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Synthesis and use of *N*-Fmoc-L-fluoroalanine

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ABSTRACT

We report a practical synthesis of *N*-Fmoc protected L-fluoroalanine **6** from L-serine. The key step involves a deoxofluorination reaction which was best achieved using XtalFluor-E in the presence of triethylamine trihydrofluoride. We also report the use of **6** in solid-phase peptide synthesis for the preparation of a model tripeptide demonstrating the possibility of incorporating a fluorinated probe in a peptide without elimination. Furthermore, cleavage of the dipeptide permitted to verify that **6** has a high enantiopurity.

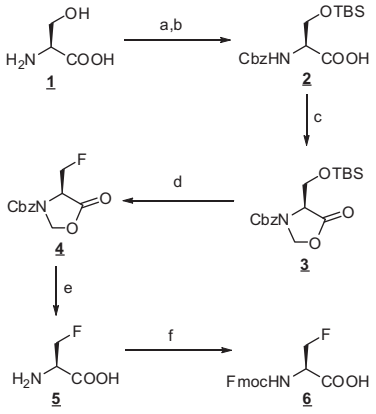
Introduction

Incorporation of fluorine atoms at different positions on bioactive molecules has been shown to improve significantly their properties, including potency.[1] This phenomenon has been thoroughly described and reviewed in the literature.[2–4] Likewise, due to the strong NMR signal of ^{19}F, introducing such atoms into peptides and proteins is a useful strategy that enables specific biophysical studies.[5–8] Particularly, solution and solid state NMR of fluorinated peptides and proteins can provide unique information on their interactions with bilayer membranes not possible to obtain otherwise. Hence, developing fluorinated amino acids as biomolecular probes is of great interest.

Toward that goal, elegant approaches have been reported.[9–14] Of special interest is the work of Hoveyda and Pineault who reported the synthesis of enantiopure L-fluoroalanine from L-serine.[15] They also reported the introduction of L-fluoroalanine in a dipeptide, though with modest yields and in solution phase synthesis. Therefore, there is a need to develop enantiopure monofluorinated alanine under a form that is compatible with solid-phase peptide synthesis.[16] Hereafter, we described such a fluorenylmethyloxycarbonyl (Fmoc) derivative and its use in the preparation of a model tripeptide by solid-phase peptide synthesis.

Synthesis of *N*-Fmoc-L-fluoroalanine 6

Based on literature precedents, we focus our synthetic efforts exploiting L-serine as starting material. Our synthetic strategy is illustrated in Scheme 1.



Scheme 1. Synthesis of Fmoc-fluoroalanine **6**. (a) CbzCl, pH 8–10, H_2O/acetone, 3 h; (b) TBSCl, DMAP, TEA, CH_2Cl_2; (c) *para*-formaldehyde, *p*-TsOH, toluene; (d) TEA-3HF, Xtalfluor-E, CH_2Cl_2, 24 h; (e) BCl_3, CH_2Cl_2, 25 °C, 30 min; (f) Fmoc-OSu, Na_2CO_3, dioxane/H_2O.

L-Serine was first protected efficiently to *N*-benzyloxycarbonyl-L-serine using benzylchloroformate in a water/acetone mixture (15/2). The side chain alcohol was then protected as a dimethyl-*t*-butylsilyl ether with the appropriate silyl chloride in dry dichloromethane to yield 51% of the desired protected compound **2** having a free carboxylate. The work-up has been investigated in order to optimize the yield. Compound **2** was then treated with paraformaldehyde and a catalytic amount of *p*-toluenesulfonic acid in toluene in a Dean Stark apparatus. This reaction produced efficiently oxazolidinone **3** in quantitative yield.

