DOI: 10.1002/ejoc.200700952

Synthesis and Biological Evaluation of Novel Rigid 1,4-Benzodiazepine-2,5-dione Chimeric Scaffolds

Ana C. Araújo,[a] Francesco Nicotra,[b] Cristina Airoldi,[b] Barbara Costa,[b] Gabriella Giagnoni,[b] Pietro Fumagalli,[c] and Laura Cipolla*[b]

Keywords: Benzodiazepine / Carbohydrates / $GABA_A$ receptor / Lead generation / Biological activity studies

Novel, conformationally constrained 1,4-benzodiazepine-2,5-diones containing both a monosaccharide, fructose, and a proline moiety have been synthesized as chimeric scaffolds; preliminary biological evaluation as $GABA_A$ receptor ligands is reported.

Introduction

Benzodiazepines are among the most widely dispensed drugs. Besides the well-known anxiolytic, sedative, anticonvulsant, myorelaxant and hypnotic activities,[1] benzodiazepines have been shown to act as peripheral cholecystokinin (CCK-A) receptor agonists,[2] α-thrombin inhibitors,[3] antitubercular drugs,[4] endothelin receptor antagonists[5] and can be cytotoxic against transformed T-cells.[6] Indeed, several of these nitrogen-containing heterocycles have been identified as antitumour antibiotics,[7] anti-HIV[8] and antithrombotic agents.[9]

Moreover, because of both their structural motifs and physicochemical properties, the benzodiazepine scaffold has been considered among novel non-peptide peptidomimetics, acting as a mimic of peptide secondary structures such as γ- and β-turns.[10] Particularly useful in medicinal chemistry are proline-derived benzodiazepines (pyrrolo[2,1-*c*][1,4]-benzodiazepines),[11] showing promise as anxiolytic drug candidates (i.e. **1**; Figure 1) and as starting materials for the synthesis of anthramycin-inspired anticancer drugs (e.g. **2**),[11c] DNA-cross-linking agents (e.g. **3**),[11d] and α5-selective $GABA_A$ receptor ligands (e.g. **4**).[11e]

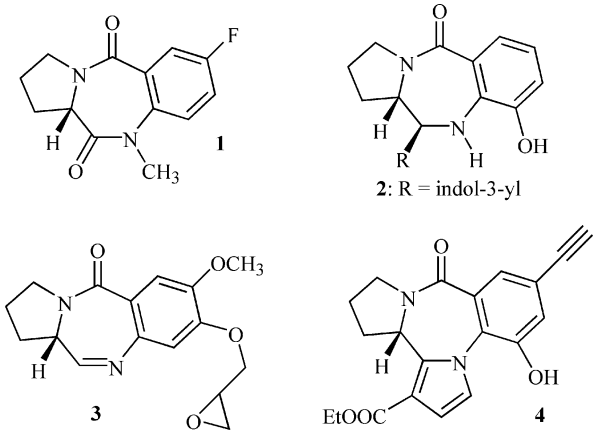


Figure 1. Chemical structures of some biologically active pyrrolo[2,1-*c*][1,4]benzodiazepines.

Recently, the design and synthesis of new chimeric scaffolds, that are hybrids of benzodiazepines with sugars wherein the latent hydroxy groups of the carbohydrate moiety permit diverse, controlled derivatization at different sites, have been proposed (i.e. compounds **5** and **6**,[12] **7** and **8**,[13] **9**;[14] Figure 2).

In addition, great efforts have been devoted to the synthesis of conformationally constrained benzodiazepine derivatives,[11a,15] since conformational changes in the benzodiazepine ring system have a strong effect on binding affinities to the receptor complex.[16]

Herein we describe the synthesis and preliminary biological evaluation as $GABA_A$ receptor ligands of novel, conformationally constrained 1,4-benzodiazepine-2,5-diones as chimeric scaffolds, containing both a monosaccharide, fructose, and a proline moiety. The free hydroxy groups on the sugar moiety offer the possibility of further

[a] Departamento de Química e Bioquímica, Universidade de Lisboa e CQB,
C8, Campo Grande, 1749-016 Lisboa, Portugal
[b] Department of Biotechnology and Biosciences, University of Milano-Bicocca,
P.za della Scienza 2, 20126 Milano, Italy
Fax: +39-02-64483565
E-mail: laura.cipolla@unimib.it
[c] Department of Environemental Sciences, University of Milano-Bicocca,
P.za della Scienza 1, 20126 Milano, Italy
Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.

