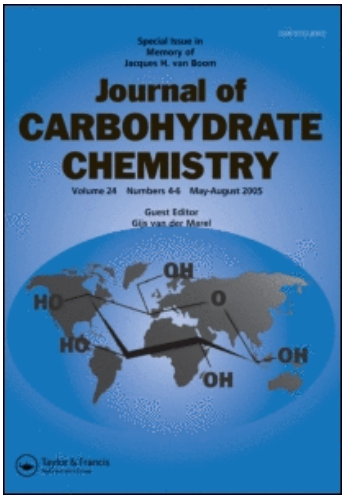

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PYRANOSYL SUGAR AMINO ACID CONJUGATES: THEIR BIOLOGICAL ORIGINS, SYNTHETIC PREPARATIONS, AND STRUCTURAL CHARACTERIZATION*

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INTRODUCTION

Sometimes it is true that everything old is new again, which can be disheartening for the originators of an idea who see others receiving credit years later. In some cases, an idea consistently evolves over time and early insights are ''rediscovered'' many years later. In other situations, ideas are born before their time, when the tools required to assure their continued development are lacking. And, in some circumstances, the creators of an idea fail to fully realize its potential. There are elements of all these factors in this chapter on the use of pyranosyl sugar amino acids as amino acid equivalents.

Perusal of the literature suggests that the idea of making pyranosyl sugar amino acids originated in the laboratories of Heyns and Paulsen, who synthetically prepared the first pyranosyl sugar amino acid in 1955. The accuracy with which the literature records the moment of inception is somewhat suspect, since reports of naturally occurring pyranosyl sugar amino acids quickly followed the syntheses. Natural product identification was facilitated by comparisons to synthetic materials presenting the possibility that collaborative efforts prompted the synthetic work. This seems especially plausible because there were relatively few syntheses published between 1955 and the late 1960s. This period was primarily dominated by reports on the isolation of several different pyranosyl sugar amino acids from natural sources. Hanessian and Haskell authored a prophetic disclosure describing the isolation and characterization of a

*Reprinted from *Glycochemistry: Principles, Synthesis, and Applications*; Wang, P.G.; Bertozzi, C.R., Eds.; Marcel Dekker, Inc.: New York, 2001, 493–534.

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pyranosyl sugar amino acid amidically linked to alanine. The possibility that the alanine could bridge sugar amino acids in a similar fashion to α-peptides did not go unnoticed by these researchers.

In the late 1960s and into the mid-1970s new synthetic reports appeared in the literature. Most of these focused on the total synthesis of naturally occurring pyranosyl sugar amino acid conjugates. Then, in 1976, Fuchs and Lehmann published the first synthesis of sugar amino acids amidically linked in a fashion similar to α-amino acids. Surprisingly, this disclosure received little attention for nearly 20 years.

In the past 5 years, the chemistry of pyranosyl sugar amino acids has evolved into a new science at the forefront of chemical design, synthesis, and structural characterization. Parallel work by Fleet and coworkers has elegantly exploited furanosyl sugar amino acids. At the same time, the sugar amino acids are only a small part of a larger science encompassing the use of unnatural amino acids in the synthesis of new materials with defined secondary structures.

NATURALLY OCCURRING PYRANOSYL SUGAR AMINO ACIDS

Glycopeptides are a large class of naturally occurring molecules containing a carbohydrate glycosidically linked to an α-amino acid, which is typically a component of a peptide or protein. There are both *N*- and *O*-linked glycopeptides. *N*-Acetylgalactosamine linked α to serine is the major *O*-glycosidic linkage, whereas *N*-acetylglucosamine linked β to asparaginine characterizes *N*-linked glycopeptides (Figure 1).[1]

Sugar amino acids are carbohydrates containing both amine and carboxylic acid functionalities in place of hydroxyls and are structurally distinct from glycopeptides. Naturally occurring sugar amino acids come in several types, consisting of both amino furanosiduronic and pyranosiduronic acids. This chapter includes the latter class of compounds, often referred to as amino hexuronic acids. Hexuronic acids are six-carbon monosaccharides containing both an aldehyde and a chain-terminating carboxylic acid. The amino hexuronic acids have amine functionalities in place of hydroxyl groups normally found in sugars.

2-Acetamido-2-deoxy-D-galacturonic acid, a major immunogenic component of *Salmonella typhosa*,[2] was the first naturally occurring amino hexuronic acid identified.[3] In 1963 Perkins reported that the cell wall of *Micrococcus lysodeikticus* contained 2-amino-2-deoxymannuronic acid,[4] and shortly thereafter, *Hemophilus influenzae*

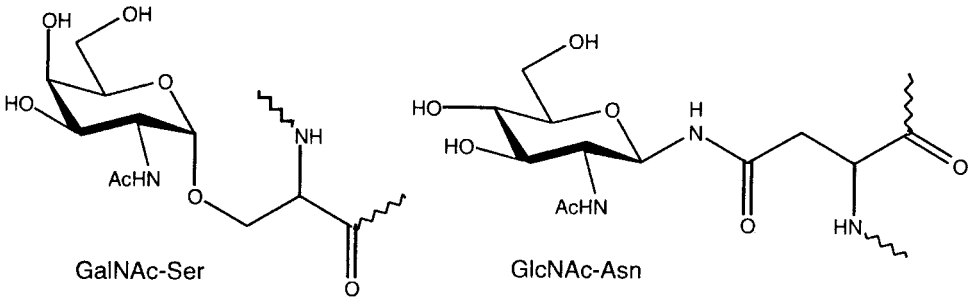

Figure 1.

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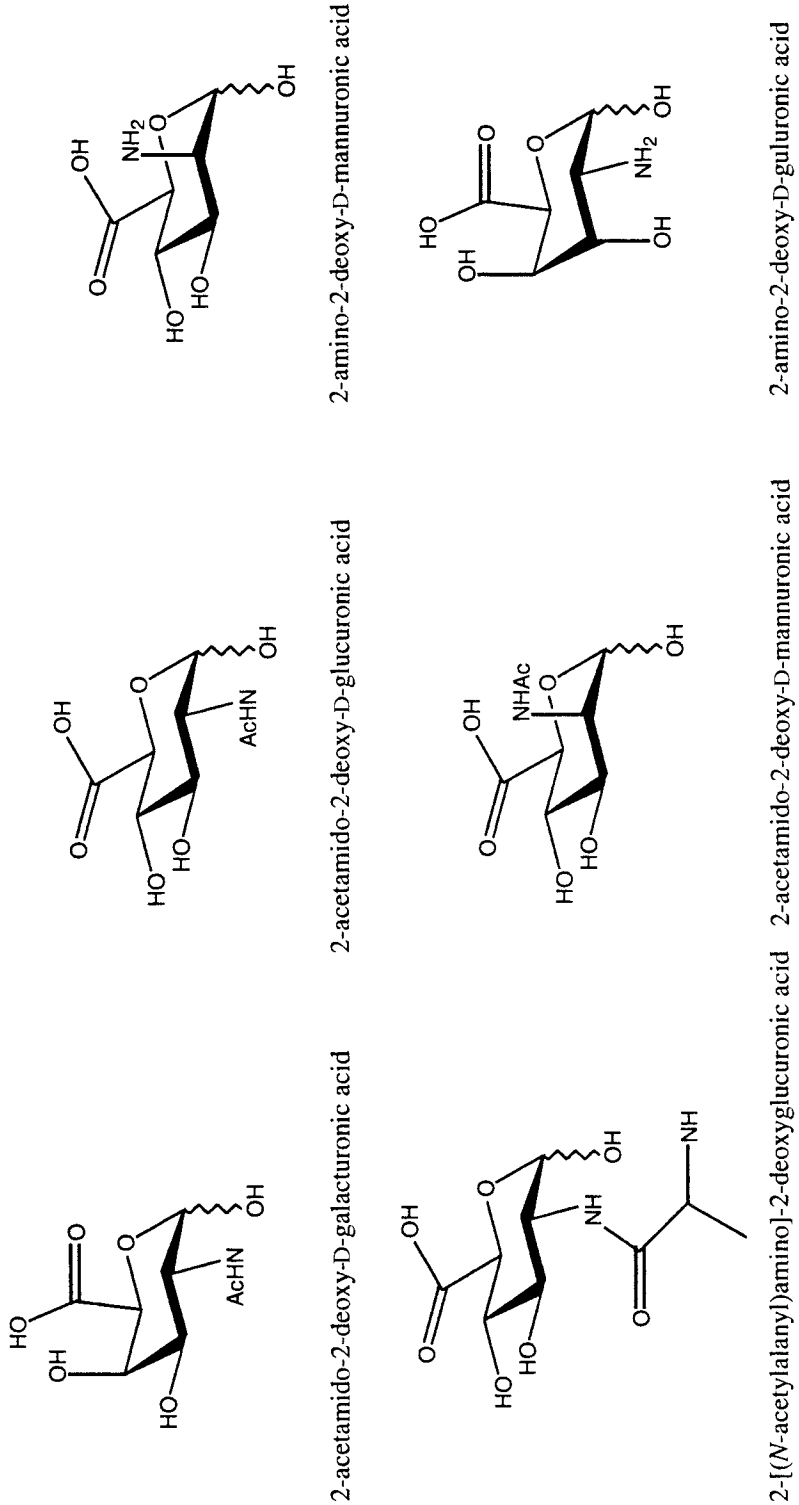

Figure 2.

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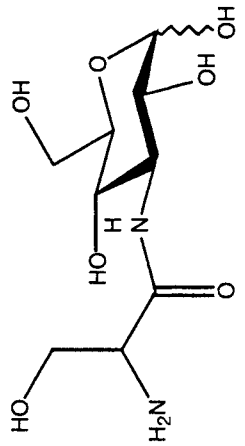
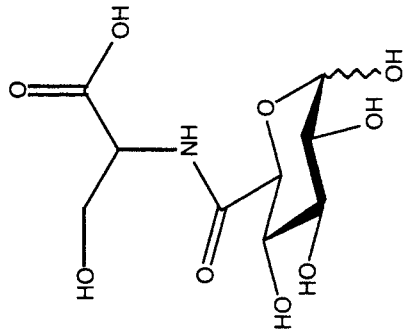
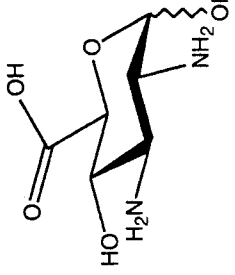


3-(*N*-acetyl-L-seryl)aminoglucose



N-glucuronyl-serine

Figure 3.



2,3-diamino-2,3-dideoxy-glucuronic Acid

type d was shown to contain 2-acetamido-2-deoxy-D-glucuronic acid.[5] At about the same time, an antigenic staphylococcal polysaccharide was shown to be composed of 2-acetamido-2-deoxy-glucuronic acid glycosidically linked to 2-[(*N*-acetylalanyl)amino]-2-deoxyglucuronic acid (Figure 2).[6] It would appear that this was the first identification of a naturally occurring sugar amino acid amidically conjugated to an α-amino acid.[7]

All possible hexose configurations have been found as amino hexuronic acids in bacteria, with the possible exception of idose.[8] In some cases, more than one hydroxyl is replaced by amine functionality; for example, 2,3-diamino-2,3-dideoxyglucuronic acid was isolated from the cell wall of *Propionibacterium acnes*.[9] Several more polysaccharides containing hexuronic acids linked to α-amino acids such as alanine, serine, and threonine have been characterized.[7] There are also examples of amino sugars linked to the carboxy terminus of α-amino acids (Figure 3).[10]

Ezomycin A1 and gougerotin are natural nucleoside antibiotics comprising sugar amino acids conjugated to α-amino acids through amide bonds. Ezomycin A1 contains two pyranoses, both having γ-amino acid functionalities, whereas gougerotin is a carbohydrate-based β-amino acid (Figure 4).[11] Ezoaminuroic acid was the first naturally occurring 3-amino-3-deoxy-hexuronic acid to be identified.[12] Structure elucidation studies, chemical syntheses, and biochemical properties of these and related compounds have been reviewed.[13,14]

Amino glyculosonic acids are members of another class of sugar amino acids that are 2-keto sugars with a C1 carboxylic acid. One of the most abundant representatives of this class of molecules is neuraminic acid, a nine-carbon sugar amino acid. There are several known derivatives of neuraminic acid, which are collectively called the sialic acids.[15] They are components of bacterial and viral capsular polysaccharides, and they are important constituents of mammalian cellular recognition elements. Besides the sialic acids, two other amino nonulosonic acids have been isolated from bacteria. These compounds are 5,7-diamino-3,5,7,9-tetradeoxynonulosonic acids. The amine functionalities of pseudaminic acid (Figure 5) have been found acylated, formylated, and conjugated to serine in nature.[16]

SYNTHETIC STUDIES OF PYRANOSYL SUGAR AMINO ACIDS

Syntheses of Natural Amino Hexuronic Acids

Several syntheses targeting amino hexuronic acids have been reported. Heyns and Paulsen synthesized the first sugar amino acids in 1955.[17] In the event, benzyl-2-*N*-carbobenzyloxy-2-deoxy-α-D-glucopyranoside was reacted with oxygen and platinum to provide 2-*N*-carbobenzyloxy-2-deoxy-D-glucuronic acid, which after removal of the nitrogen protecting group yielded a δ-sugar amino acid. (Figure 6) 2-Amino-2-deoxy-galacturonic acid was prepared in a similar fashion.[18] It is noteworthy that these synthetic efforts predated the identification of sugar amino acids in nature.

The syntheses of α- and β-phenylglycosides of 2-*N*-acetyl-2-deoxyglucuronic acid were described in 1958,[19] and in 1961 Weidmann and Zimmerman reported several different reactions of 2-amino-2-deoxyglucuronic acids.[20] These combined reports set the foundation for subsequent studies by Yoshimura et al., who prepared several derivatives of 2-amino-2-deoxyglycuronic acids.[21]

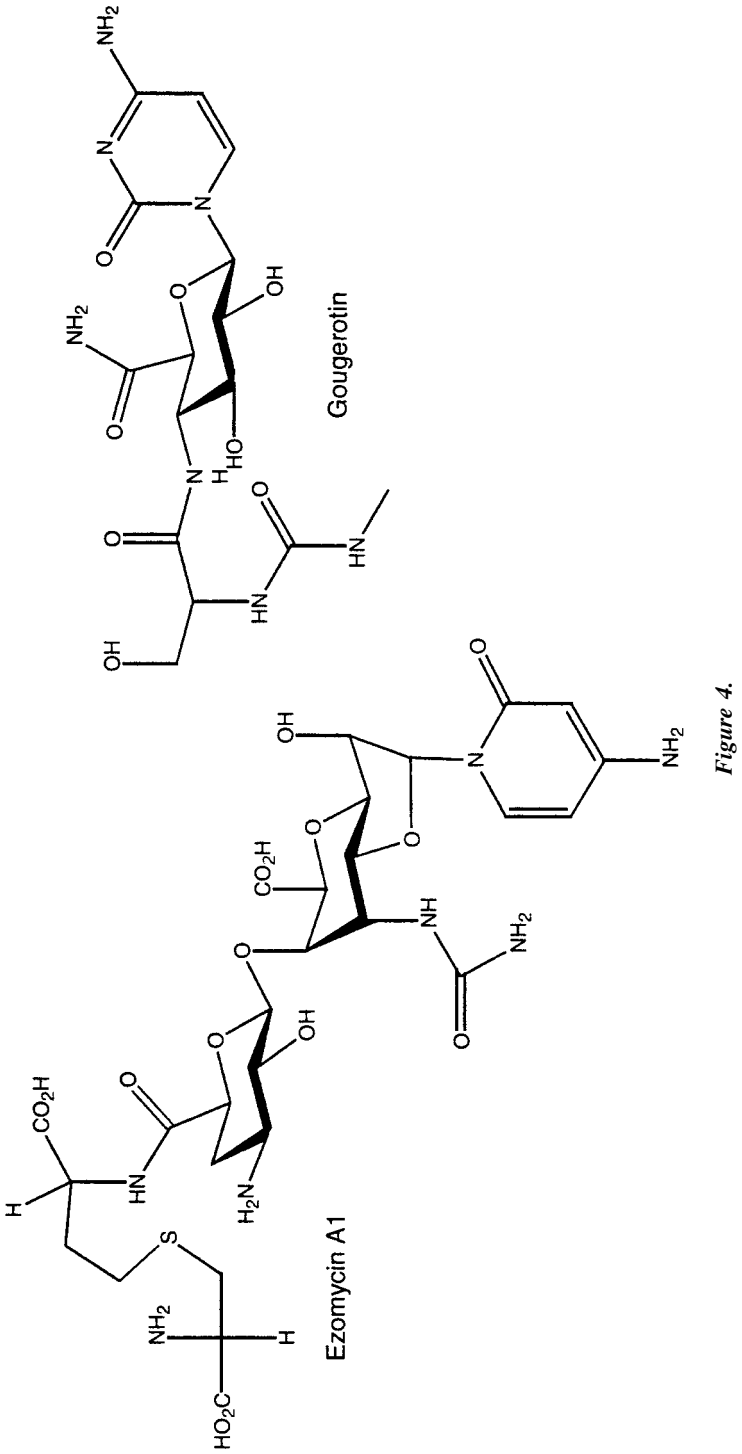
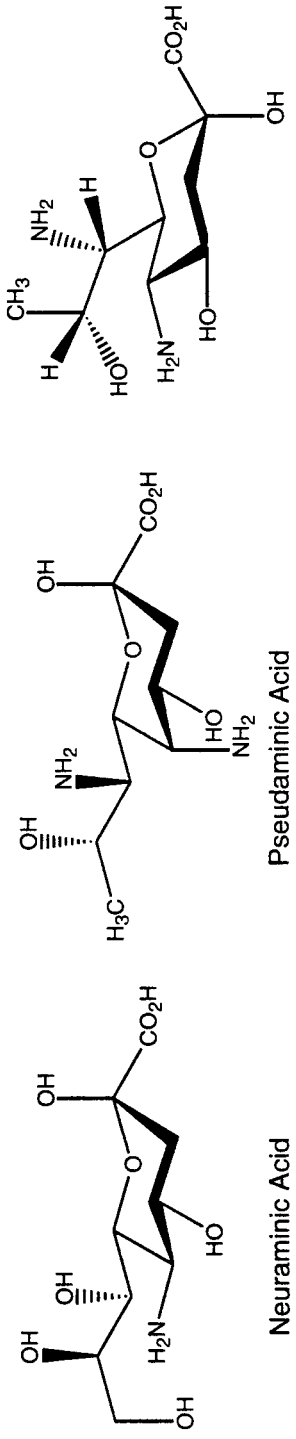


Figure 4.



