Peptides. VII. Some Sulfonates of Strongly Acidic *N*-Hydroxy Compounds as Novel Coupling Reagents[1)]

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Sulfonates of strongly acidic *N*-hydroxy compounds such as 6-chloro-1-(*p*-chlorophenylsulfonyloxy)benzotriazole have been found to be excellent coupling reagents for amide bond formation. Amides can be prepared through either an active ester or a mixed anhydride according to the chosen procedure. Several active esters and protected di- and tripeptides prepared are listed in the Tables. Racemization, possible side reactions, and reaction pathways of the amide bond formation have also been discussed.

Many reports on coupling methods and reagents for peptide synthesis have been published. Many of these reagents can be added directly to a mixture of a carboxyl and an amino components to simplify the technique. Of these, dicyclohexylcarbodiimide (DCC) is the most versatile and widely utilized one, although it has the following disadvantages:[2)] 1) the rearrangement of the active intermediate, *O*-acyl-*N*,*N*′-dicyclohexylisourea, to the inactive *N*-acyl-*N*,*N*′-dicyclohexylurea, 2) the contamination of products by *N*,*N*′-dicyclohexylurea which is to a certain extent soluble in many organic solvents, 3) the dehydration from the side chain amide group of *N*-protected asparagine or glutamine to the corresponding cyano group, 4) the racemization *via* oxazolone formation, and 5) the allergic property to humans. A recent investigation revealed that DCC might lead histidine-containing peptides to their N^{im}-(*N*,*N*′-dicyclohexylamidino) derivatives.[3)] The present authors wish to report some new sulfonate-type coupling reagents which promise the simple operation, high purity and high yield of products, and seem to be superior to DCC at the points mentioned above.

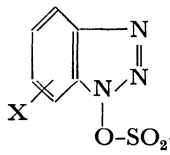
It has been reported that some carbonates of strongly acidic *N*-hydroxy compounds activated carboxylic acids by the formation of mixed anhydride-type intermediates which were subsequently transformed to esters in good yield by eliminating carbon dioxide. One of these carbonates, ethyl 2-(isobutoxycarbonyloxyimino)-2-cyanoacetate, may be employed as a coupling reagent in the mixed anhydride method.[4)] These results prompted the preparation of ethyl 2-(methylsulfonyloxyimino)-2-cyanoacetate (**3a**)[5)] and its potency as a coupling reagent was examined. Preliminary experiments showed that the coupling of a carboxylic acid with an amine proceeded smoothly by the use of **3a** and triethylamine at room temperature affording an amide in excellent yield. To demonstrate the superiority of **3a** to methanesulfonyl chloride (**1a**) itself or a combination of **1a**/ethyl 2-hydroxyimino-2-cyanoacetate (**2a**), ethyl *N*-benzyloxycarbonyl-L-prolyl-L-leucinate (**5**) was prepared using the above mentioned reagents. *N*-Benzyloxycarbonyl-L-proline (**6**) was allowed to react with the reagents in the presence of triethylamine in chloroform for 15 min at 0 °C, then a solution of ethyl L-leucinate (**7**) in chloroform was added to the resulting mixture. After stirring for 18 h at room temperature, **5** was obtained in 61.5, 76.9, and 80.2% yield by the use of **1a**, the combination of **1a**/**2a**, and **3a**, respectively. These results indicated the value of preparing other sulfonates of *N*-hydroxy compounds. The sulfonates were prepared by the reaction of alkane- or arenesulfonyl chlorides (**1**) with *N*-hydroxy compounds (**2**) under the conditions of the Schotten-Baumann reaction, and were stable, easy to prepare, and highly reactive crystalline materials. Some sulfonates were difficult to isolate such as 1-(trichloromethylsulfonyloxy)-benzotriazole. The sulfonates of *N*-hydroxy compounds prepared are listed in Tables 1 and 2. Most of the sulfonates are stable crystalline materials unless

Table 1. Some sulfonates of various *N*-hydroxy compounds

$$R^1\text{-}SO_2\text{-}O\text{-}N\begin{matrix}R^2\\R^3\end{matrix} \quad (3)$$

	R^1	R^2 R^3	Solvent	Base	Yield(%)	Mp(°C)	Recrysted from
3a	Me	NC-C(-)-COOEt	C_6H_6	TEA	96.3	79—81	C_6H_6/pet. ether
b	Me-C$_6$H$_4$-	NC-C(-)-COOEt	EtOAc	TEA	93.9	84—87	EtOAc/c. hexane
c	Me	NC-C(-)-$CONH_2$	H_2O/EtOAc	NaOH	76.4	143—145	Diox./EtOAc/pet. ether
d	Me	$-CO(CH_2)_2-CO-$	C_6H_6/Diox.	TEA	32.6	150—152	EtOAc/pet. ether
e	Me	-CO-C$_6$H$_4$-N=N-	C_6H_6	TEA	77.2	132—134	C_6H_6/pet. ether
f	C$_6$H$_5$-	-CO-C$_6$H$_4$-N=N-	H_2O/EtOAc	NaOH	88.9	132—134	C_6H_6

Table 2. Sulfonates of 1-hydoxybenzotriazoles



(4)

