SUPPORTING INFORMATION

Efficient and Straightforward Synthesis of Two United States Pharmacopeia Sitagliptin Impurities: 3-Desamino-2,3-Dehydrositagliptin and 3-Desamino-3,4-Dehydrositagliptin

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Figure S1

*^{1}H NMR spectra of **9** in $CDCl_3$*

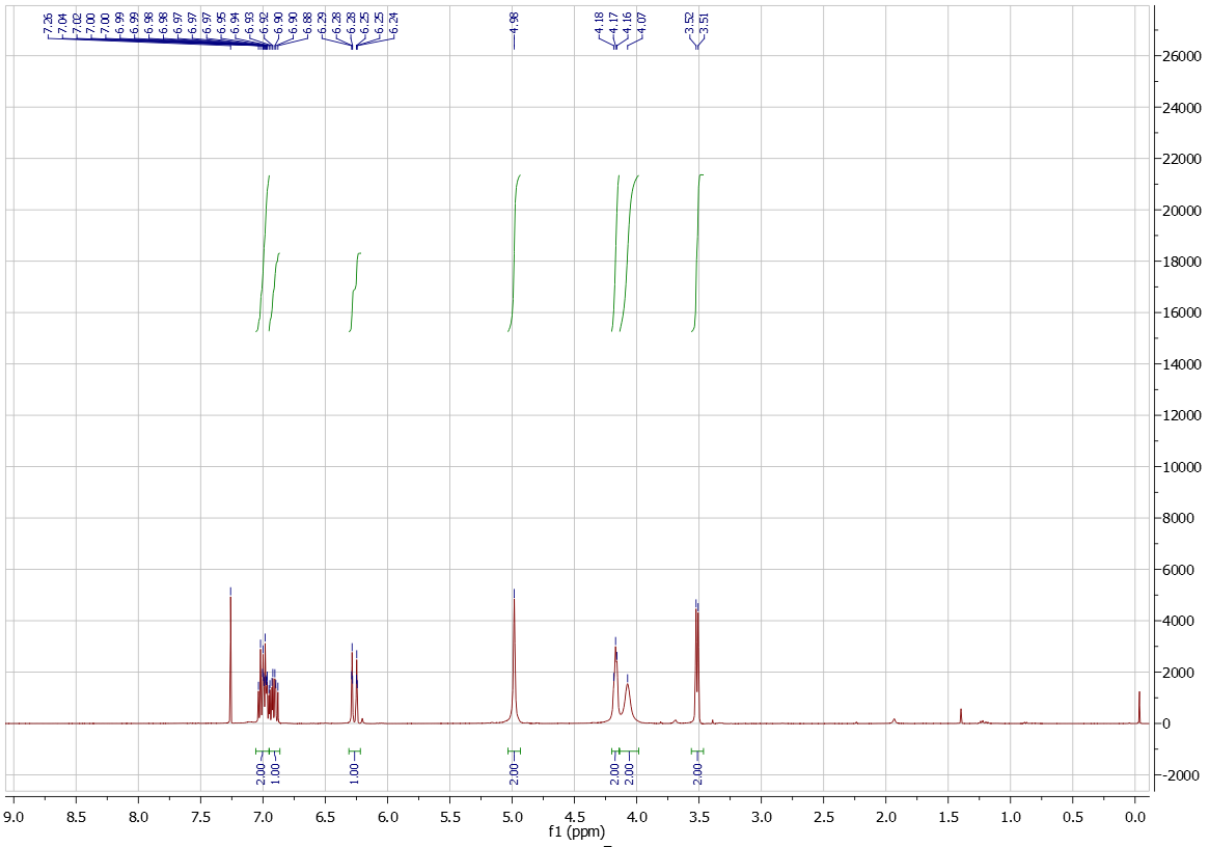


Figure S2

*^{13}C NMR spectra of **9** in $CDCl_3$*

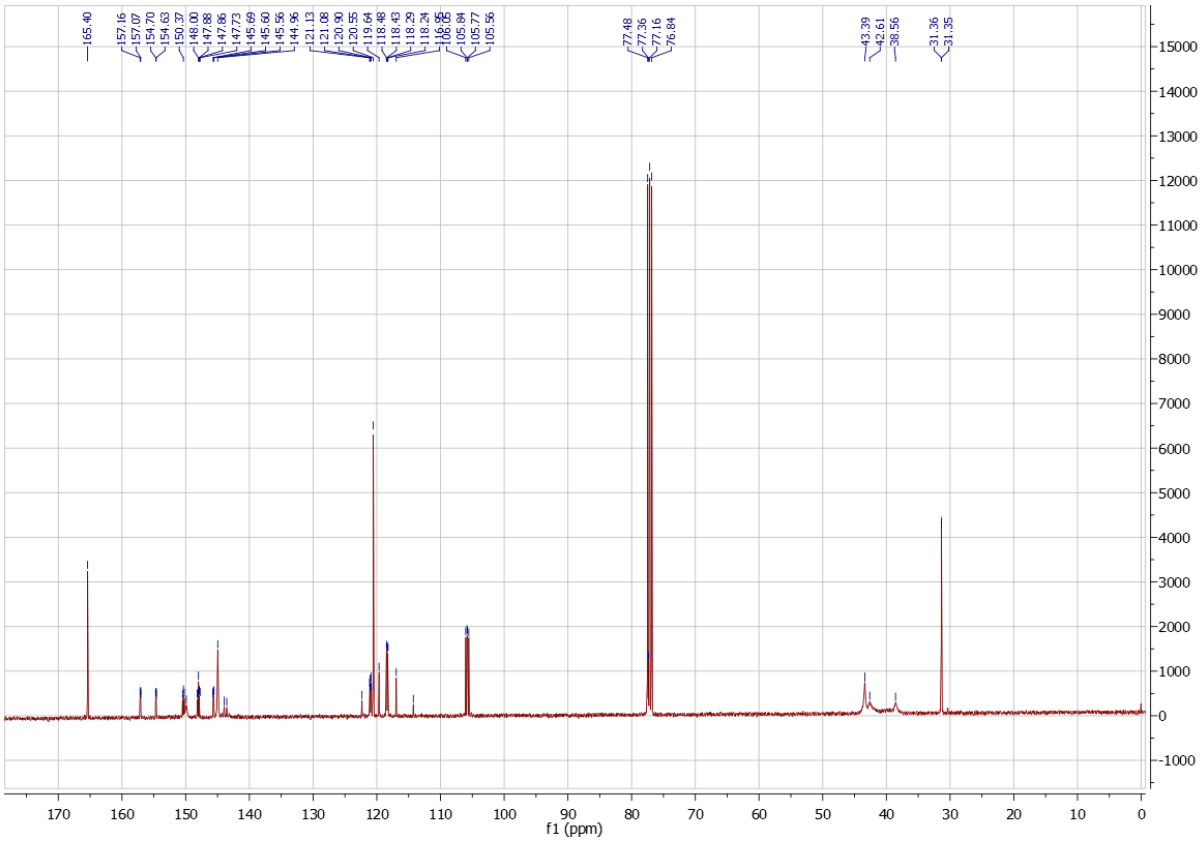


Figure S3

*HSQC of **9***

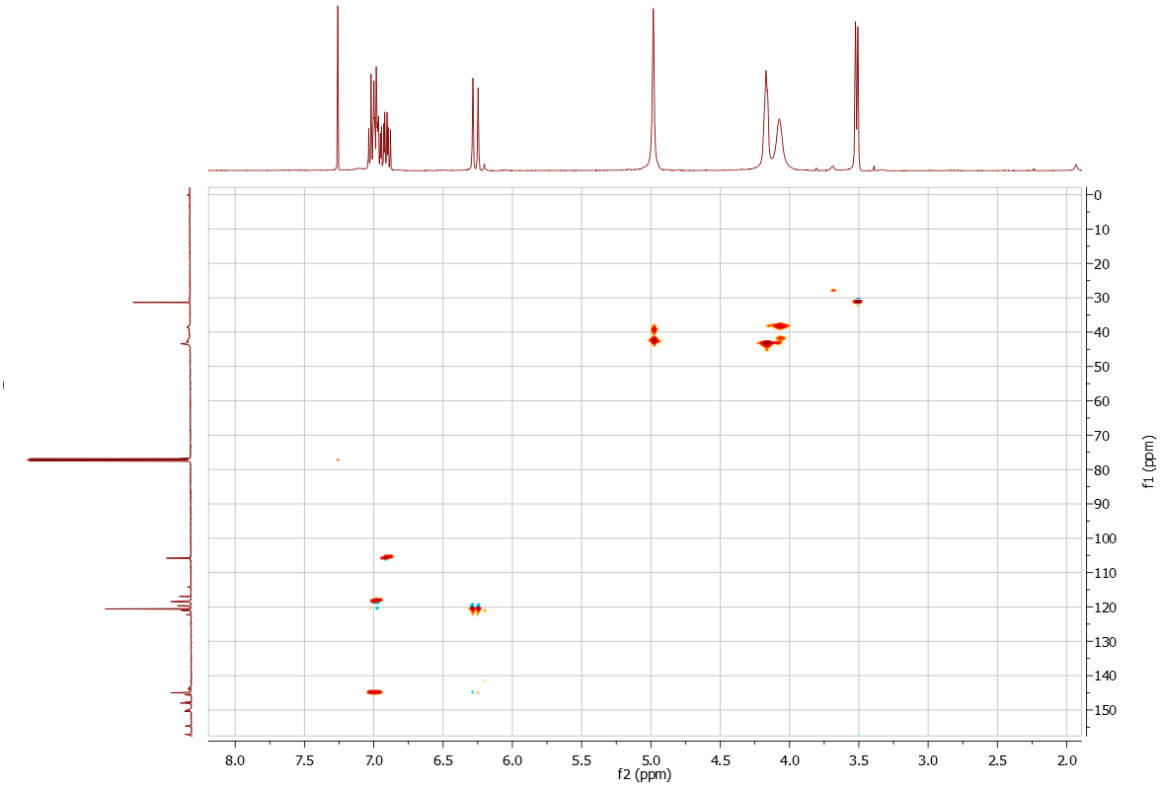


Figure S4

*HMBC of **9***

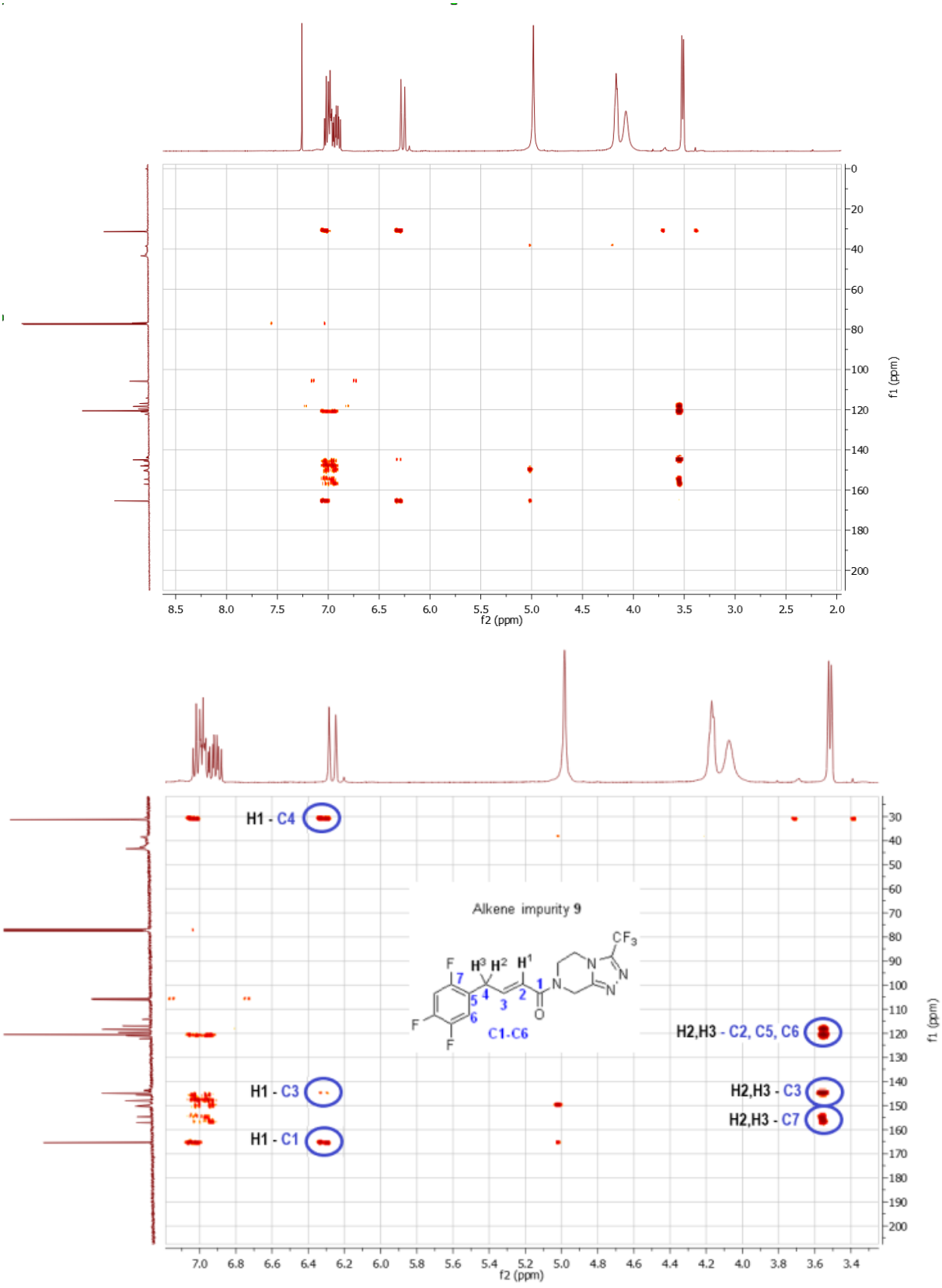
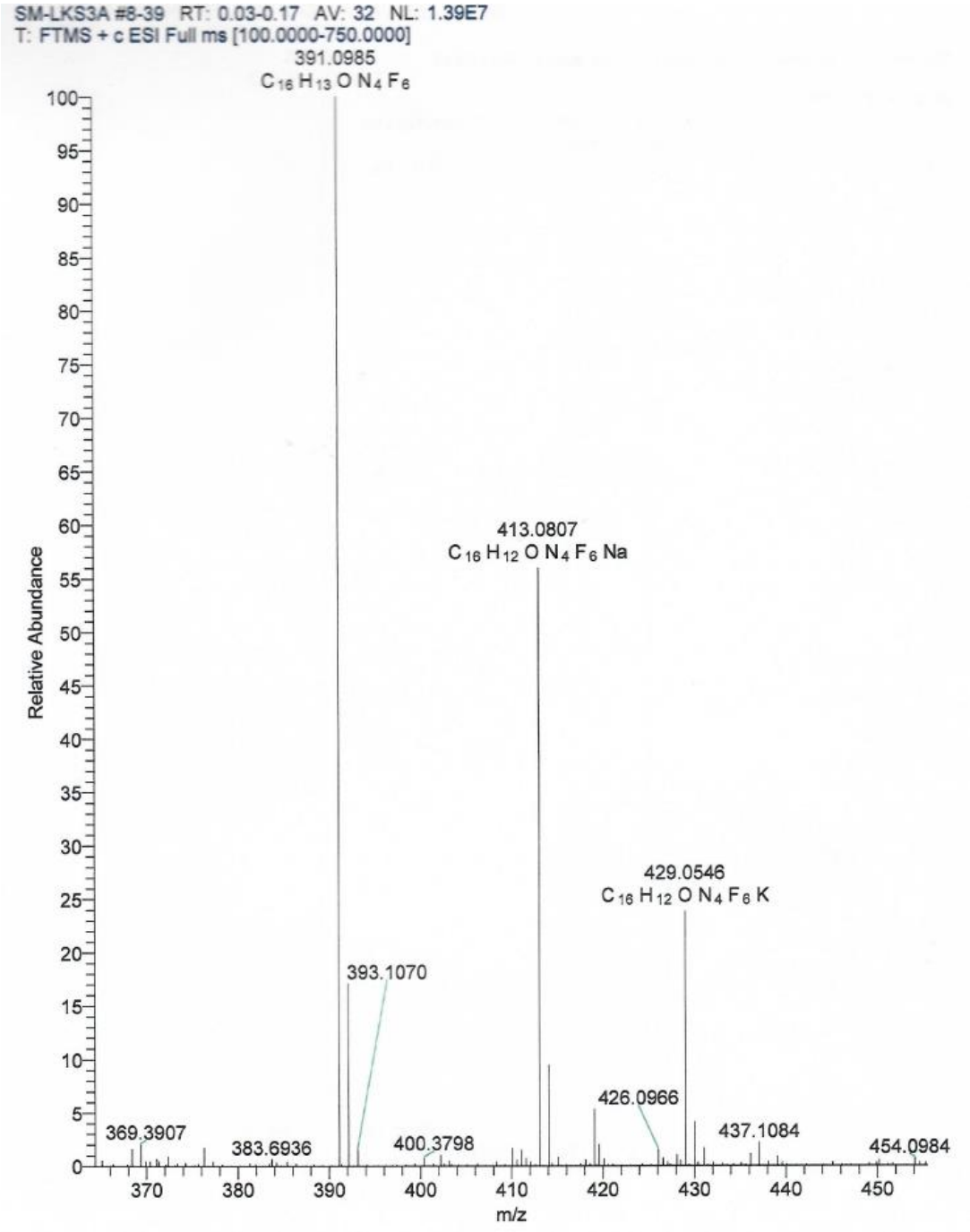


