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An Exploration of the Effects of L- and D-Tetrahydroisoquinoline-3-carboxylic Acid Substitutions at Positions 2, 3 and 7 in Cyclic and Linear Antagonists of Vasopressin and Oxytocin and at Position 3 in Arginine Vasopressin

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Abstract: We have investigated the effects of mono-substitutions with the conformationally restricted amino acid, 1,2,3,4 tetrahydroisoquinoline-3-carboxylic acid (Tic) at position 3 in arginine vasopressin (AVP), at positions 2, 3 and 7 in potent non-selective cyclic AVP V_2/V_{1a} antagonists, in potent and selective cyclic and linear AVP V_{1a} antagonists, in a potent and selective oxytocin antagonist and in a new potent linear oxytocin antagonist Phaa-D-Tyr(Me)-Ile-Val-Asn-Orn-Pro-Orn-NH_2 (10). We report here the solid-phase synthesis of peptide 10 together with the following Tic-substituted peptides: 1, [Tic^3]AVP; 2, d$(CH_2)_5$[D-Tic^2]VAVP; 3, d$(CH_2)_5$[D-$Tyr(Et)^2Tic^3$]VAVP; 4, d$(CH_2)_5$[Tic^2Ala-$NH_2{}^9$]AVP; 5, d$(CH_2)_5$[$Tyr(Me)^2$,Tic^3,Ala-$NH_2{}^9$]AVP; 6, d$(CH_2)_5$[$Tyr(Me)^2$,Tic^7]AVP; 7, Phaa-D-Tyr(Me)-Phe-Gln-Asn-Lys-Tic-Arg-NH_2; 8, desGly-NH_2,d$(CH_2)_5$[Tic^2,Thr^4]OVT; 9, desGly-NH_2d$(CH_2)_5$[$Tyr(Me)^2Thr^4$, Tic^7]OVT; 11, Phaa-D-Tic-Ile-Val-Asn-Orn-Pro-Orn-NH_2, using previously described methods. The protected precursors were synthesized by the solid-phase method, cleaved, purified and deblocked with sodium in liquid ammonia to give the free peptides 1–11 which were purified by methods previously described. Peptides 1–11 were examined for agonistic and antagonistic potency in oxytocic (*in vitro*, without Mg^{2+}) and AVP antidiuretic (V_2-receptor) and vasopressor (V_{1a}-receptor) assays. Tic^3 substitution in AVP led to drastic losses of V_2, V_{1a} and oxytocic agonistic activities in peptide 1. L- and D-Tic^2 substitutions led to drastic losses of anti-V_2/anti-V_{1a} and anti-oxytocic potencies in peptides 2, 4, 8 and 11 (peptide 2 retained substantial anti-oxytocic potency; pA_2=7.25±0.025). Whereas Tic^3 substitution in the selective V_{1a} antagonist d$(CH_2)_5$[$Tyr(Me)^2$,Ala-$NH_2{}^9$]AVP(C) led to a drastic reduction in anti-V_{1a} potency (from anti-V_{1a} pA_2 8.75 to 6.37 for peptide 5, remarkably, Tic^3 substitution in the V_2/V_{1a} antagonist d$(CH_2)_5$[D-$Tyr(Et)^2$]VAVP(B) led to full retention of anti-V_2 potency and a 95% reduction in anti-V_{1a} potency. With an anti-V_2 pA_2=7.69±0.05 and anti-V_{1a} pA_2=6.95±0.03, d$(CH_2)_5$[D-$Tyr(Et)^2$,Tic^3]VAVP exhibits a 13-fold gain in anti-V_2/anti-V_{1a} selectivity compared to (B). Tic^7 substitutions are very well tolerated in peptides 6, 7 and 9 with excellent retention of the characteristic potencies of the parent peptides. The findings on the effects of Tic^3 substitutions reported here may provide promising leads to the design of more selective and possibly orally active V_2 antagonists for use as pharmacological tools and as therapeutic clinical agents for the treatment of the syndrome of the inappropriate secretion of antidiuretic hormone (SIADH).

Keywords: Vasopressin; oxytocin; antagonist; Tic; receptor

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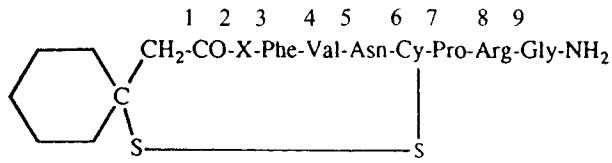
Abbreviations

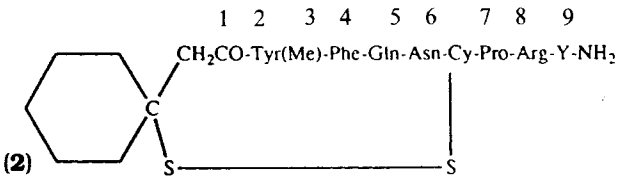
All amino acids are in the L-configuration unless otherwise noted. AVP, arginine vasopressin; LVP, lysine vasopressin; OT, oxytocin; Tic, 1,2,3,4-tetrahydroisoquinoline 3-carboxylic acid. $d(CH_2)_5$[D-Phe^2]VAVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid), 2-D-phenylalanine, 4-valine] arginine vasopressin (A); $d(CH_2)_5$[D-Tic^2]VAVP, D-Tic^2 analogue of (A); $d(CH_2)_5$[D-Tyr(Et)^2]VAVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid), 2-O-ethyl-D-tyrosine, 4-valine] arginine vasopressin (B); $d(CH_2)_5$[D-Tyr(Et)^2,Tic^3]VAVP, Tic^3 analogue of (B). $d(CH_2)_5$[Tyr(Me)^2, Ala-NH_2^{9}]AVP, [1-(β-mercapto-β,β-pentamethylenepropionic acid), 2-O-methyltyrosine, 9-alanine amide] arginine vasopressin (C); $d(CH_2)_5$, Tic^2, Ala-NH_2^{9}]AVP, Tic^2 analogue of (C); $d(CH_2)_5$ [Tyr(Me)^2, Tic^3, Ala-NH_2^{9}]AVP, Tic^3 analogue of (C); $d(CH_2)_5$[Tyr(Me)]AVP], [1-(β-mercapto-β,β-pentamethylenepropionic acid), 2-O-methyltyrosine] arginine vasopressin (D); $d(CH_2)_5$[Tyr(Me^2), Tic^7]AVP, Tic^7 analogue of (D); desGly-$NH_2$$d(CH_2)_5$[Tyr(Me)^2,Thr^4]OVT, des-9-glycineamide[1-(β-mercapto-β♀β-pentamethylenepropionic acid), 2-O-methyltyrosine, 4-threonine] ornithine-vasotocin (F); desGly-NH_2d $(CH_2)_5$[Tic^2,Thr^4]OVT, Tic^2 analogue of (F); desGly-$NH_2$$d(CH_2)_5$ [Tyr(Me)^2,Thr^4,Tic^7]OVT, Tic^7 analogue of (F); Phaa, phenylacetyl; ACE, angiotensin-converting enzyme; V_2, antidiuretic; V_{1a}, vasopressor; SIADH, syndrome of the inappropriate secretion of antidiuretic hormone.

