JOC Article

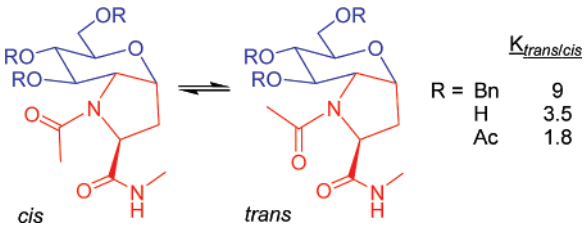
Tuning of the Prolyl *trans*/*cis*-Amide Rotamer Population by Use of *C*-Glucosylproline Hybrids

Neil W. Owens, Craig Braun, and Frank Schweizer*

Department of Chemistry, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada

schweize@ms.umanitoba.ca

Received January 15, 2007



We describe the synthesis of a fused bicyclic *C*-glucosylproline hybrid (GlcProH) from commercially available 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose. The GlcProH was incorporated into the model peptides Ac-GlcProH-NHMe and Ac-Gly-GlcProH-NHMe. Postsynthetic modifications can be introduced via derivatization of the carbohydrate scaffold. Conformational analysis of the GlcProH-modified model peptides shows that while the conformation of GlcProH remains fixed, the prolyl *N*-terminal amide equilibrium ($K_{t/c}$) can be varied with different modifications of the carbohydrate scaffold. Simple *N*-acyl derivatives studied by NMR spectroscopy showed that in CD_3OD there was an increase in the *cis*-amide content as the sugar substituents changed from benzyl (10%) to hydroxyl (22%) to acetate (36%). Similar effects were observed in DMSO-d_6. The exact nature of the influence is unclear, but it most likely arises through intramolecular interactions between sugar groups and the peptidic amide backbone. Overall, our GlcProH demonstrates variation in $K_{t/c}$ through tuning of the carbohydrate scaffold: a new concept in proline peptidomimetics.

Introduction

Proline is uniquely endowed with a side chain that is fused onto the peptide backbone. This trait restricts the rotation about its ϕ dihedral angle, thereby reducing the energy difference between the prolyl amide *cis* and *trans* isomers, making them nearly isoenergetic. Thus, while most peptide amide bonds exist almost exclusively in the trans form, proline has a much greater propensity to form *cis*-amide bonds; this makes proline crucial for inducing a reversal in peptide backbone conformation.[1] Also, *cis*−*trans* isomerization of proline is of importance because it becomes the rate-determining step in the folding pathways of peptides and proteins.[2]

Variation of the *trans*/*cis* ratio is of interest for understanding the behavior of peptides and proteins. Over the years a number of proline analogues have been developed to study the structural and biological properties of proline surrogates in peptides. This is done by controlling prolyl *N*-terminal amide isomerization, and thus reverse turn formation. Examples of amide geometry controlled through steric or stereoelectronic influences includes C^{β}-, C^{γ}-, and C^{δ}-substituted prolines,[3−6] including 4-fluoroproline,[7] azaprolines,[8] and pseudoprolines.[9]

While these analogues have proved useful for inducing specific constraints on prolyl *N*-terminal amide isomerization, no *one* analogue can lay claim to the ability to shift the prolyl amide equilibrium toward both the *cis* and *trans* isomers. In other words, different proline analogues are required to induce a desired bias in $K_{t/c}$. This is in part because none of the present

* Corresponding author: fax (+1) 204-474-7608.

(1) Wilmot, C. M.; Thornton, J. M. *J. Mol. Biol.* **1988**, *203*, 221−232.
(2) Fischer, G.; Schmid, F. X. *Biochemistry* **1990**, *29*, 2205−2212.
(3) Beausoleil, E.; Lubell, W. D. *J. Am. Chem. Soc.* **1996**, *118*, 12902−12908 and references therein.
(4) Delaney, N. G.; Madison, V. *J. Am. Chem. Soc.* **1982**, *104*, 6635−6641.
(5) Samanen, J.; Zuber, G.; Bean, J.; Eggleston, D.; Romoff, T.; Kopple, K.; Saunders, M.; Regoli, D. *Int. J. Pept. Protein Res.* **1990**, *35*, 501−509.
(6) Sharma, R.; Lubell, W. D. *J. Org. Chem.* **1996**, *61*, 202−209.
(7) Eberhardt, E. S.; Panasik, N., Jr.; Raines, R. T. *J. Am. Chem. Soc.* **1996**, *118*, 12261−12266.
(8) Che, Y.; Marshall, G. R. *J. Org. Chem.* **2004**, *69*, 9030−9042 and references therein.
(9) Tam, J. P.; Miao, Z. *J. Am. Chem. Soc.* **1999**, *121*, 9013−9022 and references therein.

10.1021/jo0700833 CCC: $37.00
Published on Web 05/31/2007

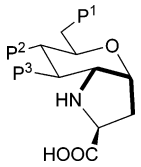
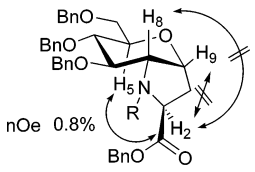


FIGURE 1. General structure of glucose−proline hybrid (GlcProH). The carbohydrate scaffold is used as a template to constrain C_β, C_γ, C_δ, and N of the pyrrolidine ring (shown in bold) of L-proline or (2*S*,4*R*)-hydroxyproline; P^1, P^2, and P^3 are hydroxy-derived substituents used to manipulate the steric and electronic properties of the GlcProH.



8 R = Boc

FIGURE 2. Conformationally relevant NOE interactions observed for **8**.

building blocks have strategic functional groups positioned for further derivatization in order to alter the amide equilibrium. This led us toward the development of a proline analogue building block in which prolyl *cis*−*trans* isomerization could be tuned through simple chemistry, even after incorporation into a peptide.

Our concept for a proline analogue 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 carbohydrate scaffold (furan or pyran).[10] The relative rigidity of the furan or 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. While there are many examples of *C*-glycosylglycine, -alanine, -serine, and -asparagine,[10c] few proline-based GAAs exist.[11]

We report here on the resulting novel fused bicyclic glucoproline hybrid (GlcProH) GAA for use as a peptidomimetic to tune prolyl *N*-terminal amide isomerization. Our bicyclic GlcProH rigidly combines the molecular elements of carbohydrates (pyran-based polyol) with the unique features of proline (Figure 1). The resulting GlcProH is a rigid, polyfunctional building block, which may find use as a proline mimetic, glycomimetic, or scaffold for combinatorial synthesis. Many conformationally constrained L-proline analogues have been developed,[12,13] but recently, fused bicyclic prolines have attracted interest due to their increased rigidity, which may permit better control of the *trans*/*cis* ratio.[14−16] For instance, bicyclic proline analogues have been studied as angiotensin[17] and thyroliberin[18] analogues, which have served as building blocks for the synthesis of peptidomimetics.[19] Because of its stable chair conformation, glucose provides an ideal scaffold to template proline since it freezes the orientation of four proline atoms (C_β, C_γ, C_δ, and N). Furthermore, the sugar hydroxyl groups amend themselves to derivatization of the building block as potential sites for influencing the peptide backbone conformation. It has been shown that hydroxylated amino acids can induce novel secondary structures in small peptides. For instance, incorporation of unprotected sugar amino acids into small peptides (opioid peptides and gramicidin S) prohibited the formation of the targeted secondary structural motif.[20,21] Appearing instead were unusual turn structures stabilized by intramolecular hydrogen bonds between sugar hydroxyl groups and the peptidic amide backbone.[22]

We envision that similar effects may also be observed with the GlcProH. For instance, we hope to influence peptide geometry via intramolecular polar interactions (H-bonding) between the sugar substituents and the peptide backbone. Furthermore, through derivatization of the polyhydroxylated carbohydrate scaffold, we hope to demonstrate the capacity to tune prolyl *N*-terminal amide isomerization. To test the effects of chemically modifying the carbohydrate hydroxyl groups on prolyl *cis*−*trans* isomerization, we will study simple *N*-acyl and *N*-glycyl model compounds. Modifications of the glycine−proline peptide motif are of particular interest since it is present in collagen[23] as well as in small peptides found to have neuroprotective properties[24] and the ability to inhibit HIV replication.[25] Furthermore, the Gly-Pro-Gly peptide sequence is known to induce reverse turns.[26]

Results

Synthesis of *N*-Boc-GlcPro-carboxylic Acid 1. The synthesis of GlcProH **1** (Scheme 1) started from known amine 2,[27]

(10) For recent reviews, see (a) Dondoni, A.; Marra, A. *Chem. Rev.* **2000**, *100*, 4395−4421. (b) Schweizer, F. *Angew. Chem., Int. Ed.* **2002**, *41*, 230−264. For recent reviews on the use of glycosyl amino acids as peptidomimetics. see (c) Gruner, S. A. W.; Locardi, E.; Lohof, E.; Kessler, H. *Chem. Rev.* **2002**, *102*, 491−514. (d) Chakraborty, T. K.; Ghosh, S.; Jayaprakash, S. *Curr. Med. Chem.* **2002**, *9*, 421−435.

(11) Two examples have been reported: (a) Zhang, K.; Schweizer, F. *Synlett* **2005**, *20*, 3111−3115. (b) Cipolla, L.; Redaelli, C.; Nicotra, F. *Lett. Drug Des. Discovery* **2005**, *2*, 291−293.

(12) Manzoni, L.; Colombo, M.; May, E.; Scolastico, C. *Tetrahedron* **2001**, *57*, 249−255.

(13) Petter, R. C. *Tetrahedron Lett.* **1989**, *30*, 399−402.

(14) Jeannotte, G.; Lubell, W. D. *J. Org. Chem.* **2004**, *69*, 4656−4662.

(15) (a) Wagaw, S.; Rennels, R. A.; Buchwald, S. L. *J. Am. Chem. Soc.* **1997**, *119*, 8451−8458. (b) Kuwano, R.; Sato, K.; Kurokawa, T.; Karube, D.; Ito, Y. *J. Am. Chem. Soc.* **2000**, *122*, 7614−7615. (c) Viswanathan, R.; Prabhakaran, E. N.; Plotkin, M. A.; Johnston, J. N. *J. Am. Chem. Soc.* **2003**, *125*, 163−168.

(16) Koep, S.; Gais, H.-J.; Raabe, G. *J. Am. Chem. Soc.* **2003**, *125*, 13243−13251 and references therein.

(17) Blankley, C. J.; Kaltenbronn, J. S.; DeJohn, D. E.; Werner, A.; Bennett, L. R.; Bobowski, G.; Krolls, U.; Johnson, D. R.; Pearlman, W. M.; Hoefle, M. L; Essenberg, A. D.; Cohen, D. M.; Kaplan, H. R. *J. Med. Chem.* **1987**, *30*, 992−998.

