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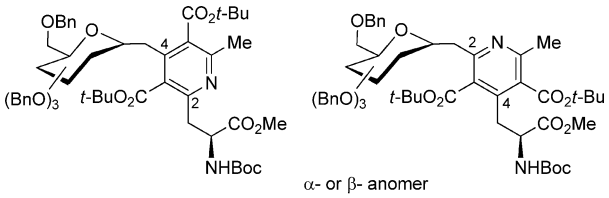
Hantzsch-Type Three-Component Approach to a New Family of Carbon-Linked Glycosyl Amino Acids. Synthesis of *C*-Glycosylmethyl Pyridylalanines†,‡

Alessandro Dondoni,* Alessandro Massi,* and Mohammad Aldhoun

Dipartimento di Chimica, Laboratorio di Chimica Organica e Centro RMN, Università di Ferrara, Via L. Borsari 46, I-44100 Ferrara, Italy

adn@unife.it; msslsn@unife.it

Received June 8, 2007



α- or β- anomer

gluco-galacto series

C-Glycosylmethyl pyridylalanines reported in this paper constitute a novel family of glycosyl amino acids that contain a pyridine ring linking the carbohydrate and amino acid residues. These amino acids may serve to prepare nonnatural glycopeptides displaying firmly bound carbohydrate fragments through a rigid and highly stable tether. A viable route to these new hybrid molecules has been opened via thermally induced Hantzsch-type cyclocondensation using an aldehyde−ketoester−enamino ester system. To one of these reagents was attached a *C*-glycosyl residue, while to another was bound an amino acid fragment. In a one-pot optimized methodology, the dihydropyridine was not isolated while its purification was carried out by removal of unreacted material and side products using polymer-supported scavengers. Then the dihydropyridine (mixture of diastereoisomers) was oxidized by a polymer-bound oxidant to give the target pyridine bearing the two bioactive residues. In this way a range of eight compounds (58−68% yield) was prepared in which the elements of diversity were (i) the gluco and galacto configurations of the pyranose ring, (ii) the α- and *β*-configurations at the anomeric center, and (iii) the positions of the carbohydrate and amino acid sectors in the pyridine ring. The orthogonal functional group protection in these amino acids allowed their easy incorporation into oligopeptides via sequential amino and carboxylic group coupling.

Introduction

Because of their potential use as probes in glycobiology and leads in carbohydrate-based drug discovery, synthetic glycopeptides in which the native *O*- or *N*-glycosidic linkages have been replaced by *C*- or *S*-linkages have garnered in recent years an increasing attention by researchers in industry and academia.[1] This modification is considered to provide substantial resistance to chemical and enzymatic deglycosylation while retaining the original biological properties of the peptide. Hence, several methods for the synthesis of *C*-glycosyl[2,3] and *S*-glycosyl[1i] amino acids have been reported in view of their use in co-translational modification of natural glycopeptides. Moreover, in order to introduce in peptides a more substantial modification which may

† This work is part of the lecture presented by A.D. in the *Symposium on Asymmetric Synthesis of Amino Acids* at the 233rd ACS National Meeting, Chicago, March 25−29, 2007.

‡ The paper is dedicated to the memory of the late Professor Yoshihiko Ito, Kyoto University, a good friend to A.D. and a respected colleague.

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10.1021/jo071221r CCC: $37.00
Published on Web 09/06/2007

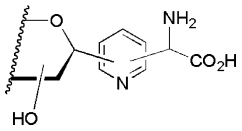


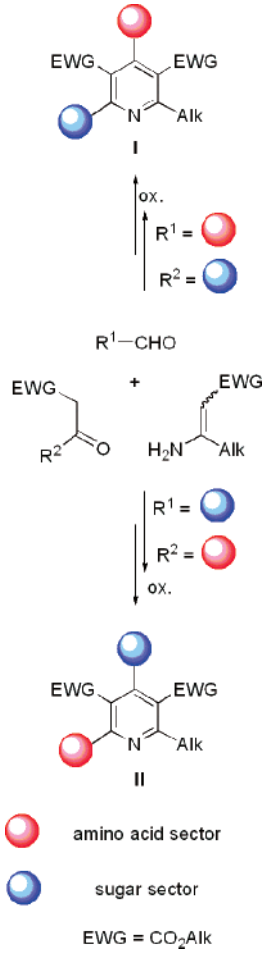
FIGURE 1. Basic structure of designed pyridine-tethered *C*-glycosyl amino acids.

induce changes in physicochemical and biological properties, *C*-glycosyl amino acids with rigid linkers such the acetylene group[4] and phenyl ring[5] have been prepared. In this context, we have recently reported[6] on the first synthesis of *C*-glycosyl amino acids with isoxazole and triazole bridges via alkyne−nitrile oxide and azide−alkyne cycloaddition (Huisgen-type reactions),[7] respectively. The latter reaction was carried out both thermally and under Cu(I) catalysis according to the recent discovery of Sharpless and Meldal and their co-workers.[8] A similar work was reported almost at the same time by another group.[9] We report here the synthesis of a novel family of heterocycle-linked *C*-glycosyl amino acids in which the flat and rigid pyridine ring holds the two chiral bioactive entities (Figure 1). The presence of nitrogen heterocycles bearing carbohydrate residues in *C*-glycopeptides may be quite beneficial since these heteroaromatic bridges can participate in hydrogen-bonding and dipole interactions, which can favor the binding to biomolecular targets and improve solubility as well as membrane transport properties.[10] Only one natural *C*-glycosyl amino acid, identified as an α-*C*-mannopyranosyltryptophan,[11] has been found so far in biologically important glycoproteins. Therefore, quite interestingly, this compound features the rigid heteroaromatic indole ring holding the sugar and amino acid moieties. Very recently, the *β*-isomer and analogues of this natural product have been synthesized by the Nishikawa and Isobe group.[12]

Results and Discussion

Multicomponent one-pot reactions[13] have emerged in recent years as a useful tool to maximize synthetic efficiency,[14] an aspect of increasing relevance in modern chemistry toward the ideal synthesis.[15] Hence, we decided to approach the construction of carbohydrate−heterocycle−amino acid hybrids depicted in Figure 1 by the three-component version of the pyridine

SCHEME 1. Synthesis Plan via Hantzsch Reaction for Pyridine-Tethered *C*-Glycosyl Amino Acid Regioisomers



Hantzsch synthesis[16] comprising the aldehyde−ketoester−enamino ester system (Scheme 1). To one of these reagents is bound the *C*-glycosyl residue and to another the amino acid. With the carbohydrate linked to the ketoester and the amino acid as a part of the aldehyde, the dihydropyridine formed from the cyclocondensation and the pyridine **I** obtained afterward will bear the amino acid at C-4 and the carbohydrate at C-2 of the ring. The regioisomer **II** will be instead obtained using a *C*-glycosyl aldehyde and a ketoester with incorporated the glycinyl group. We planned to use in both routes the same enamino ester with a methyl group adjacent to the amino group in order to keep as low as possible the steric encumbrance in this reagent. Evidently, this methodology will lead to a densely substituted pyridine ring. However, we considered the presence of the two ester groups as an advantage because these func-

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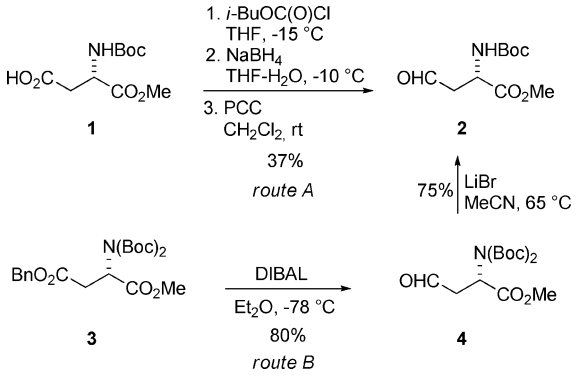
tionalities can be varied in library preparations or serve as reactive points for the introduction of the glycosyl amino acid in molecular frameworks other than peptides. We were spurred to adopt the Hantzsch multicomponent reaction in this project as a logical evolution of our recent research on the synthesis of dihydropyridine *C*-glycoconjugates and piridylalanines.[14b,17] The information collected in the earlier work constituted a precious guide in the choice of suitable reagents and conditions to perform the construction of the pyridine ring bearing both sugar and amino acid residues.

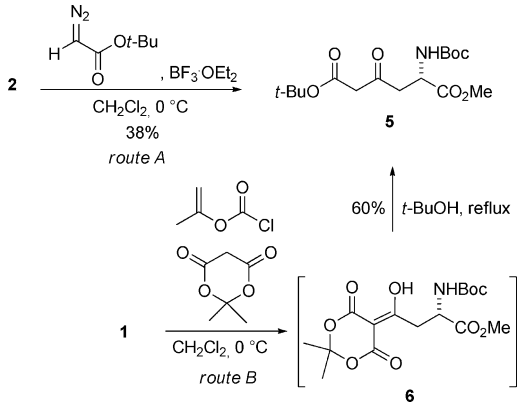
A. Design and Synthesis of Reagents for the Three-Component Hantzsch Reaction (3CHR). The first issue to be addressed in this project was to design reagents with functional protective groups which are stable under the reaction conditions and orthogonal in the product. The latter condition was crucial for the elaboration of the product via selective reactions at each functional group at will. In particular, the protection of the NH_2 of the glycinyl group should preserve the configurational integrity of the α-stereocenter and permit the introduction of the product into peptides via sequential carboxylic and amino group coupling reactions. Toward this goal, the *N*-Boc and methyl ester protection of the glycinyl moiety was previously demonstrated to be a quite convenient combination.[17c,e] Moreover, the *tert*-butyl group in the ketoester and enamino ester appeared to be a convenient choice as well because it differentiated the carboxylic groups of the ring from that of the amino acid and avoided the lactam formation via intramolecular reaction with the free amino acid group.[17c] Finally, the *O*-benzyl protective group in the carbohydrate moiety was demonstrated in several instances to be orthogonal with the *N*-Boc methylglycinate[1g,2,6] and proved to be readily removed in the presence of the pyridine ring by Pd-catalyzed hydrogenolysis.[17e]

Following the above reasoning, we first examined the synthesis of aldehyde and ketoester starting reagents bearing the *N*-Boc methyl glycinate group. Because of the easy elimination of this group from the dihydropyridine ring in the oxidation step to pyridine,[17c] we decided to prepare reagents holding the glycinate residue through a methylene spacer. Hence, the *N*-Boc-*O*-methyl aspartate semialdehyde **2** was prepared in two steps by a reduction−oxidation sequence from the readily available aspartate monoester **1**[18] (Scheme 2). However, this method afforded the aldehyde in low yield because of the lactonization of the alcohol intermediate.[19] Hence, the preparation of **2** was more conveniently carried out in gram quantities, starting from the aspartate diester **3**[20] according to the procedure of Martin and co-workers.[21] However, it is worth noting that the optical rotation value of **2** ($[\alpha]_D = 34.5$) obtained by both routes was more than double that previously registered by these authors (lit.[21b] $[\alpha]_D = 16.4$).[22]

SCHEME 2. Synthesis of *N*-Boc-*O*-methyl Aspartate Semialdehyde 2



SCHEME 3. Synthesis β-Keto Ester 5



With the aldehyde **2** available in gram scale, the synthesis of the β-ketoester **5** was first carried by reaction of **2** with *tert*-butyl diazoacetate and $BF_3{\cdot}Et_2O$ (Scheme 3) according to the procedure already employed in our laboratory.[17c,e] In contrast to previous results, the yield of isolated **5** was only 38%. Hence, an alternative synthesis of **5** was performed starting from the *N*-Boc aspartic acid monoester **1**[18] by a procedure similar to that described by Frank and co-workers.[23] This method involves the transformation of **1** into the Meldrum's acid derivative **6** and then treatment of this crude material with refluxing *t*-BuOH. The overall yield of **5** was 60%, and its optical rotation value ($[\alpha]_D = 25.4$) was identical to that of the product obtained by the other route from **2**.

