"Mediator Methodology" for the Synthesis of Peptides in a Two-Polymeric System

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Abstract: A novel methodology is described for a potential automated self-controlled synthesis of peptides. The method is based on transferring an N-protected amino acid from one insoluble polymer (donor) such as a polymeric *o*-nitrophenyl ester (a "bank" of active ester) to an insoluble polymer-bound amino acid (acceptor), with the aid of a soluble mediator molecule (shadchan).[2] The method gives high yields of pure peptides by guaranteeing stoichiometric supply of the active ester and allowing self-monitoring. When the polymer-bound active ester was replaced by polymer-bound (dimethylamino)pyridinium salts, the condensation reaction was expended to also include sulfonation and particularly phosphorylation reactions.

Reagents bound covalently to insoluble polymers will not interact although reaction between the same reagents may occur readily in solution; such polymer-bound reagents can be made to interact, however, by the action of a soluble mediator molecule. In the past, such multiphase systems have mostly been used for mechanistic studies.[3,4]

We now report the successful application of such a multiphase system to peptide synthesis. The system consists of two insoluble polymers, donor I and acceptor II, and a circulating soluble mediator molecule i.e., imidazole (Figure 1).

Polymer I carriers an N-protected amino acid bound as an active ester.[5] Polymer II is a Merrifield-type polymer carrying an amino acid or peptide with a free amino group.

The procedure involves circulating a mediator solution between the two polymers, polymer I (carrying 0.5–0.8 mequiv/g Boc-amino acid as a nitrophenyl ester) and polymer II (carrying 0.2–0.5 mequiv/g of free amino group of the attached peptide or amino acid). The mediator (imidazole) interacts with polymer I to release the soluble acylimidazole.[6] The latter reacts with polymer II to elongate the peptide by an additional amino acid. The concurrently released imidazole is now free to repeat the cycle, until polymer II is saturated. On-line monitoring of the reaction progress is possible by comparing the UV absorption of the circulating solution when entering and leaving polymer II; zero difference indicates the end of the reaction (Figure 2). Polymer II is then washed thoroughly, the Boc protecting group is removed, and the polymer is neutralized and is ready for the next coupling step.

The results of typical runs are summarized in Table I.

Thus, when polymer I was used, the methyl ester of the protected Leu[5]-enkephalin was obtained in 85% yield and 99% purity directly from the polymer without further purification. This procedure involved four cycles of transacylation, followed by alcoholysis in methanol and triethylamine (removal of the Boc group after each cycle was performed at −10 °C). On the other hand, the deprotected free enkephalin was obtained in 75% yield and 80% purity by cleavage with hydrogen bromide in trifluoracetic acid (the Boc group was removed after each step with TFA at 25 °C).

Practically quantitative yields of coupling were obtained within 24 h.[7] However, when the concentration of the mediator and the contact time (decreased pump rate) were increased, the reaction time could be shortened.

The high yield synthesis of peptides such as enkaphalin obtained by this system encouraged us to expand the "mediator methodology" to other than acylation reactions, i.e., sulfonation, phosphorylation, etc. Toward this end, it was necessary to develop a "bank" polymer to store these groups in an active form.

Polymeric 4-(dimethylamino)pyridine (DMAP), **1**, proved to be the polymer of choice, forming 1-substituted pyridinium salts, i.e., **3** for subsequent transfer to a mediator ("shadchan") molecule (Figure 3).

The DMAP **1** was prepared according to Shinkai[9] from *N*-(methylamino)methylpolystyrene and 4-chloropyridine. When acylated with an acyl chloride and exhaustively washed with anhydrous methylene chloride, a stable 1-acylpyridinium adduct remained on the polymer with negligible leakage. With benzoyl chloride, derivitization was in the range of 0.5–0.8 mequiv/g. Acetyl chloride, tosyl chloride, isobutyl chloroformate, 2-chlorophenyl isopropyl phosphorochloridate, and other electrophilic species were similarly retained by the polymer.

The applicability of polymer DMAP as a storable acylium "bank" and its versatility were demonstrated by the following experiments (Figure 3).

