Organic & Biomolecular Chemistry

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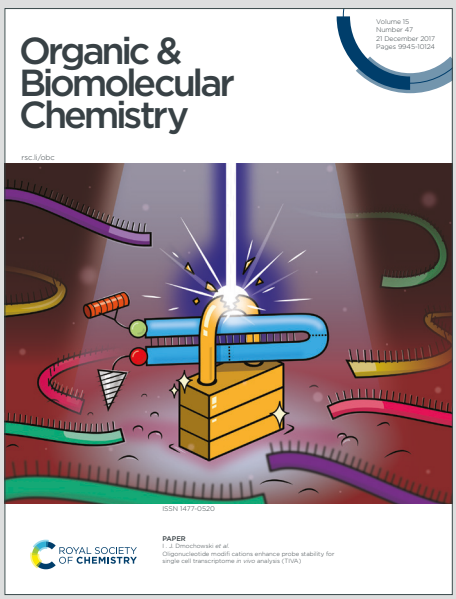

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[3-(trifluoromethyl)-1*H*-1,2,4-triazol-5-yl]methylamine derivatives: synthesis, ways of modification and use for peptides labeling

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DOI: 10.1039/D5OB01080B

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Keywords: 1,2,4-Triazole; cyclization; trifluoromethyl; labeling; peptide.

Abstract

The synthesis of new 3-(trifluoromethyl)-1*H*-1,2,4-triazoles is reported, starting from ethyl trifluoroacetimidate and Boc-protected amino acid hydrazides. The influence of elongation or branching of a linker between the hydrazide and amino group on cyclization of amidrazones is established. The scale-up of the synthesis of *tert*-butyl ((3-(trifluoromethyl)-1*H*-1,2,4-triazol-5-yl)methyl)carbamate (**4a**) starting with 1 mole of corresponding hydrazide and trifluoroacetonitrile was successfully demonstrated. Additionally, **4a** and other derivatives were further elaborated, underlining their prospects as building-blocks for organic synthesis. In particular, 2-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4]triazolo[1,5-a]pyrazine (**23**) – a component in the synthesis of Fuzuloparib - was successfully obtained in good yields (56.4%). Finally, we showed that 2-(5-(aminomethyl)-3-(trifluoromethyl)-1*H*-1,2,4-triazol-1-yl)acetic acid hydrochloride (**19**) can be incorporated into short peptides using solid phase peptide synthesis, serving as a fluorinated Gly-Gly analogue for peptide ^{19}F NMR studies.

Introduction

1,2,4-triazole containing compounds are known for their wide application in medicinal chemistry.[1] Voriconazole, Terconazole, Fluconazole are triazole-containing medications used to treat serious fungal infections (Figure 1). These compounds have been successfully used from the early 1990s and contain *N*-1 substituted 1,2,4-triazole.[2,3] Aminomethyl 1,2,4-triazole is found in the seven membered ring heterocycle-containing benzodiazepine antidepressants, such as Alprazolam and Etizolam (Figure 1).[4] Another important class of biologically active compounds is based on 1,2,4-triazolone scaffold. These are Nefazodone[5] and Aprepitant,[6] which are used as antidepressant and antiemetic agents, respectively. The latter examples show that substituted 1,2,4-triazoles are promising building-blocks for the synthesis of biologically active privileged structures in medicinal chemistry.[7,8] Another example of the use of substituted aminomethyl 1,2,4-triazole in medicinal synthesis is Sitagliptin - the first drug used for type-2 diabetes management[9] - promoting significant research into developing analogous compounds.[10,11] Particularly, the structural diversity of compounds was achieved by varying of 3-(trifluoromethyl)-tetrahydro-[1,2,4]triazolopyrazines and

related cyclic amines.[12,13] Recently another compound containing the 3-(trifluoromethyl)-tetrahydro-[1,2,4]triazolopyrazin motif, isomeric to those used for Sitagliptin, was approved in China for the treatment of platinum-sensitive recurrent ovarian cancer, fallopian tube cancer or primary peritoneal cancer under the commercial name Fuzuloparib.[14] To our surprise the literature data dedicated to the synthesis or properties of 3-(trifluoromethyl)-[1,2,4]triazoles derivatives containing akylamino group, not as part of a piperazine ring, is rather limited. Taking into account that in both cases (Sitagliptin and Fuzuloparib) 3-(trifluoromethyl)-tetrahydro-[1,2,4]triazolopyrazines were used as building-blocks for the construction of the title compounds, we believe that expanding the chemical space of aminoalkyl derivatives of 3-(trifluoromethyl)-[1,2,4]triazoles is an important topic in the context of new drugs discovery. Another emerging application for fluorinated compounds is labeling of fragments for ^{19}F NMR screening for drug discovery[15] and to interrogate the conformational status of peptides and proteins.[16] Generally, fluorine is widely used for the bioisosteric substitution of hydrogen atoms in drug and agrichemical agents, endowing altered chemical, physical and biological properties to the target compounds.[17] Therefore, we turned our attention to the synthesis of trifluoromethyl 1,2,4-triazoles for incorporation into peptides as conformational ^{19}F NMR reporters.

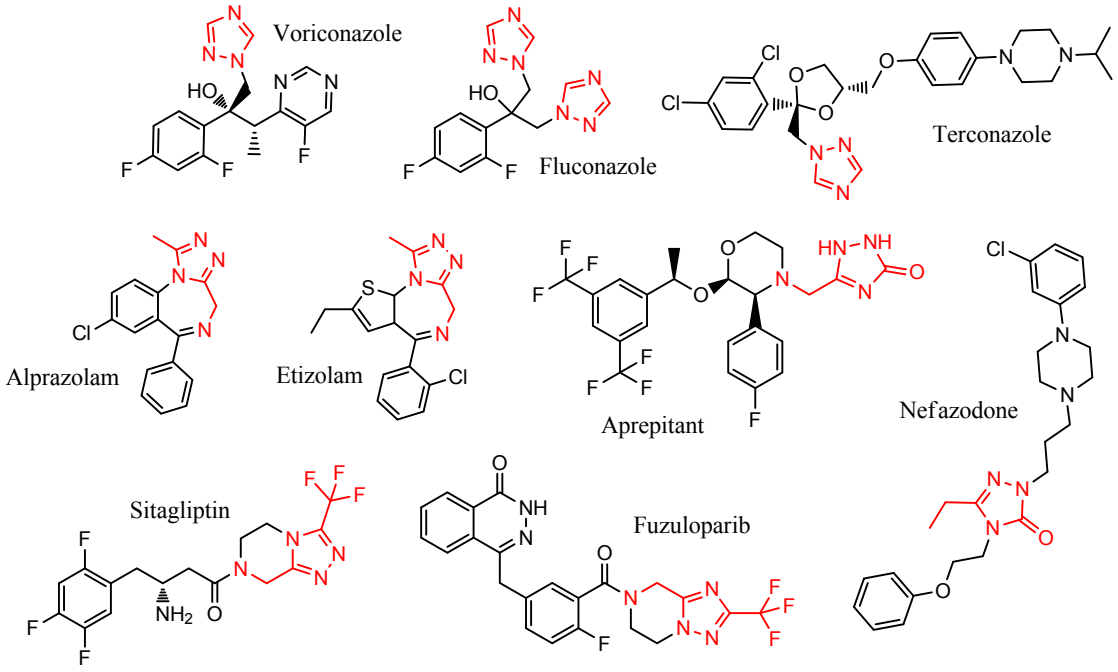
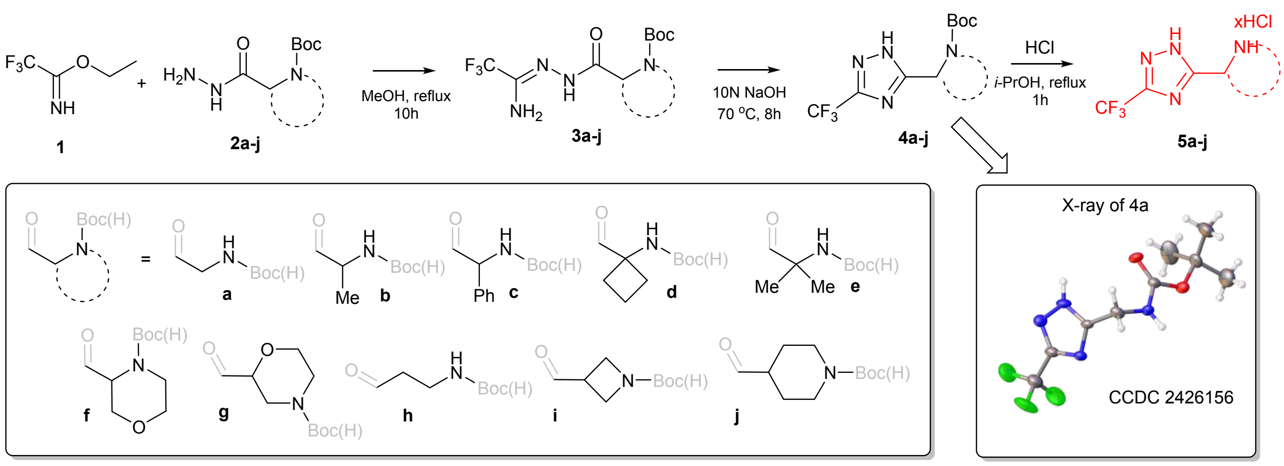


Figure 1. Representatives of marketed 1,2,4-triazole derivatives.

Results and discussion

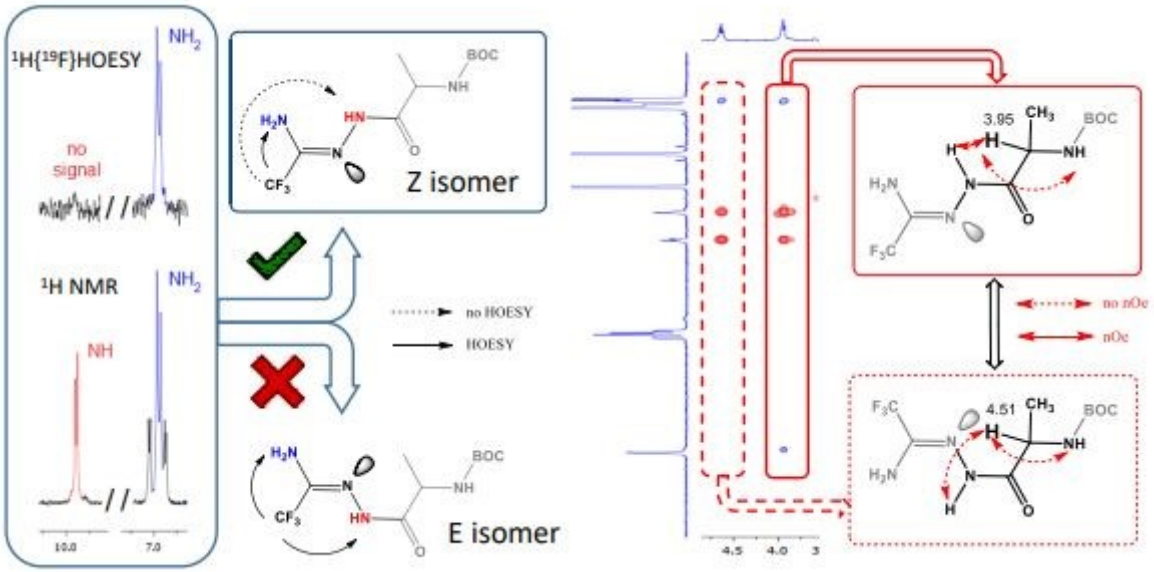
The construction of the 3-(trifluoromethyl)-1*H*-1,2,4-triazol-5-yl core was realized by using ethyl trifluoromethylacetimidate (**1**) as the C-N component, and Boc protected amino acid hydrazides (**2a-j**) as the C-N-N component. The interaction between the above-mentioned reagents proceeds in boiling methanol with a slight excess of **1**, producing the corresponding acyl amidrazones **3a-j** in quantitative yield. Since ethyl trifluoromethylacetimidate is low-boiling, excess reagent was easily removed simultaneously with methanol by evaporation. The cyclization of **3a-j** into the 1,2,4-triazole proceeds in alkali solution is close to those used for synthesis of 1,2,4-triazol-3-ones[18] and their thio-analogues.[19] In most cases, isolated yields of these steps exceed 80%. Lower yields were observed for **4d**,**e**, which could be explained by steric hindrance occurring in the cyclization step originating from substituents in close proximity to the carbonyl group. Deprotection of **4a-j** proceeded in a solution of HCl in *i*-PrOH, producing crystalline hydrochlorides of **5** in quantitative yields in all cases, except **5i** and **5f**. This is due to **5i** undergoing partial collapse of the azetidine cycle, whilst **5f** contains up to 10% of contaminants of unknown nature and thus was subjected to the reaction with NaOH, subsequently transforming into pure free base, which slightly reduced the isolated yields. It should also be noted that even huge excess of HCl used on deprotection step did not result in the formation of dihydrochloride salts, due to the remarkable decrease of basicity influenced by the CF_3 group.



Scheme 1. Synthesis of **5a-j**.

