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1,3-DIPOLAR CYCLOADDITION REACTIONS TO GALANTHAMINE NITRONE: AN ENTRY TO 4a,5,9,10-TETRAHYDRO-6*H*,14a*H*-BENZOFURO[3a,3,2-*ef*]ISOXAZOLO[3,2-*a*]-[2]BENZAZEPINES

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Abstract – Starting with galanthamine nitrone as the 1,3-dipole derivatives of the novel 4a,5,9,10-tetrahydro-6*H*,14a*H*-benzofuro[3a,3,2-*ef*]isoxazolo[3,2-*a*][2]-benzazepine ring system have been prepared by cycloaddition.

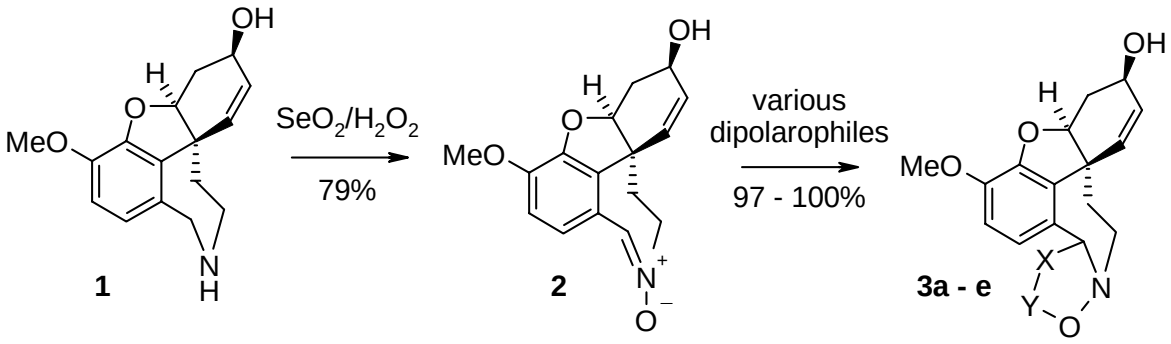
INTRODUCTION

Galanthamine (galantamine, Reminyl®) has been approved for anti-Alzheimer therapy in several countries and has been found to exhibit a dual mechanism by both AChE inhibition[1] and positive allosteric modulation of nicotinic receptors.[2]

For structure activity relationship studies of galanthamine derivatives and analogs the new annellated compounds (**3a - e**) have been prepared.

RESULTS AND DISCUSSION

(-)-Galanthamine nitrone (**2**) was prepared from (-)-norgalanthamine[3] (**1**) by treatment with a solution of hydrogen peroxide in acetone in the presence of catalytic amounts of selenium dioxide[4] as a colorless solid in 79% yield. 1,3-Dipolar cycloaddition to various alkenyl and alkynyl dipolarophiles gave rise to the isoxazole annellated compounds (**3a - e**) (see Scheme 1).



Scheme 1.

Such cycloadditions of nitrones are known to be broadly applicable for the construction of heterocyclic ring systems,[5] but bear the disadvantage of a lack of stereo- and regiochemical control.[6]

When galanthamine nitrone (**2**) and methyl propiolate were reacted in refluxing toluene, one defined compound (**3a**) was isolated in quantitative yield. By COSY and HMQC NMR spectra it could be proven that the ester moiety is attached to C-14. 1D NOESY experiments revealed a *vice versa* interaction of H-1 with H-14a and of H-4a with H-9 (see Figure 1).

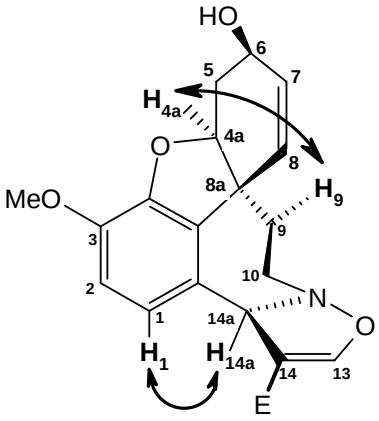


Figure 1. NOE interactions in **3a**.

Similarly, no formation of geometric isomers was detected in the reaction of **2** with cyano acetylene, yielding **3b** with identical regio- and stereochemistry to **3a** (reaction details see Table 1).

