Lesson 9 Part 1

## Síntesis de oligonucleótidos

**THE BASIC TOOLS OF GENE EXPLORATION**

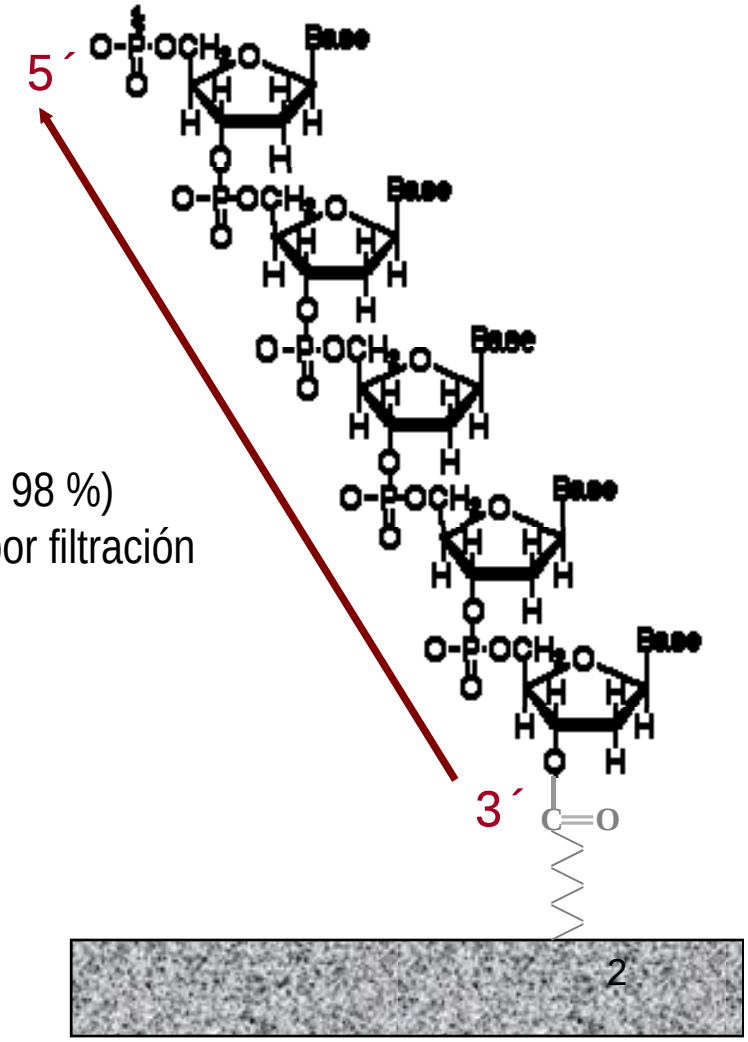
**DNA Probes and Genes Can Be Synthesized by Automated Solid-Phase Methods**

Hebras de DNA se pueden sintetizar por adición secuencial de monómero activados a una cadena en crecimiento unida a un soporte insoluble

La SÍNTESIS se realiza en FASE SÓLIDA

SOPORTES: Partículas de poliestireno
vidrio derivatizado con funciones –COOH

VENTAJAS: reacción cuantitativa (rendimiento de 95 al 98 %)
usa exceso de reactivos que se eliminan por filtración
es fácilmente automatizable
menos riesgo de contaminación

