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# Design and synthesis of glucose-templated proline–lysine chimera: polyfunctional amino acid chimera with high prolyl *cis* amide rotamer population

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ABSTRACT

We describe the synthesis of two glucose-templated proline–lysine chimeras (GlcTProLysCs) that differ in the stereochemistry of the hydroxymethyl substituent at the *C*-5′ position of the pyrrolidine ring. The key synthetic steps involve C-glycosylation of an exocyclic glucose-based epoxide with allyltributylstannane, which affords functionalized *C*-ketosides containing an α-hydroxy ester moiety; introduction of an amino group at *C*-2 through stereoselective reductive amination; and regioselective installation of the azide group at *C*-6 on the glucose scaffold. Incorporation of these chimeras into the model peptides Ac-GlcTProLysC-NHMe and Ac-GlcTProLysC-OMe demonstrates that the stereochemistry of the hydroxymethyl substituent at the C-5′ position has a profound effect on the equilibrium constant of prolyl amide *cis*/*trans* isomerization. The equilibrium constant $K_{c/t}$ for the peptide mimic Ac-GlcTProLysC-NHMe with *C*-5′(*R*) stereochemistry was determined to be 3.03 ± 0.04, while the $K_{t/c}$ for the C-5′(*S*) diastereoisomer was 0.56 ± 0.04 in $D_2O$. Temperature coefficient experiments indicate that the origin of these effects is derived from two critical hydrogen bonds involving the C-5′ hydroxymethyl substituent: one to the N-terminal amide carbonyl group, and the other to the primary amino group in the glucose moiety.



## 1. Introduction

Conformationally constrained amino acids have found wide applications as building blocks to study and probe the bioactive conformation of peptides when binding to receptors.[1–3] Among all naturally occurring amino acids, proline is the only amino acid with a side chain fused onto the peptide backbone. Its cyclic structure restricts the rotation about its $\phi$ dihedral angle, thereby reducing the energy difference between the prolyl amide *cis* and *trans* isomers. Thus, while most peptide amide bonds exist almost exclusively in the *trans* form, proline has a much greater propensity to form *cis* amide bonds. A variety of factors influence *cis*/*trans* isomerization of proline; these include electron-withdrawing groups attached to the pyrrolidine ring,[4] $n\rightarrow\pi^*$ interaction,[5] Ar–Pro interactions,[6] and steric effects.[7] In particular, incorporation of bulky substituents into the δ-position of proline has been shown to enhance the prolyl amide *cis* population significantly. *cis*/*trans* Isomerization of proline plays an important role in the formation of secondary structures in peptides and proteins because proline induces a reversal in backbone conformation resulting in the formation of reverse turns and disruption of helices and sheets in proteins. Besides the occurrence of proline in β-turns, proline-rich sequences also exist as extended helices[8] (polyproline-I and polyproline-II) and antimicrobial peptides.[9] Furthermore, proline undergoes post-translational modifications to form 4-(*R*)-hydroxyprolines, which are known to contribute to the enhanced stability of the polyproline-II conformation in both collagenous proteins and peptides,[4] and in plant cell wall glycoproteins.[10]

Proline analogues displaying the characteristics of other amino acids are referred to as proline-amino acid chimeras, and have been used to study the spatial requirements for receptor affinity and biological activity of both natural amino acids[11,12] and peptides.[13–15] For example, β-substituted-prolines such as 3-carboxyproline,[11a] 3-phenylproline,[16] and 3-di-methylproline[17] combine amino acid side chain functionality with proline's conformational rigidity. In these cases, replacement of the natural amino acids in peptides by proline-amino acid chimeras provided better understanding of the bioactive conformations of peptides binding to receptors.[13–15] While these analogues have proved useful for inducing specific constraints into amino acids and peptides, their structures do not permit additional derivatization; a trait that is often required in drug discovery and lead optimization. Polyfunctional proline-amino acid chimera may overcome these drawbacks.

Our concept for developing such polyfunctional proline-amino acid chimeras was derived from glycosyl amino acids (GAAs) which are defined by an α-amino acid group [$CH(NH_2)CO_2H$] either directly attached or carbon-linked to the anomeric carbon of a

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carbohydrate scaffold.[18] The relative rigidity of the pyran ring combined with the polyfunctional nature of the carbohydrate scaffold has inspired the design of unusual and conformationally constrained amino acids and novel peptidomimetics.[18] Although there are many examples of *C*-glycosyl-glycine, -alanine, -serine, and -asparagine,[18a] few proline-based GAAs exist.[19]

We report here the design and synthesis of spirocyclic glucose-templated proline–lysine chimeras (GlcTProLysCs), and describe their properties in peptide mimics. Spirocyclic GlcTProLysCs were selected on the basis of previous synthetic methodology.[20] Bicyclic GlcTProLysCs combine the molecular features of glucose (pyran-based polyol) with the unique characteristics of proline or 3-hydroxyproline and L-lysine (Fig. 1). The characteristics of the lysine side chain including relative length and presence of amino function are presented on the pyrrolidine ring and are further constrained by incorporation into the 6-amino-6-deoxy-D-glucose scaffold. Proline–lysine chimeras were selected due to their frequent occurrence in cationic antimicrobial peptides with polyproline conformation.[9] To control the prolyl amide *cis*/*trans* isomerization, we were interested in developing GlcTProLysC analogues that contain hydrogen bond forming substituents like a hydroxymethyl group at the δ-position of proline. Previous studies have shown that bulky substituents at the δ-position (including δ-*t*-butyl proline[7] and δ,δ-dimethyl proline[3]) enhance the prolyl amide *cis* isomer population. However, hydrogen bond forming groups at the δ-position have never been investigated. In addition, chemical manipulations and derivatization of the polyol scaffold provide an opportunity to adjust the chemical, physical, and pharmacodynamic properties of proline-containing peptides.[21] This may provide a novel tool to functionalize extended helical structures including PP1 and PP2.[22] Moreover, incorporation of polyhydroxylated amino acids have been shown to induce novel secondary structures in small peptides. For instance, incorporation of unprotected sugar amino acids into small peptides such as gramicidin S[23] and opioid peptides[24] has prohibited the formation of the targeted secondary structural motif. Instead, unusual turn structures were stabilized by intramolecular hydrogen bonds between sugar hydroxyl groups and the peptidic amide backbone.[25] Similar effects may also be observed with GlcTProLysC.

## 2. Results and discussion

The synthesis started with the readily available D-glucose-based lactone **1**[26] (Scheme 1), which reacts with the enolate of methyl bromoacetate generated from lithium bis-(trimethylsilyl)amide ($LiN(SiMe_3)_2$) in tetrahydrofuran (THF) at −78 °C to produce the exocyclic epoxide **2** in 80% yield as a single stereoisomer.[27] Trimethylsilyl trifluoromethanesulfonate (TMSOTf)-promoted C-glycosylation of epoxide **2** with allyltributylstannane in dichloromethane followed by hydrolysis of the TMS-ether with trifluoroacetic acid (TFA)-containing wet THF produced a mixture containing alcohols **3** and **4** (ratio **3**:**4** = 9:1) in a combined yield of 89%. Regioselective opening of epoxide **2** proceeded via formation of oxonium ion (intermediate **A**) that subsequently underwent α-selective C-glycosylation favored by stereoelectronic factors as observed for similar C-glycosylation reactions.[28] It is noteworthy that slow addition of epoxide **2** to allyltributylstannane is crucial

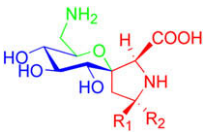
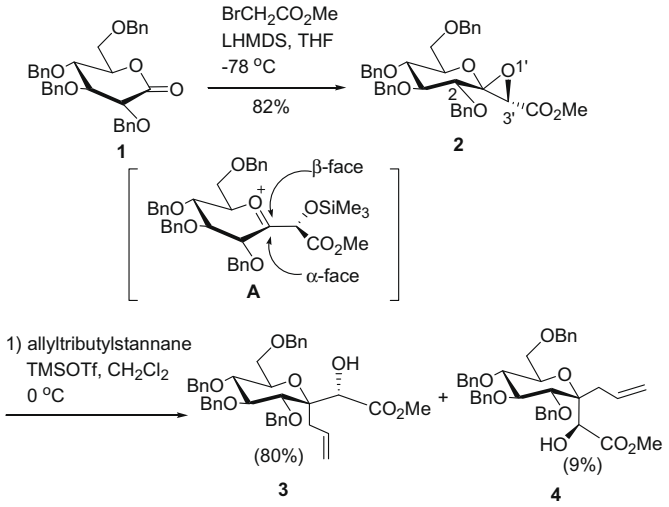



**Figure 1.** Glucose-templated proline–lysine chimera (GlcTProLysC).




**Scheme 1.** Synthesis of the α-allylic intermediate **3**.

for optimal yield of target compound **3**. The configuration at the anomeric position in compound **4** was deduced on the basis of observed/unobserved NOE[29] contacts (Fig. 2).

Compound **3** served as starting material for the installation of the amino function at *C*-2 (Scheme 2). Initially, we attempted to convert the hydroxyl group at *C*-2 into an amino function. However, nucleophilic substitution of *C*-2-activated sulfonate ester (triflate) with a variety of nucleophiles including benzylamine, *p*-methoxybenzylamine, lithium, and sodium azide at low and elevated temperatures resulted only in trace amounts of the desired amine. In these cases, unreacted starting material was recovered (>90%). To avoid these complications, we decided to explore a reductive amination approach. Alcohol **3** was oxidized to ketone **5** at −78 °C using a mixture containing trifluoroacetic anhydride, triethylamine, and dimethylsulfoxide in dichloromethane to produce ketone **5** in 95% isolated yield.[30] In order to confirm the configuration of the product, we performed NOE experiments (Fig. 2). For instance, subjecting one of the allylic protons to a one-dimensional GOESY experiment showed inter-proton effects to H-3 (7.9% NOE[29]) and H-5 (7.1%). This is consistent with the structure **5** bearing an allylic group at the axial position. Subsequently, the ketone **5** was converted into the amino ester **7** in a two-step procedure. At first, compound **5** was exposed to titanium tetrachloride-promoted imination using benzylamine in ether to afford the imine **6** in 96% after chromatographic purification.[31] The imine **6** was stereoselectively reduced to amino ester **7** in quantitative yield using sodium cyanoborohydride in acidified MeOH at 0 °C. The high diastereoselectivity could be explained in the Felkin model (Fig. 3),[32] in which the nucleophile approached the imine ion from the less hindered side (*Re* face) and resulted in the formation of *S*-configuration at C-2 position of compound **7**, which was confirmed by the following NOE experiments.

