Available free online at www.medjchem.com

Mediterranean Journal of Chemistry **2014**, 3(4), 935-946

The synthesis of a glucoconjugate of the peptidic fragment of cryptophycin-24

Abdelmoumen Mezrai[1], David Lesur[2], Anne Wadouachi[2], Florence Pilard[2] and Joseph Kajima Mulengi[1,*]

[1]Laboratory of Organic Chemistry, Natural Products and Analyse. University of Tlemcen. P.O Box 119. 13 000 Tlemcen, Algeria

[2] Laboratoire des Glucides. FRE CNRS 3517. Université de Picardie Jules Verne, 33 rue Saint-Leu, F-80039 Amiens Cedex, France.

Abstract: A novel glucoconjugate of the peptidic fragment of cryptophycin-24 was prepared through the replacement of the leucic acid residue with *L*-leucine and the functionalisation of tyrosine residue with glucose. Those modifications lead to a novel protected tripeptide fragment of cryptophycin-24.

Keywords: Hybrids; Cryptophycins; Cytotoxicity; bioconjugates; cyclopeptides.

Introduction

Chemistry in the nature provides many hybrid molecules or bioconjugates that are made of a variety of moieties. Among the latter, peptides may be encountered as peptidoglycans and peptidolipids, among others. Cryptophycin-24 (Figure 1) may be considered as a macrocyclic hybrid that is made of two important fragments, a partially conjugated carbonyl derivative and a peptide moiety.

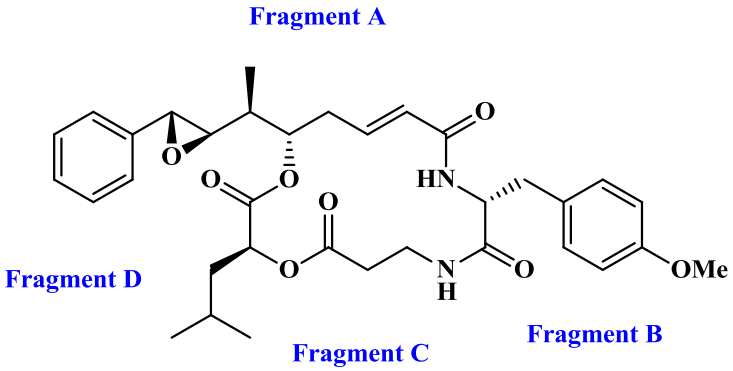
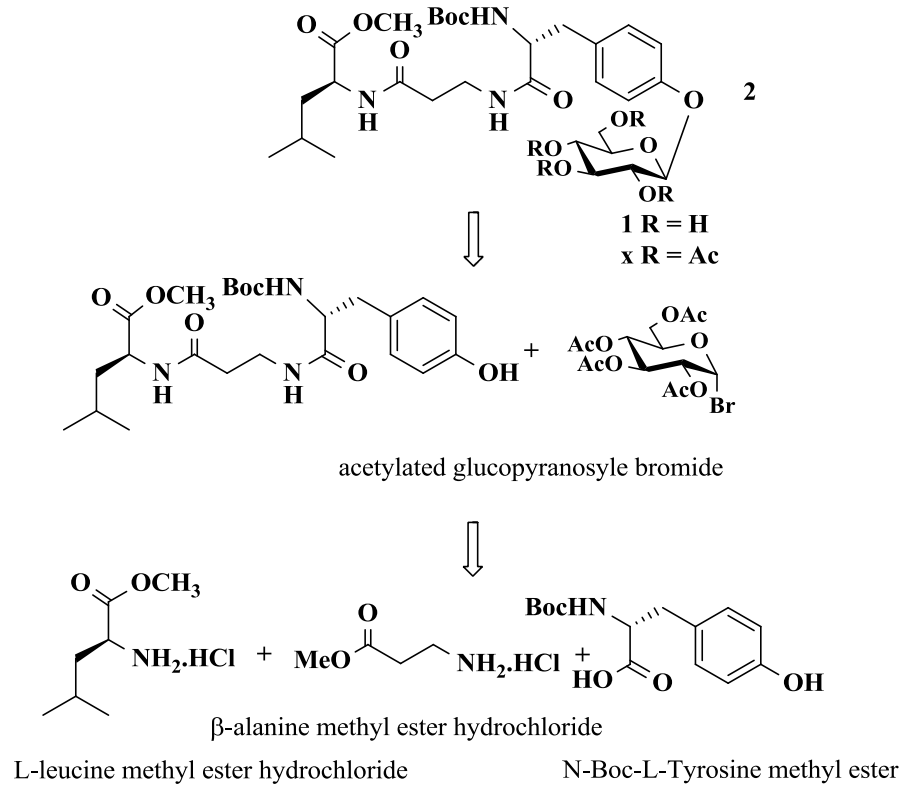


Figure 1. Cryptophycin-24

An impressive work has already been performed since the first isolation of cryptophycins from Blue-Green Algae[1-5] and many topics have been dealt with including total synthesis[6-21], as well as the synthesis of structural analogues and fragments[22-37] devoted to test their biological activities[38-46]. Our own interest in the chemistry of peptides of biological interest[47] prompted us to focus on the peptidic fragment of cryptophycins that we presume might

**Corresponding author: Joseph Kajima Mulengi*
E-mail address: cosnalab@yahoo.fr
DOI: http://dx.doi.org/10.13171/mjc.3.4.2014.04.07.15

contribute to the biological activity of the compound by means of its tyrosine residue. As a matter of fact, the latter has been recognised to be the most effective among amino acids for mediating molecular recognition[48].For this reason, we focused on the synthesis of a novel peptidic derivative of Cryptophycin-24 through the modification of fragments B and D. The target was compound 1, shown in Scheme 1, where L-tyrosine would be *O*-alkylated with a glucose derivative (fragment B) and leucic acid would be replaced with L-Leucine methyl ester (fragment D).The retrosynthetic analysis of the peptidic fragment of Cryptophycin-24 suggested that the novel hybrid could be represented by structure **2** (Scheme 1).



Scheme 1. Retrosynthetic analysis of target **2**

.

The latter would be made of L-leucine, β-alanine and *O*-glucosylated L-tyrosine that would be used as their commercially available derivatives, namely *t*-butoxycarbonyl (Boc-) or benzyloxycarbonyl (Cbz) derivatives, except for *β*-alanine that would beused as its methyl ester hydrochloride.

