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Synthesis of Leu-Enkephalin Peptidomimetics Containing Trifluoromethylalkenes as Amide Isopolar Mimics

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Abstract

Fluorinated peptidomimetics are valuable substrates for exploring peptide backbone conformations and for perturbing physicochemical properties of probe compounds. However, in some cases synthetic limitations restrict installation of the fluorinated peptidomimetics into the desired probe compounds. For instance, trifluoromethylalkenes have served as amide isopolar mimics, but are rarely utilized, because many standard peptide-coupling conditions promote the isomerization of the alkene to thermodynamically favored positions. To address this challenge, we report the conversion of a naturally occurring amino acid to a Tyr¹- ψ [(CF₃C=CH)]-Gly² dipeptide mimetic, and notably, successful peptide coupling reactions that avoid alkene isomerization. Using this strategy, we generated trifluoromethylalkene-containing Leu-enkephalin peptidomimetics in high purity and good yield. This sequence suggests that the trifluoromethylalkene peptidomimetics can be incorporated into other target molecules with appropriate optimization.

Graphical Abstract

Keywords

Leu-Enkephalin; Trifluoromethylalkene; Peptidomimetics; Peptide coupling; Trifluoromethylation

1. Introduction

Peptides are key messengers in biological signaling pathways and are therapeutically useful for modulating several pathological conditions [1]. Unfortunately, many peptide-based drug candidates have struggled clinically, because of poor physicochemical properties and *in vivo* stability, which restrict systemic distribution and half-lives [2-3]. These drawbacks can be

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jfluchem.XXXX.XX.XXX>

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primarily attributed to the peptide backbones that make strong H-bonding interactions with H₂O, thus disfavoring desolvation, and that are subject to rapid enzymatic hydrolysis [4]. To improve the drug-like properties of peptide-based probes, the labile amide bonds can be replaced with stable amide isosteric- or isopolar-mimics. One such strategy involves fluorinated substructures that can possess similar dipole moments, electrostatic potentials, and steric-, resonance-, and inductive-properties to amides [5], but are not substrates for endogenous peptidases [6-9]

Amongst fluorinated peptidomimetics, the trifluoromethylalkene group (CF₃-alkene) can serve as an isopolar replacement for the amide linkage and can be used to probe chemical biology [10-11]. This substructure closely mimics the electrostatic potential and direction of polarization of the carbonyl group, as well as the sp²-hybridization of the carbonyl carbon (Figure 1A) [12]. However, incorporation of the CF₃-alkene group would impart greater thermal, proteolytic and conformational stability, and greater lipophilicity to a polypeptide [10, 13]. This substructure has been exploited in few probe molecules, including (a) a trifluoromethylalkene analogue of Gramicidin S that showed similar solution-state and solid-state conformations to the natural peptide [12], and (b) an X-ray crystallographic study of the CF₃-alkene analogue of the L-Ala-D-Ala dipeptide, in which the CF₃-alkene analogue adopted a considerably more organized structure than other peptidomimetics (Figure 1A) [5].

Beyond these intriguing examples, exploitation of CF₃-alkene peptidomimetic biological probes remains limited, presumably because of the potential for the alkene to isomerize from the β,γ-position to the thermodynamically favorable α,β-position of the amide during peptide coupling (Figure 1B). For the two successful examples, the bulky α-substituents of Gramicidin S and L-Ala-D-Ala CF₃-alkene may disfavor the base-mediated isomerization of the double bond, which might enable coupling (Figure 1A, blue highlight).

To investigate synthetic routes for incorporating the CF₃-alkene into a peptide backbone, while minimizing alkene isomerization, we selected Leu-enkephalin (**1**, Figure 1C) as a model substrate. Leu-enkephalin is an endogenous pentapeptide that exhibits moderate selectivity for the δ-opioid receptor (DOR), and several preclinical studies have demonstrated that selective activation of the DOR induces analgesia without promoting the undesirable side effects (tolerance, dependence, and constipation) typically associated with stimulation of the μ-opioid receptor (MOR) [14]. However, as with many peptides, the poor proteolytic stability and bioavailability of Leu-enkephalin limit *in vivo* therapeutic utility [15-17]. Previous studies of Leu-enkephalin identified the Tyr¹-Gly² amide bond as both metabolically labile and amenable to modification with minimal loss of opioid activity (Figure 1C) [18]. Therefore, we elected to replace the Tyr¹-Gly² peptide bond of Leu-enkephalin with a CF₃-alkene, which might improve the pharmacokinetic and physicochemical properties of Leu-enkephalin. As part of our ongoing effort to probe the therapeutic potential of fluorinated peptidomimetics, we herein describe the synthesis of Tyr¹-ψ[CF₃C=CH]-Gly² peptidomimetic analogs of Leu-enkephalin (**2a-b**) [19-20]. These syntheses overcame the isomerization of the alkene that has likely limited the use of CF₃-alkenes as peptidomimetics.

2. Results and Discussion

Our retrosynthetic analysis of enkephalin-mimic **2** involved an initial peptide bond-disconnection to provide trifluoromethylalkene **10** and known tripeptide Gly-Phe-Leu-OR **11** (Scheme 1) [21]. Trifluoromethylalkene **10** could be constructed *via* a Wittig olefination reaction between trifluoromethylketone **6** and phosphonium ylide **7**. Trifluoromethylketone **6** would be accessed through a series of functional group transformations from commercially available *L*-tyrosine.

Initial unsuccessful attempts to prepare key building block, trifluoromethylketone **6** involved reactions of various tyrosine derivatives under Dakin–West reaction conditions using trifluoroacetic anhydride and pyridine (Scheme 2A) [22]. However, these reactions generally decomposed the substrate and produced complex mixtures of products. Next, using Kolb's modification of the Dakin–West reaction [23] we accessed only a hydrate form (**3a**) of the CF₃-ketone intermediate which under various conditions, failed to dehydrate to the corresponding ketone, and instead cyclized to produce oxazole derivatives (Scheme 2B) [24–25]. Subsequent attempts to couple CF₃-keto hydrate **3a** via Wittig reaction with ylide **7**, generally decomposed the substrate. Next, we explored a strategy involving the Ruppert–Prakash reagent (TMSCF₃), which can convert esters [26] or Weinreb amides [27] into trifluoromethyl ketones. However, these reactions generated inseparable undesired products (Scheme 2C), presumably by 1,2-addition of [−]CF₃ to the carbonyl of phthalimide. Over the course of this preliminary work, we realized that the above-mentioned general strategies would not afford the desired trifluoromethyl ketone.

Our revised synthesis of CF₃ ketone **6** involved nucleophilic addition of TMSCF₃ and subsequent oxidation as key steps. In practice, amino alcohol **4** (prepared according to reference 28, but also available commercially) was protected as a phthalimide, and subsequent Swern oxidation afforded tyrosine-derived aldehyde **5** (Scheme 2D) [28]. Next, we investigated the nucleophilic addition of TMSCF₃ to aldehyde **5** under various conditions. Reactions of **5** with TMSCF₃ in the presence of catalytic amount of TBAF at room temperature gave an inseparable mixture of products resulting from the competitive 1,2-addition of [−]CF₃ to the aldehyde and phthalimide carbonyl groups. However, lowering the reaction temperature to −78 °C, and slowing the addition of TBAF improved the 1,2-addition of [−]CF₃ to the aldehyde, and afforded a diastereomeric mixture of alcohols, which was oxidized using Dess–Martin periodinane (DMP) to provide CF₃-ketone **6**. However, compound **6** was obtained as a hydrate form that converted to CF₃-ketone **6** in 63% yield by refluxing for 2 h in dry toluene with molecular sieves (4 Å).