(18) Li, W.; Moeller, K. D. *J. Am. Chem. Soc.* **1996**, *118*, 10106−10112.

(19) Hanessian, S.; Mcnaughton-Smith, G.; Lombart, H.-G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789−12854 and references 3a−h and 4a−k in ref 17.

(20) Grotenbreg, G. M.; Timmer, M. S. M.; Llamas-Saiz, A. L.; Verdoes, M.; van der Marel, G. A.; van Raaij, M. J.; Overkleeft, H. S.; Overhand, M. *J. Am. Chem. Soc.* **2004**, *126*, 3444−3446.

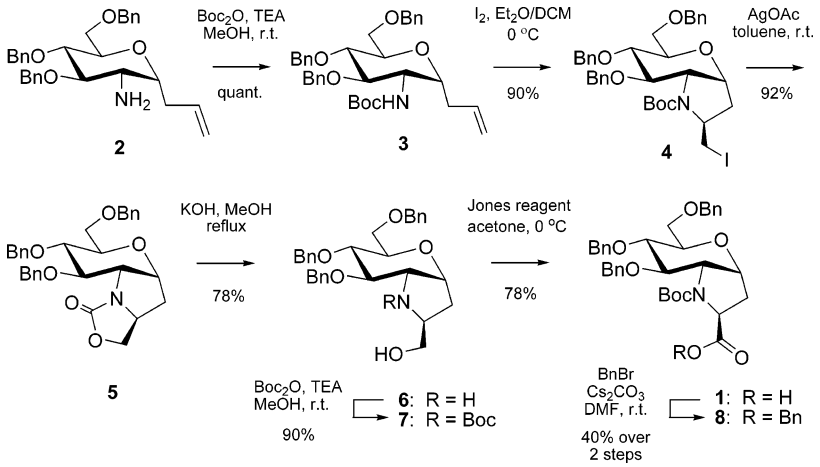
(21) Chakraborty, T. K.; Jayaprakash, S.; Diwan, P. V.; Nagaraj, R.; Jampani, S. R. B.; Kunwar, A. C. *J. Am. Chem. Soc.* **1998**, *120*, 12962−12963.

(22) It has been reported that substitution of D-proline by *cis*-3-hydroxy-D-proline (HypC3-OH) in the sequence Boc-Leu-Pro-Gly-NHMe resulted in a novel pseudo-β-turn-like nine-membered ring structure involving an intramolecular hydrogen bond between LeuNH: Chakraborty, T. K.; Srinivasu, P.; Vengal Rao, R.; Kiran Kumar, S.; Kunwar, A. C. *J. Org. Chem.* **2004**, *69*, 7399−7402.

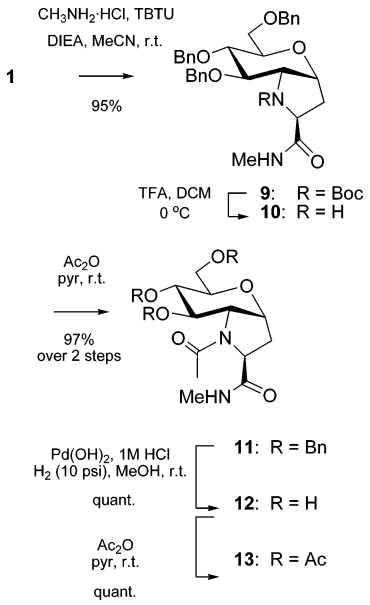
(23) Vitagliano, L.; Beriso, R.; Mazzarella, L.; Zagari, A. *Biopolymers* **2001**, *58*, 459−464.

(24) (a) Brimble, M. A.; Trotter, N. S.; Harris, P. W. R.; Sieg, F. *Bioorg. Med. Chem.* **2005**, *13*, 519−532. (b) Alonso, De Diego, S. A.; Munoz, P.; Gonzalez-Muniz, R.; Herranz, R.; Martin-Martinez, M.; Cenarruzabeitia, E.; Frechilla, D.; Del, Rio, J.; Jimeno, M. L.; Garcia-Lopez, M. T. *Bioorg. Med. Chem.* **2005**, *15*, 2279−2283. (c) Ioudina, M.; Uemura, E. *Exp. Neurol.* **2003**, *184*, 923−929.

SCHEME 1. Synthesis of *N*-Boc-GlcPro-Carboxylic Acid 1



SCHEME 2. Synthesis of *N*-Acetyl-GlcPro *N*′-Methylamides 11−13



which was synthesized in seven steps from commercially available 2,3,4,6 tetra-*O*-benzyl-D-glucopyranose in an overall yield of 40%. Protection of the amino function as *tert*-butyloxycarbamate (Boc) afforded **3** in quantitative yield. Installation of the pyrrolidine ring was achieved via aminoiodocyclization in 50% CH_2Cl_2/ether to afford the bicyclic iodo deriviative **4** in 90% yield as a single stereoisomer together with 5% unreacted **3**.[28] Attempts to substitute the iodo function in **4** by hydroxide ion (KOH) or acetate failed and produced complex mixtures containing tricyclic carbamate **5**. However, high yields (92%) of **5** could be obtained by exposure of **4** to silver acetate in toluene.[29] Hydrolysis of **5** by potassium hydroxide in methanol at elevated temperature provided the amino alcohol **6** in 78% isolated yield. Protection of the amino function in **6** was accomplished by use of di-*tert*-butyl dicarbonate to yield the Boc-protected proline analogue **7** in 90% yield. Subsequently, the alcohol **7** was subjected to Jones oxidation to afford protected GlcProH **1** in 78% yield. To assign the stereochemistry of **1**, esterfication with benzyl bromide and cesium carbonate in DMF afforded the protected GlcProH **8** in 40% yield from **7**.

Assignment of Stereochemistry for *N*-Boc-GlcPro-benzyl Ester 8. The *S*-configuration at the 2-position of protected GlcProH **8** was identified on the basis of observed/unobserved NOE contacts shown in Figure 2. For instance, subjection of the H-2 signal in **8** to a one-dimensional GOESY[30] experiment showed interproton effect to H-5 [0.8% NOE[31] relative to the H-2 (dd) signal]. By comparison, no interproton effects were observed between H-2 and H-9 or H-2 and H-8.

Synthesis of *N*-Acetyl-GlcPro *N*′-Methylamides 11−13 and Determination of $K_{t/c}$. With GlcProH **1** in hand, we then explored the use of the building block in peptide chemistry. Initially, we focused on analogues for comparison with Ac-Pro-NHMe **17**, which has frequently served as a model to study the *cis*−*trans* isomerization of proline.[4,32] The synthesis of GlcProH-modified amides **11**−**13** is outlined in Scheme 2. Acid **1** was directly coupled to methylamine by use of *O*-benzotriazolyl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) as coupling reagent in MeCN to produce amide **9** in 95% yield. Deprotection of the amine (giving **10**) followed by acylation produced diamide **11** in 97% yield over two steps. In addition, removal of the benzyl ether protecting groups by catalytic hydrogenolysis in methanol provided the polyhydroxylated diamide **12**, which was further acylated to afford diamide **13** in quantitative yield.

For each compound **11**−**13**, the ratio of *trans*/*cis* isomers was calculated by integrating as many well-resolved peaks as

(25) (a) Su, J.; Andersson, E.; Horal, P.; Naghavi, M. H.; Palm, A.; Wu, Y.-P.; Eriksson, K.; Jansson, M.; Wigzell, H.; Svennerholm, B.; Vahlne, A. *J. Hum. Virol.* **2001**, *4*, 1−7. (b) Laczko, I.; Hollosi, M.; Urge, L.; Ugen, K. E.; Weiner, D. B.; Mantsch, H. H.; Thurin, J.; Otvos, L., Jr. *Biochemistry* **1992**, *31*, 4282−4288.

(26) (a) Hayashi, T.; Asai, T.; Ogoshi, H. *Tetrahedron Lett.* **1997**, *38*, 3039−3042. (b) Zerkout, S.; Dupont, V.; Aubry, A.; Vidal, J.; Collet, A.; Vicherat, A.; Marraud, M. *Int. J. Pept. Protein Res*. **1994**, *44*, 378−387.

(27) Cipolla, L.; Lay, L.; Nicotra, F. *J. Org. Chem.* **1997**, *62*, 6678−6681.

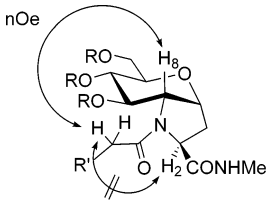
(28) NMR analysis of the crude reaction mixture containing **5** did not indicate the formation of the other diastereoisomer.

(29) Davies, S. G.; Nicholson, R. L.; Price, P. D.; Roberts, P. M.; Smith, A. D. *Synlett* **2004**, *5*, 901−903.

(30) GOESY is a 1D selective NOESY experiment. It provides quantitative information and much higher sensitivity compared to NOESY. First appeared in Stonehouse, J.; Adell, P.; Keeler, J.; Shaka, A. J. *J. Am. Chem. Soc*. **1994**, *116*, 6037−6038.

(31) A 40-ms Gaussian pulse with a 560-ms mixing time was used.

(32) (a) Taylor, C. M.; Hardre, R.; Edwards, P. J. B.; Park, J. H. *Org. Lett.* **2003**, *5*, 4413−4416. (b) Madison, V.; Kopple, K. D. *J. Am. Chem. Soc.* **1980**, *102*, 4855−4863.



11-13 R = Bn, H, Ac; R' = H
14-16 R = Bn, H, Ac; R' = CH_3CONH

FIGURE 3. Representative conformationally relevant NOE interactions observed for GlcProH-derived model compounds. Interproton effects for the *trans*-amide rotamer were observed between H-8 and the *N*-acyl methyl group (compounds **11**−**13**) or glycinyl protons (compounds **14**−**16**). By comparison, very small or no interproton effects were observed between H-2 and the *N*-acyl methyl group (compounds **11**−**13**) or glycinyl protons (compounds **14**−**16**).

TABLE 1. ***Trans/cis*** **Ratio[a] (** $K_{t/c}$**) and % *cis* Isomer for 11−13 in Various Solvents**

	solvent			
compd	$CDCl_3$	D_2O	CD_3OD	DMSO-d_6
11 (R = Bn)	>30 (<3%)	ns[b]	9 (10%)	6.7 (13%)
12 (R = H)	ns	5.7 (15%)	3.5 (22%)	4 (20%)
13 (R = Ac)	6.7 (13%)	ns	1.8 (36%)	2 (33%)

[a] Determined by 500 MHz NMR at 25 °C. [b] Not soluble.

