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Review Article

Glycopeptide analgesics

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Summary

Excluding insulin, the global market for peptide pharmaceuticals is expected to grow from $350 million in 1999 to over $800 million by 2005 (1). Despite their advantages of potency, specificity and low toxicity, peptides have not gained the rapid acceptance by the pharmaceutical industry that one might have predicted in the 1970s, chiefly due to difficulties in peptide manufacture and poor bioavailability. Results from several studies now demonstrate that O-linked glycosylation of peptides can promote the serum stability of several classes of peptides *in vivo,* and can promote the transport of enkephalin analogs across the blood-brain barrier (BBB).

Recent work with glycosylated enkephalin analogs suggests that the incorporation of a glycosylated serine or threonine residue promotes transport across the BBB, which resulted in an AUC increase of over 30-fold in rats. Antinociception assays with mice demonstrate that glycosylated enkephalins can compete with morphine in efficacy, even when administered peripherally. Proper placement of the glycoside moiety is necessary in order to avoid perturbation of the mu/delta selectivity of the parent peptide pharmacophore.

Introduction

Peptide pharmaceuticals have gained some acceptance as therapeutics, with oxytocin (2), insulin (3), ciclosporin (4) and salmon calcitonin (5-7) being widely used in childbirth, diabetes, immunosuppression and osteoporosis, respectively. In addition to these drugs, a number of new peptide drugs are beginning to enter the marketplace: abarelix (8) and goserelin (9, 10) for the treatment of prostate cancer and endometriosis (GnRH antagonists), pramlintide acetate for the treatment of diabetes (11), as well as T-20 and T-1249 for the treatment of resistant strains of HIV (12). One might have predicted a more rapid introduction of peptide pharmaceuticals, but problems with manufacturing (costs) and bioavailability (oral activity) have led to a cautious approach to peptides by major pharmaceutical companies. It is expected that peptides will gain acceptance as advances in synthetic methodologies are made and as drug delivery problems are solved.

The presumption that there was a receptor or receptors within the brain that mediated the effects of opium existed long before experimental proof did. The endogenous opiates (the endorphins and enkephalins) first appeared on the horizon in the early 1970s. In 1975 Kosterlitz *et al.* (13) isolated the enkephalins from the brains of pigs. This discovery was followed in 1976 by the hypothesis by Martin *et al.* (14) that there must be discrete opiate receptor classes to distinguish between morphine-like agonists and the enkephalins. In 1977, the theory of multiple opiate receptors was supported by the work of Kosterlitz and Lord (15). A new era of understanding brain function and the mechanisms of pain modulation had begun. The endogenous neuropeptide β-endorphin was isolated and shown to promote analgesia similar to morphine, and may represent the body's endogenous μ agonist. In 1979 the κ agonist dynorphin A was isolated by Goldstein *et al.* (16), which defined the three classes of opiate receptor agonists, μ, δ and κ. The enkephalins, with either a leucine or a methionine at the C-terminus (5th position), are now accepted as the endogenous δ-opiate receptor ligands. All of these peptides are collectively termed the opioid peptides.

Despite immense success with the design of potent μ- or δ-selective opioid peptides, one problem still exists: how can peptide drugs be delivered to the site of action? Glycosylation of peptides has been shown to dramatically extend their serum lifetimes and alter their biodistribution *in vivo.* Given the fact that nature uses glycosylation to dispatch glycopeptide hormones and large

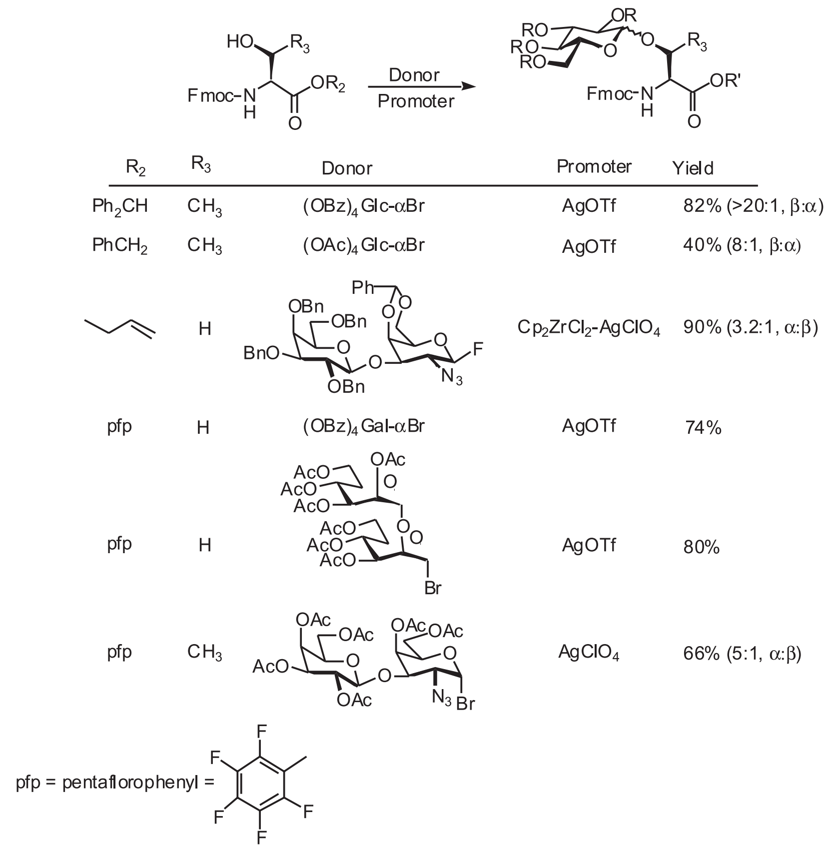


Fig. 1. Glycosylation of Fmoc esters.

glycoproteins to various intracellular compartments, different extracellular membranes and remote receptor targets, it should not be surprising that a small peptide (MW < 3000) should be greatly affected by the introduction of one or more glycosides. Initially, work in this area was hampered by a lack of effective synthetic methods.

Since the works of Emil Fischer at the turn of the century, carbohydrate chemistry has gained acceptance within the organic community, but only by fits and starts. Recently, carbohydrate chemistry was referred to as “a hostile scientific frontier” (17). Glycosylation reactions can be performed with a wide range of substrates and glycosyl donors, providing variable yields and anomeric selectivities of the product (18-20). This field has been extensively reviewed (21-23). Thus, only methods specific to O-linked glycopeptides will be discussed here.

In vivo, the glycosylation of peptides and proteins occurs cotranslationally in the endoplasmic reticulum (N-linked glycoproteins), or posttranslationally in the Golgi apparatus (O-linked glycoproteins). *In vitro*, however, glycosylation of intact peptides has not proven to be a successful approach (24). Thus, glycopeptide construction can be classified into two separate, but equally important aspects: 1) construction of the requisite glycosylated amino acid building blocks and 2) assembly of the amino acids into a glycopeptide in a stepwise fashion, typically using solid-phase methods.

Synthesis of glycosyl amino acids

Several effective syntheses of glycosyl amino acids from serine or threonine derivatives have been disclosed. With respect to the final glycopeptide assembly, an Fmoc solid-phase synthetic strategy is optimal. Boc strategies have been far less successful, as the repeated acid deprotections required generally cleave the acid-labile glycosidic linkages. Likewise, benzyloxycarbonyl protection (Cbz- or Z-protection) has not proven to be useful. Consequently, glycopeptide assembly based on Fmoc amino acids or activated Fmoc amino esters have been most successful (Fig. 1). Fmoc benzyl and benzhydryl esters have been utilized as effective glycosyl acceptors (25-28), in addition to Fmoc-allyl esters (29) and pentafluorophenoxy esters (30). While each method has advantages and disadvantages, pentafluorophenoxy esters are activated esters and can be directly incorporated into solid-phase synthesis schemes, although they seem to be less active than HOBT esters formed during amino acid activation *in situ.*

