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ASYMMETRIC ONE-POT SYNTHESIS OF CYCLOPROPANES

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Cycloaddition of the diazoalkanes to electron-deficient olefins (*in situ*) affords polysubstituted cyclopropanes in high yields (up to 85%). Deprotection of the ketal protecting group provided water-soluble cyclopropane-bearing carbohydrate in good yields. Antimicrobial activity screening of the synthesized compounds **8** and **9**, utilizing a variety of Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus fecalis*), Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) and yeast (*Candida albicans*), showed that all of the prepared analogues acquire promising activities against both Gram-positive and Gram-negative bacteria, especially compounds **9b** and **9c** (antimicrobial active agents against Gram-negative bacteria).

Keywords: asymmetric synthesis; carbohydrate; cyclopropane; antimicrobial

АСИМЕТРИЧНА СИНТЕЗА ВО ЕДЕН САД НА ЦИКЛОПРОПАНИ

Циклоадицијата на дизоалкани со олефини што имаат недостиг на електрони *in situ* дава полисупституирани циклопропани во голем принос (до 85%). Со отстранување на кеталната заштитна група се добиваат во вода растворливи циклопропански јаглехидрати со добар принос. Анализата на антимикробното дејство на синтетизираните соединенија **8** и **9** врз разни Грам-позитивни (*Staphylococcus aureus* и *Enterococcus fecalis*) и Грам-негативни бактерии (*Escherichia coli* и *Klebsiella pneumoniae*), како и врз квасец (*Candida albicans*), покажува дека сите приготвени аналози можат да дејствуваат врз Грам-позитивни и Грам-негативни бактерии, особено соединенијата **9b** и **9c** (антимикробни средства против Грам-негативни бактерии).

Клучни зборови: асиметрична синтеза; јаглехидрати; циклопропан; антимикробно дејство

1. INTRODUCTION

The cyclopropane ring is a common unit in a diverse range of both naturally occurring and synthetic compounds [1, 2] and many biologically active compounds contain the cyclopropane moiety [3, 4]. The cyclopropane sub-unit is also found in a wide variety of naturally occurring compounds including terpenes, pheromones, fatty acid metabolites, and unnatural amino acids [5, 6], as well as in

rationally designed pharmaceutical agents. Owing to the unique combination of a rigid strained structure and consequent susceptibility to electrophilic attack, the cyclopropane ring often constitutes a key element in complex compounds with a defined orientation of pendant functional groups [7]. Thus, over the past few decades, considerable attention has been devoted to the development of asymmetric methodology for the synthesis of highly functionalized cyclopropanes [8]. Also, carbohydrates constitute a rich source of materials for asymmetric syntheses amplified by a history of well-documented functional group transformations [9, 10]. Carbon-branched sugars [11] are found in numerous natural products. They have been used as starting materials for the preparation of indole alkaloid [12], commonly occurring in the glycon portion of many antibiotics [13], and have been studied during the elucidation of biochemical pathways. Additionally, naturally occurring macrolides often contain branched polyol sequences, a structural motif that portends an origin from a C-branched sugar [14]. One of the most important advances in cyclopropane chemistry over the last decade has been the integration of cyclopropanes and carbohydrates [15]. Carbohydrates have an exciting history in organic and medicinal chemistry [16]. Not only are they ubiquitous natural products occurring throughout the biosphere, but also they provide key functional sub-units for rational drug designs [17]. They are inexpensive yet powerful members of the chiral pool, which makes them attractive platforms for asymmetric synthesis [18]. We recently reported a new method for the generation of aryldiazomethanes from stable tosylhydrazone derivatives. This procedure has proven to be a highly effective and safe alternative technique to handle aryldiazomethanes [19] and has been employed in the cyclopropanation of alkenes [20]. The present article reports our success in achieving this goal and the development of a new user-friendly, one-pot procedure for the preparation of cyclopropanes from ketones in which not only the diazoi compound but also the precursor tosylhydrazones are generated *in situ.*

2. EXPERIMENTAL SECTION

Melting points (Mp) were determined in open capillaries on a Buchi-510 Melting Point apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer IR-197 spectrometer. NMR spectra were obtained on a Bruker AC 300 spectrometer operating at 300 MHz for ^{1}H and at 75.47 MHz for ^{13}C. Mass spectra were acquired in ESI mode. Optical rotations were measured at 589 nm. TLC was performed on pre-coated plates (0.2 mm, silica gel 60 F254). All solvents were distilled and purified prior to use.