Above NOE interactions indicate a cisoid arrangement of the isoxazole moiety relative to C-8 and a boat conformation of the azepine ring for both **3a** and **3b**. The X-Ray analysis of **3a** verified this structural proposal (Figure 2). Further details on this crystal structure are given in the EXPERIMENTAL.

By using vinyl acetate as the dipolarophile, the cycloaddition again occurred with unequivocal regiochemistry in agreement with the literature,[7] but together with the major product (**3c**) traces of a second stereoisomer were formed. As we failed to obtain crystals of **3c** suitable for X-Ray analysis, a direct proof for its stereochemistry was not possible. However there is no reason why the approach of the vinyl group towards the nitrone moiety should not take place from the same (sterically less hindered) site

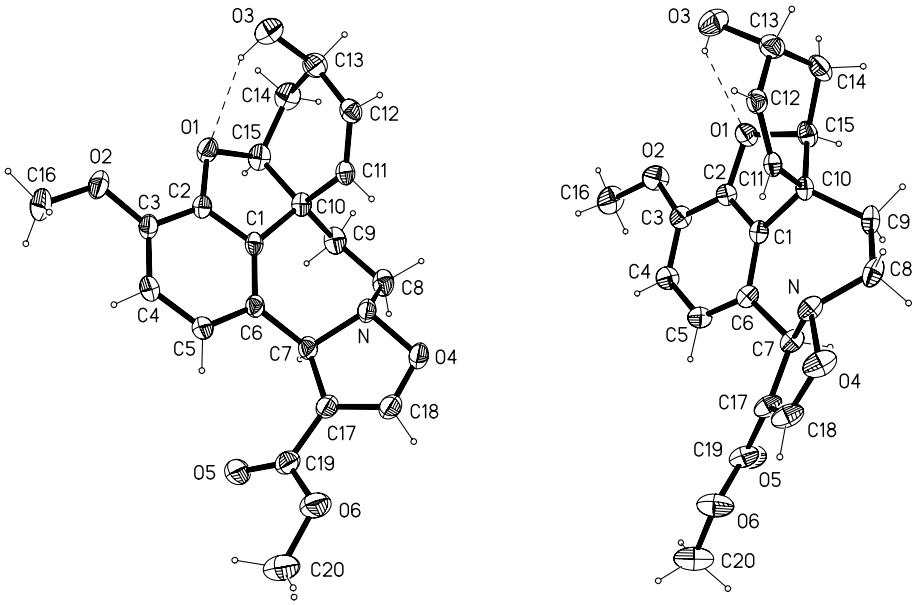


Figure 2. Molecular structure of **3a** in crystalline state (20% ellipsoids, crystallographic numbering) depicted in two views.

as observed for the acetylene group in the reactions leading to **3a** and **3b**. Therefore an analogous orientation of the isoxazole ring can be assumed for **3c**. The analysis of the coupling pattern of the isoxazole protons (which were assigned by a SELTOCSY experiment) and the results of NOE measurements are in full accordance with this assumption, additionally indicating an upward orientation of the acetoxy group (NOE between H-14 and H-14a, $J_{H\text{-}13,\ H\text{-}14} = 4.4$ Hz, $J_{H\text{-}13,\ H\text{-}14'} < 0.5$ Hz in accordance with a dihedral angle of ~ 90° in molecular modelling; see Figure 3).

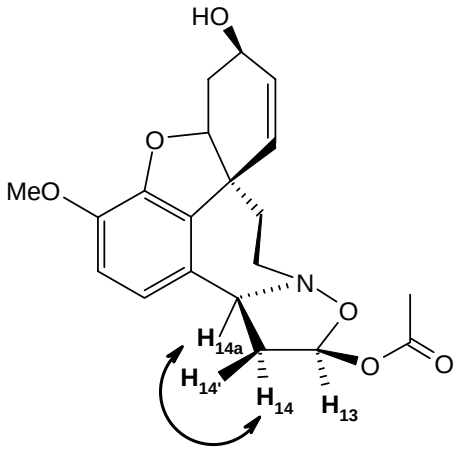


Figure 3. Structure of **3c**.

When methyl acrylate was reacted with galanthamine nitrone, quantitative conversion was obtained, but **3d** was isolated as mixture of four inseparable stereo- and regioisomers. No satisfying analysis and assignment of the obtained NMR spectra to determine the composition of the complex mixture was possible. A similar result was observed when acrylonitrile was employed for the preparation of **3e** (reaction details see Table 1).

