Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1701–1705

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

One-Pot Synthesis and Biological Evaluation of Aspergillamides and Analogues

Barbara Beck,[a,b] Sibylle Hess[a] and Alexander Dömling[a,b,*]

[a]*Morphochem AG, Gmunderstr. 37-37a, 81379 München, Germany*
[b]*Institut für Organische Chemie und Biochemie der Technischen Universität München, Lichtenbergstr. 4, 85747 Garching, Germany*

Received 13 March 2000; accepted 26 May 2000

Abstract—A one-pot total synthesis of aspergillamide and analogues by a solution phase Ugi multi component reaction (MCR) is described. The reaction is easily performed in 96-well plates and offers a facile access to diverse aspergillamide analogue compound libraries. The antibiotic and cytotoxic activity of these compounds is measured. Several of them are more potent than the natural product.

The indole-3-ethenamide substructure is a common element in many bioactive natural products. Several examples are shown in Figure 1. Recently described natural products of that class are the aspergillamides isolated by Fenical et al. from *Aspergillus spec.* from a salt lake located on the Bahamas. These compounds show moderate cytotoxicity with an IC_{50} (HCT 116) = 16 μg ml^{-1}.[1]

As part of our program to discover new anticancer drugs and antibiotics with novel mechanisms of action we recognised the presence of the indole-3-ethenamide substructure in many biological active natural products as a possible pharmacophore. Herein we describe the total synthesis of one of these molecules, aspergillamide "via" a one-pot multi component reaction. This method of synthesis allows us to generate libraries of aspergillamide analogues with five points of diversity. The compounds were screened against two cancer cell lines and their antibiotic activities were evaluated. Some initial results are reported.

Complex natural products are normally synthesised by a sequence of steps that can be distinguished by convergent and divergent synthesis routes.[2]

Convergent synthesis routes show advantages over divergent or linear approaches with respect to speed, time, yields and reproducibility. On the level of reactions, usually one or two component reactions are applied to assemble more complex products from their simpler precursor. Reactions with an assembly of more than two starting materials are called multi component reactions (MCRs).[6,7] Often, MCRs are advantageous over two component reactions in terms of time, yield, complexity and diversity of products. Therefore MCRs are convergent in contrast to the divergent or linear two component reactions.

The most famous and versatile MCR is the Ugi four component (U-4CR) and related reactions.[8] Inspection of the backbone of aspergillamide reveals that it is ideally suited for MCR chemistry of the Ugi-type (Scheme 1).

Thus aspergillamide can be synthesised from *N*-acetylleucin, methylamine, phenylacetaldehyde and *E*/*Z*-3-(2-isocyanoethen)-indole. *E*/*Z*-3-(2-isocyanoethen)-indole can be readily synthesised from commercially available 3-formylindole and isocyanomethylphosphonic acid diethylester.[9] In order to study the influence of the substituents on the cytotoxic and antiproliferative activity we synthesised a library of aspergillamide and its analogues in 96-well plates (Scheme 2).[10]

In order to introduce more variability at the indole residue we synthesised seven different isocyanides based on similar heterocycles. Isocyanides in Table 1, **23**–**26**, were synthesised with a modified Horner–Emmons reaction and could be obtained as mixtures of *E*/*Z*-isomers.[11] Isocyanide **27** was prepared by condensation of TOSMIC with 3-formylindole according to the van Leusen procedure.[12]

All the 224 possible combinations of Schiff-bases, carboxylic acids, and isocyanides have been carried out as

*Corresponding author. Fax: +1-4989-7800-5555; e-mail: alexander.doemling@morphochem.de

