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New design concepts for constraining glycosylated amino acids

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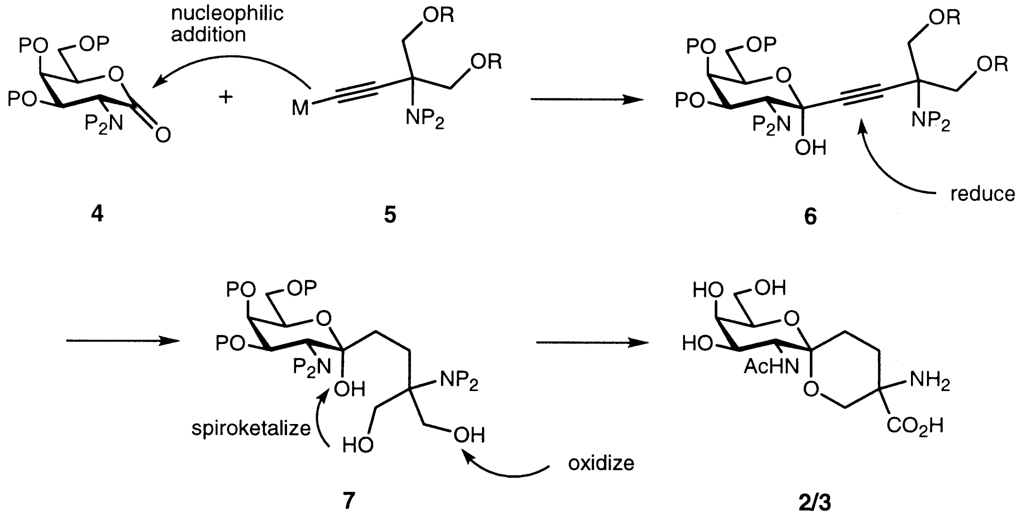
Abstract—In an effort to probe the mechanism by which glycosyltransferases recognize glycoproteins and assemble the core structures of O-linked oligosaccharides, two constrained glycopeptides based on the α-*N*-acetylgalactosaminyl serine substructure were chosen for synthesis. These compounds representing one of the two possible gauche conformations of the D-serine and L-serine configurations of the parent substructure were successfully prepared in reasonable overall yield through a convergent strategy. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Glycosylation is a ubiquitous posttranslational modification of proteins and is associated with a number of processes both within cells and at cell surfaces. These include protein transport, cell adhesion, and signal transduction.[1] Aberrant glycosylation of cellular proteins and glycolipids is associated with various diseases, including cancerous and inflammatory conditions.[2] Therefore, an understanding on a molecular level of the structural features of glycoproteins that are recognized by various enzymes and receptors would be valuable in developing inhibitor-based strategies to control carbohydrate-mediated cellular processes. This fundamental understanding could, in turn, lead to new therapeutic strategies for conditions that are characterized by abnormal glycosylation.

The ground state conformations of small glycopeptide fragments have been studied in some detail by NMR spectroscopy;[3] however, relatively little is known about the conformations that are recognized by various enzymes and receptors. The preferred torsional angle about the exocyclic C1–O bond (ϕ) of the carbohydrate moiety in the ground state is somewhat rigid, as predicted by the exoanomeric effect.[4] However, other torsional angles within the side chain, including χ_2, are somewhat more flexible, as



Scheme 1. Synthesis strategy for **2/3**.

Keywords: glycoproteins; constrained glycopeptides; spiroketalization.

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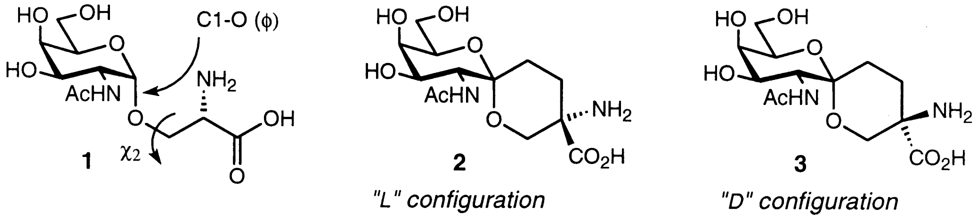


Figure 1.

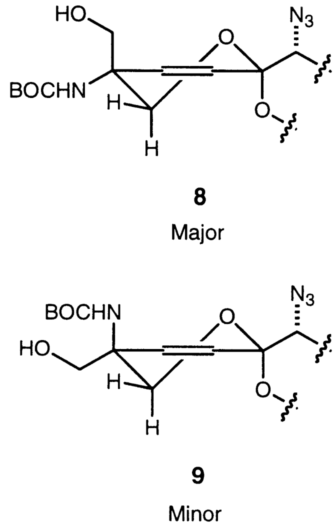


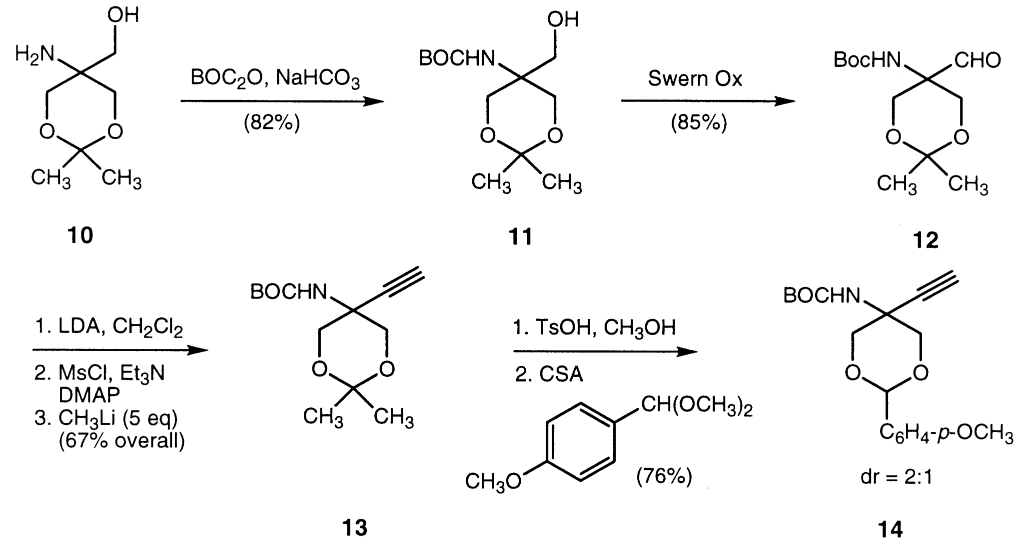
Figure 2. Predicted diastereomeric spiroketalization preferences.

illustrated in the α-*N*-acetylgalactosaminyl serine substructure **1**.