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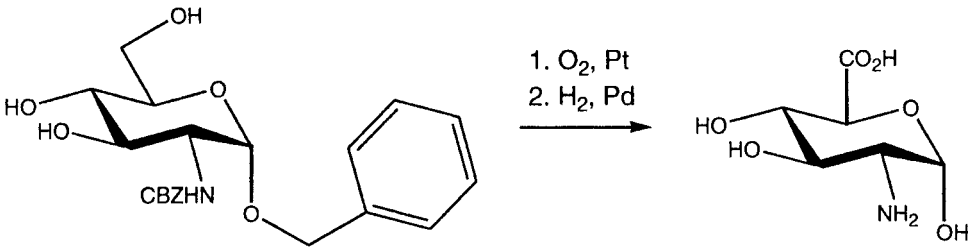


Figure 6.

Tsuji and coworkers were the first to synthesize a 3-amino-3-deoxyhexuronic acid, in 1968.[22] They began with an isopropylidene glucofuranosiduronic ester and introduced the nitrogen functionality via hydrazone formation and subsequent reduction. Removal of the isopropylidene resulted in formation of amino allouronic acid (Figure 7). Paulsen and coworkers used a very similar strategy in making 3-amino-3-deoxyglucuronic acid.[23]

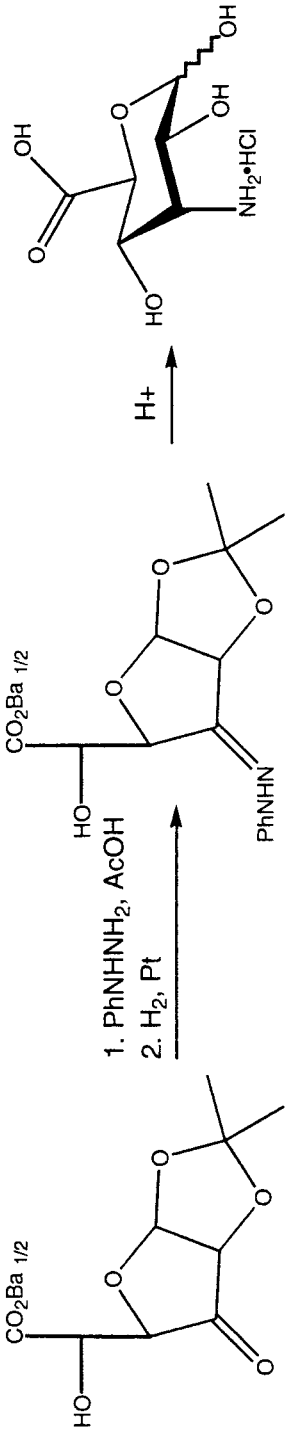
Ogawa later published the synthesis of a 3-amino-3,4-dideoxyhexuronic acid in route to ezomycin.[24] In that account, a 1,6-anhydro epoxy sugar was reacted with sodium azide followed by antimony pentachloride to give methyl 3-azido-2-*O*-benzoyl-α-D-glucospyranoside. Oxidation of the primary alcohol was achieved with potassium permanganate, and reduction of the azide was accomplished with hydrogenation (Figure 8).

Finally, in 1979 Horton reported the synthesis of 3-amino-2,3-dideoxyhexuronic acids. In six steps, methyl α-D-mannopyranoside was converted to a highly functionalized 3-acetamido-6-azido-2,3,6-trideoxy derivative. Photochemical activation of the azide provided an imine, which was subsequently hydrolyzed to the aldehyde. Bromine was used to oxidize the aldehyde to the acid, which was esterified with methyl iodide (Figure 9).

In their studies directed toward the synthesis of gougerotin, Watanabe and coworkers prepared methyl-4-*O*-mesyl galactopyranoside and reacted it with sodium azide; after deprotection, methyl 4-azido-4-deoxyglucopyranoside was obtained.[25] This compound was oxidized with platinum and oxygen to afford the 4-azido glucuronic acid (Figure 10).[26] The focus of these early synthetic studies was to prepare amino hexuronic acid derivatives that could be joined through *O*-glycosidic linkages, which are found in nature.

Syntheses of Unnatural Sugar Amino Acids

Fuchs and Lehmann demonstrated that *C*-glycoside amino acids could be prepared from selective ring opening of sugar-based anhydrides.[27] In the reaction, the anhydride was regioselectively reacted with ammonia to give a *C*-glycoside amide, which was subsequently converted to a nitrile by the action of tosyl chloride in pyridine. The nitrile was reduced with catalytic hydrogenation, and the major product resulted from migration of the C3 acetate to the C1 amine. The *O*- and *N*-acetyls were



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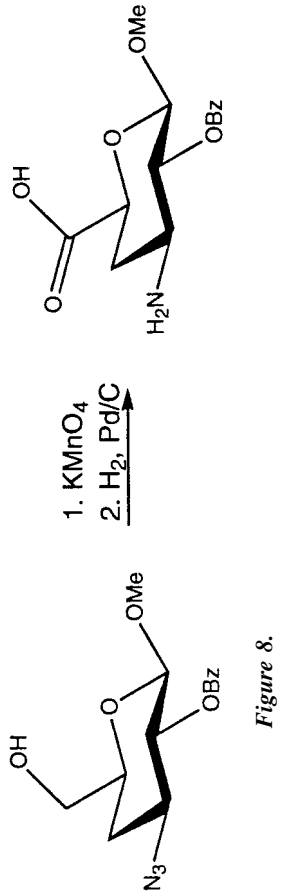

Figure 8.

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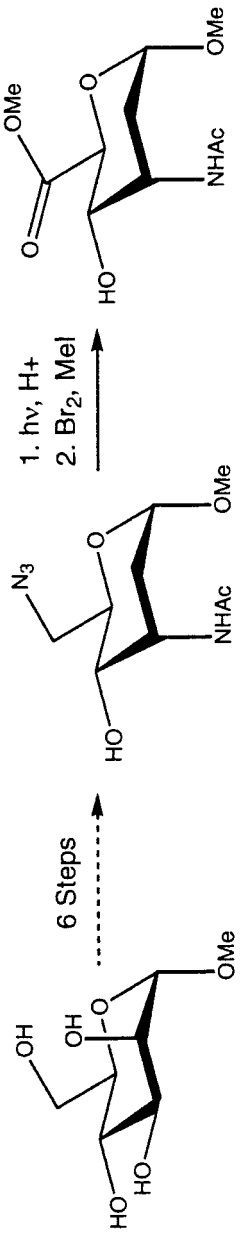

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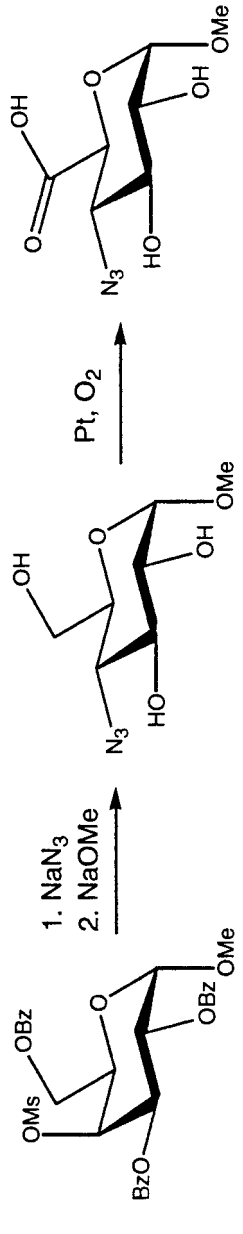


Figure 10.

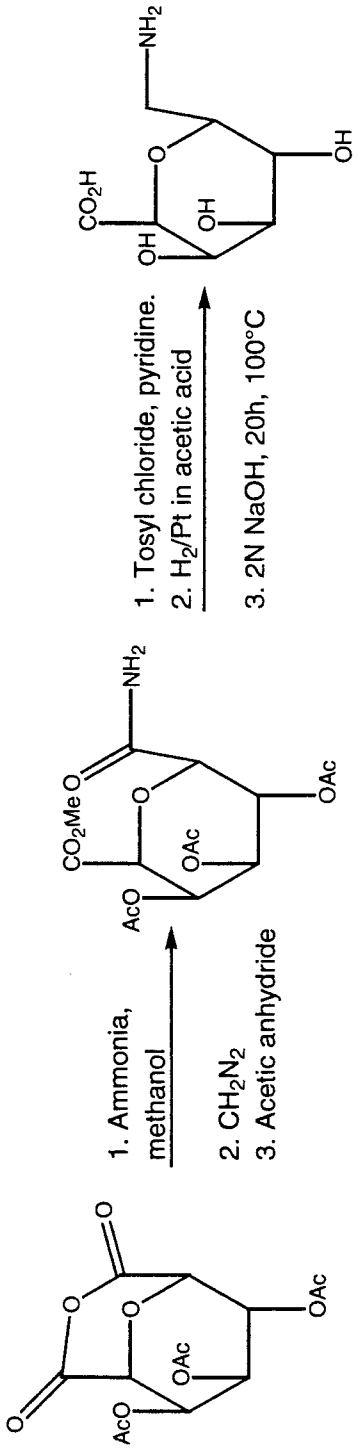


Figure 11.

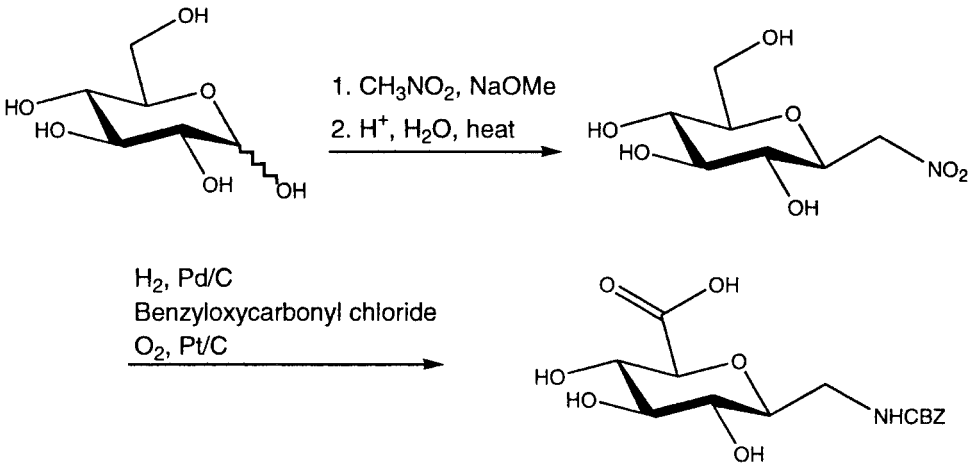
removed by saponification, using 2 N sodium hydroxide at 100°C (Figure 11). In 1994 a related heptonic amino acid was prepared by nitromethane addition to glucose, reduction of the nitro group, and oxidation of the primary hydroxyl group (Figure 12).[28]

Fuchs and Lehmann also prepared 7-amino *C*-glycoside carboxylic acids from the corresponding *C*-glycoside nitriles, which were hydrolyzed to carboxylic acids. The C6 hydroxyls were selectively converted to azides and subsequently reduced with catalytic hydrogenation.[29] Although Hanessian and Haskell mentioned the possibility that naturally occurring sugar amino acids could be linked via amide bonds rather than glycosides, Fuchs and Lehmann were the first to reduce this to practice in a synthetic arena (see below).

Kim and Hollingsworth prepared a *C*-glycoside of *N*-acetylglucosamine by alkylation of a pyranosyl bromide with malonate anion, giving an *N*-acetyl γ-sugar amino acid after decarboxylation (Figure 13).[30]

Galantinic acid is a somewhat related compound that can be considered to be a *C*-glycoside pyranosyl amine. It is an ε-amino acid that was thought to be a component of Galantin I, a naturally occurring peptide-based antibiotic. In 1992, however, Sakai and Ohfune showed that galantinic acid is a by-product of the isolation procedure, rather than a component of the natural product.[31] In earlier work, Ohfune and Kurokawa reported a stereocontrolled synthesis of galantinic acid from a serine-derived epoxide. Cuprate opening of the epoxide gave a conjugated ester that underwent Michael addition upon deprotection (Figure 14).[32] This example illustrates a subtle relationship between α-amino acids and sugar amino acids, since galantinic acid has structural entities related to both classes of compounds.

Perhaps the interplay between sugar amino acids and α-amino acids is more clearly demonstrated in the synthesis of α-D-glucosyl-(*R*)-alanine reported by Axon and Beckwith.[33] In the reaction, (2*R*)-methyleneoxazolidinone was treated with 2,3,4,6-tetra-*O*-acetyl glucosyl iodide in the presence of sodium cyanoborohydride and tributyltin chloride to give the α-*C*-glycoside in 88% yield (Figure 15).



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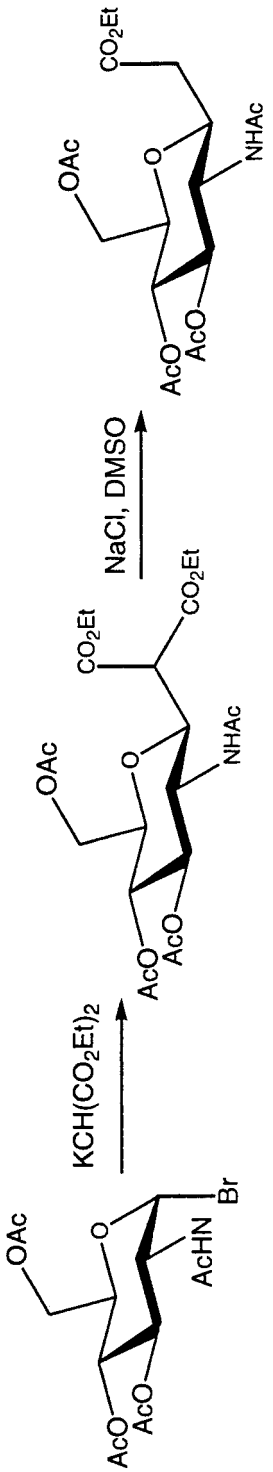

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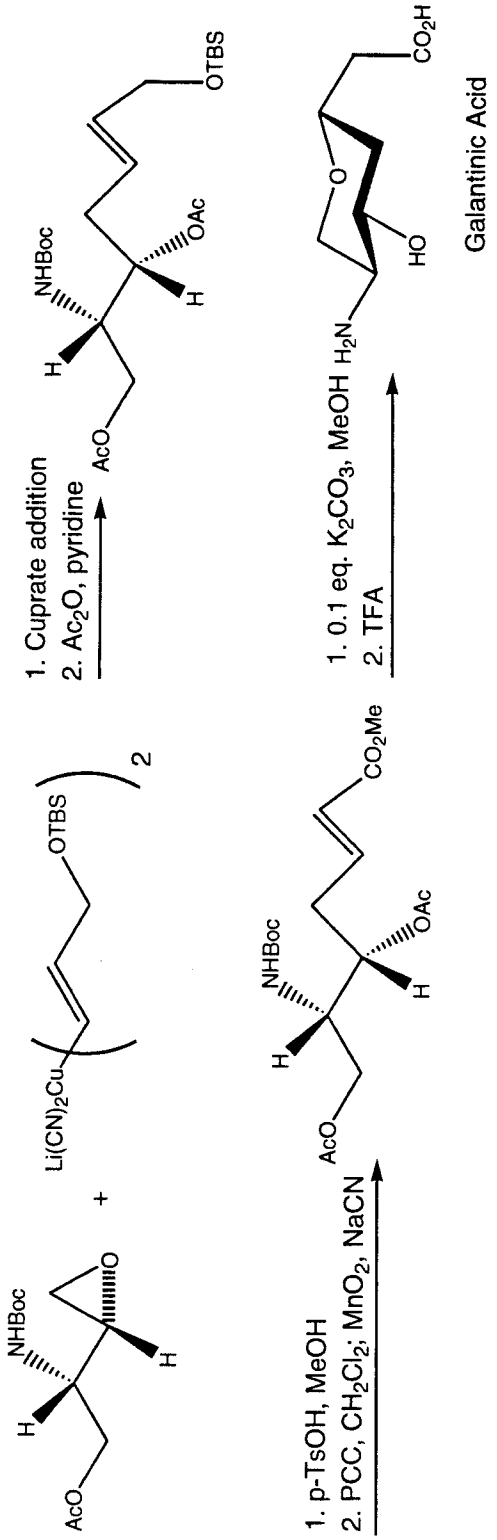

Figure 14.

