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Design and synthesis of an all-in-one 3-(1,1-difluoroprop-2-ynyl)-3*H*-diazirin-3-yl functional group for photo-affinity labeling

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ABSTRACT

A novel radioisotope-free photo-affinity probe containing the 3-(1,1-difluoroprop-2-ynyl)-3*H*-diazirin-3-yl functional group was designed and synthesized. This very compact functionality is envisaged to allow photochemically-induced coupling of a compound to its target followed by click reaction coupling with an azido-biotin reagent in order to facilitate purification of the labeled target. In a proof-of-concept study we have shown that 3-(1,1-difluoroprop-2-ynyl)-3*H*-diazirin-3-yl functional group could be photolyzed to efficiently furnish the methanol adduct **23** and that the generated highly unstable carbene does not react with the neighboring acetylene moiety. A subsequent click reaction with the azido-biotin derivative **25** proceeded smoothly to give triazole **26**. This chemical probe should thus be of unique value for facilitating identification of the molecular structure of the target of a bioactive compound.

1. Introduction

Photo-affinity labeling (PAL) is an efficient and reliable tool used to identify, isolate and characterize novel biological molecules and potential drug targets, particularly when the target molecule is unknown.[1–10] In PAL, a ligand incorporates a photoreactive group in its structure which can be irradiated when it complexes with its target. Irradiation gives rise to a highly reactive intermediate, which in turn forms a covalent bond between the ligand and the target protein. Even though many types of photosensitive groups have been used such as azides, diazirine, and benzophenones that are suitable for PAL, aromatic diazirines have enjoyed widespread application and are considered to be the most effective PAL functional group because they generate carbenes, which react with many functional groups including C–H bonds[11,12] and they can be activated at a long wavelength (λ >300 nm), thus, preventing damage or competing activation of the examined biological system.

In order to identify and facilitate the isolation of the photo-labeled product, a reporter group such as a radioactive label, a biotin tag or a fluorescent tag is often incorporated into the PAL ligand.[7] Radioactive labeling is often used for tracing the tagged molecules because of detection sensitivity and because radioactive labels do not need to employ sterically demanding tracer groups that may perturb interactions of the biological target and the labeled reagent. However, the hazardous nature of the radioactive ligand inhibits use and complicates the direct analysis of the tagged molecules. In addition, radioactive tagging methods do not offer a ready handle for target molecule enrichment and isolation. To address these disadvantages, biotin tagging has been developed which takes advantage of the high affinity between biotin for avidin to allow affinity purification of the probe-target adduct. However, the large and highly polar biotin unit often has unfavorable effects on the bioactivity of the probe.[13] To address this issue, a number of 'fishing' techniques have been devised wherein ligands are made which incorporate either an alkyl azide or terminal alkyne instead of biotin. Once the PAL ligand–target complex is photo-reacted, the azide or the alkyne group can be used as a tag for the consecutive attachment of a detectable group (e.g., biotin) using either azido-targeting or alkyne-targeting bioconjugation reactions such as Cu(I) catalyzed 1,3-dipolar cycloaddition (click chemistry).[14–20]

Recently, Hosoya and co-workers have prepared a compact 'all-in-one' (3-azidodifluoromethyl-3*H*-diazirin-3-yl)benzene derivative **1** to be used for radioisotope-free PAL.[21] Unfortunately, azido-containing compounds are potentially explosive, and in our hands a derivative (**3**) of compound **1** exploded when it was either treated with palladium, Cu(I), or Cu(II) catalyst (Fig. 1).[22] In a separate attempt to make the 3-azidodifluoromethyl-3*H*-diazirin-3-yl group, the azido-*O*-tosyl-oxime **4** decomposed to give the undesired nitrile **5** when it was treated with ammonia under reaction conditions similar to those reported by Hosoya and co-workers. Given that 3-azidodifluoromethyl-3*H*-diazirin-3-yl group appeared to be unstable and more importantly, explosive in nature under our reaction conditions, we envisioned that a compound bearing an acetylene moiety instead of an azide should be more suitable.

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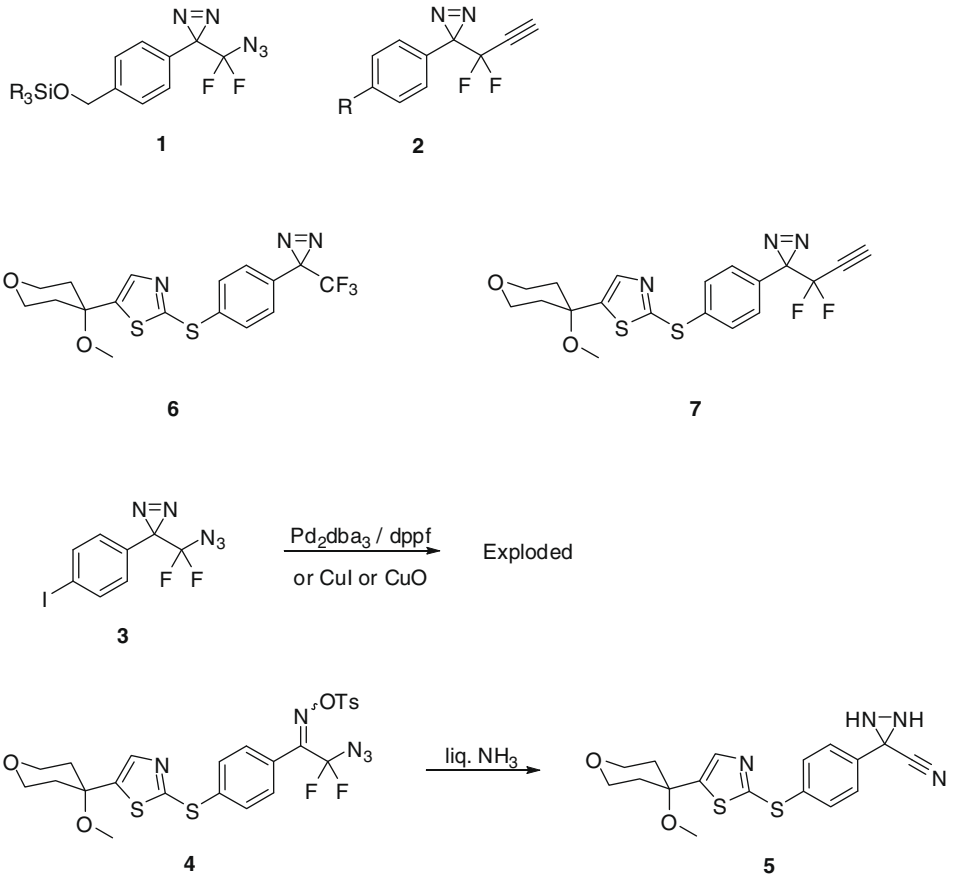


Figure 1. Decomposition of the azido group.

