Pergamon

Tetrahedron Letters 40 (1999) 3355–3358

TETRAHEDRON LETTERS

Solid Phase Decarbamoylation of Monoalkylureas and N-Carbamoylpeptides Using Gaseous NO_x : A New Easy Deprotection Reaction With Minimum Waste

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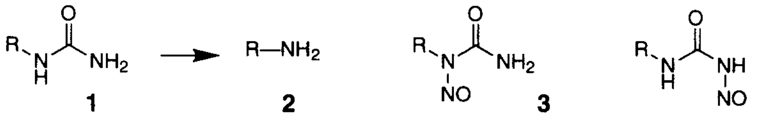
Received 23 December 1998; accepted 8 February 1999

Abstract : Treating solid monoalkylureas (including N-carbamoylpeptides) when hydrated by ca. 1 eq. water, by gaseous NOx (nitrogen peroxide or a mixture of nitric oxide and oxygen) in stoichiometric excess, deprotects quantitatively the amino function at room temperature in less than 30 minutes. The reaction was exemplified for the N-carbamoyl-Leucine-Glycine dipeptide and N-benzylurea, and turns out to be a promising method for removing an N-carbamoyl group from various monosubstituted ureas, with no waste other than nitrogen and carbon dioxide.

Introduction :

The urea group is not yet known in the literature as an N-protective group in peptide chemistry. Indeed, its hydrolysis requires concentrated basic solutions, incompatible with peptide chemistry because of α-carbon racemization, or enzymatic systems[1]. Extending our previous studies on N-carbamoylaminoacid activation[2,3], we have investigated the nitrosation of selectively converting monoalkylureas **1** (including N-carbamoylpeptides) into the corresponding amino derivatives **2**.



1 **2** **3** **4**

Nitrosation of alkylureas **1** in organic solution has already been described in the literature. Since the work of Huisgen[4], N-Nitrosoalkylureas **3** are known to be thermally unstable and to decompose into numerous compounds including the decarbamoylated compound **2**, most probably through an isocyanate intermediate. Although the internal N-nitroso compound **3** is the most stable, both nitroso compounds **3** and **4** are in equilibrium especially under acidic conditions, where nitrosation is a reversible reaction. This last fact has been established at least for N,N'-disubstituted ureas[5,6] as well as for secondary amides[7].

N-carbamoylaminoacid decarbamoylation through nitrosation has also been described in anhydrous organic solvents[8], but releases hydroxyacids as side-products. Recent improvements were found[9], but the reaction conditions used — namely micellar/microemulsion of diluted H_2SO_4, sodium dodecylsulfate, sodium nitrite — remain too sophisticated to be considered as convenient in peptide chemistry. Though nitrosation is an interesting route for the controlled decomposition of monoalkylureas and N-carbamoylpeptides, problems arise in either organic or aqueous solvents partly

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PII: S0040-4039(99)00326-3

due to intrinsic instability of the isocyanate intermediate. Therefore we decided to investigate the nitrosation reaction without any solvent, as we have previously used for the solid-phase N-carbamoylaminoacid conversion into N-carboxyanhydrides[3].

Results and discussion

The decarbamoylation reaction was investigated on the protected dipeptide N-carbamoyl-Leu-Gly **5** at room temperature under atmospheric pressure. The crystalline substrate **5** was exposed to gaseous N_2O_4 under an inert atmosphere, either in anhydrous conditions or in presence of a known small amount of water[10]. The results were checked by ^{1}H-NMR at 250 MHz. **5** was essentially converted into the decarbamoylated N-protonated dipeptide **6**, the remainder being unreacted starting material accompanied with traces of internal nitrosocarbamoyl compound **7**. The reaction conditions and conversions in **6** are listed in Table 1.

