-- Supporting Information --

Sequential Prodrug Strategy to Target and Eliminate ACPA-Selective Autoreactive B cells

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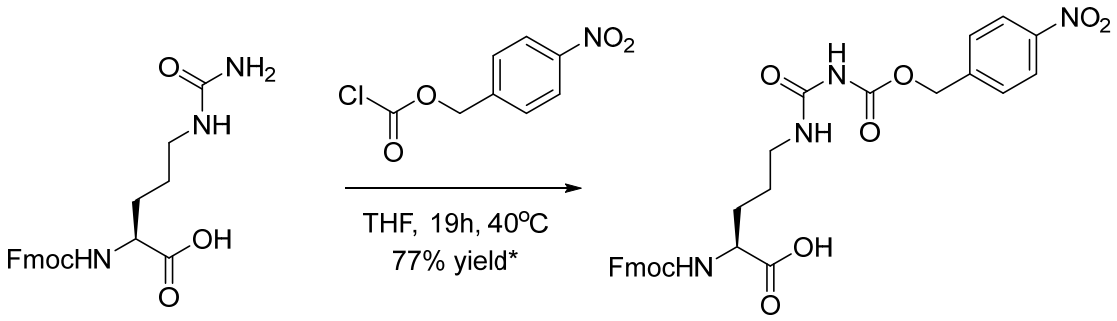
General methods and materials. Unless stated otherwise, all chemicals were used without further purification. Amino acids were obtained from Bachem (Bubendorf, Switzerland) or Novabiochem (EMD Chemicals, Gibbstown, USA). Solvents were purchased from J.T. Baker, Biosolve, Fisher Scientific and Merck Millipore and used as received. If no further details are given the reaction was performed under ambient atmosphere and temperature. Analytical thin layer chromatography (TLC) was performed on silica gel-coated plates (Merck, 60 F254) with the indicated solvent mixture, visualization was done using ultraviolet (UV) irradiation (λ = 254 nm) and/or staining with aqueous $KMnO_4$. Purification by column chromatography was carried out using silica gel 60 (Merck, 0.040-0.063 mm). ^{1}H-NMR chemical shifts (δ), recorded on a Bruker 500 MHz Avance III spectrometer equipped with a Prodigy BB cryoprobe, are reported in parts per million (ppm) relative to a residual proton peak of the solvent, δ = 7.26 for $CDCl_3$. ^{13}C-NMR chemical shifts (δ), are reported in parts per million (ppm) relative to $CDCl_3$ (δ = 77.16). Coupling constants are reported as a J-value in Hertz (Hz). Low-resolution mass spectra (LRMS) were recorded on Thermo LCQ Advantage Max (ESI). A Thermo Finnigan LCQ Fleet ESI ion-trap mass spectrometer, which is equipped with a Shimadzu HPLC (C18-column, particle size 3 µm, acetonitrile/water gradient 5 - 100%, in 16 minutes and a flow of 0.2 ml/min) and a PDA detector, was used to separate organic compounds and record low-resolution mass spectra. High-resolution mass spectra (HRMS) of small molecules were recorded on a JEOL AccuTOF JMS-T100CS (ESI). Preparative HPLC was performed on a Shimadzu LC-20A Prominence system (Shimadzu, 'sHertogenbosch, The Netherlands) equipped with a Gemini NX-C18 column, 150 × 21.2 mm, particle size 10 µm (Phenomenex, Utrecht, The Netherlands). Gradient used was acetonitrile/water 5-50%, in 30 minutes and a flow of 6 ml/min. Analytical HPLC measurements were performed on a Shimadzu LC-20A Prominence system (Shimadzu, 's-Hertogenbosch, The Netherlands) equipped with a Gemini NX-C18 column, 150 × 3 mm, particle size 3 µm (Phenomenex, Utrecht, The Netherlands). Gradient used is acetonitrile/water 5 - 100%, in 30 minutes and a flow of 0.4 ml/min.

Injected peptides were monitored at 254 nm and 215 nm and the desired peaks were integrated manually using a LabSolutions software package (Shimadzu, ‘sHertogenbosch, The Netherlands).



Scheme S1. Citrulline protection with carboxy-*p*-nitrobenzyl. *Yield after recovered starting material.

