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New general strategy of dimerization of bioactive molecules

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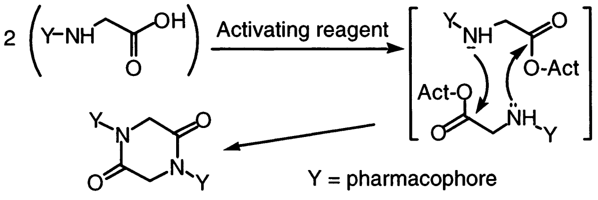
Abstract—We describe a new way of obtaining dimeric structures based on intermolecular diketopiperazine formation. The bioactive substance to dimerize was first linked to a glycine moiety. Then a coupling step using DMAP in stoichiometric quantity resulted in the cyclization involving both C-terminal carboxylic functions and the amide nitrogen. This general strategy has been applied to peptide and non-peptide bioactive molecules. © 2001 Published by Elsevier Science Ltd.

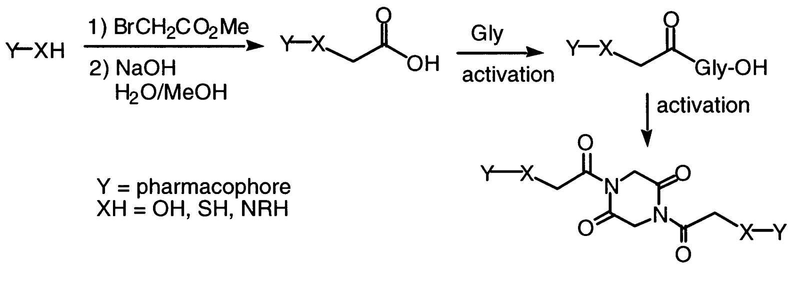
Dimerization of an active compound often results in enhanced binding and improved pharmacological properties. This potency is attributed to a higher concentration of pharmacophores in proximity to recognition sites. Usually, this bivalent ligand approach supposes to use a symmetrical bifunctional linker X to anchor two substrates P, giving rise to the general structure P-X-P.[1]

Diketopiperazines (2,5-piperazinediones, Dkps), which are the smallest cyclic peptides, are common motifs in several natural products with therapeutic properties, including inhibitors of mammalian cell cycle,[2] plasminogen activator-1[3] and topoisomerase,[4] as well as competitive substance P antagonists at the neurokinin-1 receptor.[5] Furthermore, Dkps have been shown to be useful scaffolds for the rational design of several drugs.[6] Whereas the intramolecular diketopiperazine cyclization as a side reaction in peptide synthesis is well documented,[7,8] the intermolecular formation has not yet been explored. The challenge was to build different dimeric structures, involving a Gly-Gly diketopiperazine scaffold, by activating C-terminal glycine monomers (Scheme 1).



Scheme 1.

Our strategy started with introducing a C-terminal glycine moiety on a pharmacophore, followed by an activation step to provide the diketopiperazine bearing the two substrates. Two pathways have to be considered depending on the nature, peptide or non-peptide, of the active substance. In the case of a peptide, the targeted molecule was synthesized as a C-teminal glycine extended form protected on N-terminal and side



Scheme 2.

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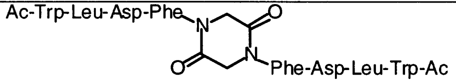
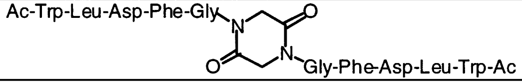
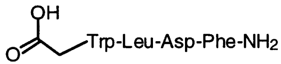
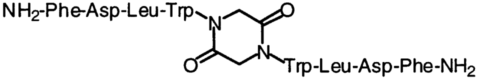
Table 1. Binding to the CCK_B receptor (human JURKAT T cells) of CCK tetrapeptide dimers

Entry	Compounds	IC_{50} (nM)	Dimerization yields (%)
1	Ac-Trp-Leu-Asp-Phe-NH_2	1	
2		106	55
3		640	71
4		400	
5		55	82

chain functions. For the dimerization of a non-peptide moiety, a bifunctional linker, the methyl bromoacetate, was inserted between the active molecule and the glycine, according to Scheme 2.

