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N,O-Heterocycles Synthesis

Synthesis of 1,2,3-Triazoles Bearing a 4-Hydroxyisoxazolidine Moiety from 4,5-Unsubstituted 2,3-Dihydroisoxazoles

Radka Štadániová,[a] Michal Sahulčík,[a] Jana Doháňošová,[b] Ján Moncol,[c] Ľuboš Janotka,[d] Kristína Šimoničová,[d] Lucia Messingerová,*[d,e] and Róbert Fischer*[a]

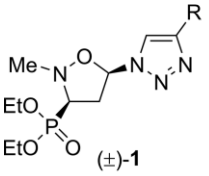
Abstract: A synthetic approach towards new 1,2,3-triazoles bearing the 3-hydroxymethylated 4-hydroxyisoxazolidine moiety has been described. The strategy has relied on dihydroxylation and epoxidation reactions of 4,5-unsubstituted 2,3-dihydroisoxazoles, allowing the introduction of the hydroxy group at the isoxazolidine ring in a *trans* stereoselective manner with respect to the substituent at C-3 carbon atom. The requisite 5-azidoisoxazolidines have been prepared from activated isoxazolidines possessing a good leaving group at C-5 carbon atom by treatment with trimethylsilyl azide and Lewis acid (isoxazolidinyl benzoates) or with sodium azide (chloroisoxazolidines). The 1,2,3-triazole moiety has been synthesized through copper(I)-catalyzed azide-alkyne cycloaddition.

Introduction

Targeted syntheses of functionalized bioactive heterocycles are continuously of high interest for organic and medicinal chemists. Many of such molecules are potent enzyme inhibitors that decrease enzyme activity by binding to its active site. Consequently, the preparation of intriguing heterocyclic compounds and the development of new synthetic methods constitute a great part of research in medicinal chemistry, e.g. for the performance of general screening,[1] for fragment-based screening libraries design,[2] or for the synthesis of ligands for decoration of the lead compound during QSAR studies.[3]

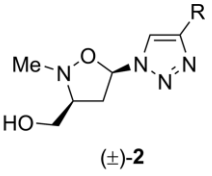
Isoxazolidines and their more complex derivatives are undeniably an important part of a large group of such biologically active structures.[4,5] They have been found to be promising drug candidates due to their interesting biological properties including anticancer[6] and antiviral.[7]

Recently, 1,2,3-triazole-containing isoxazolidines[8] (Figure 1) have been prepared as base analogs of well-known biologically effective N,O-nucleosides in which the furanose ring has been replaced by an isoxazolidine scaffold.[9] Among them, a series of 5-(1*H*-1,2,3-triazole)isoxazolidines has been synthesized either through 1,3-dipolar cycloadditions of azidoisoxazolidines with selected alkyne derivatives (exemplified by compound **1**),[8a] or by the direct nitrone 1,3-dipolar cycloadditions with 1-vinyl triazoles (exemplified by compound **2**).[8b] Biological activity of the phosphonylated isoxazolidinyl triazoles was evaluated in vitro against a variety of DNA and RNA viruses and cancer cells. No inhibitory activity against any virus was detected for the evaluated compounds at 250 μM. For evaluation of cytotoxic activity, several triazoles were able to inhibit cell proliferation at concentrations ranging from 40 to 78 μM for HEL cells, and from 120 to 250 μM for L1210, CEM, and HeLa cells. On the other hand, triazolyl isoxazolidines, bearing hydroxymethyl group, were all able to inhibit proliferation of follicular (FTC-133) and anaplastic (8305C) human thyroid cancer cell lines, with IC_{50} values ranging from 3.87 to 8.76 μM.



(±)-**1**

R = Ph
CC_{50} = 54 μM (HEL)
CC_{50} = 172 μM (L1210)



(±)-**2**

R = Ph
IC_{50} = 6.33 μM (FTC-133)
IC_{50} = 8.76 μM (8305C)

Figure 1. Known biologically active isoxazolidinyl triazoles exemplified by compounds **1**[8a] and **2**.[8b]

[a] *R. Štadániová, M. Sahulčík, Dr. R. Fischer*
Institute of Organic Chemistry, Catalysis and Petrochemistry, Slovak University of Technology in Bratislava, Radlinského 9, 81237 Bratislava, Slovak Republic
E-mail: robert.fischer@stuba.sk
http://www.fchpt.stuba.sk/
[b] *Dr. J. Doháňošová*
Central Laboratories, Slovak University of Technology in Bratislava, Radlinského 9, 81237 Bratislava, Slovak Republic
[c] *Prof. J. Moncol*
Institute of Inorganic Chemistry, Technology and Materials, Slovak University of Technology in Bratislava, Radlinského 9, 81237 Bratislava, Slovak Republic
[d] *Ľ. Janotka, K. Šimoničová, Dr. L. Messingerová*
Institute of Molecular Physiology and Genetics, Centre of Biosciences, Slovak Academy of Sciences, Dúbravská cesta 9, 84505 Bratislava 4, Slovak Republic
E-mail: lucia.messingerova@stuba.sk
http://www.umfg.sav.sk/obc/
[e] *Dr. L. Messingerová*
Institute of Biochemistry and Microbiology, Slovak University of Technology in Bratislava, Radlinského 9, 81237 Bratislava, Slovak Republic

Supporting information and ORCID(s) from the author(s) for this article are available on the WWW under https://doi.org/10.1002/ejoc.202000737.

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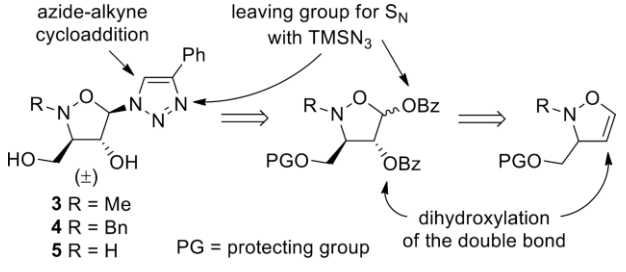
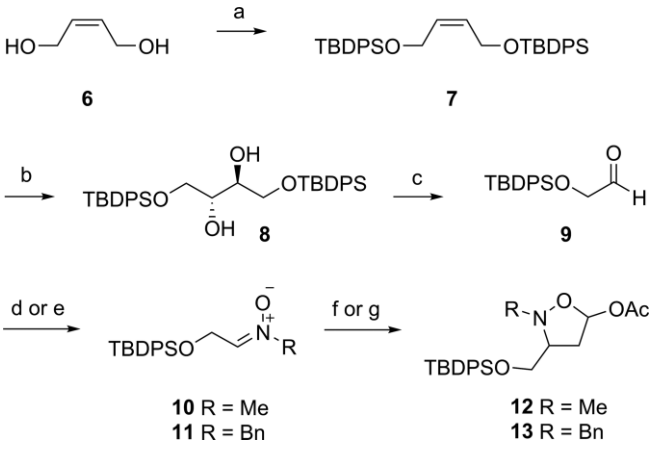
These beneficial findings inspired us to design modified triazoles **3**–**5** bearing 3-hydroxymethylated 4-hydroxyisoxazolidine moiety (Scheme 1) and evaluate their anticancer activity using AML (acute myeloid leukemia) MOLM-13 cell line. Despite a vast number of isoxazolidine analogues of bioactive molecules built up so far, to the best of our knowledge such structural modification has not yet been involved. In line with our ongoing research in the field of reactivity of 4,5-unsubstituted 2,3-dihydroisoxazoles we envisioned that the hydroxy group could be introduced into the isoxazolidine ring by dihydroxylation reaction of the double bond.[10] Upon acylation of the isoxazolidine-4,5-diol the resulting dibenzoate could act as a powerful electrophile in acid-catalyzed nucleophilic substitution with trimethylsilyl azide giving 5-azidoisoxazolidine as a suitable intermediate for triazole ring construction by copper(I)-catalyzed azide-alkyne cycloaddition with an alkyne. The *N*-unprotected isoxazolidine could be obtained in the final stage by *N*-debenzylation of the corresponding *N*-benzyl derivative.



Scheme 1. Retrosynthetic analysis of the 4-hydroxyisoxazolidinyl analogs **3**–**5**.

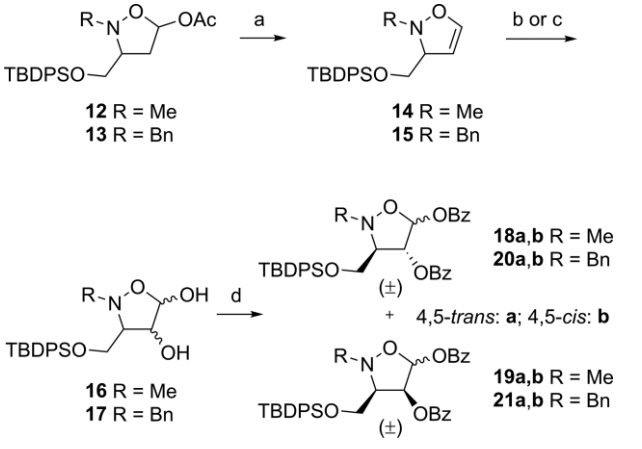
Results and Discussion

For the synthesis of *N*-methyl- and *N*-benyl-2,3-dihydroisoxazoles we decided to use 5-acetoxyisoxazolidines **12** and **13** generated by the 1,3-dipolar cycloaddition of known (*Z*)-configured nitrones **10**[11] and **11**[12] with vinyl acetate. As depicted in Scheme 2, nitrones **10** and **11** were readily synthesized in four steps starting from (*Z*)-but-2-ene-1,4-diol (**6**). The reaction of **6** with *tert*-butyldiphenylchlorosilane gave silylated alkenol **7** in 97 % yield.[13] Its treatment with a catalytic amount of $K_2OsO_4{\cdot}2H_2O$ in the presence of aqueous NMO afforded diol **8** in 95 % yield which was subjected to oxidative cleavage with sodium periodate in THF/water. Aldehyde **9** was formed in 90 % yield. Subsequent condensation of **9** with *N*-methyl- and *N*-benzylhydroxylamine hydrochloride under basic conditions in the presence of anhydrous $MgSO_4$ provided nitrones **10** and **11** in 98 and 89 % yields, respectively.[14,15] The 1,3-dipolar cycloaddition of nitrone **10** directly in neat vinyl acetate at room temperature afforded 5-acetoxyisoxazolidine **12** in 88 % combined yield.[16] More stable nitrone **11** reacted with vinyl acetate at 40 °C faster to give adduct **13** in 80 % combined yield. Cycloadditions proceeded regioselectively through *exo* transition state. In both cases, two diastereoisomers **a** (3,5-*cis*) and **b** (3,5-*trans*) were formed with good 3,5-*cis* selectivity (**12a**/**12b**, 75:25) and (**13a**/**13b**, 80:20). The relative configuration of new adducts **13a** and **13b** was established by comparison of the NMR data with those previously reported for the known compounds **12a**,**b**.[16]



Scheme 2. Synthesis of 5-acetoxyisoxazolidines **12** and **13**. Reaction conditions: (a) TBDPSCl, Et_3N, DMAP, CH_2Cl_2, r.t., 12 h, 97 %; (b) $K_2OsO_4{\cdot}2H_2O$, NMO (aq. 50 % w/w), acetone/water (4:1), r.t., 12 h, 95 %; (c) $NaIO_4$, THF/water (3:1), 0 °C, 30 min. then r.t. 12 h, 90 %; (d) $CH_3NHOH{\cdot}HCl$, $NaHCO_3$, $MgSO_4$, CH_2Cl_2, r.t., 12 h, **10** 98 %; (e) $BnNHOH{\cdot}HCl$, $NaHCO_3$, $MgSO_4$, CH_2Cl_2, r.t., 12 h, **11** 89 %; (f) vinyl acetate, r.t., 4 d, **12a**,**12b** combined yield 88 %, **12a** (3,5-*cis*)/**12b** (3,5-*trans*), 75:25; (g) vinyl acetate, 40 °C, 17 h, **13a**,**13b** combined yield 80 %, **13a** (3,5-*cis*)/**13b** (3,5-*trans*), 80:20, diastereomeric ratios were determined from the ^1H NMR spectra of the crude product mixture.

Elimination reactions of 5-acetoxyisoxazolidines **12a**,**b** and **13a**,**b** with a catalytic amount of TMSOTf in the presence of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) in anhydrous *N*-methyl-2-pyrrolidone (NMP) at room temperature afforded 2,3-dihydroisoxazoles **14** and **15** in very good 72 and 89 % yields, respectively (Scheme 3).[17]



Scheme 3. Synthesis of benzoylated isoxazolidine-4,5-diols. Reaction conditions: (a) BSTFA, TMSOTf, NMP, r.t., 2 h, **14** 72 %, **15** 89 %; (b) from **14**, $K_2OsO_4{\cdot}2H_2O$, NMO (aq. 50 % w/w), acetone/water (6:1), 4 °C, 20 h; (c) from **15**, $K_2OsO_4{\cdot}2H_2O$, NMO (aq. 50 % w/w), acetone/water (6:1), r.t., 7 h; (d) BzCl, pyridine, DMAP, CH_2Cl_2, r.t., 16 h; **18a**,**b**/**19a**,**b** combined yield 62 % over two steps; **20a**,**b**/**21a**,**b** combined yield 67 % over two steps.

