Synthesis and Characterization of Enantiomerically Pure cis- and trans-3-Fluoro-2,4-dioxa-8-aza-3-phosphadecalin 3-Oxides as γ-Homoacetylcholine Mimetics and Inhibitors of Acetylcholinesterase

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The title compounds, the P(3)-axially and P(3)-equatorially substituted cis- and trans-configured 8-benzyl-3-fluoro-2,4-dioxa-8-aza-3-phosphadecalin 3-oxides (= 8-benzyl-3-fluoro-2,4-dioxa-8-aza-3-phosphabicyclo[4.4.0]decane 3-oxides = 2-fluorohexahydro-6-(phenylmethyl)-4H-1,3,2-dioxaphosphorino-[5,4-c]pyridine 2-oxides) were prepared (ee > 98%) and fully characterized (Schemes 2 and 3). The absolute configurations were established from that of their precursors, the enantiomerically pure cis- and trans-1-benzyl-4-hydroxypiperidine-3-methanols which were unambiguously assigned. Being configuratively fixed and conformationally constrained phosphorus analogues of acetyl γ-homocholine (= 3-(acetyloxy)-N,N,N-trimethylpropan-1-aminium), they are suitable probes for the investigation of molecular interactions with acetylcholinesterase. As determined by kinetic methods, all of the compounds are weak inhibitors of the enzyme.

1. Introduction. – In the preceding report [1], we have presented the synthesis and characterization of the optically active P(3)-axially and P(3)-equatorially substituted cis- and trans-configured 9-benzyl-3-fluoro-2,4-dioxa-9-aza-3-phosphadecalin 3-oxides of type 1 (Scheme 1). Continuing our investigations on the inhibition of serine hydrolases (chymotrypsin, acetylcholinesterase) by azadecalin-type organophosphates, we discuss now the preparation and full characterization of the eight stereoisomeric, enantiomerically pure 8-benzyl-3-fluoro-2,4-dioxa-8-aza-3-phosphadecalin 3-oxides (= 8-benzyl-3-fluoro-2,4-dioxa-8-aza-3-phosphabicyclo[4.4.0]decane 3-oxides = 2-fluorohexahydro-6-(phenylmethyl)-4H-1,3,2-dioxaphosphorino[5,4-c]pyridine 2-oxides) 10 and 11 representing type-II inhibitors (cf. Schemes 1 and 3) [2]. The compounds are mimetics of γ-homoacetylcholine (= 3-(acetyloxy)-N,N,N-trimethylpropan-1-aminium)1) and as such are considered to be suitable probes for the investigation of molecular interactions with acetylcholinesterase (AChE) [3][4] and the stereochemical course of the inhibition reaction by 31P-NMR spectroscopy [5 – 7].

2. Synthesis and Characterization of the 3-Fluoro-2,4-dioxa-8-aza-3-phosphadecalins. – 2.1. Precursor Diols 2 and 3. Following the protocol for the preparation of the

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1) The trivial name ‘γ-homoacetylcholine’ is neither systematic nor is it common in the current literature. We have introduced this expression for the homologue of acetylcholine [3] and made use of it since then. Further common names used in the literature are acetyl homocholine and acetyl-γ-homocholine.
racemic compounds [3], the optically active (+)- and (−)-trans-, and (+)- and (−)-cis-1-benzyl-3-(hydroxymethyl)piperidin-4-ols ((+)- and (−)-2, and (+)- and (−)-3, resp.) were obtained after reduction of ethyl 1-benzyl-4-oxopiperidine-3-carboxylate (1) with NaBH4 and LiAlH4 (Scheme 2). The resulting mixture (ca. 2 : 1) of the diols (−)-2/(+)-3 was transformed in situ into the acetonides (−)-4/(+)-5 that could easily be separated by column chromatography (SiO2) into the pure trans- and cis-diastereoisomers (−)-4 and (−)-5, respectively [3][8]. Preparative HPLC (Chiralcel OD) of (±)-4 afforded (+)-4 ([α]D = +29.2, ee > 99%), and (−)-4 ([α]D = −28.7, ee > 98%) 2), and the

2) Although the method had been optimized (R5 > 4) [2], enantiomerically pure (−)-4 ([α]D = −29.3, ee > 99%) could be obtained only in relatively small amounts, whereas bigger fractions of (−)-4 did never exceed ee > 98%. As a consequence, the precursor (+)-2 and the corresponding phosphadecalins (−)-10a and (−)-10b had only ee > 98% [2]. The [α]D values were determined in acetone (c = 1).
analogous procedure with (±)-5 gave (+)-5 ([$\alpha$]$_D$ = +66.1, ee > 99%), and (−)-5 ([$\alpha$]$_D$ = −66.5, ee > 99%). Hydrolysis of the acetonides yielded the trans- and cis-1-benzyl-3-(hydroxymethyl)piperidin-4-ols (±)-2 ([$\alpha$]$_D$ = +9.2, ee > 98%$^f$), (−)-2 ([$\alpha$]$_D$ = −9.8, ee > 99%), (±)-3 ([$\alpha$]$_D$ = +16.7, ee > 99%), and (−)-3 ([$\alpha$]$_D$ = −16.6, ee > 99%) (Scheme 2). As recently evidenced in detail [8], the absolute configurations were inferred by means of the high-field 1H-NMR Mosher method [9] applied to the (R)- and (S)-MTPA ester couples 6a/6b, 7a/7b, 8a/8b, and 9a/9b (Scheme 2).

Scheme 2$^2$)

![Diagram](image)

2.2. 3-Fluoro-2,4-dioxa-8-aza-3-phosphadecalins 10 and 11. Applying the established protocol [7], the trans-8-benzyl-3-fluoro-2,4-dioxa-8-aza-3-phosphadecalin 3-oxides 10 (Scheme 3) were prepared from (+)- or (−)-2 by reaction with POCl$_3$ and chromatographic separation of the resulting P(3)-epimer mixture (axial/equatorial ca. 1:1) gave the pure axial epimers (+)-10a (ee > 99%) and (−)-10a (ee > 98%)$^g$ and the pure equatorial epimers (±)-10b (ee > 99%) and (−)-10b (ee > 98%)$^h$). Similarly,
starting from (+)- or (−)-3, the cis-8-benzyl-3-fluoro-2,4-dioxa-8-aza-3-phosphadecalin 3-oxides (+)-11a and (−)-11a, and (+)-11b and (−)-11b were obtained (ee > 99%) (Scheme 3). However, because the P(3)-equatorially substituted congeners 10b and 11b had a pronounced tendency to epimerize, the chromatographic separation of the epimers was not as straightforward as for the type-I [1] and type-IV [10] compounds [2], see Exper. Part. Moreover, due to both facile epimerization at the P-atom and conformational changes of (+)- and (−)-10b and (+)- and (−)-11b [11][12] (see Section 2.3), the magnitude of the [α]D values differed in the enantiomer pairs despite of equal ee (ee > 99% and ee > 98%, resp.).