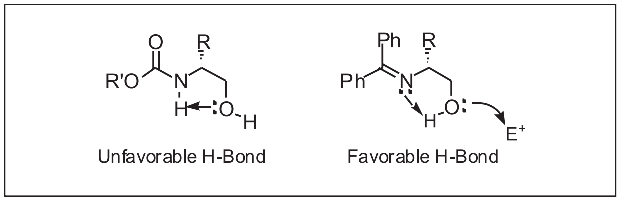
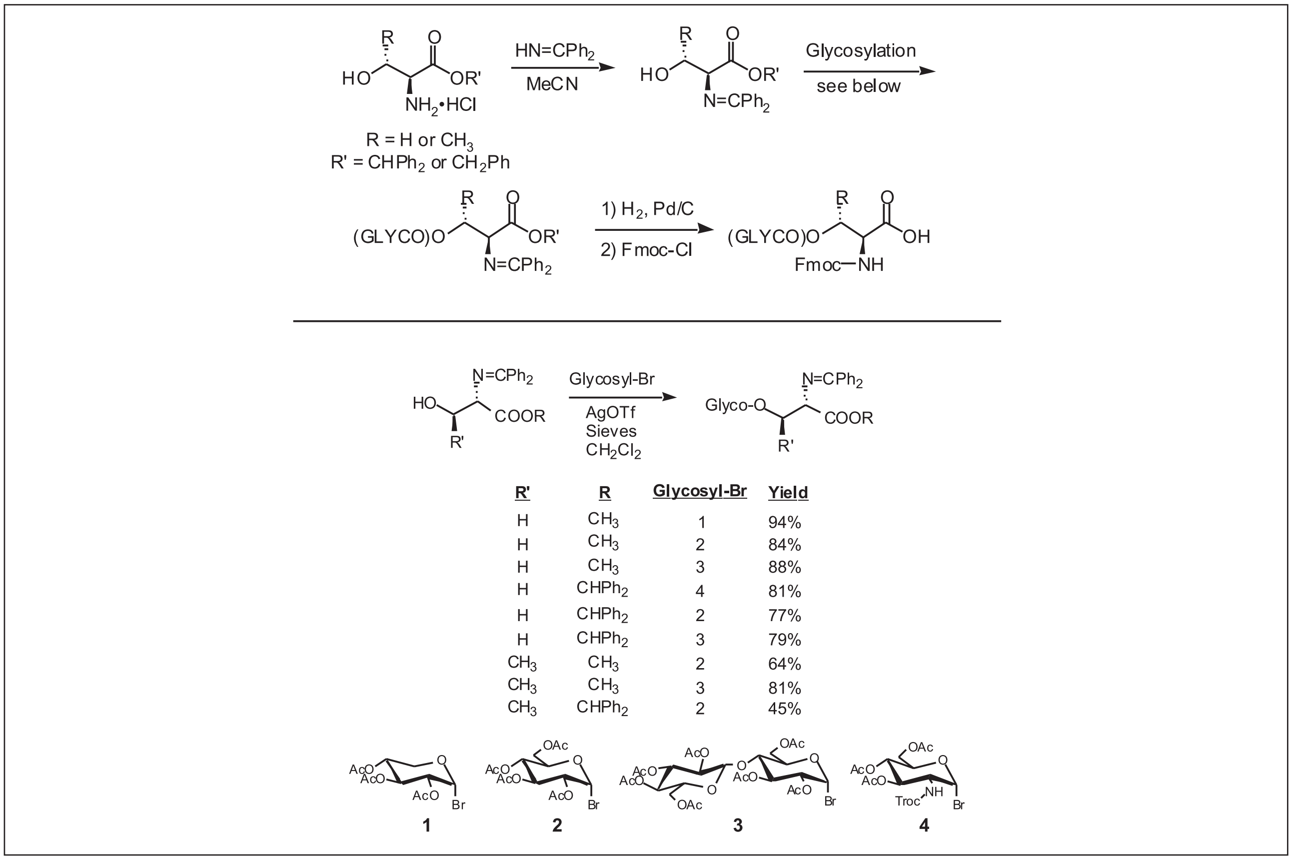


Fig. 2. Hydrogen bonding of imines *versus* carbamates.

Unfortunately, while Fmoc protection is nearly ideal for glycopeptide assembly, it is not always ideal for glycosylation of the amino acid. Fmoc protection will sometimes provide lower yields, when compared to other nitrogen protecting groups. In these instances, alternate N-protection, followed by conversion to the Fmoc amino acid after glycosylation can be the most effective route (31). Benzophenone-derived imines have proven to be excellent choices for this process (32). This functionality not only creates a more nucleophilic acceptor by favorable hydrogen bonding (Fig. 2), but can also be removed simply by hydrogenolysis or by acid-catalyzed hydrolysis. The by-products ($PhCH_2Ph$ or $Ph_2C{=}O$, respectively) are easily separated from the desired amino acid glycosides.

The synthesis of these glycosyl acceptors begins with serine or threonine (Fig. 3) protected as the benzyl or benzhydryl ester (33). The amino acids can then be further protected as the Schiff base derivative by the use of commercial diphenylketimine in CH_2Cl_2, MeCN or DMF (34). In this sequence, glycosylations were conducted with a glycosyl bromide as the donor and silver triflate as the promoter, although other promoters (*e.g.*, Hg or Zn salts) have been used. Deprotection proceeded by hydrogenolysis, which not only served to cleave the benzyl/benzhydryl ester but also to remove the imine in high yield. The nitrogen was then reprotected in the same reaction vessel with Fmoc-Cl to provide the target glycosyl amino acid, now ready for Fmoc solid-phase peptide synthesis. This strategy has been performed with an array of carbohydrates, with glycosylation yields ranging from 63-94%. While the N-protecting group switch adds steps to the synthesis, the yields are generally high, making subsequent purifications straightforward. In addition, this method has also been shown to be effective in the synthesis of 2-acetamido-2-deoxy α-glycosides (35).

Kihlberg and coworkers have synthesized glycosyl amino acids directly from the Fmoc carboxylic acid. Using Fmoc serine, threonine or cysteine and a Lewis acid promoter such as $BF_3{\cdot}Et_2$ or $SnCl_4$ (Fig. 4), the corresponding glycosylations proceeded in 33-58% yield (36). This approach was also used for the glycosylation of Fmoc protected tyrosine and hydroxyproline (37). While



R'	R	Glycosyl-Br	Yield
H	CH_3	1	94%
H	CH_3	2	84%
H	CH_3	3	88%
H	$CHPh_2$	4	81%
H	$CHPh_2$	2	77%
H	$CHPh_2$	3	79%
CH_3	CH_3	2	64%
CH_3	CH_3	3	81%
CH_3	$CHPh_2$	2	45%

Fig. 3. Schiff base glycosylation route.

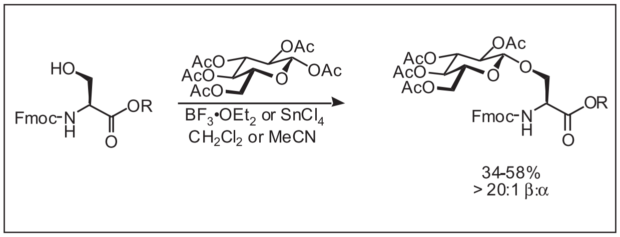


Fig. 4. Direct glycosylation of Fmoc amino acids.

this procedure eliminates the need for expensive and toxic heavy metal promoters and an ester deprotection step, it generally results in lower yields than the previous examples.

Glycopeptide assembly

Once glycosyl amino acids are in hand, stepwise assembly can commence. Numerous procedures for the synthesis have been devised. Most involve a solid support, which is cleaved at the end of the synthesis to yield the target glycopeptide. Many resins are commercially available and suitable for this purpose, including, but not limited to Wang (38), Pal (39), Rink amide, Rink acid (40) and Sasrin (41) resins. The amino acid and glycosyl amino acid couplings can be accomplished with a variety of coupling agents also, such as DCC/HOBT, BOP/HOBT and HATU. However, care must be taken during synthesis, as there are a number of potential pitfalls: 1) glycosides are acid-labile and subject to cleavage under many conditions, 2) glycosides can undergo β-elimination under strongly basic conditions, 3) glycosyl amino acids are very bulky residues, which can lead to incomplete coupling of the glycosylated residue and the following amino acid residue, as well as longer coupling times and 4) longer coupling times can lead to increased racemization of the amino acid residues.

Paulsen et al. reported an elegant glycopeptide synthesis using Fmoc amino anhydrides and Wang resin as a solid-support (42) (Fig. 5). The first coupling relied on Fmoc alanine amino acid anhydride and DMAP. The residue was then deprotected with piperidine and acylated with the glycosyl amino acid using DCC/HOBT. The Fmoc deprotection was then repeated, and the remaining amino acids were coupled to the resin in a similar fashion. All remaining amino acids were Fmoc protected, except for the final one, which was Boc protected. Cleavage from the resin was then effected using 95% TFA, which not only gave the protected peptide but also cleaved the N-terminal BOC group to yield the free glycopeptide. It was notable that the final acid cleavage proceeded in high yield, with no apparent glycoside cleavage.

