

Oligonucleotide Synthesis by KNAUER - KNAUER Devices can do even more than Liquid Chromatography

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WHY SYNTHESIS OF OLIGONUCLEOTIDES?

The oligonucleotide market is growing and gaining a significant impact in pharmaceutical applications. Therapeutic oligonucleotides include antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), anti-micro RNAs (anti-miRNAs), aptamers and mRNA. Mostly these Oligonucleotides consist of 15-40 nucleobases and can be conveniently synthesized chemically. The chemical synthesis is commonly done automated on a solid support using an oligonucleotide synthesizer consisting of pumps, valves and detectors to choose and deliver chemical reagents and surveille the ongoing synthesis.

KNAUER DEVICES CAN DO EVEN MORE THAN LIQUID CHROMATOGRAPHY

KNAUER offers all components needed to build a reliable Oligonucleotide Synthesizer and as the demand for such devices is growing, we set out to test our established Liquid Chromatography devices in a proof-of-concept oligonucleotide synthesis setup.

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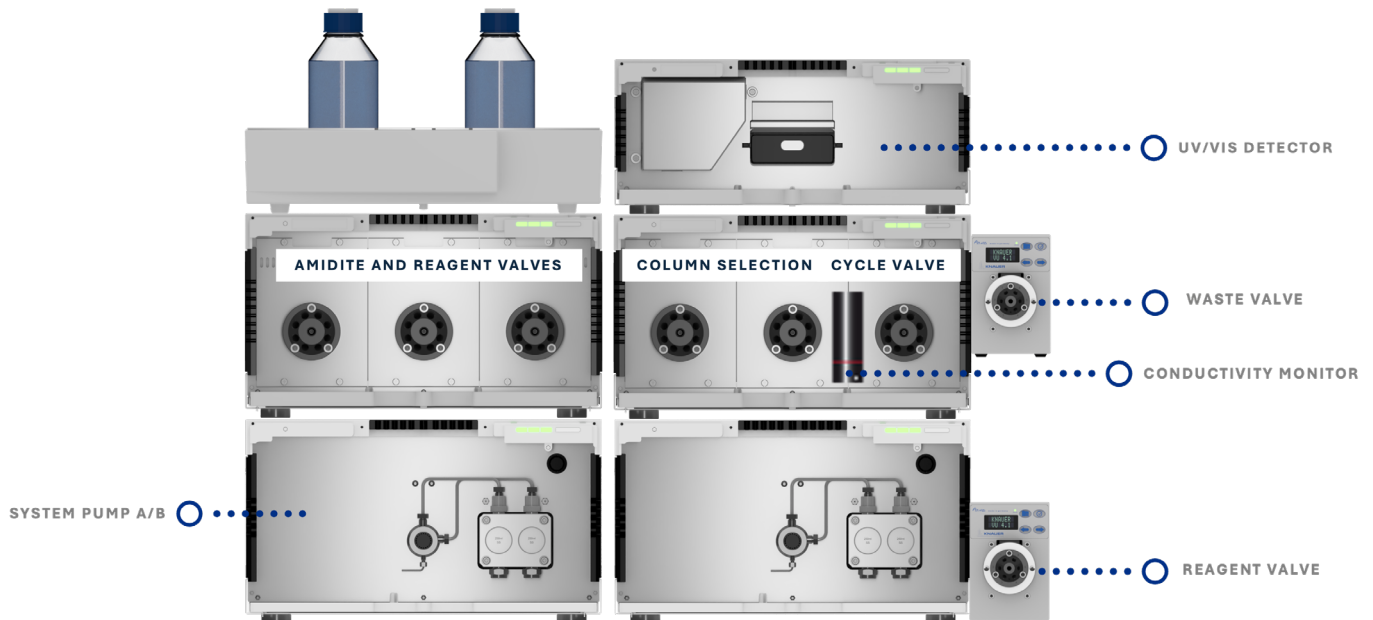


Fig. 1 System configuration

Our system consists of two pumps with maximum flow rates of 250 ml/min each, four chemical selection valves, with capacity for 12 amidites and 11 reagents and solvents, column selection of up to 7 columns and one

bypass as well as a recirculation valve and a waste valve with 7 waste outlets. Detection of detritylation, oxidation and thiolation is carried out using a multiwavelength UV Detector and a conductivity monitor.