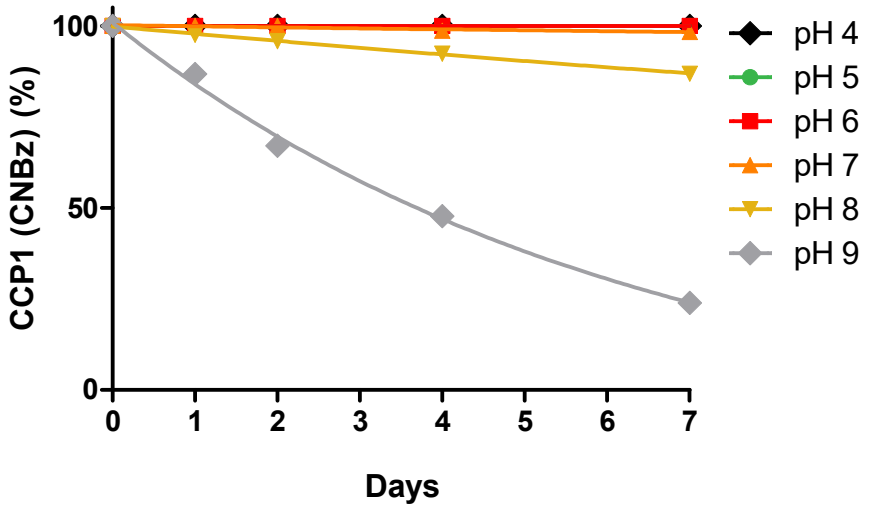


Figure S1. The stability of the CNBz linker of the protected CCP1 in pH 4-9 (McIlvaine buffer) for 1-7 days at 37°C and 600rpm.

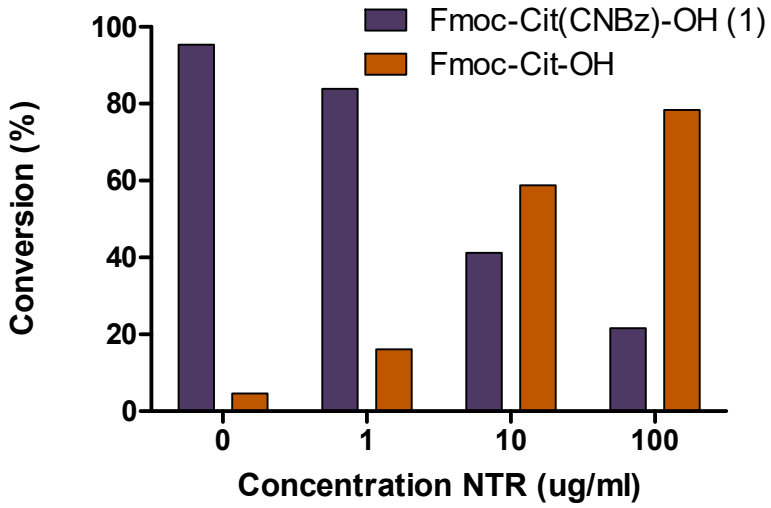


Figure S2. Removal of CNBz from Fmoc-Cit(CNBz)-OH using nitroreductase at t = 5min at 37 °C in PBS pH 7.4 analyzed by analytical HPLC. Y-axis represents the percentage of material, either Fmoc-Cit(CNBz)-OH starting material (purple) or Fmoc-Cit-OH deprotection product (orange) observed. Different amounts of nitroreductase were added and conversion were measured after 5 minutes. Using 100 µg/ml NTR (represents 0.3 equiv.) a near to complete citrulline peptide was observed.

