Design and Synthesis of a Novel Peptide β-Turn Mimetic

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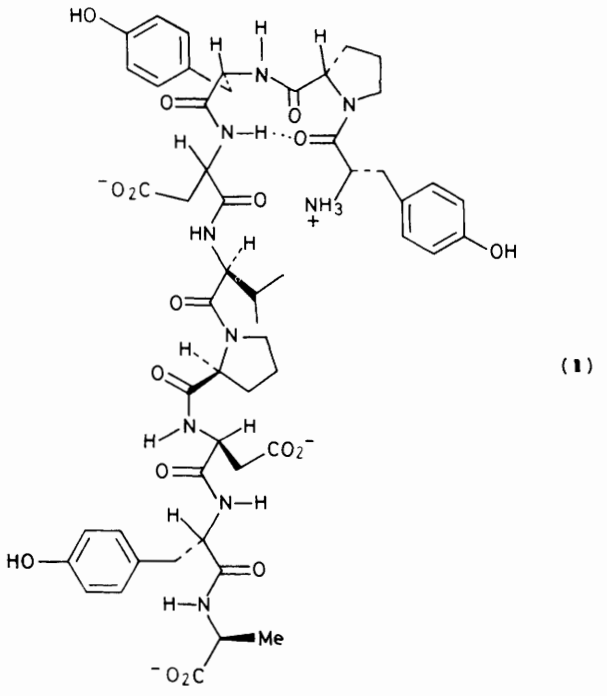
The stereospecific synthesis of the novel spirocyclic unit (**7**), and its use in the construction by solid phase methods, of a conformationally locked analogue of the immunodominant nonapeptide (**1**) is described.

The adoption of highly populated conformations containing secondary structure by short oligopeptides *in aqueous solution* has long been viewed as unlikely.[1] However, ^{1}H n.m.r. studies upon the nonapeptide (**1**) indicate that this linear peptide, in water, exists in folded forms including predominantly one containing a β-turn conformation [as in (**1**)].[2—4] More recent work has delineated the dependence of the presence of this reverse turn upon amino acid sequence.[5] These observations are important because they imply that similar conformations should exist in polypeptide chains under the *in vivo* conditions required for protein folding. In addition, the nonapeptide sequence (**1**) has been identified as the immunodominant site of the 36-residue immunogen (**2**), suggesting that the presence of local secondary structure may be an important factor in peptide-antibody recognition. We report here on the synthesis and properties of the nonapeptide (**3**), containing a novel β-turn constraint, and (**4**) having *N*-methylalanine in place of Pro-2, which mimic the extreme situations of the peptide (**1**) existing in a locked β-turn,[6] or in an extended open chain conformation, respectively, within the Tyr-Pro-Tyr-Asp segment. These materials are valuable for studies of peptide conformation in general, and afford reference spectroscopic data in this system for comparisons to those from the native peptide.



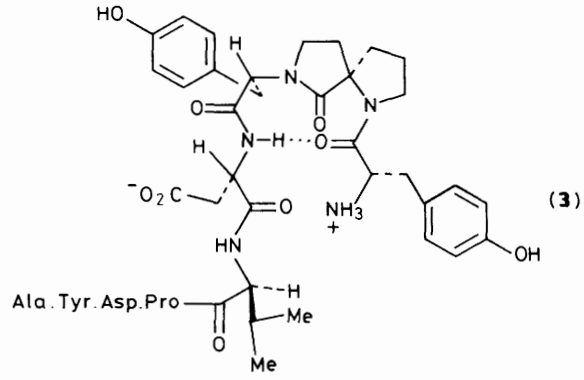
(**1**)

NH_2.His.Cys.Asp.Gly.Phe.Gln.Asn.Glu.Lys.Trp.Asp.Leu.Phe.–Val.Glu.Arg.Ser.Lys.Ala.Phe.Ser.Asn.Cys.Tyr.Pro.Tyr.Asp.–Val.Pro.Asp.Tyr.Ala.Ser.Leu.Arg.Ser.CO_2H

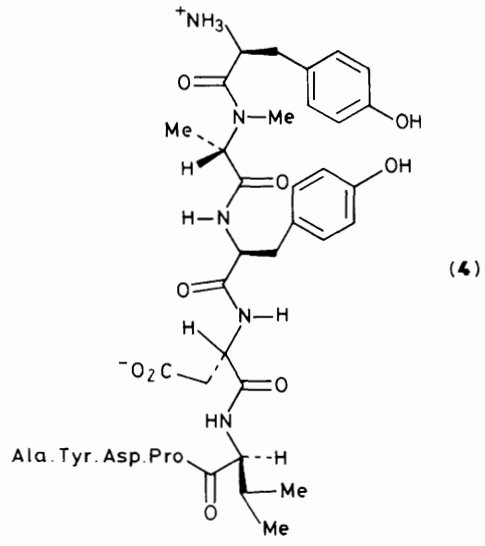
(**2**)

† Information, together with detailed co-ordinate data, about the modelling studies can be obtained from N. G. J. R., while correspondence relating to the n.m.r. and synthetic work should be addressed to J. A. R.