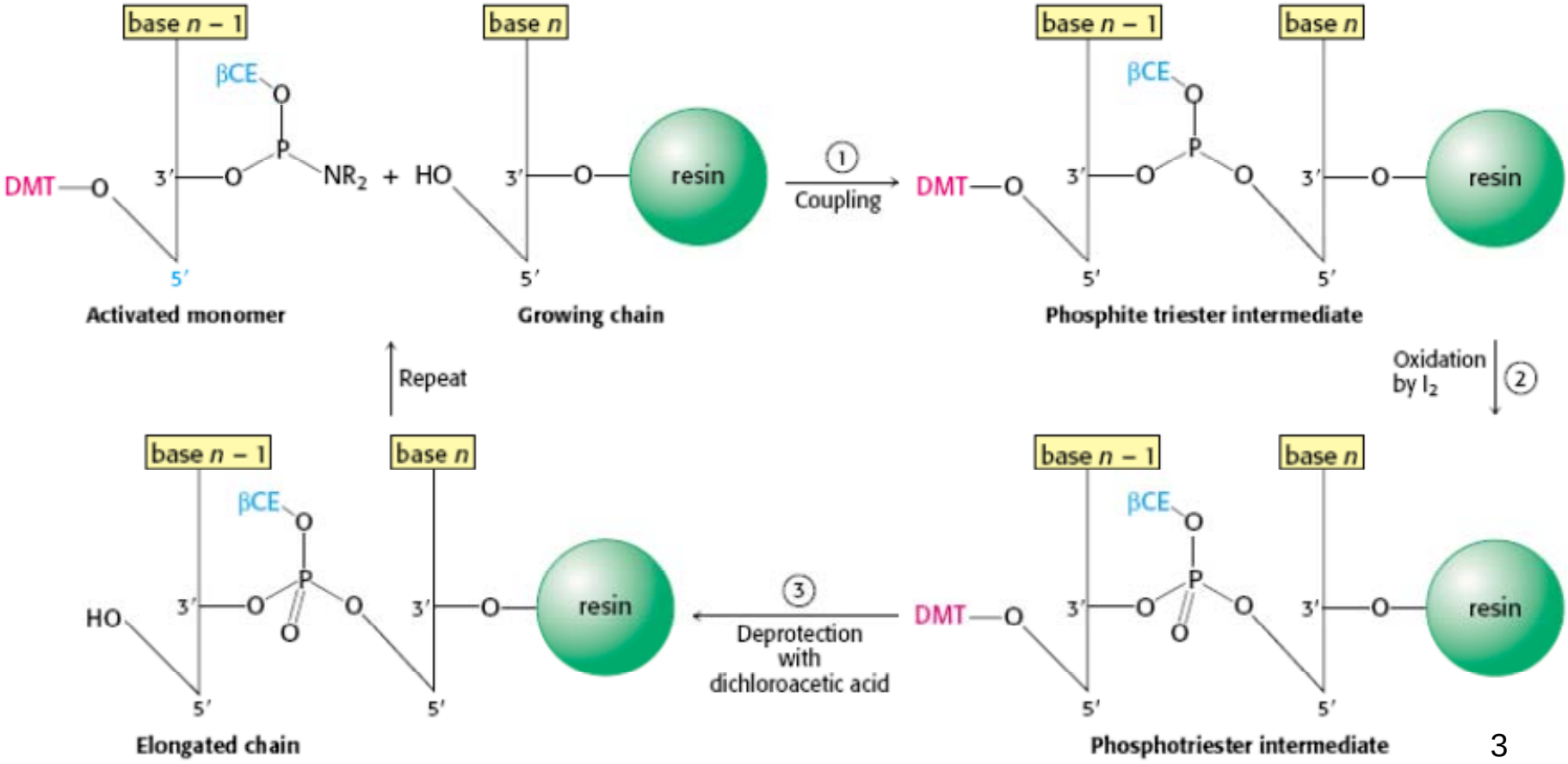



Sintetizador automático de DNA
ESCALA: umol-nmol de producto
oligonucleótidos de 100 ( y hasta 200) mer
Existen muchos sistemas comerciales

# THE BASIC TOOLS OF GENE EXPLORATION

## DNA Probes and Genes Can Be Synthesized by Automated Solid-Phase Methods

**Solid-phase synthesis of a DNA chain by the phosphite triester method.** The activated monomer added to the growing chain is a deoxyribonucleoside 3′-phosphoramidite containing a DMT protecting group on its 5′ oxygen atom, a β-cyanoethyl (βCE) protecting group on its 3′ phosphoryl oxygen, and a protecting group on the base.