For reasons given below in section B, aldehydes and ketoesters with a directly linked *C*-glycosyl group appeared to be unsuitable reagents in the planned 3CHR. On the other hand, *C*-glycosylmethyl derivatives turned out to be the reagents of choice. Fortunately enough, the *C*-glucosyl (Glc) acetaldehydes α-**7a**[24] and β-**7a**[24,25] as well as the *C*-galactosyl (Gal) derivatives

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(22) In a control experiment, the aldehyde **2** was reduced to alcohol ($NaBH_4$, MeOH, 0 °C) which spontaneously cyclized (CH_2Cl_2, rt) to give in quantitative overall yield the known (*S*)-*tert*-butyl 2-oxotetrahydrofuran-3-ylcarbamate whose optical rotation value $[\alpha]_D = -29.2$ was identical to that reported in the literature ($[\alpha]_D = -29.5$).[19]

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(25) Postema, M. H. D.; Piper, J. L.; Liu, L.; Shen, J.; Faust, M.; Andreana, P. *J. Org. Chem.* **2003**, *68*, 4748−4754.

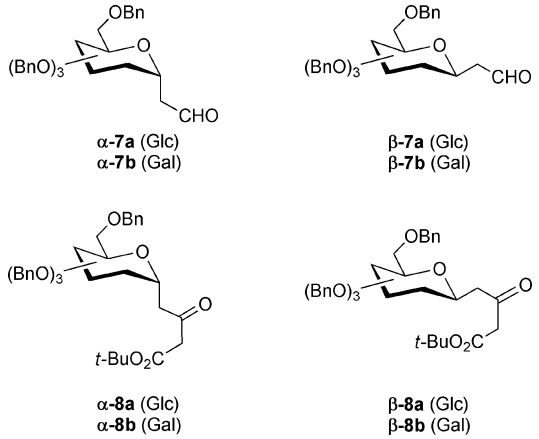
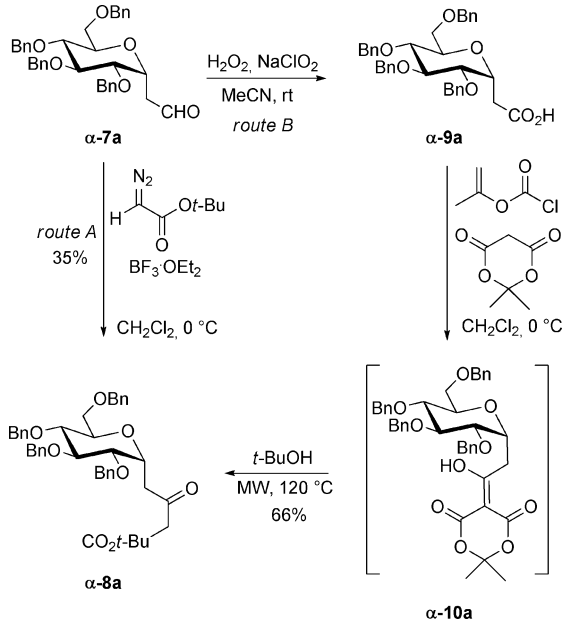


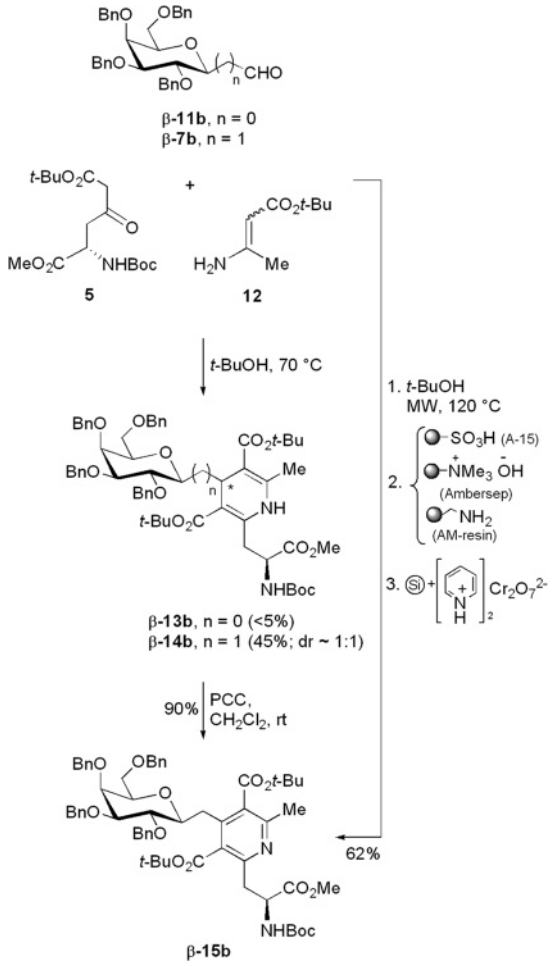
FIGURE 2. *C*-Glucosyl (Glc) and *C*-galactosyl (Gal) acetaldehydes **7** and ketoesters **8**.

SCHEME 4. Synthesis of Sugar *β*-Keto Esters α-8a



α-**7b**[26] and *β*-**7b**[26] (Figure 2) were readily available by double-bond oxidative cleavage of the corresponding *C*-allyl glycosides.[24b,26,27] Then, the four sugar aldehydes **7** were used for the preparation of the ketoesters **8** (Figure 2). As an example, the synthesis of one of these compounds is illustrated in Scheme 4. In one approach, the aldehyde α-**7a** was transformed into the ketoester α-**8a** by reaction with *tert*-butyl diazoacetate as described for other sugar ketoesters.[28] However, this method afforded α-**8a** in low yield (35%), and therefore, another procedure was followed. This involved the nearly quantitative oxidation of α-**7a** to the carboxylic acid α-**9a** and treatment of the latter with Meldrum's acid in the presence of isopropenyl chloroformate. Then the crude product α-**10a** was cleaved by treatment with *t*-BuOH and MW irradiation at 120 °C. The crude ester α-**8a** obtained in this way was purified and isolated in fairly good overall yield (66%). All of the sugar ketoesters shown in Figure 2 were prepared in good yields by this two-step route.

SCHEME 5. Optimization of Reaction Conditions for the Synthesis of 4-(*C*-Galactosylmethyl)-2-(alaninyl)pyridine *β*-15b



B. Synthesis of 4-(*C*-Glycosylmethyl)-2-alaninylpyridines 15. In recent disclosures from this laboratory, we reported that *C*-glycosyl formaldehydes readily available via thiazole-mediated chemistry[29] take part in 3CHR to give the corresponding *C*-glycosyl dihydropyridines.[17a,b] Hence we considered a model reaction constituted of the *C*-galactosyl formaldehyde *β*-**11b**,[29] the *β*-ketoester **5**, and *tert*-butyl aminocrotonate **12** (Scheme 5). Under the usual standard conditions used in our previous work of Hantzsch reactions (*t*-BuOH, 70 °C, 24 h) and even under MW irradiation at 120 °C for 4 h, only traces of the desired dihydropyridines *β*-**13b** were detected by MALDI-TOF analysis of the crude reaction mixture. Instead, various side products were isolated including the glycal arising from debenzylation of the aldehyde *β*-**11b** and carboxylic acids derived from the hydrolysis of the ester groups of **5** and **12**. Therefore, we considered the use of the *C*-galactosyl acetaldehyde *β*-**7b** with the hope that the presence of a methylene spacer between the formyl group and the carbohydrate residue would reduce the steric congestion of the system and the cyclocondensation could take place. The change turned out to be quite

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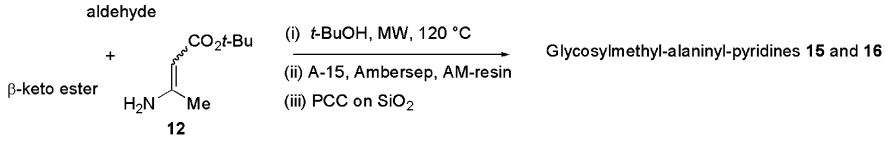
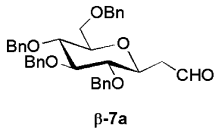
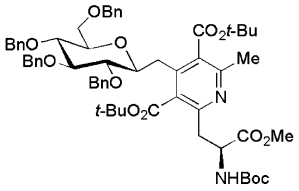
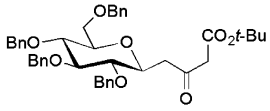
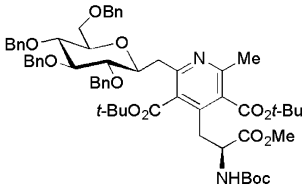
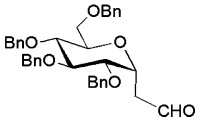
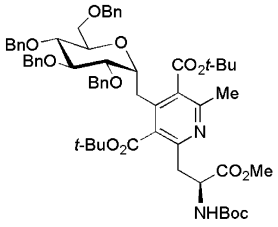
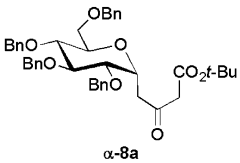
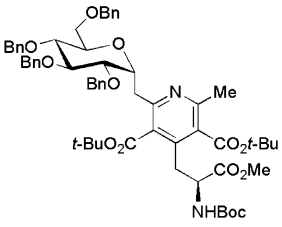
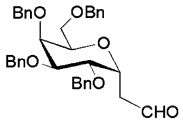
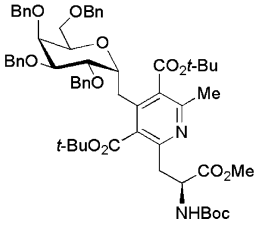
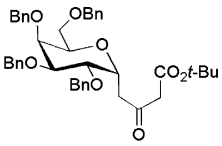
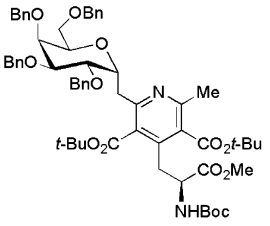
TABLE 1. Glycosylmethylalaninylpyridines Prepared[a]

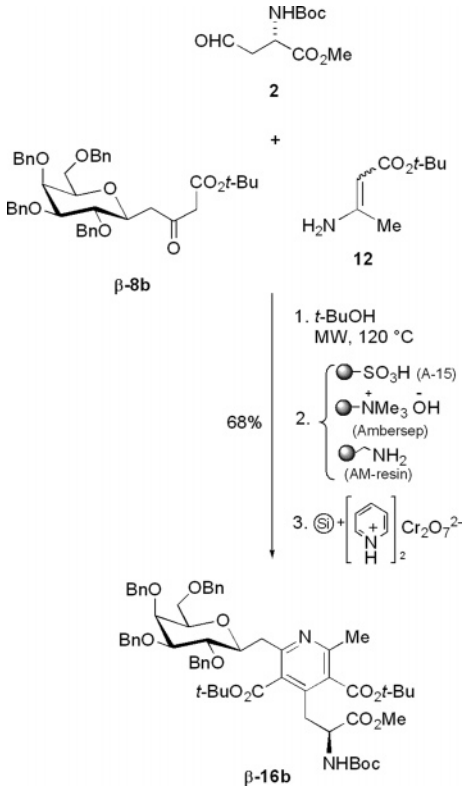


aldehyde and ketoester	product[b,c]	ketoester and aldehyde	product[c,d]
 β-**7a** and **5**	 β-**15a** (61%)	 β-**8a** and **2**	 β-**16a** (62%)
β-**7b** and **5**	β-**15b** (62%) (see Scheme 5)	β-**8b** and **2**	β-**16b** (68%) (see Scheme 6)
 α-**7a** and **5**	 α-**15a** (55%)	 α-**8a** and **2**	 α-**16a** (58%)
 α-**7b** and **5**	 α-**15b** (58%)	 α-**8b** and **2**	 α-**16b** (62%)