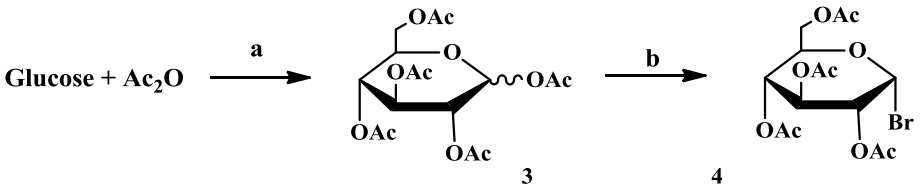
We believe that the presence of a glucose residue in the target compound could result in adding supplementary molecular recognition properties to this compound. As a matter of fact, sugars play an important role in the causative agents of infectious and bacterial diseases, since polysaccharides mediate host-pathogen interactions[49].

Moreover, carbohydrates are present in many bioactive compounds[50], and they are the basis of many therapeutic and diagnostic strategies, thus making glycoconjugates among the most challenging tools to be developed to this end[51]. On the other hand, sugars are being used in vaccine development[52] and probes[53]. Besides, groups of investigators have shown that many proteins in the human glycome are involved in binding to carbohydrates, thus inducing glycational modifications of carbohydrates and recognition of modified sugars[54]. For example, sulphated glycosaminoglycans are found on cell surfaces and within the

extracellular matrix where they mediate binding interactions and provide structural support[55]. At the same time, acylation of glycans leads to highly specific inhibitors of carbohydrate modifying transferases, and the products of acylation can play various key roles in human immunology, disease pathogenesis and cancer progression.

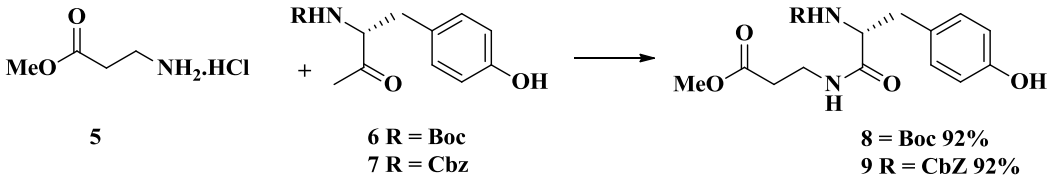
Results and Discussion

The novelty in this work is the functionalization of the tyrosine hydroxyl group and, to the best of our knowledge, such *O*-glucosylation of tyrosine in this class of compounds has not been described yet. The synthesis of 2,3,4,6-*O*-acetate-α-D-glucopyranosyl bromide **4** was achieved in two steps: first peracetylation of D-glucose was carried out with acetic anhydride (Ac_2O) in pyridine to afford 1,2,3,4,6-penta-*O*-acetyl-D-glucopyranose **3** in 75% yield and this latter was converted into the corresponding 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl bromide **4** in 98% yield using a commercial 33% HBr / CH_3COOH solution according to the literature or with slight modifications of the protocols indicated in the cited literature[57, 58] (Scheme 2). Analytical data of **4** were consistent with those of the literature[58b].



Scheme 2. (a) pyridine, 24h, 75%; (b) HBr / CH_3COOH 33%, 30 min, 98%.

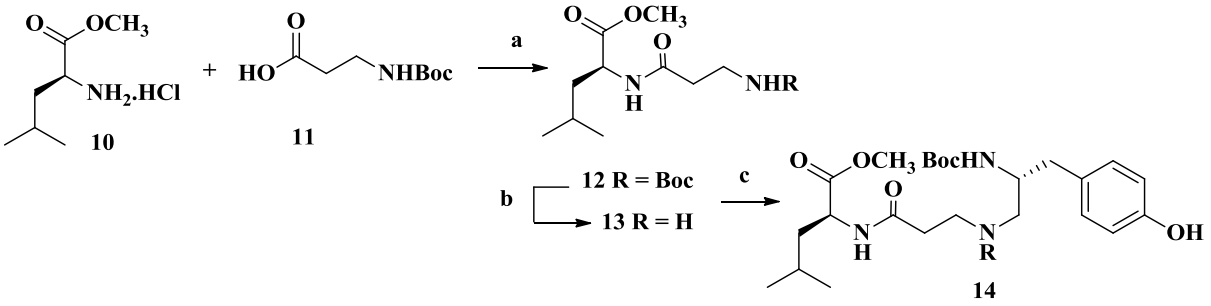
The first peptidic synthon, *N*-Boc-L-tyrosyl-β-alanine methyl ester **8** (*N*-Boc-L-Tyr-*β*-Ala-methyl ester) was synthesised in 92% yield through the coupling of commercially available *β*-alanine methyl ester hydrochloride **5** with Boc-L-tyrosine **6** (Boc-L-Tyr) using dicyclocyclohexylcarbodiimide (DCC) in the presence of hydroxybenzotriazole (HOBt) and triethylamine (TEA) in dichloromethane (DCM). The reaction afforded the corresponding dipeptide in 92% yield. It is useful to single out that this compound was previously described by Vanaman and Watt[59] and our analytical data matched quite well with theirs (Scheme 3**)**.



Scheme 3. DCC, HOBt, TEA, CH_2Cl_2, RT, 24h, 92%

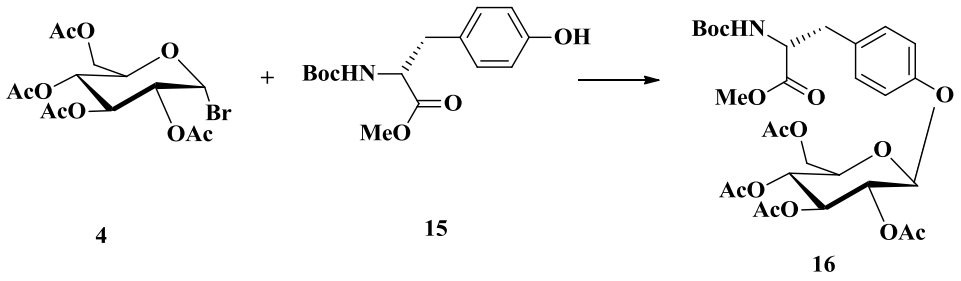
The Cbz-analogue was synthesised in the same manner from Cbz-L-Tyrosine **6** to afford **9** in 92% yield. Another approach to access the tripeptide consisted in coupling L-leucine methyl ester hydrochloride **10** and Boc-*β*-alanine **11** using the experimental conditions described above, so that a non-glucosylated dipeptide was generated first. This reaction led to dipeptide **12** (L-leucine methyl ester *N*-Boc-*β*-alanine), obtained in 88% yield. The compound displayed spectroscopic characteristics as found in the literature[60].