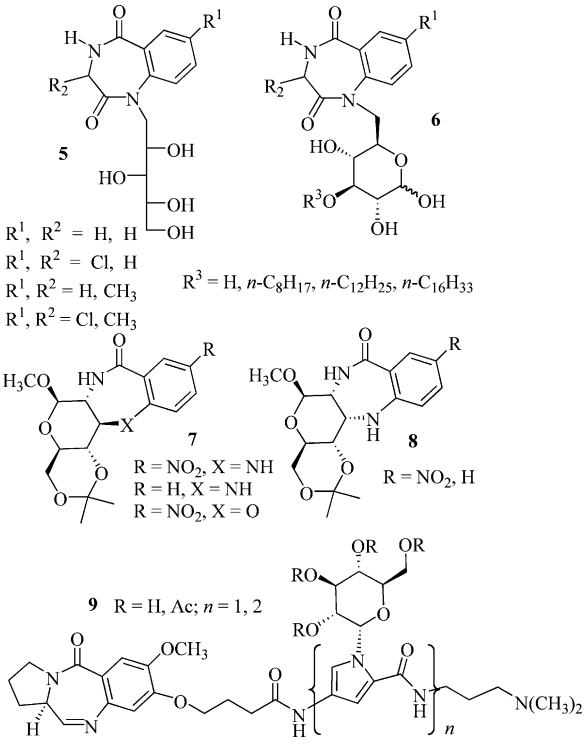


Figure 2. Examples of glyco-benzodiazepines.

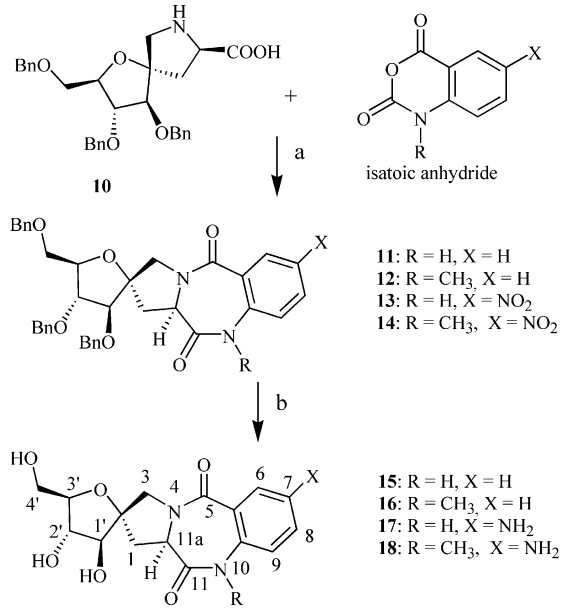
functionalisation for tuning the pharmacokinetic properties and the biological activity. The D-proline moiety connected to fructose through a spiro junction gives high conformational rigidity to the hybrid scaffolds, as confirmed by NMR spectroscopic data (see below).

Results and Discussion

The synthesis of the 1,4-benzodiazepine-2,5-dione derivatives **11**–**14** was accomplished by heating suitably functionalised isatoic anydride in dry DMF, with the proline-fructose building block **10** (Scheme 1).[17] Subsequent hydrogenolysis resulted in deprotection and reduction of the nitro group to afford the water-soluble 1,4-benzodiazepine-2,5-diones **15**–**18**.

Preliminary biological evaluation of compounds **11**–**18** as $GABA_A$ receptor ligands has been performed. In particular, we tested their ability to displace [^{3}H]Flunitrazepam from the receptor by using rat cortical membranes to perform a classical competition binding assay (Table 1). Data show that compounds **14**, **15** and **18** possess affinity for $GABA_A$ receptor significantly inhibiting [^{3}H]Flunitrazepam binding in the μM range. However, the biological activity of compounds **14**, **15** and **18** is actually far from that of classical $GABA_A$ modulators, which falls in the nM range; nevertheless, this result indicates that compounds **14**, **15** and **18** can be considered as lead compounds.