Figure S5

*Mass spectra of **9** (ESI+)*



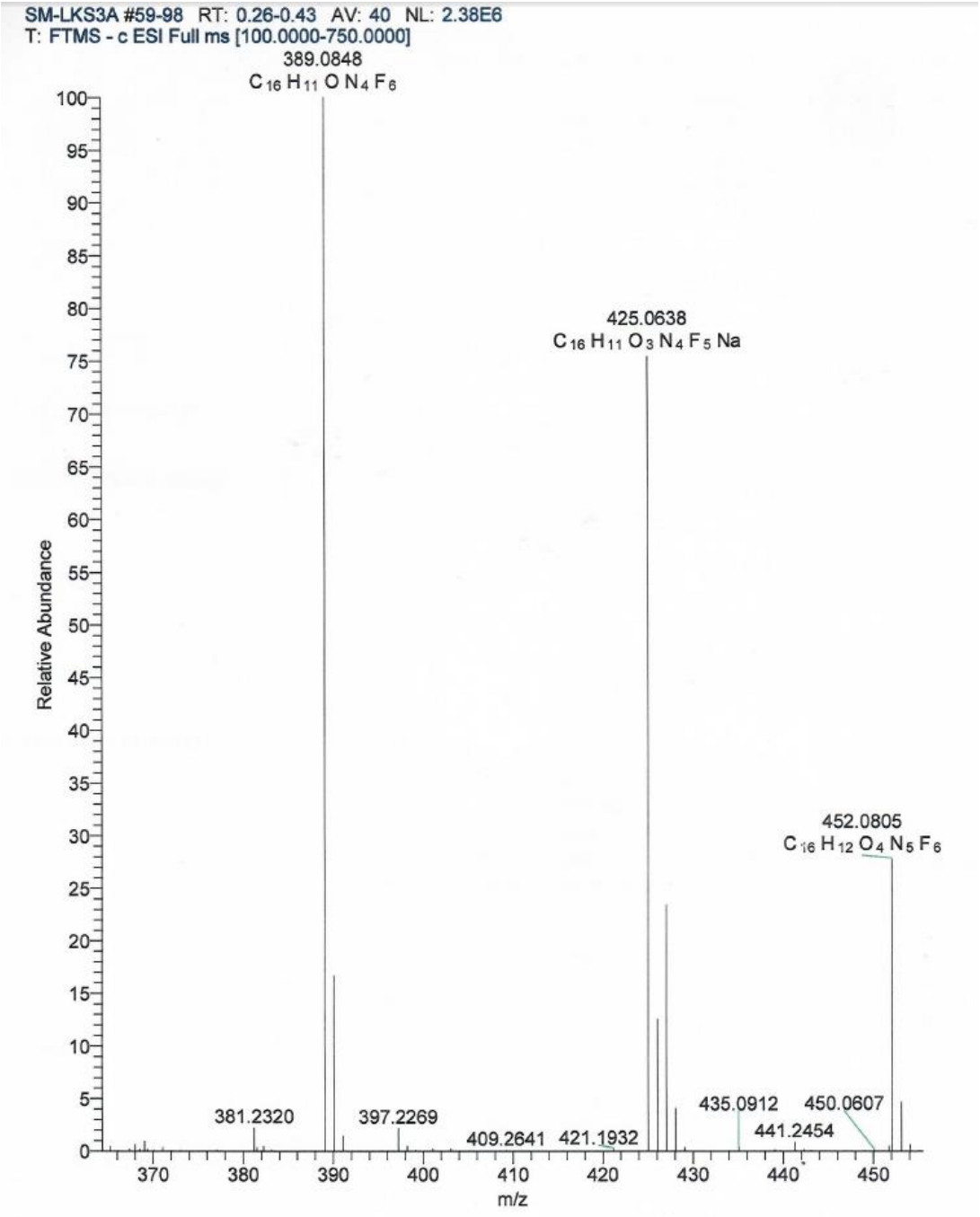
Elemental composition search on mass 391.0985

m/z= 386.0985-396.0985

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
391.09853	391.09881	-0.71	9.5	$C_{16}H_{13}ON_4F_6$

Figure S6

*Mass spectra of **9** (ESI-)*



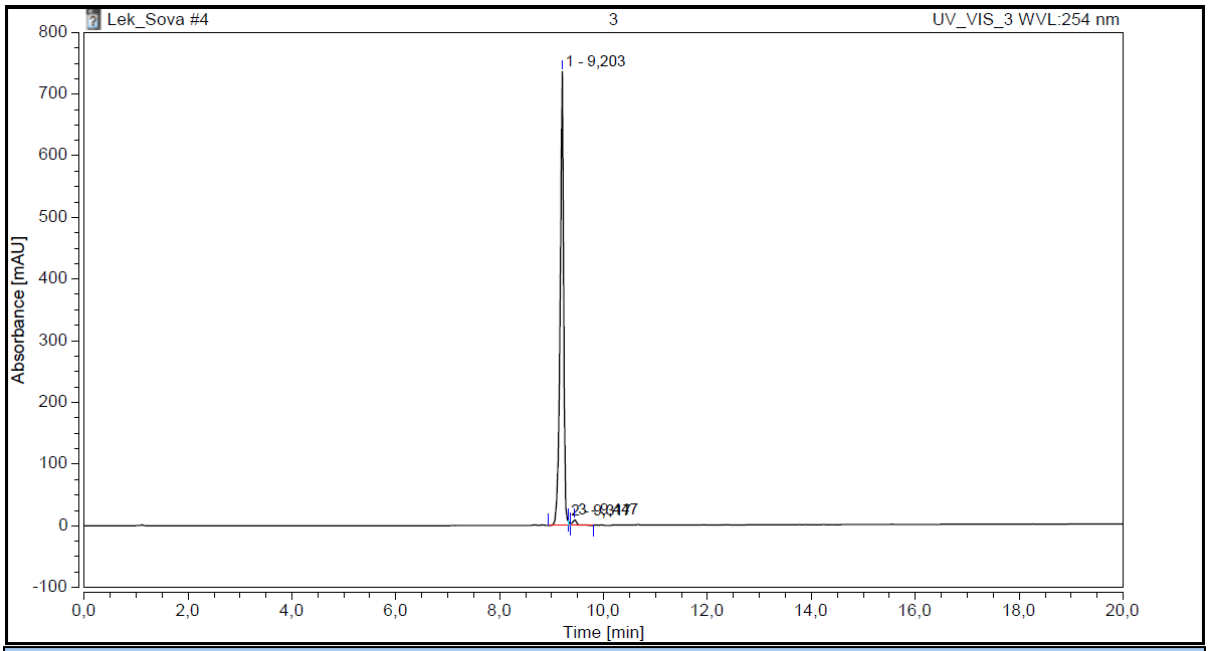
Elemental composition search on mass 389.0848

m/z= 384.0848-394.0848

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
389.08476	389.08425	1.30	10.5	$C_{16}H_{11}ON_4F_6$
	389.08812	-8.63	11.0	$C_{16}H_{12}ON_5F_4Na$

Figure S7

*HPLC chromatogram of **9***



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount n.a.
1		9,203	57,683	735,623	98,26	97,86	n.a.
2		9,317	0,253	7,009	0,43	0,93	n.a.
3		9,447	0,771	9,040	1,31	1,20	n.a.
Total:			**58,707**	**751,673**	**100,00**	**100,00**	

- Retention time of **9**: **9.203 min**
- HPLC purity of **9**: *57.683/58.707*= **98.26%**

