Table I. Kinetic Data for the Cycloadditions of TCNE to 5 (k_1^{II}) and 7 (k_2^{II}) in Toluene[9]

k_1^{II}[dm^3 mol^{-1} s^{-1}] = 0.57 (263 K), 1.02 (273 K), 1.50 (283 K), 250 (293 K), 3.86 (303 K); $\Delta H_1^{\ddagger}$ = 6.9 ± 0.84 kcal/mol, $\Delta S_1^{\ddagger}$ = −33 ± 2 cal K^{-1} mol^{-1}
k_1^{II} = 3.1 at 298 K
k_2^{II} = 0.0051 (283 K), 0.0074 (293 K), 0.0127 (303 K), 0.0194 (313 K), 0.0293 (323 K); $\Delta H_2^{\ddagger}$ = 7.5 ± 0.9 kcal/mol, $\Delta S_2^{\ddagger}$ = −42 ± 3 cal K^{-1} mol^{-1}
k_2^{II} = 0.01 at 298 K

washed with 1 N HCl (10 mL, 4 times) and then with H_2O (10 mL, 3 times). After drying ($MgSO_4$), the solvent was evaporated in vacuo and the residue recrystallized from CH_2Cl_2/hexane, yielding 210 mg (79%), yellow crystals: IR ($CHCl_3$) 3030, 3000, 2050, 1995, 1975, 1450, 1365, 1340, 1170, 1145, 1020, 970, 925, 890; ^{1}H NMR ($CDCl_3$) 5.41 (m, HC(2)), 3.90 (d, J = 3 Hz, HC(1)), 3.51 (t, J = 3 Hz, HC(4)), 3.07 (s, CH_3), 2.74 (m, HC(3) trans to C–O), 2.17 (m, HC(3) cis to C–O), 2.18, 2.10, 1.94, 1.88, 0.67, 0.63, 0.41, 0.33 (8 d, J = 3 Hz); ^{13}C NMR ($CDCl_3$) 111.8, 107.0, 105.1, 102.3 (4 s), 79.2 (d, 150, C(2)), 47.3 (d, 140, C(1)), 42.4 (d, 140, C(4)), 40.2, 38.2, 37.6 and 36.4 (4 t, 160), 39.5 (t, 137), C(3)), 38.5 (q, 139, CH_3); MS (70 eV), m/e relative intensity 504 (9, M^+ − 28), 476 (31), 448 (12), 420 (3), 392 (6), 382 (7), 364 (46), 241 (43), 214 (20), 213 (100). Anal. Calcd for $C_{19}H_{16}O_9SFe_2$ (532.09): C, 42.89; H, 3.03. Found: C, 42.64; H, 3.00.

***trans*-μ-[(1*RS*,2*RS*,3*SR*,5*RS*,6*SR*,7*RS*)-*C*,2,3,*C*-η:*C*,6,7,*C*-η-(2,3,6,7-Tetrakis(methylene)bicyclo[3.2.1]octane)]bis(tricarbonyliron) (3).** $NaBH_4$ (55 mg, 1.4 mmol) was dissolved in $(CF_3)_2CHOH$ (2 mL). The mesylate **2** (20 mg, 0.040 mmol) was added and the mixture stirred at 20 °C for 8 min. After filtration on silica gel (1 g), the solvent was evaporated in vacuo and the residue purified by column chromatography on silica gel (1 g, hexane), yielding 12 mg (73%), yellow crystals after recrystallization from hexane: mp 98–99 °C; UV (isooctane) 285 (sh, 5900); IR (KBr) 2990, 2960, 2920, 2040, 1990, 1970, 1955, 1470, 1450, 1440, 1420, 1285, 1260, 1250, 1230, 1190, 1020, 1010, 950, 925, 890, 800, 730, 715; ^{1}H NMR ($CDCl_3$) 3.17 (dd, J = 17, 4.5, HC(4-exo)), 3.05 (d, J = 4.5, HC(1)), 2.90 (t, J = 4.5, HC(5)), 2.75 (dt, J = 11, 4.5 HC(8-anti)), 2.70 (d, J = 17, HC(4-endo)), 2.36 (d, J = 11, HC(8-syn)), 2.07, 1.90, 1.89, 1.57, 0.58, 0.56, 0.54, 0.07 (8 d, J = 3); ^{13}C NMR ($CDCl_3$) 115.7, 109.0, 108.7, 101.5 (4 s), 43.3 (d, 146, C(1)), 41.3 (t, 134, C(4)), 39.2 (d, 146, C(5)), 41.1, 36.5, 35.9 and 35.4 (t, 161), 33.3 (t, 130, C(8)); MS (70 eV), m/e (relative intensity) 438 (4), 410 (25), 408 (7), 382 (63), 380 (15), 354 (56), 352 (15), 326 (13), 298 (74), 296 (13), 270 (100), 268 (28), 244 (9), 242 (17), 240 (9), 214 (48), 212 (19), 210 (9). Anal. Calcd for $C_{18}H_{14}O_6Fe_2$ (438.00): C, 49.36; H, 3.22. Found: 49.40; H, 3.28.