2.1. *General procedure for the synthesis of 4-methyl-1-phenyl-2-(2,2,7,7-tetramethyl-tetrahydro-bis[1,3]dioxolo[4,5-b;4',5'-d]pyran-5-ylmethyl)-1,2-dihydro-pyridazine-3,6-diones **3***

Pyridazine-3,6-dione **2** (1.0 mmol, 222 mg) was added to a stirred solution (spin bar) of 1',2':3',4'-di-*O*-isopropylidene-α-D-galactopyranose **1** (1.1 mmol, 286 mg) and triphenyl-phosphine (1.1 mmol, 262 mg) in anhydrous THF under a nitrogen atmosphere at 0 °C. To the resulting suspension (or solution) was added diethyl azodicarboxylate (1.1 mmol, 174 mg) and the reaction mixture was then stirred at room temperature until completion of the reaction, as indicated by TLC on silica gel TLC (hexane–EtOAc, 80:20). The precipitated diethyl hydrazodicarboxylate was collected and the filtrate was evaporated *in vacuo*. Addition of cyclohexane to the oily residue caused the precipitation of triphenylphosphine oxide which was collected. Evaporation of the filtrate under reduced pressure gave a residue that was purified by column chromatography on silica gel to afford the pure compounds.

(1'*R*,2'*S*,3'*S*,4'*S*)-4-methyl-1-phenyl-2-(2,2,7,7-tetramethyl-tetrahydro-bis[1,3]dioxolo[4, 5-b; 4', 5'-d]pyran-5-ylmethyl)-1,2-dihydro-pyridazine-3,6-dione (3).

Yield: (355 mg, 90%), white solid, mp. 103–104 °C. $[\alpha]^{22}_{D}$ = +27 (*c* = 1, CH_2Cl_2), R_f = 0.4 (cyclohexane/AcOEt 4:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.22-6.76 (m, 5H, H_{arom}), 6.66 (s, 1H, H_5), 6.12 (d, $J_{H1'-2'}$ = 3.5, 1H, $H_{1'}$), 5.58 (dd, $J_{H3'-4'}$ = 3.7, $J_{H3'-2'}$ = 2.4, 1H, $H_{3'}$), 5.18–5.10 (m, 2H, $H_{2',4'}$), 4.41–4.37 (m, 1H, $H_{5'}$), 4.20–4.16 (m, 1H, $H_{6'}$), 4.00–3.94 (m, 1H, $H_{6'}$), 2.05, 1.96, 1.91, 1.84, 1.21 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.2, 168.1, 141.2, 133.1, 130.0, 121.3, 118.7, 112.6, 110.4, 108.5, 97.2, 71.0, 70.9, 70.0, 67.6, 64.4, 27.5, 25.7, 25.6, 23.4, 22.6. HRMS: Calcd for $C_{23}H_{28}N_2O_7$ 444.1897. Found: 444.1894.

Anal. Calcd for $C_{23}H_{28}N_2O_7$: C, 62.15, H, 6.35, N, 6.30. Found: C, 62.13, H, 6.37, N, 6.27.

2.2. *General procedure for the addition of diazoalkanes to 1-phenyl-1,2-dihydropyridazine-3,6-dione N-glycoside derivatives **3***

A mixture of the ketone (1.5 mmol) and *p*-toluenesulfonylhydrazide (279 mg, 1.5 mmol) in 10 ml. of 95% ethanol was stirred for 3 h at room temperature. Then, a 5N NaOH (300 µl, 1.5 mmol) solution was added and the mixture was stirred for a further 20 min. The pyridazinedione **3** (7.5

mmol) was then added as a solid, and the mixture was heated up to 50 °C and stirred at this temperature for 48 h. After cooling, the volatiles were evaporated under reduced pressure and the residue was dissolved in a 1:1 mixture of water-ethyl acetate (70 ml). The organic layer was separated and dried over $MgSO_4$. Filtration and evaporation of the filtrate under reduced pressure led to the crude material which was purified by flash column chromatography (hexane–EtOAc, 85:15).

(1'*R*,2'*S*,3'*S*,4'*S*)-1-methyl-4,7,7-triphenyl-3-(2,2,7,7-tetramethyl-tetrahydro-bis[1,3]dio-xolo[4,5-b;4',5'-d]pyran-5-ylmethyl)-3,4-diaza-bicyclo[4.1.0]heptane-2,5-dione (7a)

Yield: (594 mg, 90%), white solid. Mp = 135–136 °C. $[\alpha]^{22}_D$ = + 19 (*c* = 1, CH_2Cl_2), R_f = 0.5 (cyclohexane/AcOEt 4:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07–6.70 (m, 15H, H_{arom}), 6.10 (d, $J_{H1'-2'}$ = 3.6, 1H, $H_{1'}$), 5.61 5.55 (dd, $J_{H3'-4'}$ = 3.7, $J_{H3'-2'}$ = 2.3, 1H, $H_{3'}$), 5.13–5.09 (m, 2H, $H_{2',4'}$), 4.37–4.33 (m, 1H, $H_{5'}$), 4.21–4.17 (m, 1H, $H_{6'}$), 4.10–3.96 (m, 1H, $H_{6'}$), 2.15 (s, 1H, H_6), 2.05, 1.96, 1.93, 1.81, 1.56 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 167.3, 139.9, 132.9, 129.1, 120.3, 117.8, 117.6, 112.5, 110.4, 108.5, 107.9, 97.2, 71.0, 70.8, 70.1, 67.4, 63.9, 38.5, 35.2. 34.4, 27.5, 25.7, 25.6, 23.3, 22.7. HRMS Calcd for $C_{36}H_{38}N_2O_7$ 610.2679. Found: 610.2676.