Removal of the Boc-group using trifluoroacetic acid (TFA) in dichloromethane afforded the corresponding amino derivative **13** in quantitative yield. The latter was coupled without further purification and under the same experimental conditions with Boc-L-tyrosine to provide tripeptide **14** in 85% yield. This latter represented the first modification of the peptidic fragment of Cryptophycin-24, where leucic acid was replaced with the L-leucyl methyl ester moiety (Scheme 4).



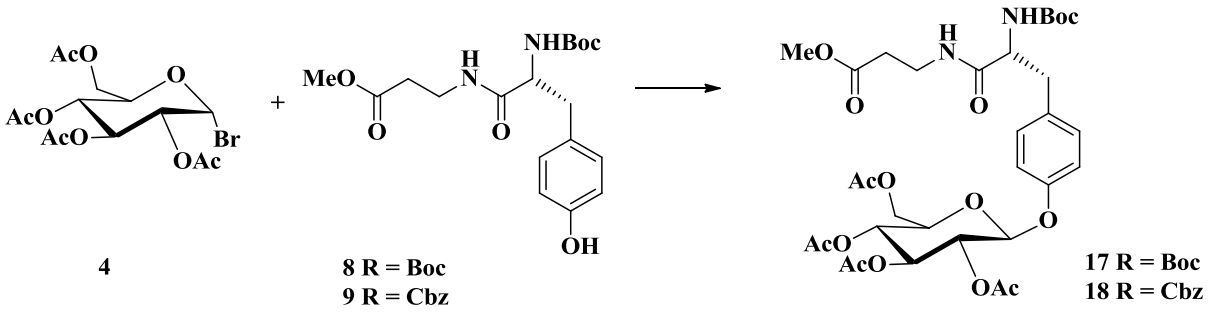
Scheme 4. (a) DCC, HOBt, TEA, CH_2Cl_2, RT, 24h, 88%; (b) TFA, CH_2Cl_2, 2h, 88%; (c) Boc-L-Tyr 5, DCC, HOBt, CH_2Cl_2, 24h, 85%.

The second step in this work consisted of the *O*-alkylation of tyrosine. It is useful to point out that Cbz-derivatives did not dissolve easily in the reaction media. This necessitated a slight modification of the usual protocol for the reaction to run smoothly. For this purpose, Cbz derivatives needed to be previously dissolved in THF before the usual protocol was implemented, i.e. reaction was performed in an acetone and water solution. 2,3,4,6-Tetra-*O*-acetate-α-D-glucopyranosyl bromide **4** was used for the *O*-alkylating of the tyrosine moiety in tyrosine containing compounds. The reaction of **4** with commercial Boc-Tyr-OMe **15** led to compound **16**, obtained as an off-white solid in 62% yield (Scheme 5).



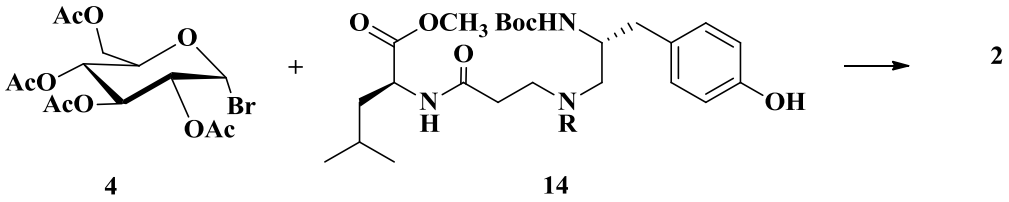
Scheme 5. NaOH, acetone / H_2O, 48h, 62%.

The first modified dipeptides were synthesised by coupling of **4** with dipeptides **8** and **9** under the above-described conditions. Reaction with dipeptide **8** led to compound **17** in 79% yield, whereas dipeptide **9** afforded the glucoconjugate **18** in 77% yield (Scheme 6). However, because of poor water solubility of both **8** and **9**, NaOH pellets were previously dissolved in a solution of water and acetone (v/v), and then reactions of **8** and **9** were carried out in this medium at room temperature.



Scheme 6. NaOH pellets in acetone / H_2O (v/v), 72h, 79% for **17** and 77% for **18**.

Finally the initially-targeted tripeptide could be reached through the reaction of **4** with tripeptide **14**. The tetraaceytate *O*-glucosylated tripeptide **2** was isolated in 77% yield (Scheme 7).



Scheme 7. NaOH, acetone / H_2O, 72h, 77%.

Conclusion

We have achieved a simple and affordable synthesis of a modified peptide that is to be involved in the synthesis of a modified cryptophycin. The novelty of this synthesis is the introduction of a glucose moiety onto the tyrosine residue of the tripeptide so that the whole hybrid would be a frame for a compound with improved molecular recognition. This could lead to improved selectivity of the biological activity of the new compound.

Acknowledgements

We are grateful to the General Directorate for Scientific Research and Technological Development (Ministry of Higher Education / Algeria) for financial support. We are deeply indebted to the Laboratoire des Glucides, Université de Picardie Jules Verne (France) for complementary syntheses, NMR, chiral HPLC and Mass spectrometry facilities granted to our PhD student as well as for valuable discussion of the results of this work.

Experimental Section

All the reactions with dry solvents were carried out under dry argon. NMR spectra were recorded in $CDCl_3$ on a Bruker 300MHz instrument. Chemical shifts are given in δ (ppm) and coupling constant (*J*) values in Hertz (Hz). The multiplicity of signals is given as usual except for broad singlet that is noted br-s. GC analysis was performed on a Shimadzu 17A CPG chromatograph using a 30m DB-35 column. Melting points were determined on an Electrothermal T1A F3.15A instrument. Column chromatography was performed on silica gel 230-270 mesh (Merck) using solvents or mixtures of solvents as appropriate. Specific

rotation values were collected on Perkin Elmer Model 343 Polarimeter instrument using 0.1 or 1g of each sample solution in an appropriate solvent. ESI-MS data were recorded in the positive ion mode on a quadrupole instrument (Waters-Micromass ZQ). The source and desolvation temperatures were kept at 120 and 250°C, respectively. Nitrogen was used as a drying and nebulizing gas at flow rates of 350 and 50 L/h, respectively. The capillary voltage was 3.5 kV. The mass range was 50-1000 Da and the spectra were recorded at 1s/scan in the profile mode. Calibration of the instrument was performed using cluster ions of sodium iodide (NaI) nNa^+. Data acquisition and processing were performed with MassLynx V4.1 software.