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00305-X

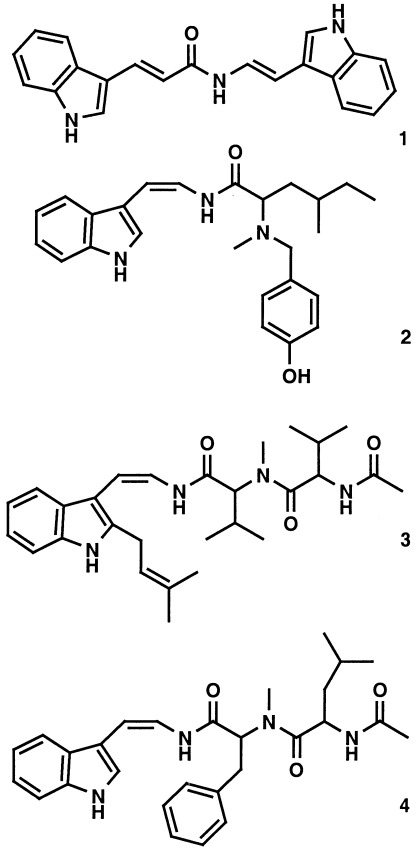


Figure 1. Some naturally occuring bioactive indole-3-ethenamides. Antifungal and cytotoxic chondriamide **1**,[3] fragilamide **2**,[4] cell cycle inhibitor terpeptin **3**,[5] and cytotoxic aspergillamide A **4**.[1] The structural similarity between the aspergillamides and terpeptin suggest they also act as cell cycle inhibitors.

one-pot reactions. The formation of the corresponding products were studied by HPLC-MS. Representative compounds have been also synthesised on a larger scale, purified and fully characterised.[13]

The expected products were formed in each well according to HPLC-MS, with the exception of **27**, which did not react under the conditions used.[14] With these compounds the biological screens were performed. In order to check for the ability of the compounds to reduce cell proliferation, the activity of acidic phosphatase was determined.[15] More than 50% of the compounds inhibited cell proliferation (< 50 μM). To determine an approximate IC_{50} selected compounds were screened at five different concentrations. The observed IC_{50} of the compounds screened with the cell line MCF-7 range between 100 μM and 100 nM (Table 2). Generally, they show a good fit with the activities recently described for Aspergillamides.[1] Microscopic inspection of the cell cultures showed the cytotoxic activity of the inspected compound class (Scheme 3).

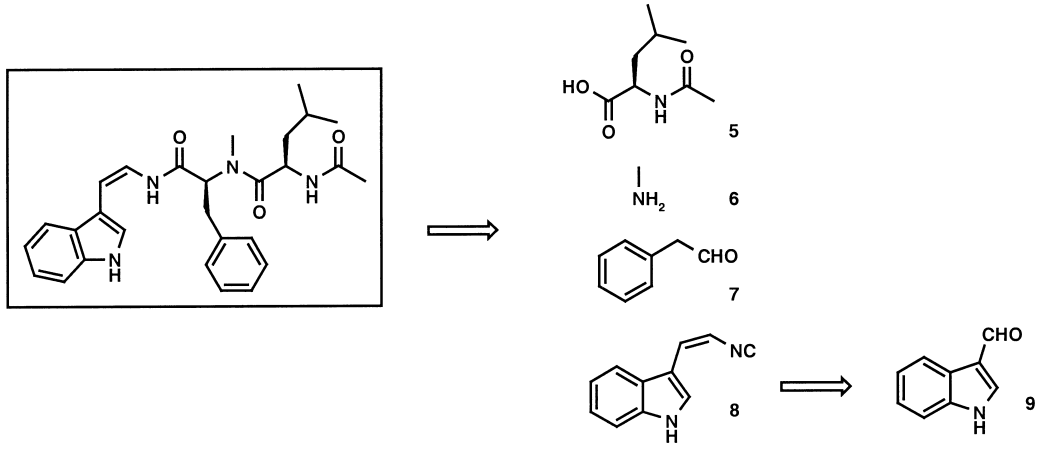
General cytotoxicity is often associated with lysis of the cell membranes. Therefore, we quantified the cytosolic enzyme lactate dehydrogenase released in the supernatant after incubation of MCF-7 with the compounds.[16] Interestingly, most of the compounds showed no general cytotoxic effects. Only several compounds that derive from the heterocyclic indole analogue isocyanide **23** show general cytotoxicity (Scheme 4).

Some of the compounds also exhibited strong antifungal and antibacterial activity. They were screened for their antibiotic activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Candida albicans*. Some of the compounds showed good activity (100% growth inhibition at a concentration lower than 100 μM) against the Gram positive *S. aureus* and *B. subtilis* (Scheme 5). Also some compounds were identified being *C. albicans* inhibitors (100% growth inhibition at a concentration lower than 100 μM) (Scheme 5).

