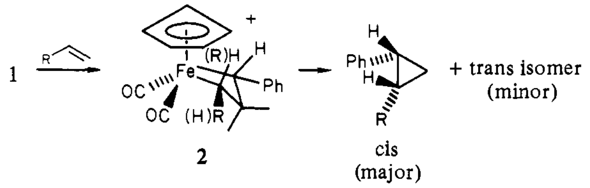
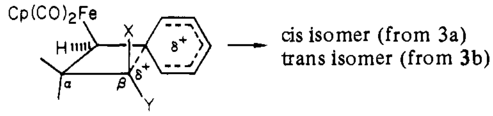
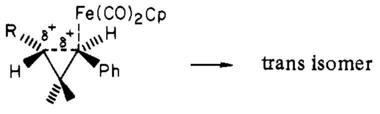
configuration around the double bond is maintained in the product (compare *cis*- and *trans*-2-butenes). Recent evidence has implicated metallacyclobutanes, formed from reactions of metal carbenes and olefins, as intermediates in olefin metathesis.[15] This suggests that a metallacyclobutane intermediate such as **2** (or at least a metallcyclobutane-like transition state) might be involved in the reaction of **1** with alkenes. Models suggest that the Cp ring would direct substituents to lie preferentially cis to one another and anti to the Cp ring in the metallacyclobutane-like transition state, thus ultimately leading selectively to cis products. Two experimental observations speak against this model. First, as the size of the alkyl substituent increases in a series of monosubstituted olefins $CH_2{=}CHR$ ($R = CH_3, CH_2CH_3, CH(CH_3)_2$; see Table I), the selectivity *decreases*. Secondly, we have examined the reaction of the *p*-tolyl complex $Cp(CO)_2FeCH(p\text{-}CH_3C_6H_4)^+$ with propene at 0 °C and have found that the p-CH_3 substituent has a significant effect on selectivity, giving a 10:1 cis:trans ratio of (*p*-tolylmethyl)cyclopropane as compared to 7.8:1 for the unsubstituted phenyl case. The effect of the p-CH_3 substituent must be electronic and not steric in nature and is inconsistent with the metallacyclic model, where steric interactions control the selectivity.[16]



The most attractive mechanistic model is one proposed by Casey[2] for reactions of $(CO)_5WCHC_6H_5$, in which a special stabilizing role is ascribed to the phenyl ring. Briefly put, the positive charge which develops at the β carbon upon interaction of the alkene with the electrophilic carbene center can be stabilized in the transition state via interaction with the ipso carbon of the phenyl ring, as shown in **3**. In this cyclobutane-like transition state the substituent R prefers to lie predominantly trans to $Cp(CO)_2Fe$, as in **3a**. Upon formation of cyclopropane by electrophilic attack of C_β on the iron–carbon bond, the substituent R ends up cis to the phenyl substituent.[17] To explain the decrease in selectivity with increasing size of alkyl groups in monosubstituted olefins, it is necessary to postulate an "open" transition state, as shown by **4**, which leads to higher fractions of trans product and in which the substituent R is sterically less crowded than in **3**. Thus, in this scheme, as R increases in size, more product is formed via transition state **4**, giving higher fractions of trans cyclopropane. The increased cis:trans selectivity of the *p*-tolyl carbene complex is also consistent with this Casey model since the p-CH_3 substituent will clearly stabilize transition state **3** (which leads predominantly to cis product) relative to transition state **4**.

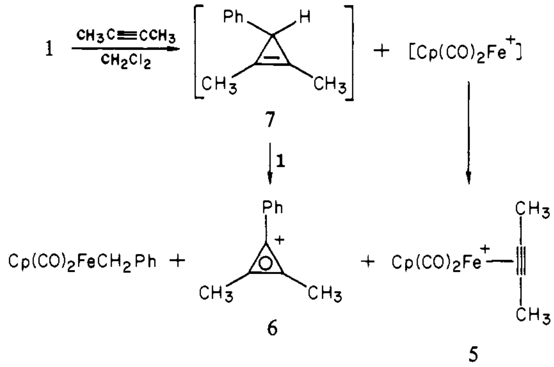


3a, X = H; Y = R
b, X = R; Y = H



4

Preliminary experiments indicate that **1** is also highly reactive toward alkynes. Relatively few reactions of metal carbenes with alkynes have been reported[2b,18] and the reactivity patterns observed here appear unprecedented. Treatment of **1** with 3 equiv of 2-butyne in methylene chloride at −78 °C followed by warming to 25 °C results in formation of equimolar ratios of $Cp(CO)_2FeCH_2C_6H_5$, $Cp(CO)_2Fe(CH_3C{\equiv}CCH_3)^+PF_6^-$ (**5**),[19] and 1,2-dimethyl-3-phenylcyclopropenium hexafluorophosphate (**6**) (isolated yields of **5** and **6** were ca. 75%). The reaction likely occurs by initial carbene transfer to form phenyldimethylcyclopropene (**7**) followed by hydride transfer to unreacted **1**.