Attempts to react acetonitrile,[8] diethyl azodicarboxylate, di-*tert*-butyl azodicarboxylate, maleic anhydride,[6] benzonitrile,[8] and ethyl acetylenedicarboxylate[7] with **2** failed.

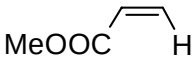
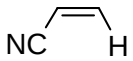
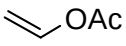
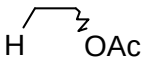
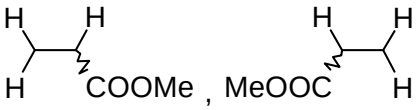
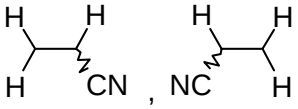
Compound	Dipolarophile	X--Y	Reaction time [h]	Yield [%]
3a			0.2	quant.
3b			168	97
3c			96	98
3d			48	99
3e			2	97

Table 1.

CONCLUSION

In summary, we have developed a strategy towards isoxazole annellated galanthamine derivatives as potential AChE inhibitors. Compounds (**3a - e**) exhibited neither acetylcholine- (AChE) nor butyryl-choline esterase (BChE) activity, whereas nitrone (**2**) showed low, but selective AChE inhibition (see Table 2).

Compound	IC_{50}-AChE [μmol]	IC_{50}-BChE [μmol]
Reminyl	2.26	18.00
2	50.00	no activity

Table 2. Biological data of **2**.

EXPERIMENTAL

General: Melting points were measured on a Kofler micro hot stage. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-200 or a Bruker Avance 400 FT-NMR spectrometer in $CDCl_3$ or DMSO-d_6 using tetramethylsilane as an internal standard. TLC was performed on precoated plates (Merck TLC

aluminum sheets silica 60 F_{254}) with detection by UV light or with phosphomolybdic acid in aqueous EtOH by heating. All reactions were magnetically stirred under an argon atmosphere. All liquid reagents were freshly distilled prior to use.

[4a*S*-(4aα,6β,8a*R)]-4a,5,9,10-Tetrahydro-3-methoxy-6*H*-benzofuro[3a,3,2-*ef*][2]benzazepin-6-ol 11-oxide (2).** Norgalanthamine (**1**) (4.25 g, 15.6 mmol) was dissolved in 35% H_2O_2 (7 mL)/acetone (63 mL) and cooled to 0 °C. SeO_2 (85 mg, 0.77 mmol) was added and stirred for 20 min at this temperature and then for 4 h at ambient temperature. The colorless precipitate was collected by filtration and triturated with acetone (2 x 5 mL). A second fraction of **2** was obtained by the following procedure: Water (10 mL) was added to the filtrate, the mixture was concentrated *in vacuo* to a volume of 15 mL, and the aqueous layer was extracted with CH_2Cl_2 (7 x 10 mL). The combined organic layer was washed with brine (1 x 20 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was recrystallized from acetone (1 mL). The combined crude product was triturated with i-Pr_2O (2 x 1 mL). Yield: colorless crystals (3.53 g, 79%); mp 232 - 233°C (i-Pr_2O); TLC: $CHCl_3$: MeOH : conc. NH_4OH = 89 : 10 : 1, R_f = 0.4; Anal. Calcd for $C_{16}H_{17}NO_4 \cdot 0.25\ H_2O$: C, 65.85; H, 6.04; N, 4.80. Found: C, 66.11; H, 6.05; N, 4.73. ^{1}H-NMR (200 MHz, DMSO-d_6): δ 7.82 (s, 1 H), 6.90 (s, 2 H), 5.81 (dd, *J* = 10.1, 4.4 Hz, 1 H), 5.54 (d, *J* = 10.1 Hz, 1 H), 4.64 (br s, 1 H), 4.36 (d, *J* = 5.5 Hz, 1 H), 4.14-4.02 (m, 2 H), 3.79 (s, 3 H), 2.39-1.99 (m, 4 H); ^{13}C-NMR (50 MHz, DMSO-d_6): δ 146.1 (s), 144.6 (s), 134.6 (d), 131.8 (s), 128.3 (d), 127.6 (d), 122.4 (d), 118.3 (s), 112.6 (d), 86.7 (d), 61.8 (d), 59.1 (t), 55.7 (q), 45.3 (s), 34.2 (t), 29.7 (t).