Tricarbonyl[(1*RS*,2*RS*,3*SR*,5*RS*)-*C*,2,3,*C*-η-(2,3,6,7-tetrakis(methylene)bicyclo[3.2.1]octane)]iron (4). A mixture of **3** (150 mg, 0.34 mmol) and freshly sublimed trimethylamine oxide (400 mg, 5.33 mmol) in anhydrous acetone (38 mL) was stirred at 20 °C for 3 h. After removal of the precipitate by filtration, H_2O (50 mL) was added and the mixture extracted with hexane (50 mL, 3 times). After drying ($MgSO_4$), the solvent was evaporated in vacuo and the residue purified by column chromatography on silica gel (3 g, CH_2Cl_2): yield 90 mg (88%); yellow oil (dried over P_2O_5 and paraffin); UV (EtOH 95%) 307 (sh, 1400) 218 (18 600); IR (CH_2Cl_2) 3085, 2960, 2900, 2890, 2880, 2840, 2040, 1980, 1790, 1630, 1470, 1455, 1420, 1330, 1195, 1130, 1115, 1040, 1020, 960, 895, 885, 850; ^{1}H NMR ($CDCl_3$) 5.43, 5.13, 5.08 and 4.77 (4 s), 3.21 (d, J = 4, C(1)), 3.15 (t, J = 5, HC(5)), 3.9 (dd, J = 16, 5, HC(4-exo)), 2.46 (d, J = 16, HC(4-endo)), 2.06 (dddd, J = 11, 5, 4.5, 1, HC(8-anti)), 2.02 (d, J = 11, HC(8-syn)), 1.75, 1.39, 0.34 and 0.16 (4 d, J = 3); ^{13}C NMR ($CDCl_3$) 154.0, 151.4, 110.3 (3 s), 105.1 and 101.9 (2 t, 158), 99.8 (s), 46.3 (d, 142, C(1)), 40.7 (d, 142, C(5)), 39.8 (t, 158), 39.7 (t, 130, C(4)), 36.3 (t, 134, C(8)), 34.3 (t, 159); MS (70 eV), m/e (relative intensity) 298 (1), 270 (5), 242 (36), 214 (100), 212 (18), 158 (16), 148 (19), 91 (17). Anal. Calcd for $C_{15}H_{14}O_3Fe$ (298.124): C, 60.43; H, 4.74. Found: C, 60.56, H, 4.85.