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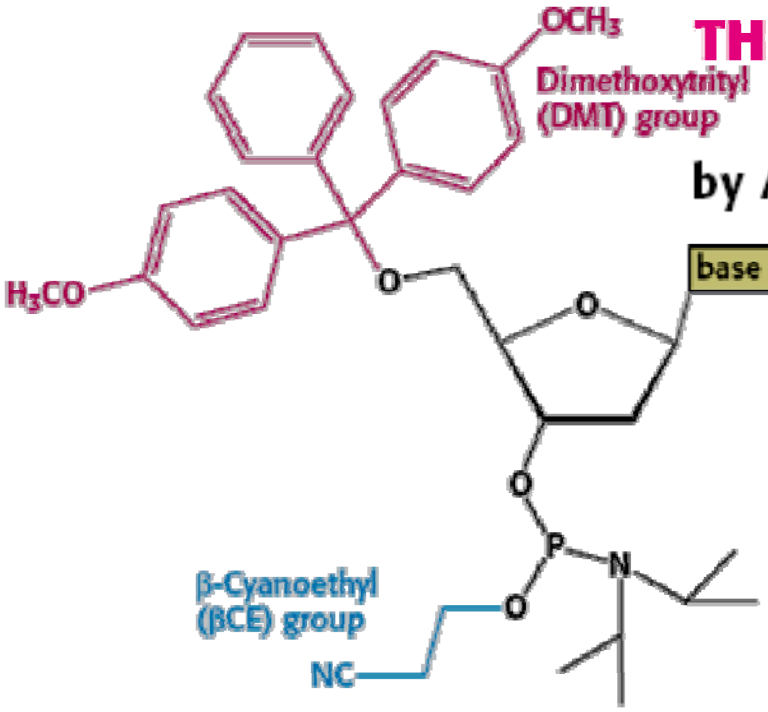
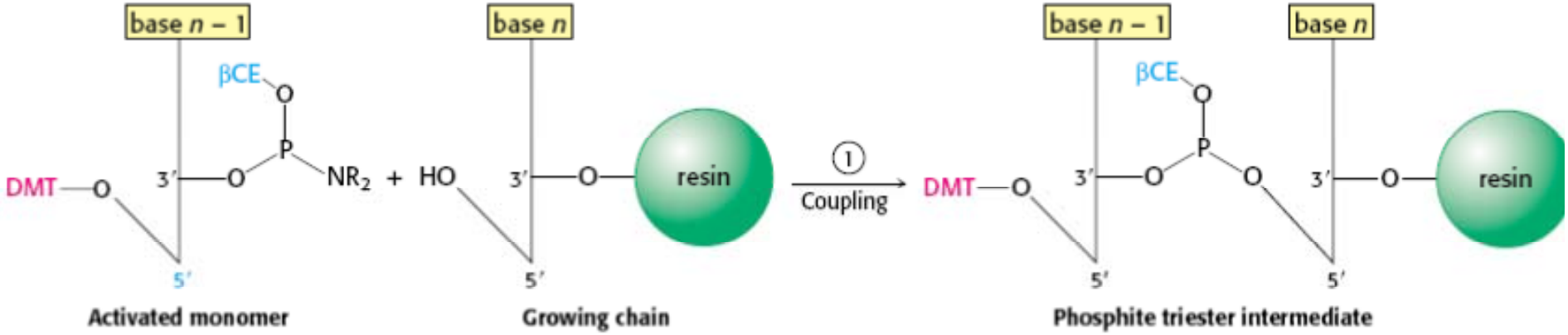
## DNA Probes and Genes Can Be Synthesized by Automated Solid-Phase Methods



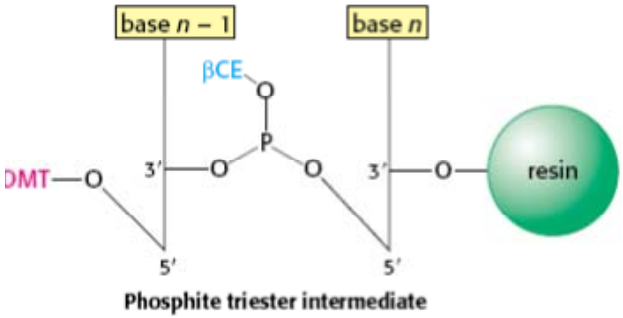

DNA strands, like polypeptides (Section 4.4), can be synthesized by the sequential addition of activated monomers to a growing chain that is linked to an insoluble support. The activated monomers are protonated *deoxyribonucleoside 3′-phosphoramidites*. In step 1, the 3′ phosphorus atom of this incoming unit becomes joined to the 5′ oxygen atom of the growing chain to form a *phosphite triester* (Figure 6.7). The 5′-OH group of the activated monomer is unreactive because it is blocked by a dimethoxytrityl (DMT) protecting group, and the 3′-phosphoryl group is rendered unreactive by attachment of the β-cyanoethyl (βCE) group. Likewise, amino groups on the purine and pyrimidine bases are blocked.