Conformation restriction has been shown to be a powerful tool for the design of selective agonists and antagonists of a number of biologically active peptides [1]. 1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid was discovered early in this century [2, 3] and is a conformationally restricted surrogate for both Phe and Pro. It has been utilized over the past decade in the design of angiotensin-converting enzyme (ACE) inhibitors [4] and to great effect in the design of receptor-selective agonists and antagonists of biologically active peptides [5–12]; most notable being its use in the design of a highly potent receptor antagonist of bradykinin [11, 12]. Recent findings by Hruby and colleagues [13], confirmed by independent assays reported here, show that replacement of the Tyr^2 residue in oxytocin by either L- or D-Tic gave rise to molecules which exhibited oxytocic antagonism *in vitro* and suggested the possibility that similar modifications at position 2 in some of our oxytocin (OT) antagonists might lead to the enhancement of their antagonistic potencies and/or selectivities. By contrast, a report by Lebl and colleagues [14] showing that L-Tic substitutions at positions two and three in lysine vasopressin (LVP) led to drastic losses in agonistic activities but did not produce antagonists, appeared to suggest that Tic substitutions at positions 2 or 3 would be of limited value in AVP V_2/V_{1a} antagonist design. Nonetheless, we expanded our investigation to explore the effects of Tic substitutions not only at position 2 in OT antagonists but also at positions 3 and 7 in AVP antagonists and also at position 7 in OT antagonists. The non-selective AVP V_2/V_{1a} antagonists: $d(CH_2)_5$[D-Phe^2]VAVP (A) [15] and $d(CH_2)_5$ [D-Tyr(Et)^2]VAVP (B) [16] which have the following general structure (**1**):



(**1**)

In (A) X = D-Phe; in (B) X = D-Tyr(Et) were modified at positions 2 and 3 to give the D-Tic^2 and L-Tic^3 analogues of (A) and (B) respectively (peptides 2 and 3, Table 1). The potent and selective cyclic V_{1a} antagonists: $d(CH_2)_5$[Tyr(Me)^2,Ala-NH_2^{9}]AVP (C) [17] and $d(CH_2)_5$Tyr(Me)^{2}AVP (D) [18] which have the following general structure (**2**):



(**2**)

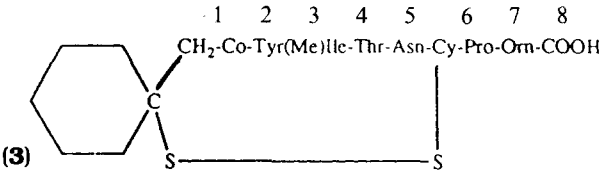
In (C): Y = Ala; in (D): Y = Gly were modified at positions 2 and 3 (C) and at position 7 (D) to give the Tic^2 and Tic^3 analogues of (C) (peptides 4 and 5, Table 2) and the Tic^7 analogue of (D) (peptide 6, Table 2). The potent and selective linear V_{1a} antagonist, Phaa-D-Tyr(Me)-Phe-Gln-Asn-Lys-Pro-Arg-NH_2 (E) [19] (where Phaa = Phenylacetyl) was modified at position 7 to give the Tic^7 analogue (E) (peptide 7, Table 2). The potent and selective OT antagonist desGly-$NH_2$$d(CH_2)$[Tyr(Me)^2,Thr^4]-OVT (F) [20] which

Table 1. Effects of D-Tic^2 and L-Tic^3 Substitutions in the Potent Non-selective V_2/V_{1a} Antagonists: d$(CH_2)_5$[D-Phe^2]VAVP and d$(CH_2)_5$D-Tyr(Et)VAVP Respectively

No.	Peptide	Anti-antidiuretic (Anti-V_2)		Antivasopressor (Anti-V_{1a}		Antioxytocic (*in vitro*) pA_2 no Mg^{2+}
		Effective Dose (ED)[a] (nmol/kg)	pA_2[b]	Effective Dose (ED)[a] nmol/kg	pA_2[b]	
A.	d$(CH_2)_5$[D-Phe^2]VAVP[d]	0.67 ± 0.13[c]	8.07 ± 0.09	0.58 ± 0.04	8.06 ± 0.03	7.74 ± 0.06
2.	d$(CH_2)_5$[D-Tic^2]VAVP[f]	mixed ~0.004 U/Mg	~5.8	53.0 ± 3.4	6.10 ± 0.03	7.25 ± 0.05
B.	d$(CH_2)_5$[D-Tyr(Et)^2]VAVP[e]	1.1 ± 0.15	7.81 ± 0.07	0.45 ± 0.11	8.22 ± 0.12	8.32 ± 0.10
3.	d$(CH_2)_5$[D-Tyr(Et)^2, Tic^3]VAVP[f]	1.38 ± 0.15	7.69 ± 0.05	7.53 ± 0.58	6.95 ± 0.03	7.54 ± 0.05

[a] The effective dose is defined as the dose (in nmol/kg) that reduced the response seen with $2x$ units of agonist to equal the response seen with x units of agonist administered in the absence of the antagonist.
[b] Estimated *in vivo* pA_2 values represent the negative logarithms of the effective dose divided by the estimated volume of distribution (67ml/kg).
[c] Means ± SE.
[d] Data from Manning *et al.* [15].

has the following structure (**3**):



(**3**)

was modified at positions 2 and 7 to give the Tic^2 and Tic^7 analogues of (F) (peptides 8 and 9, Table 3). A new potent linear OT antagonist Phaa-D-Tyr(Me)-Ile-Val-Asn-Orn-Pro-Orn-NH_2 (peptide 10, Table 3), reported here for the first time, was modified at position 2 by a D-Tic/D-Tyr(Me) interchange to give the D-Tic^2 analogue of 10, (Peptide 11, Table 3). The Tic^3 analogue of AVP ([Tic^3]AVP) (peptide 1, Table 4) was also synthesized to determine the effects of a Tic^3/Phe^3 interchange in AVP itself. We report here the synthesis by the solid phase method of the following eleven new peptides:

1. [Tic^3]AVP
2. d$(CH_2)_5$[D-Tic^2]VAVP
3. d$(CH_2)_5$[D-Tyr(Et)^2,Tic^3]VAVP
4. d$(CH_2)_5$[Tic^2,Ala-$NH_2{}^9$]AVP
5. d$(CH_2)_5$[Tyr(Me)^2,Tic^3, Ala-$NH_2{}^9$]AVP
6. d$(CH_2)_5$[Tyr(Me)^2,Tic^7]AVP
7. Phaa-D-Tyr(Me)-Phe-Gln-Asn-Lys-Tic-Arg-NH_2
8. desGly-NH_2d$(CH_2)_5$[Tic^2,Thr^4]OVT
9. desGly-NH_2d$(CH_2)_5$[Tyr(Me)^2,Thr^4,Tic^7]OVT
10. Phaa-D-Tyr(Me)-Ile-Val-Asn-Orn-Pro-Orn-NH_2
11. Phaa-D-Tic-Ile-Val-Asn-Orn-Pro-Orn-NH_2

Peptide Synthesis

Starting from Boc-Gly-resin, Boc-Ala-resin, Boc-Arg(Tos)-resin, or Boc-Orn(Tos)-resin, we synthesized the protected precursors (I–XI) of the free peptides (1–11), entirely by the solid-phase method [22–26]. HCl (1 M)/AcOH was used in all the deprotection steps except those involving Boc-Gln in which TFA was employed [22, 26]. Neutralizations were carried out with 10% Et_3N/CH_2Cl_2. Coupling reactions were mediated primarily by DCCI/HOBt [27] in CH_2Cl_2/DMF except for Boc-Asn and Boc-Gln which were incorporated as their *p*-nitrophenyl esters [28] in DMF. Cleavage from the acylpeptide resin was either by ammonolysis in methanol [22, 26, 29], with the normal DMF extraction or a modified MeOH extraction procedure (necessitated by the unusual solubility of the Tic^2 and Tic^3 protected peptides II–V in DMF/H_2O) to give protected peptide amides, or by HBr/TFA [24, 25, 30, 31] to give the protected desGly-NH_2 precursor peptides (VIII, IX). Na in NH_3 [32, 33] was used to deblock each protected precursor as previously described [34–39] and the resulting disulphydryl compounds were oxidatively cyclized with $K_3[Fe(CN)_6]$ using the normal procedure [41] or a modified reverse procedure [42]. The free peptides were desalted and purified by gel filtration on Sephadex G15 and Sephadex LH20 in a two-step procedure using 50% and 2 M AcOH as eluents, respectively as previously described [43]. After the

Table 2. Effects of L-Tic^2, L-Tic^3 and L-Tic^7 Substitutions in Potent and Selective Cyclic and Linear AVP/V_{1a} Receptor Antagonists.