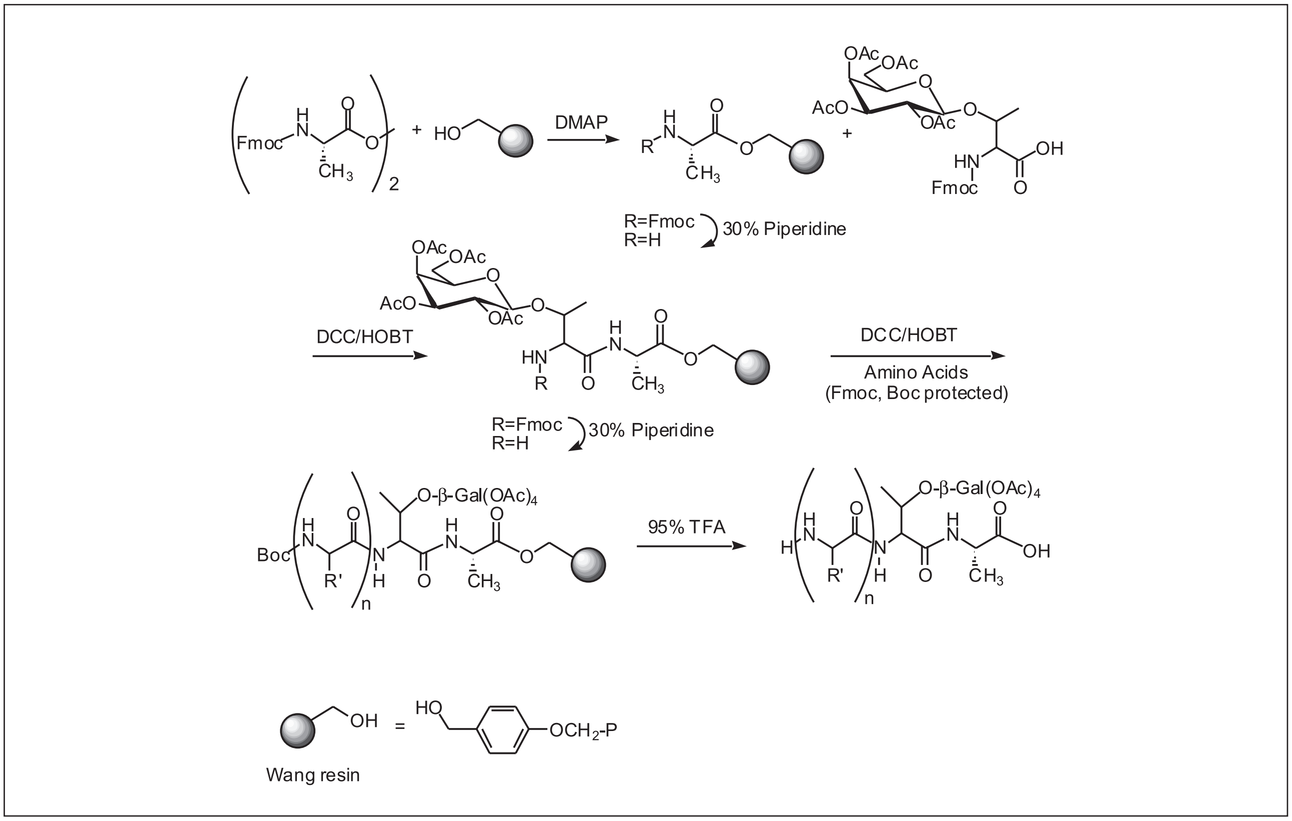


Fig. 5. Paulsen glycopeptide synthesis.

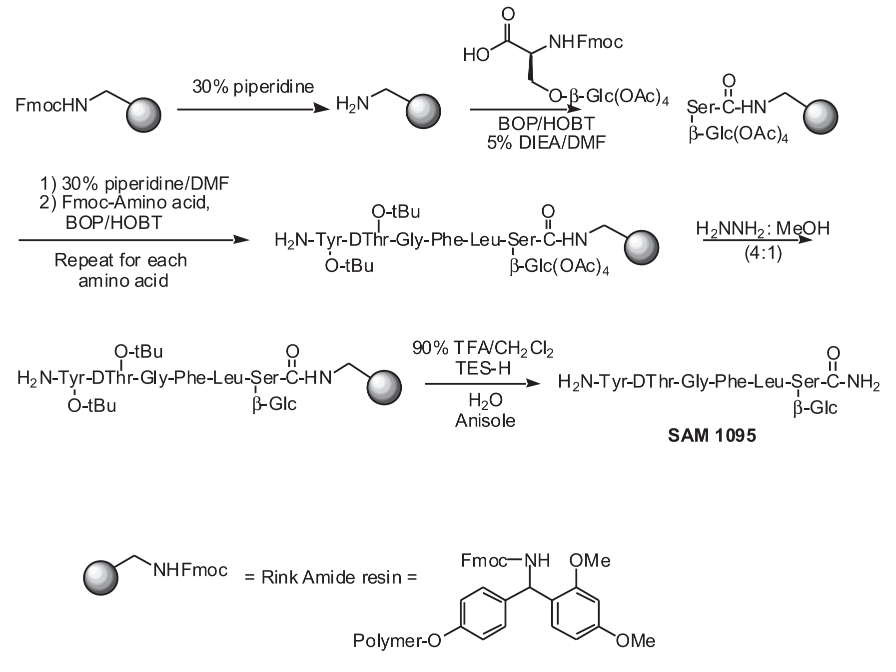


Fig. 6. SAM-1095 synthesis.

One drawback to the Paulsen approach is the use of NaOMe/MeOH for cleavage of the acetate protecting groups on the carbohydrate after cleavage from the resin support. Use of aqueous hydrazine for hydroxyl deprotection can be applied while the assembled glycopeptide is still on the resin, and was first used for this purpose by the Kunz group (43). Generally speaking, benzoyl protection is more robust than acetate and should be avoided. In our laboratories, Rink amide resin has been utilized and assembly has been conducted in a fashion similar to the Paulsen synthesis, but has been modified by the incorporation of hydrazine treatment to deprotect the carbohydrate while still on the resin. The synthesis of SAM-1095, a potent acyclic glycopeptide analgesic, is presented (Fig. 6) as an example for the synthesis of compounds of this type. The pharmacology of this drug and related compounds will be discussed in due course.

After Fmoc deprotection of the resin, acylation of the free amine then proceeded with serine β-glucoside using BOP/HOBT. Glycoside couplings are generally slower than unglycosylated amino acid couplings and were allowed to react for 2 h. The resin was then washed and the deprotection/acylation cycle was repeated for all remaining amino acids. After the final residue had been coupled, the Tyr Fmoc was cleaved and acetate removal was achieved using 4:1 hydrazine hydrate:methanol. In the final step, the resin was subjected to 90% TFA/CH_2Cl_2. This not only cleaved the crude glycopeptide from the resin to produce the C-terminal amide but also removed the *tert*-butyl protection from Tyr and D-Thr, to provide the crude glycopeptide which was then purified by reversed phase HPLC.

Message-address segment theory

Many studies of bioactive peptides invoke the “message-address” theory, the pioneering work in this area having been done by Robert Schwyzer (44). This theory of membrane-catalyzed peptide-receptor interactions states that opioid receptor agonists have two segments: a “message” and an “address” segment. An amphipathic helix may act as the address portion to guide the drug to the receptor. This is achieved by reversible insertion into the membrane to which the receptor is bound. Once at the proper address, the message is delivered and subsequent binding to the receptor leads to initiation of the neurological response (45). In opioid receptor activation, part of the drug selectivity (μ *vs.* δ *vs.* κ) depends on the address provided. According to Schwyzer, the δ receptor (binding site for enkephalins and endorphins) projects farthest into the aqueous exterior of the cell, the μ receptor (morphine binding site) resides in the anionic phospholipid head region and the κ receptor (dynorphin binding site) is buried within the lipid portion of the membrane (Fig. 7). Geographic differentiation between the receptors is thought to be one of the defining features of drug selectivity.

The message-address theory of peptide-substrate interactions is not just confined to opioid activation and has been demonstrated to be operative for antimicrobial peptides such as the cecropins and magainins, as well as other systems. Membrane compartmentalization is most clearly exemplified by studies on the bee venom peptide melittin, which is a very well documented example of the message-address dichotomy (46). Numerous

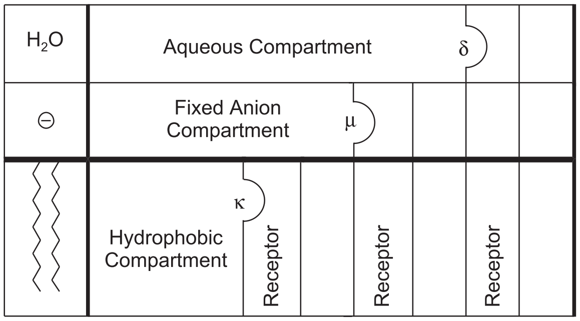


Fig. 7. Schwyzer's membrane compartment hypothesis.

researchers have illustrated that the 26 amino acid residues in melittin and related analogs can be assigned to either a very basic 6-residue message segment or an amphipathic 20-residue α-helical address segment. Studies have shown that both portions are necessary for full hemolytic activity: if the message segment is removed, membrane association still occurred but all hemolytic activity was lost, while removal of the address segment resulted in less specific membrane disruption (47). NMR and lipid bilayer studies have provided further evidence of the proposed mechanism (48).

Furthermore, this theory is not only confined to α-helical address segments. A glycoside can also impart a high degree of amphiphilicity on a peptide due to its hydrophilic nature. Not only is this type of amphipath much more water-soluble than most peptides, it is also much easier to synthesize and purify than many of its more complex analogs. In addition, these glycopeptide amphipaths still retain the full agonist activity of the parent peptide and can possess many other favorable traits, such as higher enzymatic stability and better transport profiles *in vivo*.

