APPENDICES

APPENDIX A

Table A.1 Calibration curve of NAM in 0.01 M phosphate buffer, pH 7 containing 10% EtOH

[NAM] mM	Absorbance (λ_{max} = 262 nm)			
	1st	2nd	3rd	Average
0.0625	0.2496	0.2495	0.2502	0.2497
0.125	0.4545	0.4456	0.4669	0.4557
0.25	0.8621	0.8712	0.8741	0.8691
0.50	1.6889	1.6738	1.6895	1.6841
0.75	2.4896	2.4907	2.4902	2.4901

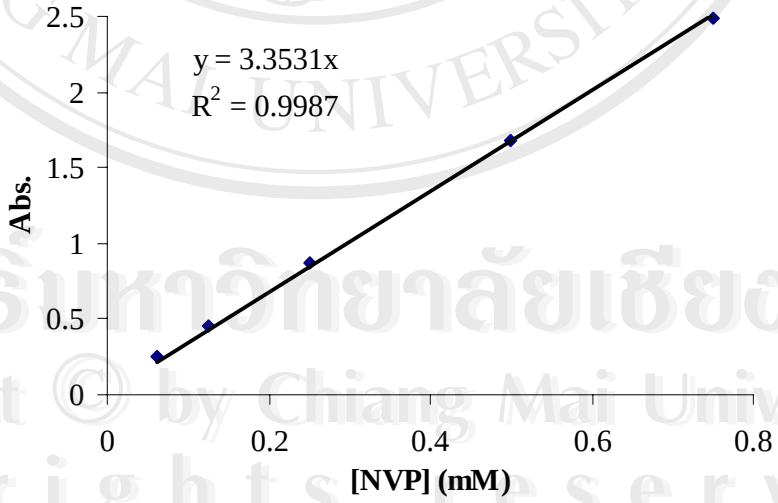

Figure A.1 Calibration curve of NAM in 0.01 M phosphate buffer, pH 7 containing 10% EtOH

Table A.2 Calibration curve of NAM in ACN

[NAM] mM	Absorbance (λ_{max} = 262 nm)			
	1st	2nd	3rd	Average
0.0625	0.1731	0.1802	0.1798	0.1777
0.125	0.3348	0.3401	0.3304	0.3351
0.25	0.6492	0.6549	0.6558	0.6533
0.50	1.2711	1.2697	1.2705	1.2704
0.75	1.8510	1.8501	1.8496	1.8502

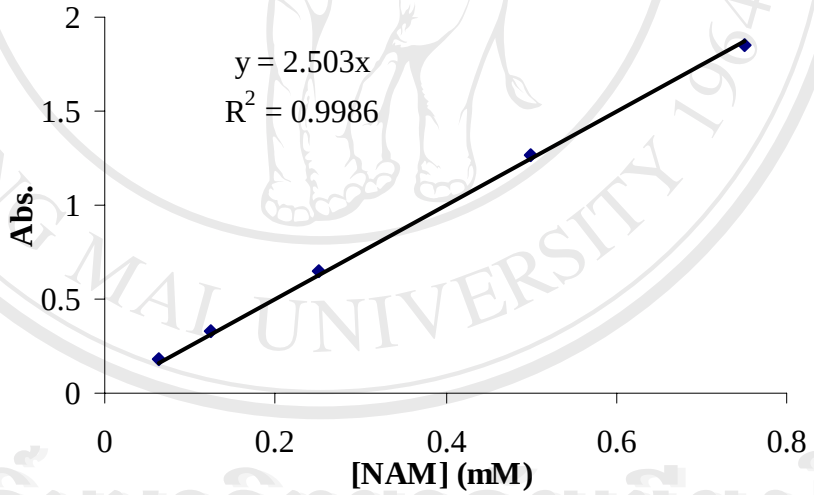

Figure A.2 Calibration curve of NAM in ACN

Table A.3 Calibration curve of NVP in 0.01 M phosphate buffer, pH 7 containing 10% EtOH

[NVP] mM	Absorbance (λ_{max} = 281 nm)			
	1st	2nd	3rd	Average
0.025	0.1877	0.1858	0.1799	0.1845
0.05	0.3957	0.4003	0.3968	0.3976
0.10	0.7496	0.7512	0.7541	0.7516
0.20	1.3721	1.3759	1.3685	1.3722
0.25	1.7097	1.7254	1.7100	1.7150