It has been reported that binding affinities at the $GABA_A$ receptor are exquisitely sensitive to the conformation



Scheme 1. Synthesis of fructose-proline-containing 1,4-benzodiazepine-2,5-diones; (a) dry DMF, reflux; (b) H_2, $Pd(OH)_2$, MeOH/AcOEt.

Table 1. Effects of test compounds in the [^{3}H]Flunitrazepam specific binding to $GABA_A$ receptor performed on rat cortical membranes.

Compound	% [^{3}H]Flunitrazepam specific binding[a]	Significance vs. control[b]
Control	100.00 ± 2.00	
11	111.81 ± 2.33	n.s.
12	96.44 ± 8.85	n.s.
13	101.01 ± 2.10	n.s.
14	74.89 ± 1.50	$P < 0.05$
15	78.22 ± 7.43	$P < 0.05$
16	93.04 ± 12.77	n.s.
17	80.17 ± 9.43	n.s.
18	70.63 ± 2.36	$P < 0.01$

[a] Values are means±SEM determined from at least three independent experiments. [b] Statistical analysis is performed with Kruskal–Wallis ANOVA for non-parametric values followed by Dunns test.

[(*M*) or (*P*)] of the benzodiazepine ring (Figure 3), which is governed by the pseudoequatorial preference and absolute stereochemistry of the C11a[18] substituent.[18,19]

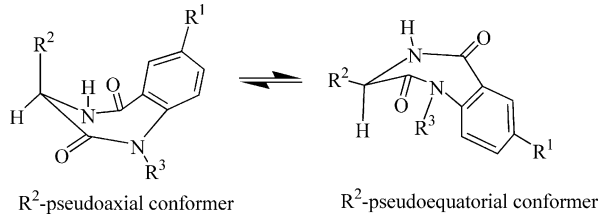


Figure 3. Conformational equilibrium present in the 1,4-benzodiazepine ring.

Hence, conformational analysis of sample benzodiazepines **15** and **16** was performed by molecular modelling calculations and experimental NMR studies at variable tem-

