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Synthesis of pyrazolidinone analogs of β-lactam antibiotics

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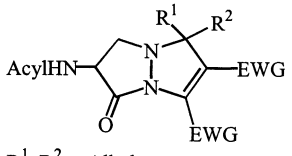
Abstract—5-Substituted pyrazolidin-3-ones, easily available by the conjugate addition–rearrangement of hydrazine to the 2,3-unsaturated sugar 1,5-lactones, react with 2 Ms equiv. of dimethyl acetylenedicarboxylate (DMAD) to give corresponding 2,3-dihydro-5,6,7-trimethoxycarbonyl-5-methoxycarbonylmethyl-1-oxo-1*H*,5*H*-pyrazolo[1,2-*a*]pyrazoles. Reaction proceeds via addition of the amine group of pyrazolidin-3-one to DMAD, rearrangement of the adduct leading to the azomethine-imine ylide followed by the 1,3-dipolar cycloaddition of second DMAD molecule. The cycloaddition product may undergo intramolecular addition of hydroxy group from the polyol side chain to the double bond to give tricyclic compounds. Reaction of pyrazolidin-3-one with DMAD is accompanied by the formation of several by-products, identification of which made possible to propose reaction pathway.

1. Introduction

The continuing and widespread emergence of bacterial antibiotic resistance necessitates the search for the new active entities. The reports of novel classes of non-β-lactam analogs of penicillin and cephalosporin have generated high levels of interest in many laboratories.[1,2] Considerable attention has been directed at the pyrazolo-pyrazolone derivatives **1**, since in some cases they exhibit high antibacterial activity.[2]



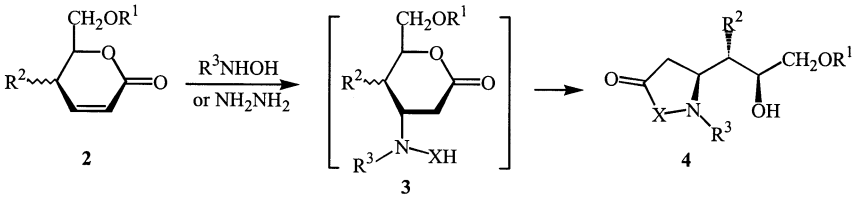
R^1, R^2 = Alkyl

1

We have reported the highly stereoselective conjugate addition–rearrangement of hydroxylamines[3] and hydrazines[4] to the α,β-unsaturated sugar δ-lactones. We established that the nucleophile approaches lactone molecule *anti* to the terminal C-6 carbon atom (Scheme 1) which leads to the formation of Michael adducts **3**.

Michael adducts **3** rearrange immediately to the corresponding isoxazolidin-5-ones **4** (for X=O)[3] or to the pyrazolidin-3-ones **4** (for X=NH).[4] The ability to control the absolute configuration at C-3 or C-5 and the presence of polyol side chain at C-5 or C-3, respectively, has prompted us to investigate the possibility of transformation of compounds **4** into the non-β-lactam analogs of cephams and/or penams **5**–**7** (Chart 1).

Recently, several derivatives of **5** were synthesized and evaluated for the antibacterial activity.[5] However, these compounds have not shown promising antibacterial properties. Moreover, the most interesting compound **5** (with R^3=CO_2*n*-Bu) exhibited low stability, particularly in the presence of base.[5]



2 **3** **4**

Scheme 1. R^1=Bn, Ac, TBS; R^2=H, OBn, OAc, OTBS; R^3=H, Bn; X=O, NH.

Keywords: pyrazolidinone; 1,3-dipolar cycloaddition; azomethine-imine ylide.
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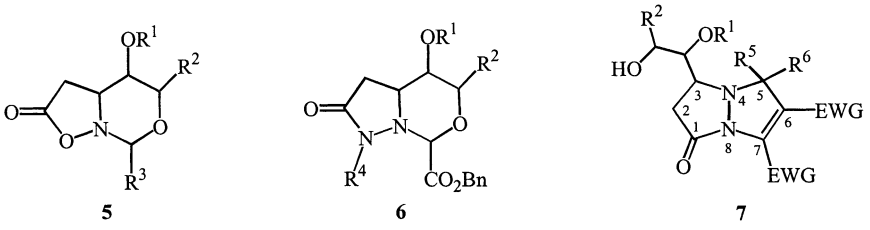


Chart 1. R^1=H, Bn, Ac, TBS; R^2=CH_3, CH_2OR^1; R^3=CH_3, CH_2OBn, CO_2*n*-Bu; R^4=H. Me, Bn; R^5, R^6=H, CH_3, CO_2*n*-Bu; EWG=electron withdrawing group.

It remained of interest, however, to synthesize and evaluate derivatives of pyrazolo-oxazine **6** and/or pyrazolo-pyrazolone **7**. The structure of the latter, incidentally, is closely related to that of **1**. We expect that compounds such as **6** and/or **7** should be more stable than their isoxazolidinone congeners. Considering that intermediary lactones **2** are easily available from either D-glucose or L-rhamnose it was thought that the compounds with general structures **6** and **7** could potentially be obtained in both enantiomeric forms.

2. Results and discussion

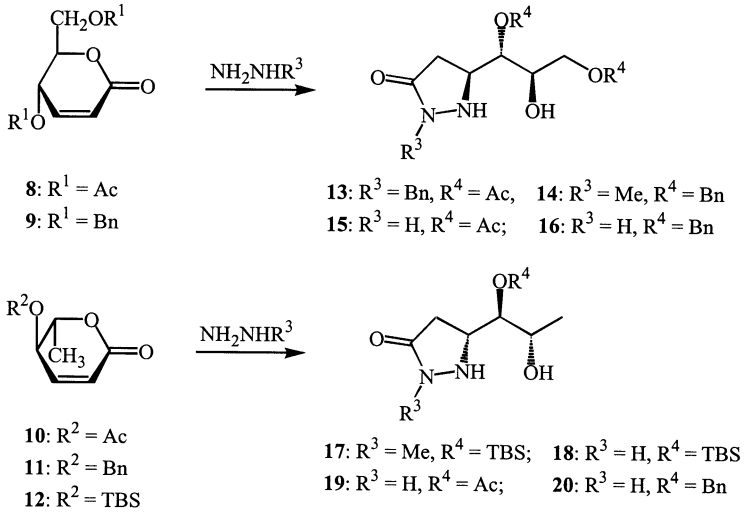
For the present study we selected the 5-substituted pyrazolidin-3-ones **13**–**20** which were obtained directly from the corresponding lactones **8**–**12** in reaction with either hydrazine or *N*-methylhydrazine, according to the known methodology (Scheme 2).[4,6] We anticipated that pyrazolidin-3-ones **13**–**20** would be more stable as the isoxazolidin-5-ones discussed earlier.

Pyrazolidin-3-ones with 2-*N*-methyl or 2-*N*-benzyl substituents (compounds **13**, **14** and **17**) were reacted with butyl glyoxylate to give corresponding acetals **21**–**24**. Structure and configuration of the acetal **21** was established by the X-ray crystallography (Fig. 1), whereas structures of corresponding compounds **23** and **24** were assigned on the basis of similarity of their respective ^{1}H NMR spectral data with that recorded for compound **21**. Compound **21** was accompanied by a small amount of its C-2 epimer **22**. As was the case with isoxazolidino-oxazines **5** investigated earlier,[5] their pyrazolidine analogs **21**, **23** and **25** did not show inhibitory activity towards DD-carboxypeptidase (Chart 2).

Bipyrazolidine antibiotics **1** were obtained from pyrazolidin-3-ones **26** by a two-step reaction sequence involving formation of an azomethine-imine ylide **27** which subsequently reacted in situ with the acetylene derivative (Scheme 3).[2,7,8]

A similar reaction sequence carried out with pyrazolidin-3-one **18**, using *n*-butyl glyoxylate and dimethyl acetylenedicarboxylate (DMAD) in boiling toluene, and in the presence of acid catalyst, led to the formation of three products: a pair of diastereomers **28** and **29** and a cyclic aminal **30** in a ratio 1:3:1.5, respectively. This mixture was subsequently separated into the individual components. The structure and configuration of **28** and **29** was assigned with the help of NOE experiments which showed a spin–spin interaction between protons in the vicinity of amine nitrogen atom, H-2 and H-8 (Scheme 4). Consequently, the (*S*)-configuration was assigned to the C-2 proton. In the case of compound **28**, the value of vicinal coupling constant ($J_{2,3}$=8.1 Hz) between protons of pyrazolidine ring was indicative of their *cis* arrangement, whereas the respective coupling constant value ($J_{2,3}$=13.5 Hz) found for compound **29** indicated the *trans* configuration.

Stereochemistry of the cyclic aminal **30** and its desilylated derivative **31** was assigned by analogy to compound **21**. It can be expected that an effective formation of the bipyrazolidines from pyrazolidin-3-ones **13**–**20** having a



Scheme 2.

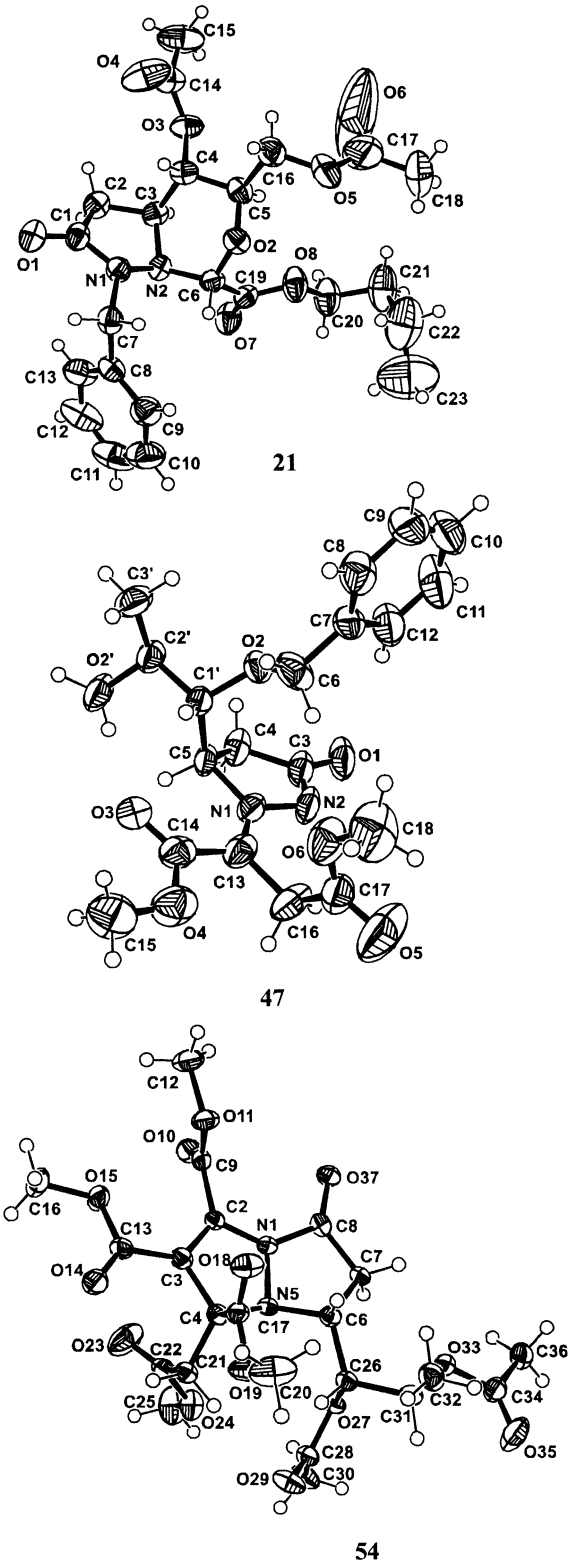


Figure 1. X-Ray structures of cephams **21**, **47**, and **54** with crystallographic numbering scheme.

free hydroxy group in the side chain would require prior hydroxy group protection, otherwise *N*,*O*-acetals can be formed. The selective *O*-silylation can be achieved by carrying out the reaction in acetonitrile solution (if the DMF solution is used the silylation of hydroxyl group is accompanied by the simultaneous formylation of the N-1 nitrogen). The double *N*- and *O*-protection was exemplified by the reaction of **17** with TBSCl in DMF, which yielded **32** (Scheme 5).