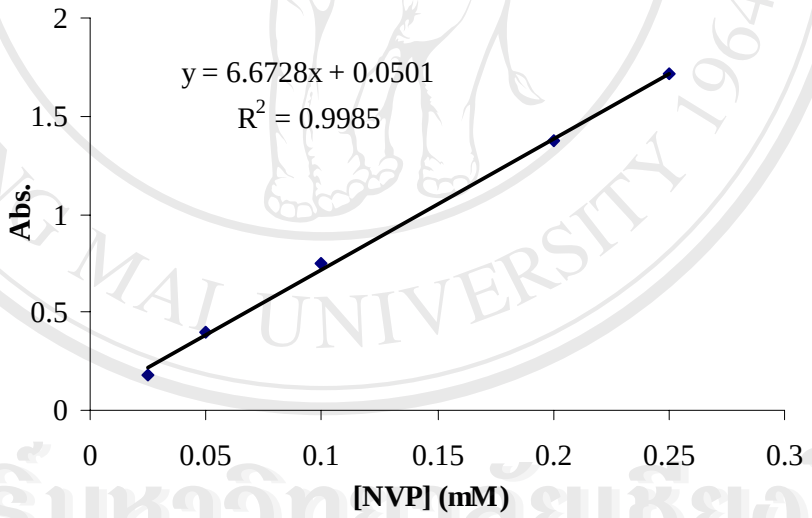

Figure A.3 Calibration curve of NVP in 0.01 M phosphate buffer, pH 7 containing 10% EtOH

Table A.4 Calibration curve of NVP in ACN

[NVP] mM	Absorbance (λ_{max} = 281 nm)			
	1st	2nd	3rd	Average
0.025	0.1886	0.1901	0.1986	0.1924
0.05	0.3832	0.3781	0.3832	0.3815
0.10	0.7408	0.7415	0.7482	0.7435
0.20	1.5441	1.5544	1.5359	1.5448
0.25	1.8508	1.8489	1.8522	1.8506

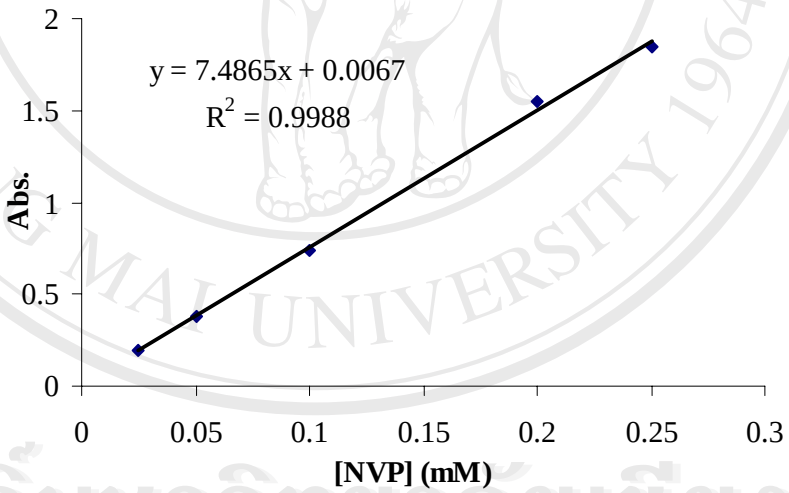

Figure A.4 Calibration curve of NVP in ACN

Table A.5 Rebinding of 0.5 mM NAM in ACN with polymers (P1-P11); polymer 40.0 mg/ml

Polymer	Absorbance (λmax = 262 nm)				% Bound	SD
	1st	2nd	3rd	Average		
P1	0.2434	0.1539	0.2216	0.2063	16.48	0.0466
P1'	0.0784	0.0674	0.0854	0.0770	6.17	0.0090
P2	0.1601	0.1016	0.1186	0.1267	10.12	0.0300
P2'	0.0642	0.0752	0.0685	0.0693	5.53	0.0055
P3	0.4551	0.4461	0.473	0.4580	36.60	0.0136
P3'	0.1204	0.1452	0.2031	0.1562	12.48	0.0424
P4	0.5101	0.5904	0.528	0.5428	43.37	0.0421
P4'	0.2085	0.2618	0.2593	0.2432	19.43	0.0300
P5	0.2596	0.2265	0.204	0.2300	18.38	0.0279
P5'	0.0659	0.0743	0.1064	0.0822	6.56	0.0213
P6	0.3763	0.5319	0.3156	0.4079	32.59	0.1115
P6'	0.1464	0.1753	0.2068	0.1761	14.07	0.0302
P7	0.1377	0.0864	0.0792	0.1011	8.078	0.0319
P7'	0.1061	0.063	0.0578	0.0756	6.043	0.0265
P8	0.8426	0.875	0.7647	0.8274	66.11	0.0566
P8'	0.3198	0.3798	0.265	0.3215	25.69	0.0574
P9	0.7167	0.7681	0.8023	0.7623	60.91	0.0430
P9'	0.4232	0.4899	0.5064	0.4731	37.80	0.0440
P10	0.6658	0.5182	0.5246	0.5695	45.50	0.0834
P10'	0.4298	0.3688	0.3805	0.3930	31.40	0.0323
P11	0.8654	0.8483	0.8952	0.8696	69.48	0.0237
P11'	0.4609	0.3781	0.4809	0.4399	35.15	0.0545