4	R^1	X	Solvent	Base	Yield (%)	Mp (°C)	Crystd from
a	Me	H	H_2O/EtOAc	NaOH	99.0	90—92	A
b	Bu(*n*)	H	C_6H_6	TEA	96.8	oil	
c	CH_2-(DL)	H	CH_2Cl_2	TEA	35.8	148—150	B
d	CH_2-	H	H_2O/ether	NaOH	46.7	65—67	A
e	-	H	H_2O	NaOH	85.5	83—84	B
f	-Me	H	H_2O/ether	NaOH	86.1	84—86	C
g	-Cl	H	C_6H_6	TEA	98.6	94—95	D
h	$-NO_2$	H	H_2O	NaOH	43.8	119.5—121.5	E
i	Me_3(2,4,6)	H	CH_2Cl_2	TEA	68.9	130—131	F
j	N	H	H_2O/ether	NaOH	70.0	98	
k	-CH=CH-	H	CH_2Cl_2	TEA	75.0	102—103	B
l	Me	NO_2(6)	H_2O/EtOAc	NaOH	72.0	117—118	B
m	CH_2-(DL)	NO_2(6)	CH_2Cl_2	TEA	89.0	157—159	B
n	-	NO_2(6)	EtOAc	TEA	53.1	122—124	B
o	-Me	NO_2(6)	EtOAc	TEA	56.1	144	B
p	Me	Cl(6)	C_6H_6	TEA	82.9	174—175(dec)	D
q	Bu(*n*)	Cl(6)	EtOAc	TEA	88.9	72—74	G
r	CH_2-(DL)	Cl(6)	C_6H_6	TEA	89.6	151—153	H
s	CH_2-	Cl(6)	H_2O/ether	NaOH	57.3	126.5—127.5	H
t	-	Cl(6)	H_2O/ether	NaOH	83.0	110—110.5	H
u	-Me	Cl(6)	C_6H_6	TEA	77.8	94—96	I
v	-Cl	Cl(6)	H_2O/ether	NaOH	91.4	125—127	A
w	Me	Cl(4)	C_6H_6	TEA	79.2	71—74	D

Recrystallization solvents: A=benzene/petroleum ether; B=ethyl acetate/hexane; C=ethanol; D=ethyl acetate/peteoleum ether; E=benzene; F=dichloromethane/hexane; G=tetrachloromethane/petroleum ether; H=benzene/hexane; I=terachloromethane.

exposed to atmospheric moisture for a long period, and are storable in a closed brown bottle without decomposition for three years. Some representative sulfonates were subjected to a rough comparison of their reactivity in the coupling of **6** or *N*-benzyloxycarbonyl-L-phenylalanine (**8**) with **7** at room temperature (Table 3). It is apparent from the table that the reactivity of each sulfonate is largely dependent on the acidity of both the parent *N*-hydroxy compound and the sulfonic acid. Electron-withdrawing substituents on the nuclei of both benzotriazole and benzenesulfonic acid increased the reactivity of the corresponding sulfonate. In contrast with the reactive sulfonates, the sulfonates of phenols such as the methanesulfonate[6] of 2,4-dinitrophenol (pK_a 4.11) showed far less reactivity.

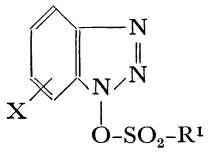
Table 3. An approximate comparison of the reactivity of representative sulfonates

Z-AA-OH + H-Leu-OEt ⟶ Z-AA-Leu-OEt

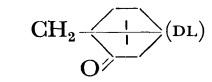
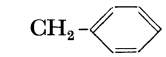
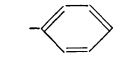
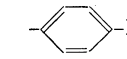
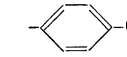
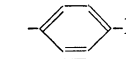
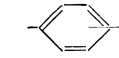
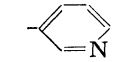
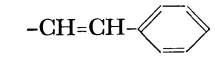
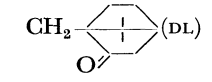
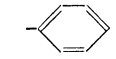
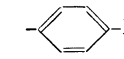
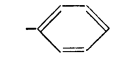
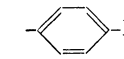
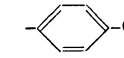
Z-AA-OH	Reagent	Procedure	Base	Solvent	Yield (%)
Z-Phe-OH	**3a**	A	TEA	CH_2Cl_2	80
Z-Phe-OH	**3a**	B	TEA	CH_2Cl_2	80
Z-Phe-OH	**3d**	A	TEA	CH_2Cl_2	trace
Z-Phe-OH	**4a**	B	TEA	CH_2Cl_2	83
Z-Phe-OH	**4e**	B	TEA	CH_2Cl_2	80
Z-Phe-OH	**4v**	B	TEA	CH_2Cl_2	75
Z-Pro-OH	**3a**	B	TEA	$CHCl_3$	72
Z-Pro-OH	**3b**	B	TEA	$CHCl_3$	trace
Z-Pro-OH	**3e**	B	NMM	$CHCl_3$	67
Z-Pro-OH	**3f**	B	NMM	$CHCl_3$	67

NMM = *N*-Methylmorpholine.

Table 4. Comparison of reactivity and stability of the sulfonates

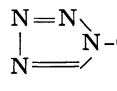
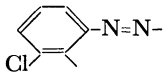
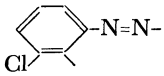
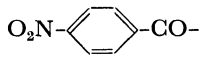
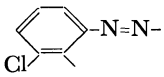
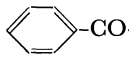
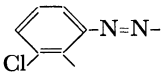
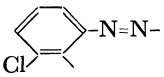
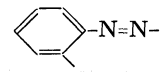
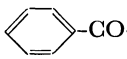
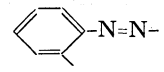
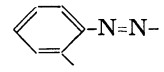
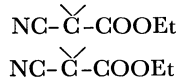


(**4**)

4	R^1	X	Reaction time required (min)	Stability to[a] moisture
a	Me	H	10	poor
c	 (DL)	H	180	excellent
d		H	—	poor
e		H	60	poor
f		H	360	—
g		H	25	good
h		H	20	—
i		H	120	—
j		H	20	—
k		H	45	poor
m	 (DL)	NO_2(6)	3	excellent
n		NO_2(6)	10	good
o		NO_2(6)	10	excellent
p	Me	Cl(6)	10	excellent
t		Cl(6)	20	good
u		Cl(6)	45	—
v		Cl(6)	5	good

a) Poor: decomposed within 3 days; good: unchanged after more than 3 days; excellent: unchanged after more than a week.

