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Double Bond Isosteres of the Peptide Bond: Synthesis and Biological Activity of Cholecystokinin (CCK) C-Terminal Hexapeptide Analogs§

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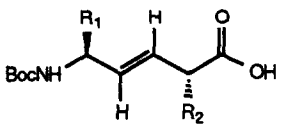
Abstract—New and existing methodologies were used to prepare a series of modified CCK analogs in which each amide bond was replaced by a *trans*-alkene unit. The data indicate that every amide linkage at C-terminal tetrapeptide (CCK-4) region is crucial for biological activity. While the amide bond beyond the Trp residue in the N-terminal direction can be replaced by a *trans*-alkene and still retain most of the binding potency and functional activity.

Cholecystokinin (CCK) is a family of peptides found throughout the brain and periphery[1,2] of many mammalian species. Studies have indicated that CCK may induce satiety,[3] anxiety,[4] regulate analgesia,[5,6] dopamine release[7] and enhance cognitive function.[8] Two distinct CCK receptor subtypes have been characterized and labeled as CCK-A (alimentary) and CCK-B (brain). The latter is the major receptor subtype in the brain, and its ligand binding profile toward various CCK ligands is similar to that of the peripheral gastrin receptor.[1,2] The pharmacological basis for the two distinct CCK receptor subtypes was based on their differences in binding affinity toward various CCK fragments. In particular, the N-terminal protected CCK tetrapeptide, Boc-Trp-Met-Asp-PheNH$_2$ (Boc-CCK-4), binds to the CCK-B receptor with an IC_{50} value of 25 nM, and exhibits 70-fold selectivity for the CCK-B over the CCK-A receptor (Table 3). This C-terminal tetrapeptide represents a lead tetrapeptide fragment of CCK that exhibits high affinity toward the CCK-B/gastrin receptor. In an effort to gain insight into the bioactive conformations of CCK and to circumvent some of the therapeutic limitations of peptides, we embarked upon a study to systematically replace each of the amide linkages with a *trans*-carbon–carbon double bond (*E*-CH=CH). By nature, the amide bond can rotate and furthermore is capable of hydrogen bonding interactions with the receptor. In contrast, the *trans*-double bond, an isosteric replacement of the amide bond, fixes the replaced peptide linkage at its lowest energy *trans* geometry and mimics the size, geometry, bond angle and bond length of the peptide bond in its *trans* configuration.[9,10] Therefore, the *E*-CH=CH replacement analogs will provide valuable information concerning the role of each amide bond in a peptide. Several *trans*-carbon–carbon double bond replacement analogs have been prepared that retain the functional properties of the parent peptides, including enkephalin,[10–12] Substance p[12] and renin substrate[13] analogs. Since it has been shown that either Leu or Nle is an acceptable replacement for the methionine of Boc-CCK-4 (Table 3), the corresponding Leu or Nle peptide isostere analogs of CCK were synthesized for this study. Five fully protected *E*-CH=CH dipeptide isosteres, Boc-Leuψ[*E*-CH=CH]Gly-OH **Ia**, Boc-Glyψ[*E*-CH=CH]Trp-OH **Ib**, Boc-Trpψ[*E*-CH=CH]Nle-OH **Ic**, Boc-Leuψ[*E*-CH=CH]Asp(β-*O*-*t*Bu)-OH **Id**, and Boc-Asp(β-*O*-*t*Bu)ψ[*E*-CH=CH]Phe-OH **Ie** (Table 1) were synthesized and incorporated into analogs of CCK by using standard peptide coupling procedures.

Table 1.



	R_1	R_2
Ia	$CH_2CH(CH_3)_2$	H
Ib	H	3-Indolylmethyl
Ic	3-Indolylmethyl	n-Butyl
Id	$CH_2CH(CH_3)_2$	CH_2CO_2-tBu
Ie	CH_2CO_2-tBu	CH_2Ph

Chemistry: Syntheses of Boc-Leuψ[*E*-CH=CH]Gly-OH Ia, Boc-Glyψ[*E*-CH=CH]Trp-OH Ib, Boc-Trpψ[*E*-CH=CH]Nle-OH Ic, and Boc-Asp(β-*O*-*t*Bu)ψ[*E*-CH=CH]Phe-OH Ie

Boc-Leuψ[*E*-CH=CH]Gly-OH **Ia** was prepared according to the known procedure.[13] Boc-Glyψ[*E*-CH=CH]Trp-OH **Ib** and Boc-Asp(β-*O*-*t*Bu)ψ[*E*-CH=CH]Phe-OH **Ie** were synthesized according to the methodology[14] developed in

§ Dedicated to Professor Fa-Chin Chen on the occasion of his 80th birthday.

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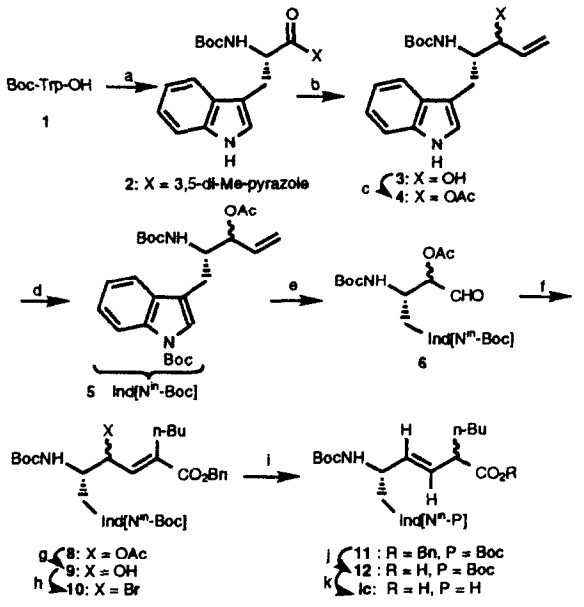
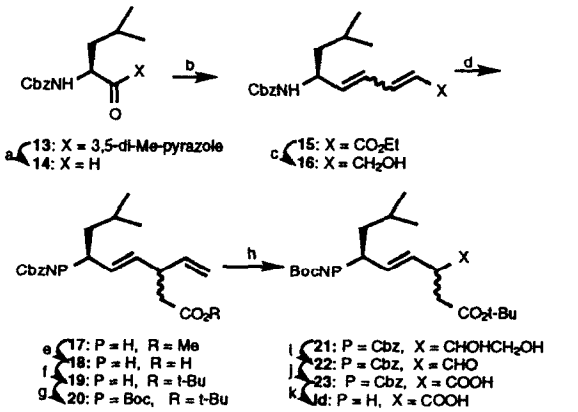
our laboratory. The most challenging analog to synthesize was the Trp-Nle segment in which the Trp is the first residue of the dipeptide fragment. The synthesis of the Boc-Trpψ[*E*-CH=CH]Nle-OH isostere **Ic** followed the route reported previously[14] for the corresponding Boc-Asp(β-*O*-*t*Bu)ψ[*E*-CH=CH]Phe-OH isostere **Ie** with some modified conditions to accommodate the indole moiety of Trp (Scheme I). In order to allow the preparation of aldehyde **6** from olefin **5** under oxidative conditions, the indole nitrogen was protected with Boc, and the oxidation step was carried out in a two-phase system, which afforded aldehyde **6** in fair yield. The crude aldehyde **6** was treated with phosphorane **7**[16] to produce the desired Wittig adduct **8** as a mixture of diastereomers in 19% overall yield from allylic acetate **5**. The Wittig product **8** was carried on to the final isosteric analog Boc-Trpψ[*E*-CH=CH]Nle-OH **Ic** using the identical reaction sequence employed for Boc-Gly ψ[*E*-CH=CH]Trp-OH **Ib** and Asp(β-*O*-*t*Bu)ψ[*E*-CH=CH]Phe-OH **Ie**.[14]



Scheme I. Reagents: a. 3,5-di-Me-pyrazole/EDCI, b. LAH/-78 °C then vinyl–MgBr, c. Ac_2O/Py, d. Boc_2O/DMAP, e. NMO/$NaIO_4$/OsO_4(cat.), f. $Ph_3P{=}C(n\text{-}Bu)CO_2Bn$ **7**, g. Na_2CO_3/CH_3OH, h. Ph_3P/CBr_4/THF, i. Zn/HOAc, j. 1,4-cyclohexadiene/Pd/C/MeOH, k. K_2CO_3/MeOH.

Synthesis of Boc-Leuψ[*E*-CH=CH]Asp (β-*O*-*t*Bu)-OH Id

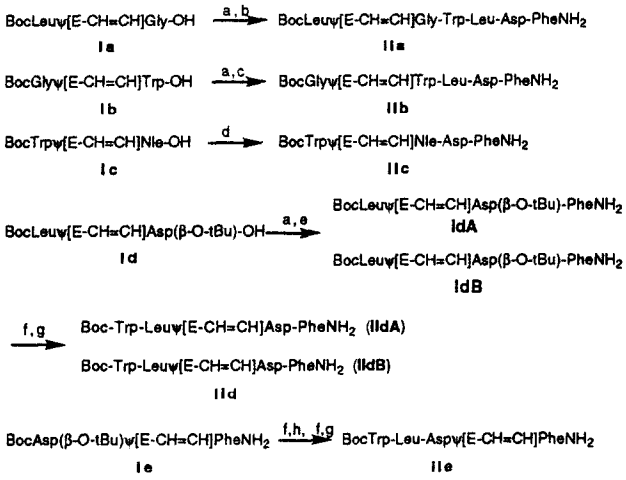
Although this analog can be prepared by following the same methodology[14] as for **Ia**, **Ib**, and **Ie**, some difficulties were encountered due to the presence of the Asp side chain as the second residue. An alternate route was developed specifically for the synthesis of this analog (Scheme II). Optically pure Cbz-L-leucinal **14**, derived from its corresponding dimethyl-pyrazolide **13** via a LAH reduction, was treated with the anion of triethyl 4-phosphonocrotonate to give the desired diene–ester **15**. The *E*-configuration of the newly formed γ–δ double bond was confirmed at a later stage. Dibal reduction of the diene–ester **15** gave the desired diene–alcohol **16**, from which an *ortho*-ester Claisen rearrangement of **16** gave rearranged methyl ester **17**, which was further converted to its corresponding *t*-butyl ester **19** through acid **18**. The degradation of the terminal olefin was best carried out on doubly-protected diene–ester **20** via a three-step sequence to give the desired acid **23** in a 41% overall yield from **20**. Basic hydrolysis of acid **23** provided the pseudodipeptide **Id** as a pair of inseparable diastereomers (ca. 1:1 at the α -center corresponding to Asp based on the yield of the separated diastereomeric tripepetides **IdA** and **IdB**; see Experimental Section).