On the basis of the results presented here, the readily and inexpensively generated carbene complexes of the type $Cp(CO)_2Fe{=}CHR^+$ appear to have substantial synthetic utility for carbene-transfer reactions and we are continuing to examine the chemistry of such species.

Acknowledgement is made to the National Science Foundation (Grant MPS75-01239) and the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research.

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(15) For reviews of olefin metathesis see: Calderon, N.; Lawrence, J. P.; Ofstead, E. A. *Adv. Organomet. Chem.* **1979**, *17*, 449–93. Katz, T. J. *Ibid.* **1977**, *16*, 283–317. Grubbs, R. H. *Prog. Inorg. Chem.* **1976**, *24*, 1–50. Calderon, N.; Ofstead, E. A.; Judy, A. W. *Angew. Chem.* **1976**, *88*, 433–42. Hains, R. J.; Leigh, G. *Chem. Soc. Rev.* **1975**, *4*, 155–88.

(16) (a) Although $(CO)_5WC(C_6H_5)_2$ reacts with alkenes to form metathesis products (presumably via CO dissociation and formation of a metallacyclobutane intermediate),[15b] we see no evidence for metathesis-like products from reaction of **1** with olefins. CO dissociation from **1** under the reaction conditions is quite slow. Generation of a stable metallacycle directly from **1** would require formation of a formally iron(IV) dicarbonyl species which is an electronically unattractive intermediate. (b) Casey, C. P.; Burkhardt, T. J. *J. Am. Chem. Soc.* **1974**, *96*, 7808–9.

(17) As pointed out by Casey,[2b] such a model also explains the high selectivity for 2-methyl-2-butene in that the single methyl group on the α carbon in **3** would prefer to lie trans to Fp.

(18) Dötz, K. H.; Dietz, R. *Chem. Ber.* **1978**, *111*, 2517–26. *Ibid.* **1977**, *110*, 1555–1563. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 644–5.

(19) (a) The properties of this salt were identical with those reported for this complex prepared by a different route.[17b] (b) Reger, D. L.; Coleman, C. J.; McElligott, P. J. *J. Organomet. Chem.* **1979**, *171*, 73–84.

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Acepleiadylene Dianion and Tetraanion

Sir:

Cyclohept[*fg*]acenaphthylene (acepleiadylene) (**1**), synthesized in 1956 by Boekelheide,[1] has always attracted special attention since, from thermochemical, spectroscopic, and theoretical arguments, it is strongly believed to exist as a vinyl-bridged [14]-annulene[2] and to thus constitute an example of Platt's perimeter

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(1) V. Boekelheide and G. K. Vick, *J. Am. Chem. Soc.*, **78**, 653 (1956).

(2) (a) R. B. Turner, W. S. Lindsay, and V. Boekelheide, *Tetrahedron*, **27**, 3341 (1971); (b) A. J. Jones, P. D. Gardner, D. M. Grant, W. M. Litchman, and V. Boekelheide, *J. Am. Chem. Soc.*, **92**, 2395 (1970); (c) A. DasGupta and N. K. DasGupta *Theor. Chim. Acta*, **33**, 177 (1974).

