molecule. We conclude that this reaction and those of proton attack discussed above are frontier-controlled and not charge controlled.[21]

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Synthesis of the Tritetracontapeptide Corresponding to the Entire Amino Acid Sequence of Gastric Inhibitory Polypeptide[1]

Sir:

We wish to report the synthesis of a tritetracontapeptide corresponding to the entire amino acid sequence of porcine gastric inhibitory polypeptide (GIP), the structure of which was determined by Brown and Dryburgh[2,3] in 1971. To date only partial syntheses of GIP have been described.[4-6]

In our present synthesis (Figure 1), amino acid derivatives bearing protecting groups removable by hydrogen fluoride[7] were employed. The α-amino function of intermediates was protected by the TFA labile Z(OMe) group.[8] Anisole containing 2% ethanedithiol[9] rather than mercaptoethanol was employed to minimize destruction of the Trp residue during the various TFA deblocking steps. No brown color was produced under these conditions. The Trp content of intermediates was estimated in 3 *N* Tos-OH hydrolysates.[10]

Figure 1. Synthetic route to GIP.

Nine relatively small peptide fragments served as the building blocks for construction of the entire amino acid sequence of GIP. Of these Z(OMe)-Phe-Val-NHNH$_2$ (IV) is a known compound.[11] This strategy was adopted for the reason that these acylating agents could be readily removed by washing or precipitation following each coupling step.

The *N*-terminal octapeptide hydrazide, Z(OMe)-Tyr-Ala-Glu(O-*t*-Bu)-Gly-Thr-Phe-Ile-Ser-NHNH$_2$ (I, mp 249–255°; $[\alpha]^{25}$D −6.7° in DMSO; Anal. Calcd for $C_{54}H_{76}N_{10}O_{16}$: C, 57.84; H, 6.83; N, 12.49. Found: C, 57.62; H, 7.08; N, 12.48), was obtained by treatment of the corresponding methyl ester with hydrazine. The ester resulted from the DCC plus HOBT condensation[12] of Z(OMe)-Tyr-Ala-Glu(O-*t*-Bu)-Gly-OH and H-Thr-Phe-Ile-Ser-OMe. Z(OMe)-Tyr-Ser-Ile-Ala-Met-NHNH$_2$ (II, mp 247–251°; $[\alpha]^{25}$D −2.0° in DMSO; Anal. Calcd for $C_{35}H_{51}N_7O_{10}S$: C, 55.17; H, 6.74; N, 12.87. Found: C, 54.89; H, 6.73; N, 12.93) was prepared by the azide condensation[13] of Z(OMe)-Tyr-Ser-NHNH$_2$ and H-Ile-Ala-Met-OMe followed by treatment of the resulting protected pentapeptide ester with hydrazine hydrate. Next, Z(OMe)-Lys(Z)-Ile-Arg(Tos)-NHNH$_2$ (III, mp 177–181°; $[\alpha]^{26}$D −7.4° in DMF; Anal. Calcd for $C_{42}H_{59}N_9O_{10}S$: C, 57.18; H, 6.74; N, 14.29. Found: C, 57.17; H, 6.76; N, 14.24) was synthesized by the stepwise elongation method starting with H-Arg(Tos)-OMe. The 5-chloro-8-quinolyl ester procedure[14] served to introduce Z(OMe)-Lys(Z)-OH.

