Synthesis of Non-Natural *O*-Glycosylamino Acids Derived from *n*-Pentenyl Glycosides; Model Studies and Proof of Principle for Glycopeptide Synthesis

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Dedicated to Professor Horst Kunz for his Continuing, Unique Contributions at the Interface of Organic Chemistry and Glycobiology on the Occasion of his 60th Birthday

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Abstract. Model studies on the transformation of the olefinic unit contained in *n*-pentenyl glycosides (NPGs) to glycoamino acids is described. The methodology involves a Horner-Emmons olefination with a protected glycine derived phosphonate, followed by asymmetric hydrogenation using DuPHOS catalyst system. A variety of protecting group schemes have been investigated and their stereoselectivity in the hydrogenation reaction determined. With *N*-Boc and *C*-TSE ester protection, the diastereoselectivity in the reaction was measured by ^{1}H NMR analysis with "racemic" product as a comparison. These modified glycoamino acids are also useful for peptide synthesis. The methodology appears to be general and was extended to include the synthesis a glycoamino acid containing the complex hexasaccharide Globo-H.

Glycoproteins and glycopeptides play an important role in a number of cellular recognition events including tumor metastasis, immune surveillance, cell-cell communication, cell growth and chemotaxis [1]. The systematic study of glycopeptides as structural elements found in cellular glycoproteins has emerged as a powerful way to investigate the role of glycosylation in protein structure and function. In hoping for an entry into the potential value of glycopeptide assemblies as potential therapeutic agents, it would be necessary to gain access to such agents through chemical synthesis.

Our laboratory has been pursuing the synthesis of complex oligosaccharides, glycopeptides and glycoconjugates [2]. The search for synthetic methodology of general applicability for the preparation of oligosaccharides in the form of glycoproteins, glycolipids and glycopeptides remains a significant goal. Current interest is in the synthesis of glycoproteins and glycopeptides that mimic components of tumor cell surfaces. Toward this end, we have been focusing on the chemical synthesis of glycoconjugates and glycopeptides carrying tumor-associated antigens for the purpose of evaluating these constructs in an anti-cancer vaccine setting [3].

Native glycoproteins and glycopeptides are typically glycosylated through the side chain hydroxyl groups of either serine or threonine with an α-glycosidic linkage. A particularly striking example is that of mucin glycoproteins. Mucins possess amino acid sequences with a very high percentage of serine and threonine residues wherein the first carbohydrate moiety is prevailingly α-*O*-linked *N*-acetylgalactosamine [4]. In addition, Tn, TF, STn, ST and glycophorin, comprise a class of tumor-associated antigens carrying this α-*O*-linkage. Such compounds have received much synthetic and immunological attention [5]. Primary synthetic effort directed at *O*-linked glycopeptides has centered on the stereoselective construction of the naturally required α-glycosidic linkage. The creative, pioneering work of Kunz [6], Paulsen [7], Schmidt [8]and others [9] has provided a framework for organic chemists to continue to progress toward a general and processable synthesis of native glycopeptides. In fact, solid phase synthesis of glycopeptides and the synthesis of combinatorial libraries containing glycopeptides has recently been accomplished [10, 11]. Synthetic studies involving glycopeptides in our own laboratory have thus far relied on a "cassette" modality rather than a maximally convergent approach [12, 13].

Compelling issues in biology and immunology have recently inspired equal efforts toward the synthesis of more readily available and more chemically stable glycopeptide analogs [14]. For example, as mimics of *O*-linked glycopeptides, *C*-linked and *S*-linked oligosaccharides have been attractive targets. Non-natural linkages avoid problems associated with the chemical and enzymatic lability of *O*-glycosyl serine. These synthetic endeavours have enabled studies demonstrating that well designed glycopeptide mimics confer biological activity similar to their native counterparts and consequently, have provided more potent therapeutic agents [15]. Given these successes, we recognized that the possibility of glycopeptides carrying tumor-associated antigens derived from non-natural amino acids being therapeutically useful in an anti-tumor vaccine setting has never been addressed [16].

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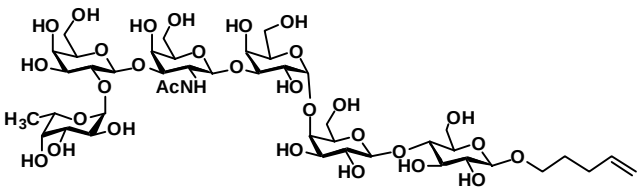
Background

The MBr1 antigen, Globo-H, was isolated and characterized as a cell surface antigen associated with breast cancer [17]. Globo-H has subsequently been further characterized as being over-expressed in other types of carcinomas including colon, prostate, lung, ovary and small cell lung cancers [18]. A first generation total synthesis of this antigen in our laboratory [19] has advanced a fully synthetic Globo-H glycoconjugate vaccine to phase II and III human clinical trials against prostate and breast cancer [20]. Following the successful synthesis of another of our oligosaccharide antigens as its *n*-pentenyl glycoside (NPG) [21], we completed a second-generation synthesis of Globo-H [22] as its corresponding NPG, **1** (NPG). The synthesis of **1** was much improved. Furthermore, we have related efficient procedures for its conjugation to yield a glycoconjugate vaccine.

