Introduction of 9-fluorenylmethyloxycarbonyl, trichloroethoxycarbonyl, and benzyloxycarbonyl amine protecting groups into O-unprotected hydroxyamino acids using succinimidyl carbonates[1]

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9-Fluorenylmethyl succinimidyl, pentachlorophenyl, and benzotriazole-1-yl carbonates were prepared and their reactivity with L-serine and L-serine benzyl ester was compared. The most efficient reagent, 9-fluorenylmethyl succinimidyl carbonate, was used for the preparation of 9-fluorenylmethyloxycarbonyl derivatives of other hydroxyamino acids and hydroxyamino acid esters in high yields. The use of trichloroethyl and benzyl succinimidyl carbonates for an efficient conversion of hydroxyamino acids and their esters into the corresponding *N*-trichloroethoxycarbonyl and benzyloxycarbonyl derivatives is described.

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On a préparé les carbonates de fluorènyl-9 methylsuccinimidile, de pentachlorophényle et de benzotriazolyle-1 et on a comparé leur réactivité vis-à-vis la L-sérine et son ester benzylique. On a utilisé le réactif le plus efficace, le carbonate du fluorènyl-9 méthylsuccinimidile, pour préparer avec d'excellents rendements des dérivés fluorènyl-9 méthyloxycarbonyles d'autres acides hydroxyaminés et de leurs esters. On décrit l'utilisation des carbonates de trichloroéthyle et de benzyle succinimidile pour la transformation efficace des acides hydroxyaminés et de leurs esters en dérivés *N*-trichloroéthoxycarbonyle et benzyloxycarbonyle correspondants.

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Amino acids bearing urethane-type protecting groups are commonly used in peptide synthesis because they can be coupled without racemization. Except for *N-tert*-butoxycarbonylamino acids, they are usually prepared using the alkoxycarbonyl chloride under Schotten–Baumann conditions. An alternative reagent which does not require the presence of base is the mixed carbonate containing a substituted phenolic group (1) and more recently the succinimidyl group (1–3). New protecting groups are continually being proposed for use in peptide and general organic chemistry, and methods for their introduction are necessary. This paper describes a new method of preparing 9-fluorenylmethyloxycarbonyl (Fmoc-),[2] trichloroethoxycarbonyl (Tec-), and benzyloxycarbonyl (Z-) amino acids with particular attention to the hydroxyamino acids using succinimidyl carbonates as reagents and the free amino acids or amino acid esters as substrates. The possibility of using other carbonates was also examined.

The Fmoc group (4) which is labile to secondary amines is being promoted for use both in solid phase (5, 6) and solution (7) peptide synthesis, particularly because it eliminates the need for a potentially harmful acid deprotection cycle. Fmoc-amino and hydroxyamino acid derivatives have been obtained using Fmoc-chloride in the presence of aqueous sodium carbonate (8). Fmoc-amino acid esters have been obtained by subsequent esterification (4, 8). The Tec group is an attractive protecting group as it can be cleaved by zinc in acetic acid (9) or organic solvents (10, 11) leaving other functional groups unaffected. However, it has been little used in peptide chemistry possibly because of the uncertainty over its stability to hydrogenolytic conditions (12). It also has been reported that an attempt to convert serine into Tec-Ser-OH using trichloroethoxycarbonyl chloride in the presence of 1 *N* sodium hydroxide failed (11).

Mixed carbonates have been obtained by reaction of alkyl chloroformates with alcohols in pyridine (1) and more recently with the thallium salt of *N*-hydroxysuccinimide in organic solvents (3). But thallium salts are toxic and their preparation involves handling of the unstable and toxic thallium ethoxide. We have now found that the dicyclohexylammonium salts of alcohols, which are readily obtained by mixing the two components in an organic solvent, serve as well for the preparation of mixed carbonates from alkyl chloroformates. Thus 9-fluorenylmethyl, trichloroethyl, and benzyl succinimidyl carbonates **1**, **4**, and **5** were prepared in high yields in this manner. As well, 9-fluorenylmethyl pentachlorophenyl carbonate **2** and benzo-

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[2]*Abbreviations*: nmr, nuclear magnetic resonance, ir, infrared. Other abbreviations are those recommended by the IUPAC–IUB Commission on Biochemical Nomenclature J. Biol. Chem. **247**, 977–983 (1972). Amino acid symbols represent the L-isomers.