[a] All Hantzsch cyclocondensations were run with 0.50 mmol of each component (see the Experimental Section). [b] Time of microwave irradiation was 1.5 h. [c] Isolated yield by chromatography. [d] Time of microwave irradiation was 2.5 h.

beneficial because this new substrate combination upon heating at 70 °C in *t*-BuOH for 24 h afforded the Hantzsch dihydropyridine *β*-**14b** in fair yield (45%) as a mixture of diastereoisomers in 1:1 ratio. The lack of asymmetric induction by the chiral sugar fragment is very likely due to its distance from the newly formed stereocenter and the harsh reaction conditions employed. The mixture of diastereoisomers was oxidized with PCC to give the pyridine *β*-**15b** as a single product (40% overall yield), thus confirming, as already observed in our earlier work,[17e] the conservation of the configurational integrity of the amino acid stereocenter during the Hantzsch cyclocondensation. In order to achieving higher efficiency and establish the conditions for an automatable process, the above reaction sequence was performed in one-pot using polymer-bound reagents. Hence, the cyclocondensation of the aldehyde *β*-**7b**, ketoester **5**, and aminocrotonate **12** in a 1:1:1 ratio was carried out by MW irradiation at 120 °C, which reduced the reaction time from 24 to 1.5 h. Then, the reaction mixture was treated with a mixed-resin bed constituted of three polymer-bound reagents, each one acting as specific scavenger of unreacted material and side products. Specifically, the supported sulfonic acid A-15 removed the residual enamine **12**, the strong hydroxylic base Ambersep sequestered the ketoester **5**, and the aminomethylated polystyrene (AM-resin) subtracted the aldehyde *β*-**7b**. This supported amine removed also the Knoevenagel adduct formed as initial condensation product between the aldehyde and the ketoester.[17e] After filtration of the resins, the diastereomeric dihydropyridines were oxidized to pyridine with PCC supported on silica gel. Also this operation was quite convenient because the noxious chromium salts anchored to the resin were easily removed by filtration. A final column chromatography of the crude product afforded pure *C*-galactosylmethyl-pyridylalanine methyl ester *β*-**15b** in 62% yield. This compound was identical in all respect (NMR, $[\alpha]_D$) to that obtained via the two-step procedure illustrated above and involving the isolation of the dihydropyridine *β*-**14b**. Accord-

SCHEME 6. Synthesis of 2-(*C*-Galactosylmethyl)-4-(alaninyl)pyridine *β*-16b



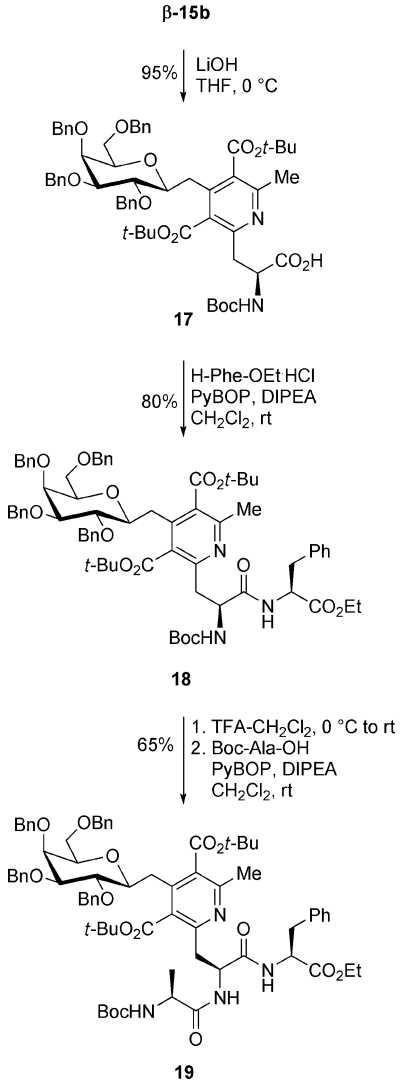
ingly, treatment of crude *β*-**14b** with the set of resins employed in the direct route afforded again authentic *β*-**15b**. This observation opens the route to an automatable and operatively simple synthesis with total preservation of stereocenter integrity and functional group protection.

Three more sugar amino acids with the same substitution pattern in the pyridine ring as in *β*-**15b**, i.e. the sugar sector linked to C-4 and the amino acid fragment linked to C-2, were prepared using the *C*-glycosyl acetaldehydes *β*-**7a**, α-**7a**, and α-**7b** with carbohydrate configuration corresponding to *β*-Glc, α-Glc, and α-Gal respectively (Table 1). The reactions were carried out by MW irradiation and processed by the use of polymer-bound reagents as described above. The three further amino esters *β*-**15a**, α-**15a**, α-**15b** thus prepared were isolated in comparable yields (55−61%) and stereochemically pure form as judged by NMR analysis.

C. Synthesis of 2-(*C*-Glycosylmethyl)-4-alaninylpyridines 16. The approach to this class of compounds started by examining a three-component model reaction (Scheme 6) constituted of the methyl *C*-galactosylacetoacetate *β*-**8b**, the *N*-Boc-*O*-methyl aspartate semialdehyde **2**, and the already exploited valuable aminocrotonate **12**.[30] In this case, the two-step procedure involving the isolation of the dihydropyridine was not explored. Instead, the same reaction conditions and workup operations of the one-pot process illustrated in Scheme 5 were used. In this way, the sugar−pyridine−amino acid *β*-**16b** was isolated in more than 90% purity. This product was mainly

(30) A parallel experiment involving the microwave-assisted 3CHR of **2**, **12**, and *tert*-butyl 3-oxo-3-(2′,3′,4′,6′-tetra-*O*-benzyl-*β*-D-galactopyranosyl)propanoate, i.e. the lower homologous of *β*-**8b**, resulted in no isolation of the corresponding dihydropyridines due to the steric congestion of the system, as previously observed in the preparation of regioisomers *β*-**13b** (Scheme 5).

SCHEME 7. Synthesis of Tripetide 19



contaminated by the residual sugar ketoester *β*-**8b** because the basic resin Ambersep turned out to be a scarcely efficient scavenger toward this bulky compound. However, filtration of the crude mixture through a short column of silica (cyclohexane−AcOEt) afforded the analytically pure amino ester *β*-**16b** in 68% isolated yield. This product resulted to be stereochemically pure as judged by NMR analysis. By changing the configuration of the carbohydrate moiety in the ketoester, the stereoisomers *β*-**16a**, α-**16a**, and α-**16b** (Table 1) were prepared in comparable fair yields (52−68%).

D. Synthesis of Pyridine-Tethered Glycopeptides. In order to demonstrate the potential of the *C*-glycosylmethyl amino esters **15** and **16** as orthogonally protected building blocks for the co-translational modification of glycopeptides, the product *β*-**15b** was selected as a prototype in this crucial validating test (Scheme 7). Toward this goal, we first carried out the selective hydrolysis of the ester functionality of the glycinyl group. The use of very mild conditions (LiOH in THF at 0 °C) afforded the *N*-Boc alanine **17** in excellent yield (95%). Then, the condensation of this product with H-Phe-OEt under activation of the condensation agent (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) and the presence of the Hünig base diisopropylethylamine (DIPEA), afforded the dipeptide **18** in fair yield. The *N*-Boc group in this compound

was removed under slightly acid conditions (diluted TFA), thus liberating the NH_2 group that was used in the condensation with Boc-Ala-OH under the same coupling conditions illustrated above. The tripeptide **19** featuring a pendant galactosylmethyl residue linked through a rigid pyridine ring to the peptide backbone was isolated in 65% yield.

Conclusions

After about one and half century after its discovery,[31] the Hantzsch pyridine synthesis continues to manifest its prolific nature and potential as a tool in synthetic organic methodology. In the present work, we have further validated these exceptional prerogatives by assembly in one-pot procedure, without added catalysts and under the simple cooperation of a clean physical energy, such as MW irradiation, three densely functionalized substrates, such as a carbohydrate, a pyridine, and an amino acid. This simple yet efficient chemistry was performed with the assistance of an orchestrated sequence of polymer supported reagents. In this way, only *one* chromatographic purification of the final product was required. The configurational integrity of the chiral reagents was preserved throughout the whole synthetic procedure. In this way a collection of eight news optically pure *C*-glycosylmethyl pyridine amino acids (*C*-glycosyl pyridylalanines) was prepared. It is noteworthy that all products have the sugar residue linked to the pyridine ring through an all carbon tether, thus providing high stability toward glycosidases. Moreover, the orthogonal protection of the various functional groups allows the use of these amino acids in glycopeptide co-translational modification. The way has been now paved for the preparation of a larger library of these amino acids by the change of the carbohydrate and amino acid residues in the reagents employed in the Hantzsch cyclocondensation. This work has been carried out with a view to biological and pharmaceutical applications. In this context, focus on the heterocycle-based ligation strategy of carbohydrate fragments to a peptide backbone via Cu(I)-catalyzed azide−alkyne cycloaddition has been recently brought by Danishefsky and co-workers in their continuous efforts to develop carbohydrate-based anticancer vaccines.[32]

Experimental Section

Aspartates **1**[18] and **3**[20] and aldehydes **2**,[21b] **4**,[21a] α-**7a**,[24] β-**7a**,[24,25] α-**8a**,[26] and β-**8a**[26] are known compounds. Spectroscopic data of intermediate acids β-**9a** and β-**9b** were identical to those reported.[33,25] PCC immobilized on silica gel was prepared according to the procedure described by Eynde and co-workers.[34]

(2*S*)-Methyl 2-(*tert*-Butoxycarbonylamino)-4-oxobutanoate (2). Route A. To a cooled (−15 °C), stirred solution of Boc-L-Asp-OMe **1**[18] (1.00 g, 4.05 mmol) in anhydrous THF (2 mL) were added 4-methylmorpholine (0.45 mL, 4.05 mmol) and isobutyl chloroformate (0.53 mL, 4.05 mmol). The suspension was stirred at −15 °C for an additional 10 min, and then salts were filtered off and washed thoroughly with cold THF (2 × 15 mL). The combined filtrates were cooled to −10 °C, and then a solution of $NaBH_4$ (230 mg, 6.08 mmol) in H_2O (1.5 mL) was added in one portion. The solution was stirred for an additional 10 min and then diluted with AcOEt (75 mL) and H_2O (50 mL). The separated organic layer was washed with 10% citric acid (10 mL), saturated aqueous $NaHCO_3$ (10 mL), and brine (10 mL) and then dried ($NaSO_4$) and concentrated. A mixture of the resulting crude alcohol, activated 4-Å powdered molecular sieves (1.00 g), and anhydrous CH_2Cl_2 (40 mL) was stirred at room temperature for 15 min, and then pyridinium chlorocromate (1.64 g, 7.40 mmol) was added in one portion. The suspension was stirred for 45 min, and then cyclohexane (40 mL) and Et_2O (80 mL) were added. The mixture was stirred for an additional 30 min, filtered through a pad of silica gel, and concentrated. The residue was eluted from a column of silica gel with 1.5:1 cyclohexane−Et_2O to afford **2**[21b] (347 mg, 37%) as a yellow oil: $[\alpha]_D = 34.0$ (*c* 2.1, $CHCl_3$) [lit.[21b] $[\alpha]_D = 16.4$ (*c* 5, $CHCl_3$)].