This is indeed the case and we report herein the first synthesis of the photo-affinity probe containing a 3-(1,1-difluoroprop-2-ynyl)-3*H*-diazirin-3-yl group (**2**).

In order to illustrate the potential use of the 'all-in-one' 3-(1,1-difluoroprop-2-ynyl)-3*H*-diazirin-3yl group we report the synthesis of a derivative (**7**) of a known 5-lipoxygenase (5-LO) inhibitor **6**[23] and we also describe a sequence of carbene-trapping followed by click chemistry using an azido-biotin reagent **25**.

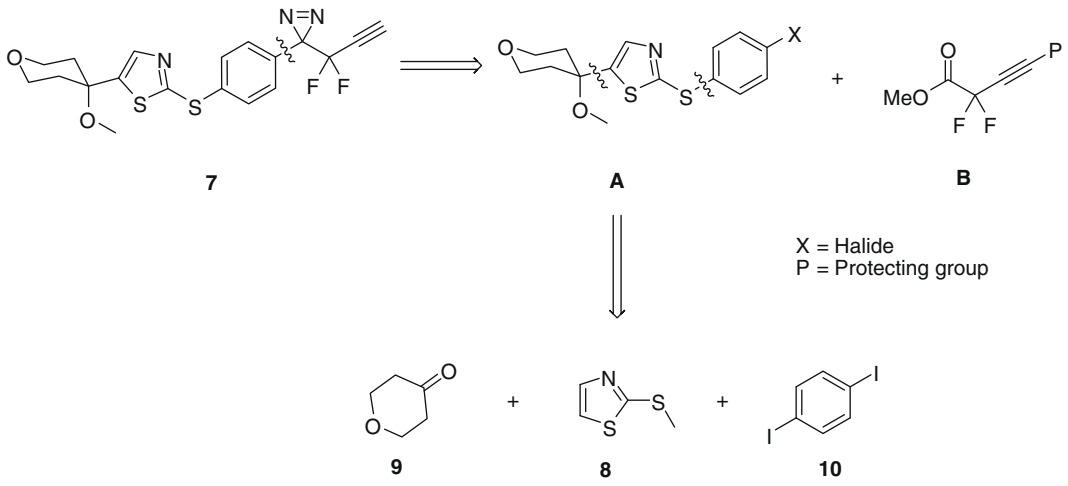
Leukotrienes (LTs) are lipid mediators implicated in numerous diseases, including inflammation, atherosclerosis, and respiratory diseases such as asthma[24–32] and 5-LO plays a key role in the biosynthesis of LTs from arachidonic acid. Therefore, blocking LT biosynthesis could lead to the development of novel therapies for such diseases. There are some indications of multiple protein complexes involved in LT biosynthesis in cells[33] and compounds such as **6** have shown anomalous LT inhibitory activity in cells[34] and may interact with other undefined protein targets. Hence, an affinity ligand such as compound **7** bearing the 3-(1,1-difluoroprop-2-ynyl)-3*H*-diazirin-3-yl group could be used to 'fish out' 5-LO or any other protein this molecule might interact with within the LT biosynthesizing cells. The results of these studies will be published elsewhere.

2. Results and discussion

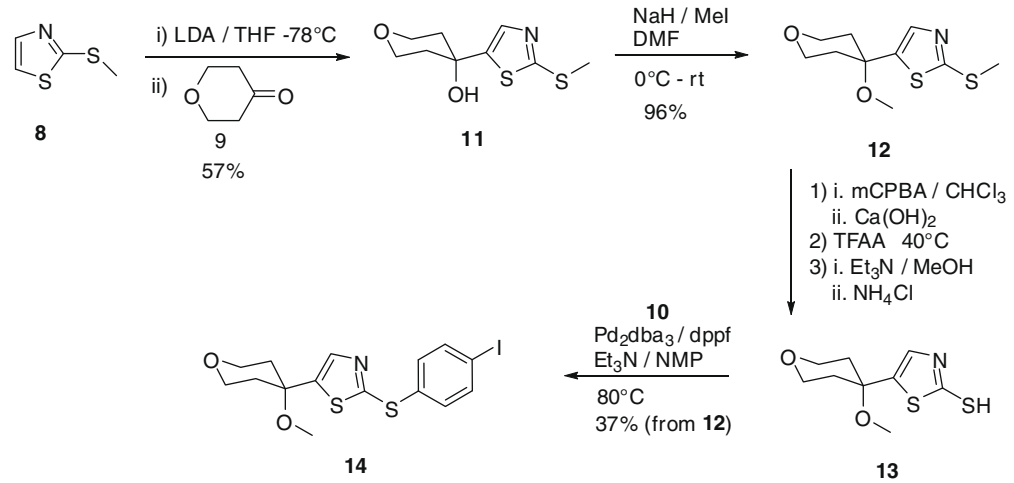
Retrosynthetic analysis indicated that target compound **7** could be synthesized by coupling aryl halide **A** with ester **B** (Scheme 1). The key intermediate **A** could, in turn, be prepared from commercially available 2-(methylthio)thiazole (**8**), tetrahydropyran-4-one (**9**), and 1,4-diiodobenzene (**10**). This route was chosen in order to provide flexibility of adding the key 3-(1,1-difluoroprop-2-ynyl)-3*H*-diazirin-3-yl group to any aryl halide. The key intermediate **14**, corresponding to **A**, was prepared from commercially available 2-(methylthio)thiazole (**8**) (Scheme 2). The most acidic H-5 proton was abstracted with LDA and upon treatment with tetrahydropyran-4-one (**9**) gave alcohol **11** which was subsequently methylated to give ether **12**. The methyl group on the sulfide was removed using a mild, one-pot, three step procedure via Pummerer rearrangement of the corresponding sulfoxide to give thiol **13**. Palladium catalyzed cross-coupling[35] of thiol **13** with 1,4-diiodobenzene (**10**) gave the target intermediate **14**.

We next turned our attention to the coupling of compound **14** with methyl 2,2-difluoro-4-(triisopropylsilyl)-3-butynoate **15**,[36] corresponding to **B** (Scheme 3). Initially, compound **14** was treated with *n*BuLi followed by addition of ester **15** at −78 °C, however, there were competing side reactions which gave undesired byproducts **17** and **18** as judged by high-resolution mass spectrometry and TLC. The ratio of the desired product **16**, increased when **14** and **15** were premixed in THF followed by addition of *n*BuLi at −78 °C, yet there was still considerable amount of byproduct **18**. However, when the same reaction was performed at −110 °C, byproduct **18** was not detected by either MS or TLC.