2,3,6,7-Tetrakis(methylene)bicyclo[3.2.1]octane (5). $Fe(NO_3)_3$ (800 mg, 1.98 mmol) was added portionwise to a stirred solution of complex **3** (50 mg, 0.11 mmol) in CH_2Cl_2 (0.1 mL) and EtOH (0.9 mL). After the end of the addition (90 min), the precipitate was filtered off on silica gel (1 g) and H_2O (20 mL) was added. The mixture was extracted with hexane (50 mL, 3 times). After drying ($MgSO_4$) the extract, the solvent was evaporated in vacuo and the rsidue purified by column chromatography on silica gel (1 g, hexane), yielding 13 mg (72%), colorless oil: UV (EtOH 95%) 259 (sh, 5400), 249 (sh, 8350), 238 (9450), 224 (9600); UV (isooctane) 259 (sh, 5600), 246 (sh, 9200), 239 (9900), 223 (10 000); IR (CH_2Cl_2) 3080, 2960, 2900, 2880, 1790, 1630, 1620, 1460, 1420, 1380, 1190, 1125, 1110, 1040, 1020, 970, 895, 885, 845; ^{1}H NMR ($CDCl_3$) 5.40, 5.35, 4.98 and 4.9 (4 s, H_2C=C(6) and H_2C=C(7)), 5.19 and 4.76 (2 br t, J = 2.5, H_2C=C(3)), 4.94 and 4.69 (2 br d, J = 2, H_2C=C(2)), 3.34 (d, J = 5, HC(1)), 2.92 (ddd, J = 5, 3, 2.6, HC(5)), 2.60 (ddt, J = 14, 3, 2.5, HC(4-exo)), 2.37 (dt, J = 14, 2.6, HC(4-endo)), 1.95 (dtd, J = 11, 5, 2.6, HC(8-anti)), 1.70 (d, J = 11, HC(8-syn)); ^{13}C NMR ($CDCl_3$) 152.4, 150.8, 150.6 and 143.4 (4 s), 112.0, 104.6, 103.5 and 103.4 (4 t, 157), 52.1 (d, 144, C(1)), 42.7 (d, 142, C(5)), 42.0 (t, 130, C(4)), 37.6 (t, 134, C(8)); MS (70 eV); m/e (relative intensity) 158 (71), 143 (67), 142 (29), 141 (25), 129 (79), 128 (93), 127 (31), 117 (43), 115 (64), 105 (32), 104 (31), 103 (28), 101 (23), 91 (100).

10,11-Bis(methylene)tricyclo[7.2.1.0^{2,7}]dodec-2(7)-ene-4,4,5,5-tetracarbonitrile (7). A mixture of tetraene **5** (13 mg, 0.08 mmol) and TCNE (11 mg, 0.08 mmol) in anhydrous benzene (1 mL) was stirred at 20 °C for 20 min. The solvent was evaporated in vacuo and the residue recrystallized from CH_2Cl_2/hexane; yield 22 mg (93%), white solid, mp 167–170 °C dec: UV (EtOH 95%) 239 (7100); UV (isooctane) 244 (6400), 238 (6400), 226 (6000), 221 (6000); IR (KBr) 3090, 2990, 2960, 2910, 2890, 2830, 2250, 1620, 1460, 1440, 1425, 1260, 1235, 1200, 1175, 1130, 1100, 1085, 1020, 1010, 895, 885, 820; ^{1}H NMR ($CDCl_3$) 5.45, 5.18, 5.12, 4.83 (4 s), 3.20–2.80 (m, H_2C(3) and H_2C(6)), 3.04 (m, HC(9)), 2.82 (dd, J = 4, 1, HC(1)), 2.54 (m, HC(8-exo)), 2.02 (m, HC(8-endo)), 1.91 (dddd, J = 11, 5, 4.5, 1, HC(12-anti)), 1.79 (d, J = 11, HC(12-syn)); MS (70 eV), m/e (relative intensity) 286 (100), 272 (42), 260 (26), 259 (30), 245 (22), 244 (32), 231 (24), 221 (25), 206 (51). Anal. Calcd for $C_{18}H_{14}N_4$ (286.337): C, 75.51; H, 4.93. Found: C, 75.51; H, 5.04.