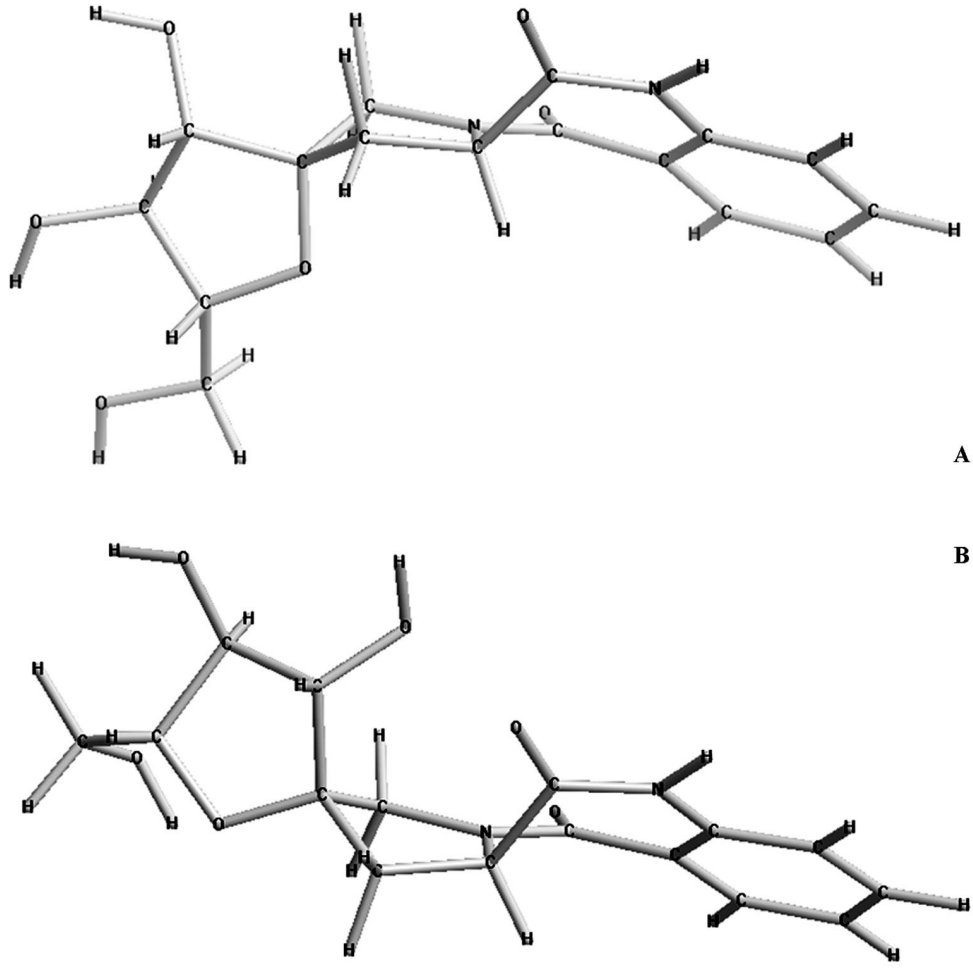


Figure 4. Oxygen-substituent pseudo-axial (**A**) and pseudo-equatorial (**B**) conformers of pyrrolo ring in compound **15**. Pseudo-axial conformer: total energy = 25.028 kcal/mol, dihedral angle C5a–C9a–N10–C11 = +16.91°; pseudo-equatorial conformer: total energy = 22.319 kcal/mol, dihedral angle C5a–C9a–N10–C11 = +17.32°.

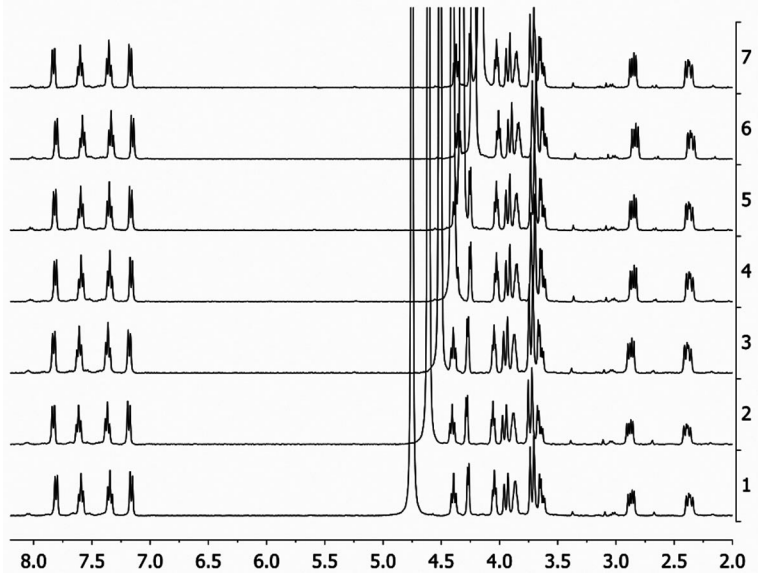


Figure 5. DNMR spectra (363–298 K) of molecule **15** dissolved in D_2O; spectrum 1: 298 K, 32 scans, spectrum 2: 313 K, 32 scans, spectrum 3: 323 K, 32 scans, spectrum 4: 333 K, 32 scans, spectrum 5: 343 K, 32 scans, spectrum 6: 353 K, 32 scans, spectrum 7: 363 K, 32 scans.

perature (dynamic NMR, DNMR). Molecular modelling and NMR spectroscopic data show a unique preferential conformation of the benzodiazepine ring, where 11a-H adopts the pseudoaxial position (Figure 4 and Supporting Information). In addition, NMR experiments run at variable temperature (Figure 5 and Supporting Information), until a maximum value of 363 K indicate that even at high temperature no conformational equilibrium of the benzodiazepine ring is present, in contrast to quaternary 1,4-benzodiazepin-2-ones, which exist as mixtures of the (*M*) and (*P*) conformers.[11a,15a] Actually, when sterically demanding substituents are present on the diazepine ring, they assume a pseudo-equatorial orientation, and in all reported cases,[20] the coalescence temperature for the pseudo-equatorial/pseudo-axial equilibrium is always around 60 °C (333 K). The dihedral angle C5a–C9a–N10–C11 was also determined and was found to be +17° for compound **15** and +24° for compound **16**, corresponding to helical chirality (*P*). However, conformers of the pyrrolo ring do exist, as illustrated in Figures 4a and b, keeping the (*P*) conformation of the diazepine. In order to best characterise this equilibrium, DNMR studies were performed also at low temperature for compound **15**, down to 173 K (Supporting Information). These studies indicate that the barrier for inversion of the pyrrolo ring is very low, as suggested also by molecular modelling (Figure 4).