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Table I. ^1H and ^{13}C Chemical Shifts[a] of 1, **2**, **3**, 4, and 5 (Ions As Lithium Salts) (THF-d_8, -30 °C)

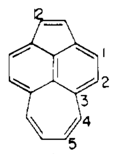
	1,10	2,9	3,8	4,7	5,6	11,14	12,13	15	16
δ_H (1)	7.95	8.33		7.79	6.89		7.83		
δ_H (2)	1.26	−0.33		−2.05	−2.05		1.53		
δ_H (3)	5.96	4.90		3.56	4.28		4.44		
δ_C (1)	125.8	127.4	134.9	126.9	137.0	138.2	126.2	127.0	126.6
δ_C (2)	98.6	131.0	121.4	155.8	127.6	132.1	86.8	146.1	173.2
$\delta_C(2)^b$	98.3	131.3	120.4	155.8	127.9	131.3	89.4	142.8	174.4
δ_C (3)	74.3	86.4	94.0	127.2	100.3	113.7	58.4	139.1	102.3
$q\pi(2)^c$	1.23	1.03	1.34	0.91	1.26	1.04	1.27	1.08	0.76
δ_H (4)	6.66	6.27		5.75	5.04		3.04		
δ_H (5)	6.36	6.73		3.45	4.28		3.23		
δ_C (4)	119.5	127.4	135.3	125.4	137.9	143.4	29.1	142.4	136.0
δ_C (5)	115.7	117.1	122.0	68.4	99.5	140.3	30.8	101.5	135.9

[a] Signal assignments for the neutral compounds 1 and 4 have been taken from ref 2b. For the ionic species ^1H-signal assignments have been made from splitting patterns, ^{13}C-signal assignments from selective ^1H decoupling and from relative signal intensities. It is not possible from experimental evidence to make a distinction between signals of nuclei (^1H, ^{13}C) 1 and 2 (as well as 4 and 5). In those cases assignments have been deduced from corresponding (PPP) π-charge densities. [b] ^{13}C-NMR spectrum of **2** obtained after addition of a tenfold excess of TMEDA. [c] π-charge densities resulting from a PPP calculation. An idealized molecular geometry has been assumed with $R_{CC} = 1.4$ Å. [d] Only π centers have been considered.

rule.[3] When describing the bonding situation of annulenes, inclusion of the corresponding ionic derivatives appears to be a most significant approach. We have, therefore, extended this concept to polycyclic π systems and reduced compound **1** with lithium to obtain a dianion, **2**, and—surprisingly enough for a C_{16} hydrocarbon—a tetraanion, **3**.

Spectroscopic, MO-theoretical, and chemical evidence can be combined to create a consistent view of the charge distribution and conjugation within the ions. The analogy with the annulene field becomes particularly obvious from the fact that compound **2**, although unlikely both from electrostatic reasons and from a naive application of Hückel's rule, exists as a weakly perturbed $4n$ π perimeter.

When the reduction of **1** (lithium, fully deuterated tetrahydrofuran, -80 °C) is monitored by ^1H NMR spectroscopy, one observes the appearance of a new diamagnetic species (well-resolved signals at $\delta = \sim 0$), which, on further metal contact, is transformed into another product absorbing at $\delta = \sim 5$ (see supplementary material). Reoxidation by dry oxygen at any stage of the reduction yields the starting material. This outcome, the analysis of the ^1H and ^{13}C NMR spectra along with the results of quenching experiments, can be interpreted in such a way that in the course of the reduction the lithium salts of a dianion (**2**) and of a tetraanion (**3**) have been formed. Kept in sealed NMR tubes, solutions of **2** or **3** remain unchanged even at room temperature.[4] Protonation of **2** with methanol provides the dihydro derivative acepleiadiene **4** already described by Boekelheide.



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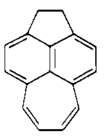

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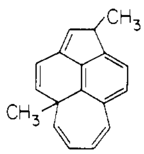

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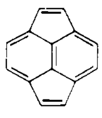

7

Reaction of **2** with excess dimethyl sulfate at −70 °C affords a 50% yield of an easily polymerizing dimethyl adduct which from its molecular weight as well as from its ^1H and ^{13}C NMR spectra is assigned structure **6**.[5] Particularly revealing in the NMR spectra is the absence of symmetry as well as the relative intensities of aromatic, olefinic, allylic, and aliphatic ^1H signals. The attack of one methyl group on a quaternary (naphthalene) position is also evidenced by the lack of a vicinal H,H coupling in the corresponding ^1H signal. Methylation of **3** in the same fashion gives rise to a mixture of unstable tetramethyl adducts identified by mass spectroscopy ($m/z = 262$), thus indicating the nature of **3** as a quadruply charged species.

