International Journal of Scientific and Technical Advancements
ISSN: 2454-1532

O-Glycopeptides: Pd(0) Catalyzed Stereoselective β-*O*-Glycosidation of Serine and Threonine, Enroute to Generation of New Class of Glycopeptides

Anita Brar, Y. D. Vankar

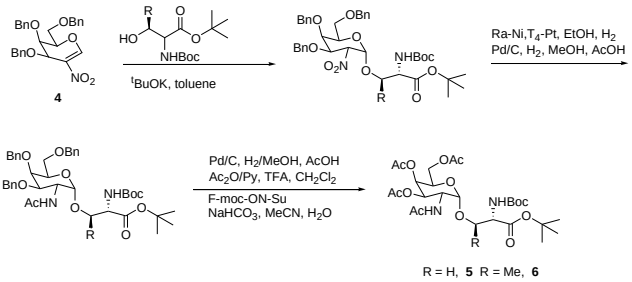
I. INTRODUCTION

Glycobiology as an area of immense importance in medicinal and biological chemistry is well known[1] nowadays. Among various glyconjugates, the glycopeptides,[2] the characteristic partial structures of the glycoproteins, have gained importance in bio-organic and medicinal research due to involvement in various cellular, biological and pathological process. Glycopeptides plays an important role in number of cellular recognition events which includes cell adhesion, cell growth regulation, cancer cell metastasis, cell differentiation, chemotaxis,[3] immunosurveillance and inflammation. It also increases proteolytic stability,[4] bioavailability,[5] improves water solubility,[6] confomational change of protein backbone and promotes blood brain barrier permeability.[7] Changes in such cellular event are responsible for various diseases such as cancer, rheumatoid arthritis.

Two types of glycosidic linkages exist in natural glycoproteins. One involves either oxygen in the side chain of serine and threonine, the so called *O*-glycoproteins. The other involves the nitrogen of the side chain of asparagines called as *N*-glycoproteins. The *O*-glycoprotein is often found in nuclear and cytoplasmic proteins. Oxygen linked glycosylation is one of the primary modes for the attachment of glycosides to proteins.

Approaches towards the synthesis of glycopeptides have gained considerable interest due to their involvement in various cellular and biological processes. The synthesis of *O*-glycoprotiens[8] is difficult due to the acid lability of the glycosidic bond and base lability of the amino acid residue. Also,the poor reactivity of the N-acylated (eg. Boc, Cbz protection) serine and threonine substrates require harsh reaction conditions for their synthesis. As a result, synthesis of *O*- and *N*- glycopeptides is an area of intense study.[9]

Schmidt et al,[10] reported the synthesis of N-Fmoc-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-L-serine **5** and **6**, valuable building blocks for *O*-glycopeptide synthesis, by using Michael addition of protected serine and threonine derivatives to 3,4,6-tri-*O*-benzyl-2-nitro-α-D-galactopyranosides **4** in good yields and stereoselectivities (Scheme 1).

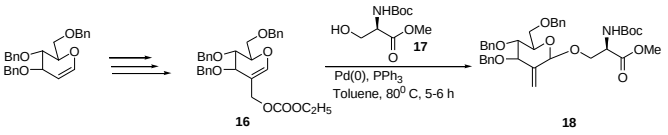


II. RESULTS AND DISCUSSION

Most of the natural glycoproteins have β-linkage of the peptide motif at the anomeric centre, it will also be of interest to develop methods for the synthesis of β-linked *O*-glycopeptides. But, as discussed in the introduction part, the synthesis of *O*-glycoprotein is complicated. So, an alternative procedure for developing *O*-glycosidic bond, we planned to make use of 3, 4, 6-tri-*O*-benzylated glucal allylic carbonate 16 as glycosyl donor.

Under the given palladium-catalyzed reaction conditions, glycosylation of serine and threonine derivatives with the glycosyl donor results in the formation of new class of 3, 4, 6-tri-*O*-benzyl-2-deoxy-2-exomethelene-β-glycopeptides in good yields.