Conclusions

We can conclude that the synthesised hybrid fructose-proline-benzodiazepines adopt a rigid conformation around the benzodiazepine ring. Noteworthy, no conformational equilibrium of the benzodiazepine ring is present, which displays a rigid pseudoequatorial orientation of the C11a substituent. However, the D-proline ring induces a (*P*)-helical conformation, which is the opposite to that required for best binding to the $GABA_A$ receptor.[18]

Nevertheless, compounds **14**, **15** and **18** show a significant affinity for the $GABA_A$ receptor, hence they can be considered as leads. Strategies that allow synthesis of L-proline analogues linked to fructose able to induce a rigid (*M*) conformation, relevant for biological activity, are under investigation and will be reported in due course.

Experimental Section

General Procedure for Benzodiazepine Synthesis: To a solution of **10** in dry DMF, suitably functionalised isatoic anhydride was added under argon, and the resulting mixture was stirred under reflux until complete consumption of the starting material. The solvent was then removed under reduced pressure and the residue dissolved in dichloromethane and washed with 5% aqueous HCl. The organic layer was dried with sodium sulfate, filtered, and the solvents were evaporated. The crude residue was purified by silica gel flash chromatography to give the desired product.

General Hydrogenolysis Procedure: Compounds **11**–**14** were dissolved in MeOH/AcOEt (4:1) mixture and a few drops of HCl (37%) and $Pd(OH)_2/C$ (10%, w/w) was added. The flask was purged 3 times with Ar and then filled with H_2. After 48 h, the catalyst was removed by filtration, and the filtrate concentrated under reduced pressure to afford compounds **15**–**18**.

$GABA_A$ Receptor Binding Assay: Membranes were prepared from rat cortex as described by Ahboucha et al.[21] and were used for displacement studies. [^{3}H]Flunitrazepam (1 nM) served as radioligand and nonspecific binding was determined in the presence of Flumazenil (100 μM) and represented about 1–2% of the total binding. Compounds **15**–**18** were diluted in TrisHCl buffer (50 mM; pH = 7.4) to obtain the final concentration of 100 μM, whereas stock solutions of compounds **11**–**14** were prepared in ethanol and then diluted in TrisHCl buffer to obtain the final concentration of 100 μM. The binding reaction consisted in 0.3 mg of membranes incubated with the radioligand in the presence or absence of the test compounds and was stopped after 90 min at 4 °C by rapid filtration under vacuum through a glass-fiber filter (GF/B; Whatman). The filters were washed twice with 2-mL portions of ice-cold TrisHCl buffer and then dissolved in 8 mL of scintillation fluid (UltimaGold). Filter-bound radioactivity was determined by a scintillation counter. For all compounds three independent experiments were performed in triplicate. Results are expressed in terms of percentage of control [^{3}H]Flunitrazepam specific binding and analyzed by GraphPad Prism, using the Kruskal–Wallis ANOVA for nonparametric data followed by Dunns test for specific comparisons.

Molecular Modelling: Conformational analysis of compounds **15** and **16** was performed by applying an MM2 force field in vacuo. Each model was built with ChemDraw, the energy was minimized (minimum RMS gradient = 0.010), and the dihedral angle C5a–C9a–N10–C11 value calculated by using Chem3D. A Molecular Dynamics Computation was performed (step interval = 2 fs; frame interval = 1 fs; heating/cooling rate = 1.000 kcal/atom ps; target temperature = 300 K) by monitoring the same dihedral angle. The average value was calculated; in both the cases it corresponded to that found by MM calculations.