Methyl [4a*S*-(4aα,6β,8a*R,14a*S**)]-4a,5,9,10-Tetrahydro-6-hydroxy-3-methoxy-6*H*,14a*H*-benzofuro[3a,3,2-*ef*]isoxazolo[3,2-*a*][2]benzazepine-14-carboxylate (3a). 2** (200 mg, 0.70 mmol) and methyl propiolate (59 mg, 0.70 mmol) in dry toluene (5 mL) were stirred under reflux for 10 min and then concentrated *in vacuo*. The residue was purified by flash chromatography (20 g SiO_2, $CHCl_3$: MeOH = 90 : 10) and triturated with EtOH (2 mL). Yield: yellow crystals (261 mg, quant.); mp 151 - 154°C (EtOH); TLC: $CHCl_3$: MeOH = 90 : 10, R_f = 0.7; Anal. Calcd for $C_{20}H_{21}NO_6$: C, 64.68; H, 5.70; N, 3.77. Found: C, 64.59; H, 5.89; N, 3.67. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.54 (s, 1 H), 6.96 (d, *J* = 8.4 Hz, 1 H), 6.74 (d, *J* = 8.4 Hz, 1H), 5.96-5.77 (m, 2 H), 5.67 (s, 1 H), 4.55 (br s, 1 H), 4.10 (bs, 1 H), 3.85 (s, 3 H), 3.68 (s, 3 H), 3.59 (ddd, *J* = 14.3, 6.8, 3.8 Hz, 1 H), 3.30 (ddd, *J* = 12.9, 9.3, 3.4 Hz, 1 H), 2.64 (dd, *J* = 15.7, 3.7 Hz, 1 H), 2.15 (td, *J* = 7.7, 3.4 Hz, 1 H), 2.01 (ddd, *J* = 15.6, 5.3, 1.9 Hz, 1 H), 1.54 (ddd, *J* = 15.6, 6.7, 3.5 Hz, 2 H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 163.9 (s), 154.6 (d), 146.8 (s), 145.1 (s), 133.3 (s), 130.3 (d), 126.9 (d), 125.3 (s), 123.3 (d), 111.3 (d), 109.9 (s), 89.1 (d), 68.7 (d), 61.4 (d), 55.8 (q), 52.4 (t), 51.4 (q), 47.2 (s), 29.2 (t), 28.0 (t).

X-Ray Structure Determination of 3a

Crystal data: $C_{20}H_{21}NO_6$, M_r = 371.38, monoclinic, space group $P2_1$ (no. 4), a = 11.118(4) Å, b = 5.564(2) Å, c = 15.194(5) Å, β = 103.76(2)°, V = 912.9(6) Å^3, Z = 2, D_x = 1.351 Mg/m^{-3}, λ(Mo-Kα) =

0.71073 Å, μ = 0.100 mm^{-1}, T = 295(2) K. X-Ray data collection with a Bruker SMART CCD area detector diffractometer and graphite monochromatized Mo Kα radiation. 11036 reflections with θ < 27.0° were measured, corrected for *LP* and absorption, and merged to 3977 unique reflections, R_{int} = 0.022. Structure solution with direct methods, structure refinement on F^2 using program SHELXL97.[9] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms had isotropic temperature factors and rided on the atoms to which they were bonded. The final refinement varied 250 parameters and converged at $R1 = \Sigma||F_o|-|F_c||/\Sigma|F_o| = 0.049$, $wR2 = [\Sigma(w(F_o^2-F_c^2)^2)/\Sigma(w(F_o^2)^2)]^{½} = 0.102$, and S = 1.08 for the 3977 unique reflections; $R1$ = 0.038 for the 3401 observed data [$I>2\sigma(I)$].[10]

