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A Novel Method for the Esterification of *N*-Protected Amino Acids

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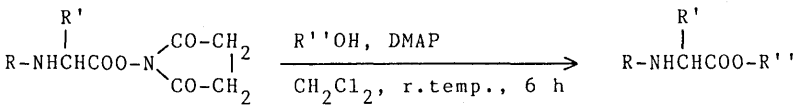
Abstract

A mild and simple procedure for the esterification of *N*-protected amino acids is described involving room temperature reaction of *N*-hydroxysuccinimide esters of a variety of *N*-protected amino acids with alcohols in the presence of 4-(*N*, *N*-dimethylamino)-pyridine. In this manner a depsipeptide was also prepared in high yield.

Mild and simple esterification procedures are of considerable interest in the synthesis and manipulation of many natural products. Esters of *N*-protected amino acids are useful intermediates not only for the peptide synthesis,[1] but also for the preparation of amino aldehydes.[2,3] The carboxyl group of amino acids is commonly protected as an alkyl ester during peptide synthesis, for which the use of methyl, ethyl, benzyl, and *tert*-butyl esters is well-documented.[1] The preparation of esters of *N*-protected amino acids is most often effected by alkylation with an alkyl halide of the triethylammonium[4] or cesium[5] salt of the corresponding carboxylate ion. Recently the use of 4-(*N*, *N*-dimethylamino) pyridine (DMAP)[6] as catalyst for carbodiimide mediated esterification of *N*-protected amino acids has been reported.[7-9] The author now reports that esters of *N*-protected amino acids are easily obtained in high yields and stereochemically pure form by reaction of the corresponding *N*-hydroxysuccinimide esters with the alcohols in the presence of DMAP under mild conditions as shown below:



Scheme 1.

The *N*-hydroxysuccinimide esters of *N*-protected amino acids are readily available and well used as active ester for acylation reaction.[10,11] The esters of *N*-protected amino acids were prepared in high yields by the treatment of the *N*-hydroxysuccinimide ester of *N*-protected amino acid with 1.5 equivalent of the appropriate alcohol and 1 equivalent

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Table 1. Isolated yield in esterification of *N*-protected amino acids and dipeptide[a]

N-Hydroxysuccinimide ester	Alcohol	Product	Yield(%)[b]
Z-Asn-ONSu	MeOH	Z-Asn-OMe (**1**)	87
Boc-Asp(OBzl)-ONSu	BzlOH	Boc-Asp(OBzl)-OBzl (**2**)	98
Boc-Cys(Bzl)-ONSu	BzlOH	Boc-Cys(Bzl)-OBzl (**3**)	85
Z-Gln-ONSu	BzlOH	Z-Gln-OBzl (**4**)	90
Boc-Glu(OBzl)-ONSu	BzlOH	Boc-Glu(OBzl)-OBzl (**5**)	93
Boc-Gly-ONSu	BzlOH	Boc-Gly-OBzl (**6**)	81
Boc-Phe-ONSu	BzlOH	Boc-Phe-OBzl (**7**)	96
Boc-Tyr(Bzl)-ONSu	MeOH	Boc-Tyr(Bzl)-OMe (**8**)	98
Z-Gly-Gly-ONSu	MeOH	Z-Gly-Gly-OMe (**9**)	80
Z-Gly-Gly-ONSu	EtOH	Z-Gly-Gly-OEt (**10**)	84
Z-Gly-Gly-ONSu	BzlOH	Z-Gly-Gly-OBzl (**11**)	90
Boc-Ala-ONSu	H-Hmb-Phe-OBzl	Boc-Ala-Hmb-Phe-OBzl (**12**)	82[c]

[a] The amino acids and hydroxy acid used, except Gly, are all of L-configuration. [b] Yields reported are for the products purified by recrystallization. [c] Yield is for product purified by silica gel column chromatography.

of DMAP in CH_2Cl_2 at room temperature for 6 h. The esters (**1-8**) prepared by this method are summarized in Table 1. Both *N*-benzyloxycarbonyl and *N-tert*-butyloxycarbonyl amino acids readily undergo esterification with methanol, ethanol, or benzyl alcohol to furnish the corresponding esters in satisfactory to high yields. Unfortunately, *tert*-butyl esters of *N*-protected amino acid could not be obtained by this method.

