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Practical and Straightforward Stereoselective Synthesis of (*S*)-5,5,5,5',5',5'-hexafluoroleucine

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Introduction

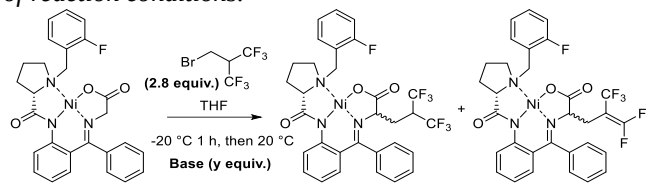
Fluorinated amino acids in drug design and protein science are currently the focus of intensive research [1,2]. The hydrophobic properties of fluorine combined with its small Van der Waals radius are interesting features for modulating lipophilicity of hydrophobic amino acids while retaining the morphology of the parent version. In addition, polyfluorinated versions of proteinogenic hydrophobic amino acids have proved particularly well suited to study the structure and function of proteins using ^{19}F NMR experiments [3]. In this respect, 5,5,5,5',5',5'-hexafluoroleucine which bears six fluorine atoms and displays a significantly higher hydrophobicity than the corresponding canonical version is a key fluorinated amino acid [4,5]. Only few synthetic pathways of this enantiopure fluorinated amino acid have been reported, and these syntheses involve the use of hexafluoroacetone or hexafluorothioacetone dimer [6-9]. In this study, we report a new route to access (*S*)-5,5,5,5',5',5'hexafluoroleucine using 2-(bromomethyl)-1,1,1,3,3,3-hexafluoropropane. The synthetic pathway is based on the homologation of chiral nickel complex, notably developed and exemplified by the group of Soloshonok [10-12] and more recently by the group of Koksch [13] for the synthesis of other α-amino acids with fluorinated side chains. The protocol enables the incorporation of the hexafluoroisobutyl side chain in one step through an original cascade reaction [14]. The method is highly practical and amenable to large scale synthesis.

Results and Discussion

Optimization of reaction conditions

We started by evaluating the alkylation reaction on chiral nickel complex **1** using NaOH powder in THF at -20 C (Table 1, entry 1). Unfortunately, the desired product **2** was formed with a very low yield (6%, entry 1) and the pentafluoroalkenylated compound **3** was obtained as the predominant product (55% yield). The structure of diastereoisomers (*S*,*S*)-**2** and (*S*,*S*)-**3** was confirmed by X-ray analysis (*Fig. 1*). Despite the successful alkylation of **1**, an elimination of fluoride is thus taking place at some point in the process. We tested other alkali metal bases, LiOH, KOH and tBuOK (entries 2-4), but compound **2** was still the minor compound (6-15%). We also tested alkali metal bases in DMF to see if a more polar solvent could favour the formation of the hexafluorinated compound (Table 2, entries 5-8). However, compound **2** was not observed with NaOH, LiOH and tBuOK. In contrast, CsOH tends to favour the formation of compound **2** (23% yield) and lower the yield of compound **3**. These observations indicate the crucial role of the fluoride counter ion by dictating the solubility of the salt. The fluoride released during the reaction (1 equiv.) is trapped by alkali metals (*i.e.* LiF, NaF, KF) to form insoluble fluoride salts precluding further reaction. With CsOH, the resulting fluoride salt is more soluble and the hydrofluorination of **3** is thus more favoured. To see whether the addition of a crown ether could capture the metal and preserve the nucleophilicity of the fluoride, we tested the combination of 15-crown-6 with NaOH (Table 1, entry 9). Surprisingly, this condition favoured the formation of the elimination product **3**, which was obtained in a yield higher than without the crown ether (61% versus 55% respectively). In these conditions, only trace amount of **2** could be detected in the crude NMR compare to 6 % yield obtained without crown ether. As the pentafluoroalkene moiety should be quite electrophilic due to the presence of five electron-withdrawing fluorine atoms, we tested whether the elimination could be reversed by using a source of fluoride as a base. To our delight, compound **2** was successfully isolated with a good 66% yield using a large quantity of TBAF (10 equiv., entry 10).