Table A.6 Rebinding of 0.5 mM NAM in 0.01 M phosphate buffer pH 7 containing 10% EtOH with polymers (P1-P11); polymer 40.0 mg/ml

Polymer	Absorbance (λmax = 262 nm)				% Bound	SD
	1st	2nd	3rd	Average		
P1	0.8367	0.7889	0.8641	0.8299	49.50	0.0380
P1'	0.6611	0.6945	0.7164	0.6906	41.19	0.0154
P2	0.6387	0.6041	0.5882	0.6103	36.40	0.0258
P2'	0.5912	0.6082	0.5624	0.5872	35.05	0.0231
P3	0.8872	0.89453	0.8575	0.8797	52.47	0.0196
P3'	0.7950	0.7981	0.7501	0.7810	46.60	0.0268
P4	0.8462	0.8955	0.9325	0.8914	53.16	0.0432
P4'	0.6532	0.6345	0.697	0.6615	39.44	0.0320
P5	0.6745	0.6746	0.647	0.6653	39.58	0.0159
P5'	0.6385	0.6177	0.5874	0.6145	36.86	0.0256
P6	0.7814	0.7645	0.7201	0.7553	44.99	0.0316
P6'	0.7045	0.6241	0.671	0.6660	39.80	0.0403
P7	0.8157	0.8045	0.7205	0.7802	46.54	0.0520
P7'	0.6224	0.6711	0.7140	0.6691	39.958	0.0458
P8	1.3514	1.3752	1.432	1.3862	82.67	0.0414
P8'	1.2410	1.0273	1.1800	1.1494	68.45	0.1100
P9	0.9254	1.1177	0.854	0.9657	57.47	0.1363
P9'	0.7511	0.8115	0.9245	0.8290	49.48	0.0882
P10	0.7684	0.7452	0.7100	0.7412	44.20	0.0294
P10'	0.8121	0.6771	0.6452	0.7114	42.41	0.0885
P11	1.4058	1.3480	1.3232	1.3590	81.06	0.0423
P11'	0.8067	0.9566	1.0187	0.9273	55.31	0.1089

Table A.7 Rebinding of 0.5 mM NAM in 0.01 M phosphate buffer pH 7 containing 10% EtOH with polymers (P1-P11); polymer 20.0 mg/ml

Polymer	Absorbance (λmax = 262 nm)				% Bound	SD
	1st	2nd	3rd	Average		
P1	0.8367	0.7889	0.8641	0.8299	49.50	0.0380
P1'	0.6511	0.7045	0.7164	0.6906	41.19	0.0084
P2	0.6387	0.6041	0.5882	0.6103	36.40	0.0258
P2'	0.5912	0.6082	0.5624	0.5872	35.05	0.0231
P3	0.8872	0.8945	0.8575	0.8797	52.47	0.0196
P3'	0.7950	0.7981	0.7501	0.7810	46.60	0.0268
P4	0.8362	0.9055	0.9325	0.8914	53.16	0.0496
P4'	0.6532	0.6345	0.6970	0.6615	39.44	0.0320
P5	0.6745	0.6746	0.6470	0.6653	39.58	0.0159
P5'	0.6385	0.6177	0.5874	0.6145	36.86	0.0256
P6	0.7814	0.7645	0.7201	0.7553	44.99	0.0316
P6'	0.7045	0.6441	0.6510	0.6665	39.80	0.0330
P7	0.8157	0.8045	0.7205	0.7802	46.54	0.0520
P7'	0.6224	0.6711	0.7140	0.6697	39.95	0.0458
P8	1.3514	1.3952	1.4120	1.3862	82.67	0.0312
P8'	1.2401	1.0273	1.1800	1.1494	68.45	0.1100
P9	0.9254	1.1177	0.8540	0.9657	57.47	0.1363
P9'	0.7511	0.8515	0.8845	0.8290	49.48	0.0694
P10	0.7684	0.7452	0.7100	0.7412	44.20	0.0294
P10'	0.8121	0.6771	0.6452	0.7114	42.41	0.0885
P11	1.4058	1.3480	1.3232	1.3590	81.06	0.0423
P11'	0.8067	0.9566	1.0187	0.9273	55.31	0.1089

Table A.8 Rebinding of 0.2 mM NVP in ACN with polymers (P12-P16); polymer 20.0 mg/ml

Polymer	Absorbance (λmax = 281 nm)				% Bound	SD
	1st	2nd	3rd	Average		
P12	0.3217	0.3001	0.3089	0.3102	20.27	0.0108
P12'	0.0742	0.1292	0.1047	0.1027	6.41	0.0275
P13	0.4540	0.4398	0.4563	0.4500	29.60	0.0211
P13'	0.3781	0.3494	0.3542	0.3605	23.63	0.0039
P14	0.2160	0.1760	0.2081	0.2000	12.91	0.0089
P14'	0.1208	0.1135	0.1197	0.1180	7.43	0.0153
P15	0.5506	0.4500	0.5479	0.5161	34.02	0.0573
P15'	0.2224	0.1629	0.1886	0.1913	12.33	0.0298
P16	0.3223	0.2605	0.3195	0.3007	19.64	0.0348
P16'	0.1636	0.1699	0.1753	0.1696	10.88	0.0058