Subsequent effort enabled the conversion of CF₃-ketone **6** to the Tyr¹-ψ[CF₃C=CH]-Gly² dipeptide mimic by a sequence involving olefination, deprotection, and subsequent oxidation. First, CF₃ ketone **6** and triphenylphosphonium bromide-derived ylide **7** (prepared from the corresponding 1,3-propanediol) [29] were coupled using a Wittig olefination reaction (Scheme 3). Initial attempts to optimize the reaction to obtain high *Z*-selectivity failed over a range of temperatures (0 → −78 °C), with most of the conditions producing a 1:1 mixture of *E/Z* isomers (**8a–b**). Optimal yields were obtained by adding a THF solution of the CF₃-ketone to a solution of the *in-situ* generated ylide at −78 °C, followed by an

aqueous workup. Following this procedure, both benzyl (**8a**) and THP (**8b**) protected alkene derivatives were obtained in good yields, which were subsequently deprotected with BCl₃ or tetrabutylammonium tribromide (TBATB) respectively, to generate a 1:1 *E:Z* mixture of isomeric alcohols (**9a–b**), which were separated by SiO₂ gel chromatography.

Next, we investigated the oxidation of allylic alcohols **9** (both *E* and *Z*), bearing the sensitive CF₃–alkene, to provide acid **10**. Oxidation of **9a** (Scheme 3) with either the Dess–Martin periodinane or using Swern oxidation conditions generally decomposed the substrate, potentially *via* oxidation of the free phenol. In contrast, treatment of **9b** (R³ = benzyl) with Dess–Martin periodinane, followed by Pinnick oxidation provided an inseparable mixture of intermediate aldehyde and other undetermined side products. Subsequently, we explored a facile one-step oxidation of primary alcohol **9a** to **10a** using a CrO₃-catalyzed oxidation using H₅IO₆ in wet MeCN at 0 °C. In this reaction, the aldehyde intermediate may have been sufficiently short-lived to minimize decomposition [30]. Additionally, the mild acidic conditions presumably minimized isomerization of the trifluoromethylalkene, and other decomposition pathways. Using these conditions, **9a** converted to the corresponding acid **10a** in 30–33% yield while also leaving unreacted alcohol (~65%). Considering that free phenol might contribute to the low yield of the reaction, we subjected benzyl-protected *E*-**9b** and *Z*-**9b** to the same oxidation conditions, which provided improved yields of carboxylates *E*-**10b** and *Z*-**10b**.

Having accomplished the successful synthesis of Tyr-ψ[CF₃C=CH]-Gly dipeptide mimics **10**, the next task involved the peptide coupling reaction with the tripeptide (**11**) to form the desired trifluoromethylalkene containing Leu-enkephalin mimetic. For this step, mild conditions would be required to inhibit the base catalyzed isomerization of the CF₃–alkene to the thermodynamically favored α,β-unsaturated position. Hence, we optimized the reaction conditions to achieve the desired coupling product (Table 1). Using standard 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) coupling conditions, the attempted coupling of *Z*-**10a** and **11a** generated a complex mixture of unreacted acid *Z*-**10a**, along with isomerized product **13** and isomerized acid **14** (Table 1, entries 1–3 & 6). As the moderate acidity of the free phenol of *Z*-**9a** might catalyze the isomerization of the CF₃–alkene, we next evaluated the coupling reaction of benzyl protected analog *Z*-**10b**. Attempts to couple *Z*-**10b** with tripeptide **11a** using a variety of BOP [(benzotriazol-1-yl)oxy]tris(dimethylamino)phosphonium hexafluorophosphate]-derived coupling reagents (entries 4, 5, 7 & 8) provided similar results. Further optimization using dual functional reagents such as 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM, entry 9) [31] and 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT, entry 10) [32], that act as both *in-situ* acid activator and coupling reagents, was not fruitful.

Considering the low conversion and high recovery of isomerized peptide **13** and isomerized acid **14**, we investigated the cause of isomerization. As determined by ¹⁹F NMR and MS analysis, 67% of substrate *Z*-**10b** isomerized to **14** (12 h, rt, THF). However, subsection of the substrate to weak bases (e.g. imidazole) accelerated the isomerization, leaving no detectable substrate at 20 min. These results prompted us to explore less basic or base-free coupling conditions that might avoid the isomerization.

Further attempts to minimize isomerization included the use of base-free conditions and uronium salt-derived coupling reagents. Employment of a recent ynamide coupling reagent that avoids the use of base provided the desired product (**Z-12**) in low yield together with a mixture of isomerized peptide (**13**) and unreacted isomerized acid (**14**, entry 11) [33] though purification of this reaction mixture was not fruitful. Next, we explored various uronium salts as coupling reagents. The use of COMU [(1-cyano-2-ethoxy-2-oxoethylidenaminoxy)dimethylamino-morpholino-carbenium hexafluorophosphate] afforded 30% of the desired peptide **Z-12** (entry 12). Encouraged by this result, we screened other uronium salts. The use of HBTU [(2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] in DCM and THF provided the desired peptide **Z-12** in 34% (1 h) and 10% (12 h) respectively (entries 13–14). Surprisingly, the use of HCTU [2-(6-chloro-1-H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate] in DMF generated 80% of isomerized acid **14** with no evidence of productive coupling (entry 15). Conversely, changing the solvent to DCM afforded 87% of isomerized coupled peptide **13** (entry 16). Considering these results, a general solvent–isomerization relationship emerged, more polar solvents promoting the isomerization of the CF₃-alkene in **Z-10**, potentially because of the improved ability to stabilize the charged cation-anion pair (Figure 1B). Thus, we continued our optimization studies exclusively in DCM. In this solvent, HATU {1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate} finally provided coupled CF₃-alkene **Z-12** as the major product (64% yield, 2:1 selectivity with **13**, entry 17). Although this reaction provided ~60% of the desired product, chromatographic separation of **13** proved challenging, and a pure product was not obtained.

Subsequent efforts eventually provided optimized conditions for generating **Z-12** that minimized formation of isomerized products **13** and **14**. Specifically, treatment of acid **Z-10b** with excess thionyl chloride (SOCl₂) in anhydrous DCM at ambient temperature, followed by addition to **11a** at 0 °C readily afforded **Z-12b** in 78% yield (entry 20), but also produced 11% of the inseparable isomerized peptide **13**. To improve the ratio of products, we reversed the addition strategy (adding a mixture of base and tripeptide to the solution of acid chloride) and shortened the reaction time (3 h → 1 h) to provide **Z-12b** in 84% yield with only 4% of isomerized peptide **14** and 11% unreacted acid **Z-10b** (entry 21). With these conditions, the product mixture was separable by column chromatography (HPLC purity = 97.0%). Employing the same conditions, the other isomer **E-10b** was successfully coupled to afford the *t*-Bu ester variant of **E-12b** in 83% yield (HPLC purity = 99.5%; eq. 1).

Having obtained the CF₃-alkene Leu-enkephalin peptidomimetic, we briefly explored the late-stage removal of the phthalimide, benzyl ether, and C-terminal alkyl ester groups. However, our attempted efforts either decomposed the substrate or isomerized the trifluoromethylalkene [34-36]. We speculate that alternate protecting strategies that avoid basic conditions might provide the desired analogs.

3. Conclusion

In conclusion, we successfully coupled phthalimide protected Tyr- ψ [CF₃C=CH]-Gly dipeptide mimics to a C-terminal tripeptide to obtain a new protected Leu-enkephalin analog. The optimized mild conditions minimize isomerization of the double bond from the β,γ -position to thermodynamically favored α,β -position. The amide coupling reactions delivered products bearing both acid-labile and base-labile protecting groups, which provides multiple strategies for late stage deprotection of the final peptides. Most importantly, these successful coupling reactions suggest that, with appropriate optimization, other CF₃-alkene peptidomimetics might be effectively coupled to access target analogs of a lead peptide. Ongoing work in our laboratory focuses on deprotecting both the C- and N-termini to provide analogs of Leu-enkephalin, and on characterizing pharmacodynamic and physicochemical perturbations imparted by the trifluoromethyl alkene isopolar mimic.