No.	Peptide	Antivasopressor (Anti-V_{1a})		Anti-antidiuretic (Anti-V_{2a})		Antioxytocic (*in vitro*) pA_2 no Mg^{2+} pA_2[b]
		Effective Dose[a] (nmol/kg)	pA_2[b]	Effective Dose[a] (nmol/kg)	pA_2[b]	
C.	d$(CH_2)_5$[Tyr(Me)^2,Ala-$NH_2{}^9$]AVP[d]	0.13 ± 0.03[c]	8.75 ± 0.08	mixed ~77	~6.0	
4.	d$(CH_2)_5$[Tic^2,Ala-$NH_2{}^9$]AVP[e]	17.1 ± 1.8	6.59 ± 0.04	agonist < 0.001 U/mg		6.37 ± 0.03
5.	d$(CH_2)_5$[Tyr(Me)^2,Tic^3,Ala-$NH_2{}^9$]AVP[e]	28.4 ± 0.24	6.37 ± 0.03	mixed < 0.001 U/mg	~5.2	6.26 ± 0.04
D.	d$(CH_2)_5$[Tyr(Me)^2]AVP[e,f]	0.39 ± 0.05[c] 0.16 ± 0.01[f]	8.27 ± 0.05[e] 8.62 ± 0.03[f]	agonist[f] 0.31 U/mg[f]		7.44 ± 0.44[e] 8.15 ± 0.20[f]
6.	d$(CH_2)_5$[Tyr(Me)^2,Tic^7]AVP[e]	0.44 ± 0.05	8.19 ± 0.05	agonist < 0.01 U/mg		7.54 ± 0.05
E.	Phaa-D-Tyr(Me)-Phe-Gln-Asn-Lys-Pro-Arg-NH_2[g]	0.083 ± 0.008	8.93 ± 0.05	> 24	< 6.5	
7.	Phaa-D-Tyr(Me)-Phe-Gln-Asn-Lys-Tic-Arg-NH_2[e]	0.24 ± 0.03	8.46 ± 0.05	agonist < 0.05 U/mg		7.72 + 0.02

[a] The effective dose is defined as the dose (in nmol/kg) that reduced the response seen with $2x$ units of agonist to equal the response seen with x units of agonist administered in the absence of the antagonist.
b Estimated in *in vivo* pA_2 values represent the negative logarithms of the effective dose divided by the estimated volume of distribution (67ml/kg).
[c] Means ± SE.
[d] Data from Manning *et al.* [17].
[e] This publication.
[f] Data from original synthesis, Kruszynski *et al.* [18]; differences may reflect the fact that the assays were carried out on different preparations in different laboratories.
[g] Data from Manning *et al.* [19].

Table 3. Effects of L-Tic^2 and L-Tic^7 Substitutions in the Potent and Selective Cyclic Oxytocin Antagonist desGly-NH_2d$(CH_2)_5$[Tyr(Me)^2,Thr^4]OVT and of D-Tic^2 in the New Linear OT Antagonist (10)

No.	Peptide	Antioxytocic *in vitro*, $pA_2{}^9$		Antivasopressor (Anti-V_{1a})		Anti-antidiuretic (Anti-V_2)	
		no Mg^{2+}	0.5mM Mg^{2+}	Effective Dose[a] (nmol/kg)	pA_2[b]	Effective Dose[a] (nmol/kg)	pA_2
F.	desGlyNH_2d$(CH_2)_5$[Tyr(Me)^2, Thr^4]OVT[d,e,f]	7.95 ± 0.07[c,d] 7.89 ± 0.04[f]	8.24 ± 0.09	25.1 ± 4.6[d] 23 ± 4[f]	6.46 ± 0.09[d] 6.48 ± 0.08[f]	(< 0.05 U/mg[d]) ~300[f]	< 5.5[d] ~5.3[f]
8.	desGlyNH_2d$(CH_2)_5$[Tic^2,Thr^4] OVT[d]	5.64 ± 0.05		ND[g]		ND[g]	
9.	desGlyNH_2d$(CH_2)_5$[Tyr(Me)^2, Thr^4,Tic^7]OVT[d]	7.63 ± 0.05		18.2 ± 0.9	6.57 ± 0.02	< 0.003 U/mg	< 5.0
10.	Phaa-D-Tyr(Me)-Ile-Val-Asn-Orn-Pro-Orn-NH_2[d]	7.90 ± 0.03	7.87 ± 0.06	2.9 ± 0.08	7.40 ± 0.09	mixed ~330	~5.3
11.	Phaa-D-Tic-Ile-Val-Asn-Orn-Pro-Orn-NH_2[d]	6.11 ± 0.04		392 ± 34	5.24 ± 0.04	< 0.001 U/mg	< 5.0

[a] The effective dose is defined as the dose (in nmol/kg) that reduced the response seen into $2x$ units of agonist to equal the response seen with x units of agonist administered in the absence of the antagonist.
[b] Estimated *in vivo* pA_2 values represent the negative logarithms of the 'effective dose' divided by the estimated volume of distribution (67 ml/kg).
[c] Means ± SE.
[d] This publication.
[e] Assays carried out in WYC Laboratory on a new preparation of this peptide.
[f] Data from Manning *et al.* [20].
[g] Non-detectable agonist or antagonist activity.

Table 4. Effects of Tic^2 (L- and D-) and Tic^3 Substitutions in the Vasopressins and in Oxytocin

No.	Peptide	Biological Activity (units/mg ± Standard Error of the Assay)		
		Rat Uterus (no Mg^{2+})	Vasopressor	Antidiuretic
	AVP[a,b]	13.5 ± 0.5	369 ± 6	323 ± 16
1.	[Tic^3]AVP[c]	0.02	0.25 ± 0.03	0.91 ± 0.08
	LVP[b]	10.1 ± 0.3	284 ± 39	270 ± 15
	[Tic^2]LVP[d]	< 0.0045	< 0.01	0.005
	[Tic^3]LVP[d]	< 0.0045	< 0.01	0.046
	OT[e]	450 ± 30	5 ± 1	5 ± 1
	[Tic^2]OT[f,c,g]	antagonist	ND[h]	ND[h]
		pA_2 = 5.46 ± 0.03[c,g]		
		< 5.6[f]	Nil[f]	
	[D-Tic^2]OT[c,g]	antagonist	ND[h]	ND[h]
		pA_2 = 6.49 ± 0.04[c,g]		
		= 6.7[f]		

[a] Abbreviations: AVP, arginine vasopressin; Tic, 1,2,3,4-tetrahydroisoquinoline 3-carboxylic acid; LVP, lysine vasopressin; OT, oxytocin.
[b] Data from Manning *et al.* [21]
[c] This publication.
[d] Data from Prochazka *et al.* [14].
[e] Data from Manning *et al.* [22].
[f] Data from Lebl *et al.* [13].
[g] Assays carried out on samples provided by Professor V.H. Hruby on materials reported by Lebl *et al.* [13].
[h] Non-detectable agonist or antagonist activity.

deblocking of the linear peptides (7, 10 and 11), the usual oxidation step was omitted prior to gel filtration. The purity of the free peptides (1–11) was checked by thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), amino acid analysis and mass spectrometry.

