Protection of Hydroxy Groups by Silylation: Use in Peptide Synthesis and as Lipophilicity Modifiers for Peptides

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A survey of a series of organosilyl derivatives of serine and tyrosine has shown that they have a satisfactory stability profile for use in peptide synthesis. Only when alkaline conditions were used did side-reactions appear. A range of stability profiles have been determined from a study of organosilyl derivatised dipeptides under different conditions, giving $t_{\frac{1}{2}}$-values for hydrolysis ranging from 41 to 465 min in acid conditions, yet giving long-term stability at pH-values near to neutrality.

The application of the *tert*-butyldiphenylsilyl (Bu^tPh_2Si) group[1] as a reversible masking group for serine in the synthesis[2] of human cholecystokinin-33 has prompted us to report our own investigations on model studies relating to the further development and application of such a protecting group. The prospect of using silyl reagents in peptide synthesis and as potential lipophilicity modifiers for peptides, giving derivatives with useful half-lives in the pro-drug context, provided the impetus for investigating silyl reagents in general as side-chain-protecting groups for both seryl and tyrosyl side-chains in peptides. The successful use of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane [$Pr^i_2Si(Cl)$-O-$Si(Cl)Pr^i_2$] to bridge the Ser^2, Ser^5 residues of an enkephalin analogue to give a biologically active cyclic enkephalin has already been reported.[3]

The type of silyl protecting group suitable for derivatisation of HO-containing amino acid side-chains became obvious from exploratory stability studies on model compounds – hexanol as a model for the alcohol side-chain and *p*-cresol for the phenolic side-chain. Assessment of a series of hexanol derivatives with respect to susceptibility to hydrolysis, using GLC for analysis,[4] is recorded in Table 1. Monitoring by HPLC proved more convenient for the corresponding *p*-cresol derivatives and the results are summarised in Table 2. Reaction conditions in the series of investigations were: 1% HCl in 95% methanol at 25 °C for assessment of stability in acid, and 5% NaOH in 95% methanol at 25 °C for base stability.

The results in Table 1 show that stability reflects the trends already noted by other workers,[4,5] *i.e.* steric bulk around the silicon atom affects the hydrolytic lability of the silyl ethers. This is most emphatically demonstrated by comparing the isomers R-O-$SiBu^tMe_2$ [stable over the period of the experiment (24 h) under base hydrolysis] and R-O-$SiBu^iMe_2$ which has a $t_{\frac{1}{2}}$ of 2.5 min.

Application to Peptide Synthesis.—The results in Tables 1 and 2 confirmed that the stability of the Bu^tPh_2Si derivatives justified further work on the adaptation of the group as possible protection for hydroxy group side-chains in peptide synthesis. We have evaluated their potential by carrying out synthetic protocols usually associated with solution-phase syntheses.

t-Boc-Serine benzyl ester **1**[6] could be converted readily into *t*-Boc-Ser($SiBu^tPh_2$)OBzl **2** by using *tert*-butyl(chloro)(diphenyl)silane (1.1 mol equiv.) in the presence of imidazole (2.5 mol equiv.). Derivative **2** was then subjected to the various conditions summarised in Scheme 1. Compound **4** was obtained in 90% yield and confirmed the previous report[1] that the Bu^tPh_2Si group is resistant to hydrogenolysis conditions. The selective removal of the Boc group to give compound **3** was a greater challenge, as previous reports indicated that Bu^tPh_2Si deriv-

Table 1 Susceptibility to hydrolysis (hexanol derivatives)

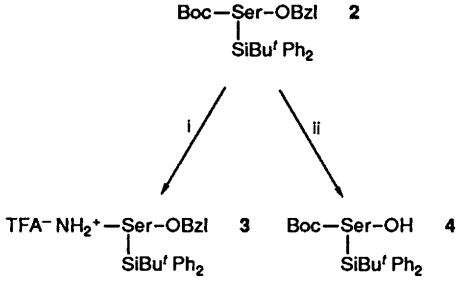
Substrate	Half-life ($t_{\frac{1}{2}}$/min) Acid hydrolysis 1% HCl in 95% MeOH	Base hydrolysis 5% NaOH in 95% MeOH
$C_6H_{13}OSiMe_3$	≤1[a]	≤1[a]
$C_6H_{13}OSiBu^iMe_2$	≤1[a]	2.5
$C_6H_{13}OSiBu^tMe_2$	≤1[a]	*b*
$C_6H_{13}OSiBu^tPh_2$	225	*b*
$C_6H_{13}OSiMePh_2$	14	≤1[a]
$C_6H_{13}OSiPr^i_3$	55	*b*

[a] A $t_{\frac{1}{2}}$ of 1 min represents the lower limit using these sampling techniques—faster rates could not be discriminated. [b] Stable over the period of the experiment (24 h).

Table 2 Susceptibility to hydrolysis (*p*-cresol derivatives)

Substrate	Half-life ($t_{\frac{1}{2}}$/min) (25 °C) Acid hydrolysis 1% HCl in 95% MeOH	Base hydrolysis 5% NaOH in 95% MeOH
p-$MeC_6H_4OSiEt_3$	≤1[a]	≤1[a]
p-$MeC_6H_4OSiBu^iMe_2$	≤1[a]	≤1[a]
p-$MeC_6H_4OSiBu^tMe_2$	273	3.5
p-$MeC_6H_4OSiBu^tPh_2$	100 (h)	6.5
p-$MeC_6H_4OSiPr^i_3$	100 (h)	188

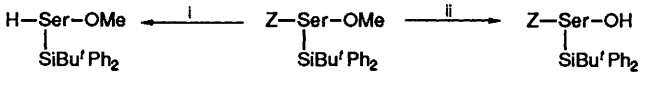
[a] A $t_{\frac{1}{2}}$ of 1 min represents the lower limit using these sampling methods—faster rates could not be discriminated.