TABLE 2. ***Trans/cis*** **Ratio[a] (** $K_{t/c}$**) and % *cis* Isomer for 14−16 in Various Solvents**

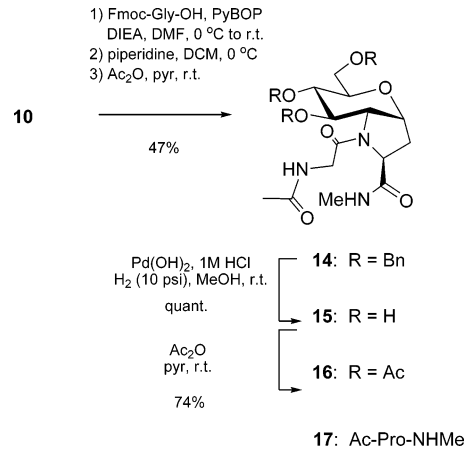
	solvent		
compd	$CDCl_3$	D_2O	CD_3OD
14 (R = Bn)	19 (5%)	ns[b]	>30 (<3%)
15 (R = H)	ns	9 (10%)	5.7 (15%)
16 (R = Ac)	4 (20%)	ns	3 (25%)

[a] Determined by 500 MHz NMR at 25 °C. [b] Not soluble.

possible for each isomer and taking the average over all peaks for respective isomers.[32a] The assignment of *N*-terminal amide geometry for both major and minor isomers of **11**−**16** was based on multiple GOESY experiments.[30] For example, selective inversion of the H-8 proton in **11** (CD_3OD) showed an interproton effect to the *N*-terminal acetate singlet (5.2%) (Figure 3). By comparison, a very small (0.4%) interproton effect was observed between H-2 and the acetate methyl singlet, and weak or no interproton effects were observed between H-8 and H-2. As H-8 and H-2 lie on opposite faces of the pyrrolidine ring, NOE contact from H-8 to the *N*-terminal acetate singlet can be assigned as the *trans* isomer. Analysis was carried out in different NMR solvents to understand how the model compounds behaved in different solvent environments. While it would be most desirable to study the GlcProHs in water, these results represent a proof of concept, and there is precedence for studying modifications of prolyl isomerization in nonaqueous environments.[13,33]

We found a significant variation of the *cis* content as the sugar substituents were varied (Table 1). In CD_3OD, there was an increase in the *cis* content as the sugar substituents changed from benzyl (**11**, 10%) to hydroxyl (**12**, 22%) to acetate (**13**, 36%). Analysis in DMSO-d_6 produced very similar results, with the *N*-terminal *cis*-amide content varying from benzyl (**11**, 13%) to hydroxyl (**12**, 20%) to acetate (**13**, 33%).

SCHEME 3. Synthesis of *N*-Acetyl-Glycyl-GlcPro *N*′-Methylamides 14−16



Overall, $K_{t/c}$ for the unprotected GlcProH **12** (3.5 in CD_3OD) was very similar to that of the L-proline model compound **17** ($K_{t/c}$ = 4),[32b] leaving the per-O-benzylated **11** favoring the *trans* conformer ($K_{t/c}$ = 9 in CD_3OD) and the per-O-acylated **13** favoring the *cis* conformer ($K_{t/c}$ = 1.8 in CD_3OD). Also, $CDCl_3$ gave higher $K_{t/c}$ than the more polar solvents ($K_{t/c}$ = 30 when R = Bn, $K_{t/c}$ = 6.7 when R = Ac).

Synthesis of *N*-Acetylglycyl-GlcPro *N*′-Methylamides 14−16 and Determination of $K_{t/c}$**.** GlcProH **10** was coupled to Fmoc-Gly-OH by use of benzotriazol-1-yloxytris(pyrrolidino)-phosphonium hexafluorophosphate (PyBOP) as coupling reagent, followed by deprotection of the amino function and acylation providing the triamide **14** in 47% yield over three steps (Scheme 3). Deblocking of the carbohydrate moiety afforded polyhydroxylated peptide **15**, which was further acylated to afford triamide **16**.

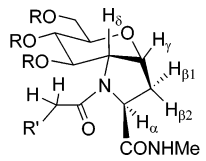
Again, the average of as many well-resolved peaks as possible for the major and minor isomers was recorded to find the $K_{t/c}$ for respective compounds in each solvent. One-dimensional GOESY experiments helped to assign the *N*-terminal amide geometry. The major isomer was assigned as the *trans* isomer on the basis of the interproton effect observed between H-8 and the glycyl α-protons (Figure 3) (for example, a 6.1% NOE was observed for **14** in CD_3OD). Furthermore, selective inversion of H-2 of the major isomer showed no interproton effect with either $H_{\alpha1(Gly)}$ or $H_{\alpha2(Gly)}$ (for example, no such NOE was observed for **14** in $CDCl_3$). Additionally, selective inversion of $H_{\alpha2(Gly)}$ of the minor isomer showed no interproton effect with H-8 of the minor isomer (for example, no such NOE was observed for **16** in CD_3OD).

We found a very similar profile of $K_{t/c}$ for the Ac-Gly-GlcPro-NHMe model compounds **14**−**16** compared to those of the Ac-GlcPro-NHMe model compounds **11**−**13** (Table 2). In CD_3OD, there was an increase in the *cis* content as the sugar substituent changed from benzyl (**14**, <3%) to hydroxyl (**15**, 15%) to acetate (**16**, 25%). These results confirm that the substituents on the sugar are influencing $K_{t/c}$.

Analysis of the coupling constants of the pyranose ring showed it exists in a chair conformation. For example, for **12** in CD_3OD, $J_{5,6}$ was 9.4 Hz, while $J_{6,7}$ was 9.6 Hz and $J_{7,8}$ was 9.0 Hz (see Figure 2 for numbering). The average values of

(33) Trabocchi, A.; Potenza, D.; Guarna, A. *Eur. J. Org. Chem.* **2004**, *22*, 4621−4627.

TABLE 3. Comparison of Average Coupling Constants for 11−16 Major and Minor Isomers[a] with Cγ-*endo* and Cγ-*exo* Puckers of 4-Fluoroproline and L-proline



11-13 R = Bn, H, Ac; R' = H
14-16 R = Bn, H, Ac; R' = CH_3CONH

compd	$^3J_{\alpha\beta}1$	$^3J_{\alpha\beta}2$	$^3J_{\gamma\beta}1$	$^3J_{\gamma\beta}2$	$^3J_{\delta\gamma}1$
11−16 major isomers	9.9 ± 0.3	1.0 ± 0.1	11.8 ± 0.3	7.4 ± 0.2	7.2 ± 0.1
11−16 minor isomers	9.3 ± 0.2	1.7 ± 0.3	10.9 ± 0.4	6.8 ± 0.2	7.1 ± 0.3
4(*R*)-fluoroproline[34] (Cγ-*endo*)	10	3	4	1	4
4(*S*)-fluoroproline[34] (Cγ-*exo*)	8	10	1	4	3
L-proline[35] (Cγ-*endo*)	6−10	2−3	5−9	8−12	6−10
L-proline[35] (Cγ-*exo*)	7−10	7−11	5−9	2−3	5−9

[a] Coupling constants are given in hertz ± standard error.

each coupling constant in comparison to the literature values match best to a Cγ-endo conformation for the pyrrolidine ring (Table 3).

Also, coupling constants indicate that the prolyl 4-position hydroxyl group (pyrano endocyclic oxygen) is oriented in an equatorial position relative to the pyrrolidine ring, which is not its preferred axial orientation.[36] Perhaps most importantly, all coupling constant values changed very little as the sugar substituents were varied, even as the solvent was varied, and between the major and minor isomers in each case. Together, these results indicate that the rigid pyranose ring is restricting the conformational freedom of the pyrrolidine ring.

Discussion

After developing a synthetic route to a novel sugar−proline analogue, we found through NMR experiments that *N*-terminal amide isomerization is dependent on the modification of the gluco scaffold but cannot be correlated with a significant variation in conformation.

There could be a steric explanation for the trend in $K_{t/c}$, as work by Lubell and co-workers[3,37] has shown that the $K_{t/c}$ for prolyl *N*-terminal amide bonds can be influenced through steric effects. Alkyl-substituted proline derivatives such as 5-*tert*-butylproline force a very high *N*-terminal *cis*-amide content (80%+) through steric repulsion of the *N*-terminal amino acid side chain and the 5-*tert*-butyl substituent on proline. Here, we chose model compounds that provide a minimal steric contribution to influence the $K_{t/c}$. Thus, the *N*-terminal acetate or glycyl groups should provide little to no steric bias between the *cis* and *trans* isomers. If there were an explanation for the trend in $K_{t/c}$ based on sterics, then the deprotected sugar−proline hybrid ($K_{t/c}$ of 3.5 in CD_3OD for **12**) should provide the lowest steric influence, while the benzyl ($K_{t/c}$ of 9 in CD_3OD for **11**) and acetate ($K_{t/c}$ of 1.8 in CD_3OD for **13**) substituents would provide greater steric bulkiness. However, the trend in $K_{t/c}$ does not follow the trend in increase in steric bulk of the sugar substituents (from 3.5 to 1.8 to 9 for **12** to **13** to **11**, respectively). This led us to consider other factors.

A large amount of interest lies in studying the conformation of proline[4,8,23,32,38] and modifications that alter the preferred conformation of the pyrrolidine ring.[3−9,36,37,39] We analyzed the coupling constants of the sugar and pyrrolidine rings in an attempt to consider the change in $K_{t/c}$ as a function of the change in the pucker of the pyrrolidine ring. Raines and co-workers[7,36] proved, through work with 4-fluoroproline model compounds, that as the stereochemistry of the 4-position EWG is varied, so is the pucker of the pyrrolidine ring (in an attempt to maximize the gauche effect). Consequently, as the pucker is changed, the degree of n $\rightarrow \pi^*$ donation from the *N*-terminal C═O to the *C*-terminal C═O is also changed, thus affecting $K_{t/c}$. In our case, one could imagine that as the substituents on the sugar are varied, the pucker of the pyrrolidine ring also changes, influencing n $\rightarrow \pi^*$ donation and $K_{t/c}$. Through NMR analysis of **11**−**16** we found several results; as the sugar substituents are varied, and even as the solvent is varied, the coupling constants are held within a very narrow range (Table 3). These results indicate that the pucker of the pyrrolidine ring does not vary as the substituents on the sugar scaffold are varied, and the change in $K_{t/c}$ cannot be explained in terms of a change in the pyrrolidine pucker.

Most likely, simple intramolecular interactions between the sugar substituents and the peptide backbone are responsible for the observed variation in the prolyl *N*-terminal amide isomerization. For compounds **12** and **15** where R = H, there is the potential for a hydrogen bond between the 7-position hydroxyl group and the prolyl *N*-terminal amide carbonyl (Figure 4a). This would stabilize the *N*-terminal *cis*-amide isomer to some extent, and would not be possible for the *trans* isomer. For

(34) Gerig, J. T.; McLeod, R. S. *J. Am. Chem. Soc*. **1973**, *95*, 5725−5729.