2,3,4,6-Penta-*O*-acetyl-α-D-glucopyranosyl bromide 4[57,58]**.**

Methyl 3-(2-(tert-butoxycarbonyl)-3-(4-hydroxyphenyl)propanamido)propanoate 8

N-Boc-L-tyrosine **6** (2g, 7.11mmol) and β-alanine hydrochloride methyl ester **5** (992mg, 7.11mmol) (Aldrich) were dissolved in DCM (10 mL). Then triethylamine (TEA) (4.94 mL, 35.55 mmol) and hydroxybenzotriazole (HOBt) (1.06 g, 7.82 mmol) were added to the solution. Stirring was kept for 10 min at 0°C, and then dicyclohexylcarbodiimide (DCC) (1.61 g, 7.82 mmol.) was added. The mixture was kept at 0°C for 30 min and then left at room temperature for 10h. After filtration with suction and removal of the solvent, the residue was dissolved in ethyl acetate (AcOEt) (10 mL) and filtered off once again. The organic layer was then washed successively with a 5% aqueous citric acid solution (10 mL), brine (10 mL), a 5% aqueous solution of potassium carbonate and finally with water (10 mL).
Finally, it was dried over anhydrous $MgSO_4$, filtered and the solvent was removed under reduced pressure. The residue was purified on a silicagel column using DCM-MeOH (98:2). After removal of the solvents, the final product was recrystallized in AcOEt to afford 2.28 g of off-white crystals (92%); mp 104-106°C; $[\alpha]^{22}_D$ +66.2°, *c* = 1 in $CHCl_3$. MS/LMRS $[ESI]^+$ *m/z* calcd (for $C_{18}H_{26}N_2NaO_6$): *m/z* =389.17, found 389.1. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.42 (s, 9H, t-Bu), 2.41 (t, 5.5 Hz, 2H, CH_2COO), 2.92 (d, 6.7 Hz, 2H, CH_2CHCO), 3.42 (t, 5.5 Hz, 2H, CH_2N), 3.77 (s, 3H, OCH_3), 5.30 (t, 5.6 Hz, 1H, CHNH), 6.33 (t, 6.3 Hz, 1H, NH), 6.88 (d, 9 Hz, 2H, Ph), 7.01(d, 9 Hz, 2H, Ph), 7.60 (br-s, 1H, OH).^{13}C NMR (300 MHz, $CDCl_3$) δ 28.3 (3C), 33.6, 34.7, 38.0, 51.8, 56.2, 80.0, 115.6 (2C), 130.4 (2C), 132.1, 154.3, 155.9, 171.2, 172.6.

Methyl 3-(2-(benzyloxycarbonyl)-3-(4-hydroxyphenyl)propanamido)propanoate 9

N-CBz-L-tyrosine **7** (1g, 3.17 mmol) and β-alanine methyl ester hydrochloride **5** (442 mg, 3.17 mmol) were dissolved in DCM (10 mL); then TEA (2.2ml, 15.85mmol) and HOBt (0.47g, 3.487 mmol) were added to the solution. After stirring for 10 min at 0°C, DCC (0.72g, 3.487 mmol) was added to the mixture, and the latter was stirred for 30 min under the same conditions and then left at room temperature for 10 h. After filtration with suction and concentration, the resulting residue was dissolved in ethyl acetate and filtered off once again. The resulting filtrate was washed successively with a 5% aqueous citric acid solution (10 mL), brine (10 mL), 5% aqueous potassium carbonate solution (10 mL) and finally with water (10 mL). The organic layer was separated and then dried over anhydrous magnesium sulphate ($MgSO_4$) filtered and concentrated under diminished pressure. The resulting residue crystallized in ether to afford **9** as an off-white product (1.17 g, 92%). mp 133°C; $[\alpha]^{22}_D$ +78.1°, *c* = 1 in $CHCl_3$. ^{1}H NMR (300 MHz, DMSO) ; δ 2.41 (t, 5.6 Hz, 2H,

CH_2COOMe), 2.80 (d, 6.7 Hz, 2H, CH_2CHNH), 3.43 (t, 5.6 Hz, 2H, CH_2NH), 3.6 (3H, s, OCH_3), 4.10 (br-s, 1H, NH), 4.90 (t, 5.6 Hz, 1H, CHNH), 5.00 (d, 7,2 Hz, 2H, CH_2Ph), 6.77 (d, 9 Hz, 2H, Ph), 7.00 (d, 9.0 Hz, 2H, Ph), 7.32 (m, 5H, Ph), 8.11 (br-s, 1H,NH), 9.20 (br-s, 1H, OH). ^{13}C NMR (300 MHz, DMSO) δ 35.3, 36.3, 37.5, 51.8, 55.2, 66.1, 115.8 (2C), 127.7 (2C), 127.6, 128.9 (2C), 129.2 (2C), 132.1, 141.6, 155.8, 156.0, 170.9, 171.4. MS/LMRS $[ESI]^+$ *m/z* calcd (for $C_{21}H_{24}N_2NaO_6$): *m/z* = 423.15, found 422.9.

***(S)*-Methyl 2-(3-(tert-butoxycarbonyl)propanamido)-4-methylpentanoate 12**

N-Boc-β-alanine **11** (2g, 10.57 mmol) and L-leucine methyl ester chlorhydrate **10** (1.92 g, 10.6 mmol) were dissolved DCM (10 mL), followed by TEA (7.34 ml, 52.88mmol) and HOBt (1.57 g, 11.6 mmol). After stirring for 10 min at 0°C, DCC (2.4 g, 11.63 mmol) was added. The mixture was left at room temperature for 10 min and then at room temperature for 10h. The mixture was then filtered under suction and the filtrate was evaporated under reduced pressure. The remaining residue was dissolved in ethyl acetate (10 mL) and then filtered again. The resulting filtrate was washed successively with a 5% aqueous citric acid solution (10 mL), brine (10 mL), 5% aqueous potassium carbonate solution (10 mL) and then with water (10 mL). Finally it was dried over anhydrous $MgSO_4$, filtered off and the solvent removed under reduced pressure to afford a residue that was purified on silicagel column using DCM-methanol (95/5). The resulting solid was recrystallised in water to afford **12** as an off-white product (3.53 g, 88%). Mp = 50-52°C. $[\alpha]^{22}_D$ +7.5°, *c* = 0.1 in $CHCl_3$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (d, 6.0 Hz, 6H, CH_3-Bu_{-i}), 1.43 (s, 9H, t-Bu), 1.54 (m, 1H, CH-Bu_{-i}), 1.64 (m, 2H, CH_2-Bu_{-i}), 2.44 (m, 2H, CH_2CH_2NBoc), 3.43 (m, 2H, CH_2NBoc), 3.74 (s, 3H, OCH_3), 4.77 (m, 1H, ($CHCO_2$), 5.22 (br-s, 1H, β-Ala- NH), 5.99(br-s, 1H, Leu-NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.9, 22.8, 24.9, 28.4, 36.3, 36.7, 41.6, 50.7, 52.3, 77.2, 156.2, 171.5, 173.5. MS/LMRS $[ESI]^+$ *m/z* calcd (for $C_{15}H_{28}N_2NaO_5$): *m/z* = 339.19, found 339.1.