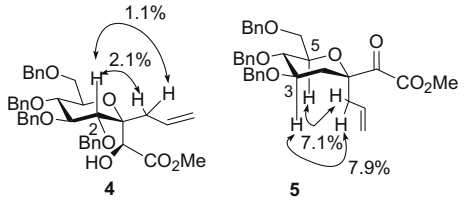
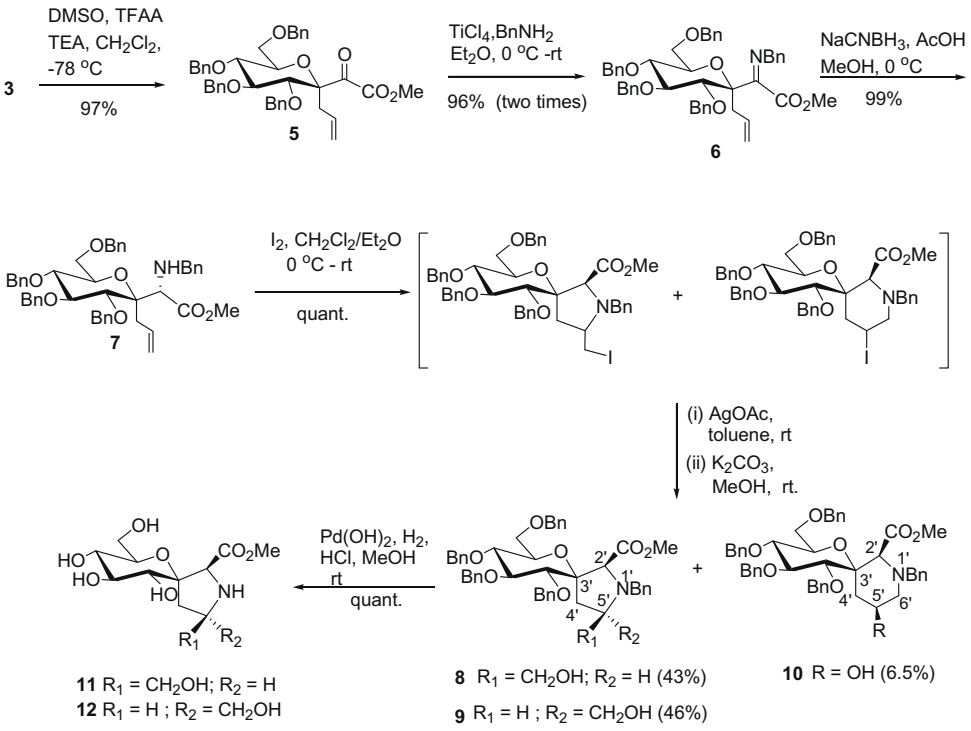



**Figure 2.** Assignment of stereochemistry at anomeric carbon in compounds **4** and **5** through 1D NOE experiments (recorded in $CDCl_3$).




**Scheme 2.** Preparation of spirocyclic glucose-based proline analogues using a reductive amination route.

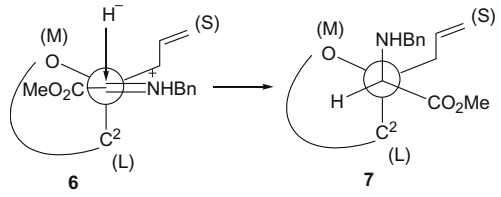
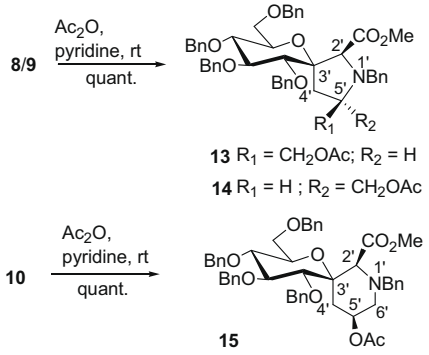



**Figure 3.** A rational explanation for stereoselective reductive amination using the Felkin model.

With amino ester **7** in hand, we installed the pyrrolidine ring by iodocyclization in dichloromethane to produce an inseparable isomeric mixture containing various iodo-compounds. To separate the compounds from each other, we converted the mixture into the alcohols **8**, **9,** and **10** via a two-step process. At first, the mixture was exposed to silver acetate in toluene[33] to produce an inseparable mixture of esters **13**, **14,** and **15** (Scheme 3) that, by treatment with potassium carbonate in MeOH, afforded the alcohols **8**, **9,** and **10** in 44%, 45%, and 6% isolated yields, respectively. Subsequently, exposure of compounds **8** and **10** to catalytic hydrogenolysis condition using Pearlman's catalyst provided the unprotected proline analogues **11** and **12** in quantitative yields, respectively.




**Scheme 3.** Acylation of compounds **8-10**.

To assign the stereochemistry at C-2′, the alcohols **8**, **9,** and **10** were converted into the acetates **13**, **14,** and **15** using standard conditions (acetic anhydride in pyridine, Scheme 3). We selected the pipecolic acid analogue **15** to assign the stereochemistry at C-2′ (Figure 4). The spirocyclic compound **15** consists of both a pyranose ring and a piperidine ring. The large coupling constants for $J_{2,3}$, $J_{3,4,}$ and $J_{4,5}$ (>9.0 Hz) in conjunction with inter-proton NOEs between H-3 and H-5, establish the $^4C_1$ chair conformation of the sugar ring. The chair conformation of the piperidine ring is deduced from the observed vicinal diaxial and long-range coupling constants. For instance, the axial position of protons H-4′$_{ax}$, H-5′$_{ax,}$ and H-6′$_{ax}$ can be deduced by their large vicinal diaxial coupling constants ($J_{4'ax,5'ax}$, $J_{5'ax,6'ax}$ > 10.5 Hz), while the observed long-range coupling constants between $J_{4'eq,6'eq}$ are equal to $J_{2'eq,4'eq}$ (~1.0 Hz), confirming the equatorial position of H-2′$_{eq}$, H-4′$_{eq,}$ and H-6′$_{eq}$ in the piperidine ring. In addition, the observed inter-proton effects (NOE) between H-5/H-5′$_{ax}$, H-5/H-3, and H-3/H-4′$_{ax}$, together with the unobserved effect between H-6′$_{ax}$/H-2′$_{eq}$ using a one-dimensional GOESY experiment, determined the C-2′ (*S*) configuration (Fig. 4).[30,34]

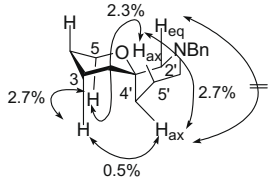



**Figure 4.** Assignment of stereochemistry at C-2′ position in compound **15** through 1D NOE experiment (recorded in $C_6D_6$). Some of the substituents in the glucose ring are omitted for clarity.

Once we had established the configuration at C-2′ in compound **15**, we turned our interest to the stereochemistry at C-5′ of the spirocyclic proline analogues **13** and **14**. Because the iodocyclization was performed on a single stereoisomer **7**, we assume that the stereochemistry at C-2′ of the proline analogues **13** and **14** remained '*S*' based on the previous assignment with piperidine analogue **15**. To discriminate between compounds **13** and **14,** we again used NOE[30] experiments (Fig. 5). As an example, the observed inter-proton effects between H-2′/H-5′ and H-5′/H-5 for compound **13** are consistent with the C-5′ (*R*) stereochemistry. By comparison, proline analogue **14** did not show any inter-proton effect between H-2′/H-5′, which is consistent with a C-5′ (*S*) configuration.

Once we had established the stereochemistry at the C-2′- and C-5′-positions in compounds **13** and **14,** we then focused on the conversion of the primary hydroxyl group on the glucose moiety into an amino function (Scheme 4). Initially, compounds **13** and **14** were exposed to catalytic hydrogenolysis condition using Pearlman's catalyst. The resulting N-debenzylated amine was protected using di-*t*-butyl dicarbonate and triethylamine in MeOH to afford the carbamates **16** and **17** in 90% and 62% yields, respectively, without acyl migration. The azido group in compounds **16** and **17** was installed by a standard two-step procedure: first, selective activation of the primary hydroxyl group as sulfonate ester; second, nucleophilic substitution with sodium azide in DMF at 80 °C, produced azides **18** and **19** in excellent yields.

Azides **18** and **19** served as starting materials for incorporation into the peptide mimics **22–25** (Scheme 5), which were used to study the thermodynamic properties of *cis*/*trans* isomerization of the glucose-templated proline–lysine chimera. In addition, we selected peptide esters **22** and **23** bearing a C-terminal methyl ester as well as methyl amides **24** and **25** as peptide mimics to study how the nature of the C-terminal group affects N-terminal prolyl amide isomerization.

Peptide esters **22** and **23** are prepared from azides **18** and **19** using the synthetic route outlined in Scheme 5. Deprotection of the *N*-Boc group in **18** and **19** with trifluoroacetic acid followed by acetylation using pyridine and acetic anhydride and O-deacetylation using sodium methoxide in MeOH produced azides **20** and **21** in 96% and 80% yields, respectively. Attempts to perform selective N-acylation failed and produced complex reaction mixtures. Catalytic hydrogenation of azides **20** and **21** using Pearlman's catalyst produced peptide esters **22** and **23** in quantitative yields. The *N*′-methylamides **24** and **25** were prepared from azides **20** and **21** through a two-step procedure: first, methyl esters **20** and **21** were treated with a concentrated methylamine solution in ethanol to afford *N*′-methylamide intermediates; second, the azido function was reduced by catalytic hydrogenation to produce peptide mimics **24** and **25** in 93% and 95% yields, respectively.