Peptide messages

To date there are several endogenous opioid peptides, all of which have the same Tyr-Gly-Gly-Phe (Y-G-G-F) N-terminal tetrapeptide pharmacophore: leu-enkephalin, met-enkephalin, β-endorphin, dynorphin A, dynorphin B and peptide E (Table I). From these native peptide sequences emerged a series of synthetic derivatives that have been designed to enhance receptor selectivity and binding affinity, and can have the additional features of enhanced stability to peptidases and enhanced serum half-lives.

While there is not a strict requirement for the Tyr-Gly-Gly-Phe for potent opiate agonist activity, Tyr^1 and Phe^4 must be conserved, and the presence of the flexible glycine residue at position 3 permits the peptide sequence to adopt a beta-turn motif. It is these two amino acids that constitute the main portion of the message segment of the majority of potent opioid agonists. The necessity of these two residues in this exact sequence becomes clearer when the structure of the active opioid peptides is compared to morphine itself. Molecular modeling clearly shows how morphine mimics endogenous opioids and thus elicits a biological response. The central residues (amino acids 2 and 3) can be varied and function mainly as a spacer between the two pharmacophore residues, so as to keep the aromatic pharmacophore residues in a proper orientation. Many examples of successful modifications on this theme are highlighted in Table I.

With a few exceptions (49), deviation from the Tyr^1/Phe^4 moiety has resulted in a multitude of peptides with low binding affinities (high K_m). In addition to this, peptides with improper or bulky spacer residues (amino acids 2 and 3) can also impede binding and lead to impotent drugs. Excessive steric bulk can also be caused by glycosylation of the message segment (50), which leads

Table I: Endogenous and synthetic opioid peptides.

	Delta-Selective Ligands
Met-Enkephalin	**Y-G-G-F**-M
DSLET	**Y**-dS-G-**F**-L-S
DTLET	**Y**-dT-G-**F**-L-T
DSTBULET	**Y**-dS(OtBu)-G-**F**-L-T
DPDPE	**Y**-dPen-G-**F**-dPen (SS)
Deltorphin	**Y**-dM-**F**-H-L-M-D-CONH$_2$
	Mu and Kappa-Selective Ligands
Leu-Enkephalin	**Y**-G-G-**F**-L
DAMGO	**Y**-dA-G-**MeF**-NH-CH$_2$CH$_2$OH
Dermorphin	**Y**-dA-**F**-G-Y-P-S-CONH$_2$
beta-Endorphin	**Y**-G-G-**F**-M-T-S-Q-T-P-L-V-T-T-L-F-K-N-A-I-I-K-N-A-Y-K-K-G-E
Dynorphin A (1-17)	**Y**-G-G-**F**-L-R-R-I-R-P-K-L-K-W-D-N-Q
Dynorphin A (1-13)	**Y**-G-G-**F**-L-R-R-I-R-P-K-L-K
Dynorphin B	**Y**-G-G-**F**-L-R-R-Q-F-K-V-V-T

to poor binding. In contrast to this, using spacer residues to constrain the peptide in a favorable geometry for receptor binding has resulted in highly potent and selective compounds. A disulfide bond between neighboring cysteine side chains is an example of this and led to the extremely successful and highly δ-selective DPDPE (51). This compound displayed up to 3000-fold selectivity for the δ receptor in mouse vas deferens (MVD) *versus* the μ-opiate receptor in the guinea pig ileum myenteric plexus (GPI) muscle contraction studies. Similarly, Schiller *et al.* (52) have designed side chain amide cyclization restraints (*e.g.*, Lys-Glu), which produced mu-selective agonists.

Peptide addresses

Unlike the message segment, which has some stringent requirements for successful receptor binding and activation, the address has been shown to be much more flexible in its sequence limitations. Modification of the address segment of cecropin A, an antimicrobial amphipathic α-helical peptide, has produced a number of interesting analogs. Merrifield has created numerous cecropin/melittin hybrids in which the message portion of cecropin A was combined with the address portion of melittin (53). As predicted, most of these peptide hybrids not only retained the potent antimicrobial activity of cecropin A but lost all hemolytic activity associated with melittin. More recently, Aldrich has continued along this line of thinking by combining the message segment of a weakly kappa-selective peptide (54) with the address segment of [D-Ala^8] DynA(1-11)-NH_2 to produce a hybrid peptide with markedly enhanced κ receptor activity (55). This demonstrates that the α-helical address segments of some peptides can be interconverted with retention or even enhancement of bioactivity.

Much of the address segment modifications in opioid chemistry has focused on β-endorphin and dynorphin A. Hruby and coworkers have performed a significant amount of work in this field. In certain dynorphin A experiments, they were able to synthesize a number of dynorphin A(1-11)-NH_2 analogs (56) with reduced psi bonds (from CONH in normal peptide bonds to CH_2NH in the reduced bonds) placed strategically within the newly created compounds. With few exceptions, when modifications were made to the address segment, binding was retained or enhanced, while changes in the message segment (between Tyr^1 and Phe^4) resulted in a large decrease in opioid binding.

Taylor and coworkers have also done a great deal of work with completely synthetic address segments of beta-endorphin (57). In these experiments, Taylor found that the *de novo* design of synthetic α-helices led to potent analogs of β-endorphin and thus illustrated that the address segment of a peptide does not need to be as highly conserved as the pharmacophore residues of the message segment.

One concern with this process for drug discovery is that although a proper address segment does not directly impede receptor binding, an unnatural address segment, such as a glycoside, could alter the entire peptide backbone conformation and lead to unfavorable pharmacophore orientations and conformations, which would in turn hamper receptor activation. Several studies have been conducted in the area of peptide-glycoside interactions. Many researchers argue that the glycoside residue acts a β-turn promoter (58), significantly alters the peptide's conformation (59, 60) or even functions as a helix breaker (61). However, NMR and molecular modeling studies performed on O-linked glycopeptide enkephalin analogs (specifically LSZ-1025) showed that the effect of the carbohydrate on peptide conformation is limited exclusively to the serine of the glycoside and the adjacent residue, Gly^7 (62), and more recent work shows only a minimal effect on α-helix formation (63). Thus, conformational biases imparted by the glycoside do not affect the message portion or hinder binding.

Glycopeptides as transport vectors

As potent and selective as these peptides may be at the desired receptor, none of them are effective central analgesics because their entry into the brain is blocked. In 1885 Dr. Paul Ehrlich was the first to define the blood-brain barrier (BBB) when he examined the effect of the dye, trypan blue, on animals after *i.v.* administration. Upon dissection he found that the brain remained a grayish-white color while all other tissues were stained blue. He reasoned that the brain and the surrounding cerebrospinal fluid (CSF) must be protected from the dye, yet at the same time the BBB must allow the passage of essential nutrients, hormones and metabolites. The BBB is mainly responsible for protecting the brain and spinal cord from materials in the blood that would be neurotoxic (64, 65). Morphologically, the BBB consists of tight-junction endothelial cells that comprise the cerebral microvascular tissue. Not only does the BBB present a physical obstacle but a metabolic one as well, possessing oxidative enzymes and numerous peptidases, including enkephalinases. This enzymatic gauntlet may be as important as the lipophilic barrier in excluding peptide pharmaceuticals.

There are several modes of transport across the endothelial cells of the brain capillaries (66) (Fig. 8). Many pharmaceuticals such as morphine penetrate the BBB by passive diffusion (67). Passive diffusion is limited by the molecular weight and by the lipophilicity of the compound. Thus, solely on the basis of passive diffusion one would not predict that large polar molecules such as peptides would ever become useful CNS drugs (68). Carrier-mediated transport may be active, in which ATP is consumed upon transport and is normally unidirectional, or may be facilitative, which is driven by concentration gradient and can function in either direction. Glucose, which is the major energy source for the brain, is facilitatively

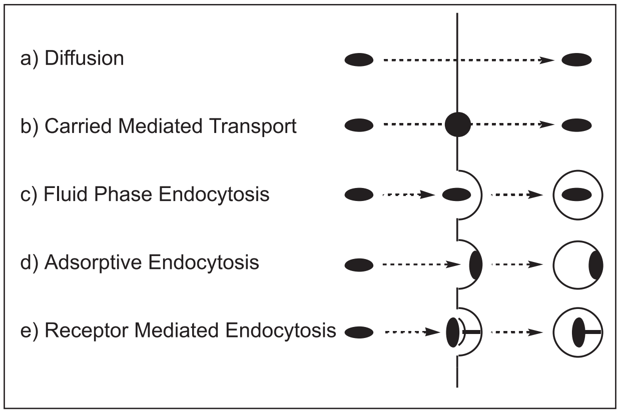


Fig. 8. Modes of transport across the BBB.

transported via the glucose transporter (GLUT-1) (69). Amino acids cross the BBB using several different transporters (70). Large neutral amino acids such as tyrosine and phenylalanine are actively transported via the large neutral amino acid transporter (LNAA transporter). Amino acids such as alanine, serine, threonine and cysteine penetrate the BBB via their own transporter, termed the Ala-Ser-Cys transporter (ASC transporter). There are other transporters that transport both small peptides (71), as well as proteins (72).