Thus *R*-2-allylproline (**5**) was prepared from *S*-proline in two steps using an enantiospecific alkylation, the configuration of the quaternary centre being assigned by analogy to previous work.[7]‡ Conversion of (**5**) to the protected dipeptide (**6**) was accomplished in two steps [$PhCH_2OCOCl$; H_2N·Tyr(OBu^t)·OMe, DIPC/HOBt (DIPC = di-isopropylcarbodiimide, HOBt = hydroxybenzotriazole); Scheme 1]. The spirocyclic lactam (**7**) was then formed by oxidative cleavage of the double bond[8] followed by cyclisation under Mitsunobu conditions[9] and cleavage of the N-protecting group by hydrogenation. Coupling of (**7**) with the protected tyrosine

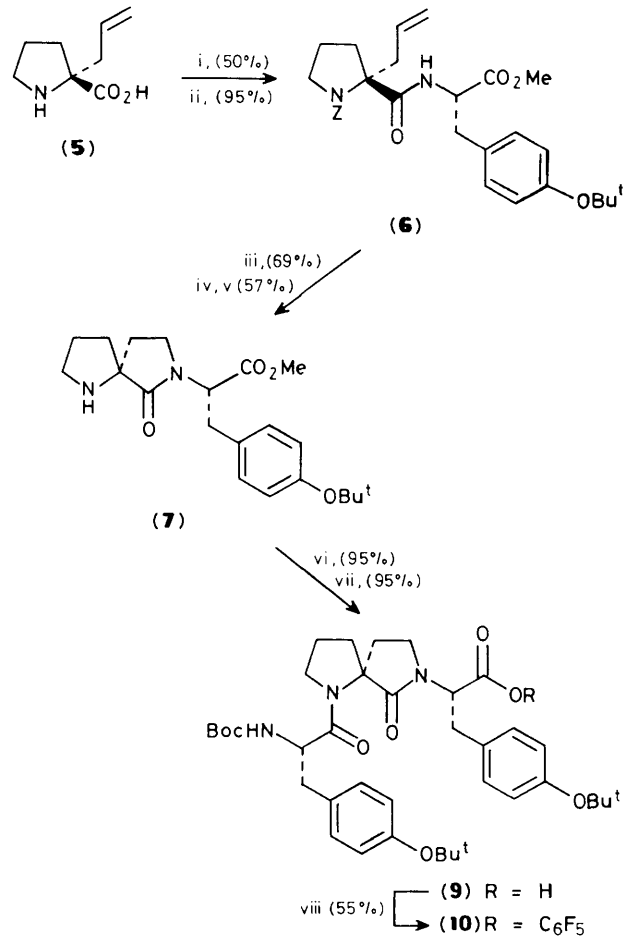


(**3**)



(**4**)

‡ All new compounds have satisfactory spectral and analytical data. Representative data for compounds (**5**)—(**7**) and (**10**): (**5**): m.p. 270 °C (decomp.); $[\alpha]_D^{19.5}$ −47.1° (*c* 1.6, H_2O); *m/z* 114 (M^+). (**6**): oil, $[\alpha]_D^{20}$ −14.1° (*c* 1.5, H_2O); fast atom bombardment (f.a.b.) m.s. (glycerol) 523 (M^+ +H); i.r.: $\nu_{max.}$ 1740 (C=O), 1690 cm^{-1} (C=O). (**7**): $[\alpha]_D^{24}$ −18.8° (*c* 0.60, $CHCl_3$); *m/z* 374.2215 ($C_{21}H_{30}N_2O_4$ requires 374.2205); i.r.: ν_{max} 1740 (C=O), 1690 cm^{-1} (C=O) (**10**): oil, $[\alpha]_D$ −21.1 (*c* 1.3, $CHCl_3$); $\nu_{max.}$ 3440 (NH), 1790, 1710, 1650 (CO) cm^{-1}; f.a.b. m.s. (glycerol) 846.3764 (M^+ + H) ($C_{44}H_{53}N_3O_8$ requires 846.3753).



