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The Mitsunobu reaction in the synthesis of α,α-difluoro-β-amino acids

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Abstract

A three-stage strategy is proposed to prepare α,α-difluoro-β-amino acids starting from aldehydes and with ethyl bromodifluoroacetate as a fluorine source. The Mitsunobu reaction as a key step was studied by ^{31}P and ^{19}F NMR for alkyl- and aryl-substituted α,α-difluoro-β-hydroxyesters and performed under optimized conditions giving the target compounds.

Keywords: Ethyl bromodifluoroacetate; Aldehydes; The Mitsunobu reaction; α,α-Difluoro-β-amino acids; NMR spectroscopy

1. Introduction

An increasing interest in β-amino acids arises from their ability to exhibit interesting pharmacological effects and their utility as components of peptides with enhanced enzymatic stability compared with α-amino acids [1]. The replacement of hydrogen by fluorine in amino acids, modifying their physical, chemical and steric properties, causes improvement or alteration of their biological activity [2]. The present work was undertaken to investigate a new route to β-amino acids with a difluoromethylene group in the α-position.

The Mitsunobu reaction is known to be a useful tool for the replacing a hydroxyl group by different nucleophiles [3]. A number of nitrogen nucleophiles have been utilized in this procedure to afford amino derivatives [3–6]. Being interested in an efficient approach to biologically important α,α-difluoro-β-amino acids from easily accessible materials, we turned our attention to this method. We also take into account that the Mitsunobu reaction mostly occurs under mild conditions and is often employed in the synthesis and further transformations of various classes of natural products. The Mitsunobu methodology have been applied for amino acids synthesis [7,8], but there were no examples of β-amino acids among them.

2. Results and discussion

Herein, we describe the three-stage strategy, which allows an efficient preparation of α,α-difluoro-β-amino acids starting from aliphatic and aromatic aldehydes **1a–e** (Scheme 1). In order to obtain the desired esters of α,α-difluoro-β-hydroxy acids, the Reformatsky reaction of ethyl bromodifluoroacetate with aldehydes **1a–e** was accomplished affording the corresponding products **2a–e** in high preparative yields (81–92%) [9]. The key step of the present methodology is the conversion of hydroxyesters **2a–e** to nitrogen-containing derivatives **3a–e**.

Despite that the Mitsunobu reaction is widely used in synthetic organic chemistry, the mechanistic details of this reaction are still a subject of debates and intensive studies. This caused by the fact that depending on the order of addition of reactants and type of substrates, different reaction pathways may take place. In the current study several factors were examined in order to improve the overall efficiency of the method for our fluorine-containing substrates **2a–e**. The reaction was conducted using a standard redox system, i.e. diethyl azodicarboxylate (DEAD)/triphenylphosphine. Phthalimide was chosen as a most commonly used nucleophilic component for the amino function introduction through the Mitsunobu procedure [7,10]. ^{31}P and ^{1}H NMR spectroscopy is usually used for mechanistic studies of the Mitsunobu reaction; the presence of the fluorine atom in the initial hydroxy esters **2** allowed us to gain additional information from the ^{19}F NMR data.

Initially, we checked whether THF, almost always used as a solvent [7,11–13] in the Mitsunobu reaction, is relevant in our case for the amination step (Scheme 1), and compared the reaction yields with those of other solvents. In these experiments a 20% solution of DEAD **5** was added dropwise at 0°C to the mixture of the hydroxyester **2a**, Ph_3P **6**, and phthalimide **7** under argon, and the reaction was left to stir at room temperature. The results are summarised in Table 1.

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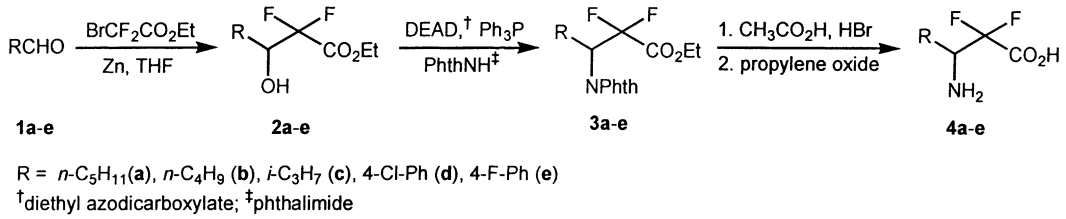


Scheme 1.

Table 1
Yields of the Mitsunobu reaction for hydroxyester **2a** in different solvents

Solvent	Yield[a] **3a** (%)	Unconverted starting material[a] **2a** (%)	Molar ratio, ROH:DEAD[b]:Ph_3P:PhthNH[b]
CH_2Cl_2	26	25	1:1:1:1
THF	66	30	1:1:1:1
Toluene	79	16	1:1:1:1

[a] Estimated from ^{19}F NMR data; measured after 20 h.
[b] DEAD: diethyl azodicarboxylate; PhthNH: phthalimide.

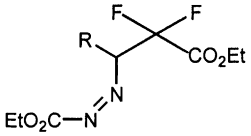
It is seen that toluene was the best solvent tested, and therefore it was employed in all further experiments.

Having a moderate conversion in all experiments, we decided to study the effect of stoichiometry and order of addition of reactants. The results of these experiments performed with hydroxyester **2a** are presented in Table 2.

It is apparent from Table 2 that method **D**, when reaction was carried out in the presence of excess of reagents, and when DEAD solution was added as a last component to the reaction mixture, is a most preparatively useful. The Mitsunobu reaction with hydroxyester **2a** was complete within an hour in that case, as monitored by ^{19}F NMR. When phthalimide **7** is added last (method **F**) the conversion is also found to be high, but yield of product **3a** is decreased by By-products.

Using the best addition order (method **D**) with the appropriate molar ratio of the reagents, we started with aromatic hydroxyester **2d** and obtained quite a low yield and low conversion to the desired product **3d**. After several experiments with different molar excess of the reagents (mixing at 0°C, Table 3), we found the optimal reaction conditions, and obtained the desired product **3d** in 32% yield. The same experiments performed at the temperatures −20 and +22°C were less successful.

