oxyshahamin C (8, 1.9 mg, 0.007% dry weight).

Dendrillolide A (4): clear oil; $[\alpha]_D$ +87.0° (*c* 0.31, $CHCl_3$); IR ($CHCl_3$) 1800, 1750, 1215, 990 cm^{-1}; ^1H NMR, see Table I; ^{13}C NMR, see Table I; HRMS (EI) *m/z* 376.2246 (M^+), $C_{22}H_{32}O_5$ requires 376.2250.

Dendrillolide D (5): crystals from diethyl ether/hexanes; mp 120 °C; $[\alpha]_D$ +35.8° (*c* 0.35, $CHCl_3$); IR ($CHCl_3$) 1730, 1370, 1230 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (s, 3 H), 0.88 (s, 3 H), 0.96 (s, 3 H), 1.01 (s, 3 H), 1.20 (m, 1 H), 1.28 (m, 1 H), 1.42 (br d, 1 H, *J* = 13.3 Hz), 1.48 (m, 1 H), 1.60 (dd, 2 H, *J* = 13.5, 2.1 Hz), 1.88 (ddd, *J* = 11.2, 5.7 4.4 Hz), 1.93 (dt, 1 H, *J* = 13.3, 2.8 Hz), 2.03 (br d, 1 H, *J* = 11.4 Hz), 2.05 (s, 3 H), 2.54 (ddd, 1 H, *J* = 11.2, 4.4, 3.7 Hz), 2.82 (dd, 1 H, *J* = 17.8, 3.7 Hz), 3.24 (dd, 1 H, *J* = 17.8, 4.4 Hz), 3.71 (s, 3 H), 3.83 (s, 1 H), 3.84 (dd, 1 H, *J* = 11.8, 5.7 Hz), 4.28 (dd, 1 H, *J* = 11.8, 4.4 Hz); ^{13}C NMR ($CDCl_3$) δ 173.2 (s), 172.2 (s), 170.6 (s), 92.7 (d), 63.3 (t), 53.0 (d), 52.0 (q), 48.9 (d), 41.7 (t), 39.4 (d), 38.8 (s), 38.4 (t), 37.1 (t), 37.1 (s), 34.7 (t), 33.1 (q), 32.9 (s), 21.4 (q), 21.0 (q), 17.8 (t), 17.7 (t), 14.6 (q), 14.0 (q); HRMS (EI) *m/z* 408.2519 (M^+), $C_{23}H_{36}O_6$ requires 408.2512.

Dendrillolide E (6): clear oil; $[\alpha]_D$ +21.4° (*c* 0.25, $CHCl_3$); IR ($CHCl_3$) 1795, 1750, 1215, 985 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.24 (m, 1 H), 0.44 (m, 1 H), 0.47 (br s, 1 H), 0.82 (s, 3 H), 0.92 (s, 3 H), 0.94 (s, 3 H), 2.07 (s, 3 H), 2.54 (dd, 1 H, *J* = 17.9, 8.9 Hz), 2.57 (dd, 1 H, *J* = 5.5, 2.7 Hz), 3.05 (dd, 1 H, *J* = 17.9, 8.9 Hz), 3.20 (m, 1 H, *J* = 8.9, 8.9, 4.8, 2.7 Hz), 6.06 (d, 1 H, *J* = 4.8 Hz), 6.46 (d, 1 H, *J* = 5.5 Hz); ^{13}C NMR ($CDCl_3$) δ 175.5 (s), 169.8 (s), 105.8 (d), 97.7 (d), 56.9 (d), 47.3 (d), 42.2 (t), 41.2 (d), 38.3 (t), 36.5 (t), 34.5 (s), 32.6 (s), 30.5 (q), 28.3 (t), 28.0 (q), 24.7 (d), 23.1 (s), 21.4 (t), 21.2 (q), 20.3 (q), 17.5 (t), 13.2 (t); HRMS (EI) *m/z* 376.2271 (M^+), $C_{22}H_{32}O_5$ requires 376.2250.

12-Desacetoxypolyrhaphin A (7): clear oil; $[\alpha]_D$ +14.3° (*c* 0.23, $CHCl_3$); IR ($CHCl_3$) 1775, 1740, 1215 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.80 (s, 3 H), 0.92 (s, 3 H), 0.96 (s, 3 H), 1.85 (m, 1 H), 2.04 (s, 3 H), 2.34 (m, 1 H), 2.43 (d, 2 H, *J* = 9.7 Hz), 2.56 (d, 1 H, *J* = 8.3 Hz), 3.07 (m, 1 H, *J* = 9.7, 8.8, 8.8, 3.7 Hz), 3.99 (dd, 1 H, *J* = 11.8, 9.1 Hz), 4.10 (t, 1 H, *J* = 8.8 Hz), 4.38 (dd, 1 H, *J* = 11.8, 4.0 Hz), 4.43 (t, 1 H, *J* = 8.8 Hz), 4.61 (d, 1 H, *J* = 1.7 Hz), 4.84 (d, 1 H, *J* = 1.7 Hz); ^{13}C NMR, see Table II; HRMS (EI) *m/z* 362.2447 (M^+), $C_{22}H_{34}O_4$ requires 362.2457.

12-Desacetoxyshahamin C (8): clear oil; $[\alpha]_D$ +54.0° (*c* 0.44, $CHCl_3$); IR ($CHCl_3$) 1745, 1230 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.92 (s, 3 H), 0.95 (s, 3 H), 1.00 (s, 3 H), 1.78 (m, 1 H), 1.82 (m, 1 H), 1.90 (m, 1 H), 2.09 (s, 3 H), 2.36 (m, 1 H), 2.48 (m, 1 H), 2.55 (m, 2 H), 2.73 (d, 1 H, *J* = 8.7 Hz), 3.84 (dd, 1 H, *J* = 11.2, 7.8 Hz), 4.19 (dd, 1 H, *J* = 11.2, 4.3 Hz), 4.21 (dd, 1 H, *J* = 11.8, 10.0 Hz), 4.32 (dd, 1 H, *J* = 11.8, 6.1 Hz), 4.63 (d, 1 H, *J* = 2.0 Hz), 4.86 (d, 1 H, *J* = 2.0 Hz); ^{13}C NMR, see Table II; HRMS (EI) *m/z* 362.2456 (M^+), $C_{22}H_{34}O_4$ requires 362.2457.