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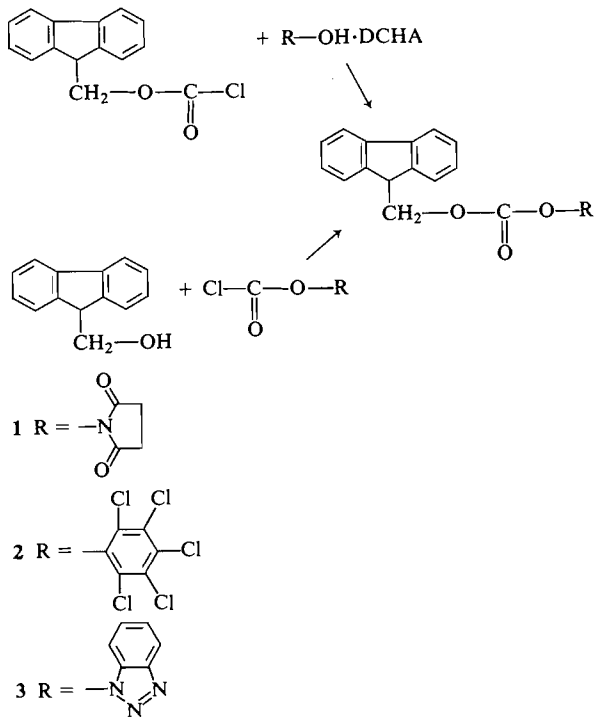

triazole-1-yl carbonate **3** were obtained from 9-fluorenylmethyloxycarbonyl chloride and the dicyclohexylammonium salt of the corresponding alcohols.

Succinimidyl carbonate **1** was also obtained by the reverse procedure, namely by reaction of 9-fluorenylmethanol with succinimidyl chloroformate. Again, the dicyclohexylammonium instead of the thallium salt (3) of *N*-hydroxysuccinimide served for the preparation of the chloroformate using phosgene. We found a literature procedure (13) to be a convenient source for 9-fluorenylmethanol.

9-Fluorenylmethyl carbonates **1–3** were reacted with one equivalent of L-serine in a water–acetone mixture in the presence of one equivalent of sodium bicarbonate, and with serine benzyl ester hydrochloride in chloroform in the presence of 1.5 equivalents of triethylamine. Under these conditions, the succinimidyl carbonate **1** gave excellent yields of Fmoc-Ser-OH and Fmoc-Ser-OBzl, the pentachlorophenyl carbonate **2** gave no Fmoc-Ser-OH and a moderate yield of the ester, and the benzotriazole-1-yl carbonate **3** gave an excellent yield of the ester but only a moderate yield of the acid derivative. The results are given in Table 1. On this basis, the succinimidyl carbonate **1** was chosen for further study. It reacted smoothly with valine and valine methyl ester giving the Fmoc-derivatives in

TABLE 1. Reactivity of reagents **1–3** with serines

	H-Ser-OH		H-Ser-OBzl	
Reagent	Time (h)	Yield (%)	Time (h)	Yield (%)
1	2	87	2	87
2	48	—	48	50
3	48	64	2	89

good yields. Other amino acid derivatives prepared are described in Table 2. The results demonstrate that the use of 9-fluorenylmethyl succinimidyl carbonate is indeed a good alternative to the use of the chloroformate for the preparation of Fmoc-derivatives. It has the advantage that it eliminates the possibility that the mixed anhydride might form during the acylation reaction. The mixed anhydride could give rise to some protected dipeptide in addition to the desired *N*-protected amino acid, a side-reaction encountered in the original synthesis of benzoylglycine (14, 15).