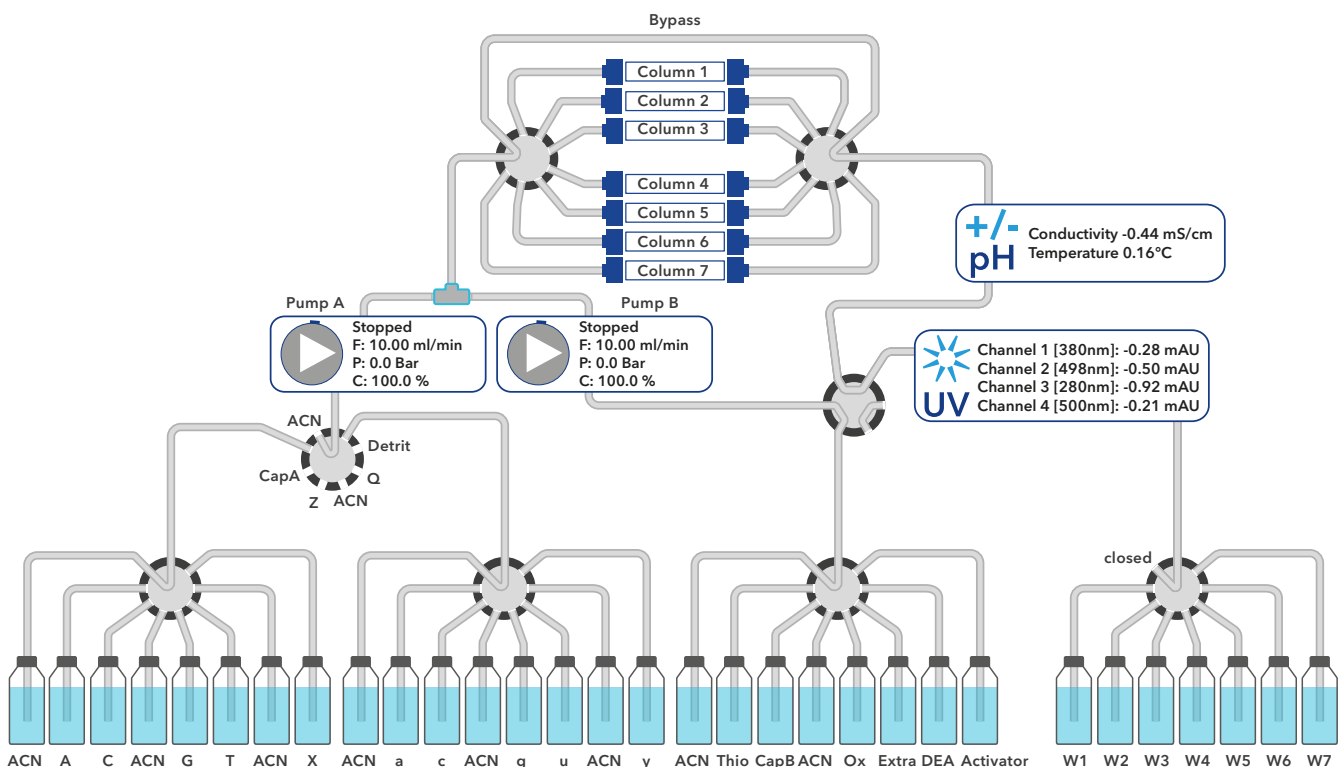


Fig. 2 System Overview in PurityChrom® 6

EXPERIMENTAL SETUP AND SCALING TESTS

With all our knowledge about pumps, valves and detectors in hand, we set out to test the optimal scaling for the devices to be used. We synthesized DNA and RNA test sequences in different scales. The DNA sequence we synthesized we could compare to another oligonucleotide synthesizer with established synthesis protocols. To our delight we could already see after minor method development excellent results for crude purities and yields compared to results for this DNA sequence using another synthesis system, which is well established in the field.

Tab. 1 Overview of crude yield and purities compared to synthesis results of the same sequence on an established synthesizer

Scaling	Relative crude yield compared to competitor synthesizer	Improvement of purity
2 mmol	123 %	6,8 %
4 mmol	116 %	6,3 %
6 mmol	123 %	4.1 %

