12c was separated from the more polar S,S,R epimer 11b by flash chromatography on silica gel using petroleum ether/ether (5:1) as eluent. The product (0.47 g, 22%) was isolated as a clear oil: NMR ($CDCl_3$) δ 1.21 (s, 9 H, $C(CH_3)_3$), 1.35–1.79 (m, 6 H, $CH_2CH_2CH_2$), 1.86–2.10 (m, 2 H, $PhCH_2CH_2$), 2.65 (t, 2 H, $PhCH_2CH_2$), 3.14 (m, 2 H, CH_2NHCO), 3.32 (t, 1 H, CH_2CHNH), 3.96 (t, 1 H, NHCHCO), 5.01–5.23 (m, 6 H, 3 × $PhCH_2O$), 6.15 (s, 1 H, heterocyclic CH), 7.07–7.44 (m, 20 H, aromatic CH).

5-*tert*-Butyl-3-[N^2-[1(*S*)-carboxy-3-phenylpropyl]-L-lysyl]-2,3-dihydro-1,3,4-thiadiazole-2(*S*)-carboxylic Acid (5c). A solution of 12c (1.1 g) in ethanol (90 mL) was stirred over 10% palladium on carbon (0.9 g) under an atmosphere of hydrogen for 1 h. The catalyst was removed by filtration and the filtrate was evaporated to dryness. Recrystallization of the residue from a mixture of wet THF and ethanol gave the hemihydrate of 5c (0.24 g, 36%) as colorless crystals: mp 180–90 °C dec; NMR (DMSO-d_6) δ 1.67 (s, 9 H, $C(CH_3)_3$), 1.32–1.70 (m, 6 H, $CH_2CH_2CH_2$), 1.80 (m, 2 H, $PhCH_2CH_2$), 2.61 (m, 2 H, $PhCH_2CH_2$), 2.72 (m, 2 H, CH_2NH_2), 3.06 (t, 1 H, CH_2CHNH), 4.14 (m, 1 H, NHCHCO), 5.78 (s, 1 H, heterocyclic CH), 7.16–7.28 (m, 5 H, aromatic CH). Anal. ($C_{23}H_{34}N_4O_5S \cdot 0.5H_2O$) C, H, N, S.

Acknowledgment. We acknowledge the contribution of T. Miranda, E. C. Kinchen, L. I. Coe, and G. H. Jones toward the syntheses. We also thank W. T. Simpson (Head of Research at Fisons throughout the period of this work) for his continual encouragement and support, N. P. Gensmantel and A. M. Davis for help with the determination of dissociation constants, D. Hunter for NMR studies, and A. A. Freer (University of Glasgow) for the X-ray crystallographic structure. The enzyme inhibition data reported in Tables I and II was generated by E. Wells and P. Killingback. Captopril (Squibb, Inc.), enalapril (Merck, Sharp and Dohme), and spirapril (Schering-Plough Corp.) were gifts from the manufacturers.

Registry No. 4a, 130324-30-0; 4b, 130324-31-1; 4b·DCHA, 130324-38-8; 4c, 130324-32-2; 4c·DCHA, 130324-39-9; 4d, 130324-33-3; 4e, 130324-34-4; 4f, 130324-35-5; 4g, 130324-36-6; 4h, 130275-77-3; 4i, 130324-37-7; 5a, 109683-61-6; 5b, 109683-79-6; 5c, 109683-62-7; 5d, 109715-05-1; 5e, 109683-63-8; 5f, 109683-80-9; 5g, 109683-82-1; 5h, 109683-84-3; 5i, 112137-37-8; 5j, 109683-78-5; 5k, 112063-18-0; 5l, 109683-81-0; 6a, 93114-01-3; 6b, 62543-18-4; 6c, 92503-30-5; 6d, 109684-20-0; 6e, 20605-40-7; 6f, 62625-55-2; 6g, 109684-16-4; 6h, 95372-07-9; 6i, 68062-22-6; 6j, 130275-76-2; 6k, 109684-10-8; 8a, 130275-78-4; 8b, 121342-42-5; 9a, 130275-79-5; 10a, 82717-96-2; 10b, 89371-42-6; 10c, 107832-08-6; 10d, 130275-80-8; 10e, 82834-12-6; 11a, 109683-69-4; 11b, 109715-01-7; 12a, 109718-93-6; 12c, 109785-11-7; 13a, 126372-01-8; 13b, 95513-34-1; 14a, 112243-70-6; 14b, 117560-14-2; 14c, 107832-07-5; 15a, 112243-71-7; 15b, 130275-81-9; 15c, 130275-82-0; ACE, 9015-82-1; OHCCOOEt, 924-44-7; $OHCCOOCH_2Ph$, 52709-42-9; $AcSCH_2CH_2COCl$, 41345-72-6; (±)-$PhCH_2CH_2CH(OH)COOH$, 111611-91-7; H-Ala-OCH_2Ph, 17831-01-5; H-Ala-OBu-*t*, 21691-50-9; H-Lys(Z)-OBu-*t*, 63628-63-7.

Non-Steroidal Antiandrogens. Design of Novel Compounds Based on an Infrared Study of the Dominant Conformation and Hydrogen-Bonding Properties of a Series of Anilide Antiandrogens

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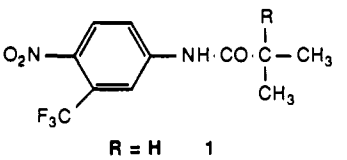
ICI Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, United Kingdom. *Received May 18, 1990*

Antiandrogenic activity is observed in anilides containing a tertiary hydroxyl group, and these compounds are used to define a pharmacophore in terms of their physicochemical properties. Infrared spectroscopy shows that these anilides exist in a single conformation, which exerts a powerful influence on the hydrogen-bond donor ability of the hydroxyl group in a model system. Arguments are presented which suggest that hydrogen-bonding ability is an important contributor to biological activity. Compounds were synthesized that reproduced these properties in series not containing an amide bond. Such compounds were found to exhibit good antiandrogen activity. We suggest that quantitative information on hydrogen bonding might also be useful in other systems.

Introduction

Androgen antagonists are a potentially useful treatment for a number of hormone-dependent conditions ranging from acne and hirsutism to prostate cancer.[1] Currently, both steroidal and non-steroidal agents (such as flutamide, 1) have shown clinical benefit in the treatment of prostate cancer, but most show a range of unpleasant side effects as well as overlapping effects on other hormonal activity.[2] Our aim was to find a peripherally selective, non-steroidal, pure antagonist that would have no effect on circulating hormone levels.[2]

Clinically the most widely studied non-steroidal antagonist is flutamide (1), although the active species in vivo was subsequently identified[3] as the hydroxylated metabolite 2. However, few details of structure–activity relationships (SAR) have been reported apart from the patent literature[4] which infers that the most active compounds contain electron-withdrawing substituents in the aromatic ring and a branched alkyl chain α to the amidic carbonyl. Consequently chemical synthesis was initially directed toward exploring structure–activity with 2 as the lead compound.



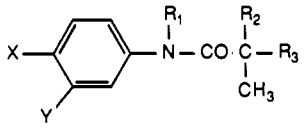
R = H 1
R = OH 2

These studies confirmed that electron-withdrawing groups in the aromatic ring are important for biological activity and that considerable variation is also possible α to the hydroxyl,[2,5] but it is not our intention to describe

(1) Rasmusson, G. H. *Ann. Rep. Med. Chem.* **1986**, *21*, 179.
(2) Glen, A. T.; Hughes, L. R.; Morris, J. J.; Taylor, P. J. *SCI-RSC Med. Chem. Symp., 3rd.* **1987**, 345 (Lambert, R. W., Ed.).
(3) Katchen, B.; Buxbaum, S. *J. Clin. Endocrinol.* **1975**, *41*, 373.
(4) G.B. Patent. 1360-001, 1971.
(5) Tucker, H.; Glen, A. T.; Hughes, L. R. Unpublished observations.