3-(4-Chloro-phenyl)-2,2-difluoro-3-(ethoxycarbonylazo)-propanoic acid ethyl ester (**8d**), formed as a result of competitive DEAD *N*-alkylation, were found as one of the main By-products. It should be noted that carrying out this process with aliphatic hydroxyester **2a** by non-optimal methods **A**–**C**, **E**, **F** (Table 2) gave the corresponding product **8a** in yield up to 5% according to ^{19}F NMR. In order to understand the differences in the Mitsunobu reaction proceeding for alkyl- and aryl-substituted α,α-difluoro-β-hydroxyesters we examined these reactions by ^{31}P NMR and ^{19}F NMR.



8a: R = *n*-C_5H_{11}

8d: R = 4-Cl-Ph

The Mitsunobu reaction is generally envisaged as proceeding through oxyphosphonium salts and phosphoranes.

Table 2
Yields of the Mitsunobu reaction for hydroxyester **2a** depending on addition order and ratio of reactants [*n*-$C_5H_{11}CH(OH)CF_2CO_2Et$ (**2a**), DEAD (**5**), Ph_3P (**6**) and phthalimide (**7**)]

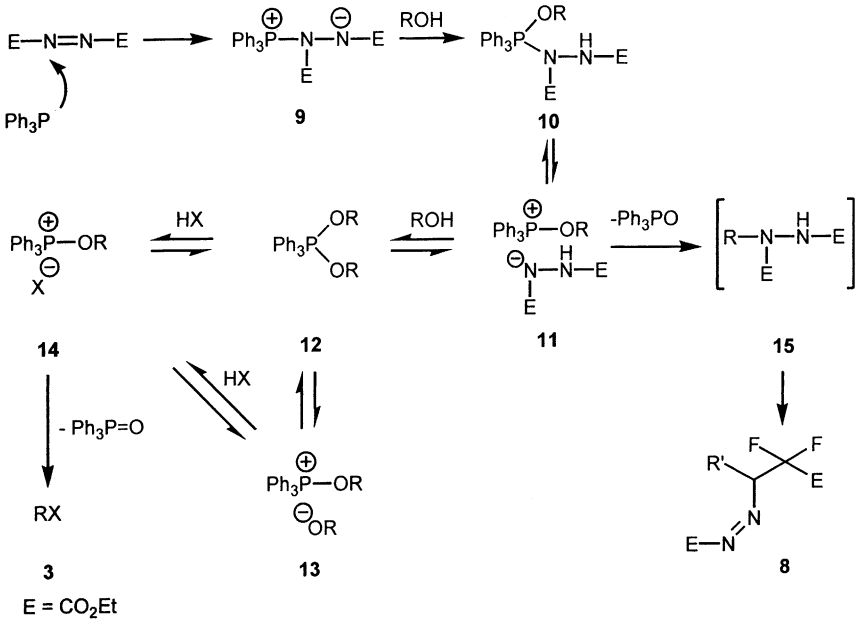
Method	Reactant addition order	Molar ratio **2a**:**5**:**6**:**7**	Yield[a] **3a** (%)	Unconverted[b] **2a** (%)
A	**2a**, **6**, **7** + **5**	1:1:1:1	79	16
B	**6** + **7** + **5** + **2a**	1:1:1:1	67	21
C	**6** + **2a** + **5** + **7**	1:1:1:1	74	11
D	**2a**, **6**, **7** + **5**	1:1.2:1.2:1.4	95	3
E	**6** + **7** + **5** + **2a**	1:1.2:1.2:1.4	77	15
F	**6** + **2a** + **5** + **7**	1:1.2:1.2:1.4	88	3

[a] Isolated yields after 20 h.
[b] Estimated from ^{19}F NMR data.

Table 3
Yields of the Mitsunobu reaction for hydroxyester **2d**

Experiment[a]	Molar excess of the reagents[b]	Yield[c] **3d** (%)	Unconverted[d] **2d** (%)
1	1.2	10	46
2	3	25	8
3	7	32	0

[a] For temperature and solvent see Section 4.12.
[b] Ph_3P, DEAD, PhthNH.
[c] Isolated yields after 20 h.
[d] Estimated from ^{19}F NMR data.



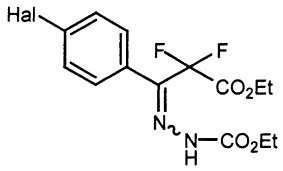
Scheme 2.

Scheme 2 shows that on adding the alcohol to the preformed betaine **9**, intermediate *O*,*N*-phosphoranes **10**, *O*,*O*-phosphoranes **12** and (or) corresponding alkoxyphosphonium hydrazide salts **11** and alkoxyphosphonium alkoxide salts **13** can be formed. Phosphoranes **10** have been postulated [14] to participate in the Mitsunobu reaction, although they have never been directly observed. Formation of dialkoxytriphenylphosphoranes **12** was suggested by Itzstein and Jenkins [15]. Walker and coworkers suggested that this compound was an intermediate in the special case when the acid was added last [11], but Hughes et al. was unable to observe any phosphoranes even in the absence of an acid [16]. Addition of the acidic component HX results in the transformation of these intermediates to alkoxyphosphonium salts **14** which can exist in equilibrium with dialkoxyphosphoranes **12** as has been previously reported [17]. As is evident from the literature, the possibility to detect by NMR any of intermediates mentioned above depends on the nature of the reagents, solvent polarity and the order of addition of the components. In some cases, when the Mitsunobu reaction was carried out with acidic components with low pK_a (>11), the alkylated hydrazine derivatives **15** were detected as the main By-products [18].