Scheme 1 *Reagents:* i, TFA; ii, H_2/Pd

atives of carbohydrates tend[1] to decompose within about 15 min in 50% trifluoroacetic acid (TFA)–1,4-dioxane mixtures whereas the Boc group normally requires *ca* 30 min for complete removal in 100% TFA. When compound **2** was subjected to the latter conditions, TLC analysis indicated complete removal of the Boc group in 5 min, and repeated azeotropic removal of excess of TFA under high vacuum (0.6 mmHg) at room temperature by using 1,4-dioxane did not result in any cleavage of the Bu^tPh_2Si group, as shown by comparison of the reaction product mixture with authentic samples of *tert*-butyldiphenylsilanol and *tert*-butyldiphenyldisiloxane, the expected hydrolysis products. Compound **3** was coupled to Bz-Tyr(Bzl)OH by using dicyclohexylcarbodiimide/hydroxybenzotriazole (DCCI/HOBt) after neutralisation with triethylamine to give Bz-Tyr(Bzl)-Ser($SiBu^tPh_2$)OBzl **5**, thereby confirming the usefulness of the silyl group for serine side-chain protection. Complete removal of the side-chain to give Bz-Tyr(Bzl)-Ser-OBzl **6** was expedited quantitatively by using tetrabutylammonium fluoride (TBAF).[1]

Conversion of Z-Ser-OMe **7** into Z-Ser($SiBu^tPh_2$)OMe **8** was also readily achieved as for the preparation of compound **2**, and the availability of compound **8** made it possible to study the stability of the silyl protecting group towards base and hydrogenation conditions as summarised in Scheme 2. Hydro-



Z = benzyloxycarbonyl

Scheme 2 *Reagents:* i, H_2, Pd/C; ii, NaOH (1.1 mol equiv.), MeOH

genation did not prove to be any different from removal of the Z-group in conventional peptide synthesis, and in the absence of any acid traps, as expected some of the corresponding dioxopiperazine was found in the reaction mixture. This side-reaction could be prevented in the presence of an acid such as toluene-*p*-sulfonic acid. Under the basic conditions used to remove the methyl ester group in compound **8**, saponification took 6 h to reach completion and TLC analysis showed that some hydrolysis of the Bu^tPh_2Si-O group had also occurred. Purification gave a 35% yield of compound **9**, but poor microanalysis results suggested contamination. This enhanced rate of removal of the protecting group was not predicted from the data in Table 1 on the hexyl ethers. We suspect that the increased reactivity may be due to neighbouring-group participation of the carboxylate anion formed during the initial hydrolysis stages as shown in Scheme 3.

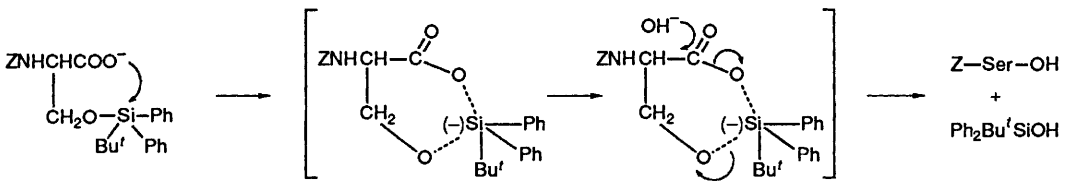
In summary, therefore, it seems likely that the $SiBu^tPh_2$ group could be used satisfactorily as a serine-protecting group in an acid/hydrogenolysis synthetic protocol. This would be particularly beneficial, *e.g.* in situations where selective protection of certain serine residues is required to enable further derivatisation by sugar moieties for the synthesis of glycopeptides. Any serine hydroxy group protected by Bu^tPh_2Si can be selectively deprotected *via* fluoride ion treatment in the presence of other protecting groups. We have not as yet tested the protecting group under the repetitive conditions of the Fmoc-protecting strategy[7] for peptide synthesis. Further evidence for the suitability of the protecting group was, however, indirectly derived from the synthesis of dipeptide derivatives, described in the next section.

Application of Silyl Derivatives as Transient 'Pro-peptide' Analogues.—To our knowledge, the most systematic study to date on the use of 'transient' silylation in the development of pro-drugs is that of F. Chiu *et al.*[8] Conclusions drawn from the study were that (a) silylamines are hydrolytically too unstable to be of use as slow-releasing agents regardless of what alkyl or aryl groups are attached to silicon; (b) silyl esters (carbamate esters in the published work) on the other hand possess the correct profile for slow release of the drug *in vivo*. Therefore the current interest[9] in modification and conformational restriction of biologically active peptides and the problems of targetting them to their receptor sites led us to investigate the potential of silyl ester derivatives as 'transient' analogues. Results reported in Tables 1 and 2 again led to the choice of Bu^tPh_2SiO for these exploratory studies. A series of dipeptide derivatives (Table 3) were synthesized using conventional solution-phase synthesis by using DCCI/HOBt for coupling. The organosilyl group was introduced onto the side-chain of the appropriate amino acid derivative prior to coupling in the case of the mono-derivatised dipeptides, but where both hydroxy groups were modified silylation was carried out on the *N*- and *C*-terminal-protected dipeptides. This gave further confirmation of the suitability of the silyl groups as protecting groups for side-chain hydroxy groups.

The hydrolysis of peptides **11**–**18** was monitored by HPLC, usually by following the disappearance of the peak due to the starting peptide derivative. Where intermediate products were formed these were also monitored *via* peaks appearing in the HPLC traces. In preliminary work it was unclear whether the Boc-protecting group in compounds **11**–**13** would survive the hydrolysis conditions, and whether the product of hydrolysis would be Boc-Tyr-Ser-OMe or H_2^+-Tyr-Ser-OMe. However, in a control experiment with Boc-Tyr-Ser-OMe it was shown that the Boc group was removed very slowly and only after 60 h was it completely removed. The main products of hydrolysis of compounds **11**–**13** were all still Boc-protected over the period of the monitored reactions.

The results of hydrolysis in 1% hydrochloric acid/methanol are summarised in Figs. 1 and 2 based on the analytical methods listed in Table 3. The raw kinetic data were fitted to the first-order rate equation by means of a modified version of the LSKIN least-squares computer program of DeTar.[10] The data confirm (Table 3) that the rate of loss of silyl group from the serine residue is faster than the loss of silyl group from the tyrosine residue. However, interestingly, when both side-chains are derivatised in Tyr-Ser dipeptides (as in compounds **11** and **14** there is more rapid decomposition of starting material relative to the corresponding mono-silylated compounds, indicating that a second triorganosilyl group influences the rate of removal of the first.