DNMR Experiments: 0.5 mg of compound **15** or **16** was dissolved in 0.6 mL of D_2O (363–293 K) or CD_3CD_2OD (293–173 K). ^{1}H and selective 1D-NOESY (data not shown) spectra were recorded at different temperatures and than processed with MestreNova (MestreLab Research s.r.l.). For spectra recorded in D_2O, the HDO chemical shift was calculated according to the equation $\delta = 5.060 - 0.0122T + (2.11 \times 10^{-5})T^2$.[22]

Supporting Information (see footnote on the first page of this article): Detailed experimental procedures and spectroscopic data for the synthesized compounds.

Acknowledgments

Work supported by Ministero dell'Università e della Ricerca under contract MIUR-PRIN 2005 n. 2005037725 and Fundação para a Ciência e Tecnologia (FCT) SFRH/BD/17815/2004.

[1] A. N. Bateson, *Sleep Med.* **2004**, *5*, suppl, 1, S9–S15.
[2] a) R. G. Sherrill, J. M. Berman, L. Birkemo, D. K. Croom, M. Dezube, G. N. Ervin, M. K. Grizzle, M. K. James, M. F. Johnson, K. L. Queen, T. J. Rimele, F. Vanmiddlesworth, E. E. Sugg, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1145–1148; b) M. G. Bock, R. M. Dipardo, B. E. Evans, K. E. Rittle, W. L. Whitter, V. M. Garsky, K. F. Gilbert, J. L. Leighton, K. L. Carson, E. C. Mellin, D. F. Veber, R. S. L. Chang, V. J. Lotti, S. B. Freedman, A. J. Smith, S. Patel, P. S. Anderson, R. M. Freidinger, *J. Med. Chem.* **1993**, *36*, 4276–4292; c) G. Hirst, C. Aquino, L.

Birkemo, D. Croom, M. Dezube, R. Dougherty, G. Ervin, M. Grizzle, B. Henke, M. James, M. Johnson, T. Momtahen, K. Queen, R. Sherill, J. Szewczyk, T. Willson, E. Sugg, *J. Med. Chem.* **1996**, *39*, 5236–5245.

[3] D. Dumas, G. Leclerc, J. J. Baldwin, S. Dale Lewis, M. Murcko, A. M. Naylor-Olsen, *Eur. J. Med. Chem.* **1998**, *33*, 471–488.

[4] A. Kamal, K. L. Reddy, V. Devaiah, N. Shankaraiah, G. S. Kumar Reddy, S. Raghavan, *J. Comb. Chem.* **2007**, *9*, 29–42.

[5] M. H. Bolli, J. Marfurt, C. Grisostomi, *J. Med. Chem.* **2004**, *47*, 2776–2795.

[6] T. M. Fran*cis*, T. B. Sundberg, J. Cleary, T. Groendyke, A. W. Opipari Jr, G. D. Glicka, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2423–2427.

[7] D. E Thurston, "Advances in the Study of Pyrrolo[2,1-*c*][1,4]-benzodiazepine (PBD) Antitumour Antibiotics", in *Molecular Aspects of Anticancer Drug–DNA Interactions*, vol. 1 (Eds.: D. Neidle, M. J. Waring), The Macmillan Press Ltd., London, **1993**, pp. 54–88.

[8] E. De Clercq, *Antiviral Res.* **1998**, *38*, 153–179.

[9] J. M. Samanen, F. E. Ali, L. S. Barton, W. E. Bondinell, J. L. Burgess, J. F. Callahan, R. R. Calvo, W. Chen, L. Chen, K. Erhard, G. Feuerstein, R. Heys, S.-M. Hwang, D. R. Jakas, R. M. Keenan, T. W. Ku, C. Kwon, C.-P. Lee, W. H. Miller, K. A. Newlander, A. Nichols, M. Parker, C. E. Peishoff, G. Rhodes, S. Ross, A. Shu, R. Simpson, D. Takata, T. O. Yellin, I. Uzsinskas, J. W. Venslavsky, C.-K. Yuan, W. F. Huffman, *J. Med. Chem.* **1996**, *39*, 4867–4870.

