Novel reagents and reactions for drug design*

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Abstract: In this presentation, we cover new results from two of our synthetic endeavors: guanidinylation reagents and novel bridged opioids. In the first part, we describe the applications of the diurethane-triflylguanidines to prepare target bioactive structures including peptides, heterocyclic drugs, and aminoglycoside derivatives. The formation of novel guanidinoglycosides led to a family of effective binders to the RNA recognition element of the HIV-1 Rev protein. In the second part, the syntheses of sulfur and amine-bridged cyclic opioids is described. These analogs exhibit enhanced binding, both *in vitro* and *in vivo*. Specifically, H-Tyr-c[D-Val$_L$-Gly-Phe-D/L-Ala$_L$]-OH (Val$_L$ and Ala$_L$ denote the lanthionine amino acid ends linked by a monosulfide bridge) is potent and highly δ-receptor selective while Tyr-c[(N$_\gamma$CH$_3$)-D-A$_2$bu-Gly-Phe-NHCH$_2$CH$_2$-] though nonselective is one of the most potent opioids prepared to date. These molecules are representative of our design of novel peptidomimetic opioids.

INTRODUCTION

An overview of the guanidinylation chemistry we have developed in our laboratory along with the design and synthesis of novel peptidomimetics will be discussed.

Guanidinylation Chemistry

The novel guanidinylation reagents, *N,N*'-diBoc-*N*''-triflylguanidine (**1**) and *N,N*'-diCbz-*N*''-triflylguanidine (**2**) have been recently developed and utilized for the preparation of guanidine-containing amino acids and peptides both in solution and on solid phase (Fig. 1, ref. 1). On solid phase, one or multiple lysine/ornithine amino acid residues in a peptide can be smoothly converted to the corresponding homo-arginine/arginine amino acids (entry 2, Table 1), even when the amino acid is proximal to a sterically demanding resin (entry 3, Table 1).
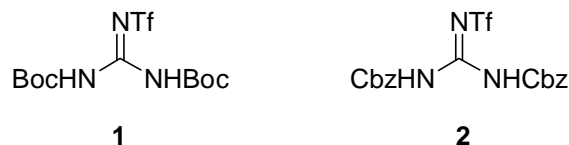

Fig. 1 Guanidinylation reagents *N,N*'-diBoc-*N*''-triflylguanidine (**1**) and *N,N*'-diCbz-*N*''-triflylguanidine (**2**).

Continuing research led to the development of improved preparations for three synthetic guanidino drugs, guanadrel (**3**), guanoxan (**4**) and guanethidine (**5**) (Fig. 2, ref. 2). As an example, guanadrel (**3**), an antihypertensive agent was prepared from the parent amine and reagent **2** in the presence of triethy-

lamine in 95% yield. The Cbz protecting groups were removed quantitatively using hydrogenolysis conditions. Additionally, we have prepared the natural product, smirnovine (**6**) a *N,N*-dialkylated guanidino-alkaloid with implications as a cold climate agrochemical using our guanidinylation method (Fig. 2, ref. 2).

Table 1 Guanidinylation of resin-bound amines using *N,N'*-diBoc-*N"*-triflylguanidine (**1**).

Entry	Amine	Product	Yield (%)[a]
1			100
2			83
3			79

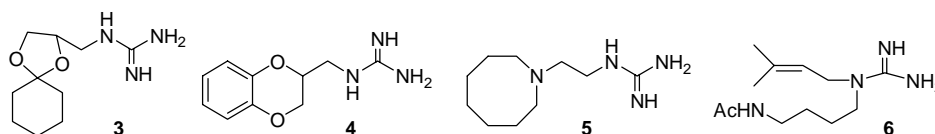[a]Percent yield based on loading capcity of resin

Fig. 2 The guanidine-containing drugs, guanadrel (**3**), guanoxan (**4**), guanethidine (**5**), and the natural product smirnovine (**6**).

Most recently, a method for the conversion of aminoglycosides to the corresponding guanidinoglycosides in aqueous media has been developed using reagent **1** [3]. For example, tobramycin (**7**) was fully guanidinylated using an excess of reagent **1** in a 1,4-dioxane/H_2O mixture to provide compound **8** in quantitative yield (Fig. 3). The guanidinylation and deprotection results of several aminoglycosides, including glucosamine, 2-deoxystreptamine, as well as kanamycin A, tobramycin (**7**), and neomycin B are summarized in Table 2.

