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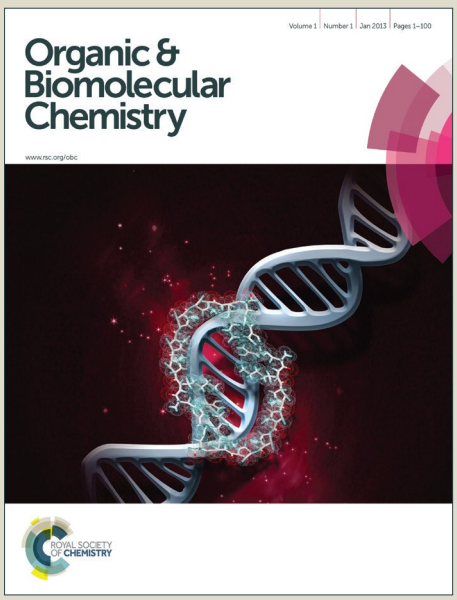

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ARTICLE

A novel strategy for the synthesis of thermally stable and apoptosis-inducing 2,3-dihydroazetes†

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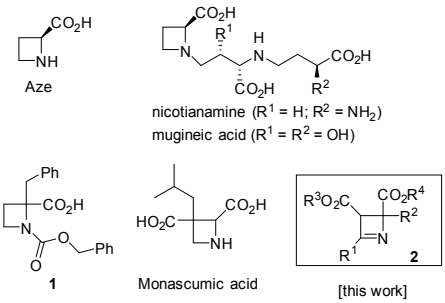
Ilia A. Smetanin,[a] Mikhail S. Novikov,*[a] Anastasiya V. Agafonova,[a] Nikolai V. Rostovskii,[a] Alexander F. Khlebnikov,[a] Igor V. Kudryavtsev,[b] Maxim A. Terpilowski,[c] Maria K. Serebriakova,[b] Andrey S. Trulioff,[b] Nikolay V. Goncharov[c]

A general and concise approach to thermally and hydrolytically stable alkyl 2,3-dihydroazete-2,3-di-/2,2,3-tricarboxylates from alkyl 2-bromoazirine-2-carboxylates or 4-bromo-5-alkoxyisoxazoles is disclosed. The synthesis involves formation of 2-azabuta-1,3-diene by reaction of rhodium carbenoid with isoxazole or azirine followed by cyclization/hydrodebromination cascade. The latter reaction is the first example of the selective hydrodehalogenation of a valence isomer under equilibrium conditions. In vitro cytotoxicity tests on THP-1 cell line revealed that the 2,3-dihydroazetes greatly differ in their ability to induce apoptosis and/or necrosis. To adequately describe and quantitatively assess these properties, the difference between the two areas under the curves of concentration dependancy of apoptosis/necrosis induction within the concentration range was used. Trimethyl 4-phenyl-2,3-dihydroazete-2,2,3-tricarboxylate was found to display the maximal apoptotic potential coupled with high cytotoxic and minimal necrotic potential.

Introduction

A four-membered aza-heterocyclic pattern is present in a wide range of natural products and synthetic bioactive compounds.[1] The majority of these strained cyclic elements have either a β-lactam or azetidine structure. Azetidine-2-carboxylic acid (Aze), a cyclic homologue of proline, is a constituent of nicotianamine[2] and mugineic acid[3], which are iron transporters in plants (Scheme 1). Aze and its substituted analogues are also used as non-protein amino acid components for the design of novel peptides and peptidomimetics.[4] Acid **1** was found to act as HCMV serine protease inhibitor.[5] Monascumic acids, azetidine-2,3-dicarboxylic acid derivatives, isolated from the extracts of Monascus pilosus-fermented rice, exhibited potent inhibitory effect on the Epstein-Barr virus early antigen (EBV-EA) activation and moderate inhibitory effects on NOR 1 activation.[6] Quite recently it was shown that some iminosugars containing the 3-fluoroazetidine unit display inhibition of growth PANC-1 cells comparable with those for gemcitabine.[7]

To further explore the biological potential of four-membered aza-heterocyclic compounds, in particular anti-cancer activity, we proposed synthesizing dehydrogenated analogues of monascumic acid, namely 2,3-dihydroazete-2,3-dicarboxylates **2**. The latter possess higher rigidity and lower N-basicity than azetidine derivatives and a different topology of the substituents due to C(sp^2) atom.



Scheme 1 Bioactive compounds containing azetidine-2-carboxylic unit.

2,3-Dihydroazetes (or 1-azetines) having no heteroatom substituent at the multiple bond are much less accessible compounds than both their hydrogenated analogues, azetidines, and cyclic unsaturated homologues: azirines and 1-pyrrolines.[1,8] A few photolytic reactions leading to 2,3-dihydroazetes (2+2)-cycloaddition of nitriles to alkenes,[9] and cyclization of N-acetyl α-dehydrophenylalanines[10] are known. 2,3-Dihydroazete derivatives were synthesized by pyrolysis of 1-azabicyclo[4.2.0]oct-3-en-2-one derivatives,[11] recyclization

[a] St. Petersburg State University, Institute of Chemistry, 7/9 Universitetskaya nab., St. Petersburg, 199034 Russia. E-mail: m.s.novikov@chem.spbu.ru. Fax: +7 812-428-6939; Tel: +7 812-428-9344
[b] Institute of Experimental Medicine, Russian Academy of Medical Sciences, ul. Acad. Pavlov 12, St. Petersburg, 197376 Russia.
[c] Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, pr. Torez 44, St. Petersburg, 194223 Russia.

† Electronic Supplementary Information (ESI) available: spectroscopic data for all new compounds, experimental procedures for the preparation of compounds **7e, 10a–h**, and bioassay details. CCDC 1449965 (**2a**) and 1449943 (**2h**). For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/x0xx00000x

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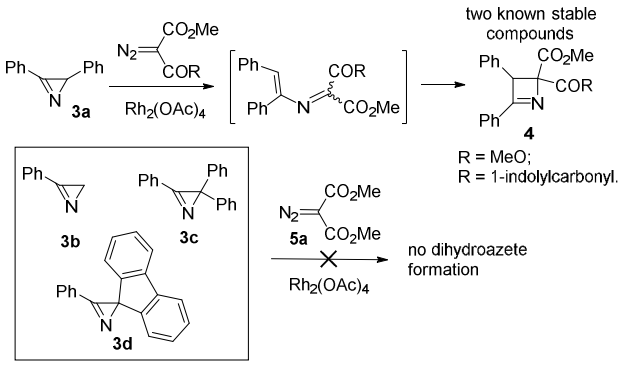
of 2-azido-1,1-dichlorocyclopropanes[12] and (3+1)-cycloaddition of the nitrilio-bis(trifluoromethyl)methanides to isonitriles.[13] All these methods have very limited application, in particular, for the preparation of the derivatives with ester and related functions. The only known approach enabling the introduction of ester groups into the 2,3-dihydroazete ring is a Rh(II)-carbenoid-mediated azirine ring expansion (Scheme 2).[14] The reaction proceeds via electrophilic attack of the rhodium carbenoid on the nitrogen of the azirine followed by 1,4-electrocyclization of the resulting 2-azabuta-1,3-diene.[15,16] Unfortunately, this reaction has serious limitations on the structure of a diazo compound. Thus, thermally stable dihydroazetes **4** can be prepared only from the diazomalonic acid esters or its amidoesters. Besides the successful results are known only for 2,3-diphenyl-2*H*-azirine (**3a**). For example, 3-monosubstituted azirine **3b** and trisubstituted azirines **3c,d** do not furnish dihydroazetes at all.[15,16] Thereby, synthetic problems mentioned above make them hitherto hardly accessible compounds with poorly known reactivity and practically unknown biological properties.

