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SYNTHESIS AND IN VITRO CYTOTOXIC ACTIVITY OF NOVEL HEXAHYDRO-2H-PYRIDO[1,2-B]ISOQUINOLINES AGAINST HUMAN BRAIN TUMOR CELL LINES

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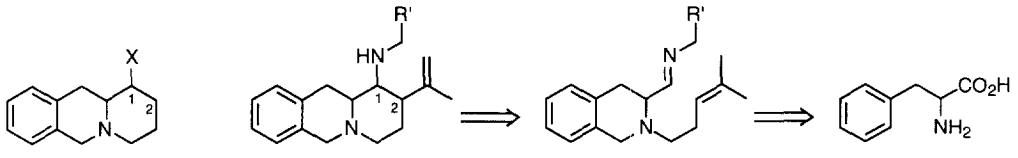
Abstract: 1-Hetero-substituted hexahydro-2H-pyrido[1,2-b]isoquinolines **8** - **12** were prepared with varying diastereoselectivities in 6 steps from L-phenylalanine employing a Pictet-Spengler reaction and a Lewis acid-catalyzed cyclization of imines **7** as the key steps. Compounds **6**, **8a,c**, **9b** and **12a,b** were tested *in vitro* against human medulloblastoma D283 Med and glioblastoma A-172 and T98G cell lines. The largest cytotoxicities were observed for **8a** (LC_{50} = 55, 29 and 42 µmol l^{-1}). © 1997 Elsevier Science Ltd.

Although considerable progress has been achieved in recent years in the development of various cancerostatic drugs, e.g. taxol,[1] *cis*-platin,[2] the chemotherapeutic treatment of brain tumors still seems to be in its infancy. Despite the promising *in vitro* activity of many cytotoxic agents their *in vivo* application against brain tumors is mainly hampered by the decreased passage through the blood brain barrier. Especially glioblastoma, the most common brain tumors among children have to be considered mostly as uncurable.[3] We therefore aimed a program toward the development of active compounds against these tumors. Our studies were focused on 1-hetero-substituted hexahydro-2H-pyrido[1,2-b]isoquinolines **1** as target molecules. Besides a few reports