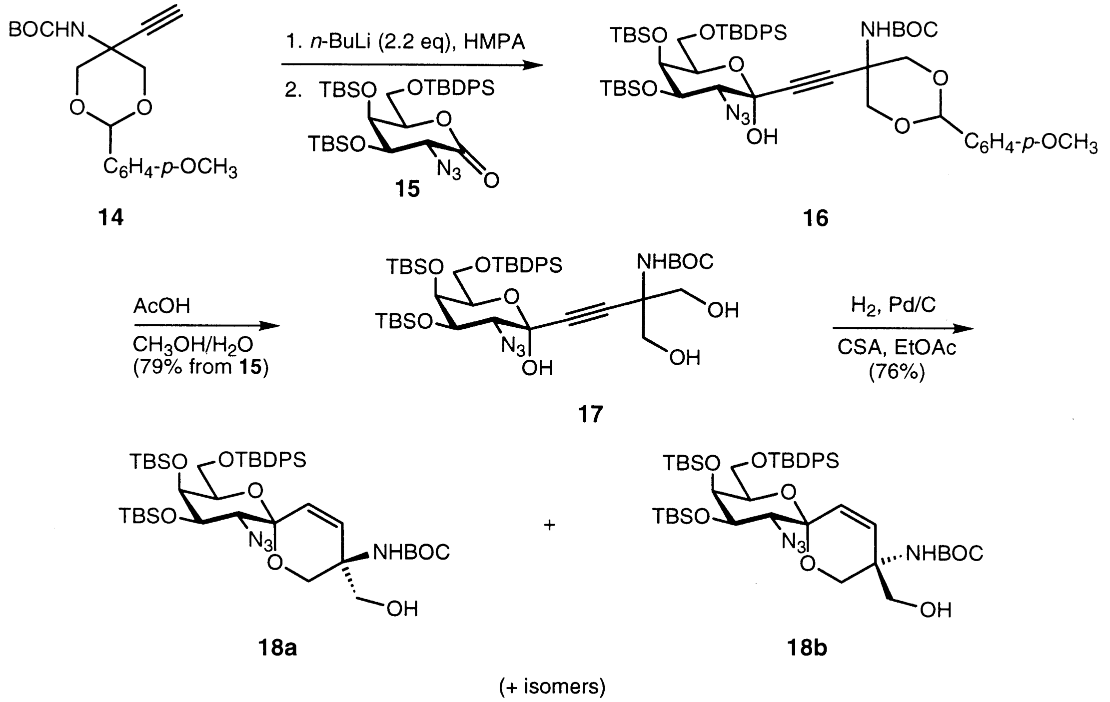
Significant research has gone into designing conformational constraints into various amino acid and peptide motifs to probe bioactive conformations.[5] However, conformationally restricted glycopeptides are relatively unexplored.[6]

In an effort to probe the mechanism by which glycosyltransferases recognize glycoproteins and assemble the core structures of O-linked oligosaccharides, a series of conformationally constrained glycopeptides was chosen for synthesis. These particular targets were identified in an effort to represent the accessible low energy conformations about χ_2 of the native structure **1**, and will ultimately be incorporated into small peptide fragments for biological evaluation. Among those chosen was compound **2** in which the α-carbon (Cα) of the amino acid component of the structure is incorporated into a six-membered ring, and the χ_2 torsional angle is effectively constrained in one of the two possible gauche conformations (Fig. 1). Additionally, the C1–O bond is locked into the low-energy exoanomeric orientation. The unnatural D-serine configuration is represented by the similarly constrained analog **3**.

The synthesis strategy for **2** (Scheme 1) was designed with the focus of preferentially establishing the desired L-serine configuration at Cα as well as the necessary anomeric orientation at the spiroketal center. Specifically, the desired anomeric configuration could arise from a thermodynamic spiroketalization event,[7] and the stereochemistry at Cα could be established through diastereomeric preferences inherent within this same process. In a general sense, the synthesis would commence with a convergent nucleophilic addition of appropriately protected versions of the carbohydrate (**4**) and amino acid (**5**) components of the target structure. Manipulations to the resulting product **6**, including reduction of the internal alkyne, would provide the spiroketal precursor **7**. Selective spiroketalization of one of the diastereotopic primary hydroxyl groups and subsequent oxidation of the other, followed by protecting group



Scheme 2. Synthesis of **14**.



Scheme 3. Acetylide addition to **15** and subsequent spiroketalization.

manipulations, should provide the analogs **2** and **3**. The unnatural D-serine analog **3** could also prove useful in our studies.