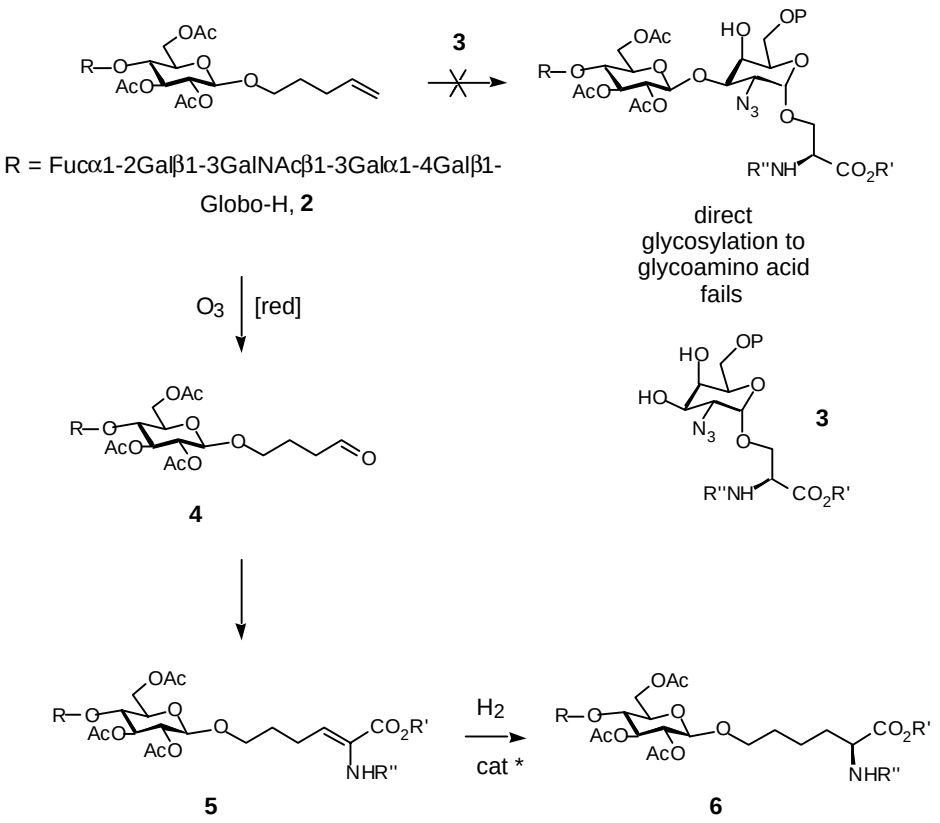
Given our continued interest in the synthesis and immunological evaluation of glycopeptide-based anti-tumor vaccines, an additional goal for our program was an efficient and general way to incorporate the complex hexasaccharide contained in Globo-H into glycopeptides. Following the work of Fraser-Reid and associates [23], our initial efforts at generating a Globo-H containing glycoamino acid were directed toward performing a direct glycosylation to yield a construct with the naturally required α-*O*-serine linkage. Unfortunately, under no conditions were we able to accomplish the [6+1] coupling of peracetylated donor **2** with cassette acceptor **3** (Scheme 1). Attempted conversion of the NPG in **2** to alternative classical glycosyl donors was also fruitless.

The transformation of the olefinic unit contained in *n*-pentenyl glycosides to various spacer functionalities has been described [24]. Using this unsaturation to achieve an amino acid attachment, would provide access to glycoamino acids derived from *n*-pentenyl glycosides such as **1**. Fortunately, precedent from Toone and co-workers suggested a plausible approach. Their work detailed methodology based on catalytic asymmetric hydrogenation of *C*-allyl glycosylated enamide



Globo-H *n*-Pentenyl Glycoside

1



Scheme 1 Proposed synthesis of glycoamino acid derivatives

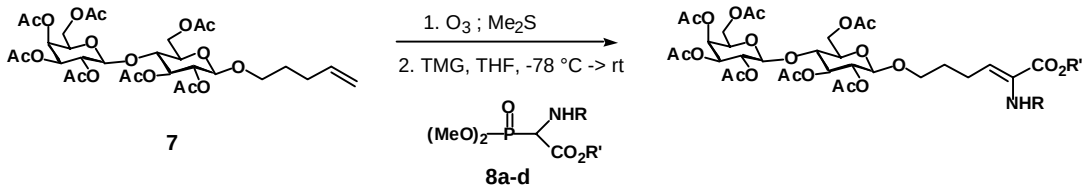
esters to produce carbon linked glycosyl serines [25]. An analogous transformation using an NPG as a starting material is presented in Scheme 1. The proposed methodology requires an ozonolysis step to give aldehyde **4**, followed by a Horner-Emmons olefination of with a suitably protected glycine derived phosphonate to give enamide ester **5**. Subsequent hydrogenation would yield glycoamino acid, **6**. Moreover, achieving the hydrogenation in an asymmetric fashion would yield diastereomerically pure glycoamino acid derivatives **6**. Following this application, the resulting glycoamino acids would represent a non-natural β-glycosidic linkage between oligosaccharide and amino acid. However, maturation of methodology to achieve such a linkage would provide an exciting synthetic possibility. We envisioned the study of the effect of these types of non-natural linkages in artificial glycopeptides as mimics of tumor cell surfaces. The disclosure herein focuses on model studies designed for the synthesis of glycoamino acids from their corresponding *n*-pentenyl glycosides and represents new general methodology for glycopeptide development.

Results and Discussion

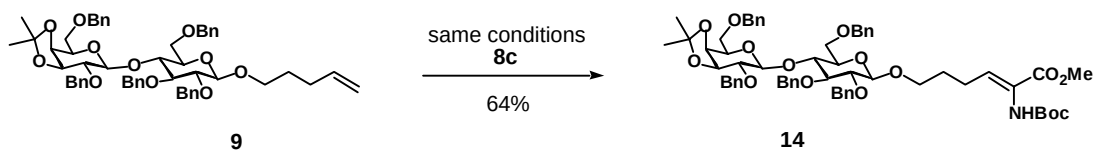
We began by conducting model studies on lactose derived NPG's. We were mindful of the fact that in the hydrogenation reaction, the newly generated stereogenic center would be five atoms removed from other chirality in the molecule. Although this separation might be favourable in terms of minimizing any substrate controlled bias in the reduction reaction, we anticipated that it could complicate our analysis of the outcome and separation of the products by chromatographic means. Accordingly, we began by surveying a variety of protecting group schemes in the enamide ester to establish the feasibility of performing the required hydrogenation, as well as of determining the diastereoselectivity of the reaction.