Compound **16,** obtained from tribenzylated glucal by the known literature procedure, on treatment with NHBoc-serine ester **17** in presence of palladium(0) in toluene at 80^0 C formed 3,4,6-tri-*O*-benzyl-2-deoxy-2-exomethylene-β-glycopeptide **18** in a good yield and β-stereoselectivity (scheme 6).



SCHEME 6

However, when the same reaction was carried out in THF solvent, the reaction doesn't occur. The above reaction afforded a mixture of α and β-*O*-glycoside in 1:10 ratio and 60% of overall yield. Unfortunately, the two anomers (α and β) could not be separated by column chromatography. The composition of the mixture was derived on the basis of its spectral data.

However, glycosylation of NHBoc-threonine ester **19** with compound **16** under palladium (0) catalyzed conditions in

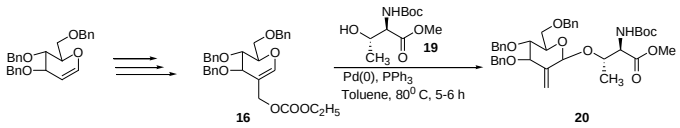
Anita Brar and Y. D. Vankar, "O-Glycopeptides: Pd(0) catalyzed stereoselective β-*O*-glycosidation of serine and threonine, enroute to generation of new class of glycopeptides," *International Journal of Scientific and Technical Advancements*, Volume 2, Issue 4, pp. 231-234, 2016.

International Journal of Scientific and Technical Advancements
ISSN: 2454-1532

toluene for 5-6 h resulted in the formation of a mixture of and -*O*-glycoside **20** in 1:8 ratio with 59% of overall yield (Scheme 7).

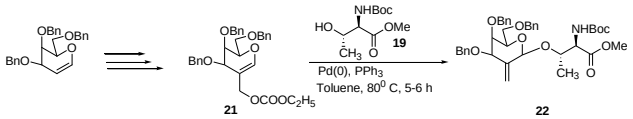
^{1}H NMR spectrum of compound **20** showed a singlet at δ 1.47 for nine protons of –Boc group. Anomeric proton for the β-anomer exist as a singlet at δ 5.11, while for the α-anomer the proton exist as singlet at δ 5.13. The two exomethylene exist as singlets at δ 5.00 and at δ 5.21. The chemical shift of various protons were assigned by cosy data. nOe and NOESY data of the compound permitted the absolute stereochemistry of the compound.

In the nOe experiment, irradiation of the peak at δ 5.12 for C-1 proton led to the enhancement of the peaks for H-5 and one of the two exomethylene protons. In addition to this, irradiation of peaks at δ 4.32 for H-5 peak led to the enhancement of peak for H-1 and H-3 protons. The data favour the structure of compound **20**. Its IR spectrum showed a strong absorption peak at ν_{max} 1718 cm^{-1} for the ester group. The mass spectrum of the molecule shows the molecular ion peak at m/z 684 (M + Na),$^+$ confirming the formation of molecule **20**.



SCHEME 7

Similarly, 3,4,6-tri-*O*-benzylated galactal allylic carbonate **21** was prepared from tribenzylated galactal by the known literature procedure. Boc protected threonine add to compound **21** in palladium (0) catalyzed condition and in toluene results in the formation of mixture of α and β-*O*-galactoside **22** in 1:10 ratio and overall yield of 66% (Scheme 8).

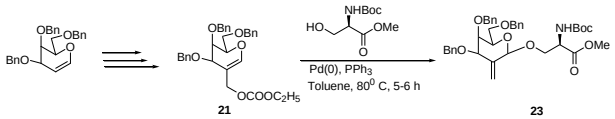


SCHEME 8

The composition of the mixture was derived on the basis of its spectral data. ^{1}H NMR spectrum of the compound **22** showed a singlet at δ 1.46 for nine protons of Boc group. Anomeric proton appeared at δ 5.13 for the β anomer where as the α-anomeric proton appeared at δ 5.14. The exomethylene protons appeared at δ 5.10 and δ 5.34 for both α and β anomeric forms. The actual position of various protons were assigned on the basis of COSY spectrum. NOESY experiment of the compound permitted the absolute stereochemical assignment.