Table A.9 Rebinding of 0.2 mM NVP in 0.01 M phosphate buffer pH 7 containing 10% EtOH with polymers (P12-P16); polymer 20.0 mg/ml

Polymer	Absorbance (λmax = 281 nm)				% Bound	SD
	1st	2nd	3rd	Average		
P12	1.1602	1.1651	1.1765	1.1672	83.73	0.0083
P12'	1.0250	1.0719	1.2467	1.1145	79.65	0.1168
P13	0.8645	0.81454	0.8011	0.8267	58.18	0.0334
P13'	0.7335	0.7025	0.7054	0.7138	49.73	0.0171
P14	1.1914	1.0991	1.1784	1.1563	82.86	0.0499
P14'	0.9441	1.2457	1.0725	1.0874	77.72	0.1513
P15	1.2112	1.1914	1.1077	1.1701	83.92	0.0549
P15'	0.4821	0.5704	0.5848	0.5457	37.21	0.0556
P16	1.2442	1.1825	1.1904	1.2057	86.59	0.0335
P16'	0.8414	0.8503	0.7914	0.8277	58.08	0.0317

Table A.10 Rebinding of 0.2 mM NVP in 0.01 M phosphate buffer pH 7 containing 10% EtOH with polymers (P12-P16); polymer 5.0 mg/ml

Polymer	Absorbance (λmax = 281 nm)				% Bound	SD
	1st	2nd	3rd	Average		
P12	0.6432	0.7414	0.7522	0.7122	49.64	0.0600
P12'	0.5231	0.5518	0.4304	0.5017	33.82	0.0634
P13	1.018	0.7985	0.8293	0.8819	62.32	0.1188
P13'	0.6921	0.7582	0.7285	0.7262	50.65	0.0331
P14	0.4527	0.3074	0.3759	0.3786	24.58	0.0726
P14'	0.2254	0.2694	0.3428	0.2792	17.16	0.0593
P15	0.9224	0.9514	1.0240	0.9659	68.55	0.0523
P15'	0.2731	0.2480	0.1953	0.2388	14.13	0.0397
P16	0.8651	0.7895	0.7965	0.8170	57.51	0.0417
P16'	0.3921	0.3558	0.2956	0.3478	22.31	0.0487

Table A.11 Selectivity of P11, P15 and NIPs

Compounds	λ_{max}	% Bound		
		P15	P11	NIPs
NVP	281	88.95	79.08	26.21
NAM	262	71.76	14.72	14.11
BAM	227	33.82	31.16	27.07
2-Apy	298	22.63	27.45	22.33
3-Apy	246	11.08	7.91	24.17
4-Apy	262	16.92	22.11	15.93
Pyridine	256	7.63	12.15	17.32
Aniline	230	42.66	40.76	40.45
OPD	231	34.68	29.56	33.99

Table A.12 Percent bound of NVP-HRP conjugate to P11, P15 and NIPs with and without tween 20

% tween 20	polymer	Absorbance at 450 nm				%Bound
		1st	2nd	3rd	Average	
no tween 20	no	1.3568	1.3154	1.3745	1.3489	
	P11	1.2270	1.3010	1.2504	1.25947	93.36
	P15	1.3102	1.2651	1.2908	1.2887	95.53
	NIP	1.1742	1.3012	1.2594	1.2449	92.29
0.05%	P11	0.6415	0.6978	0.65122	0.6635	49.18
	P15	0.6845	0.7492	0.7654	0.7330	54.34
	NIP	0.0824	0.0534	0.0452	0.0603	4.47

Table A.13 Binding of NVP–HRP relative to polymer concentration for P11, P15, and NIP in buffer pH 6

	polymer (mg/ml)	Absorbance at 450 nm				%Bound
		1st	2nd	3rd	Average	
P11	no	1.3882	1.3985	1.3961	1.3942	
	0.4	0.0522	0.0485	0.0844	0.0617	4.42
	0.6	0.2022	0.1248	0.1098	0.1456	10.44
	0.8	0.7012	0.6144	0.6041	0.6399	45.89
	1.0	1.1346	1.2012	1.1254	1.1537	82.75
	2.0	1.287	1.2554	1.2694	1.2706	91.13
	3.0	1.254	1.3645	1.3254	1.3146	94.29
	4.0	1.2703	1.3024	1.3052	1.2926	92.71
P15	0.4	0.0597	0.1054	0.0605	0.0752	5.39
	0.6	0.2303	0.3027	0.2010	0.2446	17.54
	0.8	0.7652	0.6956	0.7085	0.7231	51.86
	1.0	1.1812	1.2125	1.3011	1.2316	88.33
	2.0	1.2870	1.3054	1.2494	1.2806	91.85
	3.0	1.2885	1.2945	1.3254	1.3028	93.44
	4.0	1.2803	1.3241	1.3552	1.3259	95.10
NIP	0.4	0.0687	0.0598	0.0567	0.0617	4.42
	0.6	0.0489	0.05874	0.0524	0.0533	3.82
	0.8	0.0887	0.0718	0.0537	0.0714	5.12
	1.0	0.1909	0.07274	0.0854	0.1163	8.34
	2.0	0.0754	0.1484	0.0885	0.1041	7.46
	3.0	0.1541	0.1112	0.2944	0.1865	13.38
	4.0	0.1754	0.3024	0.1421	0.2066	14.82