The assignments of N-terminal geometry for model peptides **22–25** were made on the basis of NOE experiments in $D_2O$ (Fig. 6). For example, selective inversion of the N-terminal methyl group in the prolyl amide *cis* isomer **23a** by a selective GOESY[34] experiment showed an inter-proton effect to H-2′ (5.63% NOE). By comparison, no inter-proton effect was observed between

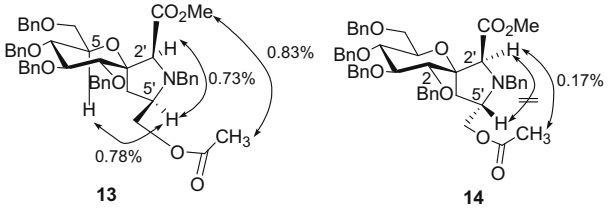
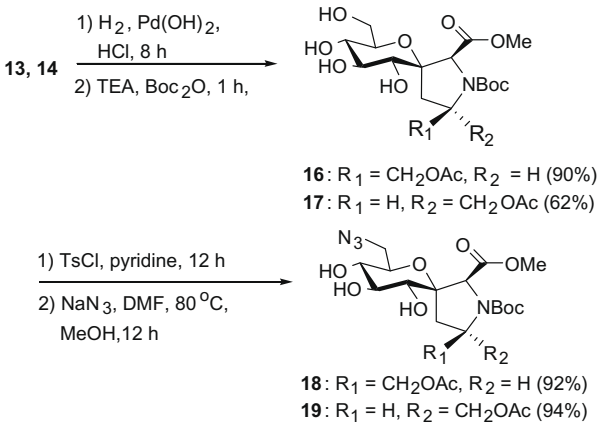



**Figure 5.** Assignment of stereochemistry at C-5′-position in compounds **13** and **14** through 1D NOE experiment (recorded in $C_6D_6$).

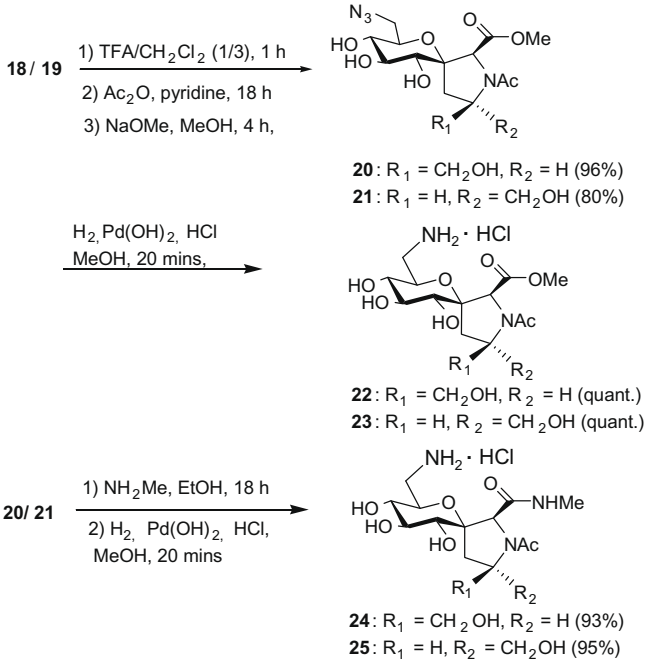



**Scheme 4.** Installation of the azide function at the C-6 position.

H-2′ and methyl group of N-terminus in *trans* isomer **23b**. Moreover, selective inversion of the methyl group of N-terminus in **23b** showed inter-proton effects to H-5′ (4.54% NOE) and H-6′ (3.80% NOE). Similar experiments were performed to assign the *cis*/*trans* isomers in compounds **22**, **24,** and **25**.

In addition, we observed that the $^{13}C$ NMR chemical shifts of the $C^{\alpha}$ atom of the *trans* rotamer in compounds **22–25** are high field shifted (0.75–1.02 ppm) relative to the *cis* isomer in water. This is consistent with previous observations made by Lubell and co-workers[7] on other proline-containing peptide mimics and may serve as a diagnostic tool to assign the *trans* isomer in cases where NOE experiments do not allow assignment due to spectral overlap.

The equilibrium constants $K_{c/t}$ for compounds **22–25** are shown in Table 1, and were determined by integrating and averaging as many distinct proton signals as possible for both the major and minor isomers in the $^1H$ NMR spectra.[35] Our results indicate that compounds **22** and **24** display a higher *cis* isomer population relative to their C-5′ diastereoisomers **23** and **25**, respectively. It appears that the stereochemistry at C-5′ has a profound effect on




**Scheme 5.** Synthesis of peptide mimics **22–25**.

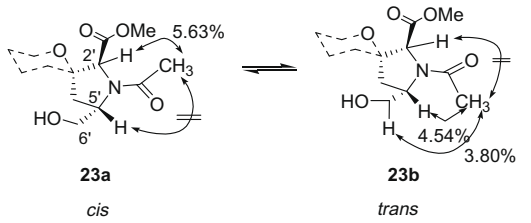



**Figure 6.** Assignment of *cis* and *trans* isomers in compound **23** in $D_2O$ using 1D NOE experiments. The same experiments were used to assign the *cis*/*trans* isomers in compounds **22**, **24,** and **25**.

the equilibrium of isomerization. Also, the *cis* prolyl amide population in esters **22** and **23** was generally lower than that in *N*′-methylamides **24** and **25**. Taylor and co-workers have proposed that the *trans* conformation of esters is stabilized relative to amides as a result of increased electron donation from the oxygen lone pair of the N-terminal amide carbonyl group to the antibonding orbital of the prolyl C-terminal carbonyl group (Fig. 7).[35]

## 2.1. Effect of pH

Because compounds **22–25** have an ionizable amino group, we were interested to study the influence of the ionization state on $K_{t/c}$. Previous studies have indicated that ionizable groups in proximity to the imide backbone can influence thermodynamics of prolyl amide *cis*/*trans* isomerization.[36] We selected three buffer ranges: pH 2.6, 7.4, and 12.4, to examine the pH effect on the isomerization of **24** and **25** (Table 2). Our study shows that the prolyl N-terminal amide *cis*/*trans* ratio is not affected by pH and the observed changes are within the experimental error. Molecular modeling suggests that the large distance of the ionizable amino function from the imide function is responsible for the absence of a pH effect.

## 2.2. Conformational analysis of compounds 24 and 25

The relatively large coupling constants for $J_{2,3}$, $J_{3,4,}$ and $J_{4,5}$ (>9.2 Hz) indicate a $^4C_1$ conformation of the pyranose ring in **24** and **25**. The conformation of the piperidine ring is expected to be $C^{\beta}$-exo based on previous studies using 3(*S*)-hydroxyproline-containing peptide mimics.[37] This conformation places the endocyclic oxygen substituent in an axial position.[37b] In this conformation, the pyrrolidine ring will be stabilized by gauche interaction and a stabilizing $\sigma(C^{\gamma}\text{-H}) \rightarrow \sigma^{*}(C^{\beta}\text{-O})$ interaction. This conformation is further supported by characteristic long-range 'W' coupling constants ($J \sim 1.0$ Hz) between H-$2'_{eq}$ and H-$4'_{eq}$ in both compounds **24** and **25** (Fig. 8).

To explain the different *cis*/*trans* ratio in compounds **24** and **25,** we considered intramolecular hydrogen bonding, which can be studied by calculating the temperature coefficients ($\Delta\delta/\Delta T$) of key exchangeable protons.[38] Previous studies have shown that ($\Delta\delta/\Delta T$) > −3.0 ppb/deg is a diagnostic tool for the detection of intramolecular H-bonding.[38] The 1D spectra of compounds **24** and **25** were analyzed between 25 and 45 °C in 5-deg steps in DMSO-$d_6$ to determine the temperature coefficients (Table 3). Our results indicate that the protons associated with $NH_2$-6, OH-6′, and NHMe exhibit the highest temperature coefficient values suggesting that these protons are involved in intramolecular H-bonding in compounds **24** and **25**. The low ($\Delta\delta/\Delta T$) values observed for OH-2 and OH-3 reflect high solvent exposure of these hydroxyl groups while the relative high value for OH-4 in **25** may indicate some H-bond interaction with one of the nitrogen lone pairs on $NH_2$-6. The relative high and nearly identical ($\Delta\delta/\Delta T$) values observed for the methyl amide proton in structures **24a**, **24b**, **25a,** and **25b** support the notion that this proton is engaged in hydrogen bonding to N-terminal carbonyl group in *cis*/*trans* isomers of both compounds **24** and **25**. Moreover, the higher ($\Delta\delta/\Delta T$) value for the $NH_2$-6 in compound **25** when compared to that of diastereomer **24** suggests that the amino group in **25** is involved in stabilization of the *trans* isomer **25b** and in the destabilization of the *cis* isomer **25a** relative to **24a** and **24b**. A similar trend is observed for the OH-6′ position. Isomers **25a** and **25b** exhibit higher ($\Delta\delta/\Delta T$) values when compared to isomers **24a** and **24b** suggesting that OH-6′ is involved in stabilization of the *trans* isomer **25b** relative to **24b**. Taken together, these results support the notion that compound **25** is stabilized by an intramolecular hydrogen bond (6′-OH–$NH_2$-6) that is absent in **24**. The hydrogen bond (OH-6′–$NH_2$-6) competes with the H-bond (6′-OH–O=C(N)$CH_3$) of the *cis* isomer **25a** resulting in a lower *cis* population of **25a** relative to **24a** (Fig. 9).