1 **2** **3** **4**

X = OH, $NHCH_2R$, N=CHR

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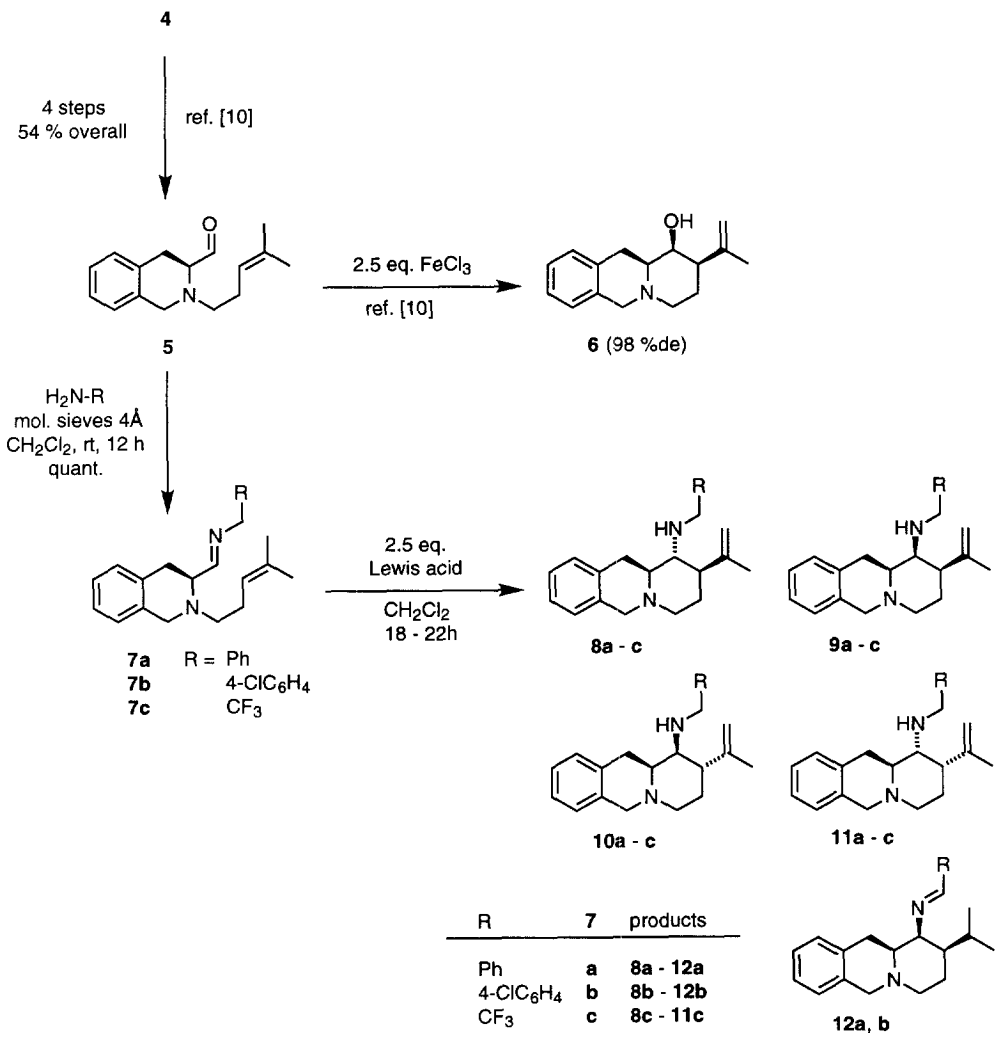
dealing with the cytotoxicity of hexahydro-2H-pyrido[1,2-b]isoquinoline derivatives bearing substructures of the antitumor antibiotic quinocarcine[4] and aza-podophyllotoxin derivatives[5] the pharmacological properties of this class of compounds remained largely unexplored. However, a series of patents decribed 1-hydroxy- and 1-amino-hexahydro-2H-pyrido[1,2-b]isoquinolines as central nervous stimulants.[6-8] These findings prompted us to extend a methodology, which was recently established in our laboratory for the synthesis of highly substituted piperidines from amino acid derivatives,[9,10] towards the synthesis of 1-amino-hexahydro-2H-pyrido[1,2-b]isoquinolines **2** from phenylalanine **4** and to test their cytoxicity. We reasoned, that a combination of cytotoxic and lipophilic properties of hexahydro-2H-pyrido[1,2-b]isoquinolines might circumvent problems with the blood brain barrier and might eventually lead to novel anticancer drugs against brain tumors. The preliminary results are described in this communication.

Scheme 1



As shown above the retrosynthetic analysis of **2** revealed a Lewis acid-induced hetero-ene cyclization of imino-substituted tetrahydroisoquinoline **3** and Pictet-Spengler cyclization of phenylalanine **4** as synthetic key steps. Following our previously established methodology (L)-phenylalanine **4** was converted in a four step sequence via Pictet-Spengler reaction and subsequent $LiAlH_4$ reduction, followed by N-alkylation of the aminoalcohol and final Swern oxidation to (*S*)-N-(4-methyl-3-pentenyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxaldehyde **5** in 54 % overall yield[10] (Scheme 1). $FeCl_3$-catalyzed cyclization of **5** gave *cis*-configured 1,3,4,6,11,11a-hexahydro-2H-pyrido[1,2-b]isoquinolin-1-ol **6** in 43 % (98 % de).[10] Aldehyde **5** was cleanly converted to the corresponding imines **7a - c** by treatment with either benzylamine, 4-chlorobenzylamine or 2,2,2-trifluoroethylamine respectively in the presence of molecular sieves 4 Å. Lewis acid-catalyzed cyclization of imines **7** resulted in the formation of four diastereomeric 1-amino-2-isopropyl-1,3,4,6,11,11a-hexahydro-2H-pyrido[1,2-b]isoquinolines **8** - **11** and the *cis*-configured 1-imino-2-isopropyl-1,3,4,6,11,11a-

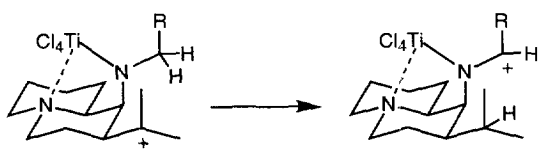
Table 1 Cyclization of Imines **7** with Various Lewis Acids[a]