The extreme low-field ^1H resonances of **1** (see Table I) point toward the existence of a peripheral diamagnetic ring current characteristic of a perimeter-type structure. The pronounced upfield shifts of the proton signals on dianion formation (by ~8 ppm) are far beyond the prediction one would have made on the basis of charge-induced shielding.[6] As can be seen from a simple MO model describing the interaction of a [14]annulene and an ethylene group, the π orbital occupied by the additional two electrons in **2** constitutes a pure perimeter MO.[7] With the excess charge localized in the periphery, the dianion **2** builds up a vinyl-bridged $16\pi = 4n$ perimeter for which one will expect the existence of a paramagnetic ring current effect. Therefore, the extreme high-field position of the ^1H resonances finds a straightforward explanation. This bonding situation, which is due to the nodal properties of the HOMO, has to be contrasted with the one in the tetraanion **3** and in the acepleiadiene dianion (**5**). The appearance of the ^1H signals of **3** and **5** at ~5 ppm downfield from those of **2** indicates that the paramagnetic ring current in **2** can be quenched both on transformation to the tetraanion and on interruption of the original perimeter as occurring in **5**.

The existence of a $4n$ π perimeter within a polycyclic π system has also been proposed by Trost for the structurally related pyracyclene (**7**).[8] In the case of the ionic species **2**, a true homologue of **7**, additional evidence in favor of the perimeter conjugation can be inferred from a knowledge of the π-charge distribution. Both calculated π-charge densities, q_π (see Table I), and experimental ^{13}C chemical shifts, δ_C, of **2**—and, in particular, the extreme low-field resonances of the bridge centers—indicate the expected charge separation between bridge and periphery. One can even conclude from the q_π and δ_C values that, resulting from some π,π polarization, a partial positive charge is established at C-16 of the dianion.[9]

Making use of the simple π-charge density/^{13}C-shift correlation,[10] one would predict for the acepleiadylene system that the injection of two extra electrons moves upfield the center of gravity of the ^{13}C signals by $\Delta\delta = 20$. The values actually measured are 2 and 32 ppm on transformation of **1** to **2** and of **1** to **3**, respectively. It appears that **3** (although from the appreciable

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(3) J. R. Platt, *J. Chem. Phys.*, **22**, 1448 (1954).

(4) After addition of a large excess of tetramethylethylenediamine (TMEDA), the ions **2** and **3** appear to be less stable, although essentially the same ^1H NMR spectra of **2** and **3** and a ^{13}C NMR spectrum of **2** with only slight signal shifts (see Table I) can be recorded. The decreased stability of **3** after addition of TMEDA does not allow ^{13}C signals to be obtained.

(5) L. D. Kershner, J. M. Gaidis, and H. H. Freedman, *J. Am. Chem. Soc.*, **94**, 985 (1972).

(6) (a) T. Schaefer and W. G. Schneider, *Can. J. Chem.*, **41**, 966 (1963); (b) H. Spiesecke and W. G. Schneider, *Tetrahedron Lett.*, 468 (1961).

(7) (a) F. Gerson and J. Heinzer, *J. Chem. Soc., Chem. Commun.*, 488 (1965); (b) K. Müllen, *Helv. Chim. Acta*, **61**, 2307 (1978).

(8) (a) B. M. Trost, G. M. Bright, C. Frihart, and D. Britteli, *J. Am. Chem. Soc.*, **93**, 737 (1971); (b) C. A. Coulson and R. B. Mallion, *ibid.* **98**, 592 (1976).

(9) (a) K. Müllen, *Helv. Chim. Acta*, **61**, 1296 (1978); (b) *ibid.*, **59**, 1357 (1976).

(10) (a) G. A. Olah and G. D. Mateescu, *J. Am. Chem. Soc.*, **92**, 1430 (1970); (b) D. G. Farnum, *Adv. Phys. Org. Chem.*, **11**, 123 (1975).

"Coulomb strain" one would have expected tighter ion pairing) behaves in a more normal fashion than **2**.[11] Similarly, the bridge carbons of **2**, not those of **3** and **5**, resonate at extremely low field. One might therefore conclude, in accordance with the above results, that the inner carbons of **2** are under the influence of a paramagnetic ring current effect.

Acknowledgment. Financial support by the Deutsche Forschungsgemeinschaft is gratefully acknowledged.

Supplementary Material Available: ^{1}H NMR spectra of acepleiadylene dianion and tetraanion and π-charge distribution (2 pages). Ordering information is given on any current masthead page.

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(11) The fact that **3** shows the typical behavior of a π-delocalized anion certainly demands further consideration of the ion-pair situation. Since the above NMR results are not particularly revealing in this respect, additional techniques, e.g., UV spectroscopy, will have to be applied.

