# 50. Specific Ligands for the Affinity Chromatography of Cholinergic Proteins

by **Gaetano Riggio, Wolfgang Herbert Hopff, Alfred André Hofmann** and **Peter Gaudenz Waser**

Institute of Pharmacology, University of Zurich, Gloriastrasse 32, CH-8006 Zurich (Switzerland)

(23.XI.79)

*Summary*

Affinity chromatography of proteins requires a ligand covalently bound to a solid support separated by a spacer of sufficient length. In the specific case of acetylcholinesterase we have reduced the conventional spacer synthesis from five to three steps.

For affinity chromatography of cholinergic proteins the ideal ligand would be acetylcholine which, however, could not be used because it is easily hydrolyzed. We synthesized hydrolysis-resistent ligands. Different specific ligands were synthesized for the affinity chromatography of serum esterase.

Affinity chromatography has achieved great importance in the last decade for the purification of biologically active proteins [1–3]. In this method a ligand bound covalently to a solid support creates an affinity for the desired protein. As proteins generally have large molecular volumes, the ligand must not be too closely bound onto the support. *Cuatrecasas* [1] first mentioned the importance of a 'spacer'. In the case of acetylcholinesterase, from both theoretical and practical experience, the ligand must be bound to a spacer of 45–58 Å of length to achieve optimum interaction with the active centre of the enzyme. The definition 'affinity chromatography' has been used differently by several authors. Unspecific elution of the desired protein with increasing ionic strength of buffer solutions after more or less specific adsorption on a support has also been defined as 'affinity chromatography' [4] [5]. We use the term affinity chromatography to mean specific adsorption and subsequent specific desorption chromatography.

An adequate support for affinity chromatography is agarose[1]). *Cuatrecasas* [1] has already described in detail the spacer synthesis on agarose. Generally the following sequence leads to good results. Bromocyano-activation of agarose, coupling with a diamine, elongation with succinic anhydride, another treatment with diamine catalyzed by water-soluble carbodiimide, again elongation with succinic anhydride, and finally, carbodiimide-catalyzed coupling of the ligand.

[1]) Agarose: Sepharose$^R$ from *Pharmacia*, CH-8034 Zürich.

0018-019X/80/2/0488-10$01.00/0 

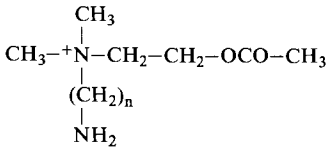
Our aim was to study the proteins of the cholinergic nervous system [6] and proteins more or less related to them[2]). We were specially interested in proteins like acetylcholine receptor, specific membrane-bound acetylcholinesterase (acetylcholine acetyl-hydrolase, EC 3.1.1.7), and serum esterase (pseudocholinesterase: acylcholine acylhydrolase, EC 3.1.1.8) which is present in free form in blood and tissues of many animals.

The natural substrate, acetylcholine, would be the ideal ligand for the purification of such proteins. Specific elution would also be effected using acetylcholine. Acetylcholine, however, is relatively unstable undergoing self-hydrolysis in aqueous solutions. Furthermore the enzymes under study usually do not allow the use of this substance, because they catalyze its hydrolysis.

For the purification of acetylcholinesterase, *Berman & Young* [7] successfully used a trimethylammoniophenyl group $(CH_3)_3N^+C_6H_4$-, and *Frank* [8] and *Schwyzer & Frank* [9] 'Acacholine' ($\varepsilon$-aminocaproylcholine) $(CH_3)_3N^+CH_2CH_2$-$OCO(CH_2)_5NH_2$. The latter authors proposed tetramethylammonium salts for specific elution. Acetylcholinesterase only weakly catalyzes the hydrolysis of propionylcholine, and not at all that of the longer-chained butyrylcholine, and acacholine. Consequently, the binding of the cationic head of the ligand alone to the anionic centre of the enzyme was effective. For this reason we synthesized $(CH_3)_3N^+(CH_2)_6NH_2$, (6-aminohexyl)trimethylammonium, which was a suitable ligand for the purification of the enzyme [10] [11].

As the conventional spacer-synthesis is relatively timeconsuming and requires water-soluble carbodiimide we examined molecules with longer chains. We treated the activated agarose with spermine [12] instead of hexamethylenediamine, and then, after coupling with succinic anhydride, with $(CH_3)_3N^+(CH_2)_{10}NH_2$, (10-aminodecyl)trimethylammonium (**1**) [13]. We achieved similar results as previously [10] [11].

For the purification of cholinergic proteins by affinity chromatography, the best ligand would probably be



However such a ligand would quickly hydrolyze in aqueous solutions. Considering the pharmophoric properties of acetylcholine and decanediammonium, we therefore undertook the synthesis of the hydrolysis-resistent compounds **2**–**4** for the affinity chromatography of the cholinergic receptor. Some results have already been reported [14]. In these investigations tetramethylammonium was used for elution of the active protein [9].

---

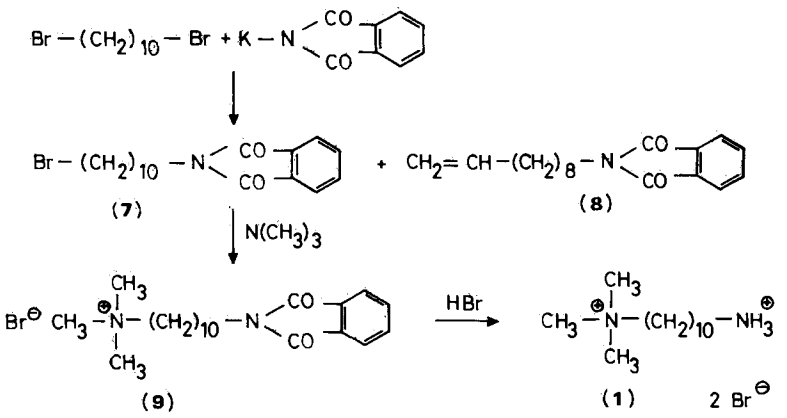
2) We would like to direct readers, who are not acquainted with the keystone of neurotransmission, to some relevant bibliography such as: Basic Neurology, *Schadé & Ford,* Elsevier, NY 1973, and/or Kurzes Lehrbuch der Pharmakologie und Toxikologie, *Kuschinsky & Lüllman*, G. Thieme, Stuttgart 1978.