Table A.14 Binding of NVP–HRP relative to polymer concentration for P11, P15, and NIP in buffer pH 7

	polymer (mg/ml)	Absorbance at 450 nm				%Bound
		1st	2nd	3rd	Average	
P11	no	1.4351	1.4921	1.4642	1.4638	
	2	0.0856	0.1211	0.1147	0.1071	7.31
	4	0.1966	0.1785	0.1634	0.1795	12.26
	6	0.2514	0.2765	0.3528	0.2935	20.05
	8	0.8024	0.7695	0.7525	0.7748	52.93
	10	1.3091	1.1170	1.2454	1.2238	83.60
	15	1.3612	1.2918	1.2717	1.3082	89.37
P15	2	0.1227	0.1694	0.1643	0.1521	10.39
	4	0.3561	0.24554	0.2654	0.2890	19.74
	6	0.4387	0.3554	0.3428	0.3789	25.88
	8	0.86612	1.1274	0.9043	0.9659	65.98
	10	1.2960	1.1858	1.1143	1.1987	81.88
	15	1.3965	1.3368	1.3040	1.3457	91.93
NIP	2	0.0356	0.0670	0.0647	0.0557	3.80
	4	0.0754	0.0813	0.0518	0.0695	4.74
	6	0.07142	0.0487	0.0754	0.0651	4.45
	8	0.1565	0.1180	0.0852	0.1199	8.19
	10	0.1654	0.12895	0.1845	0.1596	10.90
	15	0.2684	0.1146	0.1740	0.1856	12.68

Table A.15 Binding of NVP–HRP relative to polymer concentration for P11, P15, and NIP in buffer pH 8

	polymer (mg/ml)	Absorbance at 450 nm (B)				%Bound
		1st	2nd	3rd	Average	
P11	no	1.4045	1.4013	1.3894	1.3984	
	2	0.1243	0.2104	0.1025	0.1457	10.42
	4	0.1514	0.1458	0.2245	0.1739	12.43
	6	0.3440	0.19265	0.2484	0.2616	18.71
	8	0.1879	0.2552	0.2181	0.2204	15.76
	10	0.2264	0.2550	0.3987	0.2933	20.97
	15	0.2270	0.2787	0.2844	0.2633	18.83
P15	2	0.2584	0.2224	0.1785	0.2197	15.71
	4	0.19654	0.3054	0.1918	0.2312	16.53
	6	0.3931	0.2176	0.2584	0.2897	20.71
	8	0.2130	0.2878	0.4048	0.3018	21.58
	10	0.3025	0.2674	0.2621	0.2773	19.83
	15	0.3346	0.3064	0.2350	0.2920	20.88
NIP	2	0.1141	0.1254	0.2525	0.1640	11.72
	4	0.1540	0.0827	0.1245	0.1204	8.60
	6	0.0925	0.2017	0.1174	0.1372	9.81
	8	0.218	0.1948	0.1585	0.1904	13.61
	10	0.1452	0.1344	0.1477	0.1424	10.18
	15	0.2244	0.1650	0.1524	0.1806	12.91

Table A.16 The binding of NVP in 0.01 M phosphate buffer pH 6

[NVP] µg/ml	Q, µg		Q/[NVP], ml	
	P11	P15	P11	P15
25	9.03	10.59	0.34	0.42
50	13.52	17.40	0.27	0.34
100	20.77	27.52	0.20	0.27
200	30.18	40.65	0.15	0.20
500	37.14	56.58	0.07	0.11

Table A.17 The competitive ELISA using 1:500,000 dilution of NVP-HRP conjugate

polymer	[NVP] µg/ml	Absorbance at 450 nm (B)				% B/B_0
		1st	2nd	3rd	Average	
P11	no (B_0)	0.6898	0.6880	0.6892	0.6890	
	0.1	0.6923	0.7018	0.6968	0.6969	101.15
	1.0	0.6810	0.6850	0.6982	0.6880	99.86
	10	0.6830	0.6996	0.6850	0.6892	100.02
	100	0.6599	0.5476	0.60115	0.6028	87.50
	500	0.5671	0.6053	0.5320	0.5681	82.45
P15	no (B_0)	0.6790	0.6812	0.6833	0.6798	
	0.1	0.6864	0.6849	0.6768	0.6827	100.42
	1.0	0.5322	0.5429	0.5330	0.6809	100.17
	10	0.6551	0.7562	0.6800	0.6971	102.54
	100	0.5845	0.6234	0.3890	0.6156	90.55
	500	0.5109	0.5800	0.5521	0.5476	80.56