The NOESY data of the compound **22** shows that H-1 and H-5 as well as H-1 and H-3 are *cis* to each other. The mass spectrum of the compound showed the molecular ion peak at m/z 684 [M + Na]$^+$ which corresponds to the molecular weight (661) of compound **22**. In its IR spectrum strong absorption at ν_{max} 1717 cm^{-1} established the presence of ester group. This spectral data clearly established the structure of compound **22**.

In the similar fashion, Boc protected serine derivative on glycosylation with compound **21** forms exclusively β-*O*-glycosylation with compound **21** forms exclusively β-*O*-galactoside **23** in 68% yield (Scheme 9).



SCHEME 9

The ^{1}H NMR spectrum of the compound **23** showed a singlet corresponding to 9H at δ 1.43 representing ter-butyloxy carbonyl protons (-Boc). A singlet appeared at δ 5.12 for the anomeric proton. The two exomethylene protons exist at δ 5.20 and 5.39 as two singlets. Further, the formation of compound **23** as a single anomer was supported by its ^{13}C spectral data. The COSY data assigned the chemical shift of various protons followed which the n*O*e experiments permitted the absolute stereochemical assignments.

In the n*O*e experiment, irradiation of the peak at δ 5.12 for anomeric proton led to the enhancement of peaks for H-3, H-5 as well as one of the two exomethylene protons. In addition to this, irradiation of H-5 at δ 3.96 led to the enhancement of peaks for H-1, H-3 and H-4. This data favor the structure as indicated in figure 4.

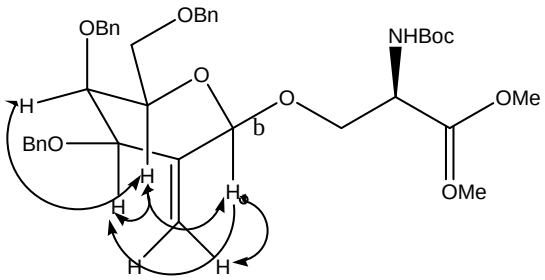


Fig. 4. nOe correlations for compound 23.

Its IR spectrum showed a strong absorption at ν_{max} 1716 cm^{-1} and its mass spectrum indicated the molecular ion peak at m/z 670 [M + Na]$^+$.

Thus, a straightforward, versatile and high yielding method has been developed for the synthesis of a novel class of *O*-glycopeptides with an exomethylene group at C-2 position, which are of tremendous biological and medicinal importance.

III. EXPERIMENTAL SECTION

General:

All the experiments were performed in oven dried glass apparatus and under nitrogen atmosphere. Commercial grade solvents were distilled before use. Dichloromethane was dried with activated $CaCl_2$ and distilled over CaH_2. $BF_3.Et_2O$ was distilled over P_2O_5 under vacuum. Toluene was dried over anhydrous $CaCl_2$ and stored over sodium wire.

Anita Brar and Y. D. Vankar, "O-Glycopeptides: Pd(0) catalyzed stereoselective β-*O*-glycosidation of serine and threonine, enroute to generation of new class of glycopeptides," *International Journal of Scientific and Technical Advancements*, Volume 2, Issue 4, pp. 231-234, 2016.

NHBoc-serine and threonine esters were purchased from Lancaster synthesis Ltd., (U.K). Triphenyl phosphine was recrystallized before use.

Thin layer chromatography was performed on prepared thin layers of Acme silicagel on microscopic slides or precoated plates (E-Merck, Germany). The visualization of spots on TLC plates was effected by exposure to iodine and spraying with 10% H_2SO_4 followed by charring. Column chromatography was performed over Acme silica gel (100-200 mesh) using hexane and ethyl acetate as eluent.