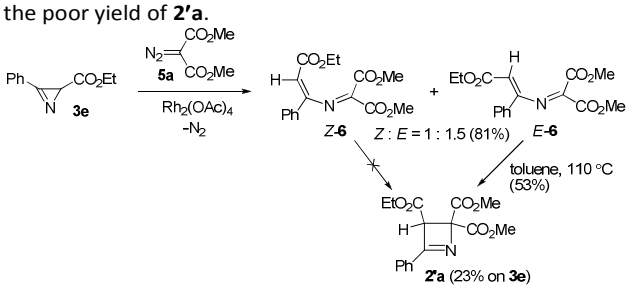


Scheme 2 One-atom azirine ring expansion to 2,3-dihydroazetes.

In the present work we report an effective two-step method for the preparation of thermally stable and differently substituted at C^2- and C^4-positions alkyl 2,3-dihydroazete-2,3-di-/2,2,3-tricarboxylates **2** that can operate both with alkyl 2-bromo-azirine-2-carboxylates and 4-bromo-5-alkoxyisoxazoles as starting material. Bioassay results obtained with THP-1 cell line on apoptosis and/or necrosis inducing activity of representative 2,3-dihydroazete derivatives are also presented.

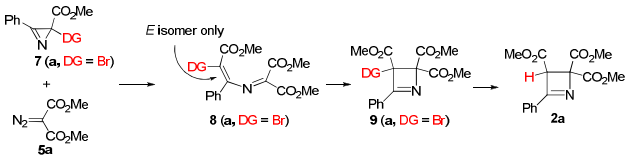
Results and discussion

The synthesis of a series of dihydroazetes **2** was begun with the 2,3-dihydroazete-2,2,3-tricarboxylates **2a, 2'a** (Scheme 3). Initially we tried to synthesize compound **2'a** directly from azirine **3e** and dimethyl diazomalonate **5a** via cyclization of azadiene **6** (Scheme 3). Heating azirine **3e** with 1.5 equiv of **5a** in the presence of $Rh_2(OAc)_4$ (1 mol %) gave a 1.5:1 mixture of *E* and *Z* isomers of azadiene **6** in 81% total yield. Assignment of the C=C bond configuration in stereoisomers **6** is based on an olefin proton signal in the ^{1}H NMR spectra which is shifted downfield for the *Z* isomer.[17] Compounds *E*-**6** and *Z*-**6** were separated by column chromatography (49 and 32% yield) and refluxed in toluene. Isomer *E*-**6** after 4 h gave dihydroazete **2'a** in 53% yield, which showed high thermal stability up to 150 °C. Isomer *Z*-**6** proved to be unreactive and did not cyclize to **2'a** even at higher temperatures. Thus, a two-step carbenoid-mediated azirine ring expansion of **3b** provided dihydroazete **2'a** in only 23% overall yield. High dependence of the 1,4-cyclization on the configuration of the C=C bond of azadiene **6** and low stereoselectivity of its formation are the reasons for the poor yield of **2'a**.



Scheme 3 Synthesis of dihydroazete **2'a** from azirine **3e**.

We supposed that the problem can be solved by introduction of efficient directing group to the azirine ring (Scheme 4, structure 7) for selective ring opening into such stereoisomer, which is capable of undergoing 1,4-cyclization into dihydroazete **9**. It is also important that a directing group should be easy to remove. Recently we have discovered that the reaction of 2-bromo-substituted azirine **7a** with **5a** under Rh(II)-catalysis occurs with full *E*-stereoselectivity, providing azadiene **8a** exclusively. Moreover, this is able to cyclize reversibly into dihydroazete **9a** at elevated temperatures.[18] A feasible alternative route to dihydroazetes **2** could, therefore, be started from bromoazirines **7** but have to involve a selective hydrodebromination of thermally unstable dihydroazetes **9**.



Scheme 4 Synthetic scheme for dihydroazete **2a**.

To verify the workability of this approach and to find suitable hydrodehalogenation conditions azadiene **8a** was synthesized from azirine **7a** and dimethyl diazomalonate **5a** according to the known procedure.[18] Tributylstannane was tried as a potential selective reagent for the hydrodebromination. The highest yields of **2a** were achieved by slow addition of a diluted Bu_3SnH solution to the solution of an equilibrium mixture of **8a** and **9a** at 110 °C. These conditions enable a selective reduction of the dihydroazete isomer **9a**. It is important that the addition of azobisisobutyronitrile (AIBN, 4 mol %) noticeably suppresses the formation of side products. These conditions allowed the preparation of **2a** in 78% yield calculated on azirine **7a** using on the first stage only a simple

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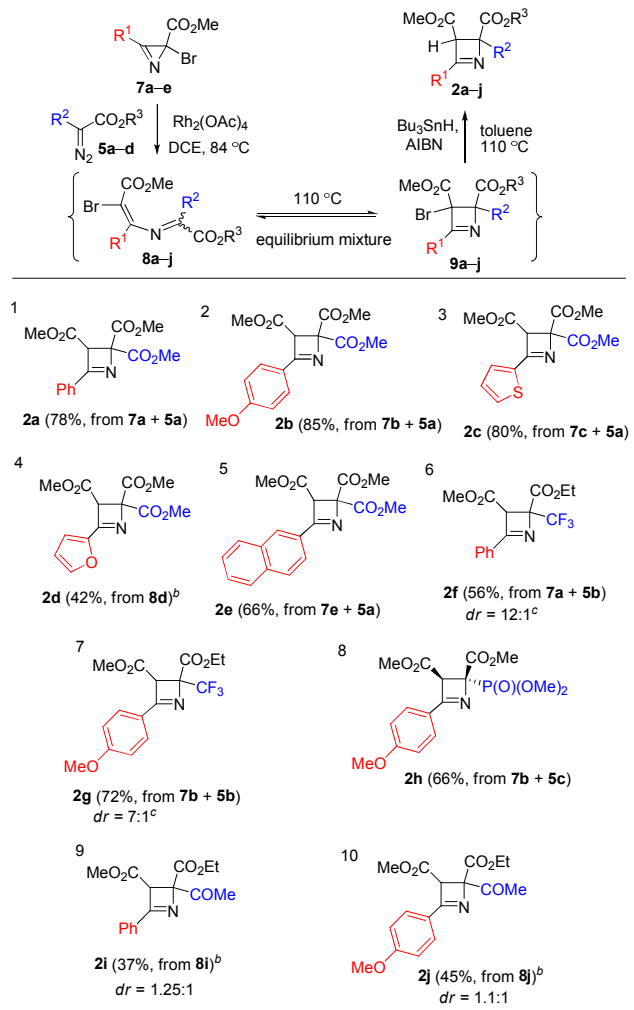
filtration of the reaction mixture through a silica gel pad. To the best of our knowledge, it is the first example of the selective hydrodehalogenation of a valence isomer under equilibrium conditions.

Next we explored the scope of the two-step "4-bromoazadiene formation/hydrodebromination" sequence with a set of azirines **7a–e** and α-diazocarbonyl compounds **5a–d** (Table 1).