Hydrolysis of butyrylcholine being catalyzed even faster than that of acetylcholine by serumesterase, we synthesized ligands **5** and **6** for specific adsorption of this enzyme. In *Schemes 1–4* we show the way to obtain the ligands described.

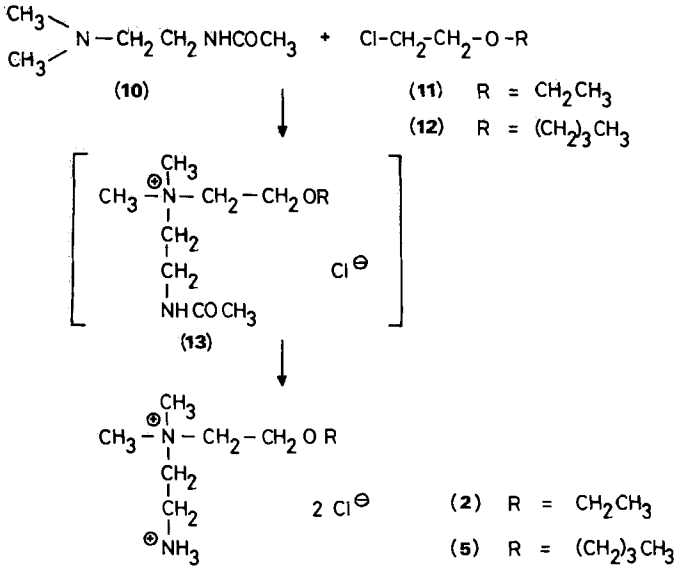
We also report the synthesis of **20**, which is expected to give in neutral solution the unstable aziridinium ion **21**, a potential irreversible inhibitor of the cholinergic receptor [15] [16].

This work was supported by the *Swiss National Science Foundation* (Project No. 3.086-0.76). We thank Mr. *H. Frohofer*, microanalytical section of the Organic Chemistry Institute, University of Zurich, for the microanalysis.

