Bioactive Surfactants containing a β-Lactam Group: Synthesis and Properties

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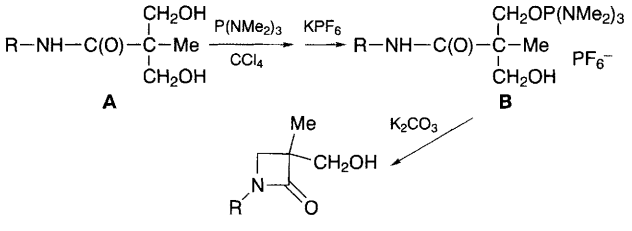
Selective activation of 3-hydroxy-2-hydroxymethyl-2-methyl propanamide with $P(NMe_2)_3$–CCl_4, and subsequent intramolecular cyclisation yields β-lactam derivatives that have both surface and antibiotic activity.

Little is known about the correlation between the surfactant properties of β-lactams and their biological activity. Here we report the synthesis and properties of a new class of β-lactam that are both bio- and surface-active materials (*i.e.* bioactive surfactants). In previous work,[1] we described the synthesis and properties of fatty amide derivatives of 3-hydroxy-2-hydroxymethyl-2-methyl propanoic acid (**A**, Scheme 1). In this study, we planned to activate only one of the hydroxy functions with $P(NMe_2)_3$–CCl_4 couple. The resulting alkoxy tris(dimethylamino)phosphonium salt (ATDP salt **B**, Scheme 1)[2,3] was obtained in good yield. A standard procedure for β-lactam synthesis involves the intramolecular reaction, under alkaline condition, of a primary or secondary amide with a nucleofuge-bearing γ-carbon.[4] Applying this cyclisation procedure, **B** was transformed into a β-lactam derivative by the use of anhydrous potassium carbonate either in acetone or dioxane, as shown below. No neopentyl rearrangement was observed.[3]

β-Lactam derivatives (type **I**) were obtained in good yield (50–55%). We also applied this procedure to the synthesis of mimics of nocardicine A, a natural β-lactam with antibiotic activity.[5–7] Thus, reaction of phenylalanine (an α-amino acid)[3] with a long-chain alcohol or amine gave the fatty ester or amide derivatives respectively. Reaction of these with 3-hydroxy-2-hydroxymethyl-2-methyl propanoic acid afforded the amido-diols, **A**, which were subsequently cyclised to type **II** β-lactams in good yields (55–65%). Both types of β-lactams have a hydrophobic long alkyl chain and a hydrophilic β-lactam ring, a condition necessary for surface activity.

Although all the compounds are only slightly soluble (*ca.* 10^{-5} mol dm^{-3}) in polar media such as water and formamide,[8] they exhibit good surfactant properties in these solvents at room temperature. Saturated aqueous solutions of both types of compounds reduce the surface tension (γ_{sat}) of water from 72 mN m^{-1} to about 35 mN m^{-1}. The solubility of **1** and **5** in water was sufficient to obtain a γ *vs.* log *c* (concentration) plot that was used to determine their critical micelle concentrations (CMCs). At room temperature, **1** and **5** have CMCs of 0.04 mmol dm^{-3} (γ_{CMC} = 28 mN m^{-1}) and 0.09 mmol dm^{-3} (γ_{CMC} = 16 mN m^{-1}) respectively. These CMCs are slightly lower than those of the corresponding derivatives of **A** that we reported in a previous paper.[1] The solubility of the compounds **1**–**11** in formamide, though higher than that in water, is only moderate (about 1 mmol dm^{-3}). However, the surface tensions of the saturated solutions in formamide, (γ_{sat}) about 33 mN m^{-1}, are comparable to those obtained in water. The compounds of the type **I** compounds, and two homologues (**7**, **8**) of the type **II** lactams are relatively more soluble in formamide than **9**, **10** and **11**. Since the amphiphilic surfactants with higher hydrophobic character are usually more soluble in formamide than in water, is expected that their CMCs are generally higher in the former.[8,9] Examination of the γ *vs.* log *c* plot for solutions of **7** in formamide gave a CMC of 178 mmol dm^{-3} and γ_{CMC} of 29 mN m^{-1}. Note that this compound does not form micelles in aqueous medium due to its low solubility (10^{-6} mol dm^{-3}).

