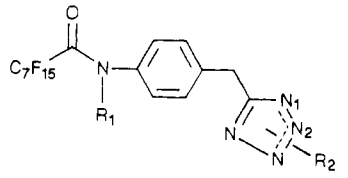
Table III. Effects of Perfluoro Anilides **2** on Plasma Glucose and Insulin in Obese Mice[a]



2

drug[b]	R_1	R_2	mp, °C	plasma glucose, mg/dL	plasma insulin, μunit/mL
vehicle (0.5% methylcellulose)				258 ± 28	232 ± 22
reference (ciglitazone)				107 ± 5*[c]	166 ± 7*
reference (**1f**; see Table I)				119 ± 4*	140 ± 15*
compd **2**					
a	H	$PhCH_2$-N_1	160-163	152 ± 8*	249 ± 12
b	H	$PhCH_2$-N_2	109-113	119 ± 4*	204 ± 9
c	Me	Me-N_1	80-82	119 ± 7*	204 ± 18
d	Me	Me-N_2	76-78	128 ± 11*	205 ± 18

[a] Compounds were administered at 20 mg/kg once daily po for 4 days. Data represent the mean ± SEM. [b] Analyses (C, H, N) were within ±0.4% of theoretical values. [c] (*) $p < 0.05$ when compared to vehicle control.

present in anilides **1** on their pharmacologic activity, tetrazole **1f** was selectively alkylated (benzyl bromide (1 equiv), K_2CO_3, acetone, 50 °C) to give a mixture (~1:1) of N_1- an N_2-alkylated tetrazoles (**2a** and **2b**, Table III), easily separable by chromatography. Reaction of **1f** with excess methyl iodide (K_2CO_3, acetone, reflux) similarly gave a mixture of dialkylated materials **2c** and **2d** (Table III).

Glucose lowering activity was retained by all the alkylated compounds (Table III) when tested in the ob/ob mouse at 20 mg/kg (once daily, 4 days, po). This result contrasts with the ciglitazone series, where alkylation of the thiazolidinedione ring abolishes hypoglycemic activity.[13]

Of further interest in Table III is that none of these alkylated materials effect insulin lowering after 4 days. These results suggest that either perfluoro anilides **1** lower plasma insulin and glucose levels by independent mechanisms[14] or that with **2** insulin lowering occurs secondarily and much slower than glucose lowering. Recently, the Upjohn group has reported that with ciglitazone administration in the ob/ob mouse, circulating insulin levels are decreased significantly prior to observed reductions in plasma glucose levels.[14] Since ciglitazone requires an acidic heterocycle for its pharmacological activity while perfluoroanilide **1f** does not, these compounds probably alter carbohydrate metabolism by different mechanisms or act at different sites. The results suggest, however, that like ciglitazone the perfluoro anilides **1** act by enhancing tissue responsiveness to insulin and that the glucose- and insulin-lowering activities may result from independent mechanisms.

Due to decreased food consumption induced by the C_9 chain in the normal rat, compounds **1c** and **1f** have been selected for evaluation in a chronic (45 days) study in genetically obese and diabetic mice. The results of these studies and further structure-activity relationships developed in the series will be the subject of a forthcoming paper.

Acknowledgment. We thank Bruce Hofmann (NMR), Marie Politowski (IR, CHN), Charles Kuhlman (HPLC), and their staffs for obtaining the analytical data. Paula Carabelli and Cheryl Delfino prepared the manuscript.

Supplementary Material Available: Experimental details for compounds **1a-j** and **2a-d** (6 pages). Ordering information is given on any current masthead page.

† Medicinal Chemistry Department.
‡ Department of Experimental Therapeutics.