(35) Cai, M.; Huang, Y.; Liu, J.; Krishnamoorthi, R. *J. Biomol. NMR* **1995**, *6*, 123−128.

(36) (a) Jenkins, C. L.; Lin, G.; Duo, J.; Rapolu, D.; Guzei, I. A.; Raines, R. T.; Krow, G. R. *J. Org. Chem.* **2004**, *69*, 8565−8573. (b) Bretscher, L. E.; Jenkins, C. L.; Taylor, K. M.; DeRider, M. L.; Raines, R. T. *J. Am. Chem. Soc.* **2001**, *123*, 777−778.

(37) (a) Halab, L.; Lubell, W. D. *J. Org. Chem.* **1999**, *64*, 3312−3321. (b) Halab, L.; Lubell, W. D. *J. Pept. Sci.* **2001**, *7*, 92−104. (c) Halab, L.; Lubell, W. D. *J. Am. Chem. Soc.* **2002**, *124*, 2474−2484.

(38) Madison, V. *Biopolymers* **1977**, *16*, 2671−2692.

(39) (a) Montelione, G. T.; Hughes, P.; Clardy, J.; Scheraga, H. A. *J. Am. Chem. Soc.* **1986**, *108*, 6765−6773. (b) Avenoza, A.; Busto, J. H.; Peregrina, J. M.; Rodriguez, F. *J. Org. Chem*. **2002**, *67*, 4241−4249. (c) Hanessian, S.; Papeo, G.; Angiolini, M.; Fettis, K.; Beretta, M.; Munro, A. *J. Org. Chem*. **2003**, *68*, 7204−7218. (d) Taylor, C. M.; Hardre, R.; Edwards, P. J. B. *J. Org. Chem*. **2005**, *70*, 1306−1315. (e) Koskinen, A. M. P.; Helaja, J.; Kumpulainen, E. T. T.; Koivisto, J.; Mansikkamaeki, H.; Rissanen, K. *J. Org. Chem*. **2005**, *70*, 6447−6453.

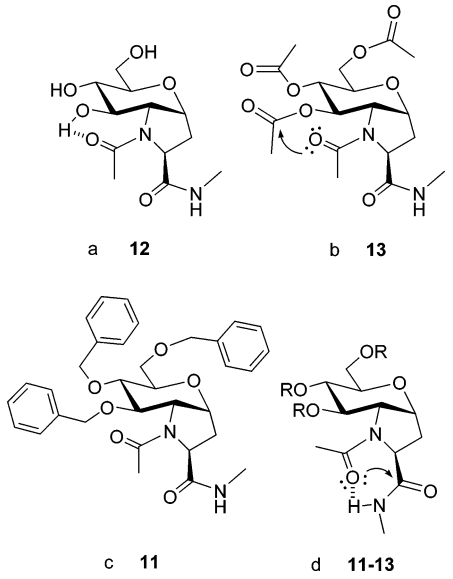


FIGURE 4. Proposed intramolecular interactions for **11**, **12**, and **13**: (a) *cis*-Amide rotamer isomer can be stabilized through an intramolecular hydrogen bond in **12**. (b) *cis*-Amide rotamer can also be stabilized through n → π^* donation from the prolyl *N*-terminal amide to the acetate ester group at the 7-position of GlcProH in **13**. (c) The interactions that stabilize the *cis*-amide rotamer in **12** and **13** are not possible for **11**. (d) *trans*-Amide rotamer for **11**−**13** can be stabilized through n → π^* donation or a hydrogen bond to the *C*-terminal amide; analogous interactions can be envisaged for **14**−**16**.

compounds **13** and **16** where R = Ac, there is the potential for n → π^* donation from the prolyl *N*-terminal amide carbonyl to the sugar 7-position acetate ester group (Figure 4b). This would stabilize the *N*-terminal *cis*-amide isomer. The relative decrease in $K_{t/c}$ for **13** and **16** ($K_{t/c}$ of 1.8 and 3, respectively, in CD_3OD) compared to **12** and **15** ($K_{t/c}$ of 3.5 and 5.7, respectively, in CD_3OD) may reflect a greater energetic stabilization of the *cis*-amide ground state from n → π^* donation relative to a hydrogen bond. Finally, for compounds **11** and **14** where R = Bn (Figure 4c), the very high *trans* content is of interest (in CD_3OD, $K_{t/c}$ is 9 for compound **11** and 30 for compound **14**). The interactions that stabilize the *cis*-amide geometry for **12** and **13** would not be possible with an ether-linked protecting group. Most likely there is a steric influence that orients the *N*-terminal amide such that it can maximize the n → π^* donation to the *C*-terminal amide, thereby stabilizing the *trans* isomer. This n → π^* donation is also probable for the *trans* isomers of **12** and **13** (Figure 4d). There is also the potential for an intramolecular hydrogen bond from the *N*-terminal carbonyl to the *C*-terminal amide. Therefore, the overall $K_{t/c}$ must represent the equilibrium established between these competing interactions.

Conclusions

In order to extend the molecular repertoire of proline analogues, we became interested in the design and synthesis of a bicyclic sugar−proline hybrid. The resulting GlcProH is a rigid, polyfunctional building block, which may find use as a proline mimetic, glycomimetic, or scaffold for combinatorial synthesis. GlcProH served as a building block in peptide synthesis and was incorporated into model peptides. Simple modifications of the sugar scaffold permitted tuning of the *trans*/*cis*-amide rotamer population in various solvents. The *cis* rotamer population increased as the substituents on the sugar scaffold were varied [*O*-benzyl < OH (unprotected) < *O*-acetyl] in CD_3OD and DMSO-d_6. Until now, the ability to vary the *N*-terminal prolyl amide isomer ratio required the synthesis of proline surrogates by independent synthetic routes. However, our results demonstrate that one-step modifications of the hydroxyl groups on the carbohydrate scaffold can be used to tune the conformational properties of GlcProH-containing model peptides.

Experimental Section

(2*S*,3a*R*,5*R*,6*R*,7*S*,7a*S*)-1-(*tert*-Butyloxycarbonyl)-6,7-di-*O*-benzyl-5-[(benzyloxy)methyl]octahydropyrano[3,2-*b*]pyrrole-2-carboxylic Acid (1). Compound **7** (0.125 g, 0.21 mmol) was dissolved in 6 mL of acetone. The solution was cooled to 0 °C. Fresh Jones' reagent (0.75 mL, 6.3 mmol) was prepared and was added dropwise. The reaction mixture was stirred for 30 min before addition of water (5 mL) and then aqueous saturated sodium bicarbonate (5 mL). The acetone was removed under reduced pressure. The product was extracted into CH_2Cl_2 (3 × 15 mL), then dried (Na_2SO_4) and concentrated under reduced pressure, and was normally used directly in the next reaction. Purification by flash chromatography with 3:1 ethyl acetate/methanol yielded **1** as a clear oil (0.100 g, 0.17 mmol) (78.4%), $[\alpha]_D^{25} = -12.8°$ (*c* 0.4, CH_3OH). ^1H NMR (500 MHz, CD_3OD, 298 K) δ = 7.09−7.37 (m, 15H, aromatic), 4.38−4.76 (m, 7H, -OCH_2Ph, H_4), 4.25 (br m, 1H, H_2, H_2 minor), 4.20 (dd, app t, 0.8H, $J_{7,8}$ = 6.0 Hz, $J_{6,7}$ = 6.3 Hz, H_7), 4.10 (dd app, 1H, $J_{8,9}$ = 6.6 Hz, H_8), [4.06−4.10 (m, 0.2H, H_8)], [4.01 (dd app t, 0.2H, H_7)], 3.80−3.89 (m, 1H, H_5, H_5 minor), 3.71 (dd, 0.8H, $J_{10a,10b}$ = 10.6 Hz, $J_{5,10a}$ = 6.0 Hz, H_{10a}), [3.67−3.73 (m, 0.2H, H_{10a})], 3.59 (dd, 0.8H, $J_{5,10b}$ = 3.4 Hz, H_{10b}), [3.57−3.62 (m, 0.2H, H_{10b})], [3.55 (dd app t, 0.2H, H_6)], 3.46 (dd app t, 1H, $J_{5,6}$ = 6.4 Hz, H_6), 2.53 (ddd, 1H, $J_{3a,3b}$ = 12.7 Hz, H_{3a}, H_{3a} minor), 1.90−1.97 (m, 1H, H_{3b}, H_{3b} minor), 1.40 (s, 7.2H, *tert*-butyl), [1.32 (s, 1.8H, *tert*-butyl)]. ^{13}C NMR (75 MHz, CD_3OD, 298 K) δ = 168.7, 156.1, 139.8, 139.7, 139.5, (81.9), 81.5, (79.8), 78.4, (76.9), 76.6, 74.9, 74.7, (74.6), (74.5), 74.3, (74.2), 74.1, (73.9), 72.9, (70.4), 70.2, (59.9), 59.8, 33.9, (28.8), 28.6. HRMS (ES) calcd for $C_{35}H_{40}NO_8$ $(M - H)^-$ 602.2759, found− 602.2755.

1-[2′-(*tert*-Butyloxycarbonyl)amino-3′,4′,6′-tri-*O*-benzyl-2′-deoxy-α-D-glucopyranosyl]-2-propene (3). Compound **2** (0.202 g, 0.43 mmol) was dissolved in 4 mL of methanol. Addition of triethylamine (0.6 mL, 4.3 mmol) was followed by addition of di-*tert*-butyl dicarbonate (0.46 g, 2.1 mmol). The reaction mixture was stirred for 16 h. All reagents and solvent were removed under reduced pressure to provide **3** as a white solid (0.244 g, 0.43 mmol) (quant), $[\alpha]_D^{25} = +11.4°$ (*c* 3.7, $CHCl_3$), mp 98−101 °C. ^1H NMR (300 MHz, $CDCl_3$, 298 K) δ = 7.20−7.40 (m, 15H, aromatic), 5.85 (dddd, 1H, *J* = 6.9, 7.0, 10.1, 17.0 Hz, −C*H*═CH_2), 5.61 (d, 1H, *J* = 9.8 Hz, N*H*Boc), 5.02−5.17 (m, 2H, −CH═CH_2), 4.46−4.87 (m, 6H, −OCH_2Ph), 4.20 (dd, 1H, *J* = 6.1, 6.2 Hz, H_5), 3.95 (ddd, 1H, *J* = 2.0, 5.6, 7.8 Hz, H_1), 3.80−3.89 (m, 2H, H_2, H_{6a}), 3.68−3.79 (m, 2H, H_3, H_{6b}), 3.55−3.60 (m, 1H, H_4), 2.17−2.39 (m, 2H, allylic), 1.45 (s, 9H, *tert*-butyl). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K) δ = 155.8, 138.3, 137.8, 137.6, 134.6, 127.4−128.6 (aromatic carbons), 117.1, 79.1, 74.9, 74.9, 73.4, 73.2, 72.1, 71.8, 68.3, 68.0, 48.9, 35.5, 28.4. MS (ES) calcd for $C_{35}H_{43}NO_6$ $(M + Na)^+$ 596.30, found 596.30. Anal. Calcd for $C_{35}H_{43}NO_6$: C 73.27, H 7.55, N 2.44. Found: C 73.43, H 7.75, N 2.19.