As for all known 4,5-unsubstituted 2,3-dihydroisoxazoles synthesized by our group so far, the double bond was characterized by the presence of the signals for the 4-H protons at 4.78–4.85 ppm and for the 5-H protons at 6.37–6.45 ppm with the small coupling constants (2.6–2.8 Hz). In ^{13}C NMR spectra, the

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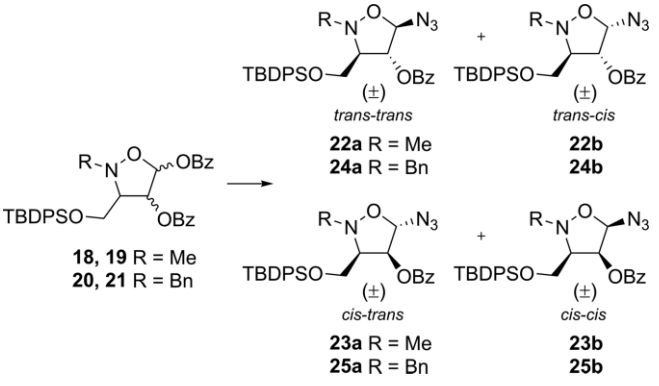
signals appeared at approximately 96 ppm (C-4) and 142 ppm (C-5). When 2,3-dihydroisoxazoles **14** and **15** were treated with a catalytic amount of $K_2OsO_4{\cdot}2H_2O$ in the presence of aqueous NMO in acetone/water, diastereomeric mixtures of isoxazolidine-4,5-diols **16** and **17** were obtained, consisting of four inseparable isomers, which were subsequently transformed without purification into the corresponding *O*-benzoylated isoxazolidines (Scheme 3). Both reactions afforded a mixture of four stereoisomeric isoxazolidines **18** (3,4-*trans*), **19** (3,4-*cis*), and **20** (3,4-*trans*), **21** (3,4-*cis*), respectively. The ratios and the combined yields over two steps from 2,3-dihydroisoxazoles are summarized in Table 1 (Entries 1, 2). To avoid problems associated with the chromatographic separation of each isomer, that has emerged during this process, all isomers **18a**,**b**/**19a**,**b** and **20a**,**b**/**21a**,**b** were isolated again as product mixtures and used in the next reaction directly. In order to clarify the stereochemistry of previous dihydroxylations, a sample of each mixture was subjected to repeated preparative thin-layer chromatography to obtain all isomers in pure form. Their relative configuration was determined by NMR spectroscopy, including NOESY experiments (see the Supporting Information). The obtained results suggest that the dihydroxylation reactions of 2,3-dihydroisoxazoles **14** and **15** proceeded with good *trans* stereoselectivity with respect to the siloxymethyl group at C-3 carbon atom, which is consistent with other dihydroxylations of 2,3-dihydroisoxazoles.[10] The *O*-benzoylated 3,4-*trans*-isoxazolidine-4,5-ols **18a**,**b** and **20a**,**b** were formed as the major ones with an approximate ratio of 3,4-*trans*/3,4-*cis*, 85:15.

Table 1. Synthesis of 5-benzoyloxy- **18**–**21** and 5-azidoisoxazolidines **22**–**25**.

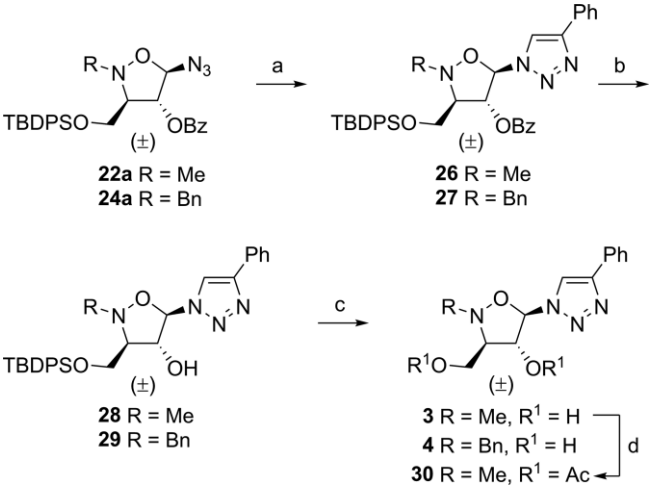
Entry	Substrates	Products	Ratio[a]	Yield [%][b]
1	**14**	**18a**, **18b**, **19a**, **19b**	13:72:10:5	62[c]
2	**15**	**20a**, **20b**, **21a**, **21b**	45:42:6:7	67[c]
3	**18a**,**b**/**19a**,**b**	**22a**, (**22b**, **23a**, **23b**)[d]	83:8:7:2	65
4	**20a**,**b**/**21a**,**b**	**24a**, (**24b**, **25a**, **25b**)[d,e]	84:9:5:2	71

[a] Diastereomeric ratios were determined from the ^{1}H NMR spectra of the crude product mixture. [b] Isolated combined yield. [c] Yield over two steps from 2,3-dihydroisoxazole. [d] Products in parenthesis were isolated as a diastereomeric mixture. [e] Diastereomers **25a**, **25b** were detected by NMR spectroscopy, however, the isolation and full characterization attempts failed.

Having benzoates **18**–**21** with a good leaving group in the C-5 position, the introduction of the azido group can be achieved by means of nucleophilic substitution with $TMSN_3$ mediated by TMSOTf. Up to now, only a few examples of the preparation of 5-azidoisoxazolidines have been reported in the literature.[8a] Recently, we reported related cyanation of *N*-benzyl-substituted 5-acetoxyisoxazolidines with TMSCN under similar reaction conditions.[10b] According to this procedure, the reactions of **18a**,**b**/**19a**,**b** and **20a**,**b**/**21a**,**b** were carried out in acetonitrile at room temperature for 24 h and afforded diastereomeric mixtures of four isoxazolidines **22a**,**b**/**23a**,**b** and **24a**,**b**/**25a**,**b** in both cases (Scheme 4). Their combined yields and ratios are summarized in Table 1 (Entries 3, 4). It is worth noting that acetonitrile and room temperature were crucial for successful reaction in terms of reactivity and yield, whereas in dichloromethane the azidation did not occur at all. For the next synthetic purpose, only the major isomers **22a** and **24a** were isolated by flash column chromatography (FCC) as pure products in 55 and 64 % yields and were fully characterized by ^{1}H and ^{13}C NMR spectroscopy, including 1D NOESY experiments. The relative configuration of the minor isomers **22b**, **23a,** and **23b** was established by inspection of the NOESY spectra of their corresponding mixtures (see the Supporting Information). The obtained results were then applied to determine the relative configuration of the corresponding *N*-benzyl derivatives. From the stereochemical point of view, we assume that the high preference for the formation of 4,5-*trans* isomers **22a** and **24a** is generally due to neighboring group participation from the 4-O protecting benzoyl group. The obtained azidoisoxazolidines **22a** and **24a** were further treated with phenylacetylene in the presence of $CuSO_4{\cdot}5H_2O$ and L-sodium ascorbate in *tert*-butyl alcohol/water (1:2) at room temperature in 12 h (Scheme 5).[8a]



Scheme 4. Synthesis of 5-azido-substituted isoxazolidines. Reaction conditions: $TMSN_3$, TMSOTf, CH_3CN, r.t., 24 h; **22a** 55 %; **24a** 64 %.



Scheme 5. Synthesis of isoxazolidinyl triazoles **3**, **4** and **30**. Reaction conditions: (a) phenylacetylene, $CuSO_4{\cdot}5H_2O$, sodium L-ascorbate, *t*BuOH/water (1:2), r.t., 12 h; **26** 93 %; **27** 84 %; (b) K_2CO_3, MeOH, r.t., 30 min; **28** 86 %; **29** 92 %; (c) TBAF, THF, r.t., 5 h; **3** 88 %; **4** 95 %; (d) Ac_2O, Et_3N, DMAP, CH_2Cl_2, r.t., 1 h, 97 %.

The reactions yielded the isoxazolidinyl triazoles **26** and **27** in very good 93 and 84 % yields as single regioisomers. De-

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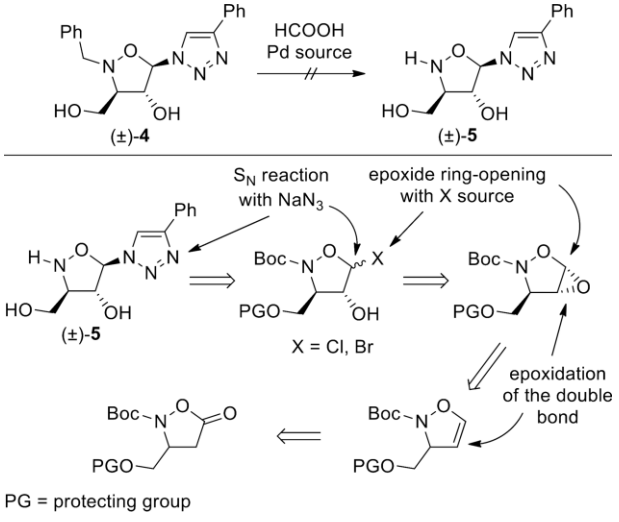
benzoylation with K_2CO_3 in methanol gave the isoxazolidin-4-ols **28** (86 % yield) and **29** (92 % yield). Finally, desilylation was performed using TBAF in THF and desired 4-hydroxyisoxazolidinyl triazoles **3** and **4** were isolated in 88 and 95 % yields.

In addition, per-*O*-acetylated isoxazolidine **30** was prepared in 97 % yield from **3** under common conditions with acetic anhydride. To confirm the 3,4-*trans*-4,5-*trans* configuration, the isoxazolidine **3** was subjected to X-ray crystallographic analysis (see the Supporting Information).

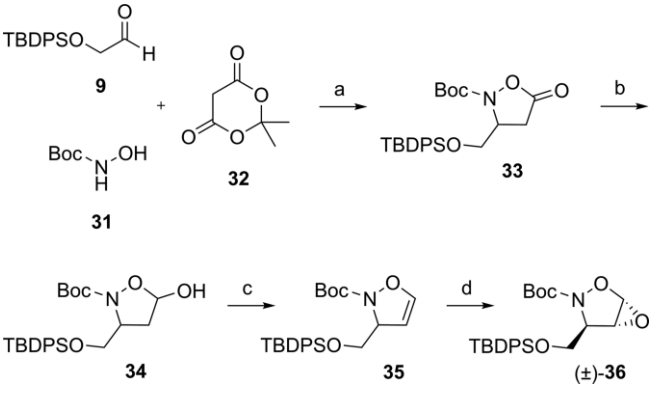
Next, our synthetic efforts were focused on the *N*-debenzylation of **4** according to the procedure using Pd-catalyzed hydrogenolysis in methanol with formic acid as the hydrogen source.[18,19] Unfortunately, we did not succeed in removing the benzyl group, observing only a complete decomposition of the starting material (Scheme 6). Therefore, in order to prepare *N*-unprotected isoxazolidinyl triazole **5**, we turned our attention to *N*-Boc-4-hydroxyisoxazolidines with a readily removable *tert*-butyloxycarbonyl group under acidic conditions. Recently, we have described the stable isoxazolidinyl epoxides, prepared from 4,5-unsubstituted *N*-Boc- and *N*-Cbz-2,3-dihydroisoxazoles, which were converted into 5-substituted 4-hydroxyisoxazolidines by carrying out the regio- and stereospecific epoxide ring-opening reactions.[20]



Scheme 6. Unsuccessful *N*-debenzylation, and new retrosynthetic analysis of the *N*-unsubstituted isoxazolidinyl triazole **5** using *N*-Boc intermediate.

