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Dipyridylamine-acetamide (Dpaa): a primary amine protecting group orthogonally cleavable under acidic conditions in the presence of *t*-butyloxycarbonyl (Boc) and *t*-butylester

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ABSTRACT

We show that dipyridylamine-acetamide (Dpaa), can be cleaved under mild acidic conditions (30% formic acid in dichloromethane). The release of the amine function is orthogonal to other acid-labile protecting groups. Calculations suggest that the ease of Dpaa cleavage relies on activation of the carbonyl function by the protonated dipyridylamine nitrogen and activation of a water molecule by a hydrogen-bond network.

The protection of chemical functions and their selective deprotection are key steps of many organic syntheses described thus far.¹ The Boc (*t*-butyloxycarbonyl) group is probably the most frequently used protecting group for the amine function due to its high chemical stability under various reaction conditions, with the notable exception of acidic conditions, leading, in general, to the release of the amine, with volatile by-product emission (e.g., carbon dioxide and isobutylene).²⁻⁵ These properties have made Boc orthogonal to many other protecting groups with higher stability under harsher acidic conditions.² However, chemically stable protective groups orthogonal to Boc under milder acidic conditions have, to date, received less attention. In this context, we report the synthesis and properties of the dipyridylamine-acetamide (Dpaa) moiety and its application as a protecting group of the amine function in the concomitant presence of Boc, as well as another acid-labile protecting group: *t*-butylester.

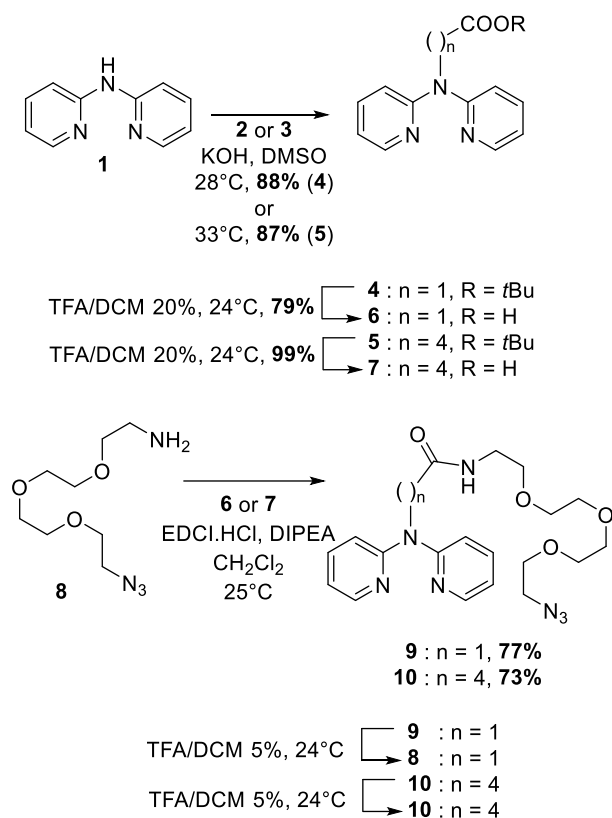
The discovery of the properties of Dpaa is the fruit of serendipity and related to the synthesis and properties of compounds **9** and **10** (Scheme 1). These compounds were synthesized as ligand for transition metals. To synthesize **9** and **10**, commercial 2,2'-dipyridyl-amine (dpa) **1** was treated with either *t*-butyl-bromoacetate **2** or *t*-butyl-bromovalerate **3** in a DMSO solution of ground KOH,^{6,7} leading to the expected *t*-butyl esters **4** and **5** isolated in 88% and 87% yield respectively. The release of the corresponding carboxylic acids **6** and **7** was obtained in respectively 79% and 99% yield using a 20% v/v solution of trifluoroacetic acid (TFA) in dichloromethane (DCM). Both dpa acids **6** and **7** were then further coupled to the terminal amine of 11-azido-3,6,9-trioxaundecane-1-amine **8** in the presence of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDCI.HCl) and *N,N*-diisopropylethylamine (DIPEA) in DCM, leading to dpa

derivatives **9** and **10** isolated in 77% and 73% yield respectively. The accidental treatment of **9** using a 5% solution of TFA in DCM during 24 h led to the unexpected complete release of amine **8**. In the same conditions **10** remains stable (Scheme 1, Figure S1 and S2 see ESI).

