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Tetrahedron Letters
Tetrahedron Letters 46 (2005) 5383–5386

N-[Chloro(dimethylamino)methylene]-*N*-methylmethanaminium chloride (TMUCl Cl), the reagent of choice for the solid-phase synthesis of anilides

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Received 24 April 2005; revised 26 May 2005; accepted 30 May 2005
Available online 20 June 2005

Abstract—An effective solid-phase preparation of anilides from supported carboxylic acids is described by their activation as the corresponding acid chlorides with TMUCl Cl.
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1. Introduction

Solid-Phase Organic Synthesis (SPOS)[1] is an emerging technology in modern organic chemistry that has its roots in the Solid-Phase Peptide Synthesis (SPPS)[2] method. The latter constitutes its main 'validator', while it also entails an important drawback. Thus, SPOS takes advantage of the methodology developed for peptide synthesis, mostly with respect to resins, proper management of protecting groups and coupling reagents.[3] On the other hand, SPOS is sometimes too 'conformist' as it relies on methods that work well for peptide synthesis. In such cases, it is difficult for SPOS to distance itself from peptide methodology by developing or adapting its own methods. This fact is made abundantly clear when an amide bond has to be prepared by solid-phase

Abbreviations: Ac, acetyl; All, allyl; Boc, *tert*-butyloxycarbonyl; CDI, 1,1′-carbonyldiimidazole; Cl–HOBt, 6-chloro-1-hydroxybenzotriazole; DCM, dichloromethane; DCT, dichlorotriazine; DepODhbt, DEPBT, 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4-(3*H*)-one; DIEA, *N*,*N*-diisopropylethylamine; DMC, 2-chloro-1,3-dimethylimidazolinium chloride; DMF, *N*,*N*-dimethylformamide; Fmoc, 9-fluorenylmethoxycarbonyl; HATU, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo-[4,5-*b*]pyridinium hexafluorophosphate 3-oxide; HCTU, 1-[bis(dimethylamino)methylene]-6-chloro-1*H*-benzotriazolium hexafluorophosphate 3-oxide; HBTU, 1-[bis(dimethylamino)methylene]-1*H*-benzotriazolium hexafluorophosphate 3-oxide; HDTU, *O*-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOAt, 1-hydroxy-7-azabenzotriazole(3-hydroxy-3*H*-1,2,3-triazolo-[4,5-*b*]pyridine); HOBt, 1-hydroxybenzotriazole; HOOBt, HODHbt, 1-oxo-2-hydroxydihydrobenzotriazine; HPLC, high performance liquid chromatography; NMM, *N*-methylmorpholine; MS, mass spectroscopy; NP, normal phase; PPHT, 2-[*N*-phenylethyl-*N*-propyl]amino-5-hydroxytetralin; Ph, phenyl; PS, polystyrene; PyAOP, (7-azabenzotriazol-1-yloxy)-tris(pyrrolidino)phosphonium hexafluorophosphate; PyBOP, benzotriazol-1-yl-*N*-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate; PyClock, 6-chloro-benzotriazol-1-yl-*N*-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate; Rink resin, peptide amide resin; SPOS, solid-phase organic synthesis; SPPS, solid-phase peptide synthesis; *t*-Bu, *tert*-butyl; TCT, 2,4,6-trichloro[1,3,5]-triazine (cyanuric chloride); TFA, trifluoroacetic acid; TFFH, tetramethylfluoroformamidinium hexafluorophosphate; TMUCl Cl, *N*-[chloro(dimethylamino)methylene]-*N*-methylmethanaminium chloride. Abbreviations used for amino acids follow the IUPAC–IUB Commission of Biochemical Nomenclature in Jones, J. H. *J. Pept. Sci.* **2003**, *9*, 1–8.

Keywords: Amide formation; Combinatorial chemistry; Coupling reagents; *p*-Nitroanilide; Peptide synthesis.

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0040-4039/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2005.05.140

methods. Although it might seem that there exists a true arsenal of coupling reagents and methods, the most popular active species are in the main *N*-hydroxylamino active esters, mostly derived from HOAt, Cl–HOBt, HOBt, HODHbt (HOOBt). These can be prepared either from carbodiimides and the corresponding hydroxylamino derivatives or from *stand-alone* reagents, such as HATU/PyAOP, HCTU, PyClock, HBTU/PyBOP, HDTU/DepODhbt (DEPBT).[4]

Compared with *O*-acylisourea, the active species when carbodiimides are used by themselves, *N*-hydroxylamino active esters are less reactive. However, their greater stability (*O*-acylisourea tends to rearrange to *N*-acylurea, which is totally unreactive) and their lower tendency to losing chirality make active esters very convenient. In spite of this fact, there are examples of couplings in which stronger activation is required and it becomes necessary to use acid chloride derivatives, which are the most active species. Although their use in SPPS is restricted to bifunctional amino acids with Fmoc N^{α}-protection, because of the incompatibility of Boc and the *tert*-butyl-based protecting groups with the conditions used to prepare these derivatives, they constitute probably the most popular amide forming methods in SPOS.[4] An additional drawback of Fmoc protected N^{α}-amino acid chlorides is that their use requires an hydrogen chloride scavenger such as DIEA or NMM, which favours the formation of the oxazolone, a clearly less reactive intermediate, which additionally is prone to racemization.