Table 5. Active esters of several carboxylic acids $R^4\text{-}COON\langle^{R^2}_{R^3}$

R^4-CO-	R^2 R^3	Reagent	Base	Solvent	Yield (%)	Mp (°C)	Recrystd from
		**4v**	TEA	$CHCl_3$	70	171—172 (dec)	$CHCl_3$
Z-Val-		**4v**	TEA	$CHCl_3$	84	96—96.5	ether/hexane
		**4v**	TEA	$CHCl_3$	69.6	198—199 (dec)	$CHCl_3$
		**4v**	TEA	CH_2Cl_2	83	132	CCl_4/ pet. ether
H_3C-CO-		**4v**	TEA	$CHCl_3$	83	141—142	EtOH
H_3C-CO-		**4a**	TEA	CH_2Cl_2	84.6	102—106	C_6H_6
		**4g**	TEA	$CHCl_3$	81	77—79	ether/ cyclohexane
Z-Phe-		**4a**	TEA	EtOAc	62	120—125	2-propanol
Z-Phe-		**3a**	TEA	EtOAc	95	oil[a]	
Z-Pro-		**3a**	TEA	EtOAc	100	oil[a]	

a) Solidifiied on standing.

In another series of preliminary experiments, it was observed that **3a**, 3-(methylsulfonyloxy)- and 3-(phenylsulfonyloxy)-1,2,3-benzotriazine-4-ones (**3e** and **3f**) induced colored impurities into the products, whereas the sulfonates of the benzotriazole series gave satisfactory results. Consequently, the reagents of the benzotriazole series were subsequently studied. To select the most prominent one from the sulfonates, the reactivity in the coupling of **6** (5 mmol) and **7** hydrochloride (2.5 mmol) by the use of triethylamine (7.5 mmol) and the sulfonates (5 mmol) were compared by recording the time required for the disappearance of free **7** in the reaction mixture. At the same time, the stability to atmospheric moisture was examined by infrared and thin layer chromatography. Most of the sulfonates adsorbed atmospheric moisture gradually during exposure and showed partial decomposition to some extent. Table 4 summarizes the results, and it is clearly shown that the stability of the reagents has no relation to their reactivity, but with their hygroscopic characters. In consideration of the reactivity, stability and accessibility, 6-chloro-1-(*p*-chlorophenylsulfonyloxy)benzotriazole (**4v**) was finally chosen as the prominent member of the series.

Using the sulfonates of acidic *N*-hydroxy compounds, amides can be prepared by the following two procedures: (A) activation of a carboxyl component with a reagent prior to the addition of an amine component, and (B) the direct addition of the reagent into a mixture of the carboxyl and the amine components. The use of an equimolar amount of tertiary amine is essential in both procedures.

Procedure A:

$$R^1\text{-}SO_2\text{-}ON\langle^{R^2}_{R^3} + R^4\text{-}COOH \xrightarrow{\text{Base}}$$

$$R^4\text{-}COON\langle^{R^2}_{R^3} + R^1\text{-}SO_3H\cdot\text{Base}$$

$$\downarrow H_2N\text{-}R^5$$

$$R^4\text{-}CONH\text{-}R^5 + HON\langle^{R^2}_{R^3}$$

Procedure B:

$$R^1\text{-}SO_2\text{-}ON\langle^{R^2}_{R^3} + R^4\text{-}COOH + H_2N\text{-}R^5 \xrightarrow{\text{Base}}$$

$$R^4\text{-}CONH\text{-}R^5 + R^1\text{-}SO_3H\cdot\text{Base} + HON\langle^{R^2}_{R^3}$$

Scheme 1.

In procedure A, the active ester formation is rapid and is usually completed within one hour using triethylamine in chloroform or ethyl acetate at room temperature. When pyridine is employed as a base, a polar solvent such as acetonitrile is necessary to produce a smooth reaction. The active ester formed in this stage can be isolated in good yield, some examples of which are listed in Table 5. Aminolysis of the active esters is also completed within a few hours at room temperature.

In procedure B, the reaction mixture tends to be

Table 6. Preparation of protected di- and tripeptides

Acylamino acid	Amino acid ester salt	Reagent	Procedure	Base/Solvent	Reaction time (h)	Yield (%)	Ref.
Boc-Arg(NO_2)-OH	Tos-OH·H-Tyr-OBzl	**4a**	B	2TEA/$CHCl_3$	20	70.3[a)]	
Z-Arg(NO_2)-OH	HCl·H-Gly-OEt	**4a**	B	2NMM/DMF-$CHCl_3$	18	73.1	8
Z-CyS(Bzl)-OH	HCl·H-Gly-OEt	**4a**	B	2TEA/$CHCl_3$	18	58.1	9
Z-Gln-OH	2HCl·H-His-OMe	**4a**	B	3TEA/$CHCl_3$	18	58.0	10
Z-Gln-OH	2HCl·H-His-OMe	**4q**	B	3TEA/$CHCl_3$	18	82.8	
Z-Gln-OH	Tos-OH·H-Tyr-OBzl	**4q**	A	2TEA/MeCN	0.5/18	71.8	11
Z-Phe-OH	HCl·H-Leu-OEt	**4a**	B	2TEA/$CHCl_3$	8	73.2	12
Z-Phe-OH	HCl·H-Leu-OEt	**4a**	B	3TEA/$CHCl_3$[b)]	2	73.2	
Z-Phe-OH	HCl·H-Ile-OEt	**4a**	B	2TEA/CH_2Cl_2	40	81.2	13
Z-Pro-OH	HCl·H-Leu-OEt	**4a**	B	2TEA/$CHCl_3$	15	74.3	14
Z-Pro-OH	HCl·H-Leu-OEt	**4f**	B	2TEA/$CHCl_3$	18	82.0	
Z-Pro-OH	HCl·H-Leu-OEt	**4q**	B	TEA-Pyr/CH_2Cl_2	18	77.4	
Z-Pro-OH	HCl·H-Ser-OMe	**4t**	B	2TEA/$CHCl_3$	18	85.7	15
Z-Val-OH	HCl·H-Gly-OEt	**4a**	B	2TEA/$CHCl_3$	65	77.3	11
Z-Val-OH	HCl·H-Val-OEt	**4a**	B	2TEA/CH_2Cl_2	72	80.4	16
Z-Val-OH	HCl·H-Val-OEt	**4o**	B	2TEA/CH_2Cl_2	48	74.0	
Z-Gly-Phe-OH	HCl·H-Gly-OEt	**4a**	B	2TEA/$CHCl_3$	18	72.5	17