When exposed to an excess of imidazole in chloroform, the benzoylated polymer was discharged to give soluble *N*-benzoylimidazole (**5**, E = PhCO, α-H = imidazole) which reacted quantitatively with benzylamine to *N*-benzoylbenzamide. Moreover, since the acyl-DMAP support is more reactive than the corresponding polymeric *o*-nitrophenyl ester, I, time required for each transfer step may be potentially reduced.

Polymer **3** reacted also with various nucleophiles in solution without the addition of imidazole or tertiary base. The reactions were carried out in methylene chloride by using 2 equiv of acylating polymer, and the concentration of the nucleophile was approximately 0.1 M (Table II). The acyl polymers were sufficiently active to form symmetric and mixed anhydrides with carboxylic acids.

The product of **1** and isobutyl chloroformate (**7**) (typically 0.72 mequiv/g) (figure 4) acts as a polymeric activating agent for carboxylic acids, to form the mixed carbonic–carboxylic anhydride, which may then acylate nucleophiles after the polymer removal.

Benzoic acid was activated as the mixed anhydride by using polymer **7** and was separated by filtration, and by following addition of excess benzylamine to the filtrate, *N*-benzoylbenzamide was isolated in 97% yield. A series of dipeptides were synthesized in solution by this activation technique.

In considerably lower yield, Boc-protected amino acids could be esterified to *o*-nitrophenyl resin activated as the mixed anhydrides by circulation over **7** in the path of circulation (Figure 3).

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(2) "Shadchan" is the Hebrew term for a matchmaker, go-between, or agent.

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(6) Depending on the particular acyl species and the temperature, (0–25 °C), the lifetime of the acylimidazole in solution (CH_2Cl_2, DMF) varies from hours to days.

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Table I. Peptides Synthesized Using the Shadchan Method

I[a]	α[b]	temp, °C	II[c]	products after cleavage[d]	coupling yield[e]
Boc-Phe-O-Ⓟ	A	0	NH_2Leu-OCH_2- P	H_2NPhe-Leu-OH	>99.8%
Boc-Phe-O-Ⓟ	A	25	NH_2Leu-OCH_2- P	H_2NPhe-Leu-OH	low yield[f]
Boc-Phe-O-Ⓟ	B	0	NH_2Leu-OCH_2- P	H_2NPhe-Leu-OH	>99.8%
Boc-Phe-O-Ⓟ	B	25	NH_2Leu-OCH_2- P	H_2NPhe-Leu-OH	>99.8%
Boc-Ala-O-Ⓟ	A	0	NH_2Leu-OCH_2- P	H_2NAla-Leu-OH	>99.8%
Boc-Ala-O-Ⓟ	A	25	NH_2Leu-OCH_2- P	H_2NAla-Leu-OH	low yield[f]
Boc-Gly-O-Ⓟ	A	0	NH_2Phe-Leu-OCH_2- P	H_2NGly-Phe-Leu-OH	>99.8%
Boc-Gly-O-Ⓟ	A	25	NH_2Phe-Leu-OCH_2- P	H_2NGly-Phe-Leu-OH	>99.8%
Boc-Gly-O-Ⓟ	B	0	NH_2Gly-Phe-Leu-OCH_2- P	H_2NGly-Gly-Phe-Leu-OH	>99.8%
Boc-Gly-O-Ⓟ	B	25	NH_2Gly-Phe-Leu-OCH_2- P	H_2NGly-Gly-Phe-Leu-OH	>99.8%
Boc-Tyr(OBz)-O-Ⓟ	A	0	NH_2Gly-Gly-Phe-Leu-OCH_2- P	Boc-Tyr(OBz)-Gly-Gly-Phe-Leu-OCH_3	>99.8%
Boc-Tyr(OBz)-O-Ⓟ	A	25	NH_2Gly-Gly-Phe-Leu-OCH_2- P	Boc-Tyr(OBz)-Gly-Gly-Phe-Leu-OCH_3	low yield[f]
Boc-Tyr(OBz-2,6-Cl)-O-Ⓟ	A	0	NH_2Gly-Gly-Phe-Leu-OCH_2- P	H_2NTyr-Gly-Gly-Phe-Leu-OH	>99.8%