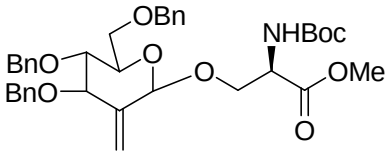
Infrared spectra were recorded on Bruker Vector 22 FT-IR spectrometer. The rotation values were recorded on Autopot II automatic polarimeter at the wavelength of the sodium D-line (589 nm) at 25 ^oC.

^1H, ^{13}C, COSY, NOESY spectra were recorded on JEOL JNM-LA 400 spectrometer in the solution of $CDCl_3$ using tetramethylsilane as the internal standard. Chemical shifts are reported in ppm downfield to tetramethylsilane. Coupling constants are reported and expressed in Hz, splitting patterns are designated as br (broad), s (singlet), d (doublet), dd (double doublet), ddd (doublet of a doublet of a doublet), q (quartet), dt (doublet of a triplet), m (multiplet). Elemental analyses (C, H, N, S and O) were done using thermoquest CE-instruments EA-1110 automatic elemental analyzer. The mass spectra were recorded on a microspcopic II triple quadrupole mass spectrometer using electrospray technique. Melting points were determined using a PEW (Pathak Electrical Works, Bombay, India) melting point apparatus.

General procedure for O-glycosylation

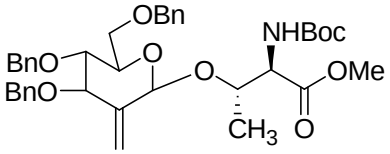
To a stirred solution of tribenzylated glucal or galactal allylic ester **16** or **21** (70 mg, 0.135 mmol) in dry toluene (2 mL) at room temperature were added serine ester **17** (27.7 mg, 1 mmol) or threonine ester **19** (29.6 mmol), triphenyl phosphine (3.54 mg, 10 mol%) and tetrakis (triphenylphosphine) palladium(0) (prepared in situ). After 10 min, the reaction mixture turned green. The reaction mixture was heated to a temperature of 80 ^oC for 5-6 h. After completion of reaction (TLC monitoring), the mixture was filtered on the pad of celite. The filtrate was evaporated and extracted with ethyl acetate (2 x 20 mL). The organic layer was washed with water, brine and then dried over anhydrous Na_2SO_4. The solvent was evaporated and the resulting residue was purified by column chromatography to give the glycosylated products (**18, 20, 22, 23**).

N-tert-butyloxycarbonyl-O-(3,4,6-tri-O-benzyl-2-deoxy-2-exomethylene-β-D-glucopyranosyl)-L-serine methyl ester (18):



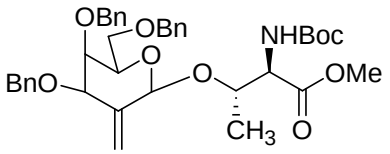
R_f = 0.40 (7:3, hexane:EtOAc); Yield = 60%; $[\alpha]_D^{25}$ = + 22.9 (*c* 2.05, $CHCl_3$); IR (CH_2Cl_2) ν_{max} = 1714cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ:1.41 (s, 9H), 3.57-3.92 (m, 6H), 3.70 (s, 3H), 4.28-4.30 (d, *J* = 8.8 Hz, 1H), 4.43-4.47 (dd, $J_{1,2}$ = 4.3 Hz, $J_{1,3}$ = 12.2 Hz, 3H), 4.52-4.83 (m, 3H), 5.07 (s, 2H), 5.25 (s, 1H), 5.43-5.45 (d, *J* = 8.5 Hz, 1H), 7.10-7.36 (m, 15H); ^{13}C NMR (100 MHz; $CDCl_3$) δ: 28.2, 52.4, 68.5, 72.0, 73.3, 73.4, 74.8, 79.6, 80.6, 102.1, 111.3, 127.6-128.4, 138.1, 141.7, 155.4, 170.8. MS (ESI): m/z = 670 $(M + Na)^+$; Anal. Calcd. for $C_{35}H_{45}NO_9$ (647.31): C, 68.61; H, 7.00; N, 2.16 % Found: C, 68.59; H, 6.99; N, 2.14 %