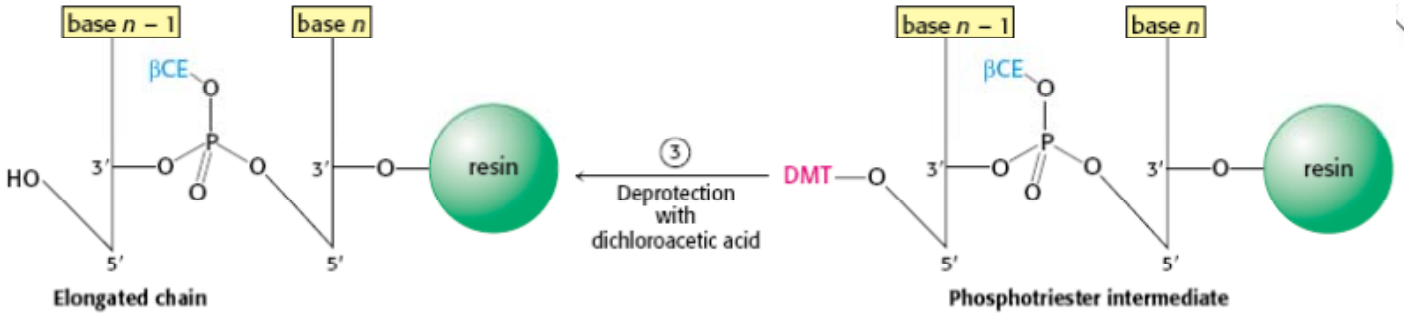


# THE BASIC TOOLS OF GENE EXPLORATION

## DNA Probes and Genes Can Be Synthesized by Automated Solid-Phase Methods

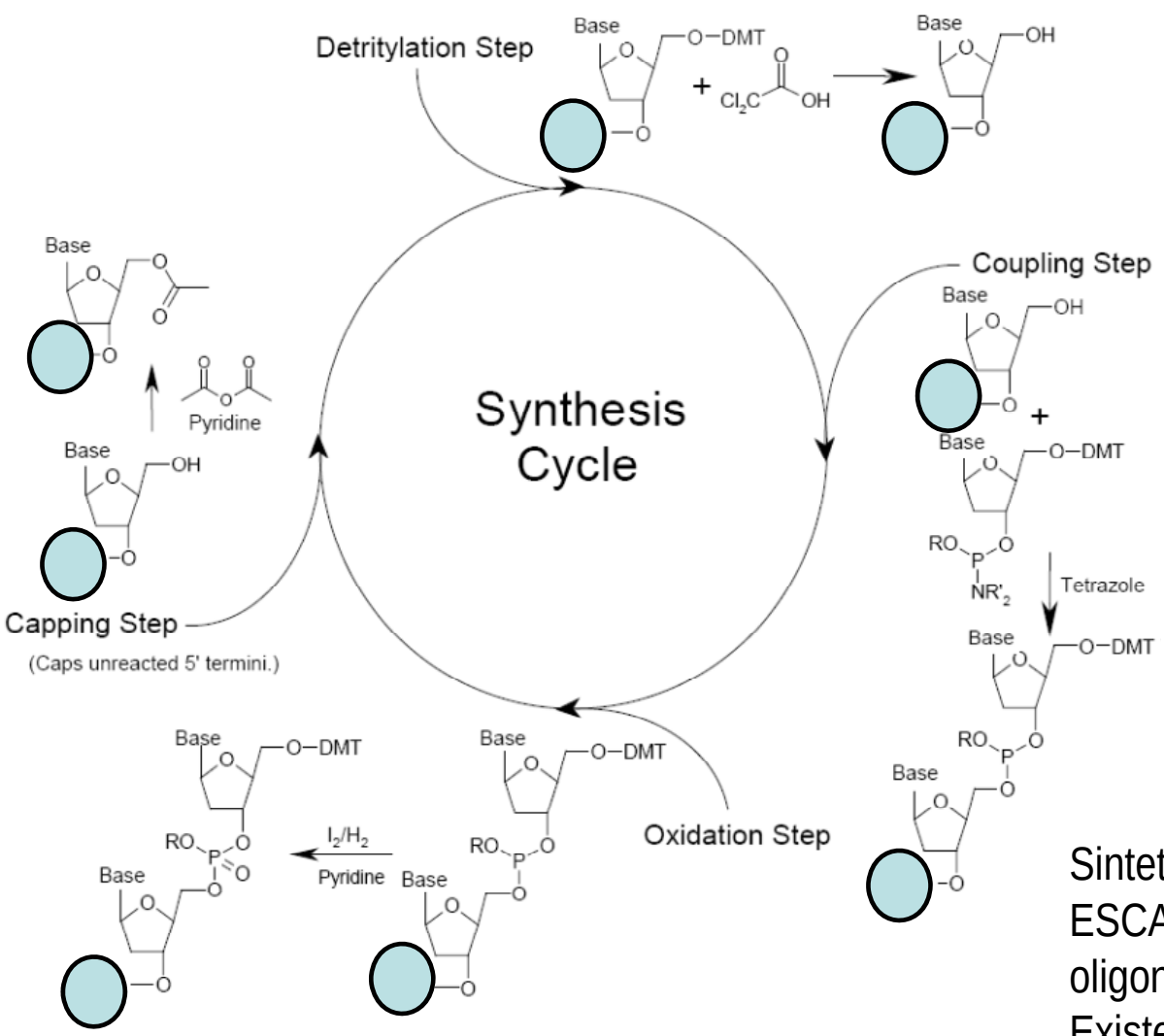
Coupling is carried out under anhydrous conditions because water reacts with phosphoramidites. In step 2, the phosphite triester (in which P is trivalent) is oxidized by iodine to form a *phosphotriester* (in which P is pentavalent). In step 3, the DMT protecting group on the 5′-OH of the growing chain is removed by the addition of dichloroacetic acid, which leaves other protecting groups intact. The DNA chain is now elongated by one unit and ready for another cycle of addition. Each cycle takes only about 10 minutes and elongates more than 98% of the chains.