[a] -O- P ≡ -O-Bz-C(=O)- P, where P is macroporous cross-linked polystyrene (XE 305 from Rohm & Haas Co.). [b] α = the "shadchan" molecule. A = imidazole; B = *N*-hydroxybenztriazole. [c] $-O-CH_2-$ P = 1% cross-linked polystyrene carrying 0.3 mequiv/g oxymethyl sites or pam-oxymethyl sites.[8] [d] Overall yields of peptides cleaved from polymer II were 70%–90% depending upon the type of polymer II used. Higher yields were obtained when using the pam resin.[8] [e] Yield was estimated on TLC plates (about 1000 μmol peptide was cleaved from the polymer, allowed detection of 0.2% impurities on TLC plate after spraying with ninhydrin). [f] The acylimidazole molecule is unstable at room temperature.

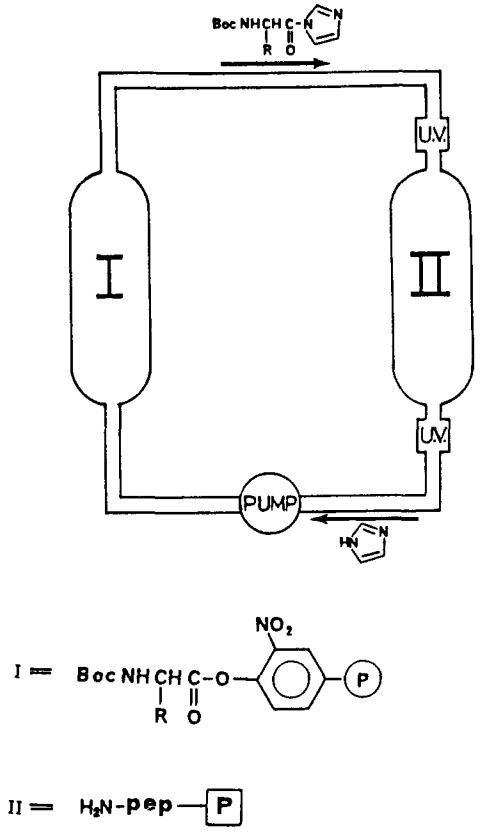


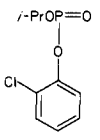
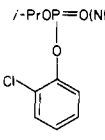
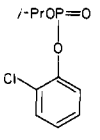
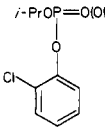
Figure 1. Schematic "shadchan" synthesis system.

The potential applicability of the mediator methodolgy to the synthesis of oligonucleotides was demonstrated by reacting polymer 1 with 5′-(dimethoxytrityl)deoxythymidinyl 3′-(2-chlorophenyl) phosphorochloridate. The incorporation on the polymer, according to dimethoxytrityl assay, was *7.3* μmol/g. Upon treatment with tetrazole carrier, in the presence of a polymer-bound 5′-free-hydroxylthymidine, transfer of the dimethoxytrityl-containing species was demonstrated qualitatively.

