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Formation and Isolation of Simple, Stable, Acyclic Di- and Tripeptide Hemiacetals.

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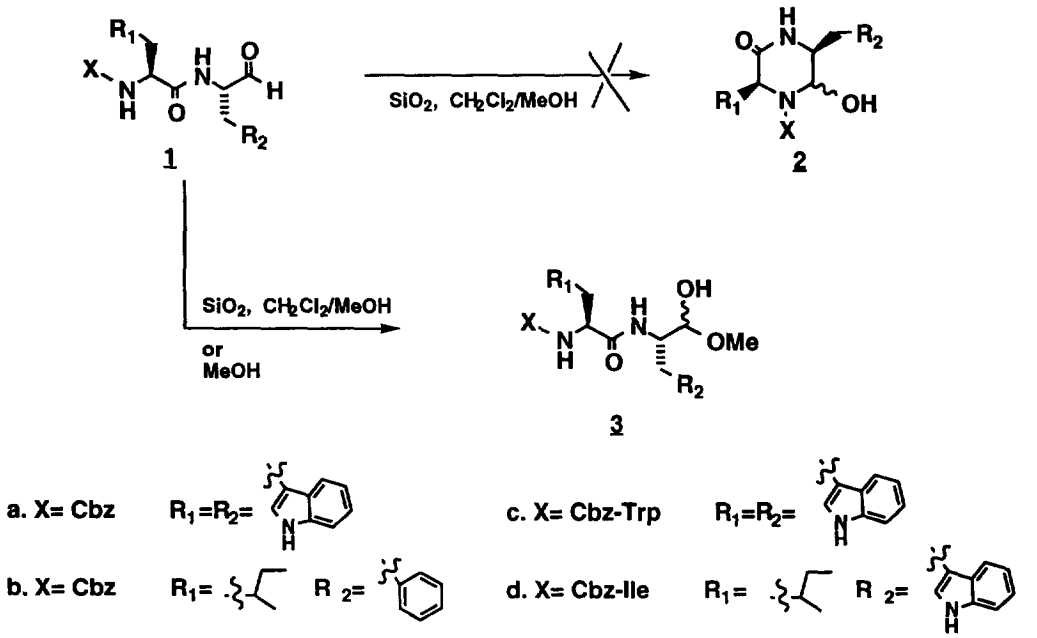
Abstract: *Di- and tripeptide aldehydes were found to undergo reaction with methanol to afford the corresponding methyl hemiacetals. These compounds, which possessed unusual stability, were isolated and characterized using NMR techniques and a chemical correlation experiment.* Copyright © 1996 Elsevier Science Ltd

Hemiacetals and hemiketals are often implicated as reactive intermediates in various processes involving aldehydes and ketones. However, while cyclic hemiacetals and -ketals are well known (many monosaccharides exist as cyclic structures), their acyclic counterparts are generally considered to be quite unstable and resistant to isolation.[1] Exceptions to this generalization include hemiacetals of polyhaloaldehydes and -ketones, and of cyclopropanones[2] and one report of the spontaneous crystallization of the dodecyl hemiacetal of dodecyl aldehyde.[2b] We now report the isolation of simple acyclic di- and tripeptide hemiacetals from the reaction of the corresponding peptide aldehydes with methanol under neutral conditions.

Our synthetic studies of serine and cysteine protease inhibitors led us to the preparation of a number of di- and tripeptide aldehydes **1** possessing a variety of amino acid substitutions. These compounds, which were typically prepared by LAH reduction of the corresponding Weinreb amides,[3] were found to undergo partial conversion to some hydroxyl-bearing species during silica gel chromatography with a CH_2Cl_2-MeOH eluent. Our initial assumption was that the aldehydes were undergoing a silica gel catalized cyclization to hemiaminals **2**. This assumption seemed reasonable in light of our earlier observation that dipeptide aldehydes undergo a facile acid-catalized cyclization / dehydration to the corresponding 5,6-dehydro 2-ketopiperazines.[4] The distinguishing spectral feature was the appearance of two hydroxyl doublets at δ 6.0-6.5 in the ^{1}H-NMR (DMSO-d_6) corresponding to the two presumed diastereomers of **2**.[5]