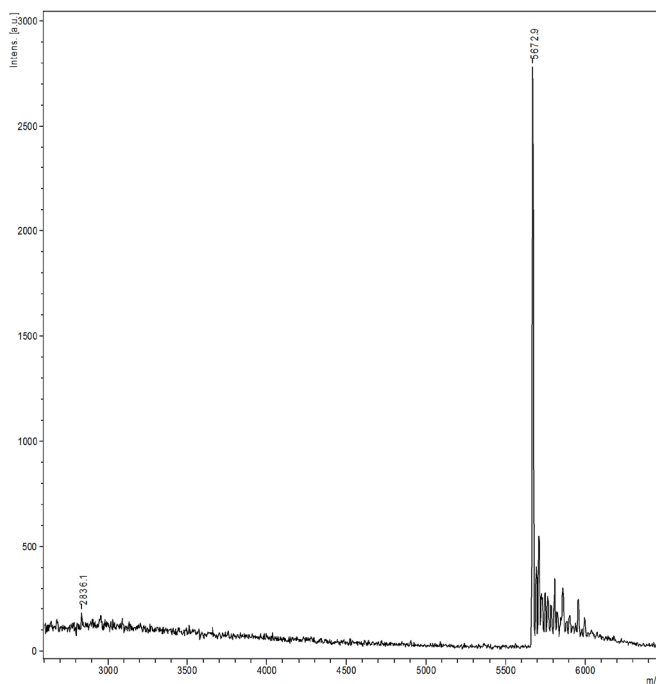


Fig. 3 MALDI of crude product

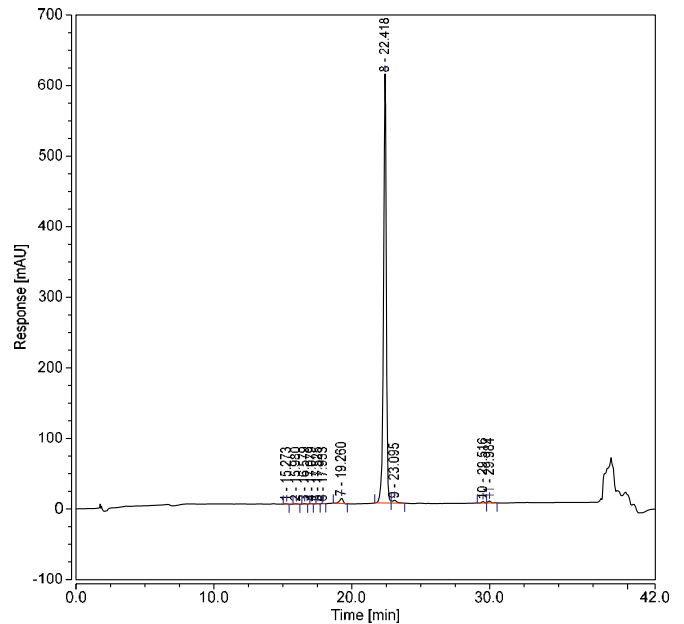


Fig. 3 LC-UV of crude product

In our set up we could not only show that we can synthesize DNA and RNA but also we could verify that in comparison to the well established synthesis protocols, we can improve crude yield and purity for a test sequence. Hence, we can say that the KNAUER devices are perfectly suited for the task of oligonucleotide synthesis in scaling from 1 mmol to 10 mmol.

CONCLUSION AND OUTLOOK

We could show that an oligonucleotide synthesis using KNAUER devices is delivering oligonucleotides with impressive purity in great yields. Thanks to our long history of continuously improving our pump, valve and detection devices we have all the tools in hand to put together an excellent synthesizer. Therefore, we are working with greatest effort on the overall KNAUER Oligonucleotide Synthesizer Package, including a dedicated Software covering all needs for the easy and convenient Oligonucleotide synthesis.

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MATERIAL AND METHODS

Instrument	Description	Article No.
Pump	AZURA® Pump P 2.1L with 250 ml/min pump head (titanium)	APE20LC
Pump accessory	Pump Head Inlet	A9868
Pump accessory	Adapter female 1/4-28 Flat Bottom to 1/2-20 UNF	A142605
Detector	AZURA Detector MWD 2.1L versatile Multi Wavelength UV-Detector	ADB01
Flow Cell	Fiber Optics Adapter Kit	AMKX8KIT
Flow Cell	Preparative UV Flow Cell, 1/8"	A4079
Conductivity Monitor	Mikron 81 with flow cell for up to 1000 ml/min	ADG61GE
ASM 2.2L	AZURA Assistant Customizable docking station for pumps, valves and detectors Left Module: Valve drive VU 4.1 Middle Module: Valve drive VU 4.1 Right Module: Valve drive VU 4.1	AY0052
Valve Drive	Valve Drive VU 4.1	AWA01XA
Valve	Multiposition valve, 8 Port for chemical and solvent selection	AVU32GE
Valve	2-position valve, 6 Port for recirculation	AVF23CE
Back Pressure	Back Pressure regulator	A5808
Software	PurityChrom® 6 – Basic License	A2680