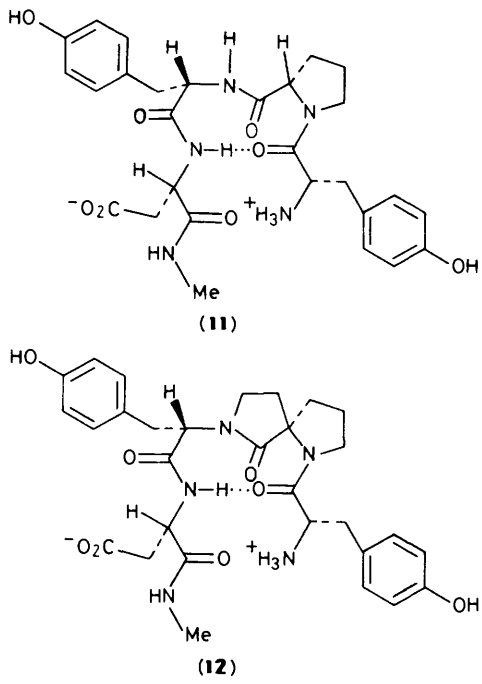
Scheme 1. *Reagents*: i, $PhCH_2OCO{\cdot}Cl$, Et_3N; ii, $H_2N{\cdot}L{\text{-}}Tyr(O\text{-}Bu^t){\cdot}CO_2Me$, $(Pr^iN{=})_2C$, HOBt, *N,N*-dimethylformamide (DMF); iii, OsO_4, $NaIO_4$, MeOH then $NaBH_4$,; iv, diethyl azodicarboxylate (DEAD), PPh_3, tetrahydrofuran (THF); v, H_2/Pd–C; vi, $Boc{\cdot}NH{\cdot}L{\text{-}}Tyr(OBu^t){\cdot}CO_2H$ (**8**), $(Pr^iN{=})_2C$, HOBt, DMF; vii, THF, aq. NaOH; viii, C_6F_5OH, $(Pr^i{}_2N)_2{=}C$, EtOAc. Boc = t-butoxycarbonyl, Z = benzyloxycarbonyl.

Table 1. Temperature coefficients (p.p.m. × 10^{-3} per K) for NH resonances in the peptides (**1**) (*trans* form), (**3**) and (**4**).

	(**1**) (*trans*)	(**3**)	(**4**)
Tyr1			
Pro(N-Me Ala)2			
Tyr3	−11.10		−10.30
Asp4	−4.28	−2.67	−7.19
Val5	−8.40	−10.48	−7.00
Pro6			
Asp7	−9.41	−10.00	−9.17
Tyr8	−8.58	−10.48	−9.72
Ala9	−8.58	−10.48	−9.72

^1H n.m.r. spectra were recorded at 360 MHz in H_2O/D_2O (90:10) (peptide conc. 15 mM), pH (uncorrected meter reading) 3.7. Coefficients were determined by least squares linear regression analysis (C_R >0.990) from 10—13 measurements over the range 276—300 K. One dimensional ^1H spectra were assigned from 2D COSY & ROESY spectra.

derivative (**8**) proceeded smoothly to give a tripeptide ester, which was then converted to the acid (**9**) using aqueous NaOH. No epimerisation was found to occur under these conditions, and so the carboxy terminus was activated through



conversion to the pentafluorophenyl ester (**10**). This is a convenient form for use in solid-phase peptide synthesis,[10] and the general utility of the spirocyclic lactam in constructing peptides of defined secondary structure is clearly evident.[11]

With the synthesis of the protected tripeptide (**10**) accomplished, the nonapeptide analogue (**3**) could be assembled using standard Fmoc solid-phase methodology (Fmoc = fluoren-9-ylmethoxycarbonyl).[10] The first six residues were loaded onto commercial Pepsyn-KA resin using preformed symmetrical anhydrides, before the N-terminus of the resin bound hexapeptide was released using piperidine. Acylation using the pentafluorophenyl ester (**10**) then smoothly generated the fully protected form of the desired resin bound nonapeptide (**3**). Cleavage of the peptide from the resin using 95% TFA/H_2O (TFA = trifluoroacetic acid) yielded the nonapeptide analogue (**3**) directly. Purification of the crude material using chromatography on LH-20 Sephadex (99:1 H_2O/AcOH) afforded material which was >98% homogeneous by reverse-phase h.p.l.c. The analogue (**4**) was assembled in a similar vein using *S*-Fmoc-*N*-methylalanine in place of proline-2.§

With these peptides in hand, their conformational properties were analysed by n.m.r. spectroscopy.¶ The ^1H n.m.r. spectrum of (**1**) shows two sets of overlapping NH resonances (in 1:2 ratio) due to *cis–trans* isomerisation about the Tyr-1 and Pro-2 amide bond, which is slow on the n.m.r. timescale. Indeed, 2D experiments have indicated that the β-turn is only populated in the *trans* isomer.[3—5] However, the ^1H n.m.r. spectrum of (**3**) and (**4**) each in 90% H_2O/D_2O each showed

