effects of *meta* and *para* unshared-pair donor substituents in side-chain reactivities of benzene are restricted to elements of the first row in even the most favorable systems.[8] We suggest that these restrictions are to be associated with the intervention of additional interaction mechanisms in the R effects which arise because of the relatively weak electronic demands placed upon the substituent by the bonded phenyl function (which acts as an available electron source). That is, the additional orbital participation by the first atom of X, *e.g.*

$$=\ddot{\underset{\cdot\cdot}{\underset{\cdot\cdot}{I}}}^{-}\ (\pi(p\text{-}d))^{9} \text{ or } -\overset{+}{S}=C=N^{-}$$

which may then be involved precludes such unshared-pair donor substituents from displaying the $\pi(p\text{-}p)$ σ_I order of R effects. Therefore the aromatic side-chain reactivities require a minimum of two substituent parameters (*e.g.*, σ_I and σ_R or σ_m and σ_p) for generalized description.[10]

(9) J. R. Hoyland and L. Goodman, *J. Phys. Chem.*, **64**, 1816 (1960); *cf.* also ref. 6, footnote 38b.

(10) Evidently at least two substituent parameters are also required to describe substituent effects in general for the methyl cation stabilization energies or the substituted benzene ionization potentials (*i.e.*, to include substituents, *e.g.*, CH_3, C_6H_5, H, CN, etc., with the unshared-pair donor substituents).

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A Rapid Synthesis of Oligopeptide Derivatives without Isolation of Intermediates

Sir:

We wish to report a novel use of a water-soluble carbodiimide [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride[1]] for the extremely rapid and facile synthesis of pure, protected oligopeptides (4–7 units) without isolation of intermediates.[2] By this technique, pure tetra- and pentapeptide derivatives (compounds **1–7**), corresponding to amino acid sequences in a strepogenin-active peptide isolated from acid digests of insulin[3] and at the active sites of certain enzymes, have been prepared in 2.5 to 3.5 days, typically in yields of 35–56% over-all. The peptides contained amino acids notoriously troublesome in peptide synthesis, including serine, threonine, methionine, and histidine.

For a typical coupling step, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.1 equiv.) was added to a solution of the N-carbobenzyloxyamino acid (1–1.1 equiv.), the amino acid ester hydrochloride or peptide ester hydrochloride (1 equiv., 5 or 10 mmoles), and triethylamine (1 equiv.) in methylene chloride (20 ml.). After 1 hr. at room temperature the solution was washed successively with water, dilute hydrochloric acid, water, sodium bicarbonate solution, and water. The dried solution was evaporated under reduced pressure and the solid (usually crystalline)

(1) J. C. Sheehan, P. A. Cruickshank, and G. L. Boshart, *J. Org. Chem.*, **26**, 2525 (1961).

(2) Throughout this work the L-forms of amino acids were used.

(3) R. B. Merrifield and D. W. Woolley, *J. Am. Chem. Soc.*, **78**, 358 (1956).

Table I

No.	Compound	Over-all yield, %	Mol. formula	M.p., °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	$[\alpha]_D$, deg.	Temp., °C.	Concn., %	Solvent
1	Z-Ser-His(Bzl)-Leu-Val-Glu-(OEt)$_2$[a]	40	$C_{44}H_{61}O_{11}N_7$	188–190	61.15	60.94	7.12	7.16	11.35	11.22	−22.7	25	2.0	DMF
2	Z-Ser-His-Leu-Val-Glu(OEt)$_2$[a–c]	11	$C_{37}H_{55}O_{11}N_7$	210–213	57.42	57.42	7.16	7.01	12.67	12.32	−47.0	25	3.0	Ethanol
3	Z-His(Z)-Leu-Val-Glu(OEt)$_2$[a]	42	$C_{43}H_{56}O_{11}N_6$	175–176	61.44	61.29	6.88	6.96	10.24	10.12	−12.6	26	2.0	DMF
4	Z-His(Bzl)-Leu-Val-Glu(OEt)$_2$[a]	54	$C_{41}H_{56}O_9N_6$	157–158	63.38	63.04	7.27	7.16	10.82	11.03	−21.5	25	2.2	DMF
5	Z-Glu(OBzl)-Ser-Ala-Gly-OEt[d]	56	$C_{30}H_{38}O_{10}N_4$	167–170	58.62	58.54	6.23	6.06	9.12	8.83	+2.7	25	2.0	DMF
6	BOC-Thr-Ser-Met-Ala-OEt[c,e]	9	$C_{22}H_{40}O_9N_5S$	100–105	49.22	49.29	7.51	7.63	10.44	10.66	−13.5	26	2.2	DMF
7	Z-Gly-Asp(OBzl)-Ser-Gly-OEt[f]	35	$C_{28}H_{34}O_{10}N_4$	135–137	57.33	57.18	5.84	5.81	9.55	9.42	−14.4	25	2.2	DMF