The esters (**9-11**) of *N*-protected dipeptide, Z-Gly-Gly-OR", were also synthesized in high yields by this procedure as shown in Table 1.

The method is applicable to the preparation of depsipeptide. Boc-L-Ala-L-Hmb-L-Phe-OBzl (**12**),[12] which is an important intermediate for the synthesis of AM-toxins,[13,14] was prepared in 82% yield from Boc-L-Ala-ONSu and 1 equivalent of H-L-Hmb-L-Phe-OBzl by this method.

The analytical parameters of the products obtained by this method with respect to melting point and specific rotation were identical with those of the literatures (see Experimental Section).

The reaction is based on a requirement for DMAP. Thus, in the absence of DMAP no reaction occurs. Probably, *N*-hydroxysuccinimide ester is converted to an acylpyridinium species, reactive intermediate, with DMAP. Nucleophilic attack by the alcohol on the acyl group of the acylpyridinium species generates the desired ester and DMAP, which consequently forms the salt with the resulting *N*-hydroxysuccinimide.

Several advantages of this new procedure should be pointed out here in comparison with the known methods. The *N*-hydroxysuccinimide esters of *N*-protected amino acids are relatively stable and may be stored for ready use, and hence the esters could be easily obtained from commercial sources. This esterification procedure is very simple and

proceeds under mild reaction conditions (CH_2Cl_2, at room temperature), so that labile protecting groups for other functional groups can be present.

This mild and simple procedure may provide a versatile synthetic method not only for the synthesis of peptides and depsipeptides but also for the preparation of many natural products. Further studies in order to demonstrate the utility of this method are now in progress.

Experimental

All the melting points were determined in open capillaries on a MP-21 Yamato melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO digital automatic polarimeter Model DIP-140 in a 1 dm microcell. Elemental analyses were carried out on a Yanagimoto CHN corder Model MT-2 instrument. TLC was performed on silica gel 60 F_{254} pre-coated plates (Merck) with the following solvent systems, the ratio in parentheses being indicated by volume: Rf^1, $CHCl_3$-MeOH (5:1); Rf^2, $CHCl_3$-MeOH-AcOH (95:5:1); Rf^3, $CHCl_3$-MeOH (10:1); Rf^4, $CHCl_3$-acetone (10:1).

Materials. The amino acids and hydroxy acid used, except of Gly, are all of L-configuration.

N-Hydroxysuccinimide esters, Z-Asn-ONSu,[15] Boc-Asp (OBzl)-ONSu,[16] Boc-Cys (Bzl)-ONSu,[10] Z-Gln-ONSu,[17] Boc-Glu (OBzl)-ONSu,[18] Boc-Gly-ONSu,[10] Boc-Phe-ONSu,[10] Boc-Tyr (Bzl)-ONSu,[19] Z-Gly-Gly-ONSu,[20] and Boc-Ala-ONSu,[10] were prepared according to the literatures.

H-Hmb-Phe-OBzl was synthesized form H-Hmb-OH[21] (3.90 g, 33.0 mmol) and H-Phe-OBzl•TosOH[22] (12.8 g, 30.0 mmol) by the DCC/HOBt method. Recrystallization from EtOAc-*n*-hexane gave the desired compound; yield, 9.44 g (89%); mp, 89.0-89.5°C; $[\alpha]_D^{20}$ -37.2° (c 1, MeOH); Rf^1 0.67, Rf^2 0.48. Found: C, 71.23; H, 7.15; N, 3.94%. Calcd for $C_{21}H_{25}O_4N$: C, 70.96; H, 7.09; N, 3.94%.