Silylation of **16** in acetonitrile followed by the reaction of resulting ether **34** with acetone and then with DMAD afforded expected compound **36** (Scheme 5).

In the course of the reaction of pyrazolidin-3-ones **13**–**20** with carbonyl compounds and DMAD, we have noticed occasional formation of products resulting from addition of two molecules of DMAD to one molecule of pyrazolidin-3-one. It should be noted that reactions of DMAD with primary or secondary amines, as well as with variety of heterocyclic compounds, have been reviewed in the past.[9–11] It has been shown that base- or acid-catalyzed additions, self condensations and rearrangements lead to formation of complex mixtures of products which, as a rule, require careful chromatographic separation prior to their identification.[9–11]

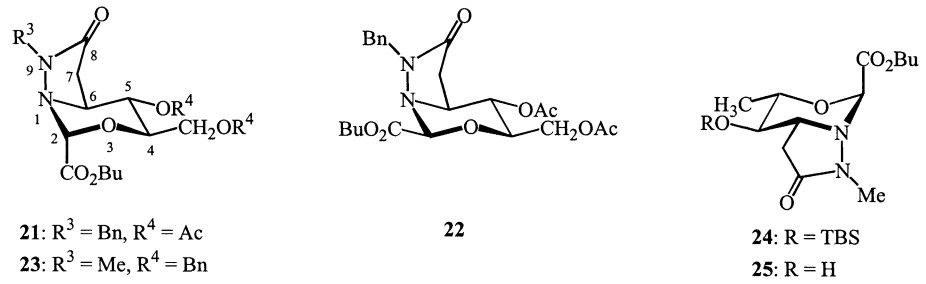
Reaction of compound **34** with 2.2 M equiv. of DMAD in acetonitrile at room temperature gave adduct **37** accompanied by the by-product **38** which was characterized as respective acetate **39**; the configuration at the double bond in **38** and **39** was not assigned (Scheme 6).

Attempted formation of a bipyrazolidine ring system by the reaction of pyrazolidin-3-ones lacking protection of the hydroxy group in the polyol side chain with DMAD at room temperature led to the mixture of unstable products. These required acetylation prior to the chromatographic purification and isolation of by-products. Thus, pyrazolidinone **16** treated with DMAD at room temperature gave a mixture of products which were characterized after acetylation as **40**–**42** (Scheme 7).

In contrast, treatment of compound **16** with DMAD in boiling benzene in the presence of *p*-TsOH led to formation of mixture of two tricyclic diastereomers **43** and **44** in a ratio 4:1, respectively (Scheme 8). Compounds **43** and **44** have similar values of vicinal coupling constants between H-6, 7, 8 and 9 protons in their ^{1}H NMR spectra, which strongly suggest presence of the same tricyclic skeleton in both products. The assignment of structure and configuration of both reaction products was based on the assumption that the configuration at C-2 is the same as it is found in compound **40** at C-6. For the more abundant product **43**, the methoxycarbonyl group at C-3 was assigned the *exo*-configuration.

In the case of compound **20**, which has the enantiomeric relation to **16**, the reaction with DMAD proceeded in analogous manner. Two isolated bipyrazolidine stereoisomers **45** and **46** were obtained in ratio 10:1, respectively. The structure and configuration of the more abundant **45** was assigned by analogy with **40**. Diastereomers **45** and **46** were accompanied by a minute amount of the crystalline azomethine-imine ylide **47**. Its structure and absolute configuration was established by the X-ray crystallography (Fig. 1) (Chart 3).

As a result of reflux in benzene in the presence of *p*-TsOH,

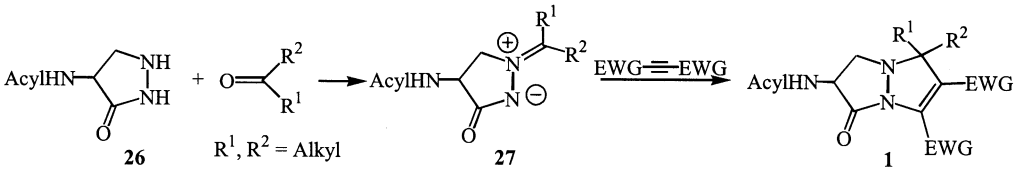


21: R^3 = Bn, R^4 = Ac
23: R^3 = Me, R^4 = Bn

22

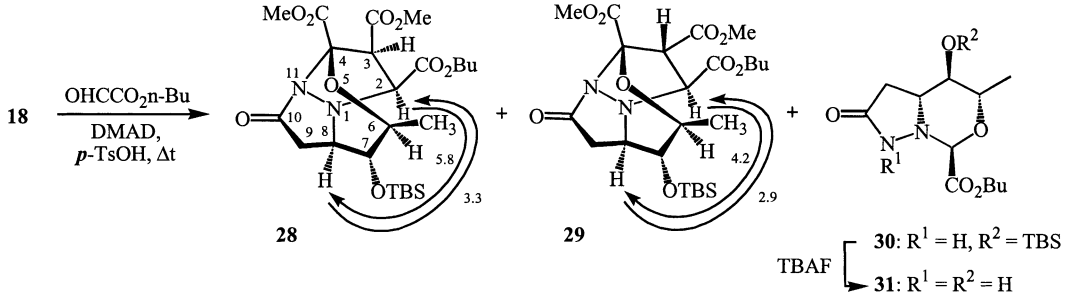
24: R = TBS
25: R = H

Chart 2.



26 **27** **1**

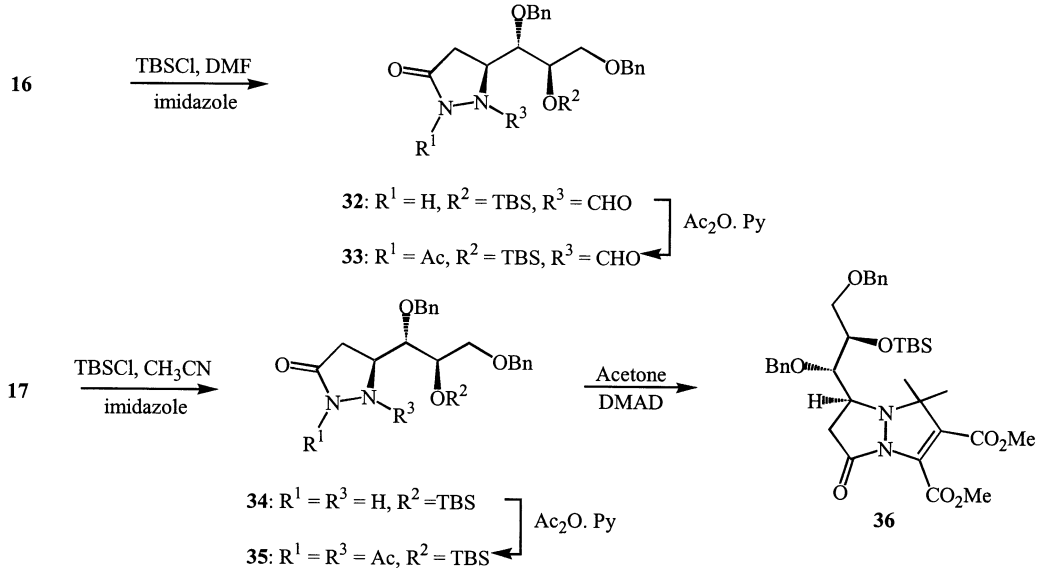
Scheme 3.



28 **29**

30: R^1 = H, R^2 = TBS
31: R^1 = R^2 = H

Scheme 4. NOE's values between H-2 and H-8 protons are shown on structures of **28** and **29**.

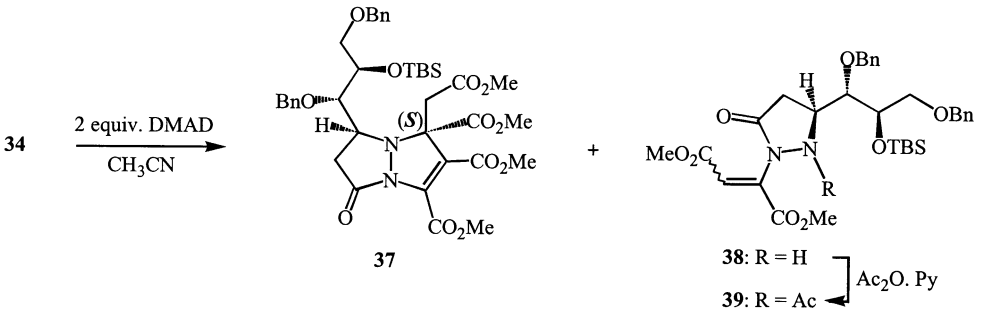


32: R^1 = H, R^2 = TBS, R^3 = CHO
33: R^1 = Ac, R^2 = TBS, R^3 = CHO

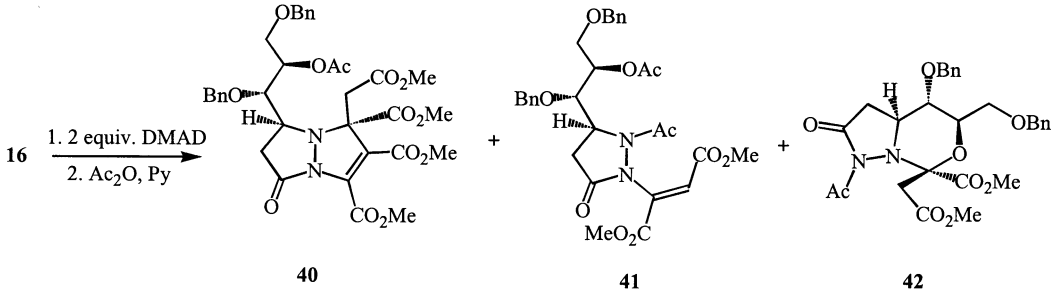
34: R^1 = R^3 = H, R^2 =TBS
35: R^1 = R^3 = Ac, R^2 = TBS

36

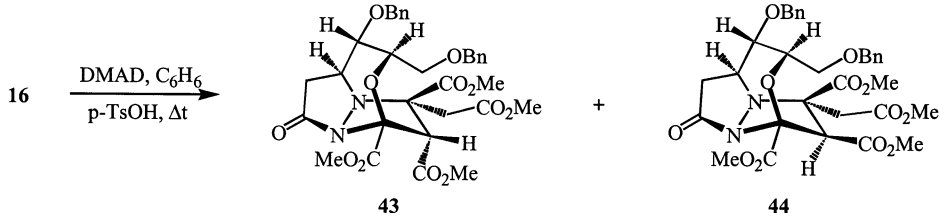
Scheme 5.



Scheme 6.



Scheme 7.



Scheme 8.

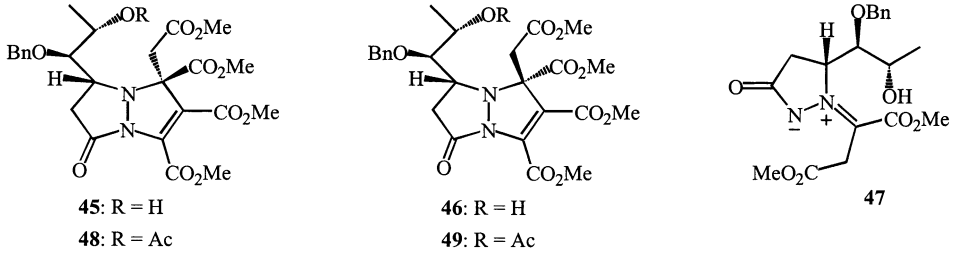
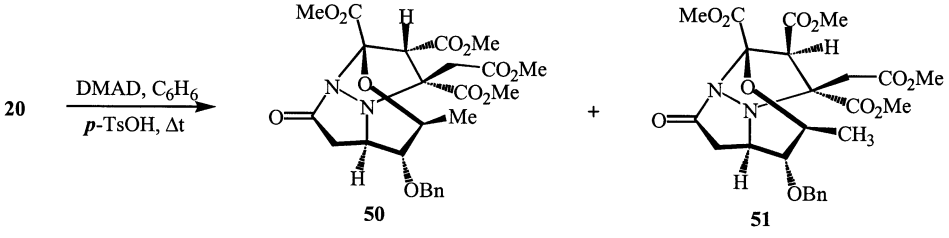


Chart 3.