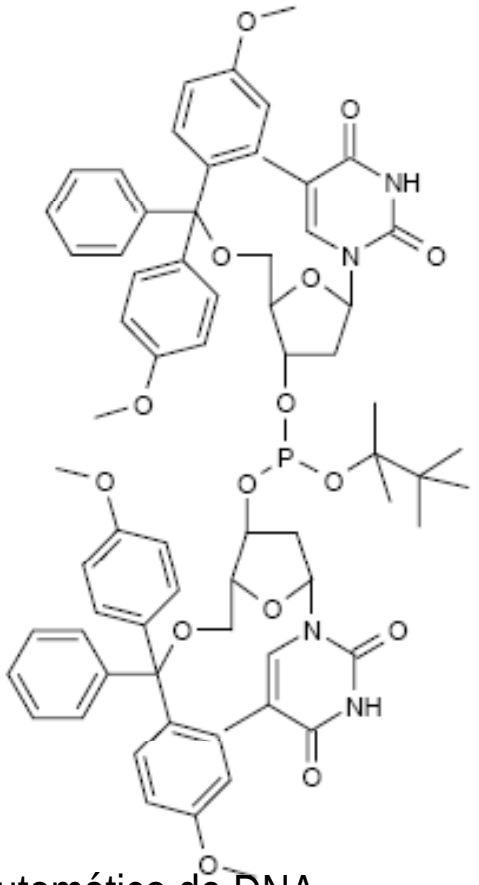


# Síntesis de oligonucleótidos. CE fosforamiditas

El monómero activado es desoxirribonucleósido 3' fosforamiditas protonadas. Se usa DMT (dimetoxitritilo) para proteger el -OH en 5´ (estable en medio básico pero lábil en medio ácido) y el grupo β-cianoetil para bloquear el grupo fosforilo en 3´. Las aminas de las bases se protegen con: benzoil, isobutiril, dimetilformamidina Finalmente, el producto se separa del soporte y se purifica: HPLC



