Synthesis and preliminary biological evaluation of carba analogues from *Neisseria meningitidis* A capsular polysaccharide

Qi Gao,\textsuperscript{a} Cristina Zaccaria,\textsuperscript{a} Marta Tontini,\textsuperscript{b} Laura Poletti,\textsuperscript{a} Paolo Costantino,\textsuperscript{b} and Luigi Lay\textsuperscript{a}\textsuperscript{*}

\textsuperscript{a}Dipartimento di Chimica Organica e Industriale and ISTM-CNR, Università degli Studi di Milano, via Venezian, 21 I-20133 Milano, Italy

\textsuperscript{b}Novartis Vaccines & Diagnostics, Vaccine Chemistry Department, Via Fiorentina 1, 51300 Siena, Italy.

Supplementary Information

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Synthesis of 1,5-Anhydro-di-O-benzyl-2,6,7-trideoxy-D-arabino-hept-1,6-dienitol (6). Freshly prepared 2-iodoxybenzoic acid\(^1\) (IBX, 89 g, 319 mmol) was added to a solution of 5 (12.3 g, 38 mmol), prepared from glucal 4 according to the procedure reported in the literature,\(^2\) in dry EtOAc (400 mL), and the suspension was stirred under nitrogen at 75 °C for 4 h. The mixture was then cooled, filtered over a Celite pad and concentrated. The crude was co-evaporated with toluene (3 × 50 mL) to obtain the aldehyde intermediate (12.3 g, 99%). NMR analysis showed the complete conversion of 5 into the aldehyde intermediate.

Freshly prepared PPh\(_3\)CH\(_3\)I (23.3 g, 57 mmol) was dissolved in dry THF (40 mL), the solution was cooled to -78 °C, then KHMDS (1M solution in THF, 57 mL, 57 mmol) was added under nitrogen. The mixture was stirred at -78 °C for 30 minutes, then another 1 h at 0 °C. A solution of the aldehyde (12.3 g, 38 mmol) in dry THF was added to the mixture at -78 °C. The reaction mixture was then stirred at room temperature. After 3 h, a saturated aqueous solution of NH\(_4\)Cl (200 mL) was added and the mixture was stirred for 10 min, then diluted with CH\(_2\)Cl\(_2\) (300 mL) and the organic layer was washed with brine, dried (Na\(_2\)SO\(_4\)), filtered, and concentrated. The crude was purified by flash chromatography (toluene:hexane 40:60) providing 6 (9.4 g, 77 %) as a clear oil. The optical rotation and the spectroscopic characterization data of compound 6 were in agreement with those previously reported.\(^3\)

Synthesis of (3R,4R,5R)-3,4-dibenzyloxy-5-(hydroxymethyl)cyclohexene (7). Compound 6 (7.5 g, 23.3 mol) was dissolved in 1,6-dichlorobenzene (25 mL) in a sealed tube and heated at 240 °C for 2 h. After cooling down, the mixture was slowly poured into a suspension of NaBH\(_4\) (500 mg, 13 mmol) in THF (100 mL) and EtOH (25 mL) and stirred for 15 min. Then water (200 mL) was added, and the mixture was extracted with CH\(_2\)Cl\(_2\) (3×100 mL). The organic layer was washed with

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brine (200 mL), dried (Na₂SO₄), filtered, and concentrated. The crude was purified by flash chromatography (EtOAc:hexane 20:80 → 50:50), providing 7 (6.5 g, 86 %) as a clear oil. The optical rotation and the spectroscopic characterization data of compound 7 were in agreement with those previously reported.³

**Synthesis of 3,4-Di-O-benzyl-5a-carba-α-D-glucopyranose (8).** Compound 7 (6.5 g, 20.8 mmol) was dissolved in a mixture of acetone (75 mL) and H₂O (25 mL), then a solution of OsO₄ (CAUTION!) (250 mg in 4.5 mL H₂O and 18 mL acetone) was added at room temperature, followed by Me₃NO (5.075 g, 46 mmol), and stirring was continued for 48 h at room temperature. A saturated aqueous solution of Na₂S₂O₃ (50 mL) was added and stirred for 30 min to reduce the OsO₄, then chloroform (300 mL) was added and the organic layer was washed with brine (200 mL), dried (Na₂SO₄), filtered, and concentrated. The crude was purified by flash chromatography (MeOH:CH₂Cl₂ 10:90), providing 8 (6.23 g, 86.3%) as a white solid. The optical rotation and the spectroscopic characterization data of triol 8 were in agreement with those previously reported.⁴

**Synthesis of 1-O-Acetyl-3,4-di-O-benzyl-6-O-thexyldimethylsilyl-5a-carba-α-D-glucopyranose (9).** To a solution of 8 (8.1 g, 22.6 mmol) and imidazole (4.6 g, 68 mmol) in THF (200 mL), thexyldimethylsilyl chloride (9.8 mL, 52 mmol) was added dropwise at 15 °C. The mixture was stirred at room temperature for 24 h, then a saturated aqueous solution of NaHCO₃ (100 mL) was added, followed by extraction with EtOAc (3 × 150 mL). The combined organic phases were dried (Na₂SO₄), filtered, and concentrated. The crude was purified by flash chromatography (EtOAc:hexane 30:70), yielding the 6-O-silylated intermediate (10.5 g, 93 %) as a colourless oil. The O-silylated intermediate was dissolved in dry CH₃CN (150 mL) under nitrogen. Trimethyl orthoacetate (6.25 mL, 50 mmol) was added at room temperature, followed by a catalytic amount of