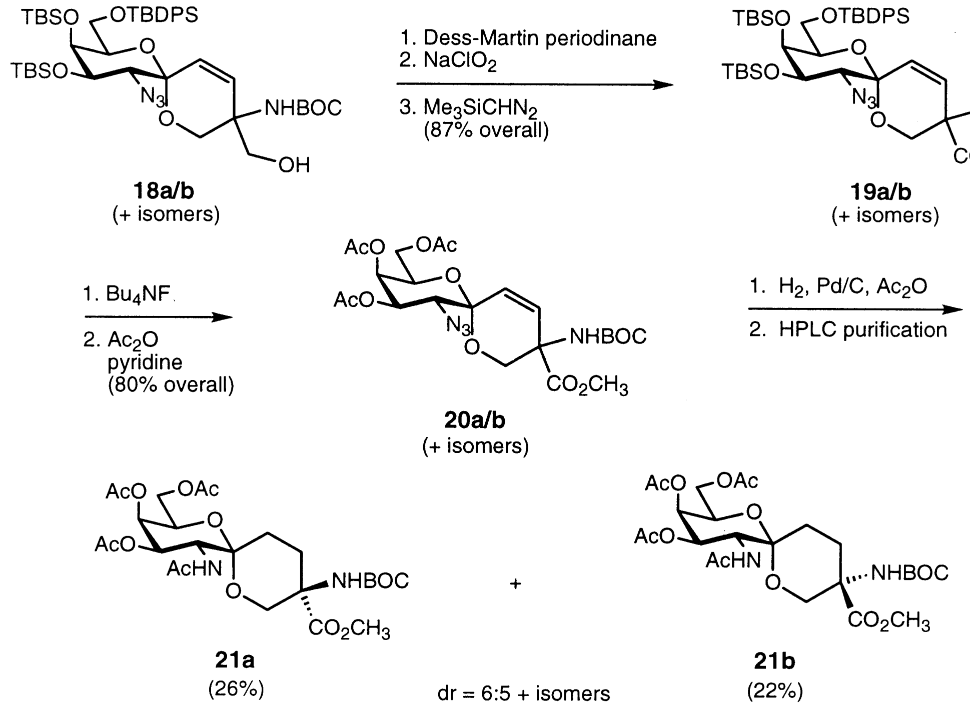
Assuming thermodynamic conditions, the spiroketalization was predicted to afford an excess of the desired L-diastereomer, **2**. This prediction was based on the presumed inherent preference of an amine bearing a bulky protecting group, such as a *tert*-butyl carbamate, over a hydroxymethyl, to reside in the pseudo-equatorial position. This preference is illustrated by the partial half-chair structures **8** and **9** (Fig. 2).

2. Results and discussion

The synthesis of the nucleophilic amino acid component **14** was accomplished in an efficient, straightforward manner (Scheme 2). The synthesis commenced with protection of the amine group of **10**[8] to give the *tert*-butyl carbamate **11** in good yield. Swern oxidation of the free primary alcohol in **11** gave the aldehyde **12**. A modified three-step alkynylation procedure adopted from a recently reported procedure[9] was applied to **12** to give the alkyne **13** in good overall yield. The acetal **14** was then obtained by a straightforward protecting group exchange sequence. The alkynyl anion of **13** also served as a reasonable nucleophile in additions to our carbohydrate acceptor. However, difficulties associated with the removal of the acetonide from the resulting product were encountered. Therefore, the acetonide was replaced with the more labile *p*-anisylidine acetal protecting group, producing compound **14** as a 2:1 mixture of diastereomers. Since both diastereomers of **14** were found to add to the carbohydrate acceptor, and since the acetal stereocenter was removed shortly thereafter, this mixture was carried to the next step without separation.

Addition of an excess of the dianion of **14** to the lactone **15**[10] afforded the desired product **16** as a complex mixture of stereoisomers (Scheme 3). In order to facilitate purification of compound **16** from unreacted **14**, the ensuing deprotection step was necessarily performed on the product mixture. Reaction of the mixture with mild acid under the conditions shown gave the desired diol **17** as a mixture of the two presumed anomers in good overall yield from **15**. The diol product resulting from the deprotection of unreacted excess **14** could be recycled if desired. Partial reduction of the alkyne group of **17** followed by acid-catalyzed spiroketalization proceeded in a one-pot reaction to give a complex mixture of products (76%), two of which were **18a** and **18b**. It is important to note that a judicious choice of solvent and acid concentration were necessary for the success of the spiroketalization. Overreduction of the internal alkyne functional group of **17** to the corresponding alkane was observed when methanol was used as a solvent or cosolvent, as well as when lower acid concentrations were employed. This alkane could not be cyclized, even under strongly acidic conditions, and only decomposition was observed in these attempts. Interestingly, the olefin groups in **18a** and **18b** were resistant to reduction to their respective alkanes even under high-pressure (1500 psi) catalytic hydrogenation conditions.

HPLC analysis of this spiroketal product mixture revealed an approximate 2:1:1 mixture of major products in addition to at least one other inseparable minor product. Partial separation of the three major compounds could be achieved by preparative HPLC. Contrary to our initial predictions, the major constituent of this mixture was shown to be **18a**, which is diastereomeric to the desired product **18b** at Cα. The configuration of **18a** was proven by X-ray analysis of an analog obtained from further transformations (unpublished results). The desired **18b** was presumed to be one of the



Scheme 4. Synthesis completion.

other major products in the mixture. Unfortunately, resubmission of **18a** to the original spiroketalization conditions resulted in no detectable equilibration to the originally observed product composition. These results seem to suggest that the spiroketalization did not give a thermodynamic product mixture, and may be reflecting kinetic preferences for the reaction. This notion is reinforced by the probable presence of other isomers, tentatively assigned as β-anomers, in the complex product mixture. Preliminary investigations into a variety of alternate conditions to equilibrate these products were unsuccessful.

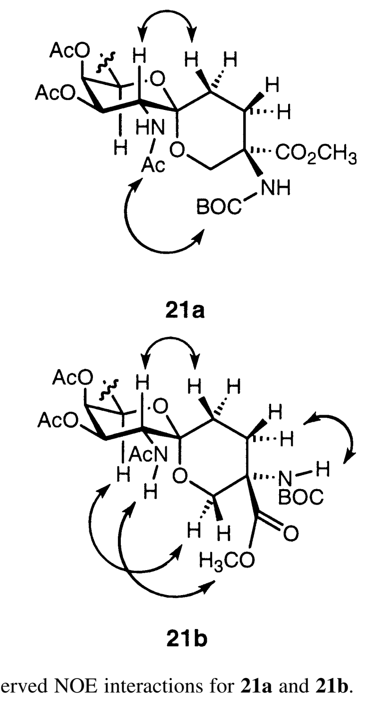


Figure 3. Observed NOE interactions for **21a** and **21b**.

Despite the complex product mixture obtained from the spiroketalization, **18a** and **18b** were transformed into the methyl esters **21a** and **21b**, respectively, thus meeting the initial goals of the project (Scheme 4). In light of the difficulties associated with separation of the various spiroketals, it was determined that purification should be executed at a late stage in the synthesis. Additionally, the product mixtures obtained following the addition reaction were complex and often inseparable. Thus, all characterizations prior to **21a** and **21b** were performed on these mixtures.