The nature of the *N*-protecting groups does not seem to influence the rates significantly, while the more polar deprotected peptides **17** and **18** had only slightly shorter half-lives than their protected counterparts. As expected,[4] in general the $SiPr^i_3$ group proved more labile than the $SiBu^tPh_2$ group under acidic conditions. Steric effects could explain the one case, **11** and **14**, where this does not hold.



Scheme 3

Table 3

Peptide derivative	Retention time (t_R/min) (eluent MeOH–water)	Half-life ($t_{\frac{1}{2}}$/min)	Rate constant (k/s^{-1})
11 Boc-Tyr(SiBu^{t}Ph$_2$)-Ser(SiBu^{t}Ph$_2$)-OMe	8.87 (90:10)	41	(2.8 ± 0.59) × 10^{-4}
12 Boc-Tyr(SiBu^{t}Ph$_2$)-Ser-OMe	6.95 (85:15)	465	(2.48 ± 0.53) × 10^{-5}
13 Boc-Tyr-Ser(SiBu^{t}Ph$_2$)-OMe	5.70 (85:15)	208	(5.54 ± 0.13) × 10^{-5}
14 Z-Tyr(SiPr$^i_3$)-Ser(SiPr$^i_3$)-OBzl	7.50 (85:15)	71	(1.62 ± 0.34) × 10^{-4}
15 Z-Tyr-Ser(SiBu^{t}Ph$_2$)-OBzl	7.19 (85:15)	225	(5.14 ± 0.14) × 10^{-5}
16 Z-Tyr-Ser(SiPr$^i_3$)-OBzl	4.10 (85:15)	133	(8.47 ± 0.23) × 10^{-4}
17 H-Tyr-Ser(SiBu^{t}Ph$_2$)-OH	3.6 (75:25)[a]	145	(7.96 ± 0.16) × 10^{-5}
18 H-Tyr-Ser(SiPr$^i_3$)-OH	6.3 (75:25)[a]	118	(9.79 ± 0.47) × 10^{-5}
19 Boc-Tyr-Ser-OMe	3.5 (85:15)		

[a] Acetonitrile–0.1% TFA.

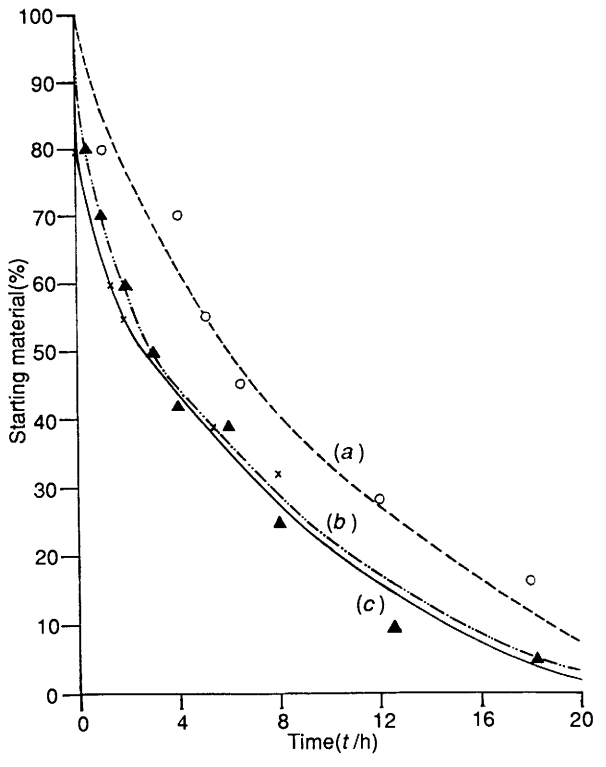


Fig. 1 Acid-catalysed hydrolysis of: (*a*) Boc-Tyr(SiBu^{t}Ph$_2$)-Ser-OMe **12**; (*b*) Boc-Tyr-Ser(SiBu^{t}Ph$_2$)-OMe **13**; (*c*) Z-Tyr-Ser(SiBu^{t}Ph$_2$)-OBzl **15**

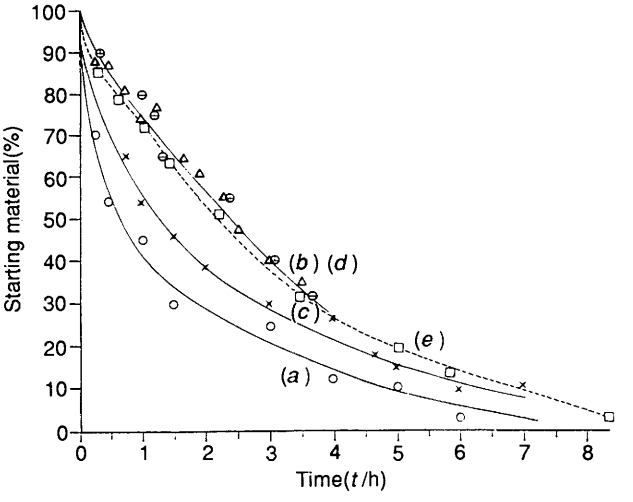


Fig. 2 Acid-catalysed hydrolysis of: (*a*) Boc-Tyr(SiBu^{t}Ph$_2$)-Ser-(SiBu^{t}Ph$_2$)-OMe **11** (⊙); (*b*) Z-Tyr-Ser(SiPr$^i_3$)-OBzl **16** (△); (*c*) Z-Tyr-(SiPr$^i_3$)-Ser(SiPr$^i_3$)-OBzl **14** (×); (*d*) H-Tyr-Ser(SiBu^{t}Ph$_2$)-OH **17** (⊕); (*e*) H-Tyr-Ser(SiPr$^i_3$)-OH **18** (□)

In order to check the stability of the compounds at more 'physiological' pH-values, they were tested at 37 °C in methanolic buffer (pH 7.47 on a pH meter reading). Over a period of 72 h both H-Tyr-Ser(SiBu^{t}Ph$_2$)-OH **17** and H-Tyr-Ser(SiPr$^i_3$)-OH **18** were found to be stable. So, while satisfactory rate profiles were obtained at the lower pH-values the aim of future work will be to pursue silyl derivatives which will have a spectrum of half-lives over a broader pH range.