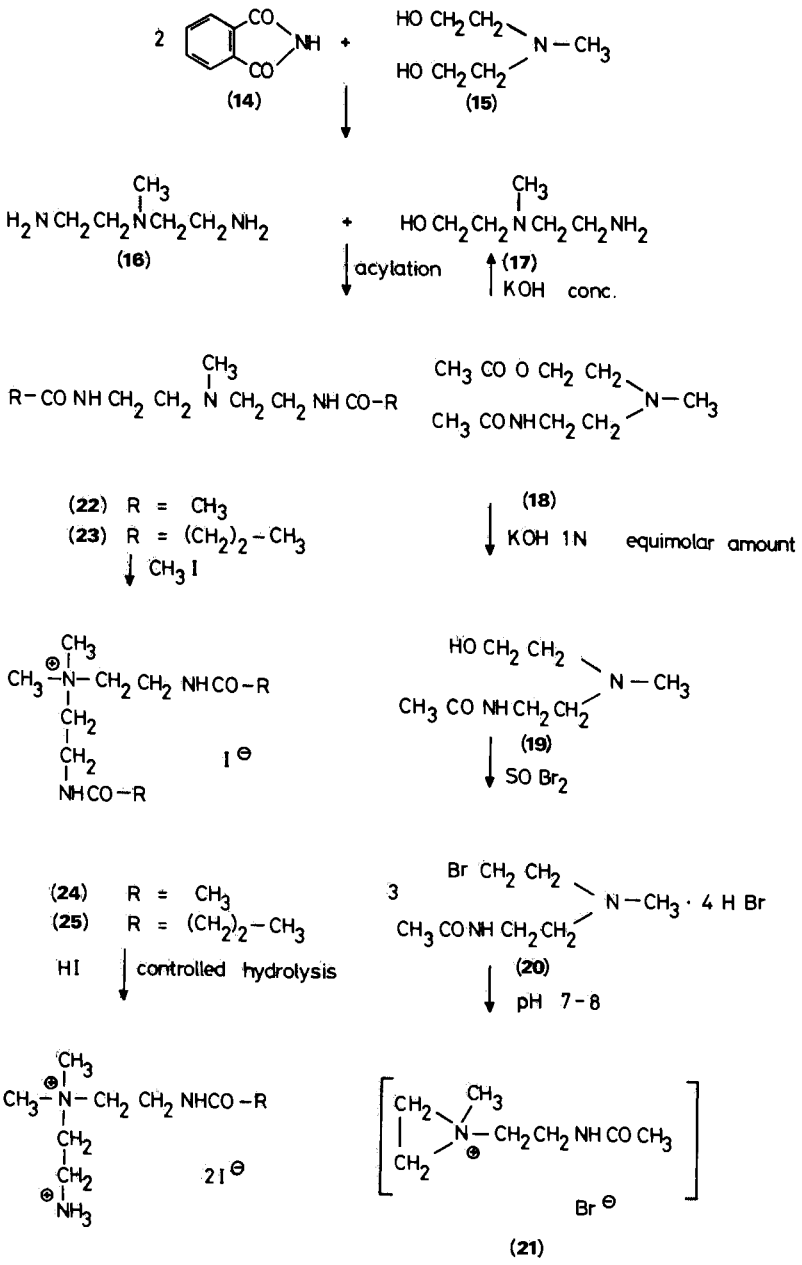
*Scheme 1*



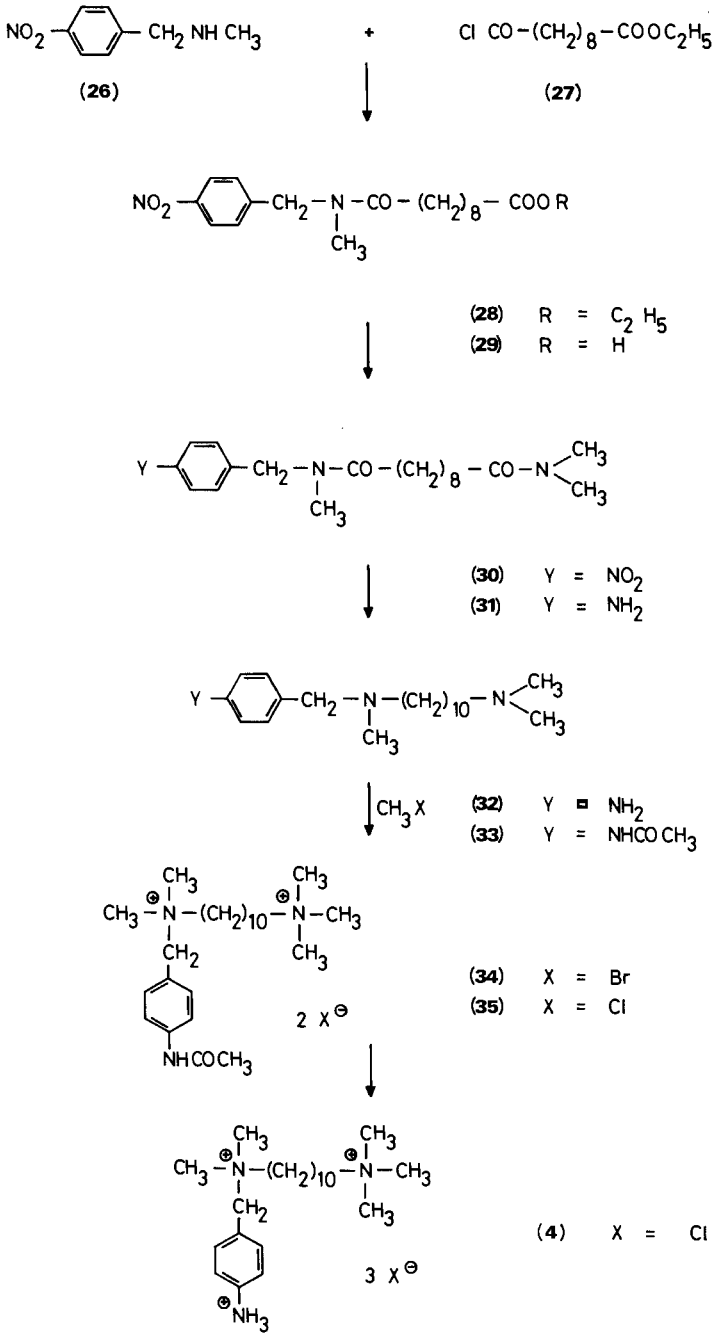
*Scheme 2*



*Scheme 3*


(14)
(15)
(16)
acylation
(17)
KOH conc.
(22) R = CH3
(23) R = (CH2)2–CH3
CH3 I
(18)
KOH 1N equimolar amount
(19)
SO Br2
(24) R = CH3
(25) R = (CH2)2–CH3
HI controlled hydrolysis
(20)
pH 7–8
(21)
(3) R = CH3
(6) R = (CH2)2–CH3

*Scheme 4*


NO2 CH2 NH CH3
(26)
+
Cl CO-(CH2)8-COOC2H5
(27)
NO2 CH2 N CO (CH2)8 COOR
CH3
(28) R = C2H5
(29) R = H
Y CH2 N CO (CH2)8 CO N CH3 CH3
CH3
(30) Y = NO2
(31) Y = NH2
Y CH2 N (CH2)10 N CH3 CH3
CH3
CH3X
(32) Y = NH2
(33) Y = NHCOCH3
CH3 N (CH2)10 N CH3
CH3 CH2 CH3 CH3
NHCOCH3
2 X⊖
(34) X = Br
(35) X = Cl
CH3 N (CH2)10 N CH3
CH3 CH2 CH3 CH3
NH3
3 X⊖
(4) X = Cl

## Experimental Part

*General remarks.* $^1$H-NMR. spectra were recorded on a *Varian* T-60 instrument. Chemical shifts ($\delta$) are given in ppm relative to TMS; abbreviations: *s* singlet, *d* doublet, *t* triplet, *qa* quartet, *m* multiplet, br. broad. IR. spectra were recorded on an *AccuLab* 4 (Beckman) ($\nu_{max}$, in $cm^{-1}$). Abbreviations: *vs* very strong, *s* strong, *m* medium, *w* weak, br. broad, sh. shoulder. Mass spectra were recorded on a *LKB* 2091-051, using a direct inlet system, mass numbers are given in m/z. Melting points (m.p.) were taken on a '*Kofler*-Mikroheiztisch' *(Reichert)* and are not corrected.

N-*(10-Bromodecyl)-phthalimide* (**7**). A mixture of 300 g (1 mol) of 1,10-dibromodecane and 56 g (0.3 mol) of potassium phthalimide was heated at 180° for 10 h. The reaction product was extracted several times with benzene, filtered and after evaporation of the solvent and the excess of dibromodecane under reduced pressure, was destilled i.V. The first fraction contained 25.5 g (30%) of *N*-(10-decenyl)-phthalimide (**8**), b.p. 128°/0.001 Torr, colorless oil,

$C_{18}H_{23}NO_2$ (285.4) Calc. C 75.75 H 8.12 N 4.91% Found C 75.53 H 8.19 N 5.18%

followed by a yellow thick oil, b.p. 210°/0.03 Torr, which became quickly solid. Recrystallization from ethanol afforded 48.3 g (44%) of **7**, m.p. 59.5–60° ([17] 57–59°).

