Monatshefte für Chemie 128, 733–736 (1997)

Monatshefte für Chemie
Chemical Monthly

Short Communication

Glucuronidation of Serine and Threonine

D. K. Alargov[1,*], **P. S. Denkova**[2], and **E. V. Golovinsky**[1]

[1] Institute of Molecular Biology, Bulgarian Academy of Sciences, BG-1113 Sofia, Bulgaria
[2] Institute of Organic Chemistry, Bulgarian Academy of Sciences, BG-1113 Sofia, Bulgaria

Summary. The synthesis of N-diphenylmethylene-O-(methyl(2,3,4-tri-O-acetyl)-β-*D*-glucuronosyl)-threonine benzyl ester (**2**) and N-diphenylmethylene-O-(methyl(2,3,4-tri-O-acetyl)-α/β-*D*-glucuronosyl)-serine benzyl ester (**3**) by *Hanessian's* modification of the *Koenigs-Knorr* reaction is presented. Highly nucleophilic benzophenone *Schiff* bases are used for the protection of the N-moiety of serine and threonine.

Keywords. β-*D*-Glucuronides; Serine; Threonine; O-Glycosyl amino acids.

Glucuronidierung von Serin und Threonin (Kurze Mitt.)

Zusammenfassung. Die Synthese von N-Diphenylmethylen-O-(methyl(2,3,4-tri-O-acetyl)-β-*D*-glucuronosyl)-threonin-benzylester (**2**) und N-Diphenylmethylen-O-(methyl(2,3,4-tri-O-acetyl)-α/β-*D*-glucuronosyl)-serin-benzylester (**3**) durch die *Hanessian*-Variante der *Koenigs-Knorr*-Reaktion wird vorgestellt. Als Schutzgruppen des N-Restes des Serins und des Threonins wurden stark nukleophile *Schiff*sche Basen (Benzophenonderivate) eingesetzt.

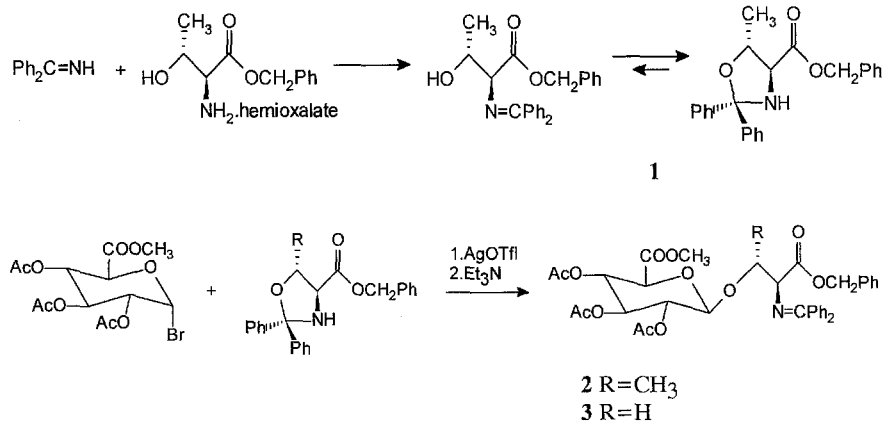
Introduction

In the recent years, biologically active O-glycosyl amino acids and peptides have gained increasing interest due to their numerous functions in the biological systems [1–3]. In order to design some potential inhibitors of UDP-glucuronosyltransferase, we wanted to find the optimal method and appropriate protection groups for O-glucuronidation of serine and threonine. The synthesized compounds may also be used as building blocks in solid phase peptide synthesis [3].