4. Experimental Section

4.1. General Methods

Unless otherwise noted, all reactions were performed using oven-dried glassware under an atmosphere of dry N₂, which were sealed with rubber septa. Stainless steel syringes were used to transfer air or moisture-sensitive liquid reagents. Reactions were monitored by thin-layer chromatography (TLC) on UNIPLATE Silica Gel HLF 250 μ m glass plates precoated with 230–400 mesh silica impregnated with a fluorescent indicator (250 nm), visualizing by quenching of fluorescence, KMnO₄ solution, or *p*-anisaldehyde solution. A Teledyne Isco CombiFlash® RF-4x purification system was used for chromatographic purifications, silica gel was purchased from Sorbent Technologies (cat. #30930M-25, 60 Å, 40–63 μ m). Commercial reagents were purchased and used as received. Anhydrous toluene (PhMe), acetonitrile (CH₃CN), methanol (MeOH), dichloromethane (DCM), tetrahydrofuran (THF), and triethylamine (NEt₃) were dispensed from a solvent purification system, in which the solvent was dried by passage through two columns of activated alumina under argon.

Proton nuclear magnetic resonance (¹H NMR) spectra were recorded at 400 or 500 MHz. Carbon nuclear magnetic resonance (¹³C NMR) spectra were recorded at 101 or 126 MHz. Fluorine nuclear magnetic resonance (¹⁹F NMR) spectra were recorded at 376 MHz. Chemical shifts (δ) for protons are reported in parts per million (ppm) down-field from trimethylsilane and are referenced to the proton resonance of residual CHCl₃ in the NMR solvent (δ = 7.27 ppm). Chemical shifts (δ) for carbon are reported in parts per million down-field from trimethylsilane and are referenced to the carbon resonances of the solvent peak (δ = 77.16 ppm). Chemical shifts (δ) for fluorine are reported in parts per million up-field from trichlorofluoromethane and are referenced to PhCF₃ (δ = –63.72 ppm). NMR data are represented as follows: chemical shift (ppm), multiplicity (s = singlet, d = doublet, t

= triplet, q = quartet, sept = septet, m = multiplet), coupling constants in hertz (Hz), and integration.

Exact mass determinations were obtained by: electrospray ionization (ESI) using a TOF mass analyzer (Waters LCT Premiere). Melting points are uncorrected and were measured on a Thomas–Hoover Capillary melting point apparatus. Optical rotation measurements were made on a Perkin–Elmer 241 polarimeter and are quoted in units of 10^{-1} deg cm² g⁻¹. Infrared spectra were measured using a Shimadzu FTIR–8400S Fourier Transform Infrared Spectrometer. HPLC preparative purification was performed using Chiralpak IC Acquity T3 C–18 column by using 0.02% formic acid, acetonitrile mobile phases, purity was determined by UV area % from HPLC analysis.

4.2. (S)–2–(1–(4–(Benzyloxy)phenyl)–3–hydroxypropan–2–yl)isoindoline–1,3–dione (4a)

Phthalic anhydride (0.82 g, 5.5 mmol) and NEt₃ (1.0 mL, 7.7 mmol) were sequentially added to a solution of (S)–2–Amino–3–(4–(benzyloxy)phenyl)propan–1–ol **4** (1.30 g, 5.0 mmol) in toluene (15 mL). The reaction was refluxed for 6 h. After the completion of the reaction, the organic solvents were removed under reduced pressure to provide a sticky oily residue. The resulting residue was purified by silica gel-column chromatography (EtOAc/hexanes, 20 % to 30 %) to obtain the title compound **4a** as a white solid (1.03 g, 53%); m.p. 119–121 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.85–7.67 (m, 4H), 7.43–7.30 (m, 5H), 7.19–7.12 (m, 2H), 6.90–6.81 (m, 2H), 5.00 (s, 2H), 4.62 (qd, *J* = 7.9, 3.5 Hz, 1H), 4.14–3.90 (m, 2H), 3.17 (d, *J* = 8.1 Hz, 2H), 2.66 (s, 1H); ¹³C NMR (126 MHz, CDCl₃) δ 169.0, 157.5, 137.0, 134.1, 131.6, 130.0, 129.7, 128.5, 127.9, 127.5, 123.3, 114.9, 69.9, 62.8, 55.3, 33.9; [α]_D²⁴ = –130.8 (*c* 0.73, CHCl₃); IR (film) 3496, 3468, 2943, 2883, 1770, 1697, 1614, 1512, 1398, 1369, 1253, 1174, 1120, 1018, 8733, 721 cm⁻¹; HRMS (ESI): *m/z* [M+Na]⁺ calc'd for C₂₄H₂₁NNaO₄: 410.1363; found 410.1370.

4.3. (S)–3–(4–(Benzyloxy)phenyl)–2–(1,3–dioxoisindolin–2–yl)propanal (5)

To a solution of oxalyl chloride (0.66 mL, 7.7 mmol) in DCM (10 mL) was added DMSO (1.1 mL, 15.4 mmol) at –78 °C, and the resulting solution was stirred at the same temperature for 30 minutes. Then, a solution of **4a** (2.0 g, 5.1 mmol) in DCM (20 mL) was added dropwise to the reaction mixture. The reaction mixture was stirred at –78 °C for 2 h, followed by the addition of NEt₃ (3.5 mL, 25.5 mmol). The cooling bath was removed after 5 min, and the reaction mixture was allowed to warm to room temperature and stirred for 2 h. The reaction was quenched with 10% aqueous citric acid solution (10 mL), and the mixture was stirred at ambient temperature for an additional 10 min and extracted with DCM (2 X 25 mL). The organic layer was washed with 1.0 N aqueous HCl solution (15 mL), followed by brine, dried over anhydrous MgSO₄, and evaporated *in vacuo*. The residue was crystallized in diethyl ether to get the title compound **5** as a white solid (1.71 g, 86 %). m.p. 152–156 °C; ¹H NMR (400 MHz, CDCl₃) δ 9.79 (s, 1H), 7.79 (ddd, *J* = 36.3, 5.5, 3.1 Hz, 4H), 7.44–7.29 (m, 5H), 7.14–7.07 (m, 2H), 6.88–6.81 (m, 2H), 4.99 (s, 2H), 4.96 (dd, *J* = 10.7, 5.6 Hz, 1H), 3.54 (dd, *J* = 14.4, 5.6 Hz, 1H), 3.33 (dd, *J* = 14.4, 10.7 Hz, 1H); ¹³C NMR (126 MHz, CDCl₃) δ 196.3, 167.6, 157.7, 136.8, 134.3, 131.5, 129.9, 128.5, 128.2, 127.9, 115.0, 69.9, 60.1, 32.2; [α]_D²⁴ = –137.6 (*c* 0.51, CHCl₃); IR (film) 2926, 1772, 1737, 1710, 1681, 1614, 1583, 1514, 1467, 1450, 1386, 1334, 1313, 1300, 1255,

1178, 1066, 1028, 721 cm^{-1} ; HRMS (ESI): m/z $[\text{M}-\text{H}]^-$ calc'd for $\text{C}_{24}\text{H}_{18}\text{NO}_4$: 384.1236; found 384.1276.

4.4. (S)-2-(1-(4-(Benzyloxy)phenyl)-4,4,4-trifluoro-3-oxobutan-2-yl)isoindoline-1,3-dione (6)

To a solution of **5** (1.0 g, 2.6 mmol) in THF (10 mL) at -78°C was added TMSCF_3 (1.2 mL, 7.8 mmol), followed by catalytic TBAF (1.0 M in THF). The reaction was stirred at room temperature for 3 h. After complete disappearance of the aldehyde, desilylation was carried out by adding 4.0 N aqueous HCl (0.5 mL) and stirred at ambient temperature for 5 h. The resulting product was extracted with EtOAc. The organic layer was washed with brine solution, dried over anhydrous MgSO_4 , and evaporated *in vacuo*. The residue was crystallized in EtOAc to provide the 1,2-addition product in 59% yield (0.70 g), which was subsequently oxidized as described below.