Small molecules (peptidomimetics) that specifically interfere with the fundamental gene regulatory elements that are essential for the HIV life-cycle are promising targets for drug discovery. Recent studies have shown that aminoglycoside antibiotics competitively block the binding of the HIV-1 Rev protein (a virally encoded sequence-specific RNA-binding protein) to its Rev-Response-Element (RRE), thereby inhibiting the production of the virus [4]. Unfortunately, these molecules are toxic and lack site-specificity [5]. However, the resulting deprotected guanidinoglycosides (Table 2) have shown enhanced affinity as compared to the parent aminoglycosides (Table 3) and selectivity in the presence of DNA and tRNA (Table 4) to the RNA recognition element of the HIV-1 Rev protein. These results were determined using a solid-phase assay developed by our collaborators in this research [6].
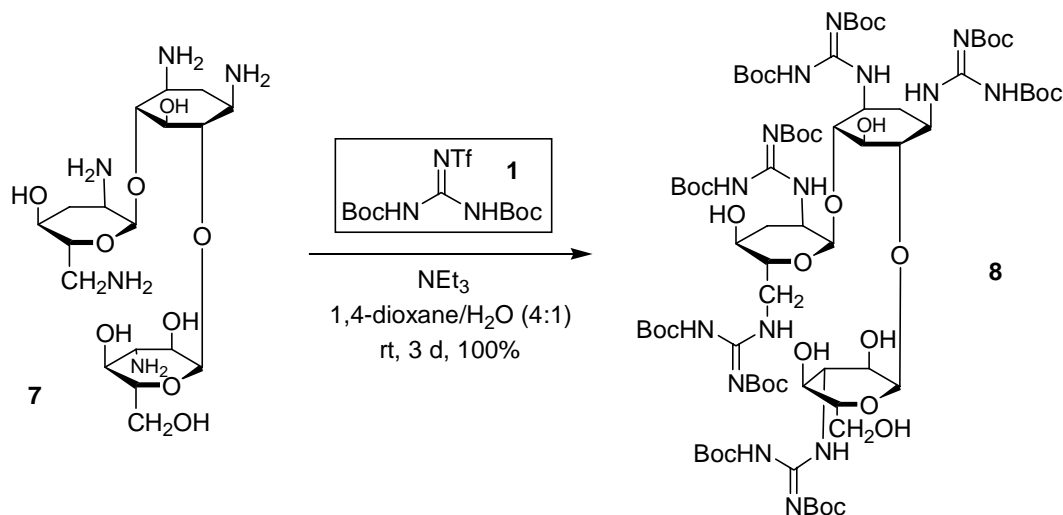

Fig. 3 Guanidinylation of tobramycin (**7**) using *N*,*N*'-diBoc-*N*"-triflylguanidine (**1**).

Table 2 Preparation and deprotection of guanidinoglycosides.

aminoglycoside	No. of amines	Eq. of **1**	Yield(%)	FAB-MS (M+H⁺)	Deprot. Conditions	Yield(%)
glucosamine[a]	1	1.1	82	422	$SnCl_4$	89
2-deoxystreptamine[b]	2	2.1	70	647	$SnCl_4$	97
kanamycin A[b]	4	8	91	1454	TFA/CH_2Cl_2	100
tobramycin (**7**)[b]	5	15	100	1678	TFA/CH_2Cl_2	99
neomycin B[c]	6	18	68	2066	TFA/CH_2Cl_2	100

[a]Reaction carried out in MeOH for 2 d with equimolar NEt_3 as **1**. [b]Reaction carried out in 4:1 mixture of 1,4-dioxane/H_2O for 3 d with equimolar NEt_3 as **1**. [c]Reaction carried out in 4:1 mixture of 1,4-dioxane/H_2O for 7 d with equimolar NEt_3 as **1**.

Table 3 The affinity of guanidinoglycosides to the RNA recognition element of HIV-1 Rev protein compared to the parent aminoglycosides.

Glycoside	IC_{50} amino form	IC_{50} guanidino form
Neomycin B	1.0 μm	0.2 μm
Tobramycin	10 μm	0.65 μm
Kanamycin A	100 μm	8.0 μm

Table 4 The specificity of guanidino-tobramycin to the RNA recognition element of HIV-1 Rev protein in the presence of DNA and tRNA compared to tobramycin.