General Procedure of Esterification. A stirred suspension of the *N*-hydroxysuccinimide ester (1 mmol) in dry CH_2Cl_2 (2 ml) were added the alcohol (1.5 mmol) or H-Hmb-Phe-OBzl (1 mmol) and then DMAP (1 mmol) at room temperature. After 6 h the clear reaction mixture was dissolved in EtOAc (60 ml). The solution was washed successively with water (15 ml), 10% aqueous citric acid (15 ml), water (15 ml), 4% aqueous $NaHCO_3$ (15 ml), water (15 ml) and brine (15 ml), dried over anhydrous $MgSO_4$, and evaporated to dryness. Recrystallization from the appropriate solvent gave the

desired ester (**1-11**). The tridepsipeptide (**12**), Boc-Ala-Hmb-Phe-OBzl, was purified by silica gel column chromatography.

Following compounds were prepared by this procedure.

Z-Asn-OMe (**1**). Recrystallization from EtOAc-ether-petroleum ether gave the compound **1**; yield, 244 mg; mp, 153.5-154.0 °C; $[\alpha]_D^{25}$ -0.4° (c 1, AcOH); Rf^1 0.40, Rf^2 0.27. Reported values; mp, 151-152 °C; $[\alpha]_D^{20}$ -0.9° (c 1, AcOH).[23] Found: C, 55.70; H, 5.75; N, 9.99%. Calcd for $C_{13}H_{16}O_5N_2$: C, 55.71; H, 5.75; N, 10.00%.

Boc-Asp(OBzl)-OBzl (**2**). Recrystallization from EtOAc-*n*-hexane gave the compound **2**; yield, 405 mg; mp, 59.5-61.0 °C; $[\alpha]_D^{20}$ -11.8° (c 1, MeOH); Rf^1 0.83, Rf^2 0.83. Found: C, 64.48; H, 6.54; N, 3.32%. Calcd for $C_{23}H_{27}O_6N \cdot 4/5H_2O$: C, 64.56; H, 6.74; N, 3.27%.

Boc-Cys(Bzl)-OBzl (**3**). Recrystallization from EtOAc-petroleum ether gave the compound **3**; yield, 340 mg; mp, 86.5-87.5 °C; $[\alpha]_D^{25}$ -39.2° (c 2, DMF); Rf^1 0.88, Rf^2 0.88. Reported values; mp, 84-86 °C; $[\alpha]_D^{27}$ -40.4° (c 2, DMF).[24] Found: C, 65.63; H, 6.73; N, 3.49%. Calcd for $C_{22}H_{27}O_4NS$: C, 65.81; H, 6.78; N, 3.49%.

Z-Gln-OBzl (**4**). Recrystallization from EtOAc-ether-petroleum ether gave the compound **4**; yield, 333 mg; mp, 130.0-131.5 °C; $[\alpha]_D^{25}$ -8.3 °(c 0.62, AcOH); Rf^1 0.43, Rf^2 0.32. Reported values; mp, 129-130 °C; $[\alpha]_D^{22}$ -7.5° (c 0.67, AcOH).[25] Found: C, 64.24; H, 5.95; N, 7.68%. Calcd for $C_{20}H_{22}O_5N_2 \cdot 1/5H_2O$:C, 64.23; H, 6.04; N, 7.49%.

Boc-Glu(OBzl)-OBzl (**5**). Recrystallization from EtOAc-*n*-hexane gave the compound **5**; yield, 399 mg; mp, 72.0-73.0 °C; $[\alpha]_D^{20}$ -22.5° (c 1, MeOH); Rf^1 0.83, Rf^2 0.83. Found: C, 67.55; H, 6.80; N, 3.20%. Calcd for $C_{24}H_{29}O_6N$: C, 67.43; H, 6.84; N, 3.28%.

Boc-Gly-OBzl (**6**). Recrystallization from EtOAc-*n*-hexane gave the compound **6**; yield, 215 mg; mp, 73.0-74.0 °C; Rf^1 0.78, Rf^3 0.68. Reported value; mp, 72-73 °C.[9] Found: C, 63.16; H, 7.23; N, 5.12%. Calcd for $C_{14}H_{19}O_4N$: C, 63.38; H, 7.22; N, 5.28%.