0022-2623/91/1834-0447$02.50/0

Table I. Physical and Biological Data for the Anilides



no.	X	Y	R_1	R_2	R_3	route	mp	formula	analysis	RBA	ED_{50}[h]	log P[i]	CLOGP[k]
1	NO_2	CF_3	H	CH_3	H	*a*	–	–	–	<0.2	0.5	3.35	3.54
2	NO_2	CF_3	H	CH_3	OH	*a*	–	–	–	2.1	0.5	2.70	2.65
3	H	H	H	CH_3	OH	*a*	–	–	–	<0.2	–		
4	Cl	H	H	CH_3	OH	*a*	–	–	–	<0.2	–	2.0	2.13
5	NO_2	H	H	CH_3	OH	*a*	–	–	–	<0.2	–	1.61	1.51
6	H	H	H	CF_3	OH	*b, c*	117–8	$C_{10}H_{10}F_3NO_2$	C,H,N	<0.2	>10		
7	Cl	H	H	CF_3	OH	*b, c*	123–5	$C_{10}H_9ClF_3NO_2$	C,H,N	<0.2	>10		
8	NO_2	H	H	CF_3	OH	*b, c*	157–9	$C_{10}H_9F_3N_2O_4$	C,H,N	0.9	5.0		
9	NO_2	CF_3	H	CF_3	OH	*b, c*	129–31	$C_{11}H_8F_6N_2O_4$	C,H,N	6.6	0.15	3.21	3.41
10	NO_2	CF_3	H	CF_3	H	*c, d*	139–42	$C_{11}H_8F_6N_2O_3$	C,H,N[f]	<0.2	0.25	3.71	4.29
11	NO_2	CF_3	H	CHF_2	OH	*c*	119–21	$C_{11}H_9F_5N_2O_4$	C,H,N	15.1	0.12	3.76	3.58
12	NO_2	CF_3	H	CH_2F	OH	*c, e*	128–30	$C_{11}H_{10}F_4N_2O_4$	C,H,N	5.9	0.15	3.50	3.26
13	NO_2	CF_3	CH_3	CH_3	OH	*g*	75–81	$C_{12}H_{13}F_3N_2O_4$	C,H,N	<0.2	–	3.06	2.99
15	NO_2	CF_3	H	pnp[i]	OH	*g*	126–27	$C_{16}H_{12}F_3N_3O_6$	C,H,N	2.5	0.5		

[a] Literature compounds, see refs 4 and 18. [b] From hydroxy acid, see ref 19. [c] General method, see Experimental Section. [d] From acid, see ref 20. [e] From hydroxy acid, see ref 21. [f] C: calcd, 40.0; found, 40.5. H: calcd, 2.4; found, 2.9. [g] See Experimental Section. [h] Subcutaneous dosing. [i] pnp = 4-nitrophenyl. [j] Measured values. [k] Calculated values from CLOGP (ref 9).

the optimization of these series here.

Our intention was to identify alternative chemical series which would, in principle, avoid the unwanted effects on hormone levels, unfortunately all the simple modifications resulted in weakly active antiandrogens.[2]

The approach we have adopted is to use 2-hydroxypropionanilides (Table I) as templates to define a pharmacophore in terms of the physicochemical requirements of the androgen receptor, with the expectation that these principles could be applied to new antagonists in different chemical series. This approach effectively ignores structural similarities between the natural agonist (testosterone) and the antagonists and concentrates instead on identifying important interactions. In any case there is no compelling reason why the antagonists should bind exactly as the agonist does.

In order to define these compounds in terms of their physical properties, a number of key issues need to be addressed. Among the most important are as follows:

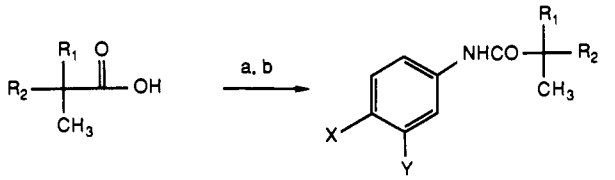
1. Is there an optimum lipophilicity range?
2. What is the role of substitution in the aromatic ring?
3. What is the dominant conformation?
4. What is the role of hydrogen bonding?

Perhaps the last of these criteria is deserving of some further comment here, because quantitative aspects of hydrogen bonding are not often considered in SAR.

Although hydrogen bonding is often used qualitatively in models of receptor binding[6] there have been few attempts to use it in a quantitative sense, yet it is recognized[7] as one of the most important forces involved in anchoring the pharmacophore to the receptor. By bearing in mind any limitations imposed by substituent size and shape, one way to obtain step-jumps in activity or to move to new chemical series may be to utilize an anchor point on the receptor that entails a well-matched hydrogen bond.

Arguably one reason for this lack of attention in the literature in the past has been the absence of suitable scales of hydrogen bonding. Recently, however such scales have appeared for both hydrogen-bond donors and acceptors,

Scheme I[a]



[a] (a) $SOCl_2$, DMA, –20 °C. (b) 3,4-Disubstituted aniline; pyridine, 23 °C.

explicitly designed for use in medicinal chemistry[8] so it is now possible to place hydrogen bonding alongside other physicochemical parameters when considering drug–receptor interactions.

Reported in this paper are details of the physicochemical requirements for antiandrogenic activity derived from 2-hydroxypropionanilides. In particular we show that a single dominant conformation has an important influence on the ability of the OH to act as a hydrogen-bond donor. The strength of this hydrogen bond to an external acceptor is shown to be an important factor in the biological activity of these compounds.

Finally, we use these and other physicochemical parameters in the design of chemically novel antiandrogens (Tables II and III).

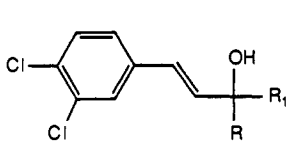
Synthetic Chemistry

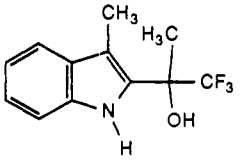
The route used to prepare the majority of the anilides listed in Table I, is shown in Scheme I. It involved coupling of the appropriate aniline and acid chloride which was prepared in situ from the acid by using thionyl chloride in dimethylacetamide at –20 °C.

The anilide **15** was prepared in a similar way, except that the hydroxyl group in the starting hydroxy acid was protected as an acetate, and in this case the coupling was carried out in pyridine. In the same way **13** was prepared by coupling the required aniline with 2-acetoxyisobutyryl chloride in DMF with sodium hydride as base, followed by hydrolysis of the acetate grouping.

(6) Ariens, E. J. *Stereochemistry in Biological Activity of Drugs*; Ariens, E. J., Soudjim, W., Timmermans, P. B. M. W. M., Eds.; Blackwell: Oxford, 1982; p 16.
(7) Albert, A. *Selective Toxicology*, 7th ed.; Chapman and Hall: London, 1985; p 498.
(8) Abraham, M. H.; Duce, P. P.; Prior, D. V. P.; Barratt, D. G.; Morris, J. J.; Taylor, P. J. *J. Chem. Soc., Perk. Trans. 2* **1989**, 1355.

Table II. Physical and Biological Data for Alkenes and Indole





19

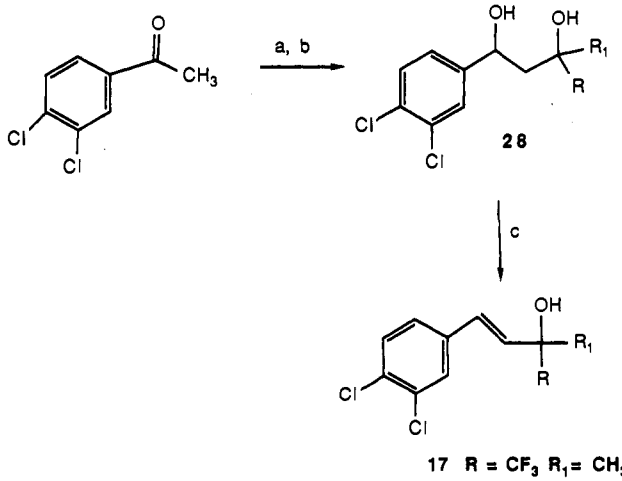
no.	R	R_1	synthetic route	mp	formula	analysis	RBA	ED_{50}
16	CH_3	CH_3	*a*	oil	–	–	<0.2	>10[g]
17	CH_3	CF_3	*b*	oil	$C_{11}H_9Cl_2F_3O$	C,H	<0.2	1.5[g]
18	CF_3	CF_3	*c*	oil	$C_{11}H_6Cl_2F_6O$	C,H	<0.2	1.5[g]
19			*b*	oil	$C_{12}H_{12}F_3NO$	C,H,N[d]	6.7	33% at 10[g]
21	CF_3	CH_2CN	*b*	116	$C_{12}H_8F_3Cl_2NO$	C,H,N[e]	3.6	2.5[f]
22	CF_3	CH_2Cl	*b*	64	$C_{11}H_8F_3Cl_3O$	C,H	1.8	2.0[f]
23	CF_3	CH_2CONH_2	*b*	126	$C_{12}H_{10}F_3Cl_2NO_2$	C,H,N	0.5	2.5[f]
24	CF_3	$COCH_3$	*b*	oil	$C_{12}H_9F_3Cl_2O_2$	C,H	0.4	0.2[f]