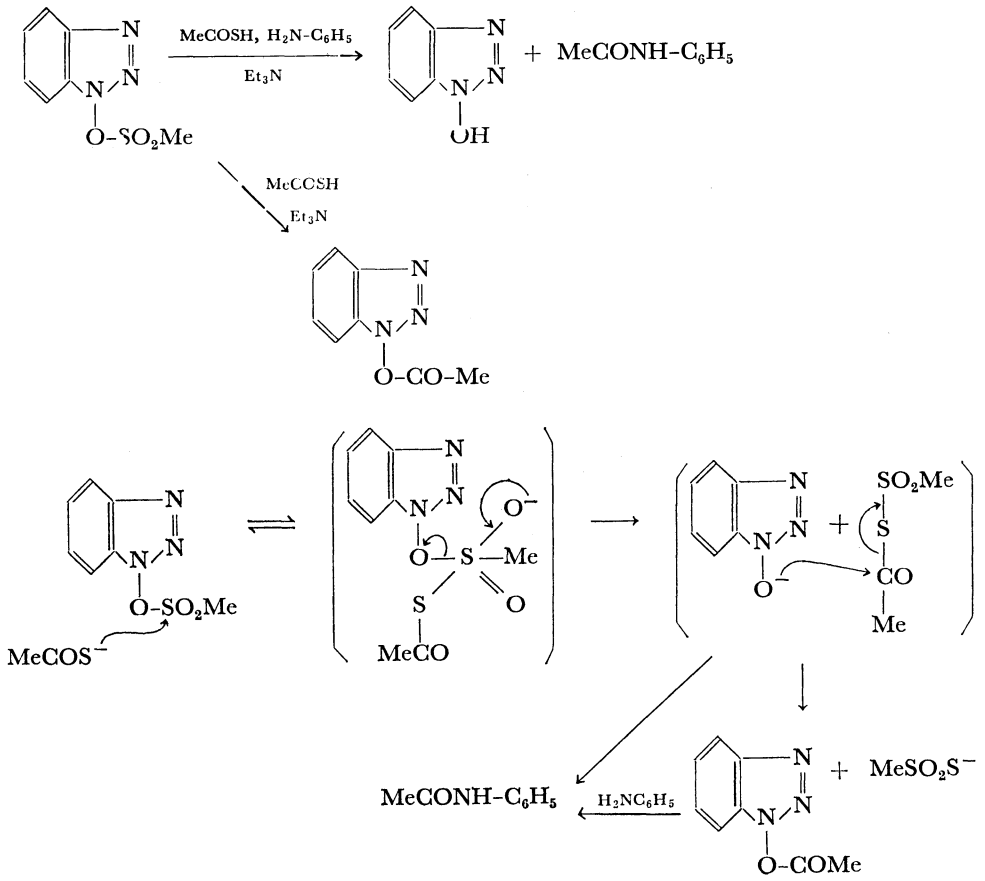
a) Hemihydrate; mp 79—80 °C(dec). b) Periodic neutralization was done.

Table 7. Preparation of model peptides using **4v**

Acylamino acid	Amino acid ester salt	Procedure	Base/Solvent	Reaction time (h)	(%) Yield	Ref.
Z-Arg(NO_2)-OH	HCl·H-Gly-OEt	B	2NMM/MeCN	2	71.2	8
Z-Arg(NO_2)-OH	HCl·H-Pro-OBzl	B	2NMM/$CHCl_3$	4	41.7	18
Boc-Arg(NO_2)-OH	Tos-OH·H-Tyr-OBzl	A	Pyr-NMM/DMF-MeCN	2/18	63.7	
Boc-Asn-OH	HCl·H-Phe-OEt	B	2TEA/DMF-$CHCl_3$	18	69.8	
Z-CyS(Bzl)-OH	HCl·H-Gly-OEt	B	2TEA/$CHCl_3$	18	85.0	9
(Z-CyS-OH)$_2$	HCl·H-Gly-OEt	B	TEA-Pyr/CH_2Cl_2	48	88.5	19
Z-Gln-OH	2HCl·H-His-OMe	B	2NMM/MeCN	18	80.9	10
Z-Gln-OH	2HCl·H-His-OMe	B	TEA-NMM/MeCN	3.5	67.1	
Z-Pro-OH	HCl·H-Leu-OEt	B	2TEA/$CHCl_3$	18	84.1	14
Z-Pro-OH	HCl·H-Leu-OEt	B	2NMM/EtOAc	8	81.8	
Z-Pro-OH	HCl·H-Ser-OMe	B	2TEA/$CHCl_3$	18	77.8	15
Boc-Pro-OH	H-Gly-OH	B	NMM/Diox-H_2O	18	16.5	
Z-Ser-OH	HCl·H-Phe-OEt	B	2Pyr/MeCN-DMF	6	48.2	
Z-Ser-OH	HCl·H-Phe-OEt	B	2Pyr/MeCN-DMF	18	72.4	
Z-Ser-OH	HCl·H-Phe-OEt	B	2Pyr/DMF	72	68.5	
Z-Val-OH	HCl·H-Gly-OEt	B	TEA-Pyr/CH_2Cl_2	60	82.1	11
Z-Val-OH	HCl·H-Val-OEt	B	2TEA/CH_2Cl_2	72	84.6	16
Z-Gly-Phe-OH	H-Gly-OEt	B	Pyr/MeCN	18	67.7	17
Boc-Pro-OH	HCl·H-Ser(Bzl)Gly-OBzl	B	2TEA/EtOAc	8	84.2	20
Z-Pro-OH	HCl·H-Leu-OEt	B	3MWA-1/CH_2Cl_2	72	85.8	
Z-Pro-OH	HCl·H-Leu-OEt	B	2MWA-1/$CHCl_3$	24	49.0	
Z-Pro-OH	HCl·H-Ser-OMe	B	3MWA-1/CH_2Cl_2-DMF	72	52.8	

MWA-1 = Dowex macroporous weak cation exchanger. The yields recorded are of recrystallized products. Melting points and optical rotations of the products are within the limits of experimental error.

acidic by the formation of the *N*-hydroxy compound resulting in a retardation of the aminolysis of active intermediates, and 6 to 8 h is required for completion of the reaction by the use of triethylamine or *N*-methylmorpholine in chloroform or ethyl acetate, and 2 to 3 h in acetonitrile. When pyridine is employed as the tertiary base, the reaction may require nearly 2 days or more for completion even in acetonitrile. In general, neutralization of the acidic reaction mixture with extra an amount of tertiary base promoted the reaction. For example, the conventional coupling of **6** and **7** by the use of 1-(methylsulfonyloxy)benzotri-



Scheme 2.

azole (**4a**) required 8 h, while the same reaction was completed within 2 h by the periodic addition of an extra amount of triethylamine.