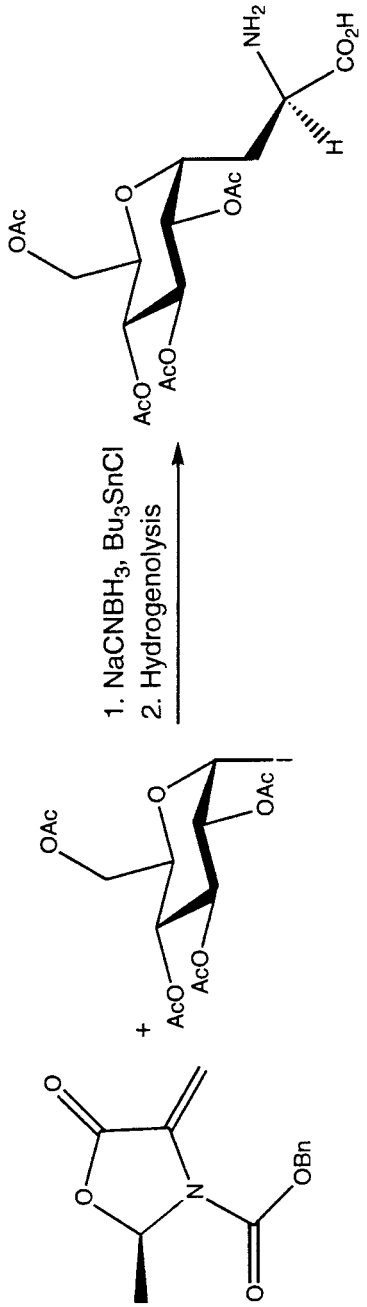

Figure 15.

β-*C*-Glycosides have also been prepared by the Lewis acid catalyzed addition of ketene acetals, derived from α-amino acids, to glycosyl bromides.[34] The acyl protecting group at C2 was critical in this reaction because it directed the β incorporation of the amino acid. However, benzoyl protecting groups were trapped by addition to the carbonyl rather than the anomeric carbon. This problem was overcome by using a pivaloyl (OCO*t*-butyl) protecting groups instead (Figure 16).

N-Linked β-amino acid conjugates of sugars were reported by Kunz et al.[35] Mannich reaction of bis(*O*-trimethylsilyl) ketene acetals and *N*-galactosylimines gave α-branched β-amino acids in high diastereomeric ratios (Figure 17).

1-Amino-1-deoxyglucuronic acids have also been prepared. In one account, 1,2,3,4-tetra-*O*-acetyl glucuronic acid was treated with iodine at 0°C, followed by the addition of trimethylsilyl azide. The acetates were removed with hydrazine and the azide was reduced with 1,2-ethanedithiol.[36] Other azido sugars have been used in the preparation of sugar amino acids. For example, Fleet and coworkers converted the isopropylidene of D-glucuronolactone into an α-azido lactone, which was subsequently reduced to an α-amino lactone.[37] Removal of the acetal protecting group unmasked the aldehyde, which underwent reductive amination and hydrolysis of the lactone to give a trihydroxypipecolinic acid (Figure 18). Fleet developed this elegant methodology as a rapid entry into several picolinic acid derivatives, which are naturally occurring L-amino acids with known biological activity. These molecules further illustrate the difficulty in defining the difference between sugar amino acids and α-amino acids. In this case, they are arguably indistinguishable.

Syntheses of Neuraminic Acid Derived Amino Acids

In his review on complex carbohydrates, Nathan Sharon stated: ''Neuraminic acid is a nine carbon sugar acid, with an amino group in the molecule.''[38] This simple and obvious declaration was an epiphany for Gervay-Hague, whose prior experience with NeuAc had been limited to the challenges of *O*-glycosylations. The realization that neuraminic acid is an amino acid presented new possibilities for its utilization in the production of novel materials. Sharon's writing inspired a program in the Gervay-Hague laboratories directed to the synthesis of amino acid equivalents derived from neuraminic acid.

Since *N*-acetyl neuraminic acid is the most abundant form of the sialic acids, it was important to first establish a method for removing the acyl group. This turned out to be remarkably difficult, since both acid and base hydrolyses led to retro aldol products rather than the desired amine. Borrowing from work published by Roy and Pon,[39] the β-methyl glycoside of NeuAc was prepared and successfully *N*-deacylated using 2 N sodium hydroxide at 100°C for 48 h. Realizing that these conditions would not be suitable for a wide variety of substrates, the investigators sought milder conditions. After much experimentation, it was found that treatment of the amide with *tert*-butoxycarbonyl (Boc) anhydride followed by mild hydrolysis with sodium methoxide provided the Boc-protected sugar amino acid in high yield.[40] This general strategy provided a reliable route to several *N*-protected neuraminic acid analogs, including α- and β-*O*-methyl glycosides and a 2,3-dehydro derivative (Figure 19).

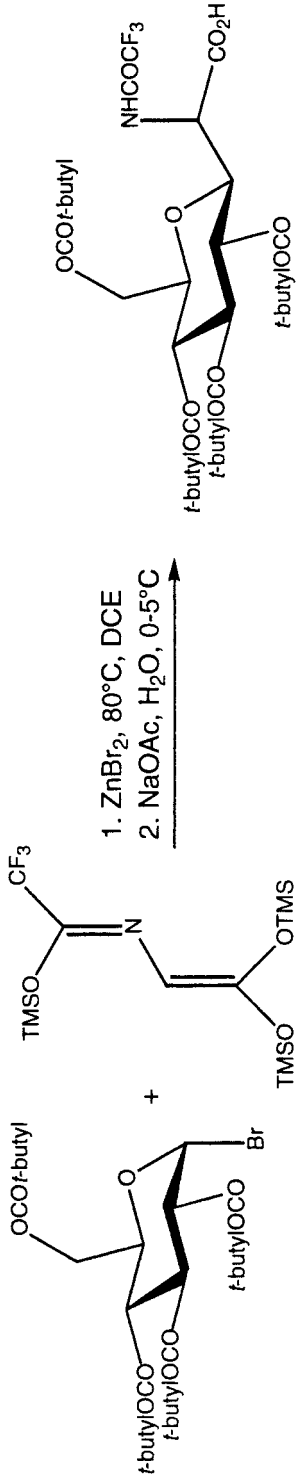

Figure 16.

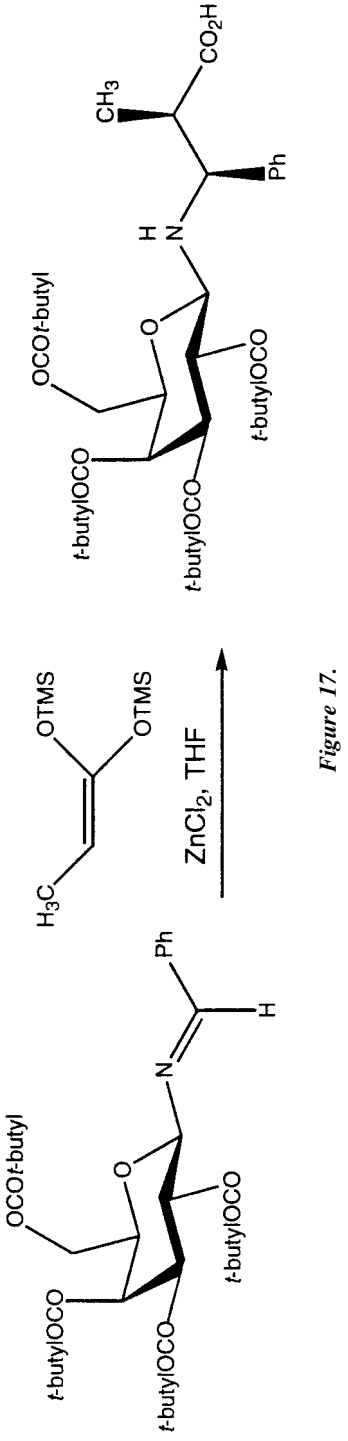

Figure 17.

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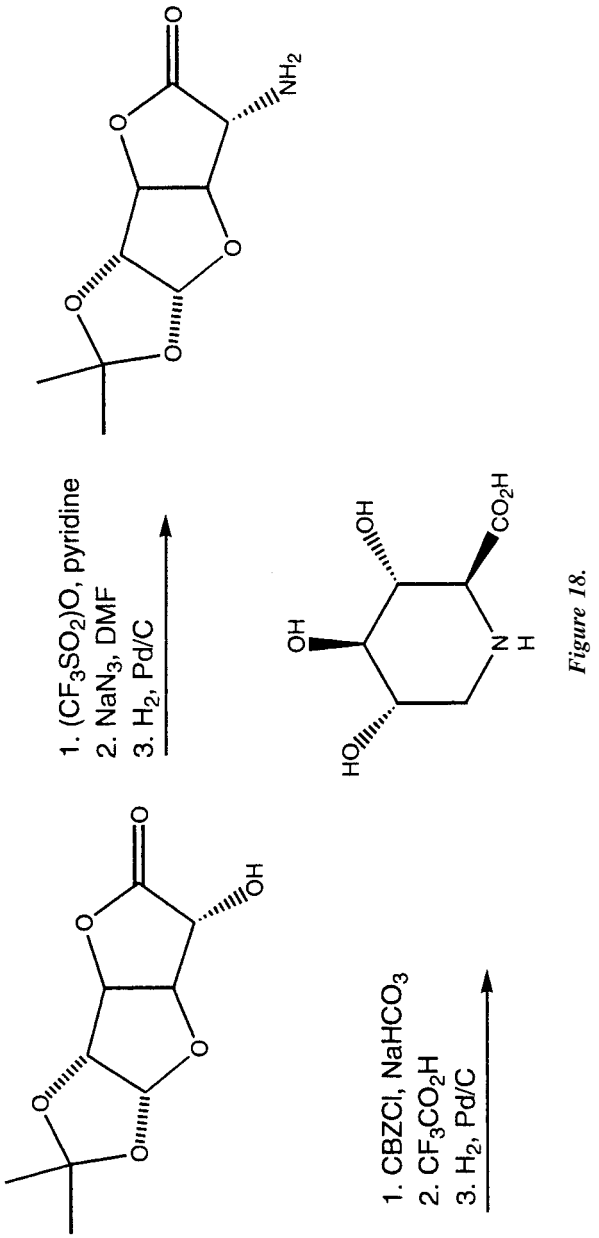


Figure 18.

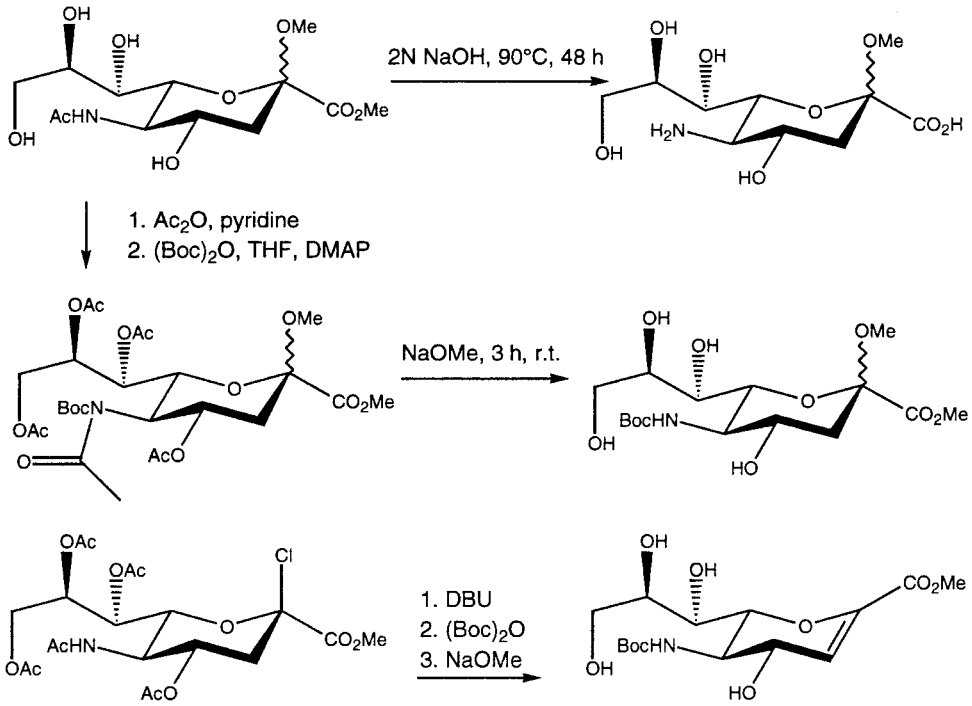


Figure 19.

2-Deoxy analogs of *N*-protected neuraminic acids were also prepared. The β-hydrido derivative was obtained by hydrogenation of *N*-acetyl-*N*-Boc-4,7,8,9-tetra-*O*-acetyl-2,3-dehydroneuraminic acid followed by treatment with sodium methoxide. The α-hydrido analog was synthesized from *N*-acetyl-2,4,7,8,9-penta-*O*-acetyl neuraminic acid benzyl ester under the action of hydrogen iodide in acetic acid. In the reaction,

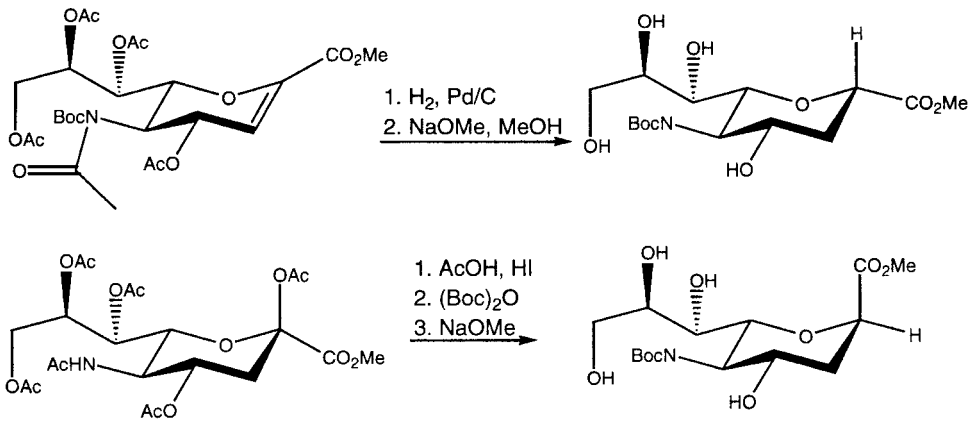


Figure 20.

the anomeric acetate was converted to an iodide, which subsequently underwent reductive elimination to give the α-hydrido compound under thermodynamic conditions (Figure 20).[41] These combined syntheses efficiently provided five new sugar amino acids with functionality suitable for typical peptide coupling reactions.

The foregoing synthetic efforts, targeting the design and syntheses of novel carbohydrate-based amino acids over the past 45 years, have established a solid foundation for an entirely new area of scientific inquiry—the use of sugar amino acids in the synthesis of novel and unnatural products.

SYNTHESIS OF SUGAR AMINO ACID CONJUGATES

The first synthetic reports of sugar amino acid conjugation appeared in the early 1970s. Most of those studies were directed to the synthesis of nucleoside antibiotics containing hexuronic acids conjugated to α-amino acids.[11] The first suggestions that unnatural compounds were of synthetic interest appeared in two separate papers published in 1976. Yoshimura and coworkers demonstrated that a hexosaminuronic acid could be conjugated to a glucosamine via an amide bond.[42] These scientists were clearly influenced by earlier work on nucleoside antibiotics. Nonetheless, they did make amido-bonded disaccharides for the first time. Because one sugar was an acid and the other an amine, these systems were self-terminating (Figure 21).

Fuchs and Lehmann first demonstrated that homo-oligomers of sugar amino acids could be prepared. In their reaction, a glucose-derived sugar amino acid posessing an anomeric carboxylate and a C4 amino group was polymerized under basic conditions.[43] After the reaction mixture had been heated for 4 h at 100°C, a water-insoluble precipitate formed (Figure 22). The products were not rigorously characterized, but there was evidence that the precipitate was composed of dimers, trimers, and tetramers.