Compound	IC_{50}	IC_{50} with DNA	IC_{50} with tRNA
Tobramycin (7)	50 μm	55 μm	110 μm
Guanidinylated Tobramycin (8)	2.5 μm	3.0 μm	3.5 μm

Novel Bridged Opioids

We have synthesized and determined the biological activities of a series of enkephalin analogs which have incorporated the lanthionine modification [7]. The lanthionine modification involves the replacement of a disulfide side chain linkage with a thioether side chain linkage. This type of linkage is observed in the naturally occurring lantibiotics [8]. Its incorporation into cyclic enkephalin sequences decreases the number of atoms in the cyclic structure compared to the disulfide counterpart (e.g., DPDPE) resulting in a more constrained structure. The lanthionine structure is also believed to be more metabolically stable than its disulfide counterpart.

Four lanthionine analogs, H-Tyr-c[D-Ala$_L$-Gly-Phe-D-Ala$_L$]-OH, H-Tyr-c[D-Val$_L$-Gly-Phe-D-Ala$_L$]-OH, H-Tyr-c[D-Ala$_L$-Gly-Phe-Ala$_L$]-OH and H-Tyr-c[D-Val$_L$-Gly-Phe-Ala$_L$]-OH (Ala$_L$ or Val$_L$ denotes each of the lanthionine amino acid ends linked by a monosulfide bridge) were synthesized by preparation of the linear peptide on solid phase followed by cyclization in solution (Fig. 4). The *in vitro* assays of these analogs using guinea pig ileum (GPI) and mouse vas deferens (MVD) preparations were carried out in the laboratories of Dr. P. W. Schiller at the Clinical Research Institute of Montreal (Table 5). The inhibition of muscle contractions evoked by electrical stimulation was investigated. The resulting IC_{50} values for GPI and MVD represent the opioid activities of the synthesized cyclic analogs at the μ- and δ-opioid receptors, respectively. The *in vivo* bioactivity tests were undertaken in the laboratories of Dr. T. L. Yaksh at the University of California, San Diego Medical School (Table 5). After injecting the analogs into the rat spinal cord, the antinociceptic effect was measured by the thermal escape assay.
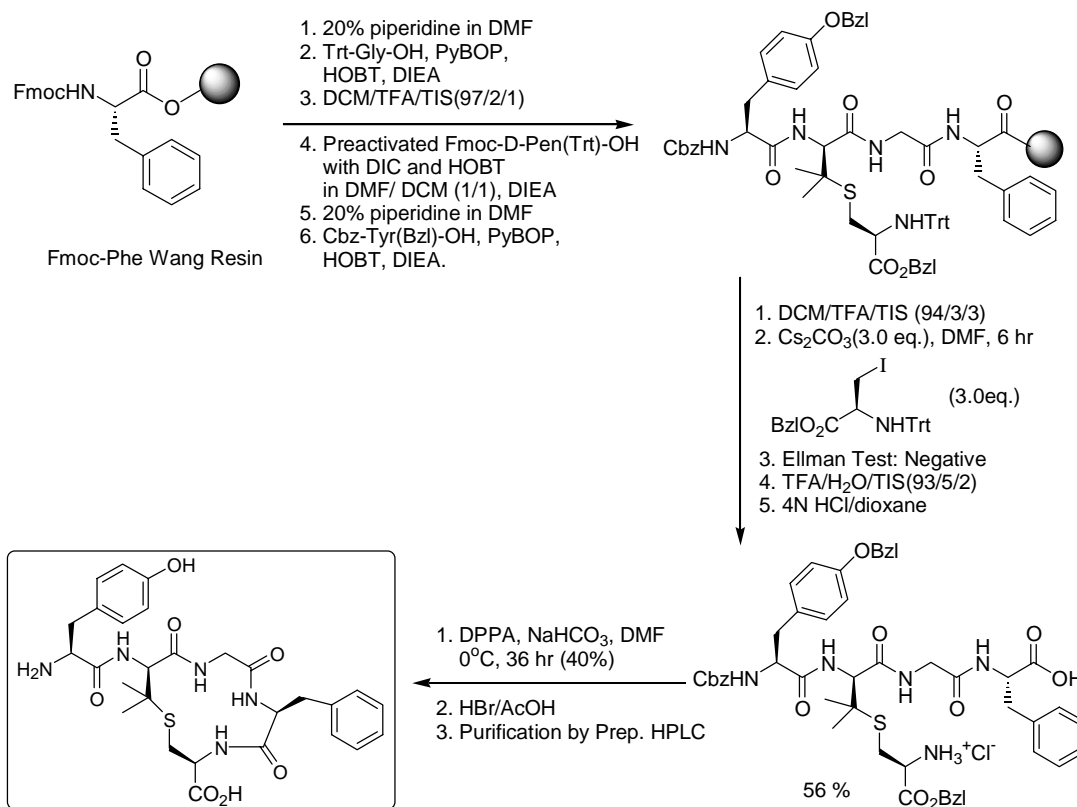
Fig. 4 Synthesis of Tyr-c[D-Val$_L$-Gly-Phe-D-Ala$_L$]-OH.