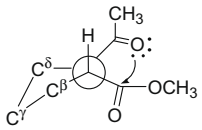



**Figure 7.** $n \rightarrow \pi^{*}$ Interaction (looking down $C^{\alpha}$–N bond).

## 3. Conclusions

We have developed a synthetic route to two spirocyclic GlcTProLysCs that differ in the stereochemistry of the hydroxymethyl substituent at the C-5′ position of the pyrrolidine ring. A key intermediate in the synthesis is the glucose-templated C-glycosyl glycine analogue **7**, which bears an additional *C*-allylic substituent at the pseudoanomeric position. Compound **7** may find future use in the synthesis of other carbohydrate-templated amino acids via derivatization of the allyl group. To study the thermodynamic properties of prolyl amide *cis*/*trans* isomerization, the two GlcTProLysC analogues were incorporated in peptide mimics Ac-GlcTProLysC-OMe and Ac-GlcTProLysC-NHMe. Our study indicates that the stereochemistry at the C-5′ position in both peptide mimics has a

**Table 1**
*Cis* population (%) and equilibrium constant $K_{c/t}$ of compounds **22–25** in $D_2O$

| Compounds | 22 | 23 | 24 | 25 |
|---|---|---|---|---|
| *cis* (±3%) | 55 | 19 | 75 | 36 |
| $K_{c/t}$ (±0.04) | 1.22 | 0.23 | 3.03 | 0.56 |

**Table 2**
pH effect on $K_{c/t}$ for compounds **24** and **25** in $D_2O$

| Compounds | pH | | |
|---|---|---|---|
| | 2.6 | 7.4 | 12.4 |
| **24** (±0.04) | 3.03 | 2.70 | 3.03 |
| **25** (±0.04) | 0.61 | 0.56 | 0.61 |

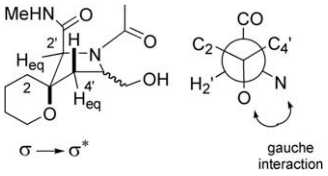



**Figure 8.** Pyrrolidine conformation in compounds **24** and **25**.

**Table 3**
Temperature coefficients ($\Delta\delta/\Delta T$, ppb/K) for compounds **24** and **25** in DMSO-$d_6$

| | | HO-2 | HO-3 | HO-4 | $NH_2$-6 | HO-6′ | NHMe |
|---|---|---|---|---|---|---|---|
| **24** | *cis* | −9.07[a] | [a] | [a] | −2.44[b] | −3.80 | −2.44[b] |
| | *trans* | −8.93[a] | [a] | [a] | −2.44[b] | −4.28 | −3.02 |
| **25** | *cis* | −8.09 | −6.90 | −4.10 | −0.92 | −3.29 | −2.68 |
| | *trans* | −7.76 | −6.80 | −4.20 | −0.92 | −2.76 | −3.26 |

[a] Not determined.
[b] Overlapped with NHMe.

profound effect on the equilibrium constant. For example, incorporation of GlcTProLysC with '*R*' configuration at C-5′ dramatically increased the *cis* population (75%) of Ac-GlcTProLysC-NHMe in water, whereas a smaller augment *cis* population (34%) was observed in GlcTProLysC with '*S*' configuration at C-5′. Temperature coefficient experiments indicate that the hydroxymethyl group at C-5′ (*S*) is involved in H-bonding with the 6-$NH_2$ and vice versa. In contrast, the same hydrogen bond is absent in the diastereomer with C-5′ (*R*) stereochemistry. Taken together, our results suggest that the *cis* isomer ratio in peptide mimic Ac-GlcTProLysC-NHMe having a C-5′ (*S*) hydroxymethyl substituent is decreased by competing H-bonding effects between 6′-OH–O=C(N)$CH_3$ and 6′-OH–6-$NH_2$.

Our work shows for the first time that polar groups capable of H-bonding in polyhydroxylated spirocyclic proline analogues can play important roles in controlling the thermodynamics of prolyl amide *cis*/*trans* isomerization. In particular, polar groups such as a hydroxymethyl group introduced at the δ-position of proline are expected to increase the prolyl N-terminal amide *cis* isomer in peptides via H-bonding to the N-terminal amide carbonyl group. Previous studies have shown that amino acid residues possessing side chains with hydrogen-bond acceptor and donor moieties are able to stabilize turn conformations when adjacent to proline.[39,40] As a result, we expect that incorporation of GlcTProLysC in bioactive peptides may induce similar effects. We are currently studying the lysine- and proline- mimetic effects of GlcTProLysC in β-turn-forming peptides.

## 4. Experimental

### 4.1. General

All solvents were obtained from a dry solvent system (alumina) and used without further drying. TLC was performed on E. Merck Silica Gel 60 F254 with detection by charring with 8% $H_2SO_4$ acid. Silica gel (0.040–0.063 mm) was used for column chromatography. Melting points are uncorrected.

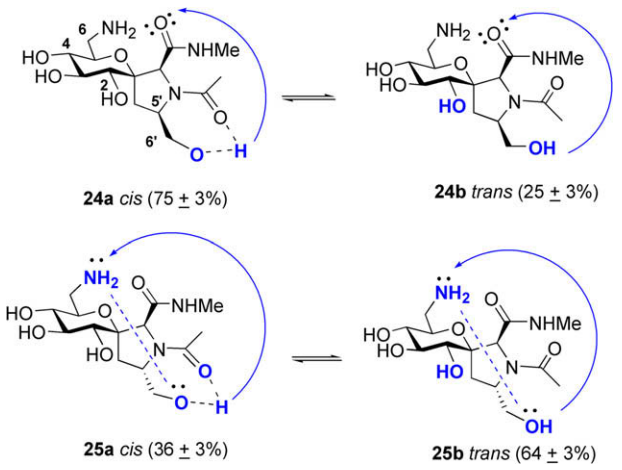



**Figure 9.** Suggested intramolecular H-bonds based on temperature coefficient experiments for compounds **24** and **25**.

### 4.2. (1*R*)-2,3,4,6-Tetra-*O*-benzyl-3′(*S*)-carboxy methyl-spiro[1,5-anhydro-D-glucitol-1,2′-oxirane] (2)

Under nitrogen atmosphere, methyl bromoacetate (4.1 mmol) was dissolved in dry THF (20 mL) and cooled to −78 °C before lithium bis(trimethylsilyl)-amide (4 mL of a 1 M solution in THF) was slowly added. The reaction mixture was kept at −78 °C for an additional 30 min. Subsequently, a THF solution (5 mL) containing the lactone **1** (1 mmol) was added over a period of 10 min and kept at −78 °C for 1 more hour. The temperature was raised to rt and stirred for 15 min before a saturated aq $NH_4Cl$ soln was added. The reaction mixture was evaporated under reduced pressure and the residue was dissolved in dichloromethane and portioned with water (3 × 20 mL). The organic layer was dried over anhydrous $Na_2SO_4$, filtered, concentrated, and purified by flash column chromatography (hexanes–EtOAc 5:1) to get **2** as a colorless oil (500 mg, 82%). $[\alpha]_D$ 99.6 (*c* 1.0, $CHCl_3$); $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$ = 3.53 (m, H-7), 3.63 (dd, H-8a, *J* = 11.2 Hz, *J* = 2.1 Hz), 3.68 (dd, H-8a, *J* = 11.2 Hz, *J* = 4.4 Hz), 3.72 (s, 3H), 3.75–3.83 (m, H-5, H-6), 3.90 (s, H-2), 3.95 (d, H-4, *J* = 9.1 Hz), 4.48 (d, 1H, *J* = 12.2 Hz), 4.55 (d, 1H, *J* = 10.7 Hz), 4.58 (d, 1H, *J* = 12.2 Hz), 4.62 (d, 1H, *J* = 11.0 Hz), 4.73 (d, 1H, *J* = 11.0 Hz), 4.82 (d, 1H, *J* = 11.2 Hz), 4.84 (d, 1H, *J* = 10.7 Hz), 4.94 (d, 1H, *J* = 11.2 Hz), 7.13–7.36 (m, 20H); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ = 52.5, 54.5, 68.3, 73.5, 74.9, 75.2, 75.7, 76.8, 77.0, 77.3, 84.7, 86.29, 127.6–128.5 (aromatic carbons), 137.4, 137.8, 137.9, 138.3, 166.7. Anal. Calcd for $C_{37}H_{38}O_8$: C, 72.77; H, 6.27. Found: C, 72.48; H, 6.54. MS (ES, $[M+Na]^+$) calcd for $C_{37}H_{38}NaO_8$ 633.25, found 633.71.

### 4.3. Methyl (2*S*)-hydroxy-2-(1-allyl-2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-ethanoate (3)

Under a nitrogen atmosphere, to a solution of allyltributylstannane (0.99 mL, 3.15 mmol) in dichloromethane (5 mL) was added dropwise the solution of trimethylsilyltrifluoromethanesulfonate (TMSOTf, 0.427 mL, 2.36 mmol) in dichloromethane (5 mL) at 0 °C followed by the syringe pump-controlled (50 μL/min) addition of the solution of epoxide **2** (480 mg, 0.79 mmol) in dichloromethane (10 mL). And then the mixture was stirred for 1 more hour at rt, the saturated sodium bicarbonate soln (10 mL) was added to quench the reaction, followed by the extraction with dichloromethane (3 × 15 mL). The organic layer was dried ($Na_2SO_4$), filtered, concentrated, and treated with trifluoroacetic acid (0.20 mL, 5 equiv) in aq tetrahydrofuran (THF–$H_2O$ 5:1) overnight. The mixture was concentrated and purified by flash column chromatography (hexanes–$CH_2Cl_2$–EtOAc from 2:1:0.2 to 2:1:0.4) to get **3** (410 mg, 80%) and **4** (46 mg, 9%). Compound **3** $[\alpha]_D$ 77.3 (*c* 1.0, $CHCl_3$); $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ = 2.77 (dd, 1H, *J* = 16.1 Hz, *J* = 6.8 Hz), 2.89 (dd, 1H, *J* = 16.1 Hz, *J* = 7.2 Hz), 3.48 (br, OH), 3.85–3.65 (m, 7H), 4.04 (dd, 1H, *J* = 9.8 Hz, *J* = 8.0 Hz), 4.13 (d, 1H, *J* = 9.8 Hz), 4.33 (s, 1H), 4.63 (d, 1H, *J* = 12.5 Hz), 4.68–4.75 (m, 2H), 4.85–4.98 (m, 3H), 5.02 (d, 1H, *J* = 10.9 Hz), 5.08 (d, 1H, *J* = 11.4 Hz), 5.28–5.16 (m, 2H), 5.89 (m, 1H), 7.25–7.49 (m, 20H); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ = 32.0, 52.2, 68.9, 73.5 (2 carbons), 73.6, 75.3, 75.3, 75.6, 78.7, 79.2, 80.6, 84.1, 118.7, 127.7–128.5 (aromatic carbons), 131.7, 138.1, 138.4, 138.4, 138.6, 173.1. Anal. Calcd for $C_{40}H_{44}O_8$: C, 73.60; H, 6.79. Found: C, 73.29; H, 7.04. MS (ES, $[M+Na]^+$) calcd for $C_{40}H_{44}NaO_8$ 675.29, found 675.40.