Anal. Calcd for $C_{36}H_{38}N_2O_7$: C, 70.80, H, 6.27, N, 4.59%. Found: C, 70.78, H, 6.24, N, 4.57%.

(1*R*,6*S*,1'*R*,2'*S*,3'*S*,4'*S*)-1-methyl-7,7-bis-(4-nitrophenyl)-4-phenyl-3-(2,2,7,7-tetramethyl-tetrahydro-bis[1,3]dioxolo[4,5-b;4',5'-d]pyran-5-ylmethyl)-3,4-diaza-bicyclo[4.1.0]-heptane-2,5-dione (7b).

Yield: (595 mg, 85%), white solid. mp 161–161 °C. $[\alpha]^{22}_D$ = +33 (*c* = 1, CH_2Cl_2), R_f = 0.7 (cyclohexane/AcOEt 4:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66–6.87 (m, 13H, H_{arom}), 6.11 (d, $J_{H1'-2'}$ = 3.6, 1H, $H_{1'}$), 5.63 5.51 (dd, $J_{H3'-4'}$ = 3.7, $J_{H3'-2'}$ = 2.3, 1H, $H_{3'}$), 5.12–5.10 (m, 2H, $H_{2',4'}$), 4.38–4.35 (m, 1H, $H_{5'}$), 4.20–4.18 (m, 1H, $H_{6'}$), 4.09–3.99 (m, 1H, $H_{6'}$), 2.17 (s, 1H, H_6), 2.06, 1.93, 1.91, 1.83, 1.57 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9, 167.3, 140.9, 132.7, 128.2, 121.7, 116.9, 115.7, 111.9, 110.2, 108.6, 107.9, 97.3, 71.5, 71.1, 70.6, 66.9, 63.3, 38.6, 35.4, 33.7, 26.9, 25.7, 25.5, 23.4, 22.5. HRMS Calcd for $C_{36}H_{36}N_4O_{11}$700.2381. Found: 700.2378.

Anal. Calcd for $C_{36}H_{36}N_4O_{11}$: C, 61.71, H, 5.18, N, 8.00%. Found: C, 61.69, H, 5168, N, 7.98.

(1*R*,6*S*,1'*R*,2'*S*,3'*S*,4'*S*)-7,7-bis-(4-methoxy-phenyl)-1-methyl-4-phenyl-3-(2,2,7,7-tetramethyl-tetrahydrobis[1,3]dioxolo[4,5-b;4',5'-d]pyran-5-ylmethyl)-3,4-diaza-bicyclo[4.1.0]heptane-2,5-dione (7c)

Yield: (636 mg, 95%), white solid. Mp. 127–128 °C. $[\alpha]^{22}_D$ = + 61 (*c* = 1, CH_2Cl_2), R_f = 0.6 (cyclohexane/AcOEt 4:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47–6.59 (m, 13H, H_{arom}), 6.10 (d, $J_{H1'-2'}$ = 3.6, 1H, $H_{1'}$), 5.61 5.49 (dd, $J_{H3'-4'}$ = 3.7, $J_{H3'-2'}$ = 2.3, 1H, $H_{3'}$), 5.17–5.12 (m, 2H, $H_{2',4'}$), 4.37–4.33 (m, 1H, $H_{5'}$), 4.21–4.18 (m, 1H, $H_{6'}$), 4.10–3.98 (m, 1H, $H_{6'}$), 3.71 (s, 6H, CH_3), 2.15 (s, 1H, H_6), 2.07, 1.91, 1.89, 1.81, 1.59 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 167.4, 159.9, 140.6, 132.6, 127.9, 116.4, 115.9, 112.1, 110.4, 108.6, 107.7, 97.2, 71.1, 69.9, 69.6, 66.6, 63.3, 55.5, 38.4, 35.6, 33.0, 26.8, 25.6, 25.4, 23.4, 21.9. HRMS Calcd for $C_{38}H_{42}N_2O_9$ 670.2890. Found: 670.2886.

Anal. Calcd for $C_{38}H_{42}N_2O_9$: C, 68.04, H, 6.31, N, 4.18%. Found: C, 68.02, H, 6.29, N, 4.16%.

2.3. *Deprotection of cyclopropanes **7***

To a solution of cyclopropanes **7** (1 mmol) in 8 ml of anhydrous methanol, was added a catalytic (20 mg) amount of sodium was added. The reaction was stirred for 2 h then neutralized by the addition of 200 mg of acidic Amberlite® IR-120 resin. The reaction mixture was filtered to remove the resin and the solvent was re-evaporated under reduced pressure to give a diastereo-isomeric mixture of **8** and **9**.