The molecular structure of **3a** in crystalline state is shown in Figure 2. The annellation of an isoxazol ring to the galanthamine skeleton has brought about a significant and unusual distortion of the azepine ring. Whereas in typical galanthamine type compounds[11] the azepine ring adopts a chair-type conformation with N, C8, and C9 (crystallographic atom designation, figure 2) located about 1 Å below the plane of the phenyl ring, these atoms are in **3a** by 0.64 Å (N) above and by 0.06 Å (C8) and 0.68 Å (C9) below this plane. The reason for this deviation from galanthamines is that the electron lone pair of nitrogen, which adopts a pyramidal coordination, is *syn*-oriented to spiro-cyclohexene ring in **3a** (pointing to C11-H11), whereas it is either *anti*- or *exo*- but not *syn*-oriented in usual galanthamines. Selected bond distances in **3a** are (Å; all e.s.d.'s 0.002-0.004 Å): O1-C2 1.377, O1-C15 1.478, O2-C3 1.366, O2-C16 1.430, O3-C13 1.443, O4-N 1.493, O4-C18 1.338, O5-C19 1.208, O6-C19 1.346, O6-C20 1.449, N-C8 1.465, N-C7 1.502, C1-C2 1.387, C1-C6 1.398, C1-C10 1.532, C2-C3 1.393, C3-C4 1.386, C4-C5 1.399, C5-C6 1.391, C6-C7 1.533, C7-C17 1.517, C8-C9 1.523, C9-C10 1.538, C10-C11 1.527, C10-C15 1.538, C11-C12 1.321, C12-C13 1.494, C13-C14 1.513, C14-C15 1.515, C17-C18 1.333, C17-C19 1.461. Hydrogen bond: O3 → O1 = 2.957 Å (intramolecular).

[4a*S*-(4aα,6β,8a*R,14a*S**)]-4a,5,9,10-Tetrahydro-6-hydroxy-3-methoxy-6*H*,14a*H*-benzofuro[3a,3,2-*ef*]isoxazolo[3,2-*a*][2]benzazepine-14-carbonitrile (3b). 2** (500 mg, 1.74 mmol) and cyanoacetylene (90 mg, 1.74 mmol) in dry toluene (10 mL) were stirred at ambient temperature for 168 h and then concentrated *in vacuo*. The residue was crystallized from MeOH (2 mL), and crude **3b** was collected by filtration. The filtrate was concentrated *in vacuo* and purified by flash chromatography (10 g SiO_2, $CHCl_3$: MeOH = 90 : 10). The combined product fractions were triturated with i-Pr_2O (2 mL). Yield: colorless crystals (570 mg, 97%); mp 137 - 139°C (i-Pr_2O); TLC: $CHCl_3$: MeOH = 90 : 10, R_f = 0.6; Anal. Calcd for $C_{19}H_{18}N_2O_4$: C, 67.45; H, 5.36; N, 8.28. Found: C, 67.17; H, 5.41; N, 8.19. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.09 (d, J = 8.6 Hz, 1 H), 6.76 (d, J = 8.6 Hz, 1 H), 5.98 (s, 2 H), 5.54 (s, 1 H), 4.52 (bs, 1 H), 4.11 (br s, 1 H), 3.83 (s, 3 H), 3.75-3.59 (m, 1 H), 3.42-3.25 (m, 1 H), 2.64 (dd, J = 15.9, 3.2 Hz, 1 H), 2.44 (d, J = 11.5 Hz, 1 H), 2.11-1.94 (m, 2 H), 1.71-1.52 (m, 1 H); ^{13}C-NMR (50 MHz, $CDCl_3$): δ 156.5

(d), 147.1 (s), 145.5 (s), 132.7 (s), 129.6 (d), 127.9 (d), 123.8 (s), 120.8 (d), 114.0 (s), 111.7 (d), 88.9 (d), 88.6 (s), 68.4 (d), 61.3 (d), 55.9 (q), 52.5 (t), 47.2 (s), 29.3 (t), 28.3 (t).