$C_{18}H_{24}BrNO_2$ (366.3) Calc. C 59.02 H 6.60% Found C 59.52 H 6.76%

*Trimethyl(10-phthalimidodecyl)ammonium bromide* (**9**). A solution of 60.9 g (0.166 mol) of **7** in 1 l of benzene in a 3-l round-bottomed flask was cooled to 5°, then 30 ml of dry trimethylamine were added and the flask was closed with a rubber balloon. After one week, the solid was filtered off and recrystallized from dry methanol/ether. Yield 44.7 g (63%), white crystals, m.p. 149–151°.

$C_{21}H_{33}BrN_2O_2$ (425.4) Calc. C 59.29 H 7.82% Found C 59.00 H 7.84%

*(10-Decylamino)trimethylammonium bromide hydrobromide* (**1**). A solution of 43.6 g (0.102 mol) of **9** in 50 ml of HBr 48% were refluxed for 50 h. After cooling, phthalic acid was filtered off, the filtrate evaporated i.V. and the solid residue recrystallized twice from 2-propanol, 23.5 g (61%), m.p. 165–167° ([18] 162–164°, ethanol/methylethylketone/ether).

$C_{13}H_{32}Br_2N_2$ (376.2) Calc. C 41.50 H 8.57 Br 42.48% Found C 41.02 H 8.84 Br 42.69%

N-*(β-Ethoxyethyl)*-N-*(β-aminoethyl)dimethylammonium chloride hydrochloride* (**2**). 32.5 g (0.25 mol) of *N*-(β-dimethylaminoethyl)-acetamide (**10**), 65 g (0.60 mol) of β-chloroethylethylether (**11**) and 100 ml abs. benzene were refluxed for 3 days. After cooling, 100 ml of water were added and the benzene layer rejected. The aqueous solution was extracted with $CHCl_3$ (10×) to eliminate starting material, and then refluxed with 40 ml of conc. HCl for 5 h. The solution was evaporated and dried i.V. until crystallization. After 3 recrystallizations from 2-propanol, 38.4 g (66%) of **2** were collected, white crystals, m.p. 142–145°. – $^1$H-NMR. ($D_2O$): 1.21 (*t*, 3 H); 3,34 (*s*, 6 H); 3.32–4.18 (*m*, 10 H).

$C_8H_{22}Cl_2N_2O$ (233.2) Calc. C 41.2 H 9.51 Cl 30.41 N 12.02%
Found „ 40.4 „ 9.78 „ 29.98 „ 12.47%

N,N-*Bis(β-acetylaminoethyl)methylamine* (**22**) and N-*(β-acetoxyethyl)*-N-*(β-acetaminoethyl)-methylamine* (**18**). To 600 g (4.07 mol) of molten phthalimide (**14**), 238 g (2 mol) of *N*-methyldiethanolamine (**15**) were added dropwise over 4 h, whereby the water distilled. The mixture was then poured into 2.5 l of water and stirred with an excess of HCl at 90° for 50 h. The phthalic acid was filtered off (RT.) and the filtrate evaporated to dryness. The residue was dissolved in enough water and 300 g of NaOH were added cautiously. The oil was extracted with 2 l of 2-propanol. This solution was dried with solid KOH and evaporated i.V. The residue containing mainly **16** and **17** was added dropwise to 550 ml (large excess) of acetic anhydride cooled in ice. The following day, water was added, then solid $K_2CO_3$ until the solution was basic. This solution was extracted with $CHCl_3$ (30×), dried ($CaCl_2$), the solvent evaporated, and the residue was distilled under reduced pressure. The first fraction contained 20.8 g (5%) of **18**, b.p. 106–110°/0.08 Torr, yellow oil[3]). – $^1$H-NMR. ($CCl_4$): 1.87 (*s*, 3 H); 2.00 (*s*, 3 H); 2.27 (*s*, 3 H); 2.58 (*t*, 4 H); 3.01–3.42 (*m*, 4 H); 6.95 (br.*s*, 1 H).

$C_9H_{18}N_2O_3$ (202.25) Calc. C 53.44 H 8.97 N 13.85% Found C 53.28 H 8.87 N 13.89%

[3]) After strong basic hydrolysis of **18** with an excess of KOH, **17** was obtained, b.p. 55°/1 Torr ([19] 103–104°/8 Torr).

The second fraction was **22**, 118.7 g (29%); b.p. 160–180°/0.03 Torr, very thick oil; white needles, m.p. 56–58° (from little ethyl acetate). – $^1$H-NMR. ($CDCl_3$): 1.95 (*s*, 6 H); 2.18 (*s*, 3 H); 2.42 (*t*, 4 H); 3.00–3.45 (*m*, 4 H); 6.95 (br.*s*, 2 H).

$C_9H_{19}N_3O_2$ (201.3) Calc. C 53.70 H 9.52 N 20.88% Found C 53.30 H 9.66 N 21.11%

When **18** was the desired compound, only 2 mol of phthalimide were used; in this case, the yield was 32% for **18** and 12% for **22.**

N,N-*Bis(β-acetylaminoethyl)dimethylammonium iodide* (**24**). A mixture of 5 g (24.8 mmol) of **22**, 20 ml of 2-propanol and 2 ml of methyliodide was kept in the dark for 12 h, then evaporated. After a long time the product crystallized, and recrystallization from abs. ethanol/ethyl acetate gave 7 g (82%) of **24**, long, white needles, m.p. 118–120°. – $^1$H-NMR. ($CD_3OD$): 2.05 (*s*, 6 H); 3.30 (*s*, 6 H); 3.49–3.86 (*m*, 8 H).