### 4.4. Methyl (2*S*)-hydroxy-2-(1-allyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-ethanoate (4)

$[\alpha]_D$ 84.5 (*c* 1.0, $CHCl_3$); $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ = 2.62–2.73 (m, 1H), 2.78–2.90 (dd, 1H, *J* = 15.3 Hz, *J* = 9.4 Hz), 3.59–3.73 (m, 6H), 3.74–3.81 (dd, 1H, *J* = 11.0 Hz, *J* = 3.7 Hz), 3.85 (d, 1H, *J* = 9.6 Hz), 3.99 (d, 1H, *J* = 1.6 Hz), 4.05–4.19 (m, 2H), 4.53 (d, 1H,

$J$ = 12.3 Hz), 4.62–4.71 (m, 2H), 4.82–4.95 (m, 4H), 5.00 (d, 1H, $J$ = 11.0 Hz), 5.10–5.24 (m, 2H), 5.88–6.05 (m, 1H), 7.20–7.40 (m, 20H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 36.6, 52.1, 69.1, 73.0, 73.4, 74.2, 75.1, 75.7, 76.2, 78.4, 78.6, 82.3, 84.0, 119.2, 127.5–128.7 (aromatic carbons), 133.4, 137.3, 138.1, 138.3, 138.5, 170.7. Anal. Calcd for $C_{40}H_{44}O_8$: C, 73.60; H, 6.79. Found: C, 73.41; H, 6.92. MS (ES, $[M+Na]^+$); calcd for $C_{40}H_{44}NaO_8$ 675.29, found 675.40.

### 4.5. Methyl 2-oxo-2-(1-allyl-2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-ethanoate (5)

Under a nitrogen atmosphere, to a solution of dry dimethyl sulfoxide (133 μL, 1.88 mmol)) in anhydrous $CH_2Cl_2$ (12 mL) cooled below –65 °C with a dry ice-acetone bath, trifluoroacetic anhydride (TFAA, 200 μL, 1.41 mmol) was slowly added with efficient stirring in ca. 10 min. After 10 min below −65 °C, a solution of compound **3** (307 mg, 0.47 mmol) in $CH_2Cl_2$ (8 mL) was added to the mixture in ca. 15 min. The rate of addition of TFAA or alcohol **3** was controlled to keep the temperature below −65 °C. The mixture was stirred below −65 °C for 40 min, followed by addition of triethylamine (394 μL, 2.82 mmol) dropwise in ca. 15 min. The reaction was kept below −65 °C for 2 more hour. The cooling bath was then removed and the reaction was allowed to warm up to rt, then quenched by the addition of $H_2O$ (10 mL), and the aqueous layer was backwashed with $CH_2Cl_2$ (2 × 15 mL). The combined organic solution was dried with anhydrous $Na_2SO_4$, filtered, concentrated, and purified by flash column chromatography (hexanes–EtOAc 6:1) to get **5** (296 mg, 97%). $[\alpha]_D$ 59.9 ($c$ 1.0, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 2.66 (dd, 1H, $J$ = 15.6 Hz, $J$ = 8.0 Hz), 3.21 (dd, 1H, $J$ = 15.6 Hz, $J$ = 5.9 Hz), 3.74 (s, 3H), 3.73–3.66 (m, 2H), 3.91–3.76 (m, 3H), 4.24 (d, 1H, $J$ = 9.6 Hz), 4.51 (d, 1H, $J$ = 11.9 Hz), 4.57 (d, 1H, $J$ = 10.3 Hz), 4.61 (d, 1H, $J$ = 12.2 Hz), 4.66 (d, 1H, $J$ = 10.7 Hz), 4.73 (d, 1H, $J$ = 10.3 Hz), 4.83–4.89 (m, 3H), 5.09–5.21 (m, 2H), 5.64 (m, 1H), 7.21–7.40 (m, 20H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 31.2, 52.3, 68.7, 73.4, 73.5, 75.3, 75.6, 75.7, 77.8, 80.3, 83.4, 84.5, 119.2, 127.4–128.5 (aromatic carbons), 130.9, 137.7, 138.0, 138.3, 138.5, 164.9, 195.8. Anal. Calcd for $C_{40}H_{42}O_8$: C, 73.83; H, 6.51. Found: C, 73.43; H, 6.39. MS (ES, $[M+Na]^+$); calcd for $C_{40}H_{42}NaO_8$ 673.28, found 673.39.

### 4.6. Methyl 2-benzyl imino-2-(1-allyl-2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-ethanoate (6)

Under a nitrogen atmosphere, to an ice-cooled solution of **5** (296 mg, 0.45 mmol) and benzylamine (148 μL, 1.36 mmol) in anhydrous $Et_2O$ (15 mL) was added dropwise $TiCl_4$ (0.23 mL of a 1 M solution in $CH_2Cl_2$, 0.23 mmol). After complete addition, the ice bath was removed and the reaction mixture was stirred for 4 h at rt. After this period, the resulting suspension was cooled at 0 °C and poured into 1 M sodium hydroxide solution. The organic layer was separated and the water layer was extracted two times with $CH_2Cl_2$ (2 × 15 mL). The combined organic layer was dried ($Na_2SO_4$), filtered, concentrated, and purified by flash column chromatography (hexanes–EtOAc 6:1) to get the mixture of **5** and **6**, which was exposed to the same procedure again to get **6** (323 mg, 96%). $[\alpha]_D$ 30.5 ($c$ 1.0, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 2.67 (dd, 1H, $J$ = 15.6 Hz, $J$ = 8.6 Hz), 3.48 (dd, 1H, $J$ = 15.6 Hz, $J$ = 5.3 Hz), 3.86–3.66 (m, 7H), 3.91 (dd, 1H, $J$ = 9.2 Hz, $J$ = 8.9 Hz), 4.16 (d, 1H, $J$ = 9.2 Hz), 4.43–4.56 (m, 3H), 4.59–5.72 (m, 4H), 4.86–4.93 (m, 3H), 5.03–5.16 (m, 2H), 5.77 (m, 1H), 7.15–7.42 (m, 25H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 32.2, 51.6, 58.7, 69.0, 72.9, 73.4, 75.3, 75.5, 75.8, 78.2, 81.5, 82.4, 84.1, 117.9, 127.7–128.5 (aromatic carbons), 132.5, 138.1, 138.2, 138.4, 138.6, 138.7, 163.7, 165.5. Anal. Calcd for $C_{47}H_{49}NO_7$: C, 76.30; H, 6.68; N, 1.89. Found: C, 75.77; H, 7.05; N, 1.86. MS (ES, $[M+Na]^+$) calcd for $C_{47}H_{49}NNaO_7$ 762.34, found 762.38.

### 4.7. Methyl (2*S*)-benzylamino-2-(1-allyl-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl) -ethanoate (7)

To an ice-cooled solution of **6** (240 mg, 0.32 mmol) in MEOH (9 mL) was added $NaCNBH_3$ (128 mg, 1.95 mmol), followed by 98% AcOH (39 μL, 0.65 mmol). The reaction mixture was stirred for 3 h at 0 °C and then quenched by the addition of water (5 mL) and extracted with $CH_2Cl_2$ (3 × 15 mL). The combined organic extracts were dried ($Na_2SO_4$), filtered, concentrated, and purified by flash column chromatography (hexanes–EtOAc 5:1) to afford **7** (239 mg, quant.). $[\alpha]_D$ 32.3 ($c$ 1.0, $CHCl_3$); $^1$H (300 MHz, $CDCl_3$): $\delta$ = 2.71 (dd, 1H, $J$ = 16.3 Hz, $J$ = 6.0 Hz), 2.84 (dd, 1H, $J$ = 16.3 Hz, $J$ = 7.4 Hz), 3.39 (d, 1H, $J$ = 12.8 Hz), 3.47 (s, 1H), 3.59–3.79 (m, 8H), 3.93 (dd, 1H, $J$ = 9.5 Hz, $J$ = 8.9 Hz), 4.28 (d, 1H, $J$ = 9.5 Hz), 4.50 (d, 1H, $J$ = 11.6 Hz), 4.58 (d, 1H, $J$ = 12.0 Hz), 4.65 (d, 1H, $J$ = 10.8 Hz), 4.67 (d, 1H, $J$ = 12.0 Hz), 4.80–4.95 (m, 4H), 5.17–5.04 (m, 2H), 5.77 (m, 1H), 7.45–7.10 (m, 26H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 32.4, 51.5, 51.8, 64.8, 69.1, 73.4, 73.5, 74.9, 75.1, 75.7, 78.8, 79.7, 80.2, 84.7, 118.1, 127.7–128.5 (aromatic carbons), 132.0, 138.2, 138.5, 138.7, 138.8, 139.7, 174.0. Anal. Calcd for $C_{47}H_{51}NO_7$: C, 76.09; H, 6.93; N, 1.89. Found: C, 75.84; H, 7.36; N, 2.37. MS (ES, $[M+Na]^+$) calcd for $C_{47}H_{51}NNaO_7$ 764.37, found 764.32.

### 4.8. (1*S*)-2,3,4,6-Tetra-*O*-benzyl-1′-*N*-benzyl-5′(*R*)-hydroxymethylene-spiro[1,5-anhydro-D-glucitol-1,3′-L-proline methyl ester] (8)

To a solution of **7** (340 mg, 0.46 mmol) in $CH_2Cl_2$ and $Et_2O$ (12 mL, 1:1) was added iodine (175 mg, 0.69 mmol) at 0 °C. The mixture was quenched by the addition of saturated sodium thiosulfate soln (5 mL) after overnight. The organic layer was separated and the aqueous layer was backwashed with $CH_2Cl_2$ (2 × 10 mL), the combined organic solution was dried with anhydrous $Na_2SO_4$ and concentrated followed by dissolution in toluene (15 mL) and treated with silver acetate (1.146 g, 6.88 mmol) overnight at rt to get an inseparable mixture of **13**, **14,** and **15** (323 mg, 93%), which was hydrolyzed with $K_2CO_3$ (73 mg, 1.3 equiv) in MeOH (8 mL) for 1 h at rt, and then quenched by the addition of saturated ammonium chloride (10 mL) and extracted with $CH_2Cl_2$ (3 × 15 mL). The combined organic solution was dried with anhydrous $Na_2SO_4$, filtered, concentrated, and purified by flash column chromatography (hexanes–EtOAc from 4:1 to 2:1) to get **8** (132 mg, 43%), **9** (141 mg, 46%), and **10** (20 mg, 6.5%). **(8)** $[\alpha]_D$ 47.7 ($c$ 1.0, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 2.13 (dd, 1H, $J$ = 13.9 Hz, $J$ = 5.4 Hz), 2.60 (dd, 1H, $J$ = 13.9 Hz, $J$ = 11.0 Hz), 3.09 (s, 3H), 3.30 (m, 1H), 3.45–3.85 (m, 11H), 4.02 (d, 1H, $J$ = 13.5 Hz), 4.38 (d, 1H, $J$ = 12.9 Hz), 4.57–4.71 (m, 4H), 4.83 (d, 1H, $J$ = 11.0 Hz), 4.86 (d, 1H, $J$ = 11.0 Hz), 5.10 (d, 1H, $J$ = 12.7 Hz), 7.10–7.45 (m, 25H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 27.2, 51.8, 59.0, 60.0, 63.0, 69.5, 72.4, 72.7, 73.5, 74.8, 75.1, 75.5, 76.7, 78.8, 86.1, 88.2, 125.8–128.9 (aromatic carbons), 138.0, 138.0, 138.3, 138.8, 138.9, 172.6; HRMS (ES) calcd for $C_{47}H_{52}NO_8$ $[M+H]^+$ 758.3693, found 758.3687.

