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- Ref: 1. Silman & Katchalski, Ann. Rev. Biochem. 35:873 (1966)
 - 2. Cuatrecasas & Anfinsen, Ann. Rev. Biochem. 40:259 (1971).
 - 3. Affinity Chromatography, Methods in Enzymology 34: Jakoby and Wilchek, Eds., Acad. Press, New York, (1975).

SIGMA already offers the following:

READY TO USE AFFINITY MEDIA

A 7013 Adenosine 5'-Diphosphate-Agarose A 7138 Adenosine 5'-Monophosphoric Acid-Agarose*

A 6888 Adenosine 5'-Triphosphate-Agarose*

C 7511 Concanavalin A-Agarose* C 4762 Cysteamine-Agarose

C 5634 L-Cysteine-CM Cellulose

C 5012 Cytidine 5'-Diphosphate-Agarose* C 5137 Cytidine 5'-Monophosphoric Acid-Agarose*

C 4887 Cytidine 5'-Triphosphate-Agarose*

G 5007 Guanosine 5'-Diphosphate-Agarose* G 5132 Guanosine 5'-Monophosphoric Acid-

Agarose*

G 5257 Guanosine 5'-Triphosphate-Agarose* N 6130 Nicotinamide Adenine Dinucleotide-

Agarose*

N 6255 Nicotinamide Adenine Dinucleotide Phosphate-Agarose*

U 9751 Uridine 5'-Diphosphate-Agarose* U 9876 Uridine 5'-Monophosphoric Acid-

Agarose* U 9626 Uridine 5'-Triphosphate-Agarose*

AMP-4B Sepharose 4B Coupled with 5'-AMP Sepharose 4B Coupled with Concanavalin A

PolyU4B Sepharose 4B Coupled with Polyuridylic Acid

*Attached to Agarose by way of Ribose Hydroxyls.

READY-TO-USE WATER-INSOLUBLE MATRICES

B 3130 **Bromoacetyl Cellulose**

C 7760 Carboxymethyl Cellulose Hydrazide

C 7510 **Cellulose Carbonate** P 8885 Polyacrylamide Hydrazide CNBr-4B Sepharose 4B, CNBr-Activated BIFUNCTIONAL SPACER ARMS

A 0638 Adipic Acid Dihydrazide A 2504 Amino-N-Caproic Acid

P 7630 Putrescine (Diaminobutane) H 7377 N-Hydroxysuccinimide

S 7626 Succinic Anhydride

WATER INSOLUBLE MATRICES

WITH SPACER ARMS CONTAINING TERMINAL FUNCTIONAL GROUPS

A 6763 €-Amino-N-Caproic Acid-Agarose

D 8260 Diaminohexane-Agarose

CH-4B Sepharose 4B Coupled with 6-Aminohexanoic Acid

Sepharose 4B Coupled with AH-4B

1, 6-Diaminohexane ACH-4B Sepharose 4B, Activated CH-4B ATH-4B Sepharose 4B, Activated Thiol

LIGANDS SPECIALLY PREPARED TO CONTAIN SPACER ARMS

A 8888 p-Aminobenzyl-1-Thio-2-Acetamido-2-Deoxy-β-D-Glucopyranoside

A 8138 8-(6-Aminohexyl)-Amino Adenosine 3':5'-Cyclic Monophosphoric Acid

A 9387 8-(6-Aminohexyl)-Amino Adenosine 5'-Monophosphoric Acid

A 9638 p-Aminophenyl a-L-Fucoside

REAGENTS

<u>E 7750</u> 1-Ethyl-3(3-Dimethylaminopropyl)-Carbodiimide

G 6257 Glutaraldehyde

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Peptide Reagents

It is in the Aldrich tradition to offer a wide variety of intermediates and reagents to the synthetic chemist. For peptide synthesis, we offer one or two reagents for each peptide coupling method described in the literature. Since each method has its advantages and disadvantages, a variety of reagents is necessary.