Reaction conditions employed and yields of model peptides are summarized in Tables 6 and 7. In the coupling of *N*-protected L-asparagine and L-glutamine with amine components, no dehydration from the ω-amide to the cyano group was detected in the infrared spectra of the crude products. Free hydroxy or imino groups did not cause any difficulty in the preparation of serine-, tyrosine- or histidine-containing peptides, and no racemate was detected in the preparation of ethyl *N*-benzyloxycarbonylglycyl-L-phenylalanylglycinate by fractional crystallization.

The use of **4v** appeared to be advantageous with oily products or products less soluble in organic solvents. Methyl *N*-benzyloxycarbonyl-L-glutaminyl-L-histidinate prepared by the DCC method was separated from *N,N'*-dicyclohexylurea by extraction with 2 M hydrochloric acid, whereas **4v** gave the product in excellent yield by a simple operation. The coupling of *N*-*t*-butoxycarbonyl-L-proline with benzyl *O*-benzyl-L-serylglycinate is another example to demonstrate the superiority of **4v** over DCC. In the DCC method the product can be isolated only with the help of counter current distribution to eliminate *N,N'*-dicyclohexylurea,[20] whereas the use of **4v** gave the pure product by the following conventional procedure.

A combination of **4v** with a macropolous cation exchange resin was examined in order to test a new technique for peptide synthesis in the liquid-solid phase method. Unlike common solid phase methods, this is designed to leave only the neutral product in the liquid phase after completion of the reaction, while the acidic by-products and unreacted carboxyl components are adsorbed on the resin. That is, a carboxylate anion on the resin is allowed to react with **4v** and forms an active intermediate first, which subsequently reacts with the free amine liberated from an amine hydrochloride on the resin affording an amide in the liquid phase. All the acidic by-products, hydrochloric acid, *p*-chlorobenzenesulfonic acid, 6-chloro-1-hydroxybenzotriazole, and unreacted carboxyl components will be retained on the resin enabling separation of the products. A mixture of an equimolar amount of **6**, the hydrochloride of **7**, **4v**, and 3 equivalents of the resin (Dowex MWA-1) in dichloromethane or chloroform was allowed to stand for 72 h with occasional stirring. The thin layer chromatogram of the liquid phase showed the presence of a large amount of **5** and trace amounts of impurities. Pure **5** was easily obtained by evaporation of the solvent and the recrystallization of the crude product. The results obtained are summarized at the bottom of Table 7.

A possible side reaction, which may occur due to the use of the sulfonates only in procedure B but not in procedure A, is sulfonamide formation from the sulfonates and the amine components. In practice the sulfonamide formation is so slow that it is not detectable in most cases. For instance, a mixture of equimolar amounts of **7** and **4a** in chloroform showed no sulfonamide formation at room temperature unless

Table 8. Racemization test

Z-Phe-Ile-OH + H-AA-OR·HCl ⟶ Z-Phe-Ile-AA-OR

Reagent	H-AA-OR	Procedure	Base/Solvent	Temp (°C)	Yield (%)	D-Alloisoleucine (%)
4g	H-Pro-OBzl	B	2NMM/DMF	r. t.	83.8	25.4
4v	H-Pro-OBzl	B	2NMM/DMF	r. t.	100	23.9
4v	H-Pro-OBzl	B	2NMM/DMF	0—+5	98	12.2
4v	H-Pro-OBzl	B	2Pyr/DMF	r. t.	66.4	18.2
4v	H-Pro-OBzl	B	2Pyr/DMF	−10	72.8	6.8
4v	H-Pro-OBzl	B	2NMM/EtOAc	r. t.	96.5	12.0
4v	H-Pro-OBzl	B	2NMM/$CHCl_3$	r. t.	93.4	14.5
4v	H-Pro-OBzl	B	2MWA-1/DMF[a]	r. t.	89.0	19.7
4v+**2a**[b]	H-Pro-OBzl	B	2NMM/DMF	r. t.	94.5	11.3
4v+**2a**	H-Pro-OBzl	B	2NMM/DMF	0—+5	92.8	7.6
4v+**2b**[c]	H-Pro-OBzl	B	2NMM/DMF	r. t.	95.1	21.3
4v	H-Pro-OBzl	A[d]	2NMM/DMF	r. t.	98.9	42.6
4v	H-Pro-OBzl	A[e]	2Pyr/DMF	r. t.	59.8	33.2
4v+**2a**	H-Pro-OBzl	A[d]	2NMM/DMF	r. t.	100	14.4
4v	H-Leu-OEt	B	2NMM/DMF	0—+5	85.5	10.4
4v	H-Leu-OEt	B	2NMM/EtOAc	0—+5	95.6	6.1
4v+**2a**	H-Leu-OEt	B	2NMM/DMF	0—+5	96.9	3.1
4v+**2a**	H-Leu-OEt	B	2NMM/$CHCl_3$	0—+5	97.2	0.5
DCC	H-Pro-OBzl	—	NMM/DMF	r. t.	95.6	43.3
DCC+**2a**	H-Pro-OBzl	—	NMM/DMF	r. t.	97.4	17.7
EEDQ	H-Pro-OBzl	—	2NMM/DMF	r. t.	57	24.0
DPPA	H-Pro-OBzl	—	2NMM/DMF	r. t.	94.4	16.3

a) The mixture was allowed to react for 24 h. b) Ethyl 2-(hydroxyimino)-2-cyanoacetate (1.2 equivalent). c) 6-Chloro-1-hydroxybenzotriazole (1.2 equivalent). d) Z-Phe-Ile-OH, **4v**, and *N*-methylmorpholine in DMF were allowed to react for 15 min before the addition of a solution of benzyl L-prolinate hydrochloride and *N*-methylmorpholine in DMF. e) Z-Phe-Ile-OH was allowed to react with **4v** for 1.5 h before the addition of benzyl L-prolinate solution.

