supernatant solution was assayed for histamine release by a fluorometric method.[10]

Chemistry. Melting points were taken in open capillary tubes on a Mel-Temp apparatus and are uncorrected. Each analytical sample was homogeneous by TLC and had IR, UV, and NMR spectra compatible with its structure. Combustion analysis for C, H, and N and Cl or Br gave results within 0.4% of theory. The procedures for the preparation of the reported compounds are listed as methods A–D and may be considered as general methods of preparation. The reported yields for the products obtained were not maximized.

Method A. 2-Hydroxy-11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxylic Acid (4). A refluxing mixture of 10.0 g (65.5 mmol) of 5-hydroxyanthranilic acid, 10.2 g (65.5 mmol) of 6-chloronicotinic acid, and 100 mL of glacial HOAc[4] was allowed to react for 15 h. The mixture was cooled and the resultant green suspension was filtered to give 9.50 g (55.2%) of crude **4**, mp 363–370 °C dec. The powder was recrystallized from pyridine, giving 4.20 g (24.4%) of analytically pure **4**, mp 375–378 °C dec.

Method B. 2-Methyl-11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxylic Acid (6). An ethanolic mixture (80 mL) containing 5.00 g (33.1 mmol) of 5-methylanthranilic acid, 5.20 g (33.1 mmol) of 6-chloronicotinic acid, and 3.0 mL (30 mmol) of hydrochloric acid was heated at reflux for 6 h. The resultant suspension was filtered, and it gave 2.10 g (25.5%) of crude **6**, mp 322–326 °C dec. One recrystallization from pyridine gave the analytical sample: yield 1.05 g (12.3%); mp 328–333 °C dec.

Method C. 3-Hydroxy-11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxylic Acid (8). Over a period of 0.5 h, 2.00 g (7.40 mmol) of **10** was added to 10 g of melted pyridine hydrochloride.[5] The resultant mixture was heated at 220 °C for 2 h. The reaction mixture was poured into ice–H_2O (200 mL) and stirred. Filtration of the resultant precipitate yielded 1.60 g (84.2%) of crude **8**, mp 363–370 °C dec. The solid material, obtained by recrystallization from pyridine, was triturated with EtOH and gave 1.00 g (52.6%) of the analytical **8**, mp 374–379 °C dec.

Method D. 2-Ethoxy-11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-8-carboxylic Acid (11). DMF (50 mL) containing 3.00 g (11.7 mmol) of **4**, 19.5 g (125 mmol) of ethyl iodide, and 2.76 g (20.0 mmol) of anhydrous K_2CO_3 was heated at 100 °C for 18 h. The resultant mixture was poured into H_2O (500 mL), and the yellow precipitate which formed was collected to give 2.35 g (70.7%) of crude **11**, mp 263–275 °C dec. The impure **11** was crystallized from MeOH to give 0.99 g (29.8%) of the analytical sample, mp 293–296 °C dec.

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Blood Glucose Lowering Sulfonamides with Asymmetric Carbon Atoms. 3.[1] Related N-Substituted Carbamoylbenzoic Acids

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Both enantiomers of 4-*N*-[1-(5-Fluoro-2-methoxyphenyl)ethyl]carbamoylmethylbenzoic acid display hypoglycemic activity. The more potent (*S*) enantiomer is approximately equipotent with the acylaminoethylbenzoic acids of the type HB 699 (Figure 1; Table I). Observations are given that suggest that these benzoic acids act at the same receptor as the hypoglycemic sulfonylureas and sulfonylaminopyrimidines and further that this receptor includes important binding sites.

Antidiabetic sulfonylamino compounds of the so-called second generation, such as glibenclamide (**2**)[2] and gliflumide [(*S*)-**5**][3] (Figure 1), display a hypoglycemic activity at much lower doses than corresponding drugs of the first generation, such as tolbutamide (**1**) and glymidine (**4**). The hypothesis was advanced that tolbutamide and glymidine are linked via their $-SO_2NH-$ group to a binding site A of a "sulfonamide" receptor, whereas, in addition, the newer drugs are bound through their –CONH– or –NHCO– group to a second binding site B and, as a result, attain higher potencies.[1,3,4]

Recently, the hypoglycemic and β-cytotropic activities of acylaminoethylbenzoic acids[5–7] have been reported; HB 699 (**3**), the most intensively investigated compound, exhibited about the same potency as tolbutamide **1**. The structure of **3** corresponds to a part of the glibenclamide molecule (part b in Figure 1). We suspect that compounds of this type act at the same receptor as the sulfonylamino compounds but via a binding site B and not via A, as in the case of tolbutamide, or via A and B, as in the case of glibenclamide.