It was noted that the [1]H NMR spectra of **3a-j** exhibited doubling of signals for all compounds except **3d**,**e**. The cause of this observation was investigated in detail for the example **3b**. Generally,

doubling of signals originates from reasons such as tautomerism, stability of rotamers due to rigidy of molecule or bonding peculiarities, for example. In our case, on the first step, we unambiguously established the geometry of amidrazone fragment using a HOESY NMR experiment. Irradiation on fluorine frequency leads to appearance of only NH_2 protons in the corresponding spectrum, indicating the Z-configuration of the amidrazone, where the NH group is distant from the trifluoromethyl substituent (Fig 2a). Given this observation, we assumed that signal doubling was the result of slow rotation of molecule around the CO-NH bond. This statement was further confirmed by COSY and NOESY experiments. It turned out that among CH signals at 4.51 and 3.95 ppm, only those in the down field region had a strong spatial interaction with the NH proton at ~9.89 ppm. At the same time, no CH signal had a spatial interaction with the neighboring respective NH signals at 7.05 and 6.87 ppm (Fig 2b). The ^{1}H, ^{13}C and ^{19}F NMR spectra of compounds **4a-j** and **5a-j** do not demonstrate these unprecedent features as depicted in the ESI.



a b

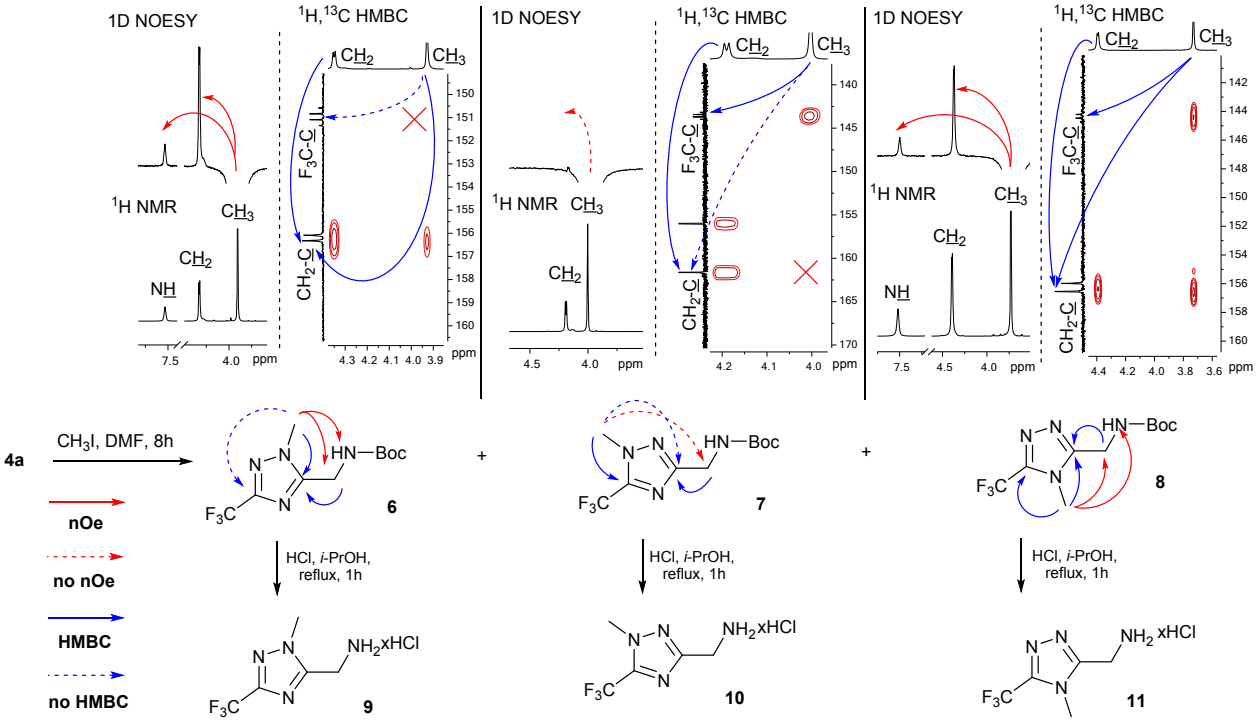
Figure 2. Fragments of 1D HOESY (a) and 2D NOESY (b) spectra used for establishing of structure of **3b**.

All the above-mentioned reactions were carried out on a 10 mmol scale with respect to the corresponding hydrazides, in most cases giving access to gram quantities of **5** as the hydrochloride salts. Since the production of multigram quantities of building-blocks is also of great interest, we

decided to extrapolate the above-mentioned protocols for hundred-fold loads. This challenging task required the simultaneous elaboration of multigram protocols for the synthesis of starting materials **1** and **2a-j**. The latter compounds could be easily produced in the required quantities. However, the synthesis of **1** from trifluoroacetonitrile and ethanol on a mole scale is inconvenient and hardly controllable for numerous reasons *i.e.* the reaction is exothermic, **CF_3CN** is a highly toxic gaseous substance and the precipitation observed during the reaction course prevents steady bubbling of **CF_3CN**. All these inconveniences forced us to find optimized conditions to obtain significant quantities of starting material. The smallest Boc-glycine hydrazide **2a** was chosen as a model substance for these trials. Taking into account the significant electrophilicity of the carbon atom of the CN group of trifluoroacetonitrile, we tried to undertake the reaction of **CF_3CN** with **2a**. It turned out that the reaction in methanol proceeded quantitatively as was observed with the corresponding imidate, but the completeness of the process required the use of a five-fold excess of **CF_3CN**. Changing methanol to aprotic THF decreased the amount of **CF_3CN** needed to a three-fold excess but overspending of reagent remain significant. Thus, we conducted subsequent reactions using methanol in the presence of catalytic amounts of sodium methylate (5%). In this case we were able to achieve full conversion of **2a** to **3a** using only 20% excess of trifluoroacetonitrile. Thus, we successfully used these conditions to complete the reaction using 1 mole of **2a**. Moreover, due to the low boiling temperature of methyl trifluoroacetimidate, its excess formed in course of the reaction could be distilled off. The synthesis of **4a** and **5a** was completed under the same conditions without any significant changes due to the hundred-fold load.

Since **5** contains two potential diversity points *i.e.* the triazole ring and the amino group, to show the prospects of using of **4** and **5** as starting materials for organic synthesis, we demonstrated its diversification using a few simple transformations. **4a** was chosen as a model substance for alkylation reactions. It was observed that its methylation leads to the formation of N1, N2 and N4 regioisomers – compounds **6, 7** and **8**, correspondingly. Their structures were established by 1D NOESY and $^{1}H,^{13}C$ HMBC experiments (Scheme 2 and ESI). Recrystalization of the mixture from petroleum ether afforded 175 g of pure **6**. The N1 and N4 isomers were isolated from the mother

liquor using column chromatography in 12.8 % and 0.4 % yields correspondingly, together with additional amounts (24 g) of **6**, making a total yield of N2 methylated regioisomer of 72.1 %. Such big difference in the yields of regioisomers could be explained by the decreased N1 nucleophilicity due to the significant electron withdrawing character of the CF_3 group. For comparison, methylation of the ethyl triazolyl carboxylate leads to the formation of approximately of N1 and N2 isomers.[20]



Scheme 2. Synthesis of **6-11** and main correlations used for establishing of structure of regioisomers.

The amino group in **5** was protected with the widely used N-protecting groups – Fmoc and Cbz, using standard protocols to produce the corresponding amides **12** and **13**. Compounds **5a** and **5h** were subjected to the reaction with glyoxal as previously reported for other examples of methylamino-[21] and ethylamino-1,2,4-triazoles,[22] leading to the formation of the same polycyclic systems **15** and **16**, respectively, in good yields. Since the deprotection of **4** was conducted in *i*-PrOH in some cases complete removal of solvent was achieved by drying of **5** in oven at elevated temperatures. In the case of **5c** it intentionally led to the formation of **17**. Most probably the driving force of this reaction is the possibility of relatively easy formation of a carbocation stabilized by two aryls (phenyl and 1,2,4-triazole), which formed as result of elimination of NH_4Cl and the subsequent

attack of the amino group from another molecule of **5c**. The ^{1}H NMR spectrum of **17** contains a double set of signals, which partially coalescence upon heating (see ESI). This could be due to existence of a mixture of tautomers, regioisomers or atropoisomers for example, and requires further investigation into NMR experiments and support by calculations, which is out of scope of present article.

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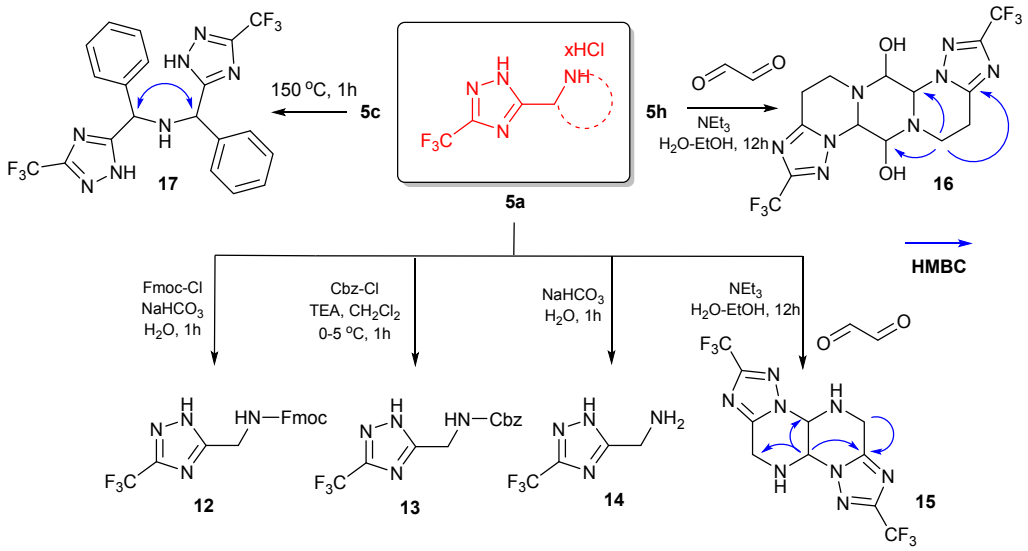

Scheme 3. Ways of modification of **5** and main correlations used for establishing of structures of **15-17**.

Hydrochloride **5a** was additionally converted into the free amine **14** and structurally characterized. The recrystallization of **14** from water solution afforded suitable single crystals for X-ray diffraction analysis of this compound (Figure 3 and ESI). Accordingly, compound **14** exhibits a molecular crystal structure, where the asymmetric part comprises three crystallographic independent zwitterions and one interstitial water molecule. The positional parameters of the hydrogen atoms, localized from different Fourier maps, and the distribution of the inter-molecular H-bonds confirms undoubtedly the presence of zwitterions in the crystal. The further analysis has revealed the numerous premises for non-covalent intermolecular interactions, due to the presence of different fragments, which are potential donors or acceptors of protons. It is worth noting, that all the potential H-bonds are completely realized in the crystal, which link all the components of the structure in a complex and dense three-dimensional supra-molecular architecture, as shown in Figure 3.

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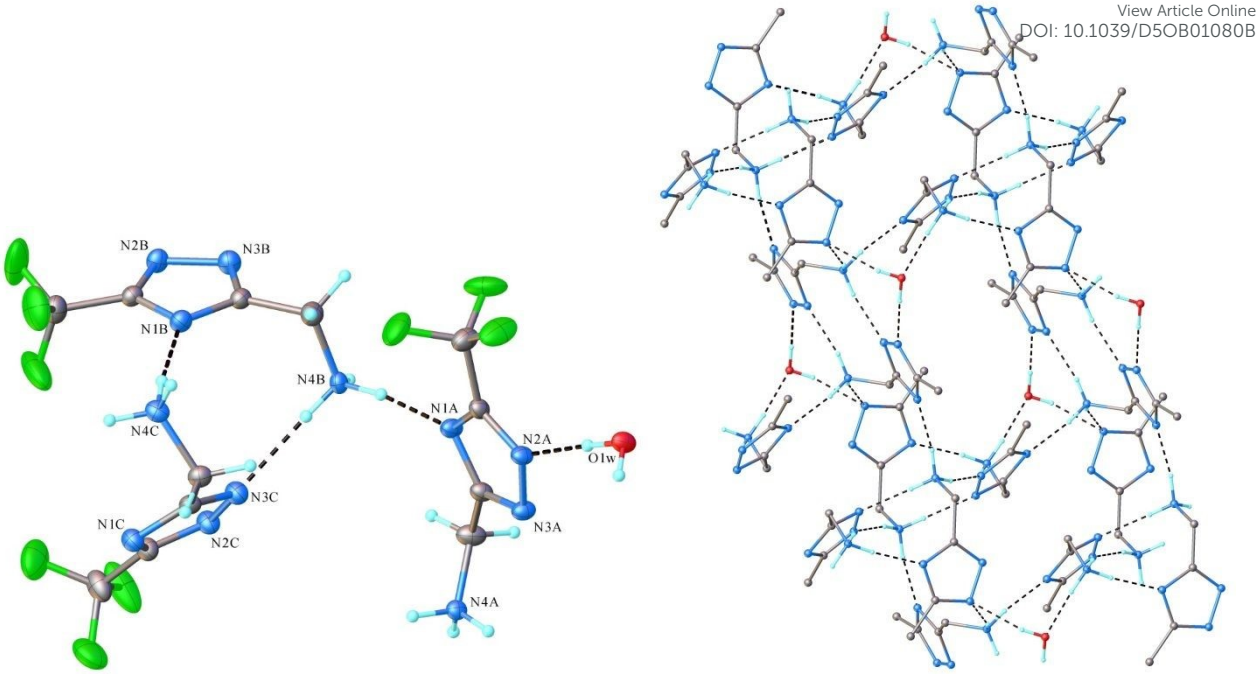


Figure 3. View of the asymmetric part in the crystal structure of **14** with selected atom labeling and thermal ellipsoids at 40% level (left) and partial view of the crystal structure packing showing the role of H-bonds into the formation of 3D supra-molecular network (right). H-bonds parameters:

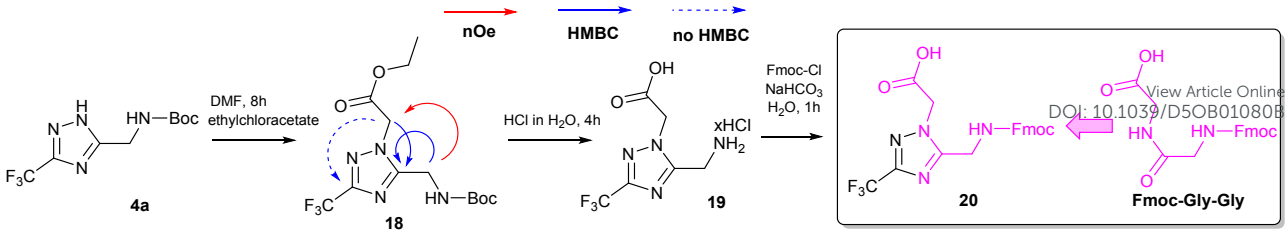
N4C-H···N1B [N4C-H 0.91Å, H···N1B 1.96Å, N4C···N1B 2.865(3)Å, N4C-H···N1B 176.3°]

N4B-H···N3C [N4B-H 0.91Å, H···N3C 1.96Å, N4B···N3C 2.870(3)Å, N4B-H···N3C 175.8°]

N4B-H···N1A [N4B-H 0.91Å, H···N1A 1.93Å, N4B···N1A 2.829(3)Å, N4B-H···N1A 170.7°]

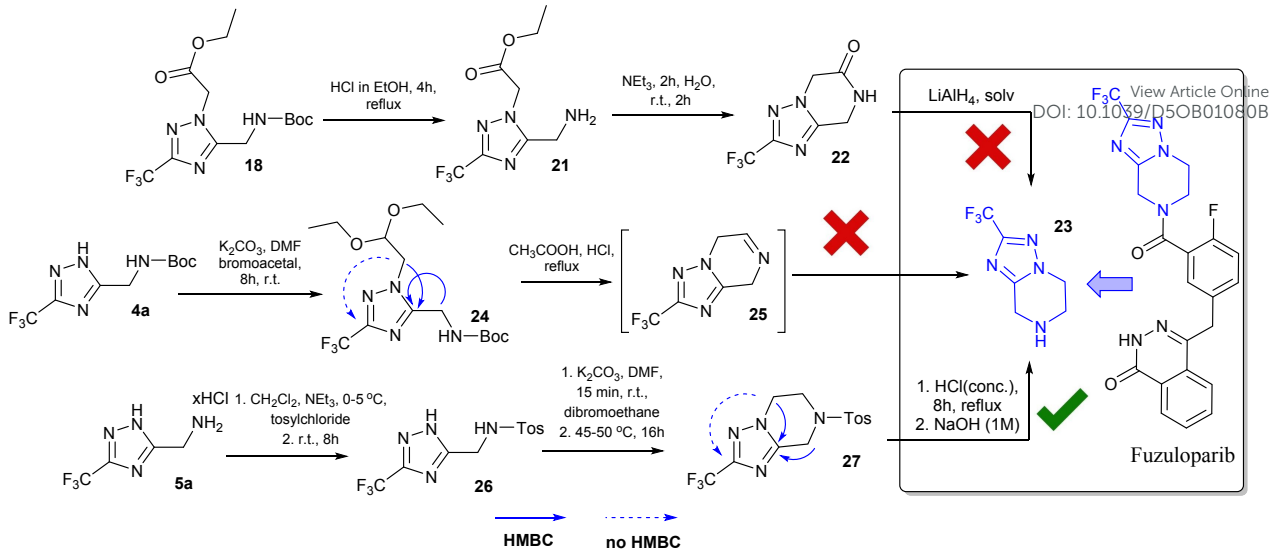
O1*w*-H···N2A [O1*w*-H 0.87Å, H···N2A 1.90Å, O1*w*···N2A 2.707(3)Å, O1*w*-H···N2A 153.5°]

The above-mentioned findings enforced us to the regioselective alkylation of **4a** with methylchloroacetate to give **12**, which was used as a precursor for two subsequent reactions. Firstly, both amide and ester groups in **12** were hydrolyzed leading to formation of amino acid **15**. Since 1,2,4-triazole is regarded as a mimetics and bioisosteric replacement of amide bonds,[23,24] **15** could be considered as a Gly-Gly analogue. Further introduction of Fmoc protection in **15** was successfully realized to afford a building block for later solid phase peptide synthesis (SPPS).



Scheme 4. Synthesis of 1,2,4-triazole containing analog (**20**) of Fmoc-Gly-Gly and main correlations used for establishing of **18**.

Secondly, we aimed to obtain amine **23** which is used in Fuzuloparib synthesis. The idea was to obtain amide **22** by intramolecular cyclization of Boc-deprotected **21** and then reduce the amide bond. The Boc-deprotection of **18** was easily completed producing **21**, which was subsequently cyclized to give **22**. The treatment of **22** with equimolar amounts of $LiAlH_4$ did not afford the reduction product, while two-fold excess of reducing agent afforded only traces of **23**. Thus, we turned our mind to the compounds that could be reduced under mild conditions. Here, on the first step **24** was obtained by alkylation of **4a** with bromoacetaldehyde diethylacetal. This product was refluxed in acetic acid containing traces of HCl, leading to a mixture of cyclization products mainly containing **25**. Despite the reduction of this mixture by $NaBH_4$ affording significant amounts of the target product, it's isolation was obstructed by other contaminants in the mixture, making this route challenging. Finally, we evaluated a method that did not require a reduction step. On the first step, the amino group in **5a** was protected by a Tosyl group in excellent yield to produce **26**, which was then subjected to double alkylation using dibromoethane leading to the formation of the tosyl-protected title amine **27**, which was easily deprotected in acidic conditions giving access to **23** in a total yield of 56.4 %, starting from **2a**.



Scheme 5. Trials of the synthesis of **23** and main correlations used for establishing of **24** and **27**.

Synthesis of peptidomimetics.

The backbone dihedral angles φ and ψ and ω define the geometry of peptide bonds. Gly-Gly motifs have significant conformational flexibility due to rotation about all of φ and ψ and ω (Figure 4) allowing it to adopt dihedral angles that are sterically forbidden for other amino acids. This allows it to adopt unusual backbone conformations and tight turns in protein structures e.g. β-turns (especially Type II and Type II′ turns).[25] Controlling the rotation about these bonds can be useful to understand the molecular origins of conformational changes in proteins. In peptide bonds, ω rotation is mostly restricted to either the relatively high-energy *cis*-amide or the relatively low energy *trans*-amide. Gly-Pro containing peptides and proteins have lower energy barriers to ω rotation and lack free rotation around the φ torsion angle due to the pyrrolidine ring. Triazoles are known to be isosteres of amide bonds mimicking both *trans*- and *cis*-amide bonds.[26,27] The trifluoromethyltriazole-containing amino acid **20** affords a possible '*cis*-locked' mimic of Gly-Gly with the triazole group in place of the standard connecting amide bond preventing ω torsional rotation (Figure 4). This adds to the toolkit of conformationally restricted amino acids to investigate the importance of *cis*-amide bond rotamers in protein and peptide flexibility. Given that Gly-Gly and Gly-Pro are common in β-turn/hairpin motifs due to their flexibility,[28] '*cis*-locked' analogues of Gly-Gly / Gly-Pro may be useful tools to study the role of amide bond *cis-trans* isomerism in β-turn/hairpin motifs. To firstly

demonstrate the compatibility of trifluoromethyltriazole-amino acid **20** with solid-phase peptide synthesis, we synthesised two model pentapeptides: Ac-YA-(**20**)-(4F-Phe)-NH_2 (**29**) and Ac-YP-(**20**)-(4F-Phe)-NH_2 (**30**). The synthesis of each peptide was performed using standard microwave-assisted SPPS procedures (DIC/Oxyma) and **20** was incorporated easily into the peptides.

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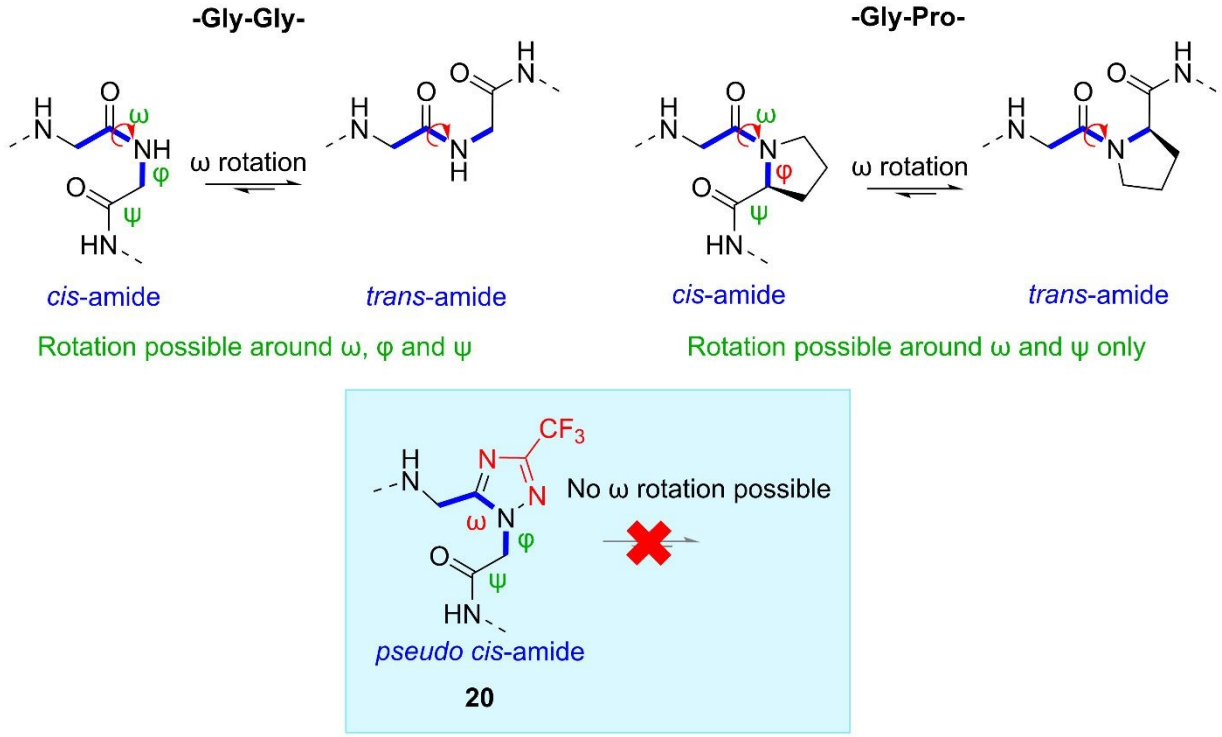

Figure 4. **20** as '*cis*-locked' analogue of Gly-Gly / Gly-Pro.

The presence of the trifluoromethyl group has the potential to tune the structural and physicochemical properties of a peptide or protein. Moreover, the trifluoromethyl group also provides a sensitive ^{19}F NMR handle that could be exploited for dynamic structural analysis or protein binding experiments. Previously we have shown that fluorinated amino acids such as 4-fluorophenylalanine are unbiased reporters of nearby prolyl bond *cis-trans* populations in short peptides by integration of the distinct ^{19}F NMR signals arising from each conformer.[16] To probe whether **20** would itself report the prolyl *cis-trans* status in a peptide, or even alter the conformational preferences at proline, two further control peptides were synthesised, replacing the trifluoromethyltriazole-amino acid with a canonical Gly-Gly dipeptide unit: Ac-YAGG-(4F-Phe)-NH_2 (**31**) & Ac-YPGG-(4F-Phe)-NH_2 (**32**). Each of the model peptides also contained a second fluorinated amino acid (4-fluorophenylalanine,

4F-Phe) as an internal reference to probe whether the presence of **20** afforded the peptides distinct conformational states.