In our experiments with hydroxyesters **2a** and **2d** we changed the order of addition of the reagents from the optimal to detect possible intermediates. Treatment of Ph_3P (1.5 equivalent) with DEAD (1.5 equivalent) in toluene under nitrogen resulted in appearance of two peaks in the ^{31}P NMR spectrum. The major peak was located at +43.48 ppm and the minor at +24.77 ppm corresponding to the betaine **9** and triphenylphosphine oxide, respectively. Addition of the hydroxyester **2a** (1 equivalent) to this mixture caused the appearance of two new peaks at −59.37 and −60.11 ppm corresponding to the phosphoranes (**12**, **10**) with the betaine **9** peak being still present together with Ph_3PO peak. We attributed the signal at −59.37 ppm to compound **12** proceeding from the stoichiometry of the reaction and relative peaks intensities. As mentioned above, phosphorane **10** was never observed previously in contrast to compound **12** [15]. Addition of phthalimide (1.5 equivalent) as an acidic component resulted in the appearance of an alkoxyphosphonium salt **14** signal at +65.29 ppm [12,17] together with peaks of betaine **9** (minor), Ph_3PO (major) and dialkoxytriphenylphosphorane **12** (−59.37 ppm, minor).

The same experiment was performed for the aryl-substituted hydroxyester **2d**. The addition of compound **2d** (1 equivalent) to the preformed betaine (1.5 equivalent) gave a ^{31}P NMR spectrum with betaine peak, Ph_3PO peak and signals at −49.68 and −47.11 ppm corresponding to the phosphoranes **10** and **12**. After the addition of phthalimide, peaks of Ph_3PO (major), betaine (minor) and phosphoranes (minor) were detected in the ^{31}P NMR spectrum, similarly to the spectral data obtained for compound **2a**. Therefore, we can conclude that there were no qualitative differences in the ^{31}P NMR spectra for hydroxyesters **2a** and **2d** at the point before the addition of the acidic component (HX) to the reaction mixture. However, the relative intensities of the observed peaks were not identical, the ratio of **9**:Ph_3PO:**12**:**10** signals for compound **2a** was approximately 7:2:4:2 and 4:2:1.5:1 for compound **2d**. Thus, before the addition of phthalimide to the mixture of Ph_3P, DEAD and ROH much more betaine relative to Ph_3PO was observed in the case of aliphatic hydroxyester **2a** than for the aromatic one. According to the ^{19}F NMR spectra (measured in parallel with ^{31}P NMR), aliphatic hydroxyester **2a** generated intermediate phosphoranes at this stage, which were transformed to the product after adding the acidic component. Reaction of Ph_3P and DEAD with aromatic hydroxyester **2d** led to the formation of the DEAD

N-alkylation product **8d** and hydrazone **16d** together with the intermediate phosphoranes. Irreversible formation of compounds **8d** and **16d** caused lowering of intensities of the peaks of betaine and phosphoranes relative to the Ph_3PO signal in the ^{31}P NMR spectrum. In the ^{19}F NMR spectrum these By-products were detected together with the target compound **3d** after addition of phthalimide.

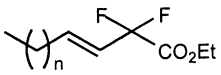


16d: Hal = Cl

16e: Hal = F

We also note that the equilibrium between phosphoranes **10**, **12** and alkoxyphosphonium salt **14** on adding phthalimide was shifted in favor of the salt **14** in the case of hydroxyester **2a** whereas for hydroxyester **2d**, phosphoranes **10** and **12** but not alkoxyphosphonium salt **14** were observed.

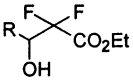
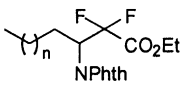
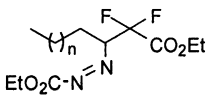
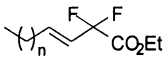
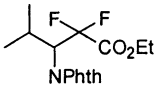
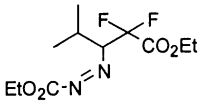
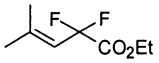
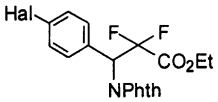
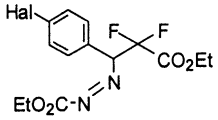
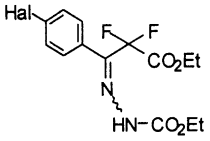
Whereas ^{31}P NMR was helpful in monitoring the process and in defining some of the phosphorus containing intermediates, ^{19}F NMR allowed us to analyze the components of the final reaction mixtures and therefore to compare the product compositions for alkyl- and aryl-substituted hydroxyesters. Following the Mitsunobu protocol for the conditions optimized for each type of the substrate, β-phthalimido derivatives were obtained starting from compounds **2a–e** (Table 4). The molar ratios of ROH:HX:Ph_3P:DEAD were 1:1.4:1.2:1.2 for the aliphatic hydroxyesters **2a–c** and 1:7:7:7 for the aromatic hydroxyesters **2d**,**e**. Some fluorine-containing By-products **8a–e**, **16d**,**e** and **17a–c** were separated by column chromatography and fully characterized. The alkylated hydrazine derivative **15**, which was described in the literature as the major By-product in the Mitsunobu reaction, was not detected in our experiments; compound **8** was found instead [18]. As was mentioned above, adducts **8a**,**b** as well as olefins **17a**,**b** were formed from hydroxyesters **2a**,**b** in the experiments under non-optimal conditions. Hydrazonoesters **16d**,**e** were detected as the main By-products from the reaction of aromatic hydroxyesters **2d**,**e**. It was noted above, that performing the Mitsunobu reaction with phthalimide being added last to the mixture of Ph_3P/DEAD/ROH, gave the hydrazone **16d** before the addition of the acidic component.