Dess–Martin oxidation[11] of the spiroketal mixture including **18a/b**, followed by further oxidation under Lindgren conditions[12] and subsequent methyl esterification, gave a mixture of products that included the methyl esters **19a/b** in good overall yield. Removal of the silyl groups of **19a/b** and peracetylation of the resulting free alcohols gave **20a/b** in addition to other minor isomers. Reduction of the azide and olefin functionalities in the presence of acetic anhydride gave **21a/b** as the usual product mixture in addition to at least two byproducts. The identities of the byproducts in this reaction have yet to be determined, and all attempts to prevent formation of these byproducts using a variety of alternate reaction conditions were unsuccessful. Final HPLC purification successfully facilitated separation of the two major products from the additional isomers in the mixture. The two major products **21a** and **21b** were isolated in a 6:5 ratio by mass in favor of **21a**. X-Ray analysis of the major diastereomer unambiguously established the structure of D-serine analog **21a**. The structure of the minor diastereomer or L-serine analog **21b** was unambiguously confirmed by NOE correlations derived from NOESY experiments. The NOE structural determination protocol was partially validated through studies performed on **21a** (Fig. 3).

3. Conclusion

In conclusion, two constrained glycopeptides were successfully synthesized in reasonable overall yield through a convergent strategy. The compounds **21a** and **21b**, representing the D-serine and L-serine configurations, respectively, were fully characterized and are poised for incorporation into peptides for subsequent biological evaluation and structural studies. These compounds illustrate a novel concept for constraining torsional angles in glycopeptides. Studies toward establishing a more efficient and selective synthesis are currently underway.

4. Experimental

4.1. General methods

Reagents obtained from commercial suppliers were used without further purification unless otherwise noted. All solvents were purified and dried by standard distillation methods. ^{1}H and ^{13}C NMR spectra are reported in parts per million (δ) relative to $CHCl_3$ (7.24 and 77.23 ppm, respectively) as the internal standard.

4.1.1. (*N-tert*-Butyloxycarbonyl-5-amino-2,2-dimethyl-[1,3]dioxan-5-yl)-methanol (11). To a solution of **10**[8] (0.310 mol, 1.00 equiv.) in 1000 mL of a 4:1 THF:H_2O solution was added $NaHCO_3$ (0.775 mol, 2.50 equiv.), followed by BOC_2O (0.388 mol, 1.25 equiv.). The resulting solution was stirred at rt for 18 h. The reaction mixture volume was reduced approximately 75% by aspirator pressure on a rotary evaporator and was subsequently diluted with 250 mL EtOAc. The layers were separated and the aq layer was extracted with 3×250 mL EtOAc. The combined organic extracts were washed with 250 mL brine, dried ($MgSO_4$), filtered, and concentrated to give the crude product as a white solid. The solid was chromatographed on silica gel (5:1 hexanes/EtOAc) to yield a powdery white solid (0.254 mol, 82%). IR (thin film): 3421, 2979, 1714 cm^{-}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.32 (s, 1H), 4.27 (s, 1H), 3.80 (AB, $\Delta\nu$=11.6 Hz, 4H), 3.70 (d, J= 6.55 Hz, 2H), 1.43 (s, 12H), 1.41 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 156.6, 98.9, 80.6, 64.9, 64.6, 53.5, 28.5, 27.0, 20.5. TLC (SiO_2, 50% EtOAc/hexanes): R_f= 0.42. mp 95–97°C. HRMS: Calcd for $C_{12}H_{23}NO_5$: 262.1654. Found: 262.1644. Anal: calcd for $C_{12}H_{23}NO_5$: C, 55.16; H, 8.87; N, 5.36. Found: C, 55.55; H, 8.68; N, 5.48.

4.1.2. *N-tert*-Butyloxycarbonyl-5-amino-2,2-dimethyl-[1,3]-dioxane-5-carbaldehyde (12). A solution of oxalyl chloride (0.534 mol, 2.0 equiv.) in 525 mL CH_2Cl_2 was cooled to −78°C in a 5 L flask equipped with a mechanical stirrer, and a solution of DMSO (0.801 mol, 3.0 equiv.) in 350 mL CH_2Cl_2 was added dropwise over 45 min. The resulting mixture was stirred an additional 15 min at −78°C. A solution of **11** (0.267 mol, 1.0 equiv.) in 525 mL of CH_2Cl_2 was then added dropwise over 45 min producing a cloudy mixture which was allowed to stir at −78°C for 2 h. Triethylamine (1.602 mol, 6.0 equiv.) was then added slowly over 30 min, and the thick solution was gradually warmed to rt over 2 h. The reaction mixture was washed with 2×300 mL saturated aq $NaHCO_3$, followed by 300 mL H_2O, and 300 mL brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated to give a tan solid which was recrystallized in a minimal amount of toluene. Multiple isolations from the mother liquor gave the product **12** as a powdery white solid (0.229 mol, 85%). IR (thin film): 3338, 2982, 1729 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.61 (s, 1H), 5.54 (s, 1H), 3.99 (AB, $\Delta\nu$=11.9 Hz, 4H), 1.44 (s, 15H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 199.5, 155.7, 98.9, 81.2, 62.9, 60.1, 28.4, 27.5, 19.8. TLC (SiO_2, 25% EtOAc/hexanes): R_f= 0.41. mp 114–115°C. HRMS: Calcd for $C_{12}H_{21}NO_5$: 260.1498. Found: 260.1510. Anal: calcd for $C_{12}H_{21}NO_5$: C, 55.58; H, 8.16; N, 5.40. Found: C, 55.64; H, 8.07; N, 5.47.