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\( p \)-toluenesulfonic acid. After 15 min, a 80% aqueous solution of acetic acid (150 mL) was added and stirring was continued for 15 min. CH\(_2\)Cl\(_2\) (200 mL) was added and the organic layer was washed with H\(_2\)O (200 mL) and a saturated aqueous solution of NaHCO\(_3\) (200 mL), dried (Na\(_2\)SO\(_4\)), filtered, and concentrated. The crude residue was purified by flash chromatography (EtOAc:hexane 25:75), providing 9 (10.45 g, 91%) as a clear oil. The optical rotation and the spectroscopic characterization data of alcohol 9 were in agreement with those previously reported.\(^4\)

**Synthesis of 1-O-Acetyl-2-azido-3,4-di-O-benzyl-2-deoxy-6-O-cteyldimethylsilyl-5a-carba-\( \alpha \)-D-mannopyranose (10).** Alcohol 9 (10.3 g, 19 mmol) was dissolved in a 5:1 mixture of CH\(_2\)Cl\(_2\)-Pyridine (360 mL), then trifluoromethanesulfonic anhydride (17 mL, 104 mmol) was added dropwise at -10 °C. The mixture was stirred at 0 °C for 60 min, then a saturated aqueous solution of NaHCO\(_3\) (150 mL) was added, and the organic layer was washed with brine (200 mL), dried (Na\(_2\)SO\(_4\)), filtered, and concentrated. The residue was then dissolved in a 19:1 mixture of DMF-H\(_2\)O (100 mL), and NaN\(_3\) (6.2 g, 95 mmol) was added. The reaction mixture was stirred overnight at 40 °C, then the solvent was evaporated and the crude residue was purified by flash chromatography (EtOAc:toluene 2:98), giving 10 (8.5 g, 79%) as an oil. The optical rotation and the spectroscopic characterization data of compound 10 were in agreement with those previously reported.\(^4\)

**Synthesis of 2-Azetamido-1-O-acetyl-3,4-di-O-benzyl-2-deoxy-6-O-thxyldimethylsilyl-5a-carba-\( \alpha \)-D-mannopyranose (11).** A mixture of 10 (9.1 g, 16 mmol) and freshly crystallized PPh\(_3\) (12.6 g, 48 mmol) in dry THF (250 mL) was stirred overnight at 60 °C under nitrogen atmosphere. After addition of water (40 mL), the reaction was stirred for further 24 h at the same temperature, then the solvent was evaporated. The residue was dissolved in MeOH (200 mL) and acetic anhydride (30 mL, 320 mmol) was added. After 24 h the solvent was evaporated and the crude
material was purified by flash chromatography (EtOAc:hexane 30:70), providing 11 (8.86 g, 95%) as an oil. The optical rotation and the spectroscopic characterization data of compound 11 were in agreement with those previously reported.\textsuperscript{4}
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 6
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 7
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 8
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 9
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 10
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 11
^1H NMR (400 MHz, CDCl₃) spectrum of compound 12
$^{13}$C NMR (100.6 MHz, CDCl$_3$) spectrum of compound 12
$^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 17
$^{13}$C NMR (100.6 MHz, CDCl$_3$) spectrum of compound 17
$^{31}$P NMR (162 MHz, CDCl$_3$) spectrum of compound 17
\(^1\)H NMR (400 MHz, CDCl\(_3\)) spectrum of compound 18
$^{13}$C NMR (100.6 MHz, CDCl$_3$) spectrum of compound 18
$^{31}$P NMR (162 MHz, CDCl$_3$) spectrum of compound 18
$^1$H NMR (400 MHz, CD$_3$OD, T = 313 K) spectrum of compound 19

[Chemical structure image]

S20
$^{13}$C NMR (100.6 MHz, CD$_3$OD, T = 313 K) spectrum of compound 19
$^{31}$P NMR (162 MHz, CD$_3$OD) spectrum of compound 19
$^1$H NMR (400 MHz, CD$_3$OD, $T = 313$ K) spectrum of compound 20
\[^{13}\text{C} \text{NMR} (100.6 \text{ MHz, CD}_3\text{OD, } T = 313 \text{ K}) \text{ spectrum of compound 20} \]
$^{31}$P NMR (162 MHz, CD$_3$OD, T = 313 K) spectrum of compound 20
$^1$H NMR (400 MHz, CD$_3$OD) spectrum of compound 21
¹³C NMR (100.6 MHz, CD₃OD) spectrum of compound 21
$^{31}$P NMR (162 MHz, CD$_3$OD) spectrum of compound 21
$^1$H NMR (400 MHz, D$_2$O, T = 313 K) spectrum of compound 1
$^{13}$C NMR (100.6 MHz, D$_2$O, T = 313 K) spectrum of compound 1
$^{31}$P NMR (162 MHz, D$_2$O, T = 313 K) spectrum of compound 1
$^1$H NMR (400 MHz, D$_2$O, T = 313 K) spectrum of compound 2
$^{13}$C NMR (100.6 MHz, D$_2$O, T = 313 K) spectrum of compound 2
$^{31}$P NMR (162 MHz, D$_2$O, T = 313 K) spectrum of compound 2
$^1$H NMR (400 MHz, D$_2$O, T = 313 K) spectrum of compound 3
$^{13}$C NMR (100.6 MHz, D$_2$O, T = 308 K) spectrum of compound 3
$^{31}$P NMR (162 MHz, D$_2$O, T = 308 K) spectrum of compound 3