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Carboxyl-protecting Groups Convertible into Activating Groups. Carbamates of *o*-Aminoanilides are Precursors of Reactive *N*-Acylureas

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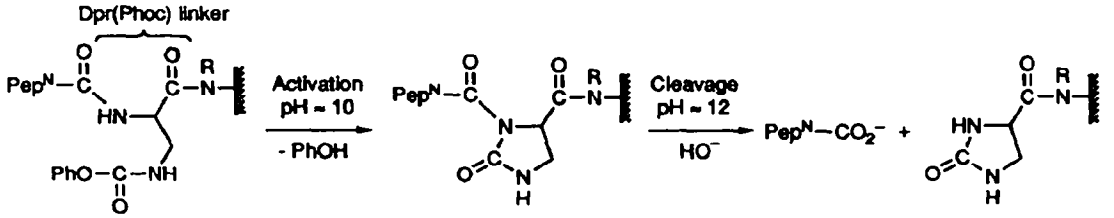
Abstract: Selected carbamates of *o*-aminoanilides of amino acid derivatives are stable under neutral and acidic aqueous conditions but undergo a quantitative base-catalysed intramolecular conversion into an 1-acylbenzimidazolin-2-one. The pH-rate profiles for this reaction and for the subsequent hydrolysis of the *N*-acylurea were established. The *N*-acylurea displays a hydrolytic reactivity reaching that of active esters and is shown to acylate leucine methyl ester.

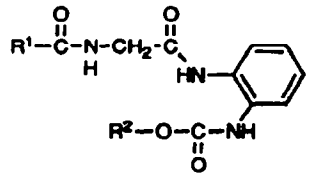
We recently described a new linker for solid-phase peptide synthesis [Dpr(Phoc) linker], which is compatible with Boc-chemistry and particularly suitable for the use of aqueous processing.[1] The release of the peptide is carried out under experimental conditions similar to those required for base-labile ester linkers, by cleavage of a cyclic *N*-acylurea which is generated by activation of the amide anchorage under milder alkaline conditions (Scheme 1).



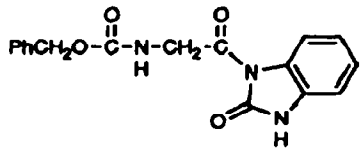
Scheme 1. Activation and cleavage of the Dpr(Phoc) linker (Dpr = L-2,3-diaminopropionic acid, Phoc = phenyloxycarbonyl, Pep^N = amino terminal part of peptide).

We are currently searching for related systems in which the *N*-acylurea reactivity would be selectively enhanced, as they may give rise to carboxyl-protecting groups readily convertible into activating groups, with various applications particularly in liquid- or solid-phase peptide synthesis, *e.g.* segment condensation (or any nucleophilic reaction), linkers that are cleavable at pH *ca.* 9-10 and thus more suited to base-sensitive peptides, side-chain protections for aspartic acid potentially useful for glycopeptide synthesis.

As a first advance in this area, we report here a substantial enhancement of reactivity with 1-acylbenzimidazolin-2-ones, such as **2** for example, and their ready and selective formation as intermediates in the anilide bond hydrolysis of *o*-phenylenediamine derivatives **1** selected as models of protected peptides.



1 a R^1 = $PhCH_2O$, R^2 = Ph
b R^1 = Ph, R^2 = CCl_3CH_2



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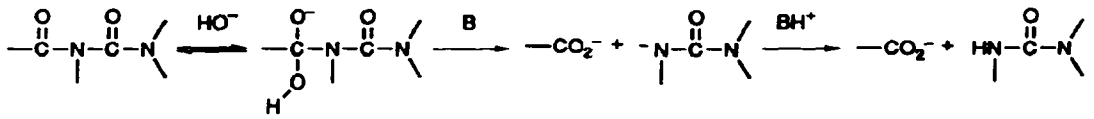
The choice of such structures resulted from an analysis of the accepted mechanism for *N*-acylurea hydrolysis[2] which involves the rate-determining expulsion of an ureide anion from the tetrahedral intermediate (Scheme 2). Thus an increase in rate was expected, owing to the leaving group ability of the anion of benzimidazolin-2-one, which must be better than that of non-conjugated ureas according to the acidities of the corresponding ureas : pK_a = 12.0 [3] and pK_a ≈ 18 [4] respectively. Moreover, as for *N*-acylimidazoles and *N*-acylbenzimidazoles,[5] the resonance in 1-acylbenzimidazolin-2-ones must markedly activate the carbonyl group, and consequently, the formation of the tetrahedral intermediate must also be facilitated.