(1*R*,6*S*,1'*S*,2'*S*,3'*S*,4'*R*)-1-methyl-4,7,7-triphenyl-3-(3,4,5,6-tetrahydroxy-tetrahydropyran-2-ylmethyl)-3,4-diaza-bicyclo[4.1.0]heptane-2,5-dione (8a).

Yield: (62%), white solid, mp. 187–188 °C. $[\alpha]^{22}_D$ = +65 (*c* = 1, CH_2Cl_2), R_f = 0.5 (MeOH/H_2O 4:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04–6.73 (m, 15H, H_{arom}), 5.31 (d, $J_{H1'-2'}$ = 3.9, 1H, $H_{1'}$), 3.98–3.86 (m, 2H, $H_{2',4'}$), 4.11–4.03 (m, 1H, $H_{5'}$), 3.82 (dd, $J_{H3'-4'}$ = 3.4, $J_{H3'-2'}$ = 10.3, 1H, $H_{3'}$), 3.72–3.68 (m, 1H, $H_{6'}$), 3.62–3.58 (m, 1H, $H_{6'}$), 2.21 (s, 1H, H_6), 1.59 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 168.6, 137.3, 135.1, 128.7, 127.9, 126.9, 123.5, 120.6, 119.7, 118.3, 114.6, 113.4, 112.7, 98.1, 74.3, 73.4, 72.2, 71.0, 64.8, 40.9, 39.4, 35.6, 34.7, 28.6.HRMS: Calcd for $C_{30}H_{30}N_2O_7$ 530.2053. Found: 530.2044.

Anal. Calcd for $C_{30}H_{30}N_2O_7$: C, 67.91; H, 5.70; N, 5.28%. Found: C, 67.88; H, 5.77; N, 5.35.

(1*R*,6*S*,1'*S*,2'*R*,3'*S*,4'*R*)-1-methyl-4,7,7-triphenyl-3-(3,4,5,6-tetrahydroxytetrahydropyran-2-ylmethyl)-3,4-diaza-bicyclo[4.1.0]heptane-2,5-dione (9a).

Yield: (33%), white solid, mp. 161–162 °C. $[\alpha]^{22}_D$ = +51 (c = 1, CH_2Cl_2), R_f = 0.45 (MeOH/H_2O 4:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03–6.71 (m, 15H, H_{arom}), 5.29 (d, $J_{H1'-2'}$ = 8.1, 1H, $H_{1'}$), 3.91- 3.87 (m, 2H, $H_{2',4'}$), 4.09–4.11 (m, 1H, $H_{5'}$), 3.79 (dd, $J_{H3'-4'}$ =

3.3, $J_{H3'-2'}$ = 10.1, 1H, $H_{3'}$), 3.70–3.69 (m, 1H, $H_{6'}$), 3.61–3.57 (m, 1H, $H_{6'}$), 2.19 (s, 1H, H_6), 1.51 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3, 168.3, 138.4, 135.3, 127.9, 127.1, 125.22, 122.7, 121.3, 120.6, 118.4, 115.3, 113.3, 112.6, 97.5, 74.4, 73.7, 72.1, 71.2, 65.6, 41.3, 39.7, 34.3, 34.1, 27.4.HRMS Calcd for $C_{30}H_{30}N_2O_7$ 530.2053. Found: 530.2039.
Anal. Calcd for $C_{30}H_{30}N_2O_7$: C, 67.91; H, 5.70; N, 5.28%. Found: C, 67.90; H, 5.75; N, 5.30.

(1*R*,6*S*,1'*S*,2'*S*,3'*S*,4'*R*)-1-methyl-7-(4-nitro-phenyl)-7-(3-nitro-phenyl)-4-phenyl-3-(3,4,5, 6)-(3,4,5, 6-tetrahydroxy-tetrahydro-pyran-2-ylmethyl)-3,4-diaza-bicyclo[4.1.0}heptane-2,5-dione (8b).
Yield: (68%), white solid, mp. 197–198 °C. $[\alpha]^{22}_D$ = + 78 (c = 1, CH_2Cl_2), R_f = 0.7 (MeOH/H_2O 4:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64-6.83 (m, 13H, H_{arom}), 5.29 (d, $J_{H1'-2'}$ = 3.9, 1H, $H_{1'}$), 4.10-4.06 (m, 1H, $H_{5'}$), 3.97–3.85 (m, 2H, $H_{2',4'}$), 3.80 (dd, $J_{H3'-4'}$ = 3.4, $J_{H3'-2'}$ = 10.3, 1H, $H_{3'}$), 3.70–3.66 (m, 1H, $H_{6'}$), 3.60–3.55 (m, 1H, $H_{6'}$), 2.19 (s, 1H, H_6), 1.53 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 168.3, 141.8, 133.7, 128.5, 123.7, 118.7, 115.7, 114.5, 113.5, 112.7, 111.9, 110.9, 110.7, 98.4, 75.3, 74.4, 72.5, 71.6, 65.4, 42.1, 40.4, 36.7, 35.8, 28.7.HRMS Calcd for $C_{30}H_{28}N_4O_{11}$ 620.1755. Found: 620.1750.
Anal. Calcd for $C_{30}H_{28}N_4O_{11}$: C, 58.06, H, 4.55, N, 9.03%. Found: C, 58.00, H, 4.59, N, 9.13.