(2*S*,3a*R*,5*R*,6*R*,7*S*,7a*S*)-1-(*tert*-Butyloxycarbonyl)-6,7-di-*O*-benzyl-2-iodomethyl-5-[(benzyloxy)methyl]octahydropyrano-[3,2-*b*]pyrrole (4). Compound **3** (0.29 g, 0.51 mmol) was dissolved in 10 mL of 1:1 CH_2Cl_2/diethyl ether. The solution was cooled to 0 °C before addition of iodine (0.39 g, 1.5 mmol). After 1 h, the reaction mixture was warmed to ambient temperature before being worked-up by the addition of 20 mL of saturated aqueous sodium

thiosulfate. With shaking, the solution became colorless. The product was extracted into CH_2Cl_2 (3 × 20 mL), dried (Na_2SO_4), concentrated, and purified by flash chromatography with 5:1 hexanes/ethyl acetate. The product **4** was isolated as a single stereoisomer as a pale yellow oil (0.32 g, 0.46 mmol) (89.6%), $[\alpha]_D^{25} = -23.9°$ (*c* 2.5, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$, 298 K) δ = 7.13−7.40 (m, 15H, aromatic), 4.32−4.78 (br m, 8H), 3.64−4.08 (broad m's, 5H), 3.55 (br m, 1H), 3.45 (br t, 2H), 2.40 (br m, 1H, H_{3a}), 1.9 (m, 1H, H_{3b}), 1.5 (s, 9H, *tert*-butyl). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K) δ = 153.8, 138.8, 138.6, 138.4, 127.4−128.7 (aromatic carbons), 80.7, 77.6, 74.2, 74.0, 73.7, 73.5, 72.5, 70.2, 69.4, 59.5, 56.8, 36.5, 28.8, 14.8. MS (ES) calcd for $C_{35}H_{43}INO_6$ $(M + H)^+$ 700.21, found 700.08; calcd for $C_{35}H_{42}INNaO_6$ $(M + Na)^+$ 722.20, found 722.01. Anal. Calcd for $C_{35}H_{42}INO_6$: C 60.09, H 6.05, N 2.00. Found: C 60.02, H 5.74, N 2.39.

(2*S*,3a*R*,5*R*,6*R*,7*S*,7a*S*)-1-(*tert*-Butyloxycarbonyl)-6,7-di-*O*-benzyl-5-[(benzyloxy)methyl]octahydropyrano[3,2-*b*]pyrrolo-[1,2-*c*]oxazol-3-one (5). Compound **4** (0.28 g, 0.41 mmol) was dissolved in 6 mL of toluene. Addition of silver acetate (0.68 g, 4.1 mmol) made the solution instantly become colorless. The reaction was stirred for 16 h at ambient temperature. The reaction mixture was diluted with 10 mL of ethyl acetate and then was filtered through Celite. The product was concentrated under reduced pressure and then purified by flash chromatography with 1:1 hexanes/ethyl acetate to yield **5** as a white solid (0.19 g, 0.37 mmol) (92.1%), $[\alpha]_D^{25} = +21.9°$ (*c* 2.0, $CHCl_3$), mp 106−111 °C. ^{1}H NMR (300 MHz, $CDCl_3$, 298 K) δ = 7.14−7.48 (m, 15H, aromatic), 4.95 (d, 1H, J = 11.1 Hz, −OCH_2Ph), 4.88 (d, 1H, J = 11.4 Hz, −OCH_2Ph), 4.75 (d, 1H, J = 11.4 Hz, -OCH_2Ph), 4.68 (ddd, 1H, $J_{3a,9}$ = 1.0 Hz, $J_{3b,9}$ = 5.7 Hz, $J_{8,9}$ = 5.7 Hz, H_9), 4.42−4.53 (m, 3H, −OCH_2Ph, H_{11a}), 4.38 (d, 1H, J = 12.2 Hz, −OCH_2-Ph), 4.08−4.20 (m, 2H, H_{11b}, H_2), 3.95 (dd, 1H, $J_{7,8}$ = 6.3 Hz, H_8), 3.72−3.82 (m, 1H, H_5), 3.64−3.72 (m, 2H, H_6, H_7), 3.53−3.59 (dd, 1H, $J_{10b,10a}$ = 10.6 Hz, $J_{5,10a}$ = 4.7 Hz, H_{10a}), 3.47−3.53 (dd, 1H, $J_{5,10b}$ = 2.8 Hz, H_{10b}), 2.10 (ddd, 1H, $J_{2,3a}$ = 5.3 Hz, $J_{3a,3b}$ = 13.2 Hz, H_{3a}), 1.59 (ddd, 1H, $J_{2,3b}$ = 10.6 Hz, H_{3b}). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K) δ = 161.1, 138.4, 138.1, 138.0, 127.6−128.4 (aromatic carbons), 80.7, 76.9, 75.4, 74.7, 74.4, 73.5, 73.2, 69.5, 66.8, 65.7, 57.5, 38.1. MS (ES) calcd for $C_{31}H_{33}NNaO_6$ $(M + Na)^+$ 538.22, found 538.22; Anal. Calcd for $C_{31}H_{33}NO_6$: C 72.21, H 6.45, N 2.72. Found: C 72.17, H 6.69, N 2.57.

(2*S*,3a*R*,5*R*,6*R*,7*S*,7a*S*)-6,7-di-*O*-Benzyl-2-hydroxymethyl-5-[(benzyloxy)methyl]octahydropyrano[3,2-*b*]pyrrole (6). Compound **5** (0.18 g, 0.35 mmol) was dissolved in 8 mL of methanol. After addition of potassium hydroxide (1.5 g, 26.2 mmol), the solution was heated to reflux for 4 h. The reaction mixture was then cooled to 0 °C, followed by acidification by addition of 5 mL of 3 M aqueous HCl. The methanol was removed under reduced pressure. The reaction mixture was brought to pH 9 by addition of 20 mL of aqueous saturated sodium bicarbonate. The product was extracted into CH_2Cl_2 (4 × 10 mL) and then dried (Na_2SO_4), concentrated under reduced pressure, and purified by flash chromatography with first 1:1 hexanes/ethyl acetate and then 5:1 ethyl acetate/methanol to yield **6** as a pale yellow solid (0.15 g, 0.27 mmol) (78.4%), $[\alpha]_D^{25} = +38.9°$ (*c* 2.8, $CHCl_3$), mp 91−94 °C. ^{1}H NMR (300 MHz, $CDCl_3$, 298 K) δ = 7.15−7.42 (m, 15H, aromatic), 4.45−4.65 (m, 6H, -OCH_2Ph), 4.25−4.35 (m, 1H, H_9), 4.05−4.11 (m, 1H, H_5), 3.82 (dd, 1H, $J_{10a,10b}$ = 10.2 Hz, $J_{5,10a}$ = 6.7 Hz, H_{10a}), 3.77 (dd app t, 1H, $J_{7,8}$ = 4.5 Hz, $J_{6,7}$ = 4.4 Hz, H_7), 3.61 (dd, 1H, $J_{5,10b}$ = 5.1 Hz, H_{10b}), 3.56 (dd app t, 1H, $J_{5,6}$ = 4.2 Hz, H_6), 3.41−3.51 (m, 2H, H_{11a}, H_2), 3.29 (br s, 0.3H, NH), 3.25 (dd, 1H, $J_{11a,11b}$ = 12.1 Hz, $J_{11b,2}$ = 7.3 Hz, H_{11b}), 3.09 (dd, 1H, $J_{8,9}$ = 3.7 Hz, H_8), 2.0 (ddd, 1H, J = 2.1, 8.0 Hz, $J_{3a,3b}$ = 14.0 Hz, H_{3a}), 1.59−1.69 (m, 1H, J = 6.0, 7.0 Hz, H_{3b}). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K) δ = 138.1, 137.8, 137.5, 127.7−128.6 (aromatic carbons), 74.4, 73.9, 73.4, 73.3, 72.8, 72.7, 71.8, 67.7, 64.1, 60.2, 57.4, 33.9. MS (ES) calcd for $C_{30}H_{36}NO_5$ $(M + H)^+$ 490.26, found 490.39; calcd for $C_{30}H_{35}NNaO_5$ $(M + Na)^+$ 512.24, found 512.36. Anal. Calcd for $C_{30}H_{35}NO_5$: C 73.59, H 7.21, N 2.86. Found: C 73.23, H 7.52, N 2.86.

(2*S*,3a*R*,5*R*,6*R*,7*S*,7a*S*)-1-(*tert*-Butyloxycarbonyl)-6,7-di-*O*-benzyl-2-hydroxymethyl-5-[(benzyloxy)methyl]octahydropyrano-[3,2-*b*]pyrrole (7). Compound **6** (0.048 g, 0.098 mmol) was dissolved in 4 mL of methanol. Addition of triethylamine (0.68 mL, 4.9 mmol) was followed by addition of di-*tert*-butyl dicarbonate (0.11 g, 0.49 mmol). The reaction mixture was stirred for 16 h. All reagents and solvent were removed under reduced pressure to provide **7** as a colorless oil (0.052 g, 0.088 mmol) (89.7%), $[\alpha]_D^{25} = -10.4°$ (*c* 2.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$, 313 K) δ = 7.14−7.41 (m, 15H, aromatic), 4.36−4.90 (m, 8H, −OCH_2Ph, H_2, H_{11a}), 3.95−4.14 (m, 3H, H_{11b}, H_9, H_7), 3.85−3.95 (m, 1H, H_8), 3.52−3.88 (m, 4H, H_5, H_6, H_{10a}, H_{10b}), 2.31 (br s, 1H, H_{3a}), 1.75 (br s, 1H, H_{3b}), 1.45 (s, 9H, *tert*-butyl). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K) δ = 155.8, 138.4, 138.0, 137.9, 126.9−128.7 (aromatic carbons), 80.9, 75.2, 73.4, 73.2, 72.7, 68.9, 66.8, 60.2, 59.8, 32.1, 29.7, 28.4, 22.7, 14.2. MS (ES) calcd for $C_{35}H_{44}NO_7$ $(M + H)^+$ 590.31, found 590.30. Anal. Calcd for $C_{35}H_{43}NO_7$: C 71.28, H 7.35, N 2.38. Found: C 71.03, H 7.59, N 2.33.