Experimental Section

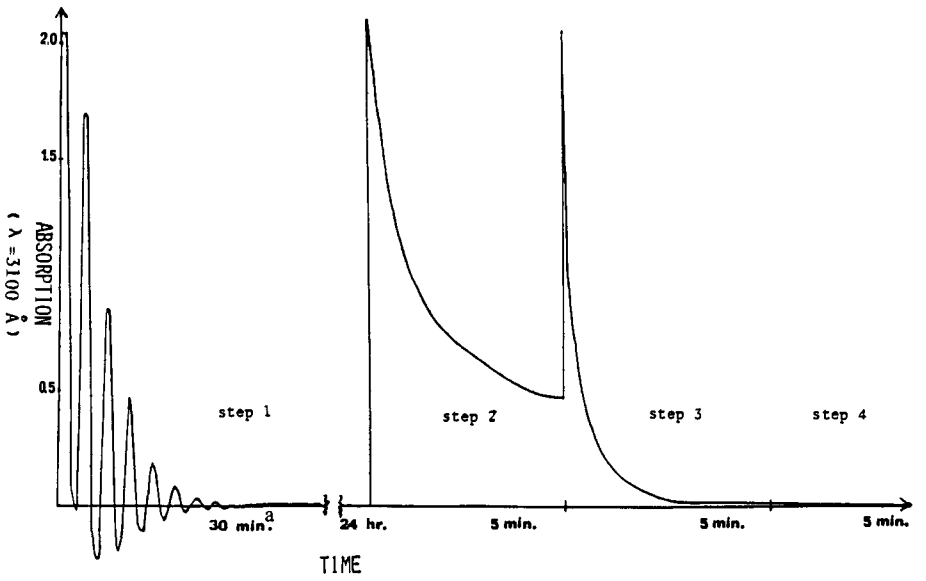
Preparation of Polymeric-Active Ester. A solution containing 2.45 mequiv of the Boc-protected amino acid and 2.5 mequiv of Et_3N in 3 mL of methylene chloride (THF was added in case of low solubility) was shaken for 1 h at 25 °C with 1 g polymer-bound 4-hydroxy-3-nitrobenzophenone (prepared according to the method of Cohen et al.[5]) containing 2.23 mequiv of available OH groups. The mixture was cooled in dry ice/isopropyl alcohol bath, and 2.9 mequiv of DCC was added. After 1 h, the temperature was set to −10–0 °C for another 10 h. The polymer was then washed with six to eight 4-mL aliquots of methylene chloride until no Boc-amino acid could be detected. Active esters of Boc-glycine, Boc-phenylalanine, Boc-(*O*-benzyl)tyrosine, and Boc-(3,6-dichloro-*O*-benzyl)tyrosine were thus prepared. Determination of the loading[5] showed that 85–98% of the available OH groups underwent esterification.

Table II. Reactions of Polymer 3 with Nucleophiles in Solution

E=	Nu-H=	product	*m/e*	% yield[b]
CH_3CO	$HOCH_2Ph$	$CH_3CO_2CH_2Ph$	150	82
PhCO	HOC_6H_4-*p*-NO_2	$PhCO_2C_6H_4$-*p*-NO_2	243	100
PhCO	HSC_6H_4-*p*-NO_2	$PhCOSC_6H_4$-*p*-NO_2	259	100
PhCO	menthol	menthol benzoate	260	30
PhCO	PhCOOH	benzoic anhydride	226	92[c]
PhCO	CH_3COOH	CH_3CO_2COPh[a]	164	54[c]
PhCO	HF (48%)	PhCOF[a]	124[c]	
i-BuOCO	H_2NCH_2Ph	*i*-BuOCONHCH$_2$Ph	207	
p-tosyl	$H_2'NCH_2Ph$	*p*-tosyl-NHCH$_2$Ph	261	100
	$HNMe_2$		277	
	MeOH		248	

[a] Benzoic anhydride present as impurity. [b] After 16 h, product is isolated unless noted. [c] Product not isolated, measured by GC.