§ Reverse-phase h.p.l.c. analysis was carried out using a C_{18} column; gradient elution H_2O/MeCN/TFA (90:10:0.2 to 10:90:0.2%) over 30 minutes. The oligopeptides (**3**) and (**4**) were characterised using standard methods, representative data being as follows: (**3**) fast atom bombardment (f.a.b.)-m.s. (Glycerol) *m/z* 1128.4873 (M^+ + 1), $C_{55}H_{70}N_9O_{17}$ requires 1128.4889; amino acid analysis, Asp 1.91, Ala 0.99, Pro 1.00, Tyr 1.43, Val 1.00. (**4**) F.a.b.-m.s. (Glycerol, Thioglycerol, TFA) *m/z* 1090 (M^+ + 1); amino acid analysis, Asp 2.00, Ala 1.00, Pro 1.07, Tyr 2.94, Val 1.10.

¶ The circular dichroism spectra of (**1**) and (**3**) are dominated by tyrosine contributions, making the relevance of any conclusions about defined secondary structure, based on these data, uncertain.

only *one major set* of NH resonances, consistent with the preponderance of a single rotamer at this peptide link. The temperature coefficients of the chemical shifts of the Asp-4 NH amide proton signals are of special interest (Table 1). The low value observed for (**3**) is in good agreement with that expected[12] for protons undergoing slow exchange with the solvent due to intramolecular hydrogen bonding (<−3.0 p.p.b./K). This is consistent with a single strong hydrogen bond in the β-turn [as shown in (**3**)], thus placing a limit on the expected coefficient in a conformationally locked system. On the other hand, since the replacement of Pro-2 by *N*-methylalanine does not significantly alter the electrostatic properties, relative to (**1**), the large increase in the coefficient for (**4**) must reflect an increased conformational freedom for the peptide, consistent now with the adoption of a random coil conformation.

Computational studies were used to evaluate further the ability of the spirocyclic lactam unit to hold the peptide backbone in a type II β-turn.[13] A full conformational analysis using a modified 'build-up' procedure,‖ together with the all-atom AMBER potential functions and parameters[14] allowed us to map the low energy structures of the tetrapeptide derivative (**11**), and the corresponding spirocyclic analogue (**12**). As would be expected, the overwhelming contribution to the low energy structures was the electrostatic interaction between the protonated amino group and the carboxylate side chain of Asp-4. Indeed, the lowest energy conformations of (**11**) and (**12**) were significantly preferred to any others.** A comparison of these structures was then undertaken using standard superimposition methods.[15] The r.m.s. deviation of the main-chain atoms in the two structures was 0.510 Å, indicating that there is a high degree of similarity in the turn region of these tetrapeptide units. In both compounds appropriate distances and torsion angles are in good agreement with those expected for type II β-turns.[16] Solvent-accessible surface area[17] and volume calculations[18] demonstrated that relative to (**11**) the constraining bridge contributes an additional 24 Å^3, approximately 4% of the total, to the volume of (**12**). These data have complemented the spectroscopic studies, and confirm that the spirocyclic unit functions as a conformational mimic of any type-II β-turn structure in the nonapeptide (**1**).

The immunological effects of altering rationally the β-turn conformation in this important nonapeptide are currently under investigation using solution phase assays with monoclonal antibodies, and our results will be reported in due course.

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‖ In this procedure, a full search was initially carried out for the L-Pro-L-Tyr dipeptide unit, and optimum type β-turn structures selected for further use. Flanking amino acid units (L-Tyr and L-Asp-NMe) were then added to each β-turn dipeptide giving a set of initial structures of (**11**). Variation of the torsion angles of this set was carried out while maintaining only the psi-phi angles of the central L-Pro-L-Tyr unit at their previously set values. Energy minimisation of all of these conformations, followed by removal of conformations greater than 20 kJ/mol above the global minimum energy structure then yielded 32 low energy structures for (**11**). Optimised conformations of (**12**) were located in an analogous fashion. For more details, see K. D. Gibson and H. A. Scheraga, *J. Comput. Chem.*, 1987, **8**, 826.

** The energy minimisation used a point-charge model for determining electrostatic energy. In our study, these partial charges were obtained from *ab initio* computations using an STO-321G basis set. We thank James Petts for performing these calculations and for useful discussions about their interpretation. Alternative methods for obtaining such charges are also under investigation. For example, see: U. C. Singh and P. A. Kollman, *J. Comput. Chem.*, 1984, **5**, 129. Full details of the modelling work will be presented elsewhere.

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