Scheme II. Reagents: a. LAH/ -78 °C, b. $(EtO)_2P(O)CH_2$-$CH{=}CHCO_2Et$/NaH, c. Dibal, d. $CH_3C(OMe)_3/PhCO_2H$/xylene/reflux, e. 2N NaOH, f. *t*-Bu-2,2,2-trichloroacetimidate/$BF_3{\cdot}Et_2O$, g. $(Boc)_2O$/DMAP/CH_3CN, h. OsO_4(cat.)/NMO, i. $NaIO_4$/MeOH, j. Jones reagent, k. 2N NaOH.

Incorporation of *trans*-Double Bond Isosteres Ia, Ib, Ic, Id and Ie into the CCK Sequence

Extension of **Ia**, **Ib**, **Ic**, **Id** and **Ie** into the target pseudopeptides was carried out either by stepwise coupling of the dipeptide isostere with the amino acids in the CCK sequence or by coupling together with CCK fragments (dipeptide or longer) via standard peptide coupling and deprotection procedures (Scheme III). The α-centers at the second residue of **Ib**, **Ic**, and **Ie** were stereorandom,



Scheme III. Reagents: a. EDCI/2,4,5-tri-Cl-phenol, b. HCl-Trp-Leu-Asp-$PheNH_2/Et_3N$/DMF, c. HCl·Leu-Asp-$PheNH_2/Et_3N$/DMF, d. IBCF/Et_3N/TFA Asp-$PheNH_2$, e. $PheNH_2$/DMF, f. HCl/HOAc, g. BocTrp-*O*Su/NMM/DMF, h. Boc-Leu symmetrical anhydride/NMM.

therefore, the target pseudopeptides **IIb, IIc, IIe**, contained a ca. 1:1 mixture of epimers as indicated by HPLC analysis. Attempts to separate these epimers were unsuccessful, thus, compounds were tested as mixtures. In the case of **Id**, coupling to $PheNH_2$ afforded a pair of diastereomeric tripeptides **IdA** and **IdB**, which were separated by flash silica gel chromatography and elaborated to the target peptides separately. The coupling constants between the two olefinic protons of these CCK peptide isosteres of various lengths are summarized in Table 2. The large coupling constants between the two vinyl protons exhibited in the synthetic CCK peptide isosteres confirm the *trans* geometry at the carbon–carbon double bond.

Table 2. NMR data

X- [*E*-CH_a=CH_b] - Y

Entry	X	Y	δCH_a	δCH_b	$J_{a,b}$(Hz)
Ic	Boc-Trp	Nle	5.46 & 5.47	5.58 & 5.63	15 & 16
Ie	Boc-Asp(β-O-tBu)	$PheNH_2$	5.51	5.69	15.8
IdA	Boc-Leu	Asp(β-O-tBu)-$PheNH_2$(A)	5.44	5.61	15
IdB	Boc-Leu	Asp(β-O-tBu)-$PheNH_2$(B)	5.34	5.45	15.7
IIa	Boc-Leu	Gly-Trp-Leu-Asp-$PheNH_2$	5.34	5.45	15
IIb	Boc-Gly	Trp-Leu-Asp-$PheNH_2$	5.53	5.70	15
IIc	Boc-Trp	Nle-Asp-$PheNH_2$	5.41	5.55	*
IIdA	Boc-Trp-Leu	Asp-$PheNH_2$	5.20	5.30	15.5
IIdB	Boc-Trp-Leu	Asp-$PheNH_2$	5.00	5.18	15.5
IIe	Boc-Trp-Leu-Asp	$PheNH_2$	5.47 & 5.50	5.71	15.5

* Unable to determine the coupling constant unequivocally due to the complexity of the spectrum which was obtained on a mixture of a pair of diastereomers. The expected coupling constant was observed in **Ic** and the final coupling procedure is unlikely to affect the geometry of the established double bond, thus ensuring the *trans*-geometry of the target peptide **IIc**.

Results and Discussion

Six amide bond replacement analogs of CCK **IIa, IIb, IIc, IIdA, IIdB** and **IIe** were tested using radioligand binding assays[19] in guinea pig cortex (CCK-B) and pancreas (CCK-A) to determine their binding potency and selectivity. Analogs with good affinity (<100 nM) for the cortical CCK-B receptors were further tested for their ability to stimulate intracellular calcium levels in NCI-H345 cells,[19] which express CCK-B/gastrin receptors.[20] As shown in Table 3, the IC_{50} values of the Leu-Gly and Gly-Trp replacement analogs **IIa** and **IIb** were 37 and 35 nM in guinea pig cortex, respectively. Their IC_{50} values in pancreas were 2700 and 1800 nM respectively. Relative to the parent peptide Boc-Leu-Gly-Trp-Leu-Asp-$PheNH_2$ and Boc-Gly-Trp-Met-Asp-$PheNH_2$, the double bond replacement analogs **IIa** and **IIb** showed only slight difference in binding affinity to both central (CCK-B) and peripheral (CCK-A) receptors. Compounds **IIa** and **IIb** proved to be full or near maximal agonists, relative to CCK-8, in stimulating calcium mobilization in NCI-H345 cells, demonstrating that the binding and functional activities of the parent peptides are preserved at the CCK-B receptor. These results indicate that the *trans*-double bond is an acceptable replacement for the amide bond at Leu-Gly region of CCK-6 and Gly-Trp site of CCK-5 in terms of maintaining the proper bioactive conformation necessary for receptor binding and activation.

The results obtained from tetrapeptide isosteres **IIc, IIdA, IIdB** and **IIe** were rather striking. The amide bond replacement analog at the Trp-Nle site (**IIc**) was inactive in the cortical binding assay; however, the IC_{50} value at the pancreatic receptor was 1300 nM, a minor improvement over the parent compound Boc-Trp-Nle-Asp-$PheNH_2$. One isomer of the Leu-Asp replacement analogs, **IIdA** failed to bind to either the CCK-A or CCK-B receptor even at 10^{-5} M, while the other isomer, **IIdB**, demonstrated a major loss (>100-fold) in binding potency at the CCK-B site. The Asp-Phe replacement analog **IIe** bound weakly to the CCK-B receptor (IC_{50} = 6900 nM), while its affinity for CCK-A receptors was 3500 nM, a slight (~2-fold) improvement over the Boc-[Leu^2]-CCK-4.

Table 3. Binding affinity of CCK peptides for the CCK-A (pancreas) and CCK-B (cortex) receptors and functional activity of CCK-B receptor

		Binding [a]		CCK-B Calcium Response[b]	
Entry	Compound	IC50(nM)		EC50(nM)	%
		CCK-B (Cortex)	CCK-A (Pancreas)	Ca^{+2} Release	max.
	Boc-Trp-Met-Asp-$PheNH_2$	25±4.5 (6)	1800±630 (5)	13±0.6 (3)	99
	Boc-Trp-Leu-Asp-$PheNH_2$	45±8 (10)	7500±1800 (4)	38±18 (3)	104
	Boc-Trp-Nle-Asp-$PheNH_2$	65±14 (6)	4000±810 (6)	nt[e]	nt
	Boc-Trp-O-Met-Asp-$PheNH_2$	>10000	>10000	nt	nt
	Boc-Gly-Trp-Met-Asp-$PheNH_2$	21±7 (3)	2800±520 (3)	49±4.6 (3)	104
	Boc-Leu-Gly-Trp-Leu-Asp-$PheNH_2$	54±12 (4)	1600±820 (3)	40±9.9 (3)	99
IIa	Boc-Leu[E-CH=CH]Gly-Trp-Leu-Asp-$PheNH_2$	37 ±7.9 (4)	2700±720 (3)	150±18 (3)	103
IIb	Boc-Gly[E-CH=CH]Trp-Leu-Asp-$PheNH_2$[d]	35±4.7 (3)	1800±600 (3)	48±7 3 (3)	88
IIc	Boc-Trp[E-CH=CH]Nle-Asp-$PheNH_2$[d]	>10,000	1300±670 (3)	nt	nt
IIdA	Boc-Trp-Leu[E-CH=CH]Asp-$PheNH_2$[c]	>10,000	>10000	nt	nt
IIdB	Boc-Trp-Leu[E-CH=CH]Asp-$PheNH_2$[c]	6500±87 (3)	>10000	nt	nt
IIe	Boc-Trp-Leu-Asp[E-CH=CH]$PheNH_2$[d]	6900±1000 (4)	3500±630 (4)	nt	nt

a. Values are mean ±SE with the number of determinations in parentheses. Each determination was conducted in duplicate with <5% sample variability. IC_{50} was determined as the concentration of peptide that inhibited 50% of the specific binding of [^{125}I]BH-CCK-8 in each tissue.
b. Measurement of intracellular calcium in NCI-H345 cells.
c. Separated pair of diastereomers at the alpha-carbon center of Asp.
d. Mixture of diastereomers at the alpha-carbon center corresponding to Trp residue in **IIb**, Nle residue in **IIc** and Phe residue in **IIe**.
e. Not tested.

