.60, 129.07, 129.26, 134.34, 138.14, 141.83, 151.07, 164.21 and 164.42; Mass (ES) 362 (MH^+), 384 (MNa^+); HRMS: calcd for $C_{21}H_{19}N_3O_3 + H^+$ 362.1506 found 362.1494.

trans-β-Lactam with di-Boc adenine (2b)

δ_H 1.41 (18H, s), 3.64 (1H, m), 4.73 (2H, d, J = 6.0 Hz), 5.04 (1H, d, J = 2.5 Hz, H-4), 7.43–7.00 (10H, m), 7.97 (1H, s), 8.44 (1H, s); Mass (ES) 571 (MH^+).

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