(2*S*,3a*R*,5*R*,6*R*,7*S*,7a*S*)-1-(*tert*-Butyloxycarbonyl)-6,7-di-*O*-benzyl-5-[(benzyloxy)methyl]octahydropyrano[3,2-*b*]pyrrole-2-carboxylic Acid Benzyl Ester (8). Compound **7** (0.018 g, 0.029 mmol) was dissolved in 3 mL of DMF. Addition of cesium carbonate (0.015 g, 0.045 mmol) was followed by addition of benzyl bromide (0.011 mL, 0.089 mmol). The reaction mixture was stirred for 1 h, and then the solvent was removed under reduced pressure. The reaction mixture was diluted with 10 mL of water followed by extraction into CH_2Cl_2 (3 × 10 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure before being purified by flash chromatography with 4:1 hexanes/ethyl acetate to yield **8** as a colorless oil (0.11 g, 0.18 mmol) (83.6%), $[\alpha]_D^{25} = -14.2°$ (*c* 0.4, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$, 298 K) δ = 7.05−7.41 (m, 20H, aromatic), 5.19 (d, 1H, J = 12.1 Hz, −OCH_2Ph), 5.11 (d, 1H, J = 12.3 Hz, −OCH_2Ph), 4.35−4.80 (m, 8H, -OCH_2Ph, H_2, H_9), 4.28 (dd app, 1H, $J_{7,8}$ = 5.6 Hz, $J_{6,7}$ = 5.7 Hz, H_7), 4.17 (dd app t, 1H, $J_{8,9}$ = 5.8 Hz, H_8), 3.83−3.93 (m, 1H, H_5), 3.70 (dd, 1H, $J_{10a,10b}$ = 10.4 Hz, $J_{5,10a}$ = 6.3 Hz, H_{10a}), 3.57 (dd, 1H, $J_{5,10b}$ = 3.4 Hz, H_{10b}), 3.48 (dd app t, 1H, $J_{5,6}$ = 5.8 Hz, H_6), 2.40−2.55 (m, 1H, H_{3a}), 1.80−2.00 (m, 1H, H_{3b}), 1.38 (s, 9H, *tert*-butyl). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K) δ = 172.9, 153.6, 138.4, 138.1, 138.1, 135.3, 127.0−128.7 (aromatic carbons), 80.4, 76.0, 74.8, 73.6, 73.5, 73.4, 72.8, 71.2, 69.0, 66.8, 58.8, 58.2, 33.0, 28.1. MS (ES) calcd for $C_{42}H_{47}NNaO_8$ $(M + Na)^+$ 716.32, found 716.11. Anal. Calcd for $C_{42}H_{47}NNaO_8$: C 70.37, H 6.61, N 1.95. Found: C 70.44, H 6.71, N 1.88.

(2*S*,3a*R*,5*R*,6*R*,7*S*,7a*S*)-1-(*tert*-Butyloxycarbonyl)-6,7-di-*O*-benzyl-5-[(benzyloxy)methyl]octahydropyrano[3,2-*b*]pyrrole-2-carboxamide *N*′-Methylamide (9). Compound **1** (0.10 g, 0.16 mmol) was dissolved in 6 mL of acetonitrile. Addition of diisopropylethylamine (0.11 mL, 0.64 mmol) was followed by addition of TBTU (0.10 g, 0.32 mmol) and methylamine hydrochloride (0.02 g, 0.32 mmol). The reaction mixture was stirred at ambient temperature for 4 h. The reaction mixture was diluted with 15 mL of water followed by extraction into CH_2Cl_2 (3 × 15 mL), dried (Na_2SO_4), and then concentrated under reduced pressure and purified by flash chromatography with 3:1 ethyl acetate/hexanes to provide **9** as a clear oil (0.086 g, 0.14 mmol) (84.3%), $[\alpha]_D^{25} = -3.5°$ (*c* 0.4, $CHCl_3$); ^{1}H NMR (300 MHz, acetone-d_6, 298 K) δ = 7.15−7.45 (m, 15H, aromatic), 4.45−4.82 (m, 8H, -OCH_2Ph, H_2, H_9), 4.39 (dd app t, 1H, $J_{7,8}$ = 5.3 Hz, $J_{6,7}$ = 5.3 Hz, H_7), 4.28 (dd, 1H, J = 5.9, 7.9 Hz, H_2), 4.11 (dd app t, 1H, $J_{8,9}$ = 5.5 Hz, H_8), 3.86−3.95 (m, 1H, H_5), 3.78 (dd, 1H, $J_{10a,10b}$ = 10.5 Hz, $J_{5,10a}$ = 6.1 Hz, H_{10a}), 3.68 (dd, 1H, $J_{5,10b}$ = 3.8 Hz, H_{10b}), 3.55 (dd app t, 1H, $J_{5,6}$ = 5.7 Hz, H_6), 2.80 (d, 3H, −NHCH_3), 2.42 (ddd, 1H, J = 6.3, 8.1, 13.4 Hz, H_{3a}), 1.89 (ddd, 1H, J = 5.8, 6.2, 12.0 Hz, H_{3b}), 1.37 (s, 9H, *tert*-butyl). ^{13}C NMR (75 MHz, acetone-d_6, 298 K) δ = 173.8, 154.4, 139.7, 139.5 (2), 128.1−129.0 (aromatic carbons), 79.4, 76.5, 75.9, 74.0, 73.4 (2), 72.9, 71.5, 69.9, 60.8,

59.3, 34.6, 28.2, 25.9. MS (ES) calcd for $C_{36}H_{44}N_2NaO_7$ (M + Na)$^+$ 639.30, found 639.27. Anal. Calcd for $C_{36}H_{44}N_2O_7$: C 70.11, H 7.19, N 4.54. Found: C 70.19, H 7.43, N 4.47.

(2*S*,3a*R*,5*R*,6*R*,7*S*,7a*S*)-6,7-di-*O*-Benzyl-5-[(benzyloxy)methyl]-octahydropyrano[3,2-*b*]pyrrole-2-carboxamide *N*′-Methylamide (10). Compound **9** (0.035 g, 0.057 mmol) was dissolved in 1.5 mL of CH_2Cl_2. The reaction mixture was then cooled to 0 °C. Trifluoroacetic acid (0.5 mL, 6.73 mmol) was added slowly. After 1 h the solution was codistilled with toluene (2 × 5 mL), and was normally used directly in the next reaction. Purification by flash chromatography with 10:1 ethyl acetate/methanol provided **10** as a clear oil (0.027 g, 0.14 mmol) (93.1%), $[\alpha]_D^{25} = -11.2°$ (*c* 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) δ = 7.58 (br q, 1H, −N*H*CH$_3$), 7.20−7.38 (m, 15H, aromatic), 4.47−4.62 (m, 6H, -OC*H*$_2$Ph), 4.18−4.27 (m, 1H, H$_2$), 4.04−4.14 (m, 1H, H$_5$), 3.78−3.91 (m, 2H, H$_9$, H$_{10a}$), 3.73 (br dd, 1H, $J_{7,8}$ = 3.9 Hz, $J_{6,7}$ = 3.8 Hz, H$_7$), 3.65 (dd, $J_{10a,10b}$ = 10.2 Hz, $J_{5,10b}$ = 5.6 Hz, H$_{10b}$), 3.59 (br dd, 1H, $J_{5,6}$ = 3.8 Hz, H$_6$), 2.89 (br dd, 1H, *J* = 3.0, 3.3 Hz, -N*H*), 2.78 (d, 3H, *J* = 5.0 Hz, -NHC*H*$_3$), 2.36 (ddd, 1H, *J* = 1.96, 9.6, 14.4 Hz, H$_{3a}$), 1.95 (ddd, 1H, *J* = 5.0, 7.0, 14.4 Hz, H$_{3b}$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K) δ = 175.3, 138.1, 137.8, 137.6, 127.6−128.6 (aromatic carbons), 74.2, 73.5, 73.3, 72.8, 72.6, 72.4, 71.6, 67.6, 60.9, 58.8, 36.7, 25.7. MS (ES) calcd for $C_{31}H_{37}N_2O_5$ (M + H)$^+$ 517.27, found 517.30. Anal. Calcd for $C_{31}H_{36}N_2O_5$: C 72.07, H 7.02, N 5.42. Found: C 72.11, H 7.13, N 5.36.

(2*S*,3a*R*,5*R*,6*R*,7*S*,7a*S*)-1-Acetyl-6,7-di-*O*-benzyl-5-[(benzyloxy)-methyl]octahydropyrano[3,2-*b*]pyrrole-2-carboxamide *N*′-Methylamide (11). Compound **10** (0.029 g, 0.056 mmol) was dissolved in 2 mL of pyridine followed by addition of acetic anhydride (0.053 mL, 0.56 mmol). The reaction mixture was stirred for 15 h, and then the solvent and reagents were removed under reduced pressure and the product was purified by flash chromatography with 10:1 ethyl acetate/methanol to provide **11** as a white solid (0.030 g, 0.054 mmol) (97% over two steps), $[\alpha]_D^{25} = +49.6°$(*c* 1.0, $CHCl_3$), decomposed at 142−147 °C. ^{1}H NMR (500 MHz, $CDCl_3$, 298 K, 0.036 M) δ = 7.23−7.41 (m, 13H, aromatic), 7.11−7.20 (m, 2H, aromatic), 5.97 (br q, 1H, −N*H*CH$_3$), 4.98 (ddd, 1H, $J_{3a,9}$ = 11.7 Hz, $J_{3b,9}$ = 7.3 Hz, $J_{8,9}$ = 7.4 Hz, H$_9$), 4.93 (d, 1H, *J* = 11.2 Hz, −OC*H*$_2$Ph), 4.80 (d, 1H, *J* = 10.8 Hz, −OC*H*$_2$Ph), 4.52−4.64 (m, 4H, −OC*H*$_2$Ph), 4.25 (dd app d, 1H, $J_{2,3a}$ = 9.5 Hz, $J_{2,3b}$ = 0.8 Hz, H$_2$), 4.03 (dd, 1H, $J_{7,8}$ = 9.2 Hz, H$_8$), 3.63−3.78 (m, 4H, H$_5$, H$_6$, H$_{10a}$, H$_{10b}$), 3.57 (dd app t, 1H, $J_{6,7}$ = 9.0 Hz, H$_7$), 2.82 (d, 3H, *J* = 4.2 Hz, -NHC*H*$_3$), 2.35 (ddd, 1H, $J_{3a,3b}$ = 12.3 Hz, H$_{3a}$), 2.15 (s, 3H, −COC*H*$_3$), 2.05 (ddd, 1H, H$_{3b}$). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K) (major conformer) δ = 172.1, 171.6, 137.8, 137.7, 137.5, 127.8−128.5 (aromatic carbons), 83.0, 78.0, 75.9, 74.9, 73.7, 73.6, 73.3, 68.8, 60.1, 57.8, 28.4, 26.4, 22.9. HRMS (ES) calcd for $C_{33}H_{39}N_2O_6$ (M + H)$^+$ 559.2802, gounf 559.2801.