Tetracyclo[7.6.1.0^{2,7}.0^{10,15}]hexadeca-2(7),10(15)-diene-4,4,5,5,12,12,13,13-octacarbonitrile (8). A mixture of **5** (15 mg, 0.09 mmol), TCNE 24 (mg, 0.19 mmol), and anhydrous benzene (1 mL) was stirred at 20 °C for 20 min. The solvent was evaporated in vacuo and the residue recrystallized from acetone, yield 37 mg (94%), white solid, mp >240 °C dec: UV (EtOH 95%) final abs. ϵ_{210} 7100; IR (KBr) 2990, 2950, 2890, 2830, 2820, 2260, 1710, 1650, 1440, 1310, 1290, 1245, 1230, 1140, 1130, 1100, 1050, 1005, 960, 840, 820; ^{1}H NMR (CD_3COCD_3) 3.78–3.44 (m, 6 H), 3.29–3.02 (m, 2 H), 2.98 (t, J = 5, HC(9)), 2.93 (d, J = 5, HC(1)), 2.46 (m, HC(8)), 2.26 (m, HC(16)), 2.12 (m, HC(8)), 1.92 (d, J = 16, HC(16)); MS (70 eV), m/e (relative intensity) 414 (58), 388 (87), 360 (100), 334 (32), 308 (23), 286 (50), 271 (40), 250 (30). Anal. Calcd for $C_{24}H_{14}N_8$ (414.43): C, 69.56; H, 3.41. Found: C, 69.34; H, 3.49.

Acknowledgment. We thank the Swiss Science Foundation, the Herbette Foundation, Lausanne, and Hoffmann-La Roche & Co., AG, Basel, for generous financial support.

Registry No. 1 (Z = CH = CH), 62234-75-7; 1 (Z = CH_2C-H(OH), 87514-93-0; **2**, 102262-09-9; **3**, 102262-10-2; **4**, 102262-11-3; **5**, 102284-76-4; **7**, 102284-77-5; **8**, 102284-78-6.

Tosylation of Alcohols

George W. Kabalka,* Manju Varma, and Rajender S. Varma

Department of Chemistry, University of Tennessee, Knoxville, Tennessee 37996-1600

Prem C. Srivastava and Furn F. Knapp, Jr.

Nuclear Medicine Group, Health and Safety Research Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

Received January 13, 1986

The tosylation (sulfonylation) of alcohols is a common transformation which is often used to facilitate subsequent

0022-3263/86/1951-2386$01.50/0

Table I. Tosylation of Decyl Alcohol[a]

entry	ROH[b] (mmol)	p-CH_3Ph-SO_2Cl (mmol)	C_6H_5N (mmol)	yield[c] (%)	alcohol recovered (%)
1	10	10	10	84	14
2	10	10	20	88	11
3	10	10	40	85	14
4	10	15	10	78	20
5	10	15	20	98	
6	10	20	20	97	
7	10	20	40	97	

[a] *p*-Toluenesulfonyl chloride was added, in portions, to an ice cold (0 °C) solution of alcohol in chloroform and pyridine. [b] Decanol. [c] Isolated yield of chromatographically pure product.

Table II. Tosylation of Alcohols in Chloroform Solution[a]

entry	alcohol	time (h)	yield[b] of arenesulfonates (%)
1	decyl alcohol	2.5	98
2	10-undecen-1-ol	2.5	92
3	10-undecyn-1-ol	3.0	93
4	12-tridecyn-1-ol	3.0	89
5	4-undecyn-1-ol	5.0 (3.0)[c]	87 (85)[c]
6	4-*tert*-butylcyclohexanol	9.0 (6.5)[c]	93 (92)[c]
7	4-hexyn-2-ol	9.5 (7.0)[c]	97 (94)[c]

[a] Using 1:1.5:2 ratio of alcohol/*p*-toluenesulfonyl chloride/pyridine. [b] Isolated yield of chromatographically pure product. [c] Using 1:2:3 ratio of alcohol/*p*-toluenesulfonyl chloride/pyridine.

nucleophillic substitution reactions. A number of alcohols have been routinely converted to the corresponding arenesulfonates by using a variety of conditions,[1a-f] yet the preparation is not troublefree. As an example, we and others[1a,2,3] have observed the formation of pyridinium salts in routine tosylation reactions in which pyridine is used as a base resulting in a concommitant loss of the desired tosylate.

We recently developed a synthesis of a series of steroidal ethers[4] and iodoalkynes[5] which involved the intermediacy of tosylate derivatives. We found that the tosylation of 12-tridecyn-1-ol in pure pyridine using 2 equiv of tosyl chloride yielded only 50% of the expected mass balance. Furthermore, the recovered material was a mixture of the desired tosylate and the corresponding chloride. Presumably, the remainder of the material was lost as the pyridinium salt during workup; the formation of such salts have been reported previously.[3] These results prompted us to more carefully investigate the tosylation reaction. We used decyl alcohol as the model compound for optimizing the reaction conditions.