Route B. To a cooled (−78 °C), stirred solution benzyl ester **3**[20] (1.23 g, 2.81 mmol) in anhydrous Et_2O (30 mL) was added dropwise DIBAL (3.2 mL, 3.20 mmol of a 1.0 M solution in hexane). The mixture was stirred at −78 °C for 15 min and then quenched with H_2O (0.5 mL). The suspension was warmed to room temperature, stirred for an additional 30 min, dried (Na_2SO_4), filtered through a pad of Celite, and then concentrated. The residue was eluted from a column of silica gel with 1.5:1 cyclohexane−AcOEt to afford **4**[21a] (745 mg, 80%) as an oil: $[\alpha]_D = -55.4$ (*c* 2.3, $CHCl_3$) [lit.[21a] $[\alpha]_D = -54.9$ (*c* 2, $CHCl_3$)]. A mixture of the aldehyde **4**[21a] (700 mg, 2.11 mmol), lithium bromide (550 mg, 6.34 mmol), and CH_3CN (20 mL) was warmed to 65 °C, stirred at that temperature for 5 h, and then cooled to room temperature and concentrated. The residue was suspended in AcOEt (50 mL) and washed with H_2O (3 × 5 mL). The organic phase was dried (Na_2SO_4), concentrated, and eluted from a column of silica gel with 1.5:1 cyclohexane−Et_2O to afford **2**[21b] (366 mg, 75%) as a yellow oil: $[\alpha]_D = 34.5$ (*c* 3.0, $CHCl_3$).

(2*S*)-6-*tert*-Butyl 1-methyl 2-(*tert*-butoxycarbonylamino)-4-oxohexanedioate (5). Route A. A mixture of aldehyde **2** (462 mg, 2.00 mmol), *tert*-butyl diazoacetate (0.33 mL, 2.40 mmol), activated 4-Å powdered molecular sieves (300 mg), and anhydrous CH_2Cl_2 (20 mL) was stirred at room temperature for 15 min and then cooled to 0 °C. To the mixture a solution of $BF_3{\cdot}Et_2O$ (127 μL, 1.00 mmol) in anhydrous CH_2Cl_2 (1 mL) was added drop by drop, controlling the N_2 evolution at a low steady rate. The mixture was stirred at 0 °C for an additional 30 min, diluted with 10% $NaHCO_3$ (10 mL), warmed to room temperature, filtered through a pad of Celite, and extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was dried (Na_2SO_4) and concentrated. The residue was eluted from a column of silica gel with 2:1 cyclohexane−Et_2O to give **5** (262 mg, 38%) as a ∼10:1 mixture of ketone and enol isomers: $[\alpha]_D = 25.3$ (*c* 0.9, $CHCl_3$); ^1H NMR (DMSO-d_6, ketone isomer) $\delta = 7.22$ (d, 1 H, $J_{2,NH} = 8.0$ Hz, NH), 4.35 (ddd, 1 H, $J_{2,3a} = 5.5$ Hz, $J_{2,3b} = 8.5$ Hz, H-2), 3.62 (s, 3 H, OCH_3), 3.53 and 3.46 (2d, 2 H, $J_{5a,5b} = 14.0$ Hz, 2 H-5), 3.00 (dd, 1 H, $J_{3a,3b} = 16.5$ Hz, H-3a), 2.85 (dd, 1 H, H-3b), 1.38 (s, 9 H, *t*-Bu); MALDI-TOF MS 368.4 ($M^+ +$ Na), 384.2 ($M^+ +$ K). Anal. Calcd for $C_{16}H_{27}NO_7$ (345.39): C, 55.64; H, 7.88; N, 4.06. Found: C, 55.68; H, 7.80; N, 4.10.

Route B. A mixture of Boc-L-Asp-OMe **1**[18] (1.00 g, 4.05 mmol), Meldrum's acid (648 mg, 4.50 mmol), DMAP (1.10 g, 8.98 mmol), and anhydrous CH_2Cl_2 (10 mL) was cooled to −5 °C, and then a solution of isopropenyl chloroformate (0.51 mL, 4.66 mmol) in anhydrous CH_2Cl_2 (5 mL) was added dropwise under vigorous magnetic stirring. The mixture was stirred at −5 °C until complete disappearance of the starting material was detected (TLC analysis, ∼1 h). Then a 10% aqueous solution of $KHSO_4$ (8 mL) was added to the solution, the cooling bath was removed, and an additional portion of 10% aqueous solution of $KHSO_4$ (8 mL) was added. The mixture was diluted with CH_2Cl_2 (80 mL), and then the two phases were separated. The organic phase was washed with brine (2 × 5 mL), dried (Na_2SO_4), and concentrated to give the adduct **6**, which was used for the following reaction without any purification. A mixture of the above crude adduct **6**, anhydrous toluene

(31) Hantzsch, A. *Justus Liebigs Ann. Chem.* **1882**, *215*, 1−82.
(32) Wan, Q.; Chen, J.; Chen, G.; Danishefsky, S. J. *J. Org. Chem.* **2006**, *71*, 8244−8249.
(33) (a) Sutherlin, D. P.; Stark, T. M.; Hughes, R.; Armstrong, R. W. *J. Org. Chem.* **1996**, *61*, 8350−8354. (b) Orsini, F.; Di Teodoro, E. *Tetrahedron: Asymmetry* **2003**, *14*, 2521−2528.
(34) Eynde, J. V.; Mayence, A.; Maquestiau, A. *Tetrahedron Lett.* **1992**, *48*, 463−468.

(24 mL), and anhydrous *t*-BuOH (12 mL) was heated under reflux for 5 h and then concentrated. The residue was eluted from a column of silica gel with 2:1 cyclohexane−Et_2O to give **5** (838 mg, 60%) as a ∼10:1 mixture of ketone and enol isomers: $[\alpha]_D = 25.4$ (*c* 0.9, $CHCl_3$).

General Procedure for the Synthesis of Sugar Acids 9a,b. To a stirred solution of sugar aldehyde (1.00 mmol) in CH_3CN (10 mL) were added 35% aqueous H_2O_2 (0.2 mL), 1.2 M aqueous KH_2PO_4 (1.0 mL), and 0.17 M aqueous $NaClO_2$ (7.0 mL). The mixture was stirred at room temperature for 2 h and then acidified with 1 N aqueous HCl to pH = 2, diluted with AcOEt (150 mL), and washed with H_2O (2 × 10 mL). The aqueous phase was extracted with AcOEt (2 × 50 mL), and then the combined organic phase was dried (Na_2SO_4) and concentrated to give the corresponding crude acid **9** in almost quantitative yield and at least 95% pure as judged by ^{1}H NMR analysis.

Crude acid α-**9a**: ^{1}H NMR δ = 7.40−7.00 (m, 20 H, Ph), 4.92 and 4.80 (2 d, 2 H, J = 11.0 Hz, PhCH_2), 4.82 and 4.50 (2 d, 2 H, J = 11.2 Hz, PhCH_2), 4.72 and 4.66 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 4.67 (ddd, 1 H, $J_{2a,3}$ = 5.5 Hz, $J_{2b,3}$ = 9.0 Hz, $J_{3,4}$ = 3.0 Hz, H-3), 4.64 and 4.48 (2 d, 2 H, J = 12.0 Hz, PhCH_2), 3.84−3.62 (m, 6 H, H-4, H-5, H-6, H-7, 2 H-8), 2.82 (dd, 1 H, $J_{2a,2b}$ = 15.0 Hz, H-2a), 2.74 (dd, 1 H, H-2b). Anal. Calcd for $C_{36}H_{38}O_7$ (582.68): C, 74.21; H, 6.57. Found: C, 74.35; H, 6.44.

Crude acid α-**9b**: ^{1}H NMR δ = 7.40−7.10 (m, 20 H, Ph), 4.72 and 4.60 (2 d, 2 H, J = 12.0 Hz, PhCH_2), 4.69 and 4.57 (2 d, 2 H, J = 11.2 Hz, PhCH_2), 4.60 and 4.50 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 4.57 and 4.49 (2 d, 2 H, J = 11.8 Hz, PhCH_2), 4.46 (ddd, 1 H, $J_{2a,3}$ = 9.0 Hz, $J_{2b,3}$= 4.5 Hz, $J_{3,4}$ = 3.0 Hz, H-3), 4.16 (ddd, 1 H, $J_{6,7'}$ = 3.0 Hz, $J_{7,8a}$ = 8.0 Hz, $J_{7,8b}$ = 4.0 Hz, H-7), 4.03 (dd, 1 H, $J_{5,6}$ = 4.5 Hz, H-6), 3.95 (dd, 1 H, $J_{8a,8b}$ = 11.0 Hz, H-8a), 3.78−3.70 (m, 2 H, H-4, H-5), 3.68 (dd, 1 H, H-8b), 2.73 (dd, 1 H, $J_{2a,2b}$ = 16.0 Hz, H-2a), 2.57 (dd, 1 H, H-2b). Anal. Calcd for $C_{36}H_{38}O_7$ (582.68): C, 74.21; H, 6.57. Found: C, 74.31; H, 6.48.

General Procedure for the Synthesis of Sugar β-Ketoesters 8a,b. Route B. A mixture of crude sugar acid **9** (∼1.00 mmol), Meldrum's acid (159 mg, 1.10 mmol), DMAP (269 mg, 2.20 mmol), and anhydrous CH_2Cl_2 (8 mL) was cooled to −5 °C, and then a solution of isopropenyl chloroformate (131 μL, 1.20 mmol) in anhydrous CH_2Cl_2 (4 mL) was added dropwise under vigorous magnetic stirring. The mixture was stirred at −5 °C until the complete disappearance of the starting material was detected (TLC analysis, ∼1 h). A 10% aqueous solution of $KHSO_4$ (2 mL) was then added to the solution, the cooling bath was removed, and an additional portion of 10% aqueous solution of $KHSO_4$ (2 mL) was added. The mixture was diluted with CH_2Cl_2 (80 mL), and then the two phases were separated. The organic phase was washed with brine (2 × 5 mL), dried (Na_2SO_4), and concentrated to give the corresponding adduct **10**, which was used for the following reaction without any purification.

A 2.0−5.0 mL process vial was filled with the above crude Meldrum adduct **10** and anhydrous *t*-BuOH (2.5 mL). The vial was sealed with the Teflon septum and aluminum crimp by using an appropriate crimping tool. The vial was then placed in its correct position in the Biotage Initiator cavity where irradiation for 15 min at 120 °C was performed. After the full irradiation sequence was completed, the vial was cooled to room temperature and then opened. The mixture was diluted with AcOEt (10 mL) and then concentrated. The residue was eluted from a column of silica gel with the suitable elution system to give the corresponding sugar β-keto ester **8**.