Table A.18 The competitive ELISA using 1:20,000 dilution of NVP-HRP conjugate

Polymer	NVP, μg/ml	Absorbance at 450 nm (B)				ΔAbs.
		1st	2nd	3rd	Average	($B-B_0$)
P11	no (B_0)	1.3584	1.2638	1.3862	1.3361	
	10	1.4737	1.3682	1.2901	1.3773	0.0412
	50	1.4970	1.5615	1.6044	1.5543	0.2181
	100	1.7502	1.7608	1.7749	1.7619	0.4257
	200	1.8710	2.2622	2.2358	2.1226	0.7865
	300	2.3892	2.4994	2.3812	2.4232	1.0871
	400	2.5232	2.4566	2.7400	2.5732	1.2371
	500	2.7926	2.7614	2.8028	2.7856	1.4494
P15	no	0.91316	0.9406	0.9469	0.9335	
	10	0.9522	0.9568	0.92632	0.9451	0.0115
	50	1.2059	1.2017	0.9678	1.1251	0.1915
	100	1.2015	1.2424	1.1081	1.1840	0.2504
	200	1.5311	1.358	1.5541	1.4810	0.5475
	300	1.6896	1.7832	2.002	1.8240	0.8913
	400	2.279	1.927	2.3032	2.1697	1.2361
	500	2.2822	2.4436	2.3006	2.3421	1.4085