Z(OMe)-Leu-Leu-Ala-NHNH$_2$ (V, mp 170–173°; $[\alpha]^{25}$D −32.2° in DMF; Anal. Calcd for $C_{24}H_{39}N_5O_6$: C, 58.39; H, 7.96; N, 14.18. Found: C, 58.09; H, 7.90; N, 14.21), Z(OMe)-Gln-Gln-Lys(Z)-Gly-NHNH$_2$ (VI, mp 225–229°; $[\alpha]^{25}$D −43.0° in DMSO; Anal. Calcd for $C_{35}H_{49}N_9O_{11}$: C, 54.46; H, 6.39; N, 16.33. Found: C, 54.25; H, 6.31; N, 16.16), and Z(OMe)-Lys(Z)-Lys(Z)-Ser-NHNH$_2$ (VII, mp 198–202°; $[\alpha]^{25}$D −8.2° in DMF; Anal. Calcd for $C_{40}H_{53}N_7O_{11}$: C, 59.46; H, 6.61; N, 12.14. Found: C, 59.16; H, 6.69; N, 12.14) were assembled in a stepwise manner by the active ester procedure. Again the 5-chloro-8-quinolyl ester method was employed for the introduction of Z(OMe)-Lys(Z)-OH. Z(OMe)-Lys(Z)-His-NHNH$_2$ (VIII, mp 180–182°; $[\alpha]^{25}$D −8.3° in DMF; Anal. Calcd for $C_{29}H_{37}N_7O_7$: C, 58.47; H, 6.26; N, 16.46. Found: C, 58.41; H, 6.15; N, 16.62) and Z(OMe)-Ile-Thr-NHNH$_2$ were prepared by the DCC condensation of the respective amino acid derivatives followed by exposure of the resulting esters to hydrazine hydrate.

The crude protected dipeptide ester, Z(OMe)-Lys(Z)-His-OMe, was exposed to methanol–acetic acid to remove the contaminating dicyclohexylamidino derivative.[15]

The hydrazide was then condensed with the triethylammonium salt of Gln via the azide procedure to give Z(OMe)-Ile-Thr-Gln-OH (IX, mp 181–184°; $[\alpha]^{24}$D +7.7° in DMF;

Anal. Calcd for $C_{24}H_{36}N_4O_9$: C, 54.95; H, 6.92; N, 10.68. Found: C, 54.79; H, 7.22; N, 10.59).

The nine peptide fragments were then condensed by the azide procedure to minimize racemization. Two residues of Asn (position 24 and 40), two residues of Trp (position 25 and 37), and three residues of Asp(OBzl) (position 9, 15, and 21) were introduced by the stepwise *p*-nitrophenyl ester procedure.[16] Poor solubility in DMF prompted the use of DMSO–DMF mixtures for acylations beyond the octapeptide stage.

Purification of intermediates including the protected pentatriacontapeptide, Z(OMe)-(GIP 9-43)-OH (mp 230–233°; $[\alpha]^{25}D$ −8.4° in DMF; R_f 0.66 in $CHCl_3$–methanol–water 8:3:1; amino acid ratios in a hydrolysate with 3 *N* Tos-OH: $Asp_{6.14}Thr_{0.90}Ser_{1.78}Glu_{5.39}Gly_{1.38}Ala_{2.35}Val_{1.00}$-$Met_{0.68}Ile_{2.72}Leu_{2.51}Tyr_{0.75}Phe_{1.05}Trp_{1.33}Lys_{5.05}His_{0.77}$ recovery 86%; Anal. Calcd for $C_{273}H_{362}N_{54}O_{70}S_2 \cdot 9H_2O$: C, 57.06; H, 6.67; N, 13.16. Found: C, 57.33; H, 6.48; N, 12.86), was carried out by batchwise washing with 5% acetic acid and water followed by repeated precipitation from DMF or mixtures of DMF and DMSO with methanol or ethyl acetate. The compounds were characterized by thin layer chromatography, elemental analysis, and amino acid analyses of 3 *N* Tos-OH hydrolysates.

Z(OMe)-(GIP 9-43)-OH was deblocked with TFA in the presence of anisole containing 2% ethanedithiol and condensed with the azide corresponding to I. The resulting product, without further purification, was exposed to hydrogen fluoride for 60 min at 0°. Anisole containing 2% ethanedithiol and skatol served as scavengers to avoid alkylation. The resulting deblocked peptide was immediately converted to the corresponding acetate with Amberlite CG-400 (type 1, acetate form) and purified by column chromatography on Sephadex G-25 and CM-cellulose. To elute the desired compound, 0.2 *M* acetic acid was used in the former step and 0.01 *M* ammonium bicarbonate (pH 7.8)[17] in the latter. Absorbency at 280 mμ due to Trp served to monitor the chromatographic purification.