Bioassays

Peptides were assayed for agonistic or antagonistic activity in the rat vasopressor assay, antidiuretic assay and *in vitro* rat oxytocic assay. For agonists, the 4-point assay design [44] was used and for antagonists, Schild's pA_2 method [45] was employed. The pA_2 is the negative logarithm of the effective molar concentration of the antagonist that will reduce the response to $2x$ units of the agonist to equal to the response to $1x$ unit of the agonist in the absence of antagonist. In practice, this concentration is estimated by finding concentrations above and below the effective concentration and interpolating on a logarithmic scale. In the rat *in vivo* assays, the effective dose of antagonist is divided by an arbitrarily assumed volume of distribution of 67 ml/kg to allow estimation of its molar concentration. Synthetic arginine-vasopressin and oxytocin which had been standardized in vasopressor and oxytocic units against the USP Posterior Pituitary Reference Standard were used as working standards in all bioassays. Vasopressor assays were performed on urethane-anaesthetized and phenoxybenzamine-treated rats as described by Dekanski [46]. Antidiuretic assays were on water-loaded rats under ethanol anaesthesia as described by Sawyer [47]. Oxytocic assays were performed on isolated uteri from diethylstilbestrol-primed rats in a Mg^{2+}-free van Dyke-Hastings's solution [48]. When standard errors are presented in the tables, the means reflect results from at least four independent assay groups. The agonistic and antagonistic oxytocic (*in vitro*, O Mg^{2+}) vasopressor (V_{1a}) and antidiuretic (V_2) potencies of the four series of Tic-substituted analogues, together with those of OT, AVP and some related antagonists (A–F) are presented in Tables 1–4. We confirmed earlier reports on the effects of L-Tic^2 and D-Tic^2 in OT [13]. Both compounds exhibit *in vitro* OT antagonism in our assays (Table 4). Like [Tic^3]LVP

[14], [Tic^3]AVP exhibits drastic losses of V_2 and V_{1a} agonistic activities (Table 4). L- and/or D-Tic substitutions at position two in the non-selective V_2/V_{1a} antagonist d$(CH_2)_5$[D-Phe^2]VAVP(A), in the selective V_{1a} antagonist d$(CH_2)_5$[Tyr(Me)^2,Ala-NH_2^9]AVP(C) (Table 1), in the cyclic OT antagonist des-GlyNH$_2$d$(CH_2)_5$[Tyr(Me)^2,Thr^4]OVT(F) and in a new linear OT antagonist: Phaa-D-Tyr(Me)-Ile-Val-Asn-Orn-Pro-Orn-NH_2 (10, Table 3) have resulted in drastic losses of V_2/V_{1a}, V_{1a} and OT antagonist potencies respectively. Also, although L-Tic^3 substitution in the selective cyclic V_{1a} antagonist (C) resulted in a drastic reduction of V_{1a} antagonism, this substitution is remarkably well tolerated with respect to V_2 antagonism in the non-selective V_2/V_{1a} antagonist d$(CH_2)_5$[D-Tyr(Et)^2]VAVP(B). Tic substitutions are very well tolerated at position 7 in the selective cyclic V_{1a} antagonist, d$(CH_2)_5$ [Tyr(Me)^2]AVP(D), in a non-selective linear V_{1a} antagonist Phaa-D-Tyr(Me)-Phe-Gln-Asn-Lys-Pro-Arg-NH_2(E) and in the selective OT antagonist (F).

Effects of Tic^3 Substitutions in AVP and of L-Tic^2 and D-Tic^2 Substitutions in OT (Table 4)

Replacement of the phenylalanine residue at position 3 in AVP by its conformationally restricted surrogate 1,2,3,4-tetrahydroisoquinoline 3-carboxylic acid (Tic) resulted in drastic losses of vasopressor (V_{1a}), antidiuretic (V_2) and OT agonistic activities (Table 4). Thus, [Tic^3]AVP exhibits less than 0.1% of the V_{1a} agonism, less than 0.3% of the V_2 agonism and less than 0.15% of the OT agonism of AVP. [Tic^3]AVP did not show any detectable antagonism or partial agonism in these assays. These findings are consistent with those reported earlier for [Tic^3]LVP [14]. Biological assays on [Tic^2]OT and [D-Tic^2]OT (kindly provided by Professor V. H. Hruby, University of Arizona) were also carried out as part of this study. Our findings in the oxytocic assays are in good agreement with those previously reported for these analogues [13]. [Tic^2]OT is a weak OT antagonist (*in vitro*, no Mg^{2+}), with a $pA_2 = 5.46$. With an anti-OT $pA_2 = 6.49$, [D-Tic^2]OT exhibits significantly stronger OT antagonism than does [Tic^2]OT. Neither compound exhibits detectable agonism or antagonism in rat vasopressor or rat antidiuretic assays. It is significant to note that [Tic^2]OT and [D-Tic^2]OT are the only OT antagonists reported to date that have no detectable anti-V_{1a} activity. Clearly on the basis of these findings, it appeared that the D-Tic^2 substitution would be a promising lead for the design of OT antagonists and also possibly for the design of AVP antagonists. By contrast, the lack of V_2 or V_{1a} antagonism by [Tic^3]LVP or [Tic^3]AVP suggested that L-Tic^3 substitution had little merit for the design of AVP antagonists. The surprising findings reported in Tables 1–3 clearly show the invalidity of both assumptions and again point to the hazards of basing predictions on antagonist design solely on the results of structural modifications of agonists.

Effects of D-Tic^2 Substitution in the Potent Non-selective AVP V_2/V_{1a} Antagonist d$(CH_2)_5$(D-Phe^2)VAVP (Table 1)

The replacement of D-Phe by its conformationally restricted surrogate D-Tic at position 2 in the potent non-selective AVP V_2/V_{1a} antagonist, d$(CH_2)_5$[D-Phe^2]VAVP [15] resulted in drastic reductions in both V_2 and V_{1a} antagonism (Table 1). d$(CH_2)_5$ [D-Tic^2]VAVP exhibits only about 1% of the V_2 and V_{1a} antagonistic potencies of d$(CH_2)_5$[D-Phe^2]VAVP. The D-Tic^2/D-Phe^2 interchange in d$(CH_2)_5$[D-Phe^2]VAVP also effected a reduction in anti-OT potency. However, in this instance the effects were not nearly as dramatic as for the anti-V_2 and anti-V_{1a} potencies. With an *in vitro* (no Mg^{2+}) pA_2 of 7.25, d$(CH_2)_5$[D-Tic^2]VAVP still retains about 20–30% of the *in vitro* anti-OT potency of d$(CH_2)_5$[D-Phe^2]VAVP ($pA_2 = 7.74$). This stands in striking contrast to the effects of D-Tic^2 substitution in a cyclic and in a linear OT antagonist. Peptides 8 and 11 (Table 3) have retained less than 2% of the anti-OT *in vitro* potency of the parent peptide in each case.

