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2-Substituted *gem*-Diamines Derived from Amino Acid Amides. Their Applications to Cross-linking in Peptide Dimerization and Conjugation of Dimer to Affinity Matrix

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Treatment of five tripeptide amides Boc-Phe-Leu-NHCH(R)CO-NH_2 (R: side chain of Gly, Leu, Phe, Asp(OBzl),or Arg(Tos)) with [bis-(trifluoroacetoxy)iodo]benzene and subsequent coupling with Boc-Phe-Leu-OH afforded the dimeric peptides cross-linked with 2-substituted *gem*-diamines; (Boc-Phe-Leu-)$_2$•(-NHCH(R)NH-). *gem*-Asp(OBzl)-cross-linked dimer was further converted into enkephalin dimer, which was conjugated to Sepharose 4B gel for receptor affinity purification.

The importance of synthetic dimer ligands in elucidating the molecular mechanism between ligand and receptor has recently been pronounced for many kinds of peptide hormones and neuropeptides such as atrial natriuretic peptide,[1] enkephalin,[2] insulin,[3] neurokinins,[4] and substance P.[5] One of problems in this line of study is the very limited availability of cross-linking spanner reagents for dimerization. In particular, those having an additional functional group at the spanner moiety for chemical modifications are little commercially available. Such spanners would be greatly useful to couple the dimer with radioactive, immunogen or fluorescence core, and with gel matrix for affinity purification. This is especially important when dimer ligands interact bivalently with receptors. The bivalent receptor structure, in which two ligand binding sites exist in a receptor molecule, has recently been found or suggested for many receptors; e.g., acetylcholine receptors,[6] insulin receptors,[7] enkephalin receptors[8] and lutropin receptors.[9]

Radhakrisha *et al.*[10] reported [bis(trifluoroacetoxy)iodo]benzene (TIB) as a reagent for the Hoffmann-type rearrangement causing amide-to-amine conversion. The usefulness of TIB was immediately introduced in peptide chemistry.[11,12] In particular, the use of TIB for peptide backbone amides has frequently been reported to synthesize the so-called "retro-inverso" derivatives of bioactive peptides such as bradykinin potentiating peptide[13], dermorphins,[14] enkephalin,[15] and somatostatin.[16] In the search of cross-linking reagents having a functional group for dimeric peptides, we appreciated the usefulness of this TIB reaction for peptide backbone amides. The *gem*-diamine moiety resulting from amide-to-amine conversion can become the spanner of dimeric peptides when another peptide with the same sequence is coupled to this newly generated amine. In the

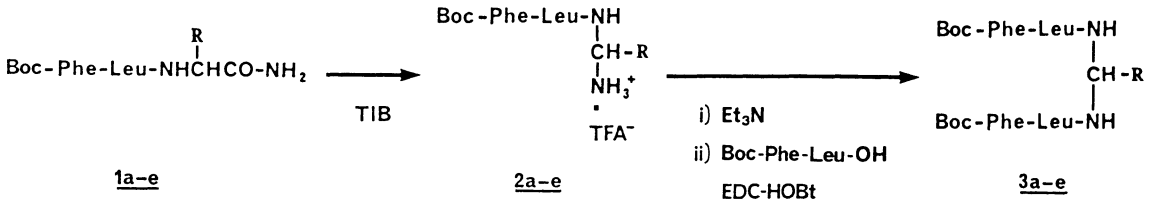

Fig. 1. Synthesis of dimeric dipeptides cross-linked with 2-substituted *gem*-diamines.

case, depending on the choice of *C*-terminal amino acid amides for TIB reaction, various substituents originating from the side chains can be introduced into a spanner moiety. We herein report the synthesis of dimeric dipeptide derivatives, (Boc-Phe-Leu-)$_2$•(-NHCH(R)NH-) 3a-e (R: a, H (Gly); b, $CH_2CH(CH_3)_2$ (Leu); c, CH_2-C_6H_5 (Phe); d, CH_2-COOBzl (Asp(OBzl)); and e, $(CH_2)_3NHC(=NH)NHTos$ (Arg(Tos))), and also the use of 3d for preparation of affinity gel loading a dimeric enkephalin.

Five tripeptide amide precursors, Boc-Phe-Leu-NHCH(R)CO-NH_2 (1a-e), were prepared from Boc-Phe-Leu-OH and amino acid amides $NH_2CH(R)CONH_2$ by utilizing 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 1-hydroxybenzotriazole (HOBt) (Fig. 1). 1a-e suspended in 50% aqueous acetonitrile were treated with 1.5 equiv. of TIB for 3-6 h at room temperature. Evaporation followed by trituration with ether gave tripeptide trifluoroacetates having 2-substituted *gem*-diamine moiety, 2a-e (yield 70-90%). Each salt (0.4-0.6 mmol) in DMF and Et_3N (1 equiv.) was coupled with Boc-Phe-Leu-OH (1 equiv.) by EDC-HOBt. The resulting dimeric dipeptide 3a-e was purified on a column (1.8 x 102 cm) of Sephadex LH-20 in DMF. The products were characterized as shown in Table 1. Diaminomethane dihydrochloride is the sole diamine commercially available (Tokyo Kasei) for the present study, and thus Boc-Phe-Leu-OH was directly dimerized with this diamine by EDC-HOBt to synthesize compound 3a. The product prepared by a direct dimerization

Table 1. Characterization[a] of dimeric dipeptide cross-linked with 2-substituted *gem*-diamines, (Boc-Phe-Leu-)$_2$•(-NHCH(R)NH-) (3a-e)

	R	Yield/%	Mp/°C	$[\alpha]_D^{20}$[b]
3a	H	76	196-198	-17.4°
3b	$CH(CH_3)_2$	72	214-215	-16.4°
3c	$CH_2C_6H_5$	52	217-219	-15.8°
3d	$CH_2COOBzl$	92	190-191	-10.0°
3e	$(CH_2)_3NHC(=NH)NHTos$	70	156-157	-9.8°

a) The products were also characterized by elemental analysis, TLC and HPTLC. b) *c* 0.5, DMF.