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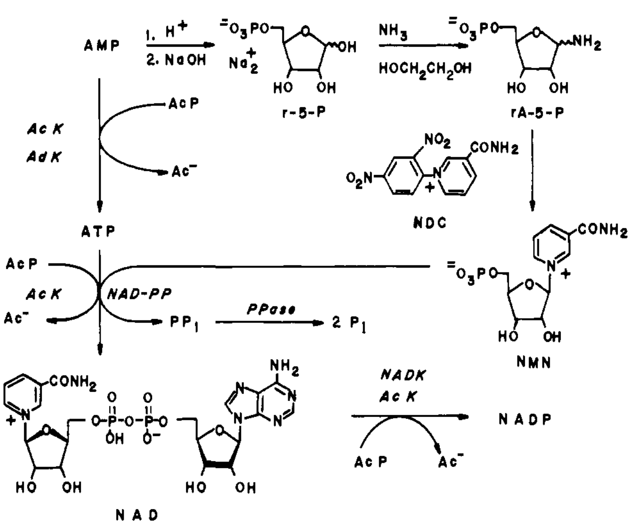
Synthesis of Nicotinamide Adenine Dinucleotide (NAD) from Adenosine Monophosphate (AMP)[1]

Sir:

The acceptance of oxoreductases as catalysts in organic synthesis[2] has been slowed by the expense of the nicotinamide cofactors required by many of these enzymes. Effective procedures for nicotinamide cofactor recycling have decreased the effective cost of these substances by allowing them to be regenerated in situ.[3] The nicotinamide cofactors are, however, intrinsically unstable in solution,[4] and the economic advantage to be gained by any recycling scheme is limited. It is thus also necessary to develop methods for producing them less expensively[5] and for stabilizing them during use. Here we report a combined cell-free enzymatic and chemical synthesis of NAD starting from readily available AMP (Scheme I). This synthesis is a step toward the development of a practical nonfermentation route to NAD and NADP.[6] It also illustrates the utility of enzymatic methods for the synthesis of useful quantities of complex substances and provides a flexible route to derivatives of NAD.

The key intermediate in this synthesis, nicotinamide mononucleotide (NMN), was prepared from AMP in three steps.[7]

Scheme I



Ribose 5-phosphate (r-5-P) was obtained by acid-catalyzed hydrolysis of AMP.[8] Treatment of r-5-P with anhydrous ammonia[9] in dry ethylene glycol provided a solution of ribosylamine 5-phosphate (rA-5-P). This substance was not isolated, but was condensed with N^1-(2,4-dinitrophenyl)-3-carbamoylpyridinium chloride (NDC)[10] to afford NMN in 25% yield based on r-5-P. The NMN (also not isolated) was coupled with ATP[11] in a step catalyzed by NAD pyrophosphorylase[12] (EC 2.7.7.1) immobilized in PAN gel.[13] This enzymatic coupling is an equilibrium reaction and was driven to completion by hydrolyzing the pyrophosphate formed by using pyrophosphatase (EC 3.6.1.1) in PAN.[13] The yield of NAD was 90–97% based on NMN.

A typical reaction sequence follows: Disodium ribose 5-phosphate (156 g, 88% pure, 500 mmol) was added to ethylene glycol (780 mL, dried over 3A molecular sieves). The slurry was cooled to 0 °C, and anhydrous NH_3 was bubbled through it for 1 h. The yellow reaction mixture was stoppered and stored for 1 week in the refrigerator at 4 °C. Excess ammonia was removed (first by using a rotary evaporator and then a vacuum pump). NDC (162 g, 0.5 mol, in 250 mL of methanol) was added as a slurry, and the reaction mixture stirred in the dark for 18 h at 25 °C. Water (1.5 L) was added, and precipitated 2,4-dinitroaniline was removed by filtration. Excess NDC was removed by adsorption on activated charcoal (Darco, 25 g) and filtration. The resulting solution contained 125 mmol of β-NMN by enzymatic assay.[14,15]

For the enzymatic coupling, a 5-L flask was charged with 20 mmol of NMN, 25 mmol of AMP, 2 mmol of ATP, and 100 mL of PAN gel containing coimmobilized NADPP (50 U), PPase (50 U), AcK (100 U), and AdK (100 U).[16] The reaction was adjusted

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[1] Supported by the National Institutes of Health, Grant GM 26543, and by grants from the Monsanto Co.