The preparation of enamide esters **10**–**14** is shown in Scheme 2. The known *n*-pentenyl lactoside **7** [21, 22] was subjected to ozonolysis to provide the four-carbon aldehyde. Subsequent reaction with a variety of protected glycine derived phosphonates, **8a**–**d**, gave enamides **10**–**13** in the yields shown [26]. In a similar effort, reaction of the aldehyde corresponding to the differentially protected NPG **9** [21] afforded enamide **14** in 64% yield for the 2-step procedure. In all cases, using tetramethyl guanidine as the base, the product enamides were formed as single (*Z*) geometric isomers.

Clearly, some of the protecting group patterns used in the synthesis of **10**–**13** would not ultimately be useful for peptide synthesis with peracylated carbohydrates. Our survey, however, was based on the assumption that diastereoselectivity in a chiral ligand-based asymmetric hydrogenation approach to these substrates might be subject to electronic, steric and coordination effects



phosphonate	R'	R	yield (2 steps)
8a	Me	CBz	**10**, 78%
8b	Me	Ac	**11**, 88%
8c	Me	Boc	**12**, 85%
8d	TSE	Boc	**13**, 88%



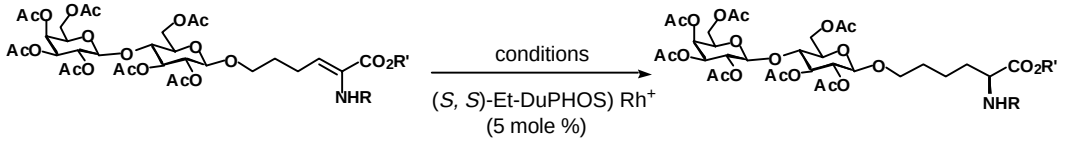
Scheme 2 Conversion to enamide ester derivatives

in the amino and acid protecting groups. This has been well documented in the case of enamide ester hydrogenations [27]. Substrate **9** is an intermediate in our synthesis of the fucosylated ganglioside GM_1 [21] and was chosen in this model study to access whether the nature of the carbohydrate protecting groups contribute any bias in the hydrogenation reaction [28]. In addition, because of the complex nature of our oligosaccharides, identifying informative regions in the ^{1}H NMR spectra of the products which would be useful for diastereoselectivity determination was not immediately straight forward. Although at this point in our investigations we did not necessarily require ultimate stereoselectivity in the hydrogenation step, we sought a general method for determination of diastereoselectivity that could ultimately be used with virtually any of our tumor-associated carbohydrate antigens.

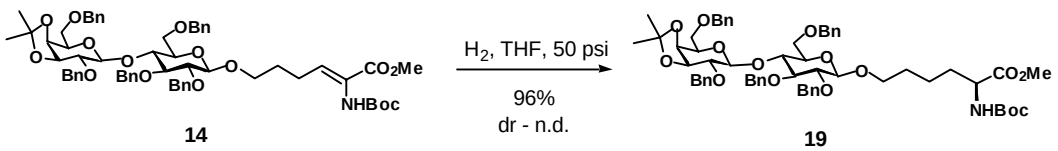
In the hydrogenation of similar *C*-glycosylated enamides, Toone had reported the use of chiral DuPHOS ligands as catalyst precursors [29] and had optimized the resulting diastereomeric excesses of the products with respect to chiral ligand and solvent. Following their report, we chose to concentrate on the Et-DuPHOS catalyst precursors. In addition, we used the (*S, S*) ligand isomer, which has been well characterized in these types of systems to give the (*S*)-isomer in the amino acid product [30]. Finally, in all cases, hydrogenation with an achiral catalyst (Pd/C or Wilkenson's catalyst) produced a 1:1 mixture of *R* and *S* configured products, providing a comparison for diastereomeric ratio (*dr*) determination [31].

The results of hydrogenation using [(*S, S*)-Et-DuPHOS]Rh^+ as the catalyst precursor are tabulated in Scheme 3. In the case of substrate **11**, with an *N*-acetamide protecting group, we found that atmospheric hydrogen pressures were acceptable, and the reduction reaction proceeded cleanly to give glycoamino acid **16** in toluene at 50 °C in excellent yield. Product **16** was determined to have been formed with a diastereomeric ratio (*dr*) of >20:1. Remarkably, hydrogenation of **11** with an achiral catalyst yields a product mixture where several of the acetate CH_3 protons are completely baseline resolved in the ^{1}H NMR [32]. In the asymmetric reaction shown in Scheme 3, the minor isomer could not be detected by ^{1}H NMR. ^{13}C analysis also shows a 1:1 diastereomeric mixture using an achiral catalyst, and supports the conclusion that the minor isomer is not formed within the limit of NMR detection in the asymmetric reduction.

The conversion of **10** and **12**–**14** to products **15** and **17**–**19** required increased hydrogen pressures to assure complete conversion of the enamide substrates. Tetrahydrofuran as solvent at 50 psi of hydrogen was found to be optimal for these reactions. In products **15** and **19**, the ^{1}H and ^{13}C NMR spectra did not show the presence of another isomer. However, the achiral reductions of



	R'	R	conditions	yield, dr * [a)]
10	Me	CBz	H_2, THF, 50 psi	**15**, 92%, n.d.
11	Me	Ac	H_2, toluene, 50 °C	**16**, 95%, >20:1
12	Me	Boc	H_2, THF, 50 psi	**17**, 98%, >20:1
13	TSE	Boc	H_2, THF, 50 psi	**18**, 98%, >20:1