Molecules can also cross the BBB via endocytosis. One approach that has been explored in several contexts is to enhance peptide delivery across the BBB by attachment of a transport vector (73, 74), which can bind to a receptor and transport the peptide pharmacophore as well. This approach, which often entails the incorporation of a linker so that the peptide can be released after transport, can become quite cumbersome and inefficient.

Originally, the GLUT-1 transporter provided the rationale for transport of the glycosylated opioid peptides (75). In mammals, β-D-glucose is the brain's primary energy substrate and is rapidly transported across the BBB by glucose transporters, such as the GLUT-1 transporter. Thus, it was hoped that glycosylation of these peptides would not interfere in the binding of the attached glucose to the transporter and allow the entire glycopeptide to be carried across the BBB and impart analgesia.

To test the hypothesis that the glycopeptide can cross the BBB, *in vivo* experiments using peripherally administered cyclic glycopeptide LSZ-1025 (further pharmacology of this and other glycopeptide drugs will be discussed in the following sections) and naloxone, an μ-receptor antagonist, were performed. When 60 mg/kg of LSZ-1025 was administered intraperitoneally (i.p.) to mice high analgesia was observed. This analgesia was blocked with 1 μg of naloxone administered intracerebroventricularly (i.c.v.), signifying that the analgesia was due to opioid receptor activation. However, when the same dose of naloxone was administered peripherally, high analgesia was again observed. The observation that LSZ-1025 provided analgesia that could be blocked centrally, but not peripherally, by naloxone signified that the glycopeptide had crossed the BBB.

However, further testing performed by the Davis group has shown that the mechanism of transport of these glycopeptides was not mediated by GLUT-1 (76). When 10 mM glucose was coadministered with LSZ-1025, the glycopeptide retained its full analgesic effects, even when GLUT-1 was saturated. Control studies were performed with 10 mM L-glucose, with the same result.

The consensus in the field was that high lipophilicity was a necessity for good BBB penetration. Glycopeptides provide an interesting exception to this rule: these are highly hydrophilic compounds that also penetrate the BBB well. Further BBB studies of LSZ-1025 analogs have also substantiated this claim.

Pharmacology of LSZ-1025

The synthesis of enkephalin glycoconjugates dates back to the middle 1980s. The glycosidic portion of those compounds was covalently linked to the C-terminus (Fig. 9). While not an endogenous motif, the carbohydrate acted as the address portion and these drugs were referred to as "neoglycopeptides" (77-79). Since then, more sophisticated analogs of these compounds possessing the natural O-glycosidic linkage have been synthesized. Following the message-address segment theory, numerous uncyclized and constrained cyclic glycopeptides were created and tested in two *in vitro* binding assays. δ- and μ-Receptor binding affinities were determined from radioligand displacement measurements from opioid receptors obtained from rat brain homogenates, or functional assays with intact opioid receptor from smooth muscle tissue were performed (GPI and MVD tissues, specific for mu and delta receptors, respectively). In brain homogenate studies, the receptors were pretreated with a high-affinity radioactive ligand specific for the receptor to be tested. Binding affinities were then calculated from the amount of radioligand displaced by the glycopeptide in question as a function of concentration of the added glycopeptide ligand. In smooth muscle assays, binding was calculated as a function of muscle contraction under an electrical stimulus. As can be seen from Table II, message-address principles had to be strictly followed in order to achieve high binding affinity. Glycopeptides **1** and **2** were synthesized with the

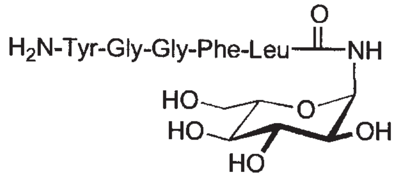
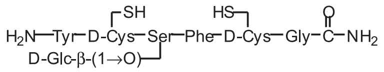
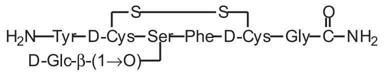
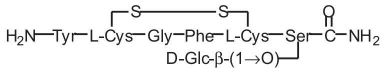
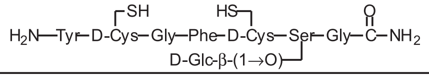
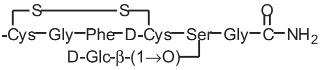
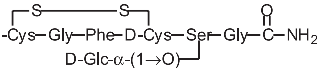
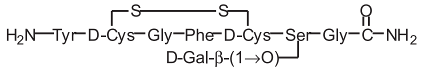
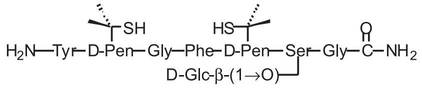
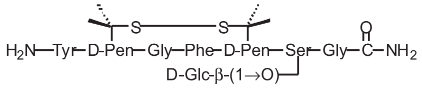
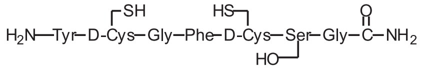
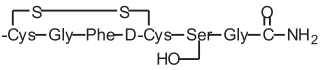
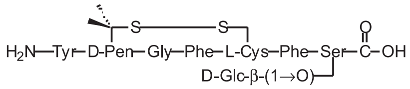
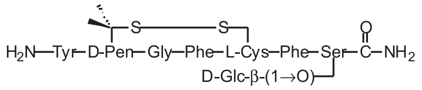
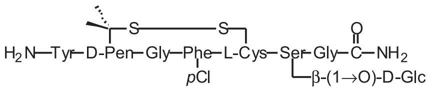


Fig. 9. Neoglycopeptide enkephalin analogs.

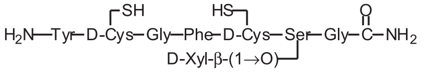
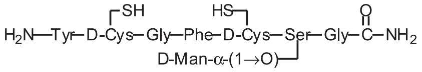
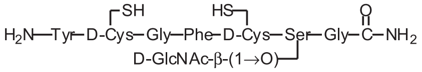
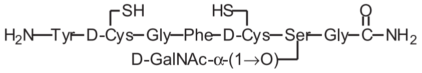
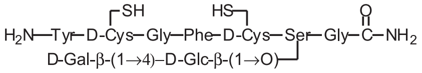
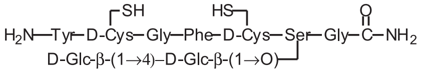
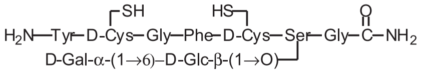
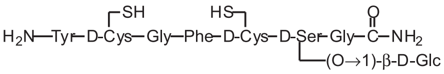
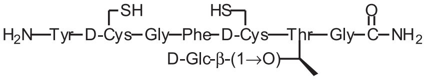
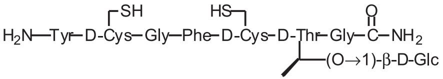
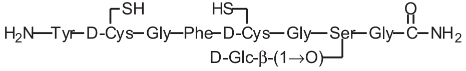
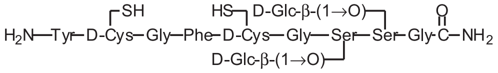
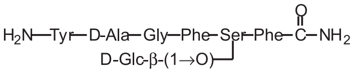
Table II: LSZ-1025 and related analogs.

	Peptide Ligand	δ (nM)	μ (nM)	MVD (nM)	GPI (nM)
1	 H_2N–Tyr–D-Cys(SH)–Ser[D-Glc-β-(1→O)]–Phe–D-Cys(SH)–Gly–C(O)–NH_2	4000	2000	1900	18,000
2	 H_2N–Tyr–D-Cys(S–)–Ser[D-Glc-β-(1→O)]–Phe–D-Cys(–S)–Gly–C(O)–NH_2	3900	7700	520	3700
3	 H_2N–Tyr–L-Cys(S–)–Gly–Phe–L-Cys(–S)–Ser[D-Glc-β-(1→O)]–C(O)–NH_2	100	48	23	28
4	 H_2N–Tyr–D-Cys(SH)–Gly–Phe–D-Cys(SH)–Ser[D-Glc-β-(1→O)]–Gly–C(O)–NH_2	9.9	42	24	110
5	**LSZ-1025**  H_2N–Tyr–D-Cys(S–)–Gly–Phe–D-Cys(–S)–Ser[D-Glc-β-(1→O)]–Gly–C(O)–NH_2	26—45	46—53	13	60
6	**LSZ-62**  H_2N–Tyr–D-Cys(S–)–Gly–Phe–D-Cys(–S)–Ser[D-Glc-α-(1→O)]–Gly–C(O)–NH_2	10	68	34	64
7	 H_2N–Tyr–D-Cys(S–)–Gly–Phe–D-Cys(–S)–Ser[D-Gal-β-(1→O)]–Gly–C(O)–NH_2	26	24—30	53—64	125—148
8	 H_2N–Tyr–D-Pen(SH)–Gly–Phe–D-Pen(SH)–Ser[D-Glc-β-(1→O)]–Gly–C(O)–NH_2	180	2400	680	125,000
9	 H_2N–Tyr–D-Pen(S–)–Gly–Phe–D-Pen(–S)–Ser[D-Glc-β-(1→O)]–Gly–C(O)–NH_2	85	48,000	560	40,000
10	 H_2N–Tyr–D-Cys(SH)–Gly–Phe–D-Cys(SH)–Ser(HO)–Gly–C(O)–NH_2	4.2	20	6.8	25
11	**LSZ-916**  H_2N–Tyr–D-Cys(S–)–Gly–Phe–D-Cys(–S)–Ser(HO)–Gly–C(O)–NH_2	6.1	30	5.5	26
12	 H_2N–Tyr–D-Pen(S–)–Gly–Phe–L-Cys(–S)–Phe–Ser[D-Glc-β-(1→O)]–C(O)–OH	1.99	419	—	—
13	 H_2N–Tyr–D-Pen(S–)–Gly–Phe–L-Cys(–S)–Phe–Ser[D-Glc-β-(1→O)]–C(O)–NH_2	< 1	> 400	2	219
14	 H_2N–Tyr–D-Pen(S–)–Gly–Phe(*p*Cl)–L-Cys(–S)–Ser[β-(1→O)-D-Glc]–Gly–C(O)–NH_2	7.8	14.2	4.9	20

glycosyl amino acid within the message segment. These compounds showed poor binding affinity, as would be predicted.