Scheme 9.

compound **20** gave two tricyclic compounds **50** and **51** in a ratio of about 3.4:1, respectively (Scheme 9).

The structure and configuration assignments of compounds **37**, **40**, **43**–**46**, **50** and **51** are based on the assumption that the configuration in the vicinity of the N=C double bond in the isolated ylide **47** represents the more stable arrangement. Furthermore, we assumed that this preferred arrangement controls the stereochemical course of all reactions between pyrazolidin-3-ones and DMAD. Consequently, the subsequent (1,3)-dipolar cycloaddition involving the ylide and the next molecule of DMAD should proceed *anti* to the polyol side chain. Such an assumption is strongly supported by the X-ray analysis of crystalline compound **54** (Fig. 1).

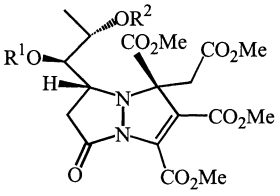
The reaction of pyrazolidin-3-one **19** with DMAD proceeds slowly, but analogously to the reactions reported above. The complicated mixture of products **52**, **53**, **55**, **57**, **59**, **61**, **63** and **64** can be, however, separated into the individual components. Acetylation leading to the corresponding acetates **54**, **56**, **58**, **60** and **62** simplified structural assignments (Chart 4).

After prolonged reflux in toluene in the presence of *p*-TsOH, compound **63** gave crystalline tricyclic compound **64**.

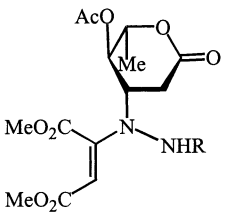
Fortuitously, a number of crystalline acetates were obtained in some of the reactions discussed earlier. As a result we were able to establish the stereochemistry of **54** (Fig. 1), **56**, **62**, and **64** (Fig. 2) by the X-ray structure analysis. It should be noted that the unequivocal structure assignment of these intermediates provided a sound base for the proposed pathway of the reaction between the pyrazolidinones having polyol side chain and DMAD.

Condensation of pyrazolidin-2-ones or acyl hydrazines with aldehydes or ketones is a well-known process that produces azomethine-imines that are active in the 1,3-dipolar cycloadditions.[7,8,12–15] However, the formation of analogous azomethine-imines during the reaction of pyrazolidin-3-ones with acetylene dicarboxylate esters has not been reported previously.

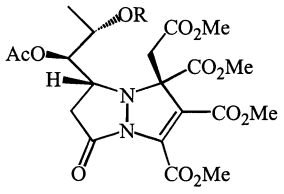
The results presented above allowed us to propose the general pathway for the reaction between pyrazolidin-3-one and DMAD. The diagram provided below (Scheme 3) uses pyrazolidin-3-one **20** as an example. The first molecule of DMAD can add to the N-1 (amine) or to N-2 (amide) nitrogen atom of pyrazolidin-3-one **20** to produce diesters **65** and/or **66**, respectively. Addition to the more nucleophilic N-1 nitrogen atom is significantly preferred.[16] Adduct **65** can rearrange to the ylide **47**. The *E*-configuration of the C=N double bond is preferred. The second molecule of DMAD approaches ylide **47** *anti* to the polyol side chain to generate preferentially the bis-pyrazolidine skeleton **45**. The tetraester **67** might be formed directly from compound



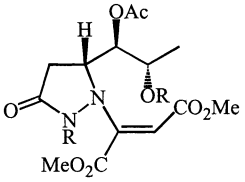
52: R^1 = Ac, R^2 = H
53: R^1 = H, R^2 = Ac
54: R^1 = R^2 =Ac



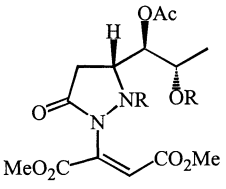
55: R = H
56: R = Ac



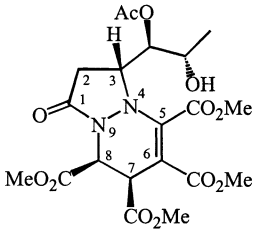
57: R = H
58: R = Ac



59: R = H
60: R = Ac



61: R = H
62: R = Ac



63

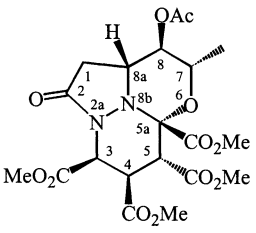


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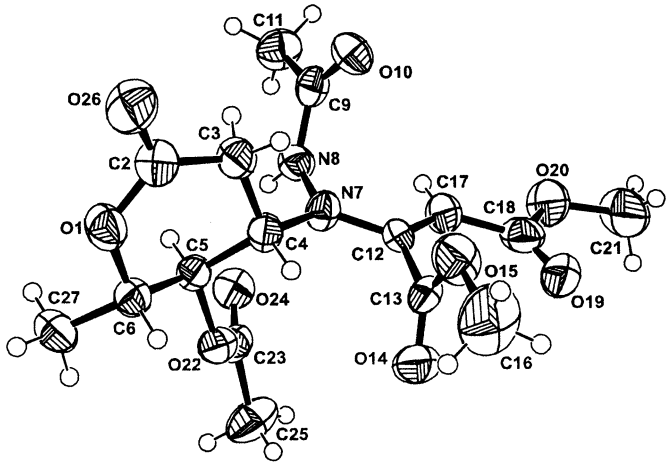
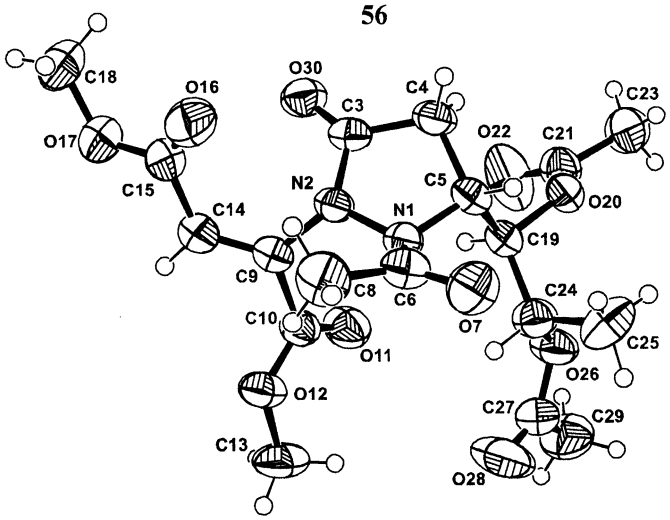
Chart 4.



56



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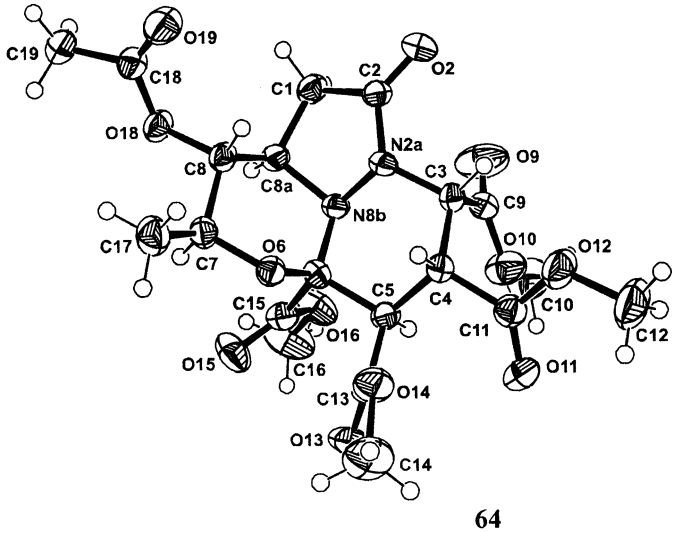
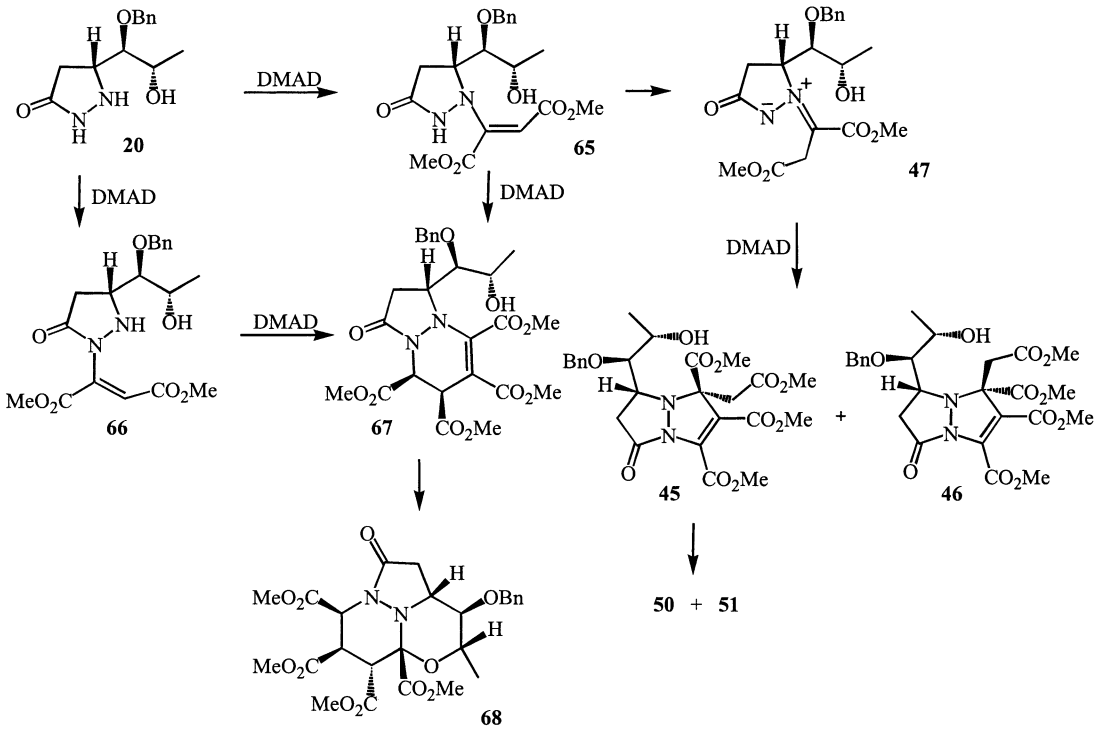


64

Figure 2. X-Ray structures of cephams **56**, **62**, and **64** with crystallographic numbering scheme.

65 by the addition of a second molecule of DMAD, although this path appears to be less likely. Tetraester **67** is derived from **66**, which cannot rearrange to the corresponding ylide. Compound **45** undergoes intramolecular cyclo-addition of the hydroxy group to yield the diastereomeric pair **50** and **51**. The *exo*-configuration of methoxycarbonyl group at C-3 is preferred. Compound **67** as a result of the intramolecular cyclization by addition of hydroxy group to the double bond can form diaza-oxa-perhydroacenaphtene **68**. Compounds **65**–**68** were not isolated and not even detected. Their existence is inferred by the assumed similarity to the analogous, isolated and characterized compounds **59**, **61**, **63**, and **64**.



Scheme 10.

With notable exception of compound **55** (L-arabino configuration), the formation of all other products can be easily explained following Scheme 3. The pathway leading to the formation of **55** is unclear and may possibly be associated with the presence of a minute amount of the alternative diastereomer (resulted from less probable *cis*-approach) which accompanied formation of pyrazolidin-3-ones **4** (X=NH) during the reaction of lactone **2** and hydrazine. Such adduct has been observed previously only in the case of *N*-benzyl-hydrazine addition to the *erythro*-lactone **2** (R^1=CH_2OAc, R^2=OAc).[4,6] The alternative path leading to the formation of **55** that involves epimerization at C-5 of the pyrazolidin-3-one skeleton via the intramolecular retro-Michael reaction is forbidden by the Baldwin rules (Scheme 10).

Bi- and tricyclic pyrazolidinones **21**, **23**, **25**, **37**, **40**, **43**, **48**, **50**, **52**, **53** and **54** were evaluated for their biological activity. An inhibition of the DD-carboxypeptidase activity was measured.[17–19] These compounds, however, did not exhibit any significant inhibitory activity. It can be speculated that the polyol side chain that is present in all investigated compounds might be responsible for the observed lack of biological activity.