17a: n = 3

17b: n = 2

Table 4
Products of the Mitsunobu reaction for *β*-hydroxyesters **2a–e**

Hydroxyester,  **2**	Product **3** (yield, %)	By-product **8** (yield, %)	By-product **16** (yield, %)	By-product **17** (yield, %)
a: R = *n*-C_5H_{11}				
b: R = *n*-C_4H_9	**a**: n = 3; (95) **b**: n =2; (90)	**a**: n = 3; (< 2) **b**: n =2; (< 3)	–	**a**: n = 3; (<3) **b**: n =2; (<7)
c: R = *i*-C_3H_7	 (44)	 (15)		 (34)
d: R = 4–Cl–Ph				
e: R = 4–F–Ph	**d**: (32) **e**: (53)	**d**: (30) **e**: (32)	**d**: (37) **e**: (14)	–

As it is seen from the Table 4, decreasing yields of phthalimido adducts **3c–e** were found relative to adducts **3a**,**b**. Presumably this is due to the steric hindrance of hydroxyesters **2c–e** compared to compounds **2a**,**b**. The facilitation the process and therefore improvement in the yields of the desired products possibly lies in using the more acidic HX [19].

The final step of our protocol consists in the transformation of the N-protected esters **3** to free amino acids **4**. Hydrazinolysis of the phthalimido derivatives, which is traditionally accomplished [10] for their transformation to amines, did not lead to the desired amino acids. We removed the phthalimido moiety together with the ester function under the acidic conditions [20]. Phthalimidoesters were heated under reflux in a HBr/HOAc system and, after subsequent propylene oxide treatment, the desired α,α-difluoro-β-amino acids **4a**,**b**,**d** were obtained. The transformation of the N-protected esters **3** to free amino acids **4** was performed in 53–57% yields.

3. Conclusions

α,α-Difluoro-β-amino acids are now available in three steps from aldehydes and ethyl bromodifluoroacetate. The key Mitsunobu amination step was studied for α,α-difluorinated β-aryl- and β-alkyl-β-hydroxyesters. It was observed by ^{31}P NMR that phosphoranes of the type $Ph_3P(OR)_2$, $Ph_3P(OR)N(CO_2R')NHCO_2R'$ and oxyphosphonium salt $[Ph_3P(OR)]^+X^-$ were involved as intermediates. The reaction conditions were optimised for all substrates. Solvent was shown to play a significant role in reaction efficacy; the best yields were obtained in toluene solution. The correct sequence for adding the reagents was found to be important, the DEAD solution has to be added to the reaction mixture last in order to reduce or eliminate conversion and regioselectivity problems. For aromatic hydroxyesters a large excess of DEAD, Ph_3P and phthalimide should be used to achieve satisfactory preparative yields of desired products. The detailed ^{19}F NMR studies allow observation of azoesters **8** and hydrazonoesters **16** as new By-products in the Mitsunobu reaction.

4. Experimental details

4.1. General

^{1}H NMR (299.9 MHz), ^{13}C NMR (75 MHz) and ^{19}F NMR (282.2 MHz) spectra were recorded on a Varian VXR-300 spectrometer. ^{31}P NMR (36.2 MHz) spectra were recorded on a Jeol FX-90Q spectrometer. ^{19}F NMR spectra were recorded with external $CFCl_3$ and ^{1}H NMR with external tetramethylsilane as references. GC/MS were measured on Hewlett-Packard 5890/5870. IR spectra were obtained with a Specord IR-75 spectrometer in $CHCl_3$ solution. Melting points were determined in open capillaries and are uncorrected.

4.2. Materials

THF was purified by distillation from sodium ketyl. DEAD and deuterochloroform were dried by standing over molecular sieves. Benzene and toluene were dried by distillation from phosphorus pentoxide. Column chromatography was performed using silica gel 60 (0.040–0.063 mm) Merck, and for TLC silica gel 60 F_{254} Merck plates were used.

4.3. 2,2-Difluoro-3-hydroxy-octanoic acid ethyl ester (*2a*)

Obtained by procedure 4.5 from hexanal with yield 82%. Spectral data are identical to those in the literature [21].

4.4. 2,2-Difluoro-3-hydroxy-heptanoic acid ethyl ester (*2b*)

Obtained by procedure 4.5 from valeraldehyde with yield 79%. Spectral data are identical to those given in the literature [22].

4.5. 2,2-Difluoro-3-hydroxy-4-methylpentanoic acid ethyl ester (*2c*)

To a refluxing suspension of 1.9 g (25.6 mg atom) of freshly activated Zn dust in 22 ml of dry THF was added a solution of 5 g (24.6 mmol) of ethyl bromodifluoroacetate and 1.42 g (19.7 mmol) of isobutyraldehyde in 22 ml THF at a rate so as to maintain vigorous reflux. After 30 min the reaction mixture was cooled, quenched with saturated aq. $NaHCO_3$, 1 M aq. $KHSO_4$ and ether and left to stir for 15 min. After this time the layers were separated and the aqueous phase was extracted with ether. The combined organic layers were washed with brine, dried over $MgSO_4$, evaporated, and distilled to yield 2.51 g (65%) **2c** as a colourless liquid, bp 60–63°C/0.06 mm Hg. ^{1}H NMR (δ, $CDCl_3$): 1.04 (t, $J_{HH} = 6.6$ Hz, 6H); 1.36 (t, $J_{HH} = 7.2$ Hz, 3H); 1.93–2.1 (m, 1H); 2.77 (bs, 1H); 3.76–3.88 (m, 1H); 4.35 (q, $J_{HH} = 7.2$ Hz, 2H); ^{19}F NMR (δ, $CDCl_3$): −112.17, −121.36 (ABX, $J_{FF} = 266$ Hz, $J_{HF} = 17.5$, 7.6 Hz, CF_2). IR: $\nu = 1760$, 2840–3000 cm^{-1}. Anal. calc. for $C_8H_{14}F_2O_3$: C, 48.98; H, 7.19. Found: C, 49.09; H, 7.23%.

4.6. 3-(4-Chloro-phenyl)-2,2-difluoro-3-hydroxy-propionic acid ethyl ester (*2d*)

Obtained by procedure 4.5 from 4-chlorobenzaldehyde with yield 93%. Spectral data are identical to those in the literature [22].