Figure S8

*^{1}H NMR spectra of **10** in $CDCl_3$*

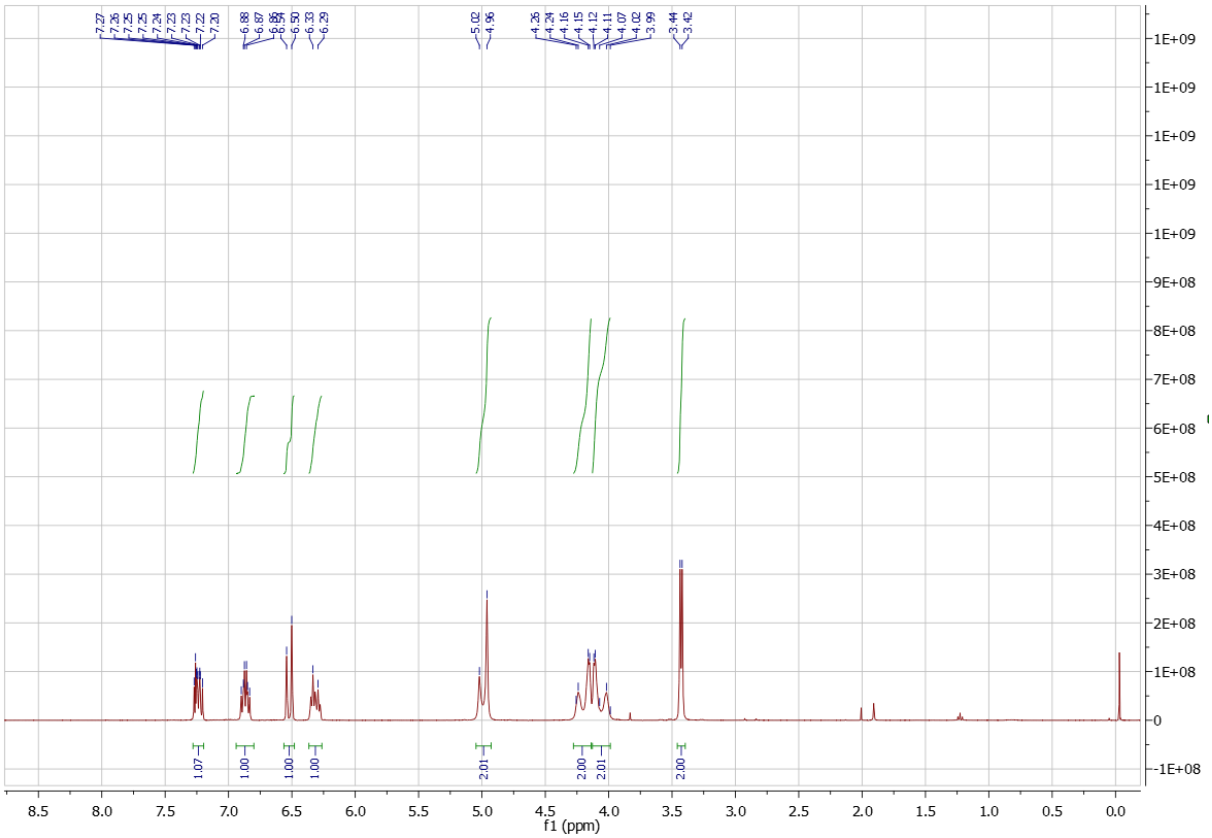


Figure S9

*^{13}C NMR spectra of **10** in $CDCl_3$*

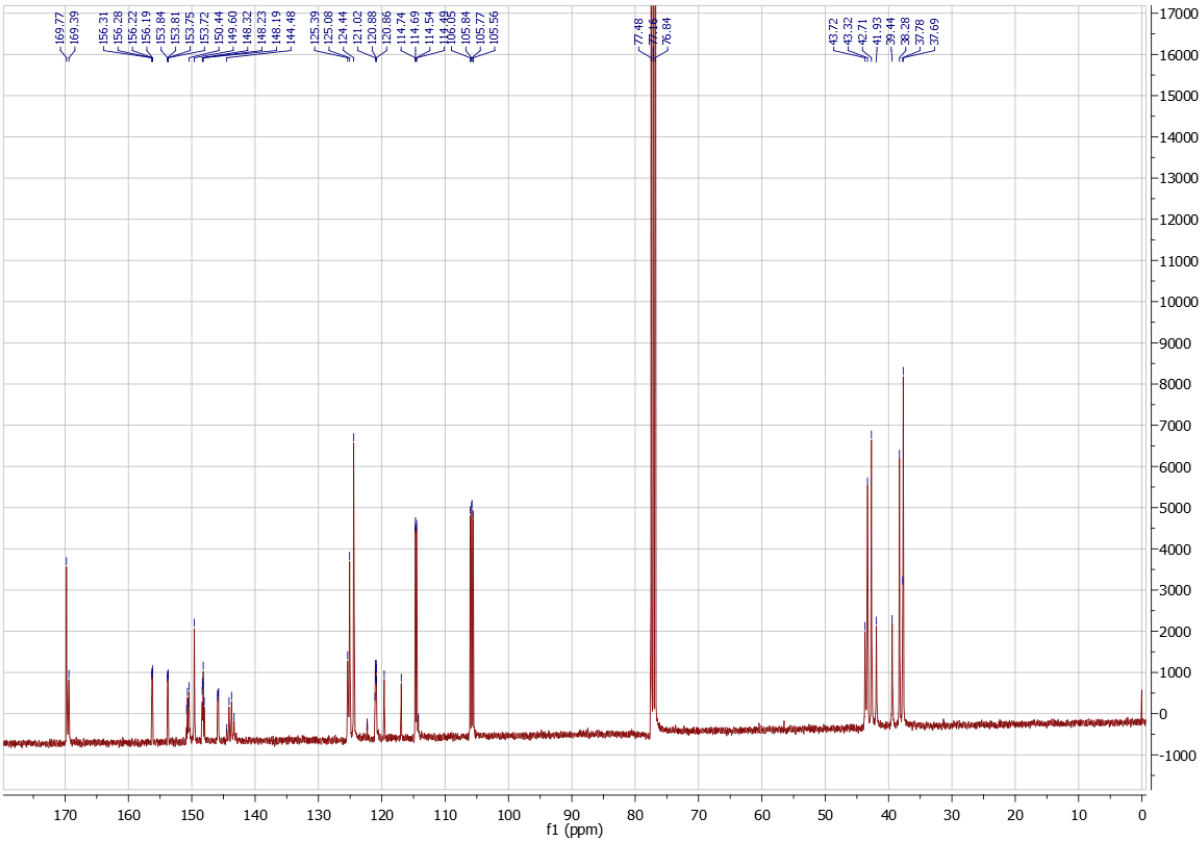


Figure S10

*HSQC of **10***

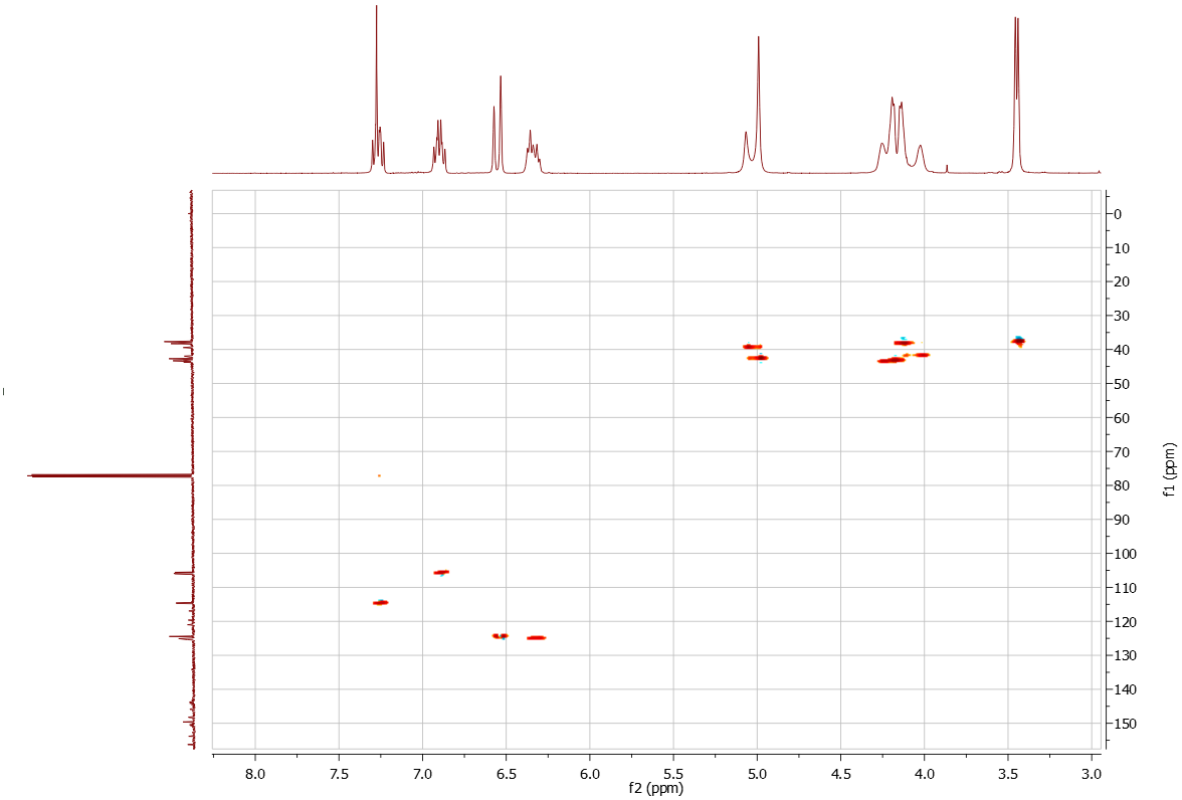


Figure S11

*HMBC of **10***

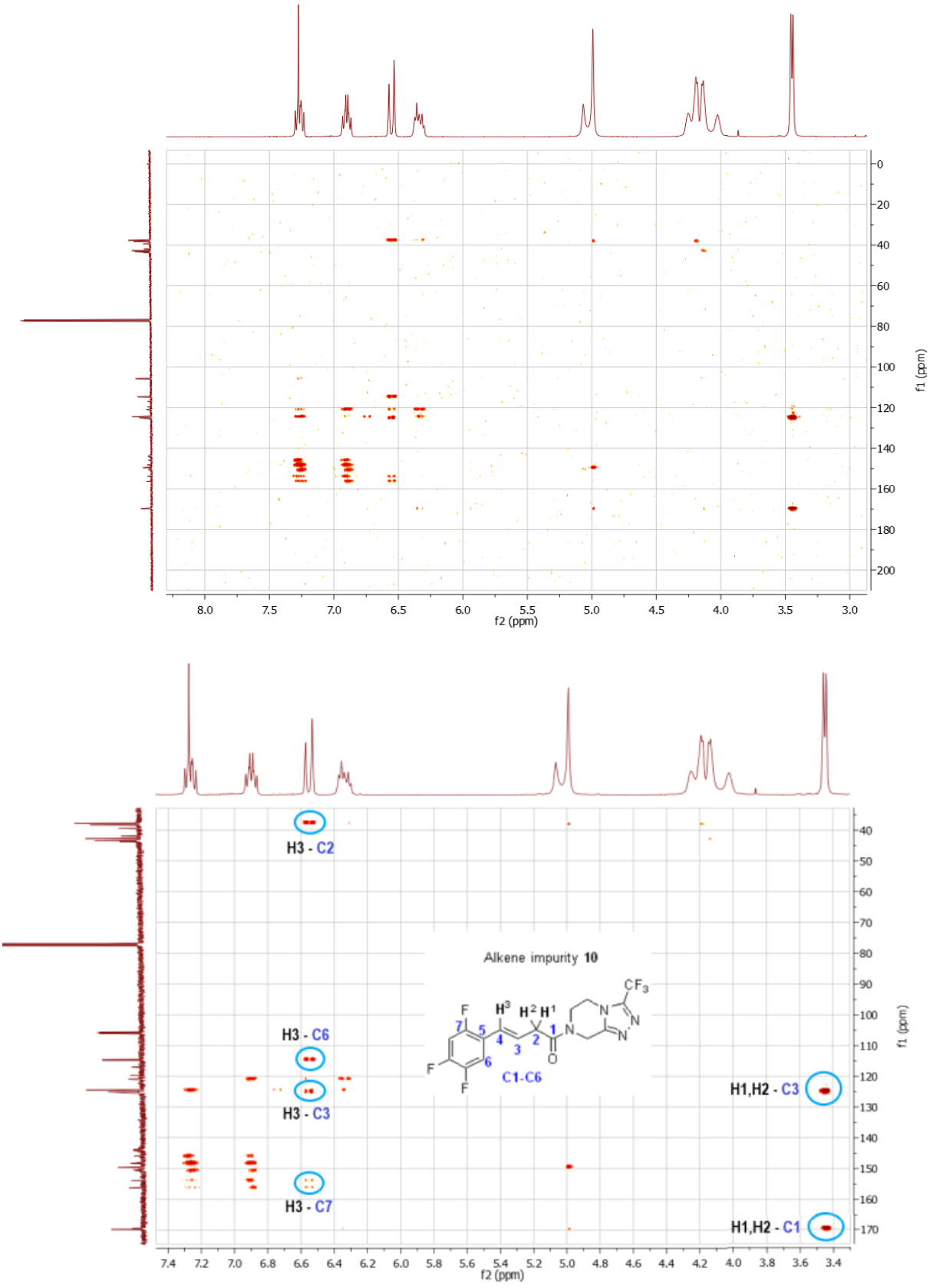