(1*R*,6*S*,1'*S*,2'*R*,3'*S*,4'*R*)-1-methyl-7-(4-nitro-phenyl)-7-(3-nitro-phenyl)-4-phenyl-3-(3,4,5,6–tetrahydroxytetrahydro-pyran-2-ylmethyl)-3,4-diaza-bicyclo[4.1.0]heptane-2,5-dione (9b)
Yield (17%), white solid. Mp = 171–172 °C. $[\alpha]^{22}_D$ = +60 (*c* = 1, CH_2Cl_2), R_f = 0.6 (MeOH/H_2O 4:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67-6.84 (m, 13H, H_{arom}), 5.27 (d, $J_{H1'-2'}$ = 8.1, 1H, $H_{1'}$), 4.12-4.08 (m, 1H, $H_{5'}$), 3.96-3.82 (m, 2H, $H_{2',4'}$), 3.78 (dd, $J_{H3'-4'}$ = 3.3, $J_{H3'-2'}$ = 10.1, 1H, $H_{3'}$), 3.67–3.61 (m, 1H, $H_{6'}$), 3.57–3.50 (m, 1H, $H_{6'}$), 2.10 (s, 1H, H_6), 1.48 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 169.0, 141.4, 132.2, 127.8, 124.1, 119.6, 115.6, 113.9, 113.2, 112.4, 112.1, 111.1, 110.6, 97.5, 74.9, 74.3, 71.4, 71.1, 66.6, 43.4, 41.4, 37.7, 36.3, 26.9.HRMS Calcd for $C_{30}H_{28}N_4O_{11}$ 620.1755. Found: 620.1747.
Anal. Calcd for $C_{30}H_{28}N_4O_{11}$: C, 58.06, H, 4.55, N, 9.03%. Found: C, 58.10, H, 4.58, N, 9.11.

(1*R*,6*S*,1'*S*,2'*S*,3'*S*,4'*R*)-7,7-bis-(4-methoxyphenyl)-1-methyl-4-phenyl-3-(3,4,5,6-tetrahydroxy-tetrahydro-pyran-2-ylmethyl)-3,4-diaza-bicyclo[4.1.0] heptane-2,5-dione (8c)
Yield (66%), white solid. Mp = 138–139 °C. $[\alpha]^{22}_D$ = +69 (*c* = 1, CH_2Cl_2), R_f = 0.5 (MeOH/H_2O 4:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–6.64 (m, 13H, H_{arom}), 5.28 (d, $J_{H1'-2'}$ = 3.9, 1H, $H_{1'}$), 4.09–4.01 (m, 1H, $H_{5'}$), 3.95-3.84 (m, 2H, $H_{2',4'}$), 3.81-3.76 (dd, $J_{H3'-4'}$ = 3.4, $J_{H3'-2'}$ = 10.3, 1H, $H_{3'}$), 3.70–3.67 (m, 1H, $H_{6'}$), 3.63–3.57 (m, 1H, $H_{6'}$), 2.18 (s, 1H, H_6), 1.51 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.3, 169.5, 159.7, 142.4, 133.5, 128.8, 120.4, 119.7, 118.9, 117.7, 115.9, 114.7, 114.5, 113.6, 99.0, 75.1, 73.7, 72.5, 72.0, 65.7, 55.5, 41.2, 40.1, 36.5, 34.6, 29.5.HRMS Calcd for $C_{32}H_{34}N_2O_9$ 590.2264. Found: 590.2255.
Anal. Calcd for $C_{32}H_{34}N_2O_9$: C, 65.07, H, 5.80, N, 4.74%. Found: C, 65.14, H, 5.85, N, 4.67%.