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Synthesis of an N^1-Phosphotryptophan-Containing Tripeptide: Glutamyl-N^1-phosphotryptophylleucine[1]

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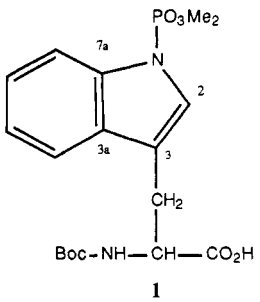
The novel protected amino acid N^α-(*tert*-butyloxycarbonyl)-N^1-(dimethylphosphono)tryptophan, or Boc-Trp(Dmop)-OH, was used in the solution-phase synthesis of the tripeptide Z-Glu(OBzl)-Trp(Dmop)-Leu-OBzl. The N^1-phosphotryptophan-containing peptide H-Glu-Trp(PO_3H_2)-Leu-OH was obtained by the selective deprotection of the protected tripeptide with CF_3SO_3H/CF_3CO_2H/*m*-cresol/DMS or thioanisole. The dimethylphosphate group was also evaluated as a new protecting group for the tryptophan indole moiety and was found to effectively suppress *tert*-butylation of the indole ring during acidolytic removal of the Boc group and to be stable to "high" HF conditions, palladium-catalyzed hydrogenation, and CF_3SO_3H/CF_3CO_2H/*m*-cresol treatment. The Dmop group was cleaved by 1 M NaOH, and the N^1-(methylphosphono)tryptophan peptide, H-Glu-Trp($MeOPO_2H$)-Leu-OH, was obtained by mild base (e.g., piperidine) treatment of H-Glu-Trp-(Dmop)-Leu-OH.

We recently reported[2] the synthesis of the N^1-(dimethylphosphono)tryptophan derivative, Boc-Trp-(Dmop)-OH (1), which can be deprotected selectively to give the novel amino acid N^1-phosphotryptophan, *P*Trp (2), or fully to yield the parent amino acid. Modern peptide synthesis methods permit the efficient incorporation of tryptophan into peptides without difficulties. However, decomposition of tryptophan frequently occurs during the acidolytic removal of side-chain protection and cleavage from the resin in solid-phase peptide synthesis of tryptophan-containing peptides. Relatively little research has been concerned with the development of indole protecting groups[3,4] to overcome the problems of oxidative degrada-



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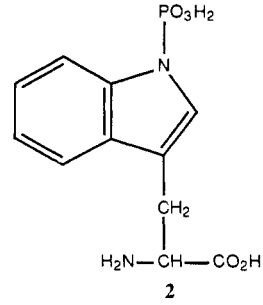

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(1) The abbreviations for natural amino acids and nomenclature for peptide structures follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature. Other abbreviations used: Dmop = dimethylphosphono, TFA = trifluoroacetic acid, TFMSA = trifluoromethanesulfonic acid, Z = benzyloxycarbonyl, Boc = *tert*-butyloxycarbonyl, DMS = dimethyl sulfide, Bzl = benzyl, EDT = ethane-1,2-dithiol, THF = tetrahydrofuran.

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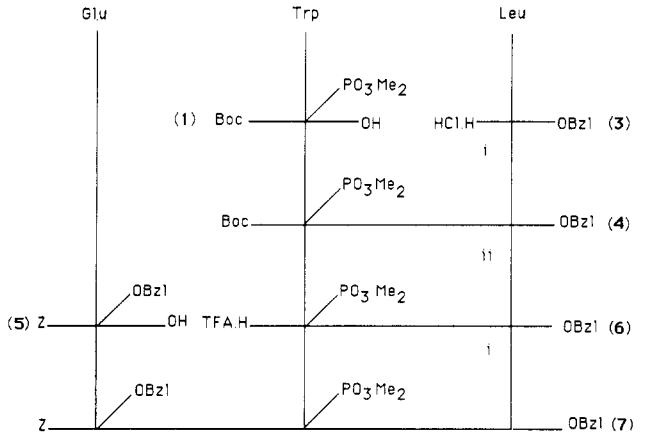

Figure 1. Synthesis of Z-Glu(OBzl)-Trp(Dmop)-Leu-OBzl: (i) NMM, IBCF (−20 °C, 2 h); (ii) 40% TFA/CH_2Cl_2.

tion[5] and alkylation[6] of the indole nucleus at low pH. Although N^1-(mesitylene-2-sulfonyl)tryptophan, Trp(Mts), is commercially available, N^1-formyltryptophan, Trp(CHO), is still the most widely used protected tryptophan derivative employed in peptide synthesis. The appropriate choice of scavengers, such as 1,2-ethanedithiol,[7] DMS,[8] or thioanisole,[9] can suppress oxidation and alkylation in the synthesis of smaller tryptophan-containing peptides, but repeated TFA/scavenger treatment of larger peptides, especially those containing carboxy-terminal tryptophan, may cause degradation and loss of indole protection. An indole protecting group resistant to TFA treatment that is easily removed under mild conditions would be useful in the synthesis of more complicated sequences involving tryptophan. Tryptophan is highly susceptible to electrophilic attack by cations formed during the acidic deprotection step in peptide synthesis[6] and also undergoes oxidation[5] and dimerization[10] at low pH. These side reactions can be minimized by the introduction of an electron-withdrawing substituent at the indole nitrogen atom. In this report, we describe the application of Boc-Trp(Dmop)-OH in the solution-phase synthesis of glutamyl-N^1-phosphotryptophylleucine, Glu-*P*Trp-Leu, and examine several deprotection strategies compatible with its use.