We offer isobutyl and ethyl chloroformates for the classic "mixed anhydride" method.2 Generally, isobutyl chloroformate is preferred for the synthesis of peptides of moderate to high molecular weights, and ethyl chloroformate for the synthesis of dipeptides.3 The carbodiimide method2 is another versatile and convenient method. The very popular DCC (N, N'-dicyclohexylcarbodiimide) is a highly reactive coupling agent and can be used in solid-phase peptide synthesis (SPPS). The related reagents, 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-p-toluenesulfonate (morpho-CDI) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, are water-soluble diimides which permit simplified purification of the peptide products because the corresponding ureas are water-soluble. Racemization in the DCC method can be minimized or completely suppressed by using an additive, such as N-hydroxysuccinimide, N-hydroxyphthalimide, N-hydroxypiperidine or 1-hydroxybenzotriazole. The last-named compound is exceptionally good at suppressing racemization, prohibiting N-acylurea formation and improving yields of high-purity peptides.4 These additives can, of course, be used in SPPS.

The use of DCC-pentachlorophenol (DCC-PCP) and DCC-pentafluorophenol (DCC-PFP) complexes in the preparation of acylpeptide-PCP and -PFP active esters.5 as well as other uses of DCC, has been described.

Another versatile reagent, EEDO (N-ethoxycarbonyl-2ethoxy-1,2-dihydroquinoline), has many more advantages: it gives volatile and easily-removable by-products, there is practically no racemization, and it is useful with O-unprotected hydroxy amino acids. EEDQ and its more reactive analog, IIDQ (2-isobutoxy-1-isobutoxycarbonyl-1,2-dihydroquinoline),7 another Aldrich first, can also be used for SPPS. Woodward's Reagents K and L offer similar advantages: they afford water-soluble by-products, yields are high and they are useful with O-unprotected hydroxy amino acids.

An oxidation-reduction condensation method⁸ employs Aldrithiol-2 (2,2'-dipyridyl disulfide) and triphenylphosphine as coupling reagents. This system can be used in a variety of solvents at a wide range of temperature, affords high yields with little racemization,8 and can be used in SPPS.9

Other reagents include trimethylacetyl chloride, 10 triphenylphosphine with CCl₄ or CBr₄, 11 triphenyl phosphite and imidazole.¹² 1.1'-carbonyldiimidazole.² α.α-dichloromethyl methyl ether, chloroacetonitrile, *1 p-nitrophenyl tri-fluoroacetate, *13 2-hydroxypyridine*14 and 2-mercaptopyridine.*15

Listed below are six of the many peptide reagents we offer. Please send for a free list and/or consult our current Catalog-Handbook for packaging and pricing information on the reagents and blocked amino acids.

References:

*Active ester reagents

- 1) For a summary of some reviews, symposia and books on this subject see J.M. Stewart, Ann. Rep. Med. Chem., 7, 293 (1972). See also: Y.S. Klausner and M. Bodansky, Synthesis, 453 (1972); Y.S. Klausner and M. Bodansky, ibid., 549 (1974); M. Bodansky and Y.S. Klausner, Chem. Polypeptides, 21 (1973).
- 2) Review: N.F. Albertson, Org. React., 12, 157 (1962).
- 3) T. Wieland and H. Bernhard, Ann. Chem., 572, 190 (1951).
- 4) W. König and R. Geiger, Chem. Ber., 103, 788 (1970).
- 5) a) J. Kovacs, L. Kisfaludy, and M.Q. Ceprini, J. Amer. Chem. Soc., 89, 183 (1967).
 - b) J. Kovacs, L. Kisfaludy, M.Q. Ceprini, and R.H. Johnson, Tetrahedron, 25, 2555 (1969).
- 6) F. Kurzer and K. Douraghi-Zadeh, Chem. Rev., 67, 107 (1967).
- 7) Y. Kiso and H. Yajima, Chem. Commun., 942 (1972).
- 8) T. Mukaiyama, R. Matsueda, and M. Suzuki, Tetrahedron Lett., 1901 (1970).
- T. Mukaiyama, R. Matsueda, and H. Marayama, Bull. Chem. Soc. Japan, 43, 1271 (1973).
- 10) L. Fieser and M. Fieser, "Reagents for Organic Synthesis," Vol. 1, John Wiley and Sons, Inc., New York, N.Y., 1967, p. 1229.
- 11) S. Yamada and Y. Takeuchi, Tetrahedron Lett., 3595 (1971).
- 12) Y.V. Mitin and O.V. Glinskaya, ibid., 5267 (1969).
- 13) S. Sakakibara and N. Inukai, Bull. Chem. Soc. Japan, 37, 1231
- 14) A.S. Dutta and J.S. Morley, J. Chem. Soc. C, 2896 (1971).
- 15) K. Lloyd and G.T. Young, ibid., 2890 (1971).
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