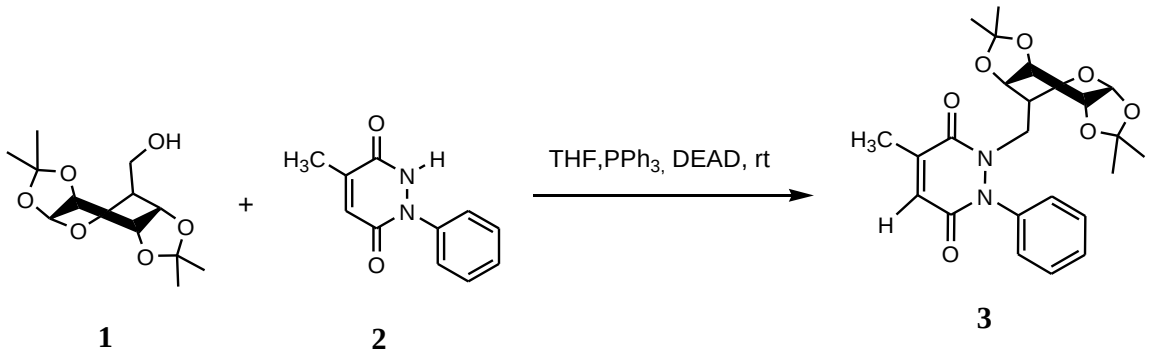
(1*R*,6*S*,1'*S*,2'*R*,3'*S*,4'*R*)7,7-bis-(4-methoxyphenyl)-1-methyl-4-phenyl-3-(3,4,5,6-tetrahydrox-tetrahydro-pyran-2-ylmethyl)-3,4-diaza-bicyclo[4.1.0] heptane-2,5-dione (9c)
Yield (28%), white solid. Mp = 141–142 °C. $[\alpha]^{22}_D$ = +34 (*c* = 1, CH_2Cl_2), R_f = 0.6 (MeOH/H_2O 4:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32–6.67 (m, 13H, H_{arom}), 5.23 (d, $J_{H1'-2'}$ = 8.1, 1H, $H_{1'}$), 4.09–4.01 (m, 1H, $H_{5'}$), 3.95–3.84 (m, 2H, $H_{2',4'}$), 3.80-3.75 (dd, $J_{H3'-4'}$ = 3.3, $J_{H3'-2'}$ = 10.1, 1H, $H_{3'}$), 3.69–3.65 (m, 1H, $H_{6'}$), 3.61–3.56 (m, 1H, $H_{6'}$), 2.19 (s, 1H, H_6), 1.54 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 168.4, 158.4, 140.3, 134.7, 127.1, 119.9, 119.2, 117.9, 117.4, 114.7, 114.7, 114.5, 113.8, 99.1, 75.6, 73.9, 72.4, 71.3, 66.8, 55.4, 41.3, 40.6, 35.9, 34.4, 27.7. HRMS Calcd for $C_{32}H_{34}N_2O_9$ 590.2264. Found: 590.2267.
Anal. Calcd for $C_{32}H_{34}N_2O_9$: C, 65.07, H, 5.80, N, 4.74%. Found: C, 65.11, H, 5.76, N, 4.77%.

2.4. *Antimicrobial activity screening*

Antimicrobial activity screening of the synthesized compounds **8** and **9** was performed by the agar dilution technique as recommended by the Clinical and Laboratory Standard Institute (CLSI) [21]. The tested compounds were dissolved in dimethyl sulfoxide (DMSO). An inoculum of about 1.5–108 colony forming unit (CFU) per spot was applied to the surfaces of Mueller–Hinton agar plates containing graded concentrations of the respective compound; plates were incubated at 37 °C for 18 h. The spot with the lowest concentration of compound showing no growth was defined as the minimum inhibitory concentration (MIC). All organisms used in this study were standard strains obtained from American Type Culture Collection (ATCC). The organisms included representatives of Gram-positive bacteria (*S. aureus* 25923 and *E. fecalis* 29212), Gram-negative bacteria (*E. coli* 25922 and *K. pneumoniae* 33495) and yeast (*C. albicans* 20260). The MIC of Ciprofloxacin and Amphotericin B was determined concurrently as reference for antibacterial activities, respectively (Table 2). Control DMSO was used with each experiment.

