

# Supplementary Materials: A Comparative Study of Enantioseparations of N<sup>α</sup>-Fmoc Proteinogenic Amino Acids on Quinine-Based Zwitterionic and Anion Exchanger-Type Chiral Stationary Phases under Hydro-Organic Liquid and Subcritical Fluid Chromatographic Conditions

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**Table S1.** Temperature dependence of retention factor of first eluting enantiomer ( $k_1$ ), separation factor ( $\alpha$ ) and resolution ( $R_s$ ) of N-Fmoc-protected protein amino acids on ZWIX(+)<sup>TM</sup> and QN-AX<sup>TM</sup> under liquid chromatographic conditions.

Compound	Column	Eluent	$k_1, \alpha, R_s$	Temperature (°C)				
				5	10	20	30	40
Fmoc-Asp(OtBu)-OH	ZWIX(+) <sup>TM</sup>	<b>k</b>	$k_1$	0.46	0.45	0.44	0.41	0.37
			$\alpha$	1.50	1.40	1.32	1.22	1.14
			$R_s$	1.30	1.04	1.04	1.08	0.91
	QN-AX <sup>TM</sup>	<b>w</b>	$k_1$	3.27	2.67	2.57	2.36	2.16
			$\alpha$	2.15	2.03	1.89	1.76	1.63
			$R_s$	11.50	11.33	10.88	10.39	9.25
Fmoc-Lys(Boc)-OH	ZWIX(+) <sup>TM</sup>	<b>k</b>	$k_1$	0.30	0.30	0.30	0.29	0.26
			$\alpha$	1.37	1.33	1.27	1.21	1.15
			$R_s$	1.03	0.93	0.94	0.65	0.76
	QN-AX <sup>TM</sup>	<b>w</b>	$k_1$	1.85	1.69	1.48	1.35	1.25
			$\alpha$	1.99	1.95	1.86	1.76	1.66
			$R_s$	10.11	10.95	10.53	7.29	7.91
Fmoc-Leu-OH	ZWIX(+) <sup>TM</sup>	<b>k</b>	$k_1$	0.30	0.30	0.30	0.29	0.26
			$\alpha$	1.47	1.43	1.38	1.33	1.27
			$R_s$	0.57	0.53	0.27	0.44	0.20
	QN-AX <sup>TM</sup>	<b>w</b>	$k_1$	2.04	1.85	1.63	1.55	1.41
			$\alpha$	2.06	1.98	1.88	1.77	1.68
			$R_s$	9.33	9.25	10.00	7.62	7.57
Fmoc-Phe-OH	ZWIX(+) <sup>TM</sup>	<b>k</b>	$k_1$	0.61	0.59	0.55	0.53	0.49
			$\alpha$	2.02	1.90	1.73	1.55	1.43
			$R_s$	3.15	3.06	2.62	1.75	2.05
	QN-AX <sup>TM</sup>	<b>w</b>	$k_1$	5.03	3.54	3.44	3.20	2.88
			$\alpha$	1.75	1.72	1.60	1.53	1.45
			$R_s$	8.76	9.29	8.85	8.16	7.33
Fmoc-Tyr(tBu)-OH	ZWIX(+) <sup>TM</sup>	<b>k</b>	$k_1$	0.77	0.74	0.66	0.59	0.57
			$\alpha$	1.47	1.38	1.26	1.12	1.02
			$R_s$	1.49	1.21	1.06	0.20	0.20
	QN-AX <sup>TM</sup>	<b>w</b>	$k_1$	5.33	4.21	4.06	3.68	3.23
			$\alpha$	1.27	1.25	1.20	1.16	1.12
			$R_s$	3.87	3.58	3.31	2.75	2.18

Chromatographic conditions: column, ZWIX(+)<sup>TM</sup> and QN-AX<sup>TM</sup>; mobile phase, **k**, H<sub>2</sub>O/MeOH (1/99 v/v) containing 3,75 mM TEA and 7,5 mM FA, **w**, MeOH/MeCN (75/25 v/v) containing 30 mM TEA and 60 mM FA; flow rate, 0.6 mL min<sup>-1</sup>; detection, 262 nm.

**Table S2.** Effect of temperature on chromatographic parameter  $k_1$ ,  $\alpha$ , and  $R_s$  of *N*-Fmoc-protected amino acids on QN-AX<sup>TM</sup> column under SFC conditions.

Compound	$k_1$ , $\alpha$ , $R_s$	Temperature (°C)			
		20	30	40	50
<b>Fmoc-Asp-(<i>t</i>Bu)-OH</b>	$k_1$	5.83	5.41	5.06	4.68
	$\alpha$	1.72	1.67	1.59	1.50
	$R_s$	8.61	8.59	8.03	7.36
<b>Fmoc-Lys(Boc)-OH</b>	$k_1$	5.65	5.35	5.06	4.78
	$\alpha$	1.59	1.57	1.51	1.49
	$R_s$	7.17	7.19	6.83	6.31
<b>Fmoc-Leu-OH</b>	$k_1$	-	4.94	4.57	4.14
	$\alpha$	-	1.73	1.62	1.53
	$R_s$	-	9.21	8.76	8.02
<b>Fmoc-Phe-OH</b>	$k_1$	12.07	11.12	10.16	9.03
	$\alpha$	1.39	1.37	1.33	1.29
	$R_s$	5.99	5.76	5.58	5.19
<b>Fmoc-Tyr(<i>t</i>Bu)-OH</b>	$k_1$	12.21	12.00	10.99	9.17
	$\alpha$	2.00	1.96	1.89	1.83
	$R_s$	10.98	11.25	11.88	12.32

Chromatographic conditions: Column, Chiralpak QN-AX<sup>TM</sup>; mobile phase, CO<sub>2</sub>/MeOH (60/40 *v/v*) containing 30 mM TEA and 60 mM FA; flow rate, 2 mL min<sup>-1</sup>; detection, 264 nm; T<sub>col</sub>, 20–40 °C; back pressure, 150 bar