Entry	Imine	R	Lewis acid	Ratio of products[b]					Yield [%]	
				8	**9**	**10**	**11**	**12**		
(1)	**7a**	Ph	$SnCl_4$	69.1	24.8	1.3	4.6	0.6	66	**8a**
(2)	**7a**	Ph	$TiCl_4$	5.7	3.7	27.0	0.7	63.0	58	**12a**
(3)	**7b**	4-ClC_6H_4	$SnCl_4$	70.0	15.9	3.1	11.1	---	47, 11	**8b, 9b**
(4)	**7b**	4-ClC_6H_4	$TiCl_4$	29.4	24.4	8.4	10.6	27.1	14	**12b**
(5)	**7c**	CF_3	$SnCl_4$	69.6	10.5	12.9	6.9	---	54	**8c**
(6)	**7c**	CF_3	$TiCl_4$	64.0	10.2	10.6	11.3	---	54	**8c**

[a] Reaction conditions: 2.5 equiv. of Lewis acid, CH_2Cl_2, rt. [b] Conversion and product ratios were determined by GC of the crude mixtures.

hexahydro-2H-pyrido[1,2-b]isoquinoline **12** respectively.[11] As shown in Table 1 a reversal of the selectivity for N-benzylimine **7a** was observed with $SnCl_4$ and $TiCl_4$ (entries 1, 2). Whereas $SnCl_4$ yielded preferably **8a**, $TiCl_4$ gave **12a** as the major product. The *p*-chloro-substituted benzylimine **7b** showed a similar diastereoselectivity when treated with $SnCl_4$ (entry 3). Again amine **8b** was the favored isomer. However, cyclization of **7b** in the presence of $TiCl_4$ was completely unselective and isomers **8b**, **9b** and **12b** were obtained in nearly equal amounts (entry 4). The benzylimino group in **7** is not necessarily required for the

Scheme 2



13 **14**

cyclization as can be seen from entries 5, 6. Treatment of trifluoroethylimine **7c** with either $SnCl_4$ or $TiCl_4$ yielded the 1-amino-hexahydro-2H-pyrido[1,2-b]isoquinoline **8c** as the major product in both cases. The less favored formation of **12b** as compared to **12a** in the presence of $TiCl_4$ and the absence of any imino product in the case of the $TiCl_4$-induced cyclization of trifluoroethylimine **7c** strongly supports our recently proposed mechanism.[10,12] That means, that the $TiCl_4$-mediated intramolecular hydride transfer (**13** → **14**) giving rise to the imino products **12** is disfavored, as soon as the newly formed cation **14** is destabilized by electron-withdrawing substituents R (Scheme 2). Whereas the *p*-chlorophenyl substituent only slightly decreases the electron density in the benzylic position, the hydride migration is completely suppressed by the trifluoromethyl group. The diastereomers were separated by preparative HPLC in order to obtain pure samples for the cytotoxicity studies. The relative configuration of diastereomers **8** - **12** was deduced from COSY- and CH-correlation experiments. Fortunately, an X-ray crystal structure of imino hexahydro-2H-pyrido[1,2-b]isoquinoline **12a** could be obtained (Figure 1) and thus the *cis*-configuration between C-1/C-2 was confirmed.[13]

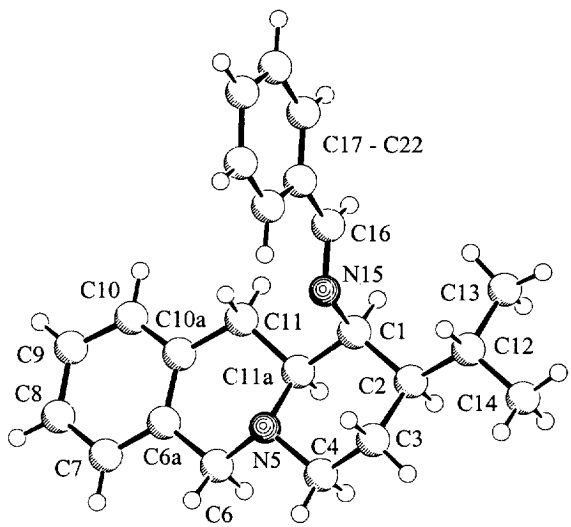


Figure 1 X-ray crystal structure of imino hexahydro-2H-pyrido[1,2-b]isoquinoline **12a**

Table 2 LC_{50} values [μmol l^{-1}] of hexahydro-2H-pyrido[1,2-b]isoquinolines against three human brain tumor cell lines[a]

cell line	hexahydro-2H-pyrido[1,2-b]isoquinolines						CDDP
	6	**8a**	**8c**	**9b**	**12a**	**12b**	
D283 Med	143	53	40	29	---	50	<1
A-172	158	58	157	55	226	97	7
T98G	---	43	172	42	222	45	3