Scheme 2. Mechanism of *N*-acylurea hydrolysis.

The reactivity of 1-acylbenzimidazolin-2-ones[6] was studied using the derivative of Z-glycine 2.[7] Its hydrolysis selectively yielded Z-glycine and benzimidazolin-2-one at any pH, as shown by HPLC analyses[8] indicating no side-products, in particular no amine resulting from the reaction of the urea carbonyl group. The pH-rate profile (Fig.1) mainly shows two mechanisms with a transition at pH 7 : a pH-independent water reaction and the reaction of HO^- with the neutral substrate (pK_a ≈ 9.8 [9]) or kinetic equivalents. The significant rate increases observed below pH 1 and above pH 12 may be attributed to an acid-catalysed hydrolysis and a reaction of HO^- with an ionized species, respectively. The second-order rate constant for the hydroxide ion reaction that predominates in moderately alkaline medium (k_{HO^-} = 50 ± 10 s^{-1} mol^{-1} dm^3 at 20.0 °C), is of the same order of magnitude as that determined for Z-glycine *p*-nitrophenylester (k_{HO^-} ≈ 130 s^{-1} mol^{-1} dm^3 at 21.0 °C [10]), and three orders of magnitude higher than that observed for the cleavage of a C-terminal glycine peptide anchored with the Dpr(Phoc) linker to a polyacrylamide support.[11] The aminolysis of *N*-acylurea 2 (1 mol dm^{-3}) was also examined : a quantitative conversion (≥ 99.5%) was observed after a 20 h treatment with leucine methyl ester (1.5 mol dm^{-3}) in DMF at 25 °C, giving selectively Z-Gly-Leu-OMe and benzimidazolinone, as shown by HPLC analysis.[12] 1-Acylbenzimidazolin-2-ones are therefore new examples of amides activated towards nucleophilic attack.

The models **1a** and **1b** [13] were used to check (i) the stability of the protection system under acidic conditions, even around neutrality for **1b**, (ii) the selectivity of the activation process at higher pH. Above pH 4, HPLC analysis [8] showed the formation of the expected end-products only : phenol and Z-glycine from **1a**, or benzoyl-glycine from **1b** (2,2,2-trichloroethanol is not detectable by UV), and benzimidazolin-2-one. An intermediate was transiently observed and was identified as *N*-acylurea **2** in the case of model **1a**, since it showed the same HPLC, UV, and kinetical features. In addition, Z- or benzoyl-glycine were very slowly formed below pH 3 with rates (k_{H^+} *ca.* 10^{-6} s^{-1} mol^{-1} dm^3 at 20 °C) compatible with a direct hydrolysis of the

