untreated enzymes. The Hill coefficients (n), as estimated from the slopes of the straight lines, are 1.9 for the untreated control and 1.1 for the PG-treated enzyme (figure 3B). Thus, treatment with PG resulted in the loss of cooperative interaction among AMP binding sites.

The presence of substrate not only protected the enzyme against PG-inactivation but also against altered response to inhibition by AMP or high substrate concentration. The mechanism of how the substrate exerts these protective effects remains to be investigated. However, the decreased sensitivity to inhibition by AMP or high substrate concentration is by no means the necessary consequence of enzyme inactivation. We induced about 50% inactivation of this enzyme by incubating it at 25 °C for 3 weeks and found that this partial inactivation was not accompanied by reduction in sensitivity to inhibition by either AMP or high substrate concentration. Partial inactivation of rabbit liver Fru-P_2ase by treatment with ADP or ATP or by incubating the enzyme at acidic pH even led to increased sensitivity to inhibition by AMP[8-10]. The altered properties induced by PG appear to be irreversible since they remained essentially unchanged after extensive dialysis or repeated washing on ultrafiltration membrane (XM-50, Amicon). Incubation of the modified enzyme (after dialysis) with substrate also failed to reverse these altered properties. It is speculated that the altered properties induced by PG might result from the formation of covalent bonds between PG and the enzyme molecule.

The molecule of PG, like those of other penicillins, contains a reactive 4-membered (β-lactam) ring. This β-lactam may be cleavaged at the CO–N bond when reacted with ε-amino group of lysyl residues in proteins to form stable benzylpenicilloyl derivatives of tissue proteins (or enzymes). This has been proposed to be the mechanism for the formation of penicillin antigen[11]. It has been reported that modification of ε-amino groups of lysyl residues of pig kidney Fru-P_2ase with pyridoxal-5'-P partially inactivated the enzyme and markedly decreased the enzyme sensitivity to inhibition by AMP and high substrate concentration[5]. These results are somewhat similar to our observations with PG treatment of turkey liver Fru-P_2ase.

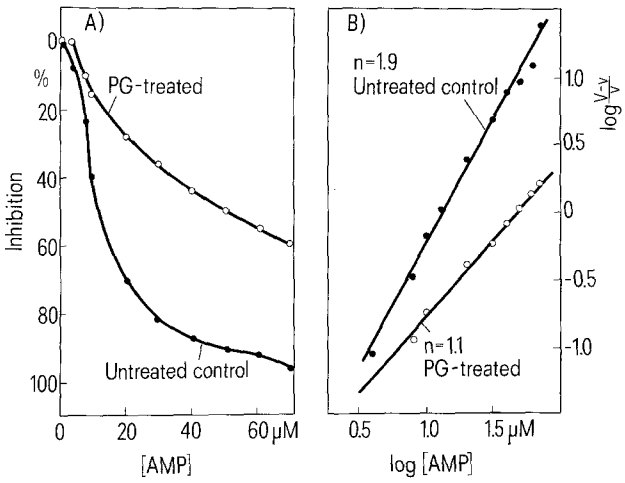

Fig. 3. Inhibition of Fru-P_2ase activity by varying concentrations of AMP (A) and Hill plots of these data performed according to Taketa and Pogell[3] (B). V is the activity without AMP and v is the activity in the presence of AMP. Hill coefficients (n) were calculated from the slopes of the straight lines. The following symbols signify: ●: untreated control; ○: treated with PG for 72 h.

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Synthesis of specific cholinergic inhibitors for affinity chromatography[1]

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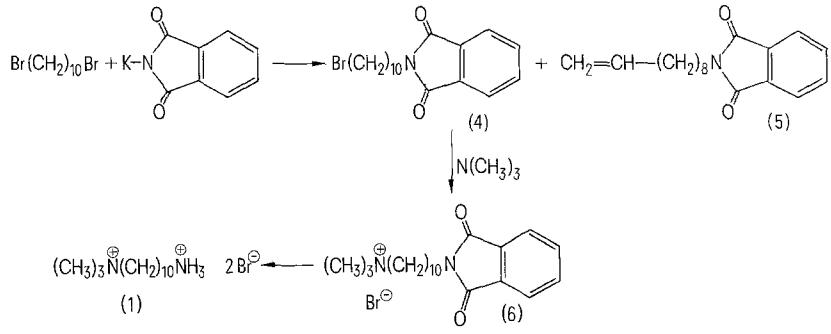
Summary. Our first aim was to simplify the spacer-synthesis for affinity-chromatography of cholinergic proteins. Furthermore we synthesized 2 new inhibitors which proved to be useful for purification of acetylcholine-receptor protein.

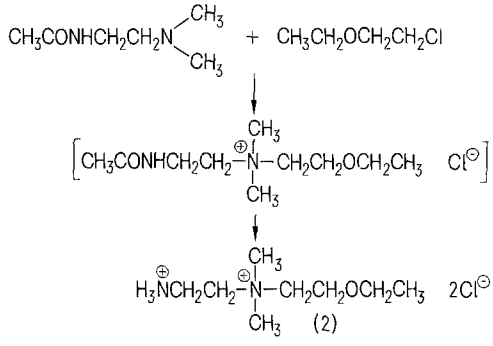
Specific proteins from the cholinergic nervous system can be highly purified by biospecific adsorption and subsequent desorption (affinity chromatography), as described in our earlier work[2-4]. The best results are obtained when the inhibitor, which has to be bound to a solid support (agarose), adequately fits the receptor, or in case of an enzyme, the active site. However the best inhibitor is useless if bound too close to the solid support. Cuatrecasas[5] first had the idea of separating the inhibitor from the solid support by a 'spacer' of considerable length. In case of acetylcholinesterase, a spacer of about 45–58 Å has been proved to be adequate.