14 **19**

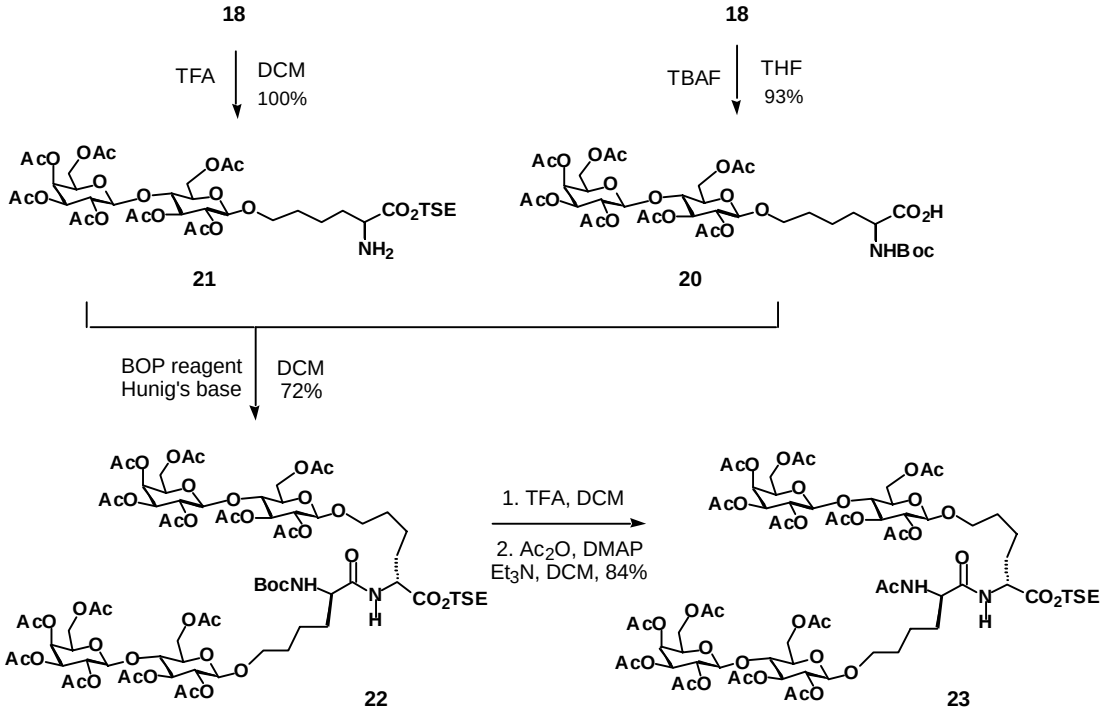
Scheme 3 Asymmetric hydrogenation reactions

[a)] key: * minor diastereomer not detected by ^{1}H NMR; dr = diastereomeric ratio; n.d. = not determinable;

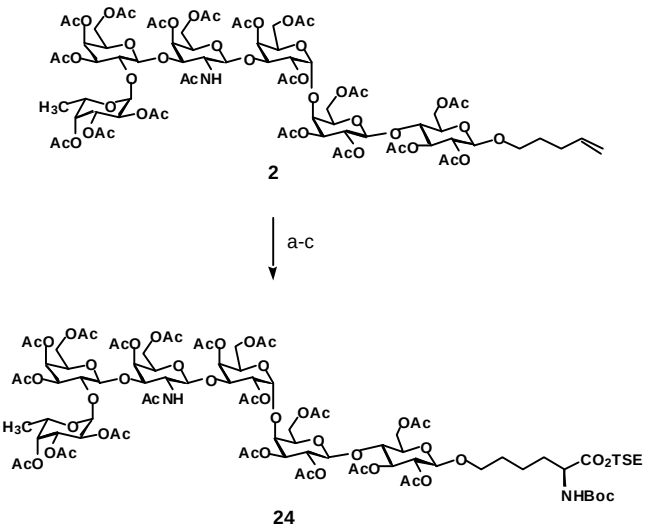
10 and **14** produced diastereomeric mixtures of **15** and **19** that were not completely baseline resolved for diastereomeric ratio determination. The methyl ester protons in **15** and **19** were obscured by other carbohydrate signals and in the case of **19**, the *t*-Boc protons were also inconclusive. Although, it is highly possible that these reductions are in fact completely stereospecific, without an accurate spectral interpretation of racemate, we conclude these substrates to be 'not determinable' (n.d.). On the other hand, products **17** and **18** were determined to be formed with excellent diastereoselectivity. The *t*-Boc protons in both products are nearly baseline resolved. With these detection limits by NMR, the DuPHOS mediated reduction does not produce any measurable amount of the minor isomer by NMR determination. In the case of **18**, the trimethylsilyl proton signals in the TSE (2-trimethylsilyl ethyl) ester are slightly separated, yet not baseline resolved. The reduction of **11** also proceeds with high diastereoselectivty (>20:1) in THF at 50 psi (not shown).

Fortunately, the successful conversion of **13** to glycoamino acid **18** allowed for its incorporation into peptide synthesis. In order to evaluate the possibility of the non-natural linkages contained in the NPG derived glycoamino acids to be substrates for peptide synthesis, we next proceeded to amino acid deprotection of **18**. As shown in Scheme 4, the peracetylated lactose model **18** was treated with TBAF in THF to give the corresponding acid **20** in 93% isolated yield. Moreover, treatment of **18** with trifuoroacetic acid (TFA) in dichloromethane yielded the corresponding amine as its TFA salt, **21**. These conversions allowed the formation of a lactose–lactose dipeptide as a model target. In the event, coupling of amine **21** and acid **20** under the agency of the BOP reagent (benzotriazol-1-oxytris(dimethylamino)phosphonium hexafluorophosphate [33]) afforded the corresponding amide **22** in 72% yield. Subsequent removal of the *N*-terminal Boc group followed by capping with acetic anhydride gave the *N*-acetylated dipeptide **23** in 84% yield for the two steps.