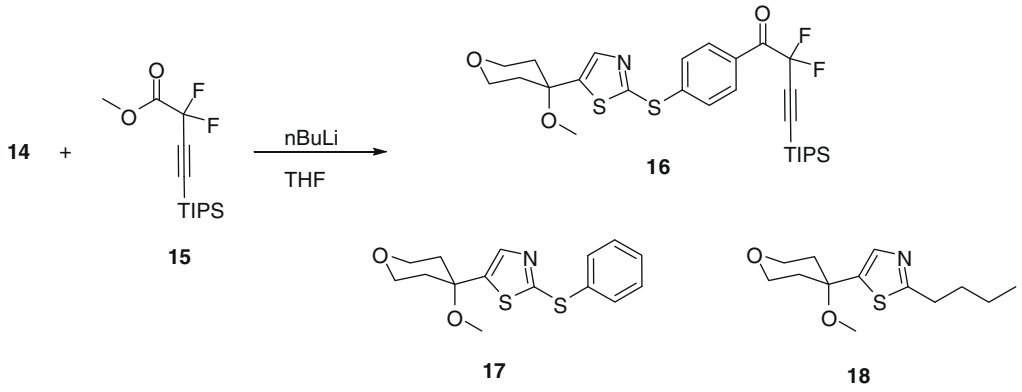
To synthesize the target compound **7**, ketone **16** was first reacted with hydroxylamine hydrochloride in the presence of pyridine to give the corresponding oxime **19** (Scheme 4). The oxime was tosylated with TsCl and upon treatment with ammonia gave diaziridine **21**. Subsequent oxidation of compound **21** using Swern oxidation gave diazirine **22**. Finally the TIPS protecting group was removed using TBAF to give the desired 'all-in-one' diazirine PAL probe **7**.



Scheme 1. Retrosynthetic analysis of compound **7**.

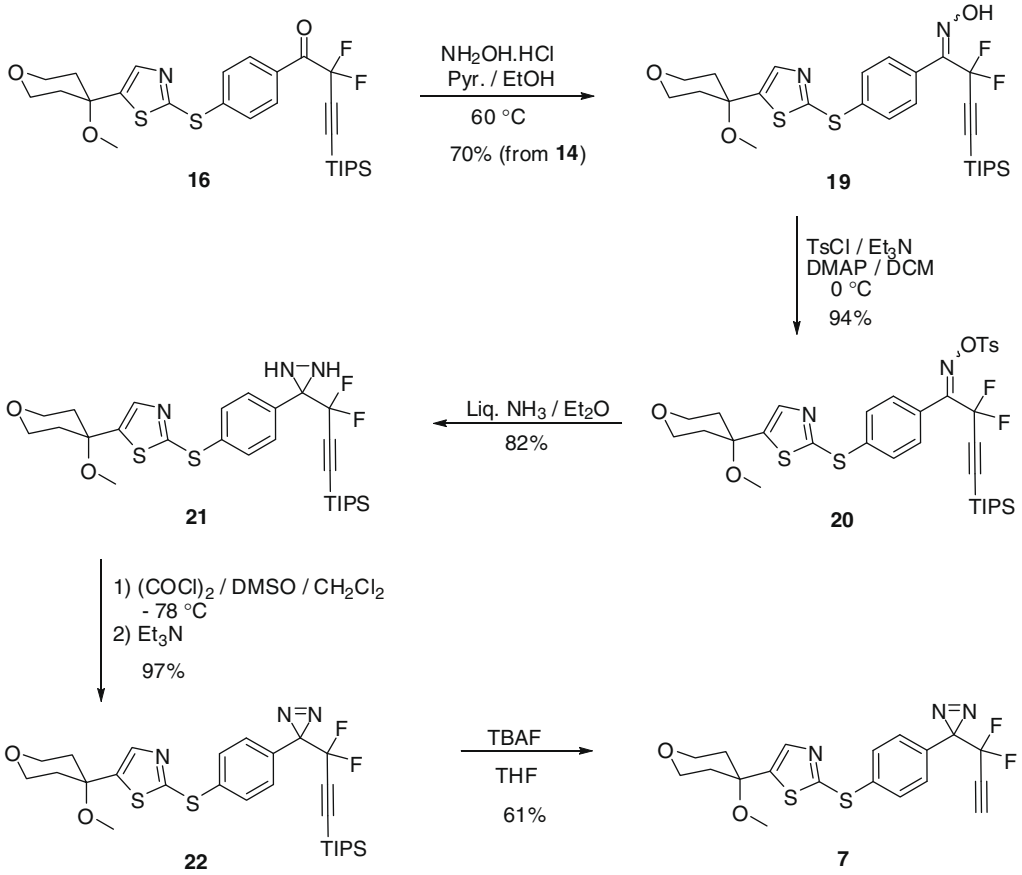


Scheme 2. Synthesis of the key intermediate **14**.

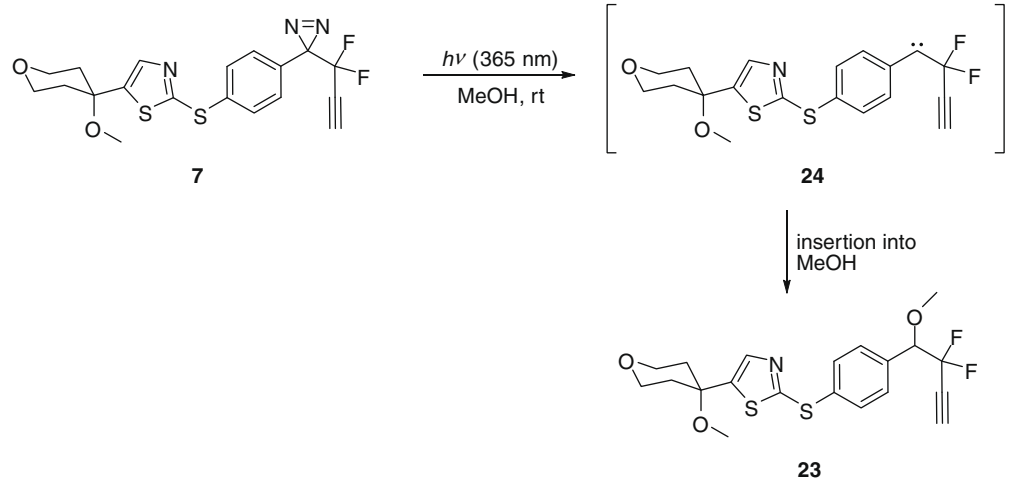


Scheme 3. Coupling reaction of compounds **14** and **15**.