N-tert-butyloxycarbonyl-O-(3,4,6-tri-O-benzyl-2-deoxy-2-exomethylene-β-D-glucopyranosyl)-L-threonine methyl ester (20):



R_f = 0.55 (8:2, hexane:EtOAc); Yield = 59%; $[\alpha]_D^{25}$ = + 25.8(*c* 2.05, $CHCl_3$); IR (CH_2Cl_2) ν_{max} = 1718cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ:1.25-1.29 (m, 3H), 1.44-1.47 (s, 9H), 1.62 (br s, 1H), 3.57-3.73 (m, 2H), 3.71 (s, 3H), 3.94-3.97 (qd, 1H), 4.30-4.38 (m, 3H), 4.45-4.56 (m, 2H), 4.59-4.86 (m, 4H), 5.00 (br s, 1H), 5.11-5.15 (br s, 2H), 5.21 (br s, 1H), 7.13-7.39 (m, 15H); ^{13}C NMR (100 MHz; $CDCl_3$) δ:18.4, 28.3, 52.53, 58.3, 68.6, 71.6, 73.4, 74.9, 75.7, 79.7, 80.7, 102.8, 110.5, 127.6-128.4, 138.1, 142.0, 156.0, 171.3; MS (ESI): m/z = 684 $(M + Na)^+$; Anal. Calcd. for $C_{38}H_{47}NO_9$ (661.33): C, 68.97; H, 7.16; N, 2.12 % Found: C, 68.94; H, 7.14; N, 2.10 %

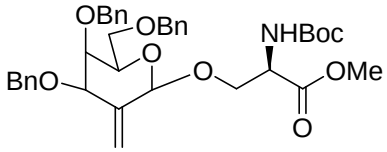
N-tert-butyloxycarbonyl-O-(3,4,6-tri-O-benzyl-2-deoxy-2-exomethylene-β-D-galactopyranosyl)-L-threonine methyl ester (22):



R_f = 0.55 (8:2, hexane:EtOAc);Yield = 66%; $[\alpha]_D^{25}$ = + 32.8 (*c* 1.25, $CHCl_3$); IR (CH_2Cl_2) ν_{max} = 1717cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.26-1.27 (d, *J* = 6.3 Hz, 3H), 1.46 (s, 9H), 3.52-3.53 (d, *J* = 6.3 Hz, 2H), 3.71 (s, 3H), 3.94-3.95 (br s, 1H), 4.08-4.12 (t, $J_{1,2}$ = 6.3, $J_{1,3}$ = 12.6 Hz, 1H), 4.29-4.48 (m, 5H), 4.59-4.71 (m, 3H), 4.86-4.89 (d, *J* = 11.9 Hz, 1H), 5.10 (s, 1H), 5.13-5.14 (s, 1H), 5.16 (s, 1H), 5.34 (s, 1H), 7.20-7.38 (m, 15H); ^{13}C NMR (100 MHz; $CDCl_3$) δ: 18.3, 28.2, 52.2, 58.2, 69.2, 71.1, 71.4, 73.4, 73.9, 75.1, 77.7, 79.9, 102.7, 111.3, 127.1-128.3, 138.2, 138.5, 140.4, 155.9, 171.3; MS (ESI): m/z = 684 $(M + Na)^+$; Anal. Calcd. for $C_{38}H_{47}NO_9$ (661.33): C, 68.97; H, 7.16; N, 2.12 % Found: C, 68.94; H, 7.14; N, 2.10 %

N-tert-butyloxycarbonyl-O-(3,4,6-tri-O-benzyl-2-deoxy-2-exomethylene-β-D-galactopyranosyl)-L-serine methyl ester (23):

Anita Brar and Y. D. Vankar, "O-Glycopeptides: Pd(0) catalyzed stereoselective β-*O*-glycosidation of serine and threonine, enroute to generation of new class of glycopeptides," *International Journal of Scientific and Technical Advancements*, Volume 2, Issue 4, pp. 231-234, 2016.