triethylamine was added. The only exception was when 2-[3,5-bis(benzyloxy)phenyl]ethylamine (**9**) was acylated with **8** by the use of the arenesulfonates of 1-hydroxybenzotriazoles and triethylamine in dichloromethane. For example, the 41% yield of *N*-(*p*-chlorophenylsulfonyl)-2-[3,5-bis(benzyloxy)phenyl]ethylamine (**10**), accompanied by the 33% yield of *N*-(*N*-benzyloxycarbonyl-L-phenylalanyl)-2-[3,5-bis(benzyloxy)phenyl]ethylamine (**11**) was obtained in the acylation of **9** with **8** using **4v**. On the other hand, the alkanesulfonates of 1-hydroxybenzotriazole such as **4a** or 6-chloro-1-(methylsulfonyloxy)benzotriazole (**4p**) produced far less sulfonamide (**10**) than **4v** did. The use of *N*-methylmorpholine or pyridine in the place of triethylamine greatly assisted the suppression of sulfonamide formation even when **4v** was used. The isolation of pure **11** in high yield posed no practical problems. The effect of weaker bases may be due to the selective activation of the carboxyl group of **8** prior to the gradual liberation of free **9** from the hydrochloride.

To elucidate the reaction path of amide formation, **4a** was treated with a mixture of either thioacetic *S*-acid and triethylamine, or thioacetic *S*-acid, aniline and triethylamine. In the former case 1-acetoxybenzotriazole[21] (**13**) was obtained in 85% yield, and in the latter, acetanilide and 1-hydroxybenzotriazole (**2b**) were obtained in 89 and 88.7% yields, respectively. These results suggest that the thioacetate anion, carboxylate anion alike, attacks the sulfur atom of the sulfonate first, forming thioacetic methanesulfonic anhydride. The mixed anhydride may directly be aminolyzed with aniline affording acetanilide and **2b**, or be converted to **13**, which is subsequently aminolyzed to acetanilide or isolated in the absence of aniline.

The active intermediate in procedure A is doubtlessly 1-acyloxybenzotriazole, but specification of the intermediate in procedure B remains difficult. As described before the active ester formation is completed within 1 h, whereas the coupling reaction in procedure B requires 6 to 8 h under the same conditions unless the mixture is neutralized periodically. This suggests that a substantial portion of amides can be produced through 1-acyloxybenzotriazole, which is also a possible active intermediate of recently reported reagents, tris(dimethylamino)(1-benzotriazolyloxy)phosphonium hexafluorophosphonate[22] and tris(dimethylamino)(1-benzotriazolyloxy)phosphonium tetrafluoroborate.[23] The reaction mechanism of **4v** and related compounds in coupling reaction has been discussed in detail by Horiki and Murakami.[24]

The tendency toward racemization of **4v** was compared with those of DCC, 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) and diphenoxyphosphinyl azide (DPPA).[26] The racemization test was conducted using a modification of Bodanszky's method reported

by Fujino *et al.*[27] Coupling of *N*-benzyloxycarbonyl-L-phenylalanyl-L-isoleucine (**14**) with benzyl L-prolinate (**15**) or **7** was carried out mainly in *N*,*N*-dimethylformamide, in which significant racemization occurs. The contents of D-alloisoleucine (**16**) in the protected tripeptide preparations were determined by subjecting the hydrolyzates to amino acid analysis. The results obtained are summarized in Table 8. As Fujino *et al.*[27] have reported, severe racemization in the coupling of **14** with **15** in DCC method, **15** caused markedly more racemization than the other amino acid esters did. DCC produced 43.3% of **16**, which was suppressed to 17.7% by the addition of **2a**. **4v** and EEDQ showed the same extent of racemization, giving 23.9 and 24.0% of **16**, respectively. The use of **2a** with **4v** lowered the formation of **16** to 11.3%, but the use of 6-chloro-1-hydroxybenzotriazole, which was an effective additive in DCC method and a component of **4v**, was not so effective. DPPA which is said to proceed the coupling reaction through carboxylic azide formation gave 16.3% of **16**. The use of pyridine in place of *N*-methylmorpholine gave more satisfactory results in racemization suppression, although it usually required a longer reaction time. An unexpected result was that severe racemization comparable to DCC method was observed in procedure A, but was again suppressed with **2a** to 14.4%.

The conditions employed are suitable only for the detection of racemization, but not for practical coupling. Reactions need to be carried out using weak bases and amine components whose *N*-terminal amino acid residue is other than proline at low temperature. These results suggest that **4v** is a much superior reagent than DCC in racemization, especially when it is used with **2a**.

Experimental

Melting points were measured on a Hoover "Uni-Melt" apparatus and are uncorrected. The optical rotations were determined with a JEOLCO Model DIP-4.

Materials. Commercially available methane-, 1-butane-, benzene-, *p*-toluene-, *p*-chlorobenzene-, *p*-nitrobenzene- and 2,4,6-trimethylbenzenesulfonyl chlorides, *N*-hydroxysuccinimide, and 3-hydroxy-1,2,3-benzotriazin-4-one were used for the preparation of the sulfonates of *N*-hydroxy compounds. Phenylmethane-,[28] *dl*-10-camphor-,[29] β-styrene-,[30] and 3-pyridinesulfonyl chloride,[31] ethyl 2-hydroxyimino-2-cyanoacetate,[32] 2-hydroxyimino-2-cyanoacetamide,[32] 1-hydroxybenzotriazole,[33] 6-chloro-1-hydroxybenzotriazole,[34] and 6-nitro-1-hydroxybenzotriazole[35] were prepared according to the literature.