If this assumption is correct, benzoic acid derivatives **6**, whose structure corresponds to the carbamoylmethylphenyl part b of the sulfonylaminopyrimidine **5**, should also have β-cytotropic activity. Further, compound **5** possesses an asymmetric carbon atom in this part of the molecule and (*S*)-**5** (gliflumide) has been found to be considerably more potent than (*R*)-**5**. If **6** is to occupy the same binding site B as **5**, an analogous stereospecificity of activity ought to be recognizable in the cases of (*S*)-**6** and

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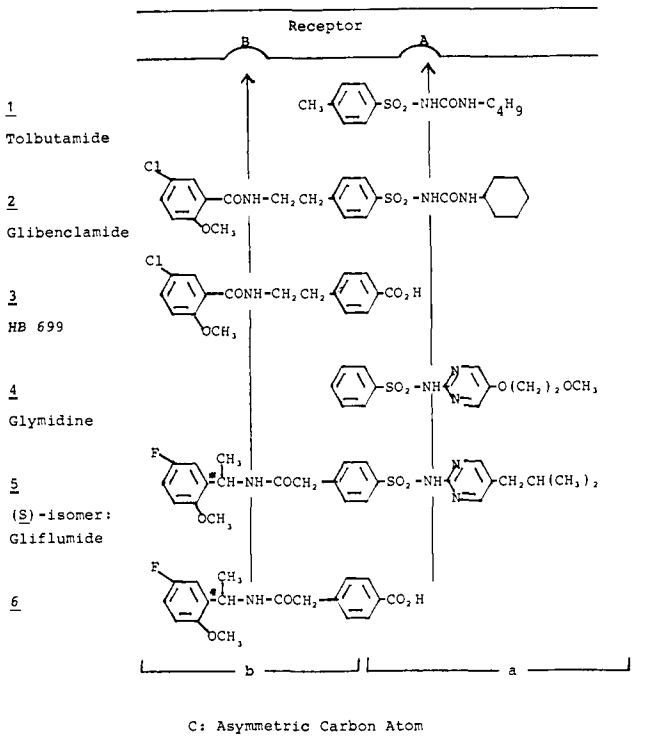
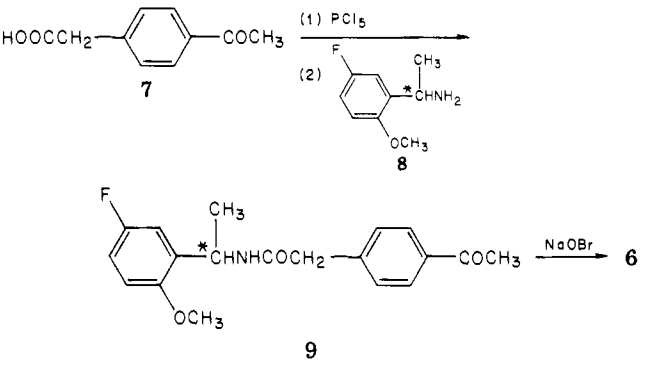


Figure 1. Antidiabetic sulfonylamino compounds and related N-substituted carbamoylbenzoic acids.

Scheme I



(*R*)-**6**. The present paper serves to verify our hypothesis.

Chemistry. The optically active carbamoylmethylbenzoic acids (*S*)-**6** and (*R*)-**6** (Scheme I) were obtained from 4-acetylphenylacetic acid[8] (**7**) and (*S*)- or (*R*)-1-(5-fluoro-2-methoxyphenyl)ethylamine (**8**)[3] (Scheme I).

Biological Results. The hypoglycemic activity of **4**, (*S*)-**5**, (*R*)-**5**, (*S*)-**6**, and (*R*)-**6**, administered as a solution of the Na salt in 0.9% saline, was investigated in fed female rats after iv injection of the doses stated in Table I.

Blood samples were taken after 1 and 3 h, blood glucose being measured enzymatically by the GOD-Perid® method[9,10] (biochemical test combination, Boehringer-Mannheim GmbH). As far as dose-dependent effects were recognizable, linear dose–response curves were calculated on the basis of relative changes from the control group (percent control value). The required doses (ED_{50}) for a 50% reduction of the mean control value ($x \pm s_x = 5.12 \pm 0.03$ mmol/L; 92.6 ± 0.6 mg/mL) were derived from these linear curves.