Surprising Effects of L-Tic^3 Substitution in the Potent Non-selective V_2/V_{1a} Antagonist: d$(CH_2)_5$(D-Tyr(Et)^2)VAVP (Table 1)

Replacement of the Phe^3 residue in the potent non-selective cyclic V_2/V_{1a} antagonist, d$(CH_2)_5$ [D-Tyr(Et)^2]VAVP [16] by its conformationally constrained surrogate L-Tic led to a full retention of anti-V_2 potency and a significant reduction in anti-V_{1a} potency, in effect leading to a substantial gain in anti-V_2/anti-V_{1a} selectively (Table 1). Anti-OT potency was also reduced but much less than anti-V_{1a} potency. With an anti-V_2 $pA_2 = 7.69 \pm 0.05$, d$(CH_2)_5$[D-Tyr(Et)^{2}Tic^3]VAVP is virtually equipotent with d$(CH_2)_5$[D-Tyr(Et)^2]VAVP (anti-V_2 pA_2=

7.81 ± 0.07). Since its anti-V_{1a}/anti-V_2 effective dose (ED) ratio = 5.45, compared to 0.4 for d(CH$_2$)$_5$[D-Tyr(Et)^2]VAVP, the Tic^3 analogue exhibits a gain in anti-V_2/anti-V_{1a} selectivity of about 13 times that of d(CH$_2$)$_5$[D-Tyr(Et)^2]VAVP. It remains to be seen if these effects on anti-V_2 potency and selectivity will be observed for a Tic^3/Phe^3 substitution in other potent linear V_2/V_{1a} antagonists and in more selective cyclic V_2 antagonists. In the meantime, the Tic^3/Phe^3 interchange looks like a promising new lead for V_2 antagonist design. The effects of the Tic^3/Phe^3 interchange in the V_2/V_{1a} antagonist d(CH$_2$)$_5$[D-Tyr(Et)^2]VAVP stands in marked contrast to its effects in AVP and on the agonistic potencies of AVP. The effects of the Tic^3/Phe^3 interchange in AVP clearly has had a deleterious effect on the bioactive agonist conformation of AVP required for a productive interaction with V_2-receptors. Remarkably, the Tic^3/Phe^3 interchange in the V_2/V_{1a} antagonist, while reducing somewhat binding to V_{1a} receptors, appears not to have perturbed the bioactive antagonist conformation required for binding to V_2 receptors. Probing this position in a variety of V_2 antagonists with other conformationally restricted amino acids combined with nuclear magnetic resonance (NMR) and molecular modelling studies may provide additional insights to the topographical features of the antagonist conformation required for optimal binding to V_2-receptors.

To our knowledge, d(CH$_2$)$_5$[D-Tyr(Et)^2, Tic^3]VAVP is the first position 3 modified V_2 antagonist to exhibit potent V_2 antagonism. This unexpected finding represents a very valuable new lead in AVP V_2 antagonist design.

Effects of L-Tic Substitutions at Positions 2, 3 and 7 in Potent and Selective Cyclic and Linear V_{1a} Antagonists (Table 2)

The data in Table 2 clearly show that Tic^2 and Tic^3 substitutions in a potent V_{1a} antagonist (C) lead to drastic losses of V_{1a} antagonism and are thus not well tolerated. Tic^7 substitution in a potent cyclic (D) and in a potent linear (E) V_{1a} antagonist although leading to some reduction in V_{1a} antagonism are much better tolerated than the Tic^2 or Tic^3 substitutions. d(CH$_2$)$_5$[Tyr(Me)^2,Ala-NH$_2$^{9}]AVP [17] is a highly potent and selective V_{1a} antagonist with an anti-V_{1a} pA_2 = 8.75. It is a very weak V_2 antagonist pA_2 = ~6.0. With anti-V_{1a} pA_2 value = 6.59 and 6.37, the Tic^2 and Tic^3 analogues (peptides 4 and 5, Table II) retain less than 1% of the V_{1a} antagonistic potency of the parent Tyr(Me)^2 peptide. Both peptides exhibit weak V_2 agonism, with the Tic^3 peptide also being a very weak V_2 antagonist pA_2 = ~5.2. With anti-OT pA_2 values = 6.37 and 6.26 both peptides 4 and 5 (Table 2) are weak *in vitro* OT antagonists.

The loss of V_{1a} antagonism by the Tic^3/Phe^3 interchange in the V_{1a} antagonist d(CH$_2$)$_5$ [Tyr(Me)^2, Ala-NH$_2$^{9}]AVP is consistent with the loss of V_{1a} antagonism exhibited by the Tic^3 analogue of the potent V_2/V_{1a} antagonist, d(CH$_2$)$_5$ [D-Tyr(Et)^2]VAVP (peptide 3, Table 1). Clearly the conformational perturbation of the aromatic residue at position 3 brought about by replacing Phe with its conformationally restricted surrogate, Tic, in either a V_2/V_{1a} or a V_{1a} antagonist is not consistent with good affinity for V_{1a} receptors. Replacement of the Pro^7 residue by Tic in the highly potent and selective cyclic V_{1a} antagonist d(CH$_2$)$_5$[Tyr(Me)^2]AVP [18] was remarkably well tolerated. With an anti-V_{1a} pA_2 = 8.19 and an anti-OT *in vitro* pA_2 = 7.54 d(CH$_2$)$_5$[Tyr(Me)^2, Tic^7]AVP (peptide 6) is virtually equipotent with d(CH$_2$)$_5$[Tyr(Me)^2]AVP in both assays. Likewise, a Tic^7/Pro^7 interchange in the potent linear antagonist: Phaa-D-Tyr(Me)-Phe-Gln-Lys-Pro-Arg-NH$_2$ (E) [19], is very well tolerated. However, in this instance the Tic^7 substituted peptide (7, Table 2) although very potent (anti-V_{1a} pA_2 = 8.46) is only about 30% as potent a V_{1a} antagonist as the Pro^7 peptide (E) (anti-V_{1a} pA_2 = 8.93). A Tic^7/Pro^7 interchange in the linear antagonist (E) effected a change from a weak V_2 antagonist (pA_2 = 6.5) to a weak V_2 agonist (<0.05 U/mg). The Tic^7 linear V_{1a} antagonist (7, Table 2) also exhibited potent *in vitro* OT antagonism (pA_2 = 7.72). We and others have previously shown that the Pro^7 residue in cyclic and linear AVP antagonists can be replaced by sarcosine and by *N*-Me-alanine with good retention of V_2, V_{1a} and OT antagonistic potencies [19, 49, 50]. It is thus not surprising that Tic^7/Pro^7 replacements are quite well tolerated in the V_{1a} and OT antagonists reported here.