A solution of compound **7** in methanol was irradiated with a 365 nm centered wavelength UV light (UVP, UVGL-25, 4 W) to cleanly give the methanol adduct **23**, presumably by insertion of the photo-generated carbene species **24** into methanol (Scheme 5). This adduct was characterized by a methoxy signal (δ = 3.46 ppm) and a methine hydrogen (δ = 4.47 ppm) which appeared as a triplet due to 2 fluorine coupling. The formation of the methanol adduct **23** confirms that the neighboring acetylene does not react with the highly reactive carbene species **24**. Finally, the photolyzed adduct **23** was treated with an azido-biotin



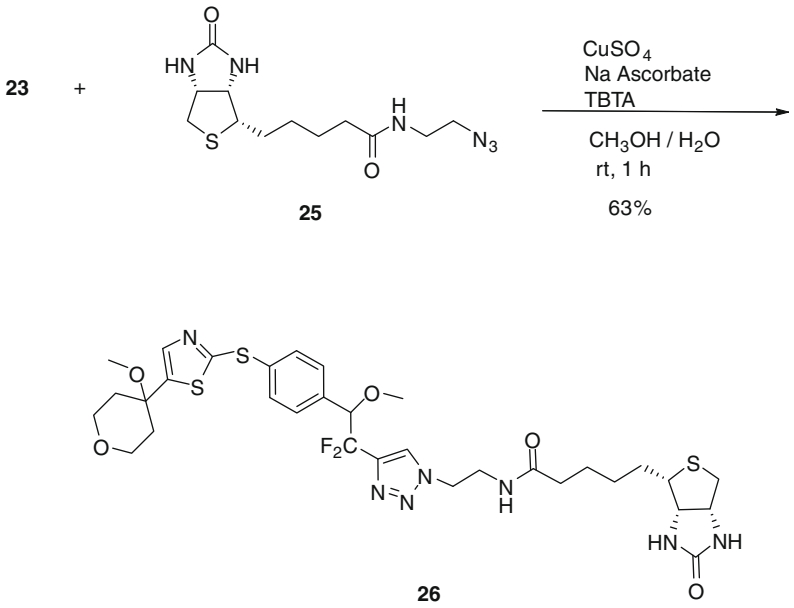
Scheme 4. Synthesis of 'all-in-one' diazirine PAL probe **7**.



Scheme 5. Photoreaction of all-in-one diazirine PAL probe **7** with MeOH.

derivative **25**[37] in the presence of $CuSO_4$, sodium ascorbate, and TBTA (triazole ligand) to promote click coupling between the azide and alkyne (Scheme 6). Consequently, the triazole **26** was isolated in pure form in moderately good yield. We also repeated the reaction where compound **7** was converted in one pot to triazole **26** without isolating intermediate **23** and its two step yield was 46%. Compounds **7**, **23**, and **26** are currently being tested against the 5-LO enzyme. Due to the smaller appendage we expect compounds **7** and **23** to be more potent than **26**.

In summary, we have prepared the first 'all-in-one' radioisotope free PAL containing the 3-(1,1-difluoroprop-2-ynyl)-3*H*-diazirin-3-yl functional group. We have shown that this photo probe could be photolyzed in methanol with 365 nm wavelength UV light to give the methanol adduct and that the highly unstable carbene thus generated does not react with the neighboring acetylene moiety. The methanol adduct reacted smoothly with the azido-biotin derivative **25** by click chemistry. The screening of compounds **7**, **23**, and **26** against 5-LO and the photolabeling studies in cells with compound **7** will be reported in due course.



Scheme 6. Click reaction of the methanol adduct **23** with the biotin azide **25**.

Due to its very compact structure and selective reactivity, compounds incorporating the 3-(1,1-difluoroprop-2-ynyl)-3*H*-diazirin-3-yl group should be useful alternatives wherever aryl-3-(trifluoromethyl)-3*H*-diazirines are now used.

3. Experiment

3.1. General methods

^{1}H and ^{13}C NMR spectra were recorded with a Bruker TCI cryoprobe at frequencies of 600 and 150 MHz, respectively. All assignments were confirmed with the aid of two-dimensional ^{1}H, ^{1}H (COSY), and ^{1}H, ^{13}C (HSQC) experiments. Processing of the spectra was performed with MestRec software. The high-resolution mass spectra were recorded in positive ion-mode with an ESI ion source on an Agilent Time-of-Flight LC/MS mass spectrometer. Analytical thin-layer chromatography (TLC) was performed on aluminum plates pre-coated with Silica Gel 60F-254 as the adsorbent. The developed plates were air-dried, exposed to UV light and/or dipped in $KMnO_4$ solution and heated. Column chromatography was performed with Silica Gel 60 (230–400 mesh). All procedures with diazirine functionality were carried out in flask/column covered by aluminum foil.

3.2. 5-(4-Hydroxytetrahydropyran-4-yl)-2-(methylthio)thiazole (11)

A solution of 2-(methylthio)thiazole **8** (5.0 g, 38.1 mmol) in THF (15 mL) was added to a stirred solution of freshly prepared LDA (*n*BuLi (22.0 mL, 2.5 M in hexane, 55.0 mmol) and diisopropylamine (7.8 mL, 55.3 mmol) in THF (10 mL)) at −78 °C under N_2. After stirring for 20 min, tetrahydropyran-4-one **9** (5 mL, 54.1 mmol) was added and the mixture was stirred at −78 °C for 2 h. The reaction mixture was partitioned between EtOAc (300 mL) and H_2O (100 mL). The organic phase was washed with brine (100 mL), dried over anhydrous Na_2SO_4, and concentrated. The crude product was recrystallized with Et_2O and hexanes to give **11** as a white solid (5.1 g, 57%). Mp 114–115 °C; ^{1}H NMR ($CDCl_3$): δ 7.46 (1H, s, H-4), 3.89 (2H, ddd, $J_{2ax',3eq'}$ = 2.2 Hz, $J_{2ax',3ax'}$ = $J_{2ax',2eq'}$ = 11.3 Hz, H-2ax′), 3.80 (2H, ddd, $J_{2eq',3ax'}$ = $J_{2eq',3eq'}$ = 4.0 Hz, $J_{2ax',2eq'}$ = 11.3 Hz, H-2eq′), 2.67 (3H, s, SCH_3), 2.14 (2H, ddd, $J_{2eq',3ax'}$ = 4.4 Hz, $J_{2ax',3ax'}$ = 11.2 Hz, $J_{3ax',3eq'}$ = 14.6 Hz, H-3ax′), 2.06 (1H, s, OH), 1.88 (2H, m, H-3eq′); ^{13}C NMR ($CDCl_3$): δ 166.3 (C-2), 147.1 (C-5), 137.3 (C-4), 68.4 (C-4′), 63.6 (C-2′), 39.7 (C-3′), 16.6 (SCH_3). HRMS calcd for $(C_9H_{13}NO_2S_2 + H)^+$ 232.0465, found 232.0461.