To investigate the methodology of synthesis of N^1-phosphotryptophan peptides, the model tripeptide sequence Z-Glu(OBzl)-Trp(Dmop)-Leu-OBzl was synthesized according to the scheme illustrated in Figure 1. The protected amino acid 1 was coupled to leucine benzyl ester in 97% yield by using the mixed anhydride coupling procedure to give Boc-Trp(Dmop)-Leu-OBzl (4). Deprotection of the amino terminus of the dipeptide ester with 40% TFA/CH_2Cl_2 followed by coupling of the dipeptide ester to the isobutoxycarbonyl mixed anhydride of Z-Glu(OBzl)-OH (5) gave Z-Glu(OBzl)-Trp(Dmop)-Leu-OBzl (7), in 97% overall yield. The peptide had no contaminants as judged by ^{13}C and ^{31}P NMR spectroscopy and was freely soluble in organic solvents such as chloroform, methanol, and diethyl ether. The presence of the dimethylphosphono group was confirmed by ^{13}C and ^{31}P NMR spectroscopy, the ^{13}C NMR spectrum exhibiting characteristic phosphorus-coupled doublet signals for carbons C2 (J_{CP} = 6.1 Hz), C3 (J_{CP} = 9.8 Hz), C7a (J_{CP} = 9.8 Hz), and C3a (J_{CP} = 4.9 Hz) of the indole system.

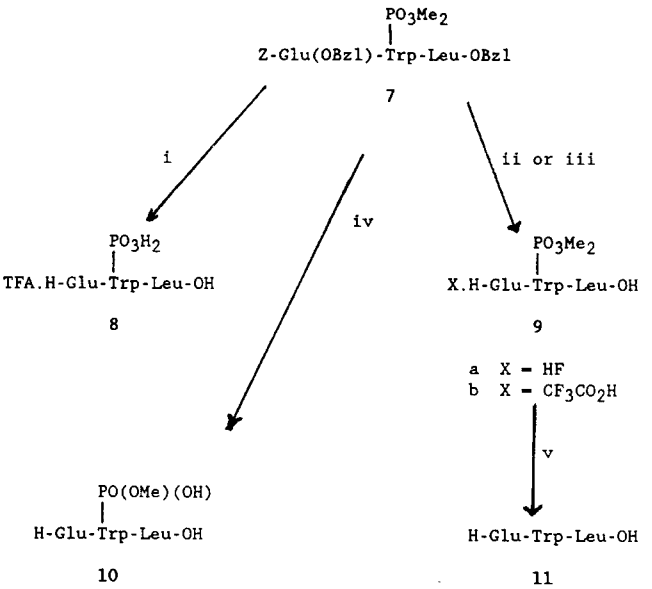

Figure 2. Reactions of Z-Glu(OBzl)-Trp(Dmop)-Leu-OBzl: (i) TFMSA/TFA/*m*-cresol/DMS (1 h, 25 °C); (ii) HF, *o*-cresol (1 h, 0 °C); (iii) H_2, 10% Pd/C, 10% MeOH/H_2O + 1 equiv of TFA; (iv) H_2, 10% Pd/C, acetic acid; (v) 1 M NaOH (1 h, 25 °C).

The phosphopeptide H-Glu-Trp(PO_3H_2)-Leu-OH (8) was then obtained in a single step by deprotection of **7** with TFMSA/TFA/*m*-cresol/DMS (10:50:30:10, "low" TFMSA;[11] Figure 2) followed by precipitation with diethyl ether. The deprotection, monitored by ^{31}P NMR spectroscopy, showed the complete disappearance within 15 min of the signal corresponding to the starting material at −1.8 ppm as the monomethylphosphono derivative (−2.5 ppm) and subsequently the dihydrogenphosphono derivative (−2.9 ppm) rapidly formed. After 45 min, only the signal corresponding to the N^1-(dihydrogenphosphono)-tryptophan peptide was observed. The reaction yield was judged to be quantitative after 2 h by ^{13}C NMR of the crude product, which contained no signals corresponding to the methyl phosphate esters or benzylic protecting groups. The ^{13}C NMR spectrum of peptide **8** displayed phosphorus-coupled doublets for carbons C2 (J_{CP} = 7.3 Hz), C3 (J_{CP} = 7.3 Hz), C7a (J_{CP} = 8.8 Hz), and C3a (J_{CP} = 4.4 Hz) of the indole moiety and an upfield chemical shift consistent with the inductive effect due to the presence of phosphorus (Table I). The phosphorus-induced shift of a signal to a higher field is also pronounced for the proton attached to C2 in the ^1H NMR of the phosphorylated peptide. The product **8** was purified by C_8 rp-HPLC, yielding a stable phosphotripeptide. Optimum yields are obtained by immediate purification, since a stability study showed that prolonged contact of the crude peptide with residual TFMSA and the salt produced on deprotection extensively degraded the peptide. The sulfonium salts produced in the deprotection reaction can act as methylating and benzylating agents,[12] even at low