Effects of L-Tic Substitutions at Positions 2 and 7 in a Cyclic OT Antagonist and of D-Tic^2 Substitution in a New Potent Linear OT Antagonist (Table 3)

The data in Table 3 clearly show that although Tic is well tolerated at position 7 in the potent and selective OT antagonist desGly-NH$_2$d(CH$_2$)$_5$

[Tyr(Me)^2, Thr^4]OVT [20] (F, Table 3), it is not well tolerated at position 2. Furthermore, D-Tic^2 is not well tolerated in the new potent linear OT antagonist: Phaa-D-Tyr(Me)-Ile-Val-Asn-Orn-Pro-Orn-NH_2 (10, Table 3). As noted above, L-Tic^2 and D-Tic^2 substitutions in oxytocin resulted on the one hand in a weak *in vitro* OT antagonist ([Tic^2]OT) and on the other ([D-Tic^2]OT) in a ten-fold more potent *in vitro* OT antagonist. It was thus hoped that these substitutions in OT antagonists would lead to enhancements of anti-OT potency. The findings in Table 3 show that neither L-Tic^2 nor D-Tic^2 substitutions in a cyclic or in a linear OT antagonist produced the expected enhancements. In fact, just the contrary, both peptides 8 and 11 are about two orders of magnitude less potent *in vitro* OT antagonists than their respective cyclic and linear parent antagonists. In the case of the Tic^2 peptide, binding to either V_{1a} or V_2 receptors has also been greatly diminished. Peptide 8 exhibits no detectable V_{1a} or V_2 agonism or antagonism. The D-Tic^2 peptide (11) also exhibits greatly diminished anti-V_{1a} antagonism (pA_2 = 5.24) relative to that of its parent peptide 10 (pA_2 = 7.40).

It may be argued that L-Tic is not an appropriate restricted surrogate for Tyr(Me)^2; since in oxytocin, a tyrosine residue was replaced by Tic, we should therefore have effected a similar Tic^2/Tyr^2 interchange in an OT antagonist. We have recently completed the synthesis of such an OT antagonist, i.e. the Tyr^2 analogue of desGly-NH_2d$(CH_2)_5$ [Tyr(Me)^2, Thr^4]OVT. Our preliminary unpublished findings show that the anti-OT potencies of the Tyr^2 and Tyr(Me)^2 peptides are virtually identical. Thus, our findings here clearly show that Tic^2 and D-Tic^2 are ineffective restricted surrogates for Tyr^2 or Tyr(Me)^2 in cyclic OT antagonists or for D-Tyr(Me) in a linear OT antagonist. It remains to be seen whether D-Tic^2 will be an effective surrogate for D-Phe^2 in the highly potent OT antagonist, desGlyNH_2 d$(CH_2)_5$[D-Phe^2,Thr^4]OVT (unpublished). However, at this point, it appears that neither L nor D-Tic substitutions at position 2 in OT antagonists are promising leads in OT antagonist design.

The profile of activities of the Tic^7 peptide (9) approximates that of its Pro^7 parent. These findings are analogous to those seen for the Tic^7/Pro^7 interchange in AVP V_{1a} antagonists (Table 2) discussed above. Thus, Tic^7 could be a useful modification in the design of orally active AVP and/or OT antagonists.

Conclusions

We have evaluated the effects on antagonistic potencies of L-Tic and D-Tic substitutions at positions 2, 3 and 7 in a number of potent and selective cyclic and linear OT and AVP V_2/V_{1a}, and V_{1a} antagonists. L-Tic^2 and D-Tic^2 substitutions led to drastic losses of antagonistic potencies in virtually all analogues studied here. Tic^2 substitutions appear not to be useful leads for OT or AVP antagonist design. Tic^7 substitutions were well tolerated in cyclic and linear V_{1a} and OT antagonists. Although L-Tic^3 was not well tolerated in a cyclic V_{1a} antagonist, surprisingly it was found to lead to full retention of anti-V_2 potency and a significant loss of anti-V_{1a} potency in the V_2/V_{1a} antagonist d$(CH_2)_5$ [D-Tyr(Et)^3,Tic^3]VAVP. With an anti-V_2 pA_2 = 7.69 ± 0.05; and an anti-V_{1a} pA_2 = 6.95 ± 0.03, this Tic^3 analogue is a virtually equipotent V_2 antagonist and 17-fold less potent a V_{1a} antagonist than d$(CH_2)_5$[D-Tyr(Et)^2]VAVP (anti-V_2 pA_2 = 7.81 ± 0.07; anti-V_{1a} pA_2 = 8.22 ± 0.12). Thus, the Tic^3 substitution has effected a 13-fold gain in anti-V_2/anti-V_{1a} selectivity. It would appear that the Tic substitution at position 3 fixes the orientation of the restricted Phe^3 residue in the proper orientation for binding to V_2 receptors but not for binding to V_{1a} receptors. Comparisons of the circular dichroism (CD) and NMR structures of d$(CH_2)_5$[D-Tyr(Et)^{2}Tic^3]VAVP with those of d$(CH_2)_5$[Tyr(Me)^{3}Tic^3, Ala-NH_2^9]AVP combined with molecular modelling should provide useful insights to the differences in the receptor-bound conformations of these molecules. The finding that Tic^3 substitution in a non-selective V_2/V_{1a} antagonist leads to full retention of V_2 antagonism and to a significant gain in anti-V_2/V_{1a} selectivity is a promising new lead to the design of potent, selective and possibly orally active V_2 antagonists for use as pharmacological tools and for potential clinical use as therapeutic agents for the treatment of SIADH [51].

EXPERIMENTAL

Amino acid derivatives were purchased from Bachem Inc. Boc-Tic [3] and [β-(benzylthio)-β,β-cyclopentamethylenpropionic acid [52] were synthesized in this laboratory. Thin-layer chromatography was run on precoated silica gel plates (60F-254, E.

Merck) with the following solvent systems: (a) 1-butanol:AcOH:H_2O (4:1:5, upper phase); (b) 1-butanol:AcOH:H_2O (4:1:1); (c) 1-butanol: AcOH:H_2O:pyridine (15:3:3:10); (d) chloroform: methanol (7:3). Loads of 10–15 μg were applied and chromatograms were developed at a minimal length of 10 cm. The chlorine gas procedure for the KI-starch reagent was used for detection [25]. Optical rotations were measured with a Rudolph Autopol III polarimeter. Amino acid analyses were done by the University of Louisville Core Facility. Molar ratios were referred to Phe, Leu or Gly = 1. Tyr(Et) and Tyr(Me) were detected as Tyr without substantial losses in the 28 h hydrolysis. The recovery of Tic was 60% under these conditions. All peptides gave the expected amino acid ratios ±5%. Analytical HPLC was performed on a Waters 810 instrument equipped with Vydac C_{18} column and UV detector. Semi-preparative HPLC purification was performed on the same instrument equipped with semi-preparative Bondapak C_{18} Waters column. All peptides were at least 95% pure. Electron spray mass spectrometry (ESMS) determinations on the free peptides were done by the University of Michigan Protein and Carbohydrate Structure Facility. ESMS spectra were in agreement with the composition of each peptide.

