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of Reliable Methods
for the Preparation
of Organic Compounds

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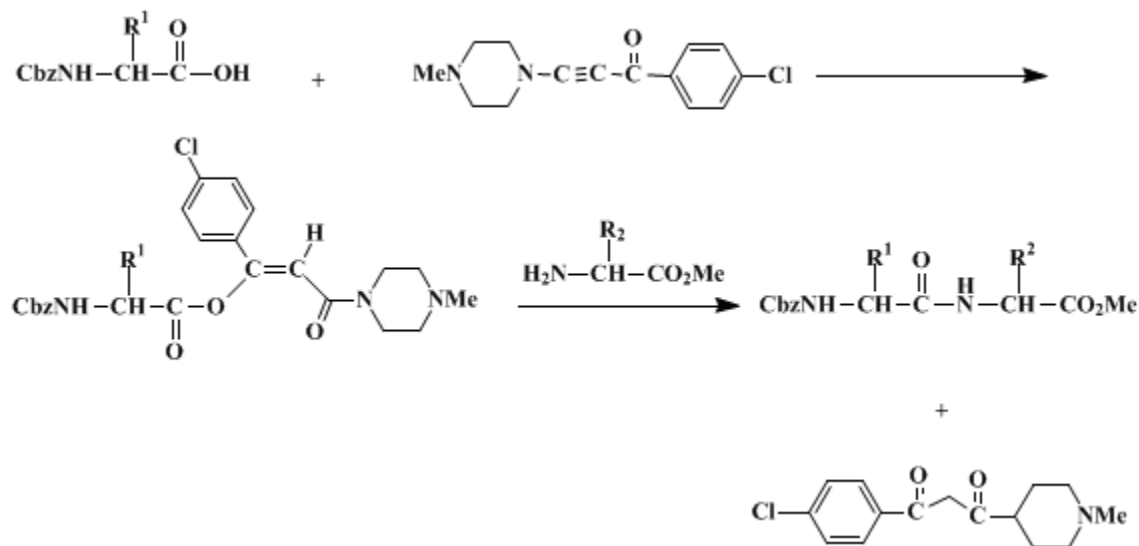
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These paragraphs were added in September 2014. The statements above do not supersede any specific hazard caution notes and safety instructions included in the procedure.

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PEPTIDE SYNTHESIS USING 1-(4-CHLOROPHENYL)-3-(4'-METHYL-1'-PIPERAZINYL)-2-PROPYN-1-ONE AS REAGENT: BENZYLOXYCARBONYL-L-ALANYL-L-CYSTEINE METHYL ESTER AND BENZYLOXYCARBONYL-L-ASPARTYL-(*tert*-BUTYL ESTER)-L-PHENYLALANYL-L-VALINE METHYL ESTER



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1. Procedure

A. *Benzyloxycarbonyl-L-alanyl-L-cysteine methyl ester*. A round-bottomed, three-necked, 100-mL flask is equipped with a magnetic stirring bar, 10-mL dropping funnel, thermometer, and nitrogen bubbler (Note 1). The apparatus is flushed with dry nitrogen and then charged with 446.5 mg (0.002 mol) of benzyloxycarbonyl-L-alanine in 10 mL of dry dichloromethane. The mixture is stirred until solution is complete and then cooled to 0°C. Within 20 min a solution of 525.5 mg (0.002 mol) of 1-(4-chlorophenyl)-3-(4'-methyl-1'-piperazinyl)-2-propyn-1-one (Note 2) in 5 mL of dry dichloromethane is added. Stirring is continued for 1 hr at 0°C and for an additional hour at room temperature (t_1). The mixture is cooled again to 0°C and a suspension of 343.3 mg (0.002 mol) of L-cysteine methyl ester hydrochloride is quickly added, followed by a solution of 202.3 mg (0.002 mol) of *N*-methylmorpholine in 5 mL of dry dichloromethane. While the nitrogen atmosphere is maintained, the mixture is allowed to warm up and is stirred for 12 hr (t_2) at room temperature. The solvent is removed by rotary evaporation, and the residue is shaken intensively with 30 mL of ethyl acetate and 10 mL of water. The organic layer is extracted two times with 10-mL portions of aqueous 10% citric acid and once with 5 mL of 1 *N* sodium hydrogen carbonate. The organic phase is dried over sodium sulfate. The solvent is removed by rotary evaporation to leave 647 mg (95%) of the crude pale-yellow dipeptide. Recrystallization from ethyl acetate provides 551 mg (81%) of colorless crystals of benzyloxycarbonyl-L-alanyl-L-cysteine methyl ester, mp 115–117°C; $[\alpha]_D^{20} - 26.4^\circ$ (CH_3OH , c 1.29) (Note 3).