4.1.3. 5-Ethynyl-2,2-dimethyl-[1,3]dioxan-(*N-tert*-butyloxycarbonyl)-5-ylamine (13). A solution of CH_2Cl_2 (191.0 mmol, 5.0 equiv.) in 150 mL THF was cooled to −78°C and lithium diisopropylamine (0.95 M in THF, 95.8 mmol, 2.5 equiv.), kept at 0°C, was added dropwise over 30 min. The resulting solution was stirred an additional 15 min, and a solution of **12** (38.3 mmol, 1.0 equiv.) in 150 mL THF was added dropwise over 25 min. The resulting mixture was stirred an additional 1 h at −78°C, and 50 mL H_2O was then added. The solution was subsequently warmed to rt, at which time 50 mL of saturated aq NH_4Cl was added. The resulting layers were separated and the aq phase was extracted with 2×100 mL EtOAc. The combined organics were washed with 100 mL brine, dried ($MgSO_4$), filtered, and concentrated to give a colorless solid (36.4 mmol), which was used without further purification. A portion of the dichloroalcohol product was purified for characterization. IR (thin film): 2981, 2360, 2342 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.78 (bs, 1H), 5.25 (s, 1H), 5.12 (bs, 1H), 4.11–3.95 (m, 5H), 1.44 (s, 12H), 1.41 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 157.4, 98.6, 81.6, 73.1, 65.4, 62.8, 55.3, 28.5, 27.8, 19.6. TLC (SiO_2, 25% EtOAc/hexanes): R_f=0.41. mp 160–162°C. HRMS: Calcd for $C_{13}H_{23}Cl_2NO_5$: 343.0953. Found: 343.0962. Anal: calcd for $C_{13}H_{23}Cl_2NO_5$: C, 45.36; H, 6.73; N, 4.07. Found: C, 45.58; H, 6.69; N, 4.16.

To a stirring solution of the above dichloroalcohol (35.8 mmol, 1.0 equiv.) in 225 mL CH_2Cl_2 at rt was added triethylamine (53.7 mmol, 1.5 equiv.), followed by methane sulfonyl chloride (53.7 mmol, 1.5 equiv.). The resulting solution was stirred for 18 h and was subsequently quenched with 75 mL H_2O. The layers were separated, and the aq phase was extracted with 2×75 mL CH_2Cl_2. The combined organics were washed with 100 mL saturated aq NH_4Cl, 100 mL H_2O, and 100 mL brine. The organics were dried ($MgSO_4$), filtered, and concentrated to give a dark orange foam (37.0 mmol), which was used without further purification. A portion of the dichloromesylate product was purified for characterization. IR (thin film): 2984, 1710 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.25 (s, 1H), 5.69 (s, 1H), 5.15 (s, 1H), 4.18 (AB, $\Delta\nu$=12.4 Hz, 2H), 4.02–3.92 (m, 2H), 3.30 (s, 3H), 1.44 (s, 12H), 1.39 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.6, 99.2, 83.7, 81.1, 69.9, 62.9, 62.0, 55.2, 39.7, 28.4, 26.7, 20.5. TLC (SiO_2, 25% EtOAc/hexanes): R_f=0.33. HRMS: Calcd for $C_{14}H_{25}Cl_2NO_7S$: 421.0729. Found: 421.0737. Anal: calcd for $C_{14}H_{25}Cl_2NO_7S$: C, 39.82; H, 5.97; N, 3.32. Found: C, 40.97; H, 5.89; N, 3.49.

To a stirring solution of the dichloromesylate intermediate (36.50 mmol, 1.0 equiv.) in 350 mL THF at −51°C was added methyllithium (1.4 M in diethyl ether, 200.75 mmol, 5.5 equiv.) dropwise over 30 min. The resulting solution was slowly warmed to 0°C over 1 h and was subsequently quenched with 25 mL H_2O, followed by 50 mL saturated aq NH_4Cl. The mixture was allowed to warm to rt, the layers were separated, and the aq phase was extracted with 3×75 mL EtOAc. The combined organics were washed with 100 mL brine, dried ($MgSO_4$), filtered, and concentrated to give a crude tan solid. The solid was chromatographed on silica gel (5:1 hexanes/EtOAc) to give **13** as a powdery white solid (24.40 mmole, 67%) from **12**. IR (thin film): 3311, 2980, 1712 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 5.13 (s, 1H), 4.02–3.94 (m, 4H), 2.40 (s, 1H), 1.45 (s, 9H), 1.43 (s, 3H), 1.40 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 154.5, 98.8, 80.5, 80.4, 73.5, 66.5, 47.4, 28.6, 28.4, 18.8. TLC (SiO_2, 25% EtOAc/hexanes): R_f=0.32. mp 113–115°C. HRMS: Calcd for $C_{13}H_{21}NO_4$: 256.1549. Found: 256.1557.

4.1.4. Addition precursor (14). To a stirring solution of **13** (20.60 mmol, 1.0 equiv.) in 400 mL MeOH at rt was added *p*-toluenesulfonic acid monohydrate (4.12 mmol, 0.2 equiv.) in one portion. The resulting solution was stirred for 12 h, and was subsequently quenched with excess triethylamine. The mixture was allowed to stir for an additional 20 min, and the volume of the solution was reduced approximately 90% by aspirator pressure rotary evaporator. The concentrated solution was diluted with 200 mL EtOAc, and was then washed with 75 mL saturated aq $NaHCO_3$. After separation of phases, the aq phase was extracted with 2×75 mL EtOAc. The combined organics were dried ($MgSO_4$), filtered, and concentrated to give the diol as a crude white solid (20.60 mmol, 100%), which was used without further purification. IR (thin film): 3399, 2978, 1696 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 5.29 (s, 1H), 3.89 (dd, J=11.5, 6.4 Hz, 2H), 3.79 (dd, J=11.5, 7.3 Hz, 2H), 3.08 (bs, 2H), 2.44 (s, 1H), 1.44 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.8, 81.5, 81.1, 73.9, 66.6, 57.2, 28.5. TLC (SiO_2, 50% EtOAc/hexanes): R_f=0.23. mp 116–118°C. HRMS: Calcd for $C_{10}H_{17}NO_4$: 216.1236. Found: 216.1240.