For the desilylation-deoxofluorination of **3**, several reagents and conditions were investigated. A combination of HF-pyridine

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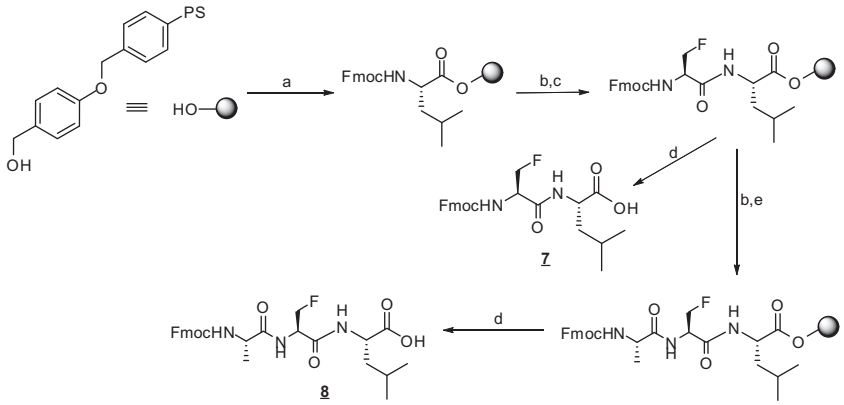

Scheme 2. Synthesis of a di- and a tripeptide using Fmoc-fluoroalanine. (a) Fmoc-Leu-OH, DIC, HOBt, DIEA DMAP, CH_2Cl_2; (b) piperidine/DMF 20%; (c) **6**, HATU, NMM, DMF; (d) TFA/H_2O 95%; (e) Fmoc-Ala-OH, HATU, NMM, DMF.

(Olah's reagent), Xtalfluor-E, and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dry dichloromethane did not yield the compound sought-after. No fluorination took place as evidenced by the absence of signal by ^{19}F NMR in the crude product of those reactions. As HF-pyridine requires notoriously complicated handling precautions, we tried substituting it for tetrabutylammonium fluoride (TBAF) as the deprotection agent and source of hydrogen fluoride. Using TBAF in tandem with Xtalfluor-E and DBU still did not yield any fluorinated compound. The deoxofluorination reagent was next changed for Deoxo-fluor with TBAF in dichloromethane, but still no trace of a fluorinated compound was found. The first success obtained was using a combination of 8 equiv of HF-pyridine, 2 equiv of triethylamine trihydrofluoride, 1.5 of Xtalfluor-E and **3** in dichloromethane, producing **4** though with a low yield. Substituting HF-pyridine for TBAF or removing completely the external source of HF from the reaction did not impact the resulting yield. The best combination of reagents was found to be 2 equiv of XtalFluor-E and 4 equiv of triethylamine trihydrofluoride in dry dichloromethane, which lead to the fluorinated oxazolidinone **4** in 59% yield. Noteworthy of mention, performing the deprotection and the deoxofluorination steps separately did not allow for the formation of the desired compound **4** under any condition tried.

Another important transformation was the deprotection and ring-opening steps to get **5**. Again, we investigated unsuccessfully different strategies, opening the ring first then deprotecting the Cbz group by catalytic hydrogenation. Ring-opening with 2 N HCl in dioxane, as proposed by Hoveyda et al., did give the linear compound with yields never exceeding 70%.[15] The following deprotection of the Cbz by catalytic hydrogenation yielded an unidentified fluorinated compound that could not be isolated. This strategy was abandoned as these two steps were best achieved with a one pot reaction using 1 M BCl_3 in DCM.[17] This quick 30 min reaction gave a white solid that was used as is for the final step of the synthesis.

The crude product was finally protected with Fmoc-OSu in a mixture of dioxane/water to yield the desired *N*-Fmoc-L-fluoroalanine **6**, which was fully characterized by 1D- and 2D-NMR spectroscopy and by mass spectrometry (see also Supporting information for salient spectroscopic data). The optical rotation of *N*-Fmoc-L-fluoroalanine **6** was determined to be −6.7 (c = 1.06, in methanol).