The synthesis of O-linked glycosyl amino acids is hampered by the acid lability of the glycosidic linkage and the base lability of the β-hydroxy amino acid residue (β-elimination). Additional complications arise due to the poor nucleophilicity of the usual N-acylated (*Boc*, *Z*, *Fmoc*) serine and threonine derivatives and the fast hydrolysis of glucuronosyl bromide, causing low yields and poor α/β selectivity [4, 5]. Here we report the synthesis of N-diphenylmethylene-O-(methyl-(2,3,4-tri-O-acetyl)-β-*D*-glucuronosyl)-threonine benzyl ester (**2**) and N-diphenylmethylene-O-(methyl(2,3,4-tri-O-acetyl)-α/β-*D*-glucuronosyl)-serine benzyl ester (**3**).

Results and Discussion

O-glucuronidation (Scheme 1) was achieved by *Hanessian's* modification of the *Koenigs-Knorr* reaction [8]. Highly nucleophilic α-imino esters (*O'Donnell's Schiff* bases) were used for the protection of the N-moiety of serine and threonine [6, 7].



Scheme 1

The preparation of N-diphenylmethylene-serine benzyl ester was carried out as described by *Polt et al.* [8]. N-Diphenylmethylene-threonine benzyl ester (**1**) was prepared in a similar way. The reaction was performed at room temperature during 24 h. Recrystallization from cyclohexane provided pure **1** in 82% yield. On the basis of its ^{1}H NMR spectrum in dimethylsulfoxide, **1** can be proposed to exist as an oxazolidine tautomeric form. The peak with the highest intensity ($m/z =$ 210.15) in its mass spectrum is probably due to the loss of the diphenylmethylene group.

The O-glycosylation of N-diphenylmethylene-threonine benzyl ester (**1**) with methyl(2,3,4-tri-O-acetyl-α-*D*-glucuronosyl bromide)-uronate [9, 10] was carried out in dichloromethane at room temperature [8]. The reaction was promoted by silver trifluoromethanesulfonate, and a reaction time of 10–12 h was needed for the completion of the glycosylation. The reaction was quenched with Et_3N, and the crude product was purified on SiO_2 by flash chromatography to afford N-diphenylmethylene-O-(methyl(2,3,4-tri-O-acetyl)-β-*D*-glucuronosyl)-threonine benzyl ester (**2**) in 71% yield. None of the corresponding 1,2-*cis* product (α-glycoside) could be detected by ^{1}H NMR or by thin layer chromatography.

In a similar way, N-diphenylmethylene-O-(methyl(2,3,4-tri-O-acetyl)-α/β-*D*-glucuronosyl)-serine benzyl ester (**3**) was obtained from N-diphenylmethylene-serine benzyl ester and methyl(2,3,4-tri-O-acetyl)-α-*D*-glucuronosyl bromide)-uronate in 64% yield. In this case, poor α/β selectivity was observed. The ^{1}H NMR

spectrum of **3** shows a pattern of overlapping, unresolved signals (3.5–5.5 ppm), indicating a mixture of α- and β-isomers. The MS peak at $m/z = 512.00$ can be explained by the loss of the diphenylmethylene group just as for **1**. It was impossible to separate the two enantiomers by flash or preparative liquid chromatography. The new derivatives were TLC pure and were characterized by ^{1}H NMR and MS analysis.

Experimental

All amino acid derivatives were purchased from Bachem Biochemica GmbH (Heidelberg). Silver triflate and D-glucurono-γ-lactone was obtained from Merck. The melting points were measured with a Kofler hot-stage apparatus. TLC analyses were performed on DC-Alufolien Kieselgel 60 F_{254} (Merck). The compounds were visualized by UV light or by spraying with the appropriate reagents (*Reindel* [11], ninhidrin). For flash chromatography, Merck Kieselgel 60 (76–230 mesh ASTM) was employed. Optical rotation was determined with a Polamat A Carl Zeiss-Jena instrument. The ^{1}H NMR spectra were recorded on a Bruker DRX 250 MHz instrument. Mass spectra were detected with a Jeol JMS 100 spectrometer.

