additional 30 min and then allowed to reach room temperature. The reaction mixture becomes dark brown and homogeneous. Acid work-up produces 2,7-dimethyloctan-3,6-dione (89%) with 3,3,6-trimethylheptan-2,5-dione (3%).

As might have been expected, coupling of lithium enolates, which were regiospecifically generated from silyl enol ethers and methyllithium,[6] by $CuCl_2$ in DMF at −78° produced the corresponding 1,4-diketones regiospecifically[3] according to eq 4 and 5.

$$(CH_3)_2CHC(OSi(CH_3)_3)=CH_2 \xrightarrow{CH_3Li} (CH_3)_2CHC(OLi)=CH_2 \xrightarrow[\text{in DMF}]{CuCl_2} (CH_3)_2CHCOCH_2CH_2COCH(CH_3)_2 \quad (4)$$

(78%)

$$CH_3C(OSi(CH_3)_3)=C(CH_3)_2 \xrightarrow{CH_3Li} CH_3C(OLi)=C(CH_3)_2 \xrightarrow[\text{in DMF}]{CuCl_2} CH_3COC(CH_3)_2C(CH_3)_2COCH_3 \quad (5)$$

(6%)

Next, the present method is successfully applicable to some cross couplings of two different methyl ketones and of methyl ketone with acetate, leading to unsymmetrical 1,4-diketones ($RCOCH_2CH_2COR'$) and γ-ketocarboesters ($RCOCH_2CH_2CO_2R'$), respectively (Table II). For instance, addition of $CuCl_2$ in DMF to a 1:3 mixture of lithium enolates of 2-octanone and acetone at −78°, prepared by addition of a 1:3 mixture of 2-octanone and acetone into lithium diisopropylamide in THF according to the above procedure, produced undecan-2,5-dione in 73% yield (based upon the starting 2-octanone) together with 3-pentylhexan-2,5-dione (4%), hexadecan-7,11-dione (8%), and hexan-2,5-dione. Besides the readily available starting materials

$$3CH_3COCH_3 + 1CH_3COC_6H_{13} \xrightarrow[2.\ 4.5CuCl_2\text{–DMF}]{1.\ 4.5LiN(i\text{-}Pr)_2}$$

$CH_3COCH_2CH_2COC_6H_{13}$ (73%)
$CH_3COCH_2CH(C_5H_{11})COCH_3$ (4%)
$C_6H_{13}COCH_2CH_2COC_6H_{13}$ (8%)
$CH_3COCH_2CH_2COCH_3$

and the manipulative simplicity, the high selectivity and good yield render this reaction most straightforward and useful in the preparation of undecan-2,5-dione, a precursor of dihydrojasmone.[7]

Cross coupling of ketone enolate with ester enolate by $CuCl_2$ was carried out as follows. First, a mixture of ketone enolate and ester enolate was prepared by the sequential additions of ketone and ester (after 15 min) to lithium diisopropylamide in THF at −78°. Then, the mixture was treated with $CuCl_2$ in DMF at the same temperature. By this procedure, condensation product[8] is almost completely excluded, resulting in the formation of coupling products. We are currently exploring the full scope of the coupling reaction of ketone enolates by $CuCl_2$ in aprotic polar solvents.

References and Notes

(1) The reaction of sodium enolate of acetophenone with $CuCl_2$ gives 1,4-diphenyl-1,4-butanedione in low yield: T. Kauffmann et al., *Angew. Chem., Int. Ed. Engl.*, **7**, 540 (1968).
(2) M. W. Rathke and A. Lindert, *J. Am. Chem. Soc.*, **93**, 4605 (1971).
(3) Y. Ito, T. Konoike, and T. Saegusa, *J. Am. Chem. Soc.*, **97**, 649 (1975).
(4) The $CuCl_2$-promoted coupling of phenyl isopropyl ketone produced 1,4-diphenyl-2,2,3,3-tetramethyl-1,4-butanedione only in 2% yield.
(5) H. O. House, "Modern Synthetic Reactions", 2nd ed, Benjamin, Menlo Park, Calif., 1972.
(6) (a) G. Stork and P. H. Hudrlik, *J. Am. Chem. Soc.*, **90**, 4462, 4468 (1968); (b) H. O. House, L. J. Czuda, M. Gall, and H. D. Olmstead, *J. Org. Chem.*, **34**, 2324 (1969).
(7) T. Ho, *Synth. Commun.*, **4**, 265 (1974).
(8) M. W. Rathke, *J. Am. Chem. Soc.*, **92**, 3222 (1970).
(9) M. S. Kharasch, H. C. McBay, and W. H. Urry, *J. Am. Chem. Soc.*, **70**, 1269 (1948).
(10) C. Chassin, E. A. Schmidt, and H. M. R. Hoffmann, *J. Am. Chem. Soc.*, **96**, 606 (1974).
(11) E. Blaise, *C. R. Acad. Sci.*, **158**, 506 (1914).
(12) T. Mukaiyama, K. Narasaka, and M. Furusato, *J. Am. Chem. Soc.*, **94**, 8641 (1972).
(13) G. A. Hill, V. Salvin, and W. T. O'Brien, Jr., *J. Am. Chem. Soc.*, **59**, 2385 (1937).