Solid-phase synthesis using 6

In order to demonstrate the utility of **6** for solid-phase peptide synthesis, we coupled it to a L-Leucine attached to Wang Resin (Scheme 2). The coupling was performed using 2 equiv of **6** and using HATU as a coupling reagent. The coupling lasted 1 h. An aliquot of the resin was then treated for 1 h with 95% TFA to cleave the dipeptide from the resin. ES-MS confirmed the preparation of the *N*-Fmoc-protected dipeptide and the purity was estimated to 60% by HPLC. Interestingly, NMR analyses were used to confirm the structure of dipeptide **7**. From those analyses, we could conclude that **6** has a high enantiopurity, although it is also possible that a kinetic resolution process leads to **7** with a high diastereomeric excess. On the other hand, as reported by others, elimination of HF leading to dehydroalanine was insignificant under the reaction conditions used.[15]

To further demonstrate the usefulness of **6**, the *N*-Fmoc-dipeptide **7** on the resin was deprotected using two 5 min treatments with 20% piperidine in DMF and the resulting free dipeptide was coupled with 2 equiv of *N*-Fmoc-L-Alanine using HATU as activating reagent. Since the ninhydrin test clearly showed an uncompleted coupling, the resin was mixed with 10 equiv of *N*-Fmoc-L-alanine and HATU for another 1 h. This second coupling resulted in a complete reaction step. This increased difficulty of carrying out the coupling can be explained by the lower nucleophilicity of the amino group of the L-fluoroalanine, which is in agreement with literature precedents.[11,18,19] Interestingly, it was not possible to observe the characteristic signals of dehydroalanine by NMR. This indicates that HF elimination leading to dehydroalanine, if any, occurred minimally during deprotection of and coupling to fluoroalanine. The obtention of tripeptide **8** demonstrates the possibility of using *N*-Fmoc-L-fluoroalanine **6** in solid-phase peptide synthesis.

Conclusion

We reported the first synthesis of *N*-Fmoc-L-fluoroalanine and its characterization. All steps have been optimized, especially the deoxofluorination, the one pot Cbz-group deprotection and ring-opening, and the Fmoc protection steps. Although HATU and a higher concentration of reagents were necessary for high yield coupling, *N*-Fmoc-L-fluoroalanine **6** has been coupled efficiently to a leucine linked to the Wang resin. Analysis of the dipeptide prepared demonstrated that the coupling proceeded with very low racemization and elimination. Addition of an alanine onto the dipeptide leads to the preparation of a tripeptide by solid-phase peptide synthesis on a classic Wang resin, demonstrating the usefulness of **6** for the preparation of fluorinated bioactive peptides.

Work is currently underway to prepare such bioactive peptides and to use them in biophysical studies with lipid membranes.

Acknowledgments

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Supplementary data

Supplementary data (these include ^{1}H, ^{13}C, ^{19}F, COSY, HRMS of compounds **6**, **7,** and **8** as well as complete characterization of all prepared compounds.) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.01.117.