2.3. Conformations of 10 and 11 in Solution. The NMR data of the 2,4-dioxa-8-aza-3-phosphadecalins 10 and 11 (see Exper. Part) display the same essential features as the type-I [1] and type-IV [10] congeners (Scheme 1). They confirm the relative configuration at the P-atom, the double chair conformations of the axial epimers 10a and 11a and distorted conformations of the 2,4-dioxa-3-phospha moiety in the equatorial
epimers 10b and 11b). Due to the strongly electronegative F-substituent, the chemical shift difference ($\Delta \delta = \delta_{eq} - \delta_{ax}$) is small in the trans-couple 10a/10b ($\Delta \delta = +0.5$ ppm) and negative in the cis-couple 11a/11b ($\Delta \delta = -0.4$ ppm) as discussed earlier [3][11].

As previously presented [1][3][5][6] and directly evidenced [11], the anomic (stereoelectronic) effect is the predominant parameter that determines the conformation of the 3-substituted 2,4-dioxo-3-phosphacladin 3-oxides. In the 3-axially substituted 3-fluoro-2,4-dioxo-8-aza-3-phosphacladin 10a and 11a, both the steric and the anomic effects act in the same direction. According to the vicinal couplings ($J(P,Heq-C(5)) = 24.8$ Hz (10a) and $J(P,Heq-C(5)) = 24.5$ Hz (11a), resp.), these compounds adopt a double-chair conformation (C-1) ($\delta_{ax}$)

In the equatorial epimers 10b and 11b, the steric and the stereoelectronic effects are opposite. Although the chair conformation is sterically favored, the anomeric preference drives the strongly electronegative F-substituent into the stereoelectronically favored axial position, hence resulting in non-chair conformations such as boat or twist-boats (i.e., B, TB-1, and TB-2) ($\delta_{eq}$) of the 2,4-dioxo-3-phospha moiety (Scheme 4) ($\delta$).

According to the vicinal-coupling data ($J(P,Heq-C(1)) = 2$, $J(P,Heq-C(5)) = 11.5$, $J(P,Heq-C(5)) = 10.2$ Hz), and on the basis of our recent conformational studies on the type-III [11 – 13] and type-IV congener (Scheme 1) that lead to the conformational assignments of the type-I inhibitors [1], we conclude in analogy that the trans-configured epimers (++) and (--) exist in an equilibrium mixture of C-I and TB-2 (Scheme 4) ($\delta$).

In the cis-configured equatorial epimers (++) and (--) of the decalin system combined with the anomeric preference of the P(3)-equatorial F-substituent render the situation significantly more complex, and additional conformations must be envisaged. In particular, the bicyclic system can undergo complete ring

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5) Generally, the $^{31}$P-NMR resonance of the axial epimer is shifted upfield with respect to the equatorial one and the chemical shift difference ($\Delta \delta = \delta_{eq} - \delta_{ax}$) is $>0$, and its magnitude is inversely proportional to the electronegativity of the substituent at the P-atom. However, the cyclic phosphorofluoridates of the cis-series of the type-I – IV compounds (Scheme 1, X = F) display $\Delta \delta <$ 0, a fact that is only explained by significant conformational changes. The magnitude of the $J(P,Heq)$ in the $^1$H-coupled $^{31}$P-NMR is indicative of the conformation of the 2,4-dioxo-3-phospha moiety: Diagnostically relevant values for the axial epimers are $J(P,Heq-C(5)) ca. 25$ Hz and $J(P,Heq-C(5)) ca. 0$, whereas the equatorial ones display $J(P,Heq-C(5)) \approx J(P,Heq-C(5)) \approx 10–15$ Hz. Hence, the axial epimers exhibit a $d$-type and the equatorial ones a $m$-type splitting pattern (see [11]).

6) The short terms for the conformations (IUPAC convention) were introduced in [11]: C = chair, B = boat, E = envelope, TB = twist-boat (see also [1])

7) There is no evidence for the completely inverted C-2 conformation in 11a. Hence, we assume that the strong anomeric preference prevents this interconversion

8) In such systems, the resulting minimum-energy conformations represent a balance between the anomeric effect favoring the axial orientation in the twist-boat and the 1,3-steric and eclipsing interactions favoring the chair conformation. This fact explains the unusual stabilization of non-chair conformations.

9) Since $J(P,Heq-C(1))$ is ca. 0, conformations B and TB-1 are excluded. At room temperature, the $^{31}$P-NMR spectra are well resolved and coalescence phenomena are not observed, i.e., the interconversion C-I $\approx$ TB-2 is fast on the NMR time scale. In contrast to [11 – 13], the conformational assignments are tentative and not corroborated by variable-temperature NMR experiments nor by X-ray crystallographic analyses.
inversion to yield the prominent C-2 arrangement (Scheme 4), which seems to be favored by both the anomeric and the steric effects. Moreover, although the piperidine moiety lacks stereoelectronic impact, the conformational equilibrium is also influenced by the N-benzyl group. According to crystal structures (see [1][11], and Fig. 2 below), the piperidine moiety adopts a chair conformation in solution with the N-benzyl group in an equatorial position. However, its steric demand certainly affects the equilibrium population of the conformers, and it may interfere with a complete ring inversion to C-2. With regard to the experimental $^3J(P,H_{ax}/C_5) = 18.5$ and $^3J(P,H_{eq}/C_5) = 7.5$ Hz, none of the conformations depicted in Scheme 4 can be excluded, nor can any be assigned. Hence, we assume that 11b exists in solution as a complex mixture of C-1/C-2, and/or TB-1/TB-2, and most likely, also B and envelope E have to be considered. A full account of our detailed conformational studies on the 3-fluoro-2,4-dioxo-3-phosphadecaline 3-oxides (types I–IV) will be presented later [12].

8) The most striking argument for the exclusion of a prominent conformation is that none of the $^3J(P,H)$ is ca. 0, see [11].