[a] Via route in ref 22. [b] See Experimental Section. [c] Method as for 17 except hexafluoroacetone used in place of trifluoroacetone, yield 35%. [d] C: calcd, 59.3; found, 59.8. [e] N: calcd, 4.3; found, 3.8. [f] ED_{50} from oral dosing. [g] ED_{50} from subcutaneous dosing.

Table III. Analysis Results for New Compounds

compd no.	theory C	theory H	theory N	found C	found H	found N
6	51.5	4.3	6.0	51.7	4.3	6.0
7	44.9	3.4	5.2	44.8	3.4	4.9
8	43.2	3.2	10.1	43.5	3.4	10.1
9	38.2	2.3	8.1	38.2	2.5	8.4
10	40.0	2.4	8.5	40.5	2.9	3.6
11	40.3	2.7	8.5	40.2	2.8	8.7
12	42.6	3.2	9.0	42.6	3.2	8.7
13	47.1	4.3	9.2	46.8	4.5	8.8
14	52.6	4.3	5.5	52.0	4.4	5.3
15	48.1	3.0	10.5	47.7	3.0	10.2
16						
17	46.3	3.2	–	46.6	3.4	–
18	38.9	1.8	–	38.1	1.9	–
19	59.3	5.0	5.8	59.8	5.0	5.6
20	49.4	3.0	3.2	49.3	2.9	2.9
21	46.5	2.6	4.5	46.2	3.0	4.6
22	41.3	2.5	–	41.2	2.7	–
23	43.9	3.0	4.3	43.8	3.1	3.8
24	46.0	2.9	–	46.1	3.0	–
26	46.7	2.5	–	46.7	2.7	–
27	48.8	2.4	–	48.8	2.5	–

Scheme II[a]

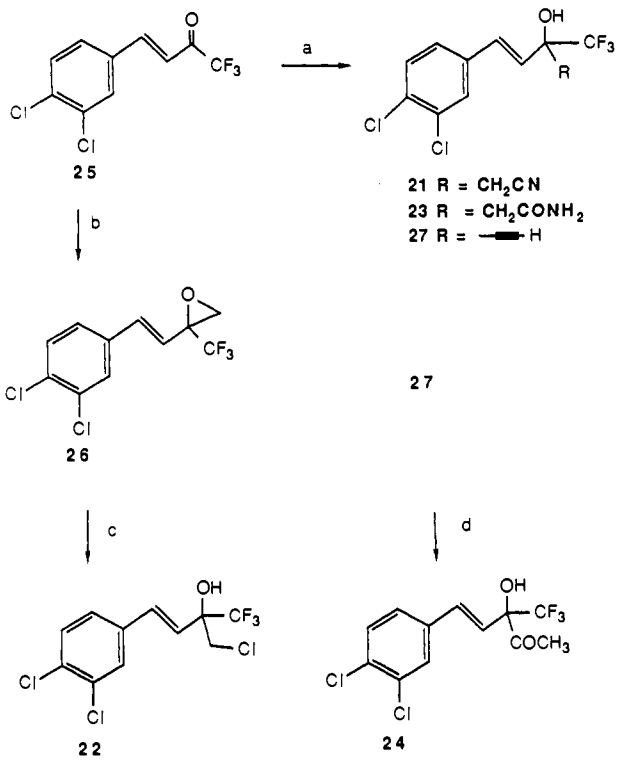


17 R = CF_3 R_1 = CH_3

[a] (a) Lithium tetramethylpiperidide, THF, $RCOR_1$, –50 °C. (b) $NaBH_4$, EtOH, 23 °C. (c) *p*-toluenesulfonic acid, 140 °C.

The alkenes (Table II) were prepared by either literature routes or as outlined in Schemes II and III. The key step in the synthesis of **17** and **18** was the acid catalyzed dehydration of the diol **28** which proceeds via the more stable benzylic carbonium ion to give the required allylic alcohol rather than the other possible regioisomer (Scheme II). Compounds **21–24** were prepared via the key enone **25** as shown in Scheme III. Addition of a suitable organometallic species gave directly the cyanomethyl **21** and carbamoylmethyl **23** analogues, while the acetyl derivative was produced by acid catalyzed hydration of the initially formed acetylene **27**.

Scheme III[a]



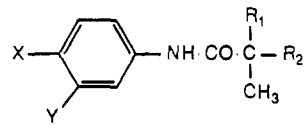
21 R = CH_2CN
23 R = CH_2CONH_2
27 R = —≡—H

[a] (a) RLi, THF, –78 °C (see Experimental Section). (b) Trimethylsulphoxonium iodide, *n*-BuLi, THF, –10 °C. (c) $MgCl_2$, Et_2O, MeOH, 23 °C. (d) HgO, 4%, H_2SO_4, MeOH, 60 °C.

The chloromethyl-substituted compound **22** was prepared by opening the epoxide **26** with magnesium chloride. The epoxide in turn was prepared by a "Corey" methylenation of the enone **25** (Scheme III).

In the synthesis of the indole **19**, advantage was taken of the ability of N-protected indoles to undergo lithiation

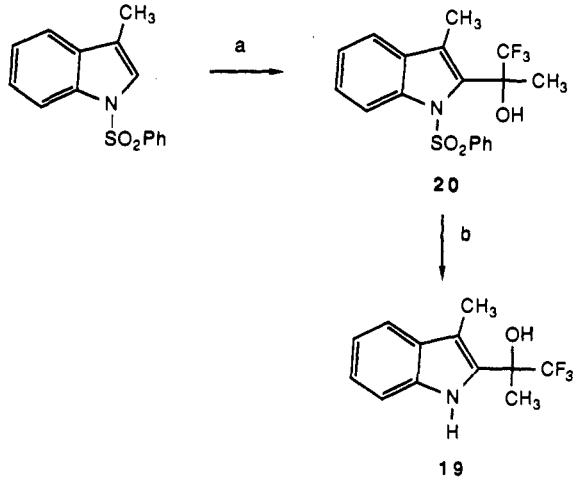
Table IV. IR Frequencies for Substituted 2-Hydroxypropionanilides



no.[a]	X	Y	R_1	R_2	IR frequencies (cm^{-1}) ν_{CO}	ν_{NH}	ν_{OH}	%OH···OC[b]
1	NO_2	CF_3	CH_3	H	1721	3450	–	–
2	NO_2	CF_3	CH_3	OH	1713	3390	3620	0
12	NO_2	CF_3	CH_2F	OH	1712	3390	3605	0
11	NO_2	CF_3	CHF_2	OH	1715	3390	3600	0
10	NO_2	CF_3	CF_3	H	1727	3395	–	–
9	NO_2	CF_3	CF_3	OH	1726	3405	3605	0
15	NO_2	CF_3	4-$NO_2(C_6H_4)$	OH	1713	3395	3610	0
3	H	H	CH_3	OH	1700	3408	3615	0
6	H	H	CF_3	OH	1712	3450	3610	60
					1700 sh		3420	
4	Cl	H	CH_3	OH	1708	3400	3612	0
7	Cl	H	CF_3	OH	1714	3450	3610	40
					1705 sh		3430	
5	NO_2	H	CH_3	OH	1712	3400	3620	0
8	NO_2	H	CF_3	OH	1723 as	3450	3605	10

[a] Numbers refer to compounds in Table I. [b] Refers to the proportion of **29** (see text).