Table 5 Biological activities of cyclic lanthionines and methylamine-bridged enkephalins.

Compounds	**GPI**	**MVD**	**GPI/MVD**	*In vivo*
	IC$_{50}$ [nM]	IC$_{50}$ [nM]	IC$_{50}$ -ratio	ED$_{50}$ [nM]
Tyr-c[D-Val$_L$-Gly-Phe-D-Ala$_L$]-OH	730	2.33	313	0.26
Tyr-c[D-Ala$_L$-Gly-Phe-D-Ala$_L$]-OH	0.56	1.58	0.35	0.0014
Tyr-c[D-Val$_L$-Gly-Phe-Ala$_L$]-OH	82.0	0.26	315	0.024
Tyr-c[D-Ala$_L$-Gly-Phe-Ala$_L$]-OH	1.06	0.35	3.03	0.12
MABE(I)	N/D	N/D	N/D	0.037
MABE(II)	1.39	1.00	1.39	0.0007
DPDPE	7300	4.10	1800	130
Leu-Enkephalin	246	11.4	21.6	>180[a]
Morphine	58.6	644	0.09	15.0

a: 40% of the maximum activity at 180 nmol dosage

The analog H-Tyr-c[D-Ala$_L$-Gly-Phe-Ala$_L$]-OH showed sub-nanomolar activity at both μ- and δ-receptors. The modification by the incorporation of two methyl groups into the β-position of D-Ala$_L$^{2} which produces H-Tyr-c[D-Val$_L$-Gly-Phe-Ala$_L$]-OH maintained the great potency of the analog H-Tyr-c[D-Ala$_L$-Gly-Phe-Ala$_L$]-OH toward the δ-receptor, while drastically decreasing in the μ-activity (Table 5). This compound is 100 times more potent than morphine *in vivo*. Thus the lanthionine-bridged enkephalins represent a new family of opioid structures. We are presently designing and synthesizing other lanthionine opioids to obtain structure–activity relationships which together with spectroscopy and molecular modeling will permit us to create novel lanthionine opioids.

In addition to sulfur-bridged cyclic peptide opioids, we have recently embarked on incorporating the nitrogen heteroatom as a bridge in the design of cyclic enkephalin analogs. In a preliminary effort, we synthesized two cyclic methylamine-bridged enkephalin analogs (MABEs): H-Tyr-c[(N$_\beta$CH$_3$)-D-A$_2$pr-Gly-Phe-NHCH$_2$CH$_2$-] [MABE(I)] **9** and H-Tyr-c[(N$_\gamma$CH$_3$)-D-A$_2$bu-Gly-Phe-NHCH$_2$CH$_2$-] [MABE(II)] **10** (Fig. 5).

Different synthetic strategies were applied for the syntheses of compounds **9** and **10**. Regioselective ring opening of the N-Cbz-D-serine β-lactone (**12**) using the secondary amine compound **11** was the key reaction for the preparation of MABE(I) **9** (Fig. 6, ref. 9). The MABE(II) analog **10** was obtained by preparation of the linear peptide on solid phase followed by cyclization in solution. Both analogs are potent but nonselective toward the μ- and δ-receptors while they exhibit only moderate activity toward the κ-receptor (Table 5). MABE(II) is among the most potent peptide opioid analogs prepared to date.
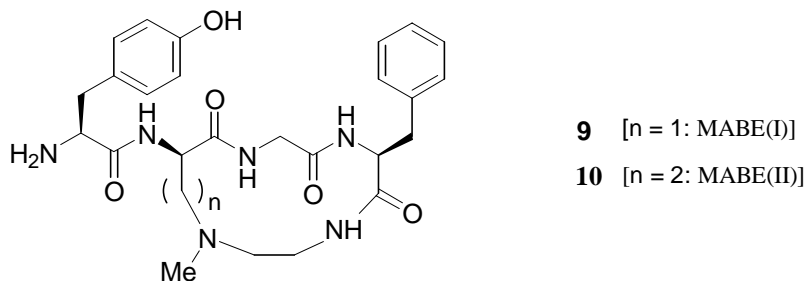

9   [n = 1: MABE(I)]

10   [n = 2: MABE(II)]

Fig. 5 Structures of MABE derivatives.