It follows from the results (Table I; ED_{50} after 1 h; after 3 h the activity had nearly disappeared) that both N-substituted carbamoylmethylbenzoic acids, (*S*)- and (*R*)-**6**, have hypoglycemic activity. As in the case of gliflumide [(*S*)-**5**] and the enantiomer (*R*)-**5**, here too the (*S*) form has a higher potency than the (*R*) enantiomer. The enantiomers differed respectively by similar factors of about 20 [(*S*)-**6**/(*R*)-**6**] and 40 [(*R*)-**5**/(*S*)-**5**]. The relative potencies of the benzoic acids are considerably lower than those of gliflumide and its enantiomers. The potency of the (*S*) form of **6** corresponds to about that of glymidine (**4**) and is, thus, more than 3000 times less that of gliflumide. The highest tested dose of the (*R*) form of **6** (937.5 mg) proved to be toxic, and no animal survived.

Table I. Hypoglycemic Potencies

compd[a]	inj doses[b] (iv), mg/kg	ED_{50},[c] mg/kg	95% conf
4	9.6, 24, 60, 150	69.7	58.0-94.0
(*S*)-**5**	0.0064, 0.016, 0.04, 0.1	0.020	0.017-0.025
(*R*)-**5**	0.16, 0.4, 1.0, 2.5	0.81	0.70-0.97
(*S*)-**6**	20, 60, 150, 375	63.9	53.7-82.5
(*R*)-**6**	40, 150, 375, 937.5	1181	461-37491

[a] See Figure 1. [b] Female rats. [c] 1 h postinjection.

After the above-mentioned working hypothesis had been basically confirmed by the present findings, another investigation was carried out to see whether upon joint administration the activities of (*S*)-**6** and glymidine (**4**) were potentiated to the point that they were equal to gliflumide [(*S*)-**5**], which contains both important structural elements in one molecule (Figure 1). This was not the case. Whereas as little as 0.04 mg/kg of gliflumide causes maximal hypoglycemia, a mixture of 0.5 mg/kg of (*S*)-**6** + 0.5 mg/kg of glymidine (**4**) had no effect on blood glucose concentration.

Conclusions. Acylaminobenzoic acids **3**, corresponding to part b of glibenclamide (Figure 1), have been reported recently to have hypoglycemic activity and potency comparable to that of tolbutamide (**1**).[5-7] Similar findings have been noted with N-substituted carbamoylmethylbenzoic acids **6** of the gliflumide (*S*)-**5** type. Since here the same relationships between steric configuration and activity are exhibited as—on a higher absolute level—are shown by gliflumide and its enantiomer, it must be suspected that this phenomenon is a general principle and, as already suspected previously,[1,3,4] that the "sulfonamide receptor" of the β cells does indeed possess two adjacent binding sites. Evidently, insulin secretion can be triggered alternatively both by isolated occupation of the first binding site A, which is specific for the $-SO_2NH-$ structure, and of the second binding site B, which is specific for the –NHCO– or –CONH– group. If both binding sites are occupied simultaneously, as is the case if gliflumide or glibenclamide is administered, there is probably a potentiation of the respective single effects resulting in the well-known high potency of these and other substances of the "second generation". This increase in activity suggests that there is positive cooperativity between both binding sites.

Since the activities of the reactive substances containing only one binding site [(*S*)-**6**, glymidine (**4**)] do not potentiate one another, it must further be suspected that the two binding sites are localized at a distance recognizable in the structure of glibenclamide or gliflumide and other substances of the same type.

Experimental Section

General. Melting points were determined in open capillary tubes and are uncorrected. Analytical data of the compounds are indicated by symbols of the elements if they were within

±0.4% of the theoretical values.

(*R*)- and (*S*)-*N*-[1-(5-Fluoro-2-methoxyphenyl)ethyl]-4-acetylphenylacetamide [(*R*)- and (*S*)-9]. A suspension of 4-acetylphenylacetic acid (**7**)[8] (3.56 g, 20 mmol) and PCl_5 (4.2 g, 20 mmol) in 30 mL of CCl_4 was stirred for 30 min at 20 °C and for a further 10 min at 50 °C. After evaporation of volatile materials from the suspension, the oily residue (4.4 g) was dissolved in 20 mL of THF. This solution was added at 10 °C to a solution of (*R*)-1-(5-fluoro-2-methoxyphenyl)ethylamine[3] [(*R*)-**8**; 3.72 g, 22 mmol] and NEt_3 (4.404 g, 40 mmol) in 20 mL of THF. After stirring the solution for 18 h at 20 °C, the solvent was removed in vacuo. Addition of ice–2 N HCl, filtration of the precipitate, and crystallization from *i*-PrOH with some drops of concentrated HCl yielded 4.05 g (61%) of (*R*)-**9**: mp 129 °C; $[\alpha]^{20}_D$ +44° (*c* 1.0, MeOH). The (*S*) isomer of **9** [from (*S*)-**8**[3]] had: mp 128 °C; $[\alpha]^{20}_D$ −42° (*c* 1.0, MeOH). Anal. ($C_{19}H_{21}FNO_3$) C, H, F, N.