Scheme IV[a]



[a] (a) *n*-BuLi, THF, CF_3COCH_3, −78 °C. (b) KOH, dioxane, 23 °C.

in the 2-position. Subsequent condensation with trifluoroacetone and deprotection (Scheme IV) gave the required product **19**.

Results and Discussion

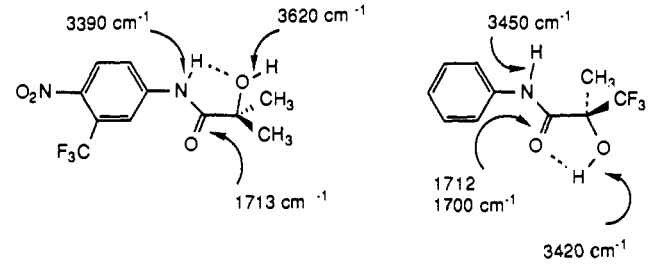
The molecular features that was investigated in this series of 2-hydroxypropionanilides are shown below.

1. **Lipophilicity**. In general these hydroxypropionanilides are quite lipophilic (Table I) and we find in vivo activity across the full range of log *P* values. Interestingly, CLOGP[9] copes surprisingly well with these structures considering that there are multiple proximity corrections to take into account (Table I).

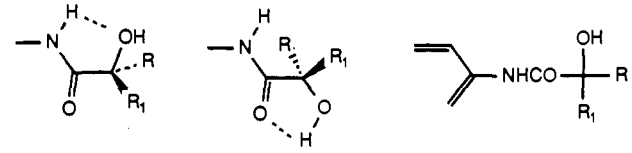
2. **Role of the Aromatic Ring**. The limited data in Table I suggests that there is a trend toward increasing activity both in vitro and in vivo with increasing electron-withdrawing ability in the aromatic ring. This is also borne out by data from other series.[2,5]

3. **Conformation of the 2-Hydroxypropionanilides**. Intuitively, the two most likely conformers both involve

Chart I



the formation of intramolecular hydrogen bonds; the first one is where the NH eclipses OH (**28**), and the other is where the OH eclipses carbonyl (**29**). The conformation of the anilides was studied by using both ab initio molecular orbital calculations and infrared spectroscopy.



28 **29** **30** R = R_1 = H

(a) Molecular Orbital Calculations. Ab initio calculations[10] carried out at the STO-3G and 4-3-1G levels on the diene derivative (**30**) clearly showed that **28** is favored over **29** by 1.5 kcal mol^{-1} in vacuo. This rather unexpected result on model compounds prompted an infrared investigation to see whether the same trends are followed in solution.

(b) Infrared Spectroscopy. IR spectroscopy is an excellent technique for studying the conformational preferences of molecules such as these because the vibrations associated with the OH, NH, and CO groups all appear separately in the spectrum (Table IV). In addition, the time needed for a bond vibration is so short compared with that for a bond rotation that any significant distribution of conformer populations might reasonably be expected to show in the spectrum as discrete bands or as shoulders on the main bands. For the compounds shown in Table IV all the bands are clean and symmetrical which

(9) CLOGP, Version 3.53; Daylight Corporation.

(10) Davies, R. H. Unpublished observations.

is consistent with the idea of a single conformer.

The frequencies shown in Table IV clearly demonstrate that the dominant conformation in solution is indeed **28**, in agreement with the theoretical calculations.

The most compelling experimental evidence is provided by a comparison of the NH frequency of **2** with the corresponding deshydroxy analogue **1**, where there is a difference ($\Delta\nu_{NH}$) of 60 cm^{-1} (Chart I and Table IV). The simplest explanation for this frequency difference is that in **2** the NH forms an intramolecular hydrogen bond (or dipolar alignment) with the oxygen of the OH as the acceptor. Consequently, the observed NH frequency for this compound corresponds to NH···OH. Clearly no such intramolecular interaction is possible in **1**, and so ν_{NH} in this case corresponds to free NH. Consistent with this interpretation is the smaller $\Delta\nu_{NH}$ (10 cm^{-1}) found for the corresponding trifluoromethyl derivatives **9** and **10** (Table IV). Here the field effect of the CF_3 reduces the acceptor ability of the OH, which in turn destabilizes the internal hydrogen bond and is reflected by the smaller $\Delta\nu_{NH}$.

Consequently any combination of substituents which can effectively destabilize the NH···OH alignment might result in a conformational switch from **28** to **29**.

The infrared frequencies in Table IV (and in Chart I) show that **29** is characterized by a split or asymmetric carbonyl band, a reduction in the relative intensity of the OH at ca. 3600 cm^{-1}, and the appearance of a new band at 3410–3450 cm^{-1} corresponding to OH···O=C. In addition, now that the NH···OH alignment is eliminated, the NH band reverts to its more typical frequency at ca. 3450 cm^{-1}, and is in line with that observed for the deshydroxy analogues **1** and **10** described above.

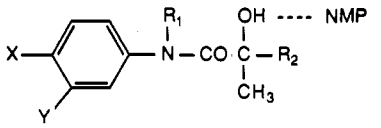
By using the absorbance of the OH as a guide, it is easy to estimate the relative proportion of each conformer (Table IV). So for example, **6** exists as a 40:60 mixture of **28** and **29**, while **3** exists exclusively as **28**.

In general, **29** occurs only when two conditions are met. There must be electron-releasing substituents in the aromatic ring combined with powerful electron-withdrawing substituents α to the OH. Electron donors in the aromatic ring weaken the hydrogen-bond donor ability of the NH, and the field effect of the α-substituent has two distinct effects. Firstly it weakens the hydrogen-bond acceptor ability of the hydroxyl oxygen, and secondly it makes the OH a better donor, both of these act to stabilize **29** at the expense of **28**. Neither substitution in the ring nor α to the OH on their own however is sufficient to tip the balance toward **29**.

It is interesting to speculate on the reasons for the predominance of **28** over **29**. Molecular modeling reveals that the angle of approach of the NH to the sp^3 lone pairs of the hydroxyl oxygen in **28** is not much better than that formed between the OH and the sp^2 lone pairs of the carbonyl oxygen in **29**. In this connection **29** only accounts for about 70% of the conformation in the N-methylated derivative **13** (where **28** cannot exist). Clearly neither of the intramolecular hydrogen bonds in **28** or **29** are optimally aligned, so it may be that the favorable dipole alignment in **28** accounts for conformational preference shown.

There are other spectroscopic features in the data in Table IV which give information about the electronic arrangement in these molecules. In particular there is an interesting effect of increasing fluoro substitution across the series **2**, **12**, **11**, and **9**. Here the response of the carbonyl frequency is essentially constant in **2**, **12**, and **11** with a sharp increase in frequency for **9**. The simplest rationalization here is that the fluorine atoms tend to orient themselves away from carbonyl if they can. However in **9**, one of the fluorine atoms has to be adjacent to carbonyl, and the resulting interaction of dipoles gives rise to the effect on carbonyl frequency.

Table V. Hydrogen Bond Donor Ability of 2-Hydroxypropionanilides



no.[a]	X	Y	R_1	R_2	$\Delta\nu_{co}$	log K_α[b]
3	H	H	H	CH_3	19	1.7 (1.6)
4	Cl	H	H	CH_3	21.4	2.0
5	NO_2	H	H	CH_3	21.2	2.0
2	NO_2	CF_3	H	CH_3	22.9	2.2
13	NO_2	CF_3	CH_3	CH_3	17.4	1.0 (0.98)
6	H	H	H	CF_3	24.6	2.5 (2.41)
7	Cl	H	H	CF_3	25.3	2.6
8	NO_2	H	H	CF_3	26.0	2.7
9	NO_2	CF_3	H	CF_3	26.9	2.8 (2.91)
phenol						2.14[c]
hexafluoropropan-2-ol						2.83[c]
propan-2-ol						0.91[c]
testosterone						1.1[d]

[a] Number refers to compound in Table I. [b] Numbers in parentheses measured directly by IR. [c] Reference 8. [d] J. J. Morris, unpublished observation.