### 4.9. (1*S*)-2,3,4,6-Tetra-*O*-benzyl-1′-*N*-benzyl-5′(*S*)-hydroxymethylene-spiro[1,5-anhydro-D-glucitol-1,3′-L-proline methyl ester] (9)

$[\alpha]_D$ −3.6 ($c$ 1.70, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 2.24 (d, 1H, $J$ = 14.6 Hz), 2.83 (dd, 1H, $J$ = 13.9 Hz, $J$ = 10.5 Hz), 3.05–3.23 (br, OH), 3.31 (s, 3H), 3.43 (d, 1H, $J$ = 11.1 Hz), 3.62–3.9 (m, 10H), 4.00 (d, 1H, $J$ = 14.6 Hz), 4.47 (d, 1H, $J$ = 12.5 Hz), 4.54–4.77 (m, 4H), 4.82–4.94 (m, 2H), 5.24 (d, 1H, $J$ = 11.8 Hz), 7.12–7.44 (m, 25H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 27.9, 51.1, 51.7, 61.3, 62.6, 69.1, 72.5, 72.9, 73.0, 73.7, 74.9, 75.7, 77.0, 78.5, 85.7, 86.2, 126.0–128.6 (aromatic carbons), 138.0, 138.2, 138.4, 138.8, 138.9,

170.5; HRMS (ES) calcd for $C_{47}H_{52}NO_8$ $[M+H]^+$ 758.3693, found 758.3696.

### 4.10. (1*S*)-2,3,4,6-Tetra-*O*-benzyl-1′-*N*-benzyl-5′(*S*)-hydroxy-spiro[1,5-anhydro-D-glucitol-1,3′-L-pipecolic methyl ester] (10)

$[\alpha]_D$ 34.7 (*c* 1.0, $CHCl_3$); $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ = 2.25–2.36 (m, 2H), 2.76 (dd, 1H, *J* = 11.0 Hz, *J* = 5.2 Hz), 3.04 (dd, 1H, *J* = 10.6 Hz, *J* = 9.6 Hz), 3.32 (s, 3H), 3.42 (s, 1H), 3.53–3.82 (m, 7H), 3.90–4.07 (m, 2H), 4.58–4.73 (m, 5H), 4.82 (d, 1H, *J* = 11.2 Hz), 4.87 (d, 1H, *J* = 10.9 Hz), 5.10 (d, 1H, *J* = 11.7 Hz), 7.12–7.39 (m, 25H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ = 29.7, 30.7, 51.0, 54.5, 59.0, 63.5, 69.5, 69.9, 72.4, 73.3, 73.9, 74.9, 75.4, 78.1, 79.1, 80.4, 84.6, 126.3–128.6 (aromatic carbons), 138.1, 138.2, 138.3, 138.6, 138.7, 170.7; HRMS (ES) calcd for $C_{47}H_{52}NO_8$ $[M+H]^+$ 758.3693, found 758.3686.

### 4.11. (1*S*)-5′(*R*)-hydroxymethylene-spiro[1,5-anhydro-D-glucitol-1,3′-L-proline methyl ester] (11)

Under the nitrogen atmosphere, to the solution of compound **8** (200 mg, 0.25 mmol) in MEOH (10 mL) were added 1 M hydrochloride acid soln (0.38 mL, 0.38 mmol) and palladium hydroxide (20 wt % Pd on carbon, 50 mg). The mixture was exposed to hydrogen ($H_2$, 10 psi) and stirred for 6 h. The solution was filtrated and evaporated in vacuum to get the product **11** (75 mg, quant.) $[\alpha]_D$ 64.1 (*c* 1.0, MeOH); $^1H$ NMR (300 MHz, $CD_3OD$): $\delta$ = 2.13 (dd, 1H, *J* = 15.0 Hz, *J* = 2.8 Hz), 2.41 (dd, 1H, *J* = 15.0 Hz, *J* = 10.8 Hz), 3.23–3.21 (m, 5H), 3.49 (m, 1H), 3.6–3.71 (m, 2H), 3.85–4.00 (m, 4H), 4.22 (s, 1H); $^{13}C$ NMR (75 MHz, $CD_3OD$): $\delta$ = 27.3, 54.3, 61.5, 61.9, 63.0, 68.7, 70.8, 71.6, 76.6, 77.0, 88.3, 168.5; HRMS (ES) calcd for $C_{12}H_{22}NO_8$ $[M+H]^+$ 308.1340, found 308.1343.

### 4.12. (1*S*)-5′(*S*)-Hydroxymethylene-spiro[1,5-anhydro-D-glucitol-1,3′-L-proline methyl ester] (12) (the detailed procedure is the same as that for 11)

$[\alpha]_D$ 75.7 (*c* 1.10, MeOH); $^1H$ NMR (300 MHz, $CD_3OD$) $\delta$ = 2.09 (m, 1H), 2.56 (dd, 1H, *J* = 10.3 Hz, *J* = 13.6 Hz), 3.24 (m, 1H), 3.30–3.40 (m, br, 1H, overlapping with solvent peak), 3.47 (m, 1H), 3.57–3.68 (m, 2H), 3.72–3.95 (m, 6H), 4.18–4.33 (m, 2H); $^{13}C$ NMR (75 MHz, $CD_3OD$): $\delta$ = 27.8, 54.2, 62.6, 63.1, 63.2, 69.1, 71.2, 71.7, 76.7, 76.9, 88.2, 167.9; HRMS (ES) calcd for $C_{12}H_{22}NO_8$ $[M+H]^+$ 308.1340, found 308.1348.

### 4.13. (1*S*)-2,3,4,6-Tetra-*O*-benzyl-1′-*N*-benzyl-5′(*R*)-methylenehydroxy acetate-spiro [1,5-anhydro-D-glucitol-1,3′-L-proline methyl ester] (13)

To a solution of **8** (60 mg, 0.079 mmol) in pyridine (1 mL) was added acetic anhydride (37 μL, 0.395 mmol) and stirred for 5 h. The pyridine was removed with high vacuum. The crude product was purified by flash column chromatography (hexanes–EtOAc 4:1) to get **13** (62 mg, quant.). $[\alpha]_D$ 50.3 (*c* 1.0, $CHCl_3$); $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ = 2.05 (s, 3H), 2.18 (dd, 1H, *J* = 13.0 Hz, *J* = 10.8 Hz), 2.33 (dd, 1H, *J* = 13.6 Hz, *J* = 5.7 Hz), 3.13 (s, 3H), 3.32 (m, 1H), 3.53 (s, 1H), 3.63–3.78 (m, 6H), 3.83 (d, 1H, *J* = 14.4 Hz), 4.08 (d, 1H, *J* = 13.7 Hz), 4.24 (d, 2H, *J* = 6.0 Hz), 4.42 (d, 1H, *J* = 12.3 Hz), 4.58–4.70 (m, 4H), 4.83 (d, 1H, *J* = 9.9 Hz), 4.86 (d, 1H, *J* = 9.9 Hz), 5.06 (d, 1H, *J* = 12.3 Hz), 7.13–7.43 (m, 25H); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ = 20.9, 30.0, 51.5, 60.4, 60.8, 67.2, 69.4, 72.5, 72.8, 73.5, 75.1, 75.5, 76.0, 76.7, 78.7, 86.1, 87.5, 126.0–128.8 (aromatic carbons),138.03, 138.04, 138.4, 138.9, 139.3, 171.0, 172.0; HRMS (ES) calcd for $C_{49}H_{54}NO_9$ $[M+H]^+$ 800.3793, found 800.3794.

### 4.14. (1*S*)-2,3,4,6-Tetra-*O*-benzyl-1′-*N*-benzyl-5′(*S*)-methylenehydroxyl acetate-spiro [1,5-anhydro-D-glucitol-1,3′-L-proline methyl ester] (14) (the detailed procedure is the same as that for 13)

$[\alpha]_D$ 6.2 (*c* 1.0, $CHCl_3$); $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ = 2.01 (s, 3H), 2.14 (dd, 1H, *J* = 15.4 Hz, *J* = 1.1 Hz), 2.82 (dd, 1H, *J* = 13.9 Hz, *J* = 10.0 Hz), 3.26 (s, 3H), 3.58–3.82 (m, 9H), 3.59–3.82 (m, 2H), 4.21 (dd, 1H, *J* = 10.7 Hz, *J* = 5.0 Hz), 4.43 (d, 1H, *J* = 12.6 Hz), 4.57–4.75 (m, 4H), 4.82 (d, 1H, *J* = 11.2 Hz), 4.86 (d, 1H, *J* = 11.2 Hz), 5.15 (d, 1H, *J* = 12.3 Hz), 7.10–7.40 (m, 25H); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ = 21.0, 27.7, 51.1, 53.0, 60.0, 67.2, 69.0, 72.9, 73.0, 73.2, 73.7, 75.1, 75.6 (2 carbons), 78.7, 85.9, 86.9, 126.0–128.5 (aromatic carbons), 137.0 (2 carbons), 138.5, 138.9, 139.5, 170.9, 170.9; HRMS (ES) calcd for $C_{49}H_{54}NO_9$ $[M+H]^+$ 800.3793, found 800.3793.