9) As discussed for 10b, the conformational assignments are tentative, and the interconversions must be fast on the NMR time scale at room temperature.
2.4. Crystallographic Analyses of (−)-10a and (−)-11a. The structures of (−)-10a and (−)-11a were solved and refined successfully. Both compounds in the crystal were enantiomerically pure, and the absolute configurations of the molecules were determined independently by the diffraction experiments. The refinement of the absolute structure parameter confidently confirmed that the refined coordinates (cf. Table 2, Exper. Part) represent the true enantiomorphs with the expected (1S,3S,6R)-configuration for (−)-10a (Fig. 1), and the (1R,3R,6R)-configuration for (−)-11a (Fig. 2). These results independently corroborate the configurational assignments of the precursors (+)-(3R,4S)-1-benzyl-3-(hydroxymethyl)piperidin-4-ol ((+)-2) and (−)-(3R,4R)-1-benzyl-3-(hydroxymethyl)piperidin-4-ol ((−)-3) by means of the high-field ¹H-NMR Mosher method. As discussed above, the six-membered ring containing the P-atom has an undistorted chair conformation with the F-atom in the axial position, and the N-benzyl group occupies the sterically favored equatorial position.

![Molecular structure of (−)-10a.](image)

Fig. 1. Molecular structure of (−)-10a ((1S,3S,6R)). Trivial numbering; 50% probability ellipsoids.

All attempts to crystallize one of the equatorial epimers (+)- or (−)-10b and/or (+)- or (−)-11b resulted in complete epimerization. Hence, no X-ray crystallographic analysis of the equatorial compounds is available.

3. Enzyme Kinetics. – 3.1. General. The inhibitory potency and the mode of action of the enantiomerically pure 3-fluoro-2,4-dioxa-8-aza-3-phosphadecalins 10 and 11 was determined according to the general considerations and procedure explicitly described in the precedent article [1]. In particular, the data acquisition was based on the Ellman assay [14]) and the mathematical evaluation was performed according to [15]. For irreversible inhibition, the simplified overall processes were considered that directly yield the covalently phosphorylated enzyme E—I (Scheme 5, mechanism a) or involve a preceding reversible step via the associative complex E—I⁺ (Scheme 5, mechanism b) [1]. However, since several congeners of the 3-fluoro-2,4-dioxa-8-aza-3-phosphadecalins were reversible inhibitors of AChE, the basic equilibria that govern the formation of the adsorptive complexes EI, ES, and ESI had to be considered, too (Scheme 5, mechanism c) [16].

[10] The full data sets are summarized in Table 2 (see Exper. Part). CCDC-838432 (−)-10a and CCDC-838433 (−)-11a contain supplementary crystallographic data. These can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.code.cam.ac.uk/data_request/cif.
3.2. Data Analysis and Results. Determination of the apparent rate constants ($k_{obs}$) from the progress curves ($A = f(t)$, see Eqn. 1 in the Exper. Part) and the mathematical evaluation of the dependence $k_{obs} = f([I])$ to evaluate the irreversible inhibition parameters ($K_D$, $k_a$, $k_r$, and $k_p$) were performed according to [15]. The reversible inhibition parameters ($K_I$ and $K'_I$) were determined from the linear primary plots ($A = f(t)$) by application of approved linearization procedures [16][17]. The mathematical equations (Eqns. 1–3) underlying Scheme 5 and further details are summarized in the Exper. Part. The secondary plot ($k_{obs} = f([I])$) exhibited a linear dependence for ($/C_6$)$-10a$, ($/+$)$-10b$, and mechanism $a$ was assigned. In the case of ($/+$)- and ($-/C_0$)-$10b$, ($-/C_0$)-$11a$, and ($/+$)- and ($-/C_0$)-$11b$, the secondary plot depended hyperbolically upon $[I]$, and mechanism $b$ was assigned for these compounds. Since the primary plots ($A = f(t)$) for ($-/C_0$)-$10a$ and ($/+$)-$11a$ clearly displayed a linear dependence, these compounds are reversible inhibitors of AChE. According to the comparison of the results of the different linearization methods [2], the linear mixed inhibition mechanism $c$ could be assigned (Scheme 5)[11]. The experimental results are summarized in Table 1.

Compared to the potent standard reference diisopropyl phosphorofluoridate (P(OR)F(OiPr)$_2$) and both the type-I [1] and type-IV [10] congeners, the investigated 3-fluoro-2,4-dioxo-8-aza-3-phosphadecalins 10 and 11 are very weak inhibitors of AChE, the most potent compound ($-/C_0$)-$10a$ being only half as active as the weakest type-I inhibitor. The P(3)-equatorially substituted congeners ($/+$)-$10a$ and ($-/C_0$)-$10b$ and ($/+$)-$11a$ react according to mechanism $b$ and do not display significant stereo-selectivity. In contrast, the P(3)-axially substituted pairs ($/+$)- and ($-/C_0$)-$10a$ and ($/+$)- and ($-/C_0$)-$11a$ are remarkably enantioselective. Whereas ($/+$)-$10a$ phosphorylates AChE directly (mechanism $a$), its enantiomer ($-/C_0$)-$10a$ is a reversible inhibitor (mechanism $c$).

11) However, the results of the linearization processes were not fully distinct [2], see Exper. Part.
**Scheme 5**

**Table 1. Kinetic Data of the Inhibition of AChE with the Enantiomerically Pure 3-Fluoro-2,4-dioxa-8-aza-3-phosphadecalins \((+)-10\) and \((-)-11\) (\(P(O)F(OiPr)\)) as reference)**

<table>
<thead>
<tr>
<th>Kinetic parameters</th>
<th>Mechanism</th>
<th>Kinetic parameters</th>
<th>Mechanism</th>
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</thead>
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<tr>
<td>((+-))-10a</td>
<td>(k_a = 4.3 \pm 0.1 \text{ m}^{-1}\text{s}^{-1})</td>
<td>((+-))-10a</td>
<td>(k_a = 195 \pm 3 \text{ m}^{-1}\text{s}^{-1})</td>
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<tr>
<td>((-))-10a</td>
<td>(K_i = 1600 \pm 20 \text{ m})</td>
<td>((-))-10a</td>
<td>(K_i = 930 \pm 5 \text{ m})</td>
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<tr>
<td>(K_i' = 1750 \pm 20 \text{ m})</td>
<td>(K_i = 7'600 \pm 30 \text{ m})</td>
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<tr>
<td>((\pm))-10a</td>
<td>(k_a = 1 \pm 0.1 \text{ m}^{-1}\text{s}^{-1})</td>
<td>((\pm))-10a</td>
<td>(k_a = 18.5 \pm 0.7 \text{ m}^{-1}\text{s}^{-1})</td>
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<tr>
<td>((+)-10b)</td>
<td>(k_i = 31.7 \pm 0.3 \text{ m}^{-1}\text{s}^{-1})</td>
<td>((+)-10b)</td>
<td>(k_i = 0.0007 \pm 0.00003 \text{ m})</td>
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<tr>
<td>(K_i = 105 \pm 6 \text{ m})</td>
<td>(K_i = 40 \pm 5 \text{ m})</td>
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<tr>
<td>(k_i = 0.0033 \pm 0.0004 \text{ s}^{-1})</td>
<td>(k_i = 0.0007 \pm 0.00003 \text{ s}^{-1})</td>
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<tr>
<td>((-))-10b</td>
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<td>((-))-10b</td>
<td>(k_i = 48.3 \pm 0.5 \text{ m}^{-1}\text{s}^{-1})</td>
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<tr>
<td>(K_i = 73 \pm 5 \text{ m})</td>
<td>(K_i = 52 \pm 5 \text{ m})</td>
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<td>(k_i = 0.0017 \pm 0.0001 \text{ s}^{-1})</td>
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<td>((+)-11a)</td>
<td>(k_i = 195 \pm 3 \text{ m}^{-1}\text{s}^{-1})</td>
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<tr>
<td>((-))-11a</td>
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<td>(k_i = 195 \pm 3 \text{ m}^{-1}\text{s}^{-1})</td>
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<tr>
<td>((-))-11b</td>
<td>(k_i = 52 \pm 5 \text{ m})</td>
<td>((-))-11b</td>
<td>(k_i = 195 \pm 3 \text{ m}^{-1}\text{s}^{-1})</td>
</tr>
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</table>