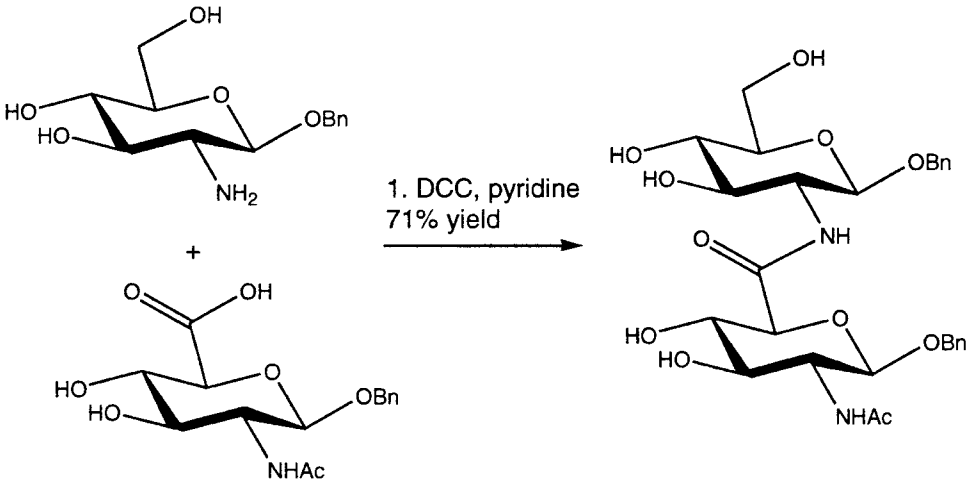


Figure 21.

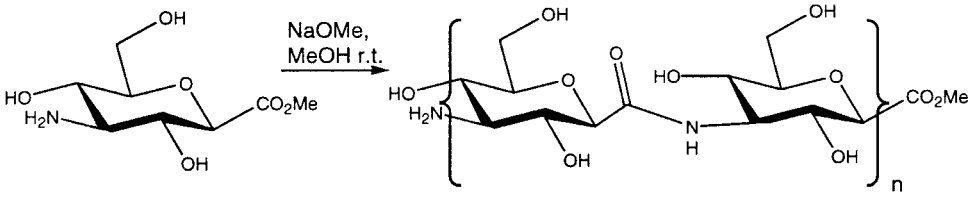


Figure 22.

Although these two reports clearly demonstrated the feasibility of using sugar amino acids in the production of novel and unnatural compounds, it was nearly 20 years before an aggressive exploration of this rich field of chemistry began. The art that followed the pioneering work of Yoshimura, Fuchs, and Lehmann can be classified into two categories: 1) syntheses involving mixed congeners, having either self-terminating residues and/or a combination of sugar amino and α-amino acids, and 2) homo-oligomeric syntheses composed of one sugar amino acid joined in consecutive amido linkages.

Syntheses of Mixed Sugar Amino Acid Conjugates

In 1994 Kessler used a sugar amino acid as a dipeptide isostere.[28] He and coworkers proposed that the *C*-glycoside amine of glucuronic acid would have torsion angles comparable to those of dipeptides composed of glycine and either serine or threonine. They incorporated the sugar amino acid into different peptides and meas-

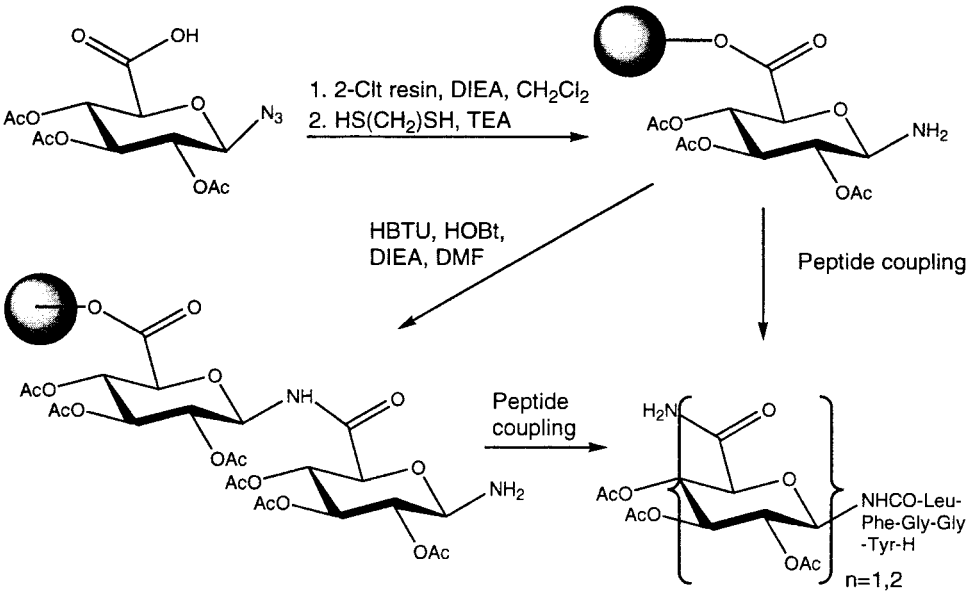


Figure 23.

ured their ability to inhibit the release of growth hormone as somatostatin analogs. The results of these experiments suggested that sugar-based peptides hold promise as potent peptidomimetics.

Toth and coworkers also prepared sugar amino acid derived peptides in route to modified enkephalins.[44] Two conjugates were prepared and studied. An azido glucuronic acid was immobilized on a trityl resin, and, after reduction, it was further conjugated to five α-amino acids (Leu-Phe-Gly-Gly-Tyr). A second derivative consisted of a sugar amino acid dimer conjugated to the same five α-amino acids. The pharmacology of these compounds was evaluated and the analog containing only one sugar amino acid was shown to be a potent and selective agonist of the δ-opioid receptor (Figure 23).

Very recently, amino deoxyglucuronic acids were used in the construction of disaccharide libraries.[45] In the report, methyl 3-azido-3-deoxy-4-*O*-methyl-β-D-glucuronic acid was prepared and glycosidically linked to a protected glucosamine, yielding a disaccharide with both amino and carboxamide functionalities. The azide was subsequently reduced and reacted with several isocyanates, providing libraries of β-linked disaccharides (Figure 24).

One incentive for making amido-linked sugars as mimics of glycosidically linked disaccharides was the possibility of effecting enzymatic resistance to glycosidic bond cleavage. Sabesan nicely illustrated this point in his work on amide-linked disaccharides containing NeuAc.[46] The 2-azido sugar was reduced and subsequently condensed with an activated glaacturonic acid to afford only the α-amino-linked disaccharide. This

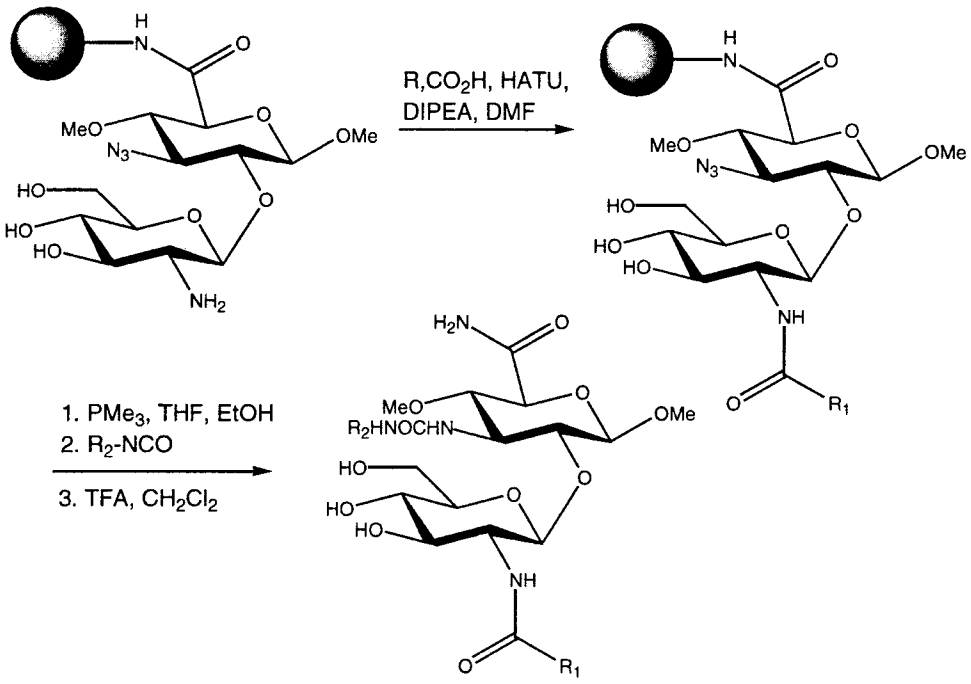


Figure 24.

compound was designed to be an isostere of the naturally occurring α-D-NeuAc-(2-6)-β-D-Gal glycosidic linkage (Figure 25).

Gervay and coworkers used two different strategies to further explore the incorporation of NeuAc into mixed sugar amino acid conjugates. In the first report, sialyllactones were prepared and condensed with glycine under thermal conditions.[47] Both five- and six-membered lactones were reacted, anticipating that the ring strain in the 1,4-lactones would promote the reaction. Unfortunately, this was not the case, and only low yields of the desired conjugates were obtained. The six-membered lactones gave improved yields, but, in general, the reactions were not efficient. In later studies, NeuAc was efficiently conjugated to several α-amino acids using BOP and HOBT activation of the acid followed by addition of an α-amino acid.[48] This protocol provided a number of sugar amino acid peptides in nearly quantitative yields (Figure 26).

Oligomeric Sugar Amino Acid Syntheses

In 1995 Nicolaou and coworkers initiated a renaissance in the construction of sugar amino acid conjugates with their synthesis of carbonucleotoids.[49] Although they did not prepare amid-linked carbohydrates, they did introduce the term ''carbopeptoid'' to designate such materials. Shortly thereafter, a number of papers directed toward the synthesis of carbopeptoids appeared. One of the earliest was reported by Wessel et al., who used *nor*-muramic acid derivatives and condensed them in solution by means of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) in DMF to construct a tetramer (Figure 27).

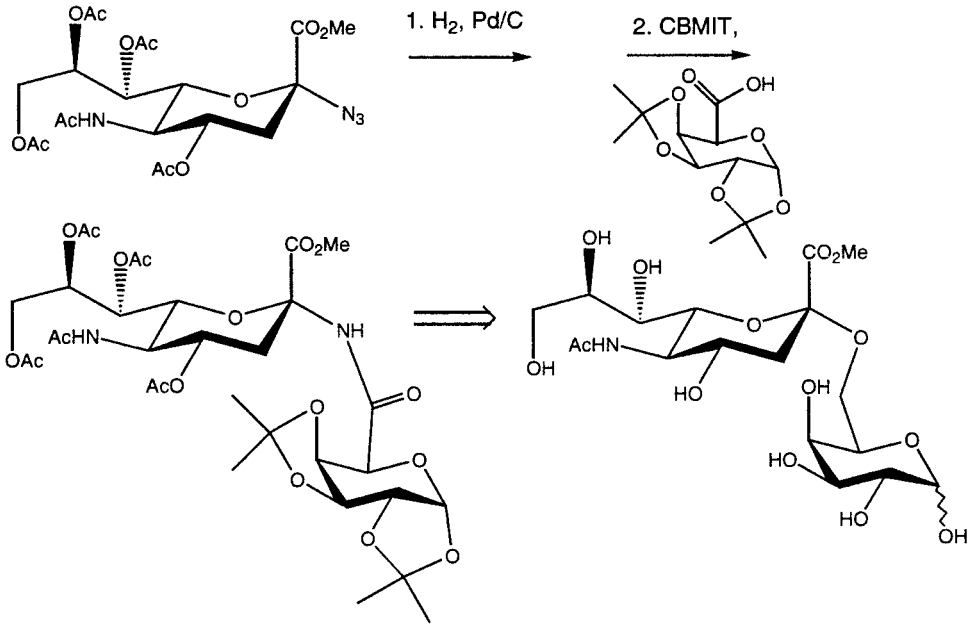


Figure 25.

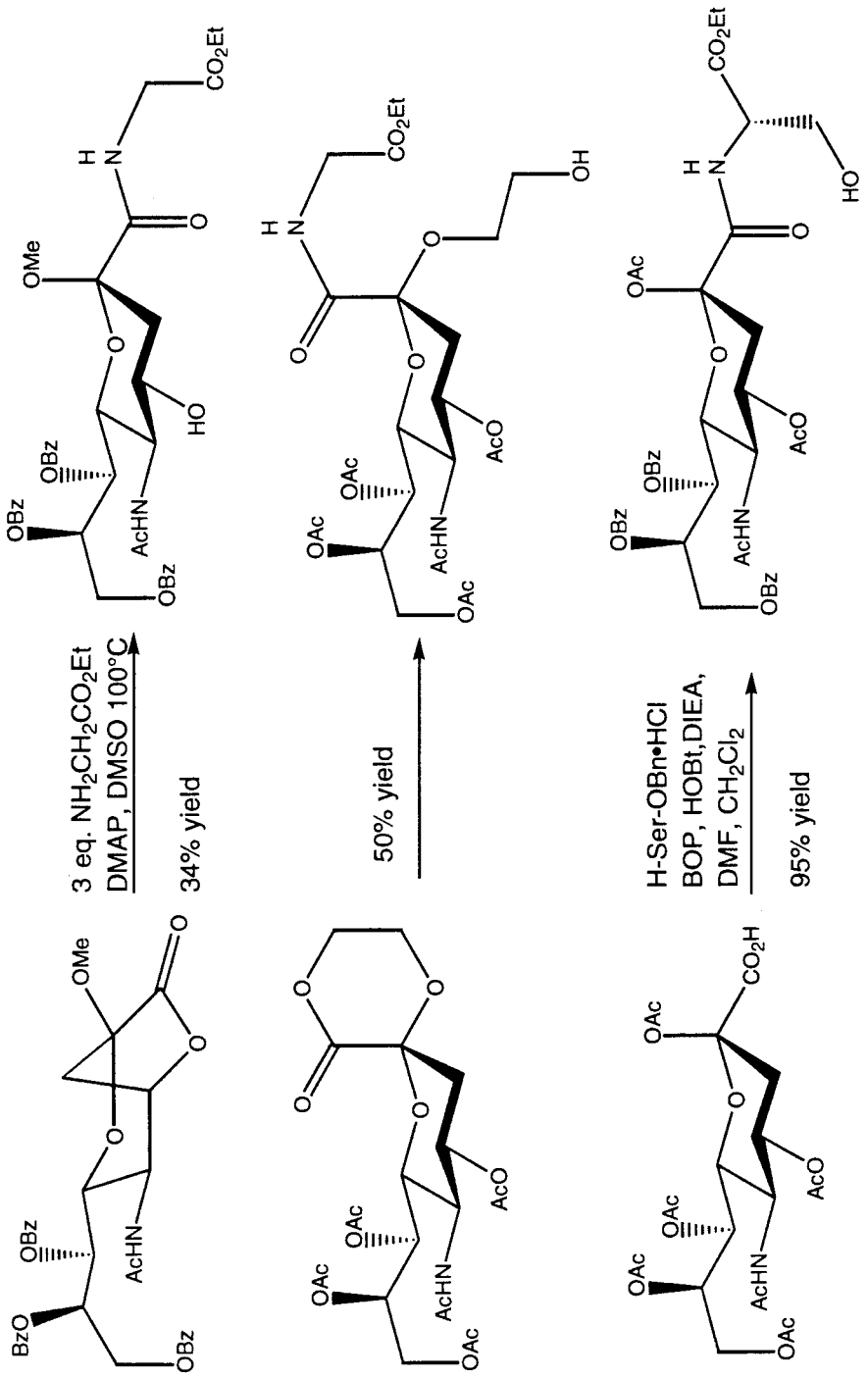


Figure 26.