4-Chloro-1-hydroxybenzotriazole. A mixture of 2,3-dichloronitrobenzene (25 g, 0.13 mol) and hydrazine hydrate (19.5 g, 0.39 mol) in 99% ethanol (40 ml) was refluxed for 24 h. After removal of the solvent *in vacuo*, the residue was dissolved in 10% sodium carbonate solution. The solution was washed with ether and acidified with concentrated hydrochloric acid to precipitate the product, which was washed with water on a filter and dried; 20.6 g (93.4%); mp 160—166 °C. A part of the product was recrystallized from methanol (MeOH); mp 170—172 °C.

Found: C, 42.74; H, 2.27; N, 24.89%. Calcd for $C_6H_4ON_3Cl$: C, 42.50; H, 2.38; N, 24.80%.

1-(Phenylsulfonyloxy)benzotriazole (4e). General Procedure for the Preparation of the Sulfonates of N-*Hydroxy Compounds*: To a solution of 1-hydroxybenzotriazole (6.6 g, 0.049 mol) in 1 M-sodium hydroxyide solution (49 ml) was added dropwise benzenesulfonyl chloride (8.8 g, 0.05 mol) with stirring under ice-cooling. After stirring for 1 h, the product was extracted with ethyl acetate (EtOAc), and the extract was washed with water and dried over magnesium sulfate. Evaporation of the solvent *in vacuo* gave a residue, which was triturated in hexane and filtered to give 11.5 g (85.2%) of **4e**; mp 83—84 °C.

Found: C, 52.30; H, 3.09; N, 15.13%. Calcd for $C_{12}H_9O_3N_3S$: C, 52.35; H, 3.30; N, 15.27%.

Other sulfonates were prepared by similar procedures under conditions cited in Tables 1 and 2. Triethylamine (TEA) was used in place of sodium hydroxide when organic solvents were employed.

1-(N-Benzyloxycarbonyl-L-phenylalanyloxy)benzotriazole. General Procedure for the Preparation of the Active Esters: To an ice-cooled solution of *N*-benzyloxycarbonyl-L-phenylalanine (1.50 g, 5 mmol) and TEA (0.70 ml, 5 mmol) in EtOAc (15 ml) was added 1-(methylsulfonyloxy)benzotriazole (1.1 g, 5 mmol). The mixture was stirred for 1 h and allowed to stand overnight. After the addition of EtOAc and water, the organic layer was washed with water and dried over magnesium sulfate. Evaporation of the solvent and subsequent trituration of the residue with ether gave the product; mp 120—125 °C; 1.3 g (62%). IR (Nujol, cm^{-1}) 3330, 1735, 1690.

General Procedures for the Coupling of Amino and Carboxyl Components. Procedure A: To a solution of *N*-benzyloxycarbonyl-L-glutamine (1.40 g, 5 mmol) and TEA (0.70 ml, 5 mmol) in dry acetonitrile (MeCN)(10 ml) was added 1-(butylsulfonyloxy)-6-chlorobenzotriazole (1.47 g, 5 mmol) at room temperature. The mixture solidified immediately. On standing for 30 min, a solution of benzyl L-tyrosinate *p*-toluenesulfonate (2.20 g, 5 mmol) and TEA (0.70 ml, 5 mmol) in dry MeCN(20 ml) was added to the mixture with stirring. The reaction mixture was allowed to react for a total of 18 h, and clarified by the addition of water. After evaporation of MeCN *in vacuo* the residue was extracted with EtOAc. The extract was washed with water, 1 M hydrochloric acid, water, 5% sodium hydrogencarbonate solution, and water, dried over magnesium sulfate, and concentarted to dryness *in vacuo*. Recrystallization of the residue from MeOH gave 1.92 g (71.8%) of benzyl *N*-benzyloxycarbonyl-L-glutaminyl-L-tyrosinate; mp 173—174 °C (lit,[11] mp 174—175 °C).

Procedure B: To an ice-cooled solution of *N*-benzyloxycarbonyl-L-proline (1.25 g, 5 mmol), methyl L-serinate hydrochloride (0.78 g, 5 mmol) and TEA (1.40 ml, 10 mmol) in dry chloroform ($CHCl_3$)(20 ml) was added 1-(phenylsulfonyloxy)-6-chlorobenzotriazole (**4t**) (1.50 g, 5 mmol). The mixture was stirred for 1 h at room temperature and allowed to stand for a further 17 h. After the addition of EtOAc and water the organic layer was worked-up in the usual manner and concentrated to dryness *in vacuo*. The residue was triturated with a mixture of ether and petroleum ether, filtered, and recrystallized from EtOAc/petroleum ether to give 1.5 g (85.7%) of methyl *N*-benzyloxycarbonyl-L-prolyl-L-serinate; mp 97—100 °C (lit,[15] mp 103—104 °C).

Other protected peptides prepared by the procedures A and B are listed in Tables 6 and 7.

N-t-*Butoxycarbonyl-L-prolylglycine.* A solution of *N*-*t*-butoxycarbonyl-L-proline (1.08 g, 5 mmol), glycine (0.375 g, 5 mmol) and *N*-methylmorpholine (NMM)(0.60 ml) in water (7.5 ml) was adjusted to pH 7.0 by the addition of a

small amount of 1-hydroxybenzotriazole. A solution of 6-chloro-1-(*p*-chlorophenylsulfonyloxy)benzotriazole (**4v**) (1.72 g, 5 mmol) in dioxane (14 ml) was added to the solution described above under ice-cooling and the whole mixture stirred for 2.5 h at room temperature. After evaporation of dioxane *in vacuo*, the residue was dissolved in water, washed with EtOAc, acidified to pH 2 and extracted with EtOAc. The extract was washed with water, dried over magnesium sulfate and concentrated to dryness. The residue was dissolved in $CHCl_3$ and adsorbed on a silica gel column (Merck Kieselgel 60, 40 g). The column was eluted with 5% MeOH-containing $CHCl_3$ (150 ml) and then with 10% MeOH-containing $CHCl_3$ (200 ml). Evaporation of the second eluate gave the product (302 mg), which was recrystallized from $CHCl_3$ and petroleunm ether,; 225 mg (16.5%); 165—167 °C (dec).