***tert*-Butyl 3-Oxo-4-(2′,3′,4′,6′-tetra-*O*-benzyl-β-D-galactopyranosyl)butanoate (β-8b).** Column chromatography with 7:1 cyclohexane−AcOEt afforded β-**8b** (476 mg, 70%) as a ∼10:1 mixture of ketone and enol isomers: $[\alpha]_D = -8.2$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (ketone isomer) δ = 7.40−7.20 (m, 20 H, Ph), 4.94 and 4.61 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 4.92 and 4.61 (2 d, 2 H, J = 11.8 Hz, PhCH_2), 4.74 and 4.65 (2 d, 2 H, J = 12.0 Hz, PhCH_2), 4.44 and 4.39 (2 d, 2 H, J = 11.2 Hz, PhCH_2), 4.01 (dd, 1 H, $J_{3',4'}$ = 3.5 Hz, $J_{4',5'}$ ∼ 0.5 Hz, H-4′), 3.74 (ddd, 1 H, $J_{1',2'}$ = 9.0 Hz, $J_{1',4a}$ = 3.5 Hz, $J_{1',4b}$ = 8.5 Hz, H-1′), 3.68 (dd, 1 H, $J_{2',3'}$ = 9.2 Hz, H-2′), 3.62 (dd, 1 H, H-3′), 3.59−3.48 (m, 3 H, H-5′, 2 H-6′), 3.35 and 3.31 (2 d, 2 H, $J_{2a,2b}$ = 15.0 Hz, 2 H-2), 2.85 (dd, 1 H, $J_{4a,4b}$ = 15.5 Hz, H-4a), 2.70 (dd, 1 H, H-4b), 1.40 (s, 9 H, *t*-Bu); MALDI-TOF MS 703.9 (M^+ + Na). Anal. Calcd for $C_{42}H_{48}O_8$ (680.83): C, 74.09; H, 7.11. Found: C, 74.15; H, 7.12.

***tert*-Butyl 3-Oxo-4-(2′,3′,4′,6′-tetra-*O*-benzyl-β-D-glucopyranosyl)butanoate (β-8a).** Column chromatography with 6:1 cyclohexane−AcOEt afforded β-**8a** (422 mg, 62%) as a ∼8:1 mixture of ketone and enol isomers: $[\alpha]_D = 2.6$ (*c* 1.2, $CHCl_3$); ^{1}H NMR (ketone isomer) δ = 7.40−7.20 (m, 20 H, Ph), 4.92 and 4.87 (2 d, 2 H, J = 10.5 Hz, PhCH_2), 4.90 and 4.56 (2 d, 2 H, J = 11.2 Hz, PhCH_2), 4.81 and 4.62 (2 d, 2 H, J = 11.0 Hz, PhCH_2), 4.58 and 4.49 (2 d, 2 H, J = 12.0 Hz, PhCH_2), 3.76 (ddd, 1 H, $J_{1',2'}$ = 8.5 Hz, $J_{1'}$,4a = 3.5 Hz, $J_{1'}$,4b = 8.5 Hz, H-1′), 3.68 (dd, 1 H, $J_{2',3'}$ = 9.0 Hz, $J_{3',4'}$ = 8.5 Hz, H-3′), 3.71−3.62 (m, 3 H, H-4′, 2 H-6′), 3.43 (ddd, 1 H, $J_{4',5'}$ = 9.0 Hz, $J_{5',6'a}$ = 3.0 Hz, $J_{5',6'b}$ = 3.5 Hz, H-5′), 3.38 and 3.33 (2 d, 2 H, $J_{2a,2b}$ = 14.5 Hz, 2 H-2), 3.32 (dd, 1 H, H-2′), 2.84 (dd, 1 H, $J_{4a,4b}$ = 15.5 Hz, H-4a), 2.66 (dd, 1 H, H-4b), 1.42 (s, 9 H, *t*-Bu); MALDI-TOF MS 703.1 (M^+ + Na), 720.0 (M^+ + K). Anal. Calcd for $C_{42}H_{48}O_8$ (680.83): C, 74.09; H, 7.11. Found: C, 74.18; H, 7.15.

***tert*-Butyl 3-Oxo-4-(2′,3′,4′,6′-tetra-*O*-benzyl-α-D-galactopyranosyl)butanoate (α-8b).** Column chromatography with 6:1 cyclohexane−AcOEt afforded α-**8b** (462 mg, 68%) as a ∼7:1 mixture of ketone and enol isomers: $[\alpha]_D = 23.8$ (*c* 2.2, $CHCl_3$); ^{1}H NMR (ketone isomer) δ = 7.40−7.20 (m, 20 H, Ph), 4.72 and 4.57 (2 d, 2 H, J = 11.8 Hz, PhCH_2), 4.68 and 4.54 (2 d, 2 H, J = 10.5 Hz, PhCH_2), 4.58 (ddd, 1 H, $J_{1',2'}$ = 3.0 Hz, $J_{1',4a}$ = 7.5 Hz, $J_{1',4b}$ = 6.0 Hz, H-1′), 4.55 and 4.47 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 4.10−3.98 (m, 2 H, H-4′, H-5′), 3.90−3.82 (m, 2 H, H-2′, H-6′a), 3.71 (dd, 1 H, $J_{2',3'}$ = 9.0 Hz, $J_{3',4'}$ = 3.5 Hz, H-3′), 3.68 (dd, 1 H, $J_{5',6'b}$ = 5.5 Hz, $J_{6'a,6'b}$ = 11.0 Hz, H-6′b), 3.42 and 3.32 (2 d, 2 H, $J_{2a,2b}$ = 15.0 Hz, 2 H-2), 2.86 (dd, 1 H, $J_{4a,4b}$ = 16.0 Hz, H-4a), 2.78 (dd, 1 H, H-4b), 1.42 (s, 9 H, *t*-Bu); MALDI-TOF MS: 703.5 (M^+ + Na), 719.8 (M^+ + K). Anal. Calcd for $C_{42}H_{48}O_8$ (680.83): C, 74.09; H, 7.11. Found: C, 74.00; H, 7.01.

***tert*-Butyl 3-Oxo-4-(2′,3′,4′,6′-tetra-*O*-benzyl-α-D-glucopyranosyl)butanoate (α-8a).** Column chromatography with 7:1 cyclohexane−AcOEt afforded α-**8a** (449 mg, 66%) as a ∼6:1 mixture of ketone and enol isomers: $[\alpha]_D = 27.7$ (*c* 2.9, $CHCl_3$); ^{1}H NMR (ketone isomer) δ = 7.40−7.05 (m, 20 H, Ph), 4.92 and 4.80 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 4.82 and 4.50 (2 d, 2 H, J = 11.0 Hz, PhCH_2), 4.74 (ddd, 1 H, $J_{1',2'}$ = 4.5 Hz, $J_{1'}$,4a = 5.5 Hz, $J_{1'}$,4b = 8.0 Hz, H-1′), 4.64 and 4.60 (2 d, 2 H, J = 11.2 Hz, PhCH_2), 4.62 and 4.48 (2 d, 2 H, J = 11.8 Hz, PhCH_2), 3.78 (dd, 1 H, $J_{2',3'}$ = 9.0 Hz, H-2′), 3.78−3.60 (m, 5 H, H-3′, H-4′, H-5′, 2 H-6′), 3.42 and 3.32 (2 d, 2 H, $J_{2a,2b}$ = 15.0 Hz, 2 H-2), 3.03 (dd, 1 H, $J_{4a,4b}$ = 15.8 Hz, H-4a), 2.85 (dd, 1 H, H-4b), 1.42 (s, 9 H, *t*-Bu); MALDI-TOF MS 703.5 (M^+ + Na), 719.8 (M^+ + K). Anal. Calcd for $C_{42}H_{48}O_8$ (680.83): C, 74.09; H, 7.11. Found: C, 74.05; H, 7.06.

Route A. A mixture of aldehyde α-**7a** (1.13 g, 2.00 mmol), *tert*-butyl diazoacetate (0.33 mL, 2.40 mmol), activated 4-Å powdered molecular sieves (300 mg), and anhydrous CH_2Cl_2 (20 mL) was stirred at room temperature for 15 min and then cooled to 0 °C. To the mixture was added a solution of BF_3•Et_2O (127 μL, 1.00 mmol) in anhydrous CH_2Cl_2 (1 mL) drop by drop, controlling the N_2 evolution at a low steady rate. The mixture was stirred at 0 °C for an additional 30 min, diluted with 10% $NaHCO_3$ (10 mL), warmed to room temperature, filtered through a pad of Celite, and extracted with CH_2Cl_2 (3 × 50 mL). The organic layer was dried (Na_2SO_4) and concentrated. The residue was eluted from a column of silica gel with 7:1 cyclohexane−AcOEt to afford α-**8a** (476 mg, 35%) as a ∼6:1 mixture of ketone and enol isomers: $[\alpha]_D = 27.9$ (*c* 2.0, $CHCl_3$).

(4*R*,2‴*S*)- and (4*S*,2‴*S*)-4-(2′,3′,4′,6′-Tetra-*O*-benzyl-β-D-galactopyranosylmethyl)-2-(2‴-*tert*-butoxycarbonylamino-2‴-meth-

oxycarbonylethyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylic Acid Di-*tert*-butyl Esters (*β*-14b). A screw-capped vial, containing a magnetic bar, was charged with aldehyde *β*-**7b** (283 mg, 0.50 mmol), *β*-ketoester **5** (173 mg, 0.50 mmol), aminocrotonate **12** (79 mg, 0.50 mmol), powdered 4 Å molecular sieves (100 mg), and *t*-BuOH (3 mL). The mixture was then vigorously stirred, degassed under vacuum, and saturated with argon (by an Ar-filled balloon) three times. The mixture was stirred at 70 °C for 24 h and then cooled to room temperature, diluted with AcOEt (10 mL), filtered through a pad of Celite, and concentrated. The residue was eluted from a column of silica gel with 4:1 cyclohexane−AcOEt to give *β*-**14b** (232 mg, 45%) as a∼1:1 mixture of C-4 epimers: ^{1}H NMR (selected data) δ = 7.40−7.20 (m, 20 H, Ph), 6.04 (bs, 0.5 H, NH), 5.89 (d, 0.5 H, J = 8.0 Hz, NH), 4.04−4.00 (m, 1 H, H-4′), 4.40−4.20 (m, 1 H, H-2‴), 3.74 and 3.72 (2 s, 3 H, OCH_3), 2.20 and 2.14 (2 s, 3 H, CH_3), 1.48−1.38 (6 s, 27 H, 3 *t*-Bu); MALDI-TOF MS 1056.5 (M^+ + Na), 1072.6 (M^+ + K). Anal. Calcd for $C_{60}H_{76}N_2O_{13}$ (1033.25): C, 69.75; H, 7.41; N, 2.71. Found: C, 69.88; H, 7.62; N, 2.65.