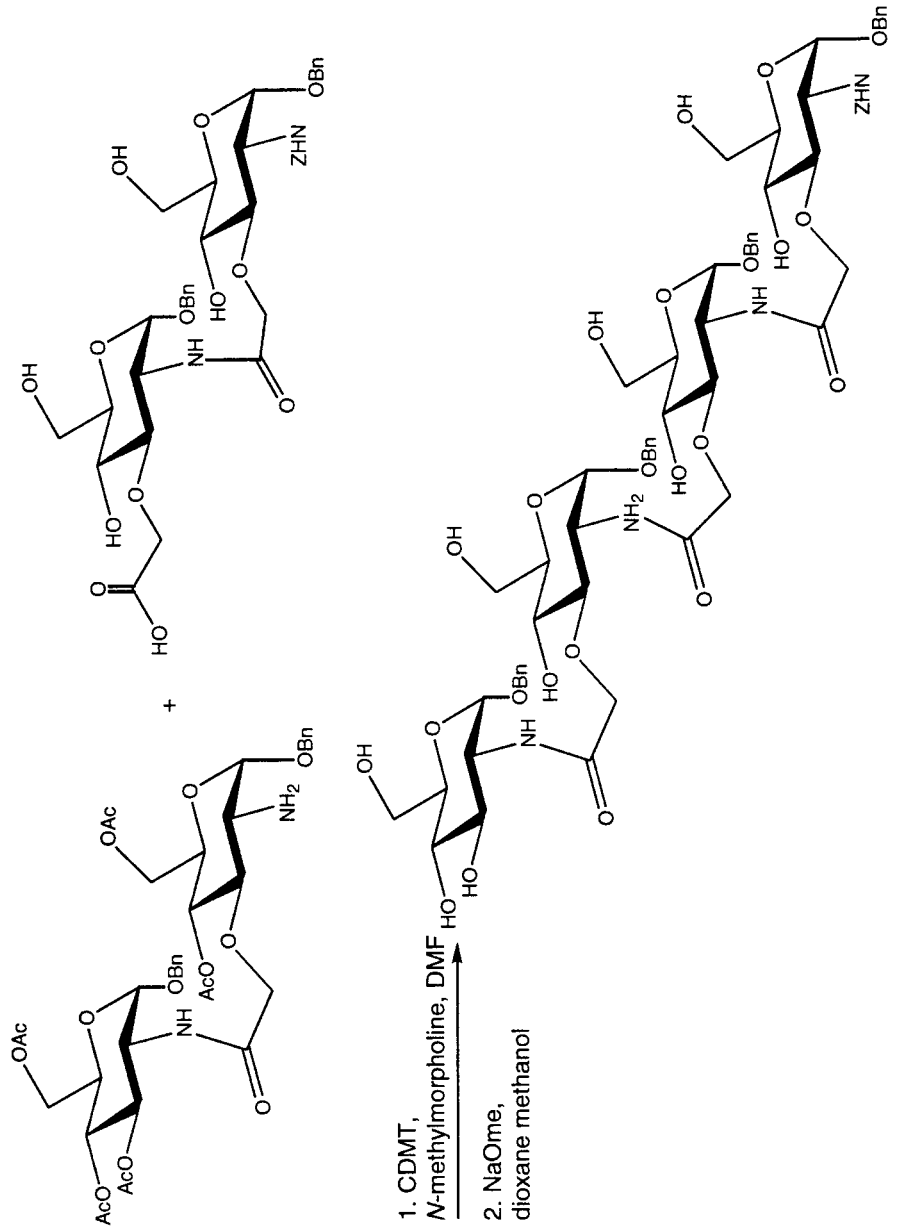


Figure 27.

Wessel later used solid phase synthesis to prepare a tetramer of amido-linked 2-amino-2-deoxyglucuronic acids.[50] In that disclosure, benzyl 2-fluoren-9-ylmethoxycarbonyl (Fmoc) amino-2-deoxy-α-D-glucuroniside was immobilized on Rink resin. After deprotection of the Fmoc group with piperidine, an Fmoc-protected amino glucuronic acid was added and amide formation was promoted by TATU [*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate]. Iteration of this procedure culminated in the construction of a tetramer. Some difficulties encountered in immobilizing the first sugar residue may have been due to steric congestion at the resin surface. Subsequent couplings readily occurred, suggesting that the first residue may have served to extend the reaction centers away from the polymer bead (Figure 28).

The Ichikawa group also made amido-linked oligomers derived from amino *C*-glycoside carboxylic acids.[51] A Boc-protected *β*-sugar amino acid was conjugated to phenylalanine and subsequently deprotected to give the free amine, which was coupled to another Boc-protected monomer. Diethylphosphoryl cyanide and triethylamine were used to activate the acid for coupling in the solution phase. A tetramer was synthesized and subsequently sulfated to increase solubility and to introduce negative charge (Figure 29).

This material was designed as a potential inhibitor of HIV replication, since it was known that sulfated polymeric carbohydrates inhibit HIV entry into T cells.

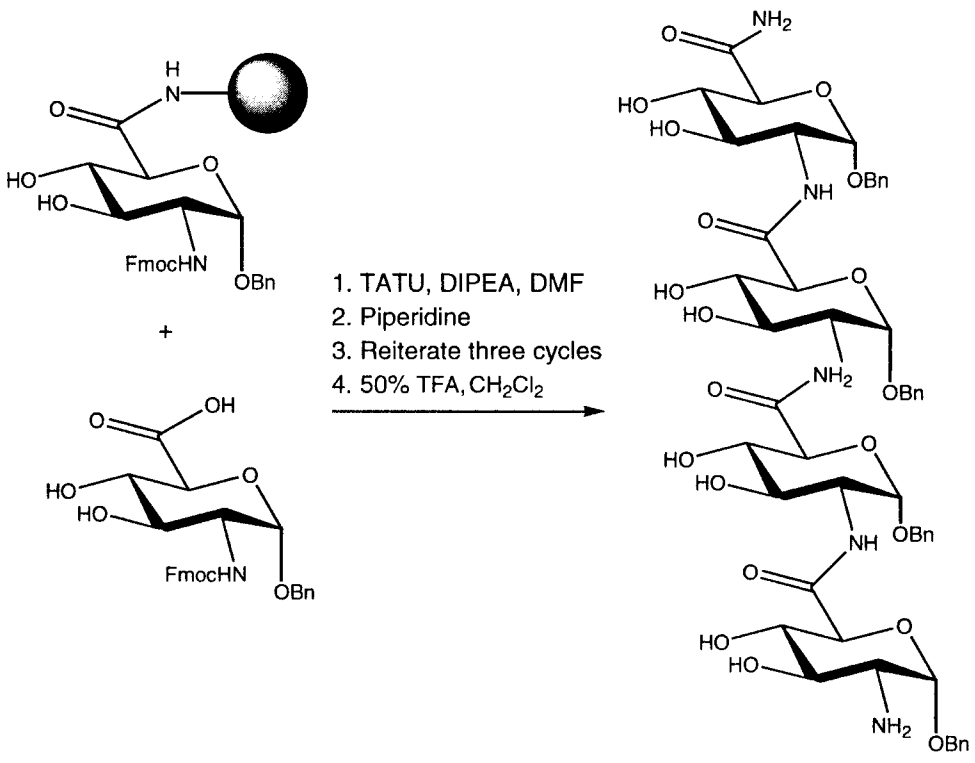


Figure 28.

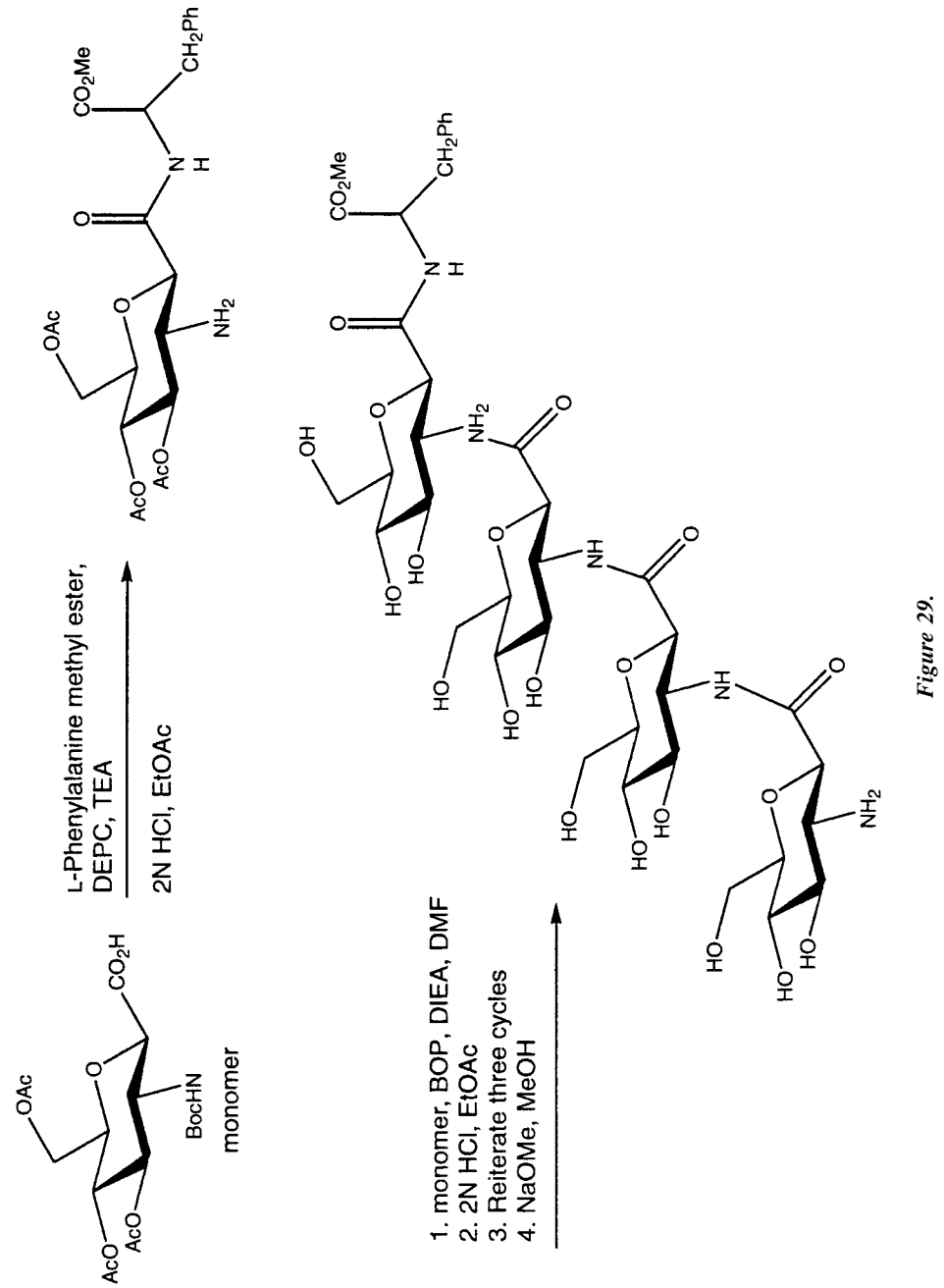


Figure 29.

Syncytium formation was completely blocked when CD4 cells were infected with HIV in the presence of 50 μM concentrations of the sulfated tetramer. Later, an even more potent inhibitor ($IC_{50} = 1$ μM) was prepared from a sulfated tetramer comprised of amido-linked C7-amino-C1 heptonic acid monomers.[52]

Goodnow et al. targeted anti-sense agents in their syntheses of amido-linked pyranosyl nucleosides.[53] Carbohydrate-based nucleic acids were sought because peptide nucleic acids self-aggregate, creating solubility and cellular entry problems. It was hoped that the rigid backbone of the amide-linked sugars would provide a scaffold allowing the bases to align in a Watson–Crick base-pairing fashion with DNA and/or RNA. Solid phase methods were used to prepare two nucleoside analogs of 2-amino-2-deoxyglucuronic acid and incorporate them into oligomers. Both the 10-residue and the 13-residue oligomers were water soluble (Figure 30). The binding affinities of the two oligomers for selected DNA and RNA oligomers were determined from duplex formation and melt temperature measurements. The 10-mer bound complementary antiparallel DNA approximately 5°C lower than a known peptide

10-mer Bases =TCACTAGATG
13-mer bases = TCTTCCTCTCTCT
Carboxy termini capped with L-lysine

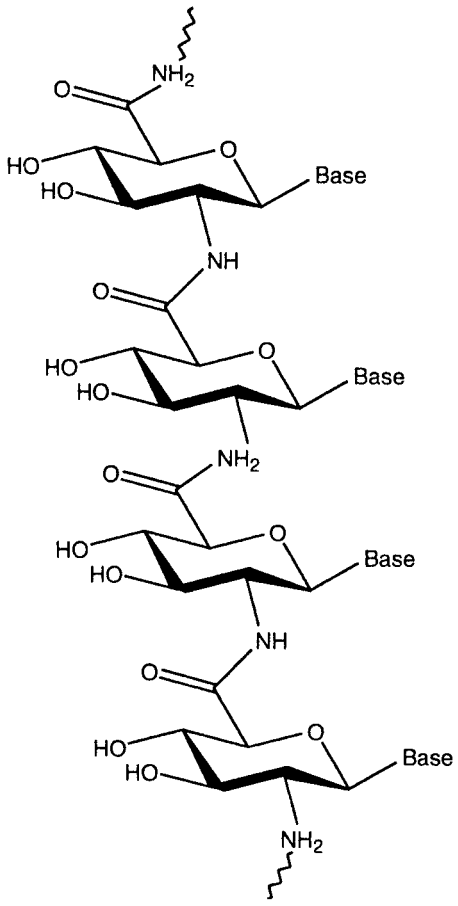
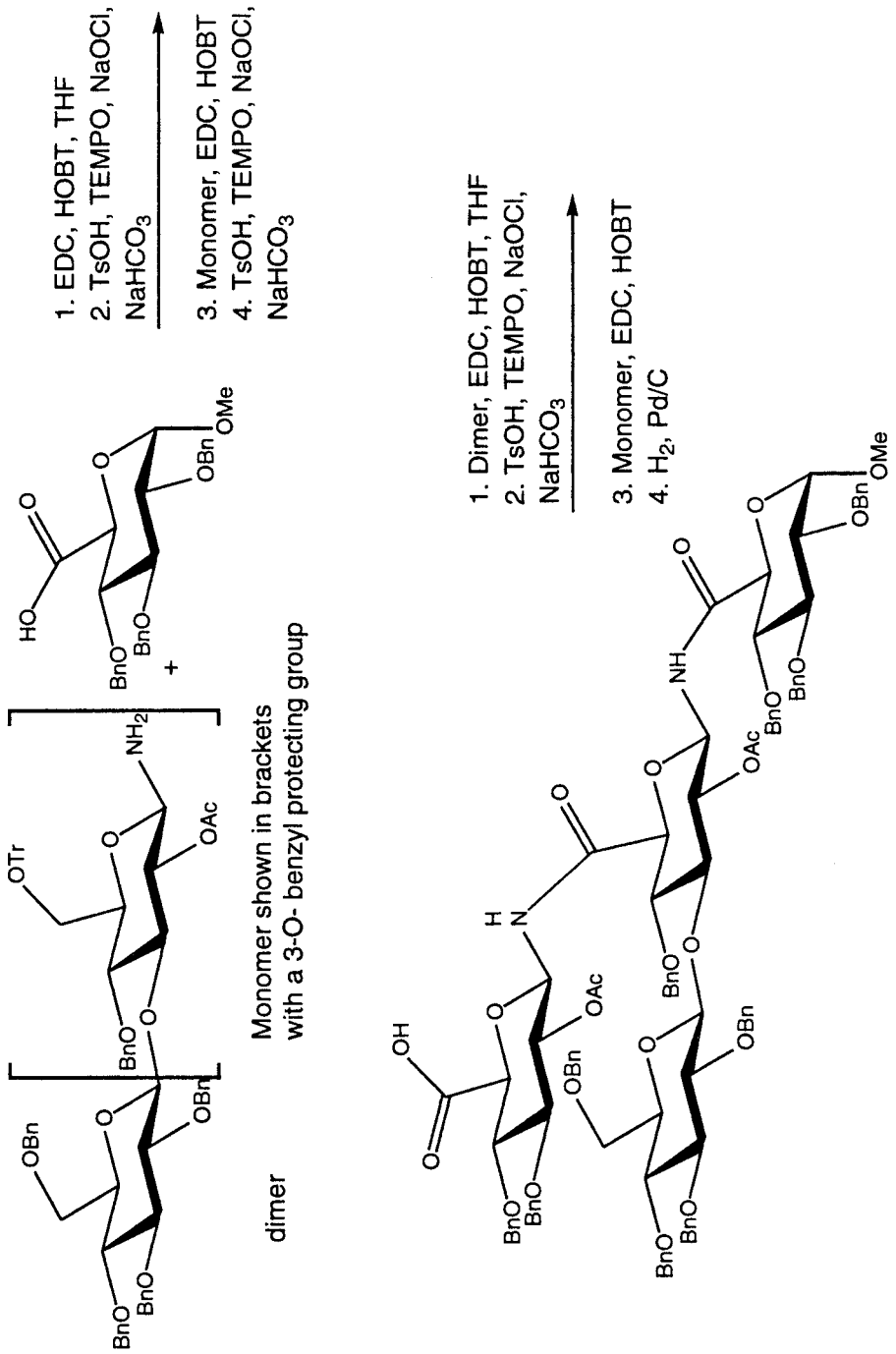


Figure 30.