Kenneth L. Kees,*,† Robert S. Cheeseman†
Dorothy H. Prozialeck,‡ Kurt E. Steiner‡
Medicinal Chemistry Department and Department of Experimental Therapeutics
Wyeth-Ayerst Research
CN 8000
Princeton, New Jersey 08543-8000
Received May 16, 1988

Benzodiazepine Gastrin and Brain Cholecystokinin Receptor Ligands: L-365,260†

Sir:

The benzodiazepines assumed medicinal importance over 25 years ago with their discovery as anxiolytic, anticonvulsant, and sedative drugs.[1] These agents bind with high affinity to specific receptors in the brain, and numerous agonist, antagonist, and partial agonist structural variants have been developed. Evidence has been advanced that the endogenous benzodiazepine receptor ligands are peptidal in nature.[2] The scope of biological activity of benzodiazepines has been expanded in recent years with the discovery that certain substitutions on the core structure produce specific ligands for other peptide receptors. For example, the 2-substituted benzodiazepine tifluadom is a potent κ opiate agonist[3] and a lower affinity

† Dedicated to Professor Richard K. Hill with gratitude, admiration, and respect on the occasion of his 60th birthday.

(1) Sternbach, L. H. *J. Med. Chem.* **1979**, *22*, 1.
(2) Guidotti, A.; Furchetti, C. M.; Corda, M. G.; Konkel, D.; Bennett, C. D.; Costa, E. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 3531.
(3) Romer, D.; Buscher, H. H.; Hill, R. C.; Mauer, R.; Petcher, T. J.; Zeugner, H.; Benson, W.; Finner, E.; Milkowski, W.; Thies, P. W. *Nature* **1982**, *298*, 759.
(4) Chang, R. S. L.; Lotti, V. J.; Chen, T. B.; Keegan, M. E. *Neurosci. Lett.* **1986**, *72*, 211.
(13) Sohda, T.; Mizuno, K.; Tawada, H.; Sugiyama, Y.; Fujita, T.; Kawamatsu, Y. *Chem. Pharm. Bull.* **1982**, *30*, 3563.
(14) Recent findings by the Upjohn group suggest that ciglitazone may reduce plasma glucose and insulin by independent mechanisms: Colca, J. R.; Wyse, B. M.; Sawada, G.; Jodelis, K. S.; Connell, C. L.; Fletcher-McGruder, B. L.; Palazuk, B. J.; Diani, A. R. *Metabolism* **1988**, *37*, 276.

0022-2623/89/1832-0013$01.50/0

cholecystokinin (CCK) antagonist.[4] Certain 3-substituted benzodiazepines, on the other hand, are highly potent CCK antagonists selective for the peripheral receptor subtype (CCK-A).[5] We now report the design of a fourth class of benzodiazepines which bind selectively to brain CCK (CCK-B) and gastrin receptors.

Cholecystokinin and gastrin are structurally related peptide hormones which have attracted considerable interest due to their actions in the gastrointestinal tract and in the central nervous system. The ligand specificities of stomach gastrin and brain CCK-B receptors have been shown to be similar.[6,7] Studies have suggested potential therapeutic utility for antagonists and/or agonists of CCK and gastrin in conditions such as pancreatitis, ulcer disease, irritable bowel syndrome, and eating disorders, as well as in the regulation of pain and amelioration of certain tumors.[8]

Investigations of the involvement of CCK and gastrin in normal and aberrant physiology have been facilitated recently by the development of selective antagonists.[8] In particular, the 3-amino-5-phenyl-1,4-benzodiazepin-2-one derivatives, typified by L-364,718 (MK-329) (1) (IC_{50} = 0.08 nM), are the most potent CCK antagonists yet described.[5] These antagonists were designed from the natural product asperlicin, which was discovered by use of receptor-based screening.[9] MK-329 has >1000-fold selectivity for the CCK-A receptor with respect to the CCK-B receptor and displays good oral activity.[10]

To further elucidate the roles of the CCK receptor subtypes, antagonists with reversed selectivity are highly desirable. The moderate affinity of MK-329 for the CCK-B and gastrin receptors (IC_{50} = 300 nM) suggested that the benzodiazepine nucleus might hold a key to selective ligands for these receptors as well. This essential information was garnered from the hundreds of analogues synthesized in connection with the development of MK-329. It was observed that subtle structural modifications resulted in a loss of receptor subtype selectivity.