$C_{10}H_{22}IN_3O_2$ (343.2) Calc. C 34.99 H 6.46% Found C 35.18 H 6.76%

N-*(β-Acetylaminoethyl)*-N-*(β-aminoethyl)dimethylammonium iodide hydroiodide* (**3**). A mixture of 35.1 g (0.102 mol) of **24**, 220 ml of water and 10.8 ml (0.095 mol) of 57% hydroiodic acid ($d = 1.69$), was refluxed in the dark under $N_2$ for 6 h and then evaporated i.V. The residue was dissolved in a little methanol (90%) and chromatographed over aluminium oxide using a gradient of $CH_2Cl_2$ with increasing percentage (0–40%) of methanol. After evaporation of the fraction with the product, the residue was diluted in 100 ml of water and the pH adjusted to 6.5 with HI (20%), then evaporated, dried at 60°/0.01 Torr and recrystallized from methanol, yield 18.1 g (41%); m.p. 185–187°, small white needles. – $^1$H-NMR. ($CD_3OD$): 2.02 (*s*, 3 H); 3.38 (*s*, 6 H); 3.49–4.02 (*m*, 8 H).

| | | | | | |
|---|---|---|---|---|---|
| $C_8H_{21}I_2N_3O$ | Calc. | C 22.39 | H 4.93 | I 59.15 | N 9.79% |
| (429.1) | Found | „ 22.22 | „ 4.92 | „ 58.91 | „ 9.96% |

N,N-*Bis(β-aminoethyl)methylamine* (**16**). See synthesis of **22**. The dark brown residue of the evaporated 2-propanol solution, containing **16** and **17**, was carefully distilled: the first fraction, 20–60°/8 Torr, a mixture of the aminoalcohol **15**, $H_2O$, **16** and **17**, was rejected. The second fraction, 60–83°/8 Torr, was redistilled and the fraction 69–71°/8 Torr was collected. It contained more than 96% of **16**; yield 89.8 g (38%) ([20] 88–89°/10 Torr; [21], 81–84°/14 Torr).

N,N-*Bis(β-butyrylaminoethyl)methylamine* (**23**). To 250 ml of stirred, ice cold, butyric anhydride, 80 g (0.684 mol) of **16** were slowly added and the mixture left overnight. Butyric acid and the excess of butyric anhydride were eliminated by distillation i.V. (70–80°/8 Torr). The residue, solid at RT., was dissolved in 250 ml of water and treated with an excess of $Na_2CO_3$ until basic (pH ~ 8.5). The solution was extracted with $CHCl_3$ (4 × 300 ml), dried ($K_2CO_3$) and filtered. After evaporation of the solvent, the residue (melted if necessary) was treated with hot diisopropyl ether, allowed to cool at RT., and left in the deep freezer. Filtration afforded 135.7 g (77%) of **23**, m.p. 73–75°, white needles.

$C_{13}H_{27}N_3O_2$ (257.4) Calc. C 60.66 H 10.58 N 16.33% Found C 60.63 H 10.94 N 15.98%

N,N-*Bis(β-butyrylaminoethyl)dimethylammonium iodide* (**25**). From **23**, in the same way as **24**. Yield 76%; m.p. 60–61° (from abs. methanol/ether).

$C_{14}H_{30}IN_3O_2$ (399.3) Calc. C 42.11 H 7.57% Found C 42.38 H 7.79%

N-*(β-Butyrylaminoethyl)*-N-*(β-aminoethyl)dimethylammonium iodide hydroiodide* (**6**). To a solution of 69.4 g (0.174 mol) of **25** in 350 ml of water, 21.5 ml of 57% hydroiodic acid ($d = 1.69$) were added and the mixture was refluxed for 6 h in the dark under $N_2$. The water was evaporated under reduced pressure and butyric acid was eliminated i.V. at 50°/0.02 Torr (6 h). The dark brown, amorphous solid was dissolved in 200 ml of hot abs. methanol and filtered. After 3 days at −18°, the separated solid was filtered off and recrystallized to afford 26.1 g (32%) of **6**; m.p. 197–199°, white-yellow crystals.

| | | | | | |
|---|---|---|---|---|---|
| $C_{10}H_{25}I_2N_3O$ | Calc. | C 26.27 | H 5.51 | I 55.52 | N 9.19% |
| (457.2) | Found | „ 26.44 | „ 5.62 | „ 56.06 | „ 9.05% |

*N-(β-Aminoethyl)-N-(β-butyloxyethyl)dimethylammonium chloride hydrochloride* (**5**). From **10** and **12**, in the same manner as for **2** but, instead of refluxing, the benzene solution was kept closed and in the dark for 18 months[4]). Yield 23.6 g (36%); m.p. 142–144°, white prisms or plates.

| | | | | | |
|---|---|---|---|---|---|
| $C_{10}H_{26}Cl_2N_2O$ | Calc. | C 45.97 | H 10.03 | Cl 27.14 | N 10.72% |
| (261.2) | Found | „ 46.16 | „ 10.14 | „ 27.53 | „ 10.84% |

*N-(β-Acetylaminoethyl)-N-(β-hydroxyethyl)-methylamine* (**19**). A mixture of 20.2 g (0.1 mol) of **18** with 102 ml of 1N KOH was kept at 60° for 1 h, then refluxed for 5 min. The solution was evaporated i.V. and the residue thoroughly extracted with $CH_2Cl_2$. The solvent was evaporated and the residue chromatographed on $Al_2O_3$. Elution with $CHCl_3$/MeOH 10:1 afforded 14.1 g (87%) of **19**, colorless thick oil. For analysis a sample was distilled i.V., b.p. 115–125°/0.01 Torr (Kugelrohr).