Table 1 Synthesis of dihydroazetes **2a–j** from azirines **7a–e**[a]

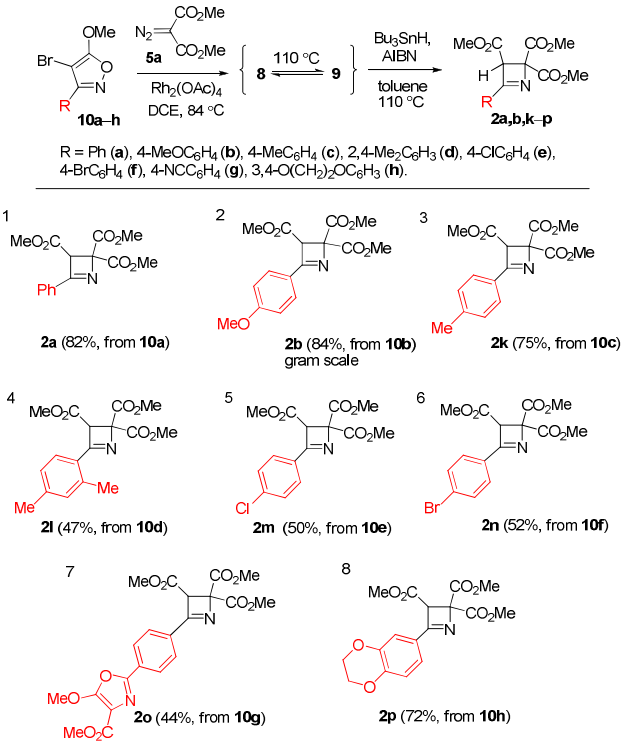


[a] Reaction conditions. Stage 1: addition of $Rh_2(OAc)_4$ (2 mol %) to solution of **7** (0.5 mmol) and **5** (0.6–1.1 mmol) in DCE (1.5 mL); Stage 2: Bu_3SnH (1 mmol), AIBN (0.02 mmol), toluene (2 mL). [b] The yields of **8d,i,j** are 74, 67 and 62%, respectively (see ESI). [c] (2*RS*,3*RS*)/(2*RS*,3*SR*)-ratio.

All synthesized dihydroazetes **2** are stable up to about 150 °C and do not undergo hydrolytic ring-opening during chromatography on silica gel. Electron-donating aryl and hetaryl substituents (entries 1–5) tolerate the reaction conditions, providing good yields of dihydroazetes **2a–e**. Functional groups such as CF_3 and $P(O)(OMe)_2$ can be introduced into the dihydroazete by use of diazo compounds **5b,c** (entries 6–8). Ethyl diazoacetoacetate **5d** is also a suitable substrate for the reaction, although it provides lower yields of dihydroazetes **2i,j** (entries 9, 10) probably due to side reactions of enolizable 4-bromo-2-azadienes **8i,j**.[18] The diastereoselectivity of the formation of **2f–j** predictably increases with the increase of the steric volume of R^2 substituent: COMe < CF_3 < $P(O)(OMe)_2$.

A limitation of the method that did appear is the inaccessibility of bromoazirines **7** with electron-withdrawing substituents in benzene ring, such as halogen atoms. In search for an alternative approach to dihydroazetes with electron-poor aryl substituents at C^4 we selected 4-bromo-5-methoxyisoxazoles **10** as potential synthetic equivalents of the corresponding 2-bromoazirines (Table 2). The formation/generation of 2-azabuta-1,3-dienes by reactions of non-halogenated isoxazoles with Rh(II)-carbenoids derived from diazo compounds[17,19] or 1-sulfonyl-1,2,3-triazoles[20] are known. The use of 4-halogeno-substituted 5-alkoxyisoxazoles in such reactions is limited by a reaction of 4-iodo-5-methoxy-3-phenylisoxazole.[18] 4-Bromoisoxazoles **10a–h** were synthesized in three steps from β-ketoesters and hydroxylamine in good yields of the products at each stage (ESI). The reaction of **10a** with **5a** in the presence of $Rh_2(OAc)_4$ followed by the hydrodebromination of the equilibrium mixture **8a**⇄**9a** with Bu_3SnH/AIBN gave practically the same yield of dihydroazete **2a** as the "azirine method" (Scheme 5, entry 1).

Table 2 Synthesis of dihydroazetes **2a,b,k–p** from isoxazoles **10a–h**[a]



[a] For reaction conditions see footnote to Table 1.

To test the practicality of this method a gram-scale reaction of 1.56 g of **10b** and 1.3 g of **5a** was carried out. Dihydroazete **2b** in this case was isolated in 84% yield, highlighting the synthetic utility of the method (entry 2). An *ortho*-substituent in the aryl

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group also tolerates the reaction conditions (entry 4). *p*-Chloro- and *p*-bromophenyl-substituted dihydroazetes were also synthesized but in somewhat lower yields (entries 5, 6). The reaction of the *p*-cyanophenyl-substituted isoxazole **10g** proceeds via a Rh(II)-carbenoid-induced transformation of the cyano-group to oxazole moiety, affording dihydroazete **2o** in 44% yield (entry 7).

The structures of compounds **2a–p** were verified by ^{1}H, ^{13}C NMR spectra, and HRMS. Structures of compounds **2a,h** were confirmed by X-ray analysis, compound **2f** – by 2D ^{1}H-^{19}F HOESY experiment (ESI).

Our studies on the anti-cancer activity of the synthesized 2,3-dihydroazetes were focused on their pro-apoptotic potential, because it is well-recognized that the failure of many anti-cancer chemotherapeutics arises from their inability to induce apoptosis at a cellular level.[21]

A cell suspension culture of THP-1 (human monocytic leukemia cells) was selected as the object for the biological studies. DNA binding dyes, YO-PRO-1 and propidium iodide, were used for the assessment of the cell viability.[22] Testing the panel of 2,3-dihydroazetes within the range of 10^{-5} to 2×10^{-4} M, we have found that they significantly differ in their ability not only to induce the cell death, but also in the way they do so, by inducing predominantly apoptosis or necrosis. To adequately describe and quantitatively assess their apoptotic and/or necrotic potential, on one hand, and their general cytotoxic potential, on the other hand, we took the difference between the two areas under the curves, apoptotic and necrotic, within the concentration range (apoptotic/necrotic difference, AND). The sense of the values obtained should not be mixed with or reduced to that of "apoptotic index" widely used for years to describe the cell death observed by various techniques.[23] According to the obtained results, dihydroazete **2a** exhibited the maximal AND on the THP-1 cell line (Figures 1, 2a); compounds **2m,n,k** (Figures 2b,c,d) showed a lower AND due to higher necrotic potential at nearly the same cytotoxicity as **2a**; dihydroazetes **2b,e** (Figures 2e,f) and **9a** (Figure 2g) showed a lower AND that significantly differ from **2a** due to higher necrotic potential and/or lower cytotoxicity; dihydroazete **2h** (Figure 2h) had both a very low AND value and a very low cytotoxicity, and dihydroazetes **9c,g** (Figures 2i,j) exhibited a negative AND due to predominantly necrotic cell death at a moderate to high level of cytotoxicity.

Many cancers can develop pro-survival adaptations and anti-apoptotic signaling pathways, and many efforts of the synthetic chemists have been made to overcome the challenge of drug resistance. To this end, it is worth mentioning a recent attempt to develop prodrugs that induce targeted necrosis as a novel strategy to circumvent apoptosis-resistance.[24] We don't share this approach because hold the opinion that necrosis should be the natural outcome of primary and fully developed apoptosis, in order to maximally avoid side effects with healthy tissues at the stage of preclinical and clinical studies. So this row of 2,3-dihydroazetes seems to be of great interest for molecular oncologists to conducting cytophysiological research with different cancer cell lines and identifying death-effector molecules. Of special importance would be additional research with normal cell lines (e.g. endothelial cells, fibroblasts, etc.) to find out if the AND is of the same level or principally different from that of malignant cells. The next stage of research is supposed to be *in vivo* experiments within the frame of preclinical studies.