In summary, we have shown that the pyrazolidin-3-ones having polyol side chain at C-5 carbon react with DMAD to form bipyrazolidine compounds. The reaction proceeds via addition of the amine nitrogen atom to a molecule of DMAD. The adduct rearranges to the ylide which adds, with a high stereoselectivity, the second molecule of DMAD. The major reaction products are accompanied by a number of by-products. Their isolation and identification allowed us to formulate the probable reaction pathway.

3. Experimental

Melting points were determined on a Koefler hot-stage apparatus. ^{1}H NMR spectra were recorded using Brucker Avance 500 and Varian Gemini AC-200 instruments. IR spectra were recorded on a Perkin–Elmer FT-IR Spectrum 2000 spectrophotometer. Mass spectra were recorded using an AMD-604 Inectra GmbH spectrometer. Column chromatography was performed using Merck Kiesel Gel (230–400 mesh).

3.1. Reaction of pyrazolidin-3-ones 13–15 and 18 with butyl glyoxylate. General procedure

Pyrazolidin-3-ones (**13**–**15**, **18**; 0.10 mmol), freshly distilled butyl glyoxylate (0.12 mmol) and *p*-TsOH (2 mg) in toluene (20 ml) were refluxed for 0.5 h while the solvent was slowly distilled off (15 ml). Subsequently the solution was washed with saturated sodium bicarbonate and water, dried and evaporated. The residue was purified by chromatography using hexane–ethyl acetate 1:1 v/v as an eluent to give products **21**–**24**.

3.1.1. (2*R*,4*S*,5*R*,6*S*)-5-Acetoxy-4-acetoxymethyl-1,9-diaza-9-benzyl-2-*n*-butoxycarbonyl-3-oxa-8-oxo-bicyclo[4.3.0]-nonane (21). 81%; Colorless crystals; mp 134–136°C; $[\alpha]_D$=+18.9 (*c* 1.0, CH_2Cl_2); IR ($CHCl_3$): ν 1744, 1700 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.34–7.25 (m, 5H, Ph), 5.18 (s, 1H, H-2), 5.06 (t, 1H, *J*=9.5, 10.0 Hz, H-5), 4.87, 4.57 (2d, 2H, *J*=16.5 Hz, Bn), 4.32 (dt, 1H, *J*=3.5, 3.5, 10.0 Hz, H-4), 4.06–4.17 (m, 1H, CO_2CH_2, CH_2OAc), 3.88 (bt, 1H, H-6), 2.73 (dd, 1H, *J*=8.0, 16.5 Hz, H-7), 2.54 (dd, 1H, *J*=16.0 Hz, H-7′), 2.10, 2.09 (2s, 6H, 2Ac), 1.57, 1.30, 0.92 (3m, 7H, C_3H_7); MS (EI/HR) *m*/*z* 462.20237, M^+; calcd for $C_{23}H_{30}N_2O_8$: 462.2002. Anal.

calcd for $C_{23}H_{30}N_2O_8$: C, 59.74; H, 6.49; N, 6.06. Found: C, 59.63; H, 6.47; N, 5.95.

3.1.2. (2*S*,4*S*,5*R*,6*S*)-1,9-Diaza-5-benzyloxy-4-benzyloxymethyl-2-butoxycarbonyl-9-methyl-3-oxa-8-oxo-bicyclo-[4.3.0]nonane (22). 6%; Colorless oil; $[\alpha]_D=-9.5$ (*c* 0.9, CH_2Cl_2); IR ($CHCl_3$): ν 1746, 1702 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.33–7.25 (m, 5H, Ph), 5.20 (dd, 1H, *J*=6.0, 10.5 Hz, H-5), 4.89, 4.03 (2d, 2H, *J*=15.0 Hz, NBn), 4.61 (s, 1H, H-2), 4.26–4.12 (m, 4H, CH_2OAc, CO_2CH_2), 4.08 (dt, 1H, *J*=6.5, 7.0, 13.5 Hz, H-6), 3.95 (ddd, 1H, *J*=3.0, 5.0, 10.5 Hz, H-4), 2.94 (dd, 1H, *J*=13.5, 16.5 Hz, H-7), 2.21 (dd, 1H, *J*=7.0, 16.5 Hz, H-7′), 2.07, 2.04 (2s, 6H, 2Ac), 1.62, 1.38, 0.94 (3m, 7H, C_3H_7); MS (EI/HR) *m/z* 462.20100, M^+; calcd for $C_{23}H_{30}N_2O_8$: 462.20021.

3.1.3. (2*R*,4*S*,5*R*,6*S*)-1,9-Diaza-5-benzyloxy-4-benzyloxymethyl-2-butoxycarbonyl-9-methyl-3-oxa-8-oxo-bicyclo-[4.3.0]nonane (23). 44%; Colorless crystals; mp 72–73°C; $[\alpha]_D=-3.0$ (*c* 0.8, CH_2Cl_2); IR ($CHCl_3$): ν 1743, 1695 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.36–7.14 (m, 10H, 2×Ph), 5.26 (s, 1H, H-2), 4.66, 4.54 (2d, 2H, *J*=12.0 Hz, Bn), 4.52, 4.48 (2d, 2H, *J*=11.0 Hz, Bn), 4.26–4.16 (m, 2H, CO_2CH_2), 4.02 (ddd, 1H, *J*=2.5, 3.5, 9.5 Hz, H-4), 3.79 (m, 1H, H-6), 3.76 (dd, 1H, *J*=3.5, 11.0 Hz, CH_AH_BOBn), 3.71 (dd, 1H, *J*=2.5, 11.0 Hz, CH_AH_BOBn), 3.63 (t, 1H, *J*=9.5, 9.5 Hz, H-5), 3.00 (s, 3H, CH_3), 2.70 (dd, 1H, *J*=8.0, 16.5 Hz, H-7), 2.43 (d, 1H, *J*=16.5 Hz, H-7′), 1.67, 1.39, 0.93 (3m, 7H, C_3H_7); MS (EI/HR) *m/z* 482.241822, M^+; calcd for $C_{27}H_{34}N_2O_6$: 482.241682. Anal. calcd for $C_{27}H_{34}N_2O_6$: C, 67.21; H, 7.05; N, 5.80. Found: C, 67.12; H, 7.05; N, 5.62.

3.1.4. (2*S*,4*R*,5*S*,6*R*)-1,9-Diaza-5-*t*-butyldimethylsiloxy-2-butoxycarbonyl-4,9-dimethyl-3-oxa-8-oxo-bicyclo-[4.3.0]nonane (24). 79%; Colorless oil; $[\alpha]_D=-23.4$ (*c* 1, CH_2Cl_2); IR ($CHCl_3$): ν 1743, 1695 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.19 (s, 3H, H-2), 4.25 (m, 2H, CO_2CH_2), 3.72 (dq, 1H, *J*=6.0, 9.0 Hz, H-4), 3.66 (bt, 2H, *J*=8.0, 9.0 Hz, H-6), 3.31 (t, 1H, *J*=9.0, 9.5 Hz, H-5), 2.97 (s, 3H, CH_3), 2.72 (dd, 1H, *J*=8.0, 16.5 Hz, H-7), 2.56 (d, H, *J*=16.5 Hz, H-7′), 1.69, 1.42, 0.96 (3m, 7H, C_3H_7), 1.25 (d, 1H, *J*=6.0 Hz, CH_3), 0.88 (s, 9H, *t*-Bu), 0.13, 0.10 (2s, 6H, 2Me); MS (EI/HR) *m/z* 400.23905, M^+; calcd for $C_{19}H_{36}N_2O_5Si$: 400.23935. Anal. calcd for $C_{19}H_{36}N_2O_5Si$: C, 57.0; H, 9.0; N, 7.0. Found: C, 57.16; H, 8.83; N, 7.34.

3.1.5. (2*S*,4*R*,5*S*,6*R*)-1,9-Diaza-2-butoxycarbonyl-5-hydroxy-4,9-dimethyl-3-oxa-8-oxo-bicyclo [4.3.0]nonane (25). To compound **24** (0.080 g, 0.2 mmol) in THF (5 ml) at room temperature was added $Bu_4NF{\cdot}3H_2O$ (0.063 g, 0.2 mmol) and resulted mixture was stirred for 0.5 h. Subsequently, solvent was removed under reduced pressure and residue purified by chromatography to give compound **25** (0.054 g, 95%); colorless crystals; mp 122–123°C; $[\alpha]_D=+7.2$ (*c* 0.5, CH_2Cl_2); IR ($CHCl_3$): ν 3400, 1741, 1692 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.20 (s, 1H, H-2), 4.24 (m, 2H, CO_2CH_2), 3.81 (dq, 1H, *J*=6.0, 9.5 Hz, H-4), 3.70 (dd, 1H, *J*=7.5, 9.5 Hz, H-6), 3.22 (t, 1H, *J*=9.5 Hz, H-5), 2.98 (s, 3H, CH_3), 2.76 (dd, 1H, *J*=7.5, 16.5 Hz, H-7), 2.66 (d, 1H, *J*=16.5 Hz, H-7′), 1.70, 1.42, 0.97 (3m, 7H, C_3H_7), 1.30 (d, 1H, *J*=6.0 Hz, CH_3); MS (EI/HR) *m/z* 287.16157, $(M+H)^+$; calcd for $C_{13}H_{23}N_2O_5$: 287.16071.

3.1.6. (2*S*,3*S*,4*R*,6*S*,7*R*,8*R*)- and (2*S*,3*R*,4*R*,6*S*,7*R*,8*R*)-1,11-Diaza-2-butoxycarbonyl-7-*t*-butyl dimethylsiloxy-3,4-dimethoxy carbonyl-6-methyl-5-oxa-tricyclo[6.3.$0^{0.0}$]-undecan-10-one (28,29) and (2*S*,4*S*,5*R*,6*R*)-1,9-diaza-2-butoxycarbonyl-5-*t*-butyldimethylsiloxy-4-methyl-3-oxa-8-oxo-bicyclo[4.3.0]nonane (30). Pyrazolidin-3-one **18** (0.15 g, 0.60 mmol) in toluene (20 ml) was treated with butyl glyoxylate (0.080 g, 0.60 mmol), DMAD (0.090 g, 0.60 mmol) and *p*-TsOH (5 mg). The mixture was refluxed for 15 min, while 12 ml of the solvent were distilled off. The remaining solution was evaporated and residue separated by chromatography using hexane–ethyl acetate 7:3 v/v as an eluent to afford **30** (0.05 g, 21%), **28** (0.04 g, 13%) and **29** (0.13 g, 40%).

Compound **28**: colorless oil; $[\alpha]_D=-63.9$ (*c* 0.7, CH_2Cl_2); IR ($CHCl_3$): ν 1740, 1702 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.10 (d, 1H, *J*=8.0 Hz, H-2), 4.46 (dq, 1H, *J*=6.5, 7.0 Hz, H-6), 4.31 (d, 1H, *J*=8.0 Hz, H-3), 4.19 (m, 2H, CO_2CH_2), 4.07 (m, 1H, H-8), 3.87 (dd, 1H, *J*=2.5, 7.0 Hz, H-7), 3.79, 3.77 (2s, 6H, $2OCH_3$), 3.24 (dd, 1H, *J*=5.0, 18.0 Hz, H-9), 2.85 (dd, 1H, *J*=6.0, 18.0 Hz, H-9′), 1.64, 1.37, 0.94 (3m, 7H, C_3H_7), 1.36 (d, 1H, *J*=6.5 Hz, CH_3), 0.89 (s, 9H, *t*-Bu), 0.13, 0.08 (2s, 6H, 2Me); MS (LSIMS/HR) *m/z* 551.22439, $(M+Na)^+$; calcd for $C_{24}H_{40}N_9O_2SiNa$: 551.24005. Anal. calcd for $C_{24}H_{40}N_9O_2Si$: C, 54.54; H, 7.57; N, 5.30. Found: C, 55.26; H, 7.83; N, 5.15.

