N-(Bromochloroacetyl)piperidine (1a). Bromochloroacetyl chloride (38.4 g, 0.2 mol) in 200 ml of dry ether was added during 15 min from a dropping funnel to a magnetically stirred, ice-cooled solution of piperidine (34 g, 0.4 mol) in 800 ml dry ether. The reaction mixture was left for 3 h at room temperature and the precipitate of piperidine hydrochloride was filtered off. The ether solution was washed with two portions of 2 M HCl, two portions of saturated $NaHCO_3$-solution and one portion of water, then dried over Na_2SO_4 and evaporated. Recrystallization of the crude product from diethyl ether gave white crystals, m.p. 60–62°C. Yield 31.7 g (66 %). (Found: C 35.08; H 4.63; Br 33.07; Cl 14.61; N 5.77. Calc. for $C_7H_{11}BrClNO$: C 35.14; H 4.60; Br 33.05; Cl 14.64; N 5.85.) IR: CO 1635 cm^{-1}. NMR 1.68 (S, CH_2); 3.64 (S, $N-CH_2$); 6.34 (S, CHBrCl). MS: *m/e* 239, 241, 243 (M).

[Bromochloro(N,N-pentamethylenecarbamoyl)methyl]phenylmercury (2a). Phenylmercury chloride (3.1 g, 0.01 mol) in THF (80 ml) and *N*-(bromochloroacetyl)piperidine (2.4 g, 0.01 mol) in THF (50 ml) were added rapidly and simultaneously from two dropping funnels to a magnetically stirred solution of unsolvated *t*-BuOK (0.01 mol) in THF (100 ml), which was maintained at −75°C during the reaction. During the addition there was always a small excess of the amide over the phenylmercury chloride. When the addition was complete the reaction mixture was kept at −75 °C for an additional 45 min. The temperature was quickly raised to +10°C, and the solvent was evaporated on a rotatory evaporator at room temperature. The residue was dissolved in benzene (200 ml), washed with distilled water (40 ml) and dried over $MgSO_4$. Crystallization from dry diethyl ether gave [bromochloro(*N,N*-pentamethylenecarbamoyl)-methyl]phenylmercury (*2a*) 2.46 g (48 %), m.p. 111–113 °C (dec.). When the reaction was performed at −40 °C, the yield was lowered to 0.55 g (11 %). A longer reaction time also lowered the yield; thus, 75 min gave 1.26 g (27 %), 120 min gave 0.94 g (18 %). (Found: C 30.7; H 3.00; Br 16.0; Cl 7.14; N 2.69. Calc. for $C_{13}H_{15}BrClHgNO$: C 30.2; H 2.94; Br 15.5; Cl 6.85; N 2.71). IR: CO 1615 cm^{-1}. NMR: 1.75 (S, CH_2); 3.90 (S, $N-CH_2$); 7.60 (S, aromatic protons).

Thermolysis of [bromochloro(N,N-pentamethylenecarbamoyl)methyl]phenylmercury (2a). 0.8 g (1.55 mmol) of *2a* was refluxed in 240 ml freshly distilled bromobenzene. The decomposition of *2a* was followed by the use of IR spectrometry (β-lactam absorption at 1760 cm^{-1}) with samples removed after 1.3 h, 1.7 h, 2 h, 2.3 h, and 3.5 h. The best result was obtained at 2.3 h. The solvent was removed *in vacuo*, ether was added and the insoluble phenylmercury bromide was filtered off. Separation of the ether soluble products was accomplished by chromatography on a column of silica gel, cooled to −20 °C by cold circulating ethanol. Elution with increasing amounts of diethyl ether in light petroleum afforded 7-chloro-8-oxo-1-azabicyclo[4.2.0]octane (*3a*) (0.125 g, 54 %). Only the *trans*-isomer of *3a* could be detected by NMR: 1.2–1.3 (M, H-3, H-5); 2.6–4.1 (M, H-2, H-6); 4.45 (D, $J = 1.3$ Hz, H-7, *cf. cis*-isomer 4.95 (two D, $J = 1.4$ and 4.4 Hz, H-7).

