1,2-Diethylthio-1-cyclohexene (Id). Similarly from 12.4 g (0.2 mole) of EtSH and 13.3 g (0.1 mole) of α-chlorohexanone we obtained 17.8 g (88%) of (Id), bp 110-115° (1 mm); n_D^{20} 1.5522. Found: C 59.25; H 8.86; S 31.52%. $C_{10}H_{18}S_2$. Calculated: C 59.35; H 8.96; S 31.69%.

1,2-Di-n-propylthio-1-cyclohexene (Ie). Similarly from 15.2 g (0.2 mole) of n-PrSH we obtained 19.8 g (86%) of (Ie), bp 138-141° (5 mm); n_D^{20} 1.5436. Found: C 62.38; H 9.57; S 27.78%. $C_{12}H_{22}S_2$. Calculated: C 62.55; H 9.62; S 27.83%.

1,2-Di-n-butylthio-1-cyclohexene (If). Similarly from 18.0 g (0.2 mole) of n-BuSH we obtained 21.9 g (85%) of (If), bp 128-132° (1 mm); n_D^{20} 1.5336. Found: C 64.95; H 10.04; S 24.65%. $C_{14}H_{26}S_2$. Calculated: C 65.05; H 10.14; S 24.81%.

CONCLUSIONS

Alkyl mercaptans react with α-chlorocyclopentanone and α-chlorocyclohexanone to give the corresponding 1,2-bisalkylthiocyclenes.

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SYNTHESIS OF N-HYDROXYSUCCINIMIDE ESTERS OF N-PROTECTED AMINO ACIDS AND PEPTIDES USING N,N'-DISUCCINIMIDYL SULFITE

A. V. Il'ina, Yu. A. Davidovich, and S. V. Rogozhin

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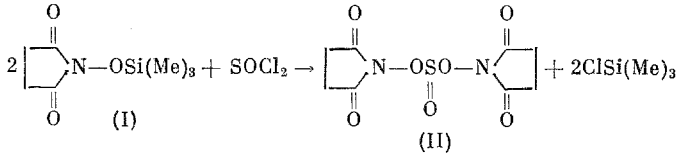
The N-hydroxysuccinimide esters of N-protected amino acids and peptides have found wide use at the present time in the synthesis of peptides [1-3], since racemization is practically not observed when they are used. The N-hydroxysuccinimide that is formed during reaction is readily soluble in water, which greatly facilitates the isolation of the end products [4]. Among the known methods for obtaining the N-hydroxysuccinimide esters of N-protected amino acids the carbodiimide method is used most frequently [5]. A disadvantage of this method is the formation of the corresponding N-acylureas, and also the high toxicity and allergenicity of dicyclohexylcarbodiimide. The methods for obtaining the N-hydroxysuccinimide esters of N-protected amino acids and peptides by the mixed anhydride method [6] or using N,N'-disuccinimidyl carbonate [7], which permit obtaining the activated esters in good yields, also have important disadvantages.

In order to create a new efficient method for obtaining the N-hydroxysuccinimide esters of N-protected amino acids and peptides we synthesized N,N'-disuccinimidyl sulfite (II) by the following scheme.



(I) (II)

The reaction was run in an inert organic solvent with cooling. The trimethylsilyl ether of N-hydroxysuccinimide (I) is a stable crystalline compound, which is easily formed by the silylation of N-hydroxysuccinimide. Compound (II) is formed in 80-85% yield, is stable when stored, and is readily soluble in most of the organic solvents used in peptide synthesis. The esterification of the N-protected derivatives of amino acids and peptides using (II) proceeds easily in up to 100% yield in the presence of organic bases in 1-1.5 h at 20°C in such solvents as DMF, DMSO, THF, MeCN, dioxane, and CH_2Cl_2.

A. N. Nesmeyanov Institute of Heteroorganic Compounds, Academy of Sciences of the USSR, Moscow.

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TABLE 1

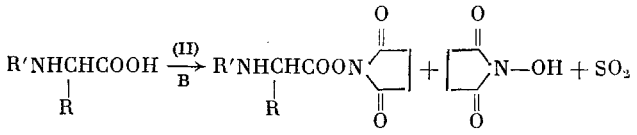
Compound	Yield, %	mp, °C	$[\alpha]_{589}^{22}$ (C, dioxane)	Literature reference
BocGlyONSu	84	167–168	–	[5]
BocProONSu	80	135–136	−55 (2,1)	[5]
BocSer(OBzl)ONSu	82	110–111	+6,2 (0,6)	[5]
BocTyrONSu	75	181–182	−12 (1,0)	[8]
BocThrONSu	84	135–136	−34 (2,0)	[9]
BocAlaONSu	98	144–145	−50,9 (2,0)	[5]
BocAlaONSu *	86	144–145	−50,9 (2,0)	[5]
BocGlu(γBzl)ONSu	83	104–105	−23 (2,0)	[10]
BocGlu(γBzl)ONSu *	80	104–105	−23 (2,0)	[10]
BocPheONSu	92	151–152	−19 (2,0)	[11]
ZAlaONSu	94	123–124	−37 (2,0)	[7]
ZProONSu	87	89–90	−55,3 (2,0)	[5]
ZAlaAlaONSu	87	155–156	−56,4 (4,0)	[7]

*As the dicyclohexylammonium salt.