B. *Benzyloxycarbonyl-L-aspartyl-(tert-butyl ester)-L-phenylalanyl-L-valine methyl ester*. A round-bottomed, three-necked, 100-mL flask is equipped with a 10-mL dropping funnel, thermometer, magnetic stirring bar, and a nitrogen bubbler. The flask is flushed with dry nitrogen and then charged with a solution of 941.1 mg (0.002 mol) of benzyloxycarbonyl-L-aspartyl-(*tert*-butyl ester)-L-phenylalanine (Note 4) in 10 mL of dry dichloromethane. The flask is maintained under a dry nitrogen atmosphere and cooled to 0°C with an ice-salt bath. The mixture is stirred and a solution of 525.5 mg

(0.002 mol) of 1-(4-chlorophenyl)-3-(4'-methyl-1'-piperazinyl)-2-propyn-1-one (Note 2) in 5 mL of dry dichloromethane is added during a period of 20 min. Stirring is continued for 1 hr at 0°C and for 5 hr at room temperature (t_1). The mixture is again cooled to 0°C and a suspension of 335.3 mg (0.002 mol) of L-valine methyl ester hydrochloride and 202.3 mg (0.002 mol) of N-methylmorpholine in 5 mL of dichloromethane is added. After 30 min the reaction mixture is allowed to warm up and is stirred overnight (18 hr; t_2) at room temperature. The solvent is removed by rotary evaporation and the residue is shaken intensively with 40 mL of ethyl acetate and 10 mL of water. The organic layer is extracted twice with 10-mL portions of aqueous 10% citric acid and once with 5 mL of 1 N sodium hydrogen carbonate.

The organic phase is dried over sodium sulfate, and solvent is removed by rotary evaporation to leave 1132 mg (97%) of the crude pale-yellow tripeptide. For further purification the crude product is dissolved in ethyl acetate, treated with some activated carbon, and filtered through Celite. Removal of the solvent and crystallization from ethyl acetate/ether/petroleum ether (ca. 2 : 1 : 1) yields 993 mg (85%) of colorless crystals of benzyloxycarbonyl-L-aspartyl-(tert-butyl ester)-L-phenylalanyl-L-valine methyl ester; mp 119–120°C (Note 5).

2. Notes

1. Cysteine derivatives are oxidized to cystine by oxygen. The nitrogen atmosphere for preparation of Cbz-alanycysteine methyl ester is therefore indispensable and is recommended for other cases as well.
2. This reagent is available from Fluka Chemical Corp.
3. The literature² value is $[\alpha]_D^{20} - 26.5^\circ$ (CH₃OH, c 1.27). The reported² mp is 116.5–118°C.
4. Benzyloxycarbonyl-L-aspartyl-(tert-butyl ester)-L-phenylalanine dicyclohexylamine salt was conveniently prepared by standard procedures.³ The salt was dissolved in ethyl acetate and extracted three times with aqueous 10% citric acid, and once with water. The organic phase was dried and solvent was removed to leave the dipeptide as an oil.
5. The product has the following physical properties: Specific rotation: $[\alpha]_D^{20} - 36.5^\circ$ (C₂H₅OH, c 2); IR (KBr), cm⁻¹: 3285, 1732, 1691, 1640, 1531, 1367, 1229, 1158, 1050, 746, 701; ¹H NMR (100 MHz, CDCl₃) δ : 0.81 (d, 3 H, $J = 7$) 0.84 (d, 3 H, $J = 7$), 1.41 (s, 9 H), 1.8–2.3 (m, 2 H), 2.62 (d of d, 1 H, $J = 17$, $J' = 6$), 2.86 (d of d, 1 H, $J = 17$, $J' = 5$), 3.08 (d, 1 H, $J = 8$), 3.71 (s, 3 H), 4.3–4.8 (m, in total 3 H), 5.11 (s, 2 H), 5.78 (d, 1 H, $J = 8$), 6.28 (d, 1 H, $J = 8$), 7.02 (d, 1 H, $J = 8$), 7.21 (s, 5 H), 7.57 (s, 5 H).

3. Discussion

This procedure illustrates the use of 1-(4-chlorophenyl)-3-(4'-methyl-1'-piperazinyl)-2-propyn-1-one^{4,5} as a reagent for peptide synthesis.⁶ The same method also gives amides in excellent yields.⁷

The preparation of Cbz-L-alanycysteine methyl ester shows the advantage of using, as the amine component, an amino acid with an unprotected sulfhydryl moiety. No problems were encountered with the use of amino acid derivatives with unprotected hydroxyl or sulfhydryl groups as either the amine⁶ or carboxyl component.⁸ This procedure is based on the pronounced selective reactivity of the enol ester, which is generated by the addition of carboxylic acids to "push-pull acetylenes." Generally, the yields of peptides are good and a broad variety of solvents (e.g., dichloromethane, tetrahydrofuran, acetonitrile, dimethylformamide) may be used, depending on the solubility of the coupling components. It is also possible to change the solvent after the activation step or to isolate the activated components. Normally, however, this is neither necessary nor recommended. Purification of the reaction mixture is simple, since the piperazine by-product is conveniently extracted with an acidic water phase.

The following peptides and further examples have been prepared⁶ by the following procedure.