N-Diphenylmethylene-L-threonine benzyl ester (**1**; $C_{24}H_{23}NO_3$)

L-Threonine benzyl ester hemioxalate (4.58 g, 20 mmol) and $Ph_2C=NH$ (3.02 ml, 18 mmol) were stirred in CH_2Cl_2 (20 ml) at room temperature for 24 h under exclusion of moisture. The reaction mixture was diluted with CH_2Cl_2 and filtered to remove the precipitated NH_4Cl. The filtrate was washed with 5% $NaHCO_3$ solution and then with water. The organic solution was dried over anhydrous Na_2SO_4 and evaporated. The resulting solid was recrystallized from cyclohexane to afford 6.12 g (82%) of **1**.

M.p.: 60–64°C; $[\alpha]_D^{20} = -36.38$ ($c = 1.0, DMF$); $R_f = 0.66$ (*PE*/EtOAc, 8:2), $R_f = 0.74$ (*PE*/EtOAc, 6:4); ^{1}H NMR ($(CD_3)_2SO$) : δ (ppm)=1.28 (d, 3H, CH_3, $J_{\alpha,CH_3} = 6.1$ Hz), 3.65 (dd, 1H, H-α, $J_{\alpha,\beta} = 7.2$ Hz, $J_{\alpha,NH} = 11.2$ Hz), 3.92 (d, 1H, NH, $J_{\alpha,NH} = 11.2$ Hz), 4.05 (m, 1H, H-β, $J_{\alpha,\beta} = 7.2$ Hz, $J_{\beta,CH_3} = 6.1$ Hz), 5.06 (dd, 2H, CH_2-benzyl, AB-system, $J_{A,B} = 12.4$ Hz), 7.5–7.2 (m, 15H, H-arom); MS (ES^+): $m/z = 210.15$ (100%), 183.15 (31%).

N-Diphenylmethylene-O-(methyl(2,3,4-tri-O-acetyl)-β-D-glucuronosyl)-L-threonine benzyl ester (**2**; $C_{37}H_{39}NO_{12}$)

N-Diphenylmethylene-*L*-threonine benzyl ester (**1**, 0.75 g, 2 mmol), methyl(2,3,4-tri-O-acetyl-α-*D*-glucuronosyl bromide)-uronate (0.95 g, 2.4 mmol), and oven-dried 4Å molecular sieve (2.0 g) were stirred at 0°C in dichloromethane (10 ml) for 10 min under argon. Silver triflate (0.62 g, 2.4 mmol) was added in portions over 10 min, and stirring was continued for 12 h at room temperature. The reaction was quenched with Et_3N (0.63 ml, 4.6 mmol), diluted with CH_2Cl_2, and filtered through Celite. The filtrate was washed with 5% $NaHCO_3$ solution and then with water. The organic solution was dried over anhydrous Na_2SO_4 and evaporated. The crude product was purified by flash chromatography on SiO_2 with *PE*/EtOAc (6:4) as eluent.

Yield: 0.98 g (71%); m.p.: 122–127°C; $[\alpha]_D^{20} = -84.22$ ($c = 1.0, DMF$); $R_f = 0.40$ (*PE*/EtOAc, 6:4); ^{1}H NMR ($(CD_3)_2SO$): δ (ppm) = 1.0 (d, 3H, CH_3), J_{α}, $CH_3 = 6.3$ Hz), 1.95 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.99 (s, 3H, OAc), 3.44 (s, 3H, OCH_3), 3.97 (d, 1H, H-α, $J_{\alpha,\beta} = 6.5$ Hz), 4.29 (m, 1H, H-β), 4.32 (d, 1H, H-5, $J_{5,4} = 10.1$ Hz), 4.72 (dd, 1H, H-2, $J_{2,1} = 8.0$ Hz, $J_{2,3} = 9.8$ Hz), 4.86 (t, 1H, H-4, $J_{4,5} = 10.1$ Hz, $J_{4,3} = 9.6$ Hz), 5.02 (d, 1H, H-1, $J_{1,2} = 8.0$ Hz), 5.03 (d, 1H) and 5.12 (d, 1H), CH_2-benzyl, AB-system, $J_{A,B} = 12.5$ Hz), 5.36 (t, 1H, H-3, $J_{3,4} = 9.6$ Hz, $J_{3,2} = 9.6$ Hz), 7.11–7.51 (m, 15H, H-arom).