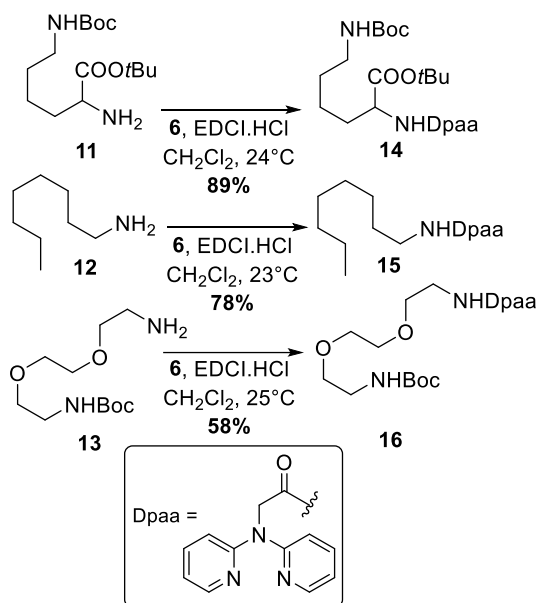
The amide bond is described to be generally cleaved under relatively harsh basic or acidic conditions.^{2,8} In this context, the lability of the Dpaa group of **9** under mild acidic conditions was unexpected and was further investigated. This intriguing lability was tested using organic acids with a higher pKa than TFA, such as formic and acetic acids, and several Dpaa-containing substrates. Thus *N* α , *N* ϵ -di-Boc-L-lysine **11**, octylamine **12**, and *tert*-butyl (2-(2-(2-amino-ethoxy)ethoxy)ethyl)carbamate **13**⁹ were reacted with dipyridylamine-acetic acid **6** in the presence of EDCI.HCl and DIPEA in DCM. The corresponding Dpaa derivatives **14**, **15**, and **16** were obtained with yields of 89, 78, and 58%, respectively (Scheme 2). Attempts to generate a Dpaa reagent ready to use for

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amine protection (e.g., NHS- or pentafluorophenyl-ester, acyl chloride) have not thus far been successful.



Scheme 1. Synthesis and reactivity of dpa-derivatives **9** and **10**.



Scheme 2. Synthesis of Dpaa derivatives **14**, **15** and **16**.

Compounds **14**, **15**, and **16**, as well as compounds **9** and **10**, were then treated with v/v 30% formic or acetic acid in DCM. The products obtained and yields are reported in Table 1. A general procedure for Dpaa removal with formic acid is provided in ESI. As expected, valerate-dpa derivative **10** (entry 2, Table 1) was not affected by either formic or acetic acid. However, the Dpaa group of compounds **9**, **14**, **15**, and **16** (entries 1, 3, 4, and 5, Table 1)

was cleaved in the presence of both acids. Under these conditions, neither the Boc nor *t*-butylester protecting groups (compounds **14** and **16**) were released (entries 3 and 5, Table 1). We also analysed the evolution of the reaction mixture by LC-MS at 30 min, and 1, 2, 4, 8, and 24 h of reaction for the hydrolysis of compounds **9**, **14**, and **15** in either v/v 30% formic acid in DCM or v/v 30% acetic acid in DCM (Figure S3 see ESI), and found that formic acid is more efficient than acetic acid for the cleavage on the same Dpaa substrate.

Table 1. Cleavage assays in acidic conditions on compounds **9**, **10**, **14**, **15** and **16**. Product isolated and yield.

