

Supplementary material.

Table S1 inactivation constants for BTL2 derivatives immobilized in presence of 20 mM DTT except for the References.

Derivative Production Conditions		At 75 °C ^a				In dioxane 80% ^a			
Immobilization step (pH)	Incubation step (pH)	k ₁ (h ⁻¹)	k ₂ (h ⁻¹)	E ₁ /E	E ₂ /E	k ₁ (h ⁻¹)	k ₂ (h ⁻¹)	E ₁ /E	E ₂ /E
7.0	7.0	1.18 × 10	3.77 × 10 ⁻²	7.52 × 10 ⁻¹	1.52 × 10 ⁻¹	3.64	6.12 × 10 ⁻²	5.44 × 10 ⁻¹	6.87 × 10 ⁻²
7.0	8.0	1.18 × 10	1.94 × 10 ⁻²	4.57 × 10 ⁻¹	4.14 × 10 ⁻¹	7.51	1.74 × 10 ⁻²	8.28 × 10 ⁻¹	2.49 × 10 ⁻¹
7.0	10.1	1.18 × 10	3.04 × 10 ⁻²	9.87 × 10 ⁻¹	4.57 × 10 ⁻¹	1.87	1.28 × 10 ⁻²	7.57 × 10 ⁻¹	2.34 × 10 ⁻¹
8.0	8.0	1.18 × 10	2.39 × 10 ⁻²	7.96 × 10 ⁻¹	2.16 × 10 ⁻¹	1.15	3.72 × 10 ⁻²	4.86 × 10 ⁻¹	1.15 × 10 ⁻¹
8.0	10.1	1.18 × 10	2.19 × 10 ⁻²	9.92 × 10 ⁻¹	4.92 × 10 ⁻¹	2.10	2.67 × 10 ⁻²	7.74 × 10 ⁻¹	2.34 × 10 ⁻¹
Reference Gx derivative (pH 9)	10.1	1.18 × 10	1.96 × 10 ⁻²	1.02	2.17 × 10 ⁻¹	1.91	3.01 × 10 ⁻²	8.41 × 10 ⁻¹	1.58 × 10 ⁻¹
Reference CNBr derivative (pH 7.0)	-	2.20 × 10	1.44 × 10 ⁻¹	5.60 × 10 ⁻¹	4.89 × 10 ⁻²	4.03	5.70 × 10 ⁻¹	6.07 × 10 ⁻¹	4.89 × 10 ⁻²

^a Values according to the best-fit model (two-stage inactivation with residual activity); k₁ and k₂ are kinetic constants for the first-order inactivation rate constants for the first and second step respectively; E₁/E and E₂/E are the fractions of the initial enzyme specific activity (E) for the forms E₁ and E₂ resulting from each inactivation step [47,49].