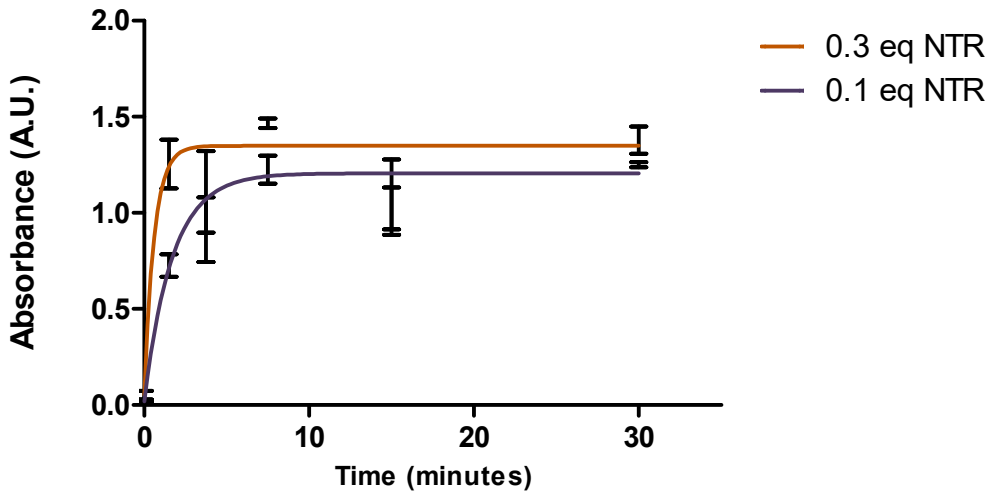


Figure S3. Activation of 10 µM CCP1(CNBz) in PBS pH 7.4 at 37 °C followed over time by ELISA. The deprotection using NTR was stopped by adding nicotinic acid (20 eq. compared to NADH, which was used in a 50 times excess over CCP1(CNBz)) at various time points, to replace the NADH necessary for NTR activity. Error bars are standard deviations calculated using GraphPad Prism. A.U. absorbance units.

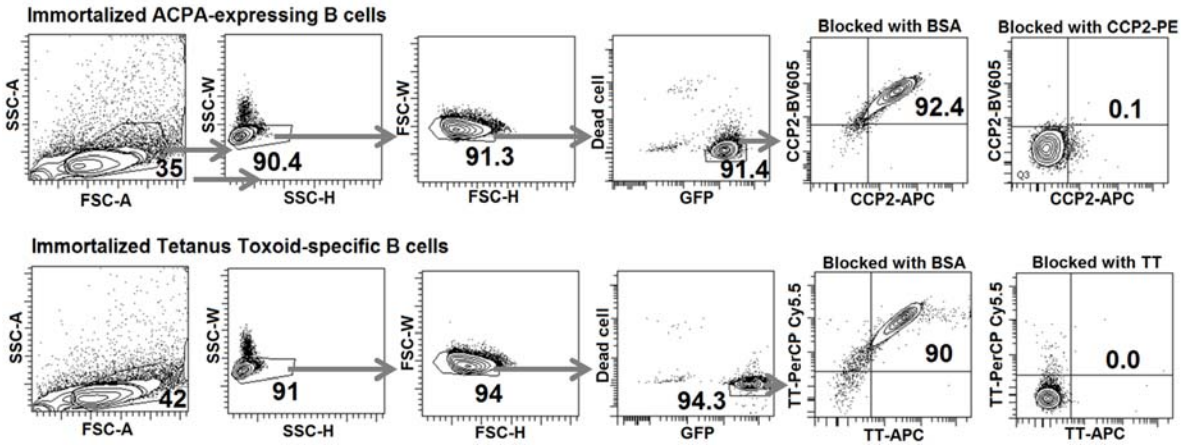


Figure S4. Antigen specificity of ACPA-expressing and TT-specific B cell clones. Primary ACPA+ and TT+ MBC were sorted and immortalized using lentiviral transduction with BCL-6 and BCL-XL. The immortalized ACPA-expressing B cell clone stains positive for CCP2-APC- and CCP2-BV605-tetramers and the signal can be blocked by pre-incubation with an excess of CCP2-PE-tetramer. Immortalized TT-specific B cells stain positive for directly labelled TT-APC and TT-PE; the signal can be blocked by pre-incubation with an excess of unlabeled TT.