The starting 2,3-dihydroisoxazoles were prepared from the corresponding isoxazolidin-5-ols through elimination reactions using Tf_2O/2-chloropyridine couple in anhydrous NMP.[21] Although direct reaction with $TMSN_3$ in the presence of nBu_3P afforded 5-azidoisoxazolidines only in very low yields, this problem was successfully overcome by preparing 5-bromoisoxazolidines which undergo reaction with sodium azide smoothly.[22]

According to the retrosynthetic analysis depicted in Scheme 6, we started with isoxazolidin-5-one **33** which was prepared by multicomponent organocatalyzed Knoevenagel-aza-Michael cyclocondensation reaction between *N*-Boc-hydroxylamine **31**, Meldrum's acid (**32**) and aldehyde **9** in 80 % yield (Scheme 7).[23] Reduction of **33** with Super-Hydride in anhydrous THF at –78 °C gave isoxazolidin-5-ol **34** in 76 % isolated yield as a mixture of two 3,5-*trans* and 3,5-*cis* anomers in an 80:20 ratio. The relative configuration of the more stable *trans* isomer **34a** was determined by comparison with already reported NMR data of related 5-hydroxyisoxazolidines.[22,24] It is important to note that the prolonged reaction time as well as the use of DIBAL-H as a reducing agent definitely caused over-reduction into the respective primary alcohol.[25] Treatment of **34** with Tf_2O in the presence of 2-chloropyridine produced 2,3-dihydroisoxazole **35** in a moderate 53 % yield. Based on our experiences with epoxidations of 4,5-unsubstituted 2,3-dihydroisoxazoles with in situ generated DMDO,[22] compound **35** was subjected to reaction with Oxone and $NaHCO_3$ in acetone/water (Scheme 7). The reaction provided isoxazolidinyl epoxide **36** in almost quantitative yield (99 %) and acceptable purity as a sole *trans* isomer (dr > 95:5, see the Supporting Information). Spectroscopic ^1H and ^{13}C NMR data were in good agreement with those previously published for related epoxides.[20,22]



Scheme 7. Synthesis of *N*-Boc-substituted isoxazolidinyl epoxide **36**. Reaction conditions: (a) DABCO, EtOAc, r.t., 17 h, 80 %; (b) Super-Hydride, THF, –78 °C, 30–50 min, combined yield 76 %, **34a** (3,5-*trans*)/**34b** (3,5-*cis*), 80:20; (c) Tf_2O, 2-chloropyridine, NMP, –20 °C to r.t., 20 h, 53 %; (d) Oxone, $NaHCO_3$, acetone/water (3:2), r.t., 30 min, 99 %, (dr > 95:5).

We have recently found out that isoxazolidinyl epoxides can react with hydrogen chloride or thionyl bromide providing 5-chloro- and 5-bromo-substituted isoxazolidin-4-ols.[20,22] However, the reaction of the epoxide **36** led only to a complex mixture under such reaction conditions. To our delight, the reaction with lithium chloride in the presence of acetic acid in THF afforded an anomeric mixture of 5-chloroisoxazolidines **37a**,**b** (4,5-*cis*-**37a**/4,5-*trans*-**37b**, 70:30) in combined yield of 95 % (Scheme 8).[26] Accordingly, the epoxide **36** was also briefly tested in reaction with lithium bromide. Unfortunately, we have only observed the formation of unidentified by-products. Next, the azidation of **37a**,**b** was examined. The reaction with sodium azide in acetonitrile at 50 °C gave 5-azidoisoxazolidines **38a** (4,5-*trans*) and **38b** (4,5-*cis*) in a ratio of 80:20 with 80 % combined yield. After separation by FCC, their relative configuration was mainly established on the basis of ^1H and ^{13}C NMR spectra. More specifically, narrow doublet for the 5-H proton of **38a** at 5.48 ppm with a small coupling constant 1.4 Hz referred to 4,5-*trans* isomer (it was later confirmed by

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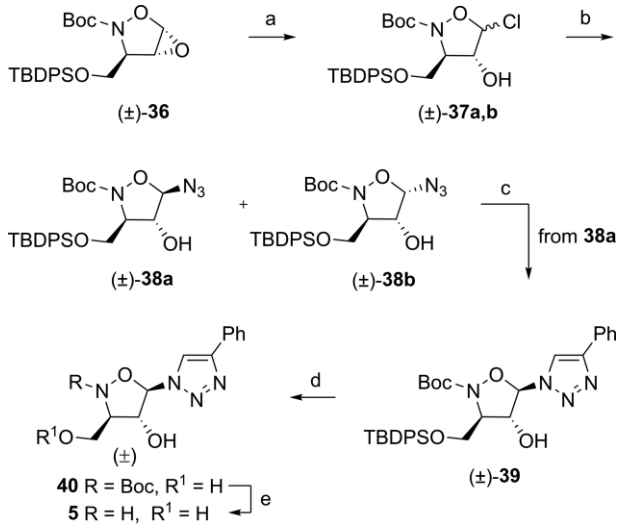
1D NOESY experiments of compound **40**; see the Supporting Information). A doublet with a larger coupling constant 5.0 Hz with chemical shift 5.59 ppm (5-H) denoted 4,5-*cis* arrangement in **38b**. The copper-catalyzed azide-alkyne cycloaddition of pure **38a** with phenylacetylene afforded isoxazolidinyl triazole **39** in 90 % yield. Subsequent desilylation of primary hydroxy group with TBAF in THF gave diol **40** in 80 % yield. Finally, the Boc protecting group was successfully removed using TFA in CH_2Cl_2.[27] Desired *N*-unprotected isoxazolidine **5** was obtained in 76 % yield.



Scheme 8. Synthesis of *N*-unsubstituted isoxazolidinyl triazole **5**. Reaction conditions: (a) LiCl, AcOH, THF, r.t., 2 h, **37a**, **37b** combined yield 95 %, **37a** (4,5-*cis*)/**37b** (4,5-*trans*), 70:30; (b) NaN_3, CH_3CN, 50 °C, 18 h; **38a**, **38b** combined yield 80 %, **38a** (4,5-*trans*)/**38b** (4,5-*cis*), 80:20; (c) phenylacetylene, $CuSO_4{\cdot}5H_2O$, sodium L-ascorbate, *t*BuOH/water (1:2), r.t., 17 h, 90 %; (d) TBAF, THF, 0 °C, 1 h, 80 %; (e) TFA/CH_2Cl_2 (1:5), 0 °C, 30 min then, r.t., 1 h, 76 %.

As mentioned above, the reaction between epoxide **36** and LiCl in the presence of acetic acid provided the mixture of two anomers **37a**,**b** in favor of the 4,5-*cis* isomer **37a** (determined on the basis of a doublet at δ = 6.28 ppm with $J_{4,5}$ = 4.3 Hz for 5-H proton) which was in accordance with our previous results.[20,22] However, we have noticed that both anomeric 5-chloroisoxazolidines were formed in nearly equimolar amounts at the early stage of the reaction. The predominance of the thermodynamically more stable 4,5-*cis* isomer was observed with progressing reaction time and graduated even after the conversion completion (for example when keeping the compound in solution for a longer time). We assume that the spontaneous isomerization of the *trans* isomer takes place probably through anomeric effect (the **37a**/**37b** ratio changes from 45:55 to 90:10, see the Supporting Information).

Biological Assay

Isoxazolidinyl triazoles **3**–**5**, **30**, and **40** were evaluated for inhibitory activity against acute myeloid leukemia cell line MOLM-13. The cell metabolic activity after treatment with these compounds was assessed by monitoring the NADH/NADPH-dependent reduction of tetrazolium dye to formazan using the MTS assay. Mode of cell death was estimated using double staining with annexin V/PI. While the metabolic activity of MOLM-13 cells after treatment with various concentrations (0.5–100 μM) of compounds **3**, **5**, and **30** did not change, a decrease in metabolic activity was observed after treatment with 100 μM concentration of compound **4**. Unlike other compounds the highest concentration of compound **40** used for cell treatment was 20 μM due to its lower solubility. A higher concentration of compound **40** would lead to the inhibitory effect of DMSO *per se*. A decrease in metabolic activity of MOLM-13 cells after treatment with 100 μM concentration of compound **4** may indicate some anticancer activity of this compound (Figure 2).

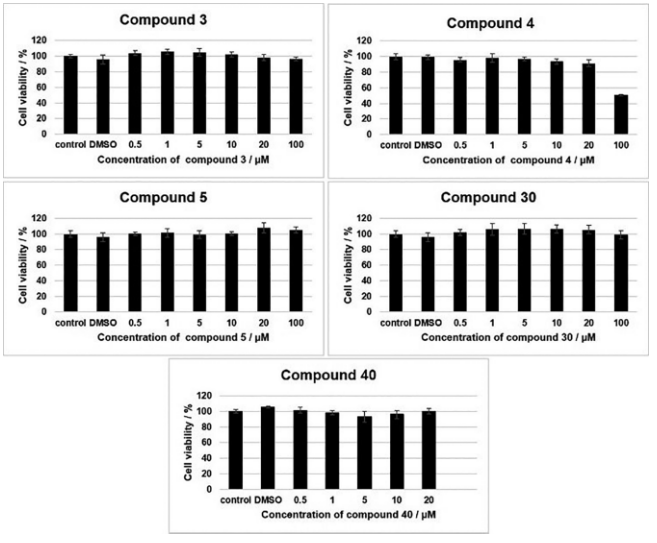


Figure 2. Cell line MOLM-13 was cultured in absence or in presence of different concentrations of prepared isoxazolidines (0; 0.5; 1; 5; 10; 20; 100 μM) for 48 h. After cultivation, metabolic activity of the cells was assessed by an MTS assay. Metabolic activity of control cells was set as 100 %. The data represent results of three independent measurements ± SD. DMSO column represents the metabolic activity of cells after treatment with the highest corresponding concentration of DMSO. Compounds **3**, **5** and **30** were prepared as 10 mM stock solutions in 1 % DMSO, compound **4** as 5 mM stock solution in 30 % DMSO and compound **40** as 2.5 mM stock solution in 50 % DMSO.

Subsequently, these results were further confirmed using double staining with annexin V (marker of cell apoptosis)/propidium iodide (cell necrosis factor). Measurement of cell death was conducted after treatment of MOLM-13 cells with 0.5–100 μM concentration of compounds **3**–**5**, **30** and 0.5–20 μM of compound **40**. Results for cells treated with the highest concentration used are summarized in Figure 3. Treatment with compounds **3**, **5**, **30** and **40** did not change the proportion of viable (unstained) cells compared to control. Induction of cell death was observed after treatment with 100 μM concentration of compound **4**.

Lower metabolic activity of MOLM-13 cells after treatment with compound **4** was also confirmed by a higher portion of cells stained with annexin V and/or PI.

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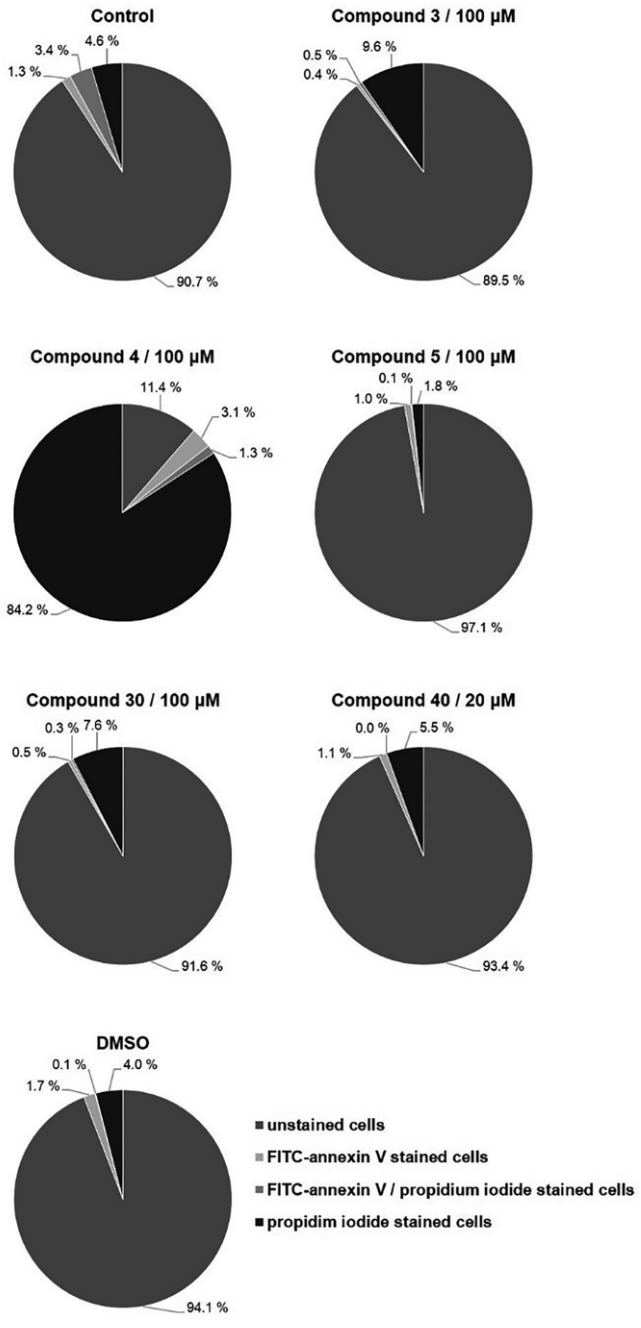

Figure 3. Pie charts represent proportion of viable (unstained), FITC-annexin V, FITC-annexin V/propidum iodide and propidium iodide stained cells after treatment with 100 µM concentration of compounds **3**–**5**, **30** and 20 µM of compound **40**. Chart for DMSO represents induction of cell death after treatment with the highest concentration of DMSO used.