To a solution of 4-methoxybenzaldehyde dimethyl acetal (20.7 mmol, 3.0 equiv.) in 20 mL DMF at rt was added the above diol (6.9 mmol, 1.0 equiv.), followed by camphorsulfonic acid (0.7 mmol, 0.1 equiv.). The resulting mixture was then placed on an aspirator pressure rotary evaporator and allowed to mix at 40°C for 4 h. The reaction was subsequently treated with excess triethylamine and concentrated to give a crude, orange residue consisting of an inseparable mixture of product, *p*-anisaldehyde, and excess 4-methoxybenzaldehyde dimethyl acetal. The residue was then redissolved in 100 mL of a 4:1 THF:H_2O solution, and 5 mL glacial AcOH was added. This solution was allowed to stir for 2 h and was then quenched with 25 mL saturated aq $NaHCO_3$. The resulting phases were separated, and the aq phase was extracted with 2×75 mL EtOAc. The combined organics were washed with 75 mL brine, dried ($MgSO_4$), filtered, and concentrated to give a crude oil consisting of an inseparable mixture of product and *p*-anisaldehyde. This oil was then dissolved in 100 mL MeOH, cooled to 0°C, and treated with sodium borohydride (20.7 mmol, 3.0 equiv.). Upon completion of the addition, the resulting mixture was allowed to warm to rt and stirred for 3 h. The mix was then quenched with 25 mL saturated aq $NaHCO_3$, and following the usual aq workup, a mixture of product and 4-methoxybenzyl alcohol was isolated as a crude oil. The oil was chromatographed on silica gel (6:1 hexanes/EtOAc) to give the product as a 2:1 mixture of diastereomers by ^1H NMR as a powdery white solid (5.3 mmol, 76%) from **13**. A portion of the major diastereomer was isolated for characterization: IR (thin film): 2977, 2360, 1717 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 7.39 (d, J=8.7 Hz, 2H), 6.89 (d, J=8.7 Hz, 2H), 5.45 (s, 1H), 5.23 (bs, 1H), 4.40 (d, J=11.3 Hz, 2H), 3.95 (d, J=11.3 Hz, 2H), 2.45 (s, 1H), 1.46 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.5, 154.5, 129.9, 127.58, 113.9, 102.0, 80.5, 79.6, 74.2, 73.2, 55.5, 47.3, 28.6. TLC (SiO_2, 25% EtOAc/hexanes): R_f's=0.22 and 0.30. mp 130–132°C. HRMS: Calcd for $C_{18}H_{23}NO_5$: 333.1576. Found: 333.1573. Anal: calcd for $C_{18}H_{23}NO_5$: C, 64.85; H, 6.95; N, 4.20. Found: C, 64.54; H, 6.97; N, 4.21.

4.1.5. Spiroketalization precursor (17). To a solution of **14** (4.48 mmol, 3.0 equiv.) in 40 mL anhydrous THF at rt was added hexamethylphosphoramide (17.90 mmol, 12.0 equiv.). The mixture was then cooled to −78°C, and *n*-butyllithium (1.6 M in hexanes, 9.39 mmol, 6.3 equiv.) was added slowly. The resulting yellow mixture was allowed to stir for 1.5 h at −78°C, at which time a solution of **15**[10] (1.49 mmol, 1.0 equiv.) in 15 mL anhydrous THF was added slowly by canula. The yellow mixture was allowed to stir for an additional 20 min at −78°C and was then quenched with 5 mL H_2O, followed by 15 mL saturated aq NH_4Cl. The heterogeneous mixture was allowed to warm to rt and was then diluted with 25 mL EtOAc. The phases were separated, and the aq phase was extracted with 2×25 mL EtOAc. The combined organic phases were washed with 50 mL brine, dried ($MgSO_4$), filtered, and concentrated. The concentrated solution was chromatographed on silica gel (6:1 then 3:1 hexanes/EtOAc) to give a moderately separable mixture of recovered **14** and product. The entirety of recovered **14** and product were subsequently combined and redissolved in 100 mL of a (8:1:1) AcOH:H_2O:MeOH solution. The resulting solution was stirred for 12 h at rt and was then diluted with excess toluene. The corresponding biphasic mixture was concentrated by high-vacuum (0.1 torr) rotary evaporator at a temperature not exceeding 35°C using additional toluene azeotropes as necessary. The resulting crude oil was immediately chromatographed on silica gel (5:1 then 2:1 then 1:1 hexanes/EtOAc) to give the product as an inseparable (~2:1) mixture of presumed anomers by ^1H NMR. A third minor compound observed by ^1H NMR was presumed to be a rapidly equilibrating ring-opened structure. Product was isolated as a foam (1.11 mmol, 79%) from **15** based on recovered **15**. Diol arising from deprotected **14** was recovered cleanly as the white solid (2.65 mmol). IR (thin film): 2930, 2857, 2114 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 7.65–7.58 (m, 4H), 7.42–7.32 (m, 6H), 5.28–5.26 ((bs (minor), δ=5.28); (bs (minor), δ=5.27); (bs (major), δ=5.26), total=1H), 4.06–3.98 (m, 1H), 3.82–3.58 (m, 11H), 1.39 (s, 9H), 1.045–1.034 ((s (minor), δ=1.045); (s (major), δ=1.038); (s (minor), δ=1.034), total=9H), 0.951–0.944 ((s (major), δ=0.951); (s (minor), δ=0.948); (s (minor), δ=0.944), total=9H), 0.837–0.811 ((s (minor), δ=0.837); (s (major), δ=0.828); (s (minor), δ=0.811), total=9H), 0.195–0.120 ((s (minor), δ=0.195); (s (major), δ=0.175); (s (minor), δ=0.170); (s (minor), δ=0.166); (s (major), δ=0.153); (s (minor), δ=0.145); (s (minor), δ=0.141); (s (major), δ=0.132); (s (minor), δ=0.120), total=9H), 0.030–0.015 ((s (minor), δ=0.030); (s (major), δ=0.024); (s (minor), δ=0.015), total=3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.8, 155.6, 135.9, 135.8, 133.7, 133.6, 133.5, 130.1, 130.0, 128.0, 127.9, 94.7, 92.2, 85.0, 82.1, 81.2, 81.0, 75.9, 73.8, 73.4, 71.5, 71.2, 70.8, 67.9, 67.0, 65.7, 62.6, 62.3, 57.2, 52.4, 28.5, 27.2, 27.1, 26.6, 26.5, 26.4, 26.3, 19.4, 19.3, 18.8, 18.7, −3.4, −3.5, −3.6, −3.7, −4.3, −4.4, −4.5. TLC (SiO_2, 50% EtOAc/hexanes): R_f*'s*=0.45 and 0.53. HRMS: Calcd for $C_{44}H_{72}N_4O_9Si_3$ $(M+Na)^+$: 907.4505. Found: 907.4494.