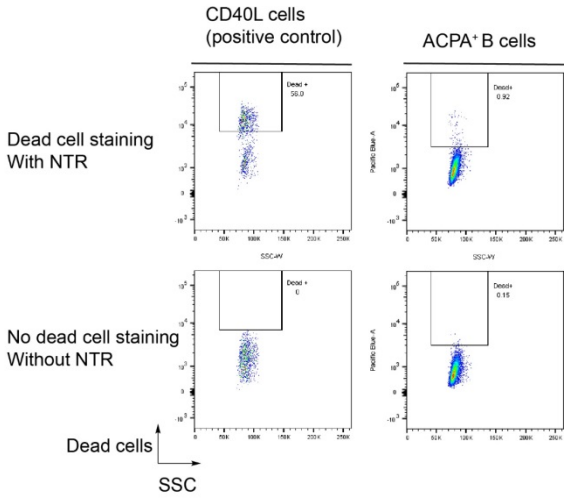
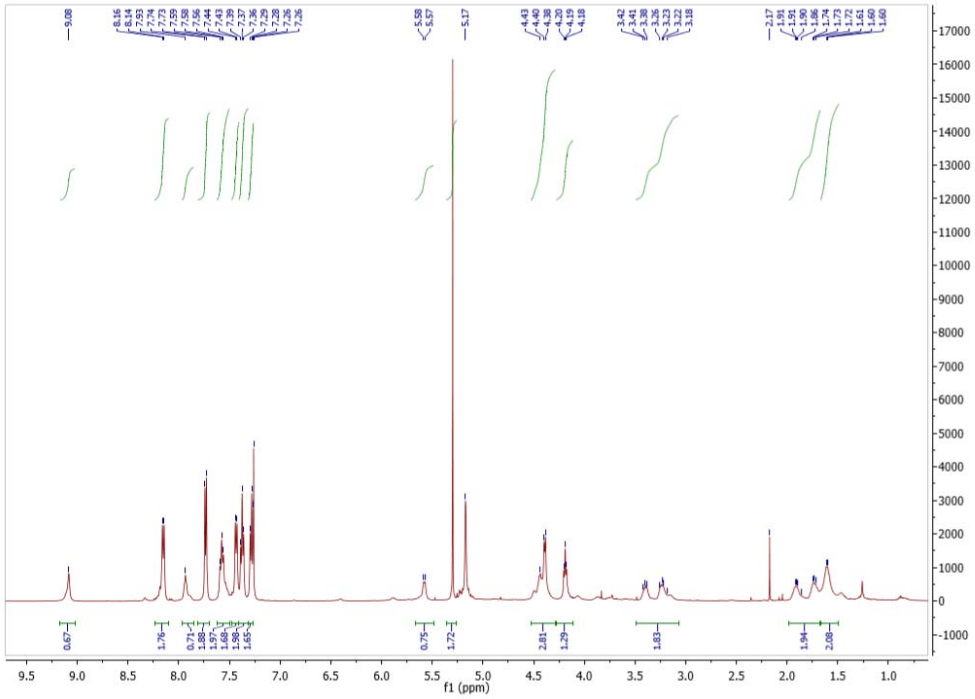


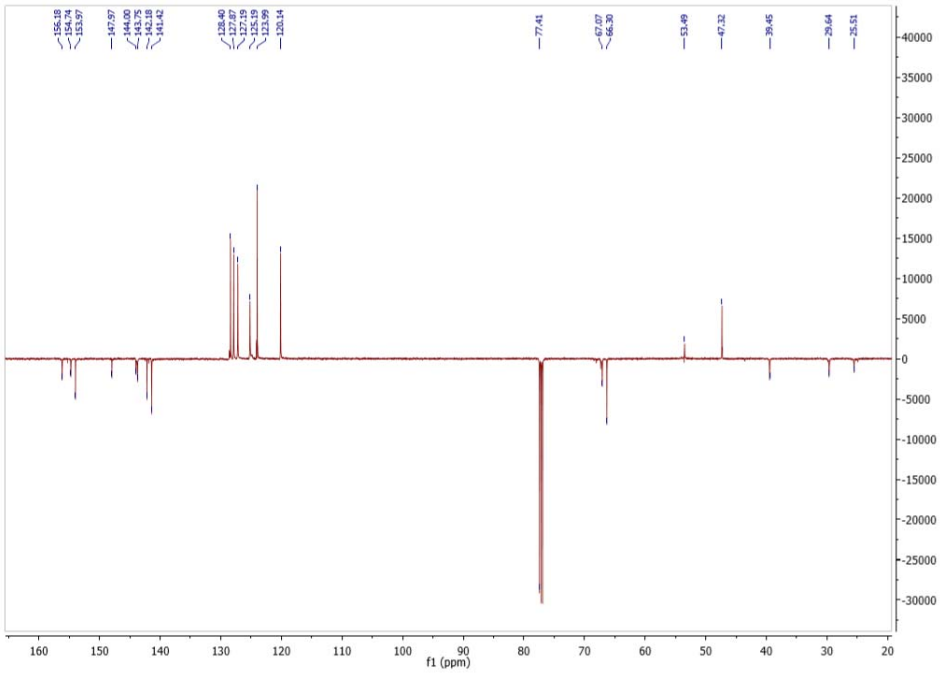
Figure S5. Less than one percent of ACPA expressing B cells is positive for dead cell staining when incubated with NTR at 37°C for 1h. As a positive control, over fifty percent of the CD40L cells (cultured with ACPA expressing cells) stains positive for death cells. This shows that NTR on its own is not toxic for B cells within 1 hour.