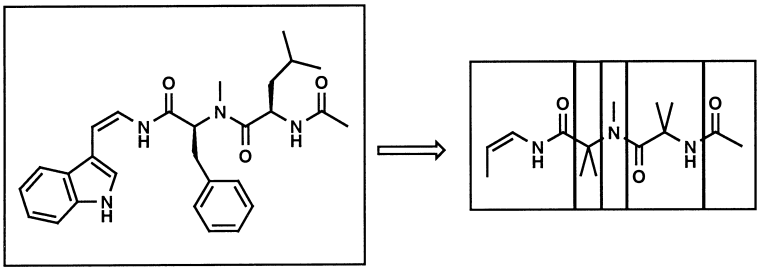
A detailed discussion of the compounds antibiotic activity is beyond the scope of this communication and will be published later.

In summary, we performed the first total synthesis of aspergillamides and analogues by a one-pot U-MCR, that is amenable to combinatorial chemistry. Their proliferating activity and the cell toxicity is in the same range of cytotoxicity as of the natural product aspergillamide.

A major draw back of the compounds synthesised is their diasteromeric nature. Therefore the measured IC_{50} values are only approximate.

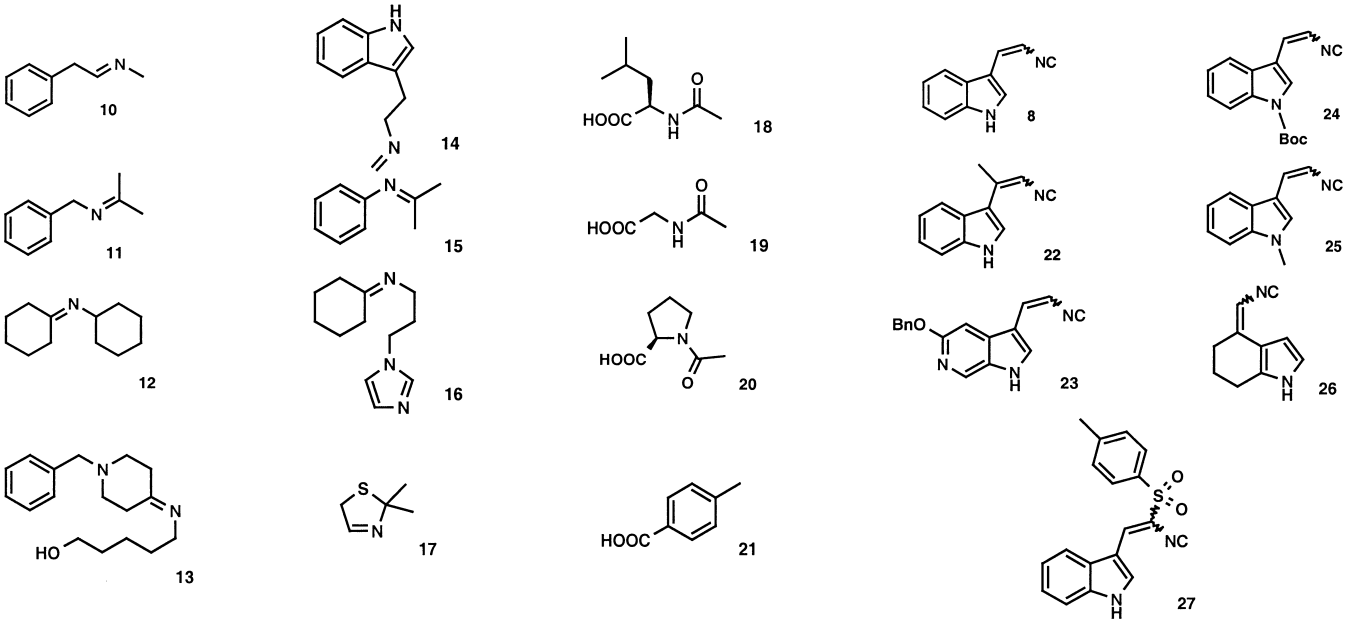


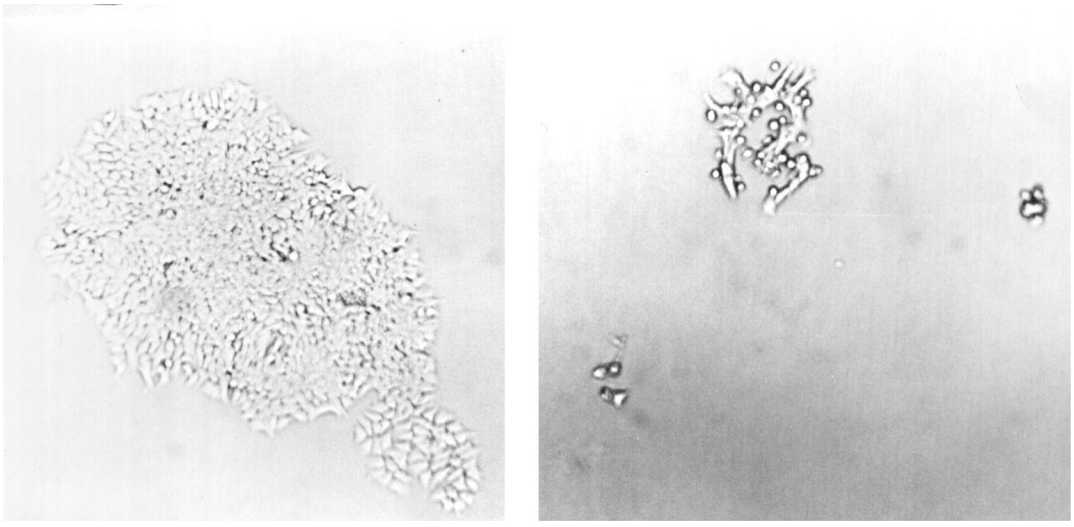
Scheme 1. Retrosynthetic analysis of the aspergillamide A total synthesis by U-MCR.