[a] Z, $C_6H_5CH_2OCO$; Bzl, $CH_2C_6H_5$; BOC, $(CH_3)_3COCO$; DMF, dimethylformamide. Peptide sequence in a strepogenin-active peptide isolated from acid digests of insulin.[3] [b] R. B. Merrifield and D. W. Woolley, *J. Am. Chem. Soc.*, **78**, 4646 (1956), report m.p. 213°, $[\alpha]^{27}D$ −46.3° (*c* 3, ethanol). [c] Low yield in final condensation. [d] Peptide sequence at active site of pseudocholinesterase and liver aliesterase (horse). [e] Peptide sequence at active site of subtilisin and mold protease. [f] Peptide sequence at active site of trypsin, chymotrypsin, thrombin, and elastase. [g] *Cf.* F. Sanger, *Proc. Chem. Soc.*, 76 (1963).

was weighed (yield about 80%). (See Table I for analyses and physical properties.)

Hydrogenolysis of the N-carbobenzyloxy group over 10% palladium on charcoal (0.4 g.) in alcoholic solution containing 1 *N* hydrochloric acid (1 equiv.) was usually complete in 1 hr. The residue obtained after removal of catalyst and solvent was partitioned between ethyl acetate and water (50 ml.:50 ml.), and the aqueous layer was concentrated (reduced pressure) and dried overnight over potassium hydroxide pellets *in vacuo*. After weighing, the residual peptide ester hydrochloride was used directly in the succeeding step. The *t*-butyloxycarbonyl group (used exclusively for compound **6**) was cleaved using hydrogen bromide in trifluoroacetic acid, and this reagent was also used for the selective removal of the carbobenzyloxy group in preference to the benzyl group in the synthesis of compounds **1** and **7**.

The simple extraction procedures described remove starting materials and all obvious by-products (for example the urea and N-acylurea), which would not be the case with such coupling methods as the active ester, the mixed anhydride, the azide, the dicyclohexylcarbodiimide, or solid-phase synthesis. The successive addition of carbobenzyloxy or *t*-butyloxycarbonyl-amino acid units from the C-terminus is a scheme known to minimize racemization.