Table 1. Optimization of reaction conditions.



Entry	*Base (y)*	*Solvent*	*Reaction Time (h)*	*Yield*		
				1	***2***[a]	***3***[a]
1	NaOH (4)	THF	4	20 %	6 %	55 %
2	LiOH (4)	THF	4	40 %	15 %	38 %
3	KOH (4)	THF	4	32 %	6 %	44 %
4	tBuOK (4)	THF	4	20 %	13 %	47 %
5[b]	NaOH (4)	DMF	4	nd	-	23 %
6	LiOH (4)	DMF	4	-	-	53 %
7[c]	tBuOK (4)	DMF	5.5	nd	-	52 %
8	CsOH (4)	DMF	4	-	23 %	35 %
9[d]	NaOH (4)	THF	4	25 %	Trace	61 %
10	TBAF (10)	THF	3	-	66%	-

*[a] Compounds **2** and **3** were obtained as a mixture of diastereoisomers, the diastereoisomeric ratio ((S,S)-**2**:(S,R)-**2** and (S,S)-**3**:(S,R)-**3**) determined by ^{19}F NMR was between 88:12 and 93:7 for all tested conditions. [b] **1**/**2**/**3** ratio: 68:1:31, determined by ^{19}F NMR. [c] 1 equiv. of 2-(bromomethyl)-1,1,1,3,3,3-hexafluoropropane was added after 4.5 h of reaction at 20 °C; **1**/**2**/**3** ratio: 25:0:75, determined by ^{19}F NMR. [d] 5 equiv. of 15-crown-5 were added*

Mechanism insights

The reactivity of the fluorinated electrophile was studied in the presence of TBAF and the reaction was followed by ^{19}F NMR (Figure 2A). After few minutes, the brominated reagent undergoes an elimination of HBr to produce hexafluoroisobutylene (HFIB), and the latter one is relatively stable in the reaction medium beyond 3 h. Indeed, this reaction is favoured due to the presence of two CF_3 groups which extensively contribute to enhancing the acidity of the central C–H bond. Consequently, the alkylating reagent in the reaction is unlikely to be the bromo derivative but rather the alkene instead. Then, we performed *in situ* NMR experiments to monitor the reaction of **1** (Figure 2B).