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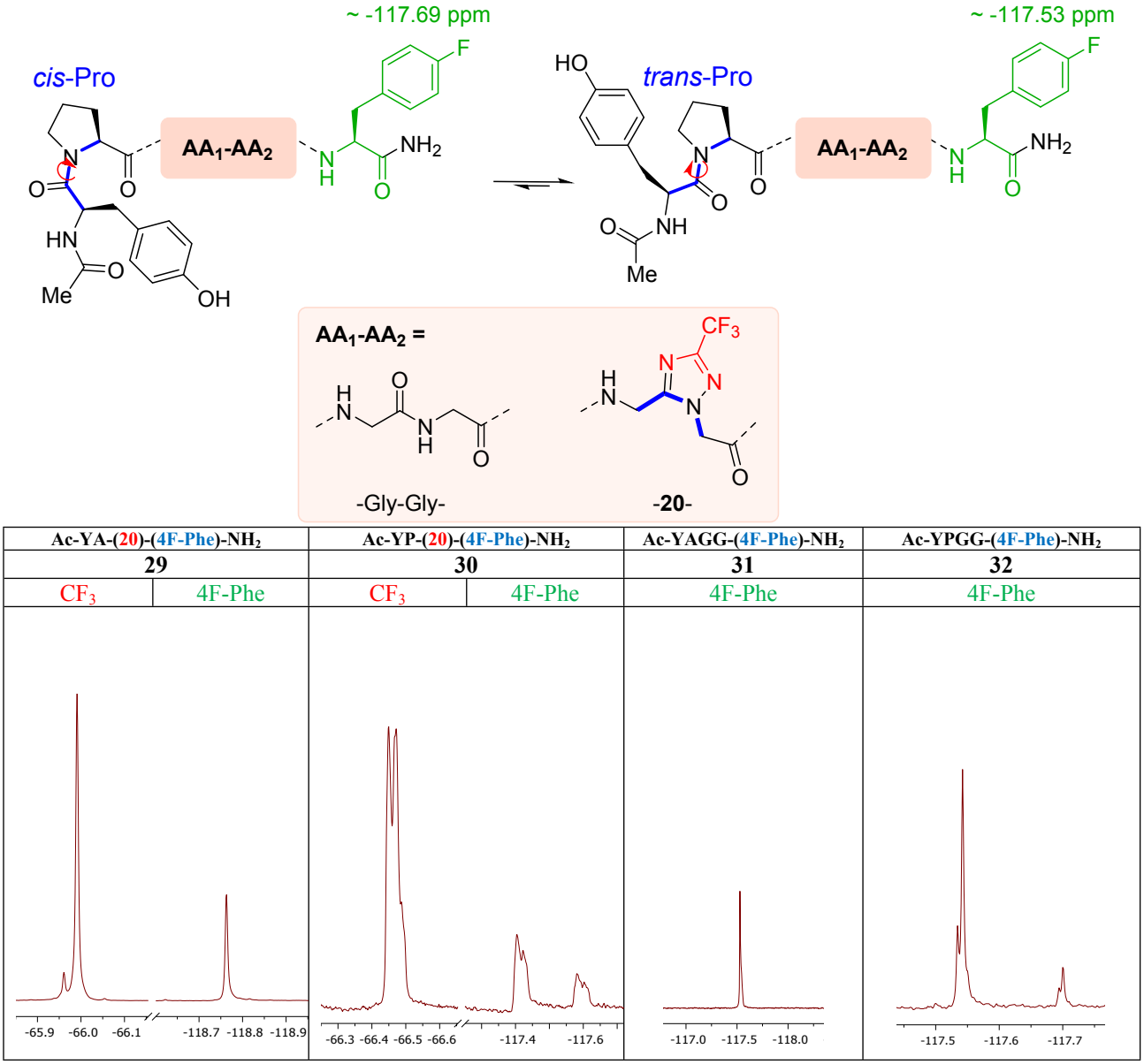

Figure 5. ^{19}F NMR spectra for the four model pentapeptides.

^{19}F NMR (proton-decoupled) room temperature analysis was carried-out on peptides at a concentration of ~1 mg/mL in PBS buffer (pH 7.4, 0.1 M, D_2O 10% *v/v*). The two control peptides containing only standard amino acids (**31** and **32**) behaved mostly as expected by ^{19}F NMR (Figure 5). Ac-YAGG-(4F-Phe)-NH_2 (**31**) exhibited a single resonance at around -117.5 ppm and showed that there were no resolvable slowly-exchanging conformers (on the NMR timescale). Whilst proline-containing Ac-YPGG-(4F-Phe)-NH_2 (**32**) displayed the expected unsymmetrical major pair of

resonances (approximately -117.53 and -117.69 ppm, respectively,) attributable to the presence of prolyl-bond *cis*-*trans* conformers. The major signal pairs were integrated to a ratio of ~25:75 (25% *cis*-Pro), which is consistent with a tyrosine stabilised prolyl-bond,[16] and there was also a smaller secondary peak-splitting observed, which may be attributable to further distinct slowly exchanging conformers that arise within Pro-Gly-Gly. Replacing the Gly-Gly motif with **20** in Ac-YA-(**20**)-(4F-Phe)-NH_2 (**29**) gave two highly dispersed singlets (~ -66 and -118.75 ppm) with a ~3:1 integral ratio for CF_3 and 4F-Phe, respectively. As expected, 4-FPhe exhibited a sharp singlet and the CF_3 signal exhibited mainly one sharp signal, indicating that **20** affords only one major conformation on the NMR timescale. However, a very small secondary signal was also observed adjacent to the CF_3 signal (~-65.96 ppm) perhaps due to the presence of a minor conformer or tautomer that was either undetectable by the distal 4-FPhe or that was introduced by **20**. Notably, when **20** was paired with Pro in Ac-YP-(**20**)-(4F-Phe)-NH_2 (**30**), both the CF_3 and 4F-Phe signals exhibited greater complexity, indicating the presence of distinct conformers and apparent changes in the relative conformer populations. The 4-FPhe signal was split into two inequivalent pairs that, like in **32**, appeared to relate to *trans*-Pro and *cis*-Pro, albeit with apparently higher % *cis*-Pro population (~33%). This suggested that the presence of the neighbouring trifluoromethyltriazole motif of **20** increased the *cis*-prolyl bond population. Both the 4-FPhe and CF_3 signals were also split into two poorly-resolved resonances in a ~1:1 ratio, which may be related to conformers present in Pro-**20** like Pro-Gly-Gly. Therefore, **20** may behave like a Gly-Gly mimic with a ^{19}F NMR reporter for peptide conformation and interaction studies.

Conclusions.

This article describes the synthesis of 3-(trifluoromethyl)-1*H*-1,2,4-triazoles containing an amino group. The methods used for their synthesis gave access to the series of compounds mostly in good yields, except for those where glycine derivatives with a doubly-substituted methylene group were used as starting materials. The scaling procedure, which was demonstrated on the model compound **4a** can be used as starting point for multigram synthesis of other derivatives of this kind.

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The prospects of using of 3-(trifluoromethyl)-1*H*-1,2,4-triazoles containing an amino function as building blocks was demonstrated not only by involving them in simple transformations (alkylation, acylation, condensation), but also by using **4a** as precursor for synthesis of 2-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4]triazolo[1,5-a]pyrazine **(23)** used in the synthesis of commercial Fuzuloparib. Since pyperazino-triazole isomeric to **23** is the part of Sitagliptin the achievements described in present work could also be useful for production of new antidiabetic agents. It should be noted separately that the trifluoromethyltriazole-amino acid derivative **20** is a valuable fluorinated peptide building block that is compatible with standard SPPS and affords a possible mimic of Gly-Gly dipeptides. Furthermore, the trifluoromethyl group provides an excellent ^{19}F NMR reporter, affording such building blocks significant potential as labels for protein and peptide conformation and interactions studies.

Experimental section

Materials, methods and synthetic procedures used for 1-27 preparation and investigation.

Materials and methods

Elemental analyses were carried out with Perkin-Elmer 2400 CHN Analyzer. Melting points (˚C, uncorrected) were measured with OptiMelt Automated Melting Point System (MPA 100). The IR spectra (KBr, pellet) were recorded with Spektrum BX Perkin Elmer spectrometer. The mass spectra were recorded on an Agilent 1100 Series high-performance liquid chromatograph equipped with a diode matrix with an Agilent LC\MSD SL mass selective detector; the ionization method is atmospheric-pressure chemical ionization (APCI).

NMR analysis

Small molecule ^{1}H, ^{19}F and ^{13}C NMR spectra of were recorded on a Bruker DRX 500 spectrometer (at 500.1 MHz, 470.6 MHz, and 125.8 MHz for ^{1}H, ^{19}F, and ^{13}C nuclei, respectively), Bruker AVANCE III 400 spectrometer (at 400.4 MHz, 376.5 MHz, and 100.7 MHz for ^{1}H, ^{19}F, and ^{13}C nuclei, respectively) and Agilent ProPulse 600 spectrometer (at 600 MHz for ^{1}H NMR and 151

MHz for ^{13}C NMR). Internal standard – signal of residual solvent protons (DMSO-*d*6 – 2.50 ppm) and carbons (DMSO-d_6 – 39.5 ppm). Trifluorochloromethane in $CDCl_3$ was used as an external standard for ^{19}F NMR spectra (0 ppm). Some of the signals in ^{13}C spectra of initial compounds are significantly broadened due to superposition from several tautomeric/rotameric forms and transition states, which makes hard or impossible their determination. In the structure elucidation of the compounds using the NMR method, a standard set of experiments from the VNMRJ 4.2 and/or TopSpin 2.1 user library was used. Using gHSQC and gCOSY experiments, the assignment of signals in the ^{1}H and ^{13}C spectra was initially conducted, after which the structure of the substance was determined using gHMBC (^{1}H-^{13}C correlation through 2 or 3 bonds) and 1D g-NOESY (^{1}H-^{1}H correlation through space).

X-ray diffraction analysis

Single-crystal X-ray diffraction data were collected using an Oxford-Diffraction XCALIBUR Eos CCD diffractometer. The unit cell determination and data integration were carried out using the CrysAlisPro package from Oxford Diffraction.[29] Multi-scan correction for absorption was applied. The structure was solved with SHELXT program using the intrinsic phasing method and refined by the full-matrix least-squares method on F^2 with SHELXL.[30,31] Olex2 was used as an interface to the SHELX programs.[32] Non-hydrogen atoms were refined anisotropically. Hydrogen atoms attached to carbon were added in idealized positions and refined using a riding model, while that attached to N or O atoms were localized from Fourier maps and their positional parameters were refined according geometry of hydrogen bonding. Selected crystallographic data and structure refinement details are provided in Table 1, ESI and the corresponding CIF-files.

Table 1. Crystal data and details of structure refinement.

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DOI: 10.1039/D5OB01080B

	14	**4a**
Emp. formula	$C_{12}H_{17}F_9N_{12}O$	$C_9H_{13}F_3N_4O_2$
Fw	516.38	266.23
T [K]	180	293
space group	*P*-1	$P2_1/c$
a [Å]	8.6652(5)	10.894(3)
b [Å]	11.6950(6)	12.028(3)
c [Å]	11.7268(6)	9.8863(18)
α [°]	65.612(5)	90
β [°]	80.451(4)	101.17(2)
γ [°]	89.034(4)	90
V [Å^3]	1065.58(10)	1270.9(5)
Z	2	4
ρ_{calcd}[g cm^{-3}]	1.609	1.391
μ[mm^{-1}]	0.165	0.129
Crystal size [mm]	0.15×0.10×0.10	0.40×0.04×0.03
2Θ range	3.83 to 58.718	3.81 to 50.05
Refls. collected	12423	5253
Indep. Refls., R_{int}	5011, 0.0333	2243, 0.0501
Data/rests./params.	5011/0/340	2243/0/166
GOF	1.002	1.068
R_1, wR_2(all data)	0.0614, 0.1368	0.0694, 0.1366
CCDC no.	2426155	2426156

Synthetic procedures

All the starting materials were obtained from Enamine Ltd. and UORSY. Analytical TLC was performed using Polychrom ESI F254 plates. Column chromatography was performed using silicagel Merck 60 (230–400 mesh) as the stationary phase. Ethyl perfluoroacetimidate **1** was prepared according procedure described for corresponding methyl perfluoroacetimidate.[33] Hydrazides **2a-j** were prepared by standard procedures starting from corresponding methyl esters. **15** and **16** were prepared using procedures analogous to previously reported for other derivatives. [20,21]

Boc protected [3-(trifluoromethyl)-1*H*-1,2,4-triazol-5-yl]alkylamines (4a-j). General procedure. Hydrazides **2a-j** (10 mmol) was added to a solution of methyl perfluoroacetimidate **1** (1.52 g, 12 mol) in MeOH (50 ml). The mixture was refluxed for 8 h. Then solvent was removed by distillation leading to the formation of white crystalline substance, which was used on the next step in the same flask without purification. 10% NaOH water solution (50 mL) was added to **3a-j** obtained on previous step and heated 8h at 70 °C. During this time complete dissolution of initial precipitate was observed. After cooling to room temperature, the reaction mixture was acidified with glacial acetic acid (app. 10 ml) maintaining inner temperature below 20 °C. Precipitation of white crystals were observed

during the adding of last portions of AcOH. The product was filtered off, washed with water and dried on air

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***tert*-butyl ((3-(trifluoromethyl)-1*H*-1,2,4-triazol-5-yl)methyl)carbamate (4a).** Scaling procedure. Three necked 5L flask, containing thermometer, bubbling tube and effective condenser was loaded with 2.5 L of methyl alcohol, where sodium (1.15 g, 0.05 mol) was subsequently dissolved upon stirring. After completing of the reaction **2a** (189.2 g, 1 mol) was added to the solution. Obtained mixture was cooled to 0 °C. Then, maintaining inner temperature 0 +5 °C, **CF_3CN** was bubbled to the reaction mixture leading to the complete dissolution of **2a** (app. 10 h) upon introducing of approximately equivalent amount of **CF_3CN**. Generally, bubbling of **CF_3CN** was continued until 115 g (1.2 mol) of **CF_3CN** was introduced into reaction mixture. Then external cooling was removed and reaction mixture allowed to heat to r.t. and additionally stirred overnight. The conversion of **2a** was monitored using NMR. In the cases when conversion was not complete additional amounts of **1** was introduced to reaction mixture, without external cooling. Further processing with reaction mixture with analogous to those described in "General procedure" for **4a-j**, with extrapolating on hundred-fold scaling. Once should be noted that 2.5 L of 20% NaOH was used for cyclization. White crystals (2.21 g, 83% - General procedure), (215 g, 81 % - Scaling procedure), m.p. 151–154 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.71 (s, 1H), 7.52 (t, J = 5.8 Hz, 1H), 4.30 (d, J = 5.8 Hz, 2H), 1.39 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.19, 155.61, 152.21 (q, J = 36.7 Hz), 119.58 (q, J = 269.1 Hz), 78.49, 36.20, 28.10; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.4; MS (m/z, CI): 265.2 $[M - H]^-$. Anal. Calcd for $C_9H_{13}F_3N_4O_2$: C, 40.60; H, 4.92; N, 21.05. Found: C, 40.45; H, 4.78; N, 21.34.