Peptide	t_1	t_2	Yield (%) ^c
Cbz-L-Ala-Gly-OMe	2 ^d	12	91
Cbz-L-Ala-L-Val-OMe	2 ^d	24	84
Cbz-L-Ala-L-Phe-OMe	2 ^d	18	88

Cbz-Gly-L-Phe-Gly-OEt	2	12	90
Cbz-L-Asp(O- <i>t</i> -Bu)-L-Phe-L-Val-OMe	6	18	85
Cbz-L-Ile-L-Ile-OBzl	18	24	75
Cbz-L-Ala-L-Ser-OMe	2 ^d	72	90
Cbz-L-Ala-L-Tyr-OMe	2 ^d	72	91
Cbz-L-Ala-L-Cys-OMe	2 ^d	12	81
Cbz-L-Ala-L-Met-OMe	2 ^d	15	85
Cbz-L-Ser-Gly-OEt	2	24	81

^a t_1 : time for activation of the carboxylic component (see procedures).

^b t_2 : time for coupling (see procedures).

^c Yield of pure recrystallized product.

^d Stirring 1 hr at 0°C, then 1 hr at 20°C.

During our experiments no side reactions were detected. This is in contrast to peptide synthesis with isoxazolium salts,^{9–10} where some side reactions, one leading to a diacyl amino compound, were observed.¹¹ In most cases, these side reactions are due to a secondary amino group in the reagent which is impossible in the case of push–pull acetylenes.

Compared with ynamines, which have also been applied to peptide synthesis,¹² push–pull acetylenes are much more selective. They do not show the side reactions observed with ynamines,¹³ and the yields are not markedly influenced by the sequence of addition of compounds in the activation step or by excess of acetylene reagent.

A crucial point in peptide synthesis is racemization of the activated amino acid. Three different tests were made to evaluate the degree of racemization. Using the Anderson test peptide¹⁴ Cbz-Gly-Phe-Gly-OEt, no racemization could be detected when the peptide was prepared in dichloromethane, acetonitrile, or tetrahydrofuran. This means racemization is below the detection limit of 1%. Benzylleucylglycine ethyl ester is used in the very sensitive Young test.¹⁵ In this test, designed to exaggerate racemization, we found 5% of racemate, when the solvent was dichloromethane. In the more polar solvent, dimethylformamide, this value rose to 12%. Therefore, racemization is in the same range as that observed for the racemization-resistant azide procedure. The coupling of Cbz-L-aspartyl(O-*t*-Bu)-L-phenylalanine with valine methyl ester is reported to be very sensitive to racemization.¹⁶ The tripeptide was prepared as described above, and the crude product was hydrolyzed. Gas-layer chromatography (GLC) showed the presence of 2–3% D-phenylalanine. Again, in contrast to the ynamine procedure,¹⁷ racemization seems to be no problem when push–pull acetylenes are used.

So far, the only observable disadvantage of the reagent is the somewhat long reaction time for the coupling of the activated amino acids (or peptides) with the amine component. The increase in reaction time t_2 could be a limiting factor, if longer peptide fragments are to be linked.

References and Notes

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Appendix
Chemical Abstracts Nomenclature (Collective Index Number);
(Registry Number)

petroleum ether

Benzyloxycarbonyl-L-aspartyl-(tert-butyl ester)-L-phenylalanyl-L-valine methyl ester

benzyloxycarbonyl-L-aspartyl-(tert-butyl ester)-L-phenylalanine

Benzyloxycarbonyl-L-aspartyl-(tert-butyl ester)-L-phenylalanine dicyclohexylamine salt

Cbz-L-Ala-Gly-OMe

Cbz-L-Ala-L-Val-OMe

Cbz-L-Ala-L-Phe-OMe

Cbz-Gly-L-Phe-Gly-OEt

Cbz-L-Asp(O-t-Bu)-L-Phe-L-Val-OMe

Cbz-L-Ile-L-Ile-OBzl

Cbz-L-Ala-L-Ser-OMe

Cbz-L-Ala-L-Tyr-OMe

Cbz-L-Ala-L-Cys-OMe

Cbz-L-Ala-L-Met-OMe

Cbz-L-Ser-Gly-OEt

Cbz-Gly-Phe-Gly-OEt

Cbz-L-aspartyl(O-t-Bu)-L-phenylalanine

acetylene (74-86-2)

ethyl acetate (141-78-6)

ether (60-29-7)

acetonitrile (75-05-8)

citric acid (77-92-9)

sodium hydrogen carbonate (144-55-8)

L-alanine (56-41-7)

sodium sulfate (7757-82-6)

oxygen (7782-44-7)

nitrogen (7727-37-9)

carbon (7782-42-5)

cystine (56-89-3)

dichloromethane (75-09-2)

Tetrahydrofuran (109-99-9)

dimethylformamide (68-12-2)

benzyloxycarbonyl

piperazine (110-85-0)

VALINE METHYL ESTER

cysteine methyl ester hydrochloride (18598-63-5)

L-valine methyl ester hydrochloride (5619-05-6)

Cbz-alanycysteine methyl ester,
Cbz-L-alanycysteine methyl ester,
Benzyloxycarbonyl-L-alanyl-L-cysteine methyl ester

Benzylleucylglycine ethyl ester

N-methylmorpholine (109-02-4)

1-(4-CHLOROPHENYL)-3-(4'-METHYL-1'-PIPERAZINYL)-2-PROPYN-1-ONE

D-phenylalanine