All glycopeptides within this series possessed two thiol-bearing amino acids (either cysteine or penicillamine) within the message portion. After cyclization (S-S bond formation) the cyclic constraint served to orient the pharmacophore residues (Tyr^1 and Phe^4) in a turn conformation favorable to δ-opioid receptor activation. When the glycopeptide was synthesized with the glycoside residing in the address portion, away from the message, all glycopeptides showed high binding affinity for the opioid receptors, with fair to high selectivity for the delta receptor. However, delta opioid activity alone was not useful for producing analgesia *in vivo.* This was evidenced when LSZ-1025 and glycopeptide **13** were compared. Glycopeptide **13**, the glycosylated analog of DPLCE, was potent and highly delta selective (564:1 δ:μ), yet failed to provide analgesia *in vivo*. This can be attributed to poor μ receptor activity, which is now thought to be crucial to *in vivo* activity of opioid drugs. LSZ-1025, while not as selective as glycopeptide **13**, is much more potent at the μ receptor and, as a consequence, provided much better analgesia profiles *in vivo*.

Table III: In vitro *binding of LSZ-1025 and analogs.*

	Peptide Ligand	δ (nM)	μ (nM)	MVD (nM)	GPI (nM)
15	 $\mathrm{H_2N{-}Tyr{-}D\text{-}Cys(SH){-}Gly{-}Phe{-}D\text{-}Cys(SH){-}Ser[D\text{-}Xyl\text{-}\beta\text{-}(1\to O)]{-}Gly{-}C(=O){-}NH_2}$	32	19	42	40
16	 $\mathrm{H_2N{-}Tyr{-}D\text{-}Cys(SH){-}Gly{-}Phe{-}D\text{-}Cys(SH){-}Ser[D\text{-}Man\text{-}\alpha\text{-}(1\to O)]{-}Gly{-}C(=O){-}NH_2}$	43	16	76	61
17	 $\mathrm{H_2N{-}Tyr{-}D\text{-}Cys(SH){-}Gly{-}Phe{-}D\text{-}Cys(SH){-}Ser[D\text{-}GlcNAc\text{-}\beta\text{-}(1\to O)]{-}Gly{-}C(=O){-}NH_2}$	62	57	52	67
18	 $\mathrm{H_2N{-}Tyr{-}D\text{-}Cys(SH){-}Gly{-}Phe{-}D\text{-}Cys(SH){-}Ser[D\text{-}GalNAc\text{-}\alpha\text{-}(1\to O)]{-}Gly{-}C(=O){-}NH_2}$	36	40	43	64
19	 $\mathrm{H_2N{-}Tyr{-}D\text{-}Cys(SH){-}Gly{-}Phe{-}D\text{-}Cys(SH){-}Ser[D\text{-}Gal\text{-}\beta\text{-}(1\to 4)\text{-}D\text{-}Glc\text{-}\beta\text{-}(1\to O)]{-}Gly{-}C(=O){-}NH_2}$	26	32	40	80
20	 $\mathrm{H_2N{-}Tyr{-}D\text{-}Cys(SH){-}Gly{-}Phe{-}D\text{-}Cys(SH){-}Ser[D\text{-}Glc\text{-}\beta\text{-}(1\to 4)\text{-}D\text{-}Glc\text{-}\beta\text{-}(1\to O)]{-}Gly{-}C(=O){-}NH_2}$	25	20	91	100
21	 $\mathrm{H_2N{-}Tyr{-}D\text{-}Cys(SH){-}Gly{-}Phe{-}D\text{-}Cys(SH){-}Ser[D\text{-}Gal\text{-}\alpha\text{-}(1\to 6)\text{-}D\text{-}Glc\text{-}\beta\text{-}(1\to O)]{-}Gly{-}C(=O){-}NH_2}$	105	14	118	186
22	 $\mathrm{H_2N{-}Tyr{-}D\text{-}Cys(SH){-}Gly{-}Phe{-}D\text{-}Cys(SH){-}D\text{-}Ser[(O\to 1)\text{-}\beta\text{-}D\text{-}Glc]{-}Gly{-}C(=O){-}NH_2}$	48	9	41	56
23	 $\mathrm{H_2N{-}Tyr{-}D\text{-}Cys(SH){-}Gly{-}Phe{-}D\text{-}Cys(SH){-}Thr[D\text{-}Glc\text{-}\beta\text{-}(1\to O)]{-}Gly{-}C(=O){-}NH_2}$	45	65	33	143
24	 $\mathrm{H_2N{-}Tyr{-}D\text{-}Cys(SH){-}Gly{-}Phe{-}D\text{-}Cys(SH){-}D\text{-}Thr[(O\to 1)\text{-}\beta\text{-}D\text{-}Glc]{-}Gly{-}C(=O){-}NH_2}$	29	6	28	23
25	 $\mathrm{H_2N{-}Tyr{-}D\text{-}Cys(SH){-}Gly{-}Phe{-}D\text{-}Cys(SH){-}Gly{-}Ser[D\text{-}Glc\text{-}\beta\text{-}(1\to O)]{-}Gly{-}C(=O){-}NH_2}$	54	12	22	201
26	 $\mathrm{H_2N{-}Tyr{-}D\text{-}Cys(SH){-}Gly{-}Phe{-}D\text{-}Cys(SH){-}Gly{-}Ser[D\text{-}Glc\text{-}\beta\text{-}(1\to O)]{-}Ser[D\text{-}Glc\text{-}\beta\text{-}(1\to O)]{-}Gly{-}C(=O){-}NH_2}$	124	26	29	310
27	 $\mathrm{H_2N{-}Tyr{-}D\text{-}Ala{-}Gly{-}Phe{-}Ser[D\text{-}Glc\text{-}\beta\text{-}(1\to O)]{-}Phe{-}C(=O){-}NH_2}$	428	20	876	201

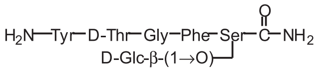
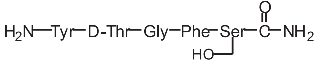
Further studies focused on the carbohydrate within the address segment (Table III). Glycopeptides **15-26** were synthesized with the uncyclized DCDCE (D-Cys D-Cys enkephalin) pharmacophore so as to avoid the synthetic problems associated with cyclization, but differed by the carbohydrate moiety. All glycopeptides were high-affinity agonists for the delta and μ receptors, with K_i values in the low nanomolar range. Opioid subtype selectivity was generally in favor of the delta receptor, with a few exceptions.

However, there were several problems with the DCDCE-type pharmacophores. First, formation of the disulfide bonds proved problematic. Overoxidation and cleavage comprised a large portion of the undesired side products. Stability on the shelf was also a problem, as the glycopeptides tended to air-oxidize and decompose over the course of a few months. Thus, the search for a new pharmacophore and a change in the message segment was desirable.

