There is a demand for synthetic peptides for investigations such as antibody binding studies, synthetic vaccines and peptide antigens. Often only small quantities of material are needed, e.g. 5-10 mg, amounts often below the useful capacity of a conventional peptide synthesizer. Where large numbers of peptides are needed, such as studies of the optimization of peptide antigens, the throughput of a peptide synthesizer is too slow to be of value. Techniques exist for the production of numbers of peptides in milligram quantities [1, 2]. These require careful operator handling plus large volumes of solvents and amino acid reagents. These methods were originally described for use with tert-butoxycarbonyl (tBoc) chemistry with its attendant problems of handling corrosive reagents. Other multi-synthesis methods [3] rely on the power of hplc to separate the various peptides from each other. Micro solid-phase synthesis on polythene pins [4, 5] can produce very large numbers of peptides; however the microgram amounts and the fact that the peptide remains on the solid-phase support can be a limiting factor in its usefulness. A manual technique is commercially available from Biotech Instruments, Luton, to make up to 48 peptides simultaneously, each on a scale of approximately 5 mg of each peptide. Although successful and easy to use, the method is labour intensive and could be prone to operator error. This paper describes the full automation of the above technique which can be used to make up to 48 different peptides simultaneously, in quantities of 2-20 mg. Solvent and reagent consumption are low; typically only 10 mg of a protected amino acid is required for each addition plus approximately 10 ml of solvent.

**Synthesis of peptides**
The fluorenylmethoxycarbonyl (FMOC) protection strategy was used throughout, as described by Atherton & Sheppard [6]. The method is applicable to any type of solid-phase support. However polystyrene based resins were used for this study; either 'Wang' resins for peptides with a free carboxyl group or 4-(2',4'-dimethoxyphenyl FMOC aminomethyl) phenoxy resins for peptide amides. Amino acid active esters, O-pentafluorophenyl or 3-hydroxy-4-oxo 3,4-dihydro-1,2,3-benzotriazine, catalysed with hydroxybenzotriazole (HOBt), were used for all the coupling reactions. Typical side chain protection is shown in Table 1.

**Typical cleavage conditions**
93% Trifluoroacetic acid, 3% anisole, 3% phenol, 1% ethane dithiol. The cleavage time was 16 h when Mtr protection was used for Arg.

The peptides were synthesized in a series of wells drilled in a polythene block, an outline of which is shown in Fig. 1. Teflon sinters in the base of each well retained the peptide/resin, while enabling solvents and reagents to be delivered into the wells then aspirated through the resin into the chamber below. The wells were arranged in a 6 x 8 grid, each well corresponding to a single peptide sequence. Before starting synthesis, software running on a personal computer edits the peptides and arranges the sequences in an array corresponding to the grid in the synthesis block. This data is stored on disc for later use by the machines' computer controller.

The peptide resin was added to each hole. To make peptide amides 5 mg of 4-(2',4'-dimethoxyphenyl FMOC aminomethyl) phenoxy resin was placed in each hole. For peptides with free C-terminal acid groups the computer programme generated a printout describing which amino acid/resin was to be placed in each well. Typically resins with a capacity of approximately 0.5 mmol/g were used for most of the work. The quantities of amino acids required for a synthesis were calculated by using a programme within the machine controller.

**Table 1**
The FMOC protection strategy

<table>
<thead>
<tr>
<th>Amino acid residue</th>
<th>Side chain protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp, Glu</td>
<td>Butyl ester</td>
</tr>
<tr>
<td>Thr, Ser, Tyr</td>
<td>Butyl ether</td>
</tr>
<tr>
<td>His, Cys</td>
<td>Trityl</td>
</tr>
<tr>
<td>Lys</td>
<td>tBOC</td>
</tr>
<tr>
<td>Arg</td>
<td>Mtr or Pmc</td>
</tr>
<tr>
<td>Asn, Gln, Pro, Gly, Ala, Val, Met, Ile, Leu, Phe, Trp</td>
<td>No protection</td>
</tr>
</tbody>
</table>

Abbreviations used: tBoc, tert-butoxycarbonyl, HOBt, hydroxybenzotriazole; FMOC, fluorenylmethoxycarbonyl.
When the amino acids were weighed out, the synthesizer was used to deliver the correct volumes of dimethylformamide into each amino acid container. The FMOC amino acid esters and the HOBt solutions were stable for a working day.

### Equipment

A Gilson 220 sample changer controlled by a personal computer, was used to position a four-way manifold over the correct set of wells in the block. The solvents were selected by a series of solenoid valves and delivered into the wells by means of gas pressure. Solvent delivery through the manifold enabled four wells to be filled simultaneously. When the required number of holes had been filled with solvent, the controller activated a vacuum pump to aspirate the reagents through the resin and into the chamber below. The spent solvents were then sucked into a waste bottle. The controller automatically washed the peptide/resins, removing the FMOC groups and washing away excess reagent.