Compound **29**: colorless oil; $[\alpha]_D=+36.7$ (*c* 1.4, CH_2Cl_2); IR ($CHCl_3$): ν 1748, 1701 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.05 (d, 1H, *J*=14.0 Hz, H-2), 4.44 (d, 1H, *J*=14.0 Hz, H-3), 4.43 (dq, 1H, *J*=6.0, 7.5 Hz, H-6), 4.12 (m, 2H, CO_2CH_2), 4.01 (ddd, 1H, *J*=3.0, 5.0, 6.5 Hz, H-8), 3.85 (dd, 1H, *J*=3.0, 7.5 Hz, H-7), 3.77, 3.70 (2s, 6H, $2OCH_3$), 3.27 (dd, 1H, *J*=5.0, 18.0 Hz, H-9), 2.87 (dd, 1H, *J*=6.0, 18.0 Hz, H-9′), 1.61, 1.37, 0.94 (3m, 7H, C_3H_7), 1.35 (d, 1H, *J*=6.5 Hz, CH_3), 0.89 (s, 9H, *t*-Bu), 0.13, 0.06 (2s, 6H, 2Me); MS (LSIMS/HR) *m/z* 551.23956, M^+; calcd for $C_{24}H_{40}N_2O_9SiNa$: 551.24005. Anal. calcd for $C_{24}H_{40}N_2O_9Si$: C, 54.54; H, 7.57; N, 5.30. Found: C, 55.63; H, 7.58; N, 5.26.

Compound **30**: colorless oil; $[\alpha]_D=-18.2$ (*c* 0.53, CH_2Cl_2); IR ($CHCl_3$): ν 3387, 1741, 1702 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.98 (s, 1H, H-2), 4.22 (m, 2H, CO_2CH_2), 3.73 (dq, 1H, *J*=6.0, 9.0 Hz, H-4), 3.67 (bt, 1H, *J*=8.0, 9.0 Hz, H-6), 3.47 (t, 1H, *J*=9.0, 9.0 Hz, H-5), 2.79 (dd, 1H, *J*=8.0, 17.0 Hz, H-7), 2.54 (d, H, *J*=17.0 Hz, H-7′), 1.68, 1.41, 0.95 (3m, 7H, C_3H_7), 1.29 (d, 1H, *J*=6.0 Hz, CH_3), 0.88 (s, 9H, *t*-Bu), 0.13, 0.11 (2s, 6H, 2Me); MS (EI/HR) *m/z* 386.22360, M^+; calcd for $C_{18}H_{34}N_2O_5Si$: 386.2237.

3.1.7. (2*S*,4*S*,5*R*,6*R*)-1,9-Diaza-2-butoxycarbonyl-5-hydroxy-4-methyl-3-oxa-8-oxo-bicyclo[4.3.0]nonane (31). Compound **31** was obtained from **30** according to the procedure described for **25**. 80%; Colorless crystals; mp 168–170°C; $[\alpha]_D=-43.8$ (*c* 0.3, CH_2Cl_2); IR ($CHCl_3$): ν 3388, 1741, 1703 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.08 (bs, 1H, NH), 4.99 (s, 1H, H-2), 4.21 (m, 2H, CO_2CH_2), 3.87 (dq, 1H, *J*=6.0, 9.0 Hz, H-4), 3.71 (dd, 1H, *J*=7.5, 9.5 Hz, H-6), 3.45 (dt, 1H, *J*=6.0, 9.5, 9.5 Hz, H-57), 2.82 (dd, 1H, *J*=7.5, 17.0 Hz, H-7), 2.63 (dd, 1H, *J*=5.0, 18.0 Hz, H-9), 3.79, 3.77 (2s, 6H, $2OCH_3$), 3.24 (dd, 1H, *J*=5.0, 18.0 Hz,

H-9), 2.85 (d, 1H, J=17.0 Hz, H-7′), 1.68, 1.40, 0.96 (3m, 7H, C_3H_7), 1.34 (d, 1H, J=6.0 Hz, CH_3); MS (LSIMS/HR) m/z 273.14504, $(M+H)^+$; calcd for $C_{12}H_{21}N_2O_5$: 273.14472.

3.1.8. (5*S*,1′*S*,2′*R*)-5-(2′-*t*-Butyldimethylsiloxy-1′,3′-dibenzyloxy)propyl-1-formyl-pyrazolidin-3-one (32). Compound **16** (0.36 g, 1.0 mmol) in DMF (2.5 ml) was treated with TBSCl (0.17 g, 1.1 mmol) and imidazole (0.08 g, 1.1 mmol) and left at room temperature for 3 h. Subsequently the mixture was poured into water and extracted with *t*-butyl methyl ether. The extract was dried and evaporated. The crude product was purified by chromatography to give **32** (0.44 g, 90%); colorless crystals; mp 102–104°C; $[\alpha]_D$=−79.0 (c 1.1, CH_2Cl_2); IR ($CHCl_3$): γ 3395, 1716, 1667 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.85 (s, 1H, CHO), 7.41–7.18 (m, 10H, 2×Ph), 4.66 (m, 1H, H-5), 4.64, 4.50 (2d, 2H, J=11.0 Hz, Bn), 4.43 (bs, 2H, Bn), 3.91 (q, 1H, H-2′), 3.82 (m, 1H, H-1′), 3.53 (m, 2H, H-3′a,3′b), 2.89 (dd, 1H, J=4.0, 17.5 Hz, H-4a), 2.77 (dd, 1H, J=9.0, 17.5 Hz, H-4b), 0.88 (s, 9H, *t*-Bu), 0.05 (2s, 6H, 2Me); MS (LSIMS) m/z 499, $(M+H)^+$. Anal. calcd for $C_{27}H_{38}N_2O_5Si$: C, 65.06; H, 7.63; N, 5.63. Found: C, 65.00; H, 7.66; N, 5.62.

3.1.9. 2-*N*-Acetyl 33. Colorless oil; $[\alpha]_D$=−6.3 (c 1.7, CH_2Cl_2); IR ($CHCl_3$): ν 1767, 1723, 1695 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.39 (bs, 1H, CHO), 7.37–7.14 (m, 10H, 2×Ph), 5.08 (bs, 1H, H-5), 4.67, 4.37 (2d, 2H, J=11.5 Hz, Bn), 4.52 (s, 2H, Bn), 4.02 (m, 1H, H-2′), 3.92 (m, 1H, H-1′), 3.58 (dd, 1H, J=5.5, 10.0 Hz, H-3′a), 3.53 (dd, 1H, J=5.0, 10.0 Hz, H-3′b), 3.04 (bd, 1H, H-4a), 2.85 (dd, 1H, J=9.0, 17.5 Hz, H-4b), 2.15 (bs, 3H, Ac), 0.90 (s, 9H, *t*-Bu), 0.07, 0.06 (2s, 6H, 2Me); MS (LSIMS) m/z 541, $(M+H)^+$. Anal. calcd for $C_{29}H_{40}N_2O_6Si$: C, 64.44; H, 7.40; N, 5.18. Found: C, 64.51; H, 7.59; N, 4.99.

3.1.10. (5*S*,1′*S*,2′*R*)-5-(2-*t*-Butyldimethylsiloxy-1′,3′-dibenzyloxy)propyl-pyrazolidin-3-one (34). Compound **17** (0.29 g, 1.0 mmol) in acetonitrile (2.5 ml) was silylated with TBSCl (0.17 g, 1.1 mmol) and imidazole (0.08 g, 1.1 mmol) and left at room temperature for 0.5 h. Work-up as for compound **32** provided **34** (0.42 g, 89%); colorless oil; $[\alpha]_D$=−21.3 (c 5.1, CH_2Cl_2); IR ($CHCl_3$): ν 3434, 3240, 1698 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.37–7.28 (m, 10H, 2×Ph), 6.50, 4.23 (2bs, 2H, 2NH), 4.77, 4.45 (2d, 2H, J=11.5 Hz, Bn), 4.51 (s, 2H, Bn), 4.10 (dt, 1H, H-2′), 3.88 (bs, 1H, H-5), 3.70 (dd, 1H, J=3.5, 4.0 Hz, H-1′), 3.52 (d, 2H, J=5.0, 10.0 Hz, H-3′a,3′b), 2.74 (dd, 1H, J=9.0, 16.5 Hz, H-4a), 2.38 (dd, 1H, J=7.0, 16.5 Hz, H-4b), 0.88 (s, 9H, *t*-Bu), 0.07, 0.06 (2s, 6H, 2Me); MS (LSIMS) m/z 471.26791, $(M+H)^+$; calcd for $C_{26}H_{39}N_2O_4Si$: 471.26933. Anal. calcd for $C_{26}H_{38}N_2O_4Si$: C, 66.38; H, 8.08; N, 5.95. Found: C, 66.29; H, 7.95; N, 5.87.

3.1.11. 1,2-Di-*N*-acetyl 35. Colorless oil; $[\alpha]_D$=+23.8 (c 0.6, CH_2Cl_2); IR ($CHCl_3$): ν 1764, 1734, 1673 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.38–7.14 (m, 10H, 2×Ph), 5.08 (bd, 1H, H-5), 4.67, 4.33 (2d, 2H, J=11.5 Hz, Bn), 4.56, 4.49 (2d, 2H, J=11.0 Hz, Bn), 4.01 (m, 1H, H-2′), 3.94 (dd, 1H, J=2.0, 4.0 Hz, H-1′), 3.59 (dd, 1H, J=5.5, 10.0 Hz, H-3′a), 3.53 (dd, 1H, J=5.0, 10.0 Hz, H-3′b), 3.03 (dd, 1H, J=1.0, 17.5 Hz, H-4a), 2.81 (dd, 1H, J=8.5, 17.5 Hz, H-4b), 2.07, 2.04 (2s, 6H, 2Ac), 0.89 (s, 9H, *t*-Bu), 0.07, 0.05 (2s, 6H, 2Me); MS (LSIMS/HR) m/z 577.27099, $(M+Na)^+$; calcd for $C_{30}H_{42}O_6N_2SiNa$: 577.27331. Anal. calcd for $C_{30}H_{42}N_2O_6Si$: C, 64.98; H, 7.58; N, 5.05. Found: C, 64.88; H, 7.35; N, 4.99.

3.1.12. (3*S*,1′*S*,2′*R*)-3-(1′,3′-Dibenzyloxy-2′-*t*-butyldimethylsiloxypropyl)-2,3-dihydro-6,7-dimethoxycarbonyl-5,5-dimethyl-1*H*,5*H*-pyrazolo[1,2-*a*]pyrazol-1-one (36). Compound **34** (0.047 g, 0.10 mmol) in methanol (2 ml) was treated with dimethoxypropane (0.048 g, 0.40 mmol), camphorsulfonic acid (2.5 mg, 0.01 mmol) and refluxed for 0.5 h. Subsequently methanol was evaporated, residue dissolved in acetonitrile (5 ml) and treated with DMAD (0.016 g, 0.11 mmol). The mixture was stirred for 3 h at room temperature, evaporated and purified by chromatography using hexane–ethyl acetate 1:1 v/v as an eluent to give **36** (0.039 g, 59%); yellow oil; $[\alpha]_D$=+171.9 (c 0.5, CH_2Cl_2); IR ($CHCl_3$): ν 1752, 1702 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.35–7.24 (m, 10H, 2×Ph), 4.95, 4.60 (2d, 2H, J=11.0 Hz, Bn), 4.49, 4.47 (2d, 2H, J=12.0 Hz, Bn), 4.08 (dd, 1H, J=8.5, 10.5 Hz, H-3), 3.92 (s, 3H, OCH_3), 3.53 (dd, 1H, H-3′a), 3.47 (dd, 1H, H-3′b), 3.32 (dd, 1H, J=10.5, 17.3 Hz, H-2a), 2.65 (dd, 1H, J=8.5, 17.5 Hz, H-2b), 1.59, 1.55 (2s, 6H, $2CH_3$), 0.86 (s, 9H, *t*-Bu), 0.03 (2s, 6H, 2Me); MS (LSIMS/HR) m/z 675.31048, $(M+Na)^+$; calcd for $C_{35}H_{48}O_8N_2SiNa$: 675.30777. Anal. calcd for $C_{35}H_{48}O_8N_2Si$: C, 64.41; H, 7.36; N, 4.29. Found: C, 64.44; H, 7.56; N, 4.08.