Characterization of compounds **1**-**4**.

^{1}H NMR Fmoc-Cit(CNBz)-OH (**1**)

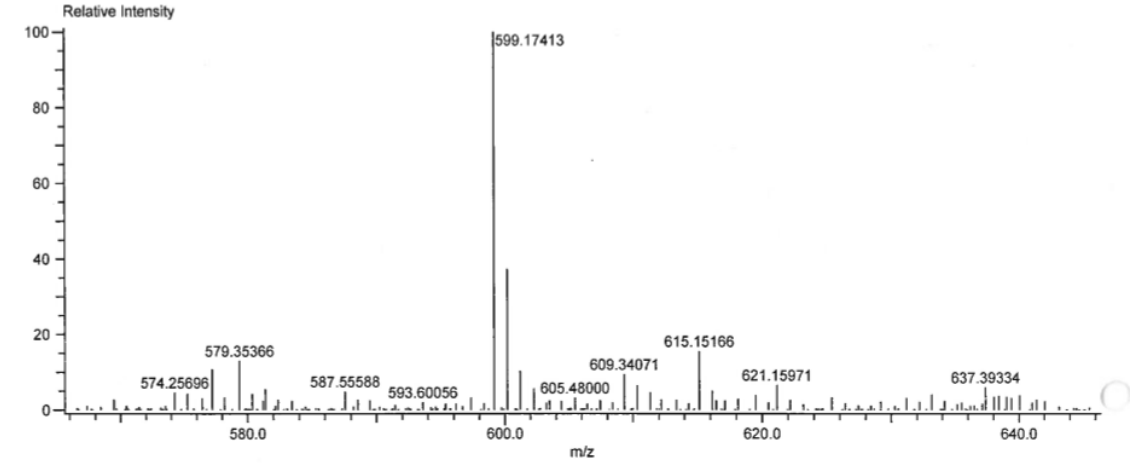


^{13}C NMR Fmoc-Cit(CNBz)-OH (**1**)



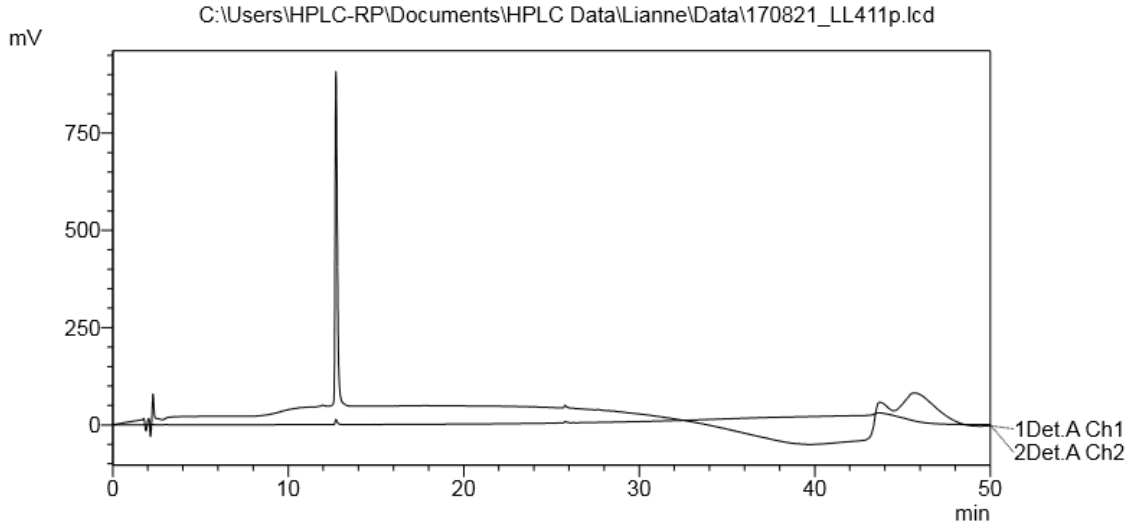
HRMS of Fmoc-Cit(CNBz) (**1**)