We report that the highest yields of pure tosylates based on starting alcohol[6] are obtained using a 1:1.5:2 ratio of alcohol/tosyl chloride/pyridine in chloroform (entry 5 in Table I). Primary alcohols react completely within 3 h whereas secondary alcohols require longer reaction times (~7 h). However, the reaction rate can be increased by using 1:2:3 ratio of alcohol/tosyl chloride/pyridine (entries 5, 6, and 7 in Table II). Our results are summarized in Tables I and II.

In initial experiments, we often recovered higher than theoretical mass balances of the tosylates. Careful observation revealed that an additional spot was always present on chromatographic analysis; the impurity appeared in close proximity to the desired tosylate on the TLC plate. This byproduct was present regardless of the alcohol used and could only be detected by careful, multiple development of the thin layer chromatograms. We identified the material as ethyl *p*-toluenesulfonate which was produced from the ethyl alcohol present as a stabilizer in the chloroform. Although, many of the tosylations reported in the literature were carried out by using chloroform as the solvent, we found no mention of this impurity being identified. Fortunately, simple filteration of the chloroform through a plug of alumina eliminates the problem and pure samples of the toluenesulfonates are obtained.

Experimental Section

Tosylation of Decyl Alcohol. General Procedure. Decyl alcohol (1.58 g, 10 mmol) was dissolved in chloroform (10 mL) and cooled in an ice bath (0 °C). Pyridine (1.62 mL, 20 mmol) was then added, followed by the addition of *p*-toluenesulfonyl chloride (2.85 g, 15 mmol) in small portions with constant stirring. The reaction was completed in 2.5 h (monitored by TLC). Ether (30 mL) and water (7 mL) were added and the organic layer was washed successively with 2 N HCl, 5% $NaHCO_3$, and water and then dried ($MgSO_4$). The solvent was removed under reduced pressure and the crude tosylate was column chromatographed (2% ether/petroleum ether) on a silica gel column to yield (3.06 g, 98%) an oil:[7] ^1H NMR ($CDCl_3$) δ 0.87 (t, 3 H, CH_3), 1.22 (br s, 14 H, alkane), 1.59 (m, 2 H, CH_2CH_2O), 2.44 (s, 3 H, CH_3), 4.02 (t, 2 H, CH_2O), 7.56 (AA'BB', 4 H, Ar H); ^{13}C NMR ($CDCl_3$) δ 144.46, 133.12, 129.65, 127.64 (Ar carbons) 70.52 (CH_2O), 31.70, 29.29, 29.24, 29.10, 28.75, 28.64, 25.17, 22.49, 21.38, and 13.94 (alkane).

10-Undecen-1-yl *p*-toluenesulfonate: following the general procedure described for decyl alcohol, from 5.73 g of 10-undecen-1-ol was obtained after purification by elution on silica gel with ether/petroleum ether (2:98 v/v) as the eluent 10.03 g (92% yield) of 10-undecen-1-yl *p*-toluenesulfonate as a thick oil.[4]

10-Undecyn-1-yl *p*-toluenesulfonate: yield 93%; thick oil; ^1H NMR ($CDCl_3$) δ 1.24 (br s, 12 H, alkane), 1.58 (m, 2 H, CH_2-CH_2O) 1.94 (t, 1 H, HC≡C), 2.17 (m, 2 H, $CH_2C\equiv$), 2.45 (s, 3 H, Ar CH_3), 4.02 (t, 2 H, CH_2O), 7.58 (AA'BB', 4 H, Ar H); ^{13}C NMR ($CDCl_3$) δ 144.63, 133.22, 129.78, 127.83 (Ar carbons), 84.63 (C≡CH), 70.65 (CH_2O), 68.16 (C≡CH), 29.18, 28.88, 28.78, 28.61, 28.39, 25.28, 21.59, and 18.35 (alkane). Anal. Calcd for $C_{18}H_{26}O_3S$: C, 67.08; H, 8.07; S, 9.94. Found: C, 66.91; H, 7.97; S, 10.14.