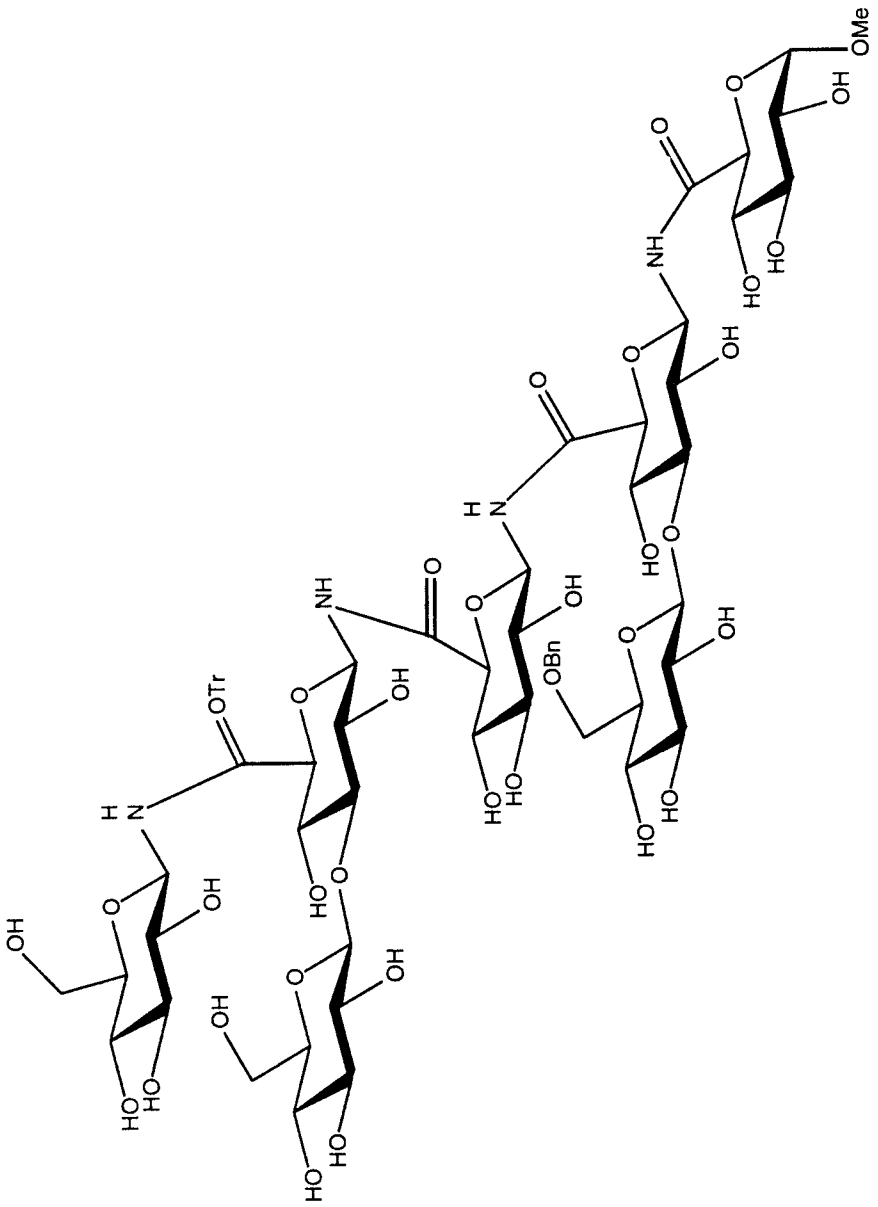


Figure 31.

nucleic acid, strongly supporting the Watson–Crick model. The amide-linked oligomers showed binding affinities for DNA that were greatly enhanced over glycosidically linked analogs.

The van Boom laboratories investigated the possibility of replacing glycosidic linkages with amide linkages to enhance the biological activity of a phytoalexin elicitor.[54] Earlier, they had shown that an amidically terminated, branched hexasaccharide was as potent as the naturally occurring sugar terminating in a reduced sugar moiety. From a series of structure-activity relationship studies, they deduced that the conformation of the glycosidic linkages along the backbone was critical for the observed activity. Based on this information, they questioned whether an amidically linked backbone structure would show improved phytoalexin elicitor activity. A glycosidically linked dimer was converted to an amino acid equivalent and amidated with a terminating sugar at the anomeric position. The C6 carboxyl functionality was readied for chain elongation with a monomeric sugar amino acid, which was subsequently condensed with a glycosidically linked dimeric sugar amino acid. Finally, the carboxy terminus was capped (Figure 31).

Subsequent biological studies showed that the amide-linked analogs were completely inactive. These results, when contrasted with the work of Ichikawa and Goodnow, suggest that amide-linked carbohydrates may serve better as unique molecular scaffolds than as glycosidic linkage mimics.

SYNTHESES AND STRUCTURAL CHARACTERIZATION OF AMIDO-LINKED OLIGOMERS WITH STABLE SECONDARY STRUCTURE

Non-Carbohydrate-Based Materials

Although this chapter is focused on carbohydrate-based compounds, it is important to briefly describe the pioneering work of Gellman and Seebach directed to the syntheses of amido-linked oligomers derived from β- and γ-amino acids that are not based on carbohydrates. These researchers and others[55] have engineered systems that adopt stable helical, sheet, and turn conformations in solution. Surprisingly, in many cases as few as four residues is sufficient to stabilize the conformation. This completely contrasts with oligomers derived from α-amino acids which typically require many more residues before conformational stability is established.[56]

Gellman introduced the term ''foldamer'' to refer to ''any polymer with strong tendency to adopt a specific compact conformation.''[57] Early work from his group exemplified the rational design process. Computer-assisted analysis of several amido-linked β-amino acids led to the proposal that *trans* 2-amino hexanoic acid and *trans* 2-amino pentanoic acid would form stable helical structures. Modeling predicted that the hexanoic system would form a 14-helix and the pentanoic system a 12-helix (Figure 32). These structures were synthesized and their conformations were experimentally verified using a combination of NH/ND exchange rates, circular dichroism (CD), and X-ray crystallography.

Seebach et al. reported the synthesis of β- and γ-peptides with stable secondary structures[58] and, independently, Hanessian et al. reported the synthesis of γ-peptides

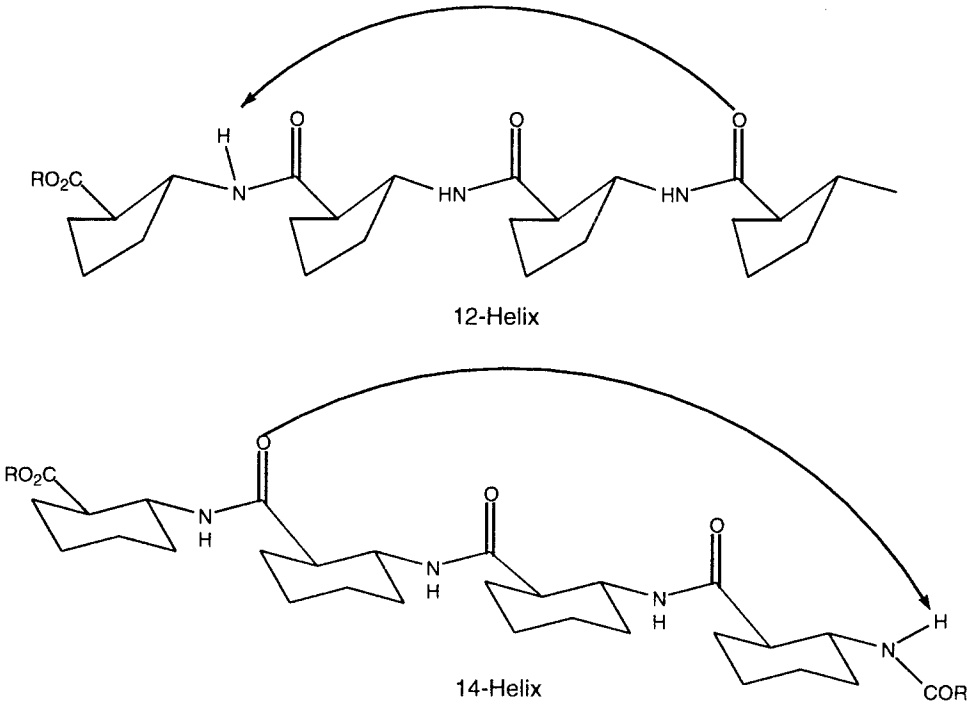


Figure 32.

that adopt helical structures in solution.[59] These combined studies showed important structural characteristics for β- and γ-peptides in contrast with α-peptides. For example, α-peptides adopt helical conformations stabilized by a 13-membered hydrogen bond between the carbonyl of the amino terminus and the amide NH of the fourth residue toward the C-terminus (3.6 residues per turn). β-Peptides formed a 14-membered hydrogen bond between the carbonyl of the C-terminus and the NH of the amide two residues toward the N-terminus (3 residues per turn) (Figure 33).[60] Seebach's γ-peptides formed a 14-membered hydrogen bond between the carbonyl of the N-terminus and the carbonyl two residues toward the C-terminus (2.6 residues per turn).[61]

Of the many elegant studies reported by Gellman and coworkers, two are particularly relevant to the discussion at hand. The first was the formation of water-soluble β-amino acid hexamers with stable helices in water.[62] Four different systems containing positively charged amine functionalities were prepared (Figure 34). The charge promoted water solubility and, at the same time, prevented aggregation.[63] Structural analyses using the aforementioned techniques confirmed that these oligomers adopted a 14-helix conformation in aqueous solution. Demonstrating that conformationally stable, water-soluble candidates could be prepared was the first step toward using these new materials to solve biologically relevant problems.

The Gellman laboratories later reported the chemical synthesis of a 17-mer based on the 12-helix forming *trans*-2-aminocyclopentanoic acids.[64] Charged amine functionalities in combination with neutral monomer units were incorporated into the oligomer to provide an amphiphilic secondary structure (Figure 35). When tested for

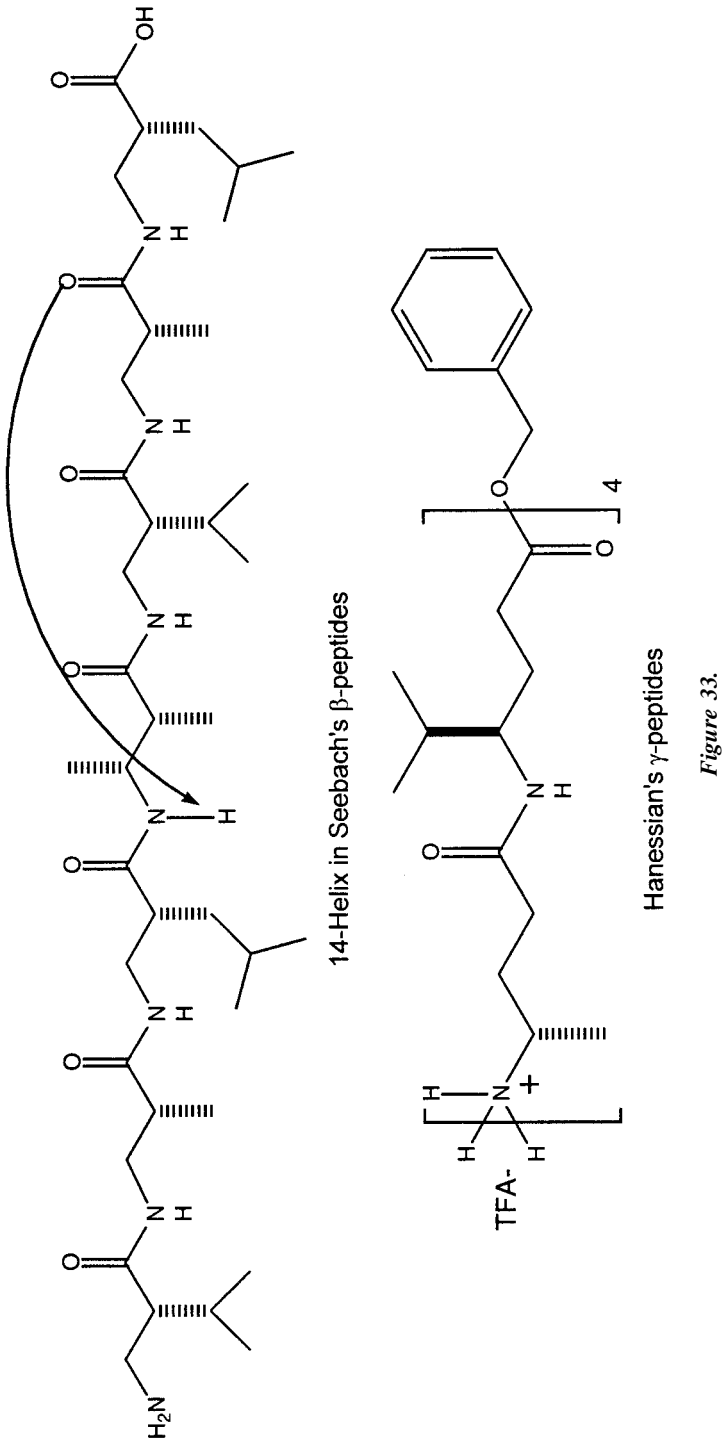


Figure 33.

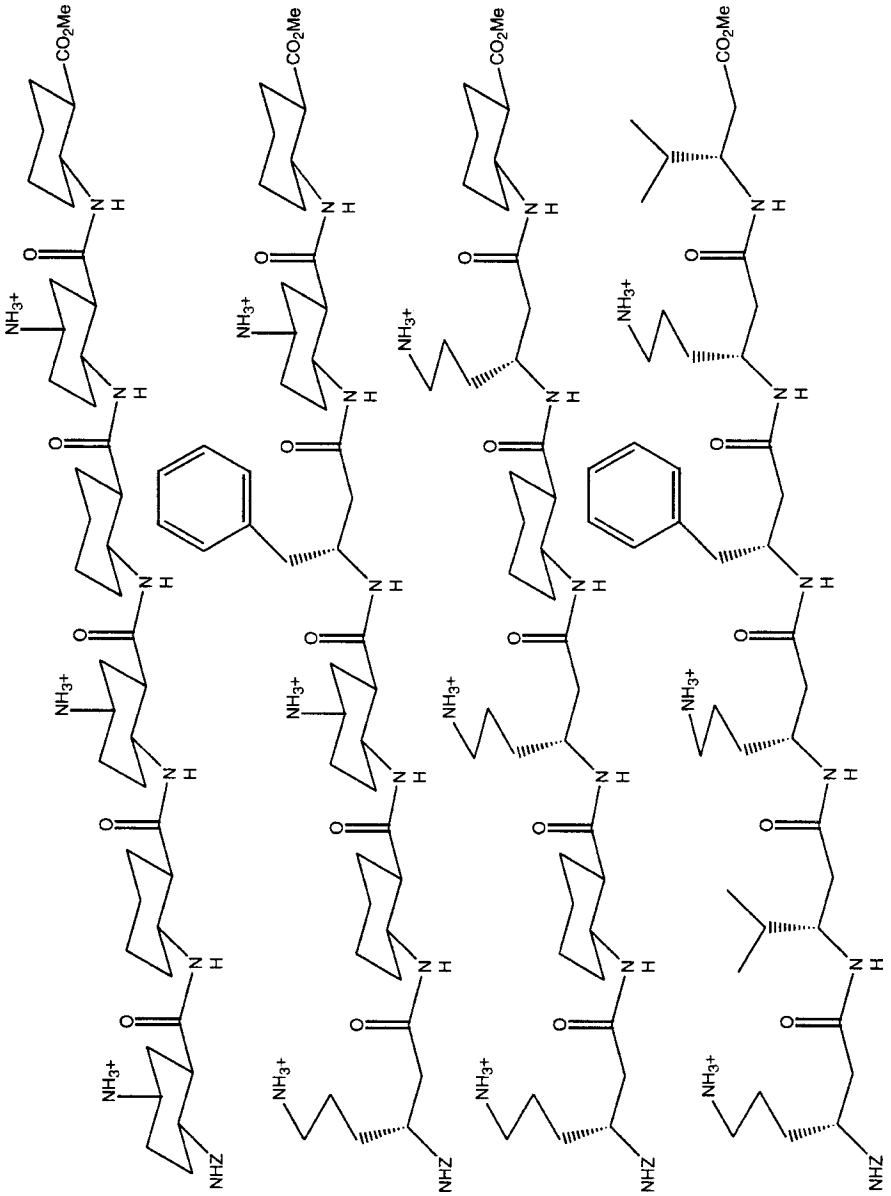


Figure 34.

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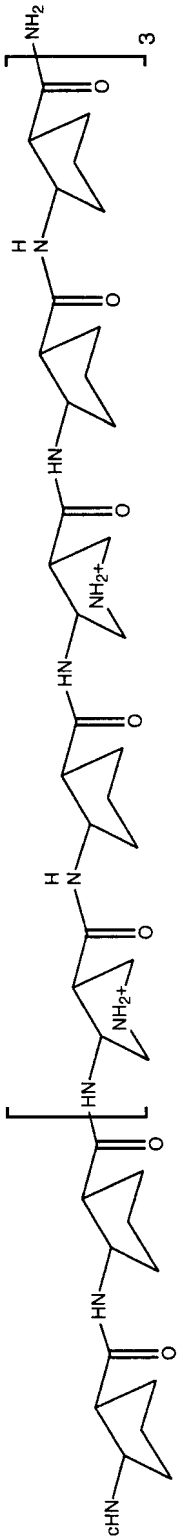

Figure 35.

bactericidal and bacteriostatic activity against a vancomycin-resistant bacterial strain, this material exhibited activity similar to that of a known 23-mer α-peptide inhibitor. Moreover, the β-peptide was less destructive to red blood cells than was the α-peptide.

In addition to their many structural studies on β- and γ-peptides, Seebach and coworkers showed that human receptors accept β-peptides that mimic natural α-peptides.[65] They modeled a cyclic β-tetrapeptide placing phenylalanine, tryptophan, lysine, and threonine-like side chains in key positions designed to mimic a cyclic α-octapeptide derived from somatostatin (Figure 36).

Standard peptide chemistry that relied on Boc and benzyl protecting group strategies was used in the synthetic construction of the tetrapeptide. The protected form of the cyclic peptide was not soluble under normal conditions required for hydrogenolysis; however, the researchers were able to reduce the benzyl protecting group in THF with 6 equiv of lithium chloride. Radioligand binding assays were conducted to measure human somatostatin receptor affinities for the β-tetrapeptide relative to the α-octapeptide. The β-peptide was active in the micromolar range, whereas only nanomolar concentrations were required for the α-peptide. Although the β-peptide was less active, it was shown to be peptidase resistant, an important consideration in terms of pursuing related compounds in the future.