Charge number:1 Tolerance:5.00(ppm), 3.00 .. (mmu) Unsaturation Number:-1.5 .. 99.0 (Fraction:Both)
Element:^{12}C:25 .. 100, ^{1}H:1 .. 200, ^{14}N:1 .. 10, ^{23}Na:0 .. 1, ^{16}O:1 .. 20

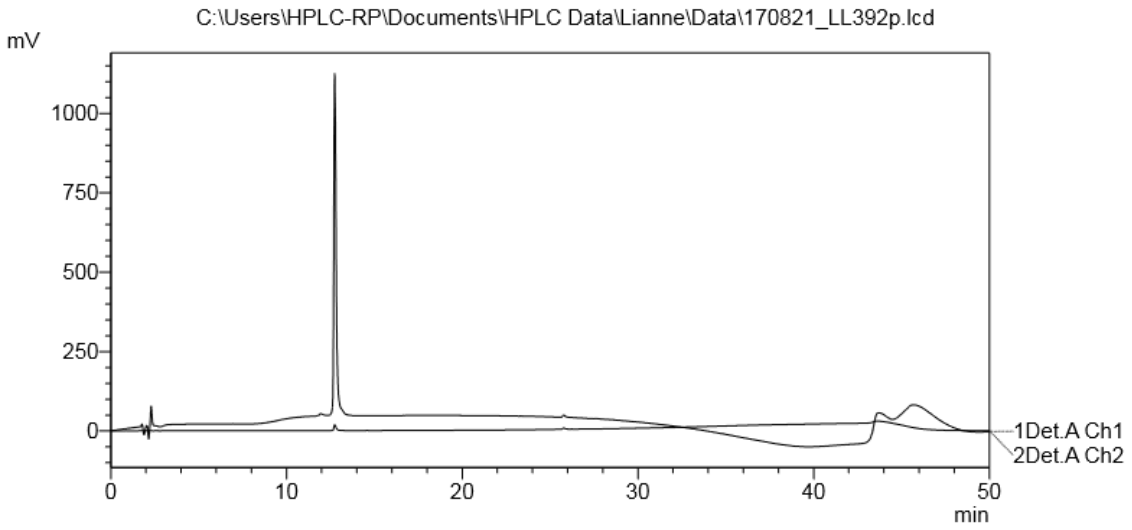


Mass	Decision	Calc. Mass	Mass Difference (mmu)	Mass Difference (ppm)	^{12}C	^{1}H	^{14}N	^{23}Na	^{16}O	Unsaturation Number
599.17413	+	599.17540	-1.26	-2.11	29	28	4	1	9	17.5
	?	599.17405	0.08	0.13	27	26	7	1	8	18.0
	?	599.17378	0.35	0.59	26	27	6		11	16.5
	?	599.17461	-0.48	-0.80	41	21	5		1	34.0
	?	599.17355	0.59	0.98	41	24	2	1	2	30.5
	?	599.17327	0.86	1.44	40	25	1		5	29.0
	?	599.17512	-0.98	-1.64	27	23	10		7	21.5
	?	599.17512	-0.99	-1.65	28	29	3		12	16.0
	?	599.17272	1.42	2.36	26	30	3	1	12	13.0
	?	599.17271	1.42	2.37	25	24	10	1	7	18.5
	?	599.17244	1.69	2.82	25	31	2		15	11.5
	?	599.17595	-1.82	-3.04	43	23	2		2	33.5
	?	599.17220	1.93	3.22	39	22	5	1	1	31.0
	?	599.17193	2.20	3.68	38	23	4		4	29.5
	?	599.17646	-2.33	-3.88	29	25	7		8	21.0
	?	599.17673	-2.60	-4.34	30	24	8	1	5	22.5
	?	599.17674	-2.61	-4.35	31	30	1	1	10	17.0

HPLC of CArgP1 (**2**)



HPLC of CCP1 (**3**)



HPLC of CCP1(CNBz) (**4**)