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Table I. ^{13}C NMR Chemical Shifts of Tripeptides 8–11

	peptide						
	8[a]		9[a]		10[a]		11[b]
	δ[c]	J_{CP}[d]	δ	J_{CP}	δ	J_{CP}	δ
Cδ Leu	21.50		21.44		21.54		21.85
	22.97		22.95		22.96		23.42
Cγ Leu	25.20		25.09		25.19		25.90
Cβ Glu	26.84		26.81		26.81		28.00
Cβ Trp	27.63		27.29		27.69		28.82
Cγ Glu	29.98		29.76		29.79		30.80
Cβ Leu	40.63		40.80		40.63		41.82
Cα Leu	52.35		51.96		52.02		52.20
Cα Glu	52.91		52.95		52.94		53.60
Cα Trp	54.82		55.11		54.89		55.55
$P(OCH_3)$			55.80	2.9	53.60	5.9	
C7	112.70	7.3	116.72	8.8	113.33	7.4	110.65
C3	114.81		114.24		114.70		112.30
C4	119.35		120.18		119.48		119.32
C5	121.62		123.67		121.99		119.89
C6	123.73		125.44		124.02		122.46
C2	128.87	7.3	127.67	7.3	129.11	5.9	124.76
C7a	130.69	8.8	131.40	10.2	130.86	8.8	128.61
C3a	138.20	4.4	137.60	4.5	137.20	4.4	138.06
C=O	169.54, 173.17, 176.84, 177.04		169.58, 172.62, 176.09, 176.87		169.47, 173.00, 176.24, 176.71		169.73, 173.60, 176.15, 176.53

[a] Sample dissolved in D_2O/CD_3CN. [b] Sample dissolved in d_4-methanol. [c] Values in ppm. [d] Values in hertz.

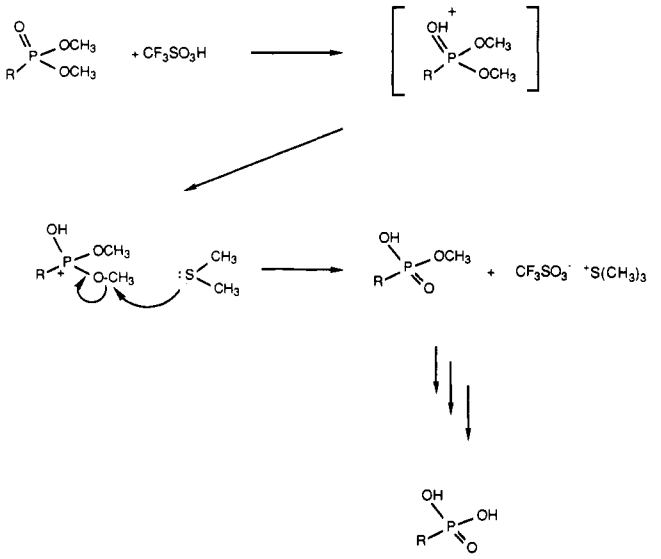

Figure 3. Thioether-mediated deprotection pathway.

temperatures (−15 °C). For example, a sample of crude peptide 8 and salt gave a mixture of products that was resolved into four major peaks in C_8 rp-HPLC after 1 month at −15 °C, none of which were characterized.

The presence of a thioether in the deprotection step mediates demethylation of the phosphonium ion intermediate by an S_N2 mechanism[11,13] (Figure 3), and thioanisole can be used in place of DMS. Monitoring the reaction of peptide 7 with TFMSA/TFA/*m*-cresol/thioanisole by ^{31}P NMR spectroscopy initially revealed only one peak at +1.1 ppm, which moved slowly upfield to +0.3 ppm over 1 h. After the first 30 min, an additional low-intensity signal appeared at +2.3 ppm, which did not correspond to the required product (as determined using TFMSA/TFA/*m*-cresol/DMS as a reaction mixture), and led to a reduced yield of peptide 8 when purified batchwise by HPLC. This result may be due to partial cleavage of the phosphate group and indicates the need for caution when using TFMSA/TFA/*m*-cresol/thioanisole for cleavage times exceeding the 30 min recommended for *tert*-butyl- and benzyl-type protecting groups.[14] In the absence of thioethers, the Dmop group is stable to strong acid: stability studies using Z-Trp(Dmop)-OBzl as a model substrate indicated that H-Trp(Dmop)-OH was recovered by treatment with TFMSA/TFA/*m*-cresol (10:50:10) for 72 h at room temperature[2] or with TFMSA/TFA/EDT (10:88:2) for 3.5 h. Thus, Trp(CHO) can be used and selectively deprotected with TFMSA/TFA/EDT in the synthesis of peptides containing multiple tryptophan residues in which N^1-phosphotryptophan substitution is required only in selected sites.

On "high" HF[15] (HF, *o*-cresol, 10:1) treatment of 7 in the absence of thioether scavengers, C_8 rp-HPLC gave a major peak, comprising 95% of the crude product, which was identified as H-Glu-Trp(Dmop)-Leu-OH (9) by ^{13}C and ^{31}P NMR spectroscopy. In contrast, treatment of 7 under high HF conditions using DMS as a scavenger (HF/*m*-cresol/DMS 80:10:10) yielded several products. The main HPLC fraction proved to be 9 by HPLC comparison with an authentic sample of 9 (prepared by treatment of 7 with TFMSA/TFA/*m*-cresol/DMS), by its characteristic ^{31}P NMR shift, and by peptide sequencing. This result is similar to that reported previously for the "low" HF[16] treatment (HF/*m*-cresol/DMS 25:10:65 v/w) of Z-Trp-(Dmop)-OBzl,[2] from which Trp(Dmop), Trp($MeOPO_2H$), Trp(PO_3H_2) (2), and tryptophan were produced. Presumably cleavage of the alkyl groups from the dimethyl phosphate ester and of the dealkylated phosphate group from the indole ring proceeds concurrently but at differing rates in the presence of both HF and DMS.

Trp(Dmop)-containing peptides are amenable to sequence analysis. The novel phenylthiohydantoin (PTH)

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of Trp(Dmop), obtained with a standard sample of H-Trp(Dmop)-OH·TFA, emerges on an Applied Biosystems 470A-120A PTH analyzer between glycine and glutamic acid on C_{18} rp-HPLC. PTH sequencing of peptide **9** gave Glu, Trp(Dmop), and Leu successively. The PTH of N^1-(methylphosphono)tryptophan, Trp($MeOPO_2H$), elutes between threonine and glycine and was observed in the second sequencing cycle of peptide **10**. N^1-Phosphotryptophan (**2**) gave a single peak corresponding to tryptophan owing to phosphate cleavage during the gas-phase TFA treatment of the phenylthiocarbamyl derivative. Sequencing of peptide **8**, however, produced two peaks for the second residue corresponding to Trp and presumably *P*Trp (broad distorted peak), eluting between glutamine and threonine, in a 1:1 ratio. In addition to sequence analysis, the presence of N^1-phosphotryptophan in peptides should be detectable by amino acid analysis[2] after enzymatic hydrolysis.