12-Tridecyn-1-yl *p*-toluenesulfonate: yield 89%; thick oil;[5] ^{13}C NMR ($CDCl_3$) δ 144.63, 133.31, 129.81, 127.89 (Ar carbons), 84.76 (C≡CH), 70.71 (CH_2O), 68.11 (C≡CH), 29.43, 29.07, 28.91, 28.83, 28.75, 28.51, 25.34, 21.65, and 18.4 (alkane).

4-Undecyn-1-yl *p*-toluenesulfonate: yield 87%; thick oil; ^1H NMR ($CDCl_3$) δ 0.88 (t, 3 H, CH_3), 1.30 (br s, 8 H, alkane), 1.81 (pentuplet, 2 H, CH_2CH_2O), 2.14 (m, 4 H, 2 × $CH_2C\equiv$), 2.43 (s, 3 H, Ar CH_3), 4.13 (t, 2 H, CH_2O), 7.53 (AA'BB', 4 H, Ar H); ^{13}C NMR ($CDCl_3$) δ 144.55, 132.98, 129.68, 127.75 (Ar carbons),

(1) (a) Sekera, V. C.; Marvel, C. S. *J. Am. Chem. Soc.* **1933**, *55*, 345. (b) Roos, A. T.; Gilman, H.; Beaber, N. J. In *Organic Synthesis*, 2nd ed.; Gilman, H., Ed.; Wiley: New York, 1941; Collect. Vol. I, pp 145–147. (c) Sekera, V. C.; Marvel, C. S. *Organic Synthesis*; Wiley: New York, 1955; Collect. Vol. III, pp 366–367. (d) Tipson, R. S. *J. Org. Chem.* **1944**, *9*, 235. (e) Newman, M. S.; Magerlein, B. J. *J. Am. Chem. Soc.* **1947**, *69*, 469. (f) Klamann, D.; Drahowzal, F. *Monatsh. Chem.* **1952**, *83*, 154 and references cited therein. (g) Dale, J.; Kristiansen, P. O. *Acta. Chem. Scand.* **1972**, *26*, 1471. (h) Quackenbush, F. W.; Grogan, W. M., Jr.; Midland, S. L.; Bell, F. P.; MacNintch, J. E.; Hutsell, T. C.; Cruzan, G.; Klauda, H. C. *Artery* **1977**, *3*, 553, *Chem. Abst.* **1978**, *89*, 283g.

(2) Ferns, J.; Lapworth, A. *J. Chem. Soc.* **1912**, *101*, 273.

(3) Nagpal, K. L.; Jain, P. C.; Srivastava, Dhar, M. M., Anand, N. *Ind. J. Chem.* **1968**, *6*, 762.

(4) Kabalka, G. W.; Varma, R. S.; Jinaraj, V. K.; Huang, L.; Painter, S. K. *J. Label. Compd. Radiopharm.* **1985**, *22*, 333.

(5) Knapp, F. F., Jr.; Srivastava, P. C.; Callahan, A. P.; Cunningham, E. B.; Kabalka, G. W.; Sastry, K. A. R. *J. Med. Chem.* **1984**, *27*, 57.

(6) In an exhaustive study, Klamann and Drahowzal[1f] noted that excess of alcohol was generally required to obtained good yield of aryl tosylates.

(7) Klamann, D.; Weiske, C. *Monatsh. Chem.* **1965**, *96*, 2025.

81.38 and 77.48 (alkyne carbons), 69.03 (CH_2O), 31.21, 28.80, 28.40, 28.21, 22.41, 21.44, 18.48, 14.86, and 13.91 (alkane). Anal. Calcd for $C_{18}H_{26}O_3S$: C, 67.08; H, 8.07; S, 9.94. Found: C, 66.96; H, 8.07; S, 10.01.