***tert*-butyl (1-(3-(trifluoromethyl)-1*H*-1,2,4-triazol-5-yl)ethyl)carbamate (4b).** White crystals (2.21 g, 79.0%), m.p. 167–168 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.66 (s, 1H), 7.52 (d, J = 7.4 Hz, 1H), 4.81 (p, J = 7.1 Hz, 1H), 1.42 (d, J = 7.1 Hz, 3H), 1.39 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.97, 155.04, 152.02 (q, J = 37.7 Hz), 119.66 (q, J = 269.3 Hz), 78.44, 43.20, 28.17, 19.33; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.3; MS (m/z, CI): 279.0 $[M - H]^-$. Anal. Calcd for $C_{10}H_{15}F_3N_4O_2$: C, 42.86; H, 5.40; N, 19.99. Found: C, 42.69; H, 5.23; N, 20.20.

***tert*-butyl (phenyl(3-(trifluoromethyl)-1*H*-1,2,4-triazol-5-yl)methyl)carbamate (4c).** White crystals (2.46 g, 71.9%), m.p. 131–134 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.96 (d, J = 8.0 Hz, 1H), 7.45 – 7.23 (m, 5H), 5.98 (d, J = 8.2 Hz, 1H), 1.39 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.02, 159.78, 154.96, 152.09 (q, J = 37.4 Hz), 138.92, 128.45, 127.74, 127.40, 119.89 (q, J = 269.3 Hz), 78.71, 51.58, 28.09; ^{19}F NMR (376 MHz, DMSO-d_6) δ -63.7; MS (m/z, CI): 341.2 [M - H]$^-$. Anal. Calcd for $C_{15}H_{17}F_3N_4O_2$: C, 52.63; H, 5.01; N, 16.37. Found: C, 52.85; H, 4.83; N, 16.18.

***tert*-butyl (1-(3-(trifluoromethyl)-1*H*-1,2,4-triazol-5-yl)cyclobutyl)carbamate (4d).** White crystals (1.07 g, 35.0%), m.p. 183–184 (subl.) °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.53 (s, 1H), 7.91 (s, 1H), 2.57 – 2.50 (m, 2H), 2.36 (q, J = 9.6 Hz, 2H), 2.05 – 1.84 (m, 2H), 1.36 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.11, 154.39, 151.86 (q, J = 37.3 Hz), 119.72 (q, J = 269.4 Hz), 78.40, 53.29, 33.22, 27.94, 14.54; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.2; MS (m/z, CI): 305.2 [M - H]$^-$. Anal. Calcd for $C_{12}H_{17}F_3N_4O_2$: C, 47.06; H, 5.59; N, 18.29. Found: C, 47.01; H, 5.50; N, 18.37.

***tert*-butyl (2-(3-(trifluoromethyl)-1*H*-1,2,4-triazol-5-yl)propan-2-yl)carbamate (4e).** White crystals (1.12 g, 38.0%), m.p. 196–198 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.51 (s, 1H), 7.45 (s, 1H), 1.53 (s, 6H), 1.33 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.03, 154.14, 151.41 (q, J = 37.3 Hz), 119.67 (q, J = 269.1 Hz), 78.14, 50.49, 27.57; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.2; MS (m/z, CI): 293.2 [M - H]$^-$. Anal. Calcd for $C_{11}H_{17}F_3N_4O_2$: C, 44.90; H, 5.82; N, 19.04. Found: C, 44.67; H, 5.64; N, 19.21.

***tert*-butyl 3-(3-(trifluoromethyl)-1*H*-1,2,4-triazol-5-yl)morpholine-4-carboxylate (4f).** White crystals (2.21 g, 68.8%), m.p. 152–153 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.80 (s, 1H), 5.20 (s, 1H), 4.28 (d, J = 12.1 Hz, 1H), 3.83 – 3.74 (m, 2H), 3.70 (d, J = 13.3 Hz, 1H), 3.45 (td, J = 11.7, 3.0 Hz, 1H), 3.15 (s, 1H), 1.38 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.48, 154.16, 152.14 (q, J = 38.8 Hz), 119.47 (q, J = 269.4 Hz), 79.86, 67.68, 65.78, 48.67, 40.35, 27.81; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.3; MS (m/z, CI): 321.2 [M - H]$^-$. Anal. Calcd for $C_{12}H_{17}F_3N_4O_3$: C, 44.72; H, 5.32; N, 17.38. Found: C, 44.66; H, 5.14; N, 17.19.

***tert*-butyl 2-(3-(trifluoromethyl)-1*H*-1,2,4-triazol-5-yl)morpholine-4-carboxylate (4g).** White crystals (2.83 g, 88.2%), m.p. 188–189 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.77 (dd, J = 10.2, 3.1

Hz, 1H), 4.10 (s, 1H), 3.95 (ddd, *J* = 11.5, 3.5, 2.0 Hz, 1H), 3.77 (dd, *J* = 13.6, 3.3 Hz, 1H), 3.64 (td, *J* = 11.4, 2.8 Hz, 1H), 3.03 (s, 2H), 1.43 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.31, 153.68, 152.06 (q, *J* = 37.7 Hz), 119.61 (q, *J* = 269.2 Hz), 79.47, 69.59, 65.68, 46.09, 42.83, 27.93; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.0; MS (m/z, CI): 321.2 [M - H]$^-$. Anal. Calcd for $C_{12}H_{17}F_3N_4O_3$: C, 44.72; H, 5.32; N, 17.38. Found: C, 44.51; H, 5.30; N, 17.43.

***tert*-butyl (2-(3-(trifluoromethyl)-1*H*-1,2,4-triazol-5-yl)ethyl)carbamate (4h).** White crystals (2.18 g, 78%), m.p. 173–175 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.5 (s, 1H), 6.99 (t, *J* = 5.8 Hz, 1H), 3.29 (q, *J* = 6.7 Hz, 2H), 2.90 (t, *J* = 7.1 Hz, 2H), 1.36 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.76, 155.46, 152.14 (q, *J* = 37.6 Hz), 119.67 (q, *J* = 269.2 Hz), 77.74, 38.10, 28.11, 26.59; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.2; MS (m/z, CI): 279.2 [M - H]$^-$. Anal. Calcd for $C_{10}H_{15}F_3N_4O_2$: C, 42.86; H, 5.40; N, 19.99. Found: C, 42.73; H, 5.36; N, 19.79.

***tert*-butyl 3-(3-(trifluoromethyl)-1*H*-1,2,4-triazol-5-yl)azetidine-1-carboxylate (4i).** White crystals (1.95 g, 67%), m.p. 209–210 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.6 (br. s, 1H), 4.24 (d, *J* = 6.8 Hz, 2H), 4.01 (s, 3H), 1.40 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.21, 155.37, 152.24 (q, *J* = 37.8 Hz), 119.55 (q, *J* = 269.2 Hz), 78.88, 53.65, 27.99, 24.78; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.3; MS (m/z, CI): 291.2 [M - H]$^-$. Anal. Calcd for $C_{11}H_{15}F_3N_4O_2$: C, 45.21; H, 5.17; N, 19.17. Found: C, 45.10; H, 5.11; N, 19.34.

***tert*-butyl 4-(3-(trifluoromethyl)-1H-1,2,4-triazol-5-yl)piperidine-1-carboxylate (4j).** White crystals (2.40 g, 75%), m.p. >250 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.97 (d, *J* = 13.2 Hz, 2H), 3.08 (tt, *J* = 11.5, 3.8 Hz, 1H), 2.89 (s, 2H), 1.99 – 1.92 (m, 2H), 1.57 (qd, *J* = 12.0, 4.1 Hz, 2H), 1.41 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.52, 153.80, 151.96 (q, *J* = 37.5 Hz), 119.65 (q, *J* = 269.2 Hz), 78.68, 42.75 (br. s), 33.19, 29.60, 28.01; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.2; MS (m/z, CI): 319.0 [M - H]$^-$. Anal. Calcd for $C_{13}H_{19}F_3N_4O_2$: C, 48.75; H, 5.98; N, 17.49. Found: C, 48.68; H, 5.85; N, 17.60.

Methylation of 4a. 4a (266 g, 1 mol) was added to the suspension of potassium carbonate (207 g, 1.5 mol) in DMF (1 L) followed by the addition of iodomethane (163 g, 1.15 mol). Resulting mixture was stirred at room temperature until the completion of the reaction (monitored by NMR). Obtained

solution was filtered off and concentrated under reduced pressure. The residue diluted with H_2O (0.5 L) and extracted with EtOAc (3∗0.5 L). The combined organic layers were dried over Na_2SO_4, filtered off and evaporated *in vacuo*. The obtained solid was subjected to recrystallization from petroleum ether. Precipitate of **6** was filtered off, washed with hexane and dried on air. Mother liquor was evaporated and subjected to column chromatography (SiO_2, 1:1 EtOAc/hexane as an eluent, Rf (**6**) = 0.43, Rf (**7**) = 0.5 and Rf (**8**) = 0.12) to afford:

***tert*-butyl ((1-methyl-3-(trifluoromethyl)-1*H*-1,2,4-triazol-5-yl)methyl)carbamate (6)**. White crystals (175 (recrystallization) + 24 (chromatography) g, 72.1%), m.p. 98–100 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.56 (t, *J* = 5.9 Hz, 1H), 4.35 (d, *J* = 5.8 Hz, 2H), 3.93 (s, 3H), 1.38 (s, 9H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 155.88, 155.64, 150.52 (q, *J* = 38.3 Hz), 119.42 (q, *J* = 269.3 Hz), 78.54, 36.04, 35.29, 28.08; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.5; MS (m/z, CI): 279.2 $[M - H]^-$. Anal. Calcd for $C_{10}H_{15}F_3N_4O_2$: C, 42.86; H, 5.40; N, 19.99. Found: C, 42.80; H, 5.31; N, 20.06.

***tert*-butyl ((1-methyl-5-(trifluoromethyl)-1*H*-1,2,4-triazol-3-yl)methyl)carbamate (7).** White crystals (35.8 g, 12.8%), m.p. 53–54 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.36 (t, *J* = 6.1 Hz, 1H), 4.19 (d, *J* = 6.1 Hz, 2H), 4.00 (s, 3H), 1.39 (s, 9H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.17, 155.55, 143.12 (q, *J* = 40.1 Hz), 118.08 (q, *J* = 270.8 Hz), 78.03, 37.34, 37.05, 28.17; ^{19}F NMR (376 MHz, DMSO-d_6) δ -62.2; MS (m/z, CI): 281.2 $[M - H]^-$. Anal. Calcd for $C_{10}H_{15}F_3N_4O_2$: C, 42.86; H, 5.40; N, 19.99. Found: C, 42.74; H, 5.29; N, 19.83.

***tert*-butyl ((4-methyl-5-(trifluoromethyl)-4*H*-1,2,4-triazol-3-yl)methyl)carbamate (8).** White crystals (1.1 g, 0.4%), m.p. 111–112 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.52 (t, *J* = 6.0 Hz, 1H), 4.39 (d, *J* = 5.8 Hz, 2H), 3.73 (s, 3H), 1.39 (s, 9H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 156.10, 155.53, 143.89 (q, *J* = 38.7 Hz), 118.49 (q, *J* = 270.0 Hz), 78.54, 34.39, 30.99, 28.09; ^{19}F NMR (376 MHz, DMSO-d_6) δ -62.7; MS (m/z, CI): 281.2 $[M - H]^-$. Anal. Calcd for $C_{10}H_{15}F_3N_4O_2$: C, 42.86; H, 5.40; N, 19.99. Found: C, 42.61; H, 5.53; N, 20.13.

[3-(trifluoromethyl)-1,2,4-triazol-5-yl]alkylamine hydrochlorides (5a-j, 9-11). General procedure. Acetyl chloride (0.4 ml, 0.0055 mol) was added to 2-propanole (25 ml) and obtained solution was stirred at room temperature for 30 min. Boc-protected amine **5a-j, 6-8** (0.005 mol) was

then added and the solution was refluxed for 4 h. The mixture was evaporated under reduced pressure to obtain compounds as hydrochlorides **5a-e, g-h**, **j, 9-11**. **5f** and **5i** were purified using procedures depicted below.

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(3-(trifluoromethyl)-1*H*-1,2,4-triazol-5-yl)methanamine hydrochloride (5a). White crystals (1.01 g, 100%), m.p. 189-191 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.2 (br. s, 1H), 8.95 (s, 3H), 4.30 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 152.94, 152.22 (q, J = 37.8 Hz), 119.47 (q, J = 269.3 Hz), 33.96; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.2; MS (m/z, CI): 167.0 $[M + H]^+$. Anal. Calcd for $C_4H_6ClF_3N_4$: C, 23.72; H, 2.99; N, 27.66. Found: C, 23.67; H, 2.94; N, 27.58.

1-(3-(trifluoromethyl)-1*H*-1,2,4-triazol-5-yl)ethanamine hydrochloride (5b). Colorless solid (1.08 g, 100%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.1 (br. s, 2H), 4.73 (q, J = 7.0 Hz, 1H), 1.64 (d, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.92, 151.91 (q, J = 38.0 Hz), 119.47 (q, J = 269.4 Hz), 42.62, 17.71; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.1; MS (m/z, CI): 181.1 $[M + H]^+$. Anal. Calcd for $C_5H_8ClF_3N_4$: C, 27.73; H, 3.72; N, 25.87. Found: C, 27.55; H, 3.57; N, 26.05.

