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Stereo-Random Synthesis of Highly Functionalized Proline Analogues by Azomethine Cycloaddition

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Abstract—Highly substituted proline analogues were synthesized on Wang-resin bearing bisprotected histidine as starting material. The proline analogues (1,5-diazabicyclo[3.3.0]octane-2-carboxylic acid) were generated by 1,3-dipolar cycloaddition of azomethine ylides with maleimides, thus creating a library of maximum stereochemical diversity. Every compound set with the same empirical formula can theoretically consist of four diastereomers and can be tested in biological assays as mixture. Additionally different methods for the acylation of the proline nitrogen were evaluated. © 2000 Elsevier Science Ltd. All rights reserved.

Combinatorial libraries offer a viable tool for the discovery of new biological active compounds. One of the most compelling approaches to highly structurally diverse molecules is multicenter reactions.[1] Especially the in situ formation of azomethine ylides and their subsequent transformation to highly substituted pyrrolidines creates a high degree of functional as well as stereochemical diversity in a single step. Stimulated by different groups[2–5] we utilized this approach for the stereo-random synthesis of a library of proline analogues **1** (Fig. 1).

To date, only bifunctional amino acids have been employed in the literature. [3,4] We were especially interested in stereodefined histidine analogues, thus requiring a protocol compatible with an imidazole protecting group such as N_{im}-trityl or N_{im}-Boc.

Thermally induced cycloaddition was ruled out, due to the putative instability of the above mentioned protecting groups under these conditions. Instead, silver induced azaylide formation as described by Gallop et al. was employed.[2]

Starting from Fmoc-His(Trt)-OH loaded Wang-resin and deprotection with 20% piperidine in DMF the liberated amino-group was reacted with ten different aromatic or heteroaromatic aldehydes under imine formation conditions.[6] Silver nitrate and triethylamine in acetonitrile[2,7–9] were added and an azomethine ylide was formed, which underwent a 1,3-dipolar cycloaddition [2,10–12] with three structurally diverse maleimides. Actually only 26 of 30 of the envisioned products were isolated after cleavage from the resin using 25% trifluoroacetic acid in dichloromethane (Scheme 1, Table 1).[13]

The crude products were purified via C18 RP-HPLC with an acetonitrile/water gradient. All fractions collected showed correct molecular mass in ion spray mass spectrometry, but ^{1}H NMR spectroscopy expectedly revealed mixtures of diastereomers with one diastereomer being predominant. Pure diastereomers were obtained from **2a**, **2b**, **2d**, **2e**, **3b**, **3c**, **3d**, **3h**, **3i**, **4b**, **4c**, **4d**, **4e**, **4g**, **4h**. However, the separation failed in the remaining cases.

In an additional set of experiments *N*-carbamoylmaleimide was introduced as the dienophile according to Scheme 1. Due to sparse solubility of the maleimide, acetonitrile had to be replaced by DMF. Only the 2-formylfuran and 2-formylpyrrole derived azomethine ylides reacted with *N*-carbamoylmaleimide yielding the desired cycloaddition products. During the cleavage from the resin with 25% trifluoroacetic acid in dichloromethane, concomitant loss of the carbamoyl moiety took place yielding the respective unsubstituted succinimides as detected by mass spectrometry and confirmed by ^{1}H- and ^{13}C NMR-spectroscopy (Scheme 2).

For introducing a further element of diversity, we envisioned acylation of the free pyrrolidine N-H. Consequently, Fmoc-Gly-OH was coupled to **4f**, using TBTU/

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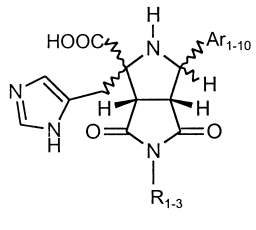