Carbohydrate-Based Oligomers with Secondary Structure

Amide-linked carbohydrate-based oligomers with defined secondary structures were first engineered by Szabo et al.[66] β-Methoxy neuraminic acid was used as the

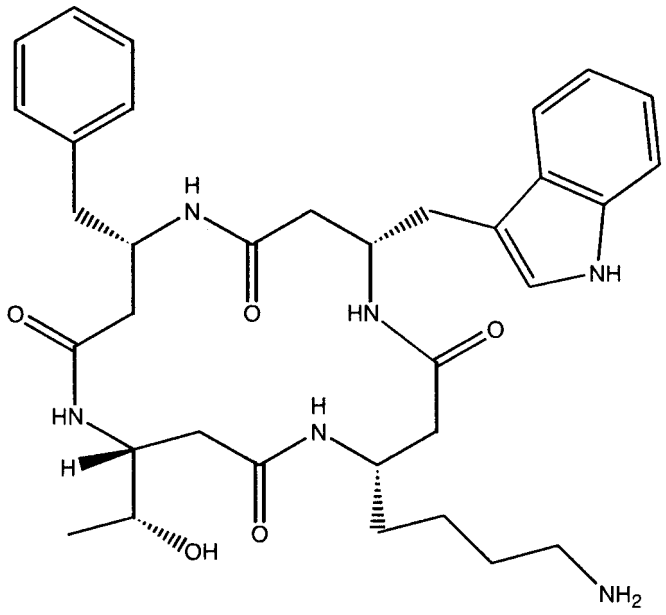

Figure 36.

monomer unit, and solid phase techniques were employed in the construction of oligomers ranging from dimer to octamer. Caproamide was incorporated at the carboxy terminus to prevent solvation of the terminal residue, a phenomenon commonly referred to as fraying (Figure 37). The hydrophobic end group provided directionality to the growing chain, fashioned after a membrane-bound oligomer. It was anticipated that these combined factors would stabilize backbone hydrogen bonding interactions and lead to relatively small molecules with stable secondary structures.

Caproamide was immobilized on Rink resin, and Fmoc-protected β-methoxy neuraminic acids were condensed by using BOP and DIEA in *N*-methylpyrrolidinone (NMP). Flaherty et al. had earlier reported that solution phase coupling of neuraminic acids was sluggish, and only poor yields were realized (48 h, 25–30%).[40] In contrast, the solid phase syntheses were completed in a few hours, as determined by the Kaiser test, and combined yields ranged from 44 to 55% indicating that each coupling step was far more efficient (Figure 38).

β-Methoxy NeuAc was used as a sugar amino acid in the construction of oligomers for three primary reasons. First, it was readily available from a naturally occurring δ-amino acid. Second, it was hypothesized that the trihydroxy side chain would increase water solubility in higher order oligomers. Finally, *O*-glycoside oligomers of NeuAc were known to have stable secondary structure in aqueous solution, and it seemed possible that amide-linked analogs would exhibit similar properties.[67]

The second hypothesis was proved by the chemical syntheses of the oligomers, which were all shown to be highly water soluble. The oligomer secondary structures were probed using a combination of NH/ND exchange studies and CD, patterned after the studies of Gellman and Seebach. The exchange studies were originally performed in dimethyl sulfoxide (DMSO) because they were too rapid to be observed in water. The half-lives of NH/ND exchange were determined for the series of oligomers ranging from dimer to octamer. The dimer exchanged rapidly (half-life $\sim$30 min). Two different exchange rates were observable for the trimer; the amino terminus amide exchanged fastest, on the same order as the dimer. The half-life of the internal amide was approximately 6 h. This was also true of the tetramer; the reducing end amide exchanged relatively quickly, but the internal amides took several hours. The rapid exchange of the reducing end amide was attributed to fraying. For the most part, as the

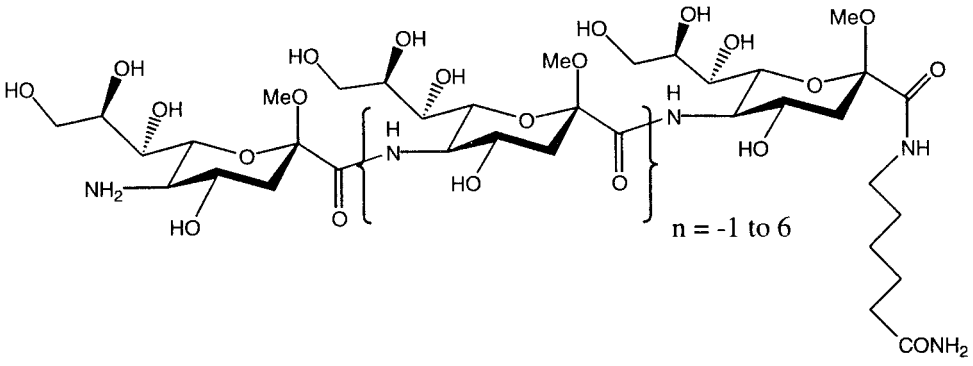

Figure 37.

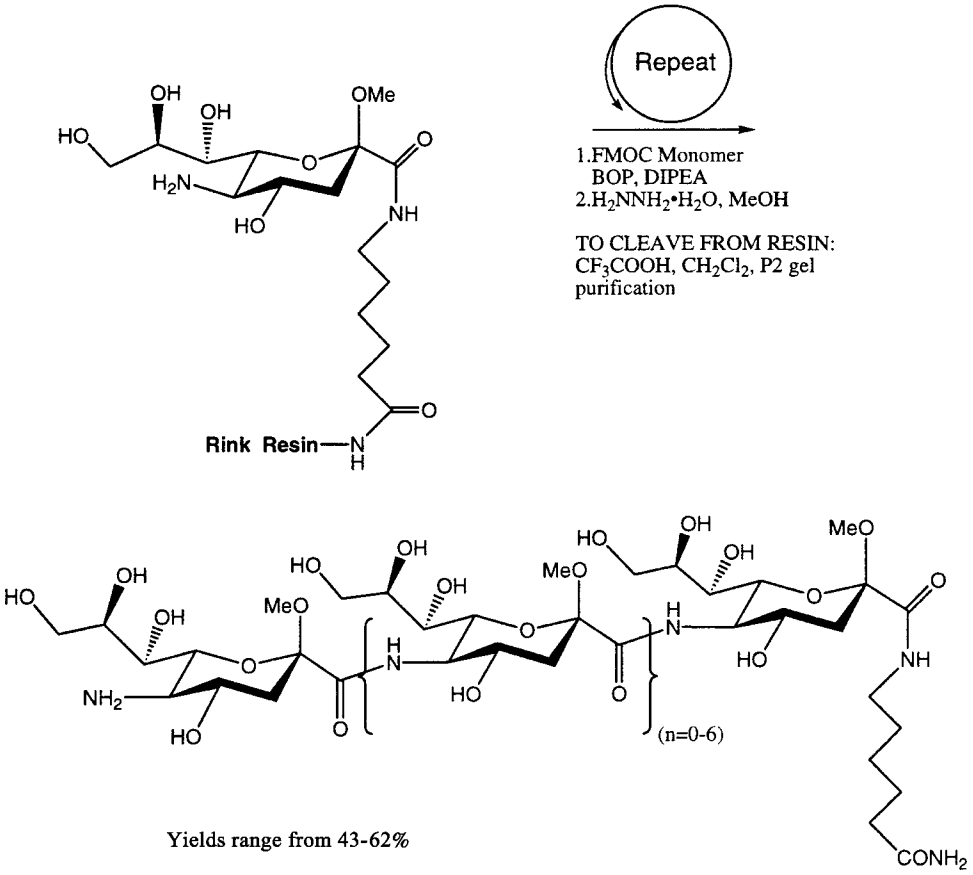

Figure 38.

oligomer length increased, the exchange rates of the internal amides slowed (the octamer was the slowest).

CD spectra were recorded in water at neutral pH, and they correlated with the NH/ND studies (performed in DMSO) surprisingly well. The dimer, lacking an internal amide, did not show a signature CD. The other oligomers displayed an absorbance maximum at $\sim$200 nm with a zero crossover at $\sim$212 nm and a minimum at 200 nm, returning to zero at $\sim$240 nm. The intensity of the maximum at 200 nm consistently increased with increasing length. These data completely correlated increasing secondary structural stability with increasing oligomer length. Shortly after this report, Fleet and coworkers reported that furanosyl-derived amindo-linked sugar amino acids also adopt stable secondary structures in solution (Figure 39).[68]

Gregar and Gervay-Hague recently prepared a series of α-methoxy NeuAc amido-linked oligomers and compared them to the β-methoxy series.[69] The first noticeable difference was the NH/ND exchange rates, which were slow enough in water (pH = 3.0 phosphate buffer) to be measured. The exchange rates were fast for dimer and trimer, and essentially disappeared before the NMR spectra were acquired. The rates slowed

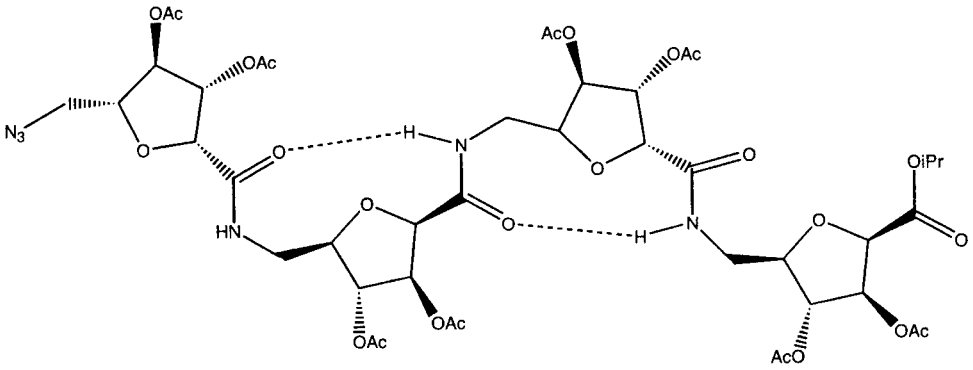

Figure 39.

for the tetramer, which exchanged similarly to the pentamer through octamer. CD spectra were also recorded in pH = 3.0 phosphate buffer solution. The CD signatures of these compounds showed a reverse trend from the β-methoxy series, with an absorbance minimum at $\sim$195 nm and a maximum at $\sim$230 nm, returning to zero at $\sim$260 nm (Figure 40). Interestingly, there was a dramatic increase in the intensity of the absorbencies in going from trimer to tetramer, but the spectra of the longer oligomers (5-mer through 8-mer) were similar to the tetramer, which correlated exactly with the NH/ND studies. The CD and NMR studies indicated that the α-methoxy series formed stable secondary structures with as few as four residues.

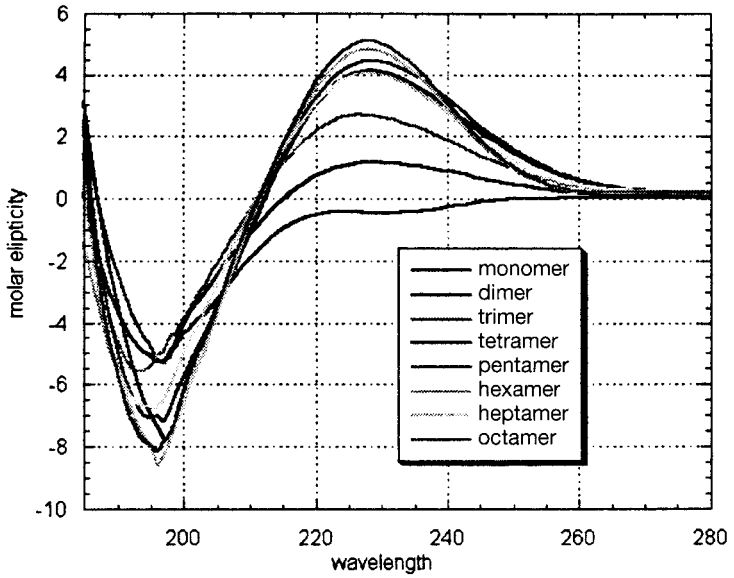

Figure 40. CD spectra of α-methoxy NeuAc amido-linked oligomers recorded in phosphate buffer solution (pH 3).

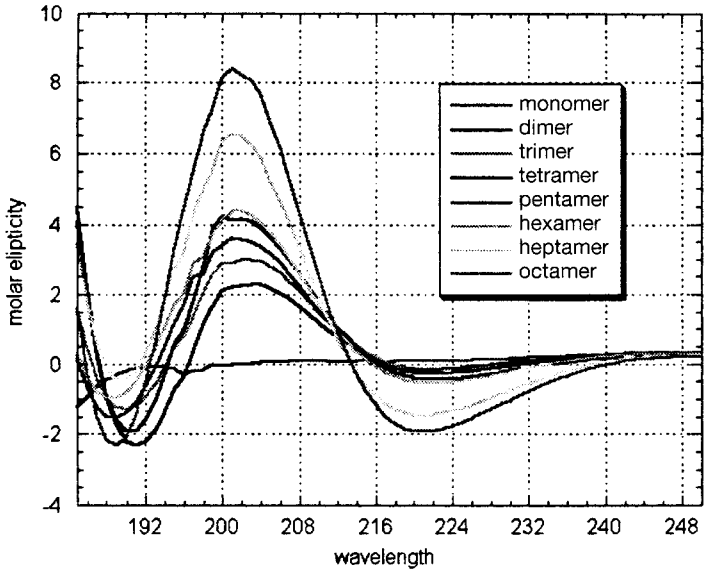

Figure 41. CD spectra of β-methoxy NeuAc amido-linked oligomers recorded in phosphate buffer solution (pH 3).

For comparison purposes, CD spectra of the β-methoxy series were recorded in pH 3.0 phosphate buffer solution. In contrast to the α-methoxy series, the octamer was clearly distinguishable from shorter oligomers, suggesting that it is significantly more stable (Figure 41). NH/ND exchange studies in pH 3.0 buffer showed that all oligomers exchanged rapidly with the exception of the octamer. These combined studies clearly showed that both α- and β-methoxy neuraminic acid-derived oligomers adopt stable secondary conformations in aqueous solution. However, the α series required only four residues for stability, whereas the β-series required eight.

THE FUTURE

There are many intended applications of the scientific inquiries described in this chapter, including 1) use of unnatural amino acids in the construction of libraries, 2) use of the oligomers as peptido mimetics, and 3) incorporation of the oligomers in artificial protein engineering (see below). Artificial amino acids have routinely been incorporated into natural peptides in pharmacophore drug design. However, rapid elimination half-lives and biochemical degradation often contribute to poor efficacy for many of these compounds. The development of nonnatural peptides may lead to drug candidates with improved bioavailability profiles, since they would be resistant to biochemical degradation through the action of proteases and peptidases.

Application to protein engineering requires the interaction of secondary structures to form tertiary structures. DeGrado and coworkers recently accomplished de novo design of helical bundles composed of natural α-peptides.[70] In earlier work, they

described three principles that guide the design process: 1) complementary packing within the hydrophobic core, 2) specific interfacial interactions to promote interhelical associations, and 3) core and interfacial interactions to destabilize potential alternate conformations.[71]

The distinctive packing of the amino acid side chains in the core of the bundle gives rise to a coiled coil, or superhelix. Coiled coils have biological relevance. For example, they are found in fibrous proteins such as fibrinogen and keratin[72] and are structural components of viral proteins.[73] De novo design of these macromolecular structures offers insight into the process of protein folding, in addition to providing new materials. The same would be true of de novo design of coiled coils composed of nonnatural peptides. As of this writing, 3° structure in nonnatural peptides has not been reported. However, Gellman has offered a blueprint for the design of coiled coils based upon β-peptides.[74]

The possibility of creating carbohydrate-based δ-peptides that adopt stable 3° structures in solution is also of great interest. Determining the conditions that promote oligomer aggregation is an important step toward engineering artificial proteins. Beyond the obvious biomedical uses as drug delivery agents, biosensors, and affinity materials, compounds with unique structural motifs could serve as chemical catalysts. For example, just as natural proteins provide a hydrophobic medium in aqueous solution, properly designed carbohydrate-based proteins could provide a hydrophilic environment in an organic medium, opening up new possibilities for chemical transformations.

It is clear that an idea born nearly 50 years ago has evolved into a rich science at the frontiers of synthetic design, structure elucidation, and materials production. Over the years, old chemistry was rediscovered and applied in new directions resulting in the birth of entirely new ideas. Many factors have contributed to the successes, including the introduction of combinatorial chemistry, the availability of biological screening assays, and the development of new and improved technologies for structure determination. Pyranosyl sugar amino acid conjugates will surely continue to provide a fertile field for scientific inquiry and discovery.