[10] For very recent examples, see: a) P. Verdié, G. Subra, L. Feliu, P. Sanchez, G. Bergé, G. Garcin, J. Martinez, *J. Comb. Chem.* **2007**, *9*, 254–262; b) M. D. Cummings, C. Schubert, D. J. Parks, R. R. Calvo, L. V. La France, J. Lattanze, K. L. Milkiewicz, T. Lu, *Chem. Biol. Drug Des.* **2006**, *67*, 201–205; c) N. Cabedo, X. Pannecoucke, J.-C. Quirion, *Eur. J. Org. Chem.* **2005**, 1590–1596.

[11] a) S. MacQuarrie-Hunter, P. R. Carlier, *Org. Lett.* **2005**, *7*, 5305–5308; b) W. Zhang, Y. Lu, C. Hiu-Tung Chen, D. P. Curran, S. Geib, *Eur. J. Org. Chem.* **2006**, 2055–2059; c) T. Nagasaka, Y. Koseki, F. Hamaguchi, *Tetrahedron Lett.* **1989**, *30*, 1871–1872; d) S. C. Wilson, P. W. Howard, S. M. Forrow, J. A. Hartley, L. J. Adams, T. C. Jenkins, L. R. Kelland, D. E. Thurston, *J. Med. Chem.* **1999**, *42*, 4028–4041; e) X. Li, J. Yu, J. R. Atack, J. M. Cook, *Med. Chem. Res.* **2004**, *13*, 259–281.

[12] a) D. Bouhlal, P. Godé, G. Goethals, M. Massoui, P. Villa, P. Martin, *Carbohydr. Res.* **2000**, *329*, 207–214; b) D. Bouhlal, P. Martin, M. Massoui, G. Nowogrocki, S. Pilard, P. Villa, G. Goethals, *Tetrahedron: Asymmetry* **2001**, *12*, 1573–1577.

[13] a) L. Abrous, P. A. Jokiel, S. R. Friedrich, J. Hynes Jr, A. B. Smith III, R. Hirschmann, *J. Org. Chem.* **2004**, *69*, 280–302; b) L. Abrous, J. Hynes Jr, S. R. Friedrich, A. B. Smith III, R. Hirschmann, *Org. Lett.* **2001**, *3*, 1089–1092.

[14] R. Kumar, J. W. Lown, *Org. Biomol. Chem.* **2003**, *1*, 3327–3342.

[15] See, for example: a) P. R. Carlier, H. Zhao, S. L. MacQuarrie-Hunter, J. C. DeGuzman, D. C. Hsu, *J. Am. Chem. Soc.* **2006**, *128*, 15215–15220; b) P. C.-H. Lam, P. R. Carlier, *J. Org. Chem.* **2005**, *70*, 1530–1538.

[16] a) J. F. Blount, R. I. Fryer, N. W. Gilman, L. J. Todaro, *Mol. Pharmacol.* **1983**, *24*, 425–428; b) G. Maksay, Z. Tegyey, M. Simonyi, *Mol. Pharmacol.* **1991**, *39*, 725–732.

[17] L. Cipolla, C. Redaelli, F. Nicotra, *Lett. Drug Discov. Des.* **2005**, *2*, 291–293.

[18] Compounds are numbered systematically as for pyrrolo-benzodiazepines.

[19] H. Qi, X. He, C. Ma, R. Liu, S. Yu, C. A. Dayer, G. R. Wenger, R. McKernan, J. M. Cook, *J. Med. Chem.* **2000**, *43*, 71–95.

[20] a) A. Avdagič, A. Lesac, V. Sunjic, *Tetrahedron* **1999**, *55*, 1407–1416; b) A. Avdagič, A. Lesac, Z. Majer, M. Hollosi, V. Sunjic, *Helv. Chim. Acta* **1998**, *81*, 1567–1582.

[21] S. Ahboucha, F. Araqi, G. Pomier Layrargues, R. F. Butterworth, *Neurochem. Int.* **2005**, *47*, 58–63.

[22] H. E. Gottlieb, V. Kotlyar, A. Nudelman, *J. Org. Chem.* **1997**, *62*, 7512–7515.

Received: October 8, 2007
Published Online: December 19, 2007