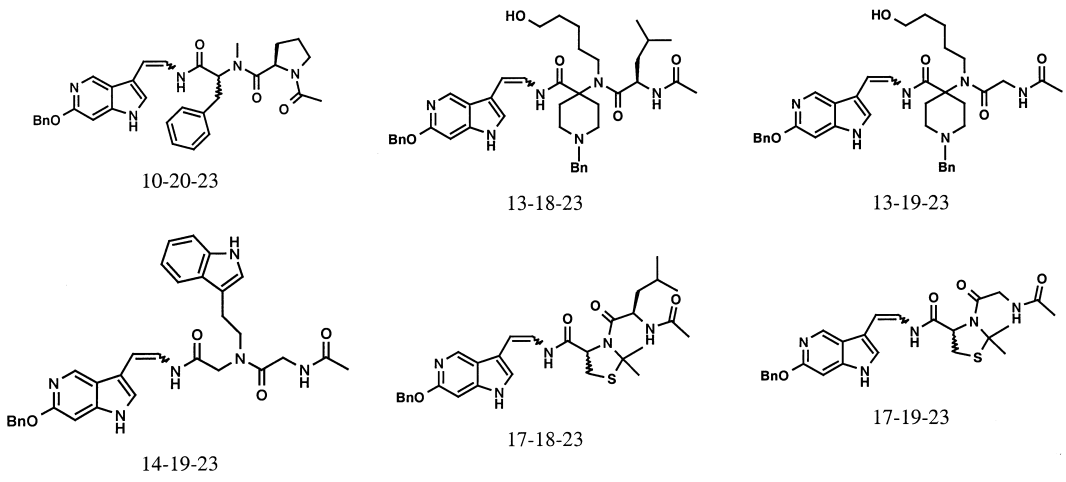
Scheme 2. The aspergillamide skeleton synthesised by a U-MCR can be divided in five parts: the α,β-unsaturated amide derived from the α,β-unsaturated isocyanide, the adjacent methylene unit which derived from the oxo component, the substituted secondary amide from the primary amine and the adjacent amino acid from the carboxylic acid and finally the acyl substituent.

Table 1. Sketch of the starting materials in order to obtain aspergillamide and their analogues. Eight different precondensed Schiff-bases **10**–**17**, four different carboxylic acids **18**–**21**, and seven different isocyanides **8**, **22**–**27** were used to prepare the library. Each of the wells were analysed by HPLC-MS and occasionally NMRs were taken[13]