$C_7H_{16}N_2O_2$ (160.2) Calc. C 52.47 H 10.07 N 17.49% Found C 52.41 H 10.26 N 17.68%

*N-(β-Acetylaminoethyl)-N-(β-bromoethyl)methylamine* (**20**). To 2 g (12.5 mmol) of **19** in 20 ml of dry $CHCl_3$, 2 ml of freshly distilled $SOBr_2$ [22] were added. A strong exothermic reaction occurred and an oil soon separated. After refluxing 0.5 h, the solution was evaporated i.V. The oily residue was dissolved in 4 ml of abs. methanol and evaporated again, then dried 1 h at 0.01 Torr (RT.). The solid was taken up in 15 ml of abs. methanol, filtered and ethyl acetate was cautiously added until slightly clouded. After 0.5 h, the solution was decanted and a further 100 ml of ethyl acetate added. A second crystallization gave a white substance, which was dried 24 h at 60°/0.01 Torr. The product contained 4 HBr for every 3 amines, and the excess of 1 HBr could not be further eliminated. Yield 1.85 g (45%). It began to melt at 118° under decomposition and ended at 132°.

| | | | | | |
|---|---|---|---|---|---|
| $C_{21}H_{49}Br_7N_6O_3$ | Calc. | C 25.39 | H 4.97 | Br 56.33 | N 8.46% |
| (993.1) | Found | „ 25.14 | „ 5.34 | „ 56.57 | „ 8.71% |

Free HBr: 20.9 mg of substance needed, in diluted $HNO_3$, 0.866 ml (theoretical 0.842) of 0.1N $AgNO_3$.

*Ethyl 9-[N-methyl-N-(p-nitrobenzyl)carbamoyl]nonanoate* (**28**). A solution of 33.4 g (0.201 mol) of *N*-methyl-*p*-nitrobenzylamine (**26**) in 200 ml of dry benzene was added dropwise over 2 h to a stirred solution of 25.8 g (0.104 mol) of ethyl 9-(chloroformyl)nonanoate (**27**) in 500 ml of dry benzene, which was cooled with water. After 2 h of further stirring, 300 ml of water were added, separated and extracted twice with benzene. The residue on evaporation of the benzene was chromatographed in 2 portions on 1 kg of $Al_2O_3$. Three impurities were eluted with diisopropyl ether, and compound **28** with diisopropyl ether/methanol 100:4. From the residue of these eluates, 34.4 g (90%) of **28**, as a straw yellow oil, could be obtained. – $^1$H-NMR. ($CCl_4$): 1.23–1.80 (*m*, 15 H, $CH_2CH_3$ and $CH_2(CH_2)_6CH_2$); 2.21 (*t*, 4 H, $CH_2CO$); 2.93 (*s*, 3 H, $NCH_3$); 4.07 (*qa*, 2 H, $CH_2CH_3$); 4.60 (*s*, 2 H, $NCH_2C_6H_4$); 7.37 (*d*, 2 H, ar.) and 8.17 (*d*, 2 H, ar.). – MS. (70 eV): 378 ($M^+$); 349 ($M^+ - C_2H_5$); 333 ($M^+ - OC_2H_5$); 213 ($C_2H_5OCO(CH_2)_8CO^+$).

$C_{20}H_{30}N_2O_5$ (378.5) Calc. N 7.40% Found N 7.21%

*9-[N-Methyl-N-(p-nitrobenzyl)carbamoyl]nonanoic acid* (**29**). A solution of 23.2 g (61.3 mmol) of **28** in 300 ml of ethanol was refluxed with 65 ml of 1N KOH, then evaporated to dryness i.V. After dissolving in 200 ml of water, the mixture was acidified at 5° with 67.5 ml of 1N HCl, then extracted with $CHCl_3$ (4×). The extract, after drying ($MgSO_4$) and evaporating at 40°, afforded an oil to which were added 200 ml of ether. The solution was left overnight at –18°. Recrystallization gave 16.3 g (76%) of **29**; white crystals, m.p. 77–78°. – MS. (70 eV): 350 ($M^+$).

$C_{18}H_{26}N_2O_5$ (350.4) Calc. N 8.00% p.eq. 350.4 Found N 7.80% p.eq. 348.2 (titr.)

*N,N,N'-Trimethyl-N'-p-nitrobenzyl-1,10-decane diamide* (**30**). To a stirred solution of 50 g (142.7 mmol) of **29** in 200 ml of dry $CHCl_3$ at 40° in a 3-necked 2-l round-bottomed flask, 60 ml of $SOCl_2$ were added over 2 h. The temperature was raised to 60° and the solution stirred for 2 further h. The solvent and the excess of $SOCl_2$ were eliminated under dry conditions and reduced pressure (RT.).

---

[4]) Experiments of accelerating the reaction through heating failed, because it was still necessary 2 weeks of heating and several by-products were obtained.

The residue was dissolved in 1000 ml of dry benzene and carefully treated with an excess of gaseous dimethylamine, the temperature being kept below 40°. After 12 h, 250 ml of water were added and the mixture worked up as usual. The pale, thick oil left from the dried benzene solution, soon crystallized and after recrystallization ($CHCl_3$) at −18°, the crystals were filtered off and washed with ether. The filtrate was evaporated and the residue chromatographed on silica gel (100 mesh, column 5×20 cm) using 800 ml of $CHCl_3$. The eluate left other crystals, which were recrystallized as before. Total yield 50.7 g (94%); white needles, m.p. 75–75.5°. – IR. ($CCl_4$): 2944*m*; 2875*m*; 1662*s*; 1530*s*; 1401*m*; 1348*s*. – MS. (70 eV): 377 ($M^+$); 333 ($M^+ - N(CH_3)_2$); 305 ($M^+ - CO - N(CH_3)_2$); 291 ($M^+ - CH_2 - CON(CH_3)_2$); 212 ($(CH_3)_2NCO(CH_2)_8CO^+$).

