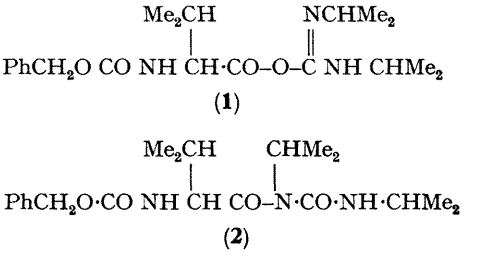
Direct Observation of an Alkoxycarbonylamino Acid *O*-Acylisourea

By Hilary S. Bates, John H. Jones,* and Michael J. Witty
(*The Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford* OX1 3QY)

Summary The reaction of equivalent amounts of benzyloxycarbonyl-L-valine and *NN'*-di-isopropylcarbodi-imide in deuteriochloroform at room temperature is very fast and clean giving an *O*-acylisourea adduct which is stable in solution for many hours and which has been characterised by n.m.r. spectroscopy.

IT seems to be generally accepted[1] that a highly reactive *O*-acylisourea is formed during the activation of *N*-protected amino-acids for peptide synthesis by means of carbodi-imides, and that if this is not consumed promptly by added nucleophile it isomerises to the corresponding unreactive *N*-acylurea or reacts with further *N*-protected amino-acid to give symmetrical anhydride and urea As far as we are aware, there have been no examples of the direct observation of *O*-acylisoureas, although the indirect evidence for their intermediacy in peptide coupling reactions is considerable [1] In fact, such adducts are stable for many hours in deuteriochloroform at room temperature Thus addition of one equivalent of *NN'*-di-isopropylcarbodi-imide to a 0 15 M solution of benzyloxycarbonyl-L-valine in deuteriochloroform at room temperature led to rapid clean and complete reaction with formation of *O*-(benzyloxycarbonyl-L-valyl)-*NN'*-di-isopropylisourea (**1**) which was characterised by its 300 MHz n m r spectrum† (see the Figure) The possibility that the spectrum observed was that of the isomeric unreactive *N*-acylurea (**2**) was eliminated by its separate preparation and characterisation, its spectrum‡ (see the Figure) was quite different The spectrum of the *O*-acylisourea remained completely unchanged for 9 h, on standing overnight it began to decompose, mainly to the *N*-acylurea, but this had only proceeded to an extent of *ca* 60% after 6 days Attempts to isolate the *O*-acylisourea, however, led to decomposition When the same experiment was performed with perdeuteriodimethylformamide as solvent sharply contrasting results were obtained In this case there was no immediate reaction Even after 30 min both reactants were more than 50% unchanged A complex mixture comprising mainly *N*-acylurea (**2**) *NN'*-di-isopropylurea and benzyloxycarbonyl-L-valine anhydride was slowly formed, at no stage were peaks attributable to the *O*-acylisourea (**1**) detected



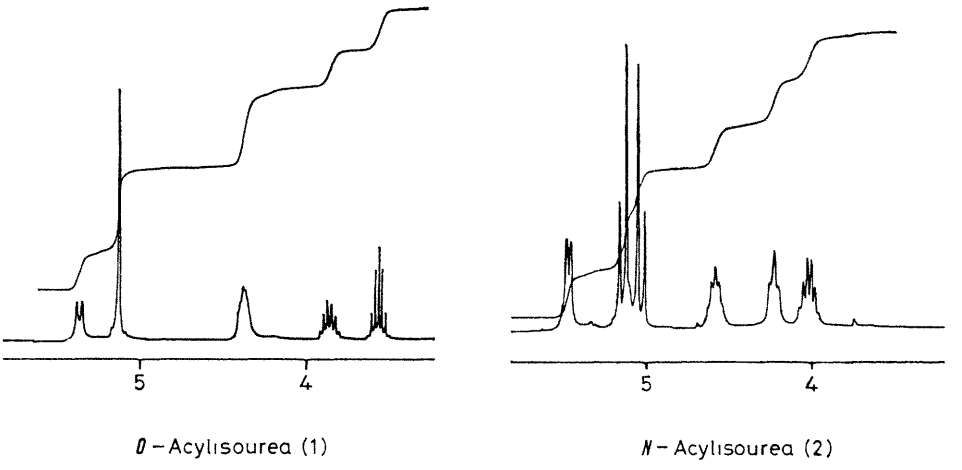


FIGURE Comparison of the n m r spectra of (**1**) and (**2**) in the region δ 3 5—5 5

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[1] For a review see D H Rich and J Singh in The Peptides ed E Gross and J Meienhofer Academic Press New York, 1979 vol 1, ch 5

† The 300 MHz n m r spectrum of a 0 15 M solution of (**1**) in $CDCl_3$ at 20 °C δ 7 35 [s 5H Ar*H*], 5 35 [d collapsing to s on irradiation of the band at 4 2—4 5 1H $PhCH_2OCONH$] 5 12 [s 2H $PhCH_2$] 4 2—4 5 [complex 2H $(CH_3)_2CHNH$ and $NHCHCO$] 3 87 [complex simplifying to a septet on irradiation of the band at 4 2—4 5 1H $(CH_3)_2CHNH$] 3 58 [septet 1H $(CH_3)_2CHN$=] 2 15—2 35 [complex 1H $(CH_3)_2CHCH$] 1 25 and 1 14 [2 × d 12H 2 × $(CH_3)_2CHN$] and 1 05 and 0 95 [2 × d 6H, $(CH_3)_2CHCH$] The 0 15 M solution was prepared by addition of 1 equiv of di isopropylcarbodi-imide (the spectrum of which consists in $CDCl_3$ of a septet at 3 57 and a doublet at 1 23 of relative intensity 1 6) as a neat liquid to a 0 15 M solution of benzyloxycarbonyl L valine in $CDCl_3$

‡ The 300 MHz n m r spectrum of a 0 15 M solution of (**2**) in $CDCl_3$ at 20 °C δ 7 5 [br 1H $NHCH(CH_3)_2$] 7 35 [s 5H Ar*H*] 5 45 [d 1H $PhCH_2OCONH$] 5 07 [ABq 2H $PhCH_2$] 4 57 [complex 1H $NHCHCO$] 4 22 [complex 1H $(CH_3)_2CHNCONH$] 4 02 [complex 1H $(CH_3)_2CHNH$] 1 93 [complex 1H $(CH_3)_2CHCH$] 1 36 and 1 27 [2 × d 6H $(CH_3)_2CHCH$] and 1 24 and 0 98 [2 × d 12H 2 × $(CH_3)_2CHN$] Compound (**2**) was isolated chromatographically from the mixture of neutral products resulting from an attempted coupling of equivalent amounts of benzyloxycarbonyl L valine and glycine ethyl ester hydrochloride in dimethylformamide by 1 equiv of di-isopropylcarbodi-imide in the presence of 1 equiv of triethylamine at 0 °C It was obtained as a semisolid material which was stable indefinitely and was homogeneous by t l c and gave i r m s and analytical data in full accord with the structure (**2**)