Other investigators[21] have shown that CH_2NH is an acceptable replacement at Trp-Leu and Leu-Asp sites of Boc-[Leu^2]-CCK-4 in peripheral gastrin binding assay on isolated mucosal cells, while the same modification at Asp-Phe site resulted in a 10-fold decrease in binding potency. In addition, the CH_2NH modified analogs at the Trp-Leu amide bond exhibited full agonist activity on gastrin induced acid secretion in rat but the compounds derived from the same modification at Leu-Asp and Asp-Phe sites were found to be antagonists. Although the CH_2NH isosteric unit contains a basic nitrogen, it is a preferred replacement moiety for Boc-[Leu^2]-CCK-4 at CCK-B/gastrin receptors compared to the *E*-CH=CH unit. Both our studies and the cited studies[21] indicate that the amide bond at the Asp-Phe site is the most sensitive toward any modification in terms of binding affinity and functional activity for the CCK-B receptor. The *E*-CH=CH replacement at the Asp-Phe site (**IIe**) showed a dramatic decrease in CCK-B affinity (>100-fold) while the binding affinity to CCK-A receptor decreases only slightly (2-fold). Apparently, the Asp-Phe portion is a critical region to differentiate the binding requirements for CCK-A and CCK-B receptors at the C-terminal peptide region.

In summary, using new and existing methodologies, we have synthesized a series of modified CCK analogs in which each amide bond was replaced by an *E*-CH=CH unit. The importance of each amide bond on receptor recognition

and signal transduction was examined. This is the first systematic study of a biologically important peptide with each of its amide moieties replaced by a *trans*-alkene unit. The importance of the hydrogen bonding ability and/or the flexibility of the amide bonds in the C-terminal tetrapeptide region is evident. In CCK-4, the amide bonds were essential for receptor recognition. However, the amide bonds beyond the Trp residue in the N-terminal direction could be replaced by a *trans*-alkene and still retain most of the binding potency and functional activity. In particular, the selectivity for the CCK-B receptor was also preserved for the Leu-Gly replacement analog **IIa**.

Experimental Section

Proton magnetic resonance spectra were recorded at 300 or 500 MHz in $CDCl_3$ unless otherwise noted. Chemical shifts are reported as δ values (ppm) relative to Me_4Si as an internal standard unless otherwise indicated. Mass spectra were obtained with Hewlett Packard HP5965 (CI) and Kratos MS50 (FAB, HRMS) spectrometers. Elemental analyses and the above determinations were performed by the Analytical Research Department, Abbott Laboratories. Optical rotation data were obtained on Perkin-Elmer model 241 polarimeter. Thin-layer chromatography (TLC) was carried out using E. Merck precoated silica gel F-254 plates (thickness 0.25 mm). Flash chromatography was carried out with Merck Silica Gel 60, 200–400 mesh. Melting points are uncorrected and were determined on a Thomas–Hoover melting point apparatus. Protected amino acids were purchased from Bachem (Torrance, CA) or Chemical Dynamics Corp. (S. Plainfield, NJ). Anhydrous solvents were purchased from Aldrich (Milwaukee, WI), and reactions requiring anhydrous conditions were performed under a nitrogen atmosphere. Final product solutions were dried over Na_2SO_4, filtered and evaporated under reduced pressure on a Buchi rotary evaporator.

General Procedure to Prepare 3,5-Dimethyl Pyrazolide from N-Protected L-α-Amino Acids

A solution of Boc or Cbz protected-L-α-amino acid in dry CH_2Cl_2 was cooled to 0 °C and treated with 3,5-dimethyl pyrazole (1 eq.), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 1 eq.) and a catalytic amount of 4-dimethylaminopyridine (DMAP). The reaction mixture was stirred at room temperature overnight. The mixture was washed with H_2O, brine, dried and concentrated *in vacuo*. Flash chromatography (EtOAc/-hexanes) afforded the product.

Boc-L-Trp-3,5-dimethylpyrazolide (2)

This compound was prepared from Boc-L-Trp-OH on an 80 mmol scale to afford 23.5 g (75%) of the product. ^1H NMR (300 MHz): δ 1.4 (s, 9H), 2.29 (s, 3H), 2.45 (s, 3H), 3.3–3.5 (m, 2H), 5.29 (m, 1H), 5.85 (br s, 1H), 6.01 (s, 1H), 6.98–7.1 (m, 2H), 7.26 (t, *J* = 7Hz, 1H), 7.35–7.4 (m, 2H), 8.08 (s, 1H). MS(EI): m/e 382 (M^+). Anal. calcd for $C_{21}H_{26}N_4O_3$: C, 65.95; H, 6.85; N, 14.65. Found: C, 66.02; H, 6.97; N, 14.73.

(3RS, 4S)-4-[(tert-Butoxycarbonyl)amino]-5-(3-indolyl)-1-penten-3-ol (3)

To a stirred solution of **2** (4 g, 10.5 mmol) in THF at -78 °C under N_2 was added 1 M $LiAlH_4$ in THF (4 mL, 4 mmol) via cannula. After 20 min the reaction was quenched with saturated aqueous $KHSO_4$ and the mixture was extracted with Et_2O. The organic extracts were washed with $NaHCO_3$, H_2O, and brine, then dried and concentrated *in vacuo* to afford 2.3 g (76%) of Boc-L-tryptophanal which was used without further purification. ^1H NMR (300 MHz): δ 1.43 (s, 9H), 3.18–3.4 (m, 2H), 4.52 (m, 1H), 5.15 (br d, *J* = 9Hz, 1H), 7.3 (d, *J* = 3Hz, 1H), 7.16 (t, *J* = 7Hz, 1H), 7.22 (t, *J* = 7Hz, 1H), 7.38 (d, *J* = 9Hz, 1H), 7.6 (d, *J* = 9Hz, 1H), 8.13 (s, 1H), 9.64 (s, 1H). MS(EI): m/e 288 (M^+). To a stirred solution 16.8 mL of 1 M vinylmagnesium bromide (16.8 mmol) in anhydrous THF at -5 to -10 °C under N_2 was added a THF solution of Boc-L-tryptophanal (1.5 g, 5.2 mmol) dropwise via cannula. After ca. 4 h, the mixture was quenched with 2 mL of MeOH and partitioned between EtOAc and saturated aqueous $KHSO_4$. The separated organic layer was washed with $NaHCO_3$, H_2O, and brine, then dried and concentrated *in vacuo*. Flash chromatography (EtOAc/hexanes, 1/3 to 1/1) afforded 1.2 g (73%) of the product (3:2 mixture of diastereoisomers) as a slightly yellow oil which solidified upon standing. ^1H NMR (500 MHz): δ 1.18 & 1.22 (2s, 9H), 2.71–2.88 (m, 2H), 2.9–3.18 (m, 1H), 3.72 (m, 1H), 4.0 & 4.09 (2m, 1H), 4.45 & 4.7 (br m, 1H), 4.98 (d, *J* = 10Hz, 1H), 5.08 (d, *J* = 15Hz) & 5.18 (d, *J* = 15Hz, 1H), 5.71 (ddd, *J* = 15, 9.5Hz) & 5.80 (ddd, *J* = 15, 10, 5Hz, 1H), 6.85 (m, 1H), 6.96 (t, *J* = 7Hz, 1H), 7.01 (t, *J* = 7Hz, 1H), 7.18 (d, *J* = 6Hz, 1H), 7.43 (m) & 7.48 (d, *J* = 6Hz, 1H), 7.88 (br m, 1H). MS (CI): m/e 317 ($M + H^+$). Anal. calcd for $C_{18}H_{24}N_2O_3 \cdot 0.5\ H_2O$: C, 66.44; H, 7.74; N, 8.61. Found: C, 66.45; H, 7.39; N, 8.32.

(3RS, 4S)-3-Acetoxy-4-[(tert-butoxycarbonyl)amino]-5-(3-indolyl)-1-pentene (4)

To a solution of the preceeding alcohol **3** (0.62 g, 1.96 mmol) in 6 mL of CH_3CN under N_2 were added 1 mL of acetic anhydride and 1 mL of pyridine. The mixture was stirred overnight then partitioned between EtOAc and saturated aqueous $NaHCO_3$. The separated organic layer was washed with H_2O and brine, dried, and concentrated *in vacuo*. Flash chromatography (EtOAc/hexanes, 1/20 to 1/10) afforded 0.58 g (83%) of the product. ^1H NMR (300 MHz): δ 1.4 & 1.43 (2s, 9H), 2.12 & 2.15 (2s, 3H), 2.92 (m, 1H), 3.02 (m, 1H), 4.2 (br s) & 4.31 (2br m, 1H), 4.55 (d, *J* = 9Hz) & 4.78 (d, *J* = 9Hz, 1H), 5.22 (d, *J* = 7Hz, 1H), 5.35 (m, 2H), 5.81 (ddd, *J* = 5, 10, 15Hz) & 5.88 (ddd, *J* = 5, 10, 15Hz, 1H), 7.02 (s, 1H), 7.15 (m, 1H), 7.2 (t, *J* = 7Hz, 1H), 7.35 (d, *J* = 9Hz, 1H), 7.6 (d, = 9Hz) & 7.68 (m, 1H), 8.07 (br s, 1H). MS (FAB^+): m/e 381 ($M + Na^+$). Anal. calcd for $C_{20}H_{26}N_2O_4$: C, 67.02; H, 7.31; N, 7.82. Found: C, 66.92; H, 7.39; N, 7.75.