Yoshihiko Ito, Toshiro Konoike, Takeo Saegusa*

Department of Synthetic Chemistry
Faculty of Engineering, Kyoto University
Kyoto 606, Japan

Received January 20, 1975

An Immunoelectrode

Sir:

Proteins in aqueous solutions are polyelectrolytes and have a net electrical charge polarity and magnitude of which depends on the isoelectric point of the protein and on the ionic composition of the solution. If the protein is an antibody which can complex with a corresponding antigen, which also can have a net electrical charge, the electrical charge of the resulting complex will be different from that of the antibody or the antigen alone.[1]

If the antibody is covalently attached to the surface of a thin layer of a hydrophobic polymer which, in turn, is deposited on a metallic conductor, then the surface charge of the polymer–solution interface will depend on the net charge of the immobilized antibody. When corresponding antigen is present in the solution and the binding site of the antibody has not been destroyed during immobilization, the immunochemical reaction will take place at the interface with a resulting change of the surface charge. This change can be measured potentiometrically against a reference electrode immersed in the same solution, using an electrometer amplifier.

It is known[2] that the reaction between immobilized antibody and free antigen, or vice versa, is an equilibrium process. The potential difference between the reference electrode and an electrode with immobilized antibody or antigen (thereafter called immunoelectrode) should, therefore, depend on concentration of the free immunochemical counterpart. The concept applies equally to the case when antigen is bound to the membrane and antibody is free.

In order to test this idea, Concanavalin A (Con A) was covalently attached to the surface of an approximately 5 μ thick, dense poly(vinyl chloride) membrane deposited on platinum wire. Although Concanavalin A is not a true antibody, it complexes certain polysaccharides[3] in the same way true antibodies do. This reaction has been used extensively as a model immunochemical system. Yeast mannan reacts with Concanavalin A, forming a strong complex[3] which is soluble between pH 2 and 4 and insoluble between pH 5 and 7. Various other polysaccharides such as, for example, agar or amyloses do not bind to Con A.

Dependence of potential of Con A immunoelectrode on concentration of yeast mannan measured against silver–silver chloride reference electrode is shown in Figure 1. Similar dependence is obtained when poly(vinyl chloride) coated platinum wire *without* Concanavalin A is used instead of a Con A immunoelectrode. This indicates that there is a considerable nonspecific adsorption of polysaccharides at the polymer surface. In order to separate the effect of adsorption from specific immunochemical interaction, the following experiments were carried out. First, the reference electrode was replaced with another Con A immunoelec-