Pharmacology of SAM-1095

The δ-selective opiate DTLES (Table IV, peptide SAM-995), designed by Roques *et al.* (80), was synthesized in glycopeptide form (SAM-1095) to examine the glycoside-mediated BBB penetration in an acyclic peptide context. These glycopeptides offered several advantages over the DCDCE-type pharmacophore (LSZ-1025, glycopeptides **1-26** in Tables II and III), the primary advantage being that cyclization and/or cleavage of the pendent disulfide bond was not a problem. This allowed for synthesis and purification on a much larger scale than before, and in turn, allowed for more *in vivo* testing. When

Table IV: In vitro *pharmacology of SAM-1095 and SAM-995.*

Peptide Ligand		δ (nM)	μ (nM)	MVD (nM)	GPI (nM)
SAM-1095	 $\mathrm{H_2N{-}Tyr{-}D\text{-}Thr{-}Gly{-}Phe{-}\underset{\text{D-Glc-}\beta\text{-}(1\to O)}{Ser}{-}\overset{O}{\overset{\|}{C}}{-}NH_2}$	2.4	7.6	1.6	34
SAM-995	 $\mathrm{H_2N{-}Tyr{-}D\text{-}Thr{-}Gly{-}Phe{-}\underset{HO}{Ser}{-}\overset{O}{\overset{\|}{C}}{-}NH_2}$	2.1	7.5	2.7	25

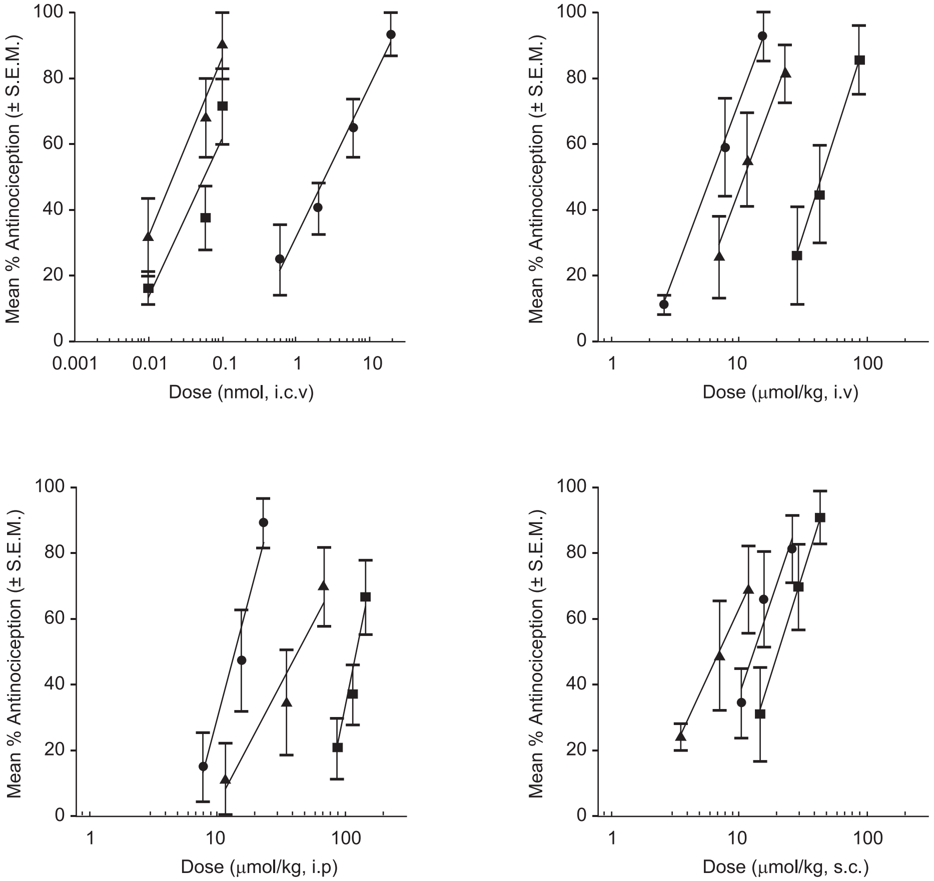


Fig. 10. Peripheral administration of morphine (●), SAM-1095 (▲) and SAM-995 (■)

Roques *et al.* tested the peptide DTLES in 1988, the reported GPI/MVD data were 0.25-99 nM, or a selectivity of 400-fold for the δ receptor (81). The corresponding C-terminal amide, SAM-995, showed much poorer selectivity in both the GPI/MVD and rat brain homogenate receptor studies. Thus, the terminal acid played a large role in maintaining the δ selectivity, which supports Schwyzer's membrane compartment hypothesis.

The glycopeptides based on this pharmacophore were quite potent, but lacked the selectivity of DTLES. The β-D-Glc-L-Ser-glucoside SAM-1095 was compared with its nonglycosylated form, SAM-995, and the cyclic disulfide LSZ-1025. Both SAM-1095 and SAM-995 were 2 nM at the δ receptor and ~30 nM at the μ receptor. Additional studies have shown that the analgesic effects of SAM-1095 are reversed by centrally administered naloxone methiodide (82, 83). Based on these preliminary results, SAM-1095 appears to be an ideal candidate for further development (84), perhaps even oral delivery, since it has been shown that some glycopeptides display absorption from the gut into the bloodstream (85, 86).

Alternative routes of administration of the glycopeptide were explored (Fig. 10) and compared to SAM-995 and morphine. As confirmed by BBB studies, an interesting i.v. profile for SAM-1095 was observed (Fig. 10b), which further indicated BBB penetration of the glycopeptide in contrast to the peptide. Surprisingly, subcutaneous (s.c.) administration of SAM-1095 provided analgesic

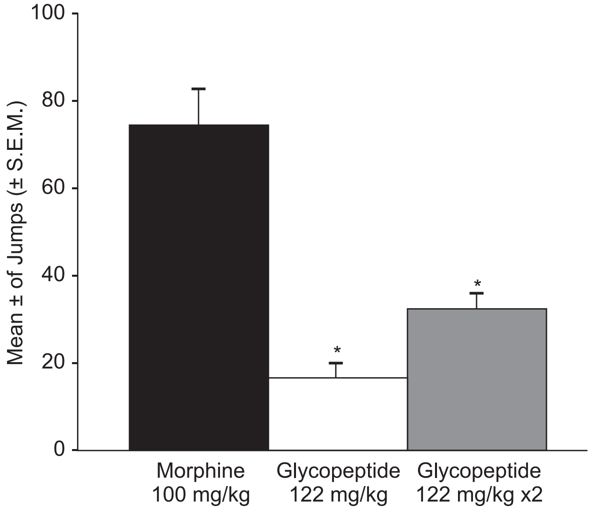


Fig. 11. Glycopeptide (SAM-1095) dependence studies.

effects superior to equimolar amounts of morphine (s.c.) (Fig. 10d).

The potent actions of glycopeptide SAM-1095, along with the ability to easily synthesize gram quantities of the drug, allowed for the assessment of the physical dependence liability using an acute model of opiate withdrawal (87). Administration of naloxone (10 mg/kg i.p.) to mice pretreated 4 h prior with a 20 X A_{50} dose (s.c.) of morphine (100 mg/kg) produced a withdrawal syndrome characterized by vertical jumping. Using a similar model, naloxone injection precipitated much less jumping in mice pretreated (–4 h) with a 20 X A_{50} dose (s.c.) of SAM-1095 (122 mg/kg). The morphine dose produced near maximal antinociception for 4 h while SAM-1095 produced near maximal analgesia for 2 h. To address these pharmacokinetic differences, a third group of mice received two injections of SAM-1095 (122 mg/kg s.c., 4 h and 2 h prior to naloxone precipitation) which maintained near maximal antinociception for the entire 4-h pretreatment period. This group of mice also jumped significantly less than the morphine control mice after withdrawal, indicating less physical dependence with SAM-1095 (Fig. 11).

Further studies on the potential side effects of SAM-1095 focused on the effect of the glycopeptide on locomotor activity in mice, which is one gauge of psychotropic activity in mice. In preliminary studies, SAM-1095 did not produce a significantly altered locomotor activity profile, even at supra-analgesic doses. In contrast, equivalent doses of morphine induced locomotor hyperactivity and stereotypical circling behavior in the mice (88).

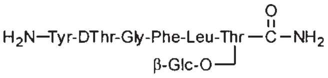
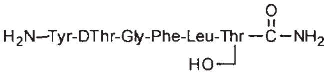
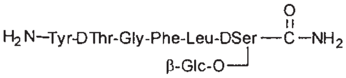
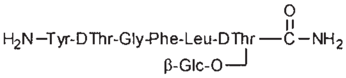
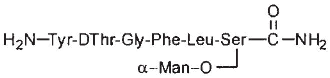
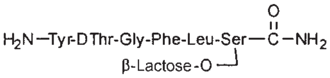
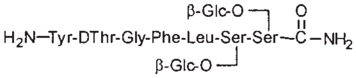
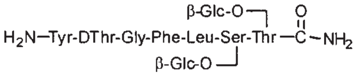
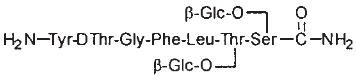
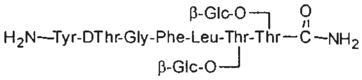
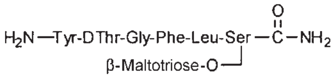
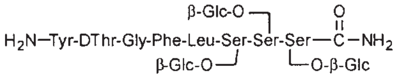
SAM-1095 analogs

Encouraged by the success of the SAM-1095 studies, further derivatives of this compound were prepared (Table V). As with the second generation of LSZ-1025 compounds, the pharmacophore of the parent drug was kept constant, while the glycoside within the address segment was varied. In contrast to the second generation of LSZ-1025 compounds, glycoside variation focused on the effect of increasing the number of attached carbohydrates and the glycosyl-amino acid linkage (serine *vs.* threonine), rather than on the monosaccharide identity. Oligosaccharyl peptides and multiple monosaccharyl peptides were synthesized and more *in vivo* studies (i.c.v. and i.v.) were performed in addition to the mu/delta binding studies and GPI/MVD functional assays. When administered i.c.v., all of the glycopeptide drugs in this series were determined to be 100-200 times more potent (20-100 pmol/mouse) than morphine (2700 pmol/mouse = 2.7 nmol/mouse). When six compounds were administered to mice i.v., the most potent acyclic compounds were the original SAM-1095 (55% the potency of morphine) and MMP-2200, the beta-lactoside (200% the potency of morphine).