(3RS, 4S)-3-Acetoxy-4-[(tert-butoxycarbonyl)amino]-5-[1-(tert-butoxycarbonyl)indol-3-yl]-1-pentene (5)

A solution of **4** (4.99 g, 13.9 mmol), di-*tert*-butyl dicarbonate (4.3 g, 19.7 mmol), and DMAP (18 mg) in 100 mL of acetonitrile was stirred at ambient temperature overnight. The mixture was concentrated and the residue chromatographed over silica gel (EtOAc/hexanes, 1/19 to 1/4) to afford the product as a colorless solid (4.95 g, 77.5%). ^{1}H NMR (300 MHz) δ 1.41 (br s, 9H), 1.66 (br s, 9H), 2.11 (s, 3H), 2.75–3.0 (m, 2H), 4.73 (br d) & 5.27 (m, 2H), 5.38 (m, 2H), 5.75–5.95 (m, 1H), 7.25 (brt, J = 7.5Hz, 1H), 7.32 (br t, J = 7.5Hz, 2H), 7.44 (m, 1H), 7.54 (m, 1H), 7.58 (m, 1H), 8.12 (br d, 1H). HRMS calcd for (M + H$^+$) $C_{25}H_{35}N_2O_6$: 459.2495. Found: 459.2499. Anal. calcd for $C_{25}H_{34}N_2O_6$: C, 65.48; H, 7.47; N, 6.11. Found: C, 65.25; H, 7.45; N, 5.98.

α-n-Butyl-carbobenzyloxymethylene triphenylphosphorane (7)

Carbobenzyloxymethyl triphenylphosphonium bromide (6.3 g, 13.7 mmol) was partitioned between EtOAc and aqueous Na_2CO_3, and the separated organic phase was washed with brine, dried and concentrated. The residue in 10 mL of dry DMF was treated with anhydrous K_2CO_3 (1.8 g, 13.7 mmol) and 1-bromobutane (2.9 mL, 27 mmol), and the mixture was stirred vigorously while immersed in an oil bath at 90 °C. At various time points, aliquots were withdrawn, diluted with EtOAc, washed with aqueous Na_2CO_3, concentrated and treated with an excess (5–10 equiv.) of benzaldehyde in $CDCl_3$ or CH_2Cl_2 for several hours. The resulting mixtures could be analyzed by TLC (9/1 hexanes/Et_2O). Additional portions of 1-bromobutane (2 equiv.) and K_2CO_3 (1 equiv.) were added after 6 h and again after 29 h as the reaction was allowed to proceed for a total of 44 h. The cooled reaction mixture was diluted with EtOAc (150 mL) and H_2O (100 mL), then the aqueous phase was adjusted to pH 10–11 with 10% aqueous Na_2CO_3. The separated aqueous phase was extracted with a fresh portion of EtOAc, then the combined organic phases were extracted with 10% aqueous citric acid (3 x 75 mL). The combined aqueous acidic fractions were washed once with a portion of Et_2O, then adjusted to pH 10–11 by addition of solid Na_2CO_3, and extracted with EtOAc (2 x 200 mL). These EtOAc extracts were combined, dried and concentrated to 4.2 g of syrupy residue. Treatment with anhydrous Et_2O afforded a solid precipitate which was removed by filtration. The filtrate was concentrated to 3.6 g (ca. 56%) of a light yellow oil composed mostly of the desired ylide. Partial ^{1}H NMR (300 MHz) δ 0.66 (t, J = 7.5Hz, 3H), 1.08 (m, 2H), 1.25 (m, 2H), 1.92 (m, 2H), 4.67–5.12 (br, 2H). MS(CI): m/e 467 (M + H$^+$).

Benzyl (4RS, 5S)-(E)-4-acetoxy-5-[(tert-butoxycarbonyl)-amino]-6-[1-(tert-butoxycarbonyl)indol-3-yl)-2-butyl-2-hexenoate (8)

To a stirred suspension of $NaIO_4$ (3.25 g, 12.8 mmol) and 4-methylmorpholine *N*-oxide ([NMO], 711 mg, 6.07 mmol) in 40 mL of pH 7 phosphate buffer at ambient temperature was added a solution of allylic acetate **5** (1.17 g, 2.54 mmol) in 40 mL of Et_2O followed by a 4% aqueous solution of OsO_4 (1.6 mL, 0.25 mmol). After stirring for 14 h an additional 0.9 mL of 4% aqueous OsO_4 was added and stirred for an additional 3 h, the mixture was diluted with H_2O and extracted twice with EtOAc. The combined organic extracts were washed with saturated aqueous $Na_2S_2O_3$ and H_2O, then dried and concentrated to afford aldehyde **6** as a light brown foam which was used without further purification. Partial ^{1}H NMR (300 MHz) δ 1.41 (br s, 9H), 1.68 (br s, 9H), 2.11 & 2.13 (2s, 3H), 5.11 (d, J = 2Hz) & 5.18 (d, J = 5Hz, 1H), 9.43 (s, 1H). HRMS calcd for $C_{24}H_{33}N_2O_7$: 461.2288. Found: 461.2272. To the crude aldehyde **6** in 20 mL of CH_2Cl_2 was added phosphorane **7** (1.075 g, ca. 2.3 mmol) and the mixture was allowed to stir at ambient temperature for 18 h. The mixture was concentrated, diluted with EtOAc, and washed with 10% aqueous citric acid, H_2O, and brine, then dried and concentrated. Flash chromatography (EtOAc/-hexanes, 1/6) afforded 196 mg of a mixture of isomers (ca. 2:7 based on $PhCH_2$ singlets at δ 5.19 and 5.13, respectively) and 92 mg of pure isomer B (combined yield 19%). Pure isomer B: ^{1}H NMR (300 MHz) δ 0.68 (t, J = 6Hz, 3H), 0.8–1.15 (m, 2H), 1.26 (m, 2H), 1.4 (s, 9H), 1.66 (s, 9H), 2.11 (s, 3H), 2.05–2.20 (m, 2H), 2.80–2.95 (m, 2H), 4.22 (m, 1H), 4.85 (d, J = 9Hz, 1H), 5.13 (s, 2H), 5.60 (dd, J = 3, 9Hz, 1H), 6.60 (d, J = 9Hz, 1H), 7.20–7.40 (m, 7H), 7.42 (s, 1H), 7.60 (d, J = 7Hz, 1H), 8.12 (d, J = 7Hz, 1H). MS (CI): m/e 666 (M + NH_4^+). Anal. calcd for $C_{37}H_{48}N_2O_8 \cdot 1.5\ H_2O$: C, 65.76; H, 7.61; N, 4.15. Found C, 65.37; H, 7.27; N, 3.86.

Benzyl (4RS, 5S)-(E)-5-[(tert-butoxycarbonyl)amino]-6-[1-(tert-butoxycarbonyl)indol-3-yl]-2-butyl-4-hydroxy-2-hexenoate (9)

Acetate **8** (mixture of isomers 337 mg, 0.52 mmol) in 5 mL of anhydrous MeOH was stirred with 1.7 g of Na_2CO_3 for 65 min. The mixture was partitioned between EtOAc (75 mL) and H_2O (50 mL), then the separated aqueous phase was extracted with a fresh portion of EtOAc. The combined organic phases were washed with brine, dried and concentrated. Flash chromatography (EtOAc/hexanes, 1/5) afforded 240 mg (76%) of the product as a mixture of isomers. A similar procedure starting from pure isomer B of **8** gave the corresponding deacetylated product. ^{1}H NMR (300 MHz) δ 0.69 (t, J = 7.5 Hz, 3H), 0.85–1.25 (m, 4H), 1.4 (s, 9H), 1.67 (s, 9H), 2.09 (t, J = 7.5Hz, 2H), 2.90 (m, 1H), 3.03 (br d, J = 7.5Hz, 1H), 3.90 (m, 1H), 4.50 (dd, J = 2, 9 Hz, 1H), 5.0 (br d, J = 7.5Hz, 1H), 5.15 (dd, J = 12, 16Hz, 2H), 6.73 (d, J = 9Hz, 1H), 7.2–7.4 (m, 7H), 7.45 (s, 1H), 7.62 (br d, J = 7.5Hz, 1H), 8.12 (br d, J = 7.5Hz, 1H). MS (CI): m/e 624 (M + NH_4^+), 607 (M + H$^+$). Anal. calcd for $C_{35}H_{46}N_2O_7 \cdot 0.5\ H_2O$: C, 68.27, H, 7.69; N, 4.55. Found: C, 68.05; H, 7.57; N, 4.55.