N-Diphenylmethylene-O-(methyl(2,3,4-tri-O-acetyl)-α/β-D-glucuronosyl)-L-serine benzyl ester (**3**; $C_{36}H_{37}NO_{12}$)

N-Diphenylmethylene-*L*-serine-benzyl ester (0.61 g, 1.7 mmol), methyl(2,3,4-tri-O-acetyl-α-*D*-glucuronosyl bromide)-uronate (0.79 g, 2.0 mmol) and oven-dried 4 Å molecular sieves (2.0 g) were stirred at 0°C in dichloromethane (10 ml) for 10 min under argon. Silver triflate (0.50 g, 2.0 mmol) was added in portions over 10 min, and stirring was continued for 12 h at room temperature. The reaction was quenched with Et_3N (0.54 ml, 3.9 mmol), diluted with CH_2Cl_2, and filtered through Celite. The filtrate was treated as described for **2**. The crude product was purified by flash chromatography on SiO_2 with *PE*/EtOAc (6:4) as eluent.

Yield: 0.74 g (64%, syrup); $R_f = 0.40$ (*PE*/EtOAc, 6:4); MS (ES^+): $m/z = 512.08$ (100%), 452.11, 317.00 (58%), 257.07, 183.14; MS (EI): $M/z = 556$, 514, 496, 450, 432, 402, 360, 342, 328, 317, 257, 224, 206, 194, 165, 127, 91 (100%), 77, 43.

Acknowledgements

This work was supported by Grant No K-602 of the *National Fund for Scientific Research* of the Bulgarian Ministry of Education and Science.

References

[1] Kunz H (1993) Pure and Appl Chem **65**: 1223
[2] Elofsson M, Walse B, Kihlberg J (1991) Tetrahedron Lett **32**: 7613
[3] Jansson AM, Meldal M, Bock K (1990) Tetrahedron Lett **31**: 6991
[4] Kunz H (1987) Angew Chem Int Ed Engl **26**: 294
[5] Gary HG, Jeanloz RW (1988) Adv Carbohydr Chem Biochem **43**: 135
[6] O'Donnell MJ, Polt RL (1982) J Org Chem **47**: 2663
[7] Polt RL, Peterson MA, De Young L (1992) J Org Chem **57**: 5469
[8] Polt RL, Szabo L, Trieberg J, Li Y, Hruby V (1992) J Am Chem Soc **144**: 10249
[9] Bollenback GN, Long JW, Benjamin DG, Lindquist JA (1955) J Am Chem Soc **77**: 3310
[10] Litvak MM, Betaneli VI, Backinowsky LV, Kochetkov NK (1982) Bioorg Khim **8**: 1133
[11] Reindel F, Hoppe W (1954) Chem Ber **87**: 1103

Received September 9, 1996. Accepted (revised) January 27, 1997

Verleger: Springer-Verlag KG, Sachsenplatz 4–6, A-1201 Wien. – Herausgeber: Österreichische Akademie der Wissenschaften. Dr.-Ignaz-Seipel-Platz 2, A-1010 Wien, und Gesellschaft Österreichischer Chemiker, Eschenbachgasse 9, A-1010 Wien. – Redaktion: Währinger Straße 38, A-1090 Wien. – Satz und Umbruch: Thomson Press Ltd., New Delhi, India. – Offsetdruck: Eugen Ketterl Gesellschaft m.b.H., Schopenhauerstraße 45, A-1180 Wien. – Verlagsort: Wien. – Herstellungsort: Wien. – Printed in Austria.