[a] LC_{50} values refer to concentrations where 50 % of the tumor cells survived. For details see ref. [14]

Compounds **6**, **8a**,**c**, **9b** and **12a**,**b** were used for the *in vitro* cytotoxicity studies against three different human brain tumor cell lines D283 Med (medulloblastoma) and A-172 and T98G (glioblastoma) (Table 2).[14] Cis-diaminodichloroplatinum(II) (CDDP) was employed as a standard. The cell lines display different sensitivity toward the same hexahydro-2H-pyrido[1,2-b]isoquinoline with the medulloblastoma being more sensitive than the glioblastoma cell lines. The highest cytotoxicity was observed for **9b**, although the LC_{50} values are about 10 times higher than those observed for CDDP. It was found that the 1-benzylamino derivatives **8a**, **8b** are more active than the corresponding 1-benzylidene-imino-substituted compounds **12a**,**b**. The 1-hydroxy and 1-trifluoroethylamino derivatives **6** and **8c** respectively also show increased LC_{50} values.

In conclusion a synthetic route towards benzylaminohexahydro-2H-pyrido[1,2-b]isoquinolines has been developed, which display promising *in vitro* cytotoxicity against human brain tumors. However, further work is necessary to increase the activity and to unravel the mode of action in order to get useful novel anticancer drugs.

Acknowledgement

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13. X-ray crystal structure analysis of **12a**: formula $C_{23}H_{28}N_2$, M = 332.247, 0.15 x 0.08 x 0.03 mm, a = 6.201(1), b = 7.884(1), c = 39.123(5) Å, V = 1912.7(5) Å^3, ρ_{calc} = 1.155 g cm^{-3}, T = 223 K, empirical absorption correction via Ψ-scan data ($0.922 \leq C \leq 0.999$), Z = 4, orthorhombic, space group

$P2_12_12_1$ (No. 19), λ = 1.54178 Å, ω/2Θ scans, 2202 reflections collected (-*h*, ±*k*, -*l*), [(sinΘ)/λ] = 0.50 $Å^{-1}$, 1956 independent and 591 observed reflections [$I \leq 2\sigma(I)$], 104 refined parameters, due to the small crystal size and weak scattering the structure was only refined isotropic, $R = 0.072$, $wR^2 = 0.130$, max. residual electron density 0.19 (-0.26) e $Å^{-3}$, Flack parameter 0(3), hydrogens calculated and riding. All data were collected on a Enraf-Nonius CAD4 diffractometer, programs used: MolEN, SHELXS-86, SHELXL-93, SCHAKAL-92. The authors have deposited atomic coordinates for the structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained on request from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ.

14. ***Cell culture materials***. Cell culture reagents were purchased from Life Technologies (Eggenstein, Germany) and *cis*-diaminodichloroplatinum(II)-solution (CDDP) was obtained from Bristol (München, Germany). Cell culture plasticware was bought from Bibby Dunn (Asbach, Germany) and Falcon (Heidelberg, Germany).

Brain tumor cell lines. The glioblastoma cell lines T98G (CRL 1690), A-172 (CRL 1620) and the medulloblastoma cell line D283 Med (HTB 185) were obtained from American Type Culture Collection. Culture medium was RPMI-1640 supplemented with 10 % (v/v) fetal calf serum, 100 U/ml penicillin, 100 µg/ml streptomycin and 2 mM L-glutamine. Cells were grown in monolayer culture at 37°C in a humidified atmosphere of 5 % CO_2.

Sensitivity tests. Cells growing in monolayer cultures were trypsinized, rinsed with PBS (w/o Ca^{2+} and Mg^{2+}) and plated into 96-well-plates (2 x 10^3 cells/well, 2 x 10^5 cells/ml). The next day test substances **6**, **8a,c**, **9b**, and **12a,b** were freshly dissolved in DMSO resulting in 200 mM stock solutions. Stock solutions were diluted in culture medium and added (100 µl/well) at various concentrations to the wells, resulting in seven final concentrations between 1 x 10^{-3} and 1 x 10^{-6} M test substance. CDDP stock solution was diluted in culture medium immediately prior to use and added (100 µl/well) at various concentrations resulting in seven final concentrations between 1 x 10^{-4} and 1 x 10^{-7} M (for all experiments, n=24 for controls, n=8 for each concentration). 72 hours later the MTT-test was performed as described to measure the cell survival [see ref. 15].

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