(2*S*,3a*R*,5*R*,6*R*,7*S*,7a*S*)-1-Acetyl-6,7-dihydroxy-5-(hydroxymethyl)octahydropyrano[3,2-*b*]pyrrole-2-carboxamide *N*′-Methylamide (12). Compound **11** (0.027 g, 0.048 mmol) was dissolved in 10 mL of methanol. Addition of Pearlman's catalyst (20% palladium hydroxide on carbon) (0.030 g, approximately 0.028 mmol) was followed by addition of 1 M aqueous HCl (0.072 mL, 0.072 mmol). The reaction mixture was stirred vigorously under hydrogen atmosphere (10 psi) for 4.5 h, after which it was flushed with nitrogen and filtered. The product was then concentrated under reduced pressure to provide **12** as a clear oil (0.014 g, 0.048 mmol) (quant), $[\alpha]_D^{25} = +20.5°$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, D_2O, 298 K, 0.035 M) δ = 4.55−4.64 (m, 0.85H, H$_9$), 4.32 (dd app d, 0.85H, $J_{2,3a}$ = 10.2 Hz, $J_{2,3b}$ = 1.3 Hz, H$_2$), [4.11 (dd, 0.15H, $J_{8,9}$ = 7.7 Hz, $J_{7,8}$ = 8.0 Hz, H$_8$)], 3.99 (dd, 0.85H, $J_{8,9}$ = 7.2 Hz, $J_{7,8}$ = 9.2 Hz, H$_8$), 3.70 (dd, 0.85H, $J_{5,10a}$ = 2.2 Hz, $J_{10a,10b}$ = 12.4 Hz, H$_{10a}$), [3.67−3.72 (m, 0.15H, H$_{10a}$)], 3.62 (dd, 0.85H, $J_{5,10b}$ = 5.0 Hz, H$_{10b}$), [3.60 (dd, 0.15H, *J* = 1.7, 12.5 Hz, H$_{10b}$)], 3.51−3.56 (m, 0.85H, H$_5$), 3.51 (dd app t, 0.85H, $J_{6,7}$ = 9.9 Hz, H$_7$, H$_7$ minor), [3.45−3.50 (m, H$_5$)], [3.35 (dd, 0.15H, $J_{5,6}$ = 9.9 Hz, H$_6$)], 3.32 (dd app t, 0.85H, $J_{5,6}$ = 9.7 Hz, H$_6$), [2.63 (s, 0.45H, −COC*H*$_3$)], 2.58 (s, 2.55H, −COC*H*$_3$), 2.55 (ddd, 0.85H, $J_{3a,9}$ = 11.7 Hz, $J_{3b,3a}$ = 13.5 Hz, H$_{3a}$, H$_{3a}$ minor), 2.10 (s, 2.55H, −NHC*H*$_3$), [1.93 (ddd, 0.15H, $J_{2,3b}$ = 1.8 Hz, $J_{3b,9}$ = 6.7 Hz, $J_{3a,3b}$ = 12.9 Hz, H$_{3b}$)], [1.83 (s, 0.45H, −COC*H*$_3$)], 1.79 (ddd, 0.85H, $J_{3b,9}$ = 7.8 Hz, H$_{3b}$, H$_{3b}$ minor). ^{13}C NMR (75 MHz, D_2O, 298 K) (major conformer) δ = 174.6, 174.4, 74.5, 73.8, 73.3, 68.6, 61.5, 61.0, 58.1, 28.3, 26.1, 22.3. HRMS (ES) calcd for $C_{12}H_{20}N_2O_6Na$ (M + Na)$^+$ 311.1212, found 311.1214.

(2*S*,3a*R*,5*R*,6*R*,7*S*,7a*S*)-1-Acetyl-6,7-di *O*-acetyl-5-[(acetyloxy)-methyl]octahydropyrano[3,2-*b*]pyrrole-2-carboxamide *N*′-Methylamide (13). Compound **12** (0.014 g, 0.048 mmol) was dissolved in 1 mL of pyridine. Acetic anhydride (0.046 mL, 0.48 mmol) was added and the reaction mixture was stirred at ambient temperature for 15 h. All solvent and reagent were removed under reduced pressure, providing compound **13** as a clear oil (0.020 g, 0.048 mmol) (quant), $[\alpha]_D^{25} = +53.3°$ (*c* 0.3, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, 298 K, 0.036 M) δ = 6.37 (br q, 0.87H, −N*H*CH$_3$), [5.97 (br q, 0.13H, −N*H*CH$_3$)], [5.25 (dd, 0.13H, H$_7$)], 5.22 (dd app t, 0.87H, $J_{7,8}$ = 9.8 Hz, $J_{6,7}$ = 10.0 Hz, H$_7$), 5.10 (ddd, 0.87H, $J_{3a,9}$ = 11.8 Hz, $J_{3b,9}$ = 7.1 Hz, $J_{8,9}$ = 7.3 Hz, H$_9$), [5.03 (dd, 0.13H, $J_{6,7}$ = 9.5 Hz, $J_{5,6}$ = 9.2 Hz, H$_6$)], 4.99 (dd app t, 0.87H, $J_{5,6}$ = 9.9 Hz, H$_6$), [4.70 (ddd, 0.13H, $J_{3a,9}$ = 11.6 Hz, $J_{3b,9}$ = 6.8 Hz, $J_{8,9}$ = 7.0 Hz, H$_9$)], [4.54 (dd, 0.13H, $J_{7,8}$ = 7.8 Hz, H$_8$)], [4.41 (dd, 0.13H, $J_{2,3a}$ = 9.4 Hz, $J_{2,3b}$ = 1.5 Hz, H$_2$)], 4.27−4.35 (m, 1.87H, H$_2$, H$_{10a}$, H$_{10a}$ minor), 4.19 (dd, 0.87H, H$_8$), 4.08 (dd, 1H, $J_{5,10b}$ = 2.4 Hz, $J_{10a,10b}$ = 12.5 Hz, H$_{10b}$, H$_{10b}$ minor), 3.98 (ddd, 0.87H, $J_{5,10a}$ = 4.3 Hz, H$_5$), [3.89 (ddd, 0.13H, $J_{5,10b}$ = 2.6 Hz, $J_{5,10a}$ = 4.9 Hz, H$_5$)], [2.84 (d, 0.39H, *J* = 4.8 Hz, −NHC*H*$_3$)], 2.81 (d, 2.61H, *J* = 4.8 Hz, −NHC*H*$_3$), [2.73 (ddd, 0.13H, $J_{3a,3b}$ = 12.8 Hz, H$_{3a}$)], 2.48 (ddd, 0.87H, $J_{2,3a}$ = 9.8 Hz, $J_{3a,3b}$ = 12.3 Hz, H$_{3a}$), 2.13 (s, 2.61H, −COC*H*$_3$), 2.09 (s, 3H, −COC*H*$_3$, −COC*H*$_3$ minor), 2.06 (s, 2.61H, −COC*H*$_3$), [2.04 (s, 0.39H, −COC*H*$_3$)], [2.03 (s, 0.39H, −COC*H*$_3$)], [2.02 (s, 0.39H, −COC*H*$_3$)], 2.00−2.05 (m, 0.87H, H$_{3b}$), 2.01 (s, 2.61H, −COC*H*$_3$), [1.86 (s, 0.39H, −COC*H*$_3$)]. ^{13}C NMR (75 MHz, $CDCl_3$, 298 K) (major conformer) δ = 171.8, 170.7, 170.3, 169.9, 169.5, 73.9, 73.8, 69.7, 68.0, 62.2, 58.6, 58.1, 28.2, 26.4, 22.0, 20.9, 20.7, 20.6. HRMS (ES) calcd for $C_{18}H_{27}N_2O_9$ (M + H)$^+$ 415.1711, found 415.1711.

***N*-Acetylglycyl-(2*S*,3a*R*,5*R*,6*R*,7*S*,7a*S*)-6,7-di-*O*-benzyl-5-[(benzyloxy)methyl]octahydropyrano[3,2-*b*]pyrrole-2-carboxamide *N*′-Methylamide (14).** Compound **10** (0.105 g, 0.203 mmol) was dissolved in 6 mL of *N*,*N*-dimethylformamide and cooled to 0 °C. The reaction was stirred under inert atmosphere. Diisopropylethylamine (0.212 mL, 1.218 mmol) and PyBOP (0.317 g, 0.609 mmol) were added and the solution was stirred for 10 min. Fmoc-Gly-OH (0.181 g, 0.609 mmol) was added and the reaction mixture was stirred for a further 5 min before being allowed to warm to ambient temperature, where it was stirred for 18 h. The red solution was diluted with ethyl acetate, washed with 1 M HCl (10 mL) and then brine (10 mL), dried, and evaporated, giving a red oil. The product was purified by flash chromatography with ethyl acetate, giving a white solid product (0.085 g) (53%), along with unreacted starting material (0.025 g) (24%). The coupled product was dissolved in 4 mL of dichloromethane, cooled to 0 °C, and treated with piperidine (1 mL). The reaction mixture was stirred for 1 h before the solvent and reagents were removed under reduced pressure, leaving a white solid. The intermediate was dissolved in 4 mL of pyridine followed by addition of acetic anhydride (0.5 mL). The reaction mixture was stirred for 15 h, and then the solvent and reagents were removed under reduced pressure and the product was purified by flash chromatography with 10:1 ethyl acetate/methanol to provide **14** as a clear oil (0.059 g, 0.096 mmol) (47.1% over three steps), $[\alpha]_D^{25} = +23.5°$(*c* 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, 298 K, 0.033 M) δ = 7.10−7.38 (m, 15H, aromatic), 6.30 (t, 1H, −N*H*$_{(Gly)}$, −N*H*$_{(Gly)}$ minor), 6.22 (q, 1H, −N*H*CH$_3$, −N*H*CH$_3$ minor), 4.95 (d, 0.95H, *J* = 11.3 Hz, −OC*H*$_2$Ph), 4.83 (ddd, 0.95H, $J_{3b,9}$ = 7.6 Hz, $J_{3a,9}$ = 12.0 Hz, $J_{8,9}$ = 7.2 Hz, H$_9$), 4.75 (d, 0.95H, *J* = 10.8 Hz, -OC*H*$_2$Ph), 4.51−4.64 (m, 4.15H,