To a solution of 2-((2S)-1-(4-(benzyloxy)phenyl)-4,4,4-trifluoro-3-hydroxybutan-2-yl)isoindoline-1,3-dione (0.70 g, 1.5 mmol) in dry DCM (20 mL) was added Dess–Martin periodinane (0.78 g, 1.8 mmol), and the mixture was stirred at ambient temperature for 3 h. The reaction mixture was then diluted with DCM (5 mL) and poured into a 0.26 M solution of sodium thiosulfate in saturated aqueous sodium bicarbonate. The layers were separated, and the organic phase was washed sequentially with saturated aqueous sodium bicarbonate and water. The combined organic phases were dried over anhydrous MgSO_4 , filtered, and the solvent was removed *in vacuo*. The crude compound was purified by silica-gel column chromatography (EtOAc/hexanes, 10% to 30%) to get a hydrate form of (S)-2-(1-(4-(benzyloxy)phenyl)-4,4,4-trifluoro-3-oxobutan-2-yl)isoindoline-1,3-dione. The obtained hydrate was refluxed in toluene (10 mL) in the presence of molecular sieves (4 Å) for 2 h. After cooling to rt, and filtering off the sieves, the solvent was evaporated to afford (S)-2-(1-(4-(benzyloxy)phenyl)-4,4,4-trifluoro-3-oxobutan-2-yl)isoindoline-1,3-dione as a white solid (0.7 g, 95%). m.p. $131\text{--}134^\circ\text{C}$; ^1H NMR (400 MHz, CDCl_3) δ 7.78–7.57 (m, 4H), 7.32–7.18 (m, 5H), 6.97 (d, $J = 8.4$ Hz, 2H), 6.75–6.67 (m, 2H), 5.31 (dd, $J = 10.2, 5.5$ Hz, 1H), 4.86 (s, 2H), 3.38 (qd, $J = 14.4, 7.9$ Hz, 2H); ^{13}C NMR (126 MHz, CDCl_3) δ 186.0 (q, $J = 35.1$ Hz), 166.8, 157.9, 136.7, 134.5, 131.1, 130.0, 128.5, 127.9, 127.4, 127.2, 117.2 (q, $J = 295.9$ Hz), 93.9 (q, $J = 32.3$ Hz), 69.9, 55.7, 32.1; ^{19}F NMR (376 MHz, CDCl_3) δ –75.33 (s, 3F); $[\alpha]_D^{24} = -6.6$ (c 0.65, CHCl_3); IR (film) 3367, 3034, 1768, 1720, 1701, 1610, 1583, 1512, 1467, 1452, 1383, 1247, 1178, 968, 719 cm^{-1} ; HRMS (ESI): m/z $[\text{M}-\text{H}]^-$ calc'd for $\text{C}_{25}\text{H}_{17}\text{NF}_3\text{NO}_4$: 452.1104; found 452.1099.

4.5. General Procedure A:

To a solution of triphenylphosphonium bromide derivative **7** (1.5 equiv.) in THF (0.04 M) at -78°C was added a solution of *n*-butyl lithium (2.5 M in hexane, 1.5 equiv.). After stirring for 1 h at the same temperature, a solution of CF_3 -ketone (1.0 equiv.) in THF (0.05 M) was added slowly over a period of 10–15 min, and the reaction was stirred at -78°C for 1 h. The cooling bath was removed, and the reaction mixture was allowed to warm to ambient temperature and stir overnight. Saturated aqueous NH_4Cl was added to the reaction, and the mixture was extracted twice with EtOAc. The organic layer was washed with brine solution,

dried over anhydrous MgSO_4 , and evaporated *in vacuo* to afford 1:1 *E/Z* mixture of trifluoromethylalkene products **8**.

4.5.1. (S)-2-(6-(Benzyloxy)-1-(4-(benzyloxy)phenyl)-3-(trifluoromethyl)hex-3-en-2-yl)isoindoline-1,3-dione (8a-Bn)—Following general procedure A, 2.40 g (4.9 mmol) of compound **7a** was reacted with 1.50 g (3.3 mmol) of **6**. Workup and purification by column chromatography (EtOAc/hexane, 20% to 30%) afforded the title compound as a colorless oil (1.20 g, 62%): ^1H NMR (400 MHz, CDCl_3) δ 7.84–7.65 (m, 4H), 7.48–7.28 (m, 10H), 7.12 (dd, $J = 27.6, 8.6$ Hz, 2H), 6.83 (dd, $J = 24.0, 8.6$ Hz, 2H), 6.69–6.43 (m, 1H), 5.36 (ddd, $J = 89.1, 10.0, 6.0$ Hz, 1H), 4.98 (d, $J = 12.7$ Hz, 2H), 4.57–4.49 (m, 2H), 3.85–3.16 (m, 4H), 2.71 (d, $J = 15.5$ Hz, 2H); ^{13}C NMR (126 MHz, CDCl_3) δ 168.0, 167.6, 157.6, 138.9 (q, $J = 3.0$ Hz), 137.2 (q, $J = 6.4$ Hz), 133.9, 131.6, 131.4, 130.1, 129.9, 128.5, 128.4, 127.9, 127.7, 127.6, 124.8 (q, $J = 276.3$ Hz), 114.8, 72.9, 69.9, 69.8, 68.7, 68.0, 50.8, 50.6, 35.2, 29.0, 28.1, 25.6; ^{19}F NMR (376 MHz, CDCl_3) δ -59.80 (3F), -62.45 (3F); $[\alpha]_{\text{D}}^{24} = -10.7$ (c 0.41, CHCl_3); IR (film) 3064, 3032, 1776, 1714, 1610, 1512, 1454, 1384, 1354, 1244, 1174, 1118, 1026, 875, 717, 698, 530 cm^{-1} ; HRMS (ESI): m/z $[\text{M}+\text{Na}]^+$ calc'd for $\text{C}_{35}\text{H}_{30}\text{F}_3\text{NNaO}_4$ 608.2019, found 608.2012.

4.5.2. 2-((2S)-1-(4-(Benzyloxy)phenyl)-6-((tetrahydro-2H-pyran-2-yl)oxy)-3-(trifluoromethyl)hex-3-en-2-yl)isoindoline-1,3-dione (8b-OTHP)—Following general procedure A, 2.10 g (5.2 mmol) of **7b**, was reacted with 1.60 g (3.5 mmol) of **6**. Workup and purification by column chromatography (EtOAc/Hexane, 20% to 30%) afforded the title compound as a colorless oil (1.50 g, 87%): ^1H NMR (400 MHz, CDCl_3) δ 7.82–7.66 (m, 4H), 7.44–7.29 (m, 5H), 7.21–7.08 (m, 2H), 6.90–6.79 (m, 2H), 6.69–6.43 (m, 1H), 5.37 (ddd, $J = 94.7, 10.3, 6.0$ Hz, 1H), 5.03–4.96 (m, 2H), 4.70–4.59 (m, 1H), 3.96–3.03 (m, 5H), 2.74–2.35 (m, 2H), 1.93–1.43 (m, 6H); ^{13}C NMR (126 MHz, CDCl_3) δ 168.0, 167.6, 157.6, 138.9, 138.8, 137.3, 137.2, 136.9, 136.8, 133.9, 131.6, 130.1, 129.9, 129.1, 128.5, 124.8 (q, $J = 274.3$ Hz), 114.8, 98.8, 69.9, 65.8, 65.4, 62.3, 62.0, 50.7, 35.3, 30.5, 29.0, 28.2, 25.4, 19.4, 19.2; ^{19}F NMR (376 MHz, CDCl_3) δ -59.78 (dd, $J = 19.0, 2.6$ Hz, 3F), -62.52 (d, $J = 10.4$ Hz, 3F); $[\alpha]_{\text{D}}^{24} = -0.86$ (c 0.46, CHCl_3); IR (film) 2945, 2359, 2341, 1712, 1691, 1678, 1629, 1583, 1562, 1546, 1512, 1383, 1352, 1330, 1244, 1165, 1118, 1033, 970, 875, 731, 717 cm^{-1} ; HRMS (ESI): m/z $[\text{M}+\text{Na}]^+$ calc'd for $\text{C}_{33}\text{H}_{32}\text{F}_3\text{NNaO}_5$: 602.2072, found 602.2084.