4.1.6. Spiroketals (18a/b). To a solution of **17** (1.055 mmol, 1.0 equiv.) in 53 mL EtOAc was added Pd/C (10%, 0.62 g) followed by camphorsulfonic acid (0.949 mmol, 0.9 equiv.). The flask was evacuated under aspirator pressure and refilled with hydrogen three times, and then stirred under hydrogen (1 atm) for 18 h. The mixture was then filtered through a bed of Celite and quenched with excess triethylamine. The solution was concentrated to give a clear, crude oil. The oil was immediately chromatographed on silica gel (3:1 then 1:1 hexanes/EtOAc) to give the product mixture as a clear foam (0.810 mmol, 76%). IR (thin film): 2931, 2111, 1698 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.64–7.58 (m, 4H), 7.44–7.32 (m, 6H), 6.09–5.93 ((dd (major), δ=6.08, *J*=9.9, 1.4 Hz); (dd (minor), δ=6.03, *J*=10.3, 1.2 Hz); (dd (minor), δ=5.96, *J*=9.9, 1.4 Hz), total=1H), 5.72–5.60 ((d (minor), δ=5.65, *J*=10.3 Hz); (d (major), δ=5.61, *J*=9.9 Hz); (d (minor), δ=5.57, *J*=9.9 Hz), total=1H), 5.39–5.08 ((bs (major), δ=5.39); (bs (minor), δ=5.17); (bs (minor), δ=5.08), total=1H), 4.66–4.38 (m, 1H), 4.20–3.40 (m, 10H), 1.43–1.38 ((s (major), δ=1.43); (s (minor), δ=1.41); (s (minor), δ=1.38), total=9H), 1.048–1.037 ((s (minor), δ=1.048); (s (major), δ=1.044); (s (minor), δ=1.037), total=9H), 0.963–0.940 ((s (major), δ=0.963); (s (minor), δ=0.944); (s (minor), δ=0.940), total=9H), 0.849–0.817 ((s (minor), δ=0.849); (s (minor), δ=0.837); (s (major), δ=0.827); (s (minor), δ=0.817), total=9H); 0.188–0.125 (s (major), δ=0.188); (s (minor), δ=0.178); (s (major), δ=0.167); (s (minor), δ=0.159); (s (minor), δ=0.143); (s (major), δ=0.134); (s (minor), δ=0.125), total=9H), 0.05–0.03 ((s (minor), δ=0.05); (s (major), δ=0.04); (s (minor), δ=0.03), total=3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 157.0, 135.9, 135.8, 133.7, 133.6, 131.5, 130.1, 130.0, 128.9, 128.0, 127.9, 127.8, 96.4, 95.9, 80.6, 77.5, 77.2, 77.0, 73.6, 72.1, 71.9, 71.5, 65.8, 65.5, 65.3, 62.7, 53.3, 28.5, 28.4, 27.1, 26.6, 26.5, 26.4, 26.3, 26.2, 19.4, 18.8, 18.7, −3.1, −3.3, −3.7, −3.8, −4.3, −4.4, −4.5. TLC (SiO_2, 25% EtOAc/hexanes): R_f's=0.37 and 0.47. HRMS: Calcd for $C_{44}H_{72}N_4O_8Si_3$ $(M+Na)^+$: 891.4556. Found: 891.4520.

4.1.7. Spiroketal methyl esters (19a/b). To a solution of **18a/b** (0.783 mmol, 1.00 equiv.) in 26 mL CH_2Cl_2 at rt was added $NaHCO_3$ (0.790 mmol, 1.00 equiv.) followed by Dess–Martin periodinane (1.570 mmol, 2.00 equiv.). The reaction mixture was stirred for 4.5 h at which time it was quenched with 10 mL of a saturated aq $NaHCO_3$ solution containing $Na_2S_2O_3$ (25g per 100 mL). After stirring for 15 min, the mixture was diluted with 50 mL EtOAc and 25 mL H_2O. The phases were separated, and the aq phase was extracted with 2×25 mL EtOAc. The combined organic phases were washed with 50 mL brine, dried ($MgSO_4$), filtered, and concentrated. The resulting product was dissolved in 25 mL of a 4:1 *t*BuOH:H_2O solution. NaH_2PO_4 (0.822 mmol, 1.05 equiv.) followed by 2-methyl-2-butene (2.0 M in THF, 3.290 mmol, 4.20 equiv.), and finally $NaClO_2$ (2.349 mmol, 3.00 equiv.) were successively added, and the solution was allowed to stir at rt for 14 h. The reaction mixture was then diluted with 25 mL H_2O and 50 mL EtOAc. The phases were separated, and the aq phase was extracted with 2×25 mL EtOAc. The combined organic phases were washed with 50 mL brine, dried ($MgSO_4$), filtered, and concentrated to give a clear oil. The oil was dissolved in 19.5 mL of a 7:2 benzene:MeOH solution and (trimethylsilyl)diazomethane (2.0 M in hexanes, 1.566 mmol, 2.00 equiv.) was added. The yellow solution was allowed to stir for 4 h at rt, at which time it was concentrated by aspirator pressure rotary evaporator to give a crude, yellow oil. The oil was immediately chromatographed on silica gel (9:1 hexanes/EtOAc) to give the product as the usual mixture of diastereomers as well as possible anomers by ^{1}H NMR. Product was isolated as a clear foam (0.683 mmol, 87%) from **18a/b**. IR (thin film): 2955, 2858, 1749 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.64–7.59 (m, 4H), 7.44–7.32 (m, 6H), 6.37–5.93 (m, 1H), 5.83–5.62 (m, 1H), 5.44–5.16 (m, 1H), 4.38–3.38 (m, 11H), 1.417–1.382 ((s (major), δ=1.417); (s (minor), δ=1.398); (s (minor), δ=1.394); (s (minor), δ=1.382), total=9H), 1.047–1.034 ((s (minor), δ=1.047); (s (major), δ=1.039); (s (minor), δ=1.034), total=9H), 0.953–0.925 ((s (major), δ=0.953); (s (minor), δ=0.933); (s (minor), δ=0.925), total=9H), 0.842–0.808 ((s (minor), δ=0.842); (s (minor), δ=0.831); (s (major), δ=0.816); (s (minor), δ=0.808), total=9H); 0.179–0.117 (s (minor), δ=0.179); (s (minor), δ=0.172); (s (major), δ=0.156); (s (minor), δ=0.131); (s (major), δ=0.122); (s (minor), δ=0.117), total=9H), 0.024–0.011 ((s (minor), δ=0.024); (s (major), δ=0.019); (s (minor), δ=0.011), total=3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.7, 135.8, 135.7, 133.8, 133.7, 133.6, 133.5, 130.8, 130.2, 130.1, 130.0, 129.9, 129.8, 129.2, 128.0, 127.9, 115.5, 96.6, 96.3, 96.1, 80.6, 77.5, 77.2, 77.0, 73.8, 73.7, 72.7, 72.6, 72.3, 71.8, 71.5, 71.4, 71.3, 65.1, 64.7, 64.4, 63.6, 62.8, 55.3, 53.1, 52.9, 28.5, 28.4, 28.3, 27.1, 26.6, 26.5, 26.4, 26.3, 26.2, 26.1, 19.4, 19.3, 19.2, 18.8, 18.7, −3.0, −3.3, −3.4, −3.5, −3.6, −4.0, −4.3, −4.4, −4.6. TLC (SiO_2, 25% EtOAc/hexanes): R_f=0.48. MS: Calcd for $C_{45}H_{72}N_4O_9Si_3$ $(M+Na)^+$: 919.45. Found: 919.48.