(S)-Methyl 2-(3-aminopropanamido)-4-methylpentanoate 13

A solution of (S)-Methyl 2-(3-(tert-butoxycarbonyl)propanamido)-4-methylpentanoate **12** (2 g, 6.32 mmol) in DCM (22.6 mL) was treated with trifluoroacetic acid (TFA) (52.66 mL) at 0 °C then stirred at room temperature for 2 h. The resulting mixture was concentrated and dried *in vacuo*. The crude product **13** (2.36 g, TFA salt) was used directly in the next reaction, without further purification.

(S)-Methyl 2-(3-aminopropanamido)-4-methylpentanoate 14

Boc-L-tyrosine (1.78 g 6.32 mmol) and **13** (2.36 g 6.32 mmol) were dissolved in DCM (10 mL). Then HOBt (0.94 g, 6.95 mmol) was added to the solution. After 10 min stirring at 0 °C, DCC (1.43 g, 6.95 mmol) was added, and the mixture was stirred at this temperature for 30 min, and then kept at room temperature for 10 h. After filtration with suction and concentration, the resulting residue was dissolved in ethyl acetate and filtered off once again. The resulting filtrate was washed successively with a 5% aqueous citric acid solution (10 mL), brine (10 mL), 5% aqueous potassium carbonate solution(10 mL) and then with water (10 mL). Finally it was dried over anhydrous $MgSO_4$, filtered off and the solvent removed under reduced pressure to afford a residue that was purified on silicagel column using DCM-MeOH (from 98:2 to 90:10) to afford **14** as an off-white solid (2.58g, 85%). MS/LMRS $[ESI]^+$ *m/z* calcd (for $C_{24}H_{37}N_3NaO_7$): *m/z* = 502.25, found 502.22. Mp = 120°C; $[\alpha]^{22}_D$ +58.3°, *c* = 1 in $CHCl_3$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (d, 6 Hz, 6H, CH_3-Bu_{-i}), 1.42 (s, 9H, t-Bu), 1.55 (m, 1H, $CH(CH_3)_2$), 1.71 (m, 2H,CH_2 $CH(CH_3)_2$), 2.4 (t, 5.5 Hz, 2H,

CH_2CON), 2.92 (d, 6.7 Hz, 2H, $CH_2CHNHCO$), 3.11 (br-s, 1H, NH), 3.55 (m, 2H, CH_2NH), 3.88 (s, 3H, OMe), 3.8 (br-s, 1H, NH), 4.62 (m, 1H, $CHCO_2Me$), 5.20 (t, 5.6 Hz, 1H, CHNHCOBoc), 6.88 (d, 9 Hz, 2H, Ph), 7.00 (br-s, 1H, NH), 7.2 (br-s, 1H, OH), 7.0 (d, 9 Hz, 2H, Ph),.^{13}C NMR (75 MHz, $CDCl_3$) δ 21.3, 22.9, 24.9, 28.3 (3C), 36.6, 36.9, 37.2, 39.8, 51.4, 52.6, 56.8, 80.3, 115.6 (2C), 130.4 (2C), 130.5, 135.4, 155.9, 172.5, 173.0, 174.9.

2,3,4,6-Tetra-*O*- acetyl-α-D-glucopyranosyl-L-tyrosine methyl ester 16

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide **4** (2.0 g, 4.86 mmol), *N*-Boc-tyr-OMe **15** (2.153g, 7.29mmol), pellets of sodium hydroxide (291 mg, 7.29 mmol), water (15 ml) and acetone (15 ml) were mixed together, and the mixture was stirred for 48 h. Then, the mixture was purified on a silicagel column using ethyl acetate-cyclohexane (8/2). Concentration of the eluate afforded a solid that was recrystallised in ethyl acetate to provide **16** as an off-white solid (1.89 g, 62%). Mp 165°C; $[\alpha]^{22}_D$ -33.6°, *c* = 1 in MeOH. MS/LMRS $[ESI]^+$ *m/z* calcd (for $C_{29}H_{39}NNaO_{14}$): *m/z* = 648.23, found 648.30. ^{1}H NMR (300 MHz, DMSO) δ ^{1}H NMR (300 MHz, DMSO) δ 1.33 (s, 9H, t-Bu), 2.02 (s, 3H, $OC(O)CH_3$), 2.03 (s, 3H, $OC(O)CH_3$), 2.11(s, 3H, $OC(O)CH_3$), 2.22 (s, 3H, $OC(O)CH_3$), 2.92 (d, 6.7 Hz, 2H, CH_2Ph), 3.65 (s, 3H, OMe), 4.11 (m, 1H, $CHOC(O)CH_3$), 4.21 (m, 2H, CH_2OAc), 5.01 (m, 2H, $CHCH_2OC(O)CH_3$, CHNHBoc), 5.22 (d, 7.2 Hz, 1H, $CHOC(O)CH_3$), 5.33 (m, 1H, $CHOC(O)CH_3$), 5.88 (d, 10 Hz, 1H, OCHOPh), 6.61 (d, 9 Hz, 2H, Ph), 6.95 (d, 9 Hz, 2H, Ph). ^{13}C NMR (300 MHz, DMSO). δ (ppm) 20.6, 20.7, 20.7, 21.0, 28.3 (3C), 36.8, 51.4, 52.6, 61.6, 68.0, 69.4, 69.9, 70.0, 89.3, 98.2, 115.4 (2C), 130.2 (2C), 130.7, 154.2, 155.8, 169.9, 169.9, 170.0, 170.5, 171.4.

2,3,4,6-Tetra-*O*- acetyl-α-D-glucopyranosyl-*N*-Boc-L-tyrosyl-β-alanine methyl ester 17

2,3,4,6-Tetra-*O*-acetyl-*α*-D-glucopyranosyl bromide **4** (1.51 g, 3.67 mmol), compound **7** (1.615 g,4.4 mmol), NaOH (176 mg, 4.4 mmol), water (10 ml) and acetone (10 ml) were mixed and stirred at room temperature for 72h. Column chromatography using AcOEt-cyclohexane (7:3) afforded **17** as an off-white solid after removal of the solvents (2.02 g, 79%). Mp 218°C. $[\alpha]^{22}_D$ +32.6°, *c* = 1 in MeOH. MS/LMRS $[ESI]^+$ *m/z* calcd for $C_{32}H_{44}N_2NaO_{15}$): *m/z* =719.26, found 719.30.