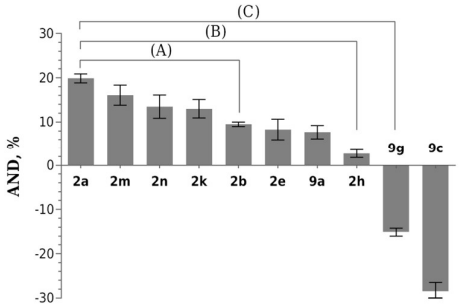
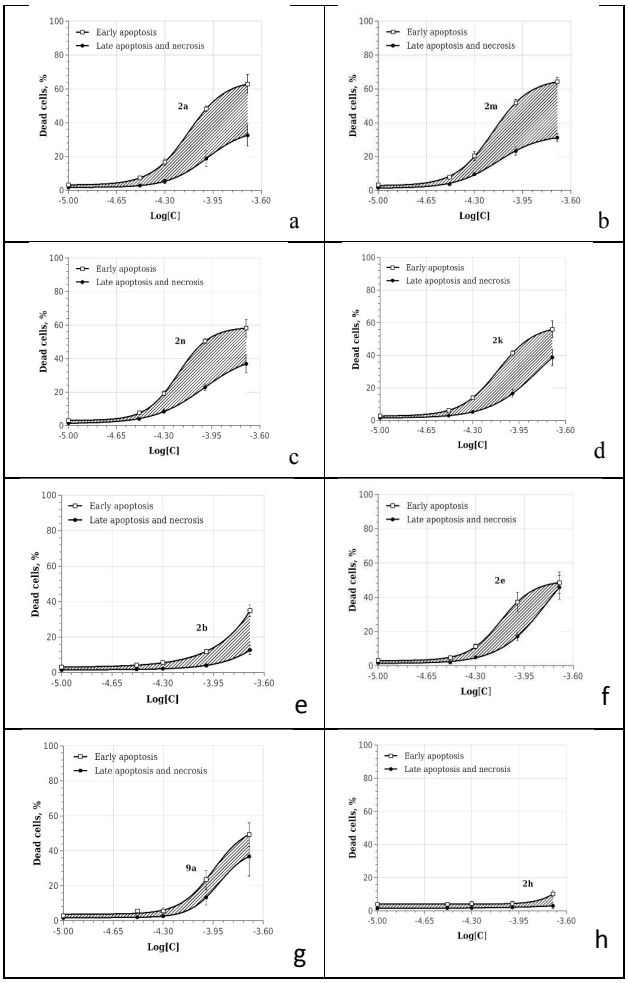


Figure 1 The apoptotic/necrotic difference (AND) of the dihydroazetes **2a,b,e,h,k,m,n** and **9a,c,g** exhibited on the THP-1 cell line ((A) $p<0.05$; (B) $p<0.01$; (C) $p<0.001$).



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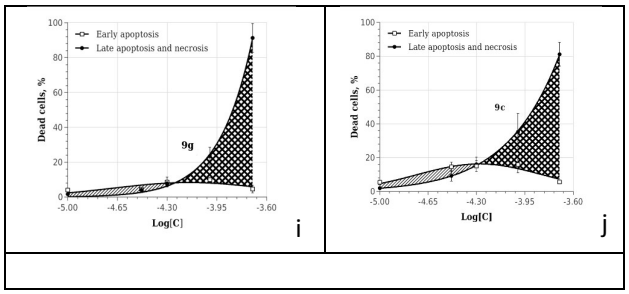

Figure 2 Concentration dependency (C = mol×L^{-1}) of apoptosis and necrosis induction. Percentage of dead cells as determined by DNA binding dyes YO-PRO-1 and propidium iodide via flow cytometry after treatment of THP-1 cells for 24 h with different concentrations of 2,3-dihydroazetes **2a,b,e,h,k,m,n** and **9a,c,g**.

Conclusions

In conclusion, we have shown that thermally and hydrolytically stable 2,3-dihydroazete-2,3-di-/2,2,3-tricarboxylates can be effectively synthesized from both 2-bromoazirines and 4-bromoisoxazoles using two-step "4-bromo-2-azadiene formation/hydrodebromination" procedure. The reduction of 3-bromo-2,3-dihydroazetes from an equilibrium mixture with 4-bromo-2-azadienes is the first example of the selective hydrodehalogenation of a valence isomer under equilibrium conditions. In vitro cytotoxicity tests on THP-1 cell line revealed that the 2,3-dihydroazete-2,3-dicarboxylates **2** greatly differ in their ability to induce apoptosis and/or necrosis. To adequately describe and quantitatively assess apoptosis/necrosis potential, the difference between the two areas under the curves of concentration dependency of apoptosis/necrosis induction within the concentration range is expedient to use. Trimethyl 4-phenyl-2,3-dihydroazete-2,2,3-tricarboxylate **2a** has got the maximal apoptotic potential coupled with high cytotoxic and minimal necrotic potential, being a good candidate for further studies of its anti-cancer activities.

Experimental

General methods

Melting points were determined on a melting point apparatus SMP30 and are uncorrected. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker AVANCE 400 spectrometer in $CDCl_3$. Electrospray ionization (ESI) mass spectra were measured on a Bruker MaXis mass spectrometer. Single crystal X-ray data were collected by means of Agilent Technologies «Supernova» and «Xcalibur» diffractometers. Crystallographic data for the structures **2a,h** have been deposited with the Cambridge Crystallographic Data Centre. Thin-layer chromatography (TLC) was conducted on aluminum sheets precoated with SiO_2 ALUGRAM SIL G/UV254. Toluene was distilled over sodium wire. 1,2-Dichloroethane was washed with concentrated H_2SO_4, water, then distilled from P_2O_5 and stored over anhydrous K_2CO_3. Commercially obtained *N*-bromosuccinimide and tributylstannane were used as received. 2*H*-Azirines **3e,**[25] **7a,**[26] **7b–d**[18] were prepared by the reported procedures.

General procedure for preparation of dihydroazetes 2a–p

$Rh_2(OAc)_4$ (2 mol % on azirine/isoxazole) was added to a refluxing solution of azirine **7a–e** (0.5 mmol) or isoxazole **10a–h** (0.5 mmol) and diazo compound **5a–d** (in amounts indicated below) in anhydrous DCE (1.5 mL) under argon. The stirred mixture was heated under reflux until nitrogen evolution stopped (10 min for **5a**, 10 s for **5b**, 50 min for **5c**, and 30 s for **5d**). The solvent was evaporated under reduced pressure, and the residue was filtered through a pad of silica gel using hexane/EtOAc mixture as eluent (pure azadiene **8d,i,j** were isolated by column chromatography, benzene/EtOAc mixture as eluent, 100:1 to 10:1). The filtrate was concentrated under reduced pressure and the residue (or pure azadiene **8d,i,j**) was dissolved in anhydrous toluene (2 mL) and refluxed for 45 min under stirring (10 min for **8i,j**). Azobisisobutyronitrile (AIBN) (0.02 mmol) was added to the resulting refluxing equilibrium mixture **8a–p**/**9a–p** and then a solution of Bu_3SnH (1 mmol, 2 equiv) in anhydrous toluene (3 mL) was added dropwise over 40 min. The reaction mixture was treated with saturated NaF solution (15 mL) and the aqueous layer was extracted with dichloromethane (2×5 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel to give dihydroazete **2a–p**.