−OCH_2Ph, −OCH_2Ph minor, H_9 minor), [4.22 (dd, 0.05H, $J_{8,9}$ = 6.5 Hz, $J_{7,8}$ = 8.2 Hz, H_8)], 4.17 (dd app d, 0.95H, $J_{2,3b}$ = 1.0 Hz, $J_{2,3a}$ = 9.7 Hz, H_2), 4.05−4.13 (m, 1.9H, H_8, $H_{\alpha1(Gly)}$, H_2 minor), 4.02 (dd, 0.95H, $J_{H\alpha2(Gly),NH}$ = 4.1 Hz, $J_{H\alpha1(Gly),H\alpha2(Gly)}$ = 17.1 Hz, $H_{\alpha2(Gly)}$), 3.68−3.76 (m, 2H, H_7, H_{10a}, H_7 minor, H_{10a} minor), 3.61−3.67 (m, 2.1H, H_5, H_{10b}, H_5 minor, H_{10b} minor, $H_{\alpha2(Gly)}$ minor, $H_{\alpha1(Gly)}$ minor), 3.53 (dd app t, 0.95H, $J_{6,7}$ = 9.7 Hz, $J_{5,6}$ = 9.4 Hz, H_6), [3.52−3.58 (m, 0.05H, H_6)], [2.80 (d, 0.15H, J = 5.0 Hz, -NHCH_3)], 2.74 (d, 2.85H, J = 5.0 Hz, -NHCH_3), 2.36 (ddd, 1H, $J_{3a,3b}$ = 12.5 Hz, H_{3a}, H_{3a} minor), 1.99 (ddd, 1H, H_{3b}, H_{3b} minor), 1.92 (s, 3H, −COCH_3, −COCH_3 minor). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K) δ = 171.5, 170.5, 169.5, 137.8, 137.6, 137.3, 127.7−128.8 (aromatic carbons), 81.8, 78.2, 75.7, 74.8, 73.8, 73.7, 73.4, 68.7, 59.0, 58.4, 42.9, 28.6, 26.4, 22.7. HRMS (ES) calcd for $C_{35}H_{41}N_3O_7Na$ $(M + Na)^+$ 638.2837, found 638.2841.

***N*-Acetylglycyl-(2*S*,3a*R*,5*R*,6*R*,7*S*,7a*S*)-6,7-dihydroxy-5-(hydroxymethyl)octahydropyrano[3,2-*b*]pyrrole-2-carboxamide *N*′-Methylamide (15).** Compound **14** (0.020 g, 0.032 mmol) was dissolved in 5 mL of methanol. Addition of Pearlman's catalyst (20% palladium hydroxide on carbon) (0.020 g, approximately 0.019 mmol) was followed by addition of 1 M aqueous HCl (0.010 mL, 0.010 mmol). The reaction mixture was stirred vigorously under hydrogen atmosphere (10 psi) for 4.5 h, after which it was flushed with nitrogen and filtered. The product was then concentrated under reduced pressure to provide **15** as a yellow oil (0.012 g, 0.035 mmol) (quant), $[\alpha]_D^{25} = -2.5°$ (*c* 0.6, $CHCl_3$). ^{1}H NMR (500 MHz, D_2O, 298 K, 0.035 M) δ = 4.57−4.66 (m, 0.9H, H_9), 4.47 (d, 0.9H, $J_{H\alpha1(Gly),H\alpha2(Gly)}$ = 17.2 Hz, $H_{\alpha1(Gly)}$), 4.35 (dd app d, 1H, $J_{2,3a}$ = 10.3 Hz, $J_{2,3b}$ = 1.0 Hz, H_2, H_2 minor), [4.14 (dd app t, 0.1H, $J_{8,9}$ = 7.2 Hz, $J_{7,8}$ = 7.7 Hz, H_8)], 4.01 (dd, 0.9H, $J_{8,9}$ = 7.2 Hz, $J_{7,8}$ = 9.0 Hz, H_8), 3.97 (d, 0.9H, $H_{\alpha2(Gly)}$), [3.87 (d, 0.1H, $J_{H\alpha1(Gly),H\alpha2(Gly)}$ = 17.1 Hz, $H_{\alpha1(Gly)}$)], 3.70 (dd, 1H, $J_{5,10a}$ = 2.3 Hz, $J_{10a,10b}$ = 12.2 Hz, H_{10a}, H_{10a} minor), 3.62 (dd, 1H, $J_{5,10b}$ = 5.0 Hz, H_{10b}, H_{10b} minor), [3.45−3.51 (m, 0.1H, H_5)], 3.50−3.58 (m, 1.9H, H_5, H_7, H_7 minor), [3.36 (dd, 0.1H, H_6)], 3.32 (dd app t, 0.9H, $J_{6,7}$ = 9.9 Hz, $J_{5,6}$ = 9.5 Hz, H_6), [2.64 (s, 0.3H, −NHCH_3)], 2.58 (s, 2.7H, −NHCH_3), 2.54 (ddd, 1H, $J_{3a,9}$ = 11.2 Hz, $J_{3a,3b}$ = 12.7 Hz, H_{3a}, H_{3a} minor), 1.90 (s, 2.7H, −COCH_3), 1.79 (ddd, 1H, $J_{3b,9}$ = 7.7 Hz, H_{3b}, H_{3a} minor). ^{13}C NMR (75 MHz, D_2O, 298 K) δ = 175.0, 174.4, 171.3, 74.5, 73.8, 73.5, 68.8, 61.1, 60.4, 58.6, 42.4, 27.9, 26.2, 22.0. HRMS (ES) calcd for $C_{14}H_{23}N_3O_7Na$ $(M + Na)^+$ 368.1428, found 368.1427.

***N*-Acetylglycyl-(2*S*,3a*R*,5*R*,6*R*,7*S*,7a*S*)-6,7-di-*O*-acetyl-5-[(acetyloxy)methyl]octahydropyrano[3,2-*b*]pyrrole-2-carboxamide *N*′-Methylamide (16).** Compound **15** (0.012 g, 0.035 mmol) was dissolved in 1 mL of pyridine. Acetic anhydride (0.034 mL, 0.35 mmol) was added and the reaction mixture was stirred at ambient temperature for 15 h. All solvent and reagent were removed under reduced pressure and the product was purified by flash chromatography with 10:1 ethyl acetate/methanol to provide **16** as a clear oil (0.014 g, 0.030 mmol) (74%), $[\alpha]_D^{25} = +37.3°$(*c* 0.3, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, 298 K, 0.03 M) δ = 6.56 (br q, 0.2H, J = 4.6 Hz, −N*H*CH$_3$), [6.39 (br dd, 0.2H, −N$H_{(Gly)}$)], 6.31 (dd, 0.8H, −N$H_{(Gly)}$), 6.16 (q, 0.8H, J = 4.6 Hz, −N*H*CH$_3$), [5.27 (dd, 0.2H, $J_{7,8}$ = 8.3 Hz, $J_{6,7}$ = 9.1 Hz, H_7)], 5.19 (dd app t, 0.8H, $J_{7,8}$ = 9.4 Hz, $J_{6,7}$ = 9.9 Hz, H_7), 5.03 (ddd, 0.8H, $J_{3a,9}$ = 11.8 Hz, $J_{3b,9}$ = 7.5 Hz, $J_{8,9}$ = 7.2 Hz, H_9), [5.00−5.05 (m, 0.2H, H_6)], 4.99 (dd app t, 0.8H, $J_{5,6}$ = 9.8 Hz, H_6), [4.84 (ddd, 0.2H, $J_{3a,9}$ = 10.9 Hz, $J_{3b,9}$ = 6.7 Hz, $J_{8,9}$ = 7.3 Hz, H_9)], [4.52 (dd app t, 0.2H, J = 7.7 Hz, H_8)], 4.44 (dd, 1H, $J_{H\alpha1(Gly),NH}$ = 6.3 Hz, $J_{H\alpha1(Gly),H\alpha2(Gly)}$ = 17.4 Hz, $H_{\alpha1(Gly)}$, H_2 minor), 4.26−4.35 (m, 1.8H, H_2, H_{10a}, H_{10a} minor), 4.23 (dd, 0.8H, H_8), 4.08 (dd, 1H, $J_{5,10b}$ = 2.3 Hz, $J_{10a,10b}$ = 12.2 Hz, H_{10b}, H_{10b} minor), 3.97 (ddd, 0.8H, $J_{5,10a}$ = 4.2 Hz, H_5), [3.86−3.92 (m, 0.4H, H_5, $H_{\alpha1(Gly)}$)], 3.86 (dd, 0.8H, $J_{H\alpha2(Gly),NH}$ = 3.1 Hz, $H_{\alpha2(Gly)}$), [3.56 (dd, 0.2H, $J_{H\alpha2(Gly),NH}$ = 3.5 Hz, $J_{H\alpha1(Gly),H\alpha2(Gly)}$ = 16.9 Hz, $H_{\alpha2(Gly)}$)], [2.83 (d, 0.6H, J = 4.6 Hz, -NHCH_3)], 2.80 (d, 2.4H, J = 4.6 Hz, -NHCH_3), [2.68 (m, 0.2H, $J_{2,3a}$ = 9.5 Hz, $J_{3a,3b}$ = 12.6 Hz, H_{3a})], 2.55 (ddd, 0.8H, $J_{2,3a}$ = 10.0 Hz, $J_{3a,3b}$ = 12.5 Hz, H_{3a}), 2.13 (s, 2.4H, −COCH_3), 2.09 (s, 3H, −COCH_3), 1.98−2.04 (m, 1H, H_{3b}, H_{3b} minor), [2.02 (s, 0.6H, −COCH_3)], 2.01 (s, 3H, −COCH_3, −COCH_3 minor), 1.99 (s, 3H, −COCH_3, −COCH_3 minor). ^{13}C NMR (75 MHz, $CDCl_3$, 298 K) δ = (major conformer) 171.4, 170.7, 170.4, 170.4, 169.4, 168.8, 73.6, 73.4, 69.8, 68.1, 62.1, 58.6, 57.1, 41.9, 28.1, 26.5, 22.9, 20.9, 20.7, 20.5. HRMS (ES) calcd for $C_{20}H_{29}N_3O_{10}Na$ $(M + Na)^+$ 494.1745, found 494.1743.

Acknowledgment. We thank the Natural Sciences and Engineering Research Council of Canada (NSERC) and the University of Manitoba (UMGF) for financial support.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of new compounds, tables of coupling constants for **11**−**16** (Tables 4 and 5), and tables of observed NOE interproton effects for **11**−**16** (Tables 6 and 7). This material is available free of charge via the Internet at http://pubs.acs.org.

JO0700833