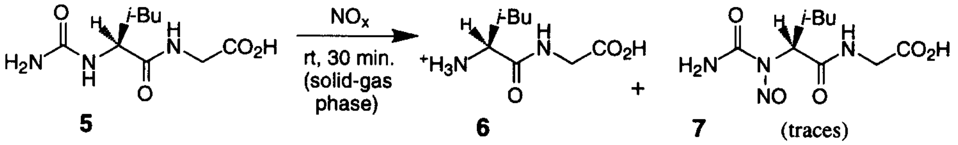


Table 1 : decarbamoylation of **5** (0.1mmol scale) using N_2O_4 under different conditions.

Entry	1	2	3	4	5	6	7	8	9	10	11	12	13
N_2O_4 (eq.)	1.12	1.12	1.49	1.49	1.86	1.86	1.12	1.12	1.49	1.49	1.86	1.86	1.86
H_2O (eq.)	0	0	0	0	0	0	1.02	2.05	1.02	2.05	1.02	2.05	3.08
T°C	20	28	20	28	20	28	20	28	20	28	20	28	28
Yield % in 6	14	14	20.4	20.6	33.5	48	25	64	34.5	100	69.7	99	100

The yield increases with the amount of N_2O_4 used, but remains low unless water (1-3 equivalent) is added. Higher temperatures also slightly increase the yield, but this effect appears to be minor and as yet ill-defined. The reactions corresponding to entries 8, 10 and 12 were repeated in the same conditions and stopped at different times to obtain kinetic information. For entry 8, the conversion slightly increased to 70% after 1h reaction. In all cases, 90% of the final conversion was reached within 10 minutes.

Anion titration on the sample entry 13 showed an excess of nitrate (1.1 eq. compared to **6**) accompanied by traces of nitrite, thus rationalizing the conditions needed for complete conversion : the material (though solid) must be acidified, as needed for classical nitrosation in organic solution. The acidification is provided in our case by hydrolysis of N_2O_4, which releases nitric and nitrous acid (Eq. I). The latter is an efficient nitrosating species[7], however unstable in acidic conditions, where it dismutates into nitric acid, water and nitric oxide (Eq. II), explaining why a complete conversion requires a stoichiometric excess of N_2O_4.

$$N_2O_4 + H_2O \longrightarrow HNO_3 + HNO_2 \qquad [I]$$
$$3\,HNO_2 \longrightarrow HNO_3 + H_2O + 2\,NO \qquad [II]$$

The decarbamoylation reaction was repeated using a 0.4/1 O_2/NO mixture as the nitrosating reagent (this O_2/NO ratio was found to be the most effective in a series of preliminary experiments performed without any water) ; the results are displayed in Table 2. Again, water (1 equivalent) was necessary for complete conversion, but could be replaced by only 0.1 equivalent trifluoroacetic acid (TFA) for the same result, what also supports the importance of an acidified medium. Anion titration on the sample entry 2 shows again a large nitrate content and traces of nitrite. The imbalance with **6** ($[NO_3^-]/[\mathbf{6}] = 0.85$) is surprising, however.

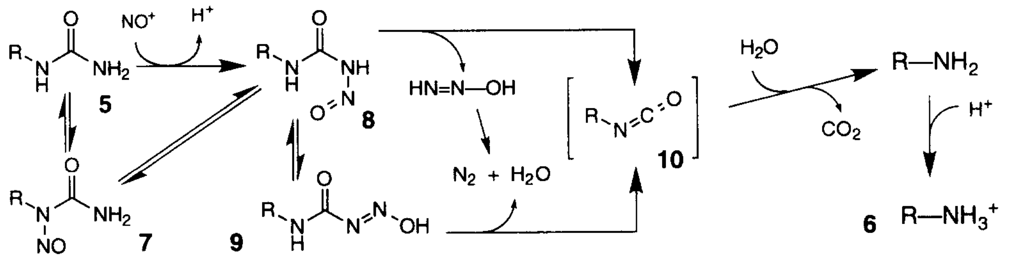
In terms of reaction rate and consumed stoichiometries, this latter nitrosating system appears to be more efficient than the previous one. Though NO reacts very fast with oxygen, yielding NO_2 then N_2O_4 (Eq. III, the reaction is actually equilibrated), the excess NO also reacts on NO_2 yielding N_2O_3 (nitrous anhydride, Eq. IV), also an efficient nitrosating species [11].