All the silyl derivatives handled in this work showed improved 'lipophilic character' as perceived by (a) higher R_f-values than their underivatised counterparts [*e.g.*, Bz-Tyr(Bzl)-Ser(SiBu^{t}Ph$_2$)-OBzl R_f 0.75 in CH_2Cl_2–10% MeOH while Bz-Tyr(Bzl)-Ser-OBzl has R_f 0.30 in the same solvent] and (b) longer retention times on a reversed-phase C_{18} HPLC column (*e.g.*, the retention times of compounds **11**–**13**) relative to that for compound **19** as recorded in Table 3.

Experimental

M.p.s were determined on a Gallenkamp capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were determined at 100 MHz with a Varian XL-100 instrument with tetramethylsilane as internal standard (except in the case of certain silylated derivatives where CH_2Cl_2 was used). Mass spectra were determined on a V.G. Analytical ZAB-E instrument, and HPLC was carried out using LDC/Milton Roy constametric III pumps, a spectromonitor D detector, a CI-10B integrator and Sekonic printer. The reversed-phase columns were Spherisorb-C_8 or -C_{18} columns (25 cm × 0.43 cm). GLC was carried out on Varian 6500 instruments with Varian CDS 400 database using Chromasorb G/3% SE UB column material. Reactions were monitored on Merck Kieselgel 60F_{254} precoated plates while compounds were purified where necessary on columns of Merck Kieselgel 60. Extra-dry glassware was used routinely and all solvents were dried and purified before use.

Preparation of Silyl Ethers of Hexan-1-ol and 4-Methylphenol-(p-Cresol).—General method. Alcohol or phenol (30 mmol), the appropriate chlorosilane (36 mmol, 12 mol equiv.), imidazole (75 mmol, 2.5 mol equiv.) and dry acetonitrile (100 cm^3) were stirred overnight under nitrogen. Solvent was removed under reduced pressure and the residue was dissolved in pentane (100 cm^3). Insoluble material was filtered off, and the pentane was washed successively with 5% HCl (3 × 30 cm^3) and water (30 cm^3). After drying over $MgSO_4$ the product was obtained by fractional distillation under reduced pressure. (For some of the cresol derivatives column chromatography on Kieselgel 60 with dichloromethane as eluent was used for purification. Analytical purity was checked by GLC (condi-

tions: A; 100 °C—Isothermal. B; 100 °C, 2 min; then 20 °C/min to 300 °C. C; 100 °C, 3 min; then 30 °C/min to 250 °C. D; 150 °C, 3 min; then 30 °C/min to 250 °C). In the NMR spectra of the hexyl derivatives proton signals for the hexyl group appeared in the same position for all derivatives: $\delta(CDCl_3)$ 3.55 (2 H, t, CH_2O), 1.30 (6 H, m, hexyl) and 0.9 (5 H, m, hexyl). In the cresol series $\delta(CDCl_3)$ for the MeC_6H_4 protons were at 2.22 (3 H, s, Me), 6.7 (2 H, d, ArH) and 6.98 (2 H, d, ArH) for silyl derivatives bearing aliphatic groups. Chemical shifts of the cresol protons were affected by the phenyl groups in p-$MeC_6H_4OSiBu^tPh_2$ and have been recorded in full in the data for this compound.

The following derivatives were thus synthesized. *Hexyloxytrimethylsilane*, b.p. 32 °C (2 mmHg) (70% yield); GLC t_R 1.9 (A); $\delta(CDCl_3)$ 0.05 (9 H, s, $SiMe_3$) [Found: $(M + H)^+$ 175.1520. $C_9H_{23}OSi$ requires (M + 1), 175.1518].

Hexyloxy(isobutyl)dimethylsilane, b.p. 68 °C (2 mmHg) (51% yield); t_R 4.41 (B); $\delta(CDCl_3)$ 1.95 (1 H, sextet, $HCMe_2$, 0.90 (6 H, d, $CHMe_2$), 0.60 (2 H, d, $SiCH_2$) and 0.30 (6 H, s, $SiMe_2$).

tert-*Butyl(hexyloxy)dimethylsilane*, b.p. 68 °C (1.5 mmHg) (83% yield); t_R 4.04 (B); $\delta(CDCl_3)$ 0.85 (9 H, s, Bu^t) and 0.05 (6 H, s, $SiMe_2$) [Found: $(M + H)^+$, 217.1990. $C_{12}H_{29}OSi$ requires (M + 1), 217.1987].

tert-*Butyl(hexyloxy)diphenylsilane*, b.p. 122 °C (0.01 mmHg) (60% yield); t_R 9.69 (B); $\delta(CDCl_3)$ 7.50–7.10 (10 H, m, 2 × SiPh), 6.85 (2 H, d, ArH), 6.63 (2 H, d, ArH) and 0.90 (9 H, s, Bu^t) [Found: $(M + H)^+$, 341.2300. $C_{22}H_{33}OSi$ requires (M + 1), 341.2301].

Hexyloxy(methyl)diphenylsilane, b.p. 116 °C (0.01 mmHg) (45% yield); t_R 9.07 (B); $\delta(CDCl_3)$ 7.90–7.30 (19 H, m, 2 × SiPh) and 0.40 (3 H, s, SiMe) [Found: $(M + H)^+$, 299.1840. $C_{19}H_{27}OSi$ requires (M + 1), 299.1831].

Hexyloxytriisopropylsilane, b.p. 122 °C (1.7 mmHg) (70% yield); t_R 6.72 (B); $\delta(CDCl_3)$ 0.80 (27 H, s, 3 × Pr^i) [Found: $(M + H)^+$, 259.2460. $C_{15}H_{35}OSi$ requires (M + 1), 259.2457].