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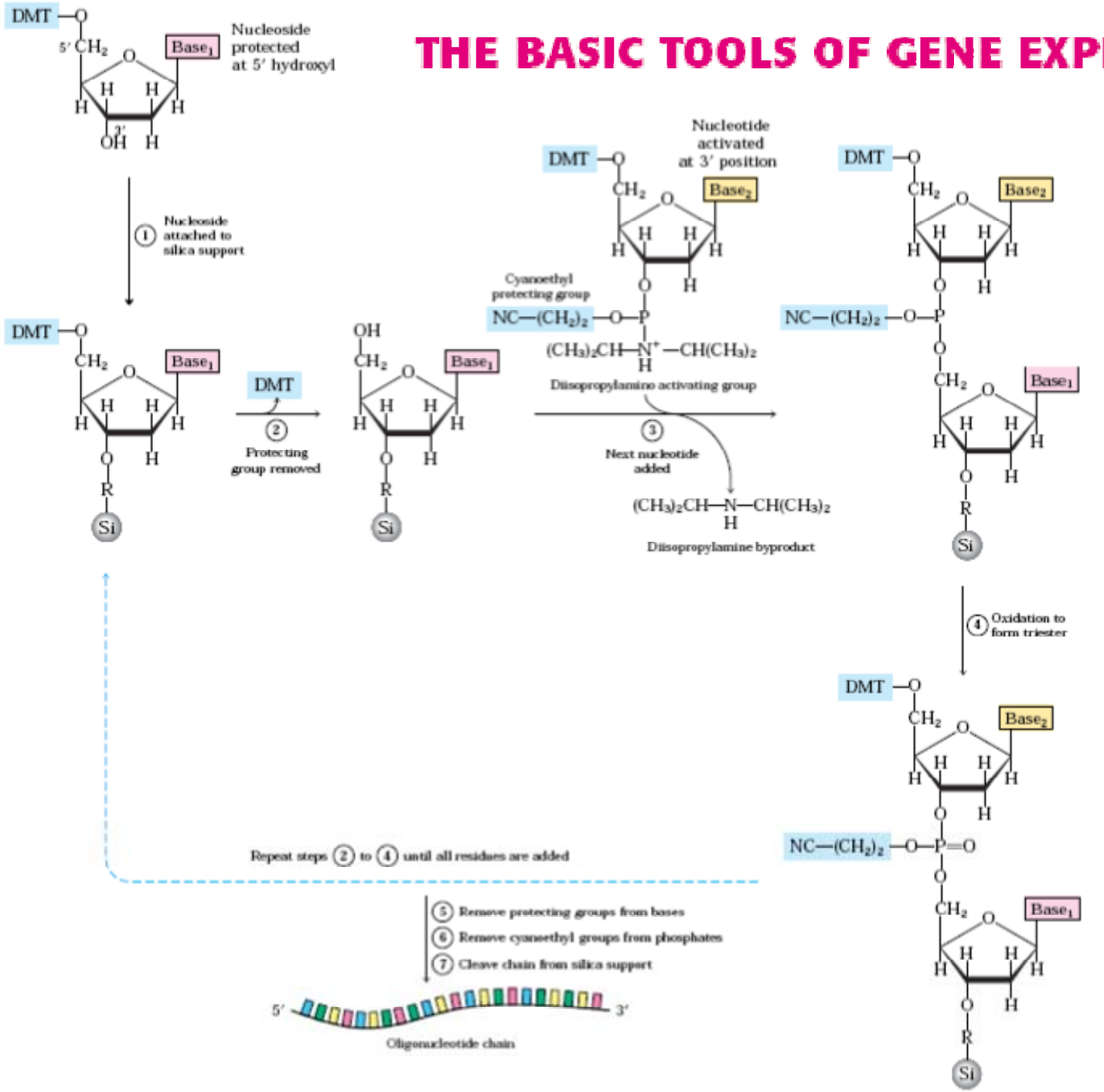
# THE BASIC TOOLS OF GENE EXPLORATION



# THE BASIC TOOLS OF GENE EXPLORATION

## DNA Probes and Genes Can Be Synthesized by Automated Solid-Phase Methods

This solid-phase approach is ideal for the synthesis of DNA, as it is for polypeptides, because the desired product stays on the insoluble support until the final release step. All the reactions take place in a single vessel, and excess soluble reagents can be added to drive reactions to completion. At the end of each step, soluble reagents and by-products are washed away from the glass beads that bear the growing chains. At the end of the synthesis, $NH_3$ is added to remove all protecting groups and release the oligonucleotide from the solid support. Because elongation is never 100% complete, the new DNA chains are of diverse lengths—the desired chain is the longest one. The sample can be purified by high-pressure liquid chromatography or by electrophoresis on polyacrylamide gels. DNA chains of as many as 100 nucleotides can be readily synthesized by this automated method.

# THE BASIC TOOLS OF GENE EXPLORATION

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The ability to rapidly synthesize DNA chains of any selected sequence opens many experimental avenues. For example, synthesized oligonucleotide labeled at one end with $^{32}P$ or a fluorescent tag can be used to search for a complementary sequence in a very long DNA molecule or even in a genome consisting of many chromosomes. The use of labeled oligonucleotides as DNA probes is powerful and general. For example, a DNA probe that can base-pair to a known complementary sequence in a chromosome can serve as the starting point of an exploration of adjacent uncharted DNA. Such a probe can be used as a *primer* to initiate the replication of neighboring DNA by DNA polymerase. One of the most exciting applications of the solid-phase approach is the *synthesis of new tailor-made genes*. New proteins with novel properties can now be produced in abundance by expressing synthetic genes. *Protein engineering* has become a reality.

## PEPTIDES CAN BE SYNTHESIZED BY AUTOMATED SOLID-PHASE METHODS

3. *Synthetic peptides can serve as drugs.* Vasopressin is a peptide hormone that stimulates the reabsorption of water in the distal tubules of the kidney, leading to the formation of more concentrated urine. Patients with diabetes insipidus are deficient in *vasopressin* (also called *antidiuretic hormone*), and so they excrete large volumes of urine (more than 5 liters per day) and are continually thirsty. This defect can be treated by administering 1-desamino-8-D-arginine vasopressin, a synthetic analog of the missing hormone (Figure 4.40). This synthetic peptide is degraded in vivo much more slowly than vasopressin and, additionally, does not increase the blood pressure.