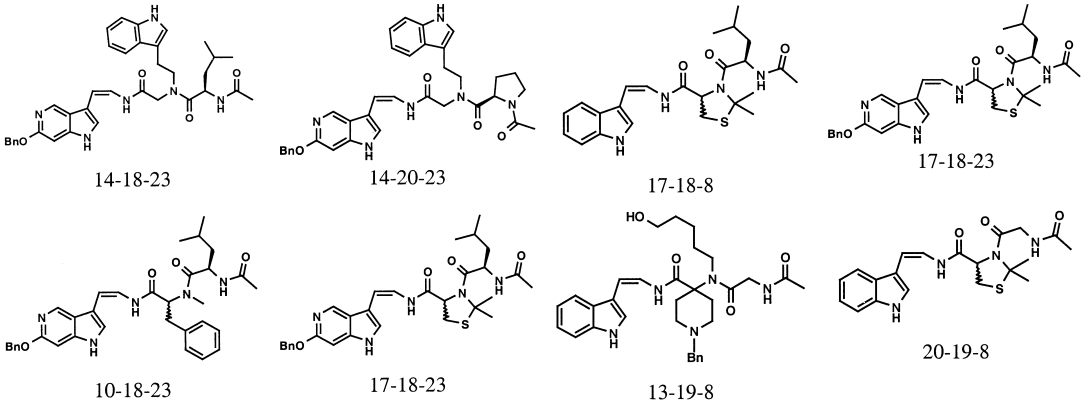




Scheme 3. Left photography (Axiovert 25 Zeiss, Jena, 1:1000); growth conditions: RPMI-1640-medium plus 5% FCS, 48 h, 37 °C, 5% CO_2: MCF-7 cells under normal growth conditions. The cells form a complete cell layer. Right photo: MCF-7 cell culture with compound 10–18–8. It shows the lack of integrity of the cell colony and change of morphology and thus attachment of cell from the substrate.



Scheme 4. Compounds showing general cytotoxicity by cell membrane lysis. This is measured by the liberation of lactate dehydrogenase. All have in common the pyridinoindole structure derived from isocyanide **23**.



Scheme 5. Compounds showing activity against Gram positive bacteria (first row) and against candida (second row).

Table 2. IC_{50} of some selected compounds against MCF-7. The compounds IDs are the numbers of the Schiff-base, the carboxylic acid and the isocyanide according to Table 1. At a concentration of 50 μM none of the compounds showed general cytotoxicity[14]

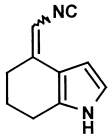
ID (SB–CA–IN)	IC_{50} (μM)
10–18–8	0.3
10–20–23	1.9
12–20–8	0.4
14–18–8	49.9
14–19–8	4.1

Studies are ongoing in our laboratory to determine the cell cycle inhibitory character of selected compounds out of this class of compounds related to the natural products aspergillamides.

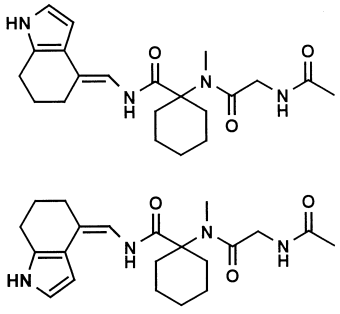
References and Notes

1. Toske, S. G.; Jensen, P. R.; Kauffman, C. A.; Fenical, W. *Tetrahedron* **1998**, *54*, 13459.
2. Nicolaou, K. C.; Sorensen, E. J. *Classics in Total Synthesis*, Wiley-VCH, New York, 1996.
3. Palermo, J. A.; Blanch Flower, P.; Seldes, A. M. *Tetrahedron Lett*. **1992**, *33*, 3097.
4. Kirkup, M. P.; Moore, R. E. *Tetrahedron Lett*. **1983**, *24*, 2087.
5. Kagamizono, T.; Sakai, N.; Arai, K.; Kobinata, K.; Osada, H. *Tetrahedron Lett*. **1997**, *38*, 1223.
6. Dömling, A.; Ugi, I. *Angew. Chem., Int. Ed. Engl*., **2000**, *39*, in press.
7. Dömling, A. *Comb. Chem. High Throughput Screening* **1998**, *1*, 1.
8. Ugi, I.; Dömling, A.; Hörl, W. *Endeavour*., **1994**, *18*, 115; Ugi, I., *J. Prakt. Chem*., **1997**, *339*, 499.
9. Hoppe, I.; Schöllkopf, U. *Liebigs Ann. Chem*., **1984**, 600.
10. Procedure (200 μM per well): 200 μL of a 100 mM solution of the aldehyde component in DCM and 200 μL of a 100 mM solution of the amine are dispensed in a 96-well plate. The solvent is evaporated over night and the Schiff-base is formed. 200 μL of a 100 mM solution of isocyanide in methanol and 200 μL of a 100 mM solution of the carboxylic acid in methanol are dispensed in the plate. The plate is shaken for 24 h and the solvent is evaporated in vacuum (Gene Vac). From the synthesis plates dilution plates are made for the HPLC-MS analytics and the biological screening.
11. General procedure for the synthesis of α,β-unsaturated isocyanides: All operations have to be done under nitrogen, free of moisture, and in the dark, to avoid photoisomerization. 3.3 mmol isocyanomethylphosphonic acid diethylester in 3 mL THF is dropped to a solution of 3 mmol sodium bistrimethylsilylamide in 5 mL THF at −78 °C. The solution is stirred for 15 min 3 mmol of a solution of the corresponding aldehyde or ketone in 30 mL THF is dropped to the above solution and the combined solutions are stirred for 20 h at 0 °C. The reaction is quenched with 3.3 mmol of acetic acid in