Found: C, 52.88; H, 7.42; N, 10.33%. Calcd for C_{12}-$H_{20}O_5N_2$: C, 52.93; H, 7.40; N, 10.29%.

The Coupling Reaction of N-*Benzyloxycarbonyl-*L-*proline with Ethyl* L-*Leucinate Using* **4v** *and the Macropolous Ion Exchanger.* To an ice-cooled mixture of *N*-benzyloxycarbonyl-L-proline (1.25 g, 5 mmol), ethyl L-leucinate hydrochloride (1.00 g, 5 mmol) and Dowex MWA-1 ion exchange resin (3.8 g, ≈15 mmol, dried over phosphorus pentoxide in a vacuum desiccator for 24 h) in dichloromethane (CH_2Cl_2) (20 ml) was added **4v** (1.72 g, 5 mmol) with occasional stirring. The mixture was allowed to react for 72 h with occasional stirring, and was then filtered. The resin was extracted five times with a small amount of CH_2Cl_2. The filtrate and the extracts were combined and evaporated to dryness, and the residue triturated with a small amount of hexane and filtrered; 1.676 g (85.8%); mp 65—67 °C.

N-(N-*Benzyloxycarbonyl-*L-*phenylalanyl)-2-[3,5-bis(benzyloxy)-phenyl]ethylamine (**11**) and* N-*(p-chlorophenylsulfonyl)-2-[3,5-bis(benzyloxy)phenyl]ethylamine (**10**).* To a mixture of *N*-benzyloxycarbonyl-L-phenylalanine (2. 99 g, 10 mmol), 2-[3,5-bis(benzyloxy)phenyl]ethylamine hydrochloride (3.69 g, 10 mmol) and TEA (2.80 ml, 20 mmol) in CH_2Cl_2 (25 ml) was added a solution of **4v** (3.44 g, 10 mmol) in CH_2Cl_2 (15 ml) under ice-cooling. After stirring at room temperature for 5.5 h, water and EtOAc were added to the reaction mixture which was subsequently filtered to remove insoluble material; mp 144—152 °C; 2.1 g. Recrystallization from MeOH gave pure **11**; mp 153—154.5 °C; 2.0 g (32. 6%).

Found: C, 75.88; H, 6.15; N, 4.56%. Calcd for C_{39}-$H_{38}O_5N_2$: C, 76.20; H, 6.23: N, 4.56%.

The filtrate was concentrated to dryness and the residue (3.0 g) was recrystallized from MeOH to give **10**; mp 102—103.5 °C; 2.1 g (41.3%).

Found: C, 65.96; H, 5.05; N, 2.80; S, 6.58; Cl, 6.96%. Calcd for $C_{28}H_{26}NO_4SCl$: C, 66.19; H, 5.16; N, 2.76; S, 6.31; Cl, 6.98%.

11 was prepared in 73.0% yield by following procedure A in MeCN.

*The Reaction of 1-(Methylsulfonyloxy)benzotriazole (**4a**) with Thioacetic* S-*Acid.* To a mixture of thioacetic *S*-acid (0.36 ml) and TEA (0.70 ml) in CH_2Cl_2 (10 ml) was added **4a** (1.07 g) under ice-cooling. The mixture was stirred for 1 h and allowed to stand overnight. Water and EtOAc were added to the mixture and the organic layer separating out was washed with 1 M sodium hydrogencarbonate solution, water, 1 M hydrochloric acid and water, dried over magnesium sulfate and evaporated to dryness. The residue was triturated with an ether–petroleum ether mixture and recrystallized from the same solvents; mp 82—100 °C; 0.6 g. The product was identical with the one obtained from the reaction between acetyl chloride and 1-hydroxybenzotriazole or from **4a** and acetic acid.

*The Reaction of **4a** with Thioacetic* S-*Acid and Aniline.* The same reaction described above was carried out in the presence of an equimolar amount of aniline (0.5 g). After the addition of water and EtOAc, the organic layer was washed with 1 M sodium hydrogencarbonate, water, 1 M hydrochloric acid, and water, dried over magnesium sulfate and evaporated *in vacuo*. The residue was triturated with petroleum ether and filtered to give acetanilide; 0.60 g; mp 110—112 °C. The 1 M sodium hydrogencarbonate washing was acidified with hydrochloric acid, and extracted with 10% dioxane-containing EtOAc. The extract was washed with water, dried over magnesium sulfate and concentrated *in vacuo*. The residue was triturated with petroleum ether and filtered to give 1-hydroxybenzotriazole; 0.6 g (88.7%); mp 151—154.5 °C.

Modified Bodanszky's Test.[27] A typical example is as follows: To a solution of benzyl L-prolinate hydrochloride (241.7 mg, 1 mmol), *N*-benzyloxycarbonyl-L-phenylalanyl-L-isoleucinate (394.5 mg, 1 mmol), ethyl 2-hydroxyimino-2-cyanoacetate (170.5 mg, 1.2 mmol) and NMM (0.22 ml, 2 mmol) in dry *N,N*-dimethylformamide (5.0 ml) was added **4v** (344.2 mg, 1 mmol) under ice-cooling. The mixture was stirred for 3 h at 0—5 °C. After evaporation of the *N,N*-dimethylformamide under nitrogen, the residue was extracted with EtOAc. The extract was washed with water, 1 M sodium hydrogencarbonate (4 times), water, 1 M hydrochloric acid and water, dried over magnesium sulfate and concentrated to dryness. A part of the residue was hydrolyzed by 6 M hydrochloric acid at 105 °C in a sealed tube for 16 h. The hydrolyzate was subjected to amino acid analysis to determine the D-alloisoleucine content. Reaction conditions and the results are given in Table 8.

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