Phenyl(3-(trifluoromethyl)-1H-1,2,4-triazol-5-yl)methanamine hydrochloride (5c). White crystals (1.36 g, 98%), m.p. 118-119 °C; ^{1}HNMR (500 MHz, DMSO-d_6) δ 15.5 (br. s, 1H), 9.48 (s, 3H), 7.59 (d, J = 6.9 Hz, 2H), 7.50 – 7.41 (m, 3H), 6.01 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 155.83, 151.90 (br.s), 133.99, 129.57, 129.12, 128.12, 119.36 (q, J = 269.4 Hz), 49.72; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.0; MS (m/z, CI): 241.1 $[M-H]^-$. Anal. Calcd for $C_{10}H_{10}ClF_3N_4$: C, 43.10; H, 3.62; N, 20.11. Found: C, 43.32; H, 3.39; N, 20.04.

1-(3-(trifluoromethyl)-1H-1,2,4-triazol-5-yl)cyclobutanamine hydrochloride (5d). White crystals (1.21 g, 100%), m.p. 208–211 °C (decomp.); ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.78 (br. s, 1H), 9.33 (s, 3H), 2.78 – 2.59 (m, 4H), 2.18 (dtt, J = 11.4, 9.5, 5.8 Hz, 1H), 2.08 – 1.95 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.83, 151.94 (br. s), 119.40 (q, J = 269.7 Hz), 52.80, 31.34, 13.80.; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.1; MS (m/z, CI): 207.2 $[M + H]^+$. Anal. Calcd for $C_7H_{10}ClF_3N_4$: C, 34.65; H, 4.15; N, 23.09. Found: C, 34.57; H, 4.02; N, 23.00.

2-(3-(trifluoromethyl)-1H-1,2,4-triazol-5-yl)propan-2-amine hydrochloride (5e). Yellowish oil that crystallized upon standing (1.15 g, 100%), m.p. 239–242 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ

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DOI: 10.1039/D5OB01080B

15.64 (br. s, 1H), 9.03 (s, 3H), 1.74 (s, 6H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.98, 151.65 (br. s), 119.36 (q, *J* = 269.5 Hz), 51.36, 25.36; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.0; MS (m/z, CI): 195.0 $[M + H]^+$. Anal. Calcd for $C_6H_{10}ClF_3N_4$: C, 31.25; H, 4.37; N, 24.29. Found: C, 31.07; H, 4.20; N, 24.42.

3-(3-(trifluoromethyl)-1H-1,2,4-triazol-5-yl)morpholine (5f). Crude hydrochloride of **5f** was dissolved in 5 ml of water, and an equimolar amount of sodium hydroxide was added. The precipitated powder was filtered to obtain white crystals, that further was dissolved in 1M HCl (10 ml) and evaporated to dryness. Colorless solid (1.03 g, 79.3%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.84 (dd, *J* = 9.1, 3.6 Hz, 1H), 4.29 (dd, *J* = 12.4, 3.6 Hz, 1H), 3.96 (dt, *J* = 12.3, 3.3 Hz, 1H), 3.90 (dd, *J* = 12.3, 9.1 Hz, 1H), 3.82 – 3.78 (m, 1H), 3.32 – 3.21 (m, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 152.70, 151.81 (q, *J* = 38.4 Hz), 119.30 (q, *J* = 269.6 Hz), 66.06, 63.17, 49.34, 42.47; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.1; MS (m/z, CI): 223.1 $[M + H]^+$. Anal. Calcd for $C_7H_{10}ClF_3N_4O$: C, 32.51; H, 3.90; N, 21.66. Found: C, 32.36; H, 3.83; N, 21.48.

2-(3-(trifluoromethyl)-1H-1,2,4-triazol-5-yl)morpholine hydrochloride (5g). White crystals (1.11 g, 100%), m.p. 114-125 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.62 (br. s, 2H), 5.25 (dd, *J* = 10.9, 2.7 Hz, 1H), 4.12 (dd, *J* = 12.4, 3.2 Hz, 1H), 4.05 (td, *J* = 12.0, 2.4 Hz, 1H), 3.60 (dd, *J* = 12.9, 2.7 Hz, 1H), 3.37 – 3.23 (m, 2H), 3.12 (td, *J* = 12.5, 4.1 Hz, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.79, 152.08 (q, *J* = 40.5 Hz), 119.40 (q, *J* = 269.0 Hz), 66.96, 63.42, 44.12, 41.90; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.2; MS (m/z, CI): 223.2 $[M + H]^+$. Anal. Calcd for $C_7H_{10}ClF_3N_4O$: C, 32.51; H, 3.90; N, 21.66. Found: C, 32.39; H, 3.75; N, 21.81.

2-(3-(trifluoromethyl)-1H-1,2,4-triazol-5-yl)ethanamine hydrochloride (5h). Yellowish oil that crystallized upon standing (1.08 g, 100%), m.p. 107–108 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.06 (s, 1H), 8.28 (s, 3H), 3.27 – 3.14 (m, 4H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 155.24, 152.10 (q, *J* = 38.1 Hz), 119.63 (q, *J* = 268.8 Hz), 36.38, 23.82; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.2; MS (m/z, CI): 181.2 $[M + H]^+$. Anal. Calcd for $C_5H_8ClF_3N_4$: C, 27.73; H, 3.72; N, 25.87. Found: C, 27.69; H, 3.66; N, 25.81.

5-(azetidin-3-yl)-3-(trifluoromethyl)-1H-1,2,4-triazole hydrochloride (5i). The precipitate occurred after cooling of the reaction mixture was filtered off and dried on air. White crystals (0.65 g, 57 %), m.p. 140–141 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.23 (s, 1H), 9.34 (s, 2H), 4.39 – 4.16 (m, 5H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 157.36, 152.14 (q, J = 38.1 Hz), 119.54 (q, J = 269.3 Hz), 49.38, 27.77; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.2; MS (m/z, CI): 193.1 $[M + H]^+$. Anal. Calcd for $C_6H_8ClF_3N_4$: C, 31.52; H, 3.53; N, 24.51. Found: C, 31.37; H, 3.38; N, 24.36.

4-(3-(trifluoromethyl)-1H-1,2,4-triazol-5-yl)piperidine hydrochloride (5j). White crystals (1.28 g, 100%), m.p. 208–210 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.98 (s, 1H), 9.26 (s, 1H), 9.05 (s, 1H), 3.35 – 3.21 (m, 3H), 3.04 (t, J = 12.6 Hz, 2H), 2.22 – 2.13 (m, 2H), 1.96 (dtd, J = 15.2, 11.7, 3.9 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.46, 152.02 (q, J = 37.6 Hz), 119.65 (q, J = 269.4 Hz), 42.11, 30.72, 26.30; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.2; MS (m/z, CI): 219.0 $[M + H]^+$. Anal. Calcd for $C_8H_{12}ClF_3N_4$: C, 37.44; H, 4.71; N, 21.83. Found: C, 37.26; H, 4.54; N, 21.75.

(1-methyl-3-(trifluoromethyl)-1H-1,2,4-triazol-5-yl)methanamine hydrochloride (9). White crystals (1.08 g, 100%), m.p. 242-245°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.00 (s, 1H), 4.36 (s, 1H), 4.01 (s, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 152.27, 150.73 (q, J = 38.8 Hz), 119.25 (q, J = 269.4 Hz), 36.61, 33.36; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.4; MS (m/z, CI): 181.0 $[M + H]^+$. Anal. Calcd for $C_5H_8ClF_3N_4$: C, 27.73; H, 3.72; N, 25.87. Found: C, 27.56; H, 3.69; N, 26.02.

(1-methyl-5-(trifluoromethyl)-1H-1,2,4-triazol-3-yl)methanamine hydrochloride (10). White crystals (1.08 g, 100%), m.p. 186–190 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.88 (s, 1H), 4.13 (s, 1H), 4.08 (q, J = 1.1 Hz, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 156.93, 143.69 (q, J = 40.3 Hz), 117.89 (q, J = 271.1 Hz), 37.50 (q, J = 1.9 Hz), 35.42; ^{19}F NMR (376 MHz, DMSO-d_6) δ -62.1; MS (m/z, CI): 181.2 $[M + H]^+$. Anal. Calcd for $C_5H_8ClF_3N_4$: C, 27.73; H, 3.72; N, 25.87. Found: C, 27.58; H, 3.65; N, 25.70.

(4-methyl-5-(trifluoromethyl)-4H-1,2,4-triazol-3-yl)methanamine hydrochloride (11). White crystals (1.08 g, 100%), m.p. >230 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.02 (s, 1H), 4.36 (s, 1H), 3.81 (s, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 153.00, 144.48 (q, J = 38.4 Hz), 118.34 (q, J = 270.1

Hz), 32.51, 31.60 (q, J = 1.9 Hz); ^{19}F NMR (376 MHz, DMSO-d_6) δ -62.7; MS (m/z, CI): 181.2 [M + H]$^+$. Anal. Calcd for $C_5H_8ClF_3N_4$: C, 27.73; H, 3.72; N, 25.87. Found: C, 27.71; H, 3.68; N, 25.90.

(9H-fluoren-9-yl)methyl ((3-(trifluoromethyl)-1H-1,2,4-triazol-5-yl)methyl)carbamate (12). 5a (2.02 g, 0.01 mol) and $NaHCO_3$ (5.25 g, 0.063 mol) were added, to a mixture of dioxane (25 ml) and water (50 ml), followed by the addition of 9-fluorenylmethyl chloroformate (2.72 g, 0.0105 mol) at room temperature. The reaction mixture was stirred at room temperature for 12 hours. Then, it was diluted with 50 ml of water and washed with dichloromethane (3 × 10 ml). The dichloromethane extract was discarded, and the aqueous phase adjusted to pH 2–3 and extracted with dichloromethane (3 × 15 ml). The organic phase was evaporated under reduced pressure and the crude residue was recrystallized from toluene to obtain compound **12**. White crystals (1.99 g, 51.3%), m.p. 213–214 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.78 (s, 1H), 8.06 (t, J = 5.9 Hz, 1H), 7.89 (d, J = 7.6 Hz, 2H), 7.71 (d, J = 7.4 Hz, 2H), 7.42 (t, J = 7.6 Hz, 2H), 7.32 (t, J = 7.5 Hz, 2H), 7.22 (s, 1H), 4.41 – 4.30 (m, 4H), 4.24 (t, J = 6.6 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.67, 157.41, 142.61, 139.45, 137.46, 128.96, 127.32, 121.42, 120.06, 109.77, 36.89, 36.70; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.31; MS (m/z, CI): 189.2 [M + H]$^+$. Anal. Calcd for $C_{19}H_{15}F_3N_4O_2$: C, 58.76; H, 3.89; N, 14.43. Found: C, 58.59; H, 3.71; N, 14.50.

Benzyl ((3-(trifluoromethyl)-1H-1,2,4-triazol-5-yl)methyl)carbamate (13). 5a (5.06 g, 0.025 mol) was dissolved in dichloromethane (50 ml), followed by the addition of triethylamine (8.1 ml, 0.058 mol). Benzyl chloroformate (4.7 g, 0.028 mol) was added dropwise at 0-5 °C to the obtained solution. Then, the mixture was slowly heated to room temperature and additionally stirred for 1 h. The obtained reaction mixture was washed with water (3 × 25 ml), dried over sodium sulfate and evaporated under reduced pressure. The crude residue was recrystallized from toluene to obtain compound **13.** White crystals (5.49 g, 74.2 %), m.p. 126–129 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.79 (s, 1H), 7.99 (t, J = 5.9 Hz, 1H), 7.46 – 7.08 (m, 5H), 5.07 (s, 2H), 4.40 (d, J = 5.9 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.97, 156.34, 152.29 (q, J = 38.1 Hz), 136.79, 128.30, 127.82, 127.76, 119.57 (q, J = 269.3 Hz), 65.77, 36.58; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.33.; MS (m/z,

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CI): 301.0 $[M + H]^+$. Anal. Calcd for $C_{12}H_{11}F_3N_4O_2$: C, 48.01; H, 3.69; N, 18.66. Found: C, 47.86; H, 3.62; N, 18.50.

(3-(trifluoromethyl)-1H-1,2,4-triazol-5-yl)methanamine (14). Sodium carbonate (2.27 g, 0.027 mol) were added to the solution of **5a** (5.06 g, 0.025 mol) in water (25 ml). The resulting mixture was stirring at room temperature for 1 hour and then evaporated under reduced pressure. The residue was dissolved in methanol (25 ml), insoluble impurities were filtered off, followed by the evaporation of mother liquor. Crude product was recrystallized from acetonitrile (10 ml) to obtain **14**. White crystals (3.04 g, 73.2%), m.p. 108–109 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.43 (s, 3H), 3.99 (s, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.01, 152.51 (q, J = 34.6 Hz), 121.57 (q, J = 268.8 Hz), 37.04; ^{19}F NMR (376 MHz, DMSO-d_6) δ -61.97; MS (m/z, CI): 167.2 $[M + H]^+$. Anal. Calcd for $C_4H_5F_3N_4$: C, 28.92; H, 3.03; N, 33.73. Found: C, 28.77; H, 2.91; N, 33.87.

2,8-bis(trifluoromethyl)-4a,5,6,10a,11,12-hexahydro-[1,2,4]triazolo[1',5':1,6]pyrazino[2,3-e][1,2,4]triazolo[1,5-a]pyrazine (15). White crystals (0.75 g, 85%), m.p. 229 °C (decomp.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.99 (d, J = 3.7 Hz, 2H), 5.07 (dt, J = 4.6, 2.5 Hz, 2H), 4.11 (dd, J = 17.0, 2.5 Hz, 2H), 3.52 (dd, J = 16.9, 2.1 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.48, 151.80 (q, J = 38.4 Hz), 119.45 (q, J = 269.6 Hz), 68.75, 36.81; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.52; MS (m/z, CI): 355.0 $[M + H]^+$. Anal. Calcd for $C_{10}H_8F_6N_8$: C, 33.91; H, 2.28; N, 31.63. Found: C, 33.86; H, 2.22; N, 31.55.