Conclusion

In summary, we have synthesized new 1,2,3-triazoles bearing 3-hydroxymethylated 4-hydroxyisoxazolidine moiety. A hydroxy group at the C-4 position of the isoxazolidine ring has been introduced by dihydroxylation and epoxidation of 4,5-unsubstituted 2,3-dihydroisoxazoles via the reactions with potassium osmate/NMO and Oxone/$NaHCO_3$/acetone with good *trans* selectivity with respect to the substituent at C-3 carbon atom. The 2,3-dihydroisoxazoles have been obtained from 5-acetoxyisoxazolidines by using a catalytic amount of TMSOTf in the presence of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide. The requisite 5-azidoisoxazolidines have been prepared either by TMSOTf-mediated nucleophilic substitution of the isoxazolidinyl benzoates with $TMSN_3$ or by the reaction of 5-chloro derivatives with sodium azide with 4,5-*trans* selectivity being favored in both cases. Finally, the 1,2,3-triazole unit has been synthesized by copper(I)-catalyzed azide-alkyne cycloaddition.

Additionally, the significance of this method lies in its variability and applicability to a concise synthesis of various other 5-substituted 4-hydroxyisoxazolidine derivatives via nucleophilic substitution reactions at the C-5 position of the isoxazolidine ring, starting from easily synthesized 4,5-unsubstituted 2,3-dihydroisoxazoles.

Isoxazolidinyl triazoles **3**–**5**, **30**, and **40** were evaluated for inhibitory activity against the MOLM-13 cell line. After treatment with compounds **3**, **5**, **30**, and **40** no inhibitory activity was observed. Moderate inhibition of metabolic activity and induction of cell death was observed after treatment with compound **4**. To confirm the anticancer activity of compound **4** further research is necessary.

To prove the anticancer activity of isoxazolidinyl triazoles **3**–**5**, **30**, and **40** in this study we used AML cell line. We did not detect the relevant anticancer activity of compounds **3**, **5**, **30**, and **40** in the treatment of this cell line, but these compounds may be active in the treatment of other cell lines (solid tumors). Further research in this field is necessary.

Experimental Section

General Methods: Flash column chromatography (FCC) was carried out with a Büchi system (Pump Manager C-615 and Fraction Collector C-660) using Normasil 60 silica gel (0.040–0.063 mm; VWR). Thin Layer Chromatography (TLC) analysis was carried out using TLC silica gel 60 F254 (aluminium sheets, Merck), and plates were visualized with UV light or by treatment with permanganate solution followed by heating. Infrared (IR) spectra were recorded as neat samples with a Nicolet 5700 FTIR spectrometer with an ATR Smart Orbit Diamond adapter (Thermo Electron Corporation). NMR spectra were recorded with a Varian INOVA-300 spectrometer (^1H, 299.95 MHz, and ^{13}C, 75.42 MHz) and a Varian VNMRS-600 instrument (^1H, 599.75 MHz, and ^{13}C, 150.81 MHz) in $CDCl_3$ (using tetramethylsilane as the internal standard) and in CD_3OD (residual [D_3]methanol, δ_H = 3.31, 4.87 ppm, δ_C = 49.00 ppm). Data are presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, ddd = doublet of doublet of doublets, td = triplet of doublets, dt = doublet of triplets, m =multiplet, bs = broad singlet), coupling constants (J/Hz) and integration. HRMS analysis was carried out with an Orbitrap Velos Pro spectrometer (Thermo Fisher Scientific). All solvents used were dried and distilled according to conventional methods.

Typical Procedure for the Synthesis of 3-[(*tert*-Butyldiphenylsilyloxy)methyl]-2-methyl-2,3-dihydroisoxazole (14): The reaction flask with 5-acetoxyisoxazolidines **12a**,**b** (1.1 g, 2.66 mmol) was sealed with a rubber septum, evacuated, and filled with argon. Anhydrous NMP (27 mL) was added followed by BSTFA (850 µL, 3.20 mmol). The stirred solution was cooled down to 0 °C, and TMSOTf (95 µL, 0.52 mmol) was added dropwise. The reaction mix-

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ture was warmed to room temperature and stirred for 2 h. After this time, TLC showed that the reaction was complete (hexanes/EtOAc, 1:1). The reaction mixture was cooled in an ice/water-bath, quenched by the addition of a sat. aq. $NaHCO_3$ (10 mL), and water was added (200 mL). The resulting mixture was extracted with Et_2O (3 × 30 mL). The combined organic layers were dried with $MgSO_4$, and the solvent was evaporated in vacuo. The residue was purified by FCC (hexanes/EtOAc, 95:5) to give 2,3-dihydroisoxazole **14** (680 mg, 1.92 mmol, 72 %) as a yellowish oil. R_f = 0.57 (hexanes/EtOAc, 7:3). IR (ATR): $\tilde{\nu}_{max}$ = 2957. 2930, 2856, 1619, 1427, 1100, 1080, 823, 739, 698, 612, 502, 487 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.70–7.65 (m, 4 H, H-Ph), 7.44–7.34 (m, 6 H, H-Ph), 6.41–6.37 (m, 1 H, 5-H), 4.78 (pseudo t, J = 2.8, 2.6 Hz, 1 H, 4-H), 3.83–3.77 (m, 1 H, 3-H), 3.66 (dd, AB, J = 10.0, 6.8 Hz, 1 H, CH_2OSi), 3.55 (dd, AB, J = 10.0, 5.7 Hz, 1 H, CH_2OSi), 2.76 (d, J = 0.7 Hz, 3 H, N-CH_3), 1.06 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 142.4, 135.7, 135.6, 133.7, 133.5, 129.6 (2 ×), 127.6 (2 ×), 96.4, 73.7, 67.3, 47.5, 26.8, 19.3 ppm. HRMS (ESI): calcd. for $C_{21}H_{28}NO_2Si$ $[M + H]^+$ 354.1884, found 354.1877.

2-Benzyl-3-[(*tert*-butyldiphenylsilyloxy)methyl]-2,3-dihydroisoxazole (15): The typical procedure described above was applied using 5-acetoxyisoxazolidines **13a**,**b** (1.3 g, 2.65 mmol), BSTFA (0.84 mL, 3.16 mmol), TMSOTf (95 μL, 0.52 mmol), and anhydrous NMP (26 mL). TLC monitoring (hexanes/EtOAc, 1:1). FCC (hexanes/EtOAc, 95:5) gave 2,3-dihydroisoxazole **15** (1.02 g, 2.37 mmol, 89 %) as a yellowish oil. R_f = 0.52 (hexanes/EtOAc, 8:2). IR (ATR): $\tilde{\nu}_{max}$ = 3068, 2929, 2856, 1620, 1427, 1111, 1065, 823, 800, 737, 613, 502, 487 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.68–7.62 (m, 4 H, H-Ph), 7.41–7.26 (m, 11 H, H-Ph), 6.45–6.41 (m, 1 H, 5-H), 4.85 (pseudo t, J = 2.8, 2.6 Hz, 1 H, 4-H), 4.16 (d, AB, J = 13.1 Hz, 1 H, N-CH_2Ph), 4.08–4.02 (m, 1 H, 3-H), 3.91 (dd, AB, J = 13.1 Hz, 1 H, N-CH_2Ph), 3.63 (dd, AB, J = 10.0, 6.8 Hz, 1 H, CH_2OSi), 3.54 (dd, AB, J = 10.0, 5.7 Hz, 1 H, CH_2OSi), 1.03 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 142.9, 137.0, 135.8, 135.7, 133.8, 133.7, 129.7 (2 ×), 129.3, 128.5, 127.8 (2 ×), 127.5, 97.0, 71.1, 67.4, 63.7, 27.0, 19.4 ppm. HRMS (ESI): calcd. for $C_{27}H_{32}NO_2Si$ $[M + H]^+$ 430.2197, found 430.2191.

Typical Procedure for the Synthesis of 3-[(*tert*-Butyldiphenylsilyloxy)methyl]-2-methylisoxazolidine-4,5-diyl Dibenzoates (18, 19): 2,3-Dihydroisoxazole **14** (1.73 g, 4.89 mmol) was dissolved in acetone/water (49 mL, 6:1 v/v), and NMO (2.03 mL, 9.78 mmol, 50 % w/w in water) was added at 0 °C followed by $K_2OsO_4{\cdot}2H_2O$ (92 mg, 0.25 mmol). The reaction mixture was warmed to room temperature and stirred for 7 h. When TLC showed that the reaction was complete (hexanes/EtOAc, 1:1), the mixture was diluted with water (200 mL) and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with a sat. aq. NaCl (50 mL), dried with $MgSO_4$, and the solvent was evaporated in vacuo. The resulting crude diols **16** were used for the next step without further purification.

To a solution of diols **16** in anhydrous CH_2Cl_2 (30 mL) at room temperature were added sequentially DMAP (80 mg, 0.65 mmol), pyridine (1.08 mL, 13.35 mmol) and benzoyl chloride (1.17 mL, 10.08 mmol). The reaction mixture was stirred for 16 h. After this time, TLC showed that the reaction was complete (hexanes/EtOAc, 1:1). The reaction mixture was diluted with H_2O (200 mL), and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layers were dried with $MgSO_4$, and the solvent was evaporated in vacuo. The residue was purified by FCC (hexanes/EtOAc, 8:2) to give a mixture of four isomers **18a**,**b** and **19a**,**b** (1.81 g, 3.04 mmol, 62 % over two steps, **18a**/**18b**/**19a**/**19b**, 13:72:10:5) as a colourless oil. With the aim to completely characterize the new compounds, a sample of the mixture was subjected to repeated preparative TLC (hexanes/EtOAc, 9:1) to give three pure isomers **18a**,**18b** and **19a**. Unfortunately, the fourth isomer (3,4-*cis*-4,5-*cis*)-**19b** was not isolated, and therefore was not characterized. **Data for (3,4-*trans*-4,5-*trans*)-18a:** Colourless oil. R_f = 0.23 (hexanes/EtOAc, 9:1). IR (ATR): $\tilde{\nu}_{max}$ = 3070, 2930, 2856, 1722, 1256, 1105, 1065, 1023, 939, 700, 502, 488 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ = 8.05–8.01 (m, 4 H, H-Ph), 7.71–7.68 (m, 4 H, H-Ph), 7.62–7.32 (m, 12 H, H-Ph), 6.49 (s, 1 H, 5-H), 5.72 (d, J = 5.1 Hz, 1 H, 4-H), 4.06–4.01 (m, 2 H, CH_2OSi), 3.11–3.09 (m, 1 H, 3-H), 2.96 (s, 3 H, N-CH_3), 1.05 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 165.4, 165.3, 135.6 (2 ×), 133.6, 133.3, 132.9 (2 ×), 130.1, 129.9, 129.8 (2 ×), 129.5, 129.0, 128.5, 128.3, 127.8 (2 ×), 98.2, 83.7, 74.9, 62.8, 46.2, 26.8, 19.2 ppm. HRMS (ESI): calcd. for $C_{35}H_{38}NO_6Si$ $[M + H]^+$ 596.2463, found 596.2455. **Data for (3,4-*trans*-4,5-*cis*)-18b:** Colourless oil. R_f = 0.19 (hexanes/EtOAc, 9:1). IR (ATR): $\tilde{\nu}_{max}$ = 3070, 2930, 2857, 1728, 1272, 1257, 1111, 1066, 1020, 975, 700, 503, 487 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ = 7.97–7.91 (m, 4 H, H-Ph), 7.70–7.66 (m, 4 H, H-Ph), 7.56–7.53 (m, 2 H, H-Ph), 7.42–7.34 (m, 10 H, H-Ph), 6.74 (d, J = 4.5 Hz, 1 H, 5-H), 5.59 (dd, J = 7.9, 4.5 Hz, 1 H, 4-H), 3.98 (dd, AB, J = 10.7, 6.8 Hz, 1 H, CH_2OSi), 3.91 (dd, AB, J = 10.7, 4.6 Hz, 1 H, CH_2OSi), 3.49–3.44 (m, 1 H, 3-H), 3.08 (s, 3 H, N-CH_3), 1.05 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 165.0, 164.9, 135.6 (2 ×), 133.5, 133.4, 132.9 (2 ×), 129.8 (3 ×), 129.7, 129.6, 128.9, 128.4 (2 ×), 127.8 (2 ×), 93.8, 76.7, 69.9, 64.0, 49.5, 26.8, 19.2 ppm. HRMS (ESI): calcd. for $C_{35}H_{38}NO_6Si$ $[M + H]^+$ 596.2463, found 596.2458. **Data for (3,4-*cis*-4,5-*trans*)-19a:** Colourless oil. R_f = 0.14 (hexanes/EtOAc, 9:1). IR (ATR): $\tilde{\nu}_{max}$ = 3070, 2929, 2856, 1724, 1243, 1091, 1051, 1023, 929, 700, 503, 486 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ = 8.08–7.99 (m, 4 H, H-Ph), 7.67–7.23 (m, 16 H, H-Ph), 6.51 (s, 1 H, 5-H), 5.98 (d, J = 4.5 Hz, 1 H, 4-H), 4.09 (dd, AB, J = 10.3, 7.1 Hz, 1 H, CH_2OSi), 3.90 (dd, AB, J = 10.3, 6.5 Hz, 1 H, CH_2OSi), 3.53–3.44 (m, 1 H, 3-H), 2.96 (s, 3 H, N-CH_3), 1.02 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 165.4, 164.7, 135.7, 135.6, 133.8, 133.7, 133.1, 132.8, 130.2, 130.1, 130.0 (2 ×), 129.5, 129.1, 128.7, 128.6, 128.0, 127.9, 98.2, 80.7, 69.8, 60.9, 49.7, 26.9, 19.2 ppm. HRMS (ESI): calcd. for $C_{35}H_{38}NO_6Si$ $[M + H]^+$ 596.2463, found 596.2454.

