each stage. Oversimplification may also result in reduced activity, reduced selectivity, and increased side effects. We shall see why in the next section (section 13.3.9).

13.3.9 **Rigidification of the structure**

Rigidification has often been used to increase the activity of a drug or to reduce its side effects. In order to understand why this tactic can work, let us consider again our hypothetical neurotransmitter from Chapter 5 (Fig. 13.54). This is quite a simple, flexible molecule with several rotatable bonds that can lead to a large number of conformations or shapes. One of these conformations is recognized by the receptor and is known as the **active conformation**. The other conformations are unable to interact efficiently with the receptor and are inactive conformations. However, it is possible that a different receptor exists which *is* capable of binding one of these alternative conformations. If this is the case, then our model neurotransmitter could switch on two different receptors and give two different biological responses, one which is desired and one which is not.

The body's own neurotransmitters are highly flexible molecules (section 4.2), but, fortunately, the body is efficient at releasing them close to their target receptors, then quickly inactivating them so that they do not make the journey to other receptors. This is not the case for drugs. They have to be sturdy enough to travel throughout the body and will interact with all the receptors that are prepared to accept them. The more flexible a drug molecule

is, the more likely it will interact with more than one receptor and produce other biological responses (side effects). Too much flexibility is also bad for oral bioavailability (section 11.3).

The strategy of rigidification is to make the molecule more rigid, such that the active conformation is retained and the number of other possible conformations is decreased. This should reduce the possibility of other receptor interactions and side effects. This same strategy should also increase activity. By making the drug more rigid, it is more likely to be in the active conformation when it approaches the target binding site and should bind more readily. This is also important when it comes to the thermodynamics of binding. A flexible molecule has to adopt a single active conformation in order to bind to its target, which means that it has to become more ordered. This results in a decrease in entropy and, as the free energy of binding is related to entropy by the equation $\Delta G = \Delta H - T\Delta S$, any decrease in entropy will adversely affect ΔG. In turn, this lowers the binding affinity (K_i), which is related to ΔG by the equation $\Delta G = -RT\ln K_i$. A totally rigid molecule, however, is already in its active conformation and there is no loss of entropy involved in binding to the target. If the binding interactions (ΔH) are exactly the same as for the more flexible molecule, the rigid molecule will have the better overall binding affinity.

Incorporating the skeleton of a flexible drug into a ring is the usual way of locking a conformation—for our model compound the analogue shown in Fig. 13.55 would be suitably rigid.

A ring was used to rigidify the acyclic pentapeptide shown in Fig. 13.56. This is a highly flexible molecule that acts as an inhibitor of a proteolytic enzyme. It was decided to rigidify the structure by linking the asparagine residue with the aromatic ring of the phenylalanine residue to form a macrocyclic ring. The resulting structure showed a 400-fold increase in activity.

Similar rigidification tactics have been useful in the development of the anti-hypertensive agent **cilazapril** (Fig. 12.12) from captopril, and the development of the
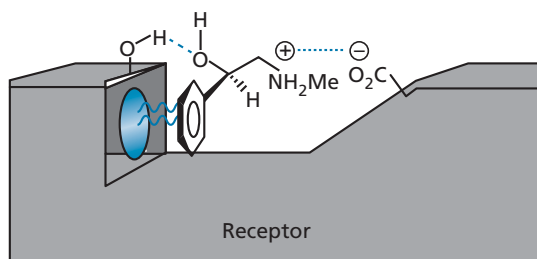
FIGURE 13.54 Active conformation of a hypothetical neurotransmitter.
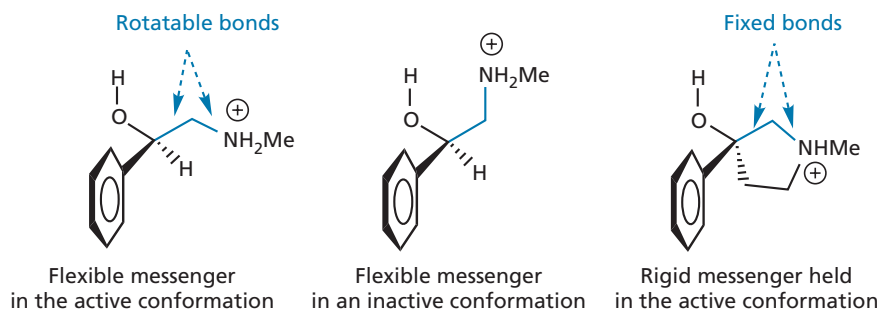
FIGURE 13.55 Rigidification of a molecule by locking rotatable bonds within a ring.
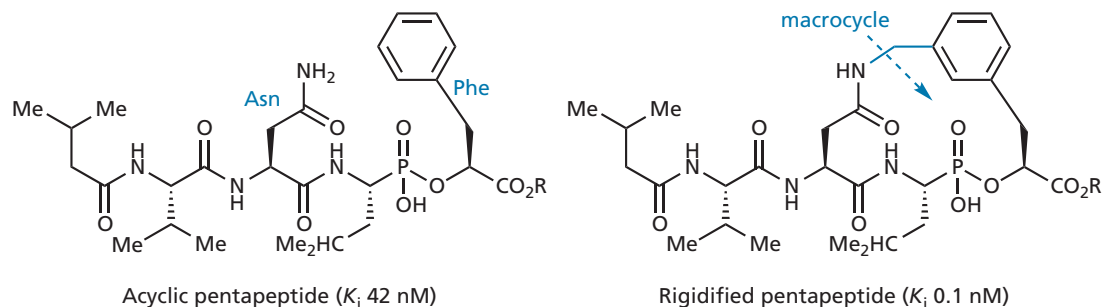
FIGURE 13.56 Rigidification of an acyclic pentapeptide.