2,10-bis(trifluoromethyl)-4,5,7,7a,12,13,15,15a-octahydro-[1,2,4]triazolo[1'',5'':3',4']pyrimido[1',2':1,6][1,4]pyrazino[3,4-b][1,2,4]triazolo[5,1-f][1,3]pyrimidine-7,15-diol (16). White crystals (1.72 g, 78%), m.p. >250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.04 (d, J = 6.4 Hz, 2H), 5.24 (d, J = 2.3 Hz, 2H), 5.17 (dd, J = 6.3, 2.4 Hz, 2H), 3.32 – 3.20 (m, 2H), 3.12 – 2.88 (m, 6H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.20, 151.62 (q, J = 38.3 Hz), 119.56 (q, J = 269.3 Hz), 79.71, 71.08, 44.52, 23.71; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.39. MS (m/z, CI): 441.2 $[M + H]^+$. Anal. Calcd for $C_{14}H_{14}F_6N_8O_2$: C, 38.19; H, 3.21; N, 25.45. Found: C, 38.46; H, 3.35; N, 25.11.

Bis(phenyl(3-(trifluoromethyl)-1H-1,2,4-triazol-5-yl)methyl)amine toluene semisolvate (17). 5c (1.39 g, 0.005 mol) was placed into 50 ml round-bottom flask and heated at oil bath to 150 °C for 1

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DOI: 10.1039/D5OB01080B

hour. The resulting brown solid was stirred with water (25 ml), filtered off dried and recrystallized from of toluene (50 ml) to obtain **17** as toluene solvate. White crystals (0.99 g, 84.4%), m.p. 136–138 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.66 (s, 3H), 7.46 – 7.29 (m, 20H), 5.01 (d, *J* = 6.7 Hz, 2H), 4.91 (d, *J* = 6.6 Hz, 2H), 4.35 (t, *J* = 6.9 Hz, 1H), 4.14 (t, *J* = 7.0 Hz, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 160.17, 159.86, 152.22 (q, *J* = 38.1 Hz), 138.85, 138.17, 137.35, 128.89, 128.82, 128.78, 128.26, 128.19, 128.16, 127.82, 127.56, 125.30, 119.53 (qd, *J* = 269.1, 4.7 Hz), 57.00, 56.74, 21.02; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.18, -64.23; MS (m/z, CI): 468.2 $[M + H]^+$. Anal. Calcd for $C_{23.5}H_{19}F_6N_7$: C 54.97; H 3.73; N 19.10. Found: C 55.22; H 3.92; N 18.88.

Ethyl 2-(5-(((tert-butoxycarbonyl)amino)methyl)-3-(trifluoromethyl)-1H-1,2,4-triazol-1-yl)acetate (18). 5a (26.6 g, 0.1 mol) was added to the suspension of K_2CO_3 (27.6 g, 0.2 mol) in DMF (100 ml), and the resulting mixture was stirred at room temperature for 15 min. Then, ethyl chloroacetate (12.9 g, 0.105 mol) of was added in one portion, and the obtained mixture was stirred at room temperature for 8 h. The resulting mixture was filtered; the filtrate was evaporated at reduced pressure, diluted with CH_2Cl_2 (100 ml) and washed with water (2 × 25 ml). Organic layer was dried over Na_2SO_4 and evaporated at reduced pressure again. The crude residue was recrystallized from hexane to obtain **18**. White crystals (29.29 g, 82.3%), m.p. 68–69 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.58 (t, *J* = 6.1 Hz, 1H), 5.35 (s, 2H), 4.35 (d, *J* = 5.8 Hz, 2H), 4.18 (q, *J* = 7.1 Hz, 2H), 1.37 (s, 9H), 1.22 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.57, 157.16, 155.68, 151.09 (q, *J* = 38.7 Hz), 119.24 (q, *J* = 269.6 Hz), 78.64, 61.65, 50.13, 35.22, 28.04, 13.87; ^{19}F NMR (376 MHz, DMSO-d_6) δ -62.72; MS (m/z, CI): 353.2 $[M + H]^+$. Anal. Calcd for $C_{13}H_{19}F_3N_4O_4$: C, 44.32; H, 5.44; N, 15.90. Found: C, 44.26; H, 5.38; N, 15.96.

2-(5-(aminomethyl)-3-(trifluoromethyl)-1H-1,2,4-triazol-1-yl)acetic acid hydrochloride (19). 18 (8.8 g, 0.025 mol) was added to the solution of NaOH (1.2 g, 0.03 mol) in 100 ml of water. The mixture was refluxed for 4 hours, then cooled to room temperature and acidified by the addition of HCl (conc.). The precipitated solid was filtered off, while mother liquor was extracted with dichloromethane (3 × 25 ml) and evaporated under reduced pressure. The resulting white solid was combined with precipitate obtained by filtration, suspended in HCl (50 ml, 1.5 N) and refluxed until

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DOI: 10.1039/D5OB01080B

the starting compound completely dissolved (estimated 1 hour). The solution was then evaporated under reduced pressure, and the residue was recrystallized from ethanol to obtain **19**. White crystals (6.22 g, 95.5%), m.p. 218–219 °C (decomp.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.74 (s, 1H), 9.00 (s, 3H), 5.38 (s, 2H), 4.35 (s, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.79, 153.40, 151.16 (q, J = 38.9 Hz), 119.11 (q, J = 269.5 Hz), 50.92, 33.49; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.54; MS (m/z, CI): 252.2 $[M + H]^+$. Anal. Calcd for $C_6H_8ClF_3N_4O_2$: C, 27.65; H, 3.09; N, 21.50. Found: C, 27.49; H, 3.24; N, 21.65.

2-(5-(((((9H-fluoren-9-yl)methoxy)carbonyl)amino)methyl)-3-(trifluoromethyl)-1H-1,2,4-triazol-1-yl)acetic acid (20). 19 (5.21 g, 0.02 mol) and $NaHCO_3$ (10.5 g, 0.125 mol) were added to the mixture of dioxane (50 ml) and water (100 ml), followed by the addition of 9-fluorenylmethyl chloroformate (5.43 g, 0.021 mol) at room temperature. The reaction solution was stirred at room temperature for 12 hours. Then, the reaction mixture was diluted with 100 ml of water and washed with dichloromethane (3 × 25 ml). The dichloromethane extract was discarded, and the aqueous phase was adjusted to pH 2–3 and extracted with dichloromethane (3 × 25 ml). The organic phase was dried over Na_2SO_4 and evaporated under reduced pressure to obtain **10.** White solid (7.57 g, 84.9%), m.p. 214–216 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.58 (s, 1H), 8.11 (t, J = 6.0 Hz, 2H), 7.89 (d, J = 7.5 Hz, 2H), 7.70 (d, J = 7.4 Hz, 2H), 7.41 (t, J = 7.5 Hz, 2H), 7.32 (t, J = 7.4 Hz, 2H), 5.26 (s, 2H), 4.43 (d, J = 5.8 Hz, 2H), 4.33 (d, J = 7.0 Hz, 2H), 4.23 (t, J = 7.1 Hz, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.00, 156.82, 156.37, 151.03 (q, J = 38.6 Hz), 143.76, 140.74, 127.64, 127.05, 125.20, 120.12, 119.29 (q, J = 269.5 Hz), 65.92, 50.33, 46.62, 35.54; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.56; MS (m/z, CI): 447.2 $[M + H]^+$. Anal. Calcd for $C_{21}H_{17}F_3N_4O_4$: C, 56.51; H, 3.84; N, 12.55. Found: C, 56.45; H, 3.78; N, 12.63.

Ethyl 2-(5-(aminomethyl)-3-(trifluoromethyl)-1H-1,2,4-triazol-1-yl)acetate hydrochloride (21). 18 (26.4 g, 0.075 mol) was dissolved in 1M solution of HCl in ethanol (100 ml). The obtained mixture was refluxing for 4 h, cooled to room temperature, and evaporated at reduced pressure. The resulting colorless residue was suspended in hexane until complete crystallization into a white powder occurred. White crystals (19.73 g, 91.2%), m.p. 117-118 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01

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DOI: 10.1039/D5OB01080B

(s, 3H), 5.49 (s, 2H), 4.39 (s, 2H), 4.19 (q, J = 7.1 Hz, 2H), 1.23 (t, J = 7.1 Hz, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.54, 153.62, 151.33 (q, J = 39.0 Hz), 119.04 (q, J = 269.8 Hz), 61.93, 50.64, 33.40, 13.91; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.62; MS (m/z, CI): 253.0 $[M + H]^+$. Anal. Calcd for $C_8H_{12}ClF_3N_4O_2$: C, 33.29; H, 4.19; N, 19.41. Found: C, 33.14; H, 4.02; N, 19.25.

2-(trifluoromethyl)-7,8-dihydro-[1,2,4]triazolo[1,5-a]pyrazin-6(5H)-one (22). 21(14.4 g, 0.05 mol) was dissolved in water (50 ml), and triethylamine (7.73 ml, 0.055 mol) was added in one portion. The resulting mixture was stirring at room temperature for 2 h, and the precipitated solid was filtered and washed with water (3 × 10 ml) to obtain **22**. Light brown crystals (7.53 g, 73.1%), m.p. >230 °C (subl.); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.65 (s, 1H), 4.86 (t, J = 2.2 Hz, 2H), 4.60 (q, J = 2.2 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 163.15, 152.44 (q, J = 38.4 Hz), 149.78, 119.32 (q, J = 269.4 Hz), 49.48, 38.45; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.66; MS (m/z, CI):205.2 $[M + H]^+$. Anal. Calcd for $C_6H_5F_3N_4O$: C, 34.96; H, 2.45; N, 27.18. Found: C, 34.82; H, 2.31; N, 27.31.

Tert-butyl ((1-(2,2-diethoxyethyl)-3-(trifluoromethyl)-1H-1,2,4-triazol-5-yl)methyl)carbamate (24). 5a (2.66 g, 0.01 mol) was added to the suspension of K_2CO_3 (2.07 g, 0.015 mol) in DMF (50 ml), and the resulting mixture was stirring at room temperature for 15 min. Then, 12.9 g (0.105 mol) of bromoacetal was added in one portion, and the obtained mixture was stirring at room temperature for 8 h. The resulting mixture was filtered; the filtrate was evaporated at reduced pressure, diluted with CH_2Cl_2 (50 ml), washed with water (3 × 10 ml). Organic layer was dried over Na_2SO_4 and evaporated at reduced pressure again. The crude residue was dissolved in 5 ml of hexane and the solution was cooled to -10 °C. The precipitate was filtered and washed with cold hexane (3 × 1 ml) to obtain **24**. White crystals (1.12 g, 29.2%), m.p. 53-54 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.52 (t, J = 6.1 Hz, 1H), 4.80 (t, J = 5.3 Hz, 1H), 4.39 (dd, J = 11.3, 5.5 Hz, 4H), 3.65 (dq, J = 9.7, 7.0 Hz, 2H), 3.44 (dq, J = 9.6, 7.0 Hz, 2H), 1.38 (s, 9H), 1.03 (t, J = 7.0 Hz, 6H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 156.97, 155.66, 150.93 (q, J = 38.3 Hz), 119.36 (q, J = 269.3 Hz), 99.97, 78.51, 62.86, 51.14, 35.36, 28.08, 14.97; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.57; MS (m/z, CI): 381.2 $[M + H]^+$. Anal. Calcd for $C_{15}H_{25}F_3N_4O_4$: C, 47.12; H, 6.59; N, 14.65. Found: C, 47.01; H, 6.73; N, 14.81.

4-methyl-N-((3-(trifluoromethyl)-1H-1,2,4-triazol-5-yl)methyl)benzenesulfonamide (26). 5a (4.06 g, 0.02 mol) of was dissolved in dichloromethane (75 ml), followed by addition of triethylamine (6.4 ml, 0.046 mol). The solution was cooled with an ice bath, and tosyl chloride (4.2 g, 0.022 mol) of was added dropwise at 0-5 °C. Then, the mixture was slowly heated to room temperature and was stirring for 8 h. The obtained solution was diluted with 50 ml of water and precipitated solid of compound **26** was filtered and washed with water (3 × 15 ml). White crystals (5.5 g, 86.0 %), m.p. 179-181 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.81 (s, 1H), 8.39 (t, *J* = 6.1 Hz, 1H), 7.65 (d, *J* = 8.3 Hz, 2H), 7.36 (d, *J* = 8.0 Hz, 2H), 4.16 (d, *J* = 6.0 Hz, 2H), 2.37 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.24, 152.24 (q, *J* = 37.6 Hz), 142.95, 136.79, 129.52, 126.58, 119.43 (q, *J* = 269.2 Hz), 38.10, 20.87; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.42; MS (m/z, CI): 321.2 $[M + H]^+$. Anal. Calcd for $C_{11}H_{11}F_3N_4O_2S$: C, 41.25; H, 3.46; N, 17.49. Found: C, 41.09; H, 3.30; N, 17.59.