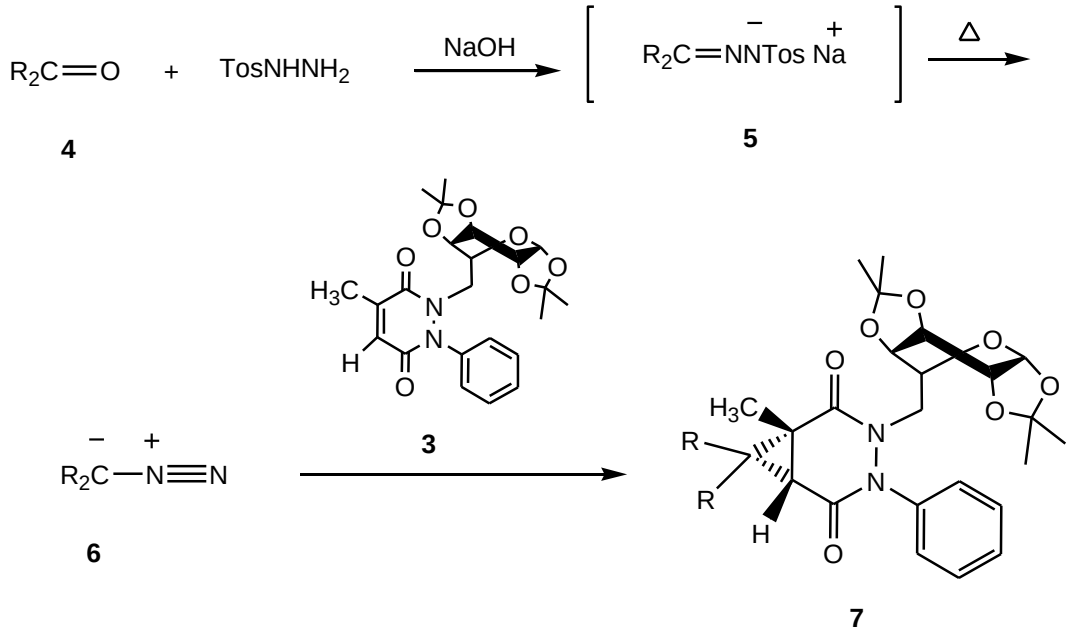
3. RESULTS AND DISCUSSION

The 4-methyl-1-phenyl-1,2-dihydropyridazine-3,6-dione derivative of D-(+)-galactose **3** was prepared according to literature procedures. Primary alcohol **1** was converted into 4-methyl-1-phenyl-1,2-dihydro-pyridazine-3,6-dione *N*-glycoside **3** in excellent yield (90%) by a Mitsunobu reaction with 4-methyl-1-phenyl-1,2-dihydro-pyridazine-3,6-dione **2** in the presence of triphenylphosphine and diethyl azodicarboxylate (Scheme 1).



Scheme 1. Substrate scope for the synthesis of glycol-pyradizine-3,6-dione **3**

Since preliminary experiments showed that diazoalkanes could also be generated *in situ* from ketones **4** in a one-pot process, condensation of *p*-tosylhydrazine with ketones followed by treatment with an aqueous solution of sodium hydroxide led to a solution of ketone tosylhydrazones sodium salt **5**, which upon warming to 50 °C gave a solution of aryldiazomethanes **6**, as heralded by formation of the characteristic deep red color [21]. For the purpose of the present investigation, 4-methyl-1-phenyl-1,2-dihydro-pyridazine-3,6-dione derivative of D-(+)-galactose **3** was added prior to warming the reaction mixture to provide the desired cyclopropanes **7** in good yields (Scheme 2).

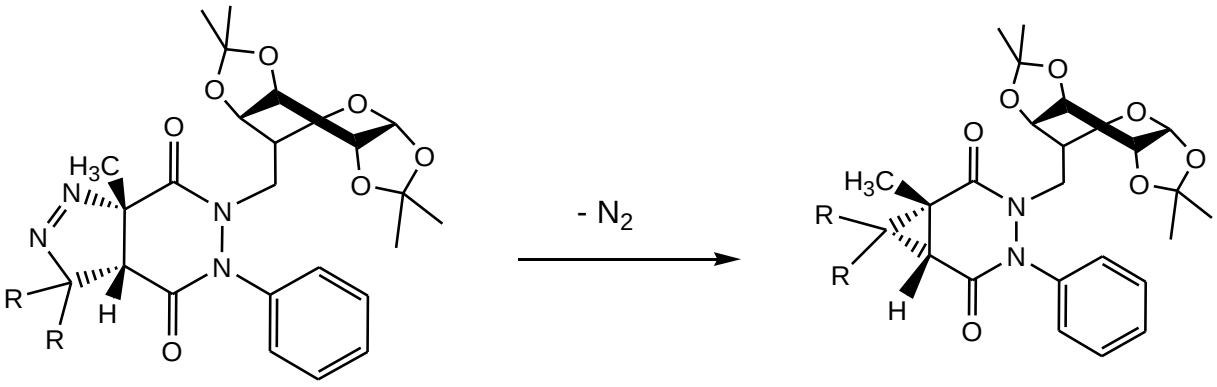


a) R = C_6H_5 b) R = p-$NO_2C_6H_4$ c) R = p-$MeOC_6H_4$

Scheme 2. Proposed synthesis of cyclopropanes using diazo compounds generated *in situ*

The intermediate of this reaction is pyrazoline which was not separated. It was found that thermal 1,3-dipolar cycloaddition followed by nitrogen extrusion gave cyclopropane [22].

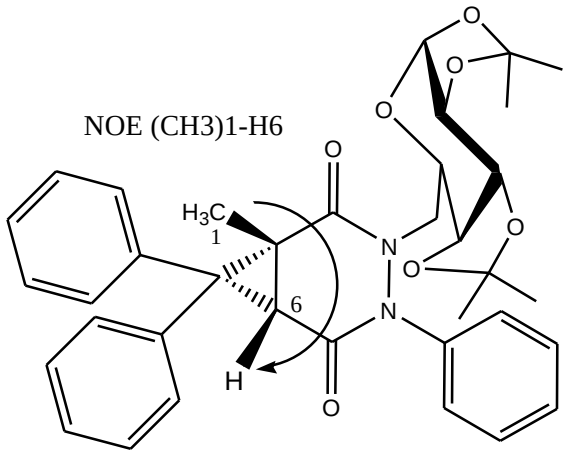
The 1,3-dipolar cycloaddition of aryldiazomethanes **6** with 4-methyl-1-phenyl-1,2-dihydropyridazine-3,6-dione derivative of D-(+)-galactose **3** was completely stereoselective; a *syn* configuration was assigned to all cyclopropanes.



intermediate pyrazoline

Scheme 3. Decomposition of the pyrazoline intermediate

The structure and stereochemistry were unambiguously established by NMR 2D. Crucial evidence for the stereochemistry was obtained by nuclear Overhauser effect spectroscopy (NOESY). The spectrum shows that protons between $(CH_3)_1$ and H_6 have strong nuclear Overhauser effect. This results confirms the *cis* relationships between protons $(CH_3)_1$ and H_6. Accordingly, the stereochemistry of **7a** was concluded (Fig. 1).