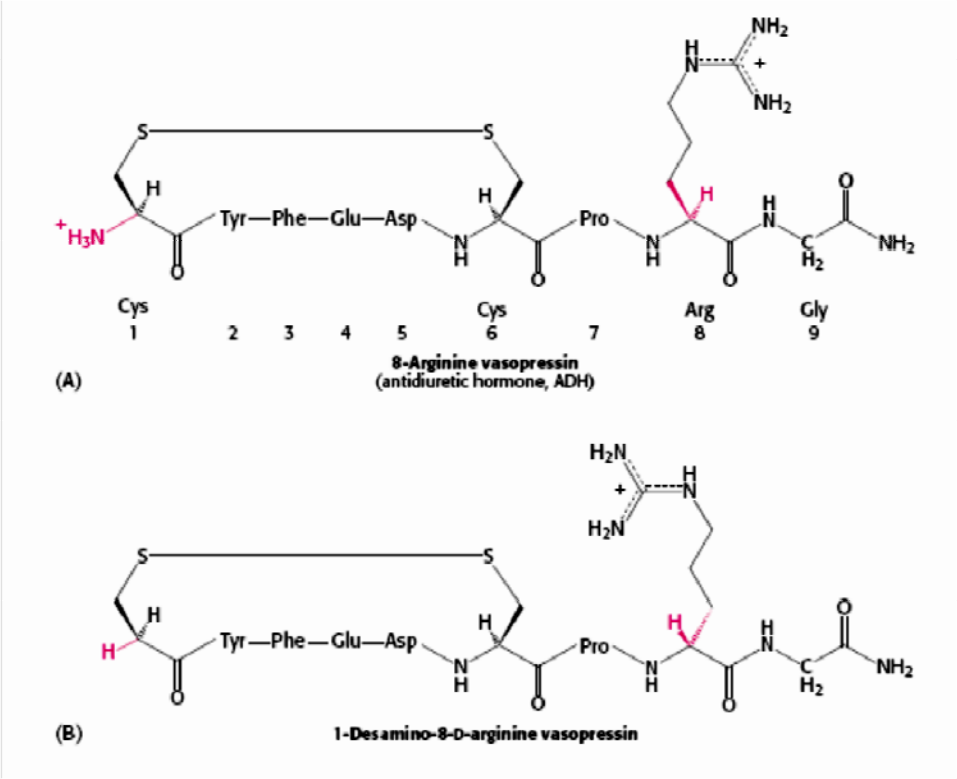


**FIGURE 4.40 Vasopressin and synthetic vasopressin.** Structural formulas of (A) vasopressin, a peptide hormone that stimulates water resorption, and (B) 1-desamino-8-D-arginine vasopressin, a more stable synthetic analog of this antidiuretic hormone.