### 4.15. (1*S*)-2,3,4,6-Tetra-*O*-benzyl-1′-*N*-benzyl-5′(*S*)-*O*-acetyl-spiro[1,5-anhydro-D-glucitol-1,3′-L-pipecolic methyl ester] (15) (the detailed procedure is the same as that for 13)

$[\alpha]_D$ 44.0 (*c* 1.0, $CHCl_3$); $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ = 2.05 (s, 3H), 2.32 (dd, 1H, *J* = 13.6 Hz, *J* = 11.9 Hz), 2.52 (dd, 1H, *J* = 13.7 Hz, *J* = 4.0 Hz), 2.90 (dd, 1H, *J* = 10.4 Hz, *J* = 5.6 Hz), 3.24 (dd, 1H, *J* = 10.4 Hz, *J* = 10.8 Hz), 3.29 (s, 3H), 3.46–3.99 (m, 9H), 4.45 (d, 1H, *J* = 12.9 Hz), 4.59–4.93 (m, 6H), 5.09 (m, 1H), 5.15 (d, 1H, *J* = 12.4 Hz), 7.10–7.44 (m, 25H); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ = 21.6, 26.4, 50.2, 50.4, 59.5, 69.7, 72.8, 73.4, 74.4 (2 carbons), 75.4 (2 carbons), 75.8, 78.7, 79.4, 80.9, 85.3, 126.50–128.90 (aromatic carbons), 138.8 (3 carbons), 139.2, 139.4, 170.7 (2 carbons); HRMS (ES) calcd for $C_{49}H_{54}NO_9$ $[M+H]^+$ 800.3793, found 800.3788.

### 4.16. (1*S*)-1′-*N*-*t*-butoxycarbonyl-5′(*R*)-methylenehydroxy acetate–spiro[1,5-anhydro-D-glucitol-1,3′-L-proline methyl ester] (16)

To a mixture of **11** (100 mg, 0.13 mmol) and $Pd(OH)_2$ (40 mg, 20 wt % on charcoal) in MEOH (10 mL) was added the solution of hydrochloride (250 μL of 1M HCl soln, 0.25 mmol) and stirred under $H_2$ (15 psi) for 8 h at rt. The catalyst was removed by filtration and the solvent was removed under the vacuum. The unprotected product was treated with triethylamine (53 μL, 0.38 mmol) and di-*t*-butyl dicarbonate (56 mg, 0.25 mmol) in MeOH (2 mL) for 1 h at rt. The solvent was removed under vacuum. The crude product was purified by flash column chromatography ($CH_2Cl_2$–MeOH 7:1) to get **16** (51 mg, 90%). $[\alpha]_D$ = 68.4 (*c* 1.3, MeOH); $^1H$ NMR (300 MHz, $CD_3OD$, two isomers): $\delta$ = 1.45 (s, 9H), 2.09 (s, 3H), 2.13–2.44 (m, 2H), 3.27–3.46 (m, 3H, partially overlapping with MeOH peaks), 3.57–3.86 (m, 6H), 4.06 (m, 1H), 4.23 (s, 1H), 4.31–4.45 (m, 1H), 4.53–4.67 (m, 1H); $^{13}C$ NMR (75 MHz, $CD_3OD$, two isomers): $\delta$ = 20.9 (2 carbons), 28.56/28.63 (6 carbons), 29.2/29.7, 52.9 (2 carbons), 56.2/56.3, 62.8 (2 carbons), 66.0/66.4, 71.2 (2 carbons), 71.4/71.8, 71.5 (2 carbons), 75.9/76.0, 77.3 (2 carbons), 81.8/82.3, 86.2/86.7, 155.6/156.2, 172.0/171.8, 172.7/172.8; HRMS (ES) calcd for $C_{19}H_{31}NNaO_{11}$ $[M+Na]^+$ 472.1795, found 472.1783.

### 4.17. (1*S*)-1′-*N*-*t*-butoxycarbonyl-5′(*S*)-methylenehydroxy acetate-spiro[1,5-anhydro-D-glucitol-1,3′-L-proline methyl ester] (17) (the detailed procedure is the same as that for 16)

$[\alpha]_D$ = 15.7 (*c* 1.35, MeOH); $^1H$ NMR (300 MHz, $CD_3OD$, two isomers): $\delta$ = 1.43 (s, 9H), 2.09 (s, 3H), 2.14–2.42 (m, 2H), 3.24–3.50 (m, 3H, partially overlapping with MeOH peaks), 3.50–3.87 (m, 6H), 4.04–4.63 (m, 4H); $^{13}C$ NMR (75 MHz, $CD_3OD$, two isomers): $\delta$ = 20.8 (2 carbons), 25.5/ 26.1, 28.5/28.7 (6 carbons), 53.0 (2 carbons), 57.2/57.3, 62.6/62.8, 65.8/65.9, 71.2 (2 carbons), 71.35 (2

carbons), 71.8/71.4, 75.8/75.9, 77.0/77.1, 81.9/82.3, 88.4/87.4, 155.8/ 156.1, 172.1/171.6, 172.6/172.4; HRMS (ES) calcd for $C_{19}H_{31}NNaO_{11}$ $[M+Na]^+$ 472.1795, found 472.1788.

### 4.18. (1*S*)-6-Azido-6-deoxy-1′-*N*-*t*-butoxycarbonyl-5′(*R*)-methylenehydroxy acetate-spiro[1,5-anhydro-D-glucitol-1,3′-L-proline methyl ester] (18)

To a solution of compound **16** (40 mg, 0.09 mmol) in pyridine (1 mL) was added *p*-tolunesulfonyl chloride (42 mg, 0.22 mmol) and stirred for 12 h at rt. The mixture was concentrated and purified by flash column chromatography ($CH_2Cl_2$–MeOH 10:1) to provide tosyl ester, which was treated with sodium azide (116 mg, 1.8 mmol) in DMF (1.5 mL) and stirred at 80 °C for 12 h. The mixture was filtered, concentrated, and purified by flash column chromatography ($CH_2Cl_2$–MeOH 15:1) to get **18** (38 mg, 92%). $[\alpha]_D$ = 31.5 (*c* 1.35, MeOH); $^1$H NMR (300 MHz, $CD_3OD$, two isomers): $\delta$ = 1.44 (s, 9H), 2.10 (s, 3H), 2.14–2.48 (m, 2H), 3.18–3.49 (m, 4H, partially overlapping with MeOH peaks), 3.58–3.77 (m, 5H), 4.01–4.17 (m, 1H), 4.21 (s, 1H), 4.32–4.48 (m, 1H), 4.52–4.64 (m, 1H); $^{13}$C NMR (75 MHz, $CD_3OD$, two isomers): $\delta$ = 20.9 (2 carbons), 28.6 (6 carbons,), 29.0/29.6, 52.6/52.8, 53.0 (2 carbons), 56.2/56.4, 66.2/66.4, 71.1 (2 carbons), 71.4/71.9, 72.4/72.8, 75.2/75.4, 77.0/77.1, 81.8/82.3, 86.4/87.0, 155.8/156.3, 171.6/171.7, 172.7/172.8; HRMS (ES) calcd for $C_{19}H_{30}N_4$ $NaO_{10}$ $[M+Na]^+$ 497.1860, found 497.1849.

### 4.19. (1*S*)-6-Azido-6-deoxy-3′-*N*-*t*-butoxycarbonyl-5′(*S*)-methylenehydroxy acetate -spiro[1,5-anhydro-D-glucitol-1,3′-L-proline methyl ester] (19) (the detailed procedure is the same as that for 18)

$[\alpha]_D$ = 29.1 (*c* 0.7, MeOH); $^1$H NMR (300 MHz, $CD_3OD$, two isomers): $\delta$ = 1.45 (s, 9H), 2.09 (s, 3H), 2.14–2.48 (m, 2H), 3.23–3.44 (m, 3H, partially overlapping with MeOH peaks), 3.50–3.78 (m, 6H), 4.06–4.34 (m, 3H), 4.39–4.63 (m, 1H); $^{13}$C NMR (75 MHz, $CD_3OD$, two isomers): $\delta$ = 20.8 (2 carbons), 25.6/26.2, 28.5/28.6 (6 carbons,), 53.0 (2 carbons), 53.0/53.1, 57.2 (2 carbons), 65.7/65.9, 71.1 (2 carbons), 71.9/71.5, 72.2/72.3, 74.7/74.8, 76.7/76.8, 82.0/82.4, 88.8/87.8, 155.8/156.1, 171.9/171.4, 172.6/172.4; HRMS (ES) calcd for $C_{19}H_{30}N_4$ $NaO_{10}$ $[M+Na]^+$ 497.1860, found 497.1852.

### 4.20. (1*S*)-6-Azido-6-deoxy-1′-*N*-acetyl-5′(*R*)-hydroxymethylene-spiro[1,5-anhydro-D-glucitol-1,3′-L-proline methyl ester] (20)

The compound **18** (30 mg, 0.063 mmol) was dissolved in a mixture of $CH_2Cl_2$ and trifluoroacetic acid (1.5 mL/0.5 mL) and stirred for 1 h at rt. The solution was concentrated at vacuum and then treated with a mixture of pyridine and acetic acid (1 mL/1 mL) and stirred for 12 h at rt and then concentrated at vacuum. After that, it was dissolved in a solution of sodium methoxide in MeOH (0.1 M, 2 mL) and stirred for 4 h at rt followed by the neutralization with Amberlite IRC-50S ion-exchange resin ($H^+$). The mixture was filtered and filtrate was concentrated and purified by the flash column chromatography (EtOAc–MeOH 6:1) to get compound **20** (22 mg, 96%). $[\alpha]_D$ = 19.2 (*c* 0.75, MeOH); $^1$H NMR (300 MHz, $CD_3OD$, two isomers): $\delta$ = 1.82 (s, 1.74H), 2.04 (s, 1.26H), 2.05–2.49 (m, 2H), 3.06–3.34 (m, 4H, partially overlapping with MeOH peaks), 3.49–3.94 (m, 7H), 4.04–4.16 (m, 1H), 4.20 (s, 0.58H), 4.42 (s, 0.42H); $^{13}$C NMR (75 MHz, $CD_3OD$, two isomers): $\delta$ = 22.8/21.6, 27.7/29.7, 52.8/52.7, 53.6/53.2, 60.6/60.1, 64.9/65.6, 71.1 (2 carbons), 72.6/71.1, 72.6/72.8, 75.4/75.7, 76.9/77.0, 87.5/86.4, 171.6/171.9, 173.6 (2 carbons); HRMS (ES) calcd for $C_{14}H_{22}N_4NaO_8$ $[M+Na]^+$ 397.1335, found 397.1351.