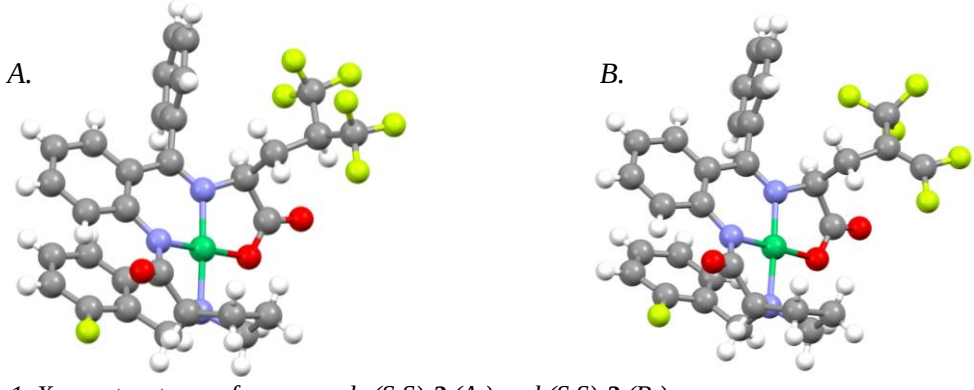


*Fig. 1. X-ray structures of compounds (S,S)-**2** (A.) and (S,S)-**3** (B.).*

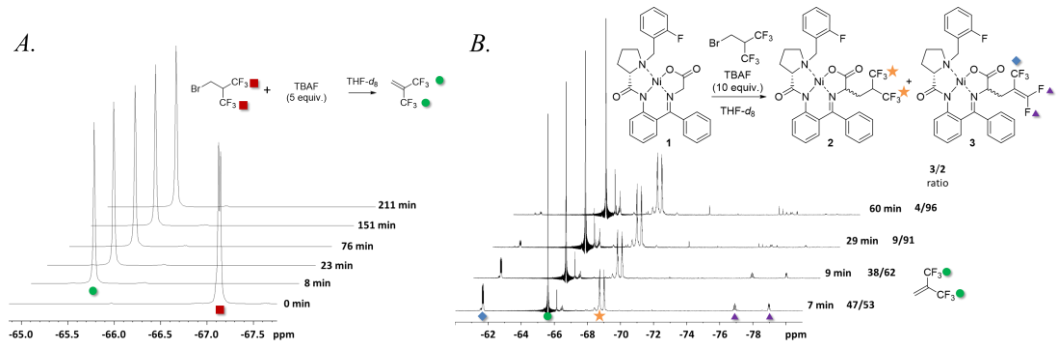


Fig. 2. In situ ^{19}F NMR experiments.

The formation of both compounds **2** and **3** was rapidly observed (after only 7 minutes). Then, the proportion of compound **3** started to decrease progressively over time with a concomitant increase in the intensity of the signal corresponding to compound **2**. These observations indicate that compound **3** is formed first and then converted into compound **2**, thus suggesting that TBAF promotes the addition of HF to the alkene.

The mechanism depicted on Figure 2 has been proposed (Figure 3). The brominated reagent rapidly undergoes an elimination of HBr under basic conditions to provide HFIB. In parallel, the deprotonation of the nickel complex leads to the formation of enolate, which then reacts with HFIB through an allylic shift (S_N2' mechanism). This provides the elimination product **3**. Then, the excess of fluoride ions efficiently reacts with **3** (hydrofluorination) to give compound **2**. In contrast, the use of alkali metal bases leads to insoluble fluoride salts and **3** is obtained predominantly.

Multi-gram scale synthesis

This methodology is compatible with a multi-gram scale procedure as shown in Figure 4. The two diastereoisomers (*S*,*S*)-**2** and (*S*,*R*)-**2** were successfully separated by flash chromatography affording pure (*S*,*S*)-**2** with a diastereomeric ratio > 99:1. The hydrolysis of the alkylated complex (*S*,*S*)-**2** afforded hexafluoroleucine (*S*)-**4** with an almost quantitative yield (Figure 4). The N-Fmoc or N-Boc protected derivatives (respectively (*S*)-**5** and (*S*)-**6**) can be obtained using FmocOSu or Boc_2O respectively, directly after hydrolysis of the alkylated nickel complex without intermediate purification. After the hydrolysis of complexes (*S*,*S*)-**2**, the chiral ligand derived from proline can be recovered quantitatively and reused to synthesise the Ni(II) complex (*S*)-**1**. Excellent enantiomeric ratios > 99:1 were found for (*S*)-**4** (Marfey's derivatization method).

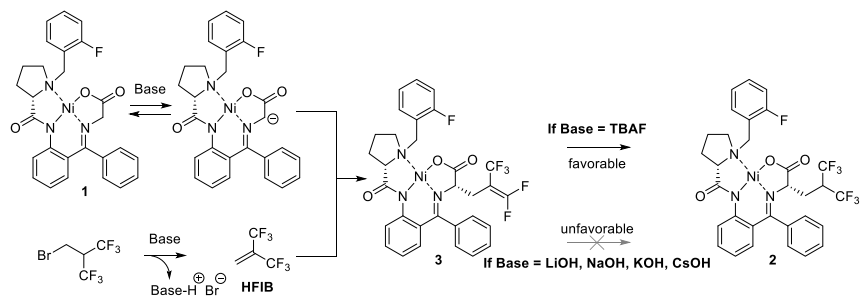


Fig. 3. Proposed mechanism.