TABLE 2

Compound	Base	Yield, %	$[\alpha]_{589}^{22}$ (C2, dioxane	Literature reference
BocAlaONSu	Pyridine	98	−50,9	[11]
The same	N-Methylmorpholine	95	−49,8	
»	Et_3N	97	−50,4	
»	DCHA*	86	−49,8	

*Dicyclohexylamine.



R'COOCH$_2$Ph(Z) or COOC(Me)$_2$(B); R = side chain substituent of amino acid; B = base; pyridine, N-methylmorpholine, triethylamine, dicyclohexylamine.

From Table 1 it follows that the use of (II) makes it possible to obtain the activated esters directly from the dicyclohexylammonium salts of the corresponding N-protected amino acids, thus eliminating the step of isolating the carbonxyl component. In this case the reaction with (II) is run without adding an external organic base, which greatly simplifies the process.

We studied the effect of the nature of the organic base on the yield and optical purity of the desired products, using a 1:1 mole ratio of the base and N-protected amino acid.

From Table 2 and the enantiomeric analysis data for the diastereomeric dipeptides, obtained from the N-hydroxysuccinimide ester of N-tert-butoxycarbonyl-L-alanine and the tert-butyl ester of L-alanine, which analysis was carried out by the ion-exchange chromatography method using an automatic analyzer, it follows that the nature of the base has little effect on the optical purity of the obtained activated esters. In all cases the degree of racemization of the carboxyl component was below 0.1%, i.e., the level detected by the indicated method.

It should be mentioned that catalytic amounts of an organic base can be used in the reaction of (II) with the N-protected derivatives of amino acids (Table 3); N-tert-butoxycarbonyl-L-alanine was used as the N-protected amino acid.

The use of a catalytic amount of a base has great importance, since it is known, for example, that when N-trifluoroacetoxysuccinimide is reacted with N-protected amino acids in aprotic solvents in the presence of tertiary amines the transesterification reaction does not always proceed unambiguously, and in a number of cases the insoluble bis-N-hydroxysuccinimide

TABLE 3

Base: N-protected amino acid (mole ratio)	Yield, %
1:1	98
3:1	93
0,1:1	94
0:1	45

ester of succinic acid is formed [12], which lowers the purity and yield of the desired products. A similar phenomenon was not observed when (II) is used.

EXPERIMENTAL

N-Hydroxysuccinimide was obtained as described in [13], the solvents were purified as described in [14], the $SOCl_2$ was distilled with triphenyl phosphite, and the N-protected L-amino acids were purchased from the Reanal Company. The melting points were determined on a Kofler stand, and the specific rotation was measured on an A1-EPO photoelectronic polarimeter using a cell 0.2-dm long. The IR spectra were taken on an IRS Hitachi 260-10 instrument.

N-Trimethylsiloxysuccinimide (I). A mixture of 23 g (0.2 mole) of N-hydroxysuccinimide and 43.5 g (0.3 mole) of trimethylsilyldiethylamine was heated at 100-120°C until the distillation of Et_2NH ceased (∿1 h) and then it was vacuum-distilled. We obtained (I) with bp 112-113° (1 mm), mp 45-46°. Infrared spectrum (ν, cm^{-1}): 1785, 1720, 1240, 1080-1010, 860. Found: C 44.82; H 6.30; N 7.48; Si 14.69%. $C_7H_{13}NSiO_3$. Calculated: C 44.89; H 6.99; N 7.48; Si 14.99%.

N,N'-Disuccinimidyl Sulfite (II). To a stirred solution of 11.1 g (0.98 mole) of $SOCl_2$ in 30 ml of ether, cooled to 0°C, was added dropwise a solution of 35.0 g (1.88 moles) of (I) in 30 ml of THF, maintaining the temperature of the mixture at −2 to ±2°C, after which it was kept for 30 min at 0°C, and the obtained precipitate was filtered, washed in succession with chilled THF (2 × 25 ml), and ether (2 × 25 ml), and dried in vacuo. We obtained 41.0 g (80%) of (II), mp 134-136°C. Infrared spectrum (ν, cm^{-1}): 1785, 1720, 1230, 1090, 1050, 1010. Found: C 35.05; H 2.79; N 10.56; S 11.41%. $C_8H_8H_2SO_7$. Calculated: C 34.79; H 2.92; N 10.14; S 11.61%.