With peptide **23** in hand, as well as appropriate confidence in our model studies, we envisioned extending this chemistry to include the preparation of a glycoamino acid containing the tumor antigen Globo-H [34]. Accordingly, following identical procedures to those described above, the hexasaccharide NPG **2** [22] was converted to the corresponding glycoamino acid **24** in the yields shown (Scheme 5). As in the case of **18**, hexasaccharide **24** was determined to be formed with a diastereomeric ratio of >20:1 by ^{1}H NMR analysis. The NPG-derived glycoamino acid **24** was synthesized with the expectation of incorporating Globo-H into a glycopeptide. Such glycopeptides will be used to provide an assessment of inducing antibody production against artificial glycopeptides in an anti-tumor vaccine setting.



Scheme 4 Synthesis of dipeptide **23**



Scheme 5 Synthesis of Globo-H derived glycoamino acid [a]Key: a) O_3, MeOH, DCM; Me_2S; b) TMG, THF, **8d** (R = TSE, R' = Boc), –78 °C, 72% 2 steps; c) (*S, S*)-Et-DuPHOS-Rh+, THF, 50 psi, 98%, dr >20:1.

Conclusions

A novel preparation of non-natural glycoamino acids starting from *n*-pentenyl glycosides has been achieved. Model studies have shown that the synthetic methodology appears to be general and, with the *N*-Ac/Me ester, *N*-Boc/Me ester or *N*-Boc/TSE ester protecting group system, the diastereomeric selectivity can be determined using NMR analysis. The olefination and asymmetric hydrogenation strategy using the DuPHOS catalysts was useful for the preparation of glycoamino acids containing lactose and the MBr1 antigen (Globo-H). These *n*-pentenyl glycoside-derived glycoamino acids, with the *N*-Boc/TSE ester protection, can also serve as units for peptide synthesis. The strategy described herein is now being applied to other complex tumor-associated antigens, as well as their incorporation into glycopeptides [35]. The synthesis and immunological studies of these constructs are well underway and will be reported in due course.

Acknowledgments

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Experimental

DuPHOS-Rh^+ catalysts were purchased from Strem Chemical Co., Newburyport, MA. All other commercial materials (purchased from Aldrich-Sigma) were used without further purification. The following solvents were obtained from a dry solvent system (passed through a column of alumina): THF, diethyl ether (Et_2O), CH_2Cl_2, toluene and benzene. All reactions were performed under an atmosphere of dry N_2, unless otherwise noted. NMR (^1H and ^{13}C) spectra were recorded on a Bruker AMX-400 MHz or Bruker Advance DRX-500 MHz and referenced to residual solvent unless otherwise noted. IR spectra were recorded with a Perkin-Elmer 1600 series-FTIR spectrometer and optical rotations were measured with a Jasco DIP-370 digital polarimeter using a 10-cm path length cell. Low-resolution mass spectral analyses were performed with a JOEL JMS-DX-303 HF mass spectrometer. Analytical TLC was performed on E. Merck silica gel 60 F254 plates and flash column chromatography was performed using the indicated solvents on E. Merck silica gel 60 (40–63 mm) or Sigma H-type silica gel (10–40 mm).

Olefination (General Procedure)

Enamide **13**

The preparation of enamide **13** is representative of this procedure. The *n*-pentenyl glycoside **7** (472 mg, 0.673 mmol) was dissolved in 10:10:1 MeOH:CH_2Cl_2:pyridine (8 mL) and cooled to –78 °C. A stream of dry ozone was passed through the reaction mixture until a pale blue colour persisted. The ozone source was removed and the reaction stirred at –78 °C for an additional 15 minutes, upon which time a stream of dry nitrogen was applied to remove excess ozone. Dimethyl sulfide (50 equivs., 2.5 mL) was added to the cooled mixture, the cooling bath was removed and the reaction was allowed to stir at rt for 4 h. The reaction was diluted with CH_2Cl_2 (100 mL), washed with water (250 mL), and back-extracted with additional CH_2Cl_2 (2×50 mL). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated. The crude aldehyde was typically not purified, but was azeotroped dry with anhydrous benzene (3×10 mL) and used directly in the next step.

Phosphonate **8d** (1.10 equivs., 283 mg) was dissolved in anhydrous THF (1.0 mL), cooled to –78 °C and tetramethyl guanidine (TMG) (1.2 equivs., 0.101 mL) was added dropwise. The reaction stirred at –78 for 30 minutes, followed by addition of the crude aldehyde in additional THF (2×0.5 mL). The reaction was allowed to stir at rt overnight (15 h), was extracted with EtOAc (50mL), washed with 0.05 M aqueous HCl (150 mL) and back-extracted with additional EtOAc (2×50 mL). The combined organic layer was dried over $MgSO_4$, concentrated and purified by flash column chromatography (25% EtOAc/hexanes -> 50% EtOAc) to yield the desired enamide ester **13** as a single isomer (570 mg, 88%): white foam; R_f = 0.45 (66% EtOAc/hexanes). – IR ($CDCl_3$ film): ν/cm^{-1} = 3407, 3146, 2954, 2898, 1752, 1654, 1233, 1167, 1055. – ^1H NMR ($CDCl_3$, 400 MHz): δ/ppm = 6.34 (m, 1H), 6.03 (bs, 1H), 5.22 (d, 1H, J = 3.2 Hz), 5.07 (t, 1H, J = 9.4 Hz), 4.99 (dd, 1H, J = 10.3, 7.9 Hz), 4.83 (dd, 1H, J = 10.5, 3.3 Hz), 4.77 (t, 1H, J = 9.3 Hz), 4.37–4.33 (m, 3H), 4.20–4.11 (m, 3H), 4.08–4.00 (m, 3H), 3.82–3.65 (m, 5H),

3.49–3.46 (m, 1H), 3.39–3.34 (m, 1H), 2.16–2.14 (m, 1H), 2.11–2.09 (m, 1H), 2.04 (s, 3H), 2.02 (s, 3H), 1.94 (s, 3H), 1.93 (s, 3H), 1.92 (s, 6H), 1.84 (s, 3H), 1.62–1.59 (m, 2H), 1.34 (s, 9H), 0.94–0.89 (m, 2H), 0.05 (s, 9H). – ^{13}C NMR ($CDCl_3$, 100 MHz): δ/ppm =170.29, 170.21, 170.03, 169.94, 169.66, 169.50, 168.97, 164.77, 153.20, 134.70, 100.97, 100.31, 80.23, 76.17, 72.69, 72.51, 71.56, 70.87, 70.52, 68.95, 68.83, 66.47, 63.54, 61.88, 60.66, 33.81, 28.05, 27.92, 24.47, 20.73, 20.68, 20.57, 20.51, 20.39, 17.21, –1.60. – HRMS (FAB) calcd. for $C_{43}H_{67}NO_{21}SiNa$ 986.4013, found 986.4029.