Fast atom bombardment mass spectrometry (FABMS), in which fragmentation ions consistent with N^1-substitution by the phosphate moiety were observed, can also be used to detect the presence of N^1-phosphotryptophan in peptides. The spectra of tripeptides **8–10** contained high-intensity [M^+] molecular ions at m/z 527, 555, and 541, respectively, and showed successive amido cleavage from the N-terminus as the major fragmentation mode, for example, loss of the glutamyl residue (129 mass units). Notably, peptide **8** displayed an additional peak at [M^+ − 80] due to loss of the phosphate moiety as PO_3H, this being indicative of the fragmentation of the phosphate group of N^1-phosphotryptophan.

A displacement of the absorption maximum to lower wavelength (of 13–23 nm) in the UV spectrum of the phosphorylated peptide salts **8** (λ_{max} 267 nm, ϵ 5.40 × 10^3), **9** (λ_{max} 257 nm, ϵ 5.33 × 10^3), and **10** (λ_{max} 260 nm, ϵ 5.71 × 10^3) is consistent with N^1-substitution and has been observed in N^1-alkyltryptophans.[18] Synthetic H-Glu-Trp-Leu-OH exhibits the characteristic strong UV absorption spectrum of tryptophan, with a maximum at 280 nm (ϵ 3.56 × 10^3) and a shoulder at 288 nm. The UV absorption spectrum of Trp(Dmop) peptides is thus diagnostic in the same way as that of N^1-formyltryptophan[19] (λ_{max} 300 nm).

Further stability studies established that the Dmop group is stable to hydrogenation. Catalytic hydrogenation of **7** at room temperature and atmospheric pressure over 10% palladium on charcoal removed the benzyloxycarbonyl and benzyl protecting groups, yielding **9**. Homogeneity of the product was found to be dependent on the reaction solvent: both **9** and the N^1-(methylphosphono)tryptophan-containing peptide **10** (5:1 ratio by HPLC) were isolated after hydrogenation of **7** in glacial acetic acid; the ratio in the crude mixture on standing changed to 1:1, indicating that slow monodemethylation of the phosphate in the acetate salt of **9** occurs in the solid state. However, pure **9** was the sole product obtained with the use of methanol/water (90:10) containing TFA (1 equiv) as a solvent. The liberated α-amino group of the free peptide is not protonated sufficiently in glacial acetic acid to prevent it from taking part in the demethylation of the phosphate ester, an effect observed by Löw et al.[17] on hydrogenation of Z-Trp(CHO)-O^{t}Bu, in which the liberated α-amino group partially removed the formyl group.

The Dmop protecting group can be removed at the completion of a synthesis by treatment with aqueous methanolic 1 M NaOH for 1 h. Only 1 equiv of NaOH is required after neutralization of acidic groups in the peptide to cleave the Dmop group. Thus H-Glu-Trp-Leu-OH·TFA (**11**) was obtained by treatment of H-Glu-Trp(Dmop)-Leu-OH·TFA with 4 equiv of NaOH followed by HPLC purification of the crude product. Tripeptide **11** was identical with synthetic H-Glu-Trp-Leu-OH by C_8 rp-HPLC co-injection and ^{13}C and ^1H NMR spectroscopy (Table I), which contained no phosphorus-coupled doublet signals. In contrast, the N^1-phosphotripeptide **8** is stable in 1 M NaOH as it forms a disodium phosphate salt. The contrasting effects of mild (e.g., amines) and strong (e.g., NaOH) bases on the Dmop group can be explained in terms of the concept of hard and soft bases. The hard nucleophile attacks the molecule at the phosphorus atom, whereas the soft nucleophile attacks at the carbon of the methyl group, removing only one alkyl group, the negative charge on the product inhibiting further nucleophilic attack.

The most interesting feature of the N^1-phosphorylated tryptophan-containing tripeptide is the marked change in its solubility conferred by the hydrophilic phosphate moiety. Tripeptide **8** is freely water soluble at 20 °C, whereas H-Glu-Trp-Leu-OH·TFA is only partly soluble in an equal volume of water.

In conclusion, this work describes the efficient synthesis of the N^1-phosphorylated tryptophan peptide Glu-*P*Trp-Leu and establishes conditions for its deprotection. The dimethylphosphono (Dmop) group is a versatile multifunctional protecting group, which can be cleaved at the completion of a synthesis or selectively deprotected to yield a peptide containing the novel amino acid **2** incorporated with total positional selectivity. The Dmop group is stable to the conditions of conventional solution-phase peptide synthesis and effectively suppresses many of the problems associated with tryptophan-containing peptide synthesis mentioned earlier. No *tert*-butylation of the indole nucleus has been observed on removal of the Boc group in Trp(Dmop) peptides in 40% TFA/CH_2Cl_2 in the absence of scavengers.

More importantly, N^1-phosphotryptophan peptides are useful tools, amenable, for example, to ^{31}P NMR studies, which can be used to yield information about molecular conformations and nonbonded interactions, in a manner similar to that suggested by Roberts and Vellaccio[20] for peptides containing fluorinated amino acids. Radiolabeling tryptophan with ^{32}P by the use of (^{32}P)dimethyl phosphorochloridate in the phosphorylation reaction is another possibility.