Triethyl-(4-methylphenoxy)silane, oily gum (75% yield); t_R 7.0 (C); $\delta(CDCl_3)$ 1.15–0.06 (15 H, m, 3 × Et) (Found: M^+, 222.1440. $C_{13}H_{22}OSi$ requires M, 222.1439).

Isobutyl(dimethyl)-(4-methylphenoxy)silane, oily gum (76% yield); t_R 7.1 (C); $\delta(CDCl_3)$ 2.0 (1 H, sextet, $CHMe_2$), 1.05 (6 H, d, $CHMe_2$), 0.80 (2 H, d, $SiCH_2$) and 0.30 (6 H, s, $SiMe_2$).

tert-*Butyl(dimethyl)-(4-methylphenoxy)silane*, b.p. 26 °C (0.03 mmHg) (72% yield); t_R 7.1 (C); $\delta(CDCl_3)$ 0.95 (9 H, s, Bu^t) and 0.20 (6 H, s, $SiMe_2$) (Found: M^+, 222.1440. $C_{13}H_{22}OSi$ requires M, 222.1440).

tert-*Butyl-(4-methylphenoxy)diphenylsilane*, b.p. 88 °C (0.03 mmHg) (34% yield); t_R 6.8 (D); $\delta(CDCl_3)$ 7.85–7.25 (10 H, m, 2 × SiPh), 6.85 (2 H, d, ArH), 6.63 (2 H, d, ArH), 2.16 (3 H, s, ArMe) and 1.05 (9 H, s, Bu^t) (Found: M^+, 346.1750. $C_{23}H_{26}OSi$ requires M, 346.1753).

Triisopropyl-(4-methylphenoxy)silane, b.p. 139 °C (6 mmHg) (65% yield); t_R 8.6 (C); $\delta(CDCl_3)$ 1.10 (27 H, s, 3 × Pri) (Found: M^+, 264.1910. $C_{16}H_{28}OSi$ requires M, 264.1909).

Hydrolytic Studies.—(a) *Silyl ethers of hexan-1-ol.* The substrate (50 mg) was dissolved in either 1% HCl–95% MeOH (0.2 cm^3) for acid hydrolysis or 5% NaOH–95% MeOH for base-catalysed hydrolysis, all solutions being thermostatted at 25 °C. Aliquots from each solution were analysed by GLC using the conditions used for the analysis of the purity of the silyl ethers recorded above.

(b) *Silyl ethers of 4-methylphenol.* The substrate (50 mg) was dissolved in either 1% HCl–95% MeOH (12.5 cm^3) for acid catalysis or 5% NaOH–95% MeOH (12.5 cm^3) for base hydrolysis, both solutions containing benzoic acid (0.015 g) as an internal standard for the HPLC analysis of the extent of reaction. Conditions used for HPLC analysis were reversed-phase (type given earlier), MeOH eluent, elution rate 1 cm^3/min; monitoring at 264 nm.

Hydrolysis results have been summarised in Tables 1 and 2.

N-tert-*Butoxycarbonyl*-O-(tert-*butyldiphenylsilyl*)-L-*serine Benzyl Ester* **2**.—*N-tert*-Butoxycarbonyl-L-serine benzyl ester **1**[6] (0.20 g, 0.712 mmol) and imidazole (0.106 g, 2.5 mol equiv.) were dissolved in dry tetrahydrofuran (THF) (0.4 cm^3) under nitrogen. *tert*-Butyl(chloro)(diphenyl)silane (203 mm^3, 1.1 mol equiv.) was added and the solution was stirred overnight. After filtration and removal of solvent under reduced pressure the product was purified by column chromatography (CH_2Cl_2 as eluent to give *N-tert*-butyloxycarbonyl-*O*-(*tert*-butyldiphenylsilyl)-L-serine benzyl ester **2** (0.29 g, 79%) as a non-crystallisable syrup, R_f 0.39 (CH_2Cl_2); $\delta(CDCl_3)$ 7.8–7.10 (15 H, m, Ph of benzyl ester and 2 × SiPh), 5.70–5.50 (1 H, d, NH), 5.10 (2 H, s, CH_2 of benzyl ester), 4.50–4.30 (1 H, m, α-H), 4.20–3.8 (2 H, dd, β-H), 1.40 (9 H, s, Bu^t of urethane) and 1.00 (9 H s, $SiBu^t$).

N-tert-*Butoxycarbonyl*-O-(tert-*butyldiphenylsilyl*)-L-*serine* **4**.—Benzyl ester **2** (0.171 g, 0.32 mmol) in methanol (10 cm^3) was hydrogenated for 3 h over Pd/C (0.017 g). The catalyst was filtered off and, on removal of solvent under reduced pressure, N-tert-*butoxycarbonyl*-O-(tert-*butyldiphenylsilyl*)-L-*serine* **4** was obtained as a syrup (0.113 g, 90%), which crystallised on trituration with ether to give a solid, m.p. 155–156 °C (Found: C, 64.6; H, 7.8; N, 3.2. $C_{24}H_{33}NO_5$ requires C, 65.0; H, 7.5; N, 3.15%); R_f 0.7 (acetone); $\delta(CDCl_3)$ 10.03 (1 H, br s, CO_2H), 7.80–7.10 (10 H, m, 2 × SiPh), 5.60–5.30 (1 H, m, NH), 4.50–4.20 (1 H, m, α-H), 4.10–3.90 (2 H, m, β-H), 1.45 (9 H, s, Bu^t of urethane) and 1.05 (9 H, s, $SiBu^t$).