Enamide **11**

88%, white foam; R_f = 0.55 (100% EtOAc). – IR ($CDCl_3$ film): ν/cm^{-1} = 3474, 3354, 3158, 2977, 2952, 2884, 1755, 1657, 1368, 1236, 1057. – ^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm = 6.97 (bs, 1H), 6.48 (m, 1H), 5.28 (d, 1H, J = 3.2 Hz), 5.07 (t, 1H, J = 9.6 Hz), 4.98 (dd, 1H, J = 10.3, 8.0 Hz), 4.83 (dd, 1H, J = 10.5, 3.4 Hz), 4.75 (t, 1H, J = 8.3 Hz), 4.38–4.32 (m, 3H), 4.04–3.93 (m, 3H), 3.76 (t, 1H, J = 8.3 Hz), 3.70 (m, 2H), 3.64 (s, 3H), 3.49–3.46 (m, 1H), 3.41–3.36 (m, 1H), 2.12–2.05 (m, 2H), 2.03 (s, 3H), 2.004 (s, 3H), 2.001 (s, 3H), 1.93 (s, 3H), 1.92 (s, 3H), 1.84 (s, 3H), 1.62–1.57 (m, 2H). – ^{13}C NMR ($CDCl_3$, 100 MHz): δ/ppm = 170.32, 170.24, 170.04, 169.95, 169.94, 168.96, 168.59, 164.89, 137.07, 125.92, 100.95, 100.45, 76.11, 72.62, 72.56, 71.56, 70.86, 70.54, 68.97, 66.48, 61.82, 60.67, 52.27, 27.78, 24.93, 23.20, 20.75, 20.70, 20.59, 20.52, 20.40. – HRMS (FAB) calcd. for $C_{35}H_{49}NO_{21}Na$ 842.2654, found 842.2694.

Enamide **12**

82%, white foam; R_f = 0.35 (50% EtOAc/hexanes). – IR ($CDCl_3$ film): ν/cm^{-1} = 3407, 3354, 3158, 2977, 2952, 2884, 1755, 1651, 1368, 1236, 1057. – ^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm = 6.46 (m, 1H), 6.11 (bs, 1H), 5.32 (d, 1H, J = 3.1 Hz), 5.16 (t, 1H, J = 9.6 Hz), 5.08 (dd, 1H, J = 10.4, 8.0 Hz), 4.92 (dd, 1H, J = 10.4, 3.3 Hz), 4.86 (dd, 1H, J = 9.4, 8.1 Hz), 4.46–4.43 (m, 3H), 4.12–4.03 (m, 3H), 3.84 (t, 1H, J = 7.1 Hz), 3.82–3.87 (m, 2H), 3.74 (s, 3H), 3.59–3.55 (m, 1H), 3.49–3.42 (m, 1H), 2.30–2.26 (m, 1H), 2.21–2.19 (m, 1H), 2.12 (s, 3H), 2.10 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.01 (s, 6H), 1.94 (s, 3H), 1.72–1.69 (m, 2H), 1.44 (s, 9H). – ^{13}C NMR ($CDCl_3$, 100 MHz): δ/ppm = 170.31, 170.21, 170.02, 169.93, 169.66, 169.48, 168.97, 165.15, 153.27, 135.20, 128.20, 100.96, 100.25, 80.29, 76.14, 72.66, 72.50, 71.54, 70.85, 70.51, 68.93, 68.62, 66.47, 61.87, 60.66, 52.14, 28.07, 27.79, 24.38, 20.70, 20.67, 20.56, 20.51, 20.39. – HRMS (FAB) calcd. for $C_{38}H_{55}NO_{22}Na$ 900.3161, found 900.3113.

Enamide **14**

64%, white foam; R_f = 0.45 (25% EtOAc/hexanes). – IR ($CDCl_3$ film): ν/cm^{-1} = 3342, 3087, 3062, 3029, 2923, 2869, 1718, 1496, 1454, 1366, 1095, 1056. – ^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm = 7.39–7.19 (m, 25H), 6.51 (t, 1H), 6.09 (bs, 1H), 4.91 (d, 1H, J = 10.6 Hz), 4.82 (d, 1H, J = 11.0 Hz), 4.75 (d, 1H, J = 11.8 Hz), 4.72 (d, 1H, J = 8.8 Hz), 4.69 (d, 1H, J = 8.3 Hz), 4.62 (d, 1H, J = 11.8 Hz), 4.54 (d, 1H, J = 12.1 Hz), 4.39 (d, 1H, J = 10.1 Hz), 4.36 (d, 1H, J = 6.3 Hz), 4.34 (d, 1H, J = 8.0 Hz), 4.29 (d, 1H, J = 12.1 Hz), 4.07 (dd, 1H, J = 5.6, 1.3 Hz), 4.01–3.98 (m, 1H), 3.93–3.84 (m, 2H), 3.80–3.76 (m, 1H), 3.72 (m, 1H), 3.70 (s, 3H), 3.67–3.61 (m, 2H), 3.56–3.46 (m, 3H), 3.39–3.29 (m, 2H), 2.38–2.23 (m, 2H), 1.82–1.72 (m, 2H), 1.40 (s, 9H), 1.37 (s, 3H), 1.32 (s, 3H). – ^{13}C NMR ($CDCl_3$, 100 MHz): δ/ppm = 165.06, 153.25, 138.81, 138.40, 138.29, 138.17, 138.06, 128.08, 127.79, 127.51, 127.25, 109.48, 103.25, 101.62, 82.67, 81.55, 80.35, 79.99, 79.11, 75.10, 74.83, 74.75, 73.38, 73.09, 72.97, 71.71, 68.66, 68.41, 68.05, 51.90, 27.99, 27.75, 26.19, 24.56.