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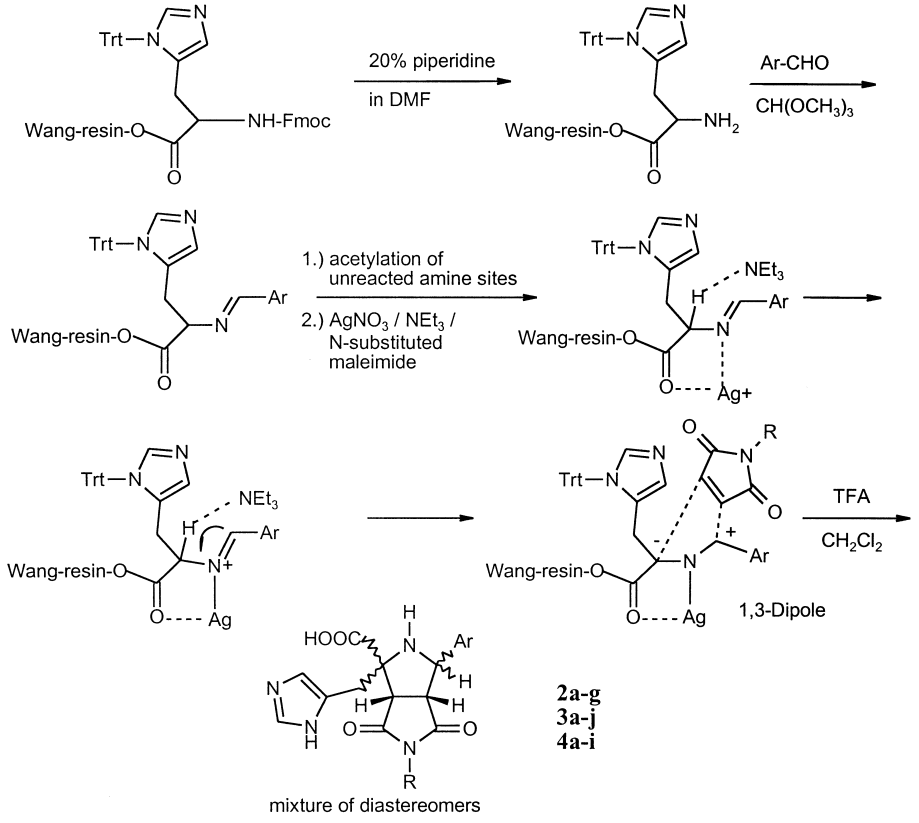
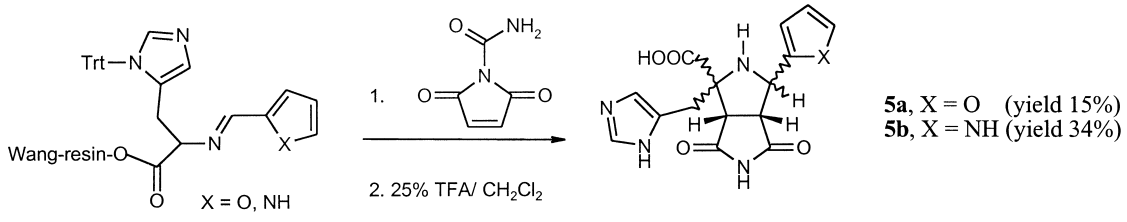
Figure 1. Field syntheses of 1,5-diazabicyclo[3.3.0]octane-2-carboxylic acids.

NMM, TFFH/NMM, HATU/NMM and DIC in DMF as coupling reagents (10-fold excess/overnight).[14] The coupling rates were determined by the method of Meienhofer yielding 21, 16, 35 and 43% of acylated product **6a** (Scheme 3).[15]

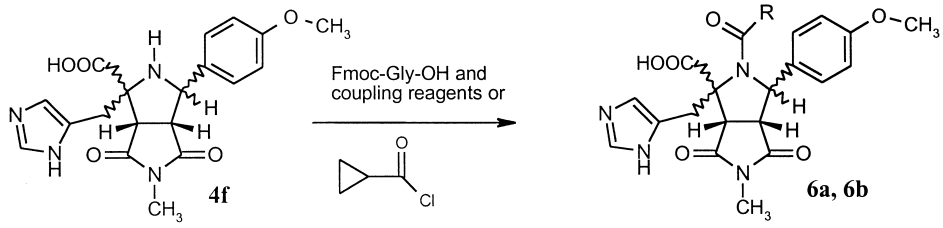
Additionally, acylation with cyclopropanecarbonyl chloride/NMM in dichloromethane was accomplished (10-fold excess/overnight), yielding **6b** (65% yield) as determined by HPLC after cleavage from the resin.



Scheme 1. Field synthesis scheme for 1,5-diazabicyclo[3.3.0]octane-2-carboxylic acids on Wang-resin, Ar = aromatic or heteroaromatic moiety (see Table 1), R see Table 1.



Scheme 2.



Scheme 3. Acylation of the proline nitrogen with Fmoc-glycine (R = CH_2NH-Fmoc, **6a**) and cyclopropanecarbonyl chloride (R = cyclopropanecarbonyl, **6b**).

Table 1. Yields (%) of the diastereomeric mixtures of the respective compounds.