Boc-Trp(N^{in}Boc)ψ[E-CH=CH]Nle-OBn (11)

A solution of carbon tetrabromide (1 g, 3.82 mmol) and triphenylphosphine (1.35 g, 4.1 mmol) in anhydrous THF

(20 mL) was stirred under N_2 at 0 °C for ca. 15 min to give a pale yellow solution. A solution of the above alcohol **9** (0.305 g, 0.50 mmol) in 5 mL of THF was added via cannula. The reaction mixture was stirred and allowed to warm to ambient temperature within 1 h then room temperature for 2 h. The mixture was poured into a solution of 10% aqueous citric acid, which was then extracted with $CHCl_3$. The combined $CHCl_3$ extracts were washed with brine, dried, filtered and concentrated. Flash chromatography (hexanes/EtOAc, 3/1 to 2/1) afforded 0.42 g of bromide **10** which was used directly in the next stage. ^1H NMR (500 MHz) indicated a ca. 2:1 mixture of diastereomers: δ 0.88 (m, 3H), 0.82–0.98 (m, 2H), 1.35–1.45 (m, 11H, includes 1.35, br s, 9H), 1.65 & 1.66 (2s, 9H), 2.09 (m) & 2.32 (m, 2H), 2.97–3.05 (m, 1H), 3.10–3.23 (m, 1H), 4.16 (m) & 4.65 (m, 1H), 4.90 (d, J = 10Hz) & 4.96 (d, J = 10Hz, 1H), 4.97–5.11 (m, 1H), 5.16 & 5.20 (2s, 2H), 6.92 (d, J = 8Hz) & 6.96 (d, J = 9Hz, 1H), 7.26 (m, 2H), 7.30–7.43 (m, 5H), 7.43–7.61 (m) & 7.7 (2m, 2H), 8.13 (br s, 1H). MS (FAB^+): m/e 591 (M + 2H–Br). An excess of zinc (4.5 g, 69.3 mmol) was added to a solution of the bromide **10** (0.42 g) in glacial acetic acid (40 mL). The reaction mixture was stirred under N_2 at ambient temperature for 3 h. The reaction mixture was filtered through Celite, and the filtrate was diluted with ether (150 mL) and washed successively with H_2O and brine, dried and concentrated. Flash chromatography (EtOAc/hexanes, 2/1 to 3/1) yielded 0.16 g (54%) of the product. ^1H NMR (500 MHz): δ 0.80–0.88 (m, 3H), 1.08–1.20 (m, 1H), 1.22–1.3 (m, 3H), 1.43 (br s, 11H), 1.67 (s, 9H), 2.84–2.95 (m, 2H), 2.99 (dd, J = 7, 15Hz, 1H), 4.5 (br m, 2H), 5.1 (s, 2H), 5.55 (m, 1H), 5.60 (dd, J = 9.5, 15.5Hz, 1H), 7.20–7.26 (m, 2H), 7.32–7.38 (m, 5H), 7.4 (s, 1H), 7.55 (t, J = 7Hz, 1H), 8.13 (br d, J = 10Hz, 1H). MS (FAB^+): m/e 591 ($M + H^+$), 613 ($M + Na^+$). HRMS calcd for $C_{35}H_{47}N_2O_6$: 591.3434. Found: 591.3428.

Boc-Trp(N^{in}Boc)ψ[E-CH=CH]Nle-OH (12)

A solution of **11** (0.155 g, 0.26 mmol) in MeOH (10 mL) was added to 0.15 g of 10% palladium on carbon wetted with methanol under a nitrogen atmosphere. Cyclohexadiene (5 mL) and a few crystals of ammonium formate were then added and the reaction mixture was stirred overnight at ambient temperature. An additional 50 mg of catalyst and 3 mL cyclohexadiene were added and stirring was continued until TLC (EtOAc/hexanes, 1/2) indicated complete consumption of starting material. The mixture was filtered through Celite and the filtrate concentrated *in vacuo*. Flash chromatography (EtOAc/hexanes/MeOH, 1/2/trace) afforded 93 mg (71%) of the product. ^1H NMR (500 MHz, CD_3OD) δ 0.83 (t, J = 7Hz) & 0.88 (m, 3H), 1.1 (m, 1H), 1.20–1.32 (m, 3H), 1.32–1.40 (m, 11H, includes 1.38, brs, 9H), 1.65 (s, 9H), 2.82–2.95 (m, 3H), 4.35 (br m, 1H), 5.52 (m, 1H), 5.61 (m, 1H), 6.7 (br m, 1H), 7.22 (m, 1H), 7.27 (t, J = 8Hz, 1H), 7.42 (d, J = 3Hz, 1H), 7.60 (t, J = 9Hz, 1H), 8.05 (br d, J = 9Hz, 1H). MS (FAB^+): m/e 501 ($M + H^+$), 523 ($M + Na^+$). Anal. calcd for $C_{28}H_{40}N_2O_6 \cdot 1.0\ H_2O$: C, 64.84; H, 8.16; N, 5.39. Found: C, 64.81; H 7.92; N, 4.99.

Boc-Trpψ[E-CH=CH]Nle-OH (1c)

Anhydrous powdered potassium carbonate (0.37 g, 2.7 mmol) was added to a solution of the above acid **12** (55 mg, 0.11 mmol) in MeOH (12 mL). The reaction mixture was stirred and heated under reflux for 6 h. The mixture was filtered and then concentrated to a volume of 1–2 mL. The residual solution was diluted with H_2O, acidified to pH 5 with 10% aqueous citric acid, and extracted with $CHCl_3$. The organic extracts were combined and washed successively with water and brine, then dried, filtered and concentrated. Flash chromatography (hexanes/EtOAc-/HOAc, 50/50/1 to 20/80/1) yielded pure product as an oil (23 mg, 53%). ^1H NMR (500 MHz, DMSO-d_6) (130 °C) δ 0.85 (m, 3H), 1.18–1.32 (m, 4H), 1.36 (s, 9H), 1.60 (m, 1H), 1.68 (m, 1H), 2.65 (m, 1H), 2.78–3.10 (m, 2H, obscured by HDO peak, visible at 90 °C), 4.20 (br m, 1H), 5.48 (m, 1H) irradiation at δ 2.65 causes collapse of these signals to 5.46 (d, J = 16Hz, 0.5H) & 5.47 (d, J = 15Hz, 0.5H), 5.58 (dd, J = 8, 16 Hz, 0.5H), 5.63 (dd, J = 8, 15Hz, 0.5H), 6.96 (t, J = 7.5Hz, 1H), 7.04 (t, J = 7.5Hz, 1H), 7.08 (d, J = 10Hz, 1H), 7.32 (d, J = 10Hz, 1H), 7.53 (dd, J = 3, 7.5Hz, 1H), 10.41 (br d, J = 12Hz, 1H). HRMS (FAB^+) calcd for $C_{23}H_{32}N_2O_4$: 400.2362. Found: 400.2359.

N-*Cbz-Leucine-3,5-dimethyl pyrazolide (13)*

This compound was prepared from Cbz-L-Leu-OH on a 34 mmol scale according to the general procedure to give 10.0 g (84%) of crystalline pyrazolide. ^1H NMR (300 MHz) δ 0.93 and 1.04 (2d, 6H), 1.55 (m, 1H), 1.76 (m, 2H), 2.22 (s, 3H), 2.50 (s, 3H), 5.11 (dd, 2H), 5.45 (br d, 1H), 5.62 (m, 1H), 5.95 (s, 1H), 7.25–7.4 (Ar–H, 5H).

Ethyl (6S)-6-[(benzyloxycarbonyl)amino]-8-methyl-2,4-nonadienoic acid (15)

To a suspension of LAH (120 mg, 3.16 mmol) in anhydrous THF (45 mL) under nitrogen at -78 °C was added dropwise over 10 min a solution of pyrazolide **13** (1.0 g, 2.91 mmol) in anhydrous THF (15 mL). Upon completion of the addition the suspension was allowed to stir for 40 min at -78 °C. The reaction mixture was subsequently quenched by the addition of EtOAc (10 mL) followed by the addition of 20–25 mL 10% citric acid solution and warming to room temperature. The resulting suspension was partitioned between dilute citric acid (300 mL) and EtOAc. The aqueous phase was extracted with EtOAc and the combined extracts were washed with H_2O then dried, and concentrated *in vacuo* to give aldehyde **14** as a clear oil. The aldehyde was sufficiently pure as isolated and further purification was not attempted. To a suspension of sodium hydride, 80% dispersion in mineral oil, (210 mg, 7.0 mmol) in anhydrous THF (20 mL) under nitrogen at 0–5 °C was added dropwise over 3 min a solution of triethyl-4-phosphonocrotonate, 80% technical grade, (2.37 g, 7.57 mmol) in anhydrous THF (5 mL). The orange red solution was allowed to stir for 0.5 h at 0 °C before a solution of the aldehyde **14** in anhydrous THF (10 mL) was added dropwise over 5 min. The reaction mixture was

allowed to stir at 0 °C for 40 min. The crude product was isolated by partitioning the reaction mixture between EtOAc and dilute citric acid solution. The aqueous phase was extracted with EtOAc. The combined organics were washed with H_2O, then dried, filtered and concentrated *in vacuo*. Flash chromatography (EtOAc/hexanes, 1/4) gave 0.60 g (60%) of the ester **15** (as a mixture of geometric isomers) as an oil. ^{1}H NMR (300 MHz) δ 0.93 (m, 6H), 1.39 (t, 3H), 1.49 (m, 2H), 1.64 (m, 1H), 4.2 (q, 2H), 4.35 (m, 1H), 4.66 (br d, 1H), 5.11 (s, 2H), 5.86 (d, J = 15Hz, 1H), 5.96 (dd, J = 6, 15Hz, 1H), 6.28 (m, 1H), 7.24 (dd, J = 10.5, 15Hz, 1H), 7.35 (Ar–H, 5H). HRMS calcd for $C_{20}H_{27}NO_4$: 346.2018 $(M + H)^+$. Found: 346.2033.