The pentachlorophenyl carbonate **2** also proved unsatisfactory for acylating valine and valine methyl ester, 50% of **2** being recovered even after 72 h.

Using the same conditions as for the preparation of the Fmoc-derivatives, trichloroethyl succinimidyl carbonate **4** and benzyl succinimidyl carbonate **5** gave good yields of the corresponding derivatives when reacted with hydroxyamino acids and esters. The compounds prepared are described in Tables 3 and 4.

All amino acid derivatives obtained gave correct elemental analyses, exhibited absorptions at 3420 (N—H), 1695–1710 (urethane carbonyl), and 1510 (urethane C—N) cm^{-1} in ir and showed amide proton signals in nmr spectra. This served as a proof that the amino group was acylated.

Experimental

Melting points (uncorrected) were taken by the capillary method. The nmr spectra were recorded on a Varian T-60 spectrometer in deuterochloroform unless stated otherwise. The ir spectra were obtained with a Beckman-IR-20 spectrometer in chloroform. Optical rotations were determined on Perkin–Elmer model 141 polarimeter. Solutions were dried over Na_2SO_4.

9-Fluorenylmethanol was prepared according to the literature (13) or purchased from Fluka, A. G. Buchs, Switzerland. Amino acids and their esters were purchased from Sigma Chemical Company (Saint Louis, Missouri, USA). Serine benzyl ester hydrochloride and tyrosine benzyl ester were prepared as indicated in the literature (16).

N-Protected amino acid esters with the exception of tyrosine esters were prepared as shown for the preparation of Fmoc-Ser-OBzl. *N*-Protected tyrosine ester derivatives were synthesized as described for the preparation of Fmoc-Tyr-OBzl.

TABLE 2. *N*-(9-Fluorenylmethyloxycarbonyl)-amino acid derivatives

Compound	Solvent for recrystallization	Yield %	Melting point °C	$[\alpha]_D^{26}$[a]	Molecular formula	Nmr data (δ)
Fmoc-Ser-OH	CH_2Cl_2–hexane	89	86–88[b]	+14.8°	—	[f]
Fmoc-Ser-OBzl	CH_2Cl_2–hexane	87	97[c]	+1.3°	—	[f]
Fmoc-Ser-OCH_3	Benzene	90	128	+4.0°	$C_{19}H_{19}NO_5$	2.4 (1H, m), 3.8 (3H, s), 3.9 (2H, m), 4.4 (4H, m), 5.8 (1H, m), 7.2–7.9 (8H, m)
Fmoc-Thr-OH·DCHA	AcOEt–Et_2O	95	165	+9.8°	$C_{31}H_{42}N_2O_5$	Not taken
Fmoc-Tyr-OCH_3	CH_2Cl_2–hexane	88	120–122[d]	+3.4°	$C_{25}H_{23}NO_5$	3.06 (2H, m), 3.76 (3H, s), 4.1–4.9 (4H, m), 5.4 (1H, m), 6.93 (4H, m), 7.33–7.93 (8H, m)
Fmoc-Tyr-OBzl	$CHCl_3$–EtOH	86	152	−11.3°	$C_{31}H_{27}NO_5$	Not taken
Fmoc-Val-OH[g]	CH_2Cl_2 – pet. ether	86	144[e]	+4.6°	—	[f]
Fmoc-Val-OCH_3	CH_2Cl_2 – pet. ether	90	95	−5°	$C_{21}H_{23}NO_4$	0.96 (6H, dd), 2.1 (1H, m), 3.8 (3H, s), 4.3 (4H, m), 5.3 (1H, m), 7.3–7.9 (8H, m)

Elemental analyses of all compounds gave satisfactory results (C ± 0.21, H ± 0.06, N ± 0.21).
[a]Determined in AcOEt except for Fmoc-Thr-OH·DCHA (DMF).
[b]Literature (8) mp 86–88°C, $[\alpha]_D^{23-25}$ +14.9° (ethyl acetate).
[c]Literature (8) mp 97–98°C, $[\alpha]_D^{23-25}$ +1.4° (ethyl acetate).
[d]126–127°C on Koffler block.
[e]Literature (8) mp 143–144°C, $[\alpha]_D^{23-25}$ +4.8° (ethyl acetate).
[f]In accord with data in literature (8).
[g]Prepared from 1.