The conventional spacer-synthesis[5], however, is relatively time-consuming, as it requires 5 steps. To overcome this difficulty, we synthesized 1-(N,N,N-trimethylammonium)-10-decylamine bromide hydrobromide (**1**), which already has a considerable length. This substance was first synthesized by Barlow[6], but the description lacks the necessary details. We prepared it (scheme 1) by selective synthesis from 1,10-dibromodecane and potassium phthalimide. The

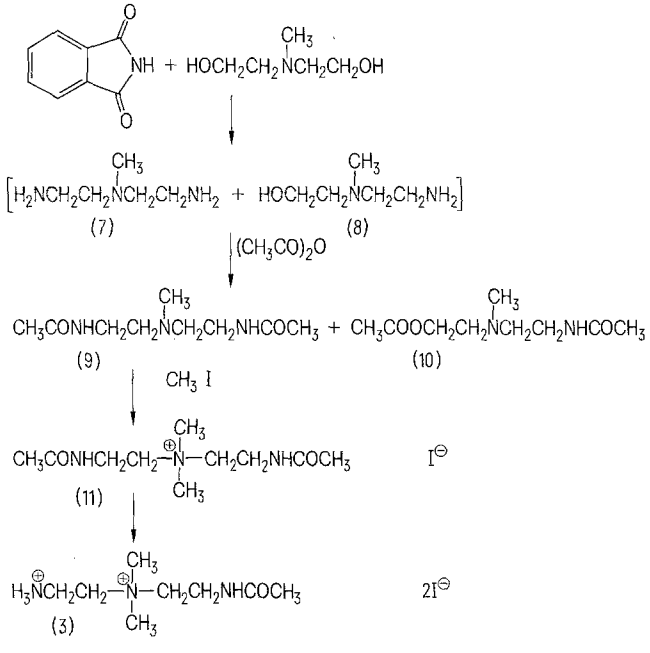
Scheme 1. Synthesis of 1-(N,N,N-trimethylammonium)-10-decylamine bromide hydrobromide (**1**).



Scheme 2. Synthesis of N-(β-ethoxyethyl)-N-(β-aminoethyl)-dimethylammonium chloride hydrochloride (**2**).



Scheme 3. Synthesis of N-(β-acetaminoethyl)-N-(β-aminoethyl)-dimethylammonium iodide hydroiodide (**3**).



resulting 1-bromodecyl-phthalimide-10 (**4**) was successively treated with trimethylamine and HBr to yield 1-(N,N,N-trimethylammonium)-10-phthalimido-decane bromide (**6**). Additionally we used spermine as suggested by Schmidt and Raftery[7] to replace the much shorter chain of hexamethylenediamine. Spermine can be coupled directly to bromocyano-activated agarose. This first step is followed by succinylation and then, after coupling of **1** with the aid of carbodiimide, the affinity-agarose was synthesized in only 3 steps. It is noteworthy that one of the eliminated steps had to be carried out by the expensive water-soluble carbodiimide.

The conventional purification of acetylcholine receptor proteins deals with relatively heavy molecules (animal toxins) bound to agarose, as discussed in the literature[7-10]. Binding of acetylcholine receptor protein to animal toxins, however, is very tight. Considerable conformational changes must be taken into account, and the liberation of the purified acetylcholine receptor again creates serious problems. For this reason Olsen et al.[11] and Schwyzer and Frank[12] suggested the use of 'small' cholinergic inhibitors. In order to purify acetylcholine receptor protein by affinity chromatography, we synthesized the acetylcholine-like compounds: N-(β-ethoxyethyl)-N-(β-aminoethyl)-dimethylammonium chloride hydrochloride (**2**) and N-(β-acetaminoethyl)-N-(β-aminoethyl)-dimethylammonium iodide hydroiodide (**3**).

2 was obtained by reacting N-(β-dimethylaminoethyl)-acetamide with ethyl-β-chloroethylether. Hydrolysis with hydrochloric acid formed the quaternary salt **2** (scheme 2).

3 was obtained by condensation of N-methyldiethanolamine with phthalimide. The resulting mixture of N,N-bis-(β-aminoethyl)-methylamine[13,14] (**7**) and N-(β-aminoethyl)-N-(β-hydroxyethyl)-methylamine[15] (**8**) after acetylation yielded pure N,N-bis-(β-acetaminoethyl)-methylamine (**9**). After quaternization with methyliodide, the formed **11** was partially hydrolized under carefully controlled conditions to give N-(β-acetaminoethyl)-N-(β-aminoethyl)-dimethylammonium iodide hydroiodide (**3**).

1 Dedicated to Prof. Peter Gaudenz Waser on the occasion of his 60th anniversary.
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