sedative **etorphine** (section 24.6.4). Other examples of rigidification can be seen in sections 21.7.1 and 25.2.8.1.

Locking a rotatable bond into a ring is not the only way a structure can be rigidified. A flexible side chain can be partially rigidified by incorporating a rigid functional group such as a double bond, alkyne, amide, or aromatic ring (see Box 13.3).

For additional material see Web article 5: the design of a serotonin antagonist as a possible anxiolytic agent.

Rigidification also has potential disadvantages. Rigidified structures may be more complicated to synthesize. There is also no guarantee that rigidification will retain the active conformation; it is perfectly possible that

BOX 13.3 Rigidification tactics in drug design

The diazepine (I) is an inhibitor of platelet aggregation, and binds to its target receptor by means of a guanidine functional group and a diazepine ring system. These binding groups are linked together by a highly flexible chain. Structures (II) and (III) are examples of active compounds where the connecting chain between the guanidine group and the bicyclic system has been partially rigidified by the introduction of rigid functional groups.

Rigidification of flexible chains.
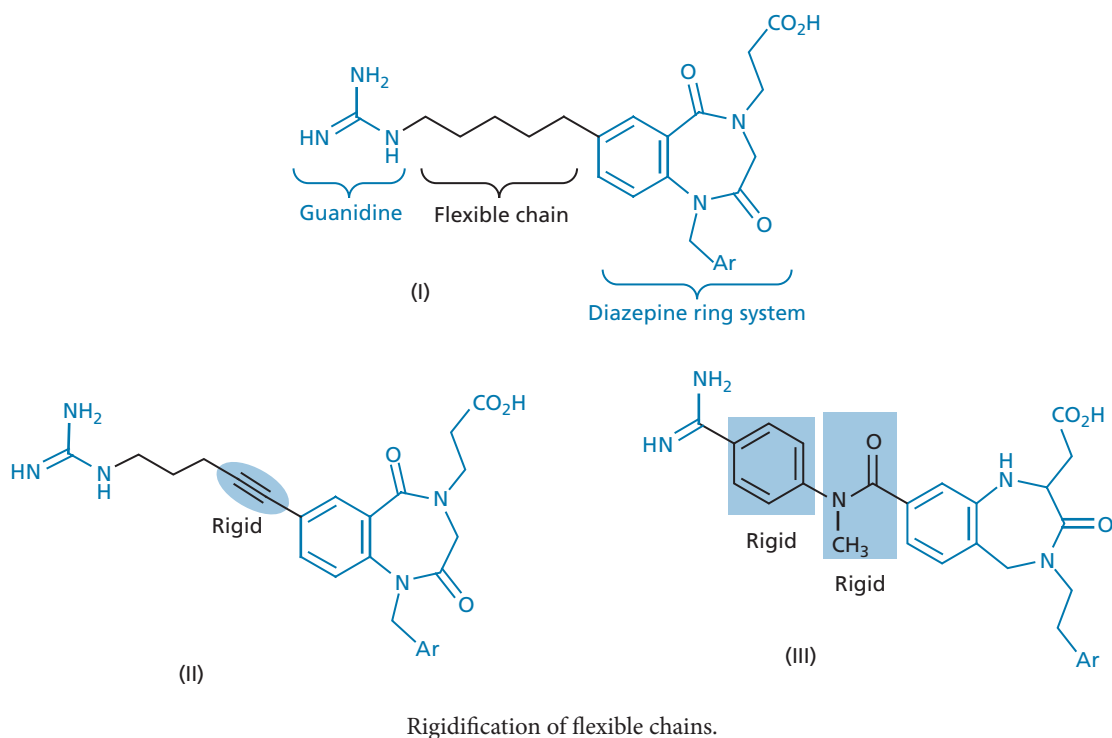
rigidification will lock the compound into an inactive conformation. Another disadvantage involves drugs acting on targets which are prone to mutation. If a mutation alters the shape of the binding site, then the drug may no longer be able to bind, whereas a more flexible drug may adopt a different conformation that *could* bind.