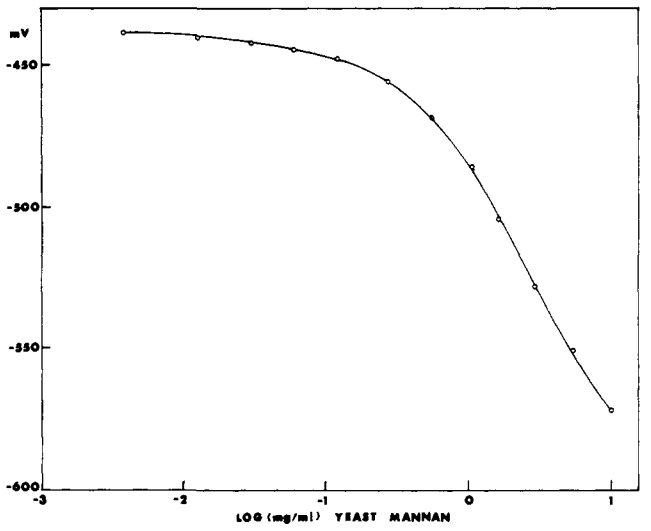


Figure 1. Dependence of the potential of Con A immunoelectrode on concentration of yeast mannan measured against Ag-AgCl (saturated KCl) reference electrode in 0.1 *M* phthalate buffer pH 3.50. The solution was thermostated at 25.00 ± 0.05° and slowly stirred. The following immobilization procedure was used: poly(vinyl chloride) membrane was dip-cast three times from solution containing 0.4 g of poly(vinyl chloride) and 0.9 g of dioctyl addipate in 10 ml of cyclohexanone. The membrane was left to dry for 24 hr and then immersed for 3 hr in solution containing 10% *n*-decanol in a 1:1 mixture of toluene and petroleum ether (30–60° fraction). The membrane was then dried under vacuum at 60° for 24 hr. The last two steps introduced -OH groups to the surface of otherwise hydrophobic membrane. These -OH groups were then used to couple covalently proteins by the epichlorohydrin coupling procedure.[4]

trode and potential difference between two identical immunoelectrodes was measured. There was a transient response to addition of yeast mannan solution, but steady state potential difference remained constant over 3 decades of concentration range (Figure 2, curve a). Binding site carboxyl groups[5] at one Con A immunoelectrode were then blocked with D-(+)-glucosamine coupled to them by 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate.[6] Concanavaline A molecules on this immunoelectrode (labeled Con A* immunoelectrode) should not complex yeast mannan, but, because the rest of the molecule is undisturbed, the amount of nonspecific adsorption should be the same as for nonmodified immunoelectrode. When Con A immunoelectrode was measured against Con A* immunoelectrode, there was indeed a dependence of steady-state potential difference on concentration of yeast mannan (Figure 2, curve b), the response saturating at 0.1 mg/ml. The measured potential changed for 30–45 minutes following an addition of yeast mannan after which period it reached a steady state value. There was no response of Con A immunoelectrode–Con A* immunoelectrode pair to addition of agar, which is a nonbinding polysaccharide.

It was interesting to know if an electrode with any immobilized nonreacting protein could be used as a reference. Such an electrode would have to be used in cases where the nature of the binding site is not known, and, therefore, a selective blocking of the binding sites is not possible. For this reason, the potential of Con A immunoelectrode was measured against an electrode with immobilized ovalbumin. The dependence of potential difference vs. the logarithm of concentration of yeast mannan is shown in Figure 2, curve c.

Apart from experiments with Concanavaline A immunoelectrode, other electrodes with immobilized antibodies or antigens were tested. When a small amount of whole rabbit antiovalbumin serum was added to the physiological

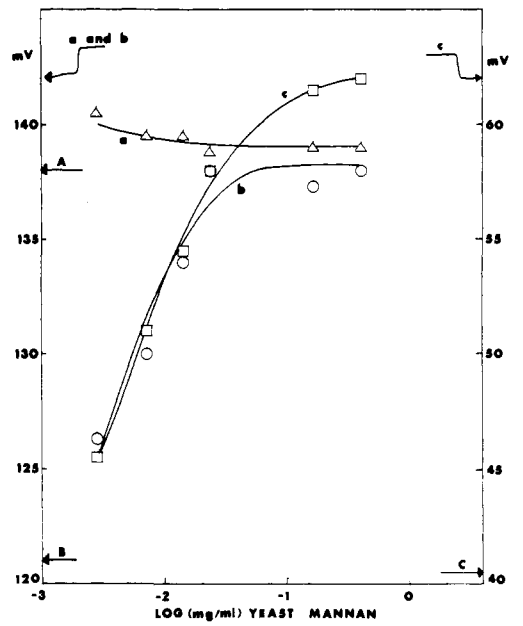


Figure 2. Potential response of Con A immunoelectrode to addition of yeast mannan solution measured against: a, Con A immunoelectrode; b, Con A* immunoelectrode; c, ovalbumin immunoelectrode. Other experimental conditions were the same as described in Figure 1. Potentials at A, B, and C correspond to zero mannan concentration for curves a, b, and c, respectively.

buffer, a small (2 mV) steady-state potential difference between ovalbumin immunoelectrode and electrode prepared by immobilization of whole rabbit serum proteins was obtained. Unfortunately, this effect was too small to be evaluated quantitatively. There was, however, no response when the same amounts of either whole rabbit serum or whole rabbit *anti*-dinitrophenolsubtilisine serum was added.