R/Ar	*p*-Bromophenyl	Phenyl	Methyl
4-Fluorophenyl	**2a**/31	**3a**/40	**4a**/56
2,4-Dichlorophenyl	—	**3b**/75	**4b**/32
2-Furanyl	**2b**/60	**3c**/34	**4c**/42
4-Carboxymethylphenyl	—	**3d**/33	**4d**/52
3-Indolyl	—	**3e**/30	**4e**/63
4-Methoxyphenyl	**2c**/51	**3f**/26	**4f**/64
4-(Dimethylamino)phenyl	**2d**/81	**3g**/82	**4g**/75
2-Thienyl	**2e**/58	**3h**/64	**4h**/52
2-Pyrrolyl	**2f**/68	**3i**/52	—
4-Pyridyl	**2g**/31	**3j**/42	**4i**/66

In conlusion we have developed a synthetic protocol for proline analogues, which allows the incorporation of amino acids bearing sensitive side-chain protecting groups. According to mass spectrometry apparently pure highly-functionalized 1,5-diazabicyclo[3.3.0]octane-2-carboxylic acids can be obtained, consisting of up to four diastereomers. Further substituent diversity can be introduced by the acylation of the proline nitrogen.

References and Notes

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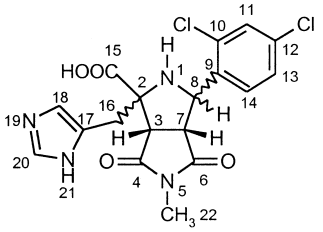
13. Typical procedure for the synthesis of **4b**, synthesis was performed on the robotic system SyRo II from MultiSynTech: Fmoc-cleavage: 200 mg Wang-resin loaded with Fmoc-His(Trt)-OH (0.085 mmol) were treated with 20% piperidine in DMF for 30 min. The resin was washed thoroughly with DMF and methanol and dried under vacuum.

Imine-formation and capping: Resin was first swollen in dichloromethane. Dichloromethane was filtered off and 0.44 g (2.5 mmol) 2,4-dichlorobenzaldehyde in 5 mL trimethylorthoformate was added to the resin. After 4 h of stirring at room temperature the reactants were filtered off and the resin washed once with trimethylorthoformate and three times with dichloromethane. Afterwards unreacted amino groups were capped with 8 mL of a mixture of acetic anhydride and *N*-ethyldiisopropylamine (3:1) for 15 min followed by several washes with dichloromethane.

Cycloaddition: 170 mg (1mmol) of silver nitrate in 2 ml of acetonitrile were added to the resin followed by the addition of 222 mg (2 mmol) of *N*-methylmaleimide in 2 mL of acetonitrile and 0.278 mL (2 mmol) of triethylamine. After 16 h of stirring the resin was washed twice with acetonitrile, five times with saturated ammonium chloride solution and three times with methanol. Cleavage and working-up: **4b** was cleaved off with 25% trifluoracetic acid in dichloromethane in 60 min. The cleavage mixture was filtered off and the resin washed with dichloromethane and methanol. The solvents were evaporated and the residue taken up in methanol. *t*-Butylmethyl ether was added to the solution forming a precipitate of crude **4b**. The precipitate was filtered off and again dissolved in methanol. Diluted hydrochloric acid was added causing a precipitate of silver chloride which was removed by filtration of the solution over diatomeous earth. The resulting solution was chromatographed by HPLC using a C18-column. Yield: 23mg (32%) **4b**.



^{1}H NMR (CD_3OD): H22 (1H, s, 2.75 ppm), H20 (1H, s, 8.80 ppm), H18 (1H, s, 7.37 ppm), H16 (2H, m, 3.55–3.60 ppm), H14 (1H, d, 7.28 ppm), H13 (1H, d, 7.47 ppm), H11 (1H, s, 7.50 ppm), H8 (1H, d, 5.15 ppm), H7 (1H, m, 4.03 ppm), H3 (1H, d, 3.39 ppm). ^{13}C NMR (CD_3OD): C4,C6 (176.8 ppm, 176.0 ppm), C15 (173.4 ppm), aromatic carbons C20, C18, C17, C9-C14 (135.9 ppm, 135.6 ppm, 135.1 ppm, 134.9 ppm, 130.1 ppm, 129.9 ppm, 129.5 ppm, 128.2 ppm, 119.3 ppm), C2 (72.2 ppm), C8 (59.5 ppm), C7 (55.9 ppm), C3 (under CD_3OD-signal), C16 (31.4 ppm), C22 (27.2 ppm).

14. DIC = *N*,*N*-diisopropylcarbodiimide, HATU = (*N*-[(dimethylamino)-1H-1,2,3-triazol[4,5-*b*]pyridin-1-ylmethylene]-*N*-methyl-methanaminium hexafluorophosphate *N*-oxide), NMM = *N*-methylmorpholine, TBTU = *O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate, TFFH = tetramethyl fluoroformamidinium hexafluorophosphate.
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