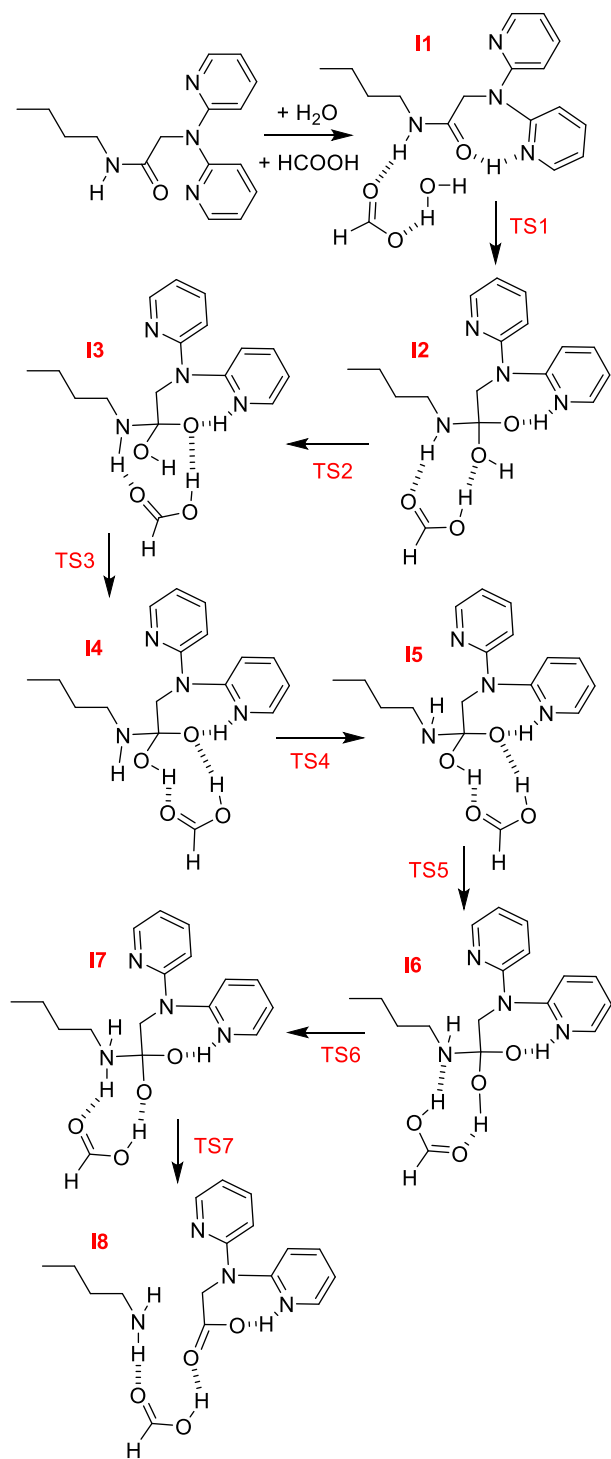
Entry	Starting material	Product, yield	
		HCOOH/CH ₂ Cl ₂ , 30%, 20°C, 24h	AcOH/CH ₂ Cl ₂ , 30%, 20°C, 24h
1	9	8 (95%)	8 (30%)
2	10	10 (92%)	10 (96%)
3	14	11 (91%)	11 (42%)
4	15	12 (100% ^a)	12 (28% ^a)
5	16	13 (93%)	13 (23%)

^a Based on LC/MS analysis due to the volatility of free amine **12** during the work-up (Figure S4 and S5 see ESI).

These data suggest the importance of the pKa and acid catalysis in the deprotection process. Moreover, the hydrolysis reactions observed for the Dpaa compounds also appeared to be related to the water content of the commercial organic acids. We thus treated compound **9** with 30% formic or acetic acid in DCM with 0%, 2%, or 10% water (biphasic medium) to evaluate the robustness of Dpaa in the presence of significant amounts of water. The presence of increasing quantities of water in the medium strongly potentiated the Dpaa removal promoted by acetic acid, whereas formic acid was more efficient, regardless of the proportion of water tested (Table S1, Figures S6 to S9 see ESI). Moreover, the Boc and *t*-butyl ester moieties of compound **14** remained unaffected in the presence of 2% water in 30% acetic acid in DCM. In the presence of 2% water in 30% formic acid in DCM, only traces of compounds resulting from cleavage of the Boc and *t*-butyl ester groups of **14** were detected by LC-MS (Table S2 and Figures S10 and S11, see ESI). The effect of inorganic acid on Dpaa group was also assessed and treatment of **9** by a mixture of HCl_{aq} 0.02 M in DCM led only to a partial release of the amine **8** (**8** was isolated in 41% yield after 24 h). This acidic condition appeared therefore more efficient than acetic acid but less than formic acid in the Dpaa cleavage.

The lability of Dpaa under mildly acidic conditions was unexpected. We thus performed calculations to investigate the basis of this reactivity. The structure of **9** was simplified by cutting between the two carbons after the first oxygen of the PEG chain. This model possesses five basic poles, and the most stable protonation site is on one of the pyridines, which forms a H-bond with the amide carbonyl. We found no path without using a water molecule for the cleavage. The mechanism was established for

three different acids (TFA, formic and acetic acids) and a model of compounds **9** (**m9**), **10** (**m10**), **14** (**m14**), and **15** (**m15**) (Figure S12, see ESI). We present the general mechanism of the cleavage for the model of **m15** with formic acid and detail the differences for the other derivatives (Scheme 3, Figure 1).