4-*tert*-Butylcyclohex-1-yl *p*-toluenesulfonate: yield 93%; obtained as semisolid[8] from mixture of isomers of 4-*tert*-butylcyclohexan-1-ol; ^{13}C NMR ($CDCl_3$) δ 144.06, 144.0, 134.58, 134.47, 129.49, 127.39 (Ar carbons), 82.11 and 78.86 (CHO), 46.79 and 46.25 (*CH*-*t*-Bu), 32.59, 32.16, 31.87, 30.97, 27.23, 27.12, 25.23, 22.36, 21.25, and 20.84 (alkane).

4-Hexyn-2-yl *p*-toluenesulfonate: yield 97%; thick oil; ^1H NMR ($CDCl_3$) δ 1.34 (d, 3 H, CH_3CH), 1.69 (d, J = 2.5 Hz, 3 H, $CH_3C\equiv$), 2.44 (s, 3 H, Ar CH_3), 4.60 (sextuplet, 1 H, CH), 7.56 (AA'BB', 4 H, Ar H); ^{13}C NMR ($CDCl_3$) δ 144.49, 133.93, 129.62, 127.54 (Ar carbons), 78.40 and 77.40 (alkyne carbons), 77.67 (CH), 26.53 (CH_2), 21.35, 19.84, and 3.16 (alkane). Anal. Calcd for $C_{13}H_{16}O_3S$: C, 61.90; H, 6.35; S, 12.70. Found: C, 61.58; H, 6.18; S, 12.72.

Acknowledgment. This work was supported by USPHS Grant HL-27012 from The National Institutes of Health and by the Office of Health and Environmental Research, U.S. Department of Energy, under contracts DE-AC05-84OR21400 and DE-AS05-80EV-10363.

(8) Winstein, S.; Holness, N. J. *J. Am. Chem. Soc.* **1955**, *77*, 5562. Winstein, S.; Lewin, A. H. *Ibid.* **1962**, *84*, 2464.

Biphasic One-Pot Synthesis of Two Useful and Separable Compounds Using Cofactor-Requiring Enzymatic Reactions: Glutamate Dehydrogenase Catalyzed Synthesis of L-α-Aminoadipate Coupled with Alcohol Dehydrogenase Catalyzed Synthesis of a Chiral Lactone

Jose R. Matos[1] and Chi-Huey Wong*[2]

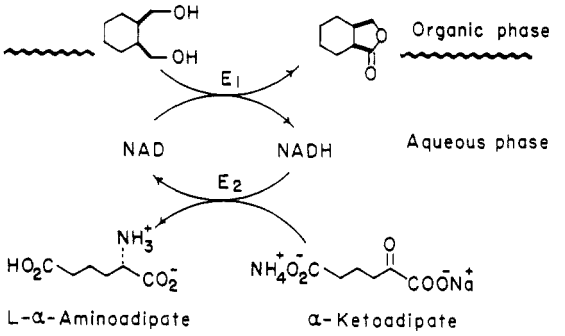
Department of Chemistry, Texas A&M University, College Station, Texas 77843

Received February 4, 1986

The most generally useful system available for in situ regeneration of NAD(P) from NAD(P)H for use in practical scale enzyme-catalyzed asymmetric oxidation is considered to be the system using 2-oxoglutarate/glutamate dehydrogenase (GluDH).[3,4] Several 70–100-mmol scale syntheses in aqueous solution or in a mixture of water and hexane have been demonstrated.[3,4] The regeneration system, however, requires a second enzyme, (GluDH), and 2-oxoglutarate, the glutamate product of which is not very useful, being currently produced in large quantities by fermentation, and also complicates workup.