13.3.10 **Conformational blockers**

We have seen how rigidification tactics can restrict the number of possible conformations for a compound. Another tactic that has the same effect is the use of **conformational blockers**. In certain situations, a quite simple substituent can hinder the free rotation of a single bond. For example, introducing a methyl substituent to the dopamine (D_3) antagonist (I in Fig. 13.57) gives structure II and results in a dramatic reduction in affinity. The explanation lies in a bad steric clash between the new methyl group and an *ortho* proton on the neighbouring ring which prevents both rings being in the same plane. Free rotation around the bond between the two rings is no longer possible and so the structure adopts a conformation where the two rings are at an angle to each other. In structure I, free rotation around the connecting bond allows the molecule to adopt a conformation where the aromatic rings are co-planar—the active conformation
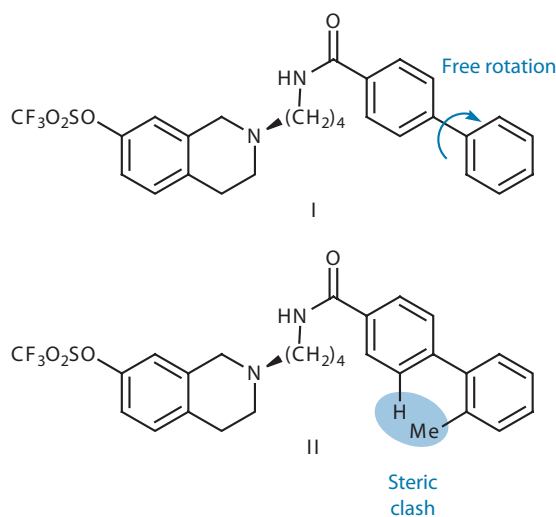

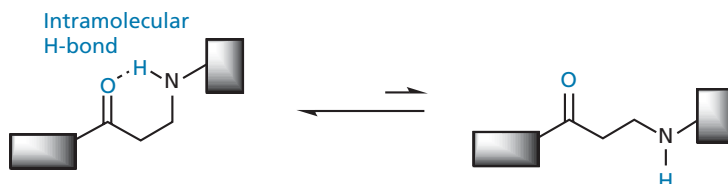**FIGURE 13.57** Introducing rigidity by conformational blocking.

for the receptor. In this case, a conformational blocker 'rejects' the active conformation. Examples of a conformational blocker favouring the active conformation can be seen with 4-methylhistamine (section 25.2.2.2), the design of a serotonin antagonist (see Web article 5), and the development of the anticancer agent imatinib (section 21.6.2.2). In the last case, conformational restraint not only increased activity, but also introduced selectivity between two similar target binding sites.

Rigidification is also possible through intramolecular hydrogen bonding, which may help to stabilize particular conformations (Fig. 13.58).

13.3.11 **Structure-based drug design and molecular modelling**

So far we have discussed the traditional strategies of drug design. These were frequently carried out with no knowledge of the target structure, and the results obtained were useful in providing information about the target binding site. Clearly, if a drug has an important binding group, there must be a complementary binding region present in the binding site of the receptor or enzyme.

If the macromolecular target can be isolated and crystallized, then it may be possible to determine the structure using X-ray crystallography. Unfortunately, this does not reveal where the binding site is, and so it is better to crystallize the protein with a known inhibitor or antagonist (ligand) bound to the binding site. X-ray crystallography can then be used to determine the structure of the complex and this can be downloaded to a computer. Molecular modelling software is then used to identify where the ligand is and thus identify the binding site. Moreover, by measuring the distances between the atoms of the ligand and neighbouring atoms in the binding site, it is possible to identify important binding interactions between the ligand and the binding site. Once this has been done, the ligand can be removed from the binding site *in silico* and novel lead compounds can be inserted *in silico* to see how well they fit. (The term *in silico* indicates that the virtual process concerned is being carried out on a computer using molecular modelling software.) Regions in the binding site which are not occupied by the lead compound can be identified and used to guide the medicinal chemist as to what modifications and additions can be made to design

FIGURE 13.58 Rigidification involving an intramolecular hydrogen bond.