Scheme 3. Mechanism of the acid promoted Dpaa hydrolysis for **m15**.

Initially, the water molecule and the deprotonated acid interact through a H-bond network to form the adduct **I1**. The first step is the attack of the water molecule (**TS1**) on the carbonyl carbon, associated with H⁺ transfer towards the formate to form **I2**. This is the rate limiting step, associated with a barrier of 30.1 kcal mol⁻¹.

For cleavage, a second proton must be present on the amide nitrogen. Then, **I2** undergoes two rearrangements of the H-bond

pattern (**TS2** and **TS3**). The associated barriers are very low (Table S3 see ESI). Once in **I4**, the configuration of the amide nitrogen is not suitable to allow H-transfer and an interconversion takes place through **TS4** to form the conformer **I5**. Then the hydrogen of formic acid makes a H-bond with the amide nitrogen to form **I6**. The proton is then transferred to the nitrogen atom (**I7**), followed by cleavage of the C-N bond (**I8**) (Scheme 3). The mechanism for **m14** is the same; the ester function of **m14** slightly increases the barrier. The computed mechanism for a cleavage of **m9** and **m10** is slightly different. The ether function just increases the barrier. The presence of the ether oxygen in the PEG chain generates a H-bond between the OH coming from the water molecule and the ether oxygen, adding several steps of H-bond network rearrangement. We identified two possible paths for **m9** and **m10**. In **m9b** the attack of the water molecule is assisted by the oxygen of the side chain. In **m9a** there are no assistance (Figure 1 and Figure S13 see ESI). The presence of the H-bond influences the barrier associated to **TS1**. The presence of the H-bond (in **m9b**, Figure S13 see ESI) stabilizes **TS1** and lower the energy by 3.5 kcal mol⁻¹ and of all subsequent structures (Table S4 see ESI) compared to the mechanism without H-bond (**m9a**).

In any case, the rate limiting step is **TS1**, the attack of the water molecule on the amide. The associated barrier varies from 26.7 (**m9**) to 32.1 kcal mol⁻¹ (**m14**). The presence of the PEG chain appears to slightly lower the barrier. The final dissociation product (Figure 1) is more stable than the initial adduct **I1** for all considered compounds, except for **m10**. The different behaviours are associated with the H-bond network. In the dissociation product, the acid generated by the cleavage of Dpaa forms an internal H-bond between the H of the acid and the nitrogen atom of one of the pyridines. This internal H-bond is stronger in “short” Dpaa (**m9**, **m14** and **m15**) than in “long” Dpaa (**m10**) by 2.0 kcal mol⁻¹, partially explaining the change in the thermodynamic balance of the reaction and the absence of cleavage for **m10**. From a kinetic point of view, the presence of the PEG chain stabilizes **TS1** by a H-bond with the water molecule, lowering the barrier in **m9**. This effect should also be present in **m14**, although in this case, **I1** is much less stable than for the other compound, counterbalancing the presence of the H-bond. Such destabilization of **I1** may be attributed to steric congestion induced by the presence of the ester function close to the targeted amide carbon atom.

We compared the effect of the nature of the acid on the first step of the reaction for **m9**. The first step depends on the formation of a H-bond between the protonated carbonyl of the amide function and one of the Dpaa pyridines. This is directly related to the pKa of the acid as shown in Table S5 (see ESI). The generated **I1** adduct is more stable with TFA than with formic acid or acetic acid. Then, the following **TS1** associated barrier varies similarly, the lowest being for TFA (fastest reaction) and the highest for acetic acid (no reaction).

The reaction with HCl slightly changes the mechanism, reducing the number of steps associated to the H-bond network rearrangement. Calculation shows that the carbonyl is not needed for the reaction. We retrieve the relationship between pKa and the attack of the water molecule. The rate determining step is not the attack of the water molecule but the transfer of the proton toward the nitrogen. The highest barrier (**I1**-**TS3**) is of 26.0 kcal/mol higher than that with formic acid (**m9a**, **I1**-**TS1**, 17.5 kcal/mol) (Figure S14, see ESI).