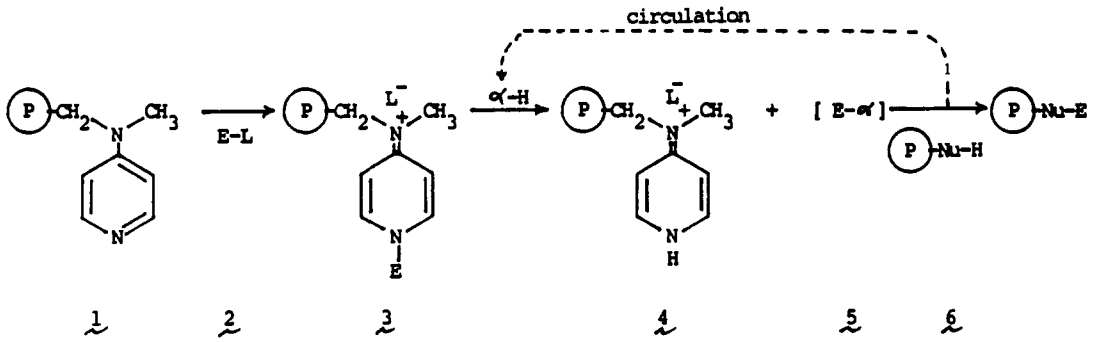
Peptide Synthesis in the Shadchan System—Synthesis of Boc-Phe-Leu-OCH_3. A one cycle of the synthesis in the shadchan system is performed as follows: The polymeric nitrophenyl active ester of Boc-Phe-OH (0.8 g, 0.2 mequiv) was placed in column I (Figure 1), and the polymer H_2-Leu-OCH_2-Ⓟ (200 mg, 0.1 mequiv) was placed in column II (both columns at 0 °C). The circulating solvent in the system was CH_2Cl_2 (total volume 10–15 mL) containing imidazole (5 mg, 0.073 mequiv) and ethyldiisopropylamine (8.7 μL, 0.05 mequiv). Circulation started with a pump rate 4 mL/min (60%–30% of the imidazole was acylated at pump rates of 0.4–4 mL/min and less than 5% at the pump rates greater than 10 cm^3/min). Monitoring of the reaction, using UV detection, was possible due to low concentration of the acylimidazole in the system and was carried out at λ 3100 Å, as generally described in the article. By this method, one flow cell of the detector was connected to the entrance (substrate cell) and another flow cell to the exit (reference cell) of column II. Because imidazole was added in a concentrated solution at the entrance of column II, the circulating solvent was not homogeneous—maximum and minimum peaks were obtained at the first cycle as shown in Figure 3. Though after 20 min more than 40% of the coupling reaction took place (as determined by using ninhydrine test[7]), the reaction was left to proceed for 24 h to assure maximum yield. After



step 1 - coupling reaction
step 2 - chloroform wash
step 3 - EtOH/chloroform wash
step 4 - chloroform wash

a - more than 40% transfer to polymer II occured within 20 minutes(4-6 cycles).

Figure 2. On-line differential UV recording to follow up the completion of the transfer Boc-Phe to polymeric acceptor and washing.



E = electrophile

∝-H = acyl carrier ("shadchan")

L = leaving group, counterion

Nu = nucleophile

Figure 3. Schematic acylation reaction carried out by the DMAP polymer as an acyl-transfer support in the "shadchan" system.

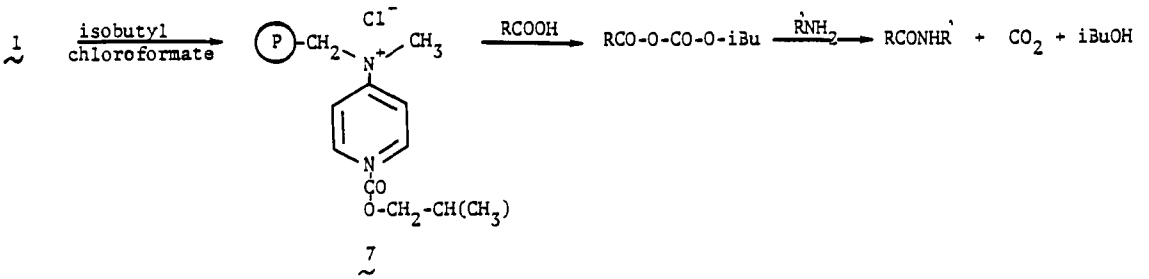


Figure 4. Direct amide formation by DMAP polymer via mixed anhydride formation.