The IR studies described above show quite clearly that the dominant conformer is that in which the NH eclipses the OH **28**. There is of course no evidence that the dominant solution conformation is that favored by the receptor, nevertheless it remains a reasonable first approximation.

One of the consequences of this NH···OH alignment is to effectively ensure that the NH, CO, and OH are all coplanar. However just achieving a planar arrangement of these groups is clearly not enough for biological activity. For example, compounds such as **6** (which exist predominantly as **29**) are also nicely planar, but are only weakly active on the androgen receptor. What is this extra factor?

4. Hydrogen-Bond Donor Ability of 12-Hydroxypropionanilides. As part of a program of work aimed at producing scales of relative hydrogen-bonding ability, we devised an experimental system to rank hydrogen bond donors.[8] Apart from the usual equilibrium constants, we also measured the infrared carbonyl frequency shift ($\Delta\nu_{CO}$) of a standard acceptor *N*-methylpyrrolidone (NMP), with a series of hydrogen-bond donors. We showed[8] that for a series of alkanols and phenols there is an excellent correlation between the measured equilibrium constant (K_α) and the IR carbonyl shift of NMP ($\Delta\nu_{CO}$ in eq 1).

$$\log K_\alpha = 0.148\Delta\nu_{CO} - 1.15 \qquad (1)$$
$$n = 11,\ r = 0.99,\ s = 0.1,\ F = 664$$

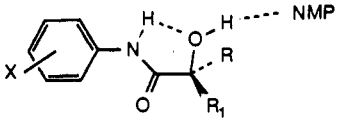
Consequently in the present study we measured the experimentally simpler $\Delta\nu_{CO}$ and used eq 1 to estimate K_α for the 2-hydroxypropionanilides (Table V) although in a few cases K_α was measured directly by IR spectroscopy and these values are also shown in Table V for comparison. To avoid complicating the interpretation, the concentration of all the donors was always maintained below the self-association limit as detected by IR spectroscopy.

Although OH is in principle an amphiprotic group, the data in Table V show that these compounds are among the

most powerful of OH donors toward NMP, and are comparable in free energy terms with phenol and hexafluoropropan-2-ol. Clearly they are also considerably stronger as hydrogen-bond donors than testosterone itself, and it is tempting to speculate that these compounds are anchored via the OH to some proton-acceptor site on the receptor by a powerful hydrogen bond.

Although good hydrogen bond donor ability is not a guarantee of good receptor binding, it is clear both from the data in Tables I and V and from work in other series[5] that weak hydrogen bond donor ability almost invariably leads to poor receptor binding. In addition, compounds which have no OH group (such as 1 and 10) do not bind to the receptor in vitro and have to rely on metabolic activation to the corresponding OH compound to show activity in vivo.[3]. Consequently we needed to understand at a molecular level the features which make this series of hydroxyanilides such powerful hydrogen-bond donors toward external acceptors.

One of the factors which makes these compounds such good hydrogen-bond donors is the presence of the NH···OH alignment which we have described above. Table V shows that the N-H derivative (2) is 1.2 log units stronger as a hydrogen-bond donor than the corresponding N-Me derivative (13) where such an alignment is impossible. The simplest rationale for this is a sort of intramolecular "cooperative effect" in which the donor properties of the OH are strengthened by acting as an acceptor to the NH hydrogen 31. This phenomenon is very well known in



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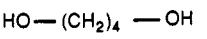
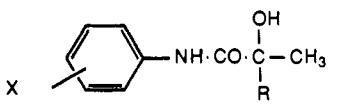


32

intermolecular interactions where it responsible for the extreme hydrogen bond donor ability of solvent water. However it is much less common in an intramolecular context, although it has been recently reported to occur in butanediol.[11]

For butanediol (32), the cooperative effect is reported to produce a 20% increase in hydrogen bond donor ability based on frequency shifts, whereas we see a factor of around 20 in free energy for these hydroxypropionanilides. Presumably in the present case since the conformation is locked in the NH···OH orientation, the unfavorable entropic component present in butanediol is effectively minimized.

Consequently factors which influence this NH···OH alignment might also be expected to influence the hydrogen bond donor ability of the OH. Perhaps then we should not be surprised to find that within series there is a relationship between log K_α and Hammett's σ for substituents in the aromatic ring. Equations 2 and 3 show the relationships obtained for 33 and 34, respectively. This long



R = CH_3 33
R = CF_3 34

$$\log K_\alpha = 1.76 + 0.39\Sigma\sigma \quad (2)$$
$$n = 4,\ r^2 = 0.98,\ s = 0.06,\ F = 47$$

$$\log K_\alpha = 2.50 + 0.28\Sigma\sigma \quad (3)$$
$$n = 4,\ r^2 = 0.98,\ s = 0.02,\ F = 87$$

range effect is accomplished through the mediation of the NH···OH alignment. As electron-withdrawing power in the ring is increased so the NH becomes a better donor and the NH···OH alignment is strengthened. The stronger this alignment (in terms of ΔH) the more the "cooperative effect" comes into play and the better the hydrogen bond donor ability of the OH toward an external acceptor. Consequently we have uncovered a mechanism by which substituents at one end of the molecule can affect hydrogen bond donor ability at the other.

In line with this hypothesis we have noted above the larger value of $\Delta\nu_{NH}$ (ν_{NH} for the OH derivative compared with ν_{NH} for the corresponding des-OH compound) for 33 compared with 34 and suggested that this frequency shift reflects the strength of the NH···OH alignment. Yet 34 as a series are the stronger donors where, apparently the NH···OH alignment is weaker. This is presumably because in 34 the contribution of the NH···OH alignment to the overall log K_α is swamped by the direct-field effect of the CF_3. Consequently, log K_α in this series is less sensitive to substitution in the ring.

It is worth reemphasising here for the discussion which follows that even though the dipolar alignment is enthalpically weak and various substituents may make it weaker, in terms of free energy 28 is still the favored conformer by a very long way. In other words we have a weak but highly favorable interaction.

In considering hydrogen-bonding interactions in these compounds, the anilide carbonyl itself is also a hydrogen-bond acceptor. However, as electron-withdrawing substituents are loaded into the aromatic ring the hydrogen bond acceptor ability of the carbonyl will decrease, yet activity in vitro is known to increase. It is reasonable to suppose therefore that the anilide carbonyl is not involved in a direct interaction with the receptor which contributes significantly to the binding energy.

It is difficult to rule out unequivocally the possibility that the NH···OH alignment does not survive at the receptor and the NH itself forms an important interaction with the receptor. However, none of the deshydroxy compounds such as 1 are active in vitro, nor indeed are the methoxy analogues.[2] So it is quite clear that although the aryl-substituted anilide NH is comparable to OH in terms of hydrogen bond donor ability.[8] It clearly cannot interact with the receptor in the same way as the OH can.

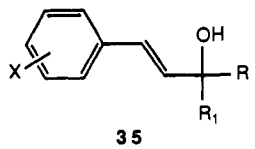
Structure–Activity Relationships. So far we have uncovered several factors which seem to be related to antiandogenic activity. These are

1. An electron-deficient aromatic ring.
2. A fixed conformation which effectively ensures that the NH–CO–OH are all coplanar.
3. A powerful hydrogen bond donor group.
4. There exists in these compounds a delicate electrical balance in which one function of the anilide moiety is to "transmit" electron density around the molecule via this NH···OH alignment, rather than form distinct interactions with the receptor itself.

Using these criteria we can now design structures which incorporate these features but which do not include the anilide group. A series of alkenes 35 and an example from a series of indoles 19 (Table II) illustrate that the compounds which bind to the androgen receptor can be designed by using this hypothesis.

Initially, replacement of the anilide by the alkene had led to weakly active compounds such as 16, but they are poor hydrogen-bond donors (Table VI). Clearly no dipolar

(11) Kleeberg, H.; Klein, D.; Luck, W. A. P. *J. Phys. Chem.* 1987, *91*, 3200.



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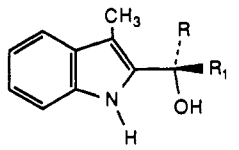
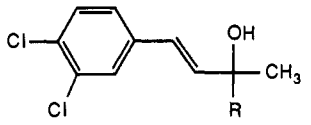


19

alignment is possible in this series, so the only way to increase proton-donor ability is by the substitution of electron-withdrawing groups α to the OH. Table II shows that for powerful donors such as 17 and 18 in vivo activity is observed.