We describe here an improved practical procedure which should reduce the cost substantially. This procedure involves an NAD-requiring asymmetric oxidation of a *meso*-diol catalyzed by horse liver alcohol dehydrogenase (HLADH) coupled with an NAD(P)H-requiring asymmetric reductive amination of 2-ketoadipate catalyzed by GluDH (Scheme I). Each of the two enzymatic reactions is *synthetically useful* and generates the proper form of the cofactor for the other. The reactions are carried out

Scheme I. Coupling of Two Nicotinamide Cofactor-Requiring Enzymatic Syntheses in a Biphasic System. E1, Horse Liver Alcohol Dehydrogenase; E2, Glutamate Dehydrogenase



in a water–organic solvent biphasic system where the chiral lactone produced is removed from the aqueous phase to separate from the other water soluble product (L-α-aminoadipate) and to minimize product inhibition.[5] The unnatural substrates used in the reactions are either readily available or easily prepared. The preparation of 2-ketoadipate is straightforward and the starting materials used for the preparation are relatively inexpensive. 2-Ketoadipate is a good substrate for GluDH[6] and the product L-α-aminoadipate produced is a component of a linear tripeptide used in the enzymatic synthesis of isopenicillin N and analogues, a precursor of penicillin antibiotics.[7] Although several methods have been described for the preparation of L-α-aminoadipate ($60/g from Sigma), each of them requires several steps and the procedures are quite tedious.[8]

The *meso*-diol used in this representative synthesis is just intended to illustrate the strategy. As shown in Jones work, a number of *meso*-diols have been enantioselectively oxidized to chiral lactones catalyzed by HLADH.[9] They should be good candidates for this system.

Although product inhibition is a severe problem in large-scale HLADH-catalyzed oxidation of alcohols, it can be lessened using *meso*-diols as substrates. The favorable kinetic parameters ($K_m/K_i < 1$) for the diols together with the use of a biphasic system to minimize product inhibition and to accomplish good separation and high turnover numbers for the cofactor and the enzymes allow preparation of chiral lactones on large scales.[3] Further, the NAD regeneration system produces a useful and separable product concurrently and thus reduces the operation cost significantly. The cofactor used in the process is no longer an expensive component.

The synthetic methodology illustrated here should be compatible with that based on non-cofactor-requiring lipase-catalyzed hydrolysis of *meso*-diol diesters which produces products with lower optical purity (67–87%) and requires another chemical step to prepare the lactone.[10]

(1) NSF Graduate Fellow, 1983–1987.
(2) Searle Scholar (1985–1988), recipient of NSF Presidential Young Investigator Award (1986–1991).
(3) Lee, L. G.; Whitesides, G. M. *J. Am. Chem. Soc.* **1985**, *107*, 6999.
(4) Wong, C.-H.; MCurry, S. D.; Whitesides, G. M. *J. Am. Chem. Soc.* **1980**, *102*, 7938. Wong, C.-H.; Pollak, A.; McCurry, S. D.; Sue, J. M.; Knowles, J. R.; Whitesides, G. M. *Method. Enzymol.* **1982**, *89*, 108.
(5) Refer to ref 3 for a detail study on product inhibition and its effect on large-scale enzymatic synthesis.
(6) The specific activity of GluDH using 2-oxoglutarate is about 20–30 U/mg.
(7) Wolfe, S.; Demain, A. L.; Jensen, S. E.; Westlake, D. W. S. *Science (Washington, D.C.)* **1985**, *226*, 1386. Konomi, T.; Herchen, S.; Baldwin, J. E.; Yoshida, M.; Hunt, N. H.; Demain, A. L. *Biochem. J.* **1979**, *184*, 427. Bahadur, G. A.; Baldwin, J. E.; Usher, J. J.; Abraham, E. P.; Jayatilake, G. S.; White, J. *J. Am. Chem. Soc.* **1981**, *103*, 7650. Baldwin, J. E.; Adlington, R. M.; Basak, A.; Flitsch, S. L.; Forrest, A. K.; Ting, H. H. *J. Chem. Soc., Chem. Commun.* **1986**, 273.
(8) Hartline, R. A. *Methods Enzymol.* **1985**, *113*, 639. Scott, A. I.; Wilkinson, T. J. *Synth. Commun.* **1980**, *10*, 127. Wolfe, S.; Johinen, M. G. *Can. J. Chem.* **1979**, *57*, 1388. Wood, T. G.; Hartline, R. A. *Anal. Biochem.* **1971**, *43*, 282.
(9) Lok, K. P.; Jakovac, I. J.; Jones, J. B. *J. Am. Chem. Soc.* **1985**, *107*, 2521 and references cited therein.

0022-3263/86/1951-2388$01.50/0