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INVESTIGATIONS OF AZAPEPTIDES AS MIMETICS OF LEU-ENKEPHALIN

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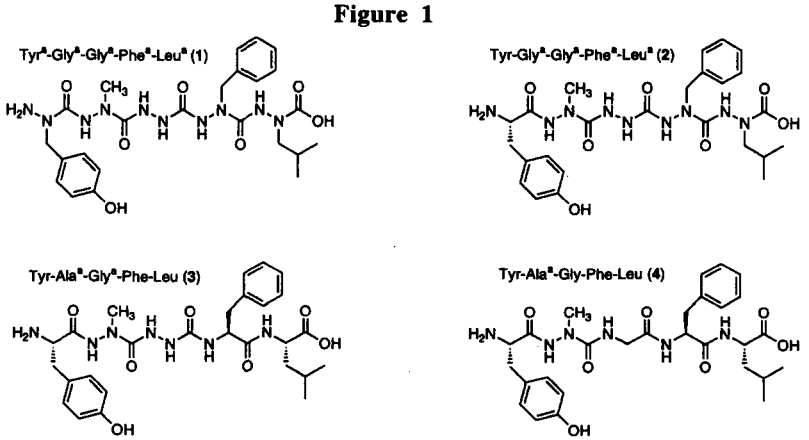
Abstract: Solution syntheses of azapeptide pentamers **2**, **3**, and **4** were accomplished. The binding affinity of these azapeptides and azatide **1** were examined in the context of monoclonal antibody 3-E7 known to strongly bind the [Leu^5]enkephalin sequence.

Leu- and Met-enkephalin are endogenous opioid peptides with morphine-like activity. Since their isolation and identification in 1975,[1] these bioactive pentapeptides have been intensely investigated in terms of their pharmacological and conformational properties. From a biochemical standpoint, investigations into the inherent mobility of the enkephalin framework, its rapid degradation in vivo[2] and the existence of multiple receptor subunits[3,4] have exposed a need for flexible synthetic routes that provide for the incorporation peptide backbone modifications.[5]

Recently, we reported an efficient method for both solution and soluble polymer syntheses of a biopolymer mimetic consisting of "α-aza-amino acids" linked in a repetitive manner to form what we term an azatide oligomer.[6] The first azatide oligomer sequence synthesized (Tyr^a-Gly^a-Gly^a-Phe^a-Leu^a **1**, Figure 1) provided us with a chance to investigate its binding affinity to monoclonal antibody 3-E7, a hybridoma raised against the antigen β-endorphin and, like the δ-opioid receptor, recognized the N-terminal portion of the protein.[7] The antibody also binds tightly to [Leu^5]enkephalin, Tyr-Gly-Gly-Phe-Leu, (K_d = 7.1 nM) and a variety of related opioid peptides.[8] However, at 1 mM the azatide pentamer **1** showed no propensity to compete with the natural

Figure 1



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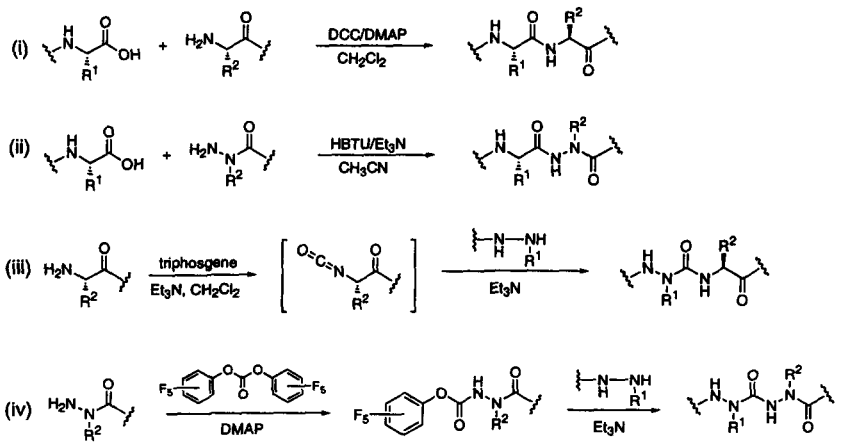
peptide for antibody 3-E7 by competition ELISA.[6,9]