4.7. 3-(4-Fluoro-phenyl)-2,2-difluoro-3-hydroxy-propionic acid ethyl ester (*2e*)

Obtained by procedure 4.5 with yield 87% as a colourless liquid, bp 122–126°C/0.06 mm Hg. ^{1}H NMR (δ, $CDCl_3$):

1.29 (t, J_{HH} = 7.2 Hz, 3H); 3.31 (bs, 1H); 4.3 (q, J_{HH} = 7.2 Hz, 2H); 5.15 (dd, J_{HF} = 15.3, 7.5 Hz, 1H); 7.0–7.1 (m, 2H); 7.3–7.4 (m, 2H); ^{19}F NMR (δ, $CDCl_3$): −121.2, −114.14 (ABX, J_{FF} = 264 Hz, J_{HF} = 15.3, 7.5 Hz, CF_2); −112.8 ... −112.6 (m, CF). IR: ν = 1605, 1760, 2950–3000 cm^{-1}. Anal. calc. for $C_{11}H_{11}F_3O_3$: C, 53.23; H, 4.47. Found: C, 53.39; H, 4.52%.

4.8. General procedure for β-alkyl-N-protected esters **3a**, **3b**, **3c** preparation by the Mitsunobu reaction

The solution of 0.19 g (1.07 mmol) of DEAD in 2 ml of dry toluene was added dropwise to a stirred and cooled (0°C) mixture of 0.89 mmol of hydroxyester **2**, 0.18 g (1.25 mmol) of phthalimide and 0.28 g (1.07 mmol) of triphenylphosphine in 3.2 ml of toluene under an argon atmosphere. The reaction mixture was allowed to warm to room temperature and after 1 h the solvent was partially evaporated and the crude mixture was chromatographed on a silica gel (eluent benzene).

4.9. 3-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-2,2-difluoro-octanoic acid ethyl ester (**3a**)

Obtained by procedure 4.8 with yield 0.29 g (95%) as a colourless oil. ^{1}H NMR (δ, $CDCl_3$): 0.84 (t, J_{HH} = 7.5 Hz, 3H); 1.17–1.4 (m, 9H); 1.8–1.93 (m, 1H); 2.53–2.7 (m, 1H); 4.22–4.39 (m, 2H); 4.7–4.86 (m, 1H); 7.72–7.8 (m, 2H); 7.84–7.91 (m, 2H); ^{19}F NMR (δ, $CDCl_3$): −111.8 ... −109.65 (m, CF_2). IR: ν = 1750, 2840–3005 cm^{-1}. Anal. calc. for $C_{18}H_{21}F_2NO_4$: C, 61.18; H, 5.99; N, 3.96. Found: C, 61.28; H, 6.05; N, 3.90%.

4.10. 3-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-2,2-difluoro-heptanoic acid ethyl ester (**3b**)

Obtained by procedure 4.8 with yield 0.27 g (90%) as a colourless oil. ^{1}H NMR (δ, $CDCl_3$): 0.82 (t, J_{HH} = 7.5 Hz, 3H); 1.14–1.4 (m, 7H); 1.7–1.83 (m, 1H); 2.5–2.67 (m, 1H); 4.2–4.37 (m, 2H); 4.69–4.85 (m, 1H); 7.71–7.79 (m, 2H); 7.84–7.92 (m, 2H); ^{19}F NMR (δ, $CDCl_3$): −111.78 ... −109.64 (m, CF_2). IR: ν = 1760, 2840–3000 cm^{-1}. Anal. calc. for $C_{17}H_{19}F_2NO_4$: C, 60.17; H, 5.64; N, 4.13. Found: C, 60.13; H, 5.60; N, 4.05%.

4.11. 3-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-2,2-difluoro-4-methyl-pentanoic acid ethyl ester (**3c**)

Obtained by procedure 4.8 with yield 0.09 g (44%) as a colourless oil. ^{1}H NMR (δ, $CDCl_3$): 0.87 (d, J_{HH} = 6.3 Hz, 3H); 1.2 (d, J_{HH} = 6.3 Hz, 3H); 1.25 (t, J_{HH} = 7.2 Hz, 3H); 2.9–3.17 (m, 1H); 4.16–4.38 (m, 2H); 4.43–4.57 (m, 1H); 7.71–7.81 (m, 2H); 7.83–7.93 (m, 2H); ^{19}F NMR (δ, $CDCl_3$): −106.36, −104.66 (ABX, J_{FF} = 260 Hz, J_{HF} = 15, 10.4 Hz, 2F); IR: ν = 1760, 2850–3000 cm^{-1}. Anal. calc. for $C_{16}H_{17}F_2NO_4$: C, 59.07; H, 5.27; N, 4.31. Found: C, 59.21; H, 5.20; N, 4.25%.

4.12. General procedure for β-aryl-N-protected esters **3d**, **3e** preparation under the Mitsunobu reaction

The solution of 0.92 g (5.31 mmol) of DEAD in 6 ml of dry toluene was added dropwise to a stirred and cooled (0°C) mixture of 0.75 mmol of hydroxyester **2**, 0.78 g (5.31 mmol) of phthalimide and 1.39 g (5.31 mmol) of triphenylphosphine in 15 ml of toluene under an argon atmosphere. The reaction mixture was allowed to warm to room temperature and after 20 h the solvent was partially evaporated and the crude mixture was chromatographed on silica gel (eluent benzene).

4.13. 2-Chloro-3-(4-chloro-phenyl)-3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-2-fluoro-propionic acid ethyl ester (**3d**)

Obtained by procedure 4.12 with yield 0.09 g (32%) as a colourless oil. ^{1}H NMR (δ, $CDCl_3$): 1.18 (t, J_{HH} = 7.2 Hz, 3H); 4.17–4.31 (m, 2H); 6.01 (dd, J_{HF} = 18.0, 13.5 Hz, 1H); 7.34 (d, J_{HH} = 8.5 Hz, 2H); 7.64 (d, J_{HH} = 8.5 Hz, 2H); 7.73–7.79 (m, 2H); 7.83–7.9 (m, 2H); ^{19}F NMR (δ, $CDCl_3$): −109.02, −105.22 (ABX, J_{FF} = 261.0 Hz, J_{HF} = 18.0, 13.5 Hz, CF_2). IR: ν = 1605, 1760, 2950–3000 cm^{-1}. Anal. calc. for $C_{19}H_{14}ClF_2NO_4$: C, 57.95; H, 3.58; N, 3.56. Found: C, 58.12; H, 3.62; N, 3.60%.