(*R*)- and (*S*)-4-*N*-[1-(5-Fluoro-2-methoxyphenyl)ethyl]-carbamoylmethylbenzoic acid [(*R*)- and (*S*)-6]. Bromine (7.2 g, 45 mmol) was dissolved in 60 mL of 2.25 N NaOH, and (*R*)-*N*-[1-(5-fluoro-2-methoxyphenyl)ethyl]-4-acetylphenylacetamide [(*R*)-**9**; 3.2 g, 9.7 mmol) in 20 mL of dioxane was added to the solution. After stirring the solution for 3 h at 90 °C, the precipitated salt was sucked off and dissolved in water. Addition of 2 N HCl yielded the crystalline product: 1.11 g (35%) of (*R*)-**6**; mp 200 °C; $[\alpha]^{20}_D$ +43° (*c* 1.0, MeOH). The (*S*) isomer of **6** [from (*S*)-**9**] had mp 198 °C; $[\alpha]^{20}_D$ −44° (*c* 1.0, MeOH). Anal. ($C_{18}H_{18}FNO_4$) C, H, F, N.

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Book Reviews

Enzyme Structure and Mechanism. By Alan Fersht. W. H. Freeman, San Francisco, Calif. 1977. xvii + 371 pp. 15.5 × 24 cm. $20.00 (cloth) and $9.95 (paperback).

This book describes the general principles and concepts which are currently used to define the relationship between enzyme structure and mechanism of catalysis. In addition, the book describes some of the experimental approaches which are used to determine the physical and chemical characteristics of an enzyme–substrate complex and the ensuing catalytic reaction. These general considerations are highlighted with specific examples of enzymes whose tertiary structures have been resolved by X-ray crystallography and for which sufficient information is available concerning their mechanisms of catalysis.

In the first two chapters of the book, the subjects of protein structure and chemical catalysis are described in fairly simple and general terms. Chapters 3–8 deal with various aspects of enzyme kinetics, including both theoretical and practical considerations. The chapters on "Measurement and Magnitude of Enzyme Rate Constants" (chapter 4) and "Practical Kinetics" (chapter 6) would be particularly informative for individuals who have limited laboratory experience in this area. Chapters 9–11, which deal with the specificity and forces involved in the formation of an enzyme–substrate complex, are quite informative. These chapters describe the general concept of enzyme–substrate complementarity and how this concept relates to the theories of enzyme catalysis. In the final chapter, the author shows how kinetic and structural work have been used to produce satisfactory descriptions of the reactions mechanisms for a selected few enzymes, whose crystal structures have been resolved at high resolution.

This book is directed toward senior undergraduate and graduate students who have had the appropriate introductory courses in organic chemistry, physical chemistry, and biochemistry. However, the book might also be recommended as a general review for chemists who are interested in updating their knowledge about the relationship of enzyme structure and mechanism. The references cited in the book are sufficiently pertinent and up-to-date to permit easy and rapid access to the more detailed literature, if so desired.

The author is to be commended for putting together a general but comprehensive review of enzyme structure and mechanism.

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Cancer—The Outlaw Cell. Edited by Richard E. La Fond. American Chemical Society, Washington, D.C. 1978. xv + 192 pp. 18 × 25.5 cm. $15.00.

This publication is based upon articles appearing in *Chemistry* during 1977. It covers basic cell biology with special emphasis on tumor cell biology.

The Forward and Introduction describe the magnitude of the cancer problem and give a very brief review of basic cell chemistry defining the roles of DNA, the various RNAs, proteins, and energy sources. The book is divided into 14 chapters which carry the reader from a definition and history of the disease through causes and modes of treatment. "Cancer—An Overview" defines the disease and introduces the medical nomenclature associated with cancer. "Tumor Growth and Spread" discusses the cellular processes involved and the factors which influence tumor cell growth, invasion, and metastasis. The cell cycle is defined in "Control of Cell Growth in Cancer". Also presented are the factors which influence and regulate normal cell growth and the manner in which normal and tumor cells differ in growth characteristics. "Cancer as a Problem in Development" discusses plant and animal tumors in terms of gene expression and alteration. Membrane structure, receptors, and the chemistry of intercell communication and its breakdown in tumor cells are outlined in the "Puzzling Role of Cell Surfaces". Known chemical carcinogens are discussed in "Cancer Causing Chemicals" relative to their molecular role in initiating cancer. In a similar vein, ionizing radiation, its interaction with genetic material, and what is known about the ways it may induce cancer is treated in "Cancer Causing Radiation". The next three chapters deal with the viral aspects of the cancer problem. "Cancer and Viruses" describes the physical appearance of various viruses and how they replicate themselves after cell invasion. "RNA Tumor Viruses" outlines