3.3. 5-(4-Methoxytetrahydropyran-4-yl)-2-(methylthio)thiazole (12)

NaH (0.31 g, 60% in oil, 7.75 mmol) was added to a stirred solution of compound **11** (1.11 g, 4.81 mmol) in DMF (20 mL) at 0 °C under N_2. After stirring for 10 min, MeI (0.36 mL, 5.78 mmol) was added and the mixture was stirred at rt for 2 h. The reaction mixture was partitioned between EtOAc (200 mL) and H_2O (100 mL). The organic phase was washed with brine (100 mL), dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified by flash chromatography (EtOAc–hexanes, 1:2) to give **12** as a white solid (1.14 g, 96%). Mp 63–65 °C; ^{1}H NMR $CDCl_3$): δ 7.45 (1H, s, H-4), 3.83 (2H, ddd, $J_{2ax',3eq'}$ = 3.6 Hz, $J_{2ax',3ax'}$ = 7.9 Hz, $J_{2ax',2eq'}$ = 11.5 Hz, H-2ax′), 3.76 (2H, ddd, $J_{2eq',3ax'}$ = $J_{2eq',3eq'}$ = 3.9 Hz, $J_{2ax',2eq'}$ = 11.5 Hz, H-2eq′), 3.10 (3H, s, OCH_3), 2.71 (3H, s, SCH_3), 2.11–2.02 (4H, m, H-3ax′, H-3eq′); ^{13}C NMR ($CDCl_3$): δ 167.4 (C-2), 143.5 (C-5), 139.8 (C-4), 72.9 (C-4′), 63.7 (C-2′), 50.1 (OCH_3), 36.7 (C-3′), 16.8 (SCH_3). HRMS calcd for $(C_{10}H_{15}NO_2S_2 + H)^+$ 246.0622, found 246.0617.

3.4. 2-(4-Iodophenylthio)-5-(4-methoxytetrahydropyran-4-yl)thiazole (14)

To a stirred solution of **12** (2.00 g, 8.16 mmol) in $CHCl_3$ (60 mL) at 0 °C was added mCPBA (1.86 g, 77%, 8.27 mmol) and the mixture was stirred for 90 min. $Ca(OH)_2$ (1.00 g, 13.48 mmol) was added and the mixture was stirred at rt for 20 min, filtered, and concentrated to give essentially pure sulfoxide. The residue was dissolved in TFAA (25 mL) and refluxed for 30 min and concentrated. The mixture was diluted with MeOH–Et_3N (1:1, 50 mL) and evaporated to dryness. The residue was dissolved in $CHCl_3$ (300 mL) and washed with satd NH_4Cl (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated to provide thiol **13**. In a separate flask, 1,4-diiodobenzene (**10**) (4.00 g, 12.12 mmol), Pd_2dba_3 (300 mg, 0.33 mmol) and dppf (740 mg, 1.34 mmol) was dissolved in NMP (40 mL) fol-

lowed by the addition of Et_3N (2.0 mL, 16.15 mmol). The mixture was purged with N_2 for 15 min and then a solution of thiol **13** (~8.16 mmol) in NMP (10 mL) was added. The reaction mixture was heated at 80 °C for 24 h and then partitioned between EtOAc (300 mL) and brine (100 mL). The organic phase was washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography (EtOAc–hexanes, 1:5→2:5) to give **14** as a white solid (1.32 g, 37%). Mp 93–94 °C; ^{1}H NMR ($CDCl_3$): δ 7.74 (2H, d, $J_{2'',3''}$ = 8.5 Hz, H-2″), 7.48 (1H, s, H-4), 7.33 (2H, d, $J_{2'',3''}$ = 8.5 Hz, H-3″), 3.78 (2H, ddd, $J_{2ax',3eq'}$ = 3.4 Hz, $J_{2ax',3ax'}$ = 9.8 Hz, $J_{2ax',2eq'}$ = 11.5 Hz, H-2ax′), 3.72 (2H, ddd, $J_{2eq',3ax'} = J_{2eq',3eq'}$ = 4.1, $J_{2ax',2eq'}$ = 11.5 Hz, H-2eq′), 3.05 (3H, s, OCH_3), 2.03–1.96 (4H, m, H-3′); ^{13}C NMR ($CDCl_3$): δ 165.2 (C-2), 145.7 (C-5), 140.4 (C-4), 138.9 (C-2″), 135.2 (C-3″), 131.3 (C-1″), 95.9 (C-4″), 72.7 (C-4′), 63.3 (C-2′), 49.9 (OCH_3), 36.4 (C-3′). HRMS calcd for $(C_{15}H_{16}INO_2S_2 + H)^+$ 433.9745, found 433.9740.

3.5. 2-(4-(2,2-Difluoro-1-hydroxyimino-4-triisopropylsilyl-but-3-ynyl)phenylthio)-5-(4-methoxytetrahydropyran-4-yl)thiazole (19)

*n*BuLi (0.41 mL, 2.5 M in hexanes, 1.03 mmol) was added to a stirred solution of **14** (300 mg, 0.69 mmol) and methyl 2,2-difluoro-4-(triisopropylsilyl)-3-butynoate (**15**) (300 mg, 1.03 mmol) in THF (20 mL) at −110 °C under Ar. The mixture was stirred at −110 °C→−78 °C for 30 min and then the reaction was quenched with 1 M aqueous HCl (20 mL). The reaction mixture was extracted with Et_2O (2 × 100 mL) and the combined organic layer was washed with H_2O (50 mL), brine (50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was dissolved in pyridine–EtOH (2:1, 15 mL), followed by addition of hydroxylamine hydrochloride (100 mg, 1.46 mmol). The mixture was stirred at 60 °C for 4 h and then partitioned between Et_2O (100 mL) and 1.0 M aqueous HCl (50 mL). The organic phase was washed with H_2O (50 mL), brine (50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography (EtOAc–hexanes, 1:3→1:2) to give **19** as colorless syrup (280 mg, 70% for the two steps) as 1:5 mixture of *E*- and *Z*-isomers. According to ^{1}H NMR and ^{13}C NMR spectra, the purified **19** contains a small amount of byproduct **17**.