It was previously reported that 4-chlorobenzoyl CCK antagonists such as **2** rival MK-329 in potency and selectivity.[11] When either the N-1-methyl substituent of **2** is substituted with an ethoxycarbonyl group (**4**) or the 3-amide linkage is replaced with a urea (**5**), the CCK-A affinity decreases substantially and the CCK-B affinity increases modestly. Combination of these two features yielded the pivotal compounds (**6** and **7**), the first nanomolar potency, nonpeptide ligands selective for the CCK-B and gastrin receptors. The potency and selectivity exhibited by **7** were furhter enhanced by transforming the N-1 group to a pyrrolidinamide. The resultant **8** is a nonpeptide with receptor affinity comparable to that of the native peptide ligands. Its potency and selectivity are comparable to those of recently reported cyclic CCK-8 analogues.[12]

A second approach to similarly selective ligands has also been discovered. The resolution of the N-1-methyl derivative of **5** (**9**) as well as the more potent **12** into their optical antipodes afforded the breakthrough. In both instances, the enantiomers of 3*S* configuration (**10** and **13**) show selectivity for the CCK-A receptor, whereas the mirror image 3*R* isomers (**11** and **14**) are selective for the CCK-B and gastrin receptors. In contrast, it is important to note that related 3-amidobenzodiazepine enantiomers **2** and **3** are both highly selective for the CCK-A receptor. The opposing selectivities for the urea enantiomers are, therefore, a consequence of both the 3-position stereochemistry and the nature of the linking group between benzodiazepine and aryl substituent. Whether or not this stereoselectivity extends to ureas with larger N-1 substituents such as **8** is under investigation.

It is noteworthy that the receptor ligands reported here do not distinguish between central CCK-B and peripheral gastrin receptors. Ligands which could discriminate between these receptors would be additional valuable pharmacological tools, but such agents remain to be developed. We are also exploring the 3-dimensional structural basis for the CCK-A and CCK-B selectivity.

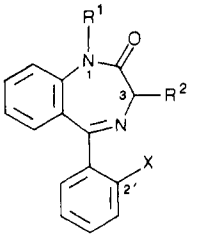
The compound of principal interest developed in these studies is **14** (L-365,260), a potent and selective CCK-B and gastrin receptor ligand. In a separate report, **14** is shown to interact competitively with these receptors and to be orally effective as an antagonist of gastrin-stimulated acid secretion in various animal models.[13]

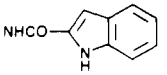
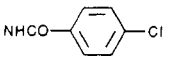
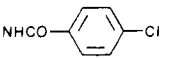
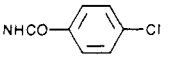
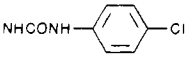
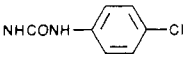
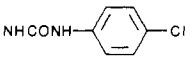
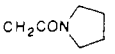
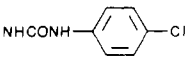
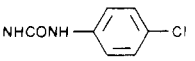
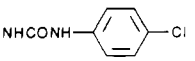
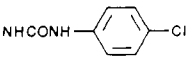
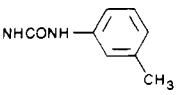
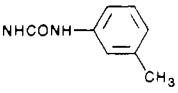
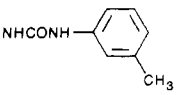
The compounds disclosed in this study constitute another example of the successful manipulation of the benzodiazepine nucleus to produce effective nonpeptidal ligands for a given peptide receptor. The mechanistic nuances of the mode of binding of these benzodiazepines to the gastrin and CCK-B receptors remain unclear, as do the reasons why benzodiazepines, in general, have affinity for seemingly disparate receptor types. One possible explanation may reside with the ontogeny of these receptors, their consequent structural relationships, and their apparent common complexation to guanine nucleotide binding proteins.[14] Whatever the reason, our results support the hypothesis that the benzodiazepine core structure is amenable to the design of ligands for a variety of peptide receptors, an effort which is continuing in these laboratories.

Compounds **2** and **3** (Table I) were prepared by acylating the corresponding enantiomers of 1,3-dihydro-3-amino-1-methyl-2*H*-1,4-benzodiazepin-2-one (**15** and **16**, respectively) with 4-chlorobenzoic acid.[11] The parent benzodiazepines **15** and **16** were synthesized according to published methods[15,16] and resolved via the crystallization