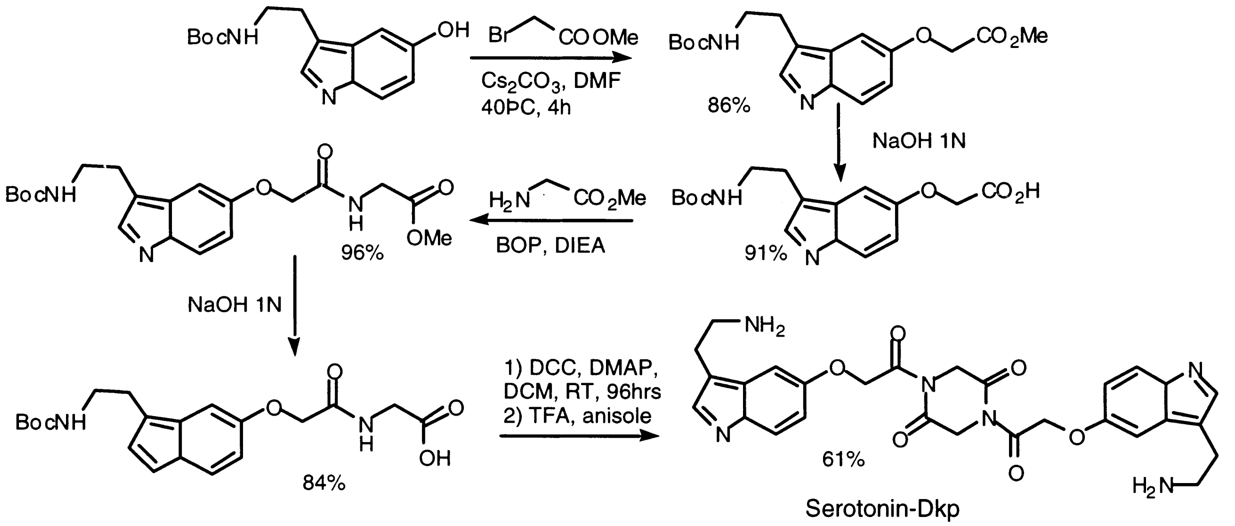
In both cases, the key reaction giving rise to the diketopiperazine scaffold is the intermolecular coupling. We have studied the activation step on a dipeptide (Boc-Leu-Gly-OH). Several activating reagents (BOP, HBTU, DCC…) were used and all produced high diketopiperazine yields when combined with DMAP. The couple DCC/DMAP, which presents a good stability in basic pH solution required for the cyclization step, produced good results despite the well-known problem of *N*-acylurea formation. Indeed this side-product formation could be limited using a high concentration of the starting monomer.

Under these reaction conditions,[9] various dipeptides (Boc-AA-Gly-OH) were dimerized in high yields (84 to >98%). However, epimerization of the carbon in the alpha position involved in the diketopiperazine formation was observed when the C-terminal Gly was replaced by chiral amino acids.

As a first application of this methodology, we synthesized several C-terminal and N-terminal cholecystokinin (CCK) tetrapeptide dimers.

In the case of the N-terminal dimer, the Gly moiety was introduced via a Williamson reaction of the N-terminal peptide on the methylbromoacetate, followed by saponification (Scheme 2, first step, XH = NRH). C-terminal dimerization (Table 1, entries 2 and 3) resulted in a decreased affinity compared to the minimum active sequence (Table 1, entry 1), since the C-terminal amide is crucial for affinity and agonist activity. However, in the case of dimerization through the N-terminal (Table 1, entry 5), we observed a 10-fold increase of activity as compared to the monomer (Table 1, entry 4).

As an example of dimerization of an active organic molecule, we selected serotonin. Serotonin 5-HT is involved in numerous physiological (e.g. thermoregulation, haemodynamics, feeding, sleeping) and pathophysiological processes (e.g. depression, hypertension, migraine, anxiety)[10] and interacts with various distinct membrane receptors. We synthesized a serotonin dimer through its hydroxyl group (Scheme 3).



Scheme 3.

Table 2. Binding to cloned 5-$HT_{1B/1D}$ and 5-HT_{1A} receptors

Compounds	K_i (nM)			EC_{50}
	5-HT_{1D}	5-HT_{1B}	5-HT_{1A}	5-HT_{1B}
Serotonin	5.06	6.76	2.48	4.82
Sumatriptan	8.5	23.1	440	77
Serotonin-Dkp	19.51	54.38	103.55	210

The agonist activity was measured on 5-$HT_{1B/1D}$ and 5-HT_{1A} receptors (Table 2). The loss of affinity and selectivity is in agreement with previous results[11,12] showing a decreased affinity when constrains rigidify the linker.

In conclusion, we have described a new dimerization of various active molecules using a diketopiperazine scaffold, resulting from an additional glycine moiety. This strategy does not require a bifunctional linker and the reaction is easy to carry out. It is worth pointing out that this dimerization reaction can be a concurrent reaction during the DCC/DMAP activation of peptide fragments on their C-terminal extremity in peptide syntheses, which is common when glycine is at the C-terminus.

Acknowledgements

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9. **Typical procedure for intermolecular cyclization**: Dicyclohexylcarbodiimide (11 mM) and 4-(dimethylamino)pyridine (11 mM) were added to the free carboxylic glycine-moiety-containing compound (10 mM) in solution in the minimum volume of dichloromethane (ca. 7 ml depending on the starting material solubility). Then the pH of the solution was adjusted to 8–9 by adding triethylamine and stirred at rt. After reaction completion, the reaction mixture was cooled down to precipitate the dicyclohexylurea which was filtered off. The resulting orange solution was concentrated in vacuo and ethyl acetate (200 ml) was added. The organic layer was washed with 1N aqueous citric acid (2×100 ml), brine (100 ml) and dried over potassium carbonate, then concentrated in vacuo. The crude product was purified by a preparative HPLC (Waters PerpLC4000 with a Delta Pack column (40×100 mm). The UV 486 detector operated at 214 nm, the flow rate was 50 ml/min. Solvents were: A, water with 0.1% TFA; B, acetonitrile with 0.1% TFA).
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