To further probe receptor-ligand interactions of this antibody with aza-mimetics, we synthesized three azapeptide pentamers and examined their binding to IgG 3-E7. The first pentamer made was Tyr-Gly^a-Gly^a-Phe^a-Leu^a, **2**, (Figure 1). This compound was synthesized on the principle knowledge that replacement of Tyrosine by other amino acids including its amide bond replacement in the Leu enkephalin peptide leads to inactive analogues. These findings were based on in vitro studies using either electrically stimulated mouse vas deferens (MVD) or guinea pig ileum (GPI) assays, with Leu-enkephalin as the reference compound.[5] In addition Dutta et al. demonstrated that replacement of Gly^2 to Ala^a or Gly^3 to Gly^a provides peptidomimetics which are more potent ligands than the parent compound.[10,11] Keeping these results in mind, a second pentamer Tyr-Ala^a-Gly^a-Phe-Leu (**3**) was synthesized in an attempt to see if cooperativity could be gained by the combination of these two aza-amino acids. The third azapeptide synthesized (Tyr-Ala^a-Gly-Phe-Leu, **4**) has been previously reported;[10] our desire to examine this compound stems from findings that **4** shows a six fold greater affinity in the GPI assay compared to Leu-enkephalin.

In general, four coupling methodologies were used in the syntheses of the three azapeptides and are detailed in Scheme 1: (1) Simple amino acid (AA) coupling was accomplished using standard DCC (1,3-dicyclohexylcarbodiimide)/DMAP (4-dimethylaminopyridine) procedures. (2) For AA and aza-amino acid (AA^a) coupling, HBTU (*O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate)/triethylamine were engaged as reagents. (3) The converse reaction of AA^a and AA coupling was accomplished using triphosgene to introduce the carbonyl group. (4) The attachment of AA^a to AA^a was performed using bis(pentafluorophenyl) carbonate as the carbonyl activating element.[12]