**E** = Free enzyme: serine hydrolase (acetylcholinesterase, chymotrypsin)

\(I\) = Inhibitor, \(S\) = substrate, \(P\) = product(s)

\(k_a, k_i\) = Association constant

\(K_i' = k_i/k_i\) = Dissociation constant (also referred to as \(K_D\))

\(k_p\) = Rate constant

\(K_i, K_i'\) = Inhibitory potency (monomolecular reaction constant)

\(K_i' = k_i/k_i\) = Inhibitory potency (bimolecular reaction constant)

\(K_i' = k_i/k_i\) = First-order rate constant

\(K_i/k_i\) = Competitive inhibition constant (also referred to as \(K_D\))

\(K_i, K_i'\) = Noncompetitive inhibition constant (\(K_i' = k_i/k_i\), also referred to as \(K_D\))

\(K_i, K_i'\) = Triplex complex association constant (\(K_i' = k_i/k_i\), also referred to as \(K_D\))

\(\alpha = (1 + [S]/[S][S]) + K_{m}\) = Modifying factor

\(K_i' = K_i', K_i' = K_i'\) = Noncompetitive inhibition

\(K_i' = K_i', K_i' = K_i'\) = Linear mixed-type inhibition

\(P(O)F(OiPr)\) = Reference compound
Interestingly, its racemate \( (\pm)-10a \) reacts irreversibly (mechanism \( a \)), thus confirming the finding that the inhibitory activity of a racemic inhibitor is roughly the arithmetic mean of the enantiomers \( 13) \). Similarly, \((-)-11a\) is an irreversible inhibitor of AChE (mechanism \( b \)), whereas \((+)-11a\) reacts reversibly with the enzyme (mechanism \( c \)). However, since all compounds are very weak inhibitors of AChE, a clear differentiation of the primary plot type \( A = f(t) \) was not always straightforward\( 11) \). In particular, in some cases its curvature was not too pronounced and rather converged towards a straight line as is characteristic for reversible inhibitors. Therefore, the interpretation and assignment of mechanism \( c \) is not fully assured\( 13) \).

4. Remarks. – Besides the investigation of the stereochemical implications of the inhibition reaction by \( ^{31}\text{P}-\text{NMR} \) spectroscopy \( 5–7\)[13], it is the aim of our research project to study the molecular interactions of AChE with acetylcholine (ACh). In particular, kinetic studies are performed to deliver insight into the physiologically active (recognition) conformation of ACh that governs the enzymatic hydrolysis process\( 14) \). Being \( \gamma \)-homoacetylcholine mimetics, the 8-benzyl-3-fluoro-2,4-dioxa-8-aza-3-phosphadecalin 3-oxides \( 10 \) and \( 11 \) (type-II inhibitors) clearly differ from the natural substrate. They cannot be compared directly with ACh and are not able to adopt a respective recognition conformation. Hence, the disclosed very weak inhibitory potency is not surprising. Moreover, the \( N \)-benzyl group as additional structural feature is considered to influence both the steric demand and the basicity of the compound. In particular, the cationic site of ACh is substituted by an electron donating group. However, in spite of these restrictions, the actual results provide some evidence of the viability of our concept\( 15) \), but they do not allow to draw significant further conclusions.

The authors are indebted to PD Dr. A. Linden, head of the X-ray department of our institute, for the high-quality X-ray crystallographic analyses. The financial support of the project by the Swiss National Science Foundation is gratefully acknowledged.

Experimental Part

1. General. See \[1\][3][6]. For the particular precautions in preparing and handling the organophosphates, see \[3\]. Determination of ee: based on the integration of the peak areas of the anal. HPLC separations of the precursor diols with optimized resolution (\( R_s > 4 \)). NMR Assignments: based on extensive 2D-NMR (see \[3\]) and selective \( ^1\text{H}-\text{decoupling} \) experiments.

\[12\] Although apparently obvious, it cannot be concluded \textit{a priori} that the inhibition constants are simply additive.

\[13\] Meanwhile, we have presented a novel, integrated approach with a reappraisal of kinetic mechanisms and diagnostic methods that enables distinct as well as subtle differentiations of generalized inhibition mechanisms \( 18) \). Because the kinetic experiments and the data evaluation for the \((+)-\) and \((-)-9\)-benzyl-3-fluoro-2,4-dioxa-8-aza-3-phosphadecalin 3-oxides \( 10 \) and \( 11 \) were performed earlier \[2\] than the recent findings \[18\], the analysis of the assay data follows the simplified procedure according to \[15\]. A complete re-interpretation of the kinetic data of \( 10 \) and \( 11 \) is in process \[19\].

\[14\] Concerning a discussion on the recognition conformation of ACh, see \[1\].

\[15\] At least, our working hypothesis is not discredited by this negative evidence.
The absolute configurations were assigned by means of the high field 1H-NMR Mosher method applied to the (R)- and (S)-MTPA ester couples 6a/6b, 7a/7b, 8a/8b, and 9a/9b; for explicit details, see [2][8].