N-*Benzoyl*-O-*benzyl*-L-*tyrosyl*-O-(tert-*butyldiphenylsilyl*)-L-*serine Benzyl Ester* **5**.—Ester **2** (0.09 g, 0.173 mmol) was stirred in TFA (0.25 cm^3) at room temperature for 5 min. TLC analysis showed that the Boc group had been completely removed after this time, so the solvent was removed under reduced pressure (0.5 mmHg; 18 °C) and the residual TFA was removed azeotropically with methanol. After storage *in vacuo* overnight the residue was taken up in dry THF (1 cm^3), HOBt (0.023 g, 1 mol equiv.), triethylamine (23 mm^3, 1 mol equiv.) and *N*-benzoyl-*O*-benzyl-L-tyrosine (0.065 g, 0.173 mmol) were added, and the resultant solution cooled to 0 °C. A solution of DCCI (0.046 g, 0.173 mmol in THF (1 cm^3) was added and the reaction mixture was stirred overnight at 0 °C. After filtration the solvent was removed under reduced pressure, to give a residue which was chromatographed on Kieselgel 60 (eluted with CH_2Cl_2 followed by 20% EtOAc–CH_2Cl_2) to yield N-*benzoyl*-O-(tert-*butyldiphenylsilyl*)-L-*serine benzyl ester* **5** (0.075 g, 55%) as a crystalline solid, m.p. 158–160 °C; R_f 0.32 (EtOAc) (Found: C, 74.7; H, 6.25; N, 3.1. $C_{49}H_{50}N_2O_6$ requires C, 74.4; H, 6.4; N, 3.5%); $\delta(CDCl_3)$ 7.85–6.86 (29 H, m, Ph of benzoyl, C_6H_4 of tyrosyl, Ph of benzyl ether, Ph of benzyl ester, and 2 × SiPh), 6.82 (1 H, d, NH amide), 6.68 (1 H, d, NH amide), 5.24 (2 H, d, $PhCH_2$), 5.04 (2 H, s, $PhCH_2$), 4.92–4.65 (2 H, m, 2 × α-CH), 4.04 (2 H, 2 × dd, Ser β-H), 3.18 (2 H, d, Tyr β-H) and 0.99 (9 H, s, $SiBu^t$).

Removal of Silyl Protecting Group by TBAF.[1]—The protocol for monitoring deprotection is typified by: *N*-benzoyl-*O*-benzyl-L-tyrosine-*O*-(*tert*-butylidphenylsilyl)-L-serine methyl ester (5 mg) in THF (R_f 0.75 in CH_2Cl_2–10% MeOH) was treated with a 1 mol dm^{-3} solution of TBAF in THF (0.012 cm^3) and left at room temperature. TLC analysis showed that within 10 min all the starting material had been converted into a new spot corresponding to authentic *N*-benzoyl-*O*-benzyl-L-tyrosine-L-serine methyl ester (R_f 0.30 in CH_2Cl_2–10% MeOH).

N-Benzyloxycarbonyl-O-(tert-butyldiphenylsilyl)-L-serine Methyl Ester **8**.—*N*-Benzyloxycarbonyl-L-serine methyl ester [11] (0.20 g, 0.787 mmol) was silylated with *tert*-butyl(chloro)-diphenylsilane (220 mm^3, 1.1 mol equiv.) with imidazole (0.133 g, 2.5 mol equiv.) as catalyst as for the preparation of compound **2** to give *N*-benzyloxycarbonyl-*O*-(*tert*-butyldiphenylsilyl)-L-serine methyl ester **8** (0.24 g, 61%) as a non-crystallisable syrup, R_f 0.32 (CH_2Cl_2); δ($CDCl_3$) 7.75–7.20 (15 H, m, Ph of urethane and 2 × SiPh), 5.70 (1 H, d, NH), 5.10 (2 H, s, CH_2 of urethane), 4.50–4.40 (1 H, m, α-CH), 4.20–3.8 (2 H, dd, β-Hs), 3.70 (3 H, s, Me) and 1.05 (9 H, s, Bu^t).

N-Benzyloxycarbonyl-O-(tert-butyldiphenylsilyl)-L-serine **9**.—Ester **8** (0.50 g, 1.01 mmol), methanol (50 cm^3) and 1 mol dm^{-3} NaOH (2 cm^3) were stirred together at room temperature for 6 h, when conversion of starting material was complete. The reaction mixture was neutralised with IR-120(H^+) resin and the solvent removed under reduced pressure to give, after column chromatography (gradient CH_2Cl_2–Me_2CO), *N*-benzyloxycarbonyl-*O*-(*tert*-butyldiphenylsilyl)-L-serine **9** (0.167 g, 35%) as crystals, m.p. 146–8 °C; R_f 0.6 (acetone) (Found: C, 66.8; H, 6.2; N, 2.8. $C_{27}H_{31}NO_5Si$ requires C, 67.9; H, 6.5; N, 2.9%); δ($CDCl_3$) 7.65–7.15 (15 H, m, Ph of urethane and 2 × SiPh), 5.65 (1 H, m, NH), 5.00 (2 H, d, CH_2 of urethane), 4.45–4.15 (1 H, m, α-CH), 4.20–3.8 (2 H, m, β-CHs) and 0.95 (9 H, s, Bu^t).

O-(tert-*Butyldiphenylsilyl*)-L-*serine Methyl Ester* **10**.—Ester **8** (0.50 g, 1.02 mmol) in methanol (10 cm^3) was hydrogenated overnight by using 10% Pd/C (0.05 g) as catalyst. TLC showed the reaction to be complete, so the catalyst was filtered off and the solvent was removed under reduced pressure. The crude product was purified on an ion-exchange column (IR-120, H^+-form), the product being eluted by 0.7 mol dm^{-3} NH_4OH to give O-(*tert*-butyldiphenylsilyl)-L-serine methyl ester **10** (0.23 g, 65%).

General Method for Synthesis of Dipeptides listed in Table 3.—The amino-protected amino acid (1 mol), DCCI (1.1 mol) and HOBt (2 mol) were stirred in dry dimethylformamide (DMF) at 0 °C. The carboxyprotected amino acid (1 mol) and triethylamine (4 mol) were added and the mixture was stirred at 0 °C for 4 h, and then refrigerated overnight. After the removal of the solvents the residue was dissolved in ethyl acetate and placed in the refrigerator for 1 h, and was then filtered to remove dicyclohexylurea. The ethyl acetate solution was washed successively with saturated aq. sodium hydrogen carbonate (2 ×), water (2 ×), 10% aq. citric acid (2 ×) and finally with water. The combined ethyl acetate extracts were washed with brine and dried over magnesium sulfate. The solvent was removed and the product was purified by column chromatography (elution gradient—chloroform to 5% methanol–chloroform).