24 h, column II was washed, and 2 mg of polymer was transesterified by using Et_3N/CH_3OH for 48 h. After the Boc group was deblocked with trifluoroacetic acid, the peptide ester was run on TLC (ethyl acetate, 6; pyridine, 5; AcOH, 1; H_2O, 3; CH_3OH, 1) and sprayed with ninhydrin. A strong spot (R_f = 0.73) of the dipeptide was seen, whereas no detectable spot (<0, 2%) of Leu was observed. Boc-Phe-Leu-OCH_3: mp 109–110 °C, R_f = 0.66,(2% hexane/ethyl acetate). Anal. Calcd for $C_{21}H_{32}N_2O_5$: C, 64.22; H, 8.26; N, 7.13. Found: C, 64.10; H, 8.20; N, 6.99. Amino acids analysis, after hydrolysis at 6 N HCl for 24 h gave Phe 1.00, Leu 0.97. Other peptides were synthesized similarly.

Polymeric 4-(Dialkylamino)pyridine. Was prepared by a modification of the method of Shinkai et al.[9] Macroporous chloromethylpolystyrene (5 mequiv/g) was prepared (from Rohm & Haas XE 305 resin) by the method of Rossey et al.[10] The dry polymer (19 g) was suspended in 30

mL and DMF and saturated with methylamine gas at 0 °C. The vessel was sealed and agitated for 1 day. The polymer was washed successively in dioxane, ethanol, 2 N NaOH/*i*-PrOH (1:1), water (until eluate neutral), ethanol, and ether. After drying in vacuo, the polymer (3.7 g/3.8 mequiv of amino groups/1 g of dry weight) was suspended in a mixture of water (1.5 mL), ethanol (0.5 mL), triethylamine (7 mL), and 4-chloropyridine hydrochloride (4.7 g) in a glass pressure vessel, sealed and heated for 4 days at 140 °C. The polymer was washed as before, and unreacted amino groups were blocked by acetylation (acetic anhydride in CH_2Cl_2, then base wash). The washed DMAP polymer was dried at 150 °C in vacuo until constant weight. Incorporation of pyridine groups was determined by potentiometric chloride titration of the hydrochloride salt bound to the polymer: 2.53 mequiv/g compared to 3.15 mequiv/g prior to acetylation.

Polymeric 1-Acyl-4-(dialkylamino)pyridinium Chlorides. In a typical experiment, the anhydrous 4-(dialkylamino)pyridine polymer was swelled in methylene chloride (freshly distilled from P_2O_5 under argon) and treated with excess benzoyl chloride at 0 °C. The polymer was filtered and washed with methylene chloride under anhydrous conditions until the washings contained negligible amounts of benzoyl chloride—by the silver nitrate test in alcohol (less than 0.1% of total pyridine groups as indicated by GC). The polymer was dried under vacuum at room temperature and was stable at −10 °C for several months. After treatment with a primary (e.g., benzyl) amine in methylene chloride, a pure amide was recovered by filtration and acid/base wash. The amount of amide corresponded to 0.8 mequiv/g of acyl substitution on the polymer.

Anhydrous manipulation as above and those involving transfer between two polymers were most conveniently carried out by using a circulating system described in Figure 1, containing Teflon columns (1-4-mL volume) joined to the solvent distillation apparatus, waste, and vacuum pump via Teflon tubing.

In summary, we have shown for the first time the possibility to perform highly efficient condensation reactions, by transferring polymer-bound electrophiles (i.e., active esters) via a mediator (shadchan) to polymer-bound nucleophiles (i.e., amines). We have also shown the possibility of on-line monitoring which is relevant for automation.

The mediator methodology developed here is believed not to be limited to acylation and related processes but to be expandable to other chemical processes that involve the creation of activated intermediates. These possibilities are currently under investigation.

Acknowledgment. We thank the Etta P. Schiff Trust and the Bantrell Fund for financial support. This work is dedicated to Prof. Arieh Berger on the 10th anniversary of his death.