Thus, the lactoside MMP-2200 was even more potent than the first glucoside, SAM-1095, and had a potency roughly twice that of morphine following i.v. administration. Morphine, being a very lipophilic compound, readily crosses the BBB. Even though morphine is much less potent i.c.v. than the glycopeptides at the receptor, it has much better transport kinetics across the BBB and produces a good i.v. profile. The addition of a disaccharide appears to improve the transport of the peptide to an even greater extent than a monosaccharide. Based on the i.v. potency of the disaccharide, we hypothesized that additional glycosylations with a monosaccharide might also enhance systemic bioavailability. This hypothesis was tested by synthesizing a number of bis-glycosylated compounds (MMP-2205 to MMP-2220). Due to a high degree of structural similarity between these compounds, a semicombinatorial approach to their synthesis was used. Houghten's "tea bag" method of simultaneous multiple peptide synthesis was the most viable alternative (89). In this procedure, solid-phase resins with different C-termini residues were placed in separate, porous polyester bags. The bags are permeable to amino acids and coupling agent solutions, but heat-sealed so as not to allow any resin beads to escape. Using this method, the four bis-monosaccharides could be synthesized in only 11 steps, instead of 36 steps.

Unfortunately, the bis-monosaccharides did not possess the *in vivo* potency of the other analogs. This leads us to believe that there is some sort of receptor-ligand occlusion, possibly due to the large steric bulk imparted by two adjacent glycosylated amino acids and in such close proximity to the message portion. However, this work did provide an additional clue to the mechanism of BBB transport. Since earlier work had ruled out diffusion and carrier-mediated transport, it may be surmised that the glycosides, if properly placed, can induce a degree of amphipathicity to the peptide moiety, which may promote transport via an endocytotic event, probably adsorptive endocytosis (90) (Fig. 8).

Table V: SAM-1095 analogs.

	Opioid Receptor Agonists	δ (nm)	μ (nm)	MVD (nm)	GPI (nm)	ICV A_{50} (pm)	IV A_{50} (nm)
	Morphine	ND	ND	258	54.7	2,700	6.30
MMP-2100	 $\text{H}_2\text{N—Tyr-DThr-Gly-Phe-Leu-}\underset{\beta\text{-Glc-O}}{\text{Thr}}\text{—}\overset{\text{O}}{\overset{\Vert}{\text{C}}}\text{—NH}_2$	16.8	39.8	0.729	24.6	20	ND
MMP-1995	 $\text{H}_2\text{N—Tyr-DThr-Gly-Phe-Leu-}\underset{\text{HO}}{\text{Thr}}\text{—}\overset{\text{O}}{\overset{\Vert}{\text{C}}}\text{—NH}_2$	9.7	11.7	2.3	24.5	40	ND
MMP-2101	 $\text{H}_2\text{N—Tyr-DThr-Gly-Phe-Leu-}\underset{\beta\text{-Glc-O}}{\text{DSer}}\text{—}\overset{\text{O}}{\overset{\Vert}{\text{C}}}\text{—NH}_2$	54.4	298	5.40	34.4	20	ND
MMP-2102	 $\text{H}_2\text{N—Tyr-DThr-Gly-Phe-Leu-}\underset{\beta\text{-Glc-O}}{\text{DThr}}\text{—}\overset{\text{O}}{\overset{\Vert}{\text{C}}}\text{—NH}_2$	24.5	31.8	2.20	48.9	20	43.0
MMP-2120	 $\text{H}_2\text{N—Tyr-DThr-Gly-Phe-Leu-}\underset{\alpha\text{–Man–O}}{\text{Ser}}\text{—}\overset{\text{O}}{\overset{\Vert}{\text{C}}}\text{—NH}_2$	22.9	15.2	3.02	23.3	40	32
MMP-2200	 $\text{H}_2\text{N—Tyr-DThr-Gly-Phe-Leu-}\underset{\beta\text{-Lactose-O}}{\text{Ser}}\text{—}\overset{\text{O}}{\overset{\Vert}{\text{C}}}\text{—NH}_2$	17.3	40.0	5.72	34.8	20	3.2
MMP-2205	 $\text{H}_2\text{N—Tyr-DThr-Gly-Phe-Leu-}\underset{\beta\text{-Glc-O}}{\text{Ser}}\text{-}\overset{\beta\text{-Glc-O}}{\text{Ser}}\text{—}\overset{\text{O}}{\overset{\Vert}{\text{C}}}\text{—NH}_2$	5.2	40	1.16	54	380	141
MMP-2210	 $\text{H}_2\text{N—Tyr-DThr-Gly-Phe-Leu-}\underset{\beta\text{-Glc-O}}{\text{Ser}}\text{-}\overset{\beta\text{-Glc-O}}{\text{Thr}}\text{—}\overset{\text{O}}{\overset{\Vert}{\text{C}}}\text{—NH}_2$	11.9	129	1.46	108	120	ND
MMP-2215	 $\text{H}_2\text{N—Tyr-DThr-Gly-Phe-Leu-}\underset{\beta\text{-Glc-O}}{\text{Thr}}\text{-}\overset{\beta\text{-Glc-O}}{\text{Ser}}\text{—}\overset{\text{O}}{\overset{\Vert}{\text{C}}}\text{—NH}_2$	7.1	60.3	2.35	161	90	ND
MMP-2220	 $\text{H}_2\text{N—Tyr-DThr-Gly-Phe-Leu-}\underset{\beta\text{-Glc-O}}{\text{Thr}}\text{-}\overset{\beta\text{-Glc-O}}{\text{Thr}}\text{—}\overset{\text{O}}{\overset{\Vert}{\text{C}}}\text{—NH}_2$	11.9	88.2	3.22	185	100	ND
MMP-2300	 $\text{H}_2\text{N—Tyr-DThr-Gly-Phe-Leu-}\underset{\beta\text{-Maltotriose-O}}{\text{Ser}}\text{—}\overset{\text{O}}{\overset{\Vert}{\text{C}}}\text{—NH}_2$	7.4	20	7.73	71.7	60	10.9
MMP-2305	 $\text{H}_2\text{N—Tyr-DThr-Gly-Phe-Leu-}\underset{\beta\text{-Glc-O}}{\text{Ser}}\text{-}\overset{\beta\text{-Glc-O}}{\text{Ser}}\text{-}\underset{\text{O-}\beta\text{-Glc}}{\text{Ser}}\text{—}\overset{\text{O}}{\overset{\Vert}{\text{C}}}\text{—NH}_2$	—Testing in Progress—				220	ND

Other glycopeptides

The enkephalins are not the only analgesic message segment to provide the basis for glycopeptide drugs. Opioid peptides isolated from amphibian skin glands carry highly potent message segments and provide excellent candidates for transformation into glycopeptide analgesics. Specifically, the deltorphins (named for high δ specificity) and the dermorphins (named for dermal morphine-like substance) have also been identified as ligands for mu opiate receptors.

Tomatis has recently synthesized a number of deltorphin- and dermorphin-related glycopeptides (91). As seen in the earlier examples, glycosylation was most effective when placed within the address segment portion. β-D-Glucosyl threonine and *tetra*-acetyl-β-D-glucosyl threonine comprised the glycosyl amino acids, with *in vitro* and *in vivo* activity of specific analogs observed to be comparable or greater than that of morphine. The ED_{50} for the most potent glycopeptide analog upon i.c.v. administration was 1.3 nM, compared to 3.2 nM for morphine i.c.v. Administration produced similar potency compared to morphine (0.53 μM s.c. glycopeptide *vs.* 10.9 μM s.c. for morphine). Further study of these compounds showed results similar to those previously disclosed (92).

Outlook

Pain is ubiquitous in medical practice, and analgesics are essential tools for any clinician (93). While there has been much research into the application of long-standing analgesic drugs, it is surprising that very few, if any, new analgesics have come into the marketplace in decades.

The past 25 years of research with enkephalin peptide analogs have generated many potent and selective opiate ligands which have proved invaluable for research in the mechanisms of pain transmission. The use of these ligands as pharmaceuticals and not simply as biochemical tools has been advanced by an understanding of the factors that govern their stability in serum and in the brain. It seems likely that one or more approaches to the problems of administration, transport and penetration of the blood-brain barrier will emerge in the years to come. Our prediction is that as the pharmacological community becomes aware of the recent advances in this field, and gains an appreciation of the potential benefits offered by peptide-based analgesics, the problems associated with the use of peptide and glycopeptide analgesics will be solved and that they will find widespread clinical application.

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