Although coupling was usually about 80% after 1 hr., it was decided to investigate the synthesis of a peptide derivative by following every reaction to completion, but again without the isolation of intermediates. The model chosen was the protected heptapeptide *t*-butyloxycarbonyl-L-methionyl-γ-*t*-butyl-L-glutamyl-*im*-benzyl-L-histidyl-L-phenylalanyl-δ-trifluoroacetyl-L-ornithyl-L-tryptophylglycine *t*-butyl ester (**8**), which comprises residues 7–13 of β-MSH, but having ornithine instead of arginine at position 11. Again the carbobenzyloxy group was used to protect each introduced amino acid (save for methionine) during coupling reactions and was subsequently removed by hydrogenolysis over palladium on charcoal. All reactions were followed to completion by thin layer chromatography on silica gel with chloroform–methanol (9:1) or with methanol as the solvent. Condensation products were detected using Ehrlich's reagent[4] and/or the *t*-butyl hypochlorite–starch–iodide reagent,[5] and hydrogenolysis products using ninhydrin. For the preparation of the hexapeptide derivative and the heptapeptide derivative it was necessary to use instead of methylene chloride as the solvent acetonitrile and an acetonitrile–dimethylformamide mixture, respectively. The time required for complete coupling varied from 24 to 66 hr. Again at several stages during the synthesis removal of solvent after washing gave crystalline residues. The protected heptapeptide **8** was obtained as a solid residue upon extraction of by-products and removal of solvent. After precipitation from methanol with ether and with water the product crystallized from methanol. Two additional crystallizations afforded the pure protected heptapeptide **8** in 42% over-all yield, m.p. 203–204° dec., $[\alpha]^{24}D$ −21.8° (*c* 1.9, dimethylformamide). *Anal.* Calcd. for $C_{65}H_{86}N_{11}O_{13}SF_3$: C, 59.2; H, 6.57. Found: C, 58.8; H, 6.64. A single Ehrlich-positive spot was observed by thin layer chromatography. Acid hydrolysis gave the amino acids methionine, glutamic acid, *im*-benzylhistidine, phenylalanine, ornithine, and glycine in equivalent amounts, together with some tryptophan.

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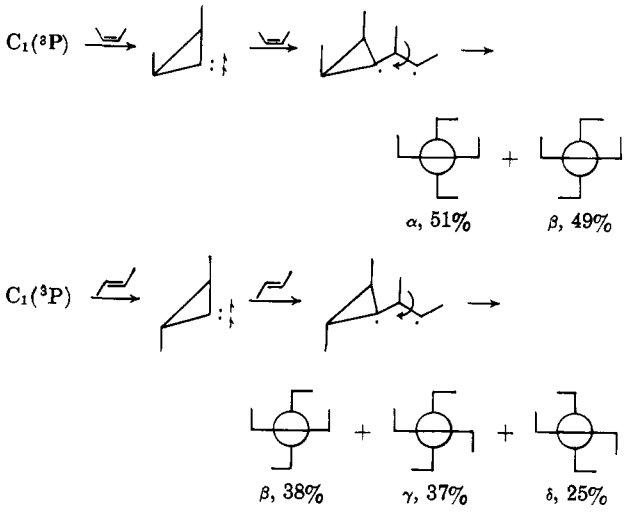
(4) I. Smith, *Nature*, **171**, 43 (1953).
(5) D. P. Schwartz and M. J. Pallansch, *Anal. Chem.*, **30**, 219 (1958).

Selectivity of Ground-State C_1 and Triplet-State Cyclopropylidene in Olefin Addition Reactions

Sir:

We have reported[1,2] that atomic carbon aged on a paraffin hydrocarbon surface is stable at −196° and reacts with olefins to form spiropentanes. With *cis*- or *trans*-2-butenes spiropentane formation was postulated to occur by a two-step sequence, the first stereospecific and the second nonstereospecific, as expected[3] for the ground state ^3P form of C. This order for the addition steps was required to explain the relative yields of the isomeric products.



An additional consequence of this hypothesis is that butadiene should show greater reactivity than monoolefins in the second step, reaction with a triplet species, and equal or lesser reactivity in the first step, reaction with a singlet species. We wish to report here the results of competition studies which have bearing on this aspect of the problem.

Aged C_1 on a paraffin surface was prepared as described earlier.[1,2] By admitting rapidly a large excess of equilibrated olefin mixture to the cold evacuated sample the matrix was melted by the heat of condensation and reaction occurred in the liquid phase at temperatures in the −100 to −150° range. Addition

(1) P. S. Skell and R. R. Engel, *J. Am. Chem. Soc.*, **87**, 1135 (1965).
(2) P. S. Skell and R. R. Engel, *ibid.*, **87**, 1135 (1965).
(3) P. S. Skell and A. Y. Garner, *ibid.*, **78**, 5430 (1956).