Trimethyl 4-phenyl-2,3-dihydroazete-2,2,3-tricarboxylate (2a). Dihydroazete **2a** (119 mg, 78%) was prepared according to the general procedure from azirine **7a** (127 mg, 0.5 mmol) and diazo compound **5a** (119 mg, 0.75 mmol) using hexane/EtOAc mixture (from 5:1 to 1:1) as eluent for chromatography. Colorless solid, mp 99–101 °C (Et_2O–hexane). δ_H (400 MHz; $CDCl_3$; Me_4Si) 3.76 (3 H, s), 3.866 (3 H, s), 3.874 (3 H, s), 5.02 (1 H, s), 7.47 (2 H, t, *J* 7.6 Hz), 7.57 (1 H, t, *J* 7.6 Hz) 7.82 (2 H, d, *J* 7.6 Hz). δ_C (100 MHz, $CDCl_3$, Me_4Si) 52.7, 53.2, 53.60, 53.64, 72.0, 126.5, 128.7, 130.3, 133.0, 166.4, 166.5, 166.8, 186.3. HRMS (ESI-TOF): $[M + Na]^+$ calcd for $C_{15}H_{15}NNaO_6^+$ 328.0792, found 328.0793. Crystal data (CCDC-1449965): $C_{15}H_{15}NO_6$, *M* = 305.28, monoclinic, space group *P* 1 21/n 1, *a* = 9.47048(18), *b* = 8.60587(17), *c* = 18.2016(4) Å, β = 98.1319(17)°, *V* = 1468.55(5) Å^3, Z = 4, *T* = 100 K, *d* = 1.381 mg mm^{-3}, F(000) = 640, μ = 1.5418 mm^{-1}. 8087 reflections measured, 2800 unique (*R*int = 0.0169) were used in all calculations. The final R_1 was 0.0322 (2555>2σ(I)) and wR_2 was 0.0827 (all data). Compound **2a** was also obtained in 82% yield from isoxazole **10a**.

Trimethyl 4-(4-methoxyphenyl)-2,3-dihydroazete-2,2,3-tricarboxylate (2b). Dihydroazete **2b** (142 mg, 85%) was prepared according to the general procedure from azirine **7b** (142 mg, 0.5 mmol) and diazo compound **5a** (119 mg, 0.75 mmol) using hexane/EtOAc mixture (from 5:1 to 1:1) as eluent for chromatography. Colorless solid, m.p. 93–95 °C (Et_2O/hexane). δ_H (400 MHz; $CDCl_3$; Me_4Si) 3.74 (3 H, s), 3.85 (3 H, s), 3.86 (6 H, s), 4.98 (1 H, s), 6.95 (2 H, d, *J* 8.8 Hz), 7.76 (2 H, d, *J* 8.8 Hz). δ_C (100 MHz, $CDCl_3$, Me_4Si) 52.7, 53.1, 53.5,

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55.4, 71.7, 114.1, 123.1, 128.5, 163.4, 166.5, 166.8, 167.1, 185.1. HRMS (ESI-TOF): $[M + H]^+$ calcd for $C_{16}H_{18}NO_7^+$ 336.1078, found 336.1079.

Gram-scale synthesis of dihydroazete 2b from isoxazole 10b. To a refluxing solution of isoxazole **10b** (1.56 g, 5.5 mmol) and dimethyl diazomalonate **5a** (1.3 g, 8.25 mmol) in anhydrous DCE (12 mL) $Rh_2(OAc)_4$ (49 mg, 2 mol %) was added under argon. The stirred mixture was refluxed until nitrogen evolution stopped (10 min). The solvent was evaporated under reduced pressure and the residue was filtered through a pad of silica gel using hexane/EtOAc 2:1 mixture as eluent. The filtrate was concentrated under reduced pressure, and the residue was dissolved in anhydrous toluene (20 mL) and refluxed for 45 min under stirring. AIBN (0.035 g, 0.22 mmol) was added to the resulting refluxing mixture and then a solution of Bu_3SnH (3.2 g, 11 mmol) in anhydrous toluene (30 mL) was added dropwise over 40 min. The reaction mixture was treated with saturated NaF solution (150 mL) and the aqueous layer was extracted with dichloromethane (2×25 mL). The combined organic layers were dried over Na_2SO4 and concentrated under reduced pressure. The residue was filtered through a pad of silica gel using hexane–EtOAc 2:1 mixture as eluent. The filtrate was concentrated under reduced pressure, the residue was washed twice with a cold Et_2O/hexane (1:2) mixture to afford pure **2b** (1.56 g, 84%).

Trimethyl 4-(2-thienyl)-2,3-dihydroazete-2,2,3-tricarboxylate (2c). Dihydroazete **2c** (124 mg, 80%) was prepared according to the general procedure from azirine **7c** (130 mg, 0.5 mmol) and diazo compound **5a** (119 mg, 0.75 mmol) using hexane/EtOAc mixture (from 5:1 to 1:1) as eluent for chromatography. Colorless solid, m.p. 88–89 °C (Et_2O–hexane). δ_H (400 MHz; $CDCl_3$; Me_4Si) 3.78 (3 H, s), 3.86 (3 H, s), 3.88 (3 H, s), 5.02 (1 H, s), 7.15 (1 H, dd, *J* 4.9, 3.8 Hz), 7.60 (1 H, dd, *J* 3.8, 1.0 Hz), 7.64 (1 H, dd, *J* 4.9,1.0 Hz). δ_C (100 MHz, $CDCl_3$, Me_4Si) 52.8, 53.2, 53.6, 54.4, 73.1, 128.0, 132.4, 132.5, 133.9, 165.9, 166.5, 166.9, 180.3. HRMS (ESI-TOF): $[M + H]^+$ calcd for $C_{13}H_{14}NO_6S^+$ 312.0536, found 312.0542.

Trimethyl 4-(furan-2-yl)-2,3-dihydroazete-2,2,3-tricarboxylate (2d). Azadiene **8d** was synthesized from azirine **7d** and diazo compound **5a** according to the known procedure in 74% yield.[18] Dihydroazete **2d** (62 mg, 42%) was prepared according to the general procedure from azadiene **8d** (187 mg, 0.5 mmol) using hexane–EtOAc mixture (from 5:1 to 1:1) as eluent for chromatography. Compound **2d**. Colorless solid, mp 86–87 °C (Et_2O–hexane). δ_H (400 MHz; $CDCl_3$; Me_4Si) 3.77 (3 H, s), 3.86 (3 H, s), 3.87 (3 H, s), 4.94 (1 H, s), 6.57 (1 H, dd, *J* 3.5, 1.7 Hz), 7.14 (1 H, d, *J* 3.5 Hz), 7.64 (1 H, d, *J* 1.7 Hz). δ_C (100 MHz, $CDCl_3$, Me_4Si) 52.7, 53.2, 53.5, 53.6, 73.9, 112.4, 117.1, 145.7, 146.9, 165.8, 166.4, 166.7, 175.9. HRMS (ESI-TOF): $[M + H]^+$ calcd for $C_{13}H_{14}NO_7^+$ 296.0765, found 296.0778.