^{1}H NMR (300 MHz, DMSO) δ 1.33 (s, 9H, t-Bu), 2.01 (s, 3H, $OC(O)CH_3$), 2.0 (s, 3H, $OC(O)CH_3$), 2.11 (s, 3H, $OC(O)CH_3$), 2.11 (s, 3H, $OC(O)CH_3$), 2.41 (m, 2H, CH_2COOMe), 2.80 (d, 6.7 Hz, 2H, CH_2CHNH), 3.40 (m, 2H, CH_2NH), 3.77 (s, 3H, OCH_3), 4.01 (m, 1H, $CHOC(O)CH_3$), 4.11 (m, 2H, $CH_2OC(O)CH_3$), 5.11 (m, 2H, $CHCH_2Ph$, $CHCH_2OC(O)CH_3$), 5.22 (d, 7.2 Hz, 1H, $CHOC(O)CH_3$), 5.2 (m, 1H, $CHOC(O)CH_3$), 5.55 (d, 10 Hz, 1H, OCHOPh), 6.77 (d, 9 Hz, 2H, Ph), 6.88 (brs, 1H, NH), 6.98 (d, 9 Hz, 2H, Ph), 7.92 (br-s, 1H, NH). ^{13}C NMR (300 MHz, DMSO) δ 20.5, 20.7, 20.8, 21.1, 28.3 (3C), 33.8, 36.7, 38.2, 51.8, 56.3, 61.6, 68.1, 69.4, 69.9, 70.0, 79.8, 98.3, 115.5 (2C), 130.0 (2C), 130.7, 154.3, 155.9, 169.9, 169.9, 170.1, 170.5, 171.3, 172.7.

2,3,4,6-Tetra-*O*- acetyl-α-D-glucopyranosyl-*N*-Cbz-L-tyrosyl-β-alanine methyl ester 18

2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide **4** (1g, 2.43 mmol) ; **9** (1.46 g, 3.65 mmol) ; NaOH pellets (146 mg, 3.65 mmol) were dissolved in a solution of acetone/water (10 ml v/v (6-4). Then **9** was added and stirred until full dissolution. Afterwards, **4** was added, and the mixture was stirred for 72h at room temperature. After removal of solvents, the residue was purified on a Silicagel column chromatography using ethyl acetate-

cyclohexane-AcOEt (7:3) to afford **18** as an off-white solid after removal of the solvents (1.77 g, 77%). mp = 134°C. $[\alpha]^{22}_{D}$ -17.2°, *c* = 1 in MeOH. MS/LMRS [ESI]$^{+}$*m/z* calcd (for $C_{35}H_{42}N_2NaO_{15}$): *m/z* =753.25, found 753.40.
^{1}H NMR (300 MHz, $CDCl_3$) δ 2.02 (s, 3H, OC(O)CH_3), 2.0 (s, 3H, OC(O)CH_3), 2.10 (s, 3H, OC(O)CH_3), 2.11 (s, 3H, OC(O)CH_3), 2.43 (t, 5.6 Hz, 2H, CH_2COOMe), 2.8 (d, 6.7 Hz, 2H, CH_2CHNH), 3.42 (t, 5.6 Hz, 2H, CH_2NH), 3.77 (s, 3H, OCH_3), 4.00 (m, 1H, CHOC(O)CH_3), 4.11 (m, 2H, CH_2CHOC(O)CH_3), 4.96 (t, 5.6 Hz,1H, CHNH), 5.11 (m, 2H, CHOC(O)CH_3), 5.22 (d, 2H, 5.6 Hz, CH_2Ph), 5.24 (m, 1H, CHOC(O)CH_3), 6.2 (d, 10 Hz, 1H, OCHOPh), 6.87(d, 9.0 Hz, 2H, Ph), 6.80 (br s, 1H, NH), 6.95 (d, 9.0 Hz, 2H, Ph), 7.00 (br-s, 1H, NH). 7.23 (m, 5H, Ph).^{13}C NMR (300 MHz, $CDCl_3$) δ 20.6, 2.7, 20.7, 21.0, 33.9, 34.8, 38.1, 51.8, 56.4, 60.4, 63.4, 67.0, 67.8, 70.4, 72.5, 92.0, 115.7, 117.3, 128.0 (2C), 128.2, 128.5 (2C), 130.4 2C), 135.5, 139.3, 155.3, 155.9, 169.5, 169.8, 169.9, 170.1, 170.7, 171.2.

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl-*N*-Cbz-L-tyrosyl-β-alanyl-L-leucine methyl ester 2

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide **4** (755 mg, 1.84 mmol), **14** (1.06g, 2.21 mmol), NaOH (88.4 mg, 2.21 mmol), water (10 mL) and acetone (10 mL) were mixed and stirred for 72 h. Silica gel column chromatography using ethyl acetate-cyclohexane (8/2) afforded **2** as an off-white solid after removal of solvents (1.14 g, 77%). mp = 108.5°C. $[\alpha]^{22}_{D}$ -14.2°, *c* = 1 in MeOH. MS/LMRS [ESI]$^{+}$*m/z* calcd(for $C_{38}H_{55}N_3NaO_{16}$): *m/z* =832.35, found 832.51. ^{1}H NMR (300 MHz, DMSO) δ 0.96 (d, 6 Hz, 6H, CH_3i-Bu), 1.32 (s, 9H, t-Bu), 1.55 (m, 1H, CH(CH_3)$_2$), 1.71 (m, 2H,CH_2 CH(CH_3)$_2$), 2.01 (s, 3H, OC(O)CH_3), 2.0 (s, 3H, OC(O)CH_3), 2.105 (s, 3H, OC(O)CH_3), 2.11 (s, 3H, OC(O)CH_3), 2.4 (t, 5.5 Hz, 2H, CH_2CON), 2.80 (d, 6.7 Hz, 2H, CH_2CHCON), 3.62 (s, 3H, OMe), 3.55 (m, 2H, CH_2NH), 4.01 (m, 1H, CHOC(O)CH_3), 4.11 (m, 2H, CH_2OAc), 4.33 (m, 1H, CHCO_2Me), 5.10 (m, 2H, CHOC(O)CH_3, CHOC(O)CH_3), 5.33 (m, 1H, CHOC(O)CH_3), 5.44 (t, 5.6 Hz, 1H, CHNHCOBoc), 6.77 (d, 9 Hz, 2H, Ph), 5.50 (d, 1H, 10 Hz, OCHOPh), 6.88 (br-s, 1H, NH), 7.00 (d, 9 Hz, 2H, Ph), 7.90 (br-s, 1H, NH), 8.30 (br-s, 1H, NH). ^{13}C NMR (300 MHz, DMSO) δ 20.5, 20.8, 20.9, 21.1, 21.8, 23.2, 24.96, 28.62 (3C), 35.6, 37.0, 39.2, 39.5, 50.7, 52.3, 56.5, 68.2, 69.4, 69.9 70.0, 71.2, 78.7, 98.3, 115.5 (2C), 128.6, 130.5 (2C), 155.6, 166.4, 169.8, 169.9, 170.1, 170.5, 171.1, 172.2, 173.6.