(5) Evans, B. E.; Bock, M. G.; Rittle, K. E.; DiPardo, R. M.; Whitter, W. L.; Veber, D. F.; Anderson, P. S.; Freidinger, R. M. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4918–4922.
(6) Rehfeld, J. F. *Am. J. Physiol.* **1981**, *240*, G255–266.
(7) Beinfeld, M. C. *Neuropeptides* **1983**, *3*, 411–427.
(8) Silverman, M. A.; Greenberg, R. E.; Bank, S. *Am. J. Gastroenterol.* **1987**, *82*, 703.
(9) Chang, R. S. L.; Lotti, V. J.; Monoghan, R. L.; Birnbaum, J.; Stapley, E. O.; Goetz, M. A.; Albers-Schönberg, G.; Patchett, A. A.; Liesch, J. M.; Hensens, O. D.; Springer, J. P. *Science* **1985**, *230*, 177–179.
(10) Chang, R. S. L.; Lotti, V. J. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4923–4926.
(11) Evans, B. E.; Rittle, K. E.; Bock, M. G.; DiPardo, R. M.; Freidinger, R. M.; Whitter, W. L.; Lundell, G. F.; Veber, D. F.; Anderson, P. S.; Chang, R. S. L.; Lotti, V. J.; Cerino, D. J.; Chen, T. B.; Kling, P. J.; Kunkel, K. A.; Springer, J. P.; Hirshfield, J. *J. Med. Chem.* **1988**, *31*, 2235.
(12) Charpentier, B.; Pelaprat, D.; Durieux, C.; Dor, A.; Reibaud, M.; Blanchard, J.-C.; Roques, B. P. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1968.
(13) The CCK-A/CCK-B selectivity of **14** and related structures is not a species effect. For example, when tested in four different species, in each case, **14** discriminates between the CCK-A and CCK-B receptors within a given species. Lotti, V. J.; Chang, R. S. L. *Eur. J. Pharmacol.*, submitted.
(14) Marx, J. L. *Science* **1987**, *238*, 615.
(15) Bock, M. G.; DiPardo, R. M.; Evans, B. E.; Rittle, K. E.; Veber, D. F.; Freidinger, R. M. *Tetrahedron Lett.* **1987**, *28*, 939.
(16) Bock, M. G.; DiPardo, R. M.; Evans, B. E.; Rittle, K. E.; Veber, D. F.; Freidinger, R. M.; Hirshfield, J.; Springer, J. P. *J. Org. Chem.* **1987**, *52*, 3232.
(17) Reider, P. J.; Davis, P.; Hughes, D. L.; Grabowski, E. J. J. *J. Org. Chem.* **1987**, *52*, 955.

Table I. Inhibition of Binding of ^{125}I-Labeled Gastrin to Guinea Pig Gastric Glands, of [^{125}I]CCK-8 to Guinea Pig Brain CCK Receptors and to Rat Pancreatic Receptors by Synthetic CCK-B/Gastrin Antagonists



no.	X	R^1	R^2	stereo	gastrin[a,d]	CCK-B[b,d]	CCK-A[c,d]
1	H	CH_3		*S*	300 ± 72	245 ± 97	0.08 ± 0.02
2	F	CH_3		*S*	2500 ± 1800	2900	0.39 ± 0.09
3	F	CH_3		*R*	7600	11000	2.9 ± 0.81
4	*F*	CH_2CO_2Et		*RS*	690	1900	110
5	F	H		*RS*	640	300	110
6	F	CH_2CO_2Et		*RS*	4.1 ± 0.7	1.2 ± 0.4	220
7	H	CH_2CO_2Et		*RS*	3.3	1.0	370
8	H			*RS*	0.5	0.28	520
9	H	CH_3		*RS*	22	23	51
10	H	CH_3		*S*	540	410	26
11	H	CH_3		*R*	12	5.5	1100
12	H	CH_3		*RS*	3.1	7.1	8.1
13	H	CH_3		*S*	130 ± 45	151 ± 12	3.0 ± 1
14	H	CH_3		*R*	1.1 ± 0.4	2.0 ± 0.3	280 ± 33
15	F	CH_3	NH_2	*S*			
16	F	CH_3	NH_2	*R*			
17	F	H	NHCbz	*RS*			
18	H	CH_3	NH_2	*RS*			
19	H	CH_3	NH_2	*S*			
20	H	CH_3	NH_2	*R*			

