example, more than four trillion decapeptide sequences were screened against a monoclonal antibody to identify candidates with increased affinity (Pinilla et al. 1994). The approach was successful and peptides with affinities ten-times higher than the natural ligand were identified using competitive ELISA.

Alternative biological approaches toward peptide libraries arose with phage display techniques (Smith 1985), and the real strength of the synthetic approach exploring the chemical space of peptide libraries was discovered by introducing chemical transformations of the peptide backbone into the synthetic schemes. Small peptides are not very well suited as drugs because of their low stability and oral availability. As a result, the "Libraries from Libraries" approach was developed by chemical modification of sublibraries (Houghten 2000; Nefzi et al. 2004; Ostresh et al. 1994). One of the first examples, permethylation of a hexapeptide library, afforded around 40 million compounds as mixtures in solution (Ostresh et al. 1994). Soon afterward, the method included heterocycle synthesis, inspired by solid-phase peptide chemistry (Fig. 2). First performed by Leznoff and Rapoport, solid-phase heterocyclic chemistry really gained momentum when applied to the synthesis of benzodiazepine analogs some 20 years later by Bunin and Ellman (Bunin and Ellman 1992; Crowley and Rapoport 1976; Leznoff and Wong 1973; Pinilla et al. 2003; Wong and Leznoff 1973). The development of a sophisticated toolbox for chemical transformations such as acylations, alkylations, and reductions accelerated the diversification of the resulting compound mixtures.

The "Libraries from Libraries" approach results in large compound collections in a mixture-based format. This strategy requires complex deconvolution schemes of successive screening of smaller subsets for the identification of hits from these

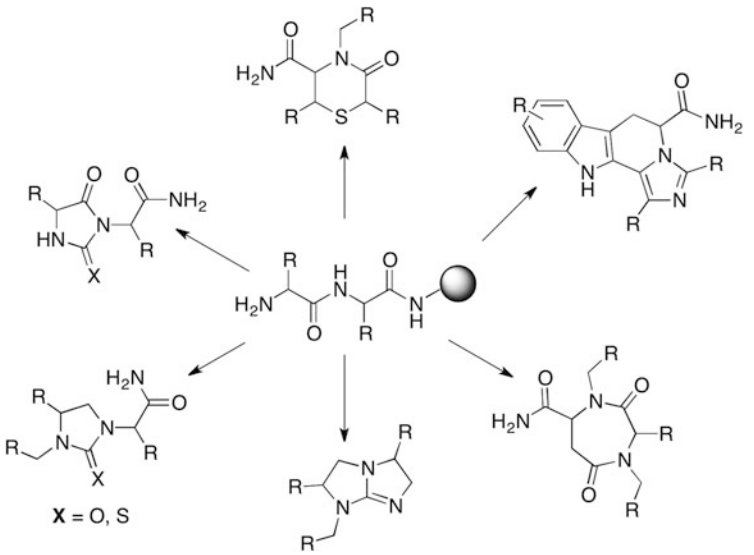

Fig. 2 Synthesis of heterocyclic compounds on solid support from dipeptides and acylated dipeptides as starting materials. Figure adapted from Houghten (2000)