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The Synthesis of N-Morphine Hapten and Production of Monoclonal Antibody

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Abstract: Those antibodies elicited by different tether site for attachment to carrier protein have different specificity. Herein we reported that a monoclonal antibody against morphine with high specificity and affinity was successfully produced by using different linkers to couple to different carrier proteins.

Keywords: Morphine, antigen, monoclonal antibody.

The drug overdose and addiction is a serious international problem. Therefore developing a rapid, accurate, convenient and cheap method for detecting morphine in urea is useful and necessary, especially for investigation of epidemiology, identification of medical jurisprudence and determination of drug addict *etc.*

There are many compounds that are similar to morphine in chemical structure and tend to have cross-reaction with the anti-morphine antibody. In order to reduce the cross reaction and obtain a specific antibody against morphine hapten, a general method is to choose an appropriate tether site for attachment to carrier protein, then couple the hapten to different proteins to gain different antigens-one is immuno-antigen for immunizing animal, the other is coating antigen for screen. However, though antibody raised in such way has high specificity, it has low affinity with free morphine sometimes. It is most likely antibody recognizes the linker and morphine as a whole hapten when coating antigen and immuno-antigen have the same linker, but, when different linkers are used, the antibody recognizing free morphine might be obtained.

In this paper, we synthesized N-morphine hapten **4** and **7**, and then coupled them to carrier proteins human gamma-globulin (HGG) and bovine serum albumin (BSA) through two different linkers, N-(diaminoethylcarboxymethyl) and N-(4-aminobutyl), shown in **Scheme 1**. The obtained antigen **5** was used as an immuno-antigen to immunize rice, while **8** as a coating antigen in ELISA for the screen of monoclonal antibody. As a result, the anti-morphine monoclonal antibody with high specificity and high affinity was successfully acquired, the titer was higher than 2×10^6 and there was no cross reaction with 19 similar compounds in ELISA and codeine lower than 10 mg/mL in our rapid semi-quantitative competitive immunochromatography test strip.

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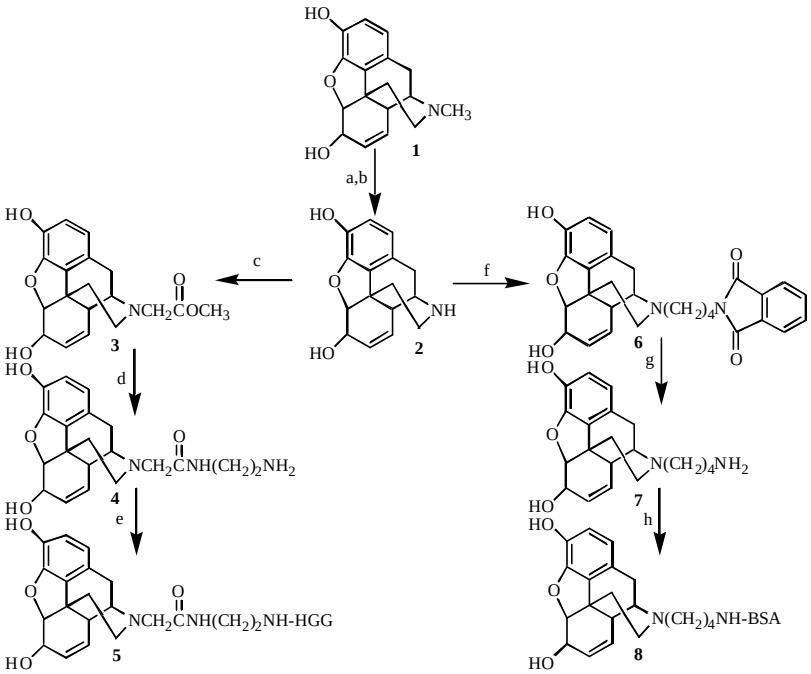
Scheme 1



Reagents and conditions: a. methyl chloroformate and $NaHCO_3$ in $CHCl_3$ refluxed 20 h[1]; b. 80% hydrazine refluxed 63 h[1] with yield 68.5%; c. methylbromacetate in dimethylformamide and sodium bicarbonate refluxed under nitrogen 4 h[2]; d. in ethylenediamine refluxed 3 h with yield 75.8%; e. HGG and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in room temperature for 24 h[3] with 1:12 molecular ratio ; f. with *N*-(4-bromobutyl)phthalimide and anhydrous sodium carbonate in dimethylformamide refluxed for 2 h[4]; g. 90% aqueous hydrazine and allyl alcohol refluxed 1 h under nitrogen[4] with yield 73.2%; h. BSA and 0.2% glutaraldehyde/ phosphate-buffered saline[5] with 1:8 molecular ratio.

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