2-Benzyl-3-[(*tert*-butyldiphenylsilyloxy)methyl]isoxazolidine-4,5-diyl Dibenzoates (20, 21): The typical procedure described above was applied using 2,3-dihydroisoxazole **15** (965 mg, 2.25 mmol), NMO (0.94 mL, 4.53 mmol, 50 % w/w in water), $K_2OsO_4{\cdot}2H_2O$ (41 mg, 0.11 mmol), and acetone/water (21 mL, 6:1 v/v). TLC monitoring (hexanes/EtOAc, 1:1). The resulting crude diols **17** were directly used for the next step after evaporation of the solvent.

The typical procedure described above was applied using diols **17**, DMAP (35 mg, 0.29 mmol), pyridine (0.47 mL, 5.84 mmol), benzoyl chloride (0.51 mL, 4.39 mmol), and anhydrous CH_2Cl_2 (15 mL). TLC monitoring (hexanes/EtOAc, 1:1). FCC (hexanes/EtOAc, 95:5) gave the mixture of four isomers **20a**,**b** and **21a**,**b** (1.01 g, 1.50 mmol, 67 % over two steps, **20a**/**20b**/**21a**/**21b**, 46:43:5:6) as a colourless oil. With the aim to completely characterize the new compounds, a sample of the mixture was subjected to repeated preparative TLC (hexanes/EtOAc, 9:1) to give two pure isomers **20a** and **20b** and a mixture of two isomers **21a** and **21b**. **Data for (3,4-*trans*-4,5-*trans*)-20a:** Colourless oil. R_f = 0.25 (hexanes/EtOAc, 9:1). IR (ATR): $\tilde{\nu}_{max}$ = 3068, 2930, 2856, 1723, 1258, 1105, 1065, 1024, 939, 698, 503, 488 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 8.04–7.96 (m, 4 H, H-Ph), 7.68–7.64 (m, 4 H, H-Ph), 7.62–7.26 (m, 17 H, H-Ph), 6.60 (s, 1 H, 5-H), 5.82 (dd, J = 3.6, 0.5 Hz, 1 H, 4-H), 4.40, (d, AB, J = 14.2 Hz, 1 H, N-CH_2Ph), 4.25 (d, AB, J = 14.2 Hz, 1 H, N-CH_2Ph), 4.03 (dd, AB, J = 10.7, 5.9 Hz, 1 H, CH_2OSi), 3.92 (dd, AB, J = 10.7, 6.8 Hz, 1 H, CH_2OSi), 3.43 (ddd, J = 6.8, 5.9, 3.6 Hz, 1 H, 3-H), 1.03 (s, 9 H, *t*Bu)

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ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.4, 165.3, 136.5, 135.7 (2 ×), 133.7, 133.4, 133.1 (2 ×), 130.1, 130.0, 129.9 (2 ×), 129.6, 129.2 (2 ×), 128.6, 128.5, 128.4, 127.9 (2 ×), 127.5, 98.4, 83.5, 71.5, 63.2, 62.8, 26.9, 19.3 ppm. HRMS (ESI): calcd. for $C_{41}H_{42}NO_6Si$ $[M + H]^+$ 672.2776, found 672.2767. **Data for (3,4-*trans*-4,5-*cis*)-20b:** Colourless oil. R_f = 0.18 (hexanes/EtOAc, 9:1). IR (ATR): $\tilde{\nu}_{max}$ = 3069, 2930, 2857, 1728, 1271, 1105, 1065, 1022, 698, 613, 503, 488 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.97–7.93 (m, 4 H, H-Ph), 7.69–7.53 (m, 6 H, H-Ph), 7.44–7.22 (m, 15 H, H-Ph), 6.78 (d, J = 4.6 Hz, 1 H, 5-H), 5.71 (dd, J = 7.0, 4.6 Hz, 1 H, 4-H), 4.43 (d, AB, J = 14.2 Hz, 1 H, *N*-CH_2Ph), 4.37 (d, AB, J = 14.2 Hz, 1 H, *N*-CH_2Ph), 3.90 (dd, AB, J = 10.6, 6.6 Hz, 1 H, CH_2OSi), 3.84 (dd, AB, J = 10.6, 5.2 Hz, 1 H, CH_2OSi), 3.73–3.67 (m, 1 H, 3-H), 1.02 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.2, 164.9, 136.6, 135.8, 135.7, 133.6, 133.5, 133.0 (2 ×), 130.0, 129.9 (3 ×), 129.7, 129.5, 129.1, 128.6 (2 ×), 128.5, 127.9 (2 ×), 127.7, 94.6, 77.0, 67.7, 65.6, 64.4, 26.9, 19.3 ppm. HRMS (ESI): calcd. for $C_{41}H_{42}NO_6Si$ $[M + H]^+$ 672.2776, found 672.2768. **Data for 21a, 21b:** Colourless oil. (**21a**/**21b**, 10:90). R_f (**21a**+**21b**) = 0.25 (hexanes/EtOAc, 9:1). Partial ^{1}H NMR data extracted from the mixture for (3,4-*cis*-4,5-*trans*)-**21a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.54 (s, 0.1 H, 5-H), 6.05 (d, J = 4.7 Hz, 0.1 H, 4-H), 3.74 (td, J = 7.1, 4.6 Hz, 0.1 H, 3-H) ppm. Partial ^{1}H NMR data extracted from the mixture for (3,4-*cis*-4,5-*cis*)-**21b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.75 (d, J = 5.2 Hz, 0.9 H, 5-H), 5.85 (dd, J = 7.9, 5.2 Hz, 0.9 H, 4-H), 3.48 (td, J = 7.2, 5.5 Hz, 0.9 H, 3-H) ppm. MS (ESI + APCI): m/z = 672.2 ($C_{41}H_{42}NO_6Si$) $[M + H]^+$.

Typical Procedure for the Synthesis of 5-Azido-3-[(*tert*-butyldiphenylsilyloxy)methyl]-2-methylisoxazolidin-4-yl Benzoate (22, 23): The reaction flask was charged with isoxazolidines **18a**,**b** and **19a**,**b** (1.2 g, 2.01 mmol), sealed with a rubber septum, evacuated, and filled with argon. Anhydrous CH_3CN (8 mL) was added followed by $TMSN_3$ (535 μL, 4.03 mmol). The stirred solution was cooled down to 0 °C, and TMSOTf (185 μL, 1.02 mmol) was added dropwise. After 5 min, the reaction mixture was warmed up to room temperature and stirred for 24 h. When TLC showed that the reaction was complete (hexanes/EtOAc, 9:1), the reaction was cooled down to 0 °C and quenched with sat. aq. $NaHCO_3$ (5 mL). The mixture was diluted with water (50 mL) and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layers were dried with $MgSO_4$, and the solvent was evaporated in vacuo. The residue was purified by FCC (hexanes/EtOAc, 95:5) to give one single isomer **22a** along with a mixture of three isomers **22b**, **23a** and **23b**. **Data for (3,4-*trans*-4,5-*trans*)-22a:** Yield: 570 mg (1.10 mmol, 55 %). Colourless oil. R_f = 0.31 (hexanes/EtOAc, 9:1). IR (ATR): $\tilde{\nu}_{max}$ = 3071, 2958, 2930, 2857, 2108, 1725, 1266, 1238, 1105, 1069, 700, 503, 488 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.01–7.98 (m, 2 H, H-Ph), 7.69–7.59 (m, 5 H, H-Ph), 7.48–7.34 (m, 8 H, H-Ph), 5.35 (s, 1 H, 5-H), 5.23 (d, J = 4.9 Hz, 1 H, 4-H), 3.96–3.91 (m, 2 H, CH_2OSi), 3.00–2.98 (m, 1 H, 3-H), 2.94 (s, 3 H, *N*-CH_3), 1.04 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 165.6, 135.6 (2 ×), 133.6 (2 ×), 132.8 (2 ×), 129.9, 129.8, 128.9, 128.5, 127.8 (2 ×), 92.1, 84.7, 74.8, 62.9, 45.7, 26.7, 19.1 ppm. HRMS (ESI): calcd. for $C_{28}H_{33}N_4O_4Si$ $[M + H]^+$ 517.2266, found 517.2261. **Data for 22b, 23a, 23b:** Yield: 105 mg (0.20 mmol, 10 %, **22b/23a/23b**, 40:50:10). Colourless oil. Partial ^{1}H NMR data extracted from the mixture for **22b**: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.65 (d, J = 5.2 Hz, 0.4 H, 5-H), 5.32 (dd, J = 7.5, 5.2 Hz, 0.4 H, 4-H), 3.31 (ddd, J = 7.5, 6.7, 4.5 Hz, 0.4 H, 3-H) ppm. Partial ^{1}H NMR data extracted from the mixture for **23a**: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.57 (d, J = 4.5 Hz, 0.5 H, 4-H), 5.47 (s, 0.5 H, 5-H), 3.41–3.34 (m, 0.5 H, 3-H) ppm. Partial ^{1}H NMR data extracted from the mixture for **23b**: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.71 (dd, J = 7.4, 5.9 Hz, 0.1 H, 4-H), 5.50 (d, J = 5.8 Hz, 0.1 H, 5-H), 3.02–2.99 (m, 0.1 H, 3-H) ppm. MS (ESI + APCI): m/z = 517.2 ($C_{28}H_{33}N_4O_4Si$) $[M + H]^+$.

5-Azido-2-benzyl-3-[(*tert*-butyldiphenylsilyloxy)methyl]isoxazolidin-4-yl Benzoate (24, 25): The typical procedure described above was applied using isoxazolidines **20a**,**b** and **21a**,**b** (690 mg, 1.03 mmol), $TMSN_3$ (275 μL, 2.07 mmol), TMSOTf (95 μL, 0.52 mmol), and anhydrous CH_3CN (4 mL). TLC monitoring (hexanes/EtOAc, 9:1). FCC (CH_2Cl_2/hexanes, 85:15) gave one single isomer **24a** and the mixture of **24a** and **24b**. The other minor diastereomers **25a** and **25b** were detected by NMR spectroscopy, however, the isolation and characterization attempts failed. **Data for (3,4-*trans*-4,5-*trans*)-24a**: Yield: 390 mg (0.66 mmol, 64 %). White solid. R_f = 0.52 (CH_2Cl_2/hexanes, 9:1). M.p. 85–86 °C. IR (ATR): $\tilde{\nu}_{max}$ = 3068.2960, 2865, 2109, 1728, 1264, 1106, 1024, 737, 695, 516, 483 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.01–7.96 (m, 2 H, H-Ph), 7.70–7.57 (m, 5 H, H-Ph), 7.50–7.27 (m, 13 H, H-Ph), 5.37 (s, 1 H, 5-H), 5.32 (dd, J = 4.1, 0.7 Hz, 1 H, 4-H), 4.47 (d, AB, J = 14.4 Hz, 1 H, *N*-CH_2Ph), 4.09 (d, AB, J = 14.4 Hz, 1 H, *N*-CH_2Ph), 3.98 (dd, J = 10.8, 5.4 Hz, 1 H, CH_2OSi), 3.93 (dd, J = 10.8, 6.8 Hz, 1 H, CH_2OSi), 3.29 (ddd, J = 6.8, 5.4, 4.1 Hz, 1 H, 3-H), 1.03 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.7, 136.7, 135.7 (2 ×), 133.8, 133.0, 132.9, 130.0 (3 ×), 129.1, 128.8, 128.6, 128.4, 127.9 (2 ×), 127.5, 91.9, 84.4, 72.4, 63.3, 62.6, 26.9, 19.3 ppm. HRMS (ESI): calcd. for $C_{34}H_{37}N_4O_4Si$ $[M + H]^+$ 593.2579, found 593.2569. **Data for 24a, 24b:** Yield: 45 mg (0.08 mmol, 8 %, **24a**/**24b**, 15:85). Colourless oil. Partial ^{1}H NMR data extracted from the mixture for **24b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.71 (d, J = 5.2 Hz, 1 H, 5-H), 5.40 (dd, J = 6.8, 5.2 Hz, 1 H, 4-H), 4.39 (d, AB, J = 13.4 Hz, 1 H, *N*-CH_2Ph), 4.33 (d, AB, J = 13.4 Hz, 1 H, *N*-CH_2Ph), 3.87 (dd, AB, J = 10.7, 6.4 Hz, 1 H, CH_2OSi), 3.78 (dd, AB, J = 10.7, 5.1 Hz, 1 H, CH_2OSi), 3.57 (td, J = 6.6, 5.1 Hz, 1 H, 3-H) ppm. MS (ESI + APCI): m/z = 593.2 ($C_{34}H_{37}N_4O_4Si$) $[M + H]^+$.