### 4.21. (1*S*)-6-Azido-6-deoxy-1′-*N*-acetyl-5′(*S*)-hydroxymethylene-spiro[1,5-anhydro-D -glucitol-1,3′-L-proline methyl ester] (21) (the detailed procedure is the same as that for 20)

$[\alpha]_D$ = 46.5 (*c* 0.55, MeOH); $^1$H NMR (300 MHz, $CD_3OD$, two isomers): $\delta$ = 1.77 (s, 1.09H), 2.08 (s, 1.91 H), 2.15–2.41 (m, 2H), 3.23–3.68 (m, 10H, partially overlapping with MeOH peaks), 3.79 (dd, 0.64 H, *J* = 5.28 Hz, *J* = 10.95 Hz), 3.96–4.23 (m, 2.36H); $^{13}$C NMR (75 MHz, $CD_3OD$, two isomers): $\delta$ = 22.04 (22.48), 26.54 (25.35), 52.97 (53.14), 53.13 (53.46), 61.52 (61.38), 64.76 (63.61), 71.14 (71.19), 72.03 (72.44), 72.27 (73.01), 74.68 (74.73), 76.71 (76.62), 87.40 (88.99), 170.78 (171.20), 173.56 (173.50); HRMS (ES) calcd for $C_{14}H_{22}N_4NaO_8$ $[M+Na]^+$ 397.1335, found 397.1348.

### 4.22. (1*S*)-6-Amino-6-deoxy-1′-*N*-acetyl-5′(*R*)-hydroxymethylene-spiro[1,5-anhydro-D-glucitol-1,3′-L-proline methyl ester] HCl salt (22)

To a solution of **20** (20 mg, 0.053 mmol) and $Pd(OH)_2$ (20 mg, 20 wt % on charcoal) in MeOH (5 mL) was added the solution of hydrochloride (800 μL of 1 M HCl soln, 0.08 mmol) and stirred under $H_2$ (15 psi) for 20 min at rt. The catalyst was removed by the regular filtration and the solvent was removed under the vacuum to afford pure product **22** (20 mg, quant.). $[\alpha]_D$ 65.2 (*c* 0.5, MeOH); $^1$H NMR (500 MHz, $D_2O$, two isomers): $\delta$ = 1.86 (s, *cis*, 1.68H), 1.98 (dd, *cis*, 0.56H, *J* = 10.39 Hz, *J* = 14.57 Hz), 2.04–2.09 (m, *trans*, 0.44H + 1.32H), 2.36 (dd, *cis*, 0.56H, *J* = 6.76 Hz, *J* = 14.56 Hz), 2.47 (dd, *trans*, 0.44H, *J* = 7.42 Hz, *J* = 14.62 Hz), 2.98 (m, 1H), 3.19–3.37 (m, 3H), 3.59–3.67 (m, 5H), 3.68–3.74 (m, 1H), 3.81–3.88 (m, 1H), 4.00–4.07 (m, *cis*, 0.56H), 4.11–4.18 (m, *trans*, 0.44H). 4.42 (s, *cis*, 0.56H), 4.44 (s, *trans*, 0.44H); $^{13}$C NMR (75 MHz, $D_2O$): *cis*, $\delta$ = 22.2, 26.7, 40.5, 53.5, 58.5, 62.4, 69.2, 69.7, 70.7, 71.2, 74.4, 86.2, 171.4, 174.4; *trans*, $\delta$ = 20.9, 28.8, 40.4, 53.2, 58.4, 63.5, 69.2, 69.7, 69.8, 70.9, 74.4, 85.1, 171.4, 174.5; HRMS (ES) calcd for $C_{14}H_{25}N_2O_8$ $[M+H]^+$ 349.1611, found 349.1623.

### 4.23. (1*S*)-6-Amino-6-deoxy-1′-*N*-acetyl-5′(*S*)-hydroxymethylene-spiro[1,5-anhydro-D-glucitol-1,3′-L-proline methyl ester] HCl salt (23) (the detailed procedure is the same as that for 22)

$[\alpha]_D$ 24.3 (*c* 0.55, MeOH); $^1$H NMR (500 MHz, $D_2O$, two isomers): $\delta$ = 1.93 (s, *cis*, 0.60H), 2.21 (s, *trans*, 2.40H), 2.29–2.46 (m, 2H), 3.20–3.59 (m, 5H), 3.63–3.82 (m, 5H), 3.85–3.94 (m, 1H), 4.33–4.41 (m, 1H), 4.43 (s, *trans*, 0.8H), 4.62 (s, 0.2H); $^{13}$C NMR (75 MHz, $D_2O$): *cis*, $\delta$ = 21.8, 25.0, 40.2, 53.6, 59.5, 62.3, 69.3, 69.6, 70.5, 71.2, 74.2, 87.6, 171.4, 174.4; *trans*, $\delta$ = 21.3, 25.9, 39.9, 53.2, 59.7, 63.2, 69.3, 69.5, 70.3 (2 carbons), 74.2, 86.3, 170.8, 174.5; HRMS (ES) calcd for $C_{14}H_{25}N_2O_8$ $[M+H]^+$ 349.1611, found 349.1618.

### 4.24. (1*S*)-6-Amino-6-deoxy-1′-*N*-acetyl-5′(*R*)-hydroxymethylene-spiro[1,5-anhydro-D-glucitol-1,3′-L-proline methyl amide] HCl salt (24)

To a solution of methylamine in ethanol (37 wt %, 1 mL) was added compound **20** (15 mg, 0.04 mmol) and stirred for 18 h at rt. The mixture was concentrated and purified by flash column chromatography ($CH_2Cl_2$–MeOH 2:1) to quantitatively afford C-terminal methyl amide intermediate, which was dissolved in a solution of $Pd(OH)_2$ (15 mg, 20 wt % on charcoal) and 1 M hydrochloride acid soln (80 μL, 0.08 mmol). The mixture was stirred under $H_2$ (15 psi) for 20 min at rt. The catalyst was removed by the regular filtration and the solvent was removed under the vacuum to afford pure product **24** (14 mg, 93%). $[\alpha]_D$ 54.8 (*c* 0.35, MeOH);

$^1$H NMR (500 MHz, $D_2O$, two isomers): $\delta$ = 2.02 (s, *cis*, 2.24H), 2.21 (s, *trans*, 0.76H), 2.27 (dd, *cis*, 0.75H, $J$ = 11.31 Hz, $J$ = 14.27 Hz), 2.35–2.44 (m, 1H), 2.53 (dd, *trans*, 0.25H, $J$ = 6.17 Hz, $J$ = 14.11 Hz), 2.67–2.83 (m, 3.75H) 2.98–3.05 (m, 0.75H), 3.25 (dd, 0.25H, $J$ = 11.31 Hz, $J$ = 14.27 Hz), 3.30–3.39 (m, 1.25H), 3.41–3.56 (m, 2H), 3.68–3.77 (m, 1.75H). 3.83 (dd, *trans*, 0.25H, $J$ = 1.81 Hz, $J$ = 12.43 Hz), 4.09–4.36 (m, 3H); $^{13}$C NMR (75 MHz, $D_2O$): *cis*, $\delta$ = 27.2, 30.1, 30.8, 46.5, 62.9 (2 carbons), 64.5, 74.6, 76.0, 77.4, 80.1, 90.3, 176.5, 179.6; *trans*, 25.9, 30.8, 32.1, 46.4, 62.8, 63.0, 66.0, 74.5, 75.9, 76.5, 78.4, 89.0, 176.6, 179.6; HRMS (ES) calcd for $C_{14}H_{26}N_3O_7$ $[M+H]^+$ 348.1771, found 348.1759.

### 4.25. (1*S*)-6-Amino-6-deoxy-1′-*N*-acetyl-5′(*S*)-hydroxymethylene–spiro[1,5-anhydro-D-glucitol-1,3′-L-proline methyl amide] HCl salt (25) (the detailed procedure is the same as that for 24)

$[\alpha]_D$ 19.2 (*c* 0.40, MeOH); $^1$H NMR (500 MHz, $D_2O$, two isomers): $\delta$ = 1.80 (s, *cis*, 1.08H), 2.08 (s, *trans*, 1.92H), 2.16–2.32 (m, 2H), 2.56 (s, *trans*, 1.92H), 2.62 (s, *cis*, 1.08H), 3.09–3.54 (m, 6H), 3.62–3.68 (m, 1H), 3.76 (dd, *trans*, 0.64H, $J$ = 5.26 Hz, $J$ = 11.50 Hz), 3.83 (dd, *cis*, 0.36 H, $J$ = 4.60 Hz, $J$ = 11.04 Hz), 4.19 (s, *trans*, 0.64H), 4.21–4.30 (m, 1.36H); $^{13}$C NMR (75 MHz, $D_2O$): *cis*, $\delta$ = 26.7, 30.1, 31.2, 45.1, 64.6, 67.3, 74.3, 74.6, 75.5, 77.4, 79.4, 92.6, 175.5, 179.2; *trans*, $\delta$ = 26.5, 31.0 (2 carbons), 44.9, 64.7, 68.2, 74.3, 74.5, 75.2, 76.6, 79.4, 91.4, 175.0, 179.1; HRMS (ES) calcd for $C_{14}H_{26}N_3O_7$ $[M+H]^+$ 348.1771, found 348.1766.

### 4.26. Measurement of equilibrium constant

The calculation was based on the integration of well-resolved peaks of the γ-protons, N-terminal methyl group, and α-proton in $^1$H NMR.

### 4.27. Temperature coefficient ($\Delta\delta/\Delta T$) experiments

1D $^1$H NMR spectra of 16 mM solutions of **24** and **25** in 100.0% $Me_2SO$-$d_6$ were recorded on Bruker AMX500 at 25 °C, and from 25 to 44 °C with increments of 5 °C, using routine techniques. Chemical shift ($\delta$) of hydroxyl and amino groups is expressed in ppm and calibrated with respect to the residual DMSO signal ($^1$H: 2.49 ppm). The chemical shift changes ($\Delta\delta$) at different temperatures were calculated with respect to the chemical shift of hydroxyl and amino groups at 25 °C.

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## Supplementary data

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