Trimethyl 4-(naphthalen-2-yl)-2,3-dihydroazete-2,2,3-tricarboxylate (2e). Dihydroazete **2e** (117 mg, 66%) was prepared according to the general procedure from azirine **7e** (152 mg, 0.5 mmol) and diazo compound **5a** (119 mg, 0.75 mmol) using hexane/EtOAc mixture (from 3:1 to 1:1) as eluent for chromatography. Colorless solid, mp 123–125 °C (Et_2O–hexane). δ_H (400 MHz; $CDCl_3$; Me_4Si) 3.79 (3 H, s), 3.89 (3 H, s), 3.90 (3 H, s), 5.15 (1 H, s), 7.52–7.63 (2 H, m), 7.85–7.95 (3 H, m), 7.96–8.02 (1 H, m), 8.21 (1 H, br. s). δ_C (100 MHz, $CDCl_3$, Me_4Si) 52.8, 53.2, 53.6, 53.8, 72.2, 121.6, 126.9, 127.8, 127.9, 128.4, 128.5, 128.7, 129.1, 132.5, 135.5, 166.4, 166.6, 166.9, 186.3. HRMS (ESI-TOF): $[M + Ag]^+$ calcd for $C_{19}H_{17}AgNO_6^+$ 462.0101, found 462.0123.

2-Ethyl 3-methyl 4-phenyl-2-(trifluoromethyl)-2,3-dihydroazete-2,3-dicarboxylate (2f). Dihydroazete **2f** (92 mg, 56%) was prepared as non-separated 12:1 mixture of two diastereomers according to the general procedure from azirine **7a** (127 mg, 0.5 mmol) and diazo compound **5b** (109 mg, 0.6 mmol) using hexane/EtOAc mixture (from 8:1 to 2:1) as eluent for chromatography. Compound **2f** (12:1 mixture). Colorless oil; HRMS (ESI-TOF): $[M + H]^+$, found 330.0956; $C_{15}H_{15}F_3NO_4^+$ requires 330.0948.

*Compound (2RS,3RS)-**2f** (major isomer)*. δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.34 (3 H, t, *J* 7.2 Hz), 3.78 (3 H, s), 4.36 (2 H, q, *J* 7.2 Hz), 4.68 (1 H, s), 7.48–7.52 (2 H, m), 7.58–7.64 (1 H, m) 7.82–7.87 (2 H, m). δ_C (100 MHz, $CDCl_3$, Me_4Si) 13.9, 51.5 (q, *J* 1.5 Hz), 52.8, 62.9, 69.7 (q, *J* 30.7 Hz), 122.5 (q, *J* 282.3 Hz), 126.7, 128.8, 129.9, 133.4, 163.9, 165.5, 187.0. δ_F (376 MHz, $CDCl_3$; $CFCl_3$): -75.10.

*Compound (2RS,3SR)-**2f** (minor isomer)*. δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.38 (3 H, t, *J* 7.2 Hz), 3.81 (3 H, s), 5.04 (1 H, s), 7.87–7.92 (2 H, m), (other signals are overlapped). δ_C (100 MHz, $CDCl_3$, Me_4Si) 52.7, 63.3, 127.3, 128.6, 130.1, 133.3, 165.0, 188.7 (signals of five carbons are overlapped). δ_F (376 MHz, $CDCl_3$; $CFCl_3$): -71.45.

2-Ethyl 3-methyl 4-(4-methoxyphenyl)-2-(trifluoromethyl)-2,3-dihydroazete-2,3-dicarboxylate (2g). Dihydroazete **2g** (129 mg, 72%) was prepared as non-separated 7:1 mixture of two diastereomers according to the general procedure from azirine **7b** (142 mg, 0.5 mmol) and diazo compound **5b** (109 mg, 0.6 mmol) using hexane/EtOAc mixture (from 8:1 to 2:1) as eluent for chromatography.

*Compound **2g** (7:1 mixture)*. Colorless oil. HRMS (ESI-TOF): $[M + H]^+$ calcd for $C_{16}H_{17}F_3NO_5^+$ 360.1053, found 360.1055.

*Compound (2RS,3RS)-**2g** (major isomer)*. δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.30 (3 H, t, *J* 7.1 Hz), 3.74 (3 H, s), 3.84 (3 H, s), 4.31 (2 H, q, *J* 7.1 Hz), 4.63 (1 H, s), 6.91–7.00 (2 H, m,), 7.74–7.80 (2 H, m,). δ_C (100 MHz, $CDCl_3$, Me_4Si) 13.7, 51.3 (q, *J* 1.5 Hz), 52.6, 55.4, 62.7, 69.4 (q, *J* 30.5 Hz), 114.2, 122.5, 122.6 (q, *J* 282.3 Hz), 128.7, 163.74, 164.1, 165.6, 185.8.

*Compound (2RS,3SR)-**2g** (minor isomer)*. δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.34 (3 H, t, *J* 7.2 Hz), 3.77 (3 H, s), 5.00 (1 H, s), 7.81–7.85 (2 H, m), (other signals are overlapped). δ_C (100 MHz, $CDCl_3$, Me_4Si) 52.5, 63.1, 72.0 (q, *J* 30.5 Hz), 114.0, 122.5 (q, *J* 282.2 Hz), 122.7, 129.3, 163.67, 164.9, 165.1, 187.4 (signals of three carbons are overlapped).

Dimethyl (2*RS*,3*SR*)-2-(dimethoxyphosphoryl)-4-(4-methoxyphenyl)-2,3-dihydroazete-2,3-dicarboxylate (2h). Dihydroazete **2h** (127 mg, 66%) was prepared according to the general procedure from azirine **7b** (142 mg, 0.5 mmol) and diazo compound **5c** (218 mg, 1.05 mmol) using hexane/EtOAc mixture (from 3:1 to 100% EtOAc) as eluent for chromatography. Colorless solid, mp 126–128 °C

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(Et_2O–hexane). δ_H (400 MHz; $CDCl_3$; Me_4Si) 3.72 (3 H, s), 3.80–3.98 (12 H, m), 4.85 (1 H, d, *J* 7.0 Hz), 6.92 (2 H, d, *J* 8.4 Hz), 7.68 (2 H, d, *J* 8.4 Hz). δ_C (100 MHz, $CDCl_3$, Me_4Si) 52.7, 53.0, 53.8 (d, *J* 3.3 Hz), 54.5 (d, *J* 6.6 Hz), 54.6 (d, *J* 6.6 Hz), 55.3, 69.3 (d, *J* 150.4 Hz), 114.1, 123.1, 128.0, 163.3, 166.5 (d, *J* 2.3 Hz), 166.7 (d, *J* 2.8 Hz), 184.0 (d, *J* 12.1 Hz). HRMS (ESI-TOF): $[M + Ag]^+$ calcd for $C_{16}H_{20}AgNO_8P^+$ 491.9972, found 491.9972. Crystal data (CCDC-1449943): $C_{16}H_{20}NO_8P$, *M* = 385.30, triclinic, space group *P*-1, *a* = 8.8135(7), *b* = 9.3935(9), *c* = 11.2015(10) Å, α = 78.735(8), β = 81.657(7), γ = 85.426(7)°, *V* = 898.60(14) $Å^3$, Z = 2, *T* = 100 K, *d* = 1.424 mg mm^{-3}, F(000) = 404, μ = 0.7107 mm^{-1}, 8555 reflections measured, 4127 unique (*R*int = 0.0267) were used in all calculations. The final R_1 was 0.0377 (3510>2σ(I)) and wR_2 was 0.0982 (all data).