Preparation of N-Hydroxysuccinimide Esters of N-Protected Amino Acids and Peptides. To a stirred solution of 10 mmoles of the N-protected amino acid (peptide) in 10 ml of a polar solvent (MeCN, DMF, THF) was added 10 mmoles of (II) and the mixture was kept at ∿20°C for 1-1.5 h, concentrated in vacuo, and the residue was diluted with 50 ml of EA and washed in succession with water (3 × 10 ml) and 5% $NaHCO_3$ solution (3 × 10 ml). The extract was dried over anhydrous Na_2SO_4, evaporated in vacuo, and recrystallized from a suitable solvent. The yield of the compounds was 80-100%, see Table 1.

CONCLUSIONS

A method was proposed for the preparation of N,N'-disuccinimidyl sulfite, a new efficient reagent for the synthesis of the N-hydroxysuccinimide esters of N-protected amino acids and peptides, which consists in treating N-trimethylsiloxysuccinimide with thionyl chloride.

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CHARACTERISTICS OF ^{95}Mo NMR IN AQUEOUS SOLUTIONS OF POLYOXOMOLYBDATES

M. A. Fedotov

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The state and structure of polyoxomolybdate anions in solution are being successfully studied by the method of the NMR of the different nuclei that enter into the polyanions, and mainly by the ^{17}O NMR method [1]. However, when studying the polyoxomolybdates the NMR of the magnetic Mo isotopes (^{95}Mo, ^{97}Mo) is practically not used, although the use of the less sensitive ^{183}W NMR for studying the similar polyoxotungstates is constantly expanding [2]. The first studies of aqueous MoO_4^{2-} [3] and oxothiomolybdate [4] solutions by the ^{95}Mo NMR method disclosed a large range in the chemical shifts (CS) of the ^{95}Mo NMR and very narrow lines of the simple MoO_4^{2-} and MoS_xO_{4-x} ions. In the polynuclear Mo(VI) ions the ^{95}Mo NMR lines proved to be much broader, and the information gained by the method in ordinary (≤ 2 T) magnetic fields is small. In addition, due to the low NMR frequency (5.86 MHz at 2.114 T) the acoustic ring in the pulse spectrometer swamps the NMR signal, which does not permit accumulating a signal with an NMR line width greater than 1 kHz. On going to high fields, which is achieved in cryomagnets (≤ 7 T), the situation is noticeably improved. (The spacing of the lines increases, the signal increases, and the duration of the ring decreases sharply.) The NMR spectra of a number of polyanions in a field of 7.05 T reveal that in a number of cases the ^{95}Mo NMR method can also be informative for polymolybdate solutions.

The informativensss of the NMR method is determined by the scale of change in the CS from some parameter when compared with the width of the NMR lines (Δf). The latter for quadrupole nuclei in solution [5]

$$\Delta f = \frac{3}{40}\frac{2I+1}{I^2(2I-1)}\left(1+\frac{\eta^2}{3}\right)\left(\frac{eQq}{\hbar}\right)^2\tau_c$$

is determined by the quadrupole coupling constant eQq/$\hbar$ and the time of the correlation of the rotational motion of the ion τ_c. Besides the generally adopted designations, here η is the asymmetry parameter ($\eta \leq 1$), q is the gradient of the electrical field on the nucleus, and Q is the quadrupole moment of the nucleus. The investigator can affect the line width only by using the τ_c parameter due to a decrease in the viscosity (selection of solvent) and an increase in the temperature. The latter noticeably narrows the ^{95}Mo NMR lines. Since the width of the ^{97}Mo NMR lines is much greater than for ^{95}Mo, due to the greater ^{97}Mo quadrupole moment, the informativeness of the ^{97}Mo NMR is correspondingly lower; consequently, only the ^{95}Mo NMR is discussed further. Examples of the spectra and structures of the studied heteropolyanions are given in Fig. 1, and the ^{95}Mo NMR data are given in Table 1. The state of the heteropolyanions (HPA) in solution was checked via the ^{17}O NMR spectra of the studied samples, since the ^{17}O NMR spectra characterize definite structures.

In the HPA with a Keggin structures (see Table 1, samples 1-9) all of the Mo ions are found in identical MoO_6 octahedra and are equivalent. Here a dependence of the CS of the charge of the HPA and the type of central atom can be expected. From Table 1 it can be seen that the charge of the HPA has a smaller effect on the CS than replacing the central atom. Since the size of HPA 1-9, the molecular weight of the HPA, and the solvent in samples 1-9 is the same, the change in τ_c from sample to sample is small, and a change in the width of the ^{95}Mo NMR lines must be attributed to the effect of the gradient of the electrical field.

Institute of Catalysis, Siberian Branch, Academy of Sciences of the USSR, Novosibirsk.
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