[a] IC_{50} (nM) for half maximal inhibition of binding of ^{125}I-labeled gastrin to guinea pig gastric glands. [b] IC_{50} (nM) for half maximal inhibition of binding of [^{125}I]CCK-8 to guinea pig cerebral cortex. [c] IC_{50} (nM) for half maximal inhibition of binding of [^{125}I]CCK-8 to receptors in rat pancreatic tissues. [d] Values without SEM were obtained from one to two separate experiments.

induced resolution–racemization sequence developed expressly for this class of compounds.[17] The benzyloxycarbonyl (Cbz) protected aminobenzodiazepine **17** was obtained by using the published synthetic route.[16] This intermediate was then deprotected and reacted with 4-chlorophenyl isocyanate to afford **5**. In an analogous fashion, the aminobenzodiazepines **18–20** were elaborated by employing the requisite aryl isocyanate to give the corresponding benzodiazepinylureas **9–14**. The R^1 substituent in compound **4** was introduced by alkylating the protected aminobenzodiazepine **17** with sodium hydride and ethyl bromoacetate. The resulting product was subsequently converted to the 4-chlorophenylamide **4** by using standard methods. Similar methodology was employed in the preparation of the analogues **6–8**. All synthetic compounds were fully characterized spectroscopically (^{1}H NMR and MS) and their chemical homogeneity was confirmed by chromatographic techniques and elemental analyses.

IC_{50} values (nM) for half-maximal inhibition of binding of [^{125}I]CCK-8 to CCK receptors in rat pancreatic tissue were obtained by using the modified Innis and Snyder procedure.[10] The [^{125}I]CCK-8 binding assay employing guinea pig cerebral cortex was performed as previously

reported.[10] ^{125}I-labeled gastrin binding in guinea pig gastric glands was determined according to literature procedures.[10,18] Details of these experimental procedures are described in a forthcoming paper.[13]

Acknowledgment. It is a pleasure to acknowledge the efforts of Dr. J. P. Springer, who established the absolute stereochemistry of compound 14 by X-ray crystallographic analysis of the related 3-bromophenyl analogue. We are indebted to Drs. V. J. Lotti and R. S. L. Chang for granting permission to publish the binding assay results presented in the table.

Mark G. Bock,* Robert M. DiPardo, Ben E. Evans
Kenneth E. Rittle, Willie L. Whitter, Daniel F. Veber
Paul S. Anderson, Roger M. Freidinger
Merck Sharp & Dohme Research Laboratories
West Point, Pennsylvania 19486
Received September 13, 1988

(18) Chang, R. S. L.; Lotti, V. J.; Keegan, M. E.; Kunkel, K. A. *Biochem. Biophys. Res. Commun.* **1986**, *134*, 895.

Articles

Rationale for the Synthesis and Preliminary Biological Evaluation of Highly Active New Antitumor Nitrosoureido Sugars

Pierre Roger,*,† Claude Monneret,‡ Jean-Paul Fournier,§ Patrick Choay,† Roselyne Gagnet,‡ Charles Gosse,‖ Yves Letourneux,⊥ Ghanem Atassi,# and Alain Gouyette‖

Département de Chimie Thérapeutique, Institut Choay, 10 rue Morel, 92120 Montrouge, France, Département de Pharmacognosie associé au CNRS 484, Faculté des Sciences Pharmaceutiques et Biologiques, 4 avenue de l'Observatoire, 75270 Paris Cedex 06, France, Département de Chimie Thérapeutique, Faculté des Sciences Pharmaceutiques et Biologiques, 4 avenue de l'Observatoire, 75270 Paris Cedex 06, France, Institut Gustave-Roussy, 39, rue Camille-Desmoulins, 94805 Villejuif Cedex, France, Laboratoire de Chimie Organique, UER des Sciences Pharmaceutiques, 1 rue Gaston-Veil, 44035 Nantes Cedex, France, and Institut Jules-Bordet, 1 rue Hêger-Bordet, 1000 Bruxelles, Belgique.
Received February 8, 1988

Various new nitrosoureido derivatives of di- or trideoxy sugars were synthesized. The influence of the hydroxyl substitution pattern, the configuration at the anomeric center, and the absolute configuration of the sugar moiety on the antitumor activity of a series of nitrosoureido derivatives of di- and trideoxy sugars was studied. All compounds showed a very significant activity in vivo against L1210 leukemia, B16 melanocarcinoma, and Lewis lung carcinoma. Methyl 3-[3-(2-chloroethyl)-3-nitrosoureido]-2,3-dideoxy-α-D-*arabino*-hexopyranoside, **24** (NSC 609224), was found to be the most active compound. When treated with **24** (NSC 609224) at 20 mg/kg on day 1, at least 90% of the L1210 leukemia and B16 melanocarcinoma bearing mice showed a survival of over 60 days for a LD50 value for this compound of 42 mg/kg.