4.6.1 (S)-2-(6-Hydroxy-1-(4-hydroxyphenyl)-3-(trifluoromethyl)hex-3-en-2-yl)isoindoline-1,3-dione (9a)—To a stirred solution of (*S*)-2-(6-(Benzyloxy)-1-(4-(benzyloxy)phenyl)-3-(trifluoromethyl)hex-3-en-2-yl)isoindoline-1,3-dione **8a-Bn** (0.50 g, 0.8 mmol) and pentamethylbenzene (0.37 g, 2.5 mmol) in dry DCM (5 mL) was added BCl_3 (1.0 M in DCM, 2.5 mL, 2.5 mmol) dropwise over 10 min via syringe at -78°C . After 30 min, the temperature was brought to -30°C and the reaction stirred for 1h. The progress of the reaction was monitored by TLC, and after the complete consumption of the starting materials, the temperature brought back to -78°C and quenched with CHCl_3 -MeOH (10:1, 20 mL). The resulting mixture was warmed to room temperature. The excess organic solvents were removed under reduced pressure. The residue was purified by column chromatography (MeOH/DCM, 5% to 10%) to separate *E* and *Z* isomers of **9a**.

4.6.1 (S,Z)-2-(6-Hydroxy-1-(4-hydroxyphenyl)-3-(trifluoromethyl)hex-3-en-2-yl)isoindoline-1,3-dione (Z-9a)—32% yield; White solid, m.p. 132–135 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.16 (s, 1H), 7.91 – 7.75 (m, 4H), 6.95 – 6.90 (m, 2H), 6.69 (t, *J* = 7.5 Hz, 1H), 6.57 – 6.48 (m, 2H), 5.07 (dd, *J* = 11.0, 4.9 Hz, 1H), 4.80 (t, *J* = 5.3 Hz, 1H), 3.54 (td, *J* = 6.5, 5.3 Hz, 2H), 3.38 (dd, *J* = 13.6, 11.0 Hz, 1H), 3.13 (dd, *J* = 13.5, 5.0 Hz, 1H), 2.50 (d, *J* = 1.9 Hz, 2H); ¹³C NMR (126 MHz, DMSO-*d*₆) δ 167.4, 156.3, 140.4 (q, *J* = 3.3 Hz), 135.2, 130.9, 130.2, 127.3, 126.1 (q, *J* = 27.2 Hz), 125.4, 124.3 (q, *J* = 276.8 Hz), 123.6, 115.5, 60.0, 50.8, 34.7, 32.4; ¹⁹F NMR (376 MHz, DMSO-*d*₆) δ –58.49 (s, 3F); [α]_D²⁴ = –5.1 (*c* 0.62, MeOH); IR (film) 3350, 3131, 1770, 1708, 1683, 1558, 1519, 1379, 1180, 1114, 1041, 713, 682 cm^{–1}; HRMS (ESI): *m/z* [M–H][–] calc'd for C₂₁H₁₇F₃NO₄: 404.1110, found 404.1119.

4.6.2. (S,E)-2-(6-Hydroxy-1-(4-hydroxyphenyl)-3-(trifluoromethyl)hex-3-en-2-yl)isoindoline-1,3-dione (E-9a)—38% yield; White solid, m.p. 136–140 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.28 (s, 1H), 7.90 (s, 4H), 7.10–7.02 (m, 2H), 6.71–6.64 (m, 2H), 6.53 (td, *J* = 7.1, 1.7 Hz, 1H), 5.42 (dd, *J* = 9.3, 7.1 Hz, 1H), 4.83 (t, *J* = 5.2 Hz, 1H), 3.72 (dd, *J* = 13.7, 9.4 Hz, 1H), 3.54–3.44 (m, 2H), 3.39–3.31 (m, 1H), 2.56–2.29 (m, 2H); ¹³C NMR (126 MHz, DMSO-*d*₆) δ 167.9, 156.4, 139.0 (q, *J* = 6.5 Hz), 135.3, 135.0, 131.4, 131.1, 130.3, 130.2, 128.6, 127.9, 126.0 (q, *J* = 26.2 Hz), 125.6 (q, *J* = 274.5 Hz), 123.6, 115.6, 115.5, 59.3, 50.9, 34.9, 31.2; ¹⁹F NMR (376 MHz, DMSO-*d*₆) δ –62.80 (s, 3F); [α]_D²⁴ = 1.08 (*c* 0.46, CHCl₃); IR (film) 3317, 3180, 1778, 2873, 1708, 1654, 1599, 1522, 1412, 1315, 1265, 1244, 1114, 1072, 905, 806, 698, 656 cm^{–1}; HRMS (ESI): *m/z* [M–H][–] calc'd for C₂₁H₁₇F₃NO₄: 404.1110, found 404.1129.

4.7. (S,Z)-2-(1-(4-(Benzyloxy)phenyl)-6-hydroxy-3-(trifluoromethyl)hex-3-en-2-yl)isoindoline-1,3-dione (9b)

2-((2S)-1-(4-(Benzyloxy)phenyl)-6-((tetrahydro-2H-pyran-2-yl)oxy)-3-(trifluoromethyl)hex-3-en-2-yl)isoindoline-1,3-dione **8b-OTHP** (0.75 g, 1.3 mmol) in methanol (20 mL) was added TBATB (0.12 g, 0.2 mmol) at ambient temperature and stirred for 2.5 h. The reaction was quenched with acetone, diluted with EtOAc and washed with 5% aqueous solution of sodium bisulfite. The organic phase was washed with brine, dried over MgSO₄, filtered, evaporated and purified by column chromatography (MeOH/DCM, 1% to 2%) to provide isomers of compound **9b**.

4.7.1. (S,Z)-2-(1-(4-(Benzyloxy)phenyl)-6-hydroxy-3-(trifluoromethyl)hex-3-en-2-yl)isoindoline-1,3-dione (Z-9b)—30% yield; Clear oil; ¹H NMR (400 MHz, CDCl₃) δ 7.76 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.69 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.41 – 7.35 (m, 4H), 7.35 – 7.29 (m, 1H), 7.09 (d, *J* = 8.3 Hz, 2H), 6.82 (d, *J* = 8.4 Hz, 2H), 6.63 (t, *J* = 7.8 Hz, 1H), 5.27 (dd, *J* = 11.0, 5.2 Hz, 1H), 4.98 (s, 2H), 3.83 (td, *J* = 6.0, 2.3 Hz, 2H), 3.54 (dd, *J* = 13.8, 10.9 Hz, 1H), 3.23 (dd, *J* = 13.8, 5.2 Hz, 1H), 2.83 – 2.56 (m, 2H); ¹³C NMR (126 MHz, CDCl₃) δ 167.6, 157.6, 139.1 (q, *J* = 3.1 Hz), 136.8, 134.0, 131.3, 129.9, 127.9 (q, *J* = 28.0 Hz), 128.8, 128.5, 127.9, 127.4, 123.6 (q, *J* = 277.0 Hz), 123.3, 114.9, 69.8, 61.6, 50.5, 35.1, 31.9; ¹⁹F NMR (376 MHz, CDCl₃) δ –59.52 (s, 3F); [α]_D²⁴ = –0.55 (*c* 0.54, MeOH); IR (film) 3518, 3475, 2931, 2879, 1776, 1712, 1610, 1512, 1467, 1454, 1379, 1354, 1244,

1174, 1116, 1039, 964, 910, 873, 790, 717, 696, 680 cm^{-1} ; HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calc'd for $\text{C}_{28}\text{H}_{25}\text{F}_3\text{NO}_4$: 496.1657, found 496.1665.