7-tosyl-2-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4]triazolo[1,5-a]pyrazine (27). 26 (3.2 g, 0.01 mol) was added to the suspension of K_2CO_3 (4.14 g, 0.03 mol) in DMF (50 ml), and the resulting mixture was stirring at room temperature for 15 min. Then, 1.2-dibromoethane (0.91 ml, 0.0105 mol) of was added in one portion, and the obtained mixture was stirring at 45-50 °C for 16 h. The obtained solution was diluted with water (100 ml) and precipitated solid of compound **27** was filtered and washed with water (3 × 15 ml). White crystals (3.18g, 92.0%), m.p. 130-132 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.85 – 7.68 (m, 2H), 7.45 (d, *J* = 8.1 Hz, 2H), 4.51 (s, 2H), 4.25 (t, *J* = 5.6 Hz, 2H), 3.71 (dd, *J* = 6.2, 4.9 Hz, 2H), 2.39 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 151.54 (q, *J* = 38.6 Hz), 150.35, 144.36, 132.87, 130.09, 127.48, 119.27 (q, *J* = 269.5 Hz), 46.43, 43.57, 42.41, 20.96; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.62.; MS (m/z, CI): 347.0 $[M + H]^+$. Anal. Calcd for $C_{13}H_{13}F_3N_4O_2S$: C, 45.09; H, 3.78; N, 16.18. Found: C, 45.03; H, 3.62; N, 16.24.

2-(trifluoromethyl)-5,6,7,8-tetrahydro-[1,2,4]triazolo[1,5-a]pyrazine (23). 27 (1.73 g, 0.005 mol) was added to HCl (25 ml, conc.), and the resulting mixture was refluxing for 8 hours. The obtained clear solution was evaporated under reduced pressure, and the residue was neutralized by the addition of aqueous solution of NaOH (5 ml, 1 M). **23** was extracted with dichloromethane (3 × 15 ml). The organic phase was evaporated under reduced pressure to obtain **23** as a white solid (0.77g, 80.2%),

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m.p. 49-52 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.13 (t, J = 5.5 Hz, 2H), 3.98 (s, 2H), 3.17 (t, J = 5.5 Hz, 2H), 2.87 (s, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 153.94, 150.81 (q, J = 38.1 Hz), 119.70 (q, J = 269.2 Hz), 47.91, 42.79, 41.79; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.49; MS (m/z, CI): 193.0 $[M + H]^+$. Anal. Calcd for $C_6H_7F_3N_4$: C, 37.51; H, 3.67; N, 29.16. Found: C, 37.34; H, 3.52; N, 29.32.

Materials, methods and procedures used for synthesis and characterization of model peptides.

Synthesis of model peptides

Fmoc solid-phase peptide synthesis was performed using an automated Liberty Blue microwave-assisted synthesiser (CEM) on Rink amide ProTide resin (0.56 mmol/g, 0.1 mmol, 179 mg), with Fmoc protected amino acids including Fmoc-trifluoromethyltriazole **20** and Fmoc-4-fluorophenylalanine (0.2 M in DMF; 5 eq.), DIC (1 M stock solution in DMF; 10 eq.) and Oxyma Pure (1 M stock solution, 5 eq.). Standard coupling procedures employed single coupling of each amino acid (2.5 min, 90 °C) and Fmoc-deprotection was achieved by treatment of the resin with morpholine (20% *v/v* in DMF, 4 mL). Following the removal of the final Fmoc-protecting group, peptides were acetylated using acetic anhydride (20 % in DMF) at 37 °C for 15 min and repeated once, followed by washing the resin with DMF three times and drying under vacuum. The resin was washed several times with Et_2O (5 mL) and the peptide was liberated by treatment with a cleavage cocktail of TFA/TIPS/H_2O (95/2.5/2.5) with rotation at ~30 rpm for 2 h at room temperature in a disposable fritted syringe. Afterwards, the suspension was filtered and precipitated in ice-cold Et_2O, centrifuged (5 min at 5000 rpm) and the pellet was resuspended with Et_2O (10 mL). This was centrifuged again (5 min 5000 rpm) and the Et_2O was discarded. Crude peptide mixtures were purified using RP-HPLC (Teledyne IscoEZPrep), equipped with a Waters XBridge C18 Prep column (19×100 mm, 5 μm, 130 Å) using a linear gradient from 5-90% acetonitrile in water (0.1% TFA) over 20 minutes at a flow rate of 8 mL/min.

Characterisation of model peptides

High-resolution mass spectrometry.

Using an Agilent 6530 accurate mass QToF system, separation was performed with an Agilent ZORBAX Eclipse Plus C18 Rapid Resolution HD analytical C18 column (1.8 µm particle size, 2.1×50 mm) at 25 °C following injection of 2 µL of sample. A mobile phase composed of water (0.1 % TFA, eluent A) and methanol (0.1 % TFA, eluent B) afforded separation using a 12.5 min linear gradient (5% to 95% B) and a flow rate of 0.5 mL/min. Monitoring was performed by UV-Vis detection at λ=215 nm. Mass spectral analysis of peptides was performed in the positive ionisation (ESI+) mode in full scan mode (*m/z* 100-3200). Operating pressures were in the range of 2000-3000 PSI. Products were identified based on the protonated ions $[M+H]^+$ and sodiated adducts $[M+Na]^+$.

Analytical HPLC.

Separation was performed on a Waters XBridge C18 analytical column (3.5 µm particle size, 4.6×50 mm) at 40 °C. Sample injection volume was 2µL and the mobile phase flow rate was 1 mL/min. The mobile phase consisted of water (0.1 % TFA, eluent A) and methanol (0.1 % TFA, eluent B). Separation used a 15 min gradient as follows: 0-10 min 95% A to 20 % A, 10-11 min to 5% A, 11-12 min hold 5% A, 12-13 min to 95% A, 13-15 min hold 95% A. Monitoring was performed by UV-Vis detection at λ=215 nm.

^{19}F NMR analysis of amide bond conformation.

Peptide were dissolved in PBS solution (1 mg/mL; pH 7.4, 10 mM by phosphate, with 10% D_2O). 1D proton decoupled ^{19}F NMR analysis was conducted using a Bruker 400 MHz NMR spectrometer operating at a frequency of 386 MHz, 28 scans, D1=10 sec, 23°C. NMR data were processed using MNova (v14.2.1-27684).

Author contributions

DMK: Methodology, Investigation, Conceptualization, Writing – review & editing. **OVV**: Writing – original draft, Visualization, Investigation. **ROD**: Investigation**,** Resources. **HVI:** Investigation, Validation. **IVR**: Visualization, Validation. **AVK**: Investigation, Visualization. **GSMH**: Investigation, Writing – original draft. **CRC**: Methodology, Investigation, Conceptualization, Writing – review & editing. **SS**: Writing – original draft, Visualization,

Investigation, Data curation. **RDL**: Supervision, Resources, Project administration, Methodology, Conceptualization.

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DOI: 10.1039/D5OB01080B

Conflict of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of ESI. The supplementary crystallographic data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223–336-033; or deposit@ccdc.ca.ac.uk).

Acknowledgements

This work was supported by the Ministry of Education and Science of Ukraine (Project No. 25BF037–02). "Prof. R. Lampeka and Dr. I. Raspertova are grateful to the Organizers of the II European Chemistry School for Ukrainians (https://acmin.agh.edu.pl/en/detail/s/ii-european-chemistry-school-for-ukrainians)".

References

1 O. Gupta, T. Pradhan and G. Chawla, *J. Mol. Struct.*, 2023, **1274**, 134487.

2 L. R. Peyton, S. Gallagher and M. Hashemzadeh, *Drugs of Today*, 2015, **51**, 705-718.

3 C. Lass-Flörl, *Drugs*, 2011, **71**, 2405–2419.

4 K. R. Manchester, E. C. Lomas, L. Waters, F. C. Dempsey and P. D. Maskell, *Drug Test. Anal.*, 2018, **10**, 37–53.

5 R. Davis, R. Whittington and H. M. Bryson, *Drugs*, 1997, **53**, 608–636.

6 T. M. Dando and C. M. Perry, *Drugs*, 2004, **64**, 777–794.

7 G. D. Madding, D. W. Smith, R. I. Sheldon and B. Lee, *J. Heterocycl. Chem.*, 1985, **22**, 1121–1126.

8 M. M. Zhao, J. M. McNamara, G.-J. Ho, K. M. Emerson, Z. J. Song, D. M. Tschaen, K. M. J. Brands, U.-H. Dolling, E. J. J. Grabowski, P. J. Reider, I. F. Cottrell, M. S. Ashwood and B. C. Bishop, *J. Org. Chem.*, 2002, **67**, 6743–6747.

9 D. Kim, L. Wang, M. Beconi, G. J. Eiermann, M. H. Fisher, H. He, G. J. Hickey, J. E. Kowalchick, B. Leiting, K. Lyons, F. Marsilio, M. E. McCann, R. A. Patel, A. Petrov, G. Scapin, S. B. Patel, R. S. Roy, J. K. Wu, M. J. Wyvratt, B. B. Zhang, L. Zhu, N. A. Thornberry and A. E. Weber, *J. Med. Chem.*, 2005, **48**, 141–151.

10 Y.-Y. Guo, J.-Y. Zhang, J.-F. Sun and H. Gao, *Eur. J. Med. Chem.*, 2024, **267**, 116185.

11 W. Hou, W. Dai, H. Huang, S.-L. Liu, J. Liu, L.-J. Huang, X.-H. Huang, J.-L. Zeng, Z.-W. Gan, Z.-Y. Zhang and J.-X. Lan, *Eur. J. Med. Chem.*, 2023, **258**, 115544.

12 S. P. France, E. A. Lindsey, E. L. McInturff, S. Berritt, D. W. Carney, J. C. DeForest, S. J. Fink, A. C. Flick, T. S. Gibson, K. Gray, A. M. Johnson, C. A. Leverett, Y. Liu, S. Mahapatra and R. B. Watson, *J. Med. Chem.*, 2024, **67**, 4376–4418.

13 J. E. Kowalchick, B. Leiting, K. D. Pryor, F. Marsilio, J. K. Wu, H. He, K. A. Lyons, G. J. Eiermann, A. Petrov, G. Scapin, R. A. Patel, N. A. Thornberry, A. E. Weber and D. Kim, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 5934–5939.

14 N. Li, Q. Liu, Y. Tian and L. Wu, *J. Gynecol. Oncol.*, DOI:10.3802/jgo.2022.33.e86.

15 R. Norton, E. Leung, I. Chandrashekaran and C. MacRaild, *Molecules*, 2016, **21**, 860.

16 P. M. Killoran, G. S. M. Hanson, S. J. M. Verhoork, M. Smith, D. Del Gobbo, L. Lian and C. R. Coxon, *Chem. – A Eur. J.*, DOI:10.1002/chem.202203017.

17 N. A. Meanwell, *J. Med. Chem.*, 2018, **61**, 5822–5880.

18 J. M. Kane, B. M. Baron, M. W. Dudley, S. M. Sorensen, M. A. Staeger and F. P. Miller, *J. Med. Chem.*, 1990, **33**, 2772–2777.

19 M. Pagacz-Kostrzewa, M. Sałdyka, A. Bil, W. Gul, M. Wierzejewska, D. M. Khomenko and R. O. Doroschuk, *J. Phys. Chem. A*, 2019, **123**, 841–850.

20 D. M. Khomenko, R. O. Doroshchuk, Y. M. Ohorodnik, H. V. Ivanova, B. V. Zakharchenko, I. V. Raspertova, O. V. Vaschenko, A. V. Dobrydnev, O. O. Grygorenko and R. D. Lampeka, *Chem. Heterocycl. Compd.*, 2022, **58**, 116–128.

21 D. M. Khomenko, R. O. Doroschuk, V. V. Trachevskii, S. Shova and R. D. Lampeka, *Tetrahedron Lett.*, 2016, **57**, 990–992.

22 D. M. Khomenko, R. O. Doroshchuk, I. V. Raspertova, J. García López, F. López Ortiz, S. Shova, O. A. Iegorov and R. D. Lampeka, *Tetrahedron Lett.*, 2019, **60**, 151089. View Article Online DOI: 10.1039/D5OB01080B

23 S. Borg, G. Estenne-Bouhtou, K. Luthman, I. Csoeregh, W. Hesselink and U. Hacksell, *J. Org. Chem.*, 1995, **60**, 3112–3120.

24 J. Cesar and M. Sollner, *Synth. Commun.*, 2000, **30**, 4147–4158.

25 S. R. Trevino, S. Schaefer, J. M. Scholtz and C. N. Pace, *J. Mol. Biol.*, 2007, **373**, 211–218.

26 E. Bonandi, M. S. Christodoulou, G. Fumagalli, D. Perdicchia, G. Rastelli and D. Passarella, *Drug Discov. Today*, 2017, **22**, 1572–1581.

27 J. Bachl, J. Mayr, F. J. Sayago, C. Cativiela and D. Díaz Díaz, *Chem. Commun.*, 2015, **51**, 5294–5297.

28 A.-S. Yang, B. Hitz and B. Honig, *J. Mol. Biol.*, 1996, **259**, 873–882.

29 2015 Rigaku Oxford Diffraction. CrysAlis Pro Software System. Rigaku Corporation: Oxford UK.

30 G. M. Sheldrick, *Acta Crystallogr. Sect. A Found. Adv.*, 2015, **71**, 3–8.

31 G. M. Sheldrick, *Acta Crystallogr. Sect. C Struct. Chem.*, 2015, **71**, 3–8.

32 O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard and H. Puschmann, *J. Appl. Crystallogr.*, 2009, **42**, 339–341.

33 H. C. Brown and C. R. Wetzel, *J. Org. Chem.*, 1965, **30**, 3729–3733.

Data availability statement

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DOI: 10.1039/D5OB01080B

The data supporting this article have been included as part of ESI. The supplementary crystallographic data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223–336-033; or deposit@ccdc.ca.ac.uk).