References

1- G. Trimurtulu, I. Ohtani, G.M.L. Patterson, R. E. Moore, T. H. Corbett, F. A. Valeriote, and L. Demchiko, J. Am. Chem. Soc. **1994**, 116, 4729-4737.
2- R. E. Moore, T. H. Corbett, G. M. L. Patterson, and F. A. Valeriote, Curr. Pharm. Des. **1996**, 2, 317- 330.
3- S. Chaganty, G. Trimurtulu, C. E. Heltzel, R. E. Moore, and W. Y. Yoshida, J. Nat. Prod. **2004,** 67, 1403-1406.
4- G. V. Subbaraju, G. Trimurtulu, G. M. L. Patterson, and R. E. Moore, J. Nat. Prod. **1997,** 60, 302-305.
5- (a) J. D. White, Jian Hong, and L. A. Robarge, J. Org. Chem. **1999,** *64,* 6206-6216.
(b) Ghosh, A. K.; Swanson, L. J. Org. Chem. **2003**, 68, 9823-9826.
6- B. H. Norman, T. Hemscheidt, R. M. Schultz, and S. I. Andis, J. Org. Chem. **1998**, 63, 5288-5294.

7- J. Liang, E. D. Moher, R. E. Moore, and D. W. Hoard, J. Org. Chem. **2000,** 65, 3143-3147.
8- A. K. Ghosh, and A. Bischoff, Org. Lett., **2000**, 2 (11), 1573-1575.
9- U. P. Dhokte, V. V. Khau, D. R. Hutchison, and M. J. Martinelli, Tetrahedron Lett. **1998**, 39, 8771-8774.
10- N. K. Tripathy, and G. I. Georg, G. I. Tetrahedron Lett. **2004,** *45*, 5309–5311.
11- M. Kobayashi, W. Wang, N. Ohyabu, M. Kurosu,.and I. Kitagawa, Chem. Pharm. Bull. **1995**, 43, 1598-1600.
12- J. D. White, J. Hong, J.; Robarge, L. A. J. Org. Chem. **1999**, 64, 6206-6216.
13- M. Eggen, C. J. Mossman, S. B. Buck, S. K. Nair, L. Bhat, S. M. Ali, E. A. Reiff, T. C. Boge, and G. I. Georg, J. Org. Chem. **2000**, 65, 7792-7799.
14- MJ. Eggen, S. K. Nair, and G. I. Georg, Org. Lett. **2001**, 3, 1813-1815.
15- M. A. Tius, Tetrahedron **2002**, 58, 4343-4367.
16- R. E. Schwartz, C. F. Hirsch, D. F. Sesin, J. E. Flor, M. Chartrain, R. E. Fromtling, G. H. Harris, M. J. Salvatore, J. M. Liesch, and K. Yudin, J. Ind. Microbiol. **1990**, 5, 113-124.
17- N. A. Magarvey, Z. Q. Beck, G. Trimurtulu, Y. Ding, U. Huber, T. K. Hemscheidt, D. Abelson, R. E. Moore, and D. H. Sherman, ACS Chem. Biol., **2006**, 1 (12), 766-779
18- S. Eissler, M. Nahrwold, B. Neumann, H-G. Stammler, and Sewald, N. Org. Lett.**2007**, 9 (5), 817-819.
19- M. Nahrwold, T. Bogner, S. Eissler, S. Verma, and N. Sewald, Org. Lett.**2010**, 12 (5), 1064-1067.
20- M. J. Eggen, and G. I. Georg, Bioorg. Med. Chem. Lett. **1998**, 8, 3177-3180.
21- a) R. Vidya, MJ. Eggen, G. I. Georga, and R. H. Himes, Bioorg. Med. Chem. Lett. **2003**, 13, 757–760.
b) C. Weiβ, T. Bogner, B. Sammett and N. Sewald *Beilstein J. Org. Chem*. **2012**, 8, 2060-2066.
22- S. B. Buck, J.K. Huff, R. H. Himes, and G. I. Georg, J. Med. Chem. **2004**, 47, 696- 702.
23- C. Shih, L. S. Gossett, J. M. Gruber, C. S. Grossman, S. L. Andis, R. M.; J. F. Schultz, Worzalla, T. H. Corbett, and J. T. Metz, Bioorg. Med. Chem. Lett. **1999,** 9, 69-74.
24- R. Vidya, MJ. Eggen, S. K. Nair, G. I. Georg, and R. H. Himes, J. Org. Chem. **2003**, 68, 9687-9693.
25- R. S. Al-awar, J. E. Ray, R. M. Schultz, S. L. Andis, J. H. Kennedy, R. E. Moore, L. Liang, G. Trimurtulu, G. V. Subbaraju, and T. H. Corbett, J. Med. Chem. **2003,** 46, 2985-3007.
26- M. Furuyama, and I. Shimizu, Tetrahedron: Asymmetry **1998**, 9, 1351-1357.
27- M. Lautens, and M. L. Maddess, Org. Lett., **2004**, 6 (12), 1883-1886.
28- A. B. Smith, Y. Shin Cho, G. R. Pettit, and R. Hirschmann, Tetrahedron **2003**, *59*, 6991-7009.
29- C. Pousset, M. Haddad, and M. Larchevêque, Tetrahedron, **2001**, 57, 7163-7167.
30- A. B. Smith, Y. Shin Cho, L. E. Zawacki, R. Hirschmann,, and G. R. Pettit, Org. Lett., **2001**, 3 (25), 4063-4066.
31 – V. F. Patel, S. L. Andis, J. H. Kennedy, J. E. Ray, and R. M. Schultz, J. Med. Chem. **1999,** 42, 2588-2603.
32- S. Raghavan, and K. A. Tony, J. Org. Chem. **2003**, 68, 5002-5005.