Registry No. Boc-Phe-OH, 13734-34-4; Boc-Gly-OH, 4530-20-5; Boc-Tyr(OBz)-OH, 2130-96-3; Boc-Tyr(OBz-2,6-Cl)-OH, 40298-71-3; Boc-Phe-Leu-OCH_3, 64152-76-7; H_2NPhe-Leu-OH, 3303-55-7; H_2NAla-Leu-OH, 3303-34-2; H_2NGly-Phe-Leu-OH, 15373-56-5; H_2NGly-Gly-Phe-Leu-OH, 60254-83-3; Boc-Tyr(OBz)-Gly-Gly-Che-Leu-OCH_3, 63631-33-4; H_2NTyr-Gly-Gly-Phe-Leu-OH, 58822-25-6; $HOCH_2Ph$, 100-51-6; HOC_6H_4-*p*-NO_2, 100-02-7; HSC_6H_4-*p*-NO_2, 1849-36-1; PhCOOH, 65-85-0; CH_3COOH, 64-19-7; H_2NCH_2Ph, 100-46-9; $CH_3CO_2CH_2Ph$, 140-11-4; $PhCO_2C_6H_4$-*p*-NO_2, 959-22-8; $PhCOSC_6H_4$-*p*-NO_2, 1219-32-5; CH_3CO_2COPh, 2819-08-1; PhCOF, 455-32-3; *i*-BuOCONHCH$_2$Ph, 69805-82-9; *p*-tosyl-NHCH$_2$Ph, 1576-37-0; methylamine, 74-89-5; 4-chloropyridine hydrochloride, 7379-35-3; benzoyl chloride, 98-88-4; menthol, 89-78-1; menthol benzoate, 612-33-9; benzoic anhydride, 93-97-0; 1-methylethyl 2-chlorophenyl dimethylphosphoramidate, 96227-79-1; 2-chlorophenyl methyl 1-methylethyl phosphorate, 96227-80-4.

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General Method of Diastereo- and Enantioselective Synthesis of β-Hydroxy-α-amino Acids by Condensation of Aldehydes and Ketones with Glycine

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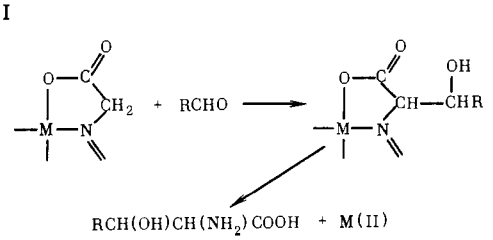
Contribution from Nesmeyanov Institute of Organo-Element Compounds, Academy of Sciences of the U.S.S.R., Moscow, U.S.S.R. *Received September 18, 1984*

Abstract: The condensation of formaldehyde with a Ni(II) complex of glycine Schiff base with (*S*)-2-[*N*-(benzylprolyl)-amino]acetophenone (**1**) or (*S*)-2-[*N*-(benzylprolyl)amino]benzophenone (**2**) in CH_3OH at 25 °C in the presence of Et_3N yields (*S*)-Ser with an enantiomeric excess (ee) of 80–98%. The same reaction gives rise to (*R*)-Ser with an ee greater than 80% in the presence of more than 0.2 N CH_3ONa, α-(hydroxymethyl)serine being formed in negligible quantities. The reaction of benzaldehyde, 3,4-(methylenedioxy)benzaldehyde, and acetaldehyde with these Gly complexes in 0.2 N CH_3ONa at 25 °C yields β-hydroxy-α-amino acids: (*R*)-β-phenylserine, (*R*)-3,4-(methylenedioxy)-β-phenylserine, and (*R*)-threonine, respectively, with a threo/allo ratio ranging from 10:1 up to over 50:1 and ee more than 80%. Condensation with acetone yields (*R*)-β-hydroxyvaline with an enantiomeric purity of 70%. The enantiomerically pure β-hydroxy-α-amino acids can be obtained from pure diastereomers, isolated by chromatography on silica or Toyopearl HW-60. The initial reagents **1** and **2** were recovered with 60–98% yield. The stereochemical mechanism of the reaction is discussed.

β-Hydroxy-α-amino acids (**3**) represent an important group of natural products. In spite of the recent progress in the field of asymmetric synthesis of amino acids in general[1] and **3** in particular,[2] convenient preparative methods for chemical enantioselective synthesis of *threo*-**3** are still not available.

Scheme I



Here we wish to describe our approach to the solution of this problem by means of aldol condensation of chiral Gly derivatives with aldehydes and ketones.

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