3. trans- and cis-8-Benzyl-3-fluoro-2-fluorohexahydro-3-aza-3-phosphadecalin 3-Oxides (= 2-Fluoroheoxydrol-6-(phenylmethyl)-4H-1,1,2-dioxaphosphorin[5,4-c]pyridine 2-Oxides; (+)- and (−)-10a, (+)- and (−)-10b, (+)- and (−)-11a, and (+)- and (−)-11b. Res. To a cooled soln. (−)-2 (136 mg, 0.61 mmol) in anh. CH2Cl2 (2.5 ml) in a glove box (N2 atmosphere), a cooled soln. (−)-2 of POCl3 [20] (62 µl, 0.68 mmol, 1.11 equiv.) in Et2O (2 ml) was added with a syringe, and the mixture was stirred for 5 min at 0°C when the mixture was withdrawn and quickly passed through SiO2 (SiO2, 60, 15–40 µm [Merck, 115111], pH 5.6 [6], Et2O). The eluate was gently evaporated (N2 stream, <30 °C), and the residue (+)-10a (+)-10b and (+)-10b (62 mg, 28%) and (+)-10b (43 mg, 25%). Applying the same procedure, phosphorylation of (+)-2 (98 mg, 0.44 mmol) afforded (−)-10a (34 mg, 27%) and (−)-10b (22 mg, 17%); the axial epimers were less polar.

Similarly, the cis-configured compounds were obtained after phosphorylation and CC (SiO2, pH 5.6 [6], hexane/AcOEt 1:2 containing 3.5% MeOH): from (+)-3 (132 mg, 0.59 mmol), (+)-11a (62 mg, 37%) and (−)-11b (39.2 mg, 41%); from (−)-3 (162 mg, 0.61 mmol), (−)-11a (81 mg, 43%) and (−)-11b (64 mg, 37%); the equatorial epimers were less polar.

In contrast to the racemic compounds [3], the optically active P(3)-equatorially substituted 3-fluoro-2-dioxahexahydro-3-aza-3-phosphadecalin compounds partially epimerized during CC. Therefore, the contact time to SiO2 had to be minimized by an optimized adsorbent substance ratio (ca. 25 mg on a 8 × 1 cm column) and high flow rates (ca. 4 ml/min). However, although the operating conditions were optimized, (+)- and (−)-10b contained ca. 4–5% of (+)- and (−)-10a [2]. The axial epimers were stable when stored as such (<0 °C, Ar), but in soln, they slowly decomposed. Although being reasonably stable when stored as such (<0 °C), Ar), the equatorial epimers gradually epimerized; in particular, the trans-congeners (+)- and (−)-10b completely epimerized during crystallization to yield (+)- and (−)-10a, resp. [2].

(+)-(1R,3S,6S)-8-Benzyl-3-fluoro-2-fluorohexahydro-3-aza-3-phosphadecalin 3-Octide (= (+)-(2R,4aS,8aR)-2-Fluoroheoxydrol-6-(phenylmethyl)-4H-1,1,2-dioxaphosphorin[5,4-c]pyridine 2-Octide; (+)-10a): Colorless prisms. M.p. 174–175.5°C. [α]D 29.3 (c = 1.00, acetone; ee >99%). IR (KBr): 3008w, 3006w, 3026m, 2961m, 2938m 2866w, 2820m, 2805m, 2778w, 2728w, 1493m, 1486w, 1471w, 1449m, 1393w, 1368w, 1322s, 1264w, 1218s, 1182m, 1142w, 1134w, 1105w, 1074w, 1045s, 997s, 869m, 841s, 846w, 799m, 786m, 741m, 698w, 663m, 535m. 1H-NMR (400 MHz, CDCl3): 7.36–7.25 (m, PhCH3); 4.29 (A of ABX-P, J = 11.0, J(5eq,P) = 24.5, J(5eq,6) = 4.5, Hα=-(5)); 4.18 (td, J(1L,6) = J(1,10a) = 12.0, J(10eq,9) = 4.8, H(−C(1))); 4.11 (B of ABX-P, J = J(5ax,6) = 11.0, J(5ax,P) = 1.0, Hα=-(5)); 3.58, 3.47 (AB, J = 13.2, PhCH3); 3.01 (ddd, J = 12.0, J(9eq,10ax) = 4.5, J(9eq,10eq) = 2.5, J(9eq,7eq) = 2.2, Hα=-(9)); 2.81 (ddd, J = 11.2, J(7eq,6) = 3.8, J(7eq,6) = 2.2, Hα=-(7)); 2.34 (X of ABXP-P, J = 12.0, J(6ax,5) = 11.0, J(6,5ax) = 11.0, J(6,7ax) = 11.2, J(6,7eq) = 3.8, H(−C(6))); 2.12 (td, J = J(9ax,10ax) = 12.0, J(9ax,10ax) = 2.5, Hα=-(9)); 2.08 (ddt, J = 12.0, J(10eq,9ax) = 4.8, J(10eq,9ax) = 2.5, Hα=-(10ax,1)); 1.94 (qd, J = J(10ax,1) = 12.0, J(10ax,9ax) = 4.5, Hα=-(10ax,9ax)); 1.70 (t, J = J(7ax,6) = 11.2, Hα=-(7)); 1.4°C-NMR (100.6 MHz, CDCl3): 137.4 (C(1)); 128.7 (C(2), C(6)); 128.3 (C(3)‘, C(3)‘); 127.4 (C(4)‘); 83.0 (d, J(1P) = 6.5, C(1)); 71.8 (d, J(5,P) = 7.6, C(5)); 62.9 (PhCH3); 51.1 (C(9)); 51.0 (C(7)); 39.6 (d, J(6,P) = 6.2, C(6)); 31.6 (d, J(10P) = 9.2, C(10)). 31P-NMR (161.9 MHz, CDCl3) = −15.9 (dd, J(1P) = 1010, J(1P,10P) = 24.5, J(1P,10P) = 1376.5 MHz, CDCl3); −85.3 (J(1P) = 1010). EI-MS: 285 (M+), 208 (8), 194 (10, [M−PhCH3]+), 186 (14), 185 (13), 172 (12), 146 (3), 132 (8), 126 (17), 118 (4), 94 (23), 92 (25), 91 (100, PhCH3), 67 (5), 65 (17), 56 (3).

(+)-(1S,3S,6R)-8-Benzyl-3-fluoro-2-fluorohexahydro-3-aza-3-phosphadecalin 3-Octide (= (+)-(2S,4aR,8aS)-2-Fluoroheoxydrol-6-(phenylmethyl)-4H-1,1,2-dioxaphosphorin[5,4-c]pyridine 2-Octide; (+)-10a): [α]D 2.59 (c = 1.00, acetone; ee >98%). All other data: identical with those of (+)-10a.