(6S)-6-[(Benzyloxycarbonyl)amino]-8-methyl-2,4-nonadien-1-ol (16)

A solution of the ethyl ester **15** (4.47 g, 12.9 mmol) in anhydrous toluene (130 mL) under nitrogen was cooled to -78 °C. To this was added pre-cooled DIBAL (1.0 M solution in toluene, 52 mL, 52 mmol) over 30 min. Upon completion of the addition the reaction mixture was warmed to -10 °C. After approximately 3 h the reaction mixture was quenched by the addition of methanol and then partitioned between dilute citric acid solution and EtOAc. The aqueous phase was extracted twice with EtOAc and the combined organics dried, filtered and concentrated *in vacuo*. Flash chromatography (EtOAc/hexanes, 1/4 to 1/1) afforded dienol **16** (1.51 g, 51%) and recovered starting material **15** (1.11 g). The dienol **16** was isolated as a mixture of geometric isomers. ^{1}H NMR (300 MHz) δ 0.92, (6H), 1.37 (m, 2H), 1.64 (m, 1H), 4.17 (d, 2H), 4.25 (m, 1H), 4.63 (br d, 1H), 5.1 (br s, 2H), 5.57 (m, 1H), 5.83 (m, 1H), 6.2 (m, 2H), 7.35 (Ar–H, 5H). HRMS calcd for $C_{18}H_{26}NO_3$: 304.1913 $(M + H)^+$. Found: 304.1911.

Methyl (3RS, 6S)-(4E)-6-[(benzyloxycarbonyl)amino]-8-methyl-3-vinyl-4-nonenoate (17)

A mixture of dienol **16** (1.51 g, 4.98 mmol), trimethyl orthoacetate (17.1 mL, 134 mmol) and benzoic acid (0.05 g, 0.41 mmol) in xylenes (75 mL) was heated at reflux for 3 h under a nitrogen atmosphere. Upon cooling the reaction mixture was washed with 5% Na_2CO_3 solution and H_2O, then dried, filtered and concentrated *in vacuo*. Flash chromatography on silica gel (EtOAc/hexanes, 1/4 to 1/2) gave the rearranged ester (1.23 g, 67%, a mixture of diastereomers) as a light yellow oil. ^{1}H NMR (300 MHz) δ 0.90 (6H), 1.32 (m, 2H), 1.6 (m, 1H), 2.41 (br d, 2H), 3.24 (m, 1H), 3.64 (2s, 3H), 4.19 (br m, 1H), 4.58 (br d, 1H), 5.01 (2t, 2H), 5.09 (br s, 2H), 5.25–5.6 (m, 2H), 5.72 (m, 1H), 7.35 (Ar–H, 5H). HRMS calcd for $C_{21}H_{30}NO_4$: 360.2175 $(M + H)^+$. Found: 360.2178.

(3RS, 6S)-6-[(Benzyloxycarbonyl)amino]-8-methyl-3-vinyl)-4-nonenoic acid (18)

To a cooled (0 °C) solution of methyl ester **17** (1.23 g, 3.42 mmol) was added 3 mL of 2N NaOH. After stirring at room temperature overnight the reaction mixture was partitioned between EtOAC and dilute aqueous hydrochloric acid. The organic phase was dried, then filtered and concentrated *in vacuo*. Flash chromatography (EtOAc/-hexanes, 1/2 to 1/1 containing 1% HOAc) gave acid **18** (1.05 g, 89%, diastereomeric mixture) as a light yellow oil. ^{1}H NMR (300 MHz) δ 0.90 (6H), 1.33 (m, 2H), 1.62 (m, 1H), 2.45 (br d, 2H), 3.23 (m, 1H), 4.2 (m, 1H), 4.63 (m, 1H), 5.05 (br d, 2H), 5.10 (br s, 2H), 5.25–5.65 (m, 2H), 5.74 (m, 1H), 7.35 (Ar–H, 5H). HRMS calcd for $C_{20}H_{28}NO_4$: 346.2018 $(M + H)^+$. Found: 346.2018.

tert-*Butyl (3RS, 6S)-(4E)-6-[(benzyloxycarbonyl)amino]-8-methyl-3-vinyl)-4-nonenoate (19)*

To a stirred solution of the acid **18** (471 mg, 1.36 mmol) in anhydrous methylene chloride (8 mL) at room temperature under a nitrogen atmosphere was added a solution of *tert*-butyl-2,2,2-trichloroacetimidate (690 mg, 3.16 mmol) in cyclohexane (8 mL) followed by BF_3 etherate (0.07 mL). After approximately 1 h the reaction mixture was concentrated *in vacuo*. Flash chromatography (EtOAc/hexane, 1/6 to 1/4) gave *tert*-butyl ester **19** (400 mg, 73%) as a clear oil. ^{1}H NMR (300 MHz) δ 0.90 (2d, 6H), 1.33 (m, 2H), 1.41 (s, 9H), 1.62 (m, 1H), 2.3 (br d, 2H), 3.2 (m, 1H), 4.2 (br m, 1H), 4.55 (br m, 1H), 4.95–5.05 (m, 2H), 5.10 (br s, 2H), 5.38 (dd, J = 6, 15Hz, 1H), 5.53 (dd, J = 6, 15Hz, 1H), 5.72 (m, 1H), 7.35 (Ar–H, 5H). HRMS calcd for $C_{24}H_{36}NO_4$: 402.2644 $(M + H)^+$. Found: 402.2642.

tert-*Butyl (3RS, 6S)-(4E)-6-[benzyloxycarbonyl(*tert-*butoxycarbonyl)amino]-8-methyl-3-vinyl)-4-nonenoate (20)*

The *tert*-butyl ester **19** (65 mg, 0.161 mmol) was dissolved in acetonitrile (1.5 mL) and treated with excess boc-dicarbonate (100 mg, 0.46 mmol) followed by a catalytic amount of DMAP and allowed to stir at room temperature overnight. The reaction mixture was applied directly to a preparative TLC plate (20 x 20 cm, 1 mm thick silica gel GF eluted with EtOAc/hexanes, 1/4) to give the bis-protected compound **20** (73 mg, 89%) as a clear oil. ^{1}H NMR (300 MHz) δ 0.86 (m, 6H), 1.42 (2-Boc, 18H), 1.49 (m, 2H), 1.73 (m, 1H), 2.29 (m, 2H), 3.18 (m, 1H), 4.77 (m, 1H), 4.95–5.08 (m, 2H) 5.2 (s, 2H), 5.53 (m, 1H), 5.65–5.80 (m, 2H), 7.3–7.45 (5 Ar–H). HRMS calcd for $C_{29}H_{43}NO_6Na$: 524.2988 $(M + Na)^+$. Found: 524.2996.

BocCbz-Leuψ[E-CH=CH]Asp(β-O-tBu)-OH (23)

To a solution of compound **20** (252 mg, 0.50 mmol) in acetone (9 mL) at room temperature was added a solution of NMO (75 mg, 0.64 mmol) in water (3.5 mL) followed by OsO_4 (0.175 mL, 2.5% solution in *tert*-butanol). After stirring for 4 h the reaction mixture was quenched by the addition of sodium thiosulfate (28 mg) followed by talc (0.7 g) and stirring for 10 min at room temperature before vacuum filtration through Celite. The filtrate was partitioned between dilute aqueous hydrochloric acid and EtOAc. The organic phase was washed with water, then dried, filtered and concentrated *in vacuo*. Flash chromatography (EtOAc/hexanes, 1/4 to 1/1) gave the diol

21 (140 mg, 52%) as a mixture of diastereomers. HRMS for this diol: calcd for $C_{29}H_{45}NO_8Na$: 558.3043 $(M + Na)^+$. Found 558.3046. To a solution of this diol (140 mg, 0.26 mmol) in methanol (6 mL) was added a suspension of $NaIO_4$ (360 mg, 1.68 mmol) in water (2.5 mL) and the mixture was allowed to stir for 15 min at room temperature. The reaction mixture was partitioned between EtOAc and brine. The organic phase was dried, then filtered and concentrated *in vacuo* to yield aldehyde **22** as a clear oil. The crude aldehyde was immediately dissolved in acetone (7 mL) and the solution cooled in an ice bath. To this was added excess (nine drops) of Jones reagent and the mixture allowed to stir for 10 min at 0 °C before quenching took place by partitioning between EtOAc and dilute aqueous HCl. The organic phase was washed with water, then dried, filtered and concentrated *in vacuo*. Flash chromatography (EtOAc/hexanes, 1/2 to 1/1 containing 1% HOAc) gave the acid **23** (127 mg, 94% from **21**) as a clear oil. ^1H NMR (300 MHz) δ 0.86 (br t, 6H), 1.42 (s, 18H), 1.49 (m, 2H), 1.76 (m, 1H), 2.42 (2dd, 1H), 2.71 (2dd, 1H), 3.45 (m, 1H), 4.78 (m, 1H), 5.2 (br s, 2H), 5.63 (m, 1H), 5.89 (m, 1H), 7.3–7.45 (Ar–H, 5H). HRMS calcd for $C_{28}H_{41}NO_8Na$: 542.2730 $(M + Na)^+$. Found: 542.2736.

*Boc-Leuψ[E-CH=CH]Asp(β-O-tBu)-OH (**Id**)*

To a solution of the doubly protected acid **23** (120 mg, 0.23 mmol) in methanol (5 mL) at 0 °C was added 2N NaOH (2 mL). The mixture was stirred for 3.5 h. The reaction mixture was concentrated *in vacuo* and the residue partitioned between EtOAc and dilute aqueous HCl. The organic phase was dried, then filtered and concentrated *in vacuo*. Flash chromatography (EtOAc/hexanes, 1/2 to 1/1 contains 1% HOAc) gave **Id** (80 mg, 87%) as a mixture of diastereomers. ^1H NMR (300 MHz) δ 0.90 (2d, 6H), 1.2–1.45 (m, 2H), 1.43 (s, 18H), 1.55–1.7 (m, 1H), 2.47 (2dd, 1H), 2.75 (2dd, 1H), 3.48 (m, 1H), 4.14 (br s, 1H), 4.4 (br s, 1H), 5.5–5.7 (m, 2H). HRMS calcd for $C_{20}H_{36}NO_6$: 386.2542 $(M + H)^+$. Found: 386.2539.