4.7.2. (S,E)-2-(1-(4-(Benzyloxy)phenyl)-6-hydroxy-3-(trifluoromethyl)hex-3-en-2-yl)isoindoline-1,3-dione (E-9b)—41% yield; Clear oil; ^1H NMR (400 MHz, CDCl_3) δ 7.76 (ddd, J = 39.1, 5.5, 3.1 Hz, 4H), 7.47–7.29 (m, 5H), 7.21–7.14 (m, 2H), 6.92–6.86 (m, 2H), 6.42 (tq, J = 8.2, 1.6 Hz, 1H), 5.46 (t, J = 8.2 Hz, 1H), 5.03 (s, 2H), 3.78 (dd, J = 14.1, 8.5 Hz, 1H), 3.68 (td, J = 6.8, 6.0, 3.8 Hz, 2H), 3.57 (dd, J = 14.0, 8.1 Hz, 1H), 2.70–2.21 (m, 2H), 1.87–1.74 (m, 1H); ^{13}C NMR (126 MHz, CDCl_3) δ 168.2, 157.6, 137.3 (d, J = 6.4 Hz), 136.9, 134.1, 131.5, 130.3, 129.7, 128.5, 127.9, 127.3, 127.1 (q, J = 27.8 Hz), 127.0, 123.8 (q, J = 275.1 Hz), 123.3, 114.9, 69.9, 61.0, 51.2, 35.4, 30.8; ^{19}F NMR (376 MHz, CDCl_3) δ -62.51 (s, 3F); $[\alpha]_{\text{D}}^{24}$ = 1.1 (c 0.49, CHCl_3); IR (film) 3518, 3475, 2931, 2879, 1776, 1712, 1610, 1512, 1467, 1454, 1379, 1354, 1244, 1174, 1116, 1039, 964, 910, 873, 790, 717, 696, 680 cm^{-1} ; HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calc'd for $\text{C}_{28}\text{H}_{25}\text{F}_3\text{NO}_4$: 496.1657, found 496.1624.

4.8. General Procedure B

To a solution of (*S*)-2-(6-Hydroxy-1-(4-hydroxyphenyl)-3-(trifluoromethyl)hex-3-en-2-yl)isoindoline-1,3-dione **Z-9a** (0.10 g, 0.2 mmol, 1.0 equiv.) in wet acetonitrile (0.5 M), (0.75 v % water), a stock solution of $\text{H}_5\text{IO}_6/\text{CrO}_3$ (1.2 mL, 0.4 mmol, 2.0 equiv.) [prepared by dissolving H_5IO_6 (11.40 g, 50.0 mmol) and CrO_3 (0.020 g, 1.2 mol %) in wet MeCN (0.75 v % water) to a volume of 114 mL] was added slowly at 0 °C for 30–60 minutes at 0 °C. The mixture was stirred at 0 °C for 0.5 h and the completion of the reaction was confirmed by ^{19}F NMR. The reaction was quenched with aqueous solution of Na_2HPO_4 (0.60 g in 10 mL of water). The reaction mixture was extracted with EtOAc (2 X 12 mL) and the organic layer was separated and washed with aqueous NaHSO_3 solution (15 mL) followed by brine (2 X 10 mL). The organic layer was concentrated *in vacuo*, and the resulting residue which was purified by silica-gel column chromatography.

4.8.1. (S, Z)-5-(1,3-Dioxoisindolin-2-yl)-6-(4-hydroxyphenyl)-4-(trifluoromethyl)hex-3-enoic acid (Z-10a)—Following general procedure B, **Z-9a** (0.10 g, 0.2 mmol, 1.0 equiv.) was reacted with stock solution of $\text{H}_5\text{IO}_6/\text{CrO}_3$ (1.2 mL, 0.4 mmol, 2.0 equiv.) Workup and chromatographic purification (MeOH/DCM, 1% to 2%) afforded compound **Z-10a** as an oil (0.03 g, 32%): ^1H NMR (400 MHz, Methanol- d_4) δ 7.78 (s, 4H), 6.99 – 6.90 (m, 3H), 6.60 – 6.56 (m, 2H), 5.20 (dd, J = 11.5, 4.8 Hz, 1H), 3.60 – 3.37 (m, 3H), 3.20 (dd, J = 13.6, 4.8 Hz, 1H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 171.4, 170.8, 167.4, 166.2, 156.4, 137.0, 135.5, 135.3, 130.9, 130.3, 130.0 (d, J = 28.6 Hz), 127.2, 127.1, 127.0, 126.9, 125.1 (q, J = 276.6 Hz), 123.7, 122.9, 115.6, 60.2, 50.6, 34.7, 33.7, 21.2, 14.5; ^{19}F NMR (376 MHz, CDCl_3) δ -59.14 (s, 3F); $[\alpha]_{\text{D}}^{24}$ = + 0.86 (c 0.65, CHCl_3); IR (film) 3745, 2360, 1770, 1708, 1683, 1558, 1519, 1379, 1361, 1259, 1190, 873, 837, 738, 713 cm^{-1} ; HRMS (ESI): m/z $[\text{M}-\text{H}]^-$ calcd for $\text{C}_{21}\text{H}_{15}\text{F}_3\text{NO}_5$: 418.0908, found 418.0887.

4.8.2. (S, E)-5-(1,3-Dioxoisindolin-2-yl)-6-(4-hydroxyphenyl)-4-(trifluoromethyl)hex-3-enoic acid (E-10a)—General procedure B was followed using (*S, E*)-2-(6-Hydroxy-1-(4-hydroxyphenyl)-3-(trifluoromethyl)hex-3-en-2-

yl)isoindoline-1,3-dione **E-9a** (0.50 g, 1.2 mmol) and added stock solution of $\text{H}_5\text{IO}_6/\text{CrO}_3$ (7.3 mL, 2.4 mmol). Workup and chromatographic purification (MeOH/DCM, 1% to 2%) afforded compound **E-10a** as an oil (0.17 g, 33%); ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 12.70 (s, 1H), 9.24 (s, 1H), 7.85 (s, 4H), 7.04 – 6.97 (m, 2H), 6.66 – 6.58 (m, 3H), 5.24 (dd, J = 9.5, 6.7 Hz, 1H), 3.68 – 3.41 (m, 2H), 3.33 – 3.19 (m, 2H); ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 171.4, 167.7, 166.5, 156.5, 135.3, 134.6, 134.6, 134.5 (q, J = 3.0 Hz), 131.1, 130.3, 130.1 (q, J = 25.6 Hz), 130.0, 127.6, 126.8, 126.6, 125.3 (q, J = 277.3 Hz), 123.7, 123.5, 115.6, 115.4, 67.4, 50.7, 34.6, 33.1, 32.7, 25.5; ^{19}F NMR (376 MHz, $\text{DMSO}-d_6$) δ -61.75 (s, 3F); $[\alpha]_D^{24}$ = -0.88 (c 0.65, CH_2Cl_2); IR (film) 3748, 2889, 2343, 1770, 1708, 1683, 1558, 1519, 1379, 1361, 1259, 1190, 873, 837, 756, 713, 682 cm^{-1} .