Major isomer: ^{1}H NMR ($CDCl_3$): δ 9.35 (1H, br s, NO*H*), 7.63 (2H, d, $J_{2'',3''}$ = 8.5 Hz, H-3″), 7.55 (1H, s, H-4), 7.52 (2H, d, $J_{2'',3''}$ = 8.4 Hz, H-2″), 3.85–3.71 (4H, m, H-2′), 3.07 (3H, s, OCH_3), 2.05–1.97 (4H, m, H-3′), 1.10–1.02 (3H, m, SiC*H*), 1.03 (18H, d, J_{CH,CH_3} = 5.0 Hz, SiCHCH_3); ^{13}C NMR ($CDCl_3$): δ 164.1 (C-2), 151.2 (1C, t, $J_{C,F}$ = 30.2 Hz, C-5″), 146.4 (C-5), 140.4 (C-4), 134.0 (C-4″), 132.5 (C-3″), 130.2 (C-2″), 128.4 (C-1″), 109.1 (1C, t, $J_{C,F}$ = 233.1 Hz, C-6″), 96.5 (1C, t, $J_{C,F}$ = 37.4 Hz, C-7″), 94.4 (1C, t, $J_{C,F}$ = 4.6 Hz, C-8″), 72.8 (C-4′), 63.4 (C-2′), 50.0 (OCH_3), 36.3 (C-3′) 18.4 (SiC*H*), 10.9 (SiCHCH_3). HRMS calcd for $(C_{28}H_{38}F_2N_2O_3S_2Si + H)^+$ 581.2139, found 581.2132.

3.6. 2-(4-(2,2-Difluoro-1-(4-methylphenylsulfonyloxy)imino-4-triisopropylsilyl-but-3-ynyl)phenylthio)-5-(4-methoxytetrahydropyran-4-yl)thiazole (20)

p-Toluenesulfonyl chloride (110 mg, 0.58 mmol), 4-(dimethylamino)pyridine (4 mg, 0.03 mmol) and Et_3N (80 μL, 0.57 mmol) were added to a stirred solution of **19** in CH_2Cl_2 (8 mL) at 0 °C under N_2. After stirring for 90 min at 0 °C, the mixture was poured into H_2O (20 mL) and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extracts were washed with H_2O (50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography (EtOAc–hexanes, 1:2) to give **20** as colorless syrup (320 mg, 94%) as 1:4 mixture of inseparable isomers. According to ^{1}H NMR and ^{13}C NMR spectra, the purified **20** contains a small amount of byproduct **17**.

Major isomer: ^{1}H NMR ($CDCl_3$): δ 7.86 (2H, d, $J_{2''',3'''}$ = 8.3 Hz, H-2‴), 7.59 (2H, d, $J_{2'',3''}$ = 8.5 Hz, H-2″), 7.57 (1H, s, H-4), 7.37 (2H, d, $J_{2'',3''}$ = 8.5 Hz, H-3″), 7.34 (2H, d, $J_{2''',3'''}$ = 8.1 Hz, H-3‴), 3.82–3.71 (4H, m, H-2′), 3.09 (3H, s, OCH_3), 2.47 (3H, s, CH_3), 2.08–1.97 (4H, m, H-3′), 1.07–1.03 (3H, m, SiC*H*), 1.04 (18H, d, J_{CH,CH_3} = 5.4 Hz, SiCHCH_3); ^{13}C NMR ($CDCl_3$): δ 161.8 (C-2), 158.1 (1C, t, $J_{C,F}$ = 30.7 Hz, C-5″), 147.4 (C-5), 145.7 (C-1‴), 140.8 (C-4), 134.1 (C-1"), 131.9 (C-4‴), 131.2 (C-2″), 130.1 (C-4″), 129.8 (C-3″), 129.7 (C-3‴), 129.1 (C-2‴), 107.8 (1C, t, $J_{C,F}$ = 236.9 Hz, C-6″), 96.0 (1C, t, $J_{C,F}$ = 4.6 Hz, C-8″), 95.2 (1C, t, $J_{C,F}$ = 36.5 Hz, C-7″), 72.8 (C-4′), 63.4 (C-2′), 50.0 (OCH_3), 36.4 (C-3′), 21.8 (CH_3), 18.4 (SiCH), 10.8 (SiCHCH_3). HRMS calcd for $(C_{35}H_{44}F_2N_2O_5S_3Si + H)^+$ 735.2227, found 735.2239.

3.7. 2-(4-(3-(1,1-Difluoro-3-triisopropylsilyl-prop-2-ynyl)diaziridin-3-yl)phenylthio)-5-(4-methoxytetrahydropyran-4-yl)-thiazole (21)

Liquid ammonia (~2 mL) was condensed into a stirred solution of **20** (310 mg, 0.42 mmol) in Et_2O (5 mL) at −78 °C under N_2 in a pressure tube. The pressure tube was sealed and the mixture was stirred at rt for 16 h. After removing excess ammonia, the mixture was diluted with Et_2O (100 mL) and washed with H_2O (50 mL), brine (50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography (EtOAc–hexanes, 1:2) to give **21** as colorless syrup (200 mg, 82%). ^{1}H NMR ($CDCl_3$): δ 7.68 (2H, d, $J_{2'',3''}$ = 8.3 Hz, H-3″), 7.61 (2H, d, $J_{2'',3''}$ = 8.4 Hz, H-2″), 7.51 (1H, s, H-4), 3.78 (2H, ddd, *J 2ax′,3eq′* = 3.4 Hz, $J_{2ax',3ax'}$ = 9.9 Hz, $J_{2ax',2eq'}$ = 11.5 Hz, H-2ax′), 3.73 (2H, ddd, $J_{2eq',3ax'} = J_{2eq',3eq'}$ = 4.1, $J_{2ax',2eq'}$ = 11.5 Hz, H-2eq′), 3.05 (3H, s, OCH_3), 2.89 (1H, d, $J_{NHa,NHb}$ = 8.6 Hz, N*H*), 2.23 (1H, d, $J_{NHa,NHb}$ = 8.7 Hz, N*H*), 2.04–1.97 (4H, m, H-3′), 1.10–1.05 (3H, m, SiC*H*), 1.06 (18H, d, J_{CH,CH_3} = 5.4 Hz, SiCHCH_3); ^{13}C NMR ($CDCl_3$): δ 164.5 (C-2), 146.1 (C-5), 140.4 (C-4), 134.4 (C-1″), 133.7 (C-4″), 132.9 (C-2″), 130.1 (C-3″), 112.1 (1C, t, $J_{C,F}$ = 237.1 Hz, C-6″), 96.6 (1C, t, $J_{C,F}$ = 38.1 Hz, C-7″), 94.6 (1C, t, $J_{C,F}$ = 4.9 Hz, C-8″), 72.7 (C-4′), 63.3 (C-2′), 60.2 (1C, t, $J_{C,F}$ = 32.3 Hz, C-5″), 49.9 (OCH_3), 36.4 (C-3′) 18.4 (SiCH), 10.8 (SiCHCH_3). HRMS calcd for $(C_{28}H_{39}F_2N_3O_2S_2Si + H)^+$ 580.2299, found 580.2291.