After the resins had been dried, a Gilson 410 syringe pump was used in conjunction with the sample changer to deliver the amino acid solutions to the appropriate wells as specified by the peptide sequences. Aliquots of 200 μl of amino acid and 50 μl of HOBt were dispensed in each case, using a five-fold molar excess of amino acid. The order of addition of the amino acids was in reverse order of their estimated reactivity. For example, Ile and Val were added first, Gly last. Reaction times of 30 min were used for short peptides, extending to 1 h when the peptides were more than ten residues long. The molar excess of amino acid was also increased for the longer peptides. The procedure of washing, deprotection, washing and reaction was performed until all the sequences had been completed. To avoid cross contamination, separate containers of HOBt were used for each amino acid and the needle was washed after each amino acid had been delivered. In addition to the 20 naturally occurring amino acids, two extra positions were available to enable other amino acids to be included.

After all the peptide had been synthesized, all the FMOC groups were removed simultaneously and the resins were then washed and dried. The synthesis block was then removed from the machine. A tray holding 48 x 4 ml glass vials was placed in the vacuum chamber, under the top block. The positions in the tray corresponded to the positions of the wells containing the peptides. The peptides were cleaved off the resins using trifluoroacetic acid together with scavengers. The acid was added in 200 μl aliquots to each well, the solution slowly draining through into the vials underneath. Approximately 2 ml of acid was added to each well over a time period of 1 h. After this time all the peptide had been cleaved off the resin and collected in the vials. If Arg(Mtr) was present in the peptides the reaction time was extended to 16 h. After reaction, the acid was removed by aspirating air through the wells in the upper block. The air flow entered the vials and removed the volatile acid, drying each vial to about 200 μl of solution. When dry, the tray was removed from the chamber and diethyl ether was added to each tube to precipitate...
Sequence-specific antibodies as probes for structural analysis of the A-type \( \gamma \)-aminobutyric acid receptors

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The amino acid, \( \gamma \)-aminobutyric acid (GABA), is the major inhibitory neurotransmitter of the mammalian central nervous system. It mediates its effects by the specific interaction with the GABA receptors of which the A-type, the GABA\(_A\) receptor, is the predominant class. Activation of GABA\(_A\) receptors results within milliseconds in the opening or gating of an integral chloride ion channel resulting, in general, in the hyperpolarization of the recipient neuron. The GABA\(_A\) receptor is an important pharmacological target since benzodiazepines, barbiturates and neurosteroids all allosterically regulate GABAergic neurotransmission by direct interaction with the protein, each at a distinct site.

Molecular cloning of GABA\(_A\) receptor genes has shown that the GABA\(_A\) receptor is a member of the ligand-gated ion channel superfamily [reviewed in 1,2]. To date 16 GABA\(_A\) receptor genes have been identified. These are classified on the basis of their amino acid sequence identity. Thus there exist five subunit types namely \( \alpha \), \( \beta \), \( \gamma \), \( \delta \) and \( \rho \) which have of the order of 35% amino acid sequence identity, whereas isoforms of one subunit class, e.g. \( \alpha_1 \) versus \( \alpha_2 \) subunit share at least 75% amino acid sequence identity. In general the divergent regions between isoforms are found at the extreme N- and C-terminal regions where the latter extends beyond transmembrane spanning region M4 and also in the putative intracellular loop regions (Fig. 1). For some of the subunit types, particularly \( \beta \), divergence in sequence even in these three domains is limited. The length of the peptide chosen is dictated partly by the high cost of peptide synthesis. Thus we have compromised between the minimum number of residues thought to be required for a satisfactory immune response and a longer peptide which has a greater probability of adopting the same conformation as that found within the native polypeptide. Successful results were obtained with peptides of length 11–18 amino acids.

Although the characteristic features of some of the GABA\(_A\) receptor genes have been determined by study of the properties of various cloned receptors [e.g. summarized in 3], the subunit complement and therefore functional properties of any one of the possible hundreds of natural GABA\(_A\) receptors has not yet been determined. To approach this goal, we have been using sequence-directed antibodies to probe the structures of native receptors. In the production of a panel of antibodies specific for each of the GABA\(_A\) receptor subunits there are several considerations to be made as well as criteria to be met to validate antibody selectivity. These are discussed below.

Firstly and most importantly there is the choice of the peptide sequence itself. Since the antibodies are required to recognize each isoform of each subunit class, the amino acid sequence selected must be from a divergent region of the respective polypeptide. As described above, isoforms of one subunit class, e.g. \( \alpha_1 \) versus \( \alpha_2 \) subunit share at least 75% amino acid sequence identity. In general the divergent regions between isoforms are found at the extreme N- and C-terminal regions where the latter extends beyond transmembrane spanning region M4 and also in the putative intracellular loop regions (Fig. 1). For some of the subunit types, particularly \( \beta \), divergence in sequence even in these three domains is limited. The length of the peptide chosen is dictated partly by the high cost of peptide synthesis. Thus we have compromised between the minimum number of residues thought to be required for a satisfactory immune response and a longer peptide which has a greater probability of adopting the same conformation as that found within the native polypeptide. Successful results were obtained with peptides of length 11–18 amino acids.

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Abbreviation used: GABA\(_A\), A-type \( \gamma \)-aminobutyric acid.