References and notes

1. Tredwell, M.; Gouverneur, V. In *Comprehensive Chirality*; Carreira, M., Yamamoto, H., Eds.; Elsevier, 2012; Vol. 1, p 70.
2. Purser, S.; Moore, P. R.; Swallow, S.; Gouverneur, V. *Chem. Soc. Rev.* **2007**, *37*, 320–330.
3. Hagmann, W. K. *J. Med. Chem.* **2008**, *51*, 4359–4369.
4. Shah, P.; Westwell, A. D. *J. Enzyme Inhib. Med. Chem.* **2007**, *22*, 527–540.
5. Buer, B. C.; Chugh, J.; Al-Hashimi, H. M.; Marsh, E. N. G. *Biochemistry-US* **2010**, *49*, 5760–5765.
6. Grage, S. L.; Dürr, U. H. N.; Afonin, S.; Mikhailiuk, P. K.; Komarov, I. V.; Ulrich, A. S. *J. Magn. Reson.* **2008**, *191*, 16–23.
7. Marsh, E. N. G. *Acc. Chem. Res.* **2014**, *47*, 2878–2886.
8. Salwiczek, M.; Nyakatura, E. K.; Gerling, U. I. M.; Ye, S.; Koksch, B. *Chem. Soc. Rev.* **2012**, *41*, 2135–2171.
9. Biava, H.; Budisa, N. *Tetrahedron Lett.* **2013**, *54*, 3662–3665.
10. Bea, H.-S.; Lee, S.-H.; Yun, H. *Biotechnol. Bioprocess. E* **2011**, *16*, 291–296.
11. Chaume, G.; Simon, J.; Caupène, C.; Lensen, N.; Miclet, E.; Brigaud, T. *J. Org. Chem.* **2013**, *78*, 10144–10153.
12. Qui, X.-L.; Meng, W.-D.; Qing, F.-L. *Tetrahedron* **2004**, *60*, 6711–6745.
13. Sutherland, A.; Willis, C. L. *Nat. Prod. Rep.* **2000**, *17*, 621–631.
14. Simon, J.; Nguyen, T. T.; Chelain, E.; Lensen, N.; Pytkowicz, J.; Chaume, G.; Brigaud, T. *Tetrahedron: Asymmetry* **2011**, *22*, 309–314.
15. Hoveyda, H. R.; Pinault, J.-F. *Org. Lett.* **2006**, *8*, 5849–5852.
16. Typical procedure for **6**: Crude compound **5** (0.542 g) was dissolved in 20 mL of aqueous 10% Na_2CO_3 and the solution was heated to 40 °C. Afterwards, dioxane (10 mL) was added. Fmoc-OSu (1 equiv, 1.71 g, 5.07 mmol) was dissolved in dioxane (10 mL) and added dropwise to the reaction vessel. The resulting mixture was stirred for 48 h at 40 °C. Then, 150 mL of icy water was added and the organic phase was discarded. The milky white aqueous layer was washed with diethyl ether (2 times 10 mL) to revert to a transparent solution and then cooled down to 0 °C before acidification to pH 0–1 with 1N HCl. The milky mixture was extracted thrice with EtOAc (75 mL) and the aqueous phase was discarded. The organic phases were combined, washed with 1N HCl and then with water, dried with $MgSO_4$ and concentrated under reduced pressure. The product was then crystallized from a EtOAc–Hexanes mixture to yield, over two steps, 0.294 g (2.74 mmol, 54%) of a white solid. ^{1}H NMR (500 MHz; $CDCl_3$), δ = 4.24–4.27 (t, 1H, *J* = 7.0, Fmoc-CH), 4.41–4.48 (q, 2H, *J* = 8.0, Fmoc-CH-CH_2), 4.61–4.68 (overlapping dd, 1H, *J* = 25.4, 8.1, CHα), 4.68–4.77 (overlapping dd, 1H, *J* = 40.1, 8.1, CH_2β), 4.86–4.97 (dd, 1H, *J* = 46.1, 8.6, CH_2β), 5.68–5.70 (d, 1H, *J* = 8.1, NH), 7.32-7.35 (t, 2H, *J* = 7.4, $FmocH_b$), 7.40–7.43 (t, 2H, *J* = 7.4, $FmocH_c$), 7.60–7.61 (d, 1H, *J* = 5.8, $FmocH_a$), 7.77–7.79 (d, 1H, *J* = 7.5, $FmocH_d$) ^{13}C NMR (125 MHz; $CDCl_3$) δ = 47.0, 67.5, 82.4, 83.8, 120.0, 125.0, 127.1, 127.8, 141.3, 143.6, 156.0, 172.5. ^{19}F NMR (470 MHz; $CDCl_3$), δ = (−230.8)–(−230.4) (m, 1F, CH_2Fβ). HRMS (ESI-TOF, *m*/*z*): calcd for $C_{18}H_{17}FNO_4$ $(M+H)^+$ = 330.1142, found 330.1134, calcd for $C_{18}H_{20}FN_2O_4$ $(M+NH_4)^+$ = 347.1407, found 347.1391, calcd for $C_{18}H_{16}FNO_4Na$ $(M+Na)^+$ = 352.0961, found 352.0962. $[\alpha]_D^{20}$ −6.7 (c 1.06, MeOH).
17. Allevi, P.; Cribiù, R.; Anastasia, M. *Tetrahedron Lett.* **2004**, *45*, 5841–5843.
18. Cho, J.-I.; Zishizono, N.; Iwahashi, N.; Saigo, K.; Ishida, Y. *Tetrahedron* **2013**, *69*, 9252–9260.
19. Chaume, G.; Lensen, N.; Caupène, C.; Brigaud, T. *Eur. J. Org. Chem.* **2009**, *33*, 5717–5724.