3.1.13. (3*S*,5*S*,1′*S*,2′*R*)-3-(1′,3′-Dibenzyloxy-2′-*t*-butyldimethylsiloxypropyl)-2,3-dihydro-5,6,7-trimethoxycarbonyl-5-methoxycarbonylmethyl-1*H*,5*H*-pyrazolo[1,2-*a*]pyrazol-1-one (37) and (5*S*,1′*S*,2′*R*)-2-acetyl-5-(1′,3′-dibenzyloxy-2′-*t*-butyldimethylsiloxy)-1-(1″,2″-dimethyloxycarbonylethylene)-pyrazolidin-3-one (39). A mixture of compound **34** (0.094 g, 0.20 mmol), DMAD (0.064 g, 0.44 mmol) and camphorsulfonic acid (2 mg, 0.01 mmol) in acetonitrile (5 ml) was stirred at room temperature for 3 h. Subsequently, solvent was evaporated and the residue separated on a silica gel column using hexane–ethyl acetate 1:1 v/v as an eluent to afford **37** (0.119 g, 79%) and **38** (7 mg, 6%), which was immediately acetylated with acetic anhydride–pyridine mixture (1:1) to give acetate **39** (8 mg).

Compound **37**: pale yellow syrup; $[\alpha]_D$=+45.4 (c 1.7, CH_2Cl_2); IR ($CHCl_3$): ν 1738, 1709 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.36–7.24 (m, 10H, 2×Ph), 4.79, 4.52 (2d, 2H, J=11.0 Hz, Bn), 4.56, 4.48 (2d, 2H, J=12.0 Hz, Bn), 3.94 (s, 3H, CO_2Me), 3.84–3.75 (m, 3H, H-5,1′,2′), 3.76, 3.76 (2s, 6H, $2CO_2Me$), 3.55 (s, 3H, CO_2Me), 3.51–3.44 (m, 2H, CH_2OBn), 3.28, 3.08 (2d, 2H, J=15.5 Hz, CH_2CO_2Me), 3.16 (dd, 1H, J=7.0, 17.5 Hz, H-4a), 2.75 (dd, 1H, J=9.5, 17.5 Hz, H-4b), 0.86 (s, 9H, *t*-Bu), 0.03, 0.00 (2s, 6H, 2Me); ^{13}C NMR ($CDCl_3$): δ 169.54, 169.37, 168.44, 162.57, 159.12, 138.19, 137.96, 135.64, 128.33, 128.26, 127.98, 127.75, 127.69, 127.65, 127.58, 117.79, 80.46, 76.88, 74.37, 73.39, 72.27, 71.59, 58.58, 53.30, 53.14, 52.14, 51.75, 37.9, 334.26, 25.80, 18.01, −4.53, −4.86. MS (LSIMS) m/z 755, $(M+H)^+$. Anal. calcd for $C_{38}H_{50}N_2O_{12}Si$: C, 60.47; H, 6.63; N, 3.71. Found: C, 60.33; H, 6.48; N, 3.67.

Compound **39**: colorless oil; $[\alpha]_D$=−19.3 (c 0.2, CH_2Cl_2); IR ($CHCl_3$): ν 1726, 1619 cm^{-1}; ^1H NMR ($CDCl_3$): δ

7.36–7.23 (m, 10H, 2×Ph), 4.94 (s, 1H, H-2″), 4.66, 4.43 (2d, 2H, *J*=11.5 Hz, Bn), 4.53, 4.48 (2d, 2H, *J*=10.0 Hz, Bn), 4.39 (m, 1H, H-5), 3.92 (m, 2H, H-1′,2′), 3.84, 3.66 (2s, 6H, $2CO_2Me$), 3.48 (m, 2H, H-3′a,3′b), 2.95 (d, 2H, H-4a,4b), 2.07 (s, 3H, Ac), 0.88 (s, 9H, *t*-Bu), 0.05, 0.04 (2s, 6H, 2Me); MS (LSIMS/HR) *m/z* 677.28730, $(M+Na)^+$; calcd for $C_{34}H_{46}N_2O_9SiNa$: 677.28703. Anal. calcd for $C_{34}H_{46}N_2O_9Si$: C, 62.38; H, 7.03; N, 4.28. Found: C, 62.42; H, 6.94; N, 4.11.

3.1.14. (3*S*,5*S*,1′*S*,2′*R*)-3-(2′-Acetoxy-1′,3′-dibenzyloxypropyl)-2,3-dihydro-5,6,7-trimethoxy carbonyl-5-methoxycarbonylmethyl-1*H*,5*H*-pyrazolo[1,2-*a*]pyrazol-1-one (40), (5*S*,1′*S*,2′*R*)-1-acetyl-5-(2′-acetoxy-1′,3′-dibenzyloxypropyl)-2-(1″,2″-dimethoxycarbonyl ethylene)-pyrazolidin-3-one (41) and (2*R*,4*R*,5*S*,6*S*)-9-acetyl-1,3-diaza-5-benzyloxy-4-benzyloxymethyl-2-methyloxycarbonyl-2-methoxycarbonylmethyl-3-oxa-8-oxo-bicyclo-[4.3.0]nonane (42). A mixture of compound **16** (0.070 g, 0.20 mmol), DMAD (0.064 g, 0.44 mmol) and camphorsulfonic acid (5 mg) in acetonitrile (5 ml) was stirred at room temperature for 3 h. Subsequently, solvent was evaporated and the residue acetylated with acetic anhydride–pyridine (1:1) mixture. The mixture of acetates was separated by chromatography using hexane–ethyl acetate 1:1 v/v as an eluent to afford three products **40** (0.103 g, 76%), **41** (0.006 g, 5%) and **42** (0.002 g, 2%).

Compound **40**: pale yellow syrup; $[\alpha]_D$=+66.5 (*c* 1.4, CH_2Cl_2); IR ($CHCl_3$): ν 1738, 1710 cm^{-1}; ^1H NMR (C_6D_6): δ 7.40–7.15 (m, 10H, 2×Ph), 5.14 (ddd, 1H, *J*=3.5, 4.5, 7.0 Hz, H-2′), 4.85, 4.60 (2d, 2H, *J*=11.0 Hz, Bn), 4.31, 4.24 (2d, 2H, *J*=12.0 Hz, Bn), 4.02 (dd, 1H, *J*=1.0, 7.0 Hz, H-1′), 3.78 (ddd, 1H, *J*=1.0, 7.5, 10.0 Hz, H-3), 3.56, 3.34, 3.30, 3.24 (4s, 12H, 4×OMe), 3.55 (dd, 1H, *J*=4.5, 10.5 Hz, H-3′a), 3.50 (dd, 1H, *J*=3.5, 10.5 Hz, H-3′b), 3.40 (s, 2H, CH_2CO_2Me), 3.17 (dd, 1H, *J*=7.5, 18.0 Hz, H-2a), 2.75 (dd, 1H, *J*=10.0, 18.0 Hz, H-2b), 1.69 (s, 3H, Ac); MS (LSIMS/HR) *m/z* 705.22992, $(M+Na)^+$; calcd for $C_{34}H_{38}N_2O_{13}Na$: 705.22716. Anal. calcd for $C_{34}H_{38}N_2O_{13}$: C, 59.82; H, 5.57; N, 4.10. Found: C, 59.77; H, 5.74; N, 4.06.

Compound **41**: colorless crystals; mp 49–51°C; $[\alpha]_D$=−50.2 (*c* 1.1, CH_2Cl_2); IR ($CHCl_3$): ν 1742, 1620 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.36–7.22 (m, 10H, 2×Ph), 5.11 (ddd, 1H, *J*=4.5, 4.5, 6.0 Hz, H-2′), 4.96 (s, 1H, H-2″), 4.67, 4.42 (2d, 2H, *J*=11.0 Hz, Bn), 4.52, 4.49 (2d, 2H, *J*=12.0 Hz, Bn), 4.30 (ddd, 1H, *J*=1.0, 2.5, 8.5 Hz, H-5), 4.04 (dd, 1H, *J*=2.5, 6.0 Hz, H-2′), 3.87, 3.67 (2s, 6H, 2×CO_2Me), 3.65 (dd, 1H, *J*=4.5, 11.0 Hz, H-2′a), 3.62 (dd, 1H, *J*=4.5, 11.0 Hz, H-2′b), 2.98 (dd, 1H, *J*=8.5, 17.5 Hz, H-4a), 2.86 (dd, 1H, *J*=1.0, 17.5 Hz, H-4b), 2.10, 2.09 (2s, 6H, 2Ac); MS (LSIMS/HR) *m/z* 605.2128, $(M+Na)^+$; calcd for $C_{30}H_{34}N_2O_{10}Na$: 605.2106. Anal. calcd for $C_{30}H_{34}N_2O_{10}$: C, 61.85; H, 5.84; N, 4.81. Found: C, 61.86; H, 6.00; N, 4.82.

Compound **42**: colorless syrup; $[\alpha]_D$=+53 (*c* 0.33, CH_2Cl_2); IR ($CHCl_3$): ν 1746, 1641 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.38–7.15 (m, 10H, 2×Ph), 4.68, 4.56 (2d, 2H, *J*=12.0 Hz, Bn), 4.61, 4.55 (2d, 2H, *J*=11.5 Hz, Bn), 4.14 (bt, 1H, H-5), 3.84, 3.66 (2s, 6H, 2×CO_2Me), 3.85–3.60 (m, 4H, H-4,6, CH_2OBn), 3.44, 3.26 (2d, 2H, *J*=16.0 Hz, CH_2CO_2Me), 3.18 (dd, 1H, *J*=8.0, 16.5 Hz, H-7a), 2.74 (dd, 1H, *J*=1.0, 16.5 Hz, H-7b), 2.23 (s, 3H, Ac); MS (LSIMS/HR) *m/z* 563.2041, $(M+Na)^+$; calcd for $C_{28}H_{32}N_2O_9Na$: 563.2007.

3.1.15. (2*R*,3*S*,4*S*,6*S*,7*S*,8*S*)- and (2*R*,3*R*,4*S*,6*S*,7*S*,8*S*)-1,11-Diaza-7-benzyloxy-6-benzyloxymethyl-2,3,4-trimethoxycarbonyl-2-methoxycarbonylmethyl-5-oxa-tricyclo[6.3.0$^{0.0}$]undecan-10-one (43 and 44). Compound **16** (0.178 g, 0.05 mmol), DMAD (0.142 g, 0.1 mmol) and *p*-TsOH (2 mg) in benzene (15 ml) were kept under reflux while the solvent (12 ml) was slowly distilled off. Subsequently, residue was separated on a silica gel using hexane–ethyl acetate 1:1 v/v as an eluent to yield two products **43** (0.06 g, 50%) and **44** (0.04 g, 30%).

Compound **43**: more polar; colorless oil; $[\alpha]_D$=+91.3 (*c* 0.4, CH_2Cl_2); IR ($CHCl_3$): ν 1740, 1701 cm^{-1}; ^1H NMR (C_6D_6): δ 7.25–7.05 (m, 10H, 2×Ph), 4.55 (ddd, 1H, *J*=2.5, 3.0, 8.5 Hz, H-6), 4.41, 4.29 (2d, 2H, *J*=11.5 Hz, Bn), 4.31, 4.17 (2d, 2H, *J*=12.0 Hz, Bn), 4.27 (m, 1H, H-8), 4.19 (s, 1H, H-3), 3.75 (dd, 1H, *J*=3.0, 8.5 Hz, H-7), 3.57 (dd, 1H, *J*=4.0, 17.5 Hz, H-9), 3.49 (dd, 1H, *J*=2.5, 11.0 Hz, CH_AH_BOBn), 3.47 (dd, 1H, *J*=3.0, 11.0 Hz, CH_AH_BOBn), 3.35, 3.32, 3.20, 3.05 (4s, 12H, 4×CO_2Me), 3.05 (2d, 2H, *J*=17.5 Hz, CH_2CO_2Me), 2.56 (dd, 1H, *J*=17.5 Hz, H-9′); MS (LSIMS/HR) *m/z* 663.21530, $(M+Na)^+$; calcd for $C_{32}H_{36}N_2O_{12}Na$: 663.21659. Anal. calcd for $C_{32}H_{36}N_2O_{12}$: C, 60.00; H, 5.62; N, 4.37. Found: C, 60.19; H, 5.81; N, 4.21.