Typical Procedure for the Synthesis of 3-[(*tert*-Butyldiphenylsilyloxy)methyl]-2-methyl-5-(4′-phenyl-1*H*-1,2,3-triazol-1-yl)-isoxazolidin-4-yl Benzoate (26): To a stirred solution of azidoisoxazolidine **22a** (450 mg, 0.87 mmol) in *t*BuOH (1.5 mL) at room temperature were added sequentially a solution of $CuSO_4$·$5H_2O$ (22 mg, 0.09 mmol) in H_2O (3 mL), sodium L-ascorbate (35 mg, 0.18 mmol) and phenylacetylene (0.1 mL, 0.91 mmol). The reaction mixture was stirred at room temperature for 12 h. When TLC showed that the reaction was complete (hexanes/EtOAc, 7:3), the mixture was diluted with H_2O (5 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried with $MgSO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by FCC (hexanes/EtOAc, 8:2) to give isoxazolidinyl triazole **26** (500 mg, 0.81 mmol, 93 %) as a white solid. M.p. 53–56 °C. R_f = 0.41 (hexanes/EtOAc, 7:3). IR (ATR): $\tilde{\nu}_{max}$ = 3070, 2930, 2857, 1725, 1428, 1264, 1105, 1068, 1025, 971, 764, 692, 503, 487 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.23 (s, 1 H, 5′-H), 8.06–8.03 (m, 2 H, H-Ph), 7.73–7.61 (m, 7 H, H-Ph), 7.49–7.47 (m, 2 H, H-Ph), 7.41–7.29 (m, 9 H, H-Ph), 6.39 (d, J = 1.2 Hz, 1 H, 5-H), 6.13 (dd, J = 5.8, 1.2 Hz, 1 H, 4-H), 4.05 (dd, AB, J = 11.4, 5.7 Hz, 1 H, CH_2OSi), 4.01 (dd, AB, J = 11.4, 3.4 Hz, 1 H, CH_2OSi), 3.08 (td, J = 5.7, 3.5 Hz, 1 H, 3-H), 2.94 (s, 3 H, *N*-CH_3), 1.03 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 165.4, 148.4, 135.7, 135.6, 133.9, 132.9, 132.7, 130.7, 130.1 (3 ×), 128.8 (2 ×), 128.7, 128.2, 128.0 (2 ×), 126.1, 118.9, 90.6, 83.7, 75.8, 61.6, 45.0, 27.0, 19.4 ppm. HRMS (ESI): calcd. for $C_{36}H_{39}N_4O_4Si$ $[M + H]^+$ 619.2736, found 619.2732.

2-Benzyl-3-[(*tert*-butyldiphenylsilyloxy)methyl]-5-(4′-phenyl-1*H*-1,2,3-triazol-1-yl)isoxazolidin-4-yl Benzoate (27): The typical procedure described above was applied using azidoisoxazolidine **24a** (380 mg, 0.64 mmol), $CuSO_4$·$5H_2O$ (17 mg, 0.07 mmol), H_2O (1.5 mL), sodium L-ascorbate (25 mg, 0.13 mmol), phenylacetylene (70 μL, 0.64 mmol), and *t*BuOH (1.0 mL). TLC monitoring (hexanes/EtOAc, 7:3). FCC (hexanes/EtOAc, 8:2) gave isoxazolidinyl triazole **27** (380 mg, 0.54 mmol, 84 %) as a colourless oil. R_f = 0.41 (hexanes/

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EtOAc, 7:3). IR (ATR): $\tilde{\nu}_{max}$ = 3070, 2930, 2857, 1726, 1263, 1105, 1069, 1026, 761, 739, 593, 503, 488 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ = 8.10 (s, 1 H, 5′-H), 8.07–8.04 (m, 2 H, H-Ph), 7.71–7.60 (m, 7 H, H-Ph), 7.51–7.46 (m, 2 H, H-Ph), 7.40–7.26 (m, 14 H, H-Ph), 6.38 (s, 1 H, 5-H), 6.20 (dd, *J* = 5.2, 1.1 Hz, 1 H, 4-H), 4.52 (d, AB, *J* = 14.6 Hz, 1 H, *N*-CH_2Ph), 4.11–4.00 (m, 3 H, *N*-CH_2Ph, CH_2OSi), 3.37 (td, *J* = 5.6, 3.6 Hz, 1 H, 3-H), 1.01 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 165.4, 148.1, 136.5, 135.7, 135.6, 133.9, 132.9, 132.7, 130.7, 130.1 (3 ×), 128.9, 128.8, 128.7 (2 ×), 128.6, 128.2, 128.0 (2 ×), 127.7, 126.0, 118.8, 90.6, 83.5, 73.5, 62.1, 61.7, 26.9, 19.4 ppm. HRMS (ESI): calcd. for $C_{42}H_{43}N_4O_4Si$ $[M + H]^+$ 695.3049, found 695.3042.

Typical Procedure for the Synthesis of 3-[(*tert*-Butyldiphenylsilyl)oxymethyl]-2-methyl-5-(4′-phenyl-1*H*-1,2,3-triazol-1-yl)isoxazolidin-4-ol (28): Isoxazolidinyl triazole **26** (560 mg, 0.91 mmol) was dissolved in anhydrous methanol (9 mL), K_2CO_3 (40 mg, 0.29 mmol) was added, and the reaction mixture was stirred at room temperature for 30 min. After this time, TLC showed that the starting isoxazolidine disappeared (hexanes/EtOAc, 7:3). The mixture was diluted with water (25 mL) and extracted with EtOAc (4 × 10 mL). The combined organic layers were dried with $MgSO_4$, and the solvent was evaporated in vacuo. The residue was purified by FCC (hexanes/EtOAc, 75:25) to give isoxazolidine **28** (400 mg, 0.78 mmol, 86 %) as a white solid. M.p. 151–153 °C. R_f = 0.26 (hexanes/EtOAc, 7:3). IR (ATR): $\tilde{\nu}_{max}$ = 3169, 2935, 2873, 1425, 1329, 1129, 1106, 1082, 954, 763, 703, 646, 493, 459 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ = 8.12 (s, 1 H, 5′-H), 7.68–7.63 (m, 6 H, H-Ph), 7.42–7.27 (m, 9 H, H-Ph), 6.07 (d, *J* = 1.6 Hz, 1 H, 5-H), 5.18 (d, *J* = 4.7 Hz, 1 H, OH), 4.89 (ddd, *J* = 6.6, 4.7, 1.6 Hz, 1 H, 4-H), 3.91 (dd, AB, *J* = 11.3, 3.4 Hz, 1 H, CH_2OSi), 3.87 (dd, AB, *J* = 11.3, 6.0 Hz, 1 H, CH_2OSi), 2.94 (td, *J* = 6.3, 3.4 Hz, 1 H, 3-H), 2.91 (s, 3 H, *N*-CH_3), 1.00 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 147.7, 135.5 (2 ×), 132.8, 132.7, 130.1, 129.9 (2 ×), 128.8, 128.2, 127.9 (2 ×), 125.7, 118.6, 93.6, 83.4, 77.2, 61.8, 45.1, 26.8, 19.2 ppm. HRMS (ESI): calcd. for $C_{29}H_{35}N_4O_3Si$ $[M + H]^+$ 515.2473, found 515.2478.

2-Benzyl-3-[(*tert*-butyldiphenylsilyloxy)methyl]-5-(4′-phenyl-1*H*-1,2,3-triazol-1-yl)isoxazolidin-4-ol (29): The typical procedure described above was applied using isoxazolidinyl triazole **27** (180 mg, 0.26 mmol), K_2CO_3 (12 mg, 0.09 mmol), and anhydrous methanol (3 mL). TLC monitoring (hexanes/EtOAc, 7:3). FCC (hexanes/EtOAc, 8:2) gave isoxazolidine **29** (140 mg, 0.24 mmol, 92 %) as a white solid. M.p. 165–166 °C. R_f = 0.25 (hexanes/EtOAc, 7:3). IR (ATR): $\tilde{\nu}_{max}$ = 3171, 2927, 2872, 1428, 1095, 958, 693, 603, 505, 487 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.91 (s, 1 H, 5′-H), 7.64–7.59 (m, 6 H, H-Ph), 7.41–7.26 (m, 14 H, H-Ph), 6.05 (d, *J* = 0.6 Hz, 1 H, 5-H), 5.25 (bs, 1 H, OH), 4.94 (d, *J* = 6.1 Hz, 1 H, 4-H), 4.52 (d, AB, *J* = 14.6 Hz, 1 H, *N*-CH_2Ph), 4.03 (d, AB, *J* = 14.6 Hz, 1 H, *N*-CH_2Ph), 3.95 (dd, AB, *J* = 11.2, 3.5 Hz, 1 H, CH_2OSi), 3.88 (dd, AB, *J* = 11.2, 6.1 Hz, 1 H, CH_2OSi), 3.23 (td, *J* = 6.1, 3.6 Hz, 1 H, 3-H), 0.98 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 147.4, 137.1, 135.6 (2 ×), 132.8 (2 ×), 130.3, 130.1 (2 ×), 129.0, 128.8, 128.5, 128.3, 128.0 (2 ×), 127.7, 125.7, 118.7, 93.7, 83.4, 75.1, 62.3, 61.9, 26.9, 19.3 ppm. HRMS (ESI): calcd. for $C_{35}H_{39}N_4O_3Si$ $[M + H]^+$ 591.2786, found 591.2782.

Typical Procedure for the Synthesis of 3-(Hydroxymethyl)-2-methyl-5-(4′-phenyl-1*H*-1,2,3-triazol-1-yl)isoxazolidin-4-ol (3): The reaction flask with isoxazolidine **28** (420 mg, 0.82 mmol) was sealed with a rubber septum, evacuated, and filled with argon. Anhydrous THF (8 mL) was added, the stirred solution was cooled down to 0 °C, and TBAF (1.23 mL, 1.23 mmol, 1 M in solution THF) was added. The reaction mixture was warmed up to room temperature and stirred for 5 h. After this time, TLC showed that the reaction was complete (hexanes/EtOAc, 1:1). The mixture was diluted with sat. aq. $NaHCO_3$ (10 mL) and extracted with EtOAc (4 × 15 mL). The combined organic layers were dried with $MgSO_4$, and the solvent was evaporated in vacuo. The residue was purified by FCC (EtOAc/hexanes, 7:3) to give isoxazolidine **3** (200 mg, 0.72 mmol, 88 %) as a white solid. M.p. 133–135 °C. R_f = 0.25 (EtOAc). IR (ATR): $\tilde{\nu}_{max}$ = 3471, 3151, 2882, 2837, 2568, 1357, 1237, 1159, 1079, 1032, 984, 763, 692, 658, 519 cm^{-1}. ^1H NMR (600 MHz, CD_3OD): δ = 8.53 (s, 1 H, 5′-H), 7.84–7.82 (m, 2 H, H-Ph), 7.45–7.33 (m, 3 H, H-Ph), 6.10 (d, *J* = 1.9 Hz, 1 H, 5-H), 4.97 (dd, *J* = 6.5, 1.9 Hz, 1 H, 4-H), 3.90 (dd, AB, *J* = 12.2, 2.8 Hz, 1 H, CH_2OH), 3.81 (dd, AB, *J* = 12.2, 5.2 Hz, 1 H, CH_2OH), 2.89 (s, 3 H, *N*-CH_3), 2.77 (ddd, *J* = 6.5, 5.2, 2.8 Hz, 1 H, 3-H) ppm. ^{13}C NMR (150 MHz, CD_3OD): δ = 149.1, 131.6, 130.0, 129.4, 126.7, 120.9, 94.6, 83.3, 79.1, 59.9, 45.0 ppm. HRMS (ESI): calcd. for $C_{13}H_{17}N_4O_3$ $[M + H]^+$ 277.1296, found 277.1299.