(+)-(1R,3S,6S)-8-Benzyl-3-fluoro-2-fluorohexahydro-3-aza-3-phosphadecalin 3-Octide (= (+)-(2R,4aS,8aR)-2-Fluoroheoxydrol-6-(phenylmethyl)-4H-1,1,2-dioxaphosphorin[5,4-c]pyridine 2-Octide; (+)-10b):
Colorless prisms. M.p. 113–114\(^{16}\)°C. \(\text{R}\left(\text{Et}_2\text{O}\right)\): 0.29. \(\alpha\text{[D]}_2^0 = +475\) (c = 1.000, acetone; ee > 99%). IR (KBr): 3065w, 3028w, 2978w, 2920w, 2869w, 2832m, 2780w, 1605v, 1498s, 1479w, 1455s, 1393m, 1368m, 1345s, 1237w, 1280w, 1205w, 1183w, 1178w, 1148w, 1131w, 1109m, 1085m, 1069m, 1042s, 999m, 975m, 959m, 915m, 897s, 849m, 792m, 705v, 655m, 575m. \(\text{H}-\text{NMR} (400\text{ MHz, CDCl}_3)\): 7.36–7.25 (m, \text{PhCH}_2); 4.37 (3 of \text{ABX}-\text{P}; \text{J} = 11.5, \text{J}(\text{Seq}) = 10.2, \text{J}(\text{Seq}) = 5.7, \text{H}_{4\text{m}}=\text{C}(5)^{15}); 4.31 (\text{dd}, \text{J}(\text{1,10axx}) = 11.5, \text{J}(\text{1,10eq}) = 4.5, \text{J}(\text{1,F}) = 2.0, \text{H}–\text{C}(1)); 4.13 (\text{B} of \text{ABX}-\text{P}; \text{J} = 14.5, \text{J}(\text{5ax,P}) = 10.5, \text{J}(\text{5ax,F}) = 3.7, \text{H}_{2\text{m}}–\text{C}(5)^{16}); 3.58, 3.46 (\text{AB}; \text{J} = 13.2, \text{PhCH}_2); 3.01 (\text{ddd}, \text{J} = 11.5, \text{J}(\text{9eq},10ax) = 4.5, \text{J}(\text{9eq},10eq) = 2.5, \text{H}_{9\text{m}}–\text{C}(9)); 2.84 (\text{ddd}, \text{J} = 11.0, \text{J}(\text{9eq}) = 3.8, \text{J}(\text{9eq},10eq) = 2.2, \text{H}_{9\text{m}}–\text{C}(7)); 2.48 (\text{X} of \text{ABX}-\text{P}; \text{J}(\text{6,1}) = 11.5, \text{J}(\text{6,5ax}) = 10.5, \text{J}(\text{6,5ax,F}) = 5.7, \text{J}(\text{6,7ax}) = 11.0, \text{J}(\text{6,7ax,F}) = 3.8, \text{H}–\text{C}(6)); 2.15 (\text{ddd}, \text{J} = 11.5, \text{J}(\text{10eq}) = 4.5, \text{J}(\text{10eq},9ax) = 2.5, \text{H}_{10\text{m}}–\text{C}(10)); 2.12 (\text{dd}, \text{J} = \text{J}(\text{9ax,10ax}) = 11.5, \text{J}(\text{9ax,10eq}) = 2.5, \text{H}_{10\text{m}}–\text{C}(9)); 1.90 (\text{qd}, \text{J} = \text{J}(\text{10ax,1}) = \text{J}(\text{10ax,9ax}) = 11.5, \text{J}(\text{10ax,eq}) = 4.2, \text{H}_{11\text{m}}–\text{C}(10)); 1.72 (\text{t}, \text{J} = \text{J}(\text{7ax,9ax}) = 11.0, \text{H}–\text{C}(7)); 13.5-\text{NMR} (100\text{ MHz, CDCl}_3): 1374 (\text{C}(1)'); 1287 (\text{C}(2)'), 1283 (\text{C}(3)'), 1274 (\text{C}(4)'); 82.3 (\text{d}, \text{J}(\text{1,P}) = 6.6, \text{C}(1)'); 71.4 (\text{d}, \text{J}(\text{5,P}) = 71.0, \text{C}(5)'); 62.0 (\text{PhCH}_3); 52.0 (\text{C}(7)'); 51.0 (\text{C}(9)'); 38.5 (\text{d}, \text{J}(\text{6,P}) = 12.6, \text{C}(6)'); 32.0; \text{J}(\text{10,P}) = 7.1 (\text{C}(10)); \text{H}-\text{NMR} (161.9\text{ MHz, CDCl}_3): -15.4 (\text{ddd}, \text{J}(\text{1,10F}) = 998, \text{J}(\text{1,10eq}) = 11.5, \text{J}(\text{1,10ax}) = 10.2, \text{J}(\text{1,10ax}) = \text{J}(\text{1,10eq}) = 2)^{17}; \text{H}-\text{NMR} (376.5\text{ MHz, CDCl}_3): -69.3 (\text{d}, \text{J}(\text{1,10F}) = 998); \text{H}–\text{MS}: 285 (22, \text{M}^+) 208 (10), 194 (32, \text{[M – PhCH}_2]^+) 186 (6), 172 (3), 160 (32), 138 (2), 126 (16), 112 (18), 94 (15), 92 (25), 91 (100, \text{PhCH}_2^+) 67 (10), 65 (15), 55 (5).

\(^{16}\) The specified m.p. corresponds to (±)-10b [3]. As discussed above, (±) and (−)-10b epimerize during the crystallization process to yield (+)- and (−)-10a, resp. (colorless needles, m.p. ca. 170–175°).

\(^{17}\) The descriptors ‘ax’ and ‘eq’ for the H-atoms at C(5) are based on their relative positions in the chair conformation of the 2,4-dioxo-3-phospho moiety. As discussed (Scheme 4), the conformation is rather a twist-boat (TB-2) than a chair in the P(3)-equatorially substituted compounds. For reasons of simplicity, the notation ‘ax’ and ‘eq’ is maintained. H\text{eq}–C(5) is always cis to H–C(1) and H\text{eq}–C(5) trans to H–C(1), see [11].
2-Fluorohexahydro-6-(phenylmethyl)-4-diagrams were drawn with ORTEPII [30].

method [23] was applied. Neutral-atom scattering factors for non-H-atoms were taken from [24], and the corrected for Lorentz factors were obtained from a least-squares refinement of the setting angles of 48331 reflections in the range 4 < \theta < 80. Plots of S vs. F were shown in Table 2. A view of the molecule is given in Fig. 1. The structure was solved by direct methods with SIR92 [31], which revealed the positions of all non-H-atoms. The mosaicity was 0.968(2). The mosaicity was 1.016(3) of all non-H-atoms. The non-H-atoms were refined anisotropically. All of the H-atoms were placed in geometrically calculated positions and refined by using a riding model where each H-atom was assigned a fixed isotropic displacement parameter with a value equal to 1.2 \sigma_{	ext{all}} of its parent atom. The refinement of the structure was carried out on F^2 by using full-matrix least-squares procedures, which minimized the function \Sigma \omega (F^2 - F_o^2). The weighting scheme was based on counting statistics and included a factor to downweight the intense reflections. Plots of \Sigma \omega (F^2 - F_o^2) vs. F_o/F(max) and resolution showed no unusual trends. A correction for secondary extinction was applied. Refinement of the absolute structure parameter [32] yielded a value of \omega = 0.06(16), which confirms that the refined model corresponds with the true enantiomorph, although the precision of the determination is low.