The above experiments confirm that interactions of charged biomacromolecules with the surface of thin hydrophobic polymeric membrane can produce measurable electrical signal. Our previous experiments showed that it is desirable that the membrane surface should have minimal interaction with water. Thus, no potential response to addition of polysaccharides was detected when Concanavalin A was immobilized on the surface of a glass pH electrode. As expected, the potential of this electrode was determined predominantly by the pH of the solution.

Measurement of the potential of an active immunoelectrode against the potential of an identical immunoelectrode with blocked binding sites can effectively eliminate the effect of nonspecific interactions. The effect of adsorption can be only partially eliminated when immunoelectrode with another nonbinding protein is used as a reference (Figure 2, curve c). At the same time, the presence of extensive adsorption indicates that the polymer surface is not completely covered with Concanavaline A molecules. A more complete coverage should not only reduce the effect of adsorption but at the same time increase the sensitivity to the level obtained with a regular reference electrode. Adsorption of serum proteins was probably also responsible for the low sensitivity of ovalbumin immunoelectrode toward rabbit antiovalbumin serum.

Since either antibody or antigen can be immobilized at the electrode surface, this device, when perfected, could find a general immunochemical application. The improvement of the efficiency of the immobilization procedure is now being studied.

Acknowledgment. The author wishes to express his thanks to Dr. William R. Gray and to Dr. Jarmila Janata for their help in the course of this work.

References and Notes

(1) E. A. Kabat, "Structural Concepts in Immunology and Immunochemistry", Holt, Rinehart and Winston, New York, N.Y., 1968, p 40.
(2) Reference 1, p 79.
(3) G. S. Hassing, and I. J. Goldstein, *Eur. J. Biochem.*, **16**, 549–556 (1970).
(4) J. Porath and L. Sundberg in "Protides of the Biological Fluids", H. Peters, Ed., Amsterdam, 1970, pp 401–407.
(5) G. S. Hassing and I. J. Goldstein, Abstracts, 156th Meeting of the American Chemical Society, Atlantic City, N.J., Sept 1968, BIOL 228.
(6) K. L. Carraway and D. E. Koshland, Jr., *Meth. Enzymol.*, **25**, 616–623 (1972).
(7) On leave from Corporate Laboratory ICI Ltd., The Heath, Runcorn, Cheshire, England, to which correspondence regarding this article should be addressed.

Jiří Janata

Department of Bioengineering, University of Utah
Salt Lake City, Utah 84112

Received January 29, 1975

A New Class of Potent Guanine Antimetabolites. Synthesis of 3-Deazaguanine, 3-Deazaguanosine, and 3-Deazaguanylic Acid by a Novel Ring Closure of Imidazole Precursors

Sir:

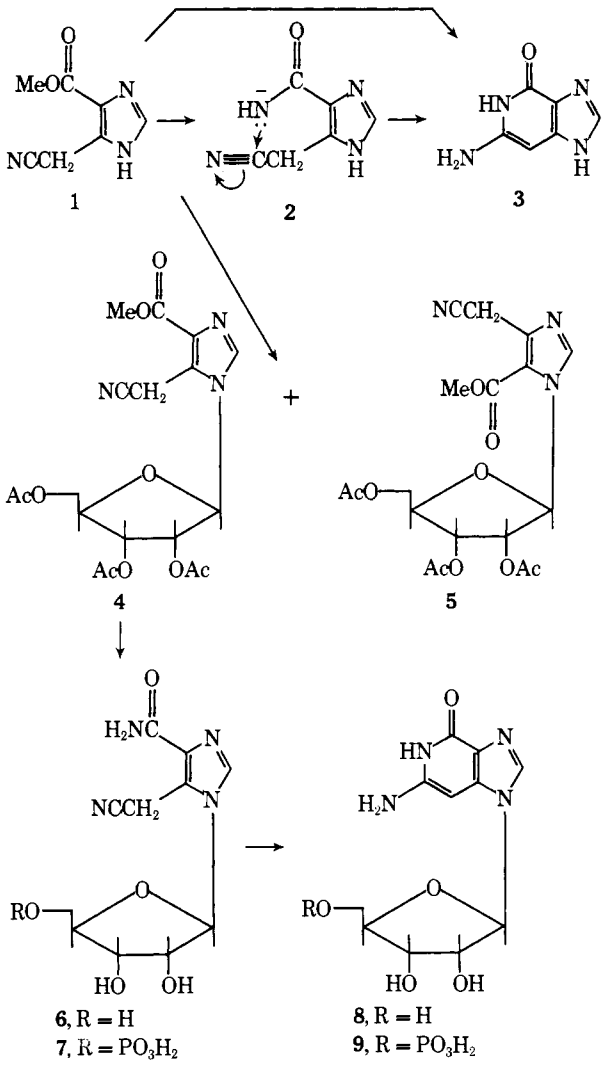
We wish to report the synthesis of 6-aminoimidazo[4,5-*c*]pyridin-4(5*H*)-one (3-deazaguanine, **3**), its nucleoside, 6-amino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridin-4(5*H*)-one (3-deazaguanosine, **8**), and the corresponding 5′-nucleotide, 6-amino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridin-4(5*H*)-one 5′-phosphate (3-deazaguanylic acid, **9**) from the requisite 5-cyanomethylimidazole-4-carboxamide which in the presence of base was found to undergo a unique ring closure to the desired 3-deazaguanine derivatives.