General Procedure for the Synthesis of 4-(*C*-Glycosylmethyl)-2-alaninylpyridines 15. A 2.0−5.0 mL process vial was filled with sugar aldehyde **7** (283 mg, 0.50 mmol), *β*-ketoester **5** (173 mg, 0.50 mmol), aminocrotonate **12** (79 mg, 0.50 mmol), powdered 4 Å molecular sieves (100 mg), and *t*-BuOH (2.5 mL). The vial was sealed with the Teflon septum and aluminum crimp by using an appropriate crimping tool. The mixture was then vigorously stirred, degassed under vacuum, and saturated with argon (by an Ar-filled balloon) three times The vial was then placed in its correct position in the Biotage Initiator cavity where irradiation for 1.5 h at 120 °C was performed. After the full irradiation sequence was completed, the vial was cooled to room temperature and then opened. The mixture was diluted with AcOEt (10 mL), filtered through a pad of Celite, and concentrated. The residue was dissolved in CH_2Cl_2 (8 mL), and then Amberlyst 15 (400 mg), Ambersep 900 OH (400 mg), and aminomethylated polystyrene (185 mg, 0.50 mmol of a 2.7 mmol g^{-1} resin) were added. The suspension was shaken for 2 h, and then the polymers were filtered off and washed thoroughly with CH_2Cl_2. The combined filtrates were concentrated to give the corresponding crude dihydropyridine derivative. A mixture of the above residue, pyridinium chlorochromate immobilized on silica gel[34] (1.87 g, ∼1.50 mmol of a ∼0.8 mmol g^{-1} resin) and anhydrous CH_2Cl_2 (8 mL) was stirred at room temperature for 12 h. Then the immobilized reagent was filtered off and washed thoroughly with CH_2Cl_2. The combined filtrates were concentrated, and the resulting residue was eluted from a column of silica gel with the suitable elution system to give the corresponding pyridine **15**.

(2‴*S*)-4-(2′,3′,4′,6′-Tetra-*O*-benzyl-*β*-D-galactopyranosyl-methyl)-2-(2‴-*tert*-butoxycarbonylamino-2‴-methoxycarbonylethyl)-6-methylpyridine-3,5-dicarboxylic Acid Di-*tert*-butyl Esters (*β*-15b). Column chromatography with 4:1 cyclohexane−AcOEt afforded *β*-**15b** (320 mg, 62%) as a white foam: $[\alpha]_D$ = 26.7 (*c* 0.9, $CHCl_3$); ^{1}H NMR δ = 7.40−7.10 (m, 20 H, Ph), 5.88 (bd, 1 H, J = 8.0 Hz, NH), 4.97 and 4.70 (2 d, 2 H, J = 11.0 Hz, PhCH_2), 4.93 and 4.50 (2 d, 2 H, J = 11.2 Hz, PhCH_2), 4.79 and 4.69 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 4.72−4.64 (m, 1 H, H-2‴), 4.41 and 4.33 (2 d, 2 H, J = 11.8 Hz, PhCH_2), 4.02 (dd, 1 H, $J_{3',4'}$ = 3.5 Hz, $J_{4',5'}$ ∼ 0.5 Hz, H-4′), 3.69 (s, 3 H, OCH_3), 3.68−3.60 (m, 2 H, H-2′, H-6′a), 3.56 (dd, 1 H, $J_{2',3'}$ = 9.0 Hz, H-3′), 3.48−3.34 (m, 3 H, H-1′, H-5′, H-6′b), 3.28 (d, 2 H, $J_{1''',2'''}$ = 7.5 Hz, 2 H-1‴), 3.20 (dd, 1 H, $J_{1',1''a}$ = 3.0 Hz, $J_{1''a,1''b}$ = 14.0 Hz, H-1″a), 2.95 (dd, 1 H, $J_{1',1''b}$ = 10.5 Hz, H-1″b), 2.42 (s, 3 H, CH_3), 1.45 and 1.42 (2 s, 27 H, 3 *t*-Bu); ^{13}C NMR δ = 173.0, 167.5, 167.1, 155.8, 154.3, 152.9, 142.9 (2 C), 139.2−130.2 (5 C), 128.9−127.6 (20 C), 85.0, 83.5, 83.1, 79.8, 79.2, 79.0, 77.4, 75.5, 75.1, 74.2, 73.6, 72.1, 68.6, 52.3, 36.8, 33.1, 28.6, (3 C), 28.2 (6 C), 23.1; MALDI-TOF MS 1032.5 (M^+ + H), 1054.2 (M^+ + Na). Anal. Calcd for $C_{60}H_{74}N_2O_{13}$ (1031.24): C, 69.88; H, 7.23; N, 2.72. Found: C, 69.88; H, 7.28; N, 2.66.

(2‴*S*)-4-(2′,3′,4′,6′-Tetra-*O*-benzyl-*β*-D-glucopyranosylmethyl)-2-(2‴-*tert*-butoxycarbonylamino-2‴-methoxycarbonylethyl)-6-methylpyridine-3,5-dicarboxylic Acid Di-*tert*-butyl Esters (*β*-15a). Column chromatography with 3:1 cyclohexane−AcOEt afforded *β*-**15a** (314 mg, 61%) as a white foam: $[\alpha]_D$ = 36.7 (*c* 0.9, $CHCl_3$); ^{1}H NMR δ = 7.40−7.10 (m, 20 H, Ph), 5.88 (bd, 1 H, J = 8.5 Hz, NH), 4.92 and 4.88 (2 d, 2 H, J = 11.0 Hz, PhCH_2), 4.91 and 4.68 (2 d, 2 H, J = 12.0 Hz, PhCH_2), 4.82 and 4.64 (2 d, 2 H, J = 10.5 Hz, PhCH_2), 4.68−4.64 (m, 1 H, H-2‴), 4.52 and 4.36 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 3.72−3.62 (m, 3 H, H-3′, H-4′, H-6′a), 3.68 (s, 3 H, OCH_3), 3.62 (dd, 1 H, $J_{5',6'b}$ = 3.5 Hz, $J_{6'a,6'b}$ = 10.5 Hz, H-6′b), 3.44 (ddd, 1 H, $J_{1',2'}$ = 9.0 Hz, $J_{1',1''}$a = 2.5 Hz, $J_{1',1''b}$ = 11.0 Hz, H-1′), 3.30−3.18 (m, 4 H, H-2′, H-5′, 2 H-1‴), 3.17 (dd, 1 H, $J_{1''a,1''b}$ = 14.5 Hz, H-1″a), 2.88 (dd, 1 H, H-1″b), 2.42 (s, 3 H, CH_3), 1.52 and 1.44 (2 s, 27 H, 3 *t*-Bu); ^{13}C NMR δ = 172.7, 167.1, 166.6, 155.6, 154.2, 152.9, 142.4 (2 C), 138.6−137.9 (5 C), 129.7−127.2 (20 C), 87.4, 83.2, 82.8, 82.5, 79.5, 79.4, 78.8, 78.4, 75.7, 75.1, 74.9, 73.0, 69.3, 52.1, 36.6, 32.8, 29.7 (3C), 28.3 (6 C), 22.3; MALDI-TOF MS 1032.8 (M^+ + H), 1054.6 (M^+ + Na). Anal. Calcd for $C_{60}H_{74}N_2O_{13}$ (1031.24): C, 69.88; H, 7.23; N, 2.72. Found: C, 69.75; H, 7.20; N, 2.75.

(2‴*S*)-4-(2′,3′,4′,6′-Tetra-*O*-benzyl-α-D-galactopyranosylmethyl)-2-(2‴-*tert*-butoxycarbonylamino-2‴-methoxycarbonylethyl)-6-methylpyridine-3,5-dicarboxylic Acid Di-*tert*-butyl Esters (α-15b). Column chromatography with 5:1 cyclohexane−AcOEt afforded α-**15b** (299 mg, 58%) as a white foam: $[\alpha]_D$ = 6.8 (*c* 0.8, $CHCl_3$); ^{1}H NMR δ = 7.40−6.90 (m, 20 H, Ph), 5.75 (bd, 1 H, J = 8.5 Hz, NH), 4.70 and 4.52 (2 d, 2 H, J = 12.0 Hz, PhCH_2), 4.63 (ddd, 1 H, $J_{1'''a,2'''}$ = 6.0 Hz, $J_{1'''b,2'''}$ = 4.5 Hz, H-2‴), 4.56 and 4.52 (2 d, 2 H, J = 11.2 Hz, PhCH_2), 4.54 and 4.41 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 4.51 and 4.40 (2 d, 2 H, J = 11.8 Hz, PhCH_2), 4.23−4.14 (m, 2 H, H-1′, H-6′a), 3.96−3.80 (m, 4 H, H-2′, H-3′, H-4′, H-6′b), 3.59 (s, 3 H, OCH_3), 3.50 (dd, 1 H, $J_{1',1''a}$ = 10.5 Hz, $J_{1''a,1''b}$ = 14.0 Hz, H-1″a), 3.45−3.41 (m, 1 H, H-5′), 3.29 (dd, 1 H, $J_{1'''a,1'''b}$ = 16.0 Hz, H-1‴a), 3.16 (dd, 1 H, H-1‴b), 2.57 (dd, 1 H, $J_{1',1''b}$ = 1.0 Hz, H-1″b), 2.36 (s, 3 H, CH_3), 1.44 and 1.38 (2 s, 27 H, 3 *t*-Bu); ^{13}C NMR δ = 172.7, 167.3, 166.9, 155.6, 154.1, 152.6, 143.5 (2 C), 139.0−137.9 (5 C), 129.8−127.0 (20 C), 83.1, 82.7, 79.6, 77.9, 75.7, 74.2, 73.7, 73.4, 72.8, 72.4, 71.8, 68.4, 65.7, 52.1, 36.6, 30.9, 28.3 (3 C), 27.9 (6 C), 22.9; MALDI-TOF MS 1032.8 (M^+ + H). Anal. Calcd for $C_{60}H_{74}N_2O_{13}$ (1031.24): C, 69.88; H, 7.23; N, 2.72. Found: C, 69.77; H, 7.29; N, 2.69.

(2‴*S*)-4-(2′,3′,4′,6′-tetra-*O*-Benzyl-α-D-glucopyranosylmethyl)-2-(2‴-*tert*-butoxycarbonylamino-2‴-methoxycarbonylethyl)-6-methylpyridine-3,5-dicarboxylic Acid Di-*tert*-butyl Esters (α-15a). Column chromatography with 5:1 cyclohexane−AcOEt afforded α-**15a** (284 mg, 55%) as a white foam: $[\alpha]_D$ = 30.5 (*c* 0.7, $CHCl_3$); ^{1}H NMR (DMSO-d_6, 120 °C) δ = 7.40−7.05 (m, 20 H, Ph), 6.42 (bs, 1 H, NH), 4.70 and 4.65 (2 d, 2 H, J = 10.5 Hz, PhCH_2), 4.68 and 4.55 (2 d, 2 H, J = 11.2 Hz, PhCH_2), 4.66 and 4.53 (2 d, 2 H, J = 11.8 Hz, PhCH_2), 4.56 (dd, 1 H, $J_{1'''a,2'''}$ = 6.0 Hz, $J_{1'''b,2'''}$ = 7.0 Hz, H-2‴), 4.32−4.26 (m, 1 H, H-1′), 4.26 (s, 2 H, PhCH_2), 3.92 (dd, 1 H, J = 4.5 Hz, J = 5.5 Hz, H-3′), 3.81 (ddd, 1 H, J = 3.5 Hz, J = 4.0 Hz, J = 8.0 Hz, H-5′), 3.67−3.52 (m, 4 H, H-2′, H-4′, 2 H-6′), 3.58 (s, 3 H, OCH_3), 3.36 (dd, 1 H, $J_{1',1''a}$ = 9.5 Hz, $J_{1''a,1''b}$ = 14.0 Hz, H-1″a), 3.22 (dd, 1 H, $J_{1'''a,1''b}$ = 15.5 Hz, H-1‴a), 3.07 (dd, 1 H, H-1‴b), 2.86 (dd, 1 H, $J_{1',1''b}$ = 4.0 Hz, H-1″b), 2.20 (s, 3 H, CH_3), 1.53, 1.51, and 1.37 (3 s, 27 H, 3 *t*-Bu); ^{13}C NMR δ = 172.7, 167.5, 167.1, 155.6, 154.5, 153.0, 143.6 (2 C), 138.6−137.9 (5 C), 129.4−127.4 (20 C), 83.6, 83.1, 81.4, 79.6, 79.5, 74.2, 74.0, 73.3, 72.9, 72.8, 72.7, 68.8, 52.2, 36.7, 30.0, 28.3 (3 C), 28.0 (6 C), 23.1; MALDI-TOF MS 1054.9 (M^+ + Na). Anal. Calcd for $C_{60}H_{74}N_2O_{13}$ (1031.24): C, 69.88; H, 7.23; N, 2.72. Found: C, 69.90; H, 7.30; N, 2.78.