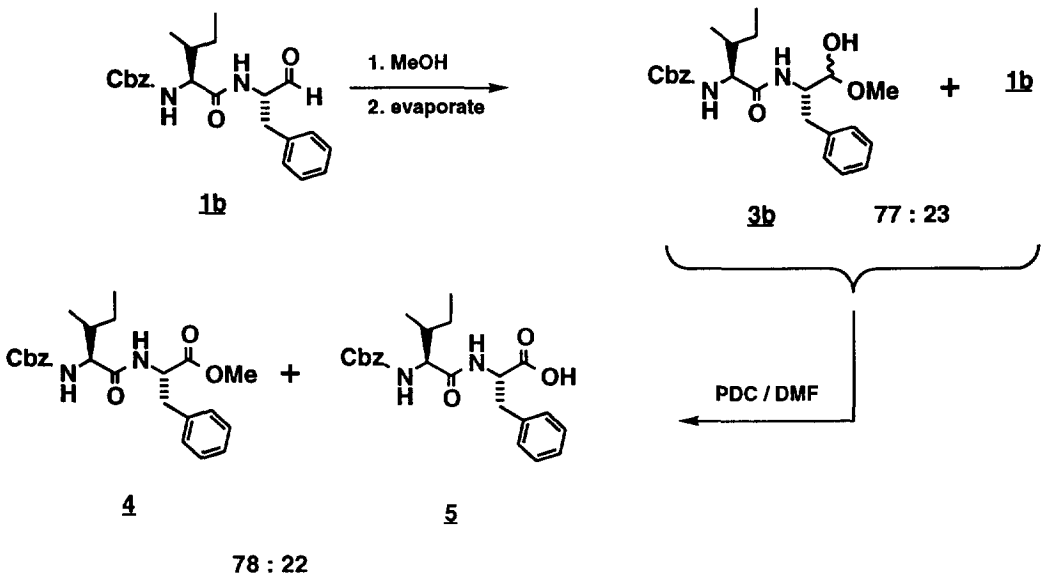


Scheme 1

More detailed spectroscopic examination of our products however revealed that the residual water peak at 3.3 ppm (DMSO-d_6) was obscurring an additional singlet which integrated for three hydrogens. The presence of this resonance and its chemical shift led us to conclude that our compounds were methyl hemiacetals **3**. In order to more fully characterize these compounds, we decided to prepare a larger batch of the putative hemiacetal **3a**. Silica gel flash chromatography (CH_2Cl_2/MeOH, 95:5) of a crude sample of Cbz-TrpTrp-H, **1a**, followed by concentration of combined fractions afforded an 85:15 mixture of methyl hemiacetal and aldehyde.[6] Rigorous structure determination was carried out using ^{1}H-NMR, 2-D NMR (proton-carbon correlation), mass spectrometry, and elemental analysis. Subsequent experiments indicated that hemiacetal formation was facile upon direct treatment of peptide aldehydes with methanol even in the absence of silica gel. Stirring a solution of **1a** in MeOH at rt for 2h followed by rotary evaporation afforded a 1:1 mixture of methyl hemiacetal and aldehyde. We felt that these reactions probably go to completion rapidly and that the aldehyde in the mixture is formed by decomposition of the hemiacetals with loss of MeOH during evaporation. To address this issue, solutions of Cbz-IlePhe-H, **1b**, Cbz-TrpTrpTrp-H, **1c**, and Cbz-IleIleTrp-H, **1d**, in CD_3OD were prepared and ^{1}H-NMR spectra obtained. All three samples showed complete conversion to hemiacetal within 1h as evidenced by the disappearance of the aldehyde C-H resonances.

Further corroboration of the hemiacetal nature of our compounds was realized through a chemical correlation experiment involving aldehyde **1b**. Stirring **1b** in MeOH for 3 days followed by

concentration *in vacuo* gave a 77: 23 mixture of hemiacetal **3b** and aldehyde.[7,8] Oxidation of the mixture with pyridinium dichromate in DMF afforded a 78:22 mixture of methyl ester **4** and carboxylic acid **5** thus demonstrating the presence of the methyl hemiacetal in the starting material.[9,10]



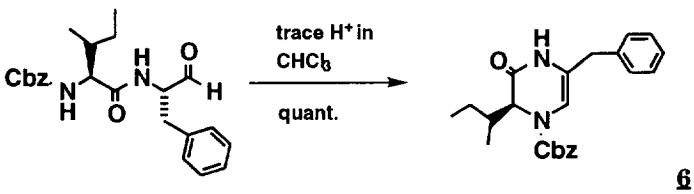
Scheme 2

In summary, we have demonstrated the formation of isolable di- and tripeptide hemiacetals by reaction of the corresponding aldehydes with methanol. Although the hemiacetal formation appears to go to completion even in the presence of only a 2-5-fold excess of methanol, to date we have not been able to isolate a 100% pure hemiacetal sample. Invariably, 10-20% of the material is converted back to the aldehyde upon evaporation of the solvent. Experiments to find conditions under which the hemiacetals crystallize, or precipitate more quantitatively are in progress. All the peptide aldehydes investigated showed similar properties with regard to ease of hemiacetal formation. These compounds, once isolated in the solid state, appear to be stable indefinitely at room temperature. The hemiacetal structure assignments were made on the basis of various spectroscopic data and a chemical correlation experiment.[11]

Acknowledgments. The authors would like to thank Mr. Ron Crouch for valuable assistance in the spectroscopic structure elucidation of our compounds.