REFERENCES

1. Kornfeld, R.; Kornfeld, S. *The Biochemistry of Glycoproteins and Proteoglycans*; Lennarz, W.J., Ed.; Plenum Press: New York, 1980; Vol. XIV, 1–33.
2. Several other sources have also been identified: Torii, M.; Sakakiba, K.; Kuroda, K. Eur. J. Biochem. **1973**, *37*, 401–405. Karakawa, W.W.; Kane, J.A. J. Immunol. **1975**, *114*, 310–315. Liau, D.; Melly, M.A.; Hash, J.H. J. Bacteriol. **1974**, *119*, 913–922. Eguchi, H.; Kaya, S.; Araki, Y. Carbohydr. Res. **1992**, *231*, 147–158. Romanows, E.; Reinhold, V. Eur. J. Biochem. **1973**, *36*, 160–166. Reddy, G.P.; Hayat, U.; Xu, Q.W.; Reddy, K.V.; Wang, Y.H.; Chiu, K.W.; Morris, J.G.; Bush, C.A. Eur. J. Biochem. **1998**, *255*, 279–288. Williamson, A.R.; Zamenhof, S. J. Biol. Chem. **1963**, *238*, 2255–2258. Jeanes, A.; Burton, K.A.; Cadmus, M.C.; Knutson, C.A.; Rowin, G.L.; Sandford, P.A. Nat. New Biol. **1971**, *233*, 259. Fournier, J.M.; Vann, W.F.; Karakawa, W.W. Infect. Immun. **1984**, *45*, 87–93. Lee, J.C.; Michon, F.; Perez, N.E.; Hopkins, C.A.; Pier, G. B. Infect. Immun. **1987**, *55*, 2191–2197. Moreau, M.; Richards, J.C.; Fournier, J.M.; Byrd, R.A.; Karakawa, W.W.; Vann,

W.F. Carbohydr. Res. **1990**, *201*, 285–297. Ichihara, N.; Ishimoto, N.; Ito, E. FEBS Lett. **1974**, *40*, 309–311. Konig, H.; Kandler, O. Arch. Microbiol. **1979**, *123*, 295–299. Kochetkov, N.K.; Kholodkova, E.V.; Stanislavsky, E.S. Eur. J. Biochem. **1987**, *167*, 549–561. Wu, T.C.M.; Park, J.T. J. Bacteriol. **1971**, *108*, 874.

3. Clark, W.R.; McLaughlin, J. J. Biol. Chem. **1958**, *230*, 81. Williamson, A.R.; Zamenhof, S.J. Biol. Chem. **1963**, *238*, 2255. Hanessian, S.; Haskell, T.H. J. Biol. Chem. **1964**, *239*, 2758. Heyns, K.; Kiessling, G.; Lindenberg, W.; Paulsen, H.; Webster, M. Chem. Ber. **1959**, *92*, 2435.
4. Perkins, H.R. Biochem. J. **1963**, *86*, 475.
5. Williamson, A.R.; Zamenhoff, S. J. Biol. Chem. **1963**, *238*, 2255. Several other sources were later cited: Braneforshelander, P.; Kenne, L.; Lindberg, B.; Petersson, K.; Unger, P. Carbohydr. Res. **1981**, *97*, 285–291. Tsui, F.P.; Schneerson, R.; Boykins, R.A.; Karpas, A.B.; Egan, W. Carbohydr. Res. **1981**, *97*, 293–306. Karakawa, W.W.; Kane, J.A. J. Immunol. **1972**, *108*, 1199. Watson, P.R.; Sandford, P.A.; Burton, K.A.; Cadmus, M.C.; Jeanes, A. Carbohydr. Res. **1976**, *46*, 259–265.
6. Fisher, M.W.; Devlin, H.B.; Erlandson, A.L. Nature **1963**, *199*, 1074. Haskell, T.H.; Hanessian, S. Nature **1963**, *199*, 1075. Haskell, T.H.; Hanessian, S. Biochim. Biophys. Acta **1964**, *83*, 35. Hanessian, S.; Haskell, T.H. J. Biol. Chem. **1964**, *9*, 2758.
7. Subsequently several other examples were reported: Hofmann, P.; Jann, B.; Jann, K. Carbohydr. Res. **1985**, *139*, 261–271. Iwasaki, H.; Araki, Y.; Kaya, S.; Ito, E. Eur. J. Biochem. **1989**, *178*, 643–648. Dengler, T.; Jann, B.; Jann, K. Carbohydr. Res. **1986**, *150*, 233–240.
8. Lindberg, B. Adv. Carbohydr. Chem. Biochem. **1990**, *48*, 279–318.
9. Cummins, C.S.; White, R.H. J. Bacteriol. **1983**, *153*, 1388.
10. Dimitriev, B.A.; Lvov, V.; Tochtamysheva, N.V.; Shashkov, A.S.; Kochetkov, N.K.; Jann, B.; Jann, K. Eur. J. Biochem. **1983**, *134*, 517.
11. Fox, J.J.; Kuwada, Y.; Watanabe, K.A. Tetrahedron Lett. **1968**, 6029. Watanabe, K.A.; Kotick, M.P.; Fox, J.J. Chem. Pharm. Bull. **1969**, *17*, 416.
12. Sakata, K.; Sakurai, A.; Tamura, S. Agric. Biol. Chem. **1973**, *37*, 697. Sakata, K.; Sakurai, A.; Tamura, S. Agric. Biol. Chem. **1974**, *38*, 1883. Sakata, K.; Sakurai, A.; Tamura, S. Agric. Biol. Chem. **1975**, *39*, 885.
13. Lichtenthaler, F.W.; Morino, T.; Winterfeldt, W.; Sanemitsu, Y. Tetrahedron Lett. **1975**, *41*, 3527, and references therein.
14. Knapp, S. Chem. Rev. **1995**, *95*, 1859.
15. Knirel, Y.A.; Vinogradov, E.V.; Shashkov, A.S.; Dmitriev, B.A.; Kochetkov, N.K.; Stanislavsky, E.S.; Mashilova, G.M. Eur. J. Biochem. **1986**, *157*, 129.
16. Kenne, L.; Lindberg, B.; Schweda, E. Carbohydr. Res. **1988**, *180*, 285.
17. Heyns, K.; Paulsen, H. Chem. Ber. **1955**, *88*, 188.
18. Heyns, K.; Beck, M. Chem. Ber. **1957**, *90*, 2443.
19. Marsh, C.A.; Levvy, G.A. Biochem. J. **1958**, *68*, 617.
20. Weidmann, H.; Zimmerman, H.K., Jr. Ann. Chem. **1961**, *639*, 198.
21. Yoshimura, J.; Sato, T.; Ando, H. Bull. Chem. Soc. Jpn. **1969**, *42*, 2352. Yoshimura, J.; Sakai, H.; Oda, N.; Hashimoto, H. Bull. Chem. Soc. Jpn. **1972**, *45*, 2027.
22. Tsuji, A.; Kinoshita, T.; Maeda, M. Chem. Pharm. Bull. **1968**, *16*, 539.
23. Paulsen, H.; Koebernick, W.; Autschbach, E. Chem. Ber. **1972**, *105*, 1524.

24. Ogawa, T.; Akatsu, M.; Matsui, M. Carbohydr. Res. **1975**, *44*, C22.
25. Kotick, M.P.; Klein, R.S.; Watanabe, K.A.; Fox, J.J. Carbohydr. Res. **1969**, *11*, 359.
26. Paulsen, H.; Koebernick, W.; Autschbach, E. Chem. Ber. **1972**, *105*, 1524.
27. Fuchs, E.-F.; Lehmann, J. Carbohydr. Res. **1975**, *45*, 135–141.
28. Graf von Roedern, E.; Kessler, H. Angew. Chem. Int. Ed. Engl. **1994**, *33*, 687.
29. Fuchs, E.-F.; Lehmann, J. Chem. Ber. **1975**, *108*, 2254–2260.
30. Kim, K.-I.; Hollingsworth, R.I. Tetrahedron Lett. **1994**, *35*, 1031.
31. Sakai, N.; Ohfune, Y. J. Am. Chem. Soc. **1992**, *114*, 998.
32. Ohfune, Y.; Kurokawa, N. Tetrahedron Lett. **1984**, *25*, 1587.
33. Axon, J.R.; Beckwith, A.L.J. J. Chem. Soc. Chem. Commun. **1995**, 549.
34. Simchen, G.; Pürkner, E. Synthesis **1990**, 525.
35. Kunz, H.; Burgard, A.; Schanzenbach, D. Angew. Chem. Int. Ed. Engl. **1997**, *36*, 386.
36. Drouillat, B.; Kellam, B.; Dekany, G.; Starr, M.S.; Toth, I. Bioorg. Med. Chem. Lett. **1997**, *17*, 2247.
37. Bashyal, B.P.; Chow, H.-F.; Fleet, G.W.J. Tetrahedron Lett. **1986**, *27*, 3205.
38. Sharon, N. *Complex Carbohydrates Their Chemistry, Biosynthesis and Function*; Addison-Wesley: London, 1975; 16.
39. Roy, R.; Pon, R.A. Glycoconj. J. **1990**, *7*, 3.
40. Gervay, J.; Flaherty, T.M.; Nguyen, C.N. Tetrahedron Lett. **1997**, *38*, 1493. Nguyen, C. Master's thesis, University of Arizona, 1998. Flaherty, T.M. Ph.D. dissertation, University of Arizona, 1997.
41. Gervay, J.; Gregar, T.Q. Tetrahedron Lett. **1997**, *34*, 5921.
42. Yoshimura, J.; Ando, H.; Sato, T.; Tsuchida, S. Bull. Chem. Soc. Jpn. **1976**, *49*, 2511.
43. Fuchs, E.-R.; Lehmann, J. Carbohydr. Res. **1976**, *49*, 267.
44. Drouillat, B.; Kellam, B.; Dekany, G.; Starr, M.S.; Toth, I. Bioorg. Med. Chem. Lett. **1997**, *17*, 2247.
45. Silva, D.J.; Wang, H.; Allanson, N.M.; Jain, R.K.; Sofia, M. J. Org. Chem. **1999**, *64*, 5926.
46. Sabesan, S. Tetrahedron Lett. **1997**, *38*, 3127.
47. Gervay, J.; Ramamoorthy, P.S.; Mamuya, N.N. Tetrahedron **1997**, *53*, 11039.
48. Ramamoorthy, P.S.; Gervay, J. J. Org. Chem. **1997**, *62*, 7801.
49. Nicolaou, K.C.; Flörke, H.; Egan, M.G.; Barth, T.; Estevez, V.A. Tetrahedron Lett. **1995**, *36*, 1775.
50. Müller, C.; Kitas, E.; Wessel, H.P. J. Chem. Soc. Chem. Commun. **1995**, 2425.
51. Suhara, Y.; Hildreth, J.E.K.; Ichikawa, Y. Tetrahedron Lett. **1996**, *37*, 1575–1578.
52. Suhara, Y.; Ichikawa, M.; Hildreth, J.E.K.; Ichikawa, Y. Tetrahedron Lett. **1996**, *37*, 2549–2552.
53. Goodnow, R.A., Jr.; Tam, S.; Pruess, D.L.; McComas, W.W. Tetrahedron Lett. **1997**, *38*, 3199.
54. Timmers, C.M.; Turner, J.J.; Ward, C.M.; van der Marel, G.A.; Kouwijzer, M.L.C.E.; Grotenhuis, P.D.J.; van Boom, J.H. Chem. Eur. J. **1997**, *3*, 920.
55. Hanessian, S.; Luo, X.; Schaum, R.; Michnick, S. J. Am. Chem. Soc. **1998**, *120*, 8569.
56. Rohl, C.A.; Baldwin, R.L. Biochemistry **1994**, *31*, 7760.
57. Gellman, S.H. Acc. Chem. Res. **1998**, *31*, 173–180.

58. (a) Seebach, D.; Ciceri, P.E.; Overhand, M.; Jaun, B.; Rigo, D.; Oberer, L.U.; Amstutz, R.; Widmer, H. Helv. Chim. Acta **1996**, *79*, 2043–2066. (b) Seebach, D.; Matthews, J.L.; Meden, A.; Wessels, T.; Baerlocher, C.; McCusker, L.B. Helv. Chim. Acta **1997**, *80*, 173–182. (c) Seebach, D.; Abele, S.; Sifferlen, T.; Hänggi, M.; Gruner, S.; Seiler, P. Helv. Chim. Acta **1998**, *81*, 2218–2243. (d) Abele, S.; Guichard, G.; Seebach, D. Helv. Chim. Acta **1998**, *81*, 2141. (e) Gademann, K.; Jaun, B.; Seebach, D.; Perozzo, R.; Scapozza, L.; Folkers, G. Helv. Chim. Acta **1999**, *82*, 1–11.
59. (a) Hannesian, S.; Luo, X.; Schaum, S.; Michnick, S. J. Am. Chem. Soc. **1998**, *120*, 8569. (b) Hannessian, S.; Luo, X.; Schaum, R. Tetrahedron Lett. **1999**, *40*, 4925–4929.
60. Appella, D.H.; Barchi, J.J., Jr.; Durell, S.R.; Gellman, S.H. J. Am. Chem. Soc. **1999**, *121*, 2309–2310.
61. Seebach, D.; Abele, S.; Gademann, K.; Guichard, G.; Hintermann, T.; Jaun, B.; Matthews, J.L.; Schreiber, J.V. Helv. Chim. Acta **1998**, *81*, 932–982. (b) Seebach, D.; Abele, S.; Gademann, K.; Jasun, B. Angew. Chem. Int. Ed. Engl. **1999**, *38*, 1595–1597.
62. Appella, D.H.; Barchi, J.J., Jr.; Durell, S.R.; Gellman, S.H. J. Am. Chem. Soc. **1999**, *121*, 2309.
63. Gung, B.W.; Zou, D.; Stalcup, A.M.; Cottrell, C.E. J. Org. Chem. **1999**, *64*, 2176. For a related study on water-soluble β-peptides see.
64. Porter, E.A.; Wang, X.; Lee, H.-S.; Weisblum, B.; Gellman, S.H. Nature **2000**, *404*, 565.
65. Gademann, K.; Ernst, M.; Hoyer, D.; Seebach, D. Angew. Chem., Int. Ed. Engl. **1999**, *38*, 1223.
66. Szabo, L.; Smith, B.L.; McReynolds, K.D.; Parril, A.L.; Morris, E.R.; Gervay, J. J. Org. Chem. **1998**, *63*, 1074.
67. Brisson, J.-R.; Baumann, H.; Imberty, A.; Pérez, S.; Jennings, H.J. Biochemistry **1992**, *31*, 4996–5004.
68. Fleet and coworkers have reported a number of successes in this area. The reader is referred to the rich literature provided by this laboratory, which is outside the scope of the present chapter: Estevez, J.C.; Arcron, H.; Wormald, M.R.; Brown, D.; Fleet, G.W.J. Tetrahedron Lett. **1994**, *47*, 8885. Estevez, J.C.; Arcron, H.; Wormald, M.R.; Brown, D.; Fleet, G.W.J. Tetrahedron Lett. **1994**, *47*, 8889. Krülle, T.M.; Watson, K.A.; Gregoriou, M.; Johnson, L.N.; Crook, S.; Watkin, D.J.; Griffiths, R.C.; Nash, R.J.; Tsitsanou, K.E.; Zographos, S.E.; Oikonomakos, N.G.; Fleet, G.W.J. Tetrahedron Lett. **1995**, *45*, 8291. Brandstetter, T.W.; Wormald, M.R.; Dwek, R.A.; Butters, T.D.; Platt, F.M.; Tsitsanou, K.E.; Zographos, S.E.; Oikonomakos, N.G.; Fleet, G.W.J. Tetrahedron Asymm. **1996**, 157. Estevez, J.C.; Smith, M.D.; Lane, A.L.; Crook, S.; Watkin, D.J.; Besra, G.S.; Brennan, P.J.; Nash, R.J.; Fleet, G.W.J. Tetrahedron Asymm. **1996**, 387. Estevez, J.C. Burton, J.W.; Estevez, R.J.; Ardron, H.; Wormald, M.R.; Dwek, R.A.; Brown, D.; Fleet G.W.J. Tetrahedron Asymm. **1998**, 2137. Smith, M.D.; Long, D.D.; Marquess, D.G.; Claridge, T.D.W.; Fleet, G.W.J. J. Chem. Soc. Chem. Commun. **1998**, 2039.
69. Gregar, T.Q.; Gervay-Hague, J. J. Am. Chem. Soc. Submitted.
70. Walsh, S.T.; Cheng, H.; Bryson, J.W.; Roder, H.; DeGrado, W.F. Proc. Natl. Acad. Sci. U.S.A. **1999**, *96*, 5486–5491.

71. Betz, S.F.; Liebman, P.A.; DeGrado, W.F. Biochemistry **1997**, *36*, 2450–2458.
72. (a) Pauling, L.; Corey, R.B. Nature **1953**, *171*, 59–61. (b) Crick, F.H.C. Acta Crystallogr. **1953**, *6*, 685–697.
73. Wilson, I.A.; Skehel, J.J.; Wiley, D.S. Nature **1981**, *289*, 366–373.
74. Appella, D.H.; Christianson, L.A.; Karle, I.L.; Powell, D.G.; Gellman, S.H. J. Am. Chem. Soc. **1999**, *121*, 6206–6212.