Solid Phase Synthesis Procedure

The procedure of solid phase synthesis conformed to that published [22–26, 30, 37, 38]. Chloromethylated resin (Bachem 1% cross-linked S-DVB, 200–400 mesh, 0.7–1.00 mmol/g) was esterified with either Boc-Gly, Boc-Ala or Boc-Orn(Tos) to an incorporation of approximately 0.5 mmol/g by the caesium salt method [53]. For the synthesis of protected peptidyl resins, eight (or seven) cycles of deprotection, neutralization and coupling were carried out by the DCC/HOBt or the active ester procedure. Ammonolysis in MeOH [22, 26, 29] (peptides I, II, III, IV, V, VI, VII, X, XI) or acidolytic cleavage with HBr/TFA [24, 30, 31] (peptides VIII, IX) was used to split the protected peptides from the resin. The protected precursors obtained by ammonolysis were purified either by extraction with warm MeOH (peptides II–V) or with hot DMF (peptides I, VI, VII, X, XI) followed by reprecipitations with Et_2O and AcOH/H_2O for the methanol-soluble peptides and with DMF/H_2O or DMF/MeOH/Et_2O for those peptides extracted with DMF, until adjudged pure by TLC. The protected peptides (I, II, III, IV, V, VI, VIII, IX, Table 5) were also deblocked with sodium in liquid ammonia [32, 33]. The resulting disulphydryl compounds were oxidatively cyclized with $K_3[Fe(CN)_6]$ using the normal [41] or a modified reverse procedure [42]. The free cyclic peptides were purified by a two-step gel filtration procedure [43] on Sephadex G-15 and Sephadex LH-20. The physicochemical properties of the free cyclic peptides (1, 2, 3, 4, 5, 6, 8, 9) are given in Table 6.

The protected peptides VII, X, XI (Table 5) were also deblocked with sodium in liquid ammonia. The usual oxidation step was omitted prior to gel filtration on Sephadex G15 and LH20 as above. The physicochemical properties of the linear free peptides (7, 10, 11) are given in Table 6.

[(β-Benzylthio)-β,β-pentamethylenepropionyl]-D-Tyr(Et)-Tic-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (III, Table 5)

Ammonolytic Cleavage. Boc-Gly-resin(1.0 g, 0.55 mmol) was subjected to eight cycles of deprotection, neutralization and coupling with Boc-Arg(Tos), Boc-Pro, Boc-Cys(Bzl), Boc-Asn-ONP, Boc-Val, Boc-Tic, Boc-D-Tyr(Et) and β-(benzylthio)-β,β-pentamethylenepropionic acid respectively. The resulting protected peptidyl resin was cleaved by ammonolysis. The protected peptide was extracted with warm methanol (35 ml) and the product precipitated by Et_2O (200 ml), collected, dried and reprecipitated from AcOH (10 ml) and H_2O (200 ml). Collection and drying *in vacuo* over P_2O_5 gave the required acyloctapeptide amide (III, Table 5). The same procedure was used for the synthesis and purification of the protected acylpeptide amides II, IV, V (Table 5). Hot DMF was utilized for the extraction of the remaining protected peptide amides to give, following purification by repeated aqueous precipitations and or reprecipitation from DMF/MeOH/Et_2O, the protected acylpeptide amides I, VI, VII, X and XI (Table 5).

[(β-Benzylthio)-β,β-Pentamethylenepropionyl]-Tic-Ile-Thr-Asn-Cys(Bzl)-Pro-Orn(Tos) (VIII, Table 5)

Acidolytic Cleavage. Boc-Orn(Tos)-resin(0.72 g, 0.6 mmol) was converted to protected acyloctapeptidyl resin in eight cycles of deprotection, neutralization, and coupling with Boc-Pro, Boc-Cys(Bzl), Boc-

Table 5. Physicochemical Properties of Protected Peptides

No.[a]	Structure	Yield[b]	M.P. (°C)	$[\alpha]^{25}D$, deg (c = 1) DMF	TLC, R_f[c]			
					a	*b*	*c*	*d*
I	Z-Cys(Bzl)-Tyr(Bzl)-Tic-Gln-Asn-Cys-(Bzl)-Pro-Arg(Tos)-Gly-NH_2	50.3	154–6	−34.6	0.43		0.73	0.92
II	d$(CH_2)_5$(Bzl)-D-Tic-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2	84.3	153–5	−25.6	0.52	0.46	0.75	
III	d$(CH_2)_5$(Bzl)-D-Tyr(Et)-Tic-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2	55.1	148–50	−26.8	0.55	0.53	0.74	
IV	d$(CH_2)_5$(Bzl)-Tic-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Ala-NH_2	66.9	163–5	−31.2	0.49	0.38	0.76	
V	d$(CH_2)_5$(Bzl)-Tyr(Me)-Tic-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Ala-NH_2	65.0	150–2	−26.0	0.49	0.39	0.67	
VI	d$(CH_2)_5$(Bzl)-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Tic-Arg(Tos)-Gly-NH_2	68.8	163–4	−21.4	0.64	0.63	0.74	0.78
VII	Phaa-D-Tyr(Me)-Phe-Gln-Asn-Lys(Tos)-Tic-Arg(Tos)-NH_2	64.0	187–8	−17.5	0.61	0.63	0.72	0.89
VIII	d$(CH_2)_5$(Bzl)-Tic-Ile-Thr(Bzl)-Asn-Cys(Bzl)-Pro-Orn(Tos)COOH	45.5	125–7	−31.8	0.69	0.67	0.68	
IX	d$(CH_2)_5$(Bzl)-Tyr(Me)-Ile-Thr(Bzl)-Asn Cys(Bzl)-Tic-Orn(Tos)COOH	93.0	153–4	−16.6	0.76	0.75	0.70	0.80
X	Phaa-D-Tyr(Et)-Ile-Val-Asn-Orn(Tos)-Pro-Orn(Tos)-NH_2	24.1	245–8	−19.0 (c = 0.5)		0.79	0.82	0.99
XI	Phaa-D-Tic-Ile-Val-Asn-Orn(Tos)-Pro-Orn(Tos)-NH_2	74.3	237–9	−10.5	0.66	0.70	0.57	

[a] The protected peptides I–XI are the immediate protected precurors for the free peptides 1–11 given in Tables 1–4 and 6.
[b] Yields were calculated on the basis of the amino acid content of the resin.
[c] Solvent systems are described in the Experimental Section.

Asn-ONP, Boc-Thr(OBzl), Boc Ile, Boc-Tic and β-benzylthio)-β,β-pentamethylenepropionic acid, respectively. The resulting protected peptidyl resin was suspended in TFA (25 ml) containing anisole (5 ml) and hydrogen bromide was bubbled through the suspension for 45 min. The filtrate was collected and the resin was resuspended in CH_2Cl_2 (12 ml), TFA (12 ml) and anisole (5 ml). HBr bubbling was resumed for a further 40 min, whereupon the filtrate was collected and the resin was washed with CH_2Cl_2-TFA (1:1, v/v, 20 ml × 2). The filtrates and the washings were combined and evaporated on the rotary evaporator. Addition of Et_2O (*ca.* 250 ml) to the residual anisole solution gave a precipitation, which, following 3 h at 4 °C was collected, washed with Et_2O and dried *in vacuo* over P_2O_5; 0.38 g. This product was dissolved in AcOH (15 ml), reprecipitated with water, collected and dried *in vacuo* over P_2O_5 to give the protected acyloctapeptide (VIII, Table V). This same procedure was utilized for the synthesis of the protected carboxylterminating protected peptide (IX, Table 5).

[1-(β-Mercapto-β,β-Pentamethylenepropionic Acid), 2-O-Ethyl-D-Tyrosine, 3-Tic, 4-Valine] Arginine Vasopressin (3, Tables 1 and 6)

A solution of protected acyloctapeptide amide (120 mg) in sodium-dried ammonia (*ca.* 400 ml) was treated at the boiling point and with stirring with sodium from a stick of metal contained in a small-bore glass tube until a light-blue colour persisted in the solution for *ca.* 30 s. NH_4Cl was added to discharge the colour. The ammonia was evaporated. Reoxidation of the deblocked disulphydryl peptide was carried out by a modified-reverse procedure [42] as follows. The residue was dissolved in 25 ml 50% AcOH and the solution was diluted with 75 ml H_2O. The peptide solution was added dropwise with stirring over a period of 15–30 min to an 800 ml aqueous solution which contained 20 ml of a 0.01 M solution of potassium ferricyanide. Meanwhile, the pH was adjusted to approximately 7.0 with concentrated ammonium hydroxide. The yellow solution was stirred for an additional 20 min.