General Procedure for the Synthesis of 2-(*C*-Glycosylmethyl)-4-alaninylpyridines 16. A 2.0−5.0 mL process vial was filled with aldehyde **2** (116 mg, 0.50 mmol), sugar *β*-ketoester **8** (340 mg, 0.50 mmol), aminocrotonate **12** (79 mg, 0.50 mmol), powdered 4

Å molecular sieves (100 mg), and *t*-BuOH (2.5 mL). The vial was sealed with the Teflon septum and aluminum crimp by using an appropriate crimping tool. The mixture was then vigorously stirred, degassed under vacuum, and saturated with argon (by an Ar-filled balloon) three times The vial was then placed in its correct position in the Biotage Initiator cavity where irradiation for 2.5 h at 120 °C was performed. After the full irradiation sequence was completed, and the vial was cooled to room temperature and then opened. The mixture was diluted with AcOEt (10 mL), filtered through a pad of Celite, and concentrated. The residue was dissolved in CH_2Cl_2 (8 mL), and then Amberlyst 15 (400 mg), Ambersep 900 OH (400 mg), and aminomethylated polystyrene (185 mg, 0.50 mmol of a 2.7 mmol g^{-1} resin) were added. The suspension was shaken for 2 h, and then the polymers were filtered off and washed thoroughly with CH_2Cl_2. The combined filtrates were concentrated to give the corresponding crude dihydropyridine derivative. A mixture of the above residue, pyridinium chlorochromate immobilized on silica gel[34] (1.87 g, ~1.50 mmol of a ~0.8 mmol g^{-1} resin) and anhydrous CH_2Cl_2 (8 mL), was stirred at room temperature for 12 h. Then the immobilized reagent was filtered off and washed thoroughly with CH_2Cl_2. The combined filtrates were concentrated, and the resulting residue was eluted from a column of silica gel with the suitable elution system to give the corresponding pyridine **16**.

(2‴*S*)-2-(2′,3′,4′,6′-tetra-*O*-Benzyl-β-D-galactopyranosyl-methyl)-4-(2‴-*tert*-butoxycarbonylamino-2‴-methoxycarbonylethyl)-6-methylpyridine-3,5-dicarboxylic Acid Di-*tert*-butyl Esters (β-16b). Column chromatography with 4:1 cyclohexane−AcOEt afforded β-**16b** (351 mg, 68%) as a white foam: $[\alpha]_D = -17.8$ (*c* 0.7, $CHCl_3$); ^{1}H NMR (DMSO-d_6, 140 °C) $\delta = 7.40-7.10$ (m, 20 H, Ph), 6.08 (bs, 1 H, NH), 4.88 and 4.55 (2 d, 2 H, $J = 11.5$ Hz, PhCH_2), 4.86 and 4.69 (2 d, 2 H, $J = 11.8$ Hz, PhCH_2), 4.81 and 4.69 (2 d, 2 H, $J = 12.0$ Hz, PhCH_2), 4.42−4.36 (m, 1 H, H-2‴), 4.38 and 4.32 (2 d, 2 H, $J = 11.8$ Hz, PhCH_2), 4.05 (dd, 1 H, $J_{3',4'} = 2.5$ Hz, $J_{4',5'} \sim 0.5$ Hz, H-4′), 3.92−3.84 (m, 1 H, H-1′), 3.78−3.72 (m, 2 H), 3.62−3.52 (m, 2 H), 3.60 (s 3 H, OCH_3), 3.46−3.40 (m, 1 H), 3.24 (dd, 1 H, $J_{1',1''a} = 3.5$ Hz, $J_{1''a,1''b} = 14.0$ Hz, H-1″a), 3.17 (dd, 1 H, $J_{1'''a,2'''} = 6.5$ Hz, $J_{1'''a,1'''b} = 14.5$ Hz, H-1‴a), 2.95 (dd, 1 H, $J_{1',1''b} = 9.0$ Hz, H-1″b), 2.89 (dd, 1 H, $J_{1'''b,2'''} = 9.5$ Hz, H-1‴b), 2.42 (s, 3 H, CH_3), 1.61, 1.52, and 1.30 (3 s, 27 H, 3 *t*-Bu); ^{13}C NMR $\delta = 172.8$, 167.7, 167.3, 157.6−154.7 (5 C), 139.1−135.0 (5 C), 129.4−127.3 (20 C), 84.8, 83.9, 79.5, 79.3, 78.9, 78.4, 75.1, 74.5, 74.0, 73.3, 72.0, 68.6, 53.7, 52.2, 38.9, 31.9, 28.0 (3 C), 27.9 (6 C), 23.1; MALDI-TOF MS 1070.9 ($M^+ + K$). Anal. Calcd for $C_{60}H_{74}N_2O_{13}$ (1031.24): C, 69.88; H, 7.23; N, 2.72. Found: C, 69.82; H, 7.23; N, 2.71.

(2‴*S*)-2-(2′,3′,4′,6′-Tetra-*O*-benzyl-β-D-glucopyranosylmethyl)-4-(2‴-*tert*-butoxycarbonylamino-2‴-methoxycarbonylethyl)-6-methylpyridine-3,5-dicarboxylic Acid Di-*tert*-butyl Esters (β-16a). Column chromatography with 4:1 cyclohexane−AcOEt afforded β-**16a** (320 mg, 62%) as a white foam: $[\alpha]_D = -23$ (*c* 0.5, $CHCl_3$); ^{1}H NMR (DMSO-d_6, 120 °C): $\delta = 7.40-7.10$ (m, 20 H, Ph), 6.25 (bs, 1 H, NH), 4.84 and 4.72 (2 d, 2 H, $J = 12.0$ Hz, PhCH_2), 4.83 (s, 2 H, PhCH_2), 4.73 and 4.60 (2 d, 2 H, $J = 11.2$ Hz, PhCH_2), 4.41−4.30 (m, 1 H, H-2‴), 4.40 and 4.33 (2 d, 2 H, $J = 11.8$ Hz, PhCH_2), 3.97 (ddd, 1 H, $J_{1',1''a} = 3.5$ Hz, $J_{1',1''b} = 8.5$ Hz, $J_{1',2'} = 9.0$ Hz, H-1′), 3.74 (dd, 1 H, $J = 8.8$ Hz, $J = 9.0$ Hz), 3.64−3.51 (m, 2 H, 2 H-6′), 3.59 (s, 3 H, OCH_3), 3.51 (dd, 1 H, $J = 8.0$ Hz, $J = 8.5$ Hz), 3.44 (dd, 1 H, $J = 8.8$ Hz, $J = 9.0$ Hz), 3.39 (m, 1 H, H-5′), 3.27 (dd, 1 H, $J_{1''a,1''b} = 14.5$ Hz, H-1″a), 3.19 (dd, 1 H, $J_{1'''a,2'''} = 8.0$ Hz, $J_{1'''a,1'''b} = 14.0$ Hz, H-1‴a), 3.26 (dd, 1 H, H-1″b), 2.88 (dd, 1 H, H-1‴b), 2.40 (s, 3 H, CH_3), 1.59, 1.56, and 1.28 (3 s, 27 H, 3 *t*-Bu); ^{13}C NMR (selected data) $\delta = 172.7$, 87.4, 84.0, 82.1, 79.4, 78.7, 75.6, 74.9, 73.2, 68.9, 52.7, 52.4, 38.5, 31.7, 28.2, 28.0, 23.0; MALDI-TOF MS 1032.4 ($M^+ + H$), 1054.2 ($M^+ + Na$). Anal. Calcd for $C_{60}H_{74}N_2O_{13}$ (1031.24): C, 69.88; H, 7.23; N, 2.72. Found: C, 69.82; H, 7.28; N, 2.71.

(2‴*S*)-4-(2′,3′,4′,6′-Tetra-*O*-benzyl-α-D-galactopyranosylmethyl)-2-(2‴-*tert*-butoxycarbonylamino-2‴-methoxycarbonylethyl)-6-methylpyridine-3,5-dicarboxylic Acid Di-*tert*-butyl Esters (α-16b). Column chromatography with 6:1 cyclohexane−AcOEt afforded α-**16b** (320 mg, 62%) as a white foam: $[\alpha]_D = -3.6$ (*c* 1.2, $CHCl_3$); ^{1}H NMR (DMSO-d_6, 140 °C): $\delta = 7.40-7.10$ (m, 20 H, Ph), 6.18 (bs, 1 H, NH), 4.70 and 4.65 (2 d, 2 H, $J = 11.5$ Hz, PhCH_2), 4.69−4.62 (m, 1 H, H-2‴), 4.68 and 4.55 (2 d, 2 H, $J = 12.0$ Hz, PhCH_2), 4.62 and 4.56 (2 d, 2 H, $J = 11.0$ Hz, PhCH_2), 4.42−4.34 (m, 1 H, H-1′), 4.38 and 4.31 (2 d, 2 H, $J = 11.5$ Hz, PhCH_2), 4.11 (ddd, 1 H, $J_{4',5'} = 1.5$ Hz, $J_{5',6'a} = 4.5$ Hz, $J_{5',6'b} = 9.0$ Hz, H-5′), 4.05 (dd, 1 H, $J_{3',4'} = 2.0$ Hz, H-4′), 3.94 (dd, 1 H, $J_{2',3'} = 6.0$ Hz, H-3′), 3.88 (dd, 1 H, $J_{1',2'} = 3.5$ Hz, H-2′), 3.76−3.68 (m, 2 H, 2 H-6′), 3.60 (s, 3 H, OCH_3), 3.24 (dd, 1 H, $J_{1',1''a} = 6.0$ Hz, $J_{1''a,1''b} = 14.0$ Hz, H-1″a), 3.12 (dd, 1 H, $J_{1'''a,2'''} = 8.0$ Hz, $J_{1'''a,1'''b} = 15.0$ Hz, H-1‴a), 3.06 (dd, 1 H, $J_{1'''b,2'''} = 6.0$ Hz, H-1‴b), 2.42 (s, 3 H, CH_3), 1.61, 1.55, and 1.30 (3 s, 27 H, 3 *t*-Bu); ^{13}C NMR (selected data): $\delta = 172.8$, 167.5, 167.2, 155.5, 155.3, 154.6, 139.0, 138.2, 138.1, 129.0−127.4 (20 C), 84.2, 84.0, 79.6, 75.6, 74.0, 73.1, 72.9, 72.5, 66.8, 53.6, 52.3, 34.0, 31.6, 28.2 (3 C), 28.0 (6 C), 23.0; MALDI-TOF MS 1032.7 ($M^+ + H$). Anal. Calcd for $C_{60}H_{74}N_2O_{13}$ (1031.24): C, 69.88; H, 7.23; N, 2.72. Found: C, 69.93; H, 7.15; N, 2.70.

