



FIGURE 6.7 Biophysical probes can be used to map ligand-binding sites and control function of membrane proteins after incorporation via unnatural mutagenesis. (a) Light is used to cross-link *p*-benzoylphenylalanine (Pba) in G protein-coupled receptors upon binding of the ligand (gray sphere) which result in a conformational change into the ligand-bound state. (b) When the photo-caged amino acid, 4,5-dimethoxy-2-nitrobenzyl-cysteine (Cmn), is incorporated into the pore of an ion channel, it blocks the channel. Exposure to UV light results in irreversible removal of the dimethoxynitrobenzyl group from cysteine, rendering the channel conductive.

by Bruce Merrifield in 1966 and since then a few other proteins have been prepared by this approach; most notably, HIV protease (99-residues) which enabled structural characterization of the protein.

However, SPPS is generally limited to the preparation of up to 40–60 amino acid peptides, whereas most proteins are considerably larger. Therefore, there has been a considerable interest in developing methods that are not confined to these restrictions and in 1994, a strategy for preparation of proteins from peptide fragments was introduced, called native chemical ligation (NCL, Figure 6.8). In NCL, two or more unprotected peptide fragments can be ligated together, generating a (native) cysteine residue in the ligation site. The ligation requires a peptide with a C-terminal thioester and a peptide with an N-terminal cysteine residue: the thiolate of the N-terminal cysteine attacks the C-terminal thioester to affect transthioesterification, followed by formation of an amide bond after *S*→*N* acyl transfer (Figure 6.8). The reaction takes place in aqueous buffer and generally proceeds in good to excellent yield.

Thus, NCL is a very useful approach for the total chemical synthesis of proteins and has been used for the preparation of numerous proteins, including glycoproteins and proteins with fluorescent labels. An example is the synthesis of an analog of erythropoietin (EPO) which was derivatized