APPENDIX B

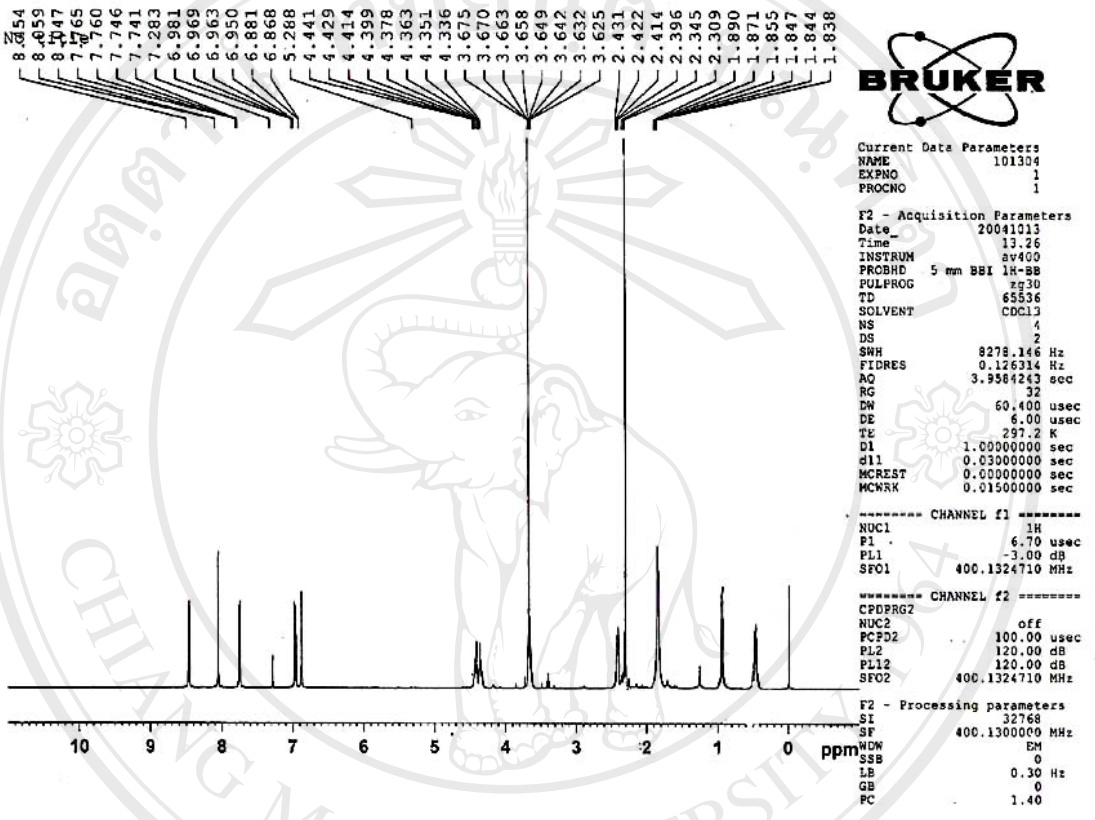

Figure B.1 The 400 MHz ^{1}H-NMR ($CDCl_3$) of compound 1

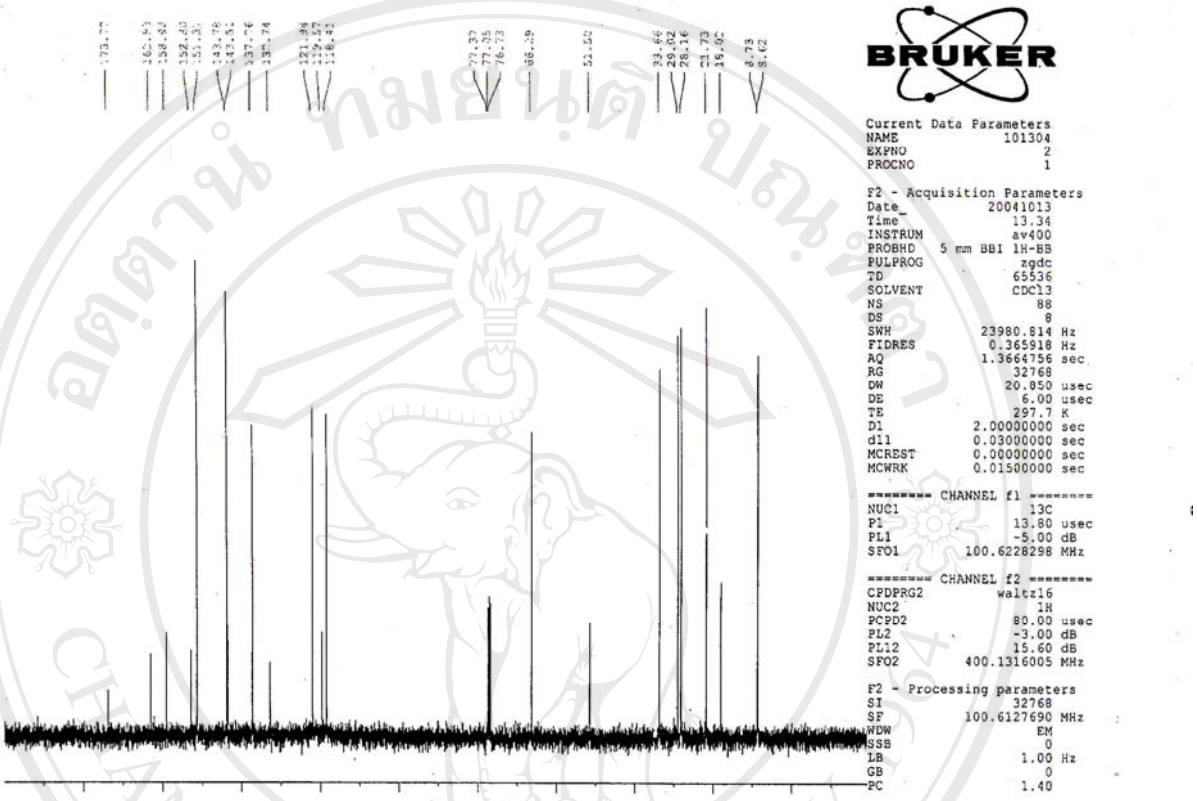

Figure B.2 The 100 MHz ^{13}C-NMR ($CDCl_3$) of compound 1