4.14. 3-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-2,2-difluoro-3-(4-fluoro-phenyl)-propionic acid ethyl ester (**3e**)

Obtained by procedure 4.12 with yield 0.15 g (53%) as a colourless oil. ^{1}H NMR (δ, $CDCl_3$): 1.16 (t, J_{HH} = 7.2 Hz, 3H); 4.16–4.3 (m, 2H); 6.03 (dd, J_{HF} = 18.0, 13.5 Hz, 1H); 7.01–7.09 (m, 2H); 7.67–7.72 (m, 2H); 7.73–7.78 (m, 2H); 7.84–7.89 (m, 2H); ^{19}F NMR (δ, $CDCl_3$): −112.12 ... −111.92 (m, CF); −109.34, −105.24 (ABX, J_{FF} = 260.0 Hz, J_{HF} = 18.0, 13.5 Hz, CF_2). IR: ν = 1600, 1760, 2950–3000 cm^{-1}. Anal. calc. for $C_{19}H_{14}F_3NO_4$: C, 60.48; H, 3.74; N, 3.71. Found: C, 60.56; H, 3.76; N, 3.66%.

4.15. General procedure for preparation of amino acids **4**

A mixture of 6 ml of 48% hydrobromic acid, 6 ml of glacial acetic acid and 6.34 mmol of ester **3** was heated under reflux for 15 h. On cooling most of the phthalic acid crystallized. After filtration, the filtrate and water washes were concentrated under reduced pressure practically to dryness. The residue was dissolved in 6 ml of water, filtered and concentrated. Diluting with water and subsequent concentration were repeated several times. To the crude amino acid hydrobromide, dissolved in methanol, 12.7 mmol of propylene oxide was added in one portion. The reaction mixture was evaporated to dryness and crystallized.

4.16. 3-Amino-2,2-difluoro-octanoic acid (**4a**)

Crystallized from 2-propanol/methanol (2/1). Yield 0.7 g (57%), mp 232–236°C. ^{1}H NMR (δ, CD_3OD): 0.92 (t, $J_{HH} = 7.5$ Hz, 3H); 1.27–1.88 (m, 8H); 3.61–3.75 (m, 1H); ^{19}F NMR (δ, CD_3OD): −127.6, −122.6 (ABX, $J_{FF} = 259$ Hz, $J_{HF} = 14.4$, 10.7 Hz, CF_2). Anal. calc. for $C_8H_{15}F_2NO_2$: C, 49.22; H, 7.75; N, 7.18. Found: C, 49.15; H, 7.79; N, 7.15%.

4.17. 3-Amino-2,2-difluoro-heptanoic acid (**4b**)

Crystallized from 2-propanol/methanol (2/1). Yield 0.65 g (57%), mp = 219–221°C. ^{1}H NMR (δ, CD_3OD): 0.94 (t, $J_{HH} = 7.5$ Hz, 3H); 1.3–1.9 (m, 6H); 3.63–3.77 (m, 1H); ^{19}F NMR (δ, CD_3OD): −127.5, −122.5 (ABX, $J_{FF} = 259$ Hz, $J_{HF} = 14.4$, 10.7 Hz, CF_2). Anal. calc. for $C_7H_{13}F_2NO_2$: C, 46.40; H, 7.23; N, 7.73. Found: C, 46.33; H, 7.26; N, 7.66%.

4.18. 3-Amino-2,2-difluoro-3-(4-chloro-phenyl)-propionic acid (**4d**)

Crystallized from methanol. Yield 0.79 g (53%), mp = 222–225°C. ^{1}H NMR (δ, CD_3OD): 5.1 (dd, $J_{HF} = 16.2$, 8.4 Hz, 1H); 7.46–7.56 (m, 4H); ^{19}F NMR (δ, CD_3OD): −114.97, −107.84 (ABX, $J_{FF} = 259$ Hz, $J_{HF} = 16.2$, 8.4 Hz, CF_2). Anal. calc. for $C_9H_8ClF_2NO_2$: C, 45.88; H, 3.42; N, 5.94. Found: C, 45.75; H, 3.46; N, 5.87%.

4.19. 2,2-difluoro-3-(ethoxycarbonylazo)-octanoic acid ethyl ester (**8a**)

Obtained by procedure 4.8. Isolated after column chromatography (eluent benzene) with yield 0.005 g (2%) as a colourless oil. ^{1}H NMR (δ, $CDCl_3$): 0.89 (t, $J_{HH} = 7.5$ Hz, 3H); 1.25–1.48 (m, 12H); 1.69–1.79 (m, 2H); 4.23 (q, $J_{HH} = 7.2$ Hz, 2H); 4.28–4.39 (m, 2H); 5.13–5.27 (m, 1H); ^{19}F NMR (δ, $CDCl_3$): −118.84, −114.95 (ABX, $J_{FF} = 266$ Hz, $J_{HF} = 12.7$, 8.7 Hz, CF_2); ^{13}C NMR (δ, $CDCl_3$): 13.83 (CH_3), 13.87 (CH_3), 14.09 (CH_3), 22.29 (CH_2), 24.38 (CH_2), 27.30 (CH_2), 31.25 (CH_2), 63.20 (CH_2), 64.79 (CH_2), 75.11 (dd, $J_1 = 29.4$ Hz, $J_2 = 34.4$ Hz, CH), 113.02 (dd, $J_1 = 298.5$ Hz, $J_2 = 309.8$ Hz, CF_2), 154.33 (CO), 162.59 (dd, $J_1 = 33.7$ Hz, $J_2 = 38.5$ Hz, CO). IR: $\nu = 1760$, 1780, 2840–3000 cm^{-1}. Anal. calc. for $C_{13}H_{22}F_2N_2O_4$: C, 50.64; H, 7.19; N, 9.09. Found: C, 50.75; H, 7.17; N, 9.13%; MS *m/z* (%): 287 (1), 263 (1), 244 (3), 223 (2), 195 (6), 186 (9), 173 (14), 164 (4), 152 (10), 144 (11), 137 (4), 124 (39), 116 (14), 113 (18), 101 (100), 91 (28), 83 (44), 69 (5), 63 (9), 55 (16).

4.20. 2,2-Difluoro-3-(ethoxycarbonylazo)-heptanoic acid ethyl ester (**8b**)

Obtained by procedure 4.8. Isolated after column chromatography (eluent benzene) with yield 0.008 g (3%) as a colourless oil. ^{1}H NMR (δ, $CDCl_3$): 0.89 (t, $J_{HH} = 7.5$ Hz, 3H); 1.25–1.47 (m, 10H); 1.7–1.79 (m, 2H); 4.23 (q, $J_{HH} = 7.2$ Hz, 2H); 4.28–4.39 (m, 2H); 5.13–5.27 (m, 1H); ^{19}F NMR (δ, $CDCl_3$): −118.85, −114.96 (ABX, $J_{FF} = 266$ Hz, $J_{HF} = 12.7$, 8.7 Hz, CF_2). IR: $\nu = 1760$, 1780, 2840–3000 cm^{-1}. Anal. calc. for $C_{12}H_{20}F_2N_2O_4$: C, 48.97; H, 6.85; N, 9.52. Found: C, 48.92; H, 6.88; N, 9.45%.

4.21. 2,2-Difluoro-3-(ethoxycarbonylazo)-3-methylpentanoic acid ethyl ester (**8c**)

Obtained by procedure 4.8. Isolated after column chromatography (eluent benzene) with yield 0.04 g (15%) as a colourless oil. ^{1}H NMR (δ, $CDCl_3$): 1.03 (d, $J_{HH} = 6.9$ Hz, 3H); 1.05 (d, $J_{HH} = 7.2$ Hz, 3H); 1.32 (t, $J_{HH} = 7.2$ Hz, 3H); 1.35 (t, $J_{HH} = 7.2$ Hz, 3H); 2.12–2.28 (m, 1H); 4.23 (q, $J_{HH} = 7.2$ Hz, 2H); 4.33 (q, $J_{HH} = 7.2$ Hz, 2H); 5.05 (ddd, $J_{HF} = 15.2$, 9.6 Hz, $J_{HH} = 5.4$ Hz, 1H); ^{19}F NMR (δ, $CDCl_3$): −112.49, −116.35 (ABX, $J_{FF} = 264$ Hz, $J_{HF} = 15.2$, 9.6 Hz, CF_2). IR: $\nu = 1760$, 1780, 2850–3000 cm^{-1}. Anal. calc. for $C_{11}H_{18}F_2N_2O_4$: C, 47.14; H, 6.47; N, 10.00. Found: C, 47.25; H, 6.45; N, 9.95%; MS *m/z* (%): 223 (1), 176 (2), 167 (3), 158 (7), 145 (18), 134 (8), 124 (32), 113 (4), 105 (34), 96 (32), 91 (43), 77 (8), 73 (100), 63 (12), 55 (11), 47 (1).

4.22. 3-(4-Chloro-phenyl)-2,2-difluoro-3-(ethoxycarbonylazo)-propanoic acid ethyl ester (**8d**)

Obtained by procedure 4.12. Isolated after column chromatography (eluent benzene) with yield 0.08 g (30%) as a yellow oil. ^{1}H NMR (δ, $CDCl_3$): 1.3 (t, $J_{HH} = 7.2$ Hz, 3H); 1.31 (t, $J_{HH} = 7.2$ Hz, 3H); 4.15–4.26 (m, 2H); 4.27–4.39 (m, 2H); 6.06 (dd, $J_{HF} = 15.0$, 8.7 Hz, 1H); 7.3–7.46 (m, 4H); ^{19}F NMR (δ, $CDCl_3$): −118.22, −113.87 (ABX, $J_{FF} = 268$ Hz, $J_{HF} = 15.0$, 8.7 Hz, CF_2); ^{13}C NMR (δ, $CDCl_3$): 13.81 (CH_3), 14.06 (CH_3), 63.41 (CH_2), 65.18 (CH_2), 75.87 (dd, $J_1 = 28.3$ Hz, $J_2 = 36$ Hz, CH), 112.14 (dd, $J_1 = 306$ Hz, $J_2 = 313$ Hz, CF_2), 128.85 (CH), 129.54 (d, $J_1 = 2.4$ Hz, C), 129.74 (CH), 135.99 (CCl), 153.42 (CO), 162.16 (dd, $J_1 = 35.6$ Hz, $J_2 = 38.6$ Hz, CO). IR: $\nu = 1605$, 1760, 1780, 2950–3000 cm^{-1}. Anal. calc. for $C_{14}H_{15}ClF_2N_2O_4$: C, 48.22; H, 4.34; N, 8.03. Found: C, 48.36; H, 4.36; N, 8.07%; MS *m/z* (%): 336 (1), 316(1), 263 (2), 246 (7), 244 (23), 216 (7), 198 (4), 174 (23), 141 (100), 113 (17), 96 (10), 77 (18), 63 (2), 51 (3).

4.23. 2,2-Difluoro-3-(ethoxycarbonylazo)-3-(4-fluoro-phenyl)-propanoic acid ethyl ester (**8e**)

Obtained by procedure 4.12. Isolated after column chromatography (eluent benzene) with yield 0.08 g (32%) as a colourless oil. ^{1}H NMR (δ, $CDCl_3$): 1.3 (t, $J_{HH} = 7.2$ Hz, 6H); 4.14–4.26 (m, 2H); 4.27–4.37 (m, 2H); 6.07 (dd, $J_{HF} = 15.0$, 9.0 Hz, 1H); 7.06–7.13 (m, 2H); 7.43–7.49 (m, 2H); ^{19}F NMR (δ, $CDCl_3$): −118.34, −114.51 (ABX,

$J_{FF} = 264$ Hz, $J_{HF} = 15.0$, 9.0 Hz, CF_2); −111.93 ... −111.73 (m, CF). IR: $\nu = 1605$, 1760, 1780, 2850–3000 cm^{-1}. Anal. calc. for $C_{14}H_{15}F_3N_2O_4$: C, 50.61; H, 4.55; N, 8.43. Found: C, 50.49; H, 4.52; N, 8.39%; MS *m/z* (%): 320 (0.5), 300 (0.5), 256 (1), 247 (1), 228 (15), 200 (5), 197 (6), 182 (4), 158 (19), 137 (2), 125 (100), 107 (5), 97 (20), 75 (2), 57 (1), 51 (1).

*4.24. 3-(4-Chloro-phenyl)-3-(ethoxycarbonyl-hydrazono)-2,2-difluoro-propionic acid ethyl ester (**16d**)*

Obtained by procedure 4.12. Isolated after column chromatography (eluent benzene) with yield 0.1 g (37%) as a colourless oil. ^{1}H NMR (δ, $CDCl_3$): 1.28 (t, $J_{HH} = 7.2$ Hz, 3H); 1.42 (t, $J_{HH} = 7.2$ Hz, 3H); 4.23 (q, $J_{HH} = 7.2$ Hz, 2H); 4.45 (q, $J_{HH} = 7.2$ Hz, 2H); 7.33 (d, $J_{HH} = 8.4$ Hz, 2H); 7.54 (d, $J_{HH} = 8.4$ Hz, 2H); ^{19}F NMR (δ, $CDCl_3$): −102.94 (s, CF_2)). IR: $\nu = 1600$, 1740, 1770, 2950–3000 cm^{-1}. Anal. calc. for $C_{14}H_{15}ClF_2N_2O_4$: C, 48.22; H, 4.34; N, 8.03. Found: C, 48.41; H, 4.38; N, 8.09%.

*4.25. 3-(Ethoxycarbonyl-hydrazono)-2,2-difluoro-3-(4-fluoro-phenyl)-propionic acid ethyl ester (**16e**)*

Obtained by procedure 4.12. Isolated after column chromatography (eluent benzene) with yield 0.04 g (14%) as a colourless oil. ^{1}H NMR (δ, $CDCl_3$): 1.28 (t, $J_{HH} = 7.2$ Hz, 3H); 1.42 (t, $J_{HH} = 7.2$ Hz, 3H); 4.24 (q, $J_{HH} = 7.2$ Hz, 2H); 4.44 (q, $J_{HH} = 7.2$ Hz, 2H); 7.02–7.12 (m, 2H); 7.4–7.5 (m, 2H); ^{19}F NMR (δ, $CDCl_3$): −108.65, (s, CF); −103.39 (s, CF_2)). IR: $\nu = 1600$, 1740, 1770, 2950–3000 cm^{-1}. Anal. calc. for $C_{14}H_{15}F_3N_2O_4$: C, 50.61; H, 4.55; N, 8.43. Found: C, 50.76; H, 4.59; N, 8.50%.

*4.26. 2,2-Difluoro-oct-3-enoic acid ethyl ester (**17a**)*

Obtained by the Mitsunobu procedure for hydroxyester **2a**. Isolated after column chromatography (eluent benzene) with yield 0.006 g (3%) as a colourless oil. ^{1}H NMR (δ, $CDCl_3$): 0.9 (t, $J_{HH} = 7.5$ Hz, 3H); 1.2–1.48 (m, 7H); 2.12–2.33 (m, 2H); 4.32 (q, $J_{HH} = 7.2$ Hz, 2H); 5.58–5.74 (m, 1H); 6.2–6.36 (m, 1H); ^{19}F NMR (δ, $CDCl_3$): −103.53 (d, $J_{HF} = 8.7$ Hz, CF_2). Anal. calc. for $C_{10}H_{16}F_2O_2$: C, 58.24; H, 7.82. Found: C, 58.15; H, 7.85%.

*4.27. 2,2-Difluoro-hept-3-enoic acid ethyl ester (**17b**)*

Obtained by the Mitsunobu procedure for hydroxyester **2b**. Isolated after column chromatography (eluent benzene) with yield 0.01 g (7%) as a colourless oil. ^{1}H NMR (δ, $CDCl_3$): 0.9 (t, $J_{HH} = 7.5$ Hz, 3H); 1.19–1.47 (m, 5H); 2.13–2.35 (m, 2H); 4.32 (q, $J_{HH} = 7.2$ Hz, 2H); 5.59–5.75 (m, 1H); 6.21–6.37 (m, 1H); ^{19}F NMR (δ, $CDCl_3$): −103.55 (d, $J_{HF} = 8.6$ Hz, CF_2). Anal. calc. for $C_9H_{14}F_2O_2$: C, 56.24; H, 7.34. Found: C, 56.15; H, 7.38%.

*4.28. 2,2-Difluoro-4-methyl-pent-3-enoic acid ethyl ester (**17c**)*

Obtained by the Mitsunobu procedure for hydroxyester **2c**. Isolated after column chromatography (eluent benzene) with yield 0.05 g (34%) as a colourless oil. ^{1}H NMR (δ, $CDCl_3$): 1.34 (t, $J_{HH} = 7.2$ Hz, 3H); 1.8–1.9 (m, 6H); 4.32 (q, $J_{HH} = 7.2$ Hz, 2H); 5.38–5.51 (m, 1H); ^{19}F NMR (δ, $CDCl_3$): −98.31 (d, $J_{HF} = 15$ Hz, CF_2). Anal. calc. for $C_8H_{12}F_2O_2$: C, 53.93; H, 6.79. Found: C, 53.85; H, 6.84%.

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