Compound **44**: less polar; colorless oil; $[\alpha]_D$=+188.0 (*c* 0.2, CH_2Cl_2); IR ($CHCl_3$): ν 1740, 1703 cm^{-1}; ^1H NMR (C_6D_6): δ 7.30–7.05 (m, 10H, 2×Ph), 5.04 (s, 1H, H-3), 4.67 (dt, 1H, *J*=2.5, 2.5, 8.0 Hz, H-6), 4.43, 4.42 (2d, 2H, *J*=11.9 Hz, Bn), 4.31, 4.13 (2d, 2H, *J*=12.0 Hz, Bn), 4.25 (dt, 1H, *J*=3.5, 5.0, 5.5 Hz, H-8), 3.79 (dd, 1H, *J*=3.5, 8.0 Hz, H-7), 3.40 (dd, 1H, *J*=2.5, 11.5 Hz, CH_AH_BOBn), 3.38 (dd, 1H, *J*=2.5, 11.5 Hz, CH_AH_BOBn), 3.37, 3.28, 3.22, 3.15 (4s, 12H, 4×CO_2Me), 3.34, 3.16 (2d, 2H, *J*=17.5 Hz, CH_2CO_2Me), 3.18 (dd, 1H, *J*=5.0, 17.5 Hz, H-9), 2.45 (dd, 1H, *J*=5.5, 17.5 Hz, H-9′); MS (LSIMS/HR) *m/z* 663.21662, $(M+Na)^+$; calcd for $C_{32}H_{36}N_2O_{12}Na$: 663.21659. Anal. calcd for $C_{32}H_{36}N_2O_{12}$: C, 60.00; H, 5.62; N, 4.37. Found: C, 60.04; H, 5.74; N, 4.27.

3.1.16. (3*R*,5*R*,1′*R*,2′*S*)- and (3*R*,5*S*,1′*R*,2′*S*)-3-(2′-Acetoxy-1′-benzyloxy-propyl)-2,3-dihydro-5,6,7-trimethoxy-carbonyl-5-methoxycarbonylmethyl-1*H*,5*H*-pyrazolo-[1,2-*a*]pyrazol-1-one (48 and 49) and (5*R*,1′*R*,2′*S*)-5-(1′-benzyloxy-2′-hydroxypropyl)-1-(1″,2″-dimethoxy carbonylethylidene)-pyrazolidin-3-one (47). Pyrazolidinone **20** (0.050 g, 0.20 mmol) was treated with DMAD (0.064 g, 0.44 mmol) and camphorsulfonic acid (5 mg) in acetonitrile (5 ml) according to the procedure described for **40**. Chromatographic separation using ethyl acetate as an eluent led to three products **45** (0.68 g, 65%), **46** (0.064 g, 6%) and **47** (0.016 g, 4%). Compounds **45** and **46** were acetylated with acetic anhydride–pyridine mixture and characterized as acetates **48** and **49**.

Compound **47**: colorless crystals; mp 135–137°C;

$[\alpha]_D$=+330.5 (*c* 1.4, CH_2Cl_2); IR ($CHCl_3$): ν 3433, 1741, 1701 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.36–7.22 (m, 5H, Ph), 6.09 (bd, 1H, *J*=9.0 Hz, H-5), 4.54, 4.44 (2d, 2H, *J*=11.0 Hz, Bn), 3.99, 3.94 (2d, 2H, *J*=16.5 Hz, CH_2CO_2Me), 3.87, 3.66 (2s, 6H, 2×CO_2CH_3), 3.84–3.84 (m, 1H, H-2′), 3.82 (dd, 1H, *J*=1.0, 6.5 Hz, H-1′), 3.07 (dd, 1H, *J*=2.0, 16.5 Hz, H-4a), 2.76 (dd, 1H, *J*=9.0, 16.5 Hz, H-4b), 1.31 (d, 3H, *J*=6.0 Hz, CH_3), 1.23 (d, 3H, *J*=6.5 Hz, CH_3); MS (LSIMS/HR) *m/z* 393.16655, $(M+H)^+$; calcd for $C_{19}H_{25}N_2O_7$: 393.16618.

Compound **48**: pale yellow oil; $[\alpha]_D$=−75.5 (*c* 0.4, CH_2Cl_2); IR ($CHCl_3$): ν 1738, 1632 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.36–7.27 (m, 5H, Ph), 4.98 (dq, 1H, *J*=5.5, 6.5 Hz, H-2′), 4.79, 4.67 (2d, 2H, *J*=11.0 Hz, Bn), 3.95, 3.82, 3.76, 3.60 (4s, 12H, 4CO_2CH_3), 3.65 (ddd, 1H, *J*=1.0, 8.5, 9.0 Hz, H-3), 3.57 (dd, 1H, *J*=1.0, 5.5 Hz, H-1′), 3.24, 3.17 (2d, 2H, *J*=16.0 Hz, CH_2CO_2Me), 3.23 (dd, 1H, *J*=8.5, 17.5 Hz, H-2a), 2.76 (dd, 1H, *J*=9.0, 17.5 Hz, H-2b), 2.04 (s, 3H, Ac), 1.25 (d, 3H, *J*=6.5 Hz, CH_3); MS (LSIMS/HR) *m/z* 599.18779, $(M+Na)^+$; calcd for $C_{27}H_{32}N_2O_{12}Na$: 599.18529.

Compound **49**: pale yellow oil; $[\alpha]_D$=+291.5 (*c* 0.7, CH_2Cl_2); IR ($CHCl_3$): ν 1738, 1612 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.36–7.27 (m, 5H, Ph), 4.92 (dq, 1H, *J*=6.0, 6.5 Hz, H-2′), 4.81, 4.66 (2d, 2H, *J*=11.5 Hz, Bn), 4.48 (ddd, 1H, *J*=1.0, 8.0, 10.5 Hz, H-3), 4.12, 3.44 (2d, 2H, *J*=18.0 Hz, CH_2CO_2Me), 3.84, 3.75, 3.68, 3.67 (4s, 12H, 4×CO_2CH_3), 3.60 (dd, 1H, *J*=1.0, 6.0 Hz, H-1′), 3.21 (dd, 1H, *J*=8.0, 17.0, 5.5 Hz, H-2a), 2.87 (dd, 1H, *J*=10.5, 17.0 Hz, H-2b), 2.01 (s, 3H, Ac), 1.23 (d, 3H, *J*=6.5 Hz, CH_3); MS (LSIMS/HR) *m/z* 577.20511, $(M+H)^+$; calcd for $C_{27}H_{33}N_2O_{12}$: 577.20335.

3.1.17. (2*S*,3*R*,4*R*,6*R*,7*R*,8*R*)- and (2*S*,3*S*,4*R*,6*R*,7*R*,8*R*)-1,11-Diaza-7-benzyloxy-2,3,4-trimethoxy carbonyl-2-methoxycarbonylmethyl-6-methyl-5-oxa-tricyclo[6.3.$0^{0.0}$]-undecan-10-one (50 and 51). Compounds **50** and **51** were obtained according to the procedure described for **43** and **44**.

Compound **50**: 78%; colorless oil; $[\alpha]_D$=−87.0 (*c* 0.5, CH_2Cl_2); IR ($CHCl_3$): ν 1737, 1703 cm^{-1}; ^1H NMR (C_6D_6): δ 7.20–7.05 (m, 5H, Ph), 4.60 (dq, 1H, *J*=6.5, 8.0 Hz, H-6), 4.26 (ddd, 1H, *J*=3.0, 4.0, 6.0 Hz, H-8), 4.19, 4.12 (2d, 2H, *J*=12.0 Hz, Bn), 4.09 (s, 1H, H-3), 3.61 (dd, 1H, *J*=4.0, 18.0 Hz, H-9a), 3.35, 3.31, 3.23, 3.04 (4s, 12H, 4CO_2CH_3), 3.06, 2.85 (2d, 2H, *J*=18.0 Hz, CH_2CO_2Me), 2.91 (dd, 1H, *J*=3.0, 8.0 Hz, H-7), 2.59 (dd, 1H, *J*=6.0, 17.7 Hz, H-9b), 1.09 (d, 3H, *J*=6.5 Hz, CH_3); MS (LSIMS/HR) *m/z* 535.19370, $(M+H)^+$; calcd for $C_{25}H_{31}N_2O_{11}$: 535.19279.

Compound **51**: 22%; colorless oil; $[\alpha]_D$=−313.3 (*c* 0.3, CH_2Cl_2); IR ($CHCl_3$): ν 1740, 1704 cm^{-1}; ^1H NMR (C_6D_6): δ 7.20–7.06 (m, 5H, Ph), 5.08 (s, 1H, H-3), 4.84 (dq, 1H, *J*=6.5, 8.5 Hz, H-6), 4.38, 4.24 (2d, 2H, *J*=11.5 Hz, Bn), 4.19 (ddd, 1H, *J*=3.5, 4.0, 6.0 Hz, H-8), 3.43, 3.34, 3.29, 3.26 (4s, 12H, 4×CO_2CH_3), 3.36, 3.23 (2d, 2H, *J*=17.5 Hz, CH_2CO_2Me), 3.21 (dd, 1H, *J*=4.0, 17.5 Hz, H-9a), 3.02 (dd, 1H, *J*=3.5, 8.5 Hz, H-7), 2.55 (dd, 1H, *J*=6.0, 17.5 Hz, H-9b), 1.15 (d, 3H, *J*=6.5 Hz, CH_3); MS (LSIMS/HR) *m/z* 557.17499, $(M+Na)^+$; calcd for $C_{25}H_{30}N_2O_{11}Na$: 557.17473.

3.1.18. (3*R*,5*R*,1′*R*,2′*S*)-3-(1′-Acetoxy-2′-hydroxypropyl)-2,3-dihydro-5,6,7-trimethoxycarbonyl-5-methoxycarbonylmethyl-1*H*,5*H*-pyrazolo[1,2-*a*]pyrazol-1-one (52), (3*R*,5*R*,1′*R*,2′*S*)-3-(2′-acetoxy-1′-hydroxypropyl)-2,3-dihydro-5,6,7-trimethoxycarbonyl-5-methoxy carbonylmethyl-1*H*,5*H*-pyrazolo[1,2-*a*]pyrazol-1-one (53), (*Z*)-4-*O*-acetyl-2,3,6-trideoxy-3-[1′-*N*-(1′,2′-dimethoxycarbonylethylene)-hydrazino]-L-arabino-hexaldono-1,5-lactone (55), (3*R*,5*S*,1′*R*,2′*S*)-3-(1′-acetoxy-2′-hydroxypropyl)-2,3-dihydro-5,6,7-trimethoxy carbonyl-5-methoxycarbonylmethyl-1*H*,5*H*-pyrazolo[1,2-*a*]pyrazol-1-one (57), (*Z*)-(5*R*,1′*R*,2′*S*)-5-(1′-acetoxy-2′-hydroxypropyl)-1-(1′,2′-dimethoxycarbony-lethylene)-pyrazolidin-3-one (59), (*Z*)-(5*R*,1′*R*,2′*S*)-5-(1′-acetoxy-2′-hydroxypropyl)-2-(1′,2′-dimethoxycarbonylethylene)-pyrazolidin-3-one (61), (3*R*,5*R*,1′*R*,2′*S*)-3-(1′-acetoxy-2′-hydroxypropyl)-2,3,7,8-tetrahydro-5,6,7,8-tetramethoxycarbonyl-1*H*-pyrazolo[1,2-*a*] pyridazin-1-one (63), (8a*R*,8*R*,7*S*,5a*S*,5*R*,4*R*,3*S*)-8-acetoxy-2a,8b-diaza-octahydro-3,4,5,5a-tetramethoxycarbonyl-7-methyl-6-oxa-acenaphthylen-2-one (64). Compound **10** (0.34 g, 0.20 mmol) and anhydrous hydrazine (0.10 g, 0.30 mmol) in methanol were stirred at room temperature for 3 h. Subsequently, methanol was evaporated and residue dissolved in acetonitrile (15 ml) and treated with DMAD (0.064 g, 0.44 mmol) and *p*-TsOH (3 mg). The mixture was refluxed for 15 min. Subsequently, it was evaporated and separated on a silica gel column using hexane–ethyl acetate 1:1 v/v mixture followed by 3:7 v/v mixture as eluents. Following products were obtained: **52** (62%), 53 (8%), 55 (5%), 57 (3%), 59 (4%), 61 (5%), 63 (3.5%).