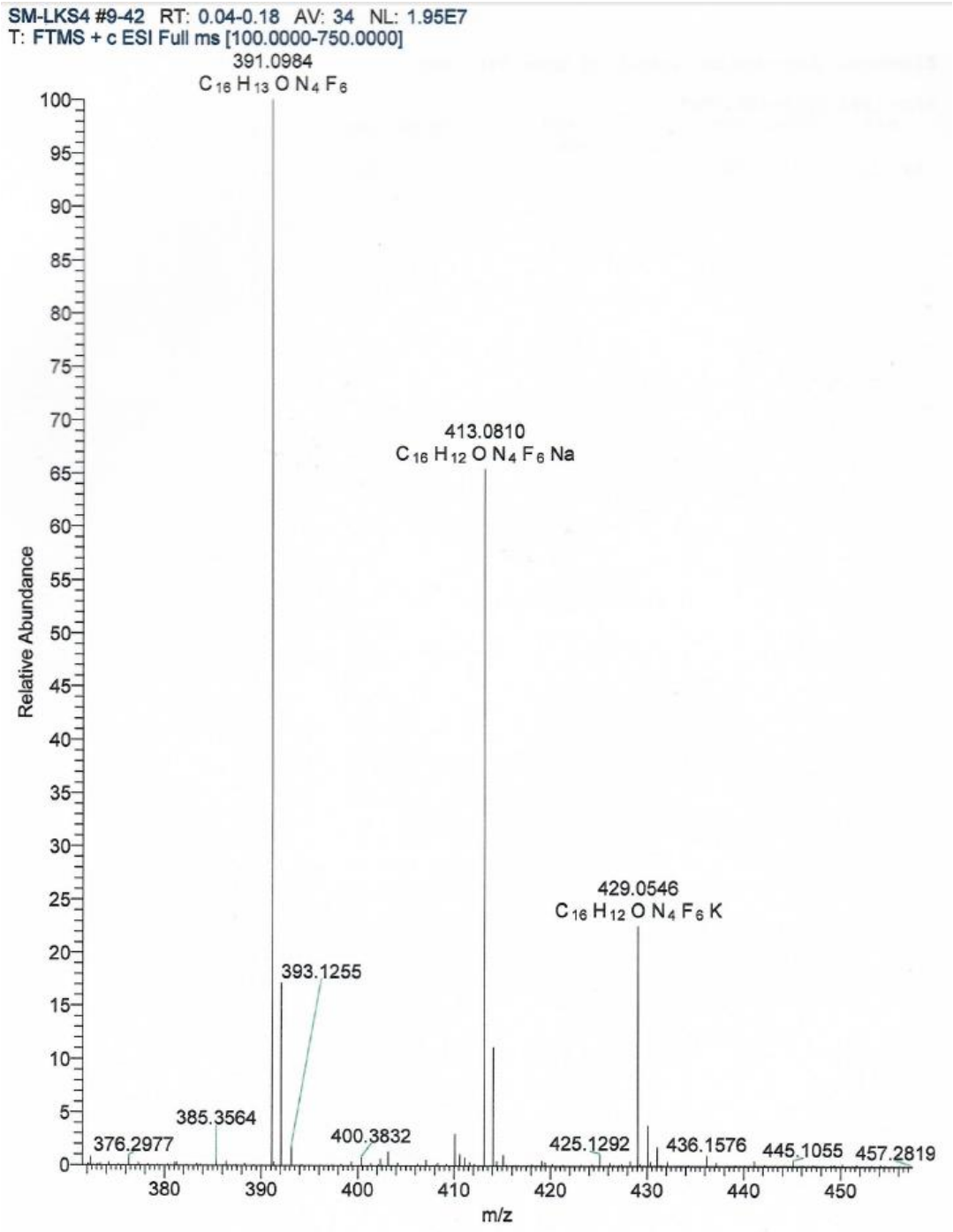


Figure S12

*Mass spectra of **10** (ESI+)*



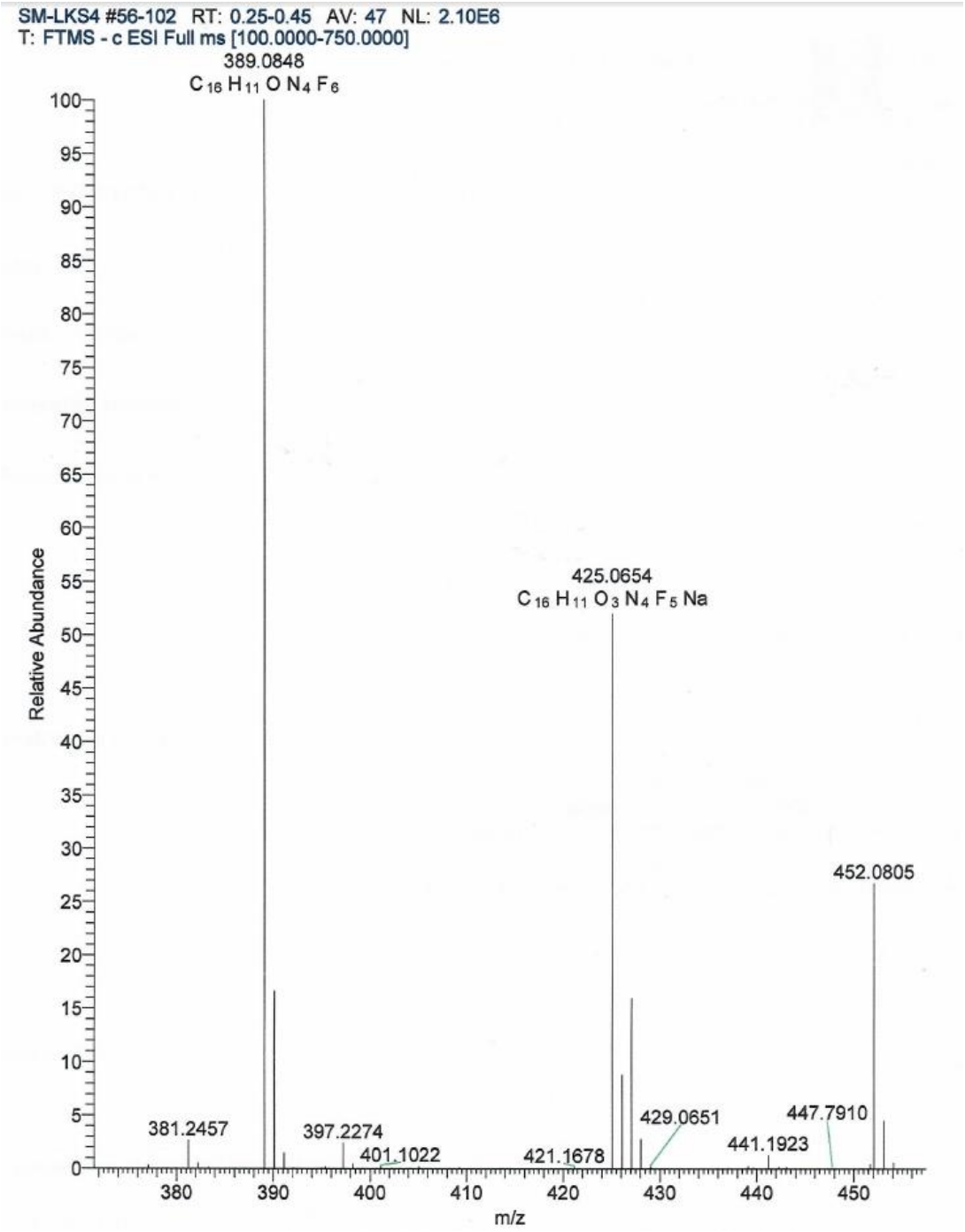
Elemental composition search on mass 391.0985

m/z= 386.0985-396.0985

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
391.09845	391.09881	-0.91	9.5	$C_{16}H_{13}ON_4F_6$