4.1.8. D-Serine and L-serine constrained glycopeptides (21a/b). To a solution of **19a/b** (0.551 mmol, 1.0 equiv.) in 11 mL THF at rt was added tetrabutylammonium fluoride (1.0 M in hexanes, 2.755 mmole, 5.0 equiv.). The reaction mixture was stirred for 5 h at which time it was quenched with 10 mL saturated aq NH_4Cl and diluted with 25 mL

EtOAc. The phases were separated, and the aq phase was extracted with 2×15 mL EtOAc. The combined organic phases were washed with 10 mL brine, dried (MgSO$_4$), filtered, and concentrated. The resulting crude oil was chromatographed on silica gel (EtOAc then 9:1 EtOAc/MeOH) to give a clear oil which was immediately taken up in 9 mL of a 2:1 pyridine:Ac$_2$O solution. The mixture was stirred for 18 h at rt, at which time the solution was concentrated by high-vacuum (0.1 torr) rotary evaporator. The resulting crude oil was chromatographed on silica gel (2:1 hexane/EtOAc) to give a clear foam (0.436 mmol, 80%) from **19a/b**. This product was observed as the usual mixture, and full characterization was not performed. The majority of the product (0.399 mmol, 1.0 equiv.) was subsequently dissolved in 10 mL MeOH, and acetic anhydride (1.995 mmol, 5.0 equiv.) followed by Pd/C (10%, 0.145 g) were added in succession. The flask was evacuated under aspirator pressure and refilled with hydrogen three times, and then stirred under hydrogen (1 atm) for 12 h. The mixture was then filtered through a bed of Celite, and concentrated to give a crude oil. The oil was immediately chromatographed on silica gel (1:1 hexanes/EtOAc then EtOAc) to give a product mixture including the two major diastereomers **21a/b**. The two major diastereomers **21a** and **21b** were purified by preparative HPLC on a silica gel column using EtOAc as the eluent. The D-isomer (**21a**) was isolated as a white, crystalline solid (0.101 mmol, 26%) from **20a/b** in greater than 95% purity. The L-isomer (**21b**) was isolated as a white foam (0.085 mmol, 22%) from **20a/b** in greater than 95% purity.

4.1.9. D-Isomer (21a). IR (thin film): 2978, 1748, 1663 cm^{-1}. ^{1}H NMR (500 MHz, CDCl$_3$): δ 5.64 (bs, 1H), 5.34 (d, J=2.6 Hz, 1H), 5.15 (dd, J=11.1, 3.4 Hz, 1H), 4.87 (bs, 1H), 4.34 (t, J=10.5 Hz, 1H), 4.16 (dd, J=10.9, 7.1 Hz, 1H), 4.10–4.05 (m, 3H), 3.85 (d, J=11.5 Hz, 1H), 3.71 (s, 3H), 2.23 (dt, J=13.9, 5.0 Hz, 1H), 2.15 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 2.00–1.93 (m, 1H), 1.82 (dt, J=13.9, 4.6 Hz, 1H), 1.66 (ddd, J=14.0, 4.6, 2.2 Hz, 1H), 1.42 (s, 9H). ^{13}C NMR (125 MHz, CDCl$_3$): δ 170.7, 170.6, 98.4, 69.2, 67.5, 67.4, 62.0, 52.7, 28.4, 25.4, 21.0, 20.9, 20.8. TLC (SiO$_2$, EtOAc): R_f=0.38. HRMS: Calcd for C$_{25}$H$_{38}$N$_2$O$_{13}$ (M+H)$^+$: 575.2452. Found: 575.2446.

4.1.10. L-Isomer (21b). IR (thin film): 1748, 1669, 1369 cm^{-1}. ^{1}H NMR (500 MHz, CDCl$_3$): δ 5.85 (d, J=10.0 Hz, 1H), 5.34 (dd, J=3.3, 0.9 Hz, 1H), 5.13 (dd, J=10.8, 3.3 Hz, 1H), 4.68 (bs, 1H), 4.38 (d, J=11.0 Hz, 1H), 4.29 (t, J=10.5 Hz, 1H), 4.12 (dd, J=11.0, 6.6 Hz, 1H) 4.08–4.00 (m, 2H), 3.71 (s, 3H), 3.37 (d, J=11.0 Hz, 1H), 2.13 (s, 3H), 2.04 (s, 3H), 2.02–1.93 (m, 1H), 1.96 (s, 3H), 1.95 (s, 3H), 1.89 (dt, J=12.8, 6.2 Hz, 1H), 1.79–1.65 (m, 2H), 1.39 (s, 9H). ^{13}C NMR (125 MHz, CDCl$_3$): δ 172.1, 170.9, 170.8, 170.7, 170.6, 97.6, 69.5, 67.5, 67.4, 64.9, 61.9, 55.4, 52.4, 50.7, 28.4, 27.3, 23.5, 21.0, 20.9. TLC (SiO$_2$, EtOAc): R_f=0.30. MS: Calcd for C$_{25}$H$_{38}$N$_2$O$_{13}$ (M+H)$^+$: 575.2. Found: 575.3.

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