Asymmetric Hydrogenation (General Procedure)

Under an inert deoxygenated atmosphere, [(COD)Rh-((*S*, *S*)–Et-DuPHOS)]$^+$OTf$^-$ (0.005 mmol, 5 mol%) and the desired enamide ester (0.100 mmol) were dissolved in deoxygenated anhydrous THF (10 mL, 0.01 M) in a Fischer-Porter tube. The reaction vessel was pressurized with 50 psi of H_2 after three vacuum/H_2 cycles and stirred at 25 °C for 24 hours. The vessel was depressurized, the mixture concentrated and purified through a short plug of silica gel to yield the glycoamino acid.

Glycoamino acid **16**

R_f = 0.55 (100% EtOAc). – ^{1}H NMR (C_6D_6, 500 MHz): δ/ppm =5.74 (d, 1H, J = 8.0 Hz), 5.66 (dd, 1H, J = 10.5, 7.9 Hz), 5.60 (d, 1H, J = 2.6 Hz), 5.51 (t, 1H, J = 9.2 Hz), 5.33 (dd, 1H, J = 9.5, 7.9 Hz), 5.24 (dd, 1H, J = 10.5, 3.4 Hz), 4.87–4.83 (m, 1H), 4.65 (dd, 1H, J = 11.9, 2.0 Hz), 4.48 (d, 1H, J = 7.8 Hz), 4.30–4.19 (m, 4H), 3.84–3.76 (m, 2H), 3.61 (t, 1H, J = 7.1 Hz), 3.43 (s, 3H), 3.40–3.33 (m , 2H), 2.09 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H), 1.85 (s, 3H), 1.84 (s, 3H), 1.78 (m, 2H), 1.77 (s, 3H), 1.75 (s, 3H), 1.66 (s, 3H), 1.50 (m, 2H). – ^{13}C NMR ($CDCl_3$, 100 MHz): δ/ppm = 172.99, 170.36, 170.31, 170.11, 170.03, 169.79, 169.73, 169.62, 169.62, 169.03, 101.03, 100.47, 76.23, 72.71, 72.61, 71.63, 70.94, 70.62, 69.44, 69.06, 66.55, 61.91, 60.74, 52.36, 51.96, 32.07, 28.50, 23.14, 21.59, 20.85, 20.77, 20.65, 20.60, 20.47. – HRMS (FAB) calcd. for $C_{35}H_{51}NO_{21}Na$ 844.2836, found 844.2851.

Glycoamino acid **17**

R_f = 0.35 (50% EtOAc/hexanes). – ^{1}H NMR (C_6D_6, 500 MHz): δ/ppm =5.54 (dd, 1H, J = 10.3, 8.0 Hz), 5.47 (d, 1H, J = 3.3 Hz), 5.39 (t, 1H, J = 9.2 Hz), 5.20 (t, 1H, J = 8.7 Hz), 5.11 (dd, 1H, J = 10.5, 3.3 Hz), 5.02 (d, 1H, J = 8.2 Hz), 4.52 (d, 1H, J = 11.4 Hz), 4.42 (m, 1H), 4.34 (d, 1H, J = 7.9 Hz), 4.16–4.07 (m, 4H), 3.66–3.62 (m, 2H), 3.49–3.45 (t, 1H), 3.27 (s, 3H), 3.20–3.18 (m, 2H), 1.97 (s, 3H), 1.94 (s, 3H), 1.89 (s, 3H), 1.73 (s, 6H), 1.64 (s, 3H), 1.53 (s, 3H), 1.44 (s, 9H), 1.31–1.29 (m, 2H). – ^{13}C NMR (C_6D_6, 100 MHz): δ/ppm = 173.54, 170.44, 170.18, 170.12, 169.74, 169.34, 155.97, 102.04, 101.04, 79.74, 77.55, 74.11, 73.24, 72.71, 71.93, 71.21, 70.11, 69.51, 67.43, 63.02, 61.31, 54.18, 52.12, 52.00, 32.56, 29.51, 28.77, 28.62, 22.38, 21.16, 20.89, 20.83, 20.74, 20.56, 20.47, 20.15. – HRMS (FAB) calcd. for $C_{38}H_{57}NO_{22}Na$ 902.3275, found 902.3269.

Glycoamino acid **18**

R_f = 0.45 (66% EtOAc/hexanes). – ^{1}H NMR (C_6D_6, 500 MHz): δ/ppm =5.54 (dd, 1H, J = 10.4, 8.0 Hz), 5.48 (d, 1H, J =

3.2 Hz), 5.39 (t, 1H, *J* = 9.2 Hz), 5.21 (dd, 1H, *J* = 6.2, 1.1 Hz), 5.12 (d, 1H, *J* = 3.1 Hz), 5.09 (d, 1H, *J* = 3.3 Hz), 4.54–4.51 (m, 2H), 4.33 (d, 1H, *J* = 7.8 Hz), 4.19–4.06 (m, 6H), 3.74–3.58 (m, 2H), 3.49–3.40 (m, 1H), 3.38 (d, 1H, *J* = 10.9 Hz), 3.23–3.16 (m, 2H), 1.96 (s, 3H), 1.94 (s, 3H), 1.90 (s, 3H), 1.74 (s, 3H), 1.73 (s, 3H), 1.64 (s, 3H), 1.51 (s, 3H), 1.45 (s, 9H), 0.91–0.88 (m, 2H), –0.10 (s, 9H). – ^{13}C NMR (C_6D_6, 100 MHz): δ/ppm = 173.26, 170.44, 170.41, 170.18, 170.09, 169.35, 156.05, 102.98, 101.06, 79.73, 77.59, 74.13, 73.21, 72.73, 71.94, 71.19, 70.10, 69.58, 67.28, 63.76, 63.01, 61.25, 54.41, 34.76, 32.76, 28.62, 28.80, 25.75, 22.45, 21.18, 20.93, 20.84, 20.76, 20.57, 20.46, 20.15, 17.83, –1.29. – HRMS (FAB) calcd. for $C_{42}H_{67}NO_{22}SiNa$ 988.3870, found 988.3821.