$C_{20}H_{31}N_3O_4$(377.5) Calc. C 63.63 H 8.28 N 11.13% Found C 63.88 H 8.26 N 10.97%

N′-p-*Acetylaminobenzyl*-N,N,N′-*trimethyl-1,10-decanediamine* (**33**). a) Reduction of the nitro group of **30**. To a stirred and refluxing suspension of 50 g (132.5 mmol) of **30**, 1 l of ethanol, 40 ml of water and 80 g of iron powder, 40 ml of acetic acid [23] were added over 3 h, then refluxed for another 10 h, then 700 ml of toluene were added and the mixture filtered at RT. over celite under slight vacuum, and the residue washed with toluene. The filtrate was concentrated to 200 ml, 300 ml of water were added followed by solid $K_2CO_3$ until pH 9. The brown solution was extracted with benzene (5×) and worked up to give 43.8 g (95%) of **31**, yellow-brown thick oil. – IR. ($CHCl_3$): 3470*w* br.; 3415*w* br.; 3010*m*; 2965*m*; 2875*m*; 1643*vs*, sh.; 1632*vs*; 1520*m*; 1405*m*.

b) Reduction of the amidegroups of **31**. A solution of 25 g (71.95 mmol) of crude **31** in 250 ml of abs. tetrahydrofuran was slowly added to an ice-cold, stirred suspension of 30 g of $LiAlH_4$ [24] in 750 ml of abs. tetrahydrofuran. The complex was refluxed for 1 h and then 250 ml of water were carefully added dropwise while keeping the temperature below 5°. The precipitate was filtered off and washed thoroughly with ether. The filtrate was concentrated, treated with 200 ml of water added and extracted with ether (3×). The organic layer was dried ($Na_2SO_4$) and evaporated, giving 17.1 g (74%) of **32**, yellow, thick oil, which was not further purified. – IR. ($CHCl_3$): 3470*w*, br.; 3405*w* br.; 2990*s*, sh.; 2940*vs*; 2860*vs*; 2830*s*; 2795*s*; 1625*s*; 1515*s*; 1465*s*; 1460*s* sh.; 1258*m*; 814*m*.

c) Acetylation of **32**. A solution of 17 g (53.2 mmol) of **32** in 200 ml of $CHCl_3$ was treated with 14 ml of acetic anhydride and left for 2 h. Then 400 ml of water and 40 g of $K_2CO_3$ were added and the mixture was stirred overnight. The organic layer was evaporated, and the oily residue chromatographed in 2 portions over 1 kg of $Al_2O_3$. Elution with $CHCl_3$ (TLC.: $CHCl_3/CH_3OH$ 30:1) afforded a faintly yellow oil, which was distilled, b.p. 210–240°/0.04 Torr (Kugelrohr). The product crystallized m.p. 38–40°, yield 14.9 g (77%). – IR. ($CHCl_3$): 3465*w*; 2940*vs*; 2867*vs*; 2838*m*; 2800*s*; 1698*vs*; 1688*s*, sh.; 1617*m*; 1603*m*; 1550*m*; sh.; 1520*vs*; 1470*m*; 1462*m*; 1410*s*; 1371*m*; 1313*s*. – $^1$H-NMR. ($CD_3OD$): 1.17–1.87 (*m*, 16 H, $CH_2(CH_2)_8CH_2$); 2.08 (*s*, 3 H, $CH_3CO$); 2.18 (*s*, 3 H, $CH_2N(CH_3)CH_2$); 2.29 (6 H, *s*, $N(CH_3)_2$); 2.30–2.67 (*m*, 4 H, $NCH_2CH_2$); 3.45 (*s*, 2 H, $CH_2$ ar.); 7.13–7.57 (*m*, 4 H, ar.). – MS: (70 eV): 361 ($M^+$); 346 ($M^+ - CH_3$); 213 ($[(CH_3)_2-N-(CH_2)_{10}-N-CH_3]^+$); 148 ($[CH_3CONH-C_6H_4CH_2]^+$); 58 ($[CH_3CONH]^+$).

$C_{22}H_{39}N_3O$ (361.6) Calc. C 73.07 H 10.87 N 11.62% Found C 73.05 H 10.56 N 10.81%

*(p-Acetylaminobenzyl)dimethyl (10-trimethylammoniodecyl)ammonium dibromide* (**34**). Through a solution of 15 g (41.5 mmol) of **33** in 250 ml of abs. benzene, dry $N_2$ was bubbled for 5 min and then a slow stream of $CH_3Br$ for 3 h. An exothermic reaction took place and a bright yellow oil separated. The reaction flask was kept closed overnight, and next day treated again with $CH_3Br$ for another hour. The following day the solvent was quickly evaporated i.V. The oil was dissolved in warm abs. ehtanol (50°), filtered through wadding under $N_2$ and ethyl acetate was added until an oil separated. The flask was firmly closed to avoid entrance of moisture, and the crystallization could be induced by stirring magnetically, while cooling in ice. After some hours a white compound precipitated, which was recrystallized under the same conditions, yield 17.3 g (76%); m.p. 173–175°. – IR. (KBr): 3660–2850*m*; 3240*w*; 3205*w*; 3175*m*; 3110*m* sh.; 3100*m*; 3030*m*; 3010*m*; 2960*m* sh.; 2945*m* sh.; 2932*s*; 2895*m*; 2890*m*; 2860*m*; 1688*s*; 1611*m* sh.; 1599*m*; 1534*s*; 1528*s*; 1510*m*; 1412*m*; 1374*s*; 1315*s*; 1256*s*; 1180*w*; 1023*w*; 956*w*; 903*w*. – $^1$H-NMR. ($CD_3OD$): 1.13–1.93 (*m*, 16 H, $CH_2(CH_2)_8CH_2$); 2.13 (*s*, 3 H, $CH_3CO$); 3.04 (*s*, 6 H, $(CH_3)_2N^+CH_2$, ar.); 3.17 (*s*, 9 H, $(CH_3)_3N^+$); 3.20–3.58 (*m*, 4 H, $CH_2CH_2N^+$ overlapped with methanol); 4.73 (*s*, 2 H, ar. $CH_2N^+$), 7.40–7.84 (*m*, 4 H, ar.).

| | | | | | |
|---|---|---|---|---|---|
| $C_{24}H_{45}Br_2N_3O$ | Calc. | C 52.27 | H 8.23 | Br 28.98 | N 7.62% |
| (551.4) | Found | „ 52.63 | „ 8.51 | „ 28.54 | „ 7.91% |

Similarly, the compound **35** as chloride was obtained, by treating **33** with $CH_3Cl$ instead of $CH_3Br$. The chloride **35** is a very hygroscopic compound, m.p. 147–151° (from abs. ethanol/benzene).