4.8.3. (S,Z)-6-(4-(Benzyloxy)phenyl)-5-(1,3-dioxoisoindolin-2-yl)-4-(trifluoromethyl)hex-3-enoic acid (Z-10b)—General procedure B was followed using (S,Z)-2-(1-(4-(Benzyloxy)phenyl)-6-hydroxy-3-(trifluoromethyl)hex-3-en-2-yl)isoindoline-1,3-dione **Z-9b** (0.10 g, 0.2 mmol) and a stock solution of $\text{H}_5\text{IO}_6/\text{CrO}_3$ (1.2 mL, 0.4 mmol). Workup and chromatographic purification (MeOH/DCM, 1% to 2%) afforded the title compound as colorless oil (0.07 g, 70%); ^1H NMR (400 MHz, CDCl_3) δ 7.77 (ddd, J = 38.0, 5.5, 3.1 Hz, 4H), 7.47 – 7.29 (m, 5H), 7.16 (d, J = 8.2 Hz, 2H), 6.89 (d, J = 8.2 Hz, 2H), 6.63 (t, J = 6.9 Hz, 1H), 5.32 (t, J = 8.2 Hz, 1H), 5.02 (s, 2H), 3.73 (dd, J = 14.1, 8.7 Hz, 1H), 3.61 – 3.50 (m, 2H), 3.33 – 3.21 (m, 1H); ^{13}C NMR (126 MHz, CDCl_3) δ 174.3, 167.8, 157.7, 136.8, 134.1, 131.7, 131.6, 131.5, 131.4 (d, J = 6.4 Hz), 130.3, 130.1, 129.2, 128.5, 128.2, 128.0 (d, J = 27.6 Hz), 127.9, 127.8, 127.4, 126.8, 125.0 (d, J = 275.0 Hz), 124.6, 122.4, 120.2, 115.0, 114.9, 69.9, 61.0, 51.2, 50.8, 35.1, 32.1, 30.7; ^{19}F NMR (376 MHz, CDCl_3) δ -63.16 (s, 3F), $[\alpha]_D^{24}$ = -1.3 (c 0.44, CH_2Cl_2); IR (film) 3034, 2827, 1772, 1716, 1508, 1456, 1375, 1361, 1329, 1317, 1301, 1286, 1184, 1120, 1097, 1085, 1045, 837, 738, 711, 696, 530, 434 cm^{-1} ; HRMS (ESI): m/z $[\text{M}-\text{H}]^-$ calc'd for $\text{C}_{28}\text{H}_{21}\text{F}_3\text{NO}_5$: 508.1377, found 508.1370.

4.8.4. (S,E)-6-(4-(Benzyloxy)phenyl)-5-(1,3-dioxoisoindolin-2-yl)-4-(trifluoromethyl)hex-3-enoic acid (E-10b)—General procedure B was followed using (S,E)-2-(1-(4-(Benzyloxy)phenyl)-6-hydroxy-3-(trifluoromethyl)hex-3-en-2-yl)isoindoline-1,3-dione **E-9b** (0.66 g, 1.3 mmol) and added stock solution of $\text{H}_5\text{IO}_6/\text{CrO}_3$ (7.9 mL, 3.3 mmol). Workup and chromatographic purification (DCM/MeOH 1:0 $\rightarrow$ 9.8:0.2) afforded the title compound as an oil (0.45 g, 67%); ^1H NMR (400 MHz, CDCl_3) δ 7.73 (ddt, J = 26.7, 5.6, 3.1 Hz, 4H), 7.43 – 7.29 (m, 5H), 7.10 (d, J = 8.3 Hz, 2H), 6.87 (t, J = 7.1 Hz, 1H), 6.81 (d, J = 8.3 Hz, 2H), 5.27 (dd, J = 11.2, 5.0 Hz, 1H), 4.98 – 4.94 (m, 2H), 3.68 – 3.51 (m, 3H), 3.26 (dd, J = 13.8, 5.1 Hz, 1H); ^{13}C NMR (126 MHz, CDCl_3) δ 174.4, 167.5, 157.6, 136.8, 134.0, 132.4 (q, J = 3.0 Hz), 132.3, 131.3, 129.9, 129.2 (q, J = 27.2 Hz), 128.9, 128.6, 128.5, 127.9, 127.4, 126.7, 124.5 (q, J = 277.3 Hz), 123.3, 122.3, 120.1, 114.9, 69.8, 50.4, 35.1, 32.8, 14.2; ^{19}F NMR (376 MHz, CDCl_3) δ -60.49 (s, 3F); $[\alpha]_D^{24}$ = -1.3 (c 0.6, CH_2Cl_2); IR (film) 3034, 2827, 1772, 1716, 1508, 1456, 1375, 1361, 1329, 1317, 1301, 1286, 1184, 1120, 1097, 1085, 1045, 837, 738, 711, 696, 530, 434 cm^{-1} ; HRMS (ESI): m/z $[\text{M}+\text{HCOOH}]^+$ calc'd for $\text{C}_{28}\text{H}_{22}\text{F}_3\text{NO}_5\text{HCOOH}$: 555.1505, found 555.1493.

4.9. Procedure for Peptide Coupling

To a solution of (*S*)-6-(4-(benzyloxy)phenyl)-5-(1,3-dioxoisindolin-2-yl) β 4-(trifluoromethyl)hex-3-enoic acid **10b** (0.10 g, 0.2 mmol, 1.0 equiv.) in DCM (6 mL) was added thionyl chloride (0.028 mL, 0.4 mmol, 2.0 equiv.) at ambient temperature and the progress of the reaction was monitored by ^{19}F NMR. After completion of the reaction, the solvent was evaporated under reduced pressure. The obtained residue is dissolved in dry DCM (6 mL), added NEt_3 (0.18 mL, 0.5 mmol, 2.5 equiv) and Gly-Phe-Leu-OR⁴ tripeptide **11** (0.15 g, 0.4 mmol, 2.0 equiv.) in DCM (5 mL) at 0 °C and the reaction mixture is stirred at the same temperature for 1 h. After the completion, quenched with 1N aqueous HCl, extracted with EtOAc, and washed with saturated NaHCO_3 solution. The combined organic phases were washed with brine, dried over MgSO_4 , filtered, evaporated and purified by silica gel chromatography (EtOAc/Hexanes 40% to 60%) to afford the respective peptide.

4.9.1. Methyl ((*S,Z*)-6-(4-(benzyloxy)phenyl)-5-(1,3-dioxoisindolin-2-yl)-4-(trifluoromethyl)hex-3-enoyl)glycyl-L-phenylalanyl-L-leucinate (**Z-12b**)—

The peptide coupling procedure was followed using (*S,Z*)-6-(4-(benzyloxy)phenyl)-5-(1,3-dioxoisindolin-2-yl)-4-(trifluoromethyl)hex-3-enoic acid **Z-10b** (0.23 g, 0.4 mmol), SOCl_2 (0.098 mL) NEt_3 (0.18 mL) and Gly-Phe-Leu-OMe tripeptide **11a** (0.34 g, 0.8 mmol). Purified by silica gel chromatography (EtOAc/Hexanes 40% to 60%) to afford the respective peptide as a white solid (0.305 g, 80%). HPLC purity (UV area percent) 97.0 %; m.p. 85–89 °C; ^1H NMR (400 MHz, CDCl_3) δ 7.83 (dq, J = 7.0, 4.3, 3.5 Hz, 2H), 7.73 (dd, J = 5.5, 3.1 Hz, 2H), 7.44–7.31 (m, 6H), 7.26–7.13 (m, 5H), 6.89 (d, J = 8.3 Hz, 2H), 6.70 (dd, J = 12.2, 6.8 Hz, 2H), 6.60–6.52 (m, 1H), 6.30 (dd, J = 10.4, 8.0 Hz, 1H), 5.35 (t, J = 8.2 Hz, 1H), 5.02 (s, 2H), 4.71 (q, J = 7.1 Hz, 1H), 4.58–4.48 (m, 1H), 3.91 (dt, J = 9.3, 4.9 Hz, 2H), 3.71 (d, J = 1.2 Hz, 4H), 3.59 (dd, J = 14.1, 8.2 Hz, 1H), 3.33 (dd, J = 16.2, 8.6 Hz, 1H), 3.19–2.95 (m, 3H), 1.62–1.43 (m, 2H), 0.89 (dd, J = 6.1, 2.3 Hz, 6H); ^{13}C NMR (126 MHz, CDCl_3) δ 172.7, 170.1, 169.3, 168.5, 168.1, 157.7, 136.7, 136.2, 134.3, 133.1, 132.9 (q, J = 6.2 Hz), 131.4, 128.6, 128.5, 128.0, 127.5, 127.1, 126.8, 124.6 (q, J = 275.2 Hz), 122.5, 120.3, 115.0, 77.2, 69.9, 54.3, 52.3, 51.1, 50.9, 43.6, 41.2, 38.0, 37.9, 35.2, 35.0, 24.7, 22.6, 21.9; ^{19}F NMR (376 MHz, CDCl_3) δ -62.90 (d, J = 9.5 Hz, 3F); $[\alpha]_{\text{D}}^{24}$ -9.5 (*c* 0.58, CHCl_3); IR (film) 3296, 3086, 3064, 3032, 2958, 2931, 1776, 1712, 1614, 1631, 1546, 1529, 1512, 1467, 1383, 1369, 1352, 1246, 1124, 1026, 732, 698, 667 cm^{-1} ; HRMS (ESI): m/z $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{46}\text{H}_{47}\text{F}_3\text{N}_4\text{O}_8\text{Na}$: 863.3244, found 863.3251.