2-Ethyl 3-methyl 2-acetyl-4-phenyl-2,3-dihydroazete-2,3-dicarboxylate (2i). Azadiene **8i** was prepared from azirine **7a** and diazo compound **5d** according to the known procedure in 67% yield.[18] Dihydroazete **2i** (56 mg, 37%) was prepared according to the general procedure from azadiene **8i** (191 mg, 0.5 mmol) using hexane/EtOAc mixture (from 5:1 to 1:1) as eluent for chromatography. Compound **2i** (non-separated mixture of two diastereomers 1:0.8). Colorless oil. δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.33 (5.4 H, t, *J* 7.2 Hz), 2.47 (3 H, s), 2.51 (2.4 H, s), 3.75 (5.4 H, s), 4.28–4.37 (3.6 H, m), 4.97 (0.8 H, s), 4.99 (1 H, s), 7.44–7.86 (9 H, m). δ_C (100 MHz, $CDCl_3$, Me_4Si) 13.9, 14.0, 25.7, 28.2, 52.1, 52.5, 52.7, 54.1, 62.5, 62.9, 76.4, 77.7, 126.1, 126.6, 128.7, 128.9, 130.4, 130.6, 132.9, 133.0, 166.4, 166.6, 166.7, 167.1, 186.4, 186.5, 199.2, 202.7. HRMS (ESI-TOF): $[M + Na]^+$ calcd for $C_{16}H_{17}NNaO_5^+$ 326.0999, found 326.0993.

Ethyl 2-[(*E*)-2-bromo-3-methoxy-1-(4-methoxyphenyl)-3-oxoprop-1-enylimino]-3-oxobutanoate (8j). A solution of azirine **7b** (250 mg, 0.88 mmol) and diazo compound **5d** (440 mg, 2.82 mmol) in anhydrous 1,2-dichloroethane (3 mL) was heated to reflux under an argon atmosphere and then $Rh_2(OAc)_4$ (8 mg, 2 mol%) was added in one portion. The mixture was stirred under reflux until nitrogen evolution stopped (ca. 30 s). The resulting mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel using benzene–EtOAc mixture (from 100:1 to 30:1) to give pure **8j** (225 mg, 62%). Orange oil. δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.29 (3 H, t, *J* 7.1 Hz), 2.54 (3 H, s), 3.79 (3 H, s), 3.84 (3 H, s), 4.28 (2 H, q, *J* 7.1 Hz), 6.91–6.97 (2 H, m), 7.49 (2 H, d, *J* 8.8 Hz). δ_C (100 MHz, $CDCl_3$, Me_4Si) 13.9, 25.6, 52.9, 55.2, 62.4, 93.8, 113.6, 127.9, 130.2, 154.2, 156.3, 160.68, 160.74, 163.3, 195.6. HRMS (ESI-TOF): $[M + Na]^+$ calcd for $C_{17}H_{18}BrNaNO_6^+$ 434.0210, found 434.0218.

2-Ethyl 3-methyl 2-acetyl-4-(4-methoxyphenyl)-2,3-dihydroazete-2,3-dicarboxylate (2j). Dihydroazete **2j** (75 mg, 45%) was prepared according to the general procedure from azadiene **8j** (206 mg, 0.5 mmol) using hexane/EtOAc mixture (from 5:1 to 1:1) as eluent for chromatography. Compound **2j** (non-separated mixture of two diastereomers 1.1:1). Colorless oil. δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.33 (6 H, t, *J* 7.1 Hz), 2.46 (3 H, s), 2.50 (3 H, s), 3.742 (3 H, s), 3.745 (3 H, s), 3.88 (6 H, s), 4.21–4.39 (4 H, m), 4.95 (1 H, s), 4.96 (1 H, s), 6.90–7.05 (4 H, m), 7.70–7.73 (2 H, m), 7.76–7.80 (2 H, m). δ_C (100 MHz, $CDCl_3$, Me_4Si) 13.9, 14.0, 25.7, 28.2, 52.0, 52.5, 52.7, 54.0, 55.45, 55.48 62.4, 62.8, 76.1, 77.4, 114.2, 114.3, 123.2, 123.4, 128.2, 128.7, 163.4, 163.5, 166.7, 166.8, 167.0, 167.3, 185.3, 185.4, 199.6, 203.1. HRMS (ESI-TOF): $[M + Na]^+$ calcd for $C_{17}H_{19}NNaO_6^+$ 356.1105, found 356.1103.

Trimethyl 4-(4-methylphenyl)-2,3-dihydroazete-2,2,3-tricarboxylate (2k). Dihydroazete **2k** (120 mg, 75%) was prepared according to the general procedure from isoxazole **10c** (134 mg, 0.5 mmol) and diazo compound **5a** (119 mg, 0.75 mmol) using hexane/EtOAc mixture (from 5:1 to 1:1) as eluent for chromatography. Colorless solid, mp 94–95 °C (Et_2O–hexane). δ_H (400 MHz; $CDCl_3$; Me_4Si) 2.42 (3 H, s), 3.75 (3 H, s), 3.86 (3 H, s), 3.87 (3 H, s), 5.00 (1 H, s), 7.27 (2 H, d, *J* 8.4 Hz), 7.71 (2 H, d, *J* 8.4 Hz). δ_C (100 MHz, $CDCl_3$, Me_4Si) 21.7, 52.7, 53.1, 53.56, 53.58, 71.9, 126.5, 127.7, 129.4, 143.9, 166.4, 166.7, 166.9, 186.0. HRMS (ESI-TOF): $[M + Ag]^+$ calcd for $C_{16}H_{17}AgNO_6^+$ 426.0101, found 426.0120.

Trimethyl 4-(2,4-dimethylphenyl)-2,3-dihydroazete-2,2,3-tricarboxylate (2l). Dihydroazete **2l** (78 mg, 47%) was prepared according to the general procedure from isoxazole **10d** (141 mg, 0.5 mmol) and diazo compound **5a** (119 mg, 0.75 mmol) using hexane/EtOAc/$CHCl_3$ mixture (from 10:4:1 to 4:4:1) as eluent for chromatography. Colorless solid, mp 68–70 °C (Et_2O–hexane). δ_H (400 MHz; $CDCl_3$; Me_4Si) 2.37 (3 H, s), 2.64 (3 H, s), 3.73 (3 H, s), 3.87 (6 H, s), 5.07 (1 H, s), 7.02–7.13 (2 H, m), 7.34 (1 H, d, *J* 7.6 Hz). δ_C (100 MHz, $CDCl_3$, Me_4Si) 21.5, 21.7, 52.7, 53.1, 53.6, 55.1, 71.8, 126.2, 126.5, 129.1, 132.5, 139.7, 142.9, 166.8 (2 C), 167.0, 187.2. HRMS (ESI-TOF): $[M + Ag]^+$ calcd for $C_{17}H_{19}AgNO_6^+$ 440.0258, found 440.0250.

Trimethyl 4-(4-chlorophenyl)-2,3-dihydroazete-2,2,3-tricarboxylate (2m). Dihydroazete **2m** (85 mg, 50%) was prepared according to the general procedure from isoxazole **10e** (144 mg, 0.5 mmol) and diazo compound **5a** (174 mg, 1.1 mmol) using hexane/EtOAc/$CHCl_3$ mixture (from 10:4:1 to 4:4:1) as eluent for chromatography. Colorless solid, mp 74–75 °C (Et_2O–hexane). δ_H (400 MHz; $CDCl_3$; Me_4Si) 3.76 (3 H, s), 3.86 (3 H, s), 3.88 (3 H, s), 4.99 (1 H, s), 7.45 (2 H, d, *J* 8.5 Hz), 7.77 (2 H, d, *J* 8.5 Hz). δ_C (100 MHz, $CDCl_3$, Me_4Si) 52.8, 53.2, 53.60, 53.64, 72.3, 127.9, 128.7, 129.1, 139.4, 166.1, 166.3, 166.7, 185.4. HRMS (ESI-TOF): $[M + Ag]^+$ calcd for $C_{15}H_{14}AgClNO_6^+$ 445.9555, found 445.9574.