Boc-Phe-OBzl (**7**). Recrystallization from EtOH-water gave the compound **7**; yield, 341mg; mp, 67.0-68.0 °C; $[\alpha]_D^{25}$ -12.4° (c 2, MeOH); Rf^1 0.83, Rf^3 0.73. Reported values; mp, 64-65 °C; $[\alpha]_D^{amb}$ -12.8° (c 2, MeOH).[9] Found: C, 70.84; H, 7.04; N, 3.93%. Calcd for $C_{21}H_{25}O_4N$: C, 70.96; H, 7.09; N, 3.94%.

Boc-Tyr(Bzl)-OMe (**8**). Recrystallization from EtOAc-petroleum ether gave the compound **8**; yield, 378 mg; mp, 56.5-57.5 °C; $[\alpha]_D^{25}$ +5.1° (c 1, MeOH); Rf^1 0.83, Rf^2 0.83. Reported value; mp, 59-60 °C.[26] Found: C, 68.48;

H, 7.08; N, 3.56%. Calcd for $C_{22}H_{27}O_5N$: C, 68.55; H, 7.06; N, 3.63%.

Z-Gly-Gly-OMe (**9**). Recrystallization from EtOAc-*n*-hexane gave the compound **9**; yield, 223 mg; mp, 66.5-68.0 °C; Rf^1 0.51, Rf^2 0.30. Reported value; mp, 63-64 °C.[27] Found: C, 55.81; H, 5.72; N, 10.01%. Calcd for $C_{13}H_{16}O_5N_2$: C, 55.71; H, 5.75; N, 10.00%.

Z-Gly-Gly-OEt (**10**). Recrystallization from EtOAc-*n*-hexane gave the compound **10**; yield, 246 mg; mp, 77.0-78.0 °C; Rf^1 0.53, Rf^2 0.30. Reported value; mp, 77-79 °C.[28] Found: C, 57.03; H, 6.13; N, 9.52%. Calcd for $C_{14}H_{18}O_5N_2$: C, 57.13; H, 6.17; N, 9.52%.

Z-Gly-Gly-OBzl (**11**). Recrystallization from EtOAc-ether-petroleum ether gave the compound **11**; yield, 319 mg; mp, 108.5-109.5 °C; Rf^1 0.59, Rf^2 0.36. Reported value; mp, 111-112 °C.[29] Found: C, 63.95; H, 5.66; N, 7.74%. Calcd for $C_{19}H_{20}O_5N_2$: C, 64.03; H, 5.66; N, 7.86%.

Boc-Ala-Hmb-Phe-OBzl (**12**). The crude product contained small amounts of reactants, so purification was carried out by silica gel chromatography using a column (2.2 x 22 cm) and a solvent of $CHCl_3$-acetone (20:1). The fractions (50-115 ml) were evaporated in vacuo. The residual solid was recrystallized from EtOAc-ether-*n*-hexane; yield, 432 mg; mp, 107.5-108.5 °C; $[\alpha]_D^{20}$ -30.8 °(c 1, $CHCl_3$); Rf^1 0.77, Rf^2 0.75, Rf^4 0.61. Reported values; mp, 86-87 °C; $[\alpha]_D^{20}$ -27.6°(c 1, $CHCl_3$).[12] Found: C, 66.03; H, 7.29; N, 5.35%. Calcd for $C_{29}H_{38}O_7N_2$: C, 66.14; H, 7.27; N, 5.32%.

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6) Abbreviations given by the IUPAC-IUB Commission (*Eur. J. Biochem.*, **138**, 9 (1984)) have been used throughout. Other abbreviations: Bzl, benzyl ether; BzlOH, benzyl alcohol; DCC, *N,N'*-dicyclohexylcarbodiimide; DMAP, 4-(*N,N*-dimethylamino) pyridine; DMF, *N,N*-dimethylformamide; Hmb, 2-hydroxy-3-methylbutanoic acid; HOBt, 1-hydroxybenzotriazole; OBzl, benzyl ester; ONSu, *N*-hydroxysuccinimide ester; TosOH, *p*-toluenesulfonic acid.
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