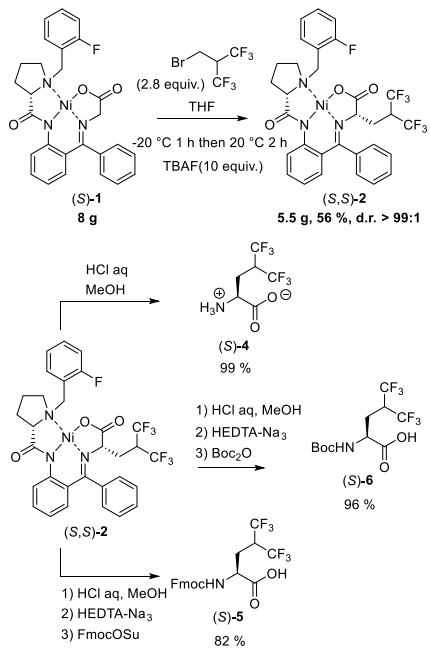


Fig. 4. Large-scale synthesis of (S)-5,5,5,5',5',5'-hexafluoroleucine and its N-Boc and N-Fmoc protected derivatives.

Conclusions

In summary, we report a hexafluoroisobutylation cascade reaction mediated by TBAF for the synthesis of enantiopure (*S*)-5,5,5,5',5',5'-hexafluoroleucine. The reaction is based on the nucleophilic attack on HFIB, rapidly formed under basic conditions from 2-(bromomethyl)-1,1,1,3,3,3-hexafluoropropane. HFIB reacts first with the deprotonated Schiff base complex through a S_N2' mechanism promoting a fluoride β-elimination affording a fluoroalkene group. Unfortunately, when using alkali metal bases, the reaction provides predominantly this undesired alkene. However, we found that the use of TBAF as a base allows the efficient and selective formation of the hexafluoro-isobutylated compounds by promoting the addition of HF to the alkene. *In situ* NMR data support the cascade elimination/allylic shift/hydrofluorination mechanism. This method is highly practical since the brominated reagent is liquid at room temperature, unlike HFIB. Hydrolysis of the nickel complex readily affords the (*S*)-fluorinated amino acid, as well as *N*-Boc and *N*-Fmoc-protected derivatives with high enantiopurity. The finding that the whole procedure is amenable to multi-gram-scale synthesis bodes well for a broader use of this polyfluorinated leucine analogue to engineer peptides and proteins for applications in medicinal chemistry and chemical biology.

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References

1. Mei, H., et al. *Eur. J. Med. Chem.* **186**, 111826 (2020), https://doi.org/10.1016/j.ejmech.2019.111826
2. Berger, A.A., et al. *Acc. Chem. Res.* **50**, 2093-2103 (2017), https://doi.org/10.1021/acs.accounts.7b00226
3. Miller, M.A., et al. *ChemBioChem* **21**, 3451-3462 (2020), https://doi.org/10.1002/cbic.202000297
4. Huhmann, S., et al. *Beilstein J. Org. Chem.* **13**, 2869-2882 (2017), https://doi.org/10.3762/bjoc.13.279
5. Marsh, E.N.G. *Acc. Chem. Res.* **47**, 2878-2886 (2014), https://doi.org/10.1021/ar500125m
6. Xing, X., et al. *Org. Lett.* **3**, 1285-1286 (2001), https://doi.org/10.1021/ol015567e
7. Anderson, J.T., et al. *Org. Lett.* **4**, 4281-4283 (2002), https://doi.org/10.1021/ol026922j
8. Chiu, H.-P., et al. *J. Am. Chem. Soc.* **128**, 15556-15557 (2006), https://doi.org/10.1021/ja0640445
9. Chiu, H.-P., et al. *Org. Lett.* **9**, 5517-5520 (2007), https://doi.org/10.1021/ol702470j
10. Wang, Y., et al. *Amino Acids* **49**, 1487-1520 (2017), https://doi.org/10.1007/s00726-017-2458-6
11. Aceña, J.L., et al. *J. Fluorine Chem.* **155**, 21-38 (2013), https://doi.org/10.1016/j.jfluchem.2013.06.004
12. Romoff, T.T., et al. *Org. Process Res. Dev.* **21**, 732-739 (2017), https://doi.org/10.1021/acs.oprd.7b00055
13. Hohmann, T., et al. *J. Org. Chem.* **87**, 10592-10604 (2022), https://doi.org/10.1021/acs.joc.2c00522
14. Delamare, A., et al. *Chem. Sci.* **13**, 9507-9514 (2022), https://doi.org/10.1039/D2SC02871A