2. Results and discussion

Anilides, which are important motifs in drug discovery programmes, are difficult to prepare owing to the poor nucleophilicity of the corresponding anilines. In the literature, solid-phase methodologies for the synthesis of anilides are mostly based on the reaction between anilines linked to a solid support and acid chlorides.[5] However, when the carboxylic acid is supported on the resin, the synthesis of anilides becomes more difficult. Activation through the formation of *N*-hydroxylamino active esters implies the use of a large excess of the corresponding reagents, which, apart from being expensive, are often difficult to remove as *N*-hydroxylamino active esters may be retained in some resins, specially in those of high hydrophobicity [polystyrene (PS)-based resins]. In addition to these methods, there are described several procedures to convert carboxylic acids into halogenated activated species by using either $POCl_3$/pyridine,[6] TCT,[7] DCT,[8] DMC,[9] TFFH.[10] These reagents may form chloride/fluoride acid derivatives, which are more reactive than the previously mentioned esters. However, they are mostly used for the activation of carboxylic acids in solution phase rather than for the activation of solid support acids. The reaction between carboxylic acids and a large excess of 1,1′-carbonyldiimidazole (CDI) can be an alternative for derivatizing acids when they are linked to a solid support.[11] In this case, an imidazolide is the active species which, in the preparation of amides, reacts with amines.[12] Despite the high reactivity of imidazolides, this method may not be efficient enough to form the amide bond when using anilines since reaction yields can be extremely low.

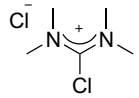
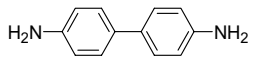
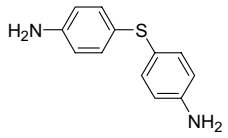
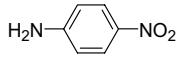


Figure 1. Structure of TMUCl Cl.

An alternative to CDI activation is the formation in the solid-phase of acid chlorides, which are more reactive species than imidazolides. TMUCl Cl (Fig. 1), which is a synthetic intermediate in the preparation of uronium/aminium salts based reagents, can react with carboxylic acids in the presence of a tertiary amine leading to acid chlorides, which can easily be converted to anilides by reaction with anilines. TMUCl Cl is not a phosgene derivative, which makes it safer, it is inexpensive and can be used in bulk also for the preparation of libraries.

In order to prove the usefulness of this method, three different anilines (benzidine, 4,4′-tiodianiline and 4-nitroaniline) were incorporated to the side-chain carboxylic acid of a glutamic acid residue anchored onto a solid support. 4-Nitroaniline plays an important role in this study as the electron-withdrawing effect of the nitro group decreases the reactivity of its amine.[13] In addition, *p*-nitroanilides are considered important motifs in

Table 1. HPLC–MS purities (220 nm) of the anilides synthesized

	CDI (2 h)	CDI (16 h)	TMUCl Cl (2 h)	TMUCl Cl (16 h)
	19%	61%	76%	90%
	25%	70%	90%	88%
	ND	9%	78%	80%

ND: no peak corresponding to the anilide was detected.

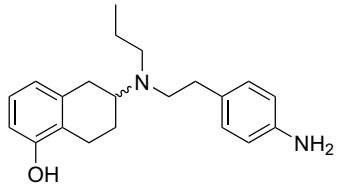


Figure 2. Structure of [±]-PPHT.

medicinal chemistry research due to their chromogenic properties.[14]

Fmoc-Glu(OAll)-OH was incorporated onto a Rink-PS-resin, the Fmoc group was removed [piperidine–DMF (2:8)], the α-amine was acetylated [Ac_2O–DIEA (1:2)], the allyl group was removed [$Pd(PPh_3)_4$ (0.1 equiv)–$PhSiH_3$ (12 equiv) in anhydrous DCM ($2 \times 20'$)] and the incorporation of the three anilines was performed by using CDI and TMUCl Cl based couplings.[15] The extent of the reaction was evaluated at 2 and 16 h by cleavage with TFA–H_2O (95:5, v/v) followed by RP-HPLC analysis.[16]

Table 1 clearly shows that superior results were obtained with TMUCl Cl compared with CDI. The contrast is sharpest in the preparation of the 4-nitroanilide.