Table 6. Physicochemical Properties of the Free Peptides

No.	Peptide	Yield (%)	$[\alpha]^{25}D$, deg (c = 1) 50% AcOH	TLC, R_f[c]			HPLC[d] t_R(min)	Formula	MW	$[M+H]^{1+}$[e]
				a	b	d				
1	[Tic^3]AVP	19.0	−21.0	0.04	0.04	0.25	15.8	$C_{47}H_{64}O_{12}N_{14}S_2$	1096.2	1097.5
2	d(CH_2)$_5$[D-Tic^2]VAVP	19.0	−12.9	0.31	0.28	0.50	31.4	$C_{52}H_{72}O_{10}N_{13}S_2$	1104.0	1105
3	d(CH_2)$_5$[D-Tyr(Et)^2,Tic^3]VAVP	33.0	−80.0	0.23	0.23	0.43	37.0	$C_{54}H_{76}O_{13}N_{13}S_2$	1147.5	1148.5
4	d(CH_2)$_5$[Tic^2,Ala-NH_2^{9}]AVP	33.3	−26.0	0.21	0.21	0.45	31.1	$C_{53}H_{73}O_{11}N_{14}S_2$	1147.3	1148
5	d(CH_2)$_5$[Tyr(Me)^2,Tic^3,Ala-NH_2^{9}]AVP	43.0	−76.0	0.23	0.22	0.47	27.6	$C_{54}H_{75}O_{12}N_{14}S_2$	1177.7	1178.5
6	d(CH_2)$_5$[Tyr(Me)^2,Tic^7]AVP	28.6	−26.0	0.25	0.27	0.42	36.4	$C_{57}H_{76}O_{12}N_{14}S_2$	1213.0	1214
7	Phaa-D-Tyr(Me)-Phe-Gln-Asn-Lys-Tic-Arg-NH_2	43.9	−36.0	0.13	0.20	0.29	29.1	$C_{58}H_{76}O_{11}N_{14}$	1144.8	1146.5
8	desGlyNH_2d(CH_2)$_5$[Tic^2,Thr^4]OVT	35.5	+56.0	0.23	0.24	0.30	29.0	$C_{45}H_{66}O_{11}N_9S_2$	973.5	974.5
9	desGlyNH_2d(CH_2)$_5$[Tyr(Me)^2,Thr^4,Tic^7]OVT	51.3	−24.0	0.28	0.24	0.33	35.8	$C_{50}H_{71}O_{12}N_9S_2$	1054.0	1055
10	Phaa-D-Tyr(Me)-Ile-Val-Asn-Orn-Pro-NH_2	49.4	−69.0	0.06	0.12	0.34	29.5	$C_{49}H_{75}O_{10}N_{11}$	978.0	979
11	Phaa-D-Tic-Ile-Val-Asn-Orn-Pro-Orn-NH_2	44.7	−78.6	0.10	0.13	0.27	27.5	$C_{48}H_{71}O_9N_{11}$	946.0	947

[a] Yields are based on the amount of protected peptide used in the reduction–reoxidation step in each case and are uncorrected for acetic acid and water content.

[b] All peptides gave the expected amino acid analysis ratios after hydrolysis ± 5%.

[c] Solvent systems and conditions are given in the Experimental Section.

[d] All peptides were at least 95% pure. For elution 60 ml linear gradient of acetonitrile from 10% to 70% TFA–water (v/v/v) with the flow rate 1 ml/min was applied.

[e] Data obtained by ESMS.

The same procedure was utilized for the reoxidation of the free cyclic peptides 1, 2, 3, 4, 5, 8 (Table 6). The remaining cyclic peptides 6 and 9 (Tables 2 and 6) were reoxidized by the normal method [41], i.e. by the addition of a solution of potassium ferricyanide (0.01 M, 20 ml) to a dilute solution of the peptide at pH 7 as previously described [38]. Following oxidation, all the free cyclic peptides were isolated and purified as described here for peptide 3 as follows: after acidification with AcOH to pH 4.5 and stirring for 20 min with anion-exchange resin (Bio-Rad, AG-3, Cl-form, 8 g damp weight), the suspension was slowly filtered and washed with 0.2 M AcOH, 3×30 ml); the combined filtrate and washings were lyophilized. The resulting powder was desalted on a Sephadex G-15 column (110 × 2.7 cm) eluting with aqueous acetic acid (50%) with a flow rate of 5 ml/h. The elute was fractionated and monitored for absorbance at 254 nm. The fractions making up the major peak were checked by TLC, pooled and lyophilized. The residue was further subjected to gel filtration on Sephadex LH-20 (100×1.5 cm) eluting with aqueous acetic acid (2 M) with a flow rate of 4 ml/h. The peptide was eluted in a single peak (absorbance at 254 nm). Lyophilization of pertinent fractions gave the desired vasopressin analogue (3, Tables 1 and 6). With the modification for reoxidation described above, this procedure was utilized to give the remaining cyclic peptides in Table 6.

Phenylacetyl-D-Tyr(Me)-Phe-Gln-Asn-Lys(Tos)-Tic-Arg(Tos)-NH_2 (VII, Table 5)

Boc-Arg(Tos)-resin (4.16 g, 1.0 mmol) was subjected to seven cycles of deprotection, neutralization and coupling using the DCC/HOBt procedure except for Boc-Gln and Boc-Asn residues which were introduced as their *p*-nitrophenyl esters. The protected peptidyl resin was ammonolysed (94 h) and the peptide was extracted with hot DMF (70 °, 25 ml). The product was precipitated by the addition of hot water (*ca.* 500 ml). After cooling, the product was collected, dried *in vacuo* over P_2O_5 and reprecipitated from hot DMF with MeOH and Et_2O to give the

desired product 870 mg (64%). The properties of the protected peptide VII are given in Table 5. The same procedure was used for the synthesis and purification of the remaining protected peptides (X, XI, Table 5).

Phenylacetyl-D-Tyr(Me)-Phe-Gln-Asn-Lys-Arg-NH_2 (7, Tables 2 and 6)

The protected acyloctapeptide amide (VII, Table 5) (100 mg) in sodium-dried ammonia (*ca.* 400 ml) was treated at the boiling point and with stirring with sodium from a stick of metal contained in a small-bore glass tube until a light-blue colour persisted in the solution for 30 s. NH_4Cl was added to discharge the colour. The ammonia was evaporated. The residue was dissolved in 50% acetic acid (*ca.* 10 ml) and desalted on a Sephadex G-15 column (110 × 2.7 cm) eluting with aqueous acetic acid 50% with a flow rate of 5 ml/h. The elute was fractioned and monitored for absorbance at 254 nm. The fractions making up the major peak were checked by TLC, pooled and lyophilized. The residue was further subjected to gel filtration on Sephadex LH 20 (100 × 1.5 cm) with aqueous acetic acid (2 M) with a flow rate of 4 ml/h. The peptide was eluted in a single peak (absorbance at 254 nm) to give peptide 7, Table 6, 35 mg (43.9%). This procedure was utilized to give the remaining linear peptides, 10, 11 (Table 6).

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