The dipeptides prepared were: Boc-Tyr-Ser-OMe **19**: m.p. 65–66 °C (lit.,[12] 64–66 °C); δ($CDCl_3$) 7.01–6.69 (4 H, dd, C_6H_4 of tyrosyl), 5.50 (1 H, d, α-H not assigned), 4.61 (1 H, d, α-H, not assigned), 4.39–3.80 (2 H, m, β-H, seryl), 3.05–2.92 (2 H, m, β-H, tyrosyl) and 1.37 (9 H, s, OBu^t).

Boc-Tyr-Ser(Bzl)-OMe: m.p. 48–50 °C; FAB-MS [(M + H^+), 473.2288. $C_{25}H_{33}N_2O_7$ requires *m/z*, 473.228]; δ($CDCl_3$) 7.81–7.18 (5 H, m, Ph of benzyl ether), 7.07–6.66 (4 H, dd, C_6H_4 of tyrosyl), 5.21–5.04 (1 H, m, NH not assigned), 4.78–4.22 (2 H, m, α-tyrosyl and α-H seryl), 4.47 (3 H, s, ester Me), 3.94–3.56 (2 H, m, β-H seryl), 3.02–2.88 (2 H, m, β-H tyrosyl) and 1.42 (9 H, s, Bu^t).

Boc-Tyr($SiBu^tPh_2$)*-Ser-OMe* **12**: m.p. 53–55 °C· FAB-MS [(M + H^+), 621.2996. $C_{34}H_{44}N_2O_7Si$ requires *m/z*, 621.2996]; δ($CDCl_3$) 7.73–7.33 (10 H, m, 2 × SiPh), 6.93–6.67 (4 H, dd, C_6H_4 of tyrosyl), 5.05 (1 H, d, α-H not assigned), 4.57–4.55 (1 H, m, α-H not assigned), 4.24–3.87 (2 H, m, β-H seryl), 3.72 (3 H, s, ester Me), 2.95–2.89 (2 H, m, β-H, tyrosyl), 1.37 (9 H, s, OBu^t) and 1.08 (9 H, s, $SiBu^t$).

Z-Tyr-Ser-OBzl: m.p. 170–171 °C; FAB-MS [(M + H^+), 493.1975. $C_{27}H_{29}N_2O_7$ requires *m/z*, 493.1975]; δ([$^2H_4$]-MeOH) 7.38–7.21 (10 H, m, 2 × Ph of benzyl ester), 7.05–6.66 (4 H, dd, C_6H_4 of tyrosyl), 5.16 (2 H, s, CH_2 of benzyl ester), 4.89 (2 H, s, CH_2 of benzyl ester), 4.58–4.37 (2 H, m, α-H tyrosyl and α-H, seryl), 3.95–3.78 (2 H, m, β-H seryl) and 3.06–2.67 (2 H, m, β-H, tyrosyl).

Z-Tyr(Bzl)-Ser-OBzl: m.p. 160–161 °C; FAB-MS [(M + H^+), 583.244. $C_{34}H_{35}N_2O_7$ requires *m/z*, 583.2444]; δ($CDCl_3$) 7.39–7.19 (15 H, m, 3 × Ph of benzyl), 7.09–6.79 (4 H, dd, C_6H_4 of tyrosyl), 5.71 (1 H, d, α-H not assigned), 5.14–4.89 (6 H, m, 2 × CH_2 of benzyl ester and 1 × CH_2 of benzyl ether), 4.67–3.87 (2 H, m, β-H, seryl) and 3.89–2.85 (2 H, m, β-H, tyrosyl).

Boc-Tyr($SiBu^tPh_2$)*-Ser(Bzl)-OMe:* an oil, FAB-MS [(M + H^+), 711.3465. $C_{41}H_{51}N_2O_7Si$ requires *m/z*, 711.3466]; δ($CDCl_3$) 7.71–7.19 (15 H, m, 2 × SiPh and 1 × Ph of benzyl ether), 6.94–6.66 (4 H, dd, C_6H_4 of tyrosyl), 4.94–4.85 (1 H, m, α-H not assigned), 4.45 (2 H, s, CH_2 of benzyl ether), 4.31–4.22 (1 H, m, α-H not assigned), 3.85–3.57 (2 H, m, β-H seryl), 2.95–2.88 (2 H, m, β-H of tyrosyl), 1.38 (9 H, s, OBu^t) and 1.08 (9 H, s, $SiBu^t$).

Synthesis of Silylated Derivatives (*Table* 3).—Methodology based on conditions described in the preparation of compound **2**: protected amino acid or peptide (1 mol equiv.), together with dry imidazole (2.5 mol equiv.) were dissolved in dry THF or DMF under nitrogen. To this solution was added *tert*-butyl-(chloro)diphenylsilane (1.1 mol equiv.) or chloro(triisopropyl)silane (1.1 mol equiv.) and the solution was stirred under nitrogen overnight. The white precipitate was then filtered off and, after removal of the solvent, the product was purified by column chromatography (chloroform ⟶ 2% methanol–chloroform as eluent).

The following silylated derivatives were prepared: *Boc-Tyr*($SiPh_2Bu^t$)*-Ser*($SiPh_2Bu^t$)*-OMe* **11**: m.p. 51–52 °C (Found: C, 69.8; H, 7.3; N, 3.0. $C_{50}H_{62}N_2O_7Si_2$ requires C, 69.89; H, 7.27; N, 3.26%); δ($CDCl_3$) 7.72–7.32 (20 H, m, 4 × SiPh), 6.9–6.66 (4 H, dd, C_6H_4 of tyrosyl), 4.88–4.78 (1 H, m, α-H not assigned), 4.62–4.59 (1 H, m, α-H not assigned), 4.10–3.79 (2 H, m, β-H seryl), 3.04–2.78 (2 H, m, β-H tyrosyl), 1.38 (9 H, s, OBu^t), 1.09 (9 H, s, $SiBu^t$ on tyrosyl) and 1.01 (9 H, s, $SiBu^t$ on seryl).