Hydrolysis of synthetic β-lactams in aqueous medium is a potential problem. Consequently, we examined the possible hydrolysis of the type **II** compounds that have both β-lactam ring and an ester function (**7**, **9**, **10** and **11**). A standard experiment was carried out by adding **7** to a mixture of $(CD_3)_2SO$–water (3 : 2) and both the ester and ring carbonyls were monitored by ^{13}C NMR spectroscopy. The carbonyl groups remained unchanged after 48 hours at room temperature. On the basis of the apparent stability of these compounds to rapid hydrolysis, we evaluated some of their biological properties. In haemocytes, saturated solutions of both type **I** and **II** lactams did not cause any haemolysis, except for **7** which produced more than 80% haemolysis. The acute toxicity of **1** was evaluated by intravenous injection in the caudal vein of five 20 g Swiss mice. Injection of 0.2 cm^3 of a 60 g dm^{-3} solution of **1** in physiological serum did not kill any of the Swiss mice. We, therefore, established that the mean lethal dose (LD_{50}) of **1** is greater than 60 mg kg^{-1} (0.27 mmol kg^{-1}). Further, we examined the aggressiveness of some of our compounds on hybridoma cells HF2 × 653. These cells are not naturally apoptotic[10] but the addition of β-lactam surfactants may alter the cell physiology. Consequently, we studied the viability and possible apoptotic or necrotic effects[10,11] of **1**, **5**, **7**, **8**, **10** and **11** on hybridoma cells incubated for one hour at different concentrations. The following trends were observed: (*i*) neither apoptosis nor necrosis was observed with **10**. This compound is completely biocompatible at the concentrations studied. (*ii*) For **1**, **5** and **11**, a decrease in the number of viable cells with increase in the concentration of surfactants was observed. It appears that apoptosis was the major cause of death because the number of necrotic cells was negligible. (*iii*) At high concentrations of **7** and **8**, a high proportion of necrotic cells was observed while the number of apoptotic cells was small.



Type **I** β-Lactams: R = C_8H_{17} **1**, $C_{10}H_{21}$ **2**, $C_{12}H_{25}$ **3**, $C_{14}H_{29}$ **4**, $C_6F_{13}CH_2CH_2$ **5**, $C_8F_{17}CH_2CH_2$ **6**

Type **II** β-Lactams: R = R^1-Z-C(O)-$CHCH_2Ph$: Z = O, R^1 = C_8H_{17} **7**; Z = NH, R^1 = C_8H_{17} **8**; Z = O, R^1 = $C_{10}H_{21}$ **9**; Z = O, R^1 = $C_{12}H_{25}$ **10**; Z = O, R^1 = $PhCH_2$ **11**

Scheme 1. Activation of amido-diols of 3-hydroxy-2-hydroxymethyl-2-methylpropanoic acid **A** by the formation of ATDP monosalt derivatives **B** and β-lactam cyclisation

The antibiotic activity on basis to the Minimum Inhibitory Concentration (MIC)[12] of some of the compounds was tested on different strains of gram-positive and -negative bacteria. All the compounds examined had antibiotic activity, though less than commercial monolactams used in chemotherapy.[13] The compounds **7**, **8**, **10** and **11** were required in concentrations >32 mg dm^{-3} in order to inhibit the growth of a range of bacteria. We note that compound **7** has a higher activity than the compounds **8**, **10** and **11**.

In summary, we have established a simple and efficient procedure for the synthesis of surfactant molecules containing a β-lactam ring, and showed that their surface properties are similar to those of known hydrophobic surfactants with higher hydrophobic character.[14] We also demonstrated that most of the compounds tested are biocompatible and that **1** has an LD_{50} > 60 mg kg^{-1} (intravenous). The search for surfactants with excellent antibiotic activity is now in progress. Those that form micelles in aqueous solutions could be used as adjuvants in the delivery of lipophilic drugs, where complementary antibiotic activity is needed.

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