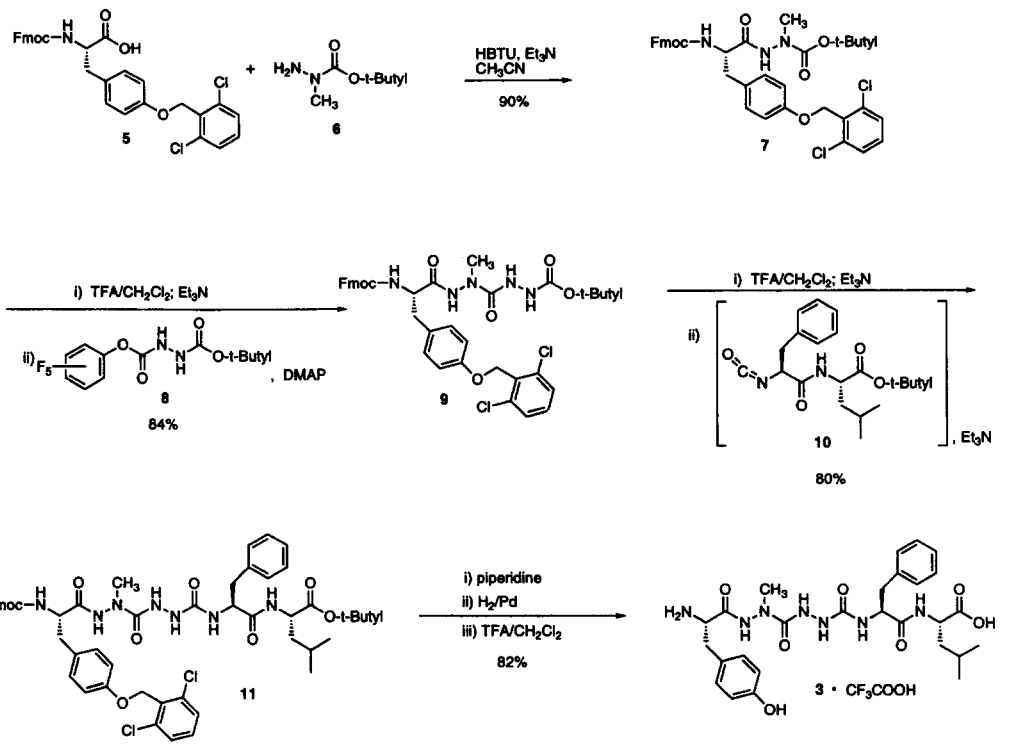
Scheme 1



(i) Between AA and AA. (ii) Between AA and AA^a. (iii) Between AA^a and AA. (iv) BetweenAA^a and AA^a.

The detailed synthesis of Tyr-Ala[a]-Gly[a]-Phe-Leu (**3**) is shown in Scheme 2. In the first step, dimer **7** was precipitated from solution and isolated in 90% yield from the protected tyrosine **5** and Boc-protected methylhydrazine using HBTU as the coupling reagent. After deprotection, dimer **7** was coupled to **8**, which was prepared from Boc-protected hydrazine and bis(pentafluorophenyl) carbonate; trimer **9** was obtained in 80% yield from these two steps. Following deprotection, **9** was coupled to **10**, which was prepared from Phe-Leu-Boc and phosgene in the presence of triethylamine. After a series of deprotection steps, **3**·CF_3COOH[13] was obtained in 82% yield. Under similar reaction conditions, compound **2**[14] and **4** weresynthesized in 55% and 50% yield, respectively.

Scheme 2



Competition ELISA[9] was used to investigate if azapeptide pentamers (**2**, **3**, and **4**) could bind IgG 3-E7. Surprisingly these azapeptides showed no propensity to compete with the natural peptide for 3-E7 at 1mM concentration. These results, especially what we observed with **4** were quite unexpected. We did not observe any correlation of compound inhibition between antibody 3-E7 and the GPI assay. The similarity of the specificity of the antibody combining site 3-E7 and that of the opioid receptor have been documented.[7] While some stereochemical specificity is clearly shared between the antibody and the opioid receptor they are not

necessarily equitable. The exploration of combinatorial azatide or azapeptide libraries may help in defining the results we have observed and will be reported in due course.

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13. Tyr-Gly^a-Gly^a-Phe^a-Leu^a (**2**)·2CF_3COOH; ^{1}H NMR (CD_3OD, 300 MHz) δ 1.04 (d, J = 6.7 Hz, 6H), 2.09 (m, 1H), 2.98 (dd, J^2 = 14.3 Hz, J = 8.1 Hz, 1H), 3.07 (m, 2H), 3.18 (dd, J^2 = 14.3 Hz, J = 6.2 Hz,1H), 4.06 (dd, J^2 = 8.0 Hz, J = 6.3 Hz, 1H), 4.23 (br s, 1H), 5.17 (br s, 1H), 6.79 (d, J = 8.5 Hz, 2H), 7.14 (d, J = 8.5 Hz, 2H), 7.33 (m, 5H); HRMS (FAB, NBA/CsI) calcd for $C_{23}H_{33}N_9O_5$·Cs^+ 648.1659, found 648.1675.

14. Tyr-Ala^a-Gly^a-Phe-Leu (**3**); ^{1}H NMR (CD_3OD, 250 MHz) δ 0.89 (d, J = 6.1 Hz, 3H), 0.94 (d, J = 6.1 Hz, 3H), 1.60-1.83 (m, 3H), 2.93 (s, 3H), 2.93-3.20 (m, 4H), 4.04 (t, J = 7.7 Hz, 1H), 4.41 (dd, J^2 = 9.1 Hz, J = 6.3 Hz, 1H), 4.53 (dd, J^2 = 9.1 Hz, J = 4.6 Hz, 1H), 6.80 (d, J = 8.5 Hz, 2H), 7.12 (d, J = 8.5 Hz, 2H), 7.15-7.33 (m, 5H); HRMS (FAB, NBA/NaI) calcd for $C_{27}H_{37}N_7O_7$·Na^+ 594.2652, found 594.2671.