*(p-Aminobenzyl)dimethyl (10-trimethylammoniodecyl)ammonium dichloride hydrochloride* (**4**). A mixture of 6.12 g (13.23 mmol) of **35** in 60 ml 1N HCl was refluxed for 10 h. After evaporation and drying at 60°/0.01 Torr, the amorphous, yellow residue was purified by dissolving in abs. ethanol and precipitating with abs. toluene (3×), and finally drying i.V. The yellow solid, an extremely hygroscopic foam, began to melt at 160° with sinterization and decomposition, and at 170° was completely liquid. Yield 3.64 g (60%). – IR. (KBr): 3700–2300*m–s*; 3040*s*; 2940*vs*; 2885*vs*; 2590*s*; 1645*m*; 1631*m* sh.; 1622*m*; 1618*m*; 1513*m*; 1491*m*, sh.; 1480*m–s*; 1210*w*; 962*w*; 903*w*. – $^1$H-NMR. ($D_2O$): 1.15–2.10 (*m*, 16 H, $CH_2(CH_2)_8CH_2$); 3.09 (*s*, 6 H, $(CH_3)_2N^+CH_2$); 3.18 (*s*, 9 H, $(CH_3)_3N^+$); 3.22–3.55 (*m*, 4 H, $N^+CH_2CH_2$); 4.53 (*s*, 2 H, $ArCH_2-N^+$); 7.26–7.73 (*m*, 4 H, arom.).

| | | | | | |
|---|---|---|---|---|---|
| $C_{22}H_{44}Cl_3N_3$ | Calc. | C 57.82 | H 9.71 | Cl 23.28 | N 9.19% |
| (457.0) | Found | „ 56.91 | „ 10.24 | „ 22.86 | „ 8.97% |

## REFERENCES


[1] *P. Cuatrecasas & C.B. Anfinsen,* Affinity Chromatography in 'Methods in Enzymology', *22*, 345–378, ed. W.B. Jakoby, Academic Press, N.Y. 1971.

[2] *W.B. Jakoby & M. Wilcheck,* Affinity Techniques in 'Methods in Enzymology', *34*, Academic Press, N.Y., 1974.

[3] *C.R. Lowe & P.D. Dean,* 'Affinity Chromatography', ed. Wiley, N.Y. 1974.

[4] *W.H. Hopff, G. Riggio & P.G. Waser,* Experientia *30*, 695 (1974).

[5] *W.H. Hopff,* Vierteljahresschrift der Naturforschenden Ges. Zürich, *121*, 223 (1976).

[6] *W.H. Hopff, G. Riggio, A. Hofmann & P.G. Waser,* Croatica Chem. Acta *47*, 309 (1975).

[7] *J.D. Berman & M. Young,* Proc. Nat. Acad. Sci. USA *68*, 395 (1971).

[8] *J. Frank,* Diss. Nr.5086 ETH Zürich, 1973.

[9] *R. Schwyzer & J. Frank,* Helv. *55*, 2678 (1972).

[10] *W.H. Hopff, G. Riggio & P.G. Waser,* FEBS Letters *35*, 220 (1973).

[11] *W.H. Hopff, G. Riggio & P.G. Waser* in 'Cholinergic Mechanisms', 293, edited by Waser, Raven Press, N.Y. 1975.

[12] *J. Schmidt & M.A. Raftery,* Biochemistry *12*, 852 (1973).

[13] *G. Riggio, W.H. Hopff & A. Hofmann,* Experientia *35*, 587 (1979).

[14] *P.G. Waser, W.H. Hopff & G. Riggio* in 'Recent Advances in Receptor Chemistry', 97, edited by Gualtieri, Giannella & Melchiorre, Elsevier/North Holland Biochemical Press 1979.

[15] *H. Graf,* Diss. Nr.5968, ETH Zürich, 1977.

[16] *C. Lebbin, A. Hofmann & P.G. Waser,* Naunyn-Schmiedeberg's Arch. Pharmacol. *289*, 237 (1975).

[17] *D.L. Klayman, M.M. Grenan & D.P. Jacobus,* J. medicin. Chemistry *12*, 510 (1969).

[18] *R.B. Barlow, H. Blaschko, Jean M. Himms & U. Trendelenburg,* Br. J. Pharmac. *10*, 116 (1956).

[19] *R.K. Preston, R.M. Peck, E.R. Breuninger, A.J. Miller & H.J. Creech,* J. medicin. Chemistry *7*, 471 (1964).

[20] *T. Tkaczynski, A. Belc, B. Ciszek & T. Kalinska,* Acta Polon. Pharm. *27*, 471 (1964).

[21] *G. Sieber,* J. prakt. Chem. *312*, 507 (1970).

[22] *O.A. Reutov, A.S. Gudkova, I.V. Petrosyan,* Izv. Akad. Nauk. SSSR, Ser. Chim. *1972*, 213., Chem. Abstr. *77*, 4793y (1972).

[23] *H. Berrie, G.T. Newbold & F. Spring,* J. chem. Soc. *1952*, 2042.

[24] *W.G. Brown,* Reduction by Lithium Aluminium Hydrid, Org. Reactions *6*, 469, edited by Wiley, N.Y. 1951.