We are currently focusing on increasing selectivities of our potent amine-bridged cyclic enkephalin analogs.

CONCLUSION

Our efforts toward the development of new guanidinylation reagents has led us to new and more efficient syntheses of guanidine-containing peptides, heterocyclic drugs, and natural products. Furthermore, preparation of guanidinoglycosides using this methodology may afford new compounds with enhanced affinity and selectivity to the RNA recognition element of the HIV-1 Rev protein. We have also described the syntheses of novel sulfur and amine-bridged cyclic opioids. Several of these compounds have displayed impressive potencies and receptor specificities.
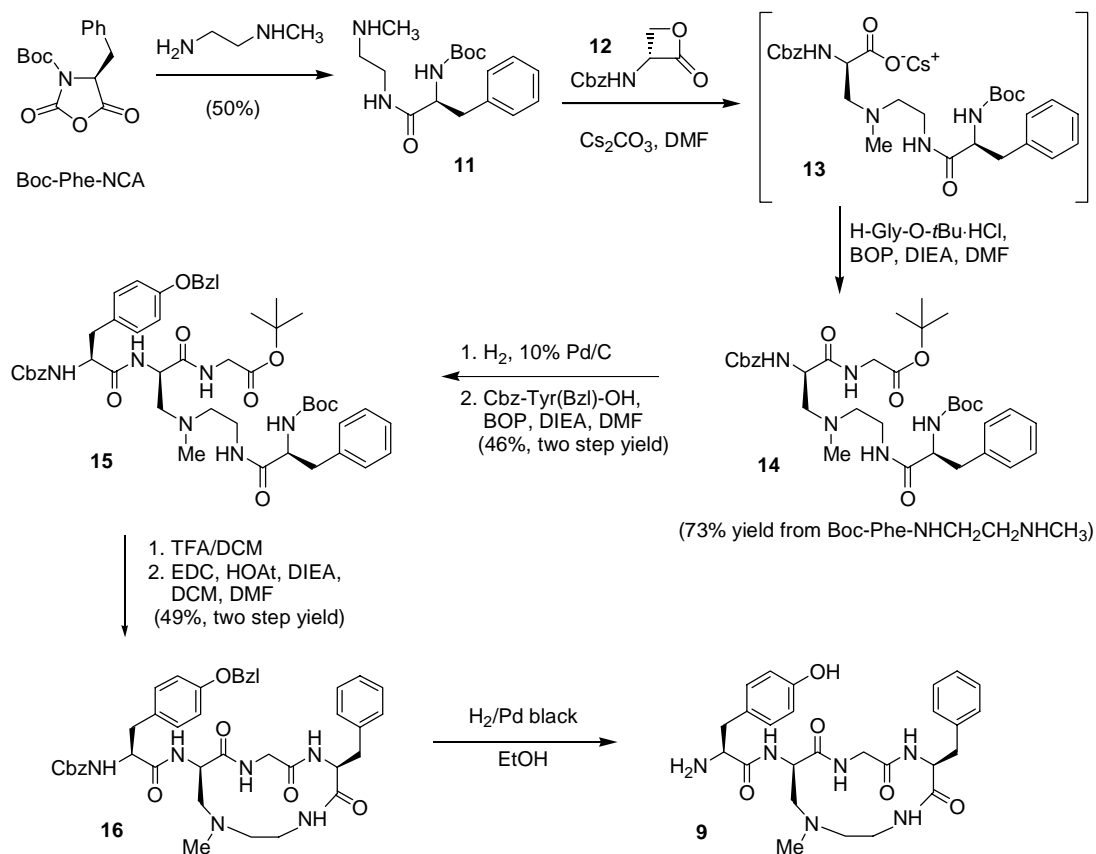
Fig. 6 Synthesis of MABE(I) **9**.

ACKNOWLEDGMENTS

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