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Synthesis of Bradykinin by Fragment Condensation on a Solid Support

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Solid phase peptide synthesis (SPPS) was introduced by Merrifield.[1] In a series of papers he developed the technique further and demonstrated the exceptional scope of his method.[2]

To simplify purification, which is the crucial step of this method, Weygand and one of the present authors [3] attempted to couple a peptide fragment instead of an amino acid derivative, *i.e.*, to use fragment condensation instead of a step-wise approach in analogy with common strategy in conventional peptide synthesis. We found that a peptide could be coupled satisfactorily with respect to the yield and with a very low degree of racemization when *N,N'*-dicyclohexylcarbodiimide (DCC) plus *N*-hydroxysuccinimide (HOSu) together [4] were used in CH_2Cl_2, the solvent of choice in SPPS, although admittedly the evidence concerning the yield was weak. Both considerations are of equally fundamental importance in this context. Since our initial work in this area, a couple of papers

on the use of fragment condensation in SPPS have appeared,[5] but to date progress has been slow.

This communication describes the preparation of bradykinin on a solid support from three protected peptide fragments (I – III below). The coupling procedure used was DCC plus HOSu when there was a risk for racemization and DCC only when that risk was absent. However, only one equivalent of HOSu was used as compared with two earlier,[3] when much more dilute solutions were used. When possible, completeness of coupling was verified using the 2-hydroxy-1-naphthaldehyde procedure [6] before proceeding to the next step. The loss in time was more than compensated for by the information gained.

The three protected peptide fragments used, Z-Arg(NO_2)-Pro (I), Z(OCH_3)-Pro-Gly-Phe (II) and Z(OCH_3)-Ser(Bzl)-Pro-Phe (III),* were prepared in step-wise fashion by the mixed anhydride procedure without a carboxyl protecting group. They all gave only one spot on thin-layer chromatography and satisfactory elemental analyses as well as, after hydrolysis, amino acid analyses. II and III are amorphous solids. The reason for selecting two peptide fragments with C-terminal phenylalanine was that racemization, if any, could be simply established and consequently compared with that obtained earlier.[3]

We started our synthesis from 1.32 g of Boc-Arg(NO_2)-resin with a substitution degree of 0.151 mmol/g of Arg. All operations were performed manually in a sintered glass vessel, and apart from those explicitly mentioned below consisted of washings with proper solvents and neutralizations of amine trifluoroacetates. Initially, Boc was removed with 50 % trifluoroacetic acid (TFA) in CH_2Cl_2. Coupling was effected in 99.5 % yield, determined as described above, with 2.5 equiv. of III, DCC and HOSu each in CH_2Cl_2 for 4 h. Z(OCH_3) was removed using 10 % TFA in CH_2Cl_2.[7] The following coupling was effected also in 99.5 % yield as before with II substituted for III. After removal of Z(OCH_3), I was attached. In this case HOSu was omitted. This coupling step was repeated once, since a preliminary experiment indicated some residual heptapeptide. Finally, the peptide was split off from the resin using HF.[8]

Crude bradykinin was obtained in 81 % yield. After hydrolysis in 6 N HCl at 110 °C for 24 h it gave the following amino acid analysis: Arg 2.00 (2), Gly 1.03 (1), Phe 2.06 (2), Pro 2.93 (3) and, after correction for decomposition during hydrolysis, Ser 0.98 (1). Another sample was hydrolyzed as just mentioned and coupled with Leu-*N*-carboxyanhydride.[9] In a parallel experiment, analytically pure, biologically fully active bradykinin was carried through the same procedure as a control. On analysis the D-Phe/L-Phe ratio was found to be 3.76 % compared to 2.91 % for the control. These determinations were performed under such conditions that the L-Leu-D-Phe peaks could be properly integrated. The difference, 0.85 %, reflects the average racemization taking place during the coupling of the two fragments II and III to the resin. The original sample of phenylalanine used in this work contained less than 0.10 % D-Phe.

The crude bradykinin was purified by ion-exchange chromatography on carboxymethyl cellulose. The recovery of pure bradykinin in this step was 87 %. This is at least 25 % higher than the average we have obtained using step-wise procedure. Less than 5 % of the total material was obtained in two minor peaks, the rest we believe are inevitable losses. Amino acid analysis after hydrolysis now gave: Arg 2.00, Gly 1.02, Phe 2.03, Pro 2.98, and Ser 0.98. Purity was further established by high voltage paper electrophoresis at pH 6.46 and elemental analysis.