7a

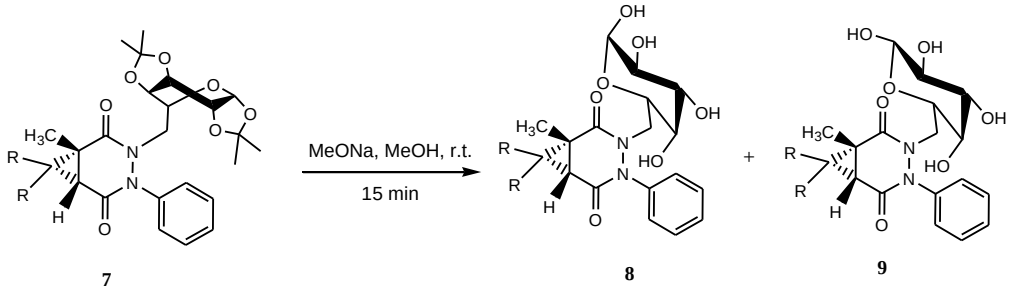
Fig. 1. 1H NOESY NMR

The choice of the protective group was important to the outcome of the cycloaddition and for the deprotection of the adducts. Deprotection of **7** by basic hydrolysis followed by acidic treatment [23] provided the known water soluble carbohydrates **8** and **9** in good yields (Table 1).

Antimicrobial activity screening of the synthesized compounds **8** and **9** was determined by the agar dilution technique, as recommended by the Clinical and Laboratory Standard Institute (CLSI) [24], utilizing a variety of Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus fecalis*), Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) and yeast (*Candida albicans*). From the obtained results (Table 1) it has been noticed that all of the tested compounds exhibit promising antimicrobial properties against both Gram-positive and Gram-negative bacteria. However, all of the tested compounds seem completely inactive against the used yeast strain (*C. albicans*). It has also generally been noticed that compounds **9** are more effective antibacterial agents than compounds **8**. Also, compounds **9b** and **9c** seem to be the most effectively prepared Gram-negative antibacterial agents. This may be attributed to the role of anisyl function attachment to cyclopropane ring system as the most observed enhancing antibacterial properties moiety comparable with the other adopted residues.

T a b l e 1

*Synthesis of cyclopropane derivatives **8** and **9***



7

a: R = Ph
b: R = p-NO_2Ph
c: R= p-OCH_3Ph

8

a: R = Ph
b: R = p-NO_2Ph
c: R= p-OCH_3Ph

9

Entry	Cyclopropane **8/9**	Ratio **8/9**[a]	yield (%)[b]
1	8a/9a	65/35	95
2	8b/9b	80/20	85
3	8c/9c	70/30	95

[a]Relative proportion determined by ^{1}H-NMR of the reaction crude.
[b]Combined yield after column chromatography.

T a b l e 2

Minimum inhibitory concentrations (MIC, μg/ml) of the tested compounds against different organisms

Compounds	**8a**	**8b**	**8c**	**9a**	**9b**	**9c**	CIP	AMP
Organisms								
S.a.	10	10	10	5	0.9	0.7	0.3	(–)
E.f.	5	10	10	2.5	4	0.6	0.3	(–)
E.c.	10	10	5	2.5	0.5	1.5	0.3	(–)
K.p	2.5	5	10	2	1.7	2.5	1.3	(–)
C.a	(–)	(–)	(–)	(–)	(–)	(–)	(–)	4

CIP = Ciprofloxacin; AMP = Amphotericin B; (–) = inactive

4. CONCLUSION

In conclusion, we have developed a simple preparation of glycol-cyclopropanes from a carbohydrate-derived precursors *via* a 1,3-dipolar cycloaddition reaction of aromatic diazo compounds generated *in situ* in a one-pot sequence from the ketones and tosylhydrazine. To the best of our knowledge, this is the first report of the synthesis of sugar-fused cyclopropanes heterocycles with an attached pyridazine-3,6-dione core by means of 1,3-dipolar cyclo-addition of aromatic diazoalkanes. Cleavage of the ketal protecting groups provided water-soluble cyclopropane-bearing carbohydrates **8** and **9**. These compounds were tested for antimicrobial properties against both Gram-positive and Gram-negative bacteria. From these studies, compounds **9b** and **9c** emerged as the lead compounds, which showed maximum antimicrobial activity. Thus, compound **9c** represents a fruitful matrix for the development of a new class of antimicrobial agent that deserves further investigation and derivatization.

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