TABLE 3. *N*-Trichloroethoxycarbonylamino acid derivatives

Compound	Solvent for recrystallization	Yield %	Melting point °C	$[\alpha]_D^{25}$[a]	Molecular formula	Nmr data (δ)
Tec-Ser-OH	H_2O	67	115	+11.7°	$C_6H_8Cl_3NO_5$	3.73 (2H, m), 4.13 (m, 1H), 4.80 (2H, s), 7.71 (1H, broad s)
Tec-Ser-OBzl	—	90	Amorphous[b]	Not taken	$C_{13}H_{14}Cl_3NO_5$	2.63 (1H, m), 4.08 (2H, m), 4.58 (1H, m), 4.8 (2H, s), 5.30 (2H, s), 6.23 (1H, m), 7.46 (5H, s)
Tec-Tyr-OBzl	CH_3Cl_2–Et_2O	85	112	−9.3°	$C_{19}H_{18}NCl_3O_5$	3.1 (2H, m), 4.9–4.63 (3H, m and s), 5.03 (2H, s), 5.66 (1H, m), 8.3 (4H, m), 7.38 (5H, s)
Tec-Val-OH	CH_2Cl_2–hexane	79	85 softens[c] 95 melts	−4.1°	—	1.0 (6H, dd), 2.2 (1H, m), 4.3 (1H, m), 4.8 (2H, s), 5.63 (1H, m)

[a]Determined in ethyl acetate except for Tec-Val-OH (ethanol).
All compounds gave satisfactory elemental analyses (C ± 0.09; H ± 0.18; N ± 0.02) except for Tec-Ser-OBzl (*Anal.* calcd.: C 42.13, H 3.80, N 3.78; found: C 41.16, H 4.27, N 3.57).
[b]Attempts to recrystallize the compound resulted in decomposition.
[c]Literature (11) mp 80.5–81°C; $[\alpha]_D^{25}$ −3.2° (ethanol).

TABLE 4. *N*-Benzyloxycarbonylamino acid derivatives

Compound	Yield %	Melting point °C	$[\alpha]_D^{26}$
Z-Thr-OH	70	102–103[a]	−5.5° (4% aq. acetic acid)
Z-Ser-OH	78[e]	120[b]	+5.7° (acetic acid)
Z-Ser-OCH_3	90	Oil[c]	−12.6° (methanol)
Z-Tyr-OBzl	92	119[d]	−14.3° (methanol)

[a]Literature (17) 103–104°, $[\alpha]_D^{28}$ −5.5° (4% aq. acetic acid).
[b]Literature (18) 119–120°, $[\alpha]_D$ +5.8° (acetic acid).
[c]Literature (19) 33–35°, $[\alpha]_D^{20}$ −13.2° (methanol).
[d]Literature (20) 118–119°, $[\alpha]_D$ −13.9° (methanol).
[e]92% yield was obtained using 2 equivalents of **4**.

Dicyclohexylammonium salts

To 0.01 mol each of *N*-hydroxysuccinimide (in acetone), pentachlorophenol (in ether), and 1-hydroxybenzotriazole (in ethyl acetate), 0.01 mol of dicyclohexylamine was added. The mixtures were stirred overnight, the precipitates isolated by filtration, washed with cold solvents used for preparation, and thoroughly dried. Elemental analyses of all products gave satisfactory results (C ± 0.38, H ± 0.02, N ± 0.03). *N*-Hydroxysuccinimide·DCHA 95%; Pentachlorophenol·DCHA, 87%; 1-hydroxybenzotriazole·DCHA, 86% yield.