References and Notes

1. Hurd, C. *J. Chem. Ed.*, **1966**,*43*, 527.
2. a. March, J. *Advanced Organic Chemistry*, 3rd Ed., Wiley-Interscience, **1985**, 790, and references cited therein. b. Erickson, J.L.E.; Campell Jr., C.R. *J. Am. Chem. Soc.*, **1954**, *76*, 4472.
3. Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.*, **1981**, *22*, 3815.
4. For example, Cbz-isoleucyl-phenylalaninal **1b**, quantitatively gave the corresponding cyclized compound **6** upon treatment with a trace amount of p-toluenesulfonic acid and standing in chloroform for 2 hours:



6

A closely related cyclization of an α-carbethoxy glycinal diethylacetal dipeptide has been reported: DiMaio, J.; Belleau, B. *J. Chem. Soc. Perkin Trans. I*, **1989**, 1687.
5. These ^{1}H-NMR signals vanished on treatment with D_2O.
6. ^{1}H-NMR (200MHz, DMSO-d_6): **3a**: δ 10.78 (br s, 2H, indole NH's), 7.82-6.91 (m, 17H, aromatic CH's, amide NH, carbamate NH), [6.44 (d, J=6.8 Hz) and 6.29 (d, J=7.0 Hz), 1H total, diastereomeric OH's], 4.96 (s, 2H, Cbz methylene), 4.50-4.22 (m, 2H, hemiacetal methine, Trp α-methine), 4.07 (m, 1H, C-teminal Trp α-methine), [3.31 (s) and 3.21 (s), 3H total, diastereomeric methoxys], 3.14-2.70 (m, 4H, Trp methylenes). Analysis: C,H,N
7. ^{1}H-NMR (200MHz, DMSO-d_6): **3b**: δ 7.67 (m, 1H, amide NH), 7.43-7.06 (m, 11H, aromatic CH's, carbamate NH), [6.43 (d, J=6.2Hz) and 6.34 (d, J=7.0 Hz), 1H total, diastereomeric OH's], 5.02 (s, 2H, Cbz methylene), 4.38 (m, 1H, hemiacetal methine),4.02-3.76 (m, 2H, Ile α-methine, Phe α-methine), [3.25 (s) and 3.24 (s), 3H total, diastereomeric methoxys], 3.02-2.71 (m, 2H, Phe methylene), 1.78-0.55 (m, 9H, Ile aliphatic CH's).
8. A sample of **1b** /CD_3OD solution was also prepared, concentrated to dryness, and examined by electrospray mass spectrometry. Prominent peaks were observed at m/e=397 (M+H for aldehyde) and m/e=455 (M+Na for trideutero methyl hemiacetal). This result eliminates the possibility that we were simply observing a cluster ion of aldehyde **1b** (M+Na+MeOH) resulting from reaction with the carrier solvent (methanol) in the spectrometer.
9. ^{1}H-NMR (200MHz, DMSO-d_6): **4**: δ 8.39 (d, J=7.0 Hz, 1H, amide NH), 7.44-7.10 (m, 11H, aromatic CH's, carbamate NH), 5.04 (s, 2H, Cbz methylene), 4.49 (m, 1H, Phe α-methine), 3.92 (m, 1H, Ile α-methine), 3.57 (s, 3H, OMe), 3.12-2.80 (m, 2H, Phe methylene), 1.78-0.55 (m, 9H, Ile aliphatic CH's). Mass Spectrum (Ion Spray): 427 $(M+H)^+$.
10. Spectral data for this compound was consistent with a commercial sample.
11. The biological activity in aqueous solution of these hemiacetals was found to be identical to that of the corresponding aldehydes in a Proteasome inhibition assay.[12]
12. Spaltenstein, A.; Leban, J.J.; Huang, J.J.; Reinhardt, K.R, Viveros, O.H.; Sigafoos, J.; Crouch, R. *Tetrahedron Lett.*, **1996**, *37*, 0000.

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