N-Boc Deprotection (General Procedure)

The desired glycoamino acid (0.100 mmol) was dissolved in CH_2Cl_2 (3.0mL) with stirring. Trifluoroacetic acid (TFA) (3.0 mL) was added dropwise and the reaction stirred at rt for 1 h. The mixture was then concentrated with a stream of dry N_2 and azeotroped with anhydrous benzene (2×5 mL) to give the crude amine as its TFA salt which was typically used without further purification.

Amine **21**

^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm = 5.32 (d, 1H, *J* = 2.7 Hz), 5.16 (t, 1H, *J* = 7.4 Hz), 5.08 (dd, 1H, *J* = 10.4. 8.0), 4.92 (dd, 1H, *J* = 10.4, 3.4 Hz), 4.85 (dd, 1H, *J* = 9.5, 7.0 Hz), 4.47–4.41 (m, 3H), 4.20–4.03 (m, 5H), 3.84 (t, 1H), 3.81–3.80 (m, 1H), 3.76 (t, 1H), 3.58–3.55 (m, 1H), 3.46–3.41 (m, 1H), 3.36 (bs, 1H), 2.12 (s, 3H), 2.09 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.94 (s, 3H), 1.61–1.54 (m, 4H), 0.99–0.96 (m, 2H), 0.03 (s, 9H).

TSE Ester Deprotection (General Procedure)

The desired glycoamino acid (0.100 mmol) was dissolved in THF (1.0–3.0 mL) and cooled to 0 °C. A 1.0 M solution of TBAF in THF (0.250 mmol, 2.5 equivs.) was added dropwise, the ice bath removed and the reaction stirred at rt for 1–2 h, as judged by TLC. The reaction mixture was diluted with CH_2Cl_2 (30mL), washed with 0.05 M aqueous HCL (50 mL), and back-extracted with additional CH_2Cl_2 (2×10 mL). The combined organic layer was dried over anhydrous Mg_2SO_4 and concentrated. The crude acid was purified by flash column chromatography (5% methanol in ethyl acetate).

Acid **20**

^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm = 5.22 (d, 1H, *J* = 2.8 Hz), 5.07 (t, 1H, *J* = 9.3 Hz), 4.98 (dd, 1H, *J* = 10.4, 5.9 Hz), 4.84 (dd, 1H, *J* = 10.4, 3.5 Hz), 4.75 (dd, 1H, *J* = 9.5, 8.0 Hz), 4.42–4.35 (m, 2H), 4.34–4.31 (m, 1H), 4.15–4.14 (m, 1H), 4.03–3.94 (m, 4H), 3.77–3.65 (m, 5H), 3.49–3.45 (m, 1H), 3.37–3.33 (m, 1H), 3.10–3.07 (m, 1H), 2.03 (s, 3H), 2.00 (s, 3H), 1.96 (s, 3H), 1.94 (s, 3H), 1.92 (s, 3H), 1.84 (s, 3H), 1.51–1.46 (m, 4H), 1.32 (s, 9H).

BOP Reagent Promoted Peptide Coupling

Amine **21** (25 mg, 0.028 mmol) and acid **20** (22 mg, 0.025 mmol) were azeotroped together with anhydrous benzene (3×3mL) and dried under high vacuum. The mixture was dissolved in CH_2Cl_2 (0.5 mL), BOP reagent (1.5 equivs.; 3.3 mg) was added and the solution cooled to 0 °C over 15 minutes. A dropwise addition of Hünig's base (15 equivs.; 66 µL) was followed by removal of the ice bath. The reaction stirred at rt for 2 h. Concentration of the reaction mixture, followed by purification by flash column chromatography (50% EtOAc/hexanes -> 75% EtOAc/hexanes) gave the dipeptide as a white foam (31 mg, 72%); R_f = 0.75 (75% EtOAc/hexanes). – ^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm = 6.60 (d, NH, 1H, *J* = 7.7 Hz), 5.32 (d, 2H, *J* = 3.2 Hz), 5.16 (t, 2H), 5.10–5.06 (m, 2H), 4.94–4.91 (m, 2H), 4.86–4.81 (m, 2H), 4.50–4.38 (m, 8H), 4.21–4.13 (m, 2H), 4.11–4.02 (m, 8H), 3.85 (t, 2H, *J* = 6.6 Hz), 3.79–3.73 (m, 6H), 3.56–3.54 (m, 2H), 3.45–4.37 (m, 2H), 2.13 (s, 6H), 2.10 (s, 6H), 2.03 (s, 6H), 2.01 (s, 6H), 1.94 (s, 6H), 1.94 (s, 6H), 1.93 (s, 6H), 1.79–1.77 (m, 2H), 1.56–1.52 (m, 4H), 1.42 (s, 9H), 1.32–1.28 (m, 4H), 0.99–0.95 (m, 2H), 0.02 (s, 9H). – HRMS (FAB) calcd. for $C_{74}H_{112}N_2O_{41}SiNa$ 1735.6402, found 1735.6333.

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[34] Detailed experimental procedures for **24** can be found in reference [35]

[35] J. R. Allen, S. J. Danishefsky, J. Am. Chem. Soc. **2000**, submitted for publication.

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