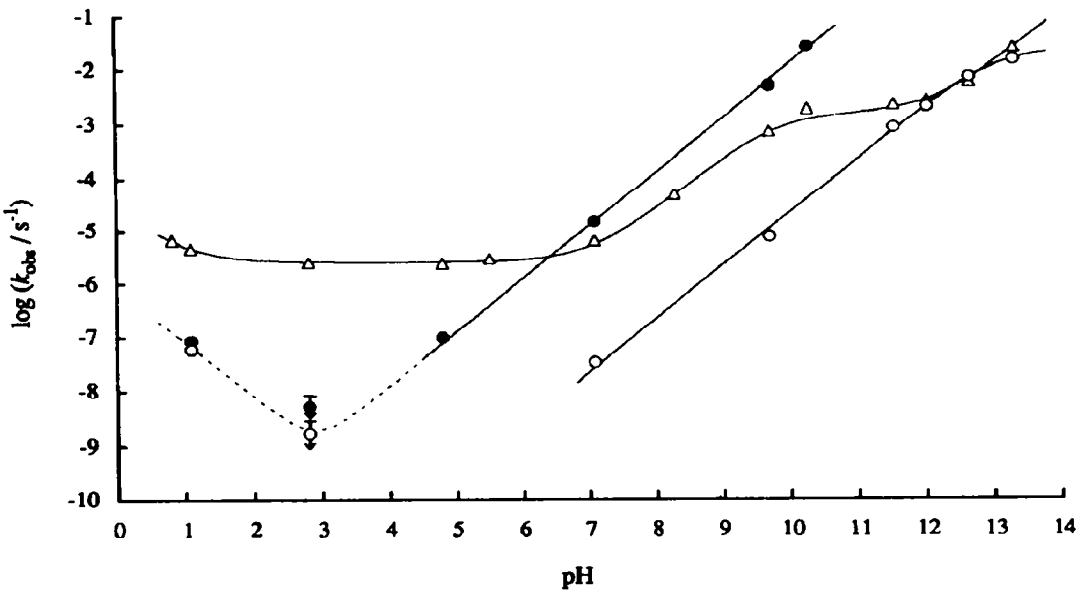


Fig. 1. pH-rate profiles for the reactions of the anilides **1a** (•) and **1b** (○), and of the *N*-acylurea **2** (Δ) in 12% (v/v) dioxane-water at 20 °C ; substrate 2.5×10^{-5} mol dm^{-3} ; $\mu = 0.2$ mol dm^{-3}, KCl ; determination by HPLC analysis ($k_{obs} < 3 \times 10^{-4}$ s^{-1}) or UV spectrometry ($k_{obs} > 3 \times 10^{-4}$ s^{-1}) at 240 nm for **2**, or at the isobestic wavelength (269 to 279 nm depending on pH) of **2** hydrolysis for **1a** and **1b**. At pH 2.8, only the upper limit of k_{obs} could be determined for **1a** and **1b**, owing to the very slow reaction of each.

anilide bond.[14] For the base-catalysed cyclization of anilides **1a** and **1b**, the pH-rate profiles (Fig. 1) show linear plots[15] with unit slopes ; the second order rate constants, $k_{HO^-} = 250$ and 0.4 s^{-1} mol^{-1} dm^3 respectively, are in agreement with those reported for the hydrolysis of unsubstituted phenyl and trichloroethyl *N*-phenyl carbamates at 25 °C, $k_{HO^-} = 54$ and 0.32 s^{-1} mol^{-1} dm^3 respectively,[16] and their higher values can easily be accounted for by the effect of the *ortho*-substituent. Consequently, the cyclization into *N*-acylurea very probably occurs through the same elimination-addition pathway involving an isocyanate intermediate formed by an $A_{xh}D_H + D_N$ (E1cB) elimination mechanism, with the same predictable structural effects ruled by the pK_a of the leaving alcohol.[16] A careful selection of that alcohol must therefore allow a fine tuning of the protection.

In addition, model **1b** was shown to be stable in organic or aqueous strongly acidic media since it was recovered with a 96% purity after 4 h treatments in TFA/CH_2Cl_2 1:1 or in aqueous 6 N HCl containing 40% (v/v) Pr^iOH. Carbamates **1a** and **1b** are also stable in many organic solvents with the important exception of dissociating solvents such as $(CH_3)_2SO$ or DMF in which a spontaneous conversion into the 1-acyl-benzimidazolin-2-one occurs within a few minutes for **1a** and more slowly for **1b** ($t_{1/2}$ *ca.* 90 min in DMF).

In conclusion, *o*-aminoanilide carbamates derived from appropriate alcohols behave as carboxyl-protecting groups in water and in some organic solvents, from neutral to strongly acidic conditions. Their conversion into highly reactive *N*-acylureas in alkaline solution allows the easy recovery of the carboxylic acid, with high selectivity at both stages of the transformation. Numerous applications can be considered but may be nevertheless limited by the carcinogenic properties of *o*-phenylenediamine and a poor protection in dissociating organic solvents, though the latter feature can be useful for activation under very mild conditions. Work is in progress to determine whether such limitations can be circumvented by structural adjustments.