General Procedure to Prepare 2,4,5-Trichlorophenol (Tcp) Ester from Fully Protected Dipeptide Isosteres

To a solution of protected dipeptide isostere (0.5 mmol) in CH_2Cl_2 was added EDCI (229 mg, 1.2 mmol) and 2,4,5-trichlorophenol (1.0 mmol) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was washed with 10% citric acid, water and brine. The organic layer was dried, then filtered and concentrated *in vacuo*. Flash chromatography on silica gel (EtOAc/hexanes) afforded the desired product.

General Procedure to Couple 2,4,5-Trichlorophenol (Tcp) Ester with CCK Fragments

A solution of Tcp ester, HCl or TFA salt of CCK fragments and base (either triethyl amine [Et_3N], 4-methylmorpholine [NMM], or *N,N*-diisopropylethylamine [DIEA], 2 eq.) in DMF was stirred at room temperature overnight. The reaction mixture was added to ice cold 10% citric acid solution with vigorous stirring. The white precipitate that formed was filtered off and was purified either by trituration with EtOAc or Et_2O, or by flash chromatography (EtOAc/Py/H_2O/HOAc) and then lyophilized to afford a fluffy powder.

General Procedure to Deprotect Boc and *t*-Butyl Ester of CCK Fragments

A solution of Boc protected psuedopeptide (1.0 mmol) in acetic acid (5 mL) was treated with 1.4N HCl/HOAc (10 mL) at ambient temperature and the flask capped with a drierite filled drying tube. After 1 h the contents of the flask were frozen and lyophilized. The crude hydrochloride was sufficiently pure for use as isolated.

*Boc-Leuψ[E-CH=CH]Gly-Trp-Leu-Asp-PheNH$_2$ (**IIa**)*

Boc-Leuψ[*E*-CH=CH]Gly-OH **Ia** (359 mg, 1.32 mmol) was converted to its corresponding Tcp active ester in 60% yield according to the general procedure. ^1H NMR (300 MHz) δ 0.93 (2d, 6H), 1.46 (s, 9H), 1.32–1.40 (m, 1H), 1.57–1.76 (m, 2H), 3.35 (d, 2H, J = 7Hz), 4.10–4.25 (br, 1H), 4.40–4.54 (br, 1H), 5.61 (dd, 1H, J = 7, 16Hz, CH_a=C), 5.78 (dt, 1H, J = 7, 16Hz, C=CH_b), 7.26 (s, 1H), 7.55 (s, 1H). The target peptide **IIa** was prepared from the above Tcp ester (200 mg, 0.44 mmol), HCl·Trp-Leu-Asp-PheNH$_2$ (273 mg, 0.44 mmol) and Et_3N (0.12 mL, 0.88 mmol) in DMF (8 mL) according to the general procedure. Following flash chromatography (EtOAc/Py/HOAc/H_2O, 12/4/0.3/0.5), the purified product was lyophilized to afford **IIa** (170 mg, 46%) as a fluffy powder. ^1H NMR (500 MHz, DMSO-d_6) δ 0.80–0.86 (overlaping d, 12H), 1.05–1.63 (m, 6H), 1.36 (s, 9H), 2.43 (dd, 1H), 2.58 (dd, 1H), 2.71–2.98 (m, 4H), 3.08 (dt, 2H), 3.85–3.96 (m, 1H), 4.21–4.37 & 4.43–4.60 (m, 4α-H), 5.34 (dd, 1H, J = 5.7, 15Hz, CH_a=C), 5.45 (dt, 1H, J = 9, 15Hz, C=CH_b), 6.90–7.31 (m, 10 Ar–H), plus 9 D_2O exchangeable protons. MS (FAB^-): m/e 830 $(M - H^+)$, Anal. calcd for $C_{44}H_{61}N_7O_9 \cdot 1.0\ H_2O$: C, 62.16; H, 7.41; N, 11.53. Found: C 61.80; H 7.47; N, 11.19.

*Boc-Glyψ[E-CH=CH]Trp-Leu-Asp-PheNH$_2$ (**IIb**)*

Boc-Glyψ[*E*-CH=CH]Trp-OH **Ib** (63 mg, 0.18 mmol) was converted to its corresponding Tcp ester in 60% yield according to the general procedure. ^1H NMR (300 MHz) δ 1.46 (s, 9H), 3.12 (dd, J = 7, 15Hz, 1H), 3.40 (dd, J = 8, 15Hz, 1H), 3.65–3.81 (m, 3H), 4.50 (br s, 1H), 5.67 (dt, J = 5, 15Hz, 1H, CH_a=C), 5.83 (dd, J = 8, 15Hz, 1H, C=CH_b), 6.89 (s, 1H), 7.07 (s, 1H), 7.15 (t, J = 7.5Hz, 1H), 7.22 (t, J = 7.5Hz, 1H), 7.38 (d, J = 7.5Hz, 1H), 7.49 (s, 1H), 7.61 (d, J = 7.5Hz, 1H), 8.10 (s, 1H). The target peptide **IIb** was prepared from the above Tcp ester (57 mg, 0.11 mmol), HCl·Leu-Asp-PheNH$_2$ (48 mg, 0.11 mmol) and DIEA (38 μL, 0.22 mmol) in DMF (1 mL) according to the general procedure. The crude product was

suspended in Et_2O, then centrifuged. This procedure was repeated twice and then the solid collected was lyophilized to give target peptide **Boc-Glyψ[*E*-CH=CH]Trp-Leu-Asp-PheNH$_2$ IIb** (46 mg, 59%). ^{1}H NMR (300 MHz, CD_3OD) δ 0.62 (dd, 3H), 0.84 (dd, 3H), 1.15–1.23 (m, 1H), 1.35–1.57 (m, 2H), 1.42 (s, 9H), 2.50–2.84 (m, 2H), 2.84–3.01 (m, 2H), 3.08–3.28 (m, 2H), 3.35–3.48 (m, 1H), 3.59 (dd, 2H), 4.18–4.29 (m, 1H), 4.48–4.59 (m, 2H), 5.53 (dt, J = 6, 15Hz, 1H, CH_a=C), 5.70 (m, 1H, C=CH_b), 6.94–7.03 (m, 2H), 7.06 (t, J = 8Hz, 1H), 7.12–7.27 (m, 5H), 7.30 (d, J = 8Hz, 1H), 7.54 (d, J = 8Hz, 1H). HRMS calcd for $C_{38}H_{51}N_6O_8$ (M + H)$^+$: 719.3768. Found: 719.3749. Anal. calcd for $C_{38}H_{50}N_6O_8 \cdot 1.5\ H_2O$: C, 61.17; H, 7.18; N, 11.27. Found: C, 61.33; H 7.05; N, 11.18.

Boc-Trpψ[E-CH=CH]Nle-Asp-Phe-NH$_2$ (IIc)

A stirred solution of Boc-Trpψ[*E*-CH=CH]Nle-OH **Ic** (22 mg, 0.055 mmol) and NMM (6.5 μL, 0.06 mmol) in THF (1 mL) under N_2 was cooled in an ice/acetone bath then treated with isobutyl chloroformate (9 μL, 0.06 mmol). After 10 min, a solution of TFA·Asp-Phe-NH_2 (32 mg, 0.08 mmol) and NMM (9 μL, 0.08 mmol) in DMF (0.5 mL) was added. And the mixture was stirred for 4 h. The mixture was concentrated to dryness and the residue was chromatographed (EtOAc/pyridine/H_2O/HOAc, 148:20:11:6). Fractions were pooled and lyophilized to afford 18 mg (52%) of fluffy powder. HPLC analysis indicated the material was <90% pure; therefore, a 12 mg portion was subjected to further purification by reverse-phase HPLC (Vydac C_{18}, 20 to 50% CH_3CN:A over 40 min ; A = 50 mM NH_4OAc, pH 4.5) which yielded 2.2 mg of material judged >98% pure by analytical HPLC (Vydac C_{18}, 30 to 80% CH_3CN:A over 15 min; A = 0.1% TFA: t_R=10.4, 10.5 min; A = 50 mM NH_4OAc, pH 4.5: t_R = 10.4, 10.5 min). HRMS (FAB$^+$) calcd for M + H$^+$ = $C_{36}H_{48}N_5O_7$: 662.3554. Found: 662.3553. ^{1}H NMR (500 MHz, CD_3OD): δ 0.8–0.95 (m) & 1.0–1.65 (2m, 18H), 2.48 (m, 1H), 2.62 (m, 1H), 2.78 (m, 1H), 2.85–2.98 (m, 3H), 3.20 (m, partially obscured), 4.30 & 4.35 (2m, 1H), 4.52 (m, 2H), 5.41 (m, 1H), 5.55 (m, 1H), 7.00 (m, 2H), 7.05 (m, 1H), 7.15 (m, 1H), 7.22 (m, 4H), 7.30 (d, J = 8Hz, 1H), 7.55 (m, 1H), 8.55 (br s, 1H).