[2] Jones, J. B. In "Enzymic and Non-Enzymic Catalysis"; Dunill, P., Wiseman, A., Blakebrough, N., Eds.; Ellis Horwood: Chichester, England, 1980; pp 54–81. Wong, C-H.; Whitesides, G. M. *J. Am. Chem. Soc.*, submitted for publication. Suckling, C. *Chem. Soc. Rev.* **1977**, *6*, 215–233.

[3] Wang, S. S.; King, C.-K. *Adv. Biochem. Eng.* **1979**, *12*, 119–146. Baricos, W.; Chambers, R.; Cohen, W. *Enzyme Technol. Dig.* **1975**, *4*, 39–53. Shaked Z.; Whitesides, G. M. *J. Am. Chem. Soc.* **1980**, *102*, 7104–7105.

[4] Lowry, O. H.; Passonneau, J. V.; Rock, M. K. *J. Biol. Chem.* **1961**, *236*, 2756–2759. Bernofsky, C. *Arch. Biochem. Biophys.* **1975**, *166*, 645–650. Oppenheimer, N. J.; Kaplan, N. O. *Biochemistry* **1974**, *13*, 4675–4685. Johnson, S. L.; Tuazon, P. T. *Biochemistry* **1977**, *16*, 1175–1183.

[5] NAD is now isolated from yeast. One pound of yeast (~$1) yields approximately 1 g of NAD: Kornberg, A. *Methods Enzymol.* **1957**, *3*, 876–879.

[6] For previous syntheses of NAD, see: Kornberg, A. *J. Biol. Chem.* **1950**, *182*, 779–793. Hughes, N. A.; Kenner, G. W.; Todd, A. R. *Ibid.* **1950**, *182*, 3733–3738. Traub, A.; Kaufman, E.; Teitz, Y. *Anal. Biochem.* **1969**, *28*, 469–473.

[7] Previous syntheses of NMN have used protecting groups to hold the ribose moiety in the furanose form and to allow selective phosphorylation of the primary hydroxyl group: Haynes, L. J.; Hughes, N. A.; Kenner, G. W.; Todd, A. R., *J. Chem. Soc.* (*London*) **1957**, 3727–3732. Atkinson, M. R.; Morton, R. K.; Naylor, R. *Ibid* **1965**, 610-15. Mellinikova, L. M.; Beresovskii, V. M. *J. Gen. Chem. USSR* (*Engl. Transl.*) **1967**, *37*, 1429–1432. Beresovskii, V. M.; Mellinikova, L. M.; Eremenko, T. V. *Ibid.* **1967**, *37*, 1433–1436. Jeck, R.; Heik, P.; Woenckhaus, C. *FEBS Lett.* **1974**, *42*, 161.

[8] The method used was adopted from Sokatch, J. R. *Biochem. Prep.* **1968**, *12*, 1–5. AMP was obtained from Kyowa Hakko Kogyo.

[9] Tipson, R. S. *J. Org. Chem.* **1961**, *26*, 2462-2464.

[10] Lettré, H.; Haede, W.; Ruhbaum, E. *Ann.* **1953**, *579*, 123–132.

[11] ATP was generated from AMP and acetyl phosphate as described previously: Baughn, R. L.; Adalsteinsson, O.; Whitesides, G. M. *J. Am. Chem. Soc.* **1978**, *100*, 304–306. Leuchs, H. J.; Lewis, J.; Rios-Mercadillo, V. M.; Whitesides, G. M. *Ibid.* **1979**, *101*, 5829–5830.

[12] NADPP was from Brewer's yeast: Kornberg, A. *J. Biol. Chem.* **1950**, *182*, 779–793.

[13] Pollak, A.; Blumenfled, H.; Wax, M.; Baughn, R. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1980**, *102*, 6324–6336.

[14] Grassl, M.; Möllering, H. In "Methods of Enzymatic Analysis", 2nd English ed.; Bergmeyer, H. U., Ed.; Academic Press: New York, 1974; Vol. 4, pp 2073–2077.

[15] Other materials present in significant quantities were unreacted r-5-P, α-NMN, and bis(5-phosphoribosyl)amine.

[16] NADPP catalyzes the hydrolysis of ATP to ADP.[14] It is, therefore, necessary to regenerate ATP continuously during the reaction which forms NAD.

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