The conclusions reached in the earlier paper [3] have now been further verified. DCC together with HOSu in CH_2Cl_2 gives a very efficient coupling to the resin for the two tripeptides II and III. This might not always be the case.[5bf] As concerns racemization, the conditions used are certainly not perfect. However, a value as low as ours can in most cases, we believe, be tolerated. It may have been lower if we had used more than one equivalent of HOSu.

Many more experiments are needed to establish the scope of this approach to SPPS. Steric hindrance or an enhanced degree of racemization may in some cases exclude its general use. Even so, however, cases can be envisaged where it would be possible to incorporate a difficult amino acid into the interior of a fragment which could then be coupled to the resin by the proposed procedure. A mixed procedure, in which some amino acids are coupled step-wise and others as oligopeptides, may in other cases be a practical alternative.

Small protected peptides with a free C-terminal carboxyl group, as needed in the present scheme, are generally simple to prepare in satisfactory quality and quantity by conventional solution procedures. Somewhat larger ones, if needed, can perhaps be made as proposed recently.[5g] A definite disadvantage of any fragment condensation approach is that time is lost in the preparation of the partial sequences. For bradykinin,[10] which is small enough to be easily purified, our procedure is hardly an improvement. Nevertheless, our experiences with this synthesis as concerns the near absence of smaller peptides in the crude product make us believe that for larger peptides the purification will be much less time-consuming, *i.e.*, make a fragment condensation approach competitive even as regards the time needed.

* All amino acids used were of L-configuration except glycine.

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Correction to "Organic Hydroxylamine Derivatives. VIII" *

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In Table 1 (p. 3254), column II, the pK_A value for compound IVc should read 8.4.

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The Oligomerization of Ethylene Oxide to Macrocyclic Ethers, Including 1,4,7-Trioxacyclononane

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Cyclic homologues of ethylene oxide, $-[-CH_2-CH_2-O-]-_n$, have attracted much attention due to their remarkable complexing power for alkali and other cations.[1-3] Laboratory methods for the preparation of the higher members ($n > 4$) have been described [2] which involve several steps and use di-, tri-, or tetraethylene glycol as starting materials; these methods fail to yield the medium rings ($n = 3$ and 4). Clearly, the simplest conceivable way to prepare this class of cyclic ethers is the direct oligomerization of ethylene oxide. In fact, it has been reported [4] that the cyclic tetramer (1,4,7,10-tetraoxacyclododecane) is obtained together with mainly dioxan and polymer from ethylene oxide in the presence of trialkylaluminium. On the other hand, although BF_3 is reported [5] to catalyse the conversion of propylene oxide to isomeric cyclic tetramers and pentamers, ethylene oxide under the same conditions gave only dioxan and polymers.

We can now report that a mixture of all the possible cyclic oligomers, including the hitherto unknown trimer 1,4,7-trioxacyclononane, m.p. 0 °C, and unaccompanied by open-chain oligomers and polymers, can be easily obtained from ethylene oxide at room temperature and atmospheric pressure in the presence of BF_3 or similar acidic fluorine compounds (PF_5, SbF_5). The important point is to exclude any substance capable of furnishing permanent end groups to polymeric chains. Thus, the common practice of using BF_3 as its etherate leads to a mixture of the rings and open-chain compounds terminated by ethoxy groups. Another common practice, to add water as a cocatalyst to speed up the reaction,[6] leads to hydroxyl-terminated open-chain compounds. Only dry HF gas proved acceptable as a cocatalyst, in accordance with the low nucleophilicity of fluoride ion. Likewise, any solvent must be inert (benzene, saturated hydrocarbons); even diethyl ether suppresses totally the formation of macrocyclic compounds, while dioxan, being one of the products, is a suitable solvent.

Other Lewis acids are either ineffective as catalysts ($AlCl_3$, $FeCl_3$) or give waxy polymers ($SnCl_4$, $SbCl_3$), while oxygen acids ($HClO_4$, *p*-toluene sulfonic acid) are rapidly transformed to half-esters of ethylene glycol.

Surprisingly, the product composition is little dependent on the concentration of monomer, so that similar products are obtained from