1.5 mL THF. The solvent is carefully evaporated in vacuum. The residue is taken up in 30 mL of ethyl acetate and washed with 15 mL phosphate buffer (pH 7) and 15 mL water. The organic phase is dried with magnesium sulfate and the solvent evaporated. The residual oil is purified by chromatography on silica gel with ether as eluent. Yields of the isocyanides: **8**: 41%, **22**: 35%, **23**: 57%, **24**: 65%, **25**: 20%, **26**: 14%. 1,5,6,7-Tetrahydro-*4H*-indol-4-vinyl-isocyanide **26**: M_w ($C_{10}H_{10}N_2$) = 158.20 g mol^{-1}. Yield: 65 mg (14%). The isomers could not be assigned. ^{1}H NMR (400 MHz:$CDCl_3$): δ = 8.00 (br, 1H, NH); 8.70 (d, 1H, pyrrol); 6.26 (d, 1H, pyrrol); 5.65 (s, 1H, vinyl-H); 2.59–1.03 (m, 6H, 3×CH_2). ^{13}C NMR (100 MHz:$CDCl_3$): δ = 140.19–114.43 (Pyrrol); 106.87 and 102.75 (**C**=CHNC, *E*- and *Z*-); 99.57 (**C**=CHNC); 29,72 (CH_2); 26.04 (CH_2); 22.97 (CH_2).



12. van Leusen, A. M.; Wildeman, J.; Oldenziel, O. H. *J. Org. Chem.* **1977**, *42*, 1153.
13. Typical procedure (1 mmol): A solution of each of precondensed Schiff-base, carboxylic acid and isocyanide in 1 mL methanol are stirred for 24 h. The solvent is evaporated and the product is extracted from the residue with ethyl acetate. The solvent is evaporated and the residue is purified by chromatography with ethyl acetate as eluent. 1-[(Acetylamino)acetyl](methyl) amino]-*N*-(1,5,6,7-tetrahydro-*4H*-indole-4-ylidenemethyl)-cyclohexanecarboxamide.M_w ($C_{21}H_{30}N_4O_3$) = 386.50 gmol^{-1}. Yield: 162.3 mg (42%) (total of *E*- and *Z*-derivative, which are formed in a 1:1 ratio). *E*-derivative: ^{1}H NMR (400 MHz:CD_3OD): δ = 6.69 (d, 1H, pyr); 6.62 (s, 1H, vinyl), 6.11 (d, 1H, pyr); 4.08 (s, 2H, CH_2); 3.73 (s, 3H, N-CH_3); 3.09 (s, 3H, CO–CH_3); 2.66–1.54 (m, 16H; CH_2, cyclohexyl). ^{13}C NMR (100 MHz:CD_3OD): δ = 173.66 (C=O); 173.24 (C=O); 171.70 (C=O); 131.17 (C); 121.28 (C); 118.47 (CH); 111.87 (CH); 104.86 (=CHNH-); 66.87 (C=CHNH); 44.65 (CH_2); 33.25 (CO–CH_2–NH); 31.84 (N-CH_3), 26.59–23.97 (CH_2); 22.76 (CO-CH_3). *Z*-derivative: ^{1}H NMR (400 MHz:CD_3OD): δ = 6.52 (d, 1H, pyr); 6.21 (s, 1H, vinyl); 6.09 (d, 1H, pyr); 4.06 (s, 2H, CH_2); 3.34 (s, 3H,N–CH_3); 3,05 (s, 3H, CO–CH_3); 2.60–1.51 (m, 16H; CH_2, cyclohexyl).



14. Products of Ugi-reaction are generally obtained as mixtures of diastereomers.
15. Yang, T.; Sinai, P.; Kain, S. R. *Analytic. Biochem.* **1996**, *241*, 103.
16. Promega-Kit, CytoTox 96® Non-Radioactive Cytotoxicity Assay, Cat.Nr.: G1780.