The increased interest in the importance of guanine nucleotide metabolism has stimulated renewed efforts to study these biochemical pathways in various microbiological and mammalian systems.[1] Antimetabolites have proved to be powerful biochemical tools employed to probe such pertinent enzymatic transformations.

de Bode and Salemink[2] recently reported a series of unsuccessful attempts to synthesize 3-deazaguanine from ring closure procedures of certain diaminopyridine derivatives. Our own approach was based on ring closure of imidazole intermediates, which had previously proved successful in the synthesis of imidazo[4,5-*c*]pyridin-4,6(5*H*,7*H*)-dione (3-deazaxanthine).[3] Preliminary success achieved in the synthesis of 6-amino-4-bromoimidazo[4,5-*c*]pyridine from 4(5)-cyano-5(4)-cyanomethylimidzole[4] did not result in the desired 3-deazaguanine (**3**) since the 4-bromo group proved to be exceptionally inert toward nucleophilic substitution.[4]

In a new approach, designed to yield 3-deazaguanine (**3**) directly by ring closure, the required key imidazole intermediate, methyl 5(4)-cyanomethylimidazole-4(5)-carboxylate[5] (**1**, Scheme I), mp 170–171° dec, was obtained in 77% yield from methyl 5(4)-carbamoylmethylimidazole-4(5)-carboxylate[3] and refluxing phosphorus oxychloride. Treatment of **1** with liquid ammonia (8 days, 100°) provided 3-deazaguanine (**3**) as light-sensitive yellow needles [75%, mp >350° (H_2O)]: $\lambda_{max}^{pH\ 1}$ 273 (ε 11,320), 311 (ε 6380); $\lambda_{max}^{pH\ 11}$ 262 (ε 9630), 298 (ε 7780). The intermediate to **3**, 5(4)-cyanomethylimidazole-4(5)-carboxamide (**2**) (mp 231–232° dec), was obtained in 77% yield by interrupting the reaction of **1** and ammonia after 48 hr. Compound **2** was smoothly cyclized to **3** with aqueous sodium carbonate. This ring closure has now been shown in our laboratory to be of general application in the synthesis of various condensed aminopyridone systems. The mechanism is visualized as occurring by base abstraction of an amide proton followed by attack of the generated anion on the nitrile carbon.

Scheme I



The requisite imidazole nucleoside, methyl 5-cyanomethyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylate (**4**) was obtained in quantitative yield from the condensation of 1 equiv of methyl 5(4)-cyanomethyl-1-trimethylsilylimidazole-4(5)-carboxylate (silylated **1**) with 1 equiv of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose in the presence of 1.44 molar equiv of stannic chloride. The yield and ratio of positional isomers in this ribosylation procedure markedly depends on the ratio of stannic chloride to silylated **1** and the blocked ribofuranose since the same condensation carried out with 0.72 molar equiv of stannic chloride afforded, after silica gel chromatography, a 29.5% yield of **4** and a 34.5% yield of the other positional isomer, methyl 4-cyanomethyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-5-carboxylate (**5**) (white needles, mp 92–93° (EtOH)).

Treatment of **4** with liquid ammonia (3 hr, 100°) provided the versatile intermediate, 5-cyanomethyl-1-β-D-ribofuranosylimidazole-4-carboxamide (**6**) (81%, mp 90–91° dec (MeOH)). When **6** was refluxed (0.5 hr) with aqueous sodium carbonate in ethanol, 3-deazaguanosine (**8**) was formed and crystallized from the reaction solution (85%, white microcrystals, mp 255–257° dec): $\lambda_{max}^{pH\ 1}$ 284 (ε 13,100), 308 sh (ε 7050); $\lambda_{max}^{pH\ 11}$ 272 (ε 11,900), 295 sh