There is a reasonable relationship (equation 4) between the hydrogen bond donor ability of the alkenes in our model system and σ_I for R in **36**, especially when we take into account the limited range of substituents However

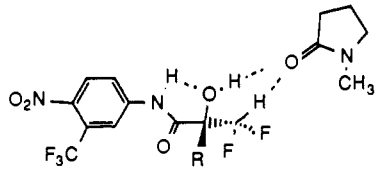


36

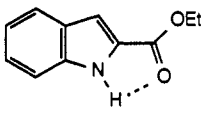
$$\Delta\nu_{CO} = 21.3 + 8.1\sigma_I \qquad n = 5, r^2 = 94\%, s = 0.3 \qquad (4)$$

it is quite clear that both **21** and **22** are much stronger donors than expected on the basis of their σ_I values. One explanation is that in the expected dominant conformer[12] (i.e. that in which the OH is gauche to the chlorine) the substituent dipoles reinforce to produce a much stronger hydrogen bond than expected. In actual fact the same phenomenon is observed in the halogenated propionanilides 11 and 12 where log K_α is also higher than expected. Here spectroscopic evidence excludes the possibility of a cyclic dimer such as **37** because $\Delta\nu_{CO}$ would be expected[13] at 35–50 cm^{-1} rather than the 15–25 cm^{-1} actually observed.

In the absence of the NH···OH alignment, the effect of substitution in the aromatic ring on the hydrogen bond donor ability of the OH is minimal. The indole, **19**, is an example of another series designed to fulfill the criteria indicated above. In this case we hoped that the indole NH might reinforce the hydrogen bond donor ability of the OH in the same way as it does in the hydroxypropionanilides. It has been shown[14] that indole NH can indeed form a weak interaction with the ester carbonyl in **38**, so despite



R = CH$_3$ 37



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the apparently appalling geometry an interaction of the type we require is clearly possible. log K_α for **19** turns out to be a factor of 3 or so higher than that found for 2,2,2-trifluoroethanol in the same system, an acceptable increase considering that the CF_3 group will destabilize the dipolar interaction just as it did for the 2-hydroxypropionanilides.

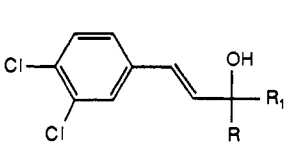
Conclusions

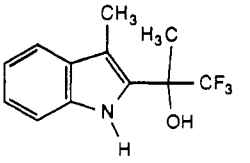
1. The physicochemical requirements for binding to the androgen receptor were defined from a series of 2-hydroxypropionanilides. The dominant conformation was found to be that where NH eclipsed OH, and this dipolar alignment had a significant effect upon the hydrogen bond donor ability of the OH.

2. While not all compounds which have good hydrogen bond donor properties are active antiandrogens, no compounds with weak hydrogen bond donor ability show activity. It is also worth making the point that if hydrogen bonding were to be treated as an indicator variable then there is effectively no difference in hydrogen-bonding ability between any of the alkenes shown in Table VI since they all contain an OH group.

3. We suggest that there may be some benefit in considering the quantitative aspects of hydrogen-bonding ability.

Table VI. Hydrogen Bond Donor Ability of some Alkene and Indole Antiandrogens





19

no.	R	R_1	$\Delta\nu_{CO}$, cm^{-1}	log K_α
16	Me	Me		1.0
17	Me	CF_3	21.4	2.0
18	CF_3	CF_3	24.8	2.5
21	CF_3	CH_2CN	26.5	2.8
22	CF_3	CH_2Cl	24.0	2.4
23	CF_3	CH_2CONH_2	26.3	2.7
24	CF_3	Ac	23.4	2.3
19			23.9	2.4

Experimental Section

Biology. Relative Binding Affinity (RBA) in Vitro. RBA for compounds were measured by using a preparation of the rat androgen receptor by the method described previously.[15]

Antiandrogenic Activity in Vivo.[16] Groups of five male rats were castrated at 24 days of age and then treated with seven daily doses of testosterone propionate (200 μg/kg) subcutaneously. The antiandrogens were dosed daily either subcutaneously or orally for the same seven days. Animals were killed 24 h after the final dose and the androgen-sensitive organs, the seminal vesicles, and ventral prostrates were weighed. The percent inhibition of the testosterone-stimulated weight was calculated by using a running castrate control, and a dose–response curve was constructed. From this curve an ED_{50} was measured. For simplicity the figures quoted in this paper refer only to the seminal vesicle weights.

Physical Chemistry. IR Spectroscopy. IR spectra were run on a Digilab FTS-20 fourier transform infrared spectrophotometer. For K_α 400 scans were collected at a nominal resolution of 4 cm^{-1}. For $\Delta\nu_{CO}$ 600 scans were collected at a resolution of 1 cm^{-1}. Temperature was maintained by the use of a water circulator (LKB Multitemp) connected to a water-jacketed cell holder and demountable cell fitted with CaF_2 plates.

All spectroscopy was carried out in the solvent 1,1,1-trichloroethane. IR spectroscopy was used to ensure that the concentration of drug used was always below that where self-association occurs (typically <0.002 mol dm^{-3}).

Hydrogen-bonding equilibrium constants were measured by following the decrease in intensity of the OH band with increasing NMP concentration.[8]

Carbonyl shifts ($\Delta\nu_{CO}$) were measured as previously described[8] by using at least two concentrations of hydrogen-bond donor which were chosen to be at either end of the complexation range with

(12) Maleknia, S.; Schwartz, M. *Spectrochim. Acta* **1983**, 265.
(13) Morris, J. J.; Prior, D. V. P. Unpublished observations.
(14) Jones, R. A.; Moritz, A. G. *Spectrochim. Acta* **1965**, *21*, 295.
(15) Wakeling, A. E.; Furr, B. J. A.; Glen, A. T.; Hughes, L. R. *J. Steroid Biochem.* **1981**, *15*, 355.
(16) Furr, B. J. A. *Management of Advanced Cancer of the Prostate and Bladder.* Motta, M., Serio, M., Eds.; A. R. Liss: New York, pp 13–26.

NMP. The concentration of NMP was usually 0.001 mol dm^{-3}. Carbonyl shifts were calculated by using both Fourier self-deconvolution methods and band synthesis techniques, and the agreement between the two methods is excellent.[8] In addition repeat experiments carried out months apart by different operators show that these shifts are reproducible to better than 0.2 cm^{-1}.

Partition coefficients were measured by using octanol-entrained HPLC.[17]

Synthetic Chemistry. All melting points were determined with use of a Kopfler Hotstage Apparatus and are uncorrected. NMR spectra were run (using either a JEOL FX 40A or Varian EM 390) on all isolated intermediates and final products and were consistent with the structural assignments. Petrol refers to petroleum ether, bp 60–80 °C. Chromatography was carried out on silica gel unless otherwise stated. Standard workup involved diluting with water, extracting with ethyl acetate, washing the organic layer with water and saturated brine, drying over $MgSO_4$, and evaporating in vacuo.

The general method of synthesis of the 2-hydroxypropionanilides is illustrated by the following examples.

2-(Difluoromethyl)-2-hydroxy-4′-nitro-3′-(trifluoromethyl)propionanilide (11). Thionyl chloride (3.8 mL; 52 mmol) was added dropwise to a stirred solution of 2-(difluoromethyl)-2-hydroxypropionic acid (7 g; 50 mmol) in dimethylacetamide (50 mL) maintained between −10 °C and −20 °C and the mixture stirred for 30 min. 4-Nitro-3-(trifluoromethyl)aniline (5.1 g; 24 mmol) was added, and the mixture was warmed to room temperature and stirred for 3 h. Standard workup with EtOAc gave a yellow oil that was purified by chromatography on Florisil with 10% EtOAc in toluene used as eluent. The solid obtained by evaporating the eluate was recrystallized from toluene to give **11**: 4.1 g (51%); mp 119–21 °C. Anal. ($C_{11}H_9F_5N_2O_4$) C, H, N.