Table 2 :
Decarbamoylation of **5** using 2.5eq. NO + 1eq. O_2 under different conditions.

Entry	1	2	3
H_2O (eq.)	0	1.0	0
TFA (eq.)	0	0	0.1
yield % in 6	24.3	97	100

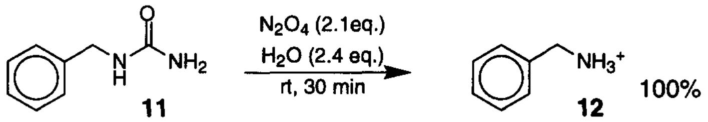
$$NO + 1/2\,O_2 \rightleftharpoons NO_2 \rightleftharpoons N_2O_4 \qquad [III]$$
$$NO_2 + NO \rightleftharpoons N_2O_3 \qquad [IV]$$

Whatever the nitrosating system used, no side-product resulting from peptide-bond break or deamination was detected in the crude reaction mixtures, conversely to literature claims concerning reactions performed in organic solution[12]. No further transformation into hydantoin or NCA was observed, probably due to the smooth solid-gas conditions we used. Though a local temporary dissolution of the solid material probably occurs (as previously observed for N-carbamoylaminoacid dry-phase nitrosation[3]), this very concentrated, strongly acidic medium is quite different from an ordinary solution. Under these conditions, we suspect that a fast equilibrium exists between the different N-nitroso species **7**-**8** as well as with the non-nitrosated **5**, the nitrosating species being probably the nitrosonium cation NO^+. However since primary amines and amides are nitrosated faster than secondary ones, the external nitroso compound **8** would also be the kinetic product. From **8** or an hydroxydiazo intermediate[4] **9**, an elimination step releasing nitrogen and water affords the isocyanate **10**. The literature suggests[13] that **8** may have the conformation depicted, favouring direct elimination to **10** (upper pathway). The water released in the previous step then hydrolyzes the isocyanate **10**, whose side-reactions with amino goups are avoided since all of them are protonated.



To check whether racemization occured during the reaction, crude deprotected L-Leu-Gly was derivatized on both amino and carboxylic groups by (-)-menthyl chloroformate[14]. Gas-chromatography analyis, comparing to (-)-menthyl derivatives of L- , D- and DL- Leu-Gly authentic samples showed that no racemization occurred during the nitrosative deprotection.

Moreover, the optimized nitrosation conditions using N_2O_4 were succesfully applied to benzylurea **11**, yielding quantitatively deprotected benzylammonium **12**, showing that this deprotective nitrosation pathway can be applied to various amines.



Conclusion

The N-decarbamoylation reaction described in this paper appears very convenient for peptide chemistry (since neither peptidic bond damage nor racemization occurs) and may become an efficient deprotection method in organic synthesis. The reaction proceeds very smoothly and with minimum waste, since no solvent is used and only carbon dioxide and nitrogen are released. Additionally, this new reaction may have important implications for chemical evolution since N-carbamoylaminoacids have been demonstrated to be realistic prebiotic molecules[15]. Details of this will be discussed elsewhere.

Acknowledgements

We thank CNRS, MRST and Degussa-Rexim Company for their support.

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10 Typical experiment : in a magnetically-stirred 50-ml glass flask containing 25mg (108µmol) of **5** spread over glass balls, and purged with nitrogen, 5ml (200µmol) gaseous N_2O_4, then 6µl (330µmol) water were introduced using syringes through a rubber cork. After 30 min gentle stirring at rt, the nitrosating gas mixture was evacuated by a rapid nitrogen flow (N_2O_4 molarity was estimated on basis of equilibrium composition NO_2/N_2O_4 = 20/80 at 25°C).

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