(2‴*S*)-4-(2′,3′,4′,6′-Tetra-*O*-benzyl-α-D-glucopyranosylmethyl)-2-(2‴-*tert*-butoxycarbonylamino-2‴-methoxycarbonylethyl)-6-methylpyridine-3,5-dicarboxylic Acid Di-*tert*-butyl Esters (α-16a). Column chromatography with 4:1 cyclohexane−AcOEt afforded α-**16a** (299 mg, 58%) as a white foam: $[\alpha]_D = 16.8$ (*c* 0.8, $CHCl_3$); ^{1}H NMR (DMSO-d_6, 120 °C) $\delta = 7.40-7.10$ (m, 20 H, Ph), 6.24 (bs, 1 H, NH), 4.81 and 4.73 (2 d, 2 H, $J = 11.0$ Hz, PhCH_2), 4.80−4.70 (m, 1 H, H-1′), 4.72 and 4.57 (2 d, 2 H, $J = 11.5$ Hz, PhCH_2), 4.68 and 4.64 (2 d, 2 H, $J = 10.8$ Hz, PhCH_2), 4.42 and 4.35 (2 d, 2 H, $J = 11.8$ Hz, PhCH_2), 4.40−4.30 (m, 1 H, H-2‴), 3.92−3.82 (m, 2 H), 3.76 (dd, 1 H, $J = 4.5$ Hz, $J = 7.5$ Hz), 3.64−3.50 (m, 3 H), 3.60 (s, 3 H, OCH_3), 3.23 (dd, 1 H, $J_{1'''a,2'''} = 6.5$ Hz, $J_{1'''a,1'''b} = 14.0$ Hz, H-1‴a), 3.18 (dd, 1 H, $J_{1',1''a} = 6.5$ Hz, $J_{1''a,1''b} = 14.5$ Hz, H-1″a), 3.12 (dd, 1 H, $J_{1',1''b} = 5.0$ Hz, H-1″b), 2.87 (dd, 1 H, $J_{1'''b,2'''} = 8.0$ Hz, H-1‴b), 2.44 (s, 3 H, CH_3), 1.62, 1.56, and 1.31 (3 s, 27 H, 3 *t*-Bu); ^{13}C NMR $\delta = 172.7$, 167.5, 167.2, 155.5−154.9 (5 C), 139.0−138.2 (5 C), 128.8−127.5 (20 C), 84.5, 84.1, 82.4, 79.7, 78.0, 75.2, 74.7, 73.7, 73.3, 72.8, 71.8, 68.5, 53.7, 52.3, 32.0, 31.7, 28.2 (3 C), 28.0 (6 C), 23.1; MALDI-TOF MS 1054.3 ($M^+ + Na$). Anal. Calcd for $C_{60}H_{74}N_2O_{13}$ (1031.24): C, 69.88; H, 7.23; N, 2.72. Found: C, 69.81; H, 7.18; N, 2.86.

(2‴*S*)-4-(2′,3′,4′,6′-Tetra-*O*-benzyl-β-D-galactopyranosylmethyl)-2-(2‴-*tert*-butoxycarbonylamino-2‴-carboxyethyl)-6-methylpyridine-3,5-dicarboxylic Acid Di-*tert*-butyl Esters (17). To a cooled (0 °C) stirred solution of methyl ester β-**15b** (103 mg, 0.10 mmol) in THF (2 mL) was added dropwise a pre-cooled 0.2 M aqueous solution of LiOH (0.60 mL, 0.12 mmol). The mixture was stirred at 0 °C until the complete disappearance of the starting material was detected (TLC analysis, ~1 h). The mixture was then acidified with 5% aqueous HCl to pH = 2, warmed to room temperature, diluted with CH_2Cl_2 (80 mL), and washed with H_2O (2 × 10 mL). The organic phase was dried (Na_2SO_4) and concentrated to give the crude acid **17** in almost quantitative yield: ^{1}H NMR $\delta = 7.40-7.10$ (m, 20 H, Ph), 5.93 (bd, 1 H, $J = 6.0$ Hz, NH), 4.98 and 4.52 (2 d, 2 H, $J = 10.5$ Hz, PhCH_2), 4.93 and 4.71 (2 d, 2 H, $J = 11.0$ Hz, PhCH_2), 4.80 and 4.68 (2 d, 2 H, $J = 11.5$ Hz, PhCH_2), 4.47 and 4.38 (2 d, 2 H, $J = 12.0$ Hz, PhCH_2), 4.38 (ddd, 1 H, $J_{1'''a,2'''} = 8.5$ Hz, $J_{1'''b,2'''} = 8.5$ Hz, H-2‴), 4.03 (dd, 1 H, $J_{3',4'} = 3.5$ Hz, $J_{4',5'} \sim 0.5$ Hz, H-4′), 3.72−3.60 (m, 2 H, H-2′, H-6′a), 3.57 (dd, 1 H, $J_{2',3'} = 9.0$ Hz, H-3′), 3.50−3.30 (m, 4 H, H-1″a, H-1′, H-5′, H-6′b), 3.22 (dd, 1 H, $J_{1',1'b} = 9.5$ Hz, $J_{1''a,1''b} = 16.5$ Hz, H-1′′b), 3.10 (d, 2 H, 2 H-1‴), 2.52 (s, 3 H, CH_3), 1.44, 1.42, and 1.28 (3 s, 27 H, 3 *t*-Bu). Anal. Calcd for $C_{59}H_{72}N_2O_{13}$ (1017.21): C, 69.66; H, 7.13; N, 2.75. Found: C, 69.75; H, 7.10; N, 2.70.

(2′*S*,2″*S*,1‴*S*)-4-(2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranosylmethyl)-2-[2′-(2″-*tert*-butoxycarbonylamino-3″-methylpropionylamino)-2′-(1‴-ethoxycarbonyl-2‴-phenylethylcarbamoyl)-ethyl]-6-methylpyridine-3,5-dicarboxylic Acid Di-*tert*-butyl Ester

(Boc-Ala-Pal-Phe-OEt) (19). To a cooled (0 °C), stirred solution of crude acid **17** (101 mg, ~0.10 mmol), L-phenylalanine ethyl ester hydrochloride (34 mg, 0.15 mmol), and (benzotriazol-1-yloxy)-tripyrrolidinophosphonium hexafluorophosphate (62 mg, 0.12 mmol) in anhydrous CH_2Cl_2 (2.0 mL) was added *N*,*N*-diisopropylethylamine (52 μL, 0.30 mmol). The solution was warmed to room temperature, stirred for an additional 2 h, and then concentrated. The residue was suspended with AcOEt (80 mL) and washed with H_2O (2 × 10 mL). The organic phase was dried (Na_2SO_4), concentrated, and eluted from a column of silica gel with 1:1 cyclohexane−AcOEt to give dipeptide **18** (94 mg, ~80%) slightly contaminated by uncharacterized byproducts: ^{1}H NMR (selected data) δ = 8.08 (bd, 1 H, J = 7.5 Hz, NH), 7.40−7.10 (m, 25 H, Ph), 6.12 (bd, 1 H, J = 8.05 Hz, NH), 4.96 and 4.68 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 4.93 and 4.50 (2 d, 2 H, J = 11.0 Hz, PhCH_2), 4.78 and 4.69 (2 d, 2 H, J = 11.2 Hz, PhCH_2), 4.43 and 4.38 (2 d, 2 H, J = 12.0 Hz, PhCH_2), 4.08 (bq, 2 H, J = 7.0 Hz, OCH_2CH$_3$), 4.02 (dd, 1 H, $J_{3,4}$ = 3.5 Hz, $J_{4,5}$ ~ 0.5 Hz, H-4s), 3.57 (dd, 1 H, $J_{2,3}$ = 9.0 Hz, H-3s), 2.33 (bs, 3 H, CH_3), 1.44, 1.42, and 1.28 (3 s, 27 H, 3 *t*-Bu), 1.22 (t, 3 H, OCH$_2$CH_3).

To a cooled (0 °C), stirred solution of dipeptide **18** (94 mg, ~0.08 mmol) in CH_2Cl_2 (2.0 mL) was slowly added a solution of TFA−CH_2Cl_2 (0.50 mL−1.50 mL). Stirring was continued at 0 °C for an additional 30 min and then warmed to room temperature. After 30 min at room temperature, the solution was neutralized at 0 °C with saturated aqueous Na_2CO_3 and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic phases were dried (Na_2SO_4) and concentrated to give the corresponding crude free amine (85 mg), which was used for the following reaction without any purification.

To a cooled (0 °C), stirred solution of the above crude amine (85 mg, ~0.08 mmol), *tert*-butoxycarbonyl-L-alanine (22 mg, 0.12 mmol), and (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (73 mg, 0.14 mmol) in anhydrous CH_2Cl_2 (2.0 mL) was added *N*,*N*-diisopropylethylamine (60 μL, 0.35 mmol). The solution was warmed to room temperature, stirred for an additional 2 h, and then concentrated. The residue was suspended with AcOEt (80 mL) and washed with saturated aqueous $NaHCO_3$ (10 mL) and brine (10 mL). The organic phase was dried (Na_2SO_4), concentrated, and eluted from a column of silica gel with 1.5:1 cyclohexane−AcOEt to give tripeptide **19** (65 mg, 65% from **35**): $[\alpha]_D$ = 23.5 (*c* 0.8, $CHCl_3$); ^{1}H NMR δ = 7.78 (bd, 2 H, J ~ 7.5 Hz, 2 NH), 7.40−7.00 (m, 25 H, Ph), 5.08 (bd, 1 H, J = 8.0 Hz, NH), 4.98 and 4.68 (2 d, 2 H, J = 11.0 Hz, PhCH_2), 4.92 and 4.48 (2 d, 2 H, J = 11.2 Hz, PhCH_2), 4.86−4.78 (m, 2 H, H-2′, H-1‴), 4.78 and 4.70 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 4.38 and 4.32 (2 d, 2 H, J = 12.0 Hz, PhCH_2), 4.20−4.12 (m, 1 H, H-2″), 4.08 (q, 2 H, J = 7.0 Hz, OCH_2CH$_3$), 4.01 (dd, 1 H, $J_{3,4}$ = 3.5 Hz, $J_{4,5}$ ~ 0.5 Hz, H-4s), 3.64 (dd, 1 H, $J_{1,2}$ = 8.8 Hz, $J_{2,3}$ = 9.0 Hz, H-2s), 3.68−3.60 (m, 1 H, H-5s), 3.57 (dd, 1 H, H-3s), 3.44 (ddd, 1 H, $J_{1,CH2a}$ = 3.0 Hz, $J_{1,CH2b}$ = 10.5 Hz, H-1s), 3.42−3.32 (m, 2 H, 2 H-6s), 3.20−3.06 (m, 5 H, CH_2a, 2 H-1′, 2 H-2‴), 2.95 (dd, 1 H, $J_{CH2a,CH2b}$ = 14.0 Hz, CH_2b), 2.36 (s, 3 H, CH_3), 1.62, 1.42, and 1.40 (3 s, 27 H, 3 *t*-Bu), 1.28 (d, 3 H, J = 7.0 Hz, CH_3), 1.15 (t, 3 H, OCH$_2$CH_3); MALDI-TOF MS 1286.7 (M^+ + Na). Anal. Calcd for $C_{73}H_{90}N_4O_{15}$ (1263.5): C, 69.39; H, 7.18; N, 4.43. Found: C, 69.45; H, 7.20; N, 4.39.

Acknowledgment. We greatly acknowledge the University of Ferrara for financial support.

Supporting Information Available: General experimental methods and NMR spectra for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO071221R