The (chloroethyl)nitrosoureas CCNU, MeCCNU, and BCNU represent an important class of antitumor agents which have a broad spectrum of activity in human cancers mainly against lymphomas, melanomas, gliomas, and a few solid tumors.[1,2] However, these drugs produce delayed and cumulative bone marrow toxicity which seriously limit their clinical application.[3,4] Since streptozocin (SZ),[5,6] the *N*-nitroso-*N*-methylurea of 2-deoxy-D-glucose, has antitumor activity with less bone marrow toxicity[7] than the noncarbohydrate nitrosoureas but suffers from diabetogenic activity,[8,9] it became of interest to prepare nitrosoureido derivatives of amino sugars. Among the numerous analogues of SZ which have been synthesized,[10,11] replacement of the methyl group with a 2-chloroethyl group, as present in nitrosoureas of the first generation, had markedly enhanced effectiveness against L1210 leukemia.[12] Furthermore diabetogenic activity in animals as reported for SZ and hepatic dysfunction were not observed.

Since then, many other nitrosoureido derivatives of amino sugars have been prepared as analogues of streptozocin including the water-soluble GANU,[13] MCNU,[14] and KCNU,[15] respectively C-1, C-6, and C-3 substituted glu-

* Proof and reprint requests to: Pierre Roger, Institut Choay, 10, rue Morel, 92120 Montrouge, France.
† Institut Choay.
‡ Département de Pharmacognosie associé au CNRS.
§ Département de Chimie Thérapeutique.
‖ Institut Gustave-Roussy.
⊥ U.E.R. des Sciences Pharmaceutiques.
Institut Jules-Bordet.

(1) Carter, S. K. *Cancer Chemother. Rep., Part 3* **1973**, *4*, 35.
(2) Weinkam, R. J.; Lin, H.-S. *Adv. Pharmacol. Chemother.* **1982**, *19*, 1.
(3) Carter, S. K.; Schabel, F. M.; Broder, L. E.; Johnston, T. P. *Adv. Cancer Res.* **1972**, *16*, 273.
(4) Wasserman, T. H.; Slavik, M.; Carter, S. K. *Cancer Treat. Rev.* **1974**, *1*, 131; **1974**, *1*, 251; *Cancer* **1975**, *36*, 1258.
(5) Vavra, J. J.; De Boer, C.; Dietz, A.; Hanka, L. J.; Sokolski, W. T. *Antibiot. Annu.* **1960**, 230.
(6) Herr, R. R.; Jahnke, H. K.; Argoudelis, A. D. *J. Am. Chem. Soc.* **1967**, *89*, 4808.
(7) Schein, P. S.; McMenamin, M. G.; Anderson, T. *Cancer Res.* **1973**, *33*, 2005.
(8) Dulin, W. E.; Lund, G. H.; Gerritsen, G. C. *Diabetes* **1967**, *16*, 512.
(9) Mansford, R. K. L.; Opie, L. *Lancet* **1968**, 670.
(10) Bannister, B. *J. Antibiot. Ser. A* **1972**, *25*, 377.
(11) Iwasaki, M.; Ueno, M.; Ninomiya, K.; Sekine, J.; Nagamatsu, Y.; Kimura, G. *J. Med. Chem.* **1976**, *19*, 918.
(12) Johnston, T. P.; McCaleb, G. S.; Montgomery, J. A. *J. Med. Chem.* **1975**, *18*, 104.
(13) Machinami, T.; Nishiyama, K. S.; Kikuchi, K.; Suami, T. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 3763.
(14) Sekido, S.; Ninomiya, K.; Iwasaki, M. *Cancer Treat. Rep.* **1979**, *63*, 961.

0022-2623/89/1832-0016$01.50/0