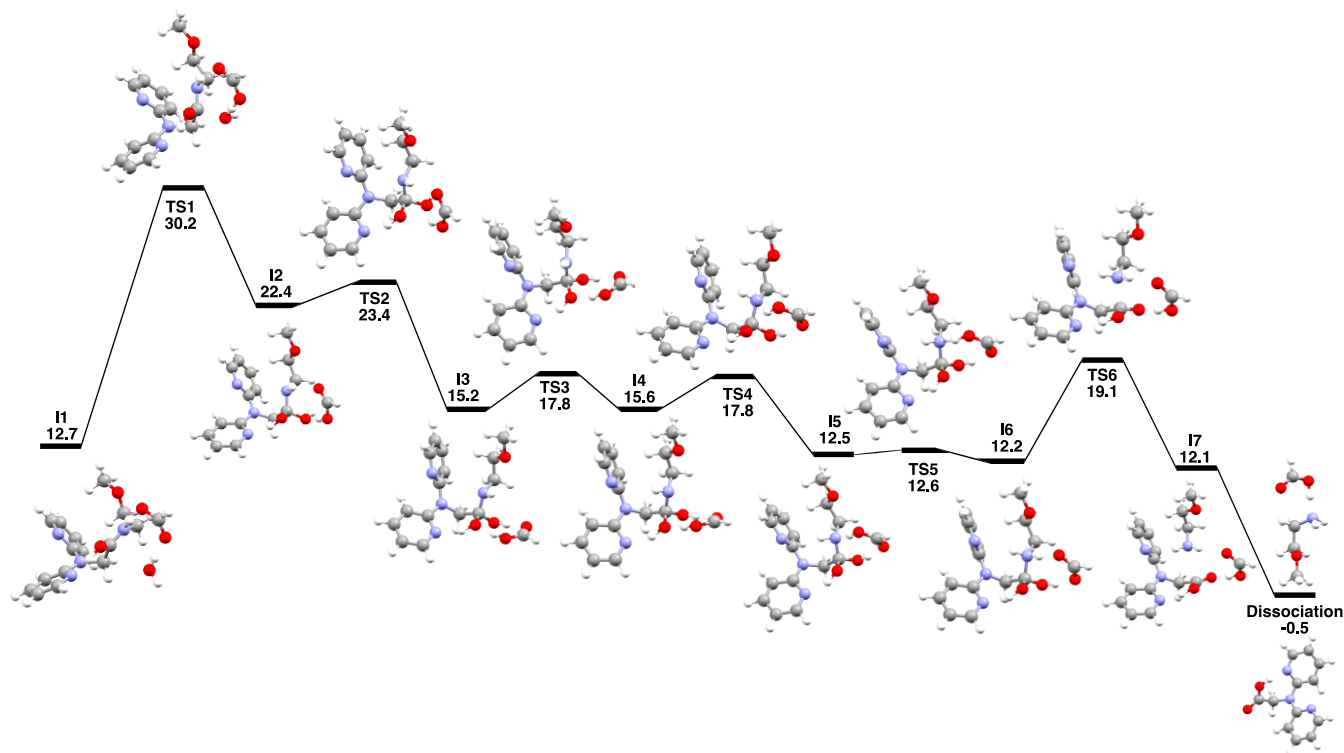


Figure 1. Mechanism for the cleavage of **m9a**. Energies are given relatively to the separated reactants (water molecule, formic acid and Dpaa) in kcal mol⁻¹.

In conclusion, we describe dipyriddyamine-acetamide (Dpaa), an organic group offering new alternatives for the protection of the primary amine function. This group is easy to install using classical peptide coupling conditions. Moreover, Dpaa is easy to remove using v/v 30% formic acid in dichloromethane, proving to also be orthogonal to other acid-labile protecting groups, namely Boc or *t*butylester. The orthogonality with other amine protecting groups is still under investigation, especially on secondary amines. Such deprotection by intramolecular acid-base catalysis promoted by a dipyriddyamine moiety is thus far unprecedented and could be an inspiration for other applications in organic and medicinal chemistry.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the present article.

Authors Contributions

ALF discovered the Dpaa reactivity. AF, FH, and NW performed the synthesis and spectral analysis of the molecules. GLAM conceived the project. CG performed calculations and modelling. GLAM and CG designed and wrote the manuscript. The manuscript was written through contributions of all authors. All authors have approved the final version of the manuscript.

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Appendix A. Supplementary Data

Synthetic protocols and spectral characterization for compounds **4**, **5**, **6**, **7**, **9**, **10**, **14**, **15** and **16**. ¹H, ¹³C and ¹⁹F NMR spectra of these compounds. Table and traces of LC-MS experiments of acid hydrolysis kinetic performed on compounds **9**, **10**, **14**, **15** and **16**. Figures and tables related to theoretical study.