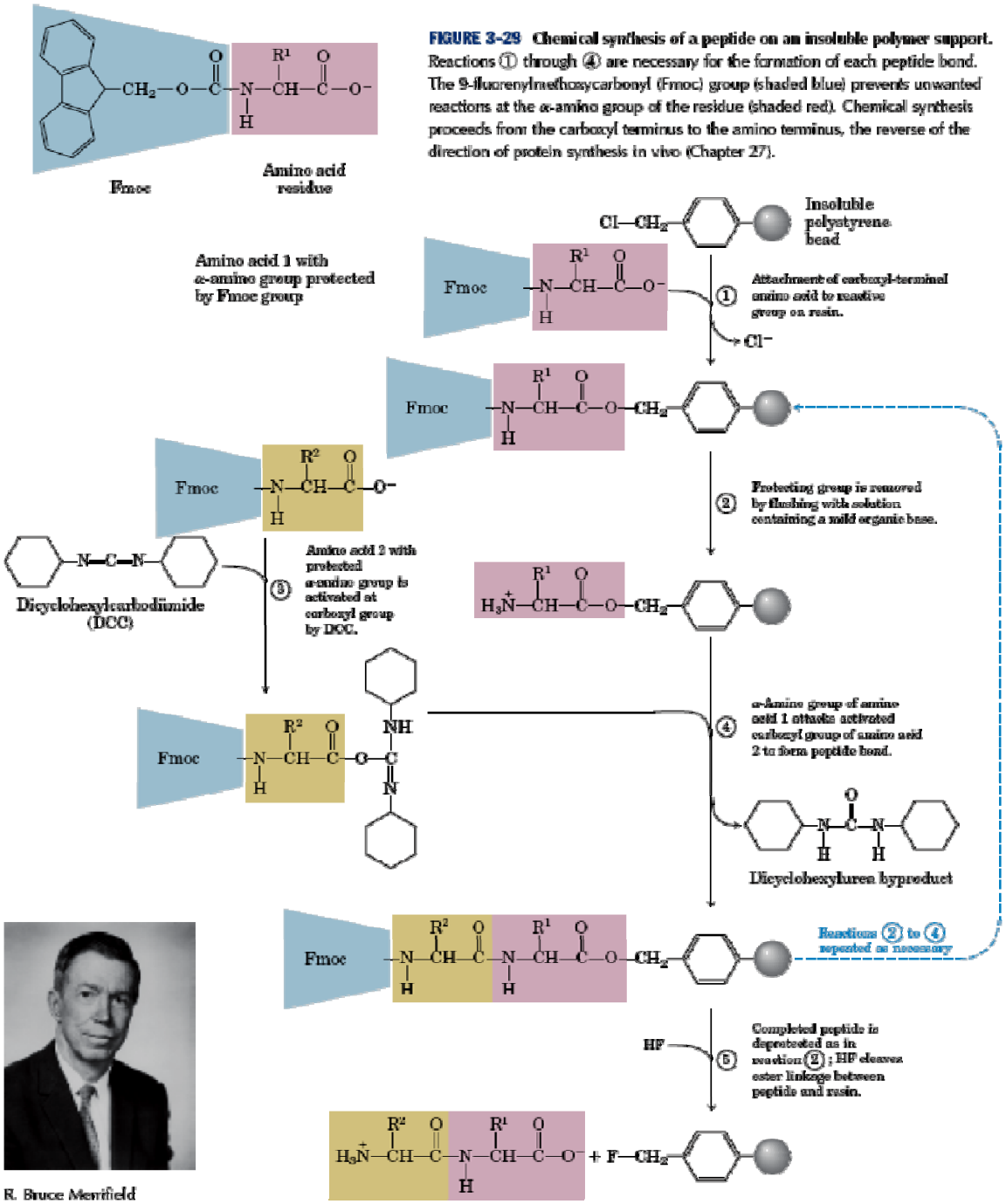


**FIGURE 3-29** Chemical synthesis of a peptide on an insoluble polymer support. Reactions ① through ④ are necessary for the formation of each peptide bond. The 9-fluorenylmethoxycarbonyl (Fmoc) group (shaded blue) prevents unwanted reactions at the α-amino group of the residue (shaded red). Chemical synthesis proceeds from the carboxyl terminus to the amino terminus, the reverse of the direction of protein synthesis in vivo (Chapter 27).



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Peptides containing more than 100 amino acids can be synthesized by sequential repetition of the preceding reactions. Linking the growing peptide chain to an insoluble matrix, such as polystyrene beads, further enhances efficiency. A major advantage of this *solid-phase method* is that the desired product at each stage is bound to beads that can be rapidly filtered and washed, and so there is no need to purify intermediates. All reactions are carried out in a single vessel, eliminating losses caused by repeated transfers of products. The carboxyl-terminal amino acid of the desired peptide sequence is first anchored to the polystyrene beads (Figure 4.42). The *t*-Boc protecting group of this amino acid is then removed. The next amino acid (in the protected *t*-Boc form) and dicyclohexylcarbodiimide, the coupling agent, are added together. After the peptide bond forms, excess reagents and dicyclohexylurea are washed away, leaving the desired dipeptide product attached to the beads. Additional amino acids are linked by the same sequence of reactions. At the end of the synthesis, the peptide is released from the beads by adding hydrofluoric acid (HF), which cleaves the carboxyl ester anchor without disrupting peptide bonds. Protecting groups on potentially reactive side chains, such as that of lysine, also are removed at this time. This cycle of reactions can be readily automated, which makes it feasible to routinely synthesize peptides containing about 50 residues in good yield and purity. In fact, the solid-phase method has been used to synthesize interferons (155 residues) that have antiviral activity and ribonuclease (124 residues) that is catalytically active.

## PEPTIDES CAN BE SYNTHESIZED BY AUTOMATED SOLID-PHASE METHODS

**TABLE 3–8** Effect of Stepwise Yield on Overall Yield in Peptide Synthesis

| *Number of residues in the final polypeptide* | *Overall yield of final peptide (%) when the yield of each step is:* | |
|---|---|---|
| | *96.0%* | *99.8%* |
| 11 | 66 | 98 |
| 21 | 44 | 96 |
| 31 | 29 | 94 |
| 51 | 13 | 90 |
| 100 | 1.7 | 82 |