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6 No kinetic data establishing the high reactivity of 1-acylbenzimidazolin-2-ones have been reported to date, but recent reports describe their thioacyl counterparts as thioacylating agents for the synthesis of thiopeptides [Boulos, Z. ; Sauvé, G.; Penney, C. *Tetrahedron*, 1993, *49*, 10489-10500; Boulos, Z.; Martel, R.; Sauvé, G.; Belleau, B. *Bioorg. Med. Chem. Lett.* 1993, *3*, 619-624].

7 1-[*N*-(Benzyloxycarbonyl)glycyl]benzimidazolin-2-one **2** was prepared in two steps from phenylene diamine by monoacylation using equimolar amounts of Z-glycine and DCC in CH_2Cl_2 or THF, and then cyclization in dioxane by reaction with carbonyldiimidazole; m.p. 188-190°C; FAB-MS *m/z* 326 ($M+H^+$); ^1H NMR (250 MHz, $(CD_3)_2SO$) δ 4.50 (d, J = 6.1 Hz, CH_2 Gly), 5.08 (s, CH_2 Z), 6.9-7.5 (m, 8H, ArH), 7.65 (t, J = 6.0 Hz, NH Gly), 7.98 (d, J = 7.4 Hz, 1H, ArH), 11.5 (s, NH).

8 HPLC conditions : Brownlee Spheri-5 RP-18 column; buffer A, 0.1% aq. TFA; B, MeCN (0.06% TFA); linear gradient 20 to 90% B over 25 min; flow 1 cm^3 min^{-1}; **1a** R_t = 18.1 min, **2** R_t = 16.2 min, Z-Gly R_t = 9.0 min, PhOH R_t = 7.2 min, benzimidazolinone R_t = 6.1 min, **1b** R_t = 17.8 min, Bz-Gly R_t = 5.6 min.

9 pK_a value determined kinetically from the data shown in Fig.1 and confirmed by a change in the UV spectrum of the substrate.

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12 HPLC conditions : Brownlee Aquapore RP-300 column; buffer A, 0.1% aq. TFA; B, MeCN (0.06% TFA); linear gradient 15 to 35% B over 40 min; flow 1 cm^3 min^{-1}; **2** R_t = 36.5 min, benzimidazolinone R_t = 5.5 min, Z-Gly-Leu-OMe R_t = 35.5 min.

13 Synthesized by reaction of the monoacylated phenylene diamine [ref. 7] with the corresponding chloroformate (1 equiv.) in the presence of pyridine (1 equiv.). **1a** : m.p. 115-117 °C; FAB-MS *m/z* 420 ($M+H^+$);^1H NMR (250 MHz, $CDCl_3$) δ 4.02 (d, J = 5.5 Hz, CH_2 Gly), 5.17 (s, CH_2 Z), 5.69 (t, J = 5.5 Hz, NH Gly), 7.17-7.55 (m, 13 H, ArH), 7.71 (d, J = 7.6 Hz, 1H, ArH), 7.84 (s, NH), 8.54 (s, NH). **1b** : m.p. 145 °C; FAB-MS *m/z* 444 with expected isotope peaks ($M+H^+$); ^1H NMR (250 MHz, $(CD_3)_2SO$) δ 4.11 (d, J = 5.7 Hz, CH_2 Gly), 4.76 (s, CH_2CCl_3), 7.12-7.25 (m, 2H, ArH), 7.4-7.7 (m, 3H, ArH), 7.85-8.1 (m, 2H, ArH), 9.45 (broad, 2H, NH), 8.97 (t, 5.6 Hz, NH Gly). The choice of *N*-benzoyl substitution in model **1b** resulted from the observation, when using the corresponding Z-derivative, of a well known side reaction of the Z-protecting group giving the hydantoic acid through an hydantoin intermediate [Bodanszky, M. *Principles of Peptide Synthesis;* Springer Verlag: Berlin, 1984; pp. 62, 174].

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15 The levelling off at high pH for model **1b**, confirmed with 1 mol dm^{-3} NaOH by HPLC analysis, can be accounted for by an ionization of the substrate (pK_a ca. 13.0), as observed for unsubstituted aryl carbamates [ref. 16a].

16 (a) Hegarty, A. F.; Frost, L. N. *J. Chem. Soc., Perkin Trans. 2* 1973, 1719-1728; (b) Williams, A. *ibid.*, 1972, 808-812; (c) 1973, 1244-1247.

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