Once we validated the advantage of using TMUCl Cl as activating reagent for supported carboxylic acids, this strategy was applied to the synthesis of a few more anilides, which were part of an *in-house* drug discovery programme. The pharmacological profile of [±]-2-[*N*-phenylethyl-*N*-propyl]amino-5-hydroxytetralin ([±]-PPHT) as a selective and potent agonist of D_2 dopamine receptor (Fig. 2) is well known.[17]

Several biological studies[18] (internalization, fluorescence) have raised the interest in derivatives of this kind of lead compounds. In the case of PPHT, this is not a straightforward task as it involves the poor nucleophilicity of an aniline. In order to compare some different coupling reagents, [±]-PPHT was linked through an amide bond to the side chain of a glutamic acid residue attached onto PS. For that purpose, CDI, TCT and TMUCl Cl were screened. Although the anilide was the major product in the three crude reaction products, HPLC–MS analysis revealed that the highest conversions were obtained when using TMUCl Cl (62%), compared to those obtained with TCT (40%) or CDI (46%).

Additionally, we undertook to evaluate the degree of racemization associated with the amide bond formation step by attaching [±]-PPHT to the carboxylic function of [Ac-Asp(&^{1})-OH][Ac-Lys(&^{1})-NH-Rink-PS][19] (Fig. 3). The analysis of the product after purification by RP-HPLC showed two peaks (each peak contained a 1:1 mixture of diastereomers) with the same mass and UV absorption spectra in a 8.5:1.5 ratio, corresponding to [Ac-L-Asp(&^{1})-(±)PPHT][Ac-Lys(&^{1})-NH_2] and [Ac-D-Asp(&^{1})-(±)PPHT][Ac-Lys(&^{1})-NH_2], respectively. These data indicate that even in this demanding case, only 15% of racemization was detected.[20]

3. Conclusions

TMUCl Cl was found to be a useful reagent for the activation of carboxylic acids attached onto a solid support. Its ready availability and its effectiveness in comparison with other carboxylic acid activation agents allowed the development of a simple and efficient method for the solid-phase synthesis of anilides. Thus, TMUCl Cl can be considered the reagent of choice for the solid-phase preparation of anilides. However, if applied, the relatively low loss of chirality should be taken into consideration.

Acknowledgements

This work was partially supported by CICYT (BQU2002-02047 and BQU 2003-00089). M.V. thanks MECD (Spain) for a predoctoral fellowship. Authors thank Yoav Luxembourg for his encouragement to perform the work.

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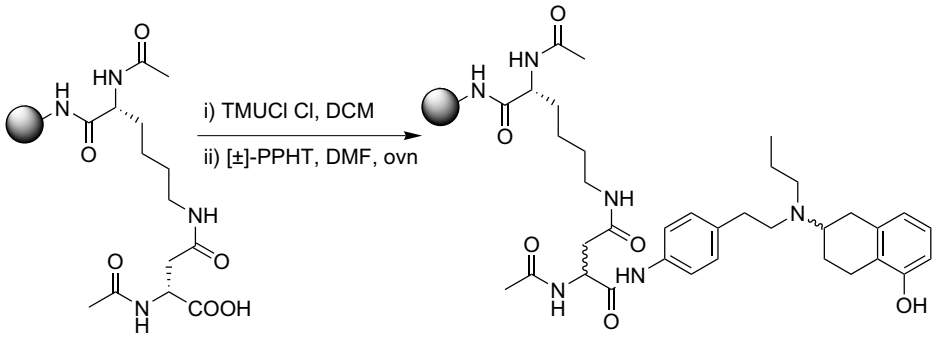

Figure 3. Scheme of [±]-PPHT modification for evaluating the racemization degree.

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15. Every experiment was carried out with 50 mg of Ac-Glu(OH)-Rink-PS-resin. Resins were swollen in DCM and the carboxylic acids were activated with CDI (25 equiv) in DMF or with TMUCl Cl (10 equiv) and DIEA (10 equiv) in DCM. Activation time varied from 20 to 30 min. Resins were filtered off and washed with DMF ($5 \times 1'$) and DCM ($5 \times 1'$). Afterwards, anilines (5 equiv) were solved in DCM–DMF (1:1) and added to the resin. Resins were under orbitalic shaking at room temperature for 2 or 16 h.
16. Cleavages were carried out at room temperature with a solution of TFA–H_2O (95:5, v/v). Reactions were complete after 1 h. RP-HPLC analysis were performed using an Alliance 2795 Waters Chromatography system with a reverse-phase symmetry C_{18} (3.9×150 mm) 5 μm column with 996 PDA detection. Mass spectra were recorded on Micromass ZQ Mass Spectrometer.
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20. In order to further separate the mixture of diastereomers from each peak, we analyzed the sample by chiral NP-HPLC using a Chiralpak AD column and a variety of elution solvents. Although the analysis by chiral NP-HPLC [hexane–ethanol–TFA (90:10:0.2)] of the starting material [±]-PPHT revealed two peaks in the same proportion, none of the assayed conditions were capable of separating the diastereomers expected in the final products.