R_f = 0.55 (8:2, hexane:EtOAc); Yield = 68%; $[\alpha]_D^{25}$ = +12.2 (*c* 2.95, $CHCl_3$); IR (CH_2Cl_2) ν_{max} = 1716 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.43 (s, 9H), 3.52-3.56 (m, 2H), 3.69 (s, 3H), 3.82-3.85 (dd, $J_{1,2}$ = 2.9 Hz, $J_{1,3}$ = 10.4 Hz, 1H), 3.96 (br s, 2H), 4.06-4.09 (t, $J_{1,2}$ = 6.6 Hz, $J_{1,3}$ = 12.9 Hz, 1H), 4.31 (br s, 1H), 4.38-4.70 (m, 6H), 4.86-4.89 (d, $J_{1,2}$ = 11.6 Hz, 1H), 5.12 (s, 1H), 5.20 (s, 1H), 5.39 (s, 1H), 5.50-5.52 (d, *J* = 8.8 Hz, 1H), 7.21-7.36 (m, 15 H); ^{13}C NMR (100 MHz; $CDCl_3$) δ: 28.2, 52.3, 53.9, 68.4, 69.0, 71.1, 71.5, 73.3, 74.0, 75.0, 79.9, 102.5, 111.9, 127.1-128.3, 138.1, 140.2, 155.4, 170.9; MS (ESI): m/z = 670 $(M + Na)^+$; Anal. Calcd. for $C_{37}H_{45}NO_9$ (647.31): C, 68.6; H, 7.0; N, 2.16 % Found: C, 68.5; H, 6.98; N, 2.14 %

REFERENCES

1. (a) Varki, A. *Glycobiology* **1993**, *3*, 97.
(b) Davis, B. G. *J. Chem. Soc. Perk. Trans I*. **1999**, 3215.
(c) Arsequell, G.; Valencia, G. *Tetrahedron Asymmetry***1999**, *10*, 3045.
2. (a) Bertozzi, C. R.; Kissling, L. *Science* **2001**, *291*, 2357.
(b) Rudd, P. M.; Elliot, T.; Crewell, P.; Wilson, I. A.; Dwek, R .A. *Science***2001**, *291*, 2370.
3. Dwek, R. A. *Chem. Rev.***1996**, *96*, 683 and references therein.
4. (a) Jiao, H.; Von, P.; Schleyer, R. *Angew. Chem. Int. Ed. Engl*. **1996**, *108*, 2548.
(b) vogler, H. *Tetrahedron Lett.***1979**, 229.
5. Diederich, F.; Staab, H. A. *Angew. Chem. Int. Ed. Engl.***1978**, *17*, 372.
6. Katritzky, A. R.; Marson, C. M. *J. Am. Chem. Soc*. **1983**, *105*, 3279.
7. (a) Ransohoff, J. E. B.; Staab, H. A. *Tetrahedron Lett.***1985**, *26*, 6179.
(b) Bell, T. W.; Firestone, A. *J. Am. Chem. Soc*.**1986,** *108*, 8109.
8. (a) Kunz, H. *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 294.
(b) Paulsen, H. *Angew. Chem. Int. Ed. Engl.***1982**, *21*, 155.
9. (a) Davis, B. G. *Chem. Rev.***2000**, *100*, 4495.
(b) Dondoni, A.; Merra, A. *Chem. Rev.* **2000**, *100*, 4395.
(c) Paluso, S.; Imperialli, B. *Tetrahedron Lett*. **2001**, *42*, 2085.
(d) Dondoni, A.; Mariotti, G.; Merra, A. *Tetrahedron Lett*. **2000**, *41*, 3483.
10. Schmidt, R. R.; Winterfeld, G. A.; Ito, Y.; Ogawa, T. *Eur. J. Org. Chem.***1999**, 1167.

Anita Brar and Y. D. Vankar, "O-Glycopeptides: Pd(0) catalyzed stereoselective β-*O*-glycosidation of serine and threonine, enroute to generation of new class of glycopeptides," *International Journal of Scientific and Technical Advancements*, Volume 2, Issue 4, pp. 231-234, 2016.