3.8. 2-(4-(3-(1,1-Difluoro-3-triisopropylsilyl-prop-2-ynyl)diazirin-3-yl)phenylthio)-5-(4-methoxytetrahydropyran-4-yl)thiazole (22)

Oxalyl chloride (60 μL, 0.69 mmol) was added to a stirred solution of dry DMSO (60 μL, 0.84 mmol) in CH_2Cl_2 (15 mL) at −78 °C under N_2. After 5 min, a solution of diaziridine **21** (300 mg, 0.52 mmol) in CH_2Cl_2 (3 mL) was added and the mixture was stirred at −78 °C for 15 min. Et_3N (0.36 μL, 2.65 mmol) was added and the mixture was allowed to warm to rt over 90 min. The reaction was quenched with H_2O (5 mL) and the organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography (EtOAc–hexanes, 1:3) to give **22** as colorless syrup (290 mg, 97%). ^{1}H NMR ($CDCl_3$): δ 7.57 (2H, d, $J_{2'',3''}$ = 8.4 Hz, H-2″), 7.50 (1H, s, H-4), 7.30 (2H, d, $J_{2'',3''}$ = 8.4 Hz, H-3″), 3.78 (2H, ddd, *J 2ax′,3eq′* = 3.3 Hz, $J_{2ax',3ax'}$ = 9.9 Hz, $J_{2ax',2eq'}$ = 11.5 Hz, H-2ax′), 3.73 (2H, ddd, $J_{2eq',3ax'} = J_{2eq',3eq'}$ = 4.0, $J_{2ax',2eq'}$ = 11.5 Hz, H-2eq′), 3.05 (3H, s, OCH_3), 2.04–1.96 (4H, m, H-3′), 1.09–1.02 (3H, m, SiC*H*), 1.03 (18H, d, J_{CH,CH_3} = 5.4 Hz, SiCHCH_3); ^{13}C NMR ($CDCl_3$): δ 164.4 (C-2), 146.2 (C-5), 140.4 (C-4), 133.2 (C-1″), 132.9 (C-2″), 132.3 (C-4″), 128.2 (C-3″), 110.2 (1C, t, $J_{C,F}$ = 235.5 Hz, C-6″), 97.5 (1C, t, $J_{C,F}$ = 5.0 Hz, C-8″), 95.6 (1C, t, $J_{C,F}$ = 38.5 Hz, C-7″), 72.7 (C-4′), 63.3 (C-2′), 49.9 (OCH_3), 36.4 (C-3′), 31.4 (1C, t, $J_{C,F}$ = 36.2 Hz, C-5″), 18.3

(SiCH), 10.7 (SiCHCH_3). HRMS calcd for $(C_{28}H_{37}F_2N_3O_2S_2Si + H)^+$ 578.2268, found 578.2276.

3.9. 2-(4-(3-(1,1-Difluoroprop-2-ynyl)diazirin-3-yl)phenylthio)-5-(4-methoxytetrahydropyran-4-yl)thiazole (7)

TBAF (0.7 mL, 1 M in THF, 0.7 mmol) was added to a stirred solution of **22** (260 mg, 0.45 mmol) in THF (15 mL) at rt under N_2. After stirring for 40 min, the mixture was concentrated and the residue was purified by flash chromatography (EtOAc–hexanes, 1:2) to give **7** as pale yellow syrup (115 mg, 61%). ^{1}H NMR ($CDCl_3$): δ 7.58 (2H, d, $J_{2'',3''}$ = 8.6 Hz, H-2″), 7.51 (1H, s, H-4), 7.28 (2H, d, $J_{2'',3''}$ = 8.5 Hz, H-3″), 3.78 (2H, ddd, $J_{2ax',3eq'}$ = 3.4 Hz, $J_{2ax',3ax'}$ = 9.8 Hz, $J_{2ax',2eq'}$ = 11.4 Hz, H-2ax′), 3.73 (2H, ddd, $J_{2eq',3ax'} = J_{2eq',3eq'}$ = 4.2, $J_{2ax',2eq'}$ = 11.5 Hz, H-2eq′), 3.06 (3H, s, OCH_3), 3.01 (1H, t, $J_{8'',F}$ = 5.1 Hz, H-8″), 2.04–1.97 (4H, m, H-3′); ^{13}C NMR ($CDCl_3$): δ 164.0 (C-2), 146.3 (C-5), 140.5 (C-4), 133.6 (C-1″), 132.8 (C-2″), 131.6 (C-4″), 128.2 (C-3″), 110.3 (1C, t, $J_{C,F}$ = 236.4 Hz, C-6″), 80.6 (1C, t, $J_{C,F}$ = 6.4 Hz, C-8″), 73.6 (1C, t, $J_{C,F}$ = 40.4 Hz, C-7″), 72.8 (C-4′), 63.3 (C-2′), 49.9 (OCH_3), 36.4 (C-3′), 31.1 (1C, t, $J_{C,F}$ = 35.4 Hz, C-5″). HRMS calcd for $(C_{19}H_{17}F_2N_3O_2S_2 + H)^+$ 422.0808, found 422.0803.