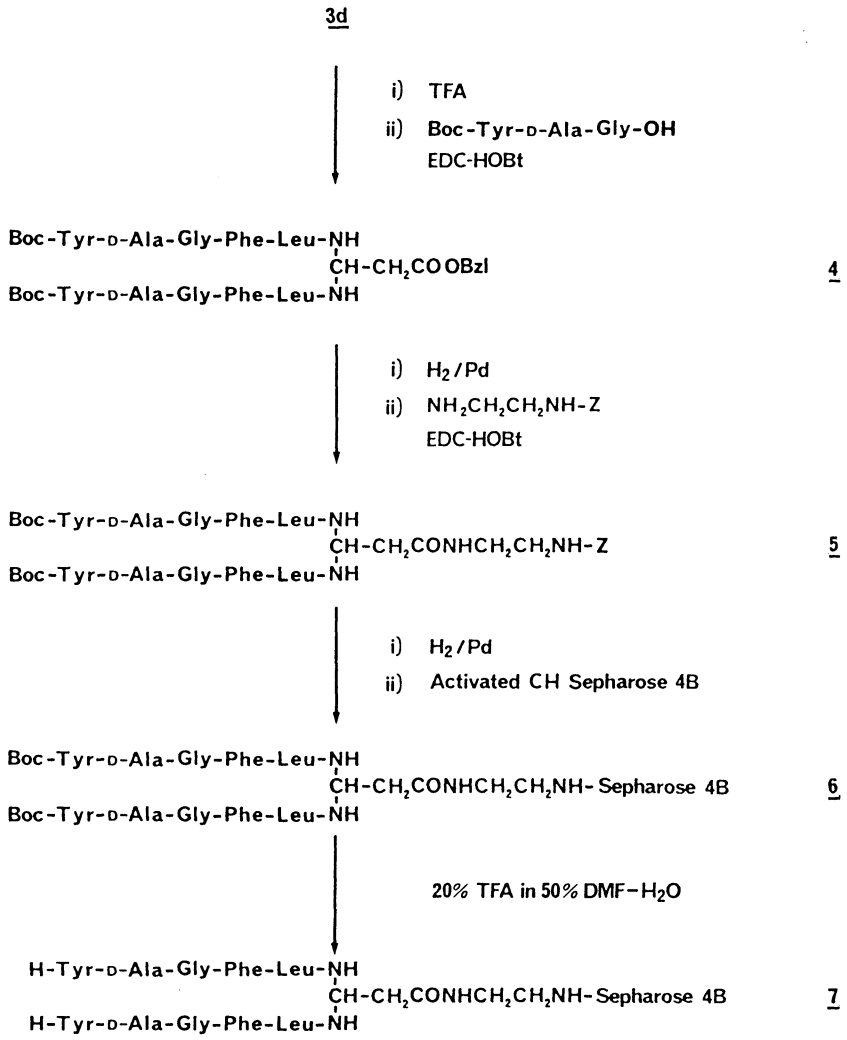

Fig. 2. Preparation of Sepharose 4B affinity gel of dimeric enkephalin analogue.

showed the same physical constants and chromatographic behaviours as those of 3a prepared as in Fig. 1.

In general, the loading of ligand onto the affinity matrix should be minimized in order to minimize the steric effects and maximize the efficiency of affinity interactions. In this sense, the usage of dimeric peptides as an affinity ligand is obviously disadvantageous. However, it is requisite to load the specific dimeric ligand onto the matrix when its receptor exist in a bivalent subunit-structure. The enkephalins might correspond to such a case. For preparation of the affinity gel of dimeric enkephalin (Fig. 2), 3d (3 mmol) was treated with trifluoroacetic acid (30 ml) to yield the ditrifluoroacetate of amino-free dimer (yield 99%). This was coupled with Boc-Tyr-D-Ala-Gly-OH (2 equiv.) by EDC-HOBt to afford the dimeric pentapeptide 4 which possesses the two sequences of [D-Ala^2,Leu^5]enkephalin (yield 82%, mp 209-211 °C, $[\alpha]_D^{20}$ -8.7° (*c* 0.5, DMF), Anal. ($C_{78}H_{104}O_{18}N_{12}\cdot H_2O$) C, H, N). Compound 4 was hydrogenated to yield the ethylenediamine by EDC-HOBt, yielding compound 5 (92%, mp 221 °C (decomp), $[\alpha]_D^{20}$ -9.1° (*c*

0.5, DMF), Anal. ($C_{81}H_{110}N_{14}O_{19}\cdot 3/2H_2O$) C, H, N). 5 was hydrogenated to yield the amino-free compound, which was incubated with the activated CH-Sepharose 4B in 50% DMF-H_2O overnight at room temperature. The Boc-protected enkephalin dimer conjugated with Sepharose 4B (6) was then treated with 20% TFA in 50% DMF-H_2O to yield the desired affinity gel 7 of dimeric enkephalin analogue.

In a qualitative radio-receptor binding assay, Boc-protected affinity gel 6 showed no binding affinity for the opiate receptors in rat brain, whereas the amine-liberated affinity gel 7 showed a distinct receptor affinity in a dose-dependent manner. It should be noted that 7 exhibited a higher preference to the delta receptors rather than to the mu receptors, consistent with the biological results obtained from dimeric pentapeptide enkephalin analogues.[17] Using this affinity gel, several biological studies are in progress in our laboratory.

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