[4a*S*-(4aα,6β,8a*R,13*S**,14a*R**)]-4a,5,9,10,13,14a-Hexahydro-3-methoxy-6*H*,14a*H*-benzofuro-[3a,3,2-*ef*]isoxazolo[3,2-*a*][2]benzazepine-6,13-diol 13-acetate (3c).** **2** (200 mg, 0.70 mmol) and vinyl acetate (60 mg, 0.70 mmol) were stirred in in dry toluene (5 mL) at ambient temperature for 96 h. Every 24 h one equivalent of vinyl acetate (60 mg, 0.70 mmol) was added. The mixture was concentrated *in vacuo*, and the residue was purified by flash chromatography (10 g SiO_2, $CHCl_3$: MeOH = 90 : 10) and recrystallized from MeOH (0.5 mL). Yield: off-white crystals (256 mg, 98%), mp 132 - 134°C; TLC: $CHCl_3$: MeOH = 90 :10, R_f = 0.7; Anal. Calcd for $C_{20}H_{23}NO_6 \cdot 0.5\ H_2O$: C, 62.82; H, 6.33; N, 3.66. Found: C, 62.59; H, 6.12; N, 3.61. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.76 (d, *J* = 8.3 Hz, 1 H), 6.68 (d, *J* = 8.3 Hz, 1 H), 6.36 (d, *J* = 4.1 Hz, 1 H), 6.30 (d, *J* = 10.6 Hz, 1 H), 6.08-5.91 (m, 1 H) , 4.50 (br s, 1 H), 4.32 (dd, *J* = 11.3, 5.6 Hz, 1 H), 4.13 (br s, 1 H), 3.84 (s, 3 H), 3.80 (dd, *J* = 19.1, 9.8 Hz, 1 H), 3.22 (ddd, *J* = 10.0, 6.8, 2.8 Hz, 1 H), 2.92 (dd, *J* = 12.3, 5.8 Hz, 1 H), 2.78-2.57 (m, 2 H), 2.09 (s, 3 H), 2.07-1.79 (m, 3 H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 169.6 (s), 145.8 (s), 143.8 (s), 134.1 (s), 129.8 (d), 128.0 (d), 127.0 (s), 118.5 (d), 111.4 (d), 95.3 (d), 88.7 (d), 61.4 (d + d), 55.7 (q), 54.5 (t), 47.3 (s), 41.7 (t), 29.7 (t), 29.3 (t), 21.0 (q).

Methyl [4a*S*-(4aα,6β,8a*R)]-4a,5,9,10,13,14a-Hexahydro-6-hydroxy-3-methoxy-6*H*,14*H*-benzofuro-[3a,3,2-*ef*]isoxazolo[3,2-*a*][2]benzazepine-13 (resp. 14)-carboxylate (3d).** **2** (175 mg, 0.61 mmol) and methyl acrylate (52 mg, 0.61 mmol) were refluxed in dry toluene (6 mL) for 48 h. The mixture was concentrated *in vacuo*, and the residue was purified by flash chromatography (20 g SiO_2, $CHCl_3$: MeOH : conc. NH_4OH = 89 : 10 : 1) and triturated with i-Pr_2O (1 mL). Yield: light brown foam (225 mg, 99%); TLC: $CHCl_3$: MeOH : conc. NH_4OH = 89 : 10: 1; R_f = 0.7; Anal. Calcd for $C_{20}H_{23}NO_6 \cdot 0.5\ H_2O$: C, 62.82; H, 6.33; N, 3.66. Found: C, 62.88; H, 6.17; N, 3.65. As **3d** was formed as a mixture of stereo- and regioisomers, no satisfying analysis and assignment of the obtained NMR spectra to determine the composition of the complex mixture was possible.

[4a*S*-(4aα,6β,8a*R)]-4a,5,9,10,13,14a-Hexahydro-6-hydroxy-3-methoxy-6*H*,14*H*-benzofuro[3a,3,2-*ef*]isoxazolo[3,2-*a*][2]benzazepine-13 (resp. 14)-carbonitrile (3e).** **2** (200 mg, 0.70 mmol) and acrylonitrile (37 mg, 0.70 mmol) in dry toluene (5 mL) were stirred under reflux for 2 h and then concentrated *in vacuo*. The residue was purified by flash chromatography (20 g SiO_2, $CHCl_3$: MeOH = 90 : 10). Yield: yellow foam (230 mg, 97%); TLC: $CHCl_3$: MeOH = 90 : 10, R_f = 0.7; Anal. Calcd for $C_{19}H_{20}N_2O_4 \cdot 0.25\ H_2O$: C, 66.17; H, 5.99; N, 8.12. Found: C, 66.22; H, 6.03; N, 7.86. As **3e** was formed as a mixture of stereo- and regioisomers, no satisfying analysis and assignment of the obtained NMR spectra to determine the composition of the complex mixture was possible.

ACKNOWLEDGEMENTS

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10. Complete crystallographic data of **3a** (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no CCDC 182130. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ (fax: +44 1223 336033, email: deposit@ccdc.cam.ac.uk).
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