The tritetracontapeptide thus purified exhibited a sharp single spot on thin layer chromatography in two different solvent systems: R_f 0.54 and 0.77 in 1-butanol–pyridine–acetic acid–water 30:6:20:24 and 30:20:6:24 respectively. Its purity was further assessed by amino acid analyses of 3 *N* Tos-OH hydrolysates and aminopeptidase AP-M digests:[18] (ratios are given in parentheses): $Tyr_{1.65(1.70)}$-$Ala_{3.16(2.97)}Glu_{6.53(0.99)}Gly_{2.14(2.24)}Thr_{1.97}Phe_{2.13(1.66)}$-$Ile_{3.71(4.02)}Ser_{2.48}Asp_{6.46(3.60)}Met_{0.63(0.64)}Lys_{5.60(5.43)}$-$Arg_{0.85(0.93)}Val_{1.00(1.00)}Trp_{1.20(1.63)}Leu_{2.37(2.15)}His_{0.79(0.90)}$-Gln + $Thr_{(6.58\ \text{calcd. as Thr})}Asn$ + $Ser_{(4.45\ \text{calcd as Ser})}$, average recovery 93 and 82%, respectively.

When administered by continuous drop infusion to Heidenhein pouch dogs, synthetic GIP (1 μg kg^{-1} hr^{-1}) suppressed gastric acid secretion stimulated by tetragastrin (4 μg/kg). The intravenous administration of synthetic GIP (1 μg/kg) to rats elicited insulin release.

References and Notes

(1) Amino acid, peptides, and their derivatives mentioned in this communication are of the L configuration. The following abbreviations are used: Z = benzyloxycarbonyl, Z(OMe) = *p*-methoxybenzyloxycarbonyl, Tos = *p*-toluenesulfonyl, OBzl = benzyl ester, O-*t*-Bu = *tert*-butyl ester, ONP = *p*-nitrophenyl ester, DMF = dimethylformamide, DMSO = dimethyl sulfoxide, TFA = trifluoroacetic acid, DCC = dicyclohexylcarbodiimide, HOBT = *N*-hydroxybenzotriazole.
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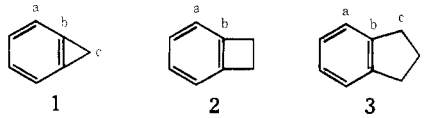
One-Bond ^{13}C–^{13}C Coupling Constants in Benzocycloalkenes[1]

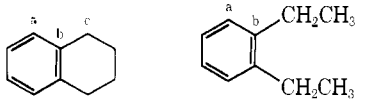
Sir:

Considerable interest has been focused recently on ^{13}C–^{13}C spin–spin coupling constants, since these parameters yield, together with ^{13}C–^{1}H coupling constants, valuable information about structure and bonding in organic molecules.[2] Whereas long range ^{13}C–^{13}C coupling constants are best accessible from ^{13}C labeled material,[2] those over one bond can be obtained in suitable cases from the ^{13}C-satellites in proton decoupled ^{13}C Fourier transform NMR spectra of compounds containing ^{13}C in natural abundance.[3,4]

We now report the results of those measurements for the benzocycloalkenes **1–4** and for 1,2-diethylbenzene (**5**). The data, including those for toluene,[5] are collected in Table I.



1 2 3



4 5

Not unexpected, the carbon–carbon coupling constants of benzocyclopropene (**1**) are exceptional, owing to the special bonding situation in the three-membered ring. Using eq 1[6]

$$^{1}J(^{13}C\text{–}^{13}C) = Ks(i)s(j) \qquad (1)$$

that relates $^{1}J(^{13}C\text{–}^{13}C)$ data to the product of the fractional s character of the two orbitals ϕ_i and ϕ_j forming the CC bond and that is well established for hydrocarbons,[4,7,8] the