Succinimidyl chloroformate

To a stirred suspension of 5.96 g (20 mmol) of *N*-hydroxy-

succinimide·DCHA in methylene chloride (100 mL) at −30°C was added liquified phosgene (20 mL) under a stream of nitrogen. The reaction was stirred for 4 h at −5°C and the excess of phosgene was removed at room temperature with a stream of nitrogen in a well vented fume-hood. Methylene chloride was added, the mixture was cooled to −2°C, the precipitate was filtered off and washed with cold methylene chloride. The combined filtrate and washings were washed with a cold 10% solution of sodium bicarbonate, water, dried, and the solvent was evaporated giving 2.85 g (80%) of the title compound; ir: 1740–1760 (chloride carbonyl), 1790–1800, and 1820 (imide carbonyl) cm^{-1}.

9-Fluorenylmethyl succinimidyl carbonate (1)

A. From 9-fluorenylmethyl chloroformate and N-hydroxysuccinimide·DCHA salt

To a stirred solution of 14.06 g of Fmoc-chloride (54.4 mmol) in chloroform (100 mL) was added 16.12 g (54.4 mmol) of *N*-hydroxysuccinimide·DCHA salt in portions. The stirring was continued overnight, the precipitate was filtered off and washed with chloroform. The filtrate and washings were combined and washed with 1/3 of the volume each of 10% citric acid, 10% sodium bicarbonate, and water, and dried. The solvent was distilled off *in vacuo* and the residue was recrystallized from chloroform–ether giving 16.5 g (90%) of the title compound, mp 151°C. *Anal.* calcd. for $C_{19}H_{15}NO_5$: C 67.65, H 4.48, N 4.15; found: C 67.85, H 4.60, N 4.05.

B. From 9-fluorenylmethanol and succinimidyl chloroformate

To a stirred solution of 7.22 g (36.8 mmol) of 9-fluorenylmethanol and 2.9 g (36.8 mmol) of pyridine in methylene chloride (7.5 mL) was added 6.54 g (36.8 mmol) of succinimidyl chloroformate in methylene chloride (6.5 mL) under cooling with tap water. The stirring was continued for 5 h at room temperature. Ice-water was added, and the organic layer was washed with 1/3 of the volume each of 10% hydrochloric acid (2 times), saturated sodium bicarbonate (2 times), water, and dried. Solvent was removed *in vacuo* and the residue recrystallized from chloroform–ether giving 8.95 g (72%) of the compound **1**, which was identical with the product obtained by method A (identical mp and ir spectra).

9-Fluorenylmethyl pentachlorophenyl carbonate (2)

This compound was obtained from 2.58 g (10 mmol) of Fmoc-chloride and 4.47 g (10 mmol) of pentachlorophenol DCHA salt as described for the synthesis A of ester **1**. Crystallization of the product from chloroform afforded 4.0 g (82%) of the title compound, mp 162–164°C. *Anal.* calcd. for $C_{21}H_{11}O_3Cl_5$: C 51.62, H 2.26, Cl 36.28; found: C 51.64, H 2.20, Cl 36.10.

9-Fluorenylmethyl benzotriazole-1-yl carbonate (3)

This compound was p ep red from 2.59 g (8.2 mmol) of 1-hydroxybenzotriazol DCHA salt and 2.12 g (8.2 mmol) of fluorenylmethyl chloroformate in methylene chloride (15 mL) as described for the synthesis of ester **1** giving 2.42 g (83%) yield, mp 180°C. *Anal.* calcd. for $C_{21}H_{15}N_3O_3$: C 70.58, H 4.23, N 11.75; found: C 70.76, H 4.39, N 11.17.

Trichloroethyl succinimidyl carbonate (4)

This compound was obtained from trichloroethyl chloroformate and the DCHA salt of *N*-hydroxysuccinimide as indicated for preparation of carbonate **1** (method A). Yield, 89%; mp 114–115°C (lit. (3) mp 113°C).