4.9.2. Tert-butyl ((*S,E*)-6-(4-(benzyloxy)phenyl)-5-(1,3-dioxoisindolin-2-yl)-4-(trifluoromethyl)hex-3-enoyl)glycyl-L-phenylalanyl-L-leucinate (**E-12b**)—

Above coupling procedure was followed using (*S,E*)-6-(4-(benzyloxy)phenyl)-5-(1,3-dioxoisindolin-2-yl)-4-(trifluoromethyl)hex-3-enoic acid **E-10b** (0.20 g, 0.4 mmol) SOCl_2 (0.085 mL, 1.1 mmol) and NEt_3 (0.18 mL, 0.8 mmol), and Gly-Phe-Leu-OMe tripeptide **11b** (0.30 g, 0.8 mmol). After workup and purification by silica gel chromatography (EtOAc/Hexanes, 40% to 60%) afforded the respective peptide as a white solid (0.28 g, 83%). HPLC purity (UV area percent) 99.5%; m.p. 80–86 °C; ^1H NMR (400 MHz, CDCl_3) δ 7.79–7.66 (m, 4H), 7.43–7.29 (m, 5H), 7.29–7.13 (m, 5H), 7.08 (dd, J = 8.6, 3.5 Hz, 2H), 6.83–6.76 (m, 4H), 6.48–6.30 (m, 1H), 5.27 (dd, J = 11.1, 4.9 Hz, 1H), 4.97 (d, J = 2.5 Hz, 2H), 4.75 (dd, J = 7.6, 3.4 Hz, 1H), 4.42 (t, J = 5.4 Hz, 1H), 4.07–3.85

(m, 1H), 3.57 – 3.43 (m, 2H), 3.40 – 3.18 (m, 2H), 3.12 (dd, J = 6.6, 4.1 Hz, 2H), 1.57 (dd, J = 8.8, 4.9 Hz, 2H), 1.46 (s, 9H), 0.91 (dd, J = 6.0, 4.1 Hz, 6H); ^{13}C NMR (126 MHz, CDCl_3) δ 171.6, 171.5, 169.9, 169.5, 169.4, 168.4, 167.6, 157.7, 136.8, 136.2, 136.1, 134.1, 133.5, 133.5, 131.2, 130.0, 129.4, 129.3, 128.6, 128.5, 127.9, 127.4, 127.0, 126.6, 124.4, 123.4, 120.0, 114.9, 81.9, 69.8, 54.2, 51.5, 50.4, 43.7, 43.6, 41.6, 38.2, 38.1, 35.4, 35.3, 35.0, 27.9, 24.8, 22.6, 22.1; ^{19}F NMR (376 MHz, CDCl_3) δ -59.79 (d, J = 17.6 Hz); $[\alpha]_{\text{D}}^{24}$ = -12.6 (c 0.58, CHCl_3); IR (film) 3280, 3086, 3064, 2958, 2931, 1776, 1712, 1614, 1631, 1546, 1529, 1512, 1467, 1383, 1369, 1352, 1246, 1124, 1026, 732, 698, 667, 401 cm^{-1} ; HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calc'd for $\text{C}_{49}\text{H}_{54}\text{F}_3\text{N}_4\text{O}_8\text{Na}$: 883.3894, found 883.3887.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- A trifluoromethylalkene isopolar peptidomimetic is introduced into the Leu-Enkephalin framework.
- A Wittig olefination strategy enables access to the trifluoromethylalkene containing dipeptide mimetic.
- Conditions were optimized to overcome a problematic amide coupling reaction and minimize undesired isomerization of the alkene.

A) Successful Examples of Trifluoromethylalkene Peptidomimetics

B) Base-promoted Isomerization of Trifluoromethylalkene

C) Target Trifluoroalkene Analogs of Leu-Enkephalin

Fig. 1.
Trifluoromethylalkene dipeptides for probing chemical biology

Scheme. 1.

Peptide coupling and Wittig olefination strategy to access Tyr¹- ψ [$\text{CF}_3\text{C}=\text{CH}$]-Gly²-Leu-enkephalin peptidomimetic **2**.

Scheme 2. Attempted strategies to synthesize Tyr-derived trifluoromethylketone **6**

Scheme 3.

Wittig olefination strategy for preparing protected Tyr¹-ψ[CF₃C=CH]-Gly² **10**

Table 1.

Coupling of Z-Tyr- ψ [CF₃C=CH]-Gly dipeptide mimics **10a–b** with Gly-Phe-Leu tripeptide ^a

entry	Z10a (or) Z10b	coupling reagent (1 equiv.)	base (2 equiv.)	additive (1 equiv.)	time (h)	solvent	¹⁹ F NMR Yield(%)			
							Z-10	Z-12	13	14
1	Z-10a	EDC	DIPEA	HOBt	12	THF	–	–	–	90
2	Z-10a	EDC	DMAP	HOBt	12	DCM	11	–	16	71
3	Z-10a	EDC	DMAP	HOBt	20	CHCl ₃	29	2	6	61
4	Z-10a	PyBOP	DIPEA	–	12	CHCl ₃	22	11	51	17
5	Z-10a	PyBOP	NMM	HOBt	12	DMF	–	14	6	78
6	Z-10b	EDC	DIPEA	HOBt	12	THF	50	–	–	50
7	Z-10b	PyBOP	DIPEA	HOBt	12	THF	19	–	–	80
8	Z-10b	BOP	NMM	–	12	DMF	Undefined mixture			
9	Z-10b	DMT-MM	–	–	5	MeOH	No Reaction			
10	Z-10b	DEPBT	DIPEA	–	5	DCM	No Reaction			
11	Z-10b	–	–	–	12	DCM	7	10	21	60
12	Z-10b	COMU	DIPEA	–	0.25	DCM	–	30	69	–
13	Z-10b	HBTU	DIPEA	–	1	DCM	14	34	51	–
14	Z-10b	HBTU	DIPEA	–	12	THF	–	10	20	64
15	Z-10b	HCTU	DIPEA	–	1	DMF	10	–	–	80
16	Z-10b	HCTU	DIPEA	–	25	DCM	–	14	87	–
17	Z-10b	HATU	DIPEA	–	1	DCM	–	64	35	–
18	Z-10b	HATU	DIPEA	–	25	DCM	–	68	31	–
19 ^b	Z-10b	SOCl ₂	TEA	–	3	DCM	–	78	11	9
20 ^b	Z-10b	SOCl₂	TEA	–	1	DCM	11	84	4	–

^aReactions were performed with 0.039 mmol of **10**, and 0.20 mL of solvent.^bReactions were performed with 0.039 mmol of **10**, 0.20 mL of solvent, 3.0 equiv. of SOCl₂, 3.0 equiv. base Conversion and yield data were determined by ¹⁹F NMR (α,α,α-trifluorotoluene as internal standard).