2-Acetoxy-2-(4-nitrophenyl)propionic Acid (14). 2-Hydroxy-2-(4-nitrophenyl)propionitrile (57.7 g; 0.27 mol) was dissolved in concentrated sulfuric acid (500 mL), stirred at ambient temperature for 2 h, diluted with water (500 mL), and heated on a steam bath for 4 h. Standard workup with EtOAc gave crude 2-hydroxy-2-(4-nitrophenyl)propionic acid which was stirred with acetic anhydride (57 mL; 0.6 mol) in pyridine (500 mL) for 17 h and then evaporated in vacuo. The resulting oil was partitioned between EtOAc and 1 N HCl and the resulting organic layer washed successively with 1 N HCl, water, and saturated brine, dried over $MgSO_4$, and evaporated to give a tan solid 45 g (64%). An analytically pure sample was obtained by dissolving this solid in saturated $NaHCO_3$ and precipitating a white solid with HCl. The solid was filtered from the solution, washed with water, and dried to give the acid, mp 160–1 °C. Anal. ($C_{11}H_{11}NO_2$) C, H, N.

2-Hydroxy-2-(4-nitrophenyl)-4′-3′-(trifluoromethyl)propionanilide (15). To a solution of (14) (3.2 g; 12.6 mmol) in dry methylene chloride at 0°C was added pyridine (1.2 mL; 14.8 mmol) followed by thionyl chloride (0.12 mL; 12.6 mmol); the reaction was warmed to ambient temperature and stirred for 30 min. The solvent was removed in vacuo, the resulting oil dissolved in pyridine (50 mL), 4-nitro-3-(trifluoromethyl)aniline (2.5 g; 12.1 mmol) added, and the mixture stirred 17 h. Removal of the solvent in vacuo gave an oil that was partioned between 1 N HCl and EtOAc, and the organic layer was washed with 1 N HCl, water, saturated $NaHCO_3$, and saturated brine, dried over $MgSO_4$, and evaporated in *vacuo*. The resulting oil was dissolved in EtOH (50 mL) with 2 N NaOH (5.8 mL; 11.6 mmol) added, the mixture stirred for 1 h at ambient temperature, the solvent removed in vacuo, and the resulting oil portioned between ethyl acetate and saturated brine. The organic layer was dried over $MgSO_4$ and evaporated in vacuo and the resulting solid recrystallized from toluene to give **15**: 2.4 g (50%); mp 126–7 °C. Anal. ($C_{16}H_{12}F_3N_3O_6$) C, H, N.

2-Hydroxy-2,*N*-dimethyl-4′-nitro-5′-(trifluoromethyl)propionanilide (13). To a solution of NaH (109 μg of 50% dispersion, 2.3 mmol) in DMF (10 mL) was added *N*-methyl-4′-nitro-3′-(trifluoromethyl)aniline (0.5 g; 2.3 mmol) and the mixture stirred for 1 h at ambient temperature. 2-Acetoxyisobutyl chloride (0.75 g; 4.6 mmol) was added and the mixture stirred for 17 h; standard workup gave a semisolid that was dissolved in EtOH (15 mL) with 2 N NaOH (2 mL; 4 mmol) added, the mixture was stirred for 1 h, and standard workup gave a yellow solid which was purified by flash chromatography on silica (eluent 20% EtOAc/petrol) and finally recrystallized from toluene to give **(13)**: 350 mg (50%); mp 85–8 °C. Anal. ($C_{16}H_{13}F_3N_2O_4$) C, H, N.

***trans*-4(3′,4′-Dichlorophenyl)-2-(trifluoromethyl)but-3-en-2-ol (17).** To a stirred solution of lithium tetramethylpiperidide in THF (50 mL at −78 °C) (from tetramethylpiperidine (0.9 mL; 5.3 mmol) and methyllithium (3.3 mL of 1.6 M; 5 mmol)) was added a solution of 3,4-dichloroacetophenone (1 g; 5.3 mmol) in THF (25 mL), the mixture stirred for 0.5 h at −78 °C, trifluoroacetone (0.6 g; 5.3 mmol) added, and the reaction allowed to warm to −50 °C for 1 h before quenching with methanol (5 mL) and aqueous ammonium chloride (20 mL). Standard workup with EtOAc gave an oil (1.3 g) that was dissolved in EtOH (75 mL); sodium borohydride (0.73 g; 19.8 mmol) was added and the mixture stirred for 1.5 h. The reaction was quenched with 2 N HCl, water added, and the mixture extracted with EtOAc. The organic layer was washed with 2 N HCl, dried ($MgSO_4$), and evaporated in vacuo. The residue was heated with 4-toluenesulfonic acid (100 mg) at 140 °C for 0.5 h, cooled, and then purified by chromatography (eluent toluene) to give **17**, 250 mg (17%) as an oil. Anal. ($C_{11}H_9Cl_2F_3O$) C, H, N.

***trans*-1-Cyano-4-(3′,4′-dichlorophenyl)-2-(trifluoromethyl)but-3-en-2-ol (21).** To a suspension of finely ground LiOH (1 g; 23.8 mmol) in EtOH (100 mL) was added a solution of 3,4-dichlorobenzaldehyde (10 g; 57 mmol) in ethanol (50 mL) followed by trifluoroacetone (20.5 mL; 0.23 mol) (injected over 10 min below the surface of the liquid) and the mixture stirred for 1 h, poured into water (600 mL), and worked up with EtOAc. (An initial wash with 2 N HCl was added.) The residue was purified by chromatography with 30% methylene chloride/petrol as eluent to give *trans*-4(3′,4′-dichlorophenyl)-1,1,1-trifluorobut-3-en-2-one (**25**), 3.3 g (21%), which was used without further purification.

To a solution of lithium diisopropylamide (10.4 mmol), prepared from diisopropylamine (1.4 mL; 10.4 mmol) and butyllithium (6.5 mL of 1.6 M solution in hexane; 10.4 mmol) in THF (50 mL) maintained at −78 °C, was added slowly a solution of acetonitrile (0.6 mL; 10.4 mmol) in THF (10 mL), the mixture stirred for 10 min, a solution of **25** (1.4 g; 5.2 mmol) in THF (40 mL) added over 10 min, and after a further 10 min the reaction quenched with saturated ammonium chloride. Workup with EtOAc gave an oil that was purified by chromatography with 10% ethyl acetate/petrol as eluent to give **21**: 600 mg (37%); mp 116 °C. Anal. ($C_{12}H_8F_3Cl_2NO$) C, H, N.

***trans*-1-Chloro-4-(3′,4′-dichlorophenyl)-2-(trifluoromethyl)but-3-en-2-ol (22).** *n*-Butyl lithium (11.6 mL of 1.6 M solution; 18.5 mmol) was added dropwise to a suspension of trimethylsulfoxonium iodide (4.1 g; 18.5 mmol) in dry THF maintained at −10 °C and the mixture stirred for 2 h and then added to a stirred solution of *trans*-4-(3′,4′-dichlorophenyl)-1,1,1-trifluorobut-3-en-2-one (**25**) (5 g; 18.5 mmol) also maintained at −10 °C. After the mixture was stirred at this temperature for 1.5 h, the reaction was quenched with saturated ammonium chloride solution and worked up with EtOAc. The resulting oil was purified by chromatography using 20% methylene chloride/petrol to give *trans*-1,2-epoxy-2-(trifluoromethyl)but-3-ene **26**, 1.2 g (13%) as an oil. Anal. ($C_{11}H_7F_3Cl_2O$) C, H.