NH_2-Ser($SiPr^i_3$)*-OBzl:* an oil; FAB-MS [(M + H^+), 352.237. $C_{19}H_{34}NO_3Si$ requires *m/z*, 352.2374]; δ($CDCl_3$) 7.35 (5 H, s, Ph of benzyl ester), 5.24–5.08 (2 H, m, CH_2 of benzyl ester), 4.25–3.91 (2 H, m, β-H, seryl), 1.08–0.93 (21 H, m, $SiPr^i$).

Z-Tyr(Bzl)-Ser($SiPr^i_3$)*-OBzl:* an oil; FAB-MS [(M + H^+), 739.3778. $C_{43}H_{55}N_2O_7Si$ requires *m/z*, 739.3778]; δ($CDCl_3$) 7.44–7.28 (15 H, m, 3 × Ph of benzyl), 7.11–6.83 (4 H, dd, C_6H_4 of tyrosyl), 5.38–4.43 (8 H, m, 2 × CH_2 of benzyl ester, 1 × CH_2 of benzyl ether, 2 × α-H), 4.19–3.74 (β-CH_2 tyrosyl) and 1.09–0.85 (21 H, m, $SiPr^i$).

Z-Tyr($SiPr^i_3$)*-Ser*($SiPr^i_3$)*-OBzl* **14**: an oil; FAB-MS [(M × H^+), 805.464. $C_{45}H_{69}N_2O_7Si_2$ requires *m/z*, 805.4643]; δ([$^2H_4$]MeOH) 7.33–7.26 (10 H, m, 2 × Ph of benzyl ester), 7.03–6.76 (4 H, dd, C_6H_4 of tyrosyl), 5.14 (2 H, s, CH_2 of benzyl ester), 5.04 (2 H, s, CH_2 of benzyl ester), 4.67 (1 H, m, α-H not assigned), 4.44 (1 H, m, α-H not assigned), 4.19–3.85 (2 H, dd, β-H, seryl), 3.10–3.02 (2 H, dd, β-H, tyrosyl) and 1.17–0.92 (42 H, m, 6 × $SiPr^i$).

Boc-Tyr-Ser($SiBu^tPh_2$)*-OMe* **13**: m.p. 58–60 °C; FAB-MS [(M + H^+), 621.2996. $C_{34}H_{45}N_2O_7Si$ requires *m/z*, 621.2996]; δ($CDCl_3$) 7.61–7.34 (10 H, m, 2 × SiPh), 7.01–6.69 (4 H, dd, C_6H_4 of tyrosyl), 6.89 (1 H, d, NH not assigned) 5.12–5.06 (1 H, br s, α-H not assigned), 4.65–4.62 (1 H, m, α-H not assigned), 4.12–3.82 (2 H, m, β-H, seryl), 3.71 (3 H, s, ester Me), 3.02–2.99

(2 H, m, β-H, tyrosyl), 1.41 (9 H, s, OBu^t) and 1.02 (9 H, s, SiBu^t).

Z-Tyr-Ser(SiBu^{t}Ph$_2$)-OBzl **15**: m.p. 56–57 °C; FAB-MS [(M + H$^+$), 731.315. $C_{43}H_{47}N_2O_7Si$ requires *m/z*, 731.3153]; δ($CDCl_3$) 7.55–7.27 (20 H, m, 2 × SiPh, 2 × Ph of benzyl esters), 6.98–6.60 (4 H, dd, C_6H_4 of tyrosyl), 5.34–5.31 (1 H, m, α-H not assigned), 5.16–5.05 (4 H, m, 2 × CH_2 of benzyl esters), 4.67–4.63 (1 H, m, α-H not assigned), 4.15–3.81 (2 H, m, β-H, seryl), 3.06–2.81 (2 H, m, β-H, tyrosyl) and 0.97 (9 H, s, SiBu^t).

Z-Tyr-Ser(SiPr$^i_3$)-OBzl **16**: an oil; δ($CDCl_3$) 7.35–7.25 (10 H, m, 2 × Ph of benzyl esters), 6.99–6.65 (4 H, dd, C_6H_4 of tyrosyl), 5.21–5.05 (4 H, m, 2 × CH_2 of benzyl esters), 4.18–3.84 (2 H, m, β-H, seryl), 3.02–2.88 (2 H, m, β-H, tyrosyl) and 1.25–0.91 (21 H, m, SiPr^i).

General Method for Deprotection of Peptide Derivatives.—The protected dipeptides were hydrogenated with 10% Pd/-charcoal in methanol or ethanol at room temperature for periods ranging from several hours to 3 days. After filtration, followed by removal of solvent, the product was isolated in crude form by trituration with ether.

The following deprotected derivatives were prepared: *H-Tyr-Ser(SiBu^{t}Ph$_2$)-OH* **17**: m.p. 117–120 °C; FAB-MS [(M + H$^+$), 507.2315. $C_{28}H_{35}N_2O_5Si$ requires *m/z*, 507.2315]; δ([$^2H_4$]-MeOH) 7.30–7.33 (10 H, m, 2 × SiPh), 7.14–6.70 (4 H, dd, C_6H_4 of tyrosyl), 4.44 (1 H, t, α-H not assigned), 4.14–3.92 (2 H, m, β-H, seryl), 3.20–2.86 (2 H, m, β-H, tyrosyl) and 1.04 (9 H, s, SiBu^t).

H-Tyr-Ser(SiPr$^i_3$)-OH **18**: m.p. 120–121 °C; FAB-MS [(M + H$^+$), 425.2472. $C_{21}H_{37}N_2O_5Si$ requires *m/z*, 425.2472]; δ-([$^2H_4$]MeOH) 7.16–6.72 (4 H, dd, C_6H_4 of tyrosyl), 4.39–4.36 (1 H, m, α-H not assigned), 4.17–3.99 (2 H, m, β-H, seryl), 3.31–2.87 (2 H, m, β-H tyrosyl) and 1.20–1.01 (21 H, m, SiPr^i).

Acknowledgements

We very much appreciate sponsorship from the SERC and Dow Corning (E. J. T.) and SmithKline Beecham (C. L. H.). The technical assistance of Ian Matthews is gratefully acknowledged.

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Paper 2/03862E
Received 20*th July* 1992
Accepted 21*st July* 1992