Compound **52**: pale yellow oil; $[\alpha]_D$=−110.4 (*c* 2, CH_2Cl_2); IR ($CHCl_3$): ν 3509, 1738 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.80 (dd, 1H, *J*=1.5, 8.0 Hz, H-1′), 4.58 (ddd, 1H, *J*=1.5, 8.5, 10.5 Hz, H-3), 3.97, 3.78, 3.70, 3.66 (4s, 12H, 4×CO_2CH_3), 2.13 (s, 3H, Ac), 1.24 (d, 3H, *J*=6.5 Hz, CH_3); MS (LSIMS/HR) *m/z* 487.15585, $(M+H)^+$; calcd for $C_{20}H_{27}N_2O_{12}$: 487.15640.

Compound **53**: pale yellow oil; $[\alpha]_D$=−108.4 (*c* 0.9, CH_2Cl_2); IR ($CHCl_3$): ν 3463, 1737, 1711 cm^{-1}; ^1H NMR (C_6D_6): δ 4.78 (dq, 1H, *J*=6.5, 7.5 Hz, H-2′), 3.82 (dd, 1H, *J*=1.5, 8.0 Hz, H-1′), 4.46 (ddd, 1H, *J*=1.5, 7.5, 11.0 Hz, H-3), 3.58, 3.30, 3.27, 3.23 (4s, 12H, 4×CO_2CH_3), 1.55 (s, 3H, Ac), 1.23 (d, 3H, *J*=6.5 Hz, CH_3); MS (LSIMS/HR) *m/z* 509.14036, $(M+Na)^+$; calcd for $C_{20}H_{26}N_2O_{12}Na$: 509.13834. Anal. calcd for $C_{20}H_{26}N_2O_{12}$: C, 49.38; H, 5.34; N, 5.76. Found: C, 49.45; H, 5.39; N, 5.83.

Compounds **52** and **53** were acetylated with acetic anhydride–pyridine (1:1) mixture and gave in each case the same product **54**: pale yellow crystals; mp 140–142°C; $[\alpha]_D$=−114 (*c* 1.6, CH_2Cl_2); IR ($CHCl_3$): ν 1741, 1709, 1635 cm^{-1}; ^1H NMR (C_6D_6): δ 5.23 (dd, 1H, *J*=1.5, 5.5 Hz, H-1′), 5.06 (dq, 1H, *J*=5.5, 6.5 Hz, H-2′), 3.77 (ddd, 1H, *J*=1.5, 8.5, 9.5 Hz, H-3), 3.56, 3.36, 3.28, 3.26 (4s, 12H, 4CO_2CH_3), 3.47, 3.39 (2d, 2H, *J*=15.5 Hz, CH_2CO_2Me), 2.95 (dd, 1H, *J*=9.5, 17.0 Hz, H-2a), 2.64 (dd, 1H, *J*=8.5, 17.0 Hz, H-2b), 1.83, 1.58 (2s, 6H, 2Ac), 0.94 (d, 3H,

J=6.5 Hz, CH_3); MS (LSIMS/HR) m/z 551.14722, $(M+Na)^+$; calcd for $C_{22}H_{28}N_2O_{13}Na$: 551.14891. Anal. calcd for $C_{22}H_{28}N_2O_{13}$: C, 50.00; H, 5.30; N, 5.30. Found: C, 50.05; H, 5.57; N, 5.15.

Compound **55** was characterized after acetylation with acetic anhydride-pyridine (1:1) mixture to give 56 as colorless crystals; mp 214–217°C; $[\alpha]_D$=−17.4 (c 1.3, CH_2Cl_2); IR ($CHCl_3$): ν 3331, 1746 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.84 (s, 1H, H-2″), 4.76 (dd, 1H, J=9.5, 10.0 Hz, H-4), 4.34 (dq, 1H, J=6.5, 9.5 Hz, H-5), 3.91, 3.64 (2s, 6H, 2×CO_2CH_3), 3.84 (m, 1H, H-3), 3.11 (dd, 1H, J=7.0, 18.5 Hz, H-2a), 2.75 (dd, 1H, J=10.0, 18.5 Hz, H-2b), 2.18, 2.08 (2s, 6H, 2Ac), 1.40 (d, 3H, J=6.5 Hz, CH_3); MS (LSIMS/HR) m/z 409.12264, $(M+Na)^+$; calcd for $C_{16}H_{22}N_2O_9Na$: 409.12230.

Compound **57** was characterized as di-acetate **58**; pale yellow oil, $[\alpha]_D$=+211.8 (c 0.4, CH_2Cl_2); IR ($CHCl_3$): ν 1745, 1621 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.06 (dd, 1H, J=1.5, 6.0 Hz, H-1′), 5.00 (quintet, 1H, H-2′), 4.48 (ddd, 1H, J=1.5, 9.0, 9.5 Hz, H-3), 3.99, 3.79, 3.70, 3.64 (4s, 12H, 4×CO_2CH_3), 3.21, 3.11 (2d, 2H, J=15.0 Hz, CH_2CO_2Me), 3.16 (dd, 1H, J=9.0, 17.5 Hz, H-2a), 2.82 (dd, 1H, J=9.5, 17.5 Hz, H-2b), 2.15, 2.06 (2s, 6H, 2Ac), 1.24 (d, 1H, J=6.0 Hz, CH_3); MS (LSIMS/HR) m/z 551.14857, $(M+Na)^+$; calcd for $C_{22}H_{28}N_2O_{13}Na$: 551.14891.

Compound **59** was characterized as tri-acetate **60**; colorless oil, $[\alpha]_D$=+25.5 (c 0.6, CH_2Cl_2); IR ($CHCl_3$): ν 1740, 1624 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.11 (s, 1H, H-2″), 5.09–5.03 (m, 2H, H-5,2′), 4.31 (dd, 1H, J=3.5, 8.5 Hz, H-1′), 3.93, 3.68 (2s, 6H, 2CO_2CH_3), 3.08 (dd, 1H, J=8.5, 17.5 Hz, H-4a), 2.69 (dd, 1H, J=0.5, 17.5 Hz, H-4b), 2.45 (s, 3H, NAc), 2.04 (s, 6H, 2Ac), 1.21 (d, 3H, J=6.5 Hz, CH_3); MS (LSIMS/HR) m/z 451.12949, $(M+Na)^+$; calcd for $C_{18}H_{24}N_2O_{10}Na$: 451.13116. Anal. calcd for $C_{18}H_{24}N_2O_{10}$: C, 50.46; H, 5.60; N, 6.54. Found: C, 50.33; H, 5.64; N, 6.54.

Compound **61** was characterized as tri-acetate **62**; colorless crystals; mp 53–55°C; $[\alpha]_D$=−11.5 (c 1.7, CH_2Cl_2); IR ($CHCl_3$): ν 1738, 1664 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.33 (dd, 1H, J=3.0, 5.5 Hz, H-1′), 5.14 (dq, 1H, J=3.0, 6.5 Hz, H-2′), 5.02 (bs, 1H, H-5), 3.81, 3.77 (2s, 6H, 2CO_2CH_3), 2.96 (dd, 1H, J=9.5, 17.0 Hz, H-4a), 2.65 (bd, 1H, J=17.0 Hz, H-4b), 2.17, 2.05, 2.04 (3s, 9H, 3Ac), 1.36 (d, 3H, J=6.5 Hz, CH_3); MS (LSIMS/HR) m/z 451.13131, $(M+Na)^+$; calcd for $C_{18}H_{24}N_2O_{10}Na$: 451.13287. Anal. calcd for $C_{18}H_{24}N_2O_{10}$: C, 50.46; H, 5.60; N, 6.54. Found: C, 50.61; H, 6.05; N, 6.34.

Compound **63**: colorless oil; $[\alpha]_D$=+42.7 (c 1.9, CH_2Cl_2); IR ($CHCl_3$): ν 3400, 1745 cm^{-1}; ^1H NMR (C_6D_6): δ 5.22 (dd, 1H, J=1.5, 8.0 Hz, H-1′), 4.51 (dt, 1H, H-4), 3.82 (d, 1H, J=3.0 Hz, H-9), 3.66, 3.45, 3.43, 3.41 (4s, 12H, 4CO_2CH_3), 3.29 (d, 1H, J=3.0 Hz, H-8), 3.16 (m, 1H, H-2′), 2.70 (dd, 1H, J=9.5, 16.5 Hz, H-3a), 2.44 (bd, 1H, J=16.5 Hz, H-3b), 1.86 (s, 3H, Ac), 0.82 (d, 3H, J=6.5 Hz, CH_3); MS (LSIMS/HR) m/z 509.14047, $(M+Na)^+$; calcd for $C_{20}H_{26}N_2O_{12}Na$: 509.13834.

3.1.19. (3*S*,4*R*,5*R*,5a*S*,7*S*,8*R*,8a*R*)-8-Acetoxy-2a,8b-diaza-octahydro-3,4,5,5a-tetramethoxy carbonyl-7-methyl-6-oxa-perhydroacenaphthylen-2-one (64). Compound **63** (0.050 g, 0.10 mmol) and *p*-TsOH (3 mg) were dissolved in toluene (15 ml) and refluxed for 0.5 h while 8 ml of toluene was distilled off. The residue was purified by chromatography to give **64** (0.039 g, 80%): colorless crystals; mp 181–183°C; $[\alpha]_D$=−56.7 (c 0.8, CH_2Cl_2); IR ($CHCl_3$): ν 1750 cm^{-1}; ^1H NMR (C_6D_6): δ 5.46 (d, 1H, J=5.0 Hz, H-3), 4.02 (ddd, 1H, J=9.5, 10.0 Hz, H-8), 4.52 (dq, 1H, J=6.5, 10.0 Hz, H-7), 3.82 (d, 1H, J=12.5 Hz, H-3), 3.66 (dd, 1H, J=5.0, 12.5 Hz, H-4), 3.40, 3.35, 3.34, 3.17 (4s, 12H, 4CO_2CH_3), 3.31 (dd, 1H, J=7.0, 9.5 Hz, H-8a), 2.48 (dd, 1H, J=7.0, 16.0 Hz, H-1a), 2.34 (d, 1H, J=16.0 Hz, H-1b), 1.50 (s, 3H, Ac), 1.10 (d, 3H, J=6.1 Hz, CH_3); MS (LSIMS/HR) m/z 487.15611, $(M+H)^+$; calcd for $C_{20}H_{27}N_2O_{12}$: 487.15640.

3.2. Assay of DD-carboxypeptidase activity

The enzyme activity was measured as described previously.[18,19] Samples for assay of the DD-carboxypeptidase activity consisted of 10 μl of DD-carboxypeptidase from *Saccharopolyspora erythraea* PZHTZ 64-575 (40 units/mg), 20 μl of substrate solution containing 4.52 mg/ml *N*α,*N*ε-diacetyl-L-lysyl-D-alanyl-D-alanine in 0.1 M phosphate buffer, pH 8.0 and 10 μl of 0.1 M phosphate buffer, pH 8.0. Standard sample contained 20 μl of D-alanine in distilled water.

Reaction mixture for assay of the DD-carboxypeptidase activity consisted of 60 μl of 0.05 mg/ml flavin adenine dinucleotide in 0.1 M phosphate buffer, pH 8.0, 10 μl of 0.05 mg/ml horseradish peroxidase (1230 units/mg) in distilled water, 5 μl of 5 mg/ml *o*-dianisidine in methanol, and 2 μl of 11.77 mg/ml D-amino acid oxidase from porcine kidney (6.7 units/mg) in 0.1 M phosphate buffer, pH 8.0.

Samples were incubated for 30 min at 37°C and then boiled for 2 min. After cooling, 77 μl of the reaction mixture was added, and all samples were incubated for 10 min at 37°C. Then to each sample was added 350 μl of mixture consisting of methanol, distilled water and sulfuric acid (5:5:6 by volume). Extinction of resulted solution was measured at 540 nm.

The inhibition of DD-peptidase 64-575 by the discussed above pyrazolidinones was evaluated.[20] Mixtures of 10 μl of DD-peptidase 64-575 (40 units/mg), 5 μl solution of pyrazolidinones in methanol and 5 μl of 0.1 M phosphate buffer, pH 8.0 were incubated for 45 min at 37°C. The concentration of pyrazolidinones in the mixture was from 0.025 to 0.00025 M. After incubation 20 μl of substrate solution was added to 20 μl of each sample and resulted mixtures were incubated again. The following pyrazolidinones were tested: **21**, **23**, **25**, **37**, **40**, **43**, **48**, **50**, **52**, **53** and **54**. At concentration of 0.025 M, none of tested compounds exhibited measurable inhibition activity.

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