2-Benzyl-3-(hydroxymethyl)-5-(4′-phenyl-1*H*-1,2,3-triazol-1-yl)-isoxazolidin-4-ol (4): The typical procedure described above was applied using isoxazolidine **29** (250 mg, 0.42 mmol), TBAF (0.63 mL, 0.63 mmol, 1 M solution in THF), and anhydrous THF (4 mL). Reaction time: 1.5 h. TLC monitoring (EtOAc). FCC (hexanes/EtOAc, 1:1) gave isoxazolidine **4** (140 mg, 0.40 mmol, 95 %) as a white solid. M.p. 156–157 °C. R_f = 0.42 (EtOAc). IR (ATR): $\tilde{\nu}_{max}$ = 3091, 3051, 2861, 2496, 1057, 1014, 961, 792, 767, 740, 693 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ = 8.31 (s, 1 H, 5′-H), 7.78–7.73 (m, 2 H, H-Ph), 7.45–7.23 (m, 8 H, H-Ph), 6.09 (d, *J* = 1.7 Hz, 1 H, 5-H), 4.97 (dd, *J* = 6.2, 1.8 Hz, 1 H, 4-H), 4.50 (d, AB, *J* = 14.5 Hz, 1 H, *N*-CH_2Ph), 4.02 (d, AB, *J* = 14.5 Hz, 1 H, *N*-CH_2Ph), 3.94 (dd, AB, *J* = 12.1, 3.3 Hz, 1 H, CH_2OH), 3.83 (dd, AB, *J* = 12.1, 5.6 Hz, 1 H, CH_2OH), 3.06 (td, *J* = 5.8, 3.3 Hz, 1 H, 3-H) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 148.8, 138.4, 131.6, 130.0 (2 ×), 129.4, 129.2, 128.4, 126.6, 120.7, 94.6, 83.2, 76.5, 62.3, 60.5 ppm. HRMS (ESI): calcd. for $C_{19}H_{21}N_4O_3$ $[M + H]^+$ 353.1609, found 353.1608.

[4-Acetoxy-2-methyl-5-(4′-phenyl-1*H*-1,2,3-triazol-1-yl)isoxazolidin-3-yl]methyl Acetate (30): To a stirred solution of isoxazolidine **4** (90 mg, 0.33 mmol) in anhydrous CH_2Cl_2 (3 mL) at 0 °C were added sequentially DMAP (9 mg, 0.07 mmol), Et_3N (0.185 mL, 1.33 mmol) and Ac_2O (95 μL, 1.00 mmol). The reaction mixture was warmed up to room temperature and stirred for 1 h. After this time, TLC showed that the reaction was complete (hexanes/EtOAc, 1:1). The mixture was quenched with sat. aq. $NaHCO_3$ (3 mL), diluted with water (5 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were dried with $MgSO_4$, and the solvent was evaporated in vacuo. The residue was purified by FCC (hexanes/EtOAc, 7:3) to give isoxazolidine **30** (115 mg, 0.32 mmol, 97 %) as a white solid. M.p. 141–143 °C. R_f = 0.25 (hexanes/EtOAc, 1:1). IR (ATR): $\tilde{\nu}_{max}$ = 3131, 2988, 1739, 1369, 1235, 1222, 1025, 969, 964, 596, 548 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ = 8.14 (s, 1 H, 5′-H), 7.86–7.84 (m, 2 H, H-Ph), 7.44–7.33 (m, 3 H, H-Ph), 6.26 (d, *J* = 0.9 Hz, 1 H, 5-H), 5.95 (dd, *J* = 5.1, 1.1 Hz, 1 H, 4-H), 4.49 (dd, AB, *J* = 12.1, 3.3 Hz, 1 H, CH_2OAc), 4.29 (dd, AB, *J* = 12.1, 5.1 Hz, 1 H, CH_2OAc), 3.03 (td, *J* = 5.1, 3.3 Hz, 1 H, 3-H), 2.93 (s, 3 H, *N*-CH_3), 2.18 (s, 3 H, $COCH_3$), 1.99 (s, 3 H, $COCH_3$) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 170.3, 169.8, 148.1, 130.4, 128.8, 128.3, 125.8, 118.6, 90.2, 83.4, 73.3, 60.5, 44.5, 20.7, 20.6 ppm. HRMS (ESI): calcd. for $C_{17}H_{21}N_4O_5$ $[M + H]^+$ 361.1507, found 361.1500.

***tert*-Butyl 3-[(*tert*-Butyldiphenylsilyloxy)methyl]isoxazole-2(3*H*)-carboxylate (35):** 5-Hydroxyisoxazolidine **34** (1.32 g, 2.88 mmol) was placed in a reaction flask, which was subsequently sealed with a rubber septum, evacuated, and filled with argon. Anhydrous NMP (30 mL) was added, the resulting solution was cooled down to −20 °C (ice/NaCl-bath). 2-Chloropyridine (1.91 mL, 20.2 mmol) and Tf_2O (0.63 mL, 3.74 mmol) were added. The reaction mixture was warmed to room temperature. When the starting material disappeared (20 h; TLC, hexanes/EtOAc; 8:2), the reaction mix-

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ture was diluted with H_2O (300 mL), and sat. aq. $NaHCO_3$ (20 mL), and the product was extracted into Et_2O (3 × 50 mL). The combined organic layers were dried with $MgSO_4$, and the solvent was evaporated in vacuo. The residue was purified by FCC (hexanes/EtOAc, 95:5) to give 2,3-dihydroisoxazole **35** (670 mg, 1.52 mmol, 53 %) as a colourless oil. R_f = 0.43 (hexanes/EtOAc, 8:2). IR (ATR): $\tilde{\nu}_{max}$ = 3074, 2931, 2857, 1712, 1367, 1111, 1079, 822, 741, 699, 613, 502, 487 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.69–7.64 (m, 4 H, H-Ph), 7.43–7.36 (m, 6 H, H-Ph), 6.57 (dd, J = 3.1, 2.0 Hz, 1 H, 5-H), 5.09 (dd, J = 3.1, 2.4 Hz, 1 H, 4-H), 5.01–4.96 (m, 1 H, H-3), 3.74 (dd, AB, J = 9.9, 4.7 Hz, 1 H, CH_2OSi), 3.64 (dd, AB, J = 9.9, 6.0 Hz, 1 H, CH_2OSi), 1.48 (s, 9 H, *t*BuO), 1.05 (s, 9 H, *t*BuSi) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.6, 142.9, 135.7 (2 ×), 133.6 (2 ×), 129.8 (2 ×), 127.8 (2 ×), 99.2, 82.7, 66.2, 65.7, 28.3, 26.9, 19.5 ppm. HRMS (ESI): calcd. for $C_{25}H_{34}NO_4Si$ $[M + H]^+$ 440.2252, found 440.2244.

***tert*-Butyl 4-[(*tert*-Butyldiphenylsilyloxy)methyl]-2,6-dioxa-3-azabicyclo[3.1.0]hexane-3-carboxylate (36):** Solid $NaHCO_3$ (1.25 g, 14.98 mmol) was placed in a reaction flask, and water was added (4.5 mL), followed by acetone (7 mL). The resulting mixture was cooled down to 0 °C and stirred for 20 min. Oxone (1.32 g, 2.14 mmol) was added in one portion, and the suspension was stirred at 0 °C for further 15 min. 2,3-Dihydroisoxazole **35** (470 mg, 1.07 mmol) was then added in one portion. The mixture was warmed up to room temperature and stirred for additional 30 min. After this time, TLC showed that the reaction was complete (hexanes/EtOAc, 8:2). The reaction mixture was diluted with water (50 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried with $MgSO_4$, and the solvent was evaporated in vacuo, to give isoxazolidinyl epoxide **36** (485 mg, 1.06 mmol, 99 %) as a colourless oil of satisfactory purity. R_f = 0.38 (hexanes/EtOAc, 8:2). IR (ATR): $\tilde{\nu}_{max}$ = 2931, 2858, 1715, 1320, 1111, 1057, 822, 738, 700, 612, 503, 488 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ = 7.66–7.63 (m, 4 H, H-Ph), 7.46–7.37 (m, 6 H, H-Ph), 5.38 (d, J = 1.5 Hz, 1 H, 5-H), 4.63 (bs, 1 H, 3-H), 3.91 (d, J = 1.8 Hz, 1 H, 4-H), 3.81 (dd, AB, J = 10.7, 5.1 Hz, 1 H, CH_2OSi), 3.67 (dd, AB, J = 10.7, 7.2 Hz, 1 H, CH_2OSi), 1.48 (s, 9 H, *t*BuO), 1.07 (s, 9 H, *t*BuSi) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 135.7 (2 ×), 133.0 (2 ×), 130.1 (2 ×), 128.0 (2 ×), 82.8, 80.3, 62.3, 62.0, 58.4, 28.2, 26.9, 19.3 ppm (the signal corresponding to the carbonyl carbon is missing). HRMS (ESI): the calcd. value for $C_{25}H_{33}NO_5$+H^+: 456.2201 was not found, however, the NMR spectroscopic data are in good agreement with those reported earlier for related isoxazolidinyl epoxides.[20,22]

***tert*-Butyl 3-[(*tert*-Butyldiphenylsilyloxy)methyl]-5-chloro-4-hydroxyisoxazolidine-2-carboxylate (37):** To a stirred solution of epoxide **36** (485 mg, 1.06 mmol) in THF (11 mL) at room temperature were added sequentially LiCl (72 mg, 1.70 mmol) and acetic acid (0.188 mL, 3.23 mmol). The reaction mixture was stirred at room temperature for 2 h. After this time, TLC showed that the reaction was complete (hexanes/EtOAc, 8:2). The mixture was diluted with water (80 mL), treated carefully with sat. aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were dried with $MgSO_4$, and the solvent was evaporated under reduced pressure, to give inseparable anomeric mixture of 5-chloroisoxazolidines **37a**,**b** (495 mg, 1.01 mmol, 95 %, **37a**/**37b**, 70:30) as a colourless oil. The resulting crude product was directly used for the next step without further purification. R_f = 0.29 (hexanes/EtOAc, 8:2). NMR data of the thermodynamically more stable product (3,4-*trans*-4,5-*cis*)-**37a** extracted from the corresponding anomeric mixture: ^1H NMR (300 MHz, $CDCl_3$): δ = 7.69–7.65 (m, 4 H, H-Ph), 7.43–7.36 (m, 6 H, H-Ph),6.28 (d, J = 4.3 Hz, 1 H, 5-H), 4.88 (ddd, J = 10.4, 7.3, 4.3 Hz, 1 H, 4-H), 4.01 (dd, AB, J = 10.6, 3.1 Hz, 1 H, CH_2OSi), 3.90 (dd, AB, J = 10.6, 4.3 Hz, 1 H, CH_2OSi), 3.84–3.78 (m, 1 H, 3-H), 2.41 (d, J = 10.4 Hz, 1 H, OH), 1.47 (s, 9 H, *t*BuO), 1.06 (s, 9 H, *t*BuSi) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 135.8, 135.6, 133.3, 132.9, 130.0 (2 ×), 127.9 (2 ×), 97.5, 83.1, 76.5, 63.6, 62.3, 28.2, 26.9, 19.4 ppm (the signal concerning to the carbonyl carbon is missing). MS (ESI + APCI): m/z = 492.2 ($C_{25}H_{35}{}^{35}ClNO_5Si$) $[M + H]^+$ 492.2.