3.10. 2-(4-(2,2-Difluoro-1-methoxy-but-3-ynyl)phenylthio)-5-(4-methoxytetrahydropyran-4-yl)thiazole (23)

A solution of compound **7** (10 mg, 23.7 μmol) in MeOH (1.5 mL) was irradiated with 365 nm wavelength UV light (UVP, UVGL-25, 4 W) at rt while monitoring by TLC. The reaction proceeded smoothly and all **7** was consumed by 90 min. The reaction mixture was concentrated and the residue was purified by flash chromatography (hexanes–EtOAc, 2:1) to give compound **23** as pale yellow syrup (7 mg, 69%). ^{1}H NMR ($CDCl_3$): δ 7.62 (2H, d, $J_{2'',3''}$ = 8.3 Hz, H-2″), 7.51 (1H, s, H-4), 7.49 (2H, d, $J_{2'',3''}$ = 8.2 Hz, H-3″), 4.47 (1H, t, $J_{5'',F}$ = 8.6 Hz, H-5″), 3.78 (2H, ddd, $J_{2ax',3eq'}$ = 3.3 Hz, $J_{2ax',3ax'}$ = 10.0 Hz, $J_{2ax',2eq'}$ = 11.4 Hz, H-2ax′), 3.73 (2H, ddd, $J_{2eq',3ax'} = J_{2eq',3eq'}$ = 4.1, $J_{2ax',2eq'}$ = 11.4 Hz, H-2eq′), 3.46 (3H, s, OCH_3), 3.06 (3H, s, OCH_3), 2.81 (1H, t, $J_{8'',F}$ = 4.2 Hz, H-8″), 2.05-1.97 (4H, m, H-3′); ^{13}C NMR ($CDCl_3$): δ 165.1 (C-2), 145.8 (C-5), 140.5 (C-4), 134.83 (C-1″), 134.81 (C-4″), 132.9 (C-2″), 129.8 (C-3″), 112.1 (1C, t, $J_{C,F}$ = 239.1 Hz, C-6″), 84.3 (1C, t, $J_{C,F}$ = 28.9 Hz, C-5″), 77.5 (1C, t, $J_{C,F}$ = 6.6 Hz, C-8″), 74.3 (1C, t, $J_{C,F}$ = 38.9 Hz, C-7″), 72.6 (C-4′), 63.4 (C-2′), 58.7 (OCH_3), 49.9 (OCH_3), 36.4 (C-3′). HRMS calcd for $(C_{20}H_{21}F_2NO_3S_2 + H)^+$ 426.1009, found 426.1001.

3.11. 2-(4-(2,2-Difluoro-1-methoxy-2-[1-(-{[5-(2-oxohexahydro-1*H*-thieno[3,4,*d*]imidazol-4-yl)pentanoyl]amino}ethyl)-1*H*-1,2,3-triazol-4-yl]ethyl)phenylthio)-5-(4-methoxytetrahydropyran-4-yl)thiazole (26)

To a stirred solution of **23** (5 mg, 11.7 μmol) in MeOH (0.5 mL) was added biotin azide (**25**) (3.5 mg, 10.7 μmol), TBTA (50 mM MeOH solution, 20 μL, 1 μmol), $CuSO_4$ (100 mM aqueous solution, 10 μL, 1 μmol), and sodium ascorbate (100 mM aqueous solution, 30 μL, 3 μmol) at rt under Ar. The mixture was stirred at rt for 1 h and concentrated. The crude product was purified by preparative TLC (CH_2Cl_2–MeOH ; 10:1) to give compound **26** as pale yellow syrup (5 mg, 63%). ^{1}H NMR (DMSO): δ 8.40 (1H, s, triazole), 7.98 (1H, t, J_{NH,CH_2} = 5.7 Hz, CON*H*), 7.73 (1H, s, H-4), 7.64 (2H, d, $J_{2'',3''}$ = 8.3 Hz, H-3″), 7.47 (2H, d, $J_{2'',3''}$ = 8.2 Hz, H-2″), 6.41 (1H, s, N*H*), 6.35 (1H, s, N*H*), 5.09 (1H, dd, $J_{5'',Fa}$ = 7.1 Hz, $J_{5'',Fb}$ = 16.1 Hz, H-5″), 4.46 (2H, t, J_{CH_2,CH_2} = 6.0 Hz, CH_2triazole), 4.29 (1H, m, SCHC*H*), 4.11 (1H, m, SCH_2CH), 3.61–3.58 (4H, m, H-2′), 3.51 (2H, dt, $J_{CH_2,CH_2} = J_{NH,CH_2}$ = 6.0 Hz, CH_2CH$_2$triazole), 3.24 (3H, s, OCH_3), 3.08 (1H, m, SC*H*), 2.10 (3H, s, OCH_3), 2.08 (1H, m, SCH_2), 2.56 (1H, d, $J_{a,b}$ = 12.4 Hz, SCH_2), 2.02 (2H, t, J_{CH_2,CH_2} = 7.0 Hz, NHCOCH_2), 1.97–1.90 (4H, m, H-3′), 1.62–1.56 (1H, m, H-5‴), 1.49–1.41 (3H, m, 2 × H-3‴, H-5‴), 1.33–1.23 (2H, m, H-4‴); ^{13}C NMR (DMSO): δ 172.5 (*C*ONH), 163.4 (C-2), 162.6 ((HN)$_2$*C*O), 145.4 (C-5), 141.2 (1C, dd, $J_{C,Fa} = J_{C,Fb}$ = 26.8 Hz, triazole-*C*-4), 135.5 (C-1″), 132.6 (C-3″), 131.4 (C-4″), 130.2 (C-2″), 124.9 (triazole-*C*-5), 116.9 (1C, dd, $J_{C,Fa}$ = 39.8 Hz, $J_{C,Fb}$ = 44.9 Hz, C-6″), 82.0 (1C, dd, $J_{C,Fa}$ = 24.6 Hz, $J_{C,Fb}$ = 31.9 Hz, C-5″), 72.4 (C-4′), 62.6 (C-2′), 60.9 (SCH_2*C*H), 59.1 (SC*H*CH), 57.5 (OCH_3), 55.3 (SCH), 49.3 (OCH_3), 49.1 (*C*H$_2$triazole), 39.8 (S*C*H$_2$), 38.5 (*C*H$_2$CH$_2$triazole), 35.8 (C-3′), 35.0 (NHCO*C*H$_2$), 28.0 (C-4‴), 27.9 (C-5‴), 25.0 (C-3‴). HRMS calcd for $(C_{32}H_{41}F_2N_7O_5S_3 + H)^+$ 738.2377, found 738.2395.

3.12. One pot synthesis of triazole 26 from compound 7

A solution of compound **7** (5 mg, 11.8 μmol) in MeOH (1 mL) was irradiated with 365 nm wavelength UV light (UVP, UVGL-25, 4 W) at rt for 90 min, followed by the addition of biotin azide (**25**) (3.5 mg, 10.7 μmol), TBTA (50 mM MeOH solution, 20 μL, 1 μmol), $CuSO_4$ (100 mM aqueous solution, 10 μL, 1 μmol), and sodium ascorbate (100 mM aqueous solution, 30 μL, 3 μmol) at rt under Ar. The mixture was stirred at rt for 1 h and concentrated. The crude product was purified by preparative TLC (CH_2Cl_2–MeOH; 10:1) to give compound **26** as pale yellow syrup (4 mg, 46%).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.06.048.

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