33- W. L. Liu, J. C. Zhang, F. Qin Jiang, and L. Fu, Arch. Pharm. Chem. Life Sci. **2009**, 342, 577-583.
34- J. M. de Muys, R. Rej, D. Nguyen, B. Go, S. Fortin, and J-F. Lavallée, Bioorg.Med. Chem. Lett., **1996**, 6, 1111-1116.
35- R. Rej, D. Nguyen, B. Go, S. Fortin, and J-F. Lavallée, J. Org. Chem. **1996,** 61, 6289-6295.
36- J. Cooksey, A. Gunn, P. J. Kocienski, A. Kuhl, S. Uppal, J. A. Christopher, and R. Bell, Org. Biomol. Chem., **2004**, 2, 1719-1731.
37- J. A. McCubbin, M. L. Maddess, and M. Lautens, Org. Lett., **2006**, 8(14), 2993-2996.
38- C. A. Mast, S. E. A. Stoncˇius, H-G. Stammler, B. Neumann, and N. Sewald, Chem. Eur. J. **2005**, 11, 4667-4677.
39- S. Eißler, T. Bogner, M. Nahrwold, and N. Sewald, Chem. Eur. J. **2009**, 15, 11273-11287.
40- R. A. Barrow, T. Hemscheidt, J. Liang, S. Paik, R. E. Moore, and M.A. Tius, J. Am. Chem. Soc. **1995**, 117, 2479-2490.
41- K. M. Gardinier, and J. W. Leahy, J. Org. Chem. **1997,** 62, 7098-7099.
42- J. Liang, R. E. Moore, E. D. Moher, J. E. Munroe, R. S. Al-awar, D. A. Hay, D. L. Varie, T. Y. Zhang, J. A. Aikins, M. J. Martinelli, C. Shih, J. E. Ray, L. L. Gibson, V. Vasu Vasudevan, L. Polin, K. White, J. Kushner, C. Simpson, S. Pugh, and T. H. Corbett, Investig. New Drugs **2005**, 23**,** 213-224.
43- S. M. Ali, and G. I. Georg, Tetrahedron Lett., **1997,** 38, 1703-1706.
44- G. I. Georg, S. M. Ali, V. J. Stella, W. N. Waugh, and R. H. Himes, Bioorg. Med. Chem. Lett. **1998**, 8, 1959-1962.
45- (a) S. B. Buck, J. K. Huff, R. H. Himes, and G. I. Georg, J. Med. Chem. **2004,** 47, 696-702.
(b) S. B. Buck, J. K. Huff, R. H. Himes, and G. I. Georg, J. Med. Chem. **2004,** 47, 3697-3699.
46- G. M. Salamonczyk, Kang Han, Zhi-wei Guo, and Charles J. Sih J. Org. Chem. **1996,** 61, 6893-6900.
47- F. Z.Baba Ahmed, H. Merzouk, S. Bouanane, N. B. Benkalfat, S. A. Merzouk, J. Kajima Mulengi,and M. Narcel, Ann. Toxicol. Anal. **2010**, DOI : 10. 1051/ata/2010017.
48- S. Koide, and S. Sidhu ACS. Chem. Biol. **2009**, 4, 325-334.
49- (a) C. Dye, and B. G. Williams, Science, **2010**, 328, 856-861.
(b) B. Cao, and S. Williams, J. Nat. Prod. Re*p*. **2010**, 27, 919-947.
(c) K. Ohtsubo, and J. D. Marth, Cell **2006**, 126, 855-867.
(d) S. M. Muthana, C. T. Campbell, and J. C. Gildersleeve, ACS Chem. Biol. **2012**, 7, 31-43.
(e) Xi Chen ACS Chem. Biol. **2011**, 6(1), 14-17.
(f) B. Cao, J. M. White, and S. J. Williams, Beilstein J. Org. Chem. **2011**, 7, 369-377.
50- (a) J. A. Singh, Chun-Xiao Xu, Xiaoming Xu, L. M. West, A. Wilines, A. Chan, E. Hamel, J. H. Miller, P. T. Northcote, and A. K. Ghosh, J. Org. Chem. **2010**, 75, 2-10.
(b) J. G. Swoboda, E. Mylonakis, B. J. Wilkinson, and S. Walker, ACS Chem. Biol. **2011**, 6, 106-116).
(c) T. J. Boltje, T. Buskas, and G. J. Boons, Nature Chemistry, **2009**, 1, 611-622.
51- G. J. L. Bermudes, B. Castagner, and P. H. Seeberg, ACS Chem Biol. **2009**, 4, 703-713.

52- L. Schofield, M. C. Hewitt, K. Evans, M. A. Siomos, and P. H. Seeberg, Nature, **2002**, 418, 785-789.
53- (a) J. A. Prescher, and C. R. Bertozzi, Cell **2006**, 126, 851-854.
(b) O. T. Keppler, R. Horstkorte, M. Pawlita, C. Schmidt, and W. Reutter, Glycobiology **2001**, 11, 11R-18R.
54- (a) I. O. Potapenko, V. D. Haaneken, T. Luders, A. Helland, I. Bukholm, T. Sorlie, V. N. Kristensen, O. C. Lingjaerde, and A. L. Borresen-Dale, Mol. Oncol. **2010**, 4, 98-118.
(b) H. Yu, and X. Chen, Org. Biomol. Chem. **2007**, 5, 865-872.
(c) M. Gutterigg, S. Burgmayr, G. Poltl, J. Rudolf, and E. Staudacher, Glycoconjugates J. **2007**, 24, 475-489.
55- C. I. Gama, S. E. Tully, N. Sotogatu, P. M. Clark, M. Rawat, N. Vaidehi, W. A. Goddard, 3rd, A. Nishi, and L. C. Hsieh-Wilson, Nat. Chem. Biol. **2006**, 2, 467-473.
57- B. S. Furniss, A. J. Hannaford, V. Rogers, P. W. G. Smith A. R. Tatchell, Vogel's Textbook of Practical Organic Chemistry, 4th Edition, Longman Group Limited, London, **1978**, ISBN 0-582-44250-8, p454-455.
58- a) B. S. Furniss, A. J. Hannaford, V. Rogers, P. W. G. Smith A. R. Tatchell, Vogel's Textbook of Practical Organic Chemistry, 4th Edition, Longman Group Limited, London, **1978**, ISBN 0-582-44250-8, p457-458)
b) S. Pearson, W. Scarano, M. H. Stenzel Chem. Commun. **2012**,
59- N. Imai, T. Kometani , P. J. Crocker, J. B. Bowdan, A. Demir, L. D. Dwyer, D. M. Mann, T. C. Vanaman, and D. S. Watt, Bioconjugate Chem. **1990**, 1(2), 138-143
60- J. G. Herandez, and E. Juaristi, J. Org. Chem. **2010**, 75, 7107-7111