In this work, an enantioselective HPLC method with UV and fluorimetric detection was developed and subsequently optimized for chiral separation of four aminoacids (D/L-alanine, D/L-valine, D/L-leucine, D/L-isoleucine) in native and particularly in derivatized form with an emphasis on enantioseparation of D-analogues. Retention and enantioseparation behavior of studied analytes was investigated on three chiral stationary phases based on teicoplanin (Chirobiotic T, Chirobiotic T2) and teicoplanin aglycone (Chirobiotic TAG). At the Chirobiotic T column, enantioseparations of underivatized aminoacids were performed with UV detection at 205 nm in the mobile phases methanol/water with different volume ratios. Baseline separation of L- and D-forms was achieved, however, the sensitivity of detection was very low. In order to increase detection sensitivity, derivatization of aminoacids was performed using 9-fluorenylmethyl chloroformate (FMOC-Cl) and the derivatization procedure was monitored on Chirobiotic T column with fluorimetric detection (λ_{Ex} = 254 nm, λ_{Em} = 314 nm) in a buffered mobile phase methanol/0.5% TEAA buffer, pH 6.0 40/60 (v/v). In terms of derivatization, volume ratio D/L-aminoacid/derivatization agent 1/1 with ten times higher concentration of derivatization agent was found to be the most suitable. Mobile phases consisting of methanol in combination with 0.5 or 1.0% water solution of TEAA buffer with pH range 4.0 – 7.0 in various volume ratios were used for study of retention and enantioseparation behavior of derivatized FMOC-aminoacids on all tested columns. Influence of methanol content as well as composition and pH value of aqueous component of mobile phase was studied. The Chirobiotic T column appeared to be the most suitable as it provided complete separation of FMOC-derivatives of D-Val, D-Ala and D-Leu and nearly complete separation all four D-aminoacid derivatives in the mixture in the mobile phase consisting of methanol/0.5 (1.0)% TEAA buffer, pH 6.0 40/60 and 38/62 (v/v), respectively. Under the optimized conditions, quantification of individual FMOC-D- and FMOC-L-enantiomers as well as FMOC-D-enantiomers in mixture was performed. Limits of detection ranged from 1.9 to 76.1 ng/ml. On Chirobiotic T2 and Chirobiotic TAG columns, no partial separation of FMOC-D-forms of aminoacids was observed for any of the tested separation systems. Only FMOC-L- and FMOC-D-enantiomers of Val, Leu and Ile were quantified on Chirobiotic TAG column.