4.2. Determination of (-)-11a. The unit cell constants and an orientation matrix for data collection were obtained from a least-squares refinement of the setting angles of 48331 reflections in the range 4 < \theta < 80. The mosaicity was 0.968(2). A total of 804 frames were collected by using \phi and \omega scans with \kappa offsets. 17 s exposure time, a rotation angle of 0.7^\circ per frame, and a crystal–detector distance of 38.6 mm. The space group was uniquely determined by the systematic absences. Equivalent reflections were merged. The data collection and refinement parameters are given in Table 2. A view of the molecule is shown in Fig. 1. The structure was solved by direct methods with SIR92 [31], which revealed the positions of all non-H-atoms. The mosaicity was 1.016(3) of all non-H-atoms. The H-atoms were placed in geometrically calculated positions and refined by using a riding model where each H-atom was assigned a fixed isotropic displacement parameter with a value equal to 1.2 \sigma_{\text{all}} of its parent atom. The refinement of the structure was carried out on F^2 by using full-matrix least-squares procedures, which minimized the function \Sigma \omega (F^2 - F_o^2). The weighting scheme was based on counting statistics and included a factor to downweight the intense reflections. Plots of \Sigma \omega (F^2 - F_o^2) vs. F_o/F(max) and resolution showed no unusual trends. A correction for secondary extinction was applied. Refinement of the absolute structure parameter [32] yielded a value of \omega = 0.06(16), which confirms that the refined model corresponds with the true enantiomorph, although the precision of the determination is low. The mosaicity was 1.016(3). A total of 322 frames were collected by using \phi and \omega scans with \kappa offsets. The mosaicity was 0.968(2). A total of 804 frames were collected by using \phi and \omega scans with \kappa offsets.
offsets, 46 s exposure time, a rotation angle of 2.0° per frame, and a crystal–detector distance of 30.0 mm. The space group was determined from packing considerations, a statistical analysis of intensity distribution, and the successful solution and refinement of the structure. Equivalent reflections, other than Friedel pairs, were merged. The data collection and refinement parameters are given in Table 2. A view of the molecule is shown in Fig. 2. The structure was solved by direct methods with SIR92 [31], which revealed the positions of all non-H-atoms. The non-H-atoms were refined anisotropically. All of the H-atoms were placed in geometrically calculated positions and refined by using a riding model where

<table>
<thead>
<tr>
<th>(→-10a)</th>
<th>(→-11a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystallized from</td>
<td>pentane/Et₂O</td>
</tr>
<tr>
<td>Empirical formula</td>
<td>C₁₃H₁₇FNO₃P</td>
</tr>
<tr>
<td>Mᵣ</td>
<td>285.25</td>
</tr>
<tr>
<td>Crystal color, habit</td>
<td>colorless, prism</td>
</tr>
<tr>
<td>Crystal dimensions [mm]</td>
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</tr>
<tr>
<td>Temperature [K]</td>
<td>160(1)</td>
</tr>
<tr>
<td>Crystal system</td>
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</tr>
<tr>
<td>Space group</td>
<td>P2₁2₁2₁ (#19)</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
</tr>
<tr>
<td>Reflections for cell determination</td>
<td>48331</td>
</tr>
<tr>
<td>2θ Range for cell determination [°]</td>
<td>4–60</td>
</tr>
</tbody>
</table>

Unit cell parameters:

- \( a \) [Å]: 5.6609(2) → 5.5530(2)
- \( b \) [Å]: 10.0766(4) → 7.7169(4)
- \( c \) [Å]: 23.0798(8) → 8.7115(4)
- \( \alpha \) [°]: 90 → 104.506(2)
- \( \beta \) [°]: 90 → 91.269(3)
- \( \gamma \) [°]: 90 → 107.293(3)
- \( V \) [Å³]: 1316.53(8) → 343.18(3)
- \( F(000) \): 600 → 150
- \( D_2 \) [g cm⁻³]: 1.439 → 1.380
- \( \mu \) (MoKα) [mm⁻¹]: 0.224 → 0.215
- Scan type: \( \phi \) and \( \omega \) → \( \phi \) and \( \omega \)
- \( 2θ_{	ext{max}} \) [°]: 60 → 55
- Transmission factors (min; max): 0.702; 0.974 → 0.873; 0.965
- Total reflections measured: 16761 → 8012
- Symmetry independent reflections: 3817 → 2854
- \( R_{int} \): 0.105 → 0.046
- Reflections with \( I > 2\sigma(I) \): 2715 → 2769
- Reflections used in refinement: 3817 → 2854
- Parameters refined: 173 → 172; 3
- Final \( R(F) \) (I > 2\( \sigma(F) \) reflections): 0.0586 → 0.0442
- \( wR(F) \) (all data): 0.1503 → 0.1079
- Weights: \( w = \frac{1}{\sigma^2(F^2) + (0.003P)^2} \)
- Goodness of fit: 1.034 → 1.096
- Secondary extinction coefficient: 0.018(3) → –
- Final \( \Delta a/a \): 0.001 → 0.001
- \( \Deltaρ \) (max; min) [e Å⁻³]: 0.45; –0.33 → 0.37; –0.24
- \( σ(d_{C–C}) \) [Å]: 0.003 – 0.005 → 0.003 – 0.004

Table 2. Crystallographic Data of (→-10a and (→-11a
each H-atom was assigned a fixed isotropic displacement parameter with a value equal to 1.2\(U_{eq}\) of its parent atom. The refinement of the structure was carried out on \(F^2\) by using full-matrix least-squares procedures, which minimized the function \(\sum w(F^2 - F_c^2)^2\). The weighting scheme was based on counting statistics and included a factor to downweight the intense reflections. Plots of \(\sum w(F^2 - F_c^2)\) vs. \(F/F_c\) resolution showed no unusual trends. A correction for secondary extinction was not applied. Refinement of the absolute structure parameter [32] yielded a value of \(-0.07(10)\), which confidently confirms that the refined model corresponds with the true enantiomorph.