***tert*-Butyl 5-Azido-3-[(*tert*-butyldiphenylsilyloxy)methyl]-4-hydroxyisoxazolidine-2-carboxylate (38):** The reaction flask with 5-chloroisoxazolidines **37a**,**b** (525 mg, 1.07 mmol) and NaN_3 (210 mg, 3.23 mmol) was sealed with a rubber septum, evacuated, and filled with argon. Anhydrous CH_3CN (11 mL) was added, and the resulting mixture was heated at 50 °C and stirred for 18 h. When TLC showed that the reaction was complete (hexanes/EtOAc, 8:2), the mixture was diluted with water (50 mL) and sat. aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were dried with $MgSO_4$, and the solvent was evaporated in vacuo. The residue was purified by FCC (hexanes/EtOAc, 9:1) to give two single 5-azidoisoxazolidines **38a** and **38b**. **Data for (3,4-*trans*-4,5-*trans*)-38a:** Yield: 305 mg (0.61 mmol, 57 %). Colourless oil. R_f = 0.16 (hexanes/EtOAc, 8:2). IR (ATR): $\tilde{\nu}_{max}$ = 3401, 2931, 2858, 2112, 1709, 1248, 1111, 1056, 823, 739, 700, 613, 502, 487 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.69–7.64 (m, 4 H, H-Ph), 7.44–7.36 (m, 6 H, H-Ph), 5.48 (d, J = 1.4 Hz, 1 H, 5-H), 4.49 (bs, 1 H, 4-H), 4.12 (ddd, J = 9.2, 6.0, 1.4 Hz, 1 H, 3-H), 3.93 (dd, AB, J = 10.2, 6.0 Hz, 1 H, CH_2OSi), 3.76 (dd, AB, J = 10.2, 9.3 Hz, 1 H, CH_2OSi), 2.57 (bs, 1 H, OH), 1.44 (s, 9 H, *t*BuO), 1.07 (s, 9 H, *t*BuSi) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.9, 135.7 (2 ×), 133.2 (2 ×), 130.0 (2 ×), 127.9 (2 ×), 95.6, 83.1, 79.4, 69.4, 62.5, 28.2, 27.0, 19.4 ppm. HRMS (ESI): calcd. for $C_{25}H_{35}N_4O_5Si$ $[M + H]^+$ 499.2372, found 499.2364. **Data for (3,4-*trans*-4,5-*cis*)-38b:** Yield: 125 mg (0.25 mmol, 23 %). Colourless oil. R_f = 0.22 (hexanes/EtOAc, 8:2). IR (ATR): $\tilde{\nu}_{max}$ = 3403, 2931, 2857, 2118, 1709, 1255, 1111, 978, 740. 700, 609, 502, 487 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ = 7.68–7.65 (m, 4 H, H-Ph), 7.44–7.35 (m, 6 H, H-Ph), 5.58 (d, J = 5.1 Hz, 1 H, 5-H), 4.64 (dt, J = 9.3, 5.3 Hz, 1 H, 4-H), 4.00–3.78 (m, 3 H, 3-H, CH_2OSi), 2.24 (d, J = 9.5 Hz, 1 H, OH), 1.50 (s, 9 H, *t*BuO), 1.06 (s, 9 H, *t*BuSi) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 158.0, 135.6, 135.5, 133.2, 132.9, 129.8 (2 ×), 127.8, 127.7, 92.0, 82.7, 76.0, 65.1, 63.2, 28.0, 26.8, 19.3 ppm. HRMS (ESI): calcd. for $C_{25}H_{35}N_4O_5Si$ $[M + H]^+$ 499.2372, found 499.2368.

***tert*-Butyl 3-[(*tert*-Butyldiphenylsilyloxy)methyl]-4-hydroxy-5-(4-phenyl-1*H*-1,2,3-triazol-1-yl)isoxazolidine-2-carboxylate (39):** To a stirred solution of azidoisoxazolidine **38a** (315 mg, 0.63 mmol) in *t*BuOH (0.7 mL) at room temperature were added sequentially a solution of $CuSO_4$•$5H_2O$ (17 mg, 0.07 mmol) in H_2O (1.4 mL), sodium L-ascorbate (25 mg, 0.13 mmol) and phenylacetylene (70 μL, 0.64 mmol). The reaction mixture was stirred at room temperature for 17 h. After this time, TLC showed that the reaction was complete (hexanes/EtOAc, 7:3). The mixture was diluted with water (15 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried with $MgSO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by FCC (hexanes/EtOAc, 8:2) to give isoxazolidinyl triazole **39** (345 mg, 0.57 mmol, 90 %) as a white solid. M.p. 121–123 °C. R_f = 0.21 (hexanes/EtOAc, 7:3). IR (ATR): $\tilde{\nu}_{max}$ = 3419, 2931, 2857, 1712, 1306, 1115, 1038, 765, 692, 612, 505, 488 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.88 (s, 1 H, 5′-H), 7.68–7.58 (m, 6 H, H-Ph), 7.41–7.28 (m, 9 H, H-Ph), 6.19 (d, J = 2.7 Hz, 1 H, 5-H), 5.45 (dt, J = 4.5, 2.7 Hz, 1 H, 4-H), 4.74 (bs, 1 H, OH), 4.29 (ddd, J = 7.2, 5.6, 2.7 Hz, 1 H, 3-H), 3.90 (dd, AB, J = 10.5, 5.6 Hz, 1 H, CH_2OSi), 3.71 (dd, AB, J = 10.5, 7.2 Hz, 1 H, CH_2OSi), 1.46 (s, 9 H, *t*BuO), 1.00 (s, 9 H, *t*BuSi) ppm. ^{13}C NMR (75 MHz,

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$CDCl_3$): δ = 157.0, 148.1, 135.6 (2 ×), 133.1, 133.0, 130.0 (2 ×), 129.7, 129.0, 128.7, 127.9 (2 ×), 125.9, 118.9, 94.1, 83.6, 80.1, 69.8, 62.5, 28.2, 26.9, 19.4 ppm. HRMS (ESI): calcd. for $C_{33}H_{41}N_4O_5Si$ $[M + H]^+$ 601.2841, found 601.2835.

***tert*-Butyl 4-Hydroxy-3-(hydroxymethyl)-5-(4-phenyl-1*H*-1,2,3-triazol-1-yl)isoxazolidine-2-carboxylate (40):** The reaction flask with isoxazolidine **39** (185 mg, 0.31 mmol) was sealed with a rubber septum, evacuated, and filled with argon. Anhydrous THF (3 mL) was added, the resulting solution was cooled down to 0 °C, and TBAF (0.47 mL, 0.47 mmol, 1 M solution in THF) was added. The reaction mixture was stirred at 0 °C for 1 h. After this time, TLC showed that the reaction was complete (hexanes/EtOAc, 1:1). The mixture was diluted with water (25 mL) and sat. aq. $NaHCO_3$ (5 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried with $MgSO_4$, and the solvent was evaporated in vacuo. The residue was purified by FCC (hexanes/EtOAc, 1:1) to give isoxazolidine **40** (90 mg, 0.25 mmol, 80 %) as a white solid. M.p. 164–166 °C. R_f = 0.11 (hexanes/EtOAc, 1:1). IR (ATR): $\tilde{\nu}_{max}$ = 3215, 2986, 2917, 1733, 1464, 1294, 1158, 1030, 963, 836, 771, 698 cm^{-1}. ^1H NMR (600 MHz, CD_3OD): δ = 8.58 (s, 1 H, 5′-H), 7.86–7.84 (m, 2 H, H-Ph), 7.46–7.35 (m, 3 H, H-Ph), 6.20 (d, J = 2.9 Hz, 1 H, 5-H), 5.22 (dd, J = 2.9, 2.2 Hz, 1 H, 4-H), 4.20 (ddd, J = 6.7, 6.2, 2.2 Hz, 1 H, 3-H), 3.84 (dd, AB, J = 11.5, 6.2 Hz, 1 H, CH_2OH), 3.77 (dd, AB, J = 11.5, 6.7 Hz, 1 H, CH_2OH), 1.54 (s, 9 H, *t*BuO) ppm. ^{13}C NMR (150 MHz, CD_3OD): δ = 159.3, 149.3, 131.2, 130.0, 129.6, 126.8, 122.1, 95.6, 84.5, 80.3, 72.4, 61.5, 28.3 ppm. HRMS (ESI): calcd. for $C_{17}H_{23}N_4O_5$ $[M + H]^+$ 363.1663, found 363.1668.

3-Hydroxymethyl-5-(4-phenyl-1*H*-1,2,3-triazol-1-yl)isoxazolidin-4-ol (5): To a solution of isoxazolidine **40** (90 mg, 0.25 mmol) in CH_2Cl_2 (2 mL) at 0 °C was added TFA (0.39 mL, 5.06 mmol), and the reaction mixture was stirred at 0 °C for 30 min and then stirred for additional 1 h allowing to reach room temperature. When TLC showed that the reaction was complete (hexanes/EtOAc, 2:3), the mixture was cooled down to 0 °C, diluted with water (10 mL) and treated carefully with sat. aq. $NaHCO_3$ (10 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were dried with $MgSO_4$, and the solvent was evaporated in vacuo. The residue was purified by FCC (CH_2Cl_2/MeOH, 95:5) to give *N*-unprotected isoxazolidine **5** (50 mg, 0.19 mmol, 76 %) as a white solid. M.p. 144–146 °C. R_f = 0.17 (EtOAc). IR (ATR): $\tilde{\nu}_{max}$ = 3193, 2937, 2881, 2370, 1434, 1328, 1154, 1083, 964, 803, 759, 688, 518, 495 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ = 8.59 (s, 1 H, 5′-H), 7.86–7.82 (m, 2 H, H-Ph), 7.47–7.32 (m, 3 H, H-Ph), 6.22 (d, J = 2.3 Hz, 1 H, 5-H), 5.13 (dd, J = 5.4, 2.3 Hz, 1 H, 4-H), 3.89 (d, J = 3.6 Hz, 2 H, CH_2OH), 3.50 (dd, J = 10.0, 4.9 Hz, 1 H, 3-H) ppm. ^{13}C NMR (150 MHz, CD_3OD): δ = 149.0, 131.4, 130.0, 129.5, 126.7, 121.6, 97.9, 82.4, 71.2, 58.8 ppm. HRMS (ESI): calcd. for $C_{12}H_{15}N_4O_3$ $[M + H]^+$ 263.1139, found 263.1141.

Crystallography: Data collection and cell refinement for **3** was carried out using Eulerian-crandle diffractometer Stoe StadiVari using Pilatus3R 300K HPAD detector at 100 K, and using CuK_α radiation (λ = 1.54186 Å, microfocus source Xenocs Genix3D Cu HF) was used for the measurement. The diffraction intensities were corrected for Lorentz, polarization, and absorption effects. The structure was solved by the direct method using SHELXT,[28] refined by a full-matrix least-squares procedure with the SHELXL (ver. 2018/3),[29] and drawn with the OLEX2 package.[30]

Crystal Data for 3: $C_{13}H_{16}N_4O_3$ (M = 276.30 g/mol): monoclinic, space group $P2_1/n$ (no. 14), a = 10.8835(5) Å, b = 10.2458(3) Å, c = 12.3935(6) Å, β = 110.306(4)°, V = 1296.11(10) Å^3, Z = 4, T = 100 K, μ(CuK_α) = 0.859 mm^{-1}, *Dcalc* = 1.416 g/cm^3, 14173 reflections measured (9.338° ≤ 2Θ ≤ 142.736°), 2469 unique (R_{int} = 0.0306, R_{sigma} = 0.0180) which were used in all calculations. The final R_1 was 0.0320 [$I > 2\sigma(I)$] and wR_2 was 0.0847 (all data).

Deposition Number 1997748 (for **3**) contains the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures service www.ccdc.cam.ac.uk/structures.

Hirshfeld surface analysis: CrystalExplorer[31] was used to calculate Hirshfeld surface and associated fingerprint plots.[32]

Cell Culture and Cultivation Conditions

In this study, AML cell line MOLM-13 (ACC 554) was used. This cell line was derived from the peripheral blood of a 20-year-old patient with AML developed from myelodysplastic syndromes supplied by Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany). Cell line was cultured in 5 mL of RPMI medium (5×10^5 cells) containing 12 % fetal bovine serum (Biotech, SR),100 000 units/L penicillin and 50 mg/L streptomycin (both from Sigma-Aldrich, USA) for one or two days at 37 °C in a humidified atmosphere containing 5 % CO_2.

Estimation of Metabolic Activity Using MTS Assay

We cultured the cell line MOLM-13 in absence or in presence of different concentrations of prepared isoxazolidines (0; 0.5; 1; 5; 10; 20; 100 μM) for 48 h. Compounds **3**, **5** and **30** were prepared as 10 mM stock solutions in 1 % DMSO, compound **4** as 5 mM stock solution in 30 % DMSO and compound **40** as 2.5 mM stock solution in 50 % DMSO. After this time, cell metabolic activity was assessed using CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS, ([3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt])) (Promega, USA) according to the manufacturer's protocol.

Detection of Cell Death in MOLM-13 Cells After Treatment with Isoxazolidines Using Double Staining with FITC-Annexin V and Propidium Iodide

After treatment with 0; 0.5; 1; 5; 10; 20; 100 μM concentration of individual isoxazolidines, cells (1×10^6 cells/mL) were incubated for 48 h under standard culture conditions. Subsequently, the proportion of viable, apoptotic, and necrotic cells was detected using an annexin V/propidium iodide kit (Calbiochem, USA). Cells were washed twice with PBS and gently re-suspended in binding buffer containing 0.5 μg/mL FITC-labelled annexin V. The mixtures were incubated for 15 min at room temperature in the dark and then centrifuged (2500 rpm, 15 min). The resulting sediments were re-suspended in binding buffer, and propidium iodide (final concentration 0.6 μg/mL) was added to each sample after which the samples were analyzed by flow cytometry using an Accuri C6 flow cytometer (BD Bioscience, USA).

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