Magnesium chloride (400 mg, 4.2 mmol) and 1,2-epoxy-2-(trifluoromethyl)but-3-ene **26** (250 mg, 0.88 mmol) were stirred in Et_2O for 4 days, MeOH (0.5 mL) was added, and the mixture was stirred for a further 2 days. Workup with methylene chloride gave an oil that was purified by chromatography with 20%

(17) Mirlees, M. S.; Moulton, S. J.; Murphy, C. T.; Taylor, P. J. *J. Med. Chem.* **1976**, *19*, 615.
(18) Bayles, R.; Johnson, M. L.; Maisey, R. F.; Turner, R. W. Synthesis **1977**, 31.
(19) Durrall, R.; Smith, F.; Stacey, M.; Tatlow, J. C. *J. Chem. Soc.* **1951**, 2329.
(20) Buxton, M. W.; Stacey, M.; Tatlow, J. C. *J. Chem. Soc.* **1959**, 306.
(21) *Chem. Abstr.* **1959**, *78*, 2078g.
(22) Parameswara Reedy, M.; Krisham Lao, G. S. *Synthesis* **1980**, 815.

methylene chloride/petrol as eluent to give **22**: 125 mg (44%); mp 64 °C. Anal. ($C_{11}H_8F_3Cl_3O$) C, H.

***trans*-5-(4,4-Dichlorophenyl)-3-hydroxy-3-(trifluoromethyl)pent-4-enamide (23).** To a solution of bis(trimethylsilyl)acetamide (0.92 mL; 3.7 mmol) in dry THF (50 mL) maintained at −78 °C was added *n*-butyllithium (2.3 mL of 1.6 M solution; 3.7 mmol), the mixture stirred for 30 min, a solution of *trans*-4-(3′,4-dichlorophenyl)-1,1,1-trifluorobut-3-en-2-one (**25**) (1 g,; 3.7 mmol) in dry THF (25 mL) added, and after stirring for an additional 30 min at −78 °C the reaction quenched with ammonium chloride solution, warmed to room temperature, and stirred for 17 h. Workup with EtOAc gave an oil that was purified by chromatography with a 1:1 mixture of EtOAc/petrol as eluent to give **23**: 0.4 g (33%); mp 126 °C. Anal. ($C_{12}H_{10}F_3Cl_2NO_2$) C, H, N.

***trans*-5-(3′,4′-Dichlorophenyl)-3-hydroxy-3-(trifluoromethyl)pent-4-en-2-one (24).** *n*-Butyllithium (3.5 mL of 1.6 M; 5.6 mmol) was added dropwise to a THF (50 mL) solution of (trimethylsilyl)acetylene (0.76 mL; 5.6 mmol) maintained at −78 °C, the mixture stirred for 1 h, a solution of *trans*-4-(3′,4′-dichlorophenyl)-1,1,1-trifluorobut-3-en-2-one (**25**) in THF (25 mL) added dropwise, and the reaction stirred for 20 min at −78 °C. After the mixture was quenched with saturated ammonium chloride solution and worked up with EtOAc, the resulting oil was dissolved in THF (15 mL), tetra-*N*-butylammonium fluoride (1.19 g; 5.6 mmol) added, and the mixture stirred at ambient temperature for 1 h, diluted with water, and worked up with EtOAc. The resulting oil was purified by chromatography with 40% methylene chloride/petrol as eluent to give *trans*-1-(3,4-dichlorophenyl)-3-(trifluoromethyl)pent-1-en-4-yn-3-ol (**27**) as an oil, 0.8 g (73%). Anal. ($C_{12}H_7F_3Cl_2O$) C, H, N. **27** (0.8 g; 2.7 mmol) dissolved in methanol (5 mL) was added to a mixture of mercuric oxide (0.6 g; 2.7 mmol) in 4% H_2SO_4 (50 mL) maintained at 60 °C and the reaction stirred at this temperature for 30 min, cooled to ambient temperature, and worked up with EtOAc. The resulting oil was purified by chromatography with 60% methylene chloride/petrol to give **24**, 180 mg (21%), as an oil. Anal. ($C_{12}H_9F_3Cl_2O_2$) C, H.

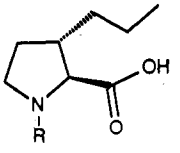
2-[1′-Hydroxy-1′-(trifluoromethyl)ethyl]-3-methylindole (19). *n*-Butyllithium (15 mL of 1.6 M solution; 24 mmol) was added dropwise to a solution of 1-(phenylsulfonyl)-3-methylindole (5.4 g; 20 mmol) (prepared from 3-methylindole by standard procedure) in THF at −78 °C, the mixture stirred for 0.5 h, trifluoroacetone (2 mL; 22 mmol) added, and the reaction stirred for an additional 1 h. Aqueous ammonium chloride was added followed by standard workup with Et_2O which gave a semisolid that was purified by chromatography with 30% methylene chloride in petrol as eluent to give **20**: 2.6 g (34%); mp 91–2 °C. Anal. ($C_{18}H_{16}F_3NO_3S$) C, H, N.

A solution of **20** (1.3 g; 3.3 mmol) in 1,4-dioxane (30 mL) was treated with KOH (22 mL; 0.22 mol) and stirred for 0.5 h at ambient temperature. It was diluted with water and extracted with Et_2O. The organic layer was washed with water, dried Na_2SO_4, and evaporated in vacuo. The residue was purified by chromatography with 70% methylene chloride/petrol as eluent to give **19**: 0.3 g (38%); mp. Anal. ($C_{12}H_{12}F_3NO$) C, H, N.

Communications to the Editor

trans-3-*n*-Propyl-L-proline Is a Highly Favorable, Conformationally Restricted Replacement for Methionine in the C-Terminal Tetrapeptide of Cholecystokinin. Stereoselective Synthesis of 3-Allyl- and 3-*n*-Propyl-L-proline Derivatives from 4-Hydroxy-L-proline

The introduction of conformational constraints into peptides has been an important approach toward studying bioactive conformations of peptides.[1,2] There are, for example, numerous cases in which conformational information has been gained by replacement of a native peptide residue with the constrained amino acid proline.[3,4] However, when such a substitution leads to a reduction in biological activity, it is not clear whether this result is more properly attributable to conformational and steric considerations or to loss of important interactions associated with the side chain of the original amino acid residue. Therefore, we have undertaken the synthesis of proline derivatives that incorporate potentially important amino acid side-chain functionality, with the expectation that replacement of appropriate residues in biologically active peptides with these analogues could lead to important conformational information and offer the potential to discover peptide analogues with improved selectivity, stability, or bioavailability. For example, *trans*-3-*n*-propyl-L-proline (3PP, 1) can be viewed as a constrained analogue of norleucine which has a two-carbon bridge from the β-carbon to the α-nitrogen. Here we wish to report the stereoselective synthesis of 1 from *trans*-4-hydroxy-L-proline and the relative effects of replacing the methionine residue in Boc-CCK$_4$ (Boc-Trp-Met-Asp-Phe-NH_2)[5] with each of alanine, norleucine, proline, and 3PP. The results indicate that replacement of Met with 3PP, which possesses conformational rigidity together with an appropriate side-chain moiety, gives a highly potent analogue that is significantly more active than either of the corresponding Nle or Pro replacement analogues.



1 R = H

2 R = Boc

We wished to prepare the N-protected derivative **2** of 3PP in a way that would allow unequivocal assignment of absolute stereochemistry at the α-position. This was successfully accomplished with *N*-Boc-4-oxo-L-proline methyl ester (**3**) as the starting material (readily prepared from 4-hydroxy-L-proline[6]) by the route shown in Scheme I. Formation of the enamine from **3** and pyrrolidine was conducted according to the procedure of Taguchi and Westheimer (room temperature, 20 h).[7,8] During allylation

(1) Momany, F. A. *Top. Curr. Phys.* **1981**, *26*, 41.
(2) Freidinger, R. M. *J. Org. Chem.* **1985**, *50*, 3631.
(3) Marshall, G. R. In *Chemical Recognition in Biological Systems*; Creighton, A. M., Turner, S., Eds.; Chemical Society: London, 1982; p 278.
(4) Momany, F. A.; Chuman, H. *Methods Enzymol.* **1986**, *124*, 3.
(5) Abbreviations used are as follows: Boc, *tert*-butyloxycarbonyl; Cbz, benzyloxycarbonyl; 4-DMAP, 4-(*N*,*N*-dimethylamino)-pyridine; AIBN, azobisisobutyronitrile; EDAC, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole.
(6) Dormoy, J.-R.; Castro, B. *Synthesis* **1986**, 81.
(7) Taguchi, K.; Westheimer, F. H. *J. Org. Chem.* **1971**, *36*, 1570.

0022-2623/91/1834-0455$02.50/0