Trimethyl 4-(4-bromophenyl)-2,3-dihydroazete-2,2,3-tricarboxylate (2n). Dihydroazete **2n** (100 mg, 52%) was prepared according to the general procedure from isoxazole **10f** (167 mg, 0.5 mmol) and diazo compound **5a** (174 mg, 1.1 mmol) using hexane–EtOAc–$CHCl_3$ mixture (from 10:4:1 to 4:4:1) as eluent for chromatography. Colorless solid, mp 66–67 °C (Et_2O–hexane). δ_H (400 MHz; $CDCl_3$; Me_4Si) 3.76 (3 H, s), 3.86 (3 H, s), 3.88 (3 H, s), 4.99 (1 H, s), 7.62 (2 H, d, *J* 8.5 Hz), 7.70 (2 H, d, *J* 8.5 Hz). δ_C (100 MHz, $CDCl_3$, Me_4Si) 52.8, 53.3, 53.6, 53.7, 72.3, 128.0, 128.0, 129.1, 132.1, 166.1, 166.3, 166.6, 185.6. HRMS (ESI-TOF): $[M + Ag]^+$ calcd for $C_{15}H_{14}AgBrNO_6^+$ 489.9050, found 489.9068.

Trimethyl 4-{4-[5-methoxy-4-(methoxycarbonyl)oxazol-2-yl]phenyl}-2,3-dihydroazete-2,2,3-tricarboxylate (2o). Dihydroazete **2o** (102 mg, 44%) was prepared according to the general procedure from isoxazole **10g** (140 mg, 0.5 mmol) and

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diazo compound **5a** (363 mg, 2.3 mmol) using hexane/EtOAc mixture (from 3:1 to 100% EtOAc) as eluent for chromatography. Colorless solid, mp 87–96 °C, 140–143 °C (Et_2O–hexane, dec.). δ_H (400 MHz; $CDCl_3$; Me_4Si) 3.76 (3 H, s), 3.86 (3 H, s), 3.87 (3 H, s), 3.92 (3 H, s), 4.29 (3 H, s), 5.02 (1 H, s), 7.90 (2 H, d, *J* 8.4 Hz), 8.07 (2 H, d, *J* 8.4 Hz). δ_C (100 MHz, $CDCl_3$, Me_4Si) 51.9, 52.8, 53.2, 53.7 (2 C), 59.9, 72.4, 108.1, 125.9, 127.0, 130.1, 131.6, 149.6, 161.6, 162.0, 166.1, 166.3, 166.6, 185.7. HRMS (ESI-TOF): $[M + Ag]^+$ calcd for $C_{21}H_{20}AgN_2O_{10}^+$ 567.0163, found 567.0188.

Trimethyl 4-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2,3-dihydroazete-2,2,3-tricarboxylate (2p). Dihydroazete **2p** (131 mg, 72%) was prepared according to the general procedure from isoxazole **10h** (156 mg, 0.5 mmol) and diazo compound **5a** (119 mg, 0.75 mmol) using hexane/EtOAc mixture (from 5:1 to 1:1) as eluent for chromatography. Colorless solid, mp 88–90 °C (Et_2O–hexane). δ_H (400 MHz; $CDCl_3$; Me_4Si) 3.75 (3 H, s), 3.85 (3 H, s), 3.86 (3 H, s), 4.25–4.32 (4 H, m), 4.95 (1 H, s), 6.92 (1 H, d, *J* 8.4 Hz), 7.28–7.35 (2 H, m). δ_C (100 MHz, $CDCl_3$, Me_4Si) 52.7, 53.1, 53.51, 53.54, 64.0, 64.6, 71.6, 115.7, 117.6, 120.6, 123.8, 143.7, 148.0, 166.5, 166.7, 167.0, 185.1. HRMS (ESI-TOF): $[M + Ag]^+$ calcd for $C_{17}H_{17}AgNO_8^+$ 470.0000, found 470.0004.

Dimethyl (*E*)- and (*Z*)-2-(3-ethoxy-3-oxo-1-phenylprop-1-enylimino)malonates (*E*-6 and *Z*-6). $Rh_2(OAc)_4$ (5 mg, 1 mol % on azirine) was added to a refluxing solution of azirine **3e** (189 mg, 1 mmol) and diazo compound **5a** (237 mg, 1.5 mmol) in anhydrous DCE under stirring. The mixture was stirred under reflux until nitrogen evolution stopped (ca. 10 min). The solvent was removed under reduced pressure and the residue was purified by column chromatography (Et_2O–hexane, from 1:25 to 1:7) to give compounds *E*-**6** (140 mg, 44%), *Z*-**6** (41 mg, 13%) and non-separated mixture of *E*-**6**/*Z*-**6** (1:4.4, 77 mg, 24%).

*Compound E-**6***. Yellow oil. δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.13 (3 H, t, *J* 7.1 Hz), 3.91 (6 H, br. s), 4.06 (2 H, q, *J* 7.1 Hz), 5.34 (1 H, s), 7.35–7.44 (3 H, m), 7.46–7.52 (2 H, m). δ_C (100 MHz, $CDCl_3$, Me_4Si) 13.8, 52.8 (br. s), 53.5 (br. s), 60.1, 102.5, 127.8, 128.5, 129.7, 132.8, 149.0, 160.8 (2C), 161.3, 164.9. HRMS (ESI-TOF): $[M + Na]^+$, found 342.0950; $C_{16}H_{17}NNaO_6^+$ requires 342.0948.

*Compound Z-**6***. Yellow oil. δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.28 (3 H, t, *J* 7.1 Hz), 3.89 (6 H, br. s), 4.17 (2 H, q, *J* 7.1 Hz), 5.60 (1 H, s), 7.37–7.45 (3 H, m), 7.49–7.56 (2 H, m). δ_C (100 MHz, $CDCl_3$, Me_4Si) 14.1, 53.2 (2 C), 60.2, 97.3, 126.4, 128.7, 130.5, 134.3, 150.4, 158.5, 165.2 (signals of two carbons are overlapped). HRMS (ESI-TOF): $[M + Na]^+$ calcd for $C_{16}H_{17}NNaO_6^+$ 342.0948, found 342.0950.

3-Ethyl 2,2-dimethyl 4-phenyl-2,3-dihydroazete-2,2,3-tricarboxylate (2a'). A solution of azadiene *E*-**6** (100 mg, 0.31 mmol) in anhydrous toluene (3 mL) was refluxed for 4 h under stirring. The solvent was removed under vacuum and the residue was purified by column chromatography (hexane–EtOAc, from 5:1 to 1:1) to give compound **2a'** (53 mg, 53%) as a colorless oil. δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.26 (3 H, t, *J* 7.1 Hz), 3.86 (3 H, s), 3.87 (3 H, s), 4.21 (2 H, q, *J* 7.1 Hz), 5.01 (1 H, s), 7.43–7.50 (2 H, m), 7.52–7.59 (1 H, m) 7.80–7.86 (1 H, m). δ_C (100 MHz, $CDCl_3$, Me_4Si) 13.9, 53.0, 53.6, 53.8, 61.9, 72.0, 126.5, 128.7, 130.3, 133.0, 165.9, 166.5, 166.8, 186.4. HRMS (ESI-TOF): $[M + Na]^+$ calcd for $C_{16}H_{17}NNaO_6^+$ 342.0948, found 342.0954.

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