5. Enzyme Kinetics. 5.1. General. For the detailed experimental procedure and the methods of data analysis, see the preceding report [1]. The following parameters are as in [1]: Apparatus and general experimental conditions, phosphate buffer pH 7.00, AChE soln., ATC and DTNB solns., inhibitor solns., and the determination of \(K_m\), P(O)F(O)PPr\(_2\) (disisopropyl fluorophosphate) was used as the standard reference.

5.2. Ellman Assay [14]. In a polystyrene cell (4 ml, \(d = 1\) cm), phosphate buffer pH 7.00 (2 ml), DTNB soln. (100 \(\mu\)l), and ATC soln. (20 \(\mu\)l) were mixed and thermostatted at 25\(^\circ\) (ca. 5 min). Then inhibitor soln. (x \(\mu\)l = 25, 15, 10, and 5 \(\mu\)l; known \([I]\)), and MeCN ((25 - x) \(\mu\)l) were added. At \(t = 0\), the AChe soln. (1 ml) was added and the mixture gently mixed for 5 s. After 10 s, the monitoring of the absorbance at 412 nm (liberated bis-anion of 5-sulfanyl-2-nitrobenzoic acid) automatically started, and 900 data points were collected for 15 min at various concentrations of the inhibitor. As in the \(K_m\) determinations, the total volume was 3.15 \(\mu\)l, the concentration of the substrate \([S]\) was 500 \(\mu\)M. Since all of the compounds were weak inhibitors, relatively high \([I]\) were necessary, and due to solubility reasons, \([I] < 300 \mu\)M had to be maintained. Per inhibitor, at least five measurements with different inhibitor concentrations were performed.

5.3. Data Analysis. a) Irreversible Inhibitors [15]. The integrated rate equation describing product generation (monitored by the absorbance at \(\lambda = 412\pm 2\) nm) and the apparent rate constants (\(k_{obs}\)) is given by Eqn. 1. It is fitted (\(R > 0.999\)) to progress curves recorded at fixed \([S]\) and variable \([I]\) (primary plot, \(A = f(t)\)) to obtain a series of \(k_{obs}\) values and their standard errors (SE). The inhibition parameters are obtained from the secondary plots (\(k_{obs} = f([I])\)) that result from weighted (SE\(^{-2}\)) linear or nonlinear regression according to Eqns. 2 or 3. The analysis of these plots enables a differentiation between the inhibition mechanisms: \(k_{obs}\) depends linearly upon the inhibitor concentration for mechanism a and hyperbolically for mechanism b (see Scheme 5). For mechanism a, the \(k_i\) values are calculated according to Eqn. 2, its slope \(k_{obs}/[I]\) is obtained from the linear regression (Scheme 5.a). The decisive plot for mechanism b is doubly reciprocal \((1/k_{obs} = f([I])/[I]),\) Eqn. 3) and the \(K_D\) and \(k_i\) values are calculated by linear regression, the slope being \((K_D/k_i)(1 + [S]/K_m)\) and the intercept \(1/k_i\). The overall inhibitory potency \((k_i)\) is expressed by \(k_i/K_m\) (see Scheme 5,b).

\[
A = \frac{v_0}{k_{obs}} (1 - e^{-kt})\]  
(1)

\[
k_i = \frac{k_{obs}}{[I]} \left(1 + \frac{[S]}{K_m}\right)\]  
(2)

\[
\frac{1}{k_{obs}} = \frac{K_D}{k_i} \left(1 + \frac{[S]}{K_m}\right)^{-1} \frac{1}{k_i}\]  
(3)\(^{18}\)

b) Reversible Inhibitors [16]. Since all the compounds were weak inhibitors, a clear differentiation of the primary plot type was not always straightforward. In particular, in some cases, the curvature of the primary plot \((A = f(t))\) was not too pronounced and rather converged towards a straight line as is characteristic for reversible inhibitors. When reversible behavior was assumed, the assays were performed each at five different inhibitor \(([I])\) and substrate concentrations \(([S])\) resulting in 25 data points per inhibitor. The inhibition parameters were evaluated from the secondary plots that were obtained by linearization methods according to Lineweaver–Burk \((1/k_{obs} = f([I])/[S])\) [16], Hanes–Woolf

\(^{18}\) The last term in Eqn. 3 was erroneously printed as a subtrahend in [1].
Comparative analysis of the individual results [2] lead to the differentiation of the various reversible inhibition mechanisms (competitive, noncompetitive, uncompetitive, linear mixed type) [16][17] (see Scheme 5, c).

5.4. Results (Table 1). The secondary plot ($k_{obs} = f([I])$) exhibited a linear dependence for (+)-10a, (+)-10a, and (P(O)/F(Pr)3). Hence, mechanism $\alpha$ was assigned. In the case of (+)- and (–)-10b, (–)-11a, and (+)- and (–)-11b, the secondary plot depended hyperbolically upon $[I]$, and mechanism $\beta$ was assigned for these compounds. Since the primary plots ($A = f(i)$) for (–)-10a and (+)-11a clearly displayed a linear dependence, these compounds are reversible inhibitors of AChE. According to the comparison of the different linearization methods [2], the linear mixed inhibition mechanism $c$ could be assigned [16] (Scheme 5). However, the results of the linearization processes were ambiguous to some extent as exemplified for (+)-11a: Data evaluation according to Lineeweaver–Burk suggested a linear mixed inhibition mechanism (intersection above the abscissa; from the intercepts ($Ax$, $Ay$) the modifying factors $\alpha$ and $\alpha'-$ were calculated ($\alpha = -\alpha'/\Delta x K_{m}, \alpha' = v_{max}/\Delta y, \text{and } K_{i} = [I]/(\alpha - 1)$) [16]: $K_{i} = 947 \mu M$, $K_{m} = 7050 \mu M (\alpha = 7.444) [2]$. A similar result was obtained from the approach according to Dixon ($K_{i} = 1/\Delta y$, slope = $1/\Delta x K_{m}, \text{and } K_{i} = \alpha K_{m}$) [17]: $K_{i} = 919 \mu M$, $K_{m} = 8100 \mu M (\alpha = 8.811) [2]$. The value in Table 1 is the rounded mean of these two approaches. The interpretation of the Hans–Woolf diagram [16] resulted in a borderline case between noncompetitive and linear mixed inhibition (intersection just above the abscissa) [2]. Linearization according to Dixon [16] was ambiguous, too: The parallel straight lines are indicative for competitive inhibition, while others suggest a linear mixed mechanism [2]. Moreover, the rather close similarity of $K_{i}$ and $K_{m}$ of (–)-10a may suggest a noncompetitive mechanism for this compound.

REFERENCES


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