Benzyl succinimidyl carbonate (5)

This synthesis was carried out from benzyl chloroformate and the DCHA salt of *N*-hydroxysuccinimide as shown for the preparation of carbonate **1**. Yield, 90%; mp 80–81°C (lit. (2) mp 79–81°C).

N-*(9-Fluorenylmethyloxycarbonyl)*-L-*serine*

To a stirred solution of 525 mg (5 mmol) of L-serine and 420 mg (5 mmol) of sodium bicarbonate in a mixture of water (7 mL) and acetone (7 mL) was added 1.68 g (5 mmol) of reagent **1**. After stirring overnight the mixture was acidified to pH 2 with concentrated hydrochloric acid and acetone was removed *in vacuo*. The product was taken up in chloroform and washed with 0.1 *N* HCl and water. The combined organic phases were dried, evaporated *in vacuo*, and the residue was crystallized.

N-*Trichloroethoxycarbonyl*-L-*serine*

To a stirred solution of 526 mg (5 mmol) of L-serine and 420 mg (5 mmol) of sodium bicarbonate in water (10 mL) and tetrahydrofurane (5 mL) was added 1.45 g (5 mmol) of reagent **4**. The mixture was stirred overnight, acidified with concentrated hydrochloric acid, the organic solvent was distilled off, and the aqueous layer was extracted with chloroform (3 × 5 mL). The combined extracts were washed with water, dried, and evaporated *in vacuo*, and the crude product was recrystallized.

N-*Trichloroethoxycarbonyl*-L-*valine*

This compound was obtained from 117 mg (1 mmol) of L-valine and (1 mmol) of carbonate **4** as shown for the synthesis of Tec-Ser-OH.

N-*Benzyloxycarbonyl*-L-*serine*

To a stirred solution of 525 mg (5 mmol) of L-serine and 420 mg (5 mmol) of sodium bicarbonate in a mixture of water (7 mL) and acetone (7 mL) was added 1.24 g (5 mmol) of ester **5**. The mixture was stirred overnight, acetone was removed *in vacuo*, and the solution was washed twice with methylene chloride (3 mL). The aqueous layer was acidified to pH 2.5 by concentrated hydrochloric acid and extracted with ethyl acetate (3 × 5 mL). The combined ethyl acetate layers were washed with water, dried, solvent removed *in vacuo*, and the residue recrystallized.

N-*(9-Fluorenylmethyloxycarbonyl)*-L-*threonine*

This compound was prepared from 119 mg (1 mmol) of L-threonine and 337 mg (1 mmol) of reagent **1** following the procedure described for the preparation of Fmoc-Ser-OH. It was crystallized as its DCHA salt by adding 181 mg (1 mmol) of dicyclohexylamine to the crude product in ethyl acetate. The mixture was stirred for 2 h, the volume was reduced *in vacuo*, ether was added, and the crystalline product was separated by filtration.

9-Fluorenylmethyloxycarbonyl-L-*valine*

This derivative was obtained from 117 mg (1 mmol) of L-valine and 337 mg (1 mmol) of reagent **1** using the procedure described for Fmoc-Ser-OH.

N-*(9-Fluorenylmethyloxycarbonyl)*-L-*serine benzyl ester*

A solution of 232 mg (1 mmol) of L-serine benzyl ester hydrochloride, 0.21 mL of triethylamine (1.5 mmol), and 337 mg (1 mmol) of ester **1** in methylene chloride (5 mL) was stirred at room temperature for 2 h. The mixture was washed with 0.1 *N* hydrochloric acid and water, the organic phase was dried, and the residue was recrystallized after removal of the solvent.

N-*(9-Fluorenylmethyloxycarbonyl)*-L-*tyrosine benzyl ester*

A solution of 271 mg (1 mmol) of tyrosine benzyl ester and 337 mg (1 mmol) of compound **1** in chloroform (5 mL) was stirred at room temperature overnight. The mixture was washed with water (3× 1 mL) and dried. The solvent was removed *in vacuo* and the residue was recrystallized.

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