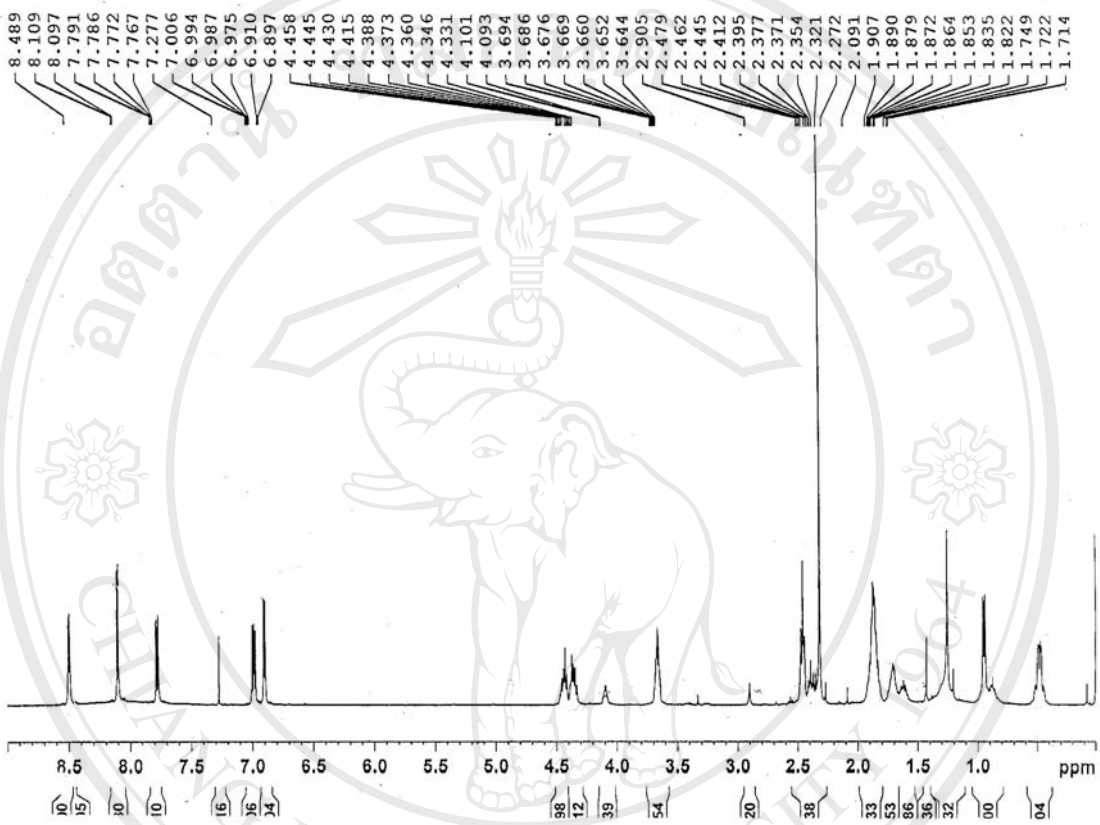

Figure B.3 The 400 MHz ^{1}H-NMR ($CDCl_3$) of compound 2

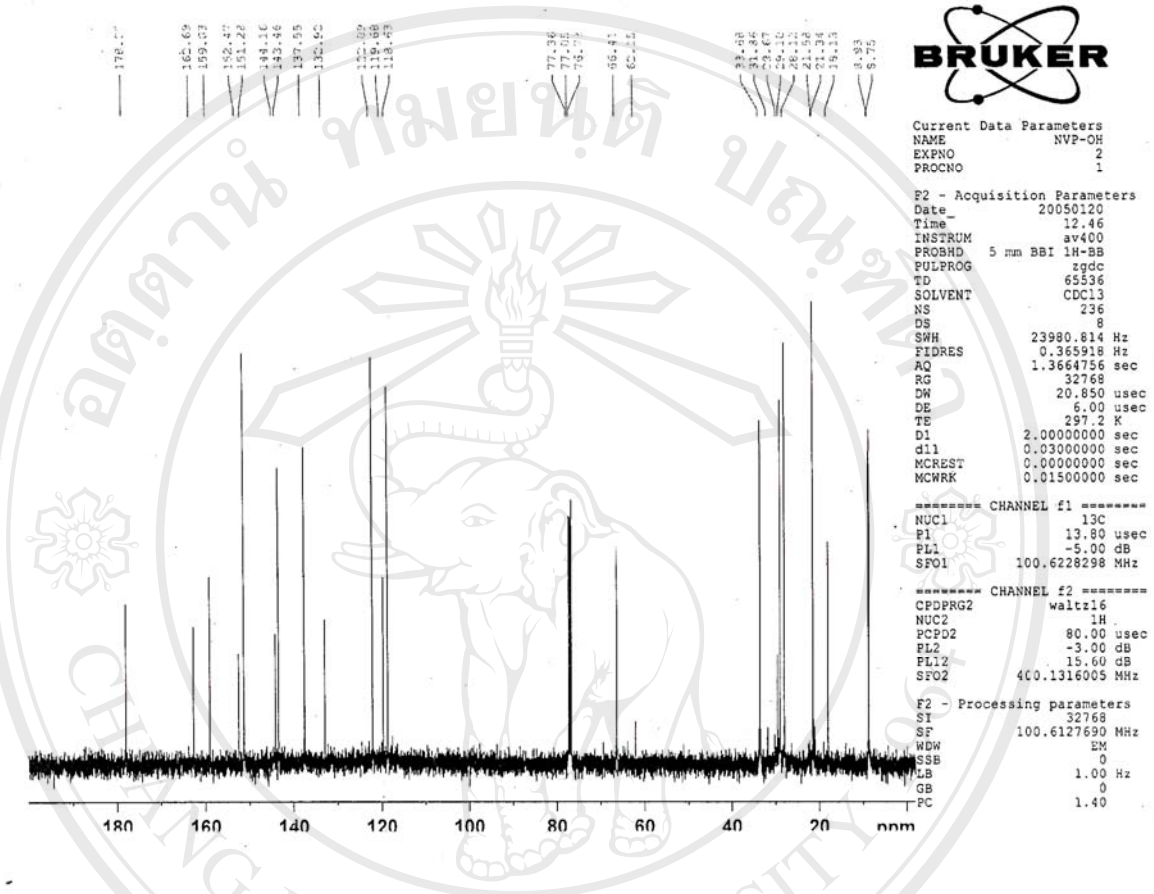

Figure B.4 The 100 MHz ^{13}C-NMR ($CDCl_3$) of compound 2

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