The ability to readily synthesize N^1-phosphotryptophan peptides should also be useful in studies on the mechanism of hormone action. Phosphorylation reactions are a key step in the cascade of events leading to the physiological response of peptide hormones.[21] In addition, the incorporation of phosphorylated tryptophan into biologically active peptides may provide analogues with enhanced or modified activities. The incorporation of hydrophilic N^1-phosphotryptophan in place of the hydrophobic tryptophan in peptide sequences should also improve the solubility characteristics of such peptides. Thus, N^1-phosphotryptophan is a new phosphoamino acid with potential applications in the study of structure–function relationships, in peptide solubilization, and in radiolabeling. Its use in larger, more complex peptides is being

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evaluated at present.

Experimental Procedures

All amino acids used were of the L configuration. Boc-Trp-(Dmop)-OH was prepared as described elsewhere.[2] TFMSA, DMS, and thioanisole were of AR grade, purchased from Fluka and used without further purification; TFA and *m*-cresol were purified by distillation. Palladium (10% on charcoal) was purchased from Aldrich Chemical Co. and Boc-Trp-OH from Chemalog. THF was distilled over potassium benzophenone ketal prior to use. ^{13}C NMR spectra were obtained on a JEOL-FX 90 Q Fourier transform (FT) instrument operating at 22.5 MHz or on a JEOL GX 400 FT instrument operating at 100.4 MHz, referenced to internal $CDCl_3$ (77.0 ppm), CD_3CN (1.3 ppm), or dioxane (66.5 ppm), ^{31}P NMR spectra on a JEOL-FX 100 FT instrument operating at 40.26 MHz, referenced to external 85% H_3PO_4 (0 ppm), and ^1H NMR spectra on a JEOL GX 400 FT operating at 399.65 MHz, referenced to internal CD_3CN (1.93 ppm), d_4-methanol (3.3 ppm), or dioxan (3.7 ppm). FAB mass spectra were obtained on a JEOL 505H mass spectrometer equipped with a FAB source using glycerol–aqueous CH_3CO_2H as a matrix. Optical rotations were measured in a Perkin-Elmer 241 MC polarimeter with a 1-dm path length in a constant-temperature jacket. UV spectra were recorded on a Varian SuperScan 3. Amino acid analyses were performed on a Beckman 6300 amino acid analyzer after hydrolysis of the peptides in 6 M HCl containing 1% phenol at 110 °C for 24 h in sealed evacuated tubes. Peptide sequencing was performed on an Applied Biosystems 470A protein sequencer equipped with an Applied Biosystems 120A phenylthiohydantoin analyzer. HPLC separations were performed on a C_8 semipreparative reverse-phase cartridge (Brownlee RP300, 250 × 4.6 mm, 7 μm), using linear gradients of 0.1% aqueous TFA (solvent A) and 0.1% TFA in CH_3CN (solvent B), at a flow rate of 3 mL/min, monitoring the eluent with a variable-wavelength UV detector operating at 280 nm.

Synthesis of Z-Glu(OBzl)-Trp(Dmop)-Leu-OBzl (7). *N*-Methylmorpholine (NMM, 51 mg, 0.5 mmol) in THF (0.5 mL) and isobutyl chloroformate (IBCF, 63 mg, 0.46 mmol) in THF (0.5 mL) were added successively to Boc-Trp(Dmop)-OH (0.216 g, 0.5 mmol) in THF (2 mL) at −30 °C (dry ice/acetone) under nitrogen, followed after 3 min by H-Leu-OBzl·HCl (92 mg, 0.36 mmol) in THF (2 mL) neutralized with NMM (36 mg, 0.36 mmol). After 2 h at −20 °C, the reaction was quenched by addition of 5% $NaHCO_3$ (0.5 mL), ethyl acetate (20 mL) added, and the organic phase washed with 5% $NaHCO_3$ (20 mL) and 1 M HCl (20 mL), dried over $MgSO_4$, filtered, and solvent evaporated under reduced pressure to give Boc-Trp(Dmop)-Leu-OBzl (**4**) as an off-white foam: yield 0.22 g, 97%; ^{31}P NMR ($CHCl_3$) δ −0.15; ^{13}C NMR (90 MHz, $CDCl_3$) δ 21.5, 22.3, 24.3, 27.9, 40.9, 50.6, 53.6 (d, J_{CP} = 4.9 Hz), 66.5, 113.2, 116.0 (d, J_{CP} = 8.6 Hz), 119.0, 121.8, 123.5, 126.6 (d, J_{CP} = 7.3 Hz), 127.8, 127.9, 128.9, 130.7 (d, J_{CP} = 9.8 Hz), 135.1, 136.9 (d, J_{CP} = 4.9 Hz), 155.2, 171.0, 172.1.

Boc-Trp(Dmop)-Leu-OBzl (**4**) was treated with 40% TFA in CH_2Cl_2 (1 mL) for 1 h at 0 °C under N_2, TFA and CH_2Cl_2 were removed under vacuum, and the residue was triturated with diethyl ether to remove residual TFA. The dipeptide ester salt neutralized with NMM (30 mg, 0.31 mmol) and coupled to the mixed anhydride of Z-Glu(OBzl)-OH (0.15 g, 0.43 mmol), obtained by addition of NMM (43 mg, 0.43 mmol) and IBCF (54 mg, 0.39 mmol) to the amino acid, for 2 h at −20 °C. Product isolation was performed as for the dipeptide: yield 0.27 g, 100% yield; ^{31}P NMR ($CHCl_3$) δ −0.09 ppm; ^{13}C NMR (90 MHz, $CDCl_3$) δ 21.6, 22.4, 24.5, 27.8, 30.0 40.9, 50.8, 53.1, 53.8 (d, J_{CP} = 4.9 Hz), 66.2, 66.7, 113.3, 115.7 (d, J_{CP} = 9.8 Hz), 119.1, 122.1, 123.7, 127.0 (d, J_{CP} = 6.1 Hz), 127.7, 127.9, 128.2, 130.7 (d, J_{CP} = 9.8 Hz), 135.2, 135.6, 136.4 (d, J_{CP} = 4.9 Hz), 156.0, 170.4, 171.2, 172.6; $[\alpha]^{22.5}_D$ −20.81° (*c* 1, $CHCl_3$); UV ($CHCl_3$) 275, (λ_{max}, ε 2.7 × 10^3), 288 nm.

H-Glu-Trp(PO_3H_2)-Leu-OH·TFA (8) by Treatment of 7 with TFMSA/TFA/*m*-Cresol/DMS. Peptide **7** (105 mg, 0.12 mmol) was dissolved in TFMSA/TFA/*m*-cresol/DMS (10:50:30:10, 2.5 mL) under N_2 in a 10-mm-diameter NMR tube. Reaction progress was monitored by ^{31}P NMR spectroscopy. After 2 h, the solution was poured into chilled ether, and the precipitate washed with several portions of cold ether. Reaction yield was judged to be >90% by analytical HPLC and ^{13}C and ^{31}P NMR of the crude product, which was then purified by HPLC. Some degradation of the products in solution was observed during consecutive semipreparative HPLC runs, resulting in a lower yield, a problem that can be circumvented by immediate purification after precipitation. Batchwise purification gave **8**: yield 16 mg, 21%; ^{31}P NMR (D_2O/CD_3CN) δ −6.36; $[\alpha]^{22.5}_D$ +1.65° (*c* 1, H_2O); UV (H_2O) 267 (λ_{max}, ε 5.4 × 10^3), 276, 287 nm; FABMS (Ar, positive mode), *m/z* (rel intensity) 527 (83), 447 (45), 318 (21), 277 (59), 239 (50), 210 (50), 132 (89); amino acid ratios (theoretical) Glu 1.00 (1), Leu 1.26 (1), Trp 0.23 (1).

H-Glu-Trp(Dmop)-Leu-OH·TFA (9) by Hydrogenation of 7. Peptide **7** (0.15 g, 0.17 mmol) was hydrogenated in methanol/water (95:5, 10 mL) containing 1 equiv of TFA (20 mg, 0.17 mmol) with 10% Pd/C (17 mg, 100 mg/mmol peptide) at 25 °C and atmospheric pressure for 3 h. The catalyst was removed by filtration, and the solvent removed under reduced pressure to give **9** as a yellow glass: yield, 0.105 g, 91%; yield after HPLC purification 23 mg; ^{31}P NMR (D_2O/CD_3CN) δ +0.85; $[\alpha]^{22.5}_D$ +9.69° (*c* 1, H_2O); UV (H_2O) 257 (λ_{max}, ε 5.3 × 10^3), 275, 287 nm; FABMS (Ar, positive mode), *m/z* (rel intensity) 555 (45), 426 (15), 278 (60), 267 (100), 252 (47), 238 (100), 224 (69), 132 (44); amino acid ratios (theoretical) Glu 1.00, (1), Leu 0.90 (1), Trp 0.16 (1).

H-Glu-Trp(MeOPO$_2$H)-Leu-OH·TFA (10) by Hydrogenation of 7. Hydrogenation of **7** (0.150 g, 0.173 mmol) for 4 h, as described for **9**, using glacial acetic acid (6 mL) as solvent, yielded a clear glass (93 mg), which on standing for a year and HPLC separation gave **10**: 41 mg (and **9**, 8 mg); ^{31}P NMR (D_2O/CD_3CN) δ −3.75; $[\alpha]^{22.5}_D$ +0.85° (*c* 1, H_2O); UV (H_2O) 260 (λ_{max}, ε 5.7 × 10^3), 276, 288 nm; FABMS (Ar, positive mode) *m/z* (rel intensity) 541 (29), 412 (12), 382 (14), 279 (24), 264 (51), 253 (100), 238 (93), 224 (100), 211 (56), 132 (100); amino acid ratios (theoretical) Glu 1.00 (1), Leu 0.90 (1), Trp 0.01 (1).

H-Glu-Trp(Dmop)-Leu-OH·HF (9) by HF Treatment of 7. Tripeptide **7** (97 mg) and *o*-cresol (1 mL) were placed in a Kel-F HF reaction vessel on an HF line. The vessel was evacuated, and doubly distilled HF was collected in the reaction vessel at −70 °C (dry ice/acetone) to a total volume of 10 mL. The reaction mixture was stirred at 0 °C for 1 h, and HF then removed rapidly at 0 °C. The residue was partitioned between water and ether, and the aqueous phase lyophilized to give **9** as a white solid: yield 53 mg, 84%; ^{31}P NMR (D_2O/CD_3CN) δ +0.54; $[\alpha]^{22}_D$ +11.20° (*c* 1, MeOH); UV (H_2O) 259 (λ_{max}, ε 5.07 × 10^3), 276, 286 nm.

Cleavage of the Dmop Group by Sodium Hydroxide. Peptide **9** (70 mg, 0.1 mmol) was dissolved in methanol (2 mL) in a 10-mm-diameter NMR tube, and 1 M NaOH (0.4 mL, 0.4 mmol) added. The reaction was monitored by ^{31}P NMR, and, after 2 h, acidification to pH 7 with 1 M HCl, and lyophilization of the crude material from aqueous solution followed by HPLC purification gave H-Glu-Trp-Leu-OH·TFA (**11**): yield 33 mg, 59%; $[\alpha]^{22.5}_D$ −2.12° (*c* 0.5, MeOH).

Synthesis of H-Glu-Trp-Leu-OH·TFA (11). Boc-Trp-OH (213 mg, 0.7 mmol) was coupled as its mixed anhydride (NMM, 71 mg, 0.7 mmol; IBCF, 89 mg, 0.65 mmol) to H-Leu-OBzl·HCl (129 mg, 0.5 mmol) neutralized with NMM (51 mg, 0.5 mmol) as described for peptide **7**; yield 0.248 g, 98%; ^{13}C NMR ($CDCl_3$) 21.9, 22.6, 24.6, 28.2, 41.4, 50.8, 55.2, 66.9, 110.4, 111.1, 118.8, 119.6, 122.1, 123.3, 127.4, 128.1, 128.5, 135.4, 136.2, 155.4, 171.4, 172.2.

The Boc group was removed with 40% TFA in CH_2Cl_2 containing 2% indole at 4 °C for 1 h, TFA and CH_2Cl_2 were removed under reduced pressure, and the residue was triturated with ether to remove residual TFA. The salt was neutralized with NMM (50 mg, 0.49 mmol), and the dipeptide was coupled to Z-Glu-(OBzl)-OH (256 mg, 0.69 mmol) mixed anhydride (NMM, 69 mg, 0.69 mmol, IBCF, 87 mg, 0.64 mmol).

Hydrogenation of the tripeptide (84 mg, 0.11 mmol) over 10% Pd/C (11 mg) in methanol (10 mL) containing 1 equiv TFA (13 mg, 0.11 mmol) for 1.75 h followed by filtration to remove catalyst and evaporation of solvent under reduced pressure gave H-Glu-Trp-Leu-OH·TFA (**11**): yield 66 mg, 100%; $[\alpha]^{22.5}_D$ −4.38° (*c* 1, MeOH); UV (MeOH) 280 (λ_{max}, ε 3.5 × 10^3), 288 nm; FABMS (Ar, positive mode), *m/z* (rel intensity) 447 (17), 429 (10), 344 (14), 301 (23), 270 (30), 215 (21), 117 (70); amino acid ratios (theoretical) Glu 1.00 (1), Leu 1.14 (1), Trp 0.10 (1).

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Registry No. 1, 118869-37-7; 3·HCl, 2462-35-3; 4, 122520-32-5; 5, 5680-86-4; 6·TFA, 122520-34-7; 7, 122539-48-4; 8·TFA, 122520-36-9; 9·TFA, 122520-38-1; 9·HF, 122520-43-8; 10·TFA, 122520-40-5; 11·TFA, 122520-42-7; BOC-Trp-OH, 13139-14-5; BOC-Trp-Leu-Obzl, 72755-34-1; H-Trp-Leu-OBzl·TFA, 122520-45-0; Z-Glu(OBzl)-Trp-Leu-OBzl, 122520-46-1.

The Reformatsky Type Reaction of Gilman and Speeter in the Preparation of Valuable β-Lactams in Carbapenem Synthesis: Scope and Synthetic Utility

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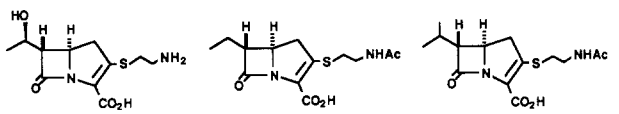
The preparation of appropriately substituted 3-alkyl β-lactams from the Reformatsky type reaction of Gilman and Speeter is described. Treatment of Schiff bases derived from α-methylcinnamaldehyde and amines with ethyl α-bromobutyrate or ethyl α-bromoisovalerate in the presence of zinc dust followed by an ozonolysis–Baeyer–Villiger sequence of the resulting β-lactams afforded 3-alkyl-4-acetoxy β-lactams as synthetic intermediates for the synthesis of PS-5 and PS-6 carbapenem compounds. The reaction between cinnamylideneamines and these α-bromoesters under Gilman and Speeter's conditions also works well to provide the expected 3-alkyl-4-styryl β-lactams suitable for further chemical manipulations by known methods. As expected, application of this procedure to the synthesis of 3-unsubstituted β-lactams starting from methyl bromoacetate was inefficient. However, activation of zinc dust by trimethylchlorosilane provided an exceedingly efficient route to 3-unsubstituted β-lactams in 70–95% yields except those derived from cinnamylideneamines. Treatment of the lithium enolate of 1-(4-methoxyphenyl)-4-(α-methylstyryl)azetidin-2-one with acetaldehyde or methyl acetate afforded the corresponding trans derivatives as synthetic precursors of (±)-thienamycin. A new diastereoselective entry for the synthesis of optically active carbapenem compounds is also described.

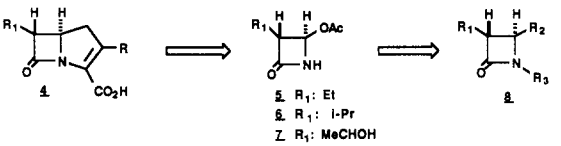
Since the discovery of novel biologically active β-lactam antibiotics[1] such as thienamycin **1** and the closely related carbapenems PS-5 **2** and PS-6 **3**, intense effort has been



1 **2** **3**

focused in the development of suitable methods for their total synthesis.[2] These compounds and related structures comprise a new family of streptomycete metabolites characterized by the presence of the 7-oxo-1-azabicyclo-[3.2.0]hept-2-ene-2-carboxylic acid system carrying an exocyclic (aminoethyl)thio substituent at C_3 of the pyrroline ring and alkyl side chains at the α-position adjacent to the β-lactam carbonyl. The novel chemical features and potent antibacterial properties of these new bicyclic β-lactams, together with the reported low-yield fermentation processes as compared to those in other noted β-lactam antibiotics, have made carbapenems attractive target molecules for many research groups.[3]

The main strategies toward carbapenem synthesis usually involve first the construction of an appropriately substituted monocyclic β-lactam **8** with the correct stereochemistry at C_3–C_4 of the β-lactam ring, followed by chemical manipulations at N_1 and C_4 and subsequent ring closure to form the bicyclic ring system **4** in the last step of the synthesis.[3,4] 4-Acetoxyazetidin-2-ones **5**–**7** are recognized as the most useful intermediates for this type of carbapenem synthesis, because the acetoxy group can



4 **5** R_1: Et; **6** R_1: i-Pr; **7** R_1: MeCHOH; **8**

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