Boc-Trp-Leuψ[E-CH=CH]Asp-PheNH$_2$ (IIdA) & (IIdB)

Boc-Leuψ[CH=CH]Asp(β-*O*-*t*-Bu)-OH **Id** (87 mg, 0.23 mmol) was converted to its corresponding Tcp ester according to the general procedure and was coupled with PheNH$_2$ (45 mg, 0.3 mmol) with Et_3N as base according to the general procedure to afford Boc-Leuψ[*E*-CH=CH]Asp(β-*O*-*t*-Bu)-PheNH$_2$ as a pair of diastereomers which were separated by flash chromatography (EtOAc/-hexanes/HOAc, 3/1/1%) to give isomer A (47 mg, 39%) and isomer B (50 mg, 44%). The NMR data of two separated tripeptide analogs are as follows: **Isomer A (IdA)**: ^{1}H NMR (500 MHz) δ 0.90 (2d, 6H), 1.25–1.32 (m, 1H), 1.40, 1.44 (2s, 18H), 1.62(m, 2H), 2.58 (dd, 1H, J = 5.5, 17Hz, Asp-β-H), 2.69 (dd, 1H, J = 5.8, 17Hz, Asp-β-H), 3.07–3.12 (m, 2H) 3.24–3.30 (m, 1H), 4.00 (dt, 1H, 6.8, 7.5Hz), 4.46 (m, 1H), 4.65–4.72 (m, 1H, Phe-α-H), 5.33 (m, 1H), 5.44 (dd, 1H, J = 6.7, 15Hz, CH_a=C), 5.61 (dd, 1H, J = 9.2, 15Hz, C=CH_b), 6.60 (2H, D_2O exchangeable), 7.18–7.33 (m, 5 Ar-H). **Isomer B (IdB)**: ^{1}H NMR (500 MHz) δ 0.90 (2d, 6H), 1.18–1.30 (m, 1H), 1.39, 1.45 (2s, 18H), 1.52–1.63 (m, 1H), 2.33 (dd, 1H, J = 5.0, 17Hz, Asp-β-H), 2.83 (dd, 1H, J = 9.5, 17Hz, Asp-β-H), 3.07–3.21 (m, 3H) 4.00 (m, 1H), 4.30 (m, 1H), 4.74 (m, 1H, Phe-α-H), 5.34 (dd, 1H, J = 5, 15.7Hz, CH_a=C), 5.45 (dd, 1H, J = 7.8, 15.7Hz, C=CH_b), 6.06 (br d, 1H), 6.62 (br s, 1H), 7.18–7.40 (m, 5 Ar–H). Boc-Leuψ[*E*-CH=CH]Asp(β-*O*-*t*-Bu)-PheNH$_2$ **IdA** (47 mg, 0.085 mmol) was deprotected with 1.4N HCl/HOAc (3 mL) according to the general procedure to give HCl·Leuψ[*E*-CH=CH]Asp-PheNH$_2$ which was coupled with Boc-Trp-Osu (72 mg, 0.18 mmol) following the general procedure. The crude product was purified by triturating it with Et_2O, then collected by centrifuge and lyophilized to give target peptide **Boc-Trp-Leuψ[*E*-CH=CH]Asp-PheNH$_2$ IIdA** (29 mg, 50%). ^{1}H NMR (500 MHz, CD_3OD) δ 0.85 (br d, 6H), 1.12–1.35 (m, 2H), 1.38 (s, 9H), 1.47–1.65 (m, 1H), 2.25 (dd, 1H, J = 5.7, 16.6Hz, Asp-β-H), 2.52 (dd, 1H, J = 8.2, 16.6Hz, Asp-β-H), 2.99 (dt, 2H, J = 5.7, 8.2Hz), 3.14 (dd, 2H, J = 6.1, 14Hz), 3.18–3.26 (m, 1H), 4.32 (t, 2H, J = 7Hz), 4.60 (dd, 1H, J = 7, 8Hz), 5.20 (dd, 1H, J = 7, 15.5Hz, CH_a=C), 5.30 (dd, 1H, J = 10.6, 15.5Hz, C=CH_b), 6.97–7.70 (m, 10 Ar–H). MS (FAB$^+$): m/e 662 (M + H$^+$): Anal. calcd for $C_{36}H_{47}N_5O_7 \cdot 1.5\ H_2O$: C, 62.76; H, 7.02; N, 10.17. Found: C, 62.66; H 6.82; N, 9.95. Boc-Trp-Leuψ[*E*-CH=CH]Asp-PheNH$_2$ **IIdB** was prepared in a 40% yield from Boc-Leuψ[*E*-CH=CH]Asp-PheNH$_2$ **IdB** in an identical fashion as for isomer A except that the crude product was triturated with EtOAc and lyophilized. ^{1}H NMR (500 MHz, CD_3OD) δ 0.85 (br d, 6H), 1.10–1.30 (m, 2H), 1.40 (s, 9H), 1.43–1.58 (m, 1H), 2.25 (dd, 1H, J = 4, 17Hz, Asp-β-H), 2.72 (dd, 1H, J = 6, 17Hz, Asp-β-H), 2.82 (dd, 1H, J = 10.4, 14Hz), 3.00 (dd, 1H, J = 6.1, 14Hz), 3.13 (m, 1H), 3.10–3.22 (m, 2H), 4.26–4.30 (m, 2H), 4.62 (dd, J = 4, 10.2Hz), 4.80 (m, 1H), 5.00 (dd, 1H, J = 7.2, 15.5Hz, CH_a=C), 5.18 (dd, 1H, J = 5.2, 15.5Hz, C=CH_b), 6.90–7.60 (m, 10 Ar–H). MS (FAB$^+$): m/e 662 (M + H$^+$): Anal. calcd for $C_{36}H_{47}N_5O_7 \cdot 1.5\ H_2O$: C, 62.76; H, 7.02; N, 10.17. Found: C, 62.90; H 6.93; N, 9.95.

Boc-Trp-Leu-Aspψ[E-CH=CH]PheNH$_2$ (IIe)

Boc-Asp(β-*O*-*t*Bu)ψ[*E*-CH=CH]PheNH$_2$ **Ie** (40 mg, 0.1 mmol) was deprotected with 1.4N HCl/HOAc (3 mL) according to the general procedure to give HCl·Aspψ[*E*-CH=CH]PheNH$_2$ which was coupled with the symmetrical anhydride derived from Boc-Leucine (100 mg, 0.4 mmol) and EDCI (38 mg, 0.2 mmol) to give Boc-Leu-Aspψ[*E*-CH=CH]PheNH$_2$ (37 mg, 73%). ^{1}H NMR (500 MHz, CD_3OD) δ 0.92 (2d, 6H), 1.42–1.52 (m, 2H), 1.44 (s, 9H), 1.61–1.72 (m, 1H), 2.46–2.57 (4dd, 2H), 2.73 (dd, 1H, J = 7.1, 13.5Hz) & 2.72 (dd, J = 7.4, 13.7Hz, 1H), 2.98 (dd, J = 7.5, 13.7Hz) & 3.02 (dd, J = 7.7, 13.5Hz,

1H), 3.15–3.22 (m, 1H), 4.02–4.05 (m, 1H), 4.66–4.70 (m, 1H), 5.18 (dd, J = 6, 15Hz) & 5.53 (dd, J = 5.8, 15.5Hz, 1H), 5.71 (dd, J = 8.5, 15.5Hz) & 7.52 (dd, J = 8.5, 15Hz, 1H), 7.15–7.22 (m, 5 Ar–H). Boc-Leu-Aspψ[*E*-CH=CH]PheNH_2 (37 mg, 0.07 mmol) from above was deprotected with 1.4N HCl/HOAc (2 mL) following the general procedure to give HCl·Leu-Aspψ[*E*-CH=CH]PheNH_2, which was coupled with Boc-Trp-Osu (56 mg) with Et_3N as base by following the general procedure. The crude product was purified by trituration with Et_2O, then collected by centrifuge and lyophilized to give target peptide **Boc-Trp-Leu-Aspψ[*E*-CH=CH]PheNH_2 IIe** (37 mg, 80%). ^{1}H NMR (300 MHz, CD_3OD) δ 0.87 (2br s, 6H), 1.15–1.32 (m, 2H), 1.37 (s, 9H), 1.43–1.55 (m, 2H), 2.40–2.52 (m, 2H), 2.72 (dd, J = 7.2, 13.4Hz), & 2.74 (dd, J = 7, 13.4Hz, 1H), 3.00 (dd, J = 2.3, 7.5Hz) & 3.04 (dd, J = 2.3, 7.5Hz, 1H), 3.05–3.20 (m, 1H), 3.20–3.30 (m, 2H), 4.30–4.40 (m, 2H), 4.60–4.68 (m, 1H), 5.47 (dd, J = 4, 15.5Hz) & 5.50 (dd, J = 5, 15.5Hz, 1H), 5.71 (br d, J = 8.4, 15.5Hz, 1H), 6.90–7.60 (m, 10 Ar–H). MS (FAB$^+$): m/e 662 (M + H$^+$): Anal. calcd for $C_{36}H_{47}N_5O_7 \cdot 2.0\ H_2O$: C, 61.95; H, 7.37; N, 10.04. Found: C, 61.61; H 6.83; N, 9.88.

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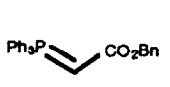
16. Preparation of phosphorane **7** was carried out by using a procedure analogous to that for the corresponding benzyl substituted phosphorane, which involved alkylation of unsubstituted phosphorane **24** with benzyl bromide.[17] Because of the more forcing conditions required for alkylation by the less reactive *n*-butyl bromide, some side reactions were observed. A major by-product was di-*n*-butyl carbonate **25** (from alkylation of the carbonate present as base), which was readily separated from the basic ylide by extractive procedures (see Experimental Section).



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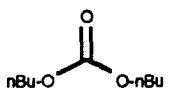

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