Figure S13

*Mass spectra of **10** (ESI-)*



Elemental composition search on mass 389.0848

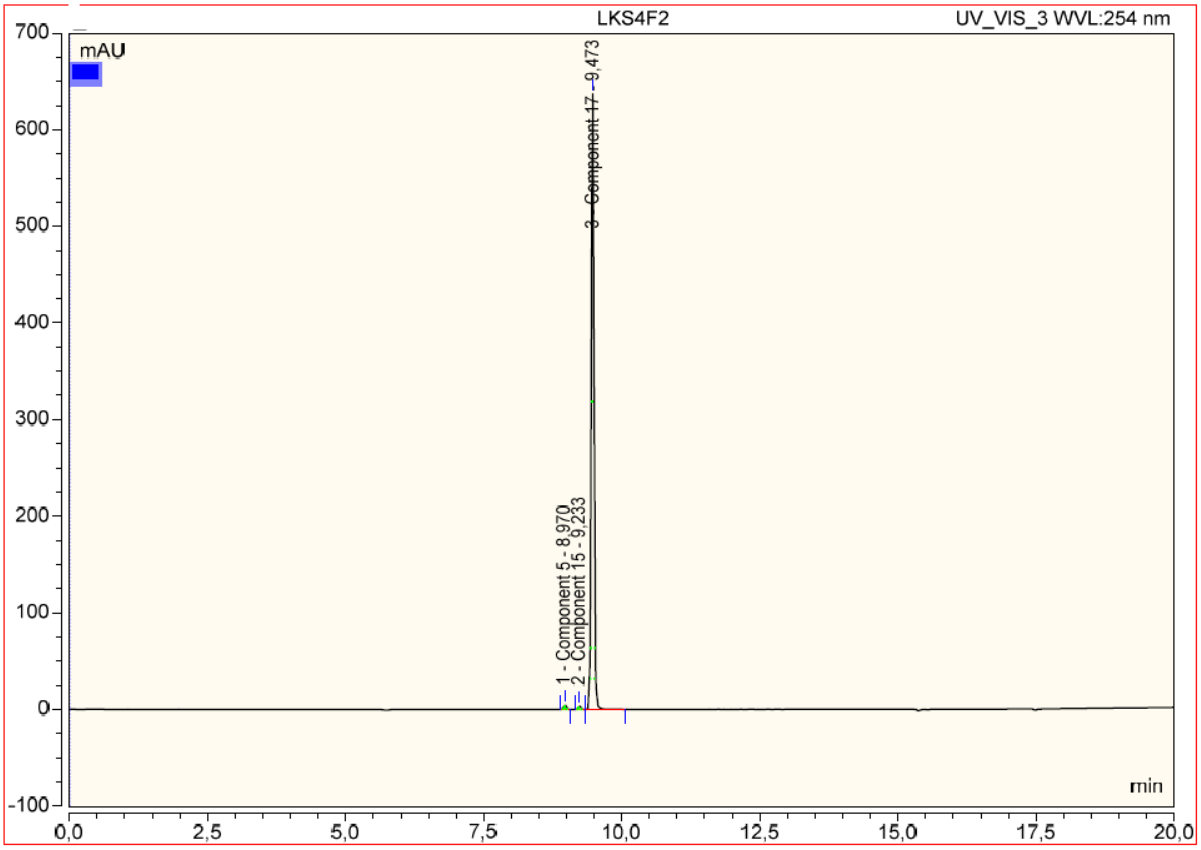
m/z= 384.0848-394.0848

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
389.08481	389.08425	1.43	10.5	$C_{16}H_{11}ON_4F_6$

Figure S14

*HPLC chromatogram of **10***

No.	Time min	Peak Name	Peak Type	Area mAU*min	Height mAU	Amount
1	8,97	**Component 5**	BMB	0,290	5,201	n.a.
2	9,23	**Component 15**	BM	0,196	3,690	n.a.
3	9,47	**Component 17**	MB	36,545	637,184	n.a.
TOTAL:				37,03	646,08	0,00

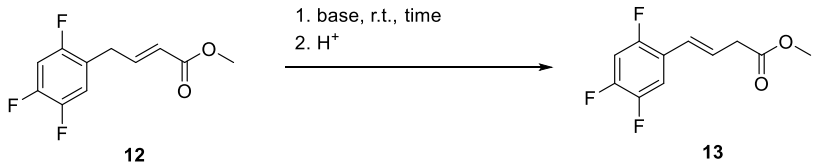


- Retention time of **10**: **9.473 min**
- HPLC purity of **10**: *36.545/37.030*= **98.69%**

Screening of appropriate base for regio-isomerization of 12 to 13

When a strong base represented by NaH was used (Table S1, entries 1 and 2), approximately 77 % conversion of **12** to **13** was achieved in one hour, however, several side products were observed in the NMR spectra of the reaction mixture. Furthermore, longer reaction time (72 h) did not improve the reaction. On the other hand, in case of commonly used base triethylamine (Et_3N), prolonged reaction time or 10 equivalents of a base (Table S1, entries 3-6) improved the conversion of **12** to **13** from 33 to 80 % (molar ratio of 1:0.5 to 0.25:1, respectively). Similar results were also obtained for NMM (Table S1, entries 8 and 9) after 72 h reaction time or excess of a base in contrast to one hour and one equivalent of NMM (Table S1, entry 7), where less than 10 % of alkene regio-isomerization occurred. The DBU was proved as inappropriate base for this type of reaction (Table S1, entries 10 and 11).

Table S1. Base screening in the alkene regio-isomerization reaction.



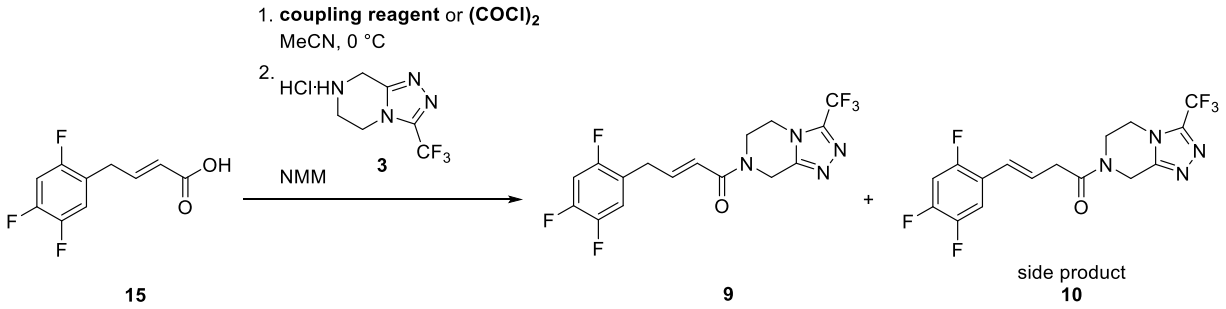
Entry	Base[a]	Equivalent(s)	Time (h)	Molar ratio **12**: **13**[b]
1	NaH	1	1	0.30 : 1
2	NaH	1	72	ND[c]
3	Et_3N	1	1	1 : 0.50
4	Et_3N	1	72	0.24 : 1
5	Et_3N	10	1	0.25 : 1
6	Et_3N	10	72	0.25 : 1
7	NMM	1	1	1 : 0.10
8	NMM	1	72	0.28 : 1
9	NMM	10	72	0.23 : 1
10	DBU	1	1	only **12**
11	DBU	1	72	ND[c]

[a] Reaction conditions: **12** (1.0 mmol), MeCN (5 mL), r.t., base (1 or 10 equiv.), time (1 or 72 h). DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene, NMM: *N*-methylmorpholine, r.t.: room temperature.
[b] Calculated by NMR analysis.
[c] ND: could not be determined, many side products detected.

Screening of optimal conditions for amide bond formation between 15 and 3

The highest yield between coupling reagents was obtained by DCC after 2 hours (Table S2, entry 2), however, the selectivity ratio was low. Further prolongation of reaction time did not improve the yield and selectivity (Table S2, entry 3). Higher selectivity ratio was achieved when DCC was replaced by EDC. After 2 hours, less than 6 % of isomer **10** was formed (Table S2, entry 5). On the other hand, in case of 24 h reaction time selectivity ratio dropped significantly (Table S2, entry 6). To improve the yield for EDC compared to DCC, we decided to add HOBt (*N*-hydroxybenzotriazole) to the reaction mixture. This indeed improved the yields (Table S2, entries 7-9), however, it significantly decreased the selectivity (ratio between **9**:**10**), for example from 16 (Table S2, entry 5) to 0.7 (Table S2, entry 8). Three other coupling reagents were also examined for amidation, namely TBTU, DPPA and CDI (Table S2, entries 10-18). The reaction using TBTU or DPPA resulted in low yields and moderate selectivity (Table S2, entries 10-15) with the exception of 24 h reaction using DPPA, where selectivity ratio was below 10 %, however, with relatively low yield of 22 % (Table S2, entry 15). CDI (Table S2, entries 16-18) improved the yield, but the main product formed was a side product **10** instead of **9**. The screening of coupling reagents conferred EDC as the most appropriate one leading to amide **9** in a satisfactory yield of 61 % with good selectivity ratio 1:16 (Table S2, entry 5). On the other hand, the highest yield for amide bond formation to **9** was obtained with oxalyl chloride (Table S2, entries 19-21). The 88 % yield and 7.8 selectivity ratio was achieved after one hour reaction at 0 °C. The prolonged reaction time lowered the yield without any significant improvement of selectivity.

Table S2. Optimization of reaction conditions for amide bond formation to alkene impurity I (**9**).



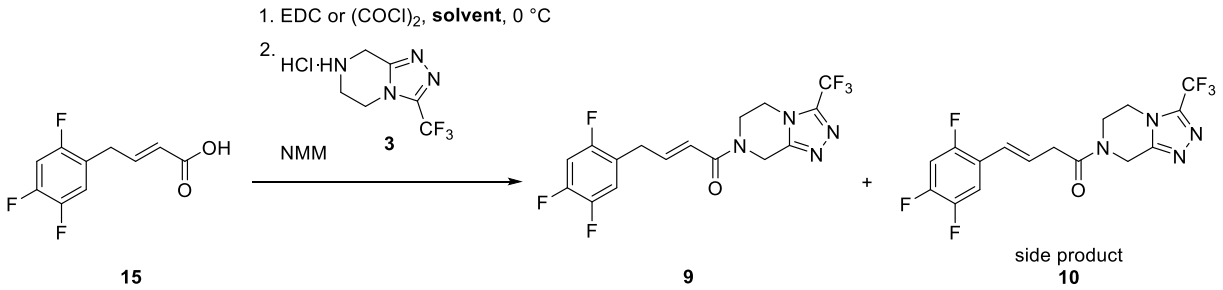
Entry	Coupling reagent or $(COCl)_2$[a]	Time (h)	Temperature	Overall Yield **9** + **10** (%)[b]	Selectivity ratio **9** : **10**[b]
1	DCC	1	0 °C	60	5.8
2	DCC	2	r.t.	77	6
3	DCC	24	r.t.	72	4.5
4	EDC	1	0 °C	56	7.8
5	EDC	2	r.t.	61	16
6	EDC	24	r.t.	51	6.3
7	EDC/HOBt	1	0 °C	61	0.65
8	EDC/HOBt	2	r.t.	67	0.7
9	EDC/HOBt	24	r.t.	68	0.76
10	TBTU	1	0 °C	23	6.3
11	TBTU	2	r.t.	30	5
12	TBTU	24	r.t.	44	3.9
13	DPPA	1	0 °C	1	/
14	DPPA	2	r.t.	10	4
15	DPPA	24	r.t.	22	10
16	CDI	1	0 °C	48	0.2
17	CDI	2	r.t.	72	0.12
18	CDI	24	r.t.	75	0.09
19	$(COCl)_2$	1	0 °C	88	7.8
20	$(COCl)_2$	2	r.t.	81	8
21	$(COCl)_2$	24	r.t.	81	4.3

[a] Reaction conditions: **Coupling reagent method**: i) **15** (0.50 mmol, 0.108 g), MeCN (5 mL), 0 °C, *coupling reagent* (0.50 mmol), 0.5 h; ii) **3** × HCl (0.5 mmol, 0.114 g), NMM (0.50 mmol, 0.055 mL), 0 °C for 1 h, then r.t. for 1-24 h. **Acid chloride method**: i) **15** (0.46 mmol, 0.10 g), CH_2Cl_2, oxalyl chloride (0.92 mmol, 0.078 mL), DMF (cat.), 0 °C, 1.5 h; ii) **3** × HCl (0.46 mmol, 0.105 g), NMM (0.92 mmol, 0.10 mL), MeCN (5 mL), 0 °C for 1 h, then r.t. for 1-24 h.
[b] Determined by HPLC.

Screening of optimal solvent for amide bond formation in coupling of 15 and 3

The 1 h reaction at 0 °C in MeCN, CH_2Cl_2, THF, EtOAc, and DMF (Table S3, entries 1, 3, 5, 7 and 9) led to low or moderate yields (from 17 % to 67 %) with the highest selectivity ratio of 31.9 obtained in DMF (Table S3, entry 9). Performing a reaction at room temperature for additional 1 h had beneficial effect on the reaction yield compared to 0 °C with very little effect on the selectivity (Table S3, entries 2, 6, 8, and 10). The only exception was CH_2Cl_2, where temperature did not influence the yield and selectivity significantly (Table S3, entries 3 and 4). Nevertheless, DMF was selected as the most optimal solvent with 77 % yield and selectivity ratio of 30.4 (Table S3, entry 10). In case of the carboxylic acid activation with oxalyl chloride (Table S3, entries 11-14), the reaction in THF was the most optimal leading to promising 84 % yield and 30.8 selectivity ratio (Table S3, entry 13). The highest yield in solvent screening was on the other hand obtained with oxalyl chloride and further amidation in MeCN at 0 °C (Table S3, entry 11), however, the selectivity ratio was very low. Therefore, THF was selected as the most optimal solvent for further optimization of an acid chloride method.

Table S3. Solvent screening for amidation to alkene impurity I (**9**).



Entry	Solvent	Activating Reagent	Time (h)	Temperature	Overall Yield **9** + **10** (%)[a,b]	Selectivity ratio **9** : **10**[b]
1	MeCN	EDC	1	0 °C	55	12.9
2	MeCN	EDC	2	r.t.	61	16.1
3	CH_2Cl_2	EDC	1	0 °C	67	14.6
4	CH_2Cl_2	EDC	2	r.t.	62	13.7
5	THF	EDC	1	0 °C	18	31.5
6	THF	EDC	2	r.t.	31	31.3
7	EtOAc	EDC	1	0 °C	17	30.2
8	EtOAc	EDC	2	r.t.	46	28
9	DMF	EDC	1	0 °C	53	31.9
10	DMF	EDC	2	r.t.	77	30.4
11	MeCN	$(COCl)_2$	1	0 °C	88	7.8
12	CH_2Cl_2	$(COCl)_2$	1	0 °C	69	13.3
13	THF	$(COCl)_2$	1	0 °C	84	30.8
14	EtOAc	$(COCl)_2$	1	0 °C	81	31

[a] Reaction conditions **Coupling reagent method**: i) **15** (0.50 mmol, 0.108 g), *solvent* (5 mL), 0 °C, EDC × HCl (0.50 mmol, 0.096 g, 0.5 h; ii) **3** × HCl (0.5 mmol, 0.114 g), NMM (0.50 mmol, 0.055 mL), 0 °C for 1 h, then r.t. for 1-24 h. **Acid chloride method**: i) **15** (0.46 mmol, 0.10 g), CH_2Cl_2, oxalyl chloride (0.92 mmol, 0.079 mL), DMF (cat.), 0 °C, 1.5 h; ii) **3** × HCl (0.46 mmol, 0.105 g), NMM (0.92 mmol, 0.10 mL), *solvent* (5 mL), 0 °C, 1 h.
[b] Determined by HPLC.

Figure S15

NMR study of regio-isomerization process of **9** to **10** in deuterated DMF in the presence of NMM as a base.

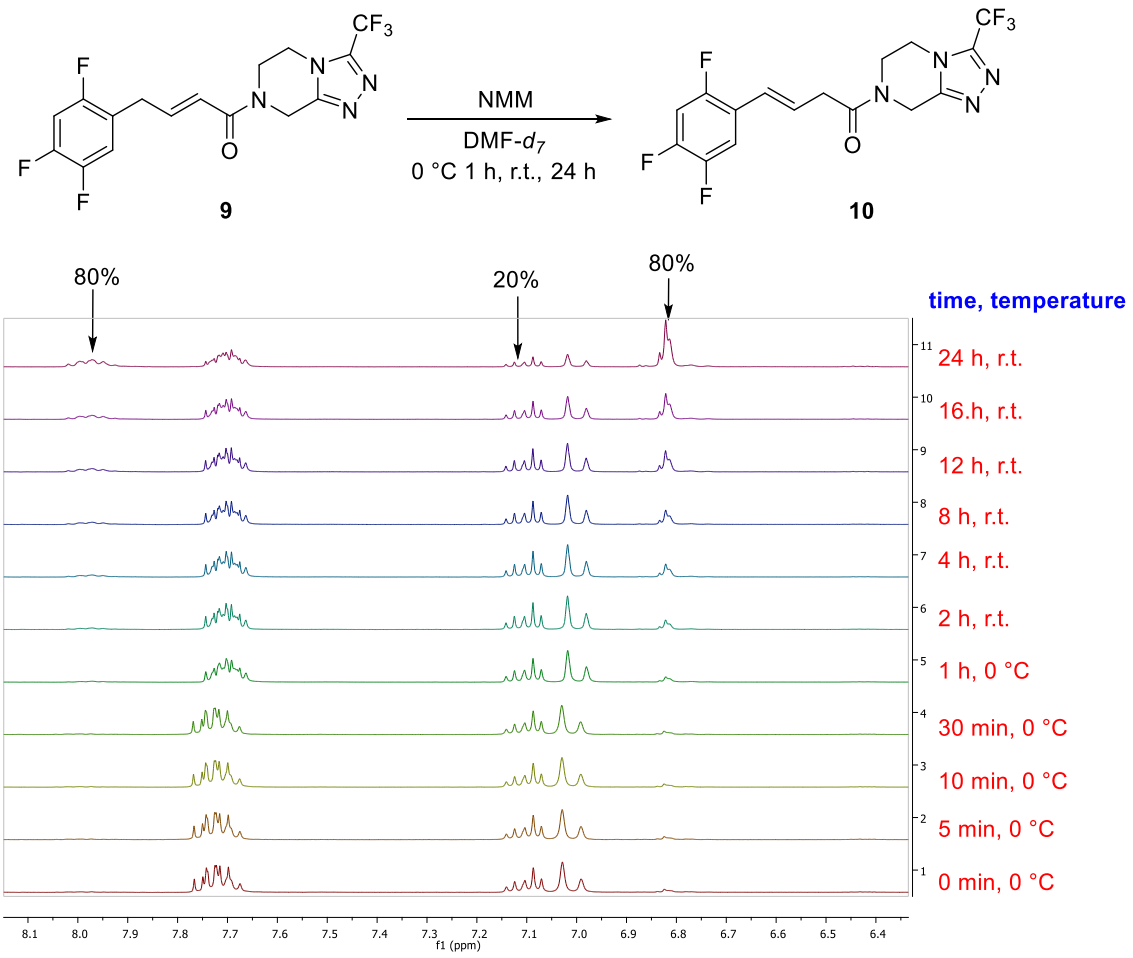
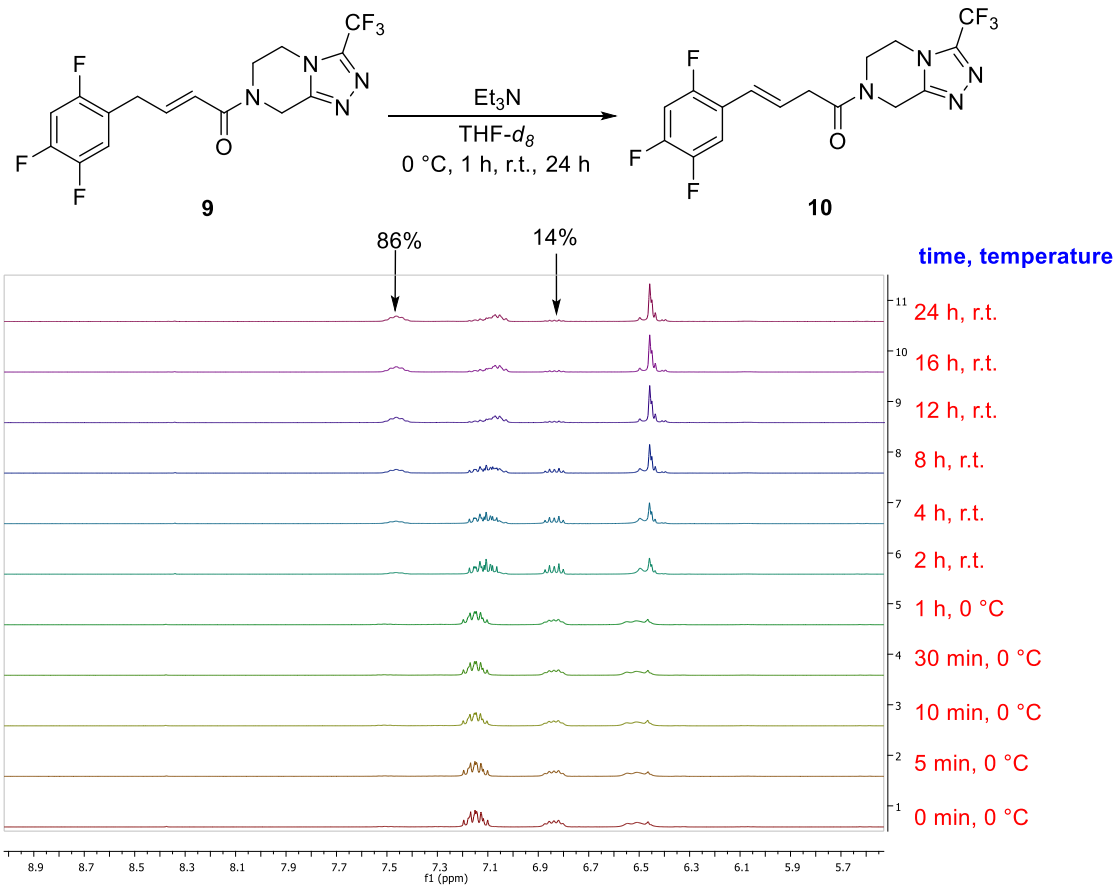


Figure S16

NMR study of regio-isomerization process of **9** to **10** in deuterated THF in the presence of Et_3N as a base.



Reactions screening procedures described in tables

*General procedure for base scan (Table **S1**):* To a solution of methyl (*E*)-4-(2,4,5-trifluorophenyl)but-2-enoate **12** (1.0 mmol, 0.23 g) in acetonitrile (5 mL) the selected *base* was added. The reaction mixture was stirred for selected *time* at room temperature. Then 1 M HCl (5 mL) was added and the solution/suspension obtained was extracted with diethyl ether (3 × 20 mL). The combined organic phases were washed with brine (25 mL), dried with anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The oily residue was dissolved in DMSO-d_6 and ^{1}H NMR spectra was recorded.

*General procedure for hydrolysis of **12** to **14**/**15** (Table **1**):* To a solution of methyl (*E*)-4-(2,4,5-trifluorophenyl)but-2-enoate **12** (1.0 mmol, 0.23 g) in 1,4-dioxane (5 mL) the selected *solvent* (H_2O/NaOH or H_2O/HCl, 5 mL) was added. The reaction mixture was stirred for selected *time* at selected *temperature*. Then pH was adjusted to 1 (if needed) with 1 M HCl (5 mL) and the solution/suspension obtained was extracted with diethyl ether (3 × 20 mL). The combined organic phases were washed with brine (25 mL), dried with anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The oily residue was dissolved in DMSO-d_6 and ^{1}H NMR spectra was recorded.

***General procedure for amide bond formation to alkene impurity I (9)** (Table **S2**):*
Screening of coupling reagents: (*E*)-4-(2,4,5-trifluorophenyl)but-2-enoic acid **15** (0.5 mmol, 0.108 g) was dissolved in acetonitrile (5 mL) and cooled to 0 °C. Selected *coupling reagent* (0.5 mmol) was added and the reaction was stirred at 0 °C for 0.5 h. Then **3** × HCl (0.5 mmol, 0.114 g) and NMM (0.5 mmol, 0.055 mL) were added. The resulting reaction mixture was stirred for 1 h at 0 °C (HPLC sample1), 1 h at room temperature (HPLC sample 2) and then 24 h at room temperature (HPLC sample 3).
Activation *via* acid chloride: (*E*)-4-(2,4,5-trifluorophenyl)but-2-enoic acid **15** (0.46 mmol, 0.10 g) was dissolved in CH_2Cl_2 (5 mL), and cooled to 0 °C. Oxalyl chloride (0.92 mmol, 0.079 mL) was added and the reaction was stirred at 0 °C for 0.5 h. The solvent was then evaporated and the residue dissolved in acetonitrile (2.5 mL).

Afterwards, the yellow solution obtained was cooled down to 0 °C and quickly added to a cooled (0 °C) solution of **3** × HCl (0.46 mmol, 0.105 g) and NMM (0.92 mmol, 0.10 mL) in acetonitrile (2.5 mL), which was stirred for 1 h at room temperature prior the addition. The resulting reaction mixture was stirred for 1 h at 0 °C (*HPLC sample 1*), 1 h at room temperature (*HPLC sample 2*) and then 24 h at room temperature (*HPLC sample 3*).

General procedure for solvent screening for amidation to alkene impurity I (9) *(Table **S3**):*

Screening of solvent (EDC method): (*E*)-4-(2,4,5-trifluorophenyl)but-2-enoic acid **15** (0.50 mmol, 0.108 g) was dissolved in the selected *solvent* (5 mL), and cooled to 0 °C. EDC × HCl (0.50 mmol, 0.096 g) was added and the reaction mixture was stirred at 0 °C for 0.5 h. Afterwards, **3** × HCl (0.50 mmol, 0.114 g) and NMM (0.50 mmol, 0.055 mL) were added. The resulting reaction mixture was stirred 1 h at 0 °C (*HPLC sample 1*) and 1 h at room temperature (*HPLC sample 2*).

Screening of solvents (acid chloride method): (*E*)-4-(2,4,5-trifluorophenyl)but-2-enoic acid **15** (0.46 mmol, 0.10 g) was dissolved in CH_2Cl_2 (5 mL), and cooled to 0 °C. Oxalyl chloride (0.92 mmol, 0.079 mL) was added and the reaction was stirred at 0 °C for 0.5 h. CH_2Cl_2 was evaporated and the residue dissolved in selected *solvent* (2.5 mL). The solution obtained was cooled down to 0 °C and quickly added to a cooled (0 °C) solution of **3** × HCl (0.46 mmol, 0.105 g) and NMM (0.92 mmol, 0.10 mL) in selected *solvent* (2.5 mL), which was stirred for 1 h at room temperature prior the addition. The resulting reaction mixture was stirred 1 h at 0 °C (*HPLC sample 1*).

General procedure for base screening for amidation to alkene impurity I (9) *(Table **2**):*

Screening of bases (EDC method): (*E*)-4-(2,4,5-trifluorophenyl)but-2-enoic acid **15** (0.50 mmol, 0.108 g) was dissolved in DMF (5 mL) and cooled to 0 °C. EDC (0.50 mmol, 0.089 g) was added and the reaction mixture was stirred at 0 °C for 0.5 h. Then **3** × HCl (0.50 mmol, 0.114 g) and selected *base* (0.50 mmol) were added. The resulting reaction mixture was stirred 1 h at 0 °C (*HPLC sample1*) and 1 h at room temperature (*HPLC sample 2*).

Screening of bases (acid chloride method): (*E*)-4-(2,4,5-trifluorophenyl)but-2-enoic acid **15** (0.46 mmol, 0.10 g) was dissolved in CH_2Cl_2 (5 mL), and cooled to 0 °C. Oxalyl chloride (0.92 mmol, 0.079 mL) was added and the reaction was stirred at 0 °C for 0.5 h. CH_2Cl_2 was evaporated and the residue dissolved in THF (2.5 mL). The solution obtained was cooled down to 0 °C and quickly added to a cooled (0 °C) solution of **3** × HCl (0.46 mmol, 0.105 g) and selected *base* (0.92 mmol